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Straightforward synthesis of functionalized cyclic polymers in high yield via RAFT and thiolactone/disulfide chemistry

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ABSTRACT

An efficient synthetic pathway toward cyclic polymers based on the combination of thiolactone and disulfide chemistry, has been developed. First, heterotelechelic linear polystyrene (PS) containing an α-thiolactone (TLa) and an ω-dithiobenzoate group was synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization, employing a newly designed TLa-bearing chain transfer agent. The subsequent reaction of this heterotelechelic polymer with an amine, which acts as a nucleophile for both the TLa and dithiobenzoate units, generated the α,ω-thiol-telechelic PS under ambient conditions without the need for any catalyst or other additive. The arrangement of thiols under a high dilution afforded single cyclic *c*-PSs through an oxidative disulfide linkage. The cyclic polystyrene (*c*-PS) disulfide ring formation was evidenced by SEC, MALDI-TOF MS and ^{1}H-NMR characterization. Moreover, we demonstrated a controlled ring opening via either disulfide reduction or thiol/disulfide exchange to enable easy and clean topology transformation. Furthermore, to illustrate the broad utility of this synthetic methodology, different amines including functional ones were employed, allowing for the one-step preparation of functionalized cyclic polymers with high yields.

INTRODUCTION

Topological polymer chemistry[1-3] has been expanding significantly with the growing need of fundamental research to address unusual polymer architectures having programmed chemical structures, and particularly with the development of controlled radical polymerization (CRP)[4] and 'click' chemistry during the last decade[5-8]. Polymer chemists can nowadays use a variety of synthetic methods, including robust, high yielding, simple covalent chemistries, to construct those unusual polymer topologies, yet with a precise control over the functionality, domain size, reactivity and solubility[9-11]. Concomitantly, such well-defined and discrete macromolecular design based on single-cyclic and multi-cyclic polymers, results in unique physical properties and functions for the derived polymer materials[2]. For example, rather than exclusively being influenced by the chemical composition, detailed investigations by Honda *et al.*[12] have recently revealed that the thermal stability of self-assembled micelles from cyclic polymers was remarkably enhanced by a topology effect. Moreover, specific properties such as their compact hydrodynamic volume, higher glass transition temperature and distinguished dynamic behavior are some of the features that are otherwise inaccessible through linear or branched topological analogs.[1]

Although the primitive single-cyclic form conceptually represents the simplest cyclic topology, the synthetic constraints in general prevent from the clean and easy production of uniform cyclic polymers, particularly when availability of a specific functional group[13] for further topological upgrade is desired[14,15]. The common syntheses of single-cyclic polymers employ end-to-end ring-closure and ring-expansion polymerization[7]. The former process entails bimolecular, *homo*-unimolecular and *hetero*-unimolecular reactions. The unimolecular processes rely on telechelic structures having identical or complementary groups for the

formation of a cycle. This has been realized by the use of a metathesis polymer cyclization[16-18] in a case of a *homo*-unimolecular reaction. On the other hand, deprotection/activation treatment of the asymmetrically functional α-acetal-ω-styryl PS telechelic precursor[19] and an intramolecular amidation reaction of α-amino-ω-carboxyl PS and poly(*tert*-butylacrylate)[20] are examples for a ring formation by *hetero*-unimolecular process. However, the recent advances in CRP[21] hold more promise in terms of easiness to access various functional telechelics prior to a cyclization step. For example, Glassner *et al.* subjected atom transfer radical polymerization (ATRP)-derived α-(furan-protected)-maleimide-ω-cyclopentadienyl poly(methyl methacrylate) and poly(*tert*-butyl acrylate) to a heat treatment to afford cyclic polymers via Diels-Alder addition.[22] Durmaz *et al.*[23] also recently reported the synthesis of cyclic homo- and block copolymers via Diels-Alder coupling of anthracene and maleimide end-groups. Furthermore, the advent of robust, efficient and orthogonal 'click' chemistries, as a toolbox complementary to CRP,[24-27] enabled Laurent *et al.*[28] to prepare cyclic PS, pioneering the use of a 'click' chemistry in polymer cyclization field. Likewise, Sugai *et al.*[9] employed azide-alkyne addition in conjuction with an *electrostatic self-assembly and covalent fixation* (ESA-CF) to effectively construct a variety of unprecedented multicyclic polymer topologies. Goldmann *et al.*[29] described the use of RAFT polymerization, followed by end-group modification to facilitate azide-alkyne 'click' chemistry as a simple and effective way to generate macrocyclic PS. Recently, Athanasios *et al*.[30] reported the synthesis of cyclic PS-*b*-polyisoprene through acetylene- and azido-'clickable' end-groups. Next to the azide-alkyne 'click' reaction, Dove and co-workers[31] employed the thiol-ene chemistry, specifically the Michael addition of thiols to maleimides, as a facile methodology for the preparation of biodegradable structures. 'Click' chemistry also enabled a programmed

polymer folding[11,32] such as doubly fused tricyclic and triply fused tetracyclic polymer topologies.[10]

One particularly attractive feature of RAFT[33-35] polymerization is the accessibility to the mercapto group as a functional handle for complementary pairing with thiol-related chemistries.[36,37] Surprisingly, until now, RAFT synthetic strategies, where a direct use of thiols is exploited, have not been extensively used to produce cyclic macromolecular topologies. An example has been reported of monocyclic PS prepared via oxidation of the α,ω-thiol-containing PS to afford one disulfide linkage per cyclic polymer chain.[38] The α,ω-thiol-containing PS was synthesized by polymerizing styrene in the presence of a difunctional RAFT agent and subsequent conversion of the dithioester end-groups to thiols through aminolysis. In contrast, He *et al*.[39] prepared cyclic poly(methyl acrylate) by monomer insertion into a cyclic dithioester-based initiator using γ-ray-induced radical polymerization at low temperatures. Attention has since turned to the application of RAFT polymerization and copper-catalyzed azide-alkyne cycloaddition to form 2- and 3-arm cyclic stars.[40]

All methodologies described so far restrict the final cyclic structure to rather simple architectures, and the synthesis of more complex polymer topologies requires a tandem use of unlike synthetic strategies. These strategies suffer from multi-step end-group modifications of the linear polymeric precursors, often proceeding with limited yields and under high temperatures. For the formation of sophisticated single cyclic molecular nanostructures, instead, one has to choose a reaction pathway with the complementary partners at individual polymer termini. Building on this prior art, in the current work, we employed an elegant synthetic approach based on RAFT polymerization and thiolactone chemistry as a precursor to a free thiol,[41] recently proposed by some of us as a facile method toward functional, linear polymers and networks. The key to this strategy is the *in situ* generation of thiols driven by

the nucleophilic ring-opening of a thiolactone with amines, inspired by a decades-old method for the introduction of sulfhydryl groups in natural proteins.[42] The initial demand to design a thiolactone-containing chain transfer agent (CTA) was therefore mandatory, providing the direct access to a thiol group at both α and ω polymer termini upon the treatment with an amine (see further *intermediate* in Scheme 2).[36,41] Then, the *in situ* produced thiol-telechelics can engage through a disulfide bonding in an intramolecular fashion to yield cyclic polymers, under high dilution and ambient conditions (open air, room temperature, without a need for a catalyst or any additive). The formed disulfide bridge is of particular interest because of thiol-disulfide exchange reactions and self-healing properties.[43-45] Inspired by those fascinating features of disulfide bonds, this work adds on one hand an example to the growing number of cyclic polymer topologies, as recently reviewed by Monteiro *et al.*[7] On the other hand, the thiolactone/disulfide approach toward *c*-PS presented herein widens the range of possible unusual topologies that have practical implications to be manufactured by available synthetic methods. The reason for that primarily lays in the potential use of functionalized amines allowing the *c*-PS to be equipped with desired functional groups for further topology upgrade, yet with the possibility to produce various topologies.

EXPERIMENTAL SECTION

Materials

4,4'-Azobis(4-cyanopentanoic acid) (≥ 98%), phosphorus pentachloride (≥ 98%), *n*-propylamine (≥ 99%), ethanolamine (≥ 98%), tri-*n*-butylphosphine (99%) were purchased from Sigma-Aldrich and used as received. Homocysteine-γ-thiolactone hydrochloride (99%) was purchased from Acros Organics and used without any further purification. Styrene (St) (99%, extra pure) (Acros Organics) was passed through a column of basic alumina to remove

the radical inhibitor. 2,2'-Azobis(isobutyronitrile) (AIBN) (Sigma-Aldrich) was recrystallized twice from methanol. Dichloromethane (DCM) (≥ 99.9%) and triethylamine (HPLC grade) were purchased from Sigma-Aldrich and distilled from CaH_2 prior to use. Ethylacetate (EtOAc) (Aldrich, HPLC grade) was used without purification. The acid chloride of 4,4'-azobis(4-cyanopentanoic acid)[46] and bis(thiobenzoyl) disulfide[47] were synthesized according to literature procedures. Silicagel (ROCC, SI 1721, 60 Å, 40 – 63 μm) was used to perform preparative column chromatography, eluting with HPLC-grade solvents. The collected fractions were analyzed by thin layer chromatography (TLC-plates, Macherey-Nagel, SIL G-25 UV254). All other solvents employed were purchased from Sigma-Aldrich (HPLC grade), and used without further purification.

Characterization

Nuclear magnetic resonance (^{1}H- and ^{13}C-NMR (Attached Proton Test, APT)) spectra were recorded at 300 or 500 MHz in $CDCl_3$ (Eurisotop) solution at room temperature on a Bruker Avance 300 or Bruker AM500 spectrometer, respectively. Chemical shifts are presented in parts per million (δ) relative to $CHCl_3$ and DMSO-d_6 (7.26 ppm and 2.50 ppm in ^{1}H- and 77.23 ppm and 39.51 ppm in ^{13}C-NMR respectively) as internal standard. Coupling constants (J) in ^{1}H-NMR are given in Hz. The resonance multiplicities are described as *d* (doublet) or *m* (multiplet).

Size Exclusion Chromatography (SEC) analyses were performed on 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.

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 ionization (MMI) source.

Matrix assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF MS) was performed on an Applied Biosystems Voyager De STR MALDI-TOF spectrometer equipped with 2 m linear and 3 m reflector flight tubes, and a 355 nm Blue Lion Biotech Marathon solid state laser (3.5 ns pulse). All mass spectra were obtained with an accelerating potential of 20 kV in positive ion mode and in linear mode. 1,8,9-Anthracenetriol (Dithranol) (20 mg/mL in THF) was used as a matrix, AgF_3Ac (1 mg/mL) was used as a cationizing agent, and polymer samples were dissolved in THF (10 mg/mL). Analyte solutions were prepared by mixing 5 μL of the matrix, 5 μL of the polymer and 10 μL of the salt 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 (M_n = 2000 g/mol) was used for calibration. All data were processed using the Data Explorer 4.0.0.0 (Applied Biosystems) software package.

UV-vis measurements were performed on an Analytik Jena AG SPECORD® 200 double-beam UV-VIS spectrophotometer. Absorbance was measured with the WinASPECT® software in the spectral range 230 – 600 nm and a speed of 5 nm/s. The internal wavelength was calibrated with a holmium oxide filter.

Synthesis

*Synthesis of compound **3** (Scheme 1)*

A suspension of homocysteine-γ-thiolactone hydrochloride (Scheme 1, **2**) (1.94 g, 12.61 mmol) in anhydrous CH_2Cl_2 (40 mL) was treated with Et_3N (4.4 mL, 31.53 mmol) at 0 °C. A solution of the freshly prepared acid chloride of 4,4'-azobis(4-cyanopentanoic acid) (**1**) (2.00 g, 6.31 mmol) in CH_2Cl_2 (20 mL) was added after 10 min at 0 °C via a cannula. The reaction was stirred overnight at room temperature. After evaporation of the solvent and column chromatography of the crude mixture (silica gel, EtOAc), the title compound was isolated (1.75 g, 3.66 mmol, 58%) as an off-white solid. LC-MS analysis (Figure S1) revealed that **3** (Scheme 1) consists of two peaks, most likely representing the *E*- and *Z*-isomers, which consequently have the same MS spectrum.

$C_{20}H_{26}N_6O_4S_2$ (478.6); *m/z* (ESI-MS) 479.1.

^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ 8.38 (*m*, 2H), 4.61 (*m*, 2H), 3.39 (*m*, 4H), 3.27 (*m*, 4H), 2.50 → 1.97 (*m*, 8H), 1.70 and 1.64 (2 *singlets*, 6H).

^{13}C-NMR (75 MHz, DMSO-d_6, ppm) δ 205.4 (C), 170.2 (C, 2 signals), 118.2 (C, 4 signals), 71.9 (C, 4 signals), 58.2 (CH), 33.0 (CH_2, 2 signals), 30.2 (CH_2), 29.9 (CH_2, 2 signals), 26.8 (CH_2), 23.2/22.9 (CH_3).

Synthesis of thiolactone-containing dithiobenzoate (**TLa-CTA**, **5**, Scheme 1).

A suspension of the azo-compound **3** (Scheme 1) (2.87 g, 6.00 mmol) and bis(thiobenzoyl) disulfide **4** (Scheme 1) (1.23 g, 4.00 mmol) in EtOAc (20 mL) was degassed and heated overnight at 80 °C. The resulting brown reaction mixture was purified by column chromatography (silica gel, eluents: CH_2Cl_2/*n*-hexane/EtOAc = 1/1/1). The pink fractions

were collected, concentrated and analyzed (Figure S2 and S3). The **TLa-CTA**, **5** (1.22 g, 3.22 mmol) was obtained in 40% yield.

$C_{17}H_{18}N_2O_2S_3$ (378.5); *m/z* (ESI-MS) 379.0. HRMS: Expected 379.0603, Found 379.0607 $[M+H]^+$.

^{1}H-NMR (300 MHz, $CDCl_3$, ppm) δ 7.90 (*m*, 2H), 7.56 (*m*, 1H), 7.39 (*m*, 2H), 6.18 (*d*, 5.5 Hz, 1H), 4.52 (*m*, 1H), 3.40 - 3.22 (*m*, 2H), 2.88 (*m*, 1H), 2.71 - 2.36 (*m*, 4H), 2.04 - 1.89 (*m*, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$, ppm) δ 226.6 (C), 205.4 (C), 171.1 (C), 144.7 (C), 133.2 (CH), 128.8 (CH), 126.9 (CH), 118.8 (C), 59.7 (CH), 46.2 (C), 34.0 (CH_2), 31.9 (CH_2), 31.7 (CH_2), 27.7 (CH_2), 24.4 (CH_3).

General polymerization procedure

All polymerizations were performed in bulk conditions and AIBN was employed as the thermal initiator. A typical polymerization procedure is as follows (Scheme 2): monomer, **TLa-CTA**, **5** and AIBN ($[M]_0/[CTA]_0/[AIBN]_0$=200/1/0.1) were placed in a Schlenk tube, degassed by three freeze-pump-thaw cycles, backfilled with nitrogen, sealed and heated in an oil bath at 70 °C. The concentrations and reaction conditions for each specific reaction are given in Table 1. The reaction mixture was quenched in liquid nitrogen. The polymerization kinetics using the various **TLa-CTA**, **5** was monitored by ^{1}H-NMR and SEC. For each single reaction, at specific time intervals during the polymerization, small aliquots were withdrawn from the polymerization solution and analyzed by ^{1}H-NMR spectroscopy for the determination of monomer conversions. SEC was employed for the determination of the molecular weights and polydispersity indices (PDI) of the resulting polymers. The purified

polymers were obtained by repeated precipitation in 10-fold excess of cold methanol. The polymer was collected by filtration and dried at least overnight *in vacuo*.

General procedure for the cyclization reaction

A typical procedure is as follows: *n*-propylamine (25 mL, 0.30 mol) or ethanolamine (18.1 mL, 0.30 mol) was added to a 1 L round-bottomed flask and dissolved in 600 mL freshly distilled DCM (0.5 M). The *l*-PS-TLa (**6**) polymer pre-solution was prepared in a separate vial by adding polymer (120 mg, 3.08 x 10^{-5} mol, M_n = 3900 g/mol, PDI = 1.07) in 10 mL DCM (0.05 mM polymer concentration). The polymer pre-solution was then transferred to a syringe prior to adding to the *n*-propylamine DCM solution via a syringe pump (12 h). Once the polymer was finished adding to the amine solution, the reaction was allowed to proceed at room temperature and under the open air for 2 additional days. The crude polymer was then concentrated *in vacuo*. The excess of *n*-propylamine was evaporated, while ethanolamine was removed by extraction with distilled water/0.1 M HCl (80/20). Finally, the polymer was precipitated in 10-fold excess cold methanol and dried overnight *in vacuo* to give a white powder (90%).

Ring opening of disulfide-containing c-PS (7a)

Reduction with tri-n-butylphosphine

To a 10 mL round-bottomed flask was added 4 mg *c*-PS (**7**) (1.54 x 10^{-6} mol) and dissolved into 1 mL THF. The flask was sealed and the solution was purged with nitrogen for 30 min followed by the addition of 500 μL (2.00 x 10^{-3} mol) reducing agent tri-*n*-butylphosphine. The solution was then allowed to stir overnight at room temperature before subjecting to SEC analysis.

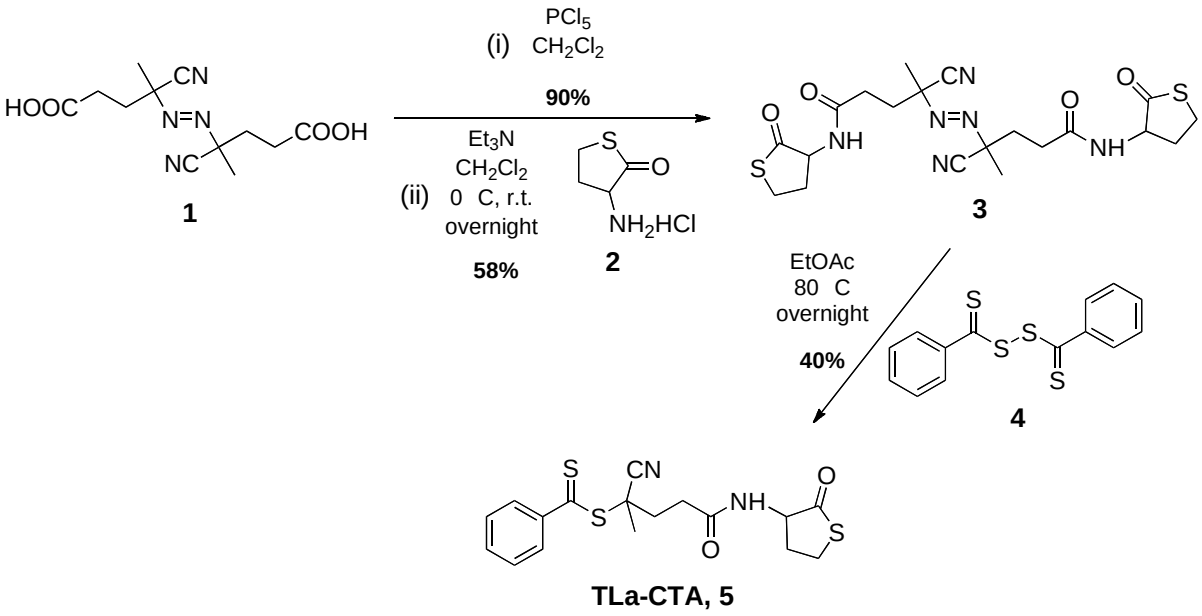
Thiol/disulfide exchange with octanethiol

To a 10 mL round-bottomed flask was added 4 mg *c*-PS (**7**) (1.54 x 10^{-6} mol) and dissolved into 1 mL DMF. The flask was sealed and 1 mL octanethiol (5.76 x 10^{-3} mol) was added to the flask. In one example, the solution was then heated to 90 °C, and allowed to stir overnight before subjecting to SEC analysis. In another example, the solution was allowed to stir overnight at room temperature before subjecting to SEC analysis.

RESULTS AND DISCUSSION

Synthesis of the thiolactone-containing chain-transfer agent (TLa-CTA, 5*)*

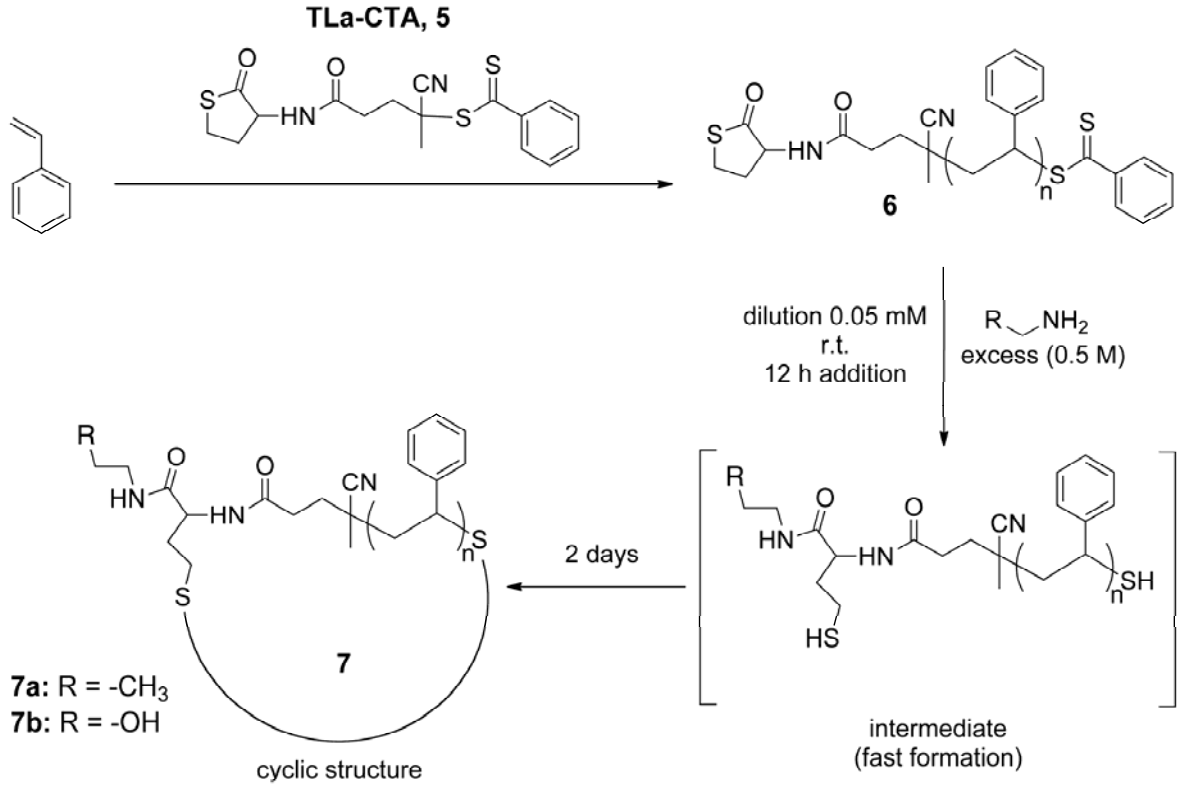
The synthesis of a thiolactone containing CTA was adapted from the procedure for the synthesis of 4-cyanopentanoic acid dithiobenzoate, a versatile RAFT controlling agent.[48] Activation of the carboxylic acid group in the commercially available 4,4'-azobis(4-cyanopentanoic acid) (Scheme 1, **1**)[46] and subsequent treatment of the corresponding acid chlorides with homocysteine-γ-thiolactone hydrochloride (Scheme 1, **2**) yielded azo-compound **3** (Scheme 1). The thermal decomposition of an 1.5 excess of **3** in the presence of bis(thiobenzoyl) disulfide **4** allowed for the preparation of the **TLa-CTA** (Scheme 1, **5**).



Scheme 1. Synthesis of the thiolactone-containing CTA (**TLa-CTA**, **5**).

Synthesis and characterization of thiolactone (TLa) containing poly(styrene) (*l*-PS-TLa, 6)

In the first step, to achieve a PS semi-telechelic chain equipped with a thiolactone unit at the α-terminus, we employed the newly designed **TLa-CTA** (**5**) to mediate RAFT polymerization of St (Scheme 2). In addition, the resulting PS bears a dithiobenzoate group at the ω-terminus, having therefore two thiol groups in a latent form, each at the opposite polymer chain-end, to encode for the α,ω-thiol-telechelic upon the nucleophilic reaction with an amine (see *intermediate*, Scheme 2).



Scheme 2. Combined RAFT and thiolactone approach toward functionalized cyclic polymers.

The crucial point during the polymerization is the inertness of the thiolactone unit against the reaction temperature and carbon-centered propagating polymeric radicals.[49] Previously conducted stability tests indicated that the thiolactone unit remained intact at moderate polymerization temperatures, *i.e.* 70 °C, and inert to a radical concentration found in a typical RAFT polymerization.[49] Hence, the RAFT polymerization was conducted at 70 °C in bulk, with a monomer-to-CTA ratio of 200 that can generate PS with targeted molecular weights, yet with a low conversion (*ca.* 25%) to maintain high TLa end-group fidelity (Table 1, Scheme 2).

Table 1. Summary of the reaction conditions and results of RAFT polymerization of St mediated by **TLa-CTA** (**5**).

Entry[a]	Time, h	Conv.,[b] %	M_n^{exp},[c] g/mol	PDI[c]	TLa fidelity,[d] %
1	6	16	3900	1.07	95
2	6	13	3300	1.09	96
3	8	22	4700	1.07	96

[a] Reaction conditions: $[St]_0/[TLa\text{-}CTA]_0/[AIBN]_0=200/1/0.1$; 70 °C, bulk;
[b] Calculated from ^{1}H-NMR;
[c] SEC, calibrated with PS standards, THF as eluent;
[d] Calculated from ^{1}H-NMR, ensuring an excellent agreement between the degree of polymerization (DP) calculated from: ^{1}H-NMR signal integration ratios and MWs observed in SEC.

Samples, periodically taken from the reaction mixture, were analyzed by SEC and ^{1}H-NMR to follow the progress of the polymerization (Table 1, Entry 1). A monomer conversion vs. time plot showed linear dependence and the desired conversion was achieved within 6 h with expected molecular weight of the isolated polymer (Figure 1a). The SEC traces indicated a linear increase of the molecular weights with conversion during the entire course of the reaction (Figure 1b), having the apparent and theoretical MW values in a good agreement. The controlled nature of the process was further confirmed by low polydispersity indices (PDI) below 1.10. The polymerization was quenched in liquid nitrogen and the polymer was purified by precipitation from cold methanol to recover **6** (Scheme 2) as a light-pink powder (Figure S4b).

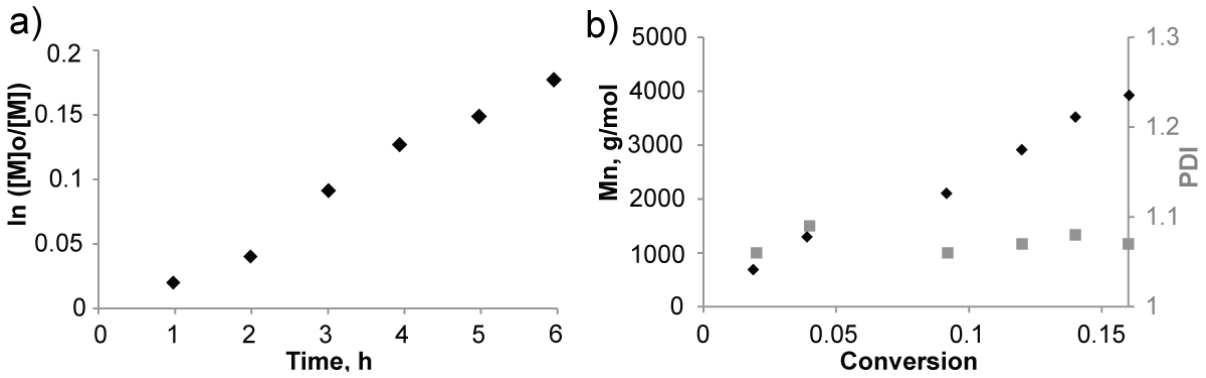


Figure 1. Polymerization of St using **TLa-CTA**, at 70 °C, in bulk and with 0.1 equiv AIBN as the radical source, St/**TLa-CTA**/AIBN = 200/1/0.1 (**Table 1**, Entry 1): a) first-order kinetic plot; b) molecular weight and PDI evolution with monomer conversion.

The polymer was characterized by 500 MHz ^{1}H-NMR, UV-vis spectroscopy and SEC. Noteworthy is that MALDI-TOF MS was not suitable to study PS made by RAFT, even though wide range of experimental conditions were examined. Concerning that, fragmentations of the dithioester end groups under MALDI-TOF MS conditions were already reported.[50,51]

At this stage, it is essential to determine the TLa content, as a quantitative amount is preferred for a successful cyclization reaction. Thus, the TLa end-group fidelity of the polymer was calculated by combining both the results from high resolution ^{1}H-NMR and SEC.[52] The protons from the thiolactone group can indeed be easily distinguished. Therefore, a detailed analysis of the integration values of the **TLa-CTA** (**5**) and its ring corresponding signals (see further Figure 4: *a*, *b*, *c*, *d* and *e*) and the PS backbone broad signal (Figure 4: *f*), combined with the results observed by SEC, revealed a TLa content at the α-terminus with 95% end-group fidelity (Table 1, Entry 1). The remaining *ca.* 5% counts for the polymer chains derived from the AIBN initiating adducts. Furthermore, as a member of the class of

thiocarbonyls, dithiobenzoate-functional RAFT polymers show a strong UV absorption band because of the π – π* transition of C=S group. Consequently, the presence of the dithiobenzoate end-group was confirmed qualitatively by UV-vis spectroscopy, which showed a maximum absorbance at *ca.* 303 nm (Figure S4a).

Synthesis and characterization of the cyclic poly(styrene) (*c*-PS, 7)

In addressing new cyclization strategies[7] to yield cyclic polymers, the role of the thiolactone chemistry emerged as a powerful tool to achieve that goal in a facile and elegant manner. The covalent disulfide linking of the individual *l*-PS-TLa (**6**) chains to afford cyclic molecules is based on two nucleophilic processes: the thiolactone ring opening at the α-terminus and the aminolysis of the dithiobenzoate unit at the ω-terminus. Given that the primary amine reacts with both thiolactone and dithiobenzoate group to yield two thiol species, our initial efforts focused on studying the cyclization involving TLa-containing PS and *n*-propylamine as a nucleophile. In general, to ensure complete reaction, a large excess of *n*-propylamine (0.5 M) was added to a flask with 600 mL DCM. Additionally, earlier observations revealed that both aminolysis and subsequent disulfide formation are promoted significantly when increasing the amine concentration in DCM.[49] Bearing in mind the oxidative coupling of free thiyl radicals to afford single *c*-PS, we anticipated that a quite low concentration of polymer would exclusively afford intramolecular cyclic product, thus avoiding intermolecular coupling reactions. Therefore, the pre-solution of *l*-TLa-PS (**6**), in 10 mL DCM, was added dropwise into a reactor filled with the amine solution via a syringe pump over extended time (10 h) with the flow rate as low as 1 mL/h, providing the final polymer concentration of 100 – 150 mg/L (0.05 mM). Thus, stirring the excess of *n*-propylamine and *l*-TLa-PS (**6**) upon the polymer addition, for two days at ambient

temperature and under the open air, followed by the removal of solvent and precipitation in cold methanol, afforded a cyclic polymer as a white powder product (Scheme 2, **7a** and Figure S4b). Isolated yields of 90% or higher were obtained in reproducible way.

The influence of the amine was apparent from the color change of the isolated polymer from a distinct pink to a white colour, indicating successful aminolysis of the dithiobenzoate group (Figure S4b). Moreover, UV-vis measurement was performed, further confirming the quantitative removal of the RAFT end-group as evidenced by a disappearance of a characteristic absorbance at *ca*. 303 nm (Figure S4a). Other than these preliminary observations, subsequent efforts were directed toward evaluating whether and to what extent a disulfide linkage, thus individual ring formation, had occurred. In this sense, SEC, ^{1}H-NMR and MALDI-TOF MS analysis were employed to facilitate the characterization of the synthesized cyclic polymer product. The success of the reaction was first evaluated by the SEC analysis of the starting *l*-PS (**6**) and final *c*-PS (**7a**) (Figure 2a). Transformation from the linear into a cyclic topology via disulfide intramolecular bridging caused an increase in the retention time and accordingly, the reduction of the measured M_p (from $M_{p,l}$ = 3500 to $M_{p,c}$ = 2700 g/mol). These results are consistent with a more compact hydrodynamic volume of a cyclic polymer in comparison to a linear counterpart. The hydrodynamic volume ratio or <G> value equals 0.77, which is in good agreement with precedent literature data.[53,54,55] Noteworthy is that a small fraction of *ca*. 5–7 % of intermolecular reaction product was observed at higher MWs, which corresponds to byproduct polymers lacking the TLa end groups (see above).

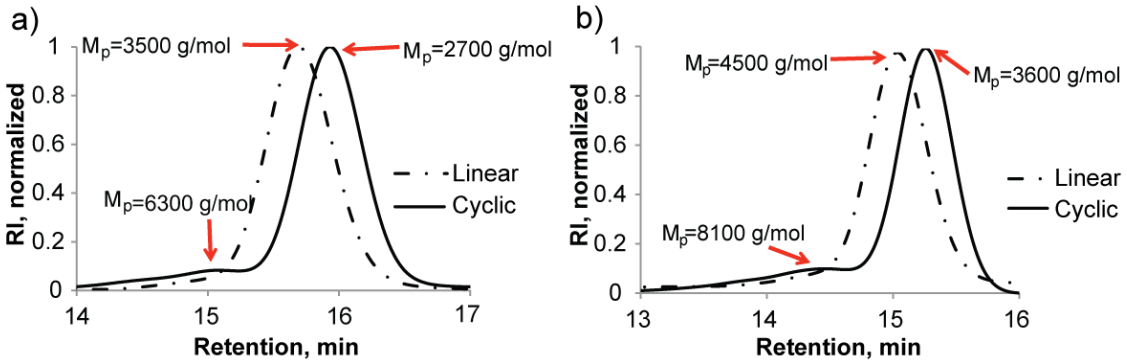


Figure 2. SEC profiles of TLa-terminated PS (*l*-PS, Scheme 2, **6**) and cyclic PS (*c*-PS, **Scheme 2**, **7**): a) reaction with *n*-propylamine (Table 1, Entry 1 and Scheme 2, **7a**); b) reaction with ethanolamine (Table 1, Entry 3 and Scheme 2, **7b**).

The cyclization through a disulfide bond was also clearly assessed by MALDI-TOF MS analysis, showing a well resolved spectrum with uniform series of peaks recorded in linear mode (Figure 3). The peaks are separated by 104 mass units corresponding to the molecular weight of a single styrene component. Moreover, each peak of the distribution matches the expected structure of a cyclic PS containing one disulfide bond and an amide group originating from a thiolactone ring (Table in Figure 3). Importantly, only the main series attributed to the cyclic PS was observed, suggesting that events such as fragmentation or side reactions at the disulfide site, occurred neither during the ring formation nor under the experimental conditions of MALDI-TOF MS measurements.[56]

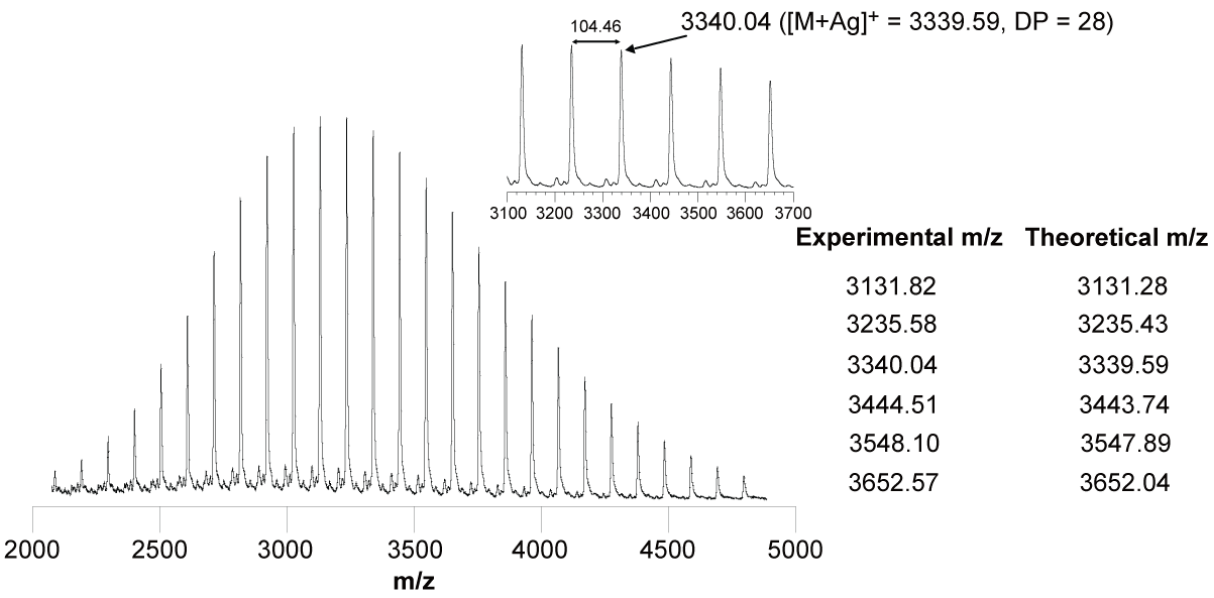


Figure 3. MALDI-TOF mass spectra of *c*-PS (**Scheme 2**, **7a**, R = -CH_3).

Finally, the ^{1}H-NMR spectrum presented in Figure 4 shows the disappearance of the characteristic signals for the protons of the TLa ring after the cyclization treatment with propyl amine (*a*, *b*, *c* and *e* labeled signals). Moreover, the appearance of *h* and *i* signals in the spectrum of a *c*-PS (**7a**) and their integration values further suggest successful ring closure via the proposed mechanism.

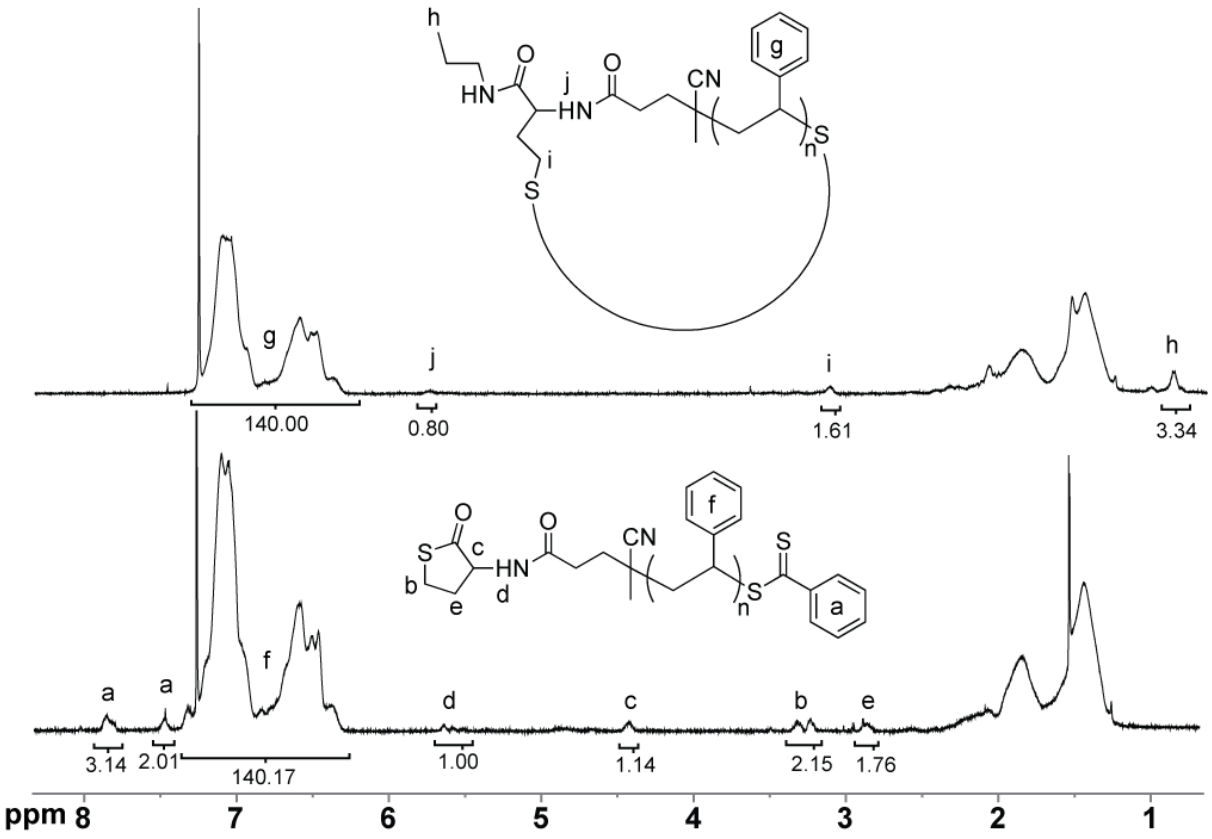


Figure 4. ^{1}H-NMR (500 MHz, $CDCl_3$) spectra of linear PS (*l*-PS, **6**, *bottom*) and cyclic PS (*c*-PS, **7a**, *top*) with corresponding signal integration values.

In contrast to the results presented above, when using a higher polymer concentration (*e.g.* 0.25 mM and 50 mM compared to the optimal 0.05 mM), a significant fraction of non-controlled intermolecular disulfide formation was observed in the SEC traces (Figure S5), yet lacking the apparent shift toward longer retentions typically observed with the ring creation. Additionally, when no amine was employed in a blank reaction, no cyclization occurred, further confirming the key role of both high dilution and the presence of amine as the only reactant to the additive-free thiolactone/disulfide synthetic strategy for the preparation of cyclic polymers.

Ring opening of disulfide-containing *c*-PS

Reduction of disulfide bond of the c-PS. One particularly attractive feature of disulfide bonds, though sufficiently stable in the extracellular environment, is their degradation in response to physiologically relevant reducing conditions.[57,58] Moreover, recent research has shown that the disulfide bridge in synthetic polymer systems can be selectively reduced[59,60] in the presence of phosphines. On the basis of this theory, it is expected that the disulfide bond of the *c*-PS reacts with a reducing agent to open a polymer ring, resulting in a thiol-telechelic *l*-PS structure similar to the starting TLa-containing PS (**6**). In order to demonstrate the viability of this reaction with *c*-PS (**7a**) made from *n*-propylamine, a 10 fold excess of tri-*n*-butylphosphine (tbp) was utilized for 12 h in THF. The decreased retention time in SEC is indicative for the increase in the hydrodynamic volume, which is supposed to occur as a result of the reaction with reducing agent and concomitant disulfide-link scission of *c*-PS (Figure S6). Furthermore, the mild nature of this reaction reduced the risk of broad polydispersity upon the polymer ring opening, and the molar mass of the obtained *l*-PS was close to the one of the parent *l*-PS-TLa (starting *l*-PS-TLa: M_p= 3500 g/mol, PDI= 1.07 ; after reduction of *c*-PS: M_p= 3400 g/mol, PDI= 1.08). Practical implications devoted to the disulfide formation during the purification did not allow for more detailed characterization.

Thiol/disulfide exchange of the c-PS. In turn, thiol/disulfide exchange is a facile reaction where protonation of thiolates and deprotonation of thiols as reacting species is pH dependent, being utilized for example in the preparation of tailor-made biodegradable hydrogels.[61] The reversible thiol/disulfide exchange is an important regulatory mechanism of protein enzymatic activities.[62] Most recently, reversible covalent cross-linking through thiol/disulfide exchange reactions was introduced as a new approach to self-healing

polymeric materials.[44] Therefore, disulfide bridges of *c*-PS (**7a**) were subjected to a thiol/disulfide exchange reaction employing octanethiol as a co-solvent in THF, at room temperature for 12 h. Following the mechanism of thiol/disulfide exchange and given the excess of octanethiol, it was expected to obtain *l*-PS having octanethiol units linked via disulfide bond at both α and ω termini. However, this initial reaction attempt failed as judged by SEC, observing no shift in retention time between starting and reacted *c*-PS. This may partly arise from both the hindered nature of the disulfide linkage in a random polymer coil and its relatively high stability at moderate temperatures. To test this hypothesis, a higher temperature was employed (*i.e.* 90 °C), increasing the polymer chain mobility and thus probability for disulfide bonds to participate in thiol/disulfide exchange events. Under these reaction conditions, SEC traces (Figure 5) showed a clear shift upon the ring opening toward the retention time of a virgin *l*-PS-TLa (**6**), compared with the *c*-PS (**7a**) before thiol/disulfide exchange (starting *l*-PS-TLa: M_p= 3500 g/mol, PDI= 1.07; after thiol/disulfide exchange of *c*-PS: M_p= 3300 g/mol, PDI= 1.09).

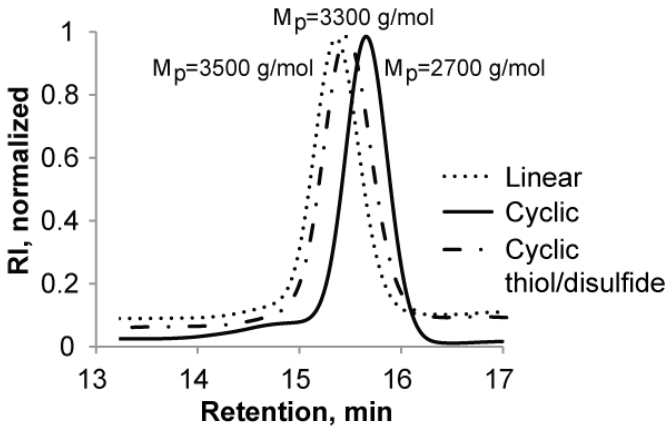


Figure 5. SEC profiles of *l*-PS (**6**), *c*-PS (**7a**) and *c*-PS after thiol/disulfide exchange.

MALDI-TOF MS confirmed the formation of disulfides on both polymer chain-ends with the peaks separated by 104 mass units corresponding to the molecular weight of a single styrene unit (Figure 6). Moreover, each peak of the distribution matched the expected linear structure, yet differing for 289.02 mass units from the starting *c*-PS (**7a**) (inset Figure 6), which corresponds to the addition of two octanethiyl units during the thiol/disulfide exchange.

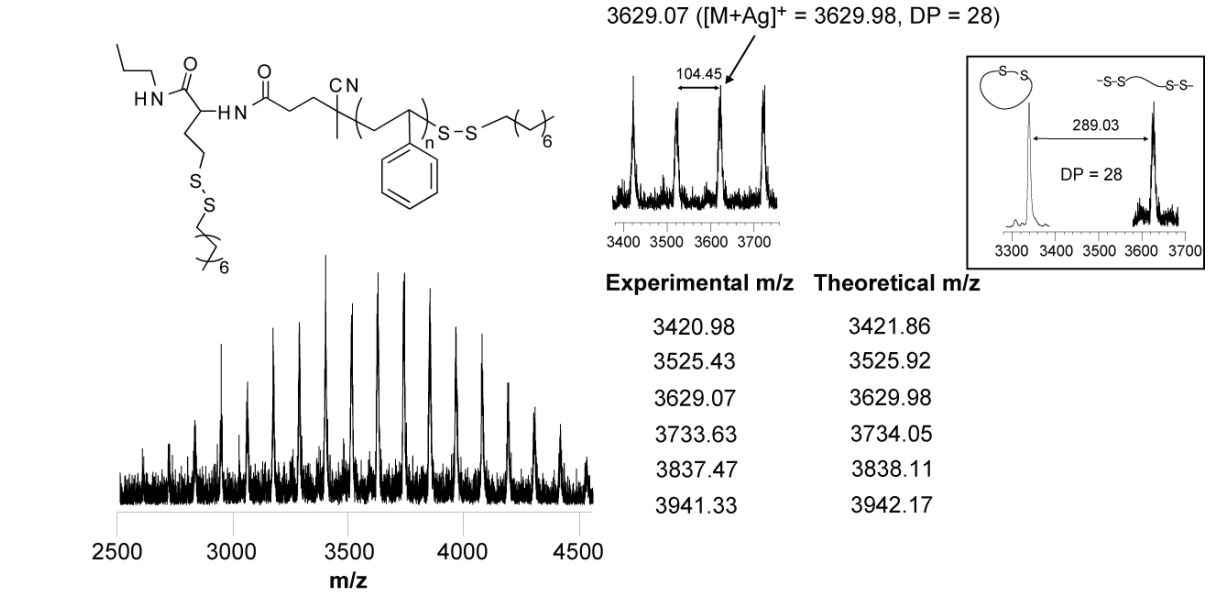


Figure 6. MALDI-TOF mass spectra of linear PS after the thiol/disulfide exchange reaction of *c*-PS (**7a**) and octanethiol. Inset: comparison of the characteristic peak distribution of *c*-PS (before reaction) and linear PS (after reaction).

Synthesis of hydroxyl-functionalized c-PS. The combined features of our synthetic strategy greatly simplify the ring polymer formation under mild and common ambient conditions, *e.g.* room temperature and oxygen, as demonstrated above. Following the same reaction protocol, while developing a modular design for the formation of cyclic polymer topologies, we employed ethanol amine to prepare a cyclic PS equipped with a hydroxyl

functional group in a single reaction step. Obviously, a profound role of the hydroxyl group offers a wealth of advantages such as the ability to design cyclic macromonomers[13] or multicyclic polymer topologies by exploiting the inherent reactivity of the pendant hydroxyl group. Therefore, in analogy to the original recipe to fabricate single ring polymers via thiolactone/disulfide approach, ethanol amine was employed instead of *n*-propylamine, yielding an OH-containing *c*-PS (Scheme 2, **7b**). On one hand, an overlay of the SEC traces of the precursor *l*-PS-TLa and *c*-PS (Figure 2b) showed a significant reduction in hydrodynamic volume (<G> = 0.80) of the resulting ring structure. On the other hand, MALDI-TOF MS (Figure 7) provided additional proof displaying a single distribution spaced by 104 Da that correspond to the mass of PS monomer unit. *m/z* Data clearly indicate that experimental and theoretical masses are consistent, possessing the expected chemical structure with the OH-group for the utilized *c*-PS (**7b**). Moreover, not only by following the color change of the reaction product through visual inspection, but also the ^{1}H-NMR analysis (Figure S7), confirmed successful preparation of functionalized *c*-PS, emphasizing the broader scope of this chemistry.

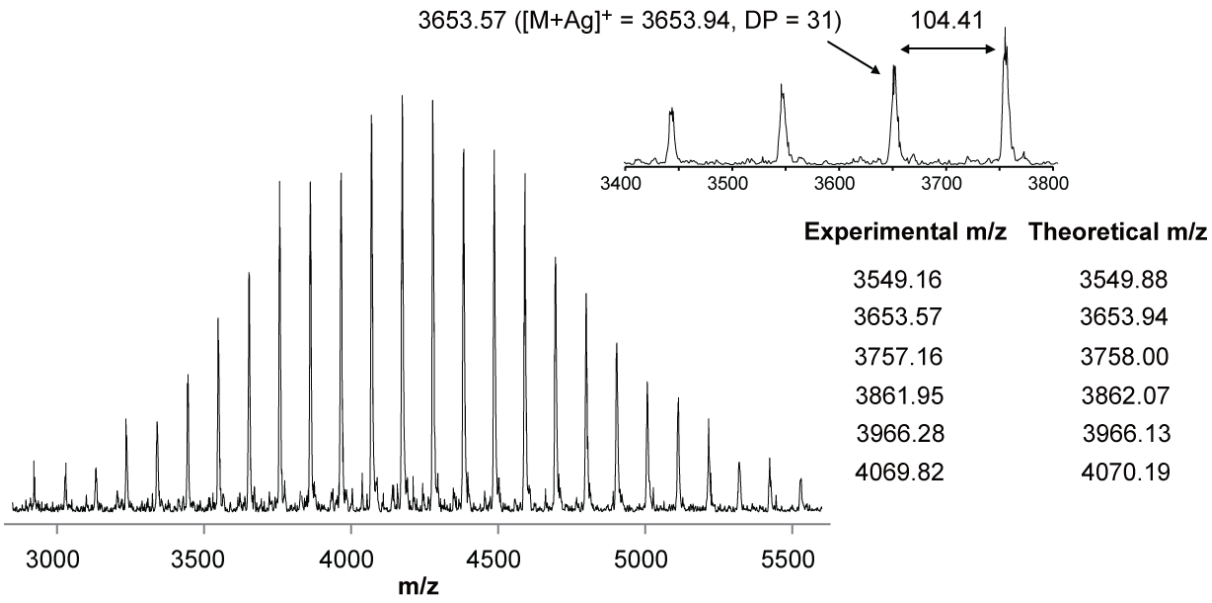


Figure 7. MALDI-TOF mass spectra of *c*-PS (**Scheme 2**, **7b**, R = -OH).

CONCLUSION

A series of high purity cyclic PS (*c*-PS, **7**) was synthesized in high yield using mild conditions, starting from a linear precursor *l*-PS-TLa (**6**) containing TLa functional group at the α and dithiobenzoate group at the ω terminus. The *l*-PS-TLa heterotelechelic was prepared via RAFT polymerization of St mediated by **TLa-CTA** (**5**). The nucleophilic reaction of primary amines with both the TLa ring and dithiobenzoate group was utilized to afford the α,ω-thiol-telechelic and its derived one-pot disulfide-promoted cyclization product. SEC, MALDI-TOF MS and ^{1}H NMR confirmed successful cyclization via a disulfide formation. Moreover, employing ethanolamine, hydroxyl-functionalized *c*-PS was obtained, demonstrating the opportunity for the preparation of cyclic polymers with the pendent functionality of choice. We have further demonstrated the disulfide ring opening in the presence of a reducing agent or through a thiol/disulfide exchange reaction, re-establishing a parent linear polymer topology. Given the unique character of the synthetic strategy

presented here, which enables the fabrication of single cyclic polymers with a desired functional group, the thiolactone/disulfide cyclization offers a key opportunity over other approaches to tailor unusual cyclic polymer topologies. An additional advantage of this system is that the mild reaction conditions are suitable for incorporating a wide range of biomolecules of interest, including systems that are temperature sensitive, such as oligo and polypeptides. Those and other possibilities ultimately serve as a platform for accessing multi-cyclic complex topological constructions and assemblies via a mild and powerful thiolactone/disulfide strategy, currently being further investigated in our laboratories.

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