“Above all, don't fear difficult moments. The best comes from them.”

— Rita Levi-Montalcini

“Science makes people reach selflessly for truth and objectivity; it teaches people to accept reality, with wonder and admiration, not to mention the deep awe and joy that the natural order of things brings to the true scientist.”

— Lise Meitner

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Faculty of Bioscience Engineering
Ghent University

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Faculty of Bioscience Engineering
Department of Sustainable Organic Chemistry

M.Sc. Martyna Jatczak

Synthesis of six- and seven-membered pyridine fused rings and their biological activity

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Science: Chemistry and Bioprocess Technology

Dutch translation of the title:
Synthese van pyridine gefuseerde zes- en zevenringen en hun biologische activiteit.

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The author:

M.Sc. Martyna Jatczak

The promoter:

Prof. dr. ir. Christian V. Stevens

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Martyna Jatczak

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ABBREVIATIONS

AcOH	acetic acid
ADDP	1,1'-(azodicarbonyl)dipiperidine
ATP	adenosine triphosphate
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Bn	benzyl
bpy	2,2´-bipyridine
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
CBr_4	tetrabromomethane
CDI	1,1′-carbonyldiimidazole
CH_2Cl_2	dichloromethane
$CHCl_3$	trichloromethane (chloroform)
CCl_4	carbon tetrachloride
CH_3SO_3H	methanesulfonic acid
$(CH_3)_2NH$	dimethylamie
CH_3NH_2	methylamine
CNS	the central nervous system
CO	carbon monoxide
C_3O_2	propa-1,2-diene-1,3-dione (carbon suboxide)
Cs_2CO_3	caesium carbonate
CsF	caesium fluoride
CsOH	cesium hydroxide
CuCl	copper(I) chloride
CuI	copper(I) iodide
CuO	copper(II) oxide
DCAD	di-(4-chlorobenzyl)azodicarboxylate
DCC	N,N´-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIPEA	*N*,*N*-diisopropylethylamine
DMA	*N*,*N*-dimethylacetamide
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DPPE	1,2-bis(diphenylphosphino)ethane
EDC	*N*-(3-dimethylaminopropyl)-*N*´-ethylcarbodiimide hydrochloride
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
eq	equivalent
Fe	iron
GABA	γ-aminobutyric acid
G2	Grubbs 2nd generation catalyst
H_2	hydrogen gas

HCl	hydrochloric acid
HMBC	Heteronuclear Multiple-Bond Correlation
HNO_3	nitric acid
H_2O_2	hydrogen peroxide
HPLC	High-Performance Liquid Chromatography
H_2SO_4	sulfuric acid
I_2	iodide
K_2CO_3	potassium carbonate
KH	potassium hydride
KI	postassium iodide
KOH	potassium hydroxide
KO*t*-Bu	potassium *tert*-butoxide
LC-MS	Liquid Chromatography–Mass Spectrometry
LDA	lithium diisopropylamide
LiHMDS	lithium bis(trimethylsilyl)amide
LiOH	lithium hydroxide
MeCN	acetonitrile
Me_2CO	acetone
MeOH	methanol
$MgSO_4$	magnesium sulfate
MsCl	methanesulfonyl chloride
N_2	nitrogen gas
$NaBH_4$	sodium borohydride
Na_2CO_3	sodium carbonate
NaH	sodium hydride
$NaHCO_3$	sodium bicarbonate
$NaOAc•3H_2O$	sodium acetate trihydrate
NaOH	sodium hydroxide
NaOMe	sodium methoxide
$Na_2S_2O_4$	sodium dithionite (sodium hydrosulfite)
NH_4Cl	ammonium chloride
NBS	*N*-bromosuccinimide
n-BuLi	*n*-butyllithium
$NiCl_2•H_2O$	nickel(II) chloride hydrate
NMP	1-methyl-2-pyrrolidinone
NR	no reaction
o.n.	overnight
Ph_2O	diphenyl ether
P_2S_5	phosphorus pentasulfide
Pd	palladium
Pd/C	palladium on carbon
$PdCl_2$	palladium(II)dichloride
Pd_2dba_3	tris(dibenzylideneacetone)dipalladium(0)
$Pd(OAc)_2$	palladium(II) acetate
$Pd(PPh_3)_2Cl_2$	bis(triphenylphosphine)palladium(II)dichloride
$Pd(OH)_2/C$	palladium hydroxide on carbon
PE	petroleum ether 40/60
PhSeCl	phenylselenyl chloride

PMDETA	*N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine
$POCl_3$	phosphorus(V) oxychloride
Red-Al	sodium bis(2-methoxyethoxy)aluminumhydride
Re(V) complex	trichlorooxobis(triphenylphosphine)rhenium(V)
$RuCl_2(PPh_3)_3$	dichlorotris(triphenylphosphine)ruthenium(II)
RuClH(CO)PPh3	carbonylchlorohydridotris(triphenylphosphine)ruthenium(II)
rt	room temperature
s-BuLi	*sec*-butyllithium
SM	starting material
$SOCl_2$	thionyl chloride
TBAHS	tetra-*n*-butylammonium hydrogen sulfate
t-BuOH	*tert*-butanol
TDA-1	tris[2-(2-methoxyethoxy)ethyl]amine
TEBA	benzyltriethylammonium chloride
TFA	perfluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
$TiCl_4$	titanium tetrachloride
TMEDA	*N*,*N*,*N*´,*N*´-tetramethylethylenediamine
TPP	triphenylphosphine
TPP-PS	diphenylphosphinopolystyrene
Ts	tosyl group (*p*-toluenesulfonyl group)
TsCl	*p*-toluenesulfonyl chloride
Xantphos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene
Zn	zinc
Δ	refluxing temperature

ABSTRACT

The current development in medical chemistry requires extended research to explore new chemical space leading to innovative active molecules. The concept of *privileged structures*, proposed by Evans in which the biological activity of synthetic benzodiazepines as cholecystokinin antagonists is discussed, is a valuable starting point. The wide range of biological activities displayed by benzodiazepine derivatives make these scaffolds one of the most important scaffolds for drug discovery. However, the modified ring systems (especially the N-analogues replacing the phenyl ring by a pyridine ring) are only scarcely studied. Some heterocycle-fused diazepine derivatives, such as the pyridodiazepines, were only rarely synthetized, but show potentially new pharmacological activity.

This PhD research is part of an IWT-SBO project funded by the agency for Innovation by Science and Technology, Flanders (2010-2014). The aim of this PhD study focused on the development of synthetic methods in order to explore the chemical space for broad screening programs against different targets (kinases, autoimmunity and invasive cancer, viruses, …) as well as for testing in phenotypic assays. The desired new compounds were selected from rarely described libraries of scaffolds. One of this unexplored class are pyridoxazepines. The synthetic methods were designed and elaborated. The synthesis of those scaffolds is very demanding, which proves the unnoticeable presence in the scientific literature. Nevertheless, the synthesis of these heterocyclic structures was successful for pyrido[4,3-*b*][1,4]oxazepines and pyrido[2,3-*b*][1,4]oxazepines, the desired pyrido[3,4-*b*][1,4]oxazepines scaffold could not be obtained. The synthesis of the pyridoxazepine scaffold includes the formation of an ether bond between hydroxynitropyridines and selected alcohols via Mitsunobu reaction or chloronitropyridine and alcohols via nucleophilic aromatic substitution. Reduction of the nitro group and intramolecular cyclization leads to the desired bicyclic molecules. Also the synthesis of six-membered rings fused to pyridine was developed. This synthesis provides a method to obtain pyrido[2,3-*d*]pyrimidines with two identical or two different groups attached to nitrogen.

The selected compounds were evaluated as potentially active agents against NPP1 (nucleotide pyrophosphatase phosphodiesterase 1) and of Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacterial strains. Although, the selected molecules were not active or slightly active, the ADME properties for the library of pyrido[2,3-*d*]pyrimidines and their precursors were performed in collaboration with the group of Prof. Patrick Augustijns (KULeuven) in order to obtain information for fragment based drug design. The biopharmaceutical profiling of a selection of pyrido[2,3-*d*]pyrimidines and their precursors reveals a broad range of structure-dependent solubility, permeability and hepatic metabolism values. 90% Of the investigated compounds showed acceptable drug-like properties.

1. Introduction and goals

Natural products play an important role in drug discovery and chemical biology.[1] Every year, the number of approved drugs based on natural products, is growing.[2][3] The current development of biology requires extended research, leading the search for new natural products-like small molecules. In order to introduce a strategy for the construction of natural product-like libraries, it can be valuable to use the concept of *privileged structures*, proposed by Evans *et al.* in which he discussed the biological activities of synthetic benzodiazepines as cholecystokinin antagonists. This concept describes the selection of structural classes that bind to multiple, unrelated protein receptors as high affinity ligands.[3][4] The tendencies of privileged structures to exhibit binding affinity toward various receptors and enzymes has made them attractive scaffolds for drug discovery. The utility of this approach is evident by the numerous libraries which are designed and constructed on such scaffolds.[5] In this regard the benzazepine- or benzodiazepine-structure is a widely explored scaffold.

The wide range of biological activities displayed by benzodiazepine derived compounds make benzodiazepine scaffolds, particularly the 1,4-benzodiazepine system, one of the most important structures for drug discovery. Benzodiazepines act by facilitating the binding of the inhibitory neurotransmitter GABA at various GABA receptors throughout the central nervous system. Changes in GABA transmission contribute to the etiology of several prominent neurological and mental disorders including epilepsy, anxiety, Angelman's syndrome and schizophrenia. Therefore, modulation of their expression, cellular distribution, and function has profound consequences for neural excitability under both physiological and pathophysiological conditions.[6] Benzodiazepines were developed in response to the need for safe and effective anxiolytics. Classical 1,4-benzodiazepines such as diazepam display a wide variety of behavioral effects, and they are clinically used as anticonvulsants, sedatives/hypnotics, anxiolytics,[7] muscle relaxants and preanesthetics. Benzodiazepines exert their action by interacting with several $GABA_A$ receptor subtypes with different pharmacological characteristics.[8][9] The majority of the pentametric $GABA_A$ receptors are believed to be composed of α, β, and γ subunits in the ratio of 2:2:1, respectively. The benzodiazepine binding site is located at the interface between α and γ subunits, and its pharmacology is thus influenced by both α and γ subunits. Most classical benzodiazepines bind to $\alpha\beta\gamma2$ receptors containing $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunits with approximately the same affinity. The insensitivity of $\alpha4$ and $\alpha6$ subunit-containing receptors to benzodiazepines is based on the presence of an arginine instead of a histidine residue at a conserved position in the benzodiazepine binding site. Anxiolytic activity of benzodiazepines is mediated by the interaction with $\alpha2$-containing $\alpha\beta\gamma2$ receptors, especially in the amygdala and hippocampus, whereas some anxiolytic activity is probably mediated by $\alpha3$-containing receptors. Muscle relaxant activity of benzodiazepines is mediated partially by each of the $\alpha\beta\gamma2$ receptor subtypes containing $\alpha1$, $\alpha2$, $\alpha3$, or $\alpha5$ subunits. In addition, hippocampal extrasynaptic α5-containing receptors are involved in learning and memory

processes.[6][10] Several 1,4-benzodiazepine derivatives have also demonstrated activity as antitumor and anti-HIV agents. A lot of efforts have been made to discover new synthetic routes to access this type of skeleton. Although, the modified ring systems (specially the N-analogues replacing the phenyl ring by a pyridine ring) is only scarcely studied. Some of heterocycle-fused diazepine derivatives, such as the pyridodiazepines, were synthetized and show potentially new pharmacological activity.[6] Pyridodiazepine activity in the central nervous system control is comparable to that of the well-known benzo-condensed analogues.[7] The pyridodiazepine derivatives are as intermediates in the preparation of anti HIV-1 substances,[11][12] antihistamines agents,[9][11][12] D1 receptor subtype of dopamine[13], analogues of galantamine[14][15] or they are known as A3 adenosine receptor antagonists which is implicated in a variety of important physiopathological processes.[16]

This PhD research focuses on the development of synthetic methods in order to explore the chemical space for broad screening programs against different targets (kinases, autoimmunity and invasive cancer, viruses, …) as well as for testing in phenotypic assays, in particular on the synthesis of bicyclic heteroarmatic structures as central scaffold. These compounds can be considered as lead compounds. To design the desired new molecules, several factors should be taken into consideration. The Lipinski rule of five can give some directions to predict whether a compound will be more permeable for membranes or can be easily absorbed by the body. In view of this rule, the designed compounds are relevant candidates. Since, the flat shape and aromaticity can cause low solubility and in fact lower permeability, which can be an issue in the ADME study, we focused on linking a non-aromatic ring to an aromatic one which may overcome these problems. The described aromatic rings can be decorated in several ways. The structures of the desired new compounds were selected from very rarely described scaffolds as checked by a SciFinder search. As mentioned above, pyridine derivatives of benzodiazepines are not widely described in the scientific literature. From the knowledge of the rarely described/synthesized library of pyridine derivatives, three pyridoxazepines were selected (Figure 1).

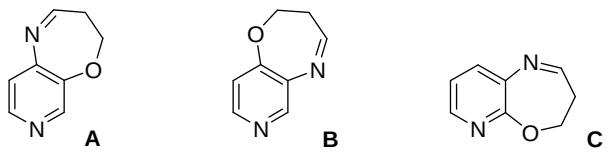
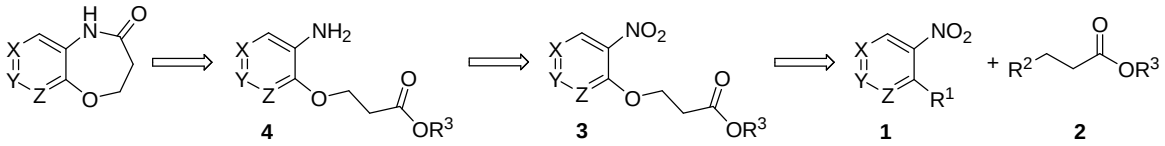


Figure 1 A: pyrido[3,4-*b*][1,4]oxazepines, B: pyrido[4,3-*b*][1,4]oxazepines, C: pyrido[2,3-*b*][1,4]oxazepines.

These scaffolds have been described, mainly in tricyclic systems, having a second aromatic ring fused to the seven-membered ring. In the scientific literature (no patents included, except for the synthesis of pyrido[2,3-*b*][1,4]oxazepines), only ten articles mention the synthesis of pyridoxazepines (four of the pyrido[3,4]oxazepines and six of the pyrido[2,3]oxazepines). That makes this unit an interesting target to further develop in synthetic organic chemistry.

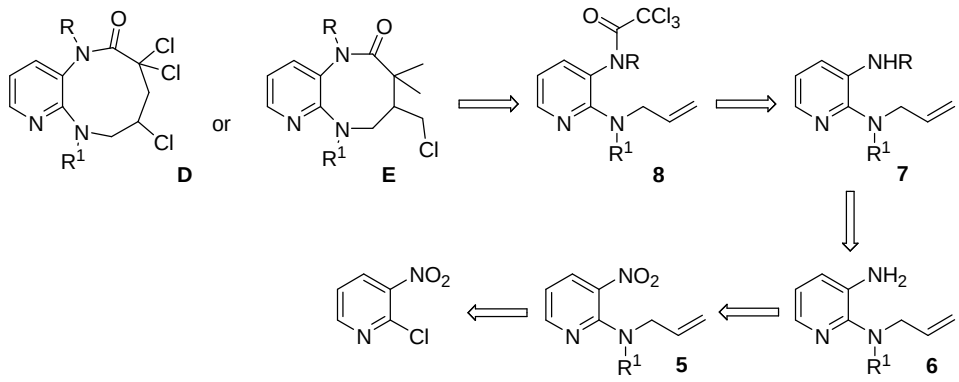
This work will be divided in four parts. In the first part, we will focus on possible pathways for the synthesis of pyrido[3,4-*b*][1,4]oxazepines **A**, pyrido[4,3-*b*][1,4]oxazepines **B**, pyrido[2,3-*b*][1,4]oxazepines **C**, and on the exploration of the saturated seven-membered ring in pyrido[2,3-*b*][1,4]oxazepines. The general strategy for the synthesis of those compounds involves a fusion of an oxazepine ring to the preformed pyridine ring (Scheme 1).



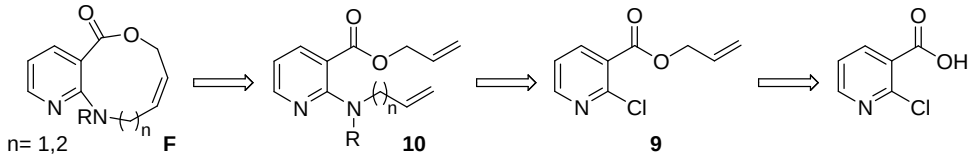
Scheme 1 Retrosynthesis of pyrido[3,4-*b*][1,4]oxazepines **A** (X=CH, Y=N, Z=CH), pyrido[4,3-*b*][1,4]oxazepines **B** (X=N, Y=CH, Z=CH) and pyrido[2,3-*b*][1,4]oxazepines **C** (X=CH, Y=CH, Z=N); R^1=OH, Cl; R^2= OH, Cl; R^3= Me, Et.

Because of the commercial availability of chloronitropyridines and hydroxynitropyridines, these compounds were chosen as starting materials for the synthesis of pyrido[4,3-*b*][1,4]oxazepines or pyrido[2,3-*b*][1,4]oxazepines. The most important part in this synthesis is the creation of the ether bond at C2 in the pyridine ring. Three options were considered. The ether bond can be created by aromatic nucleophilic substitution of chlorine in the chloropyridine (R^1=Cl, R^2=OH), by Mitsunobu reaction starting form hydroxypyridine and an alcohol (R^1=OH, R^2=OH) using TPP and DEAD/DIAD and in the third option the hydroxyl group in C2 of the pyridine ring can be alkylated (R^1=OH, R^2=Cl). In the next step the nitro group will be reduced to the corresponding amine using the common reducing agents (Pd/C, H_2; Fe; Zn). The ring closure will be performed by creation of an amide bond using strong base to activate the amine group. Because 3-hydroxy-4-nitropyridine is not commercially available, the synthesis of pyrido[3,4-*b*][1,4]oxazepines starts from 3-hydroxypyridine or 3-bromopyridine as a precursor of 3-substituted-4-nitropyridine.

Next, the synthesis of eight- or nine-membered rings fused to the pyridine ring, by atom transfer radical addition (ATRA), known as the Kharasch reaction will be discussed (Scheme 2). The nine- and ten-membered ring including an ester moiety **F**, can be generated by a metathesis reaction (Scheme 3).

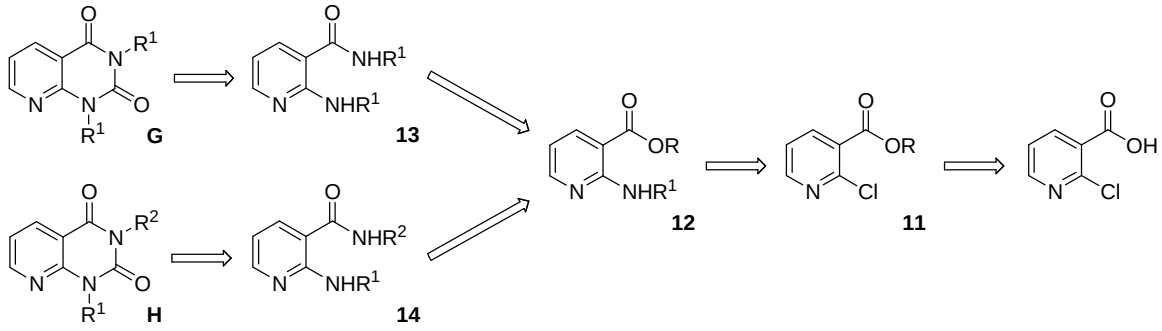


Scheme 2 Retrosynthetic scheme for the formation of nine- or eight-membered rings by Kharasch reaction, R=Me, Bn; R^1=Bn.



Scheme 3 Retrosynthetic scheme for the formation nine- and ten-membered ring generated in the metathesis reaction, R=Bn, Ts.

In the study of the pyrido[2,3-*d*]pyrimidine, the synthesis of pyrido[2,3-*d*]pyrimidines with two identical groups attached to nitrogens **G** and pyrido[2,3-*d*]pyrimidines with two different groups attached to nitrogens **H** will be presented (Scheme 4). In the literature eight synthesis methods of pyrido[2,3-*d*]pyrimidine are described. Most of those utilize expensive reagents, toxic chemicals, apply harsh reaction conditions or have a low yield of desired product. The investigated synthesis pathway starts from 2-chloropyridine-3-carboxylic acid. Through ester formation and reaction with alkyl/aromatic amines, the pyrido[2,3-*d*]pyrimidines **G** and **H** could be synthesized.



Scheme 4 Retrosynthetic scheme for the formation of pyrido[2,3-*d*]pyrimidine, R=CH_2CHCH_2; R^1=alkyl, aromatic; compound **14** and **H**: R^1=cyclohexyl, *t*-octyl, R^2=allyl, propyl, *i*-pentyl.

Finally, the results of the biological investigations of pyrido[3,4-*b*][1,4]oxazepines, pyrido[4,3-*b*][1,4]oxazepines, pyrido[2,3-*b*][1,4]oxazepines and pyrido[2,3-*d*]pyrimidine as potentially active agents against NPP1 (nucleotide pyrophosphatase phosphodiesterase 1) and of

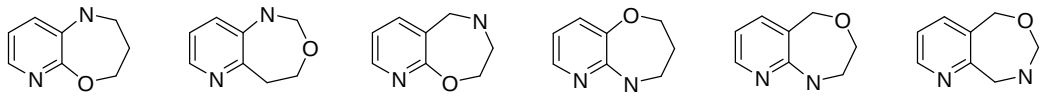
Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) strains and the ADME properties for the library of pyrido[2,3-*d*]pyrimidines and their precursors will be described in this thesis.

2. Literature overview

In this chapter, a literature overview on the published synthesis of pyrido[2,3]oxazepines and pyrido[3,4]oxazepines will be presented. Furthermore, the synthesis of the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones will be discussed.

2.1. Synthesis of pyrido[2,3]oxazepines

Pyrido[2,3]oxazepines have 20 possible isomeric structures, up to now only six have been synthesized (Figure 1).

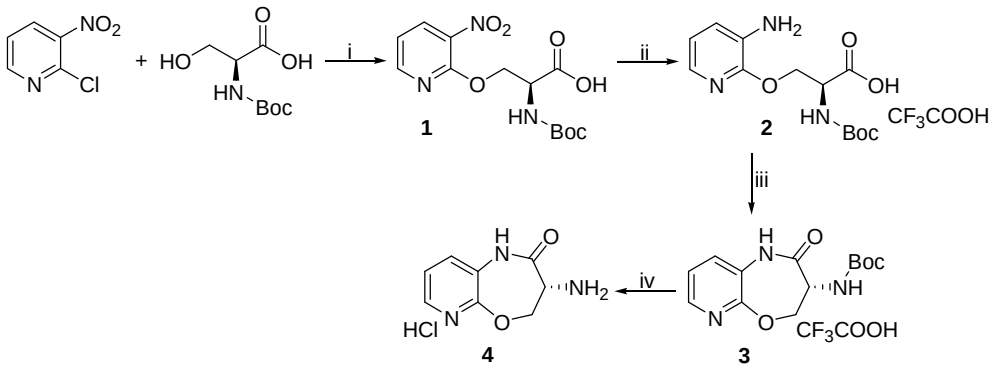


[2,3-*b*][1,4] [3,2-*d*][1,3] [3,2-*f*][1,4] [3,2-*f*][1,4] [2,3-*e*][1,4] [2,3-*f*][1,4]

Figure 1

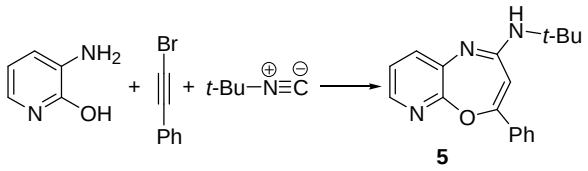
2.1.1. Pyrido[2,3-b][1,4]oxazepines

In the literature only two synthesis of pyrido[2,3-*b*][1,4]oxazepines are described. In 2004, Sher and Ellsworth[17] published a patent, in which they described the synthesis of pyrido[2,3-*b*][1,4]oxazepines. Starting from 2-chloro-3-nitropyridine and *N*-(*tert*-butoxycarbonyl)-L-serine in *N*,*N*-dimethylformamide (DMF) the 2-*tert*-butoxycarbonylamino-3-(3-nitro-pyridin-2-yloxy)propionic acid **1** was obtained in 75% yield. After reduction of the nitro group the corresponding amine was separated by preparative HPLC and the product **2** was obtained as a trifluoroacetic acid salt. For the ring closure, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 1-hydroxy-azabenzotriazole (HOAt) and diisopropylethylamine (DIPEA) in THF were used ((iii), Scheme 1). The pyrido[2,3-*b*][1,4]oxazepines trifluoroacetic acid salt **3** was obtained in 31% yield. In order to remove the Boc group from the amine, **3** was stirred in a solution of hydrogen chloride in dioxane for 16 hours.



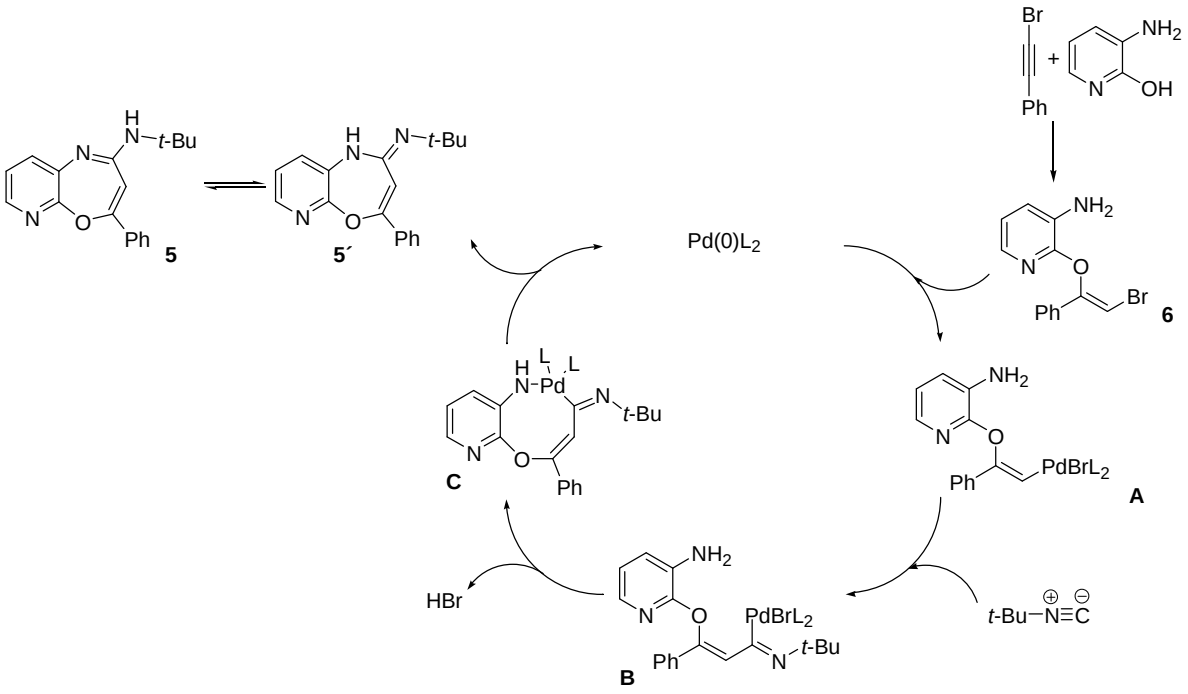
Scheme 1 Synthesis of pyrido[2,3-b][1,4]oxazepines described by Sher and Ellsworth.
Reaction condition: (i) NaH, DMF, -20°C→0°C (1h)→-20°C (1h); (ii) Pd/C, H_2, MeOH, rt (6h); (iii) EDC, HOAt, DIPEA, THF, rt (17h); (iv) HCl in dioxane, CH_2Cl_2, rt (16h).

In 2012, Liu *et al.* described the synthesis of pyrido[2,3-*b*][1,4]oxazepines in the palladium-catalyzed tandem reaction of 2-hydroxy-3-aminopyridine, 1-(2-bromoethynyl)benzene and *tert*-butylisocyanide (Scheme 2).[18]



Scheme 2 Synthesis of pyrido[2,3-*b*][1,4]oxazepines presented by Liu *et al.*[18]
The reaction conditions: $Pd(PPh_3)_2Cl_2$, TPP, Cs_2CO_3, 1,4-dioxane, 80°C.

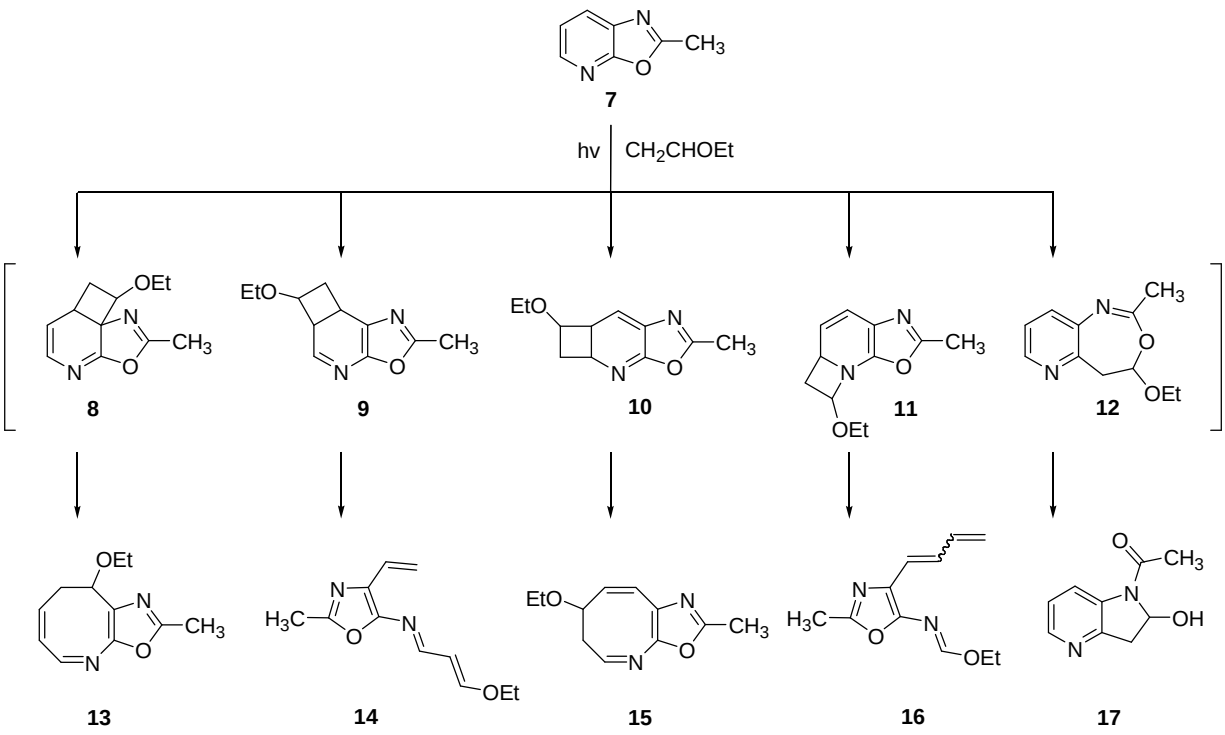
The mechanism of this reaction is depicted in Scheme 3. Initially the nucleophilic addition of 2-hydroxy-3-aminopyridine to 1-(2-bromoethynyl)benzene gave an intermediate **6**, which after oxidative addition with a Pd(0) species generates a vinyl palladium species **A**. Subsequent migratory insertion of *tert*-butylisocyanide results in the formation of intermediate **B**, which in the presence of base eliminates hydrogen bromide to generate the eight-membered intermediate **C**. In the last step, reductive elimination affords **5**, regenerating the Pd(0) catalyst.



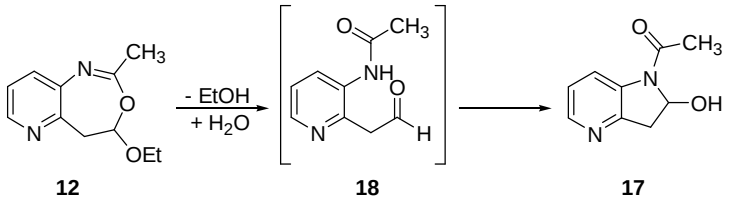
Scheme 3 Proposed mechanism by Liu *et al.*[18]

2.1.2. Pyrido[3,2-d][1,3]oxazepines

The synthesis of pyrido[3,2-*d*][1,3]oxazepines was reported by Donati *et al.*, the desired scaffold was obtained as one of the many end products of the reaction of 2-methyloxazolo[5,4-*b*]pyridine with vinyl ethyl ether (Scheme 4).[19] After the reaction, they observed five different compounds **8**-**12** in the mixture, but after chromatographic separation the compounds **13**-**17** were detected. The reaction proceeded via attack of vinyl ethyl ether at the position 7a in the oxazolopyridine, which causes the ring opening of the oxazole and the formation of **12**. The separation on the silica gel promoted the rearrangement of **12** to the 2-hydroxyindole **17** (Scheme 5).



Scheme 4 Complex mixture after photocycloaddition of vinyl ethyl ether to compound **7** (detected compounds **8**-**12**). The silica gel promoted rearrangement to compounds **13**-**17**.



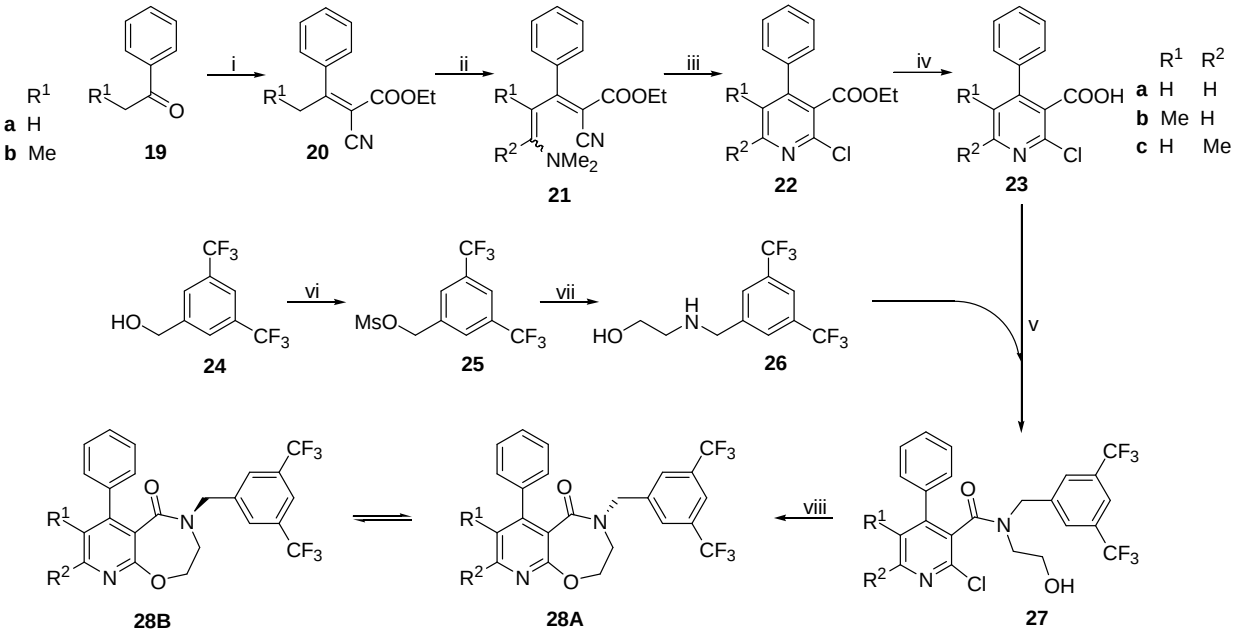
Scheme 5 Proposed mechanism of formation of compound **17** via ring opening and ring closing reactions.

2.1.3. Pyrido[2,3-b][1,5]oxazepines

Only two methods to synthesize pyrido[2,3-*b*][1,5]oxazepines have been published, via an intramolecular nucleophilic aromatic substitution or an intramolecular cyclization and ring opening.

Natsugari *et al.* have synthesized pyrido[2,3-*b*][1,5]oxazepines, as tachykinin NK_1-receptor antagonists (Scheme 6).[20] Commercially available acetophenones **19** were condensed with ethyl cyanoacetate, followed by reaction with *N*,*N*-dimethylformamide dimethyl acetal or *N*,*N*-dimethylacetamide dimethyl acetal to the enamines **21**. The reaction of **21** with anhydrous HCl caused the formation of the pyridine ring **22**, next the ester group was removed by hydrolysis affording the free acid **23**. The acid chlorides of **23** were reacted with *N*-3,5-[bis(trifluoromethyl)benzyl]aminoethanol **26**, which was prepared by the nucleophilic substitution of mesylated 3,5-bis(trifluoromethyl)benzyl alcohol **25** by 2-aminoethanol. Intramolecular

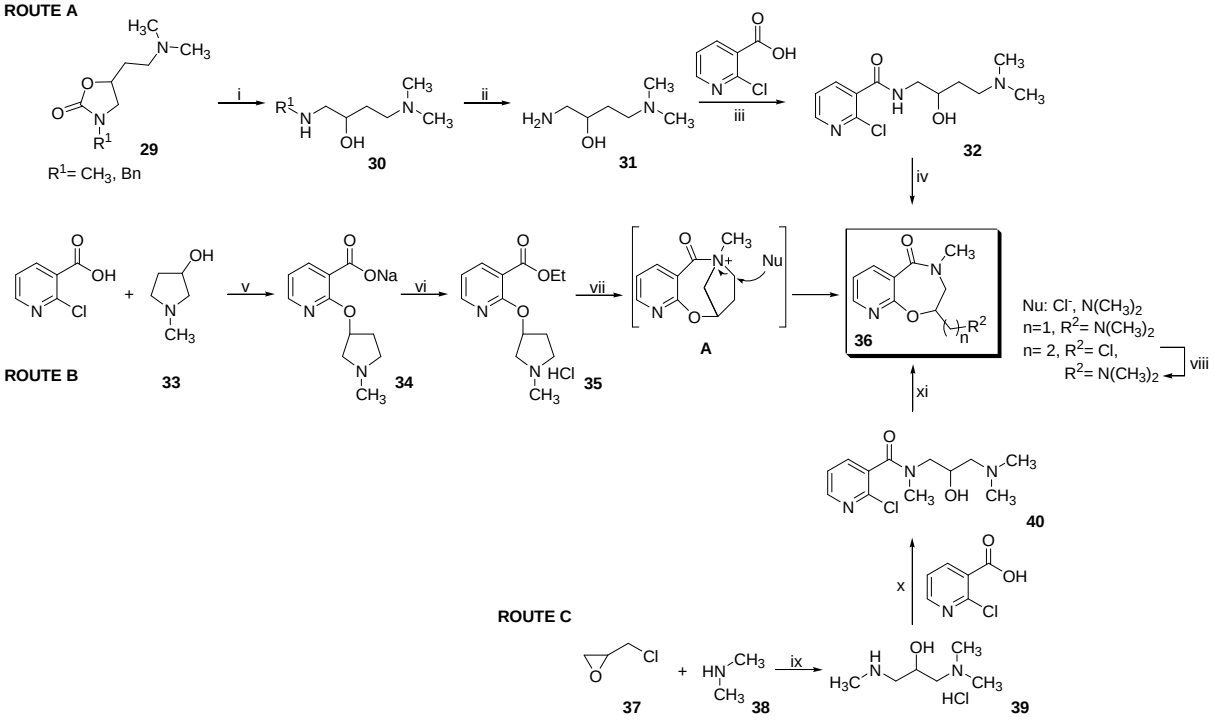
cyclization of **27** led to the formation of pyrido[2,3-*b*][1,5]oxazepine **28** existed in two stable atropisomers (**28A** and **28B**).



Scheme 6 Synthesis of pyrido[2,3-*b*][1,5]oxazepines. Reaction conditions: (i) ethyl cyanoacetate, NH_4OAc, AcOH, benzene, Δ, 10h; (ii) *N*,*N*-dimethylformamide dimethyl acetal or *N*,*N*-dimethylacetamide dimethyl acetal, rt; (iii) 4N HCl/EtOAc, rt, 30min; (iv) 4N NaOH/EtOH:1/1, Δ, 4h; (v) a) $SOCl_2$, cat. DMF, THF, Δ, 4h, b) **26**, rt, 2h; (vi) MsCl, Et_3N, THF, rt, 30min; (vii) 2-aminoethanol, THF, rt, 1h; (viii) NaH, THF, Δ, 2h.

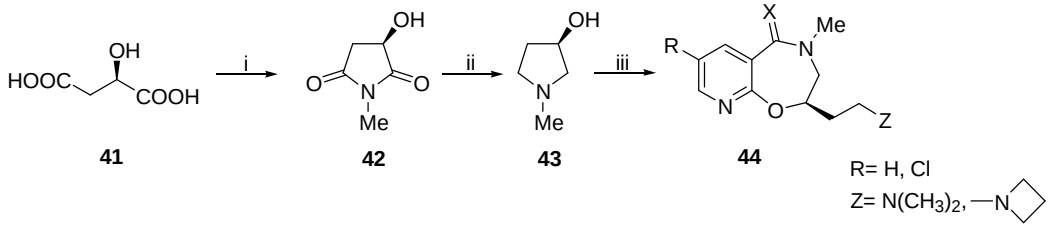
Cale *et al.* have developed the synthesis of pyrido[2,3-*b*][1,5]oxazepines as a synthesis of new H_1 histamine antagonists. They proposed three different synthesis pathways (Scheme 7).[21] **Route A** employs a cyclization to a seven-membered lactam by reaction of the chloropyridinecarboxylic acid with 1-amino-4-(dimethylamino)-2-butanol. The ring opening of3-benzyl-5-[2-(dimethylamino)ethyl]-2-oxazolidinone **29** via hydrolysis and deprotection resulted in the free amine **31**. The amide formation was achieved by coupling of a free amino group **31** with 2-chloropyridine-3-carboxylic acid by DCC, followed by the intramolecular cyclization via nucleophilic aromatic substitution of the chlorine atom leading to the desired pyrido[2,3-*b*][1,5]oxazepine **36**. The synthesis of an analogue with a shorter chloroalkyl side chain is presented by **route C**. The ring opening of epichlorohydrin **37** is carried out with dimethylamine. The bicyclic **36** was achieved through the amide formation and the ring closure by nucleophilic aromatic substitution. The **route B** started by nucleophilic aromatic substitution of the chlorine atom in 2-chloronicotinic acid by 1-methyl-3-pyrrolidinol **33**. The sodium salt of the acid **34** was treated with hydrogen chloride gas to form the hydrochloride salt **35**. The acid chloride was synthesized by the Appel reaction. The resulting rearrangement was effected by heat or by the addition of an organic base. The pyrrolidine nitrogen was involved in an intramolecular cyclization to the intermediate **A**, in which the pyrrolidine ring is opened by the attack of the chloride anion on the closest carbon connected to the ammonium nitrogen in an eight-memberd ring.

The obtained pyrido[2,3-*b*][1,5]oxazepines were converted to the thioamides by treatment with the phosphorus pentasulfide or Lawesson's reagent.



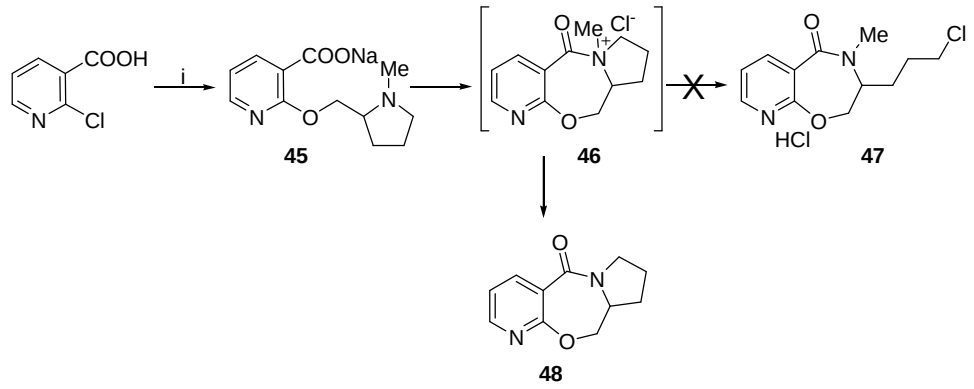
Scheme 7 Synthesis of pyrido[2,3-*b*][1,5]oxazepines. Reaction conditions: (i) NaOH, EtOH/H_2O, reflux, 3h; (ii) $Pd(OH)_2/C$, H_2 40 psi, 65°C, 4h; (iii) 1-hydroxybenzotriazole, DCC, CH_2Cl_2, rt, 72h; (iv) KH, THF, reflux, 4h; (v) NaH, DMSO, 60°C, 1h30min, N_2; (vi) a) HCl_{gas}, b) TPP, CCl_4, Δ, 4h, c) EtOH, rt, 1h; (vii) $(CH_3)_2NH$, rt, 2d; (viii) $(CH_3)_2NH$, 5°C, 2h; (ix) a) $(CH_3)_2NH$, MeOH, 5°C, 2h, b) CH_3NH_2, 5°C, 1h; (x) DCC, MeCN/H_2O, rt, overnight; (xi) NaH, toluene, reflux, 20min.

The optically pure isomers of some of the most potent compounds have also been synthesized (Scheme 8).[22] The synthesis started by the ring closure of *R*-malic acid **41** with methylamine. After reduction with red-Al and treatment with a tartrate salt, the enantiomerically pure *R*- or *S*-1-methyl-3-pyrrolidinol **43** was obtained. The remainder of the synthesis, which is the same as discussed in **route B**, was accomplished while retaining the chirality of the starting compound in the end products. The synthesis presented in Scheme 8 for the *R*-isomer was also applied for the *S*-isomer.



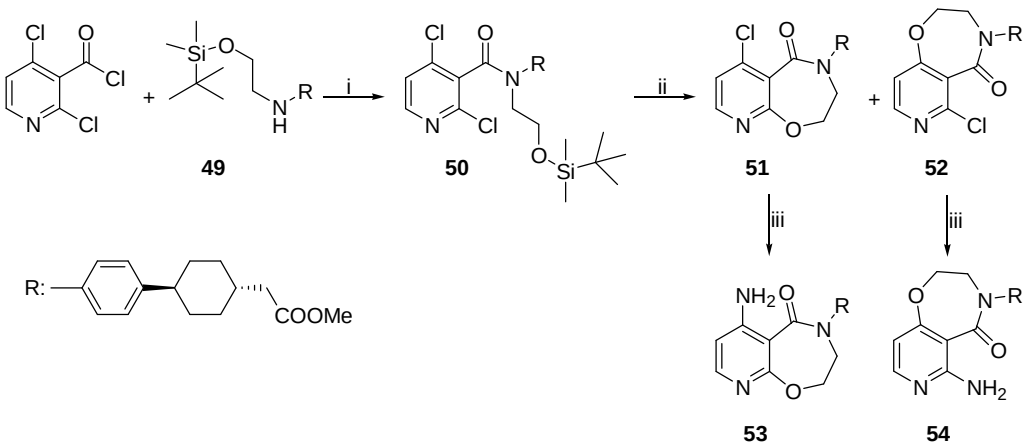
Scheme 8 Synthesis of pyrido[2,3-*b*][1,5]oxazepines. Reaction conditions: (i) CH_3NH_2, Δ, 30min; (ii) red-Al, THF, Δ, 1h; (iii) a) 2-chloronicotinic acid, NaH, THF, Δ, 2h30, b) CH_3SO_3H, TPP, CCl_4, CH_2Cl_2, Δ, 4h, c) Et_3N, P_2S_5, CH_3CN, Δ, 2h30.

The use of 2-(hydroxymethyl)-1-methylpyrrolidine and 1-methyl-3-piperidinol led to the tricyclic compound **48** (Scheme 9), by *N*-demethylation. The desired bicyclic compound **47** was not obtained or detected in the reaction mixture.



Scheme 9 Formation of tricyclic compound **48**. Reaction conditions: (i) a) 2-(hydroxymethyl)-1-methylpyrrolidine, NaH, DMF, 60°C, 1h30, b) $SOCl_2$, Δ, 2h, c) Et_3N, rt, o.n.

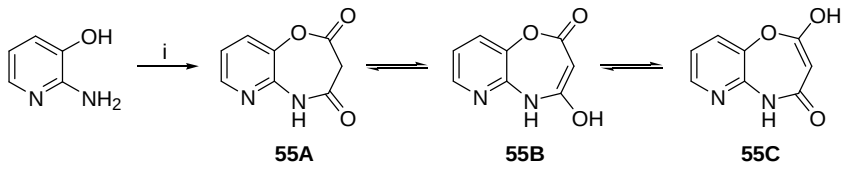
The synthesis of the pyridoxazepinone described by Dow *et al.*[23] starts from the amide bond formation by 2,4-dichloro-3-pyridinecarbonyl chloride and amine **49**. After removal of protecting group of an alcohol, and cyclization, the mixture of compounds **51** and **52** was obtained. The free amine group in **53** and **54** was introduced by condensation with 4-methoxybenzylamine followed by deprotection. Compound **51** was obtained in 55% yield, while compound **52** in 23% yield (Scheme 10).



Scheme 10 Synthesis of pyridoxazepinones **53** and **54**. The reactions conditions: (i) Et_3N, THF, 0°C; (ii) a) MeOH, HCl_{aq}, 25°C b) Cs_2CO_3, MeCN, reflux; (iii) a) 4-methoxybenzylamine, Et_3N, DMA, sealed tube, 140°C, b) TFA, 50°C, c) LiOH, H_2O, *p*-dioxane.

2.1.4. Pyrido[3,2-b][1,4]oxazepines

Bonsignore *et al.* used 2-amino-3-hydroxypyridine and carbon suboxide to synthesize the pyrido[3,2-*b*][1,4]oxazepine **55** which exist in the corresponding tautomeric forms (Scheme 11).[24] This is the only published method for pyrido[3,2-*b*][1,4]oxazepines.

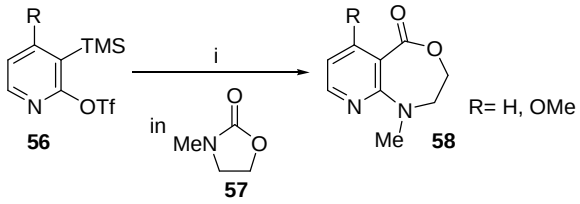


Scheme 11 Synthesis of pyrido[3,2-*b*][1,4]oxazepine **55**.
Reaction conditions: (i) C_3O_2, Et_2O, -5°C (24h)→rt (48h) or C_3O_2, rt (6 days).

2.1.5. Pyrido[2,3-e][1,4]oxazepines

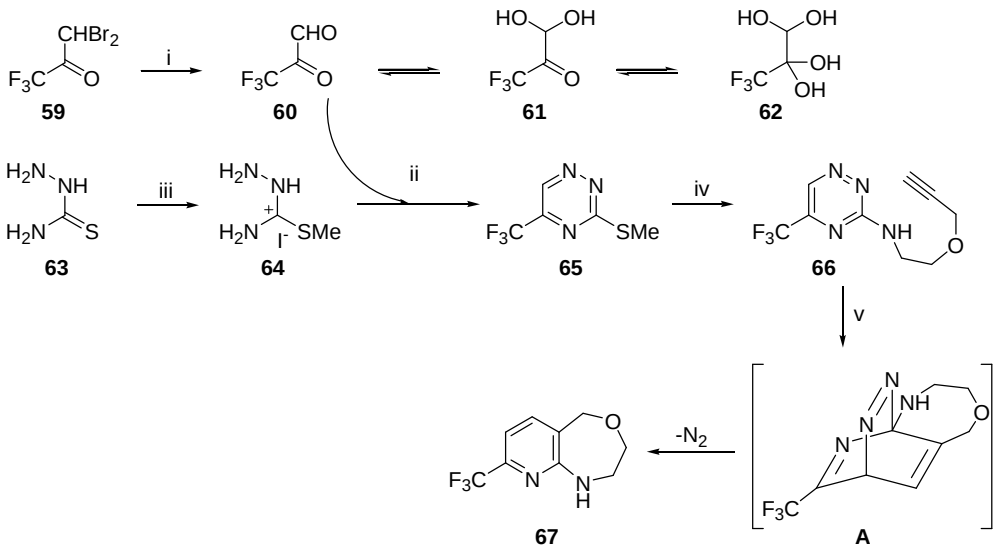
Only two methods to synthesize pyrido[2,3-*e*][1,4]oxazepines have been published, either via pyridyne formation or an intramolecular inverse electron-demand Diels Alder reaction.

Saito *et al.* have synthesized the pyrido[2,3-*e*][1,4]oxazepines **58** using 1-methyl-2-oxazolidinone **57** (Scheme 12), via a pyridyne intermediate.[25]



Scheme 12 Synthesis of pyrido[2,3-*e*][1,4]oxazepines **58**. Reaction conditions: (i) CsF, rt, 1h.

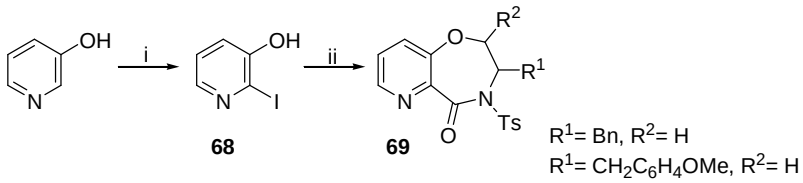
The pyrido[2,3-*e*][1,4]oxazepine **67** can be synthesized via an intramolecular inverse electron-demand Diels Alder reaction and subsequent nitrogen gas release (Scheme 13).[26]



Scheme 13 Synthesis of pyrido[2,3-*e*][1,4]oxazepine **67**. Reaction conditions: (i) $NaOAc \cdot 3H_2O$, H_2O, Δ, 45min (crude); (ii) $NaHCO_3$, 0°C, 12h; (iii) methyl iodide; (iv) 2-(prop-2-yn-1-yloxy)ethanamine, benzene, Δ, 4d; (v) Ph_2O, 230°C, 1d.

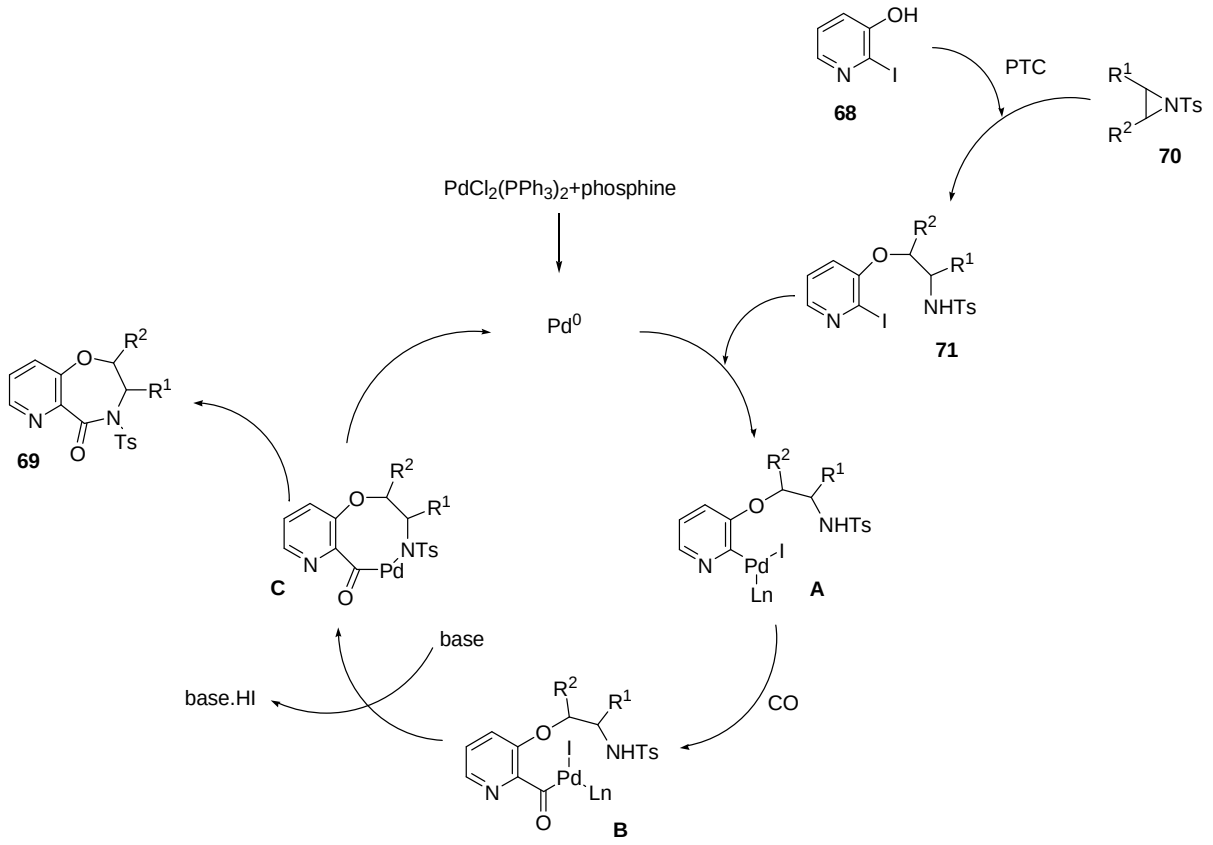
2.1.6. Pyrido[2,3-f][1,4]oxazepines

Pyrido[2,3-*f*][1,4]oxazepines could be synthesized via a domino ring opening/carboxamidation reaction of acyclic *N*-tosyl aziridines with 2-iodo-3-pyridinol **68** under phase transfer catalysis, as reported by Alper *et al.* (Scheme 14) and is the only published synthesis for pyrido[2,3-*f*][1,4]oxazepines.[27]



Scheme 14 Synthesis of pyrido[2,3-*f*][1,4]oxazepine **69**. Reaction conditions: (i) I_2, Na_2CO_3, H_2O, rt, 30h; (ii) *N*-tosyl aziridine, $Pd(PPh_3)_2Cl_2$, TPP, K_2CO_3, TEBA, CO, 30 bar, THF, 130°C, 48h.

The possible mechanism of this reaction is shown in Scheme 15. The base-catalyzed ring opening of the *N*-tosyl aziridines **70** with 2-iodo-3-pyridinol **68** under phase transfer catalysis (PTC) generated the amine **71**. The oxidative addition of **71** to the *in situ* generated Pd^0 species led to the formation of palladium complex **A**. The insertion of carbon monoxide into the aryl carbon-palladium bond of **A** afforded **B**, and the nucleophilic attack of the protected amine on an aroyl-palladium complex **B** give an eight-membered intermediate **C**. This intermediate underwent the reductive elimination to afford the pyrido[2,3-*f*][1,4]oxazepine **69** with regeneration of palladium(0).



Scheme 15 Proposed reaction mechanism for the synthesis of the pyrido[2,3-*f*][1,4]oxazepine **69**.

2.2. Synthesis of pyrido[3,4]oxazepines

The bicyclic pyridines which contain oxygen in the fused ring are not well-explored as compared to the bicyclic pyridines which contain nitrogen in the fused ring. From the possible 20 structures of pyrido[3,4]oxazepines, only 4 have been reported (Figure 2).

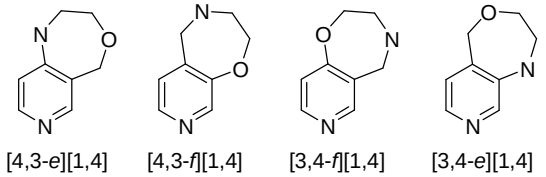
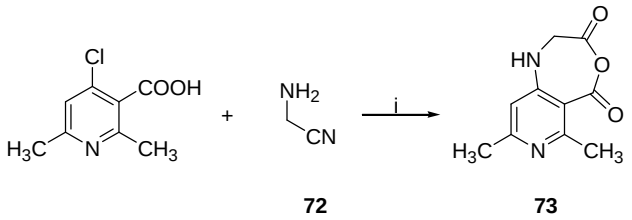


Figure 2 The structures of pyrido[3,4]oxazepine describes in the literature.

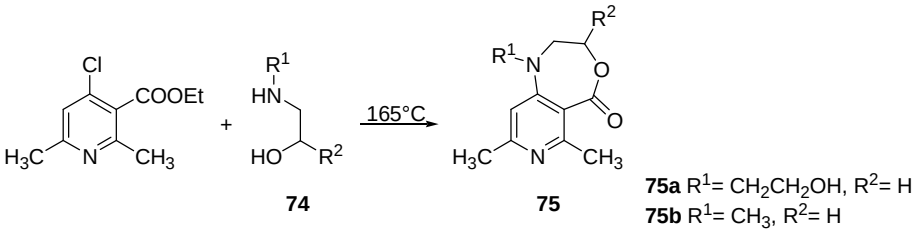
2.2.1. Pyrido[4,3-e][1,4]oxazepines

In 1927, Koenigs and Kantowitz obtained *N*-[2,6-dimethyl-pyridyl-4]-glucin-3-acid (4-[(carboxymethyl)amino]-2,6-dimethylnicotic acid cyclic anhydride) **73** in the reaction of

aminoacetonitrile **72** and chlorolutidinecarboxylic acid (Scheme 16).[28] Nearly fifty years later, Yurugi *et al*. used the ethyl ester of chlorolutidinecarboxylic acid and *N*-substituted ethanolamine **74** to obtain the cyclized pyrido[4,3-*e*][1,4]oxazepine **75** (Scheme 17).[29]

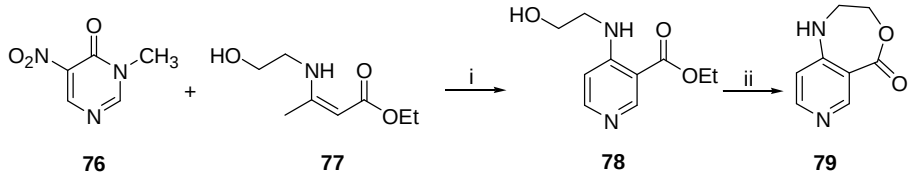


Scheme 16 Formation of cyclic anhydride **73**. Reaction conditions: (i) NaOH, EtOH, H_2O, 150°C.



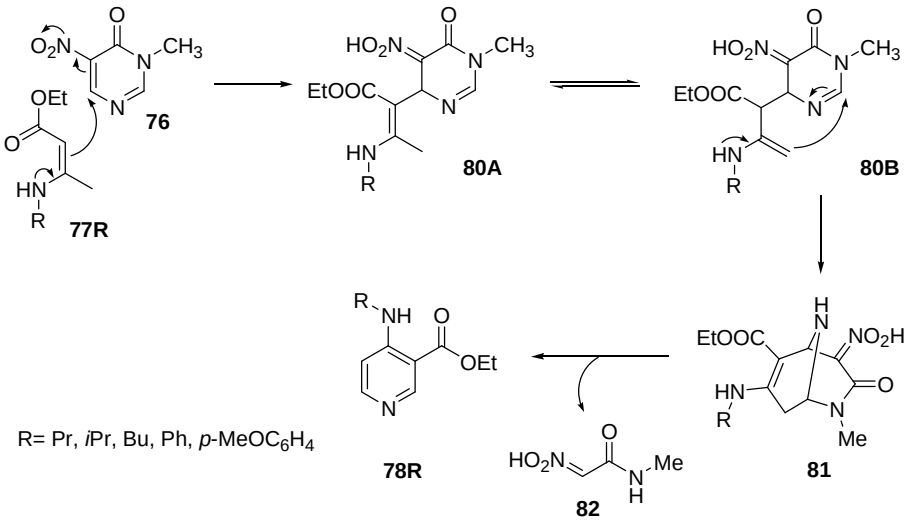
Scheme 17 Synthesis of pyrido-[4,3-*e*][1,4]oxazepine **75**.

Nishiwaki *et al*. introduced a new method for the synthesis of the pyrido[4,3-*e*][1,4]oxazepine **79** (Scheme 18).[30] The method is based on the synthesis of the pyridine **78** from nitropyrimidinone **76** with enaminone ester **77**. The nitropyrimidinone **76** is known as an excellent substrate for ring transformations and behaves as a synthetic equivalent of activated diformylamine to give azaheterocyclic compounds upon treatment with dinucleophilic reagents. An intramolecular nucleophilic addition of the alcohol function of **78** across the ester moiety on the pyridine ring in the presence of sodium hydride provided the desired pyrido[4,3-*e*][1,4]oxazepine **79**.



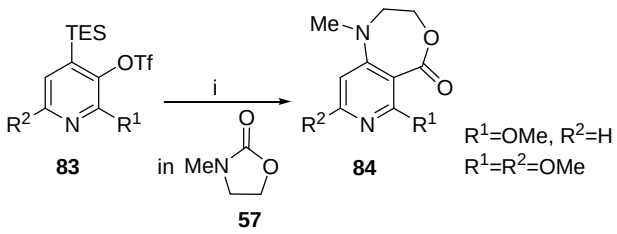
Scheme 18 Synthesis of bicyclic pyridine **79**. Reaction conditions: (i) MeOH, Δ, 1d (80%);
(ii) NaH, THF, Δ, 1d (quant.).

The potential mechanism for the synthesis of the pyridine **78R** is illustrated in Scheme 19. The enamine ester **77R** attacks the 6-position of the nitropyrimidinone **76** and forms intermediate **80**. The tautomeric enamine **80B** leads to an intramolecular cyclization to the bicyclic intermediate **81**, from which the pyridine **78R** can be obtained by the elimination of nitroacetamide **82**.



Scheme 19 Potential mechanism of the formation pyridine **78R**.

The coupling reaction of the 1-methyl-2-oxazolidone with pyridynes proceed to the pyrido[4,3-*e*][1,4]oxazepine **84** in the presence of CsF at room temperature. The pyridynes with substituents in the positions 2 or 2 and 6 gave bicyclic pyridines in 51 and 52 % yield, respectively (Scheme 20).[25]

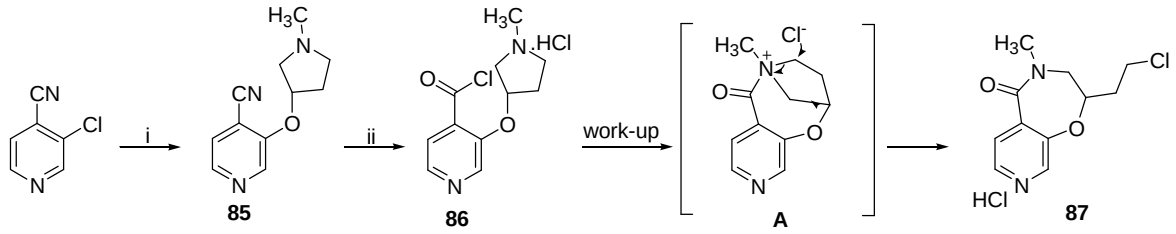


Scheme 20 Synthesis of pyrido-[4,3-*e*][1,4]-oxazepine **84**. Reaction conditions: (i) CsF, rt, 1h.

2.2.2. Pyrido[4,3-f][1,4]oxazepines

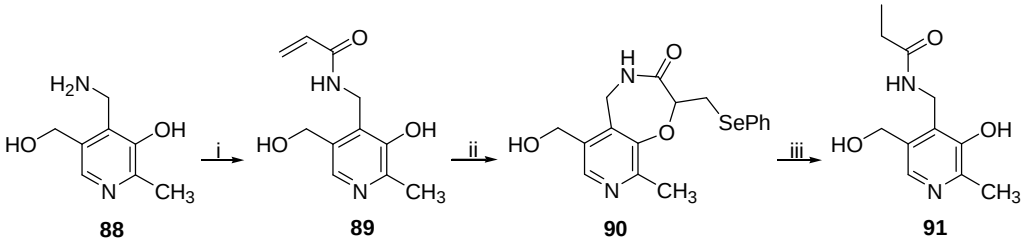
The pyrido[4,3-*f*][1,4]oxazepine scaffold can be obtain by an intramolecular cyclization and ring opening (Scheme 21) or from pyridoxamine (Scheme 22). The first pyrido[4,3-*f*][1,4]oxazepine **87** was synthesized by Cale *et al.* (Scheme 21).[21] The same synthesis route has also been used to obtain the pyrido[2,3-*b*][1,5]oxazepines **36** (Scheme 7) and the pyrido[3,4-*f*][1,4]oxazepines **95** (Scheme 23). The starting salts **86**, required for the rearrangement to **87** were prepared from the

3-chloro-4-pyridinecarbonitrile. The synthesis of the pyridine **87** was conducted in three steps. In the first step the cyanide **85** was converted to the corresponding salt using potassium hydroxide, then the same salt was suspended in chloroform and the hydrogen chloride gas was introduced to form a tertiary ammonium salt. In the last step a chlorinating reagent was added to afford compound **86**. The best method to obtain the acid chloride involved triphenylphosphine (TPP) and carbon tetrachloride.



Scheme 21 Synthesis of pyrido[4,3-*f*][1,4]oxazepine 87. Reaction conditions: (i) NaH, 1-methyl-3-pyrrolidinol, DMSO, 60°C, 1h30, N_2; (ii) a) KOH, *t*-BuOH, rt, 88h, b) HCl-gas, $CHCl_3$, c) TPP, CCl_4, Δ, 7h.

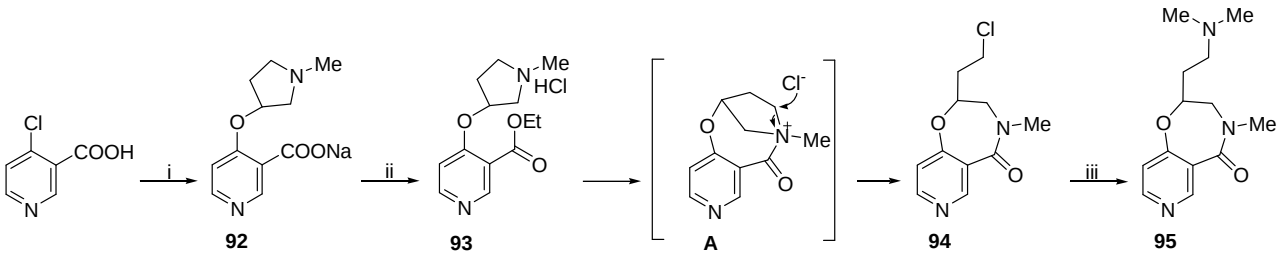
In 1990, Ueda *et al.* reported the synthesis of the pyrido[4,3-*f*][1,4]oxazepine (Scheme 22).[31] The reaction of the pyridoxamine **88** with acryloyl chloride or crotonoyl chloride gave 4-(*N*-alkenoylaminomethyl)-3-hydroxy-2-methyl-5-(hydroxymethyl)pyridine **89**. The cyclization with phenylselenenyl chloride in acetonitrile in the presence of triflic acid and water, provides the pyrido[4,3-*f*][1,4]oxazepin-3-one **90**. Deselenization of **90** was unsuccessful and led to the ring opening to **91**.



Scheme 22 Synthesis of pyridine **91** through bicyclic pyridine **90**.
Reaction conditions: (i) acryloyl chloride, 30% NaOH, rt, 1h; (ii) PhSeCl, TfOH:H_2O 1:5, MeCN, Δ, 40min; (iii) $NiCl_2{\cdot}H_2O$, $NaBH_4$, MeOH:THF 1:9, rt, 5min.

2.2.3. Pyrido[3,4-f][1,4]oxazepines

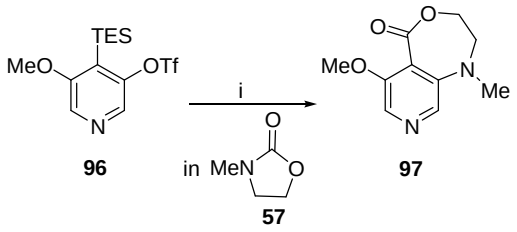
The method reported by Cale *et al.*[21] to synthesize the pyrido[2,3-*b*][1,5]oxazepines **36** (Scheme 7) and the pyrido[4,3-*f*][1,4]oxazepines **87** (Scheme 21) can also be used for the synthesis of pyrido[3,4-*f*][1,4]oxazepine **95** (Scheme 23).



Scheme 23 Synthesis of pyrido[3,4-*f*][1,4]oxazepine **95**. Reaction conditions: (i) NaH, 1-methyl-3-pyrrolidinol, DMSO, 60°C, 1h30, N_2; (ii) a) HCl-gas, b) TPP, CCl_4, Δ, 4h, c) EtOH, rt, 1h; (iii) CH_3NH_2, rt, 2d.

2.2.4. Pyrido[3,4-e][1,4]oxazepines

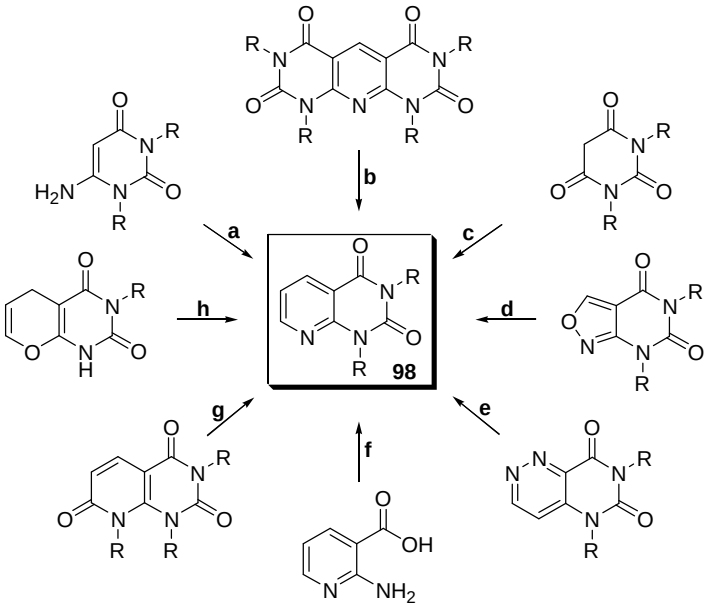
The only one reported synthesis of the pyrido[3,4-*e*][1,4]oxazepine is presented by Saito *et al.* (Scheme 24).[25] They prepared the desired pyridooxazepine **97** from 5-methoxy-4-triethylsilyl-3-trifluoromethanesulfonyloxypyridine **96** and 1-methyl-2-oxazolidone **57**. This is the same method that was used for the synthesis of the pyrido[2,3-*e*][1,4]oxazepines (Scheme 12) and the pyrido[4,3-*e*][1,4]oxazepines (Scheme 20).



Scheme 24 Reaction conditions: (i) CsF, rt, 1h.

2.3. Synthesis of pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones

Pyridines and pyrimidines have proven to be interesting subjects of research due to diverse pharmacological activities. They possess wide a range of pharmacological activities e.g. antibacterial (antibacterial agents of the nalidixic type), antitumor, antihypertensive, cardiotonic, bronchodilator, vasodilator, antialergic, antimalarial, analgestic, antifungal and CNS depressant properties.[32] Compounds containing those two rings, pyrido[2,3-*d*]pyriminides also exhibit a variety of promising pharmacological activities, such as: dihydrofolate reductase inhibition, diarrhea treatment, cyclin dependent kinase 4 inhibition and K562 cells apoptose inhibition. Compounds having pyrido[2,3-*d*]pyrimidines as a central core unit have been identified as a new class of fibroblast growth factor receptor (FGFR3) tyrosine kinase inhibitors.[33] Some pyrido[2,3-*d*]pyrimidines were found as the main metabolites of flupyrsulfon in soil.[34]



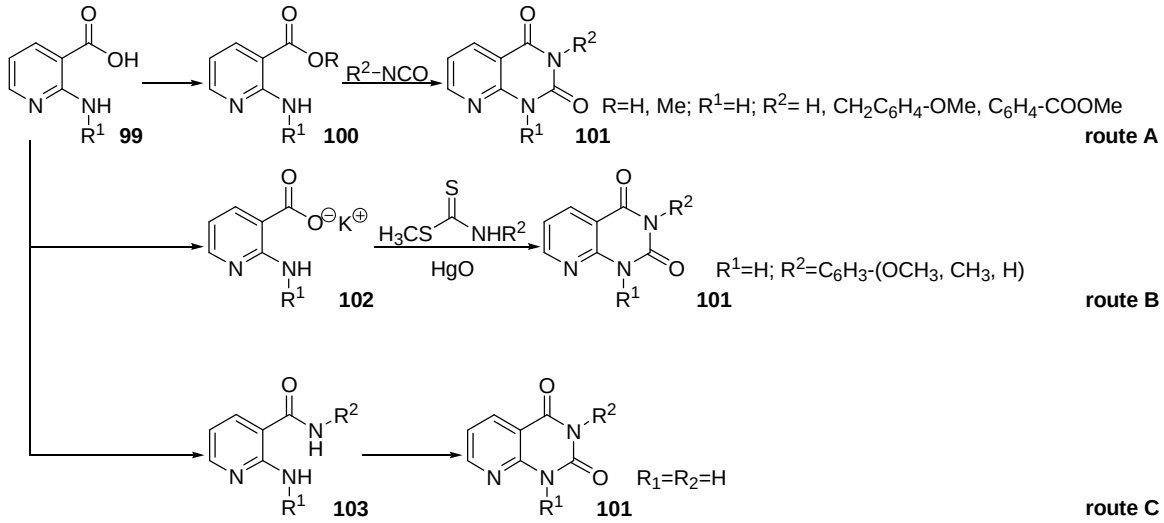
Scheme 25 Possible pathways to obtain the pyrido[2,3-*d*]pyrimidine scaffold, R=H, CH_3.

Various methods are known for the synthesis of pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones (Scheme 25): starting from 6-aminouracil[35] or 6-amino-1,3-dimethyluracil as the most convenient starting material, via condensation with α,β-unsaturated carbonyl compounds,[36] the reaction with Mannich bases,[37] the reaction with nitriles,[38] the condensation reaction of 6-amino-1,3-dimethyuracil with a dicarbonyl compound and an aldehyde in acidic conditions[39]. A three component condensation reaction between 6-amino-1,3-dimethyluracil, an aldehyde, and a nitrile (route **a**).[40] Cyclization to the desired pyrido[2,3-d]pyrimidine can be performed using palladium compound like $PdCl_2$-CuCl-O_2 complex[41] and $PdCl_2$, $Pd(OAc)_2$,[42] Pd[43] or non-palladium compounds like $TiCl_4$[44]. Some different synthetic methods with uracil derivatives provide the pyrido[2,3-*d*]pyrimidine.[45] Reaction of pyrido[2,3-*d*]pyrimidine-2,4,7(1*H*,3*H*,8*H*)-trione with $POCl_3$ leads to the desired pyrido[2,3-*d*]pyrimidine (route **g**),[46] and starting from isoxazolo[3,4-*d*]pyrimidine in the reaction with cyanoolefins in the presence of triethylamine (Et_3N) as a catalyst (route **d**),[47] arylidene derivatives of barbituric acids (route **c**),[48] pyrano[2,3-*d*]pyrimidine in reaction with ammonia (route **h**),[49] hydrolysis of pyridodipyrimidine (route **b**),[50] pyrimidotriazine with dienophiles (route **e**)[51] and pyridine-3-caboxylic acid derivatives (route **f**)[52]-[60].

Because of the performed investigations on the synthesis of the pyrido[2,3-*d*]pyrimidine scaffold in this thesis, the synthesis methods based on pyridine derivatives will be explored.

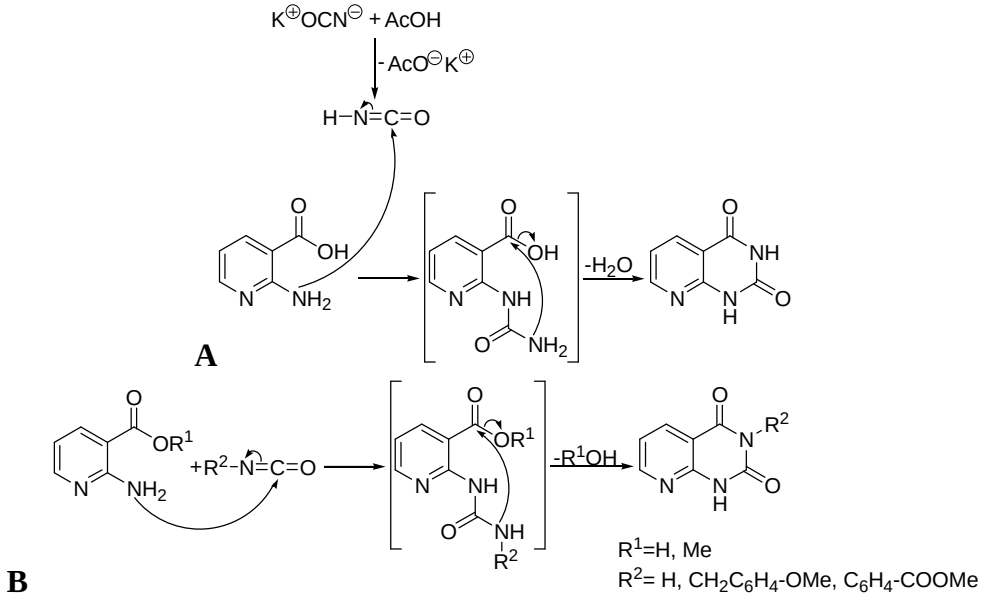
To synthesize pyrido[2,3-*d*]pyrimidine, 2-aminopyridine-3-carboxylic acid is the most popular starting material (Scheme 26). 2-Aminopyridine-3-carboxylic acid can react as a ester or free acid with isocyanates to provide the desired scaffold (route A, Scheme 26).[53] The mechanism of the reaction between 2-aminopyridine-3-carboxylic acid and isocyanate is presented in Scheme 27. The isocyanate is generated *in situ* from potassium cyanate and acetic acid. If substituted isocyanate

reacts with the ester of 2-aminopyridine-3-carboxylic acid, the alcohol is formed as a side product (route B, Scheme 27).



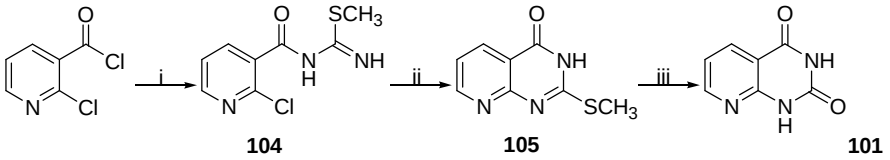
Scheme 26 The 2-aminopyridine-3-carboxylic acid as starting material for the synthesis of pyrido[2,3-*d*]pyrimidine.

The reaction of the potassium salt of 2-aminopyridine-3-carboxylic acid **102** with *N*-aryldithiocarbamate under reflux in the presence of mercury(II) oxide (HgO) in DMF, lead to the pyrido[2,3-*d*]pyrimidine after acidic hydrolysis of the 2-thioxo group.[54] As well the reaction of 2-amino-nicotinamide **103** with a carbonyl donor species (oxalyl chloride,[55] triethyl orthoacetate,[56] 1,1´-carbonyldiimidazole[57] are used to obtain the desired scaffold.



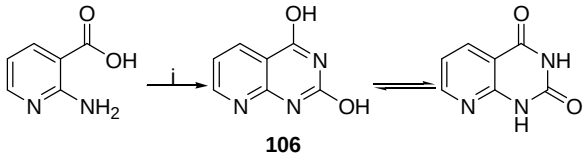
Scheme 27 Synthesis of the pyrido[2,3-*d*]pyrimidine through reaction of the free carboxylic acid (A) or the ester (B) with isocyanates.

In 1989, Monge *et al.* published the synthesis of pyrido[2,3-*d*]pyrimidine in three steps. The synthesis starts with the formation of methyl [(2-chloro-3-pyridinyl)carbonyl]-carbamimidothioate **104** from 2-chloro-3-pyridinecarboxyl chloride and 2-methylisothiourea. The substituted isothiourea was used to avoid the substitution by sulfur at C2 (Scheme 28). The cyclization was performed by heating of **104** in DMF. The compound **101** was obtained by heating compound **105** in acidic medium.[58]



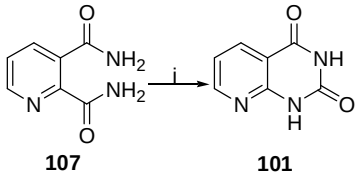
Scheme 28 Formation of pyrido[2,3-*d*]pyrimidine **101**. Reaction conditions: (i) 2-methylisothiourea, Et_3N, pyridine, $CHCl_3$, 0°C→rt, 15h; (ii) a) DMF, reflux, 15min, b) HCl in $CHCl_3$, Et_3N; (iii) HCl, reflux, 1h.

The same method to obtain the pyrido[2,3-*d*]pyrimidine scaffold was used by Palop´s group. The 2-aminopyridine-3-carboxylic acid and urea were stirred at 210°C leading to the pyrido[2,3-*d*]pyrimidin-2,4-diol **106** in 62% yield (Scheme 29).[59]



Scheme 29 Reaction conditions: (i) a) urea, 210°C, 15min, b) $NaOH_{aq}$, heated.

In 1968, Beckwith *et al.* described the synthesis of the pyrido[2,3-*d*]pyrimidine from 2,3-pyridinedicarboxamide **107** in the reaction with lead tetraacetate ($Pb(OAc)_4$) (Scheme 30). The reaction mechanism involves the formation of the isocyanate from the amide at C2 in the pyridine ring and the cyclization to pyridopyrimidine **101**. The possible isomeric product was not detected.[60]



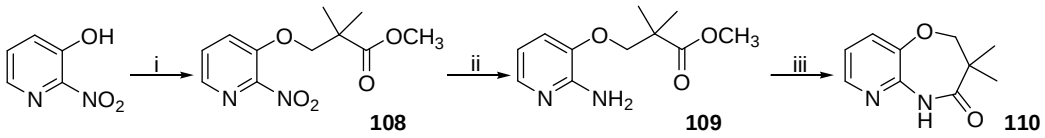
Scheme 30 Formation of the pyrido[2,3-*d*]pyrimidine **101**.
Reaction conditions: (i) DMF, $Pb(OAc)_4$, 50-60°C, 20min.

3. Results and discussion

3.1. Synthesis of pyrido[2,3-*b*][1,4]oxazepines

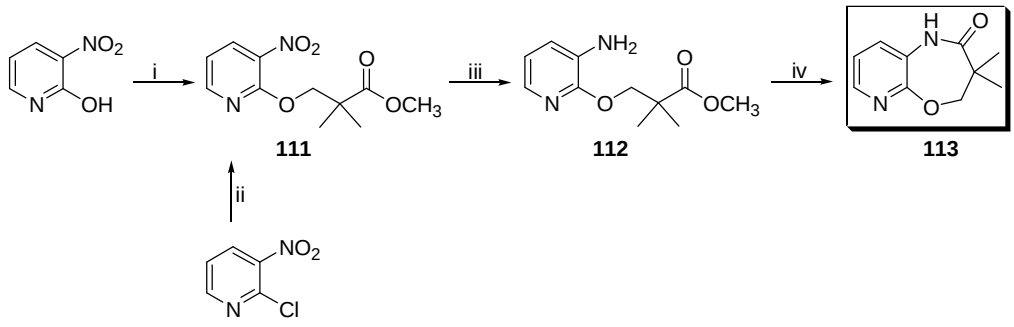
3.1.1. *Synthesis of 3,3-dimethyl-2,3-dihydropyrido[2,3-b][1,4]oxazepin-4(5H)one*

In 2008, Pauls *et al.* published the synthesis of the 3,3-dimethyl-2,3-dihydropyrido[3,2-*b*][1,4]oxazepin-4(5)-one starting from 2-nitro-3-hydroxypyridine. In the Mitsunobu reaction, the 2-nitro-3-hydroxypyridine was coupled with methyl 2,2-dimethyl-3-hydroxypropionate to obtain methyl 2,2-dimethyl-3-(2-nitropyridin-3-yloxy)propionate **108**. Then, the bicyclic 3,3-dimethyl-2,3-dihydropyrido[3,2-*b*][1,4]oxazepin-4(5)-one **110** was formed through reduction of the nitro group by H_2 and Pd/C and subsequent reaction of methyl 2,2-dimethyl-3-(2-aminopyridin-3-yloxy)propionate **109** with sodium hydride (NaH) in DMSO (Scheme 31).[61]



Scheme 31 The synthetic route presented by Pauls *et al.*[61] Reaction condition: (i) methyl 2,2-dimethyl-3-hydroxypropionate, TPP, DIAD, 1,4-dioxane, 0°C (5min) → rt (4h) → reflux (overnight), 61%; (ii) H_2, Pd/C, MeOH, rt (overnight), 100%; (iii) NaH, DMSO, rt (overnight), 94%.

To synthesize the pyrido[2,3-*b*][1,4]oxazepines, a similar synthetic route was followed. For the synthesis of methyl 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionate **111**, 2-hydroxy-3-nitropyridine and 2-chloro-3-nitropyridine was selected as starting materials.



Scheme 32 The synthesis of 3,3-dimethyl-2,3-dihydropyrido[2,3-*b*][1,4]oxazepin-4(5*H*)one **113**. Reaction conditions: (i) methyl 2,2-dimethyl-3-hydroxypropionate, TPP, DIAD, 1,4-dioxane, 0°C (5min) → rt (4h) → reflux (o.n.), (ii) methyl 2,2-dimethyl-3-hydroxypropionate, KOH, K_2CO_3, TDA-1, toluene, rt (1h) or methyl 2,2-dimethyl-3-hydroxypropionate, LiHMDS, DMF/THF, rt (19h); (iii) Fe, NH_4Cl, MeOH/H_2O, reflux (5h); (iv) NaH, DMSO, rt (18h).

Using the Mitsunobu reaction conditions, compound **111** was synthesized from 2-hydroxy-3-nitropyridine and methyl 2,2-dimethyl-3-hydroxypropionate ((i), Scheme 32). Triphenylphosphine (TPP) and diisopropyl azodicarboxylate **(**DIAD) were the reagents of choice.[62] 2,2-Dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionate **111** was obtained in 40% yield after column chromatography. Because of the low yield of **111** after coupling under the Mitsunobu conditions, an alternative approach was followed, in which the 2-chloro-3-nitropyridine and methyl 2,2-dimethyl-3-hydroxypropionate were coupled using tris(2-(2-methoxyethoxy)ethyl)amine (TDA-1)[63] or LiHMDS[64] ((ii), Scheme 32). Nucleophilic aromatic substitution of the chlorine atom in the 2-chloro-3-nitropyridine by methyl 2,2-dimethyl-3-hydroxypropionate in the presence of KOH, K_2CO_3 and TDA-1[65] gave pyridine **111** in 70% yield after column chromatography. When LiHMDS was applied as base, pyridine **111** was obtained in 50% yield after column chromatography. The ^{1}H-NMR and ^{13}C-NMR analyses of the products were compared to exclude the formation of *N*-product under the Mitsunobu reaction conditions. The products obtained by both methods showed the same shifts which proved the formation of *O*-alkylated product via Mitsunobu reaction.

For the reduction of the nitro group, two methods were adopted. The reduction using tin(II)chloride ($SnCl_2$) in ethanol under reflux for 26 hours, gave compound **112** in 80% yield.[66] Because of difficulties with the isolation of the product, a procedure using iron powder and ammonium chloride was applied ((iii), Scheme 32).[61] After 5 hours of reflux, the pure compound **112** was isolated in 90% yield. For the final ring closure, NaH in DMSO at room temperature was used,[61] and 3,3-dimethyl-2,3-dihydropyrido[2,3-*b*][1,4]oxazepin-4(5*H*)one **113** was obtained in 90% yield as a white solid.

3.1.2. *Exploration of the ring*

After the successful synthesis of bicyclic scaffold **113**, the following seven substrates were investigated (Figure 3).

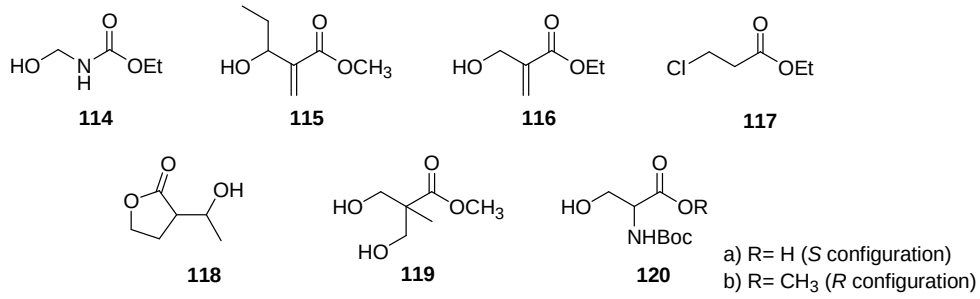
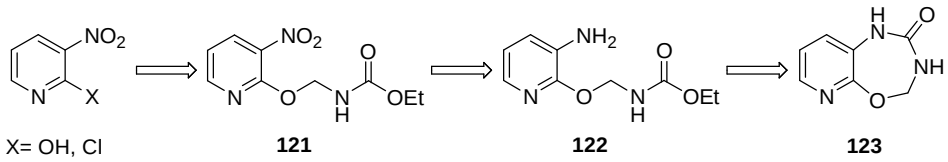


Figure 3 The investigated substrates.

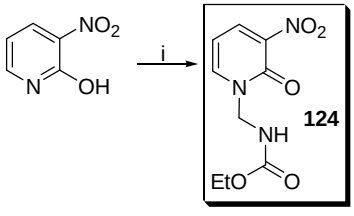
Ethyl (hydroxymethyl)carbamate **114** was synthesized from the corresponding urethane by base-catalyzed condensation with formaldehyde[67] in 40% yield. Methyl 3-hydroxy-2-methylenepentanoate **115** was synthesized by Baylis-Hillman reaction of propionaldehyde with methyl acrylate, catalyzed by 1,4-diazabicyclo[2.2.2]octane (DABCO) in 43% yield.[68] Ethyl 2-(hydroxymethyl)acrylate **116** and ethyl 3-chloropropanoate **117** are commercially available. Dihydro-3-(1-hydroxyethyl)-2(3*H*)-furanone **118** was synthesized as a mixture of isomers from the α-acetylbutyrolactone via reduction with sodium borohydride.[69] Methyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropionate **119** was synthesized by the esterification of 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid with MeOH in the presence of a catalytic amount of H_2SO_4.[70] Boc-protected serine **120a** was obtained by the reaction of serine and di-*t*-butyl dicarbonate,[71] the methyl ester of Boc-protected serine **120b** is commercially available.

The strategy for the synthesis of compound **123** is shown in Scheme 33. Starting form 2-hydroxy- or 2-chloro-3-nitropyridine and ethyl (hydroxymethyl)carbamate **114** under Mitsunobu reaction conditions or via nucleophilic aromatic substitution, product **121** could be obtained. After reduction of the nitro group and the cyclization, compound **123** can be synthetized.



Scheme 33 Retrosynthetic scheme for the formation of bicyclic compound **123**.

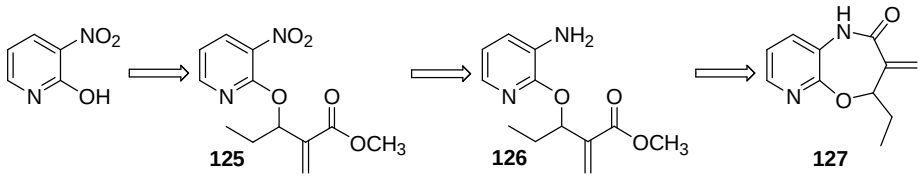
At first the Mitsunobu reaction conditions were chosen for the synthesis of **121**. DIAD and TPP were used in 1,4-dioxane. After overnight reflux the desired product was isolated in 10% yield. The low yield is due to the difficult separation of the product and triphenylphosphine oxide (TPPO). As an alternative phosphorus compound, tributylphosphine (*n*-Bu_3P) was selected. After reaction with *n*-Bu_3P and diethyl azodicarboxylate (DEAD) in THF[72] or DME,[13] the product could be obtained in 40-47% yield. However, NMR analysis (HMBC analysis) confirmed that the isolated compound is the *N*-substituted product **124** and not the *O*-substituted product **121**.



Scheme 34 The synthesis of *N*-alkylated pyridinone **124**. Reaction conditions: (i) ethyl (hydroxymethyl)carbamate **114**, DIAD, TPP, 1,4-dioxane, reflux or DEAD, *n*-Bu_3P, THF or DME, 40°C or rt.

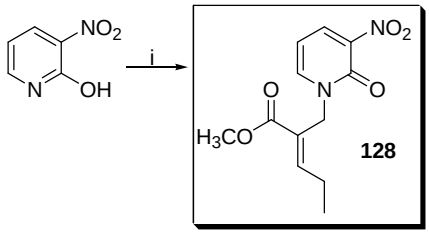
Due to the formation of *N*-substituted pyridinone **124** (Scheme 34), the 2-chloro-3-nitropyridine was used as the starting material to exclude the formation of the *N*-substituted product. Two different methods were investigated. After 1 hour stirring at room temperature of the mixture of 2-chloro-3-nitropyridine and ethyl (hydroxymethyl)carbamate in the presence of KOH, K_2CO_3 and a catalytic amount of TDA-1,[65] only starting materials were detected by ^{1}H-NMR. Also for the reaction with NaH in DMF at -20°C only starting materials were detected after 1 hour. The reason of non formation of the desired compound **121**, could be a competition between of the formation of the anion of the hydroxyl group and the nitrogen of the carbamate.

Because of the problems with formation of the *O*-alkylated product, a different substrate was selected to prepare structural derivatives, methyl 3-hydroxy-2-methylenepentanoate **115**. A proposed pathway to the bicyclic compound is shown in Scheme 35.



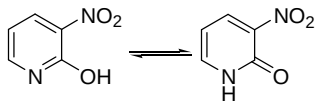
Scheme 35 Retrosynthetic scheme for the formation of bicyclic compound **127**.

2-Hydroxy-3-nitropyridine and methyl 3-hydroxy-2-methylenepentanoate **115** were coupled under Mitsunobu reaction conditions. Two solvents were used, THF and 1,4-dioxane,[62] and DIAD and TPP were employed as the promoting agents to form the betaine. After reaction, a product was isolated in 66% and 55% yield, respectively. The NMR analysis confirmed that the isolated compound is the *N*-substituted 2(1*H*)-pyridinone **128** (Scheme 36).



Scheme 36 The synthesis of *N*-alkylated pyridinone **128**. Reaction conditions: (i) methyl 3-hydroxy-2-methylenepentanoate, DIAD, TPP, THF/1,4-dioxane, rt/reflux.

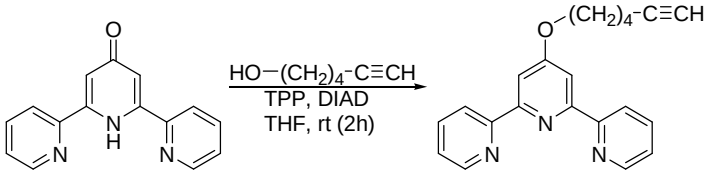
Due to the isolation of *N*-alkylated products in the reaction mixture, the equilibrium of 2-hydroxy-3-nitropyridine with 3-nitro-2(1*H*)-pyridinone **129** (Scheme 37) is shifted to the right to the 3-nitro-2(1*H*)-pyridinone.



129

Scheme 37 The equilibrium between 2-hydroxy-3-nitropyridine and 3-nitro-2(1*H*)-pyridinone.

A good example to control the formation the *O*-alkylated product was reported by Hovinen. He performed the reaction between 2,6-di(pyridine-2-yl)pyridine-4(1*H*)-one with an alcohol under Mitsunobu conditions (DIAD and TPP). The steric hindrance at the C2 and C6 of the 4(1*H*)-pyridinone causes the ether formation to be favorable instead of the *N*-alkylation (Scheme 38).[74]



Scheme 38 *O*-alkylation of 2,6-di(pyridine-2-yl)pyridine-4(1*H*)-one under Mitsunobu conditions.

It is not sure that the steric hindering group at C6 in 3-nitro-2(1*H*)-pyridinone will control the formation of the *O*-alkylated product. The steric hindrance of the alkylating agent can also have influence on the creation of the *N*-alkylated product. Comins and Jianhua used the Mitsunobu reaction as an alternative way for alkylation via silver salts.[73] In their investigations, THF or DME and *α*-benzylated alcohols were used at room temperature. *O*-alkylated products were obtained in high yields as a single product, no *N*-alkylated products were detected. In their case, steric hindrance and the solvent had a big influence for the direction of the alkylation. Ethyl (hydroxymethyl)carbamate **114** is not sterically hindered and the 6 position in the pyridine ring is well accessible[75][82] which can decrease *O*-alkylation. *N*-Alkylation was favored even with DME as a solvent.[73] On the other hand methyl 3-hydroxy-2-methylenepentanoate **115** is more sterically demanding due to the ethyl group in *α* position to hydroxyl group and methylidene in *β* should lead to *O*-alkylation. The group of Charette proposed the mechanism of formation for *γ*-attack (Figure 4).[76][77]

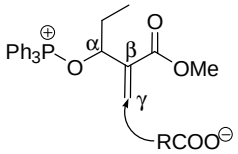
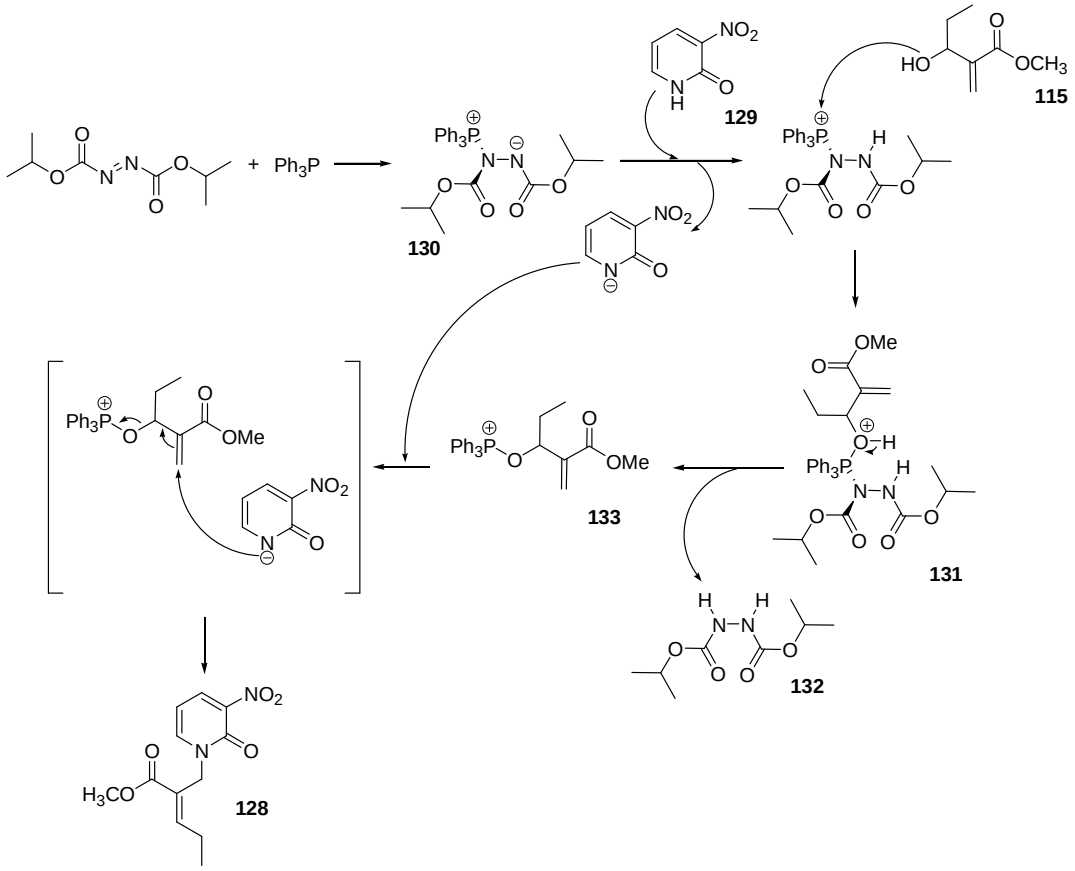


Figure 4

They performed several reactions under Mitsunobu conditions with various acids and solvents. In all reactions the major product was formed after attack of the nucleophile at the *γ* position. Using

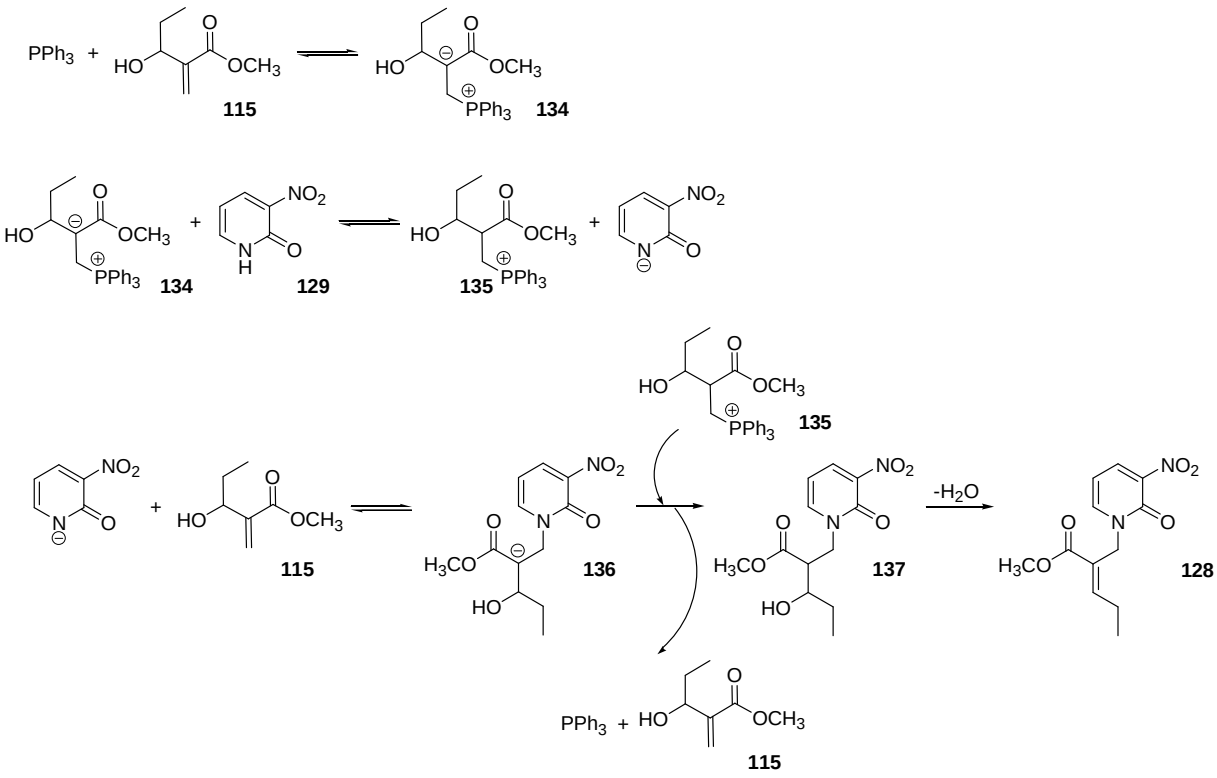
stronger carboxylic acids the ratio of *γ*:*α* attack decreased. Increasing the steric bulk of the group in *α* position of the alcohol (change the ethyl group to *i*-propyl or *t*-butyl) not only suppressed the *α*-attack, but it also considerably slowed down the *γ*-attack process.[77]

The proposed mechanism of formation of compound **128** via the Mitsunobu reaction is presented in Scheme 39. DIAD and TPP create the betaine **130** which can deprotonate 3-nitro-2(1*H*)-pyridinone **129**. The positively charged phosphorous atom in adduct **130** is attacked by the lone pair of the hydroxyl group of **15** thereupon creating **131**. After rearrangement, diisopropyl hydrazodicarboxylate **132** is removed and formed **133** is attacked by the pyridinone anion to produce **128** after losing of triphenylphosphine oxide.



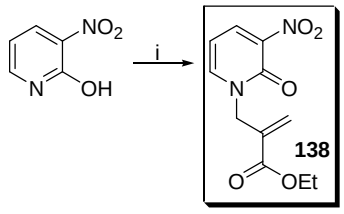
Scheme 39 The proposed mechanism for the synthesis of **128** via Mitsunobu reaction.

A second proposed mechanism involves an aza-Michael reaction catalyzed by phosphine (Scheme 40). The TPP attacks at the *β*-position of the olefin, generating the reactive intermediate **134**. The resulting anion **134** deprotonates the 3-nitro-2(1*H*)-pyridinone **129**, and the addition to the *β*-position of another olefin creating anion **136**. The formed **136** reacts with another **135** providing compound **137**. During this reaction TPP and methyl 3-hydroxy-2-methylenepentanoate **115** are released. The methyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-pent-2-enoate **128** is the result of the spontaneous elimination of H_2O forming the double bond.



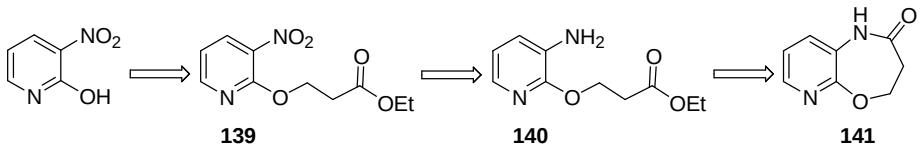
Scheme 40 The proposed mechanism for the synthesis of **128** via aza-Michael reaction catalyzed by triphenylphosphine.

Another ester with a double bond was chosen to further explore the non-aromatic ring in pyrido[2,3-*b*][1,4]oxazepines. Ethyl 2-(hydroxylmethyl)acrylate **116** reacted with 2-hydroxy-3-nitropyridine under Mitsunobu reaction conditions. Three different solvents were selected: DME,[73] 1,4-dioxane[62] and THF. As a result only *N*-alkylated pyridinone **138** was isolated (Scheme 41). The *O*-alkylated product was not detected in the reaction mixture.



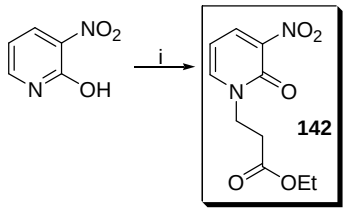
Scheme 41 The synthesis of *N*-alkylated pyridinone **138**. Reactions conditions: (i) **116**, TPP, DIAD, DME, rt (22h), 44% or **116**, TPP, DIAD, dioxane, rt (4h)- reflux (17h), 85% or **116**, TPP, DIAD, THF, rt (73h), 93%.

Further, a different alkylating agent ethyl 3-chloropropanoate **117** was chosen. The strategy is shown in Scheme 42. 2-Hydroxy-3-nitropyridine reacted with **117** in the presence of Et_3N in MeCN at room temperature for 48 hours.[78] The *N*-alkylated product **142** was isolated as yellow oil (Scheme 43). The *O*-alkylated pyridine **139** was not formed under that condition.



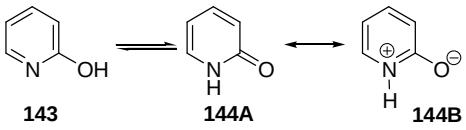
139 **140** **141**

Scheme 42 Retrosynthetic scheme for the formation of bicyclic compound **141**.



Scheme 43 The *N*-alkylation of 2-hydroxy-3-nitropyridine. Reaction conditions: (i) ethyl 3-chloro-propionate **117**, Et_3N, MeCN, rt, 48h.

In the literature, the aspect of tautomerism of 2-hydroxypyridine **143** with 2-pyridinone **144** (Scheme 44) is known.[79]



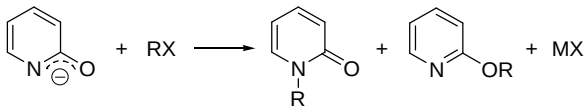
143 **144A** **144B**

Scheme 44 The tautomerism of 2-hydroxypyridine with 2-pyridinone.

Since the positive charge prefers tetravalent nitrogen and negative charge prefers oxygen, the charge-separated structure **144B**, which is also associated with an aromatic sextet, makes a significant contribution to the overall structure of 2-pyridone. Polar solvents stabilize the polar tautomer. Substituents also influence the position of the equilibrium. Electron-donating substituents favor the pyridine **144** and electro-withdrawing substituents favor the hydroxypyridine **143**. An electron-withdrawing group adjacent to a ring nitrogen atom tends to decrease its basicity, and so a tautomer with a proton at that nitrogen atom is destabilized, and the equilibrium displaced towards the isomer. Substituents may also favor one tautomer by intramolecular hydrogen bonding. The tautomeric form **129** of 2-hydroxy-3-nitropyridine is presented on Scheme 37.

The tautomerism of 2-hydroxy-3-nitropyridine and *N*-alkylation was a research subject of many research groups.[73][80] *O*-alkylation can be favored using silver salts[81]. In 1967, Hopkins *et. al.*[82] published an article about the influence of cations and the alkylating agent on *O*-alkylation of 2-pyridones (Scheme 45). According to this study the reaction with silver salts gave *O*-alkylated product as a major product or as the only product. During this investigation they found that silver salt alkylations were highly solvent sensitive and were observed in nonpolar solvents such as benzene, hexane and pentane. The procedure was applied for 3-nitro-2(1*H*)-pyridinone **129**. The silver salt of

3-nitro-2(1*H*)-pyridinone was obtained by mixing 3-nitro-2(1*H*)-pyridinone **129** with silver(I) nitrate ($AgNO_3$)[83] in 89% yield or 3-nitro-2(1*H*)-pyridinone with silver(I) oxide (Ag_2O). That prepared salt of 3-nitro-2(1*H*)-pyridinone was stirred at room temperature with ethyl 3-chloropropionate for 20-23 hours. After removal of the precipitate by filtration and washing with MeCN, the solvent was removed, however the ^{1}H-NMR analysis only showed ethyl 3-chloropropionate.



Scheme 45 The reaction of silver salt of 2-pyridones with alkylhalides.[79]

The *N*-substituted pyridinones are very promising inhibitors for cysteine proteases[84] which mediate protein hydrolysis. The best characterized *Plasmodium* cysteine proteases are falcipains, which are papain enzymes. Falcipain-2 and falcipain -3 are major hemoglobinases of *P. falciparum*.[85] Structural and functional analysis of falcipains showed that they have unique domains including a refolding domain and a hemoglobin binding domain (Figure 5, Figure 6). Overall, the complex of falcipain-2 and falcipain-3 with small and macromolecular inhibitors provides structural insight to facilitate the design or modification of effective drug treatment against malaria.

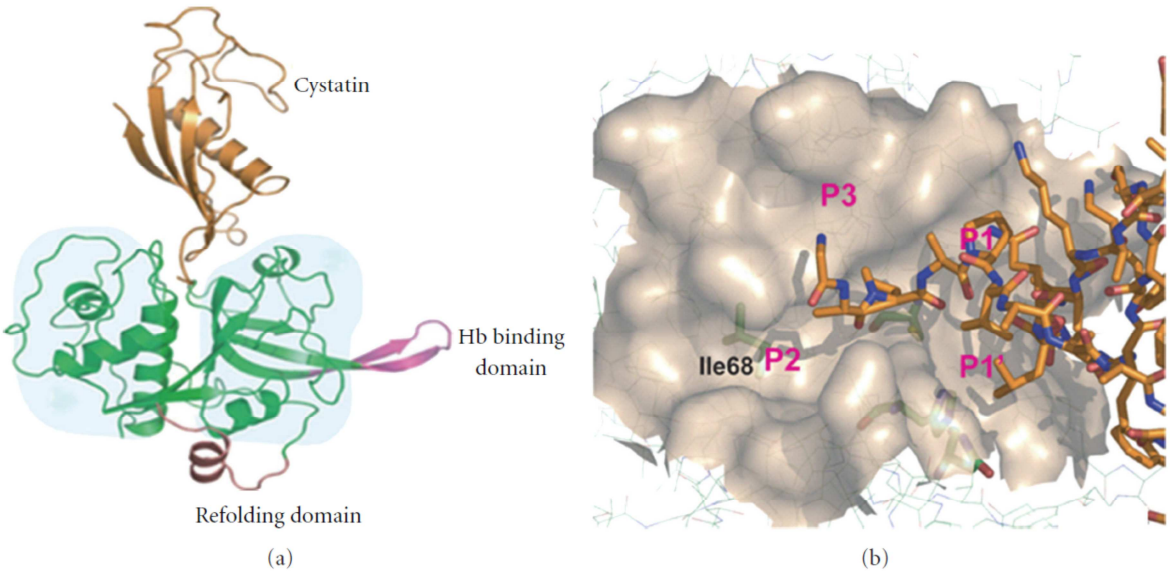


Figure 5 3D structure of falcipain-2-cystatin complex (falcipain-2 protease is green).[86]

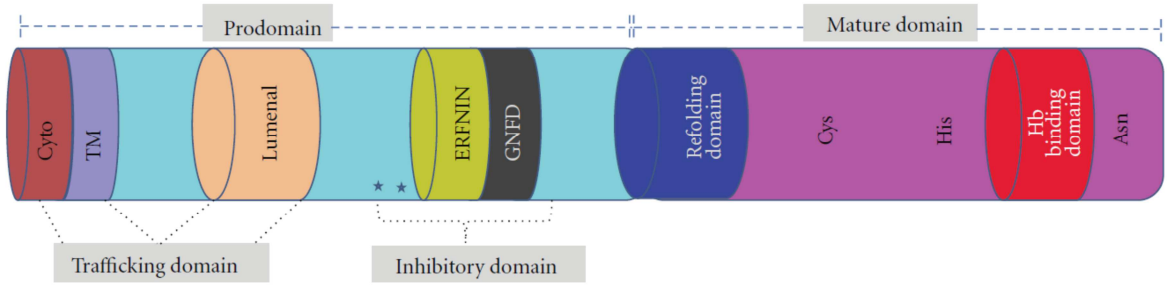


Figure 6 Domains of falcipain-2. Prodomain is made up of cytoplasmic transmembrane, luminal and inhibitor domains. The mature domain has a refolding domain, hemoglobin (Hb) binding domain and catylytic triad residues (Cys, His, Asn).[86]

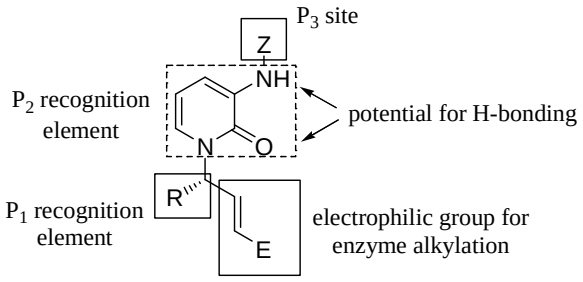
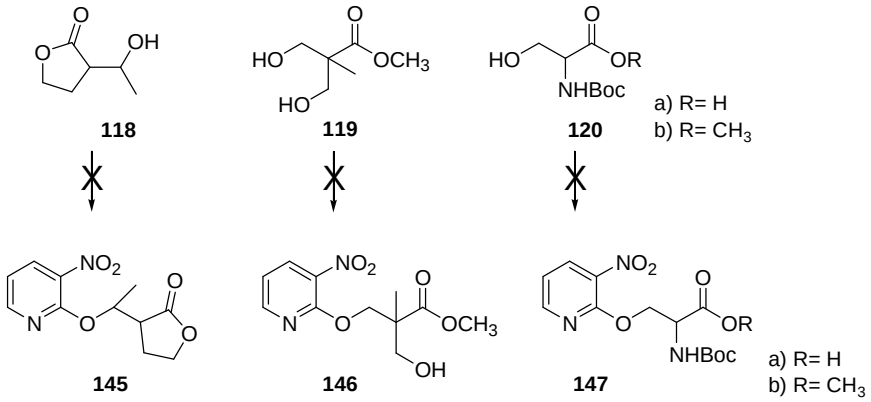


Figure 7 Structure of peptidomimetic template.[87]

The key structural aspect of these inhibitors is a moiety containing the desired substituent at the P1 position and the ester function, to allow subsequent chemical transformation (Figure 7).[87]

The next four chains were selected as a potentially good reagents for formation of ether bond with 2-hydroxy-3-nitropyridine: dihydro-3-(1-hydroxyethyl)-2(3*H*)-furanone **118**, methyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate **119**, 2-[(tert-butoxycarbonyl)amino]-3-hydroxypropionic acid **120a** and methyl 2-[(tert-butoxycarbonyl)amino]-3-hydroxypropionate **120b** (Scheme 46).



Scheme 46 Unsuccessful formation of compounds **145**-**147**.

The dihydro-3-(1-hydroxyethyl)-2(3*H*)-furanone **118** was synthesized from α-acetylbutyrolactone[69] as a mixture of isomers. Then, the Mitsunobu reaction conditions were applied. The dihydro-3-(1-hydroxyethyl)-2(3*H*)-furanone **118** was treated with DIAD and TPP or tributylphosphine (*n*-Bu_3P) in THF or 1,4-dioxane (Table 1). However, all attempts failed.

Table 1: The conditions for reaction of 2-hydroxy-3-nitropyridine with dihydro-3-(1-hydroxyethyl)-2(3*H*)-furanone **118**.

Entry	Reagents	Conditions and solvents	Result
1[62]	2-hydroxy-3-nitropyridine (1eq), DIAD (1.1eq), TPP (1.1eq)	rt (4h)→reflux (21h), 1,4-dioxane	NR
2	2-hydroxy-3-nitropyridine (1eq), DIAD (1.1eq), TPP (1.1eq)	rt (98h), THF	NR
3[72]	2-hydroxy-3-nitropyridine (1eq), DIAD (1.1eq), *n*-Bu_3P (1.1eq)	40°C (20h), THF	NR

Methyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate **119** was synthesized from 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid.[70] Table 2 present all reactions with methyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate and 2-chloro-3-nitropyridine or 2-hydroxy-3-nitropyridine.

The reaction of 2-hydroxy-3-nitropyridine with methyl 2-[(tert-butoxycarbonyl)amino]-3-hydroxypropionate **120b** under Mitunobu reaction conditions (DIAD, TPP, 1,4-dioxane) or 2-chloro-3-nitropyridine with 2-[(tert-butoxycarbonyl)amino]-3-hydroxypropionic acid **120a** in dry DMF[93] failed.

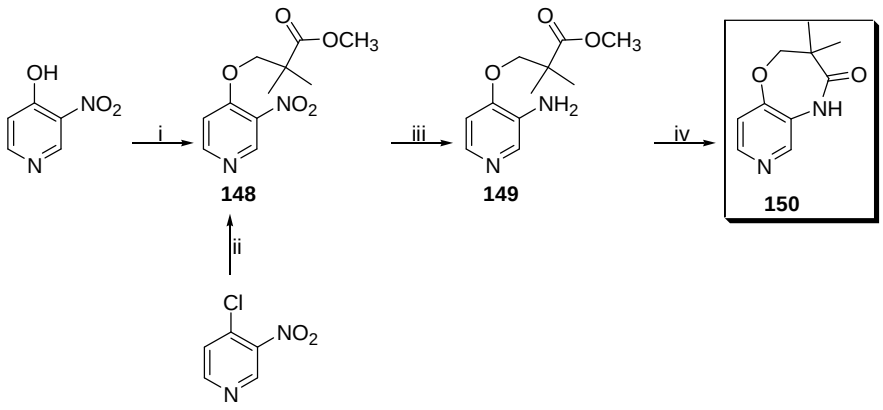
Table 2: Reactions conditions for the potential synthesis of **146**.

Entry	Reagents	Conditions and solvents	Result
1[88]	2-chloro-3-nitropyridine (1eq), LiHMDS (0.12eq)	rt (21h), THF	SM
2[62]	2-hydroxy-3-nitropyridine (1eq), DIAD (1.1eq), TPP (1.1eq)	rt (4 h)→reflux (21h), 1,4-dioxane, N_2	NR
3[62]	2-hydroxy-3-nitropyridine (1eq), DIAD (1.1eq), TPP (1.1eq)	rt (25h), THF, N_2 reflux (25h), THF, N_2	NR
4[89]	2-hydroxy-3-nitropyridine (1eq), DEAD (1.4eq), DPPE (1eq)	rt (21h), THF, N_2	NR
5[89]	2-hydroxy-3-nitropyridine (1eq), DEAD (2eq), DPPE (1eq)	rt (21h), toluene, N_2	NR
6[72]	2-hydroxy-3-nitropyridine (1eq), DEAD (3eq), *n*-Bu_3P (3eq)	40°C (18h), THF, N_2	NR
7[72]	2-hydroxy-3-nitropyridine (1eq), DIAD (3eq), *n*-Bu_3P (3eq)	40°C (23h), THF, N_2	NR
8[90]	2-hydroxy-3-nitropyridine (1eq), ADDP (1.5eq), TPP-PS (1.5eq)	rt (16h), THF, N_2	NR
9[91]	2-hydroxy-3-nitropyridine (1eq), DEAD (1.2eq), TPP-PS (1.5eq)	rt (17h), CH_2Cl_2, N_2	NR
10[91]	2-hydroxy-3-nitropyridine (1eq), DEAD (1.2eq), TPP-PS (1.5eq), Et_3N (1.4 eq)	rt (19h), THF, N_2	NR
11[72]	2-hydroxy-3-nitropyridine (1eq), ADDP (1.1eq), *n*-Bu_3P (1.1eq)	40°C (23h), THF, N_2	NR
12[92]	2-hydroxy-3-nitropyridine (0.9eq), DCAD (1.1eq), *n*-Bu_3P (1.1eq)	rt (18h), THF, N_2	NR

3.2. Synthesis of pyrido[4,3-*b*][1,4]oxazepines

3.2.1. *Synthesis of 3,3-dimethyl-2,3-dihydropyrido[4,3-b][1,4]oxazepin-4(5H)one*

The strategy to obtain pyrido[4,3-*b*][1,4]oxazepine **150** is presented in Scheme 47. To synthesize pyrido[4,3-*b*][1,4]oxazepine **150** the same synthetic route was used as for the pyrido[2,3-*b*][1,4]oxazepines. 4-Hydroxy-3-nitropyridine and 4-chloro-3-nitropyridine were selected as starting materials.



Scheme 47 The synthesis of 3,3-dimethyl-2,3-dihydropyrido[4,3-*b*][1,4]oxazepin-4(5*H*)one **150**. Reaction conditions: (i) methyl 2,2-dimethyl-3-hydroxypropionate, TPP, DIAD, 1,4-dioxane, 0°C (5min)→rt (4h)→reflux (o.n.); (ii) methyl 2,2-dimethyl-3-hydroxypropionate, KOH, K_2CO_3, TDA-1, toluene, rt (20h); (iii) Fe, NH_4Cl, MeOH/H_2O, reflux (5h); (iv) NaH, DMSO, rt (22h).

For the synthesis of methyl 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate **148**, two methods were selected. 4-Hydroxy-3-nitropyridine was coupled with methyl 2,2-dimethyl-3-hydroxypropionate under Mitsunobu reaction conditions (DIAD, TPP) in 1,4-dioxane. After stirring at room temperature for 4 hours and then overnight at reflux, the product **148** was isolated by column chromatography in 44% yield as a yellow oil ((i), Scheme 47).
The pyridine **148** was also obtained through nucleophilic substitution of chlorine atom in 4-chloro-3-nitropyridine under TDA-1 ((ii), Scheme 47).[65] The resulting yield of methyl 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate **148** was lower (21%) compared to the first conditions (44%). In the next step the nitro group was reduced to the amine. Several methods were applied (Table 3). The method with tin(II)chloride ($SnCl_2$) in ethanol under reflux for 16 hours, gave compound **149** in 80% yield.[66]. Because of difficulties with the isolation of the product, the reaction with iron powder and ammonium chloride was applied.[61] After 5 hours of reflux compound **149** was isolated in 95% yield. This method was the most efficient one. The intermolecular cyclization of compound **149** was performed using sodium hydride (NaH) in DMSO at room temperature ((iv) Scheme 47), the bicyclic pyridine **150** was obtained in 90% yield as a white solid.[61]

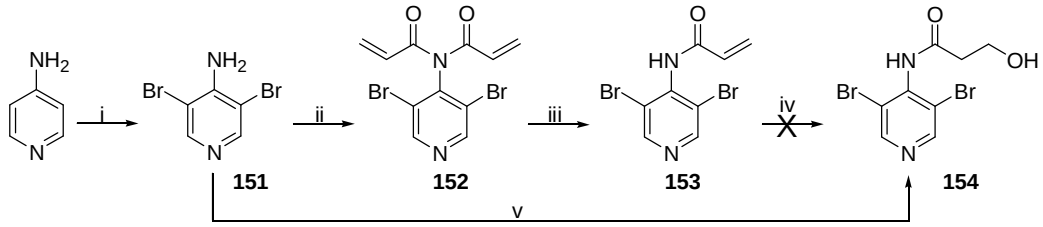
Table 3: Methods for the reduction of nitro group of compound **148**.

Entry	Substrates, solvents	Conditions	Product 149
1[61]	Fe (4eq), NH_4Cl (1.5eq), MeOH/H_2O (5:1)	reflux (5h)	95%
2[66]	$SnCl_2$ (5eq), EtOH	reflux (16h)	80%
3[94]	Fe (5eq), AcOH (0.03eq), H_2O (0.02eq), EtOH	reflux (16h)	68%
4[95]	Pd/C (10 mol%), $NaBH_4$ (2eq), H_2O/THF	rt (10-30min)	40%
5[96]	Zn (10eq), NH_4Cl (10eq), MeOH/THF (1:1)	rt (overnight)	SM
6[97]	$Na_2S_2O_4$ (4.1eq), K_2CO_3 (5eq), MeOH/H_2O (6:1)	rt (22h)	SM
7[98]	sulfur (3eq), $NaHCO_3$ (3eq), DMF	130°C (5.5h)	SM

In comparison, Mitsunobu reaction of 4-hydroxy-3-nitropyridine with methyl 2,2-dimethyl-3-hydroxypropionate gave a comparable yield to the obtained methyl 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionoate **111** under the same conditions. Although, the aromatic substitution of the chlorine atom in 4-chloro-3-nitropyridine gave a lower yield of the desired product than for 2-chloro-3-nitropyridine under the same conditions. The yield of the amine after reduction of the nitro group was slighty higher for pyridine **149**. The cyclization proceeded without difficulties with excellent yield for both pyridoxazepines. The presence of the two methyl groups are more sterically demanding than two hydrogens in α position in the chain. The repelling effect of two methyl groups reduces the internal angle and brings the two reactive units (anion created by deprotonation of amine group by NaH and methyl ester), closer together, which facilitates the cyclization (the Thorp-Ingold effect).

3.3. Synthesis of pyrido[3,4-*b*][1,4]oxazepines

In the first attempt to synthesize pyrido[3,4-*b*][1,4]oxazepines, 4-aminopyridine was chosen as starting material.



Scheme 48 Synthesis of the *N*-(3,5-dibromo-pyridin-4-yl)-3-hydroxy-propionamide.
The reaction conditions: (i) NBS, CCl_4, rt, 48h, 70%; (ii) acryloyl chloride, Et_3N, CH_2Cl_2, 0°C (2h) → rt (19h), 50%; (iii) 1M $NaOH_{aq}$, rt, 2h, 90%; (iv) MeOH, DBU, rt (9 days); (v) β-propiolactone, Et_2AlCl, CH_2Cl_2, 0°C→rt (2h), 72%.

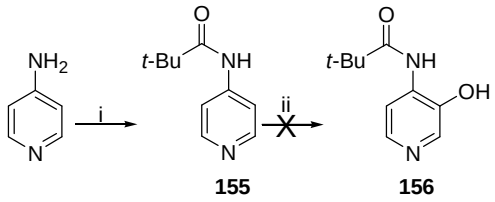
First, 4-aminopyridine was brominated with NBS to obtain 4-amino-3,5-dibromopyridine **151** in 70% yield.[99] In the next reaction 4-amino-3,5-dibromopyridine was transformed in the diamide **152** with acryloyl chloride in the presence of Et_3N.[100] To remove one of acryoyl groups, compound **152** was stirred at room temperature in a 1M aqueous solution of sodium hydroxide. To introduce the hydroxyl group onto double bond, the hydroalkoxylation was selected. Therefore, compound **153** was stirred in MeOH in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 9 days.[101] After this time, only starting material was isolated. To avoid this step, the reaction of 4-amino-3,5-dibromopyridine with *β*-propiolactone was performed in the presence of diethylaluminum chloride (Et_2AlCl), after 2 hours of stirring at room temperature *N*-(3,5-dibromo-pyridin-4-yl)-3-hydroxy-propionamide **154** was obtained ((v), Scheme 48).[102] However, different attempts for the ring closure of **154**, employing varying reaction conditions, did not lead to the desired bicyclic compound (Table 4). In all reaction mixtures only 4-amino-3,5-dibromopyridine was detected.

Table 4: Ring closure procedures of *N*-(3,5-dibromo-pyridin-4-yl)-3-hydroxy-propionamide **154**.

Entry	Substrates	Conditions and solvent	Result
1[103]	**154** (1eq), $Pd(OAc)_2$ (0.02eq), Ligand* (0.03eq), Cs_2CO_3 (1.5eq)	50°C (24h), toluene, N_2	**
2[104]	**154** (1eq), NaH (1.2eq)	80°C (2h), DMF	**
3[104]	**154** (1eq), KO*t*-Bu (1.2eq)	reflux (2h), DMF	**
4	**154** (1eq), *n*-BuLi (1.2eq)	rt (2h), THF, N_2	**
5	**154** (1eq), NaH (1.2eq)	rt (18h), THF, N_2	**

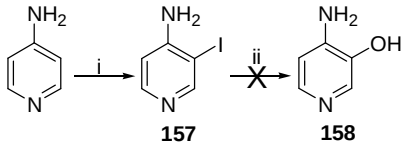
Ligand* *rac*-2-(di-*tert*-butylphosphino)-1,1′-binaphthyl;**4-amino-3,5-dibromopyridine

After all failed trials for the ring closure of **154** to the bicyclic compound, all investigations were focused on the synthesis of 4-amino-3-hydroxypyridine. Only four methods are known in literature for the synthesis of 4-amino-3-hydroxypyridine. In 1958, Boyland and Sims reported the oxidation of 4-aminopyridine to 4-aminopyridine-3-pyridyl hydrogen sulfate. After cleavage of the sulfate group, the target compound was obtained in 5% overall yield.[105] The second synthesis is based on the synthesis of 3-methoxypyridine.[106] The third method involves *ortho*-amination of 3-pyridyl *N,N*-diethylcarbamate with *p*-toluenesulfonyl azide (TsN_3) followed by cleavage of the carbamate.[107] In 1983, Turner reported regioselective metalation of 4-(pivaloylamino)pyridine at C3 position.[108] Chu-Moyer and Berger used this relation for the synthesis of 4-amino-3-hydroxypyridine.[109] The 4-(pivaloylamino)pyridine was treated with an excess of butyllithium (*n*-BuLi) in THF at 0°C for 4 hours. After this time trimethyl borate ($B(OMe)_3$) was used as electrophile, and was followed by oxidative workup. The 3-hydroxy-4-(pivaloylamino)pyridine was obtained in 81%. To follow the described procedure, 4-(pivaloylamino)pyridine **155** was synthesized from 4-aminopyridine and pivaloyl chloride in the presence of Et_3N.[110] Chu-Moyer- Berger`s procedure was repeated but only starting material was isolated.



Scheme 49 Synthesis of 3-hydroxy-4-(pivaloylamino)pyridine according to Chu-Moyer- Berger`s procedure. Reaction conditions: (i) pivaloyl chloride, Et_3N, CH_2Cl_2, 0°C→ rt, 3h, 70%; (ii) a) *n*-BuLi, THF, -78°C → 0°C (4h), b) $B(OMe)_3$, -78°C → 0°C (2h), c) AcOH, H_2O_2, rt, 3h.

In order, to introduce a hydroxyl group in the 3- position, the 4-amino-3-iodopyridine **157** was synthesized.[111]



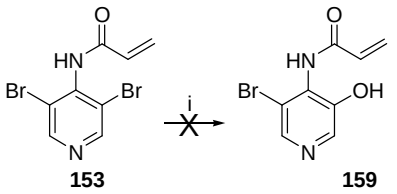
Scheme 50 Synthesis of 4-amino-3-hydroxypyridine. Reaction conditions: (i) KI, I_2, Na_2CO_3, H_2O, reflux, 2h, 26%; (ii) CsOH (50 % aq), CuI, dibenzoylmethane (ligand), DMSO/H_2O (1:1), 110°C, 24h[112]/ KOH, CuI, 8-hydroxyquinoline, DMSO/H_2O (1:1), 110°C, 48h[113].

To obtain 4-amino-3-hydroxypyridine **158**, the conditions to direct the hydroxylation of aryl iodides catalyzed by CuI/ligand system with KOH or CsOH was applied (Scheme 50). This Ullmann-type coupling of aryl iodide with phenols is widely used for formation of ethers, amines

or thioethers.[114] The coupling reaction of 4-amino-3-iodopyridine with KOH or CuOH did not lead to the desired 4-amino-3-hydroxypyridine **158**. Continuing studies on the direct hydroxylation of halopyridine, the Buchwald coupling conditions were used for reaction of 4-amino-3,5-dibromopyridine **151** with KOH in the presence of tris(dibenzylideneacetone)dipalladium(0) (Pd_2dba_3) and 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl (*t*-Bu XPhos) as a ligand.[15] After 20 hours of stirring at 100°C only starting material was isolated.

To introduce the hydroxyl group into the pyridine ring another method was applied, Cu-catalyzed benzyloxylation. The pyridine **151** was heated at 110°C with benzyl alcohol in the presence of CuI and 1,10-phenantroline as ligand.[116] After 24 hours of stirring only 4-amino-3,5-dibromopyridine was detected in the reaction mixture.

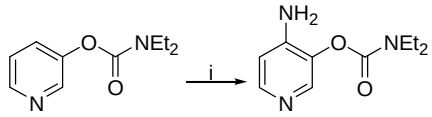
The reaction to introduce the hydroxyl group in the pyridine **153** was performed. To this purpose, pyridine **153** was reacted with KOH/CsOH catalyzed by CuI in the presence of ligand (Scheme 51). After 24 hours of stirring at 110°C, only starting material was isolated.



153 **159**

Scheme 51 The hydroxylation of **153** catalyzed by CuI in the presence of ligand. Reaction conditions: (i) CsOH (50 % aq), CuI, dibenzoylmethane (ligand), DMSO/H_2O (1:1), 110°C, 24h[112]/ KOH, CuI, 1,10-phenantroline, DMSO/H_2O (1:1), 110°C, 24h[117].

As another possibility to synthesize the 4-amino-3-hydroxypyridine, 3-hydroxypyridine was chosen as a starting material. In 1992, Shutske *et al.* published the synthesis of 4-amino-3-pyridinol *N*,*N*-diethylcarbamate **161** starting form *N*,*N*-diethylcarbamate of 3-hydroxypyridine (Scheme 52).[107]

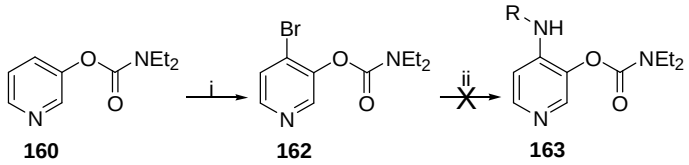


160 **161**

Scheme 52 Synthesis of 4-amino-3-pyridinol *N*,*N*-diethylcarbamate **161**.
Reaction conditions: TMEDA, THF, a) *s*-BuLi, -78°C, 1h, b) TsN_3, rt, c) TBAHS, $NaBH_4$, H_2O.

The *N*,*N*-diethylcarbamate of 3-hydroxypyridine **160** was allowed to react with *s*-BuLi in the presence of tetramethylethylenediamine (TMEDA) to achieve *ortho*-lithation. Then the lithiated pyridine was treated with tosyl azide and the product was directly reduced with $NaBH_4$ in the presence of tetra-*n*-butylammonium hydrogen sulfate (TBAHS) to obtain 4-amino-3-pyridinol *N*,*N*-diethylcarbamate **161**.[107] In order to follow the described procedure, the *N*,*N*-diethylcarbamate

of 3-hydroxypyridine **160** was synthesized by reaction of 3-hydroxypyridine with diethylcarbamoyl chloride in pyridine.[118] The procedure was repeated but after work-up, the crude mixture did not contain the desired product. Because of the unsuccesful amination of **160** with tosyl azide, a bromine atom was introduced on C4 of the pyridine ring as a good leaving group for nucleophilic substitution (Scheme 53). The *N*,*N*-diethylcarbamate of 3-hydroxypyridine **160** reacted with *s*-BuLi in the presence of TMEDA, then to the reaction mixture 1,2-dibromoethane was added. After 2 hours of stirring at -78°C, the desired bromo-derivative **162** was isolated in 20% yield after column chromatography.[119] The yield increased to 70% when the reaction was conducted without TMEDA.



Scheme 53 Synthetic pathway to compound **163**. Reaction conditions: (i) *s*-BuLi, 1,2-dibromoethane, THF, -78°C, 2h, 70%; (ii) see Table 5; R= allyl, propyl, benzyl.

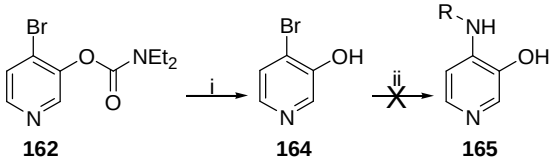
To introduce the amine group at C4 in the pyridine **162**, several conditions were tested (Table 5).

Table 5: Reaction conditions of the amination of 4-bromo-3-*O*-pyridyl *N*,*N*-diethylcarbamate **162**.

Entry	Substrates	Conditions and solvent	Result
1	propylamine	reflux (95h), propylamine	**
2[107]	propylamine (1.2eq), CuCl (0.2eq)	120°C (69h), NMP	**
3	allylamine (1eq), K_2CO_3 (1.1eq)	rt (18h), MeCN	SM
		80°C (48h), MeCN	SM
4[120]	benzylamine (1.3eq), Pd_2dba_3 (2 mol%), BINAP (6 mol%), Cs_2CO_3 (1.5eq)	100°C (20h), toluene, N_2	SM
	benzylamine (1.3eq), Pd_2dba_3 (2 mol%), Xantphos (6 mol%), Cs_2CO_3 (1.5eq)	100°C (20h), toluene, N_2	SM
5[120]	benzylamine (1.3eq), $Pd(OAc)_2$ (2 mol%), BINAP (6 mol%), Cs_2CO_3 (1.5eq)	100°C (15h), toluene, N_2	SM
	benzylamine (1.3eq), $Pd(OAc)_2$ (2 mol%), Xantphos (6 mol%), Cs_2CO_3 (1.5eq)	100°C (15h), toluene, N_2	SM
6[121]	benzylamine (1.5eq), Pd_2dba_3 (0.02eq), *n*-Bu_3P (0.02eq), KO*t*Bu (1.5eq)	85°C (17h), toluene, N_2	SM

**conversion less than 10%

Stirring 4-bromo-3-*O*-pyridyl *N*,*N*-diethylcarbamate **162** and propylamine at reflux, even after long reaction time did not lead to the desired product **163**, although the ^{1}H-NMR analysis showed small signals for the product. The Pd-catalyzed coupling of **162** with benzylamine using different sources of palladium or different ligands, did not give positive results (Table 5, entry 4, 5 and 6). In the reaction mixtures, only starting materials were detected (^{1}H-NMR or LC-MS analysis). The amination of 4-bromo-3-hydroxypyridine **164**[122] obtained in the reaction of 4-bromo-3-*O*-pyridyl *N*,*N*-diethylcarbamate **162** with sodium hydroxide, with propyl and benzylamine under different conditions is presented in Table 6.



162 **164** **165**

Scheme 54 Synthesis of 4-amino-3-hydroxypyridine **165**. Reaction conditions: (i) 2M $NaOH_{aq}$, MeOH, reflux, 40h, 90%;[122] (ii) see Table 6.

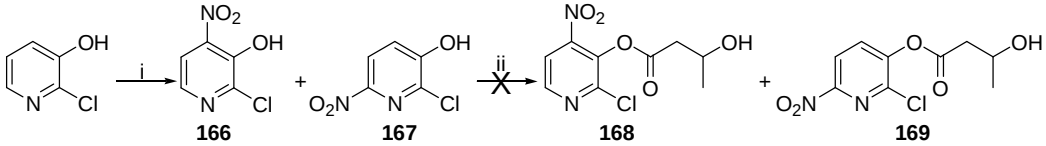
Table 6: Conditions for amination of 4-bromo-3-hydroxypyridine **164**.

Entry	Substrates	Conditions and solvent	Result
1	propylamine (1.2eq)	rt (112h), propylamine	SM
2		reflux (164h), propylamine	SM
3[120]	benzylamine (1.3eq), Pd_2dba_3 (2 mol%), BINAP (6 mol%), Cs_2CO_3 (1.5eq)	100°C (19h), toluene, N_2	SM
	benzylamine (1.3eq), Pd_2dba_3 (2 mol%), Xantphos (6 mol%), Cs_2CO_3 (1.5eq)	100°C (14h), toluene, N_2	SM
4[120]	benzylamine (1.3eq), $Pd(OAc)_2$ (2 mol%), BINAP (6 mol%), Cs_2CO_3 (1.5eq)	100°C (14h), toluene, N_2	SM
	benzylamine (1.3eq), $Pd(OAc)_2$ (2 mol%), Xantphos (6 mol%), Cs_2CO_3 (1.5eq)	100°C (14h), toluene, N_2	SM
7[121]	benzylamine (1.5eq), Pd_2dba_3 (0.02eq), *n*-Bu_3P (0.02eq), KO*t*Bu (1.5eq)	85°C (21h), toluene, N_2	SM
8[123]	benzylamine (3eq), CuI (10 mol%)	85 °C (25h), MeCN, N_2	SM

All the attempts to obtain the desired 4-alkylamino- or arylamino- 3-hydroxypyridine from 4-bromo-3-hydroxypyridine using Pd-coupling with appropriate amines failed. In the reaction mixtures only starting materials were detected (^{1}H-NMR or LC-MS analysis).

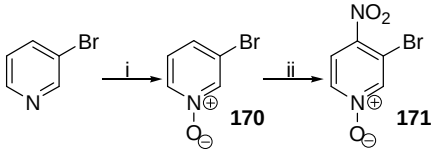
To continue the investigations, 2-chloro-3-hydroxypyridine was chosen as starting material. In the literature, nitration of 2-chloro-3-hydroxypyridine was reported in 2006 and 2007 by Jones

et al.[124] Nevertheless, the nitration of 3-hydroxypyridine was described in 1932 by Wulff. The nitration gave 3-hydroxy-2-nitropyridine in 50% yield.[125] In 1968, Selms repeated that procedure and obtained 74% yield of 3-hydroxy-2-nitropyridine and 1% of 3-hydroxy-6-nitropyridine. He also proved that substituents (group like methyl or chlorine atom) in the C2 gave 4- and 6-nitro derivatives (4:1 ratio).[126] To obtain 2-chloro-3-hydroxy-4-nitropyridine, the procedure of Jones *et al.* was repeated. The separation of the isomers by column chromatography failed, and the reaction with Et_2AlCl was performed using the mixture of 4- and 6-nitropyridine (Scheme 55).[102] After work-up, only a mixture of **166** and **167** was isolated.



Scheme 55 Attempt to obtain pyridines **168** and **169**. Reaction conditions: (i) H_2SO_4, HNO_3, 0^oC (1h) → rt (1h); (ii) Et_2AlCl, *β*-butyrolactone, CH_2Cl_2, 0^oC → rt (20 min) → 0^oC → rt (2h).

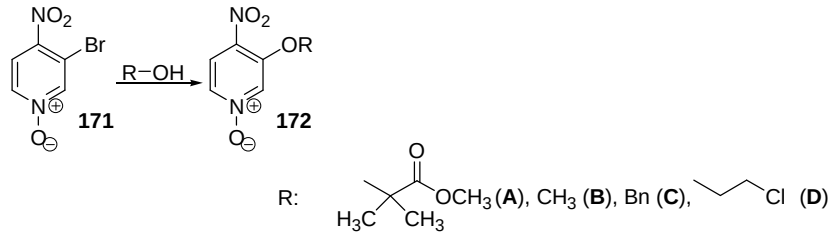
As a succeeding starting material, 3-bromopyridine was selected. Pyridine is an electron deficient molecule, and the reaction with electrophiles is difficult. To activate the pyridine, an electron rich substituent should be added and the nitrogen should be protected from acting with the electrophile.[127] Because of nucleophilicity of nitrogen, pyridine can be oxidized by H_2O_2 and AcOH. 3-Bromopyridine *N*-oxide on the other hand can react freely with electrophilic species formed in the reaction between H_2SO_4 and HNO_3 to obtain the 3-bromo-4-nitropyridine *N*-oxide **171** (Scheme 56).[128]



Scheme 56 Synthesis of 3-bromo-3-nitropyridine *N*-oxide **171**. Reaction conditions: (i) H_2O_2, AcOH, reflux (8h) → rt (o.n.), quant.; (ii) H_2SO_4, HNO_3, 90 °C, 5h, 46%.

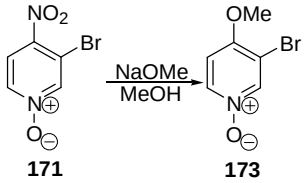
In literature, it is known that the *N*-oxide group reduces the electron density at the *α*- and *γ*-positions in the ring, which influences on the mobility of halogen substituents.[127],[129]-[132] The *N*-oxide group also activates the nitro group which can be easily replaced by various reagents, e. g. (a) in the reaction of 4-nitropyridine *N*-oxide with NaOH at 100°C leading to 4-hydroxypyridine *N*-oxide, (b) in the reaction with HBr at 120°C to 4-bromopyridine *N*-oxide, (c) in the reaction with Alkyl/Aromatic-ONa to 4-Alkyl/Aromatic-O-pyridine *N*-oxide.[127] To obtain the desired pyridine with an amino group at C4 and oxygen at C3, four alcohols were selected (Table 7). The methyl 2,2-dimethyl-3-hydroxypropionate (**A**) as an alcohol used to the synthesize 3,3-dimethyl-2,3-dihydropyrido[2,3-*b*][1,4]oxazepin-4(5*H*)one and 3,3-dimethyl-2,3-dihydropyrido[4,3-

b][1,4]oxazepin-4(5*H*)one. Benzyl alcohol (**C**) and methanol (**B**), and 3-chloro-1-propanol (**D**) possessing also the chlorine atom for further modyfication.



Scheme 57 The synthesis of compound **172**. Reaction conditions: see Table 7.

The nucleophilic substitution of bromine at C3 was successful for 3-chloro-1-propanol (**D**) and benzyl alcohol (**C**). In the reaction with sodium methoxide (NaOMe) (Table 7, entry 5) only 3-bromo-4-methoxypyridine *N*-oxide **173** was isolated (Scheme 58). This reaction was performed based on synthesis of 2-chloro-3-methoxy-4-nitropyridine *N*-oxide in the reaction of 2-chloro-3-fluoro-4-nitropyridine *N*-oxide with 1 equivalent of NaOMe. The authors, Dehmlow and Schulz observed that the reaction with 2 equivalent of NaOMe leads to 2-chloro-3,4-dimethoxypyridine *N*-oxide.[134]



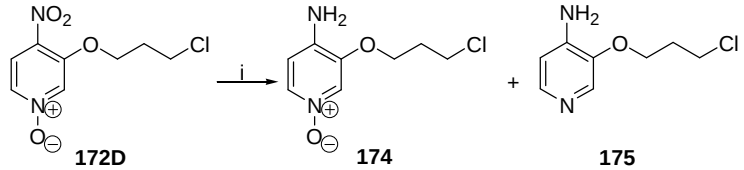
Scheme 58 Substitution of the nitro group in 3-bromo-4-nitropyridine *N*-oxide by a methoxy group.

The yield of 3-(3-chloro-propoxy)-4-nitro-pyridin-1-ol *N*-oxide **172D** after column chromatography was very low (Table 7, entry 9). All attempts to increase the yield did not give a positive effect.

Table 7: The nucleophilic substitution at C3 by methyl 2,2-dimethyl-3-hydroxypropionate (**A**), methanol (**B**), benzyl alcohol (**C**) or 3-chloro-propan-1-ol (**D**).

Entry	Substrates and solvent	Conditions	Result
1	**A** (1.1eq), K_2CO_3 (3eq), DMF	rt (23h)	SM
	A (1.1eq), K_2CO_3 (3eq), DMF	80 °C (5h)	SM
2[131]	**A** (1.25eq), NaH (1.25eq), THF/DMF	rt (15min)→ 50°C (4h)→ 40°C (14h)	SM
3[132]	**A** (1.2eq), NaOH (1eq), H_2O/acetone	reflux (16h)	SM
4[130]	**A** (1.4eq), KO*t*Bu (1eq), THF	rt (27h)	SM
5[122]	NaOMe (1eq), **B**	rt (21h)	**90 %**
6[131]	**C** (1.25eq), NaH (1.25eq), THF/DMF	rt (15min)→ 50°C (4h)→ 40°C (14h)	SM
	C (1.1eq), NaH (1.2eq), THF	0°C (5min)-rt (22h)	**25%**
7[132]	**C** (1.2eq), NaOH (1eq), H_2O/Me_2CO	reflux (16h)	SM
8[133]	**C** (1.5eq), K_2CO_3 (1eq), KOH (4eq), TDA-1 (cat.), toluene	rt (3h)	SM
9	**D** (1.2eq), NaH (1.4eq), THF	0°C (15min)-rt (16h)	**17%**

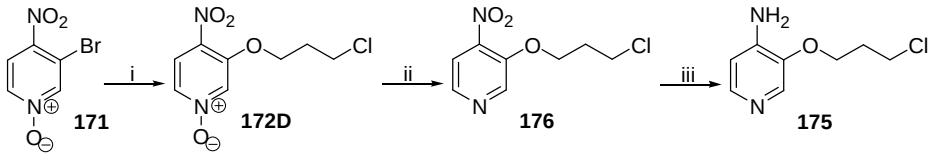
To reduce the nitro group and the *N*-oxide, the reaction of 3-(3-chloro-propoxy)-4-nitro-pyridine *N*-oxide **172D** was performed with hydrogen in the presence of Pd/C in EtOH. After 18 hours of stirring at room temperature, two compounds **174** and **175** were detected (3:2 ratio in the crude reaction mixture) (Scheme 59). Unfortunately, the isolation failed. The compounds are highly insoluble in organic solvents.



Scheme 59 The hydrogenation of 3-(3-chloro-propoxy)-4-nitro-pyridine *N*-oxide in the presence of Pd/C. Reaction conditions: (i) H_2, Pd/C, EtOH, rt (1atm), 18h.

The dominance of *N*-oxide **174** in the mixture suggests that the nitro goup is reduced first followed by removal of the *N*-oxide. To direct the reaction only to the reduction of the nitro group, the deoxygenation of **172D** was performed. In the literature, several methods to remove an *N*-oxide are

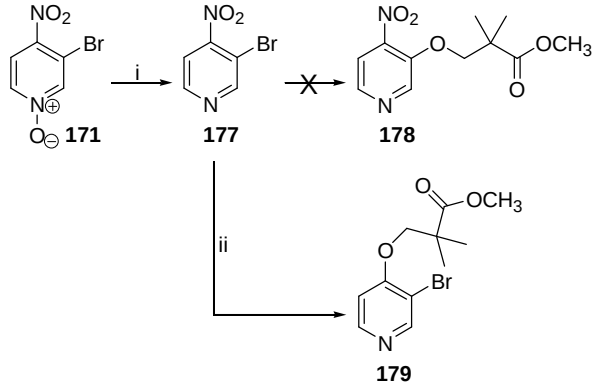
described. Those methods use a lot of different reagents, e.g. Pd complex,[135] phosphorous compounds,[136][130] systems of Lewis acids,[137] and rhenium complexes[138][139]. **172D** Reacted with TPP (1eq) in the presence of trichlorooxobis(triphenylphosphine)rhenium(V) (Re(V) complex).[139] After 20 hours of stirring at room temperature 98% of **176** was isolated (Scheme 60).



Scheme 60 Synthesis of 3-(3-chloro-propoxy)-4-amino-pyridine **175** as a precursor to pyrido[3,4-*b*][1,4]oxazepines. Reaction conditions: (i) see Table 7, entry 9; (ii) Re(V) complex (cat.), TPP, benzene, rt, 20h, 98%;[139] (iii) H_2, Pd/C, EtOH, rt (1atm), 46h.

For the reduction of the nitro group of pyridine **176**, two methods were selected. First, **176** was reacted with Fe and NH_4Cl[61] and after 25 hours of stirring at reflux, LC-MS analysis detected 3-(3-chloro-propoxy)-pyridin-4-ylamine **175** and starting material. The isolation also failed. Less than 10% of the mixture was isolated. In the second case, hydrogenation with palladium on carbon was used. **176** Was stirred at room temperature for 46 hours. The isolated mixture contained the desired product **175** and starting material. The separation of the desired compound **175** from the starting material turned out unsuccesfull. The 3-(3-chloropropoxy)-pyridin-4-ylamine **175** is insoluble in organic solvents, although the mixture of **176** and **175** can be separated from Pd/C.

To synthetize methyl 2,2-dimethyl-3-(4-nitro-pyridin-3-yloxy)-propionate **178**, the dioxygenation reaction on 3-bromo-4-nitropyridine *N*-oxide **171** with the Re(V) complex was performed.[139] The 3-bromo-4-nitropyridine **177** was obtained in 94% yield. The pyridine **177** and methyl 2,2-dimethyl-3-hydroxypropionate were stirred at room temperature with K_2CO_3 and KOH in the presence of TDA-1.[131] The desired product **178** was not detected in the reaction mixture, the isolated compound is the product of substitution of the nitro group, methyl 3-(3-bromo-pyridin-4-yloxy)-2,2-dimethyl-propionate **179** in 54% yield (Scheme 61).



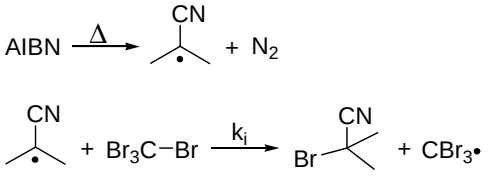
Scheme 61 Formation of methyl 3-(3-bromo-pyridin-4-yloxy)-2,2-dimethyl-propionate **179**. Reaction conditions: (i) Re(V) complex (cat.), TPP, benzene, rt, overnight, 94%;[139]; (ii) methyl 2,2-dimethyl-3-hydroxypropionate, K_2CO_3, KOH, TDA-1, toluene, rt, 3h, 54%.[131]

3.4. Synthesis of nine-membered ring pyrido annelated derivatives

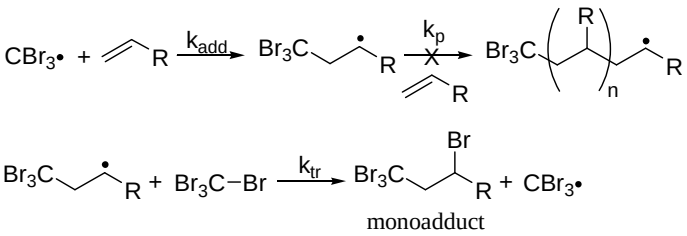
3.4.1. *The Kharasch reaction*

The efficient synthesis of cyclic systems continues to be an important area of modern organic chemistry. Increasingly, common methodologies for the formation of cyclic systems are free radical cyclization protocols. The majority of such reactions are typically mediated by organotin[140] or organosilane reagents. In the first report on atom transfer radical addition (ATRA), halogenated derivatives were directly added to olefinic bonds in the presence of radical initiators or light. Today this reaction is known as the Kharasch addition. Early work in this area involved ATRA of CCl_4 or CBr_4 to simple olefins in the presence of a radical initiator such as 2,2´-azobis(2-isobutyronitrile) (AIBN). Very high yields of monoadduct were obtained in the addition of CBr_4 to α-olefins, but this significantly decreased for more reactive monomers. The main reason for this lower yield of the monoadduct was radical-radical coupling and repeated radical addition to the growing chain, affording oligomers (Scheme 62).

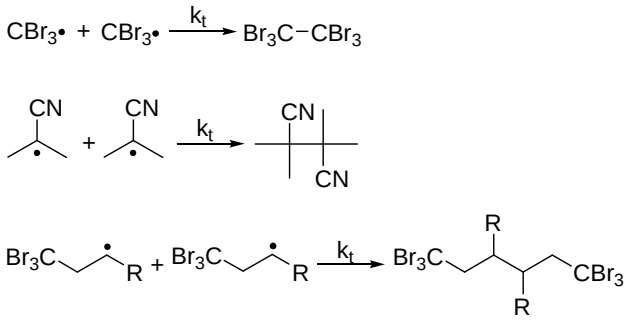
Initiation



Propagation

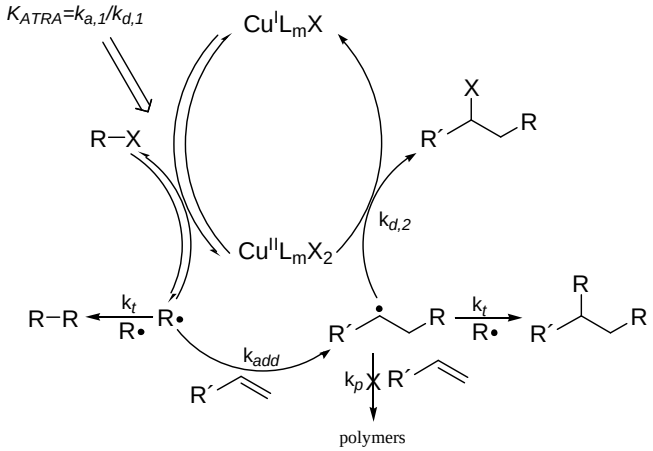


Termination (radical-radical coupling)



Scheme 62 The Kharasch addition of CBr_4 to alkene in the presence of free-radical initiator AIBN.

Starting from 1960, several groups began to investigate the use of transition metal complexes to catalyze ATRA. The major idea behind this approach is that transition-metal complexes are more effective halogen transfer agents than alkyl halides, have an increased chemoselectivity for the monoadduct and an increased speed of chain transfer (k_{tr}). A number of species were found to be particulary active in the ATRA processes,[141][144] including complexes of Cu, Fe, Ru[142][153][156] and Ni, as well as metal oxides and zero valent metals as Cu^0 and Fe^0. Based on chemo-, regio- and stereoselectivity observations, it is generally accepted that the mechanism of ATRA involves free-radical intermediates. The proposed mechanism in case of a copper complex is presented in Scheme 63.



Scheme 63 Proposed mechanism of copper catalyzed ATRA; L: complexing ligand, X-halide.

The homolytic cleavage of an alkyl halide bond by a copper(I) complex generates the corresponding copper(II) complex and an organic radical ($k_{a,1}$). The radical may terminate (k_t) or add to an alkene (k_{add}) in an inter- or intramolecular fashion or it can abstract a halogen atom from the copper(II) complex and return to the original dormant alkyl halide species ($k_{d,1}$). If the abstraction of a halogen atom occurs after the first addition to an alkene, the desired monoadduct will be formed ($k_{d,2}$). This step regenerates the corresponding copper(I) complex and, therefore, completes the catalytic cycle. The key to increase the chemoselectivity of the monoadduct in copper-mediated ATRA lies in the radical generating step. Transition metal catalyzed (TMC) ATRA reactions can be conducted intramolecularly when the alkyl halide and alkene functionalities are part of the same molecule. Intramolecular TMC ATRA or atom transfer radical cyclization (ATRC) is a very attractive synthetic tool because it enables the synthesis of functionalized ring systems that can be used as starting materials for the preparation of complex organic molecules. Furthermore, the halide functionality in the resulting product can be very beneficial because they can be easily reduced, eliminated, displaced, converted to a Grigniard reagent, or if desired, serve as a further radical precursor.[143][144]

The first successful example of a copper mediated ATRC reaction included the synthesis of trichlorinated *γ*-lactones from readily accessible alkenyl trichloroacetates.[145][147] The reaction was highly selective, but required elevated temperatures (110-130°C) and large amounts of copper catalyst (20-30 mol% relative to the substrate). The cyclization of α-*N*-allylcarbamoyl radicals is a difficult process requiring high temperatures, primarily due to the high barrier to rotation around the amide bond. As indicated in Figure 8, only the *anti* conformer can cyclize and the *N*-protecting groups typically regulate *syn-anti* equilibrium.[146] Cyclization of *γ*-lactam precursors in the presence of only $Cu^{I}Cl$ required elevated reaction temperatures (80-140°C).[147]

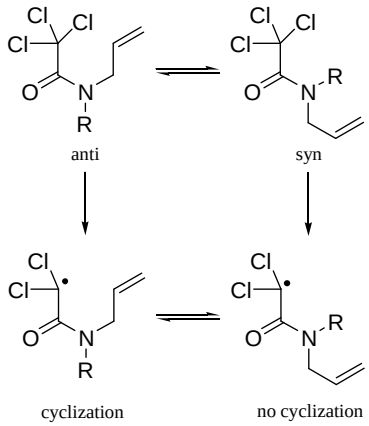


Figure 8 *Syn-anti* equilibrium in the cyclization of α-*N*-allylcarbamoyl radicals.

The role of the complexing ligands is not only to increase the solubility of the copper complex in the reaction medium, but also to regulate the equilibrium constant for atom transfer ($K_{ATRA}=k_{a,1}/k_{d,1}$). The copper(I) chloride in conjunction with 2,2´-bipyridine was also found to efficiently catalyze ATRC of several *α*-chloroglycine derivatives with a 3-alkenyl substituent at nitrogen.[148]

Copper(I) complexes with nitrogen-based ligands have been shown to be quite effective in catalyzing sequentially both ATRA and ATRC. In the case of ATRC followed by ATRA, substrates are typically chosen such that intermolecular addition reactions are slower than intramolecular ones.[149][157] The fine tuning of the transition metal complex is perhaps the most important aspect of the catalytic system because it regulates the dynamic equilibrium between dormant (alkyl halides) and propagating species (radicals). For copper catalyzed ATRA this is typically acheved utilizing bidentate, tridentate, tetradentate and multidentate nitrogen-based complexing ligands (Figure 9).

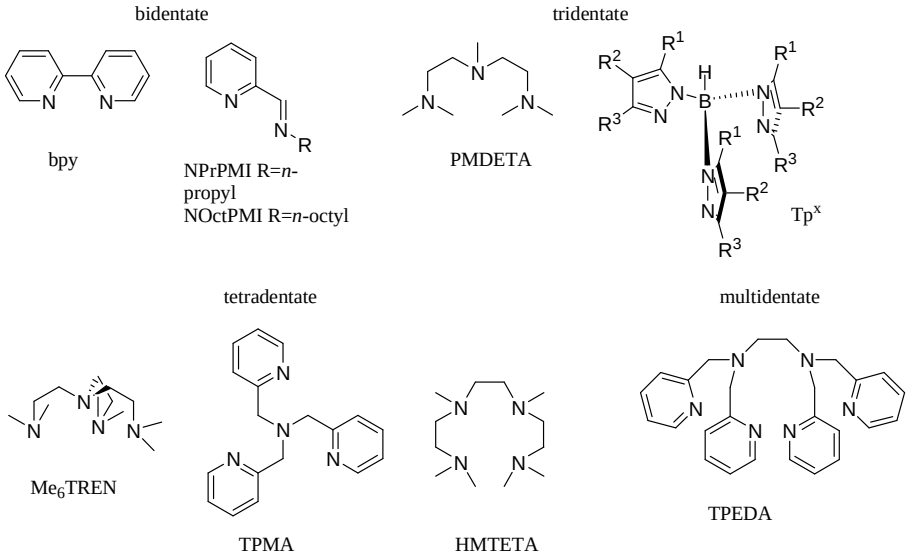
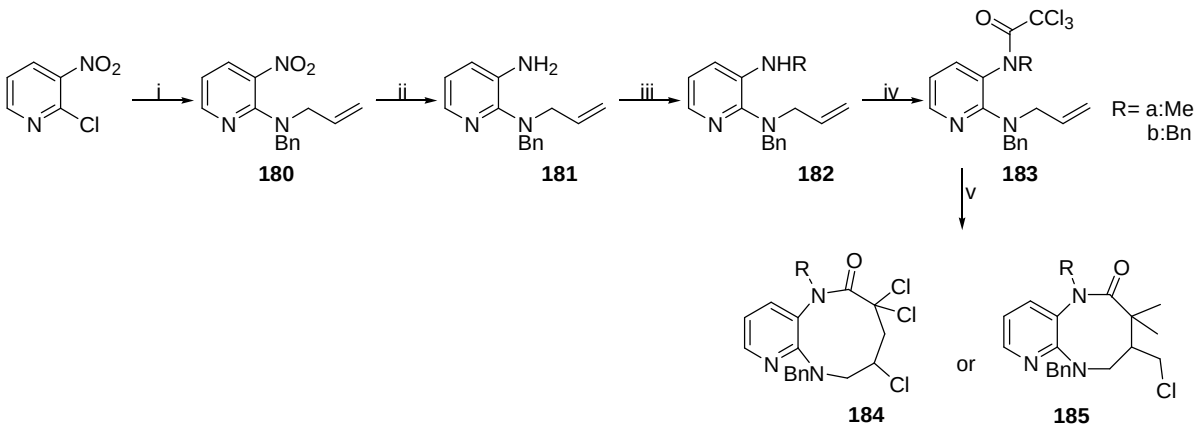


Figure 9 Nitrogen-based ligands.

In literature, there are no precedents up to now for the Kharasch reaction with pyridine species as substrate. Pyridine derivatives have been used as ligand to coordinate the involved metal.[150][157] In this part, we wanted to investigate the influence of the pyridine ring on the formation of nine- or eight-membered rings attached to the pyridine ring. For this purpose, compound **184** was synthesized (Scheme 64).



Scheme 64 Synthesis of pyrido annelated nine- and eight-membered ring attached to the pyridine. Reaction conditions: (i) *N*,*N*-allylbenzylamine, NaH, THF, 0°C→rt (22h), 82%; (ii) Fe, NH_4Cl, MeOH/H_2O, reflux, 5h, 80%; (iii) Me: *n*-BuLi, MeI, THF, -78°C (15min)→rt (2h), 69%; Bn: a) benzaldehyde, EtOH, reflux, b) $NaBH_3CN$, AcOH, MeOH, rt, 17h, 70%; (iv) Me: trichloroacetyl chloride, CH_2Cl_2, 0°C→rt (20h), 80%; Bn: trichloroacetyl chloride, CH_2Cl_2, 0°C→rt (18h), 90%; (v) see Table 8 for Bn derivative and Table 9 for Me derivative.

2-(*N*-Allyl-*N*-benzylamino)-3-nitropyridine **180** was synthesized by a nucleophilic substitution of the chlorine atom by *N*,*N*-allylbenzylamine[151] in 2-chloro-3-nitropyridine. Reduction of the nitro group was accomplished with iron powder in the presence of NH_4Cl, and after 5 hours of reflux, the desired

amine **181** was isolated. Two groups, methyl and benzyl, were selected to protect the amine functionality. Methylation was conducted by deprotonation with *n*-BuLi following by methyl iodide. Benzylated amine **183** was obtained by reductive amination. In a first step, the imine from **181** and benzaldehyde was obtained in ethanol. The reduction of imine **181** to the corresponding amine was conducted using sodium borohydride ($NaBH_4$) and sodium cyanoborohydride ($NaBH_3CN$). Only the reaction with $NaBH_3CN$ in the presence of acetic acid gave the desired secondary amine **183b**. The obtained amines **183** were reacted with trichloroacetyl chloride in CH_2Cl_2 (Scheme 64). Next, the benzyl derivative of **183** was treated with different copper catalysts (CuCl/ CuO, CuCl, CuO) in the presence of ligand (TMEDA, PMDETA) or without ligand. Different solvents were used, CH_2Cl_2 and 1,2-dichloroethane (DCE) for the benzylated amide (Table 8) and CH_2Cl_2, DCE and toluene for methylated amide. For the methyl derivative, CuCl was used in the presence of a ligand (PMDETA, 2,2´bipyridine), the ruthenium catalyst[152] ($RuCl_2(PPh_3)_3$,[153][157] Grubbs catalyst[15] or **183a** was refluxed only with copper(I) chloride (Table 9). However, cyclization to the eight- or nine-membered ring failed in all cases. Only stating material could be detected in the reaction mixtures (^{1}H-NMR and LC-MS analysis). The failure of these cyclizations is most probably related to the liganding nature of the starting material, resulting in the formation of unreactive ruthenium[155] or copper complexes.

Table 8: Kharasch reactions conditions to which compound **184** was subjected, R= benzyl.

Entry	Substrates	Solvent	Conditions	Result
1[156]	TMEDA (0.8eq), CuCl (0.4eq)	CH_2Cl_2	Ar, reflux (24h), N_2	SM
2[156]	PMDETA (0.8eq), CuCl (0.4eq)	CH_2Cl_2	Ar, reflux (24h), N_2	SM
3	CuCl (0.4eq)	CH_2Cl_2	Ar, reflux (24h), N_2	SM
4	CuO (0.4eq)	CH_2Cl_2	reflux (24h), N_2	SM
5	CuO (0.2eq), CuCl (0.2eq)	CH_2Cl_2	reflux (24h), N_2	SM
6	CuCl (0.4eq)	DCE	reflux (24h), N_2	SM
7	CuO (0.8eq)	DCE	reflux (24h), N_2	SM
8	CuO (0.8eq), CuCl (0.8eq)	DCE	reflux (24h), N_2	SM

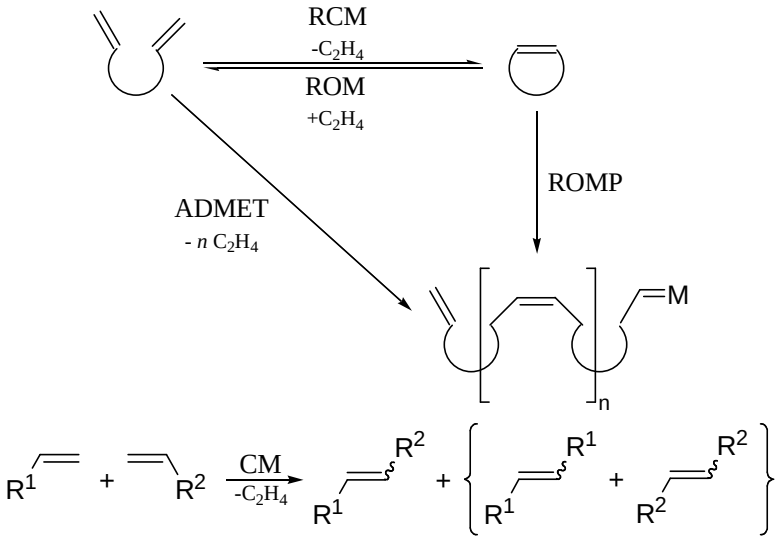
Table 9: Kharasch reactions conditions to which compound **184** was subjected, R= methyl.

Entry	Substrates	Solvent	Conditions	Result
1	CuCl (0.8eq)	CH_2Cl_2	reflux (20h), N_2	SM
2[156][153]	PMDETA (0.8eq), CuCl (1.6eq)	CH_2Cl_2	reflux (23h), N_2	*
3[157]	$RuCl_2(PPh_3)_3$ (10 mol%)	toluene	reflux (22h), N_2	SM
4	$RuCl_2(PPh_3)_3$ (10 mol%)	DCE	80°C (22h), N_2	SM
5	Grubbs 1st generation (5 mol%)	toluene	reflux (22h), N_2	SM
6[158]	CuCl-bpy (0.3eq-0.3eq)	DCE	rt (71h), N_2	SM
	CuCl-bpy (0.3eq-0.3eq)	DCE	80°C (71h), N_2	SM

* mixture of compounds (LC-MS)

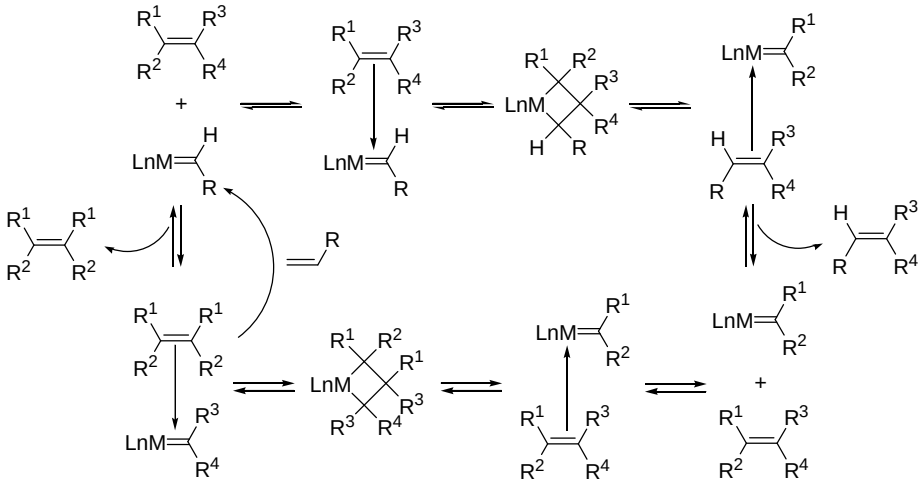
3.4.2. *The Metathesis reaction*

Olefin metathesis is a process in which alkylidene groups on alkenes are exchanged. The first metathesis reported by Anderson and Merckling, was the polymerization of norbornene by titanium(II) species. Several classes of olefin metathesis including ring closing metathesis (RCM), ring opening metathesis (ROM), cross-metathesis (CM), enyne metathesis (EM),[159] acyclic diene metathesis (ADMET) and ring opening metathesis polymerization (ROMP) have all become widely used reliable routine methods (Scheme 65).[160]



Scheme 65 Types of metathesis reactions: RCM= ring-closing metathesis; ROM= ring-opening metathesis; ROMP= ring-opening metathesis polymerization; ADMET= acyclic diene metathesis polymerization; CM= cross-metathesis.

The generally accepted catalytic cycle of transition metal catalyzed metathesis proposed by Herison and Chauvin, consists in a reversible sequence of [2+2] cycloadditions and cycloreversions, *i.e.* alkene coordination to the metallacarbene complex, cycloaddition, followed by cycloreversion to a new alkene and metallacarbene because of breaking of two different bonds. The newly formed metallacarbene complex, after coordination with a new olefin molecule, metallacyclobutene formation, and double bond reordering, gives the metathesis product and re-forms the ruthenium carbene initiator which restarts the cycle. As the product no longer participates in the catalytic cycle, the equilibrium is thus shifted towards formation of the metathesis product. At equilibrium the reverse and forward rates of all chemical reactions or all elementary steps are identical, and the reverse reaction procceds through the same series of elementary steps as the forward reaction (Scheme 66).[161][162][166]



Scheme 66 The mechanism of metathesis catalyzed by transition metal carbenes.

If two olefins of similar reactivity are subjected to CM conditions, assuming full conversion, a maximum of 50% yield of the desired product will be obtained while 25% of each of the two homocoupling products will be formed. To achieve a synthetically efficient yield of 90%, 10 equivalents of one coupling partner must be used.[162] The stereoselectivity of product formation further complicates CM. Although the thermodynamically favored *trans* olefins are usually the major products, a mixture of *E*, *Z* isomers can be obtained when the energy difference between them is small. Extensive research in this area has resulted in fruitful discoveries in more efficient catalysts and new applications. The generalization into synthetic organic chemistry has been driven primarily by the discovery of well-defined and functional group-tolerant catalysts independently by Schrock and Grubbs. In the present time there are two main types of catalyst in use (Figure 10).[163] These are the molybdenum-based complex **A**, developed by Schrock and the ruthenium-based complex[164] **B** and in particular **C**, developed by Grubbs. Complex **A** has the major disadvantage of being air- and moisture-sensitive, whereas **C** is not significantly affected by air, moisture or the reaction impurities, tolerate substrates containing free alcohols, ketones, esters, amides, epoxides, acetals, silyl ethers and sulfides.[165] Titanium carbenes such as **B**, which are more commonly utilized in olefination reactions, find occasional use. Hoveyda have reported the synthesis and some applications of ruthenium alkylidene **E** and **G**.[166]

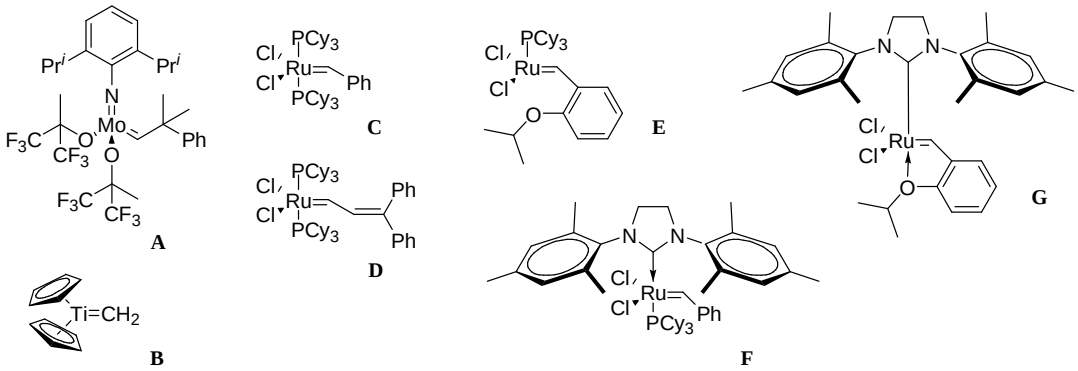
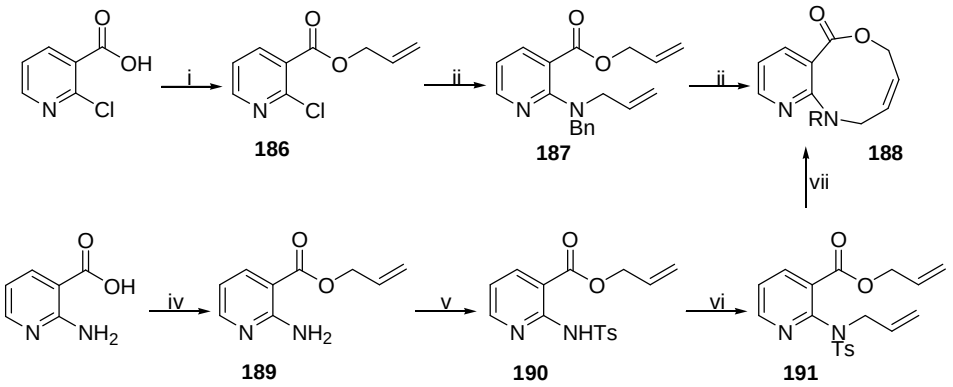


Figure 10 Catalysts used for metathesis.

Ring-closing metathesis of dienes has become one of the most important methods now in use for the assembly of cyclic organic compounds.[163][1678] First employed by Villemin[29] and by Tsuji[169], the importance of this reaction rose over the past years. RCM is used for construction of synthetically valuable building blocks such as heterocyclic rings containing phosphorous, sulfur, oxygen, or nitrogen, including aromatic heterocycles; spirocyclic, cyclophane, and polycyclic compounds; and compounds of biological and medical relevance such as peptidomimetics, carbohydrate derivatives, alkaloids, bioactive cyclic molecules, and polycyclic ethers, including macrocyclic aza-crown ethers and topologically interesting molecules.[170]

To obtain nine-membered ring compounds **187** and **191** were synthesized (Scheme 67). As a protecting group benzyl and tosyl groups were chosen. The allyl 2-(*N*-allyl-*N*-benzylamino)-3-pyridinecarboxylate **187** was synthesized from allyl 2-chloropyridine-3-carboxylate **186** by a nucleophilic substitution reaction with *N*,*N*-allylbenzylamine.[151] The synthesis of tosyl derivative **191** started with the synthesis of the allyl 2-aminopyridine-3-carboxylate **189** using the same reaction conditions as for the synthesis of ester **186**. In the next step, the amino group of allyl 2-amino-3-pyridinecarboxylate **189** was tosylated in the reaction with tosyl chloride in pyridine,[171] leading to the allyl 2-(*N*-tosylamino)-3-pyridinecarboxylate **190**. The prepared sulfonamide **190** was alkylated by allyl bromide in the presence of potassium carbonate,[171] the desired tosyl derivative **191** was isolated as a beige solid.



Scheme 67 Synthesis of nine-membered ring in metathesis of **187** and **191**. R= Bn, Ts.
Reaction conditions: (i) allyl alcohol, EDC, DMAP, CH_2Cl_2, rt, 16h, 78%; (ii) *N*,*N*-allylbenzylamine, MeCN, 80°C, 73h, 49%; (iii) see Table 10; (iv) allyl alcohol, EDC, DMAP, CH_2Cl_2, rt, 13h, 42%; (v) TsCl, pyridine, 60°C, 41h, 72%; (vi) allyl bromide, K_2CO_3, DMA, 90°C, 39h, 84%; (vii) see Table 13.

Several ruthenium catalysts, solvents and reaction conditions were investigated for the benzyl derivative **187** (Table 10). The starting material, **187**, was recovered in 75-90% yield after 16-71 hours.

Table 10: The metathesis condition reactions for benzyl derivative **187**.

Entry	Catalyst	Conditions and solvents	Result
1[172]	Grubbs 1st generation (5 mol%)	rt 24-71h), toluene, N_2	SM
2	Grubbs 1st generation (5 mol%)	rt (19h), CH_2Cl_2, N_2	SM
3[173]	[RuClH(CO)(PPh_3)] (5 mol%)	65°C (16h), toluene, N_2	SM
4	[RuClH(CO)(PPh_3)] (5 mol%)	reflux (24h), CH_2Cl_2, N_2	SM
5	Hoveyda-Grubbs 2nd generation (10 mol%)	rt (16h), toluene, N_2	SM
6[174]	G2 (4 mol%)	45°C (24h), H_2O	SM
7	G2 (12 mol%)	reflux (16h), CH_2Cl_2 or toluene, N_2	SM

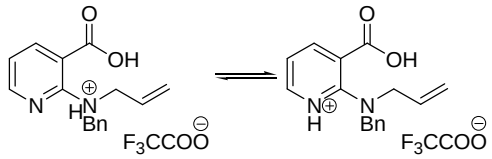
To compete with or to prevent the coordination of the nitrogen atom of the pyridine ring to the ruthenium carbene intermediate, a Lewis acid was introduced in the reaction system.[175] Grubbs 2nd generation catalyst (5% mol) and various Lewis acids (20% mol) in toluene under inert atmosphere in 80°C were evaluated (Table 11).

Table 11: The metathesis reaction of **187** in the presence of Grubbs 2nd generation catalyst in combination with a Lewis acid.

Entry	Catalyst: G2; LA	Conditions and solvent	Result
1	G2 (5 mol%), Ti(O*i*-Pr)$_4$ (20 mol%)	80°C (15h), toluene, N_2	SM
2	G2 (5 mol%), LiCl (20 mol%)	80°C (15h), toluene, N_2	SM
3	G2 (5 mol%), $ZnCl_2$ (20 mol%)	80°C (15h), toluene, N_2	SM
4	G2 (5 mol%), In(OTf)$_3$ (20 mol%)	80°C (15h), toluene, N_2	SM*
5	G2 (5 mol%), Sc(OTf)$_3$ (20 mol%)	80°C (15h), toluene, N_2	SM*

*new product detected by ^{1}H-NMR and LC-MS analysis

The presence of a Lewis acid such as titanium(IV) isopropoxide (Ti(O*i*-Pr)$_4$), lithium chloride (LiCl) or zinc chloride ($ZnCl_2$) did not improve the formation of the desired ring system. The ^{1}H-NMR and LC-MS analysis of the reactions with indium(III) trifluoromethanesulfonate (In(OTf)$_3$) and scandium(III) trifluoromethanesulfonate (Sc(OTf)$_3$) showed the presence of a new compound (Table 11). The prolongation of the reaction time to 114 hours at reflux or 6 hours at reflux and continued at room temperature for 20 days, or additional equivalents of Lewis acid (20 mol%-0.5eq) did not have any influence on the yield of the new compound (Table 12). The separation by column chromatography failed completely. Because of problems with the separation, preparative HPLC was used. Due to the presence of a 0.1% aqueous solution of trifluoroacetic acid, the compound was isolated in its salt form. Since protonation can occure on the two N-atoms, on the ^{1}H-NMR spectra the migration of proton can be visible. The ^{1}H-NMR analysis confirms the presence of a new compound (Scheme 68) 2-(*N*-allyl-*N*-benzylamino)-nicotinic acid.



Scheme 68 Deallylation during the attempted metathesis reaction.

Table 12: Optimalization conditions for ring-closing metathesis of **187** in the presence of Grubbs 2nd generation catalyst and $Sc(OTf)_3$ as a Lewis acid (LA).

Entry	**Catalyst:** G2**; LA:** $Sc(OTf)_3$	**Conditions and solvent**	**Result**
1	G2 (10 mol%), LA (20 mol%)	80°C (15h), toluene, N_2	*
2	G2 (10 mol%), LA (40 mol%)		*
3	G2 (10 mol%), LA (20 mol%)	80°C (37h), toluene, N_2	*
4	G2 (10 mol%), LA (40 mol%)		*
5	G2 (10 mol%), LA (0.5eq)	80°C (13h), toluene, N_2	*
6	G2 (5 mol%), LA (20 mol%)	reflux (16h), toluene, N_2	*
		reflux (114h), toluene, N_2	*
7	G2 (5 mol%), LA (20 mol%)	reflux (6h)- rt (476h), toluene, N_2	*

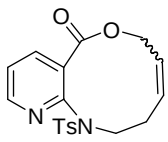
*conversion ~40%

For the tosyl derivative **191**, only three conditions were applied (Table 13). In all cases ring-closing metathesis failed. Only starting material was detected by ^{1}H-NMR and LC-MS analysis.

Table 13: The metathesis reaction conditions for the tosyl derivative **191**.

Entry	Catalyst/ LA	Conditions	Result
1	G2 (5 mol%)	rt (20h), toluene, N_2	SM
2	G2 (5 mol%), $Sc(OTf)_3$ (20 mol%)	reflux (6h)- rt (476h), toluene, N_2	SM
3	G2 (5 mol%), $In(OTf)_3$ (20 mol%)	80°C (21h), toluene, N_2	SM

Because of difficulites with the ring-closing metathesis to the nine-membered ring for derivatives **187** and **191**, a new possibility was taken into consideration. The addition of one CH_2 group to the chain in the tertiary amine should lead to a compound with a ten-membered ring **192** (Scheme 69).

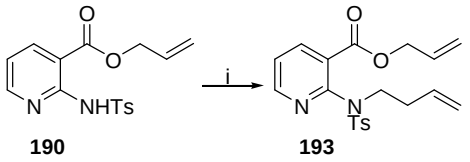


192

Scheme 69 The desired product after ring-closing metathesis reaction of **193**.

To investigate this hypothesis, the 2-propen-1-yl 2-(*N*-(3-buten-1-yl)-*N*-tosylamino)-3-pyridinecarboxylate **193** with a homo-allyl chain was synthesized (Scheme 70). Starting from pyridine

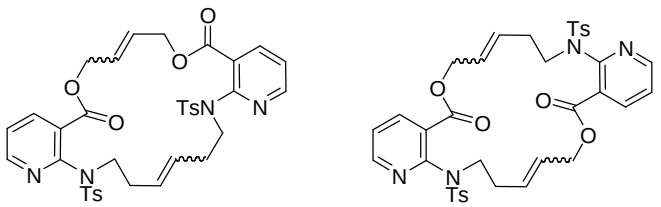
190 using 4-bromo-1-butene as an alkylating reagent in the presence of K_2CO_3 in DMA or MeCN,[171] the desired 2-propen-1-yl 2-(*N*-(3-buten-1-yl)-*N*-tosylamino)-3-pyridinecarboxylate **193** was obtained.



190 **193**

Scheme 70 Synthesis of 2-propen-1-yl 2-(*N*-(3-buten-1-yl)-*N*-tosylamino)-3-pyridinecarboxylate **193**. Reaction conditions: (i) 4-bromo-1-butene, K_2CO_3, DMA/MeCN, 90-80°C, 43-50 hrs, 84-67%.

Compound **193** could form two possible products upon ring closing metathesis: the *cis* *α,α´* ten-membered ring or the *trans* *α,α´* ten-membered ring. For the formation of ester **192**, different reaction conditions were evaluated (Table 14). Only reaction with the Grubbs 2nd generation catalyst proceeded to the mixture of compounds **194** and **195** (Figure 11).



194 **195**

Figure 11 Products after ring-closing metathesis of compound **193**.

Table 14: Reaction conditions for ring-closing metathesis of **193**.

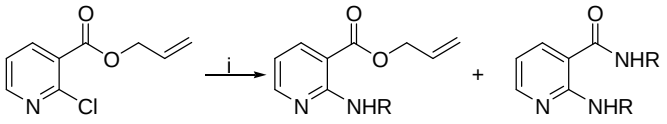
Entry	Substrates	Conditions	Result
1	G2 (10 mol%)	80°C (3h), toluene, N_2	80% conversion
2	G2 (10 mol%)	80°C (5h), toluene, N_2	100% conversion
3	G2 (10 mol%), $Sc(OTf)_3$ (20 mol%)	80°C (3h), toluene, N_2	60% conversion
4	G2 (10 mol%), $In(OTf)_3$ (20 mol%)	80°C (3h), toluene, N_2	SM
5	Grubbs 1st generation (10 mol%)	80°C (3h), toluene, N_2	SM
		rt (3h), toluene, N_2	SM

The LC-MS analysis showed three peaks, corresponding to the mass of **194**/**195**. The isolation using column chromatography failed. Only impurities were separated. The isolation of the compounds was

performed by preparative HPLC, but only three out of the four detected compounds were separated. The product was obtained as a mixture of isomers. Because of the use of 0.1% aqueous solution of trifluoroacetic acid, the compounds were isolated in their salt form. Unfortunatelly, the HPLC separation of the mixture allows only for NMR analysis and confirmation of mass (LC-MS and HRMS analysis). The yield of the separated compounds was not determined. The reaction with Grubbs 2nd generation catalyst was repeated with a higher dilution but the conversion of the reaction decreased to 70% leading to the same mixture of compounds. Changing the degree of dilution or using longer time reaction did not improve the result of this reaction.

3.5. Synthesis of pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones

The synthesis of pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones is based on the reaction of 2-propen-1-yl 2-chloro-3-pyridinecarboxylate **186** with primary amines.[176a] Alkyl amines and one aromatic amine were evaluated. Starting from commercially available 2-chloropyridine-3-carboxylic acid, reaction with allyl alcohol and the coupling reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), in the presence of a catalytic amount of DMAP, afforded 2-propen-1-yl 2-chloro-3-pyridinecarboxylate **186** in 78%.[176b] Compound **186** was further reacted with aliphatic/aromatic primary amines providing molecules **196** and **197** in good yields (Scheme 71, Table 15). Only for the reaction with 1-adamantylamine, acetonitrile (MeCN) was used as a solvent (Table 15, entry 9). Product **196** was obtained as the sole product in the reaction with sterically hindered amines like cyclopropylamine, *t*-butylamine, *t*-octylamine and 1-adamantylamine (Table 15, entries 5, 7, 8 and 9). Reaction with cyclohexylamine provided two products, 2-cyclohexylamino-nicotic acid allyl ester **196f** was obtained together with *N*-cyclohexyl-2-cyclohexylamino-nicotinamide **197f** (Table 15, entry 6). The main product **196** results from the nucleophilic aromatic substitution of chlorine, which is more favorable than the formation of amide **197**.[177]



186 **196** **197**

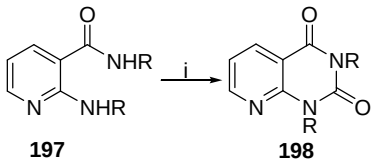
Scheme 71 Reaction of allyl 2-chloro-pyridinecarboxylate with amines.
Reaction conditions: NH_2R (solvent), reflux, see also Table 15.

Table 15: Preparation of compounds **196** and **197**.

Entry	NH_2R	Time [h]	Product			
				Yield(%)[a]		Yield(%)[a]
1	allylamine	19	**196a**	36	**197a**	61
2	propylamine	20	**196b**	29	**197b**	56
3	butylamine	23	**196c**	23	**197c**	74
4	*i*-pentylamine	17	**196d**	2	**197d**	68
5	cyclopropylamine	68	**196e**	31	**197e**	-
6	cyclohexylamine	21	**196f**	68	**197f**	30
7	*t*-butylamine	259	**196g**	63	**197g**	-
8	*t*-octylamine	27	**196h**	73	**197h**	-
9	1-adamantylamine	117	**196i**	51[b]	**197i**	-
10	benzylamine	21	**196j**	26	**197j**	56

[a] column chromatography; [b] reaction in MeCN

Using 1,1'-carbonyldiimidazole (CDI) and NaH in a mixture of THF and 1-methylpyrrolidinone (NMP), ring closure could be carried out in 16-18 hours at room temperature (Scheme 72, Table 16).[178] Under these conditions, *N*-cyclohexyl-2-(cyclohexylamino)-3-pyridinecarboxamide **197f** (Table 16, entry 5) did not react at room temperature or under reflux. For this ring closing reaction, also triphosgene was evaluated.[179] After 30 hours of stirring at room temperature, the desired product **198f** was formed in the reaction mixture; however its isolation, using column chromatography or crystallization, was not successful.



197 **198**

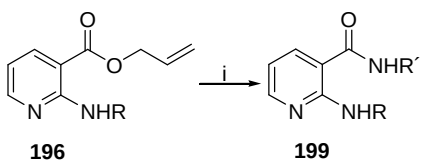
Scheme 72 Ring closure reaction of compound **197**. Reaction conditions: NaH (3eq), CDI (3eq), THF/NMP (2:3), rt, 16-18h.

Table 16: Preparation of compound **198**.

Entry	197	Time [h]	Product	Yield(%)
1	197a	18	**198a**	69%
2	197b	18	**198b**	58%
3	197c	16	**198c**	61%
4	197d	18	**198d**	91%
5	197f	16	**198f**	-[a]
6	197j	17	**198j**	60%

[a] product was not isolated

With the aim to prepare a series of pyrido[2,3-*d*]pyriminides with two different alkyl groups, compounds **196f** and **196h** were refluxed with a second amine (Scheme 73, Table 17). In the reaction of allyl 2-(cyclohexyl)-3-pyridinecaboxylate **196f** with allylamine, the yield of amide **199a** was low, even after prolonged reflux (Table 17, entry 1). The *t*-octyl derivative was obtained in higher yield (Table 17, entry 5-7). For *i*-pentyl derivatives, longer reaction times provided the same yield, as for the *t*-octyl derivative (Table 17, entries 3 and 6). For both derivatives, the reaction with cyclopropylamine and *i*-propylamine did not occur even after a prolonged period of reflux (4 days).



196 **199**

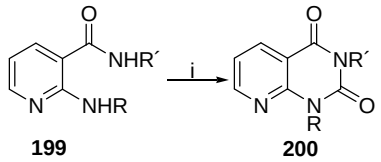
Scheme 73 Preparation of compound **199**.

Table 17: Preparation of **199**.

Entry	R	$NH_2R´$	Time [h]	Product	Yield(%)[a]
1	cyclohexyl (196f)	allylamine	189	**199a**	54%
2		propylamine	42	**199b**	55%
3		*i*-pentylamine	186	**199c**	85%
4		cyclopropylamine	192	**199d**	SM
5	*t*-octyl (196h)	allylamine	43	**199e**	74%
6		propylamine	113	**199f**	68%
7		*i*-pentylamine	88	**199g**	85%
8		cyclopropylamine	192	**199h**	SM

[a]yield after column chromatography

Compounds **199**, containing two different *N*-substituents can be easily transformed into pyrido[2,3-*d*]pyrimidines **200** using the same reaction conditions as for the ring closure of **198**. Only for the allyl derivatives, the yields are low, i.e. 47% for the cyclohexyl derivative and 49% for the *t*-octyl derivative, respectively. All other amines gave excellent yields (Table 18, entries 2, 3, 5 and 6).



199 **200**

Scheme 74 Ring closure reaction of compound **199**. Reaction conditions: NaH (3eq), CDI (3eq), THF/NMP (2:3), rt, 15-18h.

Table 18: Preparation of **200**.

Entry	199	Time [h]	Product	Yield(%)
1	199a	15	**200a**	47%
2	199b	16	**200b**	91%
3	199c	19	**200c**	97%
4	199e	19	**200e**	49%
5	199f	19	**200f**	97%
6	199g	19	**200g**	86%

In an attempt to synthesize dipyrido[2,3-*d*]pyrimidine **201**, compound **202** was prepeared.

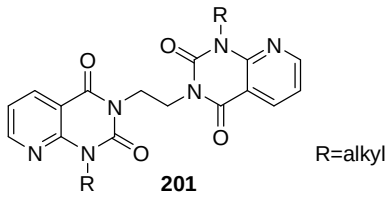
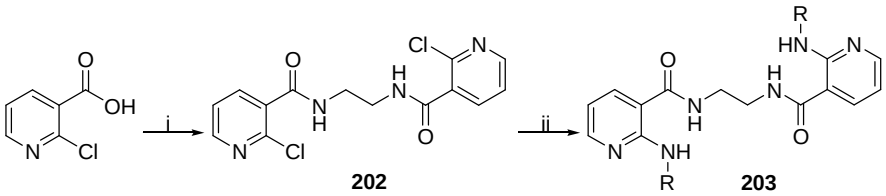


Figure 12 Structure of desired dipyrido[2,3-*d*]pyrimidine.

Starting from the 2-chloropyridine-3-carboxylic acid, 2-chloropyridinecarbonyl chloride was obtained quantitatively after 2 hours of reflux with excess of thionyl chloride. The remaining thionyl chloride was removed and ethylenediamine in CH_2Cl_2 was added, affording compound **202**. The latter molecule was refluxed with an appropriate alkylamine for 32-68 hours and the obtained products were purified by column chromatography (Table 19).



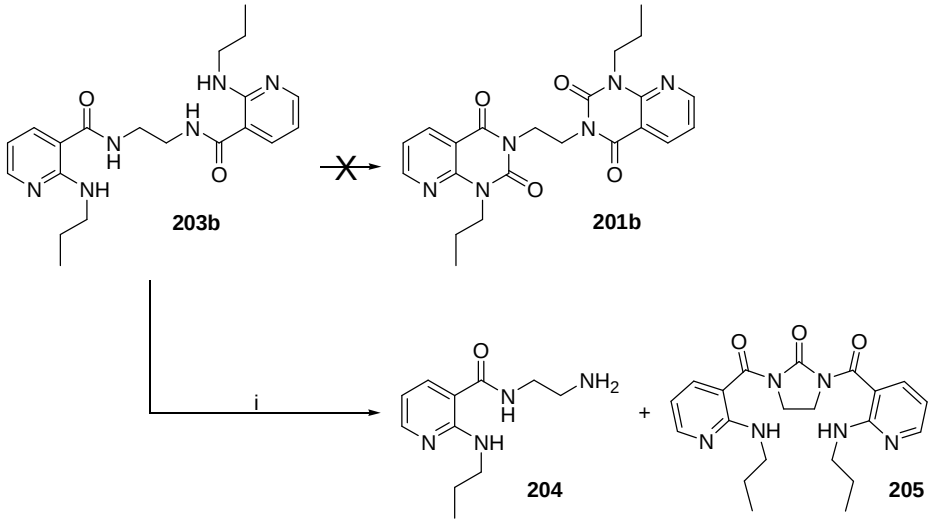
Scheme 75 Synthesis of compound **203**. Reaction conditions: (i) a) $SOCl_2$, reflux, 2h, b) ethylenediamine, CH_2Cl_2, rt, 17h, 60%; (ii) NH_2R (solvent), reflux, 32-68h.

Table 19: Preparation of compound **203**.

Entry	NH_2R	Time [h]	Product	Yield(%)[a]
1	allylamine	32	**203a**	54%
2	propylamine	68	**203b**	62%
3	*i*-pentylamine	59	**203c**	59%

[a]yield after column chromatography

Compound **203b** was treated with NaH and CDI in a mixture of THF and NMP to obtain dipyrido[2,3-*d*]pyrimidine **201b**. Unfortunately, after 17 hours of stirring at room temperature, only starting material was detected. The ring closure was subsequently attempted with triphosgene and DIPEA in CH_2Cl_2 in room temperature for 1-5 hours. However, even using the better carbonyl donor, triphosgene, dipyrido[2,3-*d*]pyrimidine **201** could not be obtained. The LC-MS analysis showed masses corresponding to three compounds: the starting material, *N*-(2-aminoethyl)-2-propylaminonicotinamide **204** and compound **205** (Scheme 76). Compound **205** was separated by preparative TLC in 9% yield and characterized.



Scheme 76 Unexpected formation of five-membered ring **205**. Reaction conditions: (i) DIPEA (6eq), triphosgene (2.4eq), CH_2Cl_2, 0°C (15min)→rt (1h), 9%.

To confirm the structure of the separated compound, the ^{1}H-NMR analysis were compared with starting material **203b**. The ^{1}H-NMR of the compound **203b** used as a starting material shows broadened signals from the amide at 6.94 ppm and a signal from the amino group at 7.52 ppm. On the ^{1}H-NMR spectrum of **205**, the broaded signal occurs at 7.55 ppm. The ^{13}C-NMR analysis showed a new signal at 151.4 ppm. This signal does not respond to any tertiary (154.2, 110.2 and 141.8 ppm) or quaternary (107.4 and 158.3 ppm) carbon from the pyridine ring (HSQC analysis). To determine the structure of **205**, the HMBC analysis was recorded. The carbon at 151.4 ppm is coupled with protons at 4.00, which corresponds with the protons of the ethyl linker. The carbon at 151.4 ppm is characteristic for the carbon from the carbonyl group of 2-imidazolidinone.[180] In conclusion, from the performed analyses the structure of the product is deduced to be **205** (Figure 13).

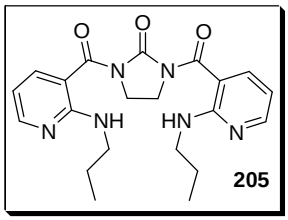
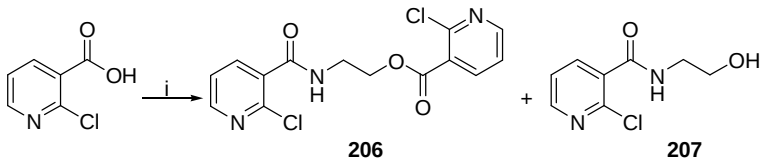


Figure 13

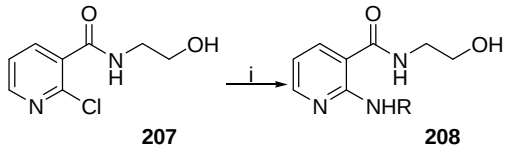
For the preparation of pyrido[2,3-*d*]pyrimidine with 2-hydroxyethyl in the 3 position, 2-chloro-*N*-(2-hydroxyethyl)-nicotinamide was synthesized from 2-chloro-3-pyridinecarbonyl chloride and ethanolamine (Scheme 77).



Scheme 77 Synthesis of 2-chloro-*N*-(2-hydroxyethyl)-nicotinamide **207**. Reaction conditions: (i) a) $SOCl_2$, reflux, 2h, b) ethanolamine, CH_2Cl_2, rt, 17h, **207**: 98%.

LC-MS analysis showed the mass of compounds **206** and **207**, while ^{1}H-NMR analysis only showed signals from **207** and ethanolamine (used in excess). Compound **207** was separated by column chromatography in 98% yield, compound **206** was isolated in less than 1% yield. In the beginning, the 2-chloro-3-pyridinecarbonyl chloride is present in excess to the ethanolamine. In the first place, the amide bond is formed and then, because of excess of acid chloride and availability of free hydroxyl group, the ester bond. When in the reaction mixture ethanolamine is in excess, the free amino group of ethanolamine can attack the carbonyl moiety of the ester **206**, and form another molecule **207**.

In order to obtain nicotinamide **208**, compound **207** was refluxed with the appropriate amines for 16-96 hours (Scheme 78, Table 20).



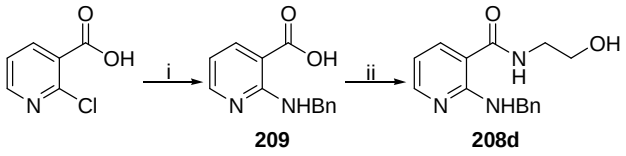
Scheme 78 Synthesis of 2-alkylamino-*N*-(2-hydroxyethyl)-nicotinamide **208**. Reaction conditions: NH_2R (solvent), reflux/100°C (19-69h).

Table 20: Synthesis of compounds **208**.

Entry	NH_2R	Conditions	Product	Yield(%)[a]
1	allylamine	reflux, 69h	**208a**	91%
2	propylamine	reflux, 26h	**208b**	91%
3	*i*-pentylamine	reflux, 16h	**208c**	98%
4	benzylamine	100°C, 20h	**208d**	95%
5	cyclohexylamine	100°C, 19h	**208e**	22%

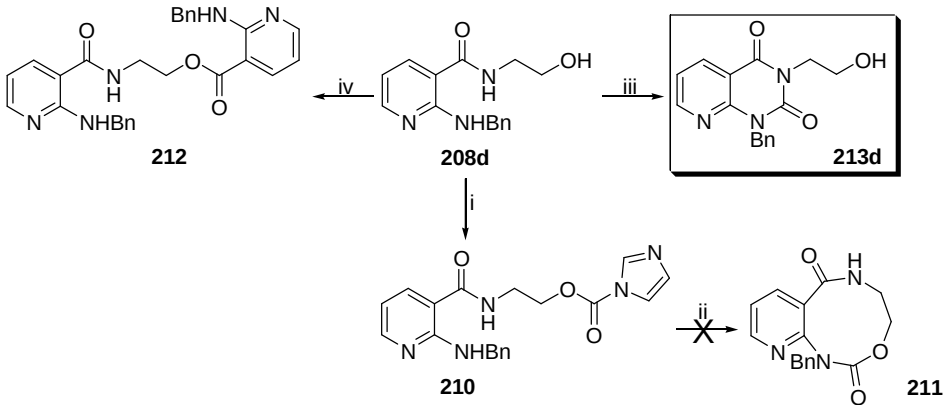
[a]yield after column chromatography

As an alternative, 2-benzyl-*N*-(2-hydroxyethyl)-nicotinamide **208d** was also synthesized from 2-benzylamine-3-pyridinecarboxylic acid **209**[181] and ethanolamine in the presence of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and Et_3N in THF at room temperature.[182] The 2-benzyl-*N*-(2-hydroxy-ethyl)-nicotinamide **208d** was separated by column chromatography.



Scheme 79 Alternative synthesis of compound **208d**. Reaction conditions: (i) $BnNH_2$, 90°C, 10h, 40%, (ii) ethanolamine, BOP, Et_3N, THF, rt, 21h, 80%.

For the ring closure, two methods were selected. The 2-benzyl-*N*-(2-hydroxyethyl)-nicotinamide **208d** was treated with CDI in THF[183] and was refluxed under a nitrogen atmosphere for 40 hours. After reaction, only **210** was present in the reaction mixture (LC-MS analysis). To cyclize compound **210** to the nine-membered molecule **211**, two methods were chosen. In the first attempt **210** was treated with NaH in THF; the second trial involved reflux in the presence of K_2CO_3. Unfortunatelly, in both cases the poor nucleophilicity of the amine did not allow the cyclization ((ii), Scheme 80). When **208d** was treated with NaH and CDI during 20 hours, only starting material and compound **212** was detected, after which **212** was isolated in 10% yield.

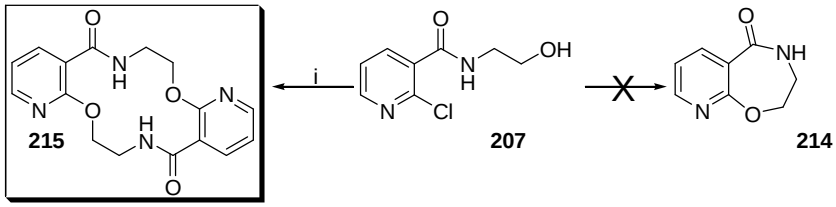


Scheme 80: Synthesis of bicyclic compound **213d**. Reaction conditions: (i) CDI, THF, reflux, 40h; (ii) NaH, THF, rt, 21h or K_2CO_3, MeCN, reflux, 22h; (iii) triphosgene, DIPEA, CH_2Cl_2, rt, 4h; (iv) NaH, CDI, THF, rt, 20h.

In a third method, 2-benzyl-*N*-(2-hydroxyethyl)-nicotinamide **208d** was treated with triphosgene in the presence of DIPEA in CH_2Cl_2. After 4 hours the desired compound **213d** was isolated in 20% yield. The ring closure using triphosgene was also performed for propyl derivative, and bicyclic **213b** was obtained in 27% yield.

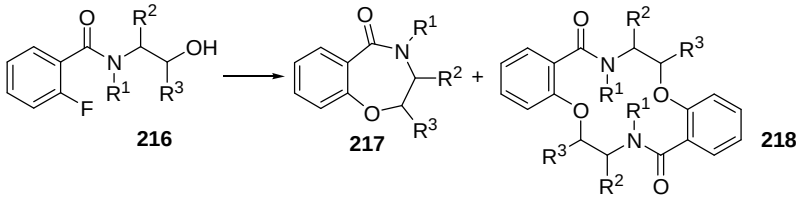
To synthesize 3,4-dihydro-pyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one **214**, 2-chloro-*N*-(2-hydroxyethyl)-nicotinamide **207** was treated with NaH in THF. After stirring at room temperature for 22 hours, a white precipitate was filtered off and washed with THF. Surprisingly the 14-membered tricyclic molecule **215** was obtained in 33% yield (Scheme 81). The desired bicyclic compound **214** was not detected in the reaction mixture. Dilution of the solution did not have any influence on the

formation of compound **214**, in the reaction mixture compounds **206** and **207** were detected by LC-MS. After reaction with TDA-1 in toluene[130] at room temperature for 2-4 hours, only starting material was detected.



Scheme 81 Unexpected formation of the 14-membered ring.
Reaction conditions: (i) NaH, THF, 0°C→rt, 22h.

In 1988, Schultz *et al.*[184] reported the synthesis of 6,7:13,14-dibenzo-1,8,4,11-dioxadiazacyclotetradecanone-5,12-dione **218** in the reaction of 2-fluoro-N-(2-hydroxymethyl)benzamide **216** with NaH in DMF, the tricyclic compound was isolated in 62%. In the reaction mixture also bicyclic 6,7-benzo-1-oxo-4-azepin-5-one was detected (4% yield). They observed that the macrocycle was formed only when 2-fluoro-*N*-(2-hydroxymethyl)benzamide was treated with NaH in THF and that only secondary amides provide the macrocycle (Table 21, entry 1-3). Tertiary amides direct an intermolecular cyclization to bicyclic compounds (Table 21, entry 4-5). Low concentration of starting material resulted in the formation of benzoxazepinone.[184]

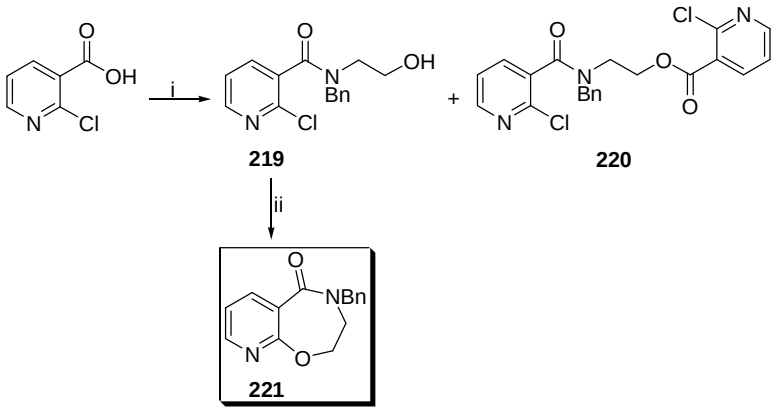


Scheme 82 Synthesis of 6,7:13,14-dibenzo-1,8,4,11-dioxadiazacyclotetradecanone-5,12-dione **218** as presented by Schultz *et al.*[184]

Table 21: Synthesis of 6,7:13,14-dibenzo-1,8,4,11-dioxadiazacyclotetradecanone-5,12-dione **218**.

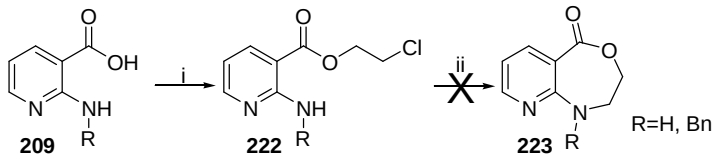
Entry	**Compound 216**			**Product (%)**	
	R^1	R^2	R^3	**217**	**218**
1	H	H	H	-	62
2	H	Me	H	4.7	69
3	H	$CHMe_2$	H	6.6	72
4	Me	H	H	90	-
5	Me	Me	Ph	60	-

Those results confirm our observation during reaction of 2-chloro-*N*-(2-hydroxyethyl)-nicotinamide **207** with NaH. Although, the dilution of the reaction mixture did not induce the formation of bicyclic compound **110**. As was mentioned before, tertiary amides direct an intermolecular cyclization to bicyclic compounds. To synthesize the 3,4-dihydro-4-benzyl-pyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one **221**, the *N*-benzyl-2-chloro-*N*-(2-hydroxyethyl)-nicotinamide **219** was treated with NaH. The seven-membered bicyclic compound **221** was obtained in 98% yield (Scheme 83).



Scheme 83 Synthesis of the seven-membered ring **221**; (i) a) $SOCl_2$, reflux, 2h, b) 2-benzylaminoethanol, CH_2Cl_2, rt, 22h; (ii) NaH, THF, rt, 16h.

To obtain the bicyclic **223**, amine-3-pyridinecarboxylic acid **209** was coupled with 2-chloroethanol using BOP and Et_3N (Scheme 84).[182] The obtained **222** was allowed to react under different conditions to potentially form the 2,3-dihydro-pyrido[2,3-*e*][1,4]oxazepin-5(1*H*)-one **223** (Table 14). The reaction of NaH to generate the anion of **222** (R=H) provided 2-aminopyridine-3-carboxylic acid as a product. The application of a weaker base (K_2CO_3), (after 41 hours of stirring at 80°C) only led to starting material. The cyclization of the benzyl derivative under different conditions did not provide the desired seven-membered compound.



Scheme 84 Attempt to obtain bicyclic **223**. Reaction conditions: (i) 2-chloroethanol, BOP, Et_3N, THF, rt (19h), 90-80%; (ii) see Table 22.

Table 22: Reaction conditions used for the potential ring closure of **222**.

Entry	R	Substrates and solvents	Conditions	Result
1	H	NaH (2eq), THF	0°C (5min) → rt (22h)	2-aminopyridine-3-carboxylic acid
2		MeCN	80°C (112h)	SM
3		K_2CO_3 (3eq), MeCN	80°C (41h)	SM
4	Bn	NaH (1.1eq), THF	0°C (5min) → rt (21h)	SM
5		MeCN	80°C (24h)	SM
6		K_2CO_3 (2.5eq), MeCN	reflux (86h)	SM
7		*n*-BuLi (1.3eq), THF	-78°C (5min) → rt (21h), N_2	SM
8		LDA (1.2eq), THF	0°C (5min) → rt (21h), N_2	SM

4. The biological activity of the selected compounds

4.1. Inhibitory activity of the selected compounds at NPP1

The screening tests for NPP1 inhibitory activity were performed in the laboratory headed by Professor Christa E. Müller (PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany).

The nucleoside pyrophosphatase/phosphodiesterases (NPPs) are widely distributed *N*-glycosylated enzymes that catalyze the hydrolysis of pyrophosphate and phosphodiester bonds of numerous nucleotides and nucleotide sugars.[185] Phosphodiesterases are classified as enzymes that hydrolyse diesters of phosphoric acid into phosphomonesters, and can be classified into two main groups; those that act on lipids or on nucleotides. Pyrophosphatases are acid anhydride hydrolases that catalyze the breakdown of diphosphate bonds and are biologically important in the cleavage of ATP.[186] Mammalian NPPs have been shown to be involved in a variety of cellular processes such as nucleotide signaling, cell differentiation, nucleotide recycling and control of the levels of nucleotides linked to glycosylation and sulfation reactions.[187] In humans the NPP family consist of five proteins of which NPP1 and NPP3 show similar structure and function and the genes encoding for these two proteins have been mapped onto the human chromosome 6q22-23.[188] The NPP1 protein is a membrane spanning homodimer and, when cleaved, the extracellular domain can function as a secreted circulating protein. *ENPP1* is expressed in a wide range of tissues including cartilage, heart, kidney, parathyroid and skeletal muscle, and it is highly expressed in vascular smooth muscle cells (VSMCs), osteoblasts and chondrocytes.[187][189] It was reported to exist in bone (osteoblast) and cartilage (chondrocytes) and has a role in regulating mineralization processes (Figure 14). Extracellular pyrophosphate (PP_i), the product of ATP hydrolysis by NPP1, is a likely source of inorganic phosphate to support hydroxyapatite formation when hydrolyzed by phosphatases and is also a potent inhibitor preventing apatite mineral deposition and growth.[190] Excessive amounts of NPP1 in the chondrocytes can lead to deposits of calcium pyrophosphate crystals in joints, the so-called calcium pyrophosphate dihydrate deposition disease, which can trigger inflammatory arthritis and joint pain. NPP1 downregulates insulin signaling by inhibiting the tyrosine kinase activity of insulin receptors, resulting in reduced insulin sensitivity.[191] NPP1 was found in human astrocytic brain tumors and was correlated with tumor gradation.[190]

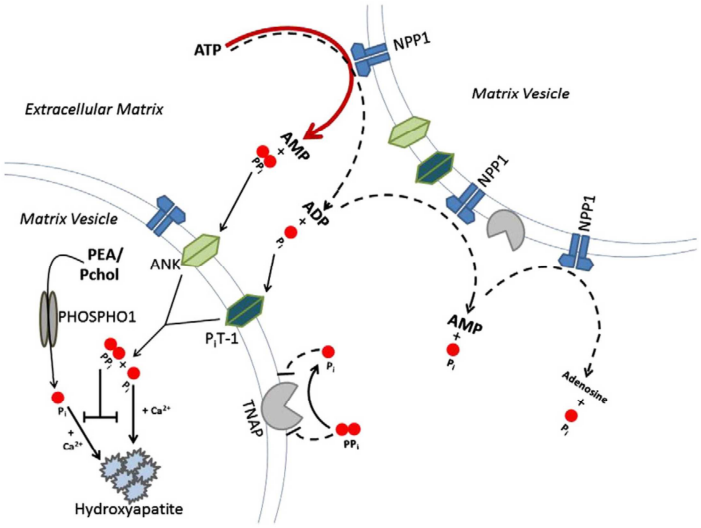


Figure 14 The role of NPP1 in ATP hydrolysis and the downstream effects of bone mineralisation.[186]

The primary function of NPP1 is the hydrolysis of ATP into AMP and PP_i, though it is involved in further degradation of pyrophosphate bonds to generate ADP, adenosine and P_i. PP_i is converted into Pi by tissue-non-specific alkaline phosphatase (TNAP) and the PP_i is transported through the cell membrane by ankylosis protein (ANK) and type III sodium-dependent P_i co-transporter (P_iT-1). The P_i is generated by phosphoethanolamine/phosphocholine phosphatase (PHOSPHO1) in the matrix vesicle by the hydrolysis of phosphoethanolamine (PEA) and phosphocholine (Pchol). PP_i inhibits hydroxyapatite formation, while P_i promotes this process, thus the balance of these two mediators is important in regulating mineralization.[186]

Studies suggested that quinazoline-4-piperidine-4-methylsulfamide is a NPP1 inhibitor lacking binding affinity for the human *ether-à-go-go*-related gene (hERG)[192] and that 1,3,4-oxadiazole(thiadiazole)-2-(3*H*)-thiones are noncompetitive human NPP1 inhibitors. In 2014, Nadel et al. reported two new potent compounds to inhibit NPP1 (Figure 15, examples A and B).[190]

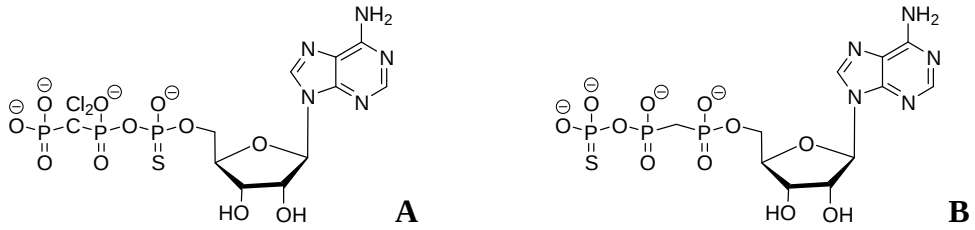
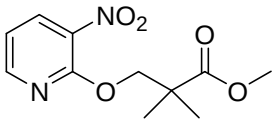
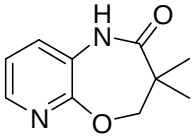
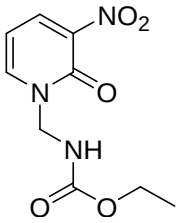
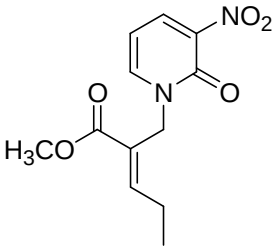
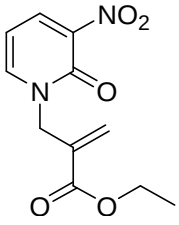
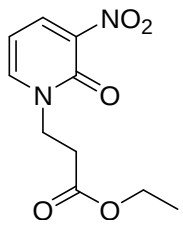
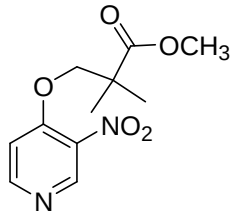
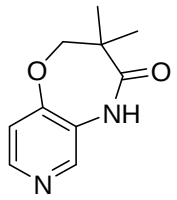
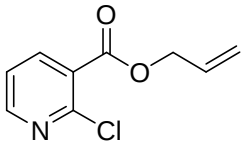
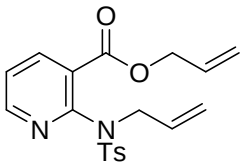
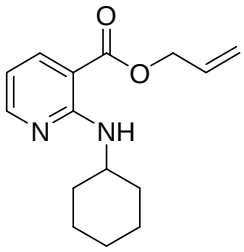


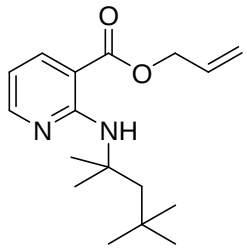
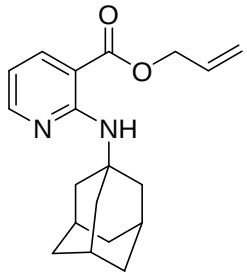
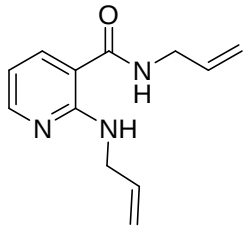
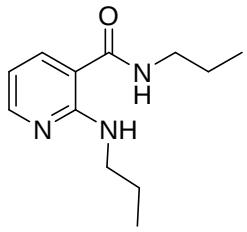
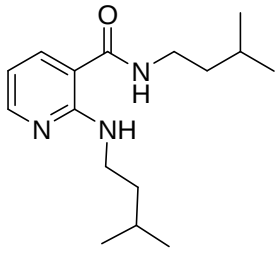
Figure 15 Dinucleotide and nucleotide analogues as potential NPP1 inhibitors: **A** adenosine 5′-Pα-thio-β,γ-(dichloromethylene)triphosphate, **B** adenosine 5′ -α,β-methylene-γ-thiotriphosphate.

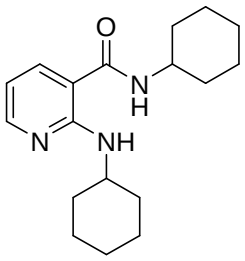
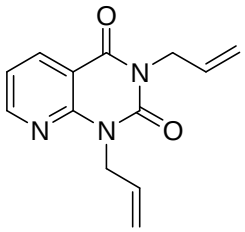
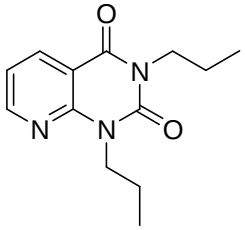
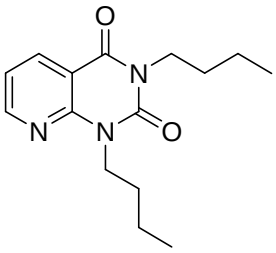
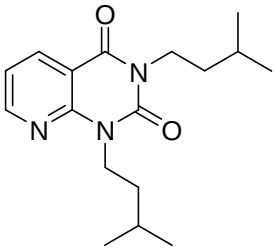
The selected compounds are showed in Table 23. Biochemical properties of NPP activity were determined by assessing nucleotide phosphodiesterase activity, using *p*-nitrophenyl 5'-thymidine monophosphate (*p*-Nph-5´-TMP) or ATP as a substrate.

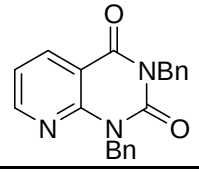
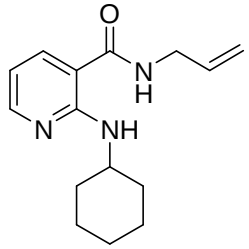
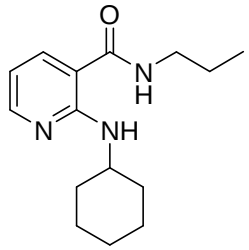
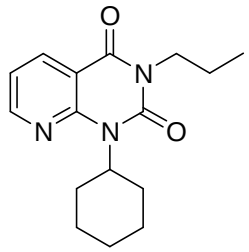
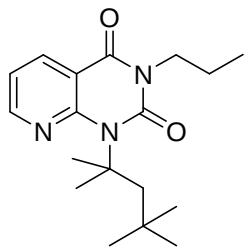
Table 23: Initial screening with p-Nph-5´-TMP and ATP for the selected compounds.

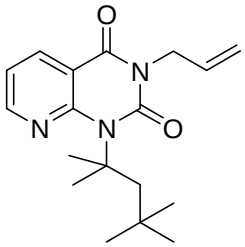
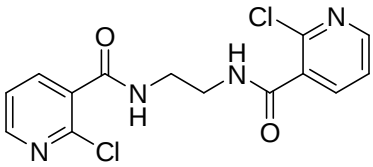
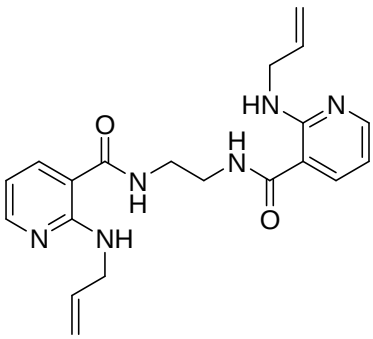
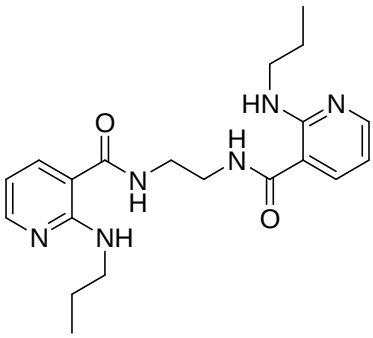
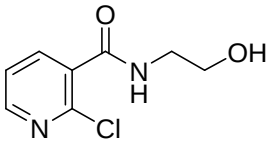
Compound	**Structure**	***p*-Nph-5´-TMP[a] Inhibition (%)**	**ATP[a´] Inhibition (%)**
111		-	11
113		**26**	**25**
124		-	13
128		-	7
138		-	5

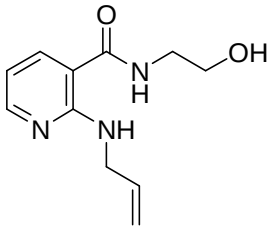
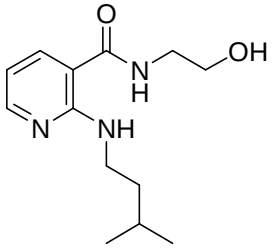
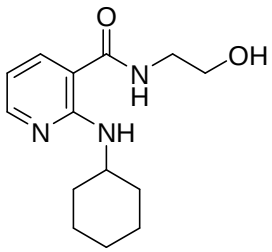
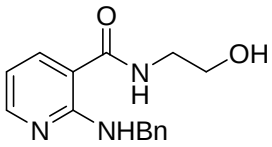
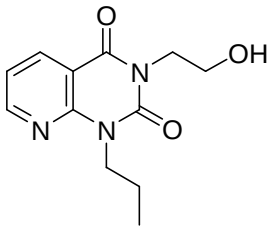
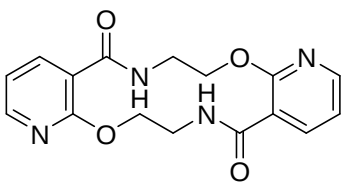
Compound	Structure	*p*-Nph-5´-TMP[a] Inhibition (%)	ATP[a´] Inhibition (%)
142		-	5
148		-	14
150		-7	-
186		-13	-
191		-	3
196f		-8	-

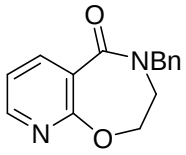
Compound	Structure	*p*-Nph-5´-TMP[a] Inhibition (%)	ATP[a´] Inhibition (%)
196h		**62**	**26**
196i		**53**	**27**
197a		-11	-
197b		-14	-
197d		-15	-

Compound	Structure	*p*-Nph-5´-TMP[a] Inhibition (%)	ATP[a´] Inhibition (%)
197f		-5	-
198a		-1	-
198b		-17	-
198c		-14	-
198d		-14	-

Compound	Structure	*p*-Nph-5´-TMP[a] Inhibition (%)	ATP[a´] Inhibition (%)
198j		-9	-
199a		-8	-
199b		**29**	**25**
200b		-21	-
200f		-	**30**

Compound	Structure	*p*-Nph-5´-TMP[a] Inhibition (%)	ATP[a´] Inhibition (%)
200e		-	20
202		-	21
203a		-	23
203b		-	18
207		-	8

Compound	Structure	*p*-Nph-5´-TMP[a] Inhibition (%)	ATP[a´] Inhibition (%)
208a		-	13
208c		-	15
208e		-	8
208d		-	6
213b		-	9
215		-	5

Compound	Structure	*p*-Nph-5´-TMP[a] Inhibition (%)	ATP[a´] Inhibition (%)
221		-	2

[a]Initial screening with the artificial substrate *p*-Nph-5´-TMP. The assay conditions were: 400µM *p*-nitrophenyl 5'-TMP, 10µM inhibitor concentration, 20 ng of human recombinant NPP1, reaction buffer (1mM $CaCl_2$, 200µM $ZnCl_2$, 50mM Tris, pH 9.0), spectrophotometric detection at 400nm and n=1.

[a´]The assay conditions were: 400µM ATP as substrate, inhibitor concentration at 10µM, 1.8ng/µl of human recombinant NPP1 (K_m = 43.2µM, own enzyme), reaction buffer (1mM $CaCl_2$, 2mM $MgCl_2$, 10mM CHES, pH 9.0), detection at 260nm (n = 3).

In the initial screening with artificial substrate (*p*-Nph-5´-TMP) only two compounds were exhibited an enzyme inhibition over 50% (compound **196h** and **196i**, 62% and 53% respectively). The screening with ATP for compounds **196h** and **196i** resulted with 26% and 27% inhibition respectively. The inhibition effect with ATP (over 25%) were detected also for compounds **199b** (25%), **200f** (30%) and **113** (25%). For investigations with the natural substrate, compounds should inhibit over 70% in the initial screening. The negative values can suggest that the products of metabolic pathway acts upon the enzyme.

4.2. Antimicrobial potency assay of the selcected compounds

The antimicrobial activity test was performed in the Laboratory of Microbiology & BCCM/LMG Bacteria Collection (Faculty of Science, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium).

The five compounds were submitted to antimicrobial activity test (Figure 16). The four strains were selected: *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis*.

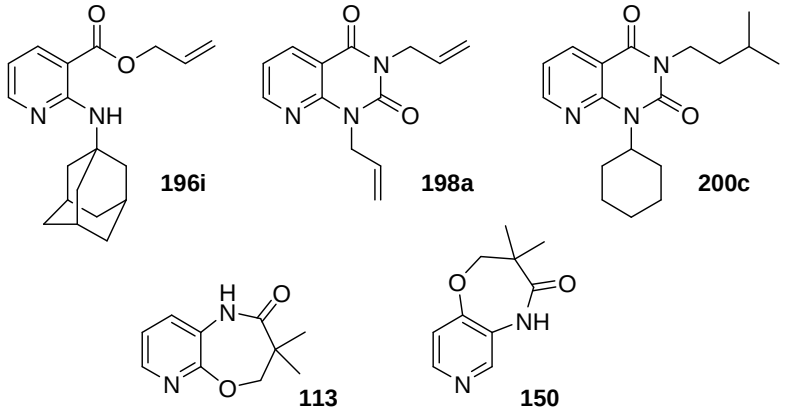


Figure 16

Escherichia coli, is a member of the bacterial family of *Enterobacteriaceae*. Also is a prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, and one of the most important pathogens responsible for a broad spectrum of diseases. The special properties of the *E. coli*, such as ease of handling, availability of the complete genome sequence, and its ability to grow under both aerobic and anaerobic condition, makes it an important host organism in biotechnology.[193] Seven major pathotypes were classified for enteric *E. coli*, and three *E. coli* pathotypes for extraintestinal strains (ExPEC). Enteric *E. coli* cause diarrhea in children, hemorrhagic colitis, traveler´s diarrhea, the extraintestinal *E. coli* case neonatal meningitis and probable source of food-borne disease.[193][194] *Klebsiella pneumoniae* is the most common organism associated with *Klebsiella pneumonia* carbapenemases (KPCs) resistance determinants. KPCs are typically reside on transferable plasmids and can hydrolyze all penicillins, cephalosporins, aztreonam, and carbapenems. Infections caused by KCPs have very limited options for treatment and often require the use of polymyxins, which fell into disuse in the 1970s due to high rates of nephrotoxicity.[195] *Staphylococcus aureus* is a gram-positive spherical bacterium. It is often found as a commensal associated with skin, skin glands, and mucous membranes. *Staphylococcus aureus* is one of the main causes of hospital- and community-acquired infections which can result in serious consequences. It can be a cause of central venous catheter-associated bacteremia and ventilator-

assisted pneumonia. It also causes serious deep-seated infections, such as endocarditis and osteomyelitis. *Staphylococcus aureus* is often responsible for toxin-mediated diseases, such as toxic shock syndrome, scalded skin syndrome and staphylococcal foodborne diseases (SFD). They are resistant to heat denaturation and proteases.[196] *Bacillus subtilis* is a Gram-positive, aerobic soil bacterium ubiquitous in the environment and commonly associate with variety of food products such as milk and dairy product, meat products, rice, pasta, and dried products such as spices. The favorable effect of *Bacillus subtilis* spores on the balance of the intestinal microflora is the rationale for its general use as a probiotic preparation in the treatment or prevention of intestinal disorders. The pathogenic potential is generally described as low or absent. Data of infections cases by *Bacillus subtilis* are incomplete due to discard these strains and also the cause-of -death statistics no data about infections are present.[197] *Bacillus subtilis* is considered as a GRAS organism (generally recognized as safe).[198] Recently *Bacillus subtilis* and other *Bacillus* have been linked to potential food poisoning issues. One of the main concerns associates with food is the low pH which is applied to prevent and control the growth of *Clostridium botulinum* can increases by *Bacillus* production of enzymes. One of the difficulties is the resistance for the pasteurization treatment. In other hand, the ability to produce enzymes have been used in a positive manner to produce the food (e.g. the Thai fermented soy products, the African fermented locus bean product).[199]

The tested compounds did not display an antimicrobial effect against both Gram-negative test strains *Escherichia coli* and *Klebsiella pneumoniae*. Four tested compounds (**198a**, **200c**, **113**, **150**) displayed a very weak growth inhibition of both Gram-positive test strains *Staphylococcus aureus* and *Bacillus subtilis*. The compound **196i** did not demonstrate any effect against Gram-negative or Gram-positive strains.

4.3. The results of the ADME study of the selected compounds

The ADME study were performed in Drug Delivery and Disposition headed by Professor Patrick Augustijns (Department of Pharmaceutical and Pahrmacological Science, KU Leuven, Campus Gasthuisberg-O&N II, Herestraat 49 – box 921, B-3000 Leuven, Belgium).

The ADME study of the synthesized library (**196i, 197a, 197d, 197f, 198a, 198b, 198d, 198j, 199e, 200a**) was published in *Bioorg. Med. Chem.* **2014**, *22*, 3947-3956.

Due to the variety of pharmacological activities of the pyridopyrimidine scaffold (Introduction), the ADME (Absorption, Distribution, Metabolism, and Excretion) study was performed on selected examples of the synthesized library (Figure 17). Key physicochemical properties were determined using Marvin Sketch (Table 24). All compounds were considered drug-like according to the Lipinski's rules concerning the molecular weight (MW), the amount of hydrogen bond donors (HBD) and acceptors (HBA) and the partition coefficient (LogP)[200] Moreover, polar surface areas were below 140 Å² which is recommended by Veber *et al.*[201]

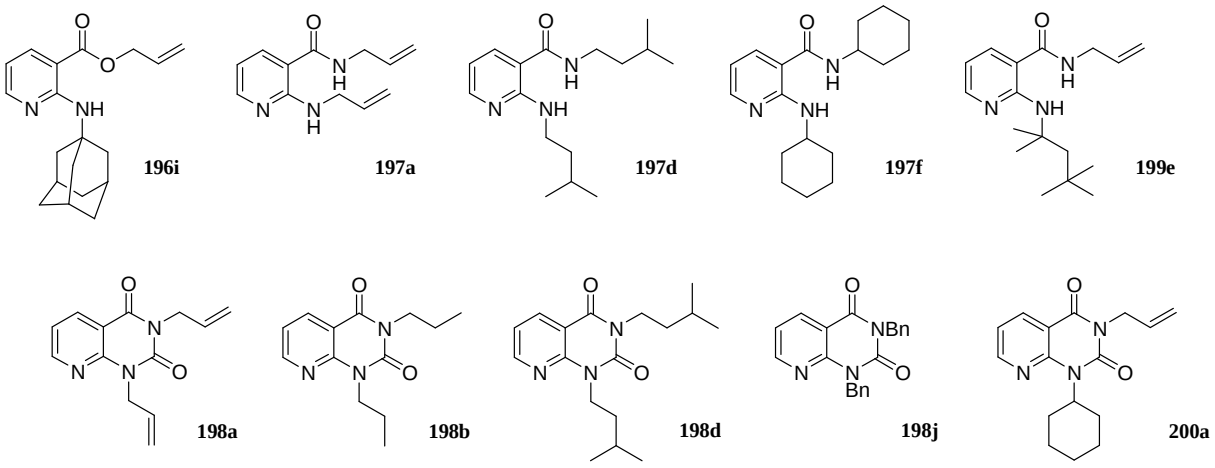


Figure 17 Selected compounds for ADME study.

With respect to the evaluation of the intestinal solubility and permeability, plain aqueous buffers are often used. However, plain aqueous buffers insufficiently represent the *in vivo* conditions, possibly resulting in too low solubilities to allow further experiments. Hence, biorelevant media are more promising, mimicking the *in vivo* environment more accurately due to the presence of mixed micelles of taurocholate and lecithin, i.e. a fasted simulated intestinal fluid (FaSSIF).[203][204] Recently, a good correlation was shown between solubility in FaSSIF and fasted state human intestinal fluid (FaHIF).[205] Table 24 and Figure 18 reveal a broad range in solubility values for this series, ranging

from 12.6 µM for compound **197f** to 13.8 mM for compound **197a**. To generally describe "solubility" the United States Pharmacopoeia (USP) uses different solubility expressions based on parts of solvent (in this case FaSSIF) required for one part of solute.[202] The selected compounds could be classified as practically insoluble (**196i, 197f, 198j, 199e, 200a**) to slightly soluble (**197a**). The use of aliphatic chains as substituent on the pyrido[2,3-*d*]pyrimidines scaffold (**198a, 198b, 198d**) resulted in a higher solubility compared with compound **198j** containing benzyl groups. The presence of ring structures as substituent generates a higher lipophilicity of the compound leading to a lower aqueous solubility. Also for the 2-aminopyridine scaffold, the use of the 2 cyclohexyl substituents (**197f**) resulted in a lower solubility compared to compounds containing aliphatic chains on the same position (**197a, 197d, 199e**). These observations suggest that aliphatic chains are preferred if solubility issues are encountered.

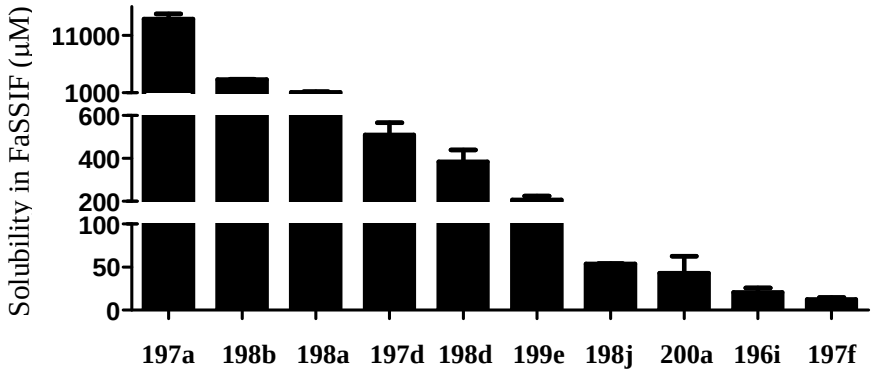


Figure 18 Solubility (µM) in fasted state human intestinal fluid (FaSSIF) of the selected pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones and their precursors is presented as the mean ± S.D. (n=3)

Table 24: Properties of pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones and their precursors.

Compound	MW (g/mol)	HB_d[a]	HB_a[b]	cLogP[c]	Log(LogP)	PSA[d] ($Å^2$)	FaSSIF Solubility (μM)	Classification[h]	P_{app} (x 10^{-6}) (cm/s)	$Cl_{int,hep,human}$ ml/min/kg.b.w.
Drug-like[e]	<500	≤5	≤10	≤5		≤140[f]				
196i	312.8	1	3	4.3	0.63	51.2	20.7 ± 5.3[g]	PI	1.2 ± 0.5	84.3 ± 6.4
197a	217.1	2	3	2.0	0.3	54.0	13801.7 ± 937.8	SS	46.8 ± 1.8	28.0 ± 3.5
197d	277.2	2	3	3.8	0.58	54.0	509.2 ± 55.9	VSS	19.8 ± 1.3	26.7 ± 8.3
197f	301.2	2	3	4.2	0.62	54.0	12.6 ± 2.4	PI	9.7 ± 2.0	152.0 ± 0.7
198a	243.1	0	3	1.7	0.23	53.5	1054.9 ± 111.1	VSS	90.7 ± 15.0	0.0 ± 0.0
198b	247.1	0	3	2.0	0.3	53.5	3265.2 ± 75.6	VSS	63.0 ± 6.2	3.6 ± 0.5
198d	303.2	0	3	3.4	0.53	53.5	384.7 ± 55.2	VSS	13.9 ± 1.7	153.2 ± 19.8
198j	343.1	0	3	3.7	0.57	53.5	53.7 ± 0.5	PI	30.5 ± 1.2	33.9 ± 5.0
199e	289.2	2	3	3.9	0.59	54.0	207.8 ± 17.2	PI	14.7 ± 1.3	159.2 ± 9.1
200a	285.1	0	3	2.7	0.43	53.5	43.1 ± 19.7	PI	32.0 ± 0.9	26.6 ± 6.3

Abbreviations: MW, molecular weight, HB_d, hydrogen bond donors, HB_a, hydrogen bond acceptors, cLogP, computational partition coefficient, PSA, polar surface area, acid dissociation constant, FaSSIF, fasted state simulated intestinal fluid, P_{app}, apparent permeability, $Cl_{int,hep,human}$, human hepatic intrinsic clearance

[a,b,c,d]Calculated with Marvin Sketch

[e]Lipinski's rule of five [200]

[f]Veber et al, 2007 [201]

[g]Standard deviation (SD), n=3

[h]Solubility classification according to the United States Pharmacopoeia (USP, 2007): PI, practically insoluble, VSS, very slightly soluble, SS, slightly soluble [202]

Similar as for the intestinal solubility, a broad range was observed for the intestinal permeability of the compounds using FaSSIF as apical medium (Table 24 and Figure 19). With exception of compound **196i**, the P_{app} values of the investigated compounds were significantly higher than that of a paracellular marker atenolol (5.3 x 10^{-6} cm/s) and lower than that of a transcellular marker indomethacin (93.3 x 10^{-6} cm/s). In literature, a P_{app} value of 10 x 10^{-6} cm/s has been reported to result in a fraction absorbed in humans of at least 90%.[206] Hence, for 9 out of 10 compounds the permeability was relatively high.

The permeability of the selected compounds was also determined in the presence of 4µM of the P-glycoprotein (P-gp) inhibitor elacridar (GF120918) to explore whether P-gp has a modulatory effect on the absorption of the selected compounds.[207] None of the compounds showed a significant increase in P_{app} when elacridar was included in the medium; this is in contrast with the 3.2-fold increase which was observed for a known P-gp substrate indinavir.[208] Hence the intestinal absorption of these compounds is not expected to be modulated by P-gp-mediated efflux transport in the intestine.

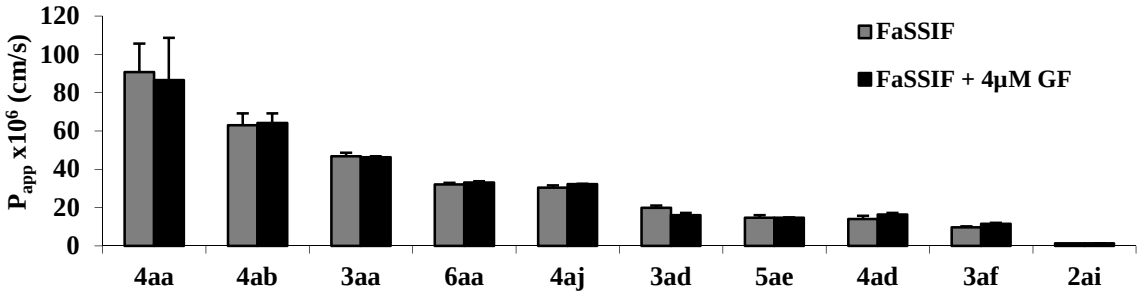


Figure 19 Absorption potential of the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones and their precursors in fasted state simulated intestinal fluid (FaSSIF), estimated as the apparent permeability coefficient (P_{app}) of the Caco-2 monolayer in presence and absence of 4µM P-glycoprotein (P-gp) inhibitor GF-120918 (data presented as the mean ± S.D., n=3)

It is important to note that solely the free concentration of the compounds is able to permeate across the epithelial membrane. Compounds with a high lipophilicity are readily incorporated in micelles present in FaSSIF and are therefore less available for absorption.[209] Figure 20 clearly illustrates the significant correlation between P_{app} and Log(LogP) in FaSSIF.

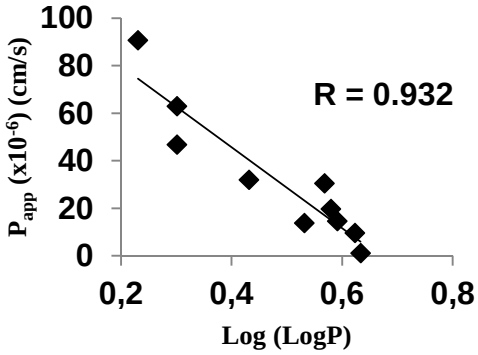


Figure 20 Relationship between the intestinal permeability (P_{app}) of the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones and their precursors *in silico* predicted lipophilicity (Log(LogP))

The Cl_{int} of the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones, determined in human liver microsomes (HLM), was clearly compound dependent, as depicted in Figure 21. Scaled values for intrinsic hepatic clearance in human ($Cl_{int,hep,human}$) varied between 0 and 159 ml/min/kg. For compound **198a,** no metabolism was observed under the conditions used. A possible explanation is that the metabolism of this compound is not mediated by cytochrome P450 enzymes (CYP) and can therefore not be determined with liver microsomes as in vitro drug metabolism model. Verapamil was used as a positive control compound for hepatic metabolism as it is known to be an extensively metabolized compound.[210][211] Compounds **199e**, **198d**, **197f** are more extensively metabolized compared to verapamil which could be useful when the metabolite shows biological activity. The metabolism of **196i** is comparable to verapamil. Five of the compounds are more stable than verapamil (**198j**, **197a**, **197d**, **200a**, **198b**). Since the biological activity of these compounds still needs to be evaluated, compounds showing intermediate metabolism **(196i, 198j, 197a, 197d and 200a)** are the most promising. Parent concentrations remained stable in incubations performed in the absence of NADPH and glucose-6-phosphate, indicating chemical stability of the compounds under the incubation conditions used.

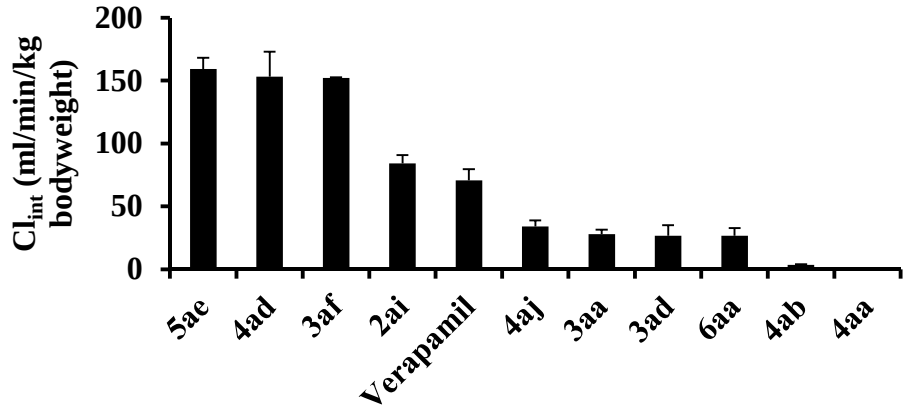
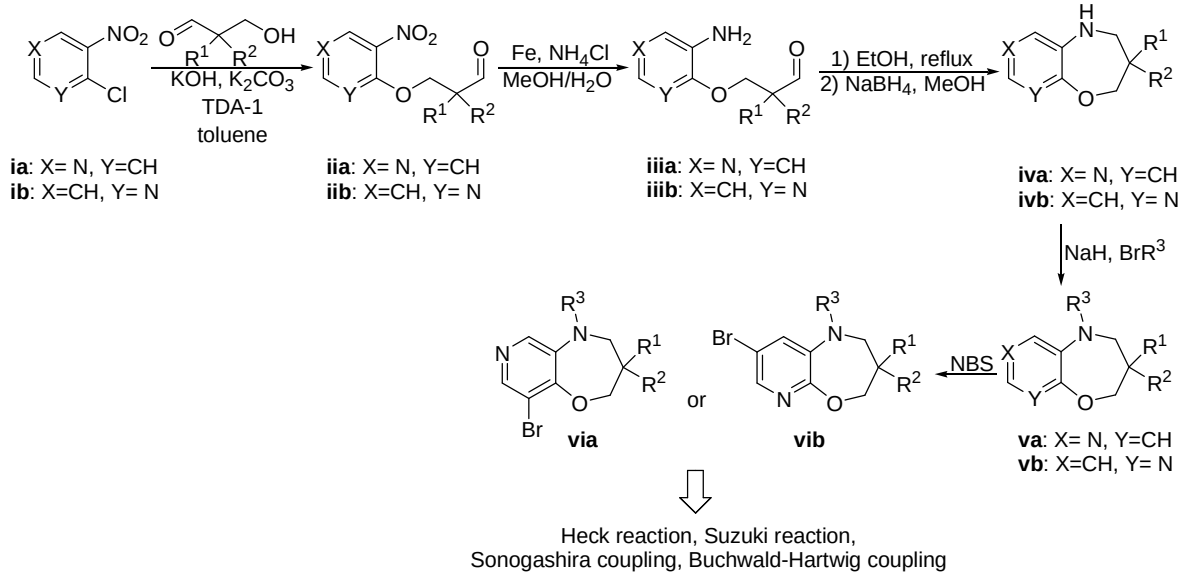


Figure 21 Mean Cl_{int} values (±S.D. n=3) for selected pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones and their precursors determined in a pool of human liver microsomes (HLM) from 45 donors.

The biopharmaceutical profiling of a selection of pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones and their precursors reveals a broad range of structure-dependent solubility, permeability and hepatic metabolism values. Taking under consideration the results of permeability and hepatic metabolism, all compounds, except for **196i**, show acceptable drug-like properties; the consequence of the hepatic metabolism study depends on the biological activity of the parent compound and its metabolites. The selected compounds show poor solubility in FaSSIF, which can be a limiting stage to further investigation.

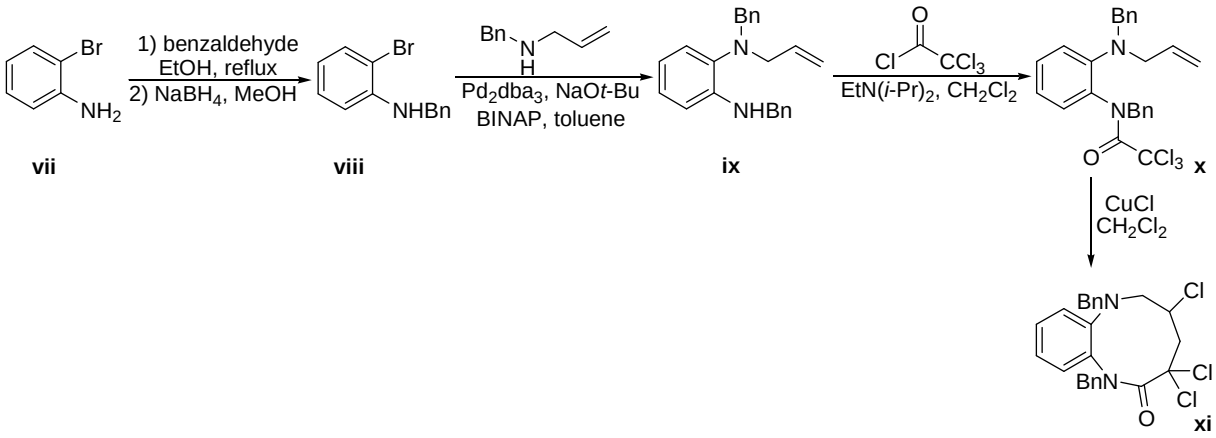
5. Perspectives

The new library of pyrido[2,3-*b*][1,4]oxazepines (**vib**) and pyrido[4,3-*b*][1,4]oxazepine (**iva**) can be synthesized using chloro derivatives (**ia** and **ib**) of pyridine. Because of the equilibrium between 2-hydroxy-3-nitropyridine and 3-nitro-2(1*H*)-pyridinone the reaction with other alcohols under Mitsunobu conditions or alkylation by chlorides could provide *N*-alkylated products. If the alcohol will possess also a carbonyl group, after reduction of the nitro group, the condensation reaction can be performed followed by the ring closure to desired pyridoxazepine scaffold. The nitrogen atom can be alkylated and the pyridine ring could be brominated at C5 with NBS or Br_2. This can open many possibilities to coupling reactions e.g. Heck reaction, Suzuki reaction, Sonogashira coupling and Buchwald-Hartwig coupling (Scheme 1).



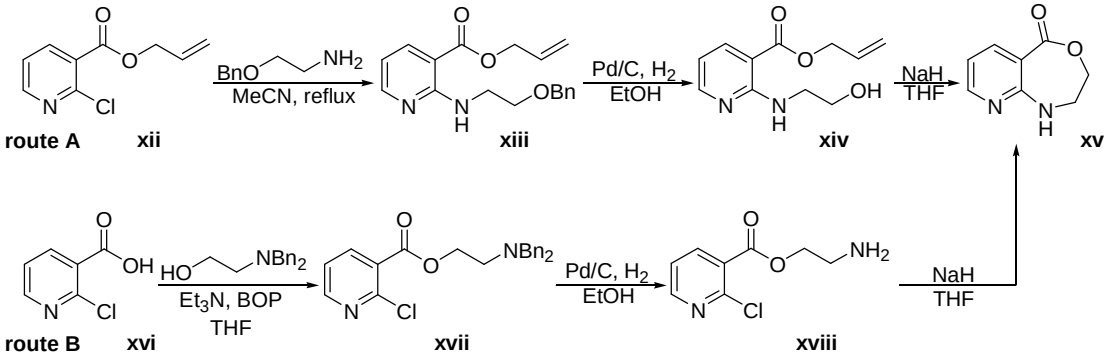
Scheme 1

The radical ring closure is more demanding. To prove the formation of the complex metal-pyridine compound, the benzyl analogue **x** should be synthesized. The synthesis can start from condensation of 2-bromoaniline **vii** and benzaldehyde to the imine, followed by reductive amination to the secondary amine **viii**. To introduce the *N*,*N*-allylbenzylamine,[151] into the 2 position, the Buchwald-Hartwig amination can be used.[212] To complete the synthesis of the starting material **x**, reaction with trichloroacetyl chloride should be performed (Scheme 2).



Scheme 2

The presented synthesis of 2,3-dihydro-pyrido[2,3-*e*][1,4]oxazepin-5(1*H*)-one started by the formation of the ester bond, however the cyclization by alkylation failed. One of the uninvestigated ways includes the synthesis of an amine at C2 followed by transesterification (Scheme 3, route A). Using allyl 2-chloropyridine-3-carboxylate the amine group can be easily introduced into C2 of the pyridine ring by substitution of the chlorine atom with *O*-benzylated ethanolamine.[213] The debenzylation can be performed with hydrogen catalyzed by Pd/C. The ring closure to the bicyclic compound **xv** could be performed by transesterification, using NaH in THF.

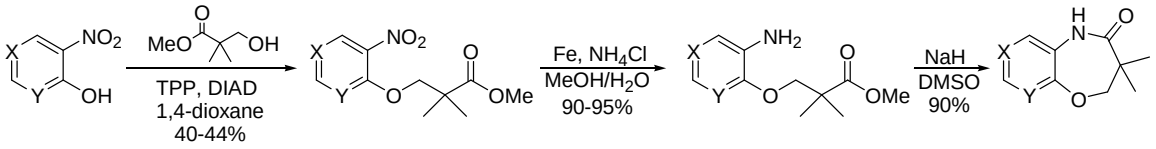


Scheme 3

The second option to the 2,3-dihydro-pyrido[2,3-*e*][1,4]oxazepin-5(1*H*)-one scaffold, starts by the formation of ester **xvii** by the reaction of 2-chloropyridine-3-carboxylic acid and *N*,*N*-dibenzylethanolamine[214] in the presence of BOP. The double substituted amine prevents the formation of amide, thus the ester will be form as the only product. The debenzylation can be conducted with hydrogen in the presence of Pd/C, provide the free amine group which in the aromatic substitution of the chlorine atom at C2 leads to the desired cyclic compound (Scheme 3, route B).

6. Summary

In conclusion, the synthesis of pyrido[2,3-*b*][1,4]oxazepines (**ivb**) and pyrido[4,3-*b*][1,4]oxazepine (**iva**) was designed and successful accomplished (Scheme 1). The most challenging step of those syntheses turned out to be the coupling of the hydroxynitropyridine with methyl 3-hydroxy-2,2-dimethylpropanoate. The 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionate (**iib**) and 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate (**iia**) were isolated in low yields. Next steps did not give any trouble, the reduction of nitro group with Fe and NH_4Cl gave the amino derivative in excellent yields. The ring closure reaction to pyridoxazepines proceeds in 90% yield for both pyridines.



ia: X= N, Y=CH
ib: X=CH, Y= N

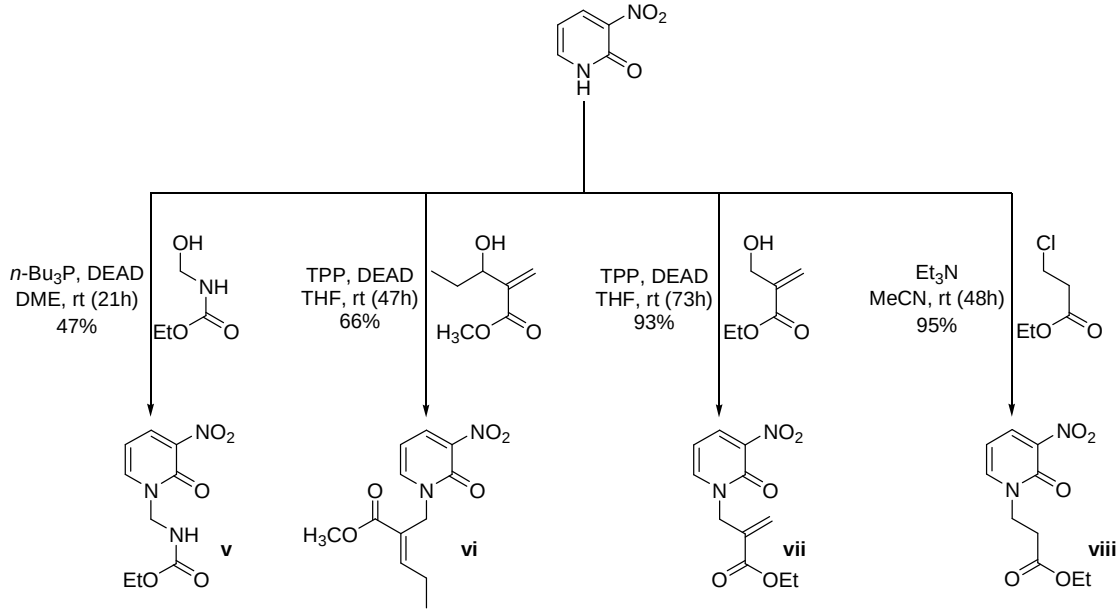
iia: X= N, Y=CH
iib: X=CH, Y= N

iiia: X= N, Y=CH
iiib: X=CH, Y= N

iva: X= N, Y=CH
ivb: X=CH, Y= N

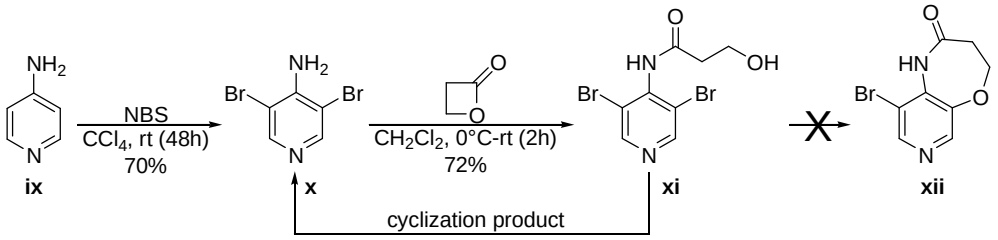
Scheme 1

To explore the seven-membered ring fused to the pyridine ring of pyrido[2,3-*b*][1,4]oxazepines, seven alcohols and a chloride derivative were selected. Due to the equilibrium between 2-hydroxy-3-nitropyridine and 3-nitro-2(1*H*)-pyridinone, the *N*-substituted products were isolated only for three alcohols and the chloride (Scheme 2). The reactions with the other alcohols did not allow to synthesize neither *O*-alkylated nor *N*-alkylated products.



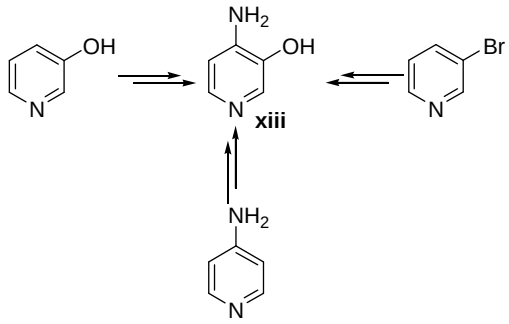
Scheme 2

The desired pyrido[3,4-*b*][1,4]oxazepine scaffold **xii** was not achieved. The cyclization of *N*-(3,5-dibromo-pyridin-4-yl)-3-hydroxy-propionamide **xi** using Buchwald coupling conditions followed by deprotonation lead to deamidation to **x**.



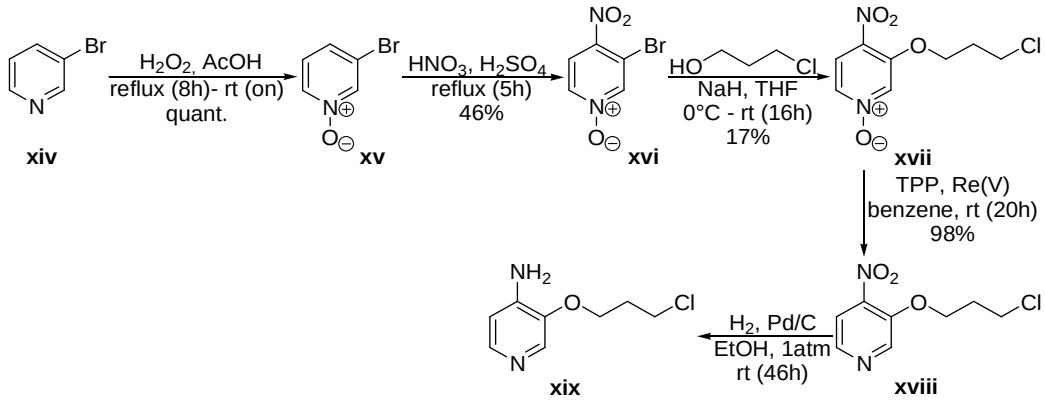
Scheme 3

Despite the fact that for the synthesis of **xii** a lot of methods were used, the starting material 4-amino-3-hydroxypyridine was not synthesized (Scheme 4).



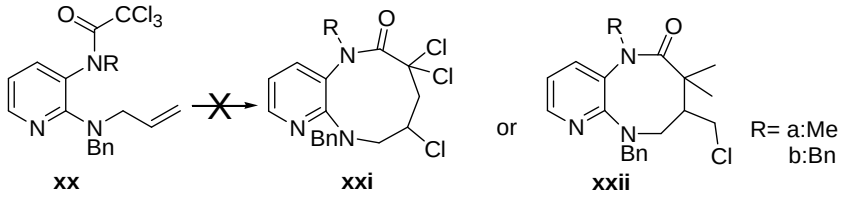
Scheme 4

The most promising pathway which includes the nucleophilic substitution of bromine at C3 did not succeed, the isolation failed because of the poor solubility of 3-(3-chloropropoxy)-pyridin-4-ylamine **xix** in organic solvents.



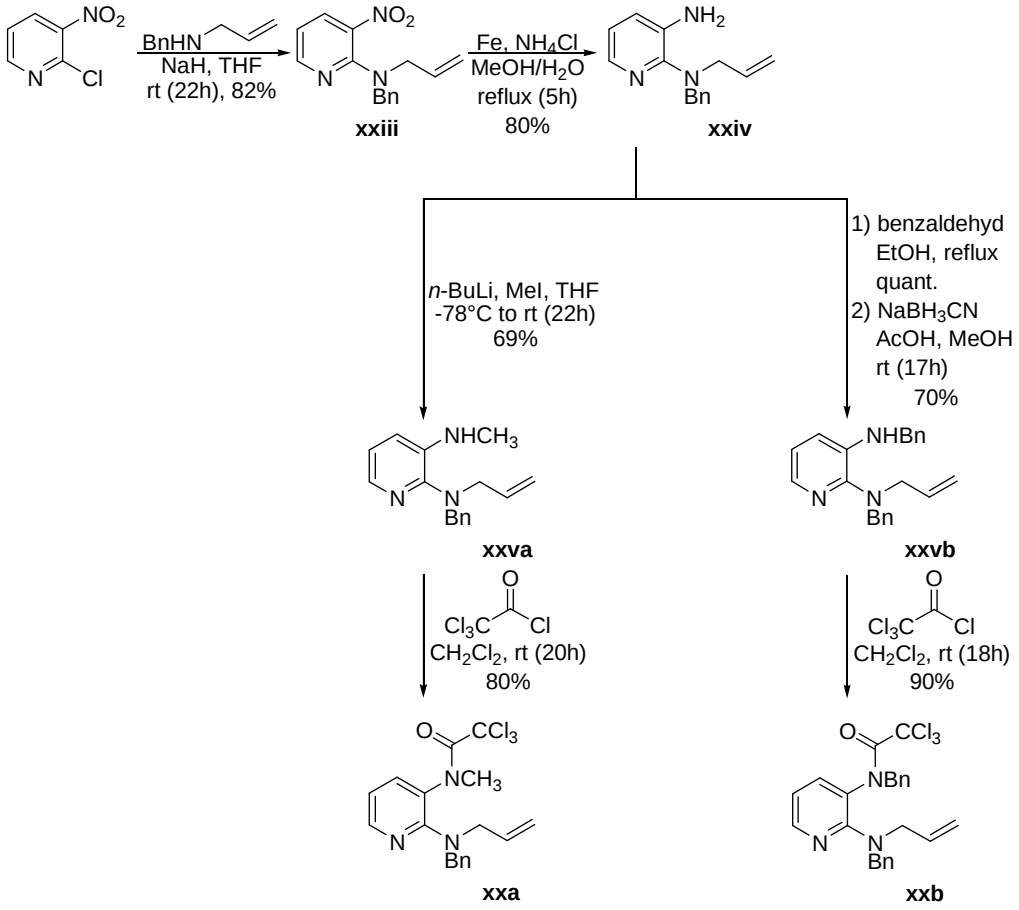
Scheme 5

The formation of the nine-membered ring of the *N*-[2-(*N*-allyl-*N*-methylamino)-pyridin-3-yl]-*N*-benzyl-2,2,2-trichloro-acetamide (**xxia**) or the *N*-[2-(*N*-allyl-*N*-benzylamino)-pyridin-3-yl]-*N*-benzyl-2,2,2-trichloro-acetamide (**xxib**) under the Kharasch reaction conditions could not be realized (Scheme 6). The synthesis of **xxa** and **xxb** is presented in Scheme 7.



xx **xxi** **xxii**

Scheme 6

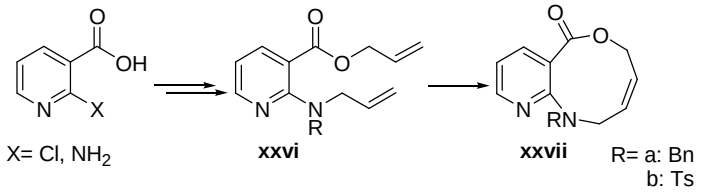


xxiii **xxiv** **xxva** **xxvb** **xxa** **xxb**

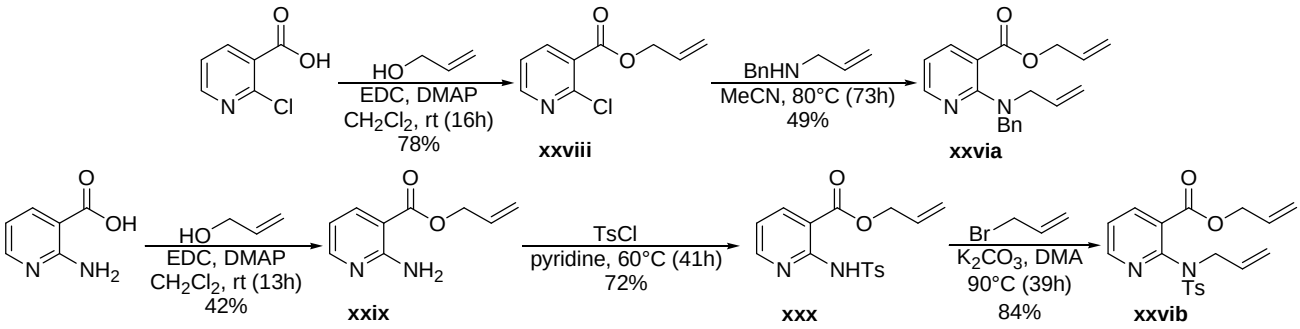
Scheme 7

The Kharasch reaction was conducted with CuCl with or without ligand (TMEDA/ PMDETA), CuO, a mixture of CuCl and CuO for the benzyl derivative and with CuCl, $Ru(PPh_3)_3Cl_2$, Grubbs 1st generation and complex CuCl-bipyridine for the methyl derivative. Different solvents (CH_2Cl_2, DCE or toluene), under reflux, 80°C or room temperature were applied. For all investigated conditions, only starting materials were recovered.

To obtain a nine-membered ring fused to pyridine, the metathesis reaction of allyl 2-(*N*-allyl-*N*-benzylamino)-3-pyridinecarboxylate (**xxvia**) and allyl 2-(*N*-allyl-*N*-tosylamino)-3-pyridinecarboxylate (**xxvib**) was performed under different conditions (Scheme 8). The synthesis of compounds **xxvi** is presented in Scheme 9. The allyl 2-(*N*-allyl-*N*-benzylamino)-3-pyridinecarboxylate (**xxvia**) was reacted with different ruthenium complexes (Grubbs 1st and 2nd generation catalyst, [RuClH(CO)(PPh_3)], Hoveyda-Grubbs 2nd generation) in toluene or CH_2Cl_2, at room temperature, reflux or 65°C. However, all the attempts led to the isolation of starting material.



Scheme 8



Scheme 9

The reaction of **xxvia** with Grubbs 2nd generation was also conducted in the presence of Lewis acids (Ti(O*i*-Pr)$_4$, LiCl, $ZnCl_2$, In(OTf)$_3$, Sc(OTf)$_3$). Only in the presence of Sc(OTf)$_3$ or In(OTf)$_3$ a new product was detected. The new compound was isolated as the trifluoroacetic acid salt after separation on preparative HPLC. The ^{1}H-NMR analysis confirms presence of the deallylated compound (Figure 1), 2-(*N*-allyl-*N*-benzylamino)-nicotinic acid or allyl 2-benzylaminopyridine-3-carboxylate.

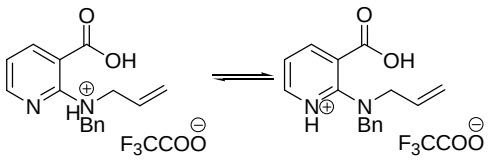
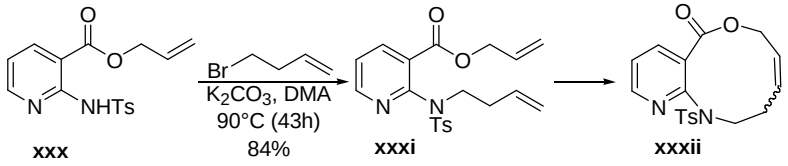


Figure 1

The ring closure of allyl 2-(*N*-allyl-*N*-tosylamino)-3-pyridinecarboxylate (**xxvib**) using Grubbs 2nd generation, and Grubbs 2nd generation catalyst with Lewis acid (In(OTf)$_3$, Sc(OTf)$_3$) did not proceed to

the bicyclic compound. Because of the deallylation and the isolation of starting material, the 2-propen-1-yl 2-(*N*-(3-buten-1-yl)-*N*-tosylamino)-3-pyridinecarboxylate (**xxxi**) was synthetized as a precursor for a ten-membered ring fused to pyridine (Scheme 10).



Scheme 10

xxxi Reacted with Grubbs 2nd generation catalyst at 80°C in toluene and after 5 h, a mixture of compounds was detected. The compounds were separated by preparative HPLC as a salt of trifluoroacetic acid (acid was added to the eluenting mixture). Unexpectedlly, under these conditions, the intramolecular cyclization products were formed (Figure 2). In the reaction mixture, the desired bicyclic ten-membered pyridine derivative was not detected.

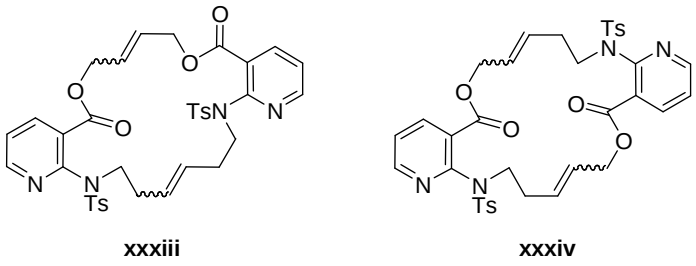
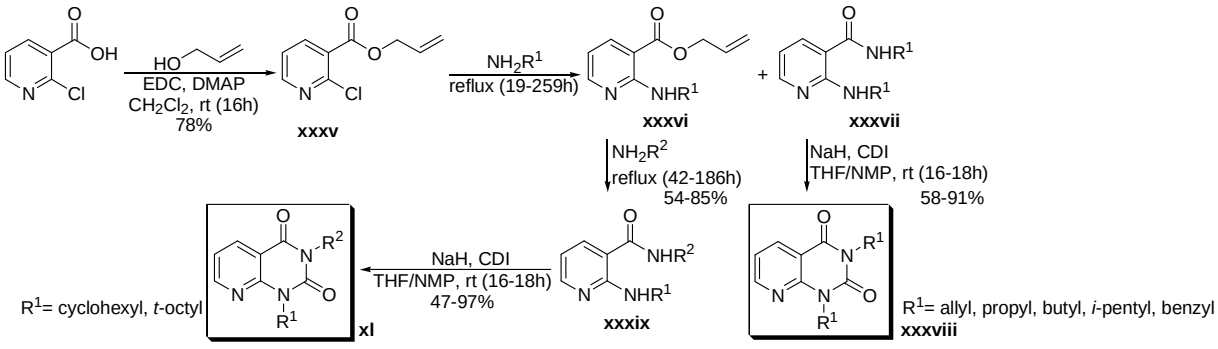


Figure 2

When more diluted conditions (0.86 mM) were applied, the conversion of the reaction decreased to 80% and in the reaction mixture, only products of intermolecular cyclization were present.

The new method for the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**xxxviii** and **xl**) is presented in Scheme 11.

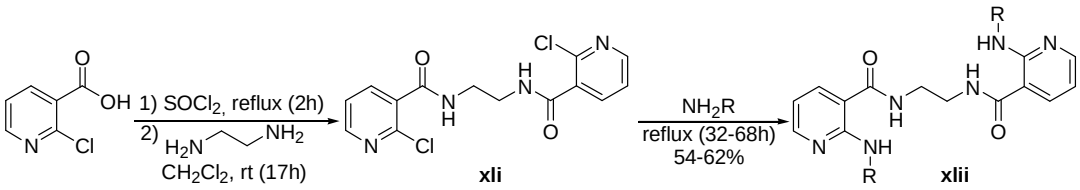


NH_2R^1= allyl, propyl, butyl, *i*-pentyl, cyclopropyl, cyclohexyl, *t*-butyl, *t*-octyl, 1-adamantyl, benzyl
NH_2R^2= allyl, propyl, *i*-pentyl

Scheme 11

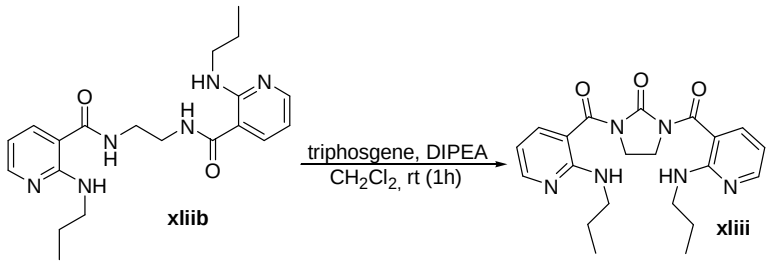
The ester **xxxv**, synthetized from 2-chloropyridine-3-carboxylic acid and allyl alcohol, in the reaction with alkyl/aromatic amines gave a mixture of products **xxxvi** and **xxxvii**, or only **xxxvi** for sterically hinder amines. The selective nucleophilic aromatic substitution with sterically hinder amines at C1 (*t*-octylamine, *t*-butylamine, 1-adamantylamine) is more difficult and needs longer reaction times than with other amines (e. g. allylamine, butylamine, *i*-pentylamine). Amines giving sterically hinder at C2, allow the synthesis of *N*-alkyl 2-(alkylamino)-3-pyridinecarboxamides in good yields. The cyclopropylamine is an exception In the reaction with cyclopropylamine, only compound **xxii** (R^1=cyclopropyl) was obtained. The synthesis of precursor **xxxix** to pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones with different groups attached to nitrogen, required longer reaction times and the yields are lower than in the case of two identical alkyl group.

To the synthesis of dipyrido[2,3-*d*]pyrimidine, the *N*,*N*'-1,2-ethanediyl-bis[2-chloro-3-pyridinecarboxamide] (**xli**) was synthesized from 2-chloropyridine-3-carboxylic acid and ethylenediamine. The obtained compound **xli** reacted with alkylamine to **xlii** (Scheme 12).



Scheme 12

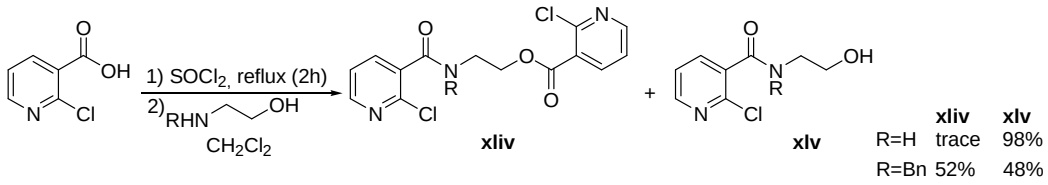
The ring closure was only performed for the propyl derivative. For this reaction, two carbonyl donor reagents were selected, CDI and triphosgene. In the reaction with CDI, only starting material was detected after 17 hours of stirring at room temperature. The reaction of *N*,*N*'-1,2-ethanediyl-bis[2-propylamino-3-pyridinecarboxamide] **xliib** with triphosgene gave unexpectedly the compound **xliii** (Scheme 13). The desired dipyrido[2,3-*d*]pyrimidine was not detected in the reaction mixture.



Scheme 13

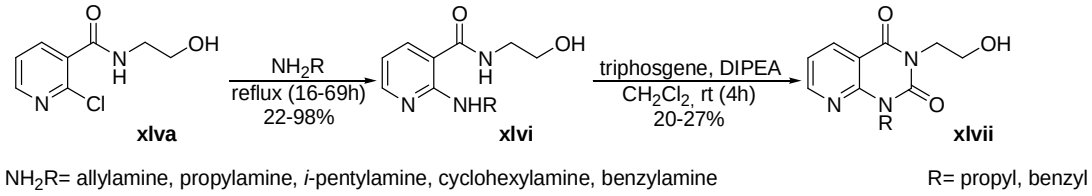
In attempt to synthesize the 3-(alkyl/aromatic)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione, the 2-chloropyridine-3-carboxylic acid was transformed into the acid chloride,

then reacted with ethanolamine or 2-benzylaminoethanol to obtain the mixture of products **xliv** and **xlv** (Scheme 14).



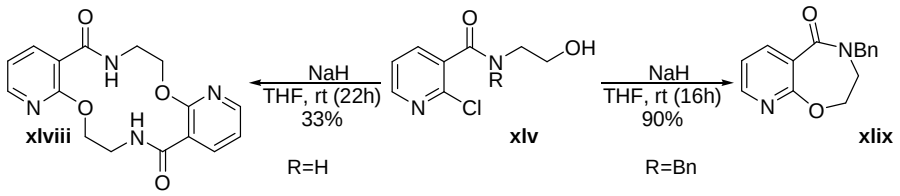
Scheme 14

The 2-chloro-*N*-(2-hydroxyethyl)-nicotinamide **xlva** in reaction with alkyl/aromatic amines gave the 2-alkyl/aromatic-*N*-(2-hydroxyethyl)-nicotinamide **xlvi** in good yields. The ring closure to pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **xlvii** (for the propyl and benzyl derivative), was performed with triphosgene as a carbonyl donor (Scheme 15). The **xlvii** were obtained in low yields, 27%¨and 20% for propyl and benzyl respectively.



Scheme 15

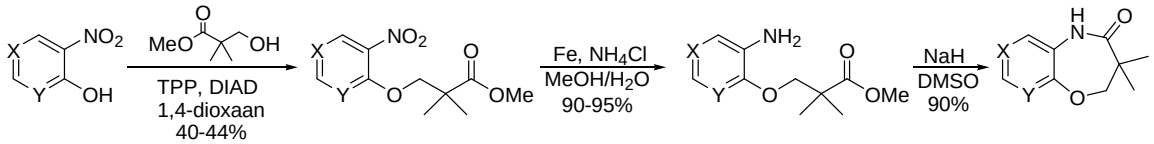
The ring closure reaction of unprotected amide **xlva** led to the intramolecular substitution and resulted into the tricyclic compound **xlviii**, whereas the reaction with the benzyl protected amide **xlvb** led to the intermolecular substitution and the formation of the seven-membered ring fused to pyridine **xlix** (Scheme 16).



Scheme 16

Dutch translation of summary

In deze doctoraatsthesis werd de synthese van pyrido[4,3-*b*][1,4]oxazepine (**iva**) en pyrido[2,3-*b*][1,4]oxazepines (**ivb**) ontwikkeld en succesvol uitgevoerd (Schema 1). In deze synthetische route bleek de grootste uitdaging de koppeling van het hydroxynitropyridine met methyl 3-hydroxy-2,2-dimethylpropanoaat te zijn. Dit resulteerde in de synthese van 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionaat (**iib**) en 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionaat (**iia**), die beiden echter geïsoleerd werden in lage rendementen. Vervolgens werden beide derivaten onderworpen aan een reductie van de nitro-groep door middel van reactie met Fe en NH_4Cl, wat resulteerde in de vorming van de overeenkomstige amino- derivaten in uitstekende rendementen. De daaropvolgende ringsluiting met vorming van de pyridoxazepines verliep voor beide derivaten in een rendement van 90%.



ia: X= N, Y=CH
ib: X=CH, Y= N

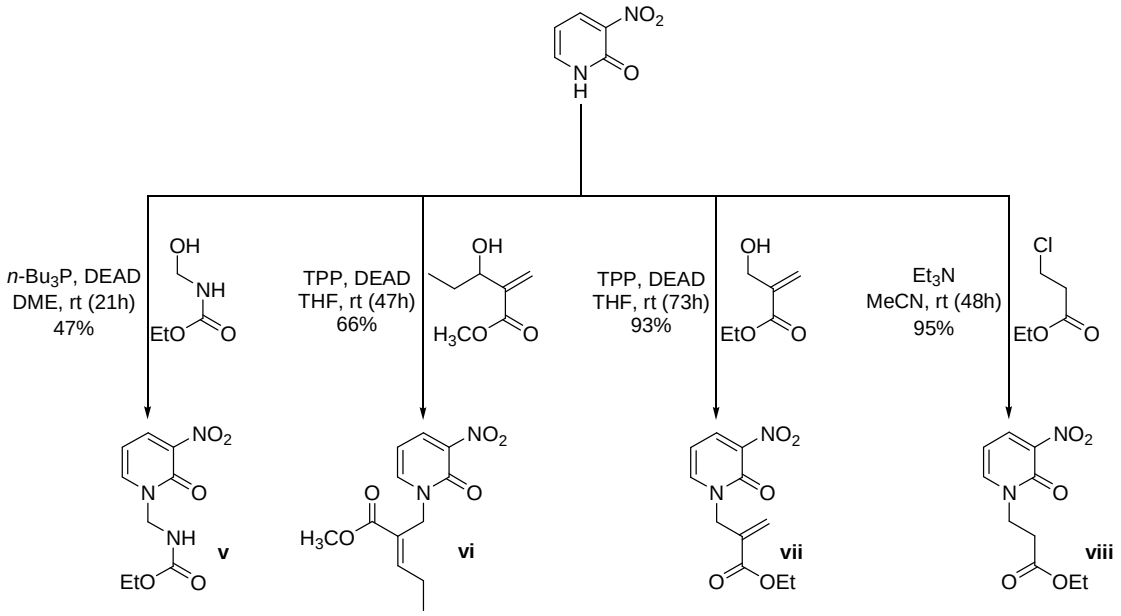
iia: X= N, Y=CH
iib: X=CH, Y= N

iiia: X= N, Y=CH
iiib: X=CH, Y= N

iva: X= N, Y=CH
ivb: X=CH, Y= N

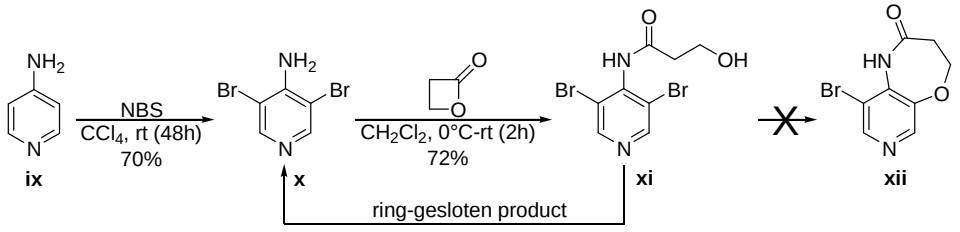
Schema 1

Met het oog op de synthese van een ruime waaier aan pyrido[2,3-*b*][1,4]oxazepines, werden zeven alcoholen en een chloride derivaat geselecteerd voor reactie met het 2-hydroxy-3-nitropyridine startproduct. Omwille van het evenwicht tussen 2-hydroxy-3-nitropyridine en 3-nitro-2(1*H*)-pyridinon, werden er enkel *N*-gesubstitueerde producten bekomen na reactie met drie van de betreffende alcoholen en tevens bij reactie met het chloride (Schema 2). De reacties met de andere alcoholen resulteerden tevens niet in de vorming van de *O*-gealkyleerde noch de *N*-gealkyleerde producten.



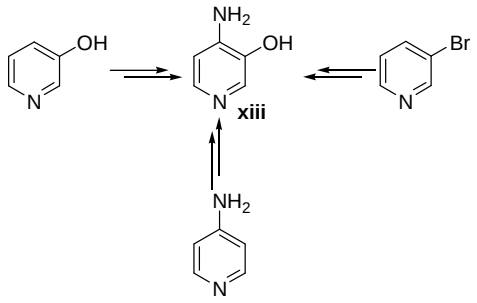
Schema 2

De synthese van de pyrido[3,4-*b*][1,4]oxazepine "scaffold" **xii** werd tevens beoogd in deze doctoraatsthesis. De cyclizatie van het *N*-(3,5-dibroom-pyridin-4-yl)-3-hydroxy-propionamide **xi** via een Buchwald koppeling, gevolgd door deprotonering, leidde echter tot een deamidatie reactie wat resulteerde in de synthese van verbinding **x**.



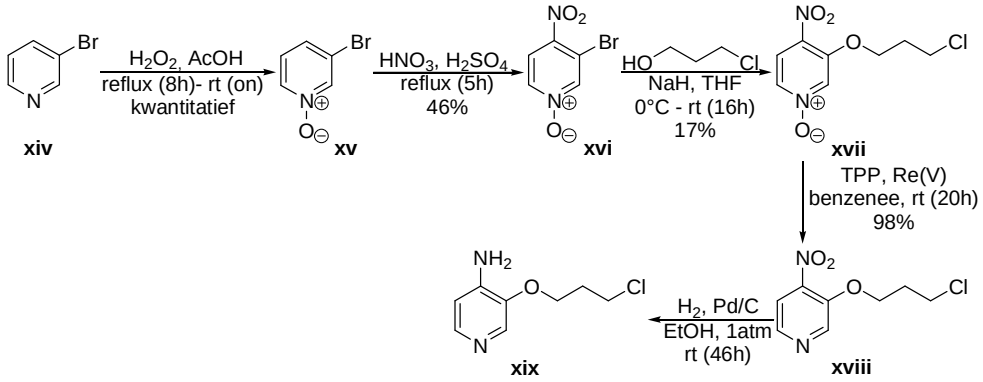
Schema 3

Ondanks het feit dat er verschillende routes zijn uitgeprobeerd voor de synthese van het 4-amino-3-hydroxypyridine startproduct, resulteerde geen enkele van deze pogingen in het gewenste resultaat. Bijgevolg kon de beoogde verbinding **xii** niet gesynthetiseerd worden (Schema 4).



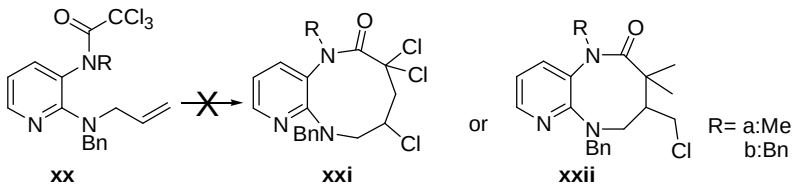
Schema 4

Misschien wel één van de meest veelbelovende “pathway's” voor de synthese van deze “scaffold” ging uit van een nucleofiele substitutie van het Br-atoom in de C3-positie. De isolatie van deze verbinding mislukte echter door de slechte oplosbaarheid van het 3-(3-chloorpropoxy)-pyridin-4-ylamine **xix** in organische solventen.

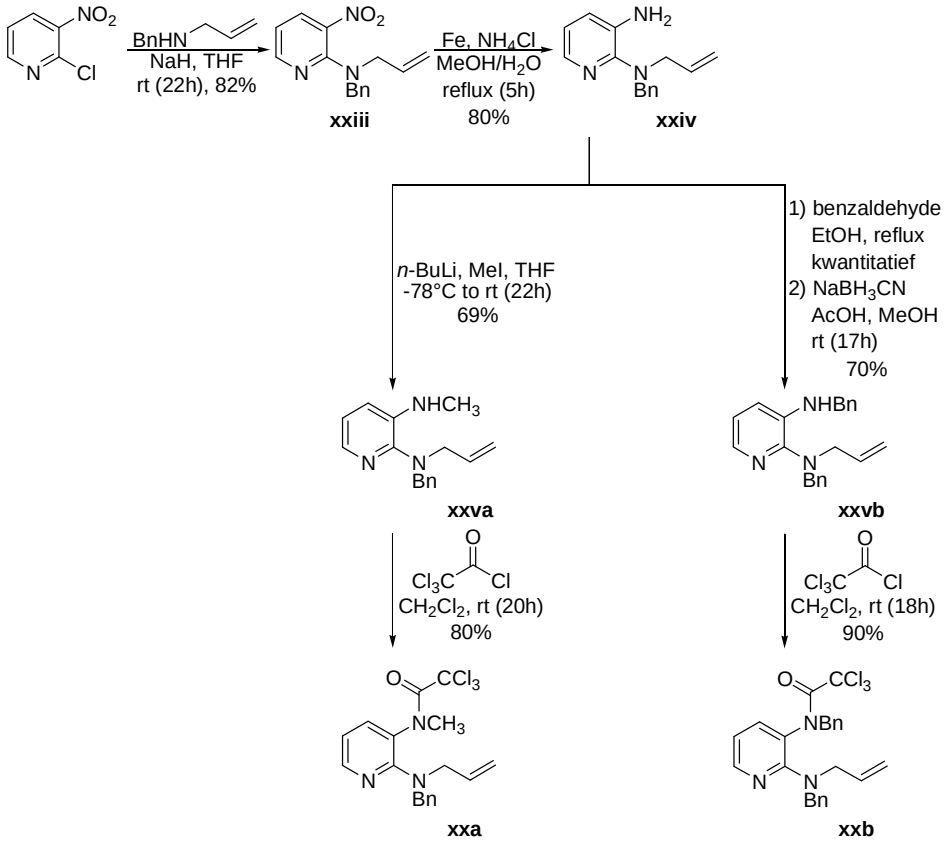


Schema 5

De synthese van het *N*-[2-(*N*-allyl-*N*-methylamino)-pyridin-3-yl]-*N*-benzyl-2,2,2-trichlooracetamide (**xxia**) of het *N*-[2-(*N*-allyl-*N*-benzylamino)-pyridin-3-yl]-*N*-benzyl-2,2,2-trichlooracetamide (**xxib**) werd tevens vooropgesteld. Geen van deze macrocyclische verbindingen kon echter bekomen worden onder de gegeven Kharasch reactie condities (Schema 6). De synthese van de startproducten **xxa** en **xxb** is weergegeven in Schema 7.



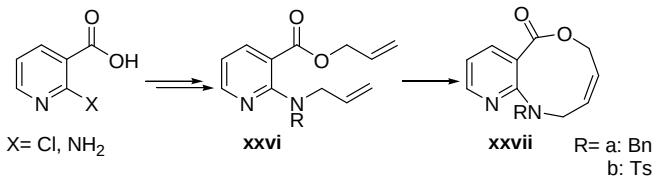
Schema 6



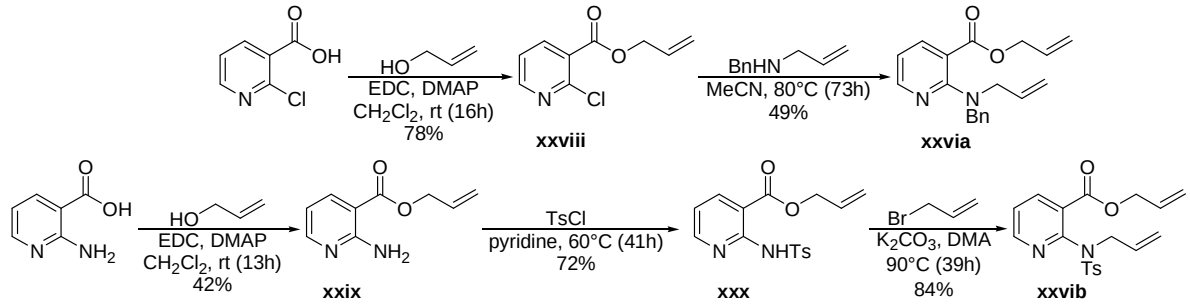
Schema 7

De Kharasch reactie werd uitgevoerd in aanwezigheid van de volgende katalysatoren: CuCl (met of zonder TMEDA/ PMDETA als ligand), CuO en een mengsel van CuCl en CuO voor het benzyl derivaat; en met CuCl, $Ru(PPh_3)_3Cl_2$, de 1ste generatie Grubbs' katalysator en CuCl-bipyridine voor het methyl derivaat. Er werdt tevens een grote verscheidenheid aan reactie condities uitgeprobeerd met verschillende solventen (CH_2Cl_2, DCE en tolueen) en temperaturen (reflux, 80°C en kamertemperatuur).

Met het oog op de synthese van een aan pyridine gefuseerde negenring, werd de metathese reactie van het allyl 2-(*N*-allyl-*N*-benzylamino)-3-pyridinecarboxylaat (**xxvia**) en het allyl 2-(*N*-allyl-*N*-tosylamino)-3-pyridinecarboxylaat (**xxvib**) tevens onderzocht (Schema 8). The synthese van de startverbindingen **xxvi** is weergegeven in Schema 9. Het allyl 2-(*N*-allyl-*N*-benzylamino)-3-pyridinecarboxylaat (**xxvia**) werd samengebracht met verschillende ruthenium reagentia (1ste en 2de generatie Grubbs' katalysatoren, $[RuClH(CO)(PPh_3)]$ of 2de generatie Hoveyda-Grubbs' katalysator) in tolueen of CH_2Cl_2 en bij verschillende temperaturen (kamer temperatuur, reflux of 65°C). Al deze pogingen leidden echter tot het opnieuw bekomen van de startproducten.

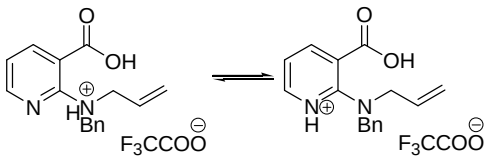


Schema 8



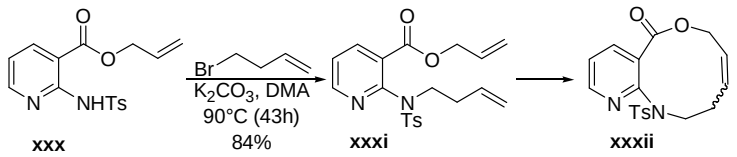
Schema 9

De reactie van verbinding **xxvia** met de 2de generatie Grubbs' katalysator werd tevens uitgevoerd in de aanwezigheid van Lewis-zuren (Ti(O*i*-Pr)$_4$, LiCl, $ZnCl_2$, In(OTf)$_3$, Sc(OTf)$_3$). Hierbij werd enkel bij reactie met Sc(OTf)$_3$ en In(OTf)$_3$ een nieuw product gedetecteerd. Na scheiding en isolatie op een preparative HPLC kolom, bleek deze nieuw component het overeenkomstige trifluorazijnzuur zout van de startverbinding te zijn.^{1}H-NMR analyse bevestigde tevens de aanwezigheid van de gedeallyleerde verbinding (Figuur 1), het 2-(*N*-allyl-*N*-benzylamino)-nicotinezuur of het allyl 2-benzylaminopyridine-3-carboxylaat.



Figuur 1

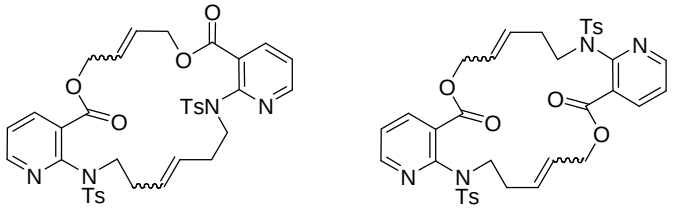
De metathese reactie van het allyl 2-(*N*-allyl-*N*-tosylamino)-3-pyridinecarboxylaat (**xxvib**) met de 2de generatie Grubbs' katalysator, al dan niet in de aanwezigheid van In(OTf)$_3$ of Sc(OTf)$_3$ als Lewis zuur, resulteerde tevens niet in de synthese van de gewenste bicyclische verbinding. Omwille van het optreden van een deallyleringsreactie en de isolatie van de startverbindingen, werd het 2-propeen-1-yl 2-(*N*-(3-buteen-1-yl)-*N*-tosylamino)-3-pyridinecarboxylaat (**xxxi**) gaangemaakt als precursor voor de synthese van een aan pyridine gefuseerde tienring (Schema 10).



xxx **xxxi** **xxxii**

Schema 10

Verbinding **xxxi** werd gereageerd met de 2de generatie Grubbs' katalysator in tolueen bij 80°C en na 5 uur reactie werd er een mengsel van verschillende verbindingen gedetecteerd. Het reactiemengsel werd opgezuiverd via preparatieve HPLC en het overeenkomstige trifluorazijnzuur zout van de startverbinding werd bekomen (trifluorazijnzuur werd toegevoegd aan het elutie mengsel). Onverhoopt, werden onder deze reactie condities de intramoleculaire cyclizatie producten gevormd (Figuur 2). In het reactiemengsel werd de beoogde bicyclische tienring echter niet gedetecteerd.

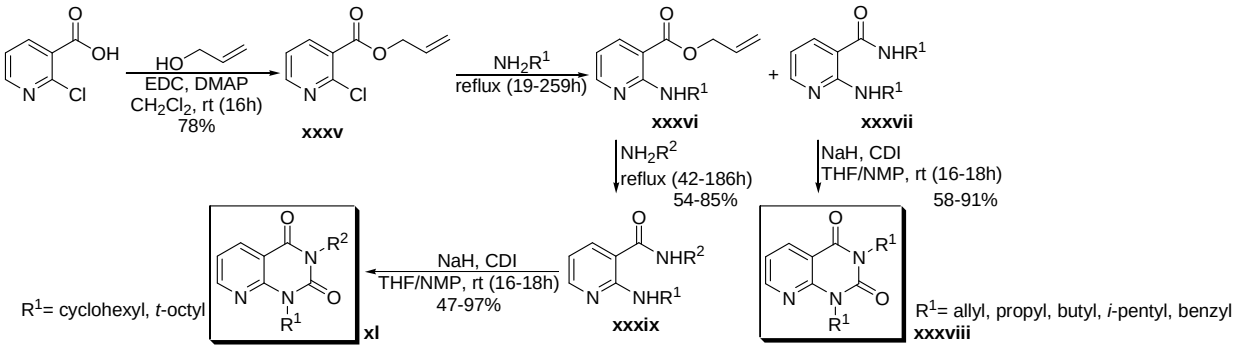


xxxiii **xxxiv**

Figuur 2

Wanneer meer verdunde reactie condities (0.86mM) werden gebruikt, daalde de conversie van de reactie naar 80% en werden er enkel intermoleculaire cyclizatie producten gevormd.

De nieuwe methode voor de synthese van de pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dionen (**xxxviii** en **xl**) is weergegeven in Schema 11.



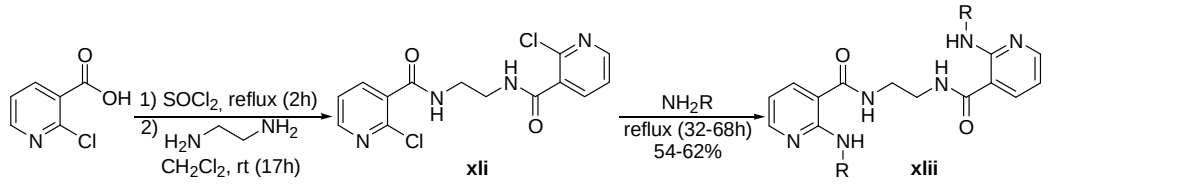
xxxv **xxxvi** **xxxvii** **xxxix** **xl** **xxxviii**

NH_2R^1= allyl, propyl, butyl, *i*-pentyl, cyclopropyl, cyclohexyl, *t*-butyl, *t*-octyl, 1-adamantyl, benzyl
NH_2R^2= allyl, propyl, *i*-pentyl

Schema 11

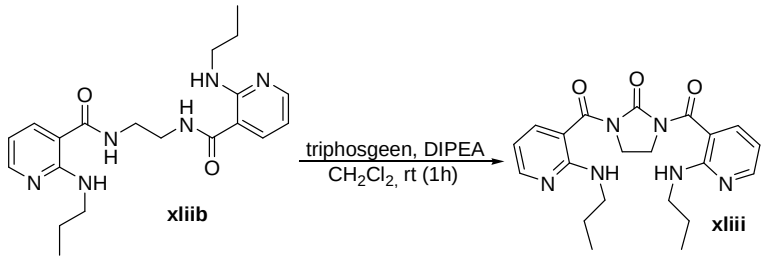
Het ester **xxxv**, gesynthetiseerd door reactie van 2-chloorpyridine-3-carbonzuur en allyl alcohol, werd gereageerd met alkyl- en aryl aminen, wat resulteerde in een mengsel van de verbindingen **xxxvi** en **xxxvii**, of selectief in verbinding **xxxvi** voor meer sterisch gehinderde aminen. Deze selectieve nucleofiele aromatische substitutie op de C1-positie wordt bemoeilijkt met sterisch gehinderde aminen (*t*-octylamine, *t*-butylamine, 1-adamantylamine) en heeft dan ook langere reactie tijden dan bij reactie met andere aminen (bvb allylamine, butylamine, *i*-pentylamine). De resulterende aminen waarvan de C2-positie meer is afgeschermd door sterische hindering, leveren de overeenkomstige *N*-alkyl 2-(alkylamino)-3-pyridinecarboxamiden in goede rendementen. Hierbij is het cyclopropylamine echter een uitzondering, aangezien na reactie enkel verbinding **xxii** (R^1= cyclopropyl) werd bekomen. De synthesis van pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dionen, uitgaande van precursor **xxxix**, met twee verschillende groepen op het *N*-atoom, vereisten langere reactie tijden en resulteerden in lagere rendementen, in vergelijking met de aminen met twee identieke groepen op het *N*-atoom.

Met het oog op synthese van het dipyrido[2,3-*d*]pyrimidine, werd het 2-chloorpyridine-3-carbonzuur gereageerd met ethyleendiamine, wat resulteerde in de vorming van *N*,*N*'-1,2-ethaandiyl-bis[2-chloor-3-pyridinecarboxamide] (**xli**). De bekomen verbinding **xli** onderging vervolgens reactie met een alkylamine, wat leidde tot de vorming van verbindingen **xlii** (Schema 12).



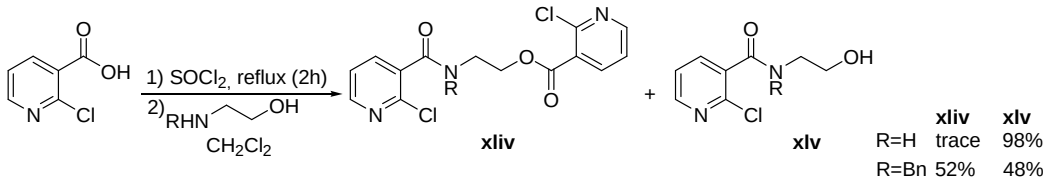
Schema 12

De daaropvolgende ringsluiting werd enkel uitgevoerd op het bekomen propyl derivaat. Met het oog op deze reactie werden twee carbonyl doneerende reagentia geselecteerd, nl. CDI and trifosgeen. Bij reactie met CDI werd na 17 uur roeren bij kamer temperatuur enkel het start product gedetecteerd. De reaction van *N*,*N*'-1,2-ethaandiyl-bis[2-propylamino-3-pyridinecarboxamide] **xliib** met trifosgeen resulteerde echter onverwacht in de synthese van verbinding **xliii** (Schema 13). Hierbij was het gewenste dipyrido[2,3-*d*]pyrimidine niet aanwezig in het reactiemengsel.



Schema 13

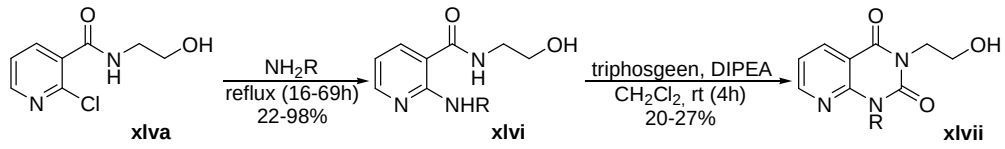
Met het oog op de synthese van de 3-(alkyl/aryl)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dionen, werd het 2-chloorpyridine-3-carbonzuur getransformeerd in het overeenkomstige zuurchloride en vervolgens gereageerd met ethanolamine of 2-benzylaminoethanol, wat resulteerde in een mengsel van de verbindingen **xliv** en **xlv** (Schema 14).



	xliv	xlv
R=H	trace	98%
R=Bn	52%	48%

Schema 14

Bij reactie van het 2-chloor-*N*-(2-hydroxyethyl)-nicotinamide **xlva** met alkyl- en aryl aminen werden de overeenkomstige 2-alkyl/aryl-*N*-(2-hydroxyethyl)-nicotinamiden **xlvi** in goede rendementen bekomen. De daaropvolgende ringsluiting tot de pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dionen **xlvii** (voor de propyl en benzyl derivaten) werd uitgevoerd met trifosgeen als carbonyl donor (Schema 15). De resulterende verbindingen **xlvii** werden echter bekomen in lage rendementen (20 - 27%).

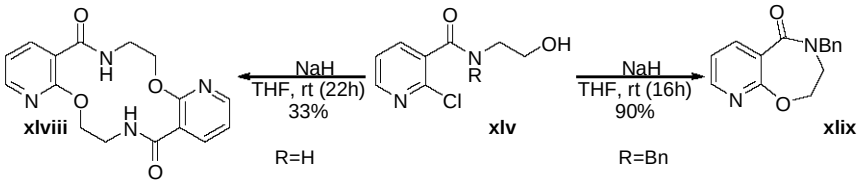


NH_2R= allylamine, propylamine, *i*-pentylamine, cyclohexylamine, benzylamine

R= propyl, benzyl

Schema 15

De ringsluitingsreactie van het vrije amide **xlva** leidde tot een intramoleculaire substitutie die resulteerde in de vorming van de tricyclische verbinding **xlviii**. Wanneer het benzyl beschermde amide **xlvb** echter werd onderworpen aan een intramoleculaire substitutie, resulteerde dit in de vorming van een aan pyridine gefuseerde zevenring **xlix** (Schema 16).



Schema 16

7. Experimental part

7.1. Reagents and equipment

All the reagents were commercially available and used without further purification. Tetrahydrofuran (THF) was distilled from sodium, dichloromethane (CH_2Cl_2) was distilled from calcium hydride and immediately prior to use. TLC (thin-layer chromatography) was carried out on glass plates coated with silica gel (Merck, Kiesegel 60F254, precoated 0.25 mm). ^{1}H (300 MHz/ 400 MHz) and ^{13}C (75 MHz/ 100.6 MHz) NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 as solvent, with a Jeol Eclipse FT spectrometer or a Bruker Avance III Nanobay 400 MHz spectrometer at room temperature. Low-resolution mass spectra were recorded using a direct inlet system with an Agilent 1100 series LC/MSD type SL with a UV detector and mass spectrometer with Electrospray Ionisation Geometry (ESI 70 eV) using a quadrupole detector. IR spectra were recorded with a Perkin–Elmer Spectrum One FTIR spectrometer with an ATR (Attenuated Total Reflectance) accessory in neat form. Melting points were measured using Kofler bench, type WME Heizbank of Wagner & Munz.

7.2. Procedures and spectra

7.2.1. Synthesis of pyrido[2,3-b][1,4]oxazepines and pyrido[3,4-b][1,4]oxazepines scaffold

7.2.1.1. Synthesis of methyl 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionate **111** and methyl 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate **148**

1 Procedure based on WO 2007067416 (**2007**)

To a cold mixture of 2-hydroxy-3-nitropyridine/ 4-hydroxy-3-nitropyridine (1eq), TPP (1.2eq) and methyl 3-hydroxy-2,2-dimethylpropionate (1.1eq) in 1,4-dioxane under nitrogen atmosphere, DIAD (1.2eq) was added dropwise over 5 min. The ice-bath was removed and the reaction mixture was stirred at room temperature for 4h and then overnight at reflux. The solvent was evaporated. The residue was dissolved in EtOAc, washed with water, dried over $MgSO_4$, filtrated and concentrated. The desired product was isolated by column chromatography, using EtOAc as eluent.

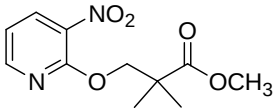
2 Procedure based on WO 2006025717 (**2006**)

To a suspension of 2-chloro-3-nitropyridine (1eq), methyl 3-hydroxy-2,2-dimethylpropionate (1.1eq), K_2CO_3 (1eq) and KOH (1eq) in dry toluene, TDA-1 (0.1eq) was added. The reaction mixture was stirred at room temperature for 1h then filtrate through celit pad, washed with toluene and methanol. The filtrate was concentrated to give 70% yield of methyl 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionate as orange oil.

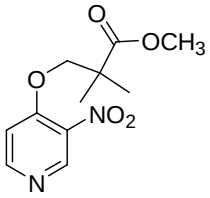
To a suspension of 4-chloro-3-nitropyridine (1eq), methyl 3-hydroxy-2,2-dimethylpropionate (1.1eq), K_2CO_3 (1eq) and KOH (1eq) in dry toluene, TDA-1 (0.1eq) was added. The reaction mixture was stirred at room temperature for 20h then filtrate through celit pad, washed with toluene and methanol. The filtrate was concentrated and the product was isolated by column chromatography using EtOAc as eluent in 21% yield of methyl 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate as yellow oil.

3 Procedure based on WO 2010116270 (**2010**)

To a methyl 3-hydroxy-2,2-dimethylpropionate (0.9eq) in dry THF, 1M LiHMDS in THF (1eq) was slowly added. After 5 min stirring at room temperature, a solution of 2-chloro-3-nitropyridine (1eq) in DMF was added. The reaction mixture was stirred at room temperature for 24h. After this time, the reaction mixture was quenched with saturated solution of NH_4Cl and extracted with EtOAc (4x). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated. The methyl 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionate was isolated by column chromatography using EtOAc as eluent in 50% yield.



Methyl 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionate (111);
40% yield; orange oil; IR: 1729 (C(O)O), 1602, 1570 (C=C), 1526 (N-O, NO_2), 1436 (C-H), 1348 (N-O, NO_2), 1305 (C-O-CH_2), 1248, 1222 (C(O)O); ^{1}H NMR ($CDCl_3$): 1.38 (s, 6H, 2xCH_3), 3.73 (s, 3H, O-CH_3), 4.19 (s, 2H, CH_2), 7.05 (d, *J*= 6.05 Hz, 1H, py), 8.63 (d, *J*= 5.50 Hz, 1H, py), 9.03 (s, 1H, py); ^{13}C ($CDCl_3$): δ22.33, 43.28, 52.46, 75.56, 109.33, 136.41, 147.24, 154.86, 158.25, 175.72; MS: m/z (%)= 255 [M+ 1]$^+$ (100), 256 (11); HRMS calcd. for $C_{11}H_{14}N_2O_5$ [M + 1]$^+$ 255.0975; found 255.0983.



Methyl 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate (148);
44% yield; yellow oil; IR: 1716 (C(O)O), 1600, 1562 (C=C), 1521 (N-O, NO_2), 1474 (C-H), 1354 (N-O, NO_2), 1310 (C-O-CH_2), 1286, 1270, 1242 (C(O)O); ^{1}H NMR ($CDCl_3$): 1.36 (s, 6H, 2xCH_3), 3.71 (s, 3H, O-CH_3), 4.50 (s, 2H, CH_2), 7.06 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 8.28 (dd, *J*= 2.20, 9.91 Hz, 1H, py), 8.38 (dd, *J*= 2.20, 7.15 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 22.48, 43.07, 52.19, 73.45, 116.73, 135.13, 151.67, 156.26, 176.26; MS: m/z (%)= 255 [M+ 1]$^+$ (100), 256 (18); HRMS calcd. for $C_{11}H_{14}N_2O_5$ [M + 1]$^+$ 255.0975; found 255.0986.

7.2.1.2. Synthesis of methyl 2,2-dimethyl-3-(3-amino-pyridin-2-yloxy)-propionate **112** and methyl 2,2-dimethyl-3-(3-amino-pyridin-4-yloxy)-propionate **149**

1 Procedure based on *Bull. Korean Chem. Soc.* **2008**, *29*, 2331-2336

The mixture of methyl 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate (1eq) and $SnCl_2$ (5eq) in ethanol was refluxed for 16h. The reaction mixture was cooled to room temperature and diluted with 10% $NaHCO_3$ and extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The desired product **149** in 80% yield.

2 Procedure based on WO 2005030213 (**2005**)

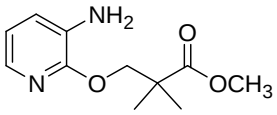
The solution of methyl 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate (1eq) in acetic acid was heated at 80°C with iron powder (5eq) overnight. Excess of acetic acid was removed; the residue was taken up in 20%$_{aq}$ NaOH solution and chloroform. The mixture was filtered through celit pad. The aqueous phase was extracted with chloroform (3x). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under vacuum. The desired product **149** was obtained in 80% yield.

3 Procedure based on Vogel´s Textbook of Practical Organic Chemistry, III Edition (Polish), WNT Warszawa, **2006**, pp 859-860

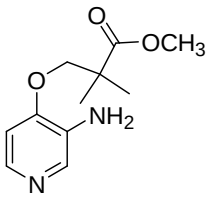
To a suspension of Pd/C in H_2O was added $NaBH_4$ (2eq) in H_2O. This mixture was stirred for 2 min and then methyl 2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate (1eq) in THF/H_2O was added. The reaction mixture was stirred at room temperature for 10 min. The reaction mixture was filtered and the filtrate was acidified with diluted HCl and then neutralized with diluted solution of NaOH in water to pH~7, extracted with Et_2O (4x). The organic phases were combined and dried over $MgSO_4$, filtrate and concentrated. The residue was washed with MeCN, organic phase was concentrated, gave the desired product **149** in 40% yield.

4 Procedure based on WO 2008046216 (**2008**)

The suspension of methyl 2,2-dimethyl-3-(3-nitro-pyridin-2-yloxy)-propionate/2,2-dimethyl-3-(3-nitro-pyridin-4-yloxy)-propionate (1eq), iron powder (4eq) and NH_4Cl (1.5eq) in the mixture of methanol and H_2O (5:1) was refluxed for 5h. The reaction mixture was cooled to room temperature and then filtrated through celit pad. The cake was washed with methanol and acetonitrile. The filtrate was concentrated under vacuum. To the residue chloroform was added and solid was removed by filtration. The solvent was removed under vacuum, gave desired product in 90% (methyl 2,2-dimethyl-3-(3-amino-pyridin-2-yloxy)-propionate) and 95% (methyl 2,2-dimethyl-3-(3-amino-pyridin-4-yloxy)-propionate) yield.



Methyl 2,2-dimethyl-3-(3-amino-pyridin-2-yloxy)-propionate (112);
dark red oil; IR: 3371 (NH_2), 1725 (C(O)O), 1614, 1594 (C=C), 1452 (C-H), 1243, 1150, 1023 (C-H); ^{1}H NMR ($CDCl_3$): 1.33 (s, 6H, 2xCH_3), 3.69 (s, 3H, O-CH_3), 4.36 (s, 2H, CH_2), 6.72 (dd, *J*= 7.44, 5.00 Hz, 1H, py), 6.87 (dd, *J*= 7.48, 1.36 Hz, 1H, py), 7.54 (dd, *J*= 6.24, 3.68 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 22.73, 43.20, 52.46, 72.38, 117.71, 120.69, 130.99, 135.12, 152.46, 176.75; MS: m/z (%)= 225 $[M+ 1]^+$ (100), 226 (12); HRMS calcd. for $C_{11}H_{16}N_2O_3$ $[M + 1]^+$ 225.1234; found 225.1233.



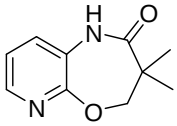
Methyl 2,2-dimethyl-3-(3-amino-pyridin-4-yloxy)-propionate (149);
light pink solid; mp. 184°C; IR: 3364, 3278 (NH_2), 1729 (C(O)O), 1631, 1557, 1508 (C=C), 1313 (=C-O-CH_2), 1261 (C-NH_2), 1149 (C(O)O)1286; ^{1}H NMR ($CDCl_3$): 1.38 (s, 6H, 2xCH_3), 3.73 (s, 3H, O-CH_3), 4.20 (s, 2H, CH_2), 5.26 (2H, NH_2), 7.00 (d, *J*= 6.05 Hz, 1H, py), 7.92 (d, *J*= 6.05 Hz, 1H, py), 8.48 (s, 1H, py);

^{13}C ($CDCl_3$): δ 22.42, 43.19, 52.62, 76.73, 106.99, 124.84, 130.61, 137.79, 156.11, 175.39; MS: m/z (%)= 225 $[M+ 1]^+$ (100), 226 (13); HRMS calcd. for $C_{11}H_{16}N_2O_3$ $[M + 1]^+$ 225.1234; found 225.1238.

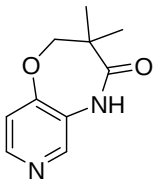
7.2.1.3.Synthesis of 3,3-dimethyl-2,3-dihydropyrido[2,3-*b*][1,4]oxazepin-4(5*H*)one **113** and 3,3-dimethyl-2,3-dihydropyrido[4,3-*b*][1,4]oxazepin-4(5*H*)one **150**

Procedure based on WO 2008009122 (**2008**)

To a suspension of NaH (1.2eq) in DMSO, methyl 2,2-dimethyl-3-(3-amino-pyridin-4-yloxy)-propionate/methyl 2,2-dimethyl-3-(3-amino-pyridin-2-yloxy)-propionate (1eq) was added in one portion. The reaction mixture was stirred at room temperature for 22h. After this time H_2O and Et_2O were added. The water phase was extracted with Et_2O (3x). The combined organic phases were washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated, gave the desired product.



3,3-Dimethyl-2,3-dihydropyrido[2,3-*b*][1,4]oxazepin-4(5*H*)one (113);
90% yield; white solid; mp. 222 °C; IR: 3209 (NH), 1660 (C(O)N), 1591 (C=C), 1474, 1450, 1431, 1409 (C-H), 1362 (-NH-pyridine), 1281, 1258, 1241 (C-H); ^1H NMR ($CDCl_3$): 1.36 (s, 6H, 2xCH_3), 4.15 (s, 2H, CH_2), 7.00 (dd, *J*= 7.71, 4.40 Hz, 1H, py), 7.24 (dd, *J*= 7.98, 1.65 Hz, 1H, py), 7.99 (dd, *J*= 4.95, 1.65 Hz, 1H, py), 8.05 (br, NH); ^{13}C ($CDCl_3$): δ 22.92, 44.09, 72.83, 119.23, 122.71, 128.84, 142.35, 153.75, 178.35; MS: m/z (%)= 193 $[M+ 1]^+$ (100), 194 (12); HRMS calcd. for $C_{10}H_{12}N_2O_2$ $[M + 1]^+$ 193.0972; found 193.0974.



3,3-Dimethyl-2,3-dihydropyrido[4,3-*b*][1,4]oxazepin-4(5*H*)one (150);
90% yield; white solid; mp. 192 °C; IR: 3206 (NH), 1656 (C(O)N), 1582, 1496, 1413, 1398 (C=C), 1362 (-NH-pyridine); ^1H NMR ($CDCl_3$): 1.32 (s, 6H, 2xCH_3), 4.08 (s, 2H, CH_2), 6.89 (d, *J*= 4.95 Hz, 1H, py), 8.13 (d, *J*= 5.50 Hz, 1H, py), 8.29 (s, 1H, py), 8.91 (br, NH); ^{13}C ($CDCl_3$): δ 22.38, 44.67, 73.08, 114.94, 123.77, 142.81, 145.18, 153.17, 178.87; MS: m/z (%)= 193 $[M+ 1]^+$ (100), 194 (15); HRMS calcd. for $C_{10}H_{12}N_2O_2$ $[M + 1]^+$ 193.0972; found 193.0975.

7.2.2. Synthesis of N-alkylated pyridinone

7.2.2.1. Synthesis of ethyl (3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-carbamate **124**

1 Procedure based on WO2007067416 (**2007**)

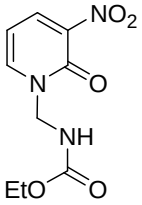
A mixture of 2-hydroxy-3-nitropyridine (0.0029mol, 1eq), ethyl (hydroxymethyl)carbamate (0.0029mol, 1eq) and TPP (0.0031mol, 1.1eq) in 1,4-dioxane (75 ml) under nitrogen atmosphere was stirred at 0°C for 5 min, then DIAD (0.0031mol, 1.1eq) was added. The reaction mixture was stirred at room temperature for 4h and then at reflux overnight. The solvent was removed under vacuum and product was isolated by column chromatography using EtOAc as eluent in 10% yield (0.070g).

2 Procedure based on *Tetrahedron* **2000**, *56*, 9121-9128

To a cold solution of 2-hydroxy-3-nitropyridine (0.0029mol, 1eq), ethyl (hydroxymethyl)carbamate (0.0029mol, 1eq) and *n*-Bu_3P (0.0031mol, 1.1eq) in dry THF (75 ml) under nitrogen atmosphere, DEAD (0.0031mol, 1.1eq) was added. The reaction mixture was stirred at 40°C for 20h. The solvent was removed under vacuum and product was isolated by column chromatography using EtOAc as eluent in 40% yield (0.279g).

3 Procedure based on *Tetrahedron Lett.* **1994**, *35*, 2819-2822

To the mixture of 2-hydroxy-3-nitropyridine (0.0007mol, 1eq), ethyl (hydroxymethyl)carbamate (0.0009mol, 1.25eq) and *n*-Bu_3P (0.0011mol, 1.5eq) in 10 ml of DME under nitrogen atmosphere, DEAD (0.0011mol, 1.5eq) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 21h. To the mixture MeOH (1ml) and H_2O (5ml) were added, and the mixture was extracted with CH_2Cl_2 (3x). The combined organic phases were washed with H_2O and brine, dried over $MgSO_4$, filtrated and concentrated. Desired product was separated by column chromatography using EtOAc as eluent, as an orange solid in 47% yield (0.079g).



Ethyl (3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-carbamate (124);

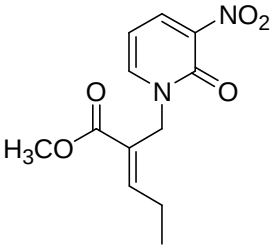
orange solid; mp. 128°C; IR: 3406 (NH), 1709 (C=O), 1680, 1596, 1502 (C=C), 1472, 1378 (C-H aliphatic), 1311 (C(O)O), 1239 (C-N); ^{1}H NMR ($CDCl_3$): 1.24 (t, *J*= 7.12 Hz, 3H, CH_3), 4.12 (q, *J*= 7.12 Hz, 2H, CH_2), 5.32 (d, *J*= 7.04 Hz, 2H, CH_2), 6.34 (dd, *J*= 14.28, 6.64 Hz, 1H, py), 6.35 (br, NH), 8.07 (d, *J*= 6.44 Hz, 1H, py), 8.38 (dd, *J*= 7.70, 2.10 Hz, 1H, py), 8.91 (br, NH); ^{13}C ($CDCl_3$): δ 14.37, 57.51, 61.89, 103.27, 138.79, 139.75, 145.08, 154.89, 156.75; MS: m/z (%)= 242 $[M+ 1]^+$ (100), 243 (12); HRMS calcd. for $C_9H_{11}N_3NaO_5$ $[M + 1]^+$ 264.0591; found 264.0594.

7.2.2.2. Synthesis of methyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-pent-2-enoate **128**

1 Procedure based on WO 2007067416 (**2007**)

A mixture of 2-hydroxy-3-nitropyridine (0.0014mol, 1eq), methyl 3-hydroxy-2-methylenepentanoate (0.0016mol, 1.1eq) and TPP (0.0017mol, 1.2eq) in 1,4-dioxane (50 ml) under nitrogen atmosphere was stirred at 0°C for 5 min. Then DIAD (0.0017mol, 1.2eq) was added and the reaction mixture was stirred at room temperature for 4h and then at reflux overnight. The solvent was removed under vacuum and the desired product was isolated by column chromatography using EtOAc as eluent. The methyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-pent-2-enoate was obtained in 55% yield (0.234g) as yellow oil.

2 A mixture of 2-hydroxy-3-nitropyridine (0.0014mol, 1eq), methyl 3-hydroxy-2-methylenepentanoate (0.0016mol, 1.1eq) and TPP (0.0017mol, 1.2eq) in THF (50 ml) under nitrogen atmosphere was stirred at 0°C for 5 min. Then DIAD (0.0017mol, 1.2eq) was added and the reaction mixture was stirred at room temperature for 47h. The solvent was removed under vacuum and the desired product was isolated by column chromatography using EtOAc as eluent. The methyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-pent-2-enoate was obtained in 66% yield (0.281g).



Methyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-pent-2-enoate (128); orange oil; IR: 1705 (C(O)), 1675 (C=C), 1537, 1518 (NO_2), 1311 (C-N), 1223 (C-N); ^{1}H NMR ($CDCl_3$): 1.14 (t, *J*= 7.52 Hz, 3H, CH_3), 2.63 (q, *J*= 7.57 Hz, 2H, CH_2), 3.74 (s, 3H, O-$\underline{CH_3}$), 4.88 (s, 2H, CH_2=), 6.26 (dd, *J*= 7.64, 6.72 Hz, 1H, N-CH), 7.16 (t, *J*= 7.56 Hz, 1H, py), 8.07 (dd, *J*= 6.70, 2.14 Hz, 1H, py), 8.29 (dd, *J*= 7.66, 2.14 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 13.14, 21.95, 22.75, 23.65, 46.72, 51.79, 52.12, 102.73, 123.84, 138.52, 138.73, 145.98, 153.71, 154.49, 167.15; MS: m/z (%)= 267 $[M+ 1]^+$ (100), 268 (13); HRMS calcd. for $C_{12}H_{15}N_2O_5$ $[M + 1]^+$ 267.0970; found 267.0975.

7.2.2.3. Synthesis of ethyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-acrylate **138**

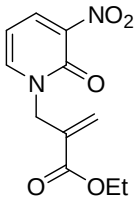
1 Procedure based on *Tetrahedron Lett.* **1994**, *35*, 2819-2822

To the mixture of 2-hydroxy-3-nitropyridine (0.0029mol, 1eq), ethyl 2-(hydroxymethyl)acrylate (0.0035mol, 1.25eq) and TPP (0.0043mol, 1.5eq) in 10 ml of DME (80 ml) under nitrogen atmosphere DIAD (0.0043mol, 1.5eq) was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 22h. The solvent was removed. Desired product was separated by column chromatography using first mixture of EtOAc and PE (1:3) and then EtOAc as eluent. The ethyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-acrylate was obtained in 44% (0.322g) yield as a yellow oil.

2 Procedure based on WO 2007067416 (**2007**)

A mixture of 2-hydroxy-3-nitropyridine (0.0029mol, 1eq), ethyl 2-(hydroxymethyl)acrylate (0.0031mol, 1.1eq) and TPP (0.0034mol, 1.2eq) in 1,4-dioxane (80 ml) under nitrogen atmosphere was stirred at 0°C for 5 min. Then DIAD (0.0034mol, 1.2eq) was added and the reaction mixture was stirred at room temperature for 4h and then at reflux for 17h. The solvent was removed under vacuum and the desired product was isolated by column chromatography using first mixture of EtOAc and PE (1:3) and then EtOAc as eluent. The ethyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-acrylate was obtained in 85% yield (0.621g) as yellow oil.

3 A mixture of 2-hydroxy-3-nitropyridine (0.0029mol, 1eq), ethyl 2-(hydroxymethyl)acrylate (0.0031mol, 1.1eq) and TPP (0.0034mol, 1.2eq) in THF (80 ml) under nitrogen atmosphere was stirred at 0°C for 5 min. Then DIAD (0.0034mol, 1.2eq) was added and the reaction mixture was stirred at room temperature for 73h. The solvent was removed under vacuum and the desired product was isolated by column chromatography using first mixture of EtOAc and PE (1:3) and then EtOAc as eluent. The ethyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-acrylate was obtained in 93% yield (0.680g).



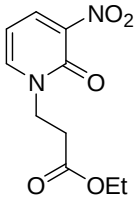
Ethyl 2-(3-nitro-2-oxo-2*H*-pyridin-1-ylmethyl)-acrylate (138);

yellow oil; IR: 1708 (C(O)O), 1673 (C(O)), 1601 (C-C), 1535 (NO_2), 1514 (C-C), 1344 (NO_2), 1297 (C(O)O); ^{1}H NMR ($CDCl_3$): 1.30 (t, *J*= 7.12 Hz, 3H, CH_3), 4.22 (q, *J*= 7.12 Hz, 2H, $\underline{CH_2}$-CH_3), 4.86 (d, *J*= 0.76 Hz, 2H, N-$\underline{CH_2}$), 6.24 (d, *J*= 0.68 Hz, 1H, =$\underline{CH_2}$), 6.30 (dd, *J*= 7.66, 6.70 Hz, 1H, py), 6.54 (d, *J*= 0.72 Hz, 1H, =CH_2), 8.01 (dd, *J*= 6.68, 2.09 Hz, 1H, py), 8.33 (dd, *J*= 7.68, 2.12 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 14.10, 51.22, 61.47, 102.97, 132.74, 133.08, 138.75, 138.89, 145.61, 154.50, 165.72; MS: m/z (%)= 253 $[M+ 1]^+$ (100), 254 (12); HRMS calcd. for $C_{11}H_{13}N_2O_5$ $[M + 1]^+$ 253.0814; found 253.0815.

7.2.2.4. Synthesis of ethyl 3-(3-nitro-2-oxo-2*H*-pyridin-1-yl)-propionate **142**

Procedure based on *Tetrahedron* **2009**, *65*, 7403-7407

To the mixture of 2-hydroxy-3-nitropyridine (0.0014mol, 1eq) and Et_3N (0.0071mol, 5eq) in MeCN (40 ml), ethyl 3-chloropropionate (0.0057mol, 4eq) was added and the reaction mixture was stirred at room temperature for 48h. The precipitate was removed by filtration and washed with MeCN, filtrate was concentrated under vacuum. The desired product was isolated by column chromatography using EtOAc as eluent in 95% yield (0.333g).



Ethyl 3-(3-nitro-2-oxo-2*H*-pyridin-1-yl)-propionate (142);

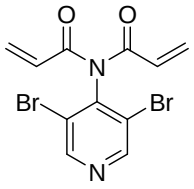
yellow oil; IR: 1722 (C(O)O), 1692 (C(O)), 1599 (C-C), 1533 (NO_2), 1505 (C-C), 1343 (NO_2), 1302 (C-N); ^{1}H NMR ($CDCl_3$): 1.24 (t, *J*= 7.15 Hz, 3H, CH_3), 2.93 (dd, *J*= 11.56, 5.50 Hz, 2H, CH_2) 4.13 (q, *J*= 7.15 Hz, 2H, $\underline{CH_2}$-CH_3), 4.31 (dd, *J*= 11.56, 5.50 Hz, 2H, CH_2), 6.29 (dd, *J*= 14.31, 6.60 Hz, 1H, py), 7.95 (dd, *J*= 8.81, 2.20 Hz, 1H, py), 8.34 (dd, *J*= 9.91, 2.20 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 14.18, 32.27, 47.79, 61.22, 103.08, 139.12, 146.16, 154.49, 171.47; MS: m/z (%)= 241 $[M+ 1]^+$ (100), 242 (15); HRMS calcd. for $C_{10}H_{13}N_2O_5$ $[M + 1]^+$ 241.0814; found 241.0829.

7.2.3. Synthesis of intermediates to the pyrido[3,4-b][1,4]oxazepines

7.2.3.1. Synthesis of *N*-acryloyl-*N*-(3,5-dibromopyridin-4-yl)-acrylamide **152**

Procedure based on *J. Pharm. Biomed. Anal.* **2000**, *53*, 179-185

To a cold solution of 4-amino-3,5-dibromopyridine (0.5g, 0.0019mol) in CH_2Cl_2 (70 ml) under nitrogen atmosphere, Et_3N (0.856g, 0.0085mol) was added. Then a solution of acryloyl chloride (0.256g, 0.0028mol) in CH_2Cl_2 (10 ml) was added dropwise. The reaction mixture was stirred at room temperature for 19h. After this time saturated solution of $NaHCO_{3aq}$ was added to pH~8, the organic layer was separated and the water layer was extracted with CH_2Cl_2 (2x), the combined organic layers were washed with H_2O, dried over $MgSO_4$, filtered and concentrated. The residue was crystallized from the mixture hexane/EtOH (5:1). The *N*-acryloyl-*N*-(3,5-dibromo-pyridin-4-yl)-acrylamide was obtained in 50% yield (0.357g).

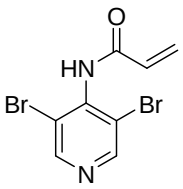


***N*-acryloyl-*N*-(3,5-dibromopyridin-4-yl)-acrylamide (152)**;

mp. 119.7-121.8°C; IR: 1702 (C(O)N), 1690, 1399 (C=C), 1212 (C-O); ^{1}H NMR ($CDCl_3$): 5.92 (dd, *J*= 10.32, 0.88 Hz, 1H, =$\underline{CH_2}$), 6.33 (dd, *J*= 17.00, 10.32 Hz, 1H, =$\underline{CH_2}$), 6.52 (dd, *J*= 17.00, 1.00 Hz, 1H,-$\underline{CH}$=CH_2), 8.69 (s, 2H, py); ^{13}C ($CDCl_3$): δ 119.91, 129.37, 129.97, 142.08, 151.39, 162.43. MS: m/z (%)= 360 $[M+ 1]^+$ (100), 358 (51), 359 (4), 361 (11), 362 (45), 363 (6); HRMS calcd. for $C_{11}H_9Br_2N_2O_2$ $[M + 1]^+$ 358.9025; found 358.9011.

7.2.3.2. Synthesis of *N*-(3,5-dibromopyridin-4-yl)-acrylamide **153**

To a flask charged with *N*-acryloyl-*N*-(3,5-dibromo-pyridin-4-yl)-acrylamide (0.2g, 0.0006mol), the 1M $NaOH_{aq}$ (30ml) was added. The reaction mixture was stirred at room temperature for 2h. The 1M HCl was added to pH~8, and the product was extracted with CH_2Cl_2 (3x). The organic phases were combined, dried over $MgSO_4$, filtered and concentrated. The pure product was obtained after crystallization from EtOAc in 90% yield (0.153g).



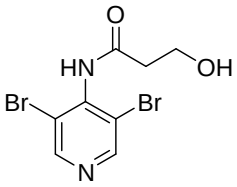
***N*-(3,5-dibromopyridin-4-yl)-acrylamide (153)**;

mp. 164.5-166.4°C; IR: 3199 (NH), 1667 (C(O)N), 1495, 1399 (C=C), 1194 (C-N), 986 (C-H); ^{1}H NMR ($CDCl_3$): 5.92 (dd, *J*= 10.32, 0.96 Hz, 1H, =$\underline{CH_2}$), 6.33 (dd, *J*= 16.92, 10.29 Hz, 1H, =$\underline{CH_2}$), 6.52 (dd, *J*= 17.00, 1.00 Hz, 1H,-$\underline{CH}$=CH_2), 8.69 (s, 2H, py); ^{13}C ($CDCl_3$): δ 119.90, 129.37, 129.98, 142.07, 151.39, 162.42. MS: m/z (%)= 306 $[M+ 1]^+$ (100), 304 (53), 307 (9), 308 (48); HRMS calcd. for $C_8H_7Br_2N_2O$ $[M + 1]^+$ 304.8920; found 304.8909.

7.2.3.3. Synthesis of *N*-(3,5-dibromopyridin-4-yl)-3-hydroxy-propionamide **154**

Procedure based on WO 2005061457 (**2005**)

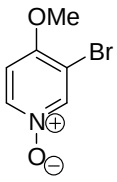
To a cold solution of 4-amino-3,5-dibromopyridine (0.756g, 0.003mol) in CH_2Cl_2 (75 ml) under nitrogen atmosphere, Et_2AlCl (1M in hexane) (0.36g, 0.003mol) was added and the mixture was stirred at room temperature for 20 min and then cooled to 0°C. The *β*-propiolactone (0.229g, 0.003mol) in CH_2Cl_2 was added dropwise, and the reaction mixture was stirred at room temperature for 2hrs. To the reaction mixture diluted HCl was added to pH~8 and the water phase was extracted with CH_2Cl_2 (3x), the combined organic phases was washed with water and dried over $MgSO_4$, filtered and concentrated by evaporation. The *N*-(3,5-dibromo-pyridin-4-yl)-3-hydroxy-propionamide was obtained as white solid after crystallization from EtOH, in 72% of yield (0.699g).



***N*-(3,5-dibromopyridin-4-yl)-3-hydroxy-propionamide (154)**;
mp. 80.9-82.4°C; IR: 3426 (NH), 1729 (C(O)N), 1620, 1565, 1484 (C=C), 1260 (C-O); ^{1}H NMR ($CDCl_3$): 3.55 (t, *J*= 5.76 Hz, 2H, CH_2), 4.28 (t, *J*= 5.76 Hz, 2H, CH_2), 5.07 (br, NH), 8.31 (s, 2H, py); ^{13}C ($CDCl_3$): δ 39.17, 58.52, 106.06, 147.70, 149.83. MS: m/z (%)= 252 $[M+ 1]^+$ (100), 250 (53), 253 (7), 254 (49). HRMS calcd. for $C_5H_5Br_2N_2$ $[M + 1]^+$ 250.88140; found 250.881.

7.2.3.4. Synthesis of 3-bromo-4-methoxypyridine *N*-oxide **173**

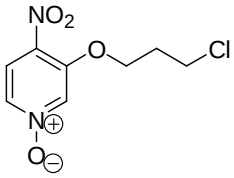
The 3-bromo-4-nitropyridine *N*-oxide (0.2g, 0.0009mol) was dissolved in MeOH (5ml) and MeONa (0.0493g, 0.0009mol) was added. The reaction mixture was stirred at room temperature for 21h. The solvent was removed and H_2O and EtOAc was added, the organic layer was separated and the water layer was extracted with EtOAc (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The 3-bromo-4-methoxypyridine *N*-oxide was isolated as white solid in 90% (0.167g).



3-Bromo-4-methoxypyridine *N*-oxide (173);
mp. 146.6°C; IR: 1643, 1613, 1485 (C=C), 1299 (N-O, *N*-oxide); ^{1}H NMR ($CDCl_3$): 3.96 (s, 3H, OCH_3), 6.78 (d, *J*= 7.24 Hz, 1H, py), 8.13 (dd, *J*= 7.23, 2.23 Hz, 1H, py), 8.36 (d, *J*= 2.18 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 56.98, 107.83, 109.97, 138.82, 141.98, 154.84; MS: m/z (%)= 204 $[M+ 1]^+$ (100), 205 (7), 206 (95), 207 (6); HRMS calcd. for $C_6H_7BrNO_2$ $[M + 1]^+$ 203.96547; found 203.9655.(Roczniki Chemii 1962, 36, 1465-1475, Talik, T.)

7.2.3.5. Synthesis of 3-(3-chloropropoxy)-4-nitro-pyridine *N*-oxide **172D**

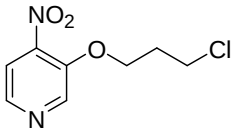
To a cold solution of 3-chloro-1-propanol (0.104g, 0.0011mol) in dry THF (30 ml) under nitrogen atmosphere, NaH (0.051g in oil, 1.2eq) was added. The mixture was stirred at 0°C for 15min. The 3-bromo-4-nitropyridine *N*-oxide (0.2g, 0.0009mol) in one portion and the reaction mixture was stirred at room temperature for 16h. After this time H_2O (few drops to hydrolyzed excess of NaH) was added and the solvents were removed by evaporation. The pure product was isolated by column chromatography using EtOAc as eluent, in 17% (0.036g) yield.



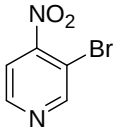
3-(3-Chloropropoxy)-4-nitro-pyridine *N*-oxide (172D);
mp. 105°C; IR: 1601, 1565, 1508 (C=C), 1322 (N-O, *N*-oxide), 1231 (C-O); ^{1}H NMR ($CDCl_3$): 2.34 (pentet, *J*= 5.72 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2), 3.82 (t, *J*= 6.02 Hz, 2H, CH_2-$\underline{CH_2}$-Cl), 4.30 (t, *J*= 5.64 Hz, 2H, CH_2-$\underline{CH_2}$-O), 7.88 (dd, *J*= 7.10, 1.74 Hz, 1H, py), 7.93 (d, *J*= 7.08 Hz, 1H, py), 8.12 (d, *J*= 1.68 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 31.45, 40.58, 67.13, 122.21, 128.36, 132.43, 134.07, 150.88. MS: m/z (%)= 233 $[M+ 1]^+$ (100), 234 (11), 235 (30); HRMS calcd. for $C_8H_{10}ClN_2O_4$ $[M + 1]^+$ 233.0324; found 233.0320.

7.2.3.6. Synthesis of 3-(3-chloropropoxy)-4-nitro-pyridine **176** and 3-bromo-4-nitropyridine **177**

General procedure; modifiied procedure from *Org. Lett.* **2000**, *2*, 3525
The pyridine *N*-oxide (1eq) was dissolved in benzene, TPP (1eq) was added and the mixture was stirred at room temperature for 5 min. To the mixture, trichlorooxobis(triphenylphosphine)rhenium(V) (6 mol%) was added and the final reaction mixture was stirred on the air overnight. The benzene was removed and the product was isolated by column chromatography.



3-(3-Chloro-propoxy)-4-nitro-pyridine (176);
98%; yellow oil; IR: 1598 (C=C), 1527 (N-O, NO_2), 1258 (C-O), 728 (C-Cl); ^{1}H NMR ($CDCl_3$): 2.32 (pentet, *J*= 5.89 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2), 3.79 (t, *J*= 6.05 Hz, 2H, CH_2-$\underline{CH_2}$-Cl), 4.43 (t, *J*= 5.71 Hz, 2H, CH_2-$\underline{CH_2}$-O), 7.65 (d, *J*= 5.37 Hz, 1H, py), 8.44 (d, *J*= 5.07 Hz, 1H, py), 8.64 (s, 1H, py); ^{13}C ($CDCl_3$): δ 31.85, 40.81, 66.61, 117.45, 138.64, 143.09, 144.27, 146.35. MS: m/z (%)= 217 $[M+ 1]^+$ (100), 218 (11), 219 (34); HRMS calcd. for $C_8H_{10}ClN_2O_3$ $[M + 1]^+$ 217.0374; found 217.0367.

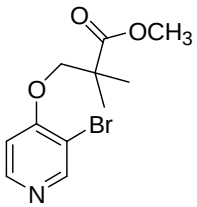


3-Bromo-4-nitropyridine (177);

94%; yellow solid, mp. 49°C; IR: 1530 (C=C), 1351 (N-O, NO_2); ^{1}H NMR ($CDCl_3$): 7.69 (d, *J*= 5.20 Hz, 1H, py), 8.76 (dd, *J*= 5.22, 0.99 Hz, 1H, py), 8.99 (d, *J*= 1.52 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 111.32, 118.26, 150.09, 154.69, 155.13.

7.2.3.7. Synthesis of methyl 3-(3-bromo-pyridin-4-yloxy)-2,2-dimethyl-propionate **179**

The mixture of 3-bromo-4-nitropyridine (0.05g, 0.00025mol), methyl 3-hydroxy-2,2-dimethylpropionate (0.036g, 0.00027mol), K_2CO_3 (0.034g, 0.00025mol) and KOH (0.014g, 0.00025mol) in dry toluene (25 ml) was stirred at room temperature, then TDA-1 (3 drops) was added. The reaction mixture was stirred at room temperature for 3h. After this time, precipitate was filtered off and washed with MeOH. The filtrate was concentrate and the product was separated by preparative TLC, using EtOAc/PE (1:2) as eluent. The product was obtained as colorless oil in 54% (0.068g).



Methyl 3-(3-bromo-pyridin-4-yloxy)-2,2-dimethyl-propionate (179);

IR: 1728 (C(O)O), 1576, 1459 (C=C), 1301 (C-O), 1147, 1024 (C-H); ^{1}H NMR ($CDCl_3$): 1.38 (s, 6H, 2xCH_3), 3.72 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 6.80 (d, *J*= 5.60 Hz, 1H, py), 8.38 (d, *J*= 5.66 Hz, 1H, py), 8.57 (s, 1H, py); ^{13}C ($CDCl_3$): δ 22.33, 52.25, 74.75, 108.25, 110.54, 149.98, 152.44, 161.01, 175.86; MS: m/z (%)= 288 $[M+ 1]^+$ (100), 289 (13), 290 (98), 291 (12); HRMS calcd. for $C_{11}H_{15}BrNO_3$ $[M + 1]^+$ 288.0230; found 288.0240.

7.2.4. Synthesis of the intermediates to the Kharasch reaction

7.2.4.1. Synthesis of 2-(*N*-allyl-*N*-benzylamino)-3-nitropyridine **180**

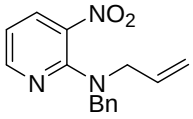
1 Procedure based on WO 2006025717 (**2006**)

To a suspension of 2-chloro-3-nitropyridine (1eq), *N*-allyl-*N*-benzylamine (1.1eq), K_2CO_3 (1eq) and KOH (1eq) in dry toluene, TDA-1 (0.1eq) was added. The reaction mixture was stirred at room temperature for 2h. Then filtrate through celit pad, and the cake was washed with EtOAc and MeCN. The filtrate was concentrated; crud product was purified by column chromatography using mixture of PE/EtOAc (9:1) as eluent. The desired product was obtained in 12% yield as yellow oil.

2 To a cold solution of *N*-allyl-*N*-benzylamine (1.1eq) in dry THF under nitrogen atmosphere, NaH (1.2eq) was added in one portion. The mixture was stirred at 0°C for 15min, the 2-chloro-3-nitropyridine (1eq) was added, the ice-bath was removed and the reaction mixture was stirred at reflux

for 22h. The solvent was evaporated and the product purified by column chromatography using mixture PE/EtOAc (9:1) as eluent, as yellow oil in 62% yield.

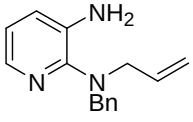
3 To a cold solution of *N*-allyl-*N*-benzylamine (1.1eq) in dry THF under nitrogen atmosphere, NaH (1.2eq) was added in one portion. The mixture was stirred at 0°C for 15min, the 2-chloro-3-nitropyridine (1eq) was added, the ice-bath was removed and the reaction mixture was stirred for 22h at room temperature. The solvent was evaporated and the product purified by column chromatography using mixture PE/EtOAc (9:1) as eluent in 82% yield.



2-(*N*-allyl-*N*-benzylamino)-3-nitropyridine (180);
yellow oil; IR: 1592 (C=C), 1555 (NO_2), 1506, 1494 (C=C), 1333 (-N=), 1231 (C-N); ^{1}H NMR ($CDCl_3$): 3.94 (t, *J*= 1.28 Hz, 1H, -$\underline{CH_2}$-CH), 3.96 (t, *J*= 1.28 Hz, 1H, -$\underline{CH_2}$-CH), 4.74 (s, 2H, $\underline{CH_2}$-benzyl), 5.17 (qdt, *J*= 2.97, 1.37, 2.67, 1.32 Hz, 2H, =$\underline{CH_2}$), 5.80 (qt, *J*= 6.15, 6.15, 6.15, 6.15 Hz, 1H, -CH=), 6.74 (dd, *J*= 4.48, 8.00 Hz, 1H, py), 7.21-7.32 (m, 5H, Bn), 8.09 (dd, *J*= 1.76, 8.68 Hz, 1H, py), 8.33 (dd, *J*= 1.76, 5.07 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 52.79, 52.97, 113.23, 119.16, 127.33, 127.89, 128.51, 132.94, 133.19, 135.39, 137.13, 151.55, 152.51; MS: m/z (%)= 270 $[M+ 1]^+$ (100), 271 (18); HRMS calcd. for $C_{15}H_{16}N_3O_2$ $[M + 1]^+$ 270.1237; found 270.1248.

7.2.4.2. Synthesis of 2-(*N*-allyl-*N*-benzylamino)-3-aminopyridine **181**

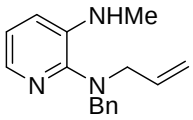
Procedure based on WO 2008046216 (**2008**)
The suspension of 2-(*N*-allyl-*N*-benzylamino)-3-nitropyridine (1eq), iron powder (4eq) and NH_4Cl (1.5eq) in the mixture of methanol and H_2O (5:1) was refluxed for 5h. The reaction mixture was cooled to room temperature, filtrated through celit pad. The cake was washed with MeOH and MeCN and the filtrate was concentrated under vacuum. To the residue chloroform was added and solid was removed by filtration. The solvent was removed under vacuum; desired product was isolated by column chromatography using mixture of PE/EtOAc (5:2) as eluent in 80% yield.



2-(*N*-allyl-*N*-benzylamino)-3-aminopyridine (181);
dark red oil; IR: 3430, 3310 (NH_2), 1601, 1584, 1451 (C=C), 1214 (C-N); ^{1}H NMR ($CDCl_3$): 3.69 (d, *J*= 6.04 Hz, 2H, -$\underline{CH_2}$-CH=), 3.88 (br, NH_2), 4.32 (s, 2H, $\underline{CH_2}$-benzyl), 5.15 (qdt, *J*= 2.95, 3.22, 1.52, 1.52 Hz, 2H, =$\underline{CH_2}$), 5.86 (qt, *J*= 6.09 Hz, 1H, -$\underline{CH}$=), 6.81 (dd, *J*= 12.48, 4.76 Hz, 1H, py), 6.92 (dd, *J*= 9.36, 1.64 Hz, 1H, py), 7.17-7.31 (m, 5H, Bn), 7.78 (dd, *J*= 6.40, 1.64 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 53.02, 53.91, 117.30, 119.69, 121.72, 126.79, 128.21, 128.45, 135.13, 136.38, 137.41, 139.19, 150.29; MS: m/z (%)= 240 $[M+ 1]^+$ (100), 241 (15); HRMS calcd. for $C_{15}H_{18}N_3$ $[M + 1]^+$ 240.1495; found 240.1498.

7.2.4.3. Synthesis of 2-(*N*-allyl-*N*-benzylamino)-3-(*N*-methylamino)-pyridine **182a**

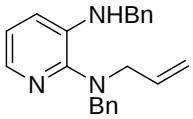
The solution of 2-(*N*-allyl-*N*-benzylamino)-3-aminopyridine (1eq) in dry THF under nitrogen atmosphere at -78°C, *n*-BuLi (1.3eq, 2.5M in hexanes) was added dropwise and the mixture was stirred at -78°C for 15 min. The methyl iodide was added and the reaction mixture was stirred at room temperature for 2h. The solvent was removed and the desired product was isolated by column chromatography using mixture PE/EtOAc (8/1) as eluent. The product was obtained in 69% yield.



2-(*N*-allyl-*N*-benzylamino)-3-(*N*-methylamino)-pyridine (182a);
light pink oil; IR: 3387 (NH), 1580, 1482, 1403 (C=C), 1223 (C-N); ^{1}H NMR ($CDCl_3$): 2.81 (d, *J*= 5.32 Hz, 3H, CH_3), 3.63 (t, *J*= 1.28 Hz, 1H, -$\underline{CH_2}$-CH=), 3.65 (t, *J*= 1.28 Hz, 1H, -$\underline{CH_2}$-CH=), 4.27 (s, 2H, $\underline{CH_2}$-benzyl), 4.48 (br, NH_2), 5.12 (ddt, *J*= 3.06, 1.66 Hz, 1H, =CH_2), 5.10-5.12 (m, 1H, =CH_2), 5.79 (qt, *J*= 6.12, 6.20, 6.20, 6.12 Hz, 1H, -CH=), 6.79 (dd, *J*= 9.40, 1.52 Hz, 1H, py), 6.91 (dd, *J*= 4.80, 12.64 Hz, 1H, py), 7.17-7.28 (m, 5H, Bn), 7.72 (dd, *J*= 1.56, 6.36 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 30.32, 53.18, 54.29, 115.65, 117.29, 120.11, 126.80, 128.18, 128.56, 135.02, 135.15, 139.13, 139.43, 150.10; MS: m/z (%)= 254 $[M+ 1]^+$ (100), 255 (18); HRMS calcd. for $C_{16}H_{20}N_3$ $[M + 1]^+$ 254.1652; found 254.1661.

7.2.4.4. Synthesis of 2-(*N*-allyl-*N*-benzylamino)-3-(*N*-benzylamino)-pyridine **182b**

The mixture of 2-(*N*-allyl-*N*-benzylamino)-3-aminopyridine (1eq) and benzaldehyde (1eq) in EtOH was refluxed till the condensation was complete (3h), the reaction was monitored by TLC. The solvent was removed and the product was used to next step without purification. To the mixture of 2-(*N*-allyl-*N*-benzylamino)-(*N*-phenylmethylene-3-amino)-pyridine (1eq) and acetic acid (1eq) in MeOH, $NaBH_3CN$ (2eq) was added. The reaction mixture was stirred at room temperature for 17h. The solvent was removed under vacuum; crud product was purified by column chromatography using mixture of PE/EtOAc (5:5) as eluent, in 70% yield.

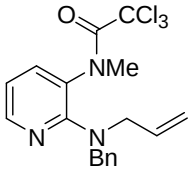


2-(*N*-allyl-*N*-benzylamino)-3-(*N*-benzylamino)-pyridine (182b);
yellow/light brown oil; IR: 3380 (NH), 1577, 1477, 1452 (C=C), 1218 (C-N); ^{1}H NMR ($CDCl_3$): 3.68 (d, *J*= 6.04 Hz, 2H, -$\underline{CH_2}$-CH=), 4.29 (s, 2H, $\underline{CH_2}$-benzyl connected to N in C2), 4.31 (d, *J*= 6.96 Hz, 2H, NH-$\underline{CH_2}$-benzyl), 5.00 (br, NH), 5.07-5.20 (m, 2H, =$\underline{CH_2}$), 5.80-5.92 (m, 1H, -CH=), 6.74 (d, *J*= 7.76 Hz, 1H, py), 6.83 (dd, *J*= 4.92, 12.56 Hz, 1H, py), 7.17-7.34 (m, 10H, 2xBn), 7.73 (dd, *J*= 2.30, 6.84 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 40.71, 53.79, 54.58, 116.61, 117.41, 120.19, 126.84, 127.09, 127.22, 128.19, 128.64, 128.68, 135.19, 135.47, 138.31, 138.96, 139.10, 150.09; MS: m/z (%)= 330 $[M+ 1]^+$ (100), 331 (25); HRMS calcd. for $C_{22}H_{24}N_3$ $[M + 1]^+$ 330.1965; found 330.1968.

7.2.4.5. Synthesis of *N*-[2-(*N*-allyl-*N*-benzylamino)-pyridin-3-yl]-*N*-methyl-2,2,2-trichloroacetamide **183a**

Procedure based on *J. Am. Chem. Soc.* **2010**, *132*, 16631-16636

To a solution of 2-(*N*-allyl-*N*-benzylamino)-3-(*N*-methylamino)-pyridine (1eq) in CH_2Cl_2 at 0°C, trichloroacetyl chloride (2.2eq) was added dropwise. The ice-bath was removed and the reaction mixture was stirred at room temperature for 20h. H_2O was added and the reaction mixture was basified with saturated solution of $NaHCO_3$ to pH~7, and extracted with CH_2Cl_2 (3x), dried over $MgSO_4$, filtered and concentrated. The product was obtained in 80% yield after column chromatography (PE/EtOAc 1:2).

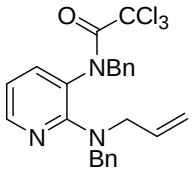


***N*-[2-(*N*-allyl-*N*-benzylamino)-pyridin-3-yl]-*N*-methyl-2,2,2-trichloroacetamide (183a)**; brown oil; IR: 1680 (N-C(O)), 1586 (C=C), 1449 (C-H), 1111 (=C-H); ^{1}H NMR ($CDCl_3$): 3.51 (s, 3H, CH_3), 3.97 (s, 2H, $\underline{CH_2}$-Bn), 4.54 (br, 1H, =CH_2), 4.71 (br, 1H, =CH_2), 5.16 (tt, *J*= 1.8 Hz, 2H, -$\underline{CH_2}$-CH=), 5.83-5.95 (m, 1H, -CH=), 6.88 (t, *J*= 5.48 Hz, 1H, py), 7.20-7.34 (m, 5H, Bn), 7.43 (dt, *J*= 1.96 Hz, 1H, py), 8.27 (dt, *J*= 2.00 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 40.54, 52.17, 52.67, 116.41, 117.62, 126.88, 128.22, 128.24, 128.46, 134.41, 137.86, 138.47, 147.25, 160.69; MS: m/z (%)= 400 $[M+ 1]^+$ (100), 398 (99), 402 (33), 399 (21), 401 (20), 403 (6); HRMS calcd. for $C_{18}H_{19}Cl_3N_3O$ $[M + 1]^+$ 398.0588; found 398.0606.

7.2.4.6. Synthesis of *N*-[2-(*N*-allyl-*N*-benzylamino)-pyridin-3-yl]-*N*-benzyl-2,2,2-trichloroacetamide **183b**

Procedure based on *J. Am. Chem. Soc.* **2010**, *132*, 16631-16636

To a solution of 2-(*N*-allyl-*N*-benzylamino)-3-(*N*-benzylamino)-pyridine (1eq) in CH_2Cl_2 at 0°C, trichloroacetyl chloride (2.2eq) was added dropwise. The ice-bath was removed and the reaction mixture was stirred at room temperature for 18h. H_2O was added and the reaction mixture was basified with saturated solution of $NaHCO_3$ to pH~7, and extracted with CH_2Cl_2 (3x), dried over $MgSO_4$, filtered and concentrated. The product was obtained in 90% yield after column chromatography (PE/EtOAc 3:1).



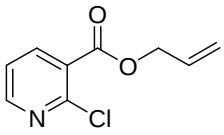
***N*-[2-(*N*-allyl-*N*-benzylamino)-pyridin-3-yl]-*N*-benzyl-2,2,2- trichloroacetamide (183b)**; brown oil; IR: 1714 (C(O)N), 1677, 1584, 1452 (C=C), 1231 (C-N); ^{1}H NMR ($CDCl_3$): 4.53 (d, *J*= 15.55 Hz, 1H, $\underline{CH_2}$-Bn), 4.86 (d, *J*= 15.55 Hz, 1H, -$\underline{CH_2}$-Bn), 5.20 (dt, *J*= 2.40 Hz, 1H, -$\underline{CH_2}$-CH=), 5.17 (qt, *J*= 3.00 Hz, 1H, -$\underline{CH_2}$-CH=), 5.60 (d, *J*= 13.96 Hz, 2H, -$\underline{CH_2}$-Bn), 5.90 (qt, *J*= 6.20 Hz, 1H, -$\underline{CH}$=), 6.56 (dd, *J*= 7.68, 4.72 Hz, 2H, -$\underline{CH_2}$-CH=), 6.95-7.03 (m, 3H, 2x1H from py and 1H from Bn), 7.14 (m, 10H, 2xBn), 8.75 (dd, *J*= 4.68, 1.72 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 52.37, 53.73, 117.98, 127.03, 127.99, 128.32, 128.34, 128.56, 129.27, 134.46, 135.14, 138.54, 140.45, 147.30, 160.83; MS: m/z (%)= 474 $[M+1]^+$ (100), 476 (95), 478 (31), 475 (27), 477 (26), 479 (6); HRMS calcd. for $C_{24}H_{23}Cl_3N_3O$ $[M+1]^+$ 474.0901; found 474.0910.

7.2.5. The metathesis reaction

7.2.5.1. Synthesis of the metathesis intermediates

7.2.5.1.1. Synthesis of 2-propen-1-yl 2-chloro-3-pyridinecarboxylate **186**

To a cold solution of 2-chloropyridine-3-carboxylic acid (0.5g, 0.003mol) and allyl alcohol (0.184g, 0.003mol) in 40 ml of dry CH_2Cl_2, EDC (0.67g, 0.0035mol) and DMAP (0.04g, 0.0003mol) were added. The reaction mixture was stirred at room temperature for 16h. Then, H_2O was added and the organic layer was washed with $NaHCO_3$ aqueous saturated solution and a saturated solution of NaCl. The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The product was purified by column chromatography, using EtOAc as eluent, to obtain 0.49g (78%) of 2-propen-1-yl 2-chloro-3-pyridinecarboxylate as colorless liquid.

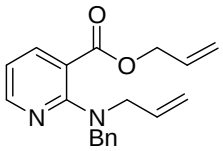


2-Propen-1-yl 2-chloro-3-pyridinecarboxylate (186);

IR: 1733 (C(O)O), 1578, 1560 (C=C), 1402 (C=CH_2), 1297, 1287, 1269, 1242 (C(O)O), 1063, 1051 (C=CH_2); ^{1}H NMR (300 MHz, $CDCl_3$): 4.86 (dt, *J*= 5.80, 1.36 Hz, 2H, CH_2), 5.32 (dt, *J*= 10.45, 2.44, 1.2 Hz, 1H, =CH_2), 5.44 (qt, *J*= 17.18, 2.91, 1.47 Hz, 1H, CH_2), 6.04 (qt, *J*= 17.18, 10.45, 5.8 Hz, CH), 7.35 (dd, *J*= 7.71, 4.95 Hz, 1H, py), 8.2 (dd, *J*= 7.71, 2.2 Hz, 1H, py), 8.53 (dd, *J*= 4.4, 1.65 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 66.65, 119.29, 122.19, 126.85, 131.41, 140.41, 150.12, 152.02, 154.78, 164.18; MS: m/z (%)= 198 $[M+1]^+$ (100), 199 (12), 200 (20); HRMS calcd. for $C_9H_8ClNO_2$ $[M+1]^+$ 198.0316; found 198.0319. Literature: M. H. Sherlock, CH 534129 A 19730413

7.2.5.1.2. Synthesis of allyl 2-(*N*-allyl-*N*-benzylamino)-3-pyridinecarboxylate **187**

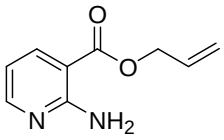
The mixture of allyl 2-chloro-3-pyridinecarboxylate (1eq) and *N*-allyl-*N*-benzylamine in MeCN was heated at 80°C for 73h. The precipitate was removed by filtration and washed with MeCN. The filtrate was concentrated and the desired product was purified by column chromatography using EtOAc as eluent in 49% yield as orange oil.



Allyl 2-(*N*-allyl-*N*-benzylamino)-3-pyridinecarboxylate (187);
IR: 1710 (C(O)O), 1582, 1554, 1471, 1442, 1414 (C=C), 1220, 1118 (C-N); ^{1}H NMR ($CDCl_3$): 3.94 (d, *J*= 6.00 Hz, 2H, =CH-$\underline{CH_2}$-N), 4.69 (s, 2H, $\underline{CH_2}$-Bn), 4.71 (dt, *J*= 5.85, 1.17 Hz, 2H, O-$\underline{CH_2}$-CH=), 5.08-5.15 (m, 2H, $\underline{CH_2}$=CH-CH_2-N), 5.27 (ddt, *J*= 10.40, 2.40, 1.16 Hz, 1H, $\underline{CH_2}$=CH-CH_2-O), 5.37 (ddt, *J*= 17.17, 1.43, 2.89 Hz, 1H, $\underline{CH_2}$=CH-CH_2-O), 5.84 (qt, *J*= 16.77, 10.70, 5.99 Hz, 1H, N-CH_2-$\underline{CH}$=), 5.97 (qt, *J*= 17.21, 10.40, 5.83 Hz, 1H, O-CH_2-$\underline{CH}$=), 6.70 (dd, *J*= 7.71, 4.68 Hz, 1H, py), 7.15-7.31 (m, 5H, Bn), 7.94 (dd, *J*= 1.94, 7.58 Hz, 1H, py), 8.26 (dd, *J*= 1.98, 4.66 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 52.70, 53.13, 65.74, 113.27, 113.37, 117.83, 118.85, 126.87, 127.83, 128.29, 132.03, 134.24, 138.29, 140.41, 150.32, 158.57, 167.29; MS: m/z (%)= 309 $[M+ 1]^+$ (100), 310 (21); HRMS calcd. for $C_9H_{21}N_2O_2$ $[M + 1]^+$ 309.1592; found 309.1610.

7.2.5.1.3. Synthesis of 2-propen-1-yl 2-amino-3-pyridinecarboxylate **189**

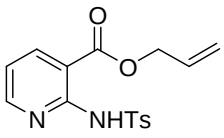
To a cold solution of 2-aminopyridine-3-carboxylic acid (1eq) and allyl alcohol (1eq) in dry CH_2Cl_2, EDC (1.1eq) and DMAP (0.1eq) were added. The reaction mixture was stirred at room temperature for 13h. The solvent was removed under vacuum. The product was purified by column chromatography, using EtOAc as eluent, to obtain white solid in 42% yield.



2-Propen-1-yl 2-amino-3-pyridinecarboxylate (189);
mp. 49°C; IR: 3429 (NH_2), 1683 (C(O)O), 1622, 1579, 1565 (C=C), 1450 (C-H), 1238, 1104, 1085 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$): 4.79 (dt, *J*= 5.67, 1.41 Hz, 2H, CH_2-CH), 5.28 (ddt, *J*= 10.44, 2.56, 1.28 Hz, 1H, CH=$\underline{CH_2}$), 5.39 (ddt, *J*= 17.21, 3.01, 1.51 Hz, 1H, CH=$\underline{CH_2}$), 6.02 (qt, *J*= 17.20, 10.47, 5.67 Hz, 1H, CH_2-$\underline{CH}$=CH_2), 6.35 (br, NH_2), 6.61 (dd, *J*= 7.80, 4.76 Hz, 1H, py), 8.15 (dd, *J*= 7.80, 1.96 Hz, 1H, py), 8.21 (dd, *J*= 4.76, 1.96 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 65.32, 106.26, 112.69, 118.31, 132.14, 139.97, 153.73, 159.57, 166.59; MS: m/z (%)= 179 $[M+ 1]^+$ (100), 180 (10); HRMS calcd. for $C_9H_{11}N_2O_2$ $[M + 1]^+$ 179.0815; found 179.0815.

7.2.5.1.4. Synthesis of 2-propen-1-yl 2-(*N*-tosylamino)-3-pyridinecarboxylate **190**

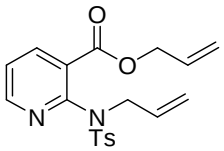
The mixture of 2-propen-1-yl 2-amino-3-pyridinecarboxylate (0.3g, 0.0017mol) and *p*-toluenesulfonyl chloride (0.483g, 0.0025mol) in pyridine (30 ml) was stirred at 60°C for 41h. The reaction mixture was cooled to room temperature and H_2O was added. The precipitate was filtered off and washed with H_2O, dried over vacuum pomp gave 0.41g (72% yield) of desired product as white solid. Crystallization form MeOH.



2-Propen-1-yl 2-(*N*-tosylamino)-3-pyridinecarboxylate (190);
mp. 126°C; IR: 3217 (NH), 1687 (C(O)O), 1591, 1579, 1462, 1450 (C=C), 1291 (C(O)O), 1146 (C-N); ^{1}H NMR ($CDCl_3$): 2.39 (s, 3H, CH_3), 4.83 (dt, *J*= 5.84, 1.32 Hz, 1H, -$\underline{CH_2}$-CH=), 5.34 (ddt, *J*= 10.37, 2.33, 1.17 Hz, 1H, =$\underline{CH_2}$), 5.41 (ddt, *J*= 17.26, 2.83, 1.45 Hz, 1H, =$\underline{CH_2}$), 6.01 (qt, *J*= 17.17, 10.40, 5.89 Hz, 2H, -$\underline{CH}$=CH_2), 6.79 (dd, *J*= 7.86, 4.82 Hz, 1H, py), 7.26 (d, *J*= 8.36 Hz, 2H, Ts), 8.05 (d, *J*= 8.36 Hz, 2H, Ts), 8.21 (dd, *J*= 7.88, 1.92 Hz, 1H, py), 8.37 (dd, *J*= 4.84, 1.92 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 21.59, 66.48, 109.47, 117.36, 119.49, 128.63, 129.13, 131.23, 137.20, 139.79, 143.87, 152.21, 152.87, 166.19; MS: m/z (%)= 333 $[M+ 1]^+$ (100), 334 (19), 335 (7); HRMS calcd. for $C_{16}H_{17}N_2O_4S$ $[M + 1]^+$ 333.0904; found 333.0912.

7.2.5.1.5. Synthesis of 2-propen-1-yl 2-(*N*-allyl-*N*-tosylamino)-3-pyridinecarboxylate **191**

The mixture of 2-propen-1-yl 2-(*N*-tosylamino)-3-pyridinecarboxylate (0.15g, 0.0005mol), allyl bromide (0.071g, 0.0006mol) and K_2CO_3 (0.249g, 0.0018mol) in DMA (15 ml) was heated at 90°C for 39h. The reaction mixture was cooled down and water/ice was added. The precipitate was filtered off and washed with H_2O, dried over vacuum pomp gave 0.14g (84% yield) of desired product as beige solid.



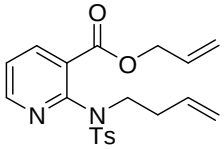
2-Propen-1-yl 2-(*N*-allyl-*N*-tosylamino)-3-pyridinecarboxylate (191);
mp. 128°C; IR: 1714 (C(O)O), 1447, 1425, 1343, 1304 (C=C), 1161 (C-N); ^{1}H NMR ($CDCl_3$): 2.40 (s, 3H, CH_3), 4.30 (dt, *J*= 6.28, 1.32 Hz, 2H, N-$\underline{CH_2}$-CH=), 4.91 (dt, *J*= 6.00, 1.28 Hz, 2H, O-$\underline{CH_2}$-CH=), 4.99 (ddt, *J*= 10.24, 2.68, 1.32 Hz, 1H, $\underline{CH_2}$=CH-), 5.16 (ddt, *J*= 17.13, 2.99, 1.51 Hz, 1H, $\underline{CH_2}$=CH-), 5.33 (ddt, *J*= 10.38, 2.44, 1.16 Hz, 1H, $\underline{CH_2}$=CH-), 5.46 (ddt, *J*= 17.17, 2.97, 1.49 Hz, 1H, $\underline{CH_2}$=CH-), 5.87 (qt, *J*= 17.18, 10.20, 6.26 Hz, 1H, CH_2=$\underline{CH}$-), 6.14 (qt, *J*= 17.22, 10.40, 5.98 Hz, 1H, CH_2=$\underline{CH}$-), 7.23 (d, *J*= 8.00 Hz, 2H, Ts), 7.29 (dd, *J*= 7.72, 4.72 Hz, 1H, py), 7.44 (d, *J*= 8.32 Hz, 2H, Ts), 8.24 (dd, *J*= 7.81, 2.05 Hz, 1H, py), 8.45 (dd, *J*= 4.76, 1.97 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 21.58, 51.39, 66.62, 118.73, 118.96, 122.25, 127.76, 128.25, 129.52,

132.07, 132.73, 135.38, 139.79, 143.65, 150.50, 150.75, 165.74; MS: m/z (%)= 373 $[M+ 1]^+$ (100), 374 (23), 375 (11); HRMS calcd. for $C_{19}H_{21}N_2O_4S$ $[M + 1]^+$ 373.1217; found 373.1220.

7.2.5.1.6. Synthesis of 2-propen-1-yl 2-(*N*-(3-buten-1-yl)-*N*-tosylamino)-3-pyridinecarboxylate **193**

1 The mixture of 2-propen-1-yl 2-(*N*-tosylamino)-3-pyridinecarboxylate (0.10g, 0.0003mol), 4-bromo-1-butene (0.053g, 0.0004mol) and K_2CO_3 (0.166g, 0.0012mol) in DMA (5 ml) was heated at 90 °C for 24h. After this time K_2CO_3 (0.128g, 0.0009mol) and 4-bromo-1-butene (0.053g, 0.0004mol) were added and the mixture was stirred at 90°C for another 19h. The reaction mixture was cooled down and water/ice was added. The precipitate was filtered off and washed with H_2O, dried over vacuum pomp and crystallized from PE gave 0.099g (84% yield) of desired product as beige solid.

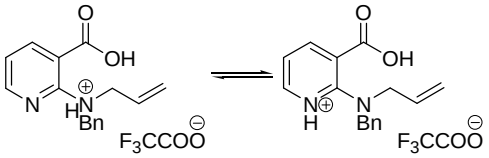
2 The mixture of 2-propen-1-yl 2-(*N*-tosylamino)-3-pyridinecarboxylate (0.10g, 0.0003mol), 4-bromo-1-butene (0.053g, 0.0004mol) and K_2CO_3 (0.166g, 0.0012mol) in MeCN (5 ml) was heated at 80 °C for 30h. After this time K_2CO_3 (0.128g, 0.0009mol) and 4-bromo-1-butene (0.053g, 0.0004mol) were added and the mixture was stirred at 80°C for another 20h. The reaction mixture was cooled to room temperature and solvent was removed. The desired product was separated by column chromatography using mixture of EtOAc and PE (1:1) as eluent. The desired product was isolated in 67% yield (0.078g) as beige solid. Crystallization was performed from PE.



2-Propen-1-yl 2-(*N*-(3-buten-1-yl)-*N*-tosylamino)-3-pyridinecarboxylate (193); mp. 122°C; IR: 1713 (C(O)O), 1424 (C=C), 1343 (C-N), 1158 (C-O); ^{1}H NMR ($CDCl_3$): 2.32 (dt, *J*= 6.64, 6.80 Hz, 2H, CH_2-$\underline{CH_2}$-CH=), 2.39 (s, 3H, CH_3), 3.73 (t, *J*= 7.62 Hz, 2H, N-$\underline{CH_2}$), 4.92-5.01 (m, 4H, O-$\underline{CH_2}$, $\underline{CH_2}$=CH-CH_2-CH_2-N), 5.33 (ddt, *J*= 10.36, 2.38, 1.10 Hz, 1H, $\underline{CH_2}$=CH-CH_2-O), 5.45 (ddt, *J*= 17.21, 3.01, 1.50 Hz, 1H, $\underline{CH_2}$=CH-CH_2-O), 5.76 (qt, *J*= 17.09, 10.31, 6.76 Hz, 1H, CH_2=$\underline{CH}$-CH_2-O), 6.15 (qt, *J*= 17.24, 10.32, 6.02 Hz, 1H, CH_2=$\underline{CH}$-CH_2-CH_2), 7.22 (d, *J*= 8.00 Hz, 2H, Ts), 7.32 (dd, *J*= 7.76, 4.93 Hz, 1H, py), 7.41 (d, *J*= 8.29 Hz, 2H, Ts), 8.26 (dd, *J*= 7.72, 7.20 Hz, 1H, py), 8.46 (dd, *J*= 4.74, 1.98 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 21.56, 32.88, 48.25, 66.67, 116.55, 119.05, 122.20, 127.68, 128.36, 129.49, 132.14, 134.96, 135.34, 139.84, 143.57, 150.57, 150.84, 165.79; MS: m/z (%)= 387 $[M+ 1]^+$ (100), 388 (24), 379 (7); HRMS calcd. for $C_{20}H_{23}N_2O_4S$ $[M + 1]^+$ 387.1373; found 387.1377.

7.2.5.2. Synthesis of 2-(*N*-allyl-*N*-benzylamino)-nicotinic acid salt of trifluoroacetic acid

To the solution of allyl 2-(*N*-allyl-*N*-benzylamino)-3-pyridinecarboxylate (0.054g, 0.0002mol) in toluene (30 ml) at room temperature under nitrogen atmosphere, $Sc(OTf)_3$ (18mg, 20 mol%) was added. The mixture was stirred at room temperature for 5 min and then 10 min at 80°C. Grubbs 2nd generation catalyst (8.5mg, 5 mol%) was added. The reaction mixture was stirred at 80°C for 15 hours. Toluene was removed and the new compound was separated by preparative HPLC using mixture of MeCN/0.1% CF_3COOH in H_2O.



2-(*N*-allyl-*N*-benzylamino)-nicotinic acid salt of trifluoroacetic acid;
^{1}H NMR ($CDCl_3$): 4.83 (dt, *J*= 5.78, 1.34 Hz, 4H, $\underline{CH_2}$-CH=, $\underline{CH_2}$-Bn), 5.36 (ddt, *J*= 10.39, 2.31, 1.13 Hz, 1H, =$\underline{CH_2}$), 5.43 (ddt, *J*= 17.17, 2.76, 1.44 Hz, 1H, =$\underline{CH_2}$), 6.02 (qt, *J*= 17.16, 10.42, 5.87 Hz, 1H, -CH=), 6.69 (br, 1H), 6.79 (t, *J*= 6.68 Hz, 1H, py), 7.30-7.37 (m, 2H, py), 7.39 (d, *J*= 4.29 Hz, 3H, Bn), 8.47 (d, *J*= 6.97 Hz, 2H, Bn), 9.01 (br, 1H); ^{13}C ($CDCl_3$): δ 46.37, 66.52, 109.59, 111.39, 119.68, 127.85, 128.11, 129.00, 131.08, 136.19, 144.22, 147.11, 154.35, 165.49; MS: m/z (%)= 269 $[M+ 1]^+$ (100), 270 (18); HRMS calcd. for $C_{16}H_{17}N_2O_2$ $[M + 1]^+$ 269.1285; found 269.1289.

7.2.5.3. Synthesis of twenty-membered ring fused to pyridine

The 2-propen-1-yl 2-(*N*-(3-buten-1-yl)-*N*-tosylamino)-3-pyridinecarboxylate (0.05g, 0.0001mol) was dissolved in dry toluene (30 ml) and the solution was flushed with nitrogen (3x), the Grubbs 2nd generation catalyst (0.011g, 0.000013mol) was added. The reaction mixture was stirred at 80°C for 3h. The solvent was removed under vacuum and the products were separated by preparative HPLC using mixture of MeCN/0.1% CF_3COOH in H_2O. Three compounds were isolated as the salt of CF_3COOH.

1st fr; ^{1}H NMR ($CDCl_3$): 2.22-2.31 (m, 2xCH_2), 2.39 (s, 2xCH_3), 3.75-3.84 (m, CH_2), 4.68 (d, *J*= 6.44 Hz, CH_2), 4.79-4.85 (m, CH_2), 4.82 (m, CH_2), 5.66-5.78 (m, 2xCH), 5.85-5.94 (m, CH), 5.94-6.04 (m, CH), 7.19-7.40 (m, 16xCH from 2xTs), 8.21 (dd, *J*= 4.16, 1.96 Hz, 2x2H from py), 8.23 (dd, *J*= 4.10, 1.98 Hz, 2x2H from py), 8.24 (d, *J*= 1.96 Hz, 2x2H from py), 8.25 (dd, *J*= 7.70, 1.98 Hz, 2H, 2x1H from py), 8.43-8.46 (m, 2x1H from py), 8.47 (dd, *J*= 4.72, 1.96 Hz, 2H, 2x1H from py); ^{13}C ($CDCl_3$): δ 21.56 (2), 26.53 (1), 30.58, 30.76, 30.94, 47.66 (1), 47.79 (2), 61.97 (2), 65.62 (2), 65.98 (1), 121.96 (2), 122.07 (1), 125.29 (2), 125.49 (1), 127.64 (1), 127.70 (1), 127.75 (2), 129.50 (1), 129.54 (2), 132.66 (1), 135.04 (1), 135.22 (2), 140.35 (2), 140.42 (1), 140.48 (1), 143.65 (1), 143.69 (2), 150.43 (2), 150.49 (1), 150.53 (2), 166.39 (C=O), 166.51 (C=O); MS: m/z (%)= 717

$[M+ 1]^+$ (100), 718 (48), 719 (26), 720 (7); HRMS calcd. for $C_{36}H_{37}N_4O_8S_2$ $[M + 1]^+$ 717.2047; found 717.2039.

(1)major compound; (2)minor compound

2nd fr; ^{1}H NMR ($CDCl_3$): 2.18 (s, 4H, 2xCH_2), 2.38 (s, 6H, 2xCH_3), 3.57 (t, *J*= 7.10 Hz, 4H, 2xCH_2), 5.01 (d, *J*= 2.72 Hz, 2H, 2xCH_2), 5.37-5.41 (m, 2H, 2xCH), 6.18-6.22 (m, 2H, 2xCH), 7.21 (d, *J*= 8.40 Hz, 4H, 2x2H from Ts), 7.35 (dd, *J*= 7.76, 4.88 Hz, 2H, 2x1H from py), 7.36 (d, *J*= 8.28 Hz, 4H, 2x2H from Ts), 8.36 (dd, *J*= 7.74, 1.94 Hz, 2H, 2x1H from py), 8.47 (dd, *J*= 4.76, 1.96 Hz, 2H, 2x1H from py); ^{13}C ($CDCl_3$): δ 21.52, 31.51, 48.67, 65.32, 122.31, 127.62, 128.23, 128.61, 129.22, 129.54, 135.01, 140.93, 143.73, 150.38, 150.44, 165.92; MS: m/z (%)= 717 $[M+ 1]^+$ (100), 718 (51), 719 (24), 720 (7); HRMS calcd. for $C_{36}H_{37}N_4O_8S_2$ $[M + 1]^+$ 717.2047; found 717.2040.

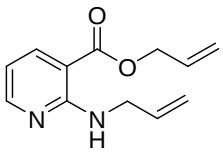
3rd fr; ^{1}H NMR ($CDCl_3$): 2.26 (s, 4H, 2xCH_2), 2.38 (s, 6H, 2xCH_3), 3.65 (t, *J*= 8.32 Hz, 4H, 2xCH_2), 5.02 (s, 4H, 2xCH_2), 5.23 (t, *J*= 4.62 Hz, 2H, 2x-CH=), 6.21-6.24 (m, 2H, 2x-CH=), 7.21 (d, *J*= 8.08 Hz, 4H, 2x2H from Ts), 7.35 (d, *J*= 8.24 Hz, 4H, 2x2H from Ts), 7.36 (dd, *J*= 7.76, 4.60 Hz, 2H, 2x1H from py), 8.34 (dd, *J*= 7.75, 1.99 Hz, 2H, 2x1H from py), 8.48 (dd, *J*= 4.76, 1.96 Hz, 2H, 2x1H from py); ^{13}C ($CDCl_3$): δ 21.53, 27.19, 48.26, 65.28, 122.50, 127.41, 127.47, 128.53, 128.73, 129.56, 135.35, 140.80, 143.63, 150.44, 150.66, 166.26; MS: m/z (%)= 717 $[M+ 1]^+$ (100), 718 (40), 719 (16), 720 (5); HRMS calcd. for $C_{36}H_{37}N_4O_8S_2$ $[M + 1]^+$ 717.2047; found 717.2033.

7.2.6. Synthesis of pyrido[2,3-d]pyrimidine-2,4(1H,3H)-diones

7.2.6.1. Synthesis of compounds **196** and **197**

General procedure

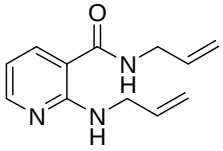
The mixture of 2-propen-1-yl 2-chloro-3-pyridinecarboxylate (0.5g, 0.003mol) in amine (~5 ml) as solvent was refluxed. The reaction mixture was cooled to room temperature, precipitate was filtered off and washed with EtOAc. Solvent was removed under vacuum and product was purified by column chromatography. The synthesis of compound **196i** was carried out in acetonitrile (20ml) as solvent.



Allyl 2-(allylamino)nicotinate (196a);

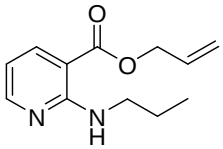
eluent: EtOAc/PE (1/4); 0.199g, 36% yield; light yellow liquid; IR: 3372 (NH), 1658 (C(O)O), 1591, 1579 (C=C), 1508 (C=C), 1241 (C-N), 1126 (C-O). ^{1}H NMR (300 MHz, $CDCl_3$): 4.18 (t, *J*= 5.50 Hz, 2H, CH_2), 4.78 (d, *J*= 5.50 Hz, 2H, CH_2), 5.10-5.46 (m, 4H, 2xCH_2), 5.93-6.11 (m, 2H, 2xCH), 6.54 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 8.07 (s, 1H, NH), 8.16 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.29 (dd, *J*= 6.05, 1.65 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 66.7 (CH_2),

119.3 (=CH_2), 122.2 (C5), 126.9 (C3), 131.4 (-CH=), 140.4 (C4), 150.1 (C2), 152.0 C6), 164.2 (C=O); MS: m/z (%)= 219 $[M+ 1]^+$ (100), 220 (12); HRMS calcd. for $C_{12}H_{15}N_2O_2$ $[M + 1]^+$ 219.1128; found 219.1135.



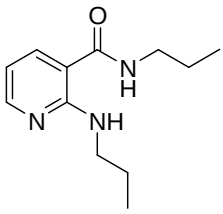
***N*-allyl 2-allylamino-3-pyridinecarboxamide (197a)**;

eluent: EtOAc/PE (1/4); 0.336g, 61% yield; orange liquid; IR: 3327 (NH), 1626 (C(O)NH), 1577 (NH), 1504 (C=C), 1257 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 4.03 (t, *J*= 5.50 Hz, 2H, CH_2), 4.13 (t, *J*= 5.50 Hz, 2H, CH_2), 5.06-5.33 (m, 4H, 2xCH_2), 5.84-6.08 (m, 2H, 2xCH), 6.23 (s, 1H, C(O)NH), 6.48 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.59 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.20 (dd, *J*= 6.05, 1.10 Hz, 1H, py), 8.20 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 42.3, 43.3, 109.9, 110.6, 115.4, 116.9, 134.0, 135.2, 135.3, 151.9, 157.9, 168.3; MS: m/z (%)= 218 $[M+ 1]^+$ (100), 219 (20); HRMS calcd. for $C_{12}H_{16}N_3O$ $[M + 1]^+$ 218.1288; found 218.1297.



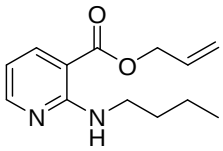
Allyl 2-(*n*-propylamino)-3-pyridinecarboxylate (196b);

eluent: EtOAc/PE (1/4); 0.162g, 29% yield; yellow liquid; IR: 3373 (NH), 1685 (C(O)O), 1593, 1580 (C=C), 1513 (C=C), 1242 (C-N), 1121 (C-O). ^{1}H NMR (300 MHz, $CDCl_3$): 1.01 (t, *J*= 7.15 Hz, 3H, CH_3), 1.67 (q, *J*= 7.15 Hz, 2H, CH_2), 3.47 (q, *J*= 6.2 Hz, 2H, CH_2), 4.77 (d, *J*= 5.50 Hz, 2H, CH_2), 5.29 (dd, *J*= 11.56, 1.10 Hz, 1H, CH_2), 5.39 (dd, *J*= 17.06, 1.1 Hz, 1H, CH_2), 6.00 (dq, *J*= 5.5, 5.5 Hz, 1H, CH), 6.50 (dd, *J*= 13.21, 4.95 Hz, 1H, py), 7.97 (s, 1H, NH), 8.14 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.29 (dd, *J*= 6.60, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 11.8, 22.8, 42.9, 65.3, 105.5, 110.7, 118.3, 132.3, 140.1, 153.9, 158.9, 167.3; MS: m/z (%)= 221 $[M+ 1]^+$ (100), 222 (14); HRMS calcd. for $C_{12}H_{17}N_2O_2$ $[M + 1]^+$ 221.1285; found 221.1293.



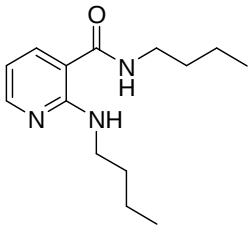
***N*-(*n*-propyl) 2-(*n*-propylamino)-3-pyridinecarboxamide (197b)**;

eluent: EtOAc/PE (1/4); 0.314g, 56% yield; yellow liquid; IR: 3325 (NH), 1625 (C(O)NH), 1577 (NH), 1509 (C-H), 1459 (C-H), 1257 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.98 (t, *J*= 7.15 Hz, 3H, CH_3), 1.00 (t, *J*= 7.15 Hz, 3H, CH_3), 6.07 (s, 1H, C(O)NH), 6.45 (dd, *J*= 12.11, 4.95 Hz, 1H, py), 7.52 (dd, *J*= 8.81, 1.1 Hz, 1H, py), 8.08 (s, 1H, NH), 8.21 (dd, *J*= 6.6, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 11.5, 11.8, 22.7, 22.9, 41.6, 42.9, 110.1, 135.1, 151.7, 158.1, 168.6; MS: m/z (%)= 222 $[M+ 1]^+$ (100), 223 (14); HRMS calcd. for $C_{12}H_{20}N_3O$ $[M + 1]^+$ 222.1601; found 222.1609.



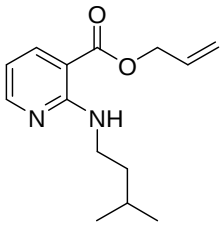
Allyl 2-(*n*-butylamino)-3-pyridinecaboxylate (196c);

eluent: EtOAc/PE (1/4); 0.136g, 23% yield; yellow liquid; IR: 3373 (NH), 2957, 2929, 2871 (C=C-H), 1685 (C(O)O), 1593, 1580 (C=C), 1513 (C-H), 1241 (C-H), 1121 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.96 (t, *J*= 7.15 Hz, 3H, CH_3), 1.38-1.52 (m, 2H, CH_2), 1.59-1.71 (m, 2H, CH_2), 3.51 (dt, *J*= 12.38, 6.88 Hz, 2H, CH_2), 4.77 (d, *J*= 5.50 Hz, 2H, CH_2), 5.29 (dd, *J*= 11.56, 1.10 Hz, 1H, CH_2), 5.39 (dd, *J*= 18.71, 1.65 Hz, 1H, CH_2), 5.94-6.10 (m, 1H, CH), 6.50 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.94 (s, 1H, NH), 8.14 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.29 (dd, *J*= 6.60, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 13.9, 20.4, 31.7, 40.8, 65.3, 105.3, 110.7, 118.3, 132.3, 140.1, 153.9, 158.9, 167.3; MS: m/z (%)= 235 $[M+ 1]^+$ (100), 236 (14); HRMS calcd. for $C_{13}H_{19}N_2O_2$ $[M + 1]^+$ 235.1441; found 235.1439.



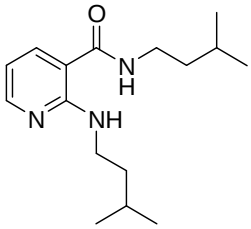
***N*-(*n*-butyl) 2-(*n*-butylamino)-3-pyridinecarboxamide (197c)**;

eluent: EtOAc/PE (1/4); 0.468g, 74% yield; orange liquid; IR: 3324 (NH), 2957, 2929, 2871 (C=C-H), 1624 (C(O)NH), 1577 (C-C), 1510 (C-H), 1258 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.95 (dt, *J*= 7.15, 7.15 Hz, 6H, 2xCH_3), 1.29-1.49 (m, 4H, 2xCH_2), 1.59 (tt, *J*= 7.15 Hz, 4H, 2xCH_2), 3.40 (qt, *J*= 7.15 Hz, 4H, 2xCH_2), 6.13 (br, C(O)NH), 6.44 (dd, *J*= 12.66, 4.95 Hz,12H, py), 7.51 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.05 (br, NH), 8.20 (dd, *J*= 6.05, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 13.8, 14.01, 31.6, 31.7, 39.6, 40.8, 110.0, 134.9, 151.8, 151.1, 168.3; MS: m/z (%)= 250 $[M+ 1]^+$ (100), 251 (17); HRMS calcd. for $C_{14}H_{24}N_3O$ $[M + 1]^+$ 250.1914; found 250.1924.

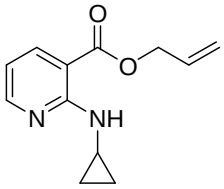


Allyl 2-(*i*-pentyloamino)-3-pyridinecaboxylate (196d);

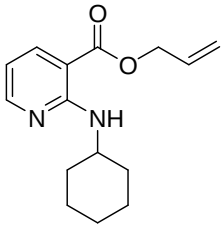
eluent: EtOAc/PE (1/5); 0.13g, 2% yield; yellow liquid; IR: 3372 (NH), 2954, 2927, 2870 (=C-H), 1686 (C(O)O), 1593, 1581 (C=C), 1513 (C-H), 1242 (C-N), 1124 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$): 0.95 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.56 (q, *J*= 7.26 Hz, 2H, CH_2), 1.69-1.79 (m, 1H, CH), 3.52 (dt, *J*= 7.29, 5.19 Hz, 2H, CH_2), 4.77 (d, *J*= 5.60 Hz, 2H, CH_2), 5.28 (ddt, *J*= 10.42, 2.64, 1.3 Hz, 1H, =CH_2), 5.39 (ddt, *J*= 17.19, 2.92, 1.42 Hz, 1H, =CH_2), 6.01 (qt, *J*= 17.17, 10.48, 5.61 Hz, 1H, -CH=), 6.49 (dd, *J*= 12.48, 4.76 Hz, 1H, py), 7.89 (s, 1H, NH), 8.14 (dd, *J*= 9.72, 2.00 Hz, 1H, py), 8.29 (dd, *J*= 6.72, 1.96 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 22.6, 25.4, 38.5, 39.2, 65.2, 105.5, 110.6, 118.2, 132.1, 139.9, 153.7, 158.8, 167.3; MS: m/z (%)= 249 $[M+ 1]^+$ (100), 250 (18); HRMS calcd. for $C_{14}H_{21}N_2O_2$ $[M + 1]^+$ 249.1598; found 249.1608.



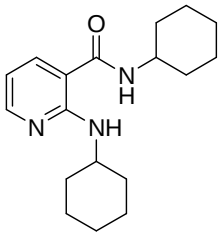
***N*-(*i*-pentyl) 2-(*i*-pentylamino)-3-pyridinecarboxamide (197d)**; eluent: EtOAc/PE (1/5); 0.478g, 68% yield; yellow liquid; IR: 3327 (NH), 2954, 2928, 2869 (=C-H), 1624 (C(O)NH), 1577 (NH), 1510 (C-H), 1257 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.94 (dd, *J*= 3.30, 0.55 Hz, 6H, 2xCH_3), 0.96 (dd, *J*= 3.30, 0.55 Hz, 6H, 2xCH_3), 1.52 (dt, *J*= 7.71, 7.71 Hz, 4H, 2xCH_2), 1.61-1.78 (m, 2H, 2xCH), 3.43 (dt, *J*= 7.15, 7.15 Hz, 4H, 2xCH_2), 6.02 (br, C(O)NH), 6.45 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.50 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.01 (s, 1H, NH), 8.21 (dd, *J*= 6.60, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 22.6, 22.7, 38.2, 38.5, 39.4, 110.0, 134.9, 151.9, 158.1, 168.2; MS: m/z (%)= 278 $[M+ 1]^+$ (100), 279 (19); HRMS calcd. for $C_{16}H_{28}N_3O$ $[M + 1]^+$ 278.2227; found 278.2237.



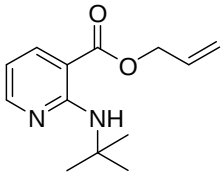
Allyl 2-cyclopropylamino-3-pyridinecaboxylate (196e);
eluent: EtOAc/PE (1/8); 0.172g, 31% yield; yellow liquid; IR: 3370 (NH), 1686 (C(O)O), 1579 (C=C), 1500 (C-H), 1251, 1230 (C-N), 1128 (C-O). ^{1}H NMR (300 MHz, $CDCl_3$): 0.52- 0.62 (m, 2H), 0.86 (dt, *J*= 12.11, 6.05 Hz, 2H, CH_2), 2.86-2.95 (m, 1H, CH), 4.76 (d, *J*= 4.95 Hz, 2H, CH_2), 5.29 (d, *J*= 10.46 Hz, 1H, =CH_2), 5.39 (d, *J*= 17.06 Hz, 1H, =CH_2), 5.93-6.09 (m, 1H, =CH-), 6.58 (dd, *J*= 12.11, 4.40 Hz, 1H, py), 8.05 (s, 1H, NH), 8.15 (d, *J*= 7.71 Hz, 1H, py), 8.39 (d, *J*= 4.40 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 7.2, 23.8, 65.4, 106.1, 111.5, 118.4, 132.2, 139.9, 154.0, 159.8; MS: m/z (%)= 219 $[M+ 1]^+$ (100), 220 (14); HRMS calcd. for $C_{12}H_{15}N_2O_2$ $[M + 1]^+$ 219.1128; found 219.1135.



Allyl 2-cyclohexylamino-3-pyridinecaboxylate (196f);
eluent: EtOAc/PE (1/6); 0.448g, 68% yield; colorless liquid; IR: 3359 (NH), 1685 (C(O)O), 1592, 1578 (C=C), 1507 (C-H), 1241 (C-N), 1106 (C-O). ^{1}H NMR (300 MHz, $CDCl_3$): 1.10-2.13 (m, 10H, cyclohexyl), 4.01-4.16 (m, 1H, CH), 4.77 (d, *J*= 5.50 Hz, 2H, =CH_2), 5.29 (dd, *J*= 11.56, 1.10 Hz, 1H, =CH_2), 5.39 (dd, *J*= 18.71, 1.62 Hz, 1H, CH_2), 5.94-6.10 (m, 1H, -CH=), 6.47 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.96 (d, *J*= 6.60, NH), 8.14 (dd, *J*= 9.91, 2.20 Hz, 1H, py), 8.27 (dd, *J*= 6.60, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 24.9, 25.9, 48.8, 65.2, 105.3, 110.5, 118.3, 132.3, 140.2, 153.9, 158.2, 167.4; MS: m/z (%)= 261 $[M+ 1]^+$ (100), 262 (15); HRMS calcd. for $C_{15}H_{21}N_2O_2$ $[M + 1]^+$ 261.1598; found 261.1607.

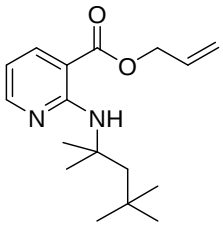


***N*-cyclohexyl 2-cyclohexylamino-3-pyridinecarboxamide (197f)**; eluent: EtOAc/PE (1/6); 0.229g, 30% yield; white solid; mp. 168°C; IR: 3355, 3260 (NH), 2924, 2851 (=C-H), 1617 (C(O)O), 1576, 1552 (C=C), 1503 (C-H), 1258 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 1.10-2.10 (m, 20H, cyclohexyl), 3.88 (tt, *J*= 3.30 Hz, 1H, CH), 4.02 (tt, *J*= 4.40 Hz, 1H, CH), 5.96 (br, C(O)NH), 6.40 (dd, *J*= 12.11, 4.95 Hz, 1H, py), 7.51(dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.11 (d, *J*= 7.15 Hz, 1H, NH), 8.18 (dd, *J*= 5.50, 1.10 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 24.9, 25.0, 25.6, 26.0, 33.2, 33.3, 48.6, 48.8, 109.8, 135.1, 151.8, 157.5, 167.7; MS: m/z (%)= 302 $[M+ 1]^+$ (100), 303 (20); HRMS calcd. for $C_{18}H_{28}N_3O$ $[M + 1]^+$ 302.2227; found 302.2227.



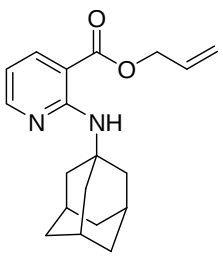
Allyl 2-(*t*-butylamino)-3-pyridinecaboxylate (196g);

eluent: EtOAc/PE (1/7); 0.374g, 63% yield; yellow liquid; IR: 3352 (NH), 1686 (C(O)O), 1591, 1528 (C=C), 1514 (C-H), 1243 (C-N), 1127 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$): 1.50 (s, 9H, 3xCH_3), 4.76 (dt, *J*= 1.57, 1.35 Hz, 1H, CH_2), 4.74 (dt, *J*= 1.57, 1.35 Hz, 1H, CH_2), 5.28 (ddt, *J*= 10.39, 2.64, 1.33 Hz, 1H, =CH_2), 5.38 (ddt, *J*= 17.25, 3.08, 1.56 1H, =CH_2), 6.01 (qt, *J*= 17.19, 10.43, 5.57 Hz, 1H, -CH=), 6.46 (dd, *J*= 12.48, 4.68 Hz, 1H, py), 8.05 (s, 1H, NH), 8.12 (dd, *J*= 7.80, 2.08, 1H, py), 8.25 (dd, *J*= 4.66, 2.06, 1H, py); ^{13}C NMR ($CDCl_3$): δ 29.2, 51.4, 65.1, 105.5, 110.2, 118.2, 132.3, 139.8, 153.2, 158.7, 167.5; MS: m/z (%)= 235 $[M+ 1]^+$ (100), 236 (14); HRMS calcd. for $C_{13}H_{19}N_2O_2$ $[M + 1]^+$ 235.1441; found 235.1447.



Allyl 2-(*t*-octylamino)-3-pyridinecaboxylate (196h);

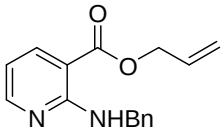
eluent: EtOAc/PE (1/10); 0.537g, 73% yield; orange liquid; IR: 3353 (NH), 1686 (C(O)O), 1592 (C=C), 1515 (C-H), 1243 (C-N), 1126 (C-O). ^{1}H NMR (300 MHz, $CDCl_3$): 0.95 (s, 9H, 3xCH_3), 1.54 (s, 6H, 2xCH_3), 1.97 (s, 2H, CH_2), 4.75 (d, *J*= 5.50 Hz, 2H, CH_2), 5.24-5.44 (m, 2H, CH_2), 5.94-6.09 (m, 1H, -CH=), 6.43 (dd, *J*= 12.11, 4.40 Hz, 1H, py), 8.06 (s, 1H, NH), 8.12 (dd, *J*= 9.91, 2.20, 1H, py), 8.24 (dd, *J*= 6.05, 1.65, 1H, py); ^{13}C NMR ($CDCl_3$): δ 30.1, 31.6, 31.8, 50.7, 55.3, 65.2, 105.4, 110.1, 118.2, 132.3, 139.9, 153.2, 158.7, 167.5; MS: m/z (%)= 291 $[M+ 1]^+$ (100), 292 (20); HRMS calcd. for $C_{17}H_{27}N_2O_2$ $[M + 1]^+$ 291.2067; found 291.2077.



Allyl 2-(1-adamantylamino)-3-pyridinecaboxylate (196i);

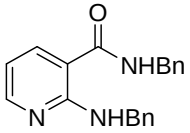
eluent: EtOAc/PE (1/7); 0.404g, 51% yield; white solid; mp. 94°C; IR: 3338 (NH), 2905, 2848 (=C-H), 1686 (C(O)O), 1588 (C=C), 1511 (C-H), 1252 (C-N), 1130 (C-O). ^{1}H NMR (300 MHz, $CDCl_3$): 1.63-1.79 (m, 6H, 3xCH_2), 2.1 (s, 3H, 3xCH), 2.19 (s, 6H, 3xCH_2), 4.75 (d, *J*= 5.50 Hz, 2H, CH_2), 5.27 (dd, *J*= 11.56, 1.10 Hz, 1H, =CH_2), 5.38 (dd, *J*= 18.16, 1.10 Hz, 1H, =CH_2), 5.93-6.07 (m, 1H, -CH=),

6.44 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.97 (s, 1H, NH), 8.11 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.21 (d, *J*= 6.60, 2.20 Hz, 1H, py), 8.39 (d, *J*= 4.40 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 29.8, 36.8, 41.9, 52.2, 65.2, 105.4, 110.3, 118.2, 132.4, 139.9, 153.2, 158.9, 167.6; MS: m/z (%)= 313 $[M+ 1]^+$ (100), 314 (24); HRMS calcd. for $C_{19}H_{25}N_2O_2$ $[M + 1]^+$ 313.1911; found 313.1914.



Allyl 2-benzylamino-3-pyridinecaboxylate (196j);

eluent: EtOAc/ PE (1/3); 0.177g, 26% yield; colorless liquid; IR: 3371 (NH), 3085, 3063, 3028, 2926, 2875 (=C-H), 1685 (C(O)O), 1592, 1578 (C=C), 1509 (C-H), 1242 (C-N), 1131 (C-O). ^{1}H NMR (300 MHz, $CDCl_3$): 4.76 (d, *J*= 5.50, 4H, CH_2 and CH_2-Bn), 5.23-5.45 (m, 2H, =CH_2), 5.92-6.08 (m, 1H, -CH=), 6.56 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.21-7.43 (m, 6H, Bn), 8.17 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.30 (dd, *J*= 6.60, 1.65 Hz, 1H, py), 8.29 (s, NH); ^{13}C NMR ($CDCl_3$): δ 44.9, 65.4, 105.7, 111.4, 118.4, 127.1, 127.6, 127.6, 128.6, 132.2, 139.5, 140.1, 153.9, 167.3; MS: m/z (%)= 269 $[M+ 1]^+$ (100), 270 (18); HRMS calcd. for $C_{16}H_{17}N_2O_2$ $[M + 1]^+$ 269.1285; found 269.1296.



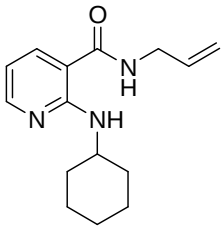
***N*-benzyl 2-benzylamino-3-pyridinecarboxamide (197j)**;

eluent: EtOAc/PE (1/3); 0.451g, 56% yield; white solid; mp. 98°C; IR: 3398, 3297 (NH), 3083, 3058, 3027, 2924, 2852, (=C-H), 1614 (C(O)N), 1573 (N-H), 1519, 1504 (C=C), 1260 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 4.53 (d, *J*= 5.50 Hz, 2H, CH_2), 4.70 (d, *J*= 5.50 Hz, 2H, CH_2), 6.45 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 6.49 (br, C(O)NH), 7.20-7.40 (m, 10H, 2xBn), 7.54 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.20 (d, *J*= 4.95 Hz, 1H, py), 8.50 (br, NH); ^{13}C NMR ($CDCl_3$): δ 43.9, 44.9, 109.8, 110.8, 127.0, 127.6, 127.8, 127.9, 128.6, 128.9, 135.3, 138.0, 139.7, 152.1, 157.9, 168.3; MS: m/z (%)= 318 $[M+ 1]^+$ (100), 319 (20); HRMS calcd. for $C_{20}H_{20}N_3O$ $[M + 1]^+$ 318.1601; found 318.1608.

7.2.6.2. Synthesis of compounds **199**

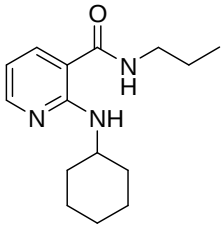
General procedure

The mixture of allyl 2-(alkylamino)-3-pyridinecaboxylate (0.5g, 0.003mol) in amine (~5 ml) as solvent was refluxed. The reaction mixture was cooled to room temperature, precipitate was filtered off and washed with EtOAc. Solvent was removed under vacuum and product was purified by column chromatography.



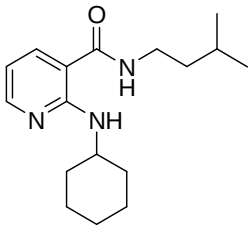
***N*-allyl 2-cyclohexylamino-3-pyridinecarboxamide (199a)**;

eluent: EtOAc/PE (1/8); 0.269g, 54% yield; white solid; mp. 96°C; IR: 3357 (NH), 3285 (NH), 1620 (C(O)N), 1573, 1551 (C=C), 1503 (C-H), 1252 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 1.19-1.49 (m, 4H, cyclohexyl), 1.53-1.80 (m, 2H), 1.92-2.07 (m, 2H), 4.03 (t, *J*= 5.50 Hz, 3H, CH_2 from CH_2-CH= and CH-cychex), 5.15-5.31 (m, 2H, =CH_2), 5.85-6.00 (m, 1H, -CH=), 6.11 (br, C(O)NH), 6.43 (dd, *J*= 12.11, 4.95 Hz, 1H, py), 7.55 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.12 (d, *J*= 6.05, NH), 8.20 (d, *J*= 3.30 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 24.9, 26.0, 33.2, 42.2, 48.8, 109.2, 109.9, 116.9, 134.1, 135.2, 152.2, 157.5, 168.4; MS: m/z (%)= 260 $[M+ 1]^+$ (100), 261 (18); HRMS calcd. for $C_{15}H_{22}N_3O$ $[M + 1]^+$ 260.1757; found 260.1761.

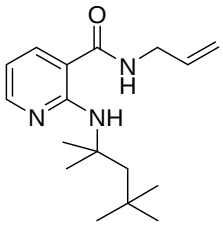


***N*-(*n*-propyl) 2-cyclohexylamino-3-pyridinecarboxamide (199b)**;

eluent: EtOAc/PE (1/6); 0.276g, 55% yield; white solid; mp. 119°C; IR: 3360 (NH), 3300 (NH), 2923, 2852 (C=C-H), 1620 (C(O)N), 1573 (C=C), 1500 (C-H), 1252 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.97 (t, *J*= 7.71 Hz, 3H, CH_3), 1.18-1.50 (m, 4H, cyclohexyl), 1.60 (dq, *J*= 14.31, 7.15 Hz, 2H, CH_2), 1.68-1.80 (m, 2H, cyclohexyl), 1.95-2.08 (m, 2H, cyclohexyl), 3.35 (dd, *J*= 19.81, 6.60 Hz, 2H, CH_2), 4.00 (tt, *J*= 3.85 Hz, 1H, CH from cyclohexyl), 6.13 (br, C(O)NH), 6.41 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.52 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.09 (d, *J*= 6.60 Hz, 1H, NH), 8.18 (dd, *J*= 6.60, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 11.5, 22.9, 24.9, 26.0, 33.2, 41.6, 48.8, 109.8, 109.8, 135.1, 151.9, 157.5, 168.6; MS: m/z (%)= 262 $[M+ 1]^+$ (100), 263 (18); HRMS calcd. for $C_{15}H_{24}N_3O$ $[M + 1]^+$ 262.1914; found 262.1918.

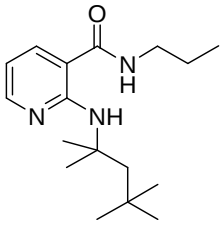


***N*-(*i*-pentyl) 2-cyclohexylamino-3-pyridinecarboxamide (199c)**; eluent: EtOAc/PE (1/7); 0.472g, 85% yield; orange oil; IR: 3320 (NH), 2954, 2928, 2854 (C=C-H), 1626 (C(O)N), 1576 (C=C), 1507 (C-H), 1257 (C-N). ^{1}H NMR (400 MHz, $CDCl_3$): 0.95 (d, *J*= 6.65 Hz, 6H, 2xCH_3 *i*-pentyl), 1.24-1.35 (m, 4H, 2xCH_2, cyclohexyl), 1.38-1.46 (m, 2H), 1.46-1.52 (m, 2H, CH_2 *i*-pentyl), 1.57-1.65 (m, 1H, *i*-pentyl), 1.70-1.78 (m, 2H, cyclohexyl), 3.41 (dt, *J*= 5.95 Hz, 2H, *i*-pentyl), 3.98-4.06 (m, 1H cyclohexyl), 5.93 (br, C(O)NH), 6.41 (dd, *J*= 12.40, 4.80 Hz, 1H, py), 7.49 (dd, *J*= 9.40, 1.80 Hz, 1H, py), 8.08 (br, NH), 8.19 (dd, *J*= 6.55, 1.75 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ ; MS: m/z (%)= 290 $[M+ 1]^+$ (100), 291 (20); HRMS calcd. for $C_{17}H_{28}N_3O$ $[M + 1]^+$ 290.2227; found 290.2238.



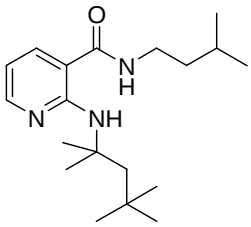
***N*-allyl 2-(*t*-octylamino)-3-pyridinecarboxamide (199e)**;

eluent: EtOAc/PE (1/8); 0.369g, 74% yield; white solid; mp. 65°C; IR: 3314 (NH), 3270 (NH), 2949, 2903, 2860 (C=C-H), 1620 (C(O)N), 1577 (C=C), 1513 (C-H), 1261 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.95 (s, 9H, 3xCH_3), 1.52 (s, 6H, 2xCH_3), 1.95 (s, 2H, CH_2), 4.02 (t, *J*= 5.5 Hz, 2H, CH_2), 5.13-5.30 (m, 2H, =CH_2), 5.84-5.96 (m, 1H, -CH=), 5.98 (s, 1H, C(O)NH), 6.39 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.51 (dd, *J*= 10.46, 2.20 Hz, 1H, py), 8.18 (dd, *J*= 6.60, 2.20 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 30.1, 31.6, 31.8, 42.2, 50.6, 55.0, 109.4, 116.8, 134.1, 134.8, 151.4, 157.9, 168.7; MS: m/z (%)= 290 $[M+ 1]^+$ (100), 291 (20); HRMS calcd. for $C_{17}H_{28}N_3O$ $[M + 1]^+$ 290.2227; found 290.2228.



***N*-(*n*-propyl) 2-(*t*-octylamino)-3-pyridinecarboxamide (199f)**;

eluent: EtOAc/PE (1/7); 0.341g, 68% yield; white solid; mp. 73°C; IR: 3348 (NH), 3276 (NH), 2954, 2871 (C=C-H), 1622 (C(O)N), 1581, 1553 (C=C), 1512 (C-H), 1263 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.95 (s, 9H, 3xCH_3), 1.52 (s, 6H, 2xCH_3), 0.96 (t, *J*= 8.26 Hz, 3H, CH_3), 1.60 (dq, *J*= 7.15, 7.15 Hz, 2H, CH_2), 1.95 (s, 2H, CH_2), 3.33 (dd, *J*= 19.81, 6.60 Hz, 2H, CH_2), 6.09 (s, 1H, C(O)NH), 6.36 (dd, *J*= 12.11, 4.40 Hz, 1H, py), 7.48 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.10 (s, 1H, NH), 8.15 (dd, *J*= 6.05, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 11.5, 22.9, 30.1, 31.6, 31.8, 41.6, 50.6, 54.9, 109.4, 110.1, 134.8, 151.1, 157.9, 168.9; MS: m/z (%)= 292 $[M+ 1]^+$ (100), 293 (20); HRMS calcd. for $C_{17}H_{30}N_3O$ $[M + 1]^+$ 292.2383; found 292.2388.

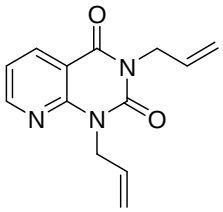


***N*-(*i*-pentyl) 2-(*t*-octylamino)-3-pyridinecarboxamide (199g)**;

eluent: EtOAc/PE (1/10); 0.482g, 85% yield; yellow solid; mp. 68°C; IR: 3286 (NH), 2955, 2870 (C=C-H), 1620 (C(O)N), 1578 (C=C), 1515 (C-H), 1264, 1223 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.95 (s, 9H, 3xCH3), 1.52 (s, 6H, 2xCH3), 1.41-1.58 (m, 2H), 1.59-1.73 (m, 1H), 1.95 (s, 2H), 3.38 (dt, *J*= 6.05, 6.05 Hz, 2H, CH_2), 6.09 (br, C(O)NH), 6.35 (dd, *J*= 12.11, 4.95 Hz, 1H, py), 7.48 (dd, *J*= 7.71 Hz, 1H, py), 8.13 (d, *J*= 6.60 Hz, 1H, py), 8.15 (br, NH); ^{13}C NMR ($CDCl_3$): δ14.3, 22.6, 26.0, 30.1, 31.6, 31.8, 38.2, 38.5, 50.6, 54.9, 109.4, 110.1, 134.8, 151.1, 157.9, 168.9; MS: m/z (%)= 320 $[M+ 1]^+$ (100), 321 (24); HRMS calcd. for $C_{19}H_{34}N_3O$ $[M + 1]^+$ 320.2696; found 320.2703.

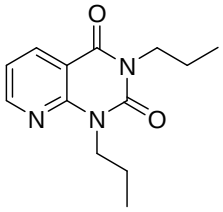
7.2.6.3. General method for the synthesis of pyrido[2,3-*d*]pyrimidines

To a solution of *N*-alkyl-2-(alkylamino)-3-pyridinecarboxamide in a mixture of dry THF and NMP (2:3 ratio), NaH (3eq) and CDI (3eq) were added. The reaction mixture was stirred at room temperature for 15-19h. After this time, solid was filtered off and washed with THF, filtrate was concentrated under vacuum. The desired product was isolated by column chromatography.



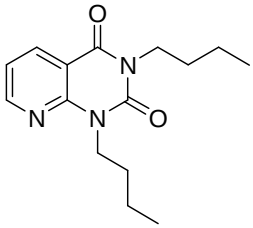
1,3-Diallyl-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (198a);

eluent: EtOAc/PE (1/3); 69% yield; white solid; mp. 91°C; IR: 2986, 2923, 2853 (C=C-H), 1708 (NC(O)N), 1657 (C(O)N), 1595 (C=C), 1482, 1462, (C=C). ^{1}H NMR (300 MHz, $CDCl_3$): 4.70 (d, *J*= 5.5 Hz, 2H, CH_2), 4.99 (d, *J*= 5.50 Hz, 2H, CH_2), 5.17-5.37 (m, 4H, 2xCH_2), 5.876.09 (m, 2H, 2x-CH=), 7.22 (dd, *J*= 12.11, 4.40 Hz, 1H, py), 8.47 (dd, *J*= 8.81, 1.10 Hz, 1H, py), 8.67 (dd, *J*= 5.50, 1.10 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 43.9, 44.5, 110.9, 117.8, 118.3, 119.1, 131.6, 132.0, 137.9, 150.5, 150.6, 154.3, 160.9; MS: m/z (%)= 244 $[M+ 1]^+$ (100), 245 (13); HRMS calcd. for $C_{13}H_{14}N_3O_2$ $[M + 1]^+$ 244.1081; found 244.1084.



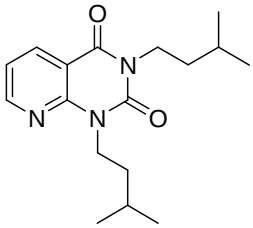
1,3-Dipropyl-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (198b);

eluent: EtOAc/PE (1/4); 58% yield; yellow liquid; IR: 1709 (NC(O)N), 1659 (C(O)N), 1597 (C=C), 1484, 1461, 1346 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 0.98 (t, *J*= 7.15 Hz, 3H, CH_3), 1.00 (t, *J*= 7.15 Hz, 3H, CH_3), 1.68-1.83 (m, 4H, 2xCH_2), 4.05 (dd, *J*= 15.41, 7.71 Hz, 2H, CH_2), 4.31 (dd, *J*= 15.41, 7.71 Hz, 2H, CH_2), 7.19 (dd, *J*= 12.11, 4.40 Hz, 1H, py), 8.46 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.65 (dd, *J*= 6.60, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 11.3, 11.4, 21.1, 21.2, 43.4, 44.1, 110.9, 118.7, 137.7, 150.8, 154.1, 161.4; MS: m/z (%)= 248 $[M+ 1]^+$ (100), 249 (17); HRMS calcd. for $C_{13}H_{18}N_3O_2$ $[M + 1]^+$ 248.1394; found 248.1405.



1,3-Dibutyl-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (198c);

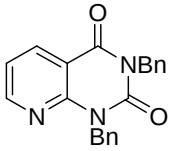
eluent: EtOAc/PE (1/3); 61% yield; yellow liquid; IR: 2958, 2930, 2871 (=C-H), 1710 ((NC(O)N), 1663 (C(O)N), 1598 (C=C), 1484, 1462, 1431, 1405, 1348 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 0.98 (dt, *J*= 3.85 Hz, 6H, 2xCH_3), 1.42 (qt, *J*= 7.15 Hz, 4H, 2xCH_2), 1.63-1.76 (m, 4H, 2xCH_2), 4.08 (t, *J*= 7.71 Hz, 2H, CH_2), 4.35 (t, *J*= 7.71 Hz, 2H, CH_2), 7.19 (dd, *J*= 12.11, 4.40 Hz, 1H, py), 8.45 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.65 (dd, *J*= 6.60, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 13.8, 13.9, 20.2, 20.3, 29.9, 30.1, 41.8, 42.5, 110.9, 118.7, 137.6, 150.8, 150.9, 154.0, 161.3; MS: m/z (%)= 276 $[M+ 1]^+$ (100), 277 (18); HRMS calcd. for $C_{15}H_{22}N_3O_2$ $[M + 1]^+$ 276.1707; found 276.1716.



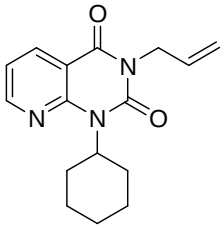
1,3-Di(*i*-pentyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (198d);

eluent: EtOAc/PE (1/5); 91% yield; white solid; mp. 48°C; IR: 2954, 2928, 2881, 2867 (=C-H), 1702 (NC(O)N), 1656 (C(O)N), 1592 (C=C), 1483, 1466, 1460, 1351 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 0.99 (t, *J*= 5.50 Hz, 12H, 4xCH_3), 1.52-1.66 (m, 4H, 2xCH_2), 1.66-1.77 (m, 4H, 2xCH_2), 4.07 (d, *J*= 6.60 Hz, 1H, CH), 4.10 (d, *J*= 6.60 Hz,14H, CH), 4.19 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 8.45 (dd, *J*= 9.36,

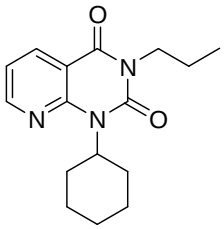
1.65 Hz, 1H, py), 8.65 (dd, *J*= 6.05, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 22.5, 22.6, 26.3, 26.4, 36.5, 36.5, 40.6, 40.9, 110.9, 118.6, 137.5, 150.7, 150.8, 154.0, 161.2; MS: m/z (%)= 304 $[M+ 1]^+$ (100), 305 (18); HRMS calcd. for $C_{17}H_{25}N_3O_2$ $[M + 1]^+$ 304.2020; found 304.2022.



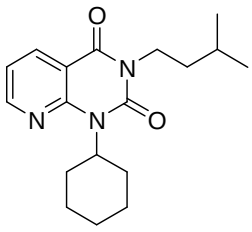
1,3-Dibenzyl-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (198j);
eluent: EtOAc/PE (1/3); 60% yield; white solid; mp. 120°C; IR: 3088, 3065, 3028 (=C-H), 1711 (NC(O)N), 1658 (C(O)NH), 1592 (C=C), 1484, 1465, 1450 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 5.25 (s, 2H, CH_2), 5.54 (s, 2H, CH_2), 7.14 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 7.18-7.33 (m, 5H, Bn), 7.43-7.55 (m, 5H, Bn), 8.43 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.61 (dd, *J*= 6.05, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 45.2, 45.5, 110.9, 119.2, 127.7, 127.8, 128.5, 128.6, 128.7, 129.2, 136.7, 137.0, 137.9, 150.8, 151.3, 154.2, 161.3; MS: m/z (%)= 344 $[M+ 1]^+$ (100), 345 (23); HRMS calcd. for $C_{21}H_{18}N_3O_2$ $[M + 1]^+$ 344.1394; found 344.1397.



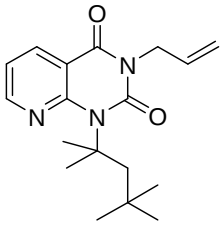
3-Allyl-1-cyclohexyl-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (200a);
eluent: EtOAc/PE (1/8); 47% yield; white solid; mp. 141°C; IR: 2925, 2852 (C=C-H), 1709 (NC(O)N), 1658 (C(O)N), 1589 (C=C), 1485, 1447, 1408 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 1.16-2.00 (m, 8H, cyclohexyl), 2.59 (dt, *J*= 11.56 Hz, 2H, cychexyl), 4.68 (d, *J*= 4.95 Hz, 2H, CH_2 from -CH_2-CH=), 5.17-5.33 (m, 2H, =CH_2), 5.39 (br, CH-cyclohexyl), 5.86-6.03 (m, 1H, -CH=), 7.19 (dd, *J*= 12.11, 4.95 Hz, 1H, py), 8.46 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.65 (dd, *J*= 6.05, 1.65); ^{13}C NMR ($CDCl_3$): δ 25.4, 26.6, 29.0, 29.8, 43.8, 55.4, 111.1, 118.1, 118.7, 131.8, 137.9, 151.2, 153.6, 161.2; MS: m/z (%)= 286 $[M+ 1]^+$ (100), 287 (16); HRMS calcd. for $C_{10}H_{10}N_3O_2$ $[M + 1]^+$ 204.0768; found 204.0773. Literature: K. Noda, A. Nakagawa, T. Motomura, S. Yamasaki, DE 2334266 A1 19740131



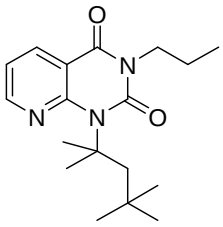
3-Propyl-1-cyclohexyl-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (200b);
eluent: EtOAc/PE (1/6); 91% yield; white solid; mp. 133°C; IR: 2925, 2852 (C=C-H), 1709 (NC(O)N), 1656 (C(O)N), 1589 (C=C), 1448 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 0.98 (t, *J*= 7.15 Hz, 3H, CH_3), 1.22-1.55 (m, 4H, cyclohexyl), 1.60-1.79 (m, 2H, CH_2), 1.81-1.97 (m, 2H, cyclohexyl), 2.59 (dt, *J*= 11.56 Hz, 2H, CH-cyclohexyl), 4.02 (t, *J*= 7.15 Hz, 2H, CH_2), 7.17 (dd, *J*= 12.11, 4.40 Hz, 1H, py), 8.46 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.64 (dd, *J*= 6.04, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 11.7, 21.2, 25.5, 26.6, 28.9; 30.4, 43.4, 111.2, 118.6, 137.8, 151.2, 153.4, 161.5; MS: m/z (%)= 288 $[M+ 1]^+$ (100), 289 (18); HRMS calcd. for $C_{16}H_{22}N_3O_2$ $[M + 1]^+$ 288.1707; found 288.1713. Literature: K. Noda, A. Nakagawa, T. Motomura, S. Yamasaki, DE 2334266 A1 19740131.



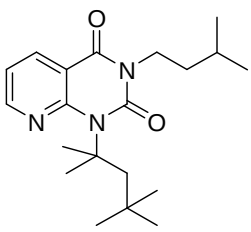
3-(*i*-Pentyl)-1-cyclohexyl-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (200c); eluent: EtOAc/PE (1/7); 97% yield; white solid; mp. 118°C; IR: 2922, 254 (C=C-H), 1708 (NC(O)N), 1656 (C(O)N), 1588 (C=C), 1484, 1449, 1411, 1381 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 0.98 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.26-1.38 (m, 1H, CH-cyclohexyl), 1.39-1.52 (m, 2H, CH_2-cyclohexyl), 1.57 (dt, *J*= 7.89 Hz, 2H, CH_2 *i*-pentyl), 1.65-1.75 (m, 4H, 2xCH_2- cyclohexyl), 1.84-1.94 (m, 2H, CH_2-cyclohexyl), 4.07 (dt, *J*= 5.20, 5.20 Hz, 2H, CH_2 *i*-pentyl), 7.18 (dd, *J*= 12.44, 4.72 Hz, 1H, py), 8.45 (dd, *J*= 9.72, 2.00 Hz, 1H, py), 8.64 (dd, *J*= 6.72, 2.00 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 22.5, 24.9, 25.9, 26.0, 33.1, 38.2, 38.4, 48.7, 109.7, 109.8, 135.0, 151.8, 157.4, 168.5; MS: m/z (%)= 316 $[M+ 1]^+$ (100), 317 (23); HRMS calcd. for $C_{18}H_{26}N_3O_2$ $[M + 1]^+$ 316.2020; found 316.2028.



3-Allyl-1-(*t*-octyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (200e); eluent: EtOAc/PE (1/8); 49% yield; yellow liquid; IR: 2986, 2950, 2868 (C=C-H), 1715 (NC(O)N), 1668 (C(O)N), 1598, 1579 (C=C), 1435 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 0.98 (s, 9H, 3xCH_3), 1.87 (s, 6H, 2xCH_3), 2.40 (s, 2H, CH_2), 4.63 (d, *J*= 5.50 Hz, 2H, CH_2), 5.15-5.31 (m, 2H, =CH_2), 5.86-6.01 (m, 1H, -CH=), 7.15 (dd, *J*= 12.38, 4.95 Hz, 1H, py), 8.39 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.61 (dd, *J*= 6.05, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 31.4, 31.9, 32.1, 43.9, 51.3, 68.1, 112.6, 117.5, 118.4, 132.0, 137.0, 151.4, 151.6, 152.7, 161.6; MS: m/z (%)= 204 $[M+ 1]^+$ (100), 205 (13); HRMS calcd. for $C_{10}H_{10}N_3O_2$ $[M + 1]^+$ 204.0768; found 20774.



3-Propyl-1-(*t*-octyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (200f); eluent: EtOAc/PE (1/10); 97% yield; light yellow liquid; IR: 2956, 2874 (C=C-H), 1715 (NC(O)N), 1667 (C(O)N), 1598, 1580 (C=C), 1430 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 0.97 (t, *J*= 8.26, 3H, CH_3), 0.98 (s, 9H, 3xCH_3), 1.69 (qd, *J*= 7.71, 7.71 Hz, 2H, CH_2), 87 (s, 6H, 2xCH_3), 2.40 (s, 2H, CH_2), 3.98 (t, *J*= 7.71Hz, 2H, CH_2), 7.14 (dd, *J*= 12.66, 4.95 Hz, 1H, py), 8.37 (dd, *J*= 9.36, 1.65 Hz, 1H, py), 8.59 (dd, *J*= 6.60, 2.20 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 11.4, 21.3, 31.4, 31.9, 32.1, 45.5, 51.3, 67.9, 76.7, 77.1, 77.5, 112.8, 118.4, 136.9, 151.5, 151.8, 152.7, 161.9; MS: m/z (%)= 206 $[M+ 1]^+$ (100); HRMS calcd. for $C_{10}H_{12}N_3O_2$ $[M + 1]^+$ 206.0924; found 206.0924.



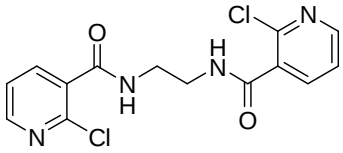
3-(*i*-Pentyl)-1-(*t*-octyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (200g); eluent: EtOAc/PE (1/7); 86% yield; yellow liquid; IR: 2922, 2854 (C=C-H), 1708 (NC(O)N), 1656 (C(O)N), 1588 (C=C), 1449 (C-H). ^{1}H NMR (400 MHz, $CDCl_3$): 0.97 (s, 3H), 0.98 (s, 9H, 3xCH_3), 0.99 (s, 3H), 1.50-1.55 (m, 1H), 1.60-1.72 (m, 1H), 1.87 (s, 6H), 2.40 (s, 2H), 4.03 (dt, *J*= 4.98, 7.80 Hz, 2H, CH_2), 7.14 (dd, *J*= 12.36, 4.68 Hz, 1H, py), 8.37 (dd, *J*= 9.76, 2.04 Hz, 1H, py), 8.59 (dd, *J*= 6.68,

2.04 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 22.5, 26.4, 31.3, 31.8, 32.0, 36.6, 40.7, 51.2, 67.8, 112.7, 118.3, 136.8, 151.4, 151.6, 152.6, 161.7; MS: m/z (%)= 234 $[M+ 1]^+$ (100), 235 (6); HRMS calcd. for $C_{12}H_{15}N_3O_2$ $[M + 1]^+$ 234.1237; found 234.1237.

7.2.6.4. Synthesis of dipyrido[2,3-*d*]pyrimidines

7.2.6.4.1. Synthesis of *N*,*N*'-1,2-ethanediyl-bis[2-chloro-3-pyridinecarboxamide] **202**

The 2-chloro-3-pyridinecarboxylic acid (1.6g, 0.0102mol) in thionyl chloride (25 ml) was stirred at reflux for 2h. The excess of thionyl chloride was removed under vacuum and the residue dried on vacuum pomp for 3h. The 2-chloro-3-pyridinecarbonyl chloride (1g, 0.0057mol) was placed in the flask, and then the flask in the ice-bath. To the stirred 2-chloro-3-pyridinecarbonyl chloride, ethylenediamine (0.17g, 0.0028mol) in CH_2Cl_2 (10 ml) was added dropwise. The mixture was stirred at room temperature for 17h. The white precipitate was filtered off, washed with CH_2Cl_2 and dried. The *N*,*N*'-1,2-ethanediyl-bis[2-chloro-3-pyridinecarboxamide] was obtained as white solid in 60% yield.

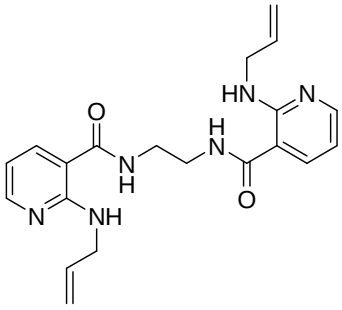


***N*,*N*'-1,2-ethanediyl-bis[2-chloro-3-pyridinecarboxamide] (202)**; mp. 232°C; IR: 3216 (NH), 1652 (C(O)N), 1542, 1404 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6): 3.43 (dd, *J*= 2.80 Hz, 4H, 2xCH_2), 7.51 (dd, *J*= 7.52, 4.84 Hz, 1H, py), 7.94 (dd, *J*= 7.52, 1.92 Hz, 1H, py), 8.47 (dd, *J*= 4.82, 1.94 Hz, 1H, py), 8.72 (br, NH, 2H); ^{13}C (DMSO-d_6): δ 36.96, 123.49, 133.56, 138.62, 146.99, 150.71, 165.73; MS: m/z (%)= 339 $[M+ 1]^+$ (100), 340 (16), 341 (65), 342 (12); HRMS calcd. for $C_{14}H_{13}Cl_2N_4O_2$ $[M + 1]^+$ 339.0405; found 339.0405.

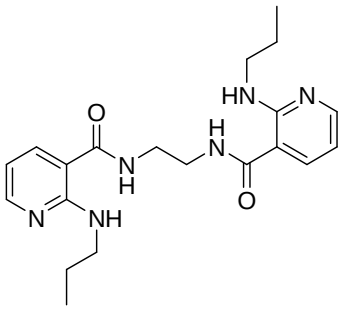
7.2.6.4.2. Synthesis of *N*,*N*'-1,2-ethanediyl-bis[2-alkylamino-3-pyridinecarboxamide] **202**

General procedure

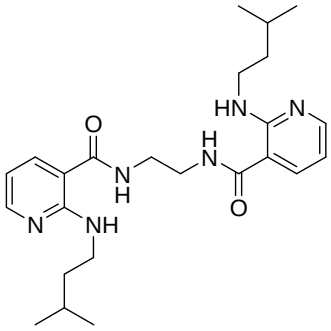
The *N*,*N*'-1,2-ethanediyl-bis[2-chloro-3-pyridinecarboxamide] (0.2g, 0.0006mol) in alkylamine (5 ml) was stirred at reflux for 32-68h. The solvent was removed and product isolated by column chgromatography.



***N,N'*-1,2-ethanediyl-bis[2-allylamino-3-pyridinecarboxamide] (203a)**; eluent: EtOAc; 0.122g, 54%; light yellow solid; mp. 136°C; IR: 3413, 3308 (NH), 1618 (C(O)NH), 1578, 1537, 1508 (C=C), 1256 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 3.66 (dt, *J*= 2.50 Hz, 4H, 2xCH_2), 4.13 (tt, *J*= 5.36, 1.68 Hz, 4H, 2x$\underline{CH_2}$-CH), 5.12 (ddt, *J*= 10.30, 3.08, 1.54 Hz, 2H, 2x =CH_2), 5.25 (ddt, *J*= 17.19, 3.33, 1.75 Hz, 2H, 2x =CH_2), 5.99 (qt, *J*= 17.19, 10.34, 5.19 Hz, 2H, 2x-CH=), 6.49 (dd, *J*= 7.68, 4.84 Hz, 2H, 2xpy), 7.00 (br, C(O)N$\underline{H}$), 7.61 (dd, *J*= 7.74; 1.78 Hz, 2H, 2xpy), 8.23 (dd, *J*= 4.80, 1.76 Hz, 2H, 2xpy), 8.26 (br, N$\underline{H}$-CH_2CH=CH_2); ^{13}C ($CDCl_3$): δ 40.84, 43.23, 109.11, 110.81, 115.33, 135.21, 135.55, 152.21, 157.79, 169.76; MS: m/z (%)= 381 $[M+ 1]^+$ (100), 382 (25); HRMS calcd. for $C_{20}H_{25}N_6O_2$ $[M + 1]^+$ 381.2028; found 381.2041.



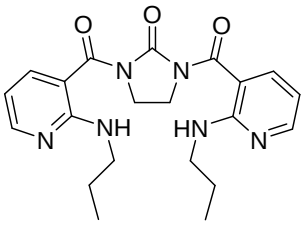
***N,N'*-1,2-ethanediyl-bis[2-propylamino-3-pyridinecarboxamide] (203b)**; eluent: EtOAc; 0.141g, 62%; light yellow solid; mp. 135°C; IR: 3243 (NH), 1635 (C(O)NH), 1574, 1538, 1508 (C=C), 1258, 1159 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.99 (t, *J*= 7.40 Hz, 6H, 2xCH_3), 1.61 (m, 4H, 2xCH_2, -$\underline{CH_2}$-CH_3), 3.42 (dt, *J*= 7.00 Hz, 4H, 2x-$\underline{CH_2}$-CH_2), 3.63 (dd, *J*= 2.84, 2.24 Hz, 4H, 2xCH_2), 6.43 (dd, *J*= 7.68, 4.80 Hz, 2H, py), 6.94 (br, C(O)-$\underline{NH}$), 7.57 (dd, *J*= 7.70, 1.82 Hz, 2H, py), 8.12 (br, NH-prop), 8.21 (dd, *J*= 4.80, 1.18 Hz, 2H, py); ^{13}C ($CDCl_3$): δ 11.59, 22.71, 40.84, 42.79, 108.92, 110.0976, 135.18, 152.26, 158.31, 169.76; MS: m/z (%)= 385 $[M+ 1]^+$ (100), 386 (24); HRMS calcd. for $C_{20}H_{29}N_6O_2$ $[M + 1]^+$ 385.2341; found 385.2349.



***N,N'*-1,2-ethanediyl-bis[2-*i*-pentylamino-3-pyridinecarboxamide] (203c)**; eluent: EtOAc; 0.154g, 59%; light yellow solid; mp. 150°C; IR: 3353 (NH), 1624 (C(O)NH), 1569, 1510 (C=C), 1260 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 0.94 (s, 6H, 2xCH_3), 0.96 (s, 6H, 2xCH_3), 1.53 (dt, *J*= 14.68 Hz, 4H, 2xCH_2), 1.68-1.78 (m, 2H, 2xCH), 3.46 (dt, *J*= 7.34 Hz, 4H, 2xCH_2), 3.64 (dt, *J*= 2.46 Hz, 4H, 2xCH_2), 6.46 (dd, *J*= 7.73, 4.80 Hz, 2H, 2xpy), 6.99 (br, C(O)N$\underline{H}$), 7.59 (dd, *J*= 7.73, 1.76 Hz, 2H, 2xpy), 8.09 (br, NH-*i*-pentyl), 8.23 (dd, *J*= 4.82, 1.78 Hz, 2H, 2xpy); ^{13}C ($CDCl_3$): δ 22.65, 26.04, 38.46, 39.30, 40.95, 108.77, 110.15, 135.30, 152.38, 158.14, 169.78; MS: m/z (%)= 441 $[M+ 1]^+$ (100), 442 (29); HRMS calcd. for $C_{24}H_{37}N_6O_2$ $[M + 1]^+$ 441.2967; found 441.2964.

7.2.6.4.3. Synthesis of 1,3-bis-(2-propylaminopyridine-3-carbonyl)-imidazolidin-2-one **205**

To the cold solution of *N*,*N*'-1,2-ethanediyl-bis[2-propylamino-3-pyridinecarboxamide (0.2g, 0.0005mol) in dry CH_2Cl_2 (30 ml), DIPEA (0.403g, 0.003mol) was added and the mixture was stirred at 0°C for 15min. The solution of triphosgene (0.371g, 0.0012mol) in CH_2Cl_2 (5 ml) was added dropwise. The reaction mixture was stirred at room temperature for 1h. The mixture of CH_2Cl_2 and H_2O was added and the organic phase was separated. The water phase was extracted with CH_2Cl_2 (2x). The combined organic phases were washed with saturated solution of NaCl, dried over $MgSO_4$, filtered and concentrated. The compound was separated by preparative TLC using EtOAC/PE (1:1) as eluent.

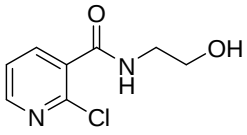


1,3-Bis-(2-propylaminopyridine-3-carbonyl)-imidazolidin-2-one (205); eluent: EtOAc/PE (1:1); 0.02g, 9%; light yellow oil; IR: 3354 (NH), 1743 (NC(O)N), 1649 (C(O)NH), 1595, 1578, 1460 (C=C), 1220 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 1.01 (t, *J*= 7.42 Hz, 6H, 2xCH_3), 1.62-1.72 (m, 4H, 2xCH_2), 3.45 (dt, *J*= 7.07 Hz, 4H, 2x-$\underline{CH_2}$-CH_2-), 4.00 (s, 4H, 2xCH_2), 6.45 (dd, *J*= 7.82, 4.74 Hz, 2H, 2x1H from py), 7.52 (t, *J*= 5.22 Hz, 2H, 2xNH), 7.64 (dd, *J*= 7.84, 1.92 Hz, 2H, 2x1H from py), 8.24 (dd, *J*= 4.72, 1.92 Hz, 2H, 2x1H from py); ^{13}C ($CDCl_3$): δ 11.66, 22.69, 40.67, 42.95, 107.39, 110.19, 141.87, 151.40, 154.18, 158.27, 170.45; MS: m/z (%)= 411 $[M+ 1]^+$ (100), 412 (24); HRMS calcd. for $C_{21}H_{27}Br_2N_6O_3$ $[M + 1]^+$ 411.21392; found 411.2127.

7.2.6.5. Synthesis of 3-(alkyl/aromatic)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione

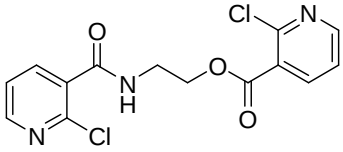
7.2.6.5.1. Synthesis of 2-chloro-*N*-(2-hydroxyethyl)-nicotinamide **207** and 2-chloronicotinic acid 2-[(2-chloropyridine-3-carbonyl)-amino]-ethyl ester **206**

The 2-chloro-3-pyridinecarboxylic acid (1.6g, 0.0102mol) in thionyl chloride (25 ml) was stirred at reflux for 2h. The excess of thionyl chloride was removed under vacuum and dried on vacuum pomp for 3h. The 2-chloro-3-pyridinecarbonyl chloride (1g, 0.0057mol) was placed in the flask, and then the flask in the ice-bath. To the stirred 2-chloro-3-pyridinecarbonyl chloride, ethanolamine (0.87g, 0.0.014mol) in CH_2Cl_2 (10 ml) was added dropwise. The mixture was stirred at room temperature for 17h. The solvent was removed and product isolated by column chromatography using EtOAc as eluent.



2-Chloro-*N*-(2-hydroxyethyl)-nicotinamide (207);

98% (1.11g); white solid; mp. 89°C; IR: 3299, 3251 (NH), 1646 (C(O)NH), 1584, 1544, 1403 (C=C), 1058 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$): 3.66 (dt, *J*= 10.28, 10.40 Hz, 2H, $\underline{CH_2}$-OH), 3.86 (dt, *J*= 9.76, 9.88 Hz, 2H, NH-$\underline{CH_2}$), 7.00 (br, C(O)NH), 7.35 (dd, *J*= 7.64, 4.76 Hz, 1H, py), 8.09 (dd, *J*= 7.64, 1.84 Hz, 1H, py), 8.46 (dd, *J*= 4.76, 1.96 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 42.79, 61.72, 122.79, 131.05, 139.73, 147.17, 151.07, 165.47; MS: m/z (%)= 201 $[M+ 1]^+$ (100), 202 (10), 203 (30); HRMS calcd. for $C_8H_{10}ClN_2O_2$ $[M + 1]^+$ 201.0425; found 201.0427.



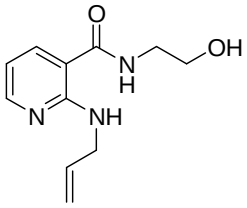
2-Chloro-nicotinic acid 2-[(2-chloro-pyridine-3-carbonyl)-amino]-ethyl ester (206); colorless oil; IR: 3276 (NH), 1733 (C(O)NH or C(O)O), 1649, 1579, 1561 (C=C), 1399 (C-H), 1272 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): 3.89 (dt, *J*= 10.68 Hz, 2H, $\underline{CH_2}$-NH), 4.59 (t, *J*= 5.24 Hz, 2H, O-$\underline{CH_2}$), 7.11 (t, *J*= 5.92 Hz, 1H, C(O)NH), 7.33 (dd, *J*= 15.69, 4.80 Hz, 1H, py), 7.33 (d, *J*= 4.76 Hz, 1H, py), 8.02 (dd, *J*= 7.62, 1.98 Hz, 1H, py), 8.20 (dd, *J*= 7.70, 1.98 Hz, 1H, py), 8.42 (dd, *J*= 4.78, 1.98 Hz, 1H, py), 8.50 (dd, *J*= 4.80, 2.00 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 39.21, 64.43, 122.29, 122.76, 126.40, 131.09, 139.41, 140.59, 147.07, 149.83, 151.03, 152.14, 164.39, 165.14; MS: m/z (%)= 340 $[M+ 1]^+$ (53), 341 (9), 342 (35), 343 (6); HRMS calcd. for $C_{14}H_{12}Cl_2N_3O_3$ $[M + 1]^+$ 340.0250; found 340.0240.

7.2.6.5.2. Synthesis of 2-(alkyl/aromatic)amino-*N*-(2-hydroxyethyl)-nicotinamide

7.2.6.5.2.1. Synthesis of 2-(alkyl/aromatic)-*N*-(2-hydroxyethyl)-nicotinamide **208**

General procedure

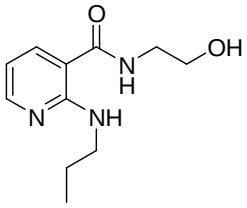
The 2-chloro-*N*-(2-hydroxyethyl)-nicotinamide (0.3g, 0.0015mol) in alkyl/aromaticamine (6 ml) was stirred at reflux for 69h. The excess of amine was removed and product was separated by column chromatography.



2-Allylamino-*N*-(2-hydroxyethyl)-nicotinamide (208a);

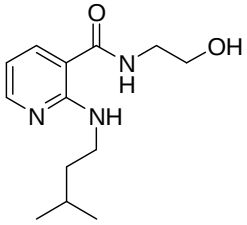
eluent: EtOAc; 0.302g, 91%; orange oil; IR: 3330 (NH), 1628 (C(O)NH), 1578, 1508 (C=C), 1258 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$): 3.53 (dt, *J*= 10.20, 5.48 Hz, 2H, $\underline{CH_2}$-OH), 3.77 (dt, *J*= 5.54, 4.58 Hz, 2H, NH-$\underline{CH_2}$), 4.09 (tt, *J*= 5.38, 1.66 Hz, 2H, O-$\underline{CH_2}$), 5.11 (ddt, *J*= 10.29, 3.03, 1.51 Hz, 1H, =$\underline{CH_2}$), 5.24 (ddt, *J*= 17.18, 3.31, 1.73 Hz, 1H, =$\underline{CH_2}$), 5.97 (qt, *J*= 17.18, 10.36, 5.20 Hz, 1H, -$\underline{CH}$=), 6.44 (dd, *J*= 7.64, 4.88 Hz, 1H, py), 6.88 (br, C(O)NH), 7.59 (dd, *J*= 7.68, 1.80 Hz, 1H, py), 8.13 (br, N$\underline{H}$-allyl), 8.16 (dd, *J*= 4.88, 1.76 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 42.42, 43.27, 61.78, 109.88, 110.69, 115.45, 135.08, 135.57, 151.77,

157.60, 169.17; MS: m/z (%)= 222 $[M+ 1]^+$ (100), 223 (13); HRMS calcd. for $C_{11}H_{16}N_3O_2$ $[M + 1]^+$ 222.1232; found 222.1242.



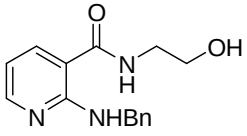
2-*n*-Propylamino-*N*-(2-hydroxyethyl)-nicotinamide (208b);

eluent: EtOAc; 0.304g, 91%; orange oil; IR: 3330 (NH), 1627 (C(O)NH), 1578 (N-H), 1513 (C=C), 1259 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$): 0.96 (t, *J*= 7.40 Hz, 3H, $\underline{CH_3}$), 1.61 (dq, *J*= 7.26 Hz, 2H, $\underline{CH_2}$-CH_3), 3.34 (dt, *J*= 7.04 Hz, 2H, $\underline{CH_2}$-OH), 3.49 (dt, *J*= 5.2 Hz, 2H, NH-$\underline{CH_2}$), 3.74 (t, *J*= 5.14 Hz, 2H, $\underline{CH_2}$-CH_2-CH_3), 6.37 (dd, *J*= 7.62, 4.90 Hz, 1H, py), 7.18 (t, *J*= 5.48 Hz, 1H, C(O)NH), 7.61 (dd, *J*= 7.68, 1.76 Hz, 1H, py), 8.04 (t, *J*= 5.10 Hz, 1H, NH-prop), 8.10 (dd, *J*= 4.90, 1.74 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 11.65, 22.55, 42.32, 42.87, 61.44, 109.80, 110.18, 135.75, 151.49, 157.83, 169.22; MS: m/z (%)= 224 $[M+ 1]^+$ (100), 225 (12); HRMS calcd. for $C_{11}H_{18}N_3O_2$ $[M + 1]^+$ 224.1394; found 224.1393.



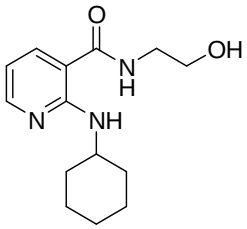
2-*i*-Pentylamino-*N*-(2-hydroxyethyl)-nicotinamide (208c);

eluent: EtOAc; 0.369g, 98%; orange oil; IR: 3322 (NH), 1628 (C(O)NH), 1578 (N-H), 1513 (C=C), 1259 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$): 0.93 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.53 (dt, *J*= 14.64 Hz, 2H, $\underline{CH_2}$-OH), 1.66-1.78 (m, 1H, CH), 3.41-3.48 (m, 2H, CH_2), 3.55 (dt, *J*= 10.04 Hz, 2H, NH-$\underline{CH_2}$), 3.79 (t, *J*= 4.98 Hz, 2H, O-$\underline{CH_2}$), 6.42 (dd, *J*= 7.62, 4.86 Hz, 1H, py), 6.91 (br, C(O)NH), 7.61 (dd, *J*= 7.68, 1.80 Hz, 1H, py), 8.01 (br, N$\underline{H}$-*i*-Pr), 8.18 (dd, *J*= 4.86, 1.74 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 22.37, 22.56, 25.96, 38.37, 39.30, 42.47, 61.75, 109.66, 110.06, 135.51, 151.75, 157.98, 169.23, 170.64; MS: m/z (%)= 252 $[M+ 1]^+$ (100), 253 (16); HRMS calcd. for $C_{13}H_{22}N_3O_2$ $[M + 1]^+$ 252.1707; found 252.1714.



2-Benzylamino-*N*-(2-hydroxyethyl)-nicotinamide 208d);

eluent: EtOAc; 0.386g, 95%; mp. 81°C; IR: 3320 (NH, OH), 1627 (C(O)NH), 1577, 1508 (C=C), 1257 (C(O)-N), 1063 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$): 3.55 (dt, *J*= 10.16, 10.08 Hz, 2H, $\underline{CH_2}$-OH), 3.78 (dt, *J*= 5.02 Hz, 2H, NH-$\underline{CH_2}$), 4.71 (d, *J*= 5.56 Hz, 2H, $\underline{CH_2}$-Ph), 6.49 (dd, *J*= 7.61, 4.88 Hz, 1H, py), 6.55 (br, C(O)NH), 7.21-7.39 (m, 5H, Ph), 7.59 (dd, *J*= 7.68, 1.80 Hz, 1H, py), 8.23 (d, *J*= 4.84, 1.76 Hz, 1H, py), 8.42 (br, N$\underline{H}$-Ph); ^{13}C ($CDCl_3$): δ 42.39, 44.90, 62.12, 109.64, 110.77, 126.95, 127.55, 128.51, 135.29, 139.58, 152.08, 157.82, 169.11; MS: m/z (%)= 272 $[M+ 1]^+$ (100), 273 (11); HRMS calcd. for $C_{15}H_{18}N_3O_2$ $[M + 1]^+$ 272.1394; found 272.1396.



2-Cyclohexylamino-*N*-(2-hydroxyethyl)-nicotinamide (208e);
eluent: EtOAc; 0.087g, 22%; mp. 102°C; IR: 3306 (NH), 1738 (C(O)NH), 1620 (N-H), 1575, 1503 (C=C), 1253 (C-O), 1063 (C-N); ^{1}H NMR (300 MHz, $CDCl_3$): 1.17-1.33 (m, 4H, cyclohexyl), 1.33-1.48 (m, 2H, cyclohexyl), 1.66-1.77 (m, 2H, cyclohexyl), 1.94-2.04 (m, 2H, cyclohexyl), 3.46 (br, OH), 3.53 (dt, *J*= 10.24 Hz, 2H, $\underline{CH_2}$-OH), 3.78 (dt, *J*= 5.58 Hz, 2H, NH-$\underline{CH_2}$), 3.92-4.03 (m, 1H, CH-cyclohexyl), 6.38 (dd, *J*= 7.64, 4.84 Hz, 1H, py), 6.85 (t, *J*= 4.94 Hz, 1H, C(O)NH), 7.58 (dd, *J*= 7.75, 1.80 Hz, 1H, py), 8.08 (d, *J*= 7.56 Hz, NH-cyclohexyl), 8.15 (dd, *J*= 4.84, 1.76 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 24.81, 25.86, 29.69, 33.08, 42.42, 48.77, 61.87, 109.24, 109.94, 135.65, 151.91, 157.29, 169.34; MS: m/z (%)= 264 $[M+ 1]^+$ (100), 265 (17); HRMS calcd. for $C_{14}H_{22}N_3O_2$ $[M + 1]^+$ 264.1707; found 264.1707.

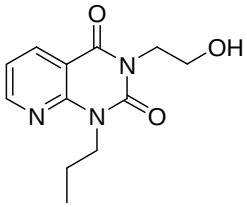
7.2.6.5.2.2. Synthesis of 2-benzylamino-*N*-(2-hydroxyethyl)-nicotinamide **208d**

Procedure based on WO 2010109123
To a suspension of 2-benzylamino-3-pyridinecarboxylic acid (0.6g, 0.0026mol) in dry THF (15 ml), Et_3N (0.8g, 0.0079mol), ethanolamine (0.19g, 0.0031mol) and BOP (1.39g, 0.0031mol) were added. The reaction mixture was stirred at room temperature for 21h. The precipitate was filtered off, washed with THF and EtOAc. The filtrate was concentrated and product was isolated by column chromatography using EtOAc as eluent. The 2-benzyl-*N*-(2-hydroxy-ethyl)-nicotinamide was obtained as light orange oil in 80% yield (0.571g).

7.2.6.5.3. Synthesis of 3-(propyl)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **213b** and 3-(benzyl)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **213d**

Synthesis of 3-(propyl)-1-(2-hydroxyethyl)-pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione

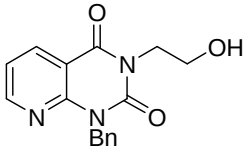
To a solution of 2-propylamino-*N*-(2-hydroxyethyl)-nicotinamide (0.2g, 0.0009mol) in dry CH_2Cl_2 (5 ml), DIPEA (0.347g, 0.0027mol) was added and the mixture was stirred at room temperature for 10min. Then the solution was cooled to 0°C and solution of triphosgene (0.319g, 0.0011mol) in dry CH_2Cl_2 (3 ml) was added dropwise. The reaction mixture was stirred at room temperature for 4h. The CH_2Cl_2 and H_2O were added and organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The desire product was separated by preparative TLC using mixture of EtOAc and PE (1:2) as eluent. The 3-(propyl)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione was obtained in 27% yield (0.06g) as colorless oil.



3-(*n*-Propyl)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (213b); eluent: EtOAc/PE (1/2); 0.06g, 27%; colorless oil; IR: 1704 (NC(O)N), 1662, 1591, 1484 (C=C), 1430 (C-H). ^{1}H NMR (300 MHz, $CDCl_3$): 1.00 (t, *J*= 7.44 Hz, 3H, CH_3), 1.77 (m, 2H, $\underline{CH_2}$-CH_3), 3.81 (t, *J*= 6.64 Hz, 2H, $\underline{CH_2}$-OH), 4.32 (dt, *J*= 9.64 Hz, 2H, -$\underline{CH_2}$- CH_2- CH_3), 4.46 (t, *J*= 6.64 Hz, 2H, N-$\underline{CH_2}$), 7.72 (dd, *J*= 7.79, 4.77 Hz, 1H, py), 8.47 (dd, *J*= 7.76, 1.96 Hz, 1H, py), 8.68 (dd, *J*= 4.76, 1.92 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 11.23, 21.13, 40.24, 42.58, 44.19, 110.59, 118.93, 137.81, 150.72, 150.81, 154.36, 161.23; MS: m/z (%)= 250 $[M+ 1]^+$ (100), 250 (13); HRMS calcd. for $C_{12}H_{16}N_3O_3$ $[M + 1]^+$ 250.1186; found 250.1185.

Synthesis of 3-(benzyl)-1-(2-hydroxyethyl)-pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione

To a solution of 2-benzylamino-*N*-(2-hydroxyethyl)-nicotinamide (0.2g, 0.0007mol) in dry CH_2Cl_2 (5 ml), DIPEA (0.0017g, 0.0027mol) was added and the mixture was stirred at room temperature for 15min. Then the solution was cooled to 0°C and solution of triphosgene (0.263g, 0.0009mol) in dry CH_2Cl_2 (3 ml) was added dropwise. The reaction mixture was stirred at room temperature for 4h. The CH_2Cl_2 and H_2O were added and organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The desire product was separated by preparative TLC using EtOAc as eluent. The 3-(benzyl)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione was obtained in 20% (0.046g) yield as yellow oil.

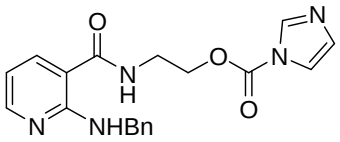


3-(Benzyl)-1-(2-hydroxyethyl)-pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (213d); eluent: EtOAc/PE (1/2); 0.046g, 20%; yellow oil; IR: 1774 (NC(O)N), 1707 (C(O)N), 1651, 1594, 1459, (C=C), 1272 (C-O). ^{1}H NMR (300 MHz, $CDCl_3$): 3.80 (t, *J*= 6.60 Hz, 2H, CH_2-OH), 4.46 (t, *J*= 6.60 Hz, 2H, N-$\underline{CH_2}$), 5.57 (s, 2H, $\underline{CH_2}$-Bn), 7.18-7.35 (m, 5H, Bn), 7.49 (d, *J*= 7.15 Hz, 1H, py), 8.47 (dd, *J*= 7.71, 1.65 Hz, 1H, py), 8.69 (dd, *J*= 4.40, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 29.79, 40.27, 42.79, 45.49, 110.75, 119.36, 127.77, 128.57, 128.69, 136.84, 138.05, 150.79, 151.04, 154.43, 161.19; MS: m/z (%)= 316 $[M+ 1 + NH_4]^+$ (100), 317 (20), 318 (36), 319 (8); HRMS calcd. for $C_{16}H_{16}N_3O_3$ $[M + 1]^+$ 298.3160; found 298.1170.

7.2.6.5.3.1. Synthesis of 2-[(2-benzylaminopyridine-3-carbonyl)-amino]-ethyl imidazole-1-carboxylate **210**

Procedure based on *J. Am. Chem. Soc.* **2002**, *124*, 1933-1940.

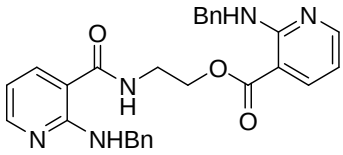
The mixture of 2-benzyl-*N*-(2-hydroxyethyl)-nicotinamide (0.55g, 0.002mol) and CDI (0.33g, 0.002mol) in dry THF (20 ml) was refluxed for 40h. The solvent was removed and product was isolated by column chromatography using EtOAc as eluent. The 2-[(2-benzylamino-pyridine-3-carbonyl)-amino]-ethyl imidazole-1-carboxylate was obtained as pale yellow oil in 35% yield (0.223g).



2-[(2-Benzylaminopyridine-3-carbonyl)-amino]-ethyl imidazole-1-carboxylate (210); eluent: EtOAc; 0.223g, 35%; pale yellow oil; IR: 3287 (NH), 2923, 2857 (C=C-H), 1766 (C(O)), 1633, 1495 (C=C), 1293 (C-O), 1241 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 3.82 (q, *J*= 5.41 Hz, 2H, $\underline{CH_2}$-NH), 4.59 (t, *J*= 5.22 Hz, 2H, O-$\underline{CH_2}$), 4.71 (d, *J*= 5.52 Hz, 2H, $\underline{CH_2}$-Ph), 6.52 (dd, *J*= 7.64, 4.84 Hz, 1H, py), 6.62 (t, *J*= 5.56 Hz, 1H, C(O)-NH), 7.05 (dt, *J*= 1.58 Hz, 1H, imidazole), 7.21-7.42 (m, 6H, Bn and proton from imidazole), 7.59 (dd, *J*= 7.72, 1.80 Hz, 1H, py), 8.11 (t, *J*= 1.00 Hz, 1H, imidazole), 8.25 (dd, *J*= 4.84, 1.76 Hz, 1H, py), 8.42 (t, *J*= 4.80 Hz, $\underline{NH}$-Ph); ^{13}C NMR ($CDCl_3$): δ 29.59, 38.98, 44.88, 66.88, 109.15, 110.68, 117.04, 126.84, 127.50, 128.42, 130.91, 134.93, 137.07, 139.69, 148.90, 152.39, 158.00, 168.65; MS: m/z (%)= 366 $[M+ 1]^+$ (100), 367 (25); HRMS calcd. for $C_{19}H_{20}N_5O_3$ $[M + 1]^+$ 366.1561.

7.2.6.5.3.2. Synthesis of 2-[(2-benzylaminopyridine-3-carbonyl)-amino]-ethyl 2-benzylaminopyridinecarboxylate **212**

To a solution of 2-benzyl-*N*-(2-hydroxyethyl)-nicotinamide (0.12g, 0.0004mol) in dry THF (15 ml) NaH (0.021g, 0.0006mol) and CDI (0.072g, 0.0004mol) were added. The reaction mixture was stirred at room tempereature for 20h. The solvent was removed and product isolated by column chromatography using mixture of EtOAc and PE (1:1) as eluent. The 2-[(2-benzylaminopyridine-3-carbonyl)-amino]-ethyl 2-benzylaminopyridinecarboxylate was isolated in 10% (0.02g) yield as a pale yellow oil.

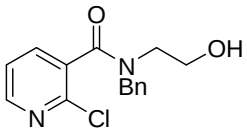


2-[(2-benzylaminopyridine-3-carbonyl)-amino]-ethyl 2-benzylaminopyridinecarboxylate (212); eluent: EtOAc/PE (1/1); 0.02g, 10%; pale yellow oil; IR: 3363 (NH), 2922, 2852 (C=C-H), 1686 (C(O)), 1633 (C(O)N), 1577, 1508 (C=C), 1243 (C-O), 1132 (C-N). ^{1}H NMR (300 MHz, $CDCl_3$): 3.74 (dt, *J*= 10.60 Hz, 2H, $\underline{CH_2}$-NH), 4.46 (t, *J*= 5.08 Hz, 2H, O-$\underline{CH_2}$), 4.69 (d, *J*= 5.66 Hz, 2H, $\underline{CH_2}$-Ph), 4.75 (d, *J*= 5.46 Hz, 2H, $\underline{CH_2}$-Ph), 6.43 (dd, *J*= 7.64, 4.84 Hz, 1H, py), 6.53 (dd, *J*= 7.80, 4.76 Hz, 1H, py), 6.54 (br, C(O)$\underline{NH}$), 7.20-7.38 (m, 10H, Ph), 7.52 (dd, *J*= 7.72, 1.80 Hz, 1H, py), 8.11 (dd, *J*= 7.78, 1.98 Hz, 1H, py), 8.22 (dd, *J*= 4.84, 1.76 Hz, 1H, py), 8.23 (br, $\underline{NH}$-Ph), 8.30 (dd, *J*= 4.76, 1.96 Hz, 1H, py), 8.44 (t, *J*= 5.24 Hz, 1H, $\underline{NH}$-Ph); ^{13}C NMR ($CDCl_3$): δ 39.53, 44.87, 44.89, 63.49, 105.32, 109.47, 110.76, 111.40, 126.93, 127.13, 127.48, 127.53, 128.51, 128.61, 135.15, 139.32, 139.58, 140.10, 152.13, 154.24, 157.85, 158.52, 167.88, 168.49; MS: m/z (%)= 482 $[M+ 1]^+$ (100), 483 (34), 484 (6); HRMS calcd. for $C_{28}H_{28}N_5O_3$ $[M + 1]^+$ 482.2181; found 482.2193.

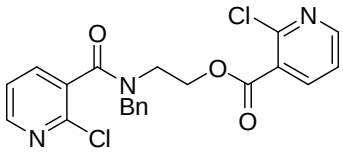
7.2.6.6. Synthesis of 3,4-dihydro-4-benzyl-pyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one **221**

7.2.6.6.1. Synthesis of *N*-benzyl-2-chloro-*N*-(2-hydroxyethyl)-nicotinamide **219** and 2-chloronicotinic acid 2-[benzyl-(2-chloropyridine-3-carbonyl)-amino]-ethyl ester **220**

The 2-chloro-3-pyridinecarboxylic acid (1.6g, 0.0102mol) in thionyl chloride (25 ml) was stirred at reflux for 2h. The excess of thionyl chloride was removed under vacuum and dried on vacuum pomp for 3h. The 2-chloro-3-pyridinecarbonyl chloride (1.73g, 0.0098mol) was placed in the flask, and then the flask in the ice-bath. To the stirred 2-chloro-3-pyridinecarbonyl chloride**,** *N*-benzylethanolamine (1.78g, 0.0118mol) in CH_2Cl_2 (10 ml) was added dropwise. The mixture was stirred at room temperature for 10min. The solvent was removed and products separated by column chromatography using EtOAc as eluent.



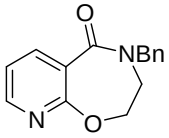
***N*-Benzyl-2-chloro-N-(2-hydroxyethyl)-nicotinamide (219)**; colorless oil; eluent: EtOAc; 1.27g, 48% yield; IR: 3390 (OH), 1732 (C(O)N), 1619 (C=C), 1396 (C-H), 1058, 1046 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): 3.26 (br, 2H, $\underline{CH_2}$-OH), 3.88 (t, *J*= 5.04 Hz, 2H, $\underline{CH_2}$-N), 4.47 (s, 2H, CH_2-Bn), 7.11 (d, *J*= 6.76 Hz, 1H, Bn), 7.20 (dd, *J*= 7.56, 4.84 Hz, 1H, py), 7.26-7.44 (m, 4H, Bn), 7.59 (dd, *J*= 7.54, 1.90 Hz, 1H, py), 8.40 (dd, *J*= 4.86, 1.82 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 48.54, 53.79, 61.27, 122.32, 127.11, 127.73, 128.02, 128.32, 128.74, 128.90, 135.89, 136.73, 137.63, 149.85, 150.08; MS: m/z (%)= 291 $[M+ 1]^+$ (100), 292 (19), 293 (34), 294 (6); HRMS calcd. for $C_{15}H_{16}ClN_2O_2$ $[M + 1]^+$ 291.0895; found 291.0903.



2-Chloronicotinic acid 2-[benzyl-(2-chloropyridine-3-carbonyl)-amino]-ethyl ester (220); colorless oil; eluent: EtOAc; 2.03g, 52% yield; IR: 1732 (C(O)N and C(O)O), 1639 (C=C), 1396 (C-H), 1241, 1063 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): 3.50 (t, *J*= 5.50 Hz, 2H, $\underline{CH_2}$-OH), 4.49 (s, 2H, CH_2-Bn), 4.63 (t, *J*= 5.34 Hz, 2H, NH-$\underline{CH_2}$), 7.09 (d, *J*= 6.80 Hz, 2H, Bn), 7.20 (dd, *J*= 7.52, 4.84 Hz, 1H, py), 7.26-7.42 (m, 4H, Bn), 7.30 (dd, *J*= 4.70, 2.86 Hz, 1H, py), 7.59 (dd, *J*= 7.54, 1.94 Hz, 1H, py), 8.19 (dd, *J*= 7.70, 1.94 Hz, 1H, py), 8.39 (dd, *J*= 4.80, 1.88 Hz, 1H, py), 8.50 (dd, *J*= 4.76, 1.92 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 46.07, 53.21, 62.83, 122.05, 122.37, 126.75, 127.91, 128.37, 128.81, 128.97, 135.56, 136.74, 139.96, 140.36, 150.13, 150.20, 151.91, 152.13, 164.26, 167.45; MS: m/z (%)= 430 $[M+ 1]^+$ (100), 431 (24), 432 (66), 433 (14), 434 (11); HRMS calcd. for $C_{21}H_{18}Cl_2N_3O_3$ $[M + 1]^+$ 430.0720; found 430.0733.

7.2.6.6.2. Synthesis of 3,4-dihydro-4-benzyl-pyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one **221**

To a cold solution of *N*-benzyl-2-chloro-*N*-(2-hydroxyethyl)-nicotinamide (0.2g, 0.0007mol) in dry THF (25 ml) under nitrogen atmosphere, NaH (0.33g in oil, 1.2eq). The reaction mixture was stirred at room temperature for 16h. The H_2O was added and the product was extracted with EtOAc (3x), the combinate organic layers were dried over $MgSO_4$, filtered and the solvent removed. The desired 3,4-dihydro-4-benzyl-pyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one was obtained in 90% yield (0.158g) as colorless oil.

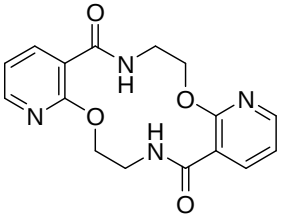


3,4-Dihydro-4-benzyl-pyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one (221); colorless oil; 0.158g, 90% yield; IR: 1630 (C(O)O), 1586, 1427 (C=C), 1050 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): 3.58 (t, *J*= 4.40 Hz, 2H, $\underline{CH_2}$-O), 4.38 (t, *J*= 4.40 Hz, 2H, N-$\underline{CH_2}$), 4.82 (s, 2H, $\underline{CH_2}$-Bn), 7.16 (dd, *J*= 7.71, 4.95 Hz, 1H, py), 7.26-7.42 (m, 5H, Bn), 8.40 (dd, *J*= 4.40, 1.65 Hz, 1H, py), 8.47 (dd, *J*= 7.71, 1.65 Hz, 1H, py); ^{13}C ($CDCl_3$): δ 46.79, 51.89, 118.87, 119.21, 128.06, 128.37, 129.01, 136.43, 143.36, 152.14, 159.77, 166.48; MS: m/z (%)= 255 $[M+ 1]^+$ (100), 256 (18); HRMS calcd. for $C_{15}H_{15}N_2O_2$ $[M + 1]^+$ 255.1128; found 255.1132.

7.2.6.7. Synthesis of 7,8,16,17-tetrahydro-dipyrido[*f*,*m*][1,8,4,11]dioxadiazacyclotetradecine-9,18(6*H*,15*H*)-dione **215**

To a cold solution of 2-chloro-*N*-(2-hydroxyethyl)-nicotinamide (0.25g, 0.0013mol) in dry THF (25 ml) under N_2 atmosphere, NaH (0.036g, 0.0015mol, 60% in oil) was added in one portion. The ice-bath was removed and the reaction mixture was stirred at room temperature for 22h. The precipitate was filtered off, washed with THF and dried. The 7,8,16,17-tetrahydro-dipyrido[*f*,*m*]

[1,8,4,11]dioxadiazacyclotetradecine-9,18(6*H*,15*H*)-dione was obtained in 33% (0.135g) as white solid.



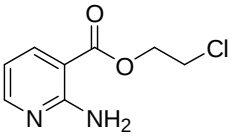
7,8,16,17-tetrahydro-dipyrido[*f*,*m*][1,8,4,11]dioxadiazacyclotetradecine-9,18(6*H*,15*H*)-dione (215); mp. >260°C; IR: 3358 (NH), 1646 (C(O)NH), 1535, 1420 (C=C), 1235 (C-N). ^{1}H NMR (300 MHz, DMSO-d_6): 3.72 (dt, *J*= 10.04 Hz, 4H, 2xCH_2-NH), 4.49 (t, *J*= 4.96 Hz, 4H, 2xCH_2-O), 7.15 (dd, *J*= 7.42, 4.90 Hz, 2H, 2xpy), 8.13 (dd, *J*= 7.42, 2.02 Hz, 2H, 2xpy), 8.30 (dd, *J*= 4.90, 1.98 Hz, 2H, 2xpy), 8.59 (br, NH); ^{13}C (DMSO-d_6): δ64.71, 118.21, 140.29, 149.47, 159.94, 164.24; MS: m/z (%)= 329 $[M+ 1]^+$ (100), 330 (19); HRMS calcd. for $C_{16}H_{17}N_4O_4$ $[M + 1]^+$ 329.1244; found 329.1243.

7.2.6.8. Synthesis of intermediates to the 2,3-dihydro-pyrido[2,3-*e*][1,4]oxazepin-5(1*H*)-one

7.2.6.8.1. Synthesis of 2-chloroethyl 2-amino-3-pyridinecarboxylate **222a**

Procedure based on WO 2010109123 (**2010**)

To a suspension of 2-aminopyridine-3-carboxylic acid (0.4g, 0.0029mol) in THF (30 ml), Et_3N (0.88g, 0.0087mol), 2-chloroethanol (0.28g, 0.0035mol) and BOP (1.54g, 0.0035mol) were added. The reaction mixture was stirred at room temperature for 19h. The solvent was removed and the 2-chloroethyl 2-amine-3-pyridinecarboxylate was isolated by column chromatography using EtOAc as eluent, as a white solid (0.52g, 90% yield).



2-chloroethyl 2-amino-3-pyridinecarboxylate (222a);

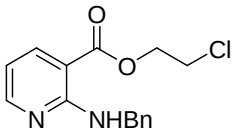
eluent: EtOAc; 0.52g, 90%; white solid; mp. 122°C; IR: 3428 (NH), 1693 (C(O)O), 1619, 1566 (C=C), 1236, 838 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$): 3.81 (t, *J*= 5.64 Hz, 2H, CH_2-Cl), 4.54 (t, *J*= 5.67 Hz, 2H, O-$\underline{CH_2}$), 6.65 (dd, *J*= 7.90, 4.78 Hz, 1H, py), 8.21 (dd, *J*= 28.69, 1.92 Hz, 1H, py), 8.21 (dd, *J*= 16.09, 1.96 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 41.65, 64.32, 105.73, 112.87, 140.38, 153.97, 1599.42, 166.47; MS: m/z (%)= 201 $[M+ 1]^+$ (100), 202 (10), 203 (31); HRMS calcd. for $C_8H_{10}ClN_2O_2$ $[M + 1]^+$ 201.04253; found 201.0426.

7.2.6.8.2. Synthesis of 2-chloroethyl 2-benzylamino-3-pyridinecarboxylate **222b**

Procedure based on WO 2010109123 (**2010**)

To a suspension of 2-benzylamino-3-pyridinecarboxylic acid (0.2g, 0.0009mol) in dry THF (15 ml), Et_3N (0.266g, 0.0026mol), 2-chloroethanol (0.08g, 0.0010mol) and BOP (0.465g, 0.0.0010mol) were

added. The reaction mixture was stirred at room temperature for 19h. The precipitate was filtered off, washed with THF and EtOAc. The filtrate was concentrated and product was isolated by column chromatography using mixture of EtOAc and PE (1:1) as eluent. The 2-chloroethyl 2-benzyl-3-pyridinecarboxylate was obtained as light orange oil in 80% yield (0.204g).



2-chloroethyl 2-benzylamino-3-pyridinecarboxylate (222b);
eluent: EtOAc/PE (1/1); 0.204g, 80%; light orange oil; IR: 3389 (NH), 1681 (C(O)O), 1590, 1578, 1507 (C=C), 1240, 733 (C-Cl). ^{1}H NMR (300 MHz, $CDCl_3$): 3.69 (t, *J*= 6.05 Hz, 2H, CH_2-Cl), 4.42 (t, *J*= 5.50 Hz, 2H, O-$\underline{CH_2}$), 4.68 (d, *J*= 5.50 Hz, 2H, $\underline{CH_2}$-Bn), 6.49 (dd, *J*= 7.71, 4.40 Hz, 1H, py), 7.14-7.32 (m, 5H, Bn), 8.09 (dd, *J*= 7.71, 2.20 Hz, 1H, py), 8.13 (br, NH), 8.24 (dd, *J*= 4.95, 1.65 Hz, 1H, py); ^{13}C NMR ($CDCl_3$): δ 41.75, 44.96, 64.30, 105.45, 111.51, 127.19, 127.62, 128.67, 139.45, 140.32, 154.25, 158.59, 167.10; MS: m/z (%)= 291 $[M+ 1]^+$ (100), 292 (17), 293 (33); HRMS calcd. for $C_{15}H_{16}ClN_2O_2$ $[M + 1]^+$ 291.0895; found 291.0900.

7.3. Characterization of the NPP inhibitors

Materials

2-(N-cyclohexylamino)ethanesulfonic acid (CHES) and Tris(hydroxymethyl)aminomethane (Tris) were obtained from Applichem (Darmstadt, Germany). Disodium hydrogen phosphate was purchased from Carl Roth (Karlsruhe, Germany). ATP, calcium chloride, dimethyl sulfoxide (DMSO), magnesium chloride, *p*-nitrophenyl 5'-thymidine monophosphate (*p*-Nph-5'-TMP), sodium chloride and sodium hydroxide were obtained Sigma (Steinheim, Germany). Human recombinant soluble NPP1, expressed in NS0 cells from murine myeloma, was obtained from R&D Systems GmbH (Wiesbaden, Germany). Human recombinant soluble NPP1, expressed in Sf9 insect cells, was prepared in the laboratory headed by Professor Christa E. Müller (PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany).

Initial screen

The initial screening of compounds for inhibition of human NPP1 was performed as previously described using a colorimetric assay with the artificial substrate *p*-nitrophenyl 5'-thymidine monophosphate (*p*-Nph-5'-TMP).[191][215] The assays were carried out at 37°C in a total volume of 100µl in a clear 96-well microplate. The reaction mixture contained 1mM $CaCl_2$, 200µM $ZnCl_2$, 50mM Tris, pH 9.0, 400µM *p*-Nph-5'-TMP, and 10µM of each test compounds. The enzyme reactions were initiated by the addition of 20ng of human NPP1 (commercial, K_m = 8.17µM), then incubated at 37°C for 15min, and subsequently terminated by the addition of 20µl of 1.0 N NaOH. The amounts of

p-nitrophenolate liberated were measured at 400nm. All experiments were performed two times in triplicate. The % inhibition of test compounds was evaluated when sets the blank (without test compound) as 100 % of enzyme activity.

ATP assays

For compounds that showed at least 70 % inhibition versus the artificial substrate, their inhibitory activities were investigated versus the natural substrate ATP at 37°C in a final volume of 100µl. The reaction mixture contained 1mM $MgCl_2$, 2mM $CaCl_2$, 10mM 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES), pH 9.0, 400µM ATP as substrate and 10µM of test compounds. The reaction was initiated by the addition of 20ng of human NPP1 (commercial, K_m = 8.17µM) or 360ng of human NPP1 (produced, K_m = 43.2µM), respectively. The mixture was incubated for 30 min, respectively and terminated by heating at 90°C for 3min. After cooling the reaction samples in ice, they were transferred into capillary electrphoresis (CE) vials and injected into the CE instrument. The previous operation conditions in CE were modified for the NPP1 detections.[216] All experiments were carried out using a P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a DAD detection system. Data collection and peak area analysis were performed by the P/ACE MDQ software 32 KARAT obtained from Beckman Coulter (Fullerton, CA, USA). The electrophoretic separations were carried out using a polyacrylamide-coated capillary (40cm [30cm effective length], x 50µm (id) obtained from CS-Chromatography (Langerwehe, Germany)). Electrokinetic injections were performed using a voltage of –6kV for 60s and separations were carried out by a voltage of – 15kV. Analytes were detected using direct UV absorbance at 260nm. The capillary temperature was kept constant at 15°C and the temperature of the storing unit was adjusted to 15°C. The running buffer consisted of 50mM phosphate buffer (pH 6.5). Between separations, the capillary was washed with water for 2min (20psi) and subsequently with running buffer for 2min (20psi) before each injection. All experiments were performed two times in triplicate. For compounds that showed at least 70 % inhibition vs. ATP, concentration-inhibition curves were determined with 6-8 different concentrations of test compound and IC_{50} values were determined by nonlinear curve fitting using the program PRISM 5.0 (GraphPad, San Diego, USA). Inhibition mechanisms were determined using five different concentrations of ATP (from 20 to 500 µM), and three different concentrations of inhibitor. The inhibition type of each inhibitor was evaluated graphically from the Lineweaver-Burk plots using PRISM 5.0.

7.4. Antimicrobial activity test methodology

Culturing of test strains

- *Escherichia coli* LMG 8063, *Klebsiella pneumonia* LMG 2095, *Staphylococcus aureus* LMG 8064, *Bacillus subtilis* LMG 13579 were routinely grown in Mueller Hinton II broth medium (BD ref 212322). All strains were incubated at 37°C for 24h under aerobic conditions, except LMG 13579 which was incubated at 28°C. Accordingly, the same broth media and incubation conditions were also used during the actual tests.
- Inocula were prepared in MH broth until a density of 10^5 CFU/mL (or a McFarland turbidity standard equivalent) was reached.

Bioassay

- The bioassay was carried out in a batch.
- Working solutions of test compounds were prepared by mixing 200µL of the supplied DMSO solutions of the compounds (app. 50mg/mL) and 800µL Mueller Hinton II (MH) culture broth.
- The bioassay was carried out in sterile 96-well microtiter plates. Per plate, the antimicrobial potency of compounds was tested in duplicate against a single test strain. Also included were duplicate wells of positive, negative and sterility controls, respectively.
- To each well, 170µL of sterile MH broth was added. Next, depending on the type of well, additional reagents are added.
- In each pair of test wells, 10µL of the working solution prepared from each test compound was added.
- In the two **positive control wells**, 10µL gentamicin sulfate solution (in a concentration similar to the compound concentration) was added.
- In the two **negative control wells**, 10µL of sterile 0.85% saline was added.
- Finally, in all wells, 20µL of bacterial inoculum was added. In the two **sterility control wells**, 30µL sterile MH broth was added.
- This way, final concentrations of app. 0.5µg/mL test compound and 10^4 CFU/mL test bacteria were obtained in a test volume of 200µL.

Depending on the test strain, plates were incubated at the respective temperatures (28 or 37°C) for 24h under aerobic conditions. Plates were covered with a seal to prevent dehydration during incubation.
Bacterial growth was scored visually. Turbidity levels comparable to those in the positive control wells were regarded as positive for antimicrobial activity of the compound in question. Turbidity levels comparable to those in the negative control wells were considered as negative for antimicrobial activity.

Remark: Originally, it was planned to also assess growth by spectrophotometric turbidity measurement at 590nm. However, because several compounds upon addition to the MH growth medium caused a ´milky´ or ´orange´coloration which may interfere with OD measurements, it was decided to only rely on visual growth inspection.

7.5. The ADME (Absorption, Distribution, Metabolism, and Excretion) study performed on selected examples of the synthesized library- methodology

Intraluminal solubility profiling: The solubility of the pyrido[2,3-*d*]pyrimidines was experimentally determined in the biorelevant medium FaSSIF, possessing mixed micelles of taurocholate and lecithin. The thermodynamic solubility was determined by adding an excess of compound to the medium (0.8mg/300µL). This suspension was shaken for 24h at 175rpm and 37°C (KS 4000i Control incubator shaker, Staufen, Germany) and afterwards centrifuged at 20,817g for 15min (5804 Centrifuge, Eppendorf, Hamburg, Germany) to remove undissolved drug. The supernatant was analyzed with HPLC and UV detection.

Intestinal permeability: Caco-2 cells were obtained from American Type Culture Collection (Manassas, VA) and were grown in DMEM$^+$ at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. Cells were passaged every 3 to 4 days (at 80–90% confluence) at a split ratio of 1:6. For transport experiments, cells were seeded at a density of 90,000 cells/cm^2 on Costar Transwell membrane inserts (3µm pore diameter, 12 mm diameter; Corning Inc., Corning, NY) and were used for experiments 17 to 18 days after seeding. Only monolayers with transepithelial electrical resistance values higher than 150 $\Omega \cdot cm^2$ were used. Transport studies were performed using a previously described method.[204] HBSS$^+$ (pH 7.4) containing 0.2% D-α-tocopheryl polyethylene glycol 1000 succinate was used in the basolateral compartment to create sink conditions; in the apical compartment, FaSSIF (pH 6.5) was used. The experiment was initiated by adding the incubation medium, containing the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones (30µM) in absence or presence of GF120918 (4µM), to the donor compartment. Due to solubility issues of compound **196i** in DMSO, an initial concentration of 20µM was used in order to obtain a non-toxic DMSO concentration below 1%. Samples were shaken at 300 rotations per minute (rpm) for 1h at 37°C (Thermostar, BMG Labtech, Offenburg, Germany). Afterwards, analysis of the samples was done with HPLC and UV detection. The apparent permeability coefficient (P_{app}) was calculated according to the following equation:

$$P_{app} = \frac{\Delta Q}{\Delta t} \times \frac{1}{A \times C_{donor}}$$

where Q is the cumulative amount of drug appearing in the acceptor compartment, A is the surface area of the Transwell membrane, and C_{donor} is the drug concentration in the donor compartment.

Human hepatic in vitro intrinsic clearance: Test compounds (8µM) were incubated with a pool of human liver microsomes (HLM) from 45 donors (KaKy-Cell, Plobsheim, France) (0.5mg microsomal protein/ml), NADPH (1mM), glucose-6-phosphate (3mM) in a total volume of 400µl phosphate buffer (0.1M) containing 3mM $MgCl_2$ at a pH of 7.4. Incubations were conducted at 37°C at 350 rpm (Thermostar, BMG Labtech, Offenburg, Germany). Reactions were commenced with the addition of NADPH and glucose-6-phosphate and after 0, 10, 20 and 30min, aliquots (75µl) were removed and added to acetonitrile (75µl) to quench the reaction. Incubations in the absence of NADPH and glucose-6-phosphate were performed as a negative control. Parallel incubation with 5µM verapamil was performed with human liver microsomes in a concentration of 0.25mg microsomal protein/ml. Intrinsic clearance (Cl_{int}) values were obtained with the 'in vitro $t_{1/2}$ method'[207] Briefly, residual concentrations of the parent compound were converted to the percentage of the drug remaining relative to the initial concentration. The slope of the linear regression from the log percentage remaining versus incubation time was used to calculate the elimination rate constant k=-slope*(ln 10). Finally, in vitro $t_{1/2}$ is calculated from k and incorporated in the following equation to obtain scaled Cl_{int} values for hepatic metabolism in human ($Cl_{int,hep,human}$):

$$\text{Scaled } Cl_{int,hep,human} = \frac{0{,}693}{\text{in vitro } t_{1/2}} \times \frac{\text{incubation volume (ml)}}{\text{mg microsomes}} \times \frac{45 \text{ mg microsomes}}{\text{g liver}} \times \frac{20 \text{ g liver}}{\text{kg bodyweight}}$$

HPLC analysis: The HPLC system used to analyze the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones samples consisted of a Waters 2790 Alliance series separations module and a Novapak C18 column under radial compression (Waters, Milford, MA). UV absorbance was monitored using a Waters 2487 detector and fluorescence using a FP-1520 intelligent fluorescence detector (Jasco, Tokyo, Japan). The observed peaks were integrated using Empower Pro (Empower 2) software. The calibration curves of the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones were linear over the concentration range of 0.39µM to 100µM. The assessment of intraday and interday reproducibility at concentrations of 1.25µM and 0.125µM for each pyrido[2,3-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione in 50:50 microsomal buffer:ACN, resulted in a RSD below 2.1 (n=5). The deviation from the theoretical concentration was lower than 3.2%. The different HPLC methods of the pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones are listed in Table 1.

Table 1 Conditions of the HPLC analysis of pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones analogues

Compound	**Mobile Phase (Methanol / 25mM acetate buffer pH 5.5)**	**UV/Fluo detection (nm)**	**t_R[a] (min)**
196i	95/5	340	11
197a	60/40	Ex:330/Em:369	4.7
197d	80/20	340	4.9
197f	90/10	Ex:256/Em:420	5.3
198a	80/20	Ex:243/Em:366	4.9
198b	80/20	Ex:243/Em:366	7.2
198d	90/10	Ex:222/Em:361	8.6
198j	80/20	340	10.1
199e	90/10	350	5.6
200a	90/10	Ex:245/Em:363	5.7
Atenolol	40/60	Ex:271/Em:302	2.8
Indomethacin	70/30	318	4.4
Indinavir	80/20	Ex:256/Em:290	5.1
Verapamil	60/40	Ex:275/Em:315	9.7

[a]Retention time

Curriculum Vitae

Personal information

Name	Martyna Jatczak
Address	ul. Slowicza 15a, 98-100 Lask, Poland
Born	January, 17th, 1986, Lask, Poland
Tel.	+32 0498352419
E-mail	jatczak_martyna@op.pl

Education

2010-2014 PhD student at Ghent University (Faculty of Bioscience Engineering, Department of Sustainable Organic Chemistry and Technology, SynBioC Research Group)

Thesis: *Synthesis of six- and seven-membered pyridine fused rings and their biological activity*

Promoter: Prof. dr. ir. Christian V. Stevens

2005- 2010 Master of Science in Chemistry (July, 2010)

University of Lodz (Faculty of Chemistry)

Thesis: *The influence of ring structures (benzene, bicyclo[2.2.2]octane, p- carborane) and fluoroalkyl chain on the generation smectic phases of two series of liquid crystals*

Promoters: Prof. dr. hab. Zbigniew H. Kudzin (University of Lodz)

Prof. dr. hab. Piotr Kaszynski (Vanderbilt University)

Career

July- December 2009	visiting student in the Organic Materials Research Laboratory, headed by Prof. Piotr Kaszynski, at the Vanderbilt University (Nashville, USA)
September 2008	student`s training in Laboratory for Analysis of Organic Compounds and Polymers Department in Centre of Molecular and Macromolecular Studies in Lodz belongs to the Polish Academy of Sciences
2008-current	member of the Polish Chemical Society
2005-2010	member of Student`s Scientific Group of Chemists of University of Lodz (2007-2009 chairwoman of Student`s Scientific Group of Chemists of University of Lodz)

Publications in international journals with peer-review

Martyna Jatczak, Koen Muylaert, Laurens M. De Coen, Janneke Keemink, Benjamin Wuyts, Patrick Augustijns, Christian V. Stevens: Straightforward Entry to Pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones and Their ADME Properties *Bioorg. Med. Chem.* **2014**, *22*, 3947-3956.

Koen Muylaert, Martyna Jatczak, Benjamin Wuyts, Laurens M. De Coen, Kristof Van Hecke, Hans Loones, Janneke Keemink, Daniel García, Sven Mangelinckx, Pieter Annaert, Christian V. Stevens: Synthesis and Early ADME Evaluation of a Novel Scaffold, Tetrahydro-6*H*-pyrido[3,2-*b*]azepin-6-one *Synlett*, **2014**, *25*, 1443-1447.

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Koen Muylaert, Martyna Jatczak, Sven Mangelinckx, and Christian V. Stevens: Synthesis of pyrido-annelated seven-membered *N*-containing heterocycles *Curr. Med. Chem.* **2014** subbmited

Conferences and posters

*14th Belgian Organic Synthesis Symposium (BOSS XIV), July 13-18, **2014**, Louvain-la-Neuve (Belgium)*

- Koen Muylaert; Sven Mangelinckx; Martyna Jatczak; Laurens M. De Coen; Christian V. Stevens: The $Cu(OTf)_2$ catalysed microwave assisted synthesis of 6,7-diaryl-7,8-dihydropyrido[4,3-*c*]pyridazin-5(6*H*)-ones. (poster)

4th Portuguese Young Chemists Meeting (4º PYCheM), April 29th -May 1st 2014, Coimbra (Portugal)

- Martyna Jatczak, Koen Muylaert, Janneke Keemink, Benjamin Wuyts, Patrick Augustijns, Christian V. Stevens: Straightforward synthesis to pyrido[2,3-*d*]pyrimidine-2,4-diones and their ADME properties. Abstract OC6 (oral presentation)

- Koen Muylaert, Martyna Jatczak, Benjamin Wuyts, Laurens M. De Coen, Hans Loones, Janneke Keemink, Daniel García, Sven Mangelinckx, Pieter Annaert, Christian V. Stevens: Synthesis and early ADME evaluation of a novel scaffold, Tetrahydro-6*H*-pyrido[3,2-*b*]azepin-6-one. Abstract P30 (poster)

*16th Sigma-Aldrich Organic Synthesis Meeting, December 6-7, **2012**, Spa (Belgium)*

- Koen Muylaert; Martyna Jatczak; Laurens M. De Coen; Daniel García; Christian C. Stevens: Synthesis of highly unexplored, biologically active scaffolds. (poster)

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