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Synthesis of novel heterocyclic hydrazine derivatives and their conjugates

UNIVERSITY OF TARTU
Press

Institute of Chemistry, Faculty of Science and Technology, University of Tartu, Estonia

The dissertation is accepted for the commencement of the degree of Doctor of Philosophy in Chemistry on 12nd June, 2018 by the Council of Institute of Chemistry, University of Tartu.

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Commencement: August 27, 2018 at 10:15, Ravila Street 14a–1020, Tartu (Chemicum)

This research was supported by institutional research funding project IUT20-17 of the Estonian Ministry of Education. This work has been supported by Graduate School of Functional materials and technologies receiving funding from the European Regional Development Fund in University of Tartu, Estonia. Dora Plus program is gratefully acknowledged for its support. Estonian Students Fund in USA Inc., Sihtasutus Tartu Ülikool and Estonian National Culture Foundation are recognized for their financial support.

ISSN 1406-0299
ISBN 978-9949-77-795-2 (print)
ISBN 978-9949-77-796-9 (pdf)

University of Tartu Press
www.tyk.ee

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LIST OF ORIGINAL PUBLICATIONS

I Ilisson, M.; Tomson, K.; Selyutina, A.; Türk, S.; Mäeorg, U. Synthesis of Novel Saccharide Hydrazones. *Synth. Commun.*, **2015**, *45*, 1367–1373.

II Ilisson, M.; Mäeorg, U. Systematic synthesis of phthalimide-protected unsaturated hydrazine heterocycles. *Synth. Commun.*, **2017**, *47*, 1231–1238.

III Ilisson, M.; Tomson, K.; Tamm, T.; Mäeorg, U. Carbon-Carbon Double Bond Isomerization in Heterocyclic Hydrazine Derivatives. *Chem. Heterocycl. Comp.*, **2018**, *accepted.*

Author's contribution:

I The author planned and performed all the syntheses, except few optimization experiments. Structural analysis and interpretation was performed by the author. The author was also responsible for manuscript preparation.

II The author planned, executed and analyzed all the experiments and prepared the manuscript.

III The author planned all the syntheses and carried out experiments for catalyst identification. Structural analysis and interpretation as well as the manuscript preparation was performed by the author.

ABBREVIATIONS

Ac	acetyl
Alk	alkyl
Ar	aryl
ATR	attenuated total reflectance
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
bp	boiling point
Bt	1-benzotriazolyl
Bu	butyl
COX-2	cyclooxygenase-2
dba	dibenzylideneacetone
DCM	dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine
DMAD	dimethyl acetylene dicarboxylate
DMEDA	*N*,*N*′-dimethylethylenediamine
DMF	*N*,*N*-dimethylformamide
eq	equivalent
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
ESI	electrospray ionization
FTIR	Fourier' transform infrared spectroscopy
Hal	halide
hfacac	hexafluoroacetylacetonate
HIV	human immunodeficiency virus
HRMS	high resolution mass spectrometry
LDA	lithium diisopropyl amide
Me	methyl
Mes	mesityl
Moc	methyloxycarbonyl
MS	molecular sieves
MTBE	methyl *tert*-butyl ether
NMR	nuclear magnetic resonance (spectroscopy)
Np	naphthyl
OTBS	*tert*-butyldimethylsilyloxy
PE	petroleum ether
PG	protective group
Ph	phenyl
Phth	phthaloyl
PTC	phase transfer catalysis
RLU	relative light unit
TBAHS	tetra-*n*-butyl ammonium hydrogen sulfate

***t*-Bu**	*tert*-butyl
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
VLP	virus-like particle

INTRODUCTION

Hydrazine is a simple compound that is most widely known for its applicability as a rocket fuel [1] and its high toxicity [2]. As a widespread belief, hydrazine's general toxicity is often extrapolated to all its derivatives, which may have hindered the development of synthetic methods for preparation of these compounds. However, N-N bond containing compounds have been isolated from living organisms and often their toxicity is selective towards various biological targets, such as viruses, bacteria, fungi or cancer cells, which is highly desirable for drug developers [3,4].

When having an insight to pharmaceuticals, numerous compounds comprising a N-N bond can be found. One of the simplest, yet most famous hydrazine-based drugs is Meldonium, which is used for its cardioprotective effect [5]. It was popular among athletes, but its use was prohibited by World Anti-Doping Agency as a metabolic modulator from 2016 [6]. COX-2 selective nonsteroidal anti-inflammatory drug Celecoxib [7], was one of Pfizer's best-selling pharmaceuticals before its availability in generic form [8]. More recently, azapeptide Atazanavir has been taken use as anti-retroviral drug due to its ability to inhibit HIV protease [9]. Hydrazine moieties can be found also in a series of anti-cancer drugs, such as Axitinib, which is used for treatment of renal cell carcinoma [10]. Structures of mentioned pharmaceuticals are presented on **Figure 1**.

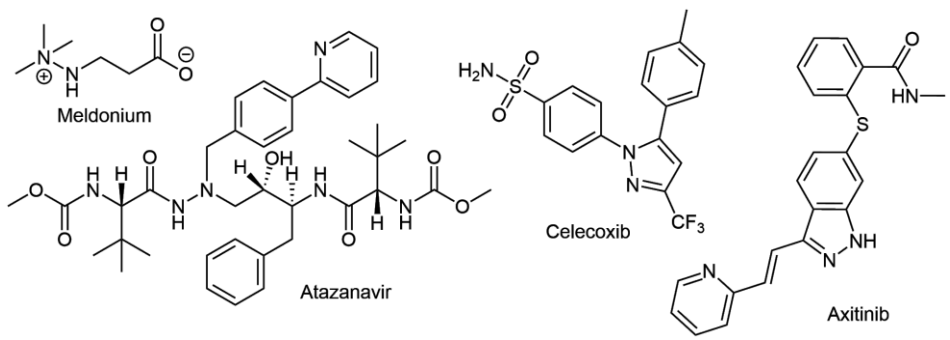
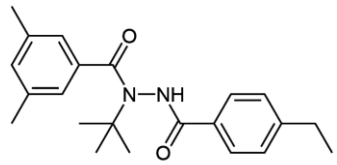


Figure 1. Examples of pharmaceuticals with a hydrazine moiety.

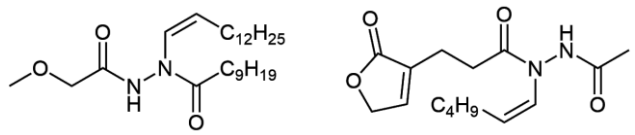
Besides their important role in pharmaceutical industry, hydrazine derivatives have also found applications elsewhere, for example in the production of dyes, polymers and agrochemicals [11]. Tebufenozide (**Figure 2**) serves a case of a well-designed insecticide, possessing high selectivity towards caterpillar pests as well as synthetic simplicity, which ultimately granted its inventors the Green Chemistry Award [12].



Tebufenozide

Figure 2. Tebufenozide, a hydrazine-based insecticide.

Enehydrazides can be considered as the analogues of enamides – compounds that are known for their high reactivity. They can be used as substrates for various reactions, such as radical, photochemical, pericyclic, electrophilic and nucleophilic reactions [13]. Enehydrazide fragment has been found from a series of natural compounds, for example elaiomycins [14], hydrazidomycins [15] and geralcins [16,17] (**Figure 3**). These compounds have shown cytotoxic properties and therefore could be anti-cancer drug candidates, making research of enehydrazides particularly promising.



Hydrazidomycin A Geralcin B

Figure 3. Two samples of naturally occurring enehydrazides possessing cytotoxic properties.

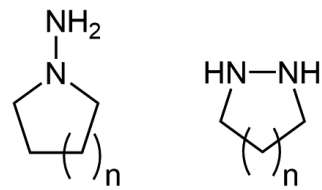
Heterocyclic compounds have an essential role in drug industry, as majority of pharmaceuticals include at least one heterocyclic ring in their composition [18] and more than 90% of these heterocyclic moieties comprise a nitrogen atom in their structure [19]. In 2012, heterocycles could be found in each of top ten brand name small molecule drugs and 8 of them had a nitrogen atom present in the ring [20]. Therefore, the development of efficient routes for the synthesis of nitrogen-containing heterocycles is very important.

There are various synthetic routes available for preparation of both acyclic and cyclic hydrazine derivatives [21,22]. For the latter, most methods allow to synthesize compounds which include N-N bond within the heterocycle. Routes for the preparation of heterocycles that comprise a N-N bond adjacent to the ring, are described much less commonly. Also, synthetic methods allowing access to enehydrazides are rather scarce – when looking for heterocyclic compounds that include these fragments within the ring, only a single example can be found [23]. For abovementioned reasons the current thesis concentrates on expanding the synthetic possibilities for preparation of these novel and hardly accessible compounds.

1. LITERATURE OVERVIEW

1.1. Synthetic methods for preparation of heterocycles with an exocyclic N-N bond

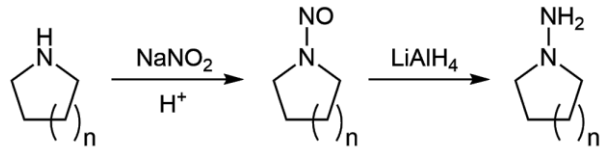
Heterocycles that include a N-N bond in their structure can be divided into two groups: one, where the N-N bond is located within the heterocycle (endocyclic N-N bond), and the other, where only one nitrogen atom is positioned in the ring and the N-N bond itself is adjacent to the heterocycle (exocyclic N-N bond). Alternatively, when no other substituents are present, these compounds can be observed as *N,N'*-disubstituted and *N,N*-disubstituted hydrazines respectively (**Scheme 1**). As the current thesis mainly concentrates on the synthesis of heterocycles with an exocyclic N-N bond, the possibilities for synthesis of these compounds are also in the focus of this chapter.



Scheme 1. Heterocyclic hydrazine derivatives, including an exocyclic N-N bond (left) and an endocyclic N-N bond (right).

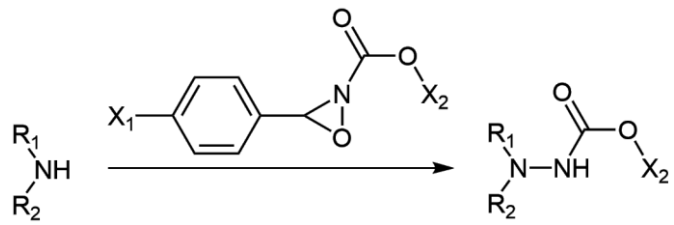
For the synthesis of heterocyclic hydrazine derivatives with an exocyclic N-N bond, two main strategies are available. The first one relies on N-N bond formation reaction between a cyclic amine and an electrophilic nitrogen donor. The second pathway involves construction of the ring on the existing hydrazine fragment. Although both strategies have their advantages and disadvantages, the first strategy is generally used more frequently.

Among N-N bond formation reactions, treating cyclic amines with *in situ* generated nitrous acid to form *N*-nitroso intermediates followed by their reduction, was pioneered by Overberger more than 60 years ago (**Scheme 2**) [24,25]. Although the nitrosation and subsequent reduction can be usually performed in high yields, this method suffers from a serious drawback, because *N*-nitroso intermediates are very likely to be carcinogenic, as more than 90% of them have shown carcinogenic properties in tests with wide variety of experimental animals [26]. However, this setback has not discouraged scientists from using this method for preparation of numerous heterocyclic hydrazine derivatives between now and then [27–30].



Scheme 2. General scheme for the synthesis of heterocyclic hydrazine derivatives by nitrosation and subsequent reduction.

Besides the previously described two-step process, direct amination with an electrophilic reagent can be used to form *N*,*N*-disubstituted hydrazine derivatives. Among simplest of them, chloramine and hydroxylamine-*O*-sulfonic acid have been used successfully [31,32]. However, chloramine is toxic and unstable even in solutions, making its use in organic synthesis rather inconvenient [33]. Stability-wise, hydroxylamine-*O*-sulfonic acid is a better choice, although byproducts, such as diazenes and tetrazenes can form during the synthesis [32]. In the early 1990-s, Vidal *et al.* developed oxaziridine-based reagents, that allow to introduce *N*-Boc or *N*-Moc fragments to various primary or secondary amines, forming protected hydrazine derivatives (**Scheme 3**) [34,35]. Preparation of more than 20 compounds are described in these publications, about half of them being heterocyclic hydrazine derivatives.

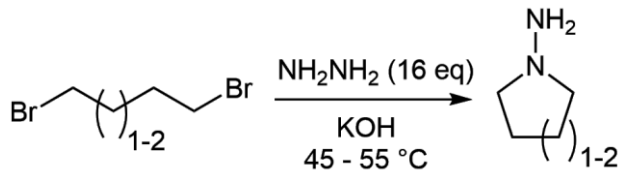


Scheme 3. Introduction of *N*-carbamate moiety to amines as described by Vidal *et al*. R_1 and R_2 represent various substituents. X_1 = -H, -CN; X_2 = -Me, -*t*-Bu.

In principle, a reaction between alkyl dihalide and the primary amino group of a hydrazine derivative should give a convenient access to corresponding heterocyclic hydrazine derivatives. Unfortunately, the effective scope of this reaction turns out to be narrow, as only limited amount of hydrazine derivatives have been synthesized this way and very often with just modest yields.

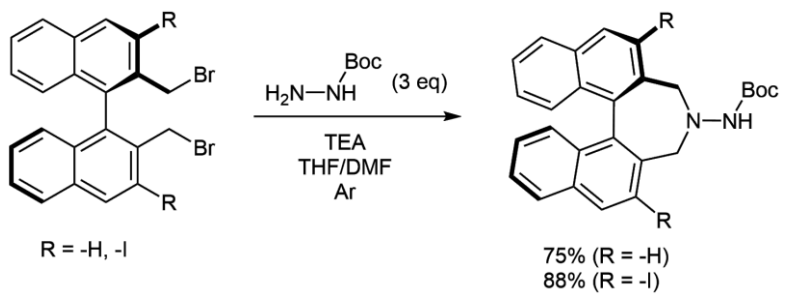
One of the first preparations of *N*-aminopyrrolidine from hydrazine hydrate and 1,4-dichlorobutane was performed by Lemal *et al.* in 1963 [27]. The preparation has not been described in detail, therefore it is difficult to speculate with the reasons for a low, 28% yield achieved in this early research. Nearly fifty years later, Levanova *et al.* discovered *N*-aminopyrrolidine and the six-

membered *N*-aminopiperidine from reaction mixtures as side products when attempting to synthesize selenium- and tellurium-containing heterocycles [36]. When running independent reactions to intentionally prepare these heterocyclic hydrazine derivatives, yields of 31% and 58% were achieved for the 5-membered- and 6-membered heterocycles correspondingly (**Scheme 4**). However, it should be stated that a massive excess (16 equivalents) of hydrazine hydrate were used in these reactions, making economic rationality of this method questionable.



Scheme 4. Synthesis of *N*-aminopyrrolidine and *N*-aminopiperidine as described by Levanova *et al.*

Slightly more success has been achieved with electrophiles which are activated (e.g. benzyl) and have a more rigid structure [37–39]. For example, in the research of Widhalm *et al.*, 7-membered rings were formed in high yields when *(R)*-2,2'-bis(bromomethyl)-1,1'-binaphthyl or its 3,3'-diiodo derivatives reacted with 3 eq of Boc-hydrazine (**Scheme 5**) [39].



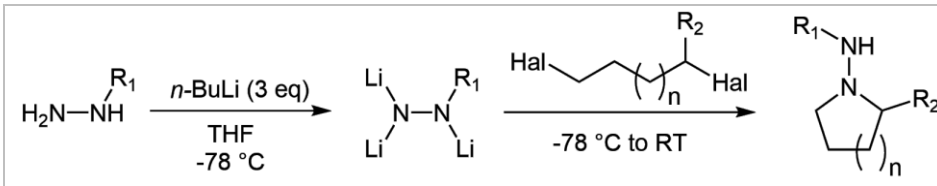
Scheme 5. Generation of 7-membered heterocyclic rings as intermediates for the synthesis of asymmetric alkylation catalysts by Widhalm *et al.*

Recently, Lebedev *et al.* experimented in our workgroup with polyanion strategy [40–42] to develop an efficient method for the synthesis of large variety of heterocyclic hydrazine derivatives [40]. For the synthesis of heterocycles with an exocyclic N-N bond, either Boc-hydrazine or phenyl hydrazine were used as substrates. These compounds were lithiated with 3 eq of *n*-BuLi at -78 °C in THF and as a result trianions were produced. Then, upon warming the reaction

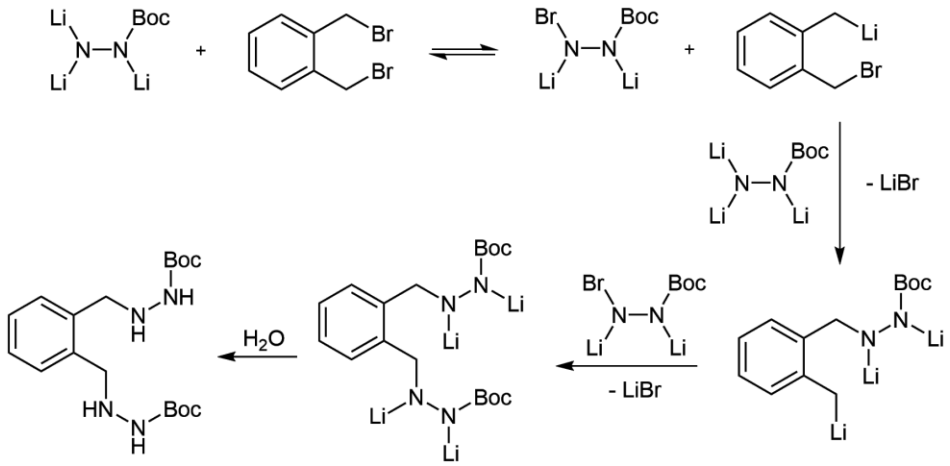
mixture, suitable dihalides were added, yielding in formation of heterocycles. Boc-protected hydrazine derivatives with 5- and 6-membered rings were isolated in good, 71–73% yields, but for the corresponding compound with a 7-membered ring, the yield reduced to 41% (**Table 1**). Yields were also lower (52%) when a branched electrophile (2,5-dibromopentane) was used. Using phenyl hydrazine instead of Boc-hydrazine also reduced the yields to ~40% for generation of 5- and 6- membered rings. In this case, authors believe that smaller difference in acidity between PhNH and NH_2 group reduces the selectivity, which is supported by the fact that significant quantity of heterocycles comprising an endocyclic N-N bond were generated in these reactions as side-products.

In the same research, an interesting phenomenon was observed. When forming a trianion from Boc-hydrazine and using this in a reaction with 1,2-bis(bromomethyl)benzene, no heterocyclic compound was isolated. Instead of that, one molecule of electrophile reacted with two trianions, forming a product with two hydrazine moieties. This outcome was explained by metal-halogen exchange between the trianion and electrophile (**Scheme 6**), which was also proved and investigated more thoroughly in a later publication [43].

Table 1. Synthesis of heterocyclic hydrazine derivatives with polyanion strategy (Lebedev *et al*). As an electrophile, corresponding dibromides were used, expect in the cases where n = 1 and R_2 = -H, then 1-bromo-4-chlorobutane was used.

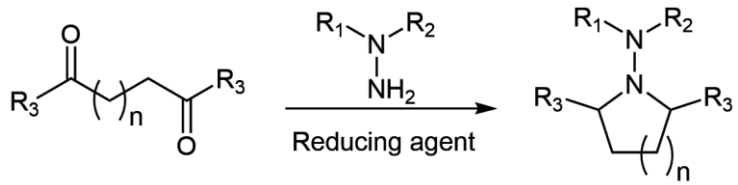


R_1	n	R_2	Yield (%)
Boc	1	-H	71
Boc	2	-H	73
Boc	3	-H	41
Boc	1	$-CH_3$	52
Ph	1	-H	45
Ph	2	-H	34



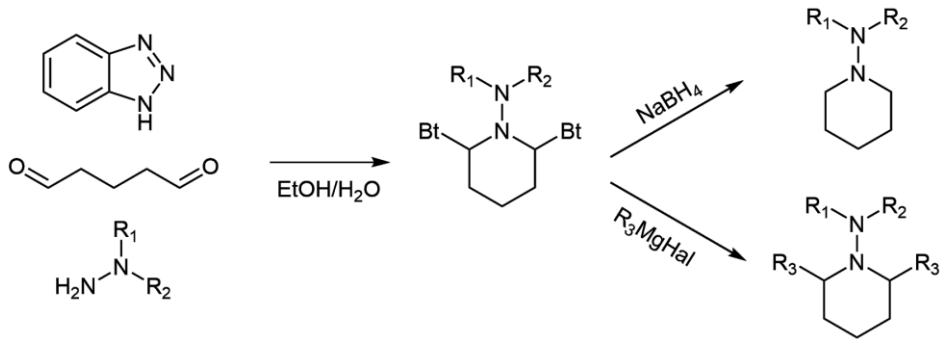
Scheme 6. Mechanism proposed by Lebedev *et al.* for the reaction of *tert*-butyl carbazate trianion with 1,2-bis(bromomethyl)benzene.

Besides dihalides, dialdehydes and diketones have been also used for the synthesis of 5- and 6-membered heterocycles with an exocyclic hydrazine moiety [44,45]. This type of synthesis proceeds via formation of imine intermediates that are subsequently reduced with $NaBH_3CN$ or $NaBH(OAc)_3$ (**Scheme 7**). In the work of Boga *et al.*, 2,5-dimethyl *N*-substituted pyrrolidines and 2,6-dimethyl *N*-substituted piperidines were synthesized [44]. However, if substrates were hydrazine derivatives, the yields remained rather low (35%) as various byproducts like hydrazones and pyrroles were formed during the reaction. Cocquet *et al.* had slightly more success with the preparation of 4-[(piperidin-1-yl)amino]ethyl-benzoate in the same way, as esterification of 4-hydrazinobenzoic acid and subsequent treatment with glutaraldehyde and sodium cyanoborohydride yielded desired heterocycle in moderate, 55% yield [45].



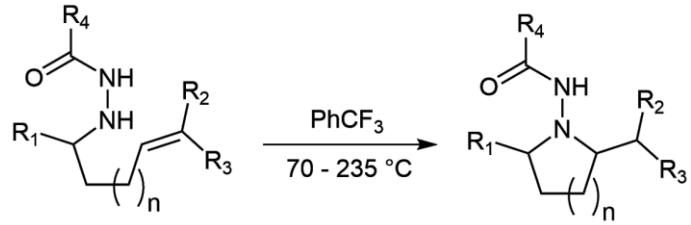
Scheme 7. Synthesis of heterocyclic hydrazine derivatives by double condensation and reduction. R_1 = -H, $-CH_3$; R_2 = $-CH_3$, 4-EtOCOPh-, R_3 = -H, $-CH_3$. Reduction agent is either $NaBH_3CN$ or $NaBH(OAc)_3$.

Remarkable improvement for this type of condensation were achieved by Katritzky *et al* [46]. They added benzotriazole in the reaction mixture alongside with glutaraldehyde and amine- or hydrazine derivative. This strategy allowed to synthesize *N*-substituted piperidines with benzotriazole substituents attached to the 2- and 6-positions in the ring, usually with yields reaching higher than 90%. Followingly, benzotriazole moieties can be either reduced with sodium borohydride to form unsubstituted piperidines or carry out reaction with Grignard reagents to introduce alkyl- or aryl groups to 2- and 6-positions in the piperidine ring (**Scheme 8**). More than 10 heterocyclic hydrazine derivatives with an exocyclic N-N bond were effectively synthesized this way.

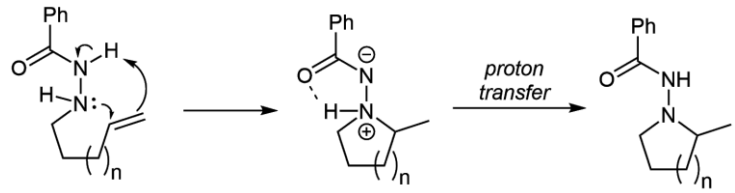


Scheme 8. Synthesis of heterocyclic hydrazine derivatives using benzotriazole as a synthetic auxiliary (by Katritzky *et al*). R_1R_2N = PhNH-, $(CH_3)_2N$-, PhCONH-, CH_3CONH-, CH_3CH_2OCONH-, -NHBoc; R_3 = -Me, -Et, -Ph, -Bn.

Another possibility to prepare heterocyclic hydrazine derivatives is hydrohydrazination, an analogue of hydroamination, which in principle is an addition of N-H bond to a multiple carbon-carbon bond. Hydroamination was pioneered by Gagné and Marks in the late 1980-s, as they applied organolanthanum catalysts for the intramolecular synthesis of nitrogen-containing heterocycles from alkenylamines [47]. Since then, hydroamination as a synthetic method rapidly gained popularity [48]. For the synthesis of heterocycles comprising an exocyclic N-N bond, the original hydroamination method has been altered by various modifications, allowing access to these molecules either by organozinc- or organoplatinum-catalysis [49,50]. Kaga *et al.* have performed t-BuOK mediated hydrohydrazination using hydrazones as substrates to produce various substituted pyrrolidines and piperidines [51]. Also, Beauchemin group has developed metal-free hydrohydrazination conditions (**Scheme 9**), as heating suitable alkenylhydrazides in *α*,*α*,*α*-trifluorotoluene leads to efficient cyclization of substrates to heterocyclic hydrazine derivatives [52–55]. This process proceeds via hydroamination, following a carbonyl-assisted proton transfer step as proposed by the authors (**Scheme 10**) [52].

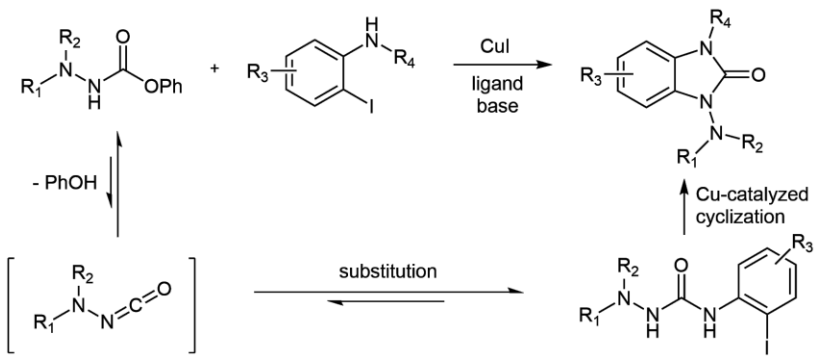


Scheme 9. Cyclization of alkenylhydrazides by hydrohydrazination as described by Beauchemin *et al*. n = 1,2; R_1 = -H, -Me; R_2, R_3 = -H, -Me, -Et. R_4 = -Alk, -Ar.



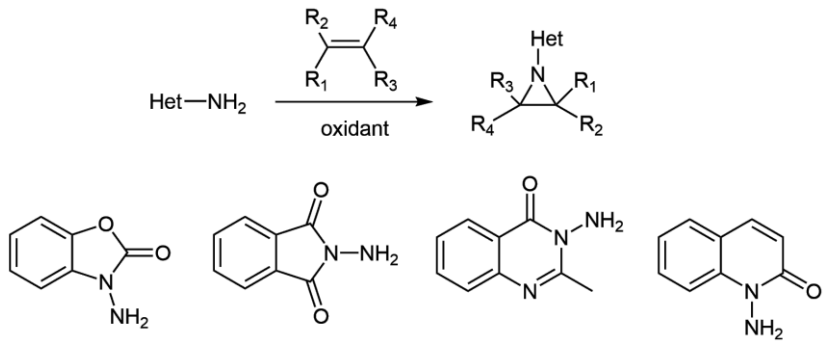
Scheme 10. Mechanism for the synthesis of 5- and 6-membered rings (n = 1,2) proposed by Beauchemin *et al*.

Besides hydrohydrazination, the Beauchemin group has applied other strategies for the synthesis of heterocyclic hydrazine derivatives as well. They have used masked isocyanates and isothiocyanates to prepare imidazolones or thiazolidines respectively by using 5-*exo*-dig cyclization with propargylamines [56]. Also, masked isocyanates have been used in substitution reactions with 2-iodoanilines, followed by Cu(I)-catalyzed cyclization yielding functionalized 1-aminobenzimidazolones (**Scheme 11**) [57]. According to more than 40 examples, both of these methods allowed to produce cyclic hydrazine derivatives with good to excellent yields.



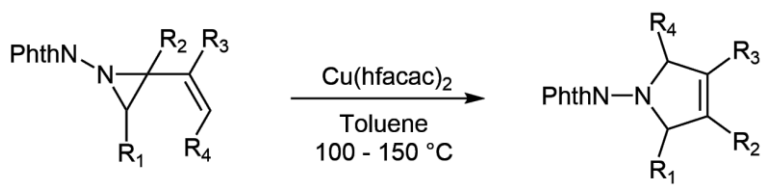
Scheme 11. Synthesis of benzimidazolones from masked isocyanates by Beauchemin *et al*. R_1, R_2 = -Alk, -Ar; R_3 = -H, -Me, -Hal, -CF_3, -COOMe; R_4 = -H, -Me.

Aziridines, the 3-membered rings, are smallest among nitrogen-containing heterocycles. These small heterocycles feature exclusively broad range of biological activities and can be found in several synthetic drugs and natural compounds [58,59]. The release of the strain energy upon cleavage of the aziridine ring is the driving force for a vast number of transformations, making aziridines particularly useful as synthetic precursors to functionalized nitrogen hetero- and acyclic compounds [58–61]. *N*-aminoaziridine derivatives are generally prepared by oxidative aminoaziridation reaction [61], pioneered by Atkinson and Rees more than half a century ago [62–64]. Classical reaction conditions involve a heterocyclic *N*-amino compound, an alkene and $Pb(OAc)_4$ as an oxidant [63,64]. Other oxidants, such as hypervalent iodine compounds [65–67] and electrochemical oxidation [68] have been also used recently (**Scheme 12**).



Scheme 12. General conditions for oxidative aminoaziridation (up) and structures of more frequently used heterocyclic *N*-amino substrates (Het-NH_2, down). R_1 – R_4 represent various substituents.

N-aminoaziridine derivatives can be used not only for ring opening reactions, but also as substrates for intramolecular ring expansion, as it was shown by Brichacek *et al* [69]. When *N*-phthalimido vinyl aziridines were heated in the presence of an organocopper catalyst, 5-membered heterocycles including a carbon-carbon double bond in their structure were formed mostly with excellent yields (**Scheme 13**).



Scheme 13. Ring-expansion of *N*-phthalimido vinyl aziridines as described by Brichacek et al. R_1 = -H, -Alk, -Ph; R_2 = -H, Alk; R_3 = -H, -Alk, -Ph, -OTBS; R_4 = -H, -Alk, -$COOCH_3$.

1.2. Synthetic methods for preparation of enehydrazines and enehydrazides

Enehydrazides and enehydrazines can be considered as analogues of enamides and enamines correspondingly and most chemical properties of the latter also apply for their hydrazine analogues. Structurally, they both include a vinylic carbon-carbon double bond and differ from each other by either having an electron-withdrawing carbonyl group attached to the vinyl-substituted nitrogen atom, or not (**Figure 4**). Enehydrazines are tautomeric with corresponding hydrazones and may spontaneously isomerize into thermodynamically more stable form, which is most widely known from Fischer indole synthesis [70–72]. Also, the localized electron pair of nitrogen atom is in conjugation with the vinylic double bond, substantially increasing its nucleophilicity. However, in enehydrazides, both of these effects are diminished as electron-withdrawing characteristic of the carbonyl group delocalizes nitrogen's free electron pair.

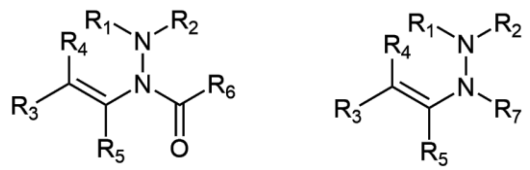
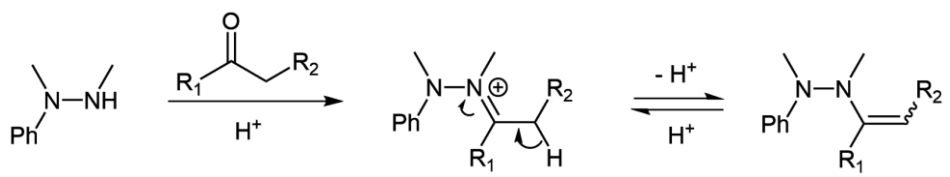


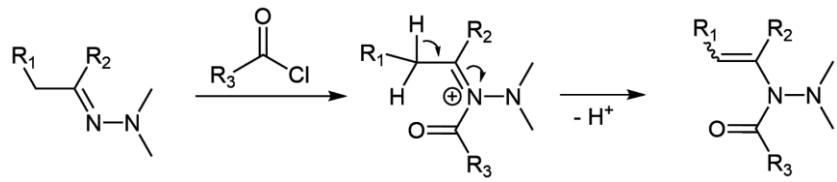
Figure 4. General structural formula of an enehydrazide (left) and an enehydrazine (right). R_1 – R_6 represent undefined functional groups, R_7 = -H, -Alk, -Ar.

Classical pathway for the synthesis of enehydrazines is a condensation reaction between a secondary amino group of a hydrazine derivative and a carbonyl compound, forming initially an ionic hydrazonium derivative which undergoes tautomerization to corresponding enehydrazine [73–75]. In the work on Schiess *et al.*, six different enehydrazines were synthesized with 50 – 86% yields (**Scheme 14**) [73]. This reaction is an analog for the one used also in enamine synthesis [76].



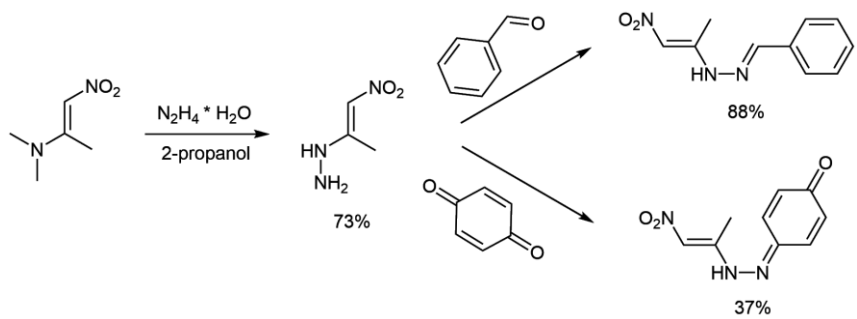
Scheme 14. Enehydrazine synthesis as described by Schiess *et al.* R_1 = -H, -Et; R_2 = -Me, -Et, -Ph or R_1 = R_2 = $-(CH_2)_3-$, $-(CH_2)_4-$, $-(CH_2)_2C_6H_4-$.

Similar approach can be used for preparation of enehydrazides. Here, a hydrazone reacts with a carboxylic acid derivative, most commonly with a corresponding anhydride or acyl halide. Again, hydrazonium intermediate is formed, which isomerizes to tautomeric enehydrazide. In this way, Lerche *et al.* were able to isolate more than 10 enehydrazides with very good yields (**Scheme 15**) [77]. Also, Miyata *et al.* have prepared various enehydrazide intermediates for following indole synthesis using this method, and achieved nearly quantitative yields in many cases [78,79].



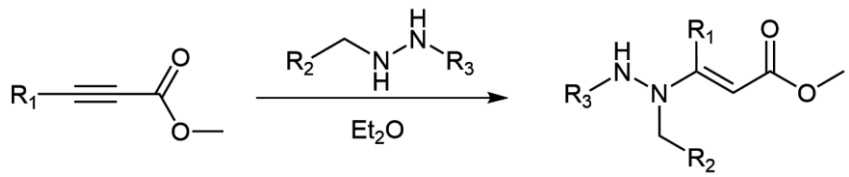
Scheme 15. Synthesis of enehydrazides by Lerche *et al*. R_1 = -H, -Me, -Ph, R_2 = -H, -Me, $-CH_2N(CH_3)_2$ or $R_1 = R_2 = -(CH_2)_3-$, $-(CH_2)_2C_6H_4-$. R_3 = -Me, $-CH_2Cl$, -Ph, $4-NO_2C_6H_4-$.

When enehydrazines and -hydrazides comprise electron-withdrawing substituents that reduce the nucleophilic nature of the enehydrazine or -hydrazide moiety, the variety of synthetic methods broadens for the preparation of these compounds. For example, in the research of Lyubchanskaya *et al.*, 1-methyl-2-nitrovinyl hydrazine was isolated with 73% yield, when 2-(dimethylamino)-1-nitroprop-1-ene was transaminated with hydrazine hydrate [80]. Furthermore, the product was successfully used in condensation reactions with 1,4-benzoquinone or benzaldehyde, as bifunctional enehydrazones were isolated (**Scheme 16**). Similarly, dialkyl dicyanofumarates or 3-(dimethylamino)-propenoate can react with hydrazine hydrate as well as its derivatives to form stable enehydrazines [81,82].



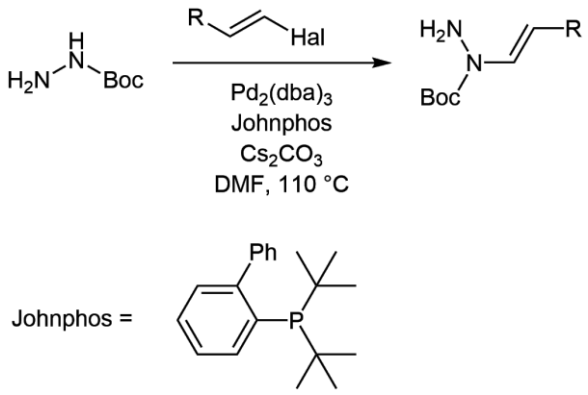
Scheme 16. Synthesis of an enehydrazine by transamination reaction and its condensation with benzaldehyde (up) and 1,4-benzoquinone (down) to form enehydrazones as described by Lyubchanskaya *et al.*

Besides substitution reactions, addition of hydrazine derivatives to electron-deficient carbon-carbon triple bonds can also be used to prepare large variety of enehydrazines, as it was shown in the review of W. Sucrow [83]. For example, this hydrohydrazination method has been applied to generate enehydrazines in a reaction between methyl propiolate or dimethyl acetylene dicarboxylate (DMAD) and methyl-, *N,N'*-dimethyl- or benzyl hydrazine (**Scheme 17**) [83,84]. Also, 50 years before that time, Diels and Reese [85,86] and Huntress *et al.* [87] followed the same reaction pathway as they reported that enehydrazine intermediates were formed in a reaction between DMAD and aryl-substituted hydrazines.



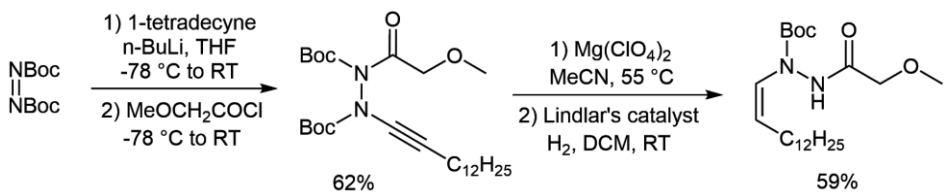
Scheme 17. Synthesis of enehydrazines by hydrohydrazination of carbon-carbon triple bonds as described by Sucrow *et al*. R_1 = -H, -COOMe; R_2 = -H, -Ph; R_3 = -H, -Me.

More recently, some methods which enable access to enehydrazides via alkylation of hydrazides with vinyl halides have been emerged [88,89]. In the earlier research of Barluenga *et al.*, *tert*-butyl carbazate was alkylated in the presence of $Pd_2(dba)_3$ catalyst, organophosphorus ligand (Johnphos) and Cs_2CO_3 as a base in DMF (**Scheme 18**) [88]. This approach allowed to prepare 11 enehydrazides in 50–75% yields and has also been successfully adapted for the synthesis of analogues of hydrazidomycin – a potent cytotoxic compound [90]. The role of organophosphorus ligand turned out to be crucial in this method, as some other ligands lead to formation of Ullmann coupling [91] products instead. The work of Zhan and Liang improves this research as they showed that alkylation can be carried out with economically much more beneficial copper catalysis as well, by using DMEDA as a ligand in refluxing toluene with a presence of inorganic base [89].

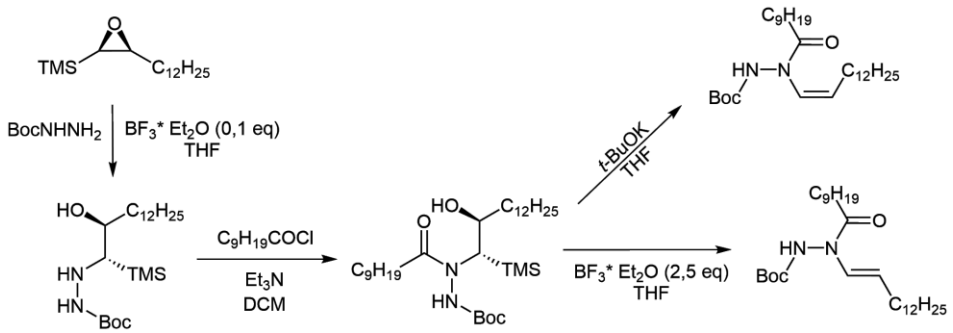


Scheme 18. Preparation of enehydrazides from vinyl halides as described by Barluenga *et al*. R represents various vinyl- or aryl substituents.

In the search of pathways for the preparation of hydrazidomycins, Beveridge and Batey have presented two different approaches for the synthesis of enehydrazides [92]. The first route involves addition of lithiated 1-tetradecyne to di-*tert*-butyl azodicarboxylate, forming a unique ynehydrazide. Afterwards, this intermediate was hydrogenated on Lindlar's catalyst to produce the desired *Z*-isomer of an enehydrazide (**Scheme 19**). In the second approach, TMS-substituted epoxide was first formed from 1-tetradecyne and this compound was conjugated with *tert*-butyl carbazate. Next, the basic nitrogen was acylated and Peterson elimination [93] was applied to produce either enehydrazides with *E*- or *Z*-configuration, depending on exact reaction conditions (**Scheme 20**).

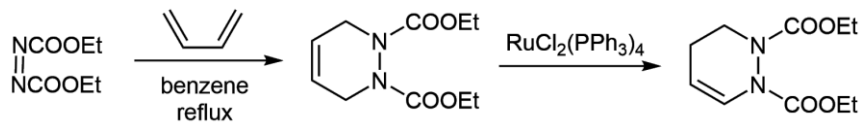


Scheme 19. Synthesis of an enehydrazide by catalytic reduction of carbon-carbon triple bond.

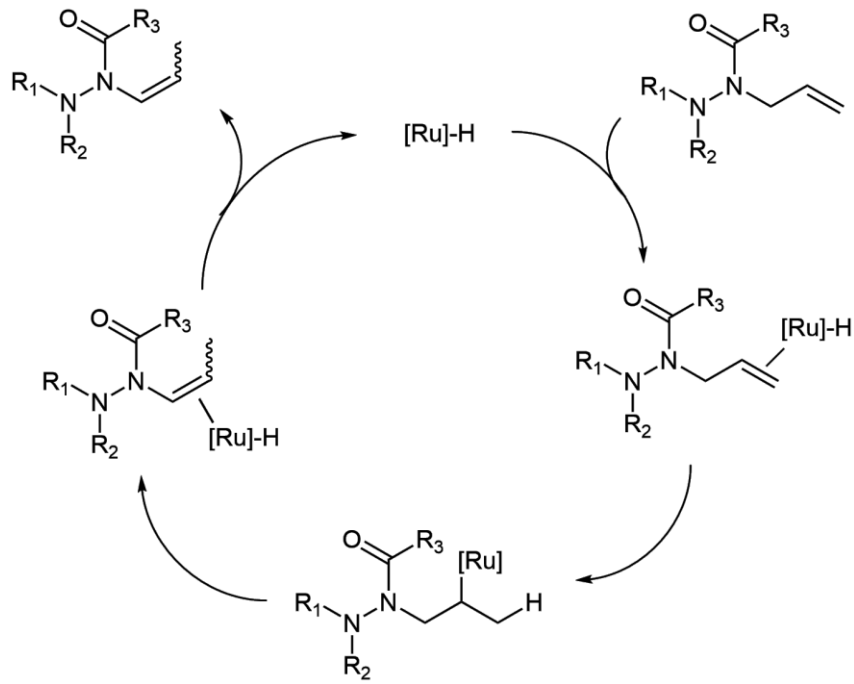


Scheme 20. Preparation of enehydrazides by Peterson elimination.

Another possibility for the synthesis of enehydrazides is to use catalytic isomerization. Although this method is widely used for the preparation of enamides [94–103], its applicability on hydrazine derivatives has been tested less often [23,104]. For example, Menchi *et al.* prepared cyclic enehydrazide by Diels-Alder reaction, following catalytic isomerization (**Scheme 21**) [23]. Usually, the isomerization is carried out by hydrides of a precious metal catalyst, most commonly a ruthenium derivative. The catalytic cycle includes addition of ruthenium hydride complex to allylic double bond, followed by elimination of ruthenium moiety with an α-hydrogen (**Scheme 22**) [100].



Scheme 21. Synthesis of cyclic enehydrazide as described by Menchi *et al*.



Scheme 22. Isomerization of allylhydrazides to enehydrazides. Adapted from the research of Alcaide *et al*. R_1 – R_3 represent various functional groups.

2. AIMS OF THE STUDY

The main objectives of the current thesis were:

1. To develop methods for the synthesis of heterocycles comprising an exocyclic N-N bond in their structure without using N-N bond formation reactions to access both saturated and unsaturated compounds.
2. Investigate possibilities to synthesize cyclic enehydrazides.
3. Conjugate synthesized heterocycles with saccharides to form corresponding hydrazones as acyclic nucleoside mimetics and measure their biological activity against various targets.

3. RESULTS AND DISCUSSION

3.1. Synthesis of saturated heterocycles with an exocyclic N-N bond

The aim for the current part of the study was to develop a method for the synthesis of *N*-aminopyrrolidine, *N*-aminopiperidine and *N*-aminoazepane, which are correspondingly 5- to 7-membered saturated heterocycles containing an exocyclic N-N bond in their structure (**Figure 5**). As the starting materials, hydrazine hydrate and aliphatic terminal dihalides were chosen in order to carry out synthesis in a single step and to avoid N-N bond formation reactions.

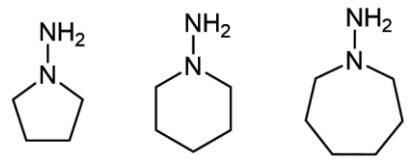
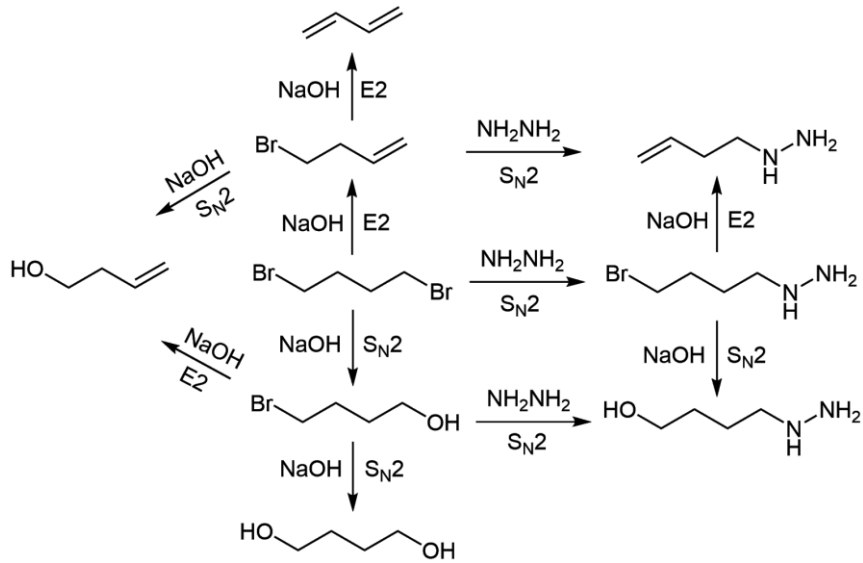


Figure 5. Structural formulas of *N*-aminopyrrolidine, *N*-aminopiperidine and *N*-aminoazepane correspondingly from left to right.

As a starting point, a method previously described in the literature was chosen [105]. In this method, dihalide and concentrated aqueous solution of NaOH were dropwise added to a slight excess (1.2 eq) of hydrazine hydrate in refluxing methanol and pH of the reaction mixture was held between 8 and 11. After the completion of the reaction, the products were purified by extraction and subsequent fractional distillation. Following this method resulted very poor yields of *N*-aminopyrrolidine (7%) and *N*-aminopiperidine (3%).

An explanation can be proposed for the low yields obtained with this method. Using NaOH can cause E2 elimination and S_N2 nucleophilic substitution reactions for dihalides, producing various undesired alcohols and alkenes in the reaction mixture (**Scheme 23**) [106]. Therefore, it is likely that using inorganic hydroxides or other strong bases is detrimental for this type of method.

However, as acidic hydrogen halides are generated during the substitution reaction of hydrazine hydrate and organic halide, a base is still required to neutralize the acid. Otherwise, hydrazine hydrate itself would become protonated, resulting in loss of nucleophilicity and reactivity. A list of suitable bases would include inorganic salts of alkali metals such as carbonates and phosphates as well as tertiary amines (TEA, DIPEA). Tertiary amines, especially when used in excess, may become problematic during the product extraction phase due to their lower polarity and in distillation phase due to their similar boiling point with products.



Scheme 23. Possible NaOH-induced reactions leading to formation of undesired byproducts. 1,4-dibromobutane serves as an example of a dihalide.

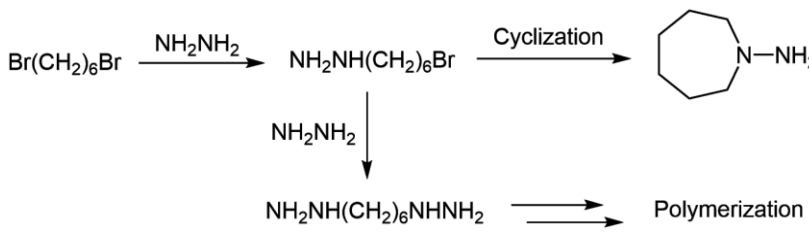
Another possibility would be to use hydrazine hydrate in large excess, as then only a part of it would become protonated during the reaction and the other part would remain reactive. Having great excess of hydrazine hydrate in the reaction mixture provides also another advantage – instead of hydrazine hydrate, the organic halide can also react with product's free amino group leading to formation of undesired bicyclic hydrazine derivatives [107]. Having large amount of hydrazine hydrate in the reaction mixture statistically reduces the chance of this disadvantageous event.

In principle, *N*,*N'*-heterocycles should also be likely byproducts in this method. In reality, almost no *N*,*N'*-heterocycles are formed during the synthesis. This can be explained by comparing nucleophilicities of hydrazine and monomethyl hydrazine. As it was shown by Nigst *et al.*, the methyl group significantly increases the nucleophilicity of α-nitrogen, while simultaneously lowering the nucleophilicity of β-nitrogen [108]. Due to this alkyl substituent effect, the formation of *N*,*N*-heterocycles is much more favorable and almost no *N*,*N'*-heterocycles and previously mentioned bicyclic hydrazine derivatives form during the synthesis.

Incorporating these changes (using more than 3 equivalents of hydrazine hydrate and no additional base) increased the yield of product to 49%. For the synthesis of *N*-aminopiperidine, the yield remained rather low (~20%) with these conditions and it seemed that during the distillation step the product started to decompose. As the product was sufficiently pure already after extraction phase, the additional purification step was omitted, and solvents were removed under reduced pressure (40 °C at 40 mbar), giving *N*-aminopiperidine

in high (74%) yield and purity (only trace amounts of methanol and Et_2O were determined with ^1H NMR).

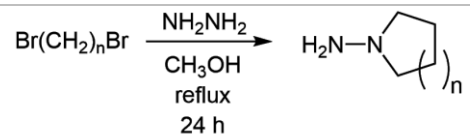
For the synthesis of *N*-aminoazepane, additional problem raised even while using optimized reaction conditions and omitting product distillation step. As the main product a white precipitate formed and only trace amount of desired *N*-aminoazepane was isolated. It is very likely that the byproduct is a polymer of hydrazine, linked together with hexamethylene bridges (**Scheme 24**). This hypothesis is supported by the fact, that by *ab initio* calculations the ring strain energy of a seven-membered ring is higher than five- and six-membered rings by approximately 2 kcal/mol and 6.5 kcal/mol respectively [109]. Additionally, the second nucleophilic substitution reaction which forms a cyclic product can only take place when the alkyl chain is in suitable conformation, resulting $-CH_2Br$ fragment being in the vicinity of the nitrogen atom. With each methylene group added to the alkyl chain, the rotational degrees of freedom increase, ultimately lowering the probability of the chain being in suitable conformation for cyclization.



Scheme 24. Cyclization and polymerization pathway in the synthesis of *N*-aminoazepane.

As the cyclization is kinetically a first-order reaction and the polymerization follow second-order kinetics, it is beneficial to lower the concentration of reagents to promote cyclization pathway and to avoid polymerization. By lowering the concentration of reagents in methanol about ten times, we were able to isolate *N*-aminoazepane in 45% yield and visually no polymerization was detected in the reaction media. Optimized reaction conditions for the synthesis of all the mentioned heterocycles are presented in **Table 2**.

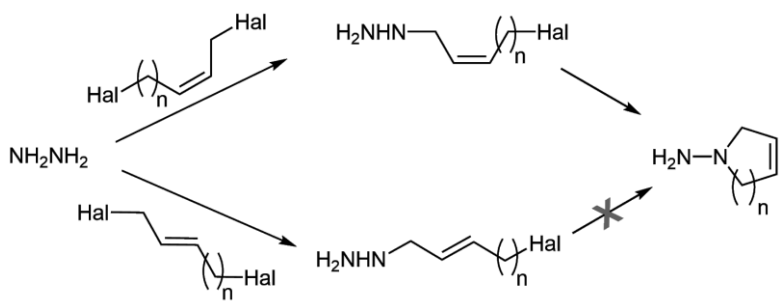
Table 2. Optimized reaction conditions for the synthesis of *N*-aminopyrrolidine, *N*-aminopiperidine and *N*-aminoazepane.



n	n(hydrazine hydrate) (mol)	n(dihalide) (mol)	V(methanol) (ml)	Yield (%)
1	0,50	0,15	40	49
2	0,30	0,10	25	74
3	0,50	0,15	360	45

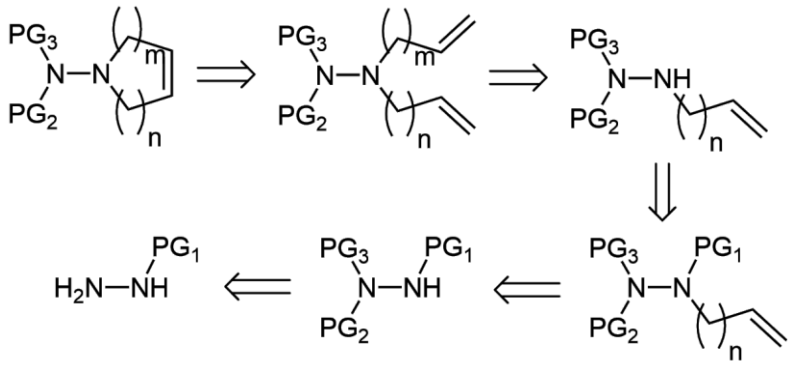
3.2. Synthesis of unsaturated heterocycles with an exocyclic N-N bond

In principle, synthesis of *N*-aminopyrrolidine, *N*-aminopiperidine and *N*-aminoazepane analogues which include a carbon-carbon double bond in their structure, could be carried out in similar conditions as described in the previous chapter. However, dihalides needed to carry out this type of cyclization must be strictly in *cis*-configuration, otherwise formation of desired 5- to 7-membered heterocycles would be geometrically impossible (**Scheme 25**) as the ring strain of *E*-cycloheptene ring is approximately 20 kcal/mol higher than its *Z*-isomer [110] and the *E*-cycloheptene itself has been never isolated [111]. Apart of *cis*-1,4-dichloro-2-butene, these dihalides are commercially not widely available. Their synthesis is possible, but being a tedious multi-step process [112,113], it was decided that other methods are used for the formation of these heterocycles.



Scheme 25. Reaction pathways of hydrazine with *cis*-dihalide (up) and *trans*-halide (down). As in the heterocycles the double bond configuration is *cis*-, no cyclization is possible when using *trans*-dihalide. n = 1-3.

We decided to use intermittent protection-deprotection and alkylation strategy, combining it with the ring-closing metathesis as a final step of the reaction sequence. The required steps can be seen in the retrosynthetical **Scheme 26**.



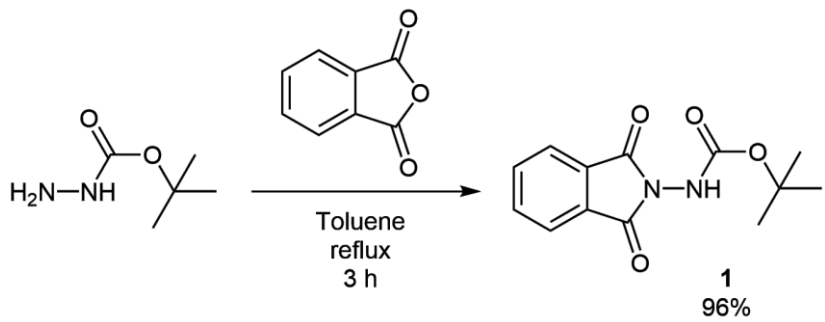
Scheme 26. Retrosynthetical scheme for the synthesis of unsaturated heterocycles comprising an exocyclic N-N bond.

Firstly, suitable protection groups (PG) were needed to be chosen for this synthesis sequence. Most important prerequisites for the protection groups were following:

1. All protective groups should withstand moderately basic conditions needed for alkylation reactions.
2. PG_2 and PG_3 should withstand conditions needed to cleave PG_1.
3. All protective groups, but especially PG_1, should be cleavable in conditions which do not interfere with the presence of carbon-carbon double bond.
4. PG_2 and PG_3 should be compatible with ring-closing metathesis conditions.
5. Ideally, PG_2 and PG_3 should be cleavable simultaneously in mild conditions.
6. For the synthesis of non-alkylated and triply protected hydrazine derivative, orthogonal protection strategy must be available.

After careful consideration, the Boc-group was chosen to fill the role of PG_1. For PG_2 and PG_3 a bidental protection in form of phthaloyl group was selected. Only contradiction with the prerequisites was that the phthaloyl group might be susceptible to basic conditions as it is usually cleaved via hydrazinolysis. However, incompability of phthaloyl group with inorganic carbonates required to carry out *N*-alkylation was not described in the literature [114].

N,*N*-phthaloyl-*N'*-*tert*-butyloxycarbonylhydrazine **1** was synthesized in a multigram scale from $BocNHNH_2$ and phthalic anhydride (**Scheme 27**) in nearly quantitative yield (96%) following a protocol from Brosse *et al* [115].



Scheme 27. Synthesis of *N,N*-phthaloyl-*N'*-tert-butyloxycarbonylhydrazine **1.**

Next step involved alkylation with alkenyl bromides. As similar alkylations had been done in PTC conditions previously [115], we tried to adapt a similar setup. Substrate **1** had low solubility in many solvents usually applied for PTC reactions, such as acetonitrile or toluene. Therefore, we carried out the reactions in DMF, which afforded good solubility. Initially, potassium carbonate was used as a base, TBAHS as the phase transfer catalyst and allyl bromide as an electrophile. In these conditions, the reaction was very slow at room temperature. The rate accelerated upon heating to 70 °C, but the reaction still required overnight stirring to achieve complete conversion. When we changed the base to cesium carbonate, the reaction rate increased substantially, giving full conversion in 2 hours even at 50 °C. This phenomenon can be explained by two factors. Firstly, the solubility of Cs_2CO_3 in DMF is greater than K_2CO_3 solubility [116], leading to higher base concentration in solution, ultimately affording more efficient deprotonation of substrate. Secondly, larger cations, in this case Cs^+, form a more "loose" ion pair with the substrate than K^+, therefore increasing reactivity of the nucleophile species [117–119].

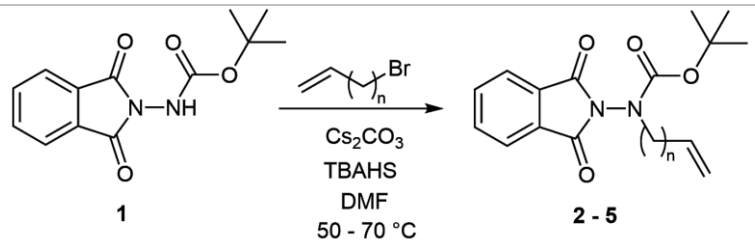
While these conditions worked well for the alkylation reaction, we were curious if TBAHS has any beneficial role in the current situation where the base is relatively well soluble in reaction media. Therefore, we carried out one experiment without TBAHS in otherwise identical conditions. Contrary to our hypothesis, the reaction rate decreased, and the time needed to complete the reaction with full conversion increased about three times. Therefore, in these particular conditions, it is advantageous to use phase transfer catalyst in order to obtain shorter reaction times. However, as it was not in the main focus of our research, we did not investigate this phenomenon more thoroughly.

As we carried out reactions with other electrophiles than allyl bromide, we noticed that the reactions proceeded much more sluggishly. To complete these reactions in a reasonable timeframe, it was necessary to raise the temperature to 70 °C. This situation can be explained by well-known fact that electrophiles containing an unsaturation at β-carbon (such as allyl- and benzyl halides) are

much more reactive, as their corresponding cations are resonance-stabilized [117,120,121].

Results and conditions for these alkylation reactions can be seen in **Table 3**. All reactions proceeded with full conversion and high yields. TLCs made after extraction of the reaction mixture revealed only very small amount of impurities, which was also confirmed by ^{1}H NMR. Therefore, no additional chromatographical purification was needed before proceeding to the next, deprotection step.

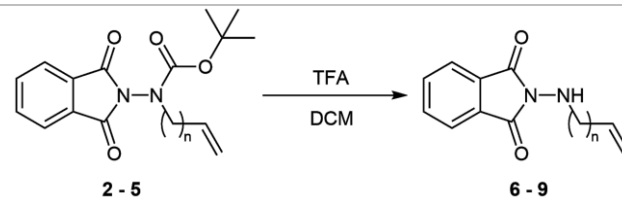
Table 3. Reaction conditions and results of alkylation reactions. 1.5 eq of alkenyl bromide, 2 eq of Cs_2CO_3 and 10 mol% of TBAHS were used in all reactions.



Entry	Substrate	Product	n	Time (h)	Temperature (°C)	Yield (%)
1	**1**	**2**	1	2	50	94
2	**1**	**3**	2	18	70	93
3	**1**	**4**	3	7	70	95
4	**1**	**5**	4	21	70	82

Second step of the synthesis sequence was removal of the Boc-group from previously alkylated nitrogen atom. Usually, acidic conditions are applied for the cleavage of Boc-group [114]. In this work we used TFA as an acid, but in a larger scale mineral acids such as HCl or H_2SO_4 would be more practical due to their cheaper price and lower corrosivity [114,122,123]. We used approximately 8% TFA solution in DCM and the acid was taken at 10-fold molar excess. With all the substrates, the Boc group was cleaved in several hours at ambient temperature. Workup included evaporation of volatiles, basification and extraction. In this step, further purification of products was necessary as they usually contained a small amount of impurities by TLC. For purification, *flash*-chromatography was the method of choice. Recrystallization was not an option, as the products were all liquids. Results of deprotection reactions can be seen in **Table 4**.

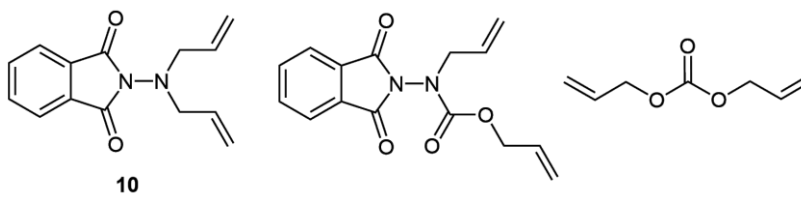
Table 4. Results of Boc-deprotection reactions. Reactions were run in 8% TFA (10 eq) solution in DCM for about 4 h at room temperature.



2 - 5 **6 - 9**

Entry	Substrate	Product	n	Yield (%)
1	**2**	**6**	1	85
2	**3**	**7**	2	91
3	**4**	**8**	3	87
4	**5**	**9**	4	76

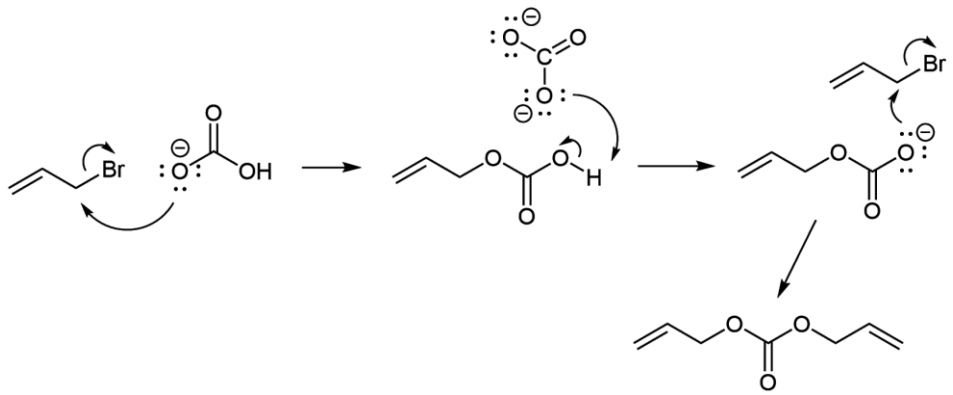
The penultimate step in the reaction sequence was second alkylation. It was a seemingly easy alkylation reaction that turned out to be rather problematic. At first, we tried to deprotonate substrate **6** with *n*-BuLi, following alkylation with allyl bromide. Although this method had previously worked well with other hydrazine derivatives [40–42], this time it lead to dephthaloylation. Deprotection was also the case when a weaker base in form of sodium hydride or LDA as a sterically hindered base were used. Next, we tried to use PTC conditions with NaOH and K_2CO_3 as bases and TBAHS as a catalyst, but incorporation of these inorganic bases yielded dephthaloylation as well. Therefore, the use of even milder bases was necessary to avoid deprotection. We repeated the previous reaction, excluding NaOH from the reaction mixture this time. Finally, we were able to isolate desired diallylated product **10** and no deprotection was observed. However, another problem arose, as byproducts in form of allyl carbamate and diallyl carbonate were also detected in the reaction mixture (**Figure 6**).



10

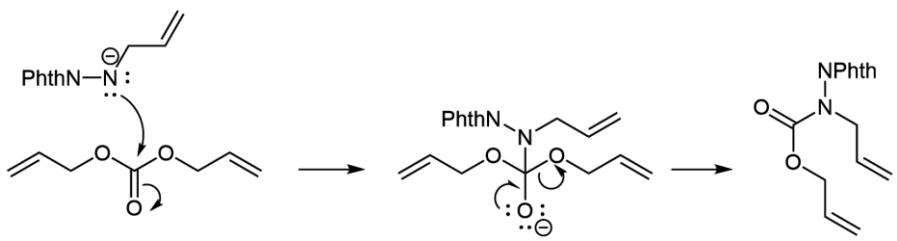
Figure 6. Products from the allylation of **6** with allyl bromide in DMF, using K_2CO_3 as a base and TBAHS as a catalyst. Diallylated main product **10** is on the left, carbamate byproduct in the middle and diallyl carbonate on the right.

The formation of organic carbonates from alkyl halides and inorganic carbonates has been described previously by Cella and Bacon [116]. As the reaction conditions in our setup were rather similar, it is likely that the generation of diallyl carbonate occurred by the same mechanism as discussed in that article (**Scheme 28**).



Scheme 28. A mechanism for the formation of diallyl carbonate. Original, generalized mechanism was proposed by Cella and Bacon.

For the generation of carbamates in these conditions, a mechanism can be proposed (**Scheme 29**). In this mechanism, carbonyl group of the diallyl carbonate is attacked by the *N*-nucleophile and tertiary intermediate complex is formed. From this complex, allyl alcohol is eliminated, and allyl carbamate is formed. This mechanism is supported by series of publications, where alkyl- and aryl carbamates were produced from amines and organic carbonates [124–126].

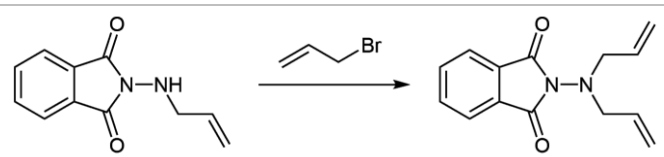


Scheme 29. Mechanism for the formation of allyl carbamates.

With the initial conditions (1.5 eq allyl bromide, 2 eq K_2CO_3, 0.1 eq TBAHS, 45 °C), the reaction proceeded rather slowly. When the temperature was raised to 70 °C, reaction rate increased, but substantial amount of dephthaloylation was detected by TLC. However, increasing the amount of electrophile and base to 4 molar equivalents lead to complete conversion in 24 hours at 45 °C. After

purifying the reaction mixture with column chromatography, desired *N,N*-phthaloyl-*N',N'*-diallylhydrazine **10** was isolated (57%) as a least polar fraction. As a second fraction corresponding allyl carbamate was also separated (32%). In the fraction of **10**, diallyl carbonate was also detected by NMR. Purifying the product from diallyl carbonate turned out to be simple task, as the organic carbonate has relatively low boiling point (74 °C at 24 mm/Hg), and was therefore removed *in vacuo* [127]. Directing the reaction so, that less carbamate and more dialkylated product is formed, turned out to be a more complex task. As in some publications crown ethers and quaternary ammonium salts were used to promote carbamate formation, we expected that omitting TBAHS might have a beneficial effect in our system [116,128]. Indeed, the ratio of alkylation to carbamate formation shifted towards desired direction (69% and 19% of dialkyl- and carbamate product were isolated correspondingly), yet reaction rate slowed down as 3 days were required to achieve full conversion. In order to restore the faster reaction rate, we exchanged the base to cesium carbonate. This change accelerated the reaction, as full conversion was reached within less than 24 hours. Also, the product ratio shifted towards advantageous direction (74% of dialkylated product and 8% of carbamate). As a downside, use of stronger base lead to some dephthaloylation, so the base should be quenched as soon as full conversion is reached. The summary of optimization for the allylation can be seen in **Table 5**.

Table 5. Optimization for the allylation of *N,N*-phthaloyl-*N'*-allylhydrazine.



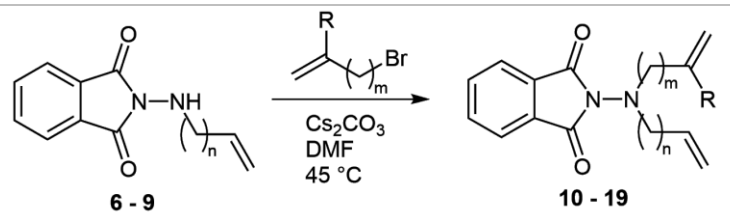
6 **10**

Entry	Solvent	Base	Catalyst	Temperature (°C)	Time (h)	Yield (%)
1[a]	THF	*n*-BuLi	-	-78 to 20	4	0
2[a]	THF	NaH	-	0 to 20	4	0
3[a]	THF	LDA	-	-78 to 20	4	0
4[a]	Toluene	NaOH/K_2CO_3	TBAHS	20	24	0
5[b]	DMF	K_2CO_3	TBAHS	45	24	57
6[b]	DMF	K_2CO_3	-	45	72	67
7[b]	DMF	K_2CO_3	-	70	24	36
8[b]	DMF	Cs_2CO_3	-	45	22	74

[a] Reaction conditions leaded to dephthaloylated products. [b] 4 eq of base and allyl bromide were used.

The optimized reaction conditions were also applicable for other substrates **7**-**9** and as well as when methallyl bromide was used as an electrophile. In these conditions yields varied from 72% to 81%. When 5-bromo-1-pentene and 4-bromo-1-butene were used as electrophiles, the yields were lower (61% and 30% correspondingly) and reactions needed more time to reach completion. Remarkably lower yield for the latter example can be explained by competitive elimination reaction. In this case it is more favored as it produces 1,3-butadiene as a conjugated elimination product. Concise results for all alkylation reactions are presented in **Table 6**.

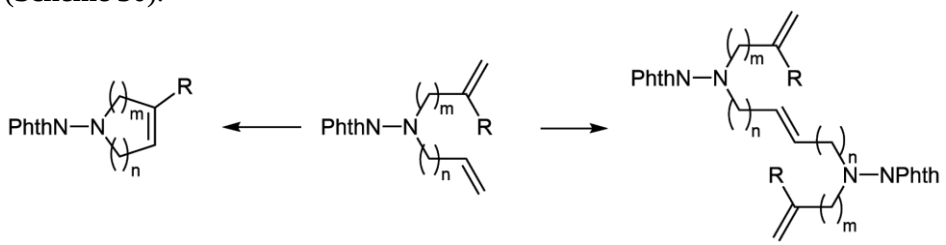
Table 6. Alkylation of *N*,*N*-phthaloyl-*N'*-alkenylhydrazines.[a]



Entry	Substrate	Product	n	m	R	Time (h)	Yield (%)
1	**6**	**10**	1	1	-H	18	74
2	**7**	**11**	2	1	-H	18	80
3	**8**	**12**	3	1	-H	18	72
4	**9**	**13**	4	1	-H	20	72
5	**6**	**14**	1	1	$-CH_3$	18	75
6	**7**	**15**	2	1	$-CH_3$	20	73
7	**8**	**16**	3	1	$-CH_3$	22	76
8	**9**	**17**	4	1	$-CH_3$	18	81
9	**7**	**18**	2	2	-H	72	30
10	**7**	**19**	2	3	-H	24	61

[a] 4 eq of alkenyl bromide and 4 eq of cesium carbonate were used in all reactions.

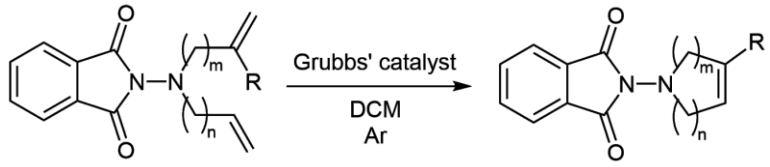
Final step of the synthesis route was the formation of heterocycles by ring-closing metathesis reaction. We started synthesis with the same reaction conditions that had previously worked well in our workgroup (5 mol% of 1st or 2nd generation Grubbs' catalyst and 20 mM substrate concentration in DCM) [41]. These conditions were applicable for the formation of 5- and 6-membered rings, as desired heterocyclic products were formed quickly in high yields. Yields dropped considerably, when the synthesis of 7-membered rings was attempted. The main cause for this was the generation of intermolecular reaction products (**Scheme 30**).



Scheme 30. Intramolecular ring-closing metathesis pathway, leading to cyclic products (left) and competitive intermolecular cross-metathesis pathway, resulting in dimerized products (right). n = 1-3, m = 1-2, R = -H, -CH_3.

This situation is remarkably similar to the problems that occurred in the synthesis of *N*-aminoazepane discussed previously. Therefore, the reactions can be redirected to cyclization pathway by lowering the concentration of the substrate [129]. In our case we noticed some improvement in yields, but reactions seemed to stall at some point even at higher catalyst loadings. For example, full conversion of substrate was never achieved when synthesizing compound **26** and all attempts to produce 8-membered heterocycles failed. This situation can be explained by catalyst poisoning, as dialkylated nitrogen atom in substrates and products acts as an electron pair donor, leading to ligation with the catalyst and ultimately resulting in formation of catalytically inactive ruthenium species [129]. Theoretically, this catalyst poisoning can be avoided by protonating substrates at first, resulting in formation of ammonium salts, and then carrying out RCM [130]. However, in our experiments this approach turned out to be unsuccessful. Results of metathesis reactions are presented in **Table 7**.

Table 7. Ring closing metathesis reactions of *N*,*N*-phthaloyl-*N'*,*N'*-dialkenylhydrazines.



10 - 19 **20 - 29**

Entry	Substrate	Product	n	m	R	Catalyst[a]	Time (h)	Yield (%)
1	**10** (20 mM)	**20**	1	1	-H	G1; 5	2	96
2	**11** (20 mM)	**21**	2	1	-H	G1; 5	18	84
3	**12** (10 mM)	**22**	3	1	-H	G1; 10	24	61
4	**13** (5 mM)	**23**	4	1	-H	G1; 10	24	0
5	**14** (20 mM)	**24**	1	1	-CH3	G2; 5	17	94
6	**15** (20 mM)	**25**	2	1	-CH3	G2; 5	19	84
7	**16** (5 mM)	**26**	3	1	-CH3	G2; 10	24	35
8	**17** (5 mM)	**27**	4	1	-CH3	G2; 10	24	0
9	**18** (20 mM)	**28**	2	2	-H	G2; 5	21	74
10	**19** (5 mM)	**29**	2	3	-H	G2; 10	24	0

[a] In the catalyst column, G1 and G2 are indicating Grubbs' 1st and 2nd generation catalysts respectively and the number behind it is marking catalyst load in mol% of substrate.

3.3. Synthesis of cyclic enehydrazines and enehydrazides

The aim for this part of the thesis was to investigate the possibilities to synthesize heterocyclic hydrazine derivatives that contain carbon-carbon double bond adjacent to nitrogen atom, therefore being analogues of enamines and enamides (**Figure 7**).

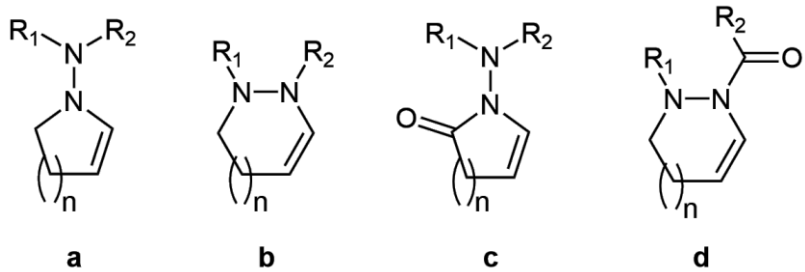
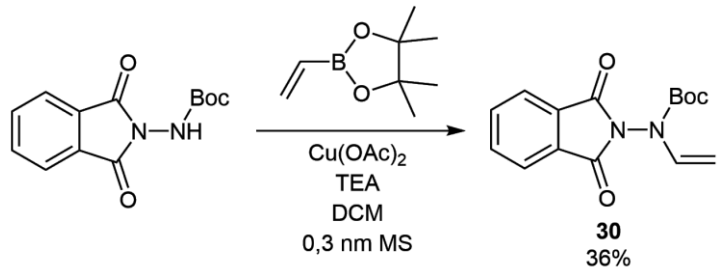


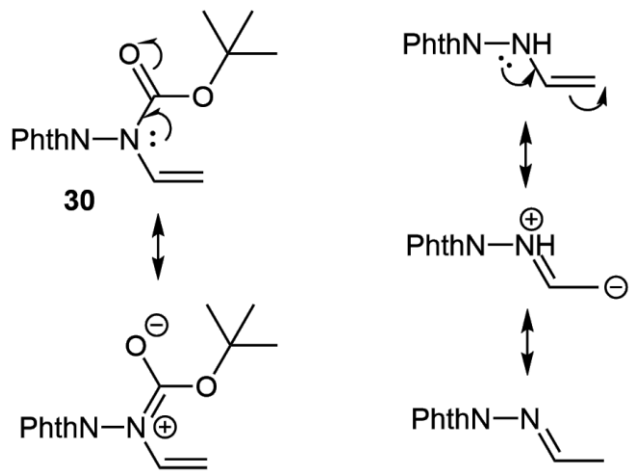
Figure 7. Variety of target molecules. Enehydrazines containing an exo- (**a**) and endocyclic (**b**) N-N bond and enehydrazides with an exo- (**c**) and endocyclic (**d**) N-N bond. R_1 and R_2 are alkyl or aryl substituents, *n* is a positive integer.

At first, we tried to synthesize type **a** compounds. For this, we attempted to implement the same strategy as for the synthesis of heterocyclic hydrazine derivatives described in the previous subchapter. Therefore, our first goal was to vinylate *N,N*-phthaloyl-*N'*-Boc-hydrazine. With conventional methods, this proved to be a difficult task. Direct alkylation with vinyl bromide failed in all cases. This can be explained by the fact that sp^2-hybridized carbon atoms have higher electronegativity than sp^3 carbons and therefore have higher attraction for the electrons of the bond. That means that corresponding sp^2 carboanions are more stable than sp^3 carboanions. In nucleophilic substitution reactions however, the leaving group carries away the electron pair, so the situation is reversed, meaning that sp^3 carbon loses the leaving group with an electron pair more easily than sp^2 carbon. This altogether results in much lower reactivity of vinylic halides [117]. Also, low boiling point (16 °C) of ethylene bromide disallows to carry out reaction in higher temperatures without using autoclave techniques. Therefore, an alternative method for vinylation was required. We implemented Chan-Lam cross-coupling reaction conditions for our substrate, using vinylboronic acid pinacol ester as a vinyl group donor [131]. This setup allowed us to isolate desired compound in 36% yield (**Scheme 31**).



Scheme 31. Synthesis of enehydrazide **30** with modified Chan-Lam coupling conditions.

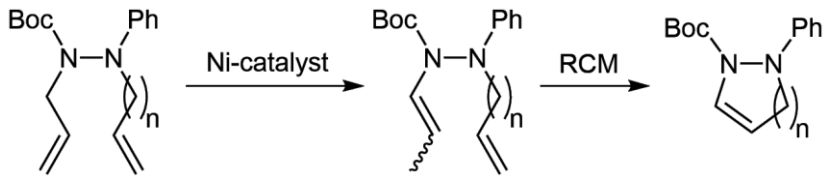
For the next step, cleavage of Boc protection was required. Using standard conditions (8% TFA in DCM) removed the Boc group but yielded a complex mixture of products. This can be explained by increased nucleophilicity of enehydrazine formed during this reaction. In substrate **30** the electron pair on nitrogen atom is withdrawn by the Boc-group. However, in enehydrazine product, this effect is cancelled, and the electron pair can delocalize towards carbon-carbon double bond, forming a carboanionic resonance structure, which can efficiently react with different electrophilic species or isomerize to thermodynamically more stable hydrazone (**Scheme 32**). Due to instability of these enehydrazines, we decided to move on and investigate possibilities for the synthesis of type **d** enehydrazides.



Scheme 32. Stabilization in enehydrazides by electron-withdrawing groups (on the left) and isomerization of enehydrazines to hydrazones (on the right).

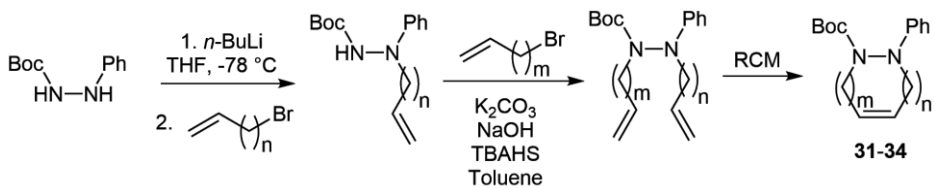
For the synthesis of type **d** enehydrazides, we used *N,N'*-dialkenyl derivatives of *N*-phenyl-*N'*-Boc-hydrazine as substrates [41]. Initially, we planned to use

nickel catalysts to isomerize allyl hydrazide fragment in substrates to corresponding enehydrazides and then carry out RCM (**Scheme 33**). For the isomerization of allylamides, various nickel catalysts such as $Ni(PPh_3)_4$ and $Ni(1\text{-}Np)(PPh_3)_2Br$ had been used recently [94,95]. As the former catalyst requires the use of glove-box techniques and the latter is air-stable, we decided to use $Ni(1\text{-}Np)(PPh_3)_2Br$ in our syntheses. Unfortunately, with our substrates no isomerization occurred and only starting material was recovered from the reaction mixture. It is difficult to say why these reactions failed, but one possible explanation is that our substrates might act as ligands for nickel and thereby adversely affect its catalytic activity.



Scheme 33. Synthesis of enehydrazides with nickel-catalytic isomerization and subsequent ring-closing metathesis.

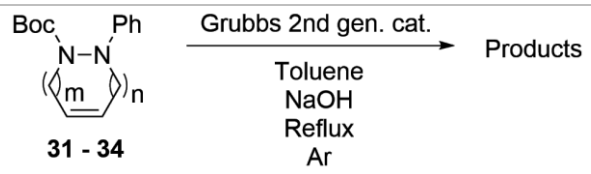
When looking further into the literature, we found that ruthenium catalysis, although economically not as advantageous as nickel catalysis, is also widely used for the synthesis of enamides and enol ethers [96–102]. In many cases, isomerization-capable ruthenium species are derived directly from Grubbs' catalysts, therefore enabling to carry out tandem RCM-isomerization reactions as an one-pot synthesis [97–99,101,102]. We first synthesized the cyclic hydrazine derivatives **31-34** by methods previously developed in our workgroup (**Scheme 34**) [41]. For isomerization (**Table 8**), we used the same reaction conditions (heating substrates with the 2nd generation Grubbs' catalyst in refluxing toluene in presence of solid sodium hydroxide) that were developed by Schmidt *et al* [102].

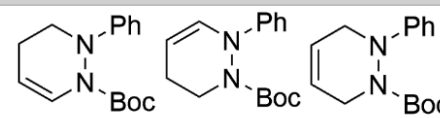
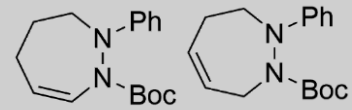
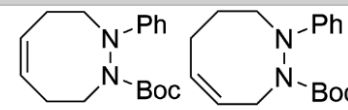
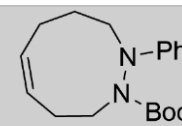


Scheme 34. Synthesis of precursors **31-34** for the isomerization reactions. n = 1-3, m = 1-2.

Considering that in similar cyclic carbamates the double bond shifted always towards nitrogen atom, we expected same results for our substrates [102]. In the 6-membered heterocycle, the double bond shifted mainly towards Boc-nitrogen, forming enehydrazide **35** (47% yield) as expected. Also, we were able to chromatographically isolate enehydrazine **36**, where the double bond had migrated towards the phenyl group (17%). Additionally, some substrate (20%) had remained in the reaction mixture and was isolated from it. These results are in general agreement with quantum chemical calculations at semi-empirical and density functional levels as they support the stabilizing role of conjugation. The total energy differences between the shifted-bond isomers of 6-membered ring were very low (< 2 kcal·mol^{-1}), while the total energy was remarkably higher for the substrate.

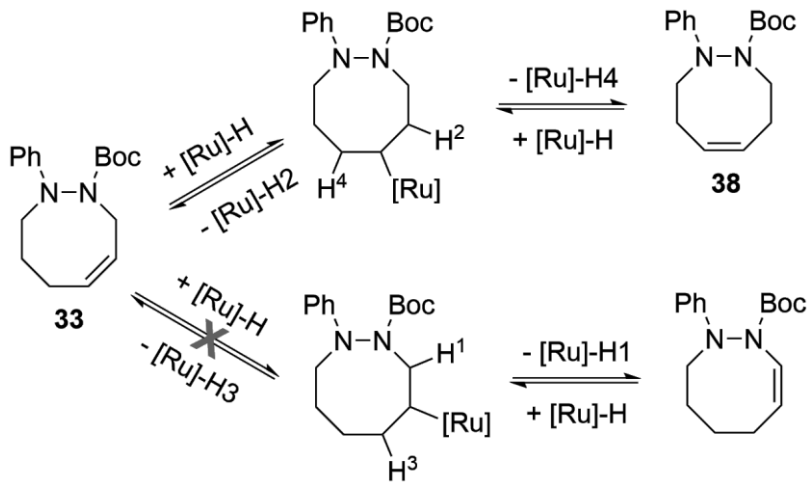
Table 8. Products of isomerization reactions. All the isomerization reactions were carried out in dry toluene solutions (0.1 M) under argon atmosphere with 5 mol% of Grubbs 2nd generation catalyst and 1.5 eq of solid sodium hydroxide as an additive. Reaction mixtures were refluxed for 24 h.



Substrate	n	m	Products
31	1	1	 **35** (47%) **36** (17%) **31** (20%)
32	2	1	 **37** (69%) **32** (trace)
33	3	1	 **38** (63%) **33** (25%)
34	3	2	 **34** (90%)

For the substrate **32** with the seven-membered ring, the isomerization results were also in good agreement with quantum chemical calculations as the double bond shifted only towards Boc-nitrogen, forming enehydrazide **37** in 69% yield. However, with 8-membered ring **33**, we received controversial results from the synthesis. Calculations suggested that the double bond should shift towards Boc-group similarly as with other substrates, but we did not detect any ene-hydrazide forming in course of the reaction. Instead, compound **38**, where the double bond migrated towards the opposite direction appeared as the main product (63%) along with some unreacted substrate **33** (25%). As the product **38** is thermodynamically less stable than corresponding enehydrazide, there must exist a specific effect that hampers the formation of the enehydrazide. We hypothesize that this effect is likely to be steric, disallowing the ruthenium complex to bind with the β-carbon (**Scheme 35**).

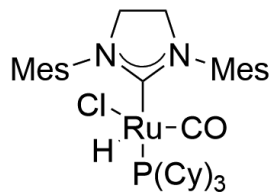
Also, we attempted to isomerize compound **34**, where the double bond is located farther from Boc-nitrogen. Similar "long-distance" isomerization for the synthesis of enamides using Ni-catalysis has proven to be successful previously [95]. Unfortunately, with our substrate and reaction conditions, we could not synthesize desired enehydrazide as we did not detect any conversion during the reaction.



Scheme 35. A possible isomerization mechanism for the 8-membered hydrazinocycle **33**. Ruthenium-hydride complex (**[Ru]-H**) addition to β-position is hampered due to steric hindrances. Therefore, the reaction can only yield compound **38** as a product.

In the literature, different ruthenium-hydride complexes as the decomposition products have been described [132,133]. However, the structure of iso-merization-capable ruthenium species generated by sodium hydroxide induced decomposition has been not published. Acquiring the exact structure of the catalyst is necessary to run more sophisticated *in silico* experiments in order to

understand mechanistic peculiarities involved with the isomerization reactions. For that, we generated the ruthenium-hydride catalyst directly in NMR-tube using deuterated toluene as a solvent and measured its ^{1}H NMR spectrum. As the ruthenium-hydride is extremely air-sensitive, the spectrum consisted mainly of signals derived from oxidized decomposition products, but a weak characteristic doublet from Ru-H was also detected at -24.99 ppm (J = 21.6 Hz). **[Ru]-H** (**Figure 8**), as the most probable catalytically active species, was generated according to Nishida protocol in deuterated toluene [133]. It had the hydride peak in same location (-25.00 ppm, J = 21.4 Hz). Therefore, it is very likely that the structure of *in situ* generated catalyst corresponds to **[Ru]-H**.



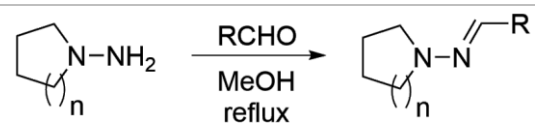
[Ru]-H

Figure 8. Structure of isomerization-capable ruthenium-hydride species, generated *in situ* from Grubbs 2nd generation catalyst.

3.4. Synthesis of saccharide hydrazones from cyclic hydrazine derivatives

Saccharide hydrazones that contain a heterocycle in their structure can be considered as acyclic nucleoside mimetics. This type of compounds have shown biological activity against many targets, such as bacteria [134–136], viruses [137,138], fungi [134,135] and cancer cells [139]. We were interested if our simple saturated heterocycles which contain an exocyclic N-N bond, also possess biological activity when conjugated with various monosaccharides. For the synthesis of these compounds, we followed procedure developed by Stroh and Scharnow [140]. In brief, a monosaccharide (L-arabinose, D-galactose, D-mannose, D-ribose, L-rhamnose or 2-deoxy-D-ribose) was mixed with 1.6 equivalents of *N*-aminopyrrolidine, *N*-aminopiperidine or *N*-aminoazepane in methanol and this mixture was refluxed until no more saccharide was detected in the mixture by TLC. Produced hydrazones were purified either by recrystallization or with column chromatography. More details for these syntheses can be seen on **Table 9**.

Table 9. Details for the synthesis of saccharide hydrazones **39**-**56**, n = 1-3



39 - 56

Entry	n	Saccharide (RCHO)	Product	Reaction time (h)	Purification method[a]	Appearance	Isolated yield (%)
1	1	L-arabinose	**39**	3	1:1	White crystals	93
2	2		**40**	3.5	1:1	White crystals	85
3	3		**41**	3	1:1	White crystals	85
4	1	D-galactose	**42**	4	1:3	White crystals	96
5	2		**43**	4.5	1:1	White crystals	77
6	3		**44**	2	2:1	White crystals	71
7	1	D-mannose	**45**	3.5	1:1	White crystals	93
8	2		**46**	4	2:1	White crystals	77
9	3		**47**	2	2:1	White crystals	68
10	1	D-ribose	**48**	3.25	CC	Yellow liquid	85
11	2		**49**	4	4:1	White crystals	83
12	3		**50**	3	1:0	White crystals	71
13	1	L-rhamnose	**51**	2.5	4:1	White crystals	80
14	2		**52**	3.75	3:1	White crystals	83
15	3		**53**	3.25	1:0	White crystals	74
16	1	2-deoxy-D-ribose	**54**	1.5	CC	Yellow liquid	88
17	2		**55**	1	CC	Yellow liquid	98
18	3		**56**	0.75	1:0	Yellow crystals	73

[a] For the purification method, MTBE:EtOH solvent ratio is written if the product was purified by recrystallization. CC stands for purification by column chromatography with ethanol-benzene-triethylamine in ratio of 29:10:1.

Synthesized hydrazones **39**-**56** were subjected to a series of biological evaluations. Their activity was examined against two strains of bacteria (*E. coli* and *S. aureus*) and *C. albicans* as a representative of fungi. In these experiments it was confirmed that the selected compounds do not have any effect on these microorganisms at used concentrations.

Next, the hydrazones were screened against HIV. For that, the toxicology tests were carried out first. These experiments revealed, that all compounds **39**-**56** are non-toxic to mammalian cells (U2OS) at the concentration of 1 mM. Then, the saccharide hydrazones were tested for anti-HIV properties. It was found out that compound **41** has a minor effect ($IC_{50} \approx 400$ μM) against HIV (**Figure 9**). Also, compound **56** showed some activity ($IC_{50} \approx 1$ mM) against HIV. Other 16 hydrazones turned out to be ineffective towards inhibiting HIV.

Although the antiviral effects observed here are not in the range to use them directly as drug candidates, it still gives an insight that even structurally simple saccharide hydrazones can act as nucleoside mimetics. Also, this research can

be used as a starting point for further structural optimizations to develop compounds with higher biological activity.

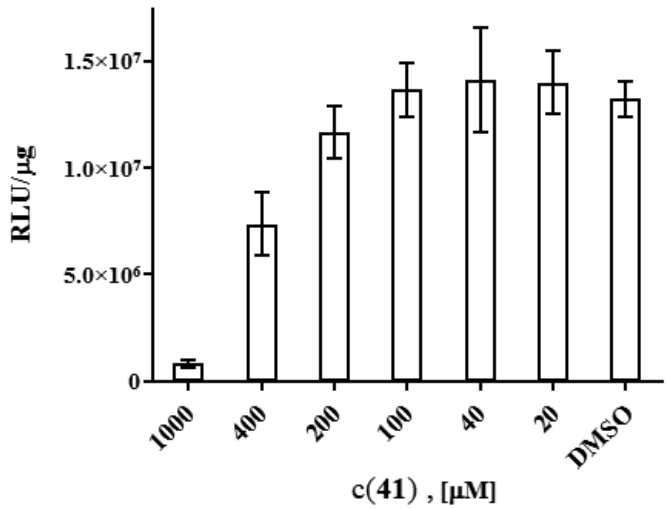


Figure 9. The effect of compound **41** to HIV virus-like particles (VLP) mediated expression of a marker protein in different concentrations. The cells were lysed 3 days after infecting with VLP-s. The activity of the marker protein was normalized to the total protein content in samples. Negative control is DMSO, which was also used as a solvent.

4. EXPERIMENTAL

4.1. Materials and methods

All the reagents used in this work were purchased from various commercial sources. All of them had at least a purity of 95% and they were used without further purification. Moisture- and air-sensitive reactions were performed under argon atmosphere in oven-dried glassware with solvents previously distilled over Na/benzophenone or CaH_2 and/or dried on molecular sieves. Thin layer chromatography was performed on Machery-Nagel Alugram® SIL G/UV 254 silica gel plates. For visualization, UV light at 254 nm, 1% phosphomolybdic acid solution in ethanol or 10% sulfuric acid in ethanol were used. Flash-chromatography was performed on Isolera One (Biotage, Sweden) with normal-phase silica gel columns. For column chromatography, Merck Kieselgel 70-230 mesh silica gel was used. FTIR spectra were measured with Perkin-Elmer Spectrum BXII FTIR spectrometer equipped with zinc selenide ATR crystal (Interspectrum, Estonia). NMR spectra were recorded with Bruker Avance III HD or Bruker Avance II 200 spectrometer. ^{1}H and ^{13}C spectra were measured at 700 MHz or 200 MHz and 176 MHz or 50 MHz correspondingly. Measurements were carried out in suitable solvents using either tetramethylsilane or residual peak of a solvent for spectrum calibration. Chemical shifts are presented in ppm, decoupling constants in Hz. For complex products, structural assignments were performed by measuring ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra. HRMS spectra were measured on Thermo Electron LTQ Orbitrap spectrometer with ESI method.

4.2. Procedures

<u>WARNING:</u> Hydrazine hydrate is a volatile, corrosive, highly toxic and carcinogenic compound and its heterocyclic *N*-amino-derivatives formed in following reactions may possess similar endangering properties. Handling of these compounds must be carried out with caution in a well-ventilated fume hood!

Procedure for synthesis of *N*-aminopyrrolidine

In a 100-ml two-necked flask, hydrazine hydrate (24.25 ml; 0.5 mol) was dissolved in 40 ml of methanol. The mixture was heated to reflux and 1,4-dibromobutane (17.91 ml; 0.15 mol) was added dropwise within 1 h. The reaction mixture was left stirring at methanol reflux for 24 h. Then, the solvent was removed by fractional distillation. Residue was basified with 75 g of 40% NaOH and extracted 10 times with diethyl ether. Combined extracts were dried on anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the product was purified by vacuum distillation (bp 46 °C at

30 mbar). This process yielded 6.374 g (49%) of *N*-aminopyrrolidine, a clear colorless liquid.
^{1}H NMR (200 MHz, DMSO-d_6): δ 2.51 (m, 4H, NC<u>H</u>$_2$); 1.64 (m, 4H, NCH$_2$C<u>H</u>$_2$).
^{13}C NMR (50 MHz, DMSO-d_6) δ 58.71 (N<u>C</u>H$_2$); 21.94 (NCH$_2$<u>C</u>H$_2$).

Procedure for synthesis of *N*-aminopiperidine
In a 100-ml two-necked flask, hydrazine hydrate (14.55 ml; 0.3 mol) was dissolved in 25 ml of methanol. The solution was heated to reflux and 1,5-dibromopentane (13.62 ml; 0.1 mol) was added dropwise within 1 h. The reaction mixture was left stirring at methanol reflux for 24 h. Solvent was removed under reduced pressure, and the residue was basified with 40 g of 40% NaOH solution. The mixture was extracted 8 times with diethyl ether and combined extracts were dried on anhydrous Na_2SO_4 and filtrated. Solvent was removed under reduced pressure (40 °C at 40 mbar). The process yielded 7.380 g (74%) of *N*-aminopiperidine, a light-yellow liquid, which was used in next experiments without further purification.
^{1}H NMR (200 MHz, DMSO-d_6): δ 2.41 (m, 4H, NC<u>H</u>$_2$); 1.49 (quint, J = 5.5 Hz, 4H, NCH$_2$C<u>H</u>$_2$); 1.29 (m, 2H, NCH$_2$CH$_2$C<u>H</u>$_2$).
^{13}C NMR (50 MHz, DMSO-d_6) δ 60.10 (N<u>C</u>H$_2$); 25.51 (NCH$_2$<u>C</u>H$_2$); 23.02 (NCH$_2$CH$_2$<u>C</u>H$_2$).

Procedure for synthesis of *N*-aminoazepane
In a 500-ml two-necked flask, hydrazine hydrate (24.25 ml; 0.5 mol) was dissolved in 360 ml of methanol. The solution was heated to reflux and 1,6-dibromohexane (23.07 ml; 0.15 mol) was added dropwise within 1.5 h. After 24 h of stirring at methanol reflux, the solvent was evaporated under reduced pressure. The residue was basified with 55 g of 40% NaOH solution and extracted 10 times with diethyl ether. Combined extracts were dried on anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure (40 °C at 25 mbar). The process yielded 7.695 g (45%) of *N*-aminoazepane, a light-yellow liquid, which was used in next experiments without further purification.
^{1}H NMR (200 MHz, DMSO-d_6): 2.67 (m, 4H, NC<u>H</u>$_2$); 1.53 (m, 8H, NCH$_2$C<u>H</u>$_2$C<u>H</u>$_2$).
^{13}C NMR (50 MHz, DMSO-d_6) δ 61.87 (N<u>C</u>H$_2$); 25.99 (NCH$_2$CH$_2$<u>C</u>H$_2$); 25.50 (NCH$_2$<u>C</u>H$_2$).

Typical procedure for alkylation of *N*,*N*-phthaloyl-*N′*-Boc-hydrazine 1
A 25-mL round bottom flask was charged with substrate **1** (1049 mg, 4 mmol) and a magnetic stirrer bar. **1** was dissolved in 10 mL DMF, and Cs_2CO_3 (2.6 g; 8 mmol; 2 eq) and TBAHS (136 mg; 0.4 mmol; 0.1 eq) were added. Mixture turned yellow. Then, alkenyl bromide (6 mmol; 1.5 eq) was added in one portion, and mixture was stirred vigorously while heating the flask in oil bath (50 °C for allyl bromide, 70 °C for other alkenyl bromides). Upon addition of alkenyl bromide, the mixture turned colorless. Reaction was monitored by TLC

(PE/EtOAc 3:1). When the reaction was complete (2 h for allyl bromide, up to 21 h for others), the mixture was poured into 120 mL of water and extracted with EtOAc (4 x 30 mL). Organic extracts were combined, washed with brine, dried on anhydrous $MgSO_4$ and filtered, and solvents were removed under reduced pressure. Residue was co-evaporated three times with small amount of toluene and dried overnight *in vacuo* to obtain *N*,*N*-phthaloyl-*N′*-Boc-*N'*-alkenylhydrazines **2-5**.

Typical procedure for Boc-deprotection of compounds 2-5

A 50-mL round-bottom flask was charged with substrate **2-5** (~3.7 mmol) and a magnetic stirrer bar. The substrate was dissolved in 3 mL of DCM. Flask was cooled on ice bath, and TFA solution (3 mL of TFA in 30 mL of DCM) was added dropwise to the flask in 20 minutes. After the addition of TFA, the ice bath was removed, and the reaction mixture was stirred at room temperature. Progress of the reaction was monitored by TLC (PE/EtOAc 3:1). When the reaction was complete (~4 h), all volatiles were removed under reduced pressure. Residue was redissolved in 10 mL of DCM and basified with saturated $NaHCO_3$ solution. Organic phase was separated and water phase was extracted with DCM (3 x 10 mL). Organic phases were combined and dried on anhydrous $MgSO_4$. Then, the extracts were filtered, and the solvent was removed under reduced pressure. Residue was purified with flash chromatography (PE/EtOAc 9:1 to 2:1) to obtain pure *N*,*N*-phthaloyl-*N'*-alkenylhydrazines **6-9**.

Typical procedure for alkylation of *N*,*N*-phthaloyl-*N′*-alkenylhydrazines 6-9

A 10-mL round-bottom flask was charged with substrate **6-9** (1 mmol) and a magnetic stirrer bar. The substrate was dissolved in 1.5 mL of DMF. Then, Cs_2CO_3 (1.3 g; 4 mmol; 4 eq) and alkenyl bromide (4 mmol; 4 eq) were added in one batch, flask was flushed with argon gas and closed with a septum that was pierced with a syringe connected to a balloon filled with argon. Reaction mixture was stirred vigorously in oil bath (45 °C). Progress of the reaction was monitored by TLC (PE/EtOAc 3:1). When the reaction was complete (~18 h when allyl- and methallyl bromide were used, up to 72 h when other electrophiles were used), the mixture was poured into 30 mL of water and extracted with EtOAc (4 x 10 mL). Organic extracts were combined, washed with brine, dried on anhydrous $MgSO_4$, filtered, and solvents removed under reduced pressure. Residue was purified with flash chromatography (PE/EtOAc 12:1 to 3:1) to yield pure compounds **10-19**.

Typical procedure for ring-closing metathesis reactions

A 50-mL round-bottom flask was charged with the substrate **10-19** (0.3 mmol) and a magnetic stirrer bar. The flask was closed with a septum, then evacuated and backfilled with argon three times. DCM was added via syringe in appropriate volume to achieve desired substrate concentration (5–20 mM). Grubbs' first- or second-generation catalyst (5–10 mol%) was dissolved in 1 mL of DCM and added also to the flask via syringe. Reaction mixture was stirred at

room temperature, and progress was monitored with TLC (PE/EtOAc 3:1). When the reaction was complete or showed no more conversion of substrate into products (2–24 h), the mixture was filtrated through a pad of celite, and the solvent was removed under reduced pressure. Residue was purified with flash chromatography (PE/EtOAc 9:1 to 2:1) to isolate pure heterocyclic compounds **20**-**29**.

Typical procedure for isomerization of compounds 31-34

Grubbs second generation catalyst (38 mg; 0.045 mmol; 0.05 eq) and solid NaOH (54 mg; 1.35 mmol; 1.5 eq) were weighed into a two-necked oven-dried flask, which was equipped with a reflux condenser. The flask and the condenser were closed with septa and the setup was vacuumed and backfilled with argon (3 times). Then, the substrate (0.90 mmol; 1 eq) was dissolved in dry toluene (9 ml) and added to flask via syringe. Reaction mixture was stirred and refluxed sluggishly overnight. The progress of the reaction was monitored by TLC (PE/EA 10:1). When TLC showed no further conversion of substrate into products, the flask was cooled to room temperature and the mixture was filtered through a pad of celite. The filtrate was concentrated and purified by flash-chromatography (PE/EA gradient, 25:1 to 10:1). Isolated compounds were analyzed with NMR (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) to identify their structural formulas.

General procedure for the synthesis of saccharide hydrazones 39-56

In a 100-ml flask, 8 mmol of heterocyclic hydrazine derivative (*N*-amino-pyrrolidine, *N*-aminopiperidine, or *N*-aminoazepane) was dissolved in 15 ml of methanol, and 5 mmol of monosaccharide was added. The mixture was stirred at methanol reflux and its progress was monitored by TLC. After 0.75–4.5 h of stirring, the reaction was complete according to TLC. Volatiles were removed under reduced pressure and the residue was recrystallized in MTBE–ethanol mixture or purified by column chromatography (eluent: ethanol–benzene–triethylamine 29:10:1) to yield pure saccharide hydrazones **39**-**56**.

5. CONCLUSIONS

The current thesis investigated possibilities for the synthesis of heterocyclic compounds, which include a N-N bond in their structure. Focus was on the synthesis of heterocycles that comprise an exocyclic N-N bond and which have either a saturated or an unsaturated ring. Some of these compounds were conjugated with various saccharides to form novel hydrazones which activity towards various biological targets were researched. Also, synthetic methods for preparation of novel cyclic enehydrazides were investigated.

The synthesis of 5- to 7-membered saturated heterocycles that include an exocyclic N-N bond was optimized in this research. Preparation of these compounds was performed in one step from simple substrates – hydrazine hydrate and a suitable alkyl dibromide. Yields of 45–74% were achieved this way, which are very good considering the simplicity and economical rationality of this process. These heterocycles were condensed with six different saccharides to form hydrazones, which in principal are acyclic nucleoside mimetics. Therefore, anti-retroviral activity of synthesized hydrazones were tested and it was found that two of them possess minor anti-HIV activity which can be used as a starting point for further structural optimization.

Similar 5- to 7-membered heterocycles, differing only by presence of carbon-carbon double bond in the ring and phthalimide protection group on the primary amino group, were also synthesized in this research. Starting from *N*,*N*-phthaloyl-*N'*-Boc-hydrazine, a 4-step synthesis route was developed. The steps included alkylation, deprotection, second alkylation and finally the ring-closing metathesis. This process yielded novel compounds in up to 58% yields. These compounds can be efficiently used as building blocks for the synthesis of more sophisticated molecules.

Finally, possibilities to prepare cyclic enehydrazides were investigated. It was found out that ruthenium-catalytic carbon-carbon double bond isomerization is generally applicable to access these novel compounds. When isomerizing substrate with 6- or 7-membered ring, the cyclic enehydrazide was formed, but with an 8-membered ring the desired enehydrazide was unexpectedly not produced as the double bond shifted towards opposite direction. This novel effect might have synthetic value, but in order to understand its mechanistic aspects, additional experiments with different substrates and sophisticated *in silico* calculations should be performed in future.

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SUMMARY IN ESTONIAN

Uudsete heterotsükliliste hüdrasiini derivaatide ja nende konjugaatide süntees

Antud doktoritöö raames uuriti võimalusi heterotsükliliste N-N sidet sisaldavate ühendite sünteesiks. Suurem tähelepanu oli eelkõige selliste küllastunud ja küllastamata heterotsüklite sünteesil, mis sisaldasid eksotsüklilist N-N sidet. Mõnedest saadud ühenditest valmistati uudseid hüdrasoone, mille aktiivsust uuriti erinevate bioloogiliste märklaudade vastu. Ühtlasi uuriti ka sünteetilisi võimalusi uudsete eenhüdrasiidide valmistamiseks.

Uurimistöö esimeses etapis täiendati viie- kuni seitsmelüliliste eksotsüklilist N-N sidet sisaldavate heterotsüklite sünteesi. Nende ühendite üheetapiliseks valmistamiseks kasutati lihtsaid lähteaineid – hüdrasiinhüdraati ja sobivat dibromoalkaani. Meetodi optimeerimise käigus saavutati 45-74% saagised, mis on selle lihtsust ja odavust arvestades väga head. Sünteesitud heterotsükleid kondenseeriti kuue erineva monosahhariidiga. Produktidena tekkis 18 uudset hüdrasooni, mida võib vaadelda kui mittetsüklilisi nukleosiidide analooge. Seetõttu uuriti antud ühendite toimet HIV vastu, kusjuures kahel hüdrasoonil avastati mõningane viirusevastane toime. Seetõttu saab antud tulemusi kasutada lähtepunktina uute ühendite struktuuri optimeerimisel.

Järgnevalt sünteesiti sarnaseid viie- kuni seitsmelülilisi heterotsükleid, mis sisaldasid tsüklisisest C-C kaksiksidet ning mille primaarne aminorühm oli kaitstud ftaloüülrühmaga. Lähtudes *N*,*N*-ftaloüül-*N'*-Boc-hüdrasiinist, koostati sünteesirada, mis koosnes neljast etapist – alküülimisest, Boc-kaitserühma eemaldamisest, teisest alküülimisest ning tsüklitekke metateesist. Väljatöötatud strateegia abil sünteesiti uudseid heterotsüklilisi ühendid, saagised ulatusid kuni 58%-ni. Saadud heterotsükleid saab kasutada lähteainetena uute ning keerulisemate ühendite sünteesiks.

Viimaks uuriti võimalusi tsükliliste eenhüdrasiidide valmistamiseks. Leiti, et selliste uudsete ühendite sünteesiks saab üldjuhul kasutada ruteenium-katalüütilist süsinik-süsinik kaksiksideme isomerisatsiooni. 6- ja 7-lüliliste heterotsükliliste lähteainete isomeriseerimisel tekkisid soovitud eenhüdrasiidid, kuid 8-lülilise tsükli korral nihkus C-C kaksikside vastupidises suunas. Sellel uudsel efektil võib olla sünteetiline väärtus, kuid antud nähtuse mehhanistlikuks tõlgendamiseks tuleks läbi viia täiendavaid eksperimente teiste lähteainetega ning uurida reaktsiooni ka arvutuskeemiliste meetoditega.

ACKNOWLEDGEMENTS

I would like to express my greatest appreciation to my supervisor Uno Mäeorg, who has mentored me in the field of science and synthesis since I was a bachelor student. He has been an excellent supervisor, encouraging independent research, but being always available when advice or troubleshooting was needed.

Also, I would like to acknowledge collaboration partners who have contributed to this thesis. Andres Merits workgroup and Silver Türk performed biological screening of synthesized compounds, Tarmo Tamm assisted with *in silico* calculations.

I would like to thank all colleagues from Institute of Chemistry, who has helped me with various instrumental methods, provided with reagents and assisted with their expert advice.

I sincerely appreciate all present and former members of our workgroup – it has been a delight working with you.

My special gratitude belongs to my chemistry teachers Anne Õunapuu and Eevi Viirsalu. Without your inspiration I would have not reached to the point I am now.

I thank my dear friends for support, chemistry-related and -unrelated discussions, and all the fun time spent together. I hope that there is more to come.

My family has always been supportive in my actions. I can never thank you enough for that.

Finally, I would like to thank Anni for being in my life. You bring the best out of me and have made me a better person.

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2011 – 2011	Henkel Balti OÜ, laborant

Scientific publications:

1. Ilisson, M.; Tomson, K.; Selyutina, A.; Türk, S.; Mäeorg, U. Synthesis of Novel Saccharide Hydrazones. *Synth. Commun.,* **2015**, *45*, 1367–1373.
2. Ilisson, M.; Mäeorg, U. Systematic synthesis of phthalimide-protected unsaturated hydrazine heterocycles. *Synth. Commun.,* **2017**, *47*, 1231–1238.
3. Ilisson, M.; Tomson, K.; Tamm, T.; Mäeorg, U. Carbon-Carbon Double Bond Isomerization in Heterocyclic Hydrazine Derivatives. *Chem. Heterocycl. Comp.,* **2018**, *accepted.*

Patent applications:

1. Waterproofing technique of polyester fibres by multi-step polymerization; Patent holders: Institute of Technology, University of Tartu; Institute of Chemisty, University of Tartu. Authors: Mäeorg, U.; Johanson, U.; Ilisson, M.; Tamm, T.; Priority number: UK1500663.8; Priority date: 15.01.2015.

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3. Ilisson, M.; Tomson, K.; Tamm, T.; Mäeorg, U. Carbon-Carbon Double Bond Isomerization in Heterocyclic Hydrazine Derivatives. *Chem. Heterocycl. Comp.,* **2018**, *vastu võetud.*

Tööstuslik omand:

1. Waterproofing technique of polyester fibres by multi-step polymerization; Patent holders: Institute of Technology, University of Tartu; Institute of Chemisty, University of Tartu. Authors: Mäeorg, U.; Johanson, U.; Ilisson, M.; Tamm, T.; Priority number: UK1500663.8; Priority date: 15.01.2015.

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