PALACKY UNIVERSITY OLOMOUC

Faculty of Science

Department of Organic Chemistry

Synthesis of D-Glucosamine Derivatives and their Reactivity Study at Position 1, 2, and 3

Ph.D. Thesis

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Declaration of originality

I hereby declare that this Ph.D. thesis represents my original work made with the best of my knowledge and that I have used no other sources except as referred by citations. Neither the thesis nor any of its substantial parts were previously used for awarding of any other academic degree.

In Olomouc, ……., 2016

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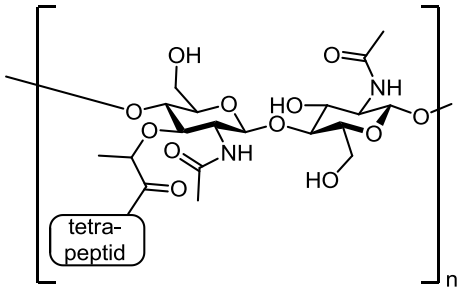
Souhrn

Předložená práce je zaměřena na syntézu a studium nových derivátů s potenciální biologickou aktivitou vycházejících ze struktury D-glukosaminu. D-glukosamin je všeobecně znám jako molekula s širokým spektrem vlastností. Je prekurzorem glykosaminoglykanů, což jsou stavební prvky chrupavek, či je obsažen v polymerní struktuře hyaluronové kyseliny. Tento polymer je důležitý pro své antibakteriální či hojivé účinky; své využití našel i v prostředcích pečující o pleť. Další, z pohledu lidstva velmi důležitou, vlastností, je inkorporace D-glukosaminu do skeletu peptidoglykanu. Tyto makromolekuly jsou základní stavební jednotkou buněčných stěn bakterií. Bylo zjištěno, že narušení tvorby buněčných stěn vede k přerušení reprodukce bakteriálních buněk. Tento princip antibakteriální léčby využívají mnohá antibiotika, jako například penicilin. Z výše zmíněných užitečných vlastností D-glukosaminu si tato práce klade za cíl přípravu nových derivátů, které by mohly rozšířit tuto stále se rozvíjející oblast.



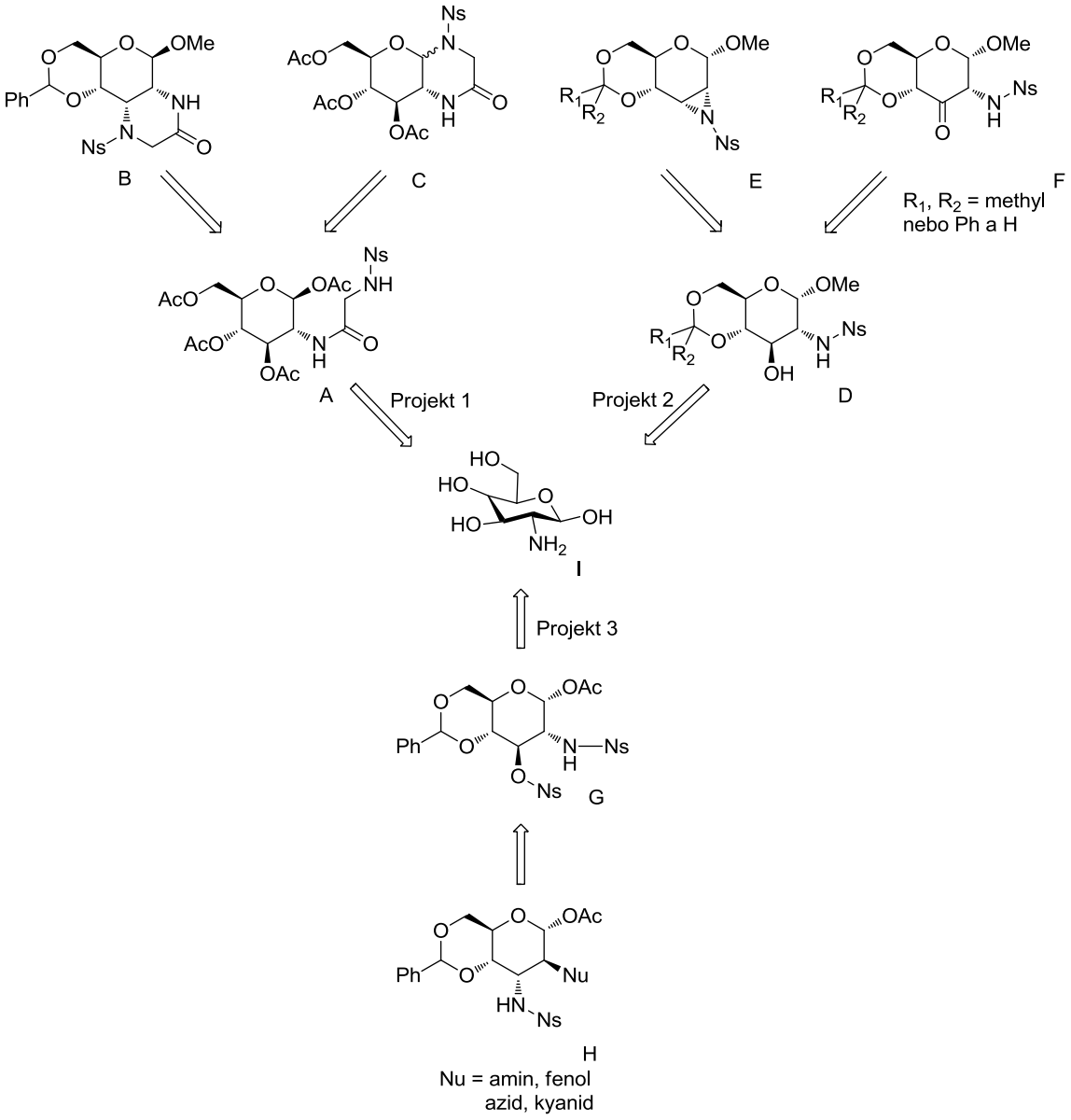
Struktura peptidoglykanu

Diskuzní část je rozdělena do tří základních částí. První část je věnována cyklizačním reakcím, kde cyklus může být uzavřen mezi dusíkem na uhlíku C2 a anomerním uhlíkem C1 (produkt **B**) anebo alternativně je cyklus uzavřen přes uhlík C3 (produkt **C**). Během výzkumu byly připraveny výchozí látky a intermediáty, které umožňovaly cyklizační reakce, avšak samotná cyklizace probíhala buďto velmi problematicky anebo prakticky vůbec. Dále byla syntéza významně komplikována tvorbou anomerů v takřka každém reakčním kroku, kde následná separace byla zdlouhavá díky podobným fyzikálně-chemickým vlastnostem látek.

Prostřední část je zaměřena na přípravu a studium reaktivity derivátu D-glukosaminu **D** s volnou hydroxy skupinou. Byly navrženy a optimalizovány dvě reakční cesty vedoucí k derivátu **D**. Následně byl meziprodukt **D** podroben alkylačním a oxidačním reakčním krokům. Během Fukuyama-Mitsunobu alkylace bylo zjištěno, že vzniká trojčetný aziridinový kruh **E** jakožto významný vedlejší produkt. Formace aziridinu nastává ovšem jen v případě,

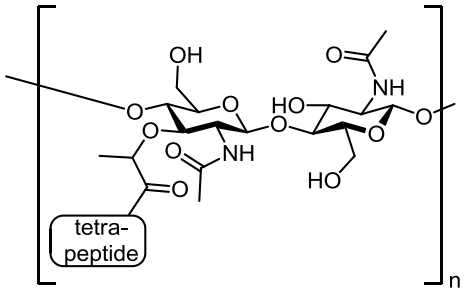
když byl k ochránění C4 a C6 hydroxy skupiny využit aceton dimethylacetal. Oxidace sloučeniny **D** vedla ke keto derivátu **F**, který byl následně podroben kondenzačním reakcím. Tyto reakce avšak nevedly k očekávaným produktům.

Třetí část práce, co do počtu nově připravených sloučenin, nejúspěšnější, je zaměřena na syntézu 2,3 - altropyranosidů **H** z dinosylovaného derivátu **G**. Tato skupina látek není nijak zvlášť prozkoumána, dosud známé syntézy zahrnují mnoho reakčních kroků s kolísavými výtěžky. Naproti tomu náš postup nabízí jednoduchou a univerzálně použitelnou metodu vycházející z komerčně dostupného *N*-acetyl glukosaminu pro přípravu 2,3-diaminoaltropyranosidů **H** s velmi dobrými výtěžky.



Abstract

The submitted thesis is focused on the synthesis and study of new potentially biologically active derivates, based on the D-glucosamine motif. D-glucosamine is well known molecule with plenty of interesting properties. It is a precursor of glucosaminoglycans, which are building units of cartilages or it can be found in the polymer structure of hyaluronic acid. This polymer is important for its antibacterial or healing effect; it is also a component of skin-care products. Moreover, the structure of D-glucosamine is incorporated into the skeleton of peptidoglycan. Such molecules are building units of bacteria cell walls. It has been found, that disruption of cell wall formation process leads to inhibition of bacteria reproduction. Lots of antibiotics, such as penicillin, use this mechanism of action. It is obvious, that D-glucosamine found the application in many aspects of our live. Therefore, this thesis is focused on the preparation of new D-glucosamine based derivates, which can extend the current knowledge of reactivity of the D-glucosamine molecule.



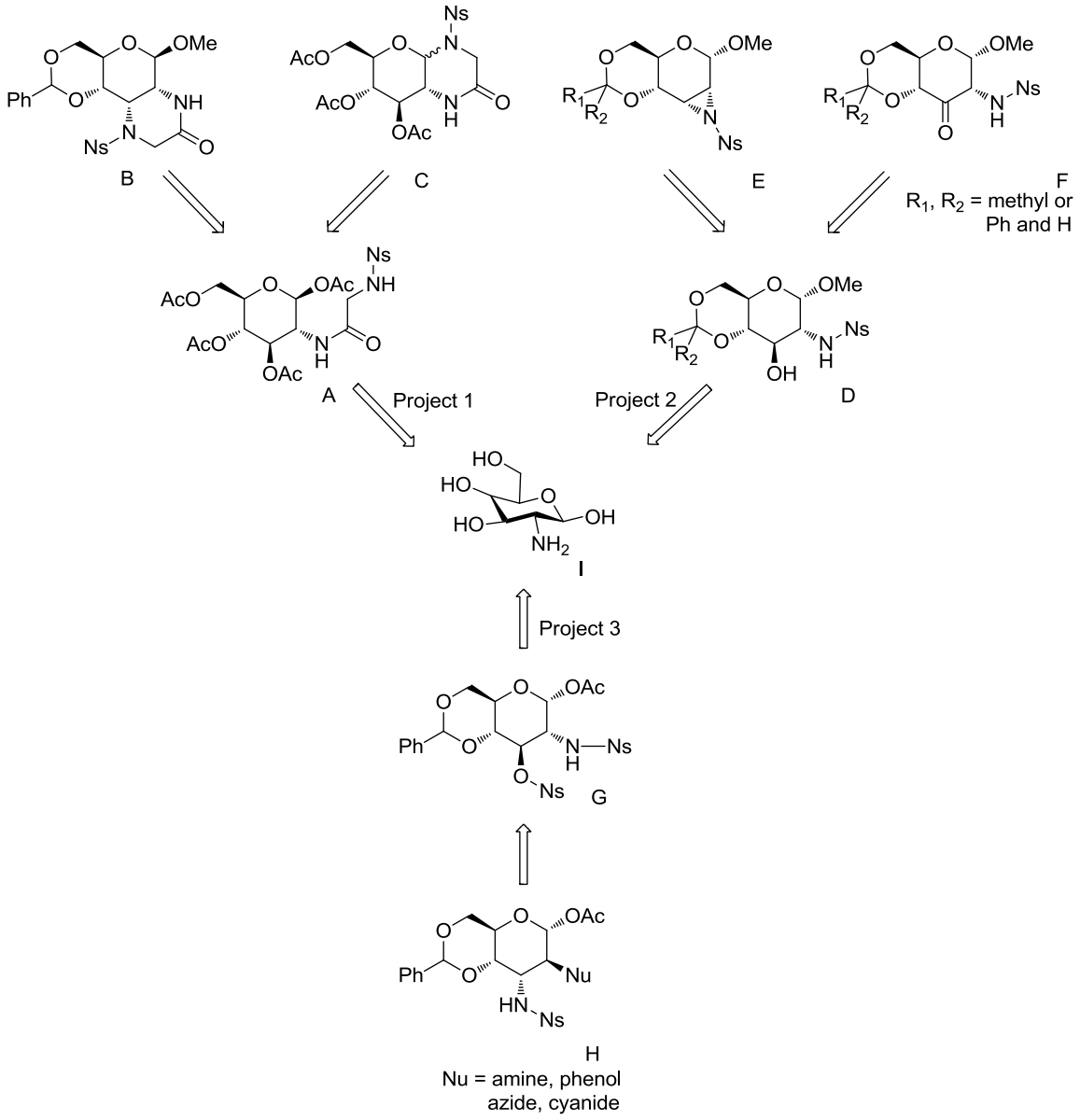
Structure of peptidoglycan

The discussion part is divided into the three chapters. The first one is dedicated to cyclization reactions, where the cycle can be formed either between the nitrogen at C2 carbon and anomeric C1 carbon (product **B**) or the cyclization occurs between the nitrogen at C2 carbon and C3 carbon (product **C**). During the research, the starting compounds and intermediates allowing the cyclization were prepared. Nevertheless, the cyclization itself was troublesome or it did not undergo at all. Moreover, the synthesis of nearly all products was significantly complicated with formation of anomers, where further separation was extremely difficult due to its similar chemical properties.

The middle part is based on the synthesis and reactivity study of D-glucosamine derivate containing the free hydroxyl group **D**. We suggested and optimized two synthetic routes leading to derivate **D**. Subsequently, intermediate **D** was used in alkylation and oxidation reactions. During Fukuyama-Mitsunobu reactions we found that three - membered

ring - aziridine as an important byproduct is easily formed. However, the aziridine formation was observed only when acetone dimethylacetal was used for the hydroxyl protection at C4 and C6 groups. The oxidation of **D** led to ketoderivate **F**, which was further tested toward the condensation reactions. These reactions did not lead to the desired products.

The last part is focused on the synthesis of 2,3-altropyranosides **H** from dinosylated derivate **G**. This family of compounds is not well explored. The known syntheses involve plenty of reaction steps with fluctuating yields. To the contrary, we developed simple and universal method utilizing commercially available *N*-acetyl glucosamine as a starting compound. This method affords 2,3-diaminoaltropyranosides in very good yields.



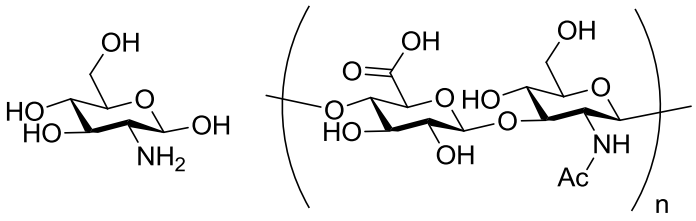
Contents

1 Introduction

D-Glucosamine (D-Glc-NH_2), an isoster of D-glucose molecule, has a long history. It was discovered by Dr. Georg Ledderhose in 1876 by acidic hydrolysis of chitine.[1] However; tumultuous development is dated in the early seventies, when a positive effect on cartilage was noticed. Since this time, it became extremely popular dietary supplement with sales of $ 200 millions per year.

Formation of D-Glc-NH_2 can be divided into synthetic or biochemical based processes. The first way - synthetic, for example, can use D-mannose as a starting material, which is in ten step synthesis transformed into desired D-Glc-NH_2.[2] However, synthetic routes leading to D-Glc-NH_2 are expensive contrary to the most usual preparation process based on the isolation from a natural material – chitin. A source of chitine for commrecial production is mostly crustacean exoskeletons.[3] D-Glc-NH_2 is biosynthetised in cells as a glucosamine-6-phosphate from fructose 6-phosphate and glutamine in the presence of glutamine-fructose-6-phosphate transaminase.[4] In the final stages, D-Glc-NH_2 is incorporated into the chitin polysaccharide chain based on *N*-acetyl-D-glucosamine units.

D-glucosamine is a common substance; it naturally occurs in a human body. Furthermore, it can be found in shells of shellfish, animal bones, bone marrow or fungi. It is also part of macromolecules, such as chitosan or chitine.



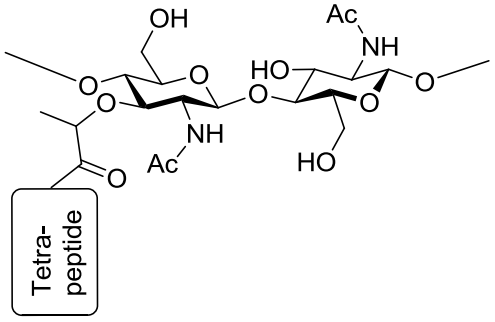
D-Glucosamine Hyaluronic acid

The list of D-Glucosamine biological properties is voluminous. Primary, D-Glc-NH_2 is a precursor of glycosaminoglycans (Mureins), which are major component of human cartilages.[5] Therefore, when arthritis occurs; D-Glc-NH_2 is indicated as a dietary supplement for arthritis, usually in the form of D-Glc-NH_2.HCl or D-Glc-NH_2.H_2SO_4. Moreover, since arthritis is often connected with obesity, D-Glc-NH_2 seems to be responsible for inducing insulin resistance in the absence of glucose or glutamine.[6]

It is also part of hyaluronic acid (HA), widely known polymer of disaccharide.[7,8] Beside *N*-acetylated D-Glc-NH_2 molecule, HA contains glucuronic acid. HA evinces plenty of biological effects; such as anti-inflammatory, antitumour or tissue healing. HA is also common part of skin-care products.

D-Glc-NH_2 can be also found in the molecules, which affect immunity system. For example, it is incorporated in macromolecules, which play a key role in a process of cell determination.[9] This process is responsible for recognition of blood type. The possible application of such structures aims to find the cure for cancer. It is believed, that molecules such as Globo-H-antigen, can particicipate in cancer cells recognition process.[10]

Last, but not least, D-Glc-NH_2 is a part of peptidoglycans, which are present in bacteria cell wall, giving to the bacteria structural strength.[11] Peptidoglycans consist of *N*-Acetyl-D-Glucosamine and *N*-Acetylmuramic acid. The free carboxy group of muramic acid is further joined to the tetrapeptide chain. The significant differences in biosynthesis between prokaryotic and eukaryotic cell walls offered an opportunity to develop very low toxic antibacterial compounds such as penicillin, which disturbs the synthesis of cell walls and inhibits the reproduction of bacteria with very high level of selectivity. [12]



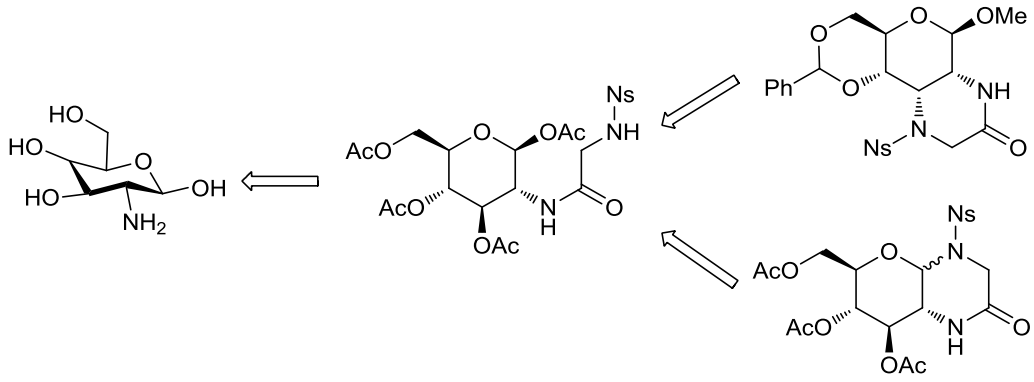
Peptidoglycan

D-Glucosamine, as it was mentioned above, is the molecule with high level of importance. It is present in human and animal cells. It founds applications predominantly in medicine especially for its role in human cartilages. D-Glucosamine is also a part of more complex molecules, such as Hyaluronic acid or peptidoglycans, which are object of study for their anti-inflammatory, antibacterial or healing properties. The development and study of D-Glucosamine based molecules has not finished. This Ph.D. thesis has an ambition to contribute with new molecules derived from D-Glucosamine and study their reactivity.

2 Aims of the work

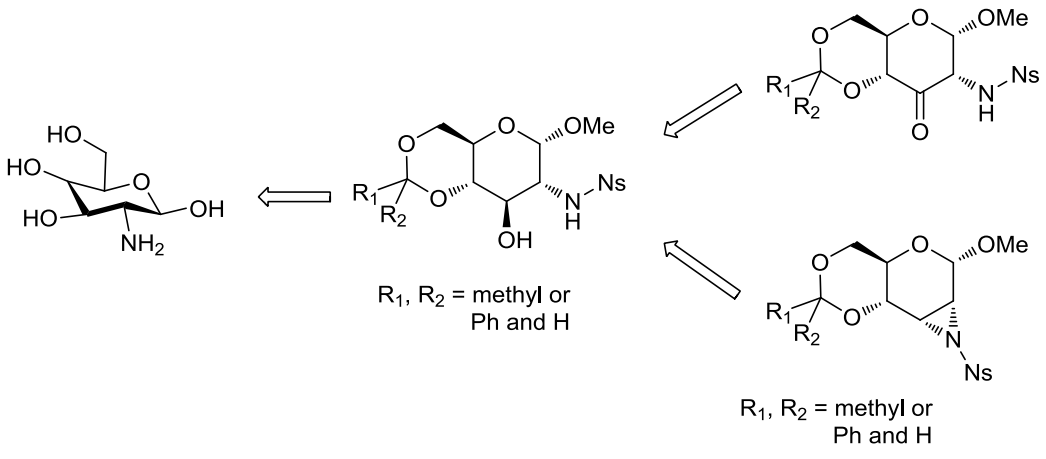
This thesis was focused on derivatization of D-Glucosamine, where 4-Nosyl group is employed for activation of the amine group. Nosylated derivatives were further tested toward different reaction paths. The whole thesis is divided into the three projects. The *Project 1* consisted in the synthesis of the glycin-containing derivate, which was subsequently tested toward cyclisation reactions.

Figure 1 - Retrosynthetic analysis of *Project 1*



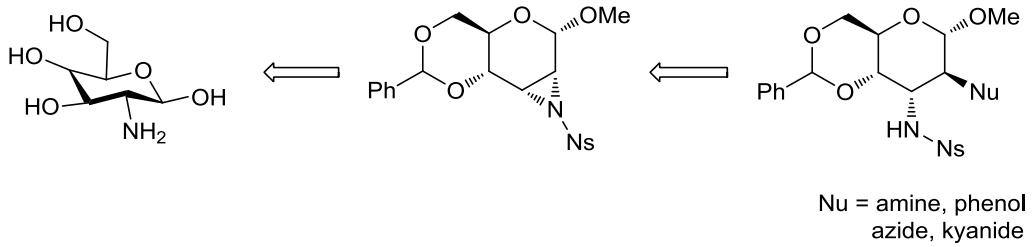
The middle part, the *Project 2*, was based on the direct nosylation of the suitably protected D-Glucosamine. The glucosamine derivate, containing the free hydroxy group, was further attempted to cyclize and oxidize. The purpose of this project was to investigate the possible formation of the aziridine ring and to oxidize the free hydroxyl to corresponding keto functionality to avoid the aziridine ring formation. Subsequently, the keto derivate was tested toward the common nucleophilic reagents.

Figure 2 - Retrosynthetic analysis of *Project 2*



The last part, the *Project 3*, utilized *in situ* formation of aziridine for preparation of a small library of diamino-altropyranosides. The aim was to investigate the reaction conditions of aziridine ring formation and its ring-opening reactions predominantly with nitrogen nucleophiles. Besides amines, also other nucleophiles such as phenols or *C*-acids were tested.

Figure 3 - Retrosynthetic analysis of *Project 3*



3 Theoretical part

This chapter is focused on theoretical background of published results. The first part briefly summarizes the most used approaches to amide bond with special emphasis on amide bond formation in carbohydrate chemistry. The second part describes the possibility of cyclization reactions in sugar containing molecules and finally the last part is dedicated to introducing of nitrogen-containing functionalities into the molecule of hexapyranosamine.

3.1 Amide bond formation in carbohydrate chemistry

Amide functionality can be found in more than 25% of known drugs.[13] The formation of amide bond belongs to the traditional and common synthetic steps. The first part of this chapter will briefly summarize the most used methods for amide bond formation, while the second part will be focused on the amides created by the reaction of D-glucosamine and its derivatives with amino acids.

3.1.1 Most common approaches for amide bond formation

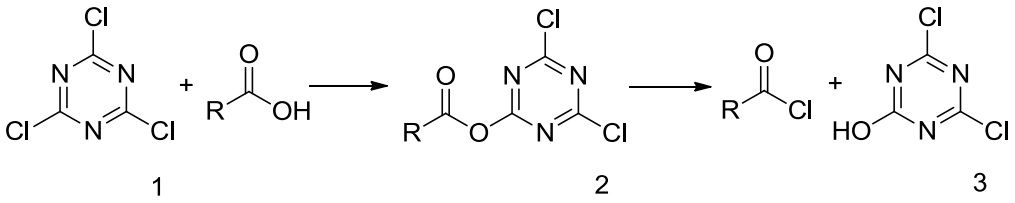
Generally, the carboxylic acid requires an activation to react with amines. The activation can be accomplished via plenty of agents. The most used ways are[14]: a) acyl halide formation, b) acyl azide formation, c) activation with carbonyl diimidazole (CDI), d) symmetric or mixed anhydrides formation, e) reaction with T3P f) active ester formation. The method of choice in carbohydrate chemistry is usually reactive ester formation giving the desired amide; therefore, it will be discussed separately in a subchapter two.

3.1.1.1 Acyl halide activation

Conversion of a carboxylic acid to the corresponding halide belongs to the traditional and the simplest methods. Since the chlorine is the most frequently used halide, bromination and fluorination will not be discussed, although some examples were described.[15,16] Thionyl chloride $SOCl_2$, oxalyl chloride $(COCl)_2$, phosphorus tri- or penta-chloride PCl_x or phosphorus oxychloride $POCl_3$ is typically used for generation of corresponding acyl chloride. The reaction can be promoted by addition of a catalytic amount of DMF.[17] The main advantages are: the mild reaction conditions and simple isolation process. However, some disadvantages can be found. One of them is the production of HCl, which can further react

with acid-labile functions or can cause corrosion of metal parts. To avoid this problem, a base is usually added. Cyanuric chloride **1** is suitable for amidation in a large scale.[18,19] The reaction can be performed in water with common inorganic bases.

Scheme 1 - Chlorination of carboxylic acid with cyanuric chloride

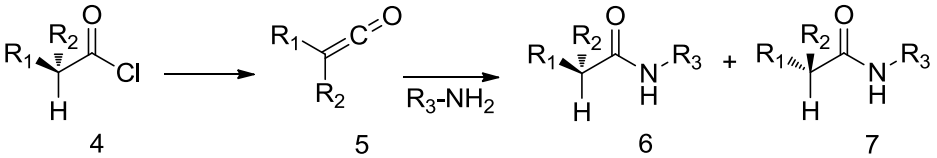


1 2 3

R = alkyl, aryl

As soon as the acyl chloride is formed, the amine is added and the amide bond is formed. It is common to add as a base trialkyl amine to trap the HCl or the reaction can be carried out in pyridine, which serves as the solvent as well as the base. It is important to mention, that hydrolysis of an acyl chloride or racemization can occur. One of the possible racemization mechanisms (Scheme 2) includes the ketene formation **5**,[20] which undergoes nucleophilic attacks of amine and racemic mixture **6** and **7** is formed.

Scheme 2 - Mechanism of racemization



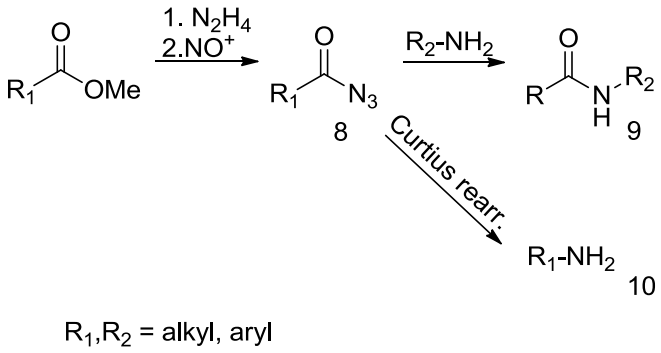
4 5 6 7

R_1, R_2, R_3 = alkyl

3.1.1.2 Acyl azide activation

Besides the acyl chloride formation, the route via acyl azide belongs to the oldest method. At first, the acyl azide was prepared via Curtius protocol,[21] where nitrosation of acylhydrazide gave acyl azide **8**, which can be transformed into desired amide **9**. The main advantage of this method is reduction of racemization; however, the acyl azide can undergo Curtius rearrangement resulting in the side product - primary amine **10**.

Scheme 3 - Azide formation

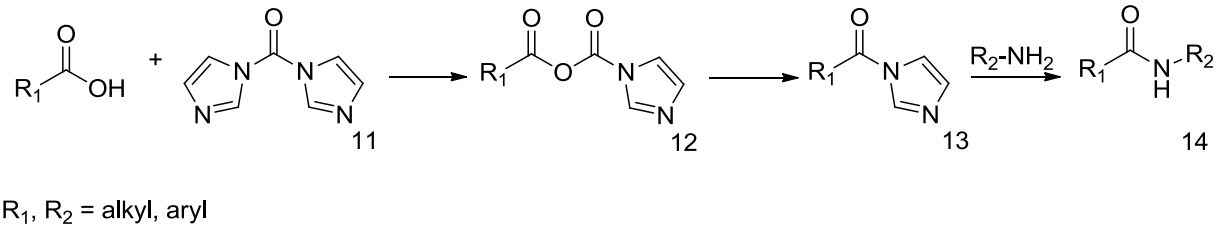


The modern method of acyl azide synthesis is based on the one-pot reaction of a carboxylate salt with diphenylphosphoryl azide (DPPA) in the presence of base.[22]

3.1.1.3 Activation with carbonyl diimidazole (CDI)

Activation of carboxylic acids by CDI **11** belongs to the favored methods due to short reaction time, no addition of a base, and one-pot reaction conditions.[23] The general mechanism (Scheme 4) includes formation of mixed anhydride **12**, which further reacts with in situ generated imidazole to give amide **13**. The addition of amine to amide **13** gives the resulting product **14**.

Scheme 4 - Amide synthesis via acylimidazole

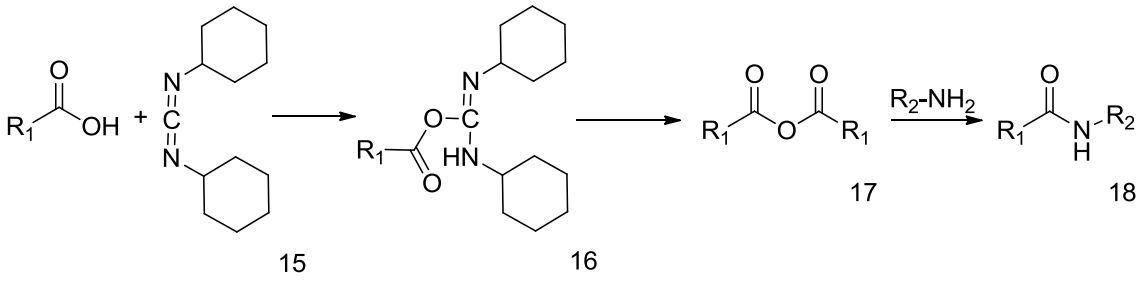


3.1.1.4 Activation via anhydride formation

Anhydrides are well known as more reactive analogues of carboxylic acids. We can distinguish symmetric and mixed anhydrides. The symmetric anhydrides can be prepared in the conventional way by heating of carboxylic acid or dehydration is mediated by dehydrating reagents. More recently, methods using carbodiimides (Scheme 5) were described.[24] New methods allow milder reaction conditions. On the other hand, the main disadvantage is low atom economy of reaction; only one half of carboxylic acid is used.

Among the most common dehydrating reagents belong carbodiimides such as dicyclohexyl carbodiimide (DCC) **15**, diisopropyl carbodiimidies (DIC) and 1-ethyl-3-(3´-dimethylamino)carbodiimide hydrochlorid (EDC). The method of choice depends on the character of amide; the carbodiimides differ particularly in the solubility of the corresponding urea.

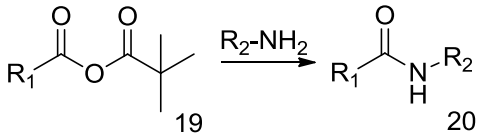
Scheme 5 - Preparation of symmetric anhydride with DCC



R_1, R_2 = alkyl, aryl

The mixed anhydrides eliminate the problem of the efficiency. The second part of anhydride is usually cheap and readily available. Unfortunately, the regioselectivity of amine nucleophilic attack is very poor. This problem can be solved by using sterically hindered anhydride, (e.g. pivalic anhydride **19**, see Scheme 6).[25]

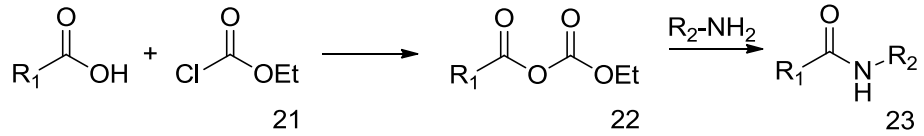
Scheme 6 - Sterically hindered anhydride



R_1, R_2 = alkyl, aryl

An alternative to the mixed carboxylic anhydride consists in mixed carbonic anhydrides. The selectivity of nucleophilic attack is caused by different reactivity of both centers, where carboxylic carbon is substantially more reactive then carbonic carbon. As an example, ethyl carbonic anhydride can be mentioned. This reagent can be prepared via reaction of carboxylic acid with ethyl chloroformate **21** (Scheme 7).[26] The resulting mixed anhydride **22** easily undergoes reaction with amine resulting in amide **23**.

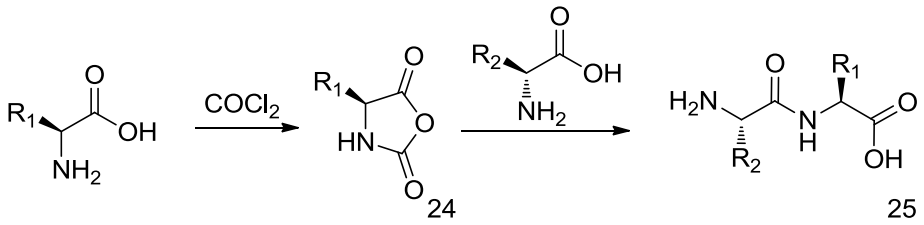
Scheme 7 - Formation of amide via mixed carbonic anhydride



R_1, R_2 = alkyl, aryl

Particularly in the peptide synthesis, *N*-carboxy- anhydrides (NCA) **24** play a key role.[27] NCA **24** can be prepared by many routes; the most common is by reaction of amino acid with phosgene or its analogues, which gives desired cyclic anhydride **24** (Scheme 8).

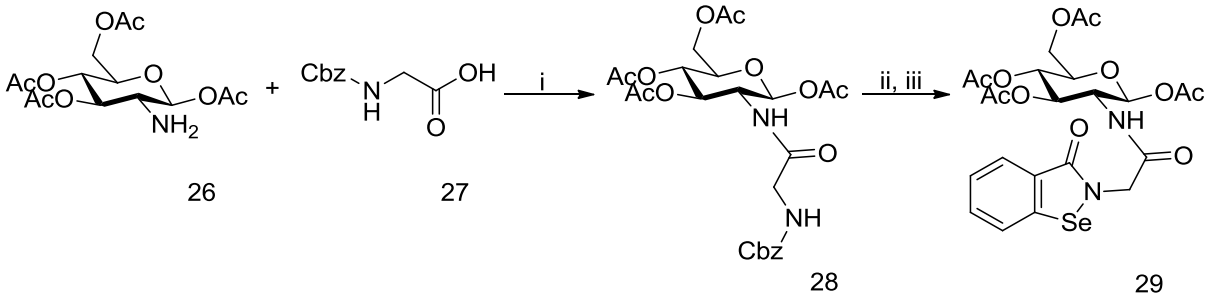
Scheme 8 - Synthesis of NCA and amide formation



R_1, R_2 = alkyl

In the carbohydrate chemistry, *N*-acylation of D-glucosamine with amino acid using carbodiimides in the absence of reactive alcohol is not frequent. However, a few examples can be found. Zhang et. al. used DCC in the first step in the synthesis of benzoselenazole derivates **29** with potential anti-inflammatory effect.[28]

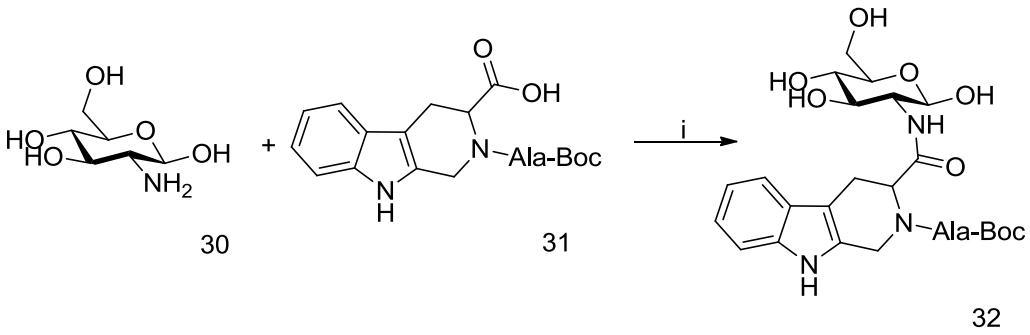
Scheme 9 - Synthesis of benzoselenazole derivates



Conditions: (i) DCC, DCM, 0°C; (ii) Pd/C, H_2; (iii) 2-(chloroseleno) benzoyl chloride, Et_3N, 0°C, overall yield 40%

Another example describes synthesis of carboline derivates,[29] where the final compound **32** evinces anti-inflammatory activity as well (Scheme 10). The key step – acylation, is accomplished with DCC in the presence of *N*-methyl morpholine.

Scheme 10 - Synthesis of carboline derivates

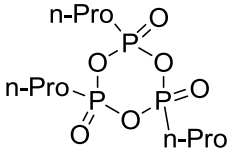


Conditions: (i) DCC, NMM, DMF, 30%

3.1.1.5 T3P activation

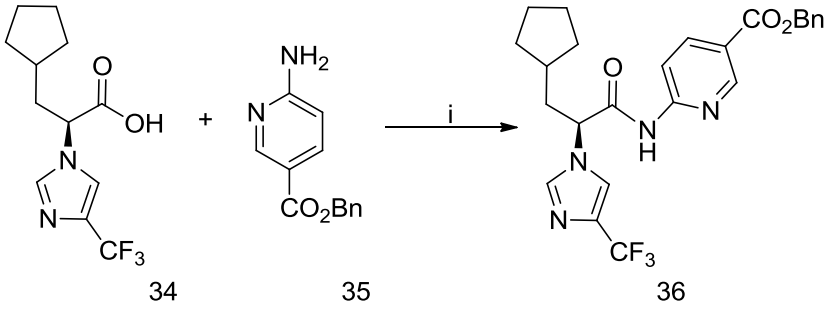
T3P reagent **33** belongs to the quite novel agents facilitating the amide bond formation (Figure 1). T3P, propylphosphonic acid anhydride, found the utilization due to the mild reaction conditions, in comparison to the other methods lower toxicity, easy work-up and high yields.[30] T3P is commercially available usually as 50% solution in EtOAc, DMF, ACN, or other solvents.

Figure 1 - Propylphosphonic acid anhydride (T3P)



Since the T3P mediated amidation is a quite new method, not so many papers have discussed this topic so far. Dunetz et.al. described synthesis of glucokinase activator **36**, where utilization of T3P provides the best results.[31]

Scheme 11 - Synthesis of glucokinase activator

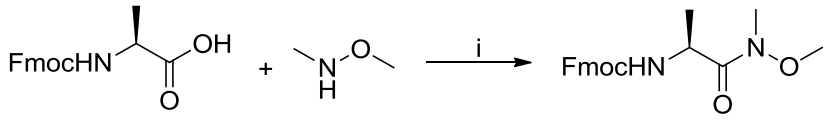


34 35 36

Conditions: (i) 50% T3P in EtOAc, pyridine, 98% ee, 80%

T3P can be used in the synthesis of Weinreb's amide **38**, which can be easily converted to the ketone, aldehyde or acetylene.[32]

Scheme 12 - Synthesis of Weinreb's amide



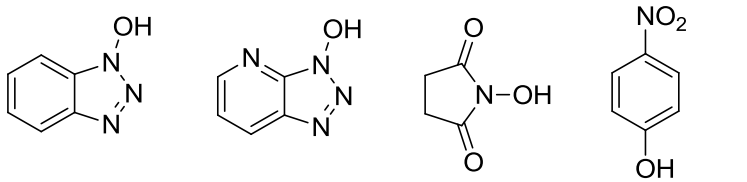
37 38

Conditions: (i) 50% T3P in ACN, pyridine, 83%

3.1.2 Amide synthesis via reactive esters

This part will be focused on the activation of carboxylic function via reactive esters with special emphasis on the carbohydrate chemistry. The alkyl esters, such as methyl, ethyl or benzyl are excluded, since their reactivity with amines require relatively harsh reaction conditions.[33] On the other hand, esterification with the reactive aromatic alcohols provides highly reactive esters towards amines and gives amide bond under mild reaction conditions. The increased reactivity is caused by the electron-withdrawing effect of the alcohol. The most common alcohols for esterification are hydroxybenzotriazole (HOBt) **39**, 1-hydroxy-7-azabenzotriazole (HOAt) **40**, *N*-hydroxysuccinimide (HOSu) **41** and *p*-nitrophenol (PNP) **42**.

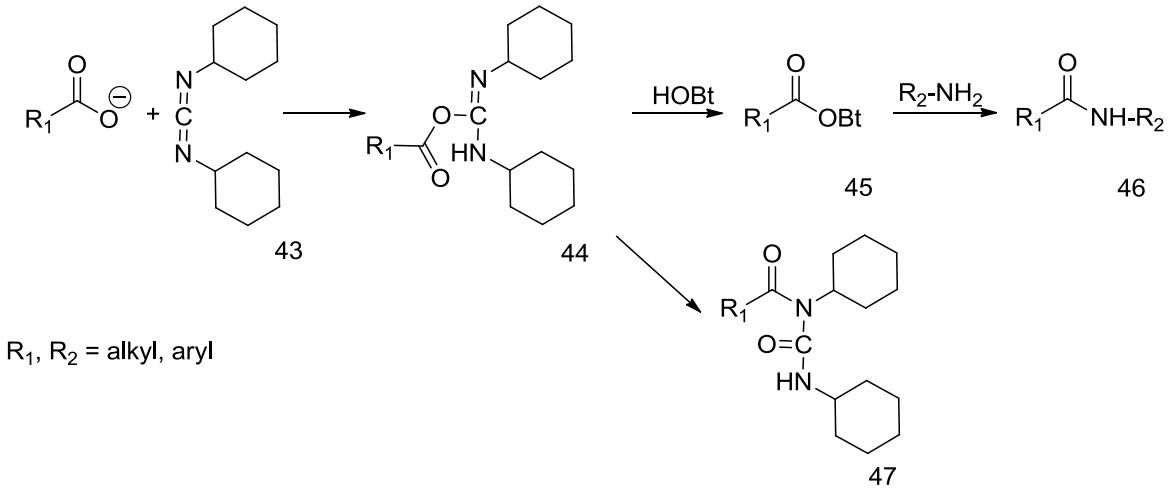
Figure 2 - Common alcohols for reactive ester preparation



HOBt, 39 HOAt, 40 HOSu, 41 PNP, 42

The first step of the mechanism of amide bond formation includes reaction of carboxylate with carbodiimide resulting in *O*-acylated urea **44**, which can rearrange into unreactive *N*-alkylated urea **47**.This may be the reason of impurities in the mixture. To prevent this undesired reaction, HOBt or similar alcohol can be added and the reaction easily proceeds to desired amide **46**.

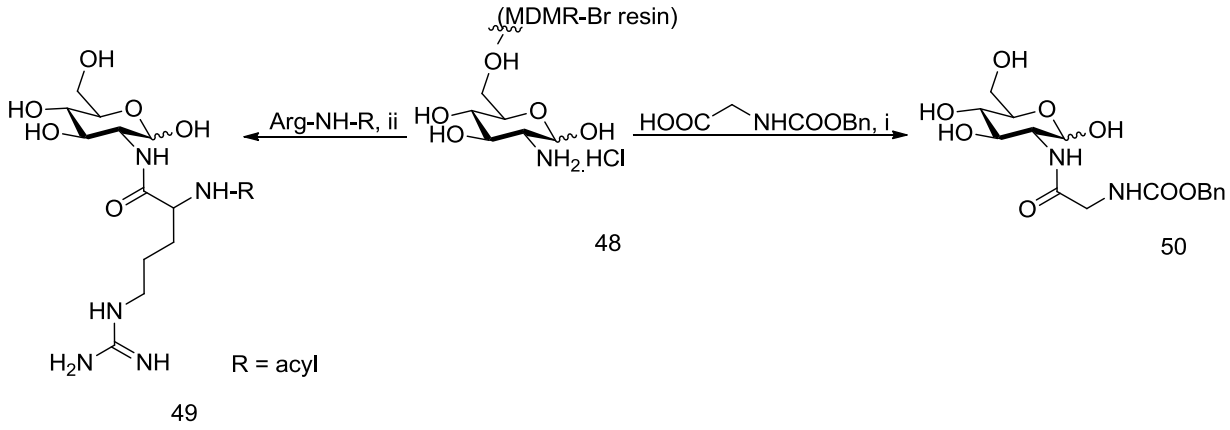
Scheme 13 - The mechanism of HOBt esterification



The choice of alcohols depends on the character of acid and reaction conditions. The HOBt, which is the most used one, is commercially available as a hydrate. The in situ formed ester **45** is moisture sensitive.[34] The HOBt is usually used together with DIC, DCC, or EDC. It found an application in solution phase as well as solid phase synthesis.[35] The reactivity of HOAt and HOSu is approximately the same as in the case of HOBt; nevertheless, when the sterically hindered amine is used, the HOAt can provide better results due to chelation of amine with pyridine nitrogen.[36]

When it comes to carbohydrate chemistry, HOBt technique is the most favored so far. Here are some recent applications. Tselios et.al. used HOBt amidation in synthesis of Thrombin receptor Glycopeptide Mimetic analogue **49** on polymer support.[37] The second example describes the key step in preparation of chiral piperazones, where the final piperazone is reached via reductive opening of hexapyranoside ring **50**.[38]

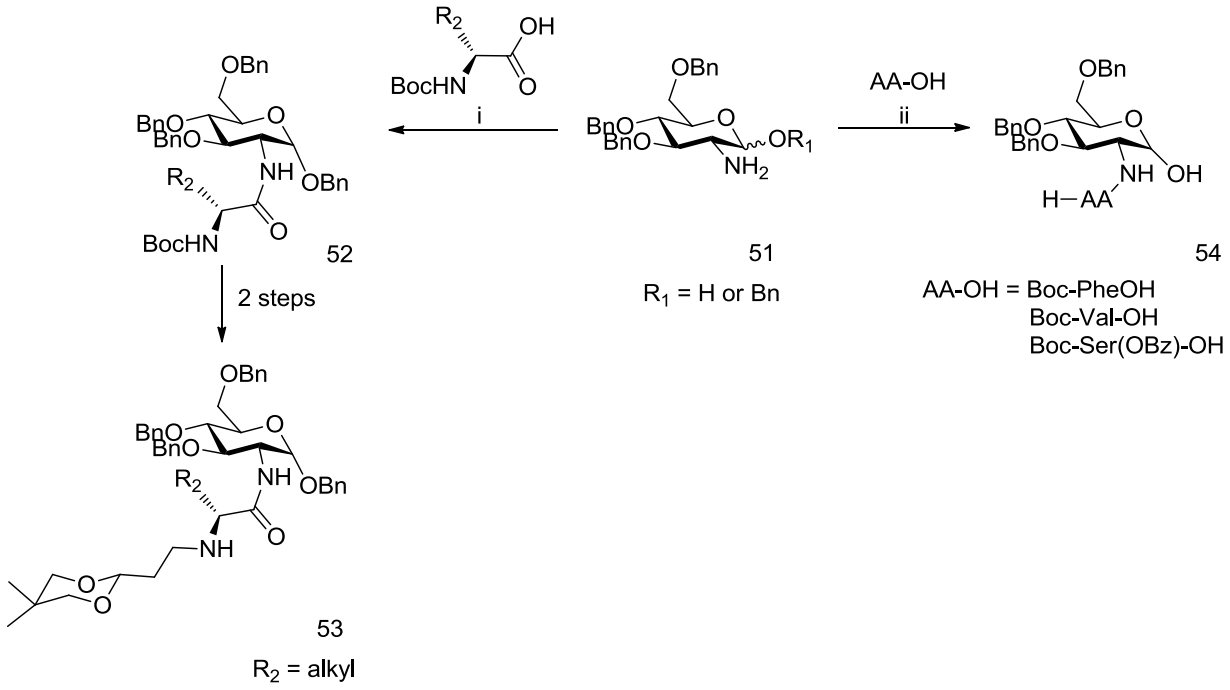
Scheme 14 - Acylation of unprotected D-glucosamine



Conditions: (i) DCC, HOBt·H_2O, DMF, 74%; (ii) DIC, HOBt·H_2O, DMF 82% (after cleavage from the resin)

The protection of hydroxy groups has no influence on the reactivity towards to acylation conditions. Reaction with Boc-protected amino acid resulted in amide **52**, which gave after two steps acylated glucosamine **53** (Scheme 15).[39] Compound **53** evinces anti-inflammatory effect. Zhang and col. used benzylated glucosamine in the synthesis of glycopeptide **54**, which can serve as a building block for more complex glycopeptide synthesis.[40]

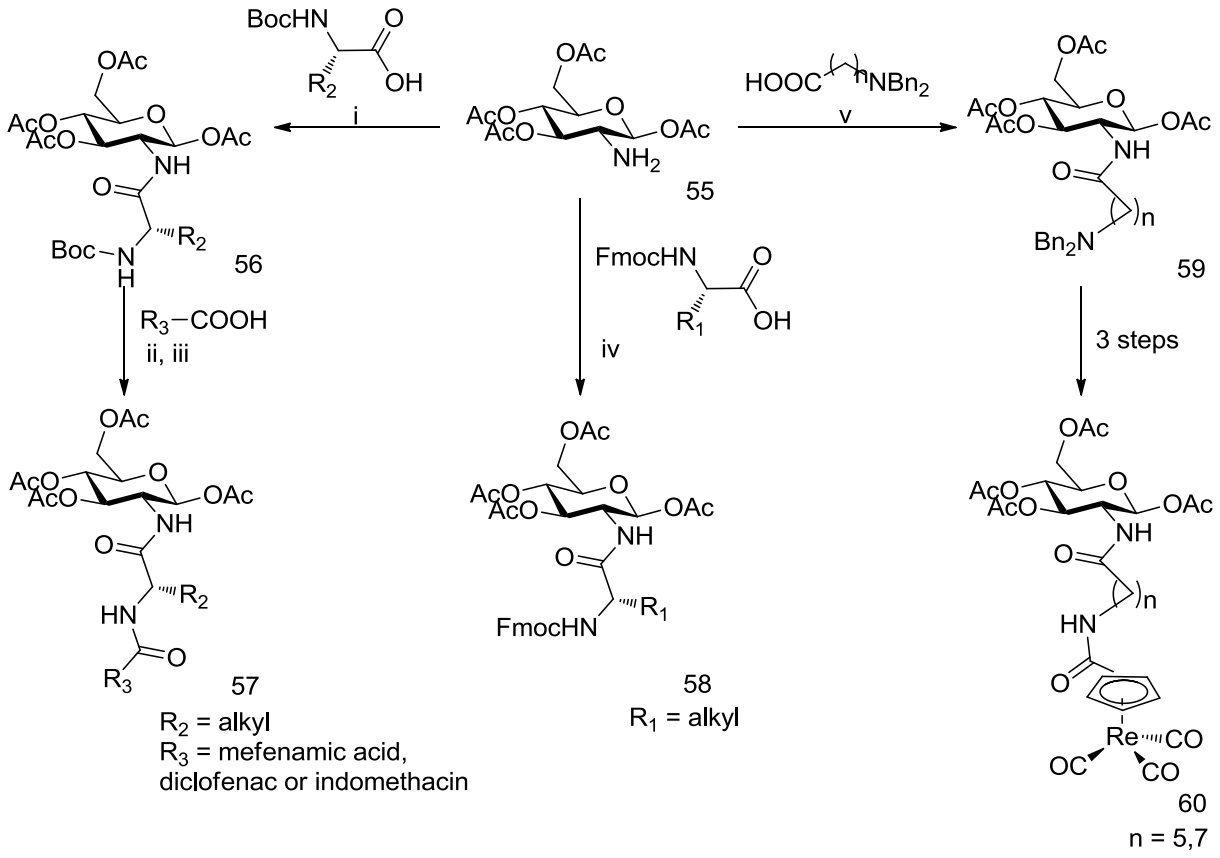
Scheme 15 - Acylation of benzylated glucosamine



Conditions: (i) DCC, HOBt·H_2O, NMM, THF, 30-56%; (ii) DCC, HOBt·H_2O, THF, 70-96%

Tetra-*O*-acetylated D-glucosamine was used as a starting material in the preparation of many compounds. Since mefenamic acid, diclofenac or indomethacin are well known non-steroidal anti-inflammatory drugs and D-glucosamine is essential for a human body, particularly for a cartilage, the collective of prof. Katritzky decided to connect these two biologically active substances via amino acid linker (Scheme 16).[41] Amide bond was achieved via HOBt/EDC technique in very good yield to give intermediate **56** and final product **57**. Wang et.al. acylated the substituted carbohydrate **55** with chiral Fmoc- amino acids where products **58** can find an application in the synthesis of more complex glycoproteins.[42] And finally, when a long chain amino acid is used and a free amino function is complexed with a metal such as ruthenium or technetium, the metal glucosamine conjugates **60** can be prepared.[43] Target compounds **60** have been found as hexokinase competitive inhibitors.

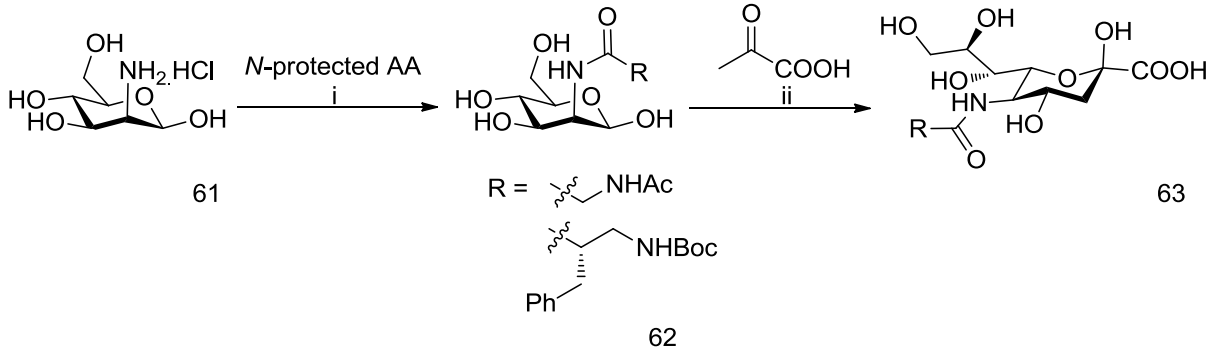
Scheme 16 - Acylation of acetylated glucosamine



Conditions: (i) EDC, HOBt·H_2O, Et_3N, THF, 0 °C to r.t., 57-89%; (ii) H_2, Pd/C, MeOH; (iii) EDC, HOBt·H_2O, DIPEA, THF, 0 °C to r.t., 36-70% (from two steps); (iv) Et_3N, DIC, HOBt·H_2O, DIPEA, THF, 32-61%; (v) EDC·HCl, HOBt·H_2O, DMAP, DMF, 55-74%

The proven alternative to HOBt consists in HOSu. Lin et.al. used combination of HOSu/EDC in the synthesis of *N*-substituted sialic acid **63**.[44] The mannosamine **61** was firstly acylated with carboxylic acid, which was subsequently transformed into sialic acid in the present of sialic acid aldolase.

Scheme 17 - Synthesis of sialic acid derivate

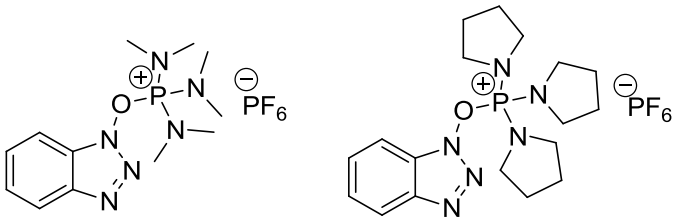


Conditions: (i) EDC, HOSu, DMF, $NaHCO_3$, 70-75%; (ii) potassium phosphate buffer (pH = 7.4), sialic acid aldolase, 55-72%

It was illustrated, that the reactive ester can be prepared by one pot reaction of carboxylic acid with carbodiimide and alcohol (HOBt, HOSu, etc.). However, there are other methods including formation of HOBt ester in one step. The hydroxy group, in most cases of HOBt and HOAt, can be converted to a salt, which can cause the better reactivity toward to amide formation. There are lots of commercially available derivatives, however, only the most significant ones will be discussed in this work.

One of the well-known and frequently employed derivate is a phosphonium salt of HOBt. The first published example is commercially available Benzotriazol-1-yl-oxytris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) **64**, also known as Castro´s reagent.[45] The main disadvantage of **64** is high toxicity of hexamethylphosphoric triamide (HMPA). Therefore, Benzotriazol-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBop) **65** was designed instead of BOP.[46]

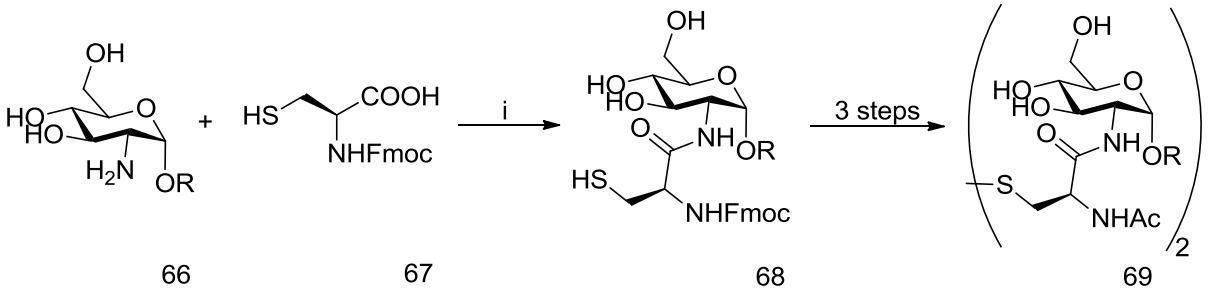
Figure 3 - Structure of Bop and PyBop



64 65

Due to the low toxicity and mild reaction conditions, PyBop **65** is predominantly used in many applications. In carbohydrate chemistry, PyBop **65** was employed in the synthesis of Mycothiol disulfide **69**, where the final product was achieved from **68** in 3 steps (Scheme 18).[47]

Scheme 18 - Application of PyBop in the synthesis of Mycothiol disulfide



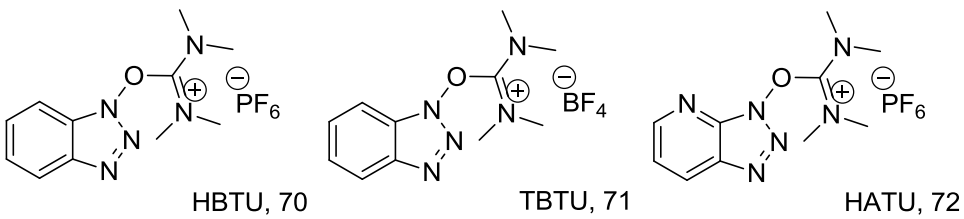
66 67 68 69

R = H, CH_3, Bn

Conditions: (i) PyBop, HOBt.H_2O, DIPEA, DMF/DCM, 46-84%

Besides phosphonium salts, also uronium salts found the application. Below, you can find several examples. Both *O*-(1*H*-benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU)[48] **70** and its tetrafluoroborate analogue (TBTU)[49] **71** can be used for amide bond formation in a laboratory scale with very good yields and racemic purity. HATU - the 1-hydroxy-7-azabenzotriazole analogue **72** of HBTU is efficient in the amidation with sterically hindered amines.[50] To prevent a potential formation of an undesired quanidine, an additional amount of HOBt (HOAt) is possible to add to the reaction mixture.

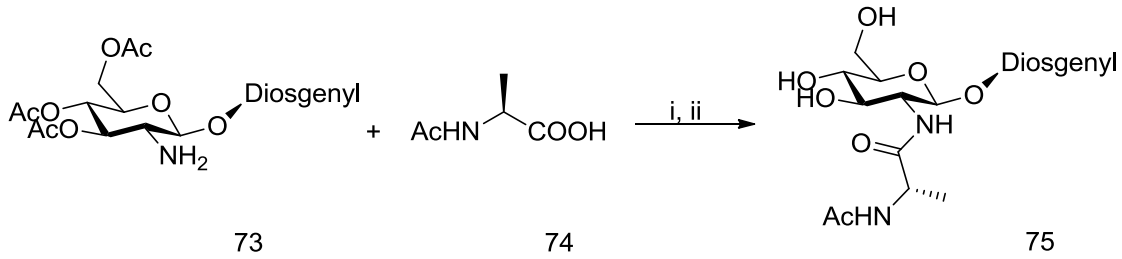
Figure 4 - Examples of uronium salts



HBTU, 70 TBTU, 71 HATU, 72

In chemistry of D-glucosamine, HBTU **70** or TBTU **71** mediated acylations of substituted glucosamines with different amino acids are not rare. Kaskiw et.al. utilized HBTU reagent in the synthesis of the diosgenyl saponine analogues **75**, which are present in traditional Chinese medicaments against cancer.[51] They described ,,classical,, HOBt/DIC method providing **75** as a racemic mixture (racemization took place on the alanine chain), while HBTU allowed only *S*- diastereomer.

Scheme 19 - Synthesis of Diosgenyl analogues

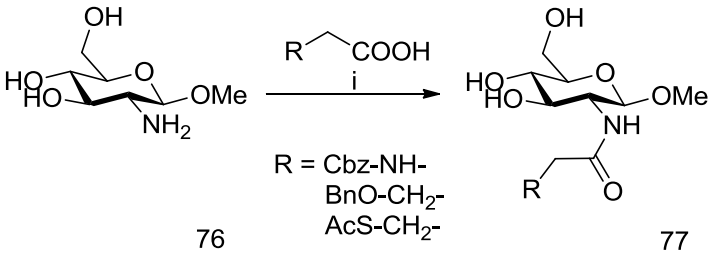


73 74 75

Conditions: (i) HBTU, DIPEA, DMF, 78%; (ii) MeONa, MeOH/DCM, 95-100%

It is possible to synthetize the less complex molecules such as glucosamine derivates **77**, where the amine group is acylated with different carboxylic acids (Scheme 20).[52] These derivates contain metal chelating groups, which can cause potential inhibition activity against poly(glycerol-adipate) butyryl ester PgaB.

Scheme 20 - Acylation of D-glucosamine with different carboxylic acids using HBTU mediation

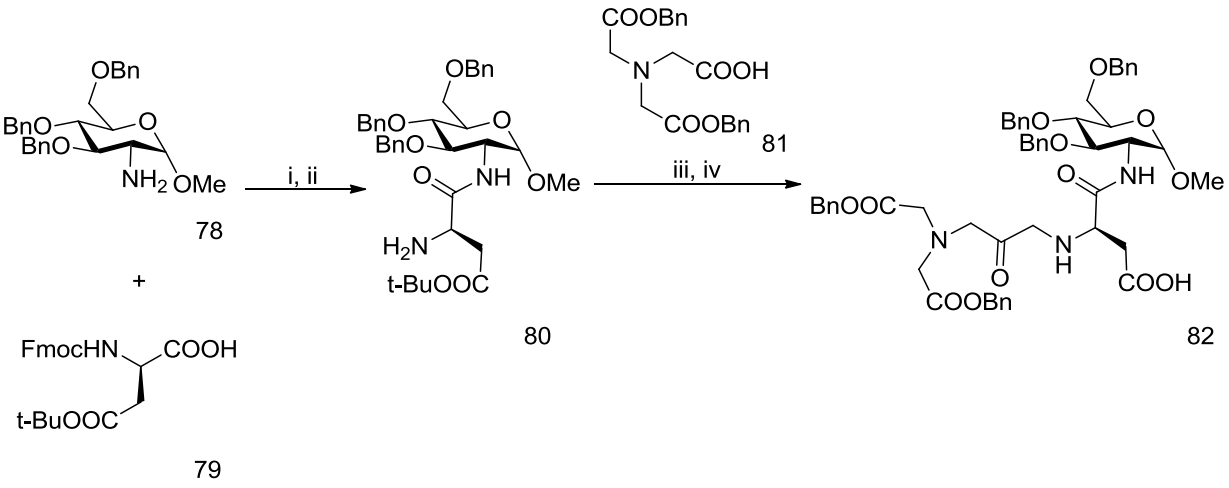


76 77

Conditions: (i) HBTU, TEA, DMF, 76%

The analogue of HBTU – TBTU, was employed in the synthesis of potential angiogenesis agent **82**.[53] The both acylations were accomplished in very good yields in the presence of additional amounts of HOBt.

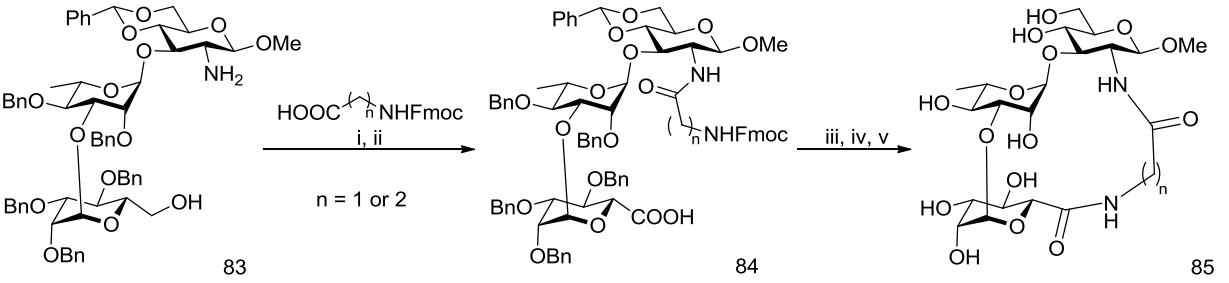
Scheme 21 - Acylation of glucosamine in the presence of HATU



Conditions: (i) HOBt, TBTU, DIPEA, DMF, N_2, 71%; (ii) 20% piperidine/DMF, 98%; (iii) HOBt, TBTU, DIPEA, DMF, N_2, (iv) TFA/DCM, 99%

HBTU acylation, again with addition of HOBt, was applied in the synthesis of macrocyclic hexokinase inhibitors containing three sugar units (Scheme 22).[54] In the first step, Fmoc-amino acid was coupled. Subsequent TEMPO catalyzed oxidation and Fmoc- deprotection allowed formation 15- and 16- membered ring **85**.

Scheme 22 - Synthesis of macrocyclic hexokinase inhibitors

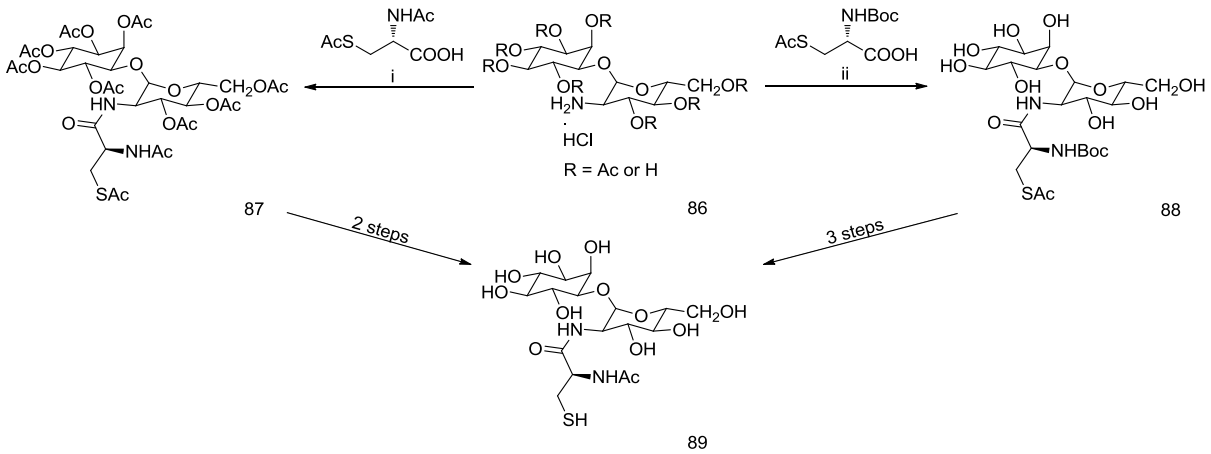


Conditions: (i) TBTU, HOBt, NEM, DMF, 86-91%; (ii) TEMPO, NaClO, *n*-Bu_4NCl, $NaHCO_3$, KBr, NaCl, 43-63%; (iii) 20% piperidine/DMF; (iv) TBTU, HOBt, NEM, DMF, 60-65%; (v) H_2, $Pd(OH)_2$, MeOH, 73-74%

As it was mentioned before, HATU mediated acylation is the method of choice for sterically hindered amines. Therefore, this reagent was employed in the synthesis of mycothiol **89** (Scheme 23). Mycothiol is a low molecular thiol contained in actinomycetes bacteria. The first total synthesis was reported by Lee et. al. The amine group of polyacetylated

carbohydrate **86** was acylated in the presence HATU.[55] Authors also described acylation using HOBt technique, however, poor yield and racemization of amino acid was observed. Later on, the mycothiol **89** was synthetized from unprotected sugar **86** under similar reaction conditions.[56,57] The final compound **89** was reached from amide **88** in three steps protocol.

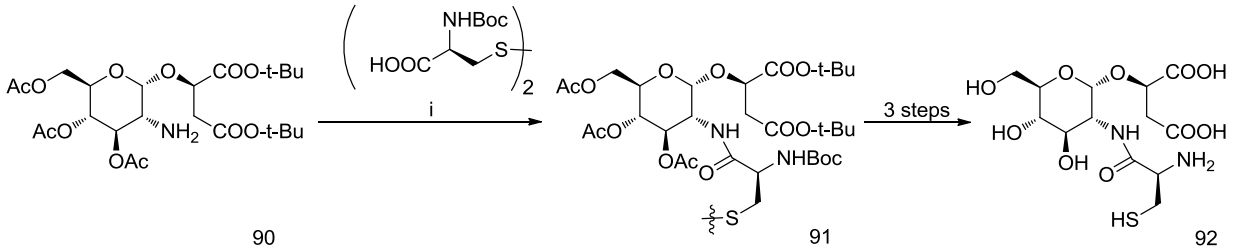
Scheme 23 - Synthesis of mycothiol



Conditions: (i) HATU, HOAt, collidine, DMF, 25%; (ii) HATU, DIPEA, DMF, 78%

Bacillithiol **92** is chemically as well as biologically similar to mycothiol **89** (Scheme 25). Acylation of **90** was achieved with *N*-Boc-cysteine disulfide, which was then reduced to desired thiol **92**.[58]

Scheme 24 - Synthesis of Bacillithiol

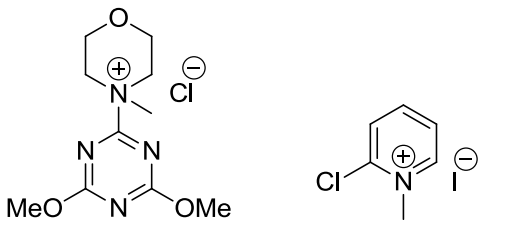


Conditions: (i) HATU, DIPEA, DMF, 80%

It was demonstrated, that *N*-acylation utilizing reactive alcohols belongs to wide used methods. However the development of amidation techniques still continues. In the last decade, there were designed ammonium salts, which can activate a carboxylic acid toward amide formation. Below, you can see a few examples of ammonium salts. Triazine based salt[59] **93** and pyridinium iodide, also known as Mukaiyama´s reagent[60] **94** found an

application mostly in the amide preparation in solution phase; neverthelessthe importance of these reagents is marginal in the chemistry of glucosamine.

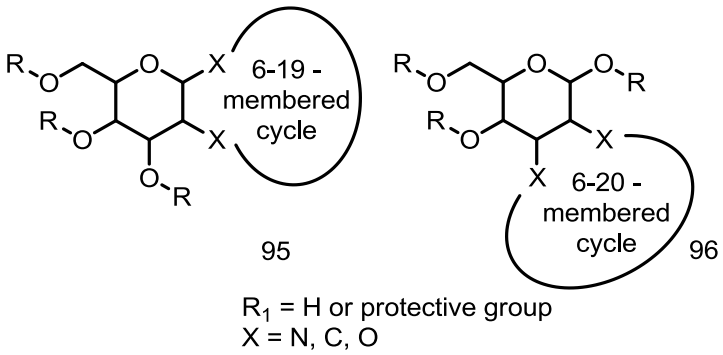
Figure 5 - Structures of ammonium salts



3.2 Synthesis of *ortho*-fused ring systems based on D-glucosamine and its epimers

While the first chapter discussed the possibility to create the amide bond from hexapyranosamine without regard to linear or cyclic amide structure, the second chapter is strictly focused on the *ortho*-fused scaffolds. Moreover, besides the cyclic amides – lactams, all cycles fused to hexapyranose ring are included. Below, you can see the general structures of discussed compounds. While the first subchapter concerns formation of *ortho*-fused ring between C1 and C2 carbon, the second subchapter analyses the *ortho*-fused ring formation between C2 and C3.

Figure 5 - General structure of *ortho*-fused ring systems containing D-hexapyranosamine



3.2.1 Cyclization resulting in the ortho-fused ring between C1 and C2 carbon

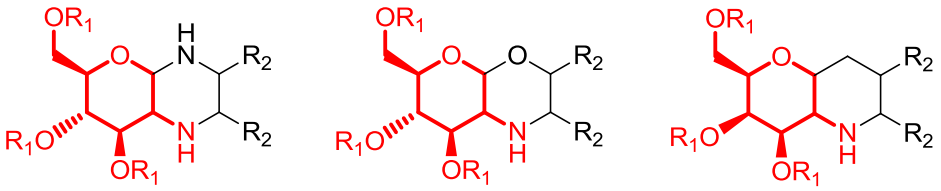
This subchapter is divided into the three parts according to size of the *ortho*-fused ring. The derivatives with *ortho*-fused six – membered rings are well explored and there are the most examples describing biological properties. The carbohydrates with fused seven –

membered ring are usually based on the oxazepine scaffold and are quite rare. Macrocycle fused ring systems containing carbohydrates mostly belong to the family of polypeptide and they will be discussed briefly in the last part.

3.2.1.1 Formation of six – membered ring

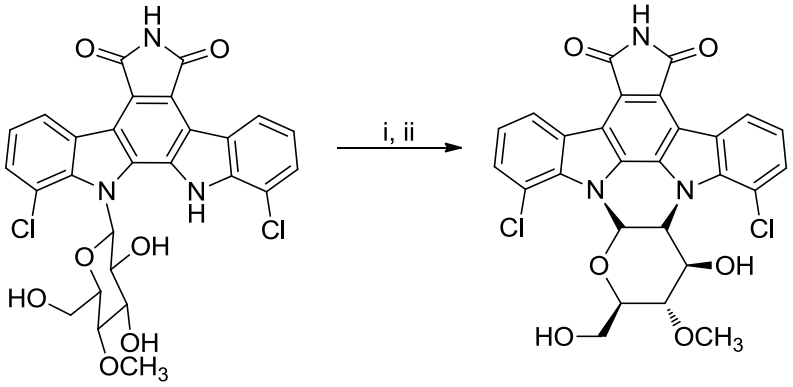
The **s**ix – membered rings fused to the hexapyranose cycle play an important role in the carbohydrate chemistry. Below, you can find the three structures. While scaffolds **97** and **98** are formally derived from the D-gluco- or mannosamine unit, **99** includes D-galactosamine motif. The first structure **97** contains fused piperazine cycle; it is the most common and will be discussed first. Structures **98** and **99**, involving morpholine, respective piperidine ring, are not so frequent; a few examples have been described.

Figure 6 - Possible structures of fused six – membered ring



Rebbecamycin **100** is well known as a microbial metabolite and evinces antiproliferative activity.[61] Therefore, a few attempts leading to derivatives of rebbecamycin can be found in the literature. All modifications are generally done on the sugar unit,[62] indole ring,[63] or the imide function.[64,65] The synthesis of **101**, starting from rebbecamycin **100**, involves activation of the hydroxy group followed by the ring closure via the azide formation.[66]

Scheme 26 - Derivatization of rebbecamycin

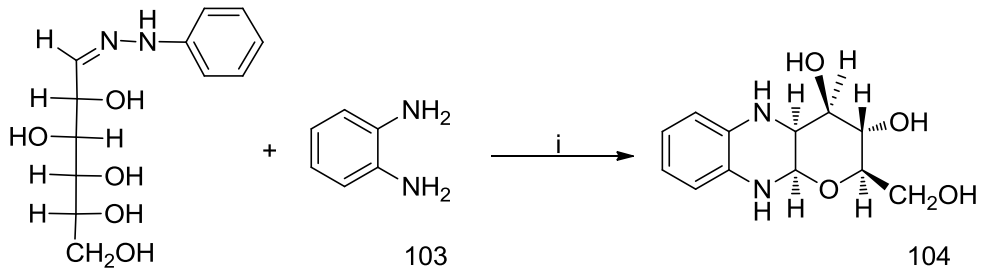


100 101

Conditions: (i) TsCl, K_2CO_3, DMF, 47%; (ii) NaN_3, DMF, 65%

The piperazine cycle is also incorporated pyrano[2,3-b]quinoxalines **104**, which can be synthetized from hydrazone **102** and phenylendiamine **103** (Scheme 27). The compound **104** is structurally similar to molybdopterin, which is an organic ligand of molybdenum cofactor.[67]

Scheme 27 - Synthesis of substituted quinoxaline **111**



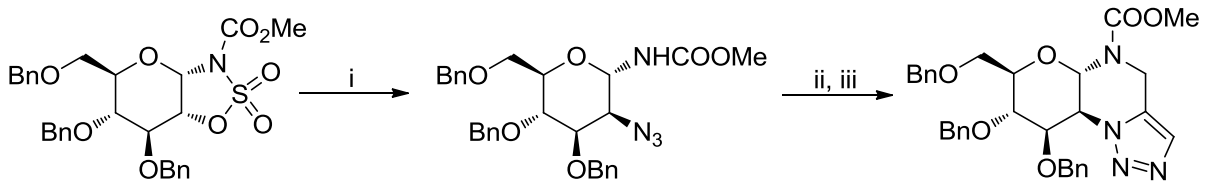
102 103 104

Conditions: (i) 5*N* HCl, 2-mercaptoethanol (cat), MeOH/water, 40%

The similar approach was used by Clinch et.al., where instead of phenylediamine and D-glucose, 2,5,6-triamino-3,4-dihydropyrimidin-4-one and D-galactose was employed.[68]

Further, Sudhir et.al. prepared the fused triazolopiperazine system, where protected D-glucose was used **105**.[69] Reaction of sulfamidate **105** with sodium azide gave azide **106**, which was subsequently converted in two steps synthesis to triazole **107** in very good yield.

Scheme 28 - Synthesis of fused triazole ring

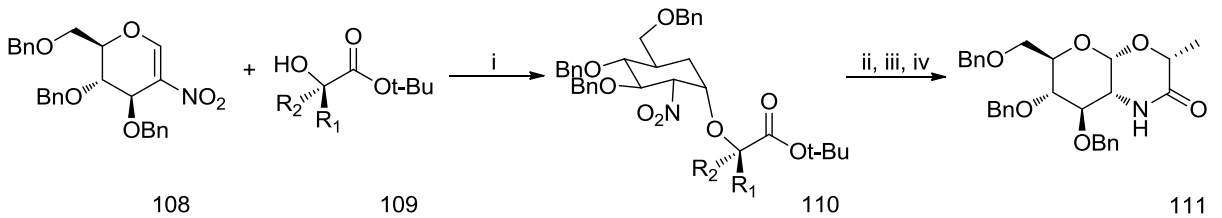


105 106 107

Conditions: (i) NaN_3, DMF, 65°C, 85%; (ii) propargyl bromide, NaH, DMF; (iii) 80°C, 80% (for two steps)

The formation of the fused morpholine ring to the hexapyranose cycle can be synthetized from nitroglucal **108**, where in the first step, the addition of the hydroxy group to the double bond affords ether **110**.[70] Subsequent the three step protocol results in the ring closure to give morpholine derivate **111**.

Scheme 29 - Synthesis of morpholine derivate



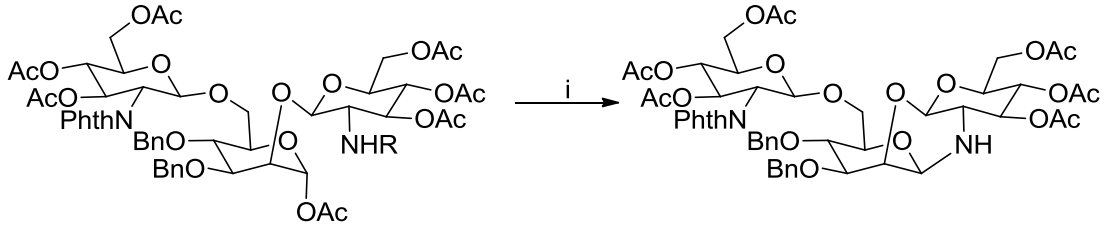
108 109 110 111

R_1, R_2 = H, Me

Conditions: (i) t-BuOK, toluene, 80%; (ii) Ra-Ni, H_2, EtOH; 78%; (iii) TFA, DCM, 96%; (iv) DPPA, Et_3N, DMF, 77%

The morpholine motif can be also found in a trisaccharide molecule, which can serve as substrates or inhibitors of GlcNAc transferase.[71] Goddat et.al. published ring closure resulting in **113** under basic conditions.

Scheme 30 - Synthesis of trisaccharide

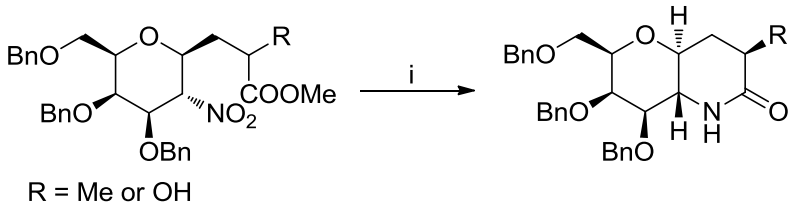


112 113

Conditions: (i) EtOH, $N_2H_4.H_2O$, 84%

The piperazine cycle is usually hidden in the lactam derivatives. The following Scheme 31 describes the lactam formation by the reaction of methyl ester and amine group **114** to give the desired sugar-based piperazinon **115**.[72,73] The product **115** is structurally similar to the polyhydroxylated piperidine alkaloids, which are well known for their antiviral biological activities.

Scheme 31 - Synthesis of piperazino derivative



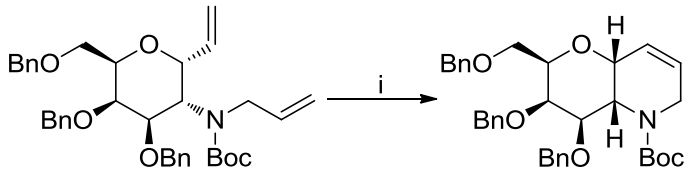
R = Me or OH

114 115

Conditions: (i) Zn/HCl, then $NaHCO_3$, 76%

The different approach was chosen in the synthesis of 1-Deoxynojirimycin analogues, where the amide **117** was formed via metathesis reaction (Scheme 32).[74] When the allyl group instead of the vinyl group is used, the seven – membered ring can be prepared under the similar conditions.

Scheme 32 - Cyclisation via metathesis reaction



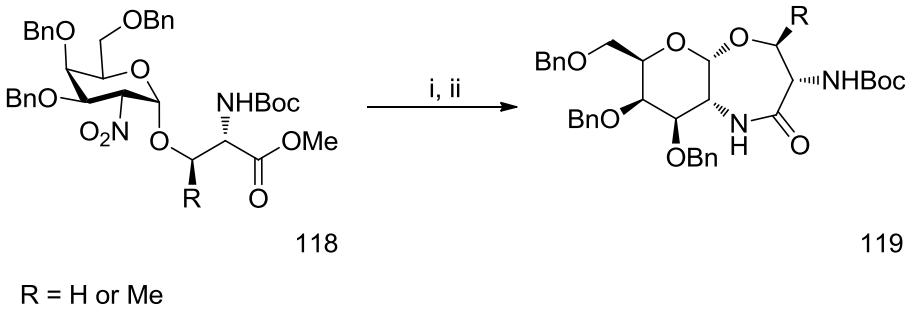
116 117

Conditions: (i) Grubbs catalyst (5 mol%), DCM, 94%

3.2.1.2 Formation of seven – membered ring

Fused seven – membered rings are quite rare in comparison to the six membered cycle. Most of the described compounds contain the oxazepine scaffold, where the last step of the synthesis is the lactam formation. This procedure was applied in the synthesis of oxazepines **119**, where serine or threonine facilitated the ring closure.[75,76]

Scheme 33 - Oxazepine ring formation



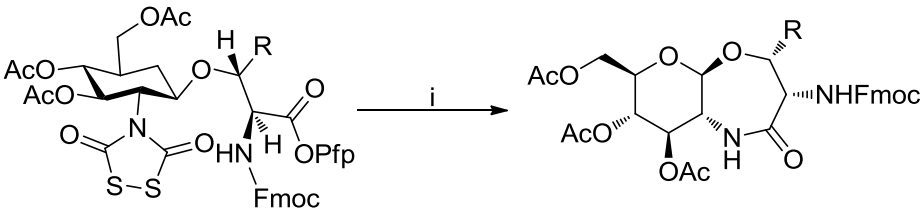
118 119

R = H or Me

Conditions: (i) Ra-Ni (T_4), H_2, EtOH, 84%; (ii) LiOH, H_2O; NEt_3, DPPA, DMF, 83%

The oxazepine ring was also observed, when the dithiosuccinyl moiety was used as a protecting group. The lactam **121** was achieved via the deprotection reaction of the dithiosuccinyl moiety and the subsequent ring closure reaction of the resulting amine group with pentafluorophenyl (Pfp) ester.[77]

Scheme 34 - Synthesis of the oxazepine ring using the dithiosuccinyl moiety



120 121

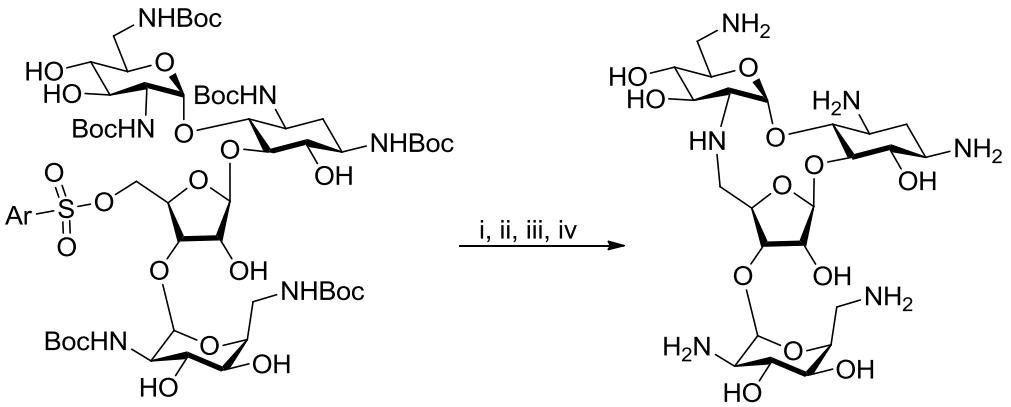
R = H or Me

Conditions: (i) Zn/AcOH, THF, 90-95%

3.2.1.3 Formation of macrocycle rings

The chemistry of the fused macrocycle rings to the D-glucosamine unit is not well explored, only a few papers described the synthesis and biological evaluation of these molecules. Mostly, the ring is created by incorporation of sugar units into the macrocycle or the sugar is a part of the polypeptide molecule. The first approach was used by Blount et.al. in the preparation of restricted Neomycin B, which is well known antibiotics.[78] The synthesis resulted in eleven – membered macrocycle **123**.

Scheme 35 - Synthesis of restricted Neomycin B

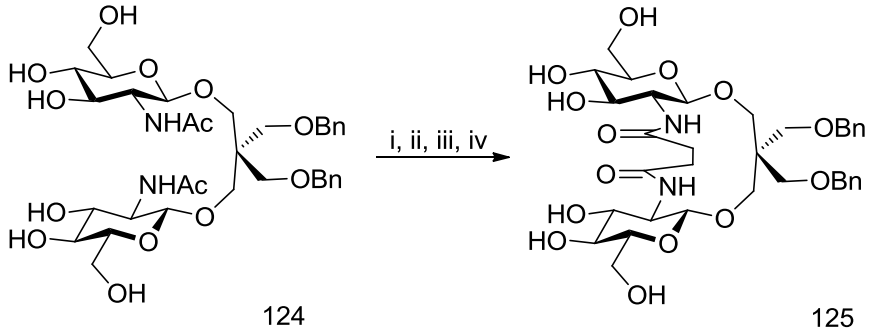


122 123

Conditions: (i) TFA/DCM; (ii) NEt_3, DMF; (iii) Boc_2O, NEt_3, MeOH, 12% (3 steps); (iv) TFA/$CHCl_3$, 97%

It is also possible to connect two D-glucosamine units with etheric chain on one side and succindiimide on the other (Scheme 36).[79] The final disaccharide **125** created fifteen - member ring, which evinces potential anti-inflammatory activity.

Scheme 36 - The synthesis of fifteen – member ring

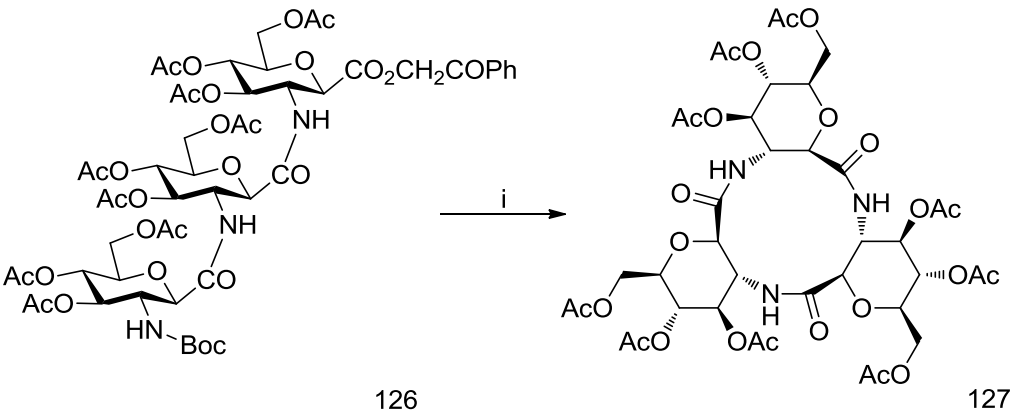


124 125

Conditions: (i) $Ba(OH)_2$, then 1 M H_2SO_4, pH=2, $(Boc)_2O$, water/dioxane, Et_3N, 82%; (ii) Ac_2O, pyridine, 90%; (iii) TFA, DCM, 92%; (iv) $ClCO(CH_2)_2COCl$, DCM, Et_3N, 42%

The second approach consists in the implementation of D-glucosamine into the polypeptide. Fujimura and col. reported the synthesis of the tripeptide, where the cyclisation is mediated by activation of carboxylic function by HOAt/Bop reagents.[80] The resulting cyclotripeptide **127** can be further substituted.

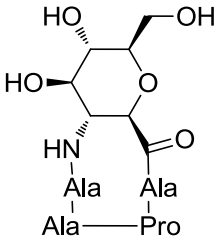
Scheme 37 - Synthesis of cyclotripeptide



Conditions: (i) BOP, HOAt, DIPEA, DMF, 70%

Polypeptide can also serve as a linker between two sides of the one sugar unit.[81] Below (Figure 7), there is an example of cyclic peptides containing glucosamine unit forming nineteen – member ring.

Figure 7 - Example of cyclic peptides



3.2.2 Formation of cyclic structure between C2 and C3 carbon

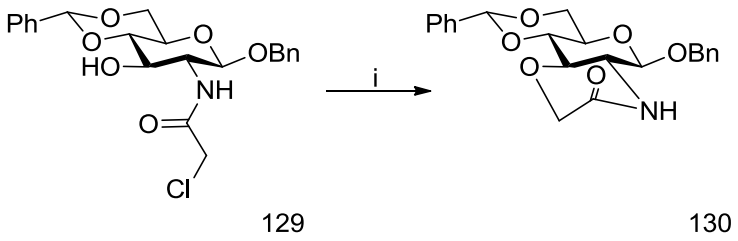
The hexopyranosides with *ortho*-fused ring between C2 and C3 carbon are quite common and biological properties of cycles are well explored. The first part is dedicated to six – membered *ortho*-fused rings, mostly to lactams. The second part describes seven – membered *ortho*-fused rings, predominantly diazepines and oxazepines, and, finally, the last part contains brief overview of macrocycle *ortho*-fused rings.

3.2.2.1 Formation of six – membered ring

Condensation of the six – membered cycle to the D-glucosamine skeleton (or to its epimers) is predominantly based on the lactam formation. These lactams can be formed on the one carbohydrate unit or the lactam function can serve as a linker between two or more sugar

units. From the synthetic point of view, the last step of cyclisation can be either C-O bond or amide bond. The first approach was applied in the structural studies of D-glucosamine, where product **130** can be further connected to another sugar unit via C1 etheric bond (Scheme 38).[82]

Scheme 38 - Synthesis of lactam via etherification as the last step

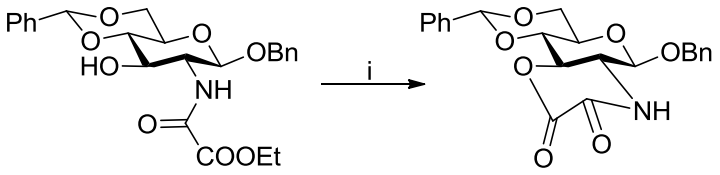


129 130

Conditions: (i) t-BuOK, DMF, 76%

When the ethyl oxamide is connected to the carbohydrate molecule, the polyfunctional bicycle **132** is formed,[83] however, the stability of **132** is significantly low.

Scheme 39 - Cyclization of ethyl oxamide derivate

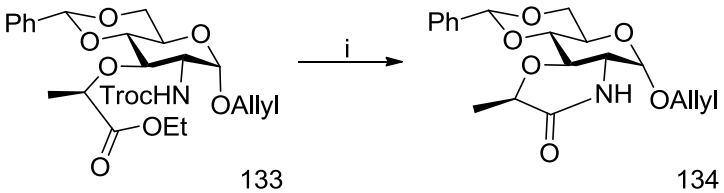


131 132

Conditions: (i) base (cat.), xylene, 85%

The second approach, where the last step of cyclization is the amide bond formation, prevails. Lactam **134** was prepared from by the intramolecular reaction of ethyl ester and the deprotected amine group in the presence of TBAF.[84]

Scheme 40 - Synthesis of the lactam ring via TBAF mediated cleavage

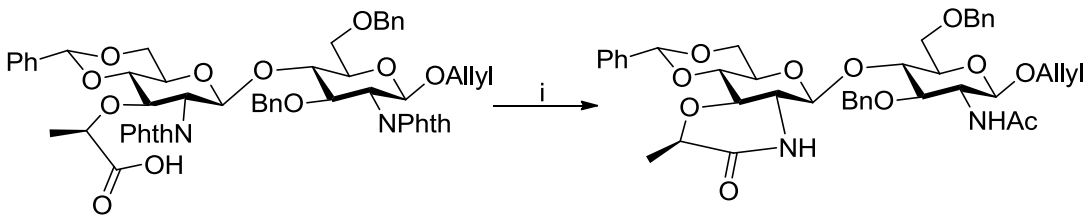


133 134

Conditions: (i) TBAF, THF, 70%

The same approach can be applied in the synthesis of disaccharides. While the Scheme 41 shows the reaction of carboxylic acid with in situ deprotected amine resulting in lactam **136**,[85] in Scheme 42, the lactam **138** is formed by azide reduction and subsequent cyclization.[86,87] Both products are structurally similar to muramic acid. Muramic acid is well known for anti-inflammatory and antitumor properties.

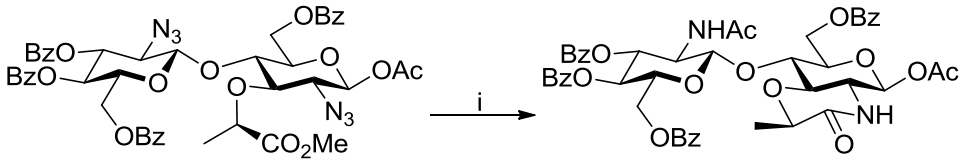
Scheme 41 - Synthesis of lactam fused to the first sugar unit



135 136

Conditions: (i) *n*-Butanol, ethylendiamine, N_2, then Ac_2O, pyridine, 76%

Scheme 42 - Synthesis of lactam fused to the second sugar unit

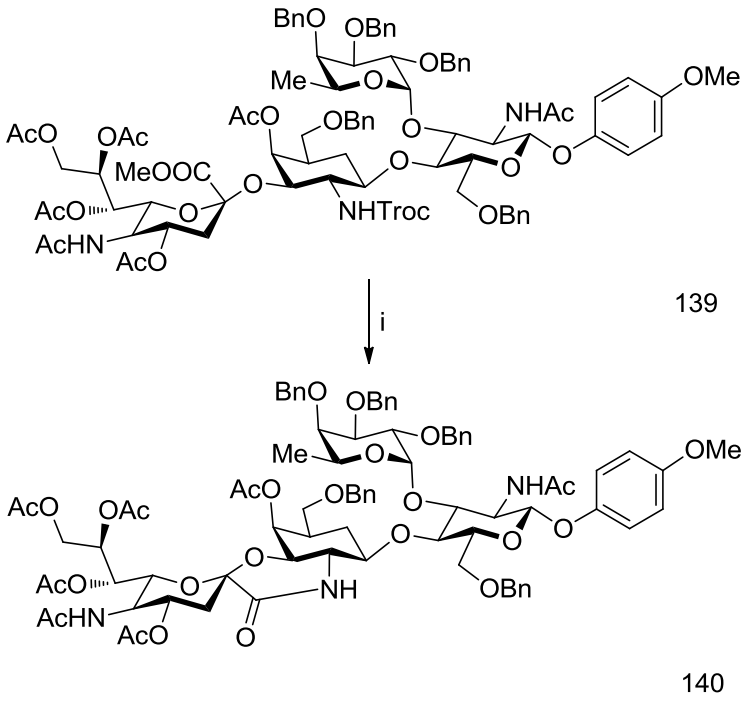


137 138

Conditions: (i) H_2S, pyridine/water, then Ac_2O, pyridine, 57%

As it was mentioned above, the lactam can connect two or more sugar units into a polysaccharide. Magnusson et.al. reported the synthesis of the lactam analogues of Sialyl Lewis tetrasaccharides **140**.[88,89] The Sialyl Lewis polysaccharides have been identified as an important structure for intercellular molecular recognition.

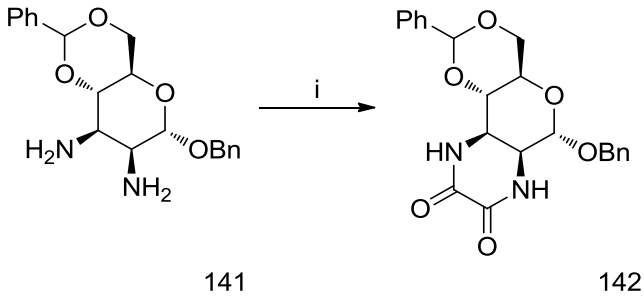
Scheme 43 - Synthesis of lactam analogues of Sialyl Lewis analogue



Conditions: (i) Zn/AcOH, then MeONa/MeOH, then H_2, $Pd(OH)_2$, EtOH, 54%

Besides the illustrated lactams, a six – membered cyclic diamide was described. The structure **142** was formed by the reaction of diethyl oxalate with 2,3 – diamino mannopyranoside **141**.[90]

Scheme 44 - Synthesis of cyclic diamide

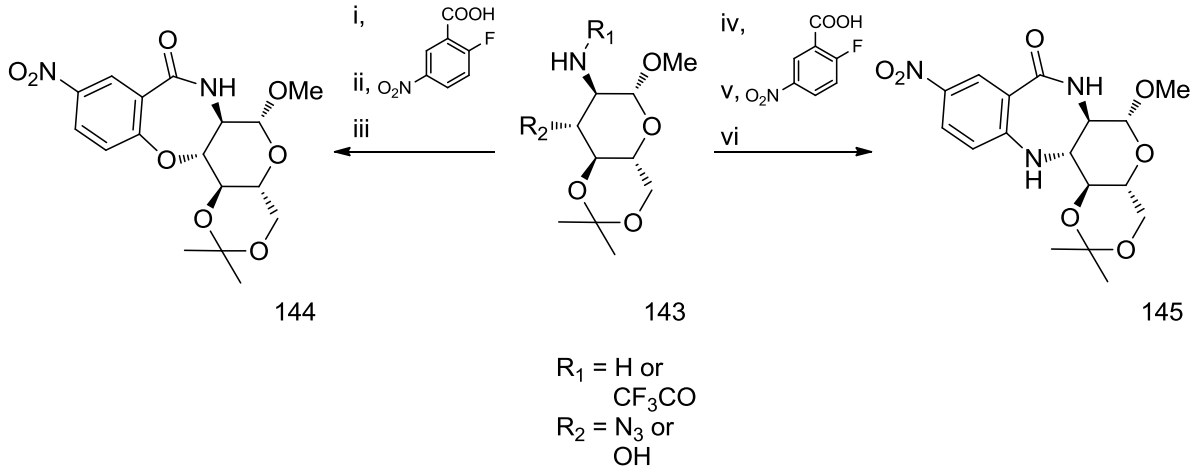


Conditions: (i) Diethyl oxalate, EtOH, 85%

3.2.2.2 Formation of seven – membered ring

The fused seven – membered rings are significantly less common than those with six membered cycle. Abrous et.al. reported two papers describing benzooxazepinone **144** and benzodiazepinone **145** derivate of D-glucosamine.[91,92] The amide bond was created in the presence of EDC and the etheric bond was formed via S_N aromatic reaction.

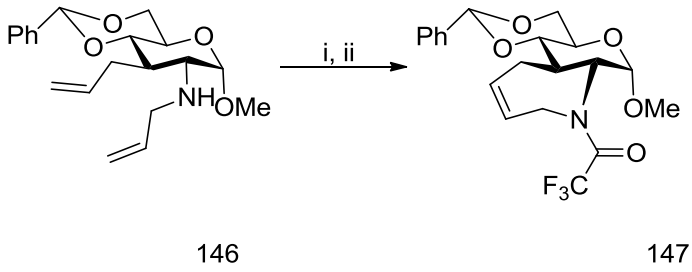
Scheme 45 - Synthesis of benzodiazepinone and benzooxazepinone scaffold



Conditions: (i) 5 M KOH, MeOH (ii) EDC, DCM, 75%; (iii) CsF, DMF, 55%; (iv) EDC, DCM, 95%; (v) Ph_3P, THF, 74%; (vi) CH_3CN, ΔT, 70%

The azepane structure **147** can be constructed via the ring closure metathesis in the presence of Grubbs catalyst, 1st generation. The possible biological application includes treatments for cancer, diabetes or HIV.[93]

Scheme 46 - Synthesis of azepane ring via metathesis reaction



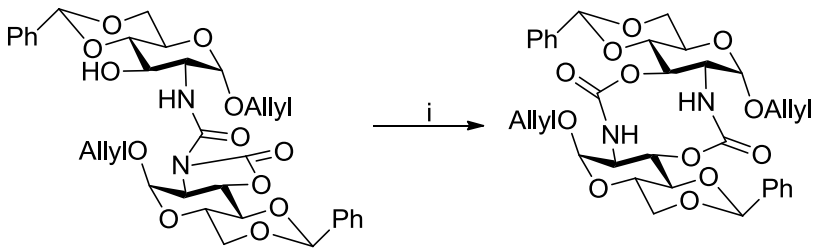
Conditions: (i) TFA, pyridine/DCM, 58%; (ii) Grubbs cat., DCM, 68%

3.2.2.3 Formation of ortho-fused macrocycle rings

This part will be focused on the ortho-fused macrocycle rings, which are, however, quite unusual and only a small number of publications has been dedicated to this topic. Dimerization of two sugar units will be included, although it was often undesired reaction.

Ten – membered ring can be found in the molecule of urea-linked disaccharide **149**, which can serve as ion transporter or organocatalyst.[94,95]

Scheme 47 - Synthesis of urea-linked disaccharide

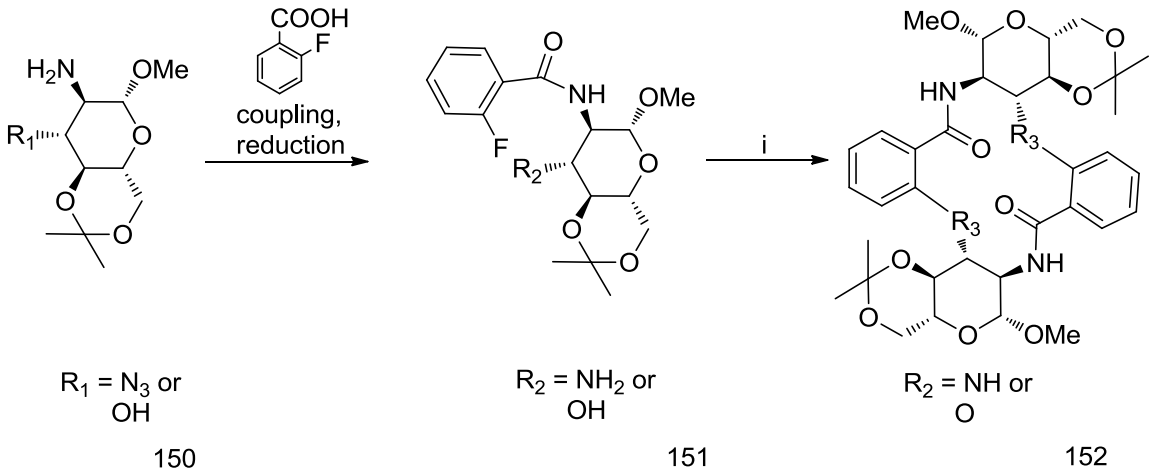


148 149

Conditions: (i) DBU, DMF, 43%

Formation of the fourteen – membered ring was observed during synthesis of benzodiazepinone and benzooxazepinone scaffolds, see ref (*91*) where due to the reaction conditions, dimer **152** was isolated either as a minor or major product. Surprisingly, dilution of a reaction mixture made just marginal difference in monomer vs. dimer ratio.

Scheme 48 - Formation of dimer product

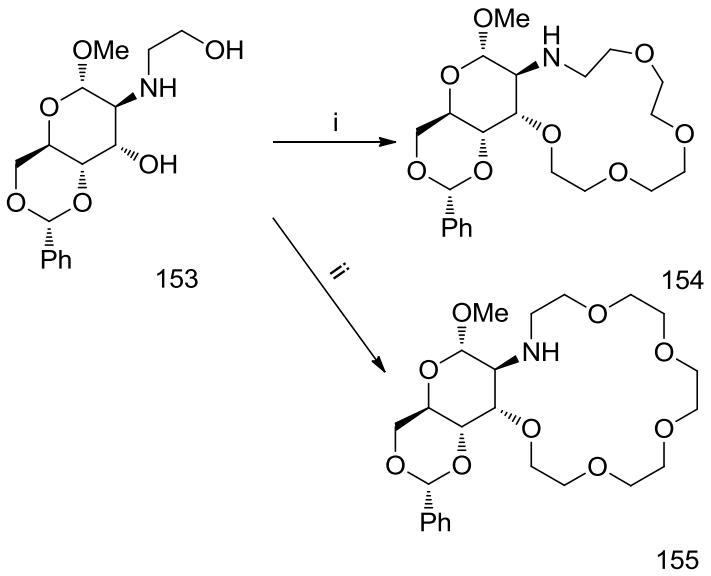


150 151 152

Conditions: (i) K_2CO_3, DMF, 75%

Aza-crown ethers are subject of interest due to their extraordinary coordination properties.[96] Bakó et.al. published the synthesis of a sugar with the incorporated fifteen and eighteen – membered aza-crown ether via the reaction of diol **153** with the appropriate polyethylene bitosylate (Scheme 49).[97] Furthermore, a formation of the fifteen – membered macrocycle ring can be reached by the HOBt mediated cyclisation. For details, see ref (*54*).

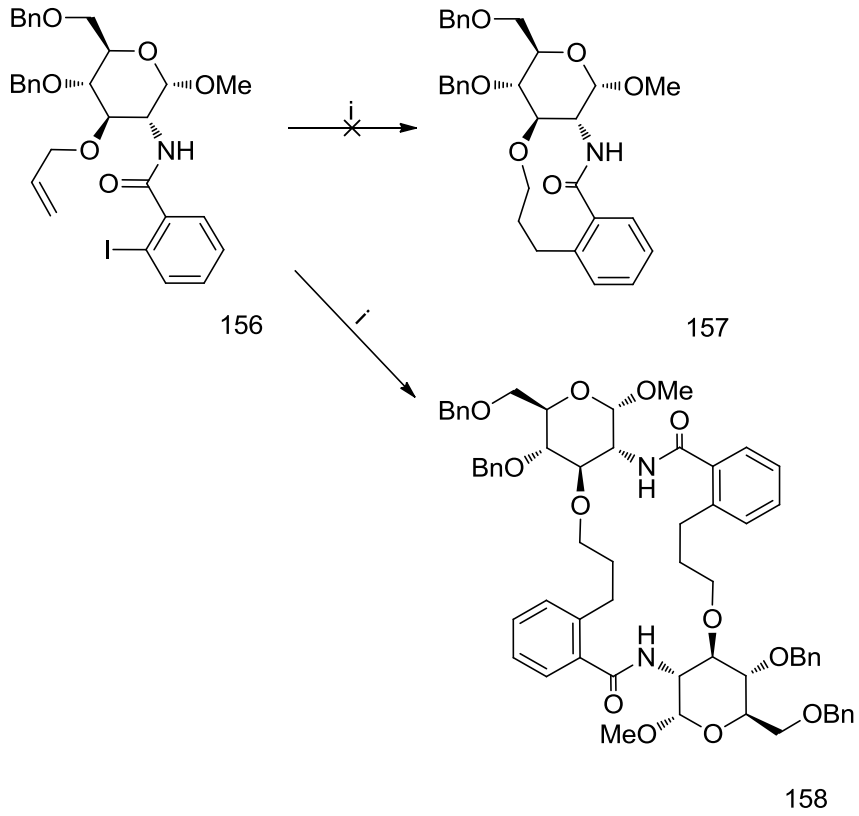
Scheme 49 - Synthesis of fifteen and eighteen – membered aza-crown ether



Conditions: (i) $TsO(CH_2CH_2O)_3Ts$, *t*-BuONa, *t*-BuOH, dioxane, 25%; (ii) $TsO(CH_2CH_2O)_4Ts$, *t*-BuONa, *t*-BuOH, dioxane, 31%

The example of an unexpected macrocycle formation is illustrated below. In the presence of Bu_3SnH/AIBN, authors intended to prepare ten – membered ring **157** by the radical cyclization, nevertheless, twenty – membered macrocycle **158** was predominantly detected (Scheme 50).[98]

Scheme 50 - Formation of the twenty - membered ring



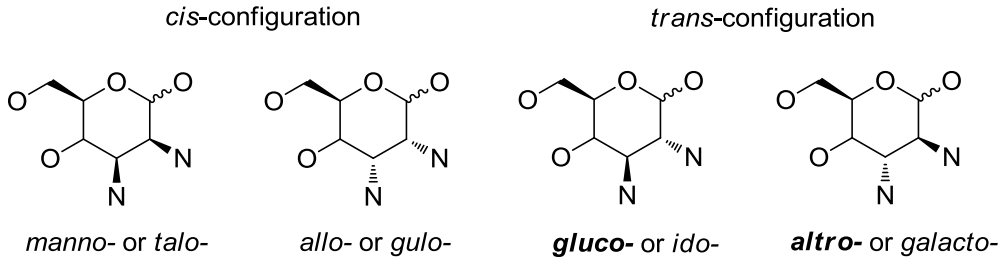
(i) Bu_3SnH, AIBN, benzene, 40%

3.3 Synthesis of D-hexopyranosides with more than one nitrogen-containing functionalities at position 2, 3, and 4

The D-hexopyranosides with more than one nitrogen-containing functionality belong to the class of compounds with a high level of importance. The list of their properties is voluminous. For illustration, we can found compounds with antibacterial, antiviral, or antitumor activity. Some of them, for example Zanamivir, are commercially available.

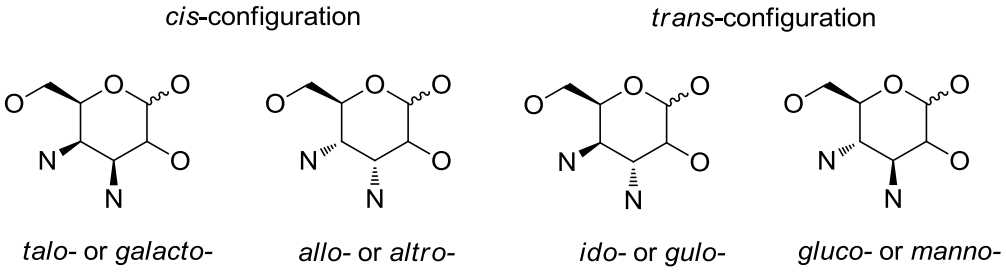
Figure 8 defines the structures of interest in 2,3-dinitrogen-substituted part with special emphasis on molecules containing *gluco*- and *altro*- configuration, since the both are the most common. 2,3–Diamino carbohydrates play an important role in the carbohydrate chemistry. They offer plenty of biological, as well as synthetical properties. Several of them are cited herein: Agelastatin A 1 precursor,[101] LPS dependent secretion enhancement,[102] ligands for Mo-catalyzed allylic alkylation,[103] and for Half-sandwich metal complexes with antitumor activity, which is easily comparable to activity of *cis*-platina[104] or precursor for glucose – benzodiazepine scaffolds.[91]

Figure 8 - Possible configurations of 2,3–disubstituted D-hexopyranosides with nitrogen-containing functionalities



The following Figure 9 shows the main motifs in the subchapter focused on the 3,4-dinitrogen D-hexopyranosides. D-Hexopyranosides with 3,4–diamino configuration are quite prevalent. *Cis*-dinitrogen-containing D-hexopyranoses can be found in derivates of Neomycin, Kanamycin and related compounds. *Trans*-dinitrogen-containing D-hexopyranoses are predominantly incorporated in the skeleton of zanamivir and its analogs.

Figure 9 - Possible configuration of 3,4-dinitrogen D-hexopyranosides



The chapter 3.3 is concluded with brief summary of tri- and tetra- nitrogen hexapyranosides, which makes the whole chapter more complex.

The development of 2,3–dinitrogen-containing carbohydrates started around 1960, when the group of professor Richardson presented a ring formation and opening of aziridine with *allo*– configuration from the corresponding activated D-glucosamine.[99]

Two decades later, Albert and col. began with derivatization of glycoside antibiotics (Neomycin B, Kanamycin, Arbekacin) toward incorporation the 3,4-diamino configuration into the molecule.[100]

These two works were a milestone in the chemistry of dinitrogen carbohydrates. This subchapter has an ambition to cover the historical as well as the present routes for the synthesis of above mentioned compounds.

3.3.1 2,3 – dinitrogen-containing D-hexopyranosides

There are several synthetic ways for the preparation of 2,3–dinitrogen-containing D-hexopyranosides. Generally, synthetic routes for 2,3–*cis* and 2,3–*trans* are different; frequently, it depends on the starting material. Both *trans*- and *cis*- derivates are discussed separately.

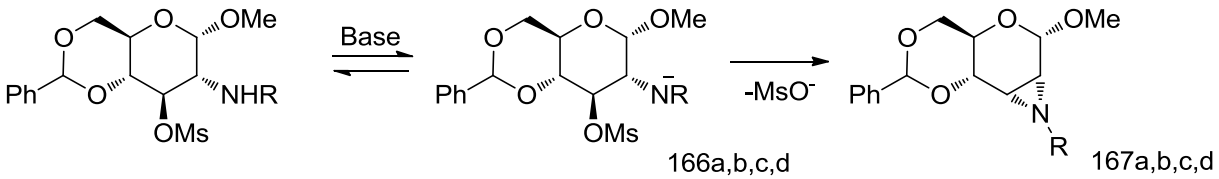
3.3.1.1 Trans – configuration

The syntheses of 2,3–*trans* dinitrogen-containing D-hexopyranosides are based on a) aziridine formation and subsequent ring-opening reaction, b) addition on the activated double bond, and c) S_N2 substitution of an activated hydroxyl group.

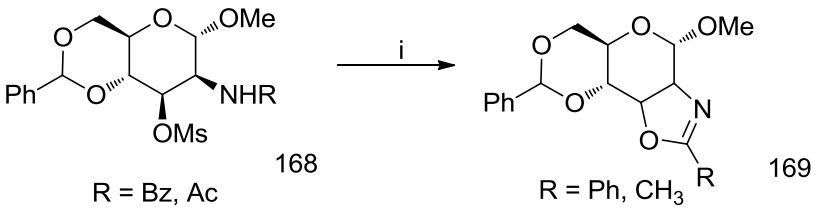
3.3.1.1.1 Via aziridine formation

In 1965, Richardson and coworkers described the formation of aziridine **167** with *allo*-configuration from substituted glucopyranoside **165**. (Scheme 51) The crucial condition of aziridine formation is *trans-diaxial* configuration on **165**. When *cis*-configuration (for example in mannopyranoside) occurs, the reaction usually results in hexopyranoside with oxazoline ring.[99] (Scheme 52) The mechanism of a base-catalyzed aziridine formation requires strong nucleophilic anion of the acylamido group at C2 carbon. The corresponding anion attacks intramolecularly C3 carbon with activated hydroxy group to yielddesired aziridines **167 a,b**. There are plenty of suitable bases; the method of choice predominantly depends on the *N*-substitution.

Scheme 51 - Formation of aziridine with *allo*-configuration



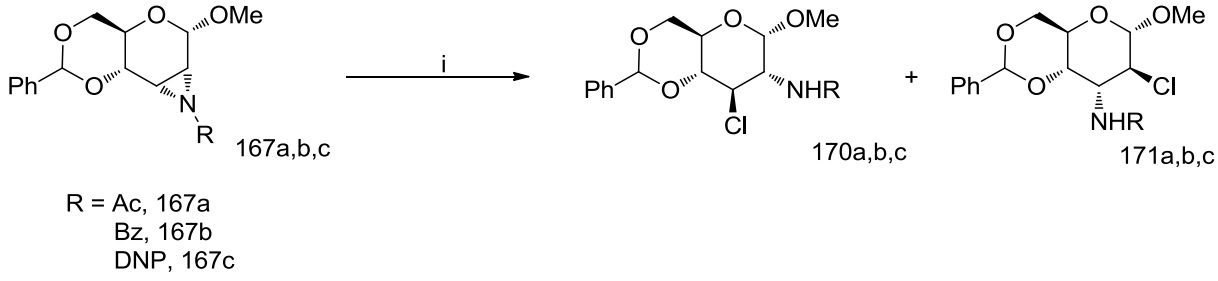
Scheme 52 - Formation of oxazoline ring



Conditions: (i) base

This paper isnpired other research groups. Later, Richardson[105] extended the substitution at C2–amine to anisoyl, dinitrophenyl (DNP), and mesyl group with desired activating effect and described the possibilities for the ring opening reactions. It was find out, that the product of the ring opening reaction strongly depends on the amine substitution at C2 carbon and nucleophile. As it was mentioned above (Scheme 51), the stronger anion is the easier is aziridine formed. The weaker anion cannot accomplish the cleavage of *O*-mesyl group and oxazoline **169** as a major side-product was easily obtained. The same publication describes the ring opening reactions of acetylated, benzoylated or DNP substituted aziridines **167** with ammonium chloride. At the very beginning, the ring-opening rections were accomplished with halogen nucleophiles. When **167a-c** is refluxed in DMF, the aziridine undergo *trans-diaxial* and *trans-dieqatorial* ring opening resulting in *altro-* and *gluco-* product.

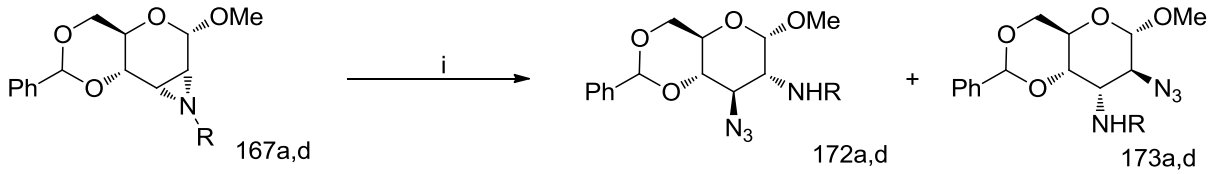
Scheme 53 - Opening of *allo*-aziridine with ammonium chloride



Conditions: (i) NH_4Cl, DMF, reflux

Treatment of **167a** with ammonium chloride provided almost exclusively **170a**, while **167b** and **167c** give the mixture of **170b,c** and **171b,c** respectively. However, when sodium azide as a stronger nucleophile is added to the mixture of DMF and NH_4Cl, the formation of chloro-derivates is suppressed and azido derivates **172** and **173** are formed.

Scheme 54 - Opening of *allo*-aziridine with sodium azide in the presence of ammonium chloride

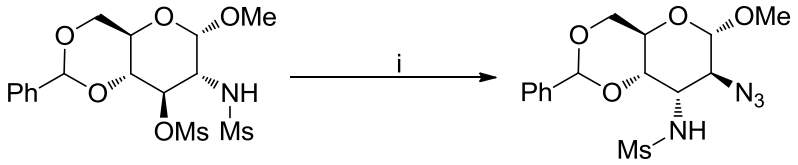


R = Ac, 167a
Ms, 167d

Conditions: (i) NaN_3, NH_4Cl, DMF, reflux

The both **167a** and **167d** in reaction with ammonium chloride – sodium azide mixture provided unsatisfactory yields due to the formation of side products or degradation of a starting compound, therefore the reaction of **165d** was performed with sodium azide. (Scheme 55) The absence of NH_4Cl in the reaction mixture allowed better yield of **173d**.

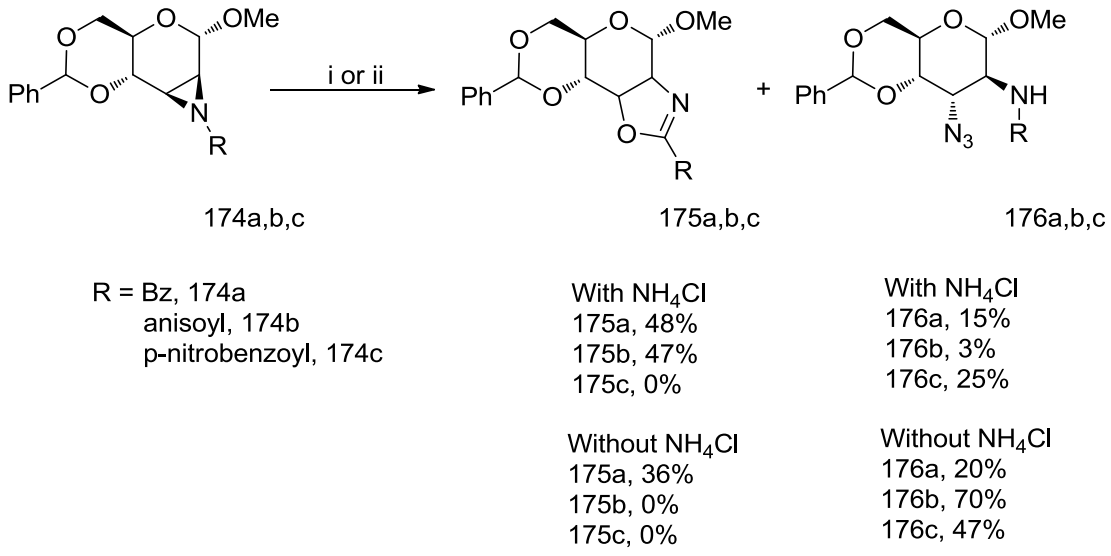
Scheme 55 - *In situ* ringopening reaction of aziridine with sodium azide



Conditions: (i) NaN_3, DMF, reflux, 65%

The reaction includes aziridine formation in situ and the *trans-diaxial* ring opening reaction resulting in **173d** with the *altro*-configuration exclusively. Together with Richardson and coworkers, the Guthrie group[106,107] investigated ring opening reactions of *manno*-aziridine (Scheme 56). It was find out, that the reaction of **174b** with NaN_3 provides the highest yields, probably due to the nucleophility of the formed anion. The similar conclusion was achieved also by zu Reckendorf,[108,109,110] where the formation of an additional *gluco*-derivate was described.

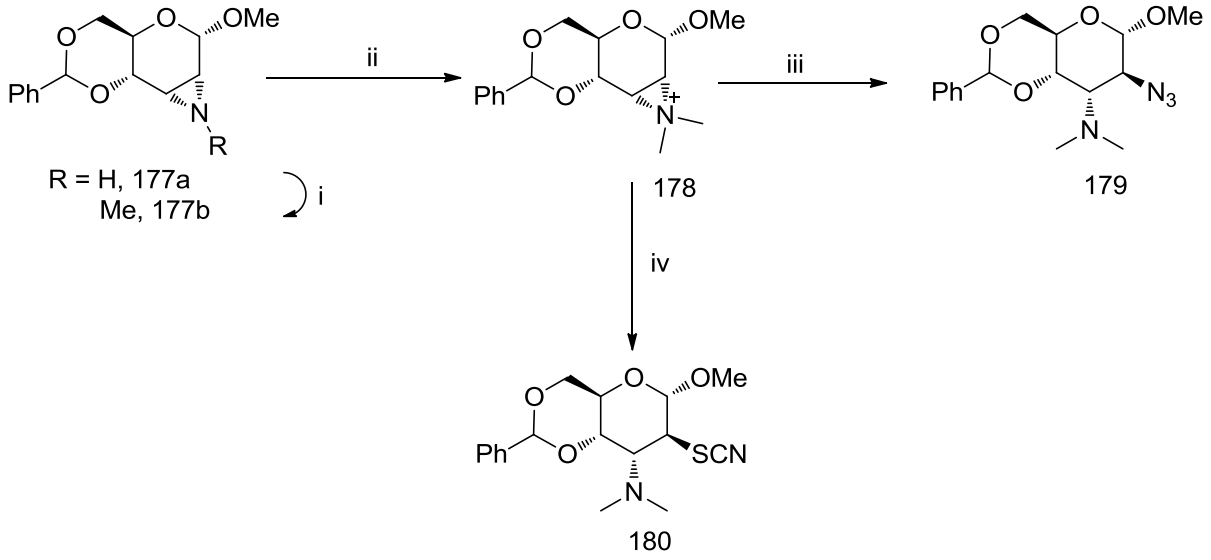
Scheme 56 – Ring-opening reaction of *manno*-aziridine with sodium azide in or without presence of ammonium chloride



Conditions: (i) NaN_3, DMF, reflux; (ii) NaN_3, NH_4Cl, DMF, reflux

It is also necessary to mention, that the synthesis of 2,3–*trans*-diamino carbohydrates can be accomplished also via aziridinium salts (Scheme 57).[111]

Scheme 57 – Ring-opening reaction of *allo*-aziridinium salt with sodium azide and potassium thiocyanate

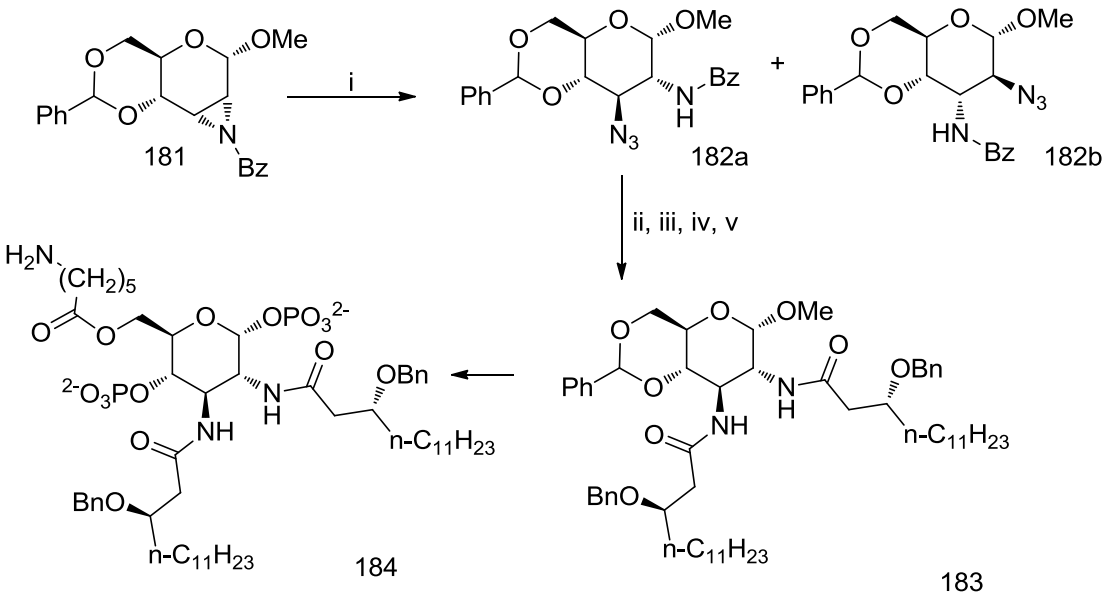


Conditions: (i) MeI, Ag_2CO_3, MeOH, 62%; (ii) MeI, silver pycrilsulphonate, CAN, 66% (iii) NaN_3, DMF, 22%; (iv) KSCN, DMF, 57%

Hydrolysis of **178** with sodium azide and potassium thiocyanate gave **179** and **180** with *altro*-configuration exclusively.

The works of Richardson, Guthrie, and zu Reckendorf laid the foundations for further applications of the ring-opening reactions. Some of them will be illustrated below. Charon and col. used aziridine ring-opening reaction in the synthesis of glycophospholipid ligands of lipopolysaccharide receptor **184**.[102] It was observed, that the ring-opening reaction of **181** give *gluco*- **182a** as a minor and *altro*- **182b** as a major product.

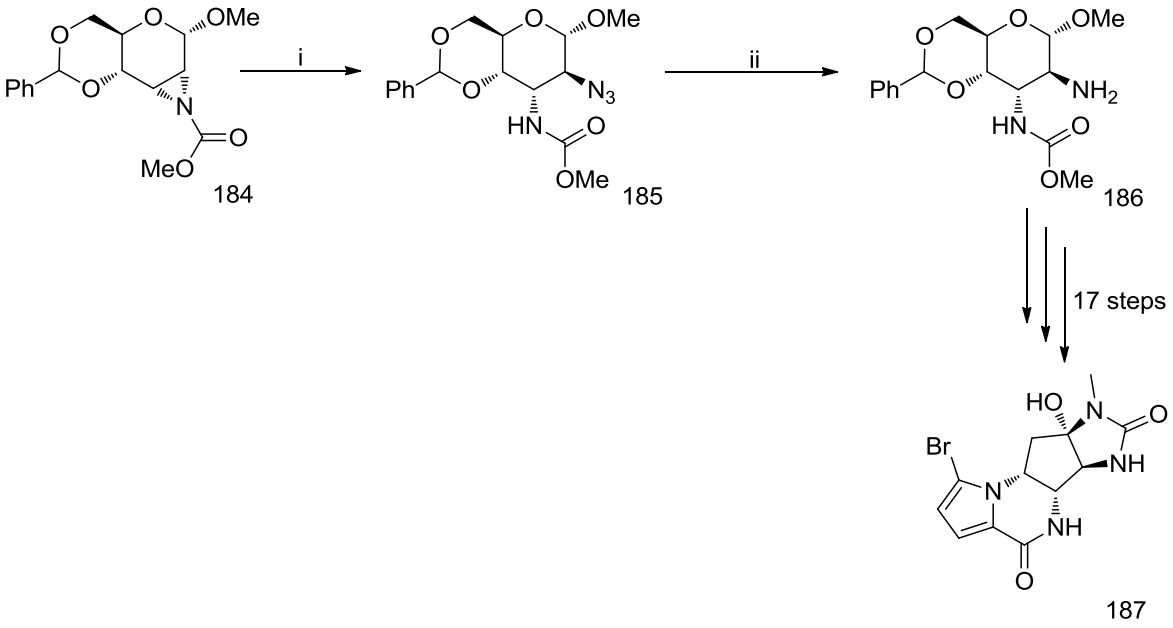
Scheme 58 - Synthesis of glycophospholipid ligands **184**



Conditions: (i) NaN_3, NH_4Cl, DMF, 25% for **182a**, 70% for **182b**; (ii) H_2, Pd/C, MeOH, (iii) AcOH, (iv) 5 N HCl, 68% (three steps); (v) *(D)*-3-OBn-Myr-O-$C_4H_2NO_2$, 43%

Hale et al used the aziridine-opening reaction in the synthesis of Agelastatin A **187**, which evince GSK-3*β* inhibition (Scheme 59).[101] Aziridine **184** was treated with sodium azide in the presence of ammonium chloride resulting in **185** as a major product. No minor product was separated. Azide **185** was subsequently reduced by $H_2/Pd(OH)_2$ giving diamine derivate **186** in very good yield. Desired Agelastatin A **187** was achieved in complex seventeen steps synthesis.

Scheme 59 - Synthesis of precursor of Agelastatin A

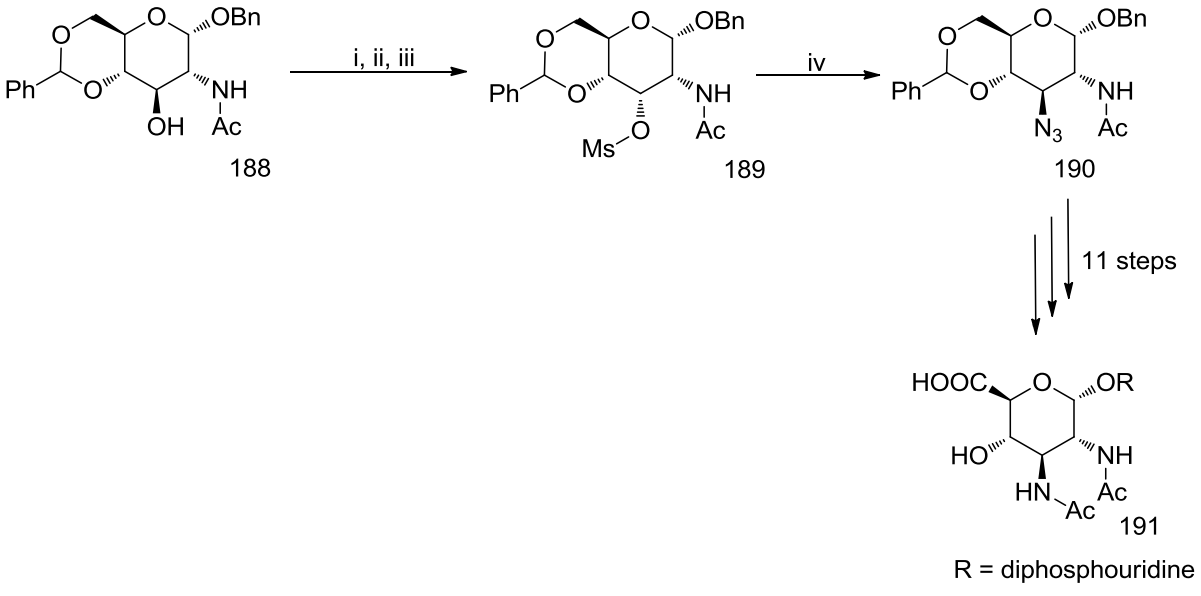


Conditions: (i) NaN_3, NH_4Cl, DMF, 88%; (ii) H_2, $Pd(OH)_2$, MeOH, 95%

3.3.1.1.2 Via substitution of activated hydroxy group

Nucleophilic substitution belongs to the traditional synthetic transformations. The activation can be reached by a reaction with mesyl, tosyl or triflate agents. The main advantage is the inversion of configuration at the carbon carrying OH group. Rejzek et al. prepared phospho- derivated glucuronic acid **191** using double inversion at C3 carbon. [112]

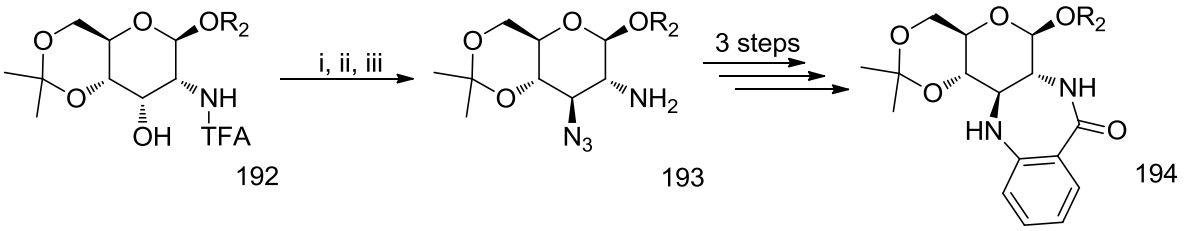
Scheme 60 - C3 double inversion in the synthesis of derivate of glucuronic acid



Conditions: (i) MsCl, pyridine; (ii) NaOAc; (iii) MsCl, pyridine; (iv) NaN_3, TBAHS, DMF, 73% (overall yield)

The similar approach was used in the synthesis of cinnamon derivates,[113] 2,3- *trans*–diamino – metal (Mn, Pt, Rh, Ru, Ir) complexes,[114-122] muramyl dipeptide analogues,[123] or ß-D-glucose benzodiazepine derivates **193**,[101] where the resulting configuration depends on the C3 hydroxy group orientation.

Scheme 61 - Formation of benzodiazepinone scaffold



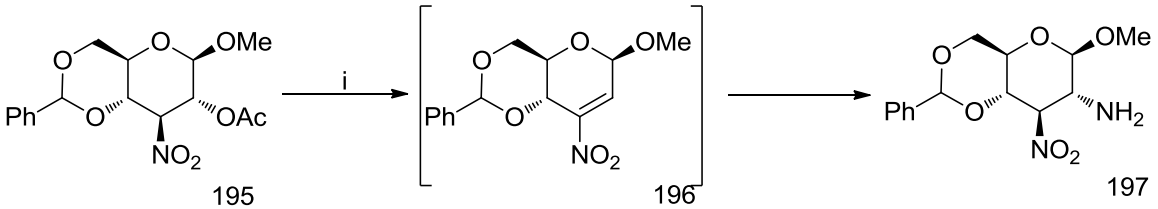
192 193 194

Conditions: (i) MsCl, TEA, DCM; (ii) NaN_3, Bu_4NHSO_4, DMF; (iii) KOH, MeOH 83% (overall yield)

3.3.1.1.3 The method utilizing addition to activated double bond - Michael Addition

The Michael addition to nitroolefines is an proven route for the synthesis of 2,3–*trans*-diamino carbohydrates. In later sixties, Professor Baer and col. published a few papers discussing the aminonitrosugar preparation. The first publication was focused on the formation of olefin followed by addition of ammonia in THF.[124] Besides the **197**, some impurity with yield less than 10% was isolated, but, not characterized.

Scheme 62 - Addition of ammonia to 3-nitroolefine



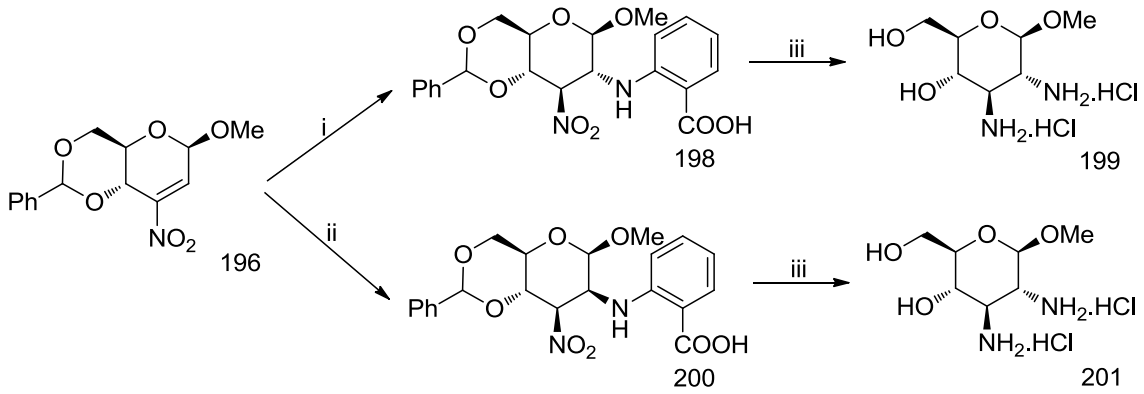
195 196 197

Conditions: (i) Ammonia, dry THF, 86%

Later, the synthesis was extended to anthranilic acid[125] and aminosugar[126] and it was described, that the configuration of diamine strongly depends on the pH of reaction (Scheme 63). When the reaction took place under basic conditions, the *gluco*- product **198**, which is

thermodynamically more stable, is almost exclusively formed. Contrary, without presence of KOH, the *manno*- product **200** can be isolated as a major. Both **198** and **200** underwent reduction in the presence of Kuhn catalyst yielding diamines **199** and **201**.

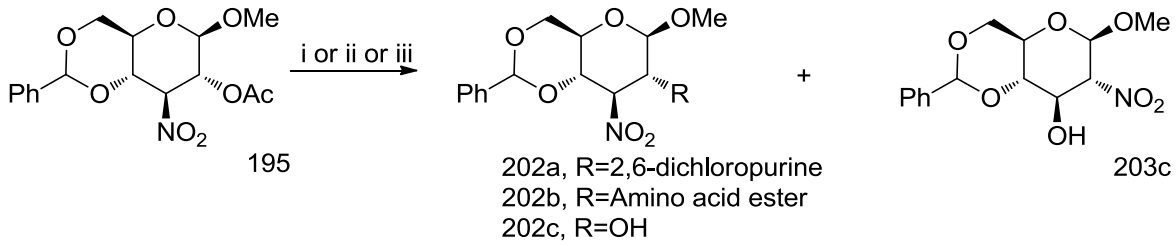
Scheme 63 - Addition of anthranilic acid to 3-nitroolefine



Conditions: (i) Anthranilic acid, KOH, dry benzene, 83%; (ii) Anthranilic acid, dry benzene, 56%; (iii) H_2, Pd, $BaSO_4$, 1 *N* HCl, 60-70%

Afterwards, papers utilizing nucleosides,[127] esters of aminoacids[128] or sodium nitrite[129] as addition agent were published. Reaction with 2,6-dichloropurine or amino acids esters (Gly, Ala, Phe, Ser, Tyr, Val) gave exclusively **202a** (C-N bond is formed beetwen C2 carbon and nitrogen in position 9) and **202b** respectively (Scheme 64). However, treating of **195** with sodium nitrite resulted in 3-nitro derivate **202c** as a major product and **203c** as a minor product. The ratio toward to **203c** can be slightly increased by addition of hexadecyltributylphosphonium bromide.

Scheme 64 - Addition of various reagents to 3-nitro derivate

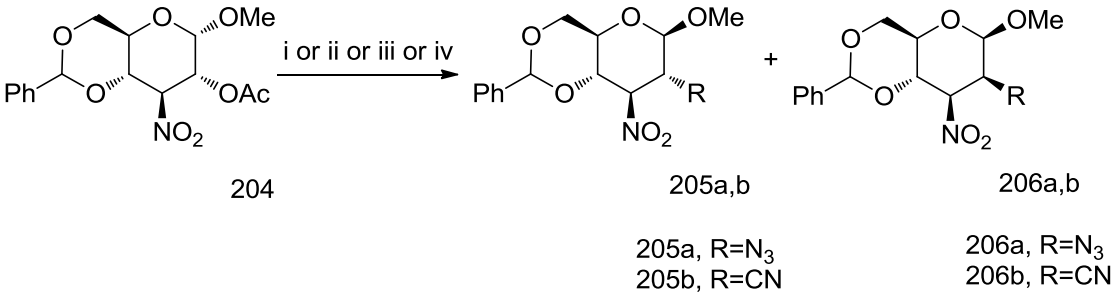


Conditions: (i) 2,6-dichloropurine, Na_2CO_3, THF, 78%; (ii) Amino acid ester, Et_3N, THF/MeOH, 73-88%; (iii) $NaNO_2$, Amberlite IRC 50, CAN/water, 70%

Sakakibara and Sudoh studied the influence of the solvent and reagent in the azide or cyanide preparation (Scheme 65).[130] When sodium azide was used, **205a** in 60% yield was isolated.

On the other hand, when hydrazoic acid in THF was added, **206a** in 79% yield was obtained. Therefore, more solvents were tried. It was find out, that solvents such as DMSO or THF in the presence of HN_3 help formation of **206a**, while chloroform or ACN affords the mixture of **205a** and **206a**. The same conclusion was achieved with HCN/KCN, where dissolving the mixture in DMSO resulted in *manno*- configuration **206b** as a major product. In other solvents (ACN), a mixture of **205b** and **206b** was achieved.

Scheme 65 - Formation of 2-azido or cyano carbohydrate



204 205a,b 206a,b

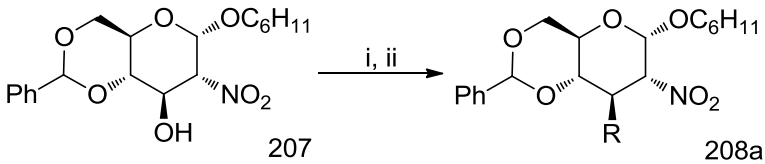
205a, R=N_3
205b, R=CN

206a, R=N_3
206b, R=CN

Conditions: (i) NaN_3 ACN/water, 60% (for **205a**); (ii) HN3, THF/water, 79% (for **206a**); (iii) HCN, KCN, ACN, 42% (for **206a**); (iv) HCN, DMSO, yield not specified

Nitro group can be localized also at C2 carbon. Vega-Peréz et al. chose the different approach, where the nitro group was placed at C2 carbon (Scheme 66). Starting compound **207** can be readily gained by oxidation of protected glucosamine with *m*-CBPA.[131] The product **208c** was further tested as a drug carrier.

Scheme 66 - Addition of primary or secondary amine to *in situ* generated 2-nitroolefine



207 208a,b,c

208a, R=t-Bu amin
208b, R=piperidine
208c, R=2-(piperazin-1-yl)ethanol

Conditions: (i) MsCl, Et_3N, DCM; (ii) amine, DCM, (two steps, 88-92%)

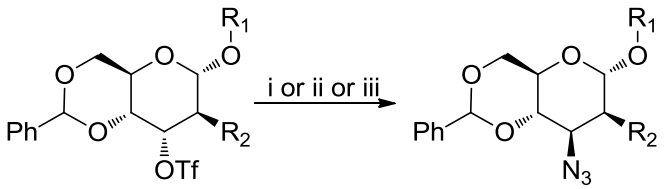
3.3.1.2 Cis–configuration

2,3–*Cis* dinitrogen configuration can be reached especially via substitution of an activated hydroxyl group with the appropriate configuration. Other synthetic methods, however not so common, are included in the subchapter miscellaneous reactions.

3.3.1.2.1 Via substitution of activated hydroxyl group

The reaction conditions of S_N2 resulting in *cis*- diamino configuration are very similar to those in chapter 3.3.1.1.2. Due to expected inversion of configuration, the hydroxy group in the starting compound must be in the *trans*- position to the amine function. Walvoort et al. used altropyranosid **209a** in the mannopyranoside uronates synthesis. The azides in **210a** were reduced at the end of the synthetic route resulting in the more complex carbohydrate skelet (Scheme 67).[132] Baer et al. prepared disaccharose of the trehalose type **210b**[133] and, finally, **210c** was synthetized as a substrate for *N*-acetylneuraminic acid aldolase .[134]

Scheme 67 - S_N2 of activated hydroxy group resulting in the mannoside derivates



209a,b,c

209a, R_1=Me, R_2=N_3
209b, R_1=sugar derivate, R_2=N_3
209c, R_1=Me, R_2=NHBoc

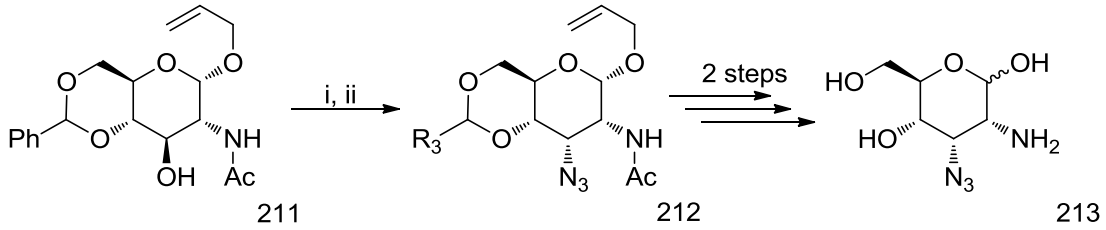
210a,b,c

210a, R_1=Me, R_2=N_3
210b, R_1=sugar derivate, R_2=N_3
210b, R_1=Me, R_2=NHBoc

Conditions: (i) NaN_3, NH_4Cl, DMF, 75%; (ii) tetramethylguanidinium azide, DCM, 68%; (iii) *n*-Bu_4NN_3, pyridine/DCM, 70%

Contrary, Posakony et al. prepared allopyranoside carbohydrate **212** from **211**, where the final product **213** can serve as catalytic cofactor analogues for glmS Rybozime (Scheme 68).[135] The desired change in the configuration was achieved by the reaction of sodium azide with the mesylated hydroxy group.

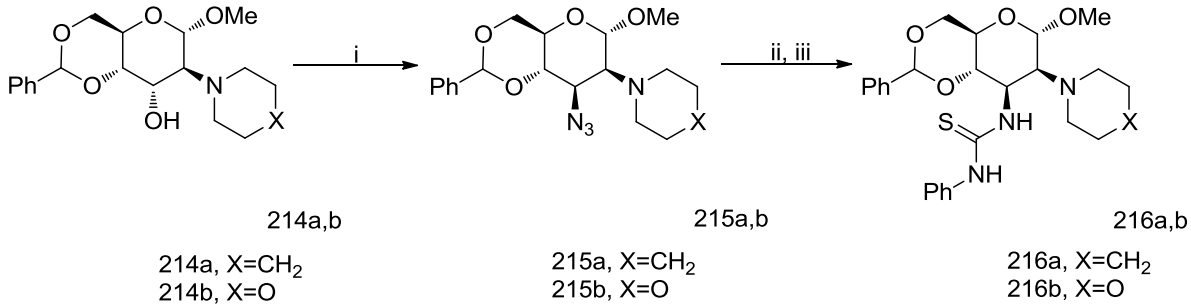
Scheme 68 - Synthesis of catalytic cofactor analogues for glmS Rybozime



Conditions: (i) Ms_2O, pyridine; (ii) NaN_3, DMF, 52% (two steps)

Alternatively the Mitsunobu reaction can be used instead of the classical S_N2 substitution of the activated hydroxy group. This powerful synthetic tool was used in the synthesis of carbohydrate organocatalysts, where the C3 hydroxy group was azidated with DPPA under Mitsunobu conditions.[136]

Scheme 69 - Using of Mitsunobu reaction in the synthesis of new organocatalysts

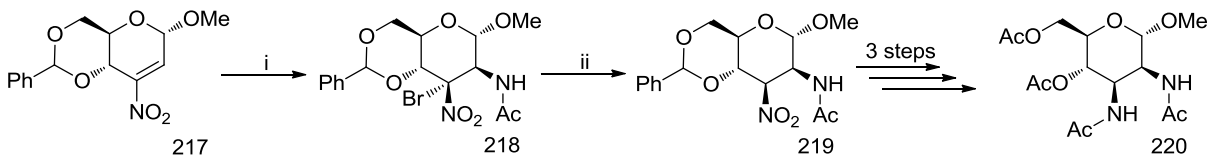


Conditions: (i) DPPA, TPP, DIAD, THF, 60-80%; (ii) TPP, THF/water; (iii) Phenylisothiocyanate, MeOH, 80% (two steps)

3.3.1.2.2 Miscellaneous methods

Besides the S_N2 reactions, the *cis*-configuration can be achieved by other reactions. For example, Rank synthetized **220**, where the key step was the addition of *N*-bromoacetamide to 3-nitroolefin **217** (Scheme 70).[137] Hydrolysis of **218** gave nitro derivate **219**, which was in three step synthesis converted to diacetylated mannopyranoside **220**. The similar results were obtained, when talopyranoside was used.

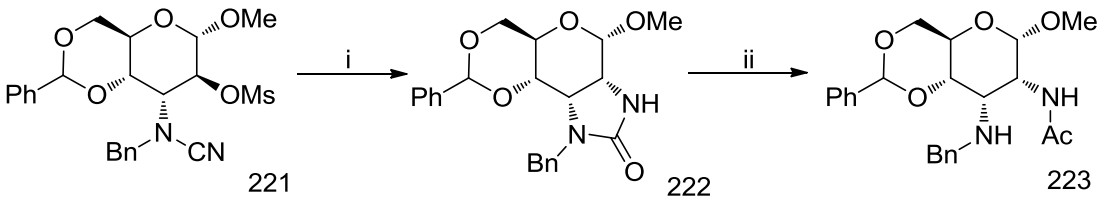
Scheme 70 - Addition of *N*-bromoacetamide



Conditions: (i) *N*-bromoacetamide, NaOAc, acetone, 76%; (ii) $NaBH_4$, EtOH, 91%

Another alternative method includes the formation of imidazoline ring and its subsequent basic hydrolysis (Scheme 71). Baker et al. published few papers describing the preparation of 2,3–diamino allopyranosides from the corresponding imidazolines, where also phenyl instead of benzyl can be attached to the imidazoline nitrogen.[138-141] The main disadvantage of these methods is long reaction times (up to 5 days) in each step.

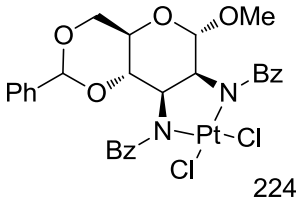
Scheme 71 - Synthesis of allopyranoside



Conditions: (i) NH_3/EtOH, 72%; (ii) KOH, Glacial CH_3COOH, Ac_2O, 34%

Later, the same author suggested the use of tosyl instead of benzyl group, but the long reaction times and low yields unfortunately remained. Recently, *allo*- or *manno*-pyranosides found an application in the chemistry of complexes, where the carbohydrate is employed as a ligand **224** and such molecules evince antitumor activity comparable with *cis*-platina.[121]

Figure 10 - Structure of Platinum complex



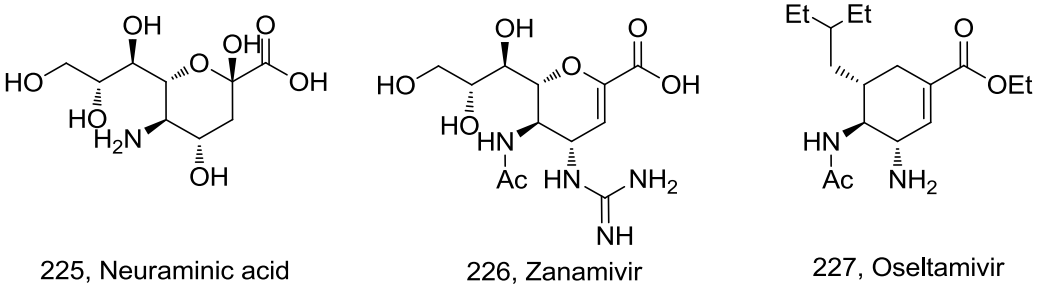
3.3.2 3,4 – dinitrogen-containing D-hexopyranosides

The first part is focused on the synthesis of the t*rans*-diaminohexopyranoses, which are incorporated mainly in the skeleton of zanamivir and its analogs. *Cis*-dinitrogen-containing D-hexopyranoses, particularly derivates of Neomycin, Kanamycin and related compounds are discussed in the second section. The last part consists of the reactions leading to *cis*- and *trans*- product, where the configuration depends on reaction conditions.

3.3.2.1 Trans-configuration

The *Trans*-3,4–diamino configuration is introduced particularly in the compounds derived from Neuraminic acid **225**, however, a few papers describing the trans configuration can be found in different structures. Some neuraminidase inhibitors, such as Zanamivir **226** or Oseltamivir **227** are commercially available. These derivates exhibit antiviral properties; therefore their substitution is a topic of many studies. There are a few synthetic approaches leading to the *trans*- 3,4–diamino configuration: a) oxazoline ring formation and opening reaction, b) via cyclization of vicinal dinitrogen-containing intermediates, c) Michael addition.

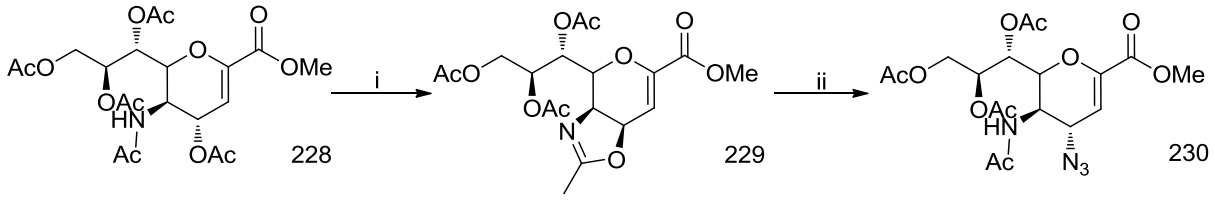
Figure 11 - Structure of neuraminic acid and inhibitors based on **225**



3.3.2.1.1 Via oxazoline ring

The formation and the ring-opening reaction of oxazoline is a proven route for the synthesis of the *trans*-3,4–diamino carbohydrates. Von Itzstein et al. prepared oxazoline **229** from *O*-acetylated derivate **228** (Scheme 72).[149] It was found, that the oxazoline ring is vulnerable against nucleophilic attack at C-O bond. Treating of **229** with lithium azide resulted in **230**.

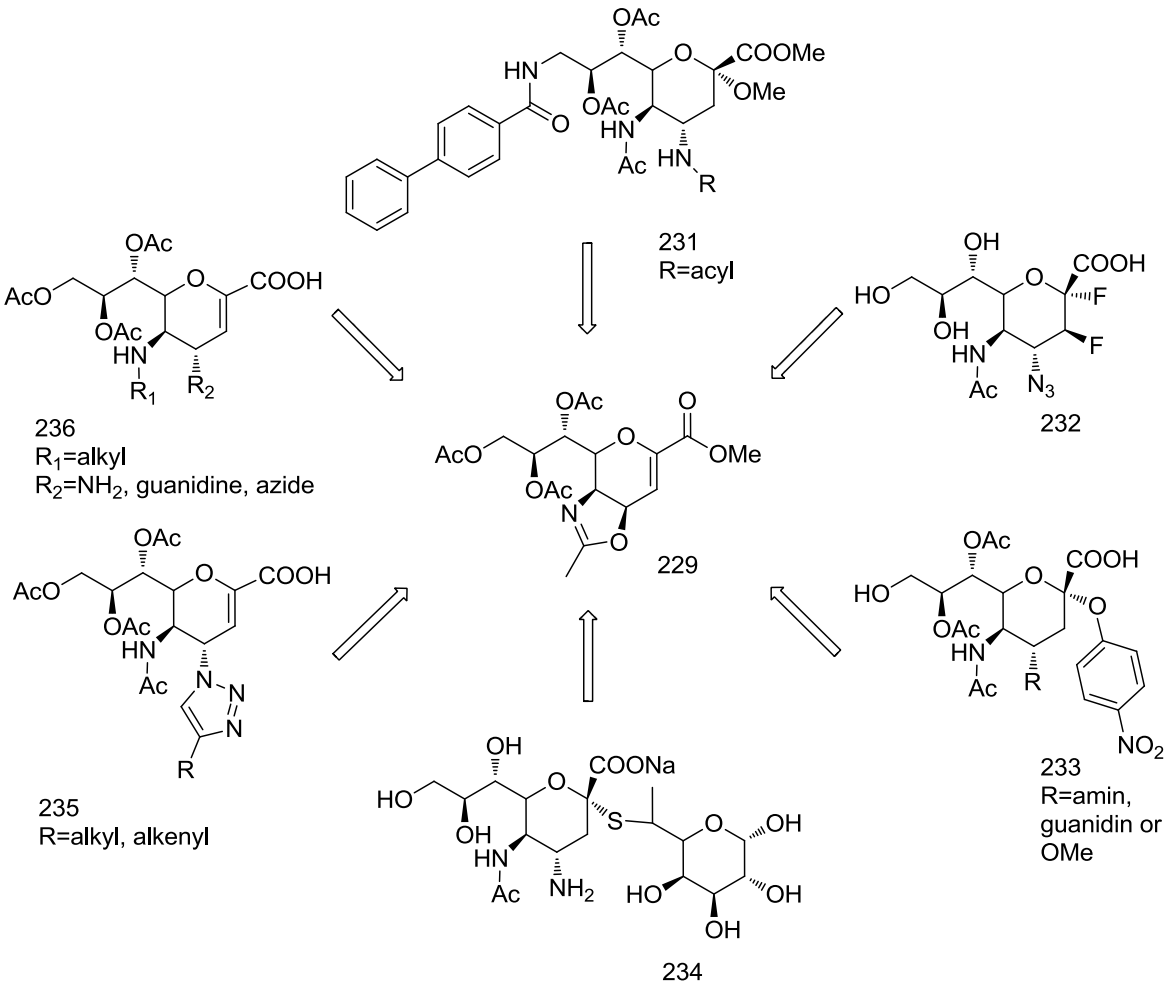
Scheme 72 - Formation and the subsequent ring-opening reaction of oxazoline ring



Conditions: (i) $BF_3.Et_2O$, DCM, MeOH, 99%; (ii) LiN_3, Dowex 50W, DMF, 96%

This method, based on opening of oxazoline with azidation reagent, was employed in the synthesis of many derivates. The substitution of deprotected primary hydroxyl group, followed by oxazoline ring opening with $TMSiN_3$ and subsequent azide reduction gave carbohydrate **231** (Figure 12).[150] Other examples differ in the further derivatization. The substitution at C3 and C4 can afford more complex nitrogen containing derivates. Click reaction of azide with different acetylens gave triazole derivate **235**. Moreover, the azide can be reduced and converted to guanidine **236**.[151-154] The substitution at the anomeric hydroxy group afforded product **233**. There is also a possibility to connect the second sugar unit via thioether bond resulting in **234**[155] or it was described a protocol for preparation of fluoroderivate **232**.[156]

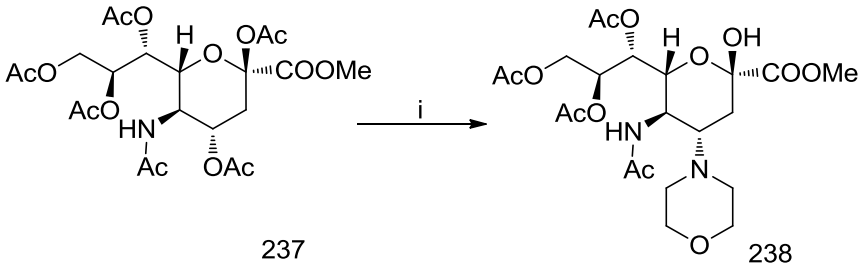
Figure 12 - Synthesis of Zanamivir derivatives via formation of the oxazoline ring and further modifications



Beside mostly used azide reagent, the oxazoline ring can be opened by other nucleophiles. Ye et al. published a protocol for introducing of the morpholine moiety

resulting in derivate **238**.[163,164] The mechanism of conversion includes *in-situ* formation of oxazoline ring, which undergoes immediately to nucleophilic attack of morpholine. The acetoxy group at position 2 is also participating in the ring-opening reaction and undergoes selective deacetylation.

Scheme 73 - Substitution of -OAc with morpholine via oxazoline intermediate

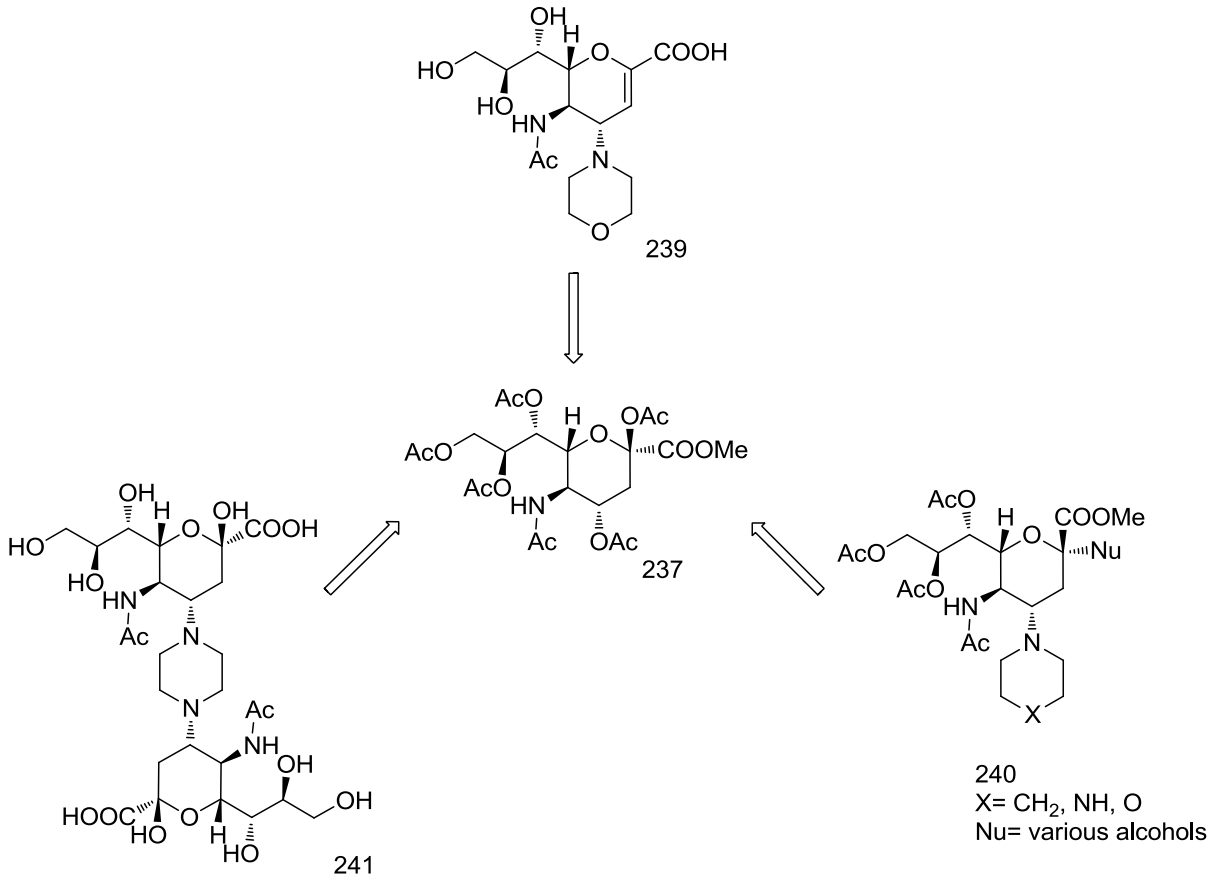


237 238

Conditions: (i) Morpholine, pyridine, 82%

Later, this method was used in the synthesis of different zanamivir derivates. Rota et al. (Figure 13) prepared zanamivir derivate **239** in the four step synthesis.[165] Further substitution can be done at the anomeric carbon of **240**,[166] where various alcohols were employed as nucleophiles. The synthesis of **241** was accomplished via the double ring-opening reactions resulting in the connection of two carbohydrate units trough the piperazine linker.[167] The linker can be prolonged with further substitution on piperazine.

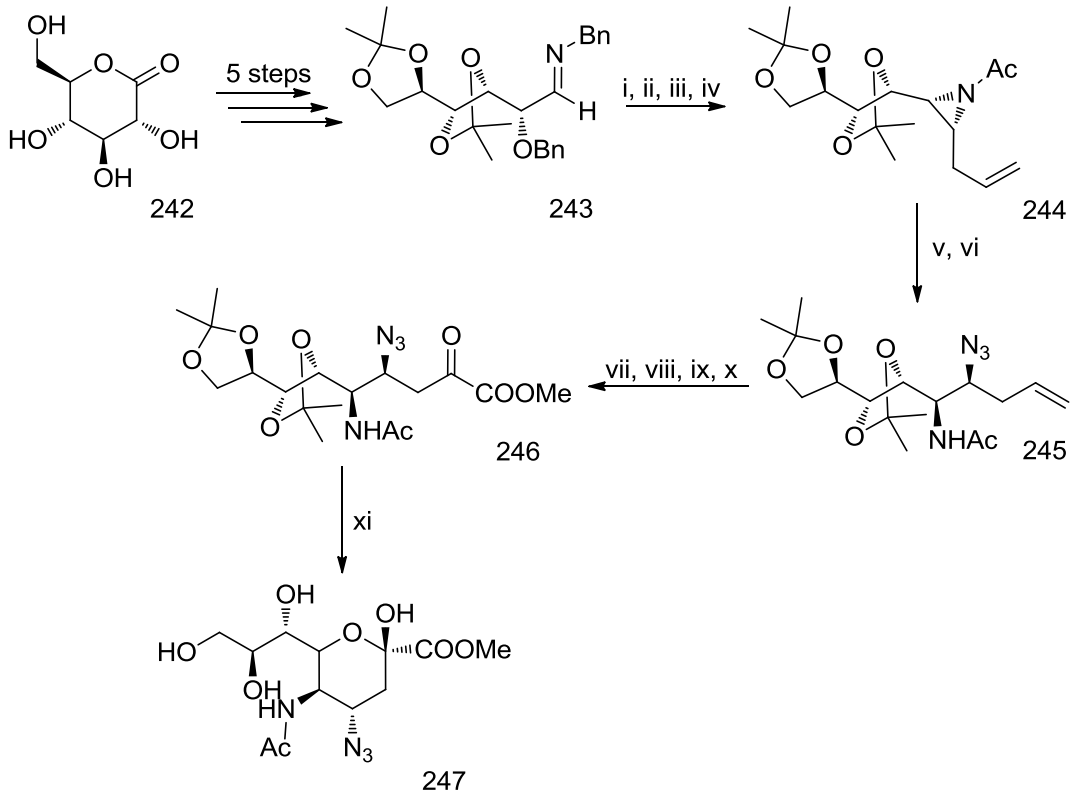
Figure 13 - Synthesis of various derivates via oxazoline ring-opening reaction



3.3.2.1.2 Via cyclization of vicinal dinitrogen-containing intermediates

The alternative to the oxazoline ring-opening reaction can be found in the 1,6-cyclization of **246** (Scheme 74). The reaction sequence starts from lactone **242**, where the synthesis follows the five step procedure resulting in imine **243**.[157] The imine **243** is then converted into aziridine **244** in the four steps synthesis.[158] The formation of 3,4–diamino intermediate **245** is reached via the aziridine ring-opening reaction and the subsequent acylation. After oxidation and *in-situ* acetonide removing, the cyclization gives **247**. Other methods differ in the way of preparation intermediate **246**.

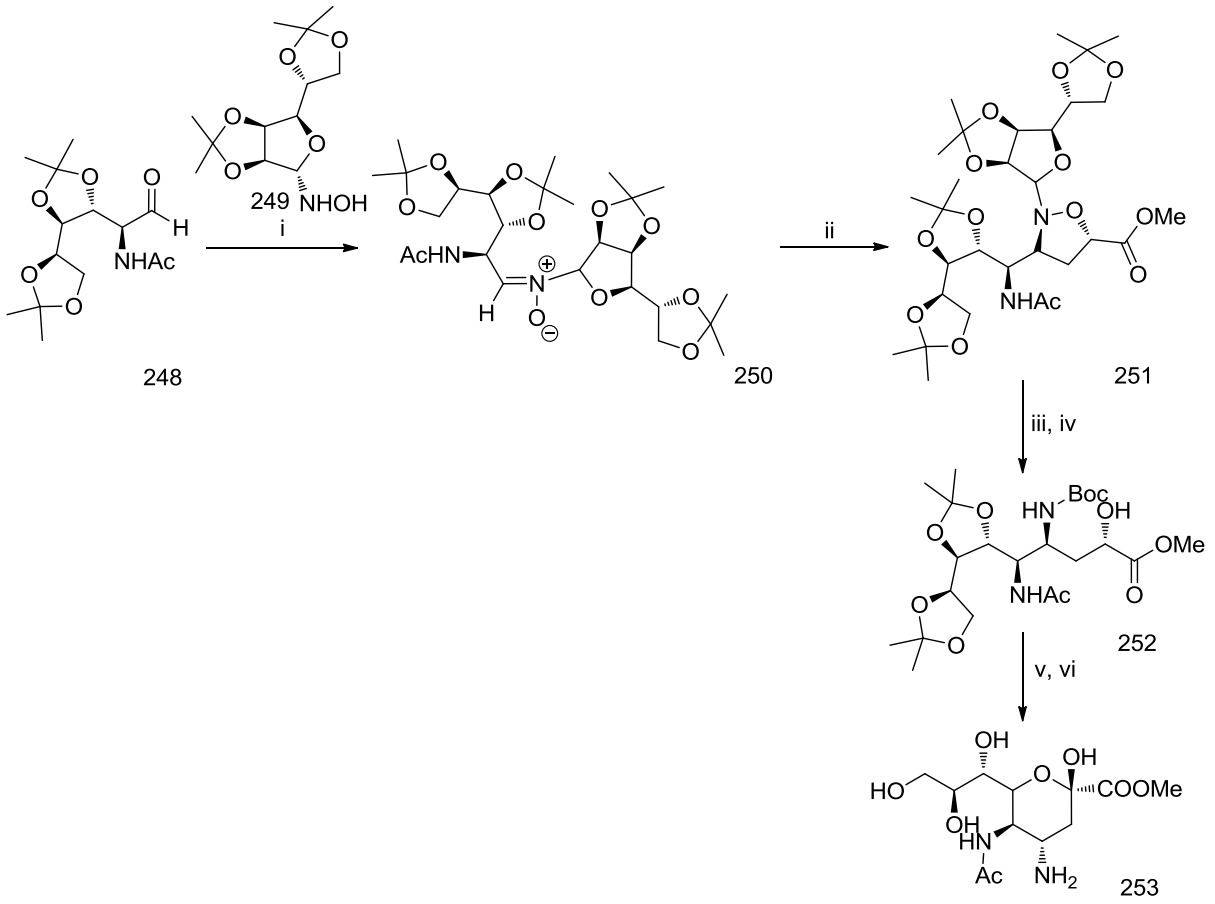
Scheme 74 - Synthesis of reactive intermediate **246**



Conditions: (i) C_3H_5MgBr, Et_2O, 56%; (ii) Ac_2O, Et_3N, DCM, 88%; (iii) Li, NH_3, THF, 82%; (iv) NaH, THF, 87%; (v) NaN_3, NH_4Cl, EtOH/water, 62%; (vi) Ac_2O, Et_3N, DMAP, DCM, 88%; (vii) OsO_4, NMO, acetone/water, 96%; (viii) KBr, TEMPO, TBAB, $Ca(ClO)_2$, DMF; (ix) MeI, K_2CO_3, DMF, 80%, (2 steps); (x) DMP, DCM; (xi) 40%HF in MeCN 52% (2steps)

Yao et al. prepared air-stable nitrone **250** by addition of hydroxylamine derivate **249** to aldehyde **248** (Scheme 75).[159,160] Heating of **250** with methyl acrylate gave isoxazolidine **251**, which was hydrolyzed and subsequently the N-O bond was cleaved to give alcohol **252**. Dess-Martin oxidation afforded the keto intermediate, which was cyclized under acidic conditions into **253**.

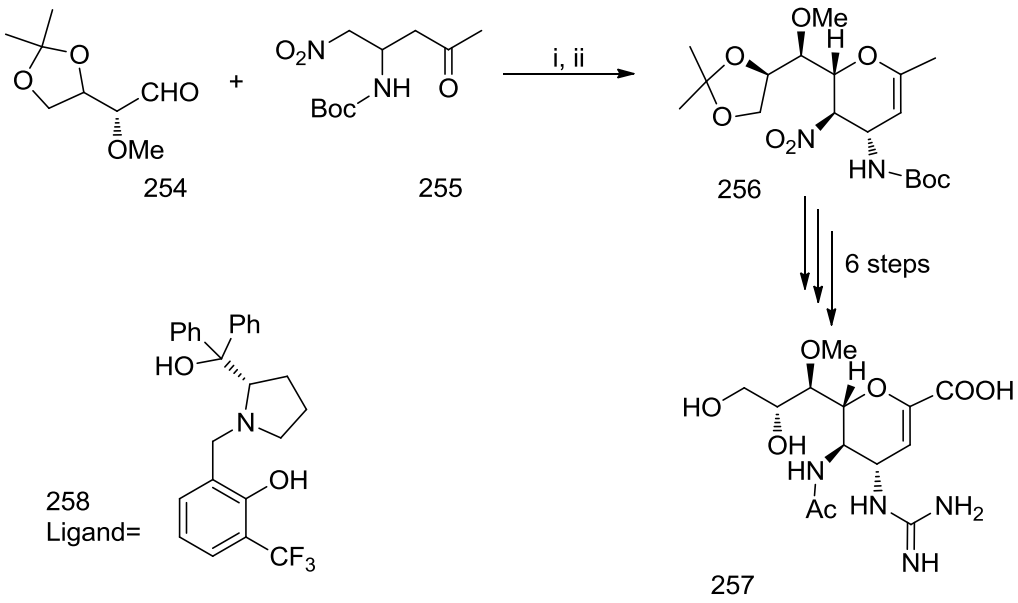
Scheme 75 - Synthesis of reactive intermediate derived from **246** via isoxazolidine



Conditions: (i) $MgSO_4$, DCM, 53%; (ii) Methyl Acrylate, toluene, 90%; (iii) NH_2OH, NaOAc, MeOH/water, 59%; (iv) $Pd(OH)_2$, then Boc_2O, 100%, (v) DMP, DCM; (vi) 4 N HCl, THF, then Et_3N, 70% (2 steps)

Another way to construct a reactive intermediate suitable for the ring closure includes Henry reaction (Scheme 76).[161] *Anti*-selective Henry reaction in the presence of ligand **258** gave cyclic intermediate, which was dehydrated by thionyl chloride to give **256**. Nitro derivate **255** was used as a starting material in the six step synthesis resulting in Zanamivir **257**. This synthesis can be carried out at a large scale.

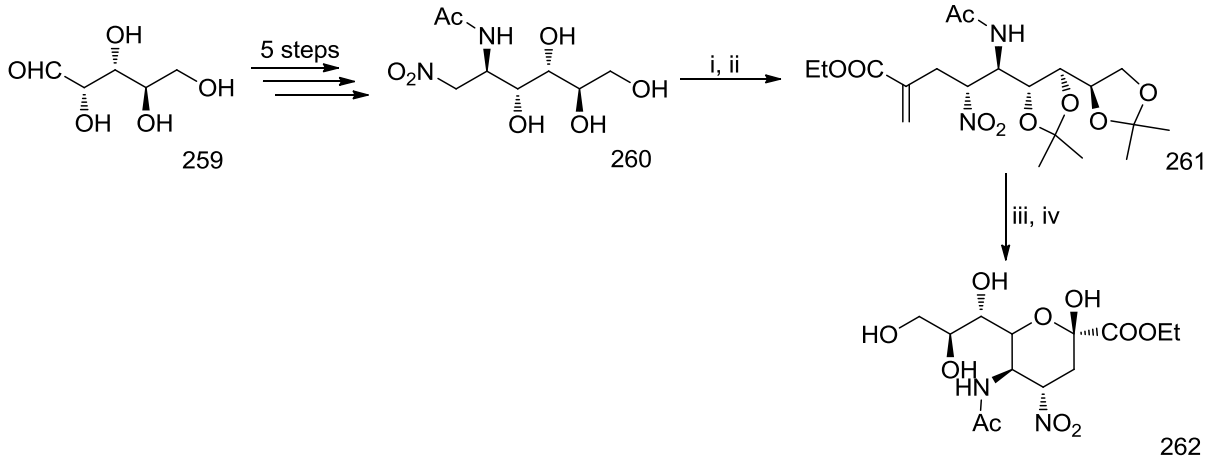
Scheme 76 - Synthesis of zanamivir **257** via reactive intermediate **256**



Conditions: (i) $CuBr_2$, ligand, Cs_2CO_3, THF; (ii) $SOCl_2$, pyridine/DCM, 76% (2 steps)

The last illustration of cyclization is based on alkylation (Scheme 77).[162] The mannitol derivate **260** was prepared from arabinose **259** in five step synthesis. Subsequent protection and alkylation with ethyl α-(bromomethyl)acrylate resulted in acyclic intermediate **261**, which after ozonolysis and subsequent reductive deprotection gave product **262**.

Scheme 77 – Synthesis of **262** via reactive intermediate **261** from arabinose **259**

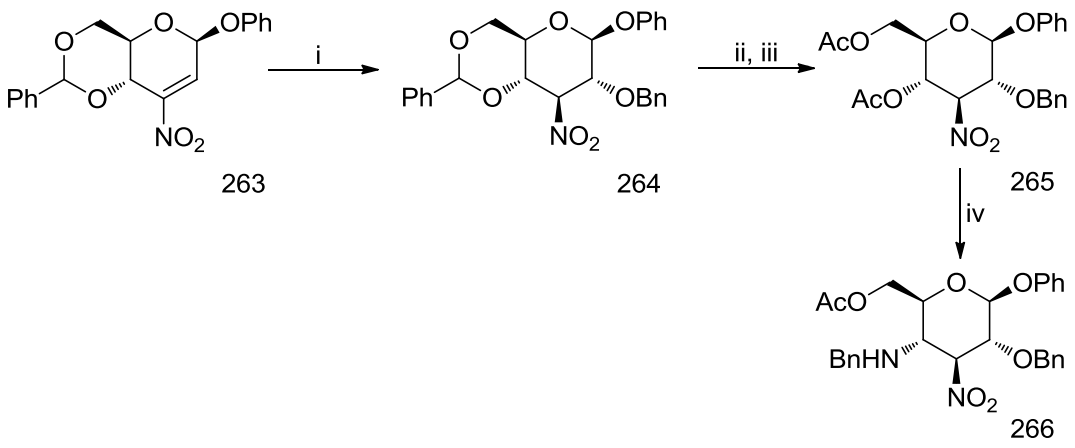


Conditions: (i) Acetone, $CuSO_4$, H_2SO_4, 74%; (ii) ethyl a-(bromomethyl)acrylate, NaOH, water, 81%; (iii) O_3, MeOH/DCM; (iv) dimethylsulfide, (55%, 2 steps)

3.3.2.1.3 Via Michael addition

Beside above described methods, it was also developed a synthesis describing introduction of primary amine into the molecule of D-glucosamine derivate.[168] The key step of synthesis of **266** (Scheme 78) involves cleavage of acetic acid followed by Michael addition of benzylamine.

Scheme 78 - Substitution of –OAc with primary amine

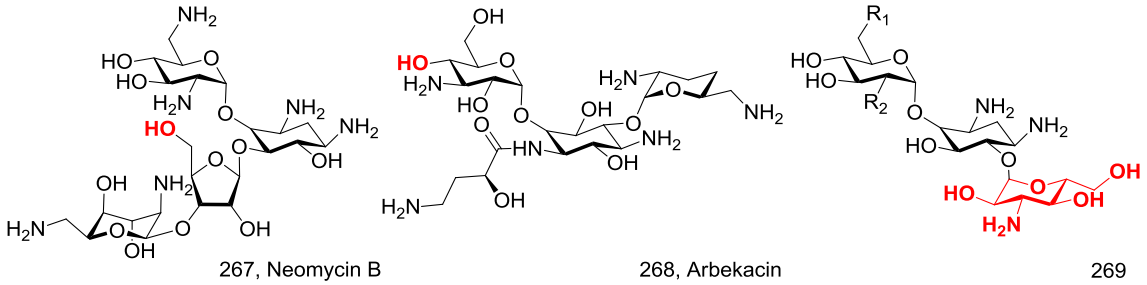


Conditions: (i) BnOH, Et_3N, toluene, 64%; (ii) 90%AcOH, 60%; (iii) Ac_2O, Pyridine, 72%; (iv) $BnNH_2$, THF, 77%

3.3.2.2 Cis-configuration

The cis configuration can be prepared mostly by S_N2 reaction. It was used primarily in the derivates of glycoside antibiotics. Arbekacin, Kanamycin B or Neomycin B can be mentioned as the most important. The biological properties of these compounds are well known. For example, Arbekacin evinces potent inhibition of methicillin resistant Staphylococcus aureus,[142] Kanamycin shows activity against gram-negative bacteria E. coli, and Klebsiella pneumonia[143] and Neomycin B can be used as a cure for liver encephalopathy.[144] In the case of Neomycin B, the derivatization was carried out at the furanose hydroxyl group (red highlighted), where the hydroxyl was alkylated with appropriate hexapyranoside. Arbekacin underwent substitution on the ring I hydroxyl group and, finally, the aza analog of Kanamycin B was prepared by *O*-glycosylation with 3,4-dinitrogen-containing carbohydrate providing the third sugar unit (red highlighted) .

Figure 14 - Structure of Neomycin B, Arbekacin, and Kanamycines



267, Neomycin B

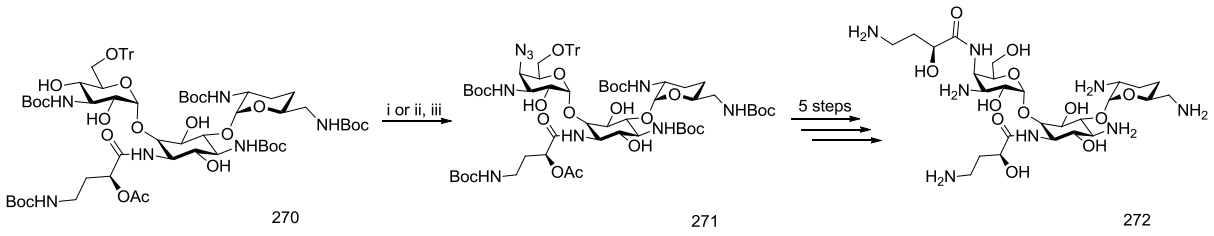
268, Arbekacin

269

269a, R_1=NH_2, R_2=OH Kanamycin A
269b, R_1=NH_2, R_2=NH_2 Kanamycin B
269c, R_1=OH, R_2=NH_2 Kanamycin C

Bimolecular nucleophilic substitution mechanism in the transition state belongs to the traditional ways of substitution of hydroxy group to amine function. Sasaki et al. activated the hydroxy group via mesylation and following substitution with sodium azide resulted in **271**, which was converted in the five steps to final product **272** (Scheme 79).[142] The biological activity of **272** was evaluated, however, the lower activity in comparison to Arbekacin **268** was observed. This paper just confirmed the results of Hiariwa et al., where the substitution of the hydroxy group with the nitrogen-contaning functionality resulted in a derivative with decreased biological activity.[145]

Scheme 79 - Activation and subsequent substitution in Arbekacin



270

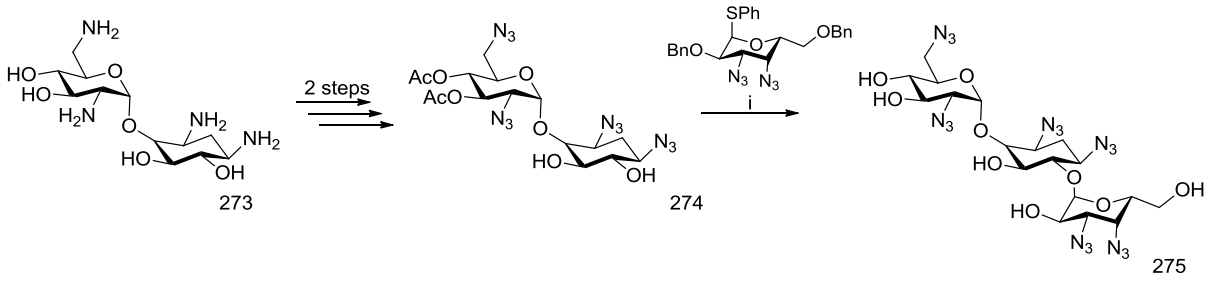
271

272

Conditions: (i) MsCl, pyridine, (ii) Tf_2O, (iii) NaN_3, 74% (two steps)

Alternative strategy using *O*-glycosylation can be used for introduction of the *cis*-configuration. Aza analog **275** of Kanamycin B was obtained after *O*-glycosylation, the azide group reduction, and deprotection of benzyl groups. However, the biological activity of **275** exhibited worse MIC than original Kanamycin B.[146]

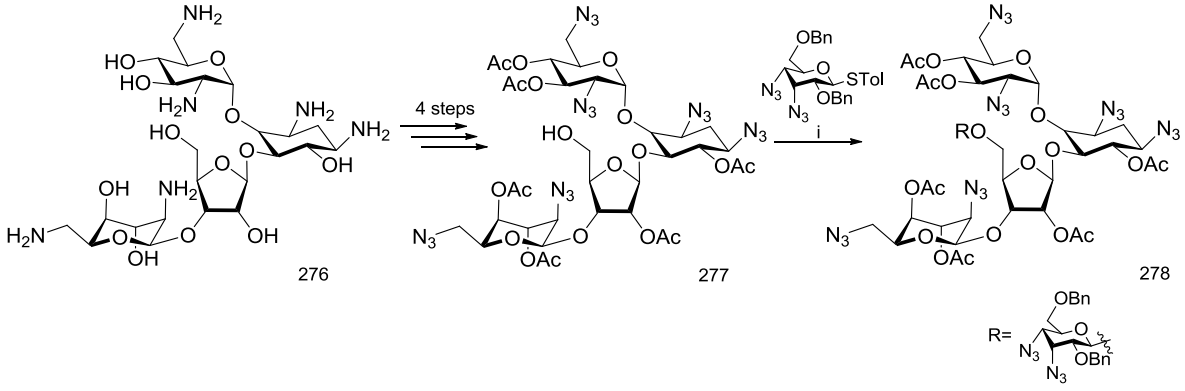
Scheme 80 - Glycosylation via anomeric thioether group resulting in Kanamycin B derivate **275**



Conditions: (i) NIS, TfOH, Et_2O/DCM, 45%

The similar approach was used in the synthesis of the Neomycin B derivates, where tolyl instead of phenyl was used (Scheme 81).[147,148] The biological testing was performed after azide reduction and deacetylation to show lower activity against bacterial Strains.

Scheme 81 - Ring connection via anomeric thioether group resulting in Neomycin B derivate

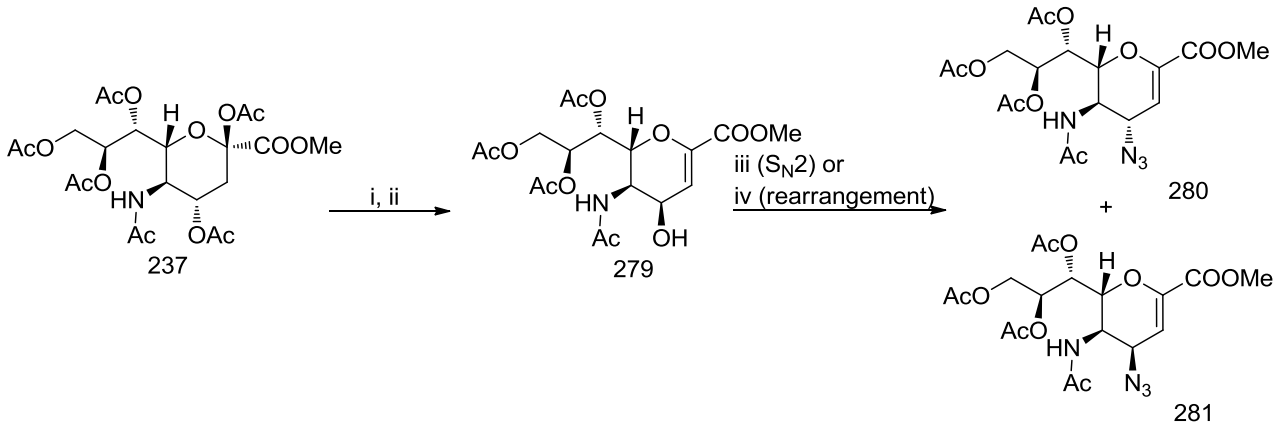


Conditions: (i) NIS, TfOH, DCM, 81%

3.3.2.3 Methods resulting in cis- and trans-configuration

In the last subchapter, it will be discussed methods giving the *trans*- and *cis*- 3,4-diamino configuration as a mixture. The major product is usually determined by the reaction conditions. Zbiral and col. published a few papers describing the incorporation of the azide function into the molecule of Neu5Ac.[169-171] The substitution of hydroxy group in **279** with azide under Mitsunobu conditions gave two isomers **280** and **281**. The ratio depends on the solvent, while toluene facilitated S_N2 reaction giving **280** (ratio of 280:281 = 3:1) as a major product, THF supported 3,3-rearrangement resulting in **281** (ratio of 280:281 = 2:3) as a major product. Both **280** and **281** were subsequently reduced via the Staudinger protocol to the corresponding amines.

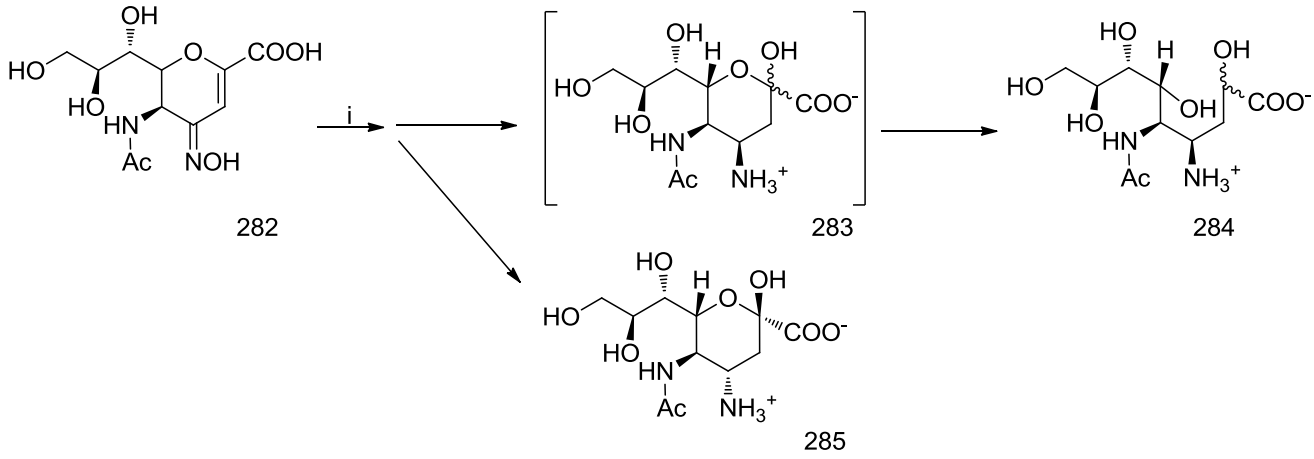
Scheme 82 - Introducing the azide function into the molecule of Neu5Ac



Conditions: (i) $CF_3SO_3Si(CH_3)_3$, ACN, 82%; (ii) TFA, ACN/water, 77%; (iii) HN_3, TPP, DEAD, toluene, 67% for **280**, 34% for **281**; (iv) HN_3, TPP, DEAD, THF 17% for **280**, 52% for **281**

Another example is based on the reduction of the oxime functionality (Scheme 83).[172] Hydrogenation of **282** gave two isomers **283** and **285**. However, **283** was unstable due to syndiaxial interactions and immediately underwent ring-opening reaction and subsequent reduction of the keto group resulting in **284**.

Scheme 83 - Reduction of oxime



Conditions: (i) H_2, Pd/C, HCl, MeOH, 85%

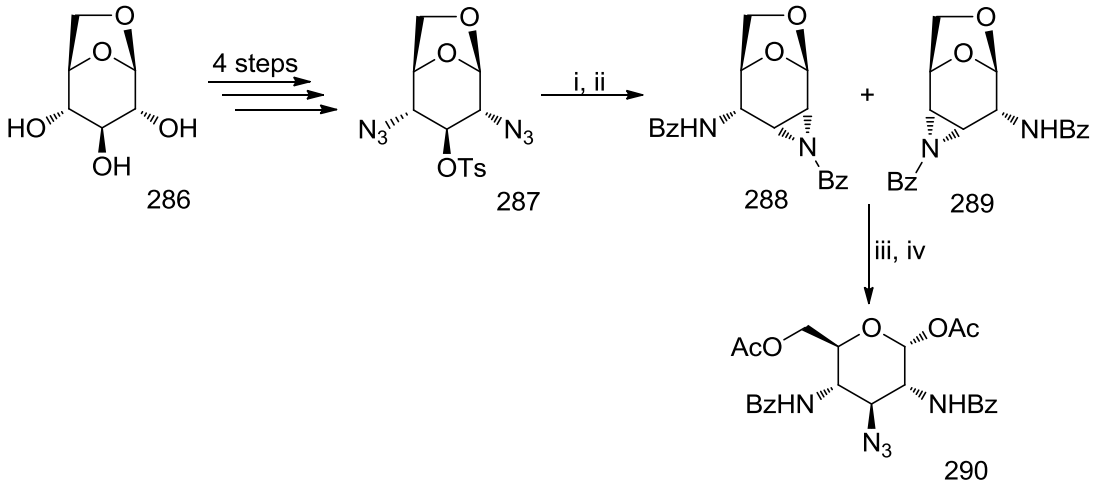
3.3.3 2,3,4-trinitrogen-containing D-hexopyranosides

This chapter is focused on the synthesis of 2,3,4–trinitrogen-containing D-hexopyranosides. In addition, two examples of the synthesis of 2,3,4,6-tetranitrogen-

containing D-hexopyranosides from trinitrogen precursors were added. The synthesis of both was motivated by the development of glycoside antibiotics containing polyaminated pyranoses.

The group of 2,3,4–trinitrogen-containing D-hexopyranosides is, in comparison to the dinitrogen ones, less complex, only a few papers described these syntheses. The first synthesis is based on the aziridine ring-opening reaction. Bailliez and col. published the synthesis of 2,3,4–trinitrogen-containing derivate of D-glucose, where levoglucosan **286** was converted to diazide **287**.[173] Subsequent reduction and benzoylation led to a mixture of isomers **288** and **289**. The mixture was treated with lithium azide and TFA giving compound **290** without further purification. The same authors described subsequent conversion to the tetranitrogen-containing derivate; more details can be found at the end of this chapter.

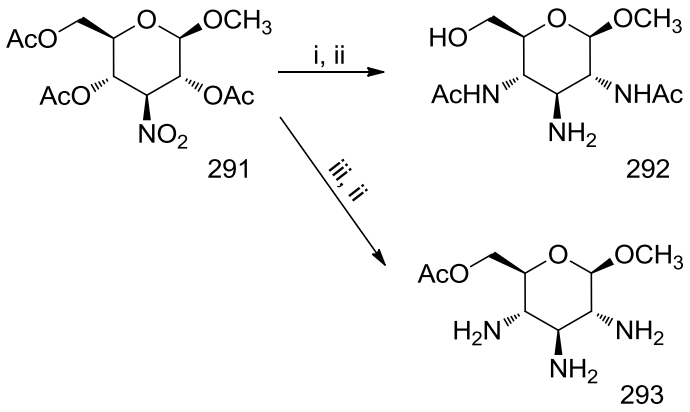
Scheme 84 - Synthesis of 2,3,4 – trinitrogen-containing carbohydrate **290** via aziridine ring-opening reaction



Conditions: (i) H_2, Pd/C, HCl, EtOAc/EtOH; (ii) Bz_2O, DMAP, pyridine/DCM, 83% (2 steps); (iii) LiN_3, Al_2O_3, DMF/toluene; (iv) TFA, Ac_2O, 79% (2 steps)

Another synthesis is based on the sequential acetoxy group elimination followed with the Michael addition with ammonia or benzylamine.[174-176] The substitution took place at C2 and C4 carbon resulting in **292** and **293**, which can be transformed into the corresponding triamine derivate.

Scheme 85 - Synthesis of 2,3,4 – trinitrogen-containing D-glucopyranosides

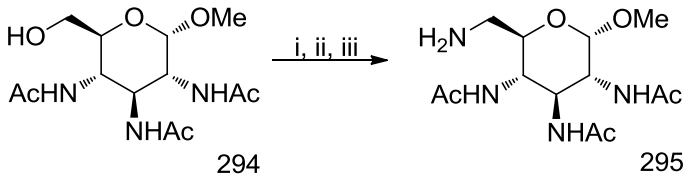


Conditions: (i) NH_3/MeOH, then Ac_2O, 57%; (ii) H_2, Pd/C, MeOH, (87-90%); (iii) $BnNH_2$, $CHCl_3$, 35%

General approach to tetranitrogen-containing sugars leads via 2,3,4-trinitrogen-containing precursors, where the hydroxyl group at C6 carbon is substituted with nitrogen-containing function. Introduction of the C-N bond at position 6 can be accomplished as the last step or it can be done simultaneously with substitution of more positions.

The first procedure was published by Baer et al.[177] The primary hydroxyl group of **294** was mesylated and substituted with the azide anion. The azide was subsequently reduced to give **295**. The similar protocol was applied by other authors.[173,178]

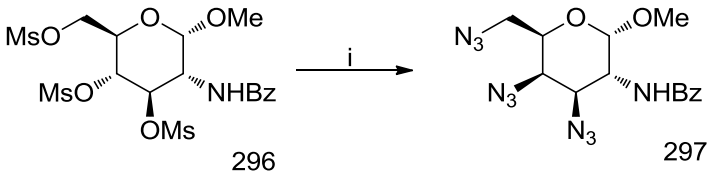
Scheme 86 - Synthesis of 2,3,4,6 – tetraamino carbohydrate



Conditions: (i) MsCl, pyridine, 56%; (ii) NaN_3, water, (51%); (iii) H_2, PtO_2, HCl, water, 67%

Ali et al. published the synthesis of tetranitrogen-containing sugar **297**, where the *galacto*-configuration arised from *in-situ* formed oxazoline ring and its ring-opening reaction with the azide anion and simultaneous two-fold mesylate substitution.[179] The similar procedure was utilized when galactopyranoside or idopyranoside instead of glucopyranosid **296** was used.[180,181]

Scheme 87 - Synthesis of 2,3,4,6 – tetranitrogen-containing carbohydrate-hexopyranosides utilizing the neighboring group participation



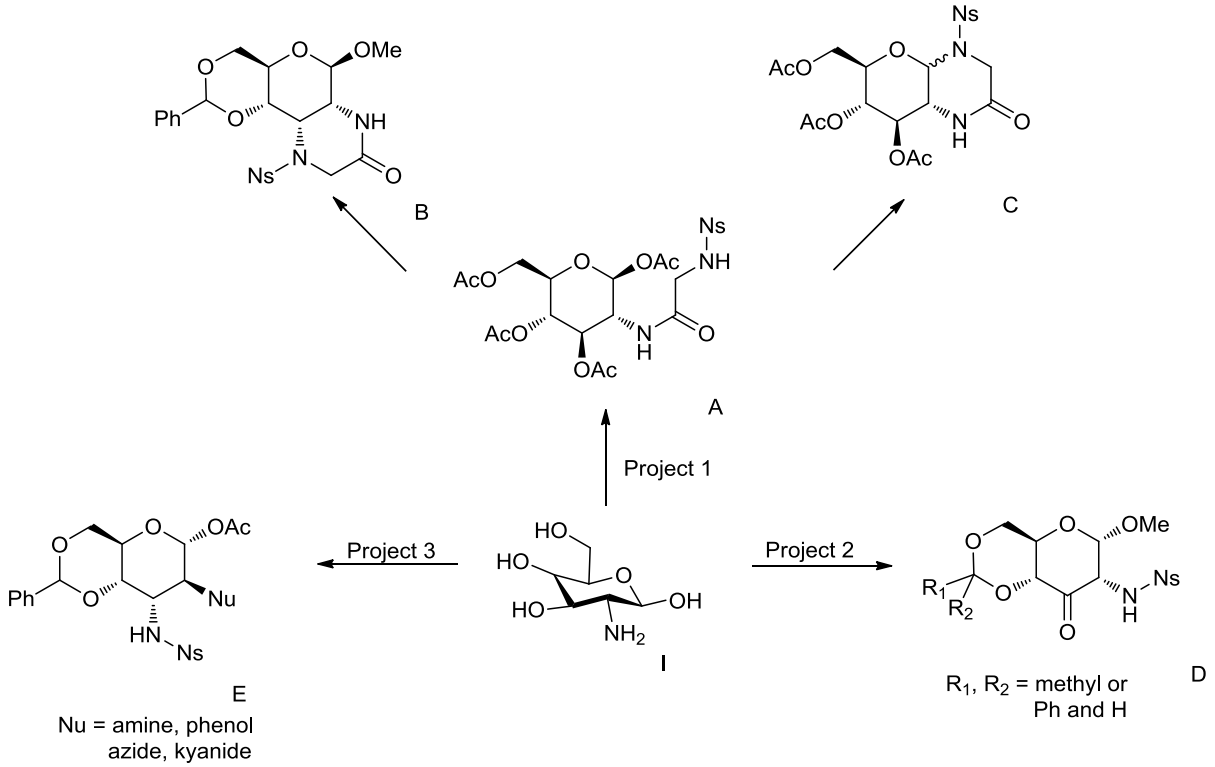
296 297

Conditions: (i) NaN_3, hexamethylphosphoric triamide, water, (10-15%)

4 Results and discussion

The dominant topic of this thesis is nosylation of D-glucosamine and its derivates. Resulting nosylated products were further tested toward alkylation and cyclisation reactions. The whole chapter is divided into the three projects. The aim of the first project was to prepare product **A**, which can further undergo ring-closure either to C1 or to C3 carbon, giving product **B** or product **C**. The second project consists in oxidation of hydroxy group at C3 carbon to corresponding ketosugar **D**. The reactivity of the resulting keto group was tested towards typical A_N reaction. This part also includes aziridine formation under Mitsunobu conditions. And the last project, is based on the preparation of 2,3-*trans*-diaminoaltropyranosides **E** via aziridine formation.

Scheme 88 - Illustration of synthetic routes

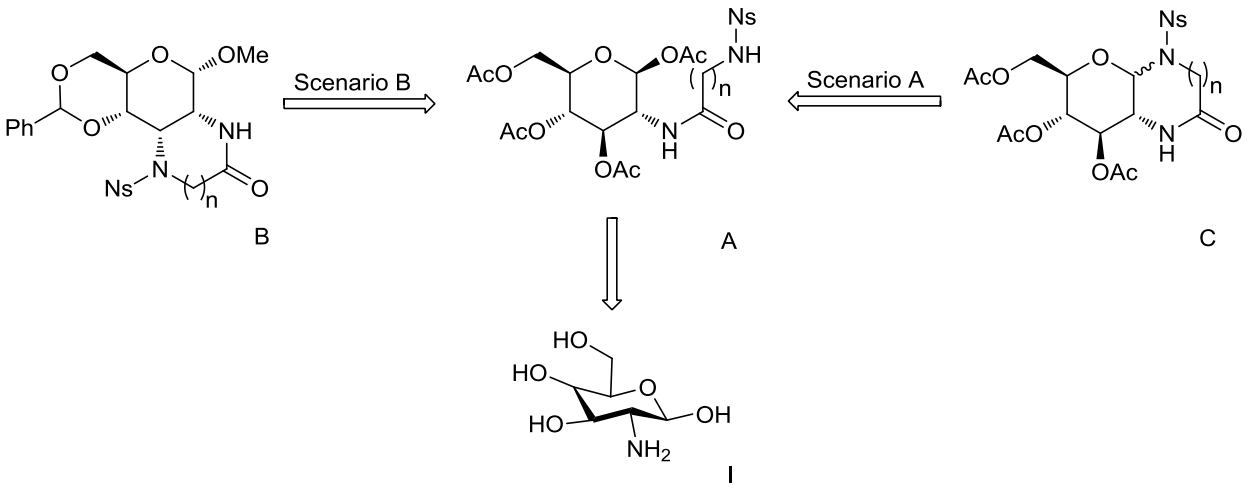


4.1 Project 1 - Synthesis of a suitable derivate for cyclization to C1 or C3 carbon

As you can see in the theoretical part, incorporation of the D-glucosamine unit into the cycle with six or more members is not so common and formation of the C1-N or C3-N bond is quite rare. Therefore, the first goal was to prepare such precursor and find reaction

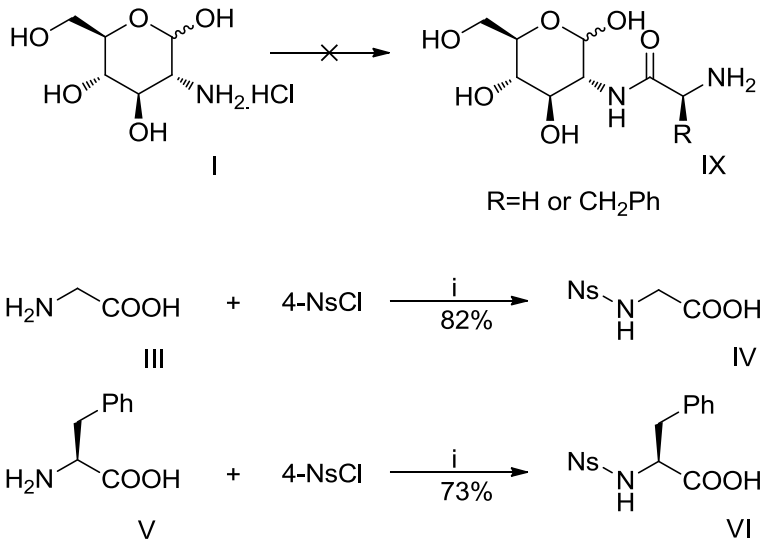
conditions allowing desired cyclization. Firstly, it was necessary to prepare the derivatives based on the structure **A**, which served as important intermediates for cyclisation reactions to C1 carbon (*Scenario A*) or to C3 carbon (*Scenario B*).

Figure 15 - Retrosynthetic analysis of *Project 1*



At the very beginning, we tried the simplest method - direct acylation of D-glucosamine hydrochloride **I** with nosylglycine **IV** or nosylphenylalanine **VI.** The reaction did not take place. Nevertheless, it is possible to find plenty of publications describing the acylation of unprotected D-glucosamine **I** with different carboxylic acids.[182-186] We used common acylation methods such as HOBt/DCC or T3P.

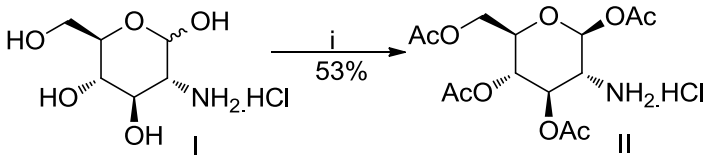
Scheme 89 - Unsuccesful acylation of unprotected D-glucosamine **I** and synthesis of nosylated amino acids



Conditions: (i) 4-Nos-Cl, amino acid, $Na_2HPO_4.12H_2O$, water, 73-82%

Since the acylation of D-glucosamine **I** did not take place, we decided to protect the free hydroxy groups via acetylation. Tetra-*O*-acetyl glucosamine **II** was prepared in the three step synthesis from readily available *D*-glucosamine **I**, following the known procedure.[187] This synthesis allows a multi gram scale (more than hundred grams).

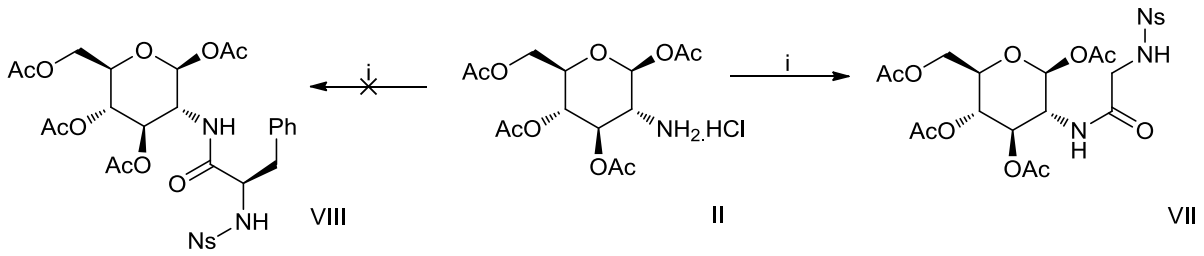
Scheme 90 – Synthesis of fully *O*-protected D-glucosamine **II**



Conditions: (i) anisaldehyde, MeOH, 1 M NaOH, then Ac_2O, Pyridine, then Acetone, 5 N HCl, 53% (three steps)

The formation of the amide bond between the amino group of D-glucosamine **II** and the carboxylate of the *N*-nosylated amino acid **IV** was surprisingly difficult. The optimization of acylation were done, plenty of different solvents were tried, unfortunately with no significant effect. The biggest problem consists in removing of the dialkyl urea, which was formed during HOBt (HOSu) mediated reaction. The best results were achieved with EDC/HOSu, where troubles with separation were suppressed due to solubility of EDC. Further, the same conditions were applied to nosylated phenylalanine **VI**. The reaction did not proceed, probably due to bulkiness of the amino acid. The configuration at C1 carbon was retained. The coupling constant of anomeric proton is ^3J ($^1H^1H$) = 8.8 Hz, which indicated the presence of ß-anomer.

Scheme 91 - Amide bond formation



Conditions: (i) Nosylated amino acid, see the table below

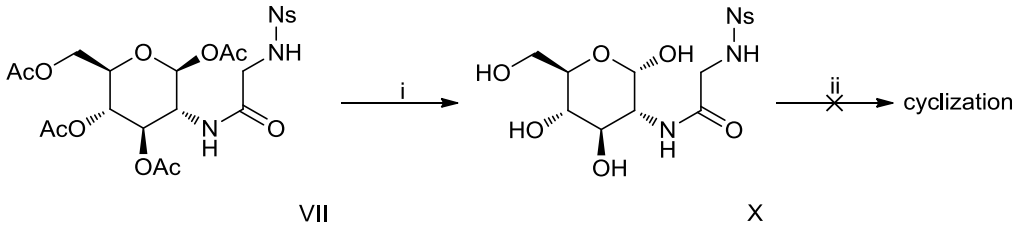
Table 1 - Optimization of the amide bond formation

agents	solvent	yield[1]	purity[1]
DIC/HOBt	py	64%	80%
DCC/HOBt	py	70%	80%
EDC/HOSu	py	34%	95%
T3P	DMF(dry)	45%	75%

[1] related to product **VII**

Following the main topic of *Project 1*, we firstly did the full deacetylation of carbohydrate **VII** with NaOMe. The purpose of this reaction was to „offer", more positions for cyclisation. The potential cyclization, performed under Fukuyama-Mitsunobu conditions, could take place either at C1 (*Scenario A*) or C3 carbon (*Scenario B*), however, only a starting material was detected.

Scheme 92 – Full deprotection of acetylated glucosamine

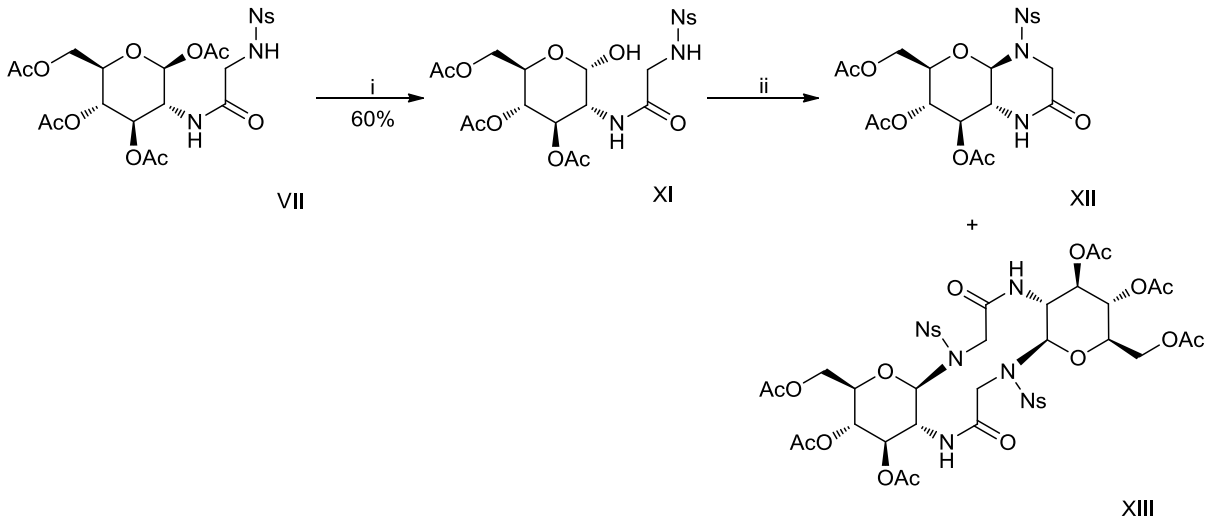


VII X

Conditions: (i) MeONa/MeOH, 85%; (ii) DIAD, TPP, dioxane

Since the cyclization of **X** does not work, a partial deprotection at the anomeric carbon of **VII** has been tried (Scheme 93). The partial deprotection of **VII** was carried out with methylamine, hydrazine acetate and a solution of ammonia in EtOH (Table 2). The best result gave the deprotection with methylamine, where the ratio of α/ß anomers was 70/30 toward α-anomer. α-Anomer was separated and characterized. The configuration at C1 was assigned according to the vicinal coupling constant $^{3}J(^{1}H^{1}H)$ = 3.9 Hz (t).

Scheme 93 - Partial deprotection followed by cyclization



Conditions: (i) CH_3NH_2 (2 M in MeOH), THF, 60%; (ii) DIAD, TPP, dioxane

Table 2 - Anomeric deprotection

Method	Conditions	Anomeric ratio (α/β)[1]
$MeNH_2$	THF, rt	70/30
$N_2H_4.AcOH$	DMF, rt	67/33
NH_3/MeOH	THF, rt	-[2]

[1] calculated from NMR

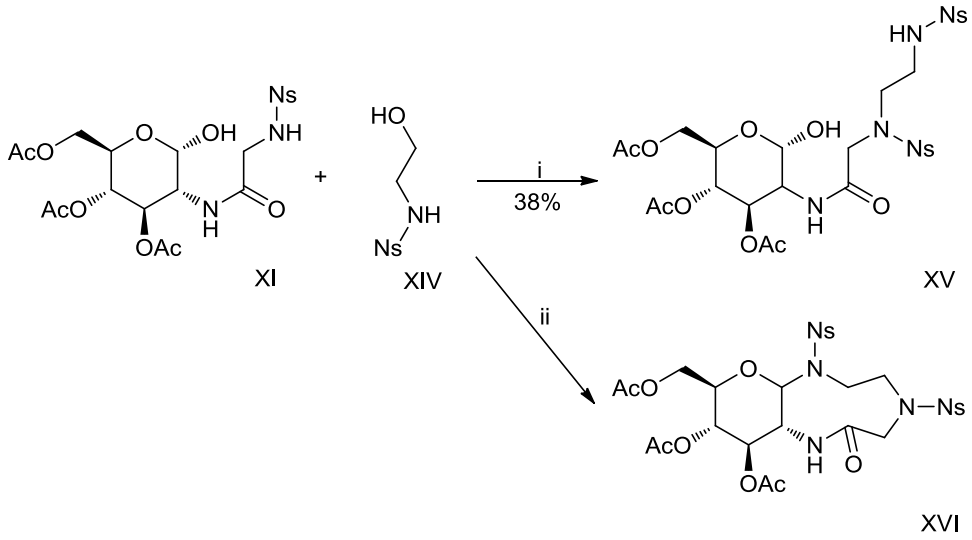
[2] mixture of partially and fully deprotected products

The subsequent cyclization was tried exclusively on α-anomer **XI** and the reaction gave a mixture of products, where the major product was probably the macrocycle **XIII** and the minor was **XII**. The structures **XII** and **XIII** were suggested on basis of LCMS chromatograms. In accordance with our observations, also other authors described the favored formation of similar macrocycles utilizing the nucleophilic aromatic substitution or even radical cyclization. For details, see *Chapter 3.2.2.3*, *Scheme 48* and *50*. *Scheme 93* does not include the possible formation of glycans, which can be formed by elimination of C1 hydroxy group. The structure confirmation was not done due to complicated mixture of products and instability of macrocycle **XII**. The change of Mitsunobu reagents had only a marginal influence on the resulting mixture. Both products were separated; however, the resulting purity does not allow the full characterization. It was obvious, that the six - membered ring formation was not favored, therefore we tried to prolong the alkyl chain with ethylamine.

The extension of the chain should result in a nine - membered ring (Scheme 94). Firstly, *N*-nosylated aminoethanol **XIV** was prepared. Afterwards, the cyclization of amide **XI** with

alcohol **XIV** was tested. It is obvious, that cyclization of **XI** with **XIV** offers plenty of possible products. Firstly, the cyclization was complicated by mutual reaction of two molecules of **XIV**. Further, we assumed that the first Mitsunobu reaction took place between the primary hydroxy group of **XIV** and nosylamide functionality of **XI**. The assumption was based on the fact, that the primary hydroxy group of **XIV** is more reactive than the anomeric hydroxyl for the steric reasons. The traditional Mitsunobu reagents (DIAD/TPP) allowed prolongation of the chain resulting in **XV**, while the use of the TMAD/Bu_3P system supported either formation of the nine - member ring **XVI** or above described dehydration resulting in glycan. The compound **XV** was separated. Its NMR spectra are not attached due to the low purity. Macrocycle **XVI** apparently decomposed during the purification.

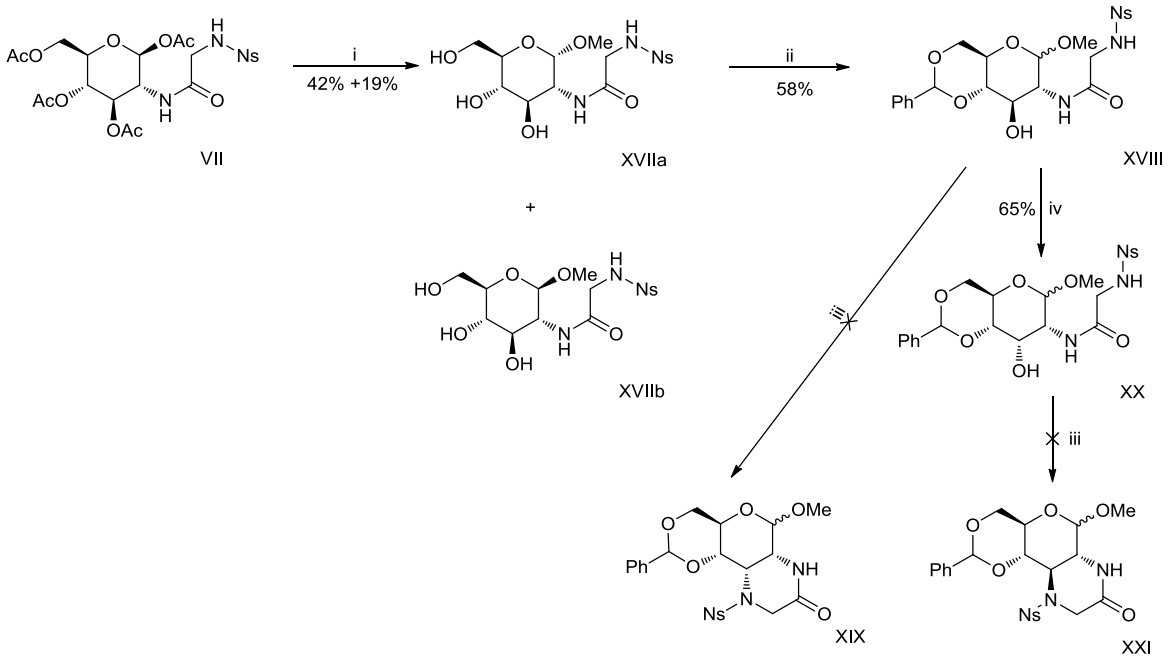
Scheme 94 - Cyclization with nosylated aminoethanol **XV**



Conditions: (i) DIAD, TPP, dioxane, 38%; (ii) TMAD, Bu_3P, dioxane

After the possibility of cyclization to C1 carbon was investigated, we focused our attention on the hydroxy group placed at the C3 carbon (*Scenario B*). First of all, we had to protect more reactive anomeric hydroxy group (see *Scheme 95*). This problem was easily solved out by the total deprotection of acetyl groups of **VII** with sodium methoxide and subsequent addition of dried Amberlite IR $120H^+$ to give the selectively methylated C1 hydroxy group. Both resulting anomers **XVII** were separated and characterized.

Scheme 95 - Cyclization to C3 carbon

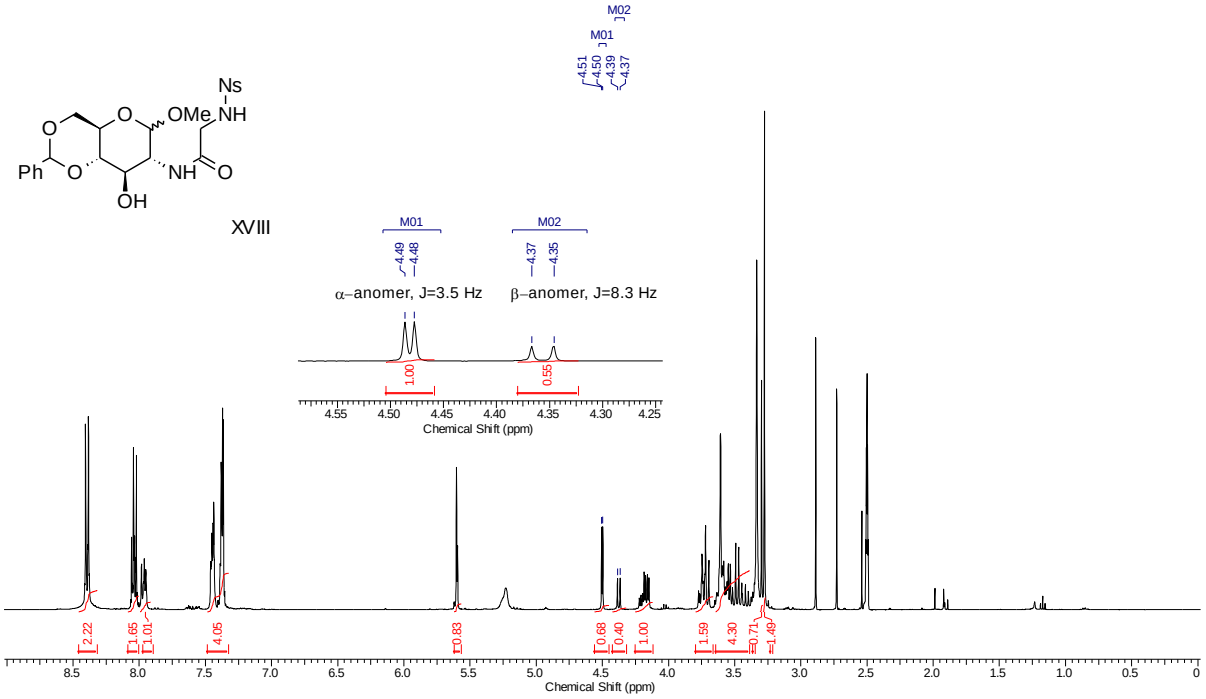


Conditions (i) MeONa/MeOH, then dry IR $120H^+$, 42% for **XVIIa** and 19% for **XVIIb**; (ii) BDA, IR $120H^+$, dioxane, 58% (mixture of isomers), (iii) DIAD, TPP, dioxane or TMAD, Bu_3P, dioxane; (iv) PhCOOH, DIAD, TPP, then K_2CO_3, MeOH, 75%

The ratio of anomers according to the ^1H NMR spectrum was 70/30 toward α-anomer. Two doublets around 4.4 ppm were assigned to C1 proton. The peak with the higher chemical shift and the coupling constant $^3J(^1H^1H)$=3.4 Hz (d) belongs to the α-anomer, while the peak with lower chemical shift and the coupling constant $^3J(^1H^1H)$=8.0 Hz (d) belongs to the ß-anomer.

The hydroxy groups at C5 and C6 carbons were protected via the acid-catalyzed reaction of benzaldehyde dimethylacetal (BDA) with glucosamine **XVIIa**. The reaction provided again a mixture of anomers; however, the isomers were not separated due to the extremely similar physical properties. The ratio of anomers was 2/1 toward α-anomer, for details, see *Figure 16.*

Figure 16 - Anomeric mixture of **XVIII**



The cyclization of **XVIII** (mixture of anomers) was tested under various Mitsunobu conditions. Applying of TPP/DIAD, TPP/DBAD, or Bu_3P/TMAD in dioxane or THF gave only the starting material; no traces of **XIX** were observed. The inversion of configuration at C3 carbon, resulting in product **XX**, unfortunately did not allow the potential cyclization leading to XXI.

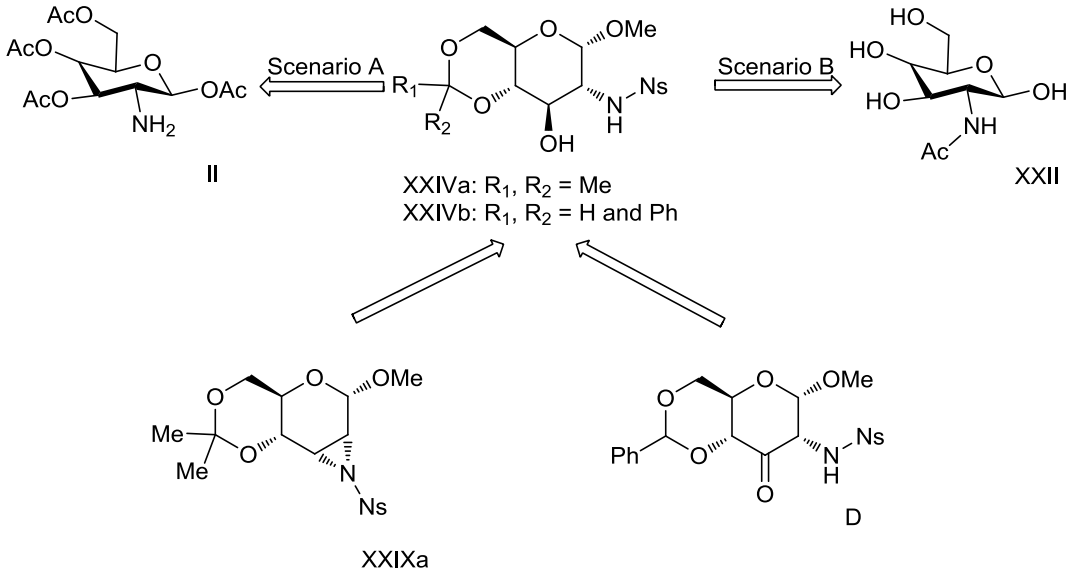
To summarize *Project 1*, all the intermediates, necessary for cyclization reactions, were prepared and their reactivity was tested. Unfortunately, the potential cyclization to C1 carbon was not proved. In addition, undesired side reactions were observed. The cyclization toward to C3 carbon did not work at all.

4.2 Project 2 - Synthesis of D-glucosamine derivate with free hydroxy group at C3 carbon and its reactivity study

Project 2 was focused on transformations of the amino and hydroxyl group of D-glucosamine at position 2 and 3 to obtain more suitable functionalities for potential formation of an *ortho*-fused ring. It was necessary to protect hydroxyls at positions 1, 4, and 6, and then modified amino and hydroxy group at position 2 and 3. A key intermediate, **XXIV**, was

prepared via two different synthetic routes, *Scenario A* and *Scenario B* (Figure 17). Firstly, it was studied the reactivity of the nosyl group toward Fukuyama-Mitsunobu alkylations and possible formation of aziridine **XXIX** as a result of a side intramolecular reaction with the hydroxyl at position 3. In the end, attention was paid to the oxidation of the hydroxyl at position 3 into the keto functionality resulting in derivative **D**. Consequently, the keto group can potentially offer other useful transformations.

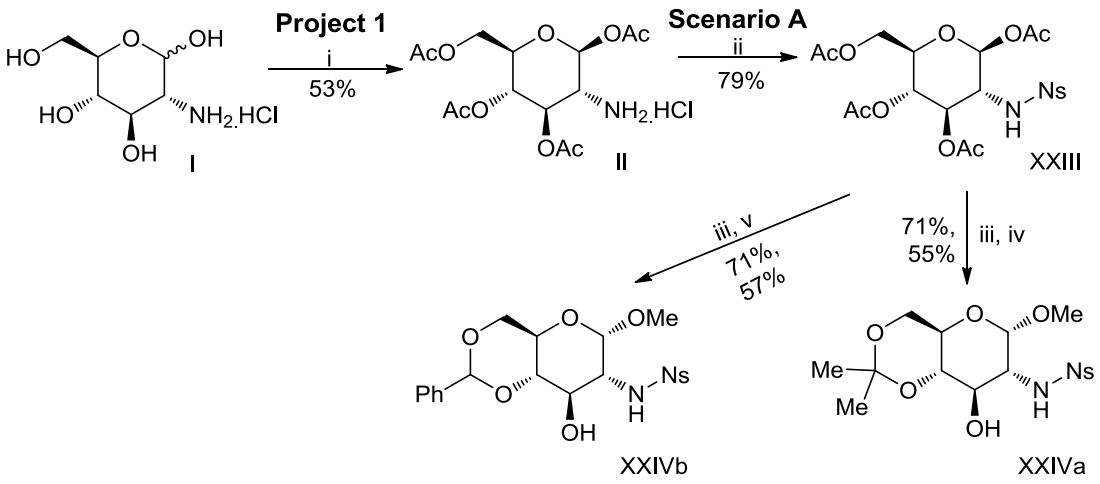
Figure 17 - Retrosynthetic analysis of Project 2



The synthesis of the key intermediate, **XXIV**, started according to *Scenario A* with tetra-*O*-acetyl glucosamine **II**, which was prepared on a large scale in *Project 1* (Scheme 96). It was anticipated the possibility to perform nosylation and deacetylation with subsequent protection in three isolation steps.

Indeed, nosylation of **II** gave *N*-nosylated derivate **XXIII** in a very good yield, which was isolated as a ß-anomer, $^{3}J(^{1}H^{1}H)$= 8.3 Hz. Conversion of **XXIII** to desired hydroxy derivates **XXIV** was done in two steps. In the first step, base-catalyzed cleavage of acetyl groups, followed by Fischer methylation of anomeric hydroxyl, gave deacetylated intermediate, which was isolated as a mixture of anomers and used for the next step. Further, anomers were treated with acetone dimethyl acetal or benzaldehyde dimethyl acetal to give **XXIVa** and **XXIVb** respectively. Both products were isolated as α-anomers ($^{3}J(^{1}H^{1}H)$= 5.2 Hz for **XXIVa** and $^{3}J(^{1}H^{1}H)$= 3.6 Hz for **XXIVb**) and fully characterized.

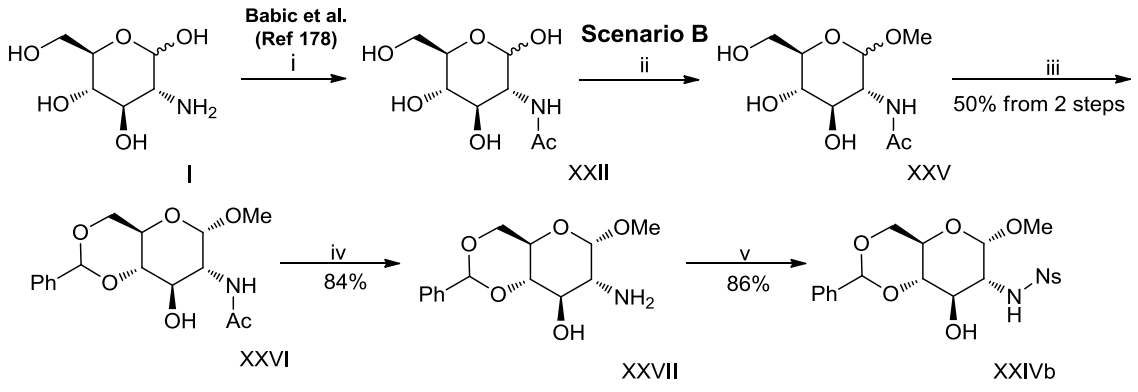
Scheme 96 - Preparation of alcohols **XXIV** via *Scenario A*



Conditions: (i) anisaldehyde, MeOH, 1 M NaOH, then Ac_2O, Pyridine, then Acetone, 5 N HCl, 53%; (ii) 4-NsCl, Et_3N, DCM/pyridine, 79%; (iii) MeONa/MeOH, then IR 120H$^+$, mixture of anomers, 71%; (iv) 2,2-dimethoxypropane, IR 120H$^+$, dioxane, 55%; (v) BDA, dioxane, IR 120H$^+$, 57%

Scenario B used a different approach for preparation of derivatives **XXIV** and started with *N*-acetylated glucosamine **XXII** (Scheme 97). The method for quantitative *N*-acetylation of D-glucosamine **I** is well described,[178] however, the price availability of **XXII**, comparable to D-glucosamine **I,** allowed purchasing **XXII** from a chemical company. The procedure resulting in **XXVII** followed known synthesis with small improvements in work-up of intermediates.[188, 189] The aminoalcohol **XXVII** was then nosylated with 4-Nosyl chloride to give **XXIVb** in a very good yield.

Scheme 97 - Synthesis of alcohol **XXIVb** via *Scenario B*



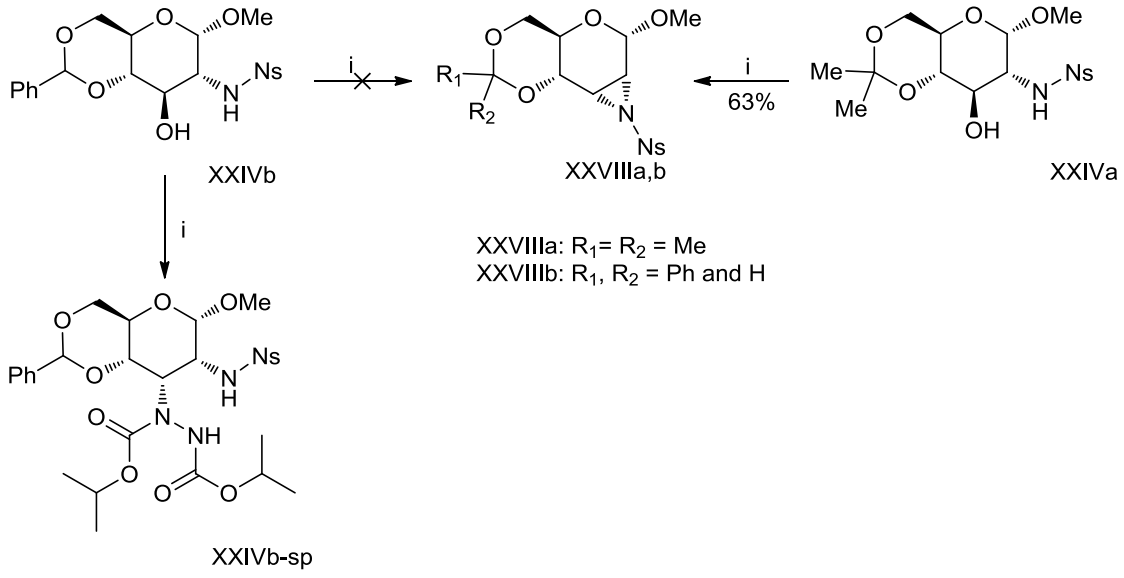
Conditions: (i) Na/MeOH, Ac_2O, quant.; (ii) MeOH, IR 120H$^+$; (iii) BDA, TsOH.H_2O, dioxane, 50% (for two steps); (iv) 4 M KOH, EtOH, 84%; (v) 4-NsCl, DCM/pyridine, 86%

When D-glucosamine **I** is considered as a starting compound, *Scenario A* gave product **XXIVb** in 17% overall yield, whereas *Scenario B* more than doubled the overall yield to 36%

of **XXIVb**. Also the number of reaction steps is lower in the case of *Scenario B* (5 steps) than in the case of *Scenario A* (6 steps). However, *Project 1* included synthesis of *O*-Acetylated D-Glucosamine **II** in a multi-gram scale (more than 100 g); therefore we could employ carbohydrate **II** as a starting compound. In such case, the overall yield of *Scenario A* was increased to 34% and the number of reaction steps was significantly decreased to three steps. Generally, from an economic as well as synthetic perspective, the *Scenario B* is more favorable, however, the availability of carbohydrate **II** from previous *Project 1* made both methods comparable.

Further, the cyclization of both **XXIVa** and **XXIVb** was tested, since the formation of aziridine **XXVIII** was possible side reaction occurring in Fukuyama-Mitsunobu alkylation (Scheme 98). Surprisingly, the cyclization under various Mitsunobu conditions (TPP/DIAD, TPP/DBAD or Bu_3P/TMAD in dioxane) gave the corresponding aziridine only in the case of derivate **XXIVa**. The reaction with **XXIVb** led to the formation of side product **XXIVb-sp** which was not isolated; however the LC-MS analysis indicated this structure with high probability. We assumed that the formation of **XXIV-sp** is caused by steric reasons. The aziridine **XXVIIIa** was fully characterized. To sum up, the substitution at C4 and C6 carbon has an important effect on the aziridine formation. It is important to mention, that aziridine **XXVIIIB** was also prepared in a different way, via substitution of the nosylated hydroxy group. The details will be discussed in the next chapter.

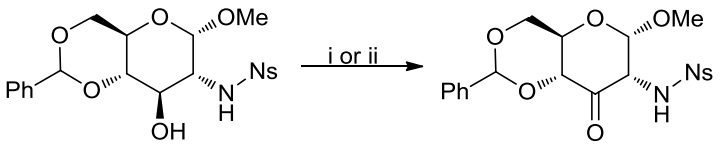
Scheme 98 - Reactivity study of **XXIV** under Mitsunobu reaction



Conditions: (i) DIAD, TPP, dioxane, 63% (for **XXVa**)

Further, the oxidation of **XXIVb** was tested. The analogous reaction with **XXIVa** was not performed. Two different methods were used for a conversion of the hydroxy group at C3 to the keto function (Scheme 99). The first one was based on oxidation with Dess Martin periodinane (DMP). This reaction provided good yields; the addition of 1.1 equivalent of water speeded up the reaction. The second approach employed modified Swern oxidation, where instead of oxalyl chloride; a 50% solution of T3P in EtOAc was used. Both methods offer good yields and easy separation; however, we decided to carry out the oxidation with T3P, which offered slightly higher yields. The ketone **XXIX** was identified by the ^{13}C NMR spectrum, where the carbonyl signal showed the chemical shift about 195 ppm, which is typical for a presence of the keto group.

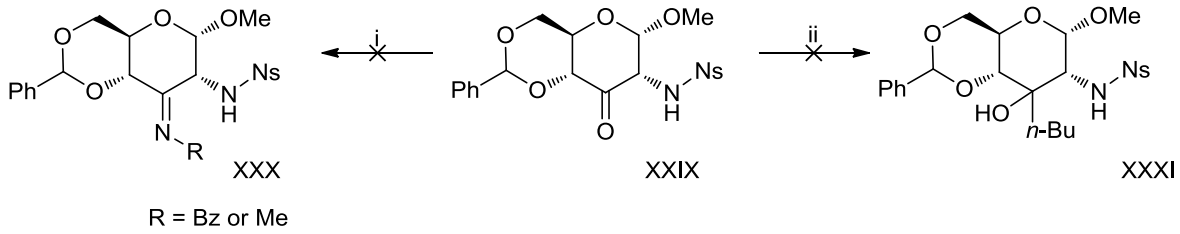
Scheme 99 - Oxidation of the hydroxy group at C3



Conditions: (i) T3P/EtOAc, DMSO, EtOAc (dry), 69%; (ii) DMP, DCM, water (1.1equiv), 55%

After the gram - scale synthesis of ketone **XXIX**, the reactivity of the keto group was investigated (Scheme 100). Surprisingly, the carbonyl group was unreactive against common reagents. The reaction of **XXIX** with two equiv. of benzylamine or methylamine gave unknown products, which did not correspond to the expected ketimine products **XXX**. Addition of *n*-BuLi or a 2 M solution of isopropylmagnesium bromide resulted in decomposition of a starting material.

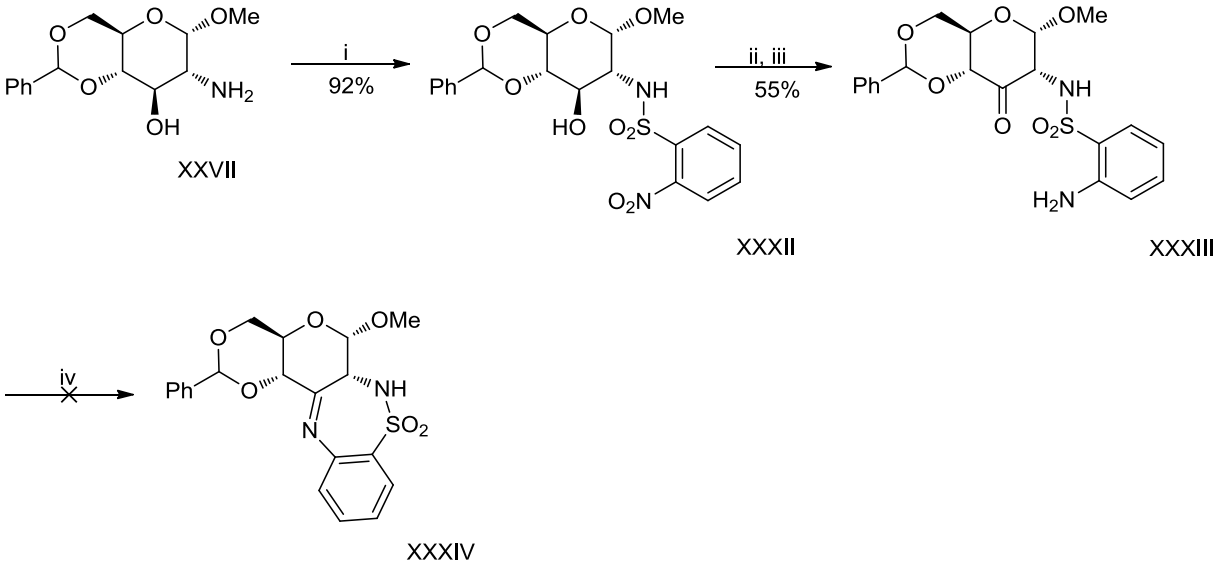
Scheme 100 - Attempted reactions with the keto group



Conditions: (i) amine, MeOH, reflux; (ii) Grignard reagent, THF (dry), 0°C

Moreover, the intramolecular cyclization was investigated. When 2-Nosyl instead of 4-Nosyl group was attached to the amine function, the reduction of the nitro group could allow the formation of seven - membered ring (Scheme 101). The reaction with 2-Nosyl group took place under the same conditions described for the 4-Nosyl group. Oxidation with T3P/EtOAc and subsequent reduction of the nitro group afforded keto derivate **XXXIII**, which was used for the final cyclization. The structure of keto derivative **XXXIII** was confirmed by the LCMS analysis; the NMR spectra of **XXXIII** are not attached due to low purity of product. Unfortunately, the closure of the cycle was not successful, even traces of the product were not detected. Further cyclization attempts were not performed due to the lack of starting materials.

Scheme 101 - Attempted intramolecular cyclization



XXVII XXXII XXXIII XXXIV

Conditions: (i) 2-NsCl, DCM/pyridine, 92%; (ii) T3P/EtOAc, DMSO, EtOAc, 78%; (iii) H_2, Pd/C, THF, 70%; (iv) Δt or base

In summary, we found and optimized two methods for the synthesis of hydroxy derivative **XXIV**. The better results were achieved with *Scenario B*. Further, Fukuyama-Mitsunobu alkylation and oxidation of **XXIV** was investigated. During alkylation reactions, it was found out, that cyclization of **XXIV** resulting in aziridine cycle took place only when 2,2 - dimethoxy propane protected hydroxyls at C4 and C6 carbons. Oxidation of **XXIVb** gave the corresponding keto derivate **XXIX**, which, unfortunately, was unreactive against common nucleophiles.

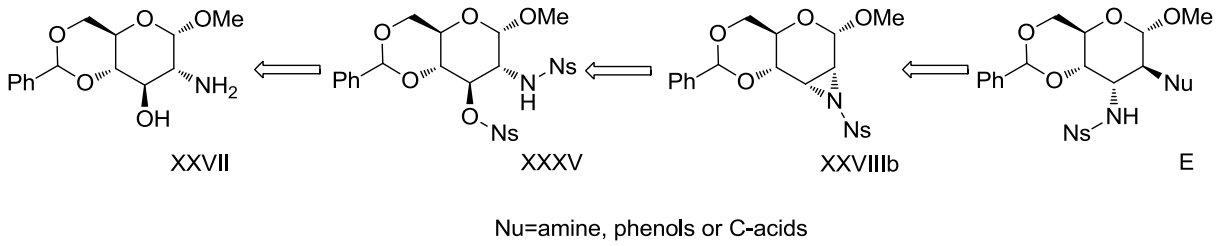
4.3 Project 3 - Preparation of 2,3 - diamino carbohydrate via aziridine formation

This part is loosely based on the publication: Ring-opening reactions of the *N*-4-nosyl Hough-Richardson aziridine with nitrogen nucleophiles. The introduction was significantly reduced. Some parts in the discussion part are extended with redundant or negative results, which were not used in the publication. The numbering and the layout were adjusted to the style of the Ph.D. thesis.

4.3.1 Introduction

Previously, we revealed in *Project 2*, that the synthesis of aziridne derivatives **XXVIII** is feasible. Consequently, we realized that the aziridine ring strain can be utilized to synthesize other useful derivatives by the ring-opening reaction. However, we chose a more practical route to synthesize above mentioned aziridine **XXVIII**. *Project 3* was focused on the preparation of altrohexopyranosides **E** via dinosylated glucosamine **XXXV** (Figure 18).

Figure 18 - Retrosynthetic analysis of *Project 3*

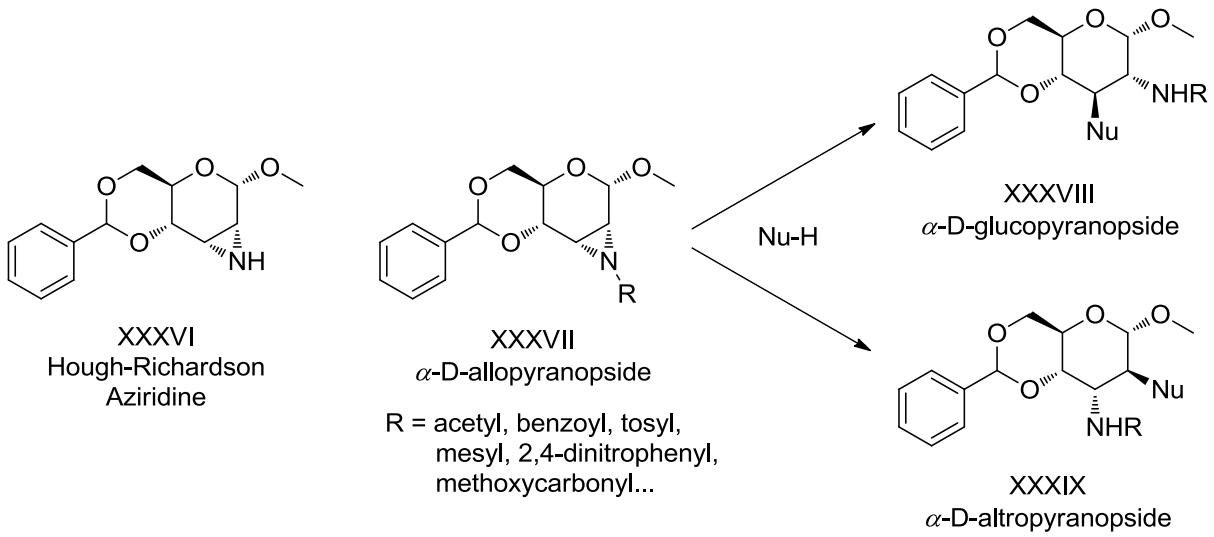


Hexopyranosides containing 2,3-diamino functionality are useful chiral synthetic intermediates in the synthesis of highly functionalized compounds with interesting biological or chemical properties. These intermediates were used directly as chiral ligands in half-sandwich metal complexes with antitumor activity[104,115] or molybdenum complexes to catalyze asymmetric allylic alkylations.[103] Further, 2,3-diaminohexopyranosides also served as a key precursor in the synthesis of glycophospholipid ligand of lipopolysaccharide receptor,[102] chimeric scaffolds with benzodiazepine moiety,[92] and Weinreb´s advanced intermediate for (-)-Agelastatin A formal total synthesis.[101]

One of the frequently used methods leading to the derivatives of 2,3-diaminohexapyranosides is the ring-opening reaction of the Hough-Richardson aziridine,[99] which is commonly

synthesized from D-glucosamine. This valuable chiral intermediate can be transformed by regioselective ring-opening reaction of strain-loaded three-membered ring into the corresponding diastereoisomers with α-D-*altro*- or α-D-*gluco*- configurations (Figure 19).

Figure 19 - The general ring-opening reaction of the Hough-Richardson aziridine



4.3.2 Scope and limitations

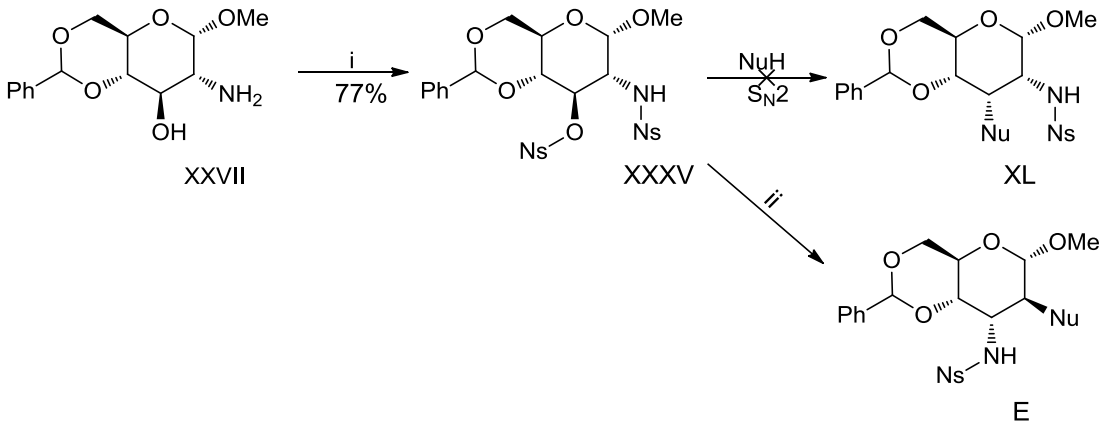
The ring opening reactions of aziridine **XXXVII** were troublesome. The yields were decreased due to the formation of two isomers (**XXXVIII**, **XXXIX**)[105,106] and the scope of the reaction was extremely narrow; from nitrogen-containing nucleophiles, only azide was tested. For more details, see *Chapter 3.1.1.1*.

In this contribution we present the first synthesis and the ring-opening reactions of the *N*-4-nosyl Hough-Richardson aziridine mostly with nitrogen nucleophiles. The *O*- and *C*-nucleophiles are included in a separate table. The electron-withdrawing effect of the nitro group in this new valuable advanced intermediate brings several significant practical advantages, especially (i) synthesis of aziridine under mild conditions and, if necessary, this intermediate can be isolated by a simple filtration in a very good yield and high purity, (ii) to perform the highly regioselective aziridine ring-opening reactions without presence of ammonium chloride resulting in products preferring the α-D-*altro*- prior to α-D-*gluco*-configuration in a ratio no less than 90:10 under more convenient conditions utilizing more practical solvent and temperature, (iii) the scope of applicable nucleophiles for the ring-opening reaction is substantially broadened, solely reported azide ion can be directly replaced

with even aqueous ammonia which eliminate the subsequent reduction step, (iv) further modification of nosylamide functionality particularly by *N*-alkylation under classical or Fukuyama-Mitsunobu protocols,[191] and (v) potential mild deprotection conditions of nosyl group in comparison to the mesyl or tosyl group.

During nosylation of **XXVII** (see Scheme 97), it was find out, that as a minor product (less than 5%) dinosylated carbohydrate **XXXV** was created. When 4.2 equivalent instead of 1.05 equivalent of 4-NsCl was added, the nosylation underwent on both the amino and hydroxy group and dinosylated product **XXXV** was formed. Subsequent conversion did not take place via S_N2 reaction to *allo*- derivate **XL**, but *altro*-pyranoside **E** was detected.

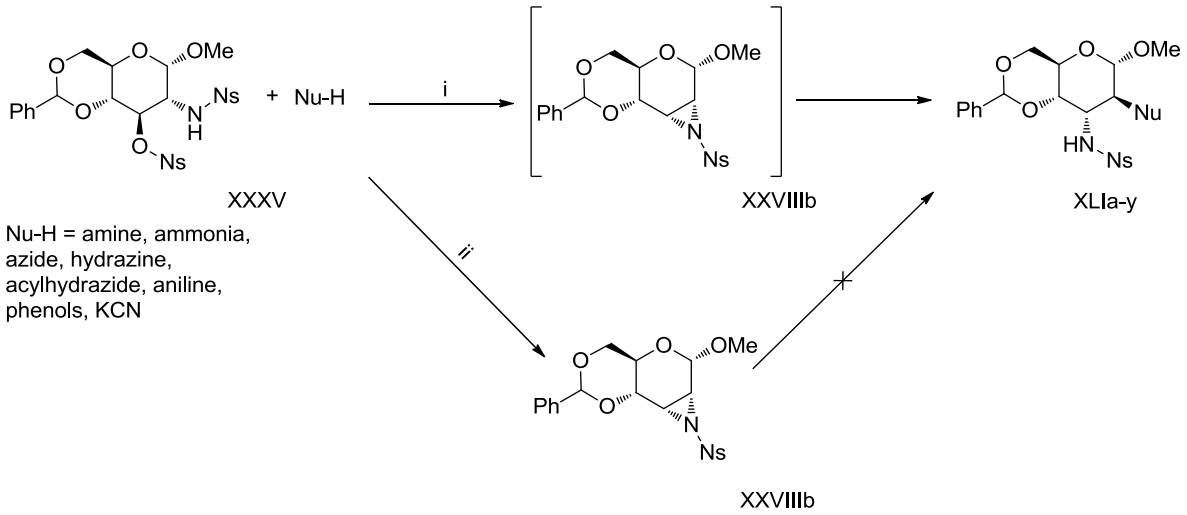
Scheme 102 - Synthesis of 2,3 – diaminohexapyranoside **E**



Conditions: (i) 4.2 equiv. 4-NsCl, DCM/pyridine, 77%; (ii) NuH, DIPEA, DMSO, 60°C

Addition of DIPEA to dinosylated glucosamine **XXXV** led to rapid formation of aziridine **XXVIIIb** (Scheme 103). If DMSO is used as a solvent, *in situ* formed aziridine **XXVIIIb** can be directly transformed at only 60 °C into 2,3-diaminoaltropyranoside **XLI** in the presence of various nucleophiles. Replacement of DMSO by 2-methoxyethanol allowed simple isolation of crude aziridine **XXVIIIb** by filtration in very good yield and high purity. Very poor solubility of aziridine **XXVIIIb** in 2-methoxyethanol practically eliminated subsequent aziridine ring-opening reaction with nitrogen nucleophile.

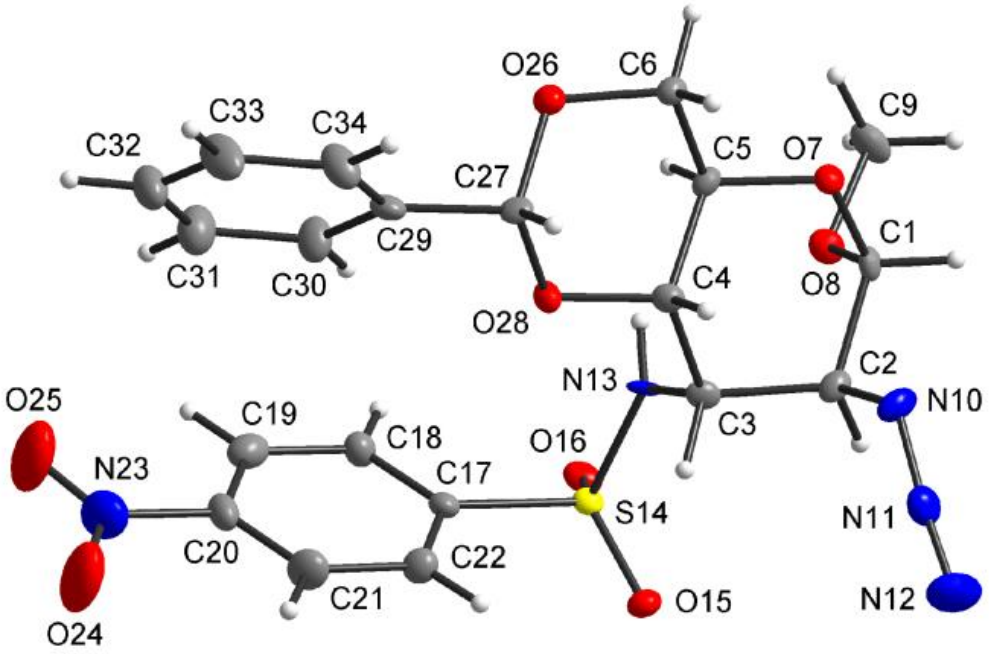
Scheme 103 - Influence of the solvent on the aziridine **XXVIIIb** ring-opening reactivity



Conditions: (i) DIPEA, DMSO, 38-91%; (ii) DIPEA, 2-methoxyethanol, 83%

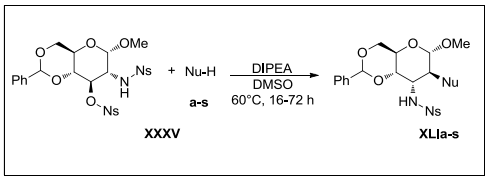
The assumed mechanism of the aziridine formation involves a transition state of nosylamide **XXXV** in the boat conformation directing the nosyl ester and nosyl amide groups in the *trans*-diaxial relationship. Formation of nosylamide anion and increased departing ability of the adjacent nosylate group was accelerated by nitro group which led to the fast aziridine ring closure. The α-D-*allo* configuration of aziridine **XXVIIIb** was confirmed by NMR spectra. Subsequent aziridine ring-opening reaction with nucleophile, resulting in the cleavage of C2-N bond, followed the Fürst-Plattner rule[192] to give *trans*-diaxial products **XLI** with α-D-*altro* configuration. Additionally, the α-D-*altro* configuration of **XLIa** was unequivocally determined by X-ray structure (Figure 20).

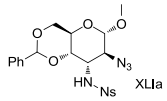
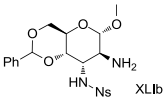
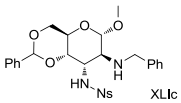
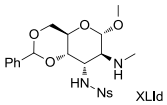
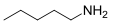
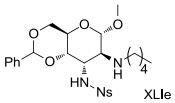
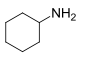
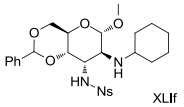
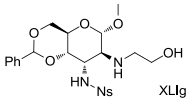
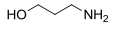
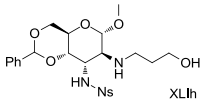
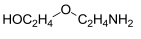
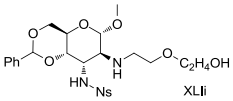
Figure 20 - X-Ray structure of **XLa**

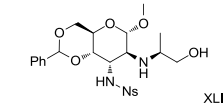
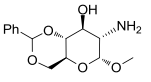
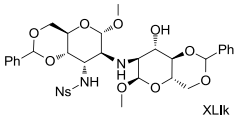
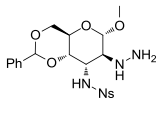
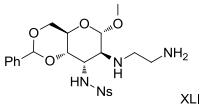
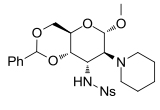
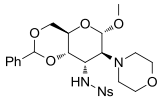
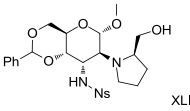
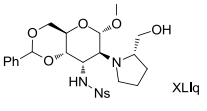
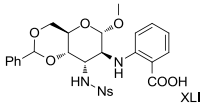
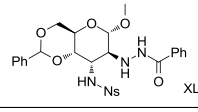


To explore the aziridine ring-opening reactivity scope with regard to the structure diversity of nitrogen nucleophile we started a set of reactions (Table 3). Besides the nitrogen nucleophiles **a-y**, also phenols and C-acids were tested, however significant limitations were observed. Since the aziridine ring closure is much faster than potential nosyl ester substitution/hydrolysis and subsequent ring-opening reaction, we utilized the direct synthesis of α-D-altropyranosides **XLI** from dinosylated pyranoside **XXXV** via *in situ* formed aziridine **XXVIIIb**.

Table 3 - Reactions of dinosylated glucosamine **XXXV** with nitrogen nucleophiles



Entry	Nu-H	Product	Time	Yield[a]
1	NaN_3		16h	72%
	a	XLIa		
2	NH_3/H_2O		16h	91%
	b	XLIb		
3			24h	59%
	c	XLIc		
4			24h	55%
	d	XLId		
5			24h	79%
	e	XLIe		
6			16h	68%
	f	XLIf		
7			16h	87%
	g	XLIg		
8			16h	59%
	h	XLIh		
9			24h	80%
	i	XLIi		

Entry	Nu-H	Product	Time	Yield[a]
10			48h	51%
	j	XLIj		
11			72h	47%
	k	XLIk		
12	$N_2H_4.H_2O$		24h	68%
	l	XLIl		
13			24h	47%
	m	XLIm		
14			24h	73%
	n	XLIn		
15			24h	69%
	o	XLIo		
16			16h	68%
	p	XLIp		
17			16h	56%
	q	XLIq		
18			24h	56%
	r	XLIr		
19			72h	38%
	s	XLIs		

[a] Isolated yields

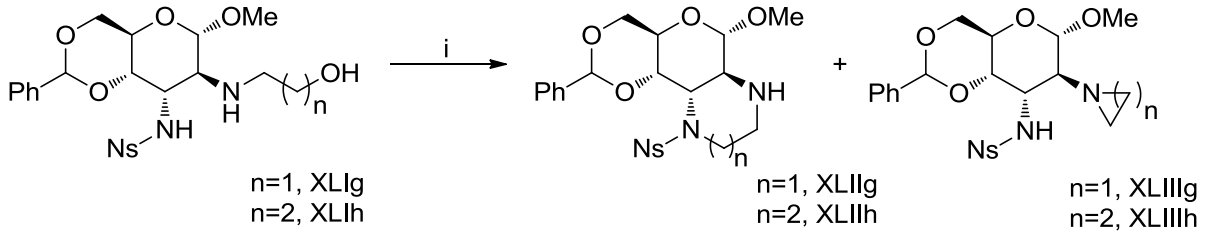
Sodium azide gave altropyranoside **XLIa** in a good yield (Entry 1). In comparison to the related *N*-substituted Hough-Richardson aziridines the ring-opening reaction was performed at 60 °C, not at conventional temperatures in a range 120-150°C.[101,106,109,110] The increased ring-opening reactivity induced by the nosyl group directly afforded amine **XLIb** with aqueous ammonia in the excellent yield (Entry 2). In comparison to classical protocols, involving the aziridine ring-opening reaction with an azide anion and essential reduction step,[101] this methodology allowed substantial simplification.

Monofunctional primary amines **c-f** provided **XLIc-f** in fair to good yield (Entry 3-6). The prolonged reaction time was utilized in reactions with **XLIc-e**. Later on, the method was extended to aminoalcohols **g-j** to yield **XLIg-j** from fair to very good yield as well (Entry 7-

10). The reaction time was necessary to extend to 48 h with amine **j**. Further increase of steric hindrance of **k** necessitated extension of reaction time to 72 h to give **XLIk** (Entry 11). The yield was predominantly reduced by the isolation process.

Preliminary attempts to cyclize nosylamides **XLIg-j** with the hydroxyl group under Fukuyama-Mitsunobu condition[192] showed the possible formation of desired products; however, significant amount of side products was observed as well (Scheme 104). We assumed that prerequisite change of the altropyranoside ring from chair to boat conformation did not occur and, consequently, both diaxialy oriented reacting groups were not redirected to the equatorial conformation. In the case of aminoalcohols **g** and **h**, the target products were detected, but the reaction mixture was complicated by formation of side products with tri- or tetra-membered rings **XLIIIg** and **XLIIIh**. Other Mitsunobu reagents such as DMEAD/TPP, DBAD/TPP or TMAD/Bu_3P did not improve the reaction. A few attempts to isolate the both products were done, but insufficient purity was achieved.

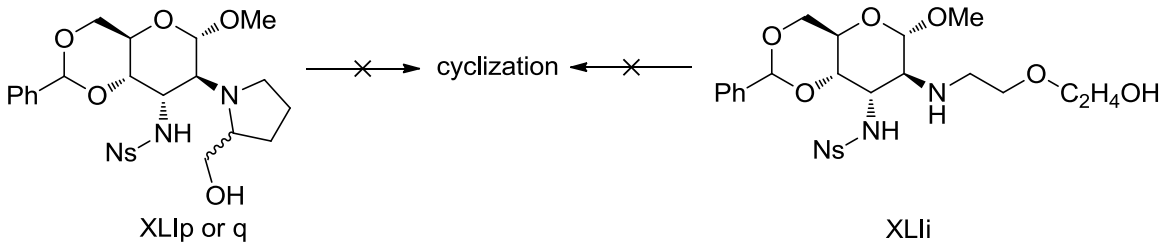
Scheme 104 - cyclization of **XLIg and XLIh**



Conditions: (i) DIAD, TPP, dioxane

In the case of prolinoles **p** and **q** and alcohol **i**, (Scheme 105) no cyclization products were observed. The unreactivity, particulary of prolinoles **p** and **q**, was caused by steric factors.

Scheme 105 - Attempted cyclization



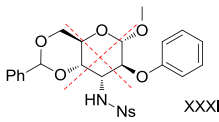
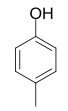
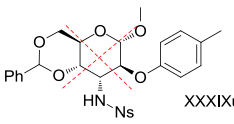
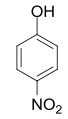
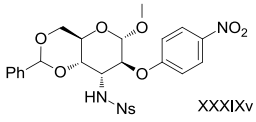
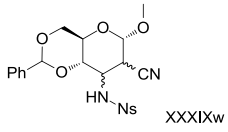
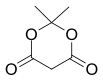
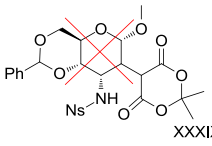
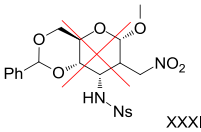
Other bifunctional nucleophiles, namely hydrazine **l** and ethylenediamine **m**, provided fair yields (Entry 12 and 13). Besides the primary amines, the reactivity of secondary amines **n-q** was also tested to give **XLIn-q** from fair to good yields (Entry 14-17). Preliminary attempts

to carry out the ring-opening reactions with anilines and aminopyridines afforded unsatisfactory yields particularly due to the low reactivity and difficulties in the isolation process. The exception was anthranilic acid **r** providing **XLIr** in fair yield (Entry 18).

In connection with the good ring-opening reactivity with hydrazine **l** (Entry 12), we also tried benzohydrazide **s** to obtain **XLIs** in a poor yield which was predominantly caused by problematic separation and decomposition during the reaction (Entry 19).

In addition to nitrogen nucleophiles, oxygen and carbon nuchleophiles were tried as well (Table 4). Since DIPEA is not a strong enough base to form correspond conjugate base, NaH instead of DIPEA was used (excluding the reaction with KCN). While phenol with electron donating group (Entry 21) provided poor yield, electron withdrawing group (Entry 22) supported reaction and product **XLIv** was isolated. In case of *C*-nucleophiles, potassium cyanide **w** allowed very good yield, however NMR analysis revealed formation of two isomers with almost identical chromatograpical data. *C*-acids **x** and **y** did not react at all.

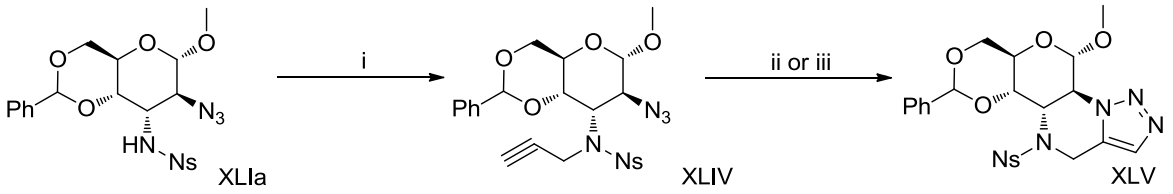
Table 4 - Reactions of dinosylated glucosamine **XXXV** with other nucleophiles

Entry	Nu	Product	Time	Yield[a]
20	 t	 XXXIXt	72h	
21	 u	 XXXIXu	72h	
22	 v	 XXXIXv	72h	40%
23	KCN w	 XXXIXw	24h	72%
24	 x	 XXXIXx	24h	
25	 y	 XXXIXy	72h	

[a] Isolated yields

To demonstrate further possible modification of nosylamide group at C3 after the ring-opening reaction, two structurally attractive compounds were tried to synthetize. The first synthesis started with azide **XLIa,** which was alkylated with propargyl alcohol under Fukuyama-Mitsunobu conditions to give **XLIV** (Scheme 105). Subsequent intramolecular copper catalyzed 1,3-dipolar cycloaddition[194] led to novel interesting scaffold **XLV** containing the 1,2,3-triazolopiperazine moiety. Similar scaffolds have been reported as glycosidase inhibitors.[195]

Scheme 105 - Synthesis of triazolopiperazine **XLV**

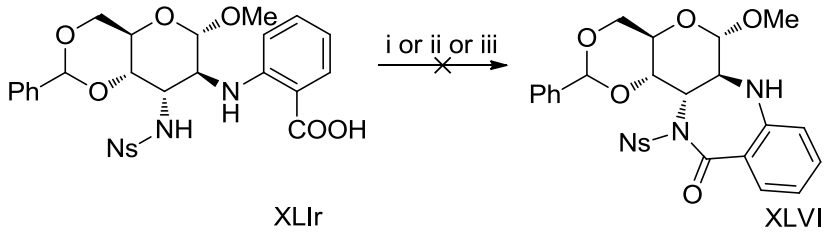


Conditions: (i) propargyl alcohol, TPP, DIAD, dioxane, 74%; (ii) Sodium Ascorbate, $CuSO_4.7H_2O$, DMF/water, 60°C, 90%; (iii) Sodium Ascorbate, $CuSO_4.7H_2O$, DMF/water, 80°C, 150W, 50%

The 1,3-dipolar cycloaddition was completed in 14 days when conventional heating was used at 60 °C. Microwave irradiation at 90 °C immensely reduced the reaction time to 3 hours, however, the yield decreased since the separation method was changed. The rate of the cycloaddition step was very likely associated with the conformation change of the pyranoside ring from chair to boat which redirects both reacting groups into the equatorial conformation.

The second attempt to modify ring-opening product was based on the preparation of benzodiazepine scaffold. Unfortunately, the target product **XLVI** was not prepared although different reaction conditions were tried.

Scheme 106 - Attempted synthesis of benzodiazepinone scaffold



Conditions: (i) DIC, DMAP (cat), DCM; (ii) CDI, DMF; (iii) T3P, DIPEA, DMF

In conclusion, the synthesis of the new *N*-*p*-nosyl Hough-Richardson aziridine was described. We demonstrated that this valuable advanced intermediate can be transformed by highly regioselective *trans*-diaxial ring-opening reactions with nitrogen nucleophiles into the corresponding α-D-altropyranosides. The increased ring-opening reactivity induced by the nosyl group allowed the direct synthesis of the aminoaltropyranoside even with aqueous ammonia in the excellent yield in comparison to the conventional methods based on the ring-opening reaction with azide anion and subsequent reduction of the azide functionality. Sodium azide, primary and secondary amines, hydrazine, and benzohydrazide provided

altropyranosides in fair to very good yield. Further work to extend the scope of the present study is in progress.

5 Conclusion

D-Glucosamine based derivatives represent highly important class of compounds with tremendous interesting properties. D-Glucosamine is a part of hyaluronic acid, well known for its anti-inflammatory properties or it is a building block of peptidoglycans, which are responsible for strength of bacteria cell walls. The significant importance of D-Glucosamine units in the biological systems iniciated the reactivity study of D-Glucosamine at position 1, 2, and 3.

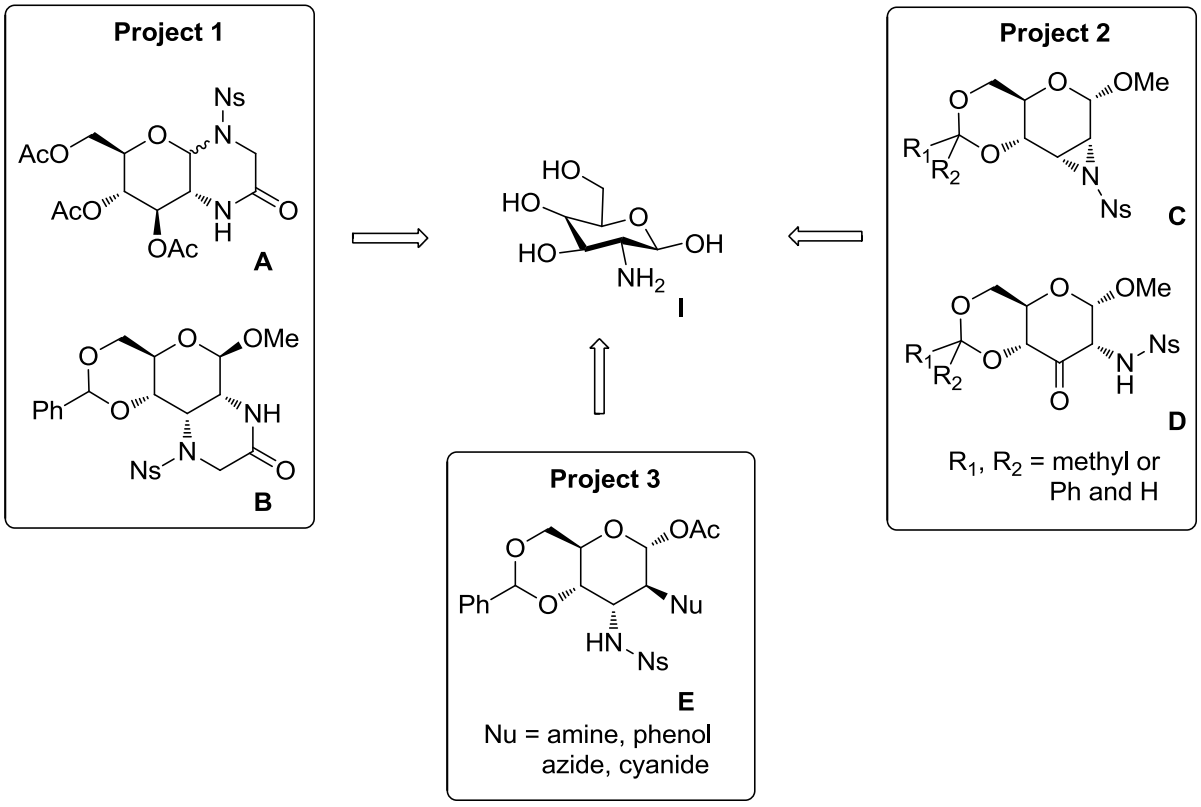
Experimental results of this thesis were divided into three main projects according to the type of the desired final product. The first project was focused on preparation of fused bicycles. The cycle could be fused to C1 and C2 carbon (Product **A**) or to C2 and C3 carbon (Product **B**). At the very beginning, it was necessary to prepare intermediate, which allows the cyclization reactions. Beside plenty of methods, HOSu/EDC mediated acylation gave the best results. Further, the cyclization reactions were investigated. It was find out, that cyclization to C1 carbon under Fukuyama-Mitsunobu conditions took place problematically, with many side products and with poor yields. In many cases, the separation was not possible due to low stability of products. When the cyclization was attempted to C3, the product **B** was not observed at all.

The second project reported the transformations of the amino and hydroxyl group of D-glucosamine at position 2 and 3 to obtain more suitable functionalities for potential formation of an *ortho*-fused ring. It was necessary to protect hydroxyls at positions 1, 4, and 6, and then modify amino and hydroxyl group at position 2 and 3. A key intermediate, containing the free hydroxyl group, was prepared via two different synthetic routes. Later, it was studied the reactivity of the nosyl group toward Fukuyama-Mitsunobu alkylation and possible formation of aziridine **C** as a result of a side intramolecular reaction with the hydroxyl at position 3. In the end, attention was paid to the oxidation of the hydroxyl at position 3 into the keto functionality resulting in derivative **D**. Subsequent condensation reactions revealed, that the reactivity of the keto derivate **D** towards common nucleophilic reagents is suppressed, probably due to steric hinderence.

The third project described the synthesis of aziridine, which was *in-situ* prepared from the corresponding 2-*O*, 3-*N*-dinosyl derivate. The addition of a nucleophile to the reaction mixture resulted in the ring-opening of the aziridine at C2 carbon and formation of 2,3-*altro*-

configuration (Product **E**). Plenty of nucleophile reagents were tried and it was find out, that this method is general for nearly all nitrogen nucleophiles. *O*- and *C*- nucleophiles required more basic conditions and afforded poorer yields.

In conclusion, we investigated the reactivity of suitably substituted D-glucosamine toward to cyclization, alkylation, oxidation and substitution reactions. It was find out, that cyclization is difficult or did not take place at all. The alkylation and oxidation reactions take place with a few limitations, however further research of condensation reactions to keto derivate will be necessary. Introducing of amine to C2 carbon is possible without any complication through *in-situ* prepared aziridine. This method is quite general and very mild conditions and high yields of reactions significantly extend the approach to 2,3-diamino derivates based on the structure **E**.



6 Experimental part

6.1 Material and methods

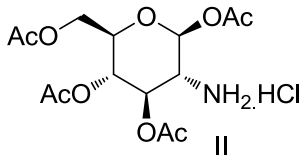
Characteristic of LC/MS: The LC/MS analyses were carried out on UHPLC-MS system consisting of UHPLC chromatograph Accela with photodiode array detector and triple quadrupole mass spectrometer TSQ Quantum Access (both Thermo Scientific, CA, USA), using Nucleodur Gravity C18 column at 30°C and flow rate of 800 μL/min (Macherey-Nagel, 1.8 μm, 2.1 × 50 mm, Germany). Mobile phase was (A) 0.1% ammonium acetate in water, and (B) 0.1% ammonium acetate in acetonitrile, linearly programmed from 10% to 80% B over 2.5 min, kept for 1.5 min. The column was re-equilibrated with 10% of solution B for 1 min. The APCI source operated at discharge current of 5 μA, vaporizer temperature of 400°C and capillary temperature of 200°C.

Melting point: Melting points were measured by capilar melt station Stuart SMP 30

Chemicals: Starting materials were purchased from commercial sources Sigma Aldrich, Carbosynth and Across.

6.2 Procedures

*(2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-aminotetrahydro-2H-pyran-2,4,5-triyl triacetate hydrochloride **II**.*



II

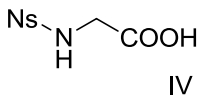
Glucosamine hydrochloride (30g, 139.3mmol) was dissolved in 1 M NaOH (137.5mL) and stirred for 5 minutes. Subsequently, solution of *p*-Anisaldehyde (16.9mL, 139.3mmol) in methanol (10mL) was carefully added and mixture was intensively stirred. The system was kept in ice bath for 2 h. Then the solid was filtered, washed by water (2x40mL) and methanol/diethylether (1:1, 2x30mL). The precipitate was dried under vacuum to yield glucose aldimine (33.3g, 81%) as a white solid.

Glucose aldimine (33.3g, 112.1mmol) was dissolved in pyridine (183mL) and cooled to 0 °C. Then Ac_2O (101.3mL) and catalytic amount of DMAP (337mg, 2.76mmol) was added. The

solid was dissolved and the reaction mixture was kept at rt. After 16 h, the reaction mixture was poured onto ice forming a white crystalline solid. The crystals were washed with water (2x30mL), diethylether (2x20mL) and dried under vacuum. The Tetra-*O*-acetylglucosealdimin was yielded as a white solid (46.9g, 76%).

Tetra-*O*-acetylglucosealdimin (46.9g, 100.9mmol) was dissolved under reflux in aceton (230mL) and subsequently 5 N HCl (23mL) was added dropwise. After the whole portion of HCl was added, the mixture was removed from heater and was kept on ice bath. The white precipitate was filtred and washed with acetone (2x30mL), ether (2x30mL) and cold ethyl acetate (3x30mL). The white precipitate was dried under vacuum. Tetra-*O*-acetylglucoseamine **II** was yielded as a white solid (28.2g, 73%). The overall yield of reaction is 52.8%. ^{1}H NMR (400MHz, DMSO-d_6) δ = 8.91 (br. s., 2 H), 5.93 (d, *J* = 8.3 Hz, 1 H), 5.37 (dd, *J* = 9.4, 10.3 Hz, 1 H), 4.92 (t, *J* = 9.4 Hz, 1 H), 4.18 (dd, *J* = 3.9, 12.3 Hz, 1 H), 4.07 - 3.92 (m, *J* = 15.8 Hz, 2 H), 3.54 (dd, *J* = 8.8, 10.5 Hz, 1 H), 2.17 (s, 3 H), 2.02 (s, 3 H), 1.99 (s, 3 H), 1.97 (s, 3 H); 13C NMR (101MHz ,DMSO-*d6*) δ = 170.0, 169.8, 169.3, 168.7, 90.1, 71.6, 70.3, 67.8, 61.3, 52.1, 21.0, 20.9, 20.5, 20.4

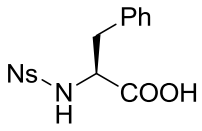
2-(4-nitrophenylsulfonamido)acetic acid ***IV.***



IV

$Na_2HPO_4.12H_2O$ (65.7g, 183mmol) was dissolved in water (460mL) and the solution was cooled to 5 °C. Subsequently, glycine (3.44g, 46mmol) and 4-NsCl (10.22g, 46mmol) was added. The reaction mixture was stirred for 2 hours; pH was kept at 9 by adding of 1 M NaOH. The crude mixture was extracted with EtOAc (3x250mL). The water layer was acidified with 1 M HCl to pH 2, then was the mixture extracted with EtOAc (3x200mL), brine (150mL) and dried with Na_2SO_4. The organic phase was evaporated; the crude product was recrystalled from MeOH affording **IV** as a yellow solid (9.76g, 82%). ^{1}H NMR (400MHz, DMSO-d_6) δ = 12.75 (br. s., 1 H), 8.48 (t, *J* = 5.3 Hz, 1 H), 8.39 (td, *J* = 2.6, 9.6 Hz, 2 H), 8.05 (td, *J* = 2.2, 9.2 Hz, 2 H), 3.70 (d, *J* = 5.3 Hz, 2 H); ^{13}C NMR (101MHz, DMSO-d_6) δ = 170.1, 149.5, 146.5, 128.1, 124.4, 43.7.

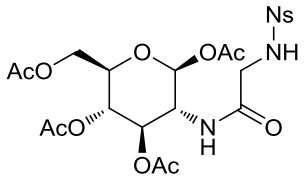
(S)-2-(4-nitrophenylsulfonamido)-3-phenylpropanoic acid ***VI.***



VI

$Na_2HPO_4.12H_2O$ (5.73g, 16mmol) was dissolved in water (40mL) and the solution was cooled to 5 °C. Subsequently, L-phenylalanine (661mg, 4mmol) and 4-NsCl (890mg, 4mmol) was added. The reaction mixture was stirred for 2 hours; pH was kept at 9 by adding of 1 M NaOH. The crude mixture was extracted with EtOAc (3x20mL). The water layer was acidified with 1 M HCl to pH 2, then was the mixture extracted with EtOAc (3x20mL), brine (15mL) and dried with Na_2SO_4. The organic phase was evaporated; the crude product was recrystallized from MeOH affording **VI** as a yellow solid (1.02g, 73%). ^{1}H NMR (400MHz, DMSO-*d6*) δ = 12.87 (br. s., 1 H), 8.74 (d, J = 9.2 Hz, 1 H), 8.20 (td, J = 2.6, 9.6 Hz, 2 H), 7.74 (td, J = 2.6, 9.2 Hz, 2 H), 7.27 - 6.85 (m, 5 H), 3.96 (dt, J = 4.8, 9.4 Hz, 1 H), 2.99 (dd, J = 4.8, 13.6 Hz, 1 H), 2.71 (dd, J = 10.1, 13.6 Hz, 1 H); ^{13}C NMR (101MHz, DMSO-d_6) δ = 172.2, 149.1, 146.6, 136.7, 129.2, 128.1, 127.7, 126.4, 124.2, 57.7, 37.6.

(2R,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(2-(4-nitrophenylsulfonamido)acetamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate ***VII***.



VII

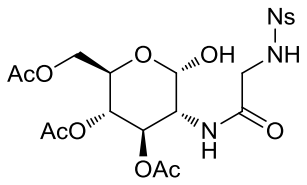
<u>The procedure with EDC/HOSu</u>

Under N_2 atmosphere, acetylated glucosamine **II** (4.03g, 10mmol) in pyridine (33mL, dry) was dissolved, DIPEA (7.33mL) was added and the mixture was stirred for 1 hour in ice bath. In the second flask, nosylated glycine **IV** (2.1g, 8.1mmol) was dissolved in pyridine (33mL, dry), then EDC (2.07mL, 12mmol) and HOSu (1.12g, 10mmol) was added and the mixture was cooled to 0 °C. After cooling, the solution with EDC/HOSu was added into the flask with glucosamine, and the mixture was stirred for 24 hours. Then pyridine was removed under reduced pressure, the residue was dissolved in EtOAc (60mL) and extracted with 5%HCl (3x90mL). The organic layer was washed with brine (30mL), dried with Na_2SO_4 and evaporated. The crude product was recrystallized from MeOH affording the title compound **VII** as a yellow solid (1.67g, 34%).

The procedure with T3P

Acetylated glucosamine **II** (1g, 2.6mmol) was dissolved in dry DMF (30mL), then DIPEA (2.7mL) and nosylated glycine (711mg, 2.7mmol) was added. The mixture was cooled to 5 °C and T3P (50% in DMF, 2.5mL) was added in one portion. The mixture was stirred for 24 hours, then the reaction was quenched with water (100mL), the mixture was acidified to pH=3 and extracted to EtOAc (3x100mL). The organic layer was washed with water (50mL), brine (50mL), dried with Na_2SO_4 and concentrated under reduced pressure. The residue was recrystallized from MeOH affording **VII** as a yellow solid (0.69g, 45%). ^{1}H NMR (400MHz, DMSO-d_6) δ = 8.50 (t, *J* = 5.9 Hz, 1 H), 8.41 (td, *J* = 2.2, 9.2 Hz, 2 H), 8.10 (d, *J* = 9.2 Hz, 1 H), 8.03 (td, *J* = 2.2, 9.2 Hz, 2 H), 5.76 (d, *J* = 8.8 Hz, 1 H), 5.24 (dd, *J* = 9.4, 10.3 Hz, 1 H), 4.87 (t, *J* = 9.4 Hz, 1 H), 4.19 (dd, *J* = 4.8, 8.3 Hz, 1 H), 4.02 - 3.88 (m, 3 H), 3.51 - 3.34 (m, *J* = 6.1, 7.9 Hz, 2 H), 2.02 (s, 3 H), 2.00 (s, 3 H), 1.97 (s, 3 H), 1.89 (s, 3 H); ^{13}C NMR (101MHz ,DMSO-d_6) δ = 170.5, 170.0, 169.7, 169.3, 168.5, 150.0, 146.3, 128.6, 124.9, 92.0, 72.3, 71.9, 68.6, 61.9, 52.3, 45.3, 21.0, 20.9, 20.8, 20.8.

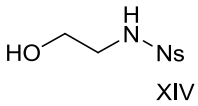
(2R,3S,4R,5R,6S)-2-(acetoxymethyl)-6-hydroxy-5-(2-(4-nitrophenylsulfonamido)acetamido)tetrahydro-2H-pyran-3,4-diyl diacetate **XI** (**α-anomer**).



XI

Amide **VII** (1g, 1.7mmol) was dissolved in dry THF (10mL) and 2 M solution of $MeNH_2$ in MeOH (2mL) was added and the mixture was stirred for 70 min. Then the solvent was removed under reduced pressure and purification on silica gel (DCM/MeOH = 50/1) allowed separation of anomers. α-anomer can be also isolated by recrystallization from MeOH. The purification on silica allowed α-anomer as a white solid (560mg, 60%). The recrystallization of residue allowed α-anomer as a white solid (550mg, 59%). ^{1}H NMR (400MHz, DMSO-*d6*) δ = 8.44 - 8.29 (m, 3 H), 8.02 (td, J = 2.2, 9.2 Hz, 2 H), 7.89 (d, J = 9.2 Hz, 1 H), 7.26 (dd, J = 1.1, 4.6 Hz, 1 H), 5.13 (dd, J = 9.4, 10.7 Hz, 1 H), 4.92 (t, J = 3.9 Hz, 1 H), 4.83 (t, J = 9.4 Hz, 1 H), 4.17 - 4.06 (m, 2 H), 4.04 - 3.87 (m, 2 H), 3.68 - 3.46 (m, 2 H), 2.00 (s, 3 H), 1.96 (s, 3 H), 1.87 (s, 3 H); ^{13}C NMR (101MHz, DMSO-*d6*) δ = 170.1, 169.8, 169.3, 167.8, 149.5, 146.3, 128.1, 124.4, 90.6, 70.2, 68.9, 66.5, 62.2, 51.5, 44.6, 20.6, 20.4, 20.4.

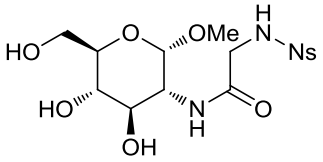
*N-(2-hydroxyethyl)-4-nitrobenzenesulfonamide **XIV**.*



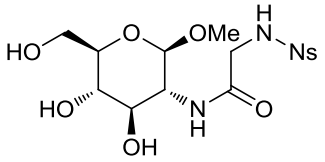
XIV

Aminoethanol (200ηL, 3.3mmol) was dissolved in dry DCM (8mL) and the mixture was cooled to 5° C. Then Et_3N (690ηL) and 4-NsCl (741mg, 3.4mmol) was added, the temperature was slowly increased to rt. After 3 hours, reaction was quenched with saturated $NaHCO_3$ (10mL), organic layer was removed and water phase was extracted with DCM (4x20mL). Combined organic phases were washed with brine (10mL), dried with Na_2SO_4 and concentrated under reduced pressure. Recrystallization from MeOH/water afforded **XIV** as a yellow solid (710mg, 87%). ^{1}H NMR (400MHz, DMSO-*d6*) δ = 8.41 (d, J = 8.7 Hz, 2 H), 8.04 (m, 3 H), 4.73 (t, J = 5.5 Hz, 1 H), 3.37 (q, J = 6.0 Hz, 2 H), 2.86 (q, J = 6.0 Hz, 2 H); ^{13}C NMR (101MHz, DMSO-*d6*) δ = 149.5, 146.3, 128.1, 124.5, 59.8, 45.1.

*N-((2S,3R,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2H-pyran-3-yl)-2-(4-nitrophenylsulfonamido)acetamide **XVIIa (α-anomer)** and N-((2R,3R,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-2-methoxytetrahydro-2H-pyran-3-yl)-2-(4-nitrophenylsulfonamido)acetamide **XVIIb (ß-anomer)**.*



XVIIa

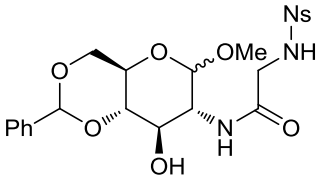


XVIIb

Amide **VII** (100mg, 0.17mmol) was dissolved in freshly prepared NaOMe (1mg~1mL MeOH, 4mL) and the mixture was stirred for 4 hours (the conversion was monitored with TLC). Then dry IR 120H$^+$ (240mg) was added and the mixture was refluxed for 3 hours. Subsequently, the resin was filtered off, the solvent was removed under reduced pressure and purification on silica gel ($CHCl_3$/MeOH=7/1) allowed the separation of anomers. **α-anomer** was gained as a white solid (31mg, 42%) and **ß-anomer** was separated as a white solid (14mg, 19%). **α-anomer:** ^{1}H NMR (500MHz, DMSO-*d6*) δ = 8.39 (d, J = 9.2 Hz, 2 H), 8.32 (br. s., 1 H), 8.04 (d, J = 9.2 Hz, 2 H), 7.81 (d, J = 8.6 Hz, 1 H), 5.00 (d, J = 5.7 Hz, 1 H), 4.79 (d, J = 5.2 Hz, 1 H), 4.50 (t, J = 5.7 Hz, 1 H), **4.40 (d, J = 3.4 Hz, 1 H)**, 3.66 - 3.59 (m, 3 H), 3.55 (m, 1 H), 3.49 - 3.35 (m, 2 H), 3.32 - 3.25 (m, 1 H), 3.21 (s, 3 H), 3.10 (dt, J = 6.0, 9.0 Hz, 1 H); ^{13}C NMR (126MHz, DMSO-*d6*) δ = 167.3, 149.5, 146.2, 128.2, 124.3, 97.7, 72.8,

70.7, 70.6, 60.7, 54.3, 53.8, 44.9. **ß-anomer:** ^{1}H NMR (500MHz, DMSO-*d6*) δ = 8.39 (d, J = 9.2 Hz, 2 H), 8.31 (br. s., 1 H), 8.05 (d, J = 9.2 Hz, 2 H), 7.82 (d, J = 8.6 Hz, 1 H), 4.99 (d, J = 5.2 Hz, 1 H), 4.88 (d, J = 5.2 Hz, 1 H), 4.52 (t, J = 6.0 Hz, 1 H), **4.16 (d, J = 8.0 Hz, 1 H)**, 3.72 - 3.63 (m, 1 H), 3.53 (dd, J = 16.6, 34.9 Hz, 2 H), 3.48 - 3.41 (m, 1 H), 3.40 - 3.34 (m, 1 H), 3.28 (s, 3 H), 3.27 - 3.20 (m, 1 H), 3.11 - 2.99 (m, 2 H); ^{13}C NMR (126MHz, DMSO-d_6) δ = 166.9, 149.5, 146.1, 128.2, 124.4, 101.6, 77.0, 74.2, 70.4, 61.0, 55.6, 55.3, 45.3.

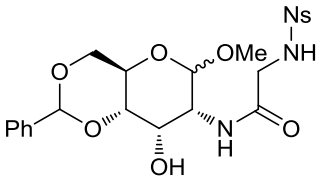
N-((4aR,7R,8R,8aS)-8-hydroxy-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)-2-(4-nitrophenylsulfonamido)acetamide ***XVIII***.



XVIII

Deprotected glucosamine **XVIIa** (265mg, 0.58mmol) was dissolved in dry DMF (12mL), then TsOH.H_2O (12.3mg, 0.065mmol) and benzaldehyde dimethylacetal was added. The mixture was refluxed for 2 hours, then TsOH.H_2O was neutralized by Et_3N (10ηL), the solvent was removed under reduced pressure and the purification of residue on silica gel (EtOAc/hex = 1/1) allowed the title compound as a mixture of isomers.

N-((4aR,7R,8S,8aS)-8-hydroxy-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)-2-(4-nitrophenylsulfonamido)acetamide ***XX***. (Inversion of configuration on C3 carbon)

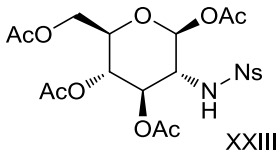


XX

Protected glucosamine **XVIII** (100mg, 0.19mmol) was dissolved in dry THF (4mL), then TPP (100mg, 0.382mmol) and DIAD (75ηL, 0.382mmol) was added and after the flask was cooled to 5 °C, benzoic acid (47mg, 0.382mmol) was poured into the flask in one portion. After 24 hours, the solvent was removed under reduced pressure and anomeric mixture of esters was isolated on silica gel. Without further characterization, the whole portion of ester was dissolved in MeOH (5mL) and K_2CO_3 was added. The mixture was refluxed for one hour, then the solvent was evaporated, the residue was extracted to EtOAc (3x10mL),

collected organic layers were washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The reaction afforded **XX** as a yellow solid (65mg, 65%).

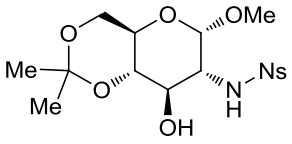
*(2S,3R,4R,5S,6R)-6-(acetoxymethyl)-3-(4-nitrophenylsulfonamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate **XXIII**.*



XXIII

Tetra-*O*-acetyl glucosamine hydrochloride **II** (20g, 52mmol) was dissolved in DCM/pyridine (300/150mL) and Et_3N (7.5mL) was added; the mixture was cooled to 5 °C and stirred for 10 minutes. Then 4-NsCl (23g, 104mmol) was poured into the flask in one portion, the temperature was increased to rt and mixture was stirred. After 20 hours, solvents were evaporated under reduced pressure and the recrystallization from MeOH/water (3/1) allowed the title compound as a yellow solid (22.05g, 79%). ^{1}H NMR (400MHz, DMSO-*d6*) δ = 8.68 (d, J = 8.8 Hz, 1 H), 8.42 (td, J = 2.6, 9.3 Hz, 2 H), 8.05 (td, J = 2.3, 9.3 Hz, 2 H), 5.57 (d, J = 8.6 Hz, 1 H), 5.11 (dd, J = 9.5, 10.3 Hz, 1 H), 4.89 (t, J = 9.7 Hz, 1 H), 4.15 (dd, J = 4.4, 12.5 Hz, 1 H), 4.08 - 3.98 (m, 1 H), 3.93 (dd, J = 2.1, 12.5 Hz, 1 H), 3.55 (td, J = 8.7, 10.3 Hz, 1 H), 1.98 (s, 3 H), 1.96 (s, 3 H), 1.78 (s, 3 H), 1.64 (s, 3 H); ^{13}C NMR (101MHz, DMSO-*d6*) δ = 170.0, 169.4, 169.2, 168.4, 149.5, 147.6, 128.0, 124.6, 91.5, 72.2, 71.4, 67.9, 61.3, 56.1, 20.5, 20.4, 20.2, 20.0.

*N-((4aR,6S,7R,8R,8aS)-8-hydroxy-6-methoxy-2,2-dimethylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)-4-nitrobenzenesulfonamide **XXIVa**.*

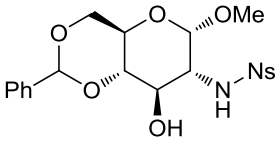


XXIVa

Nosylated tetra-*O*-acetyl glucosamine **XXIII** (0.5g, 0.95 mmol) was dissolved in dry MeOH (10mL) and MeONa/MeOH (5mL, 1mg Na/1mL MeOH) was added. The mixture was refluxed for 2.5 hours, then dry IR 120H$^+$ (1g) was added and the mixture was refluxed for another 2 hours. After TLC showed full deprotection, the mixture was filtered and solvent was evaporated. Without separation of anomers, the residue was dissolved in dry dioxane (20mL), then 2,2-dimethoxypropane (650ηL, 5.3mmol) and IR 120H$^+$ (600mg) was added and the mixture was stirred for 24 hours. The filtration and purification on silica gel

EtOAc/hexane = 2/3) afforded **XXIVa** as a white solid (0.15g, 39% from 2 steps). 1H NMR (400MHz, DMSO-*d6*) δ = 8.42 (d, J = 9.3 Hz, 1 H), 8.39 (dd, J = 2.1, 8.8 Hz, 2 H), 8.07 (dd, J = 2.6, 8.8 Hz, 2 H), 5.37 (d, J = 5.2 Hz, 1 H), 4.60 (d, J = 5.2 Hz, 1 H), 4.16 (q, J = 6.2 Hz, 1 H), 4.05 - 3.96 (m, 1 H), 3.94 - 3.84 (m, 2 H), 3.73 (dd, J = 6.7, 8.3 Hz, 1 H), 3.61 - 3.52 (m, 1 H), 2.96 (s, 3 H), 1.28 (s, 3 H), 1.23 (s, 3 H); ^{13}C NMR (101MHz, DMSO-*d6*) δ = 149.4, 147.4, 128.1, 124.2, 107.8, 100.3, 77.7, 73.3, 73.0, 65.1, 63.4, 54.6, 26.4, 25.2.

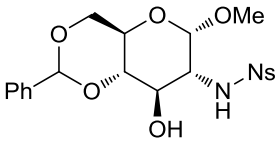
N-((4aR,6S,7R,8R,8aS)-8-hydroxy-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)-4-nitrobenzenesulfonamide ***XXIVb.****(Route A)*



XXIVb

Nosylated tetra-*O*-acetyl glucosamine **XXIII** (2g, 3.8 mmol) was dissolved in dry MeOH (30mL) and MeONa/MeOH (18.4mL, 1mg Na/1mL MeOH) was added. The mixture was refluxed for 2.5 hours, then dry IR 120H$^+$ (4.8g) was added and the mixture was refluxed for another 2 hours. After TLC showed full deprotection, the mixture was filtered and solvent was evaporated. Without separation of anomers, the residue was dissolved in dry dioxane (30mL), then BDA (2mL, 13 mmol) and IR 120H$^+$ (3g) was added and the mixture was heated for 6 hours. The filtration and purification on silica gel $CHCl_3$/MeOH = 12/1) afforded **XXIVb** as a white solid (0.7g, 40% from 2 steps). ^{1}H NMR (400MHz, DMSO-*d6*) δ = 8.48 (br. s., 1 H), 8.35 (td, J = 2.6, 8.8 Hz, 2 H), 8.08 (td, J = 2.6, 8.8 Hz, 2 H), 7.44 - 7.31 (m, 5 H), 5.54 (s, 1 H), 5.14 (d, J = 5.7 Hz, 1 H), 4.50 (d, J = 3.6 Hz, 1 H), 4.15 (dd, J = 4.7, 9.9 Hz, 1 H), 3.68 (t, J = 9.9 Hz, 1 H), 3.62 - 3.47 (m, 2 H), 3.39 (t, J = 9.3 Hz, 1 H), 3.29 - 3.21 (m, 1 H), 3.19 (s, 3 H); ^{13}C NMR (101MHz, DMSO-*d6*) δ = 149.2, 147.8, 137.6, 128.9, 128.1, 128.0, 126.3, 124.0, 100.7, 99.6, 81.6, 67.9, 67.5, 62.3, 58.6, 54.8.

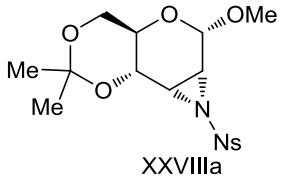
N-((4aR,6S,7R,8R,8aS)-8-hydroxy-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)-4-nitrobenzenesulfonamide ***XXIVb.****(Route B)*



XXIVb

N-acetyl glucosamine XX**II** (15g, 67.8mmol) was dissolved in dry MeOH (150mL) and dry IR 120H^+ (15g) was added. The mixture was refluxed for 8 hours, then the resin was filtered off and MeOH was evaporated under reduced pressure. The residue, which contains both α and ß anomers (5:1 toward α anomer, estimated from NMR), was dissolved in dioxane (400mL), then BDA (19mL, 126mmol) and TsOH.H_2O (2.14g, 11mmol) was added and the mixture was stirred at 60°C. After 6 hours, the solvent was evaporated under reduced pressure and recrystallization from hot EtOAc gave exclusively α-anomer **XXVI** as a white solid (9g, 50% from 2 steps). Subsequently, **XXVI** (9g, 27.8mmol) was suspended in dry EtOH (225mL) then 50g KOH was added and the mixture was refluxed for 4 hours. After TLC showed full conversion, DCM (225mL) and water (350mL) was added and the organic layer was washed with water (2x300mL) dried with Na_2SO_4 and evaporated. Purification on silica gel ($CHCl_3$/MeOH = 10/1) afforded **XXVII** as a white solid (6.6g, 84%). ^{1}H NMR (400MHz, DMSO-*d6*) δ = 7.47 - 7.42 (m, 2 H), 7.39 - 7.35 (m, 3 H), 5.58 (s, 1 H), 5.23 (br. s, 1 H), 4.62 (d, J = 3.4 Hz, 1 H), 4.16 (dd, J = 4.7, 9.9 Hz, 1 H), 3.71 (t, J = 10.1 Hz, 1 H), 3.64 - 3.55 (m, 1 H), 3.40 - 3.36 (m, 2 H), 3.32 (s, 3 H), 2.56 - 2.52 (m, 1 H), 1.41 (br. s, 2 H); ^{13}C NMR (101MHz, DMSO-*d6*) δ = 137.9, 128.8, 128.0, 126.4, 100.9, 100.8, 81.6, 71.5, 68.2, 62.8, 57.1, 54.8. *Nosylation of **XXVII***: Protected glucosamine **XXVII** (1g, 3.56mmol) was dissolved in DCM/pyridine (40/20mL) and the mixture was cooled to 5 °C. Then, 4-NsCl (828mg, 3.74mmol) was added and the mixture was stirred for 20 hours. After TLC showed full conversion, solvents were removed under reduced pressure; the residue was dissolved in EtOAc (100mL) and washed with water (3x100mL). Organic layer was dried with brine and Na_2SO_4 and evaporation under reduced pressure gave the title compound **XXIVb** as a white solid (709mg, 86%). The NMR spectra are identical to those with *Route A*.

*(4aR,6S,6aR,7aS,7bS)-6-methoxy-2,2-dimethyl-7-((4-nitrophenyl)sulfonyl)hexahydro-4H-[1,3]dioxino[4',5':5,6]pyrano[3,4-b]azirine **XXVIIIa***.

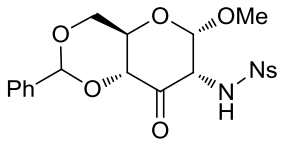


XXVIIIa

DIAD (96ηL, 0.48mmol) and TPP (125mg, 0.48mmol) were dissolved in dry dioxane (6mL) and glucosamine derivate **XXIVb** (100mg, 0.24mmol) was added. The mixture was stirred at rt for 20 hours, then solvent was removed under reduced pressure and purification on silica

gel (EtOAc/hexane = 2/1) afforded the title compound **XXVIIIa** as a yellow solid (60mg, 63%). ^{1}H NMR (400MHz, DMSO-*d6*) δ = 8.46 (td, J = 2.6, 9.3 Hz, 2 H), 8.24 (td, J = 2.1, 9.3 Hz, 2 H), 5.23 (d, J = 1.6 Hz, 1 H), 4.14 - 3.93 (m, 4 H), 3.81 (dd, J = 4.4, 9.1 Hz, 1 H), 3.66 (d, J = 5.2 Hz, 1 H), 3.24 (s, 3 H), 1.33 (s, 3 H), 1.26 (s, 3 H); ^{13}C NMR (101MHz, DMSO-*d6*) δ = 150.6, 142.7, 129.2, 124.8, 109.2, 102.7, 79.2, 75.2, 65.3, 56.1, 45.6, 44.7, 26.3, 24.8.

*N-((4aR,6S,7S,8aR)-6-methoxy-8-oxo-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)-4-nitrobenzenesulfonamide **XXIX**.*



XXIX

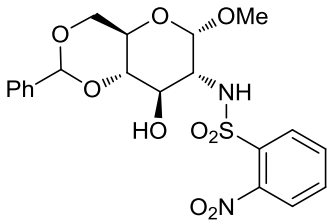
Method with T3P

XXIVb (1.18g, 2.5 mmol) was dissolved in dry EtOAc (24mL) and the solution was cooled to 5°C. Then, T3P (3.8mL, 50% in EtOAc) and DMSO (360ηL) was added and the temperature was increased to 60°C. After 2 hours, the solution was diluted with EtOAc (100mL) and water (200mL) was added. The organic layer was washed with water (2x100mL), dried with brine (100mL) and Na_2SO_4 and evaporated under reduced pressure. The purification on silica gel (EtOAc/hexane = 1/2) afforded the title compound as a white solid (0.82g, 69%).

Method with Dess Martin periodinane (DMP)

XXIVb (100mg, 0.107mmol) was dissolved in DCM (2mL) and DMP (136mg, 0.32mmol) was added. Subsequently, solution of DCM (2mL) and water (4.2ηL, 1.1equiv) was added and the mixture was stirred for 4 hours. When TLC showed full conversion, the mixture was washed with water (3x5mL), saturated $NaHCO_3$ (5mL), 10% $Na_2S_2O_3$ and organic layer was dried with Na_2SO_4. Evaporation gave the title compound **XXIX** as a white solid (55mg, 55%). 1H NMR (400MHz ,DMSO-*d6*) δ = 8.85 (d, J = 9.3 Hz, 1 H), 8.37 (td, J = 2.6, 9.3 Hz, 2 H), 8.12 (td, J = 2.1, 9.3 Hz, 2 H), 7.43 - 7.32 (m, 5 H), 5.61 (s, 1 H), 5.01 (d, J = 4.2 Hz, 1 H), 4.76 - 4.65 (m, 2 H), 4.32 (dd, J = 4.7, 9.9 Hz, 1 H), 3.90 (t, J = 9.9 Hz, 1 H), 3.78 (dt, J = 4.7, 9.9 Hz, 1 H), 3.25 (s, 3 H); 13C NMR (101MHz ,DMSO-*d6)* δ = 195.1, 149.4, 147.2, 136.9, 129.2, 128.1, 126.3, 124.2, 102.5, 100.6, 81.1, 68.3, 65.3, 61.9, 55.0.

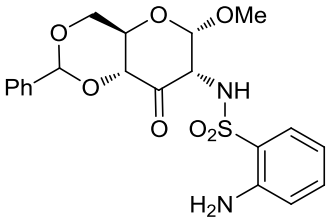
*N-((4aR,6S,7R,8R,8aS)-8-hydroxy-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)-2-nitrobenzenesulfonamide **XXXII**.*



XXXII

Protected glucosamine **XXVII** (250mg, 0.89mmol) was dissolved in DCM/pyridine (10/5mL) and the solution was cooled to 5°C. Then 2-NsCl (207mg, 0.94mmol) was added and the temperature was increased to rt. After 20 hours, solvents were removed under reduced pressure; the residue was dissolved in EtOAc (30mL) and washed with water (3x30mL). The organic layer was dried with brine (20mL) and Na2SO4, the solvent was evaporation afforded the title compound **XXXII** as a yellow solid (380mg, 92%). 1H NMR (400MHz, DMSO-d6) δ = 8.37 (br. s., 1 H), 8.24 - 8.13 (m, 1 H), 7.96 - 7.87 (m, 1 H), 7.85 - 7.75 (m, 2 H), 7.47 - 7.30 (m, 5 H), 5.57 (s, 1 H), 5.38 (d, J = 5.7 Hz, 1 H), 4.49 (d, J = 3.6 Hz, 1 H), 4.15 (dd, J = 4.7, 9.9 Hz, 1 H), 3.75 - 3.63 (m, 2 H), 3.56 (dt, J = 5.2, 9.9 Hz, 1 H), 3.44 (t, J = 9.3 Hz, 1 H), 3.32 - 3.27 (m, 1 H), 3.17 (s, 3 H); 13C NMR (101MHz, DMSO-d6) δ = 137.6, 136.1, 133.9, 133.7, 132.2, 129.9, 128.9, 128.0, 126.3, 123.9, 100.8, 99.3, 81.6, 67.8, 67.3, 62.4, 58.5, 54.9.

*2-amino-N-((4aR,6S,7S,8aR)-6-methoxy-8-oxo-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)benzenesulfonamide **XXXIII**.*

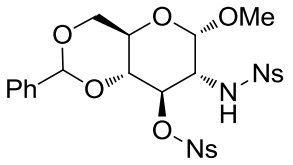


XXXIII

2-Nosylated glucosamine **XXXII** (100mg, 0.21mmol) was dissolved in EtOAc (2ml), DMSO (30ηL) was added and the solution was cooled to 5°C. Then T3P (320ηL, 50% in EtOAc) was added and the temperature was increased to 60°C. After 20 hours, EtOAc (10mL) was added and the mixture was washed with water (3x20mL). Organic layer was dried with brine and Na_2SO_4 and evaporation under reduced pressure gave oxidized product as a yellow solid (78mg, 78%). Without further characterization, the residue was dissolved in THF (2mL) and Pd/C (20mg) was carefully added. Balloon with H_2 served as a source of hydrogen. The mixture was severely stirred for 16 hours, then catalyst was filtered off, the solvent was

evaporated and purification on silica gel afforded the title compound as yellow oil (50mg, 53%). The NMR spectra are not attached due to low purity of product.

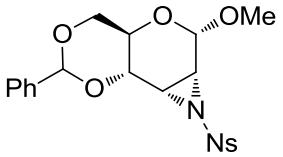
(4aR,6S,7R,8R,8aR)-6-methoxy-7-(4-nitrophenylsulfonamido)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl 4-nitrobenzenesulfonate ***XXXV.***



XXXV

N-deacylated sugar **XXVII** (6.8 g, 24.2 mmol) was dissolved in a mixture of CH_2Cl_2/pyridine (320/160 mL) and the reaction flask was cooled by ice bath to 0 °C. Subsequently, 4-nosyl chloride (4.2 equiv, 22.5 g, 101.6 mmol) was added and the mixture was heated to 60 °C. After 3 days, the solvents were removed under reduced pressure; the residue was dissolved in EtOAc (700 mL) and washed with water (3x700 mL). The organic layer was washed with brine (300 mL) and dried with $MgSO_4$. The crude product was purified on silica gel ($CHCl_3$/MeOH = 100/1) with yield 77% (12.0 g) as a pale yellow solid, mp. 149-151 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.17 (s, 3 H), 3.56-3.6 (m, 1 H), 3.62-3.69 (m, 1 H), 3.82-3.86 (m, 1 H), 3.87-3.91 (m, 1 H), 4.15 (dd, *J*=4.2, 1.0 Hz, 1 H), 4.37 (d, *J*=3.6 Hz, 1 H), 4.78 (t, *J*=9.9 Hz, 1 H), 5.32 (s, 1 H), 7.10-7,16 (m, 2 H), 7.20-7.32 (m, 3 H), 7.87-7.97 (m, 4 H), 8.09 (dt, *J*=8.8, 2.6 Hz, 2 H), 8.35 (dt, *J*=8.8, 2.1 Hz, 2 H), 8.94 (d, *J*=9.9 Hz, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 55.2, 55.9, 62.3, 67.5, 77.9, 79.4, 99.1, 100.8, 124.0, 124.5, 126.1, 127.8, 127.9, 128.7, 129.1, 136.6, 142.2, 147.4, 149.5, 149.6; HRMS (Orbitrap) *m/z* [M - H]$^-$ calcd for $C_{26}H_{25}N_3O_{13}S_2$ did not found; we found m/z [M – -SO_2-C_6H_4-NO_2 - H]$^-$
calcd for $C_{20}H_{21}N_2O_9S$ 465.0962, found 465.0967.

(4aR,6S,6aR,7aS,7bS)-6-methoxy-7-((4-nitrophenyl)sulfonyl)-2-phenylhexahydro-4H-[1,3]dioxino[4',5':5,6]pyrano[3,4-b]azirine ***XXVIIIb.***



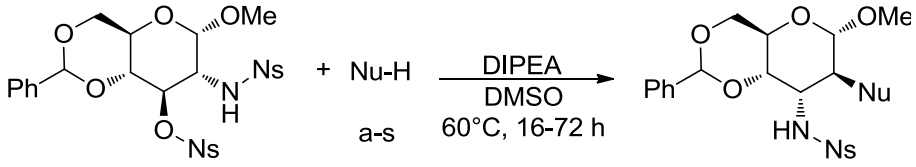
XXVIIIb

Compound **XXXV** (400.0 mg, 0.62 mmol) was suspended in 2-methoxy ethanol (12 mL) and DIPEA (0.332 mL, 1.91 mmol) was added. The mixture was heated to 60 °C and stirred for

24 hours. Then the precipitate was filtered off and washed with small portion of 2-methoxy ethanol. Compound **XXVIIIb** was obtained in 83% yield (229.0 mg) as a yellow solid, mp. 260-262 °C. ^{1}H NMR (400 MHz, DMF-d_7) δ 3.37 (s, 3 H), 3.59 (dd, *J*=7.3, 2.6 Hz, 1 H), 3.75 (dd, *J*=10.9, 9.3 Hz, 1 H), 3.77-3.84 (m, 1 H), 3.86 (dd, *J*=7.3, 4.2 Hz, 1 H), 4.08 (dd, *J*=8.8, 2.6 Hz, 1 H), 4.15 (dd, *J*=9.6, 4.4 Hz, 1 H), 5.15 (d, *J*=4.2 Hz, 1 H), 5.70 (s, 1 H), 7.24-7.42 (m, 5 H), 8.33-8.39 (m, 2 H), 8.50-8.56 (m, 2 H); ^{13}C NMR (DMF-d_7) δ 40.1, 42.3, 55.3, 60.9, 68.5, 74.7, 94.2, 101.9, 124.9, 126.5, 128.2, 129.3, 129.9, 138.2, 143.9, 151.1; HRMS (Orbitrap) *m/z* $[M + NH_4]^+$ calcd for $C_{20}H_{24}N_3O_8S$ 466.1279, found 466.1279.

In situ ring opening of aziridine

General Procedure I - nitrogen nucleophiles



Reaction was carried out on 0.15 mmol scale unless otherwise noted. Compound **XXXV** (0.15 mmol, 100.0 mg) was dissolved in DMSO (3 mL) and DIPEA (0.083 mL, 0.48 mmol) was added. The mixture was stirred for 5 minutes, then the nucleophile (2.1 equiv) was added and the mixture was heated to 60 °C. After the reaction was finished, CH_2Cl_2 (10 mL) and water (10 mL) was added and the organic layer was washed with water (3x10 mL), brine (10 mL) and dried with $MgSO_4$. The crude product was purified on silica gel using chloroform/methanol as a solvent.

N-((4aR,6S,7S,8S,8aS)-7-azido-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIa.*** Following the General procedure I on 0.6 mmol scale, the reaction with sodium azide **a** was heated for 16 hours affording **XLIa** in 72% yield (212.0 mg) as a pale yellow solid, mp. 190-191 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 3.35 (s, 3 H), 3.62 (t, J=10.1 Hz, 1 H), 3.81 (br. s., 1 H), 3.86 (dd, J=9.9, 4.7 Hz, 1 H), 3.99 (dd, J=2.6, 1.0 Hz, 1 H), 4.03 - 4.13 (m, 1 H), 4.17 (dd, J=9.9, 5.2 Hz, 1 H), 4.74 (s, 1 H), 5.50 (s, 1 H), 7.04 - 7.13 (m, 2 H), 7.17 - 7.30 (m, 3 H), 7.89 - 8.01 (m, 4 H), 8.40 (br. s., 1 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 51.8, 54.9, 58.2, 62.5, 68.1, 72.5, 97.9, 100.8, 123.7, 126.1, 127.6, 128.0,

128.7, 137.1, 146.8, 148.8; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{20}H_{22}N_5O_8S$ 492.1184, found 492.1182.

N-((4aR,6S,7S,8S,8aS)-7-amino-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIb.*** Following the General procedure I, the reaction with ammonia **b** (25% in water) was heated for 16 hours affording **X*LI*b** in 91% yield (65.0 mg) as a pale yellow solid, mp. 132-134 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.95 (br. s., 1 H), 3.00 (d, J=1.6 Hz, 1 H), 3.29 (s, 3 H), 3.64 (t, J=9.9 Hz, 1 H), 3.67 - 3.73 (m, 1 H), 3.92 - 4.09 (m, 2 H), 4.15 (dd, J=10.1, 4.9 Hz, 1 H), 4.45 (s, 1 H), 5.44 (s, 1 H), 7.09 (dd, J=8.3, 1.6 Hz, 2 H), 7.16 - 7.32 (m, 3 H), 7.95 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 54.6, 54.6, 55.6, 58.5, 68.4, 72.8, 100.8, 102.1, 123.6, 126.0, 127.5, 128.0, 128.6, 137.3, 147.4, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{20}H_{24}N_3O_8S$ 466.1279, found 466.1280.

N-((4aR,6S,7S,8S,8aS)-7-(benzylamino)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIc.*** Following the General procedure I on 0.3 mmol scale, the reaction with benzylamine **c** was heated for 24 hours affording **X*LI*c** in 59% yield (100.0 mg) as a pale yellow solid, mp. 175-177 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 2.56 (q, J=6.1 Hz, 1 H,), 2.73 (d, J=4.2 Hz, 1 H), 3.26 (s, 3 H), 3.64 (t, J=10.1 Hz, 1 H), 3.76 (qd, J=14.4, 5.7 Hz, 2 H), 3.89 (br. s., 1 H), 3.97 - 4.05 (m, 1 H), 4.08 (dd, J=9.9, 4.7 Hz, 1 H), 4.16 (dd, J=10.1, 4.9 Hz, 2 H), 4.59 (s, 1 H), 5.47 (s, 1 H), 7.09 - 7.15 (m, 2 H), 7.19 - 7.28 (m, 3 H), 7.28 - 7.36 (m, 5 H), 7.86 (br. s, 1 H), 7.90 - 7.96 (m, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 50.7, 51.6, 54.5, 58.5, 60.5, 68.4, 73.2, 100.1, 100.9, 123.6, 126.1, 126.7, 127.6, 128.0, 128.1, 128.6, 137.4, 140.3, 140.3, 147.4, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{27}H_{30}N_3O_8S$ 556.1748, found 556.1750.

N-((4aR,6S,7S,8S,8aS)-6-methoxy-7-(methylamino)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLId.*** Following the General procedure I, the reaction with methylamine **d** was heated for 24 hours affording **X*LI*d** in 55% yield (41.0 mg) as a pale yellow solid, mp. 187-189 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.23 (br. s., 1 H), 2.32 (s, 3 H), 2.65 (d, J=2.1 Hz, 1 H), 3.30 (s, 3 H), 3.60 (t, J=9.6 Hz, 1 H), 3.82 (br. s., 1 H), 3.92 - 4.06 (m, 2 H), 4.14 (dd, J=10.1, 4.4 Hz, 1 H), 4.57 (s, 1 H), 5.43 (s, 1 H), 7.04 - 7.14 (m, 2 H), 7.16 - 7.29 (m, 3 H), 7.89 (br. d, J=7.8 Hz, 1 H), 7.94 - 7.98 (m, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 34.6, 51.1, 54.6, 58.5, 64.1, 68.4, 73.2, 99.8, 100.9, 123.7, 126.1, 127.6, 128.0, 128.6, 137.3, 147.3, 148.7; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 (d, J=2.1 Hz, 1 H), 3.48 (s, 3 H), 3.73 (t, J=10.4 Hz, 1 H), 3.87 (td, J=9.9, 4.7 Hz, 1 H), 3.94 (dd, J=9.9, 4.2

Hz, 1 H), 4.11 (m, 1 H), 4.26 (dd, J=10.4, 4.7 Hz, 1 H), 4.72 (s, 1 H), 5.43 (s, 1 H), 6.05 (d, J=9.3 Hz, 1 H), 7.12 (m, 2 H), 7.25 (m, 2 H), 7.33 (m, 1 H), 7.79 (m, 2 H), 7.89 (m, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 34.7, 55.9, 59.6, 63.0, 68.9, 74.1, 77.2, 100.8, 101.8, 123.5, 125.8, 128.0, 128.2, 129.3, 136.6, 146.6, 149.3; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{21}H_{26}N_3O_8S$ 480.1435, found 480.1436.

N-((4aR,6S,7S,8S,8aS)-6-methoxy-7-(pentylamino)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIe.*** Following the General procedure I, the reaction with pentylamine **e** was heated for 24 hours affording **X*LI*e** in 79% yield (65.0 mg) as an amorphous yellow solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 0.87 (t, J=6.7 Hz, 3 H), 1.20 - 1.33 (m, 5 H), 1.33 - 1.42 (m, 2 H), 1.88 (br. s., 1 H), 2.53 - 2.59 (m, 1 H), 2.73 (br. s, 1 H), 3.30 (s, 3 H), 3.62 (t, J=9.6 Hz, 1 H), 3.78 (br. d, J=8.3 Hz, 1 H), 3.95 - 4.03 (m, 2 H), 4.14 (dd, J=9.3, 3.6 Hz, 1 H), 4.57 (s, 1 H), 5.45 (s, 1 H), 7.06 - 7.15 (m, 2 H), 7.18 - 7.29 (m, 3 H), 7.89 (d, J=9.3 Hz, 1 H), 7.96 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 14.0, 22.1, 28.9, 29.2, 47.7, 51.7, 54.5, 58.4, 62.0, 68.4, 73.1, 100.2, 100.8, 123.6, 126.1, 127.6, 128.0, 128.6, 137.3, 147.3, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{25}H_{34}N_3O_8S$ 536.2061, found 536.2064.

N-((4aR,6S,7S,8S,8aS)-7-(cyclohexylamino)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIf.*** Following the General procedure I, the reaction with cyclohexylamine **f** was heated for 16 hours affording **X*LI*f** in 68% yield (57.0 mg) as a pale yellow solid, mp. 176-178 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 0.87 - 1.04 (m, 2 H), 1.06 - 1.24 (m, 3 H), 1.49 - 1.59 (m, 1 H), 1.60 - 1.69 (m, 3 H), 1.69 - 1.82 (m, 2 H), 2.35 - 2.46 (m, 1 H), 2.84 (d, J=2.6 Hz, 1 H), 3.29 (s, 3 H), 3.64 (t, J=9.6 Hz, 1 H), 3.67 - 3.72 (m, 1 H), 3.92 - 4.05 (m, 2 H), 4.14 (dd, J=9.9, 4.2 Hz, 1 H), 4.52 (s, 1 H), 5.47 (s, 1 H), 7.05 - 7.18 (m, 2 H), 7.18 - 7.31 (m, 3 H), 7.89 (br. d, J=8.3 Hz, 1 H), 7.93-8.01 (m, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 24.3, 25.7, 32.5, 33.2, 52.6, 54.4, 54.5, 58.4, 58.4, 68.3, 73.1, 100.8, 101.0, 123.7, 126.1, 127.6, 128.1, 128.6, 137.4, 147.3, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{26}H_{34}N_3O_8S$ 548.2061, found 548.2065.

N-((4aR,6S,7S,8S,8aS)-7-((2-hydroxyethyl)amino)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIg.*** Following the General procedure I on 0.6 mmol scale, the reaction with 2-aminoethanol **g** was heated for 16 hours affording **X*LI*g** in 87% yield (272.0 mg) as a pale yellow solid, mp. 203-204 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.93 (br. s., 1 H), 2.57 - 2.72 (m, 2 H), 2.78 (d, J=1.6 Hz, 1 H), 3.30 (s, 3 H),

3.39 - 3.47 (m, 2 H), 3.62 (t, J=9.9 Hz, 1 H), 3.75 - 3.84 (m, 1 H), 3.92 - 4.07 (m, 2 H), 4.15 (dd, J=10.1, 4.9 Hz, 1 H), 4.52 (t, J=5.2 Hz, 1 H), 4.59 (s, 1 H), 5.45 (s, 1 H), 7.10 (dd, J=8.0, 1.3 Hz, 2 H), 7.15 - 7.31 (m, 3 H), 7.92 (s, 1 H), 7.94 - 7.97 (m, 4 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 50.2, 51.8, 54.6, 58.4, 60.4, 62.1, 68.4, 73.2, 100.3, 100.8, 123.6, 126.1, 127.5, 128.0, 128.6, 137.3, 147.3, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{22}H_{28}N_3O_9S$ 510.1541, found 510.1543.

N-((4aR,6S,7S,8S,8aS)-7-((3-hydroxypropyl)amino)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIh.*** Following the General procedure I on 0.6 mmol scale, the reaction with 3-aminopropanol **h** was heated for 16 hours affording **X*LI*h** in 59% yield (188.0 mg) as a pale yellow solid, mp. 171-173 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.54 (quin, J=6.6 Hz, 2 H), 1.84 - 1.98 (m, 1 H), 2.53 - 2.70 (m, 2 H), 2.74 (br. s., 1 H), 3.30 (s, 3 H), 3.45 (dd, J=6.2, 4.7 Hz, 2 H), 3.62 (t, J=9.6 Hz, 1 H), 3.75 - 3.84 (m, 1 H), 3.93 - 4.06 (m, 2 H), 4.15 (dd, J=9.6, 4.4 Hz, 1 H), 4.41 (t, J=4.9 Hz, 1 H), 4.57 (s, 1 H), 5.45 (s, 1 H), 7.10 (dd, J=8.0, 1.3 Hz, 2 H), 7.17 - 7.28 (m, 3 H), 7.89 (d, J=8.8 Hz, 1 H), 7.96 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 33.3, 45.6, 52.2, 55.1, 58.9, 59.6, 62.7, 68.9, 73.7, 100.8, 101.4, 124.2, 126.6, 128.1, 128.5, 129.1, 137.9, 147.9, 149.3; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{23}H_{30}N_3O_9S$ 524.1697, found 524.1699.

N-(8-((2-(2-hydroxyethoxy)ethyl)amino)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)-4-nitrobenzenesulfonamide ***XLIi***. Following the General procedure I, the reaction with aminoalcohol **i** was heated for 24 hours affording **X*LI*i** in 80% yield (68.0 mg) as a pale yellow solid, mp. 68-70 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) d ppm 1.98 (br. s., 1 H), 2.74 (d, J=4.7 Hz, 2 H), 2.79 (br. s., 1 H), 3.30 (s, 3 H), 3.43 (m, 4 H), 3.50 (q, J=5.2 Hz, 2 H), 3.62 (t, J=9.9 Hz, 1 H), 3.80 (m, 1 H), 4.00 (m, 2 H), 4.15 (dd, J=10.1, 4.4 Hz, 1 H), 4.57 (t, J=5.2 Hz, 1 H), 4.59 (s, 1 H), 5.46 (s, 1 H), 7.10 (m, 2 H), 7.24 (m, 3 H), 7.90 (d, J=9.3 Hz, 1 H), 7.96 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) d ppm 47.2, 51.8, 54.5, 58.4, 60.2, 62.1, 68.3, 69.8, 72.1, 73.1, 100.2, 100.8, 123.6, 126.1, 127.5, 128.0, 128.5, 137.3, 147.3, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{24}H_{32}N_3O_{10}S$ 554.1803, found 554.1806.

N-((4aR,6S,7S,8S,8aS)-7-(((S)-1-hydroxypropan-2-yl)amino)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIj.*** Following the General procedure I, the reaction with S-alaninol **j** was heated for 48 hours affording **X*LI*j** in 51% yield (41.3 mg) as a pale yellow solid, mp. 195-197 °C. ^{1}H NMR (400 MHz, DMSO-

d6) δ 0.91 (d, J=6.2 Hz, 3 H), 1.73 - 1.81 (m, 1 H), 2.69 - 2.78 (m, 1 H), 2.91 (br. d, J=6.2 Hz, 1 H), 3.19 - 3.27 (m, 1 H), 3.31 (s, 3 H), 3.62 (t, J=10.1 Hz, 1 H), 3.77 (br. s., 1 H), 3.88 - 3.95 (m, 1 H), 3.99 - 4.07 (m, 1 H), 4.15 (dd, J=10.1, 4.9 Hz, 1 H), 4.54 (s, 1 H), 4.60 (t, J=5.7 Hz, 1 H), 5.45 (s, 1 H), 7.09 - 7.15 (m, 2 H), 7.19 - 7.30 (m, 3 H), 7.94 - 7.95 (m, 1 H), 7.96 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 16.9, 51.2, 52.3, 54.5, 58.4, 58.6, 65.6, 68.4, 73.1, 100.9, 101.5, 123.6, 126.1, 127.6, 128.0, 128.6, 137.3, 147.3, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{23}H_{30}N_3O_9S$ 524.1697, found 524.1700.

N-((4aR,6S,7S,8S,8aS)-7-(((4aS,6R,7S,8S,8aR)-8-hydroxy-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)amino)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIk.*** Following the General procedure I on 0.6 mmol scale, the reaction with glucosamine **k** was heated for 72 hours affording **XLIk** in 47% yield (212.0 mg) as a pale yellow solid, mp. 130-132 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.77 (t, J=6.7 Hz, 1 H), 2.56 - 2.64 (m, 1 H), 2.98 (dd, J=6.0, 2.3 Hz, 1 H), 3.32 (s, 3 H), 3.33 (s, 3 H), 3.44 - 3.52 (m, 2 H), 3.53 - 3.68 (m, 2 H), 3.74 (t, J=9.9 Hz, 1 H), 3.95 (br. s., 1 H), 3.97 - 4.10 (m, 2 H), 4.12 - 4.22 (m, 2 H), 4.59 (s, 1 H), 4.73 (d, J=3.6 Hz, 1 H), 5.28 (d, J=4.7 Hz, 1 H), 5.44 (s, 1 H), 5.63 (s, 1 H), 7.07 - 7.13 (m, 2 H), 7.18 - 7.27 (m, 3 H), 7.35 - 7.40 (m, 3 H), 7.43 - 7.48 (m, 2 H), 7.83 (br. s., 1 H), 7.99 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 51.6, 54.6, 54.9, 58.5, 59.6, 61.3, 62.4, 68.1, 68.4, 68.6, 73.0, 79.2, 81.7, 98.9, 100.8, 100.9, 101.2, 123.6, 126.1, 126.4, 127.5, 128.0, 128.6, 128.8, 137.4, 137.8, 147.4, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{34}H_{40}N_3O_{13}S$ 730.2276, found 730.2276.

N-((4aR,6S,7S,8S,8aS)-7-hydrazinyl-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIl.*** Following the General procedure I, the reaction with hydrazine hydrate **l** was heated for 24 hours affording **XLIl** in 68% yield (50.0 mg) as a pale yellow solid, mp. 168-170 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 2.81 (d, J=2.1 Hz, 1 H), 3.30 (s, 3 H), 3.59 (t, J=9.9 Hz, 1 H), 3.90 (dd, J=4.7 Hz, 2 H), 3.96 - 4.04 (m, 1 H), 4.05 - 4.11 (m, 1 H), 4.14 (dd, J=10.1, 4.9 Hz, 1 H), 4.66 (s, 1 H), 5.43 (s, 1 H), 7.00 - 7.15 (m, 2 H), 7.16 - 7.29 (m, 3 H), 7.74 (br. s., 1 H), 7.96 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 50.8, 55.2, 59.0, 67.0, 69.0, 74.0, 99.0, 101.3, 124.1, 126.6, 128.1, 128.5, 129.1, 137.9, 148.0, 149.2; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{20}H_{25}N_4O_8S$ 481.1388, found 481.1389.

N-((4aR,6S,7S,8S,8aS)-7-((2-aminoethyl)amino)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIm.*** Following the General procedure I

except using semipreparative HPLC for purification, the reaction with ethylene diamine **m** was heated for 24 hours affording **XLIm** in 47% yield (37.0 mg) as a pale yellow solid, mp. 152-154 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 2.59 - 2.74 (m, 4 H), 2.75 (d, J=1.6 Hz, 1 H), 3.30 (s, 3 H), 3.62 (t, J=9.9 Hz, 1 H), 3.80 (br. s., 1 H), 3.96 - 4.06 (m, 2 H), 4.12 - 4.19 (m, 1 H), 4.59 (s, 1 H), 5.44 (s, 1 H), 7.02 - 7.17 (m, 2 H), 7.18 - 7.31 (m, 3 H), 7.96 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 40.6, 48.9, 52.1, 55.1, 58.9, 62.5, 68.9, 73.6, 100.9, 101.4, 124.2, 126.6, 128.1, 128.5, 129.1, 137.9, 147.9, 149.2; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{22}H_{29}N_4O_8S$ 509.1701, found 509.1703.

N-((4aR,6S,7S,8S,8aS)-6-methoxy-2-phenyl-7-(piperidin-1-yl)hexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIn.*** Following the General procedure I, the reaction with piperidine **n** was heated for 24 hours affording **XLIn** in 73% yield (60.0 mg) as a pale yellow solid, mp. 188-190 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.34 - 1.44 (m, 2 H), 1.44 - 1.54 (m, 4 H), 2.44 - 2.49 (m, 2 H), 2.62 - 2.69 (m, 2 H), 2.70 (d, J=1.0 Hz, 1 H), 3.30 (s, 3 H), 3.59 (t, J=10.1 Hz, 1 H), 3.78 - 3.90 (m, 2 H), 3.95 - 4.07 (m, 1 H), 4.16 (dd, J=9.9, 5.2 Hz, 1 H), 4.74 (s, 1 H), 5.49 (s, 1 H), 7.12 (dd, J=8.0, 1.3 Hz, 2 H), 7.17 - 7.31 (m, 3 H), 7.93 (d, J=2.1 Hz, 4 H), 8.07 (br. s., 1 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 23.9, 26.2, 49.6, 51.2, 54.4, 57.7, 68.5, 69.0, 74.3, 99.7, 100.9, 123.6, 126.2, 127.6, 128.0, 128.6, 137.4, 147.2, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{25}H_{32}N_3O_8S$ 534.1905, found 534.1906.

N-((4aR,6S,7S,8S,8aS)-6-methoxy-7-morpholino-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIo.*** Following the General procedure I, the reaction with morpholine **o** was heated for 24 hours affording **XLIo** in 69% yield (57.0 mg) as a pale yellow solid, mp. 203-205 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 2.49 - 2.53 (m, 2 H), 2.56 - 2.66 (m, 3 H), 3.27 (s, 3 H), 3.50 - 3.60 (m, 5 H), 3.76 - 3.83 (m, 1 H), 3.83 - 3.91 (m, 1 H), 3.92 - 4.03 (m, 1 H), 4.12 (dd, J=9.9, 5.2 Hz, 1 H), 4.77 (s, 1 H), 5.46 (s, 1 H), 7.02 - 7.11 (m, 2 H), 7.13 - 7.26 (m, 3 H), 7.84 - 7.96 (m, 4 H), 8.02 (d, J=7.8 Hz, 1 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 50.1, 50.7, 54.5, 57.9, 66.6, 68.2, 68.4, 73.9, 98.4, 100.8, 123.6, 126.2, 127.6, 128.0, 128.6, 137.3, 147.2, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{24}H_{30}N_3O_9S$ 536.1697, found 536.1699.

N-((4aR,6S,7S,8S,8aS)-7-((R)-2-(hydroxymethyl)pyrrolidin-1-yl)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIp.*** Following the General procedure I on 0.6 mmol scale, the reaction with (R)-pyrrolidin-2-ylmethanol **p** was heated for 16 hours affording **XLIp** in 68% yield (230.0 mg) as a pale yellow solid, mp.

106-108 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.56 - 1.81 (m, 4 H), 2.52 - 2.58 (m, 1 H), 2.83 - 2.95 (m, 2 H), 2.96 - 3.11 (m, 2 H), 3.21 - 3.31 (m, 1 H), 3.33 (s, 3 H), 3.61 (t, J=10.1 Hz, 1 H), 3.86 - 4.05 (m, 3 H), 4.16 (dd, J=10.1, 4.9 Hz, 1 H), 4.35 (t, J=5.4 Hz, 1 H), 4.76 (s, 1 H), 5.50 (s, 1 H), 7.08 (dd, J=8.0, 1.3 Hz, 2 H), 7.15 - 7.28 (m, 3 H), 7.88 (d, J=8.8 Hz, 1 H), 7.95 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 23.8, 28.1, 51.3, 52.4, 55.2, 58.7, 61.9, 64.2, 67.1, 69.0, 74.0, 100.7, 101.4, 124.2, 126.7, 128.1, 128.5, 129.1, 137.9, 147.9, 149.2; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{25}H_{32}N_3O_9S$ 550.1854, found 550.1853.

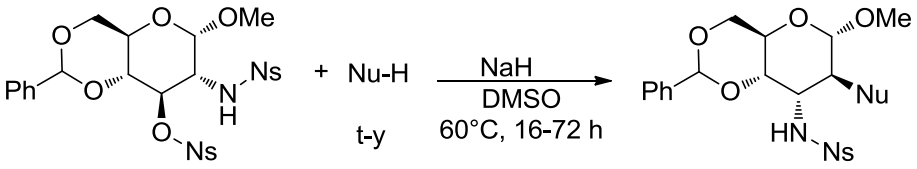
N-((4aR,6S,7S,8S,8aS)-7-((S)-2-(hydroxymethyl)pyrrolidin-1-yl)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIq.*** Following the General procedure I on 0.6 mmol scale, the reaction with (S)-pyrrolidin-2-ylmethanol **q** was heated for 16 hours affording **XLIq** in 56% yield (186.0 mg) as a pale yellow solid, mp. 67-69 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.58 - 1.74 (m, 4 H), 2.56 - 2.65 (m, 1 H), 2.83 - 2.95 (m, 3 H), 3.07 - 3.17 (m, 1 H), 3.31 (s, 3 H), 3.32 - 3.37 (m, 1 H), 3.63 (t, J=9.6 Hz, 1 H), 3.85 (br. d, J=8.8 Hz, 1 H), 3.91 - 4.06 (m, 2 H), 4.15 (dd, J=10.1, 4.4 Hz, 1 H), 4.57 (t, J=5.2 Hz, 1 H), 4.82 (s, 1 H), 5.49 (s, 1 H), 7.09 (dd, J=8.0, 1.8 Hz, 2 H), 7.16 - 7.30 (m, 3 H), 7.95 (br. s., 1 H), 7.96 (s, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 23.6, 27.4, 50.9, 53.7, 54.6, 58.0, 64.2, 64.2, 66.2, 68.4, 73.4, 98.4, 100.8, 123.6, 126.1, 127.5, 128.0, 128.6, 137.4, 147.2, 148.7; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{25}H_{32}N_3O_9S$ 550.1854, found 550.1856.

2-(((4aR,6S,7S,8S,8aS)-6-methoxy-8-(4-nitrophenylsulfonamido)-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-7-yl)amino)benzoic acid ***XLIr***. Following the General procedure I on 0.6 mmol scale, the reaction with anthranilic acid **r** was heated for 24 hours affording **XXXIXr** in 56% yield (200.0 mg) as a yellow solid, mp. 119-121 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 3.37 (s, 3 H), 3.71 (t, J=9.6 Hz, 1 H), 3.93 (m, 1 H), 4.06 (dd, J=9.7, 4.8 Hz, 1 H), 4.20 (m, 2 H), 4.71 (s, 1 H), 5.00 (dd, J=2.6, 1.0 Hz, 1 H), 5.59 (s, 1 H), 6.56 (ddd, J=8.0, 7.0, 1.0 Hz, 1 H), 6.72 (br. s, 1 H), 6.80 (dd, J=8.6, 0.8 Hz, 1 H), 7.13 (m, 2 H), 7.26 (m, 4 H), 7.80 (dd, J=8.0, 1.6 Hz, 1 H), 7.96 (dt, J=9.6, 2.3 Hz, 4 H), 8.42 (d, J=8.8 Hz, 1 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 51.4, 54.9, 58.2, 68.2, 71.4, 73.1, 97.9, 100.8, 107.5, 114.8, 116.6, 123.6, 126.2, 127.6, 128.2, 128.7, 131.0, 134.7, 137.2, 146.8, 148.8, 151.9, 165.6; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{27}H_{28}N_3O_{10}S$ 586.1490, found 586.1491.

N-((4aR,6S,7S,8S,8aS)-7-(2-benzoylhydrazinyl)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIs.*** Following the General procedure I, the

reaction with benzohydrazide **s** was heated for 72 hours affording **XLIs** in 38% yield (34.0 mg) as a amorfous yellow solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 3.20 (t, J=2.3 Hz, 1 H), 3.31 - 3.32 (s, 3 H), 3.67 (t, J=9.9 Hz, 1 H), 4.01 - 4.16 (m, 3 H), 4.20 (dd, J=10.1, 4.9 Hz, 1 H), 4.74 (s, 1 H), 5.44 (dd, J=5.4, 3.4 Hz, 1 H), 5.52 (s, 1 H), 7.16 (dd, J=7.8, 1.6 Hz, 2 H), 7.21 - 7.32 (m, 3 H), 7.45 - 7.61 (m, 3 H), 7.79 - 7.86 (m, 2 H), 7.90 - 7.93 (m, 5 H), 10.12 (d, J=5.7 Hz, 1 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 50.3, 54.6, 58.5, 63.3, 68.4, 73.2, 98.4, 100.9, 123.6, 126.1, 127.2, 127.6, 127.9, 128.4, 128.6, 131.5, 133.1, 137.4, 147.3, 148.7, 166.3; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{27}H_{29}N_4O_9S$ 585.1650, found 585.1651.

General Procedure II - oxygen and carbon nucleophiles

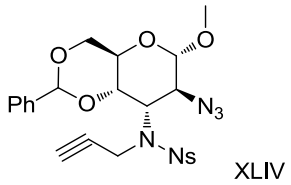


XXXV XLIt-y

Reaction was carried out on 0.15 mmol scale unless otherwise noted. Compound **XXXV** (0.15 mmol, 100.0 mg) was dissolved in DMSO (3 mL) and NaH (9.2 mg, 0.48 mmol) was added. The mixture was stirred for 5 minutes, then the nucleophile (2.1 equiv) was added and the mixture was heated to 60 °C. After the reaction was finished, CH_2Cl_2 (10 mL) and water (10 mL) was added and the organic layer was washed with water (3x10 mL), brine (10 mL) and dried with $MgSO_4$. The crude product was purified on silica gel using chloroform/methanol as a solvent.

N-((4aR,6S,7S,8S,8aS)-7-(2-benzoylhydrazinyl)-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitrobenzenesulfonamide ***XLIv.*** Following the General procedure II, the reaction with 4-nitrophenol **v** was heated for 72 hours affording **XLIv** in 40% yield (36.0 mg) as an amorphous yellow solid. ^{1}H NMR (400MHz, DMSO-*d6*) δ = 8.59 (d, J = 8.3 Hz, 1 H), 8.27 (td, J = 3.1, 9.3 Hz, 2 H), 8.00 - 7.80 (m, 4 H), 7.34 - 7.16 (m, 5 H), 7.11 - 7.02 (m, 2 H), 5.49 (s, 1 H), 4.86 (s, 1 H), 4.70 (d, J = 1.6 Hz, 1 H), 4.27 - 4.12 (m, 2 H), 4.02 - 3.86 (m, 2 H), 3.76 - 3.62 (m, 1 H), 3.40 (s, 3 H); ^{13}C NMR (101MHz, DMSO-*d6*) δ = 161.6, 149.0, 146.6, 141.8, 137.3, 128.9, 128.3, 127.8, 126.3, 126.3, 123.9, 116.1, 101.1, 98.2, 76.8, 72.9, 68.4, 58.5, 55.1, 50.5.

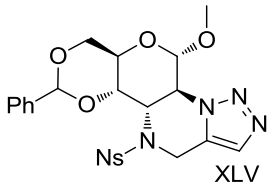
N-((4aR,6S,7S,8S,8aS)-7-azido-6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3]dioxin-8-yl)-4-nitro-N-(prop-2-yn-1-yl)benzenesulfonamide ***XLIV.***



XLIV

To solution of PPh_3 (0.40 mmol, 104 mg) and DIAD (0.40 mmol, 79 μL) in dry dioxane (4 mL) compound ***XLI*a** (0.20 mmol, 100 mg) was added. After 5 minutes, propargyl alcohol (0.40 mmol, 24 μL) was added in one portion. Reaction mixture was stirred at room temperature for 18 hours, then solvent was removed under reduced pressure and the residue was purified by silica chromatography using an chloroform/methanol (50/1) as a mobile phase affording **XLIV** in 74% yield (80 mg) as a yellow solid, mp. 99-102 °C. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 3.36 (t, J=2.3 Hz, 1 H), 3.43 (s, 3 H), 3.49 (m, 1 H), 3.86 (t, J=9.9 Hz, 1 H), 4.16 (m, 3 H), 4.32 (dd, J=19.2, 2.1 Hz, 1 H), 4.42 (dd, J=10.4, 6.7 Hz, 1 H), 4.51 (t, J=9.9 Hz, 1 H), 4.77 (d, J=6.7 Hz, 1 H), 5.15 (s, 1 H), 6.99 (d, J=7.3 Hz, 2 H), 7.18 (t, J=7.5 Hz, 2 H), 7.26 (m, 1 H), 7.97 (m, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 54.2, 55.3, 60.0, 62.7, 66.3, 68.8, 73.7, 74.8, 80.5, 100.9, 101.2, 123.7, 125.9, 127.7, 128.6, 128.8, 136.6, 144.5, 149.0; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{23}H_{24}N_5O_8S$ 530.1340, found 530.1341.

(4aS,5S,6aR,10aS,10bS)-5-methoxy-11-((4-nitrophenyl)sulfonyl)-9-phenyl-4a,5,6a,7,10a,10b,11,12-octahydro-[1,3]dioxino[4',5':5,6]pyrano[4,3-e][1,2,3]triazolo[1,5-a]pyrazine ***XLV.***



XLV

Method A: Azide **XLIV** (0.08 mmol, 40 mg) was dissolved in 1 mL of DMF and 0.3 mL of water. Then freshly prepared sodium ascorbate (1 M in water, 16 μL) and $CuSO_4.7H_2O$ (0.1 M, 16 μL) was added and reaction mixture was heated to 60 °C and stirred for 14 days. When reaction was completed, water (5 mL) was added and the mixture was extracted with chloroform (3x5 mL). Connected organic layers were dried with brine and $MgSO_4$, evaporated under reduced pressure and the residue was purified by silica chromatography using chloroform as a mobile phase affording **XLV** in 90% yield (36 mg) as a yellow solid, mp. 89-91 °C. Method B: Azide **XLIV** (0.08 mmol, 40 mg) was dissolved in 1 mL of DMF and 0.3 mL of water. Then freshly prepared sodium ascorbate (1 M in water, 16 μL) and $CuSO_4.7H_2O$

(0.1 M, 16 μL) was added and reaction mixture was heated to 90°C and microwave reactor was used (3 hours, 30 W). When reaction was completed, water (5 mL) was added and the mixture was extracted with chloroform (3x5 mL). Connected organic layers were dried with brine and $MgSO_4$, evaporated under reduced pressure and the residue was purified by semi preparative HPLC using acetonitrile/water as a mobile phase affording **XLV** in 50% yield (20 mg); ^{1}H NMR (400 MHz, DMSO-*d6*) δ 3.47 (s, 3 H), 3.91 (t, J=9.9 Hz, 1 H), 4.02 (m, 2 H), 4.12 (t, J=9.3 Hz, 1 H), 4.34 (dd, J=9.3, 4.2 Hz, 1 H), 4.48 (d, J=16.6 Hz, 1 H), 5.01 (d, J=16.6 Hz, 1 H), 5.20 (dd, J=12.5, 7.3 Hz, 1 H), 5.56 (d, J=7.3 Hz, 1 H), 5.68 (s, 1 H), 7.30 (s, 1 H), 7.36 (m, 5 H), 8.04 (m, 4 H); ^{13}C NMR (101 MHz, DMSO-*d6*) δ 54.3, 56.0, 56.0, 65.7, 69.5, 76.8, 99.9, 102.3, 124.7, 126.8, 128.5, 129.4, 129.5, 129.6, 133.0, 137.8, 143.1, 150.3, 162.8; HRMS (Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{23}H_{24}N_5O_8S$ 530.1340, found 530.1341.

7 List of abbreviations

Ac-	Acetyl protecting group
ACN	Acetonitrile
AIBN	2,2′-Azobis(2-methylpropionitrile)
Ala	Alanine
Arg	Arginine
BDA	Benzaldehyde dimethyl acetal
Bn	Benzyl protecting group
Boc-	tert-Butyloxycarbonyl protecting group
BOP	Benzotriazol-1-yl-oxytris-(dimethylamino)-phosphonium hexafluorophosphate
Bz	Benzoyl protecting group
CAN	Cerium ammoniu nitrate
Cbz	Carboxybenzyl protecting group
CDI	Carbonyl diimidazole
DBAD	Di-tert-butyl azodicarboxylate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexyl carbodiimide
DCM	Dichlormethan
DIAD	Diisopropyl azodicarboxylate
DIC	Diisopropyl carbodiimidies
DIPEA	N,N-Diisopropylethylamine
DMAP	4-Dimethyaminopyridine
DMEAD	Di-2-methoxyethyl azodicarboxylate
DMF	Dimethyl formamide
DMP	Dess-Martin periodinane
DNP	Dinitrophenyl
DPPA	Diphenylphosphoryl azide
EDC	1-Ethyl-3-(3´-dimethylamino)carbodiimide hydrochlorid
EtOAc	Ethyl acetate
Fmoc-	Fluorenylmethyloxycarbonyl protecting group
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HBTU	O-(1H-benzotriazol-1-yl)-N,N,N´,N´-tetramethyluronium hexafluorophosphate
HMPA	Hexamethylphosphoric triamide
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	Hydroxybenzotriazole
HOSu	N-hydroxysuccinimide
MDMR-Br resin	4-Methoxybenzhydryl bromide resin
MIC	Minimum inhibitory concentration
NCA	N-carboxy- anhydrides
NEM	N-ethylmorpholine
NMM	N-methylmorpholine
Pfp	Pentafluorophenyl
PgaB	Poly(glycerol-adipate) butyryl ester

Phth	Phtalimide
PNP	*p*-nitrophenol
Pro	Proline
py	Pyridine
PyBop	Benzotriazol-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate
Ra-Ni	Raney nickel
Ser	Serine
T3P	Propylphosphonic acid anhydride
TBAF	Tetrabutylammonium fluoride
TBTU	O-(1H-benzotriazol-1-yl)-N,N,N´,N´-tetramethyluronium tetrafluoroborate
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical
TFA	Trifluoracetic acid
THF	Tetrahydrofuran
TMAD	*N*,*N*,*N*′,*N*′-Tetramethylazodicarboxamide
TPP	Triphenylphosphine
Troc-	2,2,2-Trichlorethoxycarbonyl protecting group
TsCl	Tosyl chloride
Val	Valine

8 Literature

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PALACKY UNIVERSITY OLOMOUC

Faculty of Science

Department of Organic Chemistry

Synthesis of D-Glucosamine Derivatives and their Reactivity Study at Position 1, 2, and 3

Ph.D. Thesis

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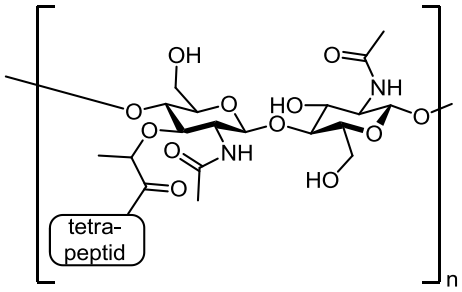
Souhrn

Předložená práce je zaměřena na syntézu a studium nových derivátů s potenciální biologickou aktivitou vycházejících ze struktury D-glukosaminu. D-glukosamin je všeobecně znám jako molekula s širokým spektrem vlastností. Je prekurzorem glykosaminoglykanů, což jsou stavební prvky chrupavek, či je obsažen v polymerní struktuře hyaluronové kyseliny. Tento polymer je důležitý pro své antibakteriální či hojivé účinky; své využití našel i v prostředcích pečující o pleť. Další, z pohledu lidstva velmi důležitou, vlastností, je inkorporace D-glukosaminu do skeletu peptidoglykanu. Tyto makromolekuly jsou základní stavební jednotkou buněčných stěn bakterií. Bylo zjištěno, že narušení tvorby buněčných stěn vede k přerušení reprodukce bakteriálních buněk. Tento princip antibakteriální léčby využívají mnohá antibiotika, jako například penicilin. Z výše zmíněných užitečných vlastností D-glukosaminu si tato práce klade za cíl přípravu nových derivátů, které by mohly rozšířit tuto stále se rozvíjející oblast.



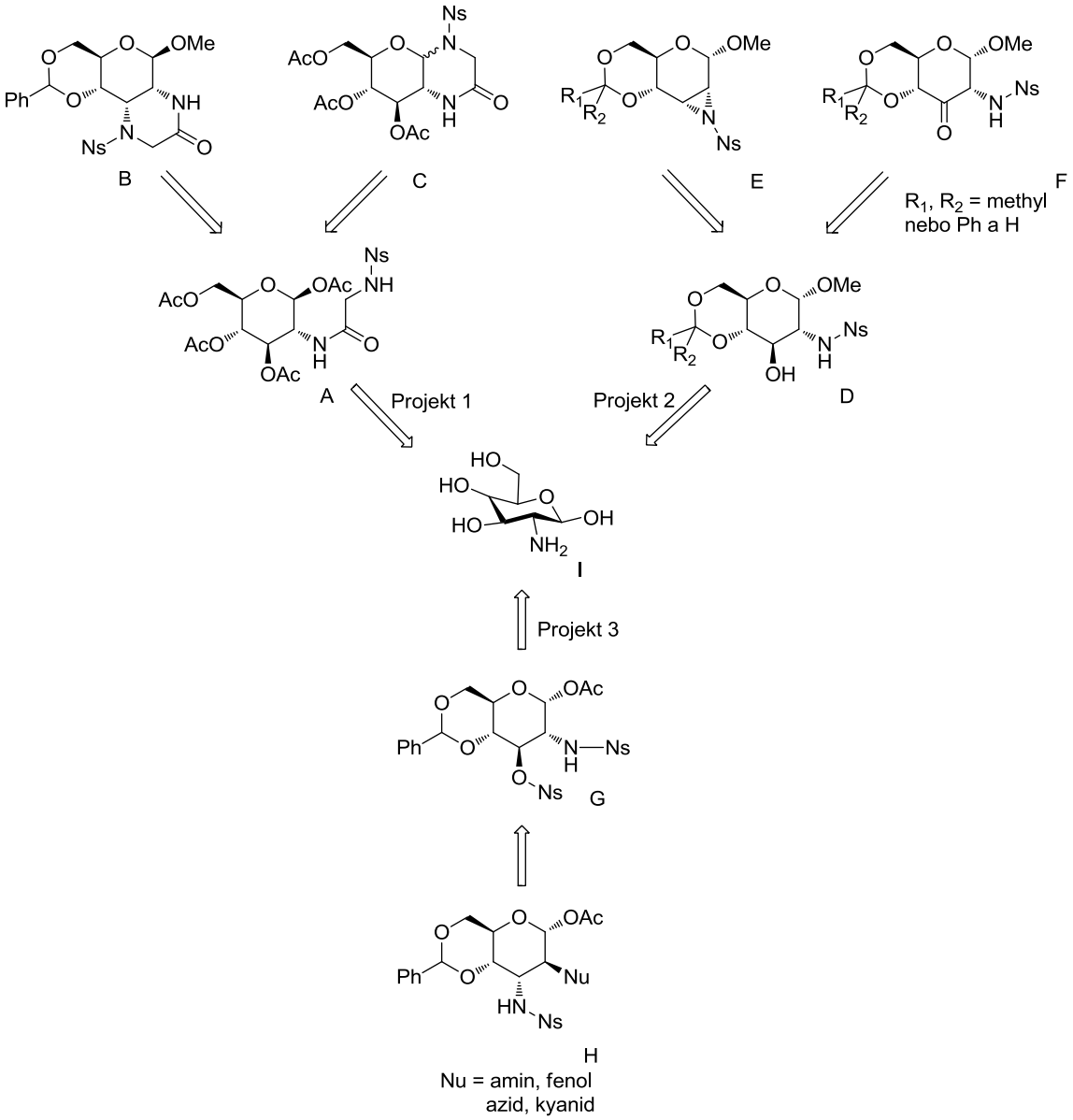
Struktura peptidoglykanu

Diskuzní část je rozdělena do tří základních částí. První část je věnována cyklizačním reakcím, kde cyklus může být uzavřen mezi dusíkem na uhlíku C2 a anomerním uhlíkem C1 (produkt **B**) anebo alternativně je cyklus uzavřen přes uhlík C3 (produkt **C**). Během výzkumu byly připraveny výchozí látky a intermediáty, které umožňovaly cyklizační reakce, avšak samotná cyklizace probíhala buďto velmi problematicky anebo prakticky vůbec. Dále byla syntéza významně komplikována tvorbou anomerů v takřka každém reakčním kroku, kde následná separace byla zdlouhavá díky podobným fyzikálně-chemickým vlastnostem látek.

Prostřední část je zaměřena na přípravu a studium reaktivity derivátu D-glukosaminu **D** s volnou hydroxy skupinou. Byly navrženy a optimalizovány dvě reakční cesty vedoucí k derivátu **D**. Následně byl meziprodukt **D** podroben alkylačním a oxidačním reakčním krokům. Během Fukuyama-Mitsunobu alkylace bylo zjištěno, že vzniká trojčetný aziridinový kruh **E** jakožto významný vedlejší produkt. Formace aziridinu nastává ovšem jen v případě,

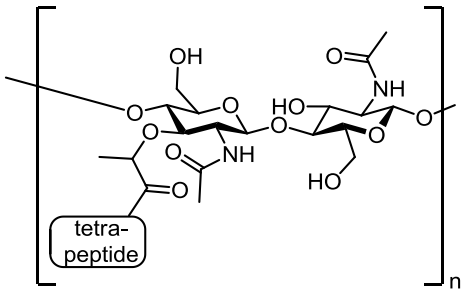
když byl k ochránění C4 a C6 hydroxy skupiny využit aceton dimethylacetal. Oxidace sloučeniny **D** vedla ke keto derivátu **F**, který byl následně podroben kondenzačním reakcím. Tyto reakce avšak nevedly k očekávaným produktům.

Třetí část práce, co do počtu nově připravených sloučenin, nejúspěšnější, je zaměřena na syntézu 2,3 - altropyranosidů **H** z dinosylovaného derivátu **G**. Tato skupina látek není nijak zvlášť prozkoumána, dosud známé syntézy zahrnují mnoho reakčních kroků s kolísavými výtěžky. Naproti tomu náš postup nabízí jednoduchou a univerzálně použitelnou metodu vycházející z komerčně dostupného *N*-acetyl glukosaminu pro přípravu 2,3-diaminoaltropyranosidů **H** s velmi dobrými výtěžky.



Abstract

The submitted thesis is focused on the synthesis and study of new potentially biologically active derivates, based on the D-glucosamine motif. D-glucosamine is well known molecule with plenty of interesting properties. It is a precursor of glucosaminoglycans, which are building units of cartilages or it can be found in the polymer structure of hyaluronic acid. This polymer is important for its antibacterial or healing effect; it is also a component of skin-care products. Moreover, the structure of D-glucosamine is incorporated into the skeleton of peptidoglycan. Such molecules are building units of bacteria cell walls. It has been found, that disruption of cell wall formation process leads to inhibition of bacteria reproduction. Lots of antibiotics, such as penicillin, use this mechanism of action. It is obvious, that D-glucosamine found the application in many aspects of our live. Therefore, this thesis is focused on the preparation of new D-glucosamine based derivates, which can extend the current knowledge of reactivity of the D-glucosamine molecule.



Structure of peptidoglycan

The discussion part is divided into the three chapters. The first one is dedicated to cyclization reactions, where the cycle can be formed either between the nitrogen at C2 carbon and anomeric C1 carbon (product **B**) or the cyclization occurs between the nitrogen at C2 carbon and C3 carbon (product **C**). During the research, the starting compounds and intermediates allowing the cyclization were prepared. Nevertheless, the cyclization itself was troublesome or it did not undergo at all. Moreover, the synthesis of nearly all products was significantly complicated with formation of anomers, where further separation was extremely difficult due to its similar chemical properties.

The middle part is based on the synthesis and reactivity study of D-glucosamine derivate containing the free hydroxyl group **D**. We suggested and optimized two synthetic routes leading to derivate **D**. Subsequently, intermediate **D** was used in alkylation and oxidation reactions. During Fukuyama-Mitsunobu reactions we found that three - membered

ring - aziridine as an important byproduct is easily formed. However, the aziridine formation was observed only when acetone dimethylacetal was used for the hydroxyl protection at C4 and C6 groups. The oxidation of **D** led to ketoderivate **F**, which was further tested toward the condensation reactions. These reactions did not lead to the desired products.

The last part is focused on the synthesis of 2,3-altropyranosides **H** from dinosylated derivate **G**. This family of compounds is not well explored. The known syntheses involve plenty of reaction steps with fluctuating yields. To the contrary, we developed simple and universal method utilizing commercially available *N*-acetyl glucosamine as a starting compound. This method affords 2,3-diaminoaltropyranosides in very good yields.

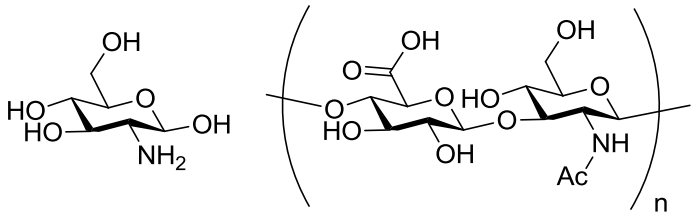
Content

1 Introduction

D-Glucosamine (D-Glc-NH_2), an isoster of D-glucose molecule, has a long history. It was discovered by Dr. Georg Ledderhose in 1876 by acidic hydrolysis of chitine.[1] However; tumultuous development is dated in the early seventies, when a positive effect on cartilage was noticed. Since this time, it became extremely popular dietary supplement with sales of $ 200 million per year.

Formation of D-Glc-NH_2 can be divided into synthetic or biochemical based processes. The first way - synthetic, for example, can use D-mannose as a starting material, which is in ten step synthesis transformed into desired D-Glc-NH_2.[2] However, synthetic routes leading to D-Glc-NH_2 are expensive contrary to the most usual preparation process based on the isolation from a natural material – chitin. A source of chitine for commrecial production is mostly crustacean exoskeletons.[3] D-Glc-NH_2 is biosynthetised in cells as a glucosamine-6-phosphate from fructose 6-phosphate and glutamine in the presence of glutamine-fructose-6-phosphate transaminase.[4] In the final stages, D-Glc-NH_2 is incorporated into the chitin polysaccharide chain based on *N*-acetyl-D-glucosamine units.

D-Glucosamine is a common substance; it naturally occurs in a human body. Furthermore, it can be found in shells of shellfish, animal bones, bone marrow or fungi. It is also part of macromolecules, such as chitosan or chitine.



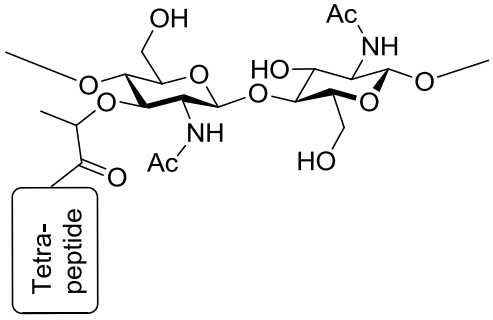
D-Glucosamine Hyaluronic acid

The list of D-Glucosamine biological properties is voluminous. Primary, D-Glc-NH_2 is a precursor of glycosaminoglycans (Mureins), which are major component of human cartilages.[5] Therefore, when arthritis occurs; D-Glc-NH_2 is indicated as a dietary supplement for arthritis, usually in the form of D-Glc-NH_2.HCl or D-Glc-NH_2.H_2SO_4. Moreover, since arthritis is often connected with obesity, D-Glc-NH_2 seems to be responsible for inducing insulin resistance in the absence of glucose or glutamine.[6]

It is also part of hyaluronic acid (HA), widely known polymer of disaccharide.[7,8] Beside *N*-acetylated D-Glc-NH_2 molecule, HA contains glucuronic acid. HA evinces plenty of biological effects; such as anti-inflammatory, antitumour or tissue healing. HA is also common part of skin-care products.

D-Glc-NH_2 can be also found in the molecules, which affect immunity system. For example, it is incorporated in macromolecules, which play a key role in a process of cell determination.[9] This process is responsible for recognition of blood type. The possible application of such structures aims to find the cure for cancer. It is believed, that molecules such as Globo-H-antigen, can particicipate in cancer cells recognition process.[10]

Last, but not least, D-Glc-NH_2 is a part of peptidoglycans, which are present in bacteria cell wall, giving to the bacteria structural strength.[11] Peptidoglycans consist of *N*-Acetyl-D-glucosamine and *N*-Acetylmuramic acid. The free carboxy group of muramic acid is further joined to the tetrapeptide chain. The significant differences in biosynthesis between prokaryotic and eukaryotic cell walls offered an opportunity to develop very low toxic antibacterial compounds such as penicillin, which disturbs the synthesis of cell walls and inhibits the reproduction of bacteria with very high level of selectivity. [12]



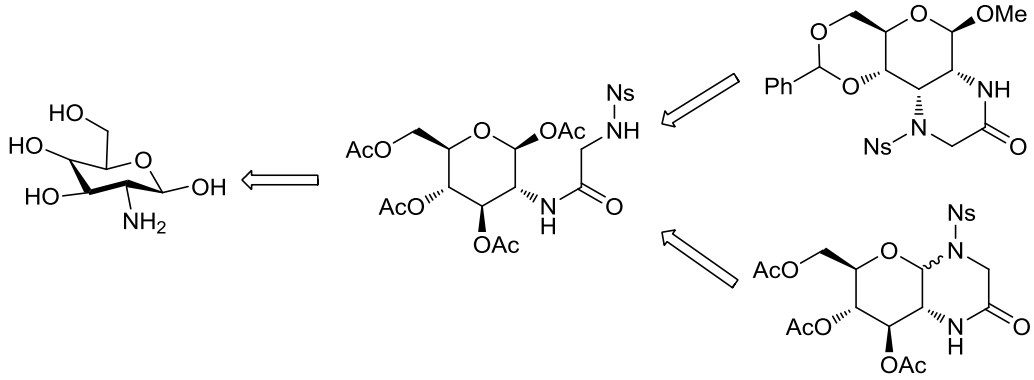
Peptidoglycan

D-Glucosamine, as it was mentioned above, is the molecule with high level of importance. It is present in human and animal cells. It founds applications predominantly in medicine especially for its role in human cartilages. D-Glucosamine is also a part of more complex molecules, such as Hyaluronic acid or peptidoglycans, which are object of study for their anti-inflammatory, antibacterial or healing properties. The development and study of D-glucosamine based molecules has not finished. This Ph.D. thesis has an ambition to contribute with new molecules derived from D-glucosamine and study their reactivity.

2 Aims of the work

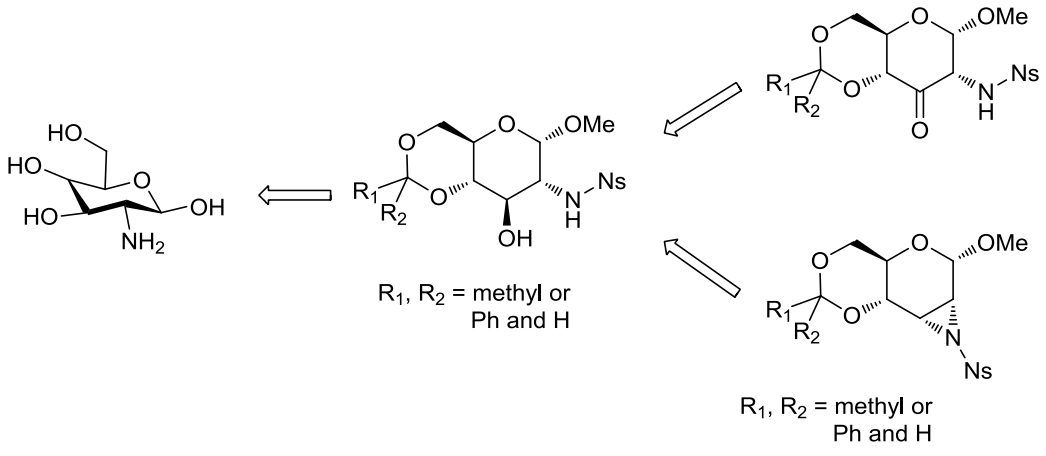
This thesis was focused on derivatization of D-glucosamine, where 4-Nosyl group is employed for activation of the amine group. Nosylated derivatives were further tested toward different reaction paths. The whole thesis is divided into the three projects. The *Project 1* consisted in the synthesis of the glycin-containing derivate, which was subsequently tested toward cyclisation reactions.

Figure 1 - Retrosynthetic analysis of *Project 1*



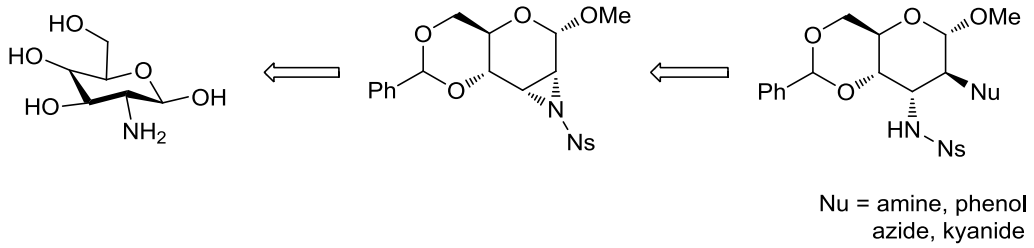
The middle part, the *Project 2*, was based on the direct nosylation of the suitably protected D-glucosamine. The glucosamine derivate, containing the free hydroxyl group, was further attempted to cyclize and oxidize. The purpose of this project was to investigate the possible formation of the aziridine ring and to oxidize the free hydroxyl to corresponding keto functionality to avoid the aziridine ring formation. Subsequently, the keto derivate was tested toward the common nucleophilic reagents.

Figure 2 - Retrosynthetic analysis of *Project 2*



The last part, the *Project 3*, utilized *in situ* formation of aziridine for preparation of a small library of diamino-altropyranosides. The aim was to investigate the reaction conditions of aziridine ring formation and its ring-opening reactions predominantly with nitrogen nucleophiles. Besides amines, also other nucleophiles such as phenols or *C*-acids were tested.

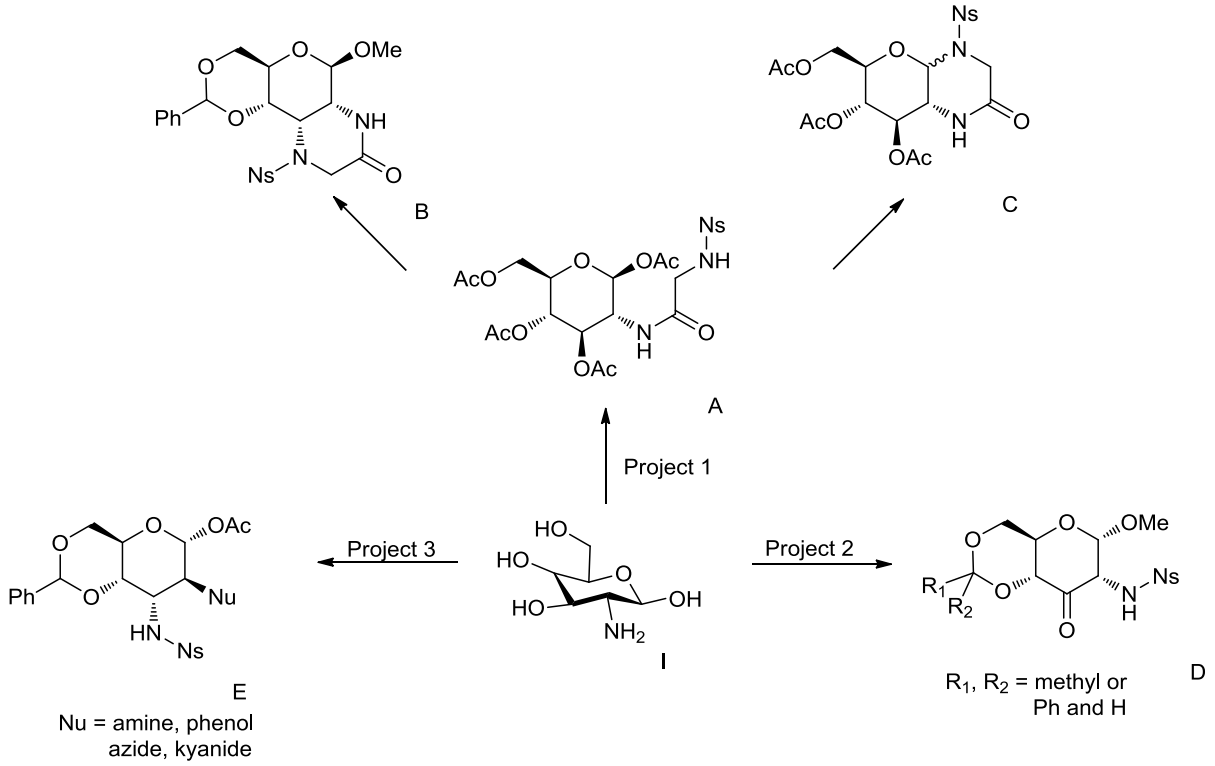
Figure 3 - Retrosynthetic analysis of *Project 3*



3 Results and discussion

The dominant topic of this thesis is nosylation of D-glucosamine and its derivates. Resulting nosylated products were further tested toward alkylation and cyclisation reactions. The whole chapter is divided into the three projects. The aim of the first project was to prepare product **A**, which can further undergo ring-closure either to C1 or to C3 carbon, giving product **B** or product **C**. The second project consists in oxidation of hydroxyl group at C3 carbon to corresponding ketosugar **D**. The reactivity of the resulting keto group was tested towards typical A_N reaction. This part also includes aziridine formation under Mitsunobu conditions. And the last project, is based on the preparation of 2,3-*trans*-diaminoaltropyranosides **E** via aziridine formation.

Scheme 1 - Illustration of synthetic routes

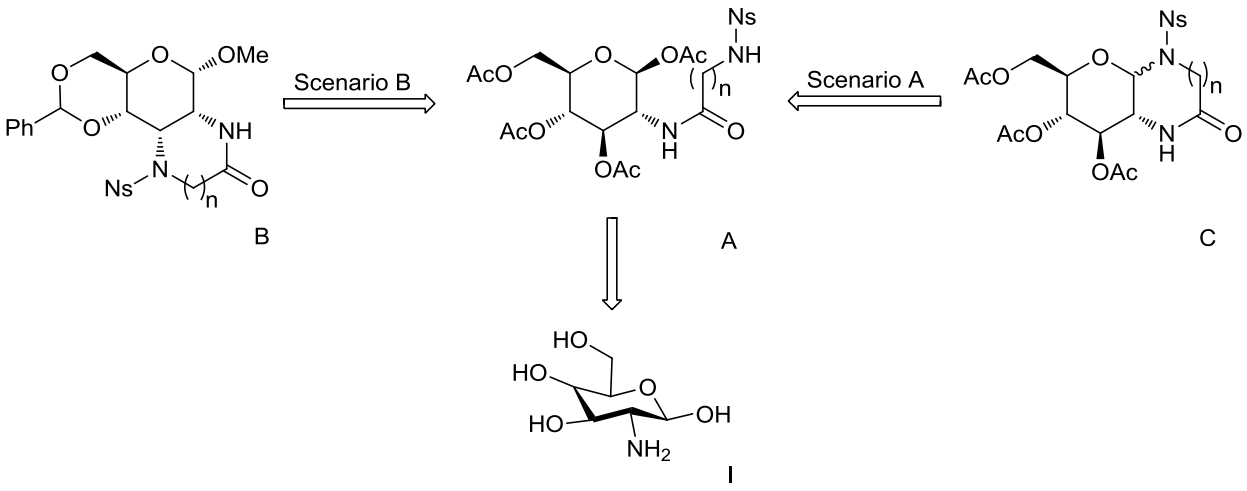


3.1 Project 1 - Synthesis of a suitable derivate for cyclization to C1 or C3 carbon

As you can see in the theoretical part of Ph.D. thesis, incorporation of the D-glucosamine unit into the cycle with six or more members is not so common and formation of the C1-N or C3-N bond is quite rare. Therefore, the first goal was to prepare such precursor

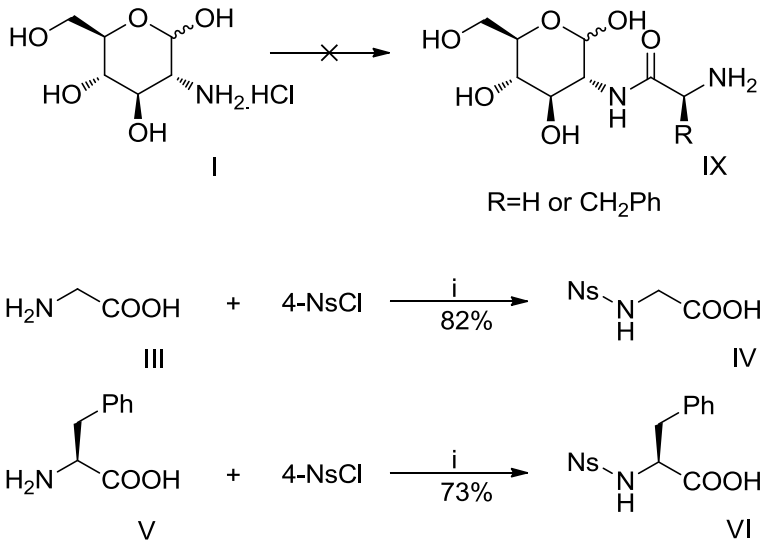
and find reaction conditions allowing desired cyclization. Firstly, it was necessary to prepare the derivatives based on the structure **A**, which served as important intermediates for cyclisation reactions to C1 carbon (*Scenario A*) or to C3 carbon (*Scenario B*).

Figure 4 - Retrosynthetic analysis of *Project 1*



At the very beginning, we tried the simplest method - direct acylation of D-glucosamine hydrochloride **I** with nosylglycine **IV** or nosylphenylalanine **VI.** The reaction did not take place. Nevertheless, it is possible to find plenty of publications describing the acylation of unprotected D-glucosamine **I** with different carboxylic acids.[13-17] We used common acylation methods such as HOBt/DCC or T3P.

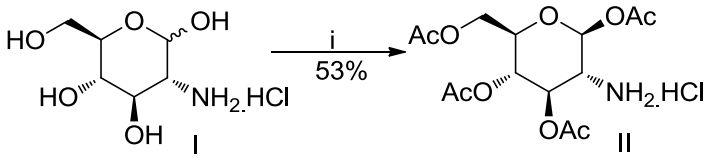
Scheme 2 - Unsuccessful acylation of unprotected D-glucosamine **I** and synthesis of nosylated amino acids



Conditions: (i) 4-Nos-Cl, amino acid, $Na_2HPO_4.12H_2O$, water, 73-82%

Since the acylation of D-glucosamine **I** did not take place, we decided to protect the free hydroxyl groups via acetylation. Tetra-*O*-acetyl glucosamine **II** was prepared in the three step synthesis from readily available D-glucosamine **I**, following the known procedure.[18] This synthesis allows a multi gram scale (more than hundred grams).

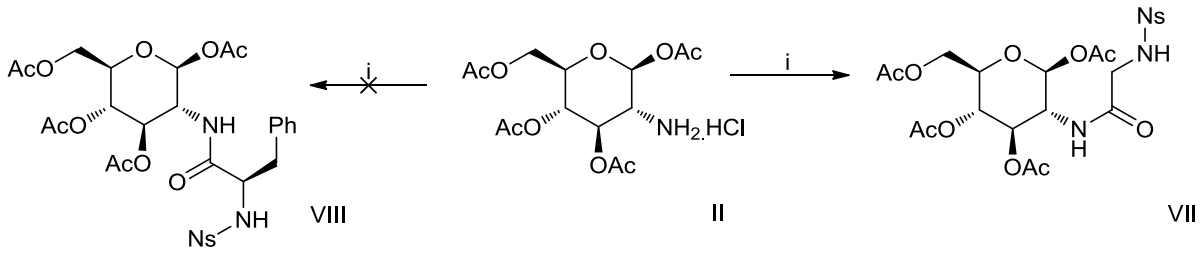
Scheme 3 – Synthesis of fully *O*-protected D-glucosamine **II**



Conditions: (i) anisaldehyde, MeOH, 1 M NaOH, then Ac_2O, Pyridine, then Acetone, 5 N HCl, 53% (three steps)

The formation of the amide bond between the amino group of D-glucosamine **II** and the carboxylate of the *N*-nosylated amino acid **IV** was surprisingly difficult. The optimization of acylation were done, plenty of different solvents were tried, unfortunately with no significant effect. The biggest problem consists in removing of the dialkyl urea, which was formed during HOBt (HOSu) mediated reaction. The best results were achieved with EDC/HOSu, where troubles with separation were suppressed due to solubility of EDC. Further, the same conditions were applied to nosylated phenylalanine **VI**. The reaction did not proceed, probably due to bulkiness of the amino acid. The configuration at C1 carbon was retained. The coupling constant of anomeric proton is ^3J ($^1H^1H$) = 8.8 Hz, which indicated the presence of ß-anomer.

Scheme 4 - Amide bond formation



Conditions: (i) Nosylated amino acid, see the table below

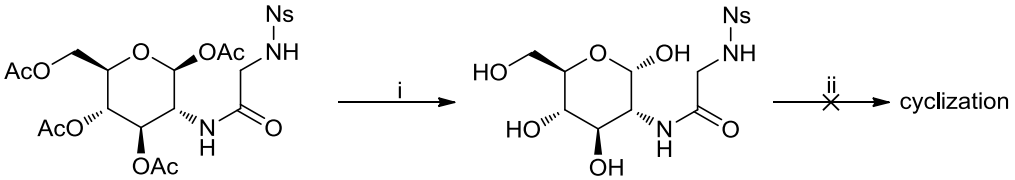
Table 1 - Optimization of the amide bond formation

agents	solvent	yield[1]	purity[1]
DIC/HOBt	py	64%	80%
DCC/HOBt	py	70%	80%
EDC/HOSu	py	34%	95%
T3P	DMF(dry)	45%	75%

[1] related to product **VII**

Following the main topic of *Project 1*, we firstly did the full deacetylation of carbohydrate **VII** with NaOMe. The purpose of this reaction was to „offer", more positions for cyclisation. The potential cyclization, performed under Fukuyama-Mitsunobu conditions, could take place either at C1 (*Scenario A*) or C3 carbon (*Scenario B*), however, only a starting material was detected.

Scheme 5 – Full deprotection of acetylated glucosamine

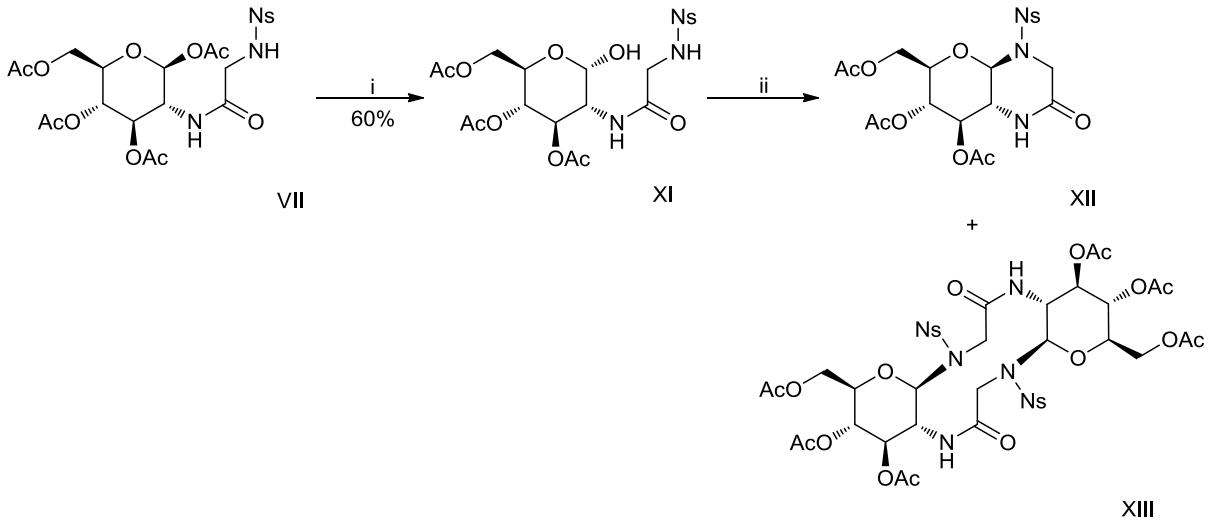


VII X

Conditions: (i) MeONa/MeOH, 85%; (ii) DIAD, TPP, dioxane

Since the cyclization of **X** does not work, a partial deprotection at the anomeric carbon of **VII** has been tried (*Scheme 6*). The partial deprotection of **VII** was carried out with methylamine, hydrazine acetate and a solution of ammonia in EtOH (*Table 2*). The best result gave the deprotection with methylamine, where the ratio of α/ß anomers was 70/30 toward α-anomer. α-Anomer was separated and characterized. The configuration at C1 was assigned according to the vicinal coupling constant $^{3}J(^{1}H^{1}H)$ = 3.9 Hz (t).

Scheme 6 - Partial deprotection followed by cyclization



Conditions: (i) CH_3NH_2 (2 M in MeOH), THF, 60%; (ii) DIAD, TPP, dioxane

Table 2 - Anomeric deprotection

Method	Conditions	Anomeric ratio (α/β)[1]
$MeNH_2$	THF, rt	70/30
$N_2H_4.AcOH$	DMF, rt	67/33
NH_3/MeOH	THF, rt	-[2]

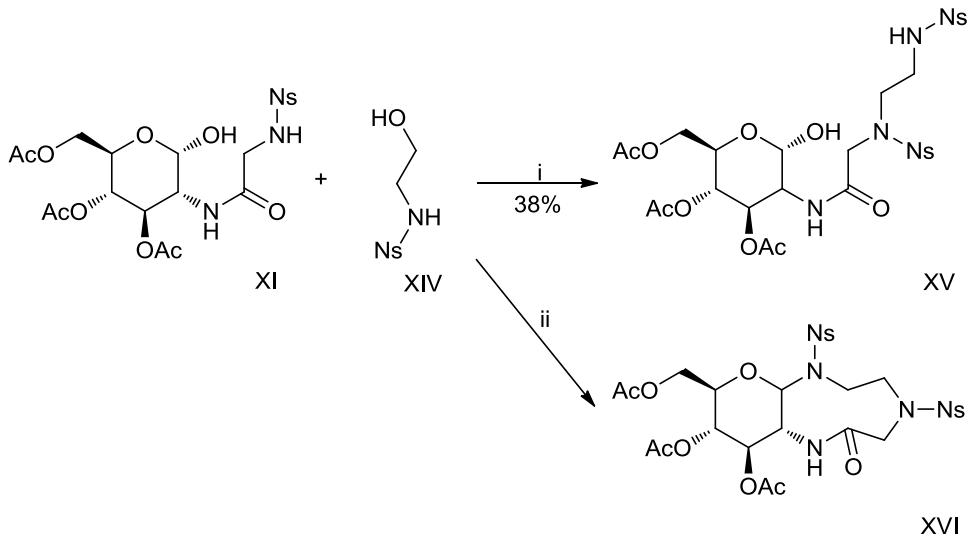
[1] calculated from NMR

[2] mixture of partially and fully deprotected products

The subsequent cyclization was tried exclusively on α-anomer **XI** and the reaction gave a mixture of products, where the major product was probably the macrocycle **XIII** and the minor was **XII**. The structures **XII** and **XIII** were suggested on basis of LCMS chromatograms. In accordance with our observations, also other authors described the favored formation of similar macrocycles utilizing the nucleophilic aromatic substitution or even radical cyclization. For details, see *Chapter 3.2.2.3*, *Scheme 48* and *50* in Ph.D. thesis. *Scheme 6* does not include the possible formation of glycans, which can be formed by elimination of C1 hydroxy group. The structure confirmation was not done due to complicated mixture of products and instability of macrocycle **XII**. The change of Mitsunobu reagents had only a marginal influence on the resulting mixture. Both products were separated; however, the resulting purity does not allow the full characterization. It was obvious, that the six - membered ring formation was not favored, therefore we tried to prolong the alkyl chain with ethylamine.

The extension of the chain should result in a nine - membered ring (*Scheme 7*). Firstly, *N*-nosylated aminoethanol **XIV** was prepared. Afterwards, the cyclization of amide **XI** with alcohol **XIV** was tested. It is obvious, that cyclization of **XI** with **XIV** offers plenty of possible products. Firstly, the cyclization was complicated by mutual reaction of two molecules of **XIV**. Further, we assumed that the first Mitsunobu reaction took place between the primary hydroxyl group of **XIV** and nosylamide functionality of **XI**. The assumption was based on the fact, that the primary hydroxyl group of **XIV** is more reactive than the anomeric hydroxyl for the steric reasons. The traditional Mitsunobu reagents (DIAD/TPP) allowed prolongation of the chain resulting in **XV**, while the use of the TMAD/Bu_3P system supported either formation of the nine - member ring **XVI** or above described dehydration resulting in glycan. The compound **XV** was separated. Its NMR spectra are not attached due to the low purity. Macrocycle **XVI** apparently decomposed during the purification.

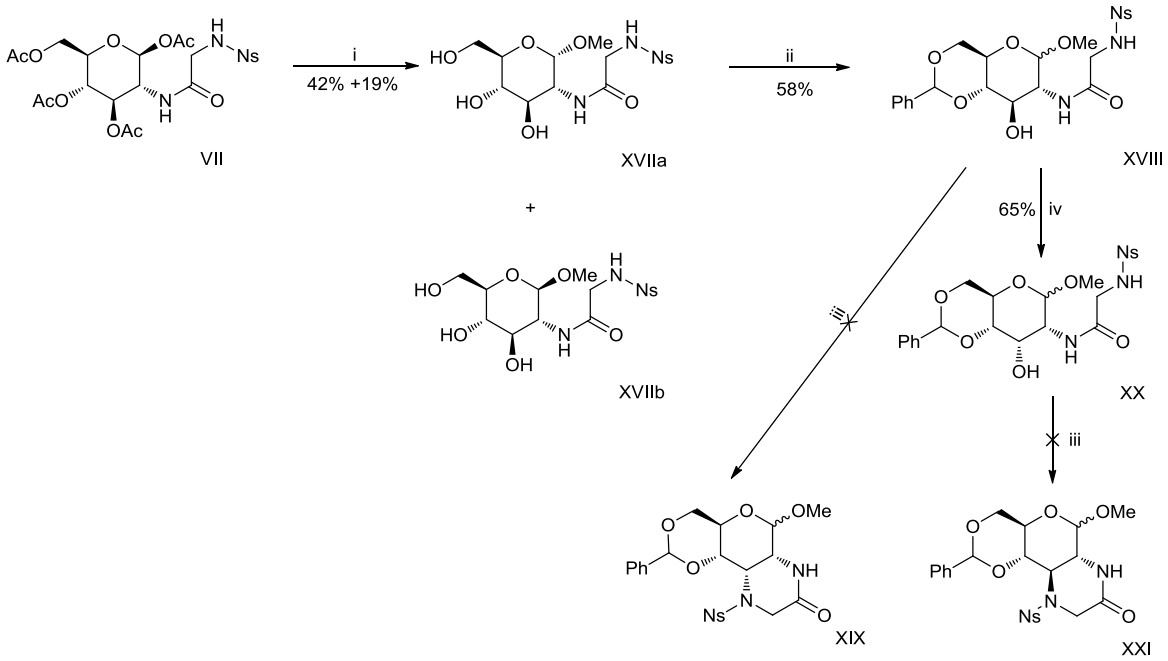
Scheme 7 - Cyclization with nosylated aminoethanol **XV**



Conditions: (i) DIAD, TPP, dioxane, 38%; (ii) TMAD, Bu_3P, dioxane

After the possibility of cyclization to C1 carbon was investigated, we focused our attention on the hydroxyl group placed at the C3 carbon (*Scenario B*). First of all, we had to protect more reactive anomeric hydroxyl group (see *Scheme 8*). This problem was easily solved out by the total deprotection of acetyl groups of **VII** with sodium methoxide and subsequent addition of dried Amberlite IR $120H^+$ to give the selectively methylated C1 hydroxyl group. Both resulting anomers **XVII** were separated and characterized.

Scheme 8 - Cyclization to C3 carbon

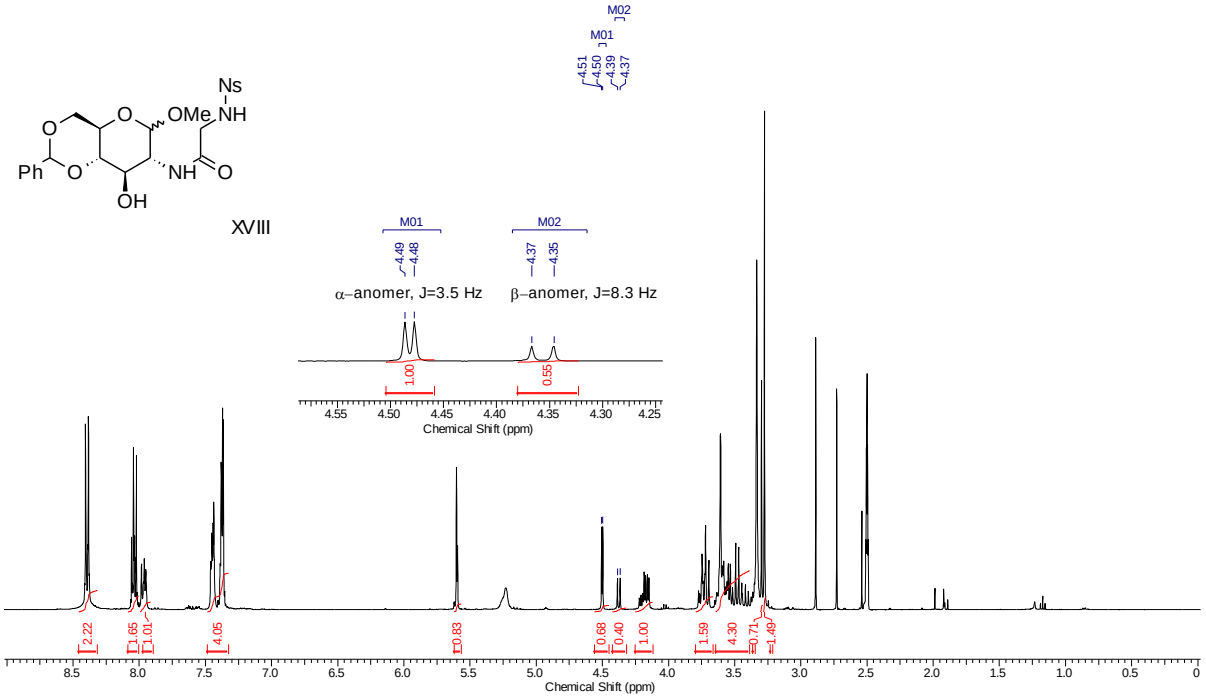


Conditions (i) MeONa/MeOH, then dry IR $120H^+$, 42% for **XVIIa** and 19% for **XVIIb**; (ii) BDA, IR $120H^+$, dioxane, 58% (mixture of isomers), (iii) DIAD, TPP, dioxane or TMAD, Bu_3P, dioxane; (iv) PhCOOH, DIAD, TPP, then K_2CO_3, MeOH, 75%

The ratio of anomers according to the ^1H NMR spectrum was 70/30 toward α-anomer. Two doublets around 4.4 ppm were assigned to C1 proton. The peak with the higher chemical shift and the coupling constant $^3J(^1H^1H)$=3.4 Hz (d) belongs to the α-anomer, while the peak with lower chemical shift and the coupling constant $^3J(^1H^1H)$=8.0 Hz (d) belongs to the ß-anomer.

The hydroxyl groups at C5 and C6 carbons were protected via the acid-catalyzed reaction of benzaldehyde dimethylacetal (BDA) with glucosamine **XVIIa**. The reaction provided again a mixture of anomers; however, the isomers were not separated due to the extremely similar physical properties. The ratio of anomers was 2/1 toward α-anomer, for details, see *Figure 5.*

Figure 5 - Anomeric mixture of **XVIII**



The cyclization of **XVIII** (mixture of anomers) was tested under various Mitsunobu conditions. Applying of TPP/DIAD, TPP/DBAD, or Bu_3P/TMAD in dioxane or THF gave only the starting material; no traces of **XIX** were observed. The inversion of configuration at C3 carbon, resulting in product **XX**, unfortunately did not allow the potential cyclization leading to XXI.

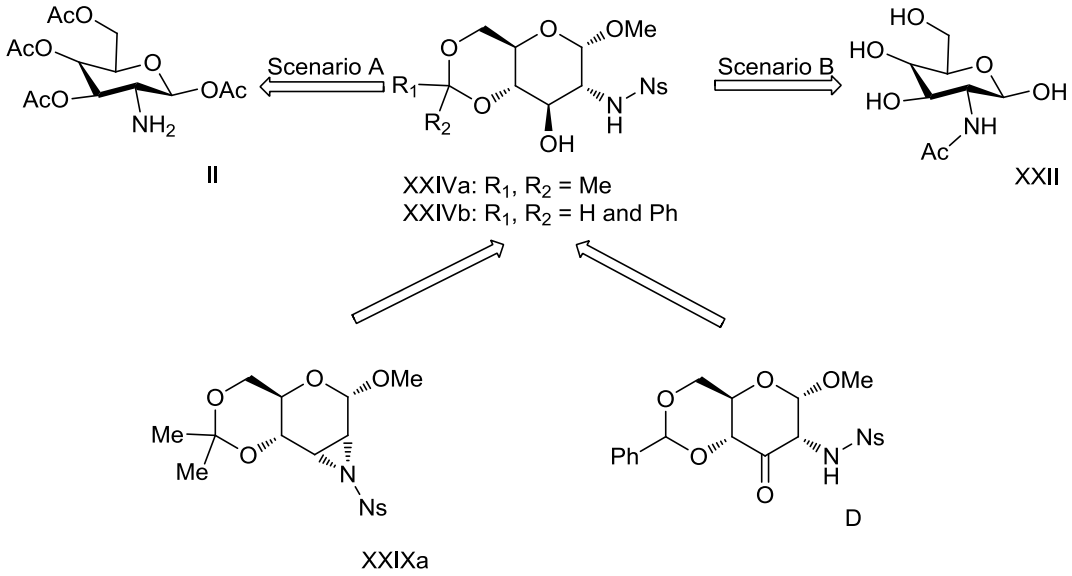
To summarize *Project 1*, all the intermediates, necessary for cyclization reactions, were prepared and their reactivity was tested. Unfortunately, the potential cyclization to C1 carbon was not proved. In addition, undesired side reactions were observed. The cyclization toward to C3 carbon did not work at all.

3.2 Project 2 - Synthesis of D-glucosamine derivate with free hydroxy group at C3 carbon and its reactivity study

Project 2 was focused on transformations of the amino and hydroxyl group of D-glucosamine at position 2 and 3 to obtain more suitable functionalities for potential formation of an *ortho*-fused ring. It was necessary to protect hydroxyls at positions 1, 4, and 6, and then modified amino and hydroxyl group at position 2 and 3. A key intermediate, **XXIV**, was

prepared via two different synthetic routes, *Scenario A* and *Scenario B* (*Figure 6*). Firstly, it was studied the reactivity of the nosyl group toward Fukuyama-Mitsunobu alkylation and possible formation of aziridine **XXIX** as a result of a side intramolecular reaction with the hydroxyl at position 3. In the end, attention was paid to the oxidation of the hydroxyl at position 3 into the keto functionality resulting in derivative **D**. Consequently, the keto group can potentially offer other useful transformations.

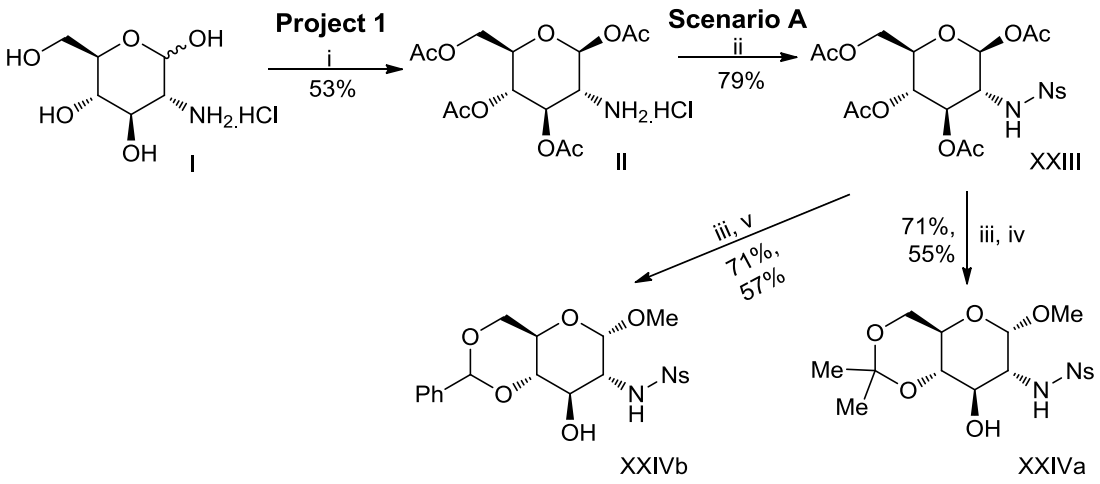
Figure 6 - Retrosynthetic analysis of Project 2



The synthesis of the key intermediate, **XXIV**, started according to *Scenario A* with tetra-*O*-acetyl glucosamine **II**, which was prepared on a large scale in *Project 1* (*Scheme 3*). It was anticipated the possibility to perform nosylation and deacetylation with subsequent protection in three isolation steps.

Indeed, nosylation of **II** gave *N*-nosylated derivate **XXIII** in a very good yield, which was isolated as a ß-anomer, $^{3}J(^{1}H^{1}H)$= 8.3 Hz. Conversion of **XXIII** to desired hydroxyl derivates **XXIV** was done in two steps. In the first step, base-catalyzed cleavage of acetyl groups, followed by Fischer methylation of anomeric hydroxyl, gave deacetylated intermediate, which was isolated as a mixture of anomers and used for the next step. Further, anomers were treated with acetone dimethyl acetal or benzaldehyde dimethyl acetal to give **XXIVa** and **XXIVb** respectively. Both products were isolated as α-anomers ($^{3}J(^{1}H^{1}H)$= 5.2 Hz for **XXIVa** and $^{3}J(^{1}H^{1}H)$= 3.6 Hz for **XXIVb**) and fully characterized.

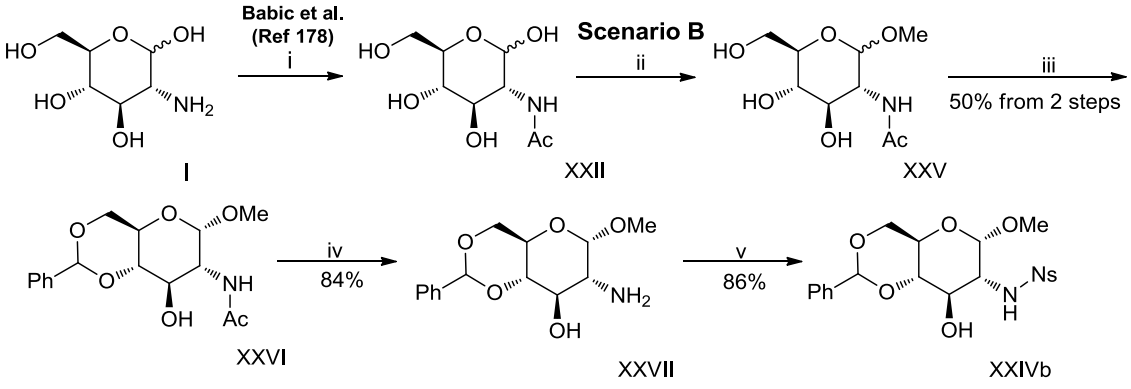
Scheme 9 - Preparation of alcohols **XXIV** via *Scenario A*



Conditions: (i) anisaldehyde, MeOH, 1 M NaOH, then Ac_2O, Pyridine, then Acetone, 5 N HCl, 53%; (ii) 4-NsCl, Et_3N, DCM/pyridine, 79%; (iii) MeONa/MeOH, then IR 120H$^+$, mixture of anomers, 71%; (iv) 2,2-dimethoxypropane, IR 120H$^+$, dioxane, 55%; (v) BDA, dioxane, IR 120H$^+$, 57%

Scenario B used a different approach for preparation of derivatives **XXIV** and started with *N*-acetylated glucosamine **XXII** (*Scheme 10*). The method for quantitative *N*-acetylation of D-glucosamine **I** is well described,[19] however, the price availability of **XXII**, comparable to D-glucosamine **I,** allowed purchasing **XXII** from a chemical company. The procedure resulting in **XXVII** followed known synthesis with small improvements in work-up of intermediates.[20,21] The aminoalcohol **XXVII** was then nosylated with 4-Nosyl chloride to give **XXIVb** in a very good yield.

Scheme 10 - Synthesis of alcohol **XXIVb** via *Scenario B*



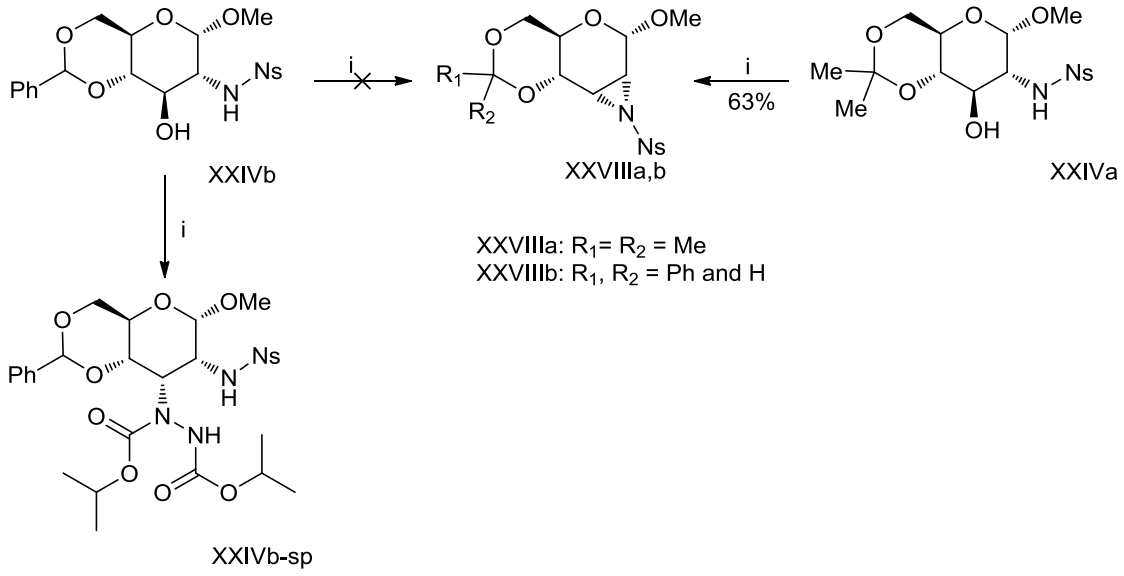
Conditions: (i) Na/MeOH, Ac_2O, quant.; (ii) MeOH, IR 120H$^+$; (iii) BDA, TsOH.H_2O, dioxane, 50% (for two steps); (iv) 4 M KOH, EtOH, 84%; (v) 4-NsCl, DCM/pyridine, 86%

When D-glucosamine **I** is considered as a starting compound, *Scenario A* gave product **XXIVb** in 17% overall yield, whereas *Scenario B* more than doubled the overall yield to 36%

of **XXIVb**. Also the number of reaction steps is lower in the case of *Scenario B* (5 steps) than in the case of *Scenario A* (6 steps). However, *Project 1* included synthesis of *O*-Acetylated D-Glucosamine **II** in a multi-gram scale (more than 100 g); therefore we could employ carbohydrate **II** as a starting compound. In such case, the overall yield of *Scenario A* was increased to 34% and the number of reaction steps was significantly decreased to three steps. Generally, from an economic as well as synthetic perspective, the *Scenario B* is more favorable, however, the availability of carbohydrate **II** from previous *Project 1* made both methods comparable.

Further, the cyclization of both **XXIVa** and **XXIVb** was tested, since the formation of aziridine **XXVIII** was possible side reaction occurring in Fukuyama-Mitsunobu alkylation (*Scheme 11*). Surprisingly, the cyclization under various Mitsunobu conditions (TPP/DIAD, TPP/DBAD or Bu_3P/TMAD in dioxane) gave the corresponding aziridine only in the case of derivate **XXIVa**. The reaction with **XXIVb** led to the formation of side product **XXIVb-sp** which was not isolated; however the LC-MS analysis indicated this structure with high probability. We assumed that the formation of **XXIV-sp** is caused by steric reasons. The aziridine **XXVIIIa** was fully characterized. To sum up, the substitution at C4 and C6 carbon has an important effect on the aziridine formation. It is important to mention, that aziridine **XXVIIIB** was also prepared in a different way, via substitution of the nosylated hydroxyl group. The details will be discussed in the next chapter.

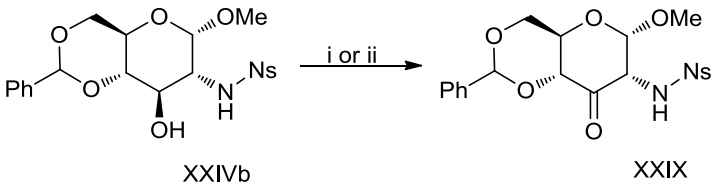
Scheme 11 - Reactivity study of **XXIV** under Mitsunobu reaction



Conditions: (i) DIAD, TPP, dioxane, 63% (for **XXVa**)

Further, the oxidation of **XXIVb** was tested. The analogous reaction with **XXIVa** was not performed. Two different methods were used for a conversion of the hydroxy group at C3 to the keto function (*Scheme 12*). The first one was based on oxidation with Dess Martin periodinane (DMP). This reaction provided good yields; the addition of 1.1 equivalent of water speeded up the reaction. The second approach employed modified Swern oxidation, where instead of oxalyl chloride; a 50% solution of T3P in EtOAc was used. Both methods offer good yields and easy separation; however, we decided to carry out the oxidation with T3P, which offered slightly higher yields. The ketone **XXIX** was identified by the ^{13}C NMR spectrum, where the carbonyl signal showed the chemical shift about 195 ppm, which is typical for a presence of the keto group.

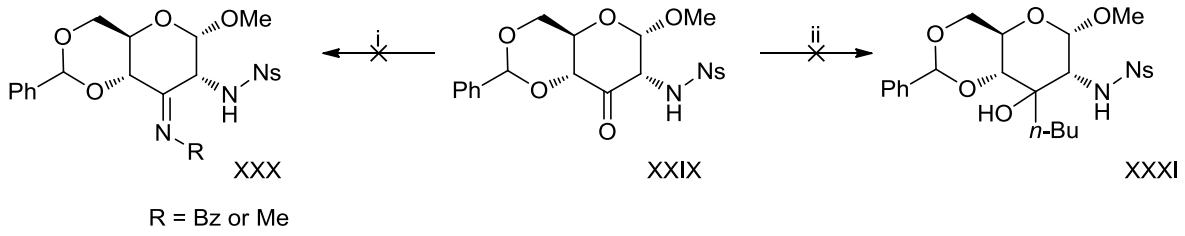
Scheme 12 - Oxidation of the hydroxyl group at C3



Conditions: (i) T3P/EtOAc, DMSO, EtOAc (dry), 69%; (ii) DMP, DCM, water (1.1equiv), 55%

After the gram - scale synthesis of ketone **XXIX**, the reactivity of the keto group was investigated (*Scheme 13*). Surprisingly, the carbonyl group was unreactive against common reagents. The reaction of **XXIX** with two equiv. of benzylamine or methylamine gave unknown products, which did not correspond to the expected ketimine products **XXX**. Addition of *n*-BuLi or a 2 M solution of isopropylmagnesium bromide resulted in decomposition of a starting material.

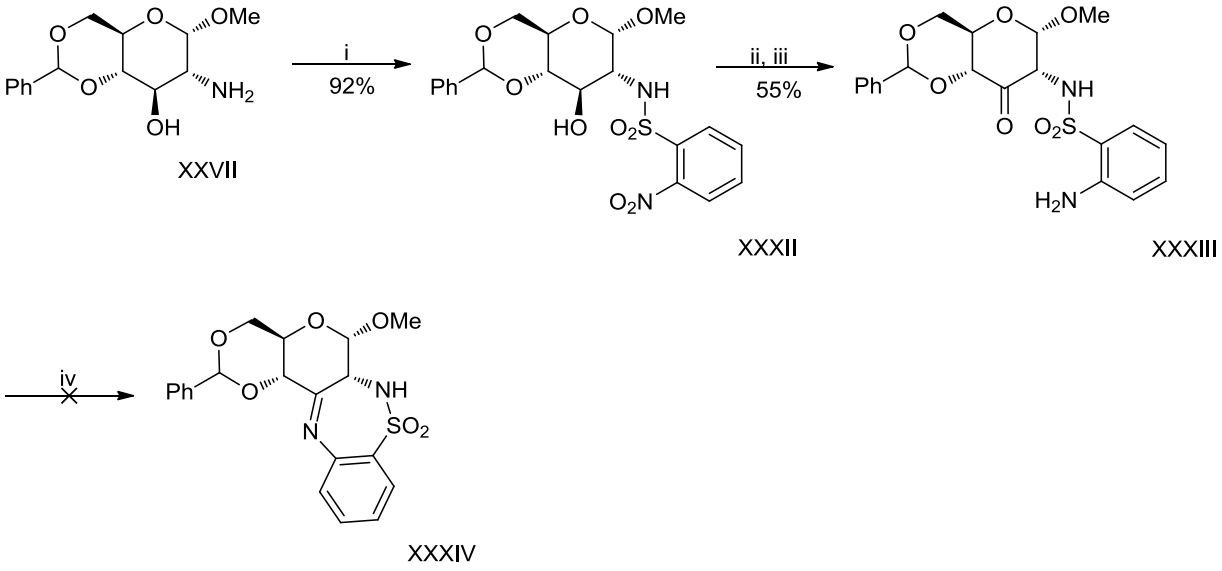
Scheme 13 - Attempted reactions with the keto group



Conditions: (i) amine, MeOH, reflux; (ii) Grignard reagent, THF (dry), 0°C

Moreover, the intramolecular cyclization was investigated. When 2-Nosyl instead of 4-Nosyl group was attached to the amine function, the reduction of the nitro group could allow the formation of seven - membered ring (*Scheme 14*). The reaction with 2-Nosyl group took place under the same conditions described for the 4-Nosyl group. Oxidation with T3P/EtOAc and subsequent reduction of the nitro group afforded keto derivate **XXXIII**, which was used for the final cyclization. The structure of keto derivative **XXXIII** was confirmed by the LCMS analysis; the NMR spectra of **XXXIII** are not attached due to low purity of product. Unfortunately, the closure of the cycle was not successful, even traces of the product were not detected. Further cyclization attempts were not performed due to the lack of starting materials.

Scheme 14 - Attempted intramolecular cyclization



XXVII XXXII XXXIII XXXIV

Conditions: (i) 2-NsCl, DCM/pyridine, 92%; (ii) T3P/EtOAc, DMSO, EtOAc, 78%; (iii) H_2, Pd/C, THF, 70%; (iv) Δt or base

In summary, we found and optimized two methods for the synthesis of hydroxyl derivative **XXIV**. The better results were achieved with *Scenario B*. Further, Fukuyama-Mitsunobu alkylation and oxidation of **XXIV** was investigated. During alkylation reactions, it was found out, that cyclization of **XXIV** resulting in aziridine cycle took place only when 2,2 - dimethoxy propane protected hydroxyls at C4 and C6 carbons. Oxidation of **XXIVb** gave the corresponding keto derivate **XXIX**, which, unfortunately, was unreactive against common nucleophiles.

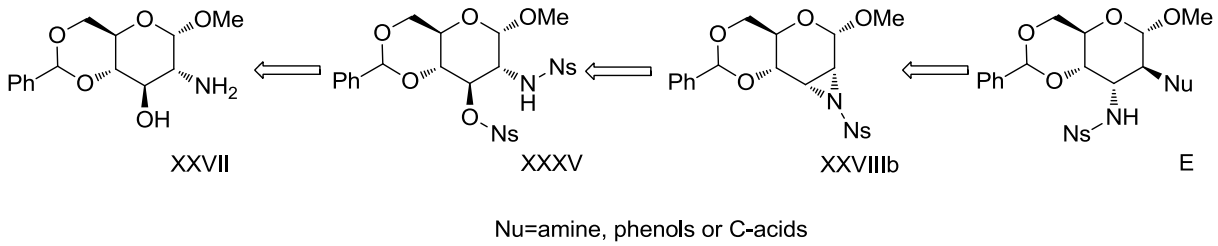
3.3 Project 3 - Preparation of 2,3 - diamino carbohydrate via aziridine formation

This part is loosely based on the publication: Ring-opening reactions of the *N*-4-nosyl Hough-Richardson aziridine with nitrogen nucleophiles. The introduction was significantly reduced. Some parts in the discussion part are extended with redundant or negative results, which were not used in the publication. The numbering and the layout were adjusted to the style of the Ph.D. thesis.

3.3.1 Introduction

Previously, we revealed in *Project 2*, that the synthesis of aziridne derivatives **XXVIII** is feasible. Consequently, we realized that the aziridine ring strain can be utilized to synthesize other useful derivatives by the ring-opening reaction. However, we chose a more practical route to synthesize above mentioned aziridine **XXVIII**. *Project 3* was focused on the preparation of altrohexopyranosides **E** via dinosylated glucosamine **XXXV** (*Figure 7*).

Figure 7 - Retrosynthetic analysis of *Project 3*

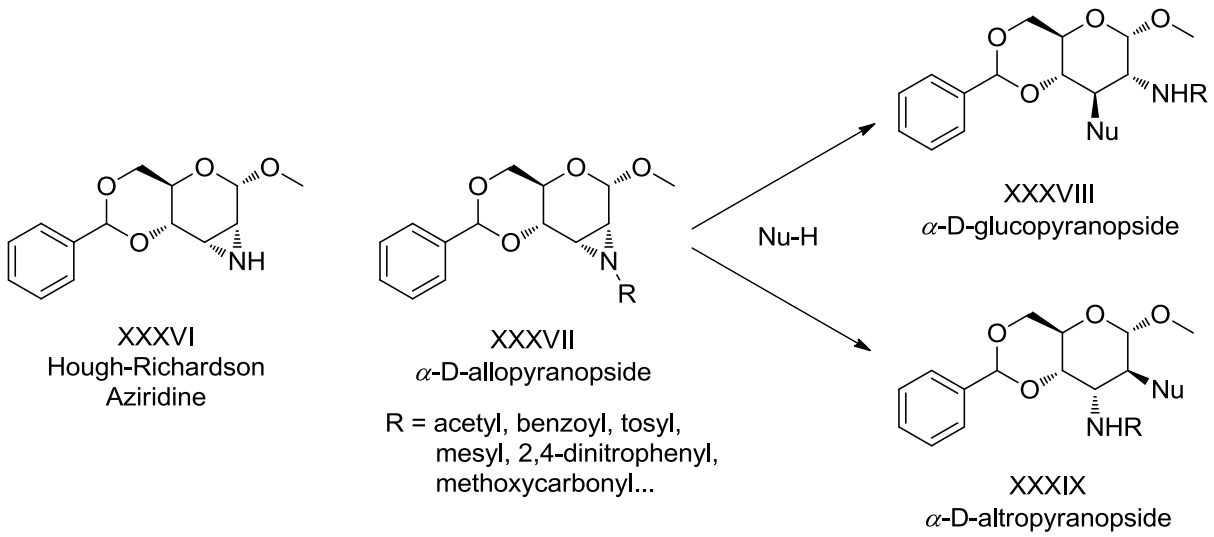


Hexopyranosides containing 2,3-diamino functionality are useful chiral synthetic intermediates in the synthesis of highly functionalized compounds with interesting biological or chemical properties. These intermediates were used directly as chiral ligands in half-sandwich metal complexes with antitumor activity[22,23] or molybdenum complexes to catalyze asymmetric allylic alkylations.[24] Further, 2,3-diaminohexopyranosides also served as a key precursor in the synthesis of glycophospholipid ligand of lipopolysaccharide receptor,[25] chimeric scaffolds with benzodiazepine moiety,[26] and Weinreb´s advanced intermediate for (-)-Agelastatin A formal total synthesis.[27]

One of the frequently used methods leading to the derivatives of 2,3-diaminohexapyranosides is the ring-opening reaction of the Hough-Richardson aziridine,[28] which is commonly

synthesized from D-glucosamine. This valuable chiral intermediate can be transformed by regioselective ring-opening reaction of strain-loaded three-membered ring into the corresponding diastereoisomers with α-D-*altro*- or α-D-*gluco*- configurations (*Figure 8*).

Figure 8 - The general ring-opening reaction of the Hough-Richardson aziridine



3.3.2 Scope and limitations

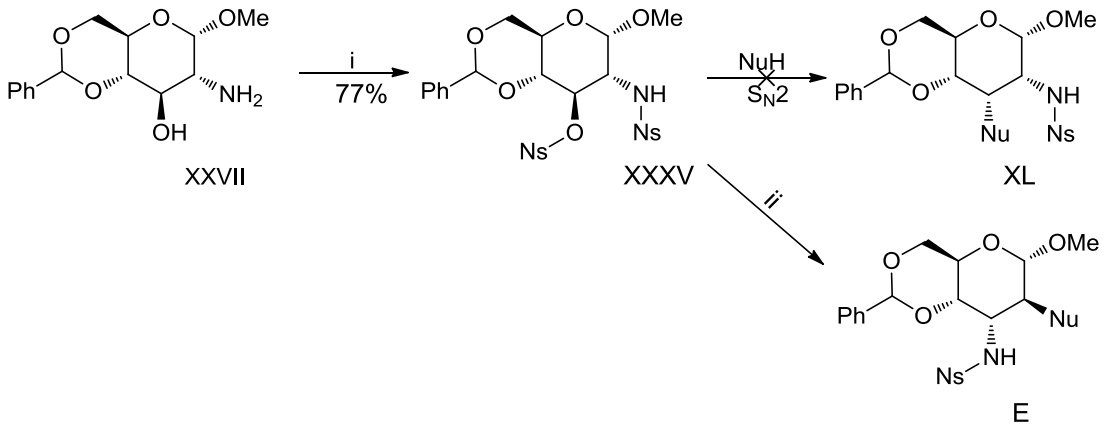
The ring opening reactions of aziridine **XXXVII** were troublesome. The yields were decreased due to the formation of two isomers (**XXXVIII**, **XXXIX**)[29,30] and the scope of the reaction was extremely narrow; from nitrogen-containing nucleophiles, only azide was tested. For more details, see *Chapter 3.1.1.1.* in Ph.D. thesis.

In this contribution we present the first synthesis and the ring-opening reactions of the *N*-4-nosyl Hough-Richardson aziridine mostly with nitrogen nucleophiles. The *O*- and *C*-nucleophiles are included in a separate table. The electron-withdrawing effect of the nitro group in this new valuable advanced intermediate brings several significant practical advantages, especially (i) synthesis of aziridine under mild conditions and, if necessary, this intermediate can be isolated by a simple filtration in a very good yield and high purity, (ii) to perform the highly regioselective aziridine ring-opening reactions without presence of ammonium chloride resulting in products preferring the α-D-*altro*- prior to α-D-*gluco*-configuration in a ratio no less than 90:10 under more convenient conditions utilizing more practical solvent and temperature, (iii) the scope of applicable nucleophiles for the ring-opening reaction is substantially broadened, solely reported azide ion can be directly replaced

with even aqueous ammonia which eliminate the subsequent reduction step, (iv) further modification of nosylamide functionality particularly by *N*-alkylation under classical or Fukuyama-Mitsunobu protocols,[31] and (v) potential mild deprotection conditions of nosyl group in comparison to the mesyl or tosyl group.

During nosylation of **XXVII** (*see Scheme 10*), it was find out, that as a minor product (less than 5%) dinosylated carbohydrate **XXXV** was created. When 4.2 equivalent instead of 1.05 equivalent of 4-NsCl was added, the nosylation underwent on both the amino and hydroxy group and dinosylated product **XXXV** was formed. Subsequent conversion did not take place via S_N2 reaction to *allo*- derivate **XL**, but *altro*-pyranoside **E** was detected.

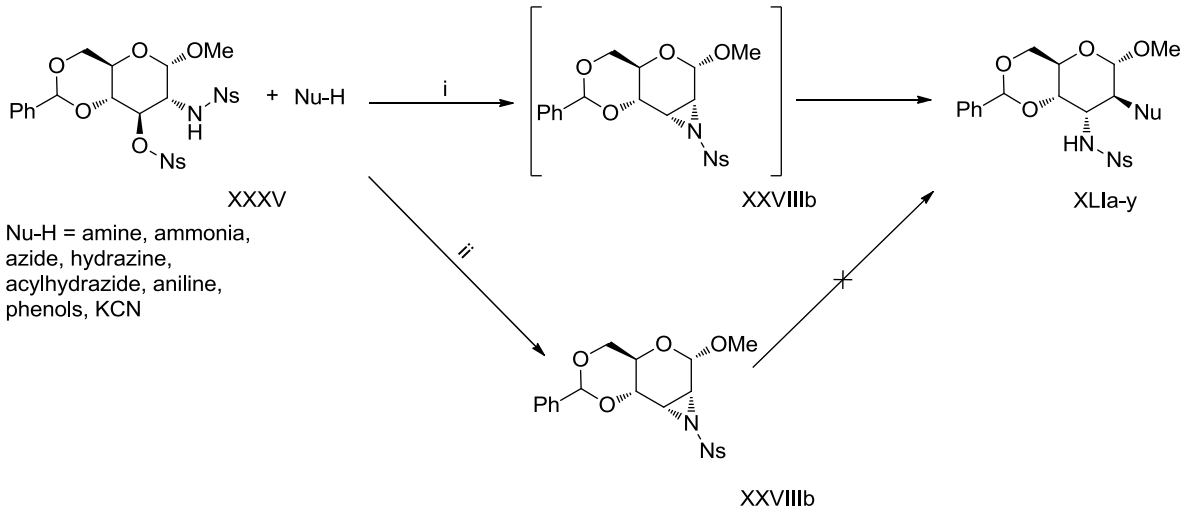
Scheme 15 - Synthesis of 2,3 – diaminohexapyranoside **E**



Conditions: (i) 4.2 equiv. 4-NsCl, DCM/pyridine, 77%; (ii) NuH, DIPEA, DMSO, 60°C

Addition of DIPEA to dinosylated glucosamine **XXXV** led to rapid formation of aziridine **XXVIIIb** (*Scheme 16*). If DMSO is used as a solvent, *in situ* formed aziridine **XXVIIIb** can be directly transformed at only 60 °C into 2,3-diaminoaltropyranoside **XLI** in the presence of various nucleophiles. Replacement of DMSO by 2-methoxyethanol allowed simple isolation of crude aziridine **XXVIIIb** by filtration in very good yield and high purity. Very poor solubility of aziridine **XXVIIIb** in 2-methoxyethanol practically eliminated subsequent aziridine ring-opening reaction with nitrogen nucleophile.

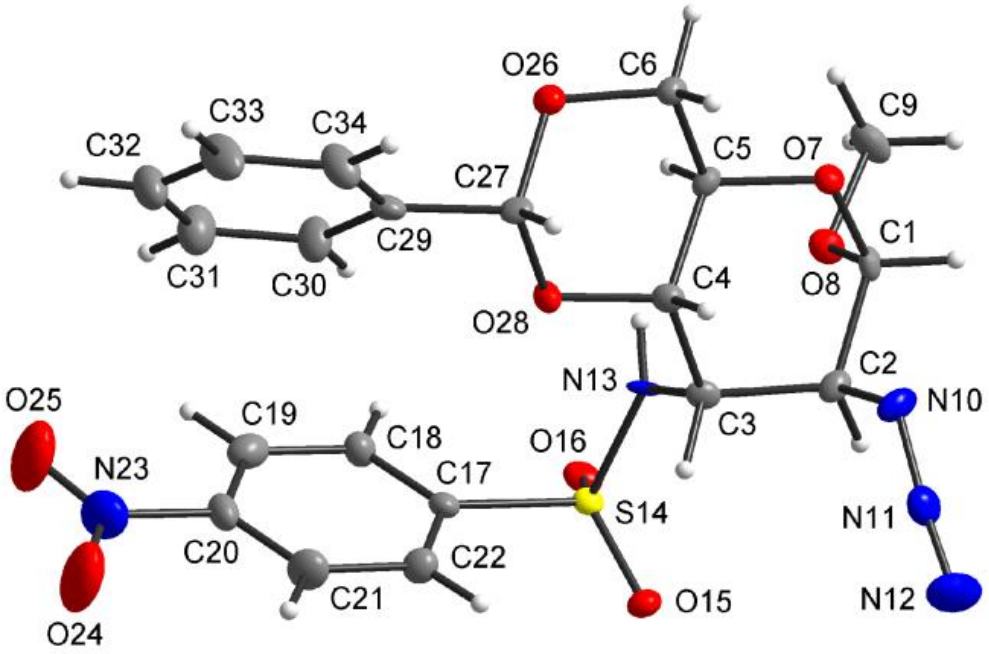
Scheme 16 - Influence of the solvent on the aziridine **XXVIIIb** ring-opening reactivity



Conditions: (i) DIPEA, DMSO, 38-91%; (ii) DIPEA, 2-methoxyethanol, 83%

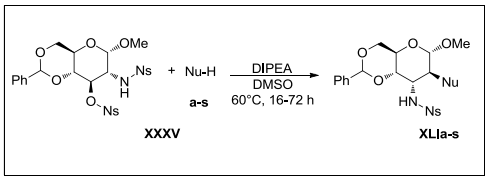
The assumed mechanism of the aziridine formation involves a transition state of nosylamide **XXXV** in the boat conformation directing the nosyl ester and nosyl amide groups in the *trans*-diaxial relationship. Formation of nosylamide anion and increased departing ability of the adjacent nosylate group was accelerated by nitro group which led to the fast aziridine ring closure. The α-D-*allo* configuration of aziridine **XXVIIIb** was confirmed by NMR spectra. Subsequent aziridine ring-opening reaction with nucleophile, resulting in the cleavage of C2-N bond, followed the Fürst-Plattner rule[32] to give *trans*-diaxial products **XLI** with α-D-*altro* configuration. Additionally, the α-D-*altro* configuration of **XLIa** was unequivocally determined by X-ray structure (*Figure 9*).

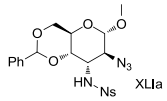
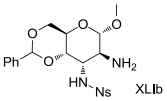
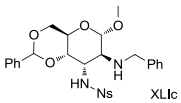
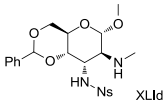
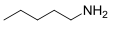
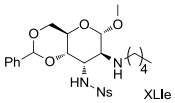
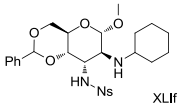
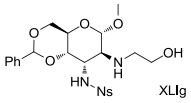
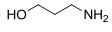
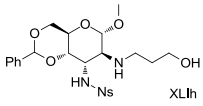
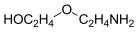
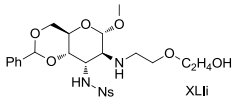
Figure 9 - X-Ray structure of **XLa**

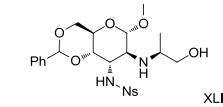
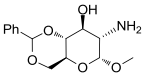
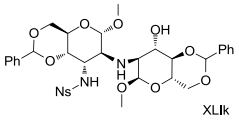
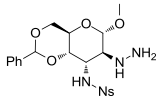
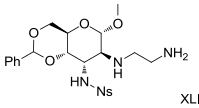
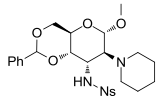
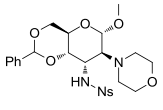
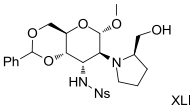
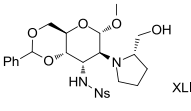
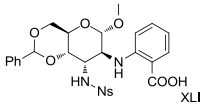
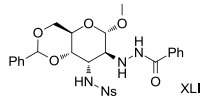


To explore the aziridine ring-opening reactivity scope with regard to the structure diversity of nitrogen nucleophile we started a set of reactions (*Table 3*). Besides the nitrogen nucleophiles **a-y**, also phenols and C-acids were tested, however significant limitations were observed. Since the aziridine ring closure is much faster than potential nosyl ester substitution/hydrolysis and subsequent ring-opening reaction, we utilized the direct synthesis of α-D-altropyranosides **XLI** from dinosylated pyranoside **XXXV** via *in situ* formed aziridine **XXVIIIb**.

Table 3 - Reactions of dinosylated glucosamine **XXXV** with nitrogen nucleophiles



Entry	Nu-H	Product	Time	Yield[a]
1	NaN_3		16h	72%
	a	XLIa		
2	NH_3/H_2O		16h	91%
	b	XLIb		
3			24h	59%
	c	XLIc		
4			24h	55%
	d	XLId		
5			24h	79%
	e	XLIe		
6			16h	68%
	f	XLIf		
7			16h	87%
	g	XLIg		
8			16h	59%
	h	XLIh		
9			24h	80%
	i	XLIi		

Entry	Nu-H	Product	Time	Yield[a]
10			48h	51%
	j	XLIj		
11			72h	47%
	k	XLIk		
12	$N_2H_4.H_2O$		24h	68%
	l	XLIl		
13			24h	47%
	m	XLIm		
14			24h	73%
	n	XLIn		
15			24h	69%
	o	XLIo		
16			16h	68%
	p	XLIp		
17			16h	56%
	q	XLIq		
18			24h	56%
	r	XLIr		
19			72h	38%
	s	XLIs		

[a] Isolated yields

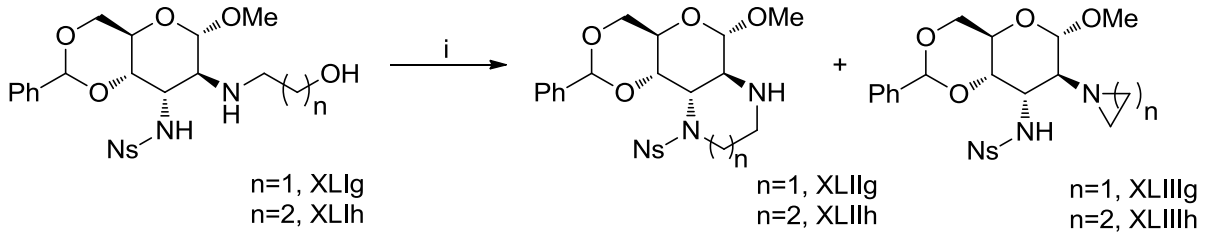
Sodium azide gave altropyranoside **XLIa** in a good yield (Entry 1). In comparison to the related *N*-substituted Hough-Richardson aziridines the ring-opening reaction was performed at 60 °C, not at conventional temperatures in a range 120-150°C.[27,30,33,34] The increased ring-opening reactivity induced by the nosyl group directly afforded amine **XLIb** with aqueous ammonia in the excellent yield (Entry 2). In comparison to classical protocols, involving the aziridine ring-opening reaction with an azide anion and essential reduction step,[27] this methodology allowed substantial simplification.

Monofunctional primary amines **c-f** provided **XLIc-f** in fair to good yield (Entry 3-6). The prolonged reaction time was utilized in reactions with **XLIc-e**. Later on, the method was extended to aminoalcohols **g-j** to yield **XLIg-j** from fair to very good yield as well (Entry 7-

10). The reaction time was necessary to extend to 48 h with amine **j**. Further increase of steric hindrance of **k** necessitated extension of reaction time to 72 h to give **XLIk** (Entry 11). The yield was predominantly reduced by the isolation process.

Preliminary attempts to cyclize nosylamides **XLIg-j** with the hydroxyl group under Fukuyama-Mitsunobu condition[32] showed the possible formation of desired products; however, significant amount of side products was observed as well (*Scheme 17*). We assumed that prerequisite change of the altropyranoside ring from chair to boat conformation did not occur and, consequently, both diaxialy oriented reacting groups were not redirected to the equatorial conformation. In the case of aminoalcohols **g** and **h**, the target products were detected, but the reaction mixture was complicated by formation of side products with tri- or tetra-membered rings **XLIIIg** and **XLIIIh**. Other Mitsunobu reagents such as DMEAD/TPP, DBAD/TPP or TMAD/Bu_3P did not improve the reaction. A few attempts to isolate the both products were done, but insufficient purity was achieved.

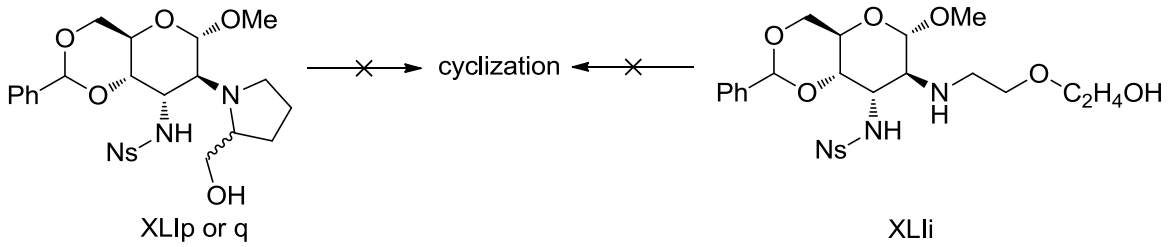
Scheme 17 - cyclization of **XLIg and XLIh**



Conditions: (i) DIAD, TPP, dioxane

In the case of prolinoles **p** and **q** and alcohol **i**, (*Scheme 18*) no cyclization products were observed. The unreactivity, particulary of prolinoles **p** and **q**, was caused by steric factors.

Scheme 18 - Attempted cyclization



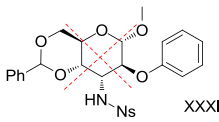
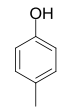
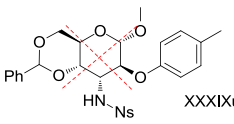
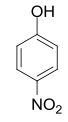
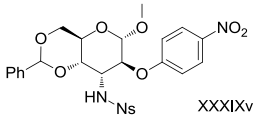
Other bifunctional nucleophiles, namely hydrazine **l** and ethylenediamine **m**, provided fair yields (Entry 12 and 13). Besides the primary amines, the reactivity of secondary amines **n-q** was also tested to give **XLIn-q** from fair to good yields (Entry 14-17). Preliminary attempts

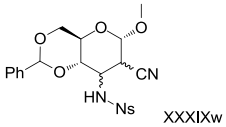
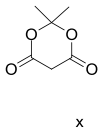
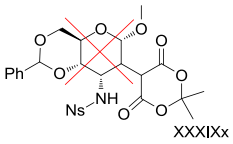
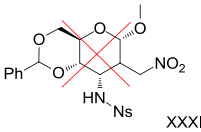
to carry out the ring-opening reactions with anilines and aminopyridines afforded unsatisfactory yields particularly due to the low reactivity and difficulties in the isolation process. The exception was anthracitic acid **r** providing **XLIr** in fair yield (Entry 18).

In connection with the good ring-opening reactivity with hydrazine **l** (Entry 12), we also tried benzohydrazide **s** to obtain **XLIs** in a poor yield which was predominantly caused by problematic separation and decomposition during the reaction (Entry 19).

In addition to nitrogen nucleophiles, oxygen and carbon nucleophiles were tried as well (*Table 4*). Since DIPEA is not a strong enough base to form correspond conjugate base, NaH instead of DIPEA was used (excluding the reaction with KCN). While phenol with electron donating group (Entry 21) provided poor yield, electron withdrawing group (Entry 22) supported reaction and product **XLIv** was isolated. In case of *C*-nucleophiles, potassium cyanide **w** allowed very good yield, however NMR analysis revealed formation of two isomers with almost identical chromatograpical data. *C*-acids **x** and **y** did not react at all.

Table 4 - Reactions of dinosylated glucosamine **XXXV** with other nucleophiles

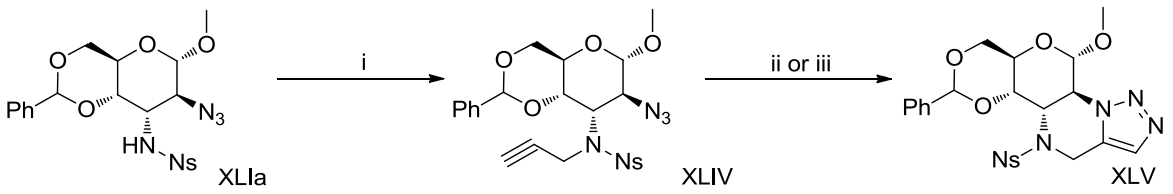
Entry	Nu	Product	Time	Yield[a]
20	 t	 XXXIXt	72h	
21	 u	 XXXIXu	72h	
22	 v	 XXXIXv	72h	40%

Entry	Nu	Product	Time	Yield[a]
23	KCN w	 XXXIXw	24h	72%
24	 x	 XXXIXx	24h	
25	 y	 XXXIXy	72h	

[a] Isolated yields

To demonstrate further possible modification of nosylamide group at C3 after the ring-opening reaction, two structurally attractive compounds were tried to synthetize. The first synthesis started with azide **XLIa,** which was alkylated with propargyl alcohol under Fukuyama-Mitsunobu conditions to give **XLIV** (*Scheme 19*). Subsequent intramolecular copper catalyzed 1,3-dipolar cycloaddition[35] led to novel interesting scaffold **XLV** containing the 1,2,3-triazolopiperazine moiety. Similar scaffolds have been reported as glycosidase inhibitors.[36]

Scheme 19 - Synthesis of triazolopiperazine **XLV**

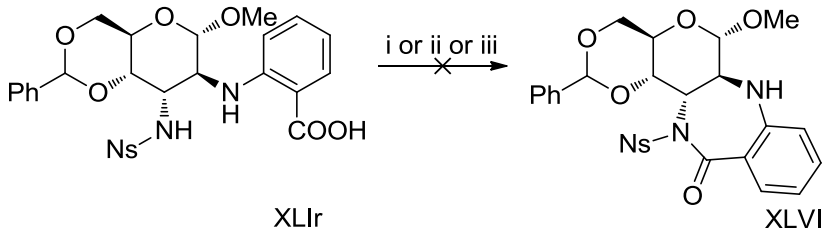


Conditions: (i) propargyl alcohol, TPP, DIAD, dioxane, 74%; (ii) Sodium Ascorbate, $CuSO_4.7H_2O$, DMF/water, 60°C, 90%; (iii) Sodium Ascorbate, $CuSO_4.7H_2O$, DMF/water, 80°C, 150W, 50%

The 1,3-dipolar cycloaddition was completed in 14 days when conventional heating was used at 60 °C. Microwave irradiation at 90 °C immensely reduced the reaction time to 3 hours, however, the yield decreased since the separation method was changed. The rate of the cycloaddition step was very likely associated with the conformation change of the pyranoside ring from chair to boat which redirects both reacting groups into the equatorial conformation.

The second attempt to modify ring-opening product was based on the preparation of benzodiazepine scaffold. Unfortunately, the target product **XLVI** was not prepared although different reaction conditions were tried.

Scheme 20 - Attempted synthesis of benzodiazepinone scaffold



Conditions: (i) DIC, DMAP (cat), DCM; (ii) CDI, DMF; (iii) T3P, DIPEA, DMF

In conclusion, the synthesis of the new *N*-*p*-nosyl Hough-Richardson aziridine was described. We demonstrated that this valuable advanced intermediate can be transformed by highly regioselective *trans*-diaxial ring-opening reactions with nitrogen nucleophiles into the corresponding α-D-altropyranosides. The increased ring-opening reactivity induced by the nosyl group allowed the direct synthesis of the aminoaltropyranoside even with aqueous ammonia in the excellent yield in comparison to the conventional methods based on the ring-opening reaction with azide anion and subsequent reduction of the azide functionality. Sodium azide, primary and secondary amines, hydrazine, and benzohydrazide provided

altropyranosides in fair to very good yield. Further work to extend the scope of the present study is in progress.

4 Conclusion

D-Glucosamine based derivatives represent highly important class of compounds with tremendous interesting properties. D-Glucosamine is a part of hyaluronic acid, well known for its anti-inflammatory properties or it is a building block of peptidoglycans, which are responsible for strength of bacteria cell walls. The significant importance of D-Glucosamine units in the biological systems initiated the reactivity study of D-Glucosamine at position 1, 2, and 3.

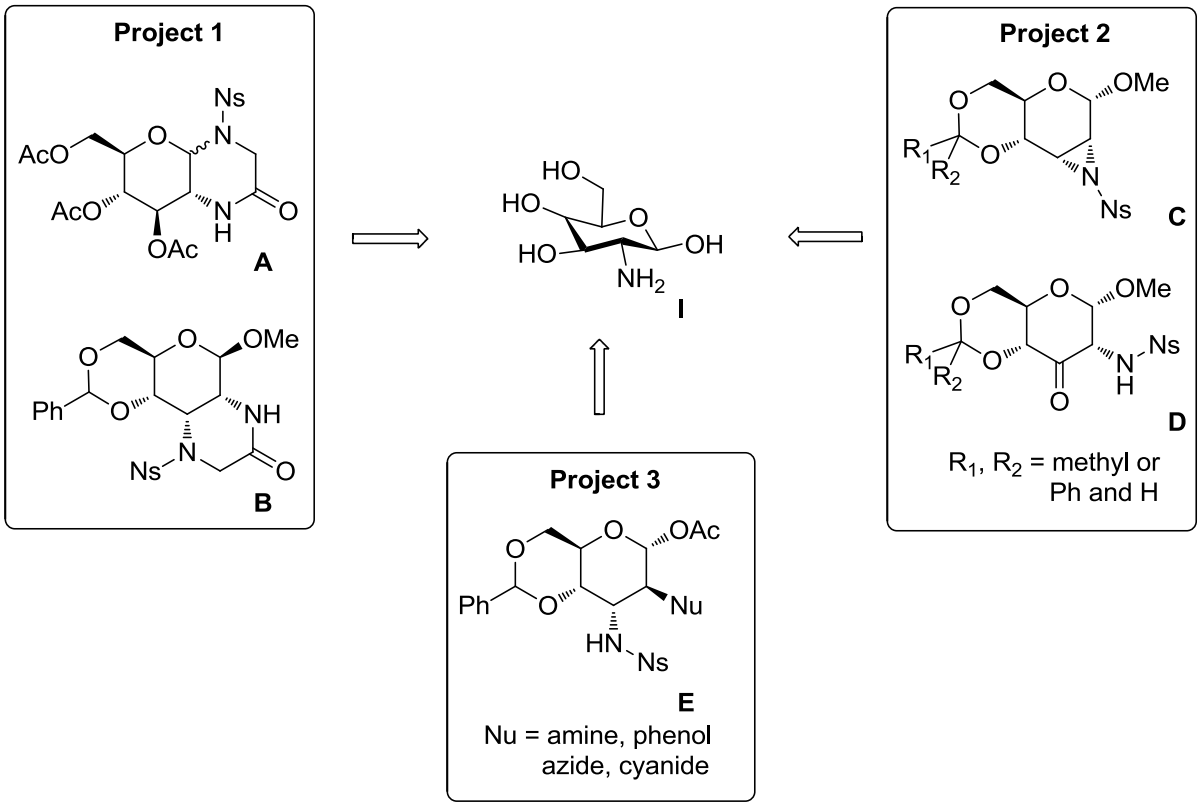
Experimental results of this thesis were divided into three main projects according to the type of the desired final product. The first project was focused on preparation of fused bicycles. The cycle could be fused to C1 and C2 carbon (Product **A**) or to C2 and C3 carbon (Product **B**). At the very beginning, it was necessary to prepare intermediate, which allows the cyclization reactions. Beside plenty of methods, HOSu/EDC mediated acylation gave the best results. Further, the cyclization reactions were investigated. It was find out, that cyclization to C1 carbon under Fukuyama-Mitsunobu conditions took place problematically, with many side products and with poor yields. In many cases, the separation was not possible due to low stability of products. When the cyclization was attempted to C3, the product **B** was not observed at all.

The second project reported the transformations of the amino and hydroxyl group of D-glucosamine at position 2 and 3 to obtain more suitable functionalities for potential formation of an *ortho*-fused ring. It was necessary to protect hydroxyls at positions 1, 4, and 6, and then modify amino and hydroxyl group at position 2 and 3. A key intermediate, containing the free hydroxyl group, was prepared via two different synthetic routes. Later, it was studied the reactivity of the nosyl group toward Fukuyama-Mitsunobu alkylation and possible formation of aziridine **C** as a result of a side intramolecular reaction with the hydroxyl at position 3. In the end, attention was paid to the oxidation of the hydroxyl at position 3 into the keto functionality resulting in derivative **D**. Subsequent condensation reactions revealed, that the reactivity of the keto derivate **D** towards common nucleophilic reagents is suppressed, probably due to steric hindrance.

The third project described the synthesis of aziridine, which was *in-situ* prepared from the corresponding 2-*O*, 3-*N*-dinosyl derivate. The addition of a nucleophile to the reaction mixture resulted in the ring-opening of the aziridine at C2 carbon and formation of 2,3-*altro*-

configuration (Product **E**). Plenty of nucleophile reagents were tried and it was find out, that this method is general for nearly all nitrogen nucleophiles. *O*- and *C*- nucleophiles required more basic conditions and afforded poorer yields.

In conclusion, we investigated the reactivity of suitably substituted D-glucosamine toward to cyclization, alkylation, oxidation and substitution reactions. It was find out, that cyclization is difficult or did not take place at all. The alkylation and oxidation reactions take place with a few limitations, however further research of condensation reactions to keto derivate will be necessary. Introducing of amine to C2 carbon is possible without any complication through *in-situ* prepared aziridine. This method is quite general and very mild conditions and high yields of reactions significantly extend the approach to 2,3-diamino derivates based on the structure **E**.



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