FACULTY OF SCIENCES

Amine-Thiolactone-Ene Conjugation: from Polymer Modification to Sequence-Controlled Polymers

Steven Martens

Promotor:
Prof. Dr. Filip Du Prez

Academic year 2018-2019

A dissertation submitted to Ghent University to obtain the degree of Doctor of Science: Chemistry

GHENT UNIVERSITY

FACULTY OF SCIENCES

Amine-Thiolactone-Ene Conjugation: from Polymer Modification to Sequence-Controlled Polymers

Steven Martens

Supervisor: Prof. Dr. Filip Du Prez

A dissertation submitted to Ghent University to obtain the degree of Doctor of Science: Chemistry

Academic year 2018-2019

GHENT UNIVERSITY

Exam commission

Prof. Dr. Richard Hoogenboom (Chair, Ghent University)

Prof. Dr. Annemieke Madder (Ghent University)

Prof. Dr. Ir. Bruno De Geest (Ghent University)

Dr. Nezha Badi (Université de Strasbourg, France)

Prof. Dr. Hans Börner (Humboldt-Universität zu Berlin, Germany)

Prof. Dr. Filip Du Prez (Promotor, Ghent University)



Research funded by the Research Foundation – Flanders

Onderzoek gefinancierd door het Fonds Wetenschappelijk Onderzoek – Vlaanderen

Dankwoord

Hier sta en schrijf ik dan. Na vijf jaar doctoreren is het laatste experiment uitgevoerd, het doctoraat geschreven, en mijn kennis doorgegeven aan opvolgers. Het ging niet altijd van een leien dakje en leidde vaak tot frustraties, maar er waren ook heel mooie momenten. Ik was altijd van mening dat een doctoraat iets was dat je op je eigen houtje moest klaren, maar ik heb (misschien een beetje te laat) beseft dat ik er ontzettend naast zat. Samenwerkingen en hulp vragen waren een noodzaak om van mijn doctoraat een pareltje te maken. Ik wil dan ook een aantal mensen bedanken die mij gedurende deze vijf jaar doctoraat heel hard gesteund hebben.

Eerst wil ik mijn dank uiten aan Prof. Filip Du Prez, die mij de kans gaf om mijn onderzoek uit te voeren in zijn groep. Ook al had ik in mijn eerste jaar geen directe funding zoals een FWO- of IWT-beurs, toch mocht ik blijven om mijn doctoraat te starten. Ik was heel dankbaar voor het vertrouwen en de kans die ik kreeg. Hij is een promotor waarbij de deur altijd openstaat voor ondersteuning en raad. Ik besef dat ik gedurende mijn doctoraat deze deur, die nochtans recht tegenover het labo lag, niet altijd vond, maar ik wist wel dat ik altijd welkom was als er iets scheelde. Filips motivatie, gedrevenheid en passie hebben geleid tot een onderzoeksgroep waarin gevarieerd, diepgaand en uitgebreid onderzoek wordt gevoerd, en dat gaat allemaal gepaard met een sterke samenwerking en veel plezier. Ik hoop ook van harte dat hij heeft kunnen genieten van mijn ‘gezang’ de laatste vijf jaar.

Dr. Pieter Espeel wil ik uit de grond van mijn hart bedanken voor zijn kritische zin, zijn inzet, de goede opvolging, de creativiteit, enzovoort. Ik kon met mijn vragen altijd bij hem terecht en mijn doctoraat zou er waarschijnlijk niet zo uitgezien hebben als hij zich er niet mee bezig had gehouden. Ik vond het heel jammer dat hij bij ons moest vertrekken, maar ik was achteraf nog altijd welkom met mijn vragen. Ik denk met plezier terug aan de restolunches, de conferenties, de vrijdagavonden om 16u50 wanneer Frank en ik met elkaars onderzoek mochten lachen... Ik ben de voorlaatste overblijver van zijn erfenis, en daar ben ik heel trots op.

Ik ben ook alle leden van de PCR-groep dankbaar voor de vijf voorbije jaren. Deze groep weet wat het is om hard te werken en elke dag (wetenschappelijke) kwaliteit te leveren, maar ook om plezier te maken tijdens happy hours, teambuildings, kerstfeestjes, housewarmings en ‘komen drinken’-avonden. Ik durf geen opsomming te maken van alle vorige en huidige leden, want het lijkt mij veel te riskant om iemand te vergeten. Hoewel ik iedereen heel dankbaar ben, wil ik toch een paar mensen extra belichten. Bernhard bijvoorbeeld. Ik denk dat buitenstaanders soms maar half beseffen hoe cruciaal deze man is voor onze doctoraten. Hij houdt de groep draaiend door zijn technisch inzicht en ondersteuning, het doorgeven van bestellingen, heel wat andere taken en natuurlijk zijn humor. De steun, de discussies en onnozele momenten samen met buddy Frank zal ik ook niet snel vergeten. Yamin en Annelies ben ik dankbaar voor het vertrouwen dat ze hadden om mij als begeleider te kiezen en ik wil hen bedanken voor hun

harde werk en hun toewijding die bijgedragen hebben aan deze thesis. De leden van labo 1 waren cruciaal voor de wetenschappelijke discussies, babbels, lachwekkende momenten en het tolereren van mijn zangtalent als ik weer eens de Soundmixshow probeerde te winnen. Ik wil ook de leden van het sequence-team bedanken. Ik ben deze reis gestart met enkel Pieter aan mijn zijde, maar nu is het een heus team geworden. Ik wens jullie het allerbeste met jullie onderzoek en hoop nog veel te horen over jullie wetenschappelijke ontdekkingen. It was nice to meet Angel and Susanne during my time at Ghent University, and I would like to thank them for the pleasant collaboration, inspiring discoveries and fun moments. In het algemeen denk ik dat Filip trots mag zijn op het onderzoeksteam dat hij had, heeft en in de toekomst nog zal hebben.

Naast de leden van de eigen groep wil ik ook andere mensen bedanken die onmisbaar waren voor dit doctoraat: Isabel, Gonzalez en Prof. Devreese voor alle MS/MS-metingen en toelichting bij de analyse van de spectra, Dorien, Willem en Prof. Madder voor de leuke samenwerkingen en wetenschappelijke discussies, Jos om mij wegwijs te maken met de synthetische robot, Prof. Dawyndt voor de ontwikkeling van de algoritmes, Tim Courtin voor de NMR-metingen en ing. Jan Goeman voor de ontelbaar vele LC-MS- en HRMS-metingen.

Mijn vrienden Pieter-Jan, Heleen, Nico, Nina, Tim, Steph, Jonas, Kim, Stan, Delphine, Reinout, Sara, Jolien, Wouter, Kyrill, Joyce, Thomas, Sara, Soetkin, Kin, Sebas, Aure, Tessie, Patriek, Kim en Bert voor het grote plezier en de steun die ze me bezorgd hebben gedurende deze vijf jaar doctoraat.

Ik wil mijn broer Bart, schoonzus Kim, mijn gekke neefjes Oscar en Marcel, mijn ouders Michel en Monique en mijn schoonouders Ignace en Rein ook ontzettend bedanken voor het geloof, de raad en het geduld dat ze hadden om mij doorheen deze opdracht te volgen. Hoewel chemie voor velen van hen iets vrij onverstaanbaars is, bleven ze mij toch steunen door alles heen.

Manon, het beste bewaar ik altijd voor het laatste en om eerlijk te zijn weet ik niet of ik kan uitdrukken hoe belangrijk jij was om dit avontuur tot een goed einde te brengen. Naast mijn thesaurus, Oxford Dictionary, spellingcorrector en andere taalgerelateerde hulpmiddelen te zijn heb je ook al mijn frustraties, pessimistisch gedrag en momenten dat het niet goed ging gedragen alsof ze de jouwe waren. Jouw nooit-ophoudende steun, geduld en liefde gepaard met een goede dosis onnozelheid (een eigenschap die we delen) loodsten mij door dit avontuur. Je bent de beste!

Gent, november 2018

Table of contents

Dankwoord ..... **i**
Table of contents ..... **iii**
List of abbreviations ..... **vii**

Chapter I – Introduction and outline **1**
I.1 Introduction 1
I.2 Aims and objectives 3
I.3 Outline 3

Chapter II – Theoretical overview **8**
II.1 Introduction to SCPs: Who is who? 9
II.2 Methods for constructing SCPs 11
II.2.1 Introduction 11
II.2.2 Biological and bioinspired methods for preparing SCPs 11
II.2.3 Synthetic methods for preparing SCPs 14
II.2.3.1 Chain-growth synthesis of SCPs 14
II.2.3.2 Step-growth synthesis of SCPs 18
II.2.3.3 Multi-step-growth synthesis of SCPs 20
II.3 Click and click-inspired chemistry within the area of SCPs: CuAAc and Diels-Alder 23
II.3.1 Introduction 23
II.3.2 Copper(I)-Catalysed Azide/Alkyne Cycloaddition 24
II.3.3 Diels-Alder: photo-triggered and thermally induced reactions 28
II.4 Click and click-inspired chemistry within the area of SCPs: Thiol-ene and thiolactone chemistries 32
II.4.1 Thiol-X chemistries 32
II.4.1.1 Radical thiol-ene chemistry 33
II.4.1.2 Nucleophilic thiol-ene chemistry 34
II.4.1.3 Thiol-ene chemistry for the synthesis and modification of SCPs 35
II.4.2 Thiolactone chemistry 40
II.4.2.1 Introduction 40
II.4.2.2 Thiolactone chemistry for the synthesis and modification of SCPs 42
II.5 Conclusions 47

Chapter III – Thiolactone-based immobilisation of PNIPAM on silica for liquid chromatography 60

III.1 Introduction 61

III.2 Preparation of PNIPAM-functionalised stationary phase with thiolactone chemistry 64

III.2.1 Synthesis of the thiolactone acrylamide 64

III.2.2 RAFT copolymerisation of thiolactone acrylamide and NIPAM 65

III.2.3 Functionalisation of the stationary phase 66

III.3 Evaluation of the TRLC columns developed 68

III.3.1 Analysis of parabens and steroids 68

III.3.2 Analysis of peptide diastereomers 73

III.4 Conclusions and outlook 75

III.5 Experimental part 77

III.5.1 Methods 77

III.5.2 Materials 79

III.5.3 Synthesis 80

III.5.4 Manipulation of the stationary phase 80

Chapter IV – Amine-thiol-ene conjugation with immobilised and end-standing thiolactones 84

IV.1 Introduction 85

IV.2 Double modification of thiolactone-containing beads 87

IV.2.1 Synthesis of thiolactone-containing linkers 87

IV.2.2 Manipulations on solid support 90

IV.3 Double modification of polymers with thiolactone end groups 99

IV.3.1 Synthesis of thiolactone end-functionalised polymers 99

IV.3.2 Manipulations of polymer end groups 104

IV.4 Conclusions and outlook 110

IV.5 Experimental part 111

IV.5.1 Methods 111

IV.5.2 Materials 114

IV.5.3 Synthesis 115

IV.5.4 Manipulation of the solid support 119

Chapter V – Thiolactone chemistry for oligopeptide polymerisation and cyclisation .. 124

V.1 Introduction 125

V.2 Polymerisation of oligopeptides based on thiolactone chemistry 127
V.2.1 Monomer synthesis and polymerisation 127
V.2.2 Oligomer synthesis and polymerisation 130
V.3 Cyclisation of oligopeptides based on thiolactone chemistry 133
V.3.1 Amine-thiol-ene conjugation for cyclisation 133
V.3.2 Lysine-induced aminolysis of thiolactone for cyclisation 138
V.4 Conclusions and outlook 143
V.5 Experimental part 145
V.5.1 Methods 145
V.5.2 Materials 146
V.5.3 Synthesis 147
V.5.4 Manipulation of the solid support 148

Chapter VI – Automated synthesis of monodisperse sequence-defined oligomers 154
VI.1 Introduction 155
VI.2 Optimisation of the protocol 157
VI.3 Synthesis of different sequences 159
VI.4 Automated synthesis 176
VI.5 Conclusions and outlook 180
VI.6 Experimental part 182
VI.6.1 Methods 182
VI.6.2 Materials 183
VI.6.3 Manipulation of the solid support 184

Chapter VII – Multifunctional sequence-defined molecules for chemical data storage 188
VII.1 Introduction 189
VII.2 Read-out of the oligomers 192
VII.2.1 Exploration of ESI and MALDI 192
VII.2.2 Development of Chemreader algorithm 197
VII.3 Writing and reading data 199
VII.3.1 Writing and reading human-readable data 199
VII.3.2 Writing and reading machine-readable data 201
VII.4 Conclusions, outlook and media coverage 203
VII.4.1 Conclusions 203

VII.4.2 Outlook 204
VII.4.3 Media coverage 205
VII.5 Experimental part 206
VII.5.1 Methods 206
VII.5.2 Materials 208
VII.5.3 Synthesis 209
VII.5.4 Manipulation of the solid support 210

Chapter VIII – From thiolactone-based sequence-defined oligomers to sequence-controlled polymers 214
VIII.1 Introduction 215
VIII.2 Polymerisation of oligomers based on thiolactone chemistry 217
VIII.2.1 Oligomer synthesis and thiol-ene polymerisation 217
VIII.2.2 Oligomer synthesis and amine-thiolactone-ene polymerisation 227
VIII.3 Conclusions and outlook 231
VIII.4 Experimental part 233
VIII.4.1 Methods 233
VIII.4.2 Materials 234
VIII.4.3 Synthesis 235
VIII.4.4 Manipulation of the solid support 236

Chapter IX – Conclusions and perspectives 239
IX.1 Conclusions 239
IX.2 Perspectives 246

Chapter X – Nederlandstalige samenvatting 252
X.1 Introductie 252
X.2 Overzicht van de resultaten 254
X.3 Vooruitblik 258

List of publications 262

List of abbreviations

^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
^{1}H-NMR	Proton Nuclear Magnetic Resonance
A	Alanine
AM PS	(Aminomethyl)polystyrene
ADMET	Acyclic Diene Metathesis
AFM	Atomic Force Microscopy
AIBN	2,2'-Azobis(isobutyronitrile)
APT	Attached Proton Test
ATR	Attenuated Total Reflection
ATRA	Atom Transfer Radical Addition
ATRC	Atom Transfer Radical Coupling
ATRP	Atom-Transfer Radical Polymerisation
$CHCl_3$	Chloroform
$CHCl_3$-*d*	Deuterated Chloroform
CH_2Cl_2	Dichloromethane
COSY	Correlation Spectroscopy
CuAAC	Copper(I)-catalysed Azide/Alkyne Cycloaddition
D	Aspartic acid
Đ	Dispersity
Da	Dalton
DBTL	Dibutyltin Dilaurate
DCC	Dicyclohexylcarbodiimide
DCTB	Trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malonitrile
DIC	Diisopropylcarbodiimide
DIPEA	N,N-Diisopropylethylamine
DMA	N,N-Dimethylacetamide
DMA-SEC	Dimethylacetamide-based Size Exclusion Chromatography
DMF	N,N-Dimethylformamide
DMPA	2,2-Dimethoxy-2-phenylacetophenone
DMPP	Dimethylphenylphosphine
DMSO	Dimethylsulfoxide
DMSO-d_6	Deuterated Dimethylsulfoxide
DNA	Deoxyribonucleic Acid
DOSY-NMR	Diffusion Ordered NMR-spectroscopy

DSC	Differential Scanning Calorimetry
DTNB	5,5'-Dithiobis-2-nitrobenzoic acid
DVB	Divinylbenzene
E	Glutamic acid
EDS	Energy-Dispersive X-ray Spectroscopy
EI-MS	Electron Ionisation Mass Spectroscopy
ESI-MS	Electrospray Ionisation Mass Spectroscopy
ESI-MS/MS	Electrospray Ionisation-Tandem Mass Spectrometry
EtOAc	Ethyl acetate
EWG	Electron Withdrawing Group
Fmoc	Fluorenylmethyloxycarbonyl
FTIR	Fourier Transform Infrared Spectroscopy
G	Glycine
GC	Gas Chromatography
GC-FID	Gas Chromatography – Flame Ionization Detector
HBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-Tetramethyluronium hexafluorophosphate
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectra
HR-MS	High-Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum coherence Spectroscopy
I	Isoleucine
IC	Internal Contamination
IEC	Ion Exchange Chromatography
IEG	Iterative Exponential Growth
IR	Infrared spectroscopy
K	Lysine
L	Leucine
LC	Liquid Chromatography
LC x LC	Two-dimensional Liquid Chromatography
LC x SEC	Liquid Chromatography x Size Exclusion Chromatography
LC-ESI-MS	Liquid Chromatography Electrospray Ionisation Mass Spectroscopy
LCST	Lower Critical Solution Temperature
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionisation-Time Of Flight
MeOH	Methanol
Me_6TREN	N,N,N',N',N'',N''-hexamethyltris(aminoethyl)amine
Mmt	Monomethoxytrityl
M_n	Number average molecular weight

mRNA	messenger Ribonucleic Acid
MS	Mass Spectroscopy
MS/MS	Tandem Mass Spectrometry
MTBE	Methyl Tert-Butyl Ether
Mtt	4-Methyltrityl
NIPAM	N-isopropyl Acrylamide
NMP	Nitroxide-Mediated Polymerisation
NMR	Nuclear Magnetic Resonance
P	Proline
P-3CR	Passerini Three-Component Reaction
PCL	Polycaprolactone
PEG	Polyethylene Glycol
PEO	Polyethylene Oxide
PET-RAFT	Photo-induced Electron/Energy Transfer RAFT
Phe (or F)	Phenylalanine
PhotoATRP	Photo-initiated Atom Transfer Radical Polymerisation
pK_a	Negative base-10 logarithm of the acid dissociation constant
PMDETA	N,N,N',N'',N''-pentamethyldiethylenetriamine
PMMA	Poly(methyl Methacrylate)
PNA	Peptide Nucleic Acid
PNIPAM	Poly(N-isopropylacrylamide)
PSS	Polystyrene Sulfonate
PVCL	Polyvinylcaprolactam
QR	Quick Response
Q-TOF	Quadrupole Time Of Flight
R	Arginine
RACP	Radical Addition-Coupling Polymerisation
RAFT	Reversible Addition-Fragmentation chain Transfer
RDRP	Reversible Deactivation Radical Polymerisations
RI	Refractive Index
RNA	Ribonucleic Acid
ROMP	Ring-Opening Metathesis Polymerisation
ROP	Ring-Opening Polymerisation
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
RPLC	Reversed-Phase Liquid Chromatography
rRNA	ribosomal Ribonucleic Acid
RSD	Relative Standard Deviation
rt	Room Temperature
S	Serine

SCP	Sequence-Controlled Polymer
SEC	Size Exclusion Chromatography
SET-LRP	Single Electron Transfer Living Radical Polymerisation
SPS	Solid Phase Synthesis
siRNA	small interfering Ribonucleic Acid
SMILES	Simplified Molecular-Input Line-Entry Specification
SPS	Solid-Phase Synthesis
SUMI	Single-Unit Monomer Insertion
TAD	1,2,4-Triazoline-3,5-dione
TDS	Total Dissolved Solids
TFA	Trifluoroacetic Acid
T_g	Glass Transition Temperature
TGA	Thermogravimetric Analysis
THF	Tetrahydrofuran
TIPS	Triisopropylsilane
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
ToFSIMS	Time Of Flight Secondary Ion Mass Spectrometry
TRLC	Temperature-Responsive Liquid Chromatography
tRNA	transfer Ribonucleic Acid
Tyr	Tyrosine
U-4CR	Ugi Four-Component Reaction
URL	Uniform Resource Locator
UV	Ultraviolet
UV-VIS	Ultraviolet–visible Spectroscopy
V	Valine
XPS	X-ray Photoelectron Spectroscopy

Chapter I
Introduction and outline

I.1 Introduction

Since the introduction of the world's first fully synthetic polymer, Bakelite, in 1907 by Leo Baekeland, chemists have continued to explore and develop the field of polymer synthesis.[1] This has in turn led to the introduction of a huge range of bulk materials that we now use daily, such as plastic bottles and bags, mattresses, polyesters in clothing, *etc*. It also resulted in the development of more complex consumer systems such as dispersants, conducting polymers, micro-electronics, *etc*. Over the last three decades, polymer chemists have endeavoured to create macromolecular architectures with even stronger structure-property relations, resulting in the evolution of new controlled polymerisation methods and the development of complex macromolecular architectures such as block copolymers, dendrimers, and graft copolymers.[2]

For all the progress that has been made in the field of polymer chemistry, biopolymers such as proteins and DNA exhibit even more extraordinary structure-property relationships.[3] These biomacromolecules are characterised by their defined primary structure or monomer order, allowing them to fold in a specific and complex form, fulfilling functions such as genetic information storage, catalysis, or bio-transport. In the last decade, chemists have delved into new approaches from a range of scientific fields such as biology, biochemistry, and organic and polymer chemistry in order to obtain more control over the monomer order in polymers, with the intention of exploring new applications and stronger correlations between primary structure and its properties.[4, 5] A new class of polymers in which the monomer order is controlled to some degree was introduced: that of *sequence-controlled polymers (SCPs)*. A subclass of these SCPs consists of *sequence-defined macromolecules*, which have a uniform and discrete structure. SCPs are expected to play a crucial role in a broad range of applications, including but not limited to data-storage[6, 7], anti-counterfeiting tags[8], catalysis[9], antimicrobial[10, 11], self-assembly and folding[12, 13], and will also be able to potentially influence material properties such as phase behaviour[14], glass transition temperature (T_g)[15] and degradation behaviour.[16]

A form of chemistry whose advances have not only had a significant impact on the synthesis, cyclisation and functionalisation of (complex) macromolecular structures, but also on biological and synthetic SCPs, is what is commonly called 'click' chemistry.[17, 18] In 2001, Sharpless, Fokin and Finn determined requirements which a reaction should meet in order to be accepted as 'click': "The reaction must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by nonchromatographic methods,

and be stereospecific (but not necessarily enantioselective). The required process characteristics include simple reaction conditions (ideally, the process should be insensitive to oxygen and water), readily available starting materials and reagents, the use of no solvent or a solvent that is benign (such as water) or easily removed, and simple product isolation. Purification - if required - must be by nonchromatographic methods, such as crystallization or distillation, and the product must be stable under physiological conditions. […] Click processes proceed rapidly to completion and also tend to be highly selective for a single product: we think of these reactions as being springloaded for a single trajectory."[19-21] Tedious chemical procedures and work-up associated with other approaches could be diminished by applying this chemistry to the synthesis of macromolecular architectures, which had been previously proven to be either impossible to prepare or only feasible on a small scale. In 2011, additional criteria more specific to the field of polymer chemistry had to be added, as certain efficient reactions were incorrectly referred to as 'click' in the literature.[21] In addition to the initial conditions, equimolarity and large-scale purification were deemed to also be essential for a click reaction involving polymers. In the last decade, which has awarded greater attention to the precise definition and control of the polymer backbone and functionality, researchers have focussed on developing up-scalable and time-efficient reactions, considered indispensable for the synthesis of SCPs.[22] Click and click-inspired chemistry have played a crucial role in the build-up, the post-modification and cyclisation of the precisely defined and controlled (multifunctional) structures.[18]

Since 2009, our research group has been exploring the combination of two efficient reactions in one pot: the aminolysis of thiolactone and the click-inspired thiol-ene reaction.[23] When a thiolactone is opened with an amine, it releases a thiol which can react in a subsequent reaction with an ene functionality. This powerful amine-thiolactone-ene conjugation has been the subject of a number of PhD theses and numerous scientific papers. It was explored by more research groups on top of our own and proved to allow for the synthesis of various functionalised polymeric architectures and the double modification of polymers.[23-27] In recent years, this conjugation has proven useful for the synthesis of sequence-controlled and -defined structures.[27-30] Although a two-step iterative protocol was developed within our group to obtain the latter structures, only tetramers could be obtained with a low purity.[28] One of the objectives of the present doctoral thesis was to obtain purer and longer functionalised oligomers, preferentially with a protocol that could be automated.

With the knowledge obtained in our group during the last years as a starting point[23], the efficient amine-thiolactone-ene conjugation is explored in depth during this PhD in the pursuit of two main goals: the double modification of polymeric systems[31, 32] and the synthesis of SCPs.[33] The sequence-defined oligomers obtained are both used to store human- and machine-readable data[6] and equipped with appropriate functional handles which can be linked together to obtain both periodic sequence-controlled polymers[34] and functionalised cyclised species.[35, 36]

I.2 Aims and objectives

The main aim of this PhD was to further explore the amine-thiolactone-ene conjugation for the double modification of polymeric systems and the synthesis of SCPs, considering the fact that this is one of the most elegant and efficient ways to incorporate functionalities. In one-pot, this multi-step conjugation allows for the incorporation of both (commercially available) functional amines and acrylates or alkenes.

While temperature-responsive liquid chromatography stationary phases can equally be prepared using different methods[37-39], this thesis aimed to maximise the efficiency of the process by double-modifying the silica with a temperature-responsive polymer and a given functionality in one-pot. This would imply that the separation behaviour of the stationary phases can be more easily tuned. In addition, the current research intended to remedy certain drawbacks of existing methods for the double modification of polymer end groups because these often require multiple steps, longer reaction times, intermediate purification, and elevated temperatures. Although several potential alternatives have been proposed[40-46], this dissertation decided to explore the one-pot conjugation previously described as an efficient method, making use of commercially available amines and acrylates.

In the last five years, since the start of the present work, the field of SCPs has seen tremendous advancements.[4, 5, 47] Multiple research groups have started to explore sequence-controlled polymers in order to develop new applications and to prove that oligomer order has a significant influence on polymer properties. In comparison to the first protocol introduced by our group (Figure II.37A)[28], this thesis aimed to contribute to this expanding endeavour by creating a protocol based on this conjugation for the easy preparation of high-purity sequence-defined structures that can be varied in both the backbone and side chains. The ability to automate this protocol is crucial as the combinatorial diversity of these defined structures can be immense. In addition to the synthesis, these precise structures could be explored as new data carriers contributing to recent endeavours to store information on macromolecules.[48, 49] A final aim of this doctorate was the installation of functional handles on these oligomers in order to cyclise and polymerise them through different approaches based on this conjugation.

I.3 Outline

A theoretical overview is provided in **Chapter II**, aiming at the recent synthetic evolutions relevant to the preparation of SCPs. This overview starts with a distribution based on the classification of both well-known and newly-developed macromolecules as belonging to one of two new categories, namely sequence-controlled and sequence-defined polymers. Next, different methods for constructing SCPs are highlighted, a distinction being made between

biological and bioinspired protocols on the one hand and synthetic protocols on the other. Lastly, this theoretical chapter describes on the use of click chemistry for the synthesis, functionalisation/post-modification and cyclisation of SCPs, focussing especially on the use of thiol-ene and thiolactone chemistry.[18]

In **Chapter III**, in order to first accustom both the researcher and the reader to the amine-thiolactone-ene conjugation, thiolactone-functionalised poly(N-isopropylacrylamide) (PNIPAM) has been immobilised on aminopropyl silica, yielding a thiol which reacts with an acrylate in the same pot. This combination of steps leads to the preparation of functionalised temperature-responsive stationary phases for liquid chromatography. The required polymer is synthesised by a copolymerisation between NIPAM and a thiolactone acrylamide which has to be synthesised. Subsequently, two different PNIPAM-functionalised silica phases are prepared with two different acrylates, benzyl and PEG acrylate, and are packed into columns. Various solutes are used to test their retention behaviour, such as diastereoisomeric peptides, which have proven to be difficult to separate in classical RPLC.[32]

Chapter IV depicts the use of the amine-thiolactone-ene conjugation for the double modification of thiolactones immobilised on a solid support or as the end group of a polymer. As the solid support is easier to handle and provides a quick understanding of the double modification of thiolactones on a polymeric system, the modification reactions are first performed on this support. The thiolactone functionality is coupled to the solid support via two different linkers. Next, the optimal reaction conditions for the amine-thiolactone-ene conjugation are determined, and various functionalities are installed on the beads via this double modification. The thiolactone end group of an on-resin oligopeptide is also modified by means of amines and acrylates. Afterwards, two different pathways are examined to incorporate a thiolactone group as an end group of a polymer. As with the previous conjugation, the ideal conditions for the double modification of these thiolactones are verified as well. Next, a set of various amines and acrylates is applied in order to install a wide range of functionalities. Finally, a polymer with an amine end group is coupled with an end-functionalised thiolactone polymer to form a block copolymer with a midchain functionality.[31]

As a logical next step, **Chapter V** continues to build on the realisation that thiolactone can be installed as an end group of an oligopeptide. This chapter reports how oligopeptides are made bearing ene and thiolactone end groups for the synthesis of sequence-controlled polymers and cyclic structures. The latter have been thoroughly explored in peptide chemistry as their properties such as solubility, aggregation and folding behaviour can be quite different in comparison to their linear analogues.[35, 36] The oligopeptides for the polymerisation are functionalised with the first group via peptoid synthesis and with the second group via a thiolactone-functionalised acid.[28, 50] SCPs are obtained through radical thiol-ene polymerisation after the aminolysis of the thiolactone on the macromonomers prepared. Next, two different strategies are explored to execute on-resin macrocyclisations with thiolactones of peptides. For the first strategy, the aminolysis of an end-standing thiolactone and subsequent

photo-induced radical thiol-ene are exploited in order to obtain cycles.[51] Different functionalities are incorporated via the aminolysis of the thiolactone. The second cyclisation strategy applies oligopeptides bearing a thiolactone and an amine functionality, the latter being introduced via a lysine unit. It is able to open the thiolactone ring, yielding a thiol which forms disulphides with another cycle to form bicyclic 'handcuff-like' oligopeptides. The disulphides are reduced to thiols in the presence of an acrylate to obtain functionalised cycles.[28]

The findings reported in Chapter IV show that the combination of the amine-thiol-ene with an immobilised thiolactone on a solid support proves to be more effective, in terms of time and purification, than the same combination on a liquid support. Multifunctional sequence-defined structures are built in **Chapter VI** by combining the nucleophilic, one-pot amine-thiolactone-ene conjugation with urethane synthesis on solid support via a protecting-group-free, two-step, iterative protocol. In the first step, the immobilised thiolactone is opened with an amino alcohol and an acrylic to install a hydroxyl and a side-chain functionality respectively. Subsequently, an isocyanate-functionalised thiolactone is coupled with the hydroxyl group to reinstall a thiolactone, which is used in the following step. First, the reaction conditions are optimised for both steps of the protocol. Next, oligomers are prepared with a wide range of various acrylics and amino alcohols to respectively modify the structure of the side chains and backbone of the defined oligomers. Finally, the protocol is translated to an automated approach which allows for the preparation of sequences in parallel. A comparison is made between the manual synthesis and this automated approach.[33]

Chapter VII highlights the data storage capacity of the sequence-defined oligomers synthesised with the two-step iterative protocol from Chapter VI. First, a single pentamer is analysed with two different tandem mass spectrometry techniques in order to compare the two techniques and determine which one is most suited for the sequence analysis of the prepared oligomers. Next, the development of an automated sequencing tool called "Chemreader" is described. This tool was based on the controlled fragmentation of the oligomers and was intended to cope with the time-consuming interpretation of the MS/MS-spectra. This algorithm is tested and improved through writing a sentence on different oligomers. Another algorithm, called "Chemcoder", is developed to automate the translation of more relevant binary data to oligomers and vice versa. Finally, a QR (Quick Response) code is translated to different sequence-defined oligomers and is read out in order to make tangible the power of these algorithms and the two-step iterative protocol.[6]

Chapter VIII, finally, develops two different strategies based on the reaction between a thiol and a norbornene group for the synthesis of periodic SCPs starting from synthetic sequence-defined oligomers. In order to efficiently incorporate the latter group on the oligomers, a norbornene-functionalised acrylate is prepared. For the first strategy, the two-step iterative protocol from Chapter VI is used to synthesise oligomers on a resin which generates thiol-functionalised products after cleavage. Next, the amine-thiolactone-ene conjugation on solid support (dealt with in Chapter IV) is applied to the thiolactone end group of the prepared

oligomers to obtain a norbornene end group. After cleavage, the oligomers contain a thiol and a norbornene end group and are polymerised via the radical thiol-ene reaction. This strategy also enables the synthesis of graft polymers by changing the position of the norbornene functionality from the end group to one of the side chains. For the second strategy, an AB'-oligomer containing a norbornene side chain and a thiolactone end group is made with the iterative protocol from Chapter VI and is polymerised with the radical amine-thiolactone-ene conjugation. The thiolactone functionality is also opened on the resin to make a comparison between the radical thiol-ene polymerisation of an AB-oligomer and the radical amine-thiolactone-ene polymerisation of an AB'-oligomer.

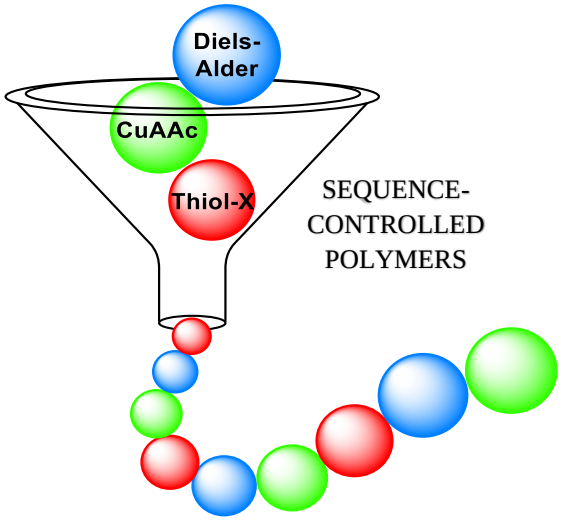
The general conclusion of this PhD and future perspectives are highlighted in **Chapter IX**.

The Dutch summary of this work is given in **Chapter X**.

References

[1] R. Mülhaupt, *Macromol. Chem. Phys.* **2013**, *214*, 159.
[2] J.-F. Lutz, J.-M. Lehn, E. W. Meijer, K. Matyjaszewski, *Nature Reviews Materials* **2016**, *1*, 16024.
[3] J. M. Berg, J. L. Tymoczko, L. Stryer, "*Biochemistry"*, W.H. Freeman, Basingstoke, 2012.
[4] J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, *38*, 1700582.
[5] J. De Neve, J. J. Haven, L. Maes, T. Junkers, *Polym. Chem.* **2018**, *9*, 4692.
[6] S. Martens, A. Landuyt, P. Espeel, B. Devreese, P. Dawyndt, F. Du Prez, *Nat. Commun.* **2018**, *9*, 4451.
[7] M. G. T. A. Rutten, F. W. Vaandrager, J. A. A. W. Elemans, R. J. M. Nolte, *Nature Reviews Chemistry* **2018**, *2*, 365.
[8] D. Karamessini, S. Poyer, L. Charles, J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, 1700426.
[9] P. Chandra, A. M. Jonas, A. E. Fernandes, *J. Am. Chem. Soc.* **2018**, *140*, 5179.
[10] A. Kuroki, P. Sangwan, Y. Qu, R. Peltier, C. Sanchez-Cano, J. Moat, C. G. Dowson, E. G. L. Williams, K. E. S. Locock, M. Hartlieb, S. Perrier, *ACS Applied Materials & Interfaces* **2017**, *9*, 40117.
[11] M. Porel, D. N. Thornlow, C. M. Artim, C. A. Alabi, *ACS Chemical Biology* **2017**, *12*, 715.
[12] J. Zhang, G. Gody, M. Hartlieb, S. Catrouillet, J. Moffat, S. Perrier, *Macromolecules* **2016**, *49*, 8933.
[13] A. S. Knight, E. Y. Zhou, M. B. Francis, R. N. Zuckermann, *Advanced Materials* **2015**, *27*, 5665.
[14] M. R. Golder, Y. Jiang, P. E. Teichen, H. V. T. Nguyen, W. Wang, N. Milos, S. A. Freedman, A. P. Willard, J. A. Johnson, *J. Am. Chem. Soc.* **2018**, *140*, 1596.
[15] J. Zhang, R. Deubler, M. Hartlieb, L. Martin, J. Tanaka, E. Patyukova, P. D. Topham, F. H. Schacher, S. Perrier, *Macromolecules* **2017**, *50*, 7380.
[16] J. Li, S. N. Rothstein, S. R. Little, H. M. Edenborn, T. Y. Meyer, *J. Am. Chem. Soc.* **2012**, *134*, 16352.
[17] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2.
[18] S. Martens, J. O. Holloway, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, 1700469.
[19] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
[20] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
[21] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angew. Chem., Int. Ed.* **2011**, *50*, 60.
[22] S. C. Solleder, R. V. Schneider, K. S. Wetzel, A. C. Boukis, M. A. R. Meier, *Macromol. Rapid Commun.* **2017**, *38*, 1600711.
[23] P. Espeel, F. E. Du Prez, *Eur. Polym. J.* **2015**, *62*, 247.
[24] S. Mommer, H. Keul, M. Möller, *Macromol. Rapid Commun.* **2014**, *35*, 1986.

[25] Y. Yan, L. Xue, J. B. Miller, K. Zhou, P. Kos, S. Elkassih, L. Liu, A. Nagai, H. Xiong, D. J. Siegwart, *Polymer* **2015**, *72*, 271.
[26] S. Montolio, O. Zagorodko, R. Porcar, M. Isabel Burguete, S. V. Luis, H. Tenhu, E. García-Verdugo, *Polym. Chem.* **2017**, *8*, 4789.
[27] Z. Zhang, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Polymer* **2015**, *64*, 221.
[28] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[29] F. Driessen, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2015**, *4*, 616.
[30] L. Yu, Z. Zhang, Y.-Z. You, C.-Y. Hong, *Eur. Polym. J.* **2018**, *103*, 80.
[31] F. Driessen, S. Martens, B. D. Meyer, F. E. Du Prez, P. Espeel, *Macromol. Rapid Commun.* **2016**, *37*, 947.
[32] A. J. Satti, P. Espeel, S. Martens, T. Van Hoeylandt, F. E. Du Prez, F. Lynen, *Journal of Chromatography A* **2015**, *1426*, 126.
[33] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[34] Z. L. Li, Z. C. Li, "Periodic Copolymers by Step-Growth Polymerization", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 349.
[35] M. Katsara, T. Tselios, S. Deraos, G. Deraos, M. T. Matsoukas, E. Lazoura, J. Matsoukas, V. Apostolopoulos, *Current Medicinal Chemistry* **2006**, *13*, 2221.
[36] D. Van Lysebetten, S. Felissati, E. Antonatou, L. L. G. Carrette, P. Espeel, E. Focquet, F. E. Du Prez, A. Madder, *ChemBioChem* **2018**, *19*, 641.
[37] F. Lynen, J. M. D. Heijl, F. E. D. Prez, R. Brown, R. Szucs, P. Sandra, *Chromatographia* **2007**, *66*, 143.
[38] T. Nishio, E. Ayano, Y. Suzuki, H. Kanazawa, T. Okano, *Journal of Chromatography A* **2011**, *1218*, 2079.
[39] B. Miserez, F. Lynen, A. Wright, M. Euerby, P. Sandra, *Chromatographia* **2010**, *71*, 1.
[40] M. Langlais, I. Kulai, O. Coutelier, M. Destarac, *Macromolecules* **2017**, *50*, 3524.
[41] M. Langlais, O. Coutelier, M. Destarac, *Macromolecules* **2018**, *51*, 4315.
[42] C. A. Figg, T. Kubo, B. S. Sumerlin, *ACS Macro Lett.* **2015**, *4*, 1114.
[43] A. Lv, X.-X. Deng, L. Li, Z.-L. Li, Y.-Z. Wang, F.-S. Du, Z.-C. Li, *Polym. Chem.* **2013**, *4*, 3659.
[44] S. C. Solleder, M. A. R. Meier, *Angew. Chem., Int. Ed.* **2014**, *53*, 711.
[45] B. Yang, Y. Zhao, C. Fu, C. Zhu, Y. Zhang, S. Wang, Y. Wei, L. Tao, *Polym. Chem.* **2014**, *5*, 2704.
[46] B. Yang, Y. Zhao, S. Wang, Y. Zhang, C. Fu, Y. Wei, L. Tao, *Macromolecules* **2014**, *47*, 5607.
[47] M. A. R. Meier, C. Barner-Kowollik, *Advanced Materials* **2018**, 1806027.
[48] J.-F. Lutz, *Accounts of Chemical Research* **2013**, *46*, 2696.
[49] H. Colquhoun, J.-F. Lutz, *Nat. Chem.* **2014**, *6*, 455.
[50] R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* **1992**, *114*, 10646.
[51] A. A. Aimetti, R. K. Shoemaker, C.-C. Lin, K. S. Anseth, *Chem. Commun.* **2010**, *46*, 4061.



Abstract

Inspired by nature's ability to define the monomer sequence in biopolymers, the urge to control and define the primary structure in synthetic polymers has increased exponentially in the last ten years. Sequence-controlled polymers (SCPs) have been prepared and characterised with various methods and chemistries imported from different scientific fields. In the previous decade, "click" chemistry and similarly efficient click-inspired reactions were introduced into the field of macromolecular chemistry. The introduction of these chemistries has already made a great impact and has enabled the synthesis of highly functionalised and complex polymeric structures, offering the prospect of many exciting further developments and applications in the near future. The present chapter provides a theoretical overview of the recent synthetic developments in the field of SCPs. This will be followed by the fruitful combination of click chemistry with different efficient chemistries and strategies for the preparation of sequence-controlled oligomeric and polymeric structures, especially the use of thiol-ene and thiolactone chemistry.

Parts of this chapter were published as:
S. Martens, J. O. Holloway, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, 1700469.

Chapter II
Theoretical overview

II.1 Introduction to SCPs: Who is who?

Nature has the amazing ability to control the order of the monomers (or primary structure) constituting biopolymers such as DNA, RNA and proteins.[1] This control of the monomer sequence allows for powerful structure-property relationships. Proteins perform different duties such as catalysing reactions and recognising specific molecules, while DNA can store genetic information and RNA executes different functions depending on its type (messenger (mRNA), transfer (tRNA), ribosomal (rRNA), micro (miRNA), small interfering (siRNA), …). Over the last 20 to 30 years, polymer chemists have made great progress and developed different techniques for making polymers with efficient structure-property relationships, such as block copolymers, dendrimers, and graft copolymers.[2-4] These macromolecules, however, do not reach the same potential as their natural counterparts.[5] In an attempt to remedy this, greater priority has been given, in the last decade, to the control of the primary structure of macromolecules in hopes of creating an even stronger correlation between their properties and structure and exploring new applications for polymers.[6]

The terms *sequence-controlled* and *sequence-defined* oligomers/polymers were introduced in the last decade to make a distinction between the different macromolecules that could be prepared with control of their monomeric order. Lutz' research group proposed a division based on the dispersity of well-known and newly-developed macromolecular structures across these two new terms (Figure II.1).[7]

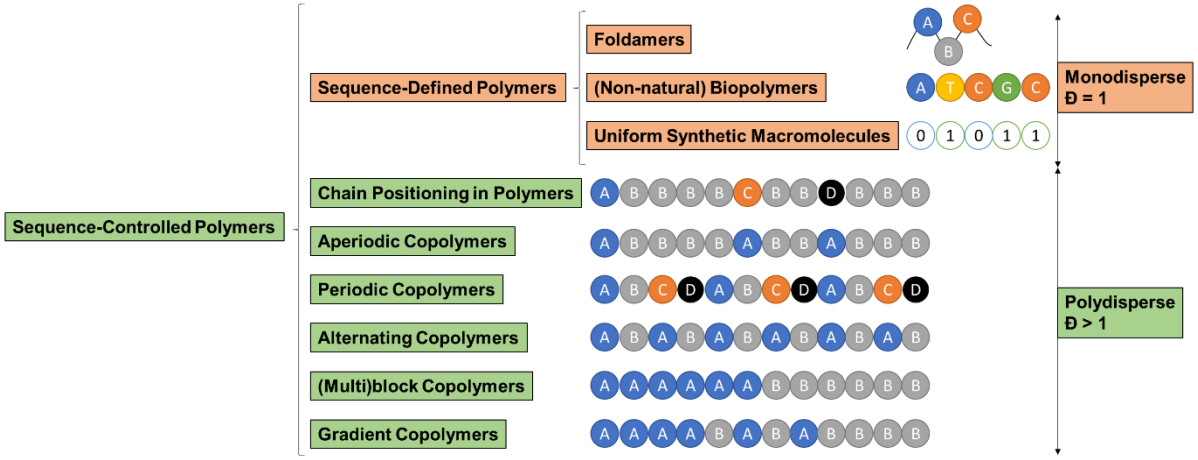


Figure II.1: Division between the different sequence-controlled and sequence-defined polymers.[7]

If the monomer sequence in the polymer is controlled to some extent, a macromolecule is called sequence-controlled.[7-9] This term encloses not only uniform or monodisperse polymers (Đ = 1), but also non-uniform or disperse ones (Đ > 1) such as block copolymers, which consist of

blocks positioned in a certain order, but with different lengths due to the polymerisation process applied. Sequence-defined polymers (Đ = 1) thus constitute a subclass of SCPs and have a defined monomer order and precise chain length.

While random copolymers do not have any control over the monomer order, different other types of copolymers have been developed over the past decades and belong to the class of SCPs (Figure II.1).[7]

Periodic copolymers consist of patterns that are periodically repeated in the polymer chain (Figure II.1).[10] The simplest periodic polymers are probably alternating copolymers, as they consist of an AB pattern repeated across the polymer chain. The protein elastin, for example, contains periodic repetitions of a pentapeptide which make it a sequence-defined periodic copolymer.[11] Guan and co-workers synthesized this pentapeptide and coupled multiple oligomers via a step-growth polymerisation, which causes the formation of disperse macromolecules.[12] The resulting polymer is thus a sequence-controlled periodic copolymer.

Other copolymers which fall under the umbrella term of SCPs are, for instance, (multi)block and gradient copolymers. The first consist of a sequence of blocks of different polymeric units which are built by completely different monomers or by the same monomer, but with a different stereochemistry.[10] In block copolymers, the comonomer composition shifts immediately from one monomer to another, while in gradient copolymers, the shift is gradual (Figure II.1).[13]

Lastly, Lutz and co-workers recently introduced a new nomenclature: that of aperiodic copolymers, which contain the same sequence order in every chain, but have an irregular monomer sequence distribution (Figure II.1).[14, 15] The A- and B-chain of insulin, for example, consist of defined oligopeptides, but do not contain any repetitions in their sequence.[16] Aperiodic copolymers are equally exemplified by sequence-controlled multiblock copolymers where all chains have the same sequence order, but the successive blocks cannot be organized in periodically repeating motifs. Within the sequence community, some scepticism arose about the use of the word “aperiodic”, as it has the connotation of “random”.[15] In a random copolymer, however, the sequence is not controlled, while an aperiodic copolymer has some degree of control (Figure II.2). This scepticism will not be further discussed in this PhD; the reader is kindly encouraged to refer to the literature for further elaboration on the use of the word aperiodic.

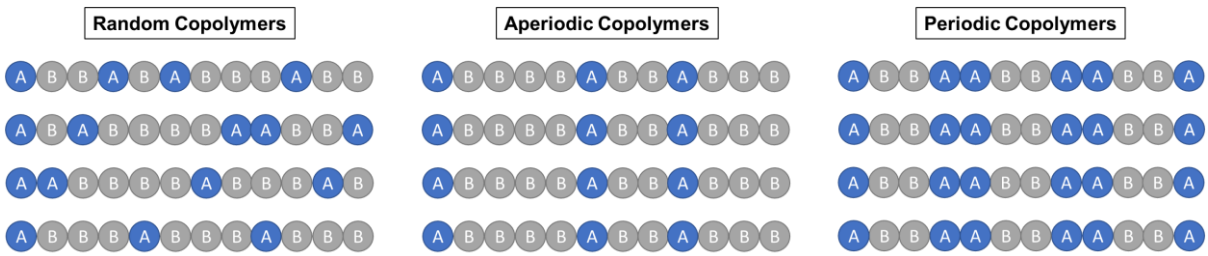


Figure II.2: Distinction between random, aperiodic and periodic (ABBA) copolymers.

II.2 Methods for constructing SCPs

II.2.1 Introduction

For the preparation of SCPs, polymer chemists have developed and examined many different methods. This required them not only to further study and explore polymer chemistry, but also to delve into other scientific fields, such as organic synthesis, biology and biochemistry.[7] In the following paragraphs, a distinction will be made between biological and bioinspired approaches for the build-up of SCPs on the one hand and synthetic approaches on the other hand.

II.2.2 Biological and bioinspired methods for preparing SCPs

Thanks to evolution, nature has been able to develop and perfect complex biocatalysts and mechanisms that exactly position amino-acids and nucleotides in a distinctive order to obtain respectively sequence-defined proteins and DNA/RNA.[1] These biocatalysts and mechanisms have been thoroughly investigated and applied by researchers for the build-up of both biological and non-biological SCPs with synthetic moieties, such as phosphate- and sugar-modified nucleic acids, new and modified nucleobases and non-natural amino acids.[7, 17, 18]

Genetic engineering has, for example, allowed for the synthesis of elastin-like and silk-like sequence-defined polypeptides with repeating oligopeptide patterns.[11] These repeating polypeptides are prepared thanks to the ribosomes of bacteria containing a gene in their DNA which is installed with recombinant DNA techniques.

Inspired by ribosomes, the research groups of Leigh and Sawamoto established synthetic approaches to the preparation of sequence-defined structures. Leigh and co-workers developed a synthetic small-molecule machine capable of making an oligopeptide (Figure II.3).[19, 20] The machine has a rotaxane structure, which is a linear macromolecule threaded through a ring. This linear macromolecule has amino acids in its side chains, which are bound together when the ring moves from one end of the linear macromolecule to the other. Sawamoto and co-workers proposed with an iterative cyclisation, cleavage and regeneration approach in order to insert monomers one-by-one (Figure II.4).[21] The starting molecule contains an initiating site and an alkene. Addition leads to the formation of a cycle which is broken by bond cleavage. A new alkene is attached to the broken bond and the iterative cyclisation-cleavage-regeneration cycle can start again.

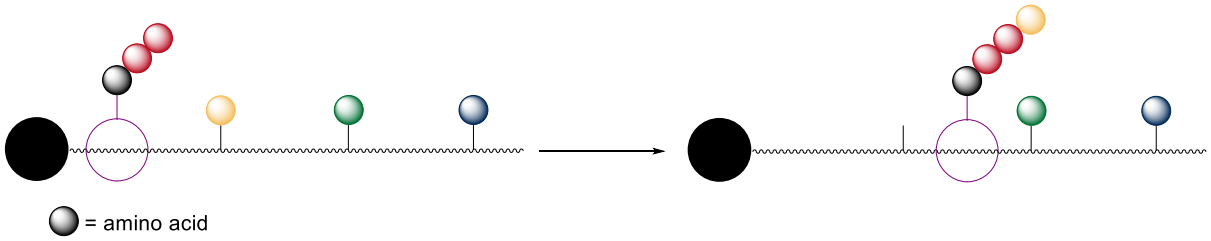


Figure II.3: Ribosome-inspired approach for the synthesis of sequence-defined oligomers based on a rotaxane machine.[19]

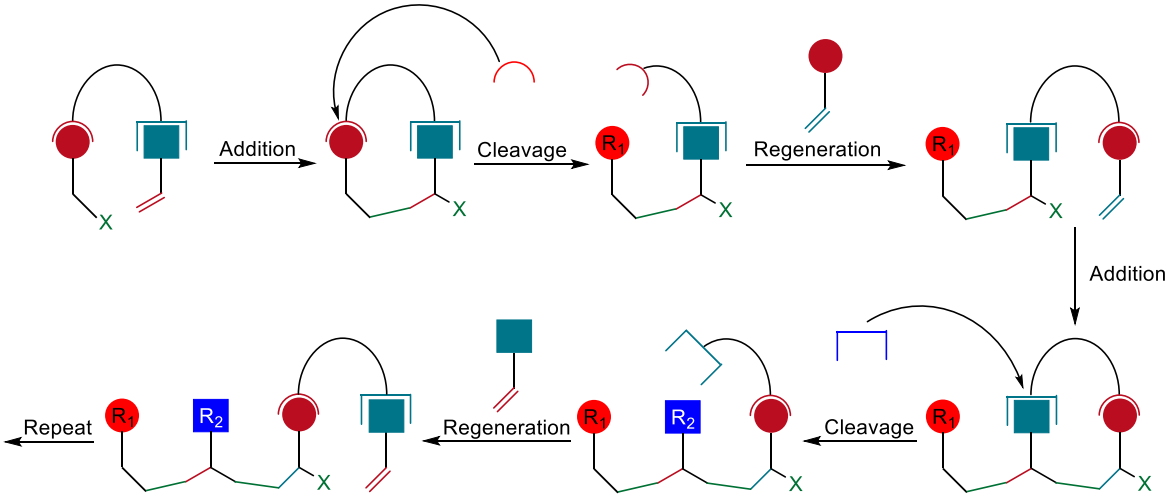


Figure II.4: Ribosome-inspired approach for the synthesis of sequence-defined oligomers based on iterative cyclisation, cleavage and regeneration (bottom).[21]

Another nature-inspired method for SCP synthesis is the spatial prearrangement of monomers and oligomers with (macro)molecular templates (Figure II.5).[17, 22-25] O'Reilly and co-workers, for example, built a sequence starting from the incomplete hybridisation between a DNA strand carrying a monomer and another strand bearing the growing sequence (Figure II.5a).[22] The monomer is coupled to the sequence and a new DNA strand is added that is fully complementary to the waste strand. The complete hybridisation between the new strand and the waste strand allows for the removal of the latter and the release of the DNA strand bearing the sequence, which can be used in a subsequent cycle. Another example of the use of DNA templates comes from Liu's group, which prepared macrocycles that can hybridise with DNA and allow the synthesis of SCPs (Figure II.5b).[24] These cycles consist of cleavable disulphide linkers, a peptide nucleic acid (PNA) pentamer which hybridises with the DNA, and an oligomer or polymer functionalised with coupling groups which can be connected to each other for the synthesis of the SCP. The DNA template coordinates the polymerisation and determines the order of the polymer. After the cleavage of the disulphide linkers and the PNA adapters, the SCP formed is still linked on one end to the DNA template.

While most research groups use DNA as a template, Sawamoto and co-workers prepared multiple synthetic templates for the synthesis of SCPs.[25] One such approach consisted in synthesising an initiator for reversible deactivation radical polymerization (RDRP) which selectively interacts with and polymerises methacrylic acid (Figure II.5c).[23] A copolymerisation was performed with benzyl methacrylate, yet results indicated that the

methacrylic acid was selectively polymerised when the template initiator was used. This template initiator was prepared via the cationic polymerisation of an azide-functionalised vinyl ether starting from an initiator with two initiating sites positioned in an ortho configuration on a benzene ring. One of these sites is suited for cationic polymerisation, the other for radical polymerisation. The polyazide formed was subsequently reduced, resulting in the polyamine-containing initiator.

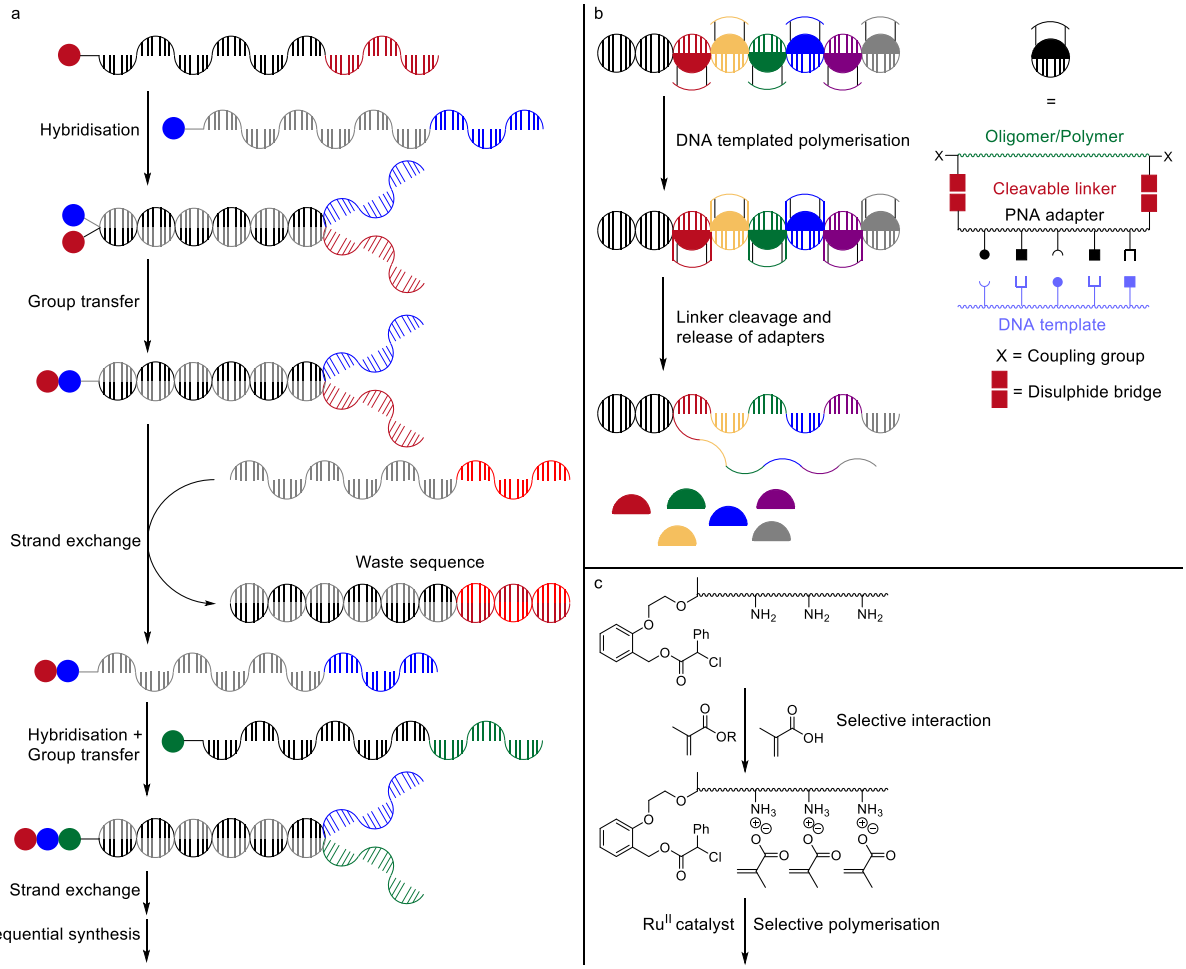


Figure II.5: Spatial prearrangement with macromolecular templates for the synthesis of SCPs: a) imperfect DNA hybridisation for the chain growth combined with perfect DNA hybridisation for the removal of waste[22], b) macrocycle consisting of cleavable disulphide linkers, oligomer/polymer with coupling groups and PNA adapter that can hybridise with the DNA template[24], c) synthetic approach for the radical polymerisation of methacrylic acid.[23]

While all these methods are based on or inspired by biological methods and processes, (polymer) chemists have spent the last decades developing synthetic ways to prepare macromolecules with control over the sequence order. The general polymerisation methods were recently divided into three categories: *chain-growth*, *step-growth* and *multistep-growth polymerisation*.[4] These three different synthetic approaches have all been applied for the synthesis of SCPs and will be discussed in the following paragraphs. The latter two polymerisation types will be used in this PhD thesis for the synthesis of SCPs.

II.2.3 Synthetic methods for preparing SCPs

II.2.3.1 Chain-growth synthesis of SCPs

Chain growth polymerisations can be performed with ionic or radical active centres, coordinative catalysts or via ring-opening procedures to incorporate monomers one by one.[7-9] As free radical polymerisations generate highly disperse polymers and do not allow any control of the molecular weight, efforts have been made in the last decades to control the molecular weight of radical polymerisations, resulting in the development of different reversible deactivation radical polymerisations. Some examples are nitroxide-mediated polymerisation (NMP), reversible addition-fragmentation chain transfer (RAFT) and copper-mediated polymerisations such as atom-transfer radical polymerisation (ATRP) and single electron transfer living radical polymerisation (SET-LRP).[26, 27] These polymerisation systems are based on the dynamic equilibrium between an active and a dormant species, which both allows for the simultaneous growth of all polymer chains and reduces termination and transfer reactions. The development of ionic polymerisation has equally made it possible to prepare polymers with a narrow distribution and control over the molecular weight.[28]

These developments and the further optimisation of the polymerisation conditions allowed for the synthesis of sequence-controlled multiblock-copolymers.[8] Perrier and co-workers were able to make functional icosablock copolymers of various acrylamides on a large scale via iterative RAFT polymerisations, carefully choosing the initiator concentration to control the termination and thoroughly examining the polymerisation process via modelling.[29-32] The research groups of Haddleton, Junkers, Hawker and Whittaker have optimized the conditions of SET-LRP and PhotoATRP to prepare linear and star-shaped multiblock copolymers of polyacrylates and acrylamides with blocks that could go up to an average of 100 monomers.[33-41] In order to efficiently scale up the synthesis of these multiblock copolymers, the use of flow reactors has been explored recently.[42-44] Via sulphur-free RAFT emulsion polymerisation, Haddleton's group prepared the longest multiblock copolymer up until now, which contains 24 individual polymethacrylate blocks.[8, 45-47]

Block copolymers can also be easily prepared via the sequential addition of monomers with ionic polymerisations and ring opening polymerisations (ROP).[48-50] To give examples of both methods, Hadjichristidis' group started from a benzyl potassium initiator to prepare a pentablock with five different monomers[51], and ROP of different substituted cyclohexene oxides and carbon dioxide was executed with a Zn-based catalyst by Coates and co-workers to obtain hexablock copolymers.[50] The combination of different chain-growth mechanisms also made it possible to synthesise block copolymers consisting of different monomers.[48, 49]

In addition, several research groups succeeded in obtaining periodic polymers through the use of ring opening metathesis polymerisations (ROMP). Hillmyer's group polymerised 3-

substituted cyclooctene derivatives, which can be regio-selectively polymerised with a second-generation Grubbs catalyst due to the steric hindrance between the substituent on the 3-position and the ligand of the catalyst.[52] After hydrogenation of the double bonds in the polymer backbone, one of the polymers obtained looks as though a periodic polymerisation was performed of vinyl acetate, styrene/isoprene and two ethylene units. The research groups of Meyer and Hawker prepared sequence-defined macrocycles which could be polymerised by means of ROMP polymerisation (Figure II.6).[53, 54] The first group obtained sequence-controlled polyesters, while the second group had repeating ester, amide, sulphonamide, aliphatic, aromatic and heterocyclic units in the polymer backbone.

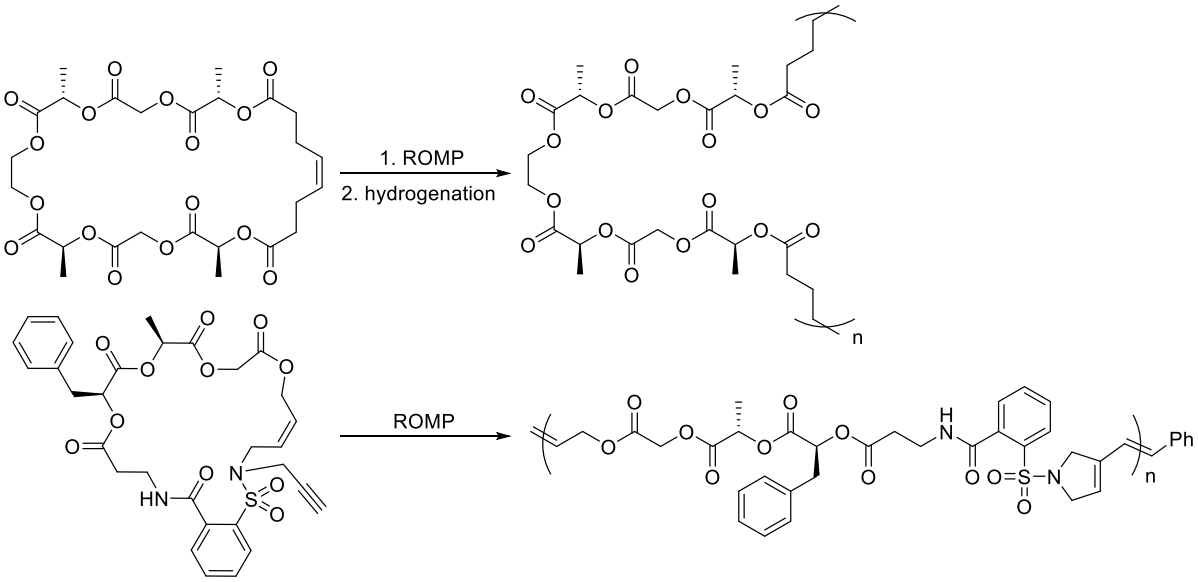


Figure II.6: ROMP syntheses starting from sequence-defined macrocycles for the synthesis of sequence-controlled polyesters (top) and ester-amide-aromatic-sulphonamide-aliphatic-heterocycle-containing polymers (bottom).[53, 54]

It equally proved possible to prepare different SCPs through the investigation and exploitation of the difference in reactivity ratios between different comonomers.[55] N-substituted maleimides, for example, have a higher tendency to cross-propagate with styrene than to homopolymerise. This difference in reactivity allows for the controlled addition of the maleimide at specific times during the RDRP of styrene and was applied in order to (automatically) create various SCPs (Figure II.7).[56, 57] In addition to using different styrene derivatives and maleic anhydride[58], this strategy allowed for the incorporation of maleimides with different functionalities.[56] These copolymerisations can be achieved through ATRP, NMP and RAFT.[58, 59]

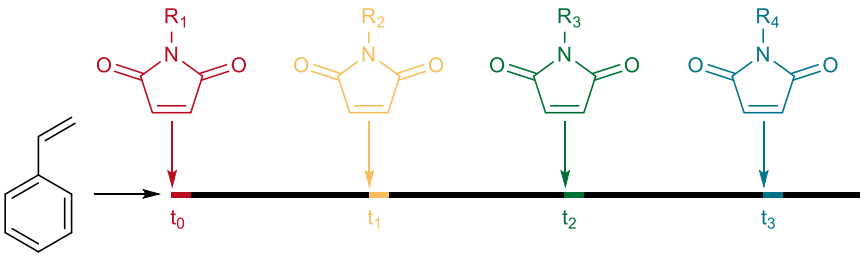


Figure II.7: Incorporation of N-substituted maleimides in polystyrene at specific times based on the difference in reactivity ratios.[55]

Instead of adding the maleimide at specific times during the polymerisation, Zhu and co-workers applied furan-protected maleimides, which release a maleimide via a temperature-stimulated retro Diels-Alder reaction. The protected maleimide was added at the start of the polymerisation and the temperature was increased at certain times during the synthesis of poly(styrene-co-N-substituted maleimide)[60] and poly(methyl methacrylate-co-N-substituted maleimide)[61] to let the released maleimide react with the monomer used. Other pairs of comonomers with different reactivities were also explored for the synthesis of SCPs, such as cyclobutene-1-carboxylate esters and cyclohexene derivatives (ROMP)[62], disubstituted cyclopropenes and low-strain cyclic olefins (ROMP)[63], endo- and exo-norbornenes (ROMP)[64], N-substituted maleimides with olefins and polyisobutene macromonomers[65] or limonene[66, 67] (free radical or RDRP), epoxides and cyclic anhydrides (ROP)[68], enol ethers and conjugated aldehydes (cationic)[69], isoeugenol and p-methoxystyrene (cationic)[70], 1,1-diphenylethylene derivatives and styrene (anionic)[49], and butadiene and styrene in the presence of a coordination catalyst.[71]

Chain polymerisation techniques were also explored in order to perform single unit additions to obtain sequence-defined oligomers. Further investigations into the kinetics of RAFT inspired polymer chemists to perform single-unit monomer insertions (SUMI) for the synthesis of sequence-defined structures.[8] Tsanaktsidis and co-workers had to screen initiator, monomer, reaction time and RAFT agent to optimise the yield of a macroRAFT agent with one styrene and one N-isopropyl acrylamide unit.[72] Members of Boyer's group, on the other hand, have applied visible-light-mediated photoinduced electron/energy transfer RAFT (PET-RAFT) for the synthesis of trimers which were linked together to make hexamers or were installed as sequence-defined grafts on a graft copolymer.[73] This group also combined the PET-RAFT technique with thiol-ene and esterification reactions for the synthesis of pentamers.[74, 75] Junkers and co-workers have optimised RAFT-based sequence-defined synthesis by applying kinetic screening and modelling, on-line monitoring via ESI-MS or FTIR, and semi-automated chromatography separations such as recycling size-exclusion chromatography and flash column chromatography.[8] All these optimisations made it possible to prepare sequence-defined decamers, which were linked together through disulphide formation to obtain monodisperse icosamers.[76] The sequences prepared could also be scaled up with flow chemistry. Instead of using RAFT, photo-induced copper-mediated radical polymerisations were also applied for SUMI.[77]

As mentioned previously, Sawamoto's group has prepared different template approaches for the synthesis of SCPs through radical chain-growth polymerisations (Figure II.4 and II.5c).[25] In one such approach, template AB- and ABA-monomers were prepared that contain two different alkene units and a carrier scaffold.[78-80] After the cyclopolymerisation of these monomers, this scaffold is removed, resulting in periodic copolymers. The last template-based method proposed by this group starts from an initiator with an initiating site and a functionality, such as an amine or lariat crown ether.[81, 82] This initiator can selectively recognise and react

with one monomer. Although this approach has not been used on the level of polymerisation, scepticism is growing as to whether this method can realistically be used for the synthesis of SCPs.[8, 25]

Recently, Sawamoto and co-workers described the single-unit addition of bulky monomers during ATRP (Figure II.8).[83] A methacrylate monomer containing both isopropyl and adamantyl groups is added once to the growing chain end, but does not allow for the incorporation of a second monomer, due to steric hindrance coming from its cumbersome functionalities. The bulky side chain is removed via selective acidolysis and replaced via esterification in order to install a new functionality and to obtain a chain end which can be used again for a new bulky monomer addition. This method was successfully applied for the creation of trimers.

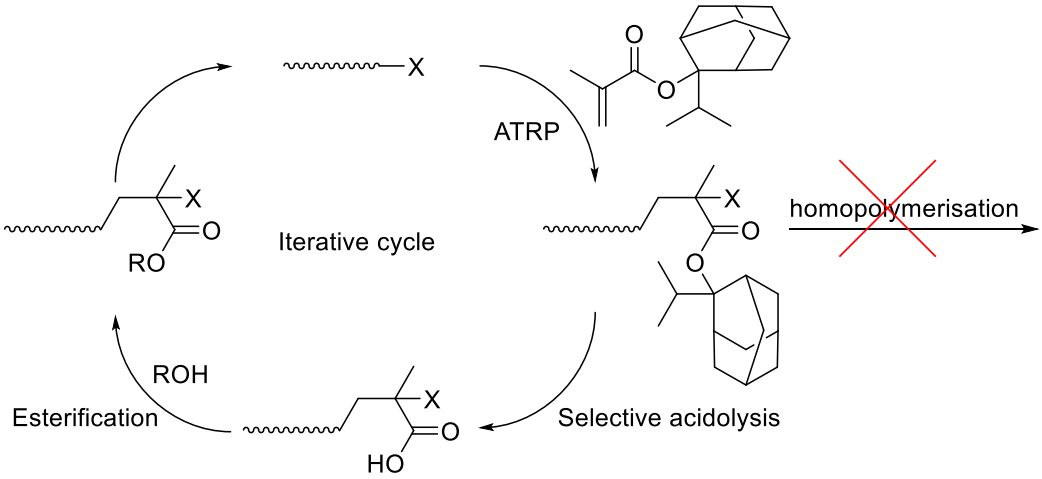


Figure II.8: Repetitive cycle of bulky monomer addition, selective acidolysis and esterification for the synthesis of sequence-defined oligomers. The bulky oligomer cannot be homopolymerised, which allows for its single insertion at the growing chain end.[83]

Another approach that can be used for the incorporation of single units based on radical-chain growth is the Kharasch addition or atom-transfer radical addition (ATRA).[84] Huang and co-workers first reacted allyl alcohol with a bromine end group of a polymer. The incorporated alcohol is transformed into an acid, which allows for the installation of a functionality via esterification. As a result of these steps, a new active chain end is obtained which can again be used for radical addition. Only one cycle has been performed so far, as chain transfer reactions occurred during the addition step of the allyl alcohol, resulting in some loss in the bromine end group. Kamigaito's group, in turn, applied iterative ATRA reactions for the synthesis of different dimers and trimers based on styrene derivatives and acrylates.[67, 85, 86] Afterwards, these oligomers were modified either with an alkene for step-growth ATRA polymerisation or with a maleimide for the copolymerisation with limonene or styrene. Instead of radical chain growth, finally, Higashimura's group performed single-unit additions with three different vinyl ethers and an initiator via cationic polymerisation to obtain a defined tetramer.[87]

II.2.3.2 Step-growth synthesis of SCPs

Step-growth polymerisations such as polyadditions and polycondensations are performed with bifunctional AB or AA and BB monomers, the A and B functionalities of which can react with each other.[7, 88] Polymer networks can also be formed by applying a trifunctional monomer or upwards during the polymerisation. Step-growth polymerisations can be easily executed, but the chain length and the dispersity of the formed polymers are difficult to control. Although some research groups have been capable of transforming stepwise polycondensations into chain-growth polymerisations and thus of obtaining polymers with low dispersities and controlled molecular weight[89], step-growth polymerisations cannot control the microstructure of a polymer.

These step-growth polymerisations have, however, proven to efficiently prepare periodic polymers by means of one of two strategies.[88] The first one is the polymerisation of symmetric or asymmetric telechelic sequence-defined oligomers, while the second is the polymerisation of a mixture of monomers. These two strategies and appropriate examples will be provided below.

The first strategy starts from functional spacers or sequence-defined oligomers with functional end groups, which can either be homopolymerised if the end groups can react with one another (A-oligomer-B) or require a reaction partner for copolymerisation if the end groups contain the same functionality (A-oligomer-A). This stepwise linkage has been achieved through different chemistries. Acyclic diene metathesis (ADMET) and subsequent hydrogenation was applied by the research groups of Wagener, Meier and Li to acquire polyolefins with side-chain functionalities which are evenly distributed across the polymer chain.[90-92] The polymers obtained display different crystalline and melting behaviour in comparison to polymers obtained through the copolymerisation of ethylene and functional vinyl monomers.

The groups of Li and Kamigaito both used ATRA to prepare monomers but made use of different approaches to polymerise them. Li and co-workers applied atom transfer radical coupling (ATRC) to obtain ABBA periodic copolymers (Figure II.9a).[93] Kamigaito's group used ATRA via two different approaches to obtain periodic polymers. In the first method, this group made sequence-defined oligomers with a chlorine and an alkene end group which could be coupled together with ATRA (Figure II.9b).[85] Another pathway started from AA and BB monomers with two alkene or two chlorine end groups to obtain polymers (Figure II.9c).[94]

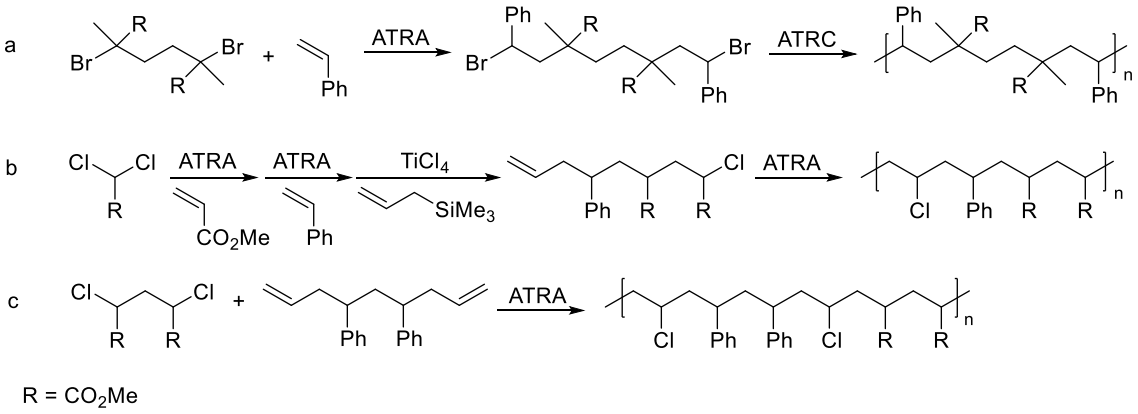


Figure II.9: ATRA and ATRC approaches for the preparation of monomers and periodic polymers.[85, 93, 94]

Another radical based approach is the radical addition-coupling polymerisation (RACP) of an asymmetric dibromo compound and nitroso adducts yielding ABCDCBAD periodic polymers (Figure II.10).[95] Trimers with carbon-centred radicals are formed which can react in a subsequent reaction with a nitroso compound to acquire the nitroxyl radical. Coupling of this nitroxyl radical with another trimer with a carbon-centred radical results in the formation of a dibromide heptamer, which can be polymerised with another nitroso compound via RACP.

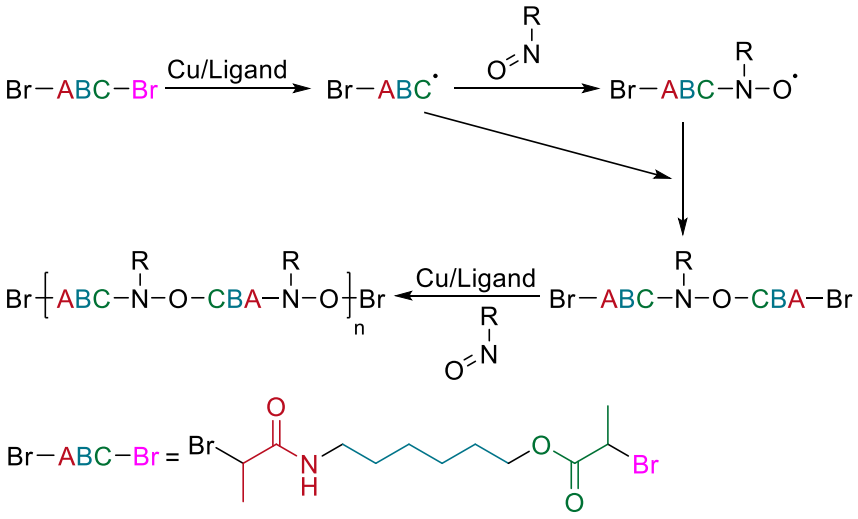


Figure II.10: RACP of an asymmetric dibromo trimer with a nitroso adduct.[95]

Instead of radical based reactions, step-growth polycondensations of sequence-defined oligomers consisting of glycolic, lactic and caprolactic units were explored by Meyer and co-workers.[96, 97] These oligomers, with an acid and an alcohol end group, were polymerised using dicyclohexylcarbodiimide (DCC) or diisopropylcarbodiimide (DIC). In comparison to their random counterparts, these periodic polymers display a slower and more constant degradation, which might be interesting for gradual drug delivery.[98]

Click reactions and the usage of thiolactone have also proven to be very effective for the coupling of functional spacers and oligomers.[99] The use of different click reactions for step-growth polymerisations will be highlighted further in this chapter.

As already mentioned, in the second strategy, periodic polymers are obtained with the stepwise polymerisation of a mixture of monomers. This can be performed by either sequentially adding and polymerising monomers in one-pot or using multicomponent reactions. Hong and coworkers prepared different periodic polymers via sequential addition based on the reactions between amines, thiols, thiolactones, methacrylates and others.[100, 101] These reactions are based on thiol-ene and CuAAC and will be discussed in detail later in this chapter.

Another option to polymerise monomer mixtures is the use of multicomponent reactions, which were exploited by different groups for the synthesis of ABAC periodic polymers. These macromolecules were prepared by the groups of Meier and Li through the use of the Passerini three-component reaction (P-3CR), starting from one monofunctional and two difunctional molecules bearing either isocyanide, aldehyde or acid groups.[102-104] By adding an amine functionality to the monomer mixture, Meier's group applied the Ugi four-component reaction (U-4CR) for the synthesis of the polymers. Two difunctional and two monofunctional monomers are required to execute this polymerisation.[105] Another promising multicomponent reaction is the Biginelli-3CR, explored, for instance by the research groups of Tao and Meier. This strategy was investigated with different urea, dialdehyde and diketone building blocks and lead to the formation of high molecular weight polymers.[106, 107]

II.2.3.3 Multi-step-growth synthesis of SCPs

Multi-step-growth polymerisations are based on iteratively repeating chemical reactions to obtain sequence-defined structures.[7, 108] Well-known examples that can be prepared with these type of polymerisations are dendrimers or star-shaped polymers. Monomers with reactive A and B functionalities are successively linked together, but precautions have to be taken to prevent their direct step-growth polymerisation. Scientists have explored different methods and reactions for the performance of multi-step-growth polymerisations. These reactions should be relatively fast and quantitative. If long polymers are targeted and every step requires multiple hours or even days to reach completion, such an approach would be very time-consuming and not useful. The general aim is to reach full conversion of each step in less than an hour.[7, 108] This last point is important, because if 95% conversion is reached every step, the overall yield after ten steps, for instance, is 60%. If a high yield is desired, the conversion of every step should also be high.

One multi-step-growth method is the one-by-one attachment of AB bifunctional monomers where one functionality is protected.[7, 108] This monomer is connected in the first step to the growing polymer chain (Figure II.11). In the second step, the protecting group is removed, allowing for the introduction of a new monomer. These two steps are repeated until a defined structure with the desired length is obtained.

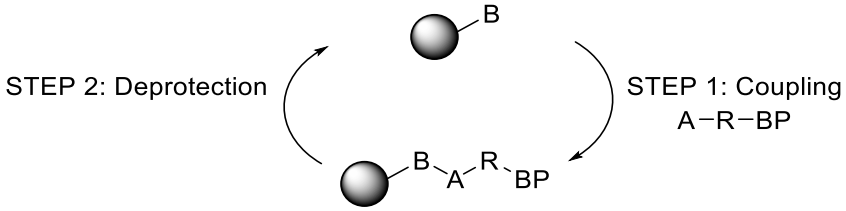


Figure II.11: General scheme for an iterative synthesis with a monoprotected molecule. After coupling, the protecting group is removed to obtain an active chain end.[7]

Although this approach can be executed in solution, tedious purifications have to be performed after every monomer addition. In 1963, Merrifield introduced the use of crosslinked polymer beads, referred as solid supports, to grow sequence-defined peptides which are covalently linked to and can be easily cleaved off these insoluble supports.[109] This shift from reactions in solution to reactions on solid support facilitated the synthesis of peptides, as an excess of reagents can easily be added to quickly drive the reaction to completion, and the beads can be simply filtered off and washed to remove the excess of reagents or soluble side-products after the reaction. The use of solid supports is not restricted to the synthesis of oligopeptides; it can also be applied to oligonucleotides, oligosaccharides and synthetic sequence-defined oligomers.[108, 110, 111] In the last decades, other supports have also been explored to perform iterative synthesis, such as soluble polymers, porous glass, solid nanoparticles and fluorous tags.[7, 108] The easier and faster parallel preparation of oligomers has been facilitated by the introduction of automated synthesizers and microwaves. Starting from monoprotected bifunctional AB monomers, many different defined structures have been obtained, such as oligoamides[112-114], oligophosphates[115-117], oligoesters[118, 119], oligoureas[120, 121] and other oligomers.[108, 122, 123]

Another method for the performance of multi-step-growth polymerisations is the orthogonal, iterative, divergent/convergent approach or iterative exponential growth (IEG), which depends upon orthogonal deprotections and couplings of end groups of diprotected monomers in order to (exponentially) increase the chain length with each repeating cycle (Figure II.12).[124, 125] The first step of this approach starts with the selective deprotection at each of the two protected end groups of the monomer. This results in the formation of two different mono-protected intermediates, one with an A- and another with a Z-end. The second step is the coupling of A and Z, forming a diprotected dimer. The latter, with the same protected end groups as the monomer, can now undergo the same cycle again. This cycle can be repeated a number of times to obtain sequences with the targeted chain length. While this method only allows for the synthesis of defined periodic structures, it makes it possible to quickly grow defined sequences.[7] This IEG strategy has been applied for the synthesis of defined oligoamides[126, 127], oligoesters[128, 129], oligoethers[130, 131], oligo(triazole)s[132, 133] and other defined oligomers.[125]

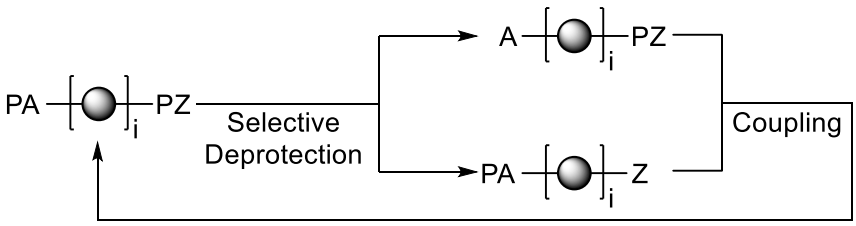


Figure II.12: General scheme for an iterative, divergent/convergent approach. A diprotected molecule is selectively deprotected and coupled to build sequences.[124, 125]

The procedures mentioned above require protecting groups. Different research groups have been exploring and combining reactions to perform orthogonal iterative procedures.

An example is the "AB+CD" mechanism (Figure II.13, left).[108] This combines two reactions in which A only reacts with D and B only with C. Consequently, protecting groups are not necessary in this approach, as monomers undergo a selective reaction with each other. This strategy was applied by several research groups, including Lutz's[134, 135], Barner-Kowollik's[136] Meier's[137, 138], and ours[139] to obtain various functionalised monodisperse structures.

The "AA+BB" mechanism is another example of a protecting-group-free strategy (Figure II.13, right).[108] In this case, large excess of every monomer has to be used in each step to avoid crosslinking between growing polymer chains. For instance, the groups of Börner and Vizzavona prepared different oligomers by combining diamines with diacids.[140, 141] Diamines can also be reacted with monosubstituted cyanuric chloride, which has two chlorine functionalities, as proposed by Daily.[142]

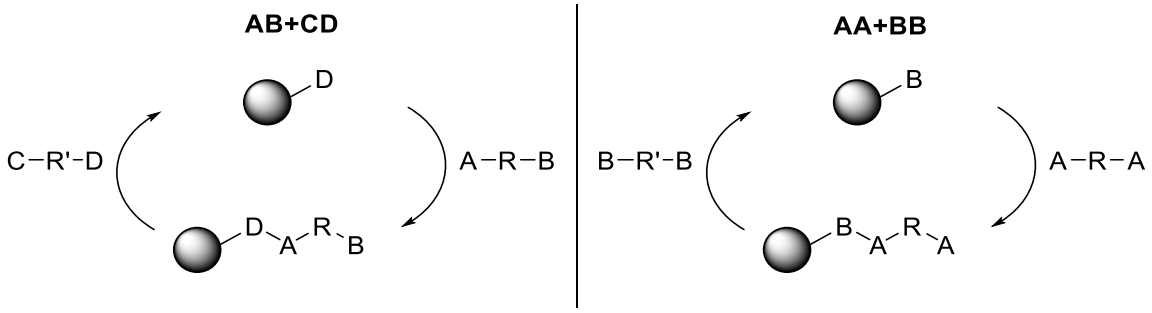


Figure II.13: General schemes for the iterative synthesis of sequence-defined structures with the "AB+CD" (left) or "AA+BB" mechanism (right).[108]

An approach that combines both strategies is the "AB+CC" mechanism.[108] A and B do not have the tendency to react with each other, but both can react with C. The research groups of Alabi and Lutz explored this method to synthesise oligothioetheramides[143], oligocarbamates[144] and oligo(alkoxyamine amide)s.[145]

A final approach that will be discussed, is the sub-monomer strategy developed in 1992 by Zuckermann and co-workers to synthesise oligopeptoids.[110] This strategy consists of two steps, which are repeated to obtain an oligomer with the desired structure and length (Figure II.14). In the first step, a DIC-activated haloacetic acid is coupled to the end-standing amine of the growing chain. Next, a primary amine is added to reinstate an amine end group and to install a

functionality by displacing the end-standing halogen from the previous step. Two submonomers, amine and haloacetic acid, are used to synthesise the N-substituted monomer on the growing chain.[146] A wide range of amines has been incorporated to obtain highly functionalised structures and the two-step procedure was automated on a robotic synthesiser in order to be able to perform the synthesis of different oligomers simultaneously.

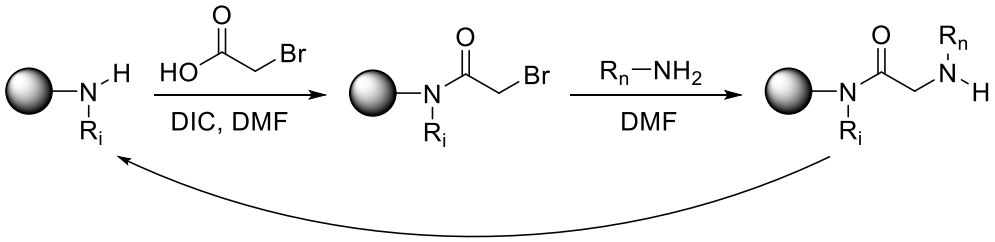


Figure II.14: Submonomer two-step iterative strategy for the synthesis of sequence-defined peptoids.[110]

In the next paragraphs, the combination of these techniques with click chemistry will be highlighted for the synthesis and modification of SCPs.

II.3 Click and click-inspired chemistry within the area of SCPs: CuAAc and Diels-Alder

II.3.1 Introduction

Since its introduction in 2001 by Sharpless, Fokin and Finn[147, 148], the concept of "click" chemistry has made a major impact in many research areas[149-158] and is now widely used by many research groups around the world. This precious label is given to reactions that are wide in scope, stereospecific, modular, form inoffensive side products which could preferentially be removed with non-chromatographic methods, and allow for high yields. The reaction should be performed with readily available starting materials, simple reaction conditions and ideally no solvents should be used. The products should be obtained via simple purification and be stable under physiological conditions. Click chemistry was quickly implemented[159-163] following its inception and led to a paradigm shift[164] in the synthesis of functional complex polymers. This revolutionary chemistry allowed for the design of macromolecular structures that were previously either impossible to make or small in scale, requiring difficult work-up and synthesis procedures.[2, 5, 165-176] Ten years after its introduction, a set of requirements were reformulated for click chemistry in polymer science[177], including the view of our group, in order to distinguish between some efficient or successful reactions between polymers that were referred to as "click", but lacked at least one of the criteria spelled out. In addition to the original requirements defined by Sharpless *et al.*, equimolarity and large-scale purification were formulated as two indispensable criteria for a click reaction concerning polymers. Recently, a

perspective by our research group highlighted the impact, advances and prospects of click chemistry in the field of macromolecular science.[158]

Click chemistry has also found its way into the synthesis of sequence-defined structures such as oligopeptides and oligonucleotides.[150, 153, 178, 179] The preparation of these macromolecules require versatile and chemoselective reactions, and the smaller scales force the need for very efficient reactions. The introduction of click reactions revolutionized the labelling/modification[178, 180-187], cyclisation[188-194] and conjugation of these biomacromolecules.[195-205] Polymer chemists adopted and modified some efficient, metal-free protocols that were first applied to these biomacromolecules to use in their own fields of research. Click chemistry equally facilitated the synthesis of peptide/protein-polymer and oligonucleotide/DNA-polymer hybrids. These syntheses also require efficient and clean conjugation reactions in order to link the natural and synthetic macromolecules together.[179, 206-225] Reasons for these requirements include the difficult installation of a specific functionality and the small available amounts of the natural compounds. These polymeric hybrids are interesting for enzyme recovery[226], nano-biomaterials[227-229], pharmaceuticals[230-233] and the modelling of peptide binding.[234]

In the last decade, the definition and control of the polymer backbone and functionality has become of great importance, for instance in the areas of data storage and single chain folding. Although there are many ways to synthesise these sequence-defined structures[7, 8, 235], the need for up-scalable and time-efficient reactions remains the most significant requirement for their preparation. This demands the installation of certain functionalities that can be easily and effectively modified. The following section will further highlight the powerful combination of click and click-inspired chemistry within the area of sequence-controlled polymer structures. This does not only include the build-up, but also the post-modification and cyclisation of the precisely defined (multifunctional) structures.

II.3.2 Copper(I)-Catalysed Azide/Alkyne Cycloaddition

The copper(I)-catalysed azide/alkyne cycloaddition (CuAAc) is the first and most well-known click reaction.[147, 148, 236, 237] Meldal and Sharpless independently improved the Huisgen 1,3-dipolar cycloaddition[238] between an azide and an alkyne moiety by adding a Cu(I) catalyst, ensuring a higher yield, reactivity and regioselectivity for this cycloaddition. As expected, the efficient CuAAc reaction quickly proved to be effective for the synthesis and post-polymerisation modification of polymers.[158, 165, 208, 239-241] This led to the synthesis of very complex structures. This section aims to highlight the extraordinary properties of CuAAc, and show how this chemistry has proven very useful in the synthesis of sequence-defined and less precise sequence-controlled structures as well as their post-modification/functionalisation and cyclisation.

CuAAc can be easily executed for the preparation of sequence-defined structures by means of various iterative protocols in combination with liquid- or solid-phase chemistry. One such method is the orthogonal, iterative, divergent/convergent approach, which resulted in the synthesis of well-defined oligo(triazoles) starting from oligo(ether)[242-244], sugar[245-247], epoxide[132, 248], nucleotide[133], polymers[249] or hydrocarbon[244, 250-253] units equipped with protected alkynes and functional groups that could be easily converted to azides. Johnson and co-workers coupled this method with multistep flow synthesis to achieve a semi-automated protocol.[244, 254] The latter allowed for the gram-scale preparation of the dimer in one hour and proved the power of this iterative, divergent/convergent approach.

Oligo(triazole amide)s were also synthesised with the "AB+CD" strategy starting from two monomers, of which the first had an alkyne (A) and carboxylic acid (B) end group and the second one an amine (C) and an azide (D) functionality (Figure II.15A).[134, 255-257] The oligomers were synthesised by means of consecutive amidation and CuAAc reactions. Lutz and co-workers used this "AB+CD" mechanism to synthesise oligomers that carried potential digital information on both solid[256] and liquid supports.[257] The same research group also combined this mechanism with peptide synthesis to create oligomers containing natural amino acids and synthetic units (Figure II.15B).[258-260] These units were formed from carboxylic acid-azide (AB) and alkyne-amine (CD) monomers. This combination demonstrates the versatility of the mechanism described and further extends the choice of building blocks for the oligomers.

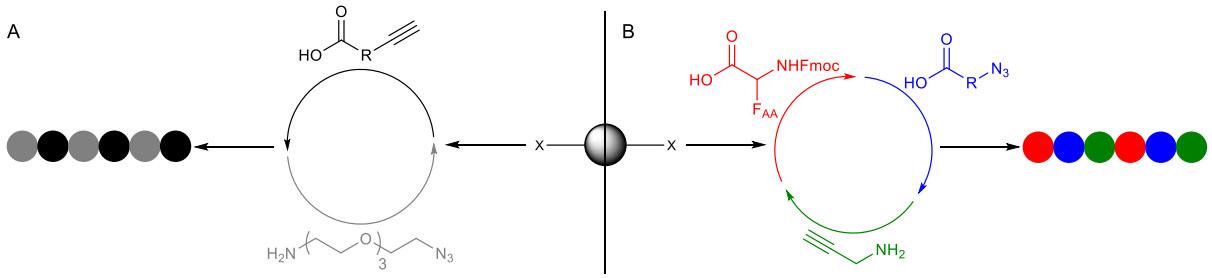


Figure II.15: "AB+CD" mechanisms on solid support for the synthesis of oligo(triazole amide)s by means of amidation and CuAAc.[256-259, 261] A) A complete synthetic backbone, B) a backbone consisting of natural and synthetic units.

Guan and co-workers performed the synthesis of SCPs via step-growth polyaddition of terminal alkynes and azides of heterotelechelic oligopeptides or polymers. They discovered that a new β-turn mimic resulted from a triazole ring formed between two oligopeptides with respectively an alkyne and azide end group.[262] This turning behaviour was also observed in oligopeptoids by Pokorski *et al.*[263] With this knowledge, defined oligopeptides that could form either β-turns or β-sheets were synthesised with an alkyne and azide end group (Figure II.16).[12, 264] The polymers resulting from these oligopeptides formed respectively elastin-mimicking structures and hierarchical nano-fibrils. The β-turn behaviour of the triazole ring did not interrupt the turning behaviour of the first polymers and helped the self-assembly and folding of the latter ones. Hartmann and co-workers similarly reported sequence-defined structures also using CuAAC to yield high molecular weight and degradable SCPs.[265]

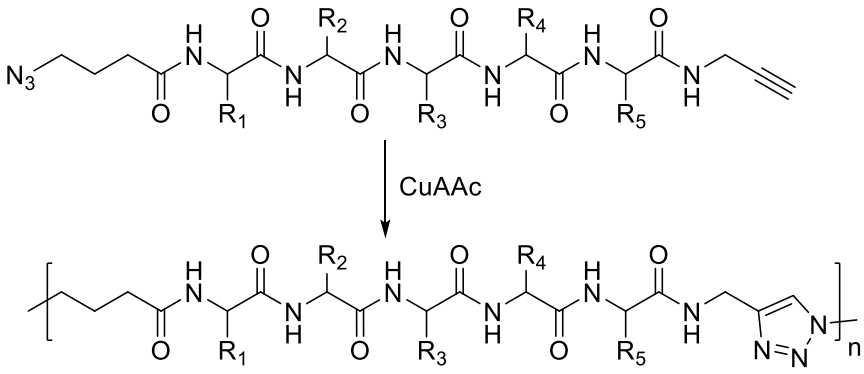


Figure II.16: Synthesis of SCPs via CuAAc starting from oligopeptides.[12, 264]

Berthet *et al.* reported a similar strategy, but instead of oligopeptides, they used well-defined functional heterotelechelic α-alkyne and ω-azido poly(styrene-*co*-*N*-substituted maleimide) precursors (Figure II.17).[266] These polystyrene precursors were made by the copolymerisation of styrene and N-substituted functional maleimides via atom transfer radical polymerisation (ATRP) initiated by a protected alkyne- and bromine-containing molecule. The difference in reactivity ratios between styrene and maleimides allows for the controlled addition of the latter during polymerisation of the first.[56] In this specific case, the functional maleimide was added during the homo-polymerisation of styrene after 50% conversion. This addition incorporates the functionality in the middle of the poly(styrene-*co*-*N*-substituted maleimide) copolymer. After the polymerisation, the bromine end group was converted to an azide and the alkyne protecting group was removed to obtain a heterotelechelic polymer with an alkyne and azide group. Finally, CuAAc polymerisation of these heterotelechelic precursors formed high-molecular-weight, periodic copolymers.

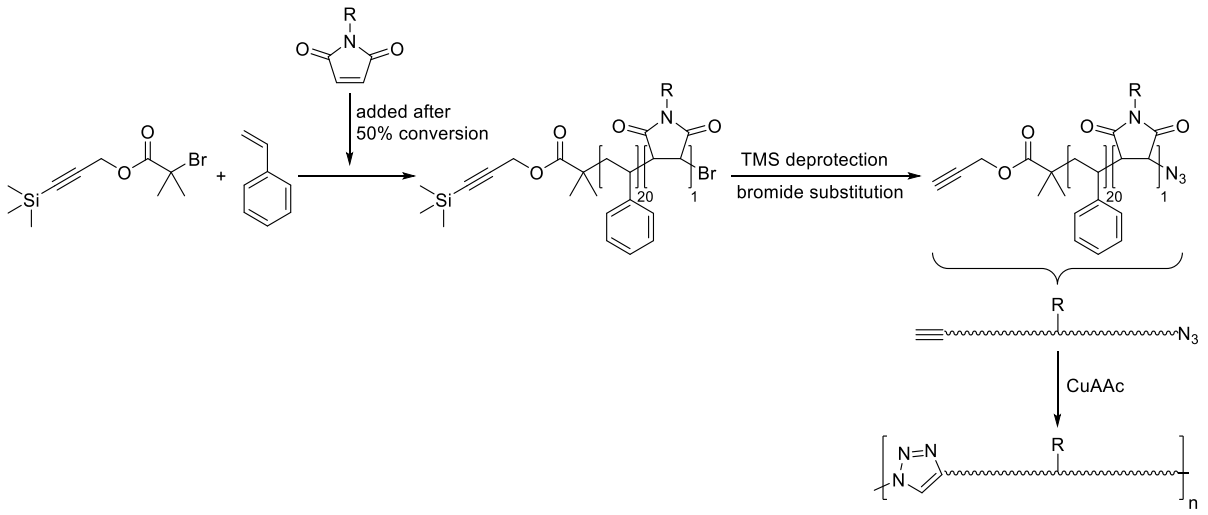


Figure II.17: Synthesis of heterotelechelic α-alkyne, ω-azido poly(styrene-co-N-substituted maleimide) precursors and polymerization to form SCPs.[266]

Xiang *et al.* prepared linear and cyclic SCPs by the combination of the Passerini three-component reaction or Ugi four-component reaction with a self-accelerating double-strain-promoted azide–alkyne cycloaddition reaction (Figure II.18).[267] The first reactions were employed for the synthesis of encoded bis-azide monomers, which could react with sym-

dibenzo-1,5-cyclooctadiene-3,7-diyne by means of the second reaction to obtain linear and cyclic SCPs.

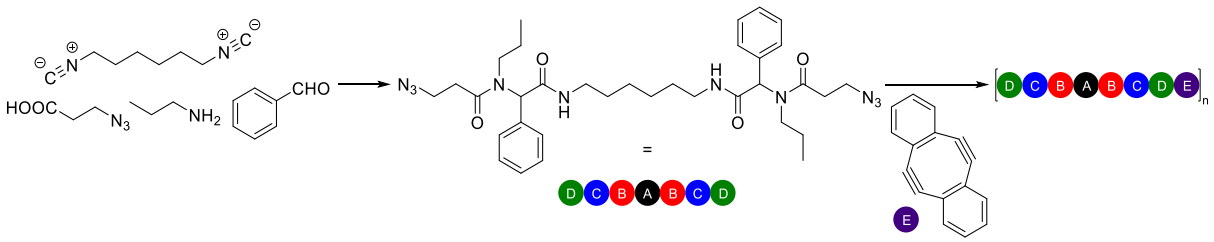


Figure II.18: Preparation of SCPs via the self-accelerating double-strain-promoted azide–alkyne cycloaddition reaction between sym-dibenzo-1,5-cyclooctadiene-3,7-diyne and encoded bis-azide monomers, which were made here with the Ugi four-component reaction.[267]

The last applications that will be discussed in this section are the functionalisations/post-modifications and cyclisations of precision structures with CuAAc. Functionalisation can be achieved by incorporating one of the required functional groups (azide or alkyne) during the synthesis of the structures in question. Alongside generally available alkynes and azides[268-274], CuAAc permits easy incorporation of an azide or alkyne containing sugar[111, 275-293], deoxyribonucleoside[193, 294], fluorophore[294], oligopeptoid[150, 294], poly(ethylene glycol) (PEG)[117, 295, 296], polystyrene[297], siRNA[298], metal complex and hormone[299, 300] moieties. This post-modification strategy was applied by Bräse and co-workers to produce defined dendrimers by reacting tetra-azides or -alkynes with alkyne- or azide-containing oligopeptoids[301] and by Baron's research group, which dimerised an oligopeptoid to mimic a lung surfactant protein B.[302] Baier *et al.* reacted sequence-defined oligo(amidoamine)s bearing azide side-chains with sugar-containing sequence-defined oligo(amidoamine)s with an alkyne end group to obtain branched precision glycomacromolecules.[303] A comparison in binding behaviour to the lectin Con A was made between linear and branched mannose-containing oligomers. Protein binding increased when the degree of branching was raised but did not change when the valency of sugars within the branches was increased for glycomacromolecules with a similar degree of branching. The latter behaviour could be attributed to a lower availability of sugar units close to the backbone.

Triazoles in the backbone of oligopeptides and -peptoids have already proved to cause structural implications on the backbone (*vide supra*)[262, 263], but Caumes *et al.* discovered that triazoles and triazoliums in the side-chain of peptoids can also control tertiary amide isomerism.[304] In both cases, the *cis*-conformation was promoted, but this effect was less significant for triazoles than for triazoliums. For cyclisation to occur, the synthesised, well-defined precursors should have either an azide and an alkyne or two alkynes/azides, which can be connected to a diazide or dialkyne moiety. Both strategies were used for the cyclisation of well-defined oligopeptoids [305, 306], oligosaccharides[307-309], polystyrene[310, 311] and oligo(ether)s[243] (Figure II.19A and B).

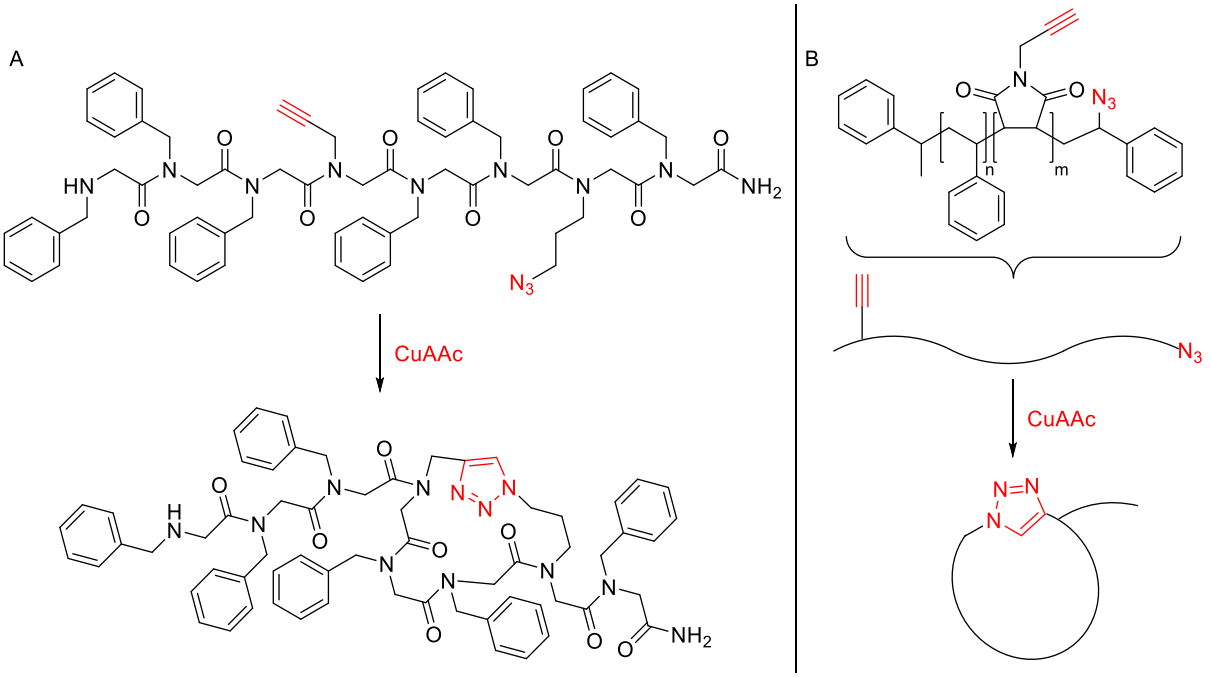


Figure II.19: A) Oligopeptoid and B) polystyrene cyclisation by means of CuAAc. [305, 310]

However, despite the many advantages, CuAAc also has considerable disadvantages, such as the use of explosive, low molar mass azides or multi-azide-containing polymers, difficult installation of either the alkyne or the azide moieties and troublesome removal of copper, which can complex with the triazole units formed.[312-314]

In this context, thiol-X[315-320] and Diels-Alder[321-331] reactions are metal-free alternatives that can counter the disadvantages mentioned and can react with different complementary reaction partners. These two reactions and their impact on SCPs will be discussed in the next paragraphs. Both reactions have proven their worth and have been elaborated in great detail in material and polymer science.[158]

II.3.3 Diels Alder: photo-triggered and thermally induced reactions

Whilst Diels-Alder reactions can be described as efficient "click" reactions[322] and are employed in a wide range of chemistries[196, 198, 199, 205, 323, 326], photo-stimulated Diels-Alder and thermally stimulated retro Diels-Alder reactions had not been investigated as potential tools for the build-up of SCP's until recently, when they were reported by the groups of Barner-Kowollik[136, 332, 333] and Zhu.[60, 61, 334] Although the groups of Grandas and Zhu reported the use of thermo-stimulated retro Diels-Alder in combination with other chemistries for the functionalisation[335] and cyclisation[193, 204] of oligopeptoids and for the synthesis of SCPs[60, 61, 334], Barner-Kowollik and co-workers were the first to report a photochemical protocol to be incorporated into the synthesis of sequence-defined, macromolecular structures. Photo-

chemistry is advantageous as it does not necessarily require high temperatures or excesses of reagents, thus reducing costs.[332]

The synthesis reported by Zydziak *et al.* begins with a bifunctional, di-maleimide core, allowing for the growth of the sequence from each side (Figure II.20).[332] This core is then reacted with the first of two hetero-bifunctional monomers that carry both a photo-reactive group and a diene by light irradiation at λ_{max} = 350 nm to render a dimer. The diene moiety of the first monomer then reacts through the phenacylsulphide group of the second monomer, again by light irradiation, to produce a tetramer. Therefore, the size of the oligomer can be increased efficiently. The maleimide unit resulting from the second monomer addition needs deprotection, which then provides a di-maleimide structure, to begin the process again until 10 repeating units are achieved. For this approach, the design of the monomers was crucial, so that ligation does not occur between the molecules as a result of the orthogonality of photo-triggered and thermally induced Diels Alder reactions. The reaction of the photo-enol of the first monomer with the di-maleimide, for example, is orthogonal to the thermally induced Diels Alder reaction that could occur between the maleimide and the open diene group of the monomer.

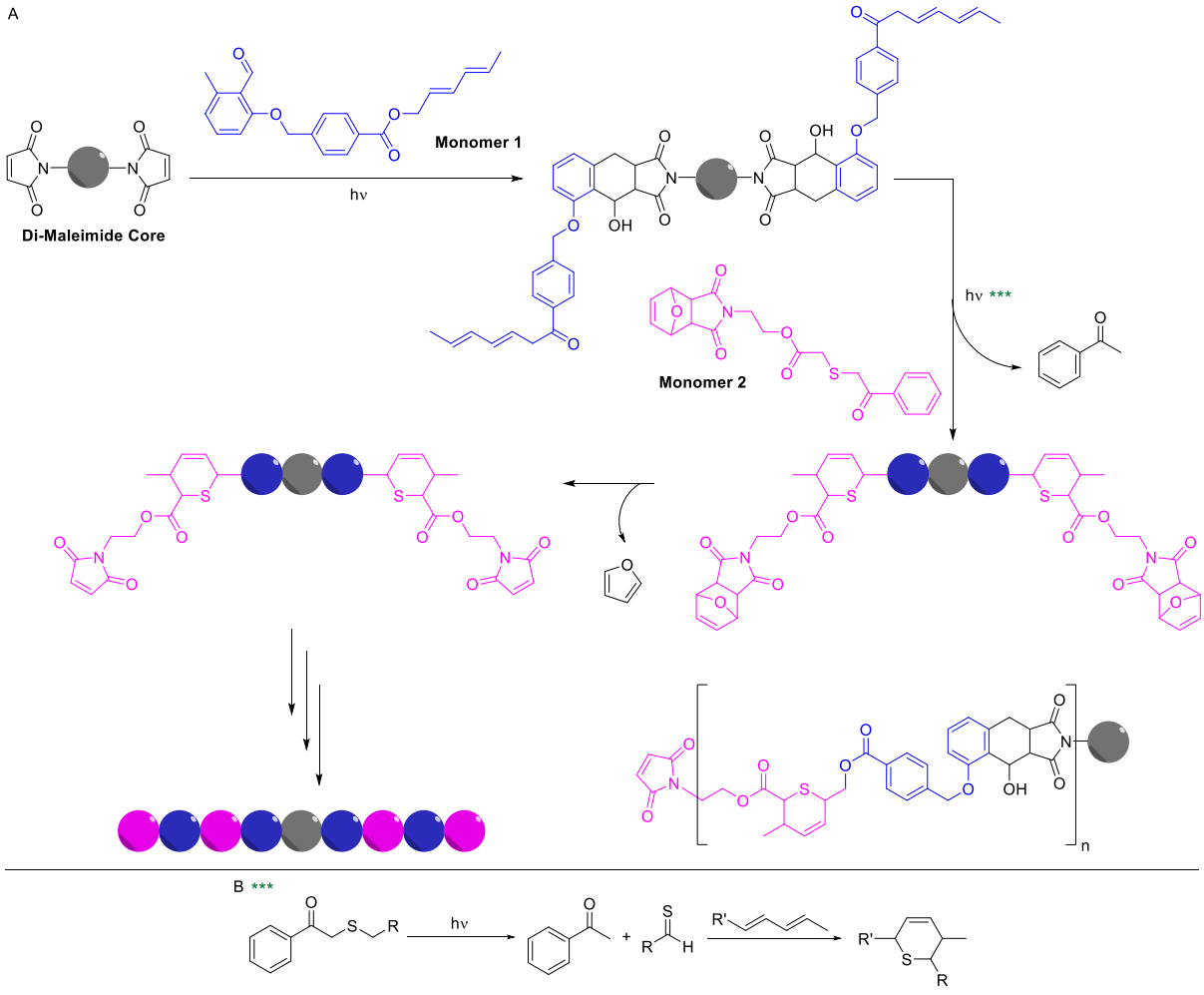


Figure II.20: Reaction schemes for A) the photo-chemical synthesis of sequence-defined oligomers based on Diels-Alder chemistry and B) the photolytic cleavage of phenacylsulphides yielding acetophenone and thioaldehyde which reacts subsequently with a diene forming the Diels–Alder adduct.[332]

A second Diels-Alder-based strategy for the preparation of sequence-defined structures was based on the reaction between the maleimide and the α-methyl benzaldehyde moiety (Figure II.21A).[333] For this method, different monomers were prepared containing an α-methyl benzaldehyde and a protected maleimide. Similar to the previous protocol, a di-maleimide core was reacted by light irradiation with the α-methyl benzaldehyde unit of the monomer used. The product obtained was heated in order to deprotect the maleimide units, which could then be reacted with a new monomer. The repetition of these reactions, coupling and deprotection, allowed the synthesis of sequence-defined decamers. The oligomers prepared could be used for data storage, as the order of the sequence could be read with tandem mass spectrometry, decoding the information that is stored on them.

Another approach that originated within the groups of Meier and Barner-Kowollik combined the Passerini three-component reaction with Diels-Alder chemistry in a two-step iterative protocol (Figure II.21B).[136] In the first step, sebacic acid was reacted through P-3CR with an aldehyde and a linker molecule bearing an isocyanide and a maleimide, the latter of which could react in a subsequent step with a second linker molecule containing an α-methyl benzaldehyde moiety and an acid. The combination of these reactions resulted in the formation of a diacid, which could again be subjected to the two-step iterative protocol. The repetition of these iterative steps allowed for the synthesis of monodisperse sequence-defined macromolecules.

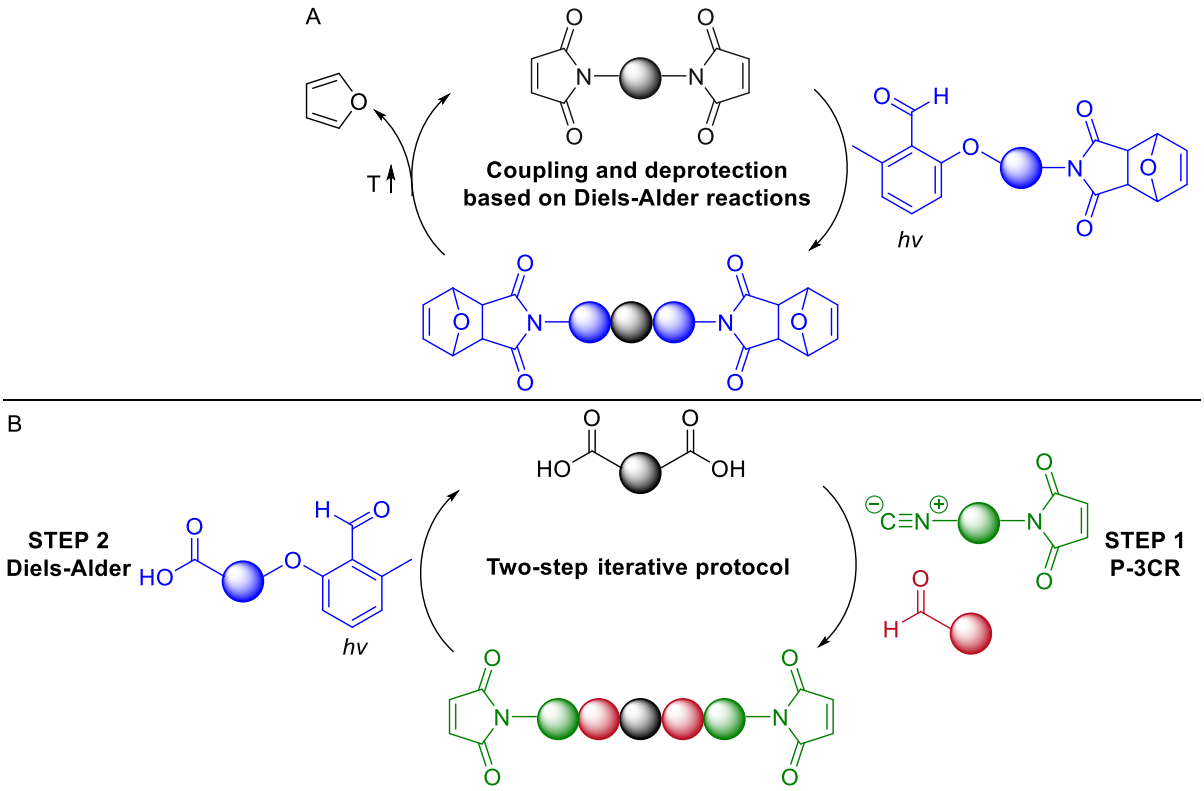


Figure II.21: Protocols for the synthesis of sequence-defined macromolecules based on Diels-Alder reactions: A) combination of a photo-triggered Diels-Alder coupling and a thermally stimulated retro Diels-Alder deprotection [333] and B) the combination of the P-3CR and Diels-Alder chemistry.[136]

Huang *et al.* prepared sequence-defined macromolecules based on the combination of nucleophilic thiol-ene and the thermo-stimulated retro Diels-Alder reaction.[334] A monomer

was prepared with a furan-protected maleimide and an acetyl-protected thiol to be used in an orthogonal, iterative, divergent/convergent approach (*vide supra*). In the first step, the selective deprotection is performed at each of the two protected end groups of the monomer. Two different mono-protected intermediates are formed, one with a thiol and another one with a maleimide, which can react with each other. The diprotected dimer obtained has the same protected end groups as the monomer and can now undergo the same cycle, which was repeated a number of times to obtain sequences with the targeted chain length.

Grandas and co-workers combined thermally stimulated retro Diels-Alder reactions with thiol-ene or Diels-Alder to functionalise[335] and cyclise[193, 204] oligopeptoids. These oligopeptoids have a (2,5-dimethylfuran)-protected maleimide end group, which can be thermally released (Figure II.22). For cyclisation [193, 204], a thiol is present in one of the side chains and reacts with the maleimide when it is released. For functionalisation[335], thiol- and diene-containing compounds are applied. An oligopeptoid-oligonucleotide conjugate could be made by using these peptoids with a diene-oligonucleotide.

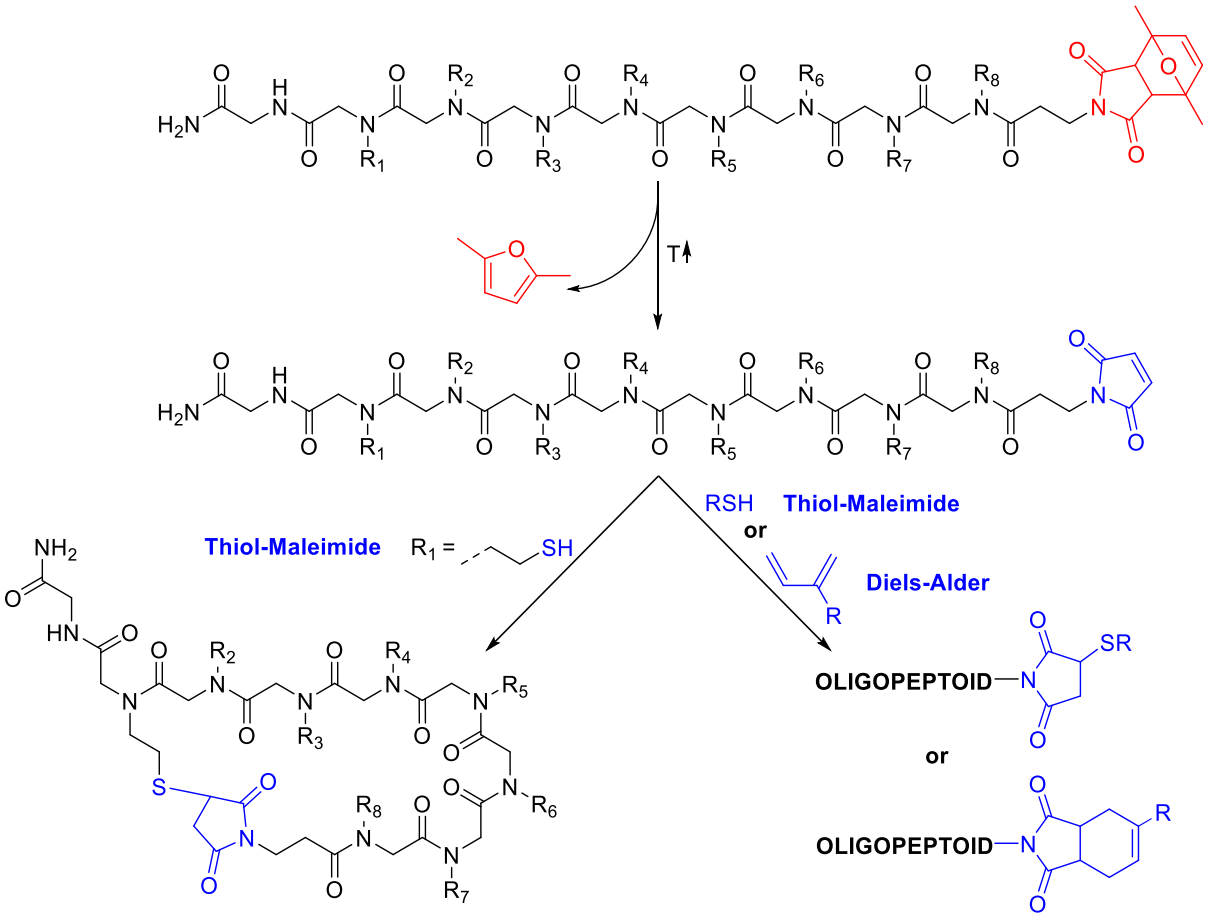


Figure II.22: Combination of retro Diels-Alder and thiol-maleimide or Diels-Alder for the cyclisation[193] and functionalisation[204, 335] of oligopeptoids.

The use of photo-reactive Diels-Alder reactions may provide a straightforward and quick way to build sequences in a “click”-like fashion. Moreover, with orthogonal thermo-responsive

groups, a wide range of side-chain diversity is possible. This technique could possibly offer a route for synthesising much longer sequences with complete monodispersity.[336, 337]

II.4 Click and click-inspired chemistry within the area of SCPs: Thiol-ene and thiolactone chemistries

In this PhD thesis, thiolactone chemistry is combined with different thiol-ene reactions to functionalise polymers and prepare sequence-controlled macromolecules. In the present section, a more detailed overview will be given of both thiol-ene and thiolactone chemistry and their use in sequence-defined chemistry.

II.4.1 Thiol-X chemistries

Thiol-X describes a wide class of efficient reactions with the thiol group being the common reaction partner (Figure II.23).[316] Thiols exhibit high versatility and high reactivity, which can be attributed either to the weak bond between the sulphur and hydrogen atoms, which allows for the easy formation of thiyl radicals, or to the relatively larger size and polarisability of the sulphur atom, which both results in a higher nucleophilicity of the thiol in comparison to amines and alcohols, and enables the smooth production of thiolate anions. Due to these properties, thiols can easily react with a wide range of functionalities. Electron-rich functional groups such as alkenes and alkynes can react fast with thiyl radicals, while the thiolate anion will swiftly react with halogens, electron-poor alkenes, isocyanates and epoxides.

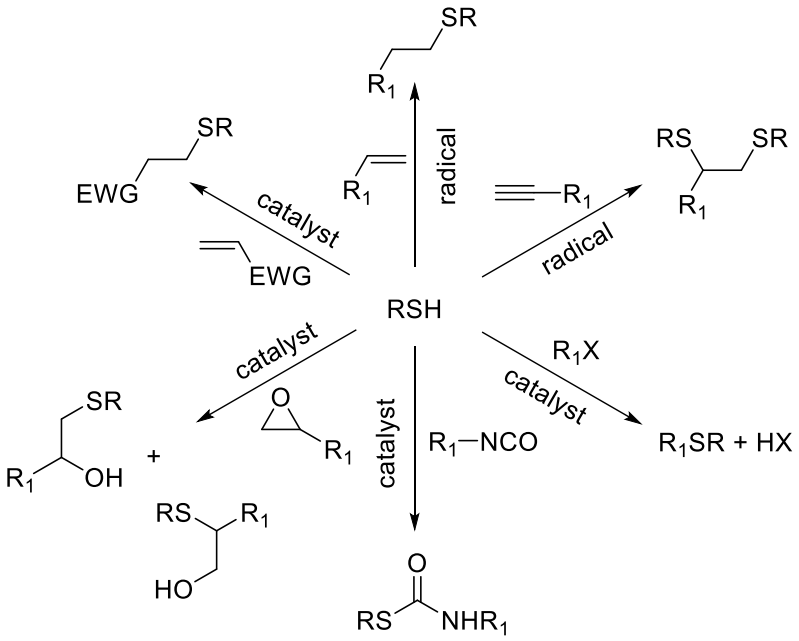


Figure II.23: Different thiol-X reactions. EWG = electron withdrawing group, X = halogen and catalyst = base.[316]

II.4.1.1 Radical thiol-ene chemistry

One notable example of the thiol-X reactions is the radical thiol-ene reaction, which has been known since 1905.[338] The characteristics of this reaction, such as high yields, speed and insensitivity to water and oxygen, allowed for its use in a wide range of applications, including post-modification and synthesis of polymers, coatings, adhesives, optical lenses, *etc*.[315, 317] The mechanism of the radical thiol-ene chemistry proceeds via a chain process, which consists of an initiation, propagation, chain transfer and termination part.[339] For the first step, the initiation, a thermal or photoinduced initiator is generally applied, yet it is not always necessary: UV- or sunlight can also be exploited for the formation of thiyl radicals (Figure II.24).[315, 316, 340-343] There are two types of photoinitiators. When Type 1 photoinitiators such as 2,2-dimethoxy-2-phenylacetophenone (DMPA) absorb a photon, they split up into two radicals, which can abstract the hydrogen from the thiol and form the thiyl radical. A Type II initiator, such as benzophenone, will undergo photon absorption and then enter the excited state, which can react with a thiol. This reaction results in the formation of a thiyl and a non-reactive ketyl radical. Thiyl radicals can also be obtained with thermal initiators, such as 2,2'-azobis(isobutyronitrile) (AIBN), which split into radicals at elevated temperature. Yagci and coworkers compared the different initiators and proved that Type I photo initiators rendered the highest efficiencies to induce the thiol-ene reaction.[343]

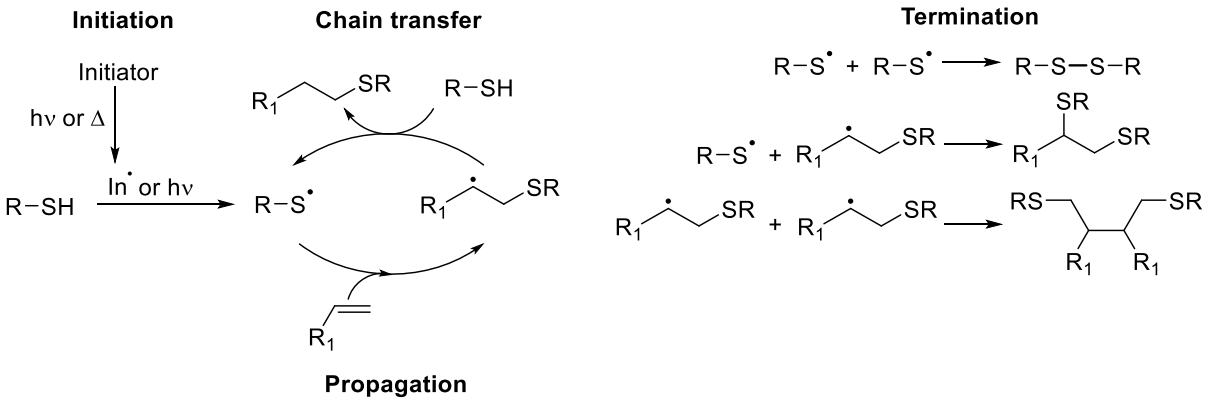


Figure II.24: Mechanism of the radical thiol-ene reaction.[317]

In the next step, the thiyl radicals formed will react with the ene functionality to form an intermediate carbon-centered radical.[317] Subsequently, the latter radical will abstract a hydrogen from another thiol with the formation of the preferred product and a new thiyl radical, which can start the cycle again and react with a new alkene (Figure II.24). The thiol-ene reaction is terminated by disulphide formation and recombination of radicals.

The structures of both the thiol and the ene play an important role to the reaction rate. The reaction rates of thiol glycolate esters and thiol propionate esters are higher in comparison to alkyl thiols.[315] The hydrogen bonding between the thiol hydrogen groups and the ester bond weakens the sulfur-hydrogen bond and allows for the easier formation of thiyl radicals.

As for the ene functionality, the substitution and the electron density are crucial parameters for its reactivity in the thiol-ene.[317] Sterical hindrance means that a more substituted double bond is less reactive than an end-standing double bond. In order to test the influence of electron density, Hoyle and co-workers tested a range of different alkenes[315] and reported the following order:

norbornene > vinyl ether > propenyl ether > alkene ≈ vinyl ester > *N*-vinyl amide > allyl ether ~ allyltriazine ~ allylisocyanurate > acrylate > unsaturated ester > *N*-substituted maleimide > acrylonitrile ~ methacrylate > styrene > conjugated diene

Generally, the reactivity increases with the electron density of the double bond, yet with a few exceptions. Norbornene reacts very fast because the addition of the thiyl radical leads to a considerable ring strain relief and the subsequent hydrogen abstraction is rapid. The last three entries in the order above (*i.e.* methacrylates, styrenes and conjugated dienes) react slower as the intermediate carbon-centered radicals are more stabilised, resulting in a slower hydrogen abstraction.

II.4.1.2 Nucleophilic thiol-ene chemistry

In addition to a radical pathway, the thiol-Michael addition is a nucleophilic-based reaction that can be performed between a thiol and an electron-poor ene.[344] This reaction has become a popular tool in both biological systems and material science.[320] Two different types of catalyst, a base or a nucleophile, can be applied, resulting in different mechanisms. Despite their differences, the formation of a thiolate anion, the use of an activated ene and the need for a catalyst can be observed in both.

When a base is used as a catalyst, the thiol is first deprotonated, which results in a conjugated acid and a thiolate anion which attacks the β-carbon of the activated alkene in the subsequent step (Figure II.25). The newly formed anion deprotonates the conjugated acid, yielding the thioether product and the base, which can be reapplied in the first step of the mechanism.

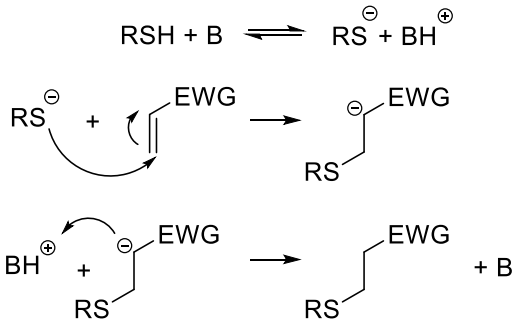


Figure II.25: The base-mediated mechanism of the thiol-Michael addition with an acrylate.[344]

Nucleophiles such as phosphines and amines can also be used as catalysts.[344-347] The activated alkene is attacked by the nucleophile, generating a strong base, which can deprotonate a thiol and start the thiol-Michael addition (Figure II.26).

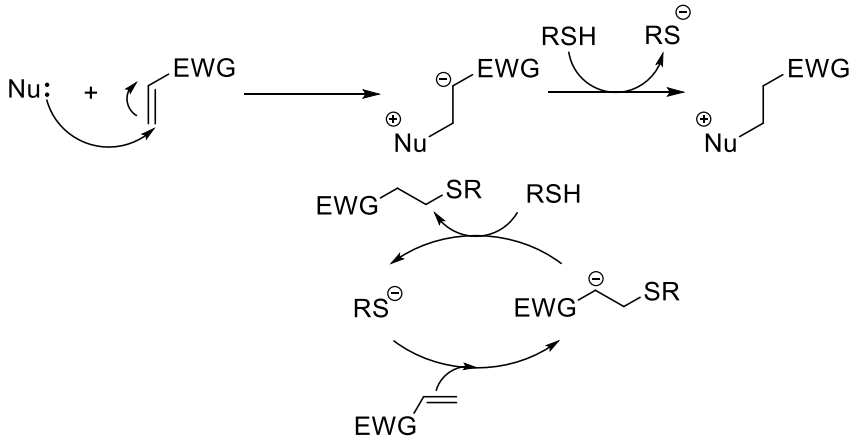


Figure II.26: The nucleophile-mediated mechanism of the thiol-Michael addition with an acrylate.[344]

The reaction rate of the thiol-Michael additions depends on the catalyst, the solvent's polarity, the thiol's pK_a and the structures of both the thiol and the ene.[344] When different nucleophilic catalysts are compared, primary amines provide higher reaction rates than secondary and tertiary amines. Tertiary phosphines, however, induce reaction rates that are 100 times larger than primary amines. Reaction rates are increased when an ene with a higher electron-deficiency and/or a thiol with a lower pK_a is chosen.

Whilst thiol-X reactions have many potential side reactions (such as disulphide formation) and radical thiol-ene has limited potential for the coupling of polymers[348] to be called 'click', their efficiency and versatility make them ideal candidates for the design of sequence-defined structures.

II.4.1.3 Thiol-ene chemistry for the synthesis and modification of SCPs

Alabi and co-workers coupled radical and nucleophilic thiol-ene chemistry for the synthesis of oligothioetheramides (Figure II.27).[143, 349-352] For this purpose, allyl acrylamide building blocks were used in combination with a soluble fluorous tag containing an alkene moiety. The latter combines advantages of both solid- and solution-phase chemistry, and can be easily removed when the required defined sequences have been formed. The functional allyl acrylamides used have two orthogonal functionalities that can react readily with thiols: the first reacts in a phosphine-catalysed Michael addition, the second in a photo-initiated thiol-ene reaction. The sequences are built via two iterative steps. First, the allyl group reacts with an excess of dithiol, resulting in a thiol end group. In the next step, this thiol can react with the acrylamide of a functional allyl acrylamide to reinstate an allyl group, after which the cycle can be continued. Biologically active oligothioetheramides were synthesised to mimic

antimicrobial peptides[350, 351], thus demonstrating the wide range of potential applications for sequence-defined chemistry.

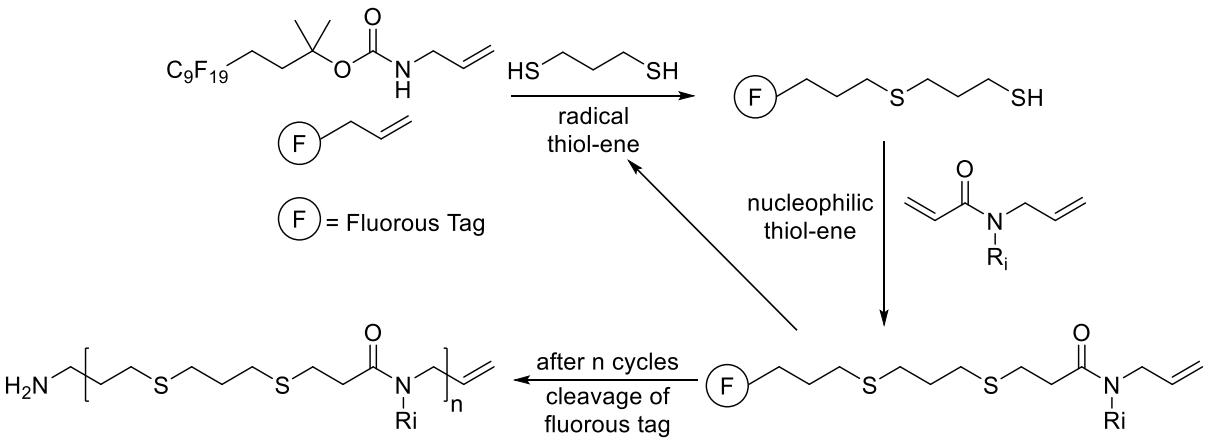


Figure II.27: Synthesis of oligothioetheramides applying both radical and nucleophilic thiol-ene chemistry using allyl acrylamides and starting from a fluorous tag containing an alkene moiety.[143, 349]

Both nucleophilic and radical thiol-ene reactions were also used by Bowman and co-workers for the synthesis of sequence-defined nucleobase-containing oligomers, which could be successfully polymerised, resulting in SCPs.[353] Complementary nucleobase-containing monomers were synthesised to incorporate a (protected) thiol and either an acrylamide or an alkene group. Monomers were connected via thiol-Michael reactions to form oligomers and then polymerised via a radical thiol-ene reaction. In this case, copolymers with PEG could also be easily synthesised and supramolecular organogels were formed as a result of reversible crosslinks between the complementary bases.

Meier and co-workers reported the combination of radical thiol-ene reaction with the efficient multi-component Passerini[137, 354] and Ugi[138] reactions for respectively single- and dual-side chain control in sequence-defined oligomers (Figure II.28), thus opening the possibility for double modification of the sequence-defined structure. In the first step of their protocols, a reaction is carried out between an acid functionality, an aldehyde that contains an end-standing alkene and an isocyanide. In case of an Ugi reaction, an amine is also added in order to introduce double modification. The use of varying isocyanides and amines allowed for the introduction of different side chains, while the aldehyde incorporates an alkene that can react with 3-mercaptopropionic acid to reinstate an acid. This acid can be used again in the Passerini reaction. In addition to well-defined oligomers, a block copolymer consisting of a PEG and a defined pentamer could be made, proving the usefulness of these interesting methods.

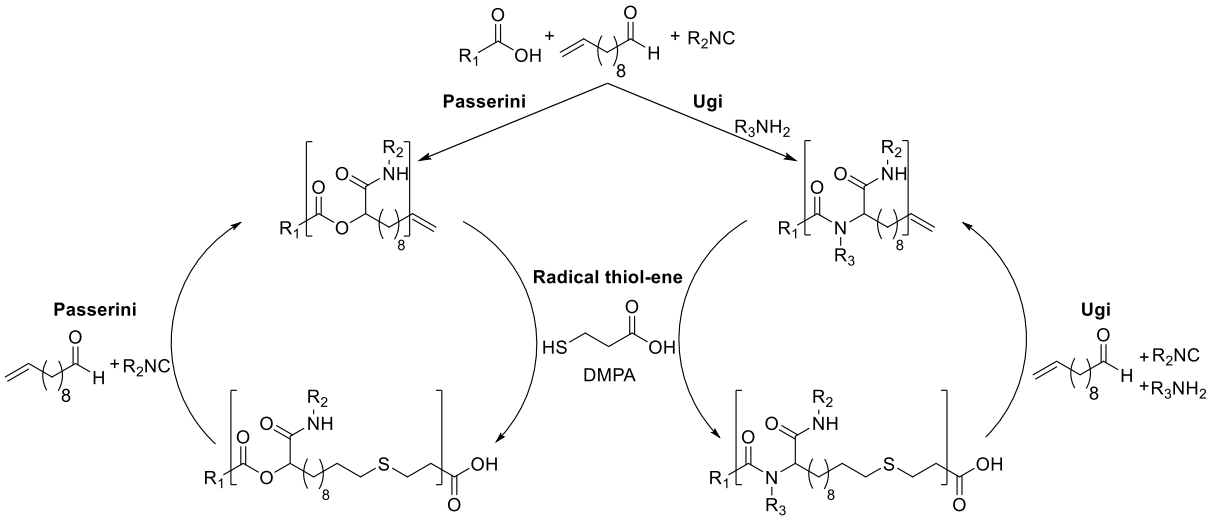


Figure II.28: Multicomponent Passerini and Ugi reactions in combination with radical thiol-ene chemistry to build defined structures.[137, 138]

Another effective combination of Passerini and thiol-ene reactions led to the synthesis of SCPs as reported by Zhang *et al.*[355] They reacted 1,6-diisocyanohexane with 10-undecylic acid and a monoaldehyde to form a diene-containing molecule. In this case, the acid incorporated the alkene, which was reacted in the consecutive step with 3-mercaptopropionic acid to reinstate an acid. A monoaldehyde and 1,6-diisocyanohexane were added to the diacid compound formed in order to prepare SCPs with a repetitive AABB sequence.

Hong and coworkers, in turn, prepared SCPs by combining the thiol-ene reaction with the amine-propiolate reaction.[356] A propiolate-methacrylate was first reacted with a diamine containing two secondary amine groups to prepare a dimethacrylate with a BAB pattern (Figure II.29). Addition of an amino thiol converted the latter to a diamine. A dipropiolate was reacted with the CBABC diamine secured to obtain SCPs.

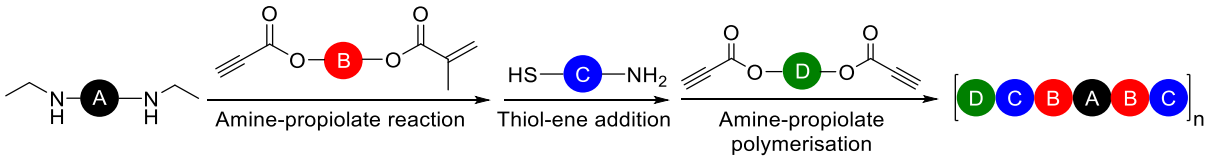


Figure II.29: Combination of thiol-ene with the amine-propiolate reaction for the synthesis of SCPs containing a repetitive DCBABC pattern.

As discussed in II.3.3, Zhu and co-workers combined the thermo-stimulated retro Diels-Alder of a furan-protected maleimide with the nucleophilic thiol-ene reaction in an orthogonal, iterative, divergent/convergent approach to obtain sequence-defined oligomers.[334] Their strategy is another interesting example of the combination of thiol-ene chemistry with another highly efficient chemistry.

Boyer's group combined thiol-ene, esterification and single-unit monomer insertion in RAFT, which is initiated with green light (Figure II.30).[75] In the first step, an acrylate or acrylamide is incorporated via SUMI. The aminolysis of the trithiocarbonate and the subsequent reaction with a hydroxy acrylate allows for the installation of an alcohol, which can react with an acid-

containing chain transfer agent. These reactions were performed three successive times to install three different functional groups in the defined oligomer.

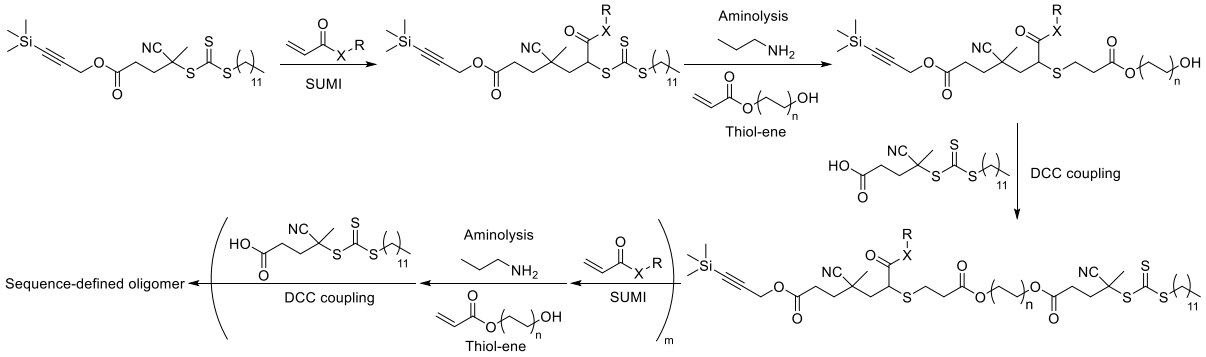


Figure II.30:Combination of SUMI, thiol-ene and DCC coupling for the synthesis of sequence-defined oligomers.[75]

Llevot *et al.* used α,ω-dienes and α,ω-dithiols to sequentially build sequence-defined oligomers on silicon surfaces.[357] Six different layers, three different dithiols and three different dienes, were installed, but the structures obtained on the surface were not monodisperse as some individual steps were not completed. A detailed characterisation was performed with techniques such as X-ray Photoelectron Spectroscopy (XPS), Time-of-Flight Secondary Ion Mass Spectrometry (ToFSIMS) and Atomic Force Microscopy (AFM) to provide evidence of the synthesis of each individual layer on the surface.

In addition to sequence synthesis, thiol-X chemistry has been used to polymerise, to post-modify or to cyclise well-defined structures. As was the case for CuAAc, the functionalisation can easily be done by incorporating a reaction partner on the side chain or the backbone. In addition to generally available thiols[125, 248, 268, 271, 274, 287, 358, 359], this strategy enabled easy incorporation of sugar units[285, 360-363], glutathione, biotin and cholesterol[335] in a post-modification strategy.

Hartmann *et al.* synthesised a series of sequence-controlled glycopolymers starting from a defined dithiol-containing hydrophilic spacer macromonomer or dithiol-functionalized PEG (Mn of 6 kDa) and different defined glycomacromonomers bearing between one to five α-D-Mannose ligands.[364, 365] The latter contained double-bond end groups which could be polymerised with the dithiol macromolecules via photoinduced thiol−ene coupling. The binding behaviour of these glyco-oligomers and -polymers toward the lectin Concanavalin A was studied in order to compare its dependence to the number of sugar moieties and overall molecular weight. The length of the defined structures did not have any effect, but it was proven that the number of mannose ligands is correlated to the Concanavalin A binding and clustering.[364] Low binding affinities were observed for the PEG-containing SCPs, because the introduction of the polymer decreased the ligand density and increased the overall molecular weight, which leads to more steric repulsion.[365]

Klok and co-workers used this strategy to modify sequence-defined oligoesters, which have alternating allyl and isobutyl side chains, with thioglycolic acid, mercaptoethanol and

cysteamine (Figure II.31A).[358] This post-modification step allowed for the preparation of hydrophilic/hydrophobic patterned oligoesters, which might be interesting as foldamers. The effect of the different side-chains on the folding behaviour could then be studied.

Unimolecular block copolymers were made by Johnson and co-workers via thiol-ene post-modification of chiral allyl-based oligomers (Figure II.31B).[248, 366] This could be done on a scale of approximately one gram. First, copolymers were prepared consisting of a block with decane-based side chains and another block with either thioglycerol- or triethyleneglycol side chains.[248] These block copolymers phase-segregate into hexagonal cylinder morphologies. This behaviour is not driven by side-chain crystallisation, but by amorphous block copolymer assembly. Next, the influence of the stereochemistry in the backbone was investigated for the defined block copolymers containing decane-based and triethyleneglycol side chains.[366] It was discovered that the phase behaviour obtained was modulated by the stereochemistry. These observations proved that unimolecular polymer self-assembly can be dependent on the stereochemistry in the sequence.

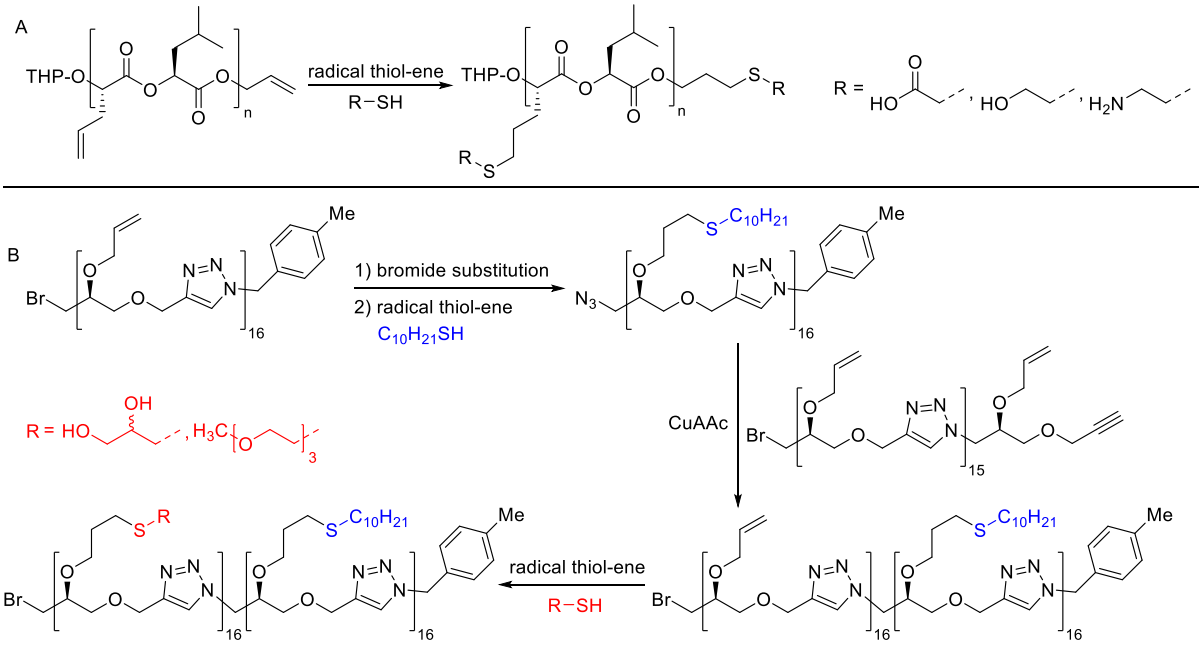


Figure II.31: Thiol-ene post-modification with different thiols of defined sequences; A) alternating allyl isobutyl oligoesters[358] (THP = tetrahydropyranyl), B) allyl-based oligomers.[248]

Like CuAAc, thiol-inspired cyclisation requires a thiol and a corresponding reaction partner, such as a maleimide. Grandas and co-workers cyclised peptoids by incorporating a thiol in the side-chain and a (2,5-dimethylfuran)-protected maleimide as an end group.[193, 204] Through an increase in temperature, the maleimide was released in a retro-Diels-Alder reaction and could react with the thiol to form a cyclic oligopeptoid (Figure II.22, *vide supra*).

Though thiols have many interesting properties, some disadvantages can be associated with their use. Thiols have a low shelf life, for instance, due to the formation of disulphide bonds. They also produce an unpleasant smell and commercial availability is highly limited. Many techniques have been developed to protect thiols and allow their release *in situ* as a result of an

external stimulus.[348, 367-383] For more details about these strategies, the reader is kindly invited to peruse a recently published review from our group.[383]

One possible atom-efficient way for *in situ* generation of thiols is through thiolactone chemistry.[384, 385]

II.4.2 Thiolactone chemistry

II.4.2.1 Introduction

Thiolactones are cyclic thioesters that can be formed by dehydratation or lactonisation of a mercapto-acid with carbodiimides or phosphorus pentoxide as dehydrating agents (Figure II.32). These molecules can easily undergo ring-opening by aminolysis generating a thiol. Depending on the ring size, a distinction can be made between the four-, five- and six-membered ring, which are respectively β-, γ- and δ-thiolactone.

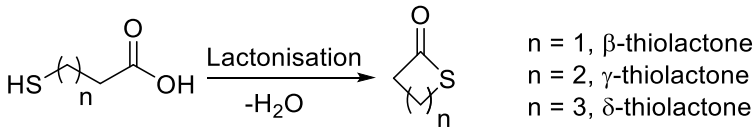


Figure II.32: Synthesis of thiolactones starting from different mercapto-acids.

The ring-opening behaviour of these thiolactones is different in comparison to their lactone counterparts.[384] Lactones are less prone to ring opening and can split at the oxygen-acyl or oxygen-alkyl bonds, while thiolactones are only opened at the sulphur-acyl bond. The stability of the thiolactone when faced with a nucleophilic attack is dependent on the ring size and the number of substituents, which provide sterical hindrance during the attack. A smaller ring has more ring strain, making it more susceptible for lysis. Keul's group also discovered that the position of a substituent can influence the ring opening (Figure II.33).[386] An asymmetric bisthiolactone was prepared with a γ-thiolactone ring carrying an amine on the α-position, also known as homocysteine thiolactone, and a γ-thiolactone ring bearing an acid on the β-position. The aminolysis of this bisthiolactone indicated that the homocysteine part of the molecule reacts faster than the other thiolactone. Modelling experiments indicated that additional hydrogen bonding between the substituent on the α-position and the attacking amine might be responsible for this behaviour.[387]

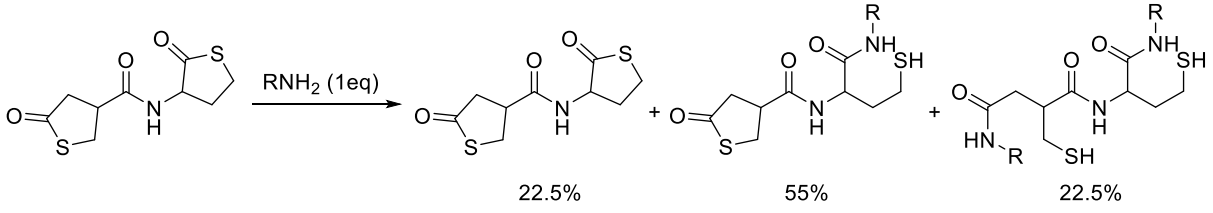


Figure II.33: Influence of the position of the substituent on the γ-thiolactone on the ring opening.

The opening of the thiolactone ring can be executed by means of nucleophiles such as amines, alcohols and water.[384] The latter two require a basic medium to be able to open the thiolactone ring, while aminolysis can be performed without additives. Research within our own group proved that the structure of the amine has an influence on the aminolysis.[388] Although secondary and tertiary amines are not able to open the thiolactone ring, the aminolysis can be performed with a cyclic secondary amine such as pyrrolidine, which reacts faster than the primary amines tested. Amines with an inductive-withdrawing group such as benzyl amine react slower than aliphatic amines, while amines with α-branching display a lower reaction rate due to sterical hindrance.[389]

As mentioned above, thiolactones can easily undergo ring-opening by aminolysis, generating a thiol and enabling the introduction of a functionality (Figure II.34).[384, 385, 390] In a subsequent step, the formed thiol can react in a thiol-X reaction to introduce a second functionality. This one-pot multi-step reaction has been used to synthesise and modify different functional polymer structures.[385] The combination of amine, γ-thiolactone and ene, especially, has proven very useful.

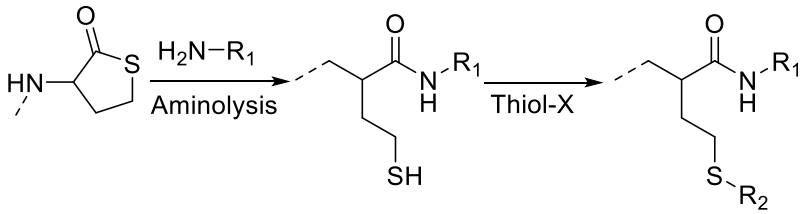


Figure II.34: Introduction of two different functionalities as a result of in-situ thiol formation via aminolysis of a thiolactone and subsequent thiol-X reaction.[385]

Different AB'-monomers with a double bond and a thiolactone were synthesised by our group and polymerised via the combination of aminolysis with the radical or nucleophilic thiol-ene reaction for the synthesis of functional linear and crosslinked polymers (Figure II.35).[388, 389, 391] Keul's group prepared an AB' monomer bearing a thiolactone and an epoxide group for the synthesis of polymers and hydrogels via the thiol-epoxide reaction.[392] This group also prepared a bisthiolactone (Figure II.33) for the synthesis of polythiols, which were modified with acrylates.[386]

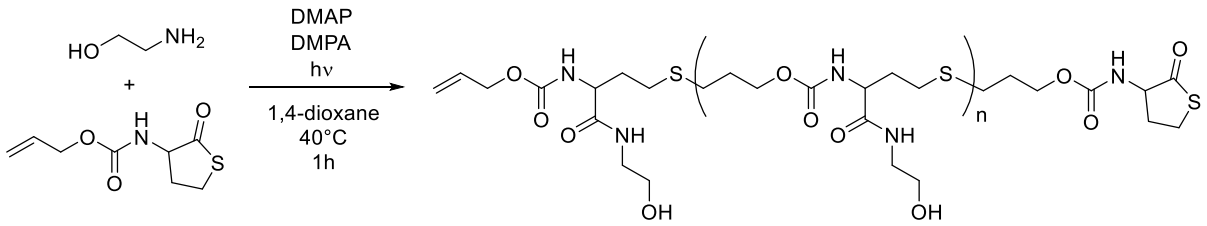


Figure II.35: Example of an AB' monomer polymerised via the photo-initiated radical thiol-ene reaction after aminolysis with ethanolamine.[391]

The thiolactone functionality could also be installed on polymers such as polystyrene and poly(N-isopropylacrylamide) (PNIPAM), which allowed for the double modification of these polymers (Figure II.36).[393-396]

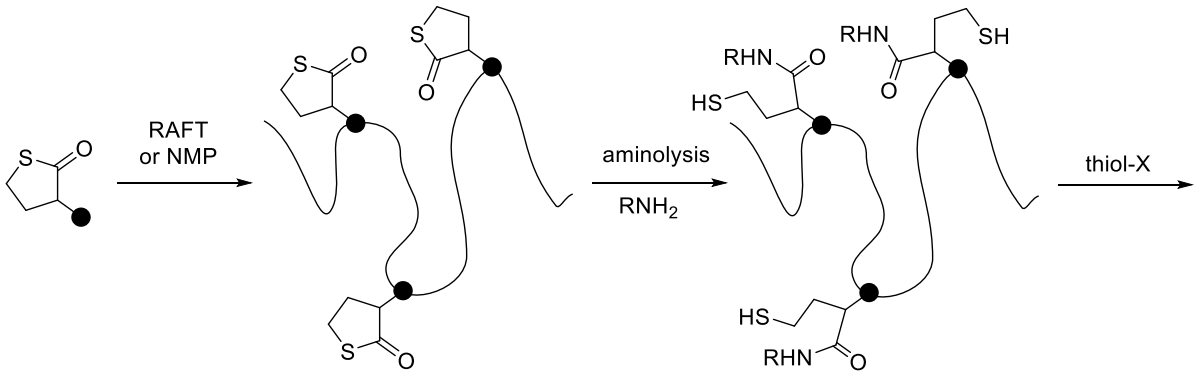


Figure II.36: Synthesis of copolymers which can be double-modified due to the amine-thiolactone-ene conjugation.[393-396]

Thiolactone derivatives have been applied for the synthesis of hyperbranched structures[397, 398], dendrimers[399], cyclic polymers[400] and oligopeptides (chapter V)[401], and the modification of silica (chapter III)[402] and polymer end groups (chapter IV).[403-405] Lately, thiolactone chemistry has also allowed for the synthesis of sequence-defined and -controlled structures.[99]

II.4.2.2 Thiolactone chemistry for the synthesis and modification of SCPs

Multifunctional, sequence-defined oligomers were prepared in our group from a single thiolactone building block via a two-step, iterative protocol on a solid support with two different approaches (Figure II.37).[406] The first one functioned as follows: In the first step, a thiolactone, immobilised on the solid support, was opened with a functional amine to generate disulphides (Figure II.37A). These disulphides were reduced in the second step to generate thiols, which could then react with a thiolactone acrylamide. Phosphines were used to reduce the disulphide bonds and catalyse the reaction between the acrylamide and the thiol. The downside of this is the difficult removal of phosphines. However, in comparison to solid-phase peptide synthesis, this method does not need any protecting groups and can be performed with one building block (*i.e.* the thiolactone derivative). In this case, the functionality originates from the different available amines that are used to open the thiolactone ring, thus introducing a wide array of functionalities, potentially higher than the 20 amino acids used for peptide synthesis.

Very recently, we modified this procedure to obtain a straightforward and versatile one, which could additionally be translated to an automated protocol in an adapted peptide synthesizer.[139, 407] This second protocol (Figure II.37B) also starts with thiolactone immobilised on the solid support, which is now opened with an amino alcohol. The aminolysis of the thiolactone releases a thiol that subsequently reacts with an available acrylic compound (acrylate or acrylamide) through a nucleophilic thiol-ene reaction, which prevents disulphide formation. The selected amino alcohol can influence the backbone of the sequence, while different functionalities can be introduced in the side chain by using abundantly available acrylics. In the following step, the remaining alcohol function of the amino alcohol reacts with an α-isocyanato-γ-thiolactone to perform the chain extension. The experimental development and detailed explanation of this

protocol will be provided in chapter VI, while the application and polymerisation of the oligomers obtained will be discussed respectively in chapters VII and VIII.[407]

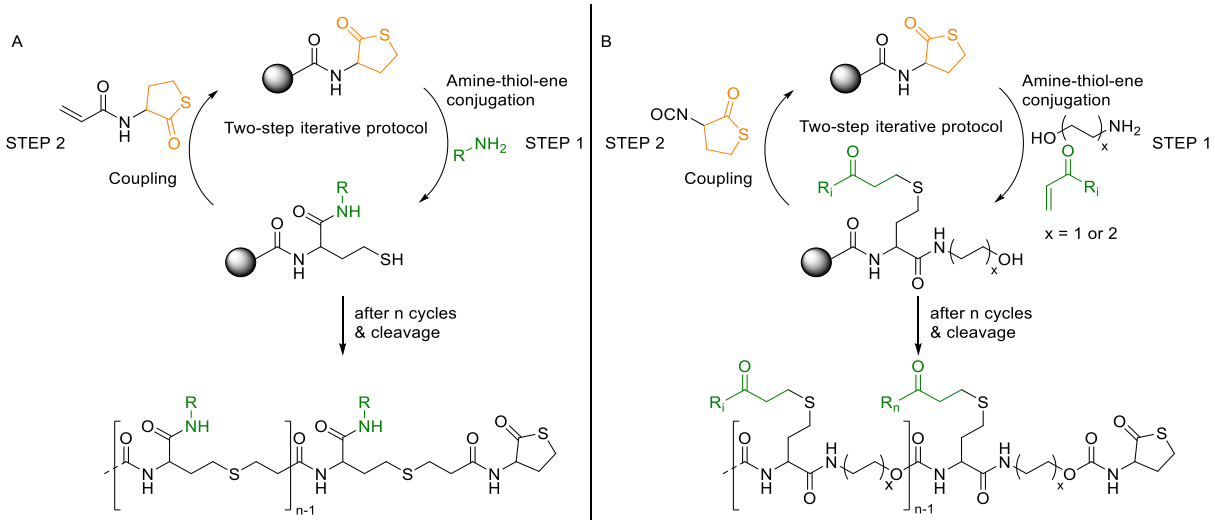


Figure II.37: Two-step, iterative protocols on solid support for the preparation of sequence-defined oligomers from a single building block: A) a thiolactone acrylamide[406] or B) an α-isocyanato-γ-thiolactone.[139]

The α-isocyanato-γ-thiolactone-based protocol has been used and modified to obtain different macromolecules. In a collaboration between our research group and Börner's, multifunctional block copolymers were synthesised with a PEG and a thiolactone-based monodisperse block.[408] To easily install the polymer, pentamers were prepared on a PEG-preloaded poly(styrene) resin. The protocol had to be modified slightly to acquire the defined block copolymers with good yields and high purity. The block copolymers obtained can potentially be used for biomedical applications.

Both Becer's and our group used thioacrylates[409] instead of acrylates and acrylamides to expand the monomeric toolbox (Figure II.38).[410] As the thioacrylate reacts very fast in an aza-Michael reaction with the amino alcohol, the first step of the existing protocol caused problems and the two-step iterative protocol had to be converted to a four-step strategy. By analogy with the original protocol, a thiolactone is immobilised on the solid support and is opened with an amino alcohol in the first step to introduce an alcohol functionality. Dimethylphenylphosphine (DMPP) was added to ensure that no disulphide formation occurred in this step. In the subsequent step, the formed thiol, generated after the aminolysis, was reacted with ethyl thioacrylate. Next, the incorporated thioester was modified with primary amines via a thiophenol-catalysed amidation. In the fourth and final step, α-isocyanato-γ-thiolactone was added to react with the alcohol functionality to reinstate the thiolactone. It was possible to prepare multifunctional oligomers with amide groups in the side-chains with a high purity.

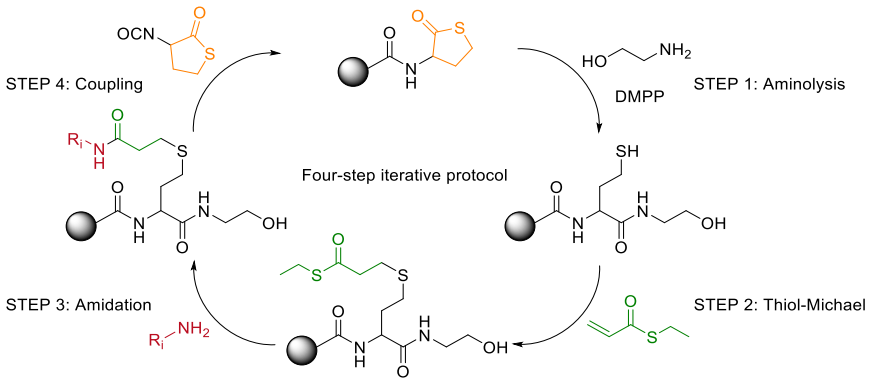


Figure II.38: Four-step, iterative protocol on solid support for the preparation of sequence-defined oligomers based on thioacrylates and a subsequent amidation.[410]

In another approach, we developed well-defined heterotelechelic polyisobornylacrylate precursors - with α-thiolactone and ω-acrylate end groups - that were used to prepare SCPs (Figure II.39).[363] Starting with a thiolactone initiator, polyisobornylacrylate was prepared via Cu(0)-mediated polymerisation. The bromide end group was converted to an alcohol with 2-mercaptoethanol using a thio-bromo reaction. The incorporated alcohol was subsequently converted to an acrylate using acryloylchloride. Addition of a functional amine to these precursors on the one hand led to the specific incorporation of a functionality and on the other hand released a thiol capable of reacting with the acrylate. This strategy allowed for the easy synthesis of both amphiphilic graft copolymers by using a PEG-amine (800 or 2000 Da) and glycosylated macromolecular line-ups in which the distance between the grafts is precisely controlled through the length of the precursor. For the glycosylated examples, allyl amine was applied to incorporate double bonds that are reactive to thiol-containing sugars by means of a radical thiol-ene reaction.

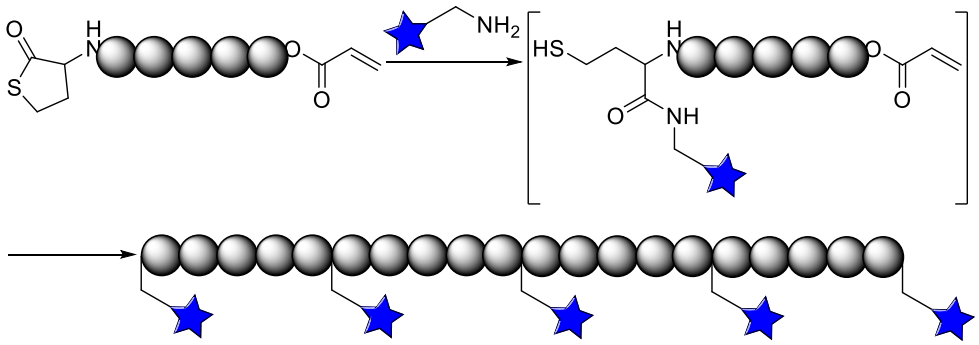


Figure II.39: Polymerisation of α-thiolactone, ω-acrylate polyisobornylacrylate precursors via aminolysis of the thiolactone end group and subsequent nucleophilic thiol-ene reaction.[363]

Other strategies to make SCPs based upon the aminolysis of thiolactone were explored by Hong and co-workers by (sequentially) adding different monomers in one pot.[101, 271, 356, 359, 411-413] This addition caused selective reactions between the different monomers and resulted in stepwise polymerisations.

The first series of polymers contained repetitive ABC or CBCA patterns (Figure II.40a and b).[101, 271, 411] The synthesis of polymers with the first pattern were prepared via two subsequent reactions (Figure II.40a)[411], the first being a Michael addition between a thiol group from

mercapto-amine and a methacrylate group from an ene-methacrylate. This resulted in the formation of an ene-amine. Next, a thiolactone and a radical initiator were added. The ene-amine opened the thiolactone, which generated a thiol. The latter-formed functionality reacted by a radical thiol-ene mechanism to create the ABC-patterned polymers. The CBCA-patterned polymers could be prepared in two different ways, with the first step for both routes being identical (Figure II.40b).[101, 271] A diamine opened two thiolactone molecules and formed a dithiol moiety. This dithiol could react with either an ene-methacrylate, followed by a radical thiol-ene [101], or a dimethacrylate to render the desired structures.[271]

Addition of the thio-bromide reaction provided CBABCD-containing polymers (Figure II.40c).[411] A dimethacrylate moiety reacted with two thiol-amines, forming a diamine. The latter reacted with two thiolactone groups, resulting in a dithiol, which subsequently reacted with a dibromide by means of a thio-bromo reaction to obtain the SCPs. This last reaction sequence (thiol-ene, aminolysis and thio-bromo), in combination with the amine-maleimide reaction, enabled the synthesis of repetitive DCBABCDE structures (Figure II.40d).[411] Instead of a dibromide, a bromomaleimide and a diamine are added sequentially to form these structures.

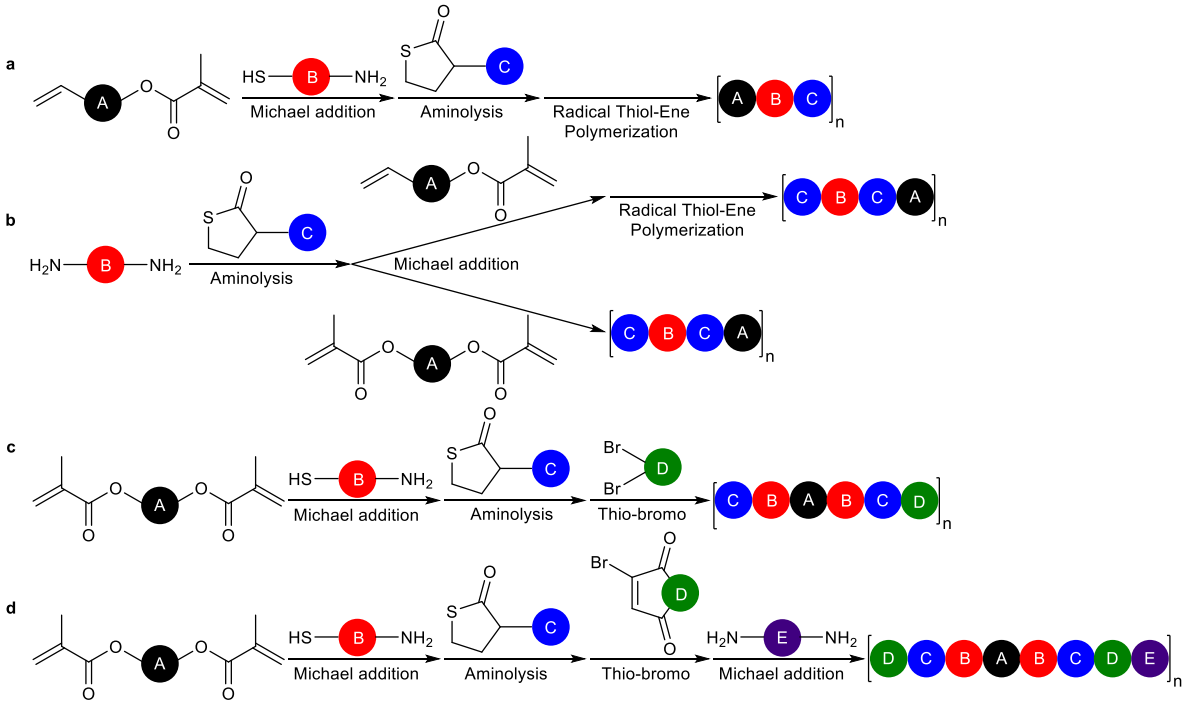


Figure II.40: Different pathways for making SCPs based on aminolysis of a thiolactone and thiol-X reactions.[101, 271, 411]

Multifunctionalised and sequence-defined oligomers could be synthesised by combining the easy aminolysis of thiolactone and the subsequent nucleophilic thiol-ene reaction of our group with the Passerini three-component reaction (P-3CR) of Meier's group.[354] First, sequence-defined trimers with a thiolactone end group were prepared in solution with the P-3CR (Figure II.28).[137] The thiolactone was opened with a (functional) amine and an acrylate-isocyanide, which can react in a subsequent P-3CR with a (functional) aldehyde and a trimer with an acid

end group. A library of sequence-defined α-ω-functionalised building blocks was prepared and used to acquire larger oligomers with up to 15 potential side chains in gram quantities.

As mentioned above, the combination of thiol-ene (and thiolactone) with the Passerini three-component reaction was fruitful in making SCPs.[354, 355] Yang *et al.* used the Passerini reaction in tandem with the amine-thiolactone-ene reaction to make polymers in one-pot (Figure II.41).[359] In the first step, a bifunctional methacrylate was made via the Passerini reaction. Subsequently, this dimethacrylate reacted either with a bis-thiolactone and a monoamine or with two thiolactones and a diamine to generate polymers via the amine-thiolactone-ene conjugation.

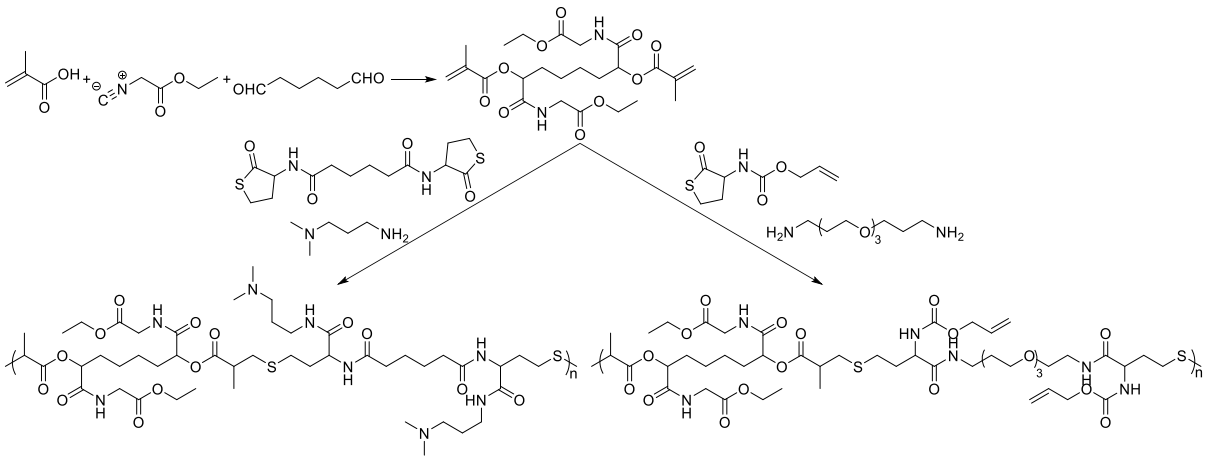


Figure II.41: One-pot preparation of SCPs by combining Passerini and thiolactone-amine-ene conjugation.[359]

The amine-thiolactone-ene reaction sequence was combined with the alkyne-acid-amine/alcohol coupling in a one-pot procedure.[412, 413] In the first of two strategies, a dialkyne with an ABCBA pattern was synthesised by means of the amine-thiolactone-ene conjugation between a diamine, an alkyne-methacrylate and a thiolactone (Figure II.42a).[412] This dialkyne reacted in a subsequent reaction with a sulfonylazide and a diamine or diol to render repetitive DABCBADE-patterned polymers. For the second method, an ABC-patterned secondary amine-alkyne was prepared and reacted afterwards with a sulfonylazide to obtain a SCP with a repetitive ABCD pattern (Figure II.42b).[413]

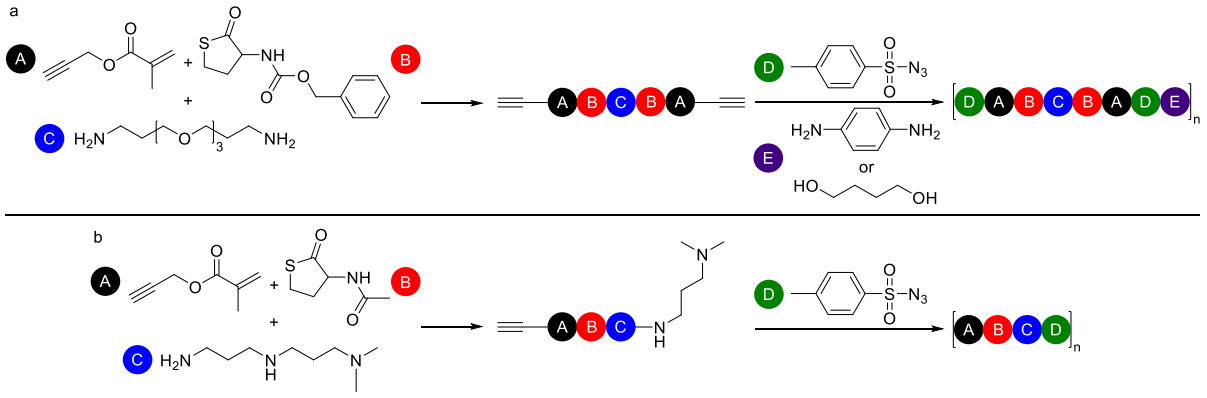


Figure II.42: Combination of amine-thiolactone-ene with alkyne-acid-amine/alcohol conjugation for the synthesis of SCPs containing repetitive DABCBADE (a) or ABCD pattern (b).[412, 413]

Finally, Hong and coworkers prepared SCPs by combining the amine-thiolactone-ene conjugation with the amine-propiolate reaction (Figure II.43).[356] First, a dimethacrylate, a thiolactone and a diamine bearing a primary and secondary amine were reacted with each other to prepare an ABCBA sequence with two secondary amine groups, which was polymerised with a dipropiolate in the next step to obtain DABCBA-patterned polymers.

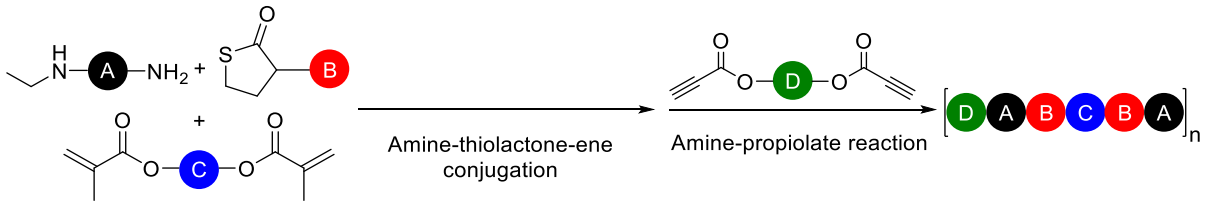


Figure II.43: Combinations of amine-thiolactone-ene conjugation with the amine-propiolate reaction for the synthesis of SCPs containing a repetitive DABCBA pattern.[356]

Madder's research group applied thiolactones for the cyclisation of oligopeptides with thiol and thiolactone end groups.[401] Aminolysis of the latter made it possible to install a functionality and to generate a thiol which can form a disulphide bond with the thiol at the other end of the oligopeptide, yielding a functionalised sequence-defined macromolecular cycle. Functionalities that can be used to further modify, label, or immobilise the macrocycle were installed. Long aliphatic amines were used to prepare cyclic lipopeptides which have antimicrobial, antitumor, surfactant, or immunosuppressant properties.[414]

II.5 Conclusions

Based upon the wide range of chemical methodologies, protocols and chemistries presented within this chapter, it can be concluded that recent years have seen great efforts towards more efficient synthesis of SCPs. Different scientific fields, such as biology, biochemistry, organic and polymer chemistry, have been explored to achieve greater control over the monomer order in a polymeric structure. Click and click-inspired chemistry, combined with the field of SCPs, has led to enormous advances in precise macromolecular control over the last decade. Thiolactone chemistry, in particular, has proven to be an effective tool for building SCPs via aminolysis and subsequent thiol-ene reactions.

In other words, the field of precise control and sequence definition within synthetic polymer chemistry has been rapidly expanding in the last ten years and can be approached using a wide variety of known chemistries. Nevertheless, the infancy of this field means that much is not yet understood about how precisely folding and structure-property relationships are influenced by structural control.[7] The aim of the present chapter was to demonstrate the specific advantage of combining "click" or similarly efficient reactions with sequence definition to achieve absolute control and a high degree of structural variation, which is necessary in applications foreseen for the near future, such as data storage, drug development or protein mimicking.[415]

Depending on the specific chemistries employed, both the nature of the chemical bonds formed (*e.g*. through additional H-bonding) and their intrinsic physical properties, such as reversibility, have an impact on the envisaged applications, and should be taken into account in light of the desired function of the materials developed.

References

[1] J. M. Berg, J. L. Tymoczko, L. Stryer, "*Biochemistry"*, W.H. Freeman, Basingstoke, 2012.
[2] D. Fournier, R. Hoogenboom, U. S. Schubert, *Chemical Society Reviews* **2007**, *36*, 1369.
[3] S. S. Sheiko, B. S. Sumerlin, K. Matyjaszewski, *Prog. Polym. Sci.* **2008**, *33*, 759.
[4] J.-F. Lutz, J.-M. Lehn, E. W. Meijer, K. Matyjaszewski, *Nature Reviews Materials* **2016**, *1*, 16024.
[5] C. J. Hawker, K. L. Wooley, *Science* **2005**, *309*, 1200.
[6] J. F. Lutz, M. Ouchi, D. R. Liu, M. Sawamoto, *Science* **2013**, *341*, 628.
[7] J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, *38*, 1700582.
[8] J. De Neve, J. J. Haven, L. Maes, T. Junkers, *Polym. Chem.* **2018**, *9*, 4692.
[9] J. K. Szymański, Y. M. Abul-Haija, L. Cronin, *Accounts of Chemical Research* **2018**, *51*, 649.
[10] R. G. Jones, E. S. Wilks, W. V. Metanomski, J. Kahovec, M. Hess, R. Stepto, T. Kitayama, "*Compendium of Polymer Terminology and Nomenclature: IUPAC Recommendations 2008"*, RSC, Cambridge, 2009.
[11] M. Haider, Z. Megeed, H. Ghandehari, *Journal of Controlled Release* **2004**, *95*, 1.
[12] Y. Chen, Z. Guan, *Journal of the American Chemical Society* **2010**, *132*, 4577.
[13] J.-S. Wang, K. Matyjaszewski, *Macromolecules* **1995**, *28*, 7901.
[14] J.-F. Lutz, *ACS Macro Lett.* **2014**, *3*, 1020.
[15] S. J. Rowan, C. Barner-Kowollik, B. Klumperman, P. Gaspard, R. B. Grubbs, M. A. Hillmyer, L. R. Hutchings, M. K. Mahanthappa, D. Moatsou, R. K. O'Reilly, M. Ouchi, M. Sawamoto, T. P. Lodge, *ACS Macro Lett.* **2016**, *5*, 1.
[16] E. Ciszak, J. M. Beals, B. H. Frank, J. C. Baker, N. D. Carter, G. D. Smith, *Structure* **1995**, *3*, 615.
[17] Z. Chen, D. R. Liu, "Nucleic Acid-Templated Synthesis of Sequence-Defined Synthetic Polymers", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 49.
[18] D. Mozhdehi, K. M. Luginbuhl, S. Roberts, A. Chilkoti, "Design of Sequence-Specific Polymers by Genetic Engineering", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 91.
[19] B. Lewandowski, G. De Bo, J. W. Ward, M. Papmeyer, S. Kuschel, M. J. Aldegunde, P. M. E. Gramlich, D. Heckmann, S. M. Goldup, D. M. D'Souza, A. E. Fernandes, D. A. Leigh, *Science* **2013**, *339*, 189.
[20] G. De Bo, S. Kuschel, D. A. Leigh, B. Lewandowski, M. Papmeyer, J. W. Ward, *J. Am. Chem. Soc.* **2014**, *136*, 5811.
[21] Y. Hibi, M. Ouchi, M. Sawamoto, *Nat. Commun.* **2016**, *7*, 11064.
[22] M. L. McKee, P. J. Milnes, J. Bath, E. Stulz, A. J. Turberfield, R. K. O'Reilly, *Angew. Chem., Int. Ed.* **2010**, *49*, 7948.
[23] S. Ida, M. Ouchi, M. Sawamoto, *Macromol. Rapid Commun.* **2011**, *32*, 209.
[24] J. Niu, R. Hili, D. R. Liu, *Nature Chemistry* **2013**, *5*, 282.
[25] M. Ouchi, "Sequence Regulation in Chain-Growth Polymerizations", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 257.
[26] W. A. Braunecker, K. Matyjaszewski, *Prog. Polym. Sci.* **2007**, *32*, 93.
[27] V. Percec, T. Guliashvili, J. S. Ladislaw, A. Wistrand, A. Stjerndahl, M. J. Sienkowska, M. J. Monteiro, S. Sahoo, *J. Am. Chem. Soc.* **2006**, *128*, 14156.
[28] W.-F. Su, "Ionic Chain Polymerization", in *Principles of Polymer Design and Synthesis*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2013, p. 185.
[29] G. Gody, T. Maschmeyer, P. B. Zetterlund, S. Perrier, *Nat. Commun.* **2013**, *4*, 3505.
[30] P. B. Zetterlund, G. Gody, S. Perrier, *Macromol. Theory Simul.* **2014**, *23*, 331.
[31] G. Gody, T. Maschmeyer, P. B. Zetterlund, S. Perrier, *Macromolecules* **2014**, *47*, 3451.
[32] G. Gody, P. B. Zetterlund, S. Perrier, S. Harrisson, *Nat. Commun.* **2016**, *7*, 10514.

[33] A. H. Soeriyadi, C. Boyer, F. Nyström, P. B. Zetterlund, M. R. Whittaker, *J. Am. Chem. Soc.* **2011**, *133*, 11128.
[34] C. Boyer, A. Derveaux, P. B. Zetterlund, M. R. Whittaker, *Polym. Chem.* **2012**, *3*, 117.
[35] A. Anastasaki, R. Whitfield, V. Nikolaou, N. P. Truong, G. R. Jones, N. G. Engelis, E. Liarou, M. R. Whittaker, D. M. Haddleton, "One-Pot Sequence-Controlled (SC) Multiblock Copolymers via Copper-Mediated Polymerization", in *Sequence-Controlled Polymers*, Wiley-vch, 2017.
[36] A. Anastasaki, C. Waldron, P. Wilson, C. Boyer, P. B. Zetterlund, M. R. Whittaker, D. Haddleton, *ACS Macro Lett.* **2013**, *2*, 896.
[37] A. Anastasaki, V. Nikolaou, G. S. Pappas, Q. Zhang, C. Wan, P. Wilson, T. P. Davis, M. R. Whittaker, D. M. Haddleton, *Chem. Sci.* **2014**, *5*, 3536.
[38] A. Anastasaki, V. Nikolaou, N. W. McCaul, A. Simula, J. Godfrey, C. Waldron, P. Wilson, K. Kempe, D. M. Haddleton, *Macromolecules* **2015**, *48*, 1404.
[39] Y.-M. Chuang, A. Ethirajan, T. Junkers, *ACS Macro Lett.* **2014**, *3*, 732.
[40] B. Wenn, A. C. Martens, Y. M. Chuang, J. Gruber, T. Junkers, *Polym. Chem.* **2016**, *7*, 2720.
[41] A. Anastasaki, B. Oschmann, J. Willenbacher, A. Melker, M. H. C. Van Son, N. P. Truong, M. W. Schulze, E. H. Discekici, A. J. McGrath, T. P. Davis, C. M. Bates, C. J. Hawker, *Angewandte Chemie* **2017**, *129*, 14675.
[42] E. Baeten, J. J. Haven, T. Junkers, *Polym. Chem.* **2017**, *8*, 3815.
[43] J. Vandenbergh, T. Moraes Ogawa, T. Junkers, *Journal of Polymer Science Part A: Polymer Chemistry* **2013**, *51*, 2366.
[44] A. Kuroki, I. Martinez-Botella, C. H. Hornung, L. Martin, E. G. L. Williams, K. E. S. Locock, M. Hartlieb, S. Perrier, *Polym. Chem.* **2017**, *8*, 3249.
[45] N. G. Engelis, A. Anastasaki, R. Whitfield, G. R. Jones, E. Liarou, V. Nikolaou, G. Nurumbetov, D. M. Haddleton, *Macromolecules* **2018**, *51*, 336.
[46] N. G. Engelis, A. Anastasaki, G. Nurumbetov, N. P. Truong, V. Nikolaou, A. Shegiwal, M. R. Whittaker, T. P. Davis, D. M. Haddleton, *Nature Chemistry* **2016**, *9*, 171.
[47] G. Nurumbetov, N. Engelis, J. Godfrey, R. Hand, A. Anastasaki, A. Simula, V. Nikolaou, D. M. Haddleton, *Polym. Chem.* **2017**, *8*, 1084.
[48] S. Aoshima, S. Kanaoka, *Chemical Reviews* **2009**, *109*, 5245.
[49] J. He, J. Ren, E. Mastan, "Sequence-Controlled Polymers by Chain Polymerization", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 281.
[50] J. G. Kim, C. D. Cowman, A. M. LaPointe, U. Wiesner, G. W. Coates, *Macromolecules* **2011**, *44*, 1110.
[51] N. Ekizoglou, N. Hadjichristidis, *Journal of Polymer Science Part A: Polymer Chemistry* **2002**, *40*, 2166.
[52] J. Zhang, M. E. Matta, M. A. Hillmyer, *ACS Macro Lett.* **2012**, *1*, 1383.
[53] W. R. Gutekunst, C. J. Hawker, *J. Am. Chem. Soc.* **2015**, *137*, 8038.
[54] R. M. Weiss, A. L. Short, T. Y. Meyer, *ACS Macro Lett.* **2015**, *4*, 1039.
[55] S. Pfeifer, J.-F. Lutz, *J. Am. Chem. Soc.* **2007**, *129*, 9542.
[56] J.-F. Lutz, B. V. K. J. Schmidt, S. Pfeifer, *Macromol. Rapid Commun.* **2011**, *32*, 127.
[57] D. Chan-Seng, M. Zamfir, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2012**, *51*, 12254.
[58] G. Moriceau, G. Gody, M. Hartlieb, J. Winn, H. Kim, A. Mastrangelo, T. Smith, S. Perrier, *Polym. Chem.* **2017**, *8*, 4152.
[59] R. K. Roy, J.-F. Lutz, *J. Am. Chem. Soc.* **2014**, *136*, 12888.
[60] Y. Ji, L. Zhang, X. Gu, W. Zhang, N. Zhou, Z. Zhang, X. Zhu, *Angewandte Chemie* **2017**, *129*, 2368.
[61] X. Gu, L. Zhang, Y. Li, W. Zhang, J. Zhu, Z. Zhang, X. Zhu, *Polym. Chem.* **2018**, *9*, 1571.
[62] A. Song, K. A. Parker, N. S. Sampson, *J. Am. Chem. Soc.* **2009**, *131*, 3444.
[63] B. R. Elling, Y. Xia, *J. Am. Chem. Soc.* **2015**, *137*, 9922.
[64] D. Moatsou, C. F. Hansell, R. K. O'Reilly, *Chem. Sci.* **2014**, *5*, 2246.
[65] M. Hisano, K. Takeda, T. Takashima, Z. Jin, A. Shiibashi, A. Matsumoto, *Macromolecules* **2013**, *46*, 7733.
[66] K. Satoh, M. Matsuda, K. Nagai, M. Kamigaito, *J. Am. Chem. Soc.* **2010**, *132*, 10003.
[67] T. Soejima, K. Satoh, M. Kamigaito, *J. Am. Chem. Soc.* **2016**, *138*, 944.
[68] M. J. Sanford, N. J. Van Zee, G. W. Coates, *Chem. Sci.* **2017**, *9*, 134.

[69] Y. Ishido, A. Kanazawa, S. Kanaoka, S. Aoshima, *Macromolecules* **2012**, *45*, 4060.
[70] K. Satoh, S. Saitoh, M. Kamigaito, *J. Am. Chem. Soc.* **2007**, *129*, 9586.
[71] F. Lin, M. Wang, Y. Pan, T. Tang, D. Cui, B. Liu, *Macromolecules* **2017**, *50*, 849.
[72] S. Houshyar, D. J. Keddie, G. Moad, R. J. Mulder, S. Saubern, J. Tsanaktsidis, *Polym. Chem.* **2012**, *3*, 1879.
[73] J. Xu, C. Fu, S. Shanmugam, C. J. Hawker, G. Moad, C. Boyer, *Angew. Chem., Int. Ed.* **2017**, *56*, 8376.
[74] J. Xu, S. Shanmugam, C. Fu, K.-F. Aguey-Zinsou, C. Boyer, *J. Am. Chem. Soc.* **2016**, *138*, 3094.
[75] C. Fu, Z. Huang, C. J. Hawker, G. Moad, J. Xu, C. Boyer, *Polym. Chem.* **2017**, *8*, 4637.
[76] J. J. Haven, J. A. De Neve, T. Junkers, *ACS Macro Lett.* **2017**, *6*, 743.
[77] J. Vandenbergh, G. Reekmans, P. Adriaensens, T. Junkers, *Chem. Sci.* **2015**, *6*, 5753.
[78] Y. Hibi, M. Ouchi, M. Sawamoto, *Angew. Chem., Int. Ed.* **2011**, *50*, 7434.
[79] Y. Hibi, S. Tokuoka, T. Terashima, M. Ouchi, M. Sawamoto, *Polym. Chem.* **2011**, *2*, 341.
[80] M. Ouchi, M. Nakano, T. Nakanishi, M. Sawamoto, *Angew. Chem., Int. Ed.* **2016**, *55*, 14584.
[81] S. Ida, T. Terashima, M. Ouchi, M. Sawamoto, *J. Am. Chem. Soc.* **2009**, *131*, 10808.
[82] S. Ida, M. Ouchi, M. Sawamoto, *J. Am. Chem. Soc.* **2010**, *132*, 14748.
[83] D. Oh, M. Ouchi, T. Nakanishi, H. Ono, M. Sawamoto, *ACS Macro Lett.* **2016**, *5*, 745.
[84] X. Tong, B.-h. Guo, Y. Huang, *Chem. Commun.* **2011**, *47*, 1455.
[85] K. Satoh, S. Ozawa, M. Mizutani, K. Nagai, M. Kamigaito, *Nat. Commun.* **2010**, *1*, 6.
[86] T. Soejima, K. Satoh, M. Kamigaito, *Polym. Chem.* **2016**, *7*, 4833.
[87] M. Minoda, M. Sawamoto, T. Higashimura, *Polymer Bulletin* **1990**, *23*, 133.
[88] Z. L. Li, Z. C. Li, "Periodic Copolymers by Step-Growth Polymerization", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 349.
[89] T. Yokozawa, Y. Ohta, *Chemical Reviews* **2016**, *116*, 1950.
[90] H. Mutlu, L. M. de Espinosa, M. A. R. Meier, *Chemical Society Reviews* **2011**, *40*, 1404.
[91] Z.-L. Li, L. Li, X.-X. Deng, L.-J. Zhang, B.-T. Dong, F.-S. Du, Z.-C. Li, *Macromolecules* **2012**, *45*, 4590.
[92] P. Atallah, K. B. Wagener, M. D. Schulz, *Macromolecules* **2013**, *46*, 4735.
[93] C.-H. Wang, Z.-Y. Song, X.-X. Deng, L.-J. Zhang, F.-S. Du, Z.-C. Li, *Macromol. Rapid Commun.* **2014**, *35*, 474.
[94] K. Satoh, T. Abe, M. Kamigaito, "Metal-Catalyzed Step-Growth Radical Polymerization of AA and BB Monomers for Monomer Sequence Regulation", in *Progress in Controlled Radical Polymerization: Mechanisms and Techniques*, K. Matyjaszewski, B.S. Sumerlin, and N.V. Tsarevsky, Eds., 2012, p. 133.
[95] C. Zhang, J. Ling, Q. Wang, *Macromolecules* **2011**, *44*, 8739.
[96] R. M. Stayshich, T. Y. Meyer, *Journal of Polymer Science Part A: Polymer Chemistry* **2008**, *46*, 4704.
[97] R. M. Weiss, E. M. Jones, D. E. Shafer, R. M. Stayshich, T. Y. Meyer, *Journal of Polymer Science Part A: Polymer Chemistry* **2011**, *49*, 1847.
[98] J. Li, S. N. Rothstein, S. R. Little, H. M. Edenborn, T. Y. Meyer, *J. Am. Chem. Soc.* **2012**, *134*, 16352.
[99] S. Martens, J. O. Holloway, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, 1700469.
[100] J.-J. Yan, D. Wang, D.-C. Wu, Y.-Z. You, *Chem. Commun.* **2013**, *49*, 6057.
[101] L. Yu, L.-H. Wang, Z.-T. Hu, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Polym. Chem.* **2015**, *6*, 1527.
[102] O. Kreye, T. Tóth, M. A. R. Meier, *J. Am. Chem. Soc.* **2011**, *133*, 1790.
[103] X.-X. Deng, L. Li, Z.-L. Li, A. Lv, F.-S. Du, Z.-C. Li, *ACS Macro Lett.* **2012**, *1*, 1300.
[104] A. Lv, X.-X. Deng, L. Li, Z.-L. Li, Y.-Z. Wang, F.-S. Du, Z.-C. Li, *Polym. Chem.* **2013**, *4*, 3659.
[105] A. Sehlinger, P.-K. Dannecker, O. Kreye, M. A. R. Meier, *Macromolecules* **2014**, *47*, 2774.
[106] A. C. Boukis, A. Llevot, M. A. R. Meier, *Macromol. Rapid Commun.* **2016**, *37*, 643.
[107] H. Xue, Y. Zhao, H. Wu, Z. Wang, B. Yang, Y. Wei, Z. Wang, L. Tao, *J. Am. Chem. Soc.* **2016**, *138*, 8690.
[108] C. Alabi, "Iterative Synthetic Methods for the Assembly of Sequence-Controlled Non-Natural Polymers", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 159.
[109] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149.
[110] R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* **1992**, *114*, 10646.

[111] Y. Abdouni, G. Yilmaz, C. R. Becer, *Macromol. Rapid Commun.* **2017**, 1700212.
[112] H. M. König, T. Gorelik, U. Kolb, A. F. M. Kilbinger, *J. Am. Chem. Soc.* **2007**, *129*, 704.
[113] G. B. Fields, *Current protocols in protein science* **2002**, *Chapter 18*, 1.
[114] N. R. Wurtz, J. M. Turner, E. E. Baird, P. B. Dervan, *Organic Letters* **2001**, *3*, 1201.
[115] S. L. Beaucage, R. P. Iyer, *Tetrahedron* **1992**, *48*, 2223.
[116] T. G. W. Edwardson, K. M. M. Carneiro, C. J. Serpell, H. F. Sleiman, *Angew. Chem., Int. Ed.* **2014**, *53*, 4567.
[117] A. Al Ouahabi, L. Charles, J.-F. Lutz, *J. Am. Chem. Soc.* **2015**, *137*, 5629.
[118] O. Brümmer, B. Clapham, K. D. Janda, *Tetrahedron Lett.* **2001**, *42*, 2257.
[119] T. M. Fyles, H. Luong, *Organic & Biomolecular Chemistry* **2009**, *7*, 725.
[120] K. Burgess, J. Ibarzo, D. S. Linthicum, D. H. Russell, H. Shin, A. Shitangkoon, R. Totani, A. J. Zhang, *J. Am. Chem. Soc.* **1997**, *119*, 1556.
[121] C. Douat-Casassus, K. Pulka, P. Claudon, G. Guichard, *Organic Letters* **2012**, *14*, 3130.
[122] S. C. Solleder, D. Zengel, K. S. Wetzel, M. A. R. Meier, *Angew. Chem., Int. Ed.* **2016**, *55*, 1204.
[123] A. C. Boukis, M. A. R. Meier, *Eur. Polym. J.* **2018**, *104*, 32.
[124] H.-F. Chow, K.-N. Lau, Z. Ke, Y. Liang, C.-M. Lo, *Chem. Commun.* **2010**, *46*, 3437.
[125] S. Binauld, D. Damiron, L. A. Connal, C. J. Hawker, E. Drockenmuller, *Macromol. Rapid Commun.* **2011**, *32*, 147.
[126] G. M. Brooke, S. Mohammed, M. C. Whiting, *Journal of the Chemical Society, Perkin Transactions 1* **1997**, 3371.
[127] S. S. Reddy, X. Dong, R. Murgasova, A. I. Gusev, D. M. Hercules, *Macromolecules* **1999**, *32*, 1367.
[128] K. Takizawa, H. Nulwala, J. Hu, K. Yoshinaga, C. J. Hawker, *Journal of Polymer Science Part A: Polymer Chemistry* **2008**, *46*, 5977.
[129] B. Huang, M. E. Hermes, *Journal of Polymer Science Part A: Polymer Chemistry* **1995**, *33*, 1419.
[130] V. Percec, P. J. Turkaly, A. D. Asandei, *Macromolecules* **1997**, *30*, 943.
[131] L. J. Markoski, J. L. Thompson, J. S. Moore, *Macromolecules* **2000**, *33*, 5315.
[132] J. C. Barnes, D. J. C. Ehrlich, A. X. Gao, F. A. Leibfarth, Y. Jiang, E. Zhou, T. F. Jamison, J. A. Johnson, *Nat. Chem.* **2015**, *7*, 810.
[133] H. Isobe, T. Fujino, N. Yamazaki, M. Guillot-Nieckowski, E. Nakamura, *Organic Letters* **2008**, *10*, 3729.
[134] S. Pfeifer, Z. Zarafshani, N. Badi, J.-F. Lutz, *J. Am. Chem. Soc.* **2009**, *131*, 9195.
[135] G. Cavallo, A. Al Ouahabi, L. Oswald, L. Charles, J.-F. Lutz, *J. Am. Chem. Soc.* **2016**, *138*, 9417.
[136] W. Konrad, F. R. Bloesser, K. S. Wetzel, A. C. Boukis, M. A. R. Meier, C. Barner-Kowollik, *Chemistry – A European Journal* **2018**, *24*, 3413.
[137] S. C. Solleder, M. A. R. Meier, *Angew. Chem., Int. Ed.* **2014**, *53*, 711.
[138] S. C. Solleder, K. S. Wetzel, M. A. R. Meier, *Polym. Chem.* **2015**, *6*, 3201.
[139] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[140] K. Rose, J. Vizzavona, *J. Am. Chem. Soc.* **1999**, *121*, 7034.
[141] L. Hartmann, S. Häfele, R. Peschka-Süss, M. Antonietti, H. G. Börner, *Chemistry – A European Journal* **2008**, *14*, 2025.
[142] J. W. Grate, K.-F. Mo, M. D. Daily, *Angew. Chem., Int. Ed.* **2016**, *55*, 3925.
[143] M. Porel, C. A. Alabi, *J. Am. Chem. Soc.* **2014**, *136*, 13162.
[144] U. S. Gunay, B. E. Petit, D. Karamessini, A. Al Ouahabi, J. A. Amalian, C. Chendo, M. Bouquey, D. Gigmes, L. Charles, J. F. Lutz, *Chem* **2016**, *1*, 114.
[145] R. K. Roy, A. Meszynska, C. Laure, L. Charles, C. Verchin, J.-F. Lutz, *Nat. Commun.* **2015**, *6*, 7237.
[146] M. A. Kline, L. Guo, R. N. Zuckermann, "Sequence-Controlled Peptoid Polymers: Bridging the Gap between Biology and Synthetic Polymers", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 183.
[147] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
[148] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
[149] J. M. Baskin, C. R. Bertozzi, *QSAR & Combinatorial Science* **2007**, *26*, 1211.

[150] J. E. Moses, A. D. Moorhouse, *Chemical Society Reviews* **2007**, *36*, 1249.
[151] C. D. Hein, X. M. Liu, D. Wang, *Pharmaceutical Research* **2008**, *25*, 2216.
[152] J. F. Lutz, Z. Zarafshani, *Advanced Drug Delivery Reviews* **2008**, *60*, 958.
[153] A. H. El-Sagheer, T. Brown, *Chemical Society Reviews* **2010**, *39*, 1388.
[154] B. J. Adzima, C. N. Bowman, *Aiche Journal* **2012**, *58*, 2952.
[155] M. A. Azagarsamy, K. S. Anseth, *ACS Macro Lett.* **2013**, *2*, 5.
[156] A. Marechal, R. El-Debs, V. Dugas, C. Demesmay, *Journal of Separation Science* **2013**, *36*, 2049.
[157] Y. Jiang, J. Chen, C. Deng, E. J. Suuronen, Z. Zhong, *Biomaterials* **2014**, *35*, 4969.
[158] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2.
[159] W. H. Binder, C. Kluger, *Macromolecules* **2004**, *37*, 9321.
[160] D. D. Díaz, S. Punna, P. Holzer, A. K. McPherson, K. B. Sharpless, V. V. Fokin, M. G. Finn, *Journal of Polymer Science Part A: Polymer Chemistry* **2004**, *42*, 4392.
[161] P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Fréchet, K. B. Sharpless, V. V. Fokin, *Angew. Chem., Int. Ed.* **2004**, *43*, 3928.
[162] N. V. Tsarevsky, K. V. Bernaerts, B. Dufour, F. E. Du Prez, K. Matyjaszewski, *Macromolecules* **2004**, *37*, 9308.
[163] B. Helms, J. L. Mynar, C. J. Hawker, J. M. J. Fréchet, *J. Am. Chem. Soc.* **2004**, *126*, 15020.
[164] C. Barner-Kowollik, A. J. Inglis, *Macromol. Chem. Phys.* **2009**, *210*, 987.
[165] M. J. Robb, C. J. Hawker, ""Click" Chemistry in Polymer Science: CuAAC and Thiol–Ene Coupling for the Synthesis and Functionalization of Macromolecules", in *Materials Science and Technology*, Wiley-VCH Verlag GmbH & Co. KGaA, 2006.
[166] W. H. Binder, R. Sachsenhofer, *Macromol. Rapid Commun.* **2007**, *28*, 15.
[167] C. J. Hawker, V. V. Fokin, M. G. Finn, K. B. Sharpless, *Australian Journal of Chemistry* **2007**, *60*, 381.
[168] H. Nandivada, X. Jiang, J. Lahann, *Advanced Materials* **2007**, *19*, 2197.
[169] W. H. Binder, R. Sachsenhofer, *Macromol. Rapid Commun.* **2008**, *29*, 952.
[170] C. R. Becer, R. Hoogenboom, U. S. Schubert, *Angew. Chem., Int. Ed.* **2009**, *48*, 4900.
[171] R. K. Iha, K. L. Wooley, A. M. Nyström, D. J. Burke, M. J. Kade, C. J. Hawker, *Chemical Reviews* **2009**, *109*, 5620.
[172] B. S. Sumerlin, A. P. Vogt, *Macromolecules* **2010**, *43*, 1.
[173] P. L. Golas, K. Matyjaszewski, *Chemical Society Reviews* **2010**, *39*, 1338.
[174] K. Kempe, A. Krieg, C. R. Becer, U. S. Schubert, *Chemical Society Reviews* **2012**, *41*, 176.
[175] A. B. Lowe, *Polymer* **2014**, *55*, 5517.
[176] W. Xi, T. F. Scott, C. J. Kloxin, C. N. Bowman, *Advanced Functional Materials* **2014**, *24*, 2572.
[177] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angew. Chem., Int. Ed.* **2011**, *50*, 60.
[178] H. Li, R. Aneja, I. Chaiken, *Molecules* **2013**, *18*, 9797.
[179] W. Tang, M. L. Becker, *Chemical Society Reviews* **2014**, *43*, 7013.
[180] F. Morvan, A. Meyer, G. Pourceau, S. Vidal, Y. Chevolot, E. Souteyrand, J.-J. Vasseur, *Nucleic Acids Symposium Series* **2008**, *52*, 47.
[181] N. Franz, L. Menin, H.-A. Klok, *Organic & Biomolecular Chemistry* **2009**, *7*, 5207.
[182] M. D. Best, *Biochemistry* **2009**, *48*, 6571.
[183] L. Markey, S. Giordani, E. M. Scanlan, *J. Org. Chem.* **2013**, *78*, 4270.
[184] L. Karmann, U. Kazmaier, *European Journal of Organic Chemistry* **2013**, *2013*, 7101.
[185] O. Brun, X. Elduque, E. Pedroso, A. Grandas, *J. Org. Chem.* **2015**, *80*, 6093.
[186] A. Meyer, M. Noël, J.-J. Vasseur, F. Morvan, *European Journal of Organic Chemistry* **2015**, *2015*, 2921.
[187] W. Liu, S.-J. Liu, Y.-Q. Kuang, F.-Y. Luo, J.-H. Jiang, *Analytical Chemistry* **2016**, *88*, 7867.
[188] R. A. Turner, A. G. Oliver, R. S. Lokey, *Organic Letters* **2007**, *9*, 5011.
[189] A. Le Chevalier Isaad, A. M. Papini, M. Chorev, P. Rovero, *Journal of Peptide Science* **2009**, *15*, 451.
[190] A. A. Aimetti, R. K. Shoemaker, C.-C. Lin, K. S. Anseth, *Chem. Commun.* **2010**, *46*, 4061.
[191] C. J. White, A. K. Yudin, *Nat. Chem.* **2011**, *3*, 509.
[192] A. Sanchez, E. Pedroso, A. Grandas, *Chem. Commun.* **2013**, *49*, 309.
[193] X. Elduque, E. Pedroso, A. Grandas, *J. Org. Chem.* **2014**, *79*, 2843.

[194] E. Scanlan, V. Corcé, A. Malone, *Molecules* **2014**, *19*, 19137.
[195] S. S. Ghosh, P. M. Kao, A. W. McCue, H. L. Chappelle, *Bioconjugate Chemistry* **1990**, *1*, 71.
[196] K. W. Hill, J. Taunton-Rigby, J. D. Carter, E. Kropp, K. Vagle, W. Pieken, D. P. C. McGee, G. M. Husar, M. Leuck, D. J. Anziano, D. P. Sebesta, *J. Org. Chem.* **2001**, *66*, 5352.
[197] R. Franke, C. Doll, J. Eichler, *Tetrahedron Lett.* **2005**, *46*, 4479.
[198] A. D. de Araújo, J. M. Palomo, J. Cramer, O. Seitz, K. Alexandrov, H. Waldmann, *Chemistry – A European Journal* **2006**, *12*, 6095.
[199] V. Marchán, S. Ortega, D. Pulido, E. Pedroso, A. Grandas, *Nucleic Acids Research* **2006**, *34*, e24.
[200] C. P. R. Hackenberger, D. Schwarzer, *Angew. Chem., Int. Ed.* **2008**, *47*, 10030.
[201] A. Ahmad Fuaad, F. Azmi, M. Skwarczynski, I. Toth, *Molecules* **2013**, *18*, 13148.
[202] A. Meyer, J.-J. Vasseur, F. Morvan, *European Journal of Organic Chemistry* **2013**, *2013*, 465.
[203] F. J. Flett, J. G. A. Walton, C. L. Mackay, H. Interthal, *Analytical Chemistry* **2015**, *87*, 9595.
[204] C. Paris, O. Brun, E. Pedroso, A. Grandas, *Molecules* **2015**, *20*, 6389.
[205] M. Pagel, R. Meier, K. Braun, M. Wiessler, A. G. Beck-Sickinger, *Organic & Biomolecular Chemistry* **2016**, *14*, 4809.
[206] B. Parrish, R. B. Breitenkamp, T. Emrick, *J. Am. Chem. Soc.* **2005**, *127*, 7404.
[207] J. Nicolas, G. Mantovani, D. M. Haddleton, *Macromol. Rapid Commun.* **2007**, *28*, 1083.
[208] J.-F. Lutz, *Angew. Chem., Int. Ed.* **2007**, *46*, 1018.
[209] M. A. Gauthier, H.-A. Klok, *Chem. Commun.* **2008**, 2591.
[210] J.-F. Lutz, H. G. Börner, *Prog. Polym. Sci.* **2008**, *33*, 1.
[211] H. G. Börner, *Prog. Polym. Sci.* **2009**, *34*, 811.
[212] H.-A. Klok, *Macromolecules* **2009**, *42*, 7990.
[213] H. Robson Marsden, A. Kros, *Macromolecular Bioscience* **2009**, *9*, 939.
[214] L. A. Canalle, D. W. P. M. Lowik, J. C. M. van Hest, *Chemical Society Reviews* **2010**, *39*, 329.
[215] M. Kwak, A. Herrmann, *Angew. Chem., Int. Ed.* **2010**, *49*, 8574.
[216] S. Dehn, R. Chapman, K. A. Jolliffe, S. Perrier, *Polymer Reviews* **2011**, *51*, 214.
[217] S. N. S. Alconcel, A. S. Baas, H. D. Maynard, *Polym. Chem.* **2011**, *2*, 1442.
[218] R. M. Broyer, G. N. Grover, H. D. Maynard, *Chem. Commun.* **2011**, *47*, 2212.
[219] C. Troiber, E. Wagner, *Bioconjugate Chemistry* **2011**, *22*, 1737.
[220] T. Schnitzler, A. Herrmann, *Accounts of Chemical Research* **2012**, *45*, 1419.
[221] R. P. Johnson, J. V. John, I. Kim, *Eur. Polym. J.* **2013**, *49*, 2925.
[222] D. E. Borchmann, T. P. Carberry, M. Weck, *Macromol. Rapid Commun.* **2014**, *35*, 27.
[223] I. W. Hamley, *Biomacromolecules* **2014**, *15*, 1543.
[224] Y. Qi, A. Chilkoti, *Polym. Chem.* **2014**, *5*, 266.
[225] M. A. Tasdelen, B. Kiskan, Y. Yagci, *Prog. Polym. Sci.* **2016**, *52*, 19.
[226] H.-A. Klok, *Journal of Polymer Science Part A: Polymer Chemistry* **2005**, *43*, 1.
[227] A. Rösler, H.-A. Klok, I. W. Hamley, V. Castelletto, O. O. Mykhaylyk, *Biomacromolecules* **2003**, *4*, 859.
[228] D. S. Goodsell, "Frontmatter", in *Bionanotechnology*, John Wiley & Sons, Inc., 2004, p. i.
[229] S. H. Waterhouse, J. A. Gerrard, *Australian Journal of Chemistry* **2004**, *57*, 519.
[230] A. Abuchowski, T. van Es, N. C. Palczuk, F. F. Davis, *Journal of Biological Chemistry* **1977**, *252*, 3578.
[231] J. M. Harris, R. B. Chess, *Nat Rev Drug Discov* **2003**, *2*, 214.
[232] R. Duncan, *Nat Rev Drug Discov* **2003**, *2*, 347.
[233] W. Zhao, F. Liu, Y. Chen, J. Bai, W. Gao, *Polymer* **2015**, *66*, A1.
[234] A. S. Hoffman, P. S. Stayton, *Macromolecular Symposia* **2004**, *207*, 139.
[235] S. C. Solleder, R. V. Schneider, K. S. Wetzel, A. C. Boukis, M. A. R. Meier, *Macromol. Rapid Commun.* **2017**, *38*, 1600711.
[236] C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057.
[237] M. Meldal, C. W. Tornøe, *Chemical Reviews* **2008**, *108*, 2952.
[238] R. Huisgen, *Angewandte Chemie International Edition in English* **1963**, *2*, 565.
[239] M. Meldal, *Macromol. Rapid Commun.* **2008**, *29*, 1016.
[240] N. V. Sokolova, V. G. Nenajdenko, *Rsc Advances* **2013**, *3*, 16212.
[241] G. Delaittre, N. K. Guimard, C. Barner-Kowollik, *Accounts of Chemical Research* **2015**, *48*, 1296.
[242] G. Lu, S. Lam, K. Burgess, *Chem. Commun.* **2006**, 1652.

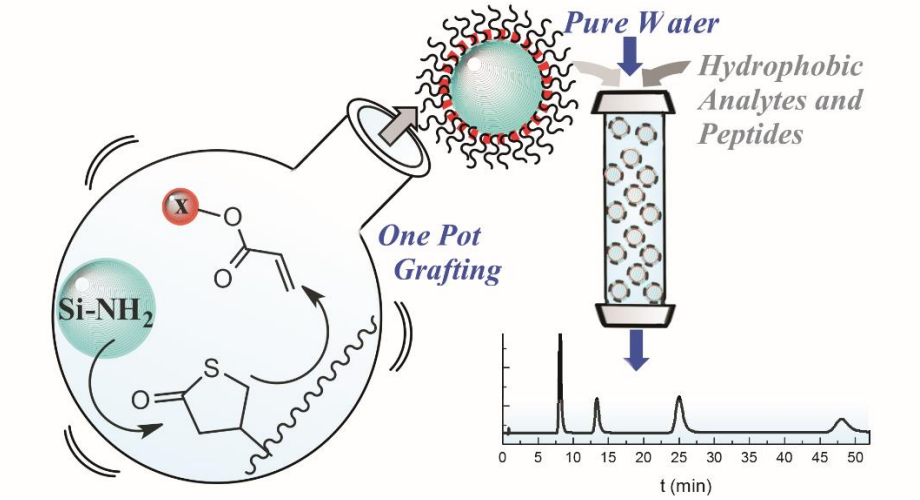
[243] S. Binauld, C. J. Hawker, E. Fleury, E. Drockenmuller, *Angew. Chem., Int. Ed.* **2009**, *48*, 6654.
[244] F. A. Leibfarth, J. A. Johnson, T. F. Jamison, *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 10617.
[245] P. Cheshev, A. Marra, A. Dondoni, *Organic & Biomolecular Chemistry* **2006**, *4*, 3225.
[246] M. Lo Conte, A. Chambery, A. Marra, A. Dondoni, *Synlett* **2009**, 2679.
[247] M. Lo Conte, A. Marra, A. Chambery, S. S. Gurcha, G. S. Besra, A. Dondoni, *J. Org. Chem.* **2010**, *75*, 6326.
[248] Y. Jiang, M. R. Golder, H. V. T. Nguyen, Y. Wang, M. Zhong, J. C. Barnes, D. J. C. Ehrlich, J. A. Johnson, *J. Am. Chem. Soc.* **2016**, *138*, 9369.
[249] F. Amir, Z. Jia, M. J. Monteiro, *J. Am. Chem. Soc.* **2016**, *138*, 16600.
[250] N. G. Angelo, P. S. Arora, *J. Am. Chem. Soc.* **2005**, *127*, 17134.
[251] O. D. Montagnat, G. Lessene, A. B. Hughes, *Tetrahedron Lett.* **2006**, *47*, 6971.
[252] N. G. Angelo, P. S. Arora, *J. Org. Chem.* **2007**, *72*, 7963.
[253] O. D. Montagnat, G. Lessene, A. B. Hughes, *J. Org. Chem.* **2010**, *75*, 390.
[254] A. C. Wicker, F. A. Leibfarth, T. F. Jamison, *Polym. Chem.* **2017**, *8*, 5786.
[255] Z. Zhang, E. Fan, *Tetrahedron Lett.* **2006**, *47*, 665.
[256] T. T. Trinh, L. Oswald, D. Chan-Seng, J.-F. Lutz, *Macromol. Rapid Commun.* **2014**, *35*, 141.
[257] T. T. Trinh, L. Oswald, D. Chan-Seng, L. Charles, J.-F. Lutz, *Chemistry – A European Journal* **2015**, *21*, 11961.
[258] D. Chan-Seng, J.-F. Lutz, *ACS Macro Letters* **2014**, 291.
[259] D. Chan-Seng, J.-F. Lutz, "Solid-Phase Synthesis as a Tool for the Preparation of Sequence-Defined Oligomers Based on Natural Amino Acids and Synthetic Building Blocks", in *Sequence-Controlled Polymers: Synthesis, Self-Assembly, and Properties*, American Chemical Society, 2014, p. 103.
[260] D. Chan-Seng, J. Louwsma, J.-F. Lutz, S. Joly, *Macromol. Rapid Commun.* **2018**, *39*, 1700764.
[261] S. Pfeifer, Z. Zarafshani, N. Badi, J. F. Lutz, *Journal of the American Chemical Society* **2009**, *131*, 9195.
[262] K. Oh, Z. Guan, *Chem. Commun.* **2006**, 3069.
[263] J. K. Pokorski, L. M. Miller Jenkins, H. Feng, S. R. Durell, Y. Bai, D. H. Appella, *Organic Letters* **2007**, *9*, 2381.
[264] T.-B. Yu, J. Z. Bai, Z. Guan, *Angew. Chem., Int. Ed.* **2009**, *48*, 1097.
[265] M. F. Ebbesen, C. Gerke, P. Hartwig, L. Hartmann, *Polym. Chem.* **2016**, *7*, 7086.
[266] M.-A. Berthet, Z. Zarafshani, S. Pfeifer, J.-F. Lutz, *Macromolecules* **2010**, *43*, 44.
[267] L. Xiang, Z. Li, J. a. Liu, J. Chen, M. Zhang, Y. Wu, K. Zhang, *Polym. Chem.* **2018**, *9*, 4036.
[268] C. Caumes, T. Hjelmgaard, R. Remuson, S. Faure, C. Taillefumier, *Synthesis* **2011**, *2011*, 257.
[269] P. J. Milnes, M. L. McKee, J. Bath, L. Song, E. Stulz, A. J. Turberfield, R. K. O'Reilly, *Chem. Commun.* **2012**, *48*, 5614.
[270] A. S. Knight, E. Y. Zhou, M. B. Francis, R. N. Zuckermann, *Advanced Materials* **2015**, *27*, 5665.
[271] Z. Zhang, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Polymer* **2015**, *64*, 221.
[272] W. Zhang, W. Xue, W. Ming, Y. Weng, G. Chen, D. M. Haddleton, *Macromol. Rapid Commun.* **2017**, *38*, 1700511.
[273] N. F. König, A. Al Ouahabi, S. Poyer, L. Charles, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2017**, *56*, 7297.
[274] Y.-Z. Wang, X.-X. Deng, L. Li, Z.-L. Li, F.-S. Du, Z.-C. Li, *Polym. Chem.* **2013**, *4*, 444.
[275] O. Roy, S. Faure, V. Thery, C. Didierjean, C. Taillefumier, *Organic Letters* **2008**, *10*, 921.
[276] A. S. Norgren, C. Budke, Z. Majer, C. Heggemann, T. Koop, N. Sewald, *Synthesis* **2009**, *2009*, 488.
[277] D. Ponader, F. Wojcik, F. Beceren-Braun, J. Dernedde, L. Hartmann, *Biomacromolecules* **2012**, *13*, 1845.
[278] D. Fürniss, T. Mack, F. Hahn, S. B. L. Vollrath, K. Koroniak, U. Schepers, S. Bräse, *Beilstein Journal of Organic Chemistry* **2013**, *9*, 56.
[279] H. O. Ham, S. H. Park, J. W. Kurutz, I. G. Szleifer, P. B. Messersmith, *J. Am. Chem. Soc.* **2013**, *135*, 13015.
[280] Q. Zhang, J. Collins, A. Anastasaki, R. Wallis, D. A. Mitchell, C. R. Becer, D. M. Haddleton, *Angewandte Chemie* **2013**, *125*, 4531.
[281] G. Yilmaz, C. R. Becer, *Eur. Polym. J.* **2013**, *49*, 3046.

[282] N. Baradel, S. Fort, S. Halila, N. Badi, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2013**, *52*, 2335.
[283] D. Ponader, P. Maffre, J. Aretz, D. Pussak, N. M. Ninnemann, S. Schmidt, P. H. Seeberger, C. Rademacher, G. U. Nienhaus, L. Hartmann, *J. Am. Chem. Soc.* **2014**, *136*, 2008.
[284] D. Ponader, S. Igde, M. Wehle, K. Märker, M. Santer, D. Bléger, L. Hartmann, *Beilstein Journal of Organic Chemistry* **2014**, *10*, 1603.
[285] F. Wojcik, D. Ponader, S. Mosca, L. Hartmann, "Recent Advances in Solid Phase Polymer Synthesis: Polyamides from Tailor-Made Building Blocks", in *Sequence-Controlled Polymers: Synthesis, Self-Assembly, and Properties*, J.F. Lutz, T.Y. Meyer, M. Ouchi, and M. Sawamoto, Eds., 2014, p. 85.
[286] D. Pussak, D. Ponader, S. Mosca, T. Pompe, L. Hartmann, S. Schmidt, *Langmuir* **2014**, *30*, 6142.
[287] Q. Zhang, A. Anastasaki, G.-Z. Li, A. J. Haddleton, P. Wilson, D. M. Haddleton, *Polym. Chem.* **2014**, *5*, 3876.
[288] G. Yilmaz, C. R. Becer, *Frontiers in Bioengineering and Biotechnology* **2014**, *2*.
[289] Q. Zhang, J. Collins, A. Anastasaki, R. Wallis, D. A. Mitchell, C. R. Becer, P. Wilson, D. M. Haddleton, "Sequence-Controlled Multi-Block Glycopolymers via Cu(0) Mediated Living Radical Polymerization", in *Sequence-Controlled Polymers: Synthesis, Self-Assembly, and Properties*, J.F. Lutz, T.Y. Meyer, M. Ouchi, and M. Sawamoto, Eds., 2014, p. 327.
[290] T. Szekely, O. Roy, S. Faure, C. Taillefumier, *European Journal of Organic Chemistry* **2014**, *2014*, 5641.
[291] V. Castro, H. Rodríguez, F. Albericio, *ACS Combinatorial Science* **2016**, *18*, 1.
[292] D. de Rochambeau, Y. Sun, M. Barlog, H. S. Bazzi, H. F. Sleiman, *J. Org. Chem.* **2018**, *83*, 9774.
[293] T. Freichel, S. Eierhoff, N. L. Snyder, L. Hartmann, *J. Org. Chem.* **2017**, *82*, 9400.
[294] H. Jang, A. Fafarman, J. M. Holub, K. Kirshenbaum, *Organic Letters* **2005**, *7*, 1951.
[295] S. Srichan, H. Mutlu, N. Badi, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2014**, *53*, 9231.
[296] C. Zhou, L. H. Deng, F. Yao, L. Q. Xu, J. Zhou, G. D. Fu, *Industrial & Engineering Chemistry Research* **2014**, *53*, 19239.
[297] A. M. Rosales, B. L. McCulloch, R. N. Zuckermann, R. A. Segalman, *Macromolecules* **2012**, *45*, 6027.
[298] R. L. Kanasty, A. J. Vegas, L. M. Ceo, M. Maier, K. Charisse, J. K. Nair, R. Langer, D. G. Anderson, *Angewandte Chemie* **2016**, *128*, 9681.
[299] J. M. Holub, H. Jang, K. Kirshenbaum, *Organic & Biomolecular Chemistry* **2006**, *4*, 1497.
[300] J. M. Holub, M. J. Garabedian, K. Kirshenbaum, *QSAR & Combinatorial Science* **2007**, *26*, 1175.
[301] K. Peschko, A. Schade, S. B. L. Vollrath, U. Schwarz, B. Luy, C. Muhle-Goll, P. Weis, S. Bräse, *Chemistry – A European Journal* **2014**, *20*, 16273.
[302] M. T. Dohm, S. L. Seurynck-Servoss, J. Seo, R. N. Zuckermann, A. E. Barron, *Peptide Science* **2009**, *92*, 538.
[303] M. Baier, M. Giesler, L. Hartmann, *Chemistry-a European Journal* **2018**, *24*, 1619.
[304] C. Caumes, O. Roy, S. Faure, C. Taillefumier, *J. Am. Chem. Soc.* **2012**, *134*, 9553.
[305] J. M. Holub, H. Jang, K. Kirshenbaum, *Organic Letters* **2007**, *9*, 3275.
[306] R. Jagasia, J. M. Holub, M. Bollinger, K. Kirshenbaum, M. G. Finn, *J. Org. Chem.* **2009**, *74*, 2964.
[307] K. D. Bodine, D. Y. Gin, M. S. Gin, *J. Am. Chem. Soc.* **2004**, *126*, 1638.
[308] K. D. Bodine, D. Y. Gin, M. S. Gin, *Organic Letters* **2005**, *7*, 4479.
[309] V. K. Tiwari, B. B. Mishra, K. B. Mishra, N. Mishra, A. S. Singh, X. Chen, *Chemical Reviews* **2016**, *116*, 3086.
[310] B. Schmidt, N. Fechler, J. Falkenhagen, J. F. Lutz, *Nature Chemistry* **2011**, *3*, 234.
[311] M. Zamfir, P. Theato, J. F. Lutz, *Polym. Chem.* **2012**, *3*, 1796.
[312] T. R. Chan, R. Hilgraf, K. B. Sharpless, V. V. Fokin, *Organic Letters* **2004**, *6*, 2853.
[313] H. Struthers, T. L. Mindt, R. Schibli, *Dalton Transactions* **2010**, *39*, 675.
[314] G. Aromí, L. A. Barrios, O. Roubeau, P. Gamez, *Coordination Chemistry Reviews* **2011**, *255*, 485.
[315] C. E. Hoyle, T. Y. Lee, T. Roper, *Journal of Polymer Science Part A: Polymer Chemistry* **2004**, *42*, 5301.
[316] C. E. Hoyle, A. B. Lowe, C. N. Bowman, *Chemical Society Reviews* **2010**, *39*, 1355.
[317] C. E. Hoyle, C. N. Bowman, *Angew. Chem., Int. Ed.* **2010**, *49*, 1540.

[318] L.-T. T. Nguyen, M. T. Gokmen, F. E. Du Prez, *Polymer Chemistry* **2013**, *4*, 5527.
[319] A. B. Lowe, *Polym. Chem.* **2014**, *5*, 4820.
[320] D. P. Nair, M. Podgorski, S. Chatani, T. Gong, W. X. Xi, C. R. Fenoli, C. N. Bowman, *Chemistry of Materials* **2014**, *26*, 724.
[321] A. J. Inglis, C. Barner-Kowollik, *Macromol. Rapid Commun.* **2010**, *31*, 1247.
[322] M. A. Tasdelen, *Polym. Chem.* **2011**, *2*, 2133.
[323] G. Hizal, U. Tunca, A. Sanyal, *Journal of Polymer Science Part A: Polymer Chemistry* **2011**, *49*, 4103.
[324] C. F. Hansell, P. Espeel, M. M. Stamenović, I. A. Barker, A. P. Dove, F. E. Du Prez, R. K. O'Reilly, *J. Am. Chem. Soc.* **2011**, *133*, 13828.
[325] A. M. Prokhorov, D. N. Kozhevnikov, *Chemistry of Heterocyclic Compounds* **2012**, *48*, 1153.
[326] A. Gandini, *Prog. Polym. Sci.* **2013**, *38*, 1.
[327] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815.
[328] B. Vonhören, O. Roling, K. De Bruycker, R. Calvo, F. E. Du Prez, B. J. Ravoo, *ACS Macro Lett.* **2015**, *4*, 331.
[329] O. Roling, K. De Bruycker, B. Vonhören, L. Stricker, M. Körsgen, H. F. Arlinghaus, B. J. Ravoo, F. E. Du Prez, *Angewandte Chemie International Edition* **2015**, *54*, 13126.
[330] S. Vandewalle, S. Billiet, F. Driessen, F. E. Du Prez, *ACS Macro Lett.* **2016**, *5*, 766.
[331] K. De Bruycker, S. Billiet, H. A. Houck, S. Chattopadhyay, J. M. Winne, F. E. Du Prez, *Chemical Reviews* **2016**, *116*, 3919.
[332] N. Zydziak, F. Feist, B. Huber, J. O. Mueller, C. Barner-Kowollik, *Chem. Commun.* **2015**, *51*, 1799.
[333] N. Zydziak, W. Konrad, F. Feist, S. Afonin, S. Weidner, C. Barner-Kowollik, *Nat. Commun.* **2016**, *7*, 13672.
[334] Z. Huang, J. Zhao, Z. Wang, F. Meng, K. Ding, X. Pan, N. Zhou, X. Li, Z. Zhang, X. Zhu, *Angew. Chem., Int. Ed.* **2017**, *56*, 13612.
[335] X. Elduque, A. Sánchez, K. Sharma, E. Pedroso, A. Grandas, *Bioconjugate Chemistry* **2013**, *24*, 832.
[336] G. Gody, T. Maschmeyer, P. B. Zetterlund, S. Perrier, *Nature communications* **2013**, *4*, 2505.
[337] B. V. K. J. Schmidt, C. Barner-Kowollik, *Nature chemistry* **2013**, *5*, 990.
[338] T. Posner, *Berichte der deutschen chemischen Gesellschaft* **1905**, *38*, 646.
[339] A. B. Lowe, *Polym. Chem.* **2010**, *1*, 17.
[340] N. ten Brummelhuis, C. Diehl, H. Schlaad, *Macromolecules* **2008**, *41*, 9946.
[341] N. B. Cramer, J. P. Scott, C. N. Bowman, *Macromolecules* **2002**, *35*, 5361.
[342] N. B. Cramer, S. K. Reddy, M. Cole, C. Hoyle, C. N. Bowman, *Journal of Polymer Science Part A: Polymer Chemistry* **2004**, *42*, 5817.
[343] M. Uygun, M. A. Tasdelen, Y. Yagci, *Macromol. Chem. Phys.* **2010**, *211*, 103.
[344] J. W. Chan, C. E. Hoyle, A. B. Lowe, M. Bowman, *Macromolecules* **2010**, *43*, 6381.
[345] J. W. Chan, H. Wei, H. Zhou, C. E. Hoyle, *Eur. Polym. J.* **2009**, *45*, 2717.
[346] J. W. Chan, C. E. Hoyle, A. B. Lowe, *J. Am. Chem. Soc.* **2009**, *131*, 5751.
[347] M. Liu, B. H. Tan, R. P. Burford, A. B. Lowe, *Polym. Chem.* **2013**, *4*, 3300.
[348] S. P. S. Koo, M. M. Stamenović, R. A. Prasath, A. J. Inglis, F. E. Du Prez, C. Barner-Kowollik, W. Van Camp, T. Junkers, *Journal of Polymer Science Part A: Polymer Chemistry* **2010**, *48*, 1699.
[349] M. Porel, J. S. Brown, C. A. Alabi, *Synlett* **2015**, *26*, 565.
[350] M. Porel, D. N. Thornlow, N. N. Phan, C. A. Alabi, *Nat Chem* **2016**, *8*, 590.
[351] M. Porel, D. N. Thornlow, C. M. Artim, C. A. Alabi, *ACS Chemical Biology* **2017**, *12*, 715.
[352] J. S. Brown, Y. M. Acevedo, G. D. He, J. H. Freed, P. Clancy, C. A. Alabi, *Macromolecules* **2017**, *50*, 8731.
[353] W. Xi, S. Pattanayak, C. Wang, B. Fairbanks, T. Gong, J. Wagner, C. J. Kloxin, C. N. Bowman, *Angew Chem Int Ed Engl* **2015**, *54*, 14462.
[354] S. C. Solleder, S. Martens, P. Espeel, F. Du Prez, M. A. R. Meier, *Chemistry – A European Journal* **2017**, *23*, 13906.

[355] J. Zhang, X.-X. Deng, F.-S. Du, Z.-C. Li, "Sequence-Regulated Polymers via Combination of Orthogonal Passerini Three-Component Reaction and Thiol-ene Reaction", in *Sequence-Controlled Polymers: Synthesis, Self-Assembly, and Properties*, American Chemical Society, 2014, p. 223.
[356] L. Yu, Z. Zhang, Y.-Z. You, C.-Y. Hong, *Eur. Polym. J.* **2018**, *103*, 80.
[357] A. Llevot, S. O. Steinmüller, B. Bitterer, B. Ridder, J. Berson, S. Walheim, T. Schimmel, S. Bräse, F. Scheiba, M. A. R. Meier, *Polym. Chem.* **2017**, *8*, 5824.
[358] N. Franz, L. Menin, H.-A. Klok, *European Journal of Organic Chemistry* **2009**, *2009*, 5390.
[359] L. Yang, Z. Zhang, B. Cheng, Y. You, D. Wu, C. Hong, *Science China Chemistry* **2015**, *58*, 1734.
[360] D. Comegna, F. D. Riccardis, *Organic Letters* **2009**, *11*, 3898.
[361] F. Wojcik, S. Lel, A. G. O'Brien, P. H. Seeberger, L. Hartmann, *Beilstein Journal of Organic Chemistry* **2013**, *9*, 2395.
[362] F. Wojcik, A. G. O'Brien, S. Götze, P. H. Seeberger, L. Hartmann, *Chemistry – A European Journal* **2013**, *19*, 3090.
[363] F. Driessen, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2015**, *4*, 616.
[364] C. Gerke, M. F. Ebbesen, D. Jansen, S. Boden, T. Freichel, L. Hartmann, *Biomacromolecules* **2017**, *18*, 787.
[365] C. Gerke, F. Jacobi, L. E. Goodwin, F. Pieper, S. Schmidt, L. Hartmann, *Macromolecules* **2018**, *51*, 5608.
[366] M. R. Golder, Y. Jiang, P. E. Teichen, H. V. T. Nguyen, W. Wang, N. Milos, S. A. Freedman, A. P. Willard, J. A. Johnson, *J. Am. Chem. Soc.* **2018**, *140*, 1596.
[367] C. G. Overberger, H. Aschkenasy, *J. Am. Chem. Soc.* **1960**, *82*, 4357.
[368] T. Moriguchi, T. Endo, *Macromolecules* **1995**, *28*, 5386.
[369] N. Kihara, C. Kanno, T. Fukutomi, *Journal of Polymer Science Part A: Polymer Chemistry* **1997**, *35*, 1443.
[370] W. Choi, F. Sanda, T. Endo, *Journal of Polymer Science Part A: Polymer Chemistry* **1998**, *36*, 1189.
[371] W. Choi, M. Nakajima, F. Sanda, T. Endo, *Macromol. Chem. Phys.* **1998**, *199*, 1909.
[372] T. Ouchi, H. Seike, H. Miyazaki, F. Tasaka, Y. Ohya, *Designed Monomers and Polymers* **2000**, *3*, 279.
[373] N. V. Tsarevsky, K. Matyjaszewski, *Macromolecules* **2002**, *35*, 9009.
[374] I. S. Alferiev, I. Fishbein, *Biomaterials* **2002**, *23*, 4753.
[375] V. Lima, X. Jiang, J. Brokken-Zijp, P. J. Schoenmakers, B. Klumperman, R. Van Der Linde, *Journal of Polymer Science Part A: Polymer Chemistry* **2005**, *43*, 959.
[376] N. V. Tsarevsky, K. Matyjaszewski, *Macromolecules* **2005**, *38*, 3087.
[377] M. R. Whittaker, Y.-K. Goh, H. Gemici, T. M. Legge, S. Perrier, M. J. Monteiro, *Macromolecules* **2006**, *39*, 9028.
[378] S. Cesana, A. Kurek, M. A. Baur, J. Auernheimer, O. Nuyken, *Macromol. Rapid Commun.* **2007**, *28*, 608.
[379] M. Li, P. De, S. R. Gondi, B. S. Sumerlin, *Journal of Polymer Science Part A: Polymer Chemistry* **2008**, *46*, 5093.
[380] A. Sudo, H. Morishita, T. Endo, *Journal of Polymer Science Part A: Polymer Chemistry* **2011**, *49*, 1097.
[381] C. Boyer, A. H. Soeriyadi, P. J. Roth, M. R. Whittaker, T. P. Davis, *Chem. Commun.* **2011**, *47*, 1318.
[382] L. Petton, A. E. Ciolino, M. M. Stamenović, P. Espeel, F. E. Du Prez, *Macromol. Rapid Commun.* **2012**, *33*, 1310.
[383] F. Goethals, D. Frank, F. Du Prez, *Prog. Polym. Sci.* **2017**, *64*, 76.
[384] P. Espeel, F. Goethals, F. E. Du Prez, "CHAPTER 9 Thiolactones as Functional Handles for Polymer Synthesis and Modification", in *Thiol-X Chemistries in Polymer and Materials Science*, The Royal Society of Chemistry, 2013, p. 195.
[385] P. Espeel, F. E. Du Prez, *Eur. Polym. J.* **2015**, *62*, 247.
[386] S. Mommer, H. Keul, M. Möller, *Macromol. Rapid Commun.* **2014**, *35*, 1986.
[387] G. B. Desmet, D. R. D'hooge, M. K. Sabbe, G. B. Marin, F. E. Du Prez, P. Espeel, M.-F. Reyniers, *J. Org. Chem.* **2015**, *80*, 8520.
[388] P. Espeel, F. Goethals, F. Driessen, L.-T. T. Nguyen, F. E. Du Prez, *Polym. Chem.* **2013**, *4*, 2449.

[389] F. Goethals, S. Martens, P. Espeel, O. van den Berg, F. E. Du Prez, *Macromolecules* **2014**, *47*, 61.
[390] P. Espeel, F. E. Du Prez, "One-Pot Double Modification of Polymers Based on Thiolactone Chemistry", in *Multi-Component and Sequential Reactions in Polymer Synthesis*, P. Theato, Ed., Springer International Publishing, Cham, 2015, p. 105.
[391] P. Espeel, F. Goethals, F. E. Du Prez, *J. Am. Chem. Soc.* **2011**, *133*, 1678.
[392] S. Mommer, K.-N. Truong, H. Keul, M. Möller, *Polym. Chem.* **2016**, *7*, 2291.
[393] P. Espeel, F. Goethals, M. M. Stamenović, L. Petton, F. E. Du Prez, *Polym. Chem.* **2012**, *3*, 1007.
[394] S. Reinicke, P. Espeel, M. M. Stamenović, F. E. Du Prez, *ACS Macro Lett.* **2013**, *2*, 539.
[395] S. Reinicke, P. Espeel, M. M. Stamenovic, F. E. Du Prez, *Polym. Chem.* **2014**, *5*, 5461.
[396] Y. Chen, P. Espeel, S. Reinicke, F. E. Du Prez, M. H. Stenzel, *Macromol. Rapid Commun.* **2014**, *35*, 1128.
[397] S. Vandewalle, S. Wallyn, S. Chattopadhyay, C. R. Becer, F. Du Prez, *Eur. Polym. J.* **2015**, *69*, 490.
[398] J.-J. Yan, J.-T. Sun, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Scientific Reports* **2013**, *3*, 2841.
[399] N. U. Kaya, F. E. Du Prez, N. Badi, *Macromol. Chem. Phys.* **2017**, *218*, 1600575.
[400] M. M. Stamenović, P. Espeel, E. Baba, T. Yamamoto, Y. Tezuka, F. E. Du Prez, *Polym. Chem.* **2013**, *4*, 184.
[401] D. Van Lysebetten, S. Felissati, E. Antonatou, L. L. G. Carrette, P. Espeel, E. Focquet, F. E. Du Prez, A. Madder, *ChemBioChem* **2018**, *19*, 641.
[402] A. J. Satti, P. Espeel, S. Martens, T. Van Hoeylandt, F. E. Du Prez, F. Lynen, *Journal of Chromatography A* **2015**, *1426*, 126.
[403] F. Driessen, S. Martens, B. D. Meyer, F. E. Du Prez, P. Espeel, *Macromol. Rapid Commun.* **2016**, *37*, 947.
[404] M. Langlais, O. Coutelier, M. Destarac, *Macromolecules* **2018**, *51*, 4315.
[405] M. Langlais, I. Kulai, O. Coutelier, M. Destarac, *Macromolecules* **2017**, *50*, 3524.
[406] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[407] S. Martens, A. Landuyt, P. Espeel, B. Devreese, P. Dawyndt, F. Du Prez, *Nat. Commun.* **2018**, *9*, 4451.
[408] S. Celasun, F. E. Du Prez, H. G. Börner, *Macromol. Rapid Commun.* **2017**, *38*, 1700688.
[409] S. Aksakal, C. Remzi Becer, *Polym. Chem.* **2016**, *7*, 7011.
[410] J. O. Holloway, S. Aksakal, F. E. D. Prez, C. R. Becer, *Macromol. Rapid Commun.* **2017**, *38*, 1700500.
[411] J.-J. Yan, D. Wang, D.-C. Wu, Y.-Z. You, *Chemical communications (Cambridge, England)* **2013**, *49*, 6057.
[412] Z. Zhang, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Macromolecules* **2015**, *48*, 3414.
[413] Z. Zhang, Z.-B. Tan, C.-Y. Hong, D.-C. Wu, Y.-Z. You, *Polym. Chem.* **2016**, *7*, 1468.
[414] J. M. Raaijmakers, I. De Bruijn, O. Nybroe, M. Ongena, *FEMS Microbiology Reviews* **2010**, *34*, 1037.
[415] J. H. Swisher, J. A. Nowalk, M. A. Washington, T. Y. Meyer, "Properties and Applications of Sequence-Controlled Polymers", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 435.



Abstract

This chapter outlines the elegant modification of aminopropyl silica with a thiolactone-functionalised PNIPAM for the development of temperature-responsive liquid chromatography stationary phases with solely water as an eluent. In a one pot chemical strategy, the thiolactone rings on the thermoresponsive polymer are opened with the primary amines of this silica and the formed thiols react in a subsequent thiol-ene reaction with an acrylate. This strategy allows for the simple immobilisation of the polymer on the stationary phase with high coupling yields and the efficient functionalisation with various acrylates to acquire different stationary phases. Benzyl acrylate- and PEG acrylate-functionalised stationary phases were prepared, packed on 10 cm x 4.6 mm columns and tested with separate mixtures of parabens, steroids and (diastereomeric) oligopeptides. Results indicated that the retentions could be adjusted as a function of temperature and the acrylate used: low retention was noticed below the LCST of PNIPAM and high retention above the LCST, and lower retentions were obtained when PEG acrylate was substituted for benzyl. Thus, the amine-thiol-ene conjugation with thiolactones can be applied to easily tune liquid chromatographic stationary phases.

Parts of this chapter were published as:
A. J. Satti, P. Espeel, S. Martens, T. Van Hoeylandt, F. E. Du Prez, F. Lynen, *Journal of Chromatography A* **2015**, 1426, 126.

Specific author contributions can be found at the end of section III.1

Chapter III
Thiolactone-based immobilisation of PNIPAM on silica for liquid chromatography

III.1 Introduction

As mentioned in Chapter II, the combination of amine-thiol-ene conjugation and thiolactones has allowed for the double-modification of polymers and the preparation of functional polymers and networks.[1-7] The five-membered thiolactone ring is opened via aminolysis, generating a thiol that can react in a subsequent reaction with an ene functionality. In this chapter, acrylates are chosen to capture the thiol. Previous research showed the use of amines and acrylates to double-modify a copolymer of N-isopropyl acrylamide (NIPAM) and thiolactone acrylamide in order to tune the polymer's cloud point[2], which is the particular temperature for a certain mixture of water and polymer at which the polymer turns from water-soluble to water-insoluble.[8] The lower critical solution temperature (LCST) is the lowest cloud point temperature below which the polymer is always soluble in water. In this chapter, the amine-thiolactone-ene conjugation in combination with a thiolactone-functionalised PNIPAM will be used to modify an amine-functionalised silica for tuneable temperature-responsive liquid chromatography (TRLC).

A range of parameters should be taken into account for the effective immobilisation of polymers on chromatographic stationary phases, including the diffusion characteristics of the polymer, the numbers of the functional groups and their corresponding location in the polymer or on the packing material, the particle size, shape, pore dimensions and area-to-weight ratio of the packing material. A quantitative coupling depends strongly on the inherent reactivity of the functional groups and requires in many cases anhydrous reaction conditions. However, yields can be enhanced by adapting the solvent, the temperature, and by incorporating spacer molecules. The grafting-from method made it possible to grow polymers on initiator-functionalised stationary phase.[9] Liquid chromatographic silica phases can also be immediately functionalised with macromolecules via direct silylation[10, 11], ring opening of an epoxide-functionalised silica[12], amidation[13, 14] or reductive amination[15] of aminopropyl silica, and click chemistries.[16-19] Although the latter chemistries are increasing in popularity because of their versatility, speed and reactivity for the modification of stationary phases with functional (macro)molecules, some limitations were noted. The usage of CuAAC is hindered by the tedious removal of toxic residual Cu (I) species from the solid phase and the explosive nature of low-molecular-weight azides. Radical thiol-ene and -yne need a thermally or photolytically cleavable initiator and Diels-Alder reactions based on furan-maleimide chemistry exhibit temperature-induced reversibility.[20] The efficient immobilisation/

functionalisation of stationary phases in fact requires chemistries that are not only metal- and trigger-free, but also allow the introduction of multiple residues in one pot. These chemistries grant the possibility to adapt the tuneable stationary phases even further. This chapter will highlight the usage of thiolactone chemistry to achieve this goal.

For the last twenty years, temperature-responsive polymeric stationary phases for liquid chromatography have been prepared and examined[13, 14, 21-29], yet they still do not perform as efficiently as the mostly applied reversed phase columns. Two polymers that have been employed for the synthesis of these phases are PNIPAM and polyvinylcaprolactam (PVCL), which both have an LCST.[13, 30] Although the polymer's LCST is influenced by its molecular weight[31], the LCST of PNIPAM is centred around 32°C[32], while the LCST of PVCL can be found between 32°C and 37°C.[33] Stationary phases were also loaded with copolymers of NIPAM and more hydrophobic monomers to reduce the LCST.[13, 34, 35]

Although elution orders similar to reversed-phase liquid chromatography (RPLC) can be attained, TRLC has the considerable advantage that it only needs water as a mobile phase, while retention can be modified by adjusting the temperature. The difference with other aqueous HPLC methods, such as ion exchange chromatography (IEC)[36] and high temperature chromatography[37-39], is that the first is restricted to the separation of charged solutes, while the second applies temperatures up to 150°C or higher, which may cause the degradation of the stationary phase and the solutes.

Theoretically speaking, TRLC is as widely applicable as RPLC, but has the added advantage of not needing organic solvents if the solutes are slightly soluble in water. In practice, however, most applications thus far analyse hydrophobic solutes, which renders this advantage more or less void. Based on (thermally regulated) hydrophobic and ionic interactions, a copolymer consisting of hydrophobic, cationic and thermoresponsive units was immobilised on a stationary phase to improve the separation of water-soluble bioactive compounds and biomacromolecules.[34, 35] As further progress in the development of retentive phases operating in the reversed-phase mode is likely and more chemistries might allow for the large-scale grafting of these kinds of polymers, TRLC might become more widely applicable. The hydrophobic retention of biomolecules is rather poor for the known TRLC columns, even at the highest working temperatures. To improve the resolution, the polymer loading and the coupled hydrophobicity at higher temperatures should be increased. Smaller particles can also be used to elevate the column efficiency, but to make TRLC more widely applicable, the retention problem should be tackled.

This chapter focusses on the immobilisation of PNIPAM and functionalisation of stationary phases with the combination of the amine-thiol-ene conjugation and thiolactone chemistry in one pot (Figure III.1). Thiolactone acrylamide was synthesised and copolymerised with NIPAM to obtain a thiolactone-functionalised polymer, which was reacted with aminopropyl silica and an acrylate to obtain the thermoresponsive stationary phase. Two different acrylates, benzyl and

PEG acrylate, were exploited to prepare two different PNIPAM-functionalised silica phases, which were packed into columns and tested with various solutes to emphasise the high retention. The benzyl acrylate-functionalised stationary phase also showed potential to retain and separate diastereoisomeric peptides, which are deemed to be one of the more difficult solutes to separate in RPLC.[40]

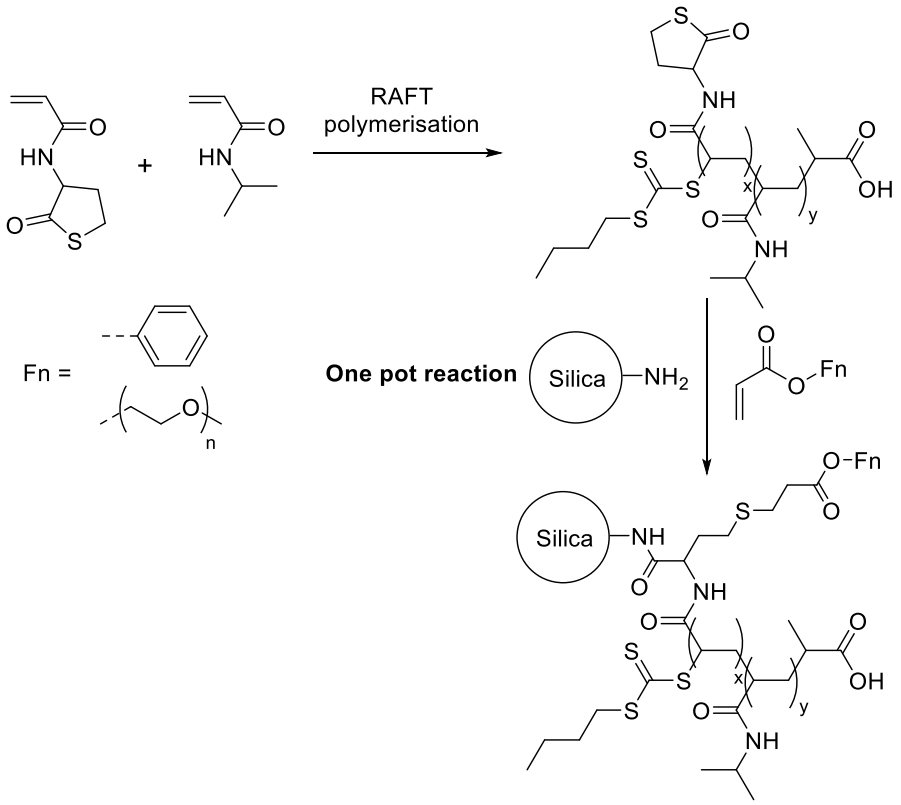


Figure III.1: Immobilisation of a thiolactone-functionalised PNIPAM, prepared via RAFT polymerisation, on aminopropyl silica and subsequent thiol-ene reaction with an acrylate to install an extra functionality.

Parts of the research described were a collaboration with Dr. Angel Satti of Universidad Nacional del Sur (Argentina), Dr. Tim Van Hoeylandt and Prof. Lynen of Ghent University. The synthesis of the thiolactone acrylamide was performed by myself. Angel Satti and I performed the polymer synthesis, the coupling of the polymer to the stationary phase and the analysis. The column packing and chromatographic experiments were performed by Angel Satti and Tim Van Hoeylandt. The results obtained were discussed, analysed and commented upon by the collaborators at all stages.

III.2 Preparation of PNIPAM-functionalised stationary phase with thiolactone chemistry

III.2.1 Synthesis of the thiolactone acrylamide

In order to immobilise the polymer on the aminopropyl silica and to be able to (extra) functionalise the stationary phase with an acrylate in one pot, thiolactone units had to be installed on PNIPAM. A thiolactone acrylamide was prepared that can copolymerise with NIPAM to obtain a thiolactone-functionalised polymer.[2, 5] DL-homocysteine thiolactone hydrochloride was reacted with acryloyl chloride to prepare the acrylamide (Figure III.2). The white solid product with a yield of 91% was purified by means of column chromatography and characterised with NMR and LC-MS analysis (Figure III.3).

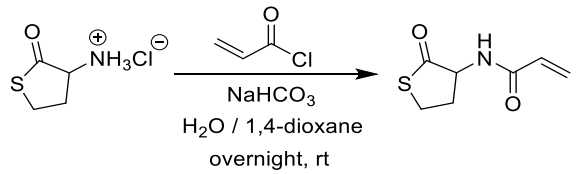


Figure III.2: Synthesis of the thiolactone acrylamide.

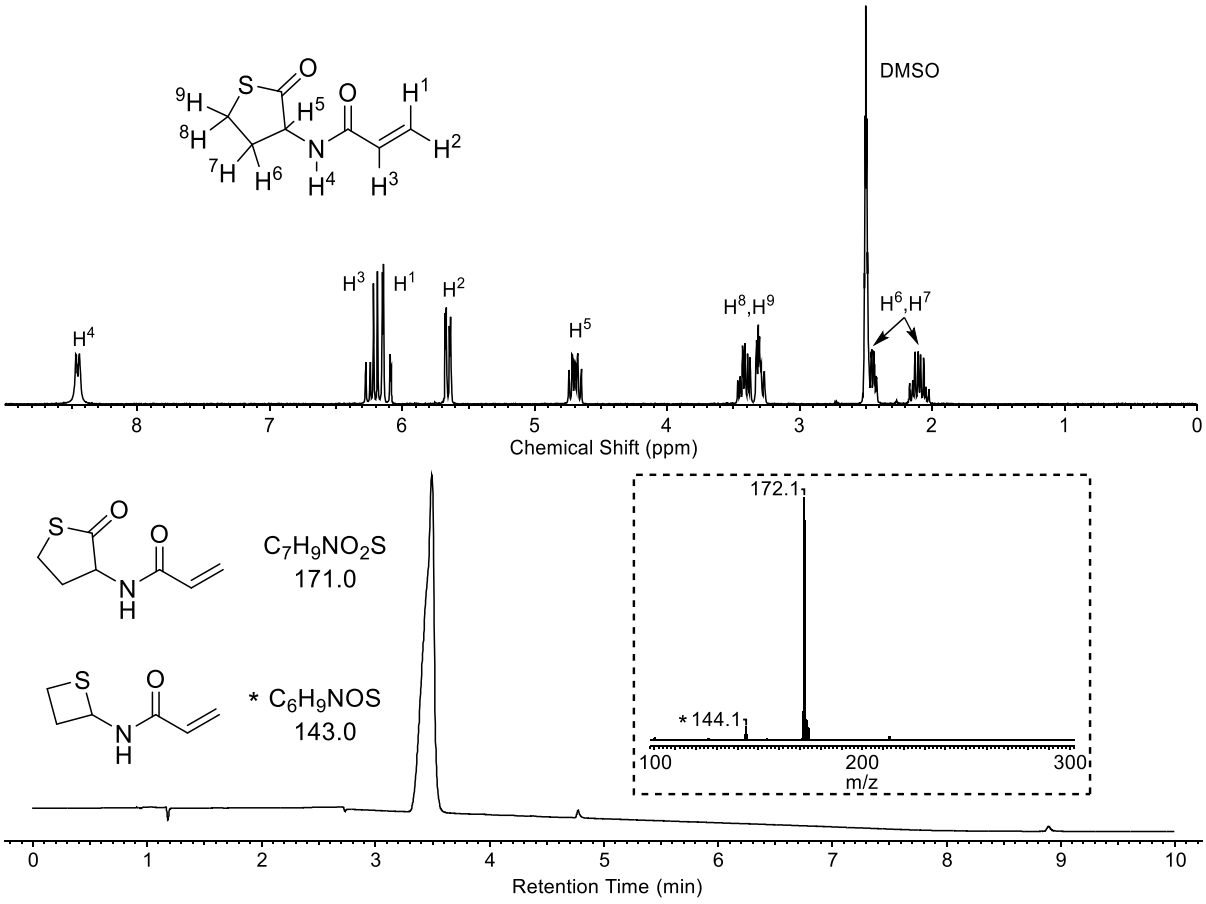


Figure III.3: ^{1}H-NMR spectrum (300 MHz, DMSO-d_6) with peak assignment (top) and HPLC trace with ESI-MS analysis (positive mode) (bottom) of the thiolactone acrylamide (fragmentation caused by carbon monoxide elimination from the thiolactone ring).[41]*

III.2.2 RAFT copolymerisation of thiolactone acrylamide and NIPAM

Before the preparation of the thiolactone-functionalised PNIPAM, two criteria had to be considered: the molecular weight and the thiolactone content of the polymer. On the one hand, the easy introduction of large amounts of the polymer on the silica material requires preparing polymers with relatively low molecular weights. Tsobukawa *et al.* discovered that increasing molecular weight of the polymer has two consequences: sterical hindrance of the already attached polymer layer on the silica increases, and the diffusion of macromolecular chains in this layer, aiming to reach reactive sites on the silica, is reduced. This results in a lower loading of the silica.[42] An optimal molecular weight for polymer coupling was determined to be between 5000 and 10000 g/mol. A high thiolactone content, on the other hand, influences the flexibility of the immobilised polymer on the silica surface, which affects the modulation of the retention time, while sufficient immobilisation on the stationary phase cannot be granted with a low content. The reaction conditions for the RAFT copolymerisation of the thiolactone acrylamide and NIPAM were chosen to take these criteria into account (Figure III.4). Based on previous research performed within our group[2] and SEC analysis, a copolymer was prepared with an M_n of about 6000 g/mol (poly(methyl methacrylate) standards) and a dispersity of 1.3. NMR and elemental analysis indicated that the thiolactone units were incorporated in the polymer and an average of two thiolactone groups could be found per polymer (Figure III.5). The latter number was calculated by applying the value obtained for the elemental analysis of sulphur (w(S) = 2.87%) to the M_n value obtained for SEC analysis divided by the molar mass of sulphur (M(S)) = 32.06). As three sulphur atoms were already present in the chain transfer agent, the obtained value (5.37) had to be subtracted by three, resulting in 2.37 sulphur atoms or thiolactone units in the polymer.

$$\text{Average number of S atoms in the polymer} = \frac{\text{w(S)} * \text{M}_\text{n}}{\text{M(S)}} = \frac{0.0287 * 6000}{32.06} = 5.37$$

$$\text{Average number of thiolactone units in the polymer}$$

$$= \text{Average number of S atoms in the polymer} - \text{number of S atoms of the RAFT agent}$$

$$= 5.37 - 3 = 2.37$$

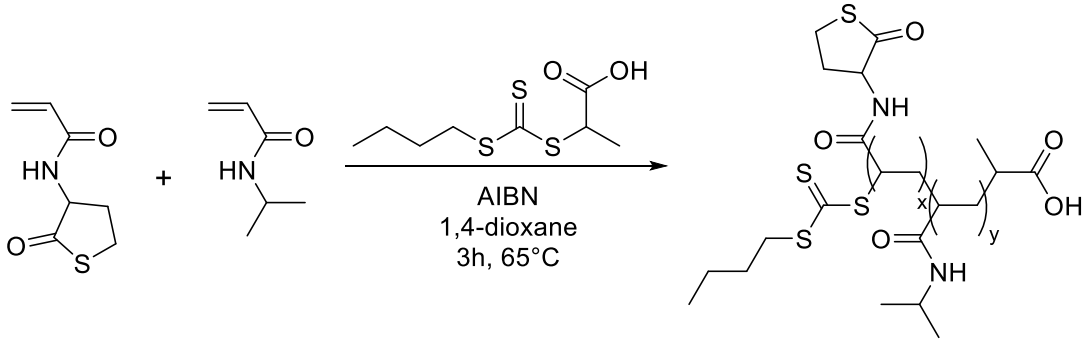


Figure III.4: RAFT copolymerisation of NIPAM and the thiolactone acrylamide.

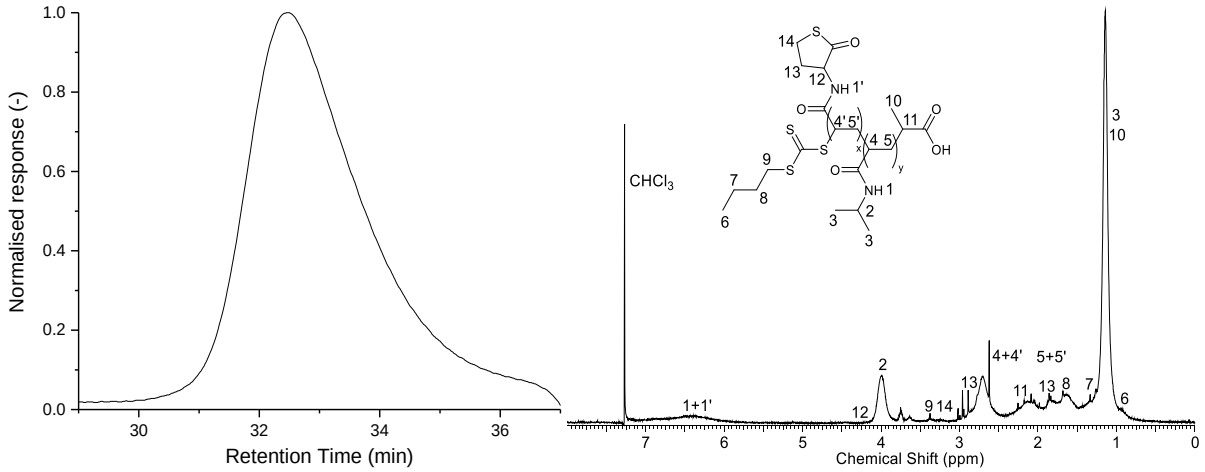


Figure III.5: SEC analysis (left) and ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) with peak assignment (right) of the prepared copolymer of NIPAM and thiolactone acrylamide.

III.2.3 Functionalisation of the stationary phase

In order to prepare the thermoresponsive stationary phase, the thiolactone-functionalised PNIPAM was reacted with aminopropyl silica and an acrylate in one pot (Figure III.1). The incorporated acrylate can have different functions: protecting the silica against hydrolysis, modifying the LCST of PNIPAM and/or altering the analytes' retention. The protective function can be explained by the fact that sterical hindrance (and hydrophobicity) of the incorporated acrylate provides an additional protection for the silica.[43] For example, C18 columns are more stable towards hydrolysis in comparison to their C8 counterparts as the first are more hydrophobic and provide more sterical hindrance to protect the silica. Benzyl or PEG acrylate (average Mn 480) were used in combination with the copolymer to obtain different stationary phases. The amine groups that remained after the reaction, were end-capped with acetic anhydride. A test with 2,4,6-trinitrobenzenesulfonic acid solution confirmed that no amine groups were still present.[44] Different techniques, such as Raman spectroscopy,[45] atomic force microscopy (AFM) and environmental scanning electron microscopy (ESEM),[46] time-of-flight secondary ion mass spectrometry (ToFSIMS),[47] x-ray photoelectron spectroscopy (XPS) and high resolution-magical angle spinning NMR (HR-MAS NMR)[48] can be applied to prove the coupling of the polymers. However, in this case, infrared spectroscopy (IR) and thermogravimetric analysis (TGA) were used, as both techniques are generally used for the analysis of modified silica[49] and permanently available in our research group. Both techniques confirmed the coupling of the polymer to the silica (Figure III.6 and III.7). The IR spectra of the stationary phase contain the distinctive bands of silica at 1100 cm^{-1} and 800 cm^{-1}, corresponding to the asymmetrical and symmetrical stretching vibrations of the Si-O-Si bonds,[45] and the bands between 1500-1700 cm^{-1}, 2800-3000 cm^{-1} and 3100-3600 cm^{-1} corresponding to respectively carbonyl stretching and N-H bending, C-H stretching, and N-H stretching (Figure III.6). TGA analysis indicated that the stationary phase consisted of over 20% of the polymer and that comparable results could be obtained for both stationary phases (Figure

III.7). This percentage can be obtained by comparing the mass percentages of the aminopropyl silica and the grafted silica at 800°C (Figure III.7, red and black curves). At this temperature, the polymer is fully degraded (Figure III.7, blue and black curves) on the grafted silica, which allows for a correct comparison. Although such elevated loadings (>20%) are not extraordinary for modified silica, the method applied allowed the immobilisation of an elevated amount of polymer in an easy and controlled way. In previous research, the coupling of aminopropyl silica with acid end-capped polymers had to be performed three times to obtain lower loading of polymer on the stationary phase.[49]

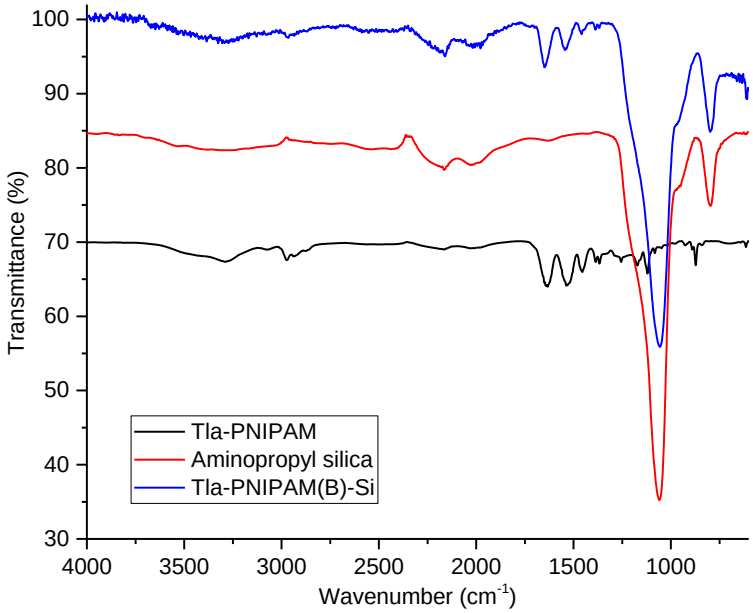


Figure III.6: IR spectra of the thiolactone-functionalised PNIPAM (Tla-PNIPAM), aminopropyl silica and silica modified with PNIPAM and benzyl acrylate (Tla-PNIPAM(B)-Si).

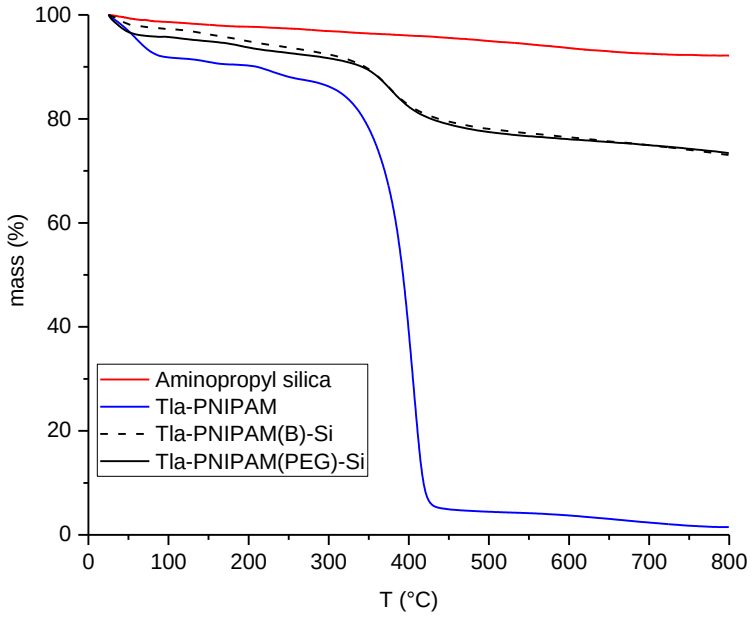


Figure III.7: TGA results of the thiolactone-functionalised PNIPAM (Tla-PNIPAM), aminopropyl silica and silica modified with PNIPAM and benzyl acrylate (Tla-PNIPAM(B)-Si) or PEG acrylate (Tla-PNIPAM(PEG)-Si).

III.3 Evaluation of the TRLC columns developed

III.3.1 Analysis of parabens and steroids

The efficiency, the retention, the peak symmetry and the temperature-responsive behaviour of columns packed with the stationary phases prepared were analysed with a mixture of parabens and steroids (Table III.1). The chromatograms obtained display elution patterns as observed for previously reported PNIPAM-containing columns (Figure III.9 and III.10).[49]

The increase in temperature at the same time also enhances some other parameters, i.e. the retention, the peak symmetry and plate numbers. Based on the theoretical plate model of chromatography,[50] the latter numbers are used as a way to express the efficiency of a column. This theoretical model splits a column up into distinct layers or plates (Figure III.8) in which solutes equilibrate between the stationary and the mobile phase. A solute is transferred through the column from one plate to another by the mobile phase. If a column consists of a large number of plates, it can improve equilibration of a solute across the stationary and the mobile phase, resulting in a more efficient separation. In other words, the higher the plate number (N), the better a separation will be. The optimal value for the plate number is 10000. Experimentally, the number of plates can be obtained from the retention time (t_R) and the peak width at half-height ($w_{1/2}$) of a solute peak via the equation in Figure III.8. Every solute of a sample has a different retention time and peak width at half-height for a certain column, so a sample will contain a range of plate numbers. Another way to express column efficiency is by the plate height (H), the theoretical length of a plate, calculated by dividing the column length (L) by N. This implies that the higher the plate number (N), the smaller the plate height and the more efficient the separation will be.

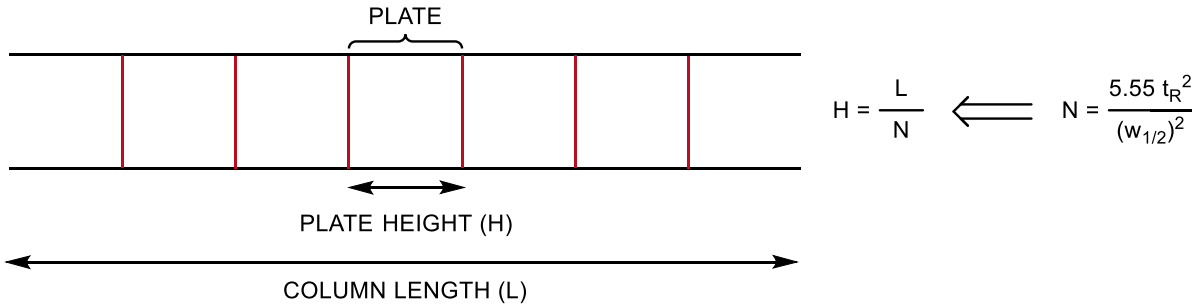
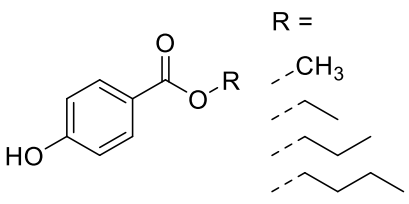
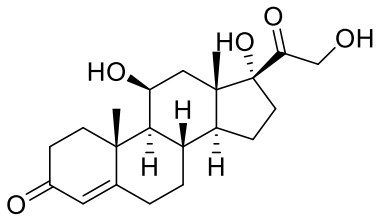
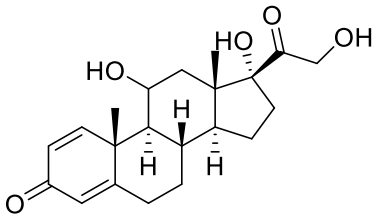
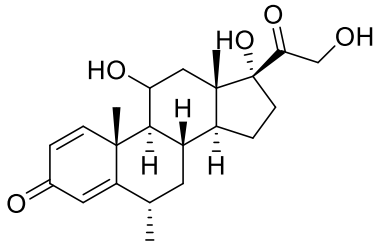
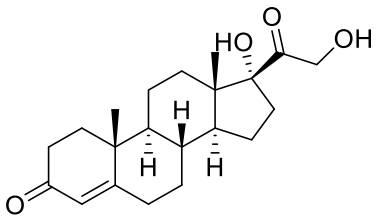
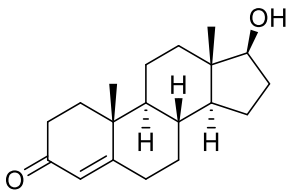


Figure III.8: Theoretical representation of a column based on the theoretical plate model of chromatography and calculations for the plate number (N) and the plate height (H).

Table III.1: Structures of the parabens and steroids used for the TRLC analysis.

1-4	 parabens	5	 hydrocortisone
6	 prednisolone	7	 methyl-prednisolone
8	 cortexolone	9	 testosterone

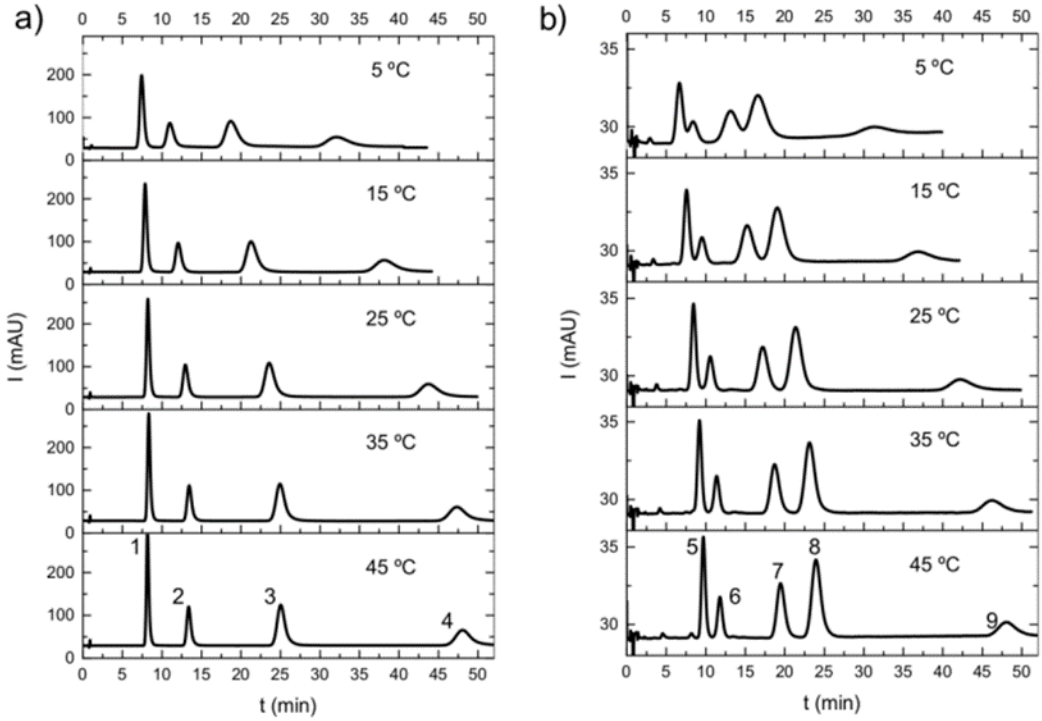


Figure III.9: TRLC analysis at different temperatures of the paraben (a) and steroid mixture (b) on the benzyl acrylate-functionalised PNIPAM column. Used chromatographic conditions: 100% Milli-Q water as mobile phase, flow rate of 1 mL/min, injection volume of 10 µL and column length of 10 cm. Peak identification: (1) methyl-paraben (20 µg/mL), (2) ethyl-paraben (20 µg/mL), (3) propyl-paraben (20 µg/mL), (4) butyl-paraben (20 µg/mL), (5) hydrocortisone (2 µg/mL), (6) prednisolone (14 µg/mL), (7) methyl-prednisolone (13 µg/mL), (8) cortexolone (8 µg/mL) and (9) testosterone (14 µg/mL).

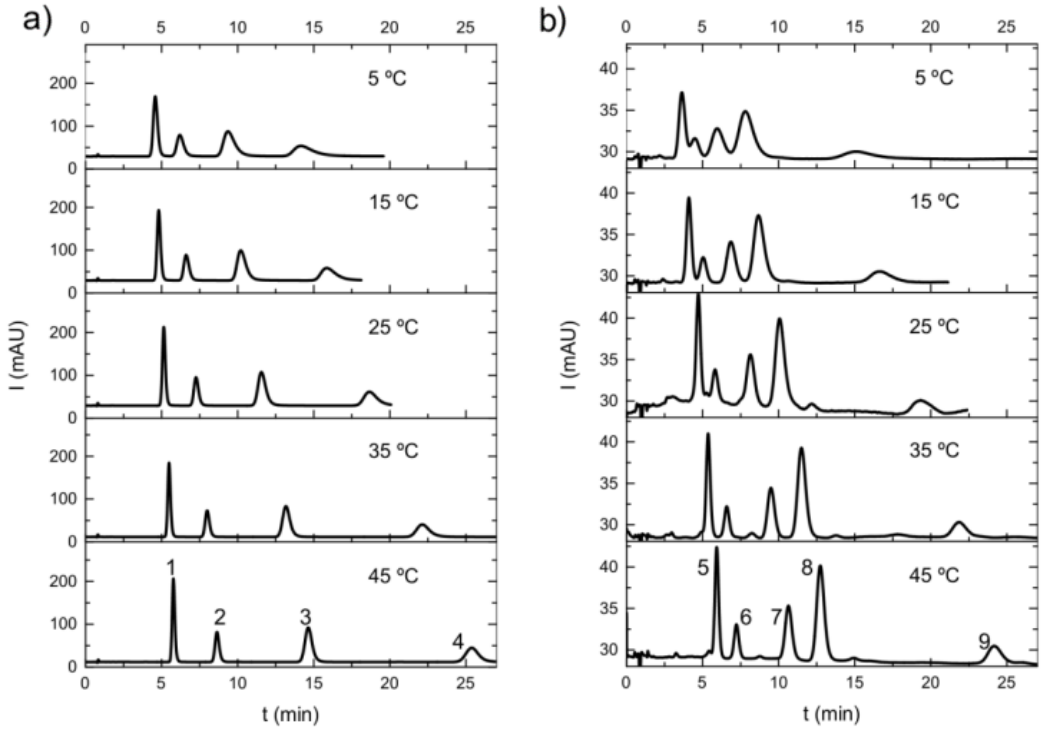


Figure III.10: TRLC analysis at different temperatures of the paraben (a) and steroid mixture (b) on the PEG acrylate-functionalised PNIPAM column. Used chromatographic conditions: 100% Milli-Q water as mobile phase, flow rate of 1 mL/min, injection volume of 10 µL and column length of 10 cm. Peak identification: (1) methyl-paraben (20 µg/mL), (2) ethyl-paraben (20 µg/mL), (3) propyl-paraben (20 µg/mL), (4) butyl-paraben (20 µg/mL), (5) hydrocortisone (2 µg/mL), (6) prednisolone (14 µg/mL), (7) methyl-prednisolone (13 µg/mL), (8) cortexolone (8 µg/mL) and (9) testosterone (14 µg/mL).

The plate numbers and the corresponding plate heights at 45°C varied respectively between 2977-3139 and 32–34 µm for the four parabens with the benzyl acrylate-functionalised stationary phase (Figure III.9a). Although the column efficiency is rather low (N < 10000) and can be boosted by the polymer's molecular weight, the reduction of the particle size, the flow rate of the mobile phase, and better packing strategies, this approach already allows for the production of effective chromatograms. A higher hydrophobic retention is observed in the chromatograms of the parabens and steroids for the benzyl acrylate-prepared stationary phase (Figure III.9a and b) in comparison to previously reported PNIPAM-containing columns.[49] The PEG acrylate did not change the elution pattern of the different solutes in the chromatograms, but the retention was more or less halved compared to the benzyl acrylate-functionalised column (Figure III.10a and b).

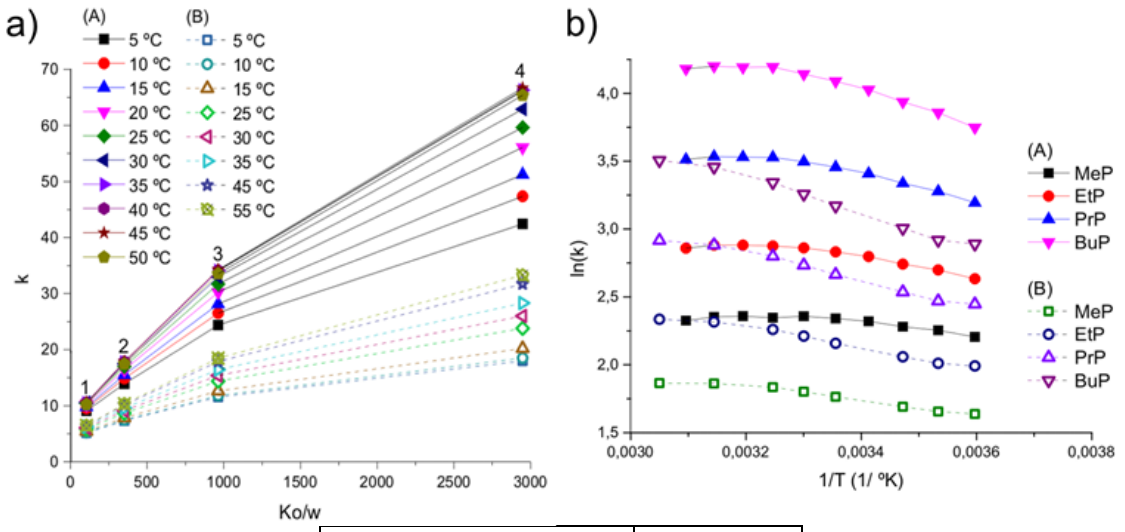
TRLC columns allow to apply a temperature gradient to improve or speed up separations.[34] For example, the separation performed on the steroids (Figure III.9b and III.10b) could be accelerated by a temperature gradient. Initially, the separation can be performed at 45°C until cortexolone (8 in Figure III.9b and III.10b) has come off. Next, the temperature can be dropped to 5°C to more quickly obtain testosterone (9 in Figure III.9b and III.10b). It could be interesting to explore this possibility of applying a gradient to other analytes in the future.

In previous research focussing on PNIPAM-containing columns, the retention factors (k) of the different parabens remained below 25.[49] At higher temperatures, butyl paraben has retention factors above 60 for the benzyl acrylate-prepared stationary phase (Figure III.11a, full lines).

The retention factor decreases by 35% when the column temperature is lowered to 5°C. Although these changes in retention are similar for all other solutes, the regular TRLC stationary phase grafted with linear PNIPAM induces stronger alterations. The behaviour observed could be attributed to the benzyl acrylate units, which generate an unchangeable hydrophobicity on the column. As mentioned above, the introduction of PEG acrylate units halves the retention factors (Figure III.11a, dashed lines). Although this reduction was expected, it is noteworthy that the incorporation of low molecular weight PEG units could cause such an effect.

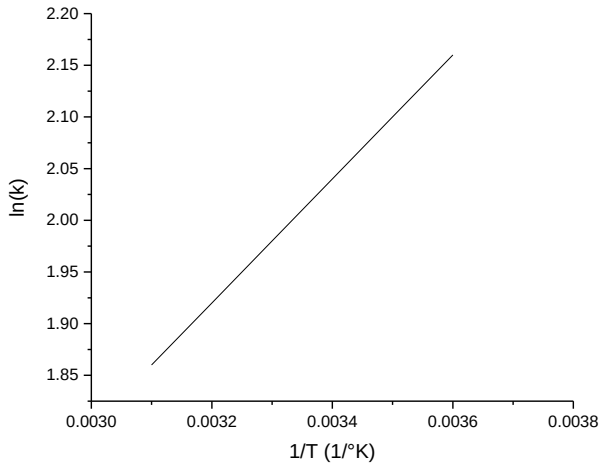
As PNIPAM has an LCST, the polymer changes from a water-soluble to water-insoluble behaviour when the temperature rises. When the column temperature is increased, the retention of the solutes is increased as well. This behaviour can be explained by the fact that the interaction mechanism with the stationary phase, PNIPAM-loaded silica, changes as a function of temperature. The Van't Hoff plots (ln k as a function of T^{-1}) for the different parabens (Figure III.11b) show different behaviour in comparison to the plots of pure RPLC (Figure III.12). The latter plots display a linear correlation with a positive slope, which corresponds to the enthalpy of the interaction between the analyte and the stationary phase.[51] For RPLC, the retention drops with increasing temperature as the interaction between the stationary phase and the solutes decreases due to the increase of thermal energy of these solutes. A gradual shift of the separation mechanism with rising temperature was caused by the formation of a hydrophobic layer on the surface of the stationary phases prepared due to PNIPAM becoming water-insoluble (Figure III.13), which resulted in the increase in retention with rising temperature. The use of different acrylates for the functionalisation of the different phases can also be observed in the Van't Hoff plots. The cloud point of the PNIPAM on the stationary phase can be found where the slope of the graph is at its maximum or the inflection point. In comparison to the benzyl-functionalised TRLC column (full lines in Figure III.11b), the plots of the PEG-functionalised TRLC column (dashed lines) show a curve inflection, which can be attributed to an induced shift in the cloud point of this second column. The benzyl-functionalised TRLC column has a lower cloud point, as the inflection point has moved to a lower temperature. This behaviour can be explained by the fact that the hydrophobic benzyl acrylate makes the silica surface more hydrophobic, which allows for the easier formation of a hydrophobic layer by PNIPAM. For the benzyl-functionalised TRLC column, a second curve inflection can be observed at higher temperature as well. At this point, the PNIPAM has fully precipitated on the silica and the column will start to behave more like an RPLC column which shows, as discussed above, a decrease in retention with increasing temperatures.

From the different chromatograms and plots (Figures III.9-11), it can be observed that the retention behaviour of the various solutes can be easily tuned by exploiting different (acrylate) units for the modification of the TRLC phase.



Analyte	Log Ko/w
Methyl-paraben	1.99
Ethyl- paraben	2.49
Propyl- paraben	2.98
Butyl- paraben	3.47

Figure III.11: Retention factors (k) versus the calculated octanol/water partition coefficient (Ko/w, estimated with the WSKOWIN software (see inserted table) (a) and corresponding Van 't Hoff plots (b) of the 4 parabens for the benzyl acrylate- (A series, full lines) and the PEG acrylate-functionalised columns (B series, dashed lines) TRLC columns.



$$\ln(k) = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln(\phi)$$

ΔH = enthalpy of interaction (exothermic)
ΔS = entropy of interaction
R = the gas constant
ϕ = phase ratio between the stationary and mobile phase

Figure III.12: Hypothetical Van't Hoff plot of an RPLC column and its corresponding equation.

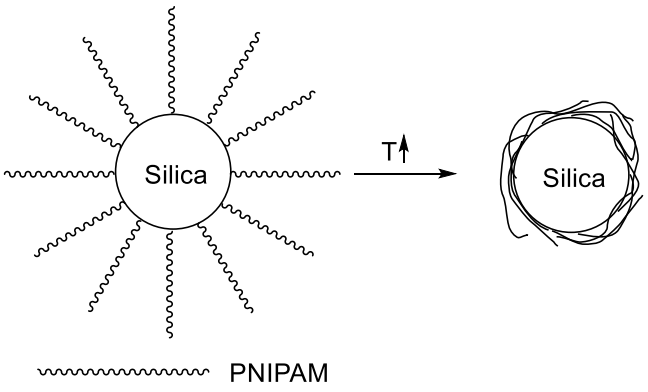


Figure III.13: Schematic representation of the formation of the hydrophobic layer on silica with increasing temperature.

The column performance did not decrease remarkably as a result of the combination of repeated injections of the various solutes and the different temperature cycles. The column stability

proved to be good as retention time variations below 1% relative standard deviation (RSD) were obtained for the inter-day precision measurements at 35°C over an interval of 6 weeks for the different parabens (Table III.2).

Table III.2: Inter-day precision measurements over an interval of 6 weeks at 35°C for the retention times of the parabens on the benzyl acrylate-functionalised column.

35°C	tr (min)			
	Me-paraben	Et-paraben	Pr-paraben	Bu-paraben
Week 1	8.28	13.50	25.32	48.56
Week 2	8.22	13.41	25,05	47.79
Week 6	8.17	13.38	25.04	48.06
average	8.23	13.43	25.18	48.14
standard deviation	0.05	0.06	0.20	0.39
RSD%	**0.65**	**0.48**	**0.79**	**0.81**

III.3.2 Analysis of peptide diastereomers

As the hydrophobic retention of biomolecules is rather poor for the known TRLC columns, even at the highest working temperatures, a peptide mixture of a dipeptide and two diastereoisomeric tripeptides was examined to test their retention and separation behaviour with the benzyl acrylate-functionalised column (Table III.3 and Figure III.14). At lower temperatures, the slower diffusion of the peptide solutes in the stationary phase causes signal broadening. With increasing temperature, the signals become more narrow and the separation of the diastereoisomeric peptides is enhanced.

Chiral chromatography of an enantiomeric (oligopeptide) mixture can be performed either by using a chiral stationary or mobile phase, or by first forming diastereomers which can be separated with RPLC.[52] The latter strategy requires to first let the mixture react with an enantiomerically pure compound to form the diastereomers. Kroeff *et al.* performed the separation of similar oligopeptides as the ones in Table III.3 with RPLC columns.[53] Before our TRLC system can be compared to the RPLC one, two elements should be taken into account. Kroeff and coworkers performed their separations on C8 columns instead of C18, while the latter normally cause better retention, and as oligopeptides are mostly charged (and polar), their separation is very difficult to perform on both RPLC and TRLC, which apply apolar stationary phases.

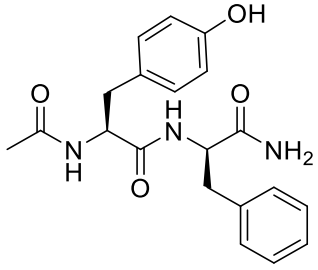
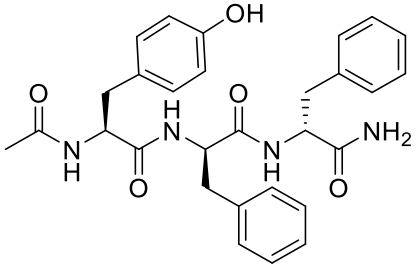
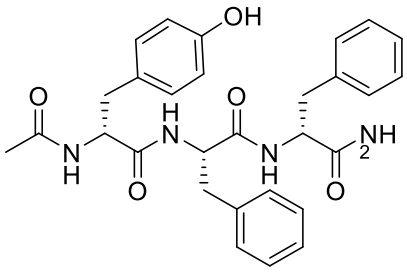
Firstly, the columns used for the RPLC separations needed to be 25 cm long and highly efficient ($N \approx 10000$). Although the applied TRLC strategy has rather low efficiencies ($N < 3200$), 10 cm columns already proved to be able to separate the diastereomers to some extent. Secondly, the pH of the mobile phase had to be reduced to 3.5 for the RPLC columns to ensure the oligopeptides carry as few charges as possible. The TRLC system could be performed with pure water as mobile phase. Finally, the retention factors were relatively low (between 0.5 and 5) for

the RPLC columns. At 45°C, the retention factors are higher (between 15 and 17) for the TRLC columns.

Although 10 cm columns were used for this research and the plate numbers are rather low, the separation of small biomolecules such as peptides looks promising for the applied stationary phase. The analysis might be improved by shifting to the more commonly applied 25 cm columns and finding ways to increase the plate numbers via the reduction of the particle size, the flow rate of the mobile phase, and better packing strategies. Although the chiral polymer applied did not have enantiomerically pure centres, which hail from the thiolactone acrylamide and the polymerisation process, the diastereomeric pair was separated to some extent at higher temperatures. It could be interesting in a future perspective to prepare stereochemically defined polymers and stationary phases by applying an enantiomerically pure thiolactone, an enantiomerically pure acrylate and a strategy to control the tacticity during the applied RAFT polymerisation.[54]

Finally, 0.1 % formic acid was added to the mobile phase to see if the prepared TRLC columns could be combined with ESI-MS analysis. The addition of this acid increased the retention slightly, but did not change the elution pattern of the solutes and did not cause any problems as no degradation of the applied columns was observed.

Table III.3: Structures of the oligopeptides used for the TRLC analysis (Tyr = Tyrosine and Phe = Phenylalanine).

1	 AcNH-L-Tyr-D-Phe-$CONH_2$	2	 AcNH-L-Tyr-D-Phe-D-Phe-$CONH_2$
		3	 AcNH-D-Tyr-L-Phe-D-Phe-$CONH_2$

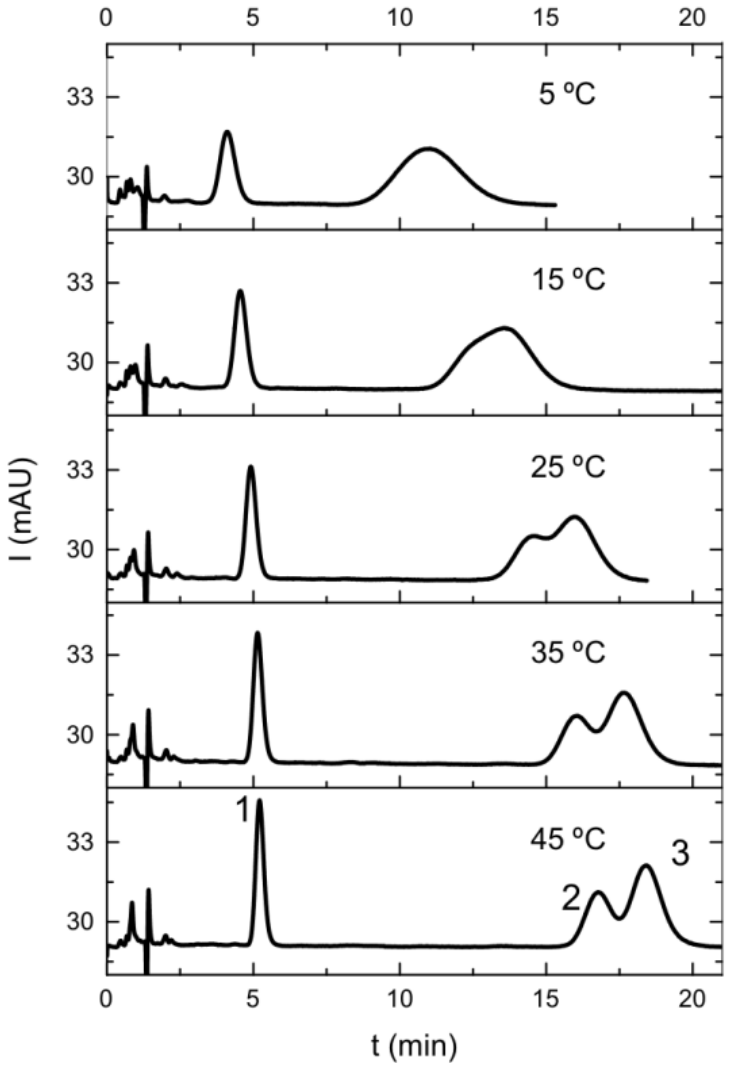


Figure III.14: TRLC analysis of a dipeptide and two diastereoisomeric tripeptides on the benzyl acrylate-functionalised column at different temperatures. Used chromatographic conditions: 100% Milli-Q water as mobile phase, flow rate of 1 mL/min, injection volume of 10 µL and column length of 10 cm. Peak identification: (1) AcNH-L-Tyr-D-Phe-CONH₂ (72 µg/mL), (2) AcNH-L-Tyr-D-Phe-D-Phe-CONH₂ (175 µg/mL) and (3) AcNH-D-Tyr-L-Phe-D-Phe-CONH₂ (160 µg/mL).

III.4 Conclusions and outlook

In this chapter, the amine-thiolactone-ene conjugation was applied to prepare tuneable TRLC stationary phases. A thiolactone acrylamide was synthesised and polymerised with NIPAM via RAFT to obtain a thiolactone-functionalised thermoresponsive polymer. The thiolactone groups of the polymer were opened by the amines of the stationary phase, resulting in a thiol that can react with an acrylate. In one pot, the stationary phase was loaded with PNIPAM and functionalised with two different acrylates in order to prepare two stationary phases.

Symmetric peaks and suitable column efficiencies were obtained for the analyses of parabens and steroids with the prepared columns with only water as mobile phase. Results indicated that the temperature used played a crucial role in the retentions of the solutes: low retention was

noticed below the LCST of PNIPAM and high retention above the LCST. Varying the acrylate also played a crucial role, as retention times were more or less halved when PEG acrylate was exploited to functionalise the stationary phase instead of benzyl acrylate. The benzyl acrylate-containing stationary phase also showed potential for the separation of diastereoisomeric peptides.

With the method applied here, based on the amine-thiol-ene conjugation with thiolactones, liquid chromatographic stationary phases can be easily tuned. In order to possibly acquire better thermoresponsive (chiral) separation in the future, the stationary phase can be loaded with other (stereochemically defined) thermoresponsive polymers and functionalised with different (enantiomerically pure) acrylates or other functionalities that can react with the formed thiol after aminolysis of the (enantiomerically pure) thiolactone.[30, 55, 56] These prepared columns could also be used in two-dimensional liquid chromatography (LC x LC), which could offer better separation of complex samples and mixtures.[27] In addition to linear polymers, more complex polymeric structures, such as hyperbranched polymers, could be coupled with this method to improve the separation of the solutes.[57, 58] Hyperbranched polymers have already been used for ion exchange chromatography (IEC)[59] and hydrophilic interaction liquid chromatography (HILIC)[60] to improve the separation of polar analytes. Thiolactone, which can be enantiomerically pure, could also be connected to the silica, which can be double-modified with an amine and an acrylate. Octadecylamine or octadecyl acrylate could be incorporated with another functionality to obtain a functionalised (chiral) RPLC column.

The analytes in this chapter were mostly apolar. TRLC columns have been applied for the separation of polar solutes, but the polymeric stationary phases consist of copolymers of NIPAM with monomers containing tertiary amines, acids or charged groups.[24, 26, 34, 35, 59] Hydrophobic monomers such as butyl methacrylate had to be incorporated as well in the copolymers to ensure that the LCST behaviour did not change too radically due to the incorporated polar functionalities. These copolymeric stationary phases are not only temperature-responsive, but also pH-responsive. Thus, the retention of the analytes can be controlled by two stimuli. Temperature gradients have also been used for these stationary phases to improve the separation of analytes.[34] The TRLC system applied in this chapter could be functionalised in the future with an acrylate that contains a tertiary amine, acid or charged group to perform the separation of polar analytes with or without the use of temperature gradients.

Alongside thiolactone chemistry, other multicomponent reactions might also allow for the modification of silica with polymers and various functionalities.[61-63] The Ugi four-component reaction between an aldehyde or a ketone, an amine, an acid and an isocyanide could perhaps be applied with the aminopropyl stationary phase used to install a (thermoresponsive) polymer and two different functionalities to prepare tuneable (TRLC) stationary phases (Figure III.15a). Another chemistry that can be used for the simple and ultrafast coupling of polymers to the stationary phase is 1,2,4-triazoline-3,5-dione chemistry (TAD), which can also provide visible

feedback (Figure III.15b).[64-66] A polymer functionalised with an indole, ene, phenol moiety or diene can be coupled to a TAD-functionalised solid support or a TAD-functionalised polymer can be coupled with an indole-, ene-, phenol- or with a diene-functionalised solid support. The indole-TAD chemistry is reversible, which might allow for the solid support to be easily rewritten with a different polymer (Figure III.15b).[67, 68] Different phenols, indoles, enes or dienes can be used to introduce various functionalities, such as the acrylates in the applied thiolactone strategy.

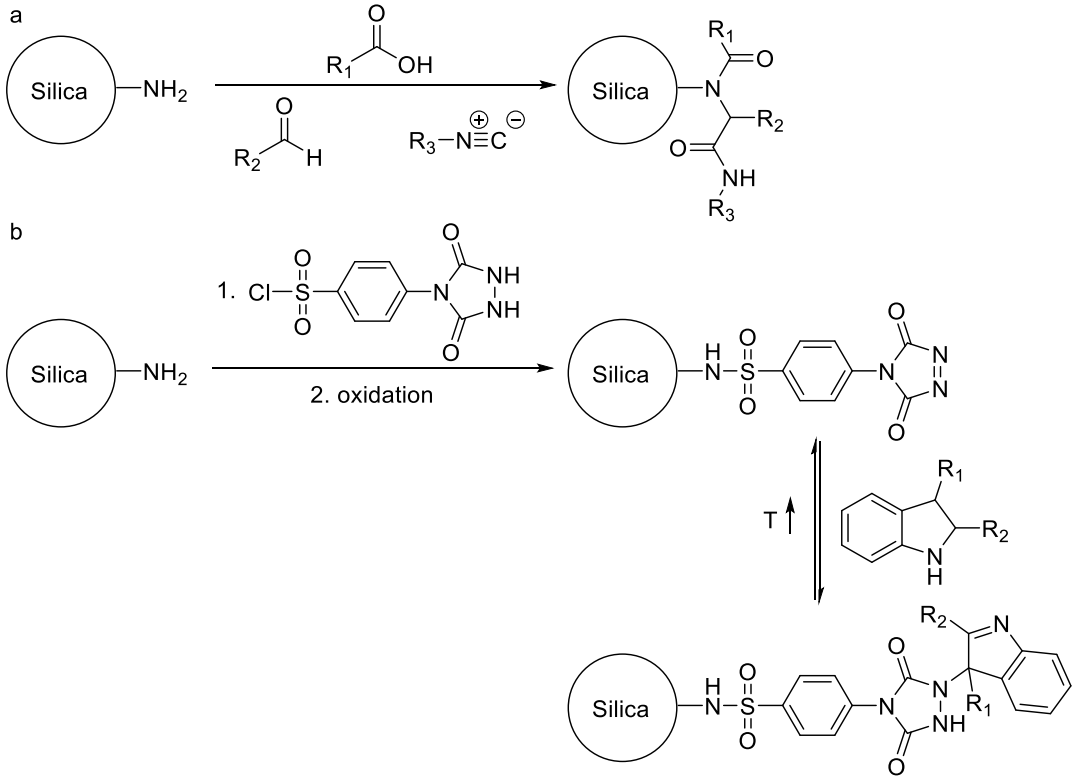


Figure III.15: Different proposed strategies for the modification of aminopropylsilica: a) Ugi reaction, and b) transformation of the amine to a TAD functionality[65] and the subsequent modification with the reversible indole-TAD chemistry.[68]

III.5 Experimental part

III.5.1 Methods

^{1}H- and ^{13}C-NMR

^{1}H- and ^{13}C-NMR (Attached Proton Test, APT) spectra were recorded on a Bruker Avance 300 at 300 MHz and 75 MHz, respectively and a Bruker Avance 500 at 500 MHz and 125 MHz, respectively. Chemical shifts are presented in parts per million (δ) relative to DMSO-d_6 or $CHCl_3$-*d* (2.50 ppm or 7.27 ppm in ^{1}H- and 39.51 ppm or 77.24 ppm in ^{13}C-NMR respectively) as internal standard. All samples were analysed with 2D-NMR techniques (COSY, HSQC and HMBC) which allowed for a full assignment of the structures. All measurements were

performed at 25°C and ACD/NMR Processor was used for the processing of all data. All spectra including 1D ^{1}H and ^{13}C, 2D COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC were recorded in a standard fashion with pulse programs available in the Bruker library.

LC-ESI-MS

An Agilent technologies 1100 series LC/MSD system equipped with a diode array detector and single quad MS detector (VL) with an electrospray source (ESI-MS) was used for classic reversed phase LC-MS (liquid chromatography mass spectroscopy) and MS analysis. Analytic reversed phase HPLC was performed with a Phenomenex C18 (2) column (5 µ, 250 x 4.6 mm) using a solvent gradient (0 → 100% acetonitrile in H_2O in 15 min) and the eluting compounds were detected via UV-detection (λ = 214 nm). High-resolution mass spectra (HRMS) were collected using an Agilent 6220 Accurate-Mass time-of-flight (TOF) equipped with a multimode ionisation (MMI) source.

IR

Infrared spectra were recorded with Attenuated Total Reflection (ATR) with a PIKE Miracle ATR unit and a Perkin Elmer FTIR SPECTRUM 1000 spectrometer. IR-software from Perkin Elmer was used for the analysis of the spectra.

SEC

Size exclusion chromatography (SEC) was performed on a Waters instrument with a Waters 2414 Refractive Index Detector (RI), equipped with 3 PSS serial columns (GRAM Analytical 30 and 1000˚A, 10 µm particle size) at 35°C. Poly(methyl methacrylate) standards were used for calibration and DMA containing LiBr (0.42 g/l) was used as an eluent at a flow rate of 1 mL/min. Molecular weights and dispersities (Đ) were determined using the Empower software.

TGA

TGA analyses were performed in nitrogen between 25 and 800°C at 10°C/min with TGA/SDTA851e of Mettler-Toledo.

Elemental analysis

Elemental analysis (CHNS) was performed on a Thermo Flash 2000 elemental analyser. During the measurement, a V_2O_5 catalyst was used.

Chromatographic conditions

A Waters Alliance 2690 system equipped with a variable wavelength UV absorbance detector was exploited to carry out the HPLC experiments, which were performed four times. 100% Milli-Q water as a mobile phase was percolated through the columns at a flow rate of 1 mL/min

and 10 µl was used as injection volume. The temperature of the column was controlled with a Julabo F10-VC water bath allowing 0.1°C accuracy. Before every run, the columns had to be equilibrated for 10 min at the various temperatures applied (5 to 50°C). The detection of the different solutes was performed at different wavelengths: parabens at 210 nm, steroids at 254 nm and peptides at 280 nm. WSKOWIN software (Environmental Protection Agency, Washington DC, USA) was employed to calculate the Log *Ko/w* values.

III.5.2 Materials

DMSO-d_6 ([2206-27-1], ≥ 99.8 %) and $CHCl_3$-*d* ([865-49-6], ≥ 99.8 %) were purchased from Euriso-top. Acetonitrile ([75-05-8], HPLC grade) and 1,4-Dioxane ([123-91-1], HPLC grade) were purchased from Acros Organics. Aminopropyl silica (Chromatorex NH2 SPS 100Ä, 5µm) was purchased from Fuji Silysia Chemical S.A.. DL-Homocysteinethiolactone hydrochloride ([6038-19-3], 99 %) was purchased from Haihang industry (Jinan City, China). Acetic anhydride ([108-24-7], ≥ 99 %) was purchased from Riedel-de Haën. Sodium bicarbonate ([144-55-8], ≥ 99.5%) was purchased from Carl Roth. Acryloyl chloride ([814-68-6], 96 %) was purchased from ABCR. Benzyl acrylate ([2495-35-4], > 97 %) was purchased from TCI and used without purification. 2-2´-Azobis(isobutyronitrile) (AIBN) ([78-67-1], ≥ 98.0 %), Butyl-paraben ([94-26-8]), Chloroform ([865-49-6], ≥ 99.8 %), Cortexolone ([152-58-9]), Dichloromethane ([75-09-2], ≥ 99.8 %), N,N-Dimethylacetamide ([127-19-5], ≥ 99.9 %), Ethyl acetate ([141-78-6], ≥ 99.7 %), Ethyl-paraben ([120-47-8]), Hydrocortisone ([50-23-7]), N-Isopropyl acrylamide ([2210-25-5], 97 %), Methanol ([67-56-1], ≥ 99.9 %), Methylprednisolone ([83-43-2]), Methyl-paraben ([99-76-3]), Prednisolone ([50-24-8]), Poly(ethylene glycol) methyl ether acrylate ([32171-39-4], average Mn 480), Propyl-paraben ([94-13-3]), Pyridine ([110-86-1], ≥ 99 %), Testosterone ([58-22-0]), Tetrahydrofuran ([109-99-9], ≥ 99 %), 2,4,6-Trinitrobenzenesulfonic acid solution ([2508-19-2], 5 % (w/v) in H_2O were purchased from Sigma-Aldrich. Before polymerisation, NIPAM was recrystallised from a mixture of toluene/n-hexane (1/1 v/v). Silicagel (ROCC, SI 1721, 60 Å, 40 – 63 µm) was used to perform preparative column chromatography, eluting with technical solvents. The collected fractions were analysed by thin layer chromatography (TLC-plates, Macherey-Nagel, SIL G-25 UV254). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). 1000 µg/mL stock solutions of the parabens and of the steroids were prepared in acetonitrile. The di- and tripeptides were synthesised in-house via standard solid phase peptide chemistry and dissolved in acetonitrile/water (80/20) at 1 mg/mL. Stock solutions were diluted with water to their final concentrations mentioned in the chapter. The thiolactone acrylamide and 2-(butylsulfanyl)-carbonothioylsulfanylpropanoic acid were prepared as previously described.[2, 69]

III.5.3 Synthesis

Synthesis of the thiolactone acrylamide

DL-homocysteine thiolactone hydrochloride (14.0 g, 91.2 mmol) was dissolved in an ice-cooled mixture of H_2O/1,4-dioxane (1/1, 200 mL) and treated with $NaHCO_3$ (38.3 g, 446.0 mmol). This mixture was stirred for 30 minutes at 0°C. In small portions acryloyl chloride (16.6 g, 183.4 mmol) was added to the mixture, which was allowed to reach room temperature during the night. After the addition of brine (200 mL), the mixture was extracted with EtOAc (3 x 400 mL). The organic fractions were dried ($MgSO_4$) and concentrated. The crude residue was purified by column chromatography (silica gel, gradient elution: 100 % CH_2Cl_2 → CH_2Cl_2 /acetone: 95/5), yielding the acrylamide as a white solid (14.2 g, 83.0 mmol, 91 %).

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 8.45 (d, 1 H, 8.2 Hz), 6.23 (dd, 1 H, 17.1, 9.7 Hz), 6.12 (dd, 1 H, 17.1, 2.6 Hz), 5.65 (dd, 1 H, 9.7, 2.6 Hz), 4.70 (ddd, 1 H, 15.3, 8.2, 7.1 Hz), 3.42 (app dt, 1 H, 11.3, 5.3 Hz), 3.30 (ddd, 1 H, 10.9, 6.9, 1.4 Hz), 2.44 (m, 1 H), 2.09 (m, 1 H).
^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 205.3 (C), 164.6 (C), 131.0 (CH), 126.2 (CH_2), 58.2 (CH), 30.3 (CH_2), 26.8 (CH_2).
IR (cm^{-1}) 3268, 3065, 2942, 2908, 1693, 1656, 1622, 1537, 1451, 1438, 1406, 1317, 1279, 1256, 1239, 1174, 1146, 1054, 1016, 988, 974, 942, 908, 842, 805, 747, 697, 684, 653, 617
ESI-MS (m/z) $[M+H]^+$ 172.1

Synthesis of thiolactone-functionalised PNIPAM via RAFT polymerisation

NIPAM (7.5 g, 66.28 mmol), thiolactone acrylamide (0.65 g, 3.31 mmol), AIBN (0.15 mmol) and 2-(butylsulfanyl)-carbonothioylsulfanylpropanoic acid (0.32 g, 1.33 mmol) were weighted in a Schlenk tube and dissolved in 1,4-dioxane (39 mL). Degasification of this mixture was performed via three freeze-pump-thaw cycles. The mixture was stirred at 65°C for 3 hours under nitrogen atmosphere. Afterwards, three precipitations in cold diethylether were performed to attain a high level of purity. The remaining diethylether was removed via evaporation overnight at 40°C in a vacuum oven.[2]

III.5.4 Manipulation of the stationary phase

Loading

The thiolactone-functionalised PNIPAM (1.51 g, 0.25 mmol) and an acrylate (1.75 mmol) were dissolved in THF (45 mL, 10 mL/1 g of stationary phase) and added to aminopropyl silica (4.5 g). After overnight shaking at room temperature, the stationary phase obtained was filtered and washed with water (400 mL) and methanol (150 mL). Next, residual amines on the stationary phase were reacted with acetic anhydride (4.6 mL, 48.7 mmol) and pyridine (1.32 mL, 16.3 mmol) in $CHCl_3$ (60 mL) to end-cap them. After 20 hours of shaking at room temperature, the

stationary phase obtained was filtered and washed with water (600 mL) and methanol (250 mL). The stationary phase was dried under vacuum. The stationary phase obtained (5.5 g) was a white solid.

Packing of the column

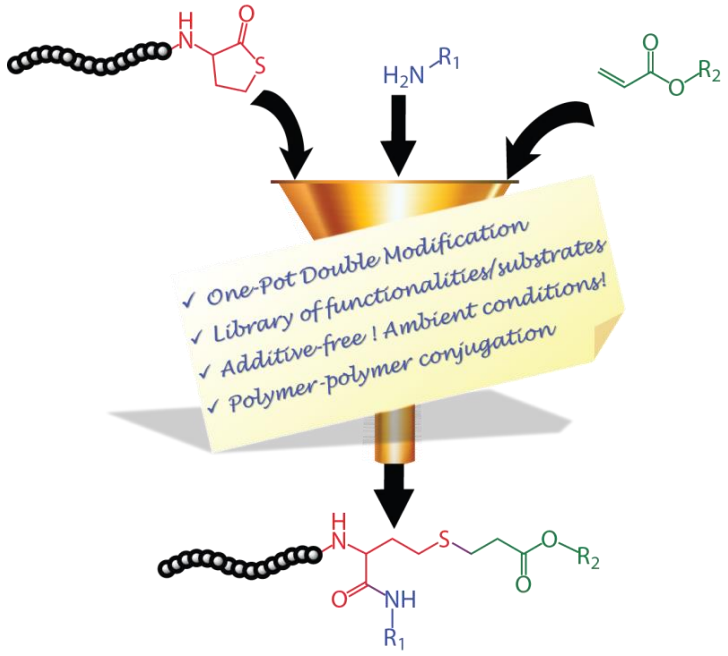
A mixture of the prepared silica (1.6 g) and water (15 mL) was well mixed in an ultrasonic bath and packed at 450 bar in a 10 cm × 4.6 mm ID stainless steel column with a Haskel air-driven pump.

References

[1] P. Espeel, F. Goethals, F. E. Du Prez, *J. Am. Chem. Soc.* **2011**, *133*, 1678.
[2] S. Reinicke, P. Espeel, M. M. Stamenović, F. E. Du Prez, *ACS Macro Lett.* **2013**, *2*, 539.
[3] P. Espeel, F. Goethals, F. Driessen, L.-T. T. Nguyen, F. E. Du Prez, *Polym. Chem.* **2013**, *4*, 2449.
[4] P. Espeel, F. Goethals, F. E. Du Prez, "CHAPTER 9 Thiolactones as Functional Handles for Polymer Synthesis and Modification", in *Thiol-X Chemistries in Polymer and Materials Science*, The Royal Society of Chemistry, 2013, p. 195.
[5] S. Reinicke, P. Espeel, M. M. Stamenovic, F. E. Du Prez, *Polym. Chem.* **2014**, *5*, 5461.
[6] F. Goethals, S. Martens, P. Espeel, O. van den Berg, F. E. Du Prez, *Macromolecules* **2014**, *47*, 61.
[7] P. Espeel, F. E. Du Prez, *Eur. Polym. J.* **2015**, *62*, 247.
[8] R. Hoogenboom, "2 - Temperature-responsive polymers: properties, synthesis and applications", in *Smart Polymers and their Applications*, M.R. Aguilar and J. San Román, Eds., Woodhead Publishing, 2014, p. 15.
[9] X.-T. Peng, B.-F. Yuan, Y.-Q. Feng, *Journal of Separation Science* **2011**, *34*, 3123.
[10] P. V. Der Voort, E. F. Vansant, *Journal of Liquid Chromatography & Related Technologies* **1996**, *19*, 2723.
[11] T. Ikegami, J. Ichimaru, W. Kajiwara, N. Nagasawa, K. Hosoya, N. Tanaka, *Analytical Sciences* **2007**, *23*, 109.
[12] A. Petri, P. Marconcini, P. Salvadori, *Journal of Molecular Catalysis B: Enzymatic* **2005**, *32*, 219.
[13] H. Kanazawa, Y. Kashiwase, K. Yamamoto, Y. Matsushima, A. Kikuchi, Y. Sakurai, T. Okano, *Analytical Chemistry* **1997**, *69*, 823.
[14] H. Kanazawa, K. Yamamoto, Y. Matsushima, N. Takai, A. Kikuchi, Y. Sakurai, T. Okano, *Analytical Chemistry* **1996**, *68*, 100.
[15] W. H. Newsome, M. Abbott, *J AOAC Int* **1999**, *82*, 666.
[16] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
[17] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2.
[18] A. Marechal, R. El-Debs, V. Dugas, C. Demesmay, *Journal of Separation Science* **2013**, *36*, 2049.
[19] A. Marechal, A. Laaniste, R. El-Debs, V. Dugas, C. Demesmay, *Journal of Chromatography A* **2014**, *1365*, 140.
[20] A. Gandini, A. Silvestre, D. Coelho, *Polym. Chem.* **2013**, *4*, 1364.
[21] H. Kanazawa, K. Yamamoto, Y. Kashiwase, Y. Matsushima, N. Takai, A. Kikuchi, Y. Sakurai, T. Okano, *Journal of Pharmaceutical and Biomedical Analysis* **1997**, *15*, 1545.
[22] H. Go, Y. Sudo, K. Hosoya, T. Ikegami, N. Tanaka, *Analytical Chemistry* **1998**, *70*, 4086.
[23] T. Yakushiji, K. Sakai, A. Kikuchi, T. Aoyagi, Y. Sakurai, T. Okano, *Analytical Chemistry* **1999**, *71*, 1125.
[24] J. Kobayashi, A. Kikuchi, K. Sakai, T. Okano, *Analytical Chemistry* **2001**, *73*, 2027.
[25] Y.-X. Song, J.-Q. Wang, Z.-X. Su, D.-Y. Chen, *Chromatographia* **2001**, *54*, 208.
[26] J. Kobayashi, A. Kikuchi, K. Sakai, T. Okano, *Analytical Chemistry* **2003**, *75*, 3244.
[27] M. Baert, S. Martens, G. Desmet, A. de Villiers, F. Du Prez, F. Lynen, *Analytical Chemistry* **2018**, *90*, 4961.

[28] F. Roohi, Y. Fatoglu, M.-M. Titirici, *Analytical Methods* **2009**, *1*, 52.
[29] Z.-J. Liu, Y.-L. Liang, F.-F. Geng, F. Lv, R.-J. Dai, Y.-K. Zhang, Y.-L. Deng, *Frontiers of Materials Science* **2012**, *6*, 60.
[30] B. Miserez, F. Lynen, A. Wright, M. Euerby, P. Sandra, *Chromatographia* **2010**, *71*, 1.
[31] Y. Xia, X. Yin, N. A. D. Burke, H. D. H. Stöver, *Macromolecules* **2005**, *38*, 5937.
[32] M. Heskins, J. E. Guillet, *Journal of Macromolecular Science: Part A - Chemistry* **1968**, *2*, 1441.
[33] F. Meeussen, E. Nies, H. Berghmans, S. Verbrugghe, E. Goethals, F. Du Prez, *Polymer* **2000**, *41*, 8597.
[34] H. Kanazawa, M. Nishikawa, A. Mizutani, C. Sakamoto, Y. Morita-Murase, Y. Nagata, A. Kikuchi, T. Okano, *Journal of Chromatography A* **2008**, *1191*, 157.
[35] T. Nishio, E. Ayano, Y. Suzuki, H. Kanazawa, T. Okano, *Journal of Chromatography A* **2011**, *1218*, 2079.
[36] N. Nakatani, D. Kozaki, M. Mori, K. Tanaka, *Analytical Sciences* **2012**, *28*, 845.
[37] J. Haun, K. Oeste, T. Teutenberg, T. C. Schmidt, *Journal of Chromatography A* **2012**, *1263*, 99.
[38] K. Tseng, G. Vanhoenacker, T. Kotsuka, P. Sandra, *Lc Gc North America* **2008**, 60.
[39] M. Ide, E. De Canck, I. Van Driessche, F. Lynen, P. Van der Voort, *Rsc Advances* **2015**, *5*, 5546.
[40] A. J. Satti, P. Espeel, S. Martens, T. Van Hoeylandt, F. E. Du Prez, F. Lynen, *Journal of Chromatography A* **2015**, *1426*, 126.
[41] D. P. Demarque, A. E. M. Crotti, R. Vessecchi, J. L. C. Lopes, N. P. Lopes, *Natural Product Reports* **2016**, *33*, 432.
[42] N. Tsubokawa, S. Yoshikawa, *Journal of Polymer Science Part A: Polymer Chemistry* **1995**, *33*, 581.
[43] S. W. Pettersson, E. Collet, U. Andersson, *Journal of Chromatography A* **2007**, *1142*, 93.
[44] W. S. Hancock, J. E. Battersby, *Analytical Biochemistry* **1976**, *71*, 260.
[45] A. Bertoluzza, C. Fagnano, M. Antonietta Morelli, V. Gottardi, M. Guglielmi, *Journal of Non-Crystalline Solids* **1982**, *48*, 117.
[46] L. Galet, Y. Ouabbas, A. Chamayou, P. Grosseau, M. Baron, G. Thomas, *KONA Powder and Particle Journal* **2010**, *28*, 209.
[47] D. Wang, J. Nakamura, G. Poologasundarampillai, T. Kasuga, J. R. Jones, D. S. McPhail, *Applied Surface Science* **2014**, *309*, 231.
[48] F. Garbassi, L. Balducci, P. Chiurlo, L. Deiana, *Applied Surface Science* **1995**, *84*, 145.
[49] F. Lynen, J. M. D. Heijl, F. E. D. Prez, R. Brown, R. Szucs, P. Sandra, *Chromatographia* **2007**, *66*, 143.
[50] A. Velayudhan, M. Ladisch, "Plate models in chromatography: Analysis and implications for scale-up", 2006, p. 123.
[51] W. Melander, D. E. Campbell, C. Horváth, *Journal of Chromatography A* **1978**, *158*, 215.
[52] K. G. Feitsma, B. F. H. Drenth, *Pharmaceutisch Weekblad* **1988**, *10*, 1.
[53] E. P. Kroeff, D. J. Pietrzyk, *Analytical Chemistry* **1978**, *50*, 1353.
[54] M. Kamigaito, K. Satoh, *Macromolecules* **2008**, *41*, 269.
[55] Y. Hiruta, R. Kanazashi, E. Ayano, T. Okano, H. Kanazawa, *Analyst* **2016**, *141*, 910.
[56] R. Vespalec, P. Boček, *Chemical Reviews* **2000**, *100*, 3715.
[57] S. Vandewalle, S. Wallyn, S. Chattopadhyay, C. R. Becer, F. Du Prez, *Eur. Polym. J.* **2015**, *69*, 490.
[58] M. Jaćkowska, S. Bocian, B. Buszewski, *Analyst* **2012**, *137*, 4610.
[59] S. Bocian, S. Studzińska, B. Buszewski, *Talanta* **2014**, *127*, 133.
[60] H. Li, X. Zhang, L. Zhang, H. Cang, F. Kong, D. Fan, W. Wang, *Analytical Sciences* **2018**, *34*, 433.
[61] G. S. Scatena, A. F. de la Torre, Q. B. Cass, D. G. Rivera, M. W. Paixão, *ChemCatChem* **2014**, *6*, 3208.
[62] B. Yang, Y. Zhao, C. Fu, C. Zhu, Y. Zhang, S. Wang, Y. Wei, L. Tao, *Polym. Chem.* **2014**, *5*, 2704.
[63] J. M. Haigh, A. Hussain, M. L. Mimmack, C. R. Lowe, *Journal of Chromatography B* **2009**, *877*, 1440.
[64] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815.

[65] J. F. W. Keana, A. P. Guzikowski, D. D. Ward, C. Morat, F. L. Van Nice, *J. Org. Chem.* **1983**, *48*, 2654.
[66] S. Vandewalle, R. De Coen, B. G. De Geest, F. E. Du Prez, *ACS Macro Lett.* **2017**, *6*, 1368.
[67] O. Roling, K. De Bruycker, B. Vonhören, L. Stricker, M. Körsgen, H. F. Arlinghaus, B. J. Ravoo, F. E. Du Prez, *Angew. Chem., Int. Ed.* **2015**, *54*, 13126.
[68] H. A. Houck, K. De Bruycker, S. Billiet, B. Dhanis, H. Goossens, S. Catak, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Chem. Sci.* **2017**, *8*, 3098.
[69] C. J. Ferguson, R. J. Hughes, D. Nguyen, B. T. T. Pham, R. G. Gilbert, A. K. Serelis, C. H. Such, B. S. Hawkett, *Macromolecules* **2005**, *38*, 2191.



Abstract

This chapter highlights the amine-thiolactone-ene conjugation with a thiolactone unit immobilised on a solid support and as a polymer end group. Firstly, two different thiolactone linkers were synthesised and immobilised on a solid support. Afterwards the ideal reaction conditions for the double modifications of the immobilised thiolactone were determined and different functionalities were installed. The thiolactone end group of an oligopeptide was also successfully double-modified on the solid support. Secondly, a thiolactone group was installed as a polymer end group on four different polymers with two different methods. For the different end-capped polymers, the ideal reaction conditions for the double modifications were examined. The double modification of the thiolactone end group allowed the incorporation of different functionalities and the synthesis of block copolymers with a specific midchain functionality.

Parts of this chapter were published as:
F. Driessen, S. Martens, B. D. Meyer, F. E. Du Prez, P. Espeel, *Macromol. Rapid Commun.* **2016**, *37*, 947.

Specific author contributions can be found at the end of section IV.1

Chapter IV
Amine-thiol-ene conjugation with immobilised and end-standing thiolactones

IV.1 Introduction

The previous chapter focussed on the amine-thiol-ene reaction in combination with thiolactone chemistry[1, 2] for the efficient double modification of silica with an immobilised amine.[3] This combination will be further explored in this chapter in light of the double modification of different polymeric systems.

The synthesis of polymers with distinct functionalities and properties has been thoroughly explored in the last decades to fulfil the needs of an ever expanding scope of application fields.[4] Although many chemical procedures can be applied to introduce functionality on polymers[4-7], only thiolactone[8-10] and some other types of chemistry have been proven to install more functionalities at the same position: the Biginelli, the Kabachnik-Fields, the Passerini and the Ugi multicomponent reactions[11-16]; sequential nucleophilic epoxide opening and alcohol modification reactions[17-19]; the Cu-catalysed three-component reaction between terminal alkyne, sulfonyl azide and secondary amine[20]; the modification with primary amines and hydrazides or hydroxylamines of a 1,1-disubstituted-2-vinylcyclopropane polymer bearing a pentafluorophenyl ester and a ketone[21]; the homodifunctionalisation of benzotrifuranone containing polymers with amines[22]; and the reaction between 2,4,6-trichloro-1,3,5-triazine and thiols or amines.[23] For some of these double modifications, an intermediate purification is needed after the first modification is performed, and longer reaction times and elevated temperatures are required for the second modification.[17, 18, 21, 23] These (tedious) extra steps and reaction conditions make these strategies less attractive for use in the double functionalisation of polymers.

The specific modification and functionalisation of polymer end groups can be elegantly and effectively employed for surface modifications, and for the preparation of complex structures and well-defined oligomers that can be utilised for coatings and segments of polyesters, polyamides, and polyurethanes.[24-27] Different methods for end group modification have been developed over the years: use of functional initiators[25], functionalisation by suitable termination in cationic and anionic polymerisations[25, 28], halogen modification after ATRP[24], transformation of a thiocarbonylthio group after RAFT polymerisation[29, 30], *etc.*[7, 22, 27, 31] Although single modifications have been well investigated, the methodologies to install two or more functional groups at the end of polymers are still very limited. Sumerlin's research group developed a method based on 2,4,6-trichloro-1,3,5-triazine, which was installed on the end of a PEO chain.[32] First, the polymer is modified with an amine or a thiol. After an intermediate

purification, another functionality can be introduced by an amine or a thiol at elevated temperatures. Although this method allows for the introduction of two functionalities in the same position, installing the triazine functionality on the starting polymer requires an intermediate purification, elevated temperatures, different solvents and nucleophilic substitution. More efficient strategies are the Passerini and Ugi multicomponent reactions performed by the groups of Li[13], Meier[14] and Tao[15, 16]. For the first reaction, an acid-functionalised PEO is reacted with an aldehyde and an isocyanide for the double modification of the end group in one-pot at room temperature.[13, 14] Tao *et al.* combined a PEO amine with an aldehyde, an acid and an isocyanide in one-pot to install different functionalities at the end of a polymer via the Ugi multicomponent reaction.[15] Afterwards, this reaction allowed the synthesis of a block copolymer with two midchain functionalities starting with PEO amine and PMMA aldehyde.[16] Recently, Destarac's research group used thiolactones for the double modifications of polymer end groups via two different strategies. The first strategy focussed on the modification of a PEO amine with various in-house made functionalised thiolactones and an acrylate.[9] For the second strategy a variety of xanthates, bromides and an alkoxyamine were prepared for the synthesis of polymers containing a thiolactone end group, which was modified with an amine and S-naphthalene ethanethiosulfonate.[10] In this chapter, the preparation of polymers with thiolactone end groups and their double modification will be described, but our approach, published before the publication of Destarac *et al.*, is different in two ways. Firstly, the thiolactone end group will be based on the commercially available homocysteine thiolactone, whereas Destarac and coworkers synthesised their own functionalised thiolactone rings. Secondly, the amine-thiolactone-ene conjugation, which was ineffective for Destarac's second strategy[10], will be applied for the double modification of the thiolactone end groups.

This chapter tackles the one-pot double modification with amines and acrylates of thiolactones immobilised on a solid support or as the end group of a polymer (Figure IV.1).

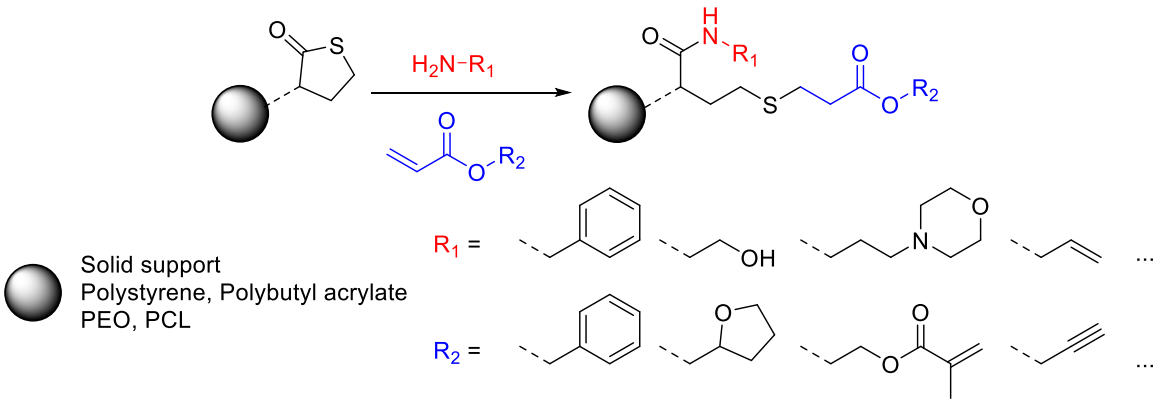


Figure IV.1: One-pot double modification with amines and acrylates of a solid support or polymers containing a thiolactone (end) group.

We started with the reactions on solid support, because of the easier handling of this support (washing and cleavage vs precipitation and drying). This allowed us to have a general idea of the double modification of thiolactone on a polymeric system. Two different linkers were prepared to connect the thiolactone to the solid support. Before different functionalities could

be introduced with this double modification, the ideal conditions had to be determined. The end group of an oligopeptide on the solid support could also be modified with this technique.

To install the thiolactone functionality as an end group of a polymer, two different pathways were explored. For this polymer-functionalised thiolactone, as well, the ideal conditions were determined for the double modification, and several combinations of amines and acrylates were used to introduce a wide set of functionalities.

Finally, two polymers, one with an amine and another with a thiolactone end group, were connected to one another to form a block copolymer with a midchain functionality.[16, 33]

Parts of the research described were a collaboration with Dr. Frank Driessen and Dr. Pieter Espeel at Ghent University. The synthesis of small thiolactone-containing molecules was performed by Pieter Espeel and myself. The latter also carried out the reactions on the solid support. The controlled radical polymerisations and the analysis of the different polymers were performed by Frank Driessen. The modification of the polymer end groups was conducted by both Frank Driessen and myself. The results obtained were discussed, analysed and commented upon by the collaborators at all stages.

IV.2 Double modification of thiolactone-containing beads

IV.2.1 Synthesis of thiolactone-containing linkers

Immobilizing the thiolactone on the solid support, a 2-chlorotrityl chloride polystyrene resin (1.6 mmol/g) (see section IV.2.2), requires functional handles that can react with this support, which was chosen because it can be easily loaded with different linkers and the needed cleavage conditions, 1% trifluoroacetic acid (TFA) in dichloromethane, are mild. Two different thiolactone linkers were prepared to achieve this immobilisation. The first was an acid-functionalised thiolactone linker, which was prepared from the reaction of the commercial DL-homocysteine thiolactone hydrochloride with glutaric anhydride (Figure IV.2). The product with a yield of 76% was purified by column chromatography and characterised with NMR and LC-MS analysis (Figure IV.3).

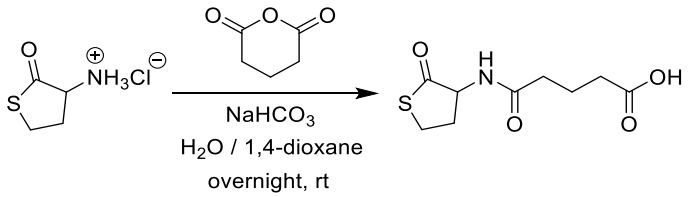


Figure IV.2: Synthesis of the acid-functionalised thiolactone linker.

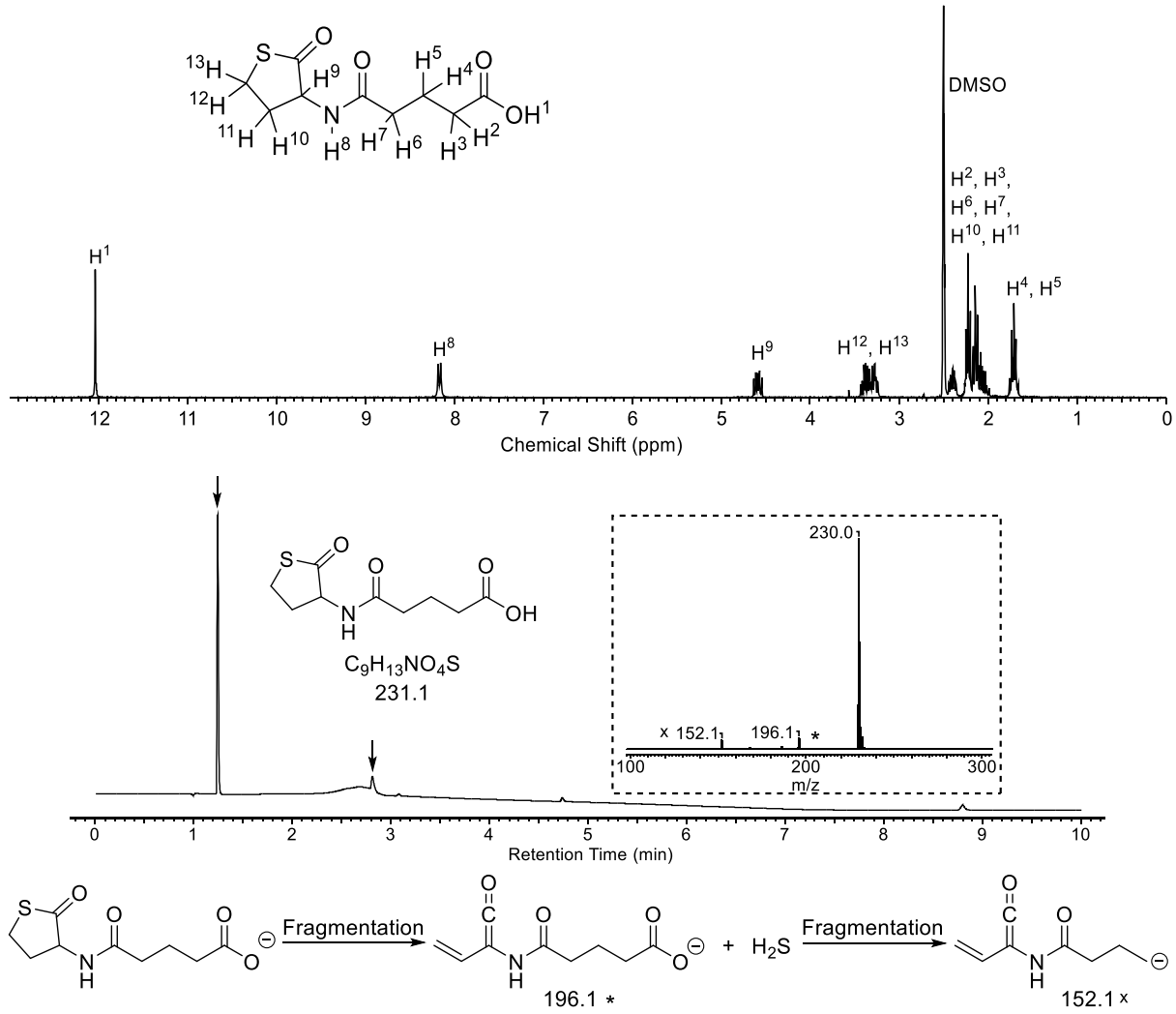


Figure IV.3: ^{1}H-NMR spectrum (300 MHz, DMSO-d_6) with peak assignment (top) and HPLC trace with ESI-MS analysis (negative mode) (bottom) of the acid-functionalised thiolactone linker (fragmentation caused by opening-chain fragmentation of the thioester followed by elimination of H_2S (*) and subsequent a-elimination of CO_2 (x)).[34, 35]

The second linker contains a hydroxyl function to connect to the solid support. For the synthesis of this hydroxyl-functionalised thiolactone linker, N-(2-bromoacetyl)homocysteine-γ-thiolactone had to be prepared first (Figure IV.4). DL-homocysteine thiolactone hydrochloride was reacted with bromoacetyl bromide and the product obtained was purified via extraction with a yield of 84%. The purity of this intermediate was analysed with NMR and LC-MS before the synthesis was continued (Figure IV.5). Next, the hydroxyl-functionalised thiolactone linker was obtained by treating the N-(2-bromoacetyl)homocysteine-γ-thiolactone with 2-mercaptoethanol. The linker was isolated by means of column chromatography and analysed with NMR and LC-MS (Figure IV.6), with a yield of 83%.

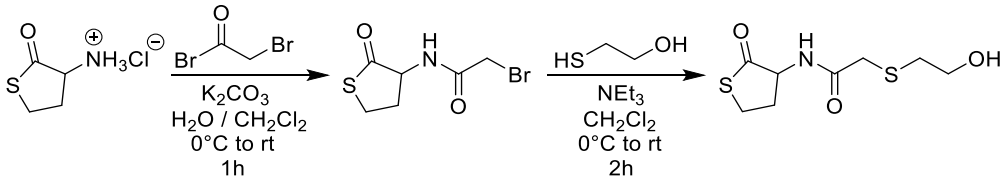


Figure IV.4: Synthesis of the hydroxyl-functionalised thiolactone linker via N-(2-bromoacetyl)homocysteine-γ-thiolactone.

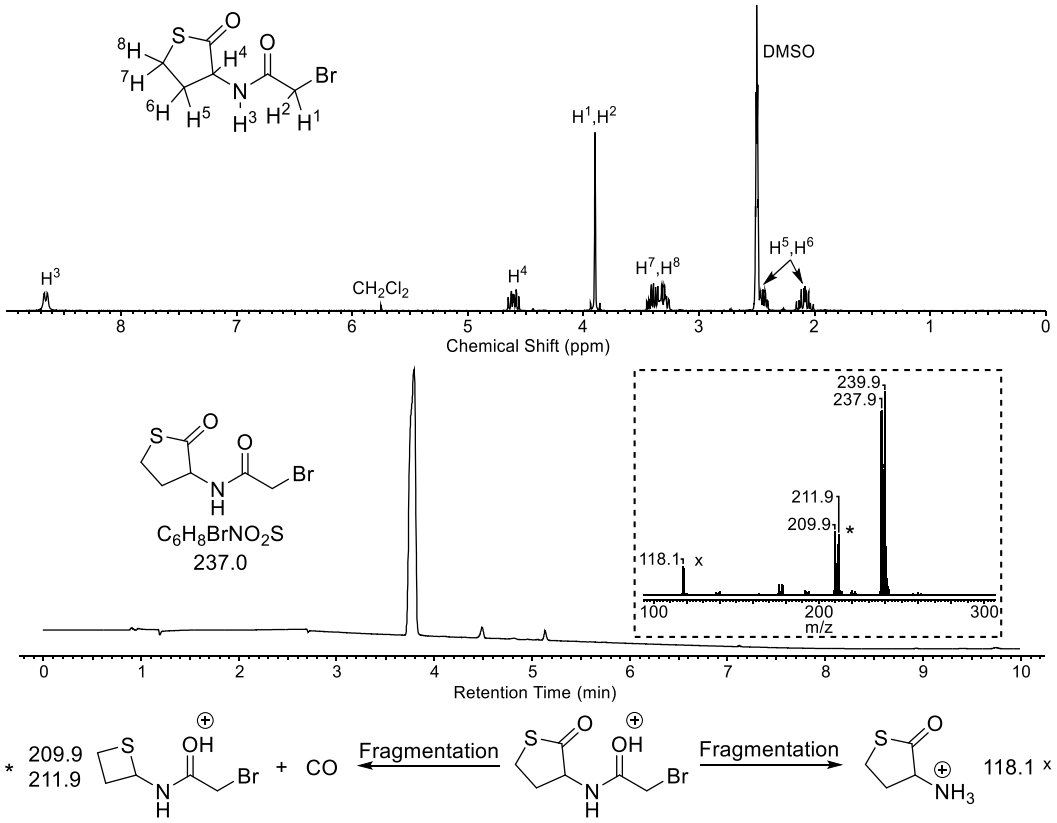


Figure IV.5: ^{1}H-NMR spectrum (300 MHz, DMSO-d_6) with peak assignment (top) and HPLC trace with ESI-MS analysis (positive mode) (bottom) of N-(2-bromoacetyl)homocysteine-γ-thiolactone (fragmentations caused by carbon monoxide elimination from the thiolactone ring (*) and amide cleavage (x)).[35]

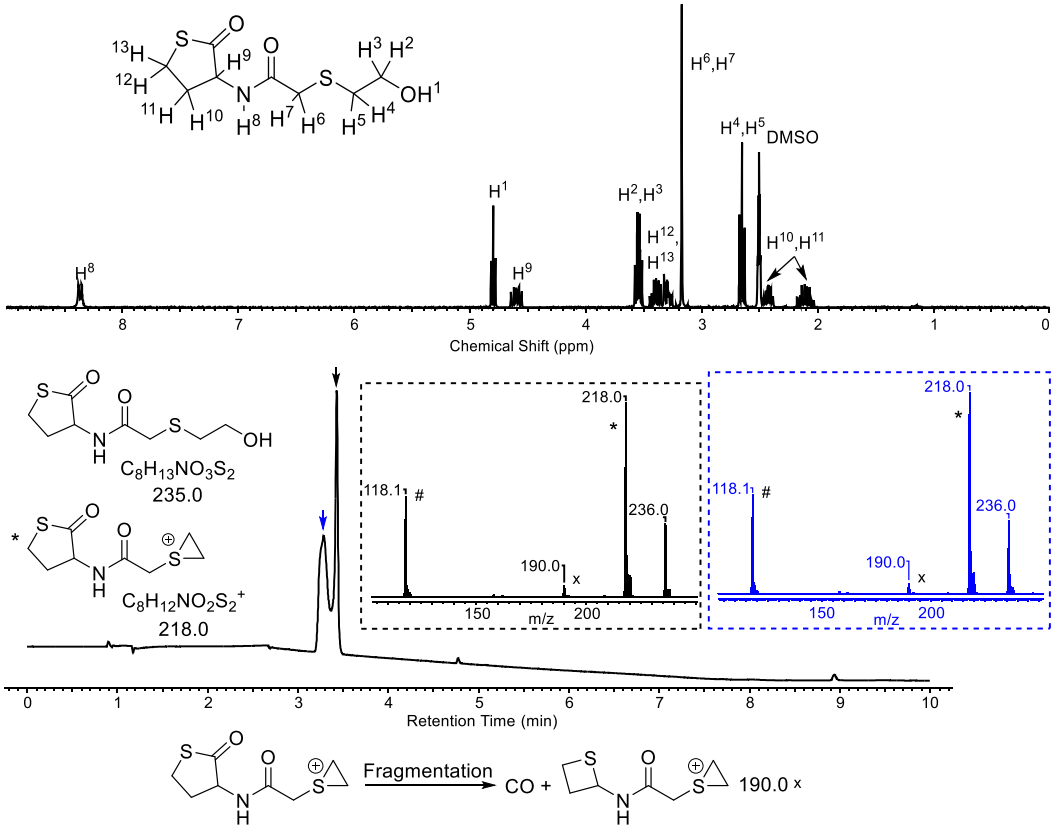


Figure IV.6: ^{1}H-NMR spectrum (300 MHz, DMSO-d_6) with peak assignment (top) and HPLC trace with ESI-MS analysis (positive mode) (bottom) of the alcohol-functionalised thiolactone linker (fragmentations caused by water elimination (*), carbon monoxide elimination from the thiolactone ring (x) and amide cleavage as in Figure IV.5(#)).[35]

IV.2.2 Manipulations on solid support

Both linkers were immobilised on a 2-chlorotrityl chloride polystyrene resin (1.6 mmol/g) (Figure IV.7).[36] The loading of the thiolactone linkers on the resin was determined by calibration curves, which were prepared through UV spectrometry at 254 nm (Figure IV.8). The loading was 1.00 and 0.85 mmol thiolactone units per gram of resin for respectively the acid and hydroxyl linker. This corresponds with 63% and 53% loading efficiency respectively. A CH_2Cl_2/MeOH/DIPEA mixture was applied to cap the remaining reactive chlorotrityl moieties (37% and 47% respectively) on the solid support.

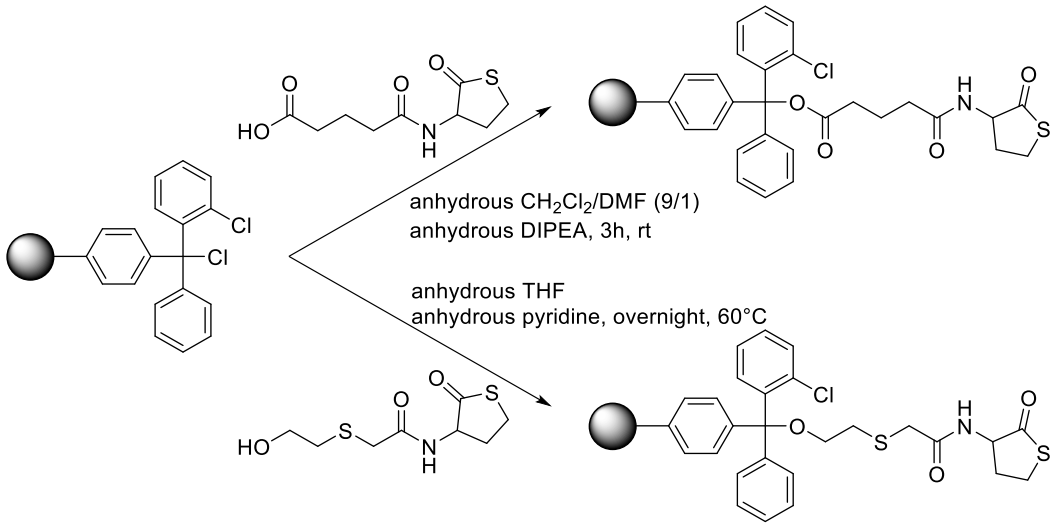


Figure IV.7: Loading of the 2-chlorotrityl chloride polystyrene resin with the thiolactone-functionalised linkers.

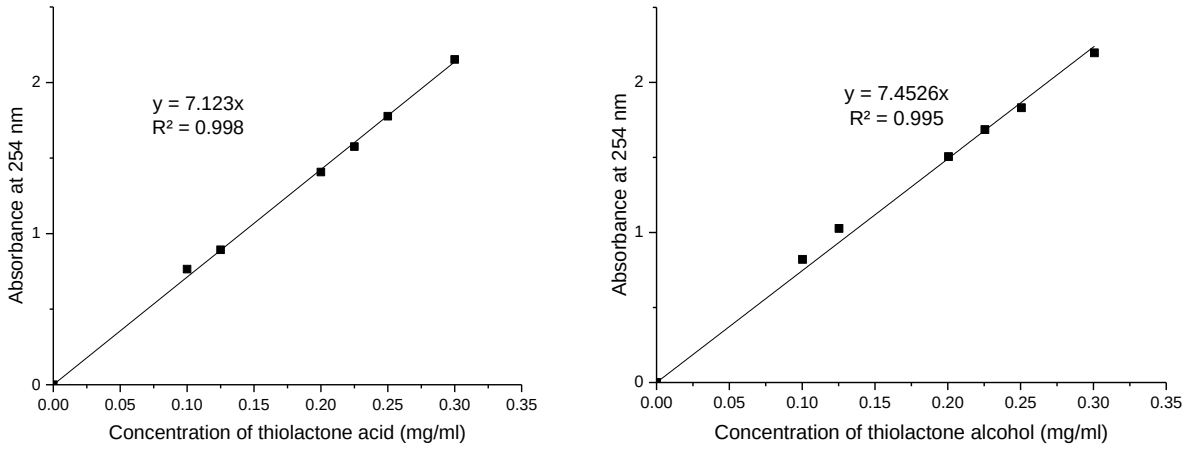


Figure IV.8: UV Calibration of the acid- (left) and alcohol-thiolactone linker (right).

After both thiolactone linkers were loaded on the resin, the optimal conditions for the aminolysis of the thiolactone and subsequent thiol-ene reaction had to be investigated.

The ratio of amine and acrylate and the equivalents of both compared to the thiolactone had to be determined. The acrylate had to be added first to ensure that, as the amine is added to the thiolactone, the formed thiol is captured and disulphide formation is avoided. This was achieved by means of benzylamine and methyl acrylate.

Next, the choice of solvent is crucial as it must promote the reaction between the three reaction partners as well as ensure the swelling of the resin. DMF, NMP and CH_2Cl_2, which are commonly applied in SPS, would cause difficulties in the present double modification: DMF and NMP promote the aza-Michael reaction between an acrylate and an amine.[37] In addition, our group has discovered earlier that, after aminolysis of the thiolactone, the formed thiol can react with CH_2Cl_2.[38] Although THF can also be used for solid-phase and peptide synthesis[39], our group discovered that peroxides present in this solvent can oxidise the thioethers formed after the performed thiol-ene reaction.[40] The solvent finally chosen was $CHCl_3$, because the occurrence of aza-Michael addition was found to be less problematic and the swelling of the resin was good. $CHCl_3$ is toxic and carcinogenic, and can form either dichlorocarbene in the presence of a base[41] or phosgene and HCl in the presence of oxygen.[42] The formation of these side products is the reason why $CHCl_3$ is preferably not used in peptide chemistry. However, no problems were observed during the reactions on the solid support or in solution when this solvent was used because the use of an excess of amine helped to capture the phosgene and HCl present in $CHCl_3$.

Two different ratios of amine/acrylate were tested overnight in chloroform: 1/1 and 1/2. For the first ratio, 5 equivalents were added compared to the immobilised thiolactone for both the amine and the acrylate. For the second ratio, two different amounts of equivalents of amine and acrylate were added: 5 and 10 equivalents, and 10 and 20 equivalents respectively. For the first ratio, the presence of the unreacted thiolactone could still be observed in the LC trace (Figure IV.9). The same applied when 5 and 10 equivalents for the second ratio were used. For the last reaction, 10 and 20 equivalents, the thiolactone was fully opened and the thiol reacted with the methyl acrylate. In the ^{1}H-NMR spectrum, the incorporation of both the aromatic and methyl functionalities (7.27 and 3.60 ppm) can be found and the signals of the closed thiolactone ring (4.58, 3.31, 2.40 and 2.10 ppm) have disappeared (Figure IV.10).

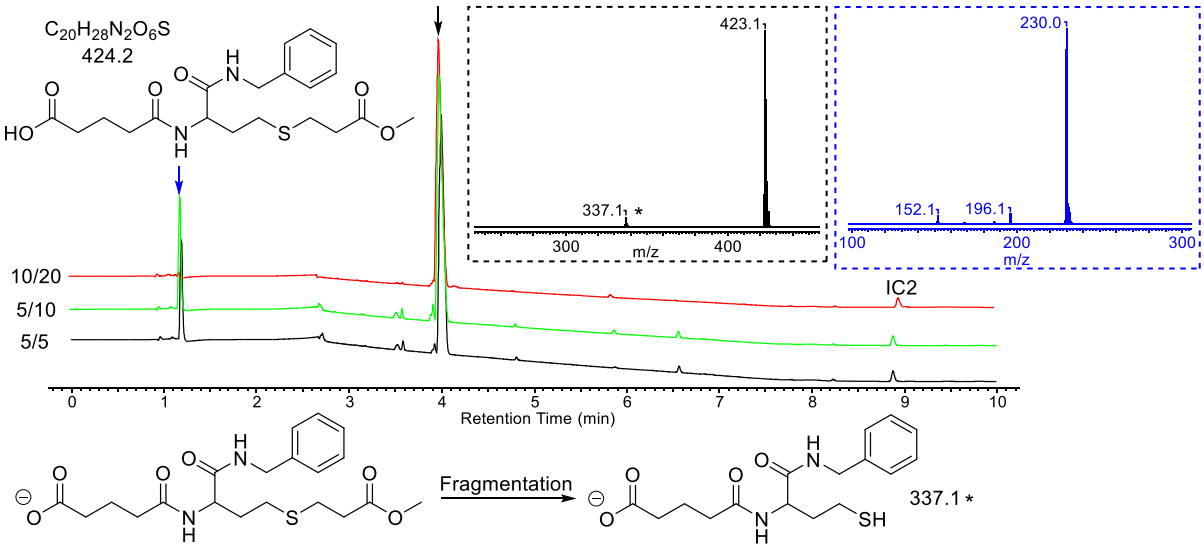


Figure IV.9: LC-ESI-MS analysis screening of different ratios and equivalents of amine and acrylate for the amine-thiolactone-ene conjugation. Inserts: ESI-MS-spectrum of dominant species (black) and starting compound (blue) (negative mode with fragmentations as in Figure IV.3 (blue MS spectrum) and methyl acrylate elimination via thioether cleavage(*)).[35] (IC2 = internal contamination (vide infra).)

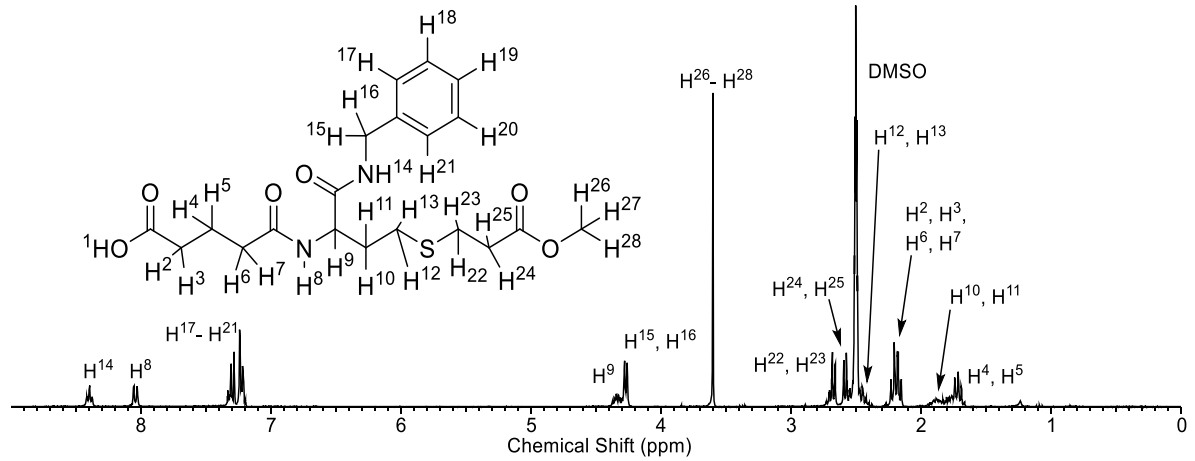


Figure IV.10: ^{1}H-NMR spectrum (300 MHz, DMSO-d_6) with peak assignment of the double modification of the immobilised acid-thiolactone linker with benzylamine and methyl acrylate.

Thus, a ratio of 1 to 2 with 10 equivalents of amine and 20 equivalents of acrylate will be used in the future to open the immobilised thiolactone on the solid support.

This double modification of thiolactone with the same equivalents of amine and acrylate was also performed with the hydroxyl-functionalised thiolactone resin. Both LC-MS and ^{1}H-NMR and analysis confirm that double modification was successful (Figures IV.11 and IV.12). It can be observed that, in addition to the product, a trifluoroacetic ester adduct is formed in small quantities, because the hydroxyl group reacts with the TFA used in the cleavage mixture (1% TFA in CH_2Cl_2).

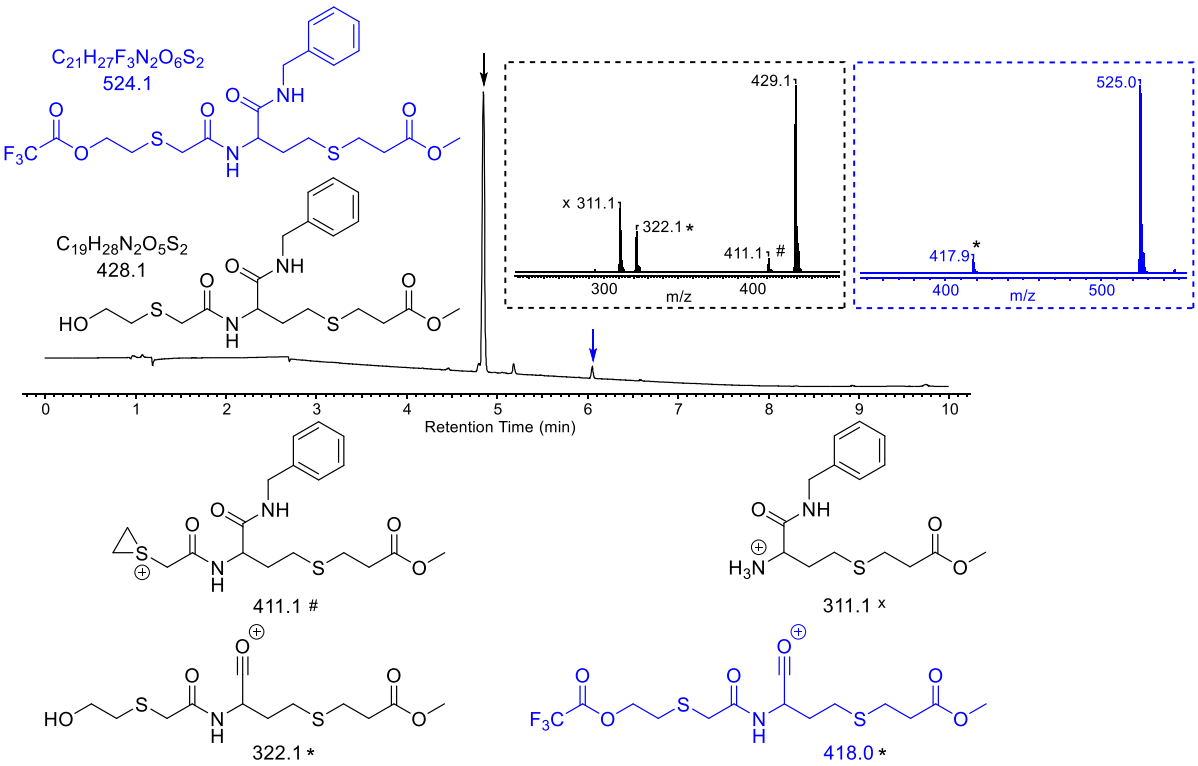


Figure IV.11: HPLC trace with ESI-MS analysis of dominant species (black) and TFA adduct (blue) (positive mode) of the immobilised alcohol-thiolactone linker with benzylamine and methyl acrylate (fragmentations caused by water elimination (#) and amide cleavages (and x)).[35]*

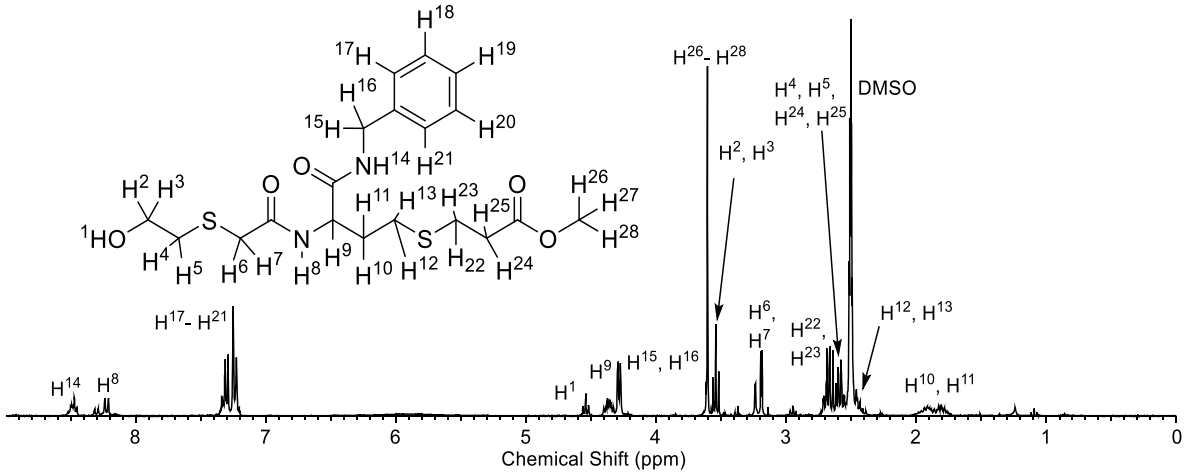


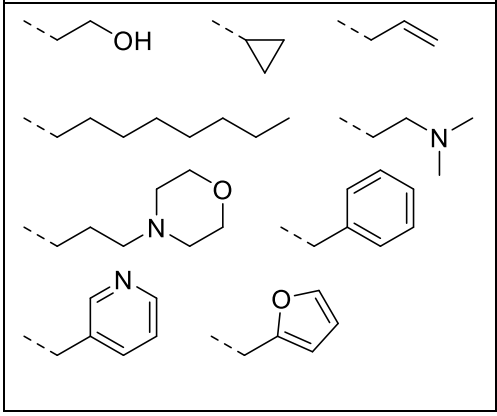
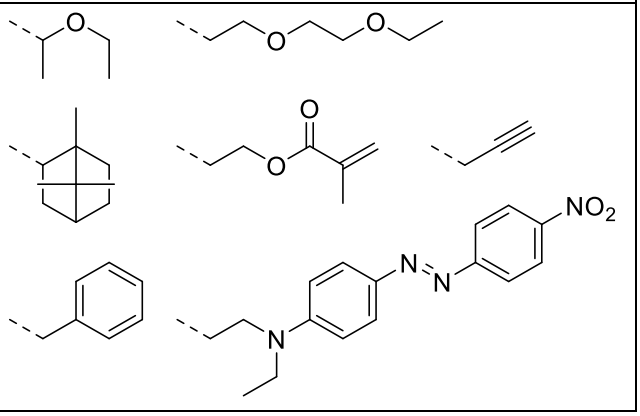
Figure IV.12: ^{1}H-NMR spectrum (300 MHz, DMSO-d_6) with peak assignment of the immobilised alcohol-thiolactone linker with benzylamine and methyl acrylate.

In order to prove the broad applicability of this double modification method, different functionalities were incorporated (aromatic, (cyclic) alkane, pyridine, acid, ether, furan, alkene, alkyne, morpholine, hydroxyl, disperse red 1, tertiary amine and methacrylate) (Table IV.1). The incorporation and full conversion of the thiolactone unit was confirmed through LC-MS analysis (Figure IV.13-21). The acid functionality was incorporated via 1-ethoxyethyl acrylate (Figure IV.15). The acidic cleavage of the product from the resin also removes the ethoxyethyl group, which results in an acid residue. Disperse red 1 acrylate was incorporated because it provides visible evidence that the acrylate has reacted with the formed thiol after aminolysis of the thiolactone. The beads that were treated with this acrylate have a distinctive red colour that is not removed after the classical washing steps of the solid support (Figure IV.21). It can be seen in the LC-ESI-MS analysis that a small amount of disulphide formation has occurred. This formation could be ascribed to the bulky disperse red 1 acrylate which might not be capable of fully capturing the formed thiol. In some LC traces (*e.g.* Figure IV.14), two internal contaminations (IC and IC2) could be observed around 4.7 min and 8.8 min (see Figure IV.47 in section IV.5.1). The first contaminant can be attributed to plasticizers that are released when (LC) solvents come into contact with falcon tubes, Eppendorf tubes, pipet tips, *etc*. The second contaminant is a mix of precipitated material such as salts that redissolve when the solvent gradient of the LC measurement switches from 100% acetonitrile to 100% water. This contamination can be caused by dirty samples that were accidentally injected onto the LC column, but can be removed by washing the column.

Some of the LC spectra obtained feature double signals. However, the mass analysis indicates the same mass spectrum for both. Initially, it was assumed that this doubling was the result of the formation of two different enantiomers, which result from the racemic thiolactone starting compound. As already discussed in III.3.2, the separation of enantiomeric compounds is normally performed either by using a chiral stationary or mobile phase, or by first forming diastereomers which can be separated with RPLC under certain conditions.[43, 44] As a result, the doubling cannot be caused by the enantiomers. Madder and coworkers suggested earlier

that this doubling could be attributed to cis/trans isomerisation of the amide bond next to the opened thiolactone.[45] Geffe and Rice also observed peak doubling for the LC analysis of amides and proved that this behaviour can indeed be attributed to cis/trans isomerisation.[46, 47] They reduced this doubling by measuring at higher temperature.

Table IV.1: Overview of the functional amines and acrylates used for the double modifications.

Primary amines	Acrylates
	

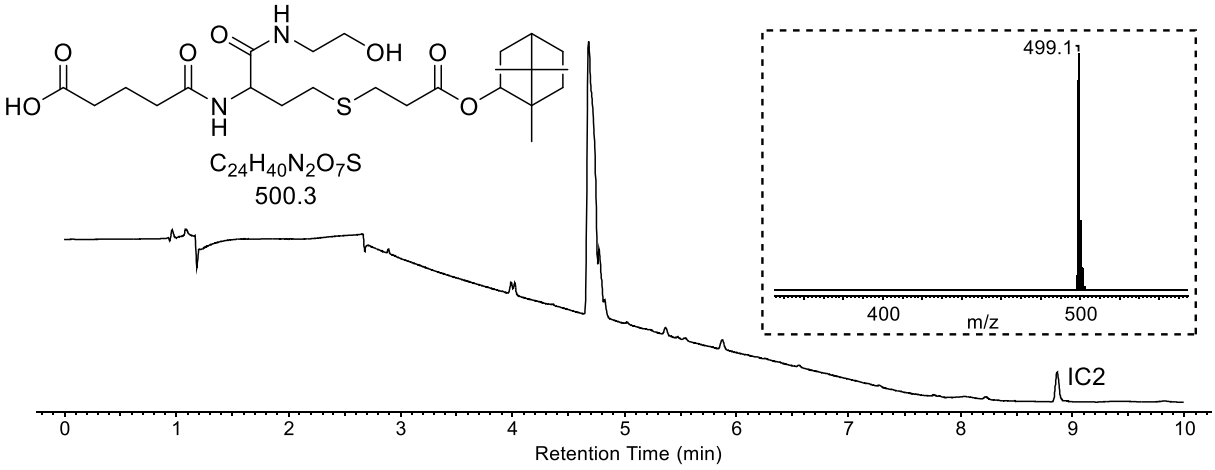


Figure IV.13: LC-ESI-MS analysis of the double modification with ethanolamine and isobornyl acrylate. Insert: ESI-MS-spectrum of dominant species (negative mode).

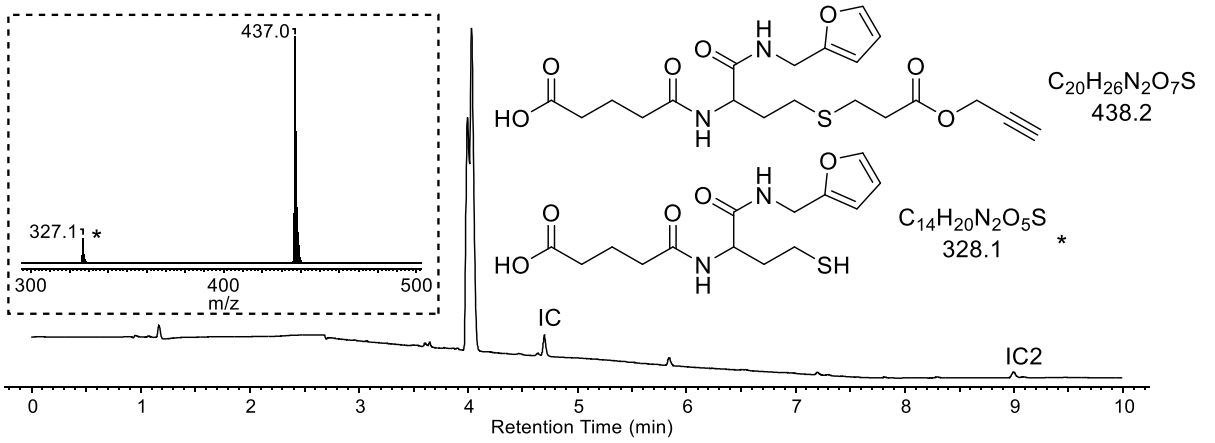


Figure IV.14: LC-ESI-MS analysis of the double modification with furfurylamine and propargyl acrylate. Insert: ESI-MS-spectrum of dominant species (negative mode).

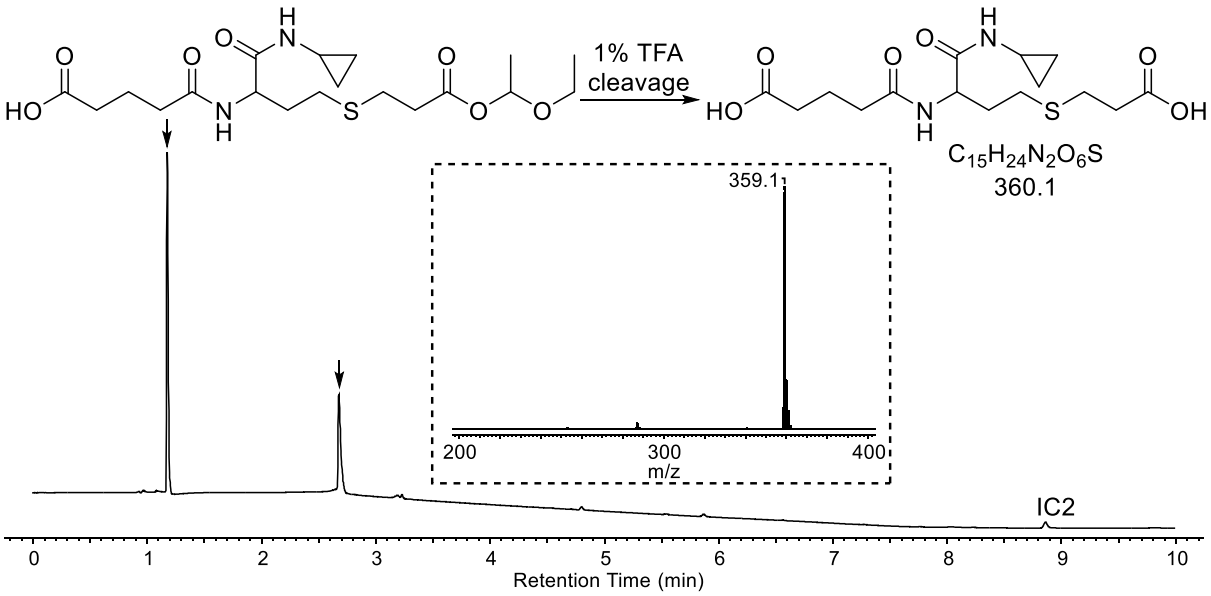


Figure IV.15: LC-ESI-MS analysis of the double modification with cyclopropylamine and 1-ethoxyethyl acrylate. Acidic cleavage removes the 1-ethoxyethyl functionality resulting in an acid. Insert: ESI-MS-spectrum of dominant species (negative mode).

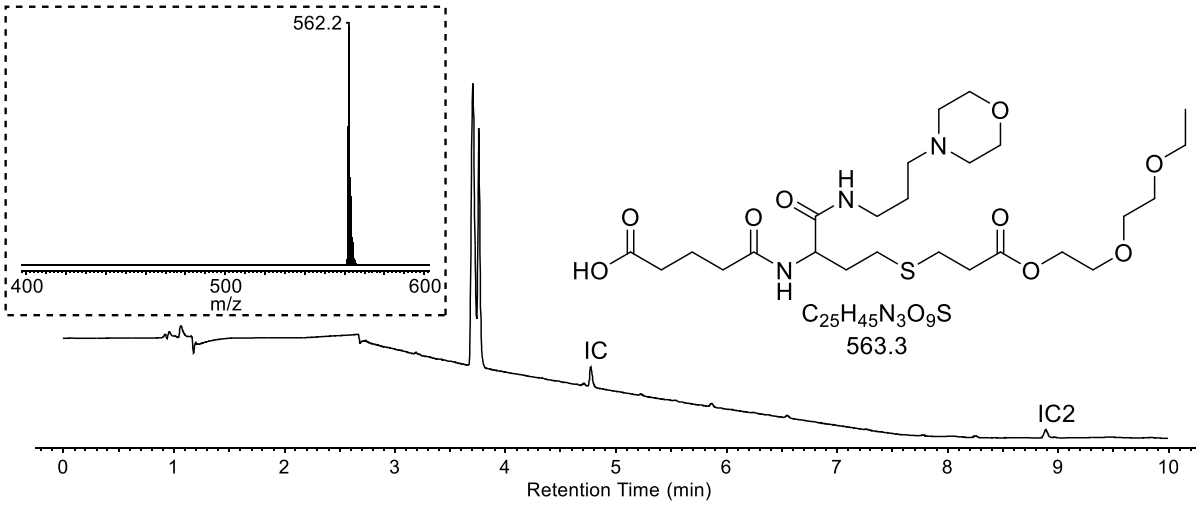


Figure IV.16: LC-ESI-MS analysis of the double modification with 3-morpholinopropylamine and 2-(2-ethoxyethoxy)ethyl acrylate. Insert: ESI-MS-spectrum of dominant species (negative mode).

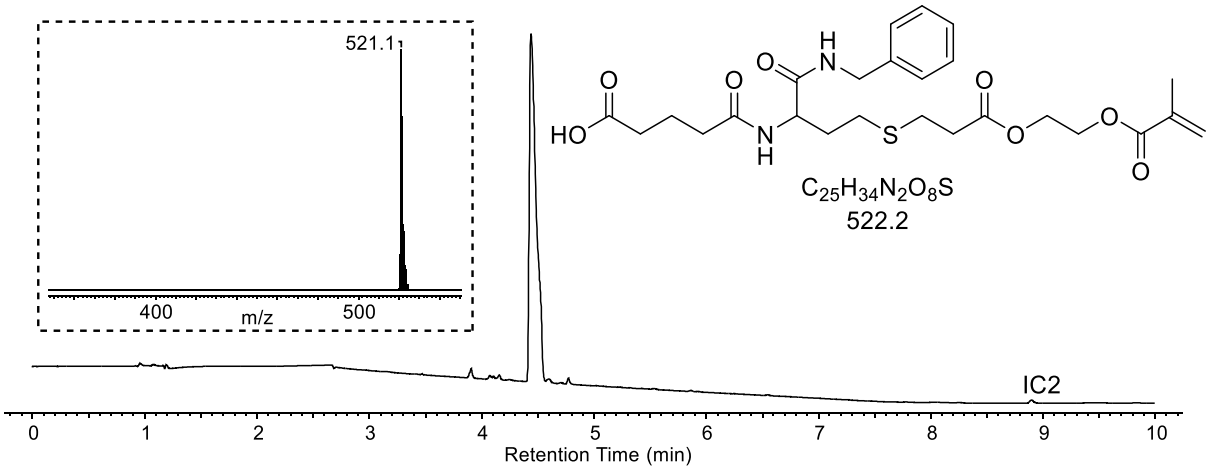


Figure IV.17: LC-ESI-MS analysis of the double modification with benzylamine and 2-(acryloyloxy)ethyl methacrylate. Insert: ESI-MS-spectrum of dominant species (negative mode).

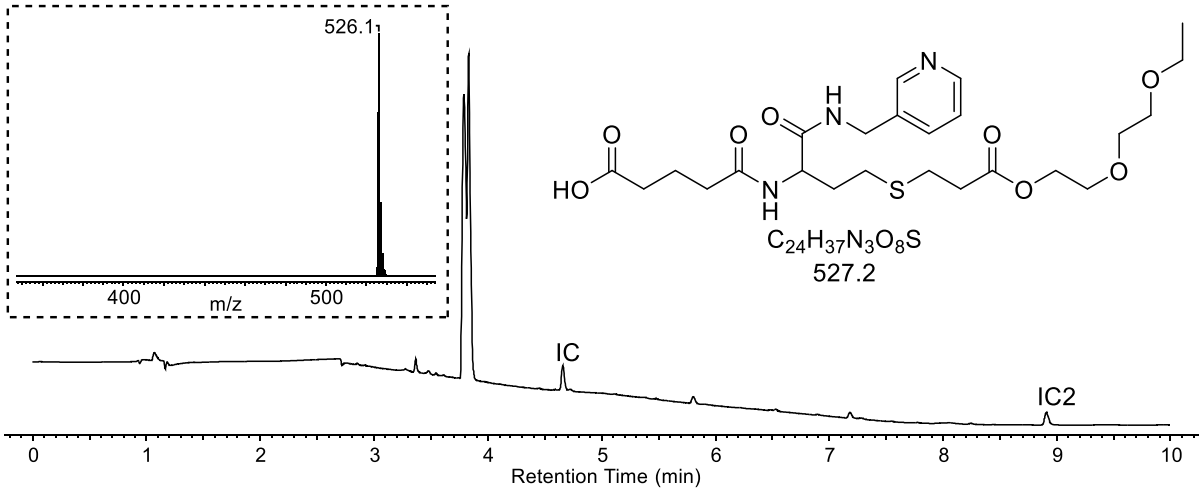


Figure IV.18: LC-ESI-MS analysis of the double modification with 3-picolylamine and 2-(2-ethoxyethoxy)ethyl acrylate. Insert: ESI-MS-spectrum of dominant species (negative mode).

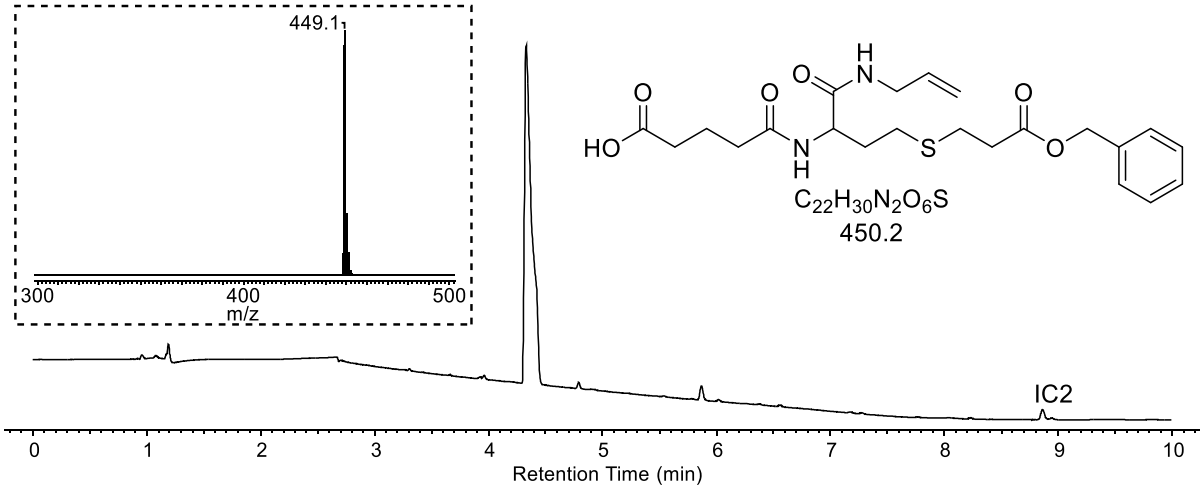


Figure IV.19: LC-ESI-MS analysis of the double modification with allylamine and benzyl acrylate. Insert: ESI-MS-spectrum of dominant species (negative mode).

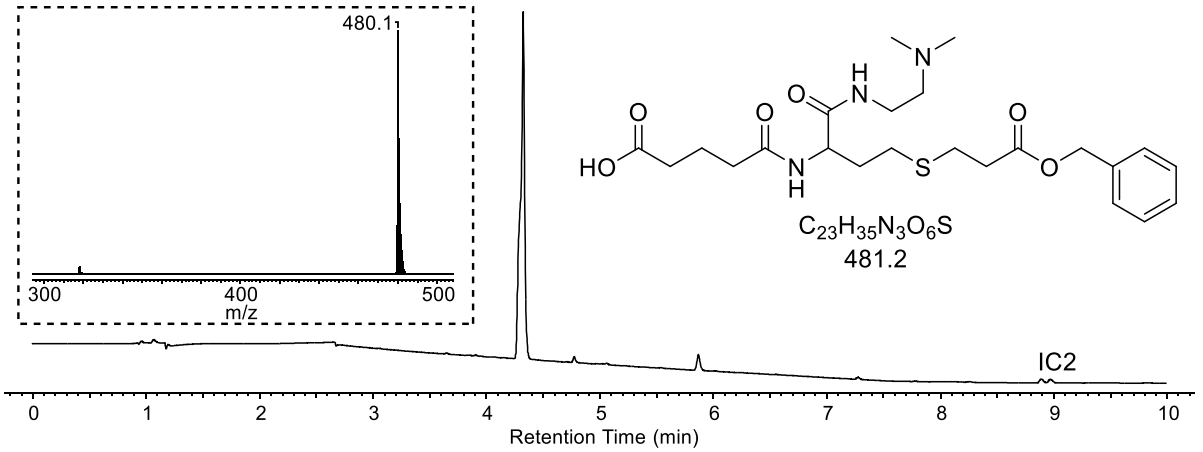


Figure IV.20: LC-ESI-MS analysis of the double modification with N,N-dimethylethylenediamine and benzyl acrylate. Insert: ESI-MS-spectrum of dominant species (negative mode).

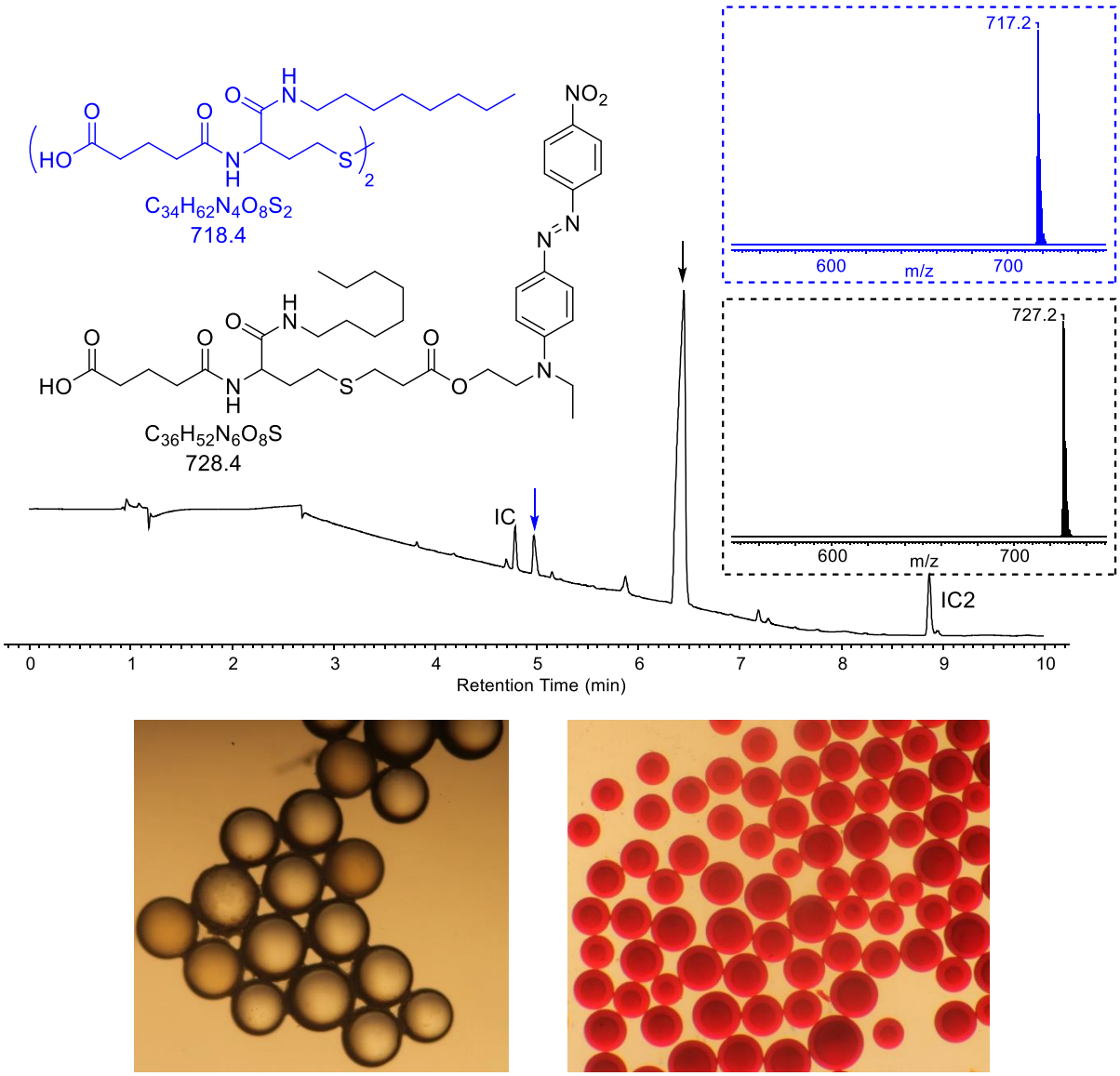


Figure IV.21: LC-ESI-MS analysis of the double modification with octylamine and disperse red 1 acrylate. Insert: ESI-MS-spectrum of dominant species (black) and disulphide (blue) (negative mode) (top) and pictures of unmodified (left) and modified beads (right) (bottom).

A tetrapeptide with a racemic thiolactone end group was made on solid support and modified to further show the strength of this method (Figure IV.22 and IV.23). When the oligopeptide is modified with benzylamine and methyl acrylate, the LC trace shows good double modification. This method could perhaps be used in the future to install certain functionalities (such as fluorescent, dye, or bio-active units) on the end of a peptide chain.

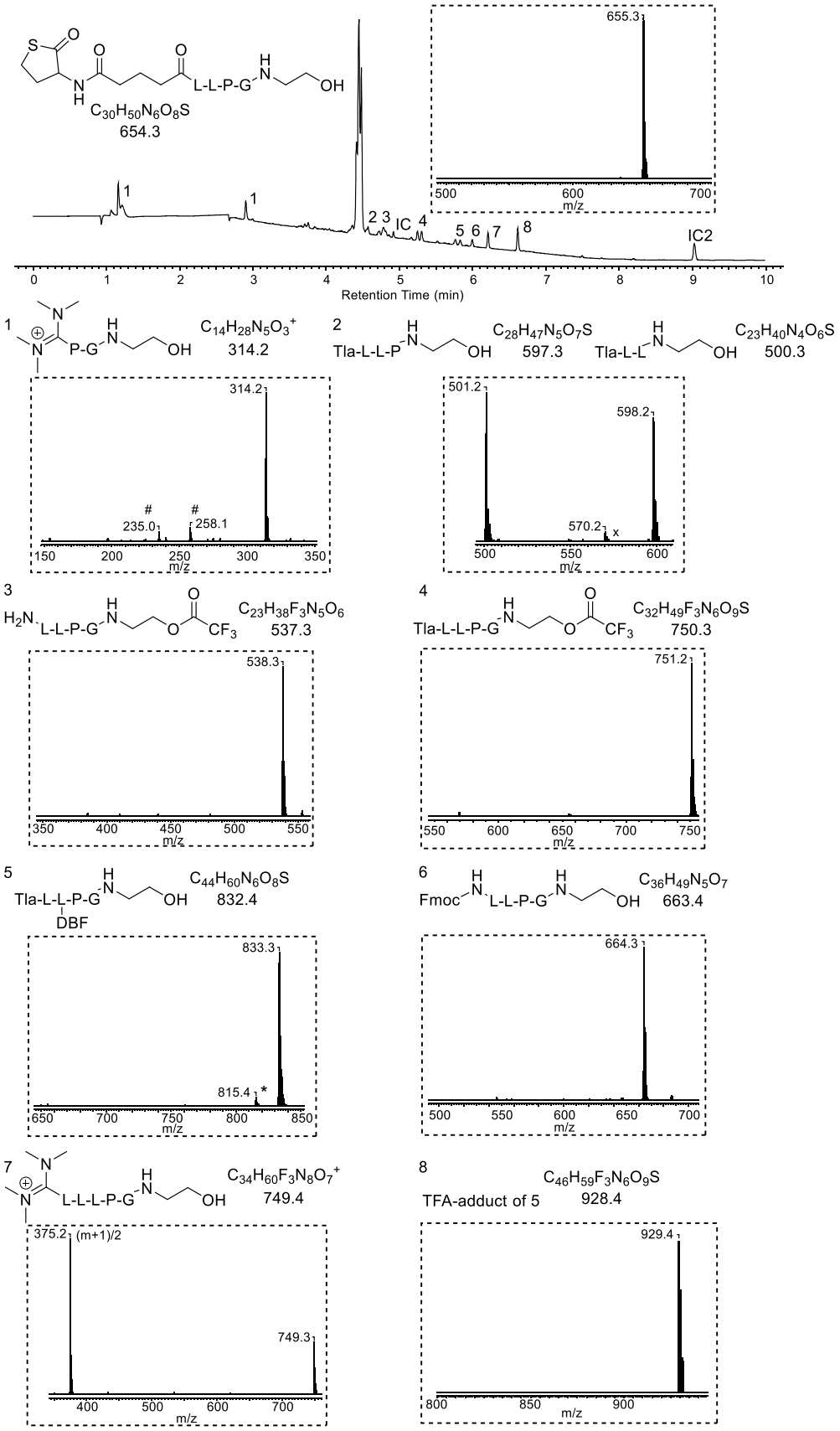


Figure IV.22: LC-ESI-MS analysis of the tetrapeptide (leucine-leucine-proline-glycine) with a thiolactone end group. Insert: ESI-MS-spectrum of dominant species (black) (positive mode).The impurities are caused by guanidinylation (1 and 7), deletions or diketopiperazine formation of GP(2), TFA-adduct (3,4 and 8) or dibenzofulvene (DBF) adduct formation (5), and incomplete reaction (3) and deprotection (6).[48, 49] (fragmentations caused by water elimination (*), carbon monoxide elimination from the thiolactone ring (x) and unknown fragmentation mechanisms (#)). [35]

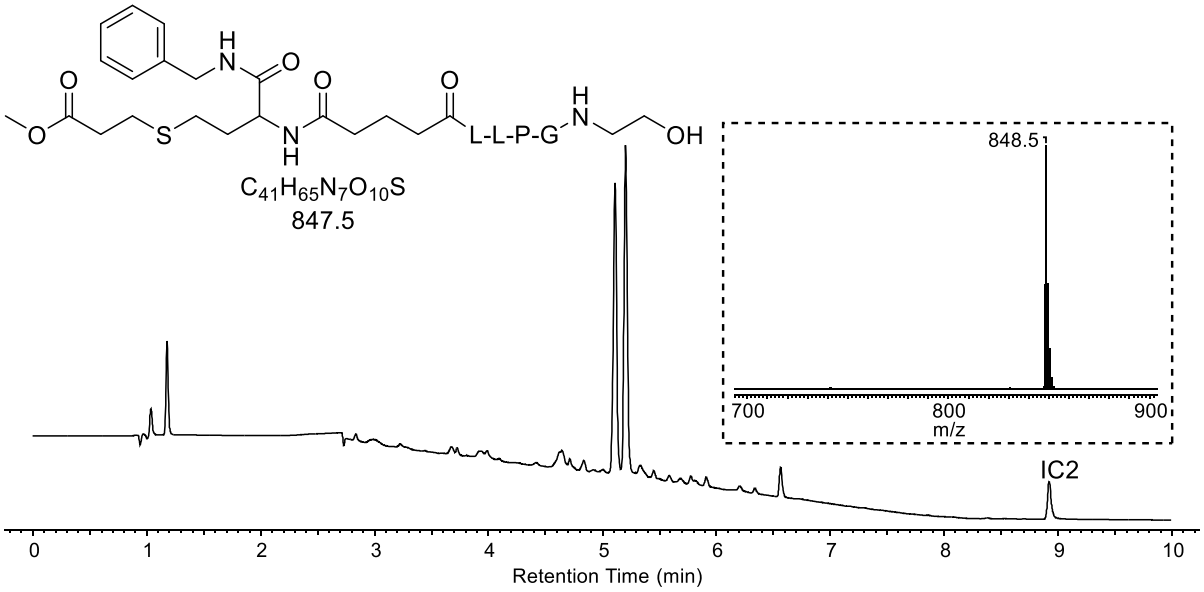


Figure IV.23: LC-ESI-MS analysis of the double modification of the tetrapeptide (leucine-leucine-proline-glycine) with benzylamine and methyl acrylate. Insert: ESI-MS-spectrum of dominant species (black) (positive mode).

IV.3 Double modification of polymers with thiolactone end groups

IV.3.1 Synthesis of thiolactone end-functionalised polymers

Two different methods were used to introduce thiolactone at the end of the polymer chain. The first method was a Cu(0)-mediated controlled radical polymerisation with a thiolactone-functionalised initiator, which was obtained after the reaction of the hydroxyl-functionalised thiolactone with α-bromoisobutyryl bromide (Figure IV.24). The initiator was purified through column chromatography with a yield of 83% and analysed by means of NMR and LC-MS analysis (Figure IV.25).

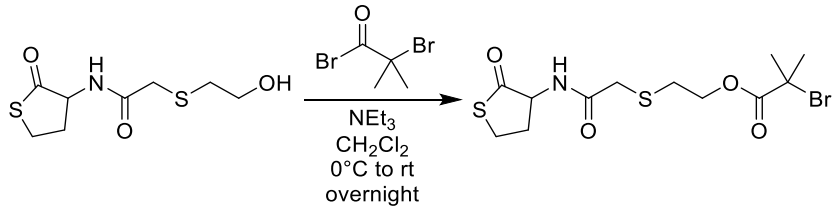


Figure IV.24: Synthesis of the thiolactone-functionalised initiator.

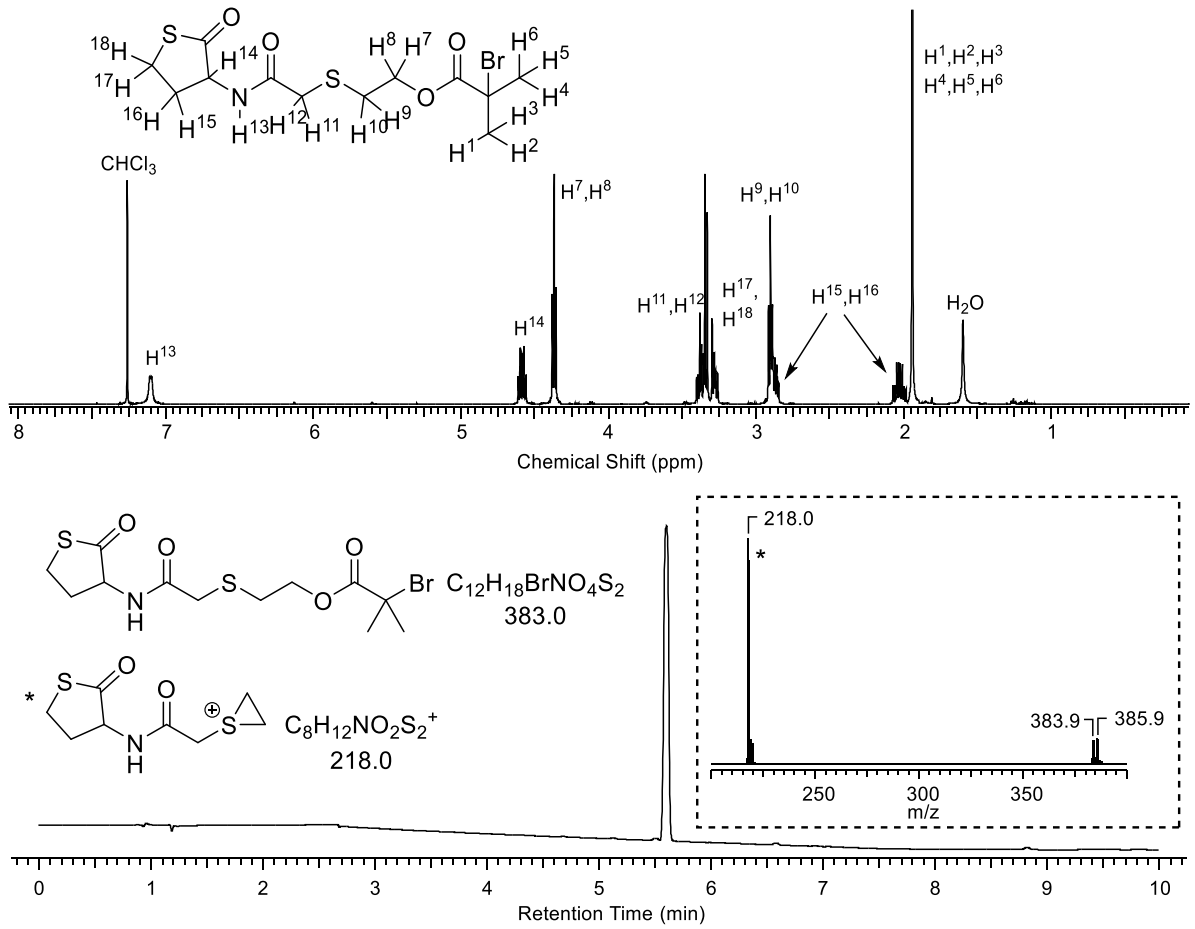


Figure IV.25: ^{1}H-NMR spectrum (300 MHz, $CHCl_3$-d) with peak assignment (top) and HPLC trace with ESI-MS analysis (negative mode) (bottom) of the thiolactone-functionalised initiator.

To illustrate that different monomers can be used for this method, two different polymers were prepared by means of a Cu(0)-mediated polymerisation with the thiolactone-functionalised initiator: polystyrene and poly(butyl acrylate) (Figure IV.26).

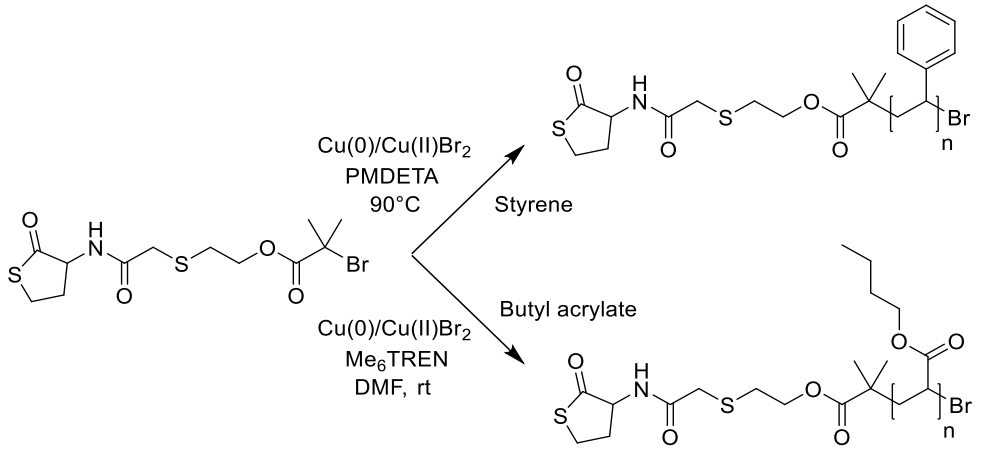


Figure IV.26: Polymerisation of styrene and butyl acrylate with the thiolactone-functionalised initiator.

For both polymerisations, the controlled nature was proven via a kinetic study (Figure IV.27). SEC and Gas Chromatography (GC) samples were periodically taken to keep track of the molecular weight and the conversion respectively. Both polymerisations proceed in a controlled

way: the molecular weight increases linearly with the monomer conversion, while the dispersity stays below 1.2.

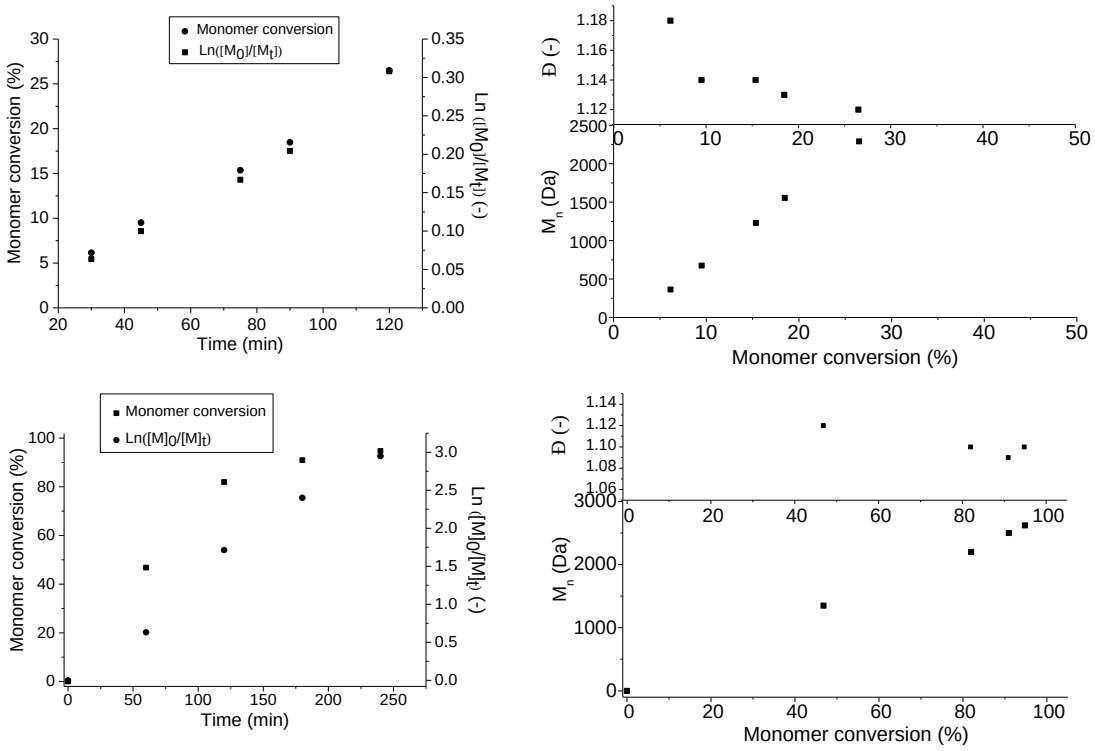


Figure IV.27: Kinetic study for the controlled radical Cu(0)-mediated polymerisations of styrene (top) and butyl acrylate (bottom) with the thiolactone-functionalised initiator: first order kinetic plots (left) and molecular weight and dispersity as a function of conversion plots (right).

^{1}H-NMR and MALDI-TOF analysis were performed to prove the presence of the thiolactone end group for both polymers (Figure IV.28 and IV.29). Signals at 2.8, 3.25 and 4.6 ppm in both NMR spectra confirm the thiolactone functionality (Figure IV.28). Additionally, the experimental molecular weights are in good correlation with the theoretical values. In the MALDI-TOF spectra both repeating units, styrene and butyl acrylate, can be noticed by means of the distinctive mass differences, respectively 104.06 Da and 128.08 Da, between two successive signals (Figure IV.29). For both polystyrene and poly(butyl acrylate) a second series can be observed in the MALDI-TOF spectra. For polystyrene, this second series can be attributed to sodium adducts. Previous research performed by our group equally observed the presence of this sodium series.[50] For poly(butyl acrylate), the second series can be attributed to the loss of HBr. The dehydrohalogenation during MALDI-TOF measurements of polystyrene has been amply reported in the literature[51] and was observed as well in the MALDI-TOF spectrum measured (Figure IV.29). Nonaka *et al.* attributed this behaviour to the instability of the benzylic halogen end group upon laser ionisation.[52] For polyacrylates, Charles and coworkers attributed this behaviour to a degradative termination reaction during the polymerisation process rather than to the MALDI process.[51] In conclusion, the NMR and MALDI-TOF results indicate that the polymers used have a thiolactone end group.

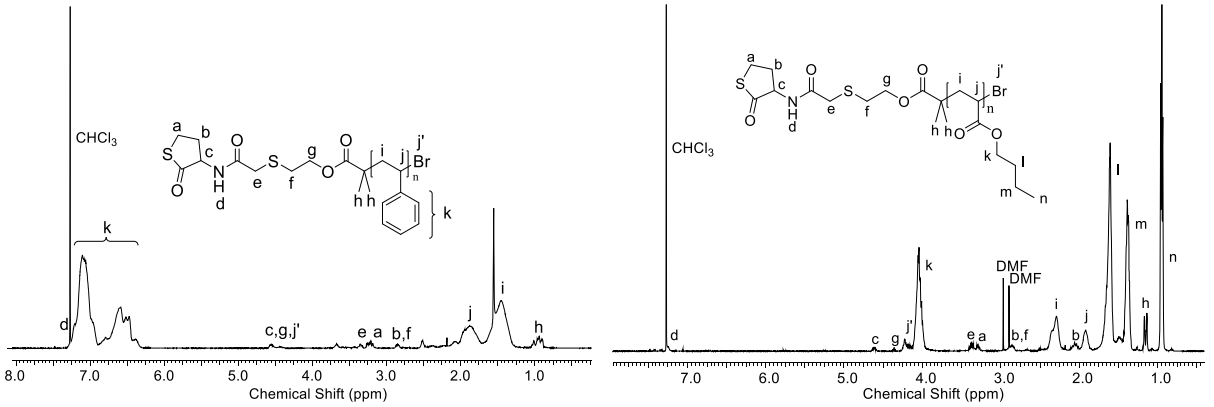


Figure IV.28: ^{1}H-NMR spectrum (300 MHz, $CHCl_3$-d) with peak assignment of polystyrene (left) and poly(butyl acrylate) (right) indicating the presence of the thiolactone end group.

Theoretical m/z (Da) (Ag^+)	Experimental m/z (Da) (Ag^+)	Theoretical m/z (Da) (Na^+)	Experimental m/z (Da) (Na^+)
3531.85	3531.26	3447.79	3447.19
3635.91	3635.31	3551.85	3551.25
3739.97	3739.36	3655.91	3655.30
3844.03	3843.41	3759.97	3759.36
3948.12	3947.48	3864.03	3863.42

Theoretical m/z (Da) (major)	Experimental m/z (Da) (major)	Theoretical m/z (Da) (minor)	Experimental m/z (Da) (minor)
2839.57	2838.69	2759.64	2758.76
2967.65	2966.76	2887.72	2886.84
3095.73	3094.84	3015.80	3014.93
3223.82	3222.93	3143.88	3143.00
3351.90	3351.02	3271.96	3271.08

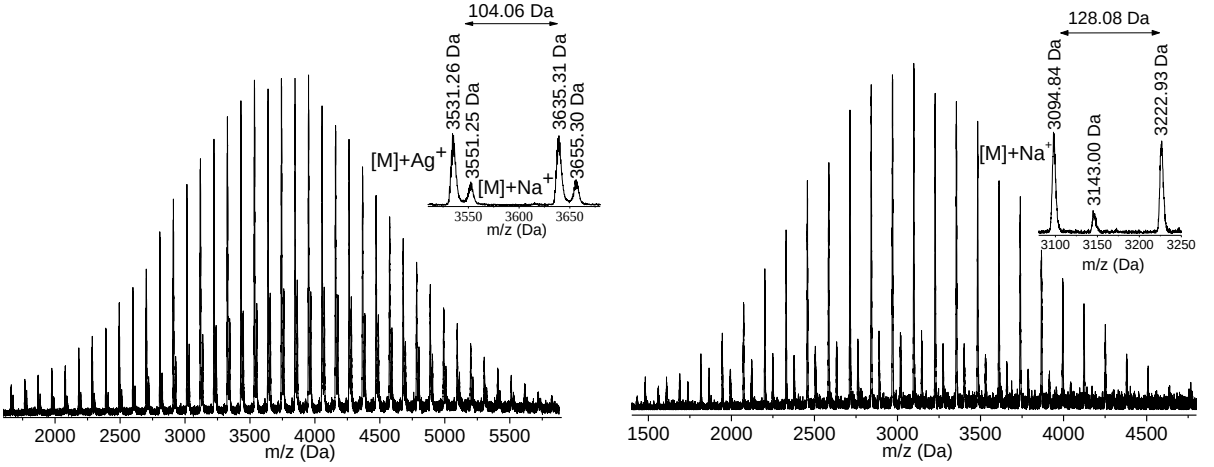


Figure IV.29: MALDI-TOF analysis of polystyrene (left) and poly(butyl acrylate) (right) indicating the presence of the thiolactone end group. The second series can be attributed to respectively sodium adducts and loss of HBr.

Another method to install thiolactones at the polymer chain ends is the modification of hydroxyl end groups with α-isocyanato-γ-thiolactone. DL-homocysteine thiolactone hydrochloride was treated with triphosgene to acquire this isocyanate-containing thiolactone (Figure IV.30). The product was purified by distillation with a yield of 87% and analysed by means of NMR analysis (Figure IV.31).

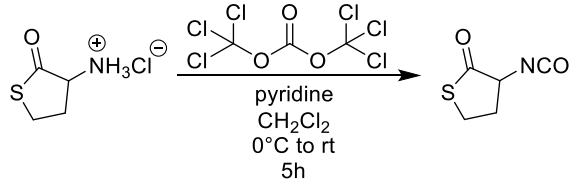


Figure IV.30: Synthesis of the α-isocyanato-γ-thiolactone.

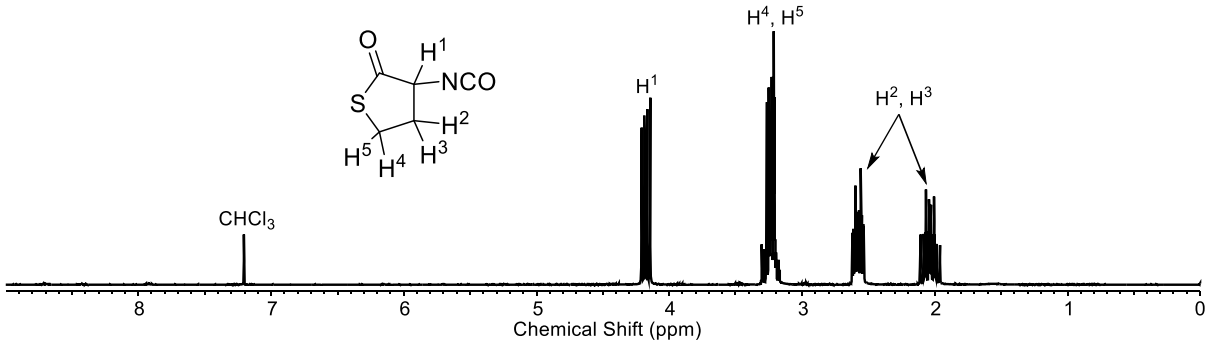


Figure IV.31: [1]H-NMR spectrum (300 MHz, $CHCl_3$-d) with peak assignment of the α-isocyanato-γ-thiolactone.

For this research, a monofunctional poly(ethylene oxide) methyl ether (PEO-OH, M_n ~ 2000 Da) and bifunctional polycaprolactone-diol (HO-PCL-OH, M_n ~ 6000 Da) were chosen, because they can be purified very elegantly via precipitation in suitable non-solvents. The hydroxyl end groups were treated with α-isocyanato-γ-thiolactone and dibutyltin dilaurate as a catalyst to attach the thiolactone to the polymers (Figure IV.32).

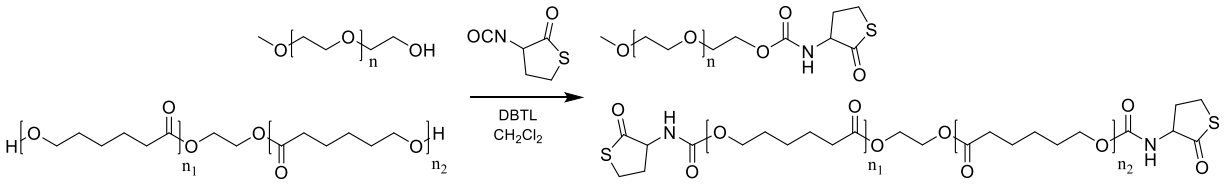


Figure IV.32: Synthesis of PEO and PCL containing end-standing thiolactone groups.

For these two polymers, as well, both [1]H-NMR and MALDI-TOF analysis were performed to prove the presence of the thiolactone end group (Figure IV.33 and IV.34). In both NMR spectra signals at 2.8, 3.25 and 4.25 ppm confirm the thiolactone moiety (Figure IV.33). Furthermore, the experimental molecular weights are in good correlation with the theoretical values, and distinctive mass differences for PEO and PCL, 44.03 Da and 114.07 Da respectively, were observed in the MALDI-TOF spectra (Figure IV.34). These results indicate that PEO has one thiolactone end group, while PCL has two.

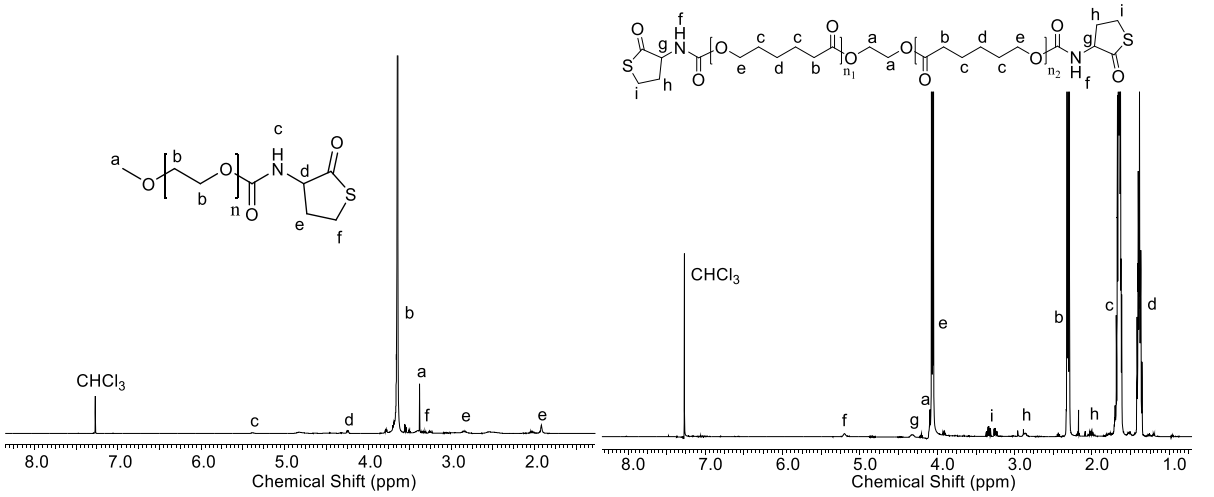


Figure IV.33: [1]H-NMR spectrum (300 MHz, $CHCl_3$-d) with peak assignment of PEO (left) and PCL (right) indicating the presence of the thiolactone end group.

Theoretical m/z (Da)	Experimental m/z (Da)
1959.07	1959.65
2003.09	2003.68
2047.12	2047.72
2091.15	2091.75
2135.18	2135.77

Theoretical m/z (Da)	Experimental m/z (Da)
4933.76	4934.13
5047.83	5048.20
5161.90	5162.27
5275.97	5276.33
5390.04	5390.41

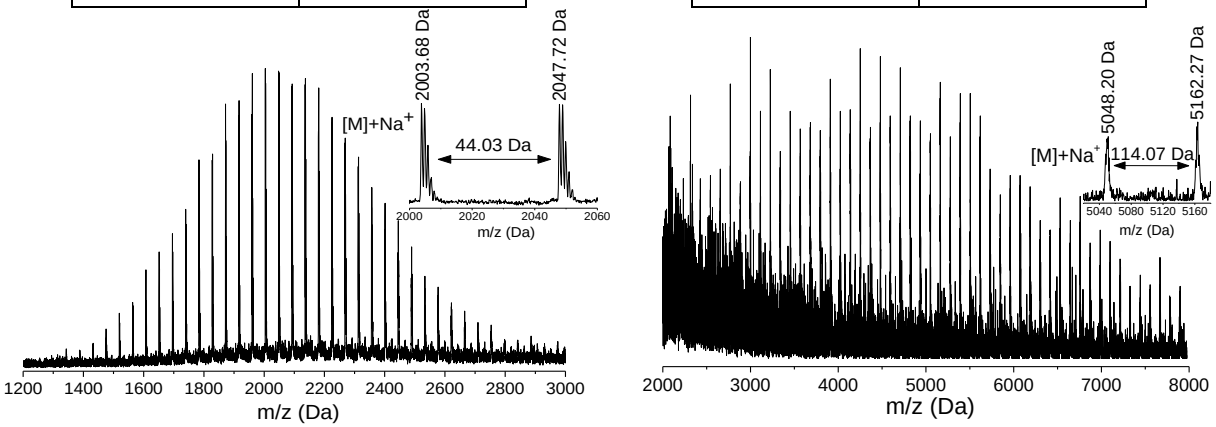


Figure IV.34: MALDI-TOF analysis of PEO (left) and PCL (right) indicating the presence of the thiolactone end group.

IV.3.2 Manipulations of polymer end groups

As with the solid support, the ratio of amine and acrylate and the equivalents of both compared to the thiolactone had to be determined for the double modification of the four different polymers. For this study, benzylamine, benzyl acrylate and chloroform as solvent were used.

For both polystyrene and poly(butyl acrylate), 10 equivalents of amine and 15 equivalents of acrylate with a reaction time of 48 hours were needed to obtain a full conversion for both the aminolysis of the end-standing thiolactone and the subsequent thiol-ene reaction (Figure IV.35).

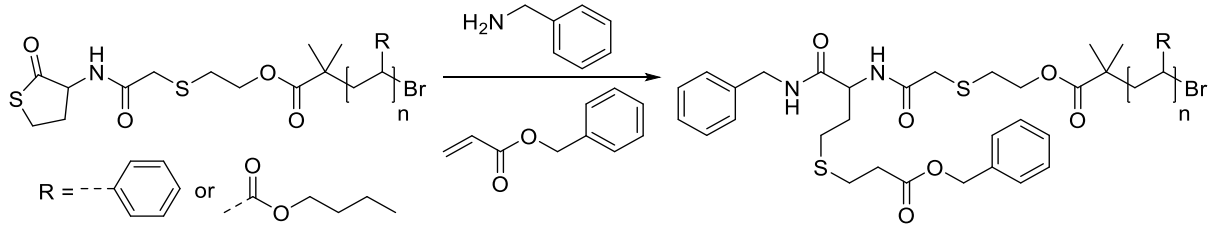


Figure IV.35: One -pot double modification with benzylamine and benzyl acrylate of polystyrene or poly(butyl acrylate) containing a thiolactone end group.

In the case of both polymers, SEC analysis indicated a unimolecular shift in molecular weight and the decrease of the dispersity after the double modification of the polymers (Figure IV.36). In the ^{1}H-NMR spectra, the disappearance of the significant thiolactone signal at 3.25 ppm and the appearance of the signals of the benzyl groups at 4.7, 5.3 and 7.3 ppm were observed (Figure IV.37). Final proof that the double modification was successful for both polymers came from the MALDI-TOF analysis (Figure IV.38). The starting polymers were no longer observed in the spectra and the experimental molecular weights were in good correlation with the theoretical

values. On the other end of these polymers a bromine group is situated, which did not react with the added amine or formed thiol: no extra distribution was observed in the mass spectra.

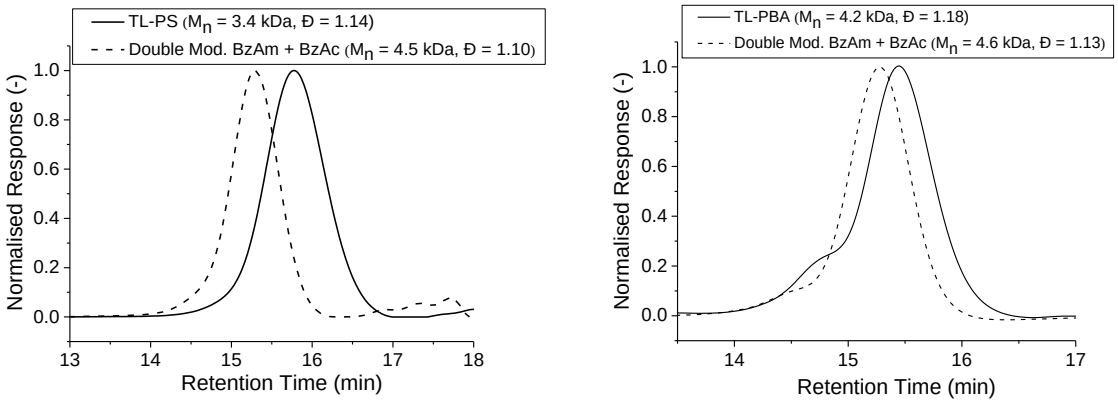


Figure IV.36: SEC analysis of the double modification reaction of polystyrene (left) and poly(butyl acrylate) (right) with benzylamine and benzyl acrylate.

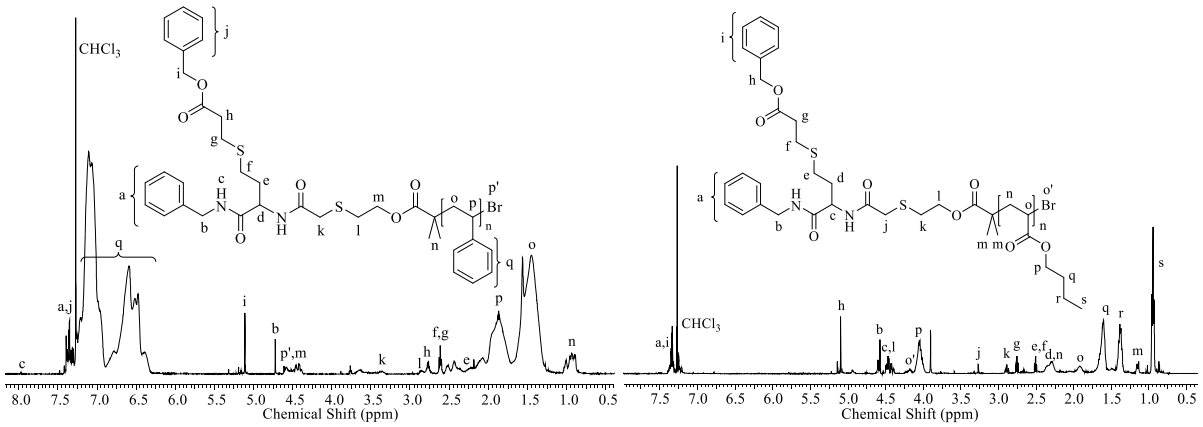


Figure IV.37: ^{1}H-NMR analysis (300 MHz, $CHCl_3$-d) with peak assignment of the double modification reaction of polystyrene (left) and poly(butyl acrylate) (right) with benzylamine and benzyl acrylate.

Theoretical m/z (Da)	Experimental m/z (Da)
4009.11	4008.17
4113.17	4112.23
4217.24	4216.29
4321.30	4320.34
4425.36	4424.41

Theoretical m/z (Da)	Experimental m/z (Da)
3236.80	3235.97
3364.88	3364.05
3492.96	3492.12
3621.04	3620.21
3749.12	3748.28

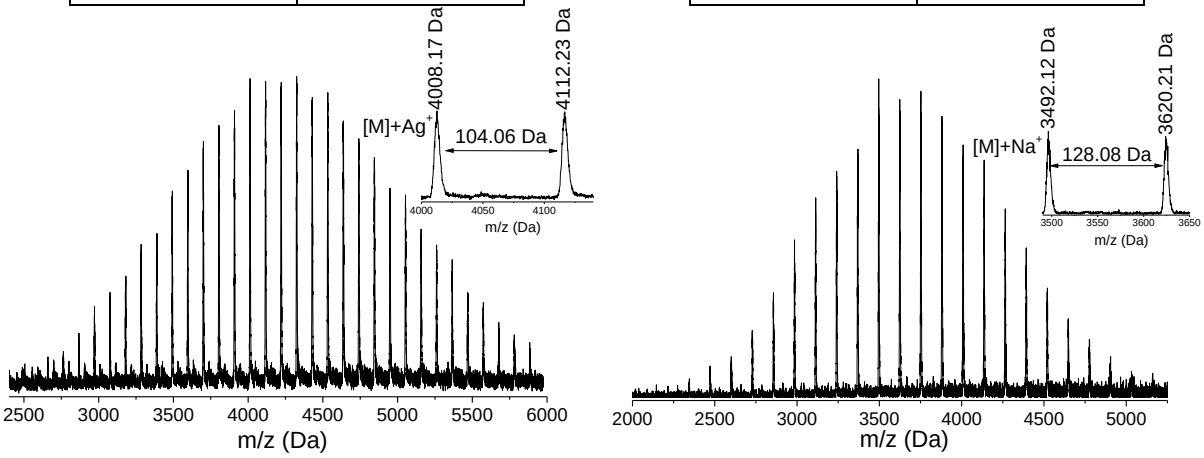


Figure IV.38: MALDI-TOF analysis of the double modification reaction of polystyrene (left) and poly(butyl acrylate) (right) with benzylamine and benzyl acrylate.

Although the same conditions were used as in the case of polystyrene and poly(butyl acrylate) to double-modify the thiolactone-functionalised PEO and PCL, the reaction did not reach full conversion after 48 hours. As was the case with the solid support, a ratio of 1 to 2 is needed for the double modifications of the thiolactone-functionalised PEO and PCL. 15 equivalents of amine and 30 equivalents of acrylate were needed for a full conversion of the thiolactone end group (Figure IV.39). Here as well, a reaction time of 48 hours was needed. The ratio and amount of equivalents necessary for the double modification seem to be dependent on the polymeric system applied. In reference to the double modification of a thiolactone immobilised on a TentaGelSRAM resin with rink amide linker or a PAP resin, preloaded with PEG anchored via a benzyl ether linker, Börner et al. found that the equivalents of amine and acrylate had to be doubled in comparison to the double modifications performed in section IV.2.2.[53] As a result, it seems fair to state that the equivalents and the ratio of the reagents for the double modification are dependent on the polymeric system.

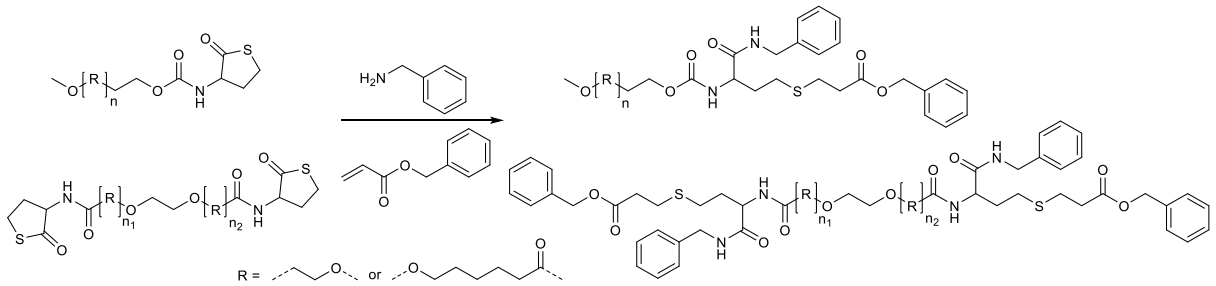


Figure IV.39: One-pot double modification with benzylamine and benzyl acrylate of PEO or PCL containing a thiolactone end group.

The SEC analysis displays a very small unimolecular shift in molecular weight and (almost) no change in dispersity (Figure IV.40). In the ^{1}H-NMR spectra, the signal at 3.25 ppm disappeared and the signals of the benzyl groups were observed at 4.4, 5.3 and 7.3 ppm (Figure IV.41). MALDI-TOF analysis also indicated that the double modification for both polymers was successful because the end-capped thiolactone polymers were absent from the spectra and the experimental and theoretical values for the molecular weights were in good correlation with each other (Figure IV.42). However, in the MALDI-TOF spectrum of PEO, an extra distribution can be observed, which can be attributed to leftover starting hydroxyl end-capped PEO (2520.48 Da). The amounts of the latter polymer must be rather low as the mass spectrum of the thiolactone end-capped polymer does not show any extra distributions of the hydroxyl end-capped polymer. The hydroxyl polymer also has lower masses than the one that is double-modified, which results in an earlier arrival at the mass detector. This in turn leads to an overestimation of the signals of the lower molecular weight polymer in the mass spectrum. Another reason for this effect is that the thiolactone functionalised PEO is probably easier to ionise than the hydroxyl end-capped PEO, while this difference in ionisation is perhaps lower for the double modified and the hydroxyl end-capped PEO.

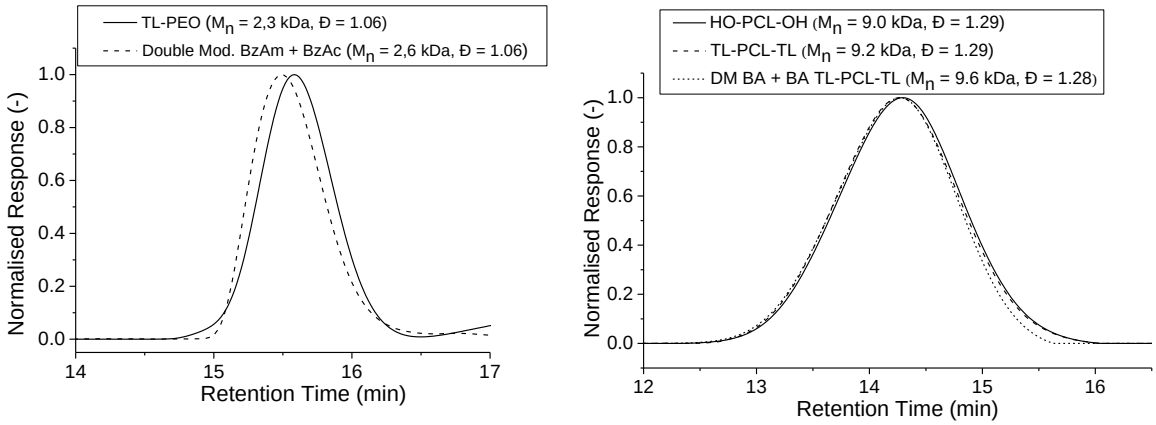


Figure IV.40: SEC analysis of the double modification reaction of PEO (left) and PCL (right) with benzylamine and benzyl acrylate.

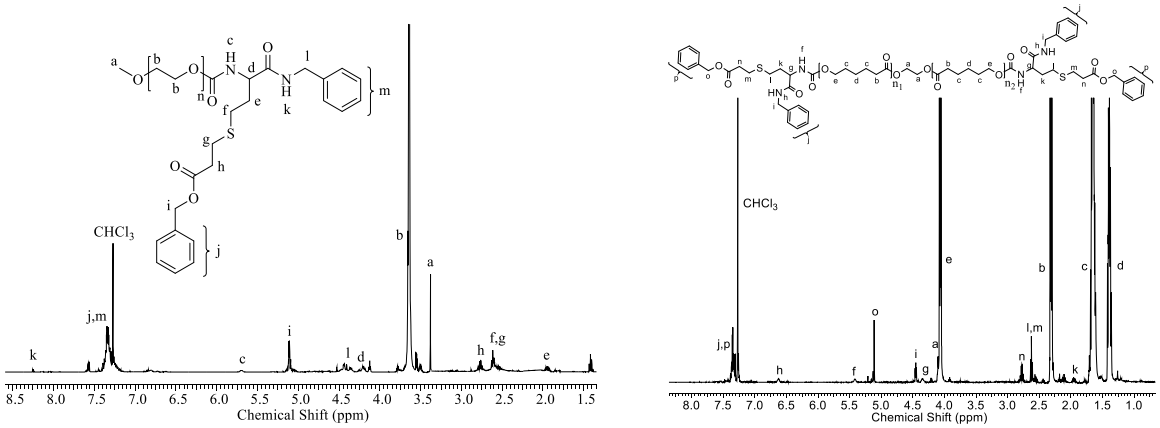


Figure IV.41: ^{1}H-NMR analysis in $CHCl_3$-d of the double modification reaction of PEO (left) and PCL (right) with benzylamine and benzyl acrylate.

Theoretical m/z (Da) (PEO-TLa)	Experimental m/z (Da) (PEO-TLa)	Theoretical m/z (Da) (PEO-OH)	Experimental m/z (Da) (PEO-OH)
2404.31	2405.05	2261.31	2262.06
2448.34	2449.09	2305.34	2306.10
2492.37	2493.12	2349.37	2350.12
2536.40	2537.14	2393.40	2394.16
2580.43	2581.17	2437.43	2438.18

Theoretical m/z (Da)	Experimental m/z (Da)
4901.70	4900.77
5015.77	5014.85
5129.84	5128.92
5243.91	5242.98
5357.98	5357.06

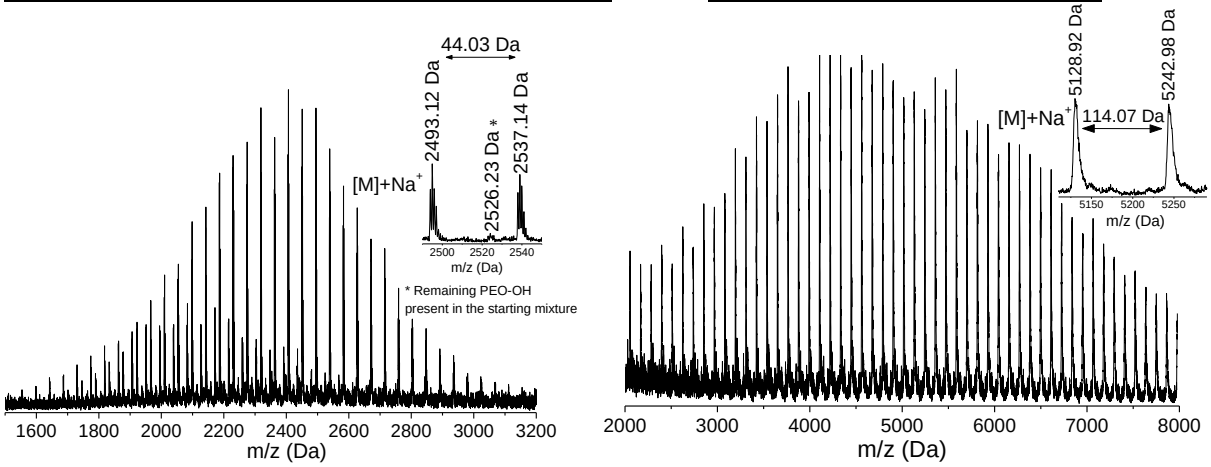


Figure IV.42: MALDI-TOF analysis of the double modification reaction of PEO (left) and PCL (right) with benzylamine and benzyl acrylate.

To prove the versatility of this approach, different functionalities (aromatic, furan, tetrahydrofurfuryl, alkene, halogen and hydroxyl) were introduced by double modification with amines and acrylates of the thiolactone end group of PEO. In order to confirm the incorporation of the functionalities, the modified polymers were analysed via SEC and MALDI-TOF. SEC analysis indicated that the dispersity did not change and the molecular weight remained unimodal after the reaction (Table IV.2, and Figures IV.43 and IV.44). To influence the isotopic distribution of the polymer in the MALDI-TOF spectrum, a PEO polymer was modified with an amine functionalised with a bromine group, 2-(4-bromophenyl)ethylamine (Figure IV.44). Bromine has two distinct stable isotopes, ^{79}Br and ^{81}Br, which cause alterations in this distribution. This alteration was proven through analysis of the theoretical and experimental isotopic distribution. The MALDI-TOF analysis also indicates that the double modification was successful, because the starting PEO with the thiolactone end group (2047 Da) was not observed. Two additional peaks can be observed, however, in the MALDI spectrum: 2036 and 2042 Da. As mentioned above, the first reflects the presence of PEO with a hydroxyl end group, while the second peak can be attributed to an unidentified fragmentation in the MALDI-TOF analysis.

Table IV.2: SEC data for the different amine-acrylate combinations for the double modification of PEO.

	Amine	Acrylate	M_n (Da)*	Đ*
start	/	/	2300	1.06
1	Benzyl	Benzyl	2600	1.06
2	Ethanolamine	Tetrahydrofurfuryl	2400	1.06
3	Benzyl	2,2,2-Trifluoroethyl	2700	1.06
4	Furfurylamine	Benzyl acrylate	2800	1.06
5	Allylamine	Isobornyl	2400	1.06
6	2-(4-Bromophenyl)ethylamine	Benzyl acrylate	2900	1.06

* Molecular weights and dispersities determined by SEC in THF vs polystyrene standards.

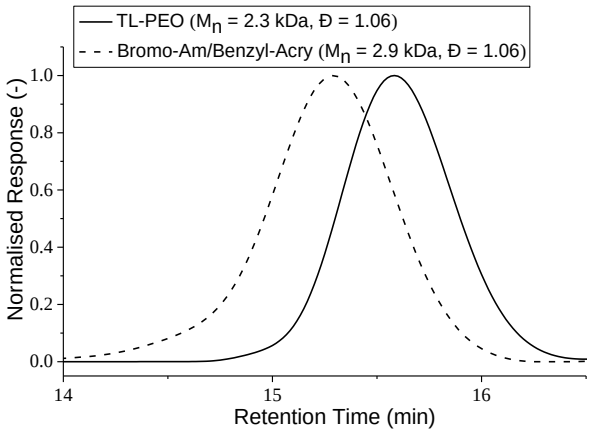


Figure IV.43: SEC analysis of the double modification reaction of PEO with 2-(4-bromophenyl)ethylamine and benzyl acrylate.

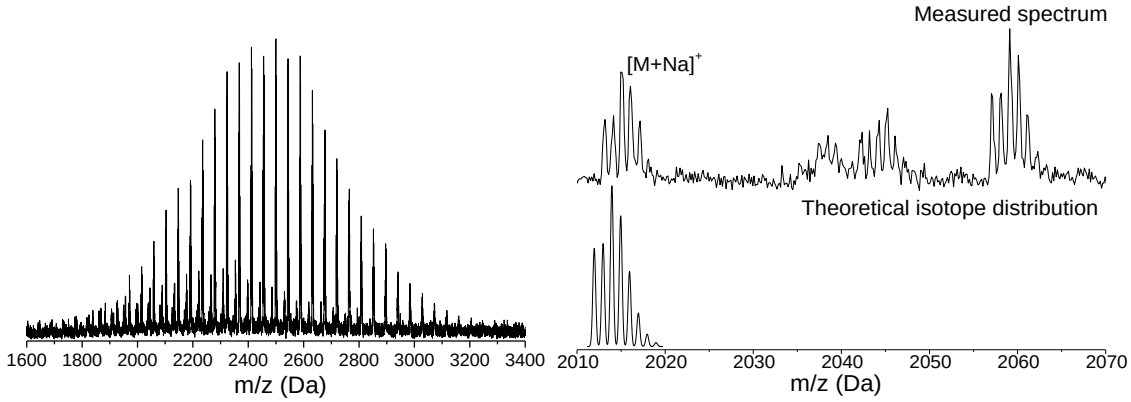


Figure IV.44: MALDI-TOF analysis of the double modification reaction of PEO with 2-(4-bromophenyl)ethylamine and benzyl acrylate.

In order to further demonstrate the strength of this method, a block copolymer consisting of a PEO and a poly(butyl acrylate) block with a methyl ester midchain functionality was prepared by means of the double modification of thiolactone end groups. 5 equivalents of a PEO amine (800 Da) were added to a mixture of the previously made poly(butyl acrylate) with a thiolactone end group, chloroform and methyl acrylate (Figure IV.45). The mixture was allowed to react for 48 hours. Comparison of the LCxSEC chromatograms before and after the reaction proves that the polymers are connected (Figure IV.46). The x-axis of these chromatograms makes a distinction based on molecular weight, while the y-axis reflects polarity. In the chromatogram representing the post-reaction situation, a few observations can be made. First, the signal of the starting poly(butyl acrylate) has disappeared and a new signal with a higher molecular weight and shift in polarity has appeared. This proves that the aminolysis was successful. Second, the signal for the PEO amine has not disappeared. The continued visibility of this signal can be explained by the fact that crude samples were used for this analysis and 5 equivalents of this amine were used at the start of this reaction. In fact, the volume fractions of the different polymer peaks should be determined to fully quantify that the coupling of the polymers was complete.[54]

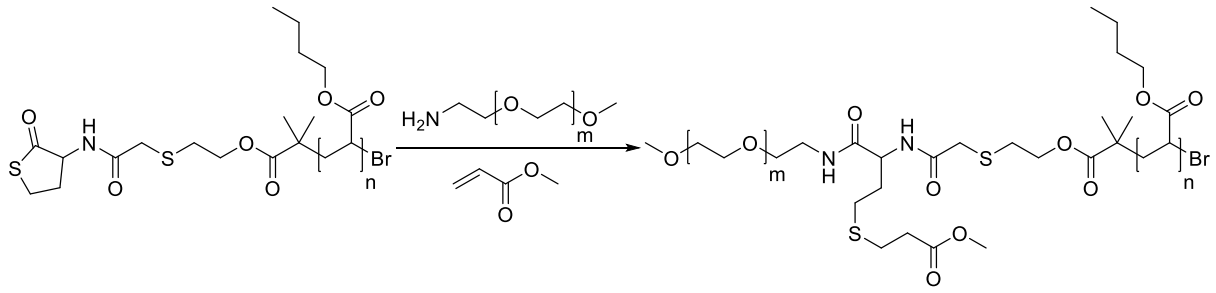


Figure IV.45: One-pot double modification with PEO amine and methyl acrylate of poly(butyl acrylate) containing a thiolactone end group.

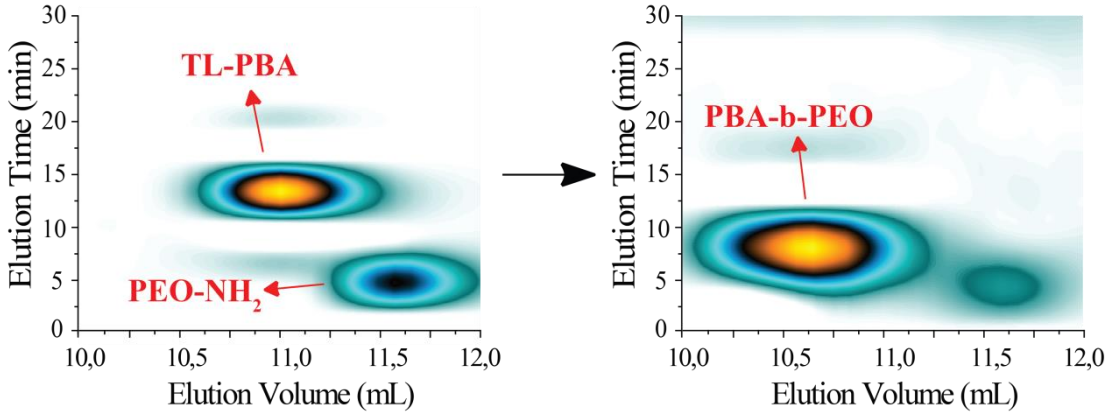


Figure IV.46: LCxSEC analysis of a mixture of the starting polymers poly(butyl acrylate) containing a thiolactone end group and PEO-NH_2 (left) and the coupled block copolymer poly(butyl acrylate)-b-PEO (right).

To prove that the methyl acrylate has reacted with the formed thiol after aminolysis, the block copolymer was added to a mixture of the Ellman's reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), N,N-diisopropylethylamine (DIPEA) and THF.[55] This mixture should become distinctively yellow when free thiols are present. No yellow colour was observed, so the thiols formed must have reacted with the methyl acrylate. LCxSEC analysis and the test with the Ellman's reagent prove that the double modification of the thiolactone end group was fully completed and that block copolymers with a specific midchain functionality, in this case methyl ester, can be created with this modification.

IV.4 Conclusions and outlook

This chapter depicted the one-pot double modification with amines and acrylates of a thiolactone immobilised on a solid support and as a polymer end group.

In the first part of this chapter, both an acid- and a hydroxyl-functionalised thiolactone linker were synthesised and immobilised on a 2-chlorotrityl chloride polystyrene resin. Next, the reaction conditions for the thiolactone opening and the subsequent thiol-ene reaction were determined. 10 equivalents of amine and 20 equivalents of acrylate in $CHCl_3$ proved to efficiently open the immobilised thiolactone on the solid support. Afterwards, different functionalities (aromatic, (cyclic) alkane, pyridine, acid, ether, furan, alkene, alkyne, morpholine, hydroxyl, tertiary amine and methacrylate) could be elegantly installed via the double modification method. The end group of an oligopeptide was also double-modified on the solid support to prove the broad applicability of this method.

The second part of this chapter focussed more strongly on the double modification of a thiolactone at the end of a polymer chain. Two methods were explored to install the thiolactone group. With the first method, a thiolactone could be introduced at the end of polystyrene and poly(butyl acrylate) by means of controlled radical polymerisation with a thiolactone-functionalised initiator. In the second method, α-isocyanato-γ-thiolactone was applied to

convert the hydroxyl end groups of PEO and PCL. Next, the ideal reaction conditions for the double modification of the different polymers were determined. Double modification in $CHCl_3$ for 48 hours proved to require 10 equivalents of amine and 15 equivalents of acrylate for polystyrene and poly(butyl acrylate), and 15 and 30 equivalents respectively for PEO and PCL. Different functionalities (aromatic, furan, tetrahydrofurfuryl, double bond, halogen and hydroxyl) were installed at the end of the polymer. Finally, this double modification method allowed the synthesis of a midchain-functionalised block copolymer with a poly(butyl acrylate) and PEO block.

The results prove that the amine-thiolactone-ene conjugation is already applicable to thiolactones immobilised on the solid support and as end groups of polymers, and that it might have some extra potential for the future. In Chapter VI this conjugation was combined with urethane synthesis for the synthesis of sequence-defined structures on solid support.[56] We concluded from the results obtained that it was optimal to build the sequences on the solid support, as this has several advantages in comparison to a liquid support: the double modification reaction is faster (overnight versus 48 hours) and the purification is easier (washing and cleavage versus precipitation).

Although only polymers were coupled with this conjugation in this chapter, the current method could perhaps be applied for the coupling of other types of oligomers and polymers. In the future, for instance, the synthesis of peptide/protein-polymer and oligonucleotide/DNA-polymer hybrids with a specific midchain functionality could be pursued. These hybrids can be used in fields such as enzyme recovery[57], nano-biomaterials[58], pharmaceuticals[59] and the modelling of peptide binding[60].

The conditions that were determined in this chapter for the aminolysis and subsequent thiol-ene reaction were also applied in our group for the monomodification of polymers which were polymerised via RAFT and have a trithiocarbonate end group.[61] UV-VIS and MALDI-TOF confirmed that the trithiocarbonate was removed and the acrylate was incorporated. The applied conditions could perhaps be used in the future to modify polymers with other thiol protecting groups such as thiocarbonylthio groups[62, 63], cyclic dithiocarbonates[64, 65] and thioesters.[66, 67]

IV.5 Experimental part

IV.5.1 Methods

^{1}H- and ^{13}C-NMR

^{1}H- and ^{13}C-NMR (Attached Proton Test, APT) spectra were recorded on a Bruker Avance 300 at 300 MHz and 75 MHz, respectively and a Bruker Avance 500 at 500 MHz and 125 MHz, respectively. Chemical shifts are presented in parts per million (δ) relative to DMSO-d_6 or

$CHCl_3$-*d* (2.50 ppm or 7.27 ppm in ^{1}H- and 39.51 ppm or 77.24 ppm in ^{13}C-NMR respectively) as internal standard. All samples were analysed with 2D-NMR techniques (COSY, HSQC and HMBC) which allowed for a full assignment of the structures. All measurements were performed at 25°C and ACD/NMR Processor was used for the processing of all data. All spectra including 1D ^{1}H and ^{13}C, 2D COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC were recorded in a standard fashion with pulse programs available in the Bruker library.

LC-ESI-MS

An Agilent technologies 1100 series LC/MSD system equipped with a diode array detector and single quad MS detector (VL) with an electrospray source (ESI-MS) was used for classic reversed phase LC-MS (liquid chromatography mass spectroscopy) and MS analysis. Analytic reversed phase HPLC was performed with a Phenomenex C18 (2) column (5 µ, 250 x 4.6 mm) using a solvent gradient (0 → 100% acetonitrile in H_2O in 15 min) and the eluting compounds were detected via UV-detection (λ = 214 nm). High-resolution mass spectra (HRMS) were collected using an Agilent 6220 Accurate-Mass time-of-flight (TOF) equipped with a multimode ionisation (MMI) source.

It was mentioned earlier that internal contaminations (IC and IC2) can be found in some LC-traces around 4.7 min and 8.8 min (Figure IV.47). When IC and IC2 were indicated in an LC trace in this chapter, it referred to these internal contaminants.

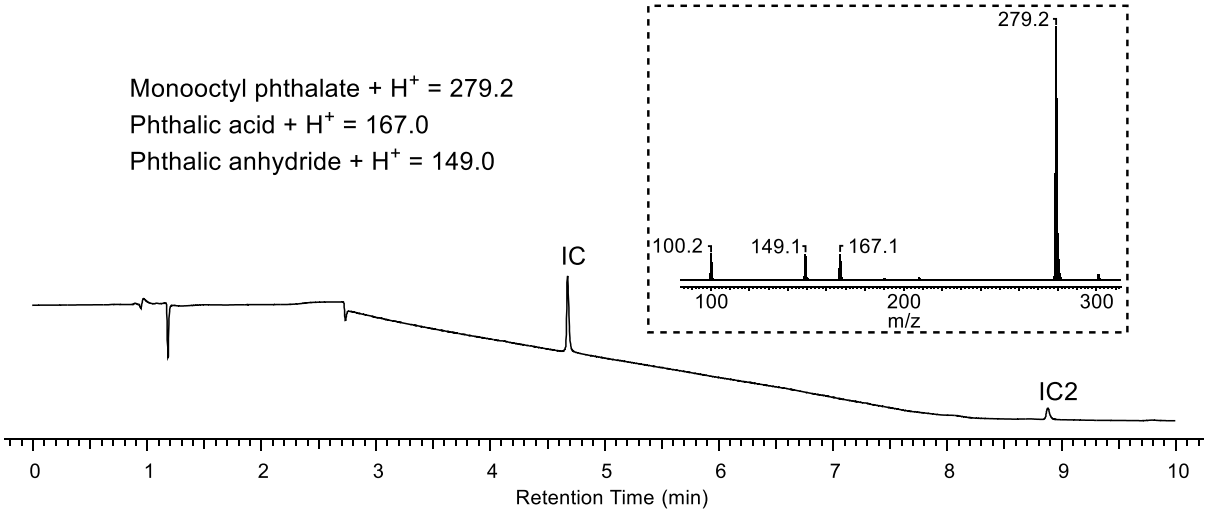


Figure IV.47: LC-ESI-MS analysis of a blank sample indicating the presence of internal contaminants. Insert: ESI-MS-spectra of dominant species (positive mode).

IR

Infrared spectra were recorded with Attenuated Total Reflection (ATR) with a PIKE Miracle ATR unit and a Perkin Elmer FTIR SPECTRUM 1000 spectrometer. IR-software from Perkin Elmer was used for the analysis of the spectra.

SEC

Size Exclusion Chromatography (SEC) was performed using a Varian PLGPC50plus instrument, using a refractive index detector, equipped with two Plgel 5 µm MIXED-D columns 40 °C. Polystyrene standards were used for calibration and THF as eluent at a flow rate of 1 mL/min. Samples were injected using a PL AS RT autosampler.

LCxSEC

For two-dimensional liquid chromatography, sample fractions from the first dimension were transferred to the second-dimension column via an electronically controlled eight-port valve system (VICI Valco instruments, Houston, TX, USA), equipped with two 100 µL sample loops. The second dimension consisted of an Agilent Infinity 1260 isocratic pump and a PSS SDV LIN M 5 µm column. Detection in the second dimension was accomplished by using an ELSD. Nitrogen was used as carrier gas in the ELSD at a flow rate of 2.5 L/min. Spray Chamber, Drift Tube and Optical Cell temperatures were set at 30 °C, 80 °C and 70 °C, respectively. The flow rates used in the first and second dimensions were 0.02 mL/min and 5 mL/min, respectively. Sample concentrations were between 0.25 and 2.0 mg/mL. an isocratic elution of methanol/hexane (70/30) was used as the solvent for the first dimension, THF was used as the solvent for the second dimension analysis. Data were recorded using PSS WinGPC Unichrom software.

MALDI-TOF

Matrix Assisted Laser Desorption Ionisation – Time of Flight (MALDI-TOF) was performed on an Applied Biosystems Voyager De STR MALDI-TOF spectrometer equipped with 2 m linear and 3 m reflector flight tubes, a nitrogen laser operating at 337 nm, pulsed ion extraction source and reflectron. All mass spectra were obtained with an accelerating potential of 20kV in positive ion mode and in reflector mode. Measurements of poly(butyl acrylate) were performed with dithranol (25 mg/mL in THF) as a matrix, sodium iodide (20 mg/mL in THF) as a cationising agent, and polymer samples were dissolved in THF (5 mg/mL). Polymer solutions were prepared by mixing 5 µL of the polymer, 10 µL of the salt, and 10 µL of the matrix solution. Subsequently, 0.5 µL of this mixture was spotted on the sample plate, and the spots were dried in air at room temperature. Measurements of polystyrene were performed with dithranol (20 mg/mL in THF) as a matrix, silver trifluoro acetate (1 mg/mL in THF) as a cationising agent, and polymer samples were dissolved in THF (10 mg/mL). Polymer solutions were prepared by mixing 5 µL of the polymer, 10 µL of the salt, and 10 µL of the matrix solution. Subsequently, 0.5 µL of this mixture was spotted on the sample plate, and the spots were dried in air at room temperature. Measurements of polyethylene oxide were performed with dithranol (10 mg/mL in THF) as a matrix, sodium trifluoro acetate (1 mg/mL in THF) as a cationising agent, and polymer samples were dissolved in THF (10 mg/mL). Polymer solutions were prepared by mixing 2 µL of the polymer, 2 µL of the salt, and 16 µL of the matrix solution. Subsequently, 0.5 µL of this mixture was spotted on the sample plate, and the

spots were dried in air at room temperature. Measurements of polycaprolactone were performed with trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malonitrile (DCTB, 20 mg/mL in THF) as a matrix, sodium iodide (1 mg/mL in THF) as a cationising agent, and polymer samples were dissolved in THF (2 mg/mL). Polymer solutions were prepared by mixing 5 µL of the polymer, 5 µL of the salt, and 10 µL of the matrix solution. Subsequently, 0.5 µL of this mixture was spotted on the sample plate, and the spots were dried in air at room temperature. A poly(ethylene oxide) standard (Mn = 2000 g/mol) was used for calibration. All data were processed using the Data Explorer 4.0.0.0 (Applied Biosystems) software package.

GC

GC-FID analyses were performed using a Carlo Erba GC800 equipped with a DB 5MS column (60mm x 0.249 mm x 0.25 µm) from J&W Scientific with 1,2-dichlorobenzene as internal standard. The carrier gas (He) was used at a flow rate of 1.4 mL/min. After sample injection, the column oven was kept at 50°C for 30 min, then heated until 260°C at a rate of 5°C/min and then finally kept at 260°C for 5 min.

IV.5.2 Materials

DMSO-d_6 ([2206-27-1], ≥ 99.8 %) and $CHCl_3$-*d* ([865-49-6], ≥ 99.8 %) were purchased from Euriso-top. Acetonitrile ([75-05-8], HPLC grade), 1,4-Dioxane ([123-91-1], HPLC grade) and Triethylamine ([121-44-8], 99 %) were purchased from Acros Organics. DL-Homocysteine thiolactone hydrochloride ([6038-19-3], 99 %) was purchased from Haihang industry (Jinan City, China). 2-(4-Bromophenyl)ethylamine ([73918-56-6], 98%) was purchased from Alfa Aesar. Polycaprolactone (average M_n ~ 6000 Da) was kindly donated by Solvay and was dried azeotropically over toluene prior to use. Potassium carbonate ([584-08-7], ≥ 99 %) and Sodium bicarbonate ([144-55-8], ≥ 99.5%) were purchased from Carl Roth. 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) ([94790-37-1], Peptide grade), 2-Chlorotrityl chloride resin ([42074-68-0], 100-200 mesh, 1% DVB, 1.6 mmol/g), MeO-PEG-NH_2 (800 Da) and Trifluoroacetic acid ([76-05-1], Peptide grade) were purchased from Iris Biotech GmbH. Allylamine ([107-11-9], ≥ 99 %), Aluminium oxide ([1344-28-1], basic), Benzylamine ([100-46-9], ≥ 99.5 %), Bromoacetyl bromide ([598-21-0], ≥ 98 %), α-Bromoisobutyryl bromide ([20769-85-1], 98 %), Butyl acrylate ([141-32-2], ≥ 99 %), Chloroform ([865-49-6], ≥ 99.8 %), Cu(0)-pellets ([7440-50-8], ≥ 99.9 %), Cu(II)Br2 ([7789-45-9], 99 %), Cyclopropylamine ([765-30-0], 98 %), 1,2-Dichlorobenzene ([95-50-1], 99 %), Dichloromethane ([75-09-2], ≥ 99.8 %), Diethylether ([60-29-7], ≥ 99.9 %), N,N-Diisopropylethylamine (DIPEA, [7087-68-5], 99 %), N,N-Dimethylethylenediamine ([108-00-9], ≥ 98.0 %), N,N-Dimethylformamide ([68-12-2], anhydrous, 99.8 %), 5,5'-Dithiobis(2-nitrobenzoic acid) ([69-78-3], ≥ 98 %), Ethanolamine ([141-43-5], ≥ 99%), Ethyl acetate ([141-78-6], ≥ 99.7 %), Furfurylamine ([617-89-0], ≥ 99 %), Glutaric anhydride ([108-55-4], 95 %),

Isobornyl acrylate ([5888-33-5], technical grade, was distilled prior to use), 2-Mercaptoethanol ([60-24-2], ≥ 99 %), Methanol ([67-56-1], ≥ 99.9 %), Methyl acrylate ([96-33-3], 99 %), 3-Morpholinopropylamine ([123-00-2], 99 %), Octylamine ([111-86-4], 99 %), 3-Picolylamine ([3731-52-0], ≥ 99 %), Propargyl acrylate ([10477-47-1], 98 %), Pyridine ([110-86-1], ≥ 99 %), N,N,N',N'',N''-Pentamethyldiethylenetriamine ([3030-47-5], 99%), Styrene ([100-42-5], ≥ 99 %), Polyethylene oxide ([9004-74-4] was dried azeotropically over toluene prior to use, average Mn ~ 2000 Da), Tetrahydrofuran ([109-99-9], ≥ 99 %) were purchased from Sigma-Aldrich. 4-Amino-1-butanol ([13325-10-5], > 98 %), Benzyl acrylate ([2495-35-4], > 97 %), Dibutyltin dilaurate ([77-58-7], > 95 %), N,N-Diethylacrylamide ([2675-94-7], > 98 %), 2-(Dimethylamino)ethyl acrylate ([2439-35-2], > 98 %), 2-(2-Ethoxyethoxy)ethyl acrylate ([7328-17-8], > 98 %), Tetrahydrofurfuryl acrylate ([2399-48-6], 98 %), 2,2,2-Trifluoroethyl acrylate ([407-37-6], > 98 %) and Triphosgene ([32315-10-9], > 98 %) were purchased from TCI and used without purification. Hydrochloric acid 36 % p. (HCl, [7647-01-0]) was purchased from Chem-Lab and used without purification. Solvents (CH_2Cl_2, DIPEA and pyridine) for the immobilisation of functionalised thiolactone linkers were distilled from CaH2 prior to use. Silicagel (ROCC, SI 1721, 60 Å, 40 – 63 μm) was used to perform preparative column chromatography, eluting with technical solvents. The collected fractions were analysed by thin layer chromatography (TLC-plates, Macherey-Nagel, SIL G-25 UV254). The Me_6TREN, 2-(acryloyloxy)ethyl methacrylate, 1-ethoxyethyl and disperse red 1 acrylate were prepared as previously described.[68-71]

IV.5.3 Synthesis

Synthesis of the acid-functionalised thiolactone linker

DL-homocysteine thiolactone hydrochloride (7.0 g, 45.6 mmol) was dissolved in an ice-cooled mixture of H_2O/1,4-dioxane (1/1, 100 mL) and treated with $NaHCO_3$ (19.15 g, 227.9 mmol). This mixture was stirred for 30 minutes at 0°C. In small portions glutaric anhydride (10.4 g, 91.2 mmol) was added to the mixture. The reaction mixture was allowed to reach room temperature during the night. Next, 12 M HCl was added dropwise to adjust the pH (pH = 1). After the addition of brine (100 mL), the mixture was extracted with EtOAc (3 x 200 mL). The organic fractions were dried ($MgSO_4$) and concentrated. The crude residue was purified by column chromatography (silicagel, gradient elution: 100 % CH_2Cl_2 → CH_2Cl_2 /acetone: 6/4), yielding linker 1 as a white solid (8.1 g, 35.0 mmol, 76 %).

^{1}H-NMR (500 MHz, DMSO-d_6, ppm) δ 12.04 (*s*, 1 H), 8.17 (*d*, 1H, 8.3 Hz), 4.58 (*ddd*, 1 H, 12.6, 8.1, 7.0 Hz), 3.31 (*m*, 2 H), 2.40 (*m*, 1 H), 2.26 to 1.99 (*m*, 5 H), 1.71 (*m*, 2 H).
^{13}C-NMR (125 MHz, DMSO-d_6, ppm) δ 205.5 (C), 174.1 (C), 171.7 (C), 58.1 (CH), 34.2 (CH_2), 32.8 (CH_2), 30.1 (CH_2), 26.7 (CH_2), 20.5 (CH_2).

IR (cm^{-1}) 3288, 3111, 2936, 2915, 2526, 1981, 1686, 1605, 1568, 1459, 1439, 1422, 1367, 1323, 1307, 1270, 1232, 1203, 1182, 1160, 1129, 1048, 1018, 974, 940, 923, 872, 853, 800, 755, 734, 714, 686, 625, 608
ESI-MS (m/z) $[M-H]^-$ 230.0

Synthesis of the hydroxyl-functionalised thiolactone linker
In an ice-cooled mixture of H_2O/CH_2Cl_2 (1/1, 400 ml) DL-homocysteine thiolactone hydrochloride (9.2 g, 60 mmol) and potassium carbonate (25 g, 180 mmol) were dissolved. A solution of bromoacetyl bromide (10.5 mL , 120 mmol) in CH_2Cl_2 (40 mL) was added dropwise to this ice-cooled mixture. After stirring the reaction for 30 min at 0°C, it was allowed to reach room temperature and stirred for 1 hour. The two layers were separated and the organic layer was washed twice with 5% aqueous citric acid (40 mL) and twice with H_2O (40 mL). The organic fraction was dried over $MgSO_4$ and concentrated in vacuo to afford N-(2-bromoacetyl) homocysteine-γ-thiolactone as a white solid (12.1 g, 51 mmol, 84%).

^{1}H-NMR (500 MHz, DMSO-d_6, ppm) δ 8.66 (*d*, 1 H, 8.1 Hz), 4.61 (*ddd*, 1 H, 12.6, 8.1, 7.2 Hz), 3.90 (*d*, 2 H, 1.3 Hz), 3.41 (*app dt*, 1 H, 11.4, 5.3 Hz), 3.30 (*ddd*, 1H, 11.1, 7.0, 1.5 Hz), 2.45 (*m*, 1 H), 2.08 (*m*, 1 H).
^{13}C-NMR (125 MHz, DMSO-d_6, ppm) δ 204.9 (C), 166.2 (C), 58.6 (CH), 30.0 (CH_2), 29.0 (CH_2), 26.8 (CH_2).
IR (cm^{-1}) 3261, 3060, 3016, 2945, 2906, 1696, 1656, 1535, 1452, 1431, 1344, 1324, 1274, 1261, 1212, 1178, 1158, 1143, 1115, 1060, 1022, 981, 921, 888, 853, 750, 684, 659, 612
ESI-MS (m/z) $[M+H]^+$ 237.9

2-Mercaptoethanol (3.86 mL, 55 mmol) was added dropwise to *N*-(2-bromoacetyl) homocysteine-γ-thiolactone (11.9 g, 50 mmol) in an ice-cooled solution of anhydrous CH_2Cl_2 (70 mL) and anhydrous Et_3N (7.7 mL, 55 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The crude residue was purified by column chromatography (silicagel, gradient elution: 100 % CH_2Cl_2 → CH_2Cl_2 / acetone: 90/10), yielding the hydroxyl-thiolactone as a white solid (9.9 g, 41.9 mmol, 83 %).

^{1}H-NMR (500 MHz, DMSO-d_6, ppm) δ 8.36 (*d*, 1 H, 8.3 Hz), 4.79 (*t*, 1 H, 5.6 Hz), 4.59 (*ddd*, 1 H, 12.7, 8.1, 7.1 Hz), 3.54 (*dt*, 2 H, 6.8, 5.7 Hz), 3.40 (*app dt*, 1 H, 11.5, 5.4 Hz), 3.28 (*ddd*, 1 H, 11.0, 7.0, 1.5 Hz), 3.17 (*s*, 2 H), 2.65 (*t*, 2 H, 6.7 Hz), 2.42 (*m*, 1 H), 2.10 (*m*, 1 H).
^{13}C-NMR (125 MHz, DMSO-d_6, ppm) δ 205.2 (C), 169.3 (C), 60.4 (CH_2), 58.4 (CH), 34.5 (CH_2), 34.4 (CH_2), 30.0 (CH_2), 26.8 (CH_2).
IR (cm^{-1}) 3383, 3281, 3245, 3071, 2957, 2915, 2858, 1678, 1639, 1618, 1554, 1427, 1407, 1323, 1281, 1226, 1189, 1170, 1161, 1139, 1078, 1043, 1018, 981, 917, 874, 848, 765, 698, 679, 621
ESI-MS (m/z) $[M+H]^+$ 236.0

Synthesis of the thiolactone containing initiator

The hydroxyl-functionalised thiolactone linker (9.9 g, 41.9 mmol) was dissolved in an ice-cooled solution of anhydrous CH_2Cl_2 (70 mL) and anhydrous Et_3N (17.5 mL, 126 mmol). Subsequently, α-bromoisobutyryl bromide (6.7 mL, 55 mmol) was added dropwise. Overnight, the reaction was allowed to warm to room temperature. Column chromatography (silicagel, gradient elution: 100 % CH_2Cl_2 → CH_2Cl_2/acetone: 95/5) was applied to purify the compound. An off-white white solid was obtained after the purification (13.3 g, 34.8 mmol, 83 %).

^{1}H-NMR (500 MHz, $CHCl_3$-*d* , ppm) δ 7.10 (*d*, 1 H, 6.0 Hz), 4.58 (*app dt*, 1 H, 13.0, 7.0 Hz), 4.36 (*app t*, 2 H, 6.5 Hz), 3.38 (app *dt*, 1 H, 11.5, 5.0 Hz), 3.36 (*d*, 1 H, 16.0 Hz), 3.31 (*d*, 1 H, 16.0 Hz), 3.27 (*ddd*, 1 H, 11.0, 7.0, 0.8 Hz), 2.88 (*m*, 3 H), 2.02 (*m*, 1 H), 1.94 (*s*, 6 H).
^{13}C-NMR (125 MHz, $CHCl_3$-*d* , ppm) δ 204.9 (C), 171.8 (C), 169.3 (C), 64.3 (CH_2), 59.7 (CH), 55.8 (C), 36.2 (CH_2), 31.7 (CH_2), 31.4 (CH_2), 30.9 (2 x CH_3), 27.7 (CH_2).
ESI-MS (m/z) $[M+H]^+$ 383.9

Synthesis of the α-isocyanato-γ-thiolactone

In ice-cooled anhydrous CH_2Cl_2 (450 mL) triphosgene (25 g, 84 mmol) was dissolved and stirred for 15 minutes, after which DL-homocysteine thiolactone hydrochloride (37 g, 241 mmol) was added. Next, pyridine (64.11 mL, 794 mmol) was added dropwise to the reaction mixture. After one hour, the reaction mixture was allowed to warm to room temperature and stirred for five hours.

To prevent the degradation of the crude isocyanate, the aqueous work-up must be done fast. The formed pyridinium hydrochloride was removed from the reaction mixture by means of filtration in a separation funnel. The collected organic phase, which went through the filter, was washed with 2M HCl solution (250 mL), brine (250 mL) and ice water (250 mL). The organic phase was dried over $MgSO_4$ and, after evaporation of the CH_2Cl_2, a brown residue was obtained. Finally, this crude residue was purified by vacuum distillation, yielding a yellow oil (30.3 g, 210 mmol, 87%).

^{1}H-NMR (500 MHz, $CHCl_3$-*d*, ppm) δ 4.24 (*dd*, 2 H, 12.6, 6.8 Hz), 3.29 (*m*, 2 H), 2.64 (*m*, 1 H), 2.10 (*m*, 1 H).
^{13}C-NMR (125 MHz, $CHCl_3$-*d*, ppm) δ 203.1 (C), 127.6 (C), 62.6 (CH), 32.1 (CH_2), 27.0 (CH_2).
IR (cm^{-1}) 2944, 2869, 2227, 1691, 1440, 1336, 1280, 1172, 1142, 1057, 1033, 1000, 964, 884, 840, 744, 681, 643, 616

Synthesis of polystyrene with a thiolactone end group

Two mixtures were prepared and degassed with argon for one hour in two separate flasks. In the first flask the thiolactone functionalised initiator (200.59 mg, 0.52 mmol) and styrene (3 mL, 26.10 mmol) were mixed. In the second flask styrene (3 mL, 26.10 mmol), Cu(0) (20

pellets), Cu(II)Br_2 (11.66 mg, 0.052 mmol), PMDETA (38.14 mg, 0.183 mmol) were mixed. The two mixtures were added to one another and heated to 90°C. After 2 hours, the reaction was stopped and purified of its metal salts by cooling it to room temperature, diluting it with THF (5 mL) and filtering it over a basic alumina column. The polymer itself was purified via precipitation in cold methanol (50 mL) and subsequent filtration. To ensure a high level of purity, the polymer was dissolved in THF (5 mL), precipitated in cold methanol (50 mL) and filtered off for a second time. The remaining methanol was removed via evaporation overnight at 40°C in a vacuum oven.
For the kinetic study, samples had to be taken and prepared for GC and SEC analysis. For the first analysis technique, the samples were diluted with THF which contained 1,2-dichlorobenzene as internal standard and phenothiazine as radical inhibitor. For the second analysis technique, the samples were also diluted with THF, but had to be filtered first over basic alumina to remove the metal salts before they could be measured.

Synthesis of poly(butyl acrylate) with a thiolactone end group
Two mixtures were prepared and degassed with argon for one hour in two separate flasks. In the first flask butyl acrylate (1.5 mL, 10.46 mmol), DMF (2.73 mL), Cu(0) (10 pellets) and the thiolactone functionalised initiator (201.04 mg, 0.52 mmol) were mixed. In the second flask Cu(II)Br_2 (5.84 mg, 0.03 mmol), Me_6TREN (14.46 mg, 0.06 mmol) and DMF (1 mL) were mixed. The two mixtures were added to one another and stirred for 6 hours. The reaction was stopped and purified of its metal salts by diluting it with THF (5 mL) and filtering it over a basic alumina column. The solvent was removed via evaporation and the obtained polymer dried overnight at 40°C in a vacuum oven.
The kinetic study was performed as described above for polystyrene.

Synthesis of PEO with a thiolactone end group
The PEO with a alcohol end group was first evaporated 3 times over toluene to ensure no water was present during the urethane formation. The α-isocyanato-γ-thiolactone (429.6 mg, 3 mmol) and DBTL (0.02 mL, 0.03 mmol) were added to a mixture of the dried PEO alcohol (1.5 g, 0.75 mmol) and dry dichloromethane (5 mL). The reaction was allowed to stir for 48 hours. The polymer was purified via precipitation in cold diethylether and subsequent filtration. This purification procedure was repeated to achieve a higher purity. The filtered polymer was dried overnight at 40°C in a vacuum oven to remove any remaining solvent.

Synthesis of PCL with a thiolactone end groups
The PCL with alcohol end groups was first evaporated 3 times over toluene to ensure no water was present during the urethane formation. The α-isocyanato-γ-thiolactone (286.32 mg, 2 mmol) and DBTL (0.012 mL, 0.02 mmol) were added to a mixture of the dried PCL diol (1.5 g, 0.25 mmol) and dry dichloromethane (5 mL). The reaction was allowed to stir for 48 hours.

The polymer was purified via precipitation in cold diethylether and subsequent filtration. This purification procedure was repeated to achieve a higher purity. The filtered polymer was dried overnight at 40°C in a vacuum oven to remove any remaining solvent.

One-pot double modification of polystyrene and poly(butyl acrylate) with a thiolactone end group

First, 50 mg of the polymer was dissolved in chloroform (0.25 mL). Next, the acrylate (15 equivalents) and amine (10 equivalents) were measured in separate vials and diluted in $CHCl_3$ (0.1 mL). The acrylate solution was added first to the polymer mixture followed by the amine solution. The reaction was stirred for 48 hours and the polymer was isolated via precipitation in cold methanol and subsequent filtration. This purification procedure was repeated to achieve higher purity. The double-modified polymer obtained was dried overnight at 40°C in a vacuum oven to remove any remaining solvent.

One-pot double modification of PEO with a thiolactone end group and PCL with thiolactone end groups

First, 50 mg of the polymer was dissolved in $CHCl_3$ (0.25 mL). Next, the acrylate (30 equivalents relative to the thiolactone end groups) and amine (15 equivalents relative to the thiolactone end groups) were measured in separate vials and diluted in $CHCl_3$ (0.1 mL). The acrylate solution was added first to the polymer mixture followed by the amine solution. The reaction was stirred for 48 hours and the polymer was isolated via precipitation in cold diethylether and subsequent filtration. This purification procedure was repeated to achieve a higher purity. The double-modified polymer obtained was dried overnight at 40°C in a vacuum oven to remove any remaining solvent.

Synthesis of PEO-poly(butyl acrylate) block copolymer with a midchain functionality

A solution of methyl acrylate (16.4 mg, 0.19 mmol) in $CHCl_3$ (0.1 mL) was added to a solution of poly(butyl acrylate) with a thiolactone end group (160 mg, 0.038 mmol) in $CHCl_3$ (0.5 mL). To this mixture a solution of the PEO amine (152 mg, 0.19 mmol, 800 Da) was added and was reacted for 48 hours. Afterwards, the $CHCl_3$ was evaporated and LCxSEC analysis was performed to analyse the obtained mixture.

IV.5.4 Manipulation of the solid support

Swelling

The solid support was swollen in the reaction solvent for at least 10 minutes.

Washing

The loaded resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried after each reaction.

Loading of the acid-functionalised thiolactone linker

The acid-functionalised thiolactone (1.33 g, 1.2 equivalents relative to the resin, 5.8 mmol) was loaded on a 2-chlorotrityl chloride resin (3 g, 1.6 mmol/g) in anhydrous CH_2Cl_2 (30 mL, 10 mL/1 g of resin) with anhydrous DIPEA (0.26 mL, 4 equiv. rel. to acid) and 10 vol% of DMF (3 mL), in order to dissolve the acid.[36] The reaction mixture was shaken for 3 hours at room temperature. The resin was subsequently washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 6 x 30 mL), DMF (4 x 30 mL), CH_2Cl_2 (4 x 30 mL) and diethyl ether (4 x 30mL). The resin was dried under vacuum.

Loading of the hydroxyl-functionalised thiolactone linker

Different conditions were needed for this loading as an alcohol instead of an acid functionality is attached to the thiolactone. The hydroxyl-thiolactone (1.69 g, 1.5 equivalents relative to the resin, 7.2 mmol) was loaded on a 2-chlorotrityl chloride resin (3 g, 1.6 mmol/g) in anhydrous THF (30 mL, 10 mL/1 g of resin) with anhydrous pyridine (1.16 mL, 2 equivalents relative to the alcohol).[72] The reaction was shaken overnight at 60°C. Next, the solid support was washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 6 x 30 mL), DMF (4 x 30 mL), CH_2Cl_2 (4 x 30 mL) and diethyl ether (4 x 30 mL). The resin was dried under vacuum.

Double modification on the solid support: general procedure

$CHCl_3$ was added to the solid support (1 mL reaction mixture per 100 mg resin). Next, the acrylate (20 equivalents relative to the thiolactone unit) and amine (10 equivalents) were added. This mixture was shaken overnight at room temperature. Next, the resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Cleavage

For cleavage, 2 mg of solid support was treated with 0.5 mL of 1% trifluoroacetic acid (TFA) in CH_2Cl_2 for 5 minutes. During cleavage, the color of the beads turned from yellow to red because of the formation of a trityl cation. The solid support was then filtered, the sample concentrated by evaporation and dissolved in acetonitrile for LC-ESI-MS analysis.

Peptide synthesis

Coupling of amino acids and the acid-functionalised thiolactone linker: The resin was first swollen in DMF. HBTU (5 equivalents), the Fmoc-protected amino acid or acid-thiolactone linker (5 equivalents) and DIPEA (10 equivalents) were first mixed in DMF to activate the

amino acid. The mixture was added to the resin, shaken for two hours at room temperature, and then filtered off. The resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

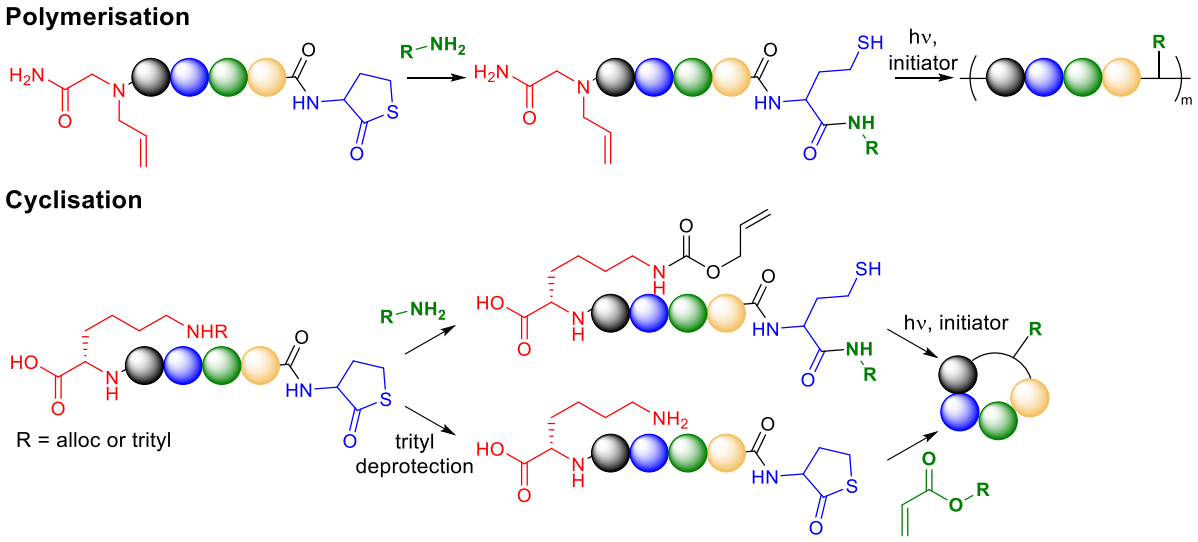
Fmoc-Deprotection: The resin was swollen in DMF. The DMF was removed by filtration and the resin was treated with a 20% piperidine solution in DMF for 2, 5, 15 and 2 times 30 minutes. Next, the resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

References

[1] P. Espeel, F. Goethals, F. Driessen, L.-T. T. Nguyen, F. E. Du Prez, *Polym. Chem.* **2013**, *4*, 2449.
[2] S. Reinicke, P. Espeel, M. M. Stamenović, F. E. Du Prez, *ACS Macro Lett.* **2013**, *2*, 539.
[3] A. J. Satti, P. Espeel, S. Martens, T. Van Hoeylandt, F. E. Du Prez, F. Lynen, *Journal of Chromatography A* **2015**, *1426*, 126.
[4] D. Braun, H. Cherdron, M. Rehahn, H. Ritter, B. Voit, "Functional Polymers", in *Polymer Synthesis: Theory and Practice: Fundamentals, Methods, Experiments*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2013, p. 375.
[5] K. A. Günay, P. Theato, H.-A. Klok, *Journal of Polymer Science Part A: Polymer Chemistry* **2013**, *51*, 1.
[6] P. Theato, "*Multi-Component and Sequential Reactions in Polymer Synthesis Preface"*, 2015, p. V.
[7] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2.
[8] P. Espeel, F. E. Du Prez, *Eur. Polym. J.* **2015**, *62*, 247.
[9] M. Langlais, I. Kulai, O. Coutelier, M. Destarac, *Macromolecules* **2017**, *50*, 3524.
[10] M. Langlais, O. Coutelier, M. Destarac, *Macromolecules* **2018**, *51*, 4315.
[11] C. Zhu, B. Yang, Y. Zhao, C. Fu, L. Tao, Y. Wei, *Polym. Chem.* **2013**, *4*, 5395.
[12] R. Kakuchi, P. Theato, *ACS Macro Lett.* **2014**, *3*, 329.
[13] A. Lv, X.-X. Deng, L. Li, Z.-L. Li, Y.-Z. Wang, F.-S. Du, Z.-C. Li, *Polym. Chem.* **2013**, *4*, 3659.
[14] S. C. Solleder, M. A. R. Meier, *Angew. Chem., Int. Ed.* **2014**, *53*, 711.
[15] B. Yang, Y. Zhao, S. Wang, Y. Zhang, C. Fu, Y. Wei, L. Tao, *Macromolecules* **2014**, *47*, 5607.
[16] B. Yang, Y. Zhao, C. Fu, C. Zhu, Y. Zhang, S. Wang, Y. Wei, L. Tao, *Polym. Chem.* **2014**, *5*, 2704.
[17] M. Benaglia, A. Alberti, L. Giorgini, F. Magnoni, S. Tozzi, *Polym. Chem.* **2013**, *4*, 124.
[18] M. Stuparu, A. Khan, "*Sequential Thiol-Epoxy and Esterification Reactions: A Facile Route to Bifunctional Homopolymer Sequences"*, 2014.
[19] B. Zhang, H. Zhang, R. Elupula, A. M. Alb, S. M. Grayson, *Macromol. Rapid Commun.* **2014**, *35*, 146.
[20] R. Kakuchi, P. Theato, *ACS Macro Lett.* **2013**, *2*, 419.
[21] D. H. Seuyep Ntoukam, G. A. Luinstra, P. Theato, *Journal of Polymer Science Part A: Polymer Chemistry* **2014**, *52*, 2841.
[22] C. Adrian Figg, A. N. Bartley, T. Kubo, B. S. Tucker, R. K. Castellano, B. S. Sumerlin, *Polym. Chem.* **2017**, *8*, 2457.
[23] T. Kubo, C. A. Figg, J. L. Swartz, W. L. A. Brooks, B. S. Sumerlin, *Macromolecules* **2016**, *49*, 2077.
[24] K. Matyjaszewski, J. Xia, *Chemical Reviews* **2001**, *101*, 2921.
[25] M. A. Tasdelen, M. U. Kahveci, Y. Yagci, *Prog. Polym. Sci.* **2011**, *36*, 455.
[26] I. Cobo, M. Li, B. S. Sumerlin, S. Perrier, *Nature Materials* **2014**, *14*, 143.
[27] T. Junkers, *Eur. Polym. J.* **2015**, *62*, 273.
[28] S. Aoshima, S. Kanaoka, *Chemical Reviews* **2009**, *109*, 5245.
[29] H. Willcock, R. K. O'Reilly, *Polym. Chem.* **2010**, *1*, 149.
[30] P. J. Roth, C. Boyer, A. B. Lowe, T. P. Davis, *Macromol. Rapid Commun.* **2011**, *32*, 1123.

[31] V. Delplace, S. Harrisson, H. T. Ho, A. Tardy, Y. Guillaneuf, S. Pascual, L. Fontaine, J. Nicolas, *Macromolecules* **2015**, *48*, 2087.
[32] C. A. Figg, T. Kubo, B. S. Sumerlin, *ACS Macro Lett.* **2015**, *4*, 1114.
[33] F. Driessen, S. Martens, B. D. Meyer, F. E. Du Prez, P. Espeel, *Macromol. Rapid Commun.* **2016**, *37*, 947.
[34] A. E. M. Crotti, T. Fonseca, H. Hong, J. Staunton, S. E. Galembeck, N. P. Lopes, P. J. Gates, *International Journal of Mass Spectrometry* **2004**, *232*, 271.
[35] D. P. Demarque, A. E. M. Crotti, R. Vessecchi, J. L. C. Lopes, N. P. Lopes, *Natural Product Reports* **2016**, *33*, 432.
[36] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[37] B. D. Mather, K. Viswanathan, K. M. Miller, T. E. Long, *Prog. Polym. Sci.* **2006**, *31*, 487.
[38] S. Reinicke, P. Espeel, M. M. Stamenovic, F. E. Du Prez, *Polym. Chem.* **2014**, *5*, 5461.
[39] Y. E. Jad, G. A. Acosta, S. N. Khattab, B. G. de la Torre, T. Govender, H. G. Kruger, A. El-Faham, F. Albericio, *Organic & Biomolecular Chemistry* **2015**, *13*, 2393.
[40] F. Goethals, S. Martens, P. Espeel, O. van den Berg, F. E. Du Prez, *Macromolecules* **2014**, *47*, 61.
[41] R. J. Ouellette, J. D. Rawn, "6 - Alkenes: Addition Reactions", in *Organic Chemistry (Second Edition)*, R.J. Ouellette and J.D. Rawn, Eds., Academic Press, 2018, p. 167.
[42] K. E. Maudens, S. M. R. Wille, W. E. Lambert, *Journal of Chromatography B* **2007**, *848*, 384.
[43] K. G. Feitsma, B. F. H. Drenth, *Pharmaceutisch Weekblad* **1988**, *10*, 1.
[44] E. P. Kroeff, D. J. Pietrzyk, *Analytical Chemistry* **1978**, *50*, 1353.
[45] D. Van Lysebetten, S. Felissati, E. Antonatou, L. L. G. Carrette, P. Espeel, E. Focquet, F. E. Du Prez, A. Madder, *ChemBioChem* **2018**, *19*, 641.
[46] M. Geffe, L. Andernach, O. Trapp, T. Opatz, *Beilstein Journal of Organic Chemistry* **2014**, *10*, 701.
[47] K. Rice, A. Brossi, *J. Org. Chem.* **1980**, *45*, 592.
[48] P. Lloyd-Williams, F. Albericio, E. Giralt, "*Chemical Approaches to the Synthesis of Peptides and Proteins"*, Taylor & Francis, 1997.
[49] O. F. Luna, J. Gomez, C. Cárdenas, F. Albericio, S. H. Marshall, F. Guzmán, *Molecules* **2016**, *21*, 1542.
[50] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815.
[51] L. Charles, *Mass Spectrometry Reviews* **2014**, *33*, 523.
[52] H. Nonaka, M. Ouchi, M. Kamigaito, M. Sawamoto, *Macromolecules* **2001**, *34*, 2083.
[53] S. Celasun, F. E. Du Prez, H. G. Börner, *Macromol. Rapid Commun.* **2017**, *38*, 1700688.
[54] C. F. Hansell, P. Espeel, M. M. Stamenović, I. A. Barker, A. P. Dove, F. E. Du Prez, R. K. O'Reilly, *J. Am. Chem. Soc.* **2011**, *133*, 13828.
[55] G. L. Ellman, *Archives of Biochemistry and Biophysics* **1958**, *74*, 443.
[56] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[57] H.-A. Klok, *Journal of Polymer Science Part A: Polymer Chemistry* **2005**, *43*, 1.
[58] A. Rösler, H.-A. Klok, I. W. Hamley, V. Castelletto, O. O. Mykhaylyk, *Biomacromolecules* **2003**, *4*, 859.
[59] R. Duncan, *Nat Rev Drug Discov* **2003**, *2*, 347.
[60] A. S. Hoffman, P. S. Stayton, *Macromolecular Symposia* **2004**, *207*, 139.
[61] S. Martens, F. Driessen, S. Wallyn, O. Türünç, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2016**, *5*, 942.
[62] B. J. Kim, J. Bang, C. J. Hawker, J. J. Chiu, D. J. Pine, S. G. Jang, S.-M. Yang, E. J. Kramer, *Langmuir* **2007**, *23*, 12693.
[63] L. Petton, A. E. Ciolino, M. M. Stamenović, P. Espeel, F. E. Du Prez, *Macromol. Rapid Commun.* **2012**, *33*, 1310.
[64] T. Moriguchi, T. Endo, *Macromolecules* **1995**, *28*, 5386.
[65] A. Sudo, H. Morishita, T. Endo, *Journal of Polymer Science Part A: Polymer Chemistry* **2011**, *49*, 1097.
[66] E. Hrsic, I. Zografou, B. Schulte, A. Pich, H. Keul, M. Möller, *Polymer* **2013**, *54*, 495.

[67] M. Liras, O. García, N. Guarrotxena, M. Palacios-Cuesta, I. Quijada-Garrido, *Polym. Chem.* **2013**, *4*, 5751.
[68] W. Van Camp, F. E. Du Prez, S. A. F. Bon, *Macromolecules* **2004**, *37*, 6673.
[69] C. Pietsch, R. Hoogenboom, U. S. Schubert, *Angew. Chem., Int. Ed.* **2009**, *48*, 5653.
[70] M. Ciampolini, N. Nardi, *Inorganic Chemistry* **1966**, *5*, 41.
[71] X. Ma, J. Tang, Y. Shen, M. Fan, H. Tang, M. Radosz, *J. Am. Chem. Soc.* **2009**, *131*, 14795.
[72] B. B. Shankar, D. Y. Yang, S. Girton, A. K. Ganguly, *Tetrahedron Lett.* **1998**, *39*, 2447.



Abstract

This chapter reports on how the polymerisation and cyclisation of oligopeptides were performed with different thiolactone-based strategies. For the polymerisation, oligopeptides were prepared containing both an ene and thiolactone end group to obtain an AB'-monomer. The aminolysis of the thiolactone generates a thiol that can react with the ene functionality via radical thiol-ene chemistry. For the cyclisation, in turn, two on-resin strategies were explored. The first cyclisation method was similar to the polymerisations executed with the aminolysis of ene-thiolactone-functionalised oligopeptides and subsequent photo-induced radical thiol-ene chemistry. For the second cyclisation strategy, oligopeptides were synthesised containing a thiolactone unit and lysine, which were able to open the thiolactone ring and generate a thiol. Subsequently, the latter formed a disulphide bond with another cycle, which resulted in the formation of bicyclic 'handcuff-like' oligopeptides. Reduction of the disulphide and the subsequent reaction with an acrylate allowed the functionalisation of the cycle.

Specific author contributions can be found at the end of section V.1

Chapter V
Thiolactone chemistry for oligopeptide polymerisation and cyclisation

V.1 Introduction

In the previous chapter, it was observed that a thiolactone unit can be easily installed as the end group of an oligopeptide and double-modified with the amine-thiol-ene conjugation. In this chapter, oligopeptides will be polymerised and cyclised via aminolysis of this thiolactone unit and subsequent thiol-ene reaction.

In recent decades, chemists have extensively prepared, developed and explored new functional polymeric architectures with tailored morphologies and structure-property relationships. Although much progress has been made in this field, the structures obtained are not yet capable of reaching the same potential as natural polymers such as DNA, RNA and proteins, which have a defined primary structure that determines their secondary, tertiary and quaternary structure. In the last decade, great efforts have been made to synthesise sequence-controlled structures.[1-3]

A specific strategy to prepare sequence-controlled polymers is to first synthesise heterotelechelic sequence-defined oligomers or polymers prepared with reversible deactivation radical polymerisation techniques, which are subsequently polymerised.[4] In order to obtain these polymers with repetitive patterns, different methods have been applied, such as ROMP (ring-opening metathesis polymerization) of sequence-defined macrocyclic structures[5, 6], ATRA (atom transfer radical addition) and ATRC (atom transfer radical coupling)[7, 8], polycondensations[9, 10], RACP (Radical Addition-Coupling Polymerisation)[11], CuAAC (copper catalyzed Huisgen-type 1,3-dipolar cycloaddition reaction)[12-15], and radical[16-18] and nucleophilic thiol-ene reactions.[19] Repetitive patterns of oligopeptides are also found in proteins such as elastine[12, 20], silk[13, 21] and the adhesive protein from the mussel *Mytilus edulis*.[22, 23] Polymers with oligopeptide-repeating units have different biomedical applications, such as drug delivery[24-26], protein purification[27], tissue engineering[28-31], and novel biomaterials.[32-34] These polymers can be genetically engineered[20] or prepared by means of polymerisation of a heterotelechelic oligopeptide.[12, 13] The latter strategy was exploited by Guan's group to prepare elastin-mimic polymers by means of CuAAC with pentapeptides containing alkyne and azido end groups.[12] Three different pentapeptides were prepared: VPGVG, GVGVP and V^{D}PGVG, which looks similar to the first, but has D-proline on the second position instead of L-proline (V, P and G are respectively valine, proline and glycine). After polymerisation of these oligomers, the polymers obtained showed differences in lower critical solution temperature (LCST) and local secondary structures. The order of the oligomeric

sequence and the chiral centres thus have an influence on the properties of the resulting polymers.

In addition to peptide-based linear polymers, another interesting class of macromolecules is that of cyclic peptides, which can be applied as drugs with antibacterial, immunosuppressive or antitumor activity.[35] In comparison to their linear counterparts, cyclic peptides have, generally, different solubility, aggregation and folding behaviour[35, 36], and a better resistance to both exo- and endoproteases[37], receptor binding[38] and cell permeability.[39] However, the latter was contradicted by a study from Kodadek's group.[40] Different methods have been developed for the (on-resin) synthesis of cyclic peptides: lactamisation[41, 42], lactonisation[41, 43], CuAAC[44, 45], ring-closing metathesis[46, 47], enzymatic cyclisation[48, 49], native chemical ligation[50-52], disulphides formation[36, 53, 54], multicomponent reactions[55, 56] and thiol-ene reaction.[57-59] The way the peptide is cyclised is also determined by the position of the functional handles: head-to-tail, head-to-side-chain, side-chain-to-tail and side-chain-to-side-chain.[41] High diluted conditions are used for cyclisations to prevent oligomerisation. Some cyclisations are performed on a solid support, because a pseudo-dilution is obtained and simple purification procedures such as washing and filtrating often suffice.[58] Although different methods and chemistries can be used to prepare cyclised oligopeptides, the amine-thiolactone-ene conjugation could elegantly provide the cyclisation and the functionalisation of an oligopeptide with a chemical handle in one-pot. These introduced handles would allow for further modification, labelling, or immobilisation.

This chapter highlights the development of sequence-controlled polymers and cycles based on oligopeptides containing an ene and a thiolactone functionality (Figure V.1). For the polymerisation, peptoid synthesis was applied to install the first functionality and the thiolactone-functionalised acid mentioned in the previous chapter was used to incorporate the second functionality. After aminolysis, the formed thiol could react in a radical thiol-ene reaction to form a periodic sequence-controlled peptide-based polymer.

Next, peptides were prepared to perform on-resin macrocyclisation with thiolactones via two different approaches. For the first one, cycles were prepared through the aminolysis of an end-standing thiolactone and subsequent photo-induced radical thiol-ene. The aminolysis of the thiolactone allowed the introduction of different functionalities. The second cyclisation method started from oligopeptides bearing a thiolactone and an amine, which was introduced via a lysine moiety. The amine will open the thiolactone ring and generate a thiol which forms disulphides with another cycle to form bicyclic 'handcuff-like' oligopeptides. Reduction of the formed disulphides generated thiols which can react with an acrylate to functionalise the cycle.

Parts of the research described in this chapter were based on the master's project of Yamin Abdouni, which was guided by myself. Co-guides were Dr. Willem Vannecke and Dorien Van Lysebetten of the research group led by Prof. Annemieke Madder. The results obtained were discussed, analysed and commented upon by the collaborators at all stages.

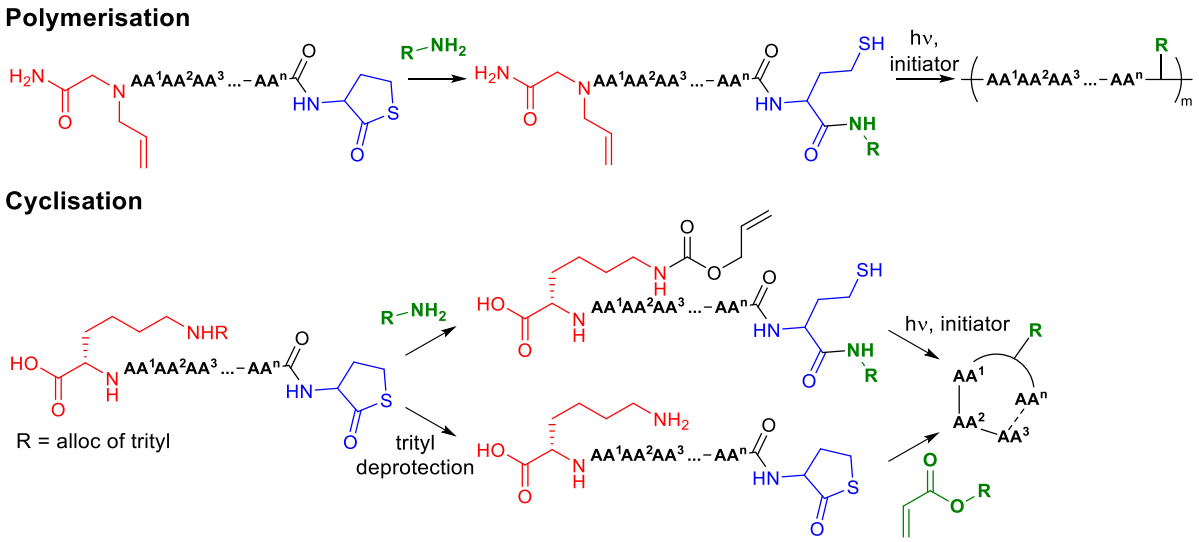


Figure V.1: Polymerisation (top) and cyclisation (bottom) strategies based on the aminolysis of a thiolactone yielding a free thiol for subsequent thiol-ene reactions.

V.2 Polymerisation of oligopeptides based on thiolactone chemistry

V.2.1 Monomer synthesis and polymerisation

Previous research in our group proved that the combination of the aminolysis of thiolactone and radical thiol-ene polymerisation is an efficient approach for the preparation of functional polymers.[60, 61] The strategies started from AB' monomers which contain an end-standing ene and thiolactone functionality. Aminolysis of the latter results in the formation of a thiol that can react with the ene functionality in a subsequent reaction (Figure V.1). AB' monomers were chosen in these approaches as they guarantee a one to one ratio of both the ene and thiolactone.

In order to ensure a one-to-one ratio of both functionalities, oligopeptides with an ene and thiolactone functionality were prepared on a solid support. The ene functionality was introduced through peptoid synthesis via a submonomer approach, as the functionality can be easily installed with allylamine and the chemicals needed come at a fairly low cost (Figure V.2).[62, 63] The word submonomer originates from the two submonomers, amine and haloacetic acid, that are needed to synthesise the N-substituted monomer.[64] The thiolactone end group was easily introduced via conventional amide coupling of the acid-functionalised thiolactone which was presented in the previous chapter.

However here, a different solid support was chosen for the AB peptidic monomer synthesis. The Rink amide (Aminomethyl)polystyrene (AM PS) resin was chosen, as it generates amide end groups instead of carboxylic acids which could cause problems during the aminolysis of

the thiolactone unit. In the first step, bromoacetic acid is coupled to the amine-containing resin (Figure V.2). In the next step, allyl amine was added to install the ene functionality.

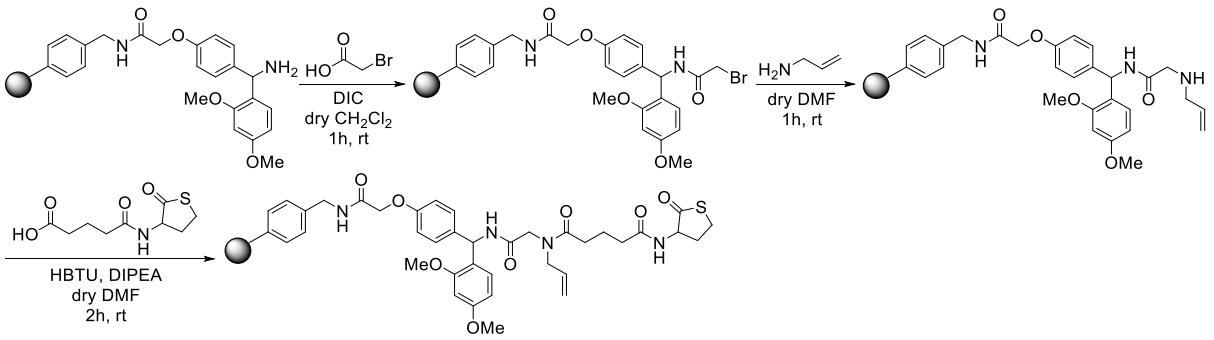


Figure V.2: Peptoid synthesis with allylamine and coupling of the acid-functionalised thiolactone on Rink amide AM PS resin.

In order to explore the polymerisation conditions, a simple monomer was first made by coupling the acid-functionalised thiolactone immediately to N-allyl-glycinamide on the solid support (Figure V.2). The product was cleaved from the solid support with a TFA/Triisopropylsilane (TIPS)/H_2O (95/2.5/2.5) mixture and precipitated in cold methyl tert-butyl ether (MTBE). The product obtained was characterised with NMR and LC-MS analysis (Figure V.3).

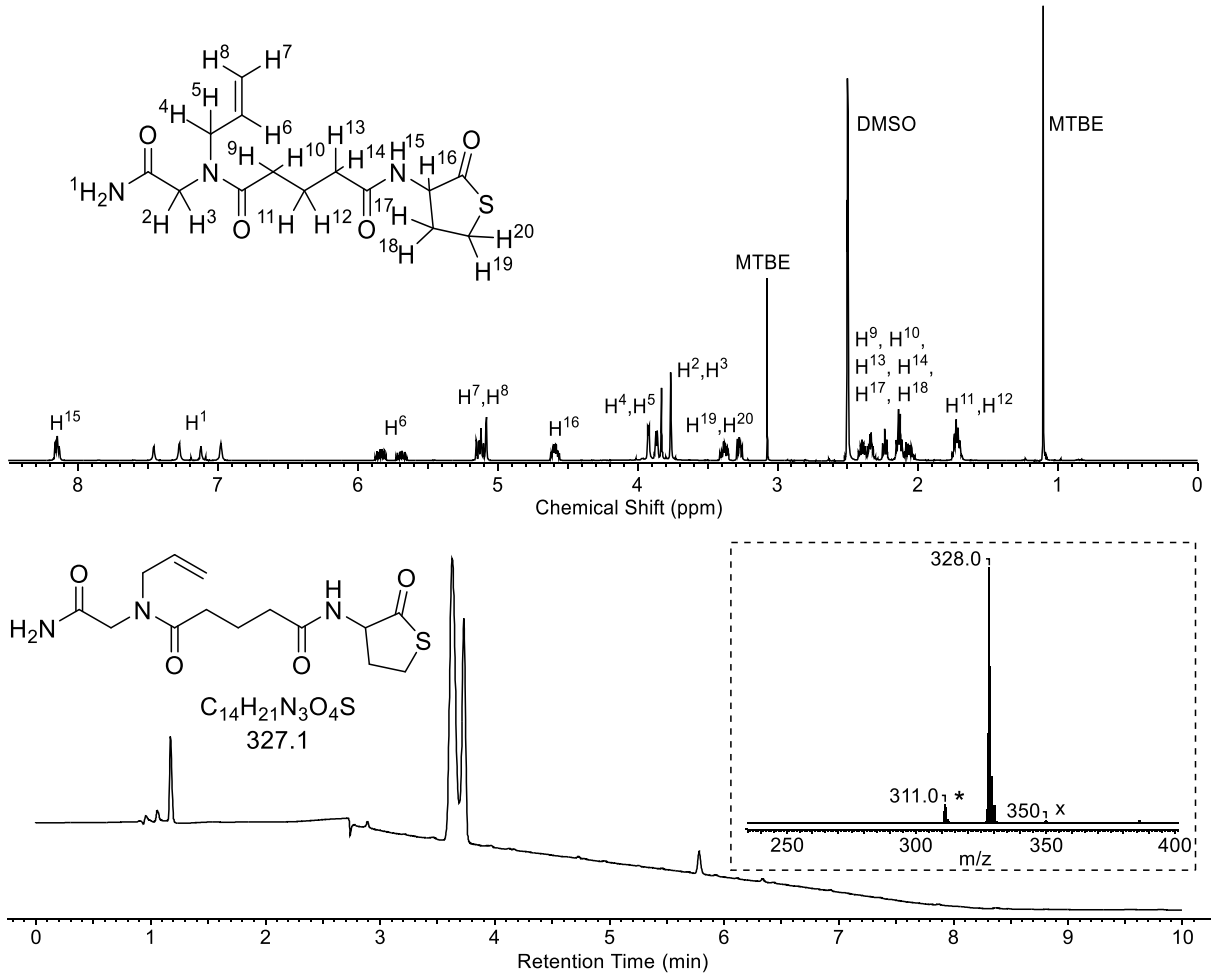


Figure V.3: ^{1}H-NMR spectrum (300 MHz, DMSO-d_6) with peak assignment (top) and HPLC trace with ESI-MS analysis (positive mode with sodium adduct (x) and fragmentation may be caused by charge migration fragmentation (via inductive cleavage assisted by adjacent heteroatoms) of the primary amide with the elimination of ammonia (*)[65]) (bottom) of the thiolactone amide N-allyl-glycinamide.

In Figure V.3, it can be observed that two peaks are visible in the LC trace for the same compound. This can possibly be attributed to cis/trans isomerisation, which was discussed in section IV.2.2.[36, 66, 67]

The test polymerisation was performed in a 1/1 solution of acetonitrile and water, because this mixture was able to dissolve various oligopeptides for LC-MS analysis samples and both solvents were used separately for radical thiol-ene polymerisations[68], so the combination was not expected to cause any problems.

Based on findings from previous research, 1.1 equivalents of butyl amine and 0.05 equivalents of 2,2-dimethoxy-2-phenylacetophenone (DMPA) were added and the polymerisation was performed for 5 hours (Figure V.4).[61] Existing literature suggested that the concentration of the monomer should be high to obtain high molecular weights.[69] As a consequence, a concentration of 1.8 M was used for the polymerisation.

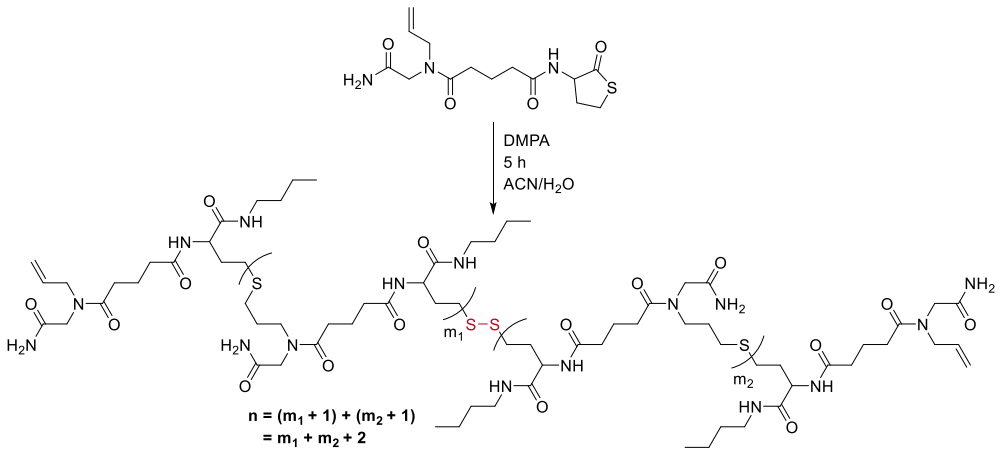


Figure V.4: Thiol-ene polymerisation and disulphide formation of the thiolactone amide N-allyl-glycinamide with butylamine.

Dimethylacetamide-based size exclusion chromatography (DMA SEC) indicated a number average molecular weight (M_n) around 4100 g/mol and a dispersity (Đ) of 1.84 (Figure V.5). In this chromatographic technique, macromolecules are separated based on their hydrodynamic volume, which can be coupled to their molar mass.[70] As low molecular weight molecules can penetrate into the pores of the crosslinked packing material of the column, they will have a higher retention time in comparison to higher molecular weight molecules. A peak can also be observed at low molecular weight (high retention time) in the SEC analysis, which could be due to unreacted monomers or the formation of disulphide (n=2 in Figure V.4) or cyclised oligomers.

MALDI-TOF analysis proved that macromolecules had been formed, but that disulphide formation had occurred as well because the expected total mass minus 2 Da was observed (Figures V.4 and V.6). In the MALDI spectrum, smaller mass peaks can be observed right before the targeted polymer peaks, which correspond to the hydrolysis of one thiolactone unit. In the future, water should be avoided as a solvent, since a basic medium, generated by the

amine, might also cause hydrolysis of the thiolactone unit.[71] Despite these side products, this approach was used for the synthesis of sequence-controlled oligopeptide-based polymers.

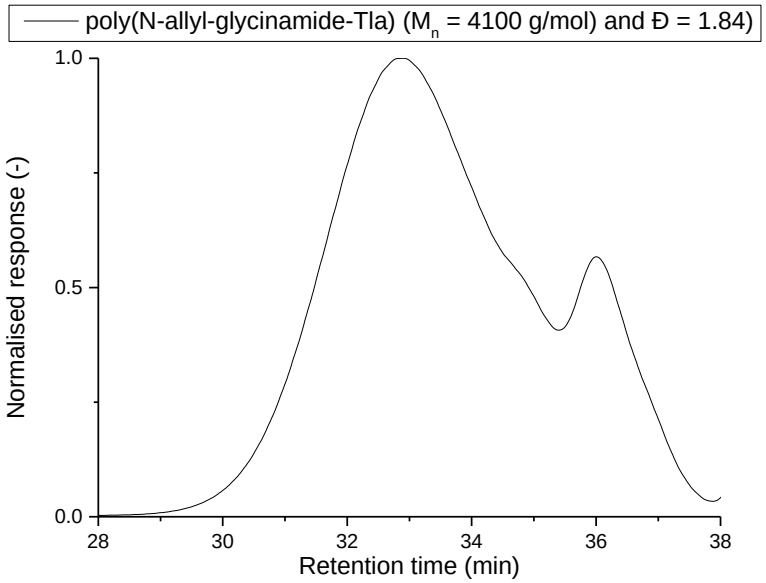
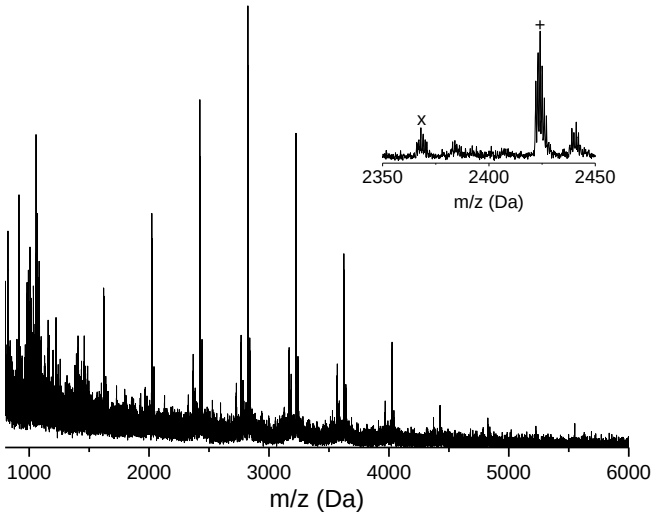


Figure V.5: SEC analysis of the thiolactone amide N-allyl-glycinamide polymer in DMA with poly(methyl methacrylate) (PMMA) standards.



	Na adduct of disulphides	
n	Theoretical m/z (Da)	Experimental m/z (Da)
3	1221.56	1221.53
4	1621.75	1621.68
5	2021.94	2021.90
6	2422.13	2422.04
7	2822.31	2822.18
8	3222.50	3222.41
9	3622.69	3622.50
10	4022.88	4022.90
11	4423.07	4423.49
12	4823.26	4823.84

Figure V.6: MALDI-TOF spectrum (left) and table with expected masses of the sodium adducts of the formed disulphides (right) of the thiolactone amide N-allyl-glycinamide polymer prepared via aminolysis with butylamine and DMPA as photoinitiator. Zooming in shows a peak due to disulphide formation (+) and peak corresponding to the polymer containing one hydrolysed position (x).

V.2.2 Oligomer synthesis and polymerisation

Inspired by Guan's work and by the fact that the repetition of the alanine-glycine (AG) motif can be found in silk[13], the hexamer AGAGAG was synthesised with the N-allyl-glycinamide and thiolactone end groups, and analysed with LC-MS (Figure V.7). The prepared oligomer was polymerised in the conditions used for the monomer, *i.e.* butylamine for aminolysis, DMPA as initiator and acetonitrile as solvent. MALDI-TOF analysis indicated that some

oligomerisation had occurred, but that disulphides were formed as well (Figure V.8). This time, no distinct hydrolysis was observed in the MALDI spectrum. Gelation was observed during polymerisation; it was assumed that this hampers the progress of the polymerisation (Figure V.9).

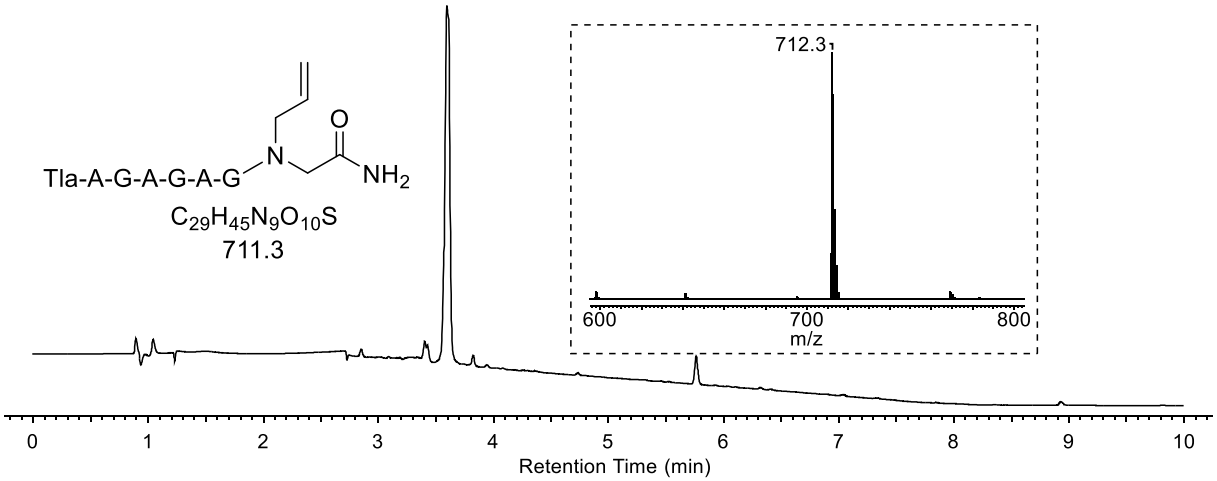
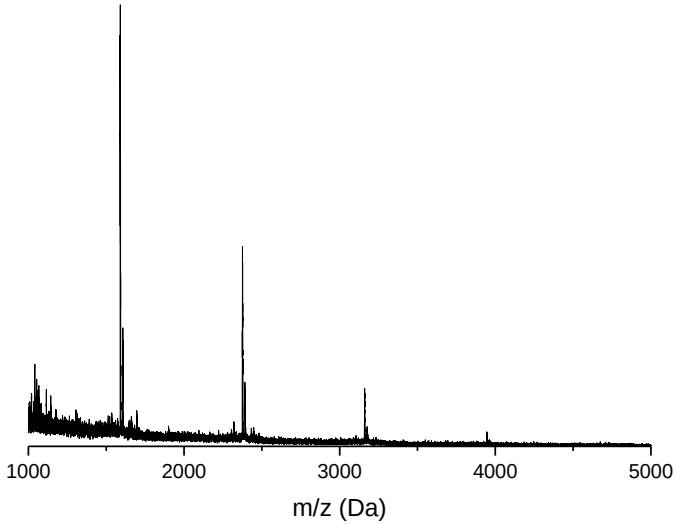


Figure V.7: HPLC trace with ESI-MS analysis (positive mode) of the thiolactone-AGAGAGG(N-allyl)-NH_2 (Tla = acid-functionalised thiolactone).



	Na adduct of disulphides	
n	Theoretical m/z (Da)	Experimental m/z (Da)
2	1589.77	1589.78
3	2374.16	2374.49
4	3158.55	3159.10
5	3942.94	3943.59

Figure V.8: MALDI-TOF analysis of the thiolactone-AGAGAGG(N-allyl)-NH_2 polymer prepared via aminolysis with butylamine and DMPA as photoinitiator. Disulphide formation was observed.

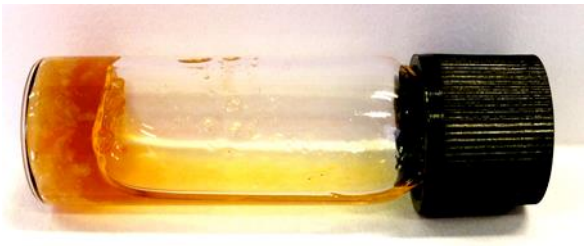


Figure V.9: Gel formation during polymerisation of the thiolactone-AGAGAGG(N-allyl)-NH_2 oligomer.

Next, a smaller tetrapeptide AGAG was synthesised with the N-allyl-glycinamide and thiolactone end groups, in order to more easily explore the polymerisation conditions (Figure V.10). The peptide synthesis and thiolactone coupling were performed on an automated peptide

synthesiser to prepare in parallel enough material to test different polymerisation conditions. Oligomerisation and disulphide formation could again be observed in the MALDI-TOF spectrum (Figure V.11, left). As the gelation had occurred again, the concentration of the monomer was halved to prevent this behaviour, yet this resulted only in dimer and trimer formation of the oligopeptide (Figure V.11, right). Different conditions and strategies were tried to improve the polymerisation, such as the addition of initiator at the beginning or during the polymerisation, more equivalents of amine, a thermal initiator (2-2´-Azobis(isobutyronitrile) (AIBN), DMA as solvent, a smaller oligopeptide (Figures V.12 and V.13), *etc.* , yet nothing seemed to improve the polymerisation. Other oligopeptides could perhaps be explored to this end in the future.

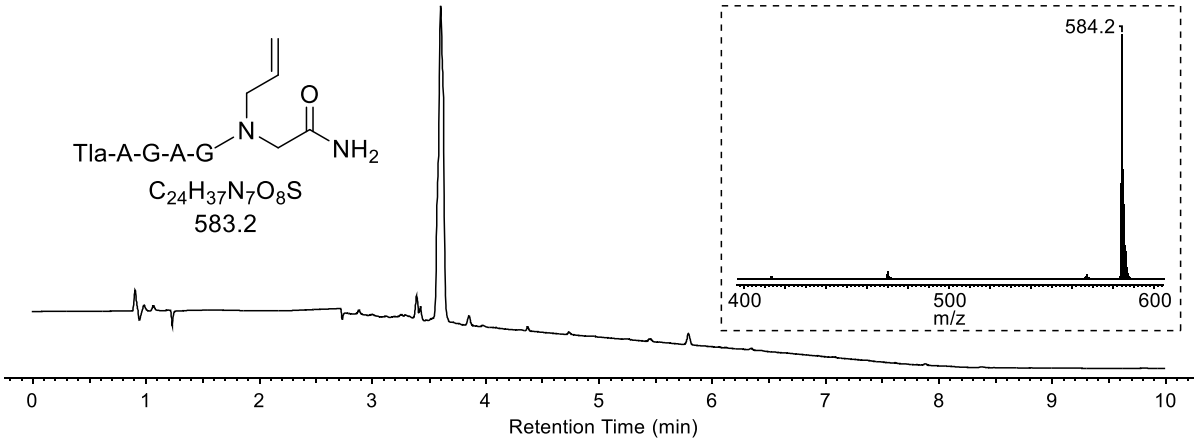
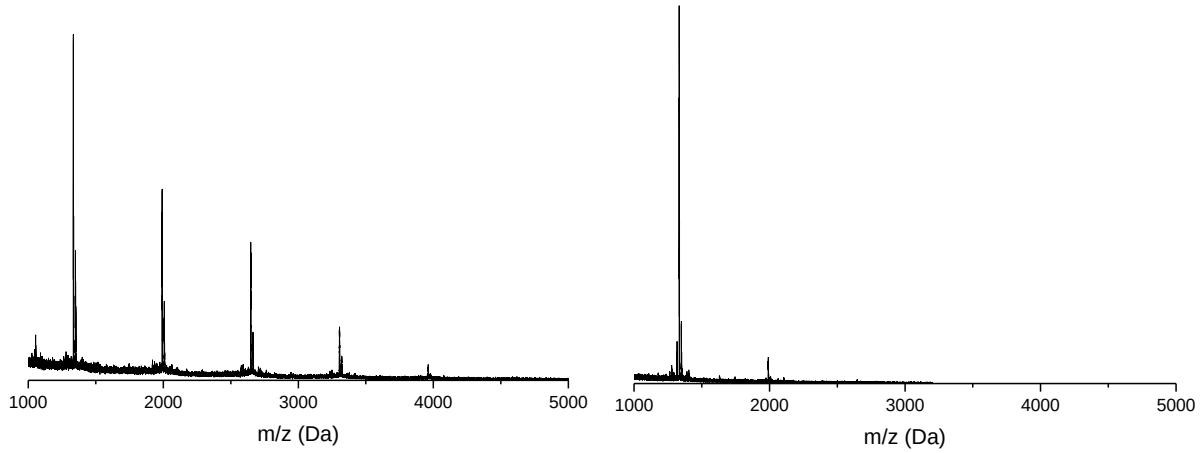


Figure V.10: HPLC trace with ESI-MS analysis (positive mode) of the thiolactone-AGAGG(N-allyl)-NH_2.



	Na adduct of disulphides	
n	Theoretical m/z (Da)	Experimental m/z (Da)
3	1333.65	1333.59
4	1989.99	1990.21
5	2646.32	2646.59
6	3302.65	3303.00
7	3958.98	3959.40

Figure V.11: MALDI-TOF analysis of the thiolactone-AGAGG(N-allyl)-NH_2 polymer with a monomer concentration of 1.8 M (left) and 0.9 M (right) prepared via aminolysis with butylamine and DMPA as photoinitiator. Disulphide formation was observed.

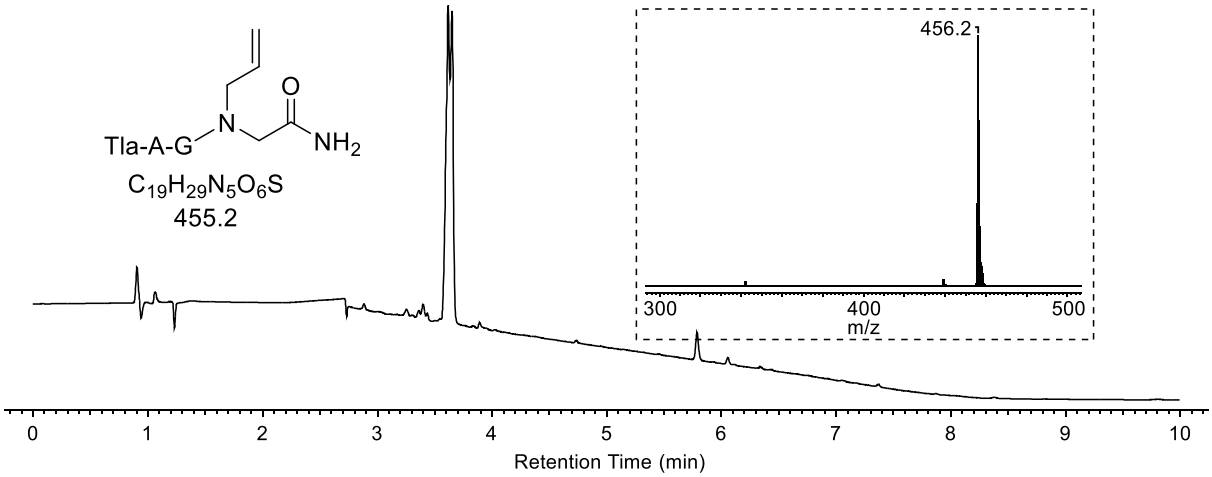
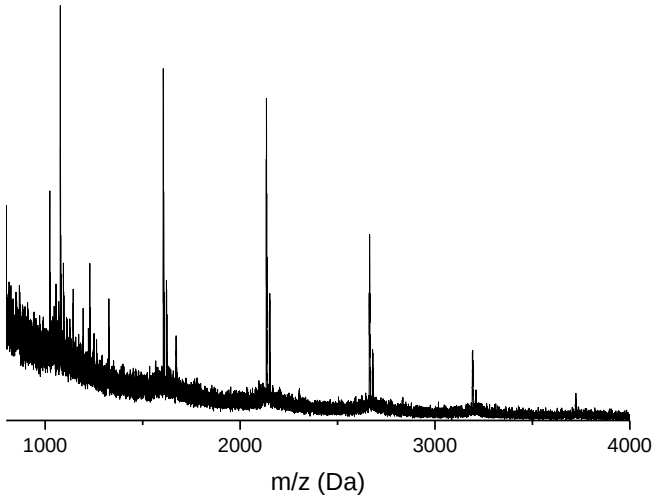


Figure V.12: HPLC trace with ESI-MS analysis (positive mode) of the thiolactone-AGG(N-allyl)-NH_2.



	Na adduct of disulphides	
n	Theoretical m/z (Da)	Experimental m/z (Da)
2	1077.54	1077.51
3	1605.81	1605.92
4	2134.08	2134.48
5	2662.36	2663.04
6	3190.63	3191.30
7	3718.90	3719.60

Figure V.13: MALDI-TOF analysis of the thiolactone-AGG(N-allyl)-NH_2 polymer prepared via aminolysis with butylamine and DMPA as photoinitiator. Disulphide formation was observed.

V.3 Cyclisation of oligopeptides based on thiolactone chemistry

V.3.1 Amine-thiol-ene conjugation for cyclisation

In this chapter, two strategies based on thiolactone chemistry were developed for the chain-to-tail cyclisation of oligopeptides. For the first strategy, the radical thiol-ene conjugation was applied in combination with a thiolactone unit which allows for the incorporation of a functionality via aminolysis. Anseth and co-workers used a radical thiol-ene reaction to cyclise oligopeptides that contain an alloc-protected lysine and a cysteine (Figure V.14, left).[58] This approach inspired us to prepare oligopeptides that contain a thiolactone group and an alloc-protected lysine which could cyclise after aminolysis via a thiol-ene reaction (Figure V.14,

right). The aminolysis also allows for the incorporation of a functionality on the cycle which can be used for immobilisation, labelling or additional modification.

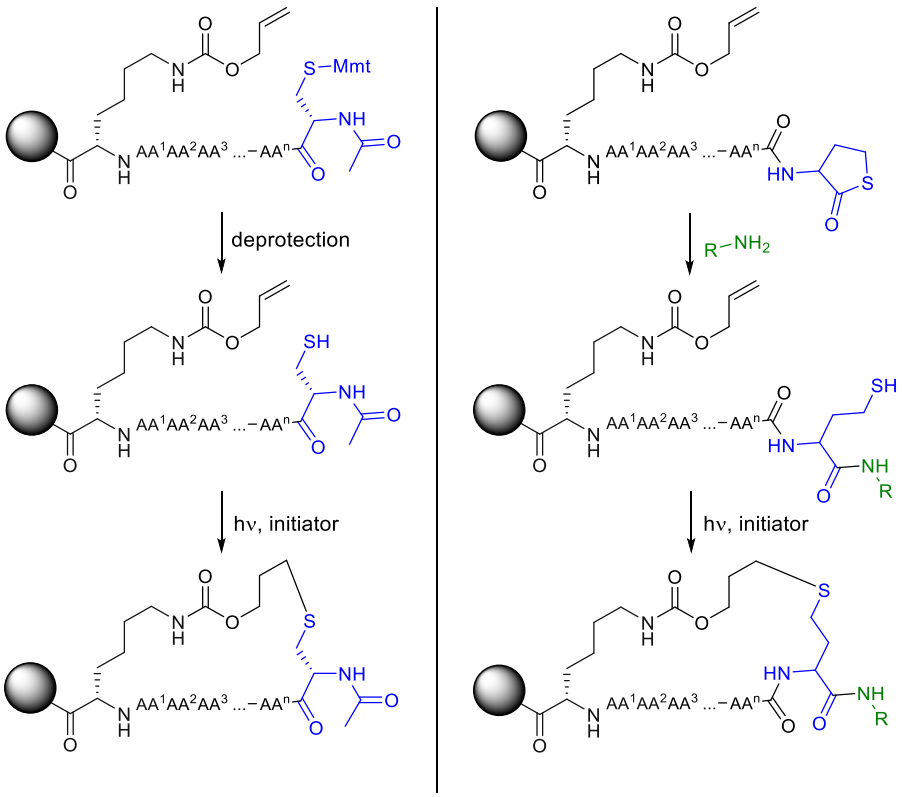


Figure V.14: Thiol-ene cyclisations of oligopeptides which contain an alloc-protected lysine and a cysteine (Mmt = monomethoxytrityl) (left) (method developed by Anseth's group)[58] or a thiolactone unit (right).

The alloc-protected lysine was immobilised on the 2-chlorotrityl chloride resin and the thiolactone end-capped oligopeptide AGAGAGK(Alloc) was prepared. The specific oligopeptide sequence (AGAGAG) was made because it had been cyclised before with a different thiolactone-based cyclisation strategy in solution, which was based on disulphide formation between a terminal thiol and a thiol that was released after the aminolysis of the thiolactone.[36] Moreover, this specific sequence was also chosen to make a parallel with the polymerization study described in the previous paragraph and to determine if conditions could be found to avoid disulphide formation.

First, four cyclisation reactions were performed on the resin in DMF with one equivalent of DMPA initiator, according to the experiments performed by Anseth and co-workers[58], and different reaction times and amounts of butylamine, which respectively varied between one or two hours and two or four equivalents (Table V.1, entries 1-4). After 2 hours and 4 equivalents of amine, some aminolysis of the thiolactone occurred, yet it resulted in undesirable disulphide formation (Table V.1, entry 4 and Figure V.15). Next, four experiments were carried out with more equivalents of amine, i.e. ten and twenty equivalents, and DMPA concentrations varying between one and two equivalents compared to the resin loading (Table V.1, entries 5-8). Complete aminolysis occurred, but resulted once more in undesirable disulphide formation.

Table V.1: Influence of reaction time and equivalents of amine on the solid-phase cyclisation reaction.

	Time (h)	DMPA (Eq)	Amine (Eq)	result
1	1	1	2	No aminolysis
2	1	1	4	No aminolysis
3	2	1	2	No aminolysis
4	2	1	4	Little aminolysis + disulphide formation
5	2	1	10	Aminolysis + disulphide formation
6	2	2	10	Aminolysis + disulphide formation
7	2	1	20	Aminolysis + disulphide formation
8	2	2	20	Aminolysis + disulphide formation

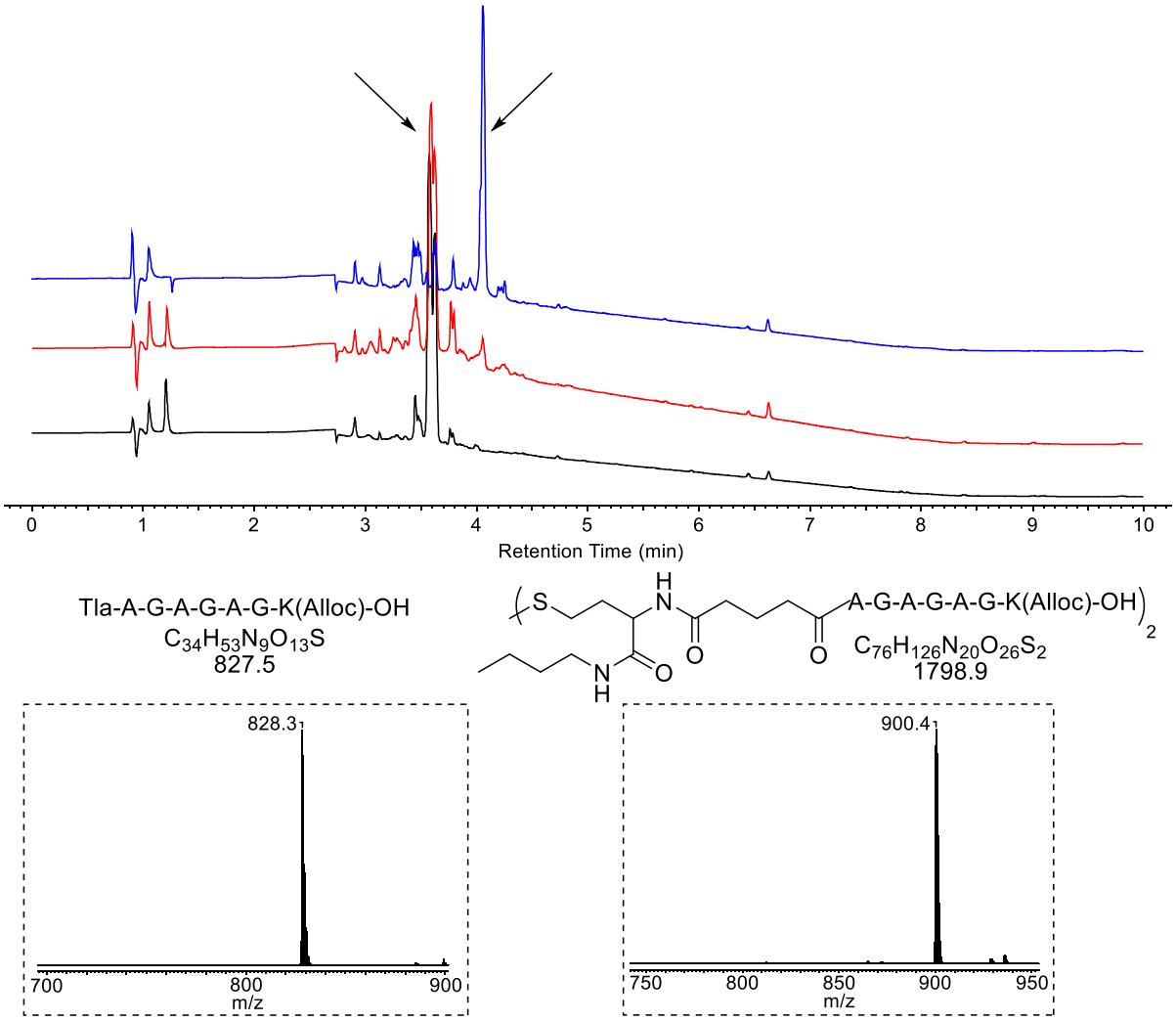


Figure V.15: HPLC trace with ESI-MS analysis (positive mode) of the oligopeptide thiolactone-AGAGAGK(Alloc) (black) and its cyclisation attempts (red and blue, entries 4 and 8 in Table V.1). Left arrow: oligopeptide with a closed thiolactone (left MS spectrum), right arrow: disulphide formation (right MS spectrum).

As the experiments with the Tla-AGAGAGK(Alloc) oligopeptide were not successful, another oligopeptide, Tla-VPGVGK(Alloc), was chosen to try the cyclisation. The oligopeptide chosen contains glycine and proline, which should stimulate the cyclisation as turn and loop structures of proteins frequently contain these amino acids.[72] Inspired by the success of Anseth's on-resin cyclisation method[58], a different resin, Rink amide AM PS resin with a lower loading

(0.69 mmol/g) compared to the 2-chlorotrityl chloride resin (1.6 mmol/g), was also chosen to promote our on-resin cyclisation. The cyclisation was performed for two hours with ten equivalents of butylamine and 2 equivalents of DMPA. After the reaction, an Ellman's test was performed to prove the presence of free thiols on the resin. This presence was indeed proven by the distinctive yellow colour that was observed (Figure V.16).



Figure V.16: Yellow colour formation during the Ellman's test.

As the mass of the linear oligopeptide and the cyclic derivative are the same, benzyl acrylate was added to react with the remaining thiols of the linear oligomer to distinguish between the two peptidic species (Figure V.17, arrows 2 and 3). The masses corresponding to cyclic peptide (and linear one before functionalization) and the modified linear oligopeptide were observed after the reaction with benzyl acrylate (Figure V.17, arrow 5). Considering that the signal of the linear oligopeptide is decreasing in the HPLC trace (Figure V.17, arrow 3 in the red and blue trace), it can be concluded that the remaining HPLC/mass signals correspond exclusively to the cyclic peptide and therefore that cyclisation can be performed via amine-thiol-ene conjugation.

In order to compare our strategy with the one proposed by Anseth (Figure V.14), another oligopeptide, Tla-RGDSFK(Alloc), was chosen for cyclisation (R, D, S, F and K are respectively arginine, aspartic acid, serine, phenylalanine and lysine).[58] This oligopeptide is certainly relevant since it contains the RGD sequence, which plays a central role in cell adhesion biology.[73] This sequence is the cell attachment site of many adhesive proteins such as fibronectin[74] and can be recognised by integrins, which are transmembrane receptors. The cyclisation of the prepared oligopeptide was done using the conditions mentioned (*vide supra*) and free thiols were again captured with benzyl acrylate (Figure V.18).

The method developed allowed, to a certain extent, the cyclisation of the last two oligopeptides. The noteworthy issue is that the basic medium generated by the excess of amine needed to perform the aminolysis results in disulphide formation[75], which prevents the thiols from participating in a radical thiol-ene reaction.

Though this method will not be further explored in this PhD thesis, it could perhaps be improved by performing the reaction in solution, so a higher dilution can be obtained. Another way to improve this method might be to reduce the disulphide bonds first, followed by a thiol-ene reaction.[76]

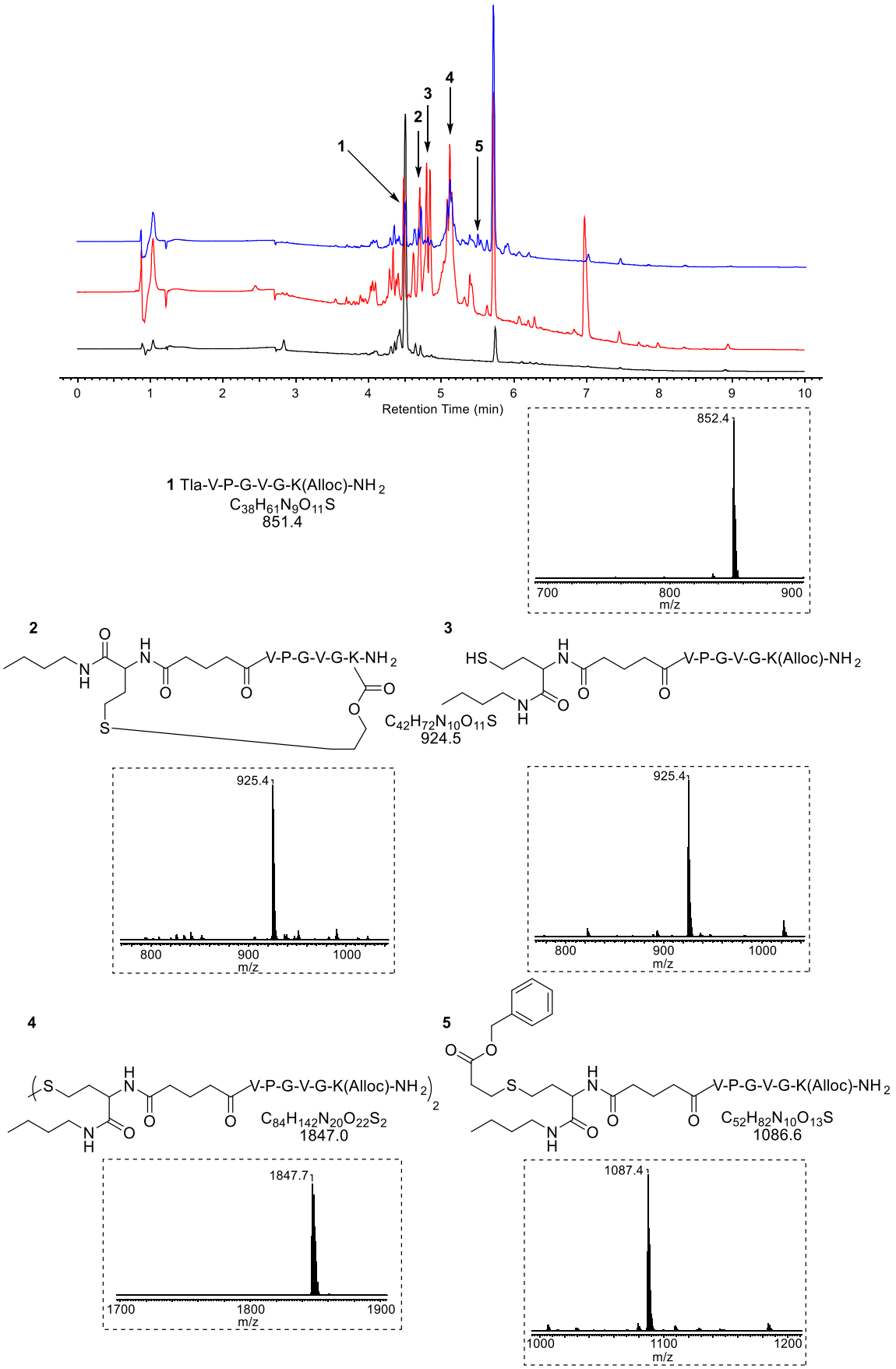


*Figure V.17: HPLC trace with ESI-MS analysis (positive mode) of the oligopeptide thiolactone-VPGVGK(Alloc) (black), its cyclisation (red) and subsequent reaction with benzyl acrylate (blue). **1)** oligopeptide with a closed thiolactone, **2)** cyclic oligopeptide, **3)** linear oligopeptide, **4)** disulphide and **5)** benzyl acrylate derivative of linear oligopeptide.*

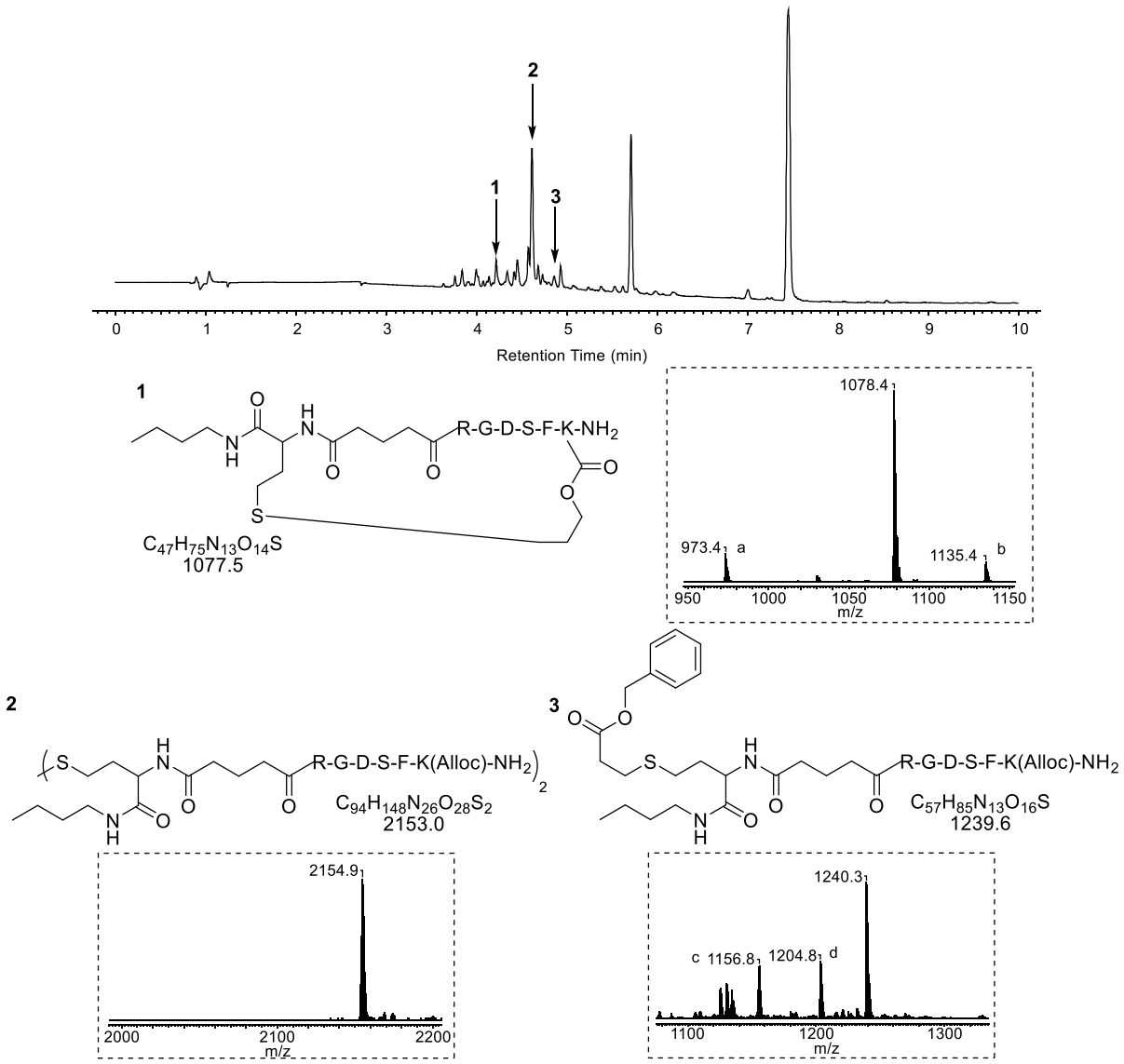


*Figure V.18: HPLC trace with ESI-MS analysis (positive mode) of the cyclisation of the oligopeptide thiolactone-RGDSFK(Alloc) and subsequent reaction with benzyl acrylate. **1**) cyclic oligopeptide, **2**) disulphide and **3**) benzyl acrylate derivative of linear oligopeptide. (Fragmentations and impurities may be caused by the eliminations of either water of serine or aspartic acid and 1-propylguanidine of arginine (a)[77], tBu-adduct (b), cleavage of the urethane bond with the formation of an amine (c)[78], and twice the elimination of water of both serine and aspartic acid with the formation of an acylium ion.[65])*

V.3.2 Lysine-induced aminolysis of thiolactone for cyclisation

For the second cyclisation approach, heterotelechelic oligopeptides were prepared with a protected lysine and a thiolactone moiety. Deprotection of the lysine results in a free primary amine that can open the thiolactone ring (Figure V.19). 4-Methyltrityl (Mtt) was chosen as a protecting group for the lysine as it can be easily and selectively removed with mild acidic conditions.

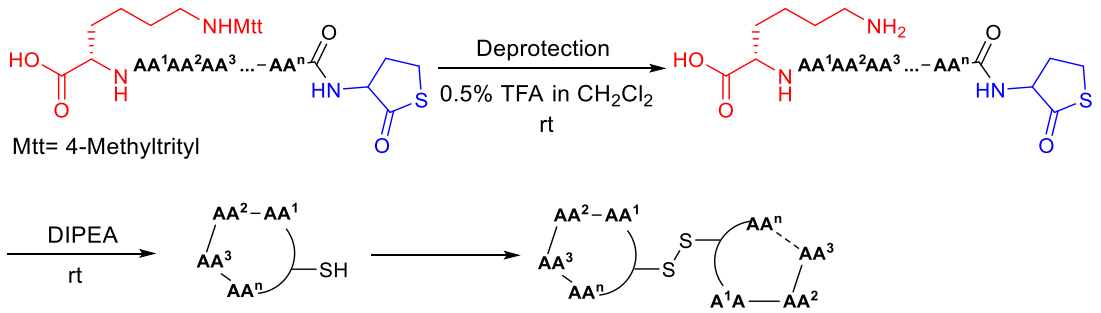


Figure V.19: Cyclisation via aminolysis of oligopeptides which contain a deprotected lysine and a thiolactone unit. Disulphide formation results in bicyclic oligopeptides.

In order to test this new strategy, the Mtt-protected lysine was first attached to the Rink amide solid support which was also used in the first strategy. Subsequently, the acid-functionalised thiolactone was coupled and the Mtt group removed with 0.5% TFA in dichloromethane. DIPEA was added to deprotonate the lysine and start the cyclisation. As the mass of the linear and the cyclic compound are the same and a racemic mixture of D- and L-acid-functionalised thiolactone was used, the linear and cyclic forms were not easily distinguishable, yet the disappearance of the signal around 1.2 min in the HPLC trace and the appearance of handcuff-like bicyclic compounds indicated that monocyclisation did occur (Figure V.20). Next to an intramolecular reaction which results in monocyclisation, an intermolecular reaction did also occur between two oligopeptides as the mass of (cyclo)dimers could also be observed in the LC-MS analysis.

As the preliminary test showed that cyclisation could occur, different RGD- and LDV-containing oligopeptides were prepared with a protected lysine and thiolactone unit. The latter tripeptide sequence can bind to integrins, just as RGD does.[79] The oligopeptide thiolactone-RGDSFK(Mtt) was prepared first on the solid support and the Mtt group was removed with a 0.5% TFA solution (Figure V.21, black trace). Afterwards, DIPEA was added and the reaction was shaken for two days (Figure V.21, red trace). In addition to the original mass of the linear compound, the bicyclic disulphide and a monocyclic-cyclodimer adduct can be found (Figure V.21, arrows 1, 2 and 3). Based on a procedure developed in our group[75], a reduction with dimethylphenylphosphine (DMPP) and a subsequent reaction with benzyl acrylate in one pot were performed to acquire functionalised monocycles instead of bicyclic compounds (Figure V.21, blue trace and Figure V.22). The phosphine reduces the disulphide and is a catalyst for the subsequent nucleophilic thiol-ene reaction. Although the cyclodimer derivative, the aza-Michael adduct and other impurities were observed in the LC trace, the functionalised monocycle was formed.

The method developed allows the synthesis of handcuff-like bicyclic peptides or functionalised monocycles (Figure V.22). This procedure might allow the incorporation of functionalities such as fluorescein, which is used in cell-penetrating studies. Two other sequences, EILDV and LDVPS, were also cyclised and functionalised with this method (Figures V.23 and V.24) (E, I and L are respectively glutamic acid, isoleucine and leucine).

While this strategy was not further explored within the scope of this PhD, it could perhaps be improved by performing the reaction in solution, thus a higher dilution can be obtained.

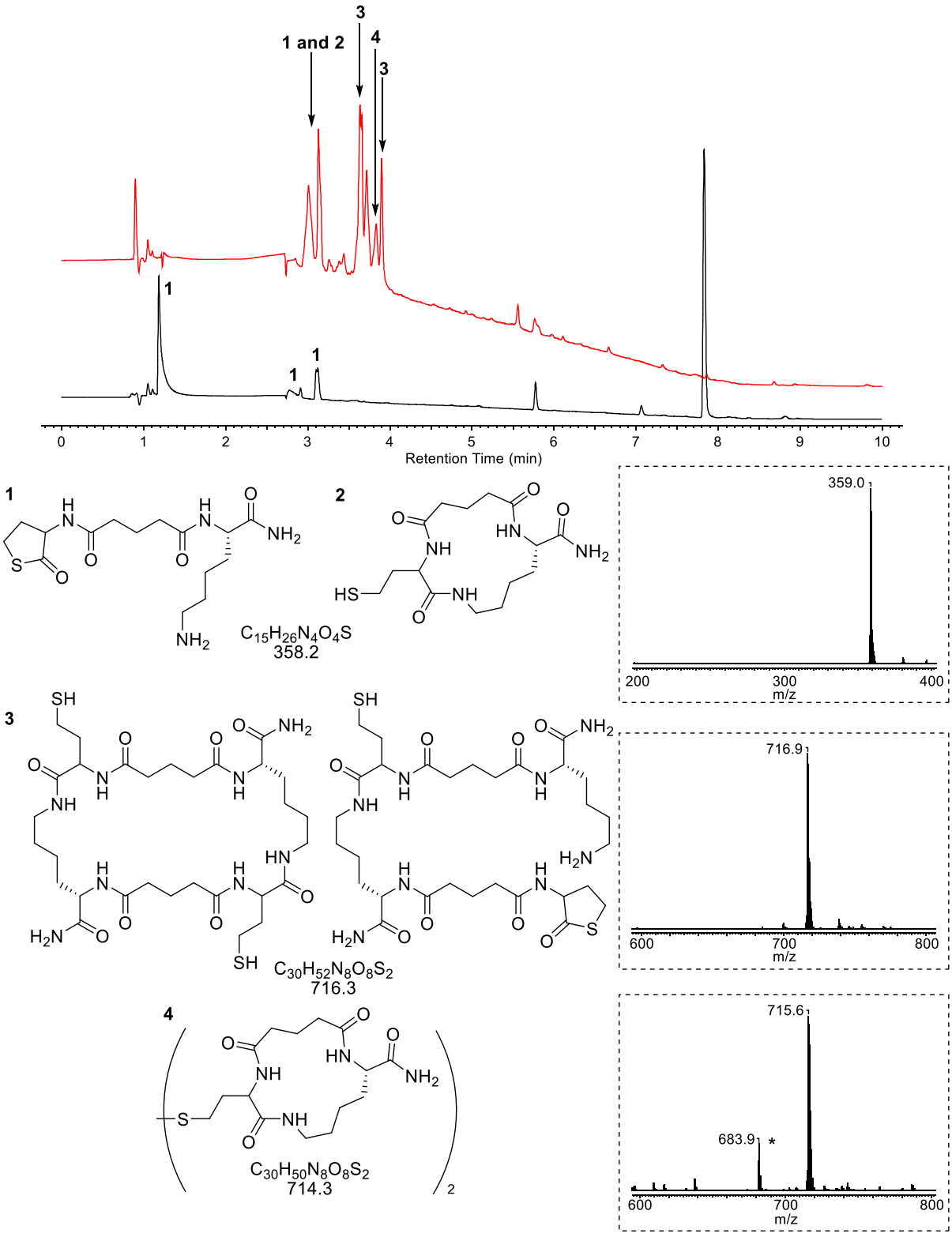


Figure V.20: HPLC trace with ESI-MS analysis (positive mode) of the lysine-thiolactone-compound (black) and its cyclisation (red). 1) Compound with a closed thiolactone, 2) cyclised compound, 3) cyclodimer or linear dimer and 4) handcuff-like disulphide bicycles. (Fragmentation may be caused by elimination of H_2S of the cyclodimer or linear dimer (*).)

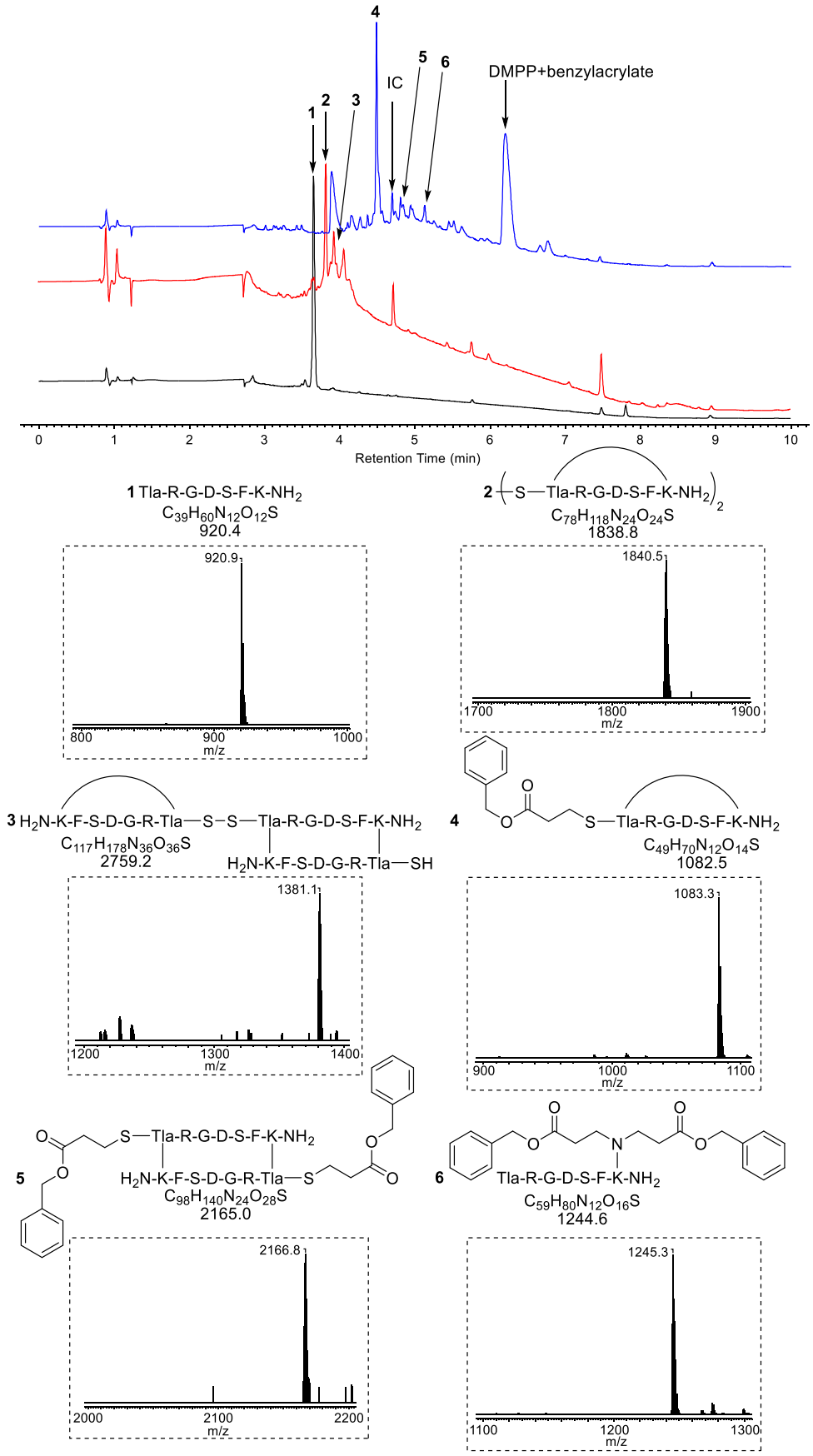


Figure V.21: HPLC trace with ESI-MS analysis (positive mode) of the oligopeptide thiolactone-RGDSFK (black), its cyclisation (red), and after the reduction and subsequent reaction with benzyl acrylate (blue). 1) oligopeptide with a closed thiolactone, 2) handcuff-like disulphide bicycles, 3) disulphide of a cyclodimer and a monocycle, 4) functionalised monocycle, 5) benzyl acrylate derivative of the cyclodimer and 6) aza-Michael adduct.

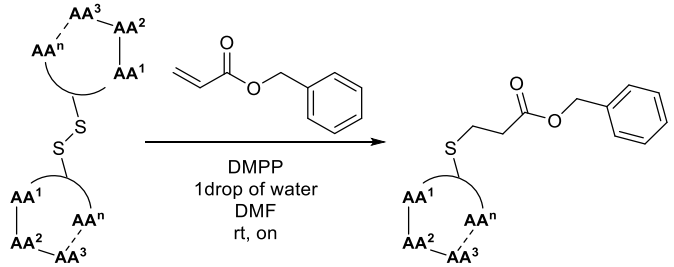


Figure V.22: Reduction of the handcuff-like bicyclic peptide and subsequent functionalisation with benzyl acrylate (on= overnight).

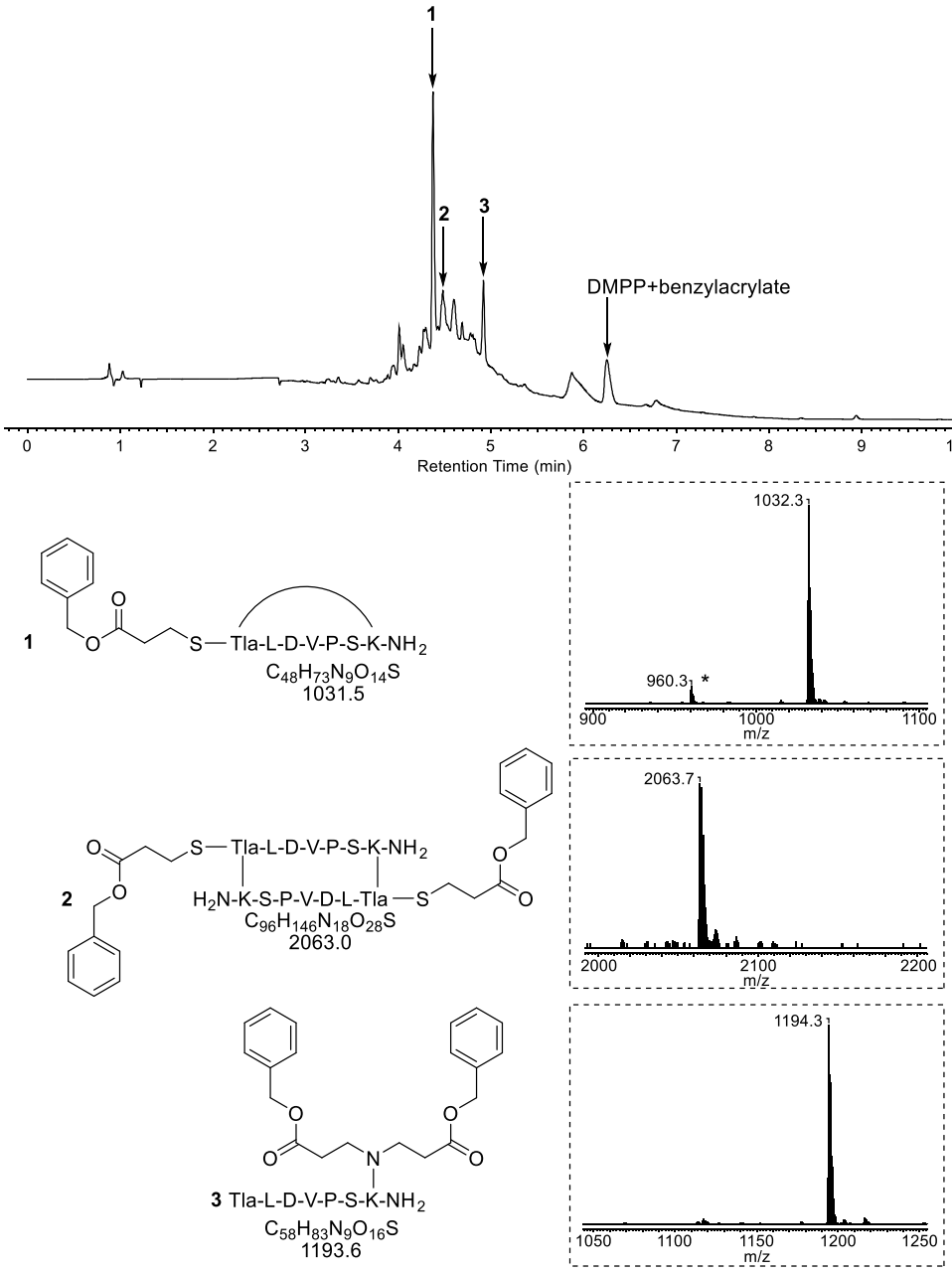


Figure V.23: HPLC trace with ESI-MS analysis (positive mode) after the cyclisation, the reduction and the subsequent reaction with benzyl acrylate of the oligopeptide thiolactone-LDVPSK. 1) Functionalised monocycle, 2) benzyl acrylate derivative of the cyclodimer and 3) aza-Michael adduct. (Fragmentation may be caused by two McLafferty rearrangements and the elimination of water of serine or aspartic acid.[65])

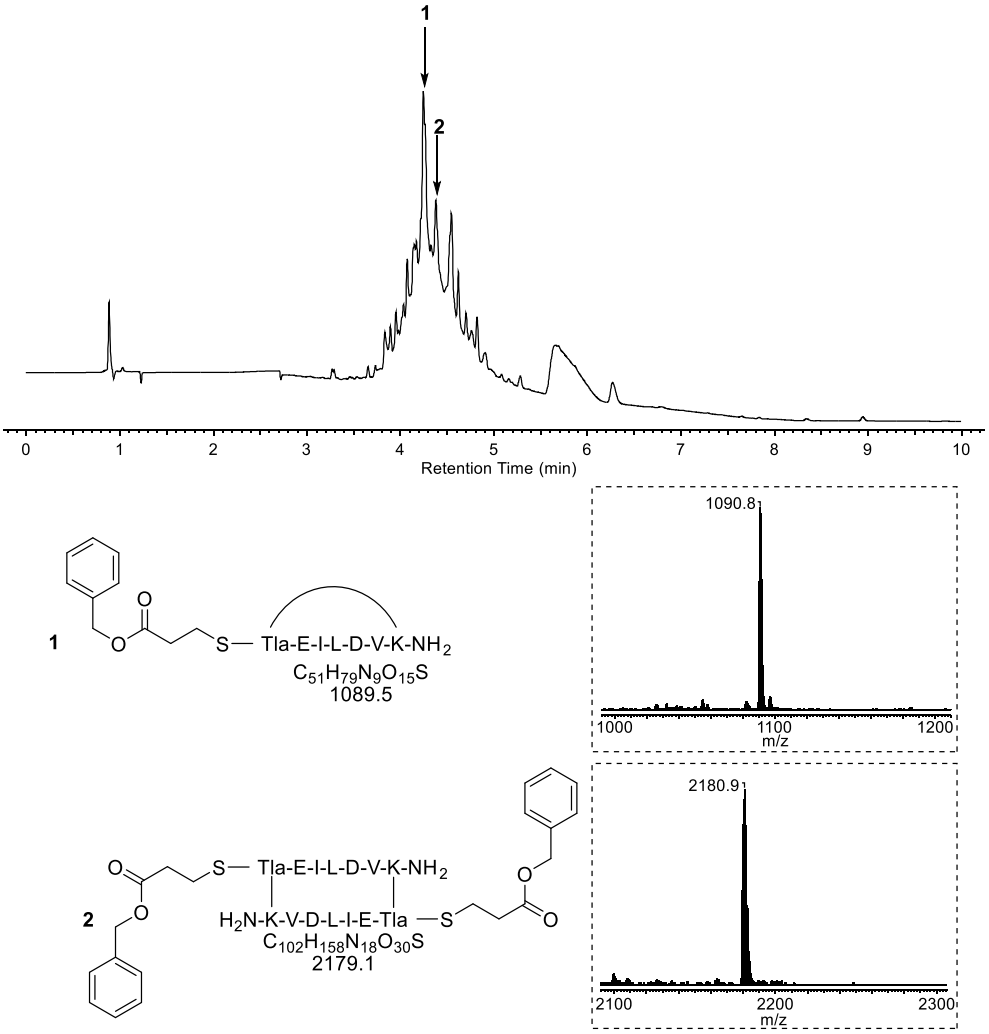


Figure V.24: HPLC trace with ESI-MS analysis (positive mode) after the cyclisation, the reduction and the subsequent reaction with benzyl acrylate of the oligopeptide thiolactone-EILDVK. 1) functionalised monocycle and 2) benzyl acrylate derivative of the cyclodimer.

These functional cyclised RGD- and LDV-containing species should be purified and biologically evaluated for their integrin-binding properties.[73, 79] At this stage, only benzyl acrylate was incorporated as a functionality, but it could be interesting to install functionalities which would be able to label, immobilise or further modify the oligopeptide cycle.

V.4 Conclusions and outlook

This chapter focussed on the polymerisation and cyclisation of oligopeptides based on the amine-thiolactone-ene conjugation.

Peptoid synthesis was applied to install the ene functionality on one side of the oligopeptide and an acid-functionalised thiolactone was incorporated on the other side to obtain AB'-functionalised oligomers able to be further polymerised. After aminolysis, the formed thiol could react with the ene functionality. It was observed that only oligomers could be formed and

that they resulted in disulphide formation. The synthesis of sequence-controlled polymers would perhaps be made possible by the use of different oligopeptides or synthetic oligomers and by further exploration of this strategy. In chapter VIII, the polymerisation of thiolactone-based sequence-defined oligomers[80] is studied in greater detail, with different strategies and a norbornene functionality.

Next, two different approaches were developed to perform on-resin macrocyclisation based on thiolactone chemistry of oligopeptides. The first approach was based on the radical thiol-ene reaction between the double bond of an alloc-protected lysine and the thiol of a thiolactone opened via aminolysis. The amounts of amine that were needed to open the thiolactone resulted in the inevitable formation of disulphide. Cyclisation was also dependent on the structure of the oligopeptide; the AGAGAG motif, for instance, only resulted in disulphide formation. The proposed cyclisation method could perhaps be improved by performing the cyclisation in solution or by first performing a reduction of the formed disulphides and subsequently a thiol-ene reaction.[76] If successful, this cyclisation method would allow for the easy installation of different functionalities via aminolysis.

The second cyclisation strategy started from oligopeptides with an Mtt-protected lysine and a thiolactone end group. Deprotection of the lysine resulted in the formation of an amine, which was able to open the thiolactone and generate a thiol. Handcuff-like bicyclic oligopeptides were formed due to disulphide formation. Functionalised monocycles could be prepared by reduction of the disulphide bond, by means of phosphines, to thiols, which could react with an acrylate via a subsequent nucleophilic thiol-ene reaction.

The approaches developed for the cyclisation could be applied in the future to polymerise the oligopeptides obtained. Another strategy that could possibly be applied for the polymerisation and cyclisation is the amine-thiol-ene conjugation with thiolactones and acrylates. In previous research performed within the group, poly(isobornylacrylate) bearing thiolactone and acrylate end groups was polymerised via aminolysis of the first functionality.[19] This method allowed for the installation of certain functionalities and spatial arrangement of side-chain residues. Installation of an acrylate on a thiolactone-functionalised oligopeptide could make possible the cyclisation or polymerisation of the prepared oligomers via aminolysis.

In parallel with the research discussed in this chapter, Madder's group in collaboration with ours developed a cyclisation method in solution based on disulphide formation between an end-standing thiol and an opened thiolactone.[36] This technique allowed for the cyclisation of different high-purity peptides. A chiral pure acid-functionalised thiolactone was made according to procedures reported in the literature and was applied for the synthesis of enantiomeric pure cycles.[75, 81] The amine used for the opening can have functional handles such as a furan, ene or yne functionality, which allow for further modification, labelling, or immobilisation. Long-chain alkyl amines were also used to synthesise lipopeptides which have surfactant, immunosuppressant, antimicrobial, or antitumor properties.[82]

Alongside thiolactone chemistry, 1,2,4-triazoline-3,5-dione chemistry (TAD), which has been developed within our group, could be interesting for the cyclisation and polymerisation of oligopeptides. Börner's group was capable of transforming the end-standing amine of an oligopeptide to a TAD group and of coupling this to a polymer with a diene end group.[83] If an oligopeptide could be made with a diene, ene, phenol or indole moiety and a TAD functionality, polymerisation or cyclisation could be performed.[84, 85] TAD chemistry is very fast (seconds to minutes) and provides visual feedback when the reaction is done.

V.5 Experimental part

V.5.1 Methods

^{1}H- and ^{13}C-NMR

^{1}H- and ^{13}C-NMR (Attached Proton Test, APT) spectra were recorded on a Bruker Avance 300 at 300 MHz and 75 MHz, respectively and a Bruker Avance 500 at 500 MHz and 125 MHz, respectively. Chemical shifts are presented in parts per million (δ) relative to DMSO-d_6 (2.50 ppm in ^{1}H- and 39.51 ppm in ^{13}C-NMR respectively) as internal standard. All measurements were performed at 25°C and ACD/NMR Processor was used for the processing of all data. All spectra were recorded in a standard fashion with pulse programs available in the Bruker library.

LC-ESI-MS

An Agilent technologies 1100 series LC/MSD system equipped with a diode array detector and single-quad MS detector (VL) with an electrospray source (ESI-MS) was used for classic reversed-phase LC-MS (liquid chromatography mass spectroscopy) and MS analysis. Analytic reversed-phase HPLC was performed with a Phenomenex C18 (2) column (5 µ, 250 x 4.6 mm) using a solvent gradient (0 → 100% acetonitrile in H_2O in 15 min) and the eluting compounds were detected via UV-detection (λ = 214 nm). High-resolution mass spectra (HRMS) were collected using an Agilent 6220 Accurate-Mass time-of-flight (TOF) equipped with a multimode ionisation (MMI) source.

SEC

Size exclusion chromatography (SEC) was performed on a Waters instrument with a Waters 2414 Refractive Index Detector (RI), equipped with 3 PSS serial columns (GRAM Analytical 30 and 1000˚A, 10 µm particle size) at 35°C. Poly(methyl methacrylate) standards were used for calibration and DMA containing LiBr (0.42 g/l) was used as an eluent at a flow rate of 1 mL/min. Molecular weights and dispersities (Đ) were determined using the Empower software.

MALDI-TOF

MALDI-TOF Mass spectrometry was carried out on an Applied Biosystems Voyager DE STR instrument equipped with a nitrogen laser at 337 nm, a pulsed ion extraction source and a reflection detector. The laser bandwidth is 3 ns and has a maximal capacity of 20 Hz. Spectra were recorded in linear mode at a potential difference of 20 kV and a delay of 100 ns. Spectra were made up of 450 different measurements and data was analysed using a Data Explorer and Polymerix software. Samples were prepared with trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malonitrile (DCTB, 30 mg/mL in dichloromethane) as a matrix, Sodium trifluoroacetate (19 mg/mL in acetone) as a cationising agent, and oligomer samples were dissolved in THF (4 mg/mL). Oligomer solutions were prepared by mixing 10 µL of the oligomer, 1 µL of the salt, and 10 µL of the matrix solution. Subsequently, 0.5 µL of this mixture was spotted on the sample plate, and the spots were dried in air at room temperature.

Automated synthesis

Automated peptide synthesis was performed on a 24-reactor Block SYRO Multiple peptide synthesizer equipped with a vortex unit (Multisynthech, Witten, Germany).

V.5.2 Materials

DMSO-d_6 ([2206-27-1], ≥ 99.8 %) and $CHCl_3$-*d* ([865-49-6], ≥ 99.8 %) were purchased from Euriso-top. N-methylpyrrolidone ([872-50-4], ≥ 99.0 %) was purchased from Biosolve. Rink amide AM PS resin ([183599-10-2], 100-200 mesh, 1% DVB, 0.69 mmol/g) was purchased from Novabiochem. Acetonitrile ([75-05-8], HPLC grade), n-Butylamine ([109-73-9], 99.5 %), 1,4-Dioxane ([123-91-1], HPLC grade), anhydrous Dimethylformamide ([68-12-2], 99.8 %) and Piperidine ([110-89-4], 99.0 %) were purchased from Acros Organics. 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) ([94790-37-1], Peptide grade), 2-Chlorotrityl chloride resin ([42074-68-0], 100-200 mesh, 1% DVB, 1.6 mmol/g), Fmoc-L-Alanine-OH ([35661-39-3], Peptide grade), Fmoc-L-Arginine(Pbf)-OH ([154445-77-9], Peptide grade), Fmoc-L-Aspartic acid(OtBu)-OH ([71989-14-5], Peptide grade), Fmoc-L-Glutamic acid(OtBu)-OH ([71989-18-9], Peptide grade), Fmoc-Glycine-OH ([29022-11-5], Peptide grade), Fmoc-L-Isoleucine-OH ([71989-23-6], Peptide grade), Fmoc-L-Leucine-OH ([35661-60-0], Peptide grade), Fmoc-L-Lysine(Alloc)-OH ([146982-27-6], Peptide grade), Fmoc-L-Lysine(Mtt)-OH ([167393-62-6], Peptide grade), Fmoc-L-Phenylalanine-OH ([35661-40-6], Peptide grade), Fmoc-L-Proline-OH ([71989-31-6], Peptide grade), Fmoc-L-Serine(tBu)-OH ([71989-33-8], Peptide grade), Fmoc-L-Valine-OH ([68858-20-8], Peptide grade) and Trifluoroacetic acid ([76-05-1], Peptide grade) were purchased from Iris Biotech GmbH. DL-Homocysteine thiolactone hydrochloride ([6038-19-3], 99 %) was purchased from Haihang industry (Jinan City, China). Sodium bicarbonate ([144-55-8], ≥ 99.5%) was purchased from Carl Roth. Acryloyl chloride ([814-68-6], 96 %) was purchased from ABCR.

Benzyl acrylate ([2495-35-4], > 97 %), Bromoacetic acid ([79-08-3], ≥ 98 %) was purchased from TCI and used without purification. Acetic acid ([64-19-7], ≥ 99.8 %), Allylamine ([107-11-9], ≥ 99.9 %), 2-2´-Azobis(isobutyronitrile) (AIBN) ([78-67-1], ≥ 98.0 %), tert-Butyl methyl ether ([1634-04-4], 99.9 %), Chloroform ([865-49-6], ≥ 99.8 %), α-Cyano-4-hydroxycinnamic ([28166-41-8], ≥ 98.0 %), Dichloromethane ([75-09-2], ≥ 99.8 %), N,N'-Diisopropylcarbodiimide (DIC) ([693-13-1], 99.0 %), N,N-Diisopropylethylamine (DIPEA) ([7087-68-5], 99.5 %), 2,2-Dimethoxy-2-phenylacetophenone (DMPA) ([24650-42-8], 99.0 %), N,N-Dimethylacetamide ([127-19-5], ≥ 99.9 %), Dimethylformamide ([69-12-2], 99.8 %), Dimethylphenylphosphine ([672-66-2], 99 %), Ethyl acetate ([141-78-6], ≥ 99.7 %), Glutaric anhydride ([108-55-4], 95 %), Lithium bromide ([7550-35-8], ≥ 99 %), Methanol ([67-56-1], ≥ 99.9 %), Octylamine ([111-68-4], 99.0 %), Propargylamine ([2450-71-7], ≥ 98.0 %), Propylamine ([107-10-8], ≥ 99.0 %), Pyridine ([110-86-1], ≥ 99.0 %), Tetrahydrofuran ([109-99-9], ≥ 99.0 %), Triethylamine ([121-44-8], 99.0 %), Trifluoroethanol ([75-89-8], ≥ 99.0 %) and Triisopropylsilane ([6485-79-6], 99.0 %)were purchased from Sigma-Aldrich. Silicagel (ROCC, SI 1721, 60 Å, 40 – 63 μm) was used to perform preparative column chromatography, eluting with technical solvents. The collected fractions were analysed by thin layer chromatography (TLC-plates, Macherey-Nagel, SIL G-25 UV254). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). The acid-functionalised thiolactone was prepared as previously described.[75]

V.5.3 Synthesis

Polymerisations with DMPA

The oligopeptide, DMPA (0.05 equivalents), DMA or acetonitrile(/H_2O) (1/1), and n-butylamine (1.1 equivalents) were added in a 2 mL vial which can be closed with a screw cap. This mixture was stirred under UV-radiation (365 nm) at 40°C for 5 hours. The obtained oligomers were purified by means of precipitation in a tenfold excess of cold MTBE. After centrifugation at 0°C for 10 minutes at 5000 rpm, the solvent was removed.

Polymerisations with AIBN

The oligopeptide, AIBN (0.05 equivalents), DMA or acetonitrile, and n-butylamine (1.1 equivalents) were added in a 2 mL vial which can be closed with a screw cap. This mixture was stirred at 75°C for 5 hours. The obtained oligomers were purified by means of precipitation in a tenfold excess of cold MTBE. After centrifugation at 0°C for 10 minutes at 5000 rpm, the solvent is removed.

V.5.4 Manipulation of the solid support

Swelling
The solid support was swollen in the reaction solvent for at least 10 minutes.

Washing
The loaded resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried after each reaction.

Loading on the 2-chlorotrityl chloride resin
The Fmoc-L-Lysine(Alloc)-OH (1.2 equivalents relative to the resin) was loaded on a 2-chlorotrityl chloride resin (1.6 mmol/g) in anhydrous CH_2Cl_2 (10 mL/1 g of resin) with anhydrous DIPEA (4 equiv. rel. to acid). The reaction mixture was shaken for 2 hours at room temperature. The resin was subsequently washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 3x), DMF (4x), CH_2Cl_2 (4x) and diethyl ether (4x). The resin was dried under vacuum.

Loading on the Rink amide aminomethylpolystyrene resin
Before the solid support was added, the dried reactor was sealed with a septum and flushed with argon. Bromoacetic acid (10 equivalents relative to the resin) and DIC (5 equiv. rel. to the resin) were first mixed in anhydrous CH_2Cl_2. The mixture (10 mL/1 g of resin) was added to the Rink amide resin (0.69 mmol/g), shaken for one hour at room temperature, and then filtered off. The resin was washed with CH_2Cl_2 (4x) and DMF (4x). Next, allyl amine (20 equiv. rel. to the resin) was mixed with anhydrous DMF. The mixture (10 mL/1 g of resin) was added to the resin, shaken for one hour at room temperature, and then filtered off. The resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x).

Peptide synthesis and modifications
Coupling of amino acids and the acid-functionalised thiolactone linker: The resin was first swollen in DMF. HBTU (5 equivalents), the Fmoc-protected amino acid or acid-thiolactone linker (5 equivalents) and DIPEA (10 equivalents) were first mixed in DMF to activate the acid. The mixture was added to the resin, shaken for two hours, and then filtered off. The resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Fmoc-deprotection: The resin was swollen in DMF. The DMF was removed by filtration and the resin was treated with a 20% piperidine solution in DMF for 2, 5, 15 and 2 times 30 minutes. Next, the resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

On-resin cyclisation with Lysine(Alloc): The resin was swollen in DMF. The DMF was removed by filtration and the resin was treated with a DMF solution containing DMPA (2 equivalents) and butylamine (10 equivalents) for 2 hours under UV-irradiation. Next, the resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Mtt-removal: A solution of 0.5% trifluoroacetic acid (TFA) in CH_2Cl_2 was added to the resin, shaken for 30 minutes and subsequently filtered off. This was repeated eight times. Afterwards the resin was washed with DIPEA/DMF (10%) (4x), DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x).

On-resin cyclisation with Lysine: The resin was swollen in DMF. The DMF was removed by filtration and a solution of DIPEA (10 equivalents) in DMF was added to the resin. The reaction was shaken for 2 days and the mixture was subsequently filtered off. Afterwards the resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x).

Phosphine reduction: The resin was swollen in DMF. The solvent was removed by filtration and a mixture (10 mL/1 g of resin) of dimethylphenylphosphine (10 equivalents), benzyl acrylate (10 equivalents) and DMF was added in the reactor with a drop of water. The reaction was shaken overnight at room temperature. The solution was filtered off and the resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x).[75]

Cleavage

For cleavage of the Rink amide resin, 2 mg of solid support was treated with a TFA/TIPS/H_2O (95/2.5/2.5) solution and shaken for 3 hours. This solution was chosen to scavenge cationic species from the protecting groups that are generated during the cleavage. The resin was then filtered and was washed with pure TFA (3x). The sample was concentrated by evaporation and precipitated 3 times via centrifugation (5000 rpm, 0°C) with cold MTBE (-20°C). For LC-ESI-MS analysis, the peptide was dissolved in acetonitrile.

For the cleavage of the 2-chlorotrityl chloride resin, 2 mg of solid support was treated with 0.5 mL of 1% trifluoroacetic acid (TFA) in CH_2Cl_2 for 5 minutes. No scavengers were needed in the cleavage mixture as the oligopeptides prepared on this resin did not carry any protecting groups. During cleavage, the color of the beads turned from yellow to red because of the formation of a trityl cation. The solid support was then filtered, the sample concentrated by evaporation and dissolved in acetonitrile for LC-ESI-MS analysis.

Ellman's Test

5 mg of resin was swollen in THF (1 mL). After 10 minutes, a 5 mM solution of 5,5'-dithiobis-2-nitrobenzoic acid (or DTNB) in methanol (1 mL) is added along with DIPEA (5 µL). This mixture should become distinctively yellow when free thiols are present.[86]

Automated peptide synthesis

100 mg of resin was used for the Peptide synthesis. For the swelling of the resin, DMF was used for 10 min and filtered off. Fmoc-deprotection was performed with a piperidine/DMF solution (40/60). HBTU, the acid-functionalised thiolactone and the different amino acids were dissolved in DMF. The following amino acids were used during the coupling steps: Fmoc-Alanine-OH, Fmoc-Aspartic acid(OtBu)-OH, Fmoc-Glutamic acid(OtBu)-OH, Fmoc-Phenylalanine-OH, Fmoc-Glycine-OH, Fmoc-Isoleucine-OH, Fmoc-Lysine(Mtt)-OH, Fmoc-Lysine(Alloc)-OH, Fmoc-Leucine-OH, Fmoc-Proline-OH, Fmoc-Arginine(Pbf)-OH, Fmoc-Serine(tBu)-OH, Fmoc-Valine-OH.

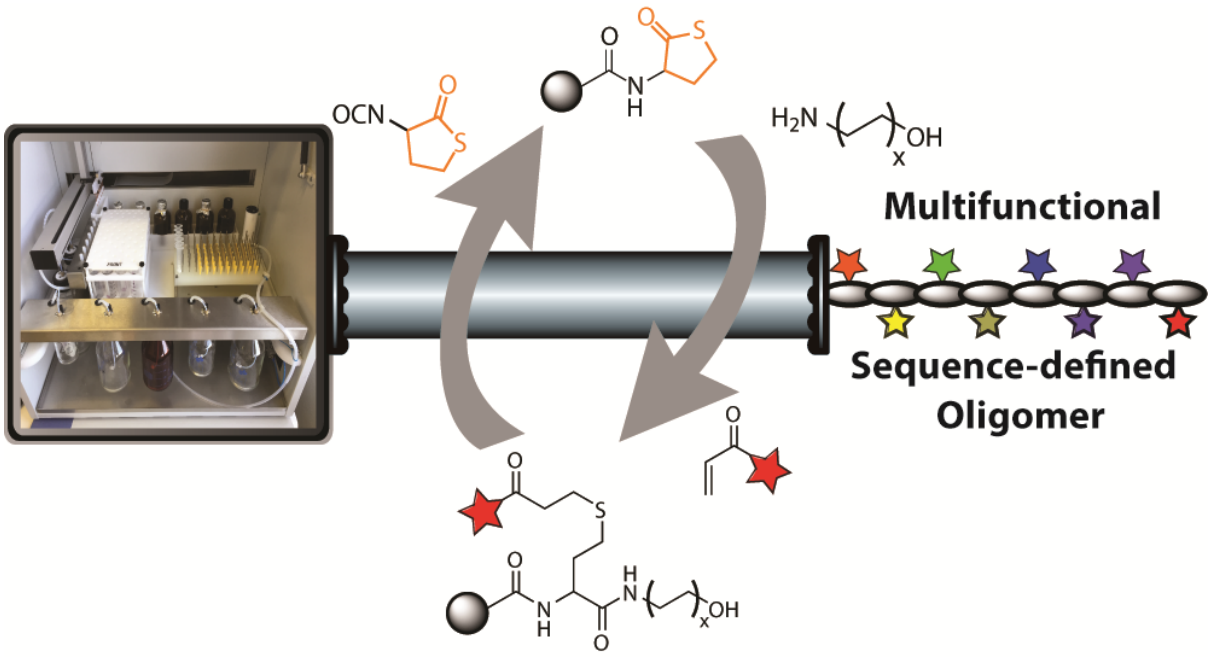
For the Fmoc-deprotection, the piperidine/DMF solution is added to the resin and shaken for 4 minutes. After filtration, the deprotection is performed for a second time, but the piperidine/DMF solution is diluted with extra DMF to obtain a 20% solution and the reaction is shaken for 12 min. The resin is filtered and washed with DMF (6x for 45 seconds). Next, a 0.5 M solution of the protected amino acids in DMF (400 µL), a 0.5 M solution of HBTU in DMF (400 µL) and a 2.0 M solution of DIPEA in NMP (200 µL) were added to the resin. The reaction was shaken for 40 minutes to perform the coupling of the amino acids or acid-functionalised thiolactone. The reaction mixture was filtered off and the resin was washed with DMF (4x for 2 minutes). A second coupling was performed to ensure full conversion. After the automated synthesis, the resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x).

References

[1] N. Badi, D. Chan-Seng, J.-F. Lutz, *Macromol. Chem. Phys.* **2013**, *214*, 135.
[2] J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, *38*, 1700582.
[3] J. De Neve, J. J. Haven, L. Maes, T. Junkers, *Polym. Chem.* **2018**, *9*, 4692.
[4] Z. L. Li, Z. C. Li, "Periodic Copolymers by Step-Growth Polymerization", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 349.
[5] W. R. Gutekunst, C. J. Hawker, *J. Am. Chem. Soc.* **2015**, *137*, 8038.
[6] R. M. Weiss, A. L. Short, T. Y. Meyer, *ACS Macro Lett.* **2015**, *4*, 1039.
[7] K. Satoh, S. Ozawa, M. Mizutani, K. Nagai, M. Kamigaito, *Nat. Commun.* **2010**, *1*, 6.
[8] C.-H. Wang, Z.-Y. Song, X.-X. Deng, L.-J. Zhang, F.-S. Du, Z.-C. Li, *Macromol. Rapid Commun.* **2014**, *35*, 474.
[9] R. M. Stayshich, T. Y. Meyer, *Journal of Polymer Science Part A: Polymer Chemistry* **2008**, *46*, 4704.
[10] J. Li, S. N. Rothstein, S. R. Little, H. M. Edenborn, T. Y. Meyer, *J. Am. Chem. Soc.* **2012**, *134*, 16352.
[11] C. Zhang, J. Ling, Q. Wang, *Macromolecules* **2011**, *44*, 8739.
[12] Y. Chen, Z. Guan, *J. Am. Chem. Soc.* **2010**, *132*, 4577.
[13] T.-B. Yu, J. Z. Bai, Z. Guan, *Angew. Chem., Int. Ed.* **2009**, *48*, 1097.
[14] M. F. Ebbesen, C. Gerke, P. Hartwig, L. Hartmann, *Polym. Chem.* **2016**, *7*, 7086.
[15] M.-A. Berthet, Z. Zarafshani, S. Pfeifer, J.-F. Lutz, *Macromolecules* **2010**, *43*, 44.
[16] W. Xi, S. Pattanayak, C. Wang, B. Fairbanks, T. Gong, J. Wagner, C. J. Kloxin, C. N. Bowman, *Angew. Chem., Int. Ed.* **2015**, *54*, 14462.
[17] C. Gerke, M. F. Ebbesen, D. Jansen, S. Boden, T. Freichel, L. Hartmann, *Biomacromolecules* **2017**, *18*, 787.

[18] C. Gerke, F. Jacobi, L. E. Goodwin, F. Pieper, S. Schmidt, L. Hartmann, *Macromolecules* **2018**, *51*, 5608.
[19] F. Driessen, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2015**, *4*, 616.
[20] M. Haider, Z. Megeed, H. Ghandehari, *Journal of Controlled Release* **2004**, *95*, 1.
[21] P. A. Guerette, D. G. Ginzinger, B. H. F. Weber, J. M. Gosline, *Science* **1996**, *272*, 112.
[22] J. H. Waite, *Journal of Biological Chemistry* **1983**, *258*, 2911.
[23] J. H. Waite, T. J. Housley, M. L. Tanzer, *Biochemistry* **1985**, *24*, 5010.
[24] E. R. Wright, V. P. Conticello, *Advanced Drug Delivery Reviews* **2002**, *54*, 1057.
[25] R. Dandu, H. Ghandehari, *Prog. Polym. Sci.* **2007**, *32*, 1008.
[26] Y. Wu, J. A. MacKay, J. R. McDaniel, A. Chilkoti, R. L. Clark, *Biomacromolecules* **2009**, *10*, 19.
[27] D. Chow, M. L. Nunalee, D. W. Lim, A. J. Simnick, A. Chilkoti, *Materials science & engineering. R, Reports : a review journal* **2008**, *62*, 125.
[28] W. F. Daamen, J. H. Veerkamp, J. C. M. van Hest, T. H. van Kuppevelt, *Biomaterials* **2007**, *28*, 4378.
[29] S. E. Grieshaber, A. J. E. Farran, S. Lin-Gibson, K. L. Kiick, X. Jia, *Macromolecules* **2009**, *42*, 2532.
[30] L. Martín, M. Alonso, M. Möller, J. C. Rodríguez-Cabello, P. Mela, *Soft Matter* **2009**, *5*, 1591.
[31] R. E. Sallach, W. Cui, F. Balderrama, A. W. Martinez, J. Wen, C. A. Haller, J. V. Taylor, E. R. Wright, R. C. Long, E. L. Chaikof, *Biomaterials* **2010**, *31*, 779.
[32] T. J. Deming, *Advanced Materials* **1997**, *9*, 299.
[33] J. C. M. van Hest, D. A. Tirrell, *Chem. Commun.* **2001**, 1897.
[34] S. Arias, F. Freire, M. Calderón, J. Bergueiro, *Angew. Chem., Int. Ed.* **2017**, *56*, 11420.
[35] M. Katsara, T. Tselios, S. Deraos, G. Deraos, M. T. Matsoukas, E. Lazoura, J. Matsoukas, V. Apostolopoulos, *Current Medicinal Chemistry* **2006**, *13*, 2221.
[36] D. Van Lysebetten, S. Felissati, E. Antonatou, L. L. G. Carrette, P. Espeel, E. Focquet, F. E. Du Prez, A. Madder, *ChemBioChem* **2018**, *19*, 641.
[37] P. K. Madala, J. D. A. Tyndall, T. Nall, D. P. Fairlie, *Chemical Reviews* **2010**, *110*, PR1.
[38] V. J. Hruby, *Nature Reviews Drug Discovery* **2002**, *1*, 847.
[39] W. M. Hewitt, S. S. F. Leung, C. R. Pye, A. R. Ponkey, M. Bednarek, M. P. Jacobson, R. S. Lokey, *J. Am. Chem. Soc.* **2015**, *137*, 715.
[40] Y.-U. Kwon, T. Kodadek, *Chemistry & Biology* **2007**, *14*, 671.
[41] C. J. White, A. K. Yudin, *Nature Chemistry* **2011**, *3*, 509.
[42] A. K. Yudin, *Chem. Sci.* **2015**, *6*, 30.
[43] Y. Li, M. Giulionatti, R. A. Houghten, *Organic Letters* **2010**, *12*, 2250.
[44] S. Punna, J. Kuzelka, Q. Wang, M. G. Finn, *Angewandte Chemie* **2005**, *117*, 2255.
[45] R. Jagasia, J. M. Holub, M. Bollinger, K. Kirshenbaum, M. G. Finn, *J. Org. Chem.* **2009**, *74*, 2964.
[46] J. Illesinghe, C. X. Guo, R. Garland, A. Ahmed, B. van Lierop, J. Elaridi, W. R. Jackson, A. J. Robinson, *Chem. Commun.* **2009**, 295.
[47] A. Baron, P. Verdié, J. Martinez, F. Lamaty, *J. Org. Chem.* **2011**, *76*, 766.
[48] R. M. Kohli, C. T. Walsh, M. D. Burkart, *Nature* **2002**, *418*, 658.
[49] A. Toplak, T. Nuijens, P. J. L. M. Quaedflieg, B. Wu, D. B. Janssen, *Advanced Synthesis & Catalysis* **2016**, *358*, 2140.
[50] P. Dawson, T. Muir, I. Clark-Lewis, S. Kent, *Science* **1994**, *266*, 776.
[51] L. Zhang, J. P. Tam, *J. Am. Chem. Soc.* **1997**, *119*, 2363.
[52] J. Tulla-Puche, G. Barany, *J. Org. Chem.* **2004**, *69*, 4101.
[53] M. Góngora-Benítez, J. Tulla-Puche, F. Albericio, *Chemical Reviews* **2014**, *114*, 901.
[54] C. Wu, J.-C. Leroux, M. A. Gauthier, *Nature Chemistry* **2012**, *4*, 1044.
[55] A. V. Vasco, C. S. Pérez, F. E. Morales, H. E. Garay, D. Vasilev, J. A. Gavín, L. A. Wessjohann, D. G. Rivera, *J. Org. Chem.* **2015**, *80*, 6697.
[56] M. C. Morejón, A. Laub, B. Westermann, D. G. Rivera, L. A. Wessjohann, *Organic Letters* **2016**, *18*, 4096.
[57] A. A. Aimetti, K. R. Feaver, K. S. Anseth, *Chem. Commun.* **2010**, *46*, 5781.
[58] A. A. Aimetti, R. K. Shoemaker, C.-C. Lin, K. S. Anseth, *Chem. Commun.* **2010**, *46*, 4061.
[59] X. Elduque, E. Pedroso, A. Grandas, *J. Org. Chem.* **2014**, *79*, 2843.
[60] P. Espeel, F. Goethals, F. E. Du Prez, *J. Am. Chem. Soc.* **2011**, *133*, 1678.

[61] F. Goethals, S. Martens, P. Espeel, O. van den Berg, F. E. Du Prez, *Macromolecules* **2014**, *47*, 61.
[62] R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* **1992**, *114*, 10646.
[63] A. S. Knight, E. Y. Zhou, M. B. Francis, R. N. Zuckermann, *Advanced Materials* **2015**, *27*, 5665.
[64] M. A. Kline, L. Guo, R. N. Zuckermann, "Sequence-Controlled Peptoid Polymers: Bridging the Gap between Biology and Synthetic Polymers", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 183.
[65] D. P. Demarque, A. E. M. Crotti, R. Vessecchi, J. L. C. Lopes, N. P. Lopes, *Natural Product Reports* **2016**, *33*, 432.
[66] K. Rice, A. Brossi, *J. Org. Chem.* **1980**, *45*, 592.
[67] M. Geffe, L. Andernach, O. Trapp, T. Opatz, *Beilstein Journal of Organic Chemistry* **2014**, *10*, 701.
[68] J. Xu, C. Boyer, *Macromolecules* **2015**, *48*, 520.
[69] J. Vandenbergh, K. Ranieri, T. Junkers, *Macromol. Chem. Phys.* **2012**, *213*, 2611.
[70] D. E. Brooks, C. A. Haynes, D. Hritcu, B. M. Steels, W. Müller, *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 7064.
[71] P. Espeel, F. Goethals, F. E. Du Prez, "CHAPTER 9 Thiolactones as Functional Handles for Polymer Synthesis and Modification", in *Thiol-X Chemistries in Polymer and Materials Science*, The Royal Society of Chemistry, 2013, p. 195.
[72] F. Krieger, A. Möglich, T. Kiefhaber, *J. Am. Chem. Soc.* **2005**, *127*, 3346.
[73] E. Ruoslahti, *Annual Review of Cell and Developmental Biology* **1996**, *12*, 697.
[74] M. D. Pierschbacher, E. Ruoslahti, *Nature* **1984**, *309*, 30.
[75] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[76] J. O. Holloway, S. Aksakal, F. E. D. Prez, C. R. Becer, *Macromol. Rapid Commun.* **2017**, *38*, 1700500.
[77] D. R. Bush, V. H. Wysocki, P. Y. Scaraffia, *Journal of mass spectrometry : JMS* **2012**, *47*, 1364.
[78] S. Martens, A. Landuyt, P. Espeel, B. Devreese, P. Dawyndt, F. Du Prez, *Nat. Commun.* **2018**, *9*, 4451.
[79] J. D. Humphries, A. Byron, M. J. Humphries, *Journal of cell science* **2006**, *119*, 3901.
[80] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[81] M. Nicola, P. A. Gatti, M. Zacche, "Process for the resolution of homocysteine-thiolactone", Google Patents, 2008.
[82] J. M. Raaijmakers, I. De Bruijn, O. Nybroe, M. Ongena, *FEMS Microbiology Reviews* **2010**, *34*, 1037.
[83] P. Wilke, T. Kunde, S. Chattopadhyay, N. ten Brummelhuis, F. Du Prez, H. Börner, *Chem. Commun.* **2016**, *53*, 593.
[84] S. Vandewalle, R. De Coen, B. G. De Geest, F. E. Du Prez, *ACS Macro Lett.* **2017**, *6*, 1368.
[85] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815.
[86] G. L. Ellman, *Archives of Biochemistry and Biophysics* **1958**, *74*, 443.



Abstract

This chapter describes a protecting-group-free two-step iterative protocol based on thiolactone chemistry and the amine-thiol-ene conjugation. This protocol is performed on solid support and allows the synthesis of multifunctional sequence-defined oligomers. The thiolactone is opened by means of aminolysis with an amino alcohol, liberating a thiol that reacts selectively with an acrylate or acrylamide, present in the same medium. In a following step, an α-isocyanato-γ-thiolactone is applied to reinstate a new thiolactone unit. Diverse functionalities can be incorporated in the side chain and backbone of the oligomers by using different acrylic compounds and amino alcohols respectively. The strategy applied was elaborately monitored to allow it to be fine-tuned and to translate it to an automated protocol.

Parts of this chapter were published as:
S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc*. **2016**, 138, 14182.

Chapter VI
Automated synthesis of monodisperse sequence-defined oligomers

VI.1 Introduction

In the previous chapters the one-pot amine-thiolactone-ene conjugation was applied for the modification of silica (chapter III), and solid supports and polymer end groups (chapter IV). In this chapter a relatively new class of macromolecules, sequence-defined oligomers, is tackled by means of this conjugation. Great progress has been made in the last decades in the preparation of functionalised and complex polymer architectures with defined structure-property relationships, yet these structures do not reach the same potential as their natural counterparts (DNA, RNA & proteins).[1-4] As a result, in the last decade, efforts have increased to achieve control over the primary structure of macromolecules in hopes of creating a stronger and/or unique correlation between properties and structure.[5] Many different methods and chemistries have been introduced to prepare sequence-controlled macromolecules.[6-26]

The fields of peptide and oligo-nucleotide chemistry were strongly revolutionised by means of solid phase synthesis (SPS)[27, 28], which enables the fast synthesis and isolation of oligomers. (Semi)automated protocols, which facilitate iterative SPS of oligonucleotides[28] and oligopept(o)ides[27, 29] have gained much attention for the preparation of defined/controlled sequences.[30, 31] This efficient automation of SPS enabled an even faster and parallel synthesis of a wide variety of natural oligomers, nowadays even up to ton scale.[32] Given these successes, Lutz *et al.* employed adapted protocols for the synthesis of sequence-defined polymers using phosphoramidite chemistry, which is classically used for the synthesis of poly- and oligonucleotides on DNA synthesisers.[31]

In previous research of our research group sequence-defined oligomers were prepared starting from an immobilised thiolactone unit enabling chain extension after on-resin aminolysis.[33] In this previous strategy, the monomer alphabet, encompassing amines and thiolactone units, is broad and protecting groups are not necessary. However, the reported approach was limited to the synthesis on a very small scale of tetramers with moderate crude purity. As a result of disulphide formation during on-resin aminolysis, treatment with phosphines as reducing agents was necessary, promoting the accumulation of side products. Therefore, in order to allow for the preparation of longer, highly functional sequences, with the additional possibility to translate it to an automated protocol, a new protecting-group-free strategy had to be developed. Features aimed at include elevated crude purity and much higher versatility in side chain functionality and backbone composition.

In chapter IV it became clear that the combination of the thiol-amine-ene with an immobilised thiolactone on a solid support proved to be more effective than the liquid support in matters of time and purification. This chapter deals with the implementation of the nucleophilic one-pot amine-thiol-ene conjugation[34] in a thiolactone-based robust and versatile protocol for solid phase synthesis of multifunctional sequence-defined oligomers and its successful automation. In the first step of the synthetic cycle of this new strategy (STEP 1, Figure VI.1), an immobilised thiolactone is selectively opened through the primary amine function of an amino alcohol, releasing a thiol that subsequently reacts with an acrylic unit through a nucleophilic thiol-ene reaction. A wide set of functionalities can be introduced by using abundantly available acrylics while the nature of the backbone can be varied through the selected amino alcohol. Moreover, in comparison to the previous reported approach to make defined oligomers, it is anticipated that disulphide formation can be completely avoided in this mild additive-free approach.[33, 34] In the next step, chain extension is performed via the reaction of the readily available and stable α-isocyanato-γ-thiolactone with the remaining alcohol function (STEP 2, Figure VI.1). This two-step protocol can be repeated until a unique oligomer with targeted length, sequence, end groups and functionality is obtained.[35]

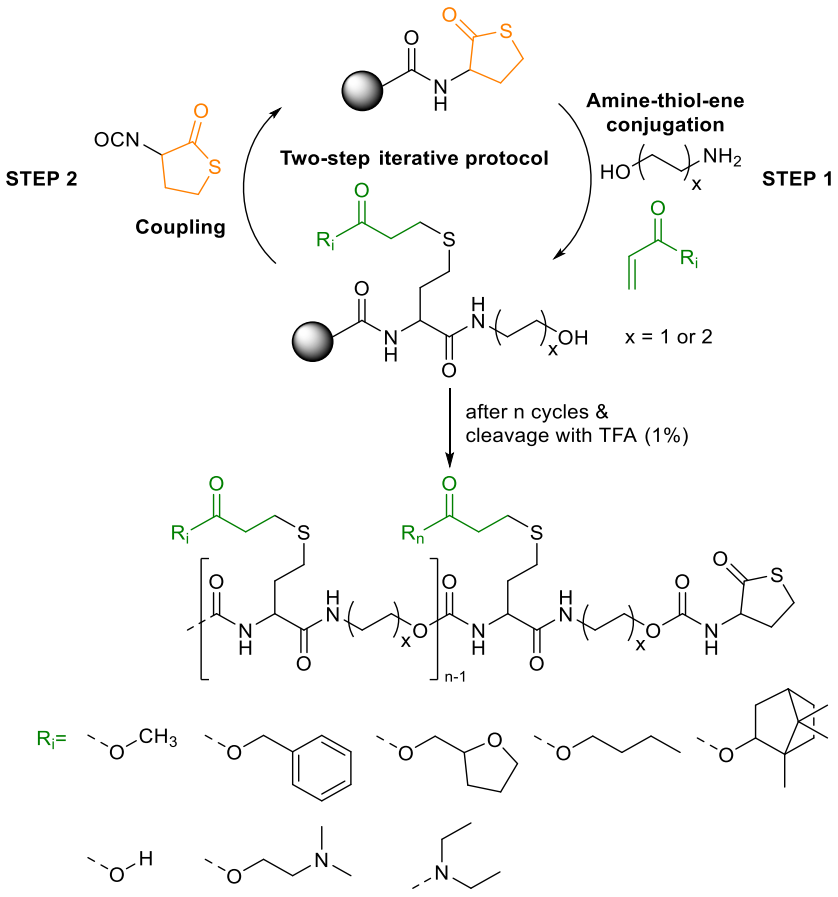


Figure VI.1: Thiolactone-based two-step iterative protocol yielding monodisperse, multifunctional oligomers on solid support. An amino alcohol opens the immobilised thiolactone and the thiol formed subsequently reacts with a functional acrylate or acrylamide (STEP 1). The thiolactone moiety is reinstated in the next step by means of the α-isocyanato-γ-thiolactone (STEP 2).

VI.2 Optimisation of the protocol

In order to optimise the protocol for the efficient synthesis of sequence-defined oligomers and to transfer this protocol to an automated approach, the reaction conditions had to be investigated thoroughly. The reaction stoichiometry, the choice of solvent and the reaction times used for each step had to be tackled. To prevent disulphide formation as much as possible, the amine/acrylate ratio used in Step 1 is important. From Chapter IV it became clear that a ratio of one to two should prevent this side reaction and that 10 equivalents of amine and 20 equivalents of acrylics should suffice for the modification of the thiolactone on the solid support. For a solvent, chloroform was chosen in accordance with Chapter IV, because the commonly applied DMF and CH_2Cl_2 promote different side reactions.[36, 37]

Finally, the reaction time is the last important parameter for Step 1. LC-ESI-MS samples were taken after 5, 15, 30 minutes' reaction and then every hour to monitor the first step of the protocol. The thiolactone, which was attached via a hydroxyl functionality to the solid support, was opened with ethanolamine and the thiol formed reacted with benzyl acrylate. With the disappearance of the peak at 3.35 min and the appearance of the peak at 4.74 min the measurements indicated that the reaction can be stopped after 15 minutes (Figure VI.2). To ensure that full conversion is also achieved for longer sequences, Step 1 is performed twice.

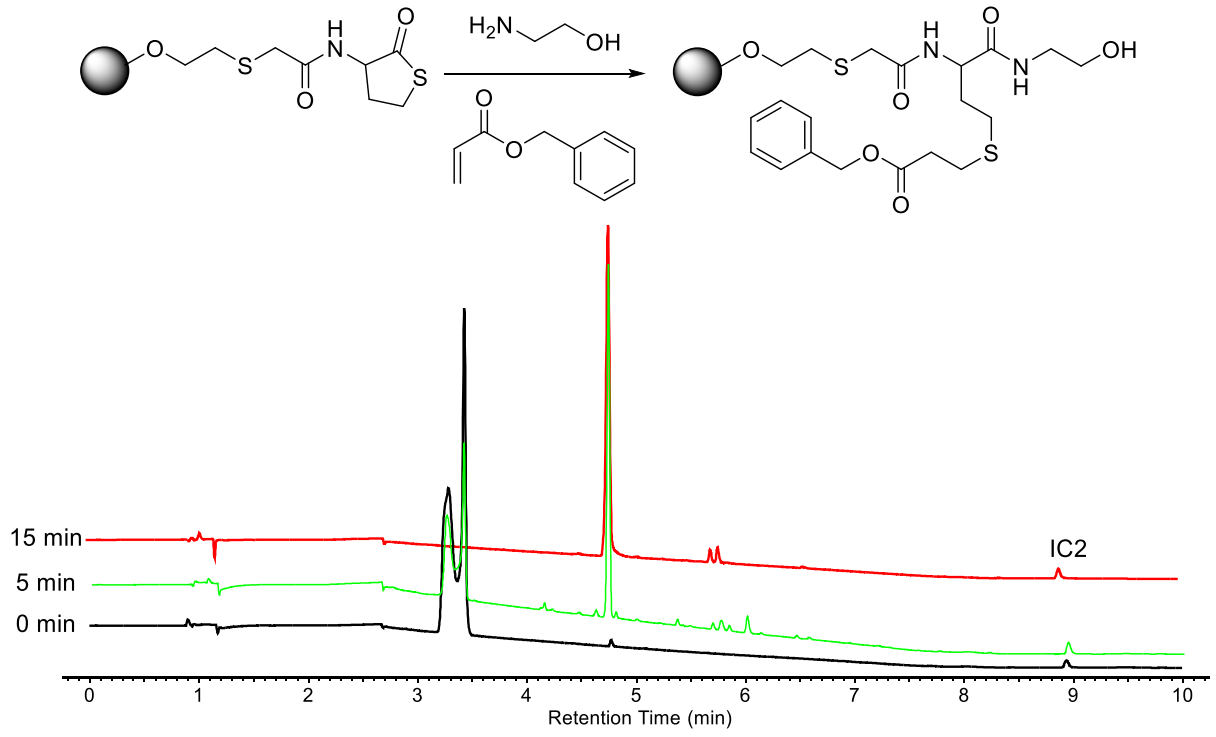


Figure VI.2: HPLC traces of the opening of thiolactone with ethanolamine and benzyl acrylate as a function of time.

In the second step of the iterative protocol (STEP 2), the alcohol, which was incorporated in the first step via aminolysis, reacts with the α-isocyanato-γ-thiolactone. During the reaction, 10 equivalents of the isocyanate and 0.025 equivalents of dibutyltin dilaurate (DBTL) which serves

as a catalyst for the reaction are applied. Subsequently, the chain extension with α-isocyanato-γ-thiolactone in three dry solvents (DMF, ethyl acetate and $CHCl_3$) was followed by taking a sample at 5, 15, 30 minutes and every hour for analysis by LC-ESI-MS. The disappearance of the peak at 4.74 min and the appearance of the peak at 5.23 min revealed for this screening of solvents that the reaction was completed after 2 hours, 24 hours and 30 minutes for ethyl acetate (Figure VI.3), DMF (Figure VI.4) and $CHCl_3$ (Figure VI.5) respectively. Because of its shorter reaction time, $CHCl_3$ was chosen. To again ensure the full conversion, the chain extension in $CHCl_3$ is made to last 1 hour.

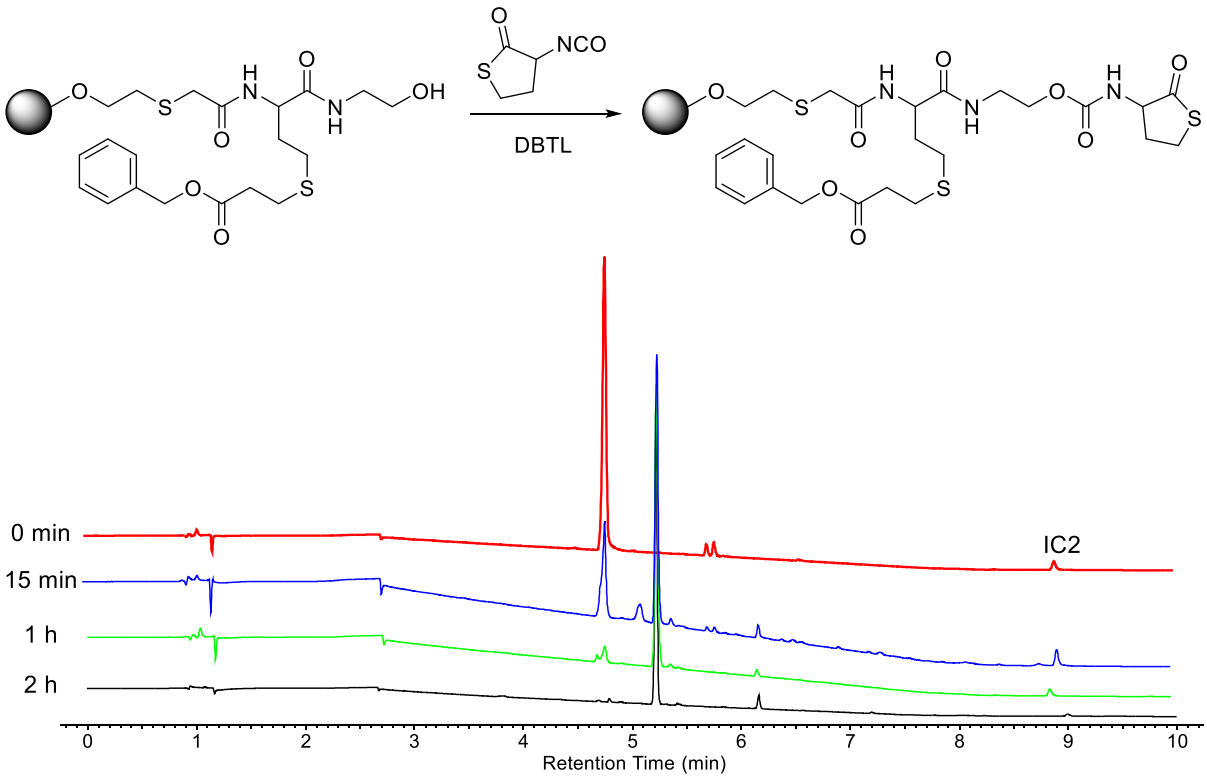


*Figure VI.3: HPLC traces of the chain extension with α-isocyanato-γ-thiolactone in **ethyl acetate** as a function of time.*

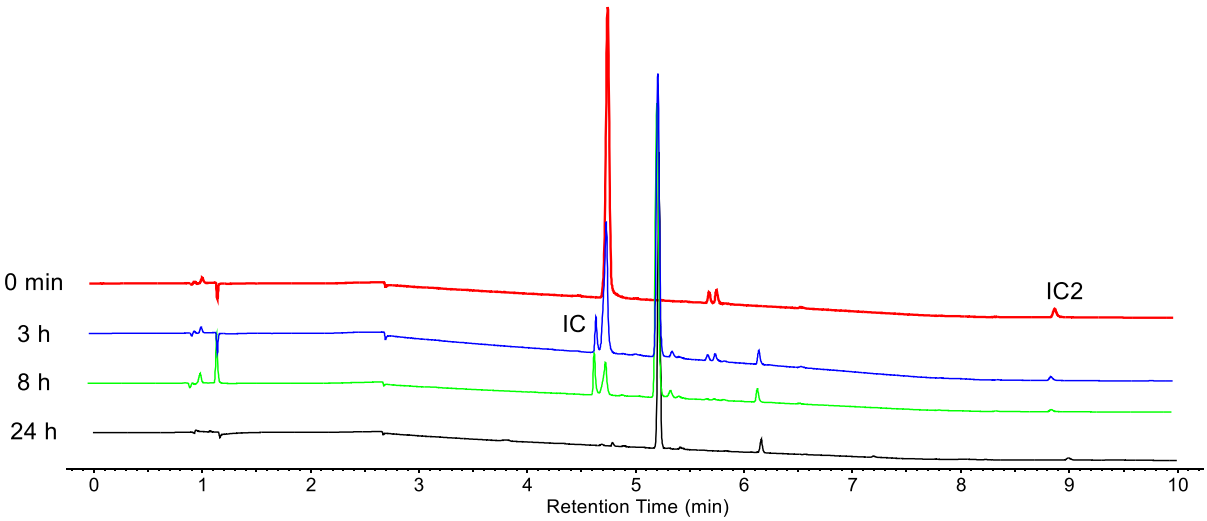


*Figure VI.4: HPLC traces of the chain extension with α-isocyanato-γ-thiolactone in **DMF** as a function of time (IC = Internal contamination, see IV.5.1).*

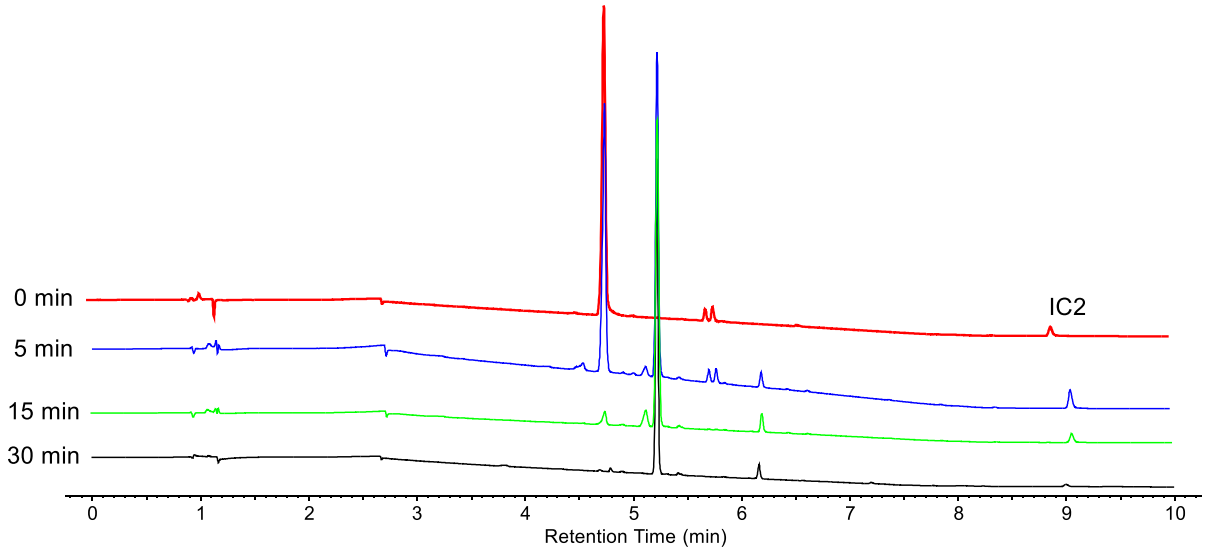


Figure VI.5: HPLC traces of the chain extension with α-isocyanato-γ-thiolactone in ***$CHCl_3$*** *as a function of time.*

VI.3 Synthesis of different sequences

First, with the optimised conditions, a trimer was built using benzyl, methyl and tetrahydrofurfuryl acrylate (C0.5-C3, Cx = performed cycles). The progress was rigorously followed using HR-MS (High Resolution Mass Spectrometry), LC-ESI-MS and NMR to verify sample purity and incorporation of side chain functionalities. One major peak that corresponded with the expected product was consistently observed in the LC traces of the crude reaction mixtures (Figure VI.6). The products display no deletions and a small amount of trifluoroacetic ester formation can be observed in the LC trace because of the reaction of the hydroxyl end group with the TFA used in the cleavage mixture (1% TFA in CH_2Cl_2) (Figure VI.7).

NMR indicates that the functionalities (benzyl, methyl and tetrahydrofurfuryl) are incorporated (Figure VI.8). The first gives signals at 5.11 and 7.36 ppm, the second at 3.6 ppm and for the third one, two distinct peaks can be found at 3.63 and 3.73 ppm. Next, the trimer was elongated to a pentamer (C5) adding two extra functionalities using butyl and isobornyl acrylate. This last structure also displayed high purity and did not show any truncated structure in LC-ESI-MS. Distinctive peaks could be observed in the NMR spectra at 0.88 and 1.33 ppm for the butyl moiety, and 0.8, 0.95, 1.05 and 1.09 ppm for the isobornyl group. Continuing with this pentamer, additional benzyl and tetrahydrofurfuryl residues were incorporated yielding a highly pure heptamer (C7).

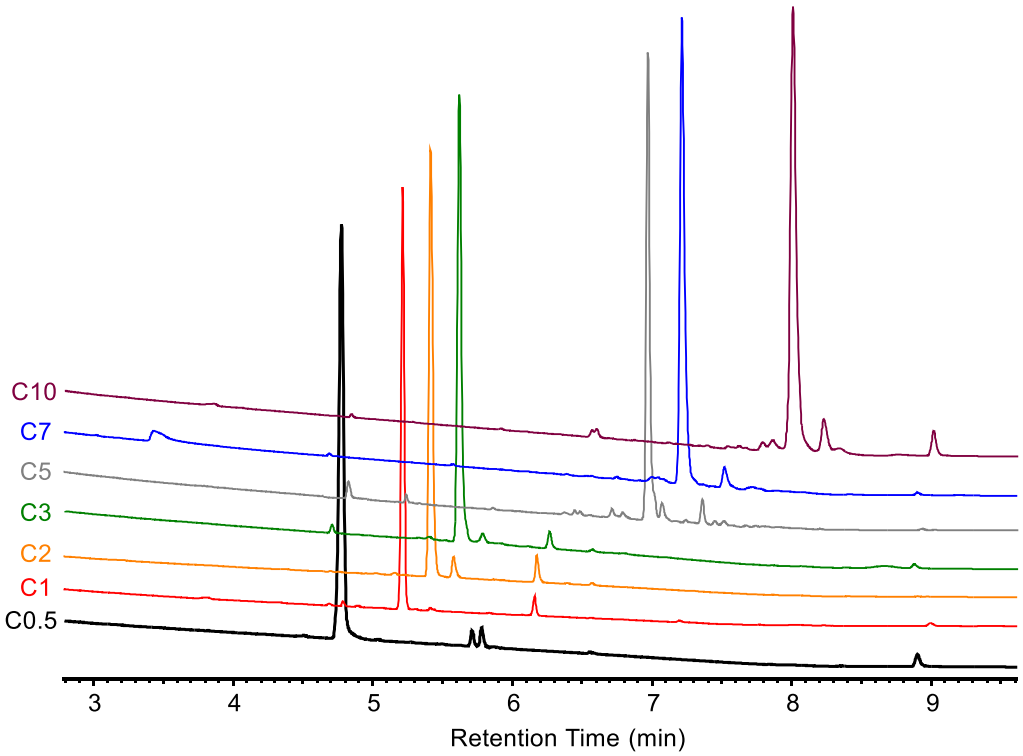


Figure VI.6: HPLC traces of sequences from an opened thiolactone (C0.5) to a decamer (C10) (Cx = performed cycles)

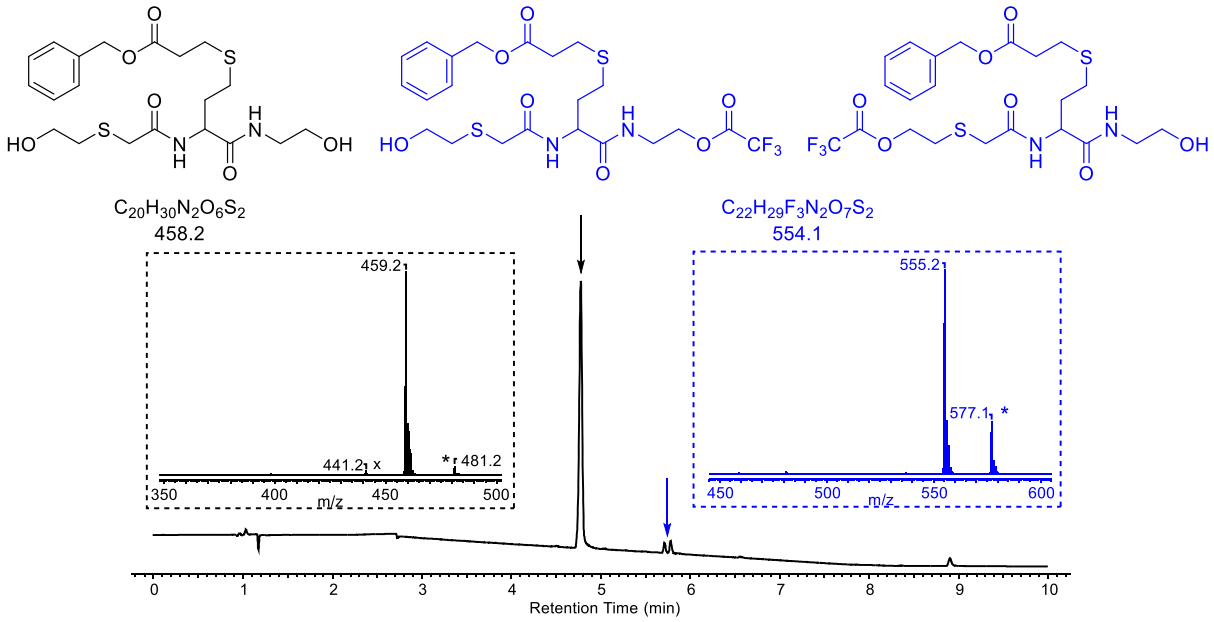


Figure VI.7: LC-ESI-MS analysis of C0.5. Inserts: ESI-MS-spectra of dominant species (black) and TFA side reaction (blue) (positive mode with sodium adducts (*) and fragmentation caused by elimination of water (x)).

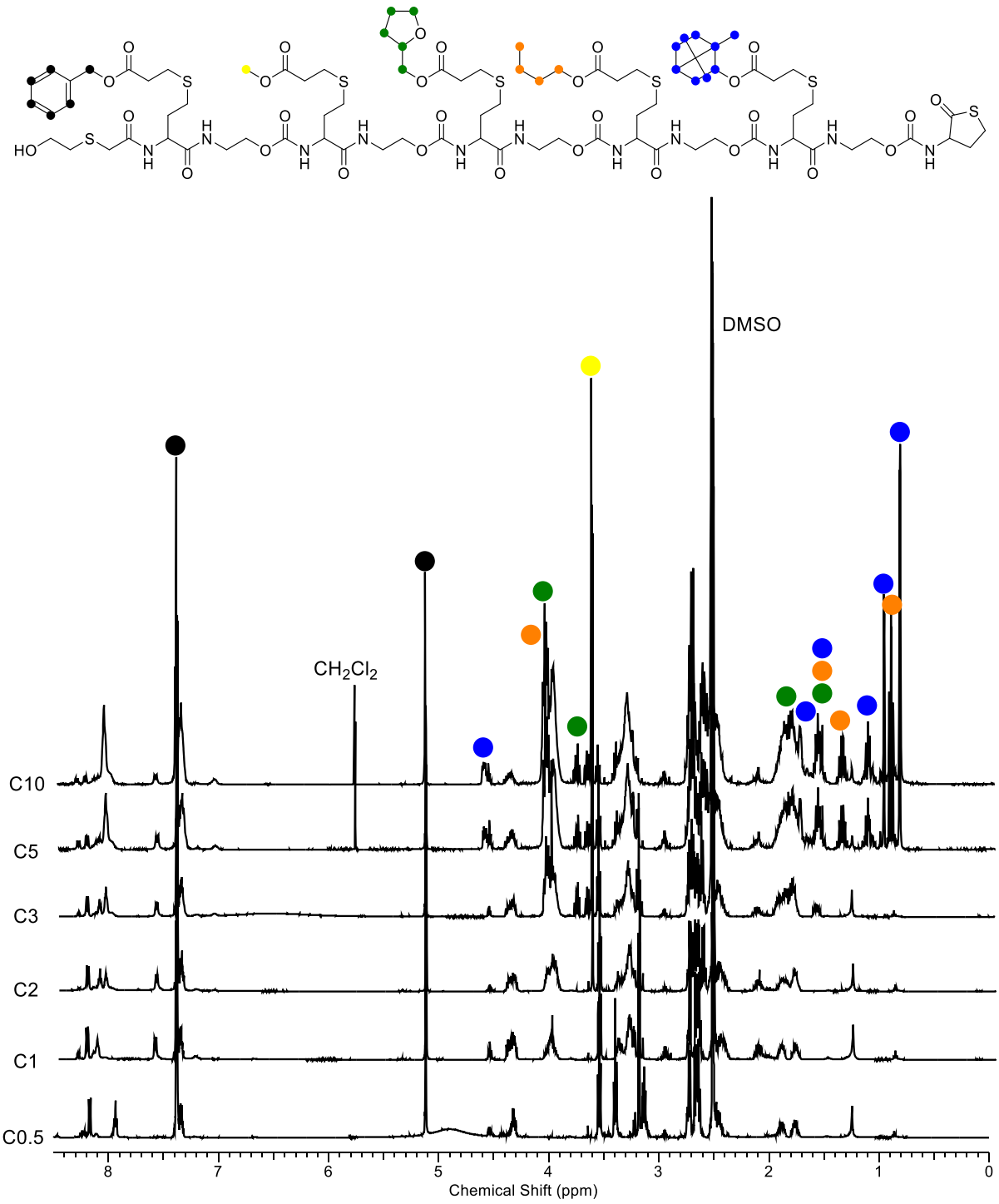


Figure VI.8: NMR spectra starting from an opened thiolactone (C0.5) to a decamer (C10). Distinctive peaks for the incorporation of certain functionalities are indicated with colored spheres on C5: benzyl (black), methyl (yellow), tetrahydrofurfuryl (green), butyl (orange) and isobornyl (blue)

Subsequently, five different decamers were made. Starting from the heptamer C7, three extra functionalities (methyl, butyl and isobornyl) were added to obtain the first decamer C10. First, a homodecamer (T10) was prepared with only tetrahydrofurfuryl acrylate as functionality. Then, the latter acrylate and benzyl acrylate were applied to synthesise the third (B5T5) and fourth ((BT)5) decamers, respectively consisting of two pentamer blocks and an alternating structure. A small retention time difference in the LC traces can be observed for both decamers

(Figure VI.9). Although the masses are the same, this difference originates from their different behaviour towards the stationary phase in RP-HPLC (Phenomenex C18 (2) column (5 µ, 250 x 4.6 mm) using a solvent gradient 0 → 100% acetonitrile in H_2O in 15 min). Finally, starting from the acid linker on solid support, the fifth decamer had a sequence similar to the first one, but an acid instead of a hydroxyl as α-end group. LC-ESI-MS spectra indicated that the purity of the different oligomers is very high and NMR confirmed the incorporation of the different acrylates or end groups.

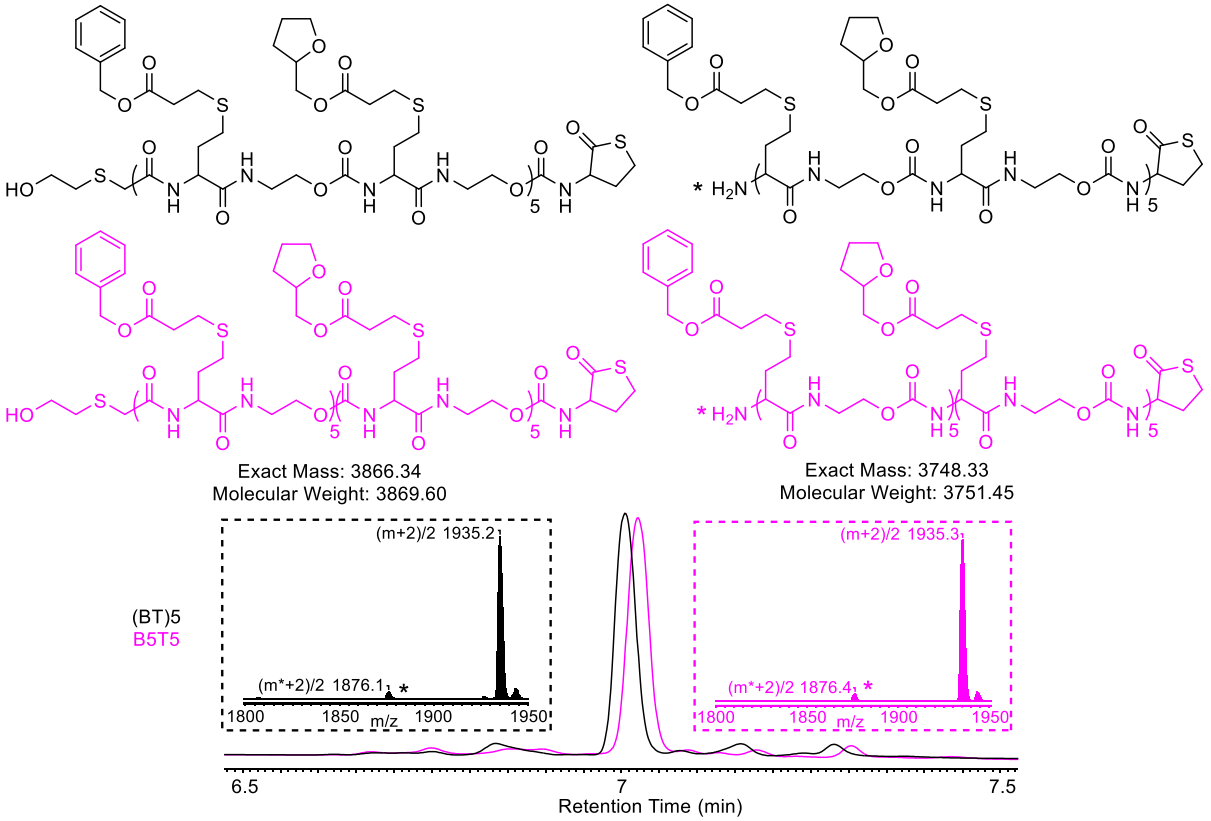


Figure VI.9: Overlay of HPLC traces of two decamers with the same mass (and amide fragmentation (*)) but with a different sequence.

Continuing with C10 and T10, a tetradecamer (C14) and a homohexadecamer (T16) respectively could be prepared. Tetrahydrofurfuryl, methyl, benzyl and butyl acrylate were incorporated in this order in the C14 oligomer. For the LC trace of C14 two peaks can be observed which correspond to the product and a tridecamer that misses a methyl acrylate unit (Figure VI.10). In the mass spectrum of T16 indications of the homopentadecamer (T15) can be found (Figure VI.11). The further build-up of both sequences was stopped, because the tridecamer and the homopentadecamer would grow together with the tetradecamer and the homohexadecamer respectively.

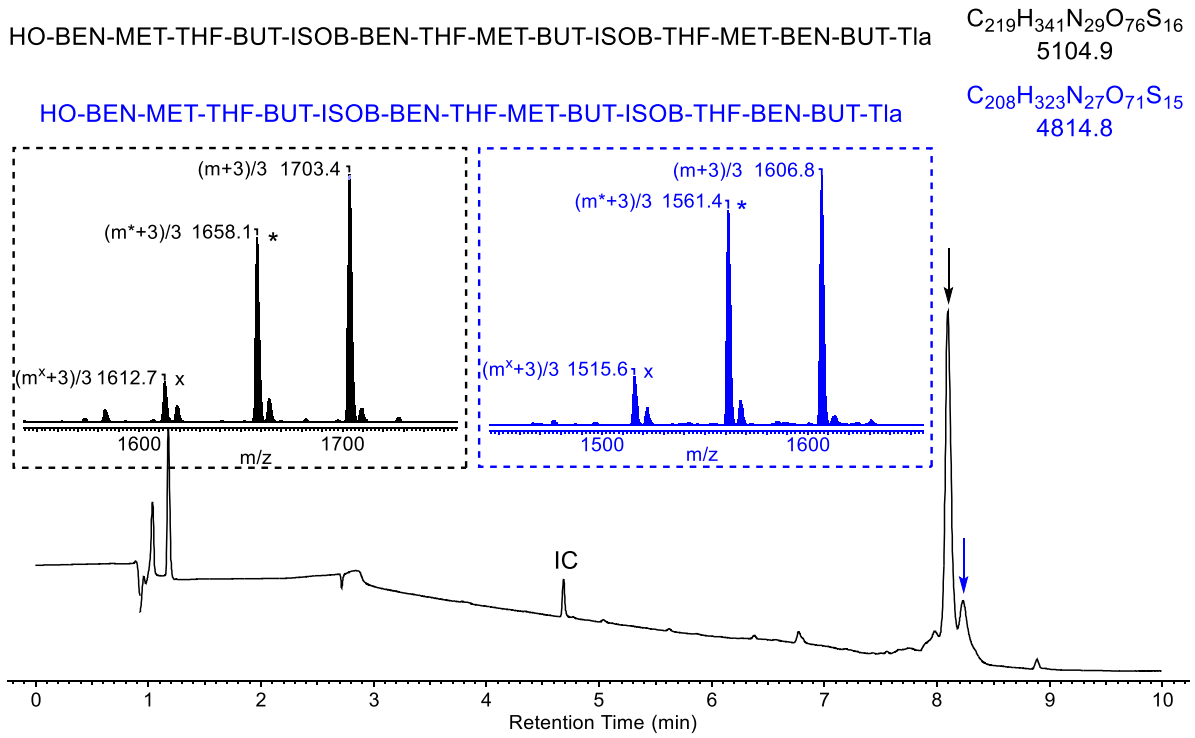


Figure VI.10: LC-ESI-MS analysis of C14. Inserts: ESI-MS-spectra of dominant tetradecamer species (black) and tridecamer missing a methyl acrylate unit (blue) (positive mode with fragmentations caused by one isobornyl (*) and two isobornyl (x) eliminations).

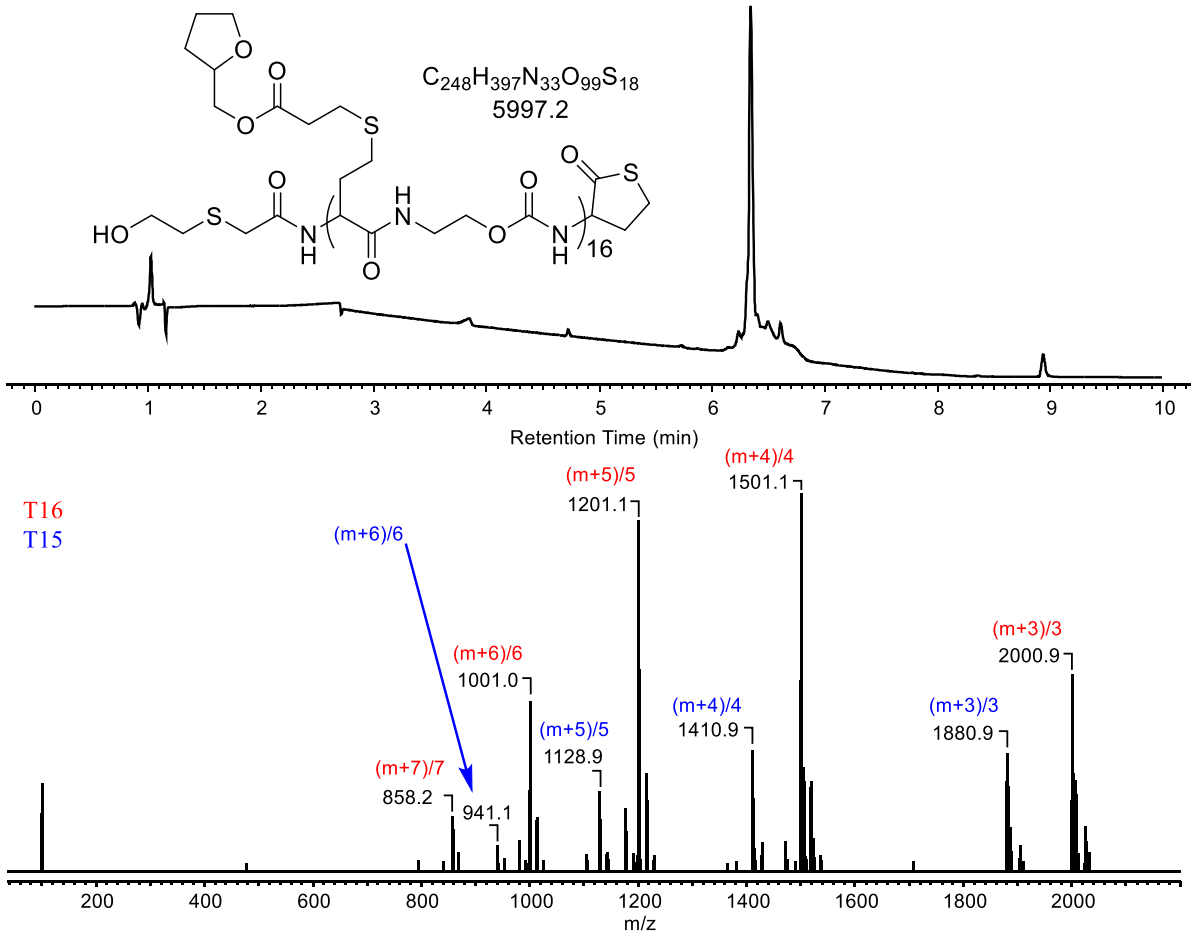


Figure VI.11: LC-ESI-MS analysis of T16. Inserts: ESI-MS-spectra of dominant hexadecamer species (black) and tridecamer missing a methyl acrylate unit (blue) (positive mode).

Within our group it is observed that with lower loading, repetition of steps and longer reaction times the purity of longer oligomers (above decamers) can be increased and the deletions are prevented. In Borner's group, this protocol was applied to a PEG preloaded poly(styrene) resin to make block copolymers consisting of a thiolactone based sequence-defined pentamer and a poly(ethylene glycol) (PEG) block.[38] It was observed that the purity of the block copolymers was improved by modifying the protocol with the measures proposed above: STEP 1: 20 eq ethanolamine and 40 eq of acrylate (2 x 30 minutes), and STEP 2: 20 eq of α-isocyanato-γ-thiolactone (3 x 1 hour). This should be taken into account when longer oligomers are required, which is now further investigated in the PhD research of Chiel Mertens, including adapted protocols.

To widen the scope of side chain functionalities, 1-ethoxyethyl and 2-(dimethylamino)ethyl acrylate were also applied to incorporate an acid and a tertiary amine in the side chains. The ethoxyethyl group is removed from the resin during the acidic cleavage, which results in an acid residue. Two trimers (EE3 and DMAE3) were prepared with the acrylates mentioned and butyl acrylate. HR-MS, LC-ESI-MS and NMR confirmed the purity of the structures and the incorporation of the functionalities (Figure VI.12).

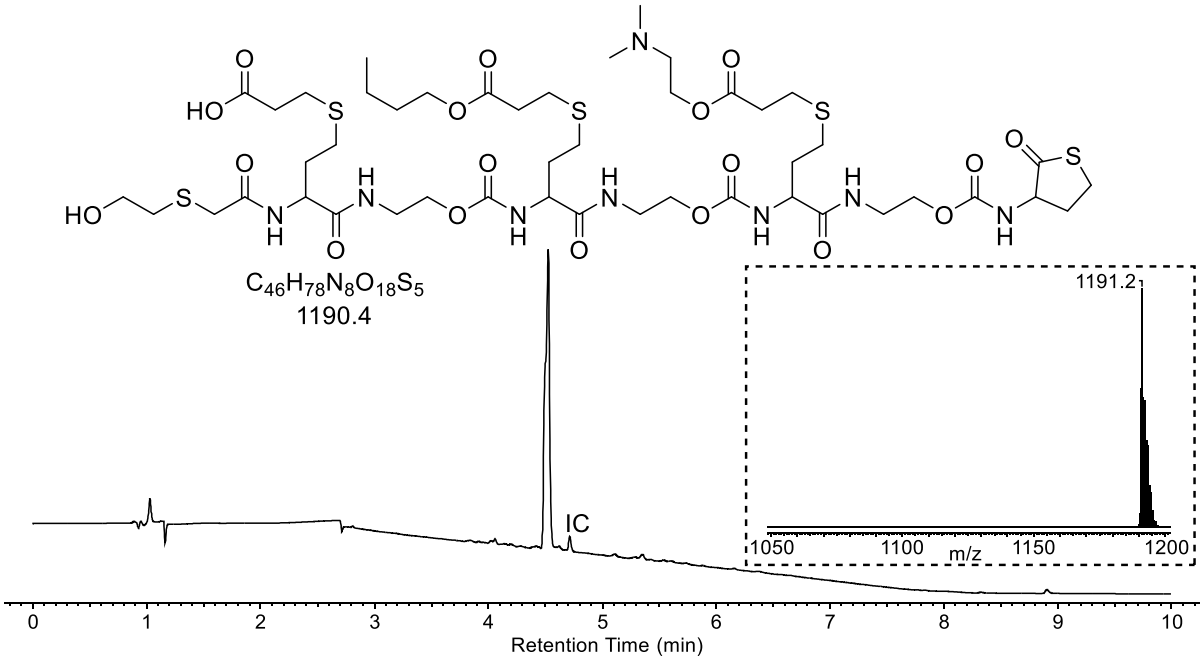


Figure VI.12: LC-ESI-MS analysis of EE3. Insert: ESI-MS-spectrum of dominant species (positive mode).

To further extend the functionality scope, acrylamides were also tested to functionalise the oligomers. Two acrylamides were tested: N-isopropyl- (NIPAM) and N,N-diethylacrylamide. For the first one, problems were encountered while building the sequence. The acrylamide did not dissolve very well in the reaction mixture, though this could be prevented by adding a drop of DMF. The formation of disulphides was also observed when reactions were performed with NIPAM. The product displays two peaks in the LC trace around 3.65 min and the disulphide around 3.52 min (Figure VI.13). The disulphide formation can be explained by the fact that acrylamides react much slower than acrylates[39, 40], and that atmospheric oxygen and a base

(ethanolamine) were present in the synthesis reactor.[41] Indeed, for this reaction, after the aminolysis of the thiolactone, the formed thiol has a higher chance to form disulphides, as it is consumed more slowly by the thiol-ene reaction with the acrylamide than it would be with an acrylate, and bases accelerate disulphide formation in the presence of atmospheric oxygen.[41] As there is an excess of amine, this acceleration can be significant. The disulphide formation could be suppressed by first working in inert conditions to avoid the presence of atmospheric oxygen during the reaction. Another strategy would be to reduce the amount of amine or add it stepwise, in order to reduce the concentration of free thiols formed due to aminolysis. The amount of acrylamide can also be increased to ensure that the formed thiols react with the acrylamide more so than forming disulphides, although this strategy could cause some solubility issues, as discussed above. A final strategy is based on a protocol which was developed recently in our group.[42] First, the thiolactone is opened with ethanolamine in the presence of a phosphine to prevent disulphide formation. In a subsequent step, the formed thiol can react with the acrylamide. This ensures that no base is present during the thiol-ene reaction and that disulphide formation is slowed down.

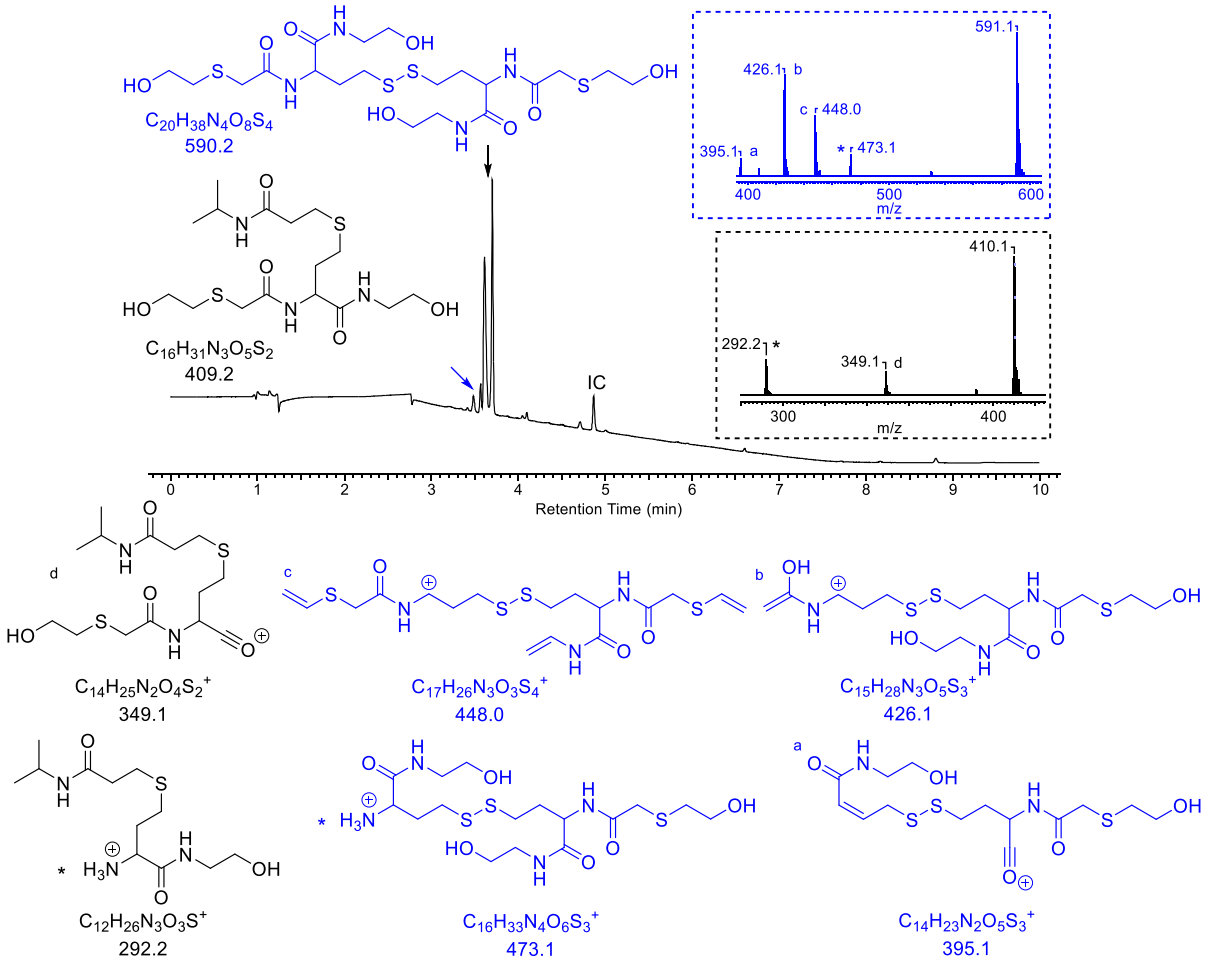


Figure VI.13: LC-ESI-MS analysis of functionalisation with NIPAM. Inserts: ESI-MS-spectra of dominant species (black) and disulphide adduct (blue) (positive mode with fragmentation as in Figure VI.9 (*), combination of a McLafferty rearrangement with either subsequent ethanolamide fragmentation (a) or α-cleavage of the ethanolamide (b), combination of α-cleavage of the ethanolamide with three times elimination of water (c), and ethanolamide fragmentation (d)).

Although disulphide formation leads to side products, the synthesis was continued in order to examine if other problems might occur. During the next step, the chain extension with α-isocyanato-γ-thiolactone, the product was formed (4.27 min), but to some extent the acrylamide was dropped from the oligomer and replaced with an isocyanate, resulting in a molecule with two thiolactone groups (4.36 min) (Figure VI.14). As expected, the disulphide side product from the previous step also continued to grow (4.39 min).

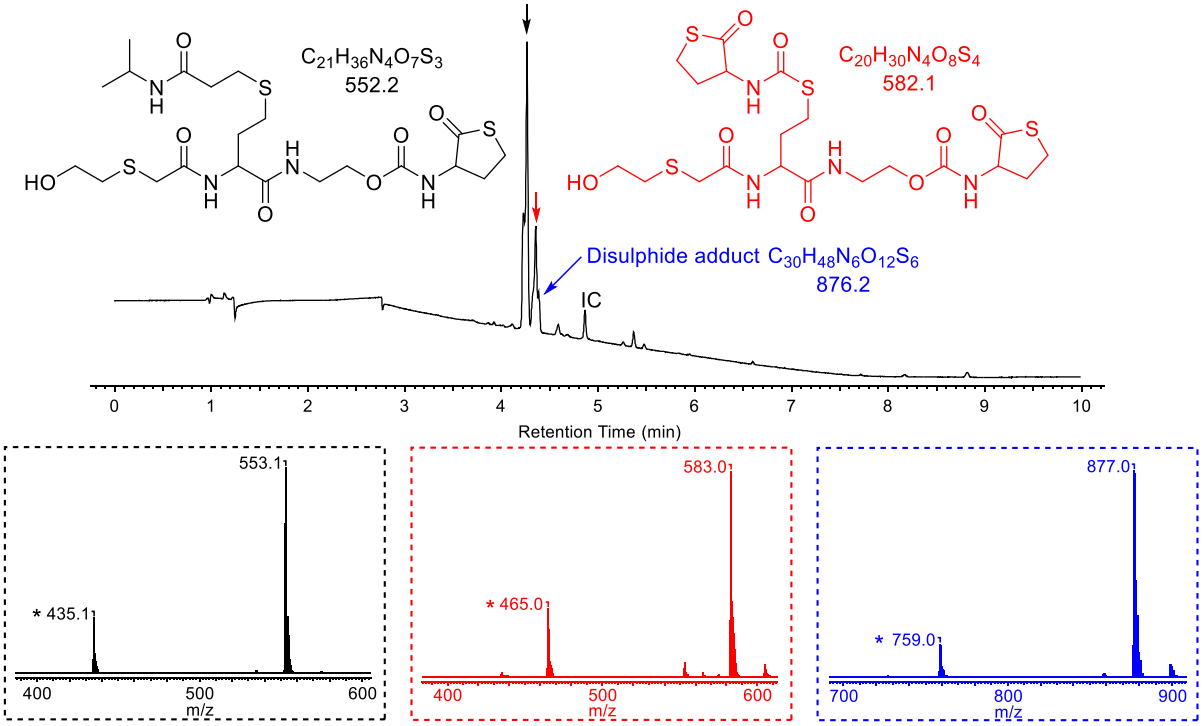


Figure VI.14: LC-ESI-MS analysis of chain extension after the NIPAM functionalisation. Inserts: ESI-MS-spectra of dominant species (black), thiolactone functionalisation (red) and disulphide adduct (blue) (positive mode with fragmentations as in Figure VI.9 (*)).

In the next protocol cycle with NIPAM as acrylic unit only two distinctive peaks can be observed: the product (4.55 min) and the disulphide side product (4.72 min) (Figure VI.15). The thiourethane must have broken during the reaction with ethanolamine with the formation of a thiol that must have reacted with NIPAM or formed a disulphide. The disulphide formation can occur between the thiols formed after aminolysis of the thiolactone or thiourethane unit, which leads to three different possible side products.

When tetrahydrofurfuryl acrylate is used instead of NIPAM after the first cycle, a branched structure (5.16 min) and a dimer with two tetrahydrofurfuryl side groups (5.04 min) can be found in addition to the desired product (4.78 min) (Figure VI.16). In this case not all thioureas were broken. It was decided not to continue with this acrylamide, because if the chain will become longer and more different acrylates are used, more side products will be formed.

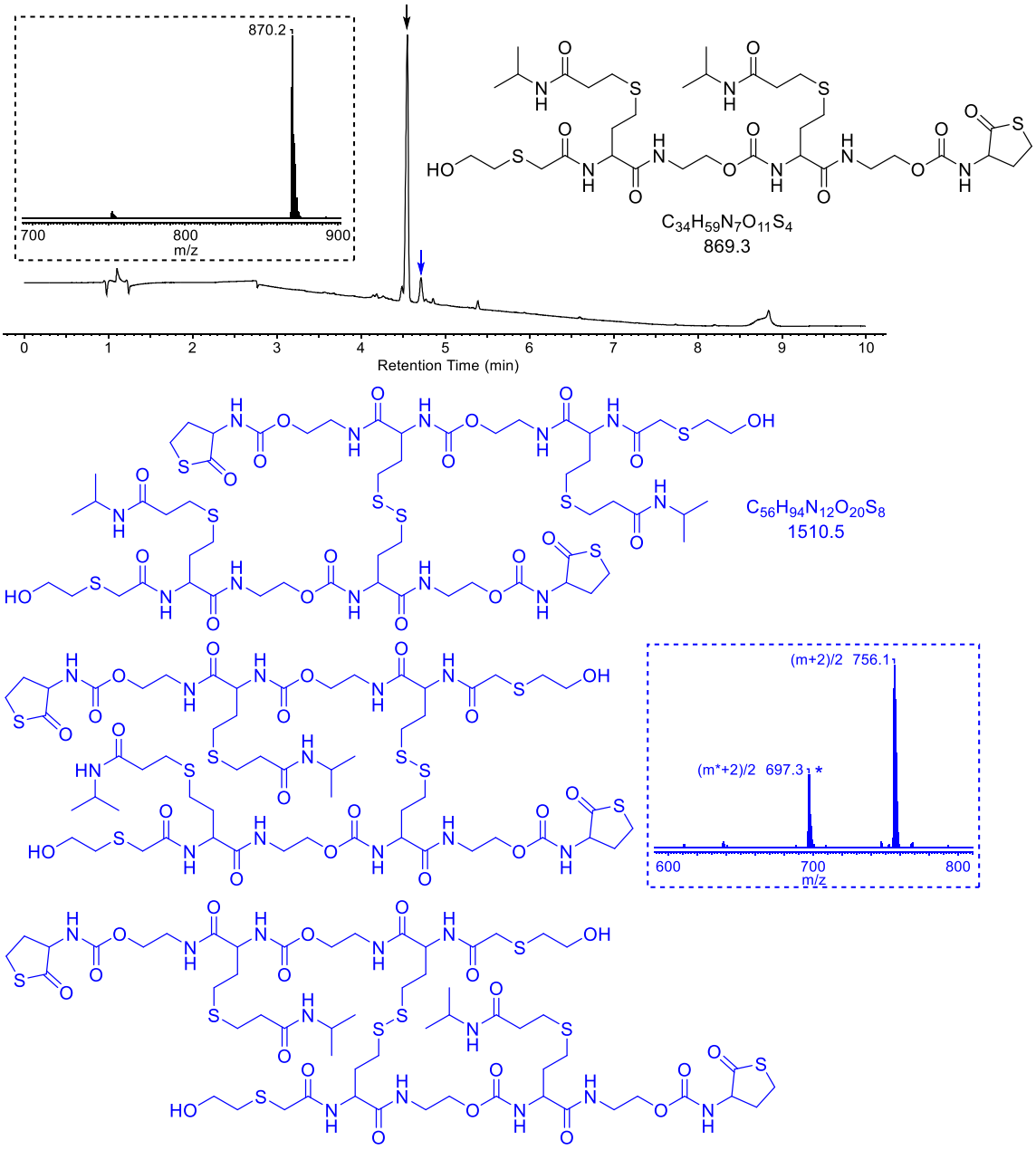


Figure VI.15: LC-ESI-MS analysis of a second NIPAM functionalisation. Inserts: ESI-MS-spectra of dominant species (black) and disulphide adducts (blue) (positive mode with fragmentations as in Figure VI.9 (*)).

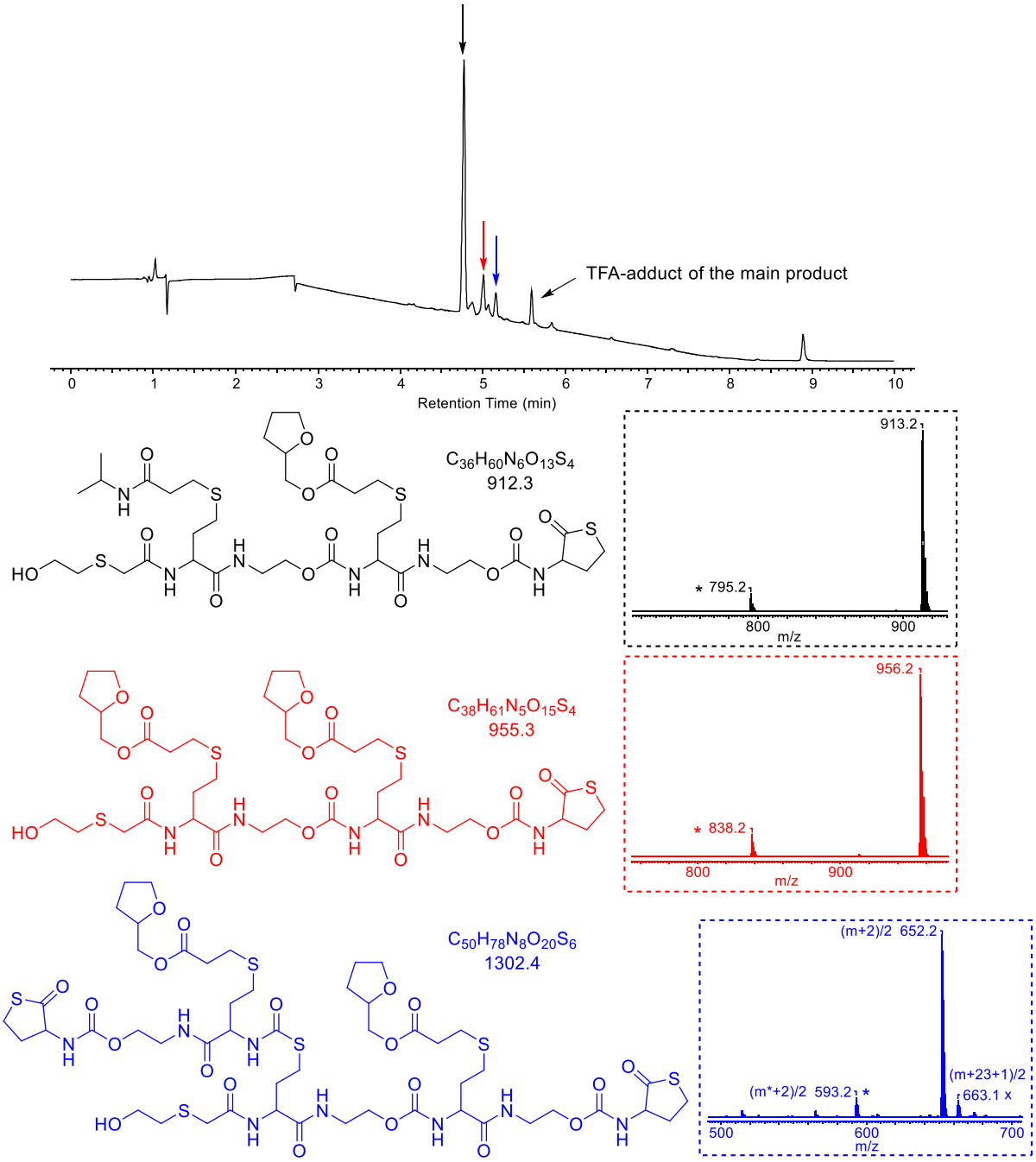


Figure VI.16: LC-ESI-MS analysis of tetrahydrofurfuryl acrylate functionalisation. Inserts: ESI-MS-spectra of dominant species (black), tetrahydrofurfuryl acrylate dimer (red) and branched adduct (blue) (positive mode with sodium adduct (x) and fragmentations as in Figure VI.9 (*)).

The use of N,N-diethylacrylamide did not cause any problems during the synthesis. In comparison to NIPAM, a trimer (DEAA3) could be formed while using tetrahydrofurfuryl acrylate in subsequent steps of the protocol. HR-MS, LC-ESI-MS (5.24 min) and NMR confirmed the purity of this oligomer and the incorporation of the functionalities (Figure VI.17). In the meantime, Joshua Holloway developed an adapted protocol to introduce amides into the side chain of the oligomers by means of thioacrylates (see II.4.2.2).[42]

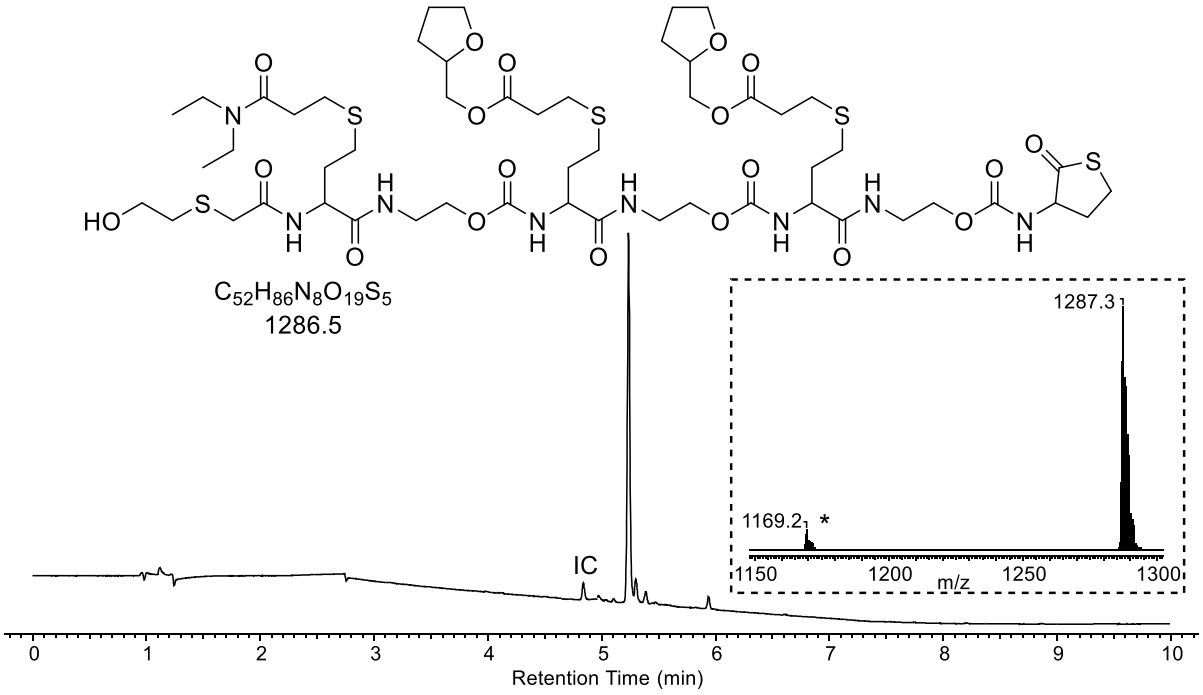


Figure VI.17: LC-ESI-MS analysis of DEAA3. Insert: ESI-MS-spectrum of dominant species (positive mode with fragmentation as in Figure VI.9 (*)).

The backbone of the oligomers can be modified by using different amino alcohols. In the previous examples ethanolamine was used, but in an attempt to illustrate the versatility of this protocol, the following amino alcohols were tested as well: DL-prolinol (2-(hydroxymethyl)pyrrolidine), trans-4-aminocyclohexanol, 4-(aminomethyl)phenol and 4-amino-1-butanol (Table VI.1). The first is a reduced form of the amino acid proline, which can be found in turn and loop structures of proteins, and was chosen for its probable potential to induce turning behaviour in the oligomer.[43] The first three have cyclised structures which could give rigidity to the backbone of the oligomer. The last amine, 4-amino-1-butanol, was chosen as it has a similar structure in comparison to ethanolamine, can give extra flexibility to the backbone and increases the distance between side-chain functionalities.

Table VI.1: Structures of the different amino alcohols that were tested in addition to ethanolamine.

DL-prolinol	trans-4-aminocyclohexanol	4-(aminomethyl)phenol	4-amino-1-butanol

The use of a different amino alcohol leads to different reaction times for aminolysis. The kinetics of the aminolysis in solution and the effect of the amine on these kinetics were explored in previous research performed in our group.[34] From a range of different amines, it was discovered that the aminolysis with the cyclic secondary amine pyrrolidine was the fastest. When we used 10 equivalents of prolinol (also a cyclic 5-membered secondary amine like pyrrolidine) for 2 x 15 minutes, similarly to ethanolamine, the opening was not fully finished (4.37 min for the product) (Figure VI.18).

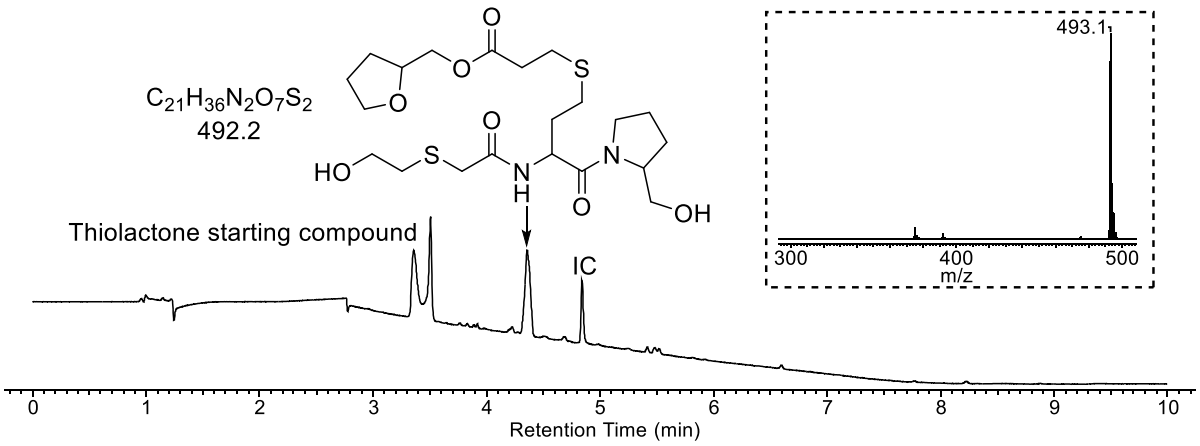


Figure VI.18: LC-ESI-MS analysis of aminolysis with prolinol. Insert: ESI-MS-spectrum of the opened adduct (positive mode).

A kinetic study was performed: LC-ESI-MS samples were taken at 5, 15, 30 minutes and every hour, and benzyl acrylate was applied to capture the formed thiol after aminolysis of the thiolactone. No big difference was observed between the LC traces of 5 minutes and 8 hours, in which both the starting compound (3.35 min) and the product (5.09 min) could be found (Figure VI.19).

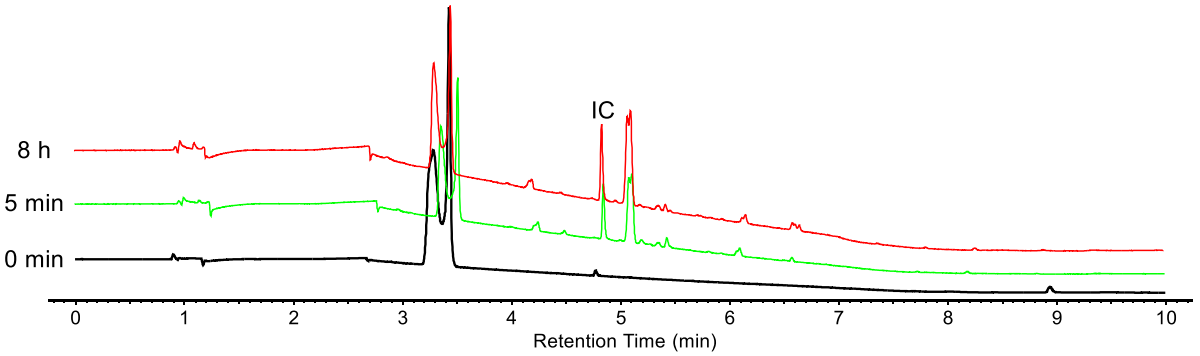


Figure VI.19: HPLC traces of the opening of thiolactone with prolinol and benzyl acrylate as a function of time.

As previously mentioned, two times 15 minutes did not suffice to open the thiolactone with prolinol. Three more iterations of this reaction still left some starting compound. The amount of amine was increased to 20 equivalents, equal to the amount of acrylate, and rendered full conversion after 3 times 15 minutes. The chain extension with α-isocyanato-γ-thiolactone resulted in two compounds: the desired product (4.92 min) and a molecule in which the THF acrylate was removed and replaced with a α-isocyanato-γ-thiolactone (4.66 min) (Figure VI.20). The next reaction with benzyl acrylate and ethanolamine led to even more different side products (Figure VI.21). Next to the expected product, unopened thiolactone was observed and the side product with the thiolactone side-chain (Figure VI.20, 4.66 min) was two times double modified with benzyl acrylate and ethanolamine (Figure VI.21, 5.55 min). A dimer with two benzyl acrylate side-chains was formed after aminolysis of the thiocarbamate and the subsequent reaction with benzyl acrylate (Figure VI.21, 5.85 min). Prolinol was thus no longer considered as a suitable amino alcohol for the protocol because of the side product formation.

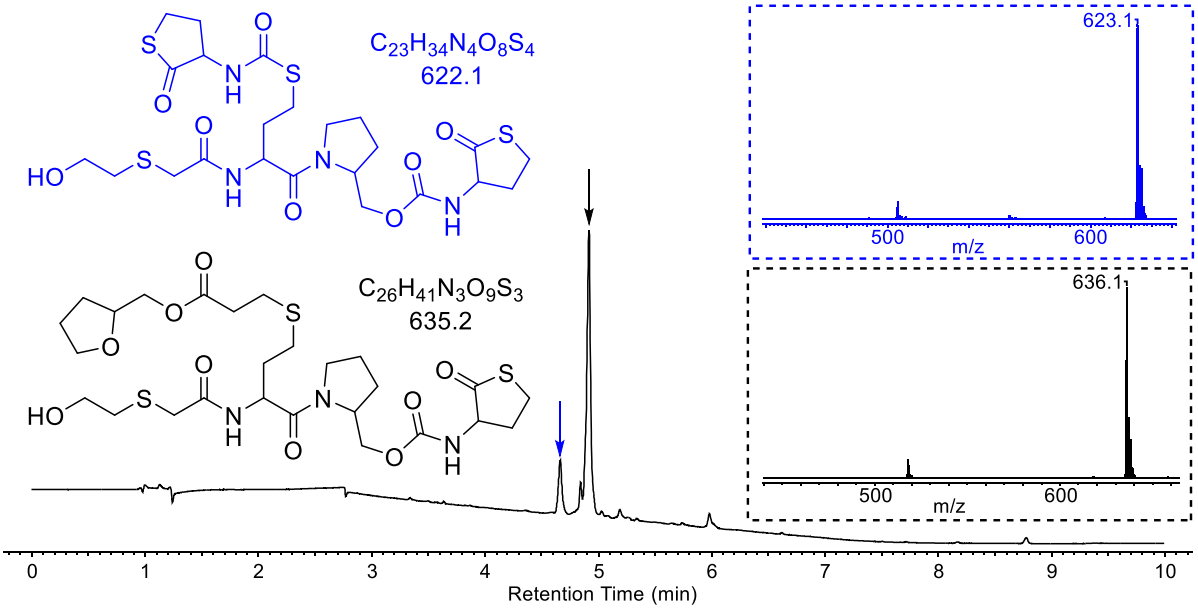


Figure VI.20: LC-ESI-MS analysis of chain extension after aminolysis with prolinol. Inserts: ESI-MS-spectra of dominant species (black) and thiolactone functionalised adduct (blue) (positive mode).

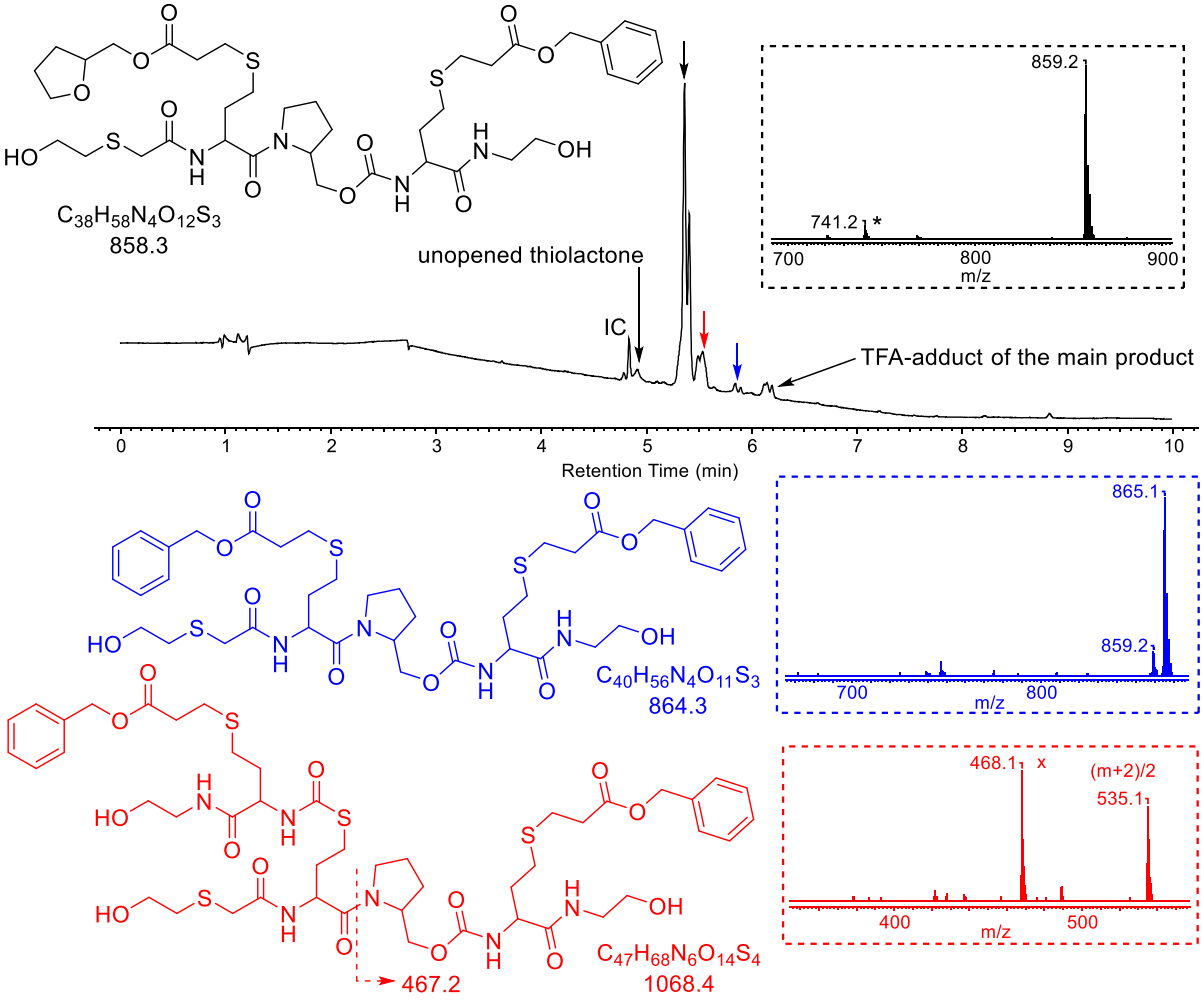


Figure VI.21: LC-ESI-MS analysis of benzyl acrylate functionalisation. Inserts: ESI-MS-spectra of dominant species (black), branched adduct (red) and benzyl acrylate dimer (blue) (positive mode with fragmentation as in Figure VI.9 (*) and fragmentation of the prolinol amide bond (x)).

Subsequently, trans-4-aminocyclohexanol was used for the aminolysis. In previous research within our group,[44] it was proven that aminolysis is slower for a primary amine next to a secondary carbon. This effect is attributed to sterical hindrance. The solubility of this amine was also very low in the reaction mixture used. The addition of more solvent, while possible, would slow down the aminolysis. To screen the solubility, a range of different solvents were used: acetone, THF, acetonitrile, DMF, NMP, methanol, ethanol, cyclohexane, dioxane, diethylether, toluene and $CHCl_3$. In this screening, NMP, DMF, methanol and ethanol proved to solubilise the amine very well. The alcohols proved to be the best option, so a mixture of methanol and $CHCl_3$ was tested first. This mixture is needed because in methanol alone the resin displays bad swelling behaviour. In addition to the formation of the main product (4.21 min), the use of methanol also resulted in the formation of a side product (4.64 min), which is the alcoholysis of the thiolactone (Figure VI.22). This alcoholysis was caused by the slower aminolysis of trans-4-aminocyclohexanol and the basic medium generated by the amine.[45] The use of methanol was not pursued any further due to this side reaction.

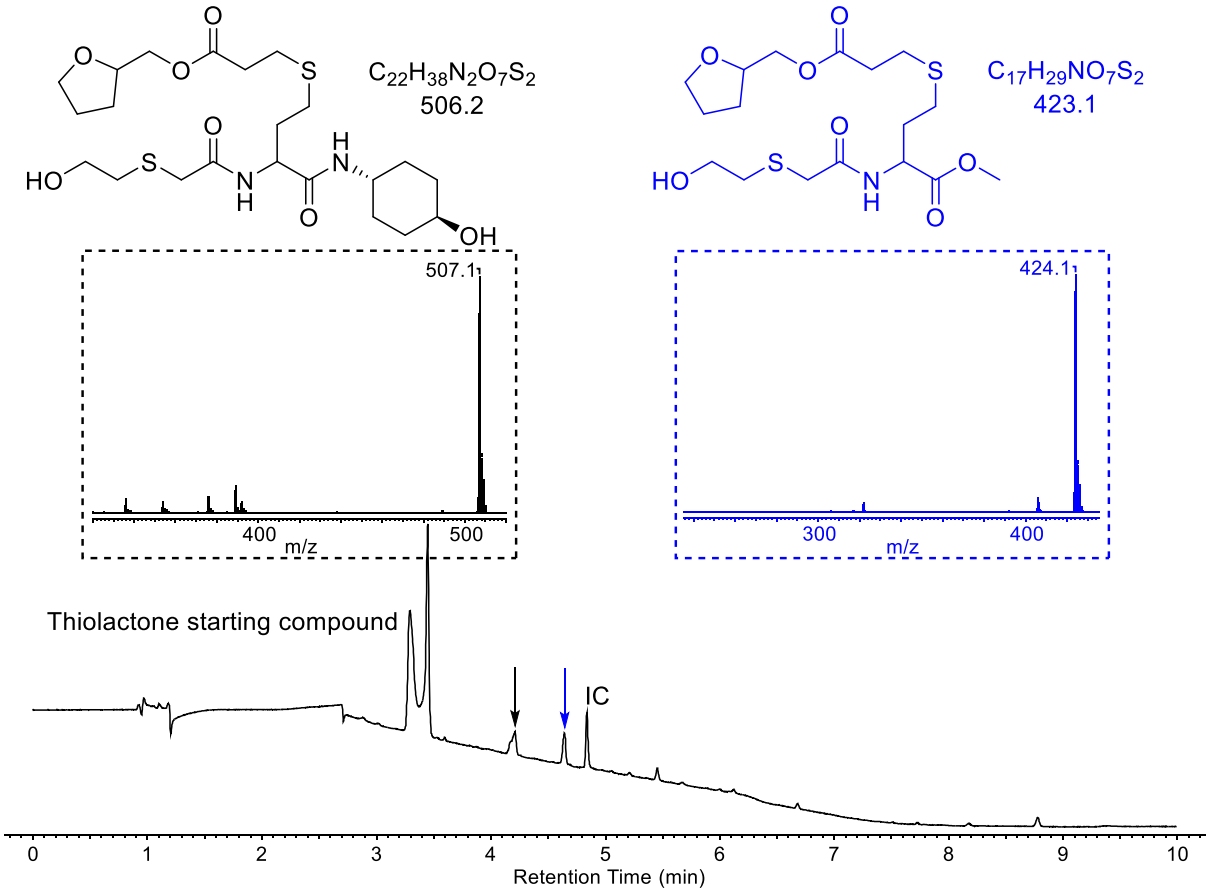


Figure VI.22: LC-ESI-MS analysis of aminolysis with trans-4-aminocyclohexanol in methanol. Inserts: ESI-MS-spectra of dominant species (black) and alcoholysis (blue) (positive mode).

Although NMP, just as DMF, can boost the aza-Michael reaction, NMP was tried next as a solvent.[36] The reaction did not reach full completion after a few iterations, modifying the ratio of amine and acrylate, and making a mixture of NMP and $CHCl_3$ to reduce the aza-Michael side reaction. In addition, a very small amount of hydrolysis of the thiolactone can be retrieved

in the LC trace due to the basic medium caused by the amine and the slower aminolysis (3.66 min) (Figure VI.23). This can perhaps be prevented with the use of dry solvents. This amine was not further investigated in this PhD thesis because of the very slow reaction kinetics and the time-consuming in-depth optimisations required to fully develop the system as we did for ethanolamine. In the future, catalysts such as DMAP could perhaps be employed to speed up the aminolysis, which was also done in previous research.[46]

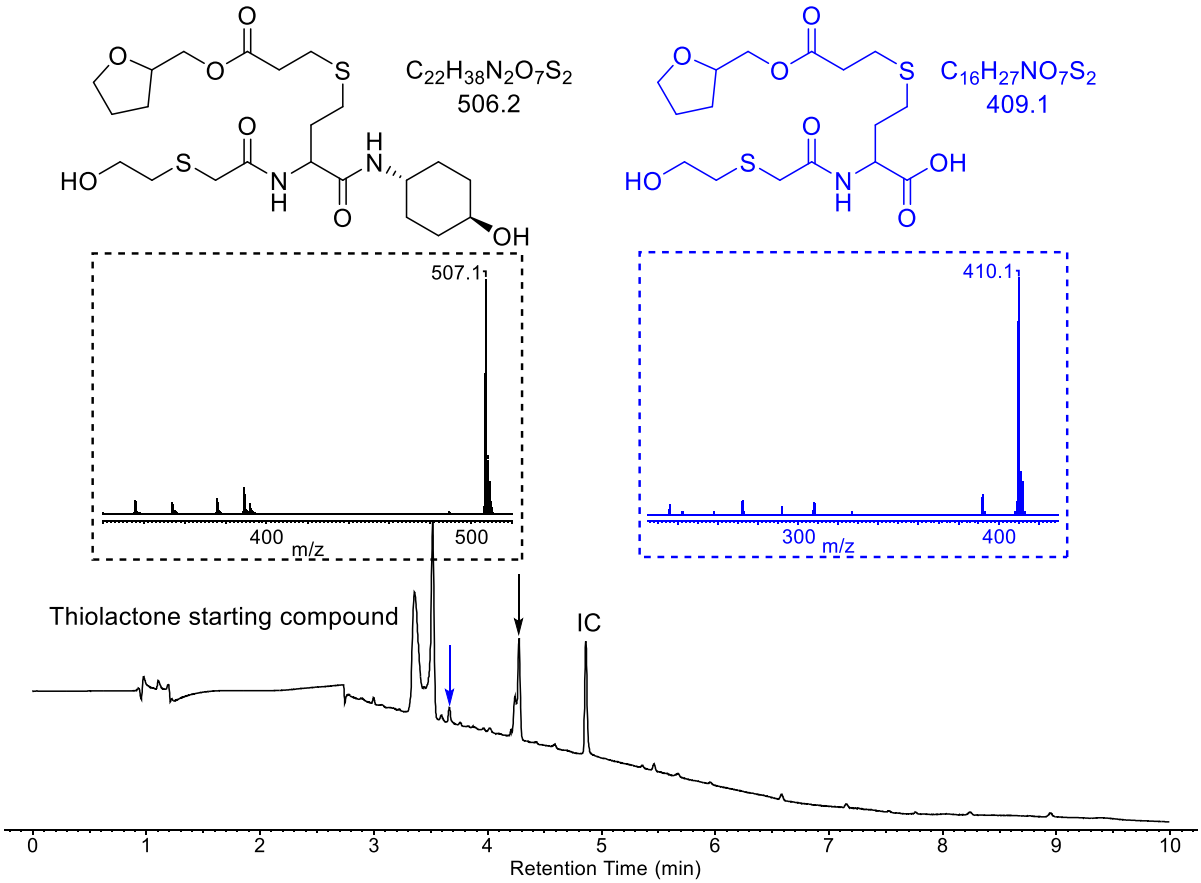


Figure VI.23: LC-ESI-MS analysis of aminolysis with trans-4-amino-1-hydroxycyclohexane in a mixture of NMP and $CHCl_3$. Inserts: ESI-MS-spectra of dominant species (black) and hydrolysis (blue) (positive mode).

Next, 4-(aminomethyl)phenol was tested. From previous research it was determined that benzylamine, which has a similar structure to 4-(aminomethyl)phenol, displays slower reaction kinetics for the aminolysis of the thiolactone.[34] The solubility of this amine in the reaction mixture used was also very low. Although DMF promotes the aza-Michael reaction,[36] it was the only option to fully dissolve this amine. A kinetic study was performed to analyse the aminolysis. The LC trace after 8 hours indicates that both the starting compound (3.35 min) and the product (5.25 min) are present (Figure VI.24). Increasing the ratio of the amine and repeating the reaction three times helped to increase the aminolysis, yet the reaction was still not fully finished (Figure VI.25). Dry solvents should also be used, because the hydrolysis of thiolactone was also observed once more. The very slow reaction kinetics and the full optimisation that would require a lot of time led to 4-(aminomethyl)phenol not being further explored within this PhD thesis. Here as well, catalysts could perhaps be used during the aminolysis to improve the reaction kinetics.[46]

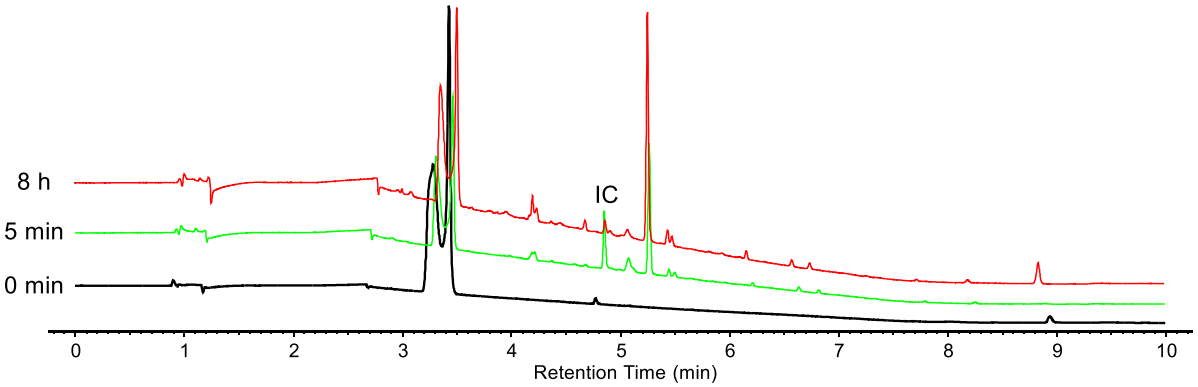


Figure VI.24: HPLC traces of the opening of thiolactone with 4-(aminomethyl)phenol and benzyl acrylate as a function of time.

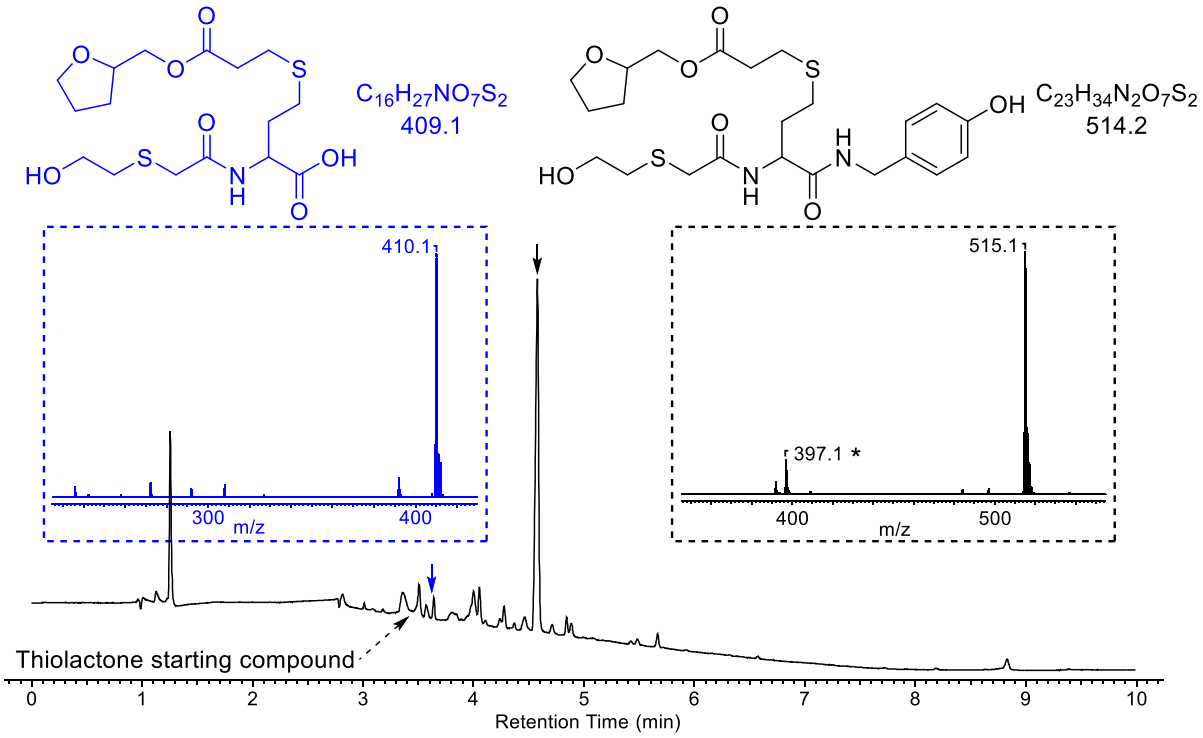


Figure VI.25: LC-ESI-MS analysis of aminolysis with 4-(aminomethyl)phenol in DMF. Inserts: ESI-MS-spectra of dominant species (black) and hydrolysis (blue) (positive mode with fragmentation as in Figure VI.9 (*)).

The final amino alcohol that was tested was 4-amino-1-butanol. A kinetic study was performed to discover the time needed for the aminolysis. Benzyl acrylate was used to capture the formed thiol. LC-ESI-MS samples were taken at 5, 15, 30 minutes and every hour. In the LC trace after 8 hours, a small amount of the starting compound could still be found (3.35 min) (Figure VI.26). This was an indication that this reaction should at least be done twice. First, similarly to ethanolamine, the aminolysis in STEP 1 of the protocol was performed for two times 15 minutes. The first trimer that was made showed a deletion, so the protocol had to be modified for this specific aminolysis to four times 15 minutes. With this adjustment, a high-purity pentamer (BUTOL1) was made with only tetrahydrofurfuryl groups as side chain functionalities (5.84 min) (Figure VI.27).

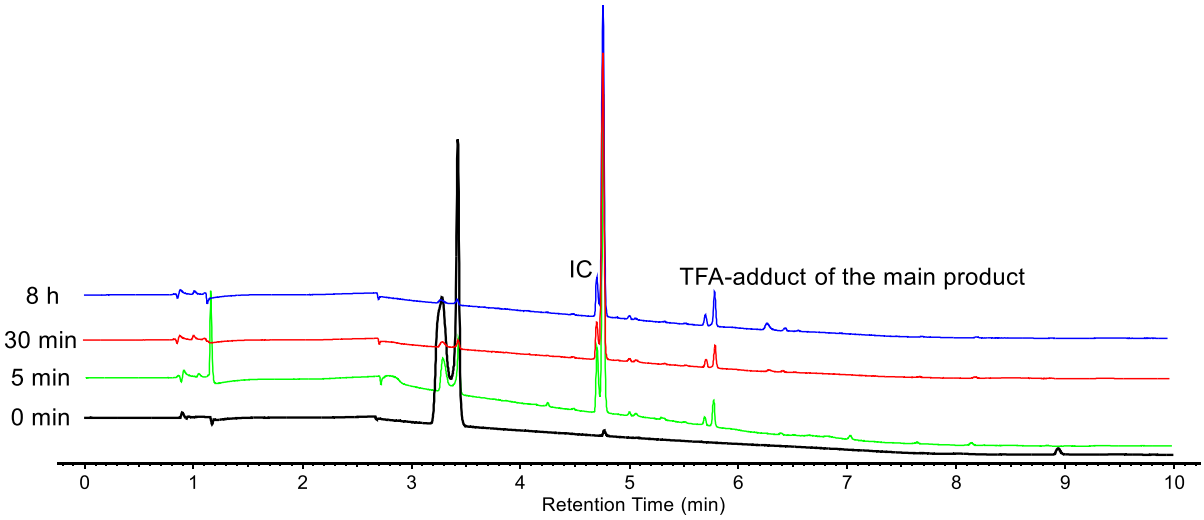


Figure VI.26: HPLC traces of the opening of thiolactone with 4-amino-1-butanol and benzyl acrylate as a function of time.

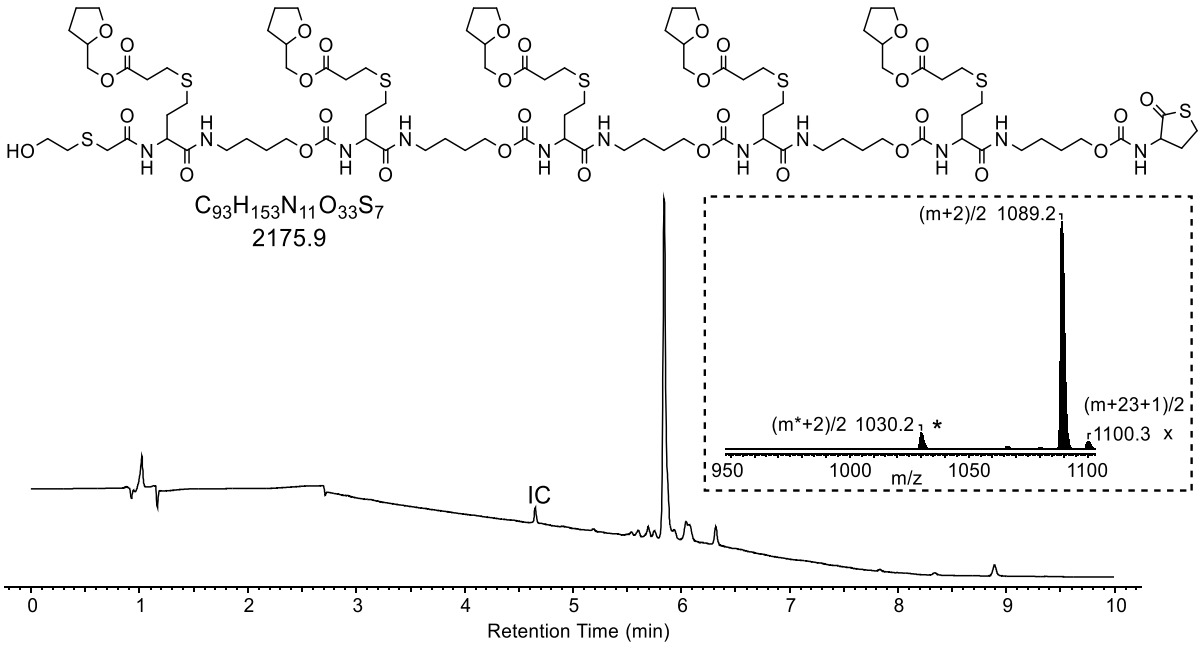


Figure VI.27: LC-ESI-MS analysis of BUTOL1. Insert: ESI-MS-spectrum of dominant species (positive mode with sodium adduct (x) and fragmentation as in Figure VI.9 (*)).

Finally, a trimer BUTOL2 was made with three different side chain functionalities and based on both ethanolamine and 4-amino-1-butanol in order to illustrate that both the backbone and side chains can be easily modified (Figure VI.28).

It can be concluded that ethanolamine and 4-amino-1-butanol allow for the variation of the backbone of the prepared oligomers. Further research should be conducted to see if other amines (including the ones tried in this chapter) could be applied for this protocol and to investigate what their influence is on the oligomer properties.

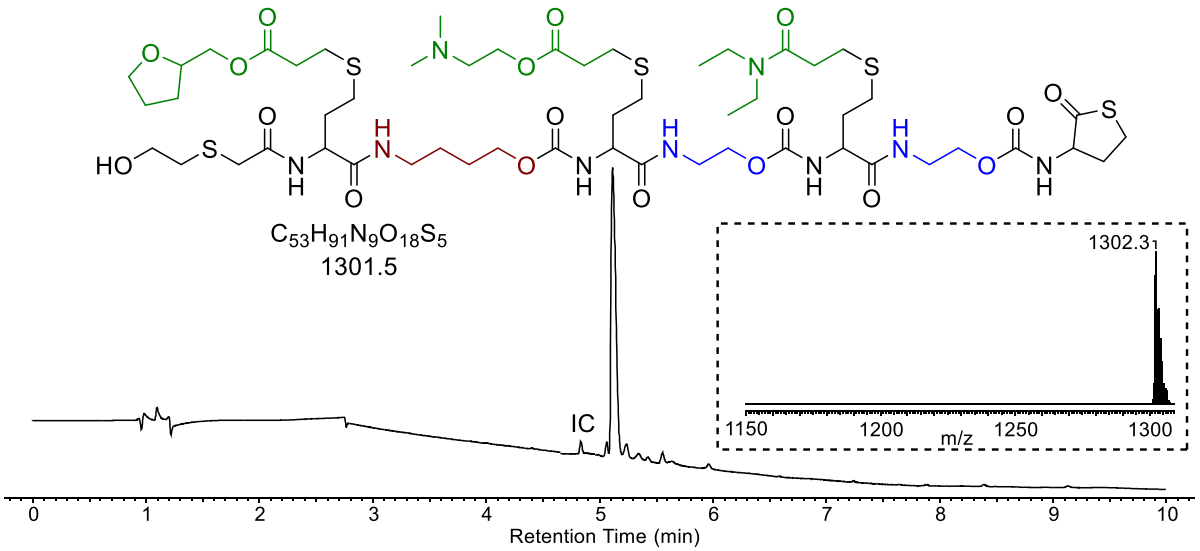


Figure VI.28: LC-ESI-MS analysis of BUTOL2. Insert: ESI-MS-spectrum of dominant species (positive mode).

VI.4 Automated synthesis

In the previous sections of this chapter, the protocol was optimised to translate it to an automated approach in order to facilitate the iterative work and to prepare different oligomers in parallel, thus significantly reducing the overall process time. This required a non-standard peptide synthesiser that has extra bottles for washing solvents and must definitely have closed vessels for the inert storage of the α-isocyanato-γ-thiolactone, DBTL and dried $CHCl_3$, because each second (urethane synthesis) step of the protocol is water-sensitive (Figure VI.29).

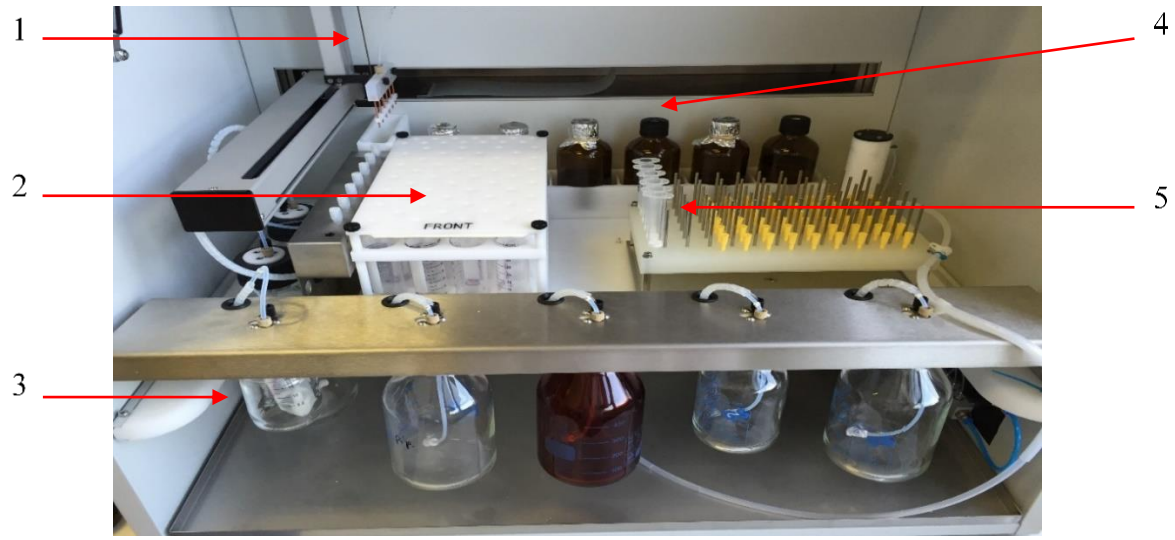


Figure VI.29: Synthesiser lay-out: needle that dispenses reagents (1), storage rack for acrylate solutions (2), reservoir for the thiolactone isocyanate protected under a nitrogen flow (3), rack with solutions, from left to right: catalyst solution, ethanolamine solution, $CHCl_3$ for washing, dry $CHCl_3$ for reaction with the thiolactone isocyanate and methanol for washing (4) and vortexing unit with reactors (5) .

Firstly, a trimer (R1) was made (Figure VI.30) in order to optimise the conditions and adjust instrument settings. Because no deletions were observed, six random hexamers (R2-R7) were

subsequently synthesised in parallel by means of benzyl, butyl and tetrahydrofurfuryl acrylate. This second screening was equally very effective, since every hexamer was characterised by a high level of purity (Figure VI.31). A nonamer (R8) was also prepared with a high abundance, but displayed traces of deletions (Figure VI.32).

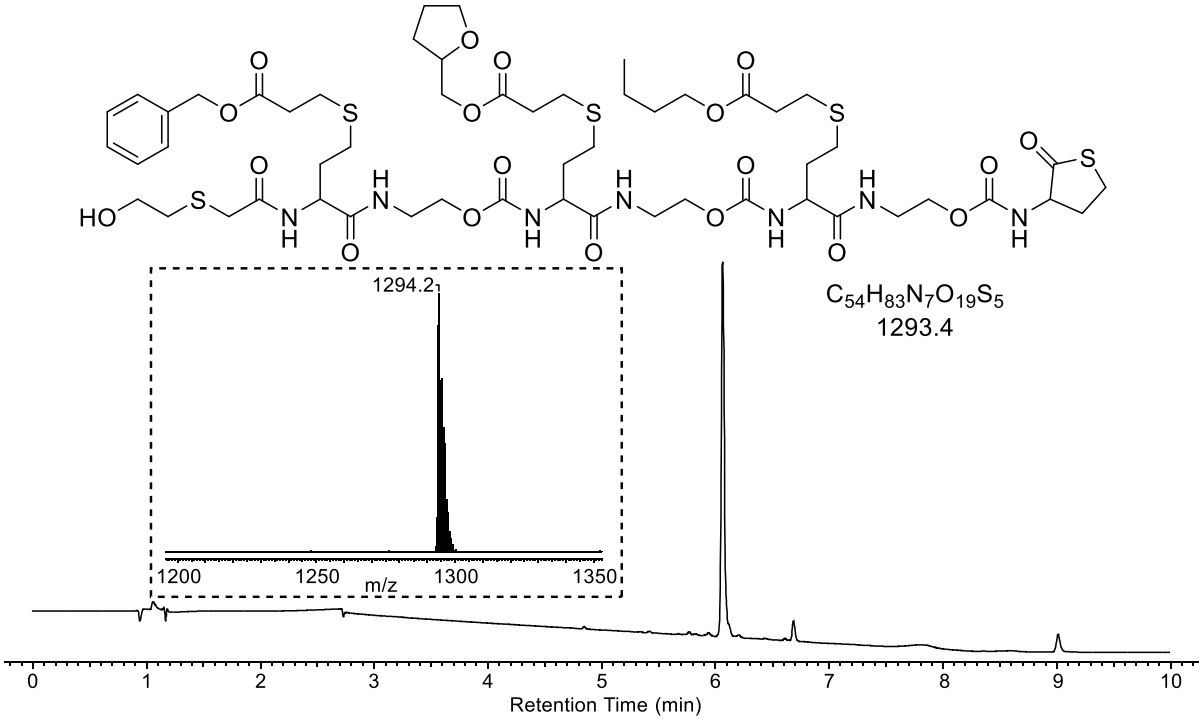


Figure VI.30: LC-ESI-MS analysis of R1. Insert: ESI-MS-spectrum of dominant species (positive mode).

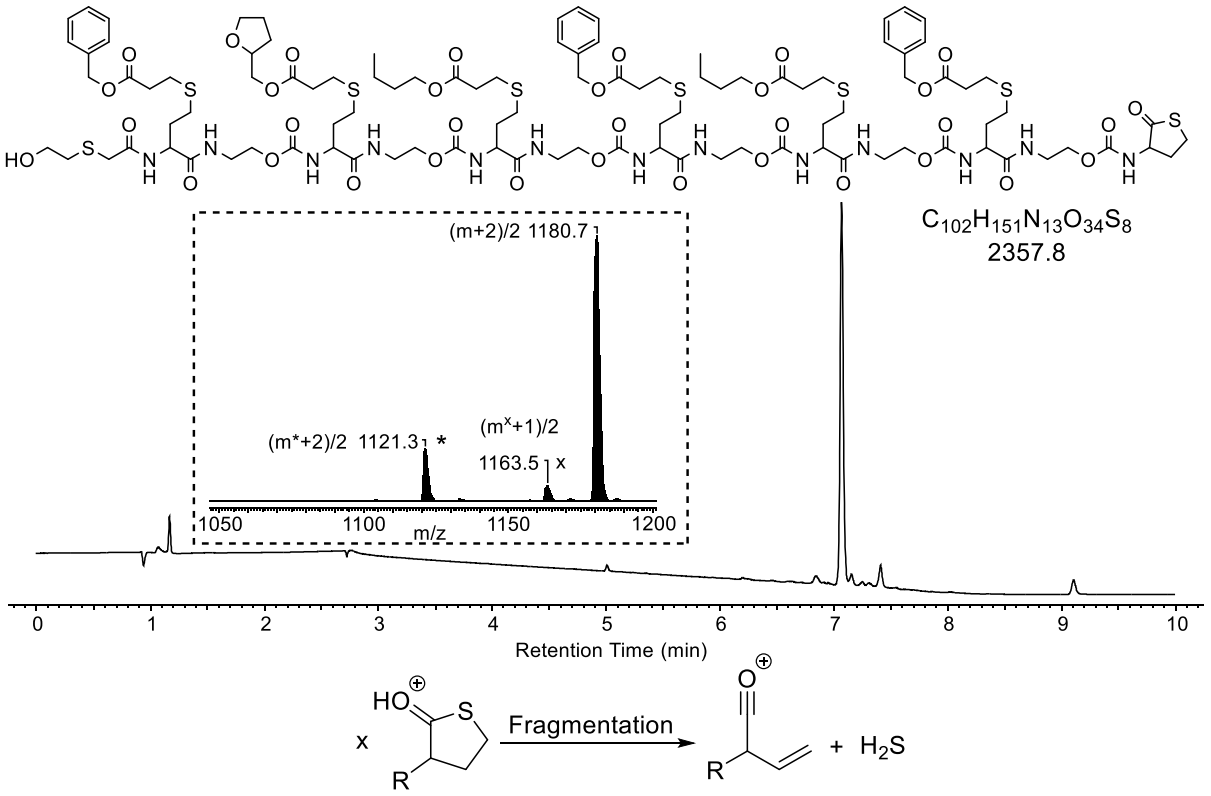


Figure VI.31: LC-ESI-MS analysis of R2. Insert: ESI-MS-spectrum of dominant species (positive mode with fragmentation as in Figure VI.9 (*) and opening-chain fragmentation of the thioester followed by loss of H_2S (x)).[47]

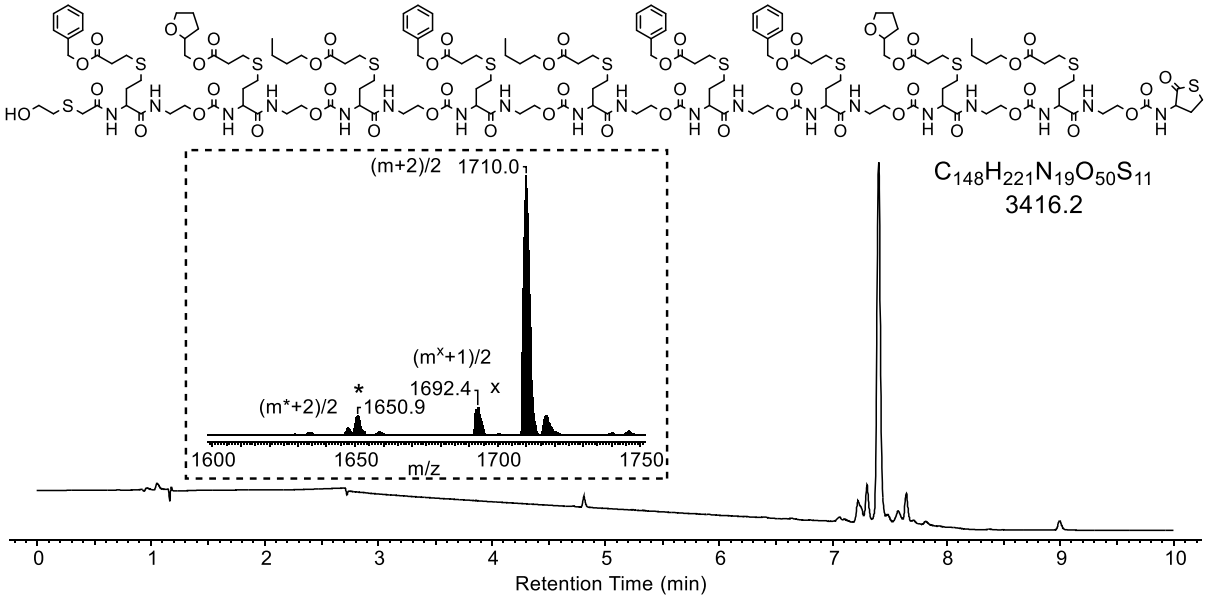


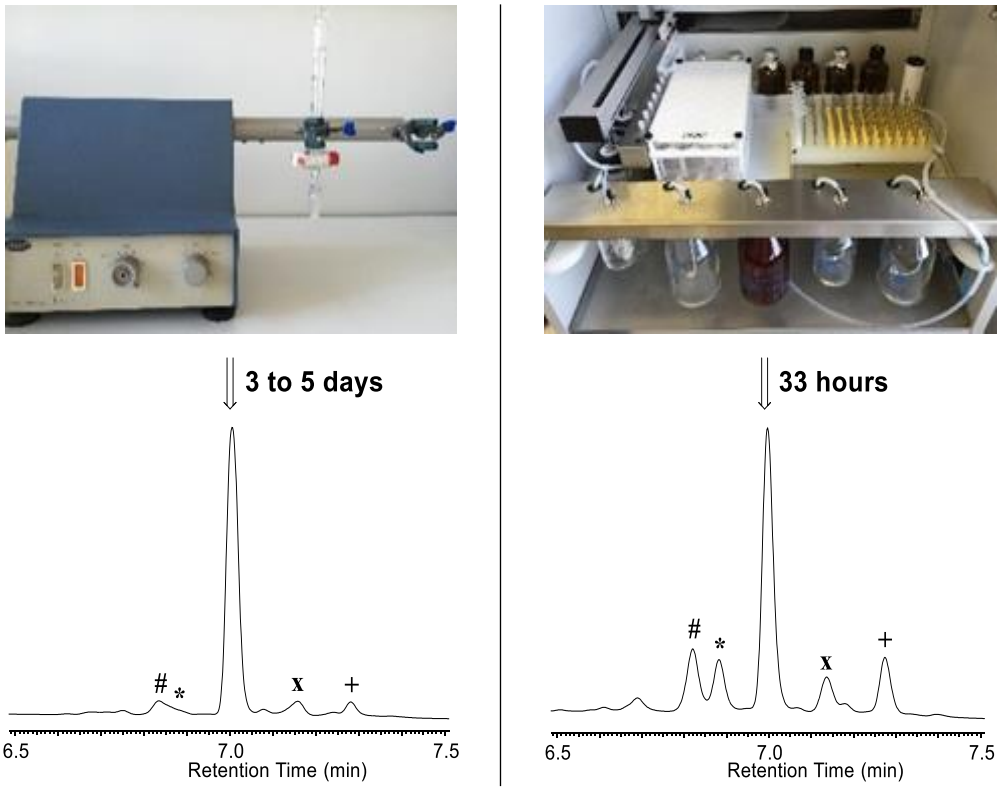
Figure VI.32: LC-ESI-MS analysis of R8. Insert: ESI-MS-spectrum of dominant species (positive mode with fragmentation as in Figure VI.9 (*) and opening-chain fragmentation of the thioester followed by loss of H_2S (x)[47]).

The final test was a comparison between (BT)5 decamers, carried out in a manual and in an automated way (R9). Both the LC traces of the manual and the robot-made oligomer display the decamer with the highest abundance, but also a nonamer (#), missing a benzyl functionality, and a decamer without the presence of a thiolactone unit (*). These two impurities could either be caused by an incomplete amine-thiolactone-ene reaction in which a thiolactone is not opened and a functionality is not built in or by an incomplete coupling reaction of the α-isocyanato-γ-thiolactone with an end-standing alcohol, resulting in the impossibility to perform the next amine-thiolactone-ene reaction. These impurities are comparable to deletions during peptide synthesis in which an oligopeptide misses one or more amino acids in its sequence. TFA-esters of * (x) and the main product (+) can be found as well (Figure VI.33). When the signals are integrated into both LC traces, the amounts of the side products are higher in case of the automated synthesis (Figure VI.33, top table). Conversion per reaction cycle can be found by averaging these integration values over ten cycles for both the manual and automated synthesis (Figure VI.33, bottom table). As the TFA-ester adduct (+) does not result from troubled synthesis, but from improper cleavage conditions, the calculated average conversion per reaction cycle also took into account the amounts of this adduct.

In comparison with the storage vessels, the reactors for the automated synthesis are open, which might cause problems during the chain extension, because isocyanates are sensitive to humidity in the air. The formation of a white precipitate, which corresponds to dithiolactone urea, can confirm this air sensitivity (Figure VI.34). On the other hand, it should be emphasised that the time for the synthesis of a decamer is obviously much shorter with the automated protocol (±33 hours versus 3 to 5 days).

The conversion per reaction cycle and the prevention of deletions could be increased by longer reaction times, the addition of more equivalents of reactants, extra repetitions of reaction steps

and inert atmosphere to avoid humidity. To prevent aggregation, which becomes problematic when the sequence length increases, the loading of the resin can be decreased, and a different resin and a different solvent or mix of solvents can be used, knowing that this can slow down the reaction (Figures VI.3-5).



Compounds	Manual (%)	Automated (%)
Nonamer missing a benzyl residue (#)	7	14
Decamer in the absence of the terminal thiolactone unit (*)		10
Main compound	85	56
TFA-ester adduct of * (x)	5	9
TFA-ester adduct of the main compound (+)	3	11

Average conversion per cycle	Without TFA (%)	With TFA (%)
Manual	98	99
Automated	94	96

*Figure VI.33: Comparison between conventional (left) and automated (peptide synthesiser) synthesis (right) of decamer (BT)5. Peak # represents a nonamer missing a benzyl residue while peak * is a decamer in the absence of the terminal thiolactone unit. Peaks x and + are TFA-ester adducts of * and the main compound. Tables express the percentages of the different compounds in the LC trace (top) and the conversion per cycle averaged over ten cycles (bottom) for both the manual and automated approach with and without the TFA-ester adducts.*

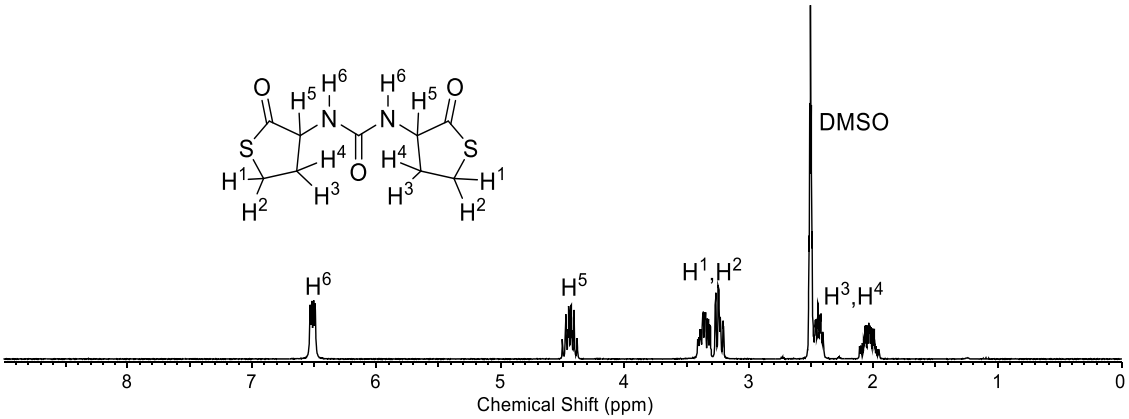


Figure VI.34: ^{1}H-NMR spectrum (300 MHz, DMSO-d_6) with peak assignment of the dithiolactone urea.

VI.5 Conclusions and outlook

This chapter described the development of a new robust protecting-group-free two-step iterative protocol for the solid-phase synthesis of multifunctional sequence-defined oligomers based on thiolactone chemistry.

First, the protocol had to be optimised. For both steps of the protocol, the reaction stoichiometry, the choice of solvent and the reaction times used were investigated. The ratio of amine/acrylate of one to two with 10 equivalents of amine and 20 equivalents of acrylics from chapter IV was chosen in the first step. In $CHCl_3$, the solvent chosen, the synthesis proved to be finished after 15 minutes for ethanolamine. This synthesis was repeated twice to ensure full conversion of the thiolactone on solid support. In the second step, 10 equivalents of the isocyanate and 0.025 equivalent of dibutyltin dilaurate (DBTL) in dry $CHCl_3$ allowed the reaction to be finished after 30 minutes. This reaction was performed for 1 hour to ensure full urethane formation.

After the optimisation, different multifunctional sequences were built. The various functional groups could be elegantly incorporated during aminolysis by means of acrylics. Functionalised oligomers, up to decamers, could be prepared with high purity. For longer sequences, the reaction conditions should be modified to prevent deletions, for instance in the sense of lower loading, repetition of steps, and longer reaction times. If amide side chains are preferred instead of esters, a modified version of the protocol developed by group member Joshua Holloway, based on thioacrylates, should be followed to prevent side reactions observed when using acrylamides with the proposed protocol.[42]

Furthermore, the proposed protocol allows the end groups, the backbone and side chains to be easily adapted. For the ω-terminus, there are two possibilities. The sequence can be terminated after step 1, which results in an alcohol, or after step 2, which results in a thiolactone. The nature of the α-end group, on the other hand, depends on the functionality used to connect the

thiolactone on the crosslinked polystyrene resin. Here we used an acid- and an alcohol-functionalised linker. To further illustrate the high level of control over the side chain and the backbone of the oligomers, a trimer was prepared with two different amines and three different acrylics. In the future, it could also be interesting to also install functionalities on the thiolactone ring itself, which was performed by Destarac and co-workers.[48]

Finally, the optimisation of the protocol allowed the efficient translation to an automated approach through an adapted peptide synthesiser, resulting in an accelerated synthesis of libraries of sequence-defined oligomers. Although the synthesis is faster, however, decamers could not be made with the same purity as during the manual synthesis. Within our group, Chiel Mertens discovered that lower loading, repetition of steps and longer reaction times help to increase the purity of longer oligomers (above decamers) without the occurrence of deletions.

The results indicate that this protocol can be applied to make multifunctional sequence-defined oligomers. An interesting application for the built oligomers is their use as data carriers. The option to store information both in the multifunctional side and/or in the main chains can result in a quite significant progress in terms of data capacity on a single oligomer, which will be discussed in the next chapter.[49]

In this chapter racemic thiolactone compounds were used for the synthesis of the oligomers. Zuckermann and others have already proven that enantiomerically pure compounds induce certain folding behaviour in oligopeptoids.[50] In the future enantiomerically pure thiolactone building blocks should be used if the impact on the folding behaviour of our oligomers is to be analysed.[51, 52] I refer to the ongoing work of Chiel Mertens and Melissa Reith to see the effect of enantiomerically pure thiolactones on the folding behaviour of the oligomers.

Börner *et al.* have applied this protocol to make block copolymers consisting of a thiolactone-based sequence-defined pentamer and a poly(ethylene glycol) (PEG) block.[38] Starting from a PEG preloaded poly(styrene) resin, a slightly modified protocol is applied to make the defined block copolymers with good yields and high purity. These block copolymers have the ability to load and release a drug. The loading of this drug is dependent on the sequence of the pentamer used, in other words sequence really matters for this application.[53] These sequences can perhaps be applied for other biomedical applications and can be installed on different polymers to prepare sequence-defined oligomer-polymer hybrid structures like peptide/protein-polymer and oligonucleotide/DNA-polymer hybrids, which can be used in fields such as enzyme recovery[54], nano-biomaterials[55], pharmaceuticals[56] and the modelling of peptide binding.[57]

In collaboration with Meier's group (KIT, Germany) the amine-thiolactone-ene conjugation was combined with the efficient Passerini three-component reaction (P-3CR) for the straightforward and convergent synthesis of other multifunctionalised and sequence-defined oligomers.[58] The thiolactone moiety was installed as an end group of sequence-defined trimers, which were prepared in solution with the P-3CR. The thiolactone was opened with a (functional) amine and an acrylate-isocyanide, which can react in a subsequent P-3CR with a

(functional) aldehyde and a trimer with an acid end group. This strategy allowed for the synthesis of a library of sequence-defined α-ω-functionalised building blocks. Thus, larger oligomers with molecular weights of up to almost 5000 g/mol were obtained in gram quantities in a convergent way, along with the introduction of independently selectable side chains (up to 15). The thiolactone double modification with acrylates and amines can perhaps be combined with other click and efficient chemistries to synthesise different sequence-defined structures.

VI.6 Experimental part

VI.6.1 Methods

^{1}H- and ^{13}C-NMR

^{1}H- and ^{13}C-NMR (Attached Proton Test, APT) spectra were recorded on a Bruker Avance 300 at 300 MHz and 75 MHz, respectively and a Bruker Avance 500 at 500 MHz and 125 MHz, respectively. Chemical shifts are presented in parts per million (δ) relative to DMSO-d_6 or $CHCl_3$-*d* (2.50 ppm or 7.27 ppm in ^{1}H- and 39.51 ppm or 77.24 ppm in ^{13}C-NMR respectively) as internal standard. All samples were analysed with 2D-NMR techniques (COSY, HSQC and HMBC), which allowed for a full assignment of the structures. All measurements were performed at 25°C and ACD/NMR Processor was used for the processing of all data. All spectra including 1D ^{1}H and ^{13}C, 2D COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC were recorded in a standard fashion with pulse programs available in the Bruker library. The NMR spectra and analysis of the compounds synthesised in this chapter can be found at https://pubs.acs.org/doi/abs/10.1021/jacs.6b07120.

LC-ESI-MS

An Agilent technologies 1100 series LC/MSD system equipped with a diode array detector and single quad MS detector (VL) with an electrospray source (ESI-MS) was used for classic reversed phase LC-MS (liquid chromatography mass spectroscopy) and MS analysis. Analytic reversed phase HPLC was performed with a Phenomenex C18 (2) column (5 µ, 250 x 4.6 mm) using a solvent gradient (0 → 100% acetonitrile in H_2O in 15 min) and the eluting compounds were detected via UV-detection (λ = 214 nm). High resolution mass spectra (HRMS) were collected using an Agilent 6220 Accurate-Mass time-of-flight (TOF) equipped with a multimode ionisation (MMI) source. The LC-ESI-MS spectra and HRMS data of the compounds synthesised in this chapter can be found at https://pubs.acs.org/doi/abs/10.1021/jacs.6b07120. In some spectra in this chapter an internal contamination (IC) could be observed, which was already discussed in IV.5.1.

IR

Infrared spectra were recorded with Attenuated Total Reflection (ATR) with a PIKE Miracle ATR unit and a Perkin Elmer FTIR SPECTRUM 1000 spectrometer. IR-software of Perkin Elmer was used for the analysis of the spectra.

Automated synthesis

Automated syntheses were performed on a 72-reactor block INTAVIS MultiPep CF Synthesiser with open 5 mL reaction columns equipped with a vortexing unit. The speed of vortexing is 550 rpm.

VI.6.2 Materials

DMSO-d_6 ([2206-27-1], ≥ 99.8 %) and $CHCl_3$-*d* ([865-49-6], ≥ 99.8 %) were purchased from Euriso-top. Acetonitrile ([75-05-8], HPLC grade), 1,4-Dioxane ([123-91-1], HPLC grade) and Triethylamine ([121-44-8], 99 %) were purchased from Acros Organics. DL-Homocysteine thiolactone hydrochloride ([6038-19-3], 99 %) was purchased from Haihang industry (Jinan City, China). Potassium carbonate ([584-08-7], ≥ 99 %) and Sodium bicarbonate ([144-55-8], ≥ 99.5%) were purchased from Carl Roth. Trifluoroacetic acid ([76-05-1], Peptide grade) and 2-Chlorotrityl chloride resin ([42074-68-0], 100-200 mesh, 1% DVB, 1.6 mmol/g) were purchased from Iris Biotech GmbH. Bromoacetyl bromide ([598-21-0], ≥ 98 %), Butyl acrylate ([141-32-2], ≥ 99 %), Chloroform ([865-49-6], ≥ 99.8 %), Dichloromethane ([75-09-2], ≥ 99.8 %), Diethylether ([60-29-7], ≥ 99.9 %), N,N-Diisopropylethylamine (DIPEA, [7087-68-5], 99 %), N,N-Dimethylformamide ([68-12-2], anhydrous, 99.8 %), Ethanolamine ([141-43-5], ≥ 99%), Ethyl acetate ([141-78-6], ≥ 99.7 %), Glutaric anhydride ([108-55-4], 95 %), Isobornyl acrylate ([5888-33-5], technical grade), N-Isopropylacrylamide ([2210-25-5], 97%), 2-Mercaptoethanol ([60-24-2], ≥ 99 %), Methanol ([67-56-1], ≥ 99.9 %), Methyl acrylate ([96-33-3], 99 %), Pyridine ([110-86-1], ≥ 99 %), and Tetrahydrofuran ([109-99-9], ≥ 99 %) were purchased from Sigma-Aldrich and used without purification, except for isobornyl acrylate, which was distilled. 4-Amino-1-butanol ([13325-10-5], > 98 %), trans-4-Aminocyclohexanol ([27489-62-9], > 98 %), 4-(Aminomethyl)phenol ([696-60-6], > 98 %), Benzyl acrylate ([2495-35-4], > 97 %), Dibutyltin dilaurate ([77-58-7], > 95 %), N,N-Diethylacrylamide ([2675-94-7], > 98 %), 2-(Dimethylamino)ethyl acrylate ([2439-35-2], > 98 %), DL-Prolinol ([498-63-5], > 95 %), Tetrahydrofurfuryl acrylate ([2399-48-6], 98 %), and Triphosgene ([32315-10-9], > 98 %) were purchased from TCI and used without purification. Hydrochloric acid 36 % p. (HCl, [7647-01-0]) was purchased from Chem-Lab and used without purification. Solvents (CH_2Cl_2, DIPEA and pyridine) for the immobilisation of functionalised thiolactone linkers were distilled from CaH_2 prior to use. Silicagel (ROCC, SI 1721, 60 Å, 40 – 63 μm) was used to perform preparative column chromatography, eluting with technical solvents. The collected fractions were analysed by thin layer chromatography (TLC-plates, Macherey-Nagel, SIL G-25 UV254).

N-(2-bromoacetyl) homocysteine-γ-thiolactone, the alcohol- and acid-functionalised thiolactone linkers and the 1-ethoxyethyl acrylate were prepared as previously described.[33, 35, 59]

VI.6.3 Manipulation of the solid support

Swelling
The solid support was swollen in the reaction solvent for at least 10 minutes.

Washing
The loaded resin was washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried after each reaction.

Loading
The acid-functionalised thiolactone linker (1.33 g, 1.2 equivalents relative to the resin, 5.8 mmol) was loaded on a 2-chlorotrityl chloride resin (3 g, 1.6 mmol/g) in dry CH_2Cl_2 (30 mL, 10 mL/1 g of resin) with dry DIPEA (0.26 mL, 4 equiv. rel. to acid) and 10 vol% of DMF (3 mL), in order to dissolve the acid. The reaction mixture was shaken for 3 hours at room temperature. The resin was subsequently washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 6 x 30 mL), DMF (4 x 30 mL), CH_2Cl_2 (4 x 30 mL) and diethyl ether (4 x 30mL). The resin was dried under vacuum.

The hydroxyl-functionalised thiolactone linker (1.69 g, 1.5 equivalents relative to the resin, 7.2 mmol) was loaded on a 2-chlorotrityl chloride resin (3 g, 1.6 mmol/g) in dry THF (30ml, 10 mL/1 g of resin) with dry pyridine (1.16 mL, 2 equivalents relative to the alcohol).[60] The reaction was shaken overnight at 60°C. Next, the solid support was washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 6 x 30 mL), DMF (4 x 30 mL), CH_2Cl_2 (4 x 30 mL) and diethyl ether (4 x 30mL). The resin was dried under vacuum.

Cleavage
For cleavage, 2 mg of solid support was treated with 0.5 mL of 1% trifluoroacetic acid (TFA) in CH_2Cl_2 for 5 minutes. During cleavage, the color of the beads turns from yellow to red because of the formation of a trityl cation. The solid support was then filtered, the sample concentrated by evaporation and dissolved in acetonitrile for LC-ESI-MS analysis.

Aminolysis and incorporation of functionalities: general procedure
$CHCl_3$ is added to the solid support (1 mL reaction mixture per 100 mg resin). Next, the acrylate (20 equivalents relative to the thiolactone unit) and ethanolamine (10 equivalents) were added. This mixture is shaken for 15 minutes at room temperature. This procedure is repeated once

more to be sure of the full conversion of the thiolactone. After this second procedure, the resin is washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Chain extension: general procedure

Dried $CHCl_3$ is added to the solid support (1 mL reaction mixture per 100 mg resin), then the α-isocyanato-γ-thiolactone (10 eq. relative to the resin-bound alcohol units) and dibutyltin dilaurate (0.025 eq. relative to the resin-bound alcohol units) are added. This mixture is shaken for 1 hour. Afterwards, the resin is washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Automated protocol: general procedure

The protocol was adapted from the conventional approach with some slight modifications. Stable stock solutions were prepared in $CHCl_3$ with either (i) the ethanolamine (7M), (ii) an acrylate (4M), (iii) the α-isocyanato-γ-thiolactone (2M) or (iv) the dibutyltin dilaurate (0.15M). 50 mg of resin was weighed in each reactor.

The resin was swollen for 5 minutes in $CHCl_3$ and afterwards the solvent was removed. 480 µL of the acrylate/$CHCl_3$ solution and 180 µL of the ethanolamine/$CHCl_3$ solution were added in this order and shaken for 15 minutes. This step was repeated for a second time, as in the manual approach. Then the resin was washed with DMF (2x), MeOH (2x) and $CHCl_3$ (2x).

Subsequently, the solid support is suspended for 5 minutes in $CHCl_3$ and afterwards the solvent is removed. 480 µL of the α-isocyanato-γ-thiolactone/$CHCl_3$ solution and 120 µL of the dibutyltin dilaurate/$CHCl_3$ solution were added in this order and shaken for 2 hours. During this reaction a white precipitate is formed. Next, 1 mL of DMF is added and shaken for 1 minute. This allows for the white precipitate that is formed to dissolve and to prevent the tubings of the robot from clogging. The resin was then washed with DMF (2x), MeOH (2x) and $CHCl_3$ (2x). After completion of the automated procedure, the resin was washed manually with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x), and dried.

Kinetics: Aminolysis of the thiolactone

The opening of the thiolactone ring was followed by LC-ESI-MS. Samples were taken after 5 and 15 minutes' reaction and then every 15 minutes. In this study, benzyl acrylate was used to react with the generated thiol.

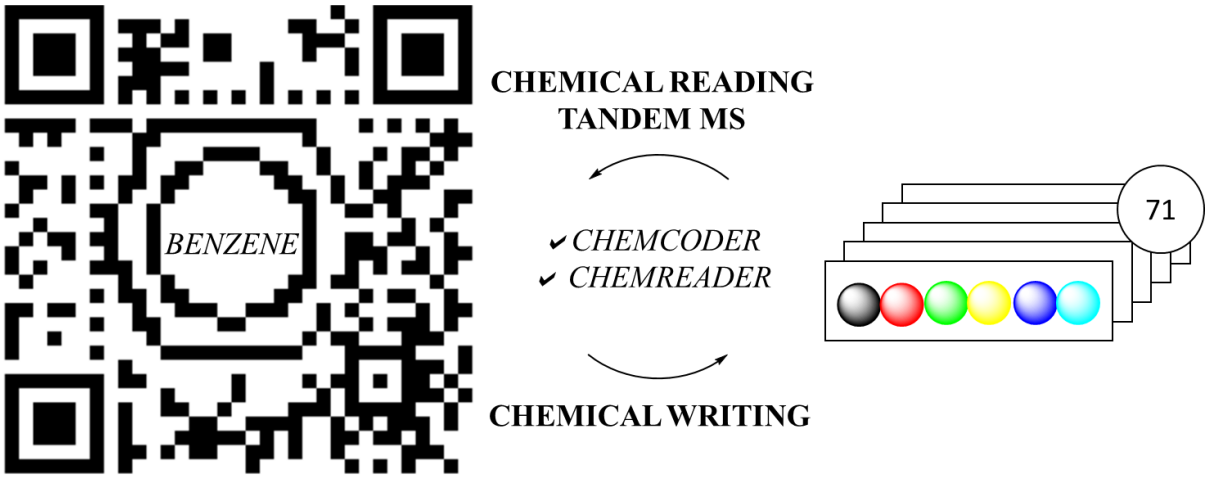
Kinetics: Chain extension

The chain extension with α-isocyanato-γ-thiolactone in different solvents was followed by taking a sample at 5, 15, 30 minutes and every hour for analysis by LC-ESI-MS. Independently of the solvent that was chosen, the product formed had a high purity. $CHCl_3$ was chosen for its shorter reaction time.

References

[1] C. J. Hawker, K. L. Wooley, *Science* **2005**, *309*, 1200.
[2] J. M. Berg, J. L. Tymoczko, L. Stryer, "*Biochemistry"*, W.H. Freeman, Basingstoke, 2012.
[3] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2.
[4] J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, *38*, 1700582.
[5] J. F. Lutz, M. Ouchi, D. R. Liu, M. Sawamoto, *Science* **2013**, *341*, 628.
[6] R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* **1992**, *114*, 10646.
[7] S. C. Solleder, R. V. Schneider, K. S. Wetzel, A. C. Boukis, M. A. R. Meier, *Macromol. Rapid Commun.* **2017**, *38*, 1600711.
[8] S. Martens, J. O. Holloway, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, 1700469.
[9] J. Vandenbergh, G. Reekmans, P. Adriaensens, T. Junkers, *Chem. Sci.* **2015**, *6*, 5753.
[10] L. Hartmann, *Macromol. Chem. Phys.* **2011**, *212*, 8.
[11] A. Al Ouahabi, L. Charles, J. F. Lutz, *J. Am. Chem. Soc.* **2015**, *137*, 5629.
[12] M. I. Amrane, D. Chouikhi, N. Badi, J. F. Lutz, *Macromol. Chem. Phys.* **2014**, *215*, 1984.
[13] G. De Bo, S. Kuschel, D. A. Leigh, B. Lewandowski, M. Papmeyer, J. W. Ward, *J. Am. Chem. Soc.* **2014**, *136*, 5811.
[14] S. Ida, M. Ouchi, M. Sawamoto, *Macromol. Rapid Commun.* **2011**, *32*, 209.
[15] F. Driessen, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2015**, *4*, 616.
[16] G. Gody, T. Maschmeyer, P. B. Zetterlund, S. Perrier, *Nat. Commun.* **2013**, *4*, 3505.
[17] M. Porel, D. N. Thornlow, C. M. Artim, C. A. Alabi, *ACS Chemical Biology* **2017**, *12*, 715.
[18] Y. Jiang, M. R. Golder, H. V. T. Nguyen, Y. Wang, M. Zhong, J. C. Barnes, D. J. C. Ehrlich, J. A. Johnson, *J. Am. Chem. Soc.* **2016**, *138*, 9369.
[19] B. van Genabeek, B. F. M. de Waal, M. M. J. Gosens, L. M. Pitet, A. R. A. Palmans, E. W. Meijer, *J. Am. Chem. Soc.* **2016**, *138*, 4210.
[20] R. M. Weiss, A. L. Short, T. Y. Meyer, *ACS Macro Lett.* **2015**, *4*, 1039.
[21] G. Cavallo, S. Poyer, J. A. Amalian, F. Dufour, A. Burel, C. Carapito, L. Charles, J. F. Lutz, *Angew. Chem., Int. Ed.* **2018**, *57*, 6266.
[22] L. Yu, Z. Zhang, Y.-Z. You, C.-Y. Hong, *Eur. Polym. J.* **2018**, *103*, 80.
[23] L. Xiang, Z. Li, J. a. Liu, J. Chen, M. Zhang, Y. Wu, K. Zhang, *Polym. Chem.* **2018**, *9*, 4036.
[24] W. Konrad, F. R. Bloesser, K. S. Wetzel, A. C. Boukis, M. A. R. Meier, C. Barner-Kowollik, *Chemistry – A European Journal* **2018**, *24*, 3413.
[25] A. C. Boukis, M. A. R. Meier, *Eur. Polym. J.* **2018**, *104*, 32.
[26] J. De Neve, J. J. Haven, L. Maes, T. Junkers, *Polym. Chem.* **2018**, *9*, 4692.
[27] S. L. Beaucage, R. P. Iyer, *Tetrahedron* **1992**, *48*, 2223.
[28] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149.
[29] A. S. Knight, E. Y. Zhou, M. B. Francis, R. N. Zuckermann, *Advanced Materials* **2015**, *27*, 5665.
[30] L. Hartmann, E. Krause, M. Antonietti, H. G. Borner, *Biomacromolecules* **2006**, *7*, 1239.
[31] A. A. Ouahabi, M. Kotera, L. Charles, J.-F. Lutz, *ACS Macro Lett.* **2015**, *4*, 1077.
[32] B. L. Bray, *Nat Rev Drug Discov* **2003**, *2*, 587.
[33] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[34] P. Espeel, F. Goethals, F. Driessen, L.-T. T. Nguyen, F. E. Du Prez, *Polym. Chem.* **2013**, *4*, 2449.
[35] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[36] B. D. Mather, K. Viswanathan, K. M. Miller, T. E. Long, *Prog. Polym. Sci.* **2006**, *31*, 487.
[37] S. Reinicke, P. Espeel, M. M. Stamenovic, F. E. Du Prez, *Polym. Chem.* **2014**, *5*, 5461.
[38] S. Celasun, F. E. Du Prez, H. G. Börner, *Macromol. Rapid Commun.* **2017**, *38*, 1700688.
[39] B. P. Sutherland, B. M. El-Zaatari, N. I. Halaszynski, J. M. French, S. Bai, C. J. Kloxin, *Bioconjugate Chemistry* **2018**, *29*, 3987.
[40] J. W. Chan, C. E. Hoyle, A. B. Lowe, M. Bowman, *Macromolecules* **2010**, *43*, 6381.
[41] F. Goethals, D. Frank, F. Du Prez, *Prog. Polym. Sci.* **2017**.
[42] J. O. Holloway, S. Aksakal, F. E. D. Prez, C. R. Becer, *Macromol. Rapid Commun.* **2017**, *38*, 1700500.
[43] F. Krieger, A. Möglich, T. Kiefhaber, *J. Am. Chem. Soc.* **2005**, *127*, 3346.

[44] F. Goethals, S. Martens, P. Espeel, O. van den Berg, F. E. Du Prez, *Macromolecules* **2014**, *47*, 61.
[45] P. Espeel, F. Goethals, F. E. Du Prez, "CHAPTER 9 Thiolactones as Functional Handles for Polymer Synthesis and Modification", in *Thiol-X Chemistries in Polymer and Materials Science*, The Royal Society of Chemistry, 2013, p. 195.
[46] P. Espeel, F. Goethals, F. E. Du Prez, *J. Am. Chem. Soc.* **2011**, *133*, 1678.
[47] A. E. M. Crotti, T. Fonseca, H. Hong, J. Staunton, S. E. Galembeck, N. P. Lopes, P. J. Gates, *International Journal of Mass Spectrometry* **2004**, *232*, 271.
[48] M. Langlais, I. Kulai, O. Coutelier, M. Destarac, *Macromolecules* **2017**, *50*, 3524.
[49] S. Martens, A. Landuyt, P. Espeel, B. Devreese, P. Dawyndt, F. Du Prez, *Nat. Commun.* **2018**, *9*, 4451.
[50] R. N. Zuckermann, *Peptide Science* **2011**, *96*, 545.
[51] D. Van Lysebetten, S. Felissati, E. Antonatou, L. L. G. Carrette, P. Espeel, E. Focquet, F. E. Du Prez, A. Madder, *ChemBioChem* **2018**, *19*, 641.
[52] M. Nicola, P. A. Gatti, M. Zacche, "Process for the resolution of homocysteine-thiolactone", Google Patents, 2008.
[53] S. Celasun, D. Remmler, T. Schwaar, M. G. Weller, F. Du Prez, H. G. Börner, *Angew. Chem., Int. Ed.* **2018**, *0*.
[54] H.-A. Klok, *Journal of Polymer Science Part A: Polymer Chemistry* **2005**, *43*, 1.
[55] A. Rösler, H.-A. Klok, I. W. Hamley, V. Castelletto, O. O. Mykhaylyk, *Biomacromolecules* **2003**, *4*, 859.
[56] R. Duncan, *Nat Rev Drug Discov* **2003**, *2*, 347.
[57] A. S. Hoffman, P. S. Stayton, *Macromolecular Symposia* **2004**, *207*, 139.
[58] S. C. Solleder, S. Martens, P. Espeel, F. Du Prez, M. A. R. Meier, *Chemistry – A European Journal* **2017**, *23*, 13906.
[59] W. Van Camp, F. E. Du Prez, S. A. F. Bon, *Macromolecules* **2004**, *37*, 6673.
[60] B. B. Shankar, D. Y. Yang, S. Girton, A. K. Ganguly, *Tetrahedron Lett.* **1998**, *39*, 2447.



QR code to the Wikipedia page of August Kekulé

Abstract

This chapter depicts the potential use of multifunctional sequence-defined oligo(amide-urethane)s as a storage medium. As a proof-of-principle, short text fragments (human-readable data) and a QR code (machine-readable data) were encoded as a collection of oligomers, which could be reconstructed as the original data. The amide-urethane-containing oligomers were generated using an automated thiolactone-based, protecting-group-free, two-step iterative protocol, which was highlighted in the previous chapter. Detailed analysis of the oligomers was performed by means of tandem mass spectrometry techniques. Two generic software tools were developed to facilitate and automate the process of chemical writing and reading: Chemcoder for encoding and decoding binary data as a collection of multifunctional macromolecules and Chemreader for reconstructing oligomer sequences from mass spectra.

Parts of this chapter were published as:
S. Martens, A. Landuyt, P. Espeel, B. Devreese, P. Dawyndt, F. Du Prez, *Nat. Commun.* **2018**, *9*, 4451.

Specific author contributions can be found at the end of section VII.1

Chapter VII
Multifunctional sequence-defined macromolecules for chemical data storage

VII.1 Introduction

The previous chapter showed that different sequence-defined oligomers could be prepared with a protecting-group-free, two-step iterative protocol based on the amine-thiol-ene conjugation with thiolactone chemistry.[1] This chapter will focus on the storage capacity of these oligomers and on software tools specifically developed to facilitate the writing and reading of these oligomers.

One of the largest technological challenges of the digital age is reliable data storage, which is already in the zettabyte (10^{21}) range and still increasing.[2] Although the current conventional storage devices are still able to cope with this increasing demand, they have some significant disadvantages, including the occupation of large floor areas in data centres, dependence on ever rarer elements and increasing energy consumption, which in itself is already a resource that is being stretched to the edge of current capacity. Encoding data at the (macro)molecular level could overcome these drawbacks, because physical maintenance is negligible, storage densities can be dramatically increased and sources of elements (C, H, N, O) as constituents of the information-containing macromolecules are abundant.

DNA, the carrier of genetic information and arguably nature's largest biopolymer, has already been used as a macromolecular carrier of information, able to archive[3-5], manage (DNA hard disk)[6] and encrypt data[7-9], which can be retrieved by well-established read-out tools.[10] Moreover, extremely high storage densities can be achieved, *i.e.* 10^6 times more information per mm^3 than in hard disk or flash memories.[11] 200 megabytes of data, which include a high-definition music video and 100 books, were stored on DNA that contained more than 1.5 billion base pairs.[12]

While the information encoded can be copied by DNA replication, this procedure is susceptible to errors, and DNA holds some other serious practical issues with respect to long-term stability and synthetic scalability. Polymer chemists have recently realised that the potential of data storage is not restricted to DNA and that sequence-defined macromolecular structures are equally applicable for data storage purposes, as indicated by recent progress in the field.[13, 14] Therefore, they have been developing different methodologies to achieve full control over the primary structure, i.e. the order of monomers in a sequence of synthetic macromolecules.[15-34] Moreover, synthetic carriers of digital information can have significantly simplified structures regarding backbone and chirality. In fact, the simplest constructs are atactic, sequence-defined

binary strings.[2] In comparison to DNA, these polymers, devised as digital information carriers, have very simple molecular structures. However, in theory they are more robust, and therefore potentially constitute the basis for future data storage technologies.

A large variety of synthetic approaches have been developed to make sequences that can be easily read with well-known technologies such as tandem mass spectrometry.[16, 35-38] Lutz and co-workers, for instance, stored digital information on sequence-defined oligourethanes[23, 24], oligo(triazole amide)s[25, 26], poly(phosphodiester)s[27, 28], oligo(alkoxyamine amide)s[29, 30] and oligo(alkoxyamine phosphodiester)s[31, 32]. In these sequences, digital information is encoded using two monomers, resulting in the binary representation commonly used by modern computer systems. While most of these approaches store data in a binary representation, recent synthetic approaches have been developed in an attempt to expand the number of functionalities that can be placed in the backbone or side-chain.[16, 17, 37, 39, 40]

After all, the amount of data that can be stored in macromolecular chains depends on two factors: the chain length and the number of different building blocks, which determine the base of the positional numeral system.[41] For example, with 20 different building blocks (base-20), up to $8x10^3$ trimers and $2.56x10^{10}$ different octamers can be made. Similarly, while only 32 different pentamers can be made with 2 different building blocks (base-2 = binary), $3.2x10^6$ pentamers are theoretically possible through the use of 20 building blocks. Thus, the use of sequence-defined structures with a large diversity of functionalities would allow for compact data storage on short macromolecular chains. The first examples of using multiple functionalities for data storage have recently been reported by the groups of Lutz[32] and Meier[42]. These approaches require, however, the multistep synthesis of different monomers that need to be coupled together to prepare oligomers. In comparison, the automated protecting-group-free, two-step iterative protocol introduced in the previous chapter only requires one synthetic building block, the α-isocyanato-γ-thiolactone, and both the backbone and the side chains of the defined oligomers can be easily tuned with (commercially available) amino alcohols and acrylates, avoiding synthetic efforts for the preparation of different monomers.[1]

This chapter depicts the storage capacity of multifunctional sequence-defined macromolecules, which were synthesised with this protocol introduced in the previous chapter. While it has been shown that this synthetic protocol also allows for modification of the oligomer backbone by using different amino alcohols, the level of functionalisation has been restricted to the side chain in order to reduce the complexity of the resulting mass spectra. First, two tandem mass spectrometry techniques were tested and compared to provide a detailed analysis for a single pentamer to determine which would be most suitable for the prepared oligomers. Next, the controlled fragmentation of the oligomers in combination with the time-consuming interpretation of the MS/MS-spectra was the inspiration for the development of an automated sequencing tool called Chemreader.

For peptide chemistry and synthetic sequences, examples can already be found of computational algorithms that revolutionised the fields of sequence-order reading, database building and *de novo* identification.[43-45] In order to test and improve the algorithm, a sentence, written on oligo(amide-urethane)s, has been decoded. Finally, the power of this algorithm and protocol was further exemplified by encoding and decoding a QR (Quick Response) code starting from short, multifunctional macromolecular structures. Another algorithm, called Chemcoder, has been developed to automate the translation of binary data (such as a QR code) to oligomers and *vice versa* (Figure VII.1).

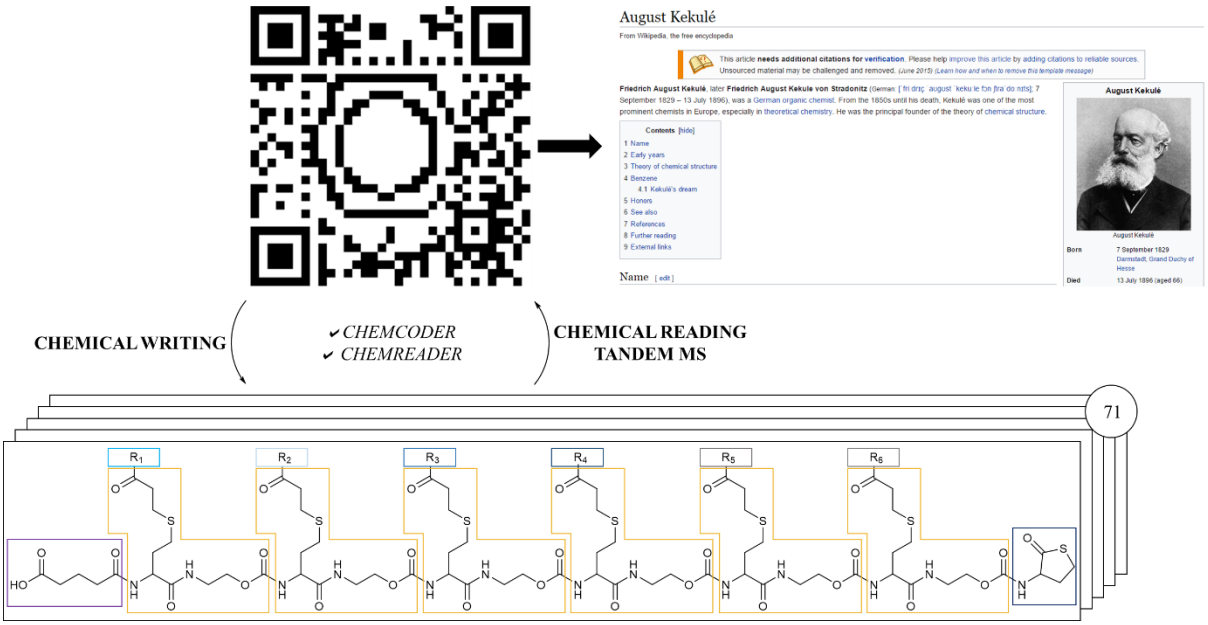


Figure VII.1: A QR code including a benzene structure, encoding the URL of the Wikipedia page of August Kekulé, who was the first to understand the structure of benzene and made a proposal for its structure, is translated to and written on 71 different sequences. Translation is done using the Chemcoder algorithm. The sequences are read afterwards by means of tandem MS and the Chemreader algorithm. Given these sequences, Chemcoder is able to reconstruct the original QR code.

Parts of the research described were a collaboration with the research groups of Prof. Peter Dawyndt (Computational Biology Lab) and Prof. Bart Devreese (Laboratory for Protein Biochemistry and Biomolecular Engineering) of Ghent University. I performed the synthesis of the oligomers, preparation of the MALDI samples and analysis of the (tandem) mass spectra obtained, which was initially guided by Prof. Bart Devreese. Isabel Vandenberghe and Gonzalez Van Driessche performed the mass measurements. Annelies Landuyt, a honour award student from the Faculty of Sciences, and Prof. Peter Dawyndt developed the software tools, Chemcoder and Chemreader, for the reading and writing of the prepared sequences. During the developmental stage, I provided details on the requirements of the software tools. The results obtained were discussed, analysed and commented upon by the collaborators at all stages.

VII.2 Read-out of the oligomers

VII.2.1 Exploration of ESI and MALDI

In order to determine on the one hand the fragmentation behaviour of the oligomers and on the other hand the most suitable mass technique for the oligo(amide-urethane)s analysis (Electrospray Ionisation (ESI) or Matrix-Assisted Laser Desorption/Ionisation-Time Of Flight (MALDI-TOF) tandem mass spectrometry), a pentamer Z5 was first prepared, starting from an acid linker, and analysed. The fragments generated in the collision cell of these mass spectrometers mainly resulted from a controlled fragmentation on the urethane bond (Figure VII.2). As can be seen in Figures VII.3 and VII.4, the pentamer can be fully read from left to right and *vice versa* for both techniques.

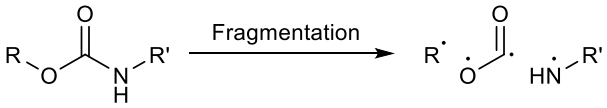


Figure VII.2: Controlled fragmentation on the urethane bond.

Although both techniques allowed for the analysis of the pentamer, MALDI-TOF was chosen over ESI for two reasons:

1) simplification of the analysis of the mass spectra, as ESI might produce multiple-charged fragments which make the spectrum more complex to analyse, as noticed during the analysis of an octamer;
2) permanent availability of MALDI-MS/MS, while the ESI-MS/MS systems were in high demand for other applications and projects such as proteomics.

ESI, however, is easier to combine with separation techniques such as liquid chromatography, which could be useful in the future.[46] In this chapter, the oligomers were analysed one by one on the MALDI-TOF/TOF MS/MS, but if a combination between LC and ESI-MS/MS were available, more complex samples, containing more oligomers, might be analysed. The first separation would then be done in the LC dimension and the analysis of the sequence order in the MS/MS dimension.

Following this initial study, six hexamers that were prepared in the previous chapter on an automated synthesiser starting from an alcohol linker[1] (R2-R7) were also deciphered with MALDI-MS/MS. These hexamers were built with benzyl-, butyl- or tetrahydrofurfuryl acrylate and contain repetitions in their sequences. Although R2 and R6 have the same mass, the order of their sequence could be easily determined and thus they could be differentiated unambiguously (Figures VII.5 and VII.6). For sequence R6, a more detailed analysis of the MS/MS spectrum was performed.

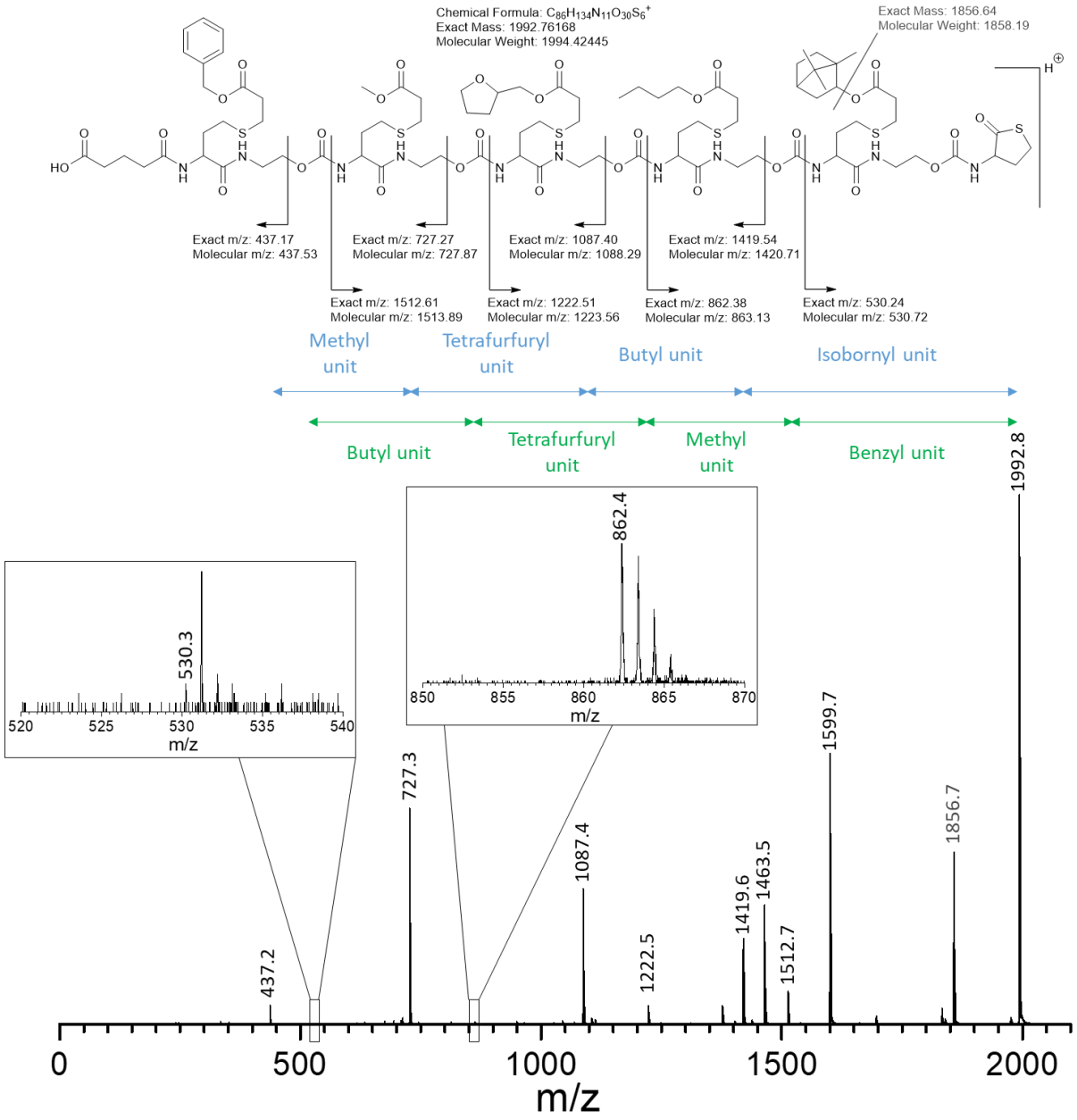


Figure VII.3: ESI-MS/MS spectrum with peak assignment of Z5.

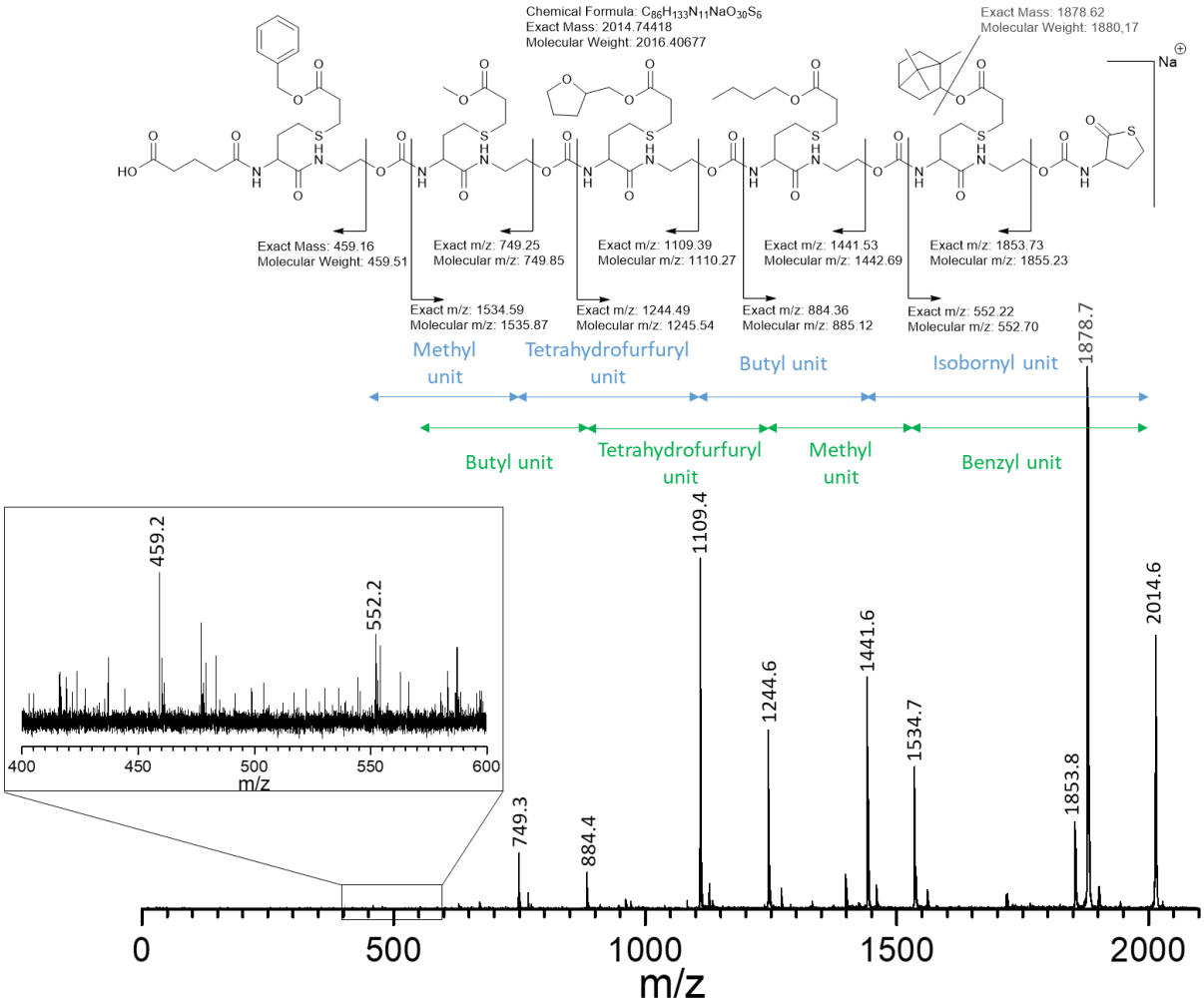


Figure VII.4: MALDI-MS/MS spectrum with peak assignment of Z5.

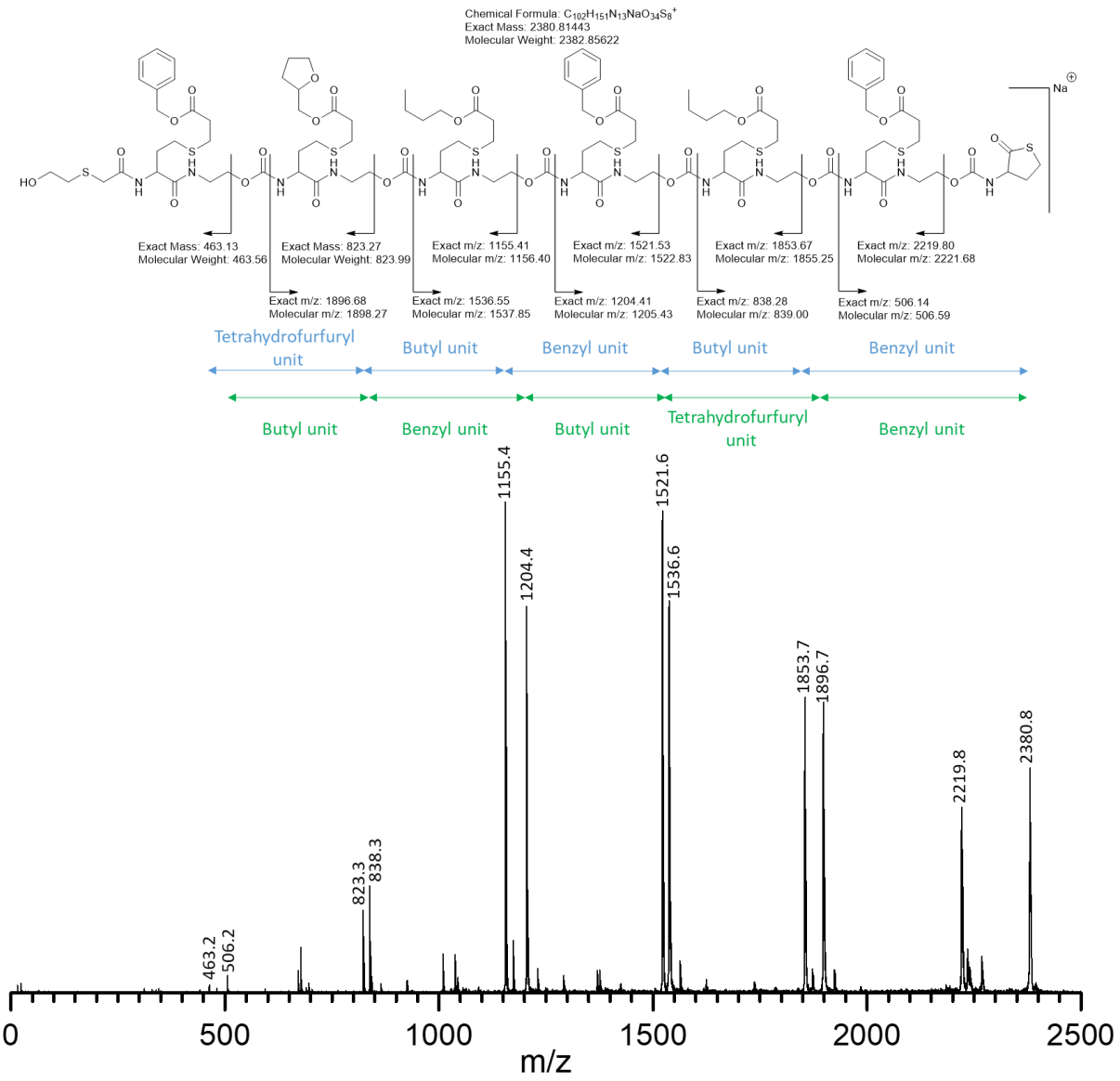


Figure VII.5: MALDI-MS/MS spectrum with peak assignment of R2.

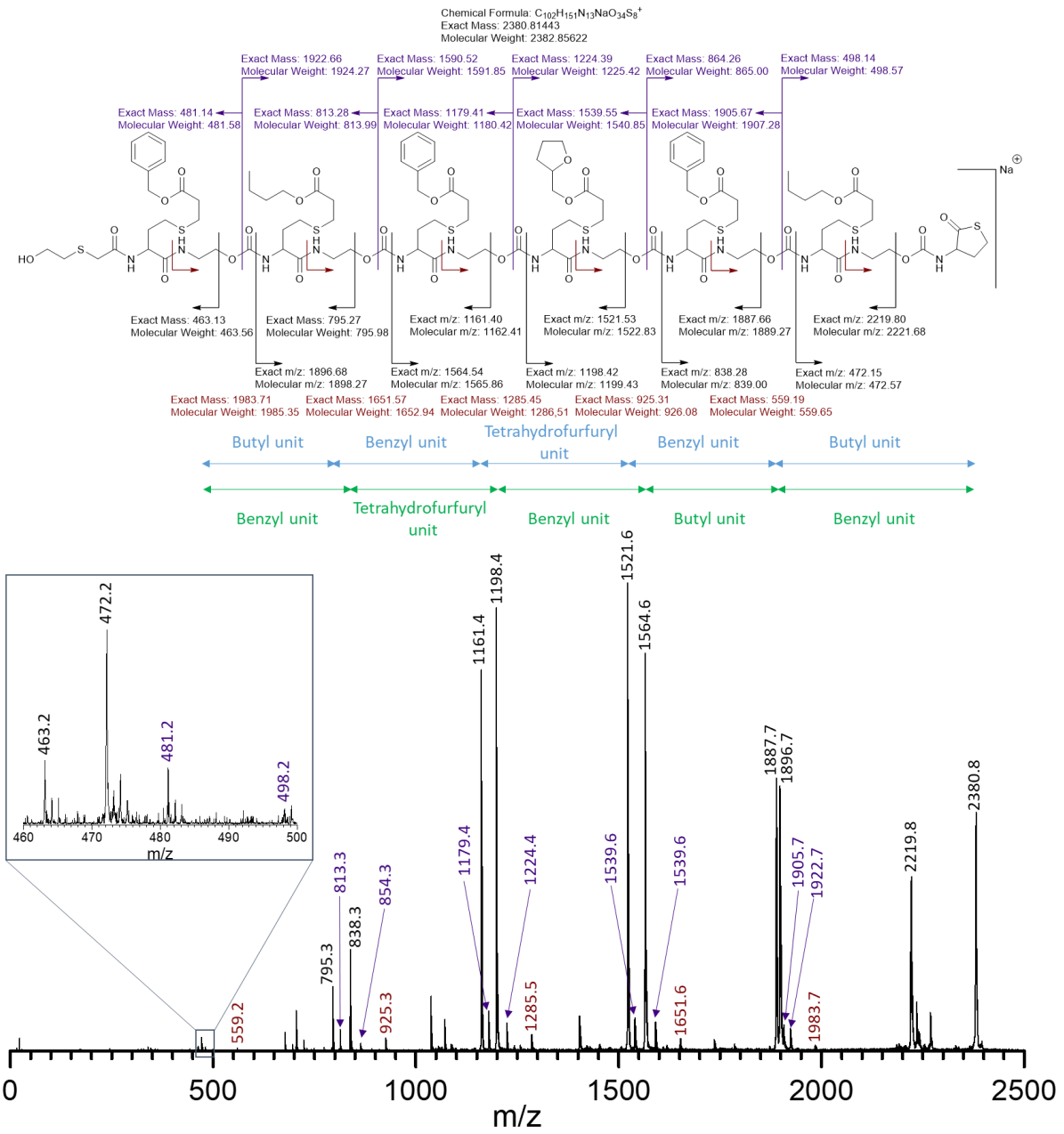


Figure VII.6: MALDI-MS/MS spectrum with peak assignment of R6.

Since the preliminary data, obtained with positive ion mode, were very promising, it was not investigated whether the same results would be achieved in negative mode. It is commonly known that MALDI-TOF MS signals (signal-to-noise ratio) are typically better in positive ion mode compared to negative ion mode.[47] Although this mode is definitely an option, as proven by Lutz et al.[48], positive mode was preferred because it works perfectly for both end groups (alcohol and acid). In addition, it was observed that in positive mode the MS/MS spectra could be read from left to right and from right to left. This interchangeability helped with the analysis and read-out of the spectra. A benefit of the negative mode could be that only the fragments with an acid end group would be visible in the MS/MS spectra, which would reduce the signals in the spectra and make them easier to analyse.

VII.2.2 Development of Chemreader algorithm

Once the easy read-out of these sequences was proven, their potential to store data was explored. To speed up, automate and simplify the read-out process, an algorithm (Chemreader) was developed, for which pentamer Z5 was initially used. The algorithm uses both the masses of the collection of functionalities and the length of the monomer sequence as input parameters. It contains three subscripts:

1) a script in which all possible theoretical fragments are generated;
2) a script that searches for matches between the theoretical fragments and the experimental peaks in the MS/MS spectrum;
3) a script in which the matched fragments are combined in order to determine the sequence.

The error in m/z, as a parameter of the software, can be adjusted at will and was set to 0.02 for our measurements, as this is the typical error in m/z for the used MS equipment. Taking into account the ionisation with a proton (ESI) or a sodium cation (MALDI), the algorithm can be applied for both ESI- and MALDI-MS/MS spectra.

A reference implementation of the Chemreader algorithm is provided in Python using the RDKit module (http://www.rdkit.org). RDKit supports all necessary functions to work with molecules, e.g. calculation of the mass, bond formation and bond fragmentation. The algorithm uses a SMILES representation (simplified molecular-input line-entry system) of molecules instead of their 2D-representation. The ESI-MS/MS spectrum of a dimer is used as an example to give a step-by-step explanation of the algorithm (Figure VII.7).

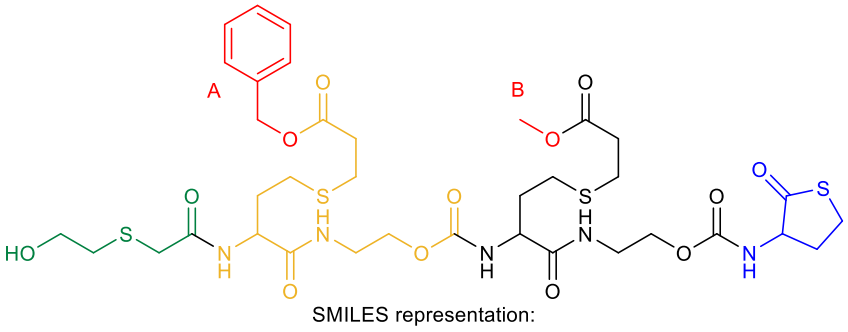


Figure VII.7: Dimer with two different functionalities and its SMILES representation. The different building blocks are indicated in different colours: start fragment (green); backbone (yellow), stop fragment (blue) and the functionalities (red).

The first script generates all possible fragments starting from a SMILES representation of the primary building blocks (as can be obtained via Chemdraw software, for instance): the backbone, the functionalities (A and B, Figure VII.7), the start fragment and the stop fragment. In case of dimers with two possible functionalities A and B, the possible fragments are: A, B, {AA}, {AB}, {BB}, *A, *B, *{AB}, *{AA}, *{BB}, A*, B*, {AA}*,{AB}*, {BB}*, *{AB}*, *{AA}* and *{BB}*. The asterisk denotes the presence of a start and/or a stop

fragment, while the brackets indicate that the actual order of the functionalities is unknown. The mass of all generated fragments is calculated.

The second script searches for all possible matches between the generated fragments and the masses in the MS/MS spectrum. For the given dimer, the only possible matches are: *{AB}*, *{AB}, *A, B*, {AB}*, A, B and {AB} (Figure VII.8). In practice it is not necessarily the case that all possible fragments are observed in the spectrum measured. Furthermore, there will also be numerous peaks in the experimental spectrum coming from fragments generated by other fragmentation patterns than the main fragmentation pattern on the urethane bond (secondary fragmentations, loss of side chain…). These phenomena might prevent an unambiguous determination of the sequence in the third part of the algorithm.

The third script reconstructs the original sequence-defined polymer from the fragments identified using Dijkstra's shortest path algorithm[49]. In order to do this, a distinction is made between fragments containing the start fragment (*{AB}*, *{AB} and *A) and fragments containing the stop fragment (*{AB}*,{AB}* and B*). Fragments without a start or a stop fragment are not used by the algorithm for now, although they could also provide useful information that will be taken into account in future versions of the algorithm. With a perfect spectrum in which all fragment peaks are present, the sequence can be recovered using only the start-containing or stop-containing fragments. However, in practice, spectra are rarely perfect and there is a considerable chance that one or more peaks will be missing due to incomplete fragmentation or because a non-ionised fragment is generated. Combining the information from the start-containing fragments (reading from left to right) with the information of the stop-containing fragments (reading from right to left) increases the chance of unambiguously determining the sequence. The start fragment (alcohol or acid linker) could be easily modified in the algorithm.

To summarise the Chemreader process: in a first step, the program generates all possible fragments that could possibly be formed; secondly, it searches for matching masses that are obtained after MS/MS analysis; finally, fragments are combined to reconstruct the original sequence. If pentamer Z5 is inspected in more detail (Figure VII.4), fragmentation on the urethane bonds leads to the fragments necessary to perform the automated sequence analysis with the Chemreader algorithm. In all cases, both the start-containing fragment (left fragment with the acid linker) and the stop-containing fragment (right fragment with the thiolactone ring) are present in the spectrum. Presence of these two fragments makes it easy for the algorithm to unambiguously translate the MS/MS spectrum into the exact pentamer structure.

The Chemreader algorithm has linear time complexity in the length of the polymers and the number of building blocks. For example, octamers with a 20-character alphabet are resolved in the order of milliseconds on a standard laptop (i5 processor).

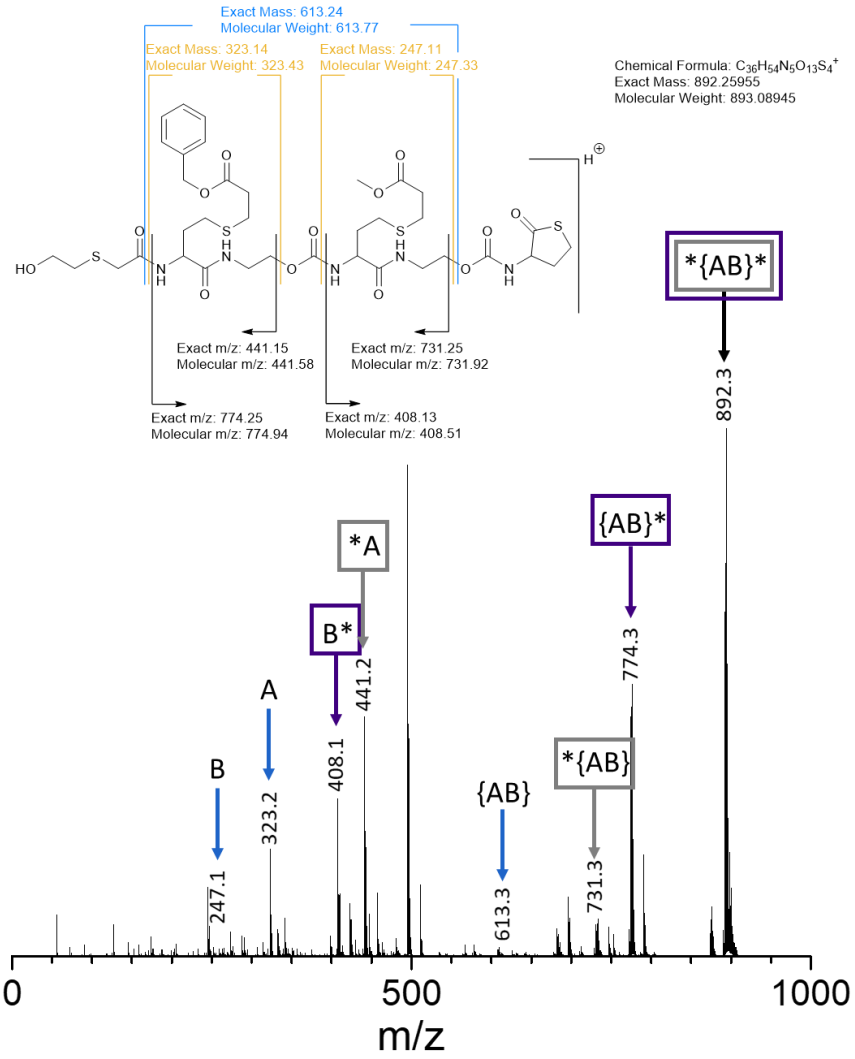


Figure VII.8: ESI-MS/MS spectrum of dimer: benzyl is A, methyl is B, the asterisk indicates the presence of a start or stop fragment and the brackets express the unknown order of functionalities in the sequence.

VII.3 Writing and reading data

VII.3.1 Writing and reading human-readable data

Before different kinds of data were stored on oligomers, various alcohols and 2-hydroxyethyl acrylate were converted respectively to their corresponding acrylates and to 2-acetoxyethyl acrylate in order to expand the number of acrylates that can be installed in the side chains of the prepared oligomers and to indicate the ease with which functionalities can be generated for the applied protocol (Figure VII.9).[1] The acrylates obtained with yields between 80 and 90% were purified by means of distillation and characterised with NMR analysis.

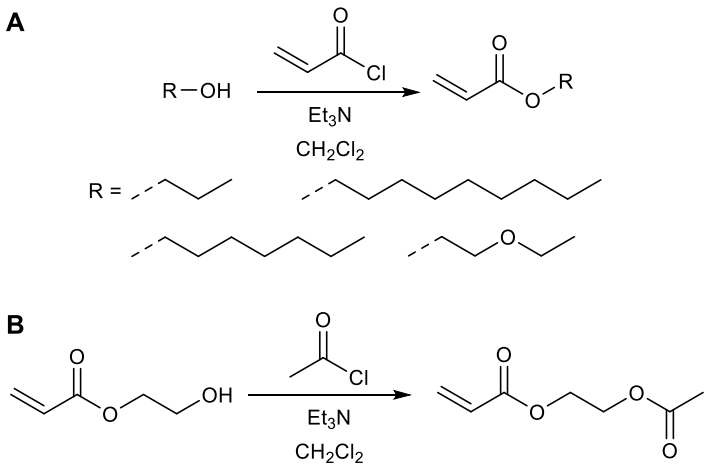


*Figure VII.9: Synthesis of different acrylates starting from different alcohols (**A**) and 2-hydroxyethyl acrylate (**B**).*

As a first proof-of-principle, the question "TO WRITE OR NOT TO WRITE ON OLIGOS?" was written on short oligomers. For this, the eight different words were converted into individual oligomers, using acrylates as a chemical alphabet to represent the individual characters. Comparable to previous research in which mass tags were added to oligomers to indicate the position of a letter in a word[50], the position of the words in the sentence has been encoded to enable the reconstruction of the words in the correct order. As a result, the sentence was actually encoded as "1TO 2WRITE 3OR 4NOT 5TO 6WRITE 7ON 8OLIGOS?" The sentence was written twice using the two different linkers, showing the versatility of the α-end groups used for writing the oligomers. The acrylates (19 in total, each with a different mass) correspond to the different letters, numbers and the question mark in the sentence (Figure VII.10 and Table VII.1).

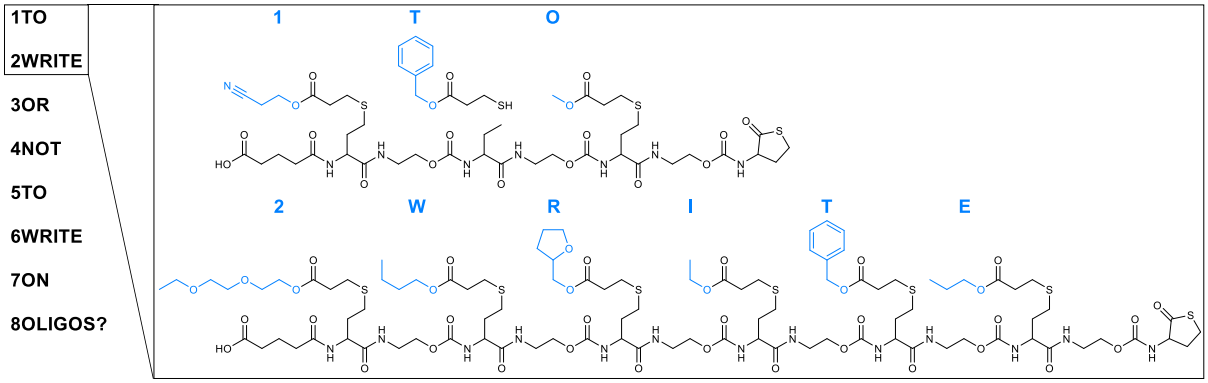


Figure VII.10: The first two words of the question '1TO 2WRITE 3OR 4NOT 5TO 6WRITE 7ON 8OLIGOS?' in their chemical form. The different functionalities, introduced in the chemical protocol via acrylates, express a different letter or number.

Table VII.1: The alphabet (the different functionalities) used to write the sentence '1TO 2WRITE 3OR 4NOT 5TO 6WRITE 7ON 8OLIGOS?' on sequence-defined poly(amide-urethane)s

Symbol	Acrylate functionality	Symbol	Acrylate functionality
1	2-Cyanoethyl	I	Ethyl
2	2-(2-Ethoxyethoxy)ethyl	L	Cyclohexyl
3	Ethylhexyl	N	Isobornyl
4	Nonyl	O	Methyl
5	Citronellyl	R	Tetrahydrofurfuryl
6	Heptyl	S	Isoamyl
7	2-Acetoxyethyl	T	Benzyl
8	Propargyl	W	Butyl
E	Propyl	?	2-ethoxyethyl
G	2-methoxyethyl		

Decoding the sentence requires knowledge about the alphabet (acrylates used), the number of words and the length of each word. Each word can be analysed separately. Given this information, Chemreader can reconstruct the original sentence. Only for the word "8OLIGOS?", one peak corresponding to the smallest fragment was absent. However, due to the redundancy in overlapping fragments and the left-right and right-left reconstruction of the data, the octamer could be correctly translated. While encoding a human-readable sentence in sequence-defined polymers provided a first proof-of-principle to demonstrate the power of the Chemreader algorithm, the encoding scheme applied is not scalable to larger text fragments due to variable-length position encoding and to larger alphabet sizes (e.g. ASCII or Unicode) as separate acrylates are needed for all characters in the alphabet.

VII.3.2 Writing and reading machine-readable data

A second and more ambitious challenge was the synthesis and analysis of different oligomers to encode a 33x33 QR code, corresponding to a square grid containing 1089 pixels. With the ever-increasing use of smartphones, QR codes have become a simple way of communicating short messages. In producing a sample QR code that encodes the URL of the Wikipedia page of August Kekulé[51], the redundancy built into these codes - for error correction purposes - was exploited to embed a visual representation of the benzene ring. Kekulé was the first to understand the structure of benzene and made a proposal for its structure (1865) during his stay at Ghent University (1858-1867).

The black and white dots in a QR code represent bits (0 and 1) in the binary numeral system. As such, a QR code is nothing more than a two-dimensional bit string. To achieve the goal of encoding the QR code in sequence-defined polymers, the bit string was converted into a

sequence of functionalities. To automate the process of encoding and decoding bit strings as collections of oligomers, a software tool called Chemcoder was developed.

Figure VII.11 schematically highlights the general outline of the Chemcoder algorithm. The encoding of a QR code bit string is done in a series of steps. In a first step, the bit string is converted into a sequence of so-called flags (= side-chain functionalities). As this sequence of flags is too long to be encoded in a single oligomer, it is split into short fixed-length fragments. To give the last fragment the same length as the other fragments, it occasionally has to be filled with a non-coding spacer region (black region in Figure VII.11). To enable reconstruction of the original bit string from the collection of fragments, an index is added to each fragment (purple region in Figure VII.11) as well as the total length of the original bit string (blue region in Figure VII.11).

Decoding can only be done if the sequence of all fragments has been determined. In that case, Chemcoder dereplicates the sequenced fragments and sorts them in their original order based on the index, removes the non-coding index and length regions, and glues the coding sections together into a single bit string, from which the spacer region is trimmed using the encoded length of the original bit string. The resulting bit string corresponds to the original QR code. The Chemcoder algorithm has linear time complexity in the length of the bit string (GB-sized files are converted in the order of milliseconds on a standard laptop).

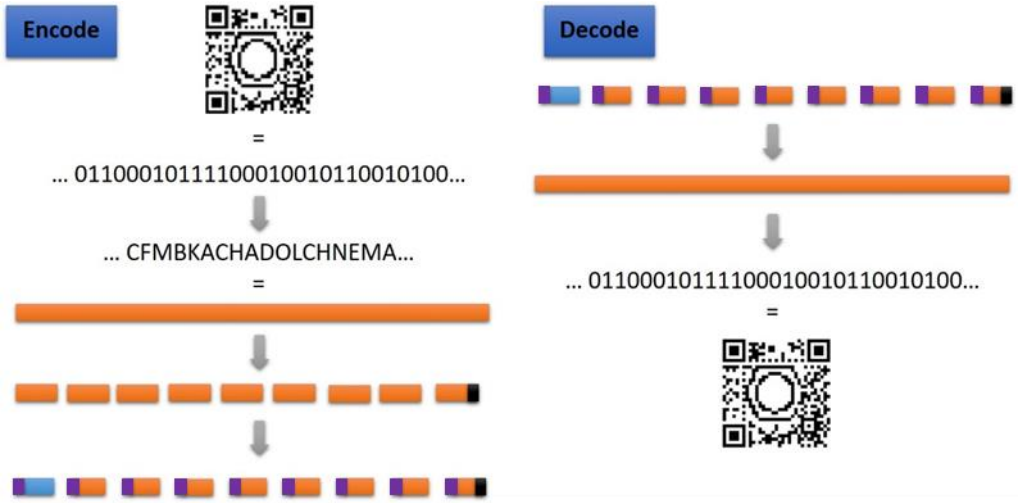


Figure VII.11: Encoding scheme (left). The bit string representing the QR code is first translated into a pentadecimal numeral system (base-20). The sequence of 'flags' is then cut into smaller pieces. In a final step, the position of each fragment (purple) and the length of the bit string (blue) is added. The last fragment may be filled with a non-coding spacer (black); Decoding scheme (right). After determination of the sequence of all fragments, they are dereplicated, sorted, trimmed and glued together. Finally, the sequence of flags is converted into the bit string that reconstructs the original QR code.

Apart from the bit string that needs to be encoded into a collection of fragments, Chemcoder needs to be configured with the maximal fragment length and the size of the chemical alphabet (available flags). Depending on these settings, a different number of oligomers must be synthesised: the longer the oligomers and the more flags that can be used, the lower the number of oligomers that needs to be synthesised. The settings for Chemcoder were chosen such that the sample QR code is translated (Figure VII.11) into a collection of 71 short oligomers (1

monomer, 11 pentamers and 59 hexamers). The automated protocol developed earlier allows for simultaneous synthesis up to 72 sequence-defined structures, which fits the 71 oligomers that are needed here.[1] To write the QR code, these fragments were synthesised using a library of 15 acrylic monomers (Table VII.2), which were labelled A, B, C… up to O to make them more human-readable. After obtaining spectra from all 71 oligomers, Chemreader reconstructed all fragments without errors, which were then converted into the original bit string by Chemcoder, yielding the original QR code.

Table VII.2: The alphabet (the different functionalities) used to write the QR code on sequence-defined poly(amide-urethanes).

Symbol	Acrylate Functionality	Symbol	Acrylate Functionality
A	Cyclohexyl	I	2-(Dimethylamino)ethyl
B	Benzyl	J	Isobornyl
C	Tetrahydrofurfuryl	K	2-(2-ethoxyethoxy)ethyl
D	Butyl	L	Propyl
E	Methyl	M	Heptyl
F	Ethyl	N	Ethylhexyl
G	Isoamyl	O	2-Methoxyethyl
H	Diethylacrylamide		

VII.4 Conclusions, outlook and media coverage

VII.4.1 Conclusions

This chapter explored the storage capacity of sequence-defined oligomers based on the amine-thiol-ene conjugation with thiolactone chemistry. Two different techniques, ESI- and MALDI-TOF/TOF MS/MS, were compared for the detailed analysis of the oligomers prepared. A controlled fragmentation on the urethane bond allowed for an easy read-out of the oligomers. An algorithm called Chemreader was developed to facilitate the read-out of these sequences, which allows one to read the information stored within sequence-defined structures in a fast and automated way on a standard laptop. The Chemreader algorithm contributes to solving the time-consuming "sequence-reading" bottleneck of sequence-defined macromolecules. In order to test the Chemreader algorithm, a sentence in natural language was first successfully written and read, followed by the more ambitious challenge of encoding a 33x33 QR code in 71 different, analysed oligomers. The software tool Chemcoder was developed to quickly encode binary data as a compact collection of oligomer fragments and to decode oligomer fragments as the original binary data. Both algorithms are extremely fast and highly configurable for application to other sets of sequence-defined polymers.

As the results obtained prove the possibilities for using these mono-disperse, multi-functional oligomers in the field of data storage, this chapter is another indication for the long-term potential that sequence-defined polymers hold for real-world applications and thus provides further validation for this rapidly developing branch of macromolecular chemistry.[52-55] As the application of the used mass analysis techniques results in the destruction of data (or oligomers), one of the main research challenges remains the further exploration of non-destructive techniques, such as nanopore sequencing, for the read-out of the sequence order in complex mixtures.[56-58]

VII.4.2 Outlook

As every oligomer had to be analysed separately, a future challenge would be to combine techniques for the analysis of much more complex samples, in order to guarantee a high data density. An example, well-known in the context of peptide analysis, consists of the coupling of liquid chromatography to tandem ESI-MS/MS equipment to separate different oligomers in the LC dimension and determine the sequence in the tandem MS dimension.[46] Another example is MALDI-TOF MS which is capable of handling complex mixtures, as is widely demonstrated in proteomics where MALDI-TOF MS is used to characterize proteins by analysing multiple tryptic peptides consequently by MS/MS.[59] This method is also adopted in the 2D technique developed by the group of Lutz.[29] A complex sample is first analysed in the MS dimension, after which the different masses are isolated and analysed in a tandem MS. For this technique, the mass difference between different parent ions should be distinctive enough in order not to cause any problems during the isolation. If the oligomer mixture becomes too complex, there are in fact two alternatives. The first is to start separating the oligomer mixture by reversed-phase high-performance LC and analyse the separated components by off-line (MALDI-TOF MS) or on-line (ESI-MS) mass spectrometry. The latter has the disadvantage that spectral complexity is increased by the generation of multiple charged ions. However, deconvolution software could be used to transform these spectra into single charged ion spectra that become amenable for analysis software. The other alternative is the use of ion mobility mass spectrometry that allows for gas phase separation. However, this comes with a considerable additional instrument cost.[60]

Another challenge would be to combine the oligomers prepared into one long chain so that the information can be stored on one polymer instead of, as with the QR code, 71 separate oligomers. For the read-out of this data-containing polymer, two tactics can be chosen: controlled fragmentations in collision-induced dissociation conditions[50] or controlled depolymerisation[61] in combination with the methods mentioned in the previous paragraph. The first technique was used by Lutz' group for the analysis of poly(phosphodiester)s chains (bit string) which contain several bytes (8 bits) of information. The structure of these information-containing polymers had two distinct molecular features: between the bytes (or

octa(phosphodiester)s) alkoxyamine groups were installed and to each byte mass tags were added to indicate the position in the bit string. First MS/MS (MS^2) conditions were applied on the poly(phosphodiester)s to cleave the weak NO-C bonds. Afterwards, MS^3 measurements were performed on each byte to decode the whole bit string or polymer.

As mentioned in the introduction, the amount of data that can be stored in macromolecular chains depends on two factors: the chain length and the number of different building blocks.[41] Since different chemistries, such as reactions with thiolactone, allow for the easy installation of different functionalities, chemists are not restricted to binary information.[1, 32, 42] Another challenge could even be to write "more with less". In this chapter, only the acrylic moiety was used to contain the information, while it was proven in the previous chapter that different amino alcohols can also be used to modify the backbone and as such store some information.[1] As another example, a thiolactone unit could be installed in the side chain of a repeating unit of an oligomer and double-modified with an amine and an acrylic unit, which would result in every repeating unit possibly containing more information in comparison to the protocol used in this chapter. Meier's group has combined the Passerini and the Biginelli reaction to produce oligomers that can store up to 24 bits per repeat unit.[42] Although more data can be stored with the mentioned approach mentioned, it could cause a more difficult mass analysis and precautions should be taken so that no mass overlap is observed.

VII.4.3 Media coverage

This research has received considerable attention in the media, as illustrated by the following web links.

http://www.avs.be/avsnews/ugent-poeder-als-databank?platform=hootsuite

https://www.vrt.be/vrtnws/nl/2018/10/29/poeder-informatie/

https://www.ugent.be/en/news-events/data-research-ghent-student-qr.htm

https://www.hln.be/nieuws/binnenland/wat-als-je-films-kon-opslaan-in-een-lepeltje-poeder-dit-moet-op-termijn-het-einde-betekenen-van-usb-sticks~aa9e8dea/

https://datanews.knack.be/ict/nieuws/qr-code-in-poedervorm-belgische-onderzoekers-ontwikkelen-een-nieuwe-manier-om-data-op-te-slaan/article-normal-1386475.html

https://www.telegraph.co.uk/technology/2018/10/30/belgian-research-could-replace-usb-sticks-data-stored-powder/

VII.5 Experimental part

VII.5.1 Methods

^{1}H- and ^{13}C-NMR

^{1}H- and ^{13}C-NMR (Attached Proton Test, APT) spectra were recorded on a Bruker Avance 300 at 300 MHz and 75 MHz, respectively and a Bruker Avance 500 at 500 MHz and 125 MHz, respectively. Chemical shifts are presented in parts per million (δ) relative to DMSO-d_6 or $CHCl_3$-*d* (2.50 ppm or 7.27 ppm in ^{1}H- and 39.51 ppm or 77.24 ppm in ^{13}C-NMR respectively) as internal standard. All samples were analysed with 2D-NMR techniques (COSY, HSQC and HMBC) which allowed for a full assignment of the structures. All measurements were performed at 25°C and ACD/NMR Processor was used for the processing of all data. All spectra including 1D ^{1}H and ^{13}C, 2D COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC were recorded in a standard fashion with pulse programs available in the Bruker library. The NMR spectra and analysis of the compounds synthesised in this chapter can be found at https://www.nature.com/articles/s41467-018-06926-3.

LC-ESI-MS

An Agilent technologies 1100 series LC/MSD system equipped with a diode array detector and single quad MS detector (VL) with an electrospray source (ESI-MS) was used for classic reversed-phase LC-MS (liquid chromatography mass spectroscopy) and MS analysis. Analytic reversed-phase HPLC was performed with a Phenomenex C18 (2) column (5 µ, 250 x 4.6 mm) using a solvent gradient (0 → 100% acetonitrile in H_2O in 15 min) and the eluting compounds were detected via UV-detection (λ = 214 nm). High-resolution mass spectra (HRMS) were collected using an Agilent 6220 Accurate-Mass time-of-flight (TOF) equipped with a multimode ionisation (MMI) source. The LC-ESI-MS spectra and HRMS data of the compounds synthesised in this chapter can be found at https://www.nature.com/articles/s41467-018-06926-3.

IR

Infrared spectra were recorded with Attenuated Total Reflection (ATR) with a PIKE Miracle ATR unit and a Perkin Elmer FTIR SPECTRUM 1000 spectrometer. IR-software from Perkin Elmer was used for the analysis of the spectra.

Automated synthesis

Automated syntheses were performed on a 72-reactor block INTAVIS MultiPep CF Synthesiser with open 5 mL reaction columns equipped with a vortexing unit. The speed of vortexing is 550 rpm.

Electrospray Tandem mass spectrometry

ESI mass spectrometry analysis was performed on a Synapt G1 HDMS mass spectrometer (Waters). Samples were diluted in 50% acetonitrile/0.1% formic acid in water and transferred into a 96-well plate. This plate was loaded into an Advion Triversa Nanomate source. From each sample 3 microliter was picked with a conductive peptide tip and moved towards the D-chip plate. Typically, 1.3 V was applied on the chip, spraying the sample in the source area of the mass spectrometer which was used in the Q-TOF mode. Tandem mass spectra were generated by collision-induced dissociation using Ar as collision gas at 30eV collision energy.

MALDI-TOF/TOF MS/MS

For MALDI analysis, measurements were performed with trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malonitrile (DCTB, 30 mg/mL in dichloromethane) as a matrix, Sodium trifluoroacetate (19 mg/mL in acetone) as a cationising agent, and oligomer samples were dissolved in THF (4 mg/mL). Oligomer solutions were prepared by mixing 10 µL of the oligomer, 1 µL of the salt, and 10 µL of the matrix solution. Subsequently, 0.5 µL of this mixture was spotted on the sample plate, and the spots were dried in air at room temperature. 0.5 microliter was spotted on a MALDI plate and loaded into the Sciex 4800 MALDI-TOF/TOF MS instrument equipped with an Nd:YAG laser (200 Hz, 355 nm) controlled by 4000 Series Explorer software version 3.5.3 (Applied Biosystems, Germany). The instrument was operated in positive ion mode with delayed extraction and an acceleration voltage of 20 kV with a grid of 15.6 kV. Fragmentation (MS/MS) was performed in positive ion mode at 1 kV using the no gas option. The 4700 Proteomics Analyser Mass Standard kit (Applied Biosystems, Germany) was prepared according to the manufacturers' recommendation and used for external calibration before analysis (mass to charge range from 800 to 4,000 Da). MS/MS calibration was based on the precursor mass of 1,570.677 Da of Glu-fibrinopeptide B. Signals were considered as interpretable if the error in m/z was not higher than 0.02; the signal-to-noise had to be higher than 5. The mass spectra of the compounds synthesised in this chapter can be found at https://www.nature.com/articles/s41467-018-06926-3.

Algorithm: "Chemreader"

The Chemreader algorithm determines the composition of a sequence-defined polymer starting from an experimental MS/MS-spectrum of the polymer, an upper limit for the length of the polymer and a list of possible functionalities in the polymer. It contains three subscripts: a first script in which all possible theoretical fragments are generated, a second script that searches for matches between the theoretical fragments and the experimental peaks in the MS/MS spectrum and a third script in which the matched fragments are combined in order to determine the sequence. The error in m/z, as a parameter of the software, can be adjusted at will and was set to 0.02 for the measurements, because this is the typical error in m/z for the MS equipment used. A reference implementation of the Chemreader algorithm is provided in Python using the RDKit module (http://www.rdkit.org). RDKit supports all necessary functions to work with

molecules, e.g. calculation of the mass, bond formation and bond fragmentation. The algorithm uses a SMILES representation of molecules instead of their 2D representation.
Both algorithms can be found at https://github.com/chemstore. The individual algorithms can be found at:
https://github.com/chemstore/chemcoder and https://github.com/chemstore/chemreader.

VII.5.2 Materials

DMSO-d_6 ([2206-27-1], ≥ 99.8 %) and $CHCl_3$-*d* ([865-49-6], ≥ 99.8 %) were purchased from Euriso-top. Acryloyl chloride ([814-68-6], 96 %) was purchased from abcr GmbH. Acetonitrile ([75-05-8], HPLC grade), 1,4-Dioxane ([123-91-1], HPLC grade), and Triethylamine ([121-44-8], 99 %) were purchased from Acros Organics. DL-Homocysteinethiolactone hydrochloride ([6038-19-3], 99 %) was purchased from Haihang industry (Jinan City, China). Magnesium sulphate hydrate [22189-08-8], ≥ 99 %), Potassium carbonate ([584-08-7], ≥ 99 %) and Sodium bicarbonate ([144-55-8], ≥ 99.5 %) were purchased from Carl Roth. Trifluoroacetic acid ([76-05-1], Peptide grade) and 2-Chlorotrityl chloride resin ([42074-68-0], 100-200 mesh, 1% DVB, 1.6 mmol/g) were purchased from Iris Biotech GmbH. Acetyl chloride ([75-36-5], ≥ 99 %), Bromoacetyl bromide ([598-21-0], ≥ 98 %), Butyl acrylate ([141-32-2], ≥ 99 %), Chloroform ([865-49-6], ≥ 99.8 %), Citronellol ([106-22-9], ≥ 95 %), Dichloromethane ([75-09-2], ≥ 99.8 %), Diethylether ([60-29-7], ≥ 99.9 %), *N*,*N*-Diisopropylethylamine (DIPEA, [7087-68-5], 99 %), *N*,*N*-Dimethylformamide ([68-12-2], anhydrous, 99.8 %), Ethanolamine ([141-43-5], ≥ 99%), Ethyl acrylate ([140-88-5], 99 %), Glutaric anhydride ([108-55-4], 95 %), 1-Heptanol ([111-70-6], 98 %), 2-Hydroxyethyl acrylate ([818-61-1], 96 %), Isobornyl acrylate ([5888-33-5], technical grade), 2-Mercaptoethanol ([60-24-2], ≥ 99 %), Methanol ([67-56-1], ≥ 99.9 %), Methyl acrylate ([96-33-3], 99 %), Phenothiazine ([92-84-2], ≥ 98 %), 1-Propanol ([71-23-8], 99.7 %), Propargyl acrylate ([10477-47-1], 98 %), Pyridine ([110-86-1], ≥ 99 %), Tetrahydrofuran ([109-99-9], ≥ 99 %) were purchased from Sigma-Aldrich and used without purification, except isobornyl acrylate which was distilled. Benzyl acrylate ([2495-35-4], > 97 %), 2-Cyanoethyl Acrylate ([106-71-8], > 95 %), Cyclohexyl Acrylate ([3066-71-5], > 98 %), Dibutyltin dilaurate ([77-58-7], > 95 %), N,N-Diethylacrylamide ([2675-94-7], > 98 %), 2-(Dimethylamino)ethyl Acrylate ([2439-35-2], > 98 %), 2-Ethoxyethanol ([110-80-5], > 99 %), 2-(2-Ethoxyethoxy)ethyl Acrylate ([7328-17-8], > 98 %), 2-Ethylhexyl Acrylate ([103-11-7], > 99 %), Isoamyl Acrylate ([4245-35-6], > 98 %), 2-Methoxyethyl Acrylate ([3121-61-7], > 98 %), 1-Nonanol ([143-08-8], > 99 %) and Triphosgene ([32315-10-9], > 98 %) were purchased from TCI and used without purification. Tetrahydrofurfuryl acrylate ([2399-48-6]) was purchased from Polysciences and used without purification. Hydrochloric acid 36 % p. (HCl, [7647-01-0]) was purchased from Chem-Lab and used without purification. Solvents (CH_2Cl_2, $CHCl_3$, DIPEA and pyridine) for the chain extension of sequences, the synthesis of α-isocyanato-γ-thiolactone or the immobilisation of functionalised thiolactone linkers were distilled from CaH_2 prior to use. Silicagel (ROCC, SI 1721, 60 Å, 40 – 63 μm) was used to

perform preparative column chromatography, eluting with technical solvents. The collected fractions were analysed by thin layer chromatography (TLC-plates, Macherey-Nagel, SIL G-25 UV254). The α-isocyanato-γ-thiolactone, the acid-functionalised and hydroxyl-functionalised thiolactone linker and 3,7-dimethyloct-6-en-1-yl acrylate (citronellyl acrylate) were prepared as previously described.[1, 62-64]

VII.5.3 Synthesis

Synthesis of different acrylates

Starting from an alcohol: The same procedure was followed for every alcohol. Acryloyl chloride (9.8 ml, 120 mmol) was added dropwise to an ice-cooled mixture of the alcohol (100 mmol), anhydrous CH_2Cl_2 (200 ml) and anhydrous Et_3N (20.9 ml ,150 mmol). The mixture was stirred for 1 hour at 0°C and allowed to reach room temperature overnight. Water (200 ml) and brine (200 ml) were used to wash the reaction. After drying the organic fraction with $MgSO_4$ and concentrating, the crude residue was distilled yielding a colorless oil. Phenothiazine was added as an inhibitor and the product was stored in the fridge.

Propyl acrylate

^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 6.39 (dd, 1H, 17.3, 1.5 Hz), 6.12 (app dd, 1H, 17.3, 10.4 Hz), 5.81 (dd, 1H, 10.4, 1.7 Hz), 4.11 (t, 2H, 6.7 Hz), 1.69 (m, 2H), 0.96 (t, 3H, 7.4 Hz).

^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 166.3 (ester), 130.4 (CH_2), 128.6 (CH), 66.1 (CH_2), 21.9 (CH_2), 10.3 (CH_3).

Boiling point 30°C at 25 mbar

Heptyl acrylate

^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 6.39 (dd, 1H, 17.3, 1.5 Hz), 6.11 (app dd, 1H, 17.3, 10.4 Hz), 5.80 (dd, 1H, 10.4, 1.5 Hz), 4.14 (t, 2H, 6.8 Hz), 1.66 (quint, 2H, 7 Hz), 1.33 (m, 2H), 1.29 (m, 6H), 0.88 (t, 3H, 7 Hz)

^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 166.3 (ester), 130.3 (CH_2), 128.6 (CH), 64.7 (CH_2), 31.7 (CH_2), 28.9 (CH_2), 28.6 (CH_2), 25.8 (CH_2), 22.5 (CH_2), 14.0 (CH_3)

Boiling point 30°C at 1,05 mbar

Nonyl acrylate

^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 6.40 (dd, 1H, 17.3, 1.7 Hz), 6.12 (app dd, 1H, 17.3, 10.4 Hz), 5.81 (dd, 1H, 10.5, 1.6 Hz), 4.15 (t, 2H, 6.8 Hz), 1.67 (quint, 2H, 7 Hz), 1.33 (m, 2H), 1.28 (m, 8H), 0.88 (t, 3H, 7 Hz)

^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 166.3 (ester), 130.4 (CH_2), 128.7 (CH), 64.7 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 28.6 (CH_2), 25.9 (CH_2), 22.6 (10), 14.1 (CH_3)

Boiling point 45°C at 1,05 mbar

2-Ethoxyethyl acrylate
^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 6.41 (dd, 1H, 17.3, 1.7 Hz), 6.14 (app dd, 1H, 17.3, 10.4 Hz), 5.81 (dd, 1H, 10.5, 1.6 Hz), 4.29 (t, 2H, 4.7 Hz), 3.65 (t, 2H, 4.9 Hz), 3.52 (q, 2H, 7 Hz), 1.20 (t, 3H, 7.1 Hz)
^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 166.1 (ester), 130.9 (CH_2), 128.2 (CH), 68.2 (CH_2), 66.6 (CH_2), 63.7 (CH_2), 15.0 (CH_3)
ESI-MS (m/z) $[M+H]^+$ 145.1
Boiling point 25°C at 0,7 mbar

Starting from 2-hydroxyethyl acrylate: Acetyl chloride (8.5 ml, 120 mmol) was added dropwise to an ice-cooled mixture of 2-hydroxyethyl acrylate (100 mmol), anhydrous CH_2Cl_2 (200 ml) and anhydrous Et_3N (20.9 ml ,150 mmol). The mixture was stirred for 1 hour at 0°C and allowed to reach room temperature overnight. Water (200 ml) and brine (200 ml) were used to wash the reaction. After drying the organic fraction with $MgSO_4$ and concentrating, the crude residue was distilled yielding a colorless oil. Phenothiazine was added as an inhibitor and the product was stored in the fridge.

2-Acetoxyethyl acrylate
^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 6.41 (dd, 1H, 17.3, 1.7 Hz), 6.12 (app dd, 1H, 17.3, 10.5 Hz), 5.84 (dd, 1H, 10.5, 1.6 Hz), 4.31 (m, 4H), 2.05 (s, 3H)
^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 170.7 (ester), 165.8 (ester), 131.3 (CH_2), 127.9 (CH), 62.2 (CH_2), 62.1 (CH_2), 20.8 (CH_3)
ESI-MS (m/z) $[M+NH_4]^+$ 176.1
Boiling point 42°C at 0,7 mbar

VII.5.4 Manipulation of the solid support

Swelling
The solid support was swollen in the reaction solvent for at least 10 minutes.

Washing
The loaded resin was washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried after each reaction.

Loading
The acid-functionalised thiolactone linker (1.33 g, 1.2 equivalents relative to the resin, 5.8 mmol) was loaded on a 2-chlorotrityl chloride resin (3 g, 1.6 mmol/g) in dry CH_2Cl_2 (30 mL, 10 mL/1 g of resin) with dry DIPEA (0.26 mL, 4 equiv. rel. to acid) and 10 vol% of DMF (3

mL), in order to dissolve the acid. The reaction mixture was shaken for 3 hours at room temperature. The resin was subsequently washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 6 x 30 mL), DMF (4 x 30 mL), CH_2Cl_2 (4 x 30 mL) and diethyl ether (4 x 30mL). The resin was dried under vacuum.

The hydroxyl-functionalised thiolactone linker (1.69 g, 1.5 equivalents relative to the resin, 7.2 mmol) was loaded on a 2-chlorotrityl chloride resin (3 g, 1.6 mmol/g) in dry THF (30ml, 10 mL/1 g of resin) with dry pyridine (1.16 mL, 2 equivalents relative to the alcohol).[65] The reaction was shaken overnight at 60°C. Next, the solid support was washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 6 x 30 mL), DMF (4 x 30 mL), CH_2Cl_2 (4 x 30 mL) and diethyl ether (4 x 30mL). The resin was dried under vacuum.

Cleavage

For cleavage, 2 mg of solid support was treated with 0.5 mL of 1% trifluoroacetic acid (TFA) in CH_2Cl_2 for 5 minutes. During cleavage, the color of the beads turned from yellow to red because of the formation of a trityl cation. The solid support was then filtered, the sample concentrated by evaporation and dissolved in acetonitrile for LC-ESI-MS analysis. For the tandem MS analysis, samples were dissolved in THF.

Aminolysis and incorporation of functionalities: general procedure

$CHCl_3$ is added to the solid support (1 mL reaction mixture per 100 mg resin). Next, the acrylate (20 equivalents relative to the thiolactone unit) and ethanolamine (10 equivalents) were added. This mixture is shaken for 15 minutes at room temperature. This procedure is repeated once more to be sure of the full conversion of the thiolactone. After this second procedure, the resin is washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Chain extension: general procedure

Dried $CHCl_3$ is added to the solid support (1 mL reaction mixture per 100 mg resin), then the α-isocyanato-γ-thiolactone (10 eq. relative to the resin-bound alcohol units) and dibutyltin dilaurate (0.025 eq. relative to the resin-bound alcohol units) are added. This mixture is shaken for 1 hour. Afterwards, the resin is washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Automated protocol: general procedure

The protocol was adapted from the conventional approach with some slight modifications. Stable stock solutions were prepared in $CHCl_3$ with either (i) the ethanolamine (7M), (ii) an acrylate (4M), (iii) the α-isocyanato-γ-thiolactone (2M) or (iv) the dibutyltin dilaurate (0.15M). 50 mg of resin was weighed in each reactor.

The resin was swollen for 5 minutes in $CHCl_3$ and afterwards the solvent was removed. 480 µL of the acrylate/$CHCl_3$ solution and 180 µL of the ethanolamine/$CHCl_3$ solution were added in

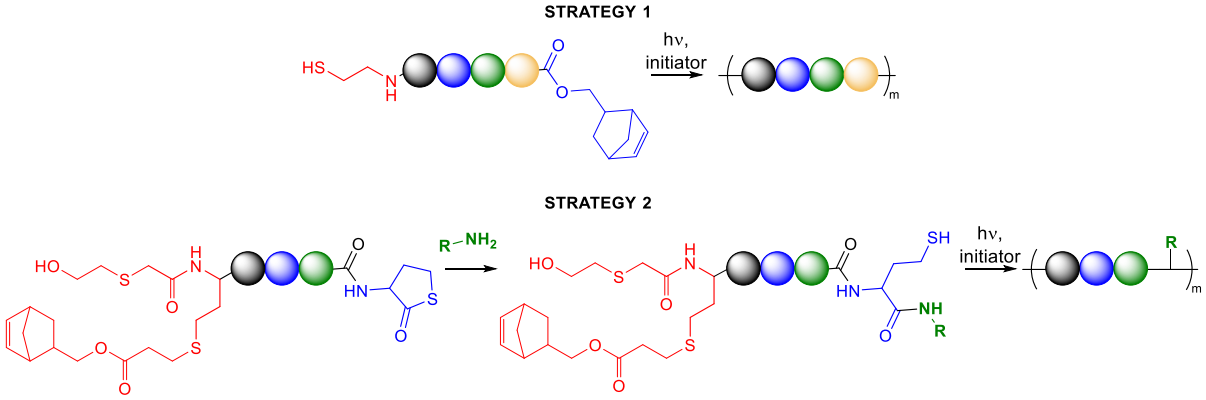
this order and shaken for 15 minutes. This step was repeated for a second time, as in the manual approach. Then the resin was washed with DMF (2x), MeOH (2x) and $CHCl_3$ (2x).
Subsequently, the solid support is suspended for 5 minutes in $CHCl_3$ and afterwards the solvent is removed. 480 µL of the α-isocyanato-γ-thiolactone/$CHCl_3$ solution and 120 µL of the dibutyltin dilaurate/$CHCl_3$ solution were added in this order and shaken for 2 hours. During this reaction a white precipitate is formed. Next, 1 mL of DMF is added and shaken for 1 minute. This allows for the white precipitate that is formed to dissolve and to prevent the tubings of the robot from clogging. The resin was then washed with DMF (2x), MeOH (2x) and $CHCl_3$ (2x). After completion of the automated procedure, the resin was washed manually with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x), and dried.

Storing of the samples
Samples, oligomers on the solid support, are stored in the fridge if not needed for reactions or cleavage.

References

[1] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[2] H. Colquhoun, J.-F. Lutz, *Nat. Chem.* **2014**, *6*, 455.
[3] N. Goldman, P. Bertone, S. Chen, C. Dessimoz, E. M. LeProust, B. Sipos, E. Birney, *Nature* **2013**, *494*, 77.
[4] V. Zhirnov, R. M. Zadegan, G. S. Sandhu, G. M. Church, W. L. Hughes, *Nat Mater* **2016**, *15*, 366.
[5] J. Bornholt, R. Lopez, D. M. Carmean, L. Ceze, G. Seelig, K. Strauss, *SIGPLAN Not.* **2016**, *51*, 637.
[6] Y.-J. Chen, N. Dalchau, N. Srinivas, A. Phillips, L. Cardelli, D. Soloveichik, G. Seelig, *Nat Nano* **2013**, *8*, 755.
[7] C. T. Clelland, V. Risca, C. Bancroft, *Nature* **1999**, *399*, 533.
[8] A. Leier, C. Richter, W. Banzhaf, H. Rauhe, *Biosystems* **2000**, *57*, 13.
[9] H. J. Shiu, K. L. Ng, J. F. Fang, R. C. T. Lee, C. H. Huang, *Information Sciences* **2010**, *180*, 2196.
[10] J. Shendure, H. Ji, *Nat Biotech* **2008**, *26*, 1135.
[11] G. M. Church, Y. Gao, S. Kosuri, *Science* **2012**, *337*, 1628.
[12] M. Peplow, *ACS Central Science* **2016**, *2*, 874.
[13] J. F. Lutz, M. Ouchi, D. R. Liu, M. Sawamoto, *Science* **2013**, *341*, 628.
[14] J.-F. Lutz, J.-M. Lehn, E. W. Meijer, K. Matyjaszewski, *Nature Reviews Materials* **2016**, *1*, 16024.
[15] R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* **1992**, *114*, 10646.
[16] M. Porel, C. A. Alabi, *J. Am. Chem. Soc.* **2014**, *136*, 13162.
[17] S. C. Solleder, D. Zengel, K. S. Wetzel, M. A. R. Meier, *Angew. Chem., Int. Ed.* **2016**, *55*, 1204.
[18] G. De Bo, S. Kuschel, D. A. Leigh, B. Lewandowski, M. Papmeyer, J. W. Ward, *J. Am. Chem. Soc.* **2014**, *136*, 5811.
[19] S. Ida, M. Ouchi, M. Sawamoto, *Macromol. Rapid Commun.* **2011**, *32*, 209.
[20] F. Driessen, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2015**, *4*, 616.
[21] G. Gody, T. Maschmeyer, P. B. Zetterlund, S. Perrier, *Nat. Commun.* **2013**, *4*, 3505.
[22] S. C. Solleder, R. V. Schneider, K. S. Wetzel, A. C. Boukis, M. A. R. Meier, *Macromol. Rapid Commun.* **2017**, *38*, 1600711.
[23] U. S. Gunay, B. E. Petit, D. Karamessini, A. Al Ouahabi, J. A. Amalian, C. Chendo, M. Bouquey, D. Gigmes, L. Charles, J. F. Lutz, *Chem* **2016**, *1*, 114.
[24] D. Karamessini, S. Poyer, L. Charles, J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, 1700426.
[25] J.-A. Amalian, T. T. Trinh, J.-F. Lutz, L. Charles, *Analytical Chemistry* **2016**, *88*, 3715.

[26] S. Pfeifer, Z. Zarafshani, N. Badi, J.-F. Lutz, *J. Am. Chem. Soc.* **2009**, *131*, 9195.
[27] A. Al Ouahabi, L. Charles, J. F. Lutz, *J. Am. Chem. Soc.* **2015**, *137*, 5629.
[28] L. Oswald, A. Al Ouahabi, L. Charles, J.-F. Lutz, *Chemistry – A European Journal* **2016**, *22*, 3462.
[29] C. Laure, D. Karamessini, O. Milenkovic, L. Charles, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2016**, *55*, 10722.
[30] R. K. Roy, A. Meszynska, C. Laure, L. Charles, C. Verchin, J.-F. Lutz, *Nat. Commun.* **2015**, *6*, 7237.
[31] G. Cavallo, A. Al Ouahabi, L. Oswald, L. Charles, J.-F. Lutz, *J. Am. Chem. Soc.* **2016**, *138*, 9417.
[32] G. Cavallo, S. Poyer, J. A. Amalian, F. Dufour, A. Burel, C. Carapito, L. Charles, J. F. Lutz, *Angew. Chem., Int. Ed.* **2018**, *57*, 6266.
[33] J. C. Barnes, D. J. C. Ehrlich, A. X. Gao, F. A. Leibfarth, Y. Jiang, E. Zhou, T. F. Jamison, J. A. Johnson, *Nat. Chem.* **2015**, *7*, 810.
[34] J. Vandenbergh, G. Reekmans, P. Adriaensens, T. Junkers, *Chem. Sci.* **2015**, *6*, 5753.
[35] J. W. Grate, K.-F. Mo, M. D. Daily, *Angew. Chem., Int. Ed.* **2016**, *55*, 3925.
[36] L. Charles, G. Cavallo, V. Monnier, L. Oswald, R. Szweda, J.-F. Lutz, *Journal of The American Society for Mass Spectrometry* **2016**, 1.
[37] N. Zydziak, W. Konrad, F. Feist, S. Afonin, S. Weidner, C. Barner-Kowollik, *Nat. Commun.* **2016**, *7*, 13672.
[38] S. Martens, J. O. Holloway, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, 1700469.
[39] N. Zydziak, F. Feist, B. Huber, J. O. Mueller, C. Barner-Kowollik, *Chem. Commun.* **2015**, *51*, 1799.
[40] M. Porel, D. N. Thornlow, N. N. Phan, C. A. Alabi, *Nat. Chem.* **2016**, *8*, 590.
[41] J.-F. Lutz, *Macromolecules* **2015**, *48*, 4759.
[42] A. C. Boukis, M. A. R. Meier, *Eur. Polym. J.* **2018**, *104*, 32.
[43] M. R. Hoopmann, R. L. Moritz, *Current Opinion in Biotechnology* **2013**, *24*, 31.
[44] A. Burel, C. Carapito, J.-F. Lutz, L. Charles, *Macromolecules* **2017**, *50*, 8290.
[45] A. C. Boukis, K. Reiter, M. Frölich, D. Hofheinz, M. A. R. Meier, *Nat. Commun.* **2018**, *9*, 1439.
[46] M. L. Biniossek, O. Schilling, *PROTEOMICS* **2012**, *12*, 1303.
[47] A. Richel, C. Vanderghem, M. Simon, B. Wathelet, M. Paquot, *Analytical Chemistry Insights* **2012**, *7*, 79.
[48] J.-A. Amalian, S. Poyer, B. E. Petit, S. Telitel, V. Monnier, D. Karamessini, D. Gigmes, J.-F. Lutz, L. Charles, *International Journal of Mass Spectrometry* **2017**, *421*, 271.
[49] E. W. Dijkstra, *Numer. Math.* **1959**, *1*, 269.
[50] A. Al Ouahabi, J.-A. Amalian, L. Charles, J.-F. Lutz, *Nat. Commun.* **2017**, *8*, 967.
[51] Wikipedia, "August Kekulé", 2018, p. 2018/.
[52] M. Porel, D. N. Thornlow, C. M. Artim, C. A. Alabi, *ACS Chemical Biology* **2017**, *12*, 715.
[53] D. Karamessini, T. Simon-Yarza, S. Poyer, E. Konishcheva, L. Charles, D. Letourneur, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2018**, *57*, 10574.
[54] J. De Neve, J. J. Haven, L. Maes, T. Junkers, *Polym. Chem.* **2018**, *9*, 4692.
[55] J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, *38*, 1700582.
[56] D. Branton, D. W. Deamer, A. Marziali, H. Bayley, S. A. Benner, T. Butler, M. Di Ventra, S. Garaj, A. Hibbs, X. Huang, S. B. Jovanovich, P. S. Krstic, S. Lindsay, X. S. Ling, C. H. Mastrangelo, A. Meller, J. S. Oliver, Y. V. Pershin, J. M. Ramsey, R. Riehn, G. V. Soni, V. Tabard-Cossa, M. Wanunu, M. Wiggin, J. A. Schloss, *Nature Biotechnology* **2008**, *26*, 1146.
[57] M. Boukhet, N. F. König, A. A. Ouahabi, G. Baaken, J.-F. Lutz, J. C. Behrends, *Macromol. Rapid Commun.* **2017**, *38*, 1700680.
[58] N. F. König, A. Al Ouahabi, S. Poyer, L. Charles, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2017**, *56*, 7297.
[59] L. Tsiatsiani, A. J. R. Heck, *The FEBS Journal* **2015**, *282*, 2612.
[60] A. B. Kanu, P. Dwivedi, M. Tam, L. Matz, H. H. Hill, *Journal of Mass Spectrometry* **2008**, *43*, 1.
[61] G. I. Peterson, M. B. Larsen, A. J. Boydston, *Macromolecules* **2012**, *45*, 7317.
[62] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[63] W. Van Camp, F. E. Du Prez, S. A. F. Bon, *Macromolecules* **2004**, *37*, 6673.
[64] C. Richter, K. Schaepe, F. Glorius, B. J. Ravoo, *Chem. Commun.* **2014**, *50*, 3204.
[65] B. B. Shankar, D. Y. Yang, S. Girton, A. K. Ganguly, *Tetrahedron Lett.* **1998**, *39*, 2447.



Abstract

This chapter describes how the polymerisation of synthetic sequence-defined oligomers was tackled with two different strategies. In the first case, oligomers were prepared bearing both a norbornene and a thiol end group to obtain AB-oligomers, which could be polymerised via the radical thiol-ene reaction. The polymers obtained were analysed by means of size exclusion chromatography (SEC), diffusion ordered NMR-spectroscopy (DOSY-NMR) and differential scanning calorimetry (DSC) measurements in order to prove the success of the polymerisation and learn more about their properties. Graft polymers could equally be prepared with this strategy through installation of the norbornene functionality on the oligomer as a side-chain. The second polymerisation strategy is based on the amine-thiolactone-ene conjugation of an AB'-oligomer that contains a norbornene and a thiolactone group. Aminolysis of the latter allows for the incorporation of a functionality and generates a thiol that can react with the norbornene unit via radical thiol-ene chemistry to prepare sequence-controlled polymers. The aminolysis of this AB'-oligomer was also performed on a solid support to prepare an oligomer with a norbornene functionality and thiol end group, and to compare the amine-thiolactone-ene polymerisation to the radical thiol-ene reaction.

Chapter VIII
From sequence-defined oligomers to sequence-controlled polymers

VIII.1 Introduction

In the previous two chapters, it was proven that sequence-defined oligomers could easily be built with the automated two-step iterative protocol developed and can be applied for data storage. In chapter V, attempts were made to prepare periodic sequence-controlled polymers starting from oligopeptides with ene and thiolactone functionalities, yet this research required further study. This chapter will describe how the synthetic oligomers obtained are polymerised via different approaches and functionalities in order to obtain periodic polymers.

Nature has the extraordinary ability of defining the primary structure of biomacromolecules. DNA, RNA and proteins can fold into a specific form and fulfil different extraordinary functions thanks to their defined structure. In the last decades, synthetic strategies have been explored and examined to obtain highly functionalised macromolecular architectures which have strong structure-property relationships and tailor-made morphologies, yet there is still a gap between these synthetic structures and their biological counterparts. Inspired by nature and guided by the scientific progress made, chemists have significantly increased their attempts in the last decade to control the primary structure of polymers to an even greater extent.[1-3]

As explained in detail in Chapter II, step-growth polymerisations have been used to prepare sequence-controlled periodic polymers starting from a sequence-defined oligomer or sequence-controlled polymer with specific end groups which can be linked together.[4] In order to synthesise these periodic structures, various chemistries have proven to be useful, such as polycondensations[5, 6], ATRA (atom transfer radical addition) and ATRC (atom transfer radical coupling)[7, 8], RACP (radical addition-coupling polymerisation)[9], ROMP (ring-opening metathesis polymerization) of sequence-defined macrocyclic structures[10, 11], CuAAC (copper catalyzed Huisgen-type 1,3-dipolar cycloaddition reaction)[12-15], and radical[16-18] and nucleophilic thiol-ene.[19] Although these polymers do not have defined, uniform structures, they exhibit interesting structure-property relationships. Guan and co-workers prepared three different pentapeptides bearing alkyne and azido end groups and polymerised them via CuAAC.[12] The three oligopeptides had the same amino acids in their primary structure, but a different order or stereochemistry. Analysis of the polymers obtained indicated that the sequence order and the chiral centres had an influence on the lower critical solution temperature (LCST) and local secondary structures. Meyer's research group performed polycondensations of sequence-defined oligomers which are based on glycolic, lactic and caprolactic units and

bear an acid and an alcohol end group.[5] The hydrolysis rates of the polymers obtained were compared to their random analogues.[6] A slower and more constant degradation was observed for the sequence-controlled polymers, which makes them interesting for gradual drug delivery. This degradation behaviour can be explained by the fact that a random copolymer can feature glycolic-rich and lactic-rich zones. As glycolic acid is less sterically hindered than lactic acid, the glycolic-rich zones will degrade faster than the lactic-rich zones. The sequence-controlled polymers are characterised by a defined balance between the glycolic and lactic units, resulting in a constant degradation. The properties of polymers can thus be tuned by varying the order and the chiral centres of the oligomers applied.

As discussed in II.4.2.2, our research group used the amine-thiolactone-ene conjugation to perform the step-growth polymerisation of well-defined heterotelechelic polyisobornylacrylate precursors - with α-thiolactone and ω-acrylate end groups - for the synthesis of sequence-controlled polymers (SCPs).[19] First, polyisobornylacrylate was prepared via Cu(0)-mediated polymerisation with a thiolactone-functionalised initiator. Next, the bromide end group was reacted with 2-mercaptoethanol to incorporate an alcohol as an end group, which was afterwards converted to an acrylate with acryloyl chloride. Aminolysis of the thiolactone allowed for the incorporation of a functionality and generated a thiol that could react with the acrylate. The functionalities are at a distance from one another which is precisely controlled through the length of the precursor polymer. This approach was used to produce amphiphilic graft copolymers and glycosylated macromolecular line-ups.

In this chapter, the preparation of periodic SCPs was explored by means of two different strategies based on synthetic oligomers bearing a norbornene and either a thiol or a thiolactone functionality (Figure VIII.1). A norbornene-functionalised acrylate was prepared to easily install this group on the oligomers.

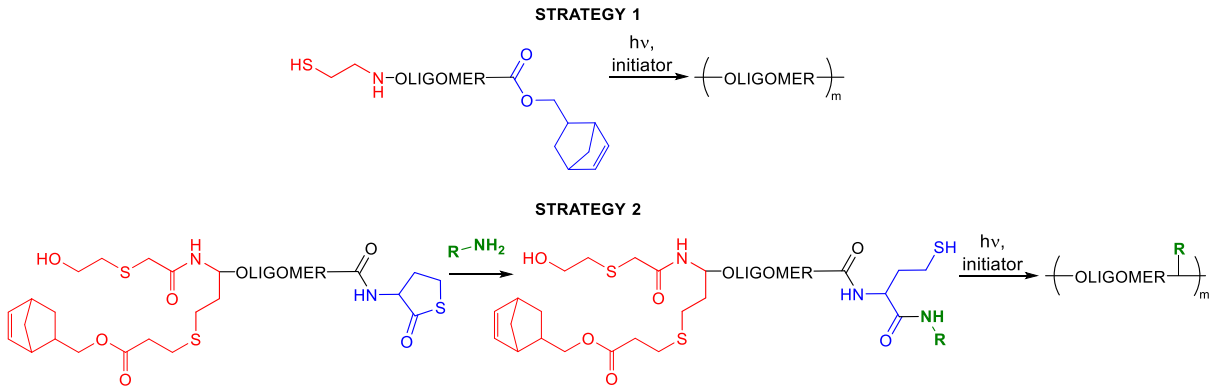


Figure VIII.1: Polymerisation of synthetic oligomers with two different strategies based on the radical thiol-ene reaction between a thiol and a norbornene group (top) or based on the amine-thiol-ene conjugation (bottom).

In the case of the first strategy, the two-step iterative protocol described in chapter VI[20] was performed on a resin which generates thiol-functionalised products after cleavage. The thiolactone-functionalised oligomers obtained were double-modified with the amine-thiol-ene conjugation on the solid support to install the norbornene functionality on the other side of the

oligomer. The oligomers were subsequently polymerised via the radical thiol-ene reaction. Graft polymers with defined branches could also be obtained with this strategy, by positioning the norbornene not at the end of the oligomer, but as one of the side chains.

Next, a second strategy was explored for the radical amine-thiolactone-ene polymerisation of an AB'-oligomer that holds a norbornene and thiolactone group. Based on the iterative protocol from chapter VI, oligomers were prepared in which the norbornene group was installed on the first side-chain and thiolactone as an end group. The latter was also opened on the resin to prepare an oligomer with a thiol end group and a norbornene unit, which allows for the comparison between the radical thiol-ene polymerisation of an AB-oligomer and the radical amine-thiolactone-ene polymerisation of an AB'-oligomer.

VIII.2 Polymerisation of oligomers based on thiolactone chemistry

VIII.2.1 Oligomer synthesis and thiol-ene polymerisation

In chapter V, oligopeptides were prepared with a thiolactone and ene end group to prepare functionalised periodic polymers via aminolysis and subsequent thiol-ene reaction. As only oligomers were observed and this approach resulted in disulphide formation, a new strategy was developed based only on radical thiol-ene chemistry, which proved to be very effective for periodic polymer synthesis.[16-18] The first objective was to prepare oligomers with ene and thiol end groups.

For that purpose, a norbornene unit was installed on one side of the oligomers. Norbornene was chosen as it is one of the fastest ene groups in radical thiol-ene reactions (see Chapter II), because the ring strain drops considerably due to the addition of the thiyl radical and because the subsequent hydrogen abstraction is fast.[21] To easy install this functionality, a norbornene-functionalised acrylate was prepared starting from 5-norbornene-2-methanol which was reacted with acryloyl chloride (Figure VIII.2).[22] The product, with a yield of 89%, was purified with column chromatography and characterised with NMR and GC-MS (Figure VIII.3). Phenothiazine was added as an inhibitor to prevent this norbornene-functionalised acrylate from polymerising.

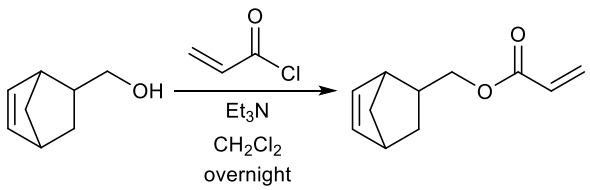


Figure VIII.2: Synthesis of bicyclo[2.2.1]hept-5-en-2-ylmethyl acrylate starting from 5-norbornene-2-methanol.

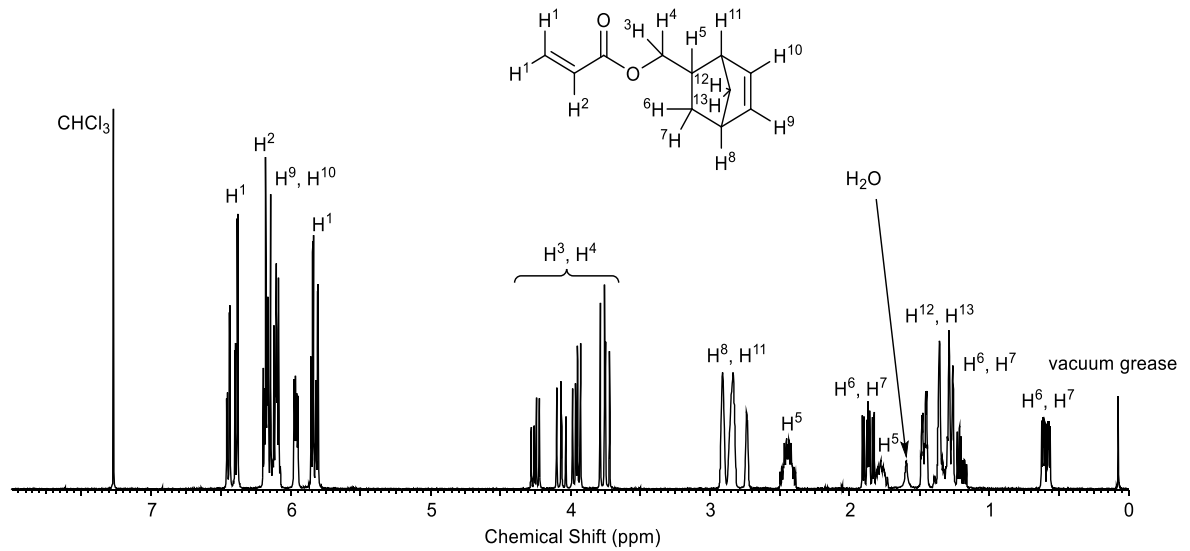


Figure VIII.3: ^{1}H-NMR spectrum (300 MHz, $CHCl_3$-d) with peak assignment (top) of purified bicyclo[2.2.1]hept-5-en-2-ylmethyl acrylate (black) and the inhibitor phenothiazine (blue).

To install a thiol on the other side of the targeted oligomer, cysteamine-4-methoxytrityl resin was used to perform its synthesis (Figure VIII.4). First, the acid-functionalised thiolactone introduced in chapter IV reacted with the amine functionality present on this resin, yielding an immobilised thiolactone from which the two-step iterative protocol introduced in chapter VI, and depicted on Figure VIII.4, can be performed.[20]

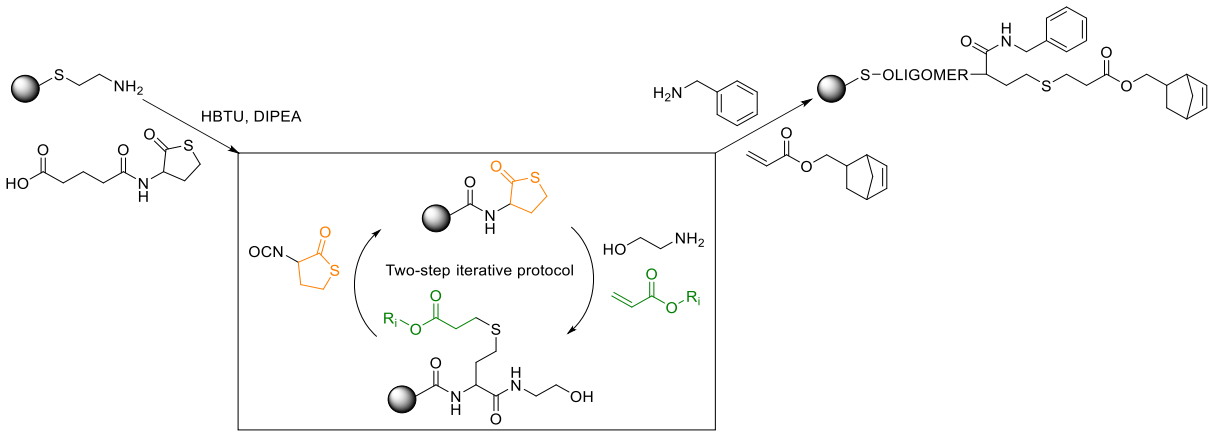


Figure VIII.4: Synthesis of oligomers with a thiol and a norbornene end group. A thiolactone unit is installed on cysteamine-4-methoxytrityl resin to be able to perform the thiolactone-based two-step iterative protocol on solid support. After the oligomer synthesis, the end-standing thiolactone is modified with benzylamine and the norbornene-functionalised acrylate.

Although the α-isocyanato-γ-thiolactone can also be exploited to transform the amine present on the solid support into a thiolactone functionality, deletions were observed during the oligomer synthesis (Figure VIII.5). These deletions were the motivation to use and explore the strategy in Figure VIII.4 based on the acid-functionalised thiolactone to immobilise the thiolactone.

For the first tests, a trimer was prepared with benzyl, methyl and tetrahydrofurfuryl acrylate, similarly to the first trimer in chapter VI (C3). After the oligomer synthesis, the amine-thiol-ene-based double modification of the thiolactone end group (cf. chapter IV) was performed

with 10 equivalents of benzylamine and 20 equivalents of the norbornene-functionalised acrylate in order to incorporate the ene functionality. Cleavage of the tetramer (oligomer 1 or O1) from the resin was performed with a 2% TFA in CH_2Cl_2 mixture and resulted in a sequence-defined macromolecule with a thiol and a norbornene end group, as proven by the LC-ESI-MS analysis (Figure VIII.6). In addition to benzylamine, other amines can be explored as well for the amine-thiolactone-ene conjugation. In this chapter, however, only benzylamine will be used to incorporate the norbornene functionality.

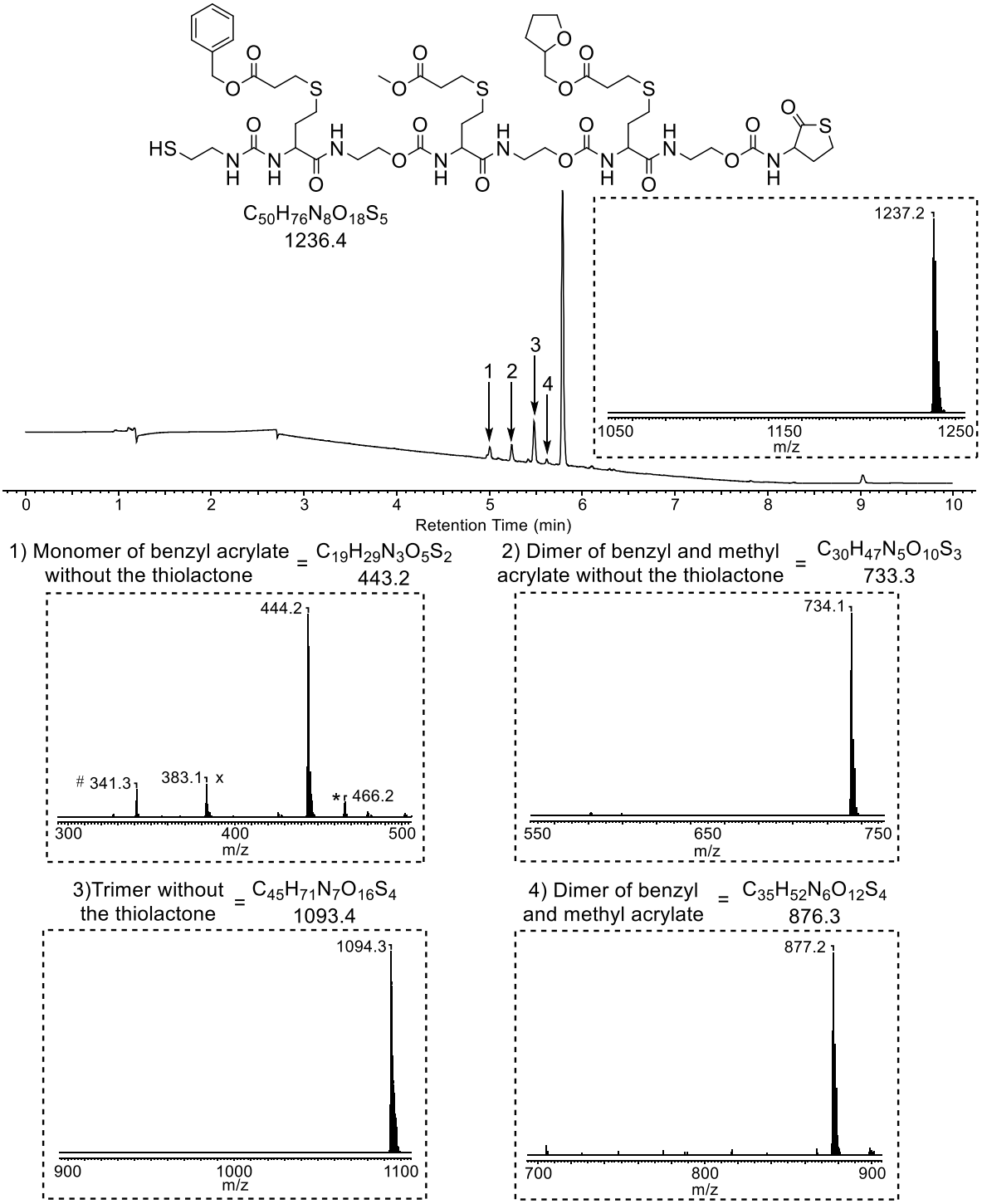


Figure VIII.5: LC-ESI-MS analysis of a trimer which was started from a thiolactone urea. Inserts: ESI-MS-spectra of dominant species and deletions (positive mode with sodium adduct (*), and ethanolamine (x) and urea fragmentations (#)).

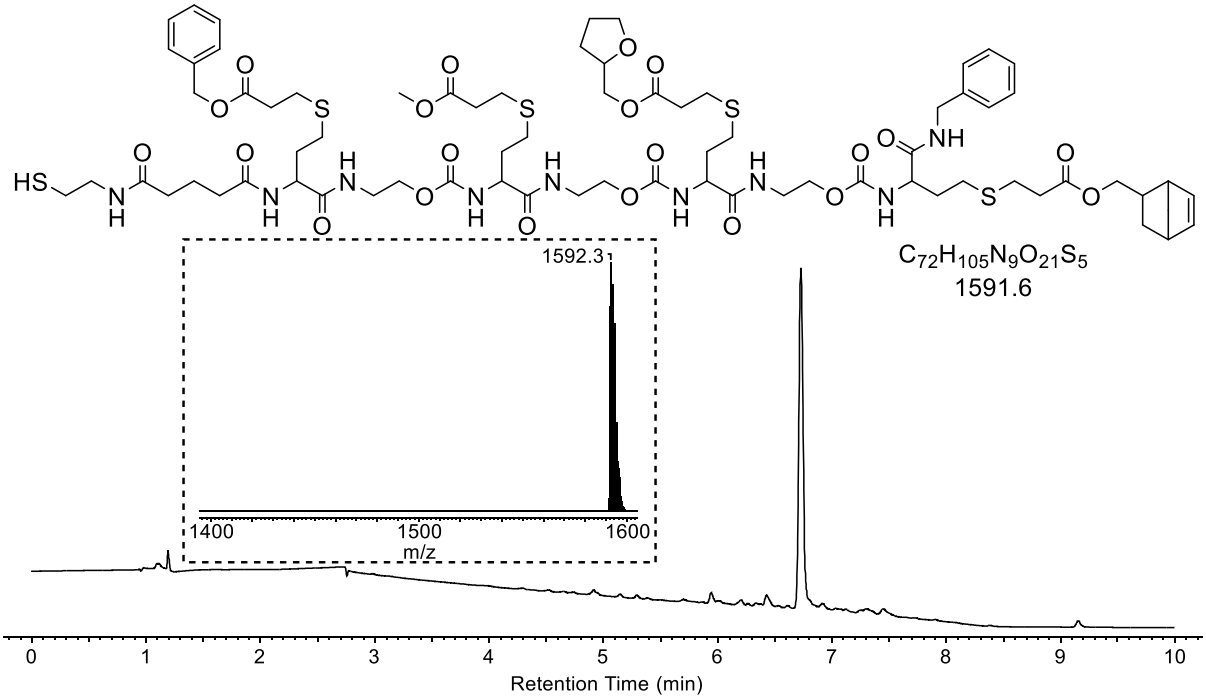


Figure VIII.6: LC-ESI-MS analysis of O1. Insert: ESI-MS-spectra of dominant species (positive mode).

O1 bearing a thiol and norbornene functionality was, afterwards, polymerised via the radical thiol-ene reaction for 2 hours in acetonitrile. This solvent was chosen because it was applied to dissolve the different oligomers for LC-MS analysis and because its use was already reported to perform radical thiol-ene polymerisations.[23] The concentration of the oligomer was 2M, since contemporary literature indicates that the monomer concentration should be as high as possible to achieve polymers with high molecular weights.[24] The polymerisation was initiated through addition of 0.03 equivalents of 2,2-dimethoxy-2-phenylacetophenone (DMPA). These equivalents are lower than the ones discussed in chapter V (0.05 equivalents), as the former amount proved to suffice based on norbornene-related research performed by our own group.[25]

First, dimethylacetamide-based SEC measurements were performed to determine the number average molecular weight (M_n) and dispersity (Đ) value of the polymer obtained in order to prove that the polymerisation took place (Figure VIII.7). The molecular weight increased and the dispersity broadened, which can be attributed to the polymerisation performed being a step-growth process. As discussed in section V.2.1, a macromolecule's retention time can be linked to its molecular weight. For example, a lower molecular weight compound has a higher retention time for a SEC measurement. A peak can still be observed at low molecular weight in the SEC analysis, which could be due to either unreacted monomers or to the formation of cyclised oligomers. At a higher molecular weight (lower retention time), another peak is visible, which could be the result of disulphide adducts. In an attempt to prove this hypothesis, the peak molecular weight (M_p) of the main peak and this peak at lower retention time were compared. Whereas M_n is a statistically average value dependent on all the polymer chains in a sample and calculated by integrating all peaks in the SEC trace, M_p is determined by the retention time of a peak's maximum. The hypothesis that the first peak is caused by disulphide formation is

contradicted by the fact that the M_p of the peak at lower retention time (> 10^6 g/mol) is much higher than the value obtained for the main peak (25100 g/mol). In principle, these peaks could be separated, with preparative SEC, to purify the polymer and to analyse all fractions. Mass spectrometry could also be performed to indicate the mass difference between two subsequent mass peaks and to see if disulphide formation had occurred. Unfortunately, these results were obtained at the very end of the PhD research and the lack of time did not permit these in-depth studies.

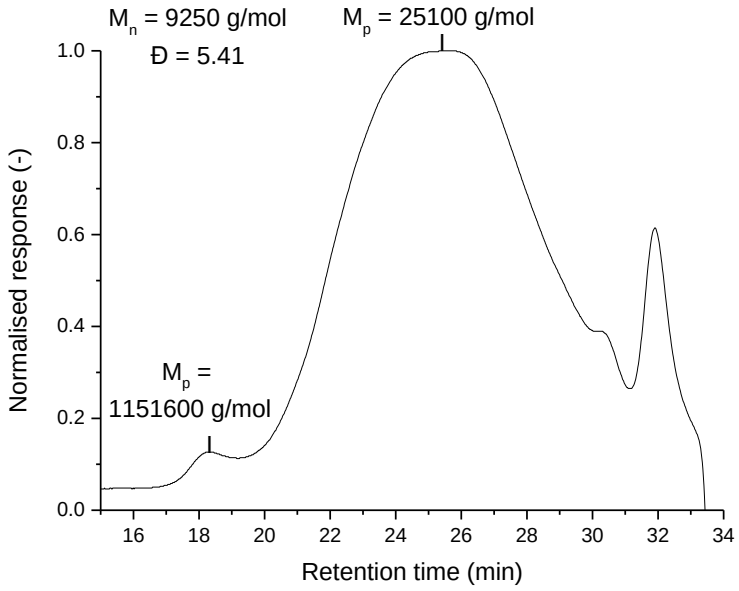


Figure VIII.7: SEC analysis of P1 in DMA with poly(methyl methacrylate) (PMMA) standards.

Next, the DOSY-NMR spectra of O1 and P1 (polymer of oligomer 1) were compared in order to further confirm that the oligomer was polymerised. In this NMR technique, the chemical shifts (x-axis) are separated according to the diffusion coefficients (y-axis) of the different species, which can be linked to their molar mass.[26] A macromolecule with high molecular weight diffuses more slowly than a compound with low molecular weight and will appear higher on the DOSY-NMR spectrum. This trend can also be observed for the O1 and the P1. The signals of the polymer (Figure VIII.8, blue) are positioned higher than those of the oligomer (Figure VIII.8, red). The oligomer also exhibits the signals from the double bond of the norbornene group between 6 and 6.5 ppm (Figure VIII.8, black box), while these signals cannot be observed for the polymer. At the bottom of the spectrum, the signal of DMSO can be found, as it has a higher diffusion coefficient in comparison to the heavier O1 and P1.

The signal of the solvent (DMSO) seems to be distorted, yet this could be explained by the fact that a mono-exponential fitting is applied during the processing of these measurements. This fitting takes into account that only one compound is present at a certain chemical shift. Due to the fact that there are also oligomer/polymer signals in very close proximity to the solvent signal, even overlapping to some extent, this fitting causes a distribution of DMSO signals which corresponds with a different diffusion coefficient each time, resulting in this distortion. A bi-exponential fitting which focusses on two compounds instead of one did not overcome

this issue. While a multi-exponential fitting is in principle required, this would demand a whole set of settings to be changed, which requires some more extensive research. A different solvent could perhaps help to be able to apply the mono-exponential fitting, providing that it does not overlap with any of the signals of the oligomer/polymer. Another solution could be the use of pure shift NMR, in which a decoupling is performed so perhaps signals will not overlap.[27]

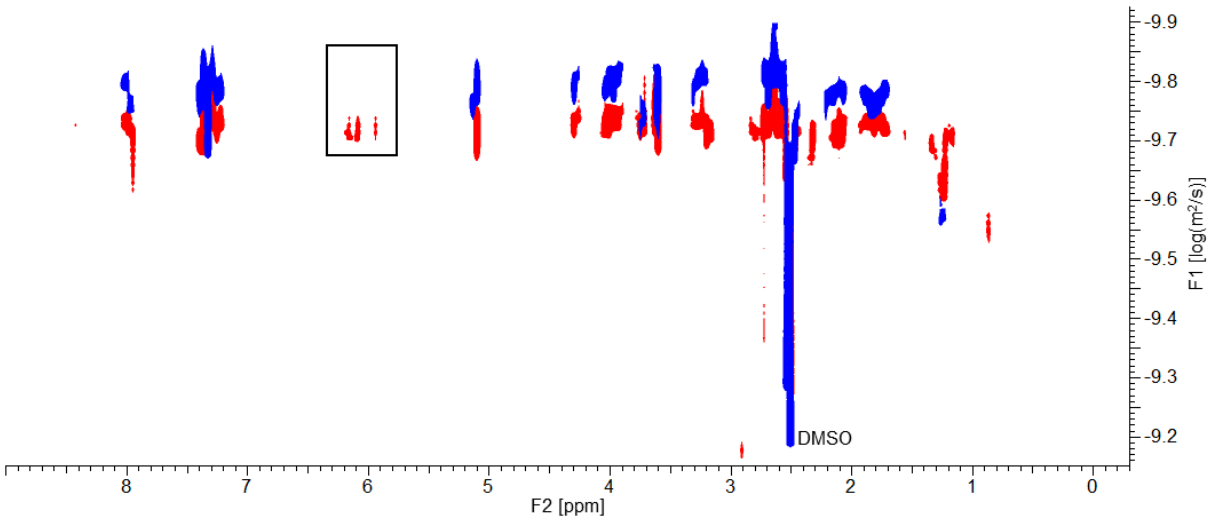


Figure VIII.8: Comparison of the DOSY-NMR (DMSO-d_6) spectra of O1 (red) and P1 (blue). The black box highlights the presence of the double bond of the norbornene functionality in the oligomer, but not in the polymer.

Finally, DSC measurements of O1 and its corresponding polymer were measured to learn more about their material properties. An increment can be observed from the tetramer's T_g (-20°C) to the polymer's T_g (-12°C) (Figure VIII.9).

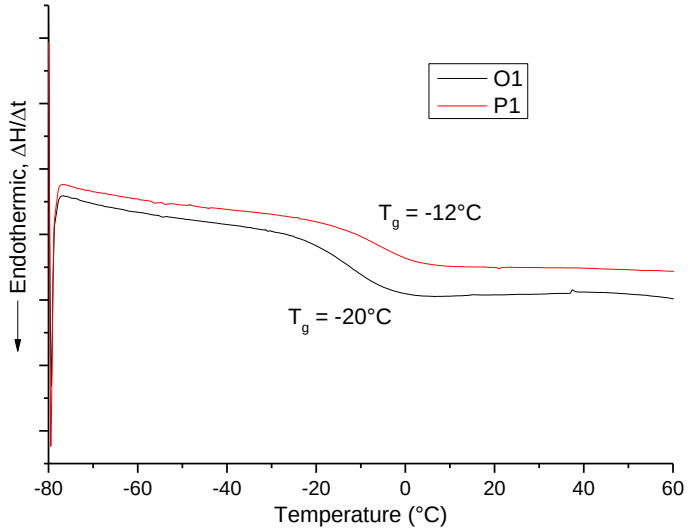


Figure VIII.9: DSC thermograms of the O1 and its corresponding polymer P1.

These three types of analysis prove that the oligomers prepared can indeed be polymerised through use of the developed radical thiol-ene strategy.

Afterwards, a hexamer (O2) bearing the thiol and norbornene end groups was built via the developed strategy (Figure VIII.4) and its purity was determined by LC-ESI-MS (Figure VIII.10). A pentamer like the original pentamer in chapter VI (C5) was prepared first on the

cysteamine-4-methoxytrityl resin and subsequently double-modified with benzylamine and the norbornene-functionalised acrylate.

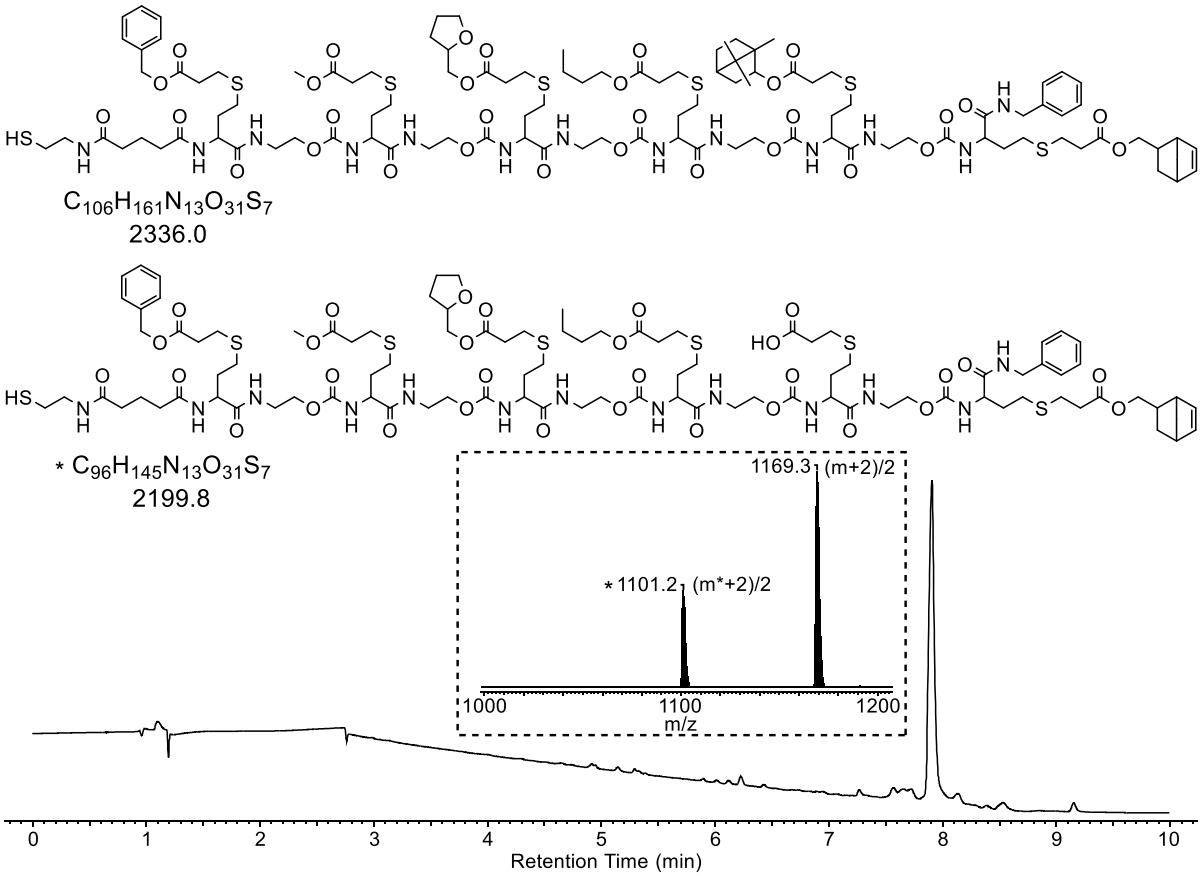


Figure VIII.10: LC-ESI-MS analysis of O2. Insert: ESI-MS-spectra of dominant species (positive mode).

O2 was polymerised with the same conditions as O1, and the polymer obtained was analysed using the same three techniques (DMA-SEC, DOSY-NMR and DSC). In comparison to the SEC analysis of the P1, the peak at low molecular weight can be observed once more, while the peak at a higher molecular weight is absent (Figure VIII.11). In order to clarify if this is due to the unreacted oligomer or to a cyclised adduct, further analysis should be carried out. The DOSY-NMR measurements also indicate that the diffusion is lower for the polymer than for the oligomer and that the signals of the double bond of norbornene are missing for the P2 (Figure VIII.12).

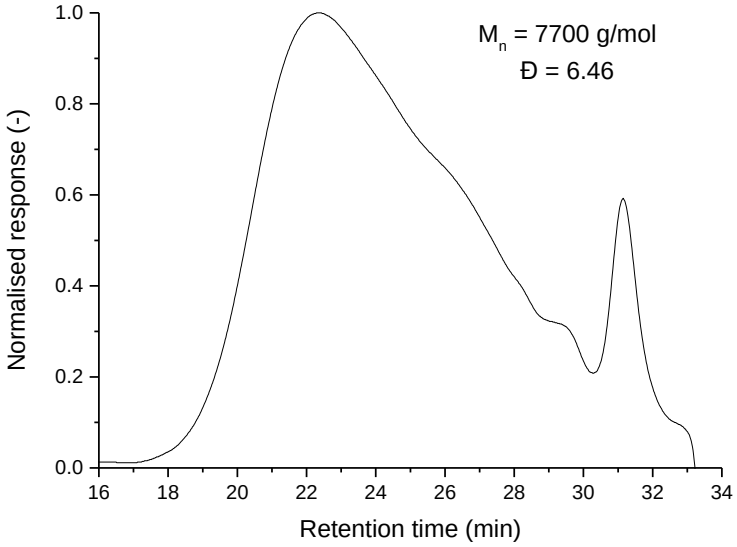


Figure VIII.11: SEC analysis of P2 in DMA with PMMA standards.

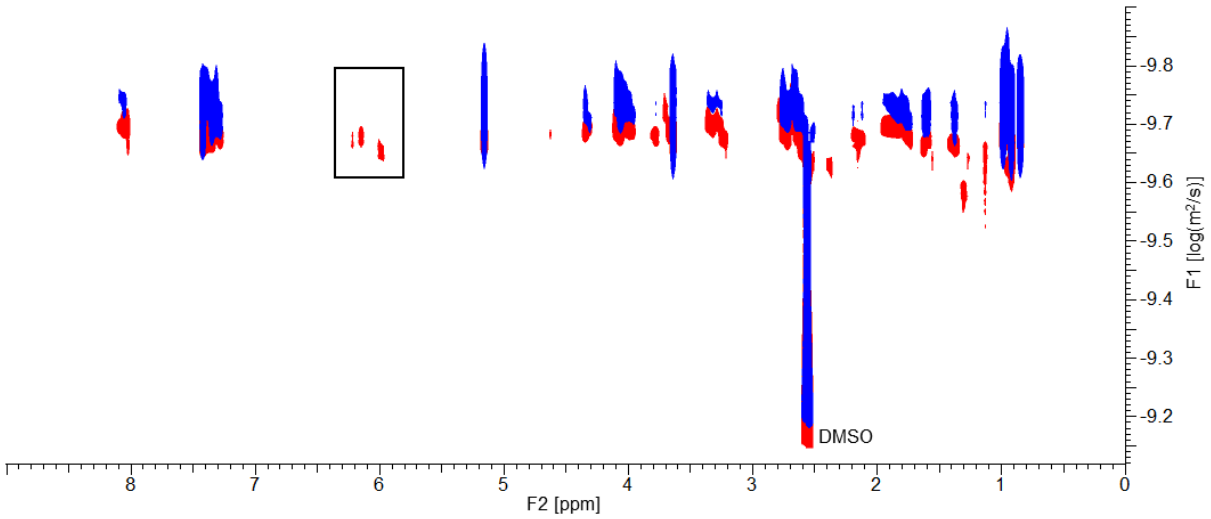


Figure VIII.12: Comparison of the DOSY-NMR (DMSO-d_6) spectra of O2 (red) and the P2 (blue). The black box highlights the presence of the double bond of the norbornene functionality in the oligomer, but not in the polymer.

Lastly, the DSC measurements do not display a strong T_g increment due to polymerisation (Figure VIII.13) as compared with O1 and P1. This could perhaps be explained by the fact that bulky (isobornyl) and flexible (butyl) units are installed in the side chains of the P2. When the size and the flexibility of the side chain increases, both the distance between polymer chains and the free volume increase as well. Although these increases should reduce the T_g, a bulky substituent impedes the rotation of the backbone, which results in a T_g increase. Another explanation for this small increment could be that the difference in (average) molecular weight between the oligomer and the corresponding polymer is not the same for both oligomers. For O1 the degree of polymerisation is roughly 6 (oligomer: 1591 g/mol and polymer: about 9250 g/mol), while for O2, this is roughly 3 (oligomer: 2336 g/mol and polymer: about 7700 g/mol). Mass analysis, such as MALDI-TOF on polymer fractions, could also be performed in order to prove the polymerisation and to investigate disulphide formation.

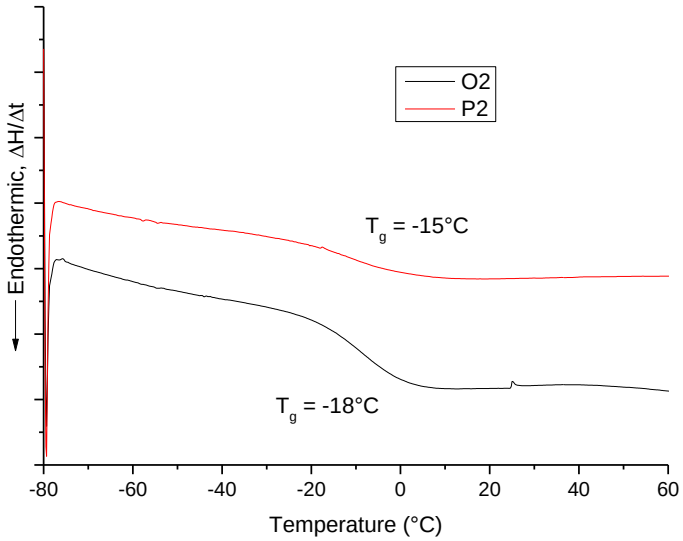


Figure VIII.13: DSC thermograms of the O2 and its corresponding polymer P2.

In the previous examples, linear polymers were prepared. In order to show the versatility in macromolecular structures that can be prepared with the thiol-ene polymerisation method developed, the two-step iterative protocol was applied on the cysteamine-4-methoxytrityl resin for the synthesis of two similar tetramers where the norbornene unit is not one of the end groups, but one of the side chains (Figures VIII.14-16). O3 has the norbornene unit on the first side chain, O4 on the second. The polymerisation of these tetramers generates graft polymers with defined branches.

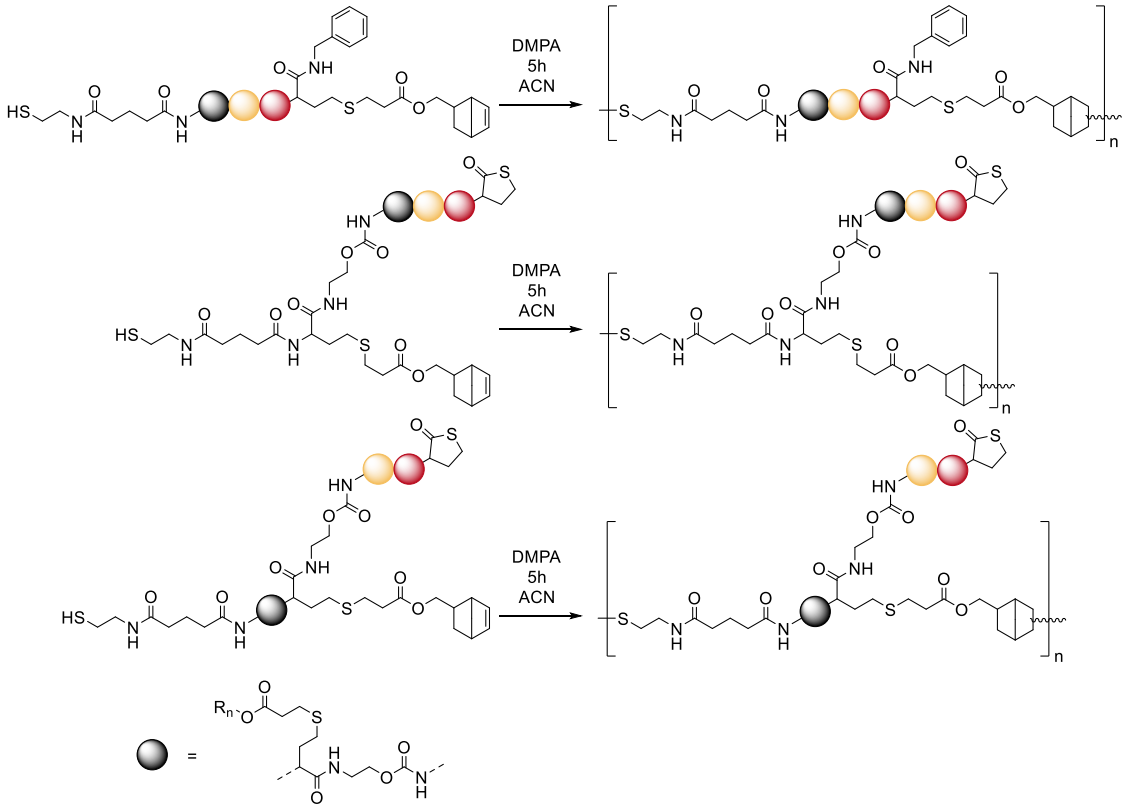


Figure VIII.14: Radical thiol-ene polymerisations of oligomers O1, O3 and O4, resulting in linear (P1) and graft polymers (P3 and P4).

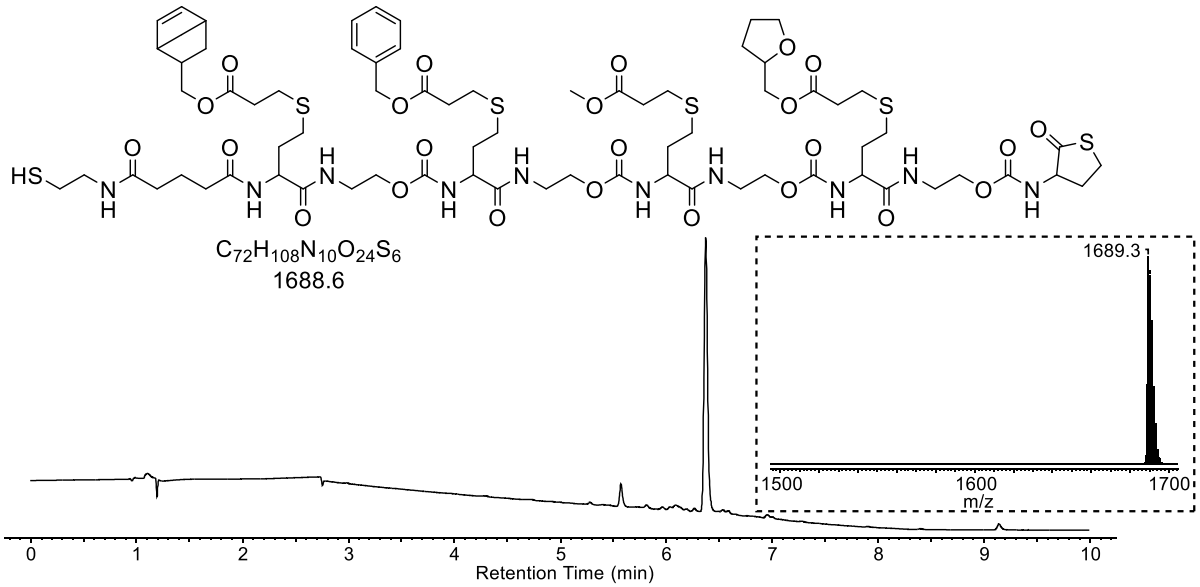


Figure VIII.15: LC-ESI-MS analysis of O3. Insert: ESI-MS-spectra of dominant species (positive mode).

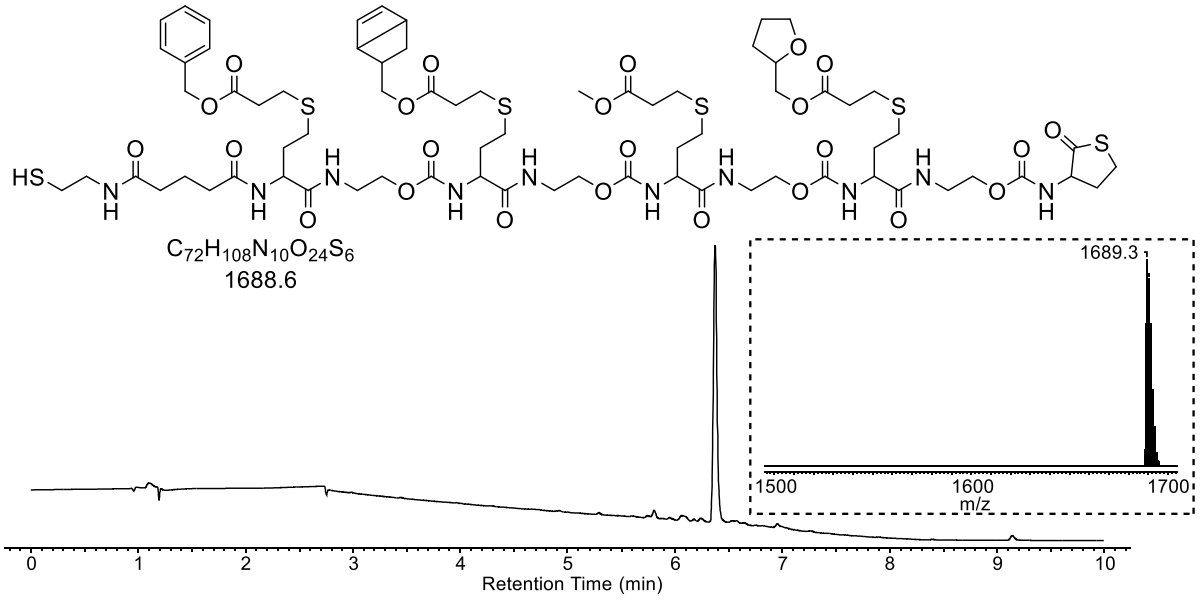


Figure VIII.16: LC-ESI-MS analysis of O4. Insert: ESI-MS-spectra of dominant species (positive mode).

These two tetramers were polymerised under the same conditions as described earlier for O1 and O2. DMA-based SEC analysis indicated that polymers were formed (Figure VIII.17). For P4, a higher average molecular weight and dispersity was observed. As with the other polymerisation, a peak at lower molecular weight can be observed once more. In comparison to O1, the norbornene functionality is closer to the thiol end group, which might stimulate cyclisation. Small peaks at higher molecular weights can be observed as well, which could be attributed to disulphide formation. This needs to be confirmed, however, with further analysis.

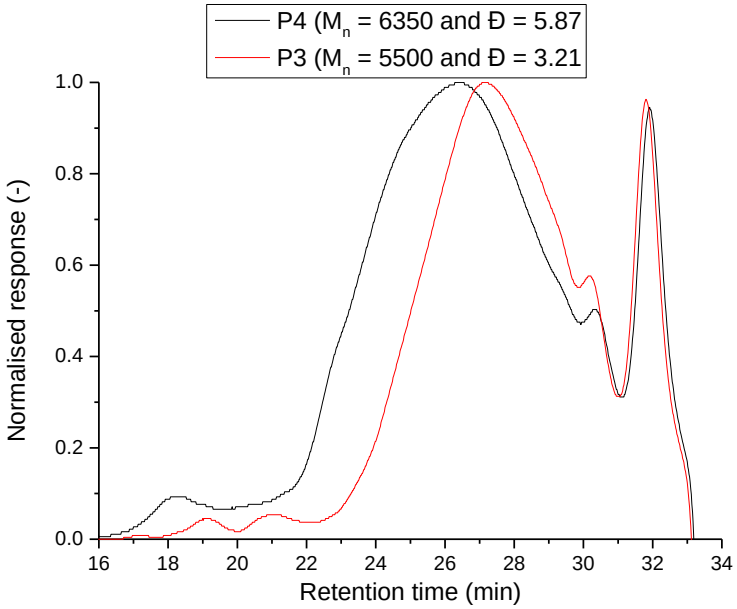


Figure VIII.17: SEC analysis of P3 (red) and P4 (black) in DMA with PMMA standards.

Due to time constraints, only SEC analysis has been performed. DOSY-NMR, DSC and mass analysis could provide extra evidence for the success of the polymerisation and disulphide formation. O3, O4 and their corresponding polymers contain unopened thiolactones in their structure, which could be interesting for further double modification or crosslinking with the amine-thiolactone-ene conjugation.

The thiol-norbornene method developed allows for the polymerisation of sequence-defined oligomers in order to synthesise periodic SCPs. In the future, in addition to polymerisation, this method could also be used to explore cyclisation. Research in the field of peptide chemistry has shown that cyclic species often have significantly different properties (solubility, aggregation, folding behaviour…) compared to their linear counterparts.[28] This strategy might perhaps also be useful for the cyclisation and polymerisation of oligopeptides and for preventing the problems that were faced in chapter V, such as disulphide and only small oligomer formation.

VIII.2.2 Oligomer synthesis and amine-thiolactone-ene polymerisation

One of the advantages of thiolactone chemistry is that various functionalised polymeric architectures can be prepared from one AB' monomer bearing an ene and thiolactone group by just changing the amine used during aminolysis.[29, 30] In chapter V, we introduced a strategy, which lead to the preparation of oligopeptides that contain an allyl functionality and a thiolactone end group. Although the polymerisation of these oligopeptides had some drawbacks, a similar approach is used here for the preparation of periodic polymers starting from synthetic oligomers bearing norbornene as an ene functionality and a thiolactone end group. These oligomers are polymerised via the amine-thiolactone-ene conjugation, following

a recent study from our group, which showed that a norbornene-functionalised thiolactone molecule could be polymerised with different amines via this technique.[25]

Through applying the two-step iterative protocol introduced in chapter VI (see Figure VIII.4) and using the hydroxyl-functionalised linker described in chapter IV, to introduce the first thiolactone unit on the 2-chlorotrityl chloride resin, a tetramer (O5) was prepared. This oligomer contains a thiolactone end group and a norbornene in the first side chain (Figure VIII.18).

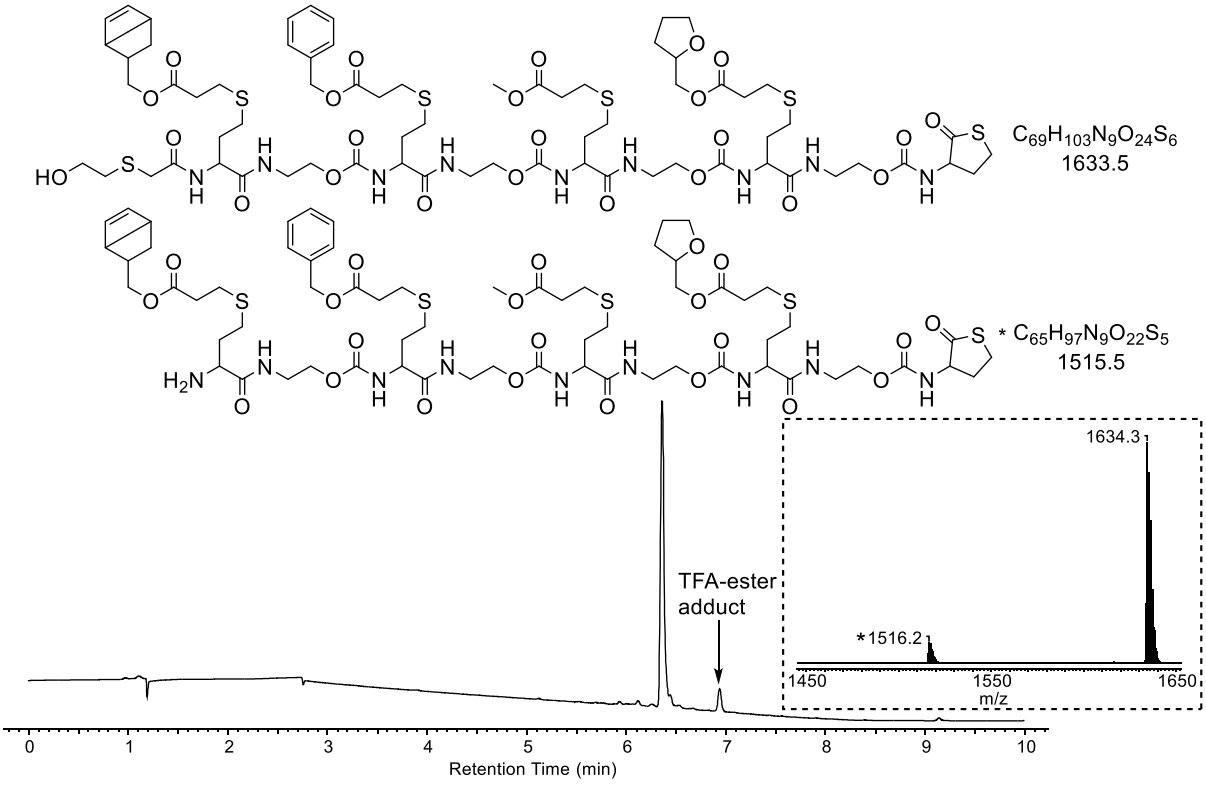


Figure VIII.18: LC-ESI-MS analysis of O5. Insert: ESI-MS-spectra of dominant species (positive mode).

Initially, the polymerisation was performed under the same conditions as the one used for the oligomers mentioned in the previous section (*i.e.* acetonitrile as a solvent, 2M oligomer concentration and 0.03 equivalents of DMPA). The only difference is the addition of 1.1 equivalents of octylamine for the aminolysis of the thiolactone (Figure VIII.19). This amine was chosen as it had proven to be fast for thiolactone aminolysis.[31]

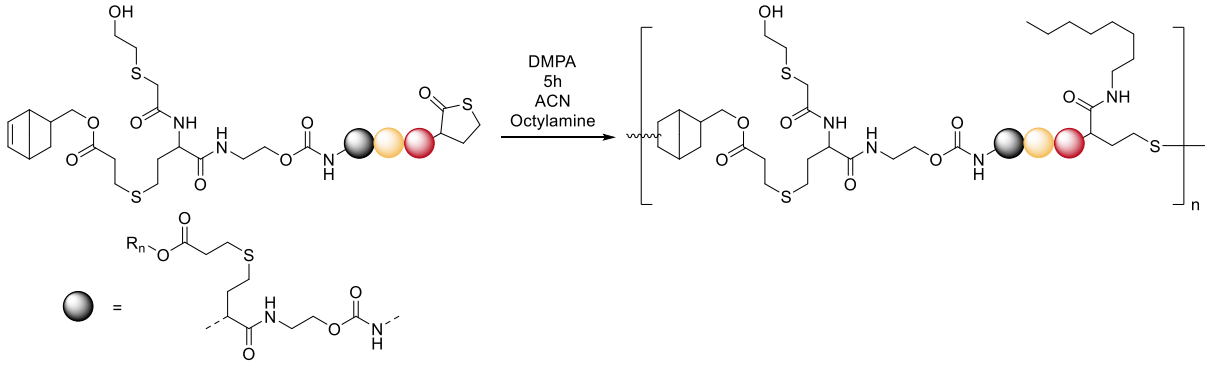


Figure VIII.19: Polymerisation of O5 via the radical amine-thiolactone-ene conjugation with octylamine.

As the polymerisation was not successful according to SEC analysis, different conditions were tested in order to optimise the polymerisation of O5, such as the equivalents of initiator (0.03 and 0.05), more equivalents of amine (between 1 and 1.5), the solvent (DMA and acetonitrile) and the concentration (between 1M and 2M), yet nothing seemed to improve the polymerisation. The best result is depicted Figure VIII.20 (black curve).

As the method developed in the previous section proved to be effective for the polymerisation of oligomers, the end-standing thiolactone was opened on the resin with benzylamine in order to obtain a thiol. Since previous research indicated that disulphides can be formed during aminolysis, the resin was treated afterwards with dimethylphenylphosphine to reduce the disulphides to thiols.[32, 33] The pentamer obtained (O6, Figure VIII.21) was cleaved off the resin and polymerised under the same conditions as the oligomers from the previous section (Figure VIII.20, blue curve). SEC analysis indicates that this polymerisation took place. A similar pentamer (O7, Figure VIII.22) was prepared on the solid support starting from the acid-functionalised linker described in chapter IV. This tetramer could also be polymerised under the established conditions (Figure VIII.20, red curve).

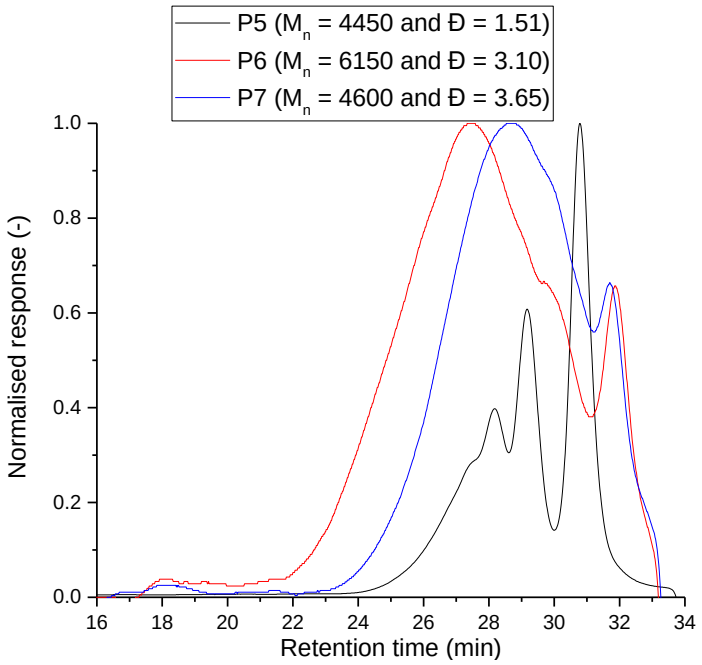


Figure VIII.20: SEC analysis of P5 (black), P6(red) and P7 (blue) in DMA with PMMA standards.

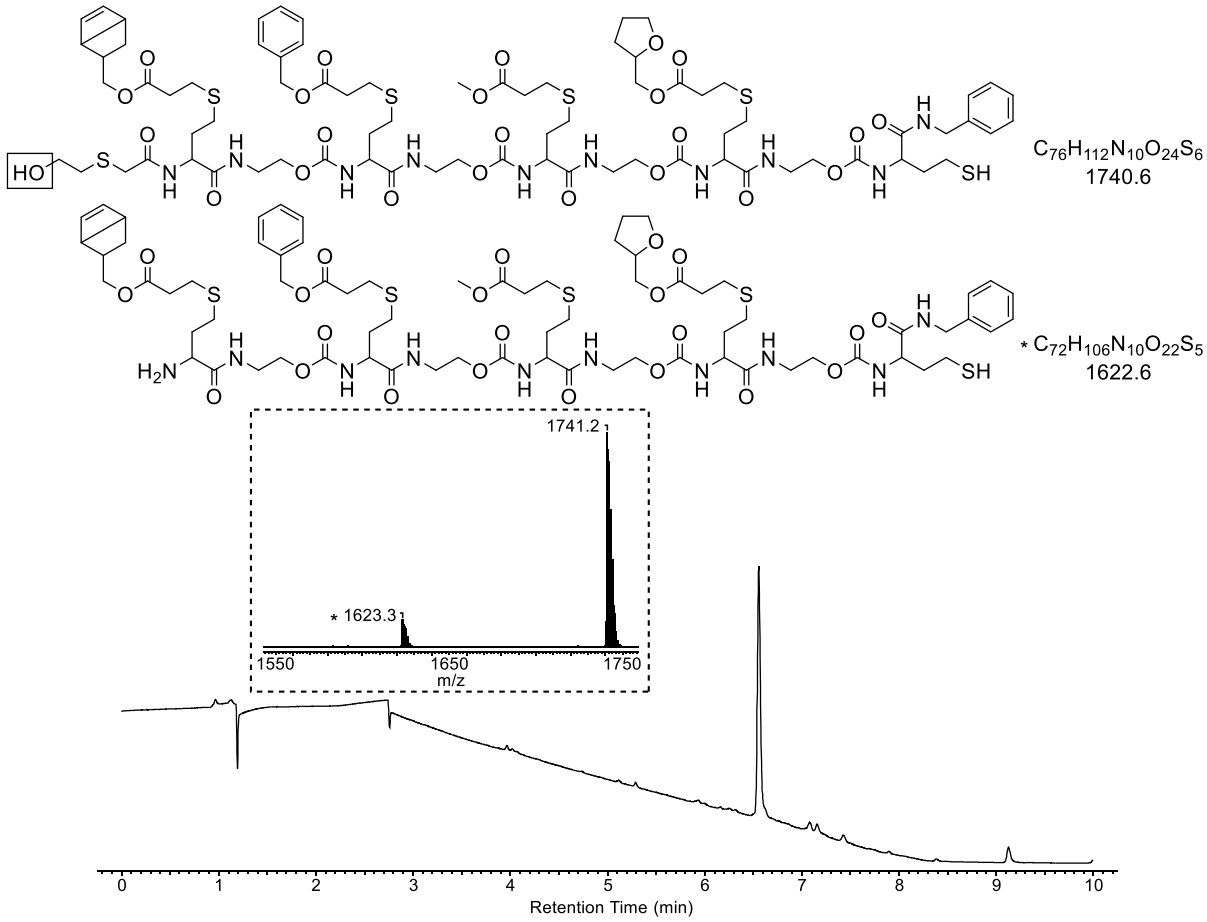


Figure VIII.21: LC-ESI-MS analysis of O6. Insert: ESI-MS-spectra of dominant species (positive mode).

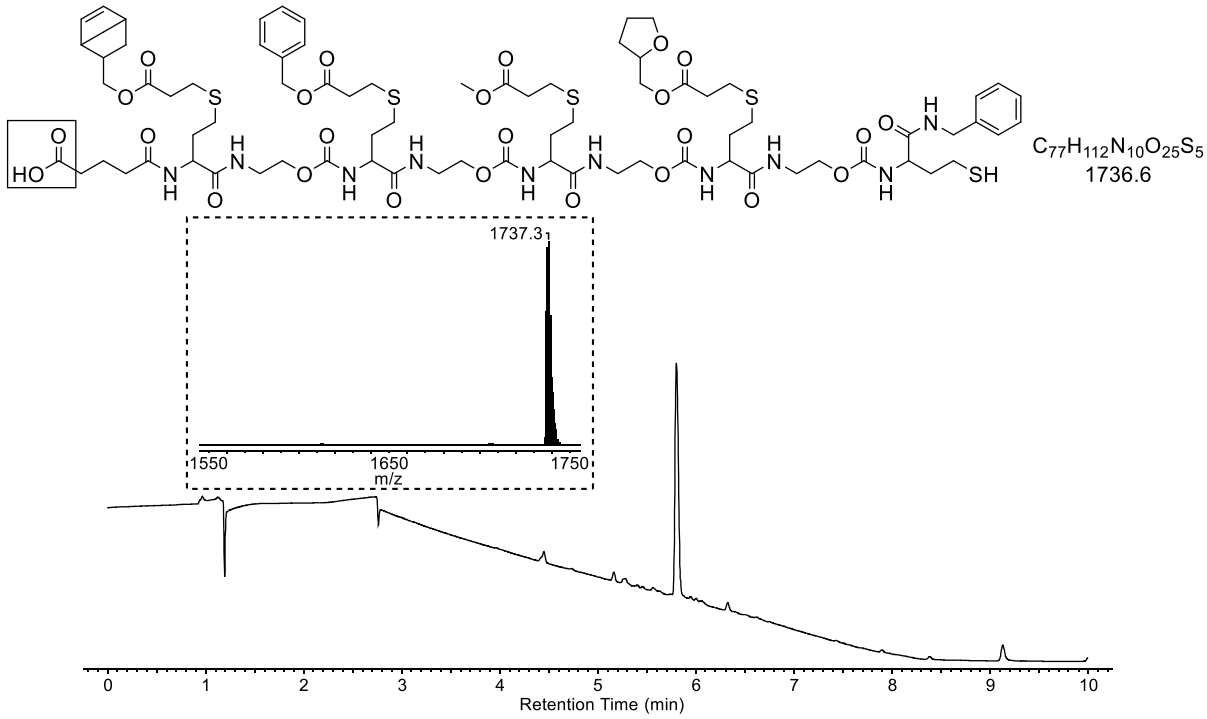


Figure VIII.22: LC-ESI-MS analysis of O7. Insert: ESI-MS-spectra of dominant species (positive mode).

While only SEC analysis has been performed so far, DOSY-NMR, DSC and mass analysis could provide extra evidence for the success of the polymerisation and could indicate whether

disulphide formation has occurred. Optimisation of the amine-thiolactone-ene polymerisation would require further investigation.

This method also holds some potential for the future. The structures of O6, O7 and their polymers respectively contain an alcohol and an acid which allow for further modification (Figure VIII.23, top). The norbornene functionality was now situated on the first side-chain, but could easily be installed in a different position in order to obtain graft polymers with defined branches (Figure VIII.23, bottom).

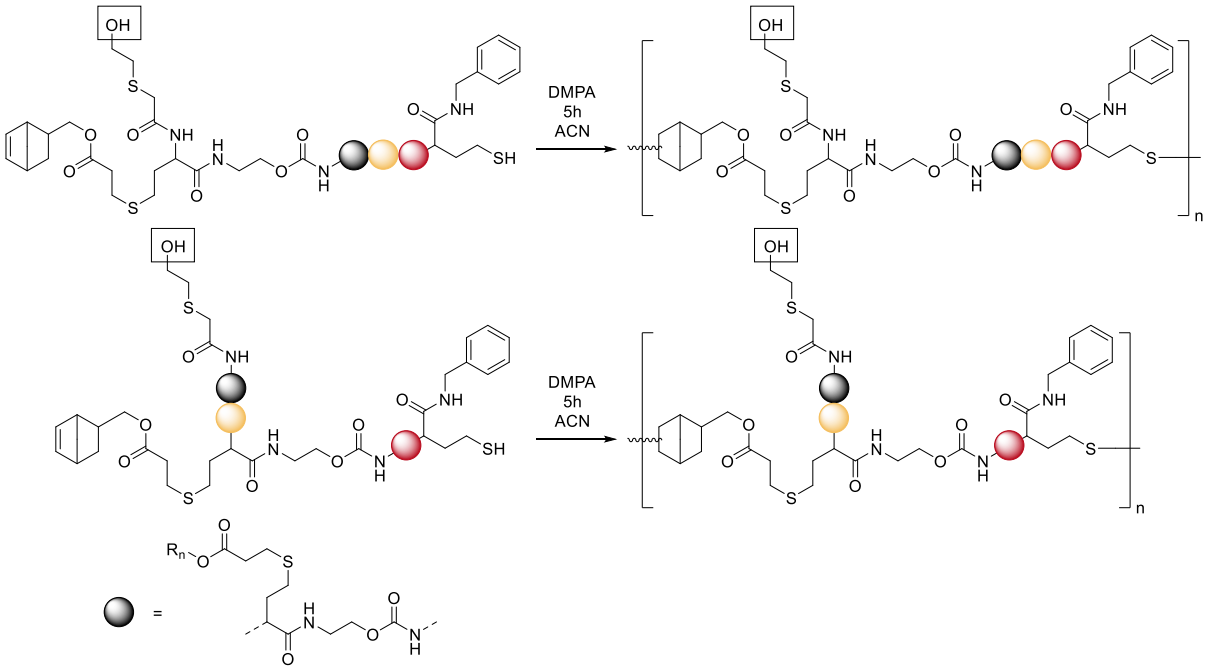


Figure VIII.23: Polymerisation of O6 which contains a functional handle (alcohol) for further functionalisation (top) and polymerisation of a hypothetical structure that results in graft polymers (bottom).

VIII.3 Conclusions and outlook

The focus of this chapter was on the polymerisation of sequence-defined oligomers via two different approaches based on the radical thiol-ene reaction between a thiol and a norbornene, which could be easily incorporated via a norbornene-functionalised acrylate.

The first strategy required the preparation of oligomers that contained a norbornene and a thiol end group. The synthesis of these oligomers started by coupling the acid-functionalised thiolactone from chapter IV on the cysteamine-4-methoxytrityl resin, which ensured that a thiol was present after cleavage. Subsequently, the two-step iterative protocol from chapter VI was performed on the prepared solid support for the synthesis of oligomers with a thiolactone end group. This latter group could be modified via the amine-thiolactone-ene conjugation with benzyl amine and the norbornene-functionalised acrylate. A tetramer and hexamer with a norbornene and a thiol end group were prepared and polymerised with the radical thiol-ene

reaction. The success of the polymerisation was confirmed by means of SEC, DOSY-NMR and DSC measurements. Graft polymers with sequence-defined branches could be prepared as well with this strategy, by installing the norbornene functionality as a side chain on the oligomer and not as an end group. Moreover, the oligomers and polymers prepared have unopened thiolactone units, which can be used for further modification.

An AB'-oligomer which contains a norbornene and a thiolactone group was prepared for the second polymerisation strategy. Via the established two-step iterative protocol, norbornene was installed on the first side chain and thiolactone was introduced as an end group. Different conditions were tried for the polymerisation of this oligomer, but to no avail. After opening of the thiolactone ring on the resin and cleavage of the oligomer off the resin, the polymerisation could be performed via the radical thiol-ene polymerisation. More analysis should be performed on these polymers in order to confirm disulphide formation and to determine the success of the polymerisation.

In the present chapter, the synthesis of the oligomers and the corresponding polymers was performed with racemic thiolactone compounds. In the future, enantiomeric pure compounds should be explored for the synthesis of these oligomers and periodic polymers in order to investigate the influence on their folding behaviour and on material properties such as melting behaviour.[34-36] In addition to chiral pure compounds, a pure exo- or endo-norbornene should be used to achieve more structural control over the backbone of the obtained SCPs, as well.

Although only the radical thiol-ene reaction was explored in this chapter, the nucleophilic thiol-ene reaction in combination with thiolactone chemistry had already been applied by our research group for the synthesis of SCPs, as mentioned in the introduction.[19] Installation of a (meth)acrylate and a thiol or thiolactone on the sequence could perhaps be explored in the future to obtain cyclic oligomers or periodic polymers. Another in-house chemistry that could be useful and interesting for the synthesis of periodic SCPs is the 1,2,4-triazoline-3,5-dione chemistry (TAD), which is very fast (seconds to minutes) and supplies visual feedback during the reaction (from pink/red to colourless). Polymerisation and even cyclisation could be performed with a sequence-defined oligomer that holds a diene, ene, phenol or indole moiety and a TAD functionality.[37, 38]

As already mentioned in this chapter, the approaches developed could also prove useful for the synthesis of cyclised oligomers. Madder and co-workers developed another thiolactone-based method for the cyclisation of oligopeptides which could also be applied to synthetic sequences.[36] This method starts from an oligopeptide bearing an end-standing thiol and an opened thiolactone, the combination of which releases a thiol after aminolysis. These two thiols can react with one another and form a disulphide bond. Instead of an oligopeptide, a synthetic sequence could be used for this cyclisation method.

VIII.4 Experimental part

VIII.4.1 Methods

^{1}H-, ^{13}C- and DOSY-NMR

^{1}H- and ^{13}C-NMR (Attached Proton Test, APT) spectra were recorded on a Bruker Avance 300 at 300 MHz and 75 MHz, respectively and a Bruker Avance 500 at 500 MHz and 125 MHz, respectively. Chemical shifts are presented in parts per million (δ) relative to DMSO-d_6 or $CHCl_3$-*d* (2.50 ppm or 7.27 ppm in ^{1}H- and 39.51 ppm or 77.24 ppm in ^{13}C-NMR respectively) as internal standard. All measurements were performed at 25°C and ACD/NMR Processor was used for the processing of all data. All spectra were recorded in a standard fashion with pulse programs available in the Bruker library. A convection-compensated sequence, double-stimulated echo with monopolar gradients with an extended-phase cycle 15 was applied for DOSY measurements, which were performed on a Bruker DRX spectrometer operating at a ^{1}H frequency of 500.13 MHz and at 25°C. A ^{1}H, ^{13}C, ^{15}N TXI-Z probe was used, with z-gradients calibrated to 56.1 G/cm. The diffusion encoding/decoding gradients were half sine bell shaped and were varied linearly between 2 % and 95 % of their maximum output over 32 increments. The duration of these gradients and the diffusion delay time were chosen in such a way that, at the highest gradient strength increment, the intensity of the signals of interest was decreased to less than 10 % of the lowest gradient strength increment.

LC-ESI-MS

An Agilent technologies 1100 series LC/MSD system equipped with a diode array detector and single-quad MS detector (VL) with an electrospray source was used for classic reversed-phase LC-MS and MS analysis. Analytic reversed-phase HPLC was performed with a Phenomenex C18 (2) column (5 µ, 250 x 4.6 mm) using a solvent gradient (0 → 100% acetonitrile in H_2O in 15 min) and the eluting compounds were detected via UV-detection (λ = 214 nm). High-resolution mass spectra (HRMS) were collected using an Agilent 6220 Accurate-Mass time-of-flight (TOF) equipped with a multimode ionisation (MMI) source.

GC-EI-MS

A Hewlett-Packard GCD-Plus equipped with an electron ionization detector and a HP-5MS (Agilent Technologies, 30m x 0.25 mm x 0.25 µm) column was exploited for GC-MS analysis. The carrier gas was He and temperature gradient was 3 min at 70°C, 17.5°C/min up to 315°C, 3 min at 315°C. Tygon tubings (0.8 mm internal diameter) were purchased from VWR international and 32G blunt needles from Ellsworth Adhesives.

SEC

Size exclusion chromatography was performed on a Waters instrument with a Waters 2414 Refractive Index Detector (RI), equipped with 3 PSS serial columns (GRAM Analytical 30 and 1000˚A, 10 µm particle size) at 35°C. Poly(methyl methacrylate) standards were used for calibration and DMA containing LiBr (0.42 g/l) was used as an eluent at a flow rate of 1 mL/min. Molecular weights and dispersities (Đ) were determined using the Empower software.

DSC

Differential scanning calorimetry was performed on a Mettler-Toledo DSC-1, equipped with an automatic sample robot, a liquid nitrogen-based cooling system and an FRS5 sensor based on a star-shaped arrangement of 56 thermocouples. DSC pans are standard Al pans of 40 µL and STARe Excellence Software was used to analyse the data.

VIII.4.2 Materials

DMSO-d_6 ([2206-27-1], ≥ 99.8 %) and $CHCl_3$-*d* ([865-49-6], ≥ 99.8 %) were purchased from Euriso-top. Acryloyl chloride ([814-68-6], 96 %) was purchased from abcr GmbH. Acetonitrile ([75-05-8], HPLC grade), 1,4-Dioxane ([123-91-1], HPLC grade), anhydrous Dimethylformamide ([68-12-2], 99.8 %) and Triethylamine ([121-44-8], 99 %) were purchased from Acros Organics. Cysteamine-4-methoxytrityl resin (200-400 mesh, 1% DVB, > 0.4 mmol/g) was purchased from CBL Patras. DL-Homocysteine thiolactone hydrochloride ([6038-19-3], 99 %) was purchased from Haihang industry (Jinan City, China). Potassium carbonate ([584-08-7], ≥ 99 %) and Sodium bicarbonate ([144-55-8], ≥ 99.5%) were purchased from Carl Roth. 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) ([94790-37-1], Peptide grade), 2-Chlorotrityl chloride resin ([42074-68-0], 100-200 mesh, 1% DVB, 1.6 mmol/g) and Trifluoroacetic acid ([76-05-1], Peptide grade) were purchased from Iris Biotech GmbH. Benzylamine ([100-46-9], 99 %), Bromoacetyl bromide ([598-21-0], ≥ 98 %), Butyl acrylate ([141-32-2], ≥ 99 %), Chloroform ([865-49-6], ≥ 99.8 %), Dichloromethane ([75-09-2], ≥ 99.8 %), Diethylether ([60-29-7], ≥ 99.9 %), N,N-Diisopropylethylamine (DIPEA, [7087-68-5], 99 %), 2,2-Dimethoxy-2-phenylacetophenone (DMPA) ([24650-42-8], 99.0 %), N,N-Dimethylacetamide ([127-19-5], ≥ 99.9 %), N,N-Dimethylformamide ([68-12-2], anhydrous, 99.8 %), Dimethylphenylphosphine ([672-66-2], 99 %), Ethanolamine ([141-43-5], ≥ 99%), Ethyl acetate ([141-78-6], ≥ 99.7 %), Glutaric anhydride ([108-55-4], 95 %), Isobornyl acrylate ([5888-33-5], technical grade), Lithium bromide ([7550-35-8], ≥ 99 %), 2-Mercaptoethanol ([60-24-2], ≥ 99 %), Methanol ([67-56-1], ≥ 99.9 %), Methyl acrylate ([96-33-3], 99 %), Octylamine ([111-68-4], 99.0 %), Pyridine ([110-86-1], ≥ 99 %), and Tetrahydrofuran ([109-99-9], ≥ 99 %) were purchased from Sigma-Aldrich and used without purification, except for isobornyl acrylate, which was distilled. Benzyl acrylate ([2495-35-4], > 97 %), Dibutyltin dilaurate ([77-58-7], > 95 %), 5-Norbornen-2-

methanol (mixture of isomers) ([95-12-5], > 98 %), Tetrahydrofurfuryl acrylate ([2399-48-6], 98 %), and Triphosgene ([32315-10-9], > 98 %) were purchased from TCI and used without purification. Hydrochloric acid 36 % p. (HCl, [7647-01-0]) was purchased from Chem-Lab and used without purification. Solvents (CH_2Cl_2, DIPEA and pyridine) for the immobilisation of functionalised thiolactone linkers were distilled from CaH_2 prior to use. Silicagel (ROCC, SI 1721, 60 Å, 40 – 63 μm) was used to perform preparative column chromatography, eluting with technical solvents. The collected fractions were analysed by thin layer chromatography (TLC-plates, Macherey-Nagel, SIL G-25 UV254). N-(2-bromoacetyl) homocysteine-γ-thiolactone, the alcohol- and acid-functionalised thiolactone linkers were prepared as previously described.[20, 32]

VIII.4.3 Synthesis

Synthesis of bicyclo[2.2.1]hept-5-en-2-ylmethyl acrylate
Acryloyl chloride (9.8 mL, 120 mmol) was added dropwise to an ice-cooled mixture of anhydrous CH_2Cl_2 (200 mL), anhydrous Et_3N (20.9 mL, 150 mmol) and 5-norbornene-2-methanol (100 mmol), and stirred for 1 hour at 0°C. The reaction mixture was allowed to reach room temperature overnight. Afterwards, the reaction was washed with water (200 mL) and $NaHCO_3$ solution (200 mL). After drying of the organic fraction with $MgSO_4$ and concentrating, the crude residue was purified by column chromatography (silicagel, hexane/ethyl acetate: 11/1) to obtain a colourless liquid. Phenothiazine was added as an inhibitor and the product was stored in the fridge.[22]

^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 6.45-6.39 (2*dd, 1H, 17.3, 1.5 Hz), 6.18-6.16 (m, 1H), 6.15-6.05 (m, 1H), 5.96 (dd, 1H, 5.7, 2.8 Hz), 5.85-5.81 (2*dd, 1H, 10.3, 1.5 Hz), 4.25-3.75 (4*dd, 2H, 10.9, 6.4 Hz; 10.9, 9 Hz; 10.8, 6.7 Hz; 10.9, 9.4 Hz), 2.91-2.74 (3*s, 2H), 2.44 (m, 1H), 1.86 (m, 2H), 1.78 (m, 1H), 1.41 (m, 2H), 1.24 (m, 2H), 0.59 (ddd, 2H, 11.7, 4.4, 2.5 Hz)
^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 166.2 (ester), 137.6 (CH), 136.9 (CH), 136.2 (CH), 132.2 (CH), 130.5 (CH_2), 130.4 (CH_2), 128.7 (CH), 128.6 (CH), 68.6 (CH_2), 68.0 (CH_2), 49.4 (CH_2), 44.9 (CH_2), 43.9 (CH), 43.7 (CH), 42.2 (CH), 41.6 (CH), 38.0 (CH), 37.8 (CH), 29.6 (CH_2), 29.0 (CH_2)
EI-MS (m/z) $[M]^+$ 178.2

Polymerisations with DMPA of oligomers with a thiol and norbornene end group
The sequence-defined oligomer, DMPA (0.03 equivalents) and acetonitrile (2 M) were added in a 2 mL vial which can be closed with a screw cap. This mixture was stirred under UV-radiation (365 nm) at 40°C for 2 hours.

Polymerisations with DMPA of oligomers with a norbornene and thiolactone end group
The sequence-defined oligomer, DMPA (0.03 or 0.05 equivalents), DMA or acetonitrile (between 1 M and 2 M), and n-octylamine (between 1 and 1.5 equivalents) were added in a 2 mL vial which can be closed with a screw cap. This mixture was stirred under UV-radiation (365 nm) at 40°C for 2 hours.

VIII.4.4 Manipulation of the solid support

Swelling
The solid support was swollen in the reaction solvent for at least 10 minutes.

Washing
The loaded resin was washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried after each reaction.

Loading on the cysteamine-4-methoxytrityl resin
The resin was first swollen in DMF. HBTU (5 equivalents), the acid-functionalised thiolactone (5 equivalents) and DIPEA (10 equivalents) were mixed in DMF to activate the acid functionality. The mixture was added to the resin, shaken for two hours, and then filtered off. The resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Loading on the 2-chlorotrityl chloride resin
The acid-functionalised thiolactone linker (1.33 g, 1.2 equivalents relative to the resin, 5.8 mmol) was loaded on a 2-chlorotrityl chloride resin (3 g, 1.6 mmol/g) in dry CH_2Cl_2 (30 mL, 10 mL/1 g of resin) with dry DIPEA (0.26 mL, 4 equiv. rel. to acid) and 10 vol% of DMF (3 mL), in order to dissolve the acid. The reaction mixture was shaken for 3 hours at room temperature. The resin was subsequently washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 6 x 30 mL), DMF (4 x 30 mL), CH_2Cl_2 (4 x 30 mL) and diethyl ether (4 x 30 mL). The resin was dried under vacuum.

The hydroxyl-functionalised thiolactone linker (1.69 g, 1.5 equivalents relative to the resin, 7.2 mmol) was loaded on a 2-chlorotrityl chloride resin (3 g, 1.6 mmol/g) in dry THF (30 mL, 10 mL/1 g of resin) with dry pyridine (1.16 mL, 2 equivalents relative to the alcohol).[39] The reaction was shaken overnight at 60°C. Next, the solid support was washed with a mixture of CH_2Cl_2/MeOH/DIPEA (17:2:1, 6 x 30 mL), DMF (4 x 30 mL), CH_2Cl_2 (4 x 30 mL) and diethyl ether (4 x 30 mL). The resin was dried under vacuum.

Cleavage

For cleavage, 2 mg of solid support was treated for 5 minutes with either 0.5 mL of 1% trifluoroacetic acid (TFA) in CH_2Cl_2 (2-chlorotrityl chloride resin) or 0.5 mL of 2% trifluoroacetic acid (TFA) in CH_2Cl_2 (cysteamine-4-methoxytrityl resin). During cleavage, the colour of the beads turns from yellow to red because of the formation of a trityl cation. For LC-ESI-MS analysis, the solid support was then filtered, the sample concentrated by evaporation and dissolved in acetonitrile. For samples that had to be polymerised, water was added and removed by freeze drying to ensure all volatile compounds were removed.

Aminolysis and incorporation of functionalities: general procedure

$CHCl_3$ is added to the solid support (1 mL reaction mixture per 100 mg resin). Next, the norbornene-functionalised or another functional acrylate (20 equivalents relative to the thiolactone unit), and ethanolamine or benzylamine (10 equivalents) were added. This mixture was shaken for 15 minutes at room temperature. This procedure was repeated once more to guarantee the full conversion of the thiolactone. After this second procedure, the resin was washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

Chain extension: general procedure

Dried $CHCl_3$ is added to the solid support (1 mL reaction mixture per 100 mg resin), then the α-isocyanato-γ-thiolactone (10 equivalents relative to the resin-bound alcohol units) and dibutyltin dilaurate (0.025 equivalents relative to the resin-bound alcohol units) were added. This mixture was shaken for 1 hour. Afterwards, the resin was washed with DMF (4x), MeOH (4x), $CHCl_3$ (4x) and diethyl ether (4x) and dried.

On resin phosphine reduction of disulphides

The resin was swollen in $CHCl_3$. The solvent was removed by filtration and a mixture (10 mL/1 g of resin) of dimethylphenylphosphine (2 equivalents) and $CHCl_3$ was added in the reactor with a drop of water. The reaction was shaken for 4 hours at room temperature. The solution was filtered off and the resin was washed with DMF (4x), methanol (4x), $CHCl_3$ (4x) and diethyl ether (4x).[33]

References

[1] J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, *38*, 1700582.
[2] J. De Neve, J. J. Haven, L. Maes, T. Junkers, *Polym. Chem.* **2018**, *9*, 4692.
[3] J. K. Szymański, Y. M. Abul-Haija, L. Cronin, *Accounts of Chemical Research* **2018**, *51*, 649.
[4] Z. L. Li, Z. C. Li, "Periodic Copolymers by Step-Growth Polymerization", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 349.
[5] R. M. Stayshich, T. Y. Meyer, *Journal of Polymer Science Part A: Polymer Chemistry* **2008**, *46*, 4704.

[6] J. Li, S. N. Rothstein, S. R. Little, H. M. Edenborn, T. Y. Meyer, *J. Am. Chem. Soc.* **2012**, *134*, 16352.
[7] K. Satoh, S. Ozawa, M. Mizutani, K. Nagai, M. Kamigaito, *Nat. Commun.* **2010**, *1*, 6.
[8] C.-H. Wang, Z.-Y. Song, X.-X. Deng, L.-J. Zhang, F.-S. Du, Z.-C. Li, *Macromol. Rapid Commun.* **2014**, *35*, 474.
[9] C. Zhang, J. Ling, Q. Wang, *Macromolecules* **2011**, *44*, 8739.
[10] W. R. Gutekunst, C. J. Hawker, *J. Am. Chem. Soc.* **2015**, *137*, 8038.
[11] R. M. Weiss, A. L. Short, T. Y. Meyer, *ACS Macro Lett.* **2015**, *4*, 1039.
[12] Y. Chen, Z. Guan, *J. Am. Chem. Soc.* **2010**, *132*, 4577.
[13] T.-B. Yu, J. Z. Bai, Z. Guan, *Angew. Chem., Int. Ed.* **2009**, *48*, 1097.
[14] M. F. Ebbesen, C. Gerke, P. Hartwig, L. Hartmann, *Polym. Chem.* **2016**, *7*, 7086.
[15] M.-A. Berthet, Z. Zarafshani, S. Pfeifer, J.-F. Lutz, *Macromolecules* **2010**, *43*, 44.
[16] W. Xi, S. Pattanayak, C. Wang, B. Fairbanks, T. Gong, J. Wagner, C. J. Kloxin, C. N. Bowman, *Angew. Chem., Int. Ed.* **2015**, *54*, 14462.
[17] C. Gerke, M. F. Ebbesen, D. Jansen, S. Boden, T. Freichel, L. Hartmann, *Biomacromolecules* **2017**, *18*, 787.
[18] C. Gerke, F. Jacobi, L. E. Goodwin, F. Pieper, S. Schmidt, L. Hartmann, *Macromolecules* **2018**, *51*, 5608.
[19] F. Driessen, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2015**, *4*, 616.
[20] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[21] C. E. Hoyle, T. Y. Lee, T. Roper, *Journal of Polymer Science Part A: Polymer Chemistry* **2004**, *42*, 5301.
[22] C. Elsner, C. Ernst, M. R. Buchmeiser, *Journal of Applied Polymer Science* **2011**, *119*, 1450.
[23] J. Xu, C. Boyer, *Macromolecules* **2015**, *48*, 520.
[24] J. Vandenbergh, K. Ranieri, T. Junkers, *Macromol. Chem. Phys.* **2012**, *213*, 2611.
[25] D. Frank, P. Espeel, N. Badi, F. Du Prez, *Eur. Polym. J.* **2018**, *98*, 246.
[26] V. Gilard, S. Trefi, S. Balayssac, M. A. Delsuc, T. Gostan, M. Malet-Martino, R. Martino, Y. Prigent, F. Taulelle, "Chapter 6 - DOSY NMR for Drug Analysis", in *NMR Spectroscopy in Pharmaceutical Analysis*, U. Holzgrabe, I. Wawer, and B. Diehl, Eds., Elsevier, Amsterdam, 2008, p. 269.
[27] M. Nilsson, G. A. Morris, *Chem. Commun.* **2007**, 933.
[28] M. Katsara, T. Tselios, S. Deraos, G. Deraos, M. T. Matsoukas, E. Lazoura, J. Matsoukas, V. Apostolopoulos, *Current Medicinal Chemistry* **2006**, *13*, 2221.
[29] P. Espeel, F. Goethals, F. E. Du Prez, *J. Am. Chem. Soc.* **2011**, *133*, 1678.
[30] F. Goethals, S. Martens, P. Espeel, O. van den Berg, F. E. Du Prez, *Macromolecules* **2014**, *47*, 61.
[31] P. Espeel, F. Goethals, F. Driessen, L.-T. T. Nguyen, F. E. Du Prez, *Polym. Chem.* **2013**, *4*, 2449.
[32] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[33] J. O. Holloway, S. Aksakal, F. E. D. Prez, C. R. Becer, *Macromol. Rapid Commun.* **2017**, *38*, 1700500.
[34] R. N. Zuckermann, *Peptide Science* **2011**, *96*, 545.
[35] M. Nicola, P. A. Gatti, M. Zacche, "Process for the resolution of homocysteine-thiolactone", Google Patents, 2008.
[36] D. Van Lysebetten, S. Felissati, E. Antonatou, L. L. G. Carrette, P. Espeel, E. Focquet, F. E. Du Prez, A. Madder, *ChemBioChem* **2018**, *19*, 641.
[37] S. Vandewalle, R. De Coen, B. G. De Geest, F. E. Du Prez, *ACS Macro Lett.* **2017**, *6*, 1368.
[38] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815.
[39] B. B. Shankar, D. Y. Yang, S. Girton, A. K. Ganguly, *Tetrahedron Lett.* **1998**, *39*, 2447.

Chapter IX
Conclusions and perspectives

IX.1 Conclusions

The aim of this doctoral thesis was the exploration of the amine-thiolactone-ene conjugation for the double modification of polymeric systems and the synthesis of sequence-controlled polymers (SCPs) (Figure IX.1). In this conjugation, an amine opens a thiolactone, generating a thiol which can react in a subsequent reaction with an ene functionality that is present in the same chemical environment.[1] In the last decade, both our PCR and other research groups have explored this combination of reactions for the synthesis of various functionalised polymeric architectures and the double modification of polymers.[1-5] This progress has ultimately enabled the synthesis of macromolecular structures with control over the monomer order, resulting in so-called SCPs.[4, 6-11] This class of polymers has been explored for the last decade to fill the gap between the classical bulk polymers and biopolymers, such as proteins and DNA, which have defined primary structures, extraordinary structure-property relationships and fulfil various functions within, for example, the human body.[12] Sequence-defined macromolecules form a subclass of these SCPs and have a uniform and discrete structure. As a previous two-step iterative thiolactone-based protocol only allowed defined tetramers with a low purity to be obtained[6], an objective of this doctoral research was to develop a new protocol which could be automated and would facilitate the synthesis of purer and longer functionalised oligomers. As it was expected that SCPs would play a crucial role in new applications such as data storage[13-15], sequence-defined oligomers were prepared with this protocol to store human- and machine-readable information. Moreover, since SCPs are expected to play a crucial role for the ultimate control of material properties[16-19], functional groups were installed on the sequence-defined oligomers in such a way that they could be coupled together to obtain both periodic sequence-controlled polymers and functionalised cyclised species.

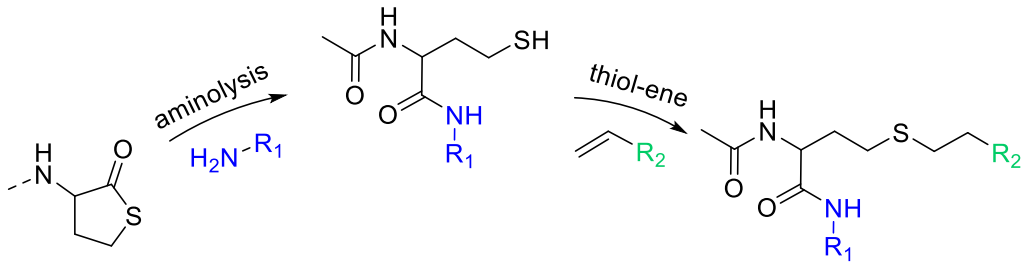


Figure IX.1: Amine-thiolactone-ene conjugation consisting of aminolysis of the thiolactone ring and subsequent thiol-ene reaction.

Each chapter within this doctoral dissertation is summarised and concluded below:

The recent progress in the construction of SCPs was depicted in a theoretical overview in **Chapter II**. First, the difference between sequence-controlled and sequence-defined polymers was clarified and both well-known and newly developed macromolecules were assigned to these two recently introduced categories of polymers. Next, a distinction was made between biological and bioinspired protocols for the preparation of SCPs on the one hand and synthetic protocols on the other hand. Finally, the usage of click chemistry was described for the synthesis, functionalisation/post-modification and cyclisation of SCPs. A special focus was given to thiol-ene and thiolactone chemistry, both of which were applied throughout the present doctoral thesis.[8]

Chapter III started by acquainting the researcher and the reader with the amine-thiolactone-ene conjugation, which was implemented for the assembly of tuneable TRLC stationary phases. A thiolactone-functionalised thermoresponsive polymer was obtained after the copolymerisation via RAFT of NIPAM and a thiolactone-functionalised acrylamide (Figure IX.2). The stationary phase bearing amine groups opened the thiolactone groups of this polymer, generating thiols, which were subsequently reacted with either benzyl or PEG acrylates. Although different methods equally exist to make temperature-responsive liquid chromatography[20-22], this procedure enabled, in the same pot, the installation of PNIPAM and functionalisation with two different acrylates in order to prepare two stationary phases. In comparison to other loading methods[20], elevated loadings (>20%) were obtained as well in an easy and controlled way without the need to repeat the coupling reaction of the polymer to the stationary phase. The analyses of parabens and steroids with the prepared columns resulted in symmetric peaks and suitable column efficiencies with only water as mobile phase. The retentions of the solutes proved to depend not only on the temperature applied as low retention was observed below the LCST of PNIPAM and high retention above the LCST, but they also depended on the acrylate used. Indeed, retention times were more or less halved when PEG acrylate was exploited instead of benzyl acrylate to functionalise the stationary phase. In comparison to other methods to prepare a stationary phase[20-22], the method applied here allows for the easy tuning of the separation behaviour. The separation of diastereoisomeric peptides could be executed to some extent with the stationary phase bearing benzyl acrylate.[23] Normally, the separation of diastereomeric oligopeptides requires specific conditions such as lower pH of the mobile phase, high column efficiency and long columns[24, 25], which were not necessary for the applied double modified stationary phases.

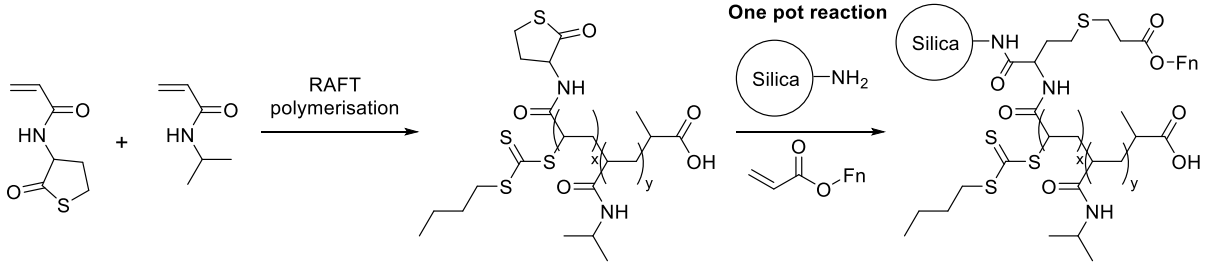


Figure IX.2: Immobilisation of a thiolactone-functionalised PNIPAM, prepared via RAFT polymerisation, on aminopropyl silica and subsequent thiol-ene reaction with an acrylate to install an extra functionality.

The focus of **Chapter IV** was on the one-pot, double modification of thiolactone which was immobilised either on a solid support or on a polymer end group, and was first ring-opened via aminolysis and followed by the subsequent thiol-ene reaction with an acrylate. The first part of this chapter focussed on the amine-thiolactone-ene conjugation on the solid support. Initially, 2-chlorotrityl chloride polystyrene resin was loaded with an acid- and a hydroxyl-functionalised thiolactone linker. Afterwards, experiments to determine the ideal reaction conditions indicated that 10 equivalents of amine and 20 equivalents of acrylate in $CHCl_3$ were required for the elegant double modification of the immobilised thiolactone on this solid support. This method allowed for the incorporation of various functionalities, such as aromatic and cyclic groups, pyridine, acid, ether, furan, alkene, alkyne, morpholine, hydroxyl, tertiary amine and methacrylate. To prove the versatility of this method, an oligopeptide with a thiolactone end group was also double-modified.

In the second part of this chapter, this double modification method was performed on a polymer with a thiolactone end group which was installed via two different methods. In the first method, styrene and butyl acrylate were polymerised by means of controlled radical polymerisation with a thiolactone-functionalised initiator to install the thiolactone functionality at the end of their corresponding polymers. With the second method, the hydroxyl end groups of PEO and PCL were converted with the α-isocyanato-γ-thiolactone. As for the solid support, the reaction conditions were determined for the thiolactone opening and the subsequent thiol-ene reaction. Double modification in $CHCl_3$ for 48 hours proved to require 10 equivalents of amine and 15 equivalents of acrylate for polystyrene and polybutyl acrylate, and 15 and 30 equivalents respectively for PEO and PCL. The polymers were functionalised with various functionalities, such as aromatic, furan, tetrahydrofurfuryl, double bond, halogen and hydroxyl. Finally, the amine-thiolactone-ene conjugation was used to obtain a midchain-functionalised block copolymer with a polybutyl acrylate and PEO block.[26] Although other methods for double modification have been investigated over the last years[27-33], some of them require multiple steps, elevated temperatures, intermediate purification, and longer reaction times. The proposed one-pot strategy remedies certain drawbacks of the existing methods and can be performed with abundantly available amines and acrylates.

From Chapter IV, it became clear that an oligopeptide can be easily functionalised with a thiolactone end group. In **Chapter V**, this realisation was a stepping stone to install different functionalities on thiolactone-functionalised oligopeptides for their polymerisation and cyclisation (Figure IX.3). Although Guan and Hartmann already developed efficient methods to prepare periodic polymers starting from oligopeptides[34-36], these strategies do not allow the incorporation of additional functionalities between two subsequent repeating macrounits. AB'-oligomers were synthesised with an ene and thiolactone functionality, which were respectively installed with peptoid synthesis and the acid-functionalised thiolactone from Chapter IV. After the aminolysis of the latter, this ene group could react with the generated thiol to form polymers

(Figure IX.3, top). Analysis indicated that disulphide formation occurred and only oligomers were formed.

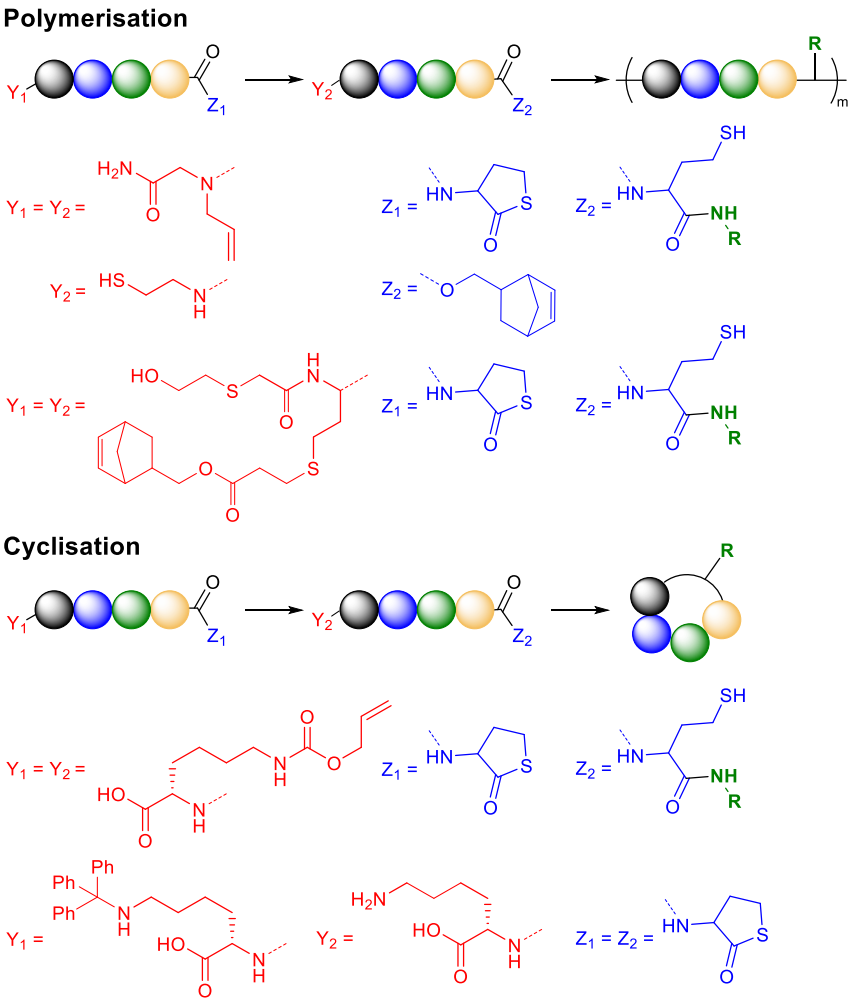


Figure IX.3: Polymerisation (top) and cyclisation (bottom) strategies of oligomers based on either the aminolysis of a thiolactone yielding a free thiol for subsequent thiol-ene reactions or the radical thiol-ene reaction between a thiol and a norbornene group.

Next, the amine-thiolactone-ene conjugation was explored for the on-resin macrocyclisation of oligopeptides via two different approaches (Figure IX.3, bottom). Anseth and coworkers also developed efficient on-resin cyclisation methods based on radical thiol-ene reactions[37, 38]. These methods, however, do not allow for the one-pot cyclisation and installation of a functionality, and require protecting groups for the thiol. For the first approach, the ene group of an alloc-protected lysine and the thiol obtained after the aminolysis of a thiolactone were reacted with each other via the radical thiol-ene reaction. Disulphide formation was observed because of the amounts of amine required to open the thiolactone. The structure of the oligopeptide also played a crucial role for the cyclisation, as only disulphide formation was noticed for the AGAGAG motif. Improvements could perhaps be done to the proposed cyclisation method by cyclising the oligopeptides in solution or by first reducing the disulphides formed and subsequently executing a thiol-ene reaction.[39] For the second cyclisation strategy, oligopeptides with an Mtt-protected lysine and a thiolactone end group were prepared. An

amine was formed after deprotection of the lysine and reacted with the thiolactone, generating a thiol. Disulphide formation resulted in handcuff-like bicyclic oligopeptides. Reduction of the disulphides with phosphines led to the formation of monocycles bearing a thiol, which could react with an acrylate via a subsequent nucleophilic thiol-ene reaction for further functionalisation. Recently, Madder and coworkers collaborated with our group in optimizing a new one-pot cyclisation method of oligopeptides based on thiolactone chemistry and disulphide formation.[40] Although this method had to be performed in solution, the aminolysis of the thiolactone allows for the elegant installation of functional groups, which can be applied for further modification, functionalisation or immobilisation.

From Chapter IV, it could be concluded that the combination of the amine-thiolactone-ene conjugation on a solid support proved to be more effective, in matters of time and purification, than the same combination on a liquid support. **Chapter VI** combined the nucleophilic one-pot amine-thiolactone-ene conjugation with urethane synthesis on solid support via a new, robust protecting-group-free, two-step, iterative protocol for the preparation of multifunctional sequence-defined oligomers (Figure IX.4). In order to easily and quickly obtain the desired oligomers, some optimisations were first required. A kinetic study indicated that the first step or the amine-thiolactone-ene step of the protocol was finished in 15 minutes for ethanolamine under the reaction conditions determined in Chapter IV. Full conversion of the thiolactone on solid support was guaranteed by repeating this step twice. For the second step or urethane formation, the reaction stoichiometry, the choice of solvent and the reaction times had to be determined. Experiments indicated that the reaction was finished after 30 minutes with 10 equivalents of the α-isocyanato-γ-thiolactone and 0.025 equivalent of dibutyltin dilaurate (DBTL) in dry chloroform. The reaction time was doubled to ensure full urethane formation. Next, different multifunctional oligomers were built as various acrylics were applied during the aminolysis. Starting from the solid support loaded with either the acid- or the alcohol-functionalised thiolactone linker from Chapter IV, this two-step protocol enabled the acquisition of functionalised decamers with a very high purity. The side chain and the backbone of the oligomers could be easily controlled, which was proven by preparing a trimer with two different amines and three different acrylics. Finally, the protocol was translated to an automated approach. Although an adapted peptide synthesiser allowed for the accelerated synthesis of libraries of sequence-defined oligomers, the purity of the automatedly made decamers was lower than that of the ones obtained during the manual synthesis.[41]

In the last five years, since the start of the present work, multiple methods have been explored to prepare sequence-defined structures.[9, 11, 42] However, many of these methods require protecting groups and/or the synthesis of different monomers, and the possibilities to automate them have only been explored for a few of these methods. The method proposed in Chapter VI only requires a single thiolactone building block and does not need protecting groups. Additionally, functionalities can be easily installed by using abundantly available acrylics and the backbone can be elegantly modified with different amino alcohols.

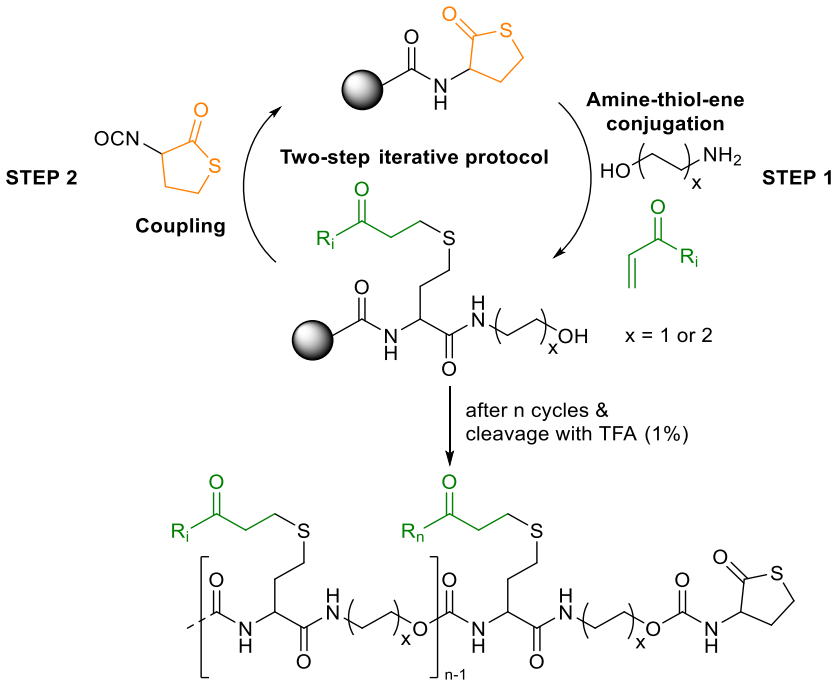


Figure IX.4: Thiolactone-based two-step iterative protocol yielding monodisperse, multifunctional oligomers on solid support. An amino alcohol opens the immobilised thiolactone and the thiol formed subsequently reacts with a functional acrylate or acrylamide (STEP 1). The thiolactone moiety is reinstated in the next step by means of the α-isocyanato-γ-thiolactone (STEP 2).

Different sequence-defined oligomeric systems have been explored for data storage.[43-52] These methods mostly focus on binary data in which every monomer corresponds to a bit or a combination of bits. As mentioned above, the monomers have to be prepared separately. For example, Meier's group has combined the Passerini and the Biginelli reaction to produce oligomers that can store up to 24 bits per repeat unit, yet this strategy requires protecting groups and every oligomer has to be made separately.[52] The data storage capacity of the sequence-defined oligomers described above was explored in **Chapter VII**. First, a comparison between two different techniques, ESI- and MALDI-TOF/TOF MS/MS, was performed for the precise analysis of the oligomers made. The read-out of these defined macromolecules was facilitated by the controlled fragmentation on the urethane bonds in their backbone. In order to speed up and automate the read-out of these defined structures on a standard laptop, an algorithm called "Chemreader" was developed, aiming at tackling the time-consuming sequence-reading bottleneck of sequence-defined macromolecules. This Chemreader algorithm was first tested by reading a sentence of which the words were written on different oligomers. Next, to increase the complexity, a 33x33 QR code corresponding to a square grid containing 1089 pixels, was stored in 71 different sequences and analysed with this algorithm. The fast encoding of binary data as a set of oligomers and the decoding of this set to the original data was executed with the software tool "Chemcoder".[13] In comparison to other research groups focussing on molecular data management, the method applied did not require any protecting groups, and data (or functionalities) could be easily built into the oligomers with available acrylics, rather than homemade monomers.

A bottleneck of the approach developed here is that every oligomer had to be analysed separately. Inspired by oligopeptide analysis[53], Lutz *et al.* explored a 2D technique based on MALDI-TOF MS for the analysis of complex oligomer mixtures.[54] A complex sample is first analysed in the MS dimension, after which the different masses are isolated and analysed in a tandem MS. For this technique, the mass difference between different parent ions should be distinctive enough in order not to cause any problems during the isolation. Another solution, well-known in the context of peptide analysis, could be the coupling of liquid chromatography to tandem ESI-MS/MS equipment in order to separate different oligomers in the LC dimension and determine the sequence in the tandem MS dimension.[55] Ion mobility mass spectrometry that allows for gas phase separation could be another alternative, but comes with a considerable instrument cost.[56]

Another way to tackle this bottleneck is to combine the oligomers into one long chain to store the information. Two approaches have been explored over the last years: controlled fragmentations in collision-induced dissociation conditions[48] or controlled depolymerisation[57] in combination with the methods mentioned in the previous paragraph. Lutz' group used the first technique for the analysis of poly(phosphodiester)s chains (bit strings) which contain several bytes (8 bits) of information. Two distinct molecular features were built into the information-containing polymers: alkoxyamine groups were installed between the bytes (or octa(phosphodiester)s) and mass tags were added to each byte to indicate its position in the bit string. The weak NO-C bonds were first cleaved via MS/MS (MS^2) conditions. Afterwards, MS^3 measurements were performed on each byte to decode the whole bit string or polymer.

A final bottleneck of the mass analysis techniques used is the destruction of data (or oligomers). Although non-destructive techniques for the read-out of the sequence order have been developed[49, 58, 59], such as nanopore sequencing, further research should be conducted to make it more generally applicable for sequence-defined macromolecules.

Chapter VIII described two different strategies for the polymerisation of sequence-defined oligomers prepared with the two-step protocol from Chapter VI (Figure IX.4), based on the radical thiol-ene reaction between a thiol group and a norbornene group (Figure IX.3, top). In order to easily install the latter group on the oligomers, a norbornene-functionalised acrylate was prepared for both strategies. The first strategy started with the synthesis of oligomers containing a norbornene and a thiol end group. The acid-functionalised thiolactone from Chapter IV was coupled on the cysteamine-4-methoxytrityl resin to guarantee that a thiol end group was obtained after cleavage. This coupling on the solid support installed a thiolactone unit that could be used for the synthesis of sequence-defined oligomers with the two-step iterative protocol from Chapter VI. The amine-thiolactone-ene conjugation with benzyl amine and the norbornene-functionalised acrylate was performed on the thiolactone end group of the oligomers. This modification allowed for the installation of norbornene as an end group on the oligomer. Afterwards, periodic SCPs were obtained after the radical thiol-ene polymerisation

of either a tetramer or a hexamer with a norbornene and a thiol end group. The success of the polymerisation was confirmed by SEC and DOSY-NMR. The material properties of the oligomers and polymers obtained were investigated with DSC measurements. Incorporation of the norbornene functionality as a side chain on the oligomer rather than as an end group resulted in the formation of graft polymers with sequence-defined branches. The second polymerisation strategy required an AB'-oligomer bearing a norbornene and a thiolactone group. The two-step iterative protocol from Chapter VI allowed for the efficient incorporation of norbornene on the first side chain and thiolactone as an end group. Although the amine-thiolactone-ene polymerisation of this oligomer was explored under different reaction conditions, nothing seemed to promote this process. On the other hand, when the aminolysis was first performed on the resin and the oligomer cleaved off the resin, radical thiol-ene polymerisation could be performed with the macromolecule obtained. Mass analysis should be performed on all the polymers made in this chapter in order to confirm disulphide formation and to determine the success of these polymerisations. Moreover, the synthesis of SCPs with the amine-thiolactone-ene polymerisation should be further studied. Although the synthesis of SCPs starting from defined oligomers has been explored with other methods, such as polycondensations[16, 60], ATRA and ATRC[61, 62], RACP[63], ROMP of sequence-defined macrocyclic structures[64, 65], CuAAC[34, 35, 66, 67], and radical[68-70] and nucleophilic thiol-ene[7], Hartmann's group made different oligomers containing two norbornene end groups.[70] These AA oligomers were combined with a dithiol to obtain polymers via an AA+BB radical thiol-ene polymerisation. However, very recently, this group also developed a way to obtain periodic polymers with a high molecular weight starting from sequence-defined AB oligomers carrying norbornene and thiol end groups.[36] Protection of the thiol was required to prevent uncontrolled polymerisation by self-initiation, and only linear polymers were prepared. In recent years, many research groups have become interested in polymerising sequence-defined structures, as the obtained polymers exhibit interesting structure-property relationships.[9, 11, 42, 71]

IX.2 Perspectives

In the conclusion section of each individual chapter, a more specific outlook was already provided for the topic tackled in the chapter at hand. This section will thus only highlight more general perspectives deriving from this doctoral thesis.

For the present PhD research, the amine-thiolactone-ene conjugation proved to be an efficient tool to modify and prepare (sequence-controlled) polymeric architectures. Although the main focus of this thesis was on the aminolysis and the subsequent thiol-ene reaction, other reaction partners might be chosen to react with the released thiol, as seen in Chapter II.[72] While the combination of thiolactones and these thiol-X reactions has already allowed for the synthesis

and functionalisation of polymeric structures[1, 33, 73, 74], it could still benefit from more detailed exploration for the synthesis of SCPs.

In addition to the thiolactone-functionalised compounds in this work, other molecules containing a thiolactone and another functionality (e.g. maleimide[75], alkyne[76], azide[77] or others[32, 78]) have been prepared and could also be interesting for double modifications and the synthesis of SCPs. The maleimide-containing thiolactone could, for instance, be useful for controlled addition at specific times during the controlled radical polymerisation of styrene[79], while the azide- or alkyne-bearing thiolactone can be incorporated in an AB+CD approach developed by Lutz and coworkers (Figure IX.5).[80-84] Combinations of the amine-thiolactone-ene conjugation with other efficient chemistries and synthetic approaches likely hold potential for the preparation of SCPs.

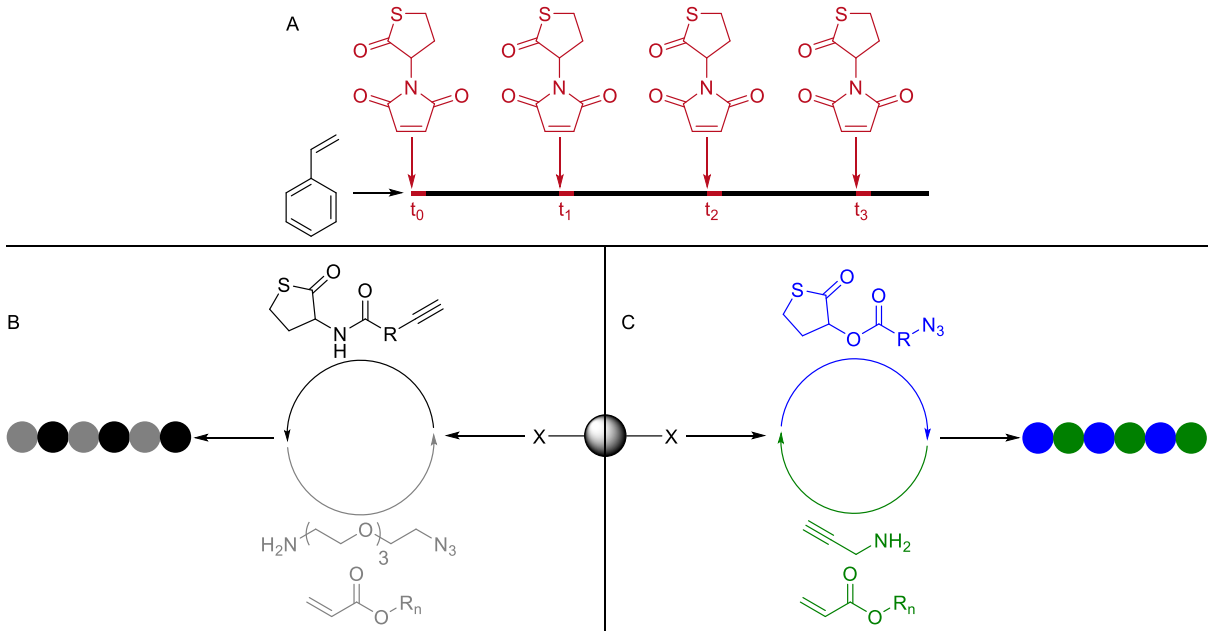


Figure IX.5: Usage of functional thiolactones for the synthesis of SCPs: maleimide-containing thiolactone for the controlled addition at specific times during the controlled radical polymerisation of styrene (A) and azide- or alkyne-bearing thiolactone as building blocks in an AB+CD approach (B and C).

This doctoral dissertation explored the capacity to store data on sequence-defined oligomers, but many other applications could potentially be tackled with the sequence-defined oligomers and sequence-controlled polymers developed in the current piece of research.[9] First, although only racemic thiolactone compounds were explored in this PhD thesis, the use of enantiomerically pure compounds could perhaps be interesting for the folding and self-assembly behaviour of these macromolecules.[40, 85] This aspect is currently taken up in other PhD research topics within the PCR group. Secondly, the installation of certain functionalities could also make them interesting for various applications: sugars and cationic groups, for example, could be installed for respectively lectin binding[86] and antibacterial activity.[87, 88] Thirdly, the polymers dealt with in Chapters V and VIII should be further explored to discover what the influence of the monomer order of a repeating oligomer is on their properties. The research groups of Meyer and Guan noted earlier that this order can influence properties such

as degradation behaviour[16], local secondary structure in solution and LCST.[35] Finally, most research groups investigating SCPs have so far focused mainly on the synthesis of linear structures and their applications, but not on branched structures. Research has revealed that the latter can be applied as dispersants, emulsifiers, drug carriers and surfactants.[89] Although some groups have started to explore sequence-controlled branched structures[90, 91], it could be interesting to further investigate the influence of monomer control on the properties of such structures, which is another topic of ongoing research within the PCR group.

In several chapters, in addition to thiolactone, the use of the 1,2,4-triazoline-3,5-dione (TAD) chemistry, developed in-house, was suggested for the functionalisation, polymerisation and cyclisation of stationary phases and sequence-controlled macromolecular structures.[92-96] This TAD group can react with phenols, indoles, enes or dienes, and this chemistry could also prove useful for the synthesis and modification of sequence-defined and -controlled structures[15], as it is ultrafast (taking only seconds to minutes) and provides visual feedback when the reaction is finished (changing from red/pink to colourless). A multi-step-growth procedure, for instance, can be very time-consuming for the preparation of a long sequence-defined structure, which could be accelerated by applying this chemistry. Existing literature indicates the great interest of reactions which could be performed in less than five minutes for the synthesis of sequence-defined structures.[9] The TAD-indole reaction is also reversible, which could be interesting for the creation of erasable sequences in the field of data storage.[45]

References

[1] P. Espeel, F. E. Du Prez, *Eur. Polym. J.* **2015**, *62*, 247.
[2] S. Mommer, H. Keul, M. Möller, *Macromol. Rapid Commun.* **2014**, *35*, 1986.
[3] Y. Yan, L. Xue, J. B. Miller, K. Zhou, P. Kos, S. Elkassih, L. Liu, A. Nagai, H. Xiong, D. J. Siegwart, *Polymer* **2015**, *72*, 271.
[4] Z. Zhang, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Polymer* **2015**, *64*, 221.
[5] S. Montolio, O. Zagorodko, R. Porcar, M. Isabel Burguete, S. V. Luis, H. Tenhu, E. García-Verdugo, *Polym. Chem.* **2017**, *8*, 4789.
[6] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[7] F. Driessen, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2015**, *4*, 616.
[8] S. Martens, J. O. Holloway, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, 1700469.
[9] J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, *38*, 1700582.
[10] L. Yu, Z. Zhang, Y.-Z. You, C.-Y. Hong, *Eur. Polym. J.* **2018**, *103*, 80.
[11] J. De Neve, J. J. Haven, L. Maes, T. Junkers, *Polym. Chem.* **2018**, *9*, 4692.
[12] J. M. Berg, J. L. Tymoczko, L. Stryer, "*Biochemistry*", W.H. Freeman, Basingstoke, 2012.
[13] S. Martens, A. Landuyt, P. Espeel, B. Devreese, P. Dawyndt, F. Du Prez, *Nat. Commun.* **2018**, *9*, 4451.
[14] M. G. T. A. Rutten, F. W. Vaandrager, J. A. A. W. Elemans, R. J. M. Nolte, *Nature Reviews Chemistry* **2018**, *2*, 365.
[15] J. K. Szymański, Y. M. Abul-Haija, L. Cronin, *Accounts of Chemical Research* **2018**, *51*, 649.
[16] J. Li, S. N. Rothstein, S. R. Little, H. M. Edenborn, T. Y. Meyer, *J. Am. Chem. Soc.* **2012**, *134*, 16352.
[17] J. Zhang, G. Gody, M. Hartlieb, S. Catrouillet, J. Moffat, S. Perrier, *Macromolecules* **2016**, *49*, 8933.

[18] J. Zhang, R. Deubler, M. Hartlieb, L. Martin, J. Tanaka, E. Patyukova, P. D. Topham, F. H. Schacher, S. Perrier, *Macromolecules* **2017**, *50*, 7380.
[19] M. R. Golder, Y. Jiang, P. E. Teichen, H. V. T. Nguyen, W. Wang, N. Milos, S. A. Freedman, A. P. Willard, J. A. Johnson, *J. Am. Chem. Soc.* **2018**, *140*, 1596.
[20] F. Lynen, J. M. D. Heijl, F. E. D. Prez, R. Brown, R. Szucs, P. Sandra, *Chromatographia* **2007**, *66*, 143.
[21] B. Miserez, F. Lynen, A. Wright, M. Euerby, P. Sandra, *Chromatographia* **2010**, *71*, 1.
[22] T. Nishio, E. Ayano, Y. Suzuki, H. Kanazawa, T. Okano, *Journal of Chromatography A* **2011**, *1218*, 2079.
[23] A. J. Satti, P. Espeel, S. Martens, T. Van Hoeylandt, F. E. Du Prez, F. Lynen, *Journal of Chromatography A* **2015**, *1426*, 126.
[24] E. P. Kroeff, D. J. Pietrzyk, *Analytical Chemistry* **1978**, *50*, 1353.
[25] K. G. Feitsma, B. F. H. Drenth, *Pharmaceutisch Weekblad* **1988**, *10*, 1.
[26] F. Driessen, S. Martens, B. D. Meyer, F. E. Du Prez, P. Espeel, *Macromol. Rapid Commun.* **2016**, *37*, 947.
[27] A. Lv, X.-X. Deng, L. Li, Z.-L. Li, Y.-Z. Wang, F.-S. Du, Z.-C. Li, *Polym. Chem.* **2013**, *4*, 3659.
[28] S. C. Solleder, M. A. R. Meier, *Angew. Chem., Int. Ed.* **2014**, *53*, 711.
[29] B. Yang, Y. Zhao, C. Fu, C. Zhu, Y. Zhang, S. Wang, Y. Wei, L. Tao, *Polym. Chem.* **2014**, *5*, 2704.
[30] B. Yang, Y. Zhao, S. Wang, Y. Zhang, C. Fu, Y. Wei, L. Tao, *Macromolecules* **2014**, *47*, 5607.
[31] C. A. Figg, T. Kubo, B. S. Sumerlin, *ACS Macro Lett.* **2015**, *4*, 1114.
[32] M. Langlais, I. Kulai, O. Coutelier, M. Destarac, *Macromolecules* **2017**, *50*, 3524.
[33] M. Langlais, O. Coutelier, M. Destarac, *Macromolecules* **2018**, *51*, 4315.
[34] T.-B. Yu, J. Z. Bai, Z. Guan, *Angew. Chem., Int. Ed.* **2009**, *48*, 1097.
[35] Y. Chen, Z. Guan, *J. Am. Chem. Soc.* **2010**, *132*, 4577.
[36] C. Gerke, P. Siegfeld, K. Schaper, L. Hartmann, *Macromol. Rapid Commun.* **2018**, 1800735.
[37] A. A. Aimetti, K. R. Feaver, K. S. Anseth, *Chem. Commun.* **2010**, *46*, 5781.
[38] A. A. Aimetti, R. K. Shoemaker, C.-C. Lin, K. S. Anseth, *Chem. Commun.* **2010**, *46*, 4061.
[39] J. O. Holloway, S. Aksakal, F. E. D. Prez, C. R. Becer, *Macromol. Rapid Commun.* **2017**, *38*, 1700500.
[40] D. Van Lysebetten, S. Felissati, E. Antonatou, L. L. G. Carrette, P. Espeel, E. Focquet, F. E. Du Prez, A. Madder, *ChemBioChem* **2018**, *19*, 641.
[41] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[42] M. A. R. Meier, C. Barner-Kowollik, *Advanced Materials* **2018**, 1806027.
[43] R. K. Roy, C. Laure, D. Fischer-Krauser, L. Charles, J.-F. Lutz, *Chem. Commun.* **2015**, *51*, 15677.
[44] A. A. Ouahabi, M. Kotera, L. Charles, J.-F. Lutz, *ACS Macro Lett.* **2015**, *4*, 1077.
[45] R. K. Roy, A. Meszynska, C. Laure, L. Charles, C. Verchin, J.-F. Lutz, *Nat. Commun.* **2015**, *6*, 7237.
[46] S. Telitel, B. E. Petit, S. Poyer, L. Charles, J.-F. Lutz, *Polym. Chem.* **2017**.
[47] D. Karamessini, S. Poyer, L. Charles, J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, 1700426.
[48] A. Al Ouahabi, J.-A. Amalian, L. Charles, J.-F. Lutz, *Nat. Commun.* **2017**, *8*, 967.
[49] M. Boukhet, N. F. König, A. A. Ouahabi, G. Baaken, J.-F. Lutz, J. C. Behrends, *Macromol. Rapid Commun.* **2017**, *38*, 1700680.
[50] A. Burel, C. Carapito, J.-F. Lutz, L. Charles, *Macromolecules* **2017**, *50*, 8290.
[51] G. Cavallo, S. Poyer, J. A. Amalian, F. Dufour, A. Burel, C. Carapito, L. Charles, J. F. Lutz, *Angew. Chem., Int. Ed.* **2018**, *57*, 6266.
[52] A. C. Boukis, M. A. R. Meier, *Eur. Polym. J.* **2018**, *104*, 32.
[53] L. Tsiatsiani, A. J. R. Heck, *The FEBS Journal* **2015**, *282*, 2612.
[54] C. Laure, D. Karamessini, O. Milenkovic, L. Charles, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2016**, *55*, 10722.
[55] M. L. Biniossek, O. Schilling, *PROTEOMICS* **2012**, *12*, 1303.
[56] A. B. Kanu, P. Dwivedi, M. Tam, L. Matz, H. H. Hill, *Journal of Mass Spectrometry* **2008**, *43*, 1.
[57] G. I. Peterson, M. B. Larsen, A. J. Boydston, *Macromolecules* **2012**, *45*, 7317.
[58] D. Branton, D. W. Deamer, A. Marziali, H. Bayley, S. A. Benner, T. Butler, M. Di Ventra, S. Garaj, A. Hibbs, X. Huang, S. B. Jovanovich, P. S. Krstic, S. Lindsay, X. S. Ling, C. H. Mastrangelo, A. Meller,

J. S. Oliver, Y. V. Pershin, J. M. Ramsey, R. Riehn, G. V. Soni, V. Tabard-Cossa, M. Wanunu, M. Wiggin, J. A. Schloss, *Nature Biotechnology* **2008**, *26*, 1146.
[59] N. F. König, A. Al Ouahabi, S. Poyer, L. Charles, J.-F. Lutz, *Angew. Chem., Int. Ed.* **2017**, *56*, 7297.
[60] R. M. Stayshich, T. Y. Meyer, *Journal of Polymer Science Part A: Polymer Chemistry* **2008**, *46*, 4704.
[61] K. Satoh, S. Ozawa, M. Mizutani, K. Nagai, M. Kamigaito, *Nat. Commun.* **2010**, *1*, 6.
[62] C.-H. Wang, Z.-Y. Song, X.-X. Deng, L.-J. Zhang, F.-S. Du, Z.-C. Li, *Macromol. Rapid Commun.* **2014**, *35*, 474.
[63] C. Zhang, J. Ling, Q. Wang, *Macromolecules* **2011**, *44*, 8739.
[64] W. R. Gutekunst, C. J. Hawker, *J. Am. Chem. Soc.* **2015**, *137*, 8038.
[65] R. M. Weiss, A. L. Short, T. Y. Meyer, *ACS Macro Lett.* **2015**, *4*, 1039.
[66] M. F. Ebbesen, C. Gerke, P. Hartwig, L. Hartmann, *Polym. Chem.* **2016**, *7*, 7086.
[67] M.-A. Berthet, Z. Zarafshani, S. Pfeifer, J.-F. Lutz, *Macromolecules* **2010**, *43*, 44.
[68] W. Xi, S. Pattanayak, C. Wang, B. Fairbanks, T. Gong, J. Wagner, C. J. Kloxin, C. N. Bowman, *Angew. Chem., Int. Ed.* **2015**, *54*, 14462.
[69] C. Gerke, M. F. Ebbesen, D. Jansen, S. Boden, T. Freichel, L. Hartmann, *Biomacromolecules* **2017**, *18*, 787.
[70] C. Gerke, F. Jacobi, L. E. Goodwin, F. Pieper, S. Schmidt, L. Hartmann, *Macromolecules* **2018**, *51*, 5608.
[71] Z. L. Li, Z. C. Li, "Periodic Copolymers by Step-Growth Polymerization", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 349.
[72] C. E. Hoyle, A. B. Lowe, C. N. Bowman, *Chemical Society Reviews* **2010**, *39*, 1355.
[73] S. Mommer, K.-N. Truong, H. Keul, M. Möller, *Polym. Chem.* **2016**, *7*, 2291.
[74] Y. Chen, P. Espeel, S. Reinicke, F. E. Du Prez, M. H. Stenzel, *Macromol. Rapid Commun.* **2014**, *35*, 1128.
[75] T. Rudolph, P. Espeel, F. E. Du Prez, F. H. Schacher, *Polym. Chem.* **2015**, *6*, 4240.
[76] J.-J. Yan, J.-T. Sun, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Scientific Reports* **2013**, *3*, 2841.
[77] R.-R. Wang, J.-J. Yan, R.-L. Yang, D.-H. Pan, W. Li, Y.-P. Xu, L.-Z. Wang, X.-Y. Wang, M. Yang, *Journal of Polymer Science Part A: Polymer Chemistry* **2017**, *55*, 2053.
[78] D. Frank, P. Espeel, S. Claessens, E. Mes, F. E. Du Prez, *Tetrahedron* **2016**, *72*, 6616.
[79] J.-F. Lutz, B. V. K. J. Schmidt, S. Pfeifer, *Macromol. Rapid Commun.* **2011**, *32*, 127.
[80] S. Pfeifer, Z. Zarafshani, N. Badi, J. F. Lutz, *Journal of the American Chemical Society* **2009**, *131*, 9195.
[81] T. T. Trinh, L. Oswald, D. Chan-Seng, J.-F. Lutz, *Macromol. Rapid Commun.* **2014**, *35*, 141.
[82] D. Chan-Seng, J.-F. Lutz, *ACS Macro Lett.* **2014**, *3*, 291.
[83] D. Chan-Seng, J.-F. Lutz, "Solid-Phase Synthesis as a Tool for the Preparation of Sequence-Defined Oligomers Based on Natural Amino Acids and Synthetic Building Blocks", in *Sequence-Controlled Polymers: Synthesis, Self-Assembly, and Properties*, American Chemical Society, 2014, p. 103.
[84] T. T. Trinh, L. Oswald, D. Chan-Seng, L. Charles, J.-F. Lutz, *Chemistry – A European Journal* **2015**, *21*, 11961.
[85] M. Nicola, P. A. Gatti, M. Zacche, "Process for the resolution of homocysteine-thiolactone", Google Patents, 2008.
[86] Y. Abdouni, G. Yilmaz, C. R. Becer, *Macromol. Rapid Commun.* **2017**, 1700212.
[87] M. Porel, D. N. Thornlow, C. M. Artim, C. A. Alabi, *ACS Chemical Biology* **2017**, *12*, 715.
[88] M. Porel, D. N. Thornlow, N. N. Phan, C. A. Alabi, *Nat. Chem.* **2016**, *8*, 590.
[89] F. Driessen, R. Herckens, P. Espeel, F. E. Du Prez, *Polym. Chem.* **2016**, *7*, 1632.
[90] M. Baier, M. Giesler, L. Hartmann, *Chemistry-a European Journal* **2018**, *24*, 1619.
[91] F. Amir, Z. Jia, M. J. Monteiro, *J. Am. Chem. Soc.* **2016**, *138*, 16600.
[92] J. F. W. Keana, A. P. Guzikowski, D. D. Ward, C. Morat, F. L. Van Nice, *J. Org. Chem.* **1983**, *48*, 2654.
[93] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815.

[94] O. Roling, K. De Bruycker, B. Vonhören, L. Stricker, M. Körsgen, H. F. Arlinghaus, B. J. Ravoo, F. E. Du Prez, *Angew. Chem., Int. Ed.* **2015**, *54*, 13126.
[95] S. Vandewalle, R. De Coen, B. G. De Geest, F. E. Du Prez, *ACS Macro Lett.* **2017**, *6*, 1368.
[96] H. A. Houck, K. De Bruycker, S. Billiet, B. Dhanis, H. Goossens, S. Catak, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Chem. Sci.* **2017**, *8*, 3098.

Chapter X
Nederlandstalige samenvatting

X.1 Introductie

Sinds Leo Baekeland in 1907 bakeliet introduceerde, ‘s werelds eerste volledig synthetisch polymeer, hebben chemici de ontwikkeling van polymeren verdergezet en uitgediept.[1] Dit heeft geleid tot een breed gamma aan bulkmaterialen die we dagelijks gebruiken, zoals plastic flessen en zakjes, matrassen en polyesters in onze kleren. Het heeft ook gezorgd voor de ontwikkeling van complexere systemen, waaronder dispergeermiddelen, geleidende polymeren en micro-elektronica. De voorbije 30 jaar hebben polymeerchemici getracht macromoleculen te bereiden met een nog sterkere link tussen hun structuur en hun eigenschappen. Dit leidde tot de evolutie van nieuwe gecontroleerde polymerisatiemethoden en de ontwikkeling van complexe macromoleculaire structuren zoals dendrimeren, blok- en graftcopolymeren.[2]

Al werd er een opmerkelijk grote vooruitgang geboekt in het onderzoek naar polymeren, biopolymeren zoals proteïnen en DNA hebben nog steeds een streepje voor, aangezien de link tussen hun structuur en eigenschappen nog beduidend sterker is.[3] Deze biomacromoleculen worden gekenmerkt door hun gedefinieerde primaire structuur of monomeervolgorde, die hen toelaat om op een specifieke en complexe manier te vouwen, waardoor ze functies kunnen vervullen zoals genetische informatieopslag, katalyse of biotransport. In het laatste decennium hebben chemici zich laten inspireren door andere wetenschapstakken, zoals biologie, biochemie, organische chemie en polymeerchemie, om nieuwe methoden te ontwikkelen die meer controle verschaffen over de monomeervolgorde in polymeren. Hiermee hopen ze nieuwe toepassingen voor polymeren te kunnen verkennen en sterkere correlaties te creëren tussen de primaire structuur en de eigenschappen van polymeren.[4, 5] Dit leidde tot de introductie van een nieuwe klasse van polymeren waarin de monomeervolgorde wordt gecontroleerd: de *sequentiegecontroleerde polymeren (SCPs)*. Een subgroep van deze SCPs bestaat uit *sequentiegedefinieerde macromoleculen*, die een uniforme en discrete structuur hebben. Er wordt verwacht dat SCPs een cruciale rol zullen spelen in een hele reeks applicaties, zoals dataopslag[6, 7], anti-namaaklabels[8], katalyse[9], antibacteriële toepassingen[10, 11], zelfassemblage en opvouwing[12, 13], en zullen mogelijk ook in staat zijn om materiaaleigenschappen te beïnvloeden, zoals het fasegedrag[14], de glastransitietemperatuur (T_g)[15] en het degradatiegedrag.[16]

Een vorm van chemie die niet alleen een significante impact heeft gehad op de synthese, cyclisatie en functionalisatie van (complexe) macromoleculaire structuren, maar ook op

biologische en synthetische SCPs, is wat algemeen 'klik'-chemie genoemd wordt.[17, 18] Sharpless, Fokin en Finn bepaalden in 2001 vereisten waaraan een reactie moet voldoen om gecatalogeerd te worden als een klikreactie: "De reactie moet algemeen toepasbaar zijn, een zeer hoge opbrengst opleveren, enkel onschadelijke nevenproducten vormen die bij voorkeur verwijderd worden met niet-chromatografische methoden, en stereospecifiek (maar niet noodzakelijk enantioselectief) zijn. De vereiste reactiekenmerken omvatten eenvoudige reactiecondities (idealiter zou het proces ongevoelig moeten zijn voor zuurstof en water), vlot beschikbaar startmateriaal en reagentia, geen gebruik van een solvent of gebruik van een solvent dat onschadelijk (zoals water) of gemakkelijk te verwijderen is, en eenvoudige productisolatie. Opzuivering, indien nodig, moet niet-chromatografisch zijn, zoals kristallisatie of destillatie, en het product moet stabiel zijn onder fysiologische omstandigheden. […] Klikreacties zijn snel afgerond en hebben de neiging om heel selectief te zijn voor één enkel product: we denken bij deze reacties aan een springplank met één traject."[19-21] Vervelende chemische procedures en opwerkingen die komen kijken bij andere methoden konden gereduceerd worden door gebruik te maken van deze chemie voor de synthese van macromoleculaire structuren, die tot dan toe onmogelijk bleken te zijn of enkel haalbaar op een kleine schaal. In 2011 moesten er specifiek voor hun gebruik in de polymeerchemie extra criteria toegevoegd worden, omdat bepaalde efficiënte reacties onterecht als 'klik' werden gecategoriseerd in de literatuur.[21] Aan de originele vereisten werden equimolariteit en grootschalige opzuiveringen toegevoegd, aangezien deze essentieel geacht werden voor een klikreactie met betrekking tot polymeren. Gedurende het voorbije decennium, waarin steeds meer aandacht ging naar de precieze definitie en controle van de polymeerhoofdketen en de functionalisering van polymeren, hebben onderzoekers de focus gelegd op het ontwikkelen van opschaalbare en tijdbesparende reacties, die als onmisbaar worden beschouwd voor de synthese van SCPs.[22] Klik- en klikgeïnspireerde reacties hebben een cruciale rol gespeeld in de opbouw, postmodificatie en cyclisatie van nauwkeurig gedefinieerde en gecontroleerde (multigefunctionaliseerde) structuren.[18]

Sinds 2009 onderzoekt onze onderzoeksgroep de combinatie van twee efficiënte reacties in hetzelfde reactiemedium: de aminolyse van thiolacton en de klikgeïnspireerde thiol-eenreactie.[23] Wanneer een thiolacton geopend wordt door een amine, stelt het een thiol vrij dat in een vervolgreactie kan reageren met een alkeen. Deze krachtige amine-thiolacton-alkeenconjunctie was het onderwerp van een aantal doctoraten en talrijke wetenschappelijke papers. Het werd onderzocht door meerdere onderzoeksgroepen naast de onze en maakte zowel de synthese mogelijk van verscheidene gefunctionaliseerde polymeerarchitecturen als de dubbele modificatie van polymeren.[23-27] Recent is gebleken dat deze conjunctie geschikt is voor de synthese van sequentiegecontroleerde en –gedefinieerde structuren.[27-30] Hoewel voor de aanvang van dit doctoraatsonderzoek binnen onze groep een iteratief tweestapsprotocol ontwikkeld werd voor het verkrijgen van de laatste structuren, konden hierdoor slechts tetrameren verkregen worden met een lage zuiverheid.[28] Een van de initiële doelstellingen van deze doctoraatsthesis was het verkrijgen van zuiverdere en langer gefunctionaliseerde oligomeren, liefst met een protocol dat geautomatiseerd kan worden.

Met de kennis die in de afgelopen jaren verzameld werd binnen onze groep[23] werd de efficiënte amine-thiolacton-alkeenconjunctie uitvoerig onderzocht, en dit met twee doelen: de dubbele modificatie van polymeersystemen[31, 32] en de synthese van SCPs.[33] De verkregen sequentiegedefinieerde oligomeren werden zowel gebruikt voor de opslag van data die door mensen en machines gegenereerd werden[6] als uitgerust met geschikte functionele groepen die aan elkaar gelinkt konden worden om periodieke sequentiegecontroleerde polymeren[34] en gefunctionaliseerde cyclische adducten[35, 36] te verkrijgen.

X.2 Overzicht van de resultaten

De recente vooruitgang in de opbouw van SCPs werd toegelicht in een theoretisch overzicht in **Hoofdstuk II**. Ten eerste werd het verschil tussen sequentiegecontroleerde en -gedefinieerde polymeren verduidelijkt en werden zowel gekende als nieuw ontwikkelde macromoleculen ingedeeld in deze twee recent geïntroduceerde polymeercategorieën. Vervolgens werd er een onderscheid gemaakt tussen enerzijds biologische en biogeïnspireerde methodes en anderzijds synthetische technieken voor de bereiding van SCPs. Tenslotte werd het gebruik van klikchemie beschreven voor de synthese, functionalisatie/postmodificatie en cyclisatie van SCPs. De focus lag hierbij vooral op thiol-eengerelateerde en thiolactongebaseerde reacties, die beide doorheen dit volledige proefschrift gebruikt werden.[18]

Om zowel de onderzoeker als de lezer vertrouwd te laten worden met de amine-thiolacton-alkeenconjunctie, beschreef **Hoofdstuk III** eerst hoe deze conjunctie geïmplementeerd wordt voor de bereiding van aanpasbare TRLC-stationaire fasen. Een thiolactongefunctionaliseerd thermoresponsief polymeer werd verkregen na de copolymerisatie van N-isopropylacrylamide (NIPAM) en een thiolactongefunctionaliseerd acrylamide. De amines op de stationaire fase openden de thiolactongroepen van dit polymeer, wat resulteerde in de vorming van thiolen die vervolgens reageerden met benzyl- of PEG-acrylaten. Deze procedure maakte de installatie van poly(N-isopropylacrylamide) (PNIPAM) en de functionalisatie met twee verschillende acrylaten mogelijk in hetzelfde reactiemedium voor de bereiding van twee stationaire fasen. De analyse van parabenen en steroïden met de bereide kolommen en met enkel water als mobiele fase resulteerde in symmetrische pieken en geschikte kolomefficiëntie. De retentie van de geanalyseerde componenten bleek deels, maar niet uitsluitend, af te hangen van de gebruikte temperatuur, waarbij lage retentie werd verkregen bij temperaturen onder de LCST en hoge retentie boven de LCST. Deze retentie was daarnaast ook afhankelijk van het gebruikte acrylaat: de retentietijden werden min of meer gehalveerd wanneer PEG-acrylaat werd gehanteerd voor de functionalisatie van de stationaire fase in plaats van benzylacrylaat. De scheiding van diastereomeren van peptiden kon in enige mate uitgevoerd worden door de stationaire fase gefunctionaliseerd met benzylacrylaat.[32]

De focus van **Hoofdstuk IV** lag op de één-pot-, dubbele modificatie van een thiolacton dat geïmmobiliseerd was op ofwel een vaste fase of een polymeereindgroep. Dit thiolacton werd eerst geopend via aminolyse, waarna het gevormde thiol reageerde met een acrylaat. Het eerste deel van dit hoofdstuk focuste op de amine-thiolacton-alkeenconjunctie van de vaste fase. Eerst werd 2-chlorotritylchloridepolystyreenhars beladen met een zuur- en alcoholgefunctionaliseerde thiolactonlinker. Vervolgens werden experimenten uitgevoerd voor het bepalen van de ideale reactiecondities, die aantoonden dat 10 equivalenten aan amine en 20 equivalenten aan acrylaat in $CHCl_3$ nodig zijn voor de efficiënte dubbele modificatie van het geïmmobiliseerde thiolacton op dit hars. Deze methode maakte het mogelijk om verscheidene functionele groepen in te bouwen, zoals aromaten, (cyclische) alkanen, pyridine, zuur, ether, furaan, alkeen, alkyn, morfoline, alcohol, tertiair amine en methacrylaat. Om de veelzijdigheid van deze methode te bewijzen werd ook een oligopeptide met een thiolactoneindgroep dubbel gemodificeerd.

In het tweede deel van dit hoofdstuk werd deze dubbele modificatiemethode toegepast op een polymeer met een thiolactoneindgroep die op twee verschillende manieren ingebouwd kon worden. Bij de eerste methode werden styreen en butylacrylaat gepolymeriseerd d.m.v. gecontroleerde radicalaire polymerisaties met een thiolactongefunctionaliseerde initiator. Zo kon een thiolactongroep als eindgroep van hun corresponderende polymeren geplaatst worden. Met de tweede methode werden de alcoholeindgroepen van PEO en PCL omgezet met het α-isocyanato-γ-thiolacton. Naar analogie met de vaste fase werden de reactiecondities voor de thiolactonopening en de daaropvolgende thiol-een reactie bepaald. Dubbele modificatie in $CHCl_3$, 48 uur lang, vereiste 10 equivalenten aan amine en 15 equivalenten aan acrylaat voor polystyreen en polybutylacrylaat, en respectievelijk 15 en 30 equivalenten voor PEO en PCL. De polymeren werden gefunctionaliseerd met een aromaat, een furaan, een tetrahydrofuraan, een alkeen, een halogeen en een alcohol. Ten slotte werd met de amine-thiolacton-alkeenconjunctie een blokcopolymeer verkregen met een functionele groep tussen een PEO-blok en polybutylacrylaatblok.[31]

Uit Hoofdstuk IV bleek duidelijk dat een oligopeptide gemakkelijk gefunctionaliseerd kan worden met een thiolactoneindgroep. In **Hoofdstuk V** was deze vaststelling het startpunt voor het installeren van verschillende functionele groepen op thiolactongefunctionaliseerde oligopeptiden, met het oog op hun cyclisatie en polymerisatie. AB'-oligomeren met een alkeen en thiolactongroep werden gesynthetiseerd d.m.v. respectievelijk peptoïdesynthese en de koppeling van het zuurgefunctionaliseerde thiolacton uit Hoofdstuk IV aan het oligopeptide. Polymeren worden gevormd wanneer deze alkeengroep reageert met het thiol dat gevormd werd na de aminolyse van het thiolacton. Analyse van de resultaten toonde aan dat disulfidevorming optrad en enkel oligomeren werden gevormd. Deze strategie zou verder onderzocht moeten worden met andere oligopeptiden of synthetische oligomeren om te bepalen of ze geschikt is voor de synthese van SCPs. Vervolgens werd de amine-thiolacton-alkeenconjunctie verkend voor de macrocyclisatie van oligopeptiden op het hars via twee verschillende methodes. De

eerste tactiek was gebaseerd op de radicalaire thiol-een reactie van het alkeen in de alloc-beschermde groep van een lysine met het thiol dat vrijkwam na aminolyse van een thiolacton. Door toedoen van de hoeveelheden amine die nodig waren om het thiolacton te openen werden disulfiden gevormd. De structuur van het oligopeptide speelde ook een cruciale rol in de cyclisatie: bij de AGAGAG-sequentie werden uitsluitend disulfiden gevormd. Mogelijke verbeteringen zouden aangebracht kunnen worden aan de voorgestelde methode door de cyclisatie uit te voeren in oplossing of door eerst de disulfiden te reduceren en daarna pas de thiol-eenreactie uit te voeren.[37] Als deze methode goed werkt, zouden verschillende functionele groepen via aminolyse geïnstalleerd kunnen worden. In het geval van de tweede cyclisatiestrategie werden oligopeptiden bereid met een lysine waarvan het amine beschermd is met een Mtt-groep en een thiolactoneindgroep. Door het verwijderen van deze beschermgroep werd een amine gevormd dat kon reageren met een thiolacton, wat leidde tot de vorming van een thiol. Disulfidevorming resulteerde in handboeivormige bicyclische oligopeptiden. Het reduceren van deze disulfiden leidde tot de vorming van monocyclische structuren met een thiol dat verder gefunctionaliseerd kon worden via een nucleofiele thiol-eenreactie met een acrylaat.

Uit Hoofdstuk IV kon ook geconcludeerd worden dat de amine-thiolacton-alkeenconjunctie van een hars op het vlak van tijd en opzuivering efficiënter bleek dan dezelfde conjugatie met een thiolactoneindgroep van een oplosbaar polymeer. In **Hoofdstuk VI** werd de nucleofiele één-pot amine-thiolacton-alkeenconjunctie gecombineerd met urethaansynthese op de vaste fase via een nieuw, robuust, iteratief tweestapsprotocol waarbij geen beschermgroepen nodig zijn voor de bereiding van multigefunctionaliseerde sequentiegedefinieerde oligomeren. Eerst moesten weliswaar een aantal optimalisaties uitgevoerd worden om de gewenste oligomeren snel en eenvoudig te verkrijgen. Een kinetische studie wees uit dat de eerste stap van het protocol of de amine-thiolacton-alkeenconjunctie voor ethanolamine na 15 minuten afgelopen was onder de reactiecondities uit Hoofdstuk IV. Volledige conversie van het thiolacton op het hars kon gegarandeerd worden door deze stap twee keer uit te voeren. Voor de tweede stap of de urethaanvorming moesten de stoichiometrie, de keuze van het solvent en de reactietijd bepaald worden. Experimenten wezen uit dat de reactie afgelopen was na 30 minuten met 10 equivalenten aan α-isocyanato-γ-thiolacton en 0,025 equivalenten aan dibutyltin dilauraat (DBTL) in droge chloroform. De reactietijd werd verdubbeld om ervoor te zorgen dat de urethaanvorming volledig was afgelopen. Vervolgens werden verschillende multigefunctionaliseerde oligomeren bereid door diverse acrylaten en acrylamiden te gebruiken gedurende de aminolyse. Vertrekkende van een vaste fase die beladen was met het zuur- of alcoholgefunctionaliseerde thiolacton uit Hoofdstuk IV konden met het tweestapsprotocol gefunctionaliseerde decameren met een heel hoge zuiverheid verkregen worden. De zij- en hoofdketen van de oligomeren kon gemakkelijk gecontroleerd worden. Om deze controle te bewijzen werd een trimeer gesynthetiseerd met twee verschillende amines, twee verschillende acrylaten en een acrylamide. Tenslotte werd het protocol geautomatiseerd. Hoewel een syntheserobot voor oligopeptidesynthese aangepast werd voor de snellere bereiding van hele

sets aan sequentiegedefinieerde oligomeren, was de zuiverheid van de automatisch bereide decameren lager dan tijdens de manuele synthese.[33]

De mogelijkheid om data op te slaan op deze sequentiegedefinieerde oligomeren werd beschreven in **Hoofdstuk VII**. Eerst werd een vergelijking gemaakt tussen twee verschillende technieken, ESI- en MALDI-TOF/TOF MS/MS, voor de nauwkeurige analyse van de bereide oligomeren. De uitlezing van deze gedefinieerde macromoleculen werd vereenvoudigd door de gecontroleerde fragmentatie van de urethaanverbindingen in hun hoofdketen. Om de uitlezing van deze gedefinieerde structuren te versnellen en te vereenvoudigen op een klassieke laptop werd een algoritme ontwikkeld dat de naam "Chemreader" kreeg. Hierbij werd vooral ingespeeld op het tijdrovende proces dat het uitlezen van sequentiegedefinieerde macromoleculen vaak is. Dit Chemreader algoritme werd eerst getest door een zin uit te lezen waarvan de woorden waren geschreven op verschillende oligomeren. Vervolgens werd een meer uitdagend probleem aangepakt: een 33x33 QR-code die overeenstemt met een vierkant raster van 1089 pixels. Deze QR-code werd opgeslagen in 71 verschillende sequenties en geanalyseerd met behulp van dit algoritme. De snelle codering van binaire data naar een set oligomeren en de decodering van deze set tot de originele data werd uitgevoerd met een algoritme genaamd "Chemcoder".[6]

Hoofdstuk VIII beschreef twee verschillende strategieën voor de polymerisatie van sequentiegedefinieerde oligomeren die bereid werden met het tweestapsprotocol uit Hoofdstuk VI. Deze strategieën werden gebaseerd op de radicalaire thiol-eenreactie tussen een thiol en een norborneengroep, die gemakkelijk geïnstalleerd kon worden door een zelfbereid norborneengefunctionaliseerd acrylaat. De eerste strategie startte met de synthese van oligomeren die een norborneen- en een thioleindgroep bevatten. Het zuurgefunctionaliseerde thiolacton uit Hoofdstuk IV werd gekoppeld op het cysteamine-4-methoxytritylhars om ervoor te zorgen dat er na afsplitsing zeker een thioleindgroep aanwezig was. Deze koppeling op de vaste fase zorgde ervoor dat op het hars een thiolacton aanwezig was dat gebruikt kon worden voor de synthese van sequentiegedefinieerde oligomeren met het iteratieve tweestapsprotocol uit Hoofdstuk VI. De amine-thiolacton-alkeenconjunctie met benzylamine en het norborneengefunctionaliseerde acrylaat werd uitgevoerd op de thiolactoneindgroep van deze oligomeren. Deze modificatie maakte de installatie van een norborneeneindgroep op het oligomeer mogelijk. Vervolgens werden periodieke SCPs verkregen na de radicalaire thiol-eenpolymerisatie van ofwel een tetrameer of een hexameer met een norborneen- en een thioleindgroep. Het succes van de polymerisatie werd bevestigd a.d.h.v. SEC-, DOSY-NMR- en DSC-metingen. Als de norborneengroep op een zijketen werd geplaatst i.p.v. op de eindgroep van het oligomeer, konden graftpolymeren met sequentiegedefinieerde vertakkingen gevormd worden. De tweede polymerisatiestrategie vereiste een AB'-oligomeer met een norborneen- en thiolactongroep. Het iteratieve tweestapsprotocol uit Hoofdstuk VI maakte het mogelijk om een norborneen in de eerste zijketen te plaatsen en het thiolacton als eindgroep. Hoewel de amine-thiolacton-alkeenpolymerisatie van deze oligomeren werd onderzocht onder

verschillende reactiecondities, bleek niets het polymerisatieproces te bevorderen. Wanneer de aminolyse eerst werd uitgevoerd op het hars en het oligomeer werd afgesplitst van dit hars, kon de radicalaire thiol-een polymerisatie van deze macromolecule uitgevoerd worden. De massa's van de bereide polymeren uit dit hoofdstuk zouden geanalyseerd moeten worden om te bepalen of er disulfidevorming optrad en of de polymerisaties succesvol waren. Bovendien zou de synthese van SCPs met de amine-thiolacton-alkeenpolymerisatie verder bestudeerd moeten worden.

X.3 Vooruitblik

Aan het slot van ieder hoofdstuk werden specifieke vooruitzichten geboden over het onderwerp waar het hoofdstuk over handelde. In wat volgt zullen daarom meer algemene perspectieven toegelicht worden met betrekking tot deze doctoraatsthesis.

Voor het huidige doctoraatsonderzoek bleek de amine-thiolacton-alkeenconjunctie een geschikte combinatie om (sequentiegecontroleerde) polymeerarchitecturen te modificeren en te bereiden. Hoewel de algemene focus van deze thesis lag op de aminolyse en de daaropvolgende thiol-eenreactie zouden er ook andere reactiepartners gekozen kunnen worden om te reageren met het gevormde thiol. In Hoofdstuk II werden deze partners aangehaald.[38] De combinatie van thiolacton met deze thiol-X-reacties heeft de synthese en functionalisatie van polymeerstructuren al mogelijk gemaakt[23, 39-41], maar zou in de toekomst ook van pas kunnen komen voor de synthese van SCPs.

Naast de thiolactongefunctionaliseerde componenten die beschreven werden in dit werk zijn er ook andere thiolactonbevattende moleculen met een bijkomende functionaliteit (maleïmide[42], alkyn[43], azide[44] of andere[45, 46]) die interessant zouden kunnen zijn voor de dubbele modificatie en synthese van SCPs. Het maleïmidebevattende thiolacton kan bijvoorbeeld ingezet worden voor de gecontroleerde additie op specifieke tijdstippen tijdens de gecontroleerde radicalaire polymerisatie van styreen[47], terwijl het azide- of alkyndragende thiolacton gebruikt kan worden in een AB+CD-protocol ontwikkeld door de Lutz' groep.[48-52] Combinaties van de amine-thiolacton-alkeenconjunctie met andere efficiënte chemische reacties en synthetische procedures hebben potentieel voor de synthese van SCPs.

In dit doctoraatsonderzoek werd de mogelijkheid om data op te slaan op sequentiegedefinieerde oligomeren nagegaan, maar de oligomeren en sequentiegecontroleerde polymeren die hiervoor ontwikkeld werden zouden ook nuttig kunnen zijn voor een heel aantal andere applicaties.[4] Ten eerste zouden chiraal zuivere componenten interessant kunnen zijn voor het opvouwen en de zelfassemblage van deze macromoleculen.[36, 53] Ten tweede kunnen bepaalde functionele groepen ingebouwd worden die interessante applicaties met zich meebrengen: suikers en kationische groepen kunnen bijvoorbeeld geïnstalleerd worden voor respectievelijk

lectinebinding[54] en antibacteriële eigenschappen.[11, 55] Ten derde zouden de polymeren uit hoofdstukken V en VIII verder onderzocht moeten worden om te ontdekken hoe hun eigenschappen beïnvloed worden door de monomeervolgorde van een herhalend oligomeer. De onderzoeksgroepen van Meyer en Guan hebben eerder al opgemerkt dat deze volgorde een invloed kan hebben op eigenschappen zoals het degradatiegedrag[16], de secundaire structuur in oplossing en de LCST.[56] Ten slotte bestuderen de meeste SCP-onderzoeksgroepen vooral de synthese van lineaire structuren en hun applicaties, geen vertakte structuren. Onderzoekers hebben aangetoond dat deze laatste groep van structuren nochtans ingezet kan worden voor toepassingen zoals dispergeermiddelen, emulgatoren, medicijndragers en surfactanten.[57] Hoewel een aantal onderzoeksgroepen sinds kort onderzoek uitvoeren naar sequentiegecontroleerde vertakte structuren[58, 59], zou het interessant kunnen zijn om dieper in te gaan op de invloed van de monomeervolgorde op de eigenschappen van deze structuren.

In sommige hoofdstukken werd in plaats van thiolacton het gebruik van 1,2,4-triazoline-3,5-dione (TAD)-chemie, ontwikkeld binnen onze eigen onderzoeksgroep, aangeraden voor de functionalisatie, polymerisatie en cyclisatie van stationaire fasen en sequentiegecontroleerde macromoleculaire structuren.[60-64] Deze TAD-groep kan reageren met fenolen, indolen, alkenen of diënen, en deze chemie kan ook nuttig blijken voor de synthese en modificatie van sequentiegedefinieerde en -gecontroleerde structuren[65]: ze is heel snel (duurt enkele seconden tot hooguit minuten) en voorziet visuele feedback wanneer de reactie is afgelopen (een verandering van rood/roze naar kleurloos). Een multistapsprocedure, bijvoorbeeld, kan heel tijdrovend zijn bij de bereiding van lange sequentiegedefinieerde structuren. Dit kan versneld worden door deze TAD-chemie te gebruiken. De huidige literatuur geeft aan dat er grote interesse is voor reacties die uitgevoerd kunnen worden in minder dan vijf minuten bij de synthese van sequentiegecontroleerde structuren.[4] De TAD-indoolreactie is daarnaast ook reversibel, wat interessant zou kunnen zijn voor de synthese van uitwisbare sequenties binnen het onderzoek naar dataopslag op oligomeren.[66]

Referenties

[1] R. Mülhaupt, *Macromol. Chem. Phys.* **2013**, *214*, 159.
[2] J.-F. Lutz, J.-M. Lehn, E. W. Meijer, K. Matyjaszewski, *Nature Reviews Materials* **2016**, *1*, 16024.
[3] J. M. Berg, J. L. Tymoczko, L. Stryer, "*Biochemistry"*, W.H. Freeman, Basingstoke, 2012.
[4] J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, *38*, 1700582.
[5] J. De Neve, J. J. Haven, L. Maes, T. Junkers, *Polym. Chem.* **2018**, *9*, 4692.
[6] S. Martens, A. Landuyt, P. Espeel, B. Devreese, P. Dawyndt, F. Du Prez, *Nat. Commun.* **2018**, *9*, 4451.
[7] M. G. T. A. Rutten, F. W. Vaandrager, J. A. A. W. Elemans, R. J. M. Nolte, *Nature Reviews Chemistry* **2018**, *2*, 365.
[8] D. Karamessini, S. Poyer, L. Charles, J.-F. Lutz, *Macromol. Rapid Commun.* **2017**, 1700426.
[9] P. Chandra, A. M. Jonas, A. E. Fernandes, *J. Am. Chem. Soc.* **2018**, *140*, 5179.
[10] A. Kuroki, P. Sangwan, Y. Qu, R. Peltier, C. Sanchez-Cano, J. Moat, C. G. Dowson, E. G. L. Williams, K. E. S. Locock, M. Hartlieb, S. Perrier, *ACS Applied Materials & Interfaces* **2017**, *9*, 40117.
[11] M. Porel, D. N. Thornlow, C. M. Artim, C. A. Alabi, *ACS Chemical Biology* **2017**, *12*, 715.

[12] J. Zhang, G. Gody, M. Hartlieb, S. Catrouillet, J. Moffat, S. Perrier, *Macromolecules* **2016**, *49*, 8933.
[13] A. S. Knight, E. Y. Zhou, M. B. Francis, R. N. Zuckermann, *Advanced Materials* **2015**, *27*, 5665.
[14] M. R. Golder, Y. Jiang, P. E. Teichen, H. V. T. Nguyen, W. Wang, N. Milos, S. A. Freedman, A. P. Willard, J. A. Johnson, *J. Am. Chem. Soc.* **2018**, *140*, 1596.
[15] J. Zhang, R. Deubler, M. Hartlieb, L. Martin, J. Tanaka, E. Patyukova, P. D. Topham, F. H. Schacher, S. Perrier, *Macromolecules* **2017**, *50*, 7380.
[16] J. Li, S. N. Rothstein, S. R. Little, H. M. Edenborn, T. Y. Meyer, *J. Am. Chem. Soc.* **2012**, *134*, 16352.
[17] P. Espeel, F. E. Du Prez, *Macromolecules* **2015**, *48*, 2.
[18] S. Martens, J. O. Holloway, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, 1700469.
[19] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
[20] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
[21] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angew. Chem., Int. Ed.* **2011**, *50*, 60.
[22] S. C. Solleder, R. V. Schneider, K. S. Wetzel, A. C. Boukis, M. A. R. Meier, *Macromol. Rapid Commun.* **2017**, *38*, 1600711.
[23] P. Espeel, F. E. Du Prez, *Eur. Polym. J.* **2015**, *62*, 247.
[24] S. Mommer, H. Keul, M. Möller, *Macromol. Rapid Commun.* **2014**, *35*, 1986.
[25] Y. Yan, L. Xue, J. B. Miller, K. Zhou, P. Kos, S. Elkassih, L. Liu, A. Nagai, H. Xiong, D. J. Siegwart, *Polymer* **2015**, *72*, 271.
[26] S. Montolio, O. Zagorodko, R. Porcar, M. Isabel Burguete, S. V. Luis, H. Tenhu, E. García-Verdugo, *Polym. Chem.* **2017**, *8*, 4789.
[27] Z. Zhang, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Polymer* **2015**, *64*, 221.
[28] P. Espeel, L. L. G. Carrette, K. Bury, S. Capenberghs, J. C. Martins, F. E. Du Prez, A. Madder, *Angew. Chem., Int. Ed.* **2013**, *52*, 13261.
[29] F. Driessen, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2015**, *4*, 616.
[30] L. Yu, Z. Zhang, Y.-Z. You, C.-Y. Hong, *Eur. Polym. J.* **2018**, *103*, 80.
[31] F. Driessen, S. Martens, B. D. Meyer, F. E. Du Prez, P. Espeel, *Macromol. Rapid Commun.* **2016**, *37*, 947.
[32] A. J. Satti, P. Espeel, S. Martens, T. Van Hoeylandt, F. E. Du Prez, F. Lynen, *Journal of Chromatography A* **2015**, *1426*, 126.
[33] S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.
[34] Z. L. Li, Z. C. Li, "Periodic Copolymers by Step-Growth Polymerization", in *Sequence-Controlled Polymers*, Wiley-vch, 2017, p. 349.
[35] M. Katsara, T. Tselios, S. Deraos, G. Deraos, M. T. Matsoukas, E. Lazoura, J. Matsoukas, V. Apostolopoulos, *Current Medicinal Chemistry* **2006**, *13*, 2221.
[36] D. Van Lysebetten, S. Felissati, E. Antonatou, L. L. G. Carrette, P. Espeel, E. Focquet, F. E. Du Prez, A. Madder, *ChemBioChem* **2018**, *19*, 641.
[37] J. O. Holloway, S. Aksakal, F. E. D. Prez, C. R. Becer, *Macromol. Rapid Commun.* **2017**, *38*, 1700500.
[38] C. E. Hoyle, A. B. Lowe, C. N. Bowman, *Chemical Society Reviews* **2010**, *39*, 1355.
[39] S. Mommer, K.-N. Truong, H. Keul, M. Möller, *Polym. Chem.* **2016**, *7*, 2291.
[40] Y. Chen, P. Espeel, S. Reinicke, F. E. Du Prez, M. H. Stenzel, *Macromol. Rapid Commun.* **2014**, *35*, 1128.
[41] M. Langlais, O. Coutelier, M. Destarac, *Macromolecules* **2018**, *51*, 4315.
[42] T. Rudolph, P. Espeel, F. E. Du Prez, F. H. Schacher, *Polym. Chem.* **2015**, *6*, 4240.
[43] J.-J. Yan, J.-T. Sun, Y.-Z. You, D.-C. Wu, C.-Y. Hong, *Scientific Reports* **2013**, *3*, 2841.
[44] R.-R. Wang, J.-J. Yan, R.-L. Yang, D.-H. Pan, W. Li, Y.-P. Xu, L.-Z. Wang, X.-Y. Wang, M. Yang, *Journal of Polymer Science Part A: Polymer Chemistry* **2017**, *55*, 2053.
[45] M. Langlais, I. Kulai, O. Coutelier, M. Destarac, *Macromolecules* **2017**, *50*, 3524.
[46] D. Frank, P. Espeel, S. Claessens, E. Mes, F. E. Du Prez, *Tetrahedron* **2016**, *72*, 6616.
[47] J.-F. Lutz, B. V. K. J. Schmidt, S. Pfeifer, *Macromol. Rapid Commun.* **2011**, *32*, 127.

[48] S. Pfeifer, Z. Zarafshani, N. Badi, J. F. Lutz, *Journal of the American Chemical Society* **2009**, *131*, 9195.
[49] T. T. Trinh, L. Oswald, D. Chan-Seng, J.-F. Lutz, *Macromol. Rapid Commun.* **2014**, *35*, 141.
[50] D. Chan-Seng, J.-F. Lutz, *ACS Macro Lett.* **2014**, *3*, 291.
[51] D. Chan-Seng, J.-F. Lutz, "Solid-Phase Synthesis as a Tool for the Preparation of Sequence-Defined Oligomers Based on Natural Amino Acids and Synthetic Building Blocks", in *Sequence-Controlled Polymers: Synthesis, Self-Assembly, and Properties*, American Chemical Society, 2014, p. 103.
[52] T. T. Trinh, L. Oswald, D. Chan-Seng, L. Charles, J.-F. Lutz, *Chemistry – A European Journal* **2015**, *21*, 11961.
[53] M. Nicola, P. A. Gatti, M. Zacche, "Process for the resolution of homocysteine-thiolactone", Google Patents, 2008.
[54] Y. Abdouni, G. Yilmaz, C. R. Becer, *Macromol. Rapid Commun.* **2017**, 1700212.
[55] M. Porel, D. N. Thornlow, N. N. Phan, C. A. Alabi, *Nat. Chem.* **2016**, *8*, 590.
[56] Y. Chen, Z. Guan, *J. Am. Chem. Soc.* **2010**, *132*, 4577.
[57] F. Driessen, R. Herckens, P. Espeel, F. E. Du Prez, *Polym. Chem.* **2016**, *7*, 1632.
[58] M. Baier, M. Giesler, L. Hartmann, *Chemistry-a European Journal* **2018**, *24*, 1619.
[59] F. Amir, Z. Jia, M. J. Monteiro, *J. Am. Chem. Soc.* **2016**, *138*, 16600.
[60] J. F. W. Keana, A. P. Guzikowski, D. D. Ward, C. Morat, F. L. Van Nice, *J. Org. Chem.* **1983**, *48*, 2654.
[61] S. Billiet, K. De Bruycker, F. Driessen, H. Goossens, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Nature Chemistry* **2014**, *6*, 815.
[62] O. Roling, K. De Bruycker, B. Vonhören, L. Stricker, M. Körsgen, H. F. Arlinghaus, B. J. Ravoo, F. E. Du Prez, *Angew. Chem., Int. Ed.* **2015**, *54*, 13126.
[63] S. Vandewalle, R. De Coen, B. G. De Geest, F. E. Du Prez, *ACS Macro Lett.* **2017**, *6*, 1368.
[64] H. A. Houck, K. De Bruycker, S. Billiet, B. Dhanis, H. Goossens, S. Catak, V. Van Speybroeck, J. M. Winne, F. E. Du Prez, *Chem. Sci.* **2017**, *8*, 3098.
[65] J. K. Szymański, Y. M. Abul-Haija, L. Cronin, *Accounts of Chemical Research* **2018**, *51*, 649.
[66] R. K. Roy, A. Meszynska, C. Laure, L. Charles, C. Verchin, J.-F. Lutz, *Nat. Commun.* **2015**, *6*, 7237.

List of publications

Publications included in this thesis

Multifunctional sequence-defined macromolecules for chemical data storage
S. Martens, A. Landuyt, P. Espeel, B. Devreese, P. Dawyndt, F. Du Prez, *Nat. Commun.* **2018**, *9*, 4451.

Click and Click-Inspired Chemistry for the Design of Sequence-Controlled Polymers
S. Martens, J. O. Holloway, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, 38, 1700469.

Automated Synthesis of Monodisperse Oligomers, Featuring Sequence Control and Tailored Functionalization
S. Martens, J. Van den Begin, A. Madder, F. E. Du Prez, P. Espeel, *J. Am. Chem. Soc.* **2016**, *138*, 14182.

Double Modification of Polymer End Groups through Thiolactone Chemistry
F. Driessen, S. Martens, B. D. Meyer, F. E. Du Prez, P. Espeel, *Macromol. Rapid Commun.* **2016**, *37*, 947.

Tunable temperature responsive liquid chromatography through thiolactone-based immobilization of poly(N-isopropylacrylamide)
A. J. Satti, P. Espeel, S. Martens, T. Van Hoeylandt, F. E. Du Prez, F. Lynen, *Journal of Chromatography A* **2015**, *1426*, 126.

Publications outside this thesis

Enhancing the Possibilities of Comprehensive Two-Dimensional Liquid Chromatography through Hyphenation of Purely Aqueous Temperature-Responsive and Reversed-Phase Liquid Chromatography
M. Baert, S. Martens, G. Desmet, A. de Villiers, F. Du Prez, F. Lynen, *Analytical Chemistry* **2018**, *90*, 4961.

Responsive Thiolactone-Derived N-Substituted Poly(Urethane-Amide)s
P. Espeel, S. Celasun, P. S. Omurtag, S. Martens, F. E. Du Prez, *Macromol. Rapid Commun.* **2017**, *38*, 1600783.

Combining Two Methods of Sequence Definition in a Convergent Approach: Scalable Synthesis of Highly Defined and Multifunctionalized Macromolecules
S. C. Solleder, S. Martens, P. Espeel, F. Du Prez, M. A. R. Meier, *Chemistry – A European Journal* **2017**, *23*, 13906.

One-Pot Modular Synthesis of Functionalized RAFT Agents Derived from a Single Thiolactone Precursor
S. Martens, F. Driessen, S. Wallyn, O. Türünç, F. E. Du Prez, P. Espeel, *ACS Macro Lett.* **2016**, *5*, 942.

Diversely Substituted Polyamide Structures through Thiol−Ene Polymerization of Renewable Thiolactone Building Blocks
F. Goethals, S. Martens, P. Espeel, O. van den Berg, F. E. Du Prez, *Macromolecules* **2014**, *47*, 61.

Book chapter

Click and Click-Inspired Chemistry for the Design of Sequence-Controlled Polymers
S. Martens, J. O. Holloway, F. E. Du Prez, "Click and Click-Inspired Chemistry for the Design of Sequence-Controlled Polymers", in Sequence-Controlled Polymers, Wiley-vch, **2017**.



In 2009, the PCR group started combining two reactions in the same pot: the aminolysis of a thiolactone and the subsequent thiol-ene reaction. The opening of the thiolactone with an amine generates a thiol which then reacts with an ene functionality. This so-called amine-thiolactone-ene conjugation has enabled several research groups, including ours, to synthesise various macromolecular architectures and to perform double modifications of polymers. Recently, this conjugation has been explored for the synthesis of sequence-controlled polymers, which form a new, interesting class of polymers in which the monomer order is controlled to a greater extent. An important subclass of these polymers consists of the sequence-defined macromolecules, which have a uniform and discrete structure.

For this doctoral dissertation, the efficient amine-thiolactone-ene conjugation was explored for the double modification of polymeric systems and the synthesis of sequence-controlled polymers. This conjugation was first applied for the synthesis of functionalised, temperature-responsive, liquid chromatography stationary phases and next used to double-modify thiolactones immobilised on a solid support or as the end group of a polymer. With the knowledge obtained from these double modifications of immobilised thiolactones, the synthesis of sequence-defined and sequence-controlled macromolecules was tackled. Multifunctional synthetic sequence-defined oligomers were prepared with a newly developed protecting-group-free two-step iterative protocol on solid support, which was also carried out later in an automated fashion. These oligomers were capable of storing human- and machine-readable data, such as a QR-code. Finally, these oligomers and oligopeptides were functionalised with specific handles, which can be linked together to obtain both periodic sequence-controlled polymers and functionalised cyclised species.

www.pcr.ugent.be

www.cmac.ugent.be

Ghent University
Polymer Chemistry Research Group (PCR)
Centre of Macromolecular Chemistry (CMaC)
Department of Organic and Macromolecular Chemistry
Krijgslaan 281, S4bis
9000 Ghent, Belgium