ADVANCES IN THE SYNTHESIS OF AMINOPYRAN-BASED CARBOHYDRATE MIMETICS AND THEIR EVALUATION AS SELECTIN INHIBITORS

Inaugural-Dissertation

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To my parents

The submitted doctoral thesis has been carried out under the supervision of Professor Dr. Hans-Ulrich Reißig in the period from August 2010 to September 2014 at the Institut für Chemie und Biochemie des Fachbereich Biologie, Chemie, Pharmazie der Freien Universität Berlin.

Content

Abbreviations

In the text, numbers of the compounds are given in bold font. In the experimental part, experiments are indicated as JNS#, as appears in the laboratory notebooks.

The following abbreviations are used throughout the entire text.

Ac	Acetyl
Ar	Aryl
Bn	Benzyl
bp	Boiling point
brs	Broad singlet
Bu	*n*-Butyl
t-Bu	*tert*-Butyl
COSY	^{1}H-NMR correlated spectroscopy
Cu/C	Copper on charcoal
CuAAC	Copper(I)-catalyzed azide alkyne cycloaddition
δ	Chemical shift
d	Day(s) or Doublet
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide
equiv.	Stoichiometric equivalent(s)
ESI-TOF	Electrospray ionization time of flight mass spectrometry
Et	Ethyl
EtOAc	Ethyl Acetate
g	Gram
GP	General Procedure
h	Hour(s)
HMQC	Heteronuclear multiple quantum correlation
HOAc	Acetic Acid
HRMS	High resolution mass spectrometry
Hz	Hertz

IR	Infrared
J	Coupling constant
lit.	Literature value
m	Multiplet
M	Molarity
MHz	Megahertz
Me	Methyl
min	Minute(s)
Nf	Nonaflyl
MS	Mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
Pd/C	Palladium on charcoal
Ph	Phenyl
ppm	Parts per million
q	Quartet
quant.	Quantitative, yield > 99.5%
R	Organic substituent
r.t.	Room temperature
s	Singlet
sLe^x	Sialyl-Lewis-X
SPR	Surface plasmon resonance
t	Triplet
TBTA	Tris-[(1-benzyl-1*H*-1,2,3-triazol-4-yl) methyl]amine
TBS	*tert*-Butyldimethylsilyl
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSE	Trimethylsilylethyl
ν	Wave number

Abstract

The aim of this dissertation was the development of new enantiopure multivalent carbohydrate mimetics and their study as potential selectin inhibitors. The main building blocks have been synthetized following a previously established synthetic route, starting from lithiated 2-(trimethylsilyl)ethoxyallene and a chiral D-glyceraldehyde-derived aldonitrone. The obtained 1,2-oxazine undergoes a stereoselective Lewis acid-promoted rearrangement leading to a bicyclic ketone which can be transformed into the corresponding carbohydrate mimetic with two straightforward steps: reduction of the carbonyl group followed by the cleavage of the *N*-benzyl and N-O bonds leads to an enantiopure aminopyran derivative. In the present work several transformations were made from this key building block including the conversion to the corresponding stable azidopyran prepared by a copper-catalyzed diazotransfer. Using the amino group and choosing adequate linkers, a library of multivalent compounds was prepared via Schotten-Baumann reaction or reductive amination. Additionally, different conditions for the Huisgen-Sharpless-Meldal cycloaddition were used to synthesize carbohydrate mimetics with different degrees of multivalency. In this approach, aspects that can influence multivalency binding were considered as linker length, symmetry and number of pyran ligands. As final and most challenging step, some of the obtained carbohydrate mimetics could successfully be polysulfated and tested with and without sulfate groups in a surface plasmon resonance (SPR) inhibition assay as potential selectin inhibitors. The tested compounds showed IC_{50} values from micro- to nanomolar range (for L- and P-selectin), which are promising results for such conjugates.

Overall, a straightforward synthetic route towards new multivalent carbohydrate mimetics was developed and the first results were obtained from the SPR inhibition assays providing some leads to further development of selectin inhibitors.

German abstract

Ziel dieser Arbeit war die Darstellung neuer enantiomerenreiner multivalenter Kohlenhydratmimetika und die Evaluierung ihre Aktivität als potentielle Selektin-Inhibitoren. Der zentrale Baustein wurde ausgehend von einer zuvor etablierten Methode aus lithiiertem 2-(Trimethylsilyl)ethoxyallen und einem chiralen von D-Glyceraldehyd abgeleiteten Aldonitron synthetisiert. Das erhaltene 1,2-Oxazin wurde durch eine stereoselektive Lewis-Säure-induzierte Umlagerung in ein bicyclisches Keton umgewandelt, welches dann in zwei direkten Schritten in das Kohlenhydratmimetika übergeführt wurde: die Reduktion der Carbonyl-Gruppe und die anschließende Spaltung der *N*-Benzyl- und der N-O-Bindungen liefert ein Aminopyran. In der vorliegenden Arbeit wurden verschiedene Transformationen mit diesem Schlüsselbaustein durchgeführt, zum Beispiel die Darstellung des entsprechenden stabilen Azidopyrans durch Kupfer-katalysierten Diazotransfer. Durch die freie Aminogruppe und adequate Linker konnte eine Vielzahl von multivalenten Verbindungen durch Schotten-Baumann-Reaktion oder durch reduktive Aminierung dargestellt werden. Zusätzlich wurden unter verschiedenen Reaktionsbedingungen der Huisgen-Meldal-Sharpless-Reaktion Verbindungen mit unterschiedlichem Grad an Multivalenz synthetisiert. Dabei wurden verschiedene Aspekte zum Einfluss auf multivalente Bindungen in Betracht gezogen, zum Beispiel Linkerlänge, Symmetrie und die Anzahl der Pyran-Liganden. Als letzten und anspruchsvollsten Schritt wurden einige der Kohlenhydratmimetika erfolgreich polysulfatiert und dann mit oder ohne Sulfatgruppen in Surface-Plasmon-Resonance- (SPR) Inhibitions-Untersuchungen als Selektin Inhibitoren getestet. Die untersuchten Verbindungen zeigten IC_{50}-Werte im mikro- bis nanomolarem Bereich (für L- und P-Selektin), was viel versprechende Ergebnisse für niedervalente Konjugate sind.

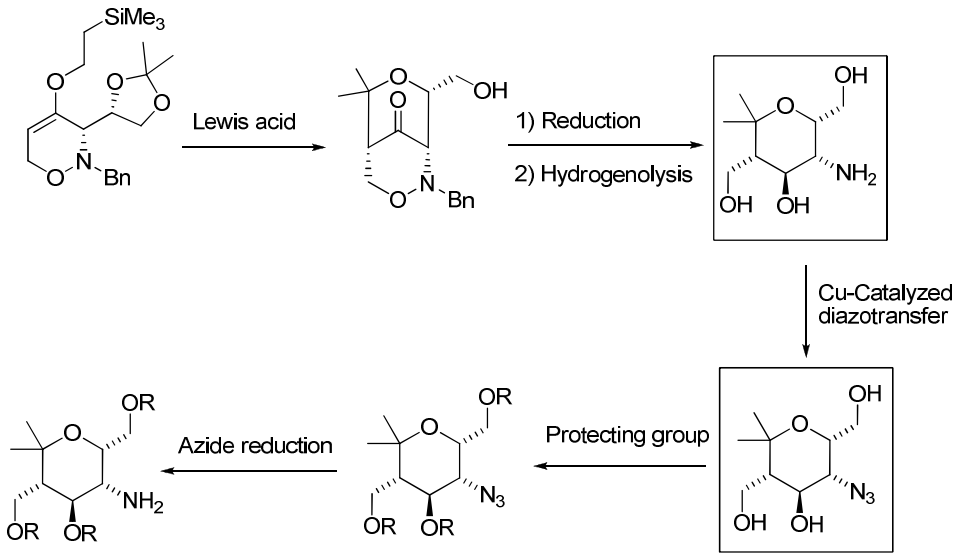
Zusammenfassend wurde eine direkte Syntheseroute für neue multivalente Kohlenhydratmimetika verwirklicht und erste Resultate der SPR-Inhibitions-Untersuchungen gaben Hinweise für die weitere Entwicklung von Selektin-Inhibitoren.

Summary

The present work includes the synthesis of a library of carbohydrate mimetics bearing an aminopyran moiety with a range from mono- to hexavalent structures. Adequate linkers that differ in their chain length, flexibility and different nature of the reactive groups were chosen.

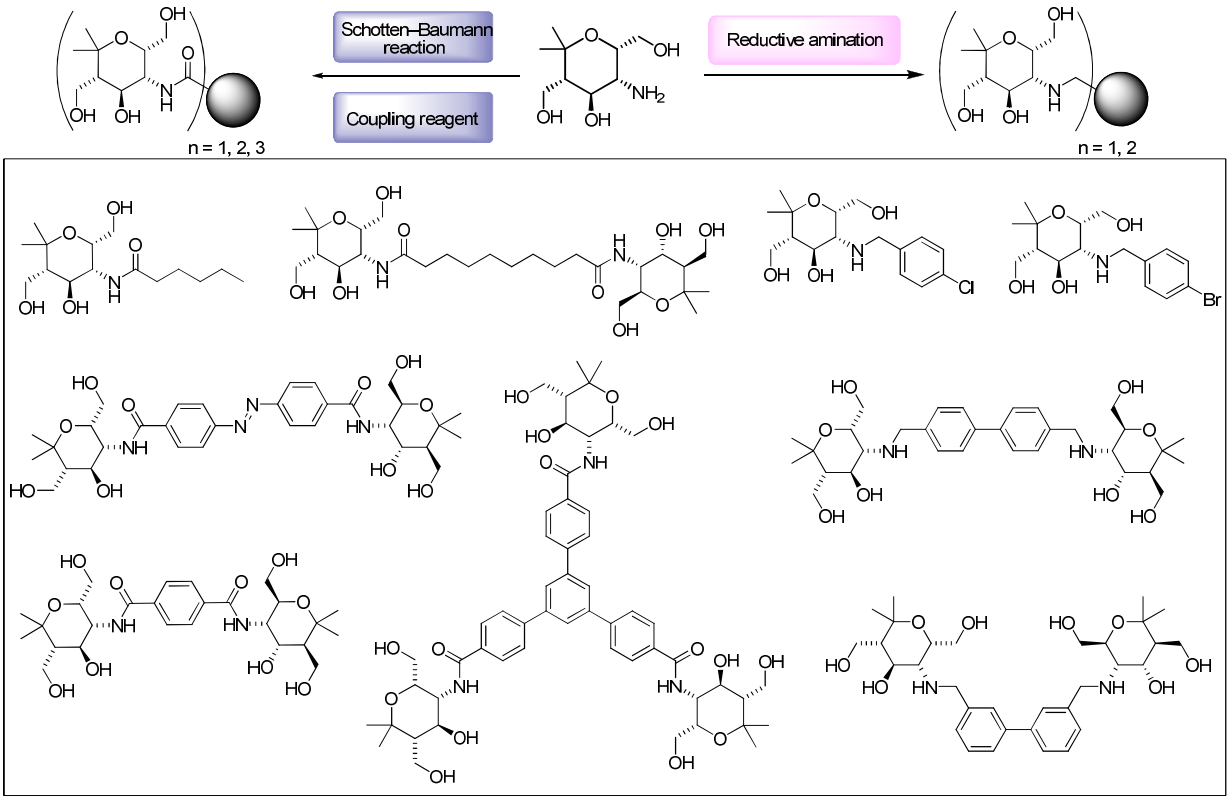
1. Synthesis of building blocks

Following the synthetic pathway previously described by Al-Harrasi, the Lewis acid-promoted rearrangement of the key 1,2-oxazine to the bicyclic ketone was performed with the commonly used $SnCl_4$ and alternatively with $TiCl_4$ that also provided the desired bicyclic ketone in very good yield. The stereoselective reduction of the carbonyl group with sodium borohydride afforded the corresponding alcohol in excellent yield. Further hydrogenolysis cleaving the *N*-benzyl and N-O bonds, provided the desired aminopyran in excellent yield. The side products generated during this step could be avoided by using isopropanol as solvent. Furthermore, the new stable azide was easily obtained by converting the amino into the azido group with retention of configuration using a mild copper-catalyzed diazotransfer protocol. The amino- and azidopyran building blocks were protected with several protecting groups. The reduction of the protected azidopyran to the corresponding aminopyran proved to be challenging and after the failure of methods like the Staudinger reaction or hydrogenolysis, it was successfully carried out using $LiAlH_4$.



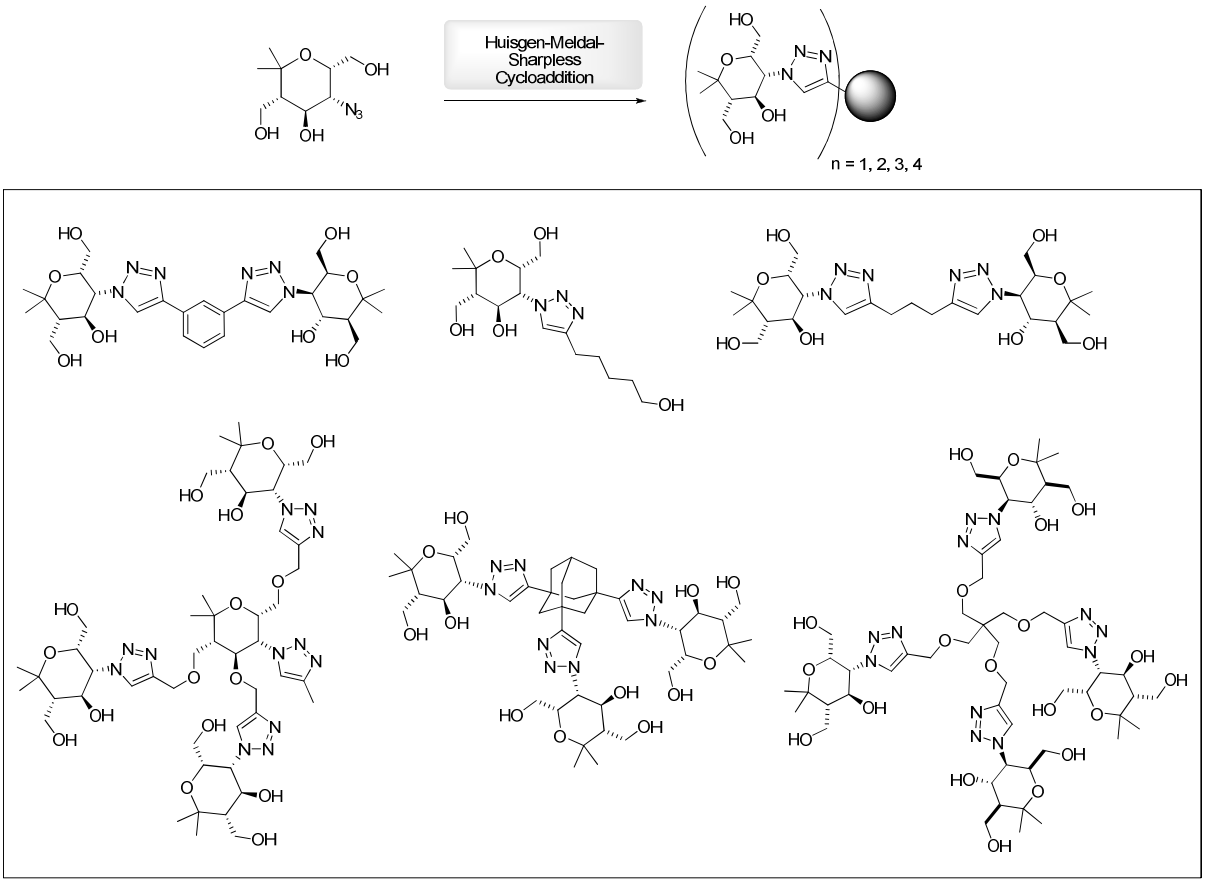
1. *Synthesis of multivalent carbohydrate mimetics with amide and amine linkage*

Starting from the easily accessible aminopyran and employing standard Schotten-Baumann reaction conditions, with adequate mono- and divalent acid chlorides, a range of carbohydrate mimetics was successfully obtained. Moreover, using the peptide coupling reagent HATU trivalent carbohydrate mimetics could be prepared. Reaction of the aminopyran with different aldehydes under reductive amination conditions afforded mono- and divalent amines.



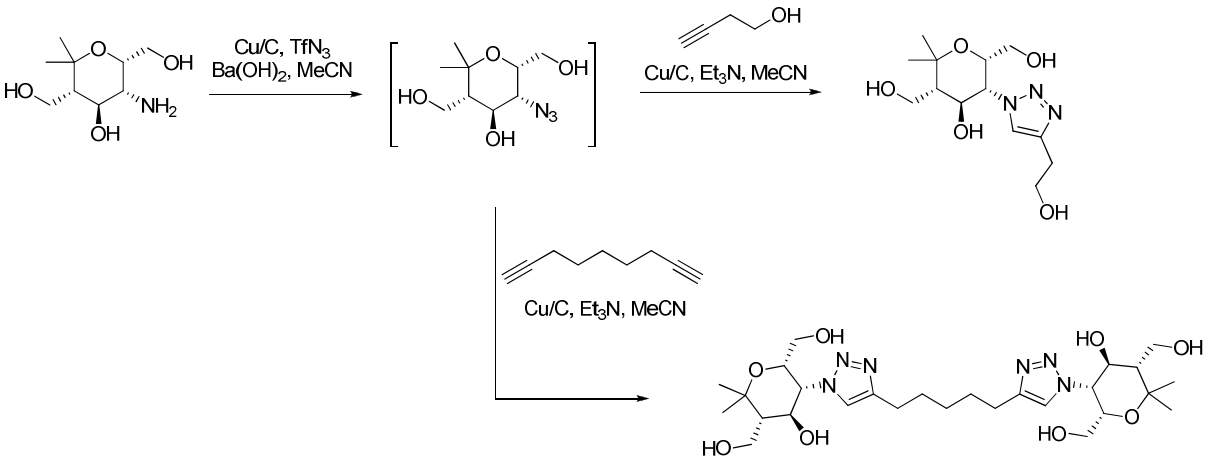
2. *Synthesis of multivalent carbohydrate mimetics by Huisgen-Meldal-Sharpless reaction*

With the stable unprotected azidopyran, different conditions for the Huisgen-Sharpless-Meldal cycloaddition were examined. Besides the commonly used conditions with CuI and TBTA as additive (previously used in the Reißig group for the synthesis of multivalent carbohydrate mimetics), the recently reported conditions of Wang et al. using CuI and an acid/base catalyzed method as well as the conditions reported by Lipshutz with Cu/C were investigated and adapted to the presented systems.

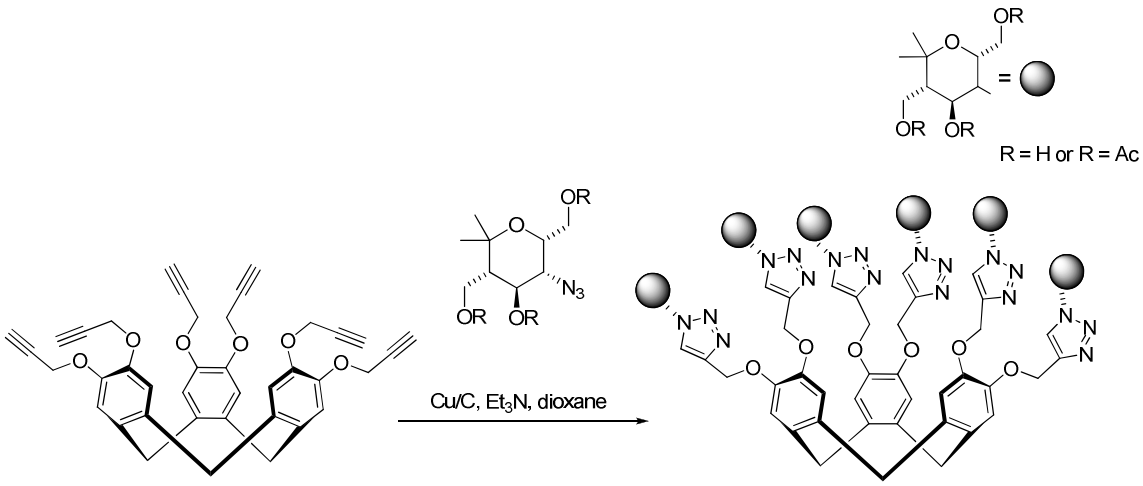


Method A: CuI, TBTA, Et_3N, MeCN, r.t.; Method B: CuI, DIPEA, HOAc, Toluene, r.t.; Method C: Cu/C, Et_3N, Dioxane, 60 °C.

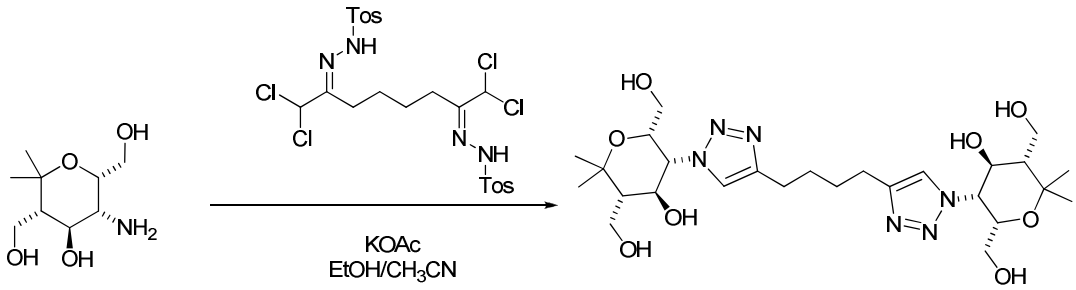
Adapting the original procedure of Lipshutz, a direct one-pot transformation from amines to the desired click products by generating the corresponding azide in situ was carried out. A mono- and a divalent compound were successfully synthetized; this is the first described example of a one-pot synthesis of a multivalent compound using Cu/C as catalyst.



The convenient conditions for the Huisgen-Sharpless-Meldal cycloaddition using Cu/C as catalyst were used for the reaction of the azidopyran with the porpargylated CTV derivative. Hexavalent carbohydrate mimetics with acetyl protecting groups or hydroxyl groups could successfully be prepared.



In the search for a copper-free method as alternative to the Huisgen-Sharpless-Meldal cycloaddition, Westermann et al. investigated the Sakai reaction for the synthesis of biologically significant compounds. These same conditions were used in the present work for the construction of a mono- and a divalent carbohydrate mimetic under copper-free “click” type conditions. This is the first report of the use of the Sakai-Westermann reaction for the synthesis of a divalent compound.

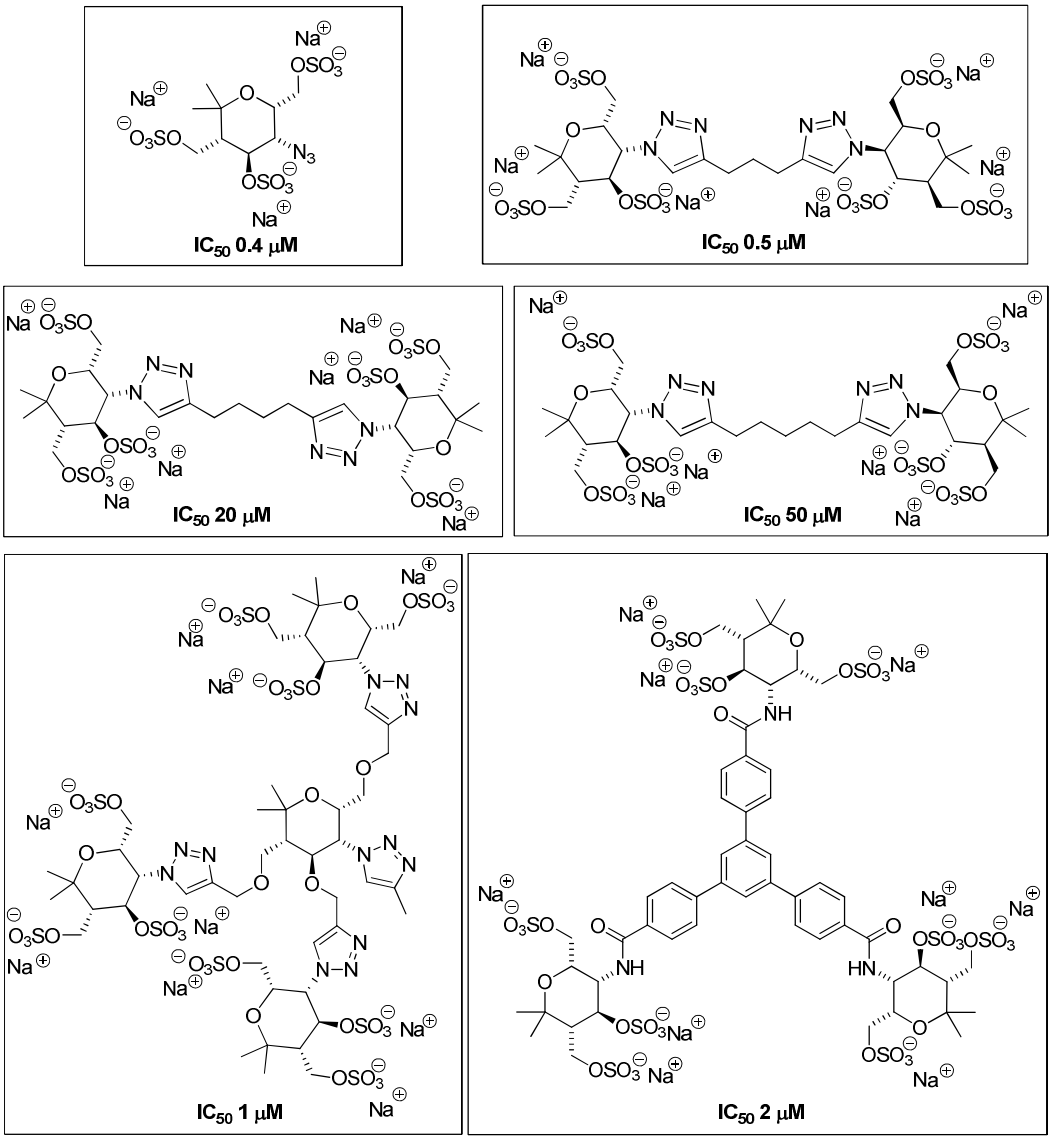


3. Polysulfation of oligovalent carbohydrate mimetics

After modifications of the previously employed procedure, mono-, di- and trivalent pyran derivatives could successfully be sulfated with the SO_3·DMF complex and isolated in pure fashion (see examples of sulfated compounds in section 5). Disappointingly, with larger molecules, fully sulfated products could not be obtained. The reaction control using the 700 MHz NMR spectrometer as well as the transformation into the corresponding sodium salts using a 0.5 M solution of sodium hydroxide proved to be the crucial steps for a good result.

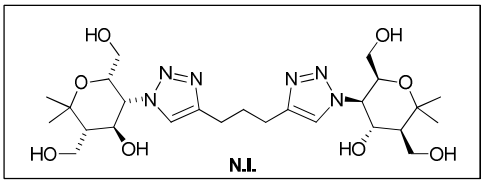
4. Oligovalent carbohydrate mimetics as selectin inhibitors

Different compounds were screened by surface plasmon resonance (SPR) and most of the non-sulfated carbohydrate mimetics proved not to be suitable candidates for testing due to their insolubility in aqueous media. All sulfated compounds chosen were water soluble and showed inhibitory activity for L-selectin. A clear multivalency effect of all the tested carbohydrate mimetics from mono- to trivalent compounds is not obvious; however, regarding the presented divalent mimetics, an influence of the linker length on the inhibitory activity was observed: the increase of the length lowered the inhibitory activity. Overall, the sulfated compounds tested proved to be very good L-selectin inhibitors with IC_{50} values in the high nM to low μM range, which are promising results for this type of compounds.



The active compounds were also screened for potential P-selectin inhibition to verify if any of the carbohydrate mimetics was selective towards one of the sulfate binding proteins. All compounds were active as P-selectin inhibitors; the three sulfated divalent triazoles showed a moderate preference to bind L-selectin and the trivalent compounds reached nearly comparable IC_{50} values for L- and P-selectin.

Furthermore, the activity of the carbohydrate mimetics was evaluated as E-selectin ligands. In this case all sulfated compounds were inadequate ligands and just a slight reduction of the binding was observed. Since E-selectin ligand binding is not charge-dependent, additional uncharged compounds were selected and tested as potential E-selectin inhibitors. None of the carbohydrate mimetics showed activity at the maximum concentration of 1 mM.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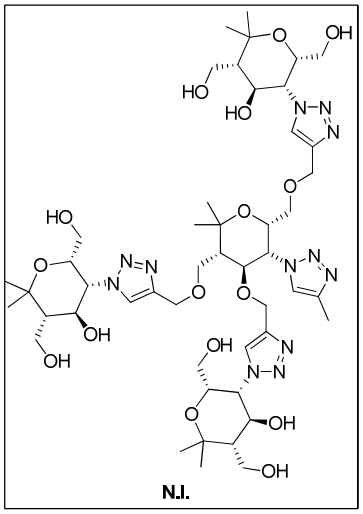
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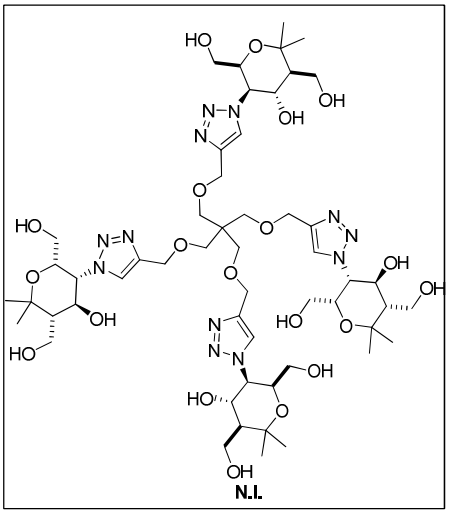
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1. Introduction

1.1. Carbohydrate chemistry

Carbohydrates are the most abundant organic molecules in nature and one of the most important classes of biomolecules allowing a wide range of structural variations due to their configurational diversity and high density of functional groups. This diversity of monosaccharides allows a vast number of different arrangements and combinations in oligosaccharides. Due to their structural diversity oligosaccharides are perfect biological information carriers and play a crucial role as source of metabolic energy, builders in cells and tissues as well as in cell-cell communication and recognition in the form of glycoconjugates such as glyco-lipids and glyco-proteins.[1] Despite their great importance and role in many complex biological processes, this class continues to be one of the least explored ones. The molecular diversity of carbohydrates offers a valuable tool for drug discovery by evaluating their structural and functional impact, but it is the same diversity which makes them so interesting that also makes their understanding and studying in biological processes so complex. Further development is often abandoned in early stages due to their metabolic instability, poor oral bioavailability and hydrophilicity which make them unsatisfactory drug targets.

1.1.1. Sulfated carbohydrates

Carbohydrate sulfates are present in a variety of organisms from bacteria to humans. It has been accepted over the years that the variety of sulfated oligosaccharides play a role in regulation and control of biological functions and it has been shown that the addition of a sulfate group to a carbohydrate can lead to its recognition by a specific receptor. The sulfate is transferred to the oligosaccharide acceptors by a coenzyme 3'-phosphoadenosine-5'-phosphosulfate (PAPS).[2] As examples of some identified sulfated monosaccharides on glycoproteins and glygolipids, the GalNAc-4-SO_4 is responsible for the circulatory half-life of the hormone lutropin (LH) and its release into the blood.[3] The 6'-sulfo-sialyl Le^x, the natural ligand of L-selectin is involved in the recruitment of leukocytes to inflammation sites and adhesion of lymphocytes to lymph nodes.[4] Recently, Hu and coworkers described the first synthesis of a sulfated tetrasaccharide that belongs to a sequence of the galactofucan isolated

from the brown algae *Sargassum polycystum*.[5] This family of sulfated polysaccharides often present activity as anticoagulants, anti-inflammatory or antitumor agents.

The field or sulfated carbohydrates is fairly unexplored since the understanding of the specific role and mode of action of these compounds is limited, furthermore the isolation of these compounds is in many cases not possible and only complex mixtures are extracted from natural sources. For this reason the development of sulfated synthetic analogs is a fascinating research field even though it is known that the chemistry of sulfated carbohydrates and the isolation of pure sulfated structures is extremely challenging.

1.1.2. Carbohydrate mimetics

The term carbohydrate mimetic is often used to describe any carbohydrate derived structure or molecules that resemble carbohydrates and present several hydroxy functionalities. Besides this broad definition, it should be taken in consideration that a carbohydrate mimetic used for drug development should not only mimic the structure of a real carbohydrate but also improve its properties. It is expected that a mimetic can have an increased stability towards endogenous degradative enzymes, improved bioavailability, and can provide a higher affinity and selectivity towards certain receptors.[6]

In the development and design of new carbohydrate mimetics, one should take into account the interactions between carbohydrates and their targets (most predominant being hydrogen bonds, interactions with metals, hydrophobic and ionic interactions). Additionally, other strategies to modify natural sugars and to create adequate and enhanced architectures can be for example, the modification or removal of certain functional groups, the introduction of new scaffolds or linkers and the introduction of hydrophobic or charged groups.[7]

1.1.3. Low molecular weight carbohydrate mimetic inhibitors

Over the past years several carbohydrate mimetic structures have been described and investigated in the drug discovery field. Some of these molecules are currently approved drugs in the market or in clinical trial phases. Glycosidases are catabolic enzymes responsible for the hydrolysis of *O*-, *N*- and *S*-linked glycosides. In Figure 1 are presented some glycosidase inhibitors of clinical relevance; for example Relenza and Tamiflu (used for targeting influenza neuraminidases) and Miglitol (used to control the levels of sugar in the blood in patients with diabetes).[8]

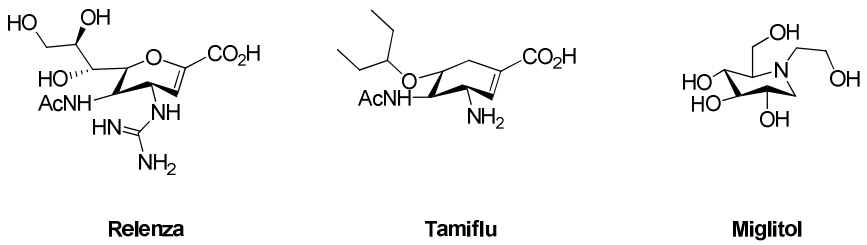


Figure 1. Examples of carbohydrate based drugs.

Focusing on selectin targets, a promising compound, Bimosiamose (TBC-1269) made it to the second phase of clinical trials for the treatment of asthma.[9] Bimosiamose (Figure 2) is an example of a low molecular weight compound that inhibits P-, L- and E-selectin. In flow chamber assays it was able to inhibit the murine leucocyte rolling, showing some anti-inflamatory activity.[10]

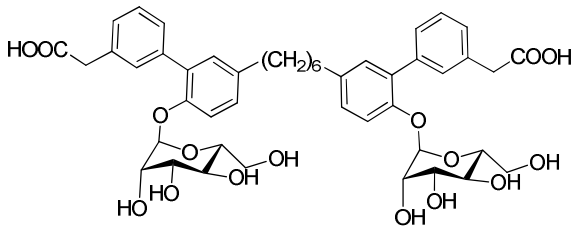


Figure 2. Structure of Bimosiamose (TBC-1269).

Recently, a collaboration between GlycoMimetics Inc. and Pfizer has called attention regarding the selectin inhibitor rivipansel[11] (GMI 1070) that is currently in the phase 3 of clinical trials (Figure 3). The companies have been developing rivipansel as a potential treatment for vaso-occlusive crisis of sickle cell disease (VOC).

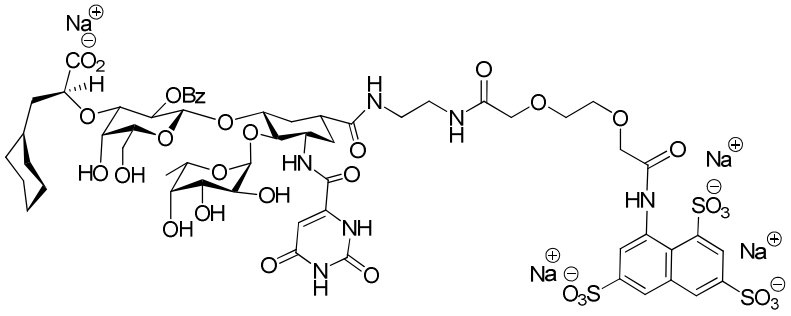


Figure 3. Structure of rivipansel (GMI 1070).

These small molecules provided promising leads in the development of carbohydrate mimetic based drugs. The interest in development of drug targets based on carbohydrates is still a growing field and over the past years it has been demonstrated that one of the most important factors that can help providing better binding affinities is the multivalency effect.

1.2. Multivalent interactions

1.2.1. Multivalent binding effects

It is known that carbohydrate-receptor interactions are generally weak with dissociation constants in the millimolar range. In nature the weak monovalent binding is enhanced by the presence of multiple carbohydrate epitopes. This principle is often referred as multivalency[12] or cluster effect[13] and this increasing in the binding affinity is achieved through different mechanisms of binding modes.

In the chelation binding mode (Figure 4, **A**) a ligand with multiple recognition elements binds to multiple binding sites on a single multivalent receptor. Examples of this type of receptors are immunoglobulins and lectins. In some other cases, a single receptor can have binding subsites; when a ligand is capable to bind to the principal interaction site and simultaneously (either with another recognition element or with another unit of its scaffold) to the subsites, a high affinity is achieved (Figure 4, **B**).

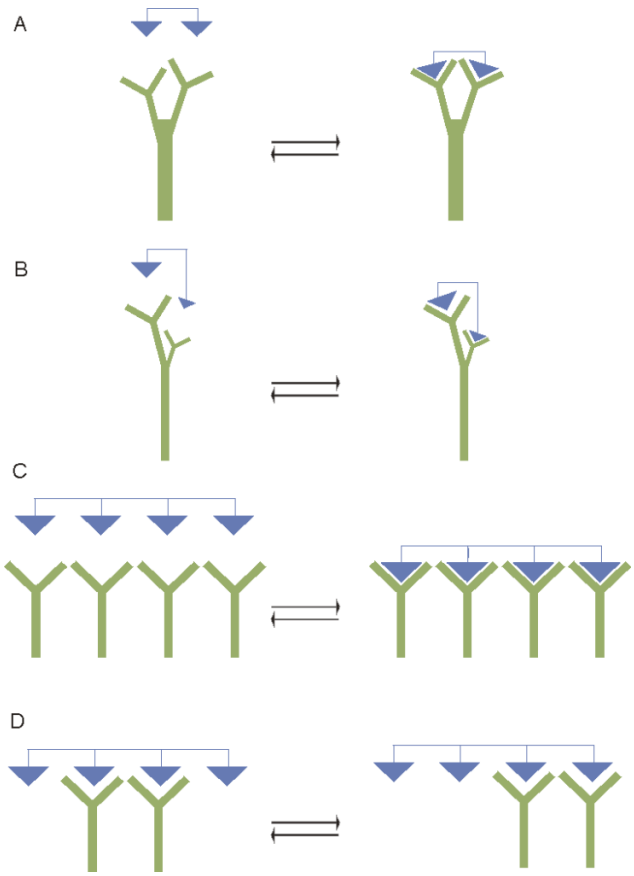


Figure 4. Different modes of multivalent ligand-receptor binding. **A** Chelation; **B** Subsite binding; **C** Cluster effect; **D** Statistical rebinding.

To obtain a strong cluster effect, a clustered receptor and a multivalent ligand are needed that can present units with specific orientation and spacing (Figure 4, **C**).[14] Finally, a high local concentration of a ligand can lead to an enhancement of the binding affinity and this process is often referred to as the statistical rebinding effect. If we consider the binding between a monomeric ligand and a receptor as an equilibrium between a bound and an unbound state, the rebinding is favored when it comes to a multivalent ligand due to the high local concentration of the binding elements (Figure 4, **D**).[15]

1.3. Selectin-carbohydrate interactions

Lectins are proteins that bind very specifically to carbohydrates (for example via hydrogen bridges or ionic interactions) and thus they play an important role in cell-biological recognition processes. The selectins belong to a subclass of lectins and are a family of three type-I cell-surface glycoproteins. They are expressed upon plasma membranes on leukocytes (L-selectin) and on the vascular endothelium (E- and P-selectin).

1.3.1. Selectin role during the inflammation process

In the case of a cellular immune response, leukocytes are involved in the repair of damaged tissue and in the defense process against microbial infection. They migrate from the blood stream to the injury sites. Selectins mediate the transport of leukocytes to inflammatory cells via binding of their respective carbohydrate ligands.[16] The recruitment of leukocytes to the site of infection or tissue injury is fundamental to the host's defense system. This inflammatory response is characterized by a series of events and it is also referred to as inflammatory cascade (Figure 5). Such a cascade initializes as the damaged tissue releases cytokines which stimulate the endothelial cells. Once the latter is stimulated, both E- and P-selectin proteins are expressed in order to recruit neutrophils (leukocytes), the most common type of white cells. Selectins recognize carbohydrate ligands on the surface of the white cells, promoting their addition. Further adhesion or "rolling" is induced by the multivalent interactions of these ligands located on the leucocytes and endothelial cells causing the slowing down of the leucocytes.[17] There is a firm adhesion caused by the high affinity of the

protein-protein interactions leading to a flattening of the leucocytes causing their migration through the endothelial layer (extravasation).[16]

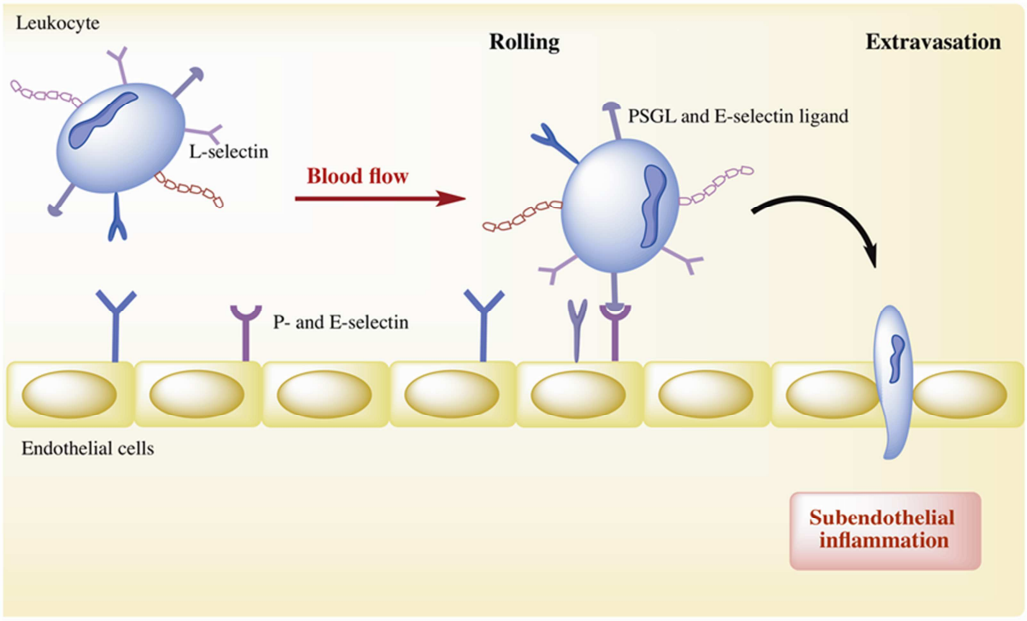


Figure 5. Inflammatory cascade process.

A deregulation of this immune response takes place for example, when too many neutrophils are recruited and a leucocyte-mediated tissue damage occurs. This dysregulation is responsible for chronic inflammation and autoimmune disorders such as asthma and arthritis.[18]

1.3.2. Selectin structure and ligands

The three selectins (P-, E- and L-) have similar structures (Figure 6). They are Ca^{2+}-dependent binding proteins. Common to them is the NH_2-terminal C-type domain, or lectin domain.[19],[20] This is followed by a EGF-like (epidermal growth factor-like) domain and a different long extended region: a different number of complement control protein (CCP) modules, or short consensus repeats (CR domain). The number of domains varies in between the three selectins: L-selectin has two domains, E-selectin six and P-selectin nine.[21] Each domain is composed of approximately 60 amino acid residues and the adjacent domains are connected in a head-to-tail fashion.[22] The consensus repeats are connected to the cytoplasmic domain through a transmembrane domain.

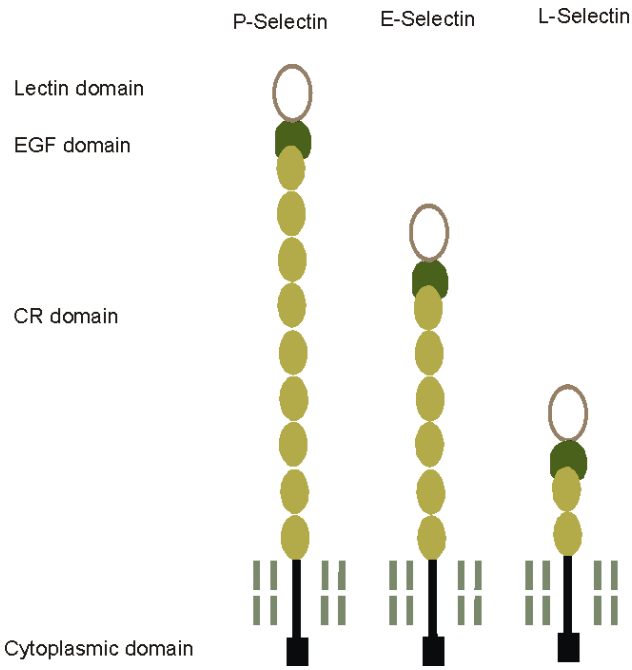


Figure 6. Simplified domain structure of selectins.

Selectins mediate the cell-cell adhesion through interactions between their lectin domains and specific glycoproteins. Since the lectin domain is similar in all three selectins this can indicate that they bind similarly to carbohydrate structures. From the oligosaccharides involved in this process an ideal ligand has not been found. However, it is known that all selectins bind to the tetrasaccharide sialyl-Lewis-X (sLe^x) (Figure 7).

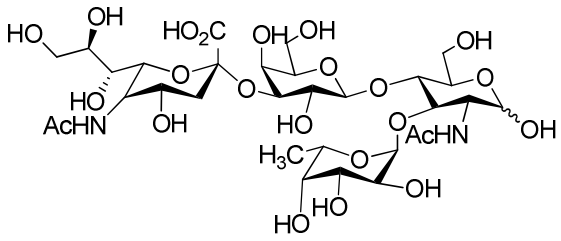


Figure 7. Structure of sialyl-Lewis-X.

Among the natural selectin binding ligands the PSGL-1 (P-selectin glycoprotein ligand-1) has been widely studied.[20],[23] PSGL-1 is a type 1 membrane protein consisting of 402 amino acids. A crystal structure analysis of the PSGL-1/P-selectin complex showed the binding of the sLe^x unit and of two sulfated tyrosine residues presented on PSGL-1. It has been shown that this ligand has a similar interaction with L-selectin.[24] On the other hand, interaction between PSGL-1 and E-selectin is not associated with any sulfate units and mainly the glycan is involved.[20]

Since selectin-carbohydrate interactions take place at an early stage of the inflammatory cascade, a new anti-inflammatory strategy has been considered where the adhesion step is inhibited. The inhibition of the adhesion step by artificial ligands can be a potential strategy to treat inflammatory symptoms. One of the most effective ligands discovered and investigated in the past years is heparin (Figure 8).[25] Heparin, a sulfated glycosaminoglycan (GAG) is a natural polysaccharide containing a large number of sulfated units. The structure of heparin consists of a carbohydrate backbone including D-glucuronic and L-iduronic acid units as well as glucosamine residues in a α-(1,4)-linked fashion.[26] Since heparin does not contain the sLe^x motif, its high affinity to selectins is attributed to the large number of sulfates that interact in a multivalent manner.

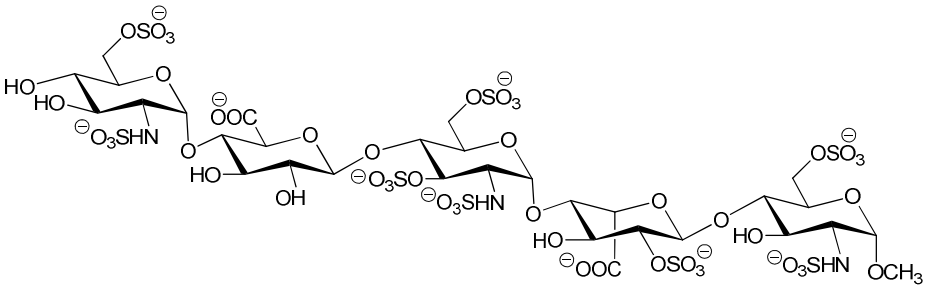


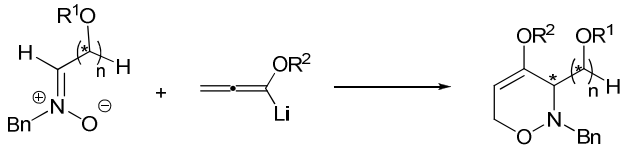
Figure 8. Pentasaccharide segment of heparin.

Heparin is an ideal example of the importance of multivalency and sulfated units. Research has been continued around heparin mimetic oligosaccharides and smaller molecules, some of those are currently in different stages of clinical development or approved drugs in the market.[27]

2. Scientific background

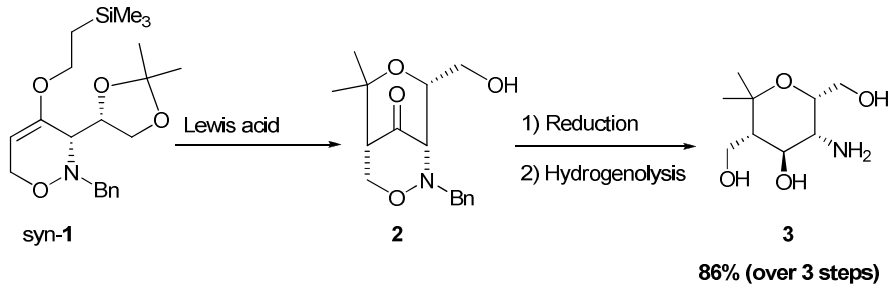
2.1. Reißig's approach to carbohydrate mimetics

Alkoxyallenes are valuable C-3 building blocks with a characteristic pattern of reactivity which are used in many syntheses of natural products and heterocycles. They can be lithiated at the C-1 and subsequently reacted with electrophiles. One example is the [3+3]-cyclization of a lithiated alkoxyallenes and aldonitrones to afford 1,2-oxazines (Scheme 1).[28]



Scheme 1. Synthesis of 1,2-oxazines by addition of lithiated alkoxyallenes to chiral aldonitrones.

One of the major research interests in the Reißig group has been the development of different approaches for the synthesis of amino sugar mimetics starting from enantiopure 1,2-oxazines. Al-Harrasi investigated and described the key step to obtain these amino sugars: a Lewis acid-promoted rearrangement of the 1,2-oxazine **1** to give bicyclic ketone **2** (Scheme 2).[29] With this intermediate and with two straightforward steps, first reduction of the carbonyl group and then cleavage of the *N*-benzyl and N-O bond, carbohydrate mimetic **3** can be obtained with excellent yields. [30],[31]



Scheme 2. Synthetic route of Al-Harrasi to carbohydrate mimetic **3**.

When comparing pyran **3** with a monosaccharide (Figure 9), and assuming that the two methyl groups replace the anomeric center, the configuration of D-idopyranose is obtained.

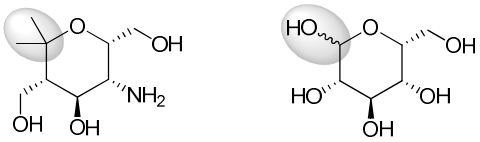
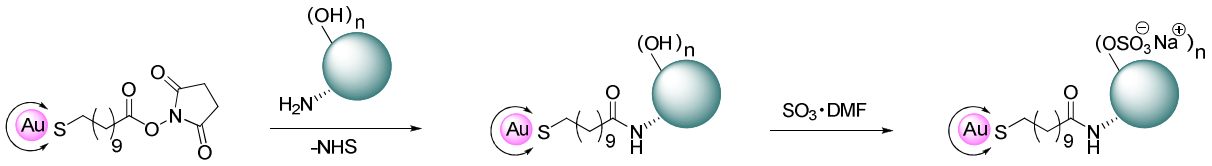


Figure 9. Comparison of Al-Harrasi's carbohydrate mimetic **3** and D-idopyranose.

Not much research has been done with D-idose, however Dromowicz and Köll reported a convenient synthesis of D-idose from D-xylose which can be easily converted into 1,6-anhydro-β-D-idopyranose.[32] The same fact can be observed for L-idopyranose, the enantiomer of D-idopyranose: neither L-idose and L-iduronic acid nor their derivatives are available commercially although L-iduronic acid is one of the components of the heparin structure.

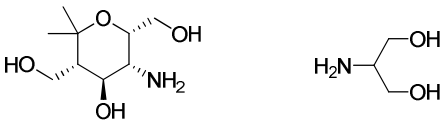
2.2. Carbohydrate mimetics as selectin inhibitors

In previous studies, a scope of monovalent carbohydrates synthesized in the Reißig group were immobilized as ligands on gold nanoparticles (Au-NP). Two types of colloids were used with different core diameters (6 nm and 14 nm) and both with a terminally functionalized thiol shell. Each colloid can carry between thousand and five thousand ligands in their shell providing a high degree of multivalency. The corresponding carbohydrate mimetics with a free amino group were coupled to the colloids with a terminal active ester forming an amide bond. As a final step the free hydroxyl groups were polysulfated using SO_3·DMF complex (Scheme 3).[18]



Scheme 3. General scheme of the route to functionalized gold nanoparticles.

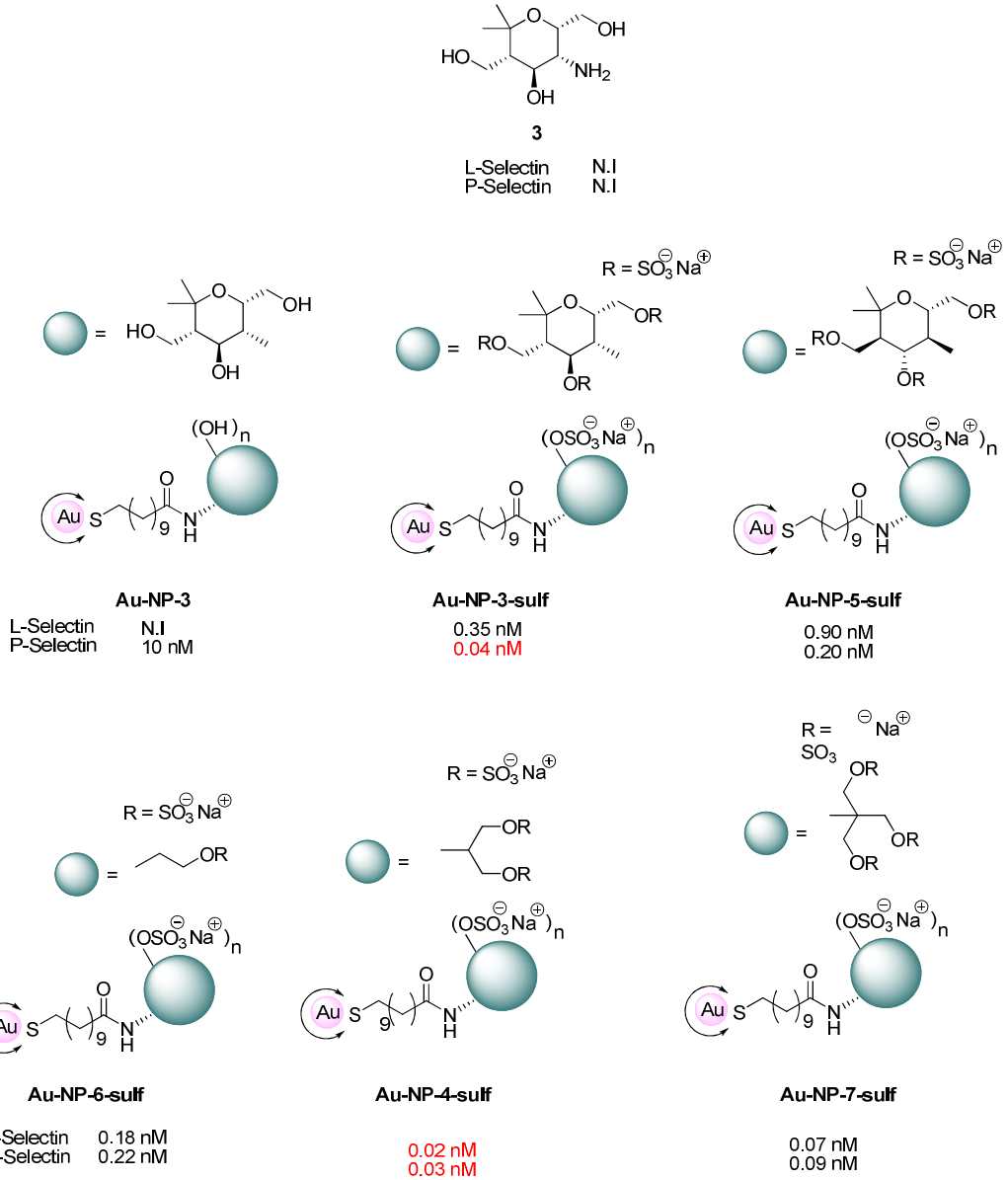
Acyclic aminopolyols and aminofurans were also investigated in order to compare different ring sizes, polarity, configuration and flexibility. Surface plasmon resonance (SPR) inhibition assays were conducted with the resulting compounds in order to obtain their IC_{50}-values as inhibitors for P- and L-selectin. Focusing on the best results, animopyran **3** and polyol **4** proved to be the best inhibitors (Figure 10).



3 **4**

Figure 10. Structure of the two end groups tested as selectin inhibitors connected to Au-NP that presented best inhibition.

Aminopyran **3** was tested alone and gave no inhibition. When tested in a multivalent fashion connected to the Au-NP and with free hydroxyl groups (Scheme 4, **Au-NP-3**), no inhibition was observed for L-selectin, but in contrast, an IC_{50}-value in the nM-range was observed.

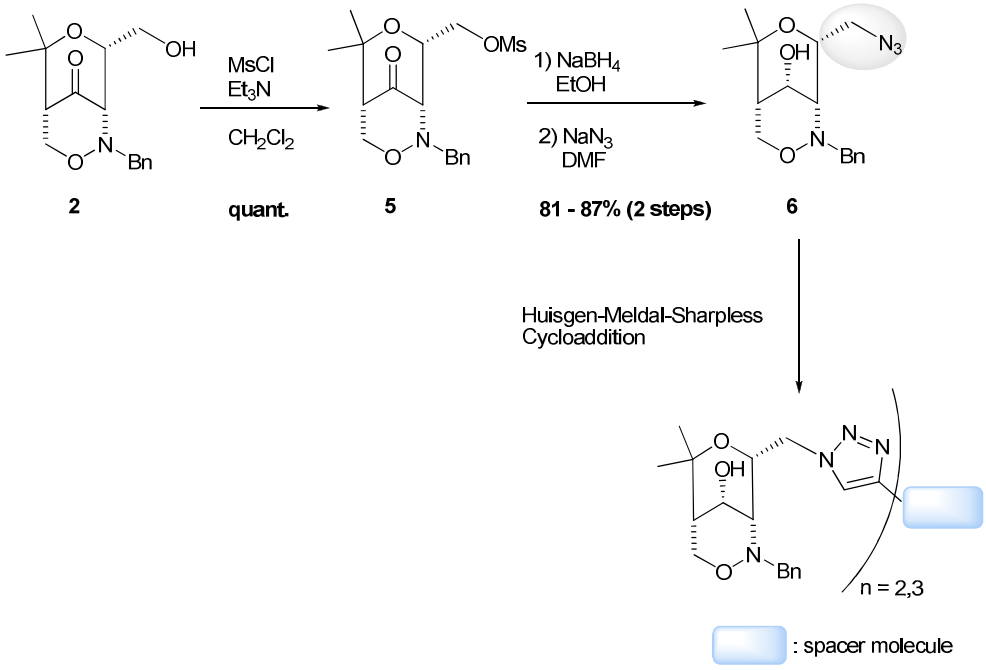


Scheme 4. IC_{50}-Values (determined by SPR) for sulfated functionalized gold colloids (6 nm) carrying different aminopyrans and acyclic aminoalcohols.

A strong improvement of the binding properties is confirmed by the multivalent presented on Au-NP structures as well as by the presence of sulfate groups. All functionalized and sulfated gold nanoparticles showed IC_{50} values in the picomolar range. A unique selectivity for P-selectin was achieved for the sulfated functionalized colloid **Au-NP-3-sulf**. Concerning the acyclic aminoalcohols, they also showed excellent binding to both selectins however, in contrast to **3**, none of these moieties showed any selectivity (Scheme 4, **Au-NP-6-sulf**, **Au-NP-4-sulf**, **Au-NP-7-sulf**).

2.3. A new approach to multivalent carbohydrate mimetics

Bicyclic ketone **2** is a very versatile building block and can be regarded as a protected aminopyran allowing several possibilities of transformations and functionalization. Using this building block, Koóš could develop a synthetic route towards multivalent carbohydrate mimetics.[33] Starting from bicyclic ketone **2**, the primary hydroxyl group was activated using MsCl providing the mesylate **5**, followed by stereoselective reduction of the carbonyl group using $NaBH_4$. The mesylate was then converted into the azide **6** in good yields over two steps. With this moiety in hand several di- and trivalent compounds could be obtained via Huisgen-Meldal-Sharpless cycloaddition with di- and trialkynes (Scheme 5). These multivalent units were still in the bicyclic form and to approach the final products further steps are needed: cleavage of the *N*-benzyl and N-O bond, and finally sulfation of the free hydroxyl groups.



Scheme 5. Approach of Koóš to multivalent carbohydrate mimetics.

Although Koóš was successful in the synthesis of some new multivalent carbohydrate mimetics, the yields were low and the subsequent polysulfation led often to mixtures of compounds and inconclusive results.

In a similar route, Al-Harrasi developed a strategy to obtain enantiopure aminooxepanes.[34] Following his investigations, Bouché was able to synthetize a series of new multivalent oxepanes. By reaction of azidooxepanes for example in Huisgen-Meldal-Sharpless reactions with different spacer units mono-, di- and trivalent seven membered ring mimetics were obtained in excellent yields (Figure 11, **7** and **8**). Furthermore, with a diazidooxepane the synthesis of macrocyclic structures like **9** were successfully achieved.[35]

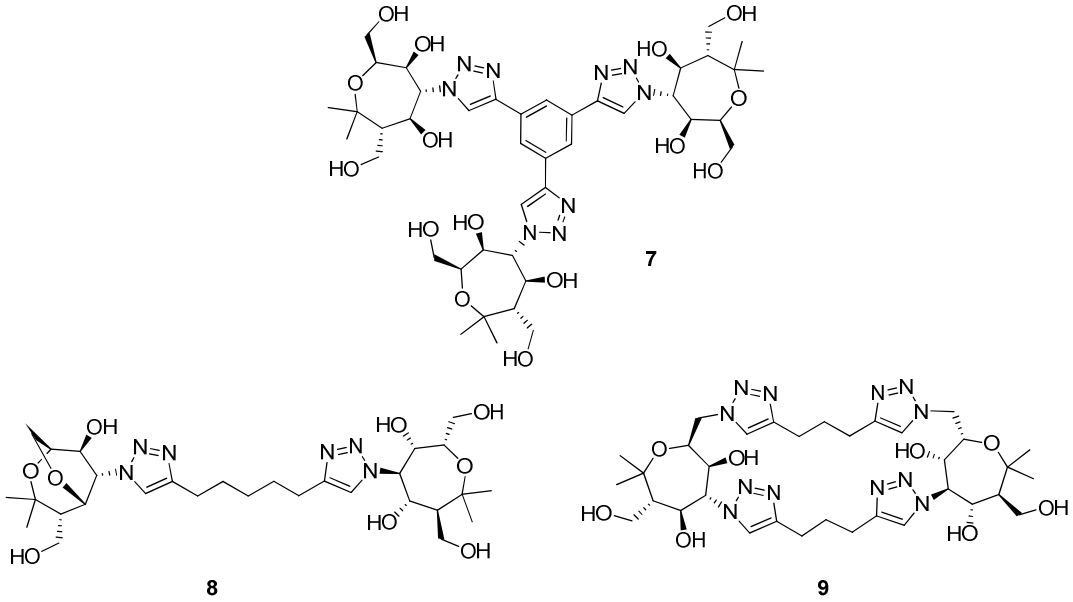


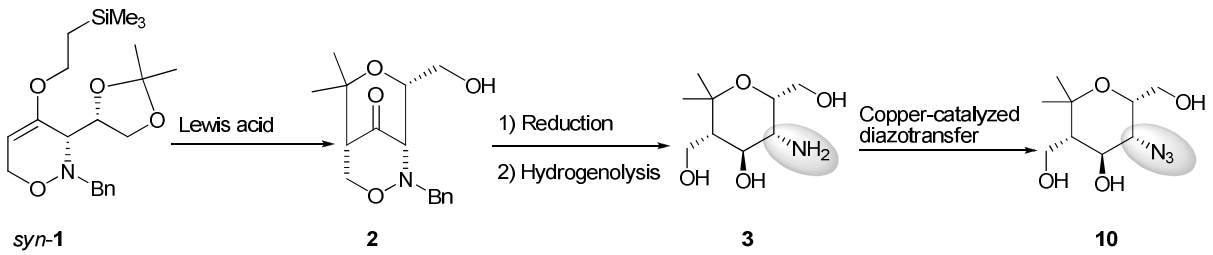
Figure 11. Examples of multivalent poly(hydroxy)oxepanes by Bouché.

Much research was carried out in the optimization of a polysulfation method for this type of compounds. Although a general method was not found, Bouché could isolate multivalent oxepanes in pure fashion that were successfully tested in SPR measurements that will be discussed further in section 4.6.

3. Aim of the present work

Encouraged by the excellent results of the SPR inhibition assays, we were motivated to further investigate aminopyran **3** and also acyclic polyol **4**. To overcome the problems of the Kooš approach and to be able to build a library of potentially active compounds, a new strategy was considered in order to obtain the new desired structures in a direct and high yielding synthetic route.

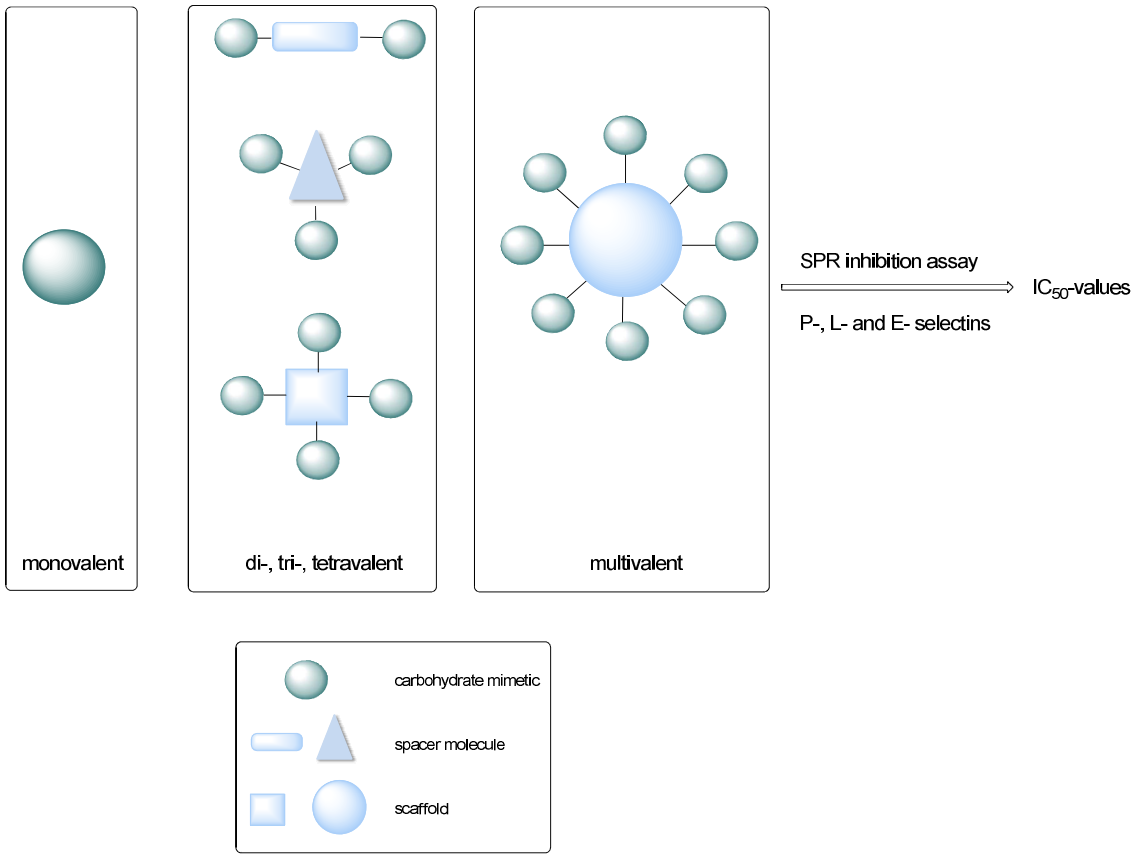
In our new approach presented in Scheme 6, aminopyran **3** will be used as fully deprotected building block. Furthermore, the stable organic azide **10** can be easily obtained by converting the amino functionality to the azido group under retention of configuration using a mild copper-catalyzed diazotransfer protocol.



Scheme 6. Proposed approach to the key building blocks **3** and **13** as precursors for multivalent carbohydrate mimetics.

With these two key building blocks in hand, not only the Huisgen-Meldal-Sharpless cycloaddition can be performed starting from the azide, but also new multivalent compounds can be achieved starting from the free amine generating multivalent units bearing amide or amine bonds.

We aim to investigate systematically the influence of multivalency, therefore, the degree of multivalency should be increased from monovalent to di-, tri-, tetravalent and more. In addition, we also want to understand selectivity between P-, E- and L-selectin, the influence of the chain length and the presence of an amide bond in the spacer molecules. To achieve this goal different spacer molecules and scaffolds were chosen with varying flexibility length and geometry (Scheme 7).



Scheme 7. General scheme for the different presentations of multivalent carbohydrate mimetics and final testing as selectin inhibitors.

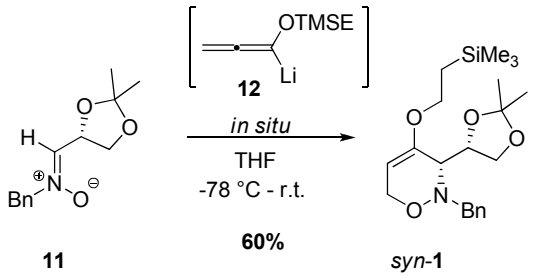
Finally, with this work we aim to development new and metabolic stable structures to be applied and evaluated in biologically active systems via SPR measurements and to start building a small rational library of biological active carbohydrate mimetics. It is our hope that the resulting compounds due to their resemblance to carbohydrates can represent useful drug candidates, overcoming the problems associated with natural oligosaccharides.

4. Results and Discussion

4.1. Synthesis of starting materials

4.1.1. syn-1,2-Oxazine synthesis

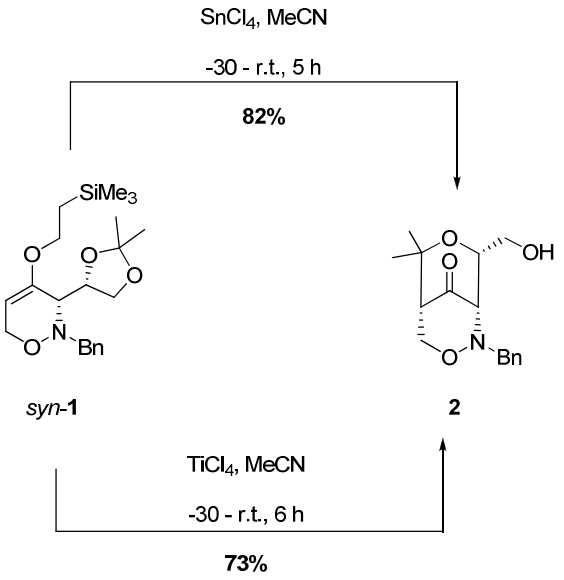
According to the literature, lithiated alkoxyallenes can be added to a variety of electrophiles such as aldehydes, imines, nitrils and nitrones in order to produce a range of heterocyclic compounds, including 1,2-oxazines. As previously reported[28],[36] the addition of lithiated alkoxyallenes to D-glyceraldehyde-derived *N*-benzyl nitrone **11** results in the formation of *syn*- or *anti*-configured 1,2-oxazine derivatives depending on the chosen reaction conditions. The 1,2-oxazine *syn*-**1** was smoothly synthesized (Scheme 8) by lithiation of 2-(trimethylsilyl)ethoxyallene (at -40 °C in THF) and subsequent addition of **12** to (*Z*)-nitrone **11** at -78 °C, affording the desired product with 60% (Lit.[28] 76%).



Scheme 8. Synthesis of 1,2-oxazine *syn*-**1**.

4.1.2. Lewis acid-induced rearrangement

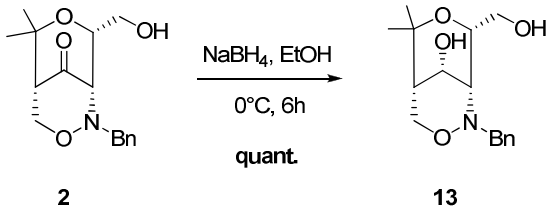
During the optimization of the key step of the presented synthesis, Al-Harrasi screened a variety of Lewis acids as promoters for this rearrangement.[37] Since then the use of $SnCl_4$ in MeCN is the method of choice for the rearrangement of the 1,2-oxazine *syn*-**1**. In the presented investigations the same conditions (Scheme 9) for the Lewis acid-promoted rearrangement of *syn*-**1** afforded the desired bicyclic ketone **2** with 82% yield (Lit.[29a] 95%). Al-Harrasi reported that when $TiCl_4$ was used with CH_2Cl_2 as solvent, a complex mixture was obtained and the desired product could only be isolated in 14%. Surprisingly, when the same reaction was performed with $TiCl_4$ and MeCN as solvent, instead of CH_2Cl_2, the yield could be improved to 73%.



Scheme 9. Lewis acid-induced rearrangement of 1,2-oxazine *syn*-**1** to bicyclic ketone **2**.

4.1.3. Stereoselective reduction with sodium borohydride

The reduction of the carbonyl group of ketone **2** with $NaBH_4$ (Scheme 10) afforded the corresponding alcohol **13** in quant. yield (Lit.[29a] 97%). In this step a new stereogenic center is formed and only the diastereomer with (*S*)-configuration is obtained in excellent yields.



Scheme 10. Stereoselective reduction of bicyclic ketone **2**.

The diastereofacial selection is based essentially on the steric shielding of the direction for the possible attack of the hydride reagent. The electrophilic carbonyl group is generally attacked by a nucleophile (hydride reagent) along the Bürgi-Dunitz angle (107°). Using the program Chem-3D a three-dimensional molecular model of the ketone was calculated. The model is shown in Figure 12. It is clear that due to the steric hindrance of the two methyl groups, the nucleophilic attack is only allowed from one side (*Re* face) giving **13** as a single diastereomer.

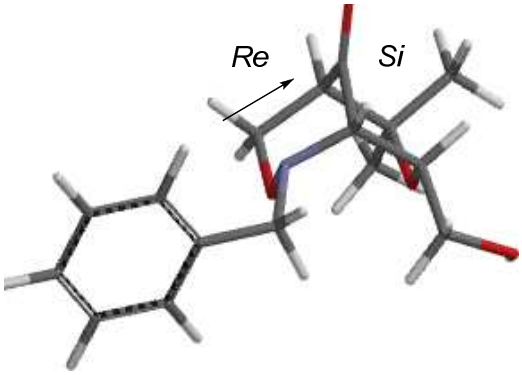
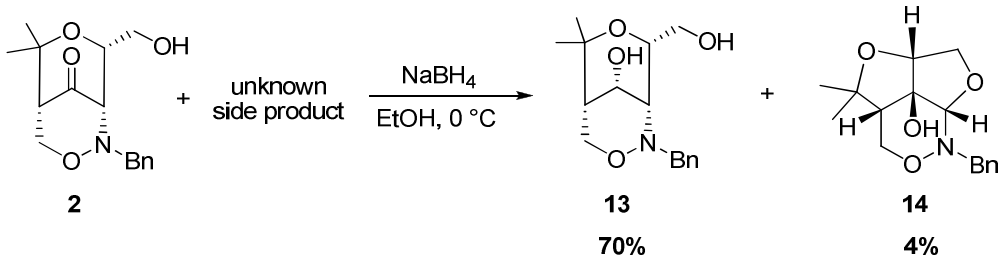


Figure 12. Three-dimensional molecular model showing the less hindered face for the nucleophilic attack from the *Re* face.

The same experiment was carried out with bicyclic ketone **2** obtained with 95%. The formation of a side product was noticed during the Lewis acid-promoted rearrangement using $SnCl_4$, but this product could not be identified by ^{1}H-NMR spectroscopy nor separated during the purification. It was decided to carry out the next reactions hoping that a separation would be possible at a later stage. When the reduction of **2** was performed with this mixture the new tricyclic compound **14** could be isolated in 4% yield and identified (Scheme 13).



Scheme 13. Isolation of the new tricyclic compound **14**.

Very characteristic in the ^{1}H-NMR spectrum of this tricyclic structure is the clear ABX system resulting from the coupling between 2-H and 2a-H (Figure 13). Also the singlet of the 7a-H was an important hint that led us to the elucidation of the correct structure.

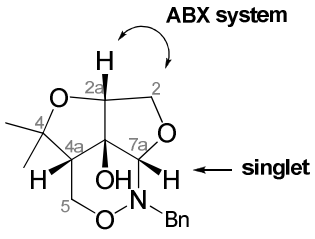
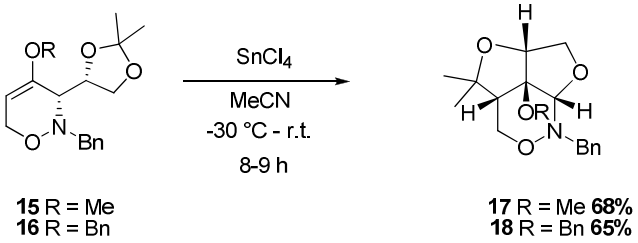


Figure 13. Relevant protons for the ^{1}H-NMR elucidation of tricyclic structure **14**.

Tricyclic compound **14** was never observed nor isolated before, however the formation of such tricyclic compounds as a result of a different rearrangement of 1,2-oxazines bearing a methoxy **15** or benzyloxy **16** group was previously reported in our group (Scheme 14).[38]

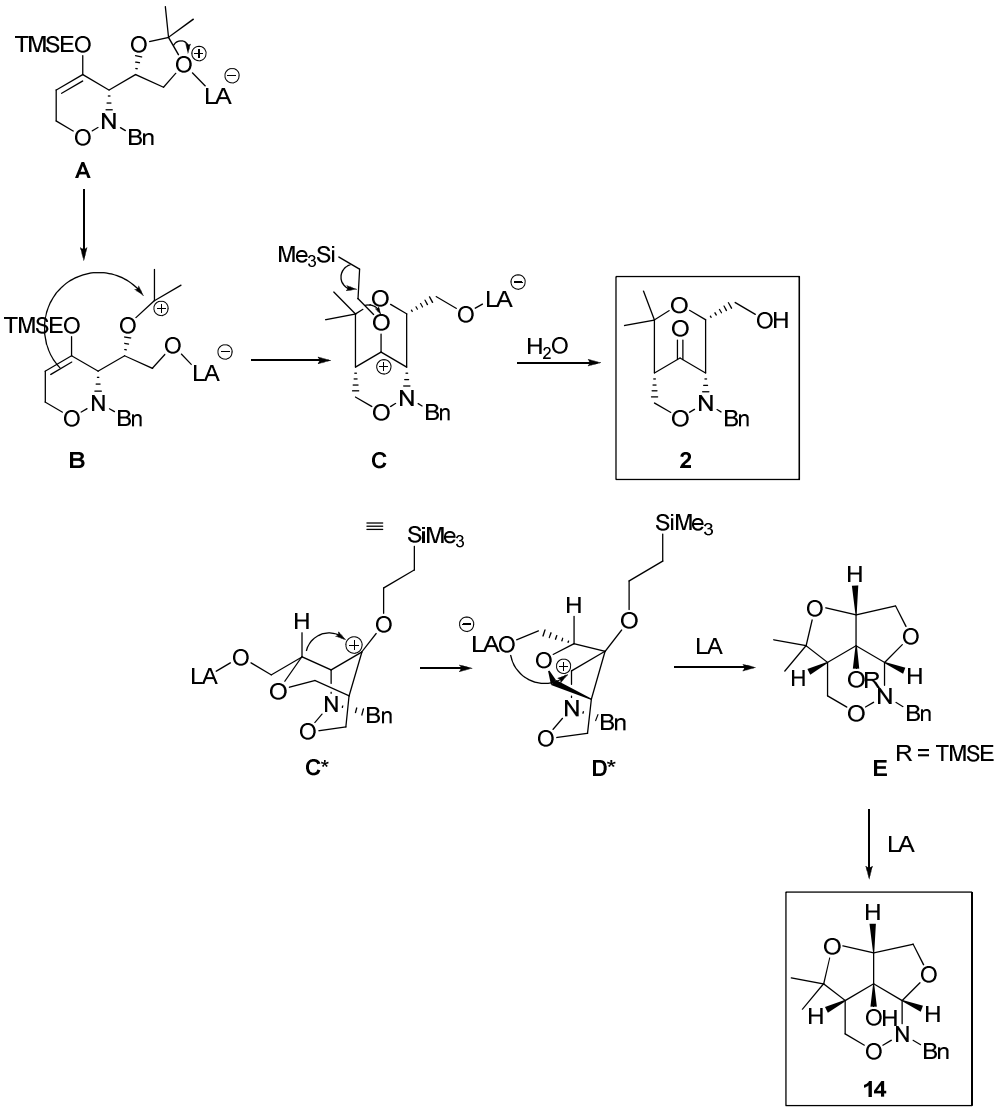


15 R = Me
16 R = Bn

17 R = Me **68%**
18 R = Bn **65%**

Scheme 14. Lewis acid-promoted rearrangements of 1,2-oxazines **15** and **16** leading to tricyclic 1,2-oxazine derivatives **17** and **18**.

One can explain the formation of such moieties by analyzing the mechanism and the intermediates formed during the Lewis acid-induced rearrangement (Scheme 15). The Lewis acid coordinates to the outer oxygen atom of dioxolane moiety since it is sterically less hindered. When this coordination occurs, the acetonide ring opens generating a stabilized carbenium ion **B** as intermediate. An intramolecular nucleophilic attack of the enol ether to the carbenium ion occurs and the six-membered ring **C** is formed. In previous reports it was discussed that in the crucial intermediate **C**, the nature of the 4-alkoxy group was decisive for the final outcome. It was thought that due to the fast fragmentation of 4-(2-trimethylsilyl)ethoxy substituted oxazine **1** the only outcome would be the bicyclic ketone **2**. With substituents like methoxy or benzyloxy not capable of undergoing such fast fragmentation, a direct cyclization is possible leading to the tricyclic product. The intermediate **C** undergoes a 1,2-shift resulting in the carbenium **D**. This stabilized intermediate can react with the remaining oxygen with displacement of the Lewis acid and fragmentation of the trimethylsilylethyl group affording tricyclic compound **14**.

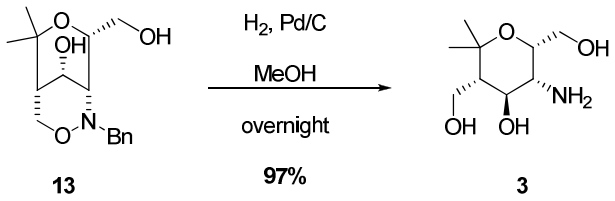


Scheme 15. Proposed mechanism for the formation of tricyclic compound **14** (*the methyl groups were omitted for clarity).

With the isolation of compound **14**, we can claim that even in small amounts, this cyclization is also possible for the OTMSE-substituted 1,2-oxazine *syn*-**1**, and can occur before the normal fragmentation.

4.1.4. Hydrogenolysis

To obtain a fully deprotected aminopyran as a final compound, two different bonds have to be cleaved: the N-O and *N*-benzyl bond. The method of choice often used in the Reißig group to cleave simultaneously these two bonds is the Pd/C-catalyzed hydrogenation. With similar substrates the *N*-benzyl bond can be selectively cleaved under these conditions,[39] however in the case of the bicyclic compound **13**, even after five minutes the fully deprotected aminopyran **3** was detected by TLC control, indicating that N-O and *N*-benzyl bond cleavage is a competing process and the cleavage of both bonds happens almost simultaneously. Hydrogenolysis of **13** with palladium on charcoal catalyst in methanol gave aminopyran **3** with 97% yield (Lit.[29a] 83%) (Scheme 16).



Scheme 16. Hydrogenolysis of bicyclic alcohol **13** affording aminopyran **3**.

One of the advantages of this method is the easy purification of polar compounds. When the reaction proceeds smoothly a simple filtration of the catalyst over Celite® is sufficient affording the desired product in pure fashion. In the majority of the cases excellent yields were obtained, however sometimes a decrease in the yield was observed as well as the formation of side products. Closer investigation led to the isolation of the by-products **19** and **20** depending on the used solvent (Figure 14).

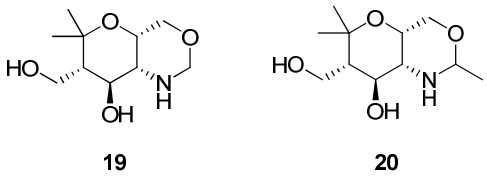


Figure 14. By-products formed during hydrogenolysis of **13**.

Such products were so far not described or isolated after hydrogenolysis of bicyclic compound **13** leading to six membered aminopyran **3**, however a similar side product was previously observed by Bouché for the seven membered analog when the reaction was carried

out in methanol. It was assumed that during the reaction (or filtration) formaldehyde is formed in situ.[40] The formation of the by-products can be explained by the reaction of the free amino and hydroxyl groups with the aldehydes corresponding to the used solvents methanol or ethanol forming the corresponding acetal. It is difficult to distinguish both possible by-products (with a new five memebred ring when the secondary hydroxyl group reacts or a six membered in the case of the primary) since they have the same mass and the expected ^{1}H-NMR spectra are very similar (Figure 15). However, by HMBC analysis of the long range couplings it was possible to elucidate the exact showing that exclusively the six-membered by-product was formed.

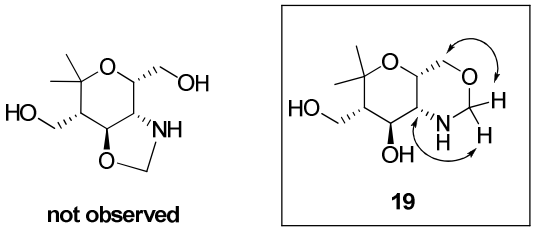
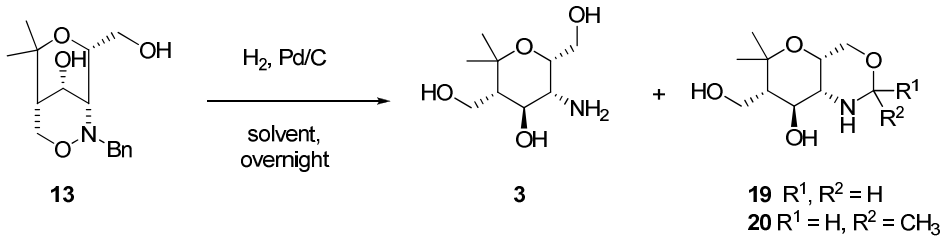


Figure 15. Observed long range couplings proving the structure of the by-product **19**.

To have a better understanding of the formation of these bicyclic compounds further investigations were carried out changing the solvent during hydrogenolysis as well as the solvent used for the filtration. These results are summarized in Table 1. The irreproducible results made it difficult to find a clear explanation for this process (even the source and time of use of the hydrogen bottle showed to have some influence in the final reaction outcome). In Table 1 entry 1 and entry 2 it can be seen that under the same reaction conditions the desired aminopyran **3** could be obtained either in excellent yields or with much lower yield together with the formation of side product **19**. To understand the influence of the solvent during the reaction time and during the filtration, a new experiment was done where the reaction was carried out in ethanol but the filtration was performed using methanol (Table 1, entry 3). Once again in this case only side product **19** was isolated. In contrast, when both reaction and filtration steps were performed in ethanol side product **20** could be isolated (Table 1, entry 4).

Table1. Solvent variation for the hydrogenolysis of **13** and subsequent filtration.

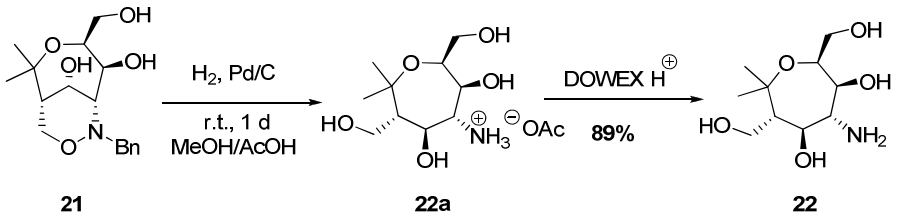


13 **3** **19** R^1, R^2 = H; **20** R^1 = H, R^2 = CH_3

Entry	**Solvent**		**Product, yield (%)**		
	Reaction	**Filtration**	**3**	**19**	**20**
1	MeOH	MeOH	97	-	-
2	MeOH	MeOH	57	16	-
3	EtOH	MeOH	57	24	-
4	EtOH	EtOH	46	-	17

In contrary to what was described by Bouché (who could detect the formation of such products during the reaction and before the filtration),[41] with this results we can propose that the by-product solely depends on the solvent used for the filtration and thus the oxidation probably occurs during the filtration through Celite®. Zhang and Wan described a catalytic process for the aerobic oxidation of alcohols using a Pd/SiO_2-Al_2O_3 catalyst, that somehow resembles the composition of Pd on Celite® as present in the filtration.[42]

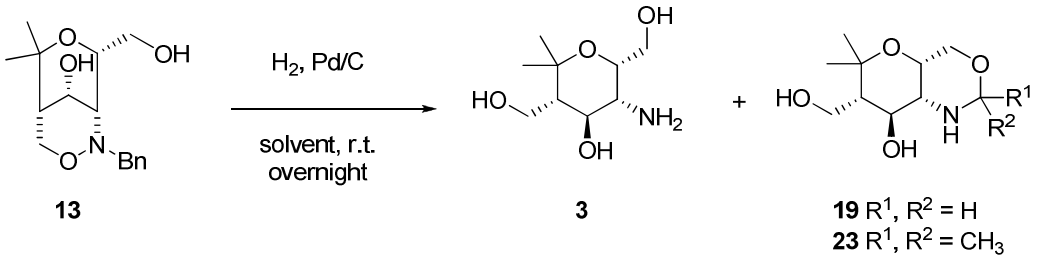
Alternative reaction conditions were tried to prevent the formation of the by-products. Bouché could avoid this problem by adding acid to the reaction mixture. In this case the amine is protonated forming an ammonium salt **22a** that therefore can no longer react with the possible formed formaldehyde. The most commonly employed acids for this purpose are hydrochloric acid and acetic acid.[43] From the different acids, acetic acid proved to be the best one and the reaction was then performed in a MeOH/AcOH = 5:1 mixture. An important aspect of this method is the regeneration of the free amine **22**. This has been realized by a filtration through an acidic ion exchange resin (Scheme 17), where the column is first washed with water until the acidic components are completely removed (controlled with pH paper) and then washed with NH_3 to obtain the free amine.



Scheme 17. Hydrogenolysis of 7-membered bicyclic compound **21** in MeOH/AcOH performed by Bouché.

These conditions were applied to bicyclic product **13** and no side product was detected, but unfortunately the desired product **3** could only be isolated in low yield (Table 2, entry 1). Probably not all the free amine was regenerated and therefore lost during the filtration. Since these conditions did not give satisfying results, a new alternative was tried where isopropanol was used as solvent for the reaction and during filtration (Table 2, entry 2). This procedure gave exceptionally good results and aminopyran **3** was isolated in excellent yield and purity after filtration through a short pad of Celite®. Isopropanol is an adequate solvent since its oxidation affords acetone and the attack of the amine to this ketone is less likely to occur.

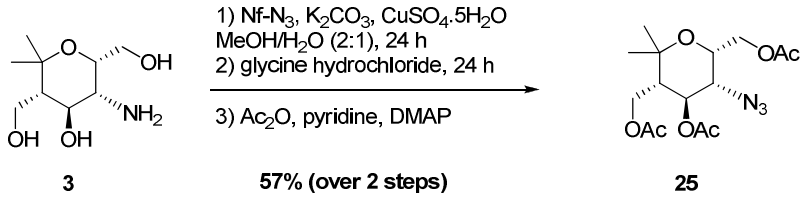
Table 2. New conditions employed for hydrogenolysis to avoid formation of side products.



Entry	Solvent		Product, yield (%)		
	Reaction	**Filtration**	**3**	**19**	**23**
1	MeOH/AcOH (5:1)	MeOH/Dowex	45	-	-
2	*i*PrOH	*i*PrOH	96	-	-

4.1.5. Copper(II)-catalyzed diazotransfer

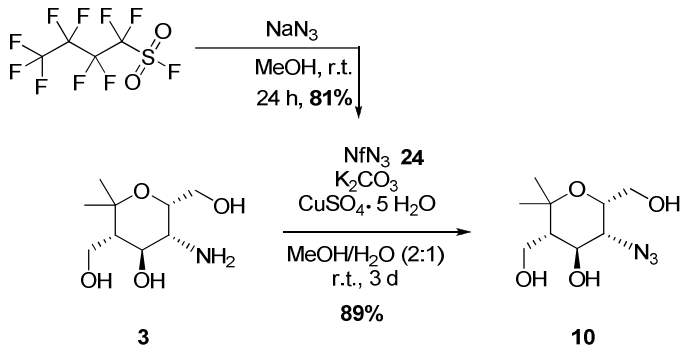
For the transformation of the aminopyran **3** into the corresponding azide, the copper-catalyzed diazotransfer was used. This reaction has been used for many years[44] and optimized by Wong[45] applying metal catalysts. In our group Yekta[29A] has developed conditions for the transformation of aminopyran **3**. Under these conditions the use of nonafluorbutanesulfonyl azide **24** (NfN_3)[46] was a great improvement once it is less explosive and volatile than the commonly used trifluoromethanesulfonyl azide (TfN_3) and therefore much easier to handle. Yekta used this procedure to generate the azide functionality and directly protect in situ all hydroxyl groups with acetic anhydride to obtain the protected azide **25** (Scheme 18). This method was also used by Bouché[35] and Koos[33] to generate a range of different azides giving excellent results.



3 **57% (over 2 steps)** **25**

Scheme 18. Conversion of the aminopyran **3** into the protected azide **25** by Yekta.

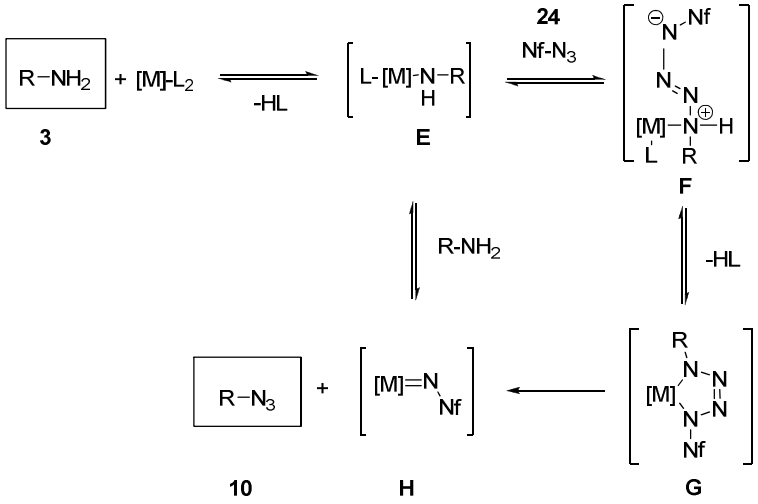
In the presented investigations the isolation of the fully deprotected azide was required (Scheme 19). The azide **24** was generated from nonafluorobutanesulfonyl fluoride (NfF) and sodium azide in methanol according to the literature procedure.[46] The freshly prepared NfN_3 **24** was then slowly added in excess to a stirred solution of aminopyran **3** in the presence of copper(II) sulfate and potassium carbonate in a methanol/water mixture during 24 hours. After completion of the reaction (3 days) glycine was added in excess to quench the excess of **24**.



Scheme 19. Synthetic route to azidopyran **10**.

The reaction mixture was then quenched with 7N ammonia methanol solution to coordinate the copper that could then be easily filtrated off. The method to prepare these azides proved to be effective and easy to perform. Furthermore, these mild conditions allow the isolation of the stable deprotected azide **10** with full retention of configuration in excellent yield.

The mechanism of this reaction is still unknown. However, Wong proposed a mechanism where zinc(II) is used and it is assumed that with copper(II) the same mechanism is plausible (Scheme 20).[47] Under basic conditions, a complexation of the amine **3** to the metal catalyst leads to the intermediate **E**. This is followed by nucleophilic attack of **E** on the highly electrophilic terminal nitrogen of **24**, followed by deprotonation and cyclization forming the tetrazene **G**. Possibly a [3+2]-cycloreversion occurs leading to the desired azide product and complex **H**.



Scheme 20. Possible mechanism for the metal-catalyzed diazotransfer reaction proposed by Wong.

4.1.6. Introduction of protecting groups

Protecting groups have been widely used over the years and in the synthesis of complex products they are often required. When in the same molecule more than one functional group is present and a reaction needs to be performed in a selective way, the other possible reaction sites should be blocked. Although the goal was to generate a straightforward route to the new multivalent compounds avoiding as much as possible the use of protecting groups in some cases it was strictly necessary. With the two principal building blocks **3** and **10** in hand (Figure 16) the hydroxyl groups should be protected selectively. For azidopyran **10** a variety of protecting groups can be applied, but in the case of aminopyran **3** one should chose a protective group that selectively protects the hydroxyl functionality in the presence of a free amine.

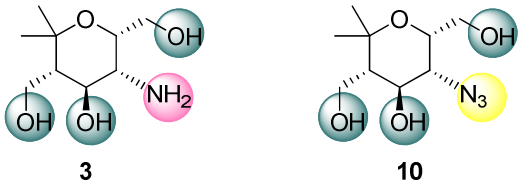
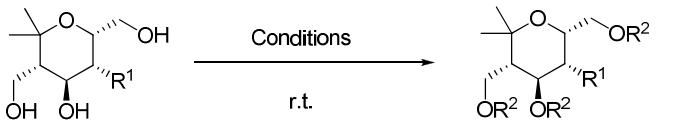


Figure 16. Main building blocks **3** and **10** used in the synthesis of multivalent carbohydrate mimetics.

In the process of choosing the adequate protecting groups, was also considered the facility of their removal in later stages. Since the final multivalent products will contain several hydroxyl functionalities, the purification of these molecules after cleavage of the respective protecting groups should be simple. Table 3 summarizes the methods used for the protection of the hydroxyl functionalities of aminopyran **3** and azidopyran **10**. As a first attempt a silyl ether type group, *tert*-butyldimethylsilyl (TBS) was chosen and the reaction was carried out under standard conditions using *tert*-butyldimethylsilyltriflate (TBSOTf) and a tertiary amine as base (Table 3, entry 1). This reaction was performed several times and the yield could be improved to 72% when the reaction time was extended to 3 days. When shorter times were applied, the yields drop to 50% (12 hours). Alternatively the methoxymethyl group MOM was chosen (Table 3, entry 2). With the usual conditions for protection using MOMCl, a complex mixture of starting material, mono- and diprotected products was obtained even with longer reaction times. Since the reagent MOMCl is considered carcinogenic, this method was discarded and the TBS group was the preferred for selective protection of the hydroxyl functionalities in aminopyran **3**. As previously mentioned, the choice of protecting groups

was easier in the case of azidopyran **10**. Both commonly used protecting groups acetyl and benzyl (Table 3, entry 3 and entry 4) proved to be efficient groups and both reactions were easily carried out leading to excellent yields.

Table 3. Reaction conditions for the hydroxyl protection of pyrans **3** and **10**.



3 $R^1 = NH_2$

10 $R^1 = N_3$

26 $R^1 = NH_2$; R^2 = TBS

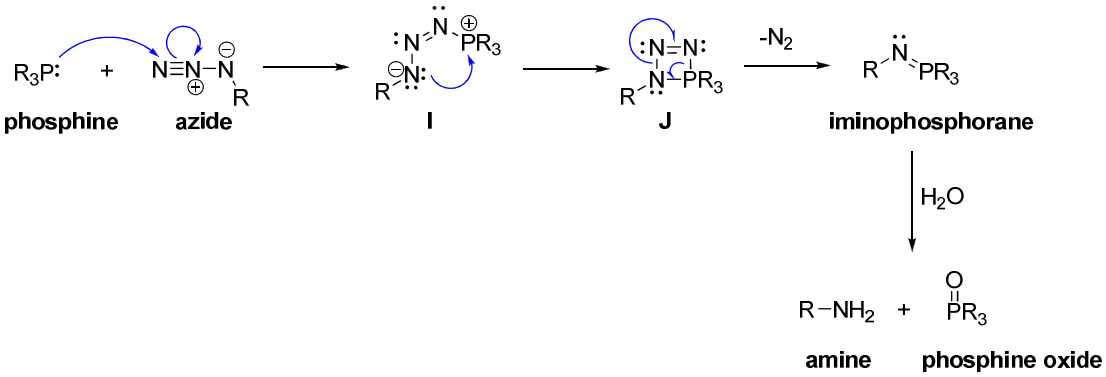
25 $R^1 = N_3$; R^2 = Ac

27 $R^1 = N_3$; R^2 = Bn

Entry	Conditions	Time	R^1	R^2	Yield (%)
1	TBSOTf, NEt_3 DMF 0 ºC - r.t.	3 days	NH_2	TBS	72
2	MOMCl, DIPEA 0 ºC - r.t.	overnight	NH_2	MOM	Complex mixture
3	Ac_2O py, DMAP r.t.	overnight	N_3	Ac	91
4	NaH, BnBr,THF 0 °C – r.t.	overnight	N_3	Bn	76

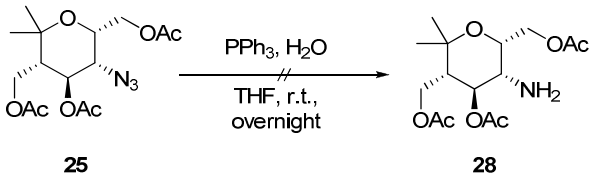
4.1.7. Conversion of azidopyrans into aminopyrans

With the easily obtained protected compounds **25** and **27** in hand, it was intended to reduce the azide functionality to the corresponding amine to access benzyl and acetyl protected aminopyrans in a direct way. Azides can be easily converted to amines for example by catalytic hydrogenation, reduction with complex metal hydrides or by the Staudinger reaction. The Staudinger reaction was discovered in 1919 by Staudinger et al.[48] In this reaction a phosphine is used to reduce an azide to the amine functionality. Scheme 21 presents the reaction mechanism where a nucleophilic addition of the phosphine to the terminal nitrogen of the azide occurs forming a phosphazide **I** which rearranges to the 4-membered-ring intermediate **J**. After loss of dinitrogen an iminophosphorane is formed which is hydrolyzed in the presence of water to yield a primary amine and a phosphine oxide.



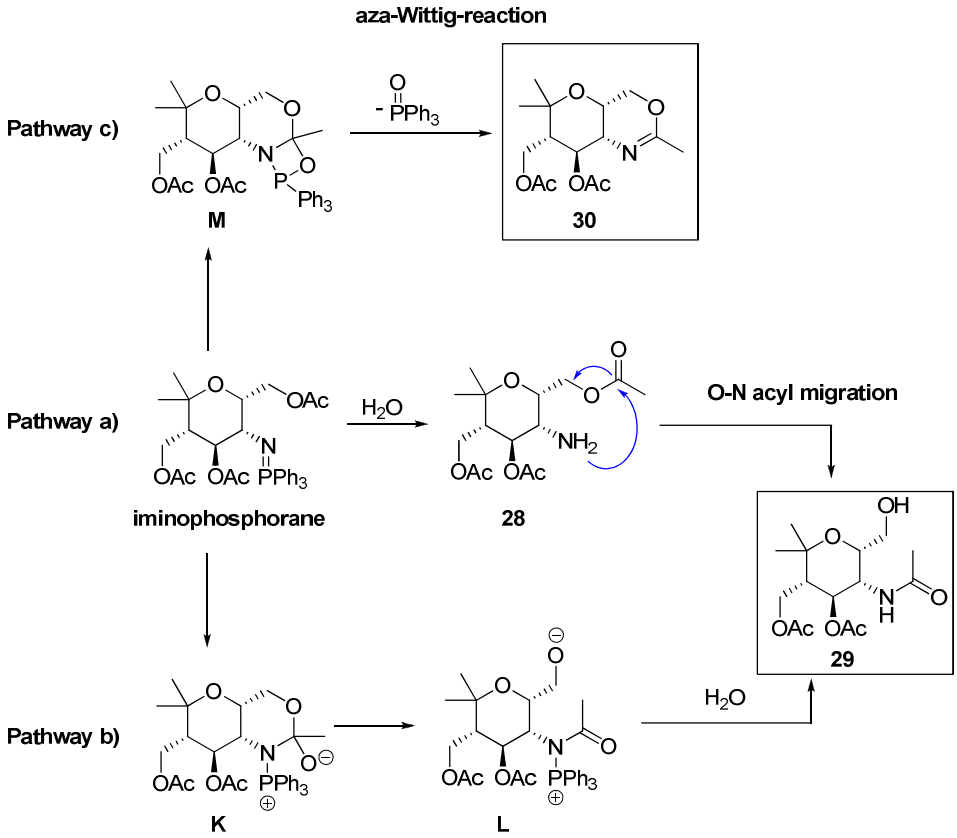
Scheme 21. Mechanism of the Staudinger reaction.

Standard Staudinger conditions were used in order to reduce azide **25** (Scheme 22). After reaction and purification by flash column chromatography with silica gel the desired product **28** could not be isolated nor identified by ESI or ^{1}H-NMR spectroscopy. Several fractions were isolated containing more than one product (during purification the formation of more products was noticed on TLC control).



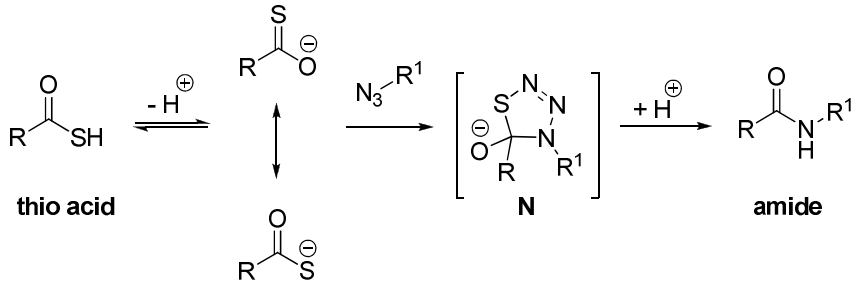
Scheme 22. Attempt to reduce azide **25** using standard Staudinger conditions.

Several products could be identified from the mixture, being a result of *O*-acetyl to *N*-acetyl migration (like **29**), acetyl cleavage and aza-Wittig product **30**. The acetyl group migration is well known in many cases but to the best of our knowledge in the literature only one report was found for the *O*-acetyl to *N*-acetyl migration under Staudinger conditions.[49] In the presented case this migration can occur not only during the reaction, but it may also be catalyzed by the acidity of the silica gel. Although the intramolecular aza-Wittig reaction is commonly used in the synthesis of heterocyclic natural products[50] the identification of such products was quite surprising. There are different possibilities for the *O*→*N* acetyl migration: once the free amine **28** is generated it can react intramolecularly with the carbonyl group leading to the migration product **29** (Scheme 23, pathway a). Alternatively, the stable and reactive iminophosphorane formed can react intramolecularly with the carbonyl group of the ester moiety prior to reduction (Scheme 23, pathway b). To form the aza-Wittig product, the resulting iminophosphorane with its highly nucleophilic nitrogen atom can also be regarded as an aza-ylide and it can react with the acetyl group leading to intermediate **M**. It is known[50],[51] that even with less reactive esters this reaction can take place, especially because it occurs here in an intramolecular manner (Scheme 23, pathway c). Without isolation in pure form and confirmation of the exact structure, it is assumed that similar to what was discussed for the hydrogenolysis side products (Section 4.1.4), the cyclized product has a six membered ring instead of the also possible five membered.



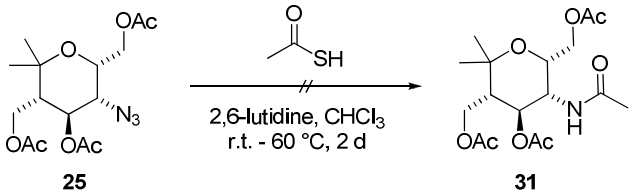
Scheme 23. Different pathways for the formation of side products and aza-Wittig product **30**.

Discouraged by the instability of the acetyl group other known conditions in the literature were examined for the formation of amide bonds from thio acids. The conditions presented by Williams et al.[52] were quite appealing since according to their proposed mechanism a thiazolidine intermediate **N** would be formed rather than a reduction from azide to amine avoiding this way the previous side reactions (Scheme 24).



Scheme 24. Mechanism for the reaction of thio acids with azides.

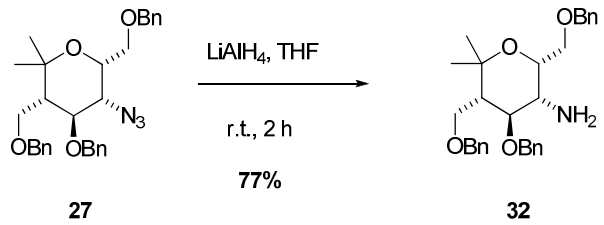
Unsuccessfully, applying the reported conditions did not lead to the formation of any product since reaction of azide **25** with thioacetic acid led only to starting material (Scheme 25).



Scheme 25. Attempt to synthesize compound **31** using the conditions applied by Williams.

Since there are literature reports on the reduction of carbohydrate azides using hydrogenolysis with Pd/C,[53] a further attempt was done to reduce the acetyl-protected azide **25** with Pd/C in isopropanol. Unfortunately, a mixture of products as observed in the Staudinger reaction was received. After these unsatisfactory results, we decided to use the benzyl protected azide **27** and to perform the standard Staudinger reaction in hope that with the higher stability of the benzyl protecting group, the respective reduced amine product would be obtained. Once again these conditions led to a mixture of unidentified products.

Enders and Müller-Hüwen described the reduction of the azido group with $LiAlH_4$ to give a primary amine in the synthesis of D-*erythro*-sphinganine.[54] This method proved to be the most efficient and adequate for compound **27** (Scheme 26). Applying these conditions, the corresponding amine **32** was obtained in good yield.



Scheme 26. Reduction of azide **27** using $LiAlH_4$.

This method proved to be efficient for the reduction of benzyl protected azide **27** but the same conditions were probably not applicable to the acetyl protected analog **25**. Recently, Nishida et al. reported on the total synthesis of (±)-lundurine A and B, involving the successful reduction of an azide intermediate in the presence of acetyl protecting group using samarium(II) iodide.[55] This could be an alternative method for the reduction of acetyl protected azide **25**.

4.2. Multivalent compounds by amide bond formation

The amide linkage is one of the most important chemical bonds in nature. Therefore in the development of new mimetics this is a key moiety when trying to mimic compounds present in proteins and involved in biological processes. In 2006 an assay of the reactions used for the preparation of drug candidates was published and acylation reactions were one of the most commonly used representing 12% of the reactions involved, of which 66% were *N*-acylations to form amides.[56] The delocalization of electrons over the N-C-O bond and the hydrogen bonding play a crucial role in the amide bond interactions in protein structures. The partial double bond nature of the peptide linkage is not rigid and therefore can exist in *cis* and *trans* configurations (Figure 17). However it has been proved that the majority of peptide bonds in protein structures exist in the more stable *trans* configuration.[57]

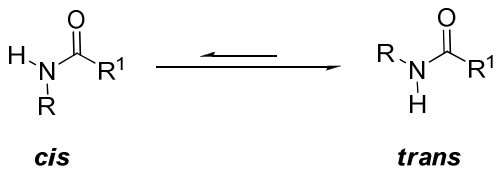


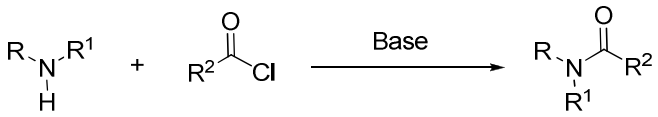
Figure 17. Possible configurations of an amide bond.

Since amide bonds are often found in natural molecules and systems this type of ligation is often the choice for the construction of multivalent architectures. In literature applications can be found for example for the construction of multivalent cellobiosylated glycoconjugates and their interaction with enzymes[53] or the synthesis of multivalent *C*-glycosidic units and their combination to scaffolds that provide for example fluorescent-labeled multivalent scaffolds.[58]

4.2.1. Amide bond formation by Schotten-Baumann reaction

Amide bonds are generally synthesized by reaction of a carboxylic acid and an amine. The reaction between this two groups does not occur spontaneously at room temperature, therefore one needs to first activate the carboxylic acid normally by converting it into a good leaving group for example in an acid chloride. The Schotten-Baumann reaction was first described in 1883 by the two german chemists Carl Schotten and Eugen Baumann[59] and became one of the most popular reactions for the synthesis of amide bonds. This reaction involves a coupling

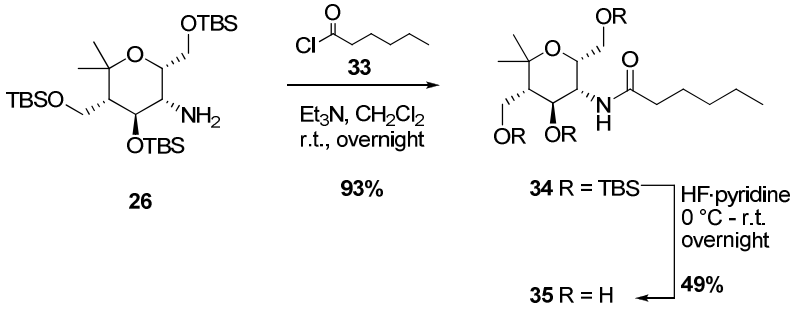
of an acid chloride with an amine affording the amide (Scheme 27). Due to the formation of hydrochloric acid the use of a base is required.



Scheme 27. The Schotten-Baumann reaction.

4.2.1.1. Synthesis of monovalent carbohydrate mimetics

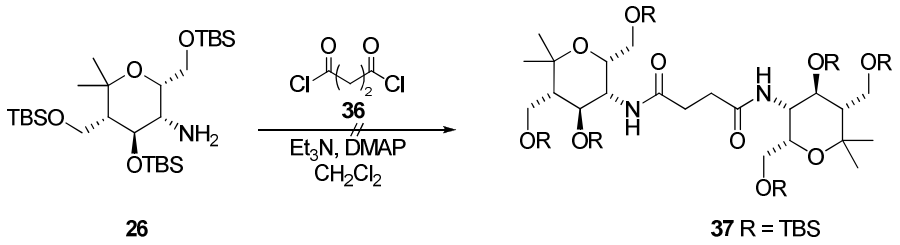
To perform such a reaction, protected aminopyran **26** was chosen since in the case of the fully deprotected analog **3** the free hydroxyl groups may compete with the amine and react under the same conditions with the acid chloride generating an ester. Using standard conditions, protected aminopyran **26** reacted with commercially available hexanoyl chloride (**33**) affording the desired amide **34** in excellent yield (Scheme 28). After cleavage of the TBS protecting groups, fully deprotected monovalent aminopyran derivative **35** was obtained in moderate yield.



Scheme 28. Synthesis of monovalent amide **34** and subsequent deprotection to **35**.

4.2.1.2. Synthesis of divalent carbohydrate mimetics

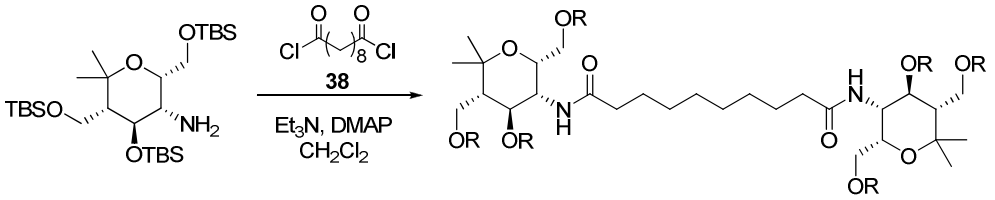
After successfully synthesis of the monovalent model compound **35**, the same conditions were tested for the synthesis of divalent structures. To generate these divalent compounds at least two equivalents of the protected aminopyran **26** are needed for one equivalent of succinyl chloride **36**. When the same conditions were applied to acid chloride **36** the desired divalent product **37** was not observed (Scheme 29).



Scheme 29. Attempt to synthetize protected divalent compound **37**.

After several attempts to generate product **37**, changing reaction time and equivalents of protected amionpyran **26** and succinyl chloride **36**, the desired product was not obtained and the formation of the corresponding pyrrolidine-2,5-dione resulting from the intramolecular reaction was also not noticed. It was quite unanticipated that we could not achieve the formation of dimer **37** since in the literature similar conditions were found for the construction of multivalent acetyl protected carbohydrates.[53] At this point it was thought that the resulting product **37** would be sterically too hindered due to the bulkiness of the TBS protecting groups. For this reason a new dicarboxylic acid with a longer chain (sebacoyl chloride **38**) was used and the desired product **39** could be isolated. The different attempts to optimize this reaction are summarized in Table 4. When performing the reaction with a large excess of aminopyran **26** (Table 4, entry 1), the yields were low since starting material **26** and the desired product **39** have the same Rf value, making the purification by column chromatography hard and tedious. Due to these unexpected problems different conditions were tried changing the equivalents of protected aminopyran **26**. Indeed the best results were obtained when the minimum amount of **26** required to form divalent product **39** were used (exactly 2.0 equivalents). Compound **39** could then be isolated with the best yield of 58%.

Table 4. Reaction optimization

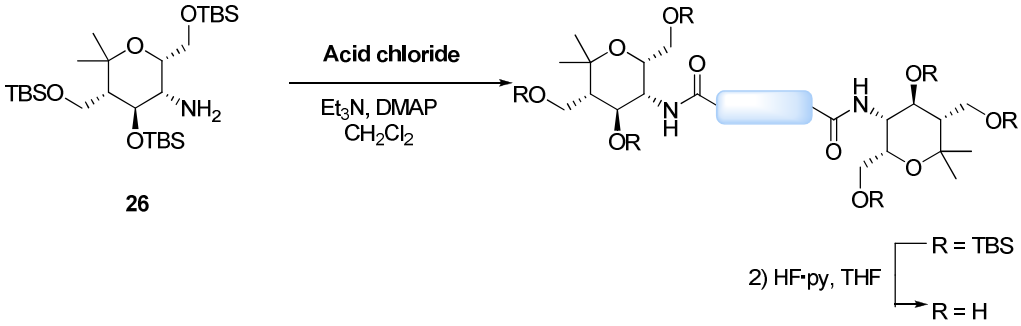


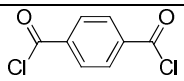
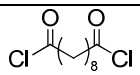
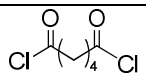
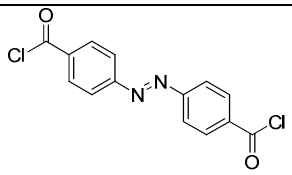
Entry	Equiv. Aminopyran	Yield (%)
1	3.3	30
2	2.7	40
3	2.1	30
4	2.0	58

With the optimized conditions in hand, several dicarboxylic acids were chosen to generate a series of divalent carbohydrate mimetics with different spacing and flexibility. The synthesized compounds are listed in Table 5. Reaction with the aromatic linker terephthaloyl dichloride **40** (Table 5, entry 1) afforded the desired protected dimer **40** with excellent yield. This was the linker who gave the best reaction results. On the other hand, reaction with the aliphatic adipoyl dichloride **45** (Table 5, entry 5) gave the lowest yields and the desired product **46** was isolated with a moderate yield of 43%. The interesting *trans*-azobenzene derivative **48** was also used and the desired divalent azo compound **49** was obtained with a yield of 55%. Azo compounds can exist in both *cis* and *trans* isomeric form, however the *trans* isomer is more stable by approximately 50 kJ/mol, and the barrier to photoisomerisation is approximately 200 kJ/mol.[60] Although no further investigations were done with azo derivative **49**, the study of the shape-influence of both *cis* and *trans* isomers would be interesting.

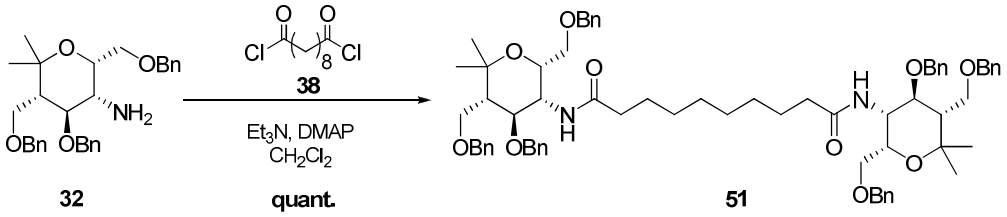
Deprotection using HF·pyridine proved to be an adequate method and all fully deprotected dimers were obtained in excellent yields.

Table 5. Yields for the different synthesized dimeric amides and the subsequent deprotection step.



Entry	Acid chloride ClOC—[ ]—COCl	R	Product	Yield (%)
1		TBS	**41**	83
2	**40**	H	**42**	quant.
3		TBS	**39**	58
4	**38**	H	**44**	80
5		TBS	**46**	43
6	**45**	H	**47**	92
7		TBS	**49**	55
8	**48**	H	**50**	quant.

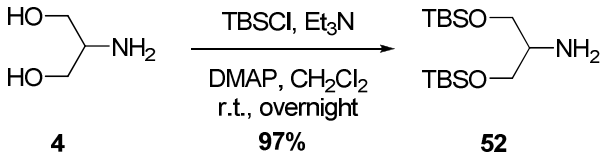
Starting from the benzyl protected aminopyran **32** and using sebacoyl chloride **38** as linker, divalent compound **51** was synthetized in quantitative yield (Scheme 30). This was a great improvement from the obtained 58% for the analog TBS protected dimer **43** (Table 5, entry 3). Besides providing an excellent yield, divalent compound **51** has the advantage of the deprotection being easily performed under hydrogenolysis conditions, which should provide a clean reaction and an easy purification.



Scheme 30. Synthesis of divalent benzyl protected compound **51**.

4.2.1.3. Synthesis of divalent 2-amino-1,3-diol derivatives

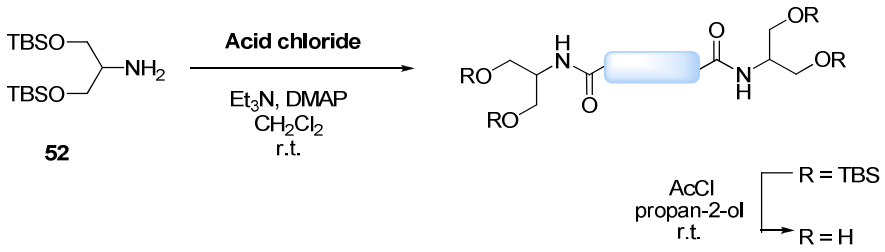
As previously mentioned, an additional goal was the investigation of multivalent compounds starting from the commercially available amino polyol **4**. Similarly to what was done for aminopyran **3**, the hydroxyl groups of **4** were first TBS protected affording protected compound **52** in excellent yield (Scheme 31).

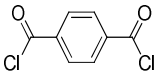
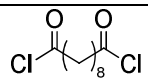
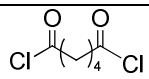
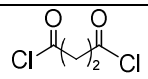


Scheme 31. TBS protection of amino polyol **4** leading to protected amine **52**.

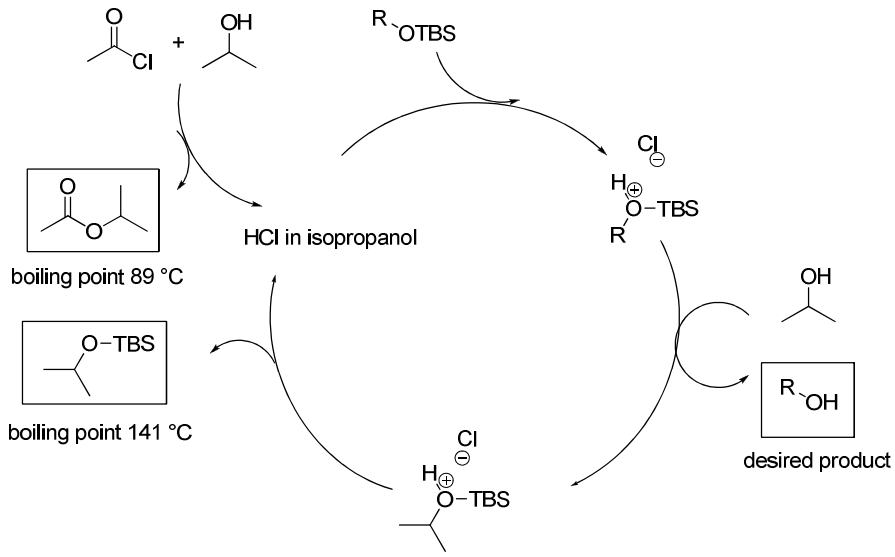
To be able to compare aminopyran **3** with aminopolyol **4** the same linkers were used to prepare divalent compounds. The TBS protected divalent compounds were obtained in excellent yields (Table 6). Interestingly, using succinyl chloride **36** and TBS protected amine **52**, the corresponding dimer **59** was obtained in excellent yield (Table 6, entry 7). Contrary to the protected aminopyran **26** that did not react with succinyl chloride **36**, in this case amine **52** is much more flexible and therefore sterically less hindered apparently allowing the formation of the desired product.

Table 6. Yields for the different synthesized dimers **53** – **60**.



Entry	Acid chloride	R	Product	Yield (%)
1		TBS	**53**	83
2	**40**	H	**54**	quant.
3		TBS	**55**	75
4	**38**	H	**56**	quant.
5		TBS	**57**	62
6	**45**	H	**58**	quant.
7		TBS	**59**	83
8	**36**	H	**60**	quant.

Although TBS deprotection using HF·pyridine proved to be an efficient method, other options were considered in search of an alternative method using milder conditions and cheaper reagents. Khan et al. reported a method where acetyl chloride in methanol was used as a source of hydrochloric acid.[61] Acid catalyzed solvolysis seems to be the method of choice, with the advantage that all the side products can easily be removed in vacuo, making no further purification necessary. First attempts were done using methanol but poor conversions were observed mainly due to the insolubility of the starting material. Isopropanol was then chosen as alternative leading to excellent results. In this case HCl is also generated in situ and both side products isopropyl acetate (product of esterification of isopropanol and acetyl chloride) and *tert*-butyl(isopropoxy)dimethylsilane have boiling points that allow their removal in vacuo (Scheme 32).



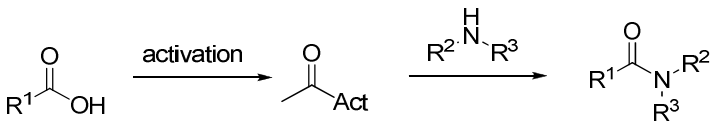
Scheme 32. Catalytic cycle for silyl ether cleavage using acetyl chloride in isopropanol.

Applying these conditions to deprotect the obtained dimers resulted in excellent yields and all fully deprotected compounds were obtained in quantitative yield without any column chromatography by simple removal of all volatiles in vacuo (Table 6).

Although this method proved to be excellent to deprotect all dimers derived from the simple aminopolyol, when the same conditions were applied to the compounds containing the aminopyran moiety, a mixture of more than two products was always obtained. Probably these conditions are sufficient to cleave the TBS groups of primary alcohols but not of secondary alcohols.

4.2.2. Amide bond formation by HATU coupling reagent

Nowadays the most common way to generate an amide bond is the use of a coupling reagent to first activate a carboxylic acid which then reacts with an amine (Scheme 33).



Scheme 33. Carboxylic acid activation in presence of a coupling reagent and reaction with an amine.

From the many known coupling reagents[62] HATU was selected, a coupling reagent using aminium salts and often used for peptide synthesis. X-ray structure determinations of HATU, shows that it crystallizes as a aminium *N*-oxide salt, rather than as a uronium salt (Figure 18) however in solution both co-exist and can react with a carboxylic acid.[63]

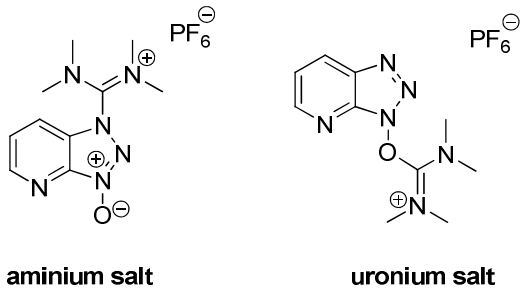
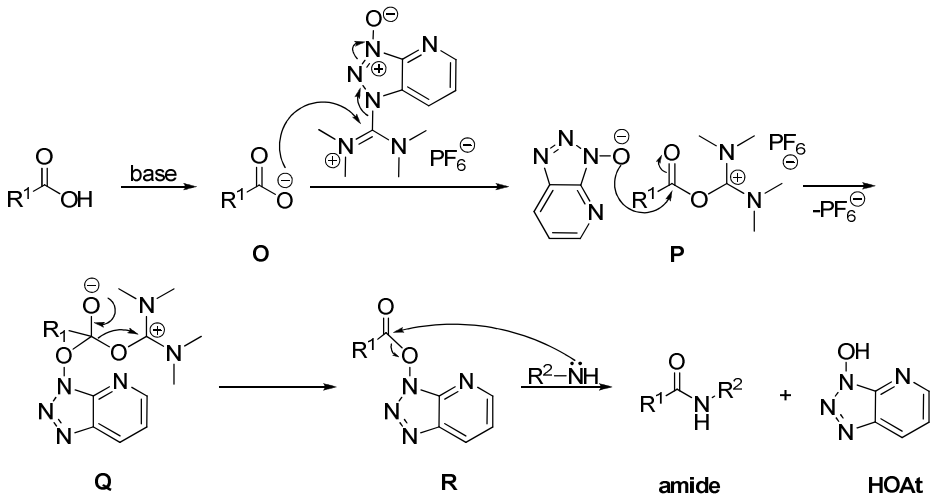


Figure 18. Aminium and uronium isomer of HATU.

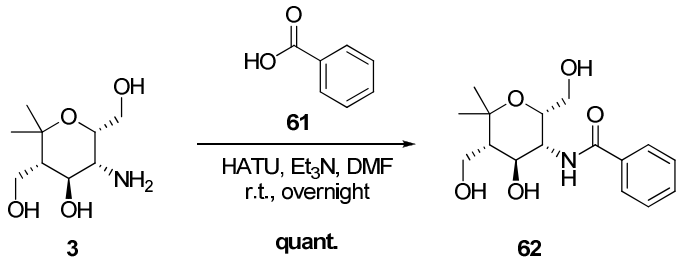
The reaction mechanism[62] of activation is shown in Scheme 34. After deprotonation of the carboxylic acid by a base, the resulting carboxylate **O** attacks the sp^2 carbon atom of HATU leading to the formation of OAt anion that attacks the salt **P** leading to the formation of the active ester **R**, that subsequently reacts with the amine to form the desired amide and HOAt as side product.



Scheme 34. Mechanism of the amide formation using HATU as reagent.

4.2.2.1. Synthesis of monovalent carbohydrate mimetics

To test the standard conditions for aminopyran **3**, benzoic acid **61** was chosen and the desired amide **62** was formed in excellent yield (Scheme 35).

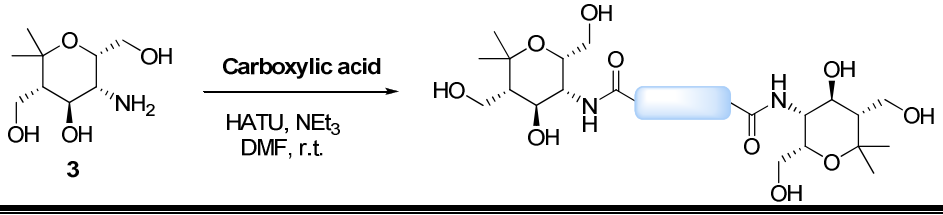


Scheme 35. Synthesis of compound **62** using HATU as coupling reagent.

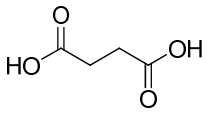
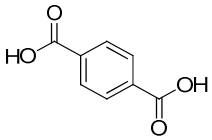
4.2.2.2. Synthesis of divalent carbohydrate mimetics

Encouraged by these results, the same method was used for the synthesis of multivalent compounds. Starting with dicarboxylic acids **63** and **65**, divalent compounds **64** and **66** were obtained (Table 7). In this case a small excess of amine **3** was used: for one equivalent of the dicarboxylic acid, only 2.5 equivalents of the amine **3** were used. To our surprise divalent compound **64** from reaction of unprotected aminopyran **3** and succinic acid **63** was isolated in excellent yield (Table 7, entry 1). This is again an indication that the TBS protected aminopyran was probably sterically too hindered to react with the acid chloride **63**. Reaction with terephthalic acid **65** afforded the desired dimeric structure **66,** but in much lower yield compared to the reaction performed with the corresponding acid chloride (Table 7, entry 2). This low yield was due to the difficult purification and the hard separation of the desired product from HOAt generated during the reaction. One should notice that even with the much lower yield, this procedure saves two synthetic steps: the protection/deprotection of aminopyran **3**.

Table 7. Synthesis of divalent aminopyran derivatives using HATU.



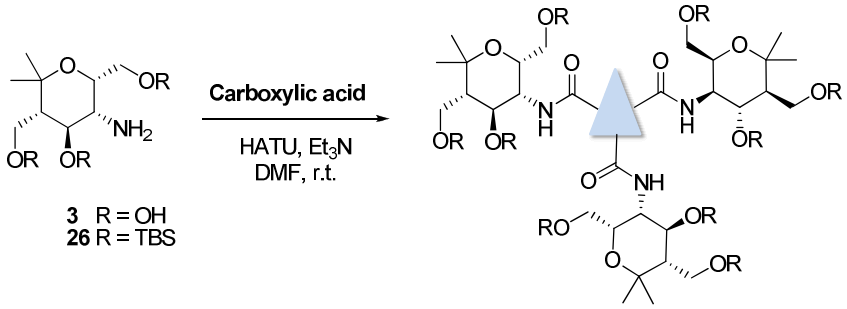
3

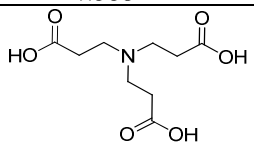
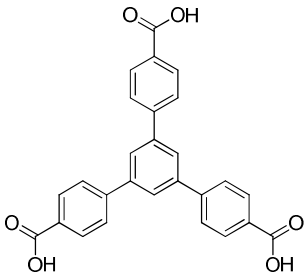
Entry	Carboxylic Acid	product	Yield (%)
1	 **63**	**64**	73
2	 **65**	**66**	21

4.2.2.3. Synthesis of trivalent carbohydrate mimetics

In addition, trivalent carbohydrate mimetics **68** and **70** were synthetized in good yields using this procedure. Once again it was noticed that the reaction proceeds efficiently even without a large excess of the corresponding aminopyran. For the synthesis of each trivalent compound 1.3 equivalents of amine per carboxylic acid unit were needed. Starting from TBS protected aminopyran **26** and using an aliphatic linker **67**, trivalent compound **68** was obtained in excellent yield (Table 8, entry 1). It is known that under these conditions the hydroxyl groups need not to be protected. However, when these conditions were applied to unprotected aminopyran **3** and linker **67**, no product could be isolated after column chromatography, most probably due to its high polarity, making it impossible to separate the product from HOAt. In contrary, when the aromatic linker **69** was chosen (Table 8, entry 2) the polarity of the final product is reduced and the reaction proceeded perfectly. Starting from the unprotected aminopyran **3** the desired trivalent compound **70** was received with excellent yield without the need of protecting groups.

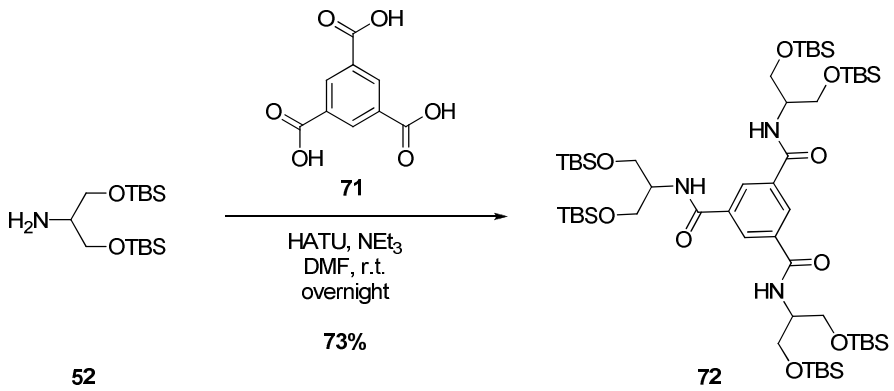
Table 8. Synthesis of trivalent aminopyran derivatives using HATU.



Entry	Carboxylic Acid	R	Product	Yield (%)
1	 **67**	TBS	**68**	83
2	 **69**	H	**70**	80

4.2.2.4. Synthesis of trivalent 2-amino-1,3-diol derivatives

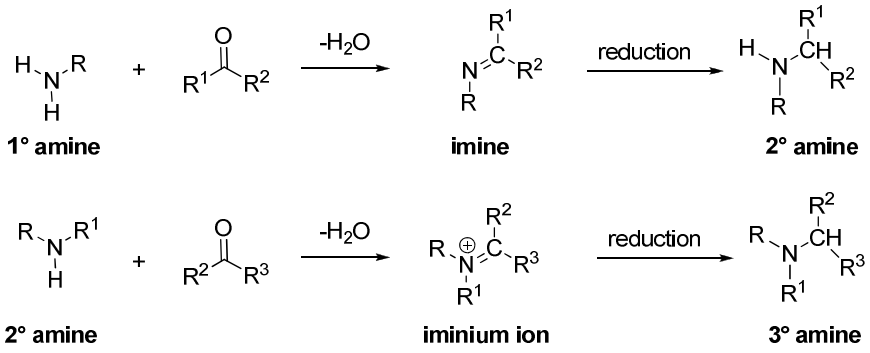
In addition, an example of a trivalent compound starting from the simple TBS protected compound **52** was prepared (Scheme 36). Once again the use of protected starting material was necessary due to purification reasons. Trivalent compound **72** was obtained in good yield.



Scheme 36. Synthesis of trivalent compound **72**.

4.3. Multivalent compounds by reductive amination

Many classes of natural products and living systems contain amines, an example of these are the alkaloids. One of a the most useful and important tools in the synthesis of different amines is the reaction between aldehydes or ketones with ammonia, primary amines, or secondary amines in the presence of a reducing agent to give a primary, secondary, or tertiary amine, respectively. This reductive amination of a primary or secondary amine involves at first stage the formation of an imine or iminium ion intermediate, respectively, that can be subsequently reduced leading to the secondary or tertiary amine (Scheme 37).[64]



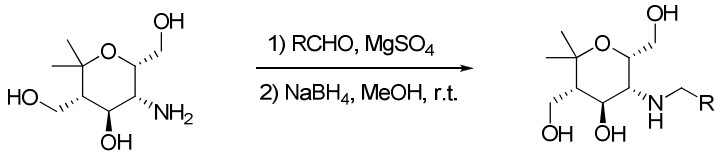
Scheme 37. Mechanism of reductive amination.

The choice of the reducing agent is very important to the success of the method, since the reducing agent must reduce imines (or iminium ions) selectively over aldehydes or ketones under the reaction conditions. Reductive amination can then be performed in two ways: a direct one pot procedure where the reducing agent is mixed initially with the carbonyl compound and the amine prior to the fully formation of the iminium salt intermediate or a stepwise version where first all the starting material should be consumed leading to full conversion to the imine intermediate and only then the reduction is performed in a separate step. Some of the used methods for direct reductive amination include for example catalytic hydrogenation with platinum or palladium catalyst.[65]

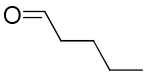
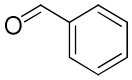
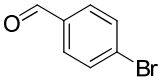
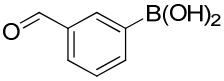
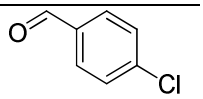
4.3.1. Synthesis of monovalent carbohydrate mimetics

For the formation of the desired amines starting from primary amine **3** with different aldehydes, it was decided to use a procedure previously employed in our research group using $NaBH_4$ as a reducing agent since it is inexpensive, safe to handle and environmentally friendly.[66] In this case it is important to make sure that the starting material is fully consumed before adding the $NaBH_4$. In this procedure $MgSO_4$ is also used as a dehydration agent helping to remove the water formed during the reaction and shifting the equilibrium to the formation of the imine intermediate. Although $NaBH_4$ is only added after formation of the imine this procedure can be seen as a one-pot method since a simple filtration is needed before adding the reducing agent and no intermediate is isolated. A range of monovalent products were synthetized and they are summarized in Table 9.

Table 9. Yields for the different synthesized *N*-alkylated aminopyrans via reductive amination.



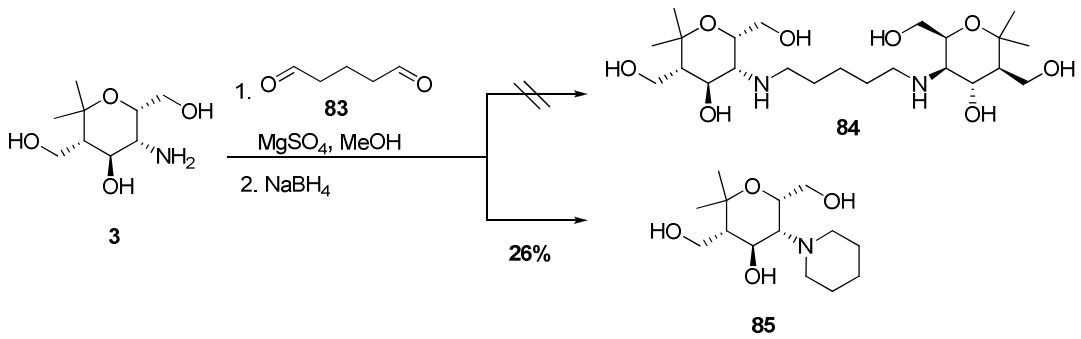
3

Entry	**1**	**2**	**3**	**4**	**5**
Aldehyde					
	73	**75**	**77**	**79**	**81**
Product	**74**	**76**	**78**	**80**	**82**
Yield (%)	45	65	71	30	51

From the commercially available aldehydes, aliphatic aldehyde pentanal (**73**) and benzaldehyde **75** were freshly distilled prior to use. The best yield was obtained for the reaction with 4-bromobenzaldehyde **77** (Table 9, entry 3). To our disappointment the reaction of aminopyran **3** with aldehyde **79** bearing a boronic acid functionality proved to be quite unreliable. Product **80** would be an interesting building block since it would allow further transformations via palladium catalyzed Suzuki reaction. Although the desired product could be isolated even with very poor yield, the reaction was not reproducible and all the further attempts to generate compound **80** did not lead to the formation of any product.

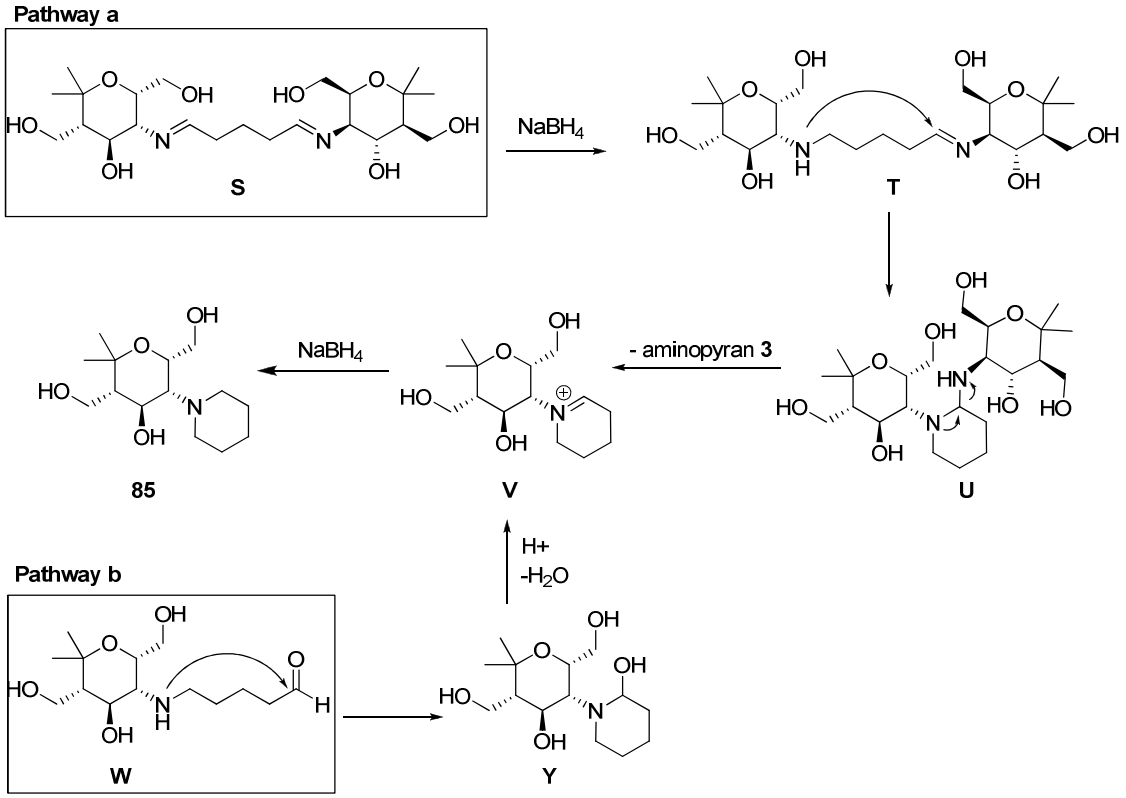
4.3.2. Synthesis of divalent carbohydrate mimetics

The attempt to use the same method to generate dimers with aliphatic linkers failed. With an aqueous solution of 1,5-pentanedial (**83**) and large excess of $MgSO_4$ compound **84** was not obtained but instead the product of a double reductive amination **85** was isolated in low yield (Scheme 38).



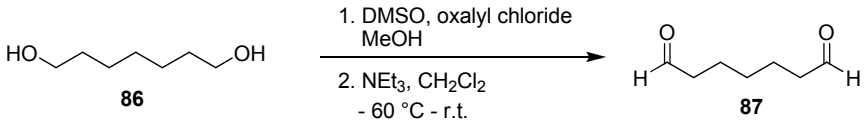
Scheme 38. Attempt to synthetize dimer **84** and isolation of the reductive amination product **85**.

A proposed mechanism for this undesired reaction is shown in Scheme 39. First an imine dimer **S** might be formed and once one of the imine units is reduced to a secondary amine **T**, followed by an intramolecular attack to the second imine. This process needs to happen faster than the second reduction that would lead to the desired product (Scheme 39, pathway a). An alternative mechanism can be considered where an imine monomer is formed that after reduction with $NaBH_4$ generates intermediate **V** that reacts intramolecularly with the second aldehyde (Scheme 39, pathway b).



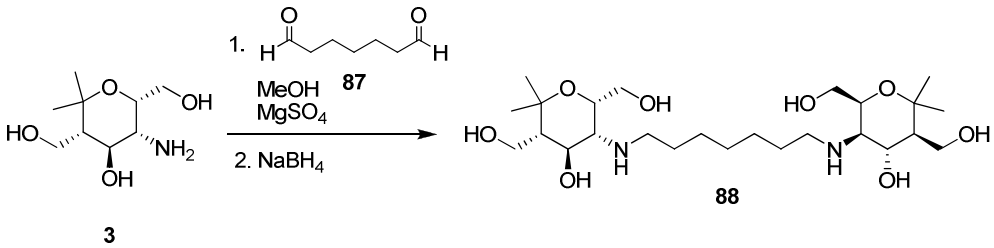
Scheme 39. Proposed mechanistic pathways for the formation of side product **85**.

To avoid the formation of a stable six membered ring we searched for an alternative aliphatic linker with a larger number of carbons. 1,7-Heptanedial (**87**) was freshly prepared by Swern oxidation of the corresponding alcohol 1,7-heptanediol (**86**) (Scheme 40).



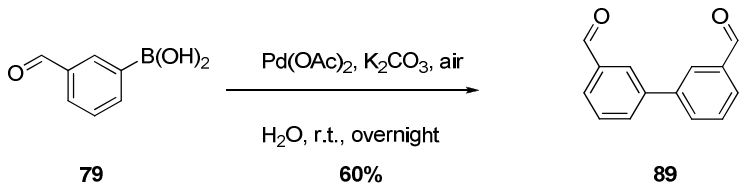
Scheme 40. Swern oxidation of 1,7-heptanediol (**86**) leading to 1,7-heptanedial (**87**).

The dialdehyde **87** was directly used in the reductive amination reaction with aminopyran **3** (Scheme 41). The desired product **88** could be identified by ^{1}H-NMR spectroscopy, but not isolated in a pure fashion: a mixture of amine **88** and aminopyran **3** was obtained. An intramolecular double reductive amination product was not found for this reaction, most probably because the formation of a resulting eight membered ring as side product is not favorable.



Scheme 41. Reductive amination using aliphatic linker **87** and formation of the product **88**.

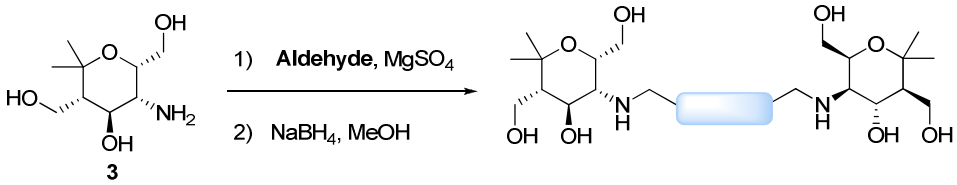
To overcome the intramolecular reductive amination the use of aromatic dialdehydes was also considered since due to their rigidity such products cannot be formed. All aldehydes were commercially available and only aldehyde **89** was synthetized according to literature procedure by the homocoupling reaction of aryl boronic acid **79** catalyzed by $Pd(OAc)_2/K_2CO_3$ in water (Scheme 42).[67]

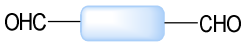
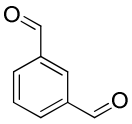
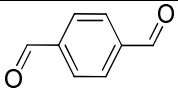
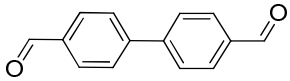
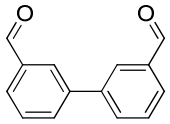


Scheme 42. Synthesis of dialdehyde **89**.

With the aromatic aldehydes the desired dimers could be synthetized (Table 10). The best yield was obtained when aldehyde **89** was used as linker (Table 10, entry 4). In contrast, with aldehyde **90** the lowest yield was received, probably due to the bulkiness of the final dimer **91** (Table 10, entry 1).

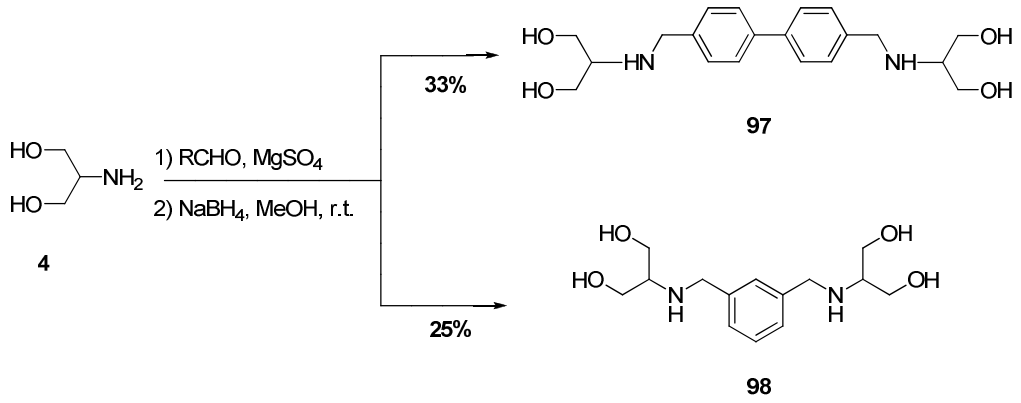
Table 10. Yields for the synthesized *N*-alkylated dimers by reductive amination.



Entry	**1**	**2**	**3**	**4**
Aldehyde 	 **90**	 **92**	 **94**	 **89**
Product	**91**	**93**	**95**	**96**
Yield (%)	32	50	43	70

4.3.3. Synthesis of divalent 2-amino-1,3-diol derivatives

The same method was applied to the simple 2-amino-1,3-diol **4** affording divalent compounds **97** and **98** in low yields (Scheme 42).



Scheme 43. Synthesis of divalent aminopolyols **97** and **98** by reductive amination.

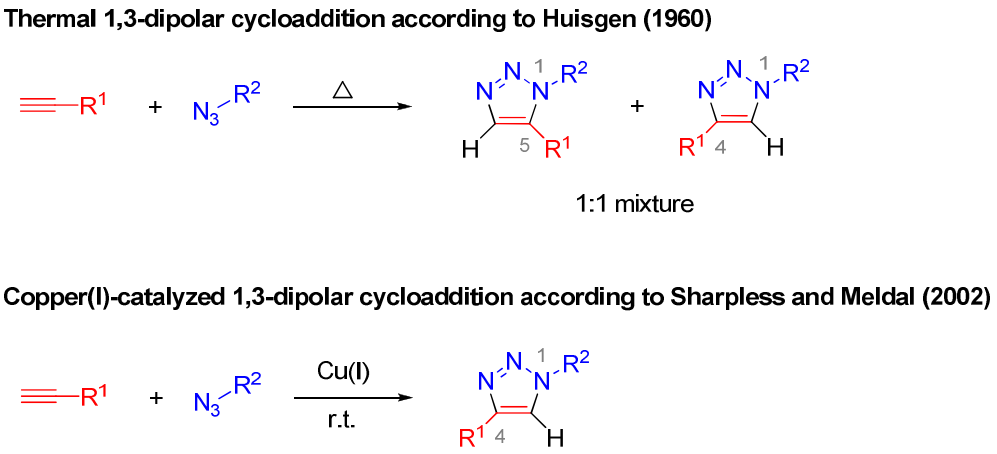
Despite the fact that a series of mono- and divalent compounds could be generated by reductive amination, in general the yields were poor to moderate and the purifications hard. An alternative to obtain better results could be the use of sodium cyanoborohydride ($NaBH_3CN$) as reducing agent, that can react selectively depending on the pH of the solution: at pH 3-4 it reduces aldehydes and ketones and at higher pH this reduction becomes slow; at pH 6-8 imines are reduced faster than aldehydes or ketones.[68] These properties make this reducing agent convenient for a direct reductive amination procedure.

4.4. Multivalent compounds by “click” chemistry

As synthetic chemists, the search and discovery of new and original reactions is a strong motive. However, chemists should also consider that to obtain a library of compounds the simplest reactions must be applied as strategy. As a promoter of this concept, Barry Sharpless introduced in 2001 the term "click" chemistry to describe thermodynamically favorable reactions that can be easily carried out in the laboratory and are able to connect two molecules efficiently in high yields.[69] According to Sharpless, to be characterized as "click" reaction a process must be fast, stereospecific (but not necessarily enantioselective) and generate harmless side products to be removed preferentially without the use of chromatography. Furthermore, a "click" reaction should be performed either without solvents or with nontoxic and inert solvents. One should also assure the use of stable and simple to obtain starting materials that can be used in a reaction that does not require any special care (the reaction should be insensitive to oxygen and water). Over the past years, the number of publications involving this term and the use of “click” reactions to generate new molecules keeps growing tremendously, and it became one of the most attractive tools to organic chemists.

4.4.1. 1,3-Dipolar cycloaddition

Among the reactions meeting “click” standards, the most attractive is the 1,3-dipolar cycloaddition, a relevant synthetic method for the construction of five membered heterocyclic systems. In 1893, Michael reported for the first time a thermal 1,3-dipolar cycloaddition of phenyl azide and diethyl acetylenedicarboxylate.[70] In the 1960s, Huisgen and coworkers investigated intensely 1,3-dipolar cycloadditions and it was found that for the variant azide-alkyne cycloaddition, due to its activation barrier, in general high temperatures were required and the reaction proceeded in a non-regioselective way leading to the formation of a mixture of 1,4- and 1,5-substituted triazoles in a 1:1 ratio (Scheme 44).[71] It was not until 2002 with a discovery made independently by Sharpless[72] and Meldal[73] that it was found that the use of a copper(I) catalyst allowed the reaction of monosubstituted alkynes to run at room temperature, and the 1,4-substituted triazoles were formed exclusively as a single regioisomer (Scheme 44). Both also recognized the importance of the combination of the copper(I) catalyst with amine-containing bases for a smooth reaction.



Scheme 44. History and progress of azide-alkyne cycloadditions.

In the literature various ways to define this type of reaction can be found: the so called "click" reaction is often mentioned as Huisgen-Sharpless-Meldal cycloaddition or copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). Due to the simple reaction conditions, easy availability of the respective starting materials as well as the excellent reaction outcome, this transformation became one of the most powerful methods with vast applications for example in the synthesis of libraries of small molecules, synthesis of natural products and for the marking of biomolecules. Besides the importance of the CuAAC, over the years 1,2,3-triazoles became relevant in medicinal chemistry since they possess for example, anti-viral and cytotoxic,[74] anti-HIV[75] and anti-cancer[76] activity. Although triazoles are not present in biological systems, they can be considered as amide bond mimics since they have similar properties with the advantage of being more stable against acidic and basic hydrolysis as well as oxidative and reductive conditions. The triazole unit is a rigid connection, with R^1 and R^2 groups at a distance of 5.0 Å (compared to a 3.8 Å distance between the same groups in an amide bond) (Figure 19).[77] 1,2,3-Triazoles have a large dipole moment (~ 5 D) that may polarize the 5-H proton which acts as hydrogen-bond donor, similar to an amide proton. Furthermore, the nitrogen atoms of positions 2 and 3 allow metal coordination and simultaneously, act as hydrogen-bond acceptors (like the carbonyl oxygen of an amide).[78]

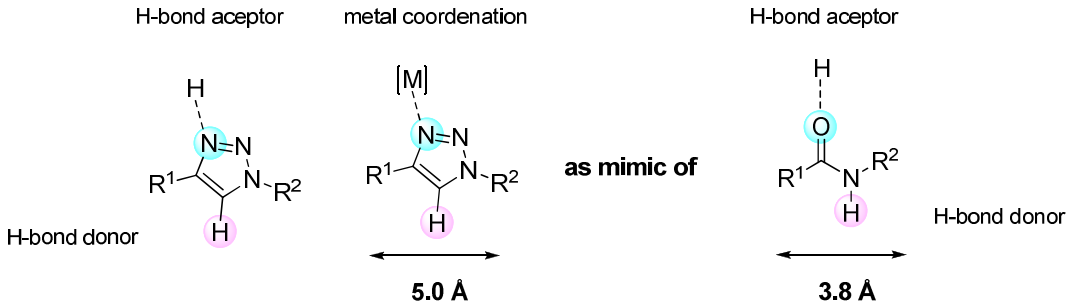
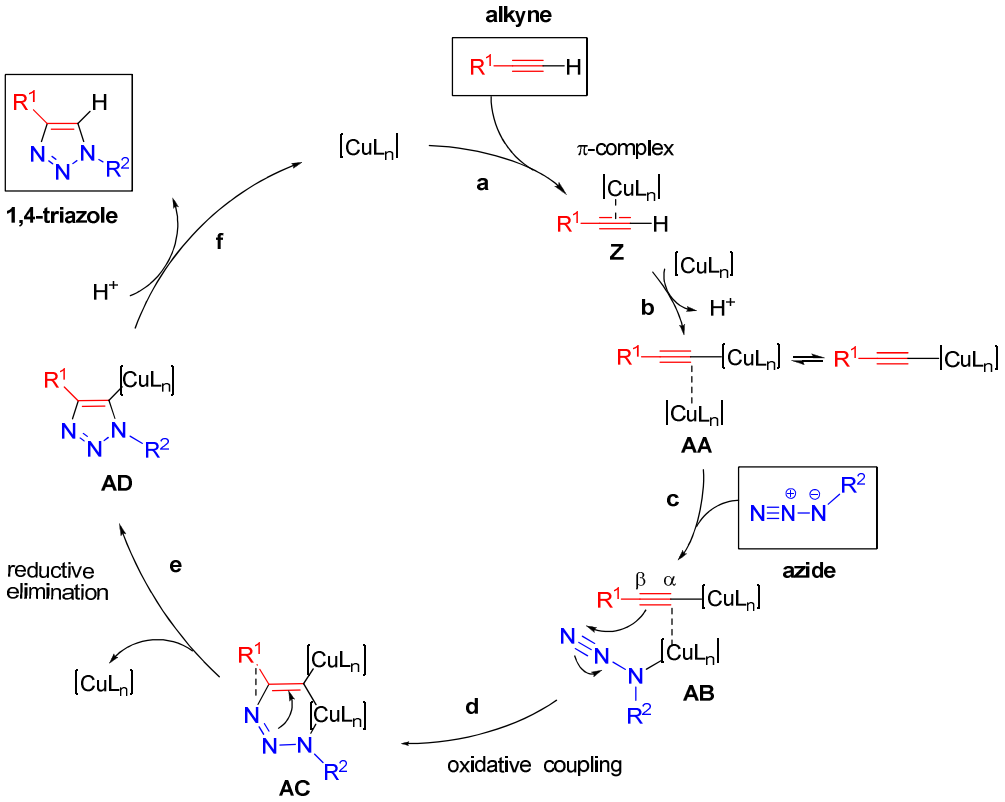


Figure 19. Comparison of 1,2,3-triazole units with amide bonds.

The mechanistic cycle for the azide-alkyne cycloaddition was proposed in 2002 by Sharpless[72] however, until today the exact mechanism and intermediates are not fully understood and several studies as well as calculations are still carried out.[78],[79] Based on the ability of copper to complex with alkynes, it is believed that the first step of this cycle consists in the π-coordination of the copper(I) catalyst to the alkyne (initially with a pKa $\approx$ 25) (Scheme 45, **a**), lowering its pKa to 15 and leading to the exothermal formation the acetylide complex **AA** (Scheme 45, **b**). Such complexes may vary depending on the reaction conditions but it has been accepted that in most of the cases a di-copper complex **AA** is involved.[80] It was suggested that a second Cu(I) species coordinates to the α-carbon of the acetylide intermediate. Subsequently, the internal nitrogen atom of the azide coordinates with the π-coordinated copper generating a N-Cu bond resulting in species **AB** (Scheme 45, **c**). Although the terminal nitrogen and internal nitrogen atoms can coordinate, it is expected that the substituted nitrogen coordinates preferentially, since it increases the electron density on the metal center facilitating by this way the oxidative coupling to intermediate **AC** (Scheme 45, **d**). This is followed by ring contraction and Cu(I) exclusion via reductive elimination leading to the formation of the Cu(I) triazolide **AD** (Scheme 45, **e**). Protonation of this intermediate forms the 1,4-substituted triazole and regenerates the copper(I) in the catalytic cycle (Scheme 45, **f**).

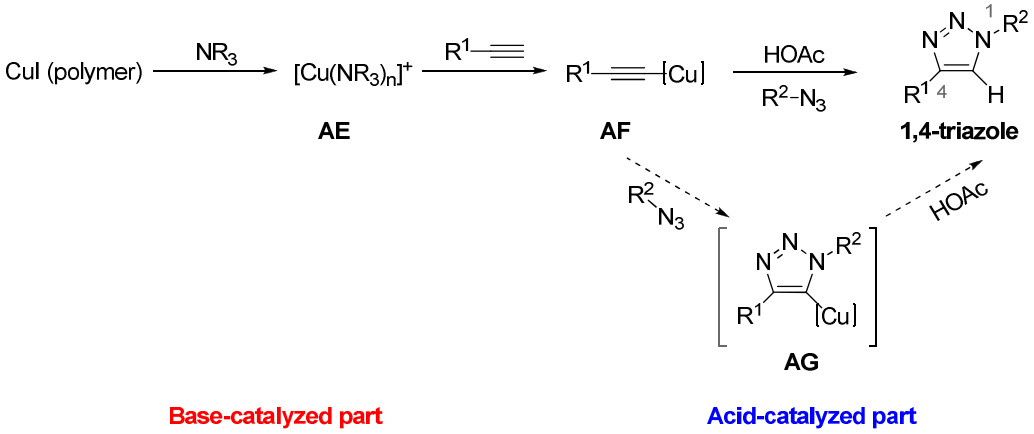


Scheme 45. Catalytic cycle for the CuAAC reaction.

As previously mentioned, to perform CuAAC Cu(I) is needed. A wide range of direct Cu(I) sources can be used, most commonly Cu(I) salts like copper(I) iodide or copper(I) bromide. Generally, the reaction with Cu(I) salts demands the use of an amine base and often heating is necessary to promote initial formation of the copper acetylide **AA**,[81] as alternative sonication has been described as a possibility to accelerate the reaction.[82] It has been found that amine-containing bases and ligands may stabilize Cu(I) species by coordination, protecting the Cu(I) from being oxidized and accelerating the reaction. From the different existing ligands,[80a] TBTA was introduced by Sharpless and is the most popular ligand used today.[72] TBTA has proved not only to be effective in catalysis, but also to be efficient in protecting Cu(I) against disproportionation. The more stable Cu(II) salts can also be used by addition of a reducing agent. The most common precursors are Cu(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) or copper(II) acetate in the presence of a weak reducing agent such as sodium ascorbate. Originally reported by Sharpless,[72] this methodology presents some important advantages: the reaction is less sensitive to atmospheric oxygen and can be performed in an open flask without the need of additives.

4.4.2. Acid/Base-catalyzed azide-alkyne cycloadditions

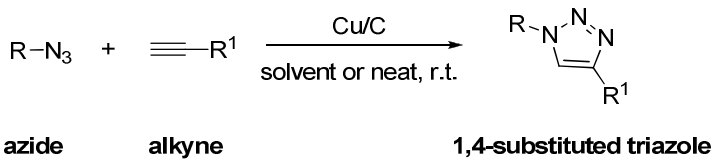
Wang et al. reported in 2011 an alternative approach to copper(I)-catalyzed azide-alkyne cycloadditions where an acid/base-promoted pathway led to a reduced reaction time and to an increase of yields.[83] It was proposed that the addition of both acid and base accelerate the reaction at different points. Firstly, the base acts as a ligand and catalyzes the copper iodide polymer dissociation, generating the active Cu(I) species **AE** that by reaction with the alkyne leads to the Cu(I) acetylide **AF** (Scheme 46, base-catalyzed part). By monitoring the reactions closely, it was noticed that the intermediate **AF** usually was formed very fast, but its conversion into the 1,4-triazole product often proceeded slowly. Trying to overcome these drawbacks, the use of an acid was applied to accelerate the conversion of the C-Cu bond-containing intermediates **AF** and **AG** (Scheme 46, acid-catalyzed part).



Scheme 46. Acid/base-catalyzed 1,3-dipolar cycloaddition.

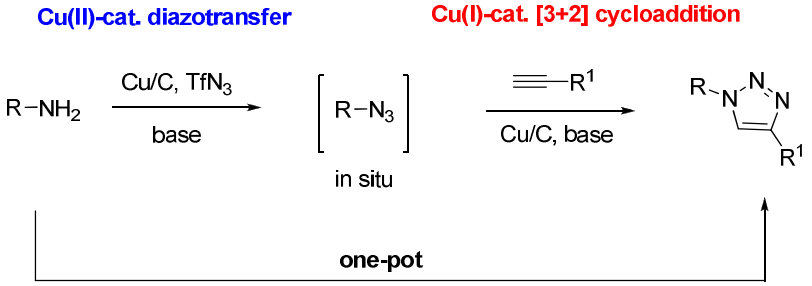
4.4.3. Copper on charcoal-catalyzed azide-alkyne cycloadditions

In 2006 Lipshutz described the use of Cu/C as an inexpensive and efficient heterogeneous catalyst for CuAAC reactions.[84] Since the Cu/C matrix contains copper in both oxidation states [Cu(II) and Cu(I)] the addition of a reducing agent is not required. Moreover, even though the presence of Et_3N proved to increase the reaction rates, it was reported that no additives like bases or ligands were necessary for an effective cycloaddition reaction that can also be performed in absence of solvent (Scheme 47).



Scheme 47. Copper on charcoal-catalyzed 1,3-dipolar cycloaddition.

An additional advantage of the method is the fact that cycloadditions carried out in the presence of Cu/C take place heterogeneously and in the matrix, avoiding this way the presence of copper in the solution, which prevents contamination of the final products with the catalyst. Moreover, the easy removal of the catalyst by simple filtration through a pad of Celite® and its reusability makes this a very attractive method. As previously mentioned in the Cu/C matrix are present copper(I) and copper(II) species. Taking advantage of this fact, Lipshutz published in 2009 a direct transformation from amines to the desired click products by generating the corresponding azide in situ in an one-pot reaction.[85] This was possible since the diazotransfer is copper(II)-catalyzed and the subsequent CuAAC is Cu(I)-catalyzed (Scheme 48).



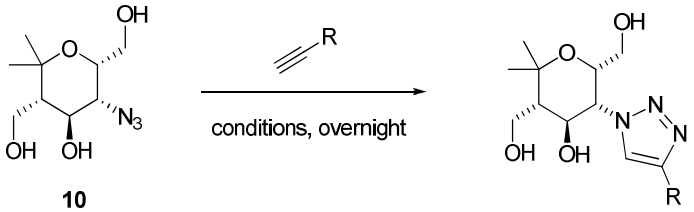
Scheme 48. Cu/C-catalyzed tandem diazotransfer and CuAAC reaction.

4.4.4. Synthesis of monovalent carbohydrate mimetics

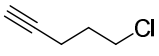
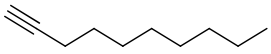
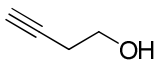
Azidopyran **10** reacted with monoalkynes **99**, **101** and **103** in copper-catalyzed azide-alkyne cycloadditions (CuAAC) (Table 11). Although it was originally reported by Lipshutz that most of the substrates reacted in excellent yields at room temperature, when his conditions were applied to azide **10** and alkyne **99**, after 24 hours at room temperature almost no product was generated and mostly starting material was present in the reaction mixture. This was also

observed by Lipshutz in some cases: with aliphatic alkynes or sterically hindered substrates the reaction rates were slower and for these cases mild heating (60 °C) was needed. Repeating the reaction at 60 °C led to excellent results and the desired product **100** was obtained in quantitative yield (Table 11, entry 1). When alkyne **101** was used a much lower yield was obtained. After filtration of the catalyst it was noticed that the desired click product **102** and azidopyran **10** have exactly the same Rf, and the desired product was isolated in a 1:2.2 mixture with starting material (Table 11, entry 2). With 2-hydroxyethylacetylene **103** and azidopyran **10** different conditions were applied; besides the Cu/C procedure (Conditions a) the acid/base-catalyzed method (Conditions b) as well as the common CuI and TBTA CuAAC (Conditions c) were performed. The reaction proceeded smoothly under the three different conditions and with conditions a) and c) the excellent yields were obtained even though with CuI and TBTA the purification is not as straightforward due to the presence of the ligand. Concerning the acid/base-catalyzed method the desired product **104** was obtained with a lower but still good yield; since extraction is not possible in this case due to the polarity of product **104**, the separation from the acid and base afforded **104** in 67%. Besides the slight difference in the final yield, the three methods proved to be efficient for the synthesis of triazole **104**.

Table 11. Synthesis of **100**, **102** and **104** by CuAAC.

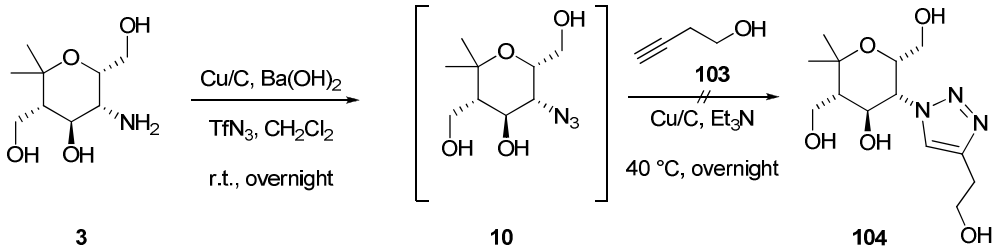


10

Entry	Conditions	Alkyne	Product	Yield (%)
1	a)	 **99**	**100**	quant.
2	a)	 **101**	**102**	33 (yield calculated according to ^{1}H-NMR spectrum)
3	a), b), c)	 **103**	**104**	a) 90 b) 67 c) 90

Conditions: a) Cu/C, Et_3N, Dioxane, 60 °C; b) CuI, DIPEA, HOAc, Toluene, r.t.; c) CuI, TBTA, Et_3N, MeCN, r.t.

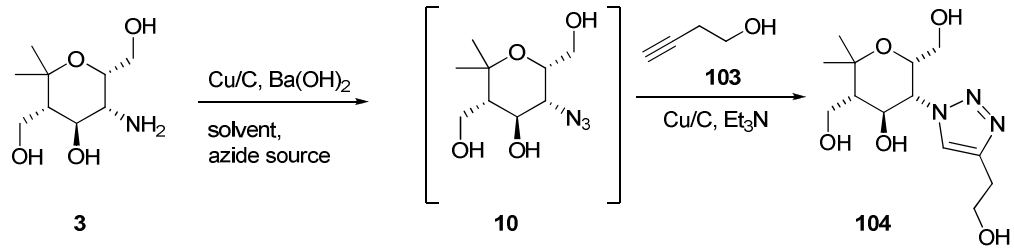
It was of great interest to apply the one-pot conditions reported by Lipshutz[85] to the synthesis of carbohydrate mimetics. As previously mentioned, under these conditions it should be possible to start from aminopyran **3** and after in situ generation the azide **10**, a chosen alkyne can be added and the desired click product should obtained in a one-pot reaction. After the problems faced with the polarity of compound **102** and azidopyran **10**, it was assured that the alkyne used in the one pot procedure would lead to a final product with a considerable difference in polarity from azidopyran **10**, therefore alkyne **103** was chosen. The first attempt to the one-pot CuAAC was performed using the conditions described by Lipshutz (Scheme 49): dichloromethane was used as solvent and TfN_3 as azide source. After formation of azidopyran **10** (controlled by TLC) alkyne **103** was added and the reaction was stirred for 31 hours. Disappointingly, click product **104** was not formed (even after addition of more Cu/C, base and alkyne **103**) and only aminopyran **3** and azidopyran **10** could be isolated.



Scheme 49. Attempt to synthetize click product **104** in a one pot procedure using Cu/C.

Trying to adapt the original procedure it was decided to use a different solvent that would allow heating of the mixture to a higher temperature, therefore acetonitrile was chosen (Table 12, entry 1). With these conditions the reaction was completed after 28 hours and the desired product **104** was obtained with 74% yield. Lipshutz also reported an acceleration of the CuAAC step using microwave radiation (MW) at 120°C; performing the reaction with MW in acetonitrile at 75 °C afforded the desired product **104** with no great improvement in the yield but the reaction time was reduced to 15 hours (Table 12, entry 2). With a working one-pot procedure, the change of azide source was considered to the less explosive and more convenient NfN_3 often used in the Reißig group. Once again the desired product **104** was obtained with a comparable yield (Table 12, entry 3).

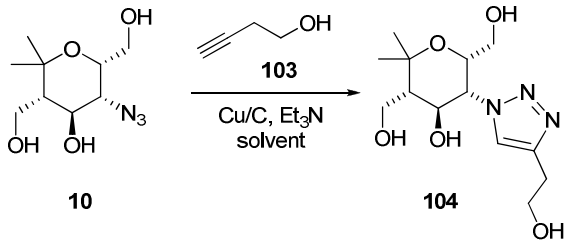
Table 12. Optimization of the conditions for the one-pot synthesis of **104** by CuAAC using Cu/C.



Entry	Solvent	Azide source	Temperature (°C)	Time (h)	MW	Yield (%)
1	MeCN	TfN_3	60	28	-	74
2	MeCN	TfN_3	75	15	300 W	76
3	MeCN	NfN_3	60	66	-	75

Even though all one-pot attempts in acetonitrile led to the formation of the desired product it was quite surprising that in dichlorometane triazole **104** was not formed. To provide more insight into the solvent influence, two additional reactions were performed starting from azidopyran **10** and alkyne **103** in dichloromethane and acetonitrile (Table 13). The different reactions led to the formation of the desired product **104** showing that even though azidopyran **10** did not dissolve completely in dichloromethane or acetonitrile, the [3+2] cycloaddition proceeded in both cases even at 40 °C (Table 13, entry 1) .

Table 13. Synthesis of **100** by CuAAC using Cu/C.



Entry	Solvent	Temperature (°C)	Time (h)	Yield (%)
1	CH_2Cl_2	40	20	61
2	MeCN	60	41	39

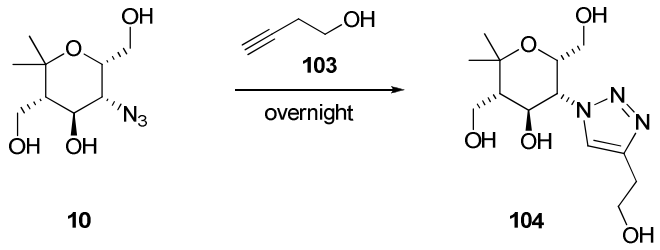
Since in all performed one-pot reactions the formation of the azidopyran **10** occur (controlled by TLC), one can assume that the solubility of aminopyran **3** does not have an impact on the reaction outcome. The reason for the failure of the one-pot procedure performed in dichloromethane is not clear; the only difference observed in this one-pot attempt was that not all aminopyran **3** was converted into azidopyran **10** (in all other attempts alkyne **103** was only added after full conversion), so in this case when alkyne **103** was added there was still aminopyran **3** present. The influence of aminopyran **3** in the reaction mixture during the CuAAC step is unknown, but it can be speculated that it influences the [3+2] cycloaddition possibly by coordination to the catalyst.

4.4.4.1. Evaluation of trace metal content by ICP-MS

Copper is an essential element in living organisms and it is involved in several metabolic events. Over the years cooper-binding proteins and their importance have been widely studied.[86] Despite this fact, there are still discussions and uncertainties around the toxicity of copper-containing compounds tested in certain cells or biological systems. Copper can be considered toxic since it can easily change its oxidation state and it is able to catalyze the formation of highly reactive hydroxyl radicals.[87]

Since the goal of this project was to generate carbohydrate mimetics to be tested in biological assays (SPR) one should assure that there is no copper present in the generated products from CuAAC. As previously reported in Table 11, using azidopyran **10** and alkyne **103**, the CuAAC reaction was performed using different conditions to generate triazole **104**. At this point the goal is not to compare or discuss the advantages and disadvantages of each method nor the efficacy or yield, but solely to compare the percentage of copper present in the final products obtained. The three samples of **104** were submitted to quantitative copper ICP-MS analyses and satisfactorily, the copper concentrations observed for all methods were too low for an accurate and representative detection with this type of measurement (in ICP-MS analysis amounts below 15 ppb are considered insignificant and not sufficient for an accurate measurement) (Table 14).

Table 14. CuAAC reaction leading to carbohydrate mimetic **100** using different methods and evaluation of the respective copper content in the product.



10 **104**

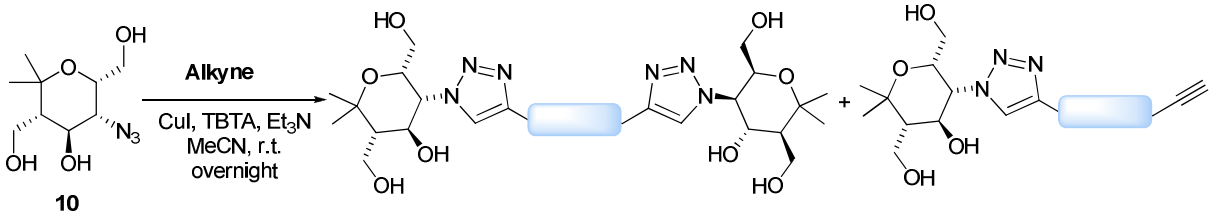
Entry	Conditions	Cu determination by ICP-MS (ppb)
1	CuI, TBTA, Et_3N, MeCN	^{63}Cu – 4.99 ^{65}Cu – 5.19
2	CuI, DIPEA, HOAc, Toluene	^{63}Cu – 7.22 ^{65}Cu – 7.54
3	Cu/C, Et_3N, Dioxane	^{63}Cu – 4.12 ^{65}Cu – 4.41

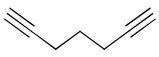
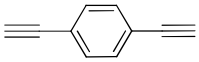
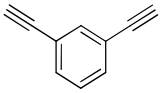
With these results one can assume that all three methods are adequate to generate the desired multivalent carbohydrate mimetics without measurable Cu contamination of the final products.

4.4.5. Synthesis of divalent carbohydrate mimetics

The first attempts for the synthesis of divalent structures were performed before the Cu/C procedure was considered as a good alternative, therefore the conditions previously used in the Reißig group (with CuI and TBTA as additive) were applied.[29a],[35] Building block **10** reacted with three different linkers, 1,6-heptadiyne (**105**), 1,4-diethynylbenzene (**108**) and 1,3-diethynylbenzene (**110**) (Table 15). In these reactions a slight excess of azide **10** was used (2.2 equivalents). Although all desired dimers were obtained, in some cases the corresponding monoadduct was also isolated (Table 15, entry 1 and entry 3), proving that these conditions were not adequate for the full transformation to the desired dimers even with an excess of azide **10**.

Table 15. Synthesis of divalent carbohydrate mimetics by CuAAC.

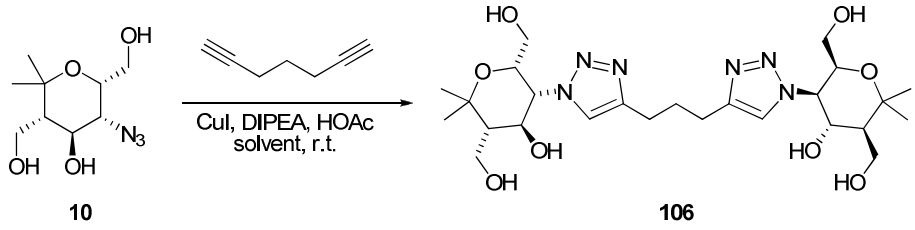


Entry	Alkyne	Disubstituted product	Monosubstituted product	Yield (%)
1	 **105**	**106**	**107**	(**106**) 61 (**107**) 8
2	 **108**	**109**	-	(**109**) 64
3	 **110**	**111**	**112**	(**111**) 53 (**112**) 4

The acid/base-catalyzed CuAAC method[83] was then used in an attempt to obtain better yields and full conversions. Aliphatic linker **105** was chosen and the results are summarized in Table 16. Using toluene as solvent and a mixture of DIPEA/HOAc led to an improvement of the yield and a much faster reaction (Table 16, entry 1). It was also described that under these

conditions the reactions can be carried out neat; without the use of any solvent the desired dimer **106** was obtained in quantitative yield (in the final ^{1}H-NMR the peaks corresponding to DIPEA could be detected). These conditions proved to be more efficient and since the use of TBTA as additive is not necessary, it also makes the purification easier; it was previously observed that the separation of the desired products from TBTA can sometimes be tedious.[35] From the experiments performed, it is important to note that with this procedure the equivalents of acid and base can be increased but an exact 1:1 ratio of acid/base is needed for the good efficacy of the reaction.

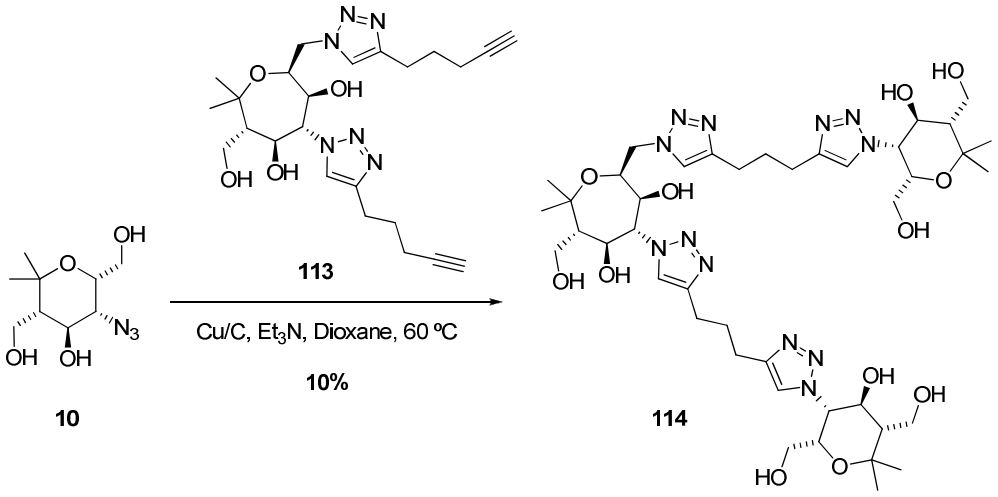
Table 16. Synthesis of dimer **113** by acid/base-catalyzed CuAAC.



10 **106**

Entry	Solvent	DIPEA (equiv.)	HOAc (equiv.)	Time (h)	Yield (%)
1	Toluene	4.0	4.0	3	71
2	-	8.0	8.0	3.5	quant.

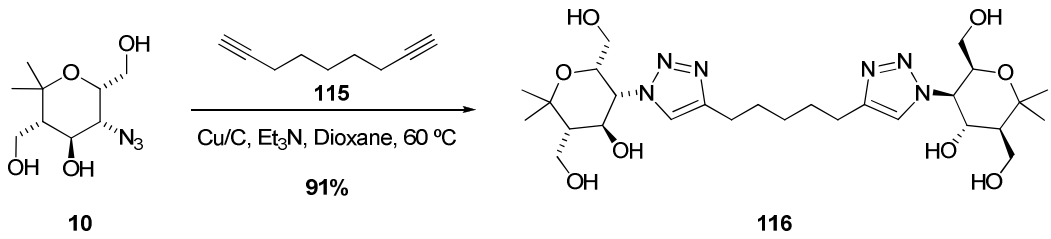
Due to good results obtained from Cu/C catalyzed CuAAC[80a] as well as easy purification of the obtained compounds, some divalent structures were also synthetized using this method. The interesting compound **114** was obtained in 10% from reaction of azide **10** and the oxepane dialkyne derivative **113** synthetized by Bouché[35] (Scheme 50). The oxepane unit made the final compound extremely polar resulting in a hard purification and a low yield.



Scheme 50. Synthesis of unsymmetrical dimer **114** using Cu/C as catalyst in CuAAC.

This unsymmetrical dimeric structure is an interesting compound to be tested in the SPR assays and since both six and seven membered carbohydrate mimetics are present it would be of interest to see if it would lead to an increase of affinity compared to the other synthetized dimers.

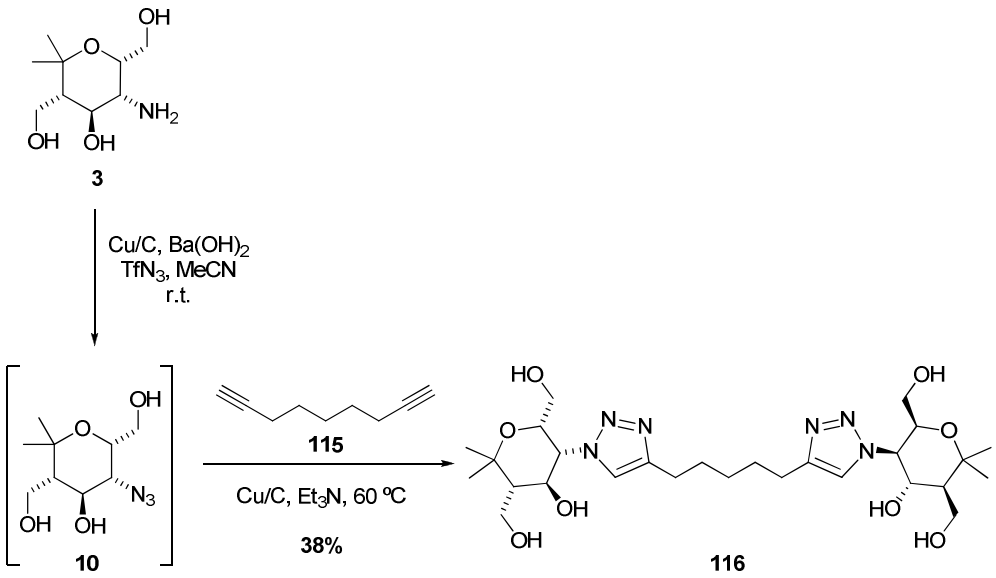
Additionally, with the commercially available alkyne **115** and azidopyran **10**, the corresponding dimer **116** was obtained in excellent yield (Scheme 51).



Scheme 51. Synthesis of dimer **116** using Cu/C as catalyst in CuAAC.

After the success of the one-pot procedure for the synthesis of monovalent compounds, it was of interest to apply these conditions to obtain multivalent compounds starting from amine **3**. The Cu/C catalyzed one-pot click reaction was performed for the synthesis of divalent carbohydrate mimetic **116**. Aminopyran **3** reacted with TfN_3 leading to the formation of azidopyran **10** in situ which was subsequently reacted with 1,8-nonadiyne (**115**) affording the desired divalent click product **116** (Scheme 52). Satisfactorily, this procedure proved to be convenient for the synthesis of multivalent structures. After filtration over Celite® the crude

product was obtained with 95% yield, but due to complications during the separation from the excess of azide **10**, the final product **116** was isolated with a much lower yield.



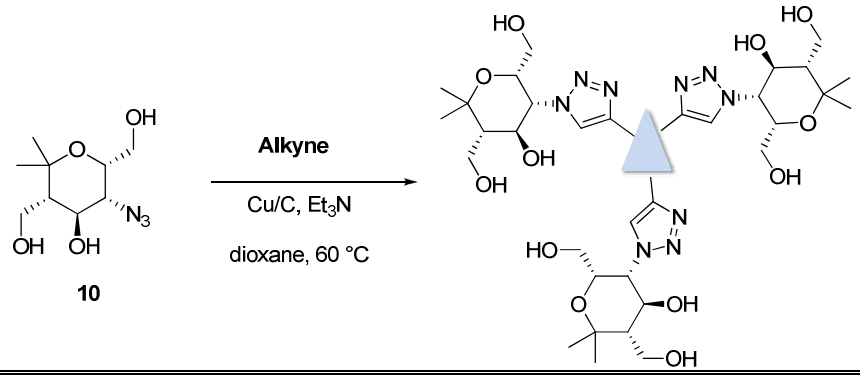
Scheme 52. One-pot synthesis of dimer **116** by CuAAc using Cu/C.

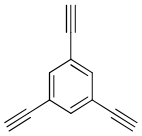
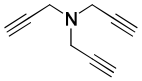
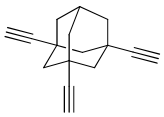
Beckmann and Wittmann also reported a one-pot procedure for the diazotransfer and azide-alkyne cycloaddition.[88] In this case, starting from an amine a Cu(II) species was used for the diazotransfer reaction that after completion was in situ reduced to Cu(I) by adding a reducing agent to perform the azide-alkyne cycloaddition. Although they were able to synthesize multivalent glycoconjugates with this one-pot procedure, it has some drawbacks: the use of MW radiation is necessary as well as the use of TBTA making the isolation of the desired product unconvenient. The Cu/C protocol seems to be a more convenient and advantageous procedure and as far as one can tell from the existing literature up to date, the synthesis of dimer **116** is the first described one pot synthesis of a multivalent compound using Cu/C as catalyst.

4.4.6. Synthesis of trivalent carbohydrate mimetics

Using the Cu/C procedure, trivalent compounds were successfully synthetized (Table 17). It was noticed that in some cases like with alkyne **117** (Table 17, entry 1) the reaction was slow and not complete even after one day. In this case the same amount of Cu/C and Et_3N was additionally added and the reaction was further stirred. The C_3-symmetric compound **118** was obtained with a good yield of 69%. The importance of C_3-symmetry in biological systems has been vastly discussed[89] and since some binding sides of enzymes have a pseudo C_3-symmetric geometry, synthetic molecules with this arrangement can have enhanced affinity. The use of such compounds as hosts for carbohydrate recognition has been previously reported.[90] A similar yield was obtained with alkyne 119 (Table 17, entry 2) that after a reaction time of 21 hours afforded trimer **120** in 60% yield. The interesting adamantanyl trisalkyne derivative **121** was also used as linker to afford a trivalent carbohydrate mimetic **122** (Table 17, entry 3). Adamantane consists of four fused cyclohexane rings in chair conformation and it is the most simple diamondoid compound commonly used in host-guest chemistry and in drug delivery systems.[91] Interesting functionalizations and adamantane derivatives tri- and tetrasubstituted have been investigated by Maison and coworkers.[92] Dondoni and Marra also used adamantane as scaffold for the synthesis of glycoclusters.[93] In the synthesis of the C_3-symmetric compound **122** low yields were received, most probably due to its rigidity and steric bulkiness (it was noticed in this reaction that there was not a full consumption of the starting materials). After obtaining **122** in 16% yield with the Cu/C procedure, the acid/base-catalyzed conditions were applied and **122** was obtained in 29% yield (79% based on consumed starting material).

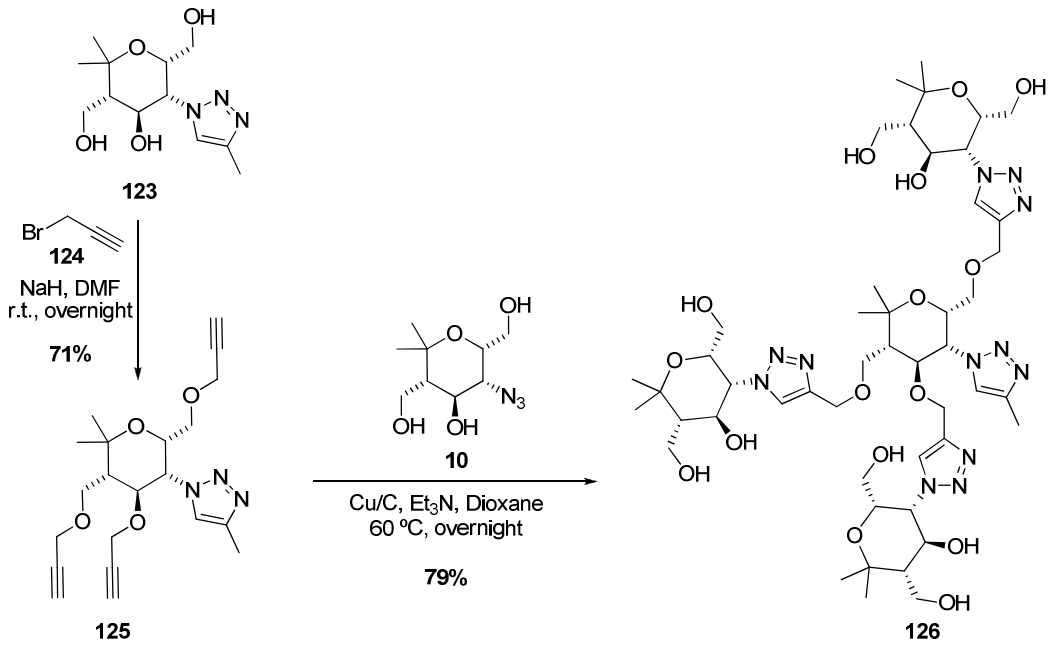
Table 17. Synthesis of trivalent carbohydrate mimetics by CuAAC with Cu/C.



Entry	Alkyne	Time (h)	Product	Yield (%)
1	 **117**	48	**118**	69
2	 **119**	21	**120**	60
3	 **121**	16	**122**	16 Method B: 29 (79% based on consumed **121**)

Method B: CuI, DIPEA, HOAc, Toluene, r.t.

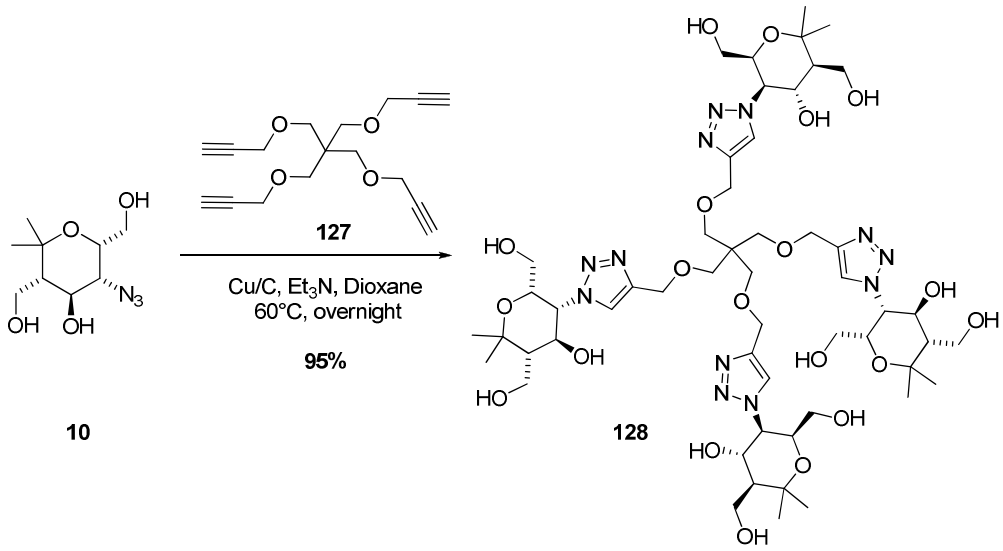
For the synthesis of an unsymmetrical trivalent carbohydrate mimetic **126**, monomer **123** (the synthesis of this derivative is described in section 4.4.9) was first propargylated using standard conditions. The resulting product **125** was subsequently used as trivalent linker in CuAAC with **10** and Cu/C affording the trivalent carbohydrate mimetic **126** in very good yield (Scheme 53).



Scheme 53. Synthesis of unsymmetrical trimer **126** using Cu/C as catalyst in CuAAC.

4.4.7. Synthesis of tetravalent carbohydrate mimetics

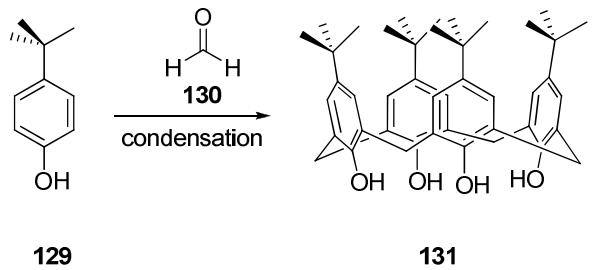
Tetravalent linker **127** was used for the successful construction of carbohydrate mimetic derivative **128** (Scheme 54). Bouché attempted the synthesis of such tetravalent compounds using the azidooxepan derivative and the standard conditions for the CuAAC with CuI and TBTA as additive, but the desired tetravalent carbohydrate mimetic was not obtained.[40] Successfully applying the Cu/C method the desired product **128** was obtained and isolated in excellent yield. This linker has been used by Bernardi et al. for the construction of fucose-based glycomimetics which presented activity against DC-SIGN, a C-type lectin involved in the infection process and HIV virus.[94] Tetravalent carbohydrate mimetic **128** was tested in the SPR inhibition assay as selectin inhibitor and it will be discussed further in section 4.6.4.



Scheme 54. Synthesis of tetravalent carbohydrate mimetic **128** using Cu/C as catalyst in CuAAC.

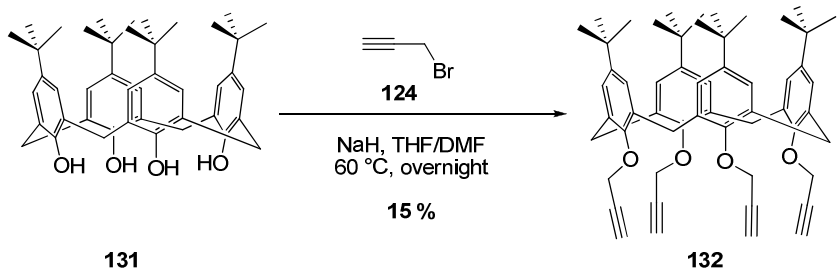
4.4.8. Synthesis of bigger architectures

Calix[*n*]arenes are often used as scaffolds for the synthesis of multivalent ligands.[95] Since these oligomers allow the arrangement of multiple ligands in a specific spatial orientation, they can lead to an optimization of events of recognition and an enhanced binding to certain receptors. Moreover their cavity can sometimes encapsulate guest species. The commercially available *tert*-butylcalix[4]arene **131** was chosen as scaffold for multivalent presentation of azidopyran **10**. This macromolecule can be synthetized in a single step by a condensation reaction between 4-*tert*-butylphenol (**129**) and formaldehyde **130** (Scheme 55),[96] and can be further functionalized at the phenolic hydroxyl groups.



Scheme 55. Synthesis of calix[4]arene **131**.

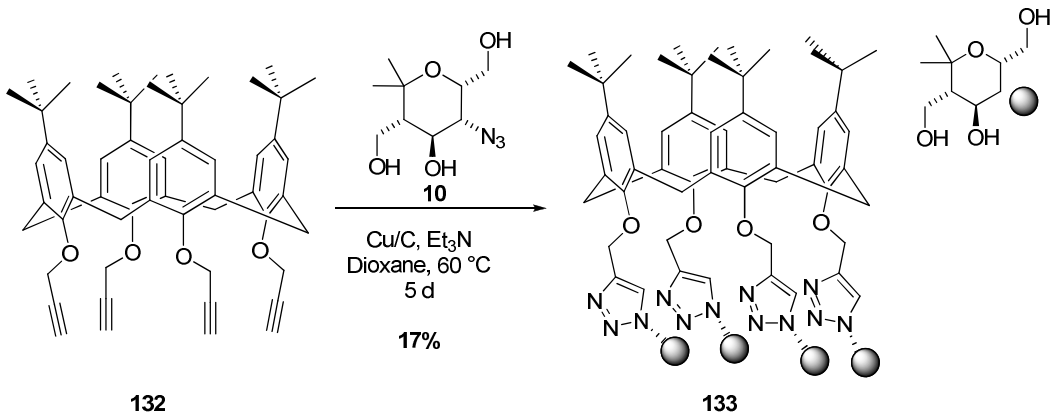
In the literature several procedures are described for the transformation of *tert*-butylcalix[4]arene **131** into the corresponding propargylated compound **132**. Applying known conditions with K_2CO_3 as a base in acetonitrile[97] led to a mixture of products and the desired tetraalkyne **132** could not be isolated. Alternatively, following the conditions applied by Ryo and Zhao[98] using NaH as base in a THF/DMF mixture, compound **132** was successfully obtained however with a much lower yield than the reported (Lit.[98] 65%).



131 132

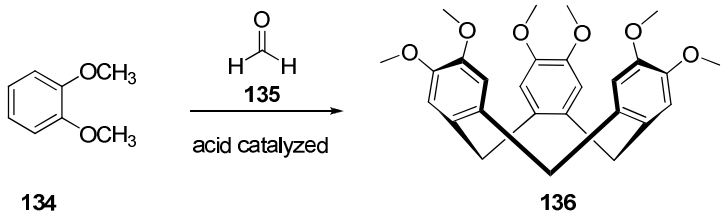
Scheme 56. Synthesis of propargylated calix[4]arene **132**.

Reacting calix[4]arene derivative **132** and azidopyran **10** in CuAAC using Cu/C as catalyst lead to formation of the desired product **133** but the same could not be isolated in pure form (Scheme 57). It was noticed that the reaction was slow even at 60 °C and the formation of several products was observed by TLC control, making the purification of the desired product **132** quite challenging. After purification by flash column chromatography several fractions with mixtures of products were obtained. The tetrasubstituted product **133** was detected in more than one fraction by ESI measurements. The yield of the reaction was calculated by ^{1}H-NMR from the fraction containing the bigger amount of desired product **133** corresponding to 17%. Similar difficulties were previously reported by Ryo and Zhao[98] that obtained a complex mixture when reacting **132** with a simple azide. Although it was never observed before in any of the CuAAC performed at 60 °C, the slightly higher temperature could promote in this case the homocoupling of the alkynes leading to the formation of side products.[73]



Scheme 57. Synthesis of tetramer **133** using Cu/C as catalyst in CuAAC.

The synthesis of cyclotriveratrylene **136** (CTV), a cyclic molecular host, was first reported in 1915 by Robinson[99] as a result of the condensation of veratrole **134** with formaldehyde **135** (Scheme 58), but it was not until 1963 that the exact structure was determined.[100]



Scheme 58. Synthesis of cyclotriveratrylene **136**.

CTV **136** is related to calix[*n*]arenes but unlike calix[*n*]arenes that have been widely used, CTV **136** did not achieve the same vast applications. Only in the last years this building block has attracted some attention and it has been used for example as fullerene host[101] or as scaffold for the attachment of biomolecules.[102] CTV is a cyclic trimer that occurs mostly in a bowl-shaped conformation and its molecular cavity allows it to serve as a host. Although two conformations are possible for CTV **136**, steric reasons favor the "crown" conformation (Figure 20, **136**). The molecular structure is pyramidal with the aromatic rings forming three sides of the pyramid.[100] Although possible, the saddle conformation (Figure 20, **136a**) is less stable and NMR studies support the "crown" conformation (NMR in chloroform); even when heated to 200 °C, no inversion of conformation is observed.[103]

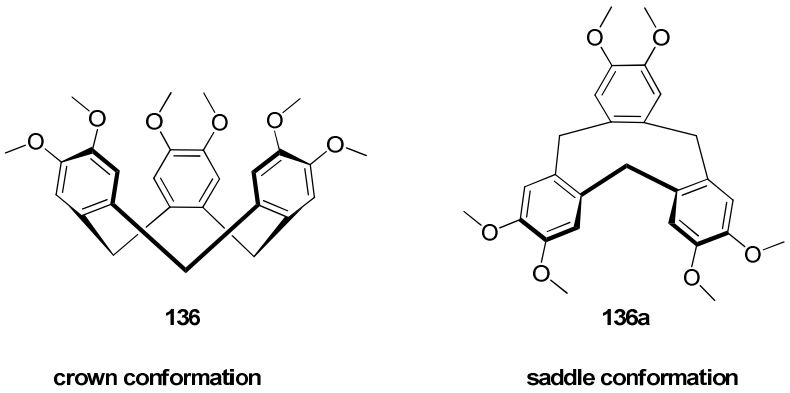


Figure 20. Possible conformations of cyclotriveratrylene **136**.

The crown conformation was confirmed by several X-ray structures [104] and it is defined by the angle $\Phi = 47\pm2°$ between the plane of each benzene ring and the C_3 axis, and by the distance d = 4.79 Å between their centers (Figure 21).

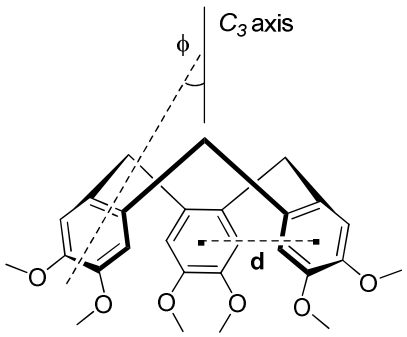


Figure 21. Geometry of cyclotriveratrylene **136**.

CTV **136** was synthetized according to the literature procedure[100] and the stable crown conformation can be confirmed by the two characteristic doublets of the methylene bridges H_a and H_e (Figure 22). As described in the literature, the pseudo axial hydrogens from the methylene bridges (H_a) are separated by 2 Å, appearing 1.2 ppm downfield with respect to the pseudo equatorial counterpart (H_e).[104c, 105]

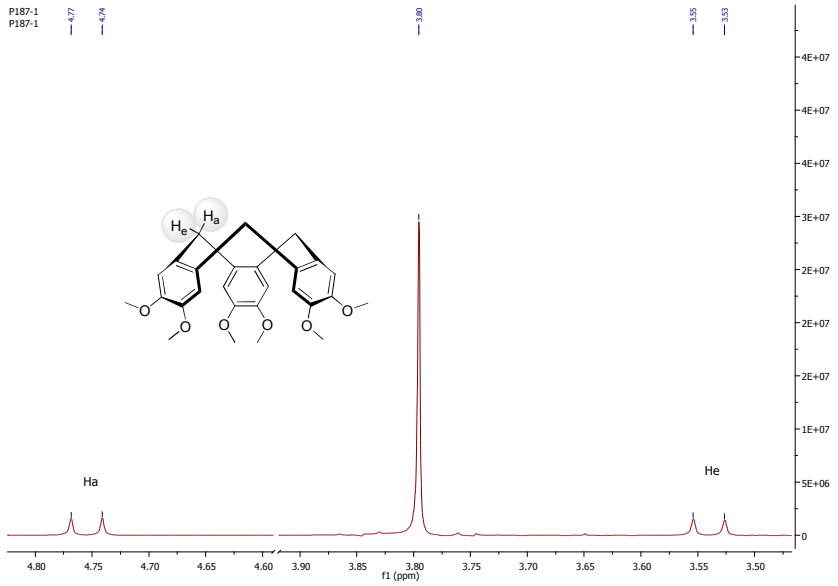
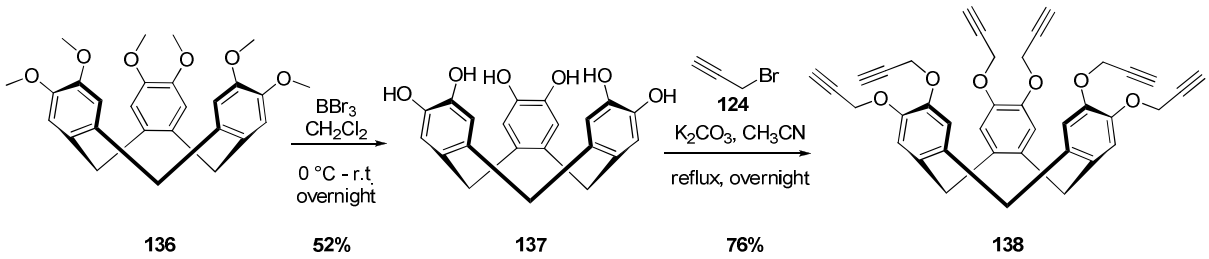


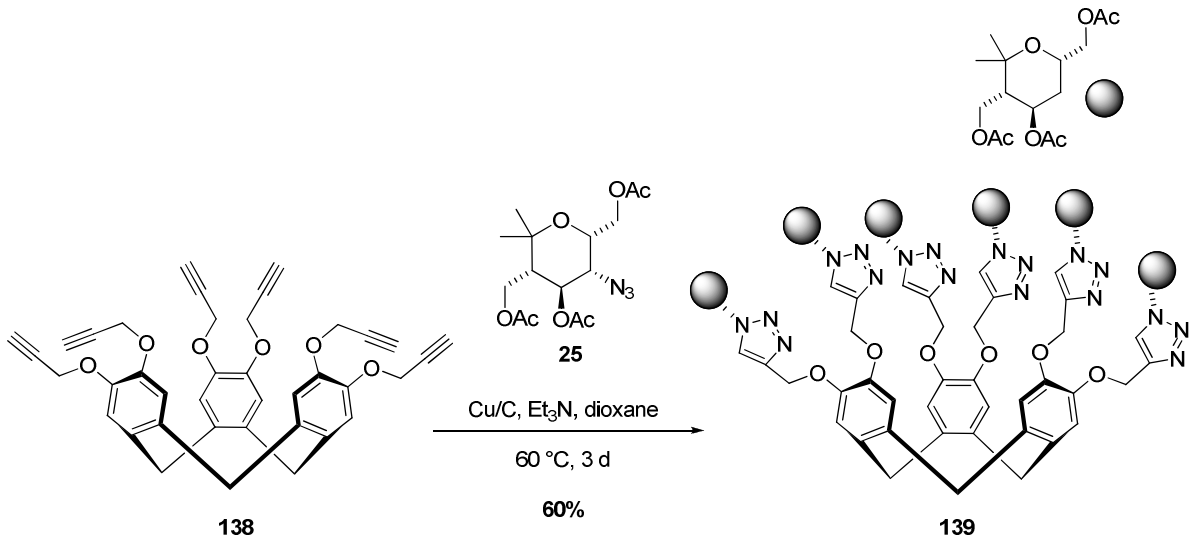
Figure 22. ^{1}H-NMR detail of CTV **136**.

CTV **136** was demethylated as previously described[106] and compound **137** could be obtained in good yield (Scheme 59). The six phenolic hydroxyl groups were then propargylated using propargyl bromide under basic conditions[101b] affording **138** in good yield.



Scheme 59. Synthetic route to CTV derivative **138**.

With the obtained compound **138**, a CuAAC was performed to functionalize this CTV derivative with six carbohydrate mimetic units. Acetyl protected azidopyran **25** was chosen for the first attempt and acid/base-catalyzed CuAAC method was applied. Although under these conditions the desired product **139** could be generated (confirmed by ^{1}H-NMR and mass spectroscopy), its isolation in pure form was not trivial. To overcome these difficulties the Cu/C method was applied since it had demonstrated before to be a cleaner method. With Cu/C as catalyst in dioxane, the desired hexavalent **139** was obtained in good yield (Scheme 60).



Scheme 60. Synthesis of hexavalent **139** by CuAAC with Cu/C as catalyst.

When analyzing the ^{1}H-NMR spectra of hexamer **139**, a clear splitting in some signals was observed, namely the signal corresponding to the aromatic protons, the signal of the 1,4-triazole 5'-H (Figure 23) and the methyl groups corresponding to the pyran unit. Each splitting resulted in two identical singlets with the exact same intensity.

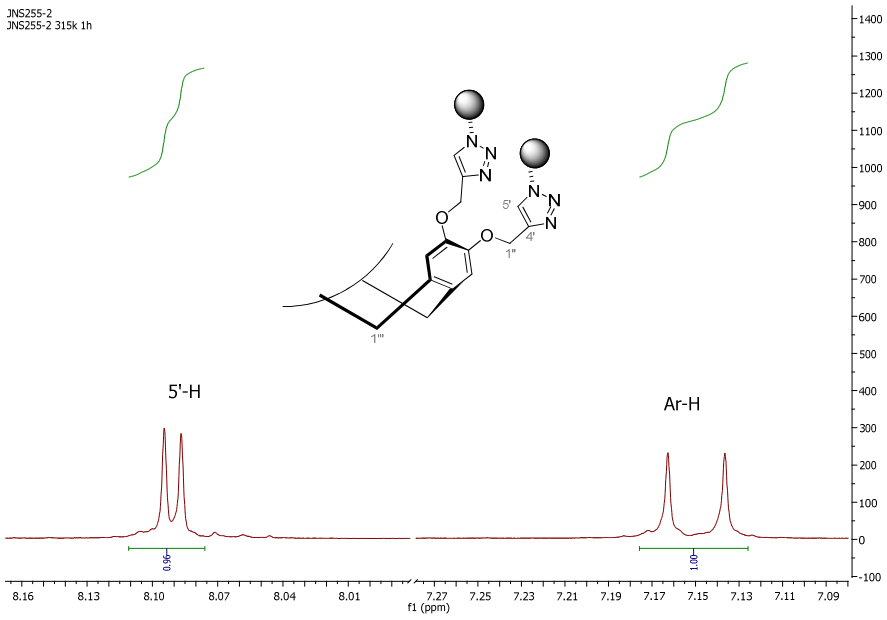


Figure 23. Excerpt of the ^{1}H-NMR spectrum of CTV derivative **139**.

This was quite surprising since the molecule should be highly symmetric and this kind of splitting was never observed before in all symmetric multivalent carbohydrate mimetics presented. First it was questioned if the right structure was really obtained. The correct structure of **139** was confirmed by mass spectrometry as well as by NMR experiments. It was thought that possibly due to the high substitution pattern and due to the bulkiness of the molecule a saddle conformation would be preferred. This hypothesis had to be discarded since in the ^{1}H-NMR no alteration was observed for the multiplicity or shifting of the methylene bridges H_a and H_e (in this case H_a is ≈ 1.1 ppm downfield from H_e compared to the ≈ 1.2 ppm in the unsubstituted CTV **136**) (Figure 24).

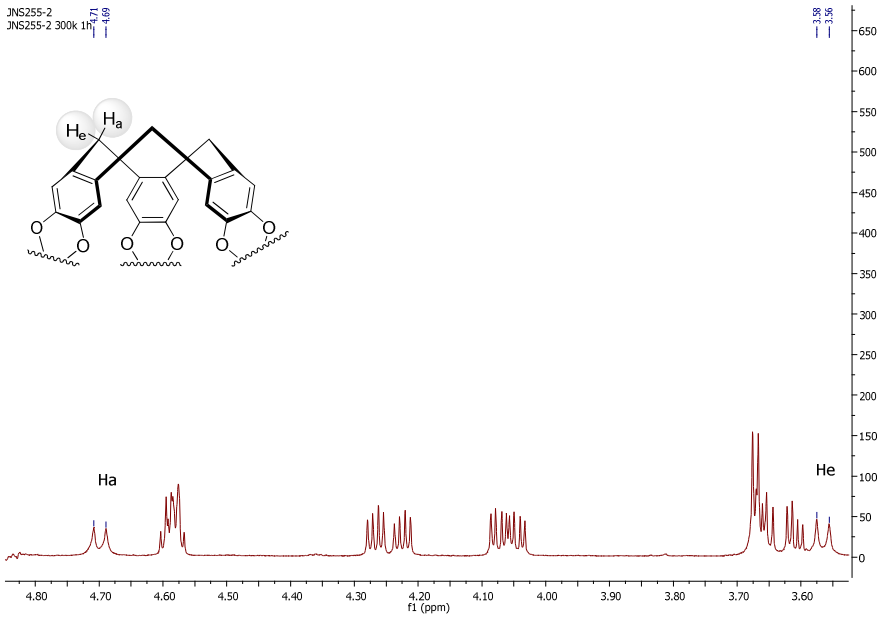


Figure 24. ^{1}H-NMR detail of **139**.

This was also an indication that the difference in the structure was in each aromatic ring unit and since the splitting was in a 1:1 ratio the pattern in each unit was repeated and the three units were similar in between themselves. Quite clear is that in the CTV derivative **139** a C_3-symmetry is retained. With Nuclear Overhauser Effect (NOE) experiments and irradiating an aromatic proton, it was possible to determine which protons are in the spatial proximity (Figure 25): the methylene bridge protons H_a and H_e, the 1’’-protons as well as the triazole proton 5’-H.

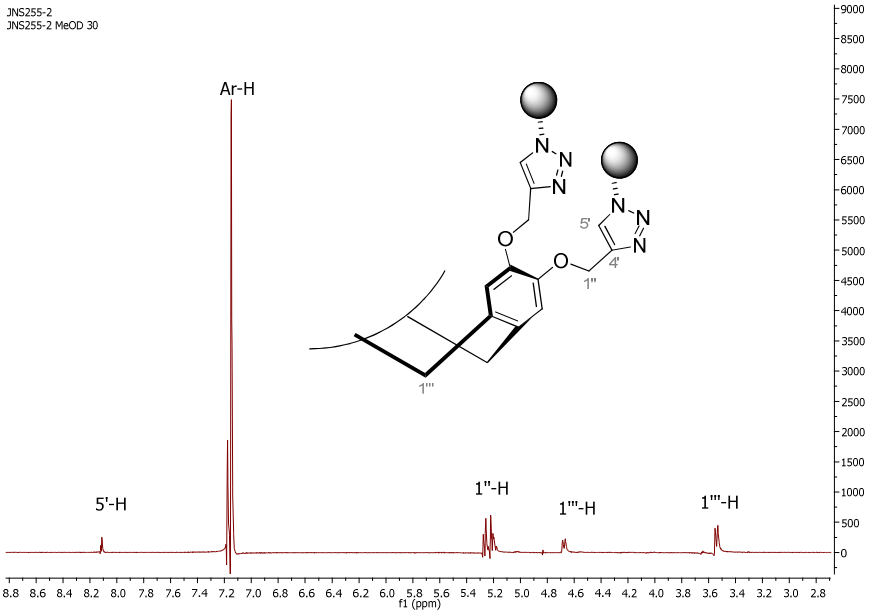


Figure 25. NOESY spectrum of CTV derivative **139**.

Without an X-ray structure one can only speculate about the possible conformation of **139**. Looking closer to the possible conformations in the aromatic rings one can see that the triazole protons can point “outside” (Figure 26, **a**) or “inside” (Figure 26, **b**). In any of these cases, if the orientation is repeated in the three aromatic units, no splitting of the signals should be observed. It can be assumed that the only possible orientation that fits to the observed NMR signals is the alternate orientation: in each aromatic ring a triazole proton points to the “inside” and the other to the “outside” (Figure 26, **c**).

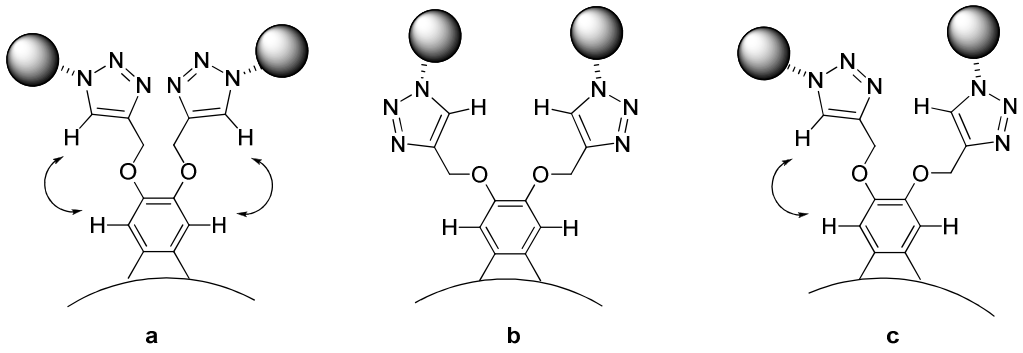
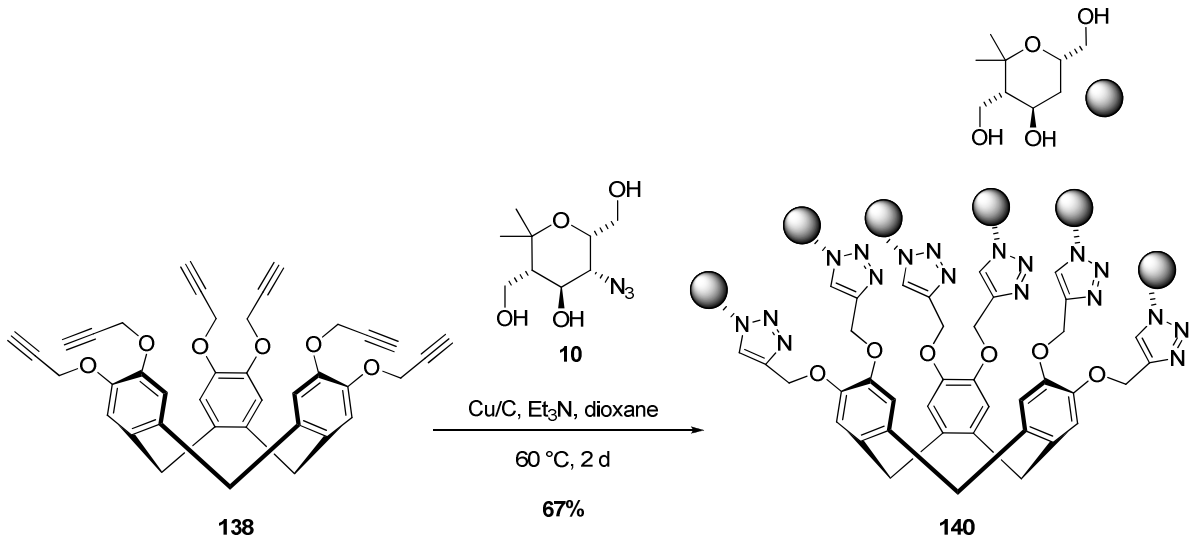


Figure 26. Simplified representation of the possible conformations of CTV derivative **139**.

There were no reports found in the literature for this kind of splitting in functionalized CTV units. In 2011 Han et al. published hexavalent CTV bearing six sugar derivatives with either acetyl protected or with free hydroxyl groups (obtained by CuAAC) but no considerations of the splitting of the signals was discussed.[101b]

Starting from **138** and unprotected azidopyran **10**, the copper(I)-catalyzed azide-alkyne cycloaddition using Cu/C as catalyst was also used in the synthesis of hexavalent **140** bearing carbohydrate mimetic units with free hydroxyl groups (Scheme 61).

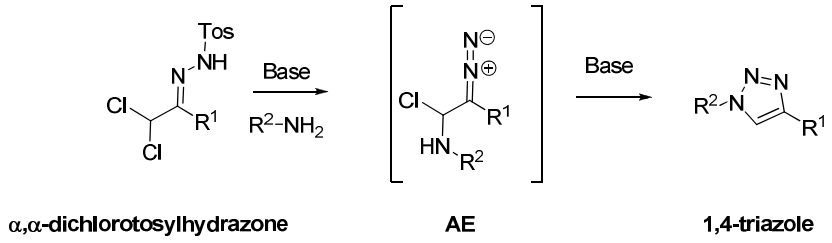


Scheme 61. Synthesis of hexavalent carbohydrate mimetic **140** by CuAAC with Cu/C as catalyst.

Spectroscopic analysis of compound **140** also showed a small splitting of the aromatic proton but in the case of the triazole proton a singlet was observed. For this molecule no further NOE measurements were done but one can assume that since **140** is sterically less hindered compared to **139** there is a higher mobility of the pyran units, leading to a different splitting and possibly another preferred conformation.

4.4.9. Metal-free 1,2,3-triazole formation

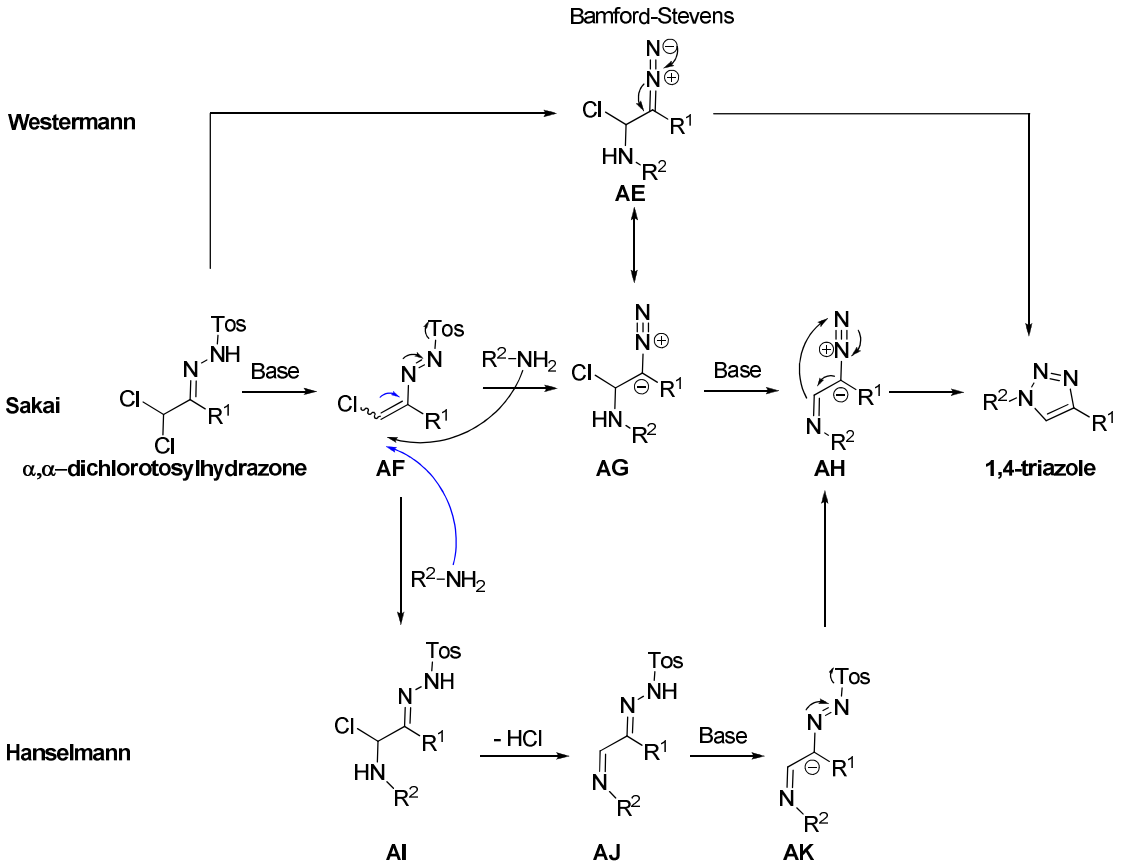
All the methods previously presented showed to be suitable methods for the [3+2] cycloaddition. However, the requirement of a metal catalyst (as discussed before) can have some drawbacks. In the search of metal free methods as an alternative for the CuAAC, several orthogonal ligation methods have been described.[107] The most common one is the strain-promoted azide-alkyne cycloaddition. This reaction was first described by Wittig and Krebs that noticed the reaction between cyclooctyne and phenyl azide led "like an explosion" exclusively to the corresponding triazole.[108] Over the years this method has attracted some attention and found applications in the marking of biomolecules.[109] However, some of the drawbacks are the formation of 1,4- and 1,5-substituted triazoles and the sometimes tedious availability of the starting materials. Sakai et al. described in 1986 the transformation of a primary amine and an α,α-dichlorotosylhydrazone to form exclusively a 1,4-substituted triazole (Scheme 62).[110]



Scheme 62. Sakai reaction leading to the formation of 1,4-triazoles.

Not many mechanistic studies have been performed for this transformation, but three different proposed pathways can be found in the literature. The first mechanism for the triazole formation was proposed by Sakai in 1986 (Scheme 63).[110] It was proposed that the first step would be the deprotonation of α,α-dichlorotosylhydrazone leading to the vinyldiazine **AF**. Subsequent attack of the amine to the electrophilic α-carbon with elimination of toluenesulfinic acid leads to intermediate **AG**. This intermediate can after deprotonation cyclize to give the 1,4-triazole. Later in 2010, Hanselmann et al.[111] proposed a slightly different mechanism as they observed and analyzed two intermediates by LC-MS. The first intermediate observed **AI** suggested that after addition of the amine to the vinyldiazine **AF** occurs without loss of the tosyl group. After loss of HCl the intermediate **AJ** is formed that only after deprotonation by the base leads to the cleavage of the tosyl group and to

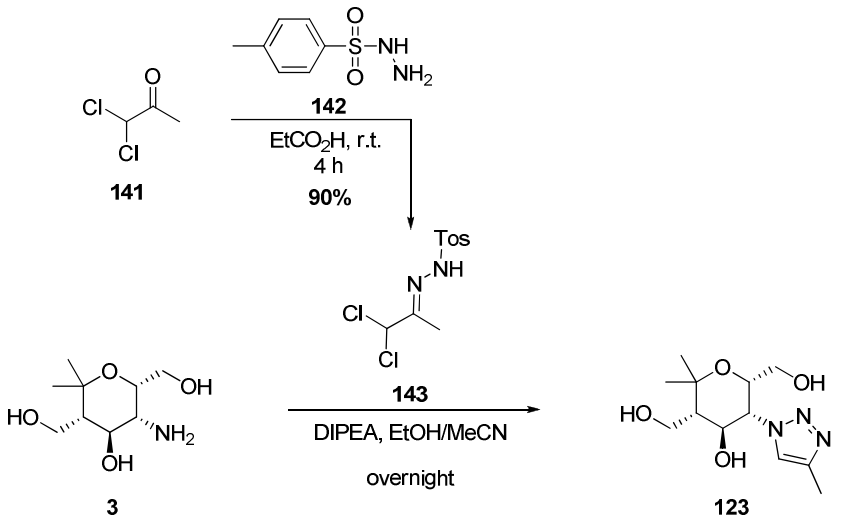
intermediate **AH** (the same proposed by Sakai). Without any further elucidation, in 2012 Westermann et al.[112] proposed the probable intermediate (Bamford-Stevens intermediate **AE**) formed through electron rearrangement of intermediate **AG**.



Scheme 63. Possible mechanistic pathways for the Sakai reaction.

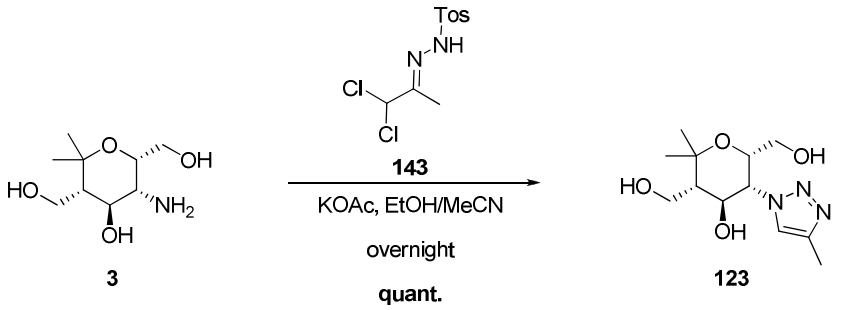
Although this transformation could overcome some of the disadvantages of the strain promoted azide-alkyne cycloaddition, this method never got the due attention. It was not only until 2012 that Westermann et al. reported applications and investigations of this procedure for the synthesis and modification of biologically significant targets.[112] After the success of the Westermann applications of such reaction, it seemed a good alternative for the construction of multivalent carbohydrate mimetics in a copper-free "click" type conditions. Another advantage of this method is that one can directly react aminopyran **3** saving the transformation of the last into the azidopyran **10**. For the first experiment the simple α,α-dichlorotosylhydrazone **143** was chosen and it was synthetized according to the literature procedure by reaction of hydrazide **142** and 1,1-dichloroacetone (**141**) in propionic acid affording **143** in 90% yield (Lit.[112] 92%) (Scheme 64). Following Westermann's reported conditions for the Sakai reaction, aminopyran **3** reacted with **143** in a solvent mixture of

acetonitrile and methanol in presence of an excess of base (6 equivalents) (Scheme 64). These simple reaction conditions afforded the desired triazole **123**, but after column chromatography the final product was still contaminated with the used Hünig base.



Scheme 64. Synthesis of α,α-dichlorotosylhydrazone **143** and further Sakai reaction leading to triazole **123**.

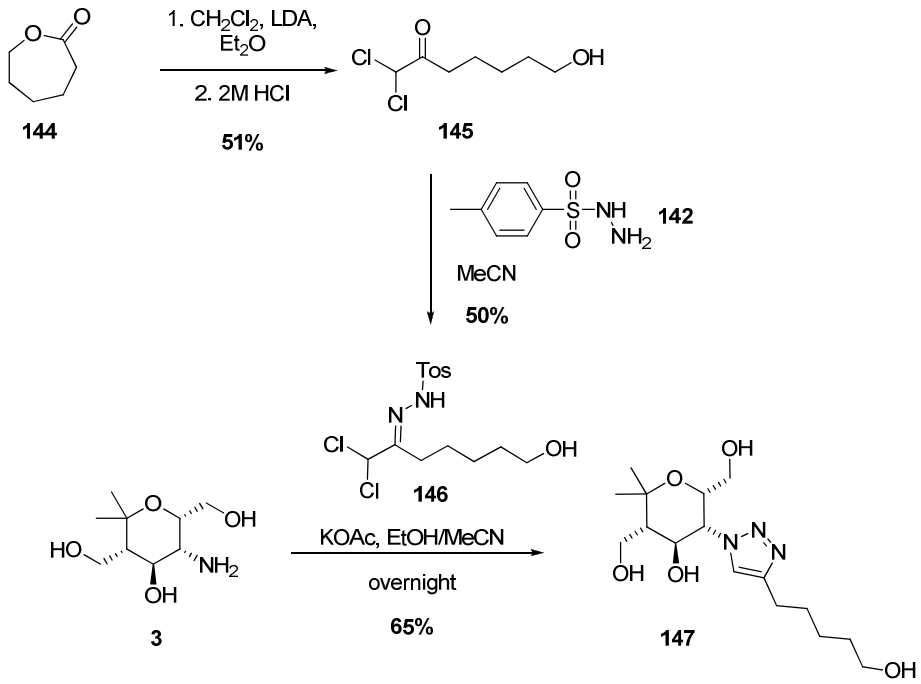
To overcome this purification difficulties and after personal communication with the authors, a different base was employed (Scheme 65). Using three equivalents of potassium acetate, the desired triazole **123** was obtained in quantitative yield and excellent purity.



Scheme 65. Sakai reaction leading to triazole **123**.

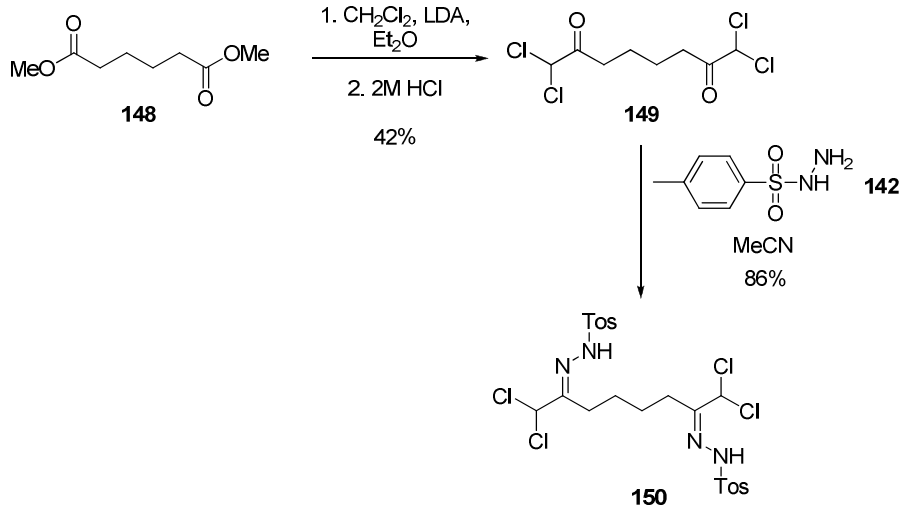
To obtain a further functionalized α,α-dichlorotosylhydrazone **146**, prodecures reported by Westermann[112] and Barluenga[113] were followed. α,α-Dichloro-7-hydroxyheptan-2-one (**145**) was synthesized by treating a solution of ε-caprolactone **144** and dichloromethane in dry diethyl ether with freshly prepared LDA at -78 °C (Scheme 66). Dichloromethane is used

as a reagent that after being deprotonated by the LDA leads to a nucleophilic dichloromethane anion. This species attacks the carbonyl group of the ε-caprolactone and by ring opening generates an acyclic anion that is protonated during the acidic workup affording **145**. Dichloro ketone **145** can be easily converted into the corresponding tosylhydrazone **146** by treatment with hydrazide **142** in 50% yield (Lit.[112] 52%). Finally, α,α-dichlorotosylhydrazone **146** reacted with aminopyran **3** under the optimized conditions for the Sakai reaction affording the desired triazole product **147** in good yield (Scheme 66).



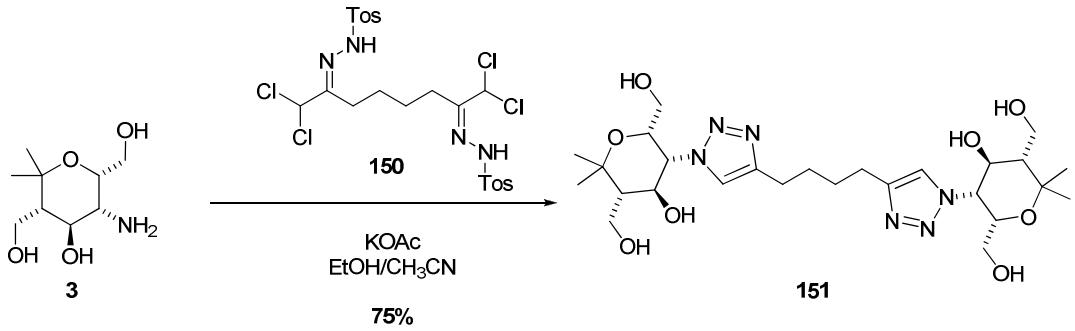
Scheme 66. Synthesis of α,α-dichlorotosylhydrazone **146** and further Sakai reaction leading to triazole **147**.

With the optimized conditions and with this very efficient and high yielding method, the synthesis of a multivalent carbohydrate mimetic was attemped. To evaluate the efficacy of this procedure, the synthesis of a divalent structure was considered therefore a bis(dichlorotosylhydrazone) was synthetized. Starting from dimethyl adipate **148** and using an excess of LDA the tetrahaloketone **149** was obtained with 42% yield (Lit. [113] 60%) using Barluengas procedure. With a slight excess of hydrazide **142** (2.1 equivalents), compound **149** was easily converted into the corresponding bis(tosylhydrazone) **150** in excellent yield (Scheme 67).



Scheme 67. Synthesis of bis-α,α-dichlorotosylhydrazone **150**.

The versatility of this metal-free strategy could be proved by the successful synthesis of divalent carbohydrate mimetic **151** in very good yield (Scheme 67).

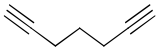
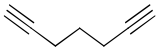
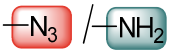
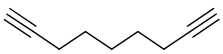
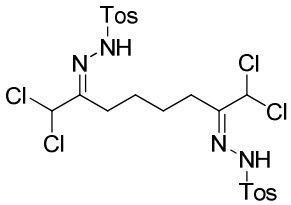


Scheme 68. Synthesis of divalent carbohydrate mimetic **151** by Sakai reaction.

This is the first report of the use of the Sakai reaction for the synthesis of a divalent compound. With easily obtained and bench stable starting materials this method proved to be a good (and until date unexplored) procedure for the synthesis of multivalent compounds bearing triazole units in a metal-free way starting from amines.

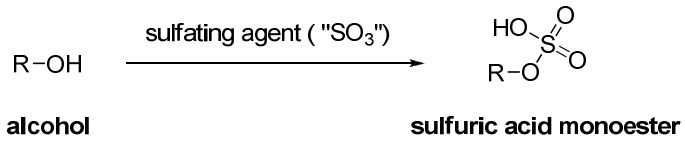
As a short summary, in Table 18 are schematically represented all the different conditions used for the synthesis of divalent carbohydrate mimetics using aliphatic linkers. Although with the metal-free method using tosylhydrazone **150** and aminopyran **3** (Table 18, entry 4) the desired dimer **151** was obtained in very good yield, this method has the disadvantage of the preparation of the bis(tosylhydrazone) **150**. This bench stable compound needs to be synthetized in a two-step procedure compared to the commercially available dialkynes **105** and **115**. If one method needed to be chosen as a favorite, from experience and the results presented, the Cu/C method would be the chosen one (Table 18, entry 3). Although the reaction times are considerably longer and heat is needed, the handling and purification of the desired products is quite straightforward. Moreover, with this method there is also the possibility of starting the synthesis with azidopyran **10** or aminopyran **3**. In general, it is not possible to affirm that one method is better than the other. From the presented results it was observed that the method of choice depends on the substrate used as well as the polarity of the final compound.

Table 18. Summary of the different methods used for the generation of 1,2,3-triazoles.

Entry	Substrate	Spacer	Product	Cu source	Base	Additive	Temperature (°C)	Yield (%)
1	 **10**	 **105**	**106**	CuI	Et_3N	TBTA	r.t.	61
2	 **10**	 **105**	**106**	CuI	DiPEA	AcOH	r.t.	71
3	 **10/3**	 **116**	**117**	Cu/C	Et_3N	-	60	91/38
4	 **3**	 **150**	**151**	-	KOAc	-	r.t.	75

4.5. Polysulfation of carbohydrate mimetics

The relevance of natural occurring sulfated molecules and their modes of action was previously mentioned in the introduction. In this chapter, the importance and challenges of synthetic sulfation of biological molecules will be discussed. Sulfation is a one-step transformation of a hydroxyl group into the corresponding sulfate (bearing a carbon-oxygen-sulfur bond) in presence of a sulfating agent (Scheme 69). In most cases, the resultant product is hydrolytically unstable and unless neutralized, can decompose to form sulfuric acid and regenerate the alcohol. Since the introduction of sulfate groups drastically changes the physical and chemical properties of the molecules, isolation of pure fully sulfated compounds continues to be a great challenge.

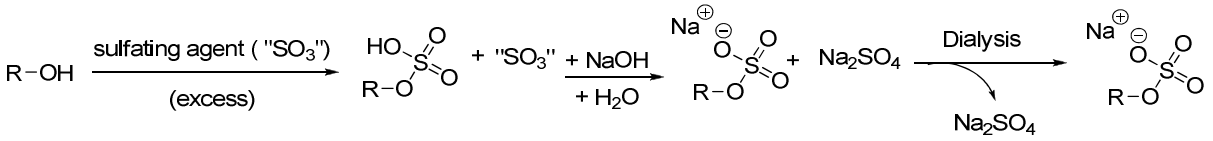


Scheme 69. Sulfation reaction.

Procedures for such transformation have been known for many years, and started to be performed using sulfuric acid (H_2SO_4) or alternatively, sulfamic acid (H_2NSO_3H).[114] This early approaches had the disadvantage of leading to a variety of side reactions like dehydration, non-selective sulfation and in some cases, product degradation. Mumma reported in 1966 the first sulfation using sulfuric acid and dicyclohexylcarbodiimide (DCC).[115] Even though this was a more convenient method, the formation of insoluble dicyclohexylurea made the purification and isolation of the desired sulfate product complicated.[116] As alternative, the use of protected sulfate groups is known for some time; however, applications of this approach have some drawbacks: the number and availability of the protecting groups is limited as well as the ease by which their introduction and removal is performed. Some examples of protecting groups are the phenyl[117] or the trifluoroethyl group.[118] The most common method currently used for sulfation (of small or larger molecules) is the use of sulfur trioxide-amine complexes namely complexes, of SO_3 with pyridine, trimethylamine, triethylamine or with DMF. For these reactions are numerous varieties of performance known, but a consistent and reproducible reaction report has not yet been found, since the conditions differently affect the molecules according to their size and number of hydroxyl

groups. As the number of hydroxyl groups increases, it seems that the sulfation becomes more complicated due to the crowding of negative charges resulting in formation of only partially sulfated products. In the synthesis and evaluation of tetrasaccharides presenting antiproliferative activity, Wessel et al. described that one of the reasons for the non-conclusive analysis of the biological data was the fact that the resulting sulfated oligosaccharides presented random degrees of sulfate groups.[119] The same type of complications was described for example by Desai et al. that tried to overcome such difficulties by applying a microwave assisted procedure.[120] Also in the synthesis of sulfated polysaccharides as potential selectin inhibitors, Nifantiev and coworkers obtained mixtures of partially sulfated polysaccharides when using the common SO_3·pyridine complex;[121] alternatively, the use of the SO_3·Et_3N complex in presence of triflic acid promoted the *O*-sulfation at lower temperature and shorter reaction times and the desired sulfated compounds could be successfully obtained. It seems that a sulfation procedure cannot be generalized and it needs to be adapted to the corresponding systems.

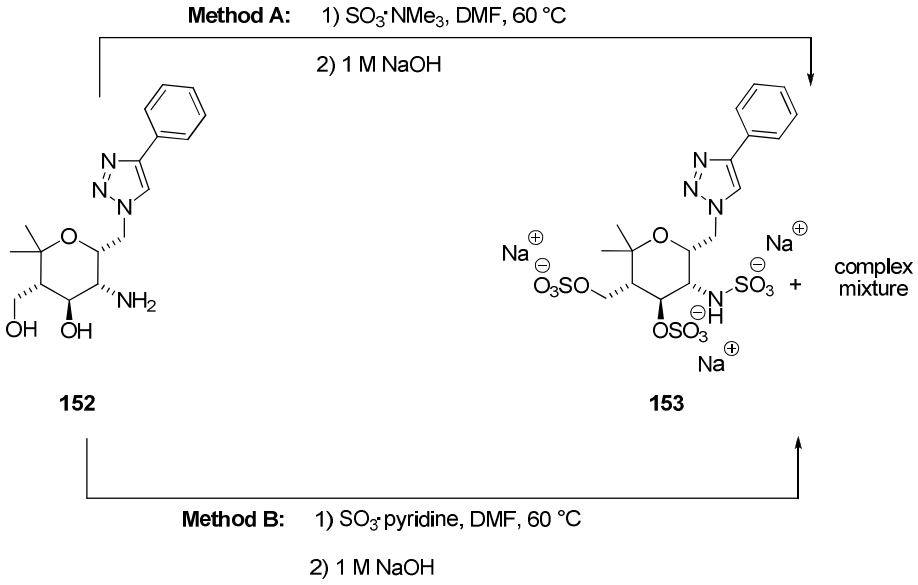
Like many literature reports, in the presented work the sulfation step also proved to be the most challenging one. In the Reißig group initial investigations for sulfation of carbohydrate mimetics were performed by Koóš[33] and Bouché.[40] Here the further developments of the initial procedures are described leading to the optimal one for the synthetized carbohydrate mimetics. Scheme 70 presents the general procedure normally used in the Reißig group for the sulfation step: after reaction with a sulfating agent (always used in excess), the sulfuric acid monoester is obtained (in a mixture with the excess of sulfating reagent). This mixture is directly converted into the corresponding sodium salts that are separated using dialysis, affording the desired product.



Scheme 70. Presentation of the sulfation procedure leading to the final sodium salt.

The first sulfation experiments performed by Koóš[33] led to inconclusive results and no product could be isolated in pure form. In the example presented in Scheme 71, for the sulfation of the simple aminopyran derivative **152**, SO_3 complexes with trimethylamine and

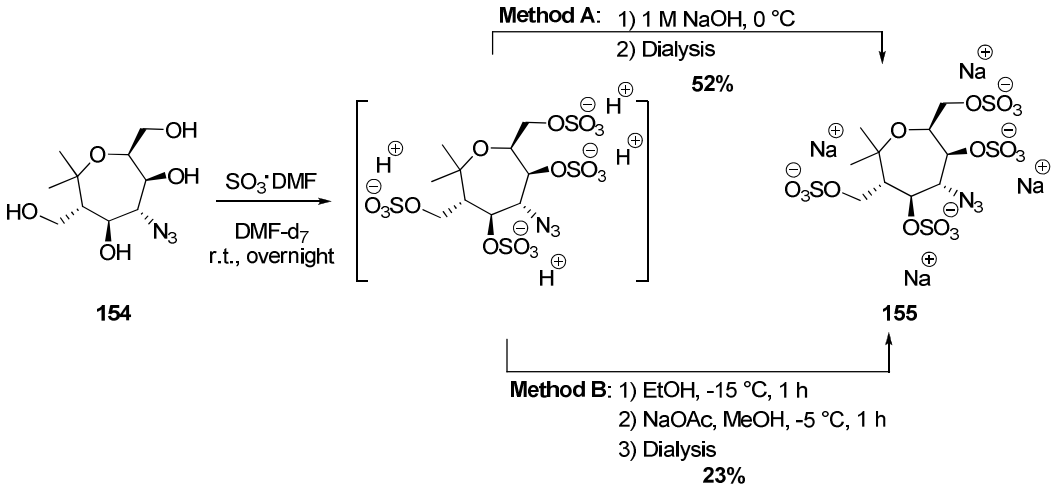
pyridine were mainly used, and in both cases the use of heating was needed. The transformation of the sulfated intermediate into the corresponding sodium salt was performed using a 1 M sodium hydroxide solution as previously reported.[122] The desired sulfated compound **153** could be detected by mass spectrometry in the mixture received, but unfortunately could not be isolated in pure form from the other products formed.



Scheme 71. Attempts to synthetize polysulfated carbohydrate mimetic **153** by Koóš.

These investigations were carried out in compounds with relatively small molecular weight which also made the reactions quite challenging (purification of small molecules using dialysis will be discussed in more detail in section 4.5.1 and 4.5.2). Due to the high polarity of the final compounds, a control of the reactions by TLC (either with normal phase or reverse phase) was not a suitable method. As reported in the literature,[121],[123] NMR control of such reactions can be an alternative and although Koóš was not able to obtain fully sulfated compounds in pure form, the performance of sulfation in deuterated solvents to allow the direct control by NMR spectroscopy was a great improvement. Since sulfation is the last step of a quite long synthesis, the reactions are usually performed in a small scale; the use of deuterated solvents allows a better detection of the signals by NMR control of the crude products. The transformation of a mixture of sulfated products to a single final product can be followed (by further addition of the sulfating agent) in the ^{1}H-NMR spectrum. However, since sodium hydroxide is used to transform the sulfated product into the corresponding sodium salt, after dialysis, the purity of the final product is not evident in the ^{1}H-NMR spectrum and the use of mass spectrometry is needed. In the search of an optimal method Bouché performed

several investigations and an alternative procedure was considered: following a literature report,[124] the neutralization step was changed and in this case after full conversion to a final product (controlled by ^{1}H-NMR spectroscopy) the solution was cooled to -20 °C and then quenched with EtOH (the excess SO_3·DMF is converted into ethyl sulfate) followed by slow addition of sodium acetate in methanol. This method allows the detection of product **155** and the excess of salts formed (methyl sulfate sodium salts) in the ^{1}H-NMR spectrum. Following this route, it is possible to have a direct confirmation of the purity of the desired product **155**. In the sulfation of azidooxepane **154** (Scheme 72), Bouché used both neutralization methods described to obtain the final sodium salt **155**.



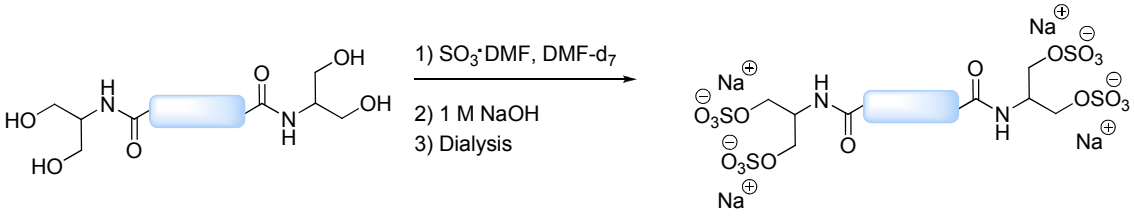
Scheme 72. Synthetic route of Bouché to polysulfated oxepane **155**.

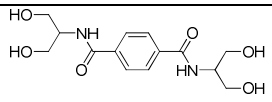
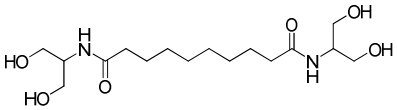
Although both procedures led to the formation of sulfated product **155**, method B was (in most of the cases) not suitable for the separation of the final product from the excess of salts and more than one dialysis was needed; in the end, the methyl sulfate sodium salts could not be completely removed. Bouché used both presented methods for the sulfation of oxepane derivatives, and from most experiments performed, fully sulfated multivalent carbohydrate mimetics bearing oxepane units were successfully isolated, however, no general and reproducible procedure could be described. It is believed that one of the reasons for the non-reproducibility of this method in our group is the difficulty to ascertain complete conversion; even using 700 MHz NMR spectrometer, for bigger and more complicated structures it is hard to have good evidence of the reaction completion. Moreover, it was noticed that in most cases two equivalents of the sulfating agent per hydroxyl group were not sufficient, leading to a large excess of the salts after the reaction.

4.5.1. 2-Amino-1,3-diol derivatives

Several attempts to sulfate the small molecules presented in this work were performed without success. In early investigations, attempts to isolate the products before the conversion to the sodium salt were not successful (most probably the resulting sulfates were not stable) and mixtures of several products were obtained. It was decided to only isolate the final products as sodium salts, however still discrepancies were observed and the results were not reproducible. A major error in these attempts was the removal of the solvent (DMF) after reaction completion; this resulted in a highly acidic environment that is likely to induce side reactions. Therefore, immediately after the end of the sulfation reaction (controlled by NMR), the solution was cooled to 0 °C and a sodium hydroxide solution (1 M) was slowly added dropwise until a basic pH was obtained (pH 12). Only after this procedure, the remaining solvents (DMF and water) were removed in vacuo. The obtained mixture of product and excess of salts (Na_2SO_4) were separated using dialysis. As presented in Table 19, entry 1, the sulfation of compound **54** was carried out using 3 equivalents of sulfating agent per hydroxyl group for 5 days. When the reaction was controlled by ^{1}H-NMR spectroscopy it was possible to detect more than one product; to lead the reaction to completion, extra equivalents of SO_3·DMF were added and this procedure was repeated every day. In the attempt presented, the desired polysulfated compound **156** could be isolated with a good yield. When the sulfation reaction was performed using diamide **56**, the reaction was much faster and after one day ^{1}H-NMR spectroscopy showed only one product. In this case no extra equivalents of the sulfating agent were added to the mixture and after dialysis the desired product **157** was isolated in very good yield (Table 19, entry 2).

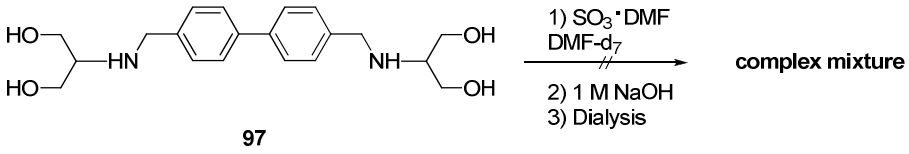
Table 19. Polysulfation of 2-amino-1,3-diol derivatives **54** and **56**.



Entry	Starting material	Equiv SO_3·DMF	Time (d)	pH	Product	Yield (%)
1	 **54**	12 (5x)	5	12.5	**156**	69
2	 **56**	12	1	12	**157**	79

The dialysis process is briefly explained here. The dialyses tubes have different pore sizes that allow small molecules to pass through keeping the bigger ones inside. This means that the relatively small salts can pass through staying in the "water phase" whereas the desired product is kept inside the tube. To obtain an efficient separation, the molecular weight of the desired product should be five times bigger than the tube cut off (the cut off indicates the molecular weight of those molecules which can still pass the membrane efficiently). The smallest tubes in the market present a cut off between 100 and 500 MW; for the small carbohydrate mimetics presented, a rule of double molecular weight of the cut off had to be applied. Regarding the sulfation of **54** (Table 19, entry 1) the resulting fully sulfated product **156** has a molecular weight of 720.5 g·mol^{-1} which is less than the double of the tube cut off. Moreover, in some attempts after the first dialyses there were still salts present and a further dialysis was needed. For such small molecules this was a big disadvantage and in some attempts, the desired final product was totally lost in the water phase after several dialyses.

An attempt was also made to sulfate diamine derivative **97** (Scheme 73). Unfortunately this reaction led to an complex mixture of products.



97

Scheme 73. Attempt to polysulfate amine **97**.

During the reaction control and comparing the ^{1}H-NMR spectrum of the reaction mixture with that of precursor **97** (precursor Figure 27 top part and mixture Figure 27 lower part) one can observe the biggest shifting of the 1-H ≈ 1.6 ppm downfield and a shifting of the 2-H ≈ 0.6 ppm upfield. Obvious in the ^{1}H-NMR spectrum of the reaction mixture are the extra signals corresponding to side products or partially sulfated compounds.

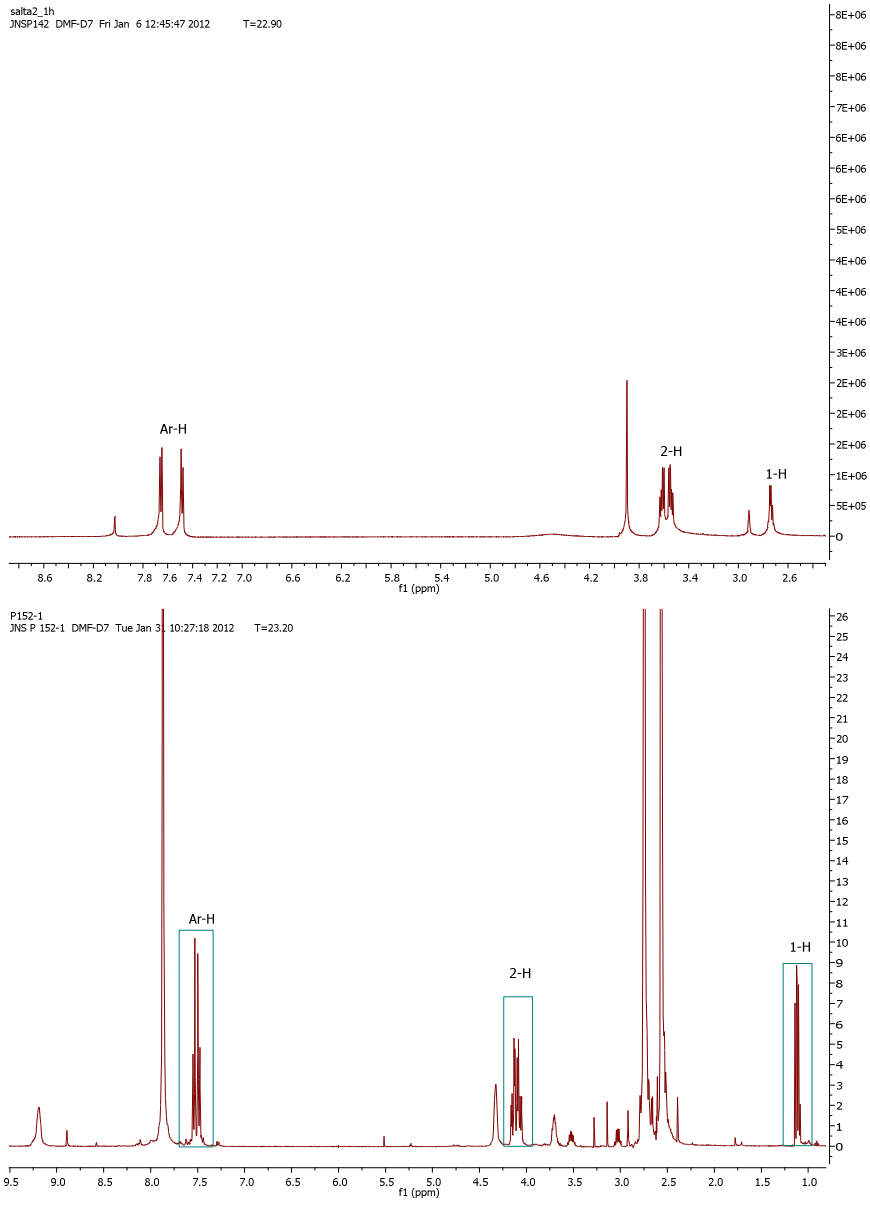
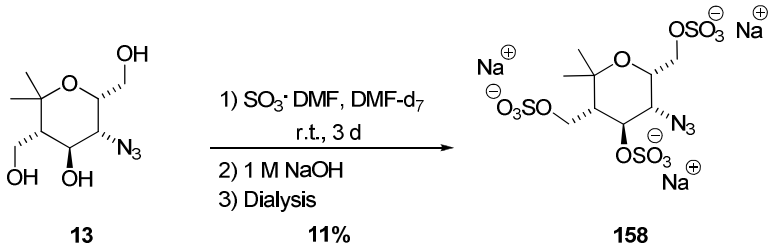


Figure 27. ^{1}H-NMR spectrum of precursor **97** (top) and ^{1}H-NMR spectrum of the polysulfation of **97** (bottom).

Even after further addition of the sulfating agent no changes were observed in the ^{1}H-NMR spectroscopy controls. This was an indication that the sulfation of amine derivatives was more complex since in this case, even though the *O*-sulfation of primary hydroxyl groups should be preferred, there is a competition between *O*- and *N*-sulfation leading to a complex mixture of products; this was observed after transformation into the corresponding sodium salts and dialysis where a mixture of several products were obtained.

4.5.2. Monovalent carbohydrate mimetics

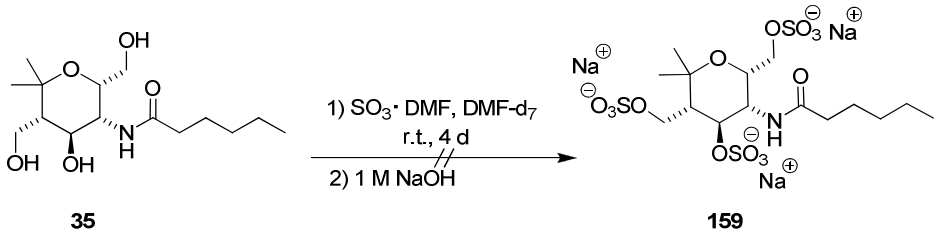
Initial sulfation experiments were performed with substrate **13** at room temperature (Scheme 74). Once again 3 equivalents of SO_3·DMF per hydroxyl group were used and the reaction was performed in DMF-d_7. After one day reaction, in the ^{1}H-NMR spectrum (400 MHz) only one product could be detected. It was previously observed by Bouché that with more complex substrates, the NMR control could be misleading (even when using the 700 MHz spectrometer). Often it was thought that only one product was obtained (due to the excess of salts and the small scales in which the reactions are performed) but after neutralization and purification, mixtures were received. Since for the control of the reaction leading to product **158** only the 400 MHz spectrometer was used, extra 1.5 equivalents of SO_3·DMF were added and the reaction was continued for two extra days. After neutralization using sodium hydroxide solution (1 M) and dialysis, the desired sulfated azidopyran **158** was obtained with a yield of 11%. The molecular weight of **158** is just slightly above the cut of the dialysis tube (**158** has a molecular weight of 537.4 g·mol^{-1} for a cut off tube 100-500 MW) which makes it difficult to separate **158** from the excess of salts without a big loss of the final product.



13 **11%** **158**

Scheme 74. Synthesis of polysulfated azide **158**.

The same procedure was applied to amide **35** and in this case the desired sulfated amide **159** was not obtained; instead a complex mixture was received (Scheme 75).



35 **159**

Scheme 75. Attempt to synthetize sulfated amide **159**.

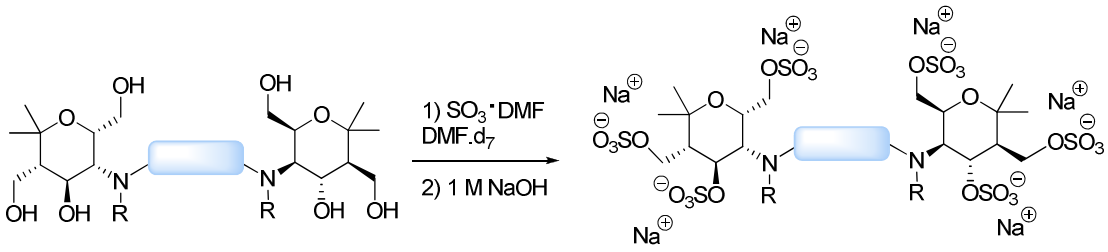
A possible explanation for the failure of this attempt can be the fact that once again the reaction was followed using a 400 MHz spectrometer and a wrong judgment was done assuming full conversion to final product **159**. At this point it was already quite clear that to obtain the best reaction control possible, the 700 MHZ spectrometer was the most adequate.

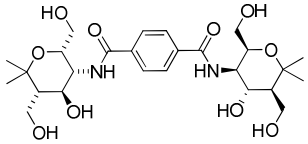
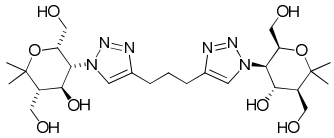
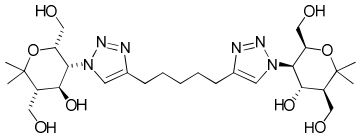
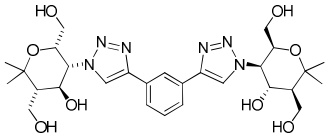
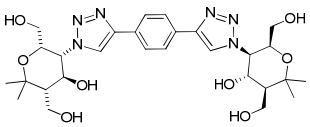
4.5.3. Divalent carbohydrate mimetics

A summary of the results concerning the sulfation of the divalent structures is presented in Table 20. A complete conversion of amide **54** with SO_3·DMF into the corresponding sulfuric acid monoester could be observed by NMR control after one day; conversion into the sodium salt using 1 M sodium hydroxide solution followed by dialysis, afforded **160** in good yield (Table 20, entry 1). Concerning triazole **106** (Table 20, entry 2), both polysulfation and the subsequent neutralization were successfully carried out applying the same conditions and the polysulfated divalent compound **161** could be isolated with 48% yield. Similarly, divalent compound **116** could be successfully sulfated and transformed into the corresponding sodium salt **162** with 39% yield (Table 20, entry 3). There is no clear explanation for the discrepancies in the yields; most probably in some cases there was a bigger loss of product during dialysis purification. From the results presented so far, the method for the neutralization step using 1 M sodium hydroxide solution seems to be quite adequate, however, several attempts to polysulfate the presented compounds **54**, **106**, **116** and others (using exactly the same procedure) failed and this method proved to be inconsistent. Two examples are presented in Table 20, entry 4 and entry 5, where a full conversion was observed by ^{1}H-

NMR spectrum (700MHz) but after neutralization and dialysis, a mixture of products was received.

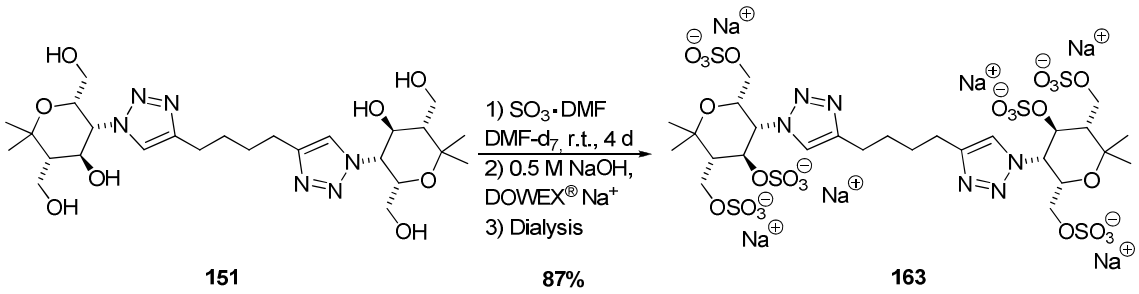
Table 20. Polysulfation of divalent carbohydrate mimetics.



Entry	Starting material	Equiv SO_3·DMF	Time (d)	pH	Product	Yield (%)
1	 **54**	18	1	10	**160**	60
2	 **106**	36	2	12	**161**	48
3	 **116**	36	2	12	**162**	39
4	 **111**	36	5	12	-	-
5	 **109**	36	4	12	-	-

At this point the reason for the non-reproducibility of the method was not clear, but in all attempts, when performing the neutralization it was noticed that even when adding small amounts of sodium hydroxide (1 M solution) to the reaction mixture, an accurate pH control was not possible with regular pH paper. An extremely fast change from highly acidic to

highly basic pH was always observed. This fast change and the resulting highly basic environment could lead to decomposition of the product or regeneration of the alcohol leading to mixtures of compounds. To overcome the described problem and in hope that the pH could be stopped at around 7, a new change was introduced in the procedure: instead of a 1 M solution of sodium hydroxide a more diluted solution (0.5 M) was used. Additionally, the obtained mixture was filtrated through an ion exchange DOWEX® Na^+ column to assure that all sulfuric acid monoesters were converted into the corresponding sodium salts as well as the excess of the sulfating agent. This new method was applied for the sulfation of compound **151** (Scheme 76). While adding sodium hydroxide solution (0.5 M) a much better control of the pH was observed, and the addition could be stopped at a pH ≈ 8. After filtration through the DOWEX® Na^+ column and purification by dialysis, the desired poly-sulfated compound **163** was obtained in excellent yield.

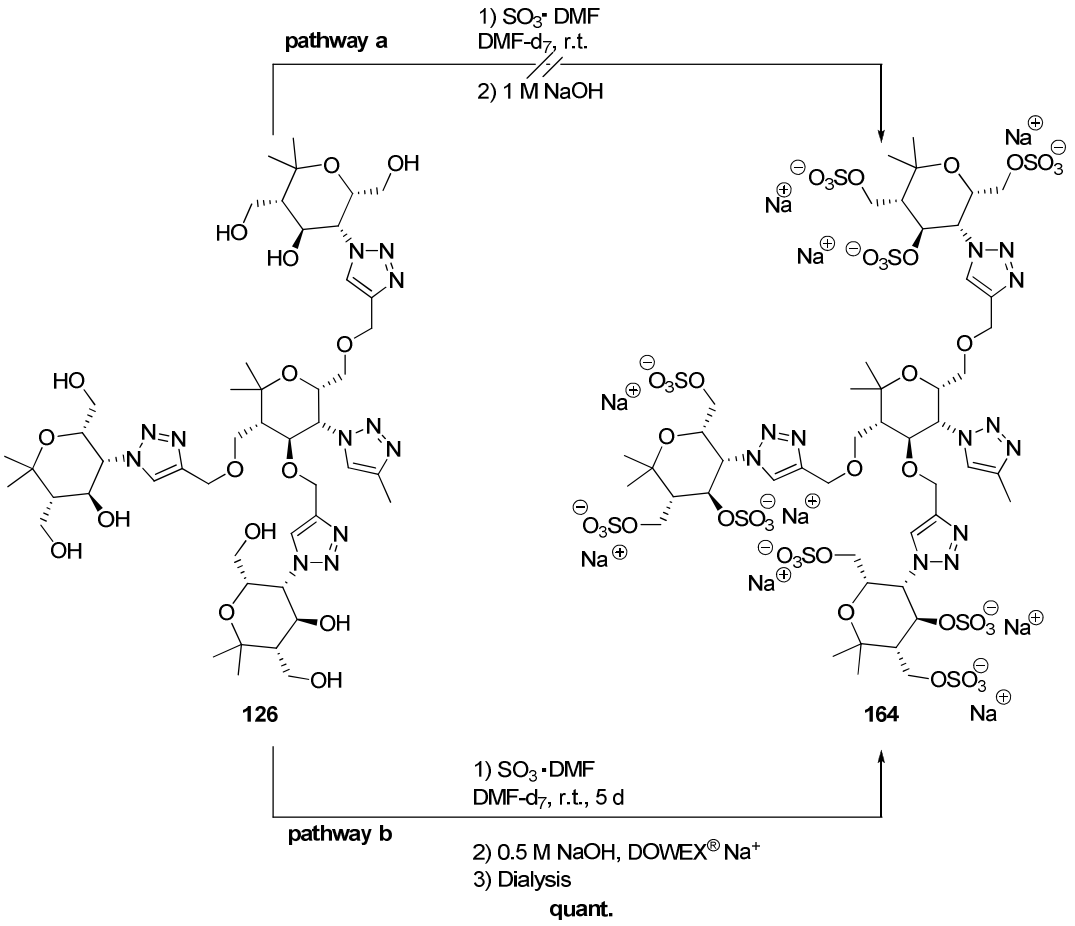


Scheme 76. Synthesis of polysulfated divalent compound **163**.

4.5.4. Trivalent carbohydrate mimetics

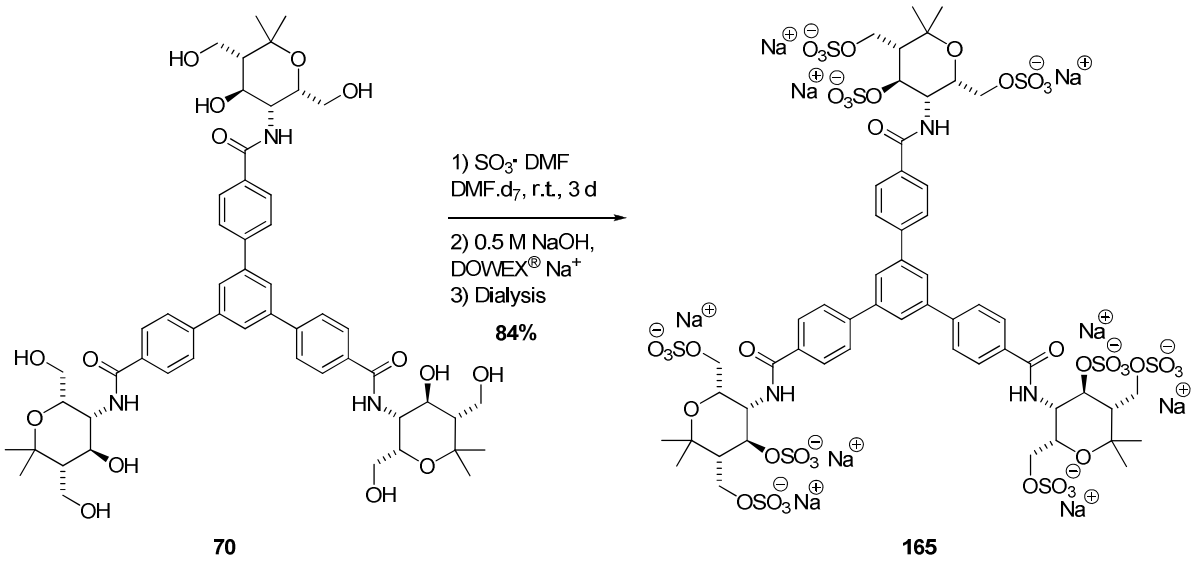
The polysulfation of unsymmetrical trivalent carbohydrate mimetic **126** and subsequent transformation into the sodium salt **164** was performed several times using 1 M sodium hydroxide (these experiments were performed before the new neutralization method was considered). In all attempts with this procedure, after neutralization and purification, mixtures of products were received (Scheme 77, pathway a). With such structure, the NMR control was quite complicated since as mentioned, the reactions were performed in small scale. The NMR of the pure starting material **126** is already quite complex making it difficult to control its full conversion during reaction. These reactions were carried out during longer times and the control was made based on the shifting of an easily recognizable peak until no shift was observed (after addition of extra sulfating agent). Applying the slightly modified method

using 0.5 M sodium hydroxide solution (until pH 9 was reached) and filtration through an ion exchange DOWEX® Na^+ column, provided the desired polysulfated product **164** in excellent yield (Scheme 77, pathway b). It should be noticed that in none of the polysulfated compounds synthetized the complete exclusion of water is assured, but residual amounts of water should not have an influence on the results of the SPR measurements.



Scheme 77. Synthesis of sulfated trimer **164**.

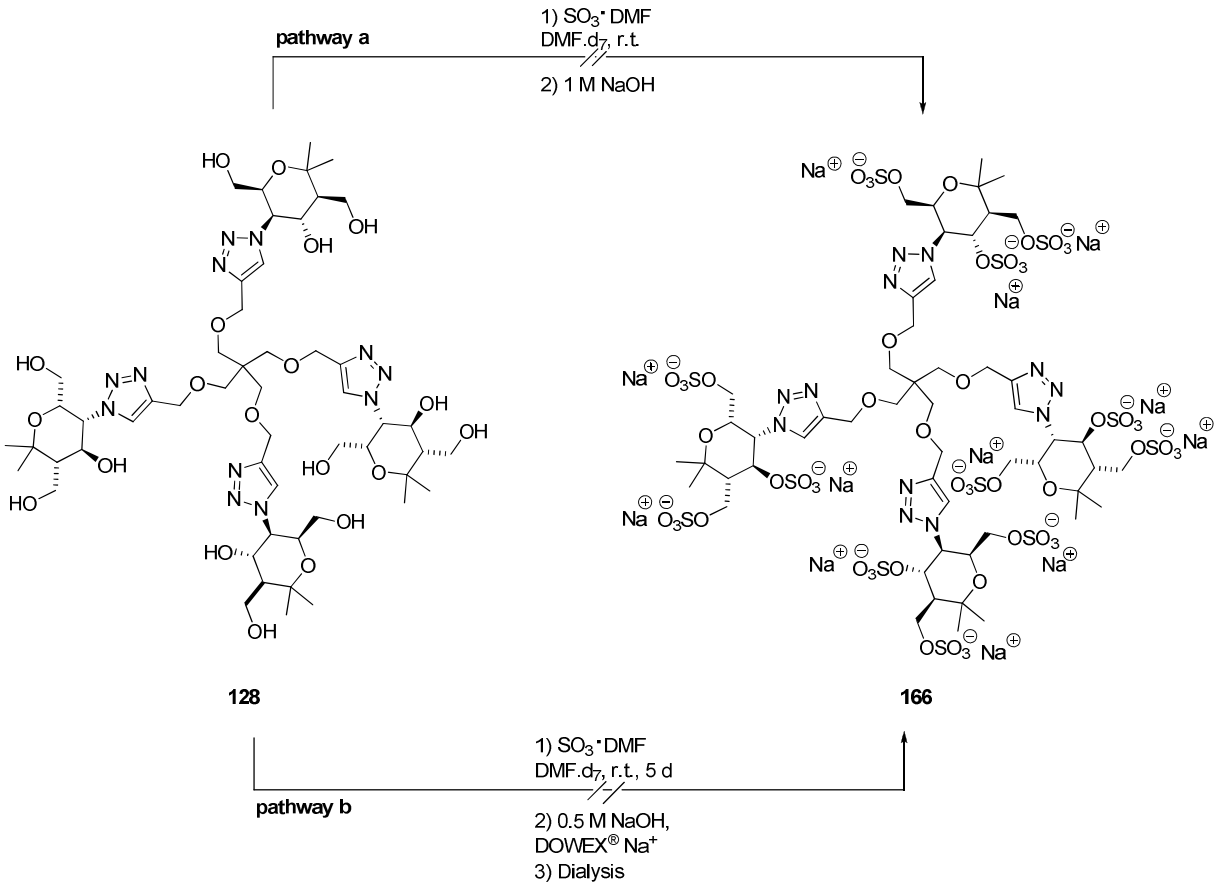
The modified procedure was also applied to the sulfation of amide **70** and the reaction was completed after 3 days (each day additional SO_3·DMF was added to the reaction mixture). Sodium hydroxide solution (0.5 M) was added until pH 9 was reached and the mixture was filtrated through an DOWEX® Na^+ column. After purification, the desired sodium salt **165** was successfully obtained in excellent yield (Scheme 78).



Scheme 78. Synthesis of polysulfated trivalent carbohydrate mimetic **165**.

4.5.5. Tetravalent carbohydrate mimetics

For the sulfation of tetramer **128**, both conditions were used for the neutralization: with 1 M sodium hydroxide solution as well as the more convenient method using 0.5 M NaOH and DOWEX® Na^+ (Scheme 79). Disappointingly, in all attempts performed, no pure compound **166** could be isolated. It was expected that with larger molecules the sulfation and purification would be easier since there are less chances of losing product during dialysis purification; this was not observed and probably in this case the large anionic crowding led to the formation of only partially sulfated products. Although it is not clear at which point the mixture was formed, NMR control (700 MHz spectrometer) indicated that full conversion was obtained and the mixture was only noticed after neutralization and purification.

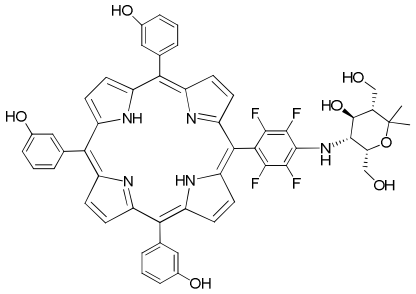
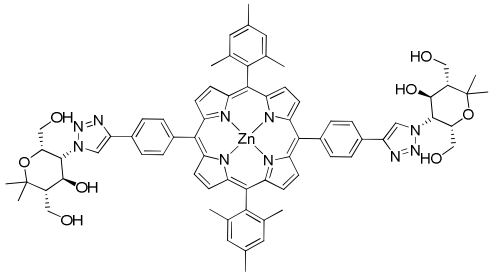
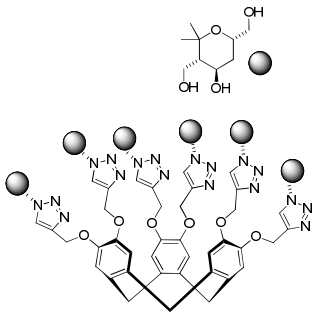


Scheme 79. Different attempts to synthetize tetravalent carbohydrate mimetic **166**.

4.5.6. Other oligovalent compounds

Table 21 summarizes some of the failed attempts to sulfate larger molecules. When controlling the reaction of porphyrins **167**[125] and **168**[126] by ^{1}H-NMR spectroscopy, a mixture of more than one product was always observed and even after addition of more sulfating agent during several days no full conversion was observed. This indicates that these reactions would not lead to a pure final compound. Moreover, regarding porphyrin **167**, the sulfation of the phenolic hydroxyl groups leads to labile sulfates, which make the isolation of a fully sulfated final product quite challenging. After purification, as expected, mixtures were obtained. With CTV derivative **140**, the NMR control was quite complex and it was not possible to extract information about a full conversion. After several days the reaction was stopped and neutralized but unfortunately once again only a mixture was isolated after purification.

Table 21. Attempts to polysulfate porphyrins **167**, **168** and CTV derivative **140**.

Entry	Starting material	Equiv SO_3·DMF	Time (d)	pH
1	 **167**	27	8	10 (1 M NaOH)
2	 **168**	18	6	12 (1 M NaOH)
3	 **140**	54	6	9 (0.5 M NaOH and DOWEX® Na^+)

Although mono-, di-, and trivalent compounds could be successfully sulfated and isolated in pure fashion, with larger molecules no fully sulfated products were obtained. Moreover, from all the sulfations performed there are several aspects that should be considered:

- The purity of the sulfating agent as well as the time of use since the opening of the commercially available bottles sometimes seem to influence the reaction outcome (the water content of SO_3·DMF is crucial since it is highly hygroscopic);
- Even though some sulfation reactions had long reaction times, in a majority of the cases, if a full conversion was not observed in the first 3 days, the addition of more sulfating agent did not lead to full conversion;

- For a precise reaction control, the 700 MHz NMR spectrometer should always be used and for more complicated cases, where a full conversion is not clear, it is helpful to control (after further addition of the sulfating reagent) the shifting of an easy recognizable peak;
- All attempts to sulfate secondary amine derivatives led to mixtures of products;
- The conversion to the corresponding sodium salts seems to be the crucial step of the procedure and the pH control should be done carefully (for a more precise outcome, the use of an accurate pH-meter could be helpful);
- The removal of the solvents (water and DMF) after neutralization should be efficient and complete; in several cases the presence of traces of DMF led to a rupture of the dialysis tube and a total loss of the products;
- Although sulfated molecules with a low molecular weight could be successfully isolated, in these cases dialysis purification was quite problematic leading to low yields and sometimes loss of the desired product;
- Overall, the neutralization using 0.5 M sodium hydroxide solution and filtration through a DOWEX® Na^+ column led to a better result and proved to be the most reproducible procedure.

4.6. Selectin inhibition

The evaluation of biological carbohydrate interactions is often performed by using specific receptors (mostly lectins) that selectively capture carbohydrates. Among these techniques carbohydrate microarrays have been widely used in the last decade[127] as well as NMR spectroscopic studies[128] and mass spectrometry.[129] In the present work surface plasmon resonance (SPR) spectroscopy was applied for the evaluation of carbohydrate mimetics that target selectin proteins. This method has the major advantage that it is label-free, allows for real time monitoring of interactions, and reveals quantitative data.

4.6.1. Surface plasmon resonance

SPR is a spectroscopic analytical method for the detection of bimolecular interactions. On a thin functionalized gold surface (sensor chip) the ligand is immobilized and coupled at one site to an internal flow cell, where binding of a floating analyte is examined. Coupling to an optical interface at the other surface site detects the changes of the refractive index of polarized light upon analyte binding and dissociation. The measurement occurs in real time and is plotted in resonance units (RU) versus time in a sensorgram that can be followed on a computer screen (Figure 28). In the association phase the analyte binds to the immobilized ligand and reaches equilibrium. The dissociation of the analyte from the ligand (obtained by injecting buffer without the analyte) leads to a decrease of the analyte concentration at the sensor surface which therefore results in a decrease of the response (Figure 28, dissociation phase). To remove all the possible remaining ligands from the surface, a regeneration procedure is applied before a new cycle of interaction can be recorded (Figure 28, regeneration).

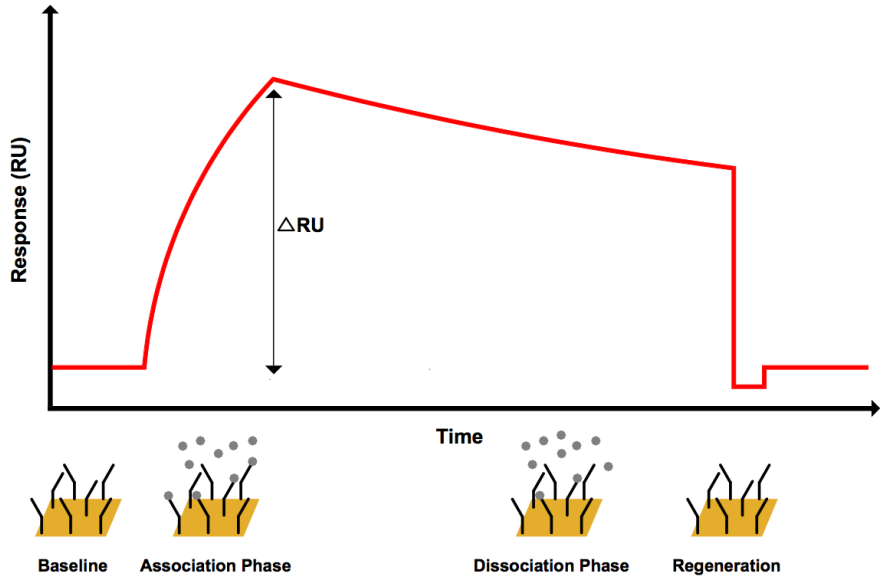


Figure 28. Schematic representation of an SPR-based interaction assay with resulting sensorgram (RU = resonance unit).

For the evaluation of the synthetized carbohydrate mimetics as potential selectin inhibitors a routine competitive binding assay previously established in the Dernedde group was applied.[130] In this assay, gold nanoparticles were prefunctionalized with selectins to mimic a leukocyte and binding to the immobilized ligand was recorded. This set-up mimics the physiological situation where leukocytes adhere to vascular endothelial cells in the blood stream (Figure 29). Using a Biacore X instrument, the natural selectin ligand sialyl-Lewis-X is immobilized on the gold surface of a sensor chip. As first measurement, selectin functionalized gold nanoparticles (AuNP) are injected in the flow chamber and the obtained sensorgram corresponds to a maximum binding. Recorded resonance units (RU) are set to 100% binding (Figure 29, control binding signal). The investigation of the potential inhibitors (carbohydrate mimetics) is done in a concentration-dependent manner and the inhibitor is pre-incubated with the selectin-functionalized AuNP at defined concentrations and injected into the flow chamber. Reduced binding signals occur in the case of successful selectin inhibition (Figure 29, reduced binding signal).

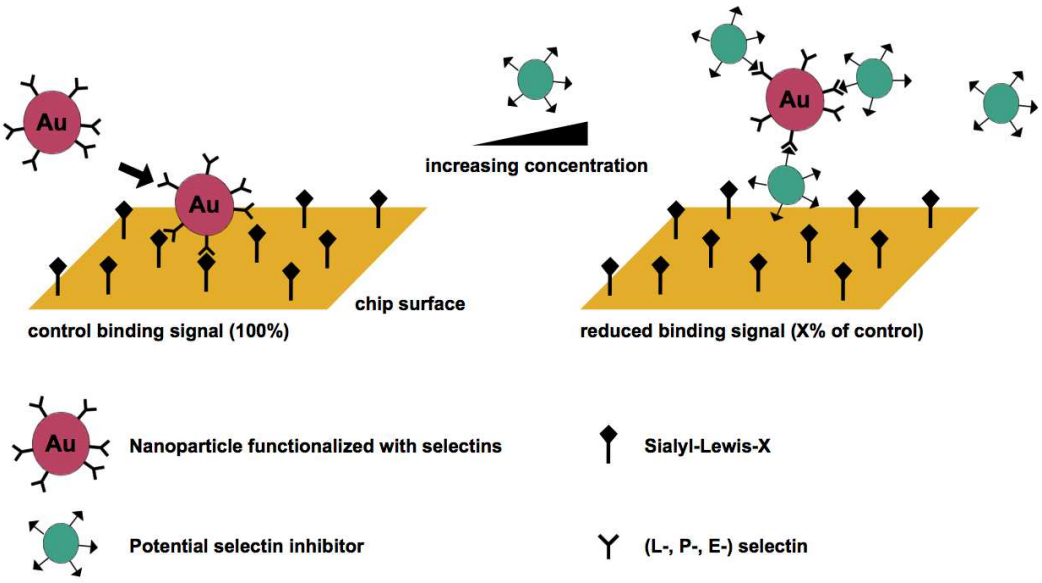


Figure 29. Competitive SPR-based in vitro selectin binding assay.

Dose response curves are recorded which allow the determination of the IC_{50} values, inhibitor concentrations at which the binding signal decrease to 50%. The highest concentration used for initial testing the inhibitor potential was set to 1 mM. The assay is described in more detail in the experimental section 6.3.1.

4.6.2. L-Selectin

4.6.2.1. Screening of compounds

A first screening was made with a range of all synthetized carbohydrate mimetics and derivatives from 2-amino-1,3-diol. In general, from the first screening it could be seen that most of the non-sulfated substances were not water soluble (it was quite surprising that compound **91** was soluble in aqueous media, but not compound **44**), therefore not applicable for testing (Figure 30, **97**, **44**, **140**). It was suspected that CTV derivative **140**, could be a good inhibitor candidate due to hexavalent ligand presentation, but unfortunately the compound also turned out to be insoluble in aqueous solution. Aminopyran derivative **91** and 2-amino-1,3-diol derivative **58** were successfully solubilized and tested in the SPR-based inhibition

assay. However, the outcome was not satisfactory: both showed no inhibitory activity for L-selectin at the maximum concentration of 1 mM.

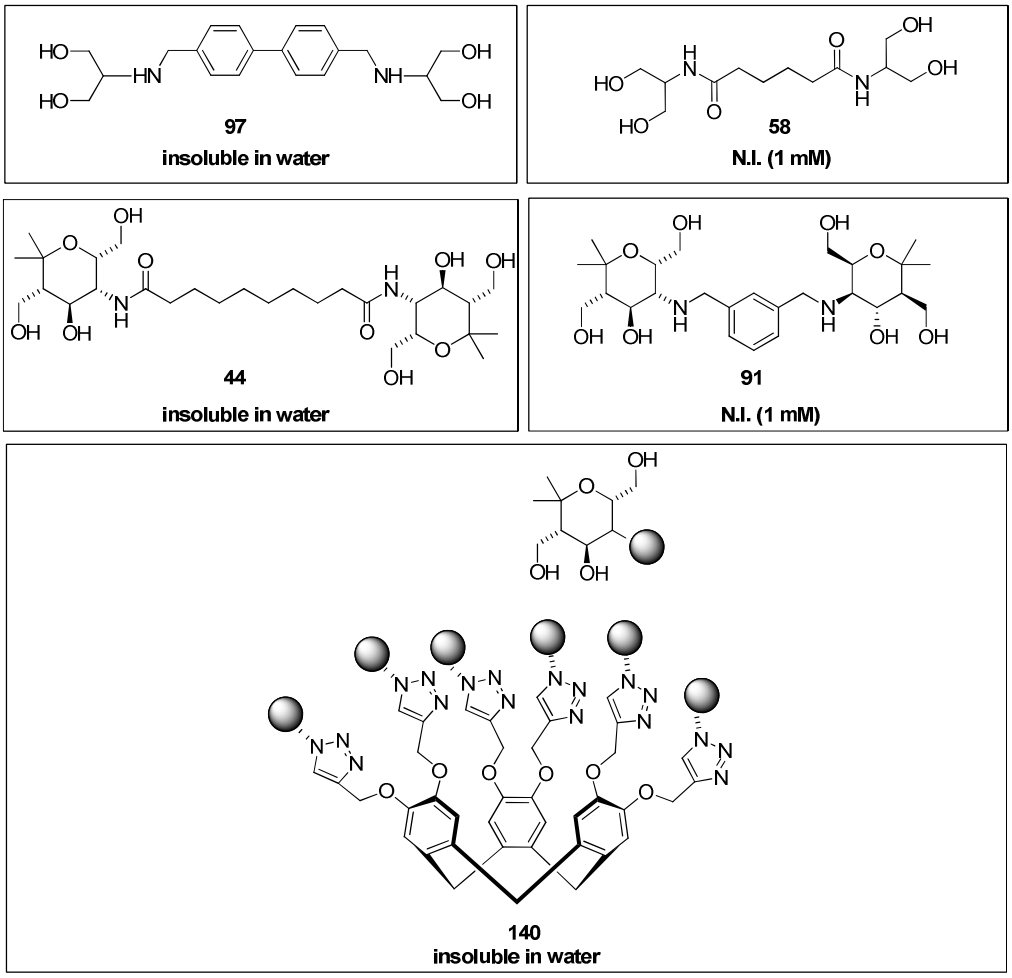


Figure 30. Initial testing of compounds for their application in SPR measurements (N.I. – no inhibition).

From these results it was assumed that the introduction of sulfate groups is important, not only for an enhancement of activity (as discussed previously), but also to overcome solubility problems. Although it would be interesting to compare the non-sulfated with the corresponding sulfated analogs, in the majority of the cases this was not possible. Therefore, a second screening was performed with fully sulfated substances (Figure 31).

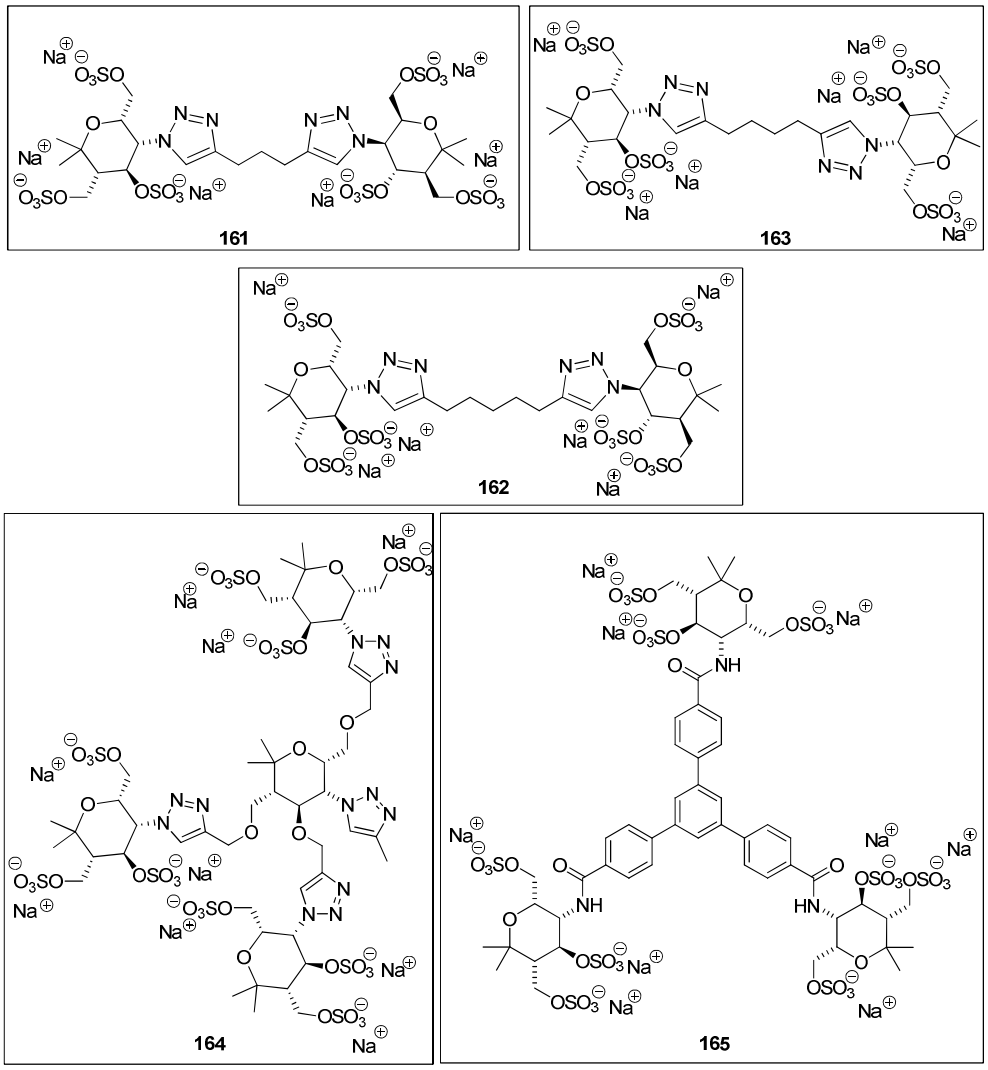


Figure 31. Structures of the sulfated carbohydrate mimetics **161 - 165** analyzed for L-selectin inhibition.

It can be seen from Figure 32 that at a maximum concentration of 1 mM all compounds reduced binding below 50%.

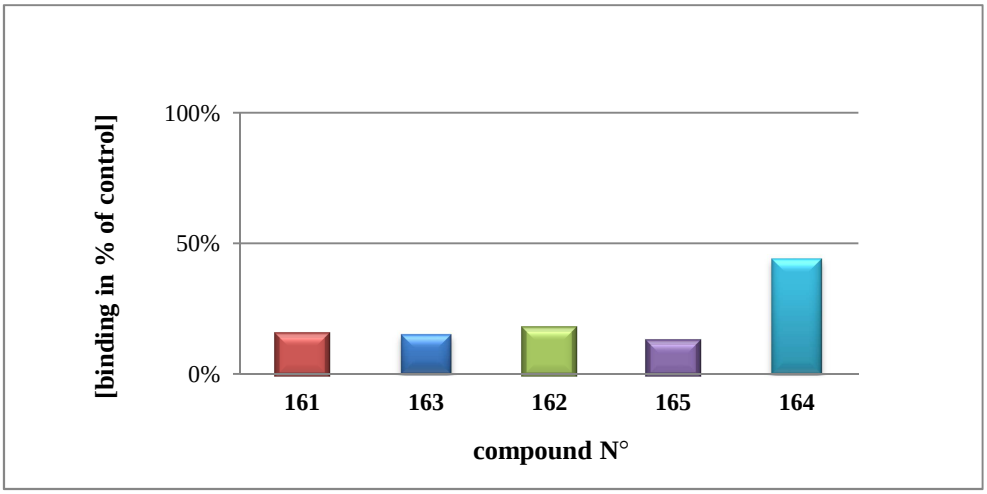
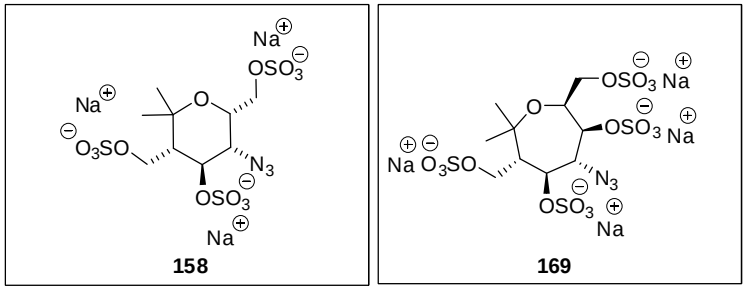


Figure 32. Inhibition of L-selectin dependent binding of compounds **161** - **165** at 1 mM concentration. Residual binding is given in % of the control (no inhibitor = 100%).

These were encouraging results and the sulfated compounds were systematically investigated in order to determine their corresponding IC_{50} values for L-selectin.

4.6.2.2. Monovalent carbohydrate mimetics

After observing that all the chosen sulfated compounds presented activity, sulfated azidopyran **158** was chosen as first test substance for a more detailed study. The determined IC_{50} for azidopyran **158** in the competitive L-selectin inhibition assay was 400 nM. This was a quite surprising result that a small molecule turned out to be a such good inhibitor. A comparison with the sulfated oxepane derivative **169** (Figure 33) which carries one additional sulfate group surprisingly revealed a ca. 10^3 worse IC_{50} value than azidopyran **158**.

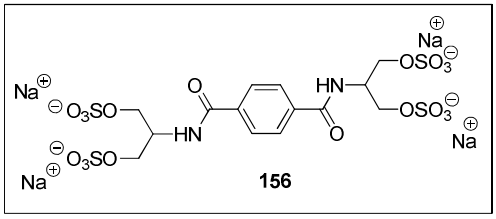


IC_{50} 0.4 µM **IC_{50} 210 µM**

Figure 33. Structure of azidopyran **158** and azidoopexane **169** and their respective IC_{50} values for L-selectin inhibition.

4.6.2.3.2-Amino-1,3-diol derivatives

The 2-amino-1,3-diol derivative **156** (Figure 34) was also tested in the competitive inhibition assay as inhibitor for L-selectin. Similar to azidopyran **158**, this small molecule showed a good inhibitory potential with an IC_{50} value of 1.0 μM.



IC_{50} 1.0 μM

Figure 34. Structure of amide **156** and respective IC_{50} value for L-selectin.

4.6.2.4. Divalent carbohydrate mimetics

The divalent carbohydrate mimetics **161**, **162** and **163** were analyzed (Figure 35). When comparing the obtained IC_{50} values of the three compounds, the linker has obviously an influence on selectin binding: an increase of the linker length apparently impairs the inhibiton. The large difference between substances **161** and **163** is quite surprising: with the difference of only one carbon atom, the inhibition potential of **163** dropped 40-fold. However, a similar effect was not observed for compounds **162** and **163**; here the further increase of the linker length by one extra carbon atom had almost no influence on the inhibitory activity.

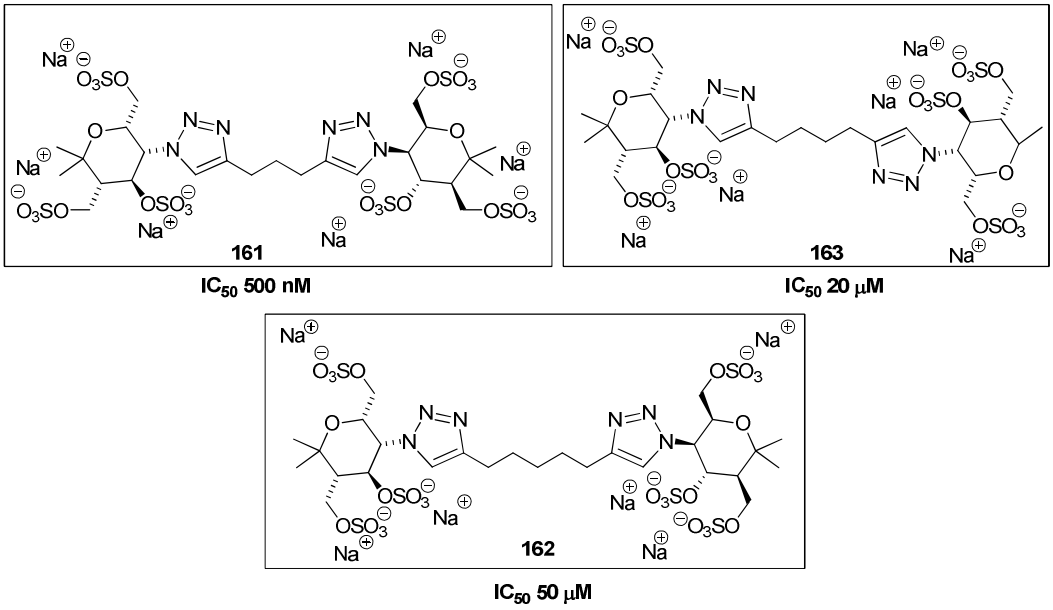


Figure 35. Structures of sulfated triazoles **161**, **163** and **162** and the respective IC_{50} values for L-selectin.

Quite interesting is the comparison of the inhibitory activity of triazole **162** bearing sulfated pyran moieties with the corresponding dimer **170** bearing oxepane units (Figure 36). The increasing number of sulfate groups would possibly lead to a better inhibition, but comparing these two similar compounds, aminopyran derivative **162** is a 10^3 times better inhibitor than the corresponding oxepane derivative **170**. This is the same activity decrease as observed for the two sulfated compounds **158** and **169** (Figure 33).

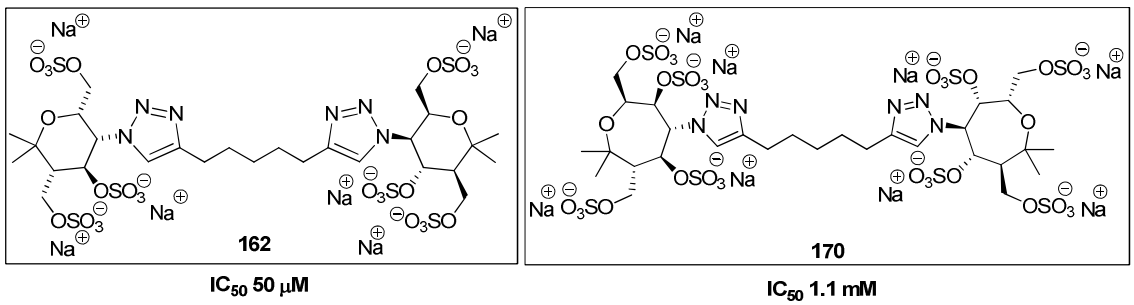


Figure 36. Structures of sulfated triazoles **162** and **170** and respective IC_{50} values for L-selectin inhibition.

Looking back to the results obtained with the functionalized gold colloids with acyclic aminoalcohols (Figure 37), a similar behavior was observed: of the mono-, di- and trisulfated compounds, the disulfate **Au-NP-4-sulf** proved to be the best one for interaction with the binding pocket. Either the reduction of one sulfate group as well as the increase of one led to a decrease of activity.

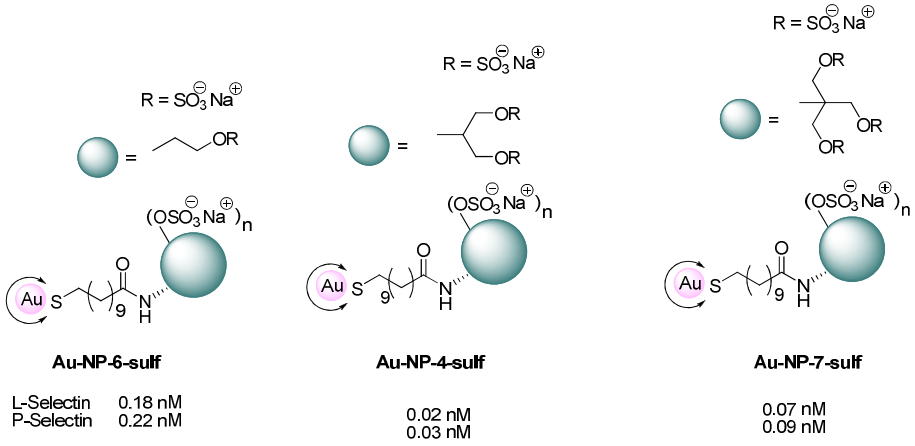


Figure 37. IC_{50}-Values for sulfated functionalized gold colloids **Au-NP-4-sulf**, **Au-NP-6-sulf** and **Au-NP-7-sulf**.

This might be the same situation observed with the pyran and oxepane compounds, where the oxepane seems not to be the right scaffold for the presentation of sulfates to selectin proteins. The overload with negative charge or the higher steric hindrance could induce this effect.

4.6.2.5. Trivalent carbohydrate mimetics

Figure 38 presents the structures of sulfated trivalent compound **164** and **165** and the corresponding IC_{50} values. Both compounds show comparable inhibitory potential regarding L-selectin inhibition. A structure activity relationship with respect to scaffold, linker or number of functional sulfate groups is not obvious.

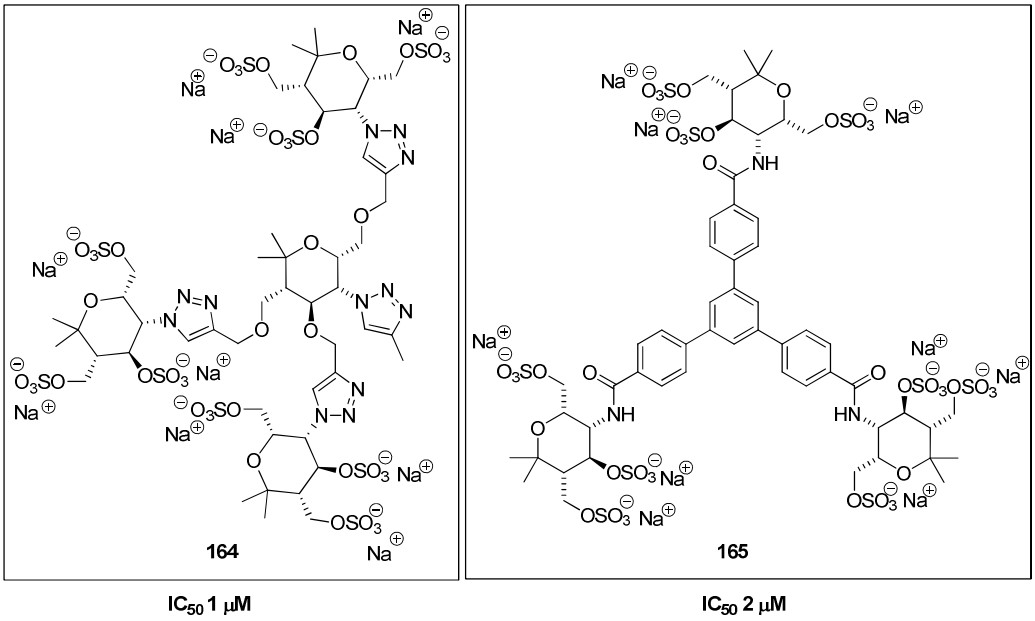


Figure 38. Structures of sulfated compounds, triazole **164** and amide **165** and their respective IC_{50} values for L-selectin.

The results obtained regarding the trivalent compounds **164** and **165** can be an indication that the triazole unit might indeed mimic the amide bond leading to similar results in the inhibition assay. It should be noticed that both structures present a remarkable difference in the central core: **164** presents a more flexible core while **165** has a planar and rigid structure; however, this difference does not have an impact on the inhibitory potential of both compounds. Once again, in comparison with the trivalent compound **171** bearing oxepane units (Figure 39), compounds **165** and **164** proved to be better inhibitors, but in this case the improvement was only a factor of 10 to 20.

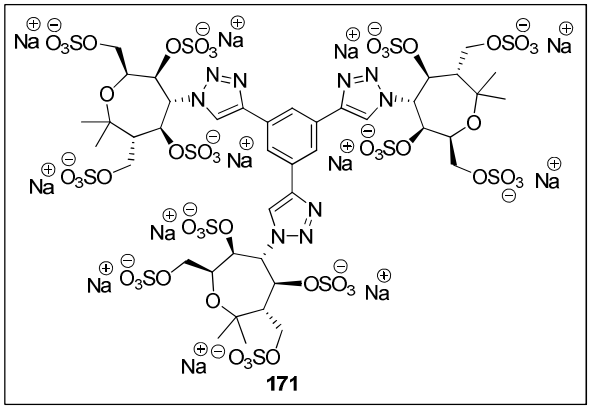


Figure 39. Structure of triazole **171** and its respective IC_{50} value for L-selectin.

Overall, some compounds proved to be very good L-selectin inhibitors with IC_{50} values in the high nM to low μM range. A comparison of the presented pyran-derived structures with the previously tested oxepane derivatives, shows that in all cases (from mono- to trivalent structures) a better inhibitory activity of the aminopyran derivatives was observed, with an improvement of up to 525-fold (see compound **158** versus **169**). Regarding the two best inhibitors sulfated azidopyran **158** and sulfated triazole **161**, it should be noticed that these substances showed a strange behavior at higher concentrations: up to a certain concentration, a further increase turned into a decrease of the inhibitory activity (see the corresponding curves in the experimental part). This behavior is so far not fully understood, but might be explained by bridging of selectin functionalized AuNP, which consequently enhance binding to the chip surface. Also quite surprising was the very good inhibitory activity of 2-amino-1,3-diol derivative **156**. When comparing all the examined compounds, a multivalency effect is not evident; more detailed analyses of the binding mode of the small compounds **158**, **161** and **156** are required for the data interpretation. Regarding the divalent triazoles it was clear that the linker length influences binding affinity in a way. Increase of the linker length lowered the inhibitory activity (see compounds **161**, **163** and **162**). This might indicate that the longer and more flexible dimers **163** and **162** were not appropriate to reach two binding sites presented on the selectin functionalized AuNP. They may be too flexible and the ligands are present in a unfavorable distance. Comparing divalent compounds **162** and **163** with the trivalent structures **164** and **165**, a multivalency effect can be seen: trivalent compounds proved to be 10 times better inhibitors.

4.6.3. P-Selectin

After the good inhibitory activity presented for L-selectin, all compounds were screened for potential inhibition of the second sulfate binding selectin, the P-selectin. Here it was of interest to analyze if any of the carbohydrate mimetics displays selectivity towards protein binding (L- versus P-selectin). The previously discussed divalent and trivalent structures were tested at the corresponding L-selectin specific IC_{50} concentrations for P-selectin inhibition (Figure 40). All compounds were also active on P-selectin, but **161**, **162**, and **163** showed a more pronounced preference to bind L-selectin. Only the trivalent compounds **164** and **165** reached nearly comparable IC_{50} values for L- and P-selectin.

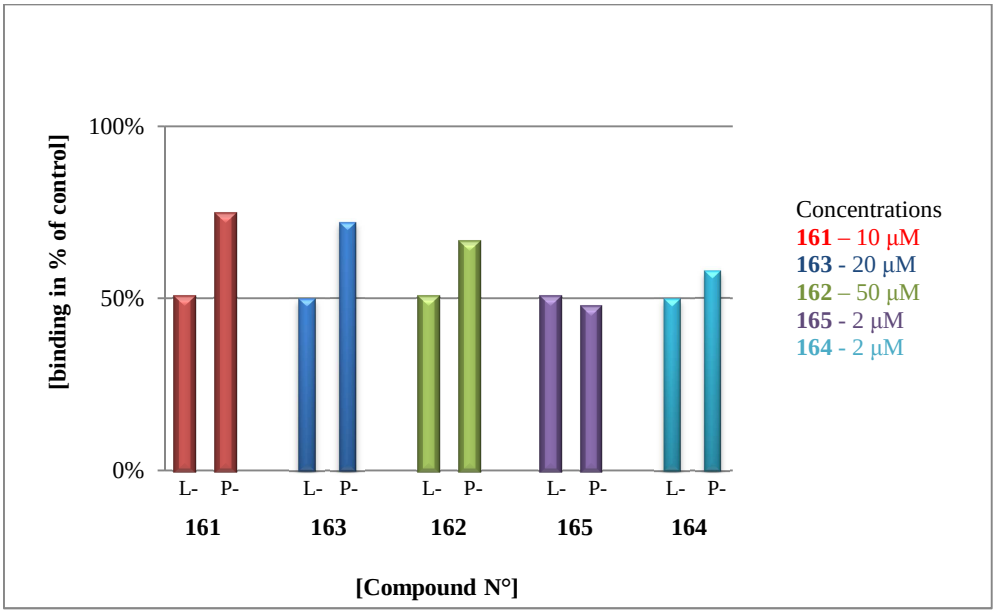


Figure 40. Selectivity screening for P-selectin inhibition of compounds **161 - 165** at compound concentrations that inhibit L-selectin to 50% (IC_{50}).

4.6.4. E-Selectin

In the following experiment also E-selectin binding was analyzed with the same competitive assay. Again selected substances were tested at the corresponding L-selectin specific IC_{50} concentrations for E-selectin inhibition (Figure 41). Here, only very moderate reduction of binding was observed. This phenomenon was not unexpected, since E-selectin ligand binding is not charge-dependent.

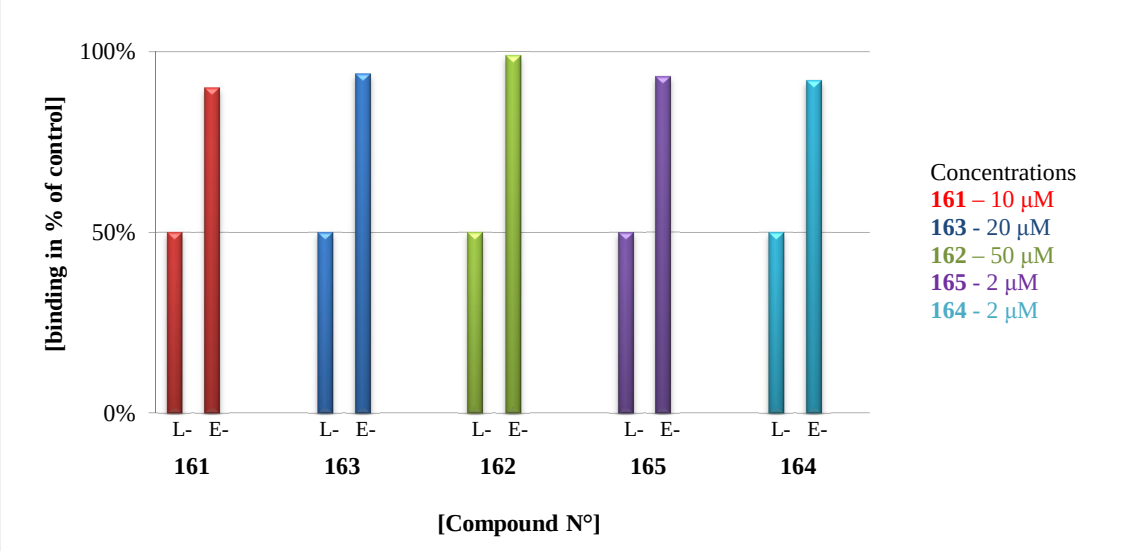


Figure 41. Selectivity screening for E-selectin inhibition of compounds **161** - **165** at compound concentrations that inhibit L-selectin to 50% (IC_{50}).

Therefore, additional compounds were chosen and tested (Figure 42). The uncharged compounds were selected based on their inhibitory effect for L-selectin. Since sulfated triazole **161** proved to be the best one relatively to the other sulfated triazoles, the corresponding compound **106** with hydroxyl groups instead of sulfates was chosen. For the trivalent compound **164** that also presented to be the slightly better one from the two tested, the corresponding precursor **126** was chosen. Moreover it was thought that tetramer **128** could be a good candidate; unsatisfactorily, none of the chosen structures presented any inhibitory activity, even when tested at the maximum concentration of 1 mM (Figure 43).

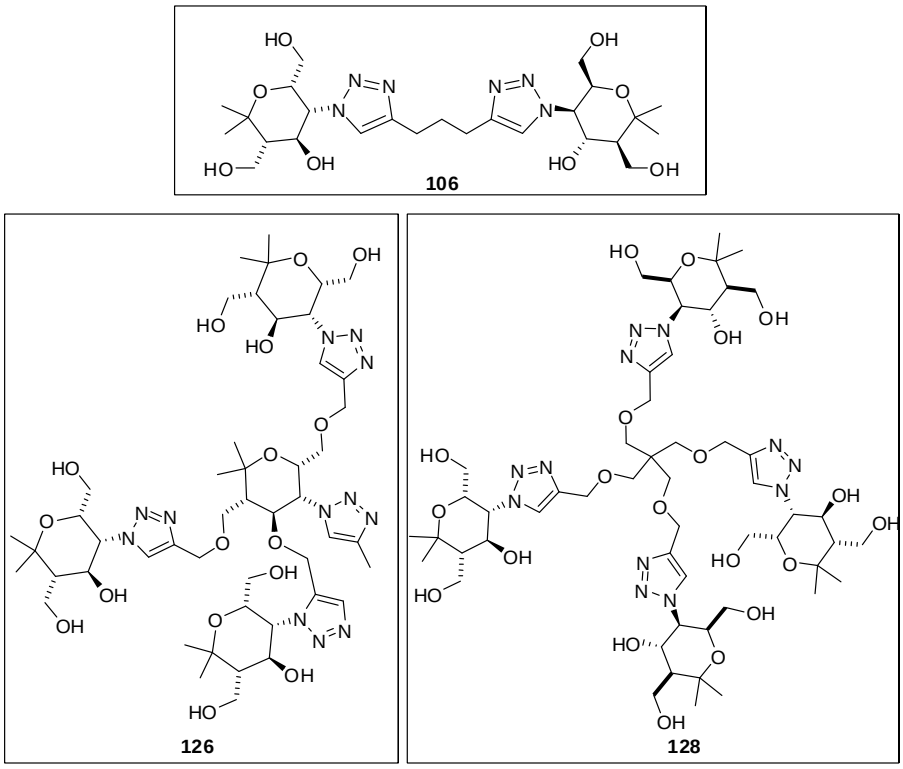


Figure 42. Structures of carbohydrate mimetics **106**, **126** and **128** screened as potential E-selectin inhibitors.

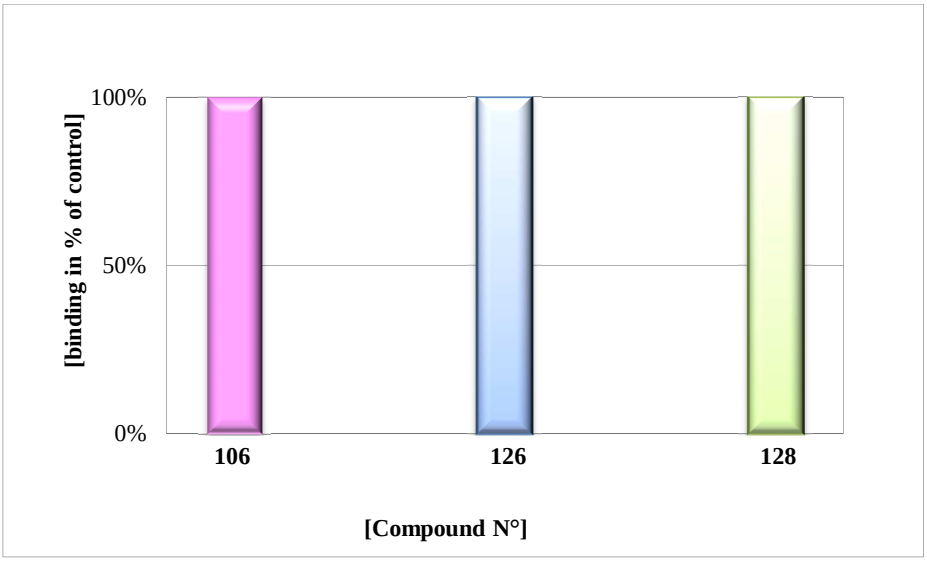
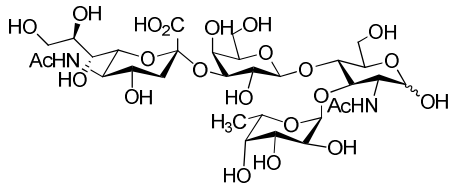
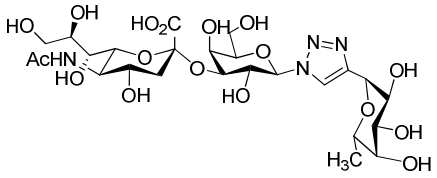
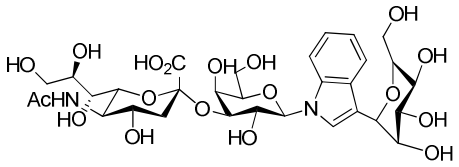


Figure 43. Influence on E-selectin dependent ligand binding of compounds **106**, **126** and **128** at 1 mM concentration.

4.6.5. Comparison with previously reported selectin inhibitors

Several compounds and their corresponding IC_{50} values as selectin inhibitors are reported in the literature however, a direct comparison of these with the presented carbohydrate mimetics is not trivial since a different assay were applied. To avoid misleading information or comparisons, the presented compounds will solely be compared with reported results obtained from the exactly same assay format. In a collaboration work between the Dernedde group and the Opatz group, different sialyl-Lewis-X mimetics were evaluated in the SPR inhibition assay as selectin inhibitors (Table 22).[131] For comparison the natural selectin ligand, the tetrasaccharide sialyl-Lewis-X was included (Table 22, entry 1). It is interesting to note, that under the applied conditions the natural ligand does not show any inhibitory activity for L- and P-selectin and only values for for E-selectin inhibition could be detected.

Table 22. IC_{50} values of sialyl-Lewis-X and Opatz mimetics **172** and **173**.

Entry	Compound	IC_{50} value (mM)		
		L-selectin	**P-selectin**	**E-Selectin**
1	 **sialyl-Lewis-X**	-	-	0.7
2	 **172**	2.3	2.2	-
3	 **173**	1.8	0.7	7.4

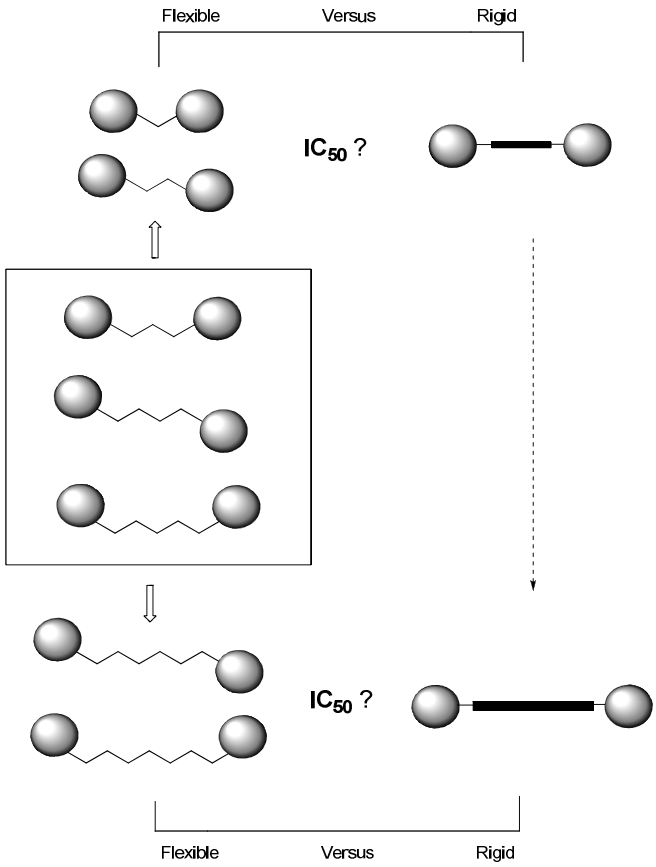
Although, a direct structure-activity between the presented carbohydrate mimetics and the Opatz mimetics is not possible due to their structural diversity, it can be seen that all the presented carbohydrate mimetics (from mono- to trivalent) exhibit an enhanced activity for L-selectin when compared to the Opatz mimetics that already show quite good inhibitory activity for compounds without any sulfate groups. Moreover, when considering mimetic **172**, it can be seen that no selectivity was obtained between L- and P-selectin and for compound **173** only a slight selectivity was observed; these results are also with agreement with those observed for the presented structures, where no considerable selectivity was obtained. Regarding E-selectin inhibition, it can be seen that comparable to the presented carbohydrate mimetics, Opatz's compound **172** also did not show any inhibitory activity. Interesting is the Opatz mimetic **173** that with a similar structure to **172** shows activity as E-selectin inhibitor. This structure can give some leads to future development of selectin inhibitor mimetics and relatively similar structures should be considered and slightly modified to achieve an improvement in the inhibitory activity. Also, a combination of the presented pyran mimetic units with real sugars joined in the same compound could bring some activity enhancement.
For a later in vivo application, the cytotoxicity of all compounds that proved to be good inhibitors should be evaluated.

5. Summarizing discussion and Outlook

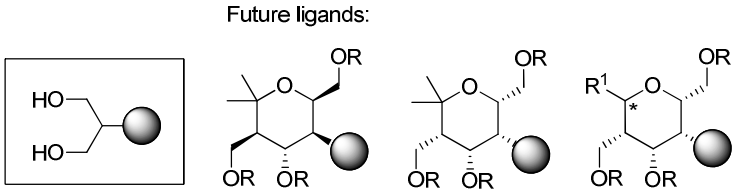
Intensive investigations of carbohydrate mimetics and their applications have been performed by the Reißig group over the last years. Their versatility and possible transformations make them easily available building blocks that can be used, as presented in this work, for the construction of multivalent structures that mimic natural carbohydrates in a reasonably simple synthetic route. A library of mono- to oligovalent compounds could successfully be prepared through three different main routes: Schotten-Baumann reaction, reductive amination and Copper(I)-catalyzed azide alkyne cycloaddition. Although reductive amination reactions led to lower yields, in general no reactivity problems were faced. Regarding the obtained amides and triazoles, even with compounds with high valency, the purification problems normally faced with polar compounds could be avoided and the desired final compounds were isolated in good yields.

The sulfation procedure could be optimized for the polysulfation of mono- to trivalent structures, and a characteristic series of compounds could successfully be obtained and tested in the SPR inhibition assay. Unfortunately this procedure seems not to be appropriate for the polysulfation of compounds with higher valency. Alternatively, the use of purification methods like HPLC or convergence chromatography (that combines LC and GC techniques) should be considered to separate the obtained mixtures. After this separation, the percentage of sulfate groups in the obtained products can be determined to test them as potential inhibitors.

Due to the complexity of biochemical processes, the evaluation of carbohydrate-protein interactions is, in most of the cases, not trivial. An exact knowledge of the in vivo interactions should be understood for a perfect development of a synthetic mimetic. The interpretation of the results obtained from the SPR inhibition assay demonstrated to be quite complex showing that this is still an essentially unexplored field. In between the divalent carbohydrate mimetics tested an influence of the linker length in the inhibitory activity was clear. The increasing of the linker length from three to five carbons lowered the inhibitory activity. To understand the optimal spacing between the pyran units, analogs bearing one and two carbons should be synthetized and tested. It is also interesting to evaluate if with longer chains the activity potential would continue to decrease. As a further study, the use of rigid linkers with the same spacing in between the pyran units could bring some insight about the optimal structure rigidity.

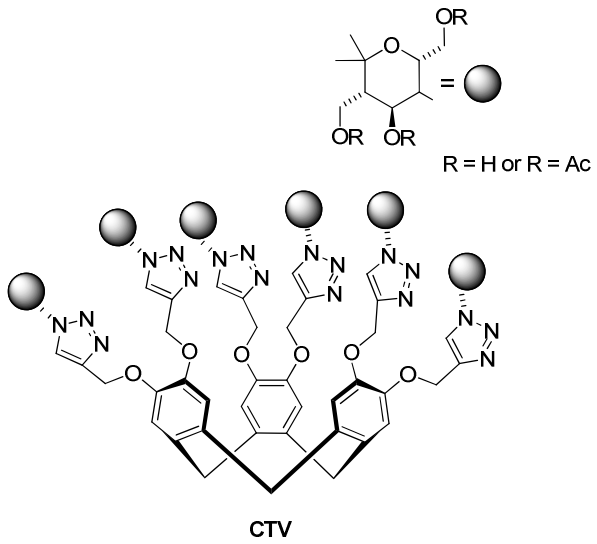


Of interest would be the sulfation and further SPR measurements of the amino polyol derivatives bearing the same liker and spacing as the aminopyran compounds tested and the use of carbohydrate mimetics similar to the aminopyran used in this study, for example, its enantiomer, diastereomer and other aminopyran derivatives bearing different groups instead of the gem-di-methyl group. The synthetic route to such analogs has previously been investigated and established in the Reißig group.[29a],[39]

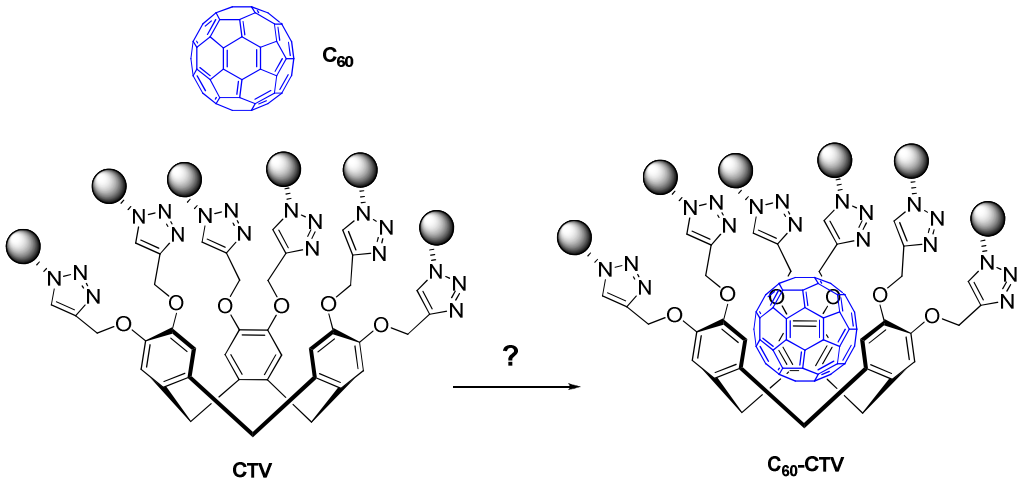


Moreover, regarding the mimetics that showed a particular behavior in the SPR assay, a molecular modeling study and the use of X-ray crystallography to obtain a crystal structure of the mimetic-selectin complex could bring further information about their mode of action. Efforts should be focused on a better understanding of each tested carbohydrate mimetic for a rational design and development of future analogs.

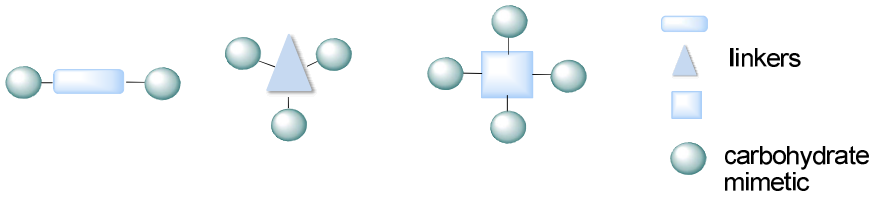
From the synthetized carbohydrate mimetics, the hexavalent CTV derivative bearing pyran units should be further studied with respect to its conformation. The derivatives bearing acetyl and hydroxyl groups have to be obtained in suitable crystalline form and the corresponding X-ray structures evaluated for a better understanding



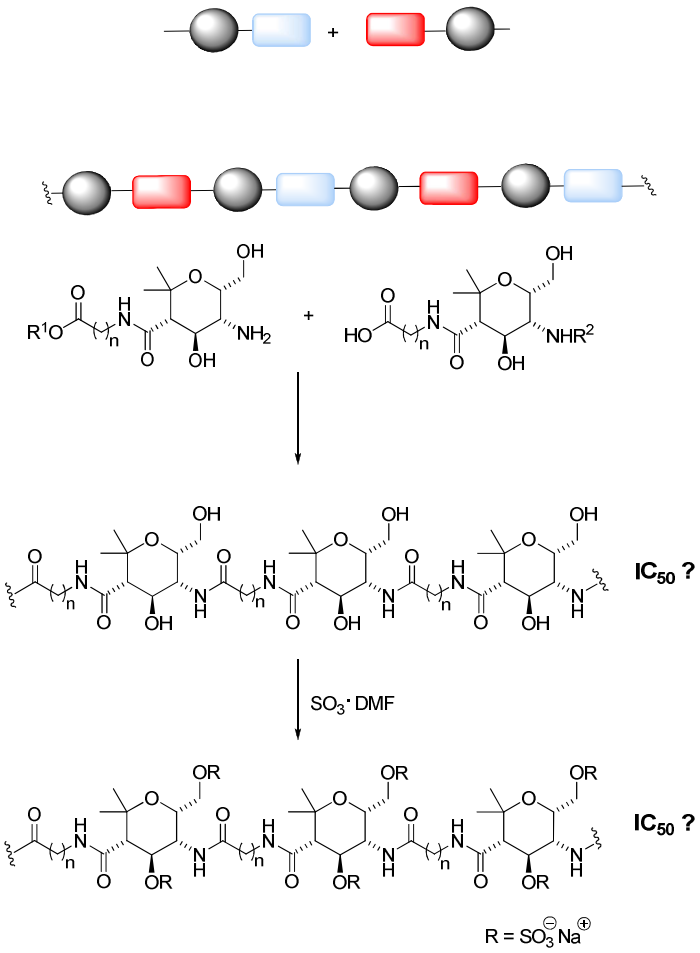
The hexavalent CTV derivative bearing free hydroxyl groups was not a suitable candidate for the SPR measurements due to its insolubility in water, but other applications could be found for this compound. A possibility could be its use as host for fullerenes.[101b] The properties of the resulting host-guest complex could be studied; however applications can also be limited due to the solubility of the final C_{60}-CTV unit; it is known that the low solubility of fullerenes in aqueous media is one of the major obstacles for its biological applications.[132]



In the presented work all multivalent carbohydrate mimetics were constructed based on central linkers that differ in their flexibility and length; according to the number of reacting sites, these units could be decorated with carbohydrate mimetics.



In a possible continuation of Prisyazhnyuk work,[29b] the construction of multivalent carbohydrate mimetics in a linear form would be of interest. In this case, the spacing and number of pyran units should be varied. The study of such structures is interesting since both sialyl-Lewis-X and heparin are oligosaccharides that present a linear structure with only few branches.



Overall, the focus on such small molecules gather new insights in the carbohydrate mimetics-selectin interactions. However, the presented results bring new challenges and show that these molecular mechanisms are still not fully understood. The continuation of these studies can lead to promissing strategies for an optimal ligand-receptor affinity.

6. Experimental Part

6.1. General information

6.1.1. Analytical methods

Melting points of solid compounds were measured with a Reichert apparatus (Thermovar) and are uncorrected.

NMR spectra were recorded on BRUKER (AV 500, AV 700) and JEOL (ECP 500) instruments at room temperature. Chemical shifts (δ) are listed in parts per million (ppm) and are reported relative to solvent residual signals: $CDCl_3$ (^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.2 ppm), CD_3OD (^{1}H: δ = 3.31 ppm, ^{13}C: δ = 49.0 ppm), DMSO-d_6 (^{1}H: δ = 2.50 ppm, ^{13}C: δ = 116.6 ppm), DMF-d_7 (^{1}H: δ = 2.75 ppm, ^{13}C: δ = 29.76 ppm), D_2O (^{1}H: δ = 4.79 ppm). Integrals are in accordance with assignments; coupling constants (*J*) are given in Hz. All ^{13}C NMR spectra are proton-decoupled. Multiplicity is indicated as follows: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), dt (doublet of triplet), td (triplet of doublet), m (multiplet), m_c (centered multiplet). For detailed peak assignments 2D spectra were measured (COSY and HMQC).

IR spectra were measured with a Jasco spectrometer (FT/IR-4100 with DLATGS Detector).

HRMS analyses were performed with Agilent 6210 (ESI–TOF, 10 μL/min, 1.0 bar, 4 kV) and Varian/Agilent Ionspec QFT-7 (ESI–FTICR, 4 μL/min, 1.0 bar, 4kV) instruments.

Elemental analyses were carried out with instruments from Elementar (Vario EL, Vario EL III).

6.1.2. Chromatography

Thin layer chromatography (TLC) analyses were performed on silica gel coated aluminium plates purchased from Merck. Products were detected by UV-activity and by using staining reagents (Cer/molybdenum reagent, $KMnO_4$ and ninhydrine).

Preparative column chromatograpy was performed on silica gel (230–400 mesh, MACHERY-NAGEL), Celite® (535 from SIGMA ALDRICH) and by RP-HPLC (Gemini®-NX C18 Phenomenex).

6.1.3. Preparative methods, solvents and reagents

Reactions were generally performed under inert atmosphere (argon) in flame-dried flasks. Solvents and reagents were added by syringe or EPPENDORF pipettes. Solvents were dried using standard procedures and were purified with a MB SPS-800-dry solvent system. Commercial available reagents were used as received without further purification unless otherwise stated. Unless otherwise stated, yields refer to analytical pure samples. Hydrogenolyses were performed with hydrogen from Air Liquide (Alphagaz 2).

Microwave reactions were performed with a MILESTONE μ-Chemist instrument (2.45 GHz, wavelength: 12.25 cm)

The following compounds were prepared analogously to literature procedures: **11**,[36] **12**,[36] **89**,[67] **136**,[100] **137**,[106] **138**,[101b] **143**,[112] **145**,[112] **146**,[112] **149**,[113] **150**.[113]

6.1.4. General procedures

Propargylation of alcohols (GP-1): To a cooled solution of starting material (1.0 equiv.) in anhydrous DMF (3.7 mL/mmol) was added NaH (1.5 equiv. per OH) under argon atmosphere. The resulting suspension was stirred at r.t. during the indicated time. After cooling to 0 °C, propargyl bromide (4 equiv. per OH) was added dropwise and the reaction mixture was stirred during the indicated time. MeOH was added and the mixture was stirred for 30 min. After removing of volatiles in vacuo, the resulting mixture was dissolved in water and extracted with EtOAc (3x). The combined organic phases were dried with Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by flash column chromatography.

Amide bond by coupling (GP-2): Under argon atmosphere, the amine (1.0 equiv.), the corresponding carboxylic acid (1.0 equiv.) and HATU (1.0 equiv.) were dissolved in DMF (8 mL/mmol). After addition of Et_3N (4.5 equiv.), the reaction mixture was stirred at r.t. during the indicated time. After removing the solvents in vacuo, the crude product was purified by flash column chromatography.

Amide bond by Schotten-Baumann reaction (GP-3): Under argon atmosphere, the amine (1.0 equiv.) was dissolved in CH_2Cl_2 (8 mL/mmol) and the solution was cooled to 0 °C. After addition of Et_3N (2.0 equiv.) and the corresponding acid chloride (1.2 equiv.) the reaction mixture was stirred from 0 °C to r.t. during the indicated time. H_2O was added to the mixture and the aqueous phase was extracted with CH_2Cl_2 (3x). The combined organic phases were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography.

TBS deprotection with HF·pyridine (GP-4): To a stirred solution of starting material (1 equiv.) in THF (9 mL/mmol) at 0 °C, was added HF·pyridine (ca 65-70% HF, 8 equiv.) under argon atmosphere. After warming up to r.t., the reaction mixture was stirred during the indicated time. H_2O was added to the mixture and the aqueous layer was extracted with CH_2Cl_2 (3x). The organic phase was dried with Na_2SO_4 and the solvents were removed in vacuo. The crude product was purified by flash column chromatography.

TBS deprotection by solvolysis (GP-5): To a stirred solution of starting material (1.0 equiv.) in isopropanol (3 mL/mmol) was added AcCl (0.6 equiv.) at 0 °C. The reaction mixture was stirred at r.t. during the indicated time. All volatiles were removed in vacuo affording the desired product without further purification.

Reductive amination (GP-6): The amine (1.0 equiv.) was dissolved in MeOH (16 mL/mmol) and the corresponding aldehyde (1.0 equiv.) was added. After addition of $MgSO_4$ (2.0 equiv.) the mixture was stirred at r.t. After complete consumption of **3** (monitored by TLC), $MgSO_4$ was filtered off and the filtrate was cooled to 0 °C. $NaBH_4$ (1.1 equiv.) was added and the mixture was stirred at r.t. during the indicated time. The reaction was quenched with H_2O and extracted with CH_2Cl_2 (3 x). The combined organic phases were washed with brine, dried with Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by flash column chromatography.

CuAAC using TBTA (GP-7): Under argon atmosphere, the azidopyran **10** (1.0 equiv.) was dissolved in MeCN (40 mL/mmol). TBTA (0.23 equiv.) and CuI (0.23 equiv.) were added, followed by the addition of the corresponding alkyne (1.2 equiv.) and Et_3N (0.23 equiv.). The resulting mixture was stirred at r.t. during the indicated time. The solution was quenched with 7N NH_3 in MeOH and the resulting mixture was filtered through a short silica gel column and washed with a mixture of CH_2Cl_2/7N NH_3 in MeOH (10:1). After removing the solvents in vacuo, the crude product was purified by flash column chromatography.

CuAAC using DIPEA/AcOH (GP-8): To a suspension of the azidopyran **10** (1.0 equiv.) in toluene (1 mL/mmol) was added CuI (0.4 equiv.), the corresponding alkyne (1.2 equiv.) followed by DIPEA (4.0 equiv.) and HOAc (4.0 equiv.). The reaction mixture was stirred at r.t. during the indicated time. The solution was quenched with 7N NH_3 in MeOH and the resulting mixture was filtered through a short silica gel column and washed with a mixture of CH_2Cl_2/7N NH_3 in MeOH (10:1). After removing the solvents in vacuo, the crude product was purified by flash column chromatography.

CuAAC using Cu/C (GP-9): To a solution of the azidopyran **10** (1.0 equiv.) in dioxane (3 mL/mmol) was added Cu/C (0.1 - 0.5 equiv.), Et_3N (1.2 - 3.0 equiv.) and the corresponding

alkyne (1.2 equiv.). The reaction mixture was stirred at 60 °C during the indicated time. The mixture was filtrated through a pad of Celite® and washed with MeOH. When indicated, the product was further purified by flash column chromatography.

Metal free "click" reaction using tosylhydrazones (GP-10): To a cooled solution of the amine (1.0 equiv.) in EtOH (12 mL/mmol), KOAc (3.0 equiv.) was added and the resulting solution was stirred for 10 min. at 0 °C. The corresponding tosylhydrazone (1.3 equiv.) was dissolved in MeCN (6 mL/mmol) and added dropwise to the reaction mixture. Stirring was continued at r.t. until the reaction was complete (monitored by TLC). All volatiles were removed in vacuo and the crude product was purified by flash column chromatography.

Polysulfation (GP-11): The polyol (1.0 equiv.) was dissolved in DMF-d_7 (0.6 – 1.0 mL). The solution was cooled to 0 °C and SO_3·DMF (3.0 equiv. per OH) was added. The reaction mixture was stirred at r.t. during the indicated time. The reaction conversion was followed by ^{1}H-NMR spectroscopy (500 MHz or 700 MHz). When indicated, additional SO_3·DMF (1.0 – 3.0 equiv. for each OH group) was added and the reaction mixture was stirred at r.t. during the additional given time until full conversion was observed. The obtained sulfated intermediates were directly converted into the corresponding sodium salts according to Method A or Method B.

Method A: The reaction mixture was cooled to 0 °C and an aq. solution of NaOH 1 M was added dropwise until pH 10 – 12 was reached. The solvents were removed in vacuo and the crude product was purified by dialysis in H_2O.

Method B: The reaction solution was cooled to 0 °C and an aq. solution of NaOH 0.5 M was added dropwise until pH 7 – 9 was reached. The reaction mixture was filtrated through an ion exchange DOWEX® Na^+ column. The solvents were removed in vacuo and the crude product was purified by dialysis in H_2O.

The final products were filtrated through a syringe filter (diam. 25 mm; pore size 0.2 μm; PTFE membrane) when indicated.

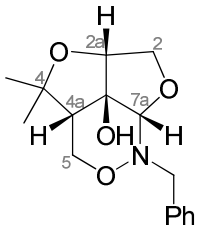
6.2. Experimental procedures

6.2.1. Synthesis of starting materials

(4a*R*,7a*S*,7b*S*)-7-Benzyl-7b-hydroxy-4,4-dimethylhexahydro-2*H*,4*H*-1,3,6-trioxa-7-azacyclopenta[*cd*]indene (**14**)

JNS103A

The ketone **2** (purity after ^{1}H NMR: 95%, 4.75 g, 16.3 mmol) was dissolved in EtOH (205 mL) under argon atmosphere and cooled to 0 °C. $NaBH_4$ (1.26 g, 33.4 mmol) was added portionwise and the reaction mixture was stirred from 0 °C to r.t. for 1 h. After 3 h, $NaBH_4$ (0.132 g, 3.50 mmol) was added at 0 °C. After stirring for 4.5 h at r.t, the solvent was removed in vacuo. The resulting residue was dissolved in CH_2Cl_2 (250 mL) and H_2O (500 mL). The aqueous phase was extracted with CH_2Cl_2 (4 x 250 mL) and with EtOAc (2 x 150 mL). The combined organic phases were dried with Na_2SO_4 and the solvent was removed in vacuo. Purification by flash column chromatography (silica gel, hexanes/EtOAc 1:1, 1:2 to pure EtOAc) afforded product **13** (3.35 g, 70%)[29a] as colorless crystals and side product **14** (212 mg, 4.4%) as light yellow crystals.



14

Chemical Formula: $C_{16}H_{21}NO_4$

Molecular Weight: 291.3 g·mol^{-1}

Melting point: 112 – 115 °C

Optical rotation: $[\alpha]_D^{22}$ = -11.7 (c = 1.32, CH_2Cl_2)

^{1}H NMR (700 MHz, $CDCl_3$): δ = 1.35, 1.41 (2 s, 3 H each, CH_3), 1.96 (m_c, 1 H, 4a-H), 2.74 (bs, 1 H, OH), 3.89 (A part of ABX system, J_{AB} = 10.7 Hz, J_{AX} = 4.8 Hz, 1 H, 2-H), 3.90 (m_c 1 H, 5-H), 3.96 (A part of AB system, J_{AB} = 14.1 Hz, 1 H, CH_2Ph), 3.98 (B part of ABX system, J_{AB} = 10.7 Hz, J_{BX} = 1.3 Hz, 1 H, 2-H), 4.08 (B part of ABX system, J_{AB} = 12.5 Hz, J_{BX} = 5.1 Hz, 1 H, 5-H), 4.22 (B part of AB system, J_{AB} = 14.1 Hz, 1 H, CH_2Ph), 4.33 (s, 1 H,

7a-H), 4.43 (dd, *J* = 1.3, 4.8 Hz, 1 H, 2a-H), 7.26 – 7.29 (m, 1 H, Ph), 7.32 – 7.34, 7.38 – 7.39 (2 m, 2 H each, Ph) ppm.

^{13}C NMR (125 MHz, $CDCl_3$,): δ = 24.3, 30.6 (2 q, CH_3), 53.8 (d, C-4a), 57.8 (t, CH_2Ph), 65.7 (t, C-5), 71.7 (t, C-2), 85.6, 86.2 (2 s, C-4, C-7b), 87.1 (d, C-2a), 95.1 (d, C-7a), 127.4, 128.5, 128.7 (3 d, Ph), 137.0 (s, Ph) ppm.

IR (ATR): ν = 3420 (OH), 2970-2865 (C-H), 1495 (C=C), 1225 (C-O), 1080 (C-O-C) cm^{-1}

HRMS (pos. ESI-TOF): $C_{16}H_{21}NO_4 \cdot Na^+$: calcd.: 314.1369
found: 314.1373

Elemental Analysis: $C_{16}H_{21}NO_4$ (291.3) calcd. (%): C 65.96, H 7.27, N 4.81
found (%): C 65.41, H 7.31, N 4.68

6.2.1.1. Hydrogenolysis

Method 1 (MeOH)

JNSV13

Compound **13** (50 mg, 0.17 mmol) was dissolved in MeOH (3 mL) and palladium on charcoal (10% Pd, 74 mg) was added. Hydrogen was bubbled through the suspension for 45 min and afterwards the reaction mixture was stirred overnight under the hydrogen atmosphere. The suspension was filtrated through a pad of Celite® and washed with MeOH. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 9:1 to 4:1). The desired aminopyran **3** (20 mg, 57 %) was obtained as a slightly yellow solid. By-product **19** (6 mg, 16 %) was isolated as slightly yellow solid.

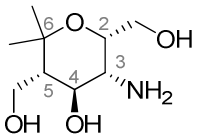
Method 2 (Isopropanol)

JNS290

Bicyclic compound **13** (1.37 g, 4.67 mmol) was dissolved in isopropanol (157 mL) and palladium on charcoal (10% Pd, 1.65 g) was added. The suspension was saturated with hydrogen for 1 h and stirred under the hydrogen atmosphere overnight. The solution was

filtrated through a short pad of Celite®, washed with isopropanol and subsequently concentrated in vacuo affording aminopyran **3** (916 mg, 96%) as a colorless solid.

(2*S*,3*R*,4*S*,5*S*)-3-Amino-4-hydroxy-6,6-dimethyltetrahydro-2*H*-pyran-2,5-diyl)-dimethanol (**3**)



3

Chemical Formula: $C_9H_{19}NO_4$

Molecular Weight: 205.3 g·mol^{-1}

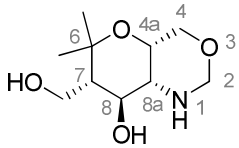
Melting point: 106 – 108 °C

^{1}H NMR (400 MHz, CD_3OD): δ = 1.24, 1.38 (2 s, 3 H each, CH_3), 1.73 (ddd, *J* = 4.9, 5.7, 7.4 Hz, 1 H, 5-H), 2.91 (dd, *J* = 3.7, 4.9 Hz, 1 H, 3-H), 3.61 – 3.69 (m, 4 H, 5-CH_2, 2-CH_2, 4-H), 3.84 (dd, *J* = 5.7, 11.4 Hz, 1 H, 5-CH_2), 4.00 (dt, *J* = 3.7, 6.2 Hz, 1 H, 2-H) ppm.

Elemental Analysis: $C_9H_{19}NO_4$ (205.3) calcd. (%): C 52.67, H 9.33, N 6.82
found (%): C 52.30, H 9.39, N 7.05

The analytical data are in agreement with those reported in the literature.[29a]

(4a*S*,7*S*,8*S*,8a*R*)-7-(Hydroxymethyl)-6,6-dimethyloctahydropyrano[3,2-d][1,3]oxazin-8-ol (**19**)



19

Chemical Formula: $C_{10}H_{19}NO_4$

Molecular Weight: 217.3 g·mol^{-1}

Melting range: 138 – 142 °C

Optical rotation: $[\alpha]_D^{22}$ = -2.5 (c = 10.3, MeOH)

^{1}H NMR (500 MHz, CD_3OD): δ = 1.11, 1.29 (2 s, 3 H each, CH_3), 1.85 (ddd, *J* = 2.7, 8.0, 10.8, 1 H, 7-H), 2.98 (dd, *J* = 3.0, 4.8 Hz, 1 H, 8a-H), 3.60 (q, *J* = 3.0 Hz, 1 H, 4a-H), 3.63 – 3.77 (m, 4 H, NH, 8-H, 7-CH_2), 3.84 (dd, *J* = 3.0, 12.6 Hz, 1 H, 4-H), 4.00 (dd, *J* = 3.0, 12.6 Hz, 1 H, 4-H), 4.16, 4.51 (2 d, *J* = 10.2 Hz, 1 H each, 2-H) ppm.

^{13}C NMR (125 MHz, CD_3OD): δ = 24.2, 26.8 (2 q, CH_3), 48.2 (d, C-7), 60.6 (d, C-8a), 63.8 (t, 7-CH_2), 63.9 (d, C-4a), 69.3 (t, C-4), 75.1 (s, C-6), 75.5 (d, C-8), 77.7 (t, C-2) ppm.

IR (ATR): ν = 3350-3250 (OH, NH), 2970, 2905 (C-H), 1090, 1035 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{10}H_{19}NO_4 \cdot Na^+$: calcd.: 218.1387
found: 218.1392

Elemental Analysis: $C_{10}H_{19}NO_4$ (217.3) calcd. (%): C 55.28, H 8.81, N 6.45
found (%): C 55.30, H 8.86, N 6.44

6.2.1.2. Copper(II)-catalyzed diazotransfer

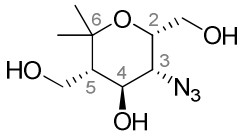
(2*S*,3*R*,4*S*,5*S*)-(3-Azido-4-hydroxy-6,6-dimethyltetrahydro-2*H*-pyran-2,5-diyl)-dimethanol (**10**)

JNS051

A mixture of nonafluorobutanesulfonyl fluoride (1.54 g, 0.90 mL, 5.11 mmol) and NaN_3 (325 mg, 5.32 mmol) was dissolved in MeOH (10 mL) and stirred at r.t. for 20 h. The mixture was poured into ice-cold water and extracted with Et_2O (3x 20 mL). The combined organic layers were dried with $MgSO_4$ and the solvent was removed at atmospheric pressure affording product **24** (1.40 g, 84%) as a colorless oil.

To a solution of aminopyran **3** (150 mg, 0.73 mmol) in MeOH:H_2O (2:1, 3 mL) at r.t. were added $CuSO_4 \cdot 5H_2O$ (18 mg, 0.073 mmol) and K_2CO_3 (101 mg, 0.73 mmol), followed by slow addition of Nf-N_3 **24** (475 mg, 1.46 mmol) via syringe. The mixture was stirred for 24 h, then

glycine hydrochloride (554 mg, 5.00 mmol) was added and the suspension was stirred for another 24 h. The solution was quenched with 7N NH_3 in MeOH and the copper salt was filtered through a short silica gel column. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2 100%, CH_2Cl_2/MeOH 95:5, 90:10) affording **10** (301 mg, 89%) as a colorless solid.



10

Chemical Formula: $C_9H_{17}N_3O_4$

Molecular Weight: 231.3 g·mol^{-1}

Melting point: 121 °C

Optical rotation: $[\alpha]_D^{22}$ = -18.4 (c = 0.76, MeOH)

^{1}H NMR (500 MHz, CD_3OD): δ = 1.14, 1.34 (2 s, 3 H each, CH_3), 1.82 (td, $J \approx 6.0$, 9.3 Hz, 1 H, 5-H), 3.51 (dd, J = 4.1, 5.5 Hz, 1 H, 3-H), 3.56, 3.63 (AB part of ABX system, J_{AB} = 11.2 Hz, J_{AX} = 5.7 Hz, J_{BX} = 7.0 Hz, 1 H each, 2-CH_2), 3.68, 3.80 (AB part of AB system, J_{AB} = 11.1 Hz, J_{AX} = 5.6 Hz, J_{BX} = 6.6 Hz, 1 H each, 5-CH_2), 3.92 (dd, J = 5.5, 9.3 Hz, 1 H, 4-H), 4.02 (ddd, J = 4.1, 5.7, 7.0 Hz, 1 H, 2-H) ppm.

^{13}C NMR (125 MHz, CD_3OD): δ = 24.8, 27.0 (2 q, CH_3), 49.5 (d, C-5), 62.1, 62.7 (2 t, 2-CH_2, 5-CH_2), 68.2 (d, C-3), 71.3 (d, C-2), 72.3 (d, C-4), 76.4 (s, C-6) ppm.

IR (ATR): ν = 3370 (OH), 2970-2930 (C-H), 2110 (N_3), 1235 (C-O), 1045 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_9H_{17}N_3O_4 \cdot Na^+$: calcd.: 254.1113
found: 254.1122

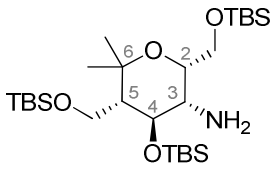
Elemental Analysis: $C_9H_{17}N_3O_4$ (231.3) calcd. (%): C 46.74, H 7.41, N 18.17
found (%): C 46.80, H 7.27, N 18.21

6.2.1.3.Introduction of protecting groups

(2*S*,3*S*,4*S*,5*S*)-4-(*tert*-Butyldimethylsiloxy)-2,5-bis[(*tert*-butyldimethylsiloxy)methyl]-6,6-dimethyltetrahydro-2*H*-pyran-3-amine (**26**)

JNS118

To a solution of aminopyran **3** (100 mg, 487 µmol) in dry DMF (5 mL), DMAP (6 mg, 49 µmol) and Et_3N (0.54 mL, 3.90 mmol) were added under argon atmosphere. After cooling to 0 °C, TBSOTf (901 mg, 0.78 mL, 3.41 mmol) was added dropwise and the reaction mixture was stirred for 1 h at 0 °C until it reached r.t. After stirring for 5 d at this temperature, a sat. aq. $NaHCO_3$ solution (15 mL) was added to the reaction mixture followed by extraction with EtOAc (3 x 40 mL). The combined organic phases were dried with Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes 100%, hexanes/EtOAc 10:1, 9:1) affording TBS protected aminopyran **26** (268 mg, 97%) as a yellow oil.



26

Chemical Formula: $C_{27}H_{61}NO_4Si_3$

Molecular Weight: 548.0 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = +0.72 (c = 1.95, $CHCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.040, 0.047, 0.053, 0.055, 0.069, 0.080 (6 s, 3 H each, CH_3), 0.87, 0.88, 0.89 (3 s, 9 H each, *t*Bu), 1.16, 1.44 (2 s, 3 H each, CH_3), 1.66 (dt, $J \approx 3.4$, 7.7 Hz, 1 H, 5-H), 2.75 (m_c, 1 H, 3-H), 3.65 – 3.66 (m, 2 H, 2-CH_2), 3.71, 3.77 (AB part of ABX system, J_{AX} = 7.5 Hz, J_{BX} = 7.9 Hz, J_{AB} = 10.2 Hz, 1 H each, 5-CH_2), 4.01 – 4.04 (m, 2 H, 4-H, 2-H) ppm.

^{13}C NMR (125 MHz, $CDCl_3$): δ = -5.5, -5.3, -5.2, -5.1, -4.7, -4.6 (6 q, $SiCH_3$), 18.1, 18.2, 18.3 [3 s, Si*C*$(CH_3)_3$], 25.9, 26.0, 26.1 [3 q, SiC(*C*$H_3)_3$], 27.4, 27.6 (2 q, CH_3), 49.1 (C-5), 52.7 (d, C-3), 62.7 (t, 2-CH_2), 62.9 (t, 5-CH_2), 68.5 (d, C-2), 72.9 (d, C-4), 74.1 (s, C-6) ppm.

IR (ATR): ν = 3390 (NH_2), 2955-2860 (C-H), 1255, 1070 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{27}H_{61}NO_4Si_3 \cdot H^+$: calcd.: 548.3981
found: 548.4013

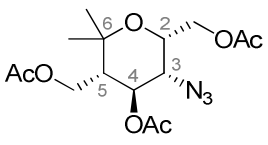
Elemental Analysis: $C_{27}H_{61}NO_4Si_3$ (548.0) calcd. (%): C 59.17, H 11.22, N 2.56
found (%): C 57.38, H 11.06, N 2.54

A better elemental analysis could not be obtained.

(2*S*,3*R*,4*S*,5*S*)-(4-Acetoxy-3-azido-6,6-dimethyltetrahydro-2*H*-pyran-2,5-diyl)bis-(methylene)diacetate (**25**)

JNS203

Azide **10** (100 mg, 0.43 mmol) was dissolved in pyridine (4 mL) and the solution was cooled to 0 °C. After addition of DMAP (2 mg, 16 μmol) and acetic anhydride (480 mg, 0.45 mL, 4.71 mmol) the reaction mixture was stirred at r.t. overnight. The residue was taken up in Et_2O (20 mL) and washed with 1M solution of HCl (1x 10 mL), brine (1x 10 mL) and sat. aq. $NaHCO_3$ solution (1x 10 mL). The organic phase was dried with Na_2SO_4 and the solvents were removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc 3:1) affording **25** (153 mg, quant.) as a colorless oil.



25

Chemical Formula: $C_{15}H_{23}N_3O_7$
Molecular Weight: 357.4 g·mol^{-1}

^{1}H NMR (500 MHz, CD_3OD): δ = 1.21, 1.37 (2 s, 3 H each, CH_3), 2.01 (ddd, *J* = 6.4, 9.0, 11.6 Hz, 1 H, 5-H), 2.08 (s, 6 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 3.60 (dd, *J* = 2.7, 3.8 Hz, 1 H, 3-H), 4.08 – 4.19 (m, 4 H, 2-CH_2, 5-CH_2, 4-H), 4.40 (dd, *J* = 6.4, 11.4 Hz, 1 H, 5-CH_2), 5.33 – 5.37 (m, 1 H, 2-H) ppm.

^{13}C NMR (125 MHz, CD_3OD): δ = 21.0, 21.1, 21.3 (3 q, $COCH_3$), 26.1, 26.5 (2 q, CH_3), 42.7 (d, C-5), 60.2 (d, C-3), 62.3 (t, 5-CH_2), 63.8 (t, 2-CH_2), 66.5 (d, C-4), 69.8 (d, C-2), 74.1 (s, C-6), 169.7, 170.8, 170.9 (3 s, C=O) ppm.

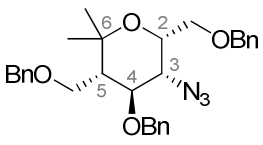
HRMS (pos. ESI-TOF): $C_{15}H_{23}N_3O_7{\cdot}Na^+$: calcd.: 380.1428
found: 380.1439

The analytical data are in agreement with those reported in the literature.[29a]

(2*S*,3*R*,4*S*,5*S*)-3-Azido-4-(benzyloxy)-2,5-bis(benzyloxymethyl)-6,6-dimethyltetrahydro-2*H*-pyran (**27**)

JNS258

Under argon atmosphere, a solution of azide **10** (79 mg, 0.34 mmol) in THF (1 mL) was added to a suspension of NaH (49 mg, 2.05 mmol) in THF (5.2 mL) at 0 °C. The reaction mixture was stirred from 0 °C to r.t. for 2 h. Benzyl bromide (351 mg, 0.24 mL, 2.05 mmol) was added and the reaction stirred at r.t. for 21 h. The mixture was then treated with sat. aq. NH_4Cl solution and extracted with CH_2Cl_2 (3x 10 mL). The combined organic phases were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 6:1) affording **27** (130 mg, 76%) as a colorless oil.



27

Chemical Formula: $C_{30}H_{35}N_3O_4$

Molecular Weight: 501.6 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = +8.3 (c = 0.89, $CHCl_3$)

^{1}H NMR (500 MHz, CD_3OD): δ = 1.21, 1.46 (2 s, 3 H each, CH_3), 2.10 (dt, *J* = 4.5, 7.1 Hz, 1 H, 5-H), 3.56 – 3.64 (m, 2 H, 2-CH_2), 3.68 (d, *J* = 7.1 Hz, 2 H, 5-CH_2), 3.69 – 3.72 (m, 1 H, 3-H), 4.05 (dd, *J* = 3.7, 4.5 Hz, 1 H, 4-H), 4.23 – 4.28 (m, 1 H, 2-H), 4.48, 4.51 (AB system, J_{AB} = 11.7 Hz, 1 H each, CH_2Ph), 4.57, 4.60 (AB system, J_{AB} = 11.8 Hz, 1 H each, CH_2Ph), 4.63 (s, 2 H, CH_2Ph), 7.30 – 7.38 (m, 15 H, Ph) ppm.

^{13}C NMR (125 MHz, CD_3OD): δ = 26.7, 27.1 (2 q, CH_3), 43.8 (d, C-5), 61.4 (d, C-3), 67.2 (d, C-2), 69.0 (t, 5-CH_2), 69.7 (t, 2-CH_2), 72.2, 73.3, 73.7 (3 t, CH_2Ph), 74.3 (s, C-6), 76.1 (d, C-4), 127.68, 127.78, 127.80, 127.85, 127.86, 128.06, 128.46, 128.50, 128.53 (9 d, Ph), 138.0, 138.1, 138.4 (3 s, Ph) ppm.

IR (ATR): ν = 2960-2850 (C-H), 2100 (N_3), 1500, 1460 (C=C), 1260, 1100, 1030 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{30}H_{35}N_3O_4 \cdot Na^+$: calcd.: 524.2520
found: 524.2548

$C_{30}H_{35}N_3O_4 \cdot K^+$: calcd.: 540.2259
found: 540.2274

Elemental Analysis: $C_{30}H_{35}N_3O_4$ (501.6) calcd. (%): C 71.83, H 7.03, N 8.38
found (%): C 70.53, H 6.73, N 7.29

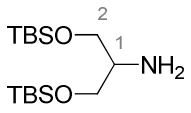
A better elemental analysis could not be obtained.

TBS protected amine (**52**)

JNS057

2-Amino1,3-diol **4** (8.02 g, 88.0 mmol) and DMAP (50 mg, 0.40 mmol) were dissolved in CH_2Cl_2 (100 mL) under argon. The reaction mixture was stirred at r.t. and Et_3N (48 ml, 344 mmol) was added. In a second flask *tert*-butyldimethylsilyl chloride (34.0 g, 255 mmol) was dissolved in CH_2Cl_2 (50 mL). The solution was added to the reaction mixture and stirred at r.t. overnight. H_2O (100 mL) was added to the mixture and the aqueous phase extracted with CH_2Cl_2 (3x 150 mL). The combined organic phases were dried with Na_2SO_4 and solvents

removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 2:1) affording **52** (27.0 g, 96%) as a colorless liquid.



52

Chemical Formula: $C_{15}H_{37}NO_2Si_2$

Molecular Weight: 319.6 g·mol^{-1}

Refraction Index at 25°C: 1.4363

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 12 H, CH_3), 0.88 (s, 18 H, *t*Bu), 2.85 (quint., $J \approx$ 5.5 Hz, 1 H, 1-H), 3.50, 3.59 (AB part of ABX system, J_{AB} = 9.8 Hz, J_{AX} = 5.2 Hz, J_{BX} = 5.7 Hz, 2 H each, 2-H) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.3, -3.4 (2 q, $SiCH_3$), 18.4 [s, Si*C*$(CH_3)_3$], 26.0 [q, SiC(*C*$H_3)_3$], 54.5 (d, C-1), 64.9 (t, C-2) ppm.

IR (ATR): ν = 3370 (NH_2), 1250 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{15}H_{37}NO_2Si_2 \cdot H^+$: calcd.: 320.2436
found: 320.2447

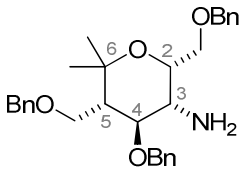
6.2.1.4. Conversion of azidopyran into aminopyran

(2*S*,3*R*,4*S*,5*S*)-4-(Benzyloxy)-2,5-bis(benzyloxymethyl)-6,6-dimethyltetrahydro-2*H*-pyran-3-amine (**32**)

JNS321

A solution of azide **27** (41 mg, 0.08 mmol) in THF (0.8 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (6.2 mg, 0.16 mmol) in THF (1 mL) at r.t. under argon. The reaction was stirred for 2 h and 10% aq NaOH solution (4.7 mL) was added dropwise and diluted with CH_2Cl_2 (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3x 10 mL). The combined organic layers were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product

was purified by flash column chromatography (silica gel, hexanes/EtOAc, 3:1 to pure EtOAc) affording **32** (25 mg, 64%) as a colorless oil.



32

Chemical Formula: $C_{30}H_{37}NO_4$

Molecular Weight: 475.6 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$= -28.2 (c = 1.11, MeOH)

^{1}H NMR (700 MHz, CD_3OD): δ = 1.25, 1.42 (2 s, 3 H each, CH_3), 1.98 – 2.02 (m, 1 H, 5-H), 2.93 – 2.96 (m, 1 H, 3-H), 3.56 (d, *J* = 6.1 Hz, 2 H, 2-CH_2), 3.61, 3.65 (AB part of ABX system, J_{AB} = 9.9 Hz, J_{AX} = 5.1 Hz, J_{BX} = 6.0 Hz, 1 H each, 5-CH_2), 3.68 (dd, *J* = 3.4, 4.3 Hz, 1 H, 4-H), 4.18 (dt, *J* = 2.6, 6.1 Hz, 1 H, 2-H), 4.45 (s, 2 H, CH_2Ph), 4.52, 4.56 (AB system, J_{AB} = 11.9 Hz, 1 H each, CH_2Ph), 4.58 – 4.60 (m, 2 H, CH_2Ph), 7.25 – 7.35 (m, 15 H, Ph) ppm.

^{13}C NMR (175 MHz, CD_3OD): δ = 27.3, 27.7 (2 q, CH_3), 45.5 (d, C-5), 52.1 (d, C-3), 69.1 (d, C-2), 71.0 (t, 5-CH_2), 71.4 (t, 2-CH_2), 72.8, 74.2, 74.3 (3 t, CH_2Ph), 75.8 (s, C-6), 81.7 (d, C-4), 128.67, 128.77 , 128.75, 128.79, 128.88, 129.00, 129.40, 129.41, 129.43 (9 d, Ph) 139.4, 139.5, 139.9 (3 s, Ph) ppm.

IR (ATR): ν = 3390 (NH_2), 2930-2860 (C-H), 1500, 1450 (C=C), 1250 (C-O), 1070 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{30}H_{37}NO_4 \cdot H^+$: calcd.: 476.2795
found: 476.2817

$C_{30}H_{37}NO_4 \cdot Na^+$: calcd.: 498.2615
found: 498.2631

Elemental Analysis: $C_{30}H_{37}NO_4$ (475.6) calcd. (%): C 75.76, H 7.84, N 2.94
found (%): C 75.50, H 7.99, N 3.06

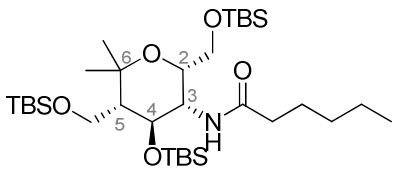
6.2.2. Amide bond formation

6.2.2.1. Schotten-Baumann reaction

(2*S*,3*R*,4*S*,5*S*)-4-(*tert*-Butyldimethylsiloxy)-2,5-bis[(tert-butyldimethylsiloxy)methyl]-6,6-dimethyltetrahydro-2*H*-pyran-3-hexanamide (**34**)

JNS036

According to **GP-3**, protected aminopyran **26** (208 mg, 0.40 mmol) was dissolved in CH_2Cl_2 (2.9 mL) and the solution was cooled to 0 °C under argon atmosphere. After addition of Et_3N (0.1 mL, 0.59 mmol) and hexanoyl chloride (61 mg, 64 µL, 0.45 mmol) the reaction mixture was stirred at 0 °C for 2.5 h, and at r.t. overnight. H_2O (5 mL) was then added to the reaction mixture and the aqueous phase was extracted with CH_2Cl_2 (3x 10 mL). The combined organic phases were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 6:1) affording **34** (227 mg, 93%) as a pale yellow oil.



34

Chemical Formula: $C_{33}H_{73}NO_4Si_3$

Molecular Weight: 632.2 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = +1.20 (c = 1.51, $CHCl_3$)

^{1}H-NMR (500 MHz, $CDCl_3$): δ =0.015, 0.018, 0.028, 0.033, 0.101, 0.174 (6 s, 3 H each, CH_3), 0.87– 0.90 (m, 30 H, *t*Bu, CH_3), 1.18 (s, 3 H, CH_3), 1.27 – 1.34 (m, 4 H, CH_2), 1.49 (s, 3 H, CH_3), 1.59 – 1.66 (m, 3 H, 5-H, CH_2), 2.03– 2.18 (m, 2 H, CH_2), 3.44 (dd, *J* = 7.2, 10.2 Hz, 1 H, 5-CH_2), 3.60 (d, *J* = 5.9 Hz, 2 H, 2-CH_2), 3.71 – 3.78 (m, 2 H, 4-H, 5-CH_2), 4.15 – 4.17 (m, 1 H, 2-H), 4.24 (m_c, 1 H, 3-H), 5.80 (d, *J* = 7.5 Hz, 1 H, NH) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -4.7, -5.0, -5.2, -5.3 (4 q, $SiCH_3$), 14.1 (q, CH_3), 18.0, 18.8, 18.4 [3 s, Si*C*$(CH_3)_3$], 22.6 (t, CH_2), 25.4 (t, CH_2), 25.9, 25.96, 26.0 [3 q, SiC(*C*$H_3)_3$], 27.3, 28.3 (2 q, CH_3), 31.7 (t, CH_2), 37.1 (t, CH_2), 49.5 (d, C-5), 50.2 (d, C-3), 62.8 (t, 5-CH_2), 63.5 (t, 2-CH_2), 67.2 (d, C-2), 68.9 (d, C-4), 74.2 (s, C-6), 172.5 (s, C=O) ppm.

IR (ATR): ν = 2960 (NH), 2930-2860 (C-H), 1680 (C=O), 1250 (C-O), 1070 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{33}H_{73}NO_4Si_3 \cdot H^+$: calcd.: 646.4713
found: 646.4705

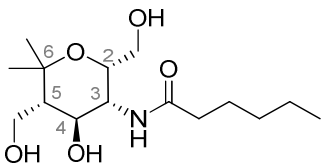
$C_{33}H_{73}NO_4Si_3 \cdot Na^+$: calcd.: 668.4532
found: 668.4528

Elemental Analysis: $C_{33}H_{73}NO_4Si_3$ (632.2) calcd. (%): C 61.34, H 11.07, N 2.17
found (%): C 61.15, H 11.07, N 1.99

(2*S*,3*R*,4*S*,5*S*)-4-Hydroxy-2,5-bis(hydroxymethyl)-6,6-dimethyltetrahydro-2*H*-pyran-3-hexanamide (**35**)

JNS043

According to **GP-4**, to a stirred solution of **34** (83 mg, 0.13 mmol) in THF (1 mL) at 0 °C, was added HF·pyridine (1.1 mL, 1.1 mmol). After warming up to r.t., the reaction mixture was stirred for 24 h. H_2O (5 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3x 10mL). The organic phase was dried with Na_2SO_4 and the solvents were removed in vacuo. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 95:5) affording **35** (49 mg, 49%) as a colorless solid.



35

Chemical Formula: $C_{15}H_{29}NO_5$

Molecular Weight: 303.4 $g \cdot mol^{-1}$

Melting point: 108 – 110 °C

Optical rotation: $[\alpha]_D^{22}$ = +42.7 (c = 1.49, MeOH)

^{1}H-NMR (500 MHz, CD_2Cl_2): δ = 0.90 (t, *J* = 6.9 Hz, CH_3), 1.06, 1.28 (2 s, 3 H each, CH_3), 1.30 – 1.36 (m, 2 H, CH_2), 1.57 – 1.65 (m, 4 H, CH_2), 1.84 – 1.89 (m, 1 H, 5-H), 2.20 – 2.25 (m, 2 H, CH_2), 3.53 – 3.60 (m, 1 H, 2-CH_2), 3.67 – 3.72 (m, 1 H, 5-CH_2), 3.75 (B part of ABX system, J_{AB} = 11.0, Hz, J_{BX} = 8.0 Hz, 1 H, 5-CH_2), 3.80 (m_c, 1 H, 4-H), 3.81 – 3.86 (m, 2 H, 2-H, 2-CH_2), 3.95 – 3.98 (m, 1H, 3-H), 7.98 (s, 1H, NH) ppm.

^{13}C-NMR (125 MHz CD_2Cl_2): δ = 14.6 (q, CH_3), 24.3 (q, CH_3), 26.0 (t, CH_2), 26.8 (q, CH_3), 27.3 (t, CH_2), 32.2 (t, CH_2), 37.4 (t, CH_2), 48.9 (d, C-5), 55.2 (d, C-3), 63.6 (t, 5-CH_2), 63.7 (2-CH_2), 71.5 (d, C-2), 74.0 (d, C-4), 76.0 (s, C-6), 129.0 (s, C=O) ppm.

IR (ATR): ν = 3450 (NH), 3310 (OH), 2960-2860 (C-H), 1620 (C=O), 1230 (C-O), 1080 (C-O-C) cm^{-1}.

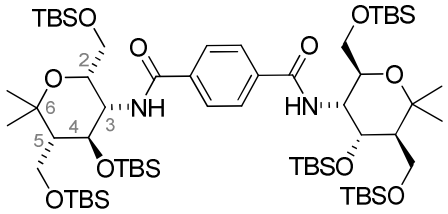
HRMS (pos. ESI-TOF): $C_{15}H_{29}NO_5 \cdot Na^+$: calcd.: 326.1938
found: 326.1966

Elemental Analysis: $C_{15}H_{29}NO_5$ (303.4) calcd. (%): C 59.38, H 9.63, N 4.62
found (%): C 59.15, H 9.63, N 4.38

Divalent amide (**41**)

JNS123

According to **GP-3**, protected aminopyran **26** (200 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (3.2 mL) and the solution was cooled to 0 °C. After addition of Et_3N (0.1 mL, 0.73 mmol) and terephthaloyl chloride **40** (45 mg, 0.22 mmol) the reaction mixture was stirred at 0 °C for 1 h and at r.t. for 20 h. H_2O (5 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3x 10mL). The combined organic layers were dried with Na_2SO_4 and the solvents were removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 6:1) affording **41** as colorless solid (223 mg, 83%).



41

Chemical Formula: $C_{62}H_{124}N_2O_{10}Si_6$

Molecular Weight: 1226.2 g·mol^{-1}

Melting range: 105 – 110 °C

Optical rotation: $[\alpha]_D^{22}$ = +33.8 (c = 0.73, $CHCl_3$)

^{1}H-NMR (500 MHz, $CDCl_3$): δ = -0.20, -0.10 (2 s, 6 H each, CH_3) -0.01 (s, 12 H, CH_3), 0.14, 0.24 (2 s, 6 H each, CH_3), 0.75, 0.83, 0.92 (3 s, 18 H each, *t*Bu), 1.21, 1.54 (2 s, 6 H each, CH_3), 1.69 (bt, $J \approx 7.6$ Hz, 2 H, 5-H), 3.50 (dd, J = 8.6, 10.1 Hz, 2 H, 5-CH_2), 3.72 (d, J = 5.5 Hz, 4 H, 2-CH_2), 3.79 (dd, J = 7.1, 10.1 Hz, 2 H, 5-CH_2), 3.92 – 3.96 (m, 2 H, 3-H), 4.26 (dt, J = 2.1, 5.5 Hz, 2 H, 2-H), 4.52 – 4.55 (m, 2 H, 4-H), 6.82 (d, J = 6.8 Hz, 2 H, NH), 7.78 (s, 4 H, Ar) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.45, -5.41, -5.28, -5.25, -4.93, -4.65 (6 q, $SiCH_3$) 18.0, 18.1, 18.5 [3 s, Si*C*$(CH_3)_3$], 25.88, 25.91, 25.95 [3 q, SiC(*C*$H_3)_3$], 27.3, 28.2 (2 q, CH_3), 49.0 (d, C-5), 51.7 (d, C-3), 62.4 (t, 5-CH_2), 64.0 (t, 2-CH_2), 66.5 (d, C-2), 67.9 (d, C-4), 74.3 (s, C-6), 127.2 (d, Ar), 137.4 (s, Ar), 166.1 (s, C=O) ppm.

IR (ATR): ν = 3380 (NH), 2860 (C-H), 1670 (C=O), 1525 (C=C), 1250 (C-O), 1075 (C-O-C) cm^{-1}.

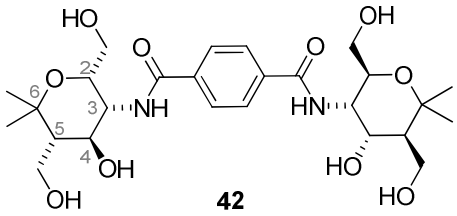
HRMS (pos. ESI-TOF): $C_{62}H_{124}N_2O_{10}Si_6 \cdot Na^+$: calcd.: 1248.7786
found: 1248.7760

Elemental Analysis: $C_{62}H_{124}N_2O_{10}Si_6$ (1226.2) calcd. (%): C 60.73, H 10.19, N 2.28
found. (%): C 60.85, H 10.24, N 2.36

Divanlent amide (**42**)

JNS134

According to **GP-4**, to a stirred solution of **41** (116 mg, 0.095 mmol) in THF (0.7 mL) at 0 °C, was added HF·pyridine (0.15 mL, 0.114 mmol). After warming up to r.t., the reaction mixture was stirred for 22 h. MeOH was added to the mixture and the solvents were removed in vacuo. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 9:1) affording **42** (52 mg, quant.) as a slightly pink solid.



42

Chemical Formula: $C_{26}H_{40}N_2O_{10}$

Molecular Weight: 540.6 g·mol^{-1}

Melting point: 210 °C

Optical rotation: $[\alpha]_D^{22}$ = +45.2 (c = 0.43, MeOH)

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.33, 1.46 (2 s, 6 H each, CH_3), 1.78 (m_c, 2 H, 5-H), 3.51, 3.56 (AB part of ABX system, J_{AB} = 11.6 Hz, J_{AX} = 4.8 Hz, J_{BX} = 6.9 Hz, 2 H, 2-CH_2), 3.67 (dd, J = 4.2, 11.1 Hz, 2 H, 5-CH_2), 3.96 – 3.99 (m, 4 H, 5-CH_2, 4-H), 4.18 – 4.21 (m, 4 H, 3-H, 2-H), 7.91 (s, 4 H, Ar) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 26.3, 27.7 (2 q, CH_3), 48.8 (d, C-5), 54.2 (d, C-3), 63.2 (t, 2-CH_2), 63.3 (t, 5-CH_2), 70.9 (d, C-2), 74.0 (d, C-4), 75.9 (s, C-6), 128.6 (d, Ar), 138.4 (s, Ar), 169.5 (s, C=O) ppm.

IR (ATR): ν = 3290 (OH, NH), 2980-2910 (C-H), 1725 (C=O), 1440 (C=C), 1250 (C-O), 1070 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{26}H_{40}N_2O_{10}$·Na^+: calcd.: 563.2575
found: 563.2581

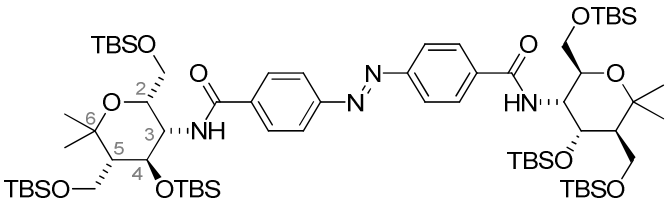
Elemental Analysis: $C_{26}H_{40}N_2O_{10}$ (540.6) calcd.(%): C 57.76, H 7.46, N 5.18
found.(%): C 52.05, H 7.54, N 8.00

A better elemental analysis could not be obtained.

Divalent amide (**49**)

JNS173

According to **GP-3**, protected aminopyran **26** (103 mg, 0.19 mmol) was dissolved in CH_2Cl_2 (2 mL) and the solution was cooled to 0 °C. After addition of Et_3N (0.1 mL, 0.72 mmol) and acid chloride **48** (26 mg, 0.08 mmol) the reaction mixture was stirred at 0 °C for 1 h and at r.t. for 24 h. H_2O (5 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3x 10 mL). The combined organic phases were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 6:1) affording **49** as a pink solid (114 mg, 55%).



49

Chemical Formula: $C_{68}H_{128}N_4O_{10}Si_6$

Molecular Weight: 1330.3 g·mol^{-1}

Melting range: 170 – 175 °C

Optical rotation: $[\alpha]_D^{22}$ = +40.2 (c = 1.13, $CHCl_3$)

^{1}H-NMR (500 MHz, $CDCl_3$): δ = -0.16, -0.08, 0.02, 0.07, 0.16, 0.26 (6 s, 6 H each, CH_3), 0.76, 0.85, 0.93 (3 s, 18 H each, *t*Bu), 1.22, 1.25 (2 s, 6 H each, CH_3), 1.66 – 1.69 (m, 2 H, 5-H), 3.53 (dd, *J* = 8.6, 10.1 Hz, 2 H, 5-CH_2), 3.74, 3.76 (AB part of ABX system, J_{AB} = 10.9 Hz, J_{AX} = 5.1 Hz, J_{BX} = 5.9 Hz, 2 H each, 2-CH_2), 3.81 (dd, *J* = 7.1, 10.1 Hz, 2 H, 5-CH_2), 3.96 – 3.99 (m, 2 H, 3-H), 4.28 (dt, *J* ≈ 2.0, 5.5 Hz, 2 H, 2-H), 4.54 (t, *J* = 2.3 Hz, 2 H, 4-H), 6.84 (d, *J* = 7.0 Hz, 2 H, NH), 7.91 (d, *J* = 8.5 Hz, 4 H, Ar), 7.98 (d, *J* = 8.5 Hz, 4 H, Ar) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.45, -5.43, -5.25, -5.22, -4.93, -4.65 (6 q, $SiCH_3$), 18.0, 18.2, 18.5 [3 s, Si*C*$(CH_3)_3$], 25.89, 25.94, 25.96 [3 q, SiC(*C*$H_3)_3$], 27.3, 28.2 (2 q, CH_3), 49.0 (d, C-5), 51.6 (d, C-3), 62.5 (t, 5-CH_2), 64.0 (t, 2-CH_2), 66.7 (d, C-2), 67.9 (d, C-4), 74.3 (s, C-6), 123.2, 128.1 (2 d, Ar), 137.1, 154.1 (2 s, Ar), 166.1 (s, C=O) ppm.

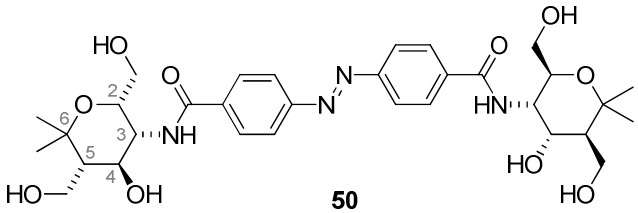
IR (ATR): ν = 3310 (NH), 2960-2850 (C-H), 1735 (C=O), 1470 (C=C), 1250 (C-O), 1060 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{68}H_{128}N_4O_{10}Si_6 \cdot Na^+$: calcd.: 1351.8133
found: 1351.8136

Divalent amide (**50**)

JNS176

According to **GP-4**, to a stirred solution of **49** (44 mg, 0.033 mmol) in THF (0.3 mL) at 0 °C, was added HF·pyridine (40 µL, 0.397 mmol). After warming up to r.t., the reaction mixture was stirred for 24 h. MeOH was added to the mixture and the solvents were removed in vacuo. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 95:5) affording **50** (27 mg, quant.) as an orange solid.



50

Chemical Formula: $C_{32}H_{44}N_4O_{10}$

Molecular Weight: 644.7 g·mol^{-1}

Melting point: 249 °C

Optical rotation: $[\alpha]_D^{22}$ = +71.8 (c = 0.66, MeOH)

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.34, 1.47 (2 s, 6 H each, CH_3) 1.79 (m_c, 2 H, 5-H), 3.54, 3.59 (AB part of ABX system, J_{AB} = 11.7 Hz, J_{AX} = 4.4 Hz, J_{BX} = 6.7 Hz, 2 H each, 2-CH_2),

3.70 (dd, *J* = 4.1, 11.2 Hz, 2 H, 5-CH_2), 3.97 – 4.02 (m, 4 H, 5-CH_2, 4-H), 4.18 – 4.23 (m, 4 H, 2-H, 3-H), 8.01 (d, *J* = 8.6 Hz, 4 H, Ar), 8.04 (d, *J* = 8.6 Hz, 4 H, Ar) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 26.3, 27.7 (2 q, CH_3), 48.8 (d, C-5), 54.2 (d, C-3), 63.2 (t, 2-CH_2), 63.3 (t, 5-CH_2), 70.9 (d, C-2), 74.1 (d, C-4), 75.9 (s, C-6), 124.0, 129.7 (2 d, Ar), 138.1, 155.6 (2 s, Ar), 169.5 (s, C=O) ppm.

IR (ATR): ν = 3320 (OH, NH), 2960-2855 (C-H), 1730 (C=O), 1440 (C=C), 1250 (C-O), 1060 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{32}H_{44}N_4O_{10}{\cdot}Na^+$: calcd.: 667.2944
found: 667.2943

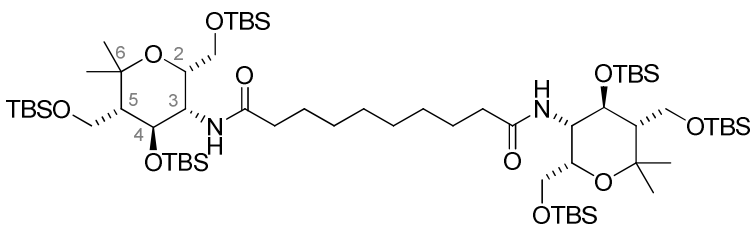
Elemental Analysis:

$C_{32}H_{44}N_4O_{10}$ (644.7) + 6 H_2O calcd. (%): C 51.05, H 7.50, N 7.44
found. (%): C 51.06, H 6.08, N 7.11

Divalent amide (**39**)

JNS079-89

According to **GP-3**, protected aminopyran **26** (200 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (3.2 mL) and the solution was cooled to 0 °C. After addition of Et_3N (0.1 mL, 0.73 mmol) and sebacoyl chloride **38** (46.7 µL, 0.219 mmol) the reaction mixture was stirred at 0 °C for 1 h and at r.t. for 20 h. Water (5 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3x 10 mL). The combined organic layers were dried with Na_2SO_4 and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 6:1) affording **39** as colorless oil (132 mg, 58%).



39

Chemical Formula: $C_{64}H_{136}N_2O_{10}Si_6$

Molecular Weight: 1262.3 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = +32.0 (c = 0.95, $CHCl_3$)

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.009, 0.013, 0.022, 0.028, 0.10, 0.17 (6 s, 6 H each, CH_3), 0.87 (s, 36 H, *t*Bu), 0.89 (s, 18 H, *t*Bu), 1.18 (s, 6 H, CH_3), 1.28 (bs, 8 H, CH_2), 1.48 (s, 6 H, CH_3), 1.57 – 1.62 (m, 6 H, CH_2, 5-H), 2.04 – 2.16 (m, 4 H, CH_2), 3.43 (dd, *J* = 7.4, 10.2 Hz, 2 H, 5-CH_2), 3.59 (d, *J* = 5.9 Hz, 4 H, 2-CH_2), 3.70 – 3.77 (m, 4 H, 5-CH_2, 3-H), 4.15 (dt, *J* = 2.1, 5.9 Hz, 2 H, 2-H), 4.22 – 4.25 (m, 2 H, 4-H), 5.80 (d, *J* = 7.8 Hz, 2 H, NH) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.32, -5.32, -5.31, -5.21, -5.06, -4.77 (6 q, $SiCH_3$), 17.9, 18.2, 18.3 [3 s, Si*C*$(CH_3)_3$], 25.7 (t, CH_2), 25.87, 25.90, 25.97 [3 q, SiC(*C*$H_3)_3$], 27.3, 28.2 (2 q, CH_3), 29.4, 29.6 (2 t, CH_2), 37.0 (t, CH_2), 49.4 (d, C-5), 50.2 (d, C-3), 62.7 (t, 5-CH_2), 63.5 (t, 2-CH_2), 67.1 (d, C-2), 68.8 (d, C-4), 74.2 (s, C-6), 172.4 (s, C=O) ppm

IR (ATR): 3275 (NH), 2970-2855 (C-H), 1640 (C=O), 1230 (C-O), 1070 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{64}H_{136}N_2O_{10}Si_6 \cdot Na^+$: calcd.: 1283.8703
found: 1283.8759

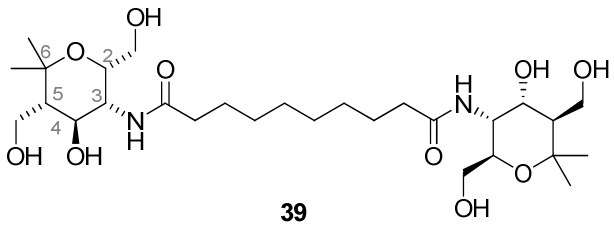
Elemental Analysis: $C_{64}H_{136}N_2O_{10}Si_6$ (1262.3) calcd. (%): C 60.90, H 10.86, N 2.22
found. (%): C 59.13, H 14.45, N 3.45

A better elemental analysis could not be obtained.

Divalent amide (**44**)

JNS140

According to **GP-4**, to a stirred solution of **39** (68 mg, 0.054 mmol) in THF (0.4 mL) at 0 °C, was added HF·pyridine (89.6 µL, 0.646 mmol). After warming up to r.t., the reaction mixture was stirred for 24 h. MeOH was added to the reaction mixture and the solvents were removed in vacuo. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH 9:1 to 4:1) affording **44** as a colorless solid (25 mg, 80%).



39

Chemical Formula: $C_{28}H_{52}N_2O_{10}$

Molecular Weight: 576.7 g·mol^{-1}

Melting point: 215 – 217 °C

Optical rotation: $[\alpha]_D^{22}$ = +54.9 (c = 1.24, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ =1.23 (s, 6 H, CH_3), 1.34 (s, 8 H, CH_2), 1.39 (s, 6 H, CH_3), 1.61 – 1.63 (m, 4 H, CH_2), 1.78 (td, *J* ≈ 5.5, 7.8 Hz, 2 H, 5-H), 2.23 (t, *J* = 7.2 Hz, 4 H, CH_2), 3.46 (d, *J* = 6.1 Hz, 4 H, 2-CH_2), 3.65 (dd, *J* = 5.4, 11.2 Hz, 2 H, 5-CH_2), 3.77 (dd, *J* = 7.8, 5.2 Hz, 2 H, 4-H), 3.83 (dd, *J* = 5.7, 11.2 Hz, 2 H, 5-CH_2), 3.91 – 3.94 (m, 2 H, 3-H), 4.05 (dt, *J* = 3.9, 6.1 Hz, 2 H, 2-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 25.4 (q, CH_3), 26.9 (t, CH_2), 27.3 (q, CH_3), 30.0, 30.2, 37.1 (3 t, CH_2), 49.5 (d, C-5), 55.1 (d, C-3), 62.9 (t, 5-CH_2), 63.0 (t, 2-CH_2), 71.1 (d, C-2), 73.6 (d, C-4), 76.0 (s, C-6), 128.8 (s)* ppm. *Signal could not be attributed. C=O singlet could not be detected.

IR (ATR): ν = 3320 (OH, NH), 2970-2890 (C-H), 1675 (C=O), 1245 (C-O), 1070 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{28}H_{52}N_2O_{10}$·Na^+: calcd.: 601.3671
found: 601.3642

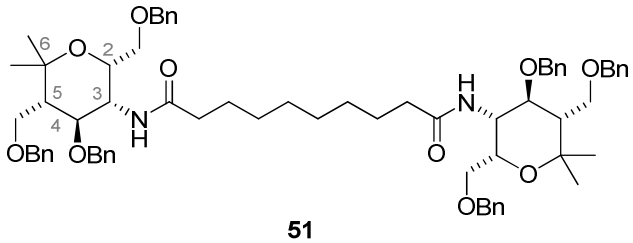
Elemental Analysis: $C_{25}H_{42}N_6O_8$ (554.6) calcd. (%): C 58.31, H 9.09, N 4.86
found. (%): C 33.80, H 5.57, N 3.26

A better elemental analysis could not be obtained.

Divalent amide (**51**)

JNS325

According to **GP-3**, protected aminopyran **32** (66 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (1.1 mL) and the solution was cooled to 0 °C. After addition of Et_3N (60 µL , 0.42 mmol) and sebacoyl chloride **38** (11.9 µL, 0.055 mmol) the reaction mixture was stirred at 0 °C for 1 h and at r.t. for 24 h. H_2O (5 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (3x 10 mL). The combined organic layers were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 3:1) affording **51** as colorless oil (64 mg, quant.).



51

Chemical Formula: $C_{70}H_{88}N_2O_{10}$

Molecular Weight: 1117.4 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = +10.2 (c = 0.45, MeOH)

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.13 – 1.14 (m_c, 8 H, CH_2), 1.27 (s, 6 H, CH_3), 1.38 – 1.44 (m, 4 H, CH_2), 1.46 (s, 6 H, CH_3), 1.89 (t, J = 7.5 Hz, 4 H, CH_2), 1.99 (dt, J = 3.5, 6.0 Hz, 2 H, 5-H), 3.43 – 3.49 (m, 4 H, 2-CH_2), 3.60, 3.62 (AB part of ABX system, J_{AB} = 10.0 Hz, J_{AX} = J_{BX} = 6.0 Hz, 4 H, 5-CH_2), 3.77 (t, J = 3.5 Hz, 2 H, 4-H), 4.14 (t, $J \approx$ 3.5 Hz, 2 H, 3-H), 4.28 (ddd, J = 3.5, 5.8, 6.6 Hz, 2 H, 2-H), 4.39, 4.43 (AB system, J_{AB} = 11.6 Hz, 2 H each, CH_2Ph), 4.47, 4.51 (AB system, J_{AB} = 12.0 Hz, 2 H each, CH_2Ph), 4.57, 4.65 (AB system, J_{AB} = 11.8 Hz, 2 H each, CH_2Ph), 7.22 – 7.26 (m, 5 H, Ph), 7.27 – 7.33 (m, 25 H, Ph) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 26.9, 27.4 (2 q, CH_3), 27.5, 30.2, 30.3, 36.9 (4 t, CH_2), 45.4 (d, C-5), 49.3 (d, C-3), 68.4 (d, C-2), 71.2 (t, 5-CH_2), 71.4 (t, 2-CH_2), 72.7, 74.1, 74.4 (3 t, CH_2Ph), 75.5 (s, C-6), 79.3 (d, C-4), 128.56, 128.63, 128.69, 128.73, 128.8, 128.9, 129.3, 129.4, 129.5 (9 d, Ph), 139.4, 139.5, 139.9 (3 s, Ph), 175.7 (s, C=O) ppm.

IR (ATR): 3320 (NH), 2925-2855 (C-H), 1645 (C=O), 1460 (C=C), 1240 (C-O), 1075 (C-O-C) cm^{-1}.

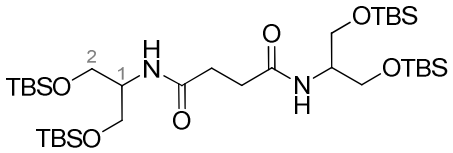
HRMS (pos. ESI-TOF): $C_{70}H_{88}N_2O_{10}{\cdot}Na^+$: calcd.: 1139.6331
found: 1139.6223

Elemental Analysis: $C_{70}H_{88}N_2O_{10}$ (1117.4) calcd. (%): C 75.24, H 7.94, N 2.51
found. (%): C 75.18, H 7.95, N 2.76

Divalent amide (**59**)

JNS071

According to **GP-3**, protected serinol **52** (100 mg, 0.313 mmol) was dissolved in CH_2Cl_2 (2.7 mL) and the solution was cooled to 0 °C. After addition of Et_3N (90 µL, 0.63 mmol) and succinyl chloride **36** (29 mg, 0.19 mmol) the reaction mixture was stirred at 0 °C for 1 h and at r.t. for 2 h. H_2O (5 mL) was added to the reaction mixture and the aqueous layer was extracted with CH_2Cl_2 (3x 10mL). The combined organic layers were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 3:1) affording **59** (93 mg, 68%) as colorless solid.



59

Chemical Formula: $C_{34}H_{76}N_2O_6Si_4$
Molecular Weight: 721.3 $g{\cdot}mol^{-1}$
Melting point: 143 – 145 ºC

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.050, 0.053 (2 s, 12 H each, CH_3), 0.88 (s, 36 H, *t*Bu), 2.50 (s, 4 H, CH_2), 3.52 (dd, *J* = 6.4, 9.6 Hz, 4 H, 2-H), 3.72 (dd, *J* = 3.5, 9.6 Hz, 4 H, 2-H), 3.92 (m_c, 2 H, 1-H), 6.01 (d, *J* = 8.5 Hz, 2 H, NH) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.34, -5.28 (2 q, $SiCH_3$), 18.4 [s, $SiC(CH_3)_3$], 26.0 [q, $SiC(CH_3)_3$], 31.8 (t, CH_2), 51.7 (d, C-1), 60.4 (t, C-2), 171.5 (s, C=O) ppm.

IR (ATR): ν = 3290 (NH), 2960-2860 (C-H), 1740 (C=O), 1250 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{34}H_{76}N_2O_6Si_4 \cdot H^+$: calcd.: 721.4853
found: 721.4881

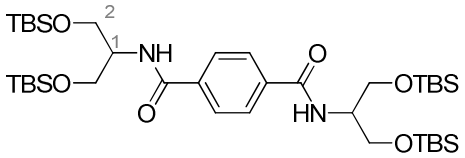
$C_{34}H_{76}N_2O_6Si_4 \cdot Na^+$: calcd.: 743.6667
found: 743.4703

Elemental Analysis: $C_{34}H_{76}N_2O_6Si_4$ (721.3) calcd. (%): C 56.61, H 10.62, N 3.88
found. (%): C 56.72, H 10.48, N 3.81

Divalent amide (**53**)

JNS094

According to **GP-3**, protected serinol **52** (200 mg, 0.626 mmol) was dissolved in CH_2Cl_2 (5.4 mL) and the solution was cooled to 0 °C. After addition of Et_3N (0.2 mL, 1.25 mmol) and terephthaloyl chloride **40** (76 mg, 0.38 mmol) the reaction mixture was stirred at 0 °C for 1 h and at r.t. for 17 h. H_2O (10 mL) was added to the reaction mixture and the aqueous layer was extracted with CH_2Cl_2 (3x 20 mL). The combined organic layers were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 5:1) affording **53** (201 mg, 83%) as colorless solid.



53

Chemical Formula: $C_{38}H_{76}N_2O_6Si_4$
Molecular Weight: 769.4 $g \cdot mol^{-1}$
Melting point: 142 ºC

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.07, 0.09 (2 s, 12 H each, CH_3), 0.91 (s, 36 H, *t*Bu), 3.65 (dd, *J* = 6.4, 9.6 Hz, 4 H, 2-H), 3.87 (dd, *J* = 3.5, 9.6 Hz, 4 H, 2-H), 4.13 – 4.19 (m, 2 H, 1-H), 6.58 (d, *J* = 8.3 Hz, 2 H, NH), 7.80 (s, 4 H, Ar) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.33, -5.25 (2 q, $SiCH_3$), 18.4 [s, $SiC(CH_3)_3$], 26.0 [q, $SiC(CH_3)_3$], 52.1 (d, C-1), 60.5 (t, C-2), 127.3 (d, Ar), 137.4 (s, Ar), 166.0 (s, CO) ppm.

IR (ATR): ν = 3275 (NH), 2880 (C-H), 1630 (C=O), 1545 (C=C), 1255 (C-O) cm^{-1}.

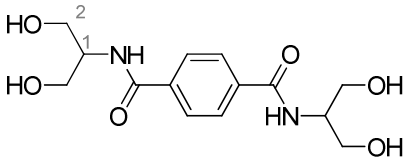
HRMS (pos. ESI-TOF): $C_{38}H_{76}N_2O_6Si_4 \cdot Na^+$: calcd.: 791.4673
found: 791.4743

Elemental Analysis: $C_{38}H_{76}N_2O_6Si_4$ (769.4) calcd. (%): C 59.32, H 9.96, N 3.64
found. (%): C 59.25, H 9.93, N 3.52

Divalent amide (**54**)

JNS102

According to **GP-5**, to a stirred suspension of **53** (200 mg, 0.267 mmol) in isopropanol (0.9 mL) was added AcCl (10 µL, 0.16 mmol) at 0 °C. The reaction mixture was stirred at r.t. for 1.5 h. All volatiles were removed in vacuo affording **54** (73 mg, 90%) as a colorless solid.



54

Chemical Formula: $C_{14}H_{20}N_2O_6$
Molecular Weight: 312.3 $g \cdot mol^{-1}$
Melting range: 178 – 182 °C

^{1}H-NMR (500 MHz, DMSO-D_6): δ = 3.47 – 3.57 (m, 8 H, 2-H), 3.93 – 4.02 (m, 2 H, 1-H), 4.68 (t, *J* = 5.7 Hz, 4 H, OH), 7.93 (s, 4 H, Ar), 8.09 (d, *J* = 8.1 Hz, 1 H, NH) ppm.

^{13}C-NMR (125 MHz, DMSO-D_6): δ = 54.0 (d, C-1), 60.4 (t, C-2), 127.2 (d, Ar), 136.8 (s, Ar), 165.6 (s, C=O) ppm.

IR (ATR): ν = 3290-3230 (OH, NH), 2970-2840 (C-H), 1740 (C=O), 1555 (C=C), 1230 (C-O) cm^{-1}.

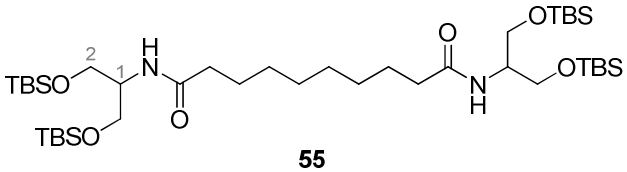
HRMS (pos. ESI-TOF): $C_{14}H_{20}N_2O_6 \cdot Na^+$: calcd.: 335.1214
found: 335.1216

Elemental Analysis: $C_{14}H_{20}N_2O_6$ (312.3) calcd. (%): C 53.84, H 6.45, N 8.97
found. (%): C 53.81, H 6.59, N 8.99

Divalent amide (**55**)

JNS078

According to **GP-3**, protected serinol **52** (200 mg, 0.626 mmol) was dissolved in CH_2Cl_2 (5.4 mL) and the solution was cooled to 0 °C. After addition of Et_3N (0.20 mL, 1.25 mmol) and sebacoyl chloride **38** (90 mg, 0.38 mmol) the reaction mixture was stirred at 0 °C for 1 h and at r.t. for 18 h. H_2O (10 mL) was added to the reaction mixture and the aqueous layer was extracted with CH_2Cl_2 (3x 20 mL). The combined organic layers were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 5:1) affording **55** (190 mg, 75%) as colorless solid.



55

Chemical Formula: $C_{40}H_{88}N_2O_6Si_4$
Molecular Weight: 805.5 $g \cdot mol^{-1}$
Appearance: colorless solid
Melting point: 75 ºC

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.05, 0.06 (2 s, 12 H each, CH_3), 0.89 (s, 36 H, *t*Bu), 1.29 (bs, 8 H, CH_2), 1.58 – 1.61 (m, 4 H, CH_2), 2.12 – 2.17 (m, 4 H, CH_2), 3.52 (dd, *J* = 6.4, 9.6 Hz, 4 H, 2-H), 3.73 (dd, *J* = 3.6, 9.6 Hz, 4 H, 2-H), 3.92 – 3.98 (m, 2 H, 1-H), 5.73 (d, *J* = 8.2 Hz, 2 H, NH) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.4, -5.3 (q, $SiCH_3$), 18.3 [s, $SiC(CH_3)_3$], 25.8 (t, CH_2), 25.9 [q, $SiC(CH_3)_3$], 29.2, 29.3, 37.0 (3 t, CH_2), 51.4 (d, C-1), 60.6 (t, C-2), 172.6 (s, C=O) ppm.

IR (ATR): ν = 3290 (NH), 2955-2855 (C-H), 1740 (C=O), 1250 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{40}H_{88}N_2O_6Si_4 \cdot Na^+$: calcd.: 827.5606
found: 827.5625

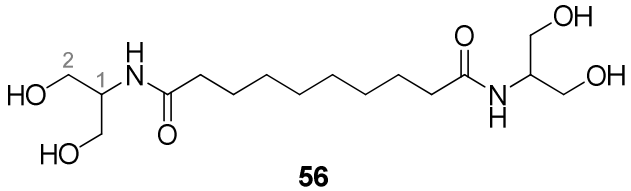
Elemental Analysis:

$C_{40}H_{88}N_2O_6Si_4$ (805.5) + 1 H_2O calcd. (%): C 58.34, H 11.02, N 3.40
found. (%): C 58.37, H 9.73, N 3.22

Divalent amide (**56**)

JNS105

According to **GP-5**, to a stirred solution of **55** (200 mg, 0.248 mmol) in isopropanol (0.9 mL) was added AcCl (10.6 µL, 0.160 mmol) at 0 °C. The reaction mixture was stirred at r.t. for 2.5 h. All volatiles were removed in vacuo affording **56** (88 mg, quant.) as a colorless solid.



56

Chemical Formula: $C_{16}H_{32}N_2O_6$
Molecular Weight: 348.4 $g \cdot mol^{-1}$
Melting point: 170 – 171 °C

^{1}H-NMR (500 MHz, DMSO-D_6): δ = 1.20 – 1.25 (m, 8 H, CH_2), 1.43 – 1.50 (m, 4 H, CH_2), 2.06 (t, *J* = 7.5 Hz, 4 H, CH_2), 3.37 (bd, *J* = 5.7 Hz, 8 H, 2-H), 3.62 – 3.73 (m, 2 H, 1-H), 4.57 (bs, 4 H, OH), 7.43 (d, *J* = 8.1 Hz, 2 H, NH) ppm.

^{13}C-NMR (125 MHz, DMSO-D_6): δ = 25.3, 28.6, 28.7, 35.4 (4 t, CH_2), 52.7 (d, C-1), 60.2 (t, C-2), 172.1 (s, C=O) ppm.

IR (ATR): ν = 3300 (OH, NH), 2920-2850 (C-H), 1730 (C=O), 1255 (C-O) cm^{-1}.

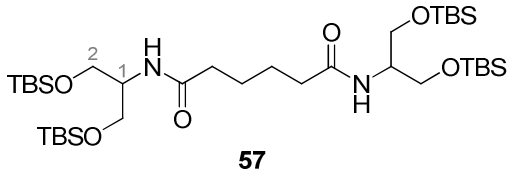
HRMS (pos. ESI-TOF): $C_{16}H_{32}N_2O_6 \cdot Na^+$: calcd.: 371.2153
found: 371.2154

Elemental Analysis: $C_{16}H_{32}N_2O_6$ (348.4) calcd. (%): C 55.15, H 9.26, N 8.04
found. (%): C 55.01, H 9.31, N 7.88

Divalent amide (**57**)

JNS109

According to **GP-3**, protected serinol **52** (2.00 g, 6.26 mmol) was dissolved in CH_2Cl_2 (54 mL) and the solution was cooled to 0 °C. After addition of Et_3N (1.75 mL, 12.5 mmol) and adipoyl chloride **45** (687 mg, 3.75 mmol) the reaction mixture was stirred at 0 °C for 1 h and at r.t. for 17 h. H_2O (100 mL) was added to the reaction and the aqueous layer was extracted with CH_2Cl_2 (3x 200 mL). The combined organic layers were dried with Na_2SO_4 and the solvents removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes/EtOAc, 5:1) affording **57** (1.45 g, 62%) as colorless solid.



57

Chemical Formula: $C_{36}H_{80}N_2O_6Si_4$
Molecular Weight: 749.4 g·mol^{-1}
Appearance: colorless solid
Melting point: 101 °C

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.04, 0.05 (2 s, 12 H each, CH_3), 0.88 (s, 36 H, *t*Bu), 1.61 – 1.68 (m, 4 H, CH_2), 2.16 – 2.19 (m, 4 H, CH_2), 3.52 (dd, *J* = 6.4, 9.6 Hz, 4 H, 2-H), 3.72 (dd, *J* = 3.7, 9.6 Hz, 4 H, 2-H), 3.94 (m_c, 2 H, 1-H), 5.77 (d, *J* = 8.5 Hz, 2 H, NH) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.35, -5.27 (2 q, $SiCH_3$), 18.4 [s, Si*C*$(CH_3)_3$], 25.3 (t, CH_2), 26.0 [q, SiC(*C*$H_3)_3$], 36.5 (t, CH_2), 51.6 (d, C-1), 60.6 (t, C-2), 172.2 (s, C=O) ppm.

IR (ATR): ν = 3295 (NH), 2960-2855 (C-H), 1710 (C=O), 1250 (C-O) cm^{-1}.

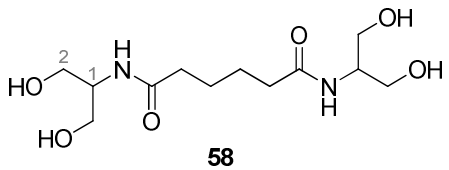
HRMS (pos. ESI-TOF): $C_{36}H_{80}N_2O_6Si_4 \cdot Na^+$: calcd.: 771.4986
found: 771.4960

Elemental Analysis: $C_{36}H_{80}N_2O_6Si_4$ (749.4) calcd. (%): C 57.70, H 10.76, N 3.74
found. (%): C 57.88, H 10.63, N 3.71

Divalent amide (**58**)

JNS120

According to **GP-5**, to a stirred solution of **57** (200 mg, 0.267 mmol) in isopropanol (0.9 mL) was added AcCl (11.4 µL, 0.160 mmol) at 0 °C. The reaction mixture was stirred at r.t. for 1 h and 40 min. All volatiles were removed in vacuo affording **58** (76 mg, quant.) as a colorless solid.



58

Chemical Formula: $C_{12}H_{24}N_2O_6$
Molecular Weight: 292.3 $g \cdot mol^{-1}$
Melting point: 174 – 175 ºC

^{1}H-NMR (500 MHz, DMSO-D_6): δ = 1.42 – 1.47 (m, 4 H, CH_2), 2.07 (t, *J* = 6.6 Hz, 4 H, CH_2), 3.38 (d, *J* = 5.7 Hz, 8 H, 2-H), 3.65 – 3.72 (m, 2 H, 1-H), 4.27 (bs, 4 H, OH), 7.45 (d, *J* = 8.1 Hz, 2 H, NH) ppm.

^{13}C-NMR (125 MHz, DMSO-D_6): δ = 25.0, 35.2 (2 t, CH_2), 52.8 (d, C-1), 60.2 (t, C-2), 172.0 (s, C=O) ppm.

IR (ATR): 3295 (OH, NH), 2965-2855 (C-H), 1635 (C=O), 1260 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{12}H_{24}N_2O_6 \cdot Na^+$: calcd.: 315.1521
found: 315.1526

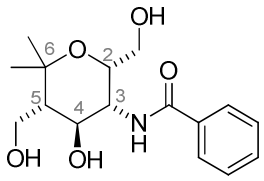
Elemental Analysis: $C_{12}H_{24}N_2O_6$ (292.3) calcd. (%): C 49.30, H 8.28, N 9.85
found. (%): C 49.36, H 8.30, N 9.55

6.2.2.2. HATU coupling reagent

(2*S*,3*R*,4*S*,5*S*)-4-Hydroxy-2,5-bis(hydroxymethyl)-6,6-dimethyltetrahydro-2*H*-pyran-3-benzamide (**62**)

JNS283

According to **GP-2**, under argon atmosphere, aminopyran **3** (50 mg, 0.24 mmol), benzoic acid **61** (29 mg, 0.24 mmol) and HATU (93 mg, 0.24 mmol) were dissolved in DMF (2 mL). After addition of Et_3N (0.2 mL, 1.1 mmol), the reaction mixture was stirred at r.t. for 24 h. The solvent was removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2 100%, CH_2Cl_2/MeOH 9:1) affording **62** (74 mg, quant.) as a colorless solid.



62

Chemical Formula: $C_{16}H_{23}NO_5$
Molecular Weight: 309.4 $g \cdot mol^{-1}$
Melting point: 80 °C
Optical rotation: $[\alpha]_D^{22}$ = +91.5 (c = 0.74, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.33, 1.46 (2 s, 3 H each, CH_3), 1.77 (m_c, 1 H, 5-H), 3.51, 3.56 (AB part of ABX system, J_{AB} = 11.6 Hz, J_{AX} = 4.9 Hz, J_{BX} = 6.8 Hz, 1 H each, 2-CH_2), 3.67 (dd, J = 4.2, 11.1 Hz, 1 H, 5-CH_2), 3.96 – 4.00 (m, 2 H, 4-H, 5-CH_2), 4.17 – 4.22 (m, 2 H, 2-H, 3-H), 7.44 – 7.49 (m, 2 H, Ph), 7.53 (m_c, 1 H, Ph), 7.83 – 7.86 (m, 2 H, Ph) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 26.3, 27.7 (2 q, CH_3), 49.0 (d, C-5), 53.9 (d, C-3), 63.1 (t, 2-CH_2), 63.3 (t, 5-CH_2), 70.8 (d, C-2), 74.1 (d, C-4), 75.8 (s, C-6), 128.4, 129.6, 132.8 (3 d, Ph), 135.5 (s, Ph), 170.5 (s, C=O) ppm.

IR (ATR): ν = 3320-3150 (OH, NH), 2890 (C-H), 1640 (C=O), 1540 (C=C), 1230 (C-O), 1070 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{16}H_{23}NO_5 \cdot Na^+$: calcd.: 332.1468
found: 332.1465

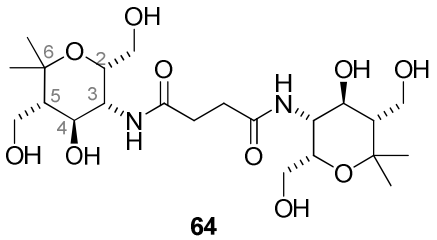
Elemental Analysis: $C_{16}H_{23}NO_5$ (309.4) calcd. (%): C 62.12, H 7.49, N 4.53
found (%): C 56.13, H 7.38, N 7.08

A better elemental analysis could not be obtained.

Divalent amide (**64**)

JNS285

According to **GP-2**, aminopyran **3** (50 mg, 0.24 mmol), succinic acid **63** (11 mg, 0.97 mmol) and HATU (93 mg, 0.24 mmol) were dissolved in DMF (2.0 mL). After addition of Et_3N (0.4 mL, 2.2 mmol), the reaction mixture was stirred at r.t. under argon atmosphere. After 24 h the solvent was removed in vacuo. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH 9:1 to 4:1) affording **64** (35 mg, 73%) as a colorless solid.



64

Chemical Formula: $C_{22}H_{40}N_2O_{10}$

Molecular Weight: 492.6 g·mol^{-1}

Melting range: 185 – 201 °C

Optical rotation: $[\alpha]_D^{22}$ = +47.2 (c =1.40, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.21, 1.40 (2 s, 6 H each, CH_3), 1.77 – 1.81 (m, 2 H, 5-H), 2.54 (bs, 4 H, CH_2), 3.48 (d, *J* = 6.1 Hz, 4 H, 2-CH_2), 3.66 (dd, *J* = 5.5, 11.2 Hz, 2 H, 5-CH_2), 3.79 – 3.83 (m, 4 H, 5-CH_2, 4-H), 3.92 (dd, *J* = 3.9, 5.2 Hz, 2 H, 3-H), 4.06 (dt, *J* = 3.9, 6.1 Hz, 2 H, 2-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 25.5, 27.3 (2 q, CH_3), 32.1 (t, CH_2), 49.4 (d, C-5), 55.2 (d, C-3), 62.7 (t, 2-CH_2), 62.8 (t, 5-CH_2), 71.0 (d, C-2), 73.1 (d, C-4), 76.0 (C-6), 175.2 (C=O) ppm.

IR (ATR): 3410-3260 (OH, NH), 2970-2890 (C-H), 1640 (C=O), 1230 (C-O), 1075 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{22}H_{40}N_2O_{10}$·Na^+: calcd.: 515.2575
found: 515.2599

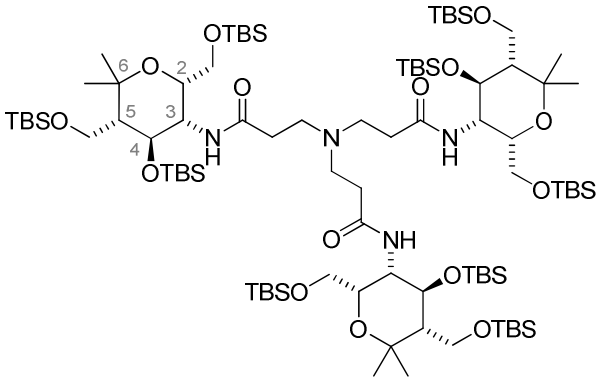
Elemental Analysis: $C_{22}H_{40}N_2O_{10}$ (492.6) calcd. (%): C 53.65, H 8.19, N 5.69
found. (%): C 45.88, H 7.94, N 7.50

A better elemental analysis could not be obtained.

Trivalent amide (**68**)

JNSW11

According to **GP-2**, aminopyran **26** (87 mg, 159 µmol), 3,3',3"-nitrilotripropanoic acid (9 mg, 39 µmol) and HATU (60 mg, 159 µmol) were dissolved in DMF (2.8 mL). After addition of Et_3N (0.1 mL, 721 µmol), the reaction mixture was stirred at r.t. for 24 h. Saturated $NaHCO_3$ solution (10 mL) was added and the aqueous layer was extracted with EtOAc (3x 20 mL). The combined organic phases were washed with saturated NaCl solution, dried with Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (silica gel, hexanes, hexanes/EtOAc 10:1 to 6:1) Affording **68** (60 mg, 83%) as a colorless oil.



68

Chemical Formula: $C_{90}H_{192}N_4O_{15}Si_9$

Molecular Weight: 1823.3 g·mol^{-1}

Appearance: colorless oil

Optical rotation: $[\alpha]_D^{22}$ = + 10.8 (c = 2.56, $CHCl_3$)

^{1}H NMR (700 MHz, $CDCl_3$): δ = 0.01 – 0.03 (m, 36 H, CH_3), 0.09 (s, 9 H, CH_3), 0.16 (s, 9 H, CH_3), 0.85 – 0.88 (m, 54 H, *t*-Bu), 0.89 (s, 27 H, *t*-Bu), 1.17, 1.47 (2 s, 9 H each, CH_3), 1.61 (m_c, 3 H, 5-H), 2.32 (t, *J* = 7.4 Hz, 6 H, CH_2CO), 2.73 (td, *J* = 7.4, 14.1 Hz, 3 H, NCH_2), 2.82 (td, *J* = 7.4, 14.1 Hz, 3 H, NCH_2), 3.45 (dd, *J* = 8.0, 10.2 Hz, 3 H, 5-CH_2), 3.52, 3.57 (AB part of ABX system, J_{AB} = 10.5 Hz, J_{AB} = J_{BX} = 6.1 Hz, 3 H each, 2-CH_2), 3.70 – 3.76 (m, 6 H, 5-CH_2, 3-H), 4.16 (dt, *J* = 1.7, 6.1 Hz, 3 H, 2-H), 4.22 (m_c, 3 H, 4-H), 6.30 (d, *J* = 8.5 Hz, 3 H, NH) ppm.

^{13}C NMR (175 MHz, $CDCl_3$): δ = -5.14, -5.11, -5.09, -5.00, -4.97, -4.65 (6 q, CH_3), 18.0, 18.2, 18.3 [3 s, Si*C*(CH_3)$_3$], 25.9, 26.0, 26.0 [3 q, SiC(*C*H_3)$_3$], 27.4 , 28.0 (2 q, CH_3), 33.3 (t,

CH_2CO), 49.0 (t, NCH_2), 49.5 (d, C-5), 50.2 (d, C-3), 62.6 (t, 5-CH_2), 63.3 (t, 2-CH_2), 67.7 (d, C-2), 68.7 (d, C-4), 74.3 (s, C-6), 171.0 (s, C=O) ppm.

IR (ATR): ν = 3440 (NH), 2960-2855 (C-H), 1660, 1505 (C=O), 1245 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{90}H_{192}N_4O_{15}Si_9 \cdot H^+$: calcd.: 1822.2387
found: 1822.2400

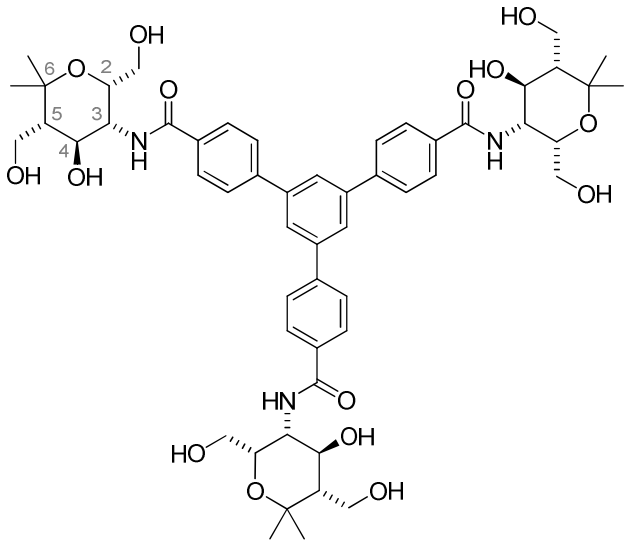
$C_{90}H_{192}N_4O_{15}Si_9 \cdot Na^+$: calcd.: 1844.2206
found: 1844.2203

Elemental Analysis: $C_{90}H_{192}N_4O_{15}Si_9$ (1823.3) calcd. (%): C 59.29, H 10.61, N 3.07
found. (%): C 59.74, H 10.49, N 2.97

Trivalent amide (**70**)

JNS289

According to **GP-2**, aminopyran **3** (50 mg, 0.244 mmol), acid **69** (27 mg, 61 μmol) and HATU (93 mg, 0.244 mmol) were dissolved in DMF (2.0 mL). After addition of Et_3N (0.6 mL, 4.4 mmol), the reaction mixture was stirred at r.t. for 24 h. The solvent was removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH 6:1 to 4:1) affording **70** (49 mg, 80%) as a colorless solid.



70

Chemical Formula: $C_{54}H_{69}N_3O_{15}$

Molecular Weight: 1000.1 g·mol^{-1}

Melting point: 245 °C

Optical rotation: $[\alpha]_D^{22}$ = +150.5 (c = 0.74, MeOH)

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.34, 1.47 (2 s, 9 H each, CH_3), 1.80 (td, *J* = 4.8, 10.0 Hz, 3 H, 5-H), 3.56, 3.61 (AB part of ABX system, J_{AB} = 11.7 Hz, J_{AX} = 4.6 Hz, J_{BX} = 6.6 Hz, 3 H each, 2-CH_2), 3.71, 4.00 (2 dd, *J* = 4.8, 11.1 Hz, 3 H each, 5-CH_2), 4.02 – 4.05 (m, 3 H, 4-H), 4.20 – 4.25 (m, 6 H, 2-H, 3-H), 7.76 (d, *J* = 8.3 Hz, 6 H, Ar), 7.82 (s, 3 H, Ar), 7.94 (d, *J* = 8.3 Hz, 6 H, Ar) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 26.3, 27.7 (2 q, CH_3), 49.9 (d, C-5), 54.2 (d, C-2), 63.2 (t, 2-CH_2), 63.3 (t, 5-CH_2), 70.9 (d, C-3), 74.1 (d, C-4), 75.9 (s, C-6), 126.6 , 128.4, 129.2 (3 d, Ar), 134.6, 142.8, 145.0 (3 s, Ar), 170.1 (s, C=O) ppm.

IR (ATR): ν = 3320 (OH, NH), 2970-2920 (C-H), 1735 (C=O), 1440 (C=C), 1230 (C-O), 1085 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{54}H_{69}N_3O_{15}$·Na^+: calcd.: 1022.4615
found: 1022.4612

Elemental Analysis: $C_{54}H_{69}N_3O_{15}$ (1000.1) calcd. (%): C 64.85, H 6.95, N 4.20
found. (%): C 55.59, H 7.00, N 7.49

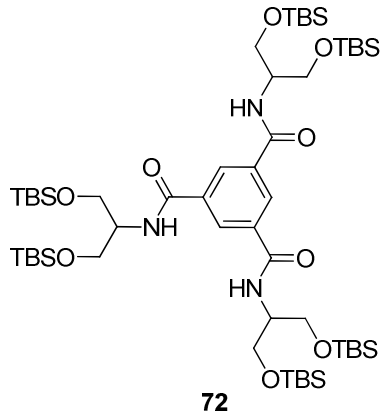
A better elemental analysis could not be obtained.

Trivalent amide (**72**)

JNS318

According to **GP-2**, under argon atmosphere, **52** (130 mg, 0.407 mmol), benzene-1,3,5-tricarboxylic acid (21 mg, 0.10 mmol) and HATU (155 mg, 0.407 mmol) were dissolved in DMF (2 mL). After addition of Et_3N (0.3 mL, 1.8 mmol), the reaction mixture was stirred at r.t. After 22 h saturated $NaHCO_3$ solution (10 mL) was added and the aqueous layer was extracted with EtOAc (3x 20 mL). The combined organic phases were washed with saturated NaCl solution, dried with Na_2SO_4 and the solvent was removed in vacuo. The crude product

was purified by flash column chromatography (silica gel, hexanes/EtOAc 6:1) affording **72** (83 mg, 73%) as a colorless oil.



72

Chemical Formula: $C_{54}H_{111}N_3O_9Si_6$

Molecular Weight: 1115.0 g·mol^{-1}

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.075, 0.083 (2 s, 18 H each, CH_3), 0.90 (s, 54 H, *t*Bu), 3.66 (dd, *J* = 9.7, 6.4 Hz, 6 H, 2-H), 3.85 (dd, *J* = 9.7, 3.7 Hz, 6 H, 2-H), 4.16 – 4.19 (m, 3 H, 1-H), 6.62 (d, *J* = 8.4 Hz, 3 H, NH), 8.28 (s, 3 H, Ar) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = -5.30, -5.25, -3.44 (3 q, $SiCH_3$), 18.4 [s, Si*C*$(CH_3)_3$], 26.0 [q, SiC(CH_3)$_3$], 52.5 (d, C-1), 60.5 (t, C-2), 128.1 (d, Ar), 135.7 (s, Ar), 165.3 (s, C=O) ppm.

IR (ATR): ν = 3320 (NH), 2950-2880 (C-H), 1665 (C=O), 1505 (C=C), 1250 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{54}H_{111}N_3O_9Si_6 \cdot H^+$: calcd.: 1136.6828
found: 1136.6865

Elemental Analysis:

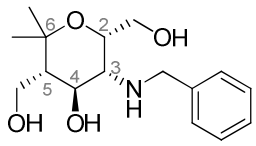
$C_{54}H_{111}N_3O_9Si_6$ (1115.0) + 2 H_2O calcd. (%): C 56.35, H 10.07, N 3.65
found. (%): C 56.98, H 10.08, N 3.53

6.2.3. Reductive amination

(2*S*,3*R*,4*S*,5*S*)-[3-(Benzylamino)-4-hydroxy-6,6-dimethyltetrahydro-2*H*-pyran-2,5-diyl] dimethanol (**76**)

JNS128

According to **GP-6**, aminopyran **3** (70 mg, 0.34 mmol) was dissolved in MeOH (5.5 mL), and freshly distilled benzaldehyde **75** (36 mg, 35µL, 0.34 mmol) was added followed by addition of $MgSO_4$ (92 mg, 0.76 mmol). The reaction mixture was stirred for 1 h. After filtration and cooling to 0 °C, $NaBH_4$ (14 mg, 0.37 mmol) was added. After stirring at r.t. for 2 h, the reaction was quenched with H_2O (5.5 mL) and extracted with CH_2Cl_2 (3x 20 mL), washed with brine, dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1) affording **76** (65 mg, 65%) as a colorless solid.



76

Chemical Formula:	$C_{16}H_{25}NO_4$
Molecular Weight:	295.4 g·mol^{-1}
Melting Point:	125 – 127 ºC
Optical rotation:	$[\alpha]_D^{22}$ = +21.8 (c = 1.04, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.32, 1.41 (2 s, 3 H each, CH_3), 1.63 – 1.66 (m, 1 H, 5-H), 2.73 – 2.75 (m, 1 H, 3-H), 3.62 (dd, *J* = 3.2, 11.5 Hz, 1 H, 5-CH_2), 3.70 (d, *J* = 5.0 Hz, 2 H, 2-CH_2), 3.78 (A part of AB system, J_{AB} = 13.0 Hz, 1 H, CH_2Ph), 3.87 (dd, *J* = 4.3, 11.5 Hz, 1 H, 5-CH_2), 3.93 (B part of AB system, J_{AB} = 13.0 Hz, 1 H, CH_2Ph), 4.03 (t, *J* = 3.9 Hz, 1 H, 4-H), 4.05 (dt, *J* = 2.9, 5.0 Hz, 1 H, 2-H), 7.38 – 7.24 (m, 5 H, Ph) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ =27.2, 28.3 (2 q, CH_3), 49.4 (d, C-5), 52.8 (t, CH_2Ph), 60.8 (d, C-3), 63.6 (t, 5-CH_2), 64.4 (t, 2-CH_2), 69.4 (d, C-2), 72.9 (d, C-4), 75.6 (s, C-6), 128.4, 129.6* (2 d, Ph), 140.2 (s, Ph) ppm. *signal with high intensity

IR (ATR): ν = 3510-3195 (OH,NH), 2915 (C-H), 1460 (C=C), 1210 (C-O), 1090 (C-O-C) cm^{-1}.

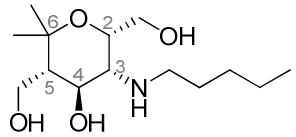
HRMS (pos. ESI-TOF): $C_{16}H_{25}NO_4 \cdot Na^+$: calcd.: 296.1856
found: 296.1863

Elemental Analysis: $C_{16}H_{25}NO_4$ (295.4) calcd. (%): C 65.06, H 8.53, N 4.74
found. (%): C 65.09, H 8.42, N 4.85

(2*S*,3*R*,4*S*,5*S*)-[4-Hydroxy-6,6-dimethyl-3-(pentylamino)tetrahydro-2*H*-pyran-2,5-diyl]dimethanol (**74**)

JNSV15

According to **GP-6**, aminopyran **3** (170 mg, 0.83 mmol) was dissolved in MeOH (10 mL), and freshly distilled pentanal **73** (71.3 mg, 88 µL, 0.83 mmol) was added followed by addition of $MgSO_4$ (200 mg, 1.66 mmol). The reaction mixture was stirred for 2 h. Pentanal **73** (7.15 mg, 8.8 µl, 0.083 mmol) was added and the reaction stirred for one additional hour. After filtration and cooling to 0 °C, $NaBH_4$ (64 mg, 1.66 mmol) was added. After stirring at r.t. for 8 h, the reaction was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (3x 10 mL), washed with brine, dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1 to 4:1) affording **74** (102 mg, 45 %) as a colorless solid.



74

Chemical Formula: $C_{14}H_{29}NO_4$
Molecular Weight: 275.4 $g \cdot mol^{-1}$
Melting point: 88 – 90 °C
Optical rotation: $[\alpha]_D^{22}$ = + 31.2 (c = 8.85, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 0.94 (t, *J* = 7.0 Hz, 3 H, CH_3), 1.34 (s, 3 H, CH_3), 1.34 – 1.41 (m, 4 H, CH_2), 1.43 (s, 3 H, CH_3), 1.57 – 1.66 (m, 2 H, CH_2), 1.70 (q, *J* = 3.6 Hz, 1 H, 5-H), 2.84 – 2.89 (m, 1 H, CH_2), 2.94 – 2.97 (m, 2 H, CH_2, 3-H), 3.61 (dd, *J* = 3.6, 11.3 Hz, 1 H, 5-CH_2), 3.79 – 3.85 (m, 2 H, 2-CH_2), 3.93 (dd, *J* = 3.6, 11.3 Hz, 1 H, 5-CH_2), 4.07 (t, *J* = 3.6 Hz, 1 H, 4-H), 4.10 (dt, *J* = 2.5, 4.5 Hz, 1 H, 2-H) ppm.

^{13}C-NMR (126 MHz, CD_3OD): δ = 14.2 (q, CH_3), 23.3 (t, CH_2), 27.1, 28.1 (2 q, CH_3), 28.8 (t, CH_2), 30.0 (t, CH_2), 48.2 (t, CH_2), 48.6 (d, C-5), 61.5 (d, C-3), 63.3 (t, 5-CH_2), 64.5 (t, 2-CH_2), 67.5 (d, C-2), 71.7 (d, C-4), 75.9 (s, C-6) ppm.

IR (ATR): ν = 3350-3250 (OH, NH), 2960-2870 (C-H), 1090 (C-O) cm^{-1}.

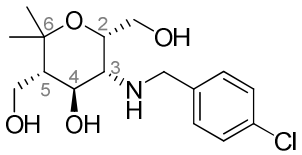
HRMS (pos. ESI-TOF): $C_{14}H_{29}NO_4 \cdot H^+$: calcd.: 276.2169
found: 276.2156

Elemental Analysis: $C_{14}H_{29}NO_4$ (275.4) calcd. (%): C 61.06, H 10.61, N 5.09
found. (%): C 60.28, H 10.33, N 5.04

(2*S*,3*R*,4*S*,5*S*)-[3-(4-Chlorobenzylamino)-4-hydroxy-6,6-dimethyltetrahydro-2H-pyran-2,5-diyl]dimethanol (**82**)

JNS205

According to **GP-6**, aminopyran **3** (100 mg, 0.48 mmol) was dissolved in MeOH (7.9 mL), and 4-chlorobenzaldehyde (68 mg, 0.48 mmol) was added followed by $MgSO_4$ (132 mg, 1.01 mmol). The reaction mixture was stirred for 1 h. After filtration and cooling to 0 °C, $NaBH_4$ (24 mg, 0.63 mmol) was added. After stirring at r.t. for 2 h, the reaction was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (3x 20 mL), washed with brine, dried and concentrated. The crude was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1) affording **82** (82 mg, 51%) as a colorless solid.



82

Chemical Formula: $C_{16}H_{24}ClNO_4$

Molecular Weight: 329.8 g·mol^{-1}

Melting point: 134 – 136 °C

Optical rotation: $[\alpha]_D^{22}$ = +26.5 (c = 0.94, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.31, 1.40 (2 s, 3 H each, CH_3), 1.64 (m_c, 1H, 5-H), 2.67 – 2.70 (m, 1 H, 3-H), 3.60 – 3.64 (m, 1 H, 5-CH_2), 3.69 (d, *J* = 5.1 Hz, 2 H, 2-CH_2), 3.77 (A part of AB system, J_{AB} = 13.2 Hz, 1 H, CH_2Ar), 3.84 – 3.86 (m, 1 H, 5-CH_2), 3.89 (B part of AB system, J_{AB} = 13.2 Hz, 1 H, CH_2Ar), 4.00 (t, *J* = 4.3 Hz, 1 H, 4-H), 4.04 (dt, *J* = 3.0, 5.1 Hz, 1 H, 2-H), 7.30 – 7.36 (m, 4 H, Ar) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 27.1, 28.2 (2 q, CH_3), 49.5 (d, C-5), 51.9 (t, CH_2Ph), 60.8 (d, C-3), 63.6 (t, 5-CH_2), 64.3 (t, 2-CH_2), 69.6 (d, C-2), 73.0 (d, C-4), 75.6 (s, C-6), 129.6, 131.2 (2d, Ar) 134.0, 139.3 (2 s, Ar) ppm.

IR (ATR): ν = 3520-3260 (OH, NH), 2930 (C-H), 1490 (C=C), 1160 (C-O), 1095 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{16}H_{24}ClNO_4 \cdot H^+$: calcd.: 330.1467
found: 330.1496

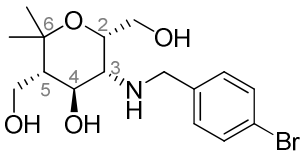
Elemental Analysis: $C_{16}H_{24}ClNO_4$ (329.8) calcd. (%): C 58.27, H 7.33, N 4.25
found. (%): C 58.33, H 7.33, N 4.26

(2*S*,3*R*,4*S*,5*S*)-[3-(4-Bromobenzylamino)-4-hydroxy-6,6-dimethyltetrahydro-2*H*-pyran-2,5-diyl]dimethanol (**78**)

JNS201

According to **GP-6**, aminopyran **3** (100 mg, 0.48 mmol) was dissolved in MeOH (7.9 mL), and 4-bromobenzaldehyde (90 mg, 0.48 mmol) was added followed by $MgSO_4$ (132 mg, 1.01 mmol). The reaction mixture was stirred for 1 h. After filtration and cooling to 0° C, $NaBH_4$ (23.9 mg, 0.63 mmol) was added. After stirring at r.t. for 2 h, the reaction was quenched with water (10 mL) and extracted with CH_2Cl_2 (3x 20 mL), washed with brine,

dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1) affording **78** (128 mg, 71%) as a colorless solid.



78

Chemical Formula: $C_{16}H_{24}BrNO_4$

Molecular Weight: 374.3 g·mol^{-1}

Melting point: 137 – 139 ºC

Optical rotation: $[\alpha]_D^{22}$ = +15.9 (c =1.70, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.31, 1.40 (2 s, 3 H each, CH_3), 1.63 – 1.66 (m, 1 H, 5-H), 2.66 – 2.69 (m, 1 H, 3-H), 3.59 – 3.64 (m, 1 H, 5-CH_2), 3.69 (d, *J* = 5.1 Hz, 2 H, 2-CH_2), 3.75 (A part of AB system, J_{AB} = 13.3 Hz, 1 H, CH_2Ar), 3.83 – 3.89 (m, 2 H, 5-CH_2, CH_2Ar), 3.99 (t, *J* = 4.0 Hz, 1 H, 4-H), 4.04 (dt, *J* = 3.0, 5.1 Hz, 1 H, 2-H), 7.26 – 7.32, 7.44 – 7.50 (2 m, 2 H each, Ar) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 27.1, 28.2 (2 q, CH_3), 49.5 (d, C-5), 52.00 (t, CH_2Ar), 60.7 (d, C-3), 63.6 (t, 5-CH_2), 64.3 (t, 2-CH_2), 69.6 (d, C-2), 73.1 (d, C-4), 75.6 (s, C-6), 122.0 (s, Ar), 131.5, 132.6 (2 d, Ar), 139.9 (s, Ar) ppm.

IR (ATR): ν = 3485-3235 (OH, NH), 2970 (C-H), 1490 (C=C), 1160 (C-O), 1095 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{16}H_{24}BrNO_4 \cdot H^+$: calcd.: 374.0961
found: 374.0965

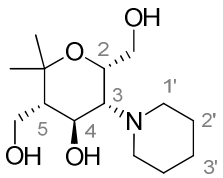
$C_{16}H_{24}BrNO_4 \cdot Na^+$: calcd.: 396.0781
found: 396.0779

Elemental Analysis: $C_{16}H_{24}BrNO_4$ (374.3) calcd. (%): C 51.35, H 6.46, N 3.74
found. (%): C 51.76, H 6.47, N 3.75

(2*S*,3*R*,4*S*,5*S*)-[4-Hydroxy-6,6-dimethyl-3-(piperidin-1-yl)tetrahydro-2*H*-pyran-2,5-diyl]dimethanol (**85**)

JNSV17

According to **GP-6**, aminopyran **3** (200 mg, 0.97 mmol) was dissolved in MeOH (8 mL) and dialdehyde **83** (25% in water, 0.175 ml, 0.443 mmol) was added followed by addition of $MgSO_4$ (680 mg, 8.31 mmol). The reaction mixture was stirred overnight. After filtration and cooling to 0 °C, $NaBH_4$ (89 mg, 2.37 mmol) was added. After stirring at r.t. for 3 h, the reaction was quenched with H_2O (10 mL), extracted with CH_2Cl_2 (5x 10 mL), washed with brine dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2, CH_2Cl_2/ MeOH 20:1, 10:1, 4:1) affording the reductive amination product **85** (32 mg, 26 %) as a colorless solid.



85

Chemical Formula: $C_{14}H_{27}NO_4$

Molecular Weight: 273.4 g·mol^{-1}

Melting range: 91 – 95 °C

Optical rotation: $[\alpha]_D^{22}$ = +10.7 (c = 0.93, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.10, 1.25 (2 s, 3 H each, CH_3), 1.44 – 1.50 (m, 2 H, 3'-H), 1.51 – 1.57 (m, 4 H, 2'-H), 1.81 (td, *J* = 6.1, 11.9 Hz, 1 H, 5-H), 2.59 – 2.66 (m, 2 H, 1'-H), 2.71 (t, *J* = 6.7 Hz, 1 H, 3-H), 2.92 (m_c, 2 H, 1'-H), 3.56 – 3.70 (m, 3 H, 2-CH_2, 5-CH_2), 3.84 (dd, *J* = 6.1, 11.2 Hz, 1 H, 5-CH_2), 3.95 – 4.01 (m, 1 H, 2-H), 4.15 (dd, *J* = 6.7, 11.9 Hz, 1 H, 4-H) ppm.

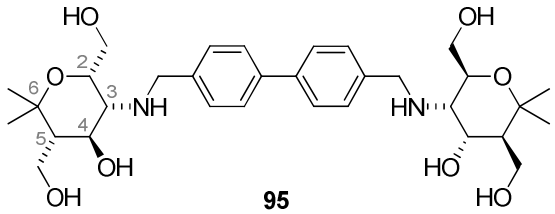
^{13}C-NMR (125 MHz, CD_3OD): δ = 22.2 (q, CH_3), 24.4 (t, C-3'), 25.5 (q, CH_3), 26.6 (t, C-2'), 49.0 (d, C-5), 52.4 (t, C-1'), 61.7 (t, 5-CH_2), 62.5 (t, 2-CH_2), 66.4 (d, C-4), 71.2 (d, C-2), 71.6 (d, C-3), 75.1 (s, C-6) ppm.

HRMS (pos. ESI-TOF): $C_{14}H_{27}NO_4 \cdot H^+$: calcd.: 274.2018
found: 274.2032

Divalent amine (**95**)

JNS137

According to **GP-6**, aminopyran **3** (200 mg, 0.97 mmol) was dissolved in MeOH (17 mL), and 4,4'-biphenyldicarboxaldehyde (97 mg, 0.46 mmol) was added followed by $MgSO_4$ (285 mg, 2.37 mmol). The reaction mixture was stirred for 2.5 h. After filtration and cooling to 0 °C, $NaBH_4$ (38 mg, 1.01 mmol) was added. After stirring at r.t. for 2 h, the reaction was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (3x 20 mL), washed with brine, dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH 9:1 to 4:1) affording **95** (103 mg, 40%) as a colorless solid.



95

Chemical Formula:	$C_{32}H_{48}N_2O_8$
Molecular Weight:	588.7 g·mol^{-1}
Melting point:	118 – 120 °C
Optical rotation:	$[\alpha]_D^{22}$ = +28.8 (c = 0.69, MeOH)

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.33, 1.42 (2 s, 6 H each, CH_3), 1.67 (q, $J \approx 3.9$ Hz, 2 H, 5-H), 2.83 – 2.85 (m, 2 H, 3-H), 3.65 (dd, J = 3.1, 11.4 Hz, 2 H, 5-CH_2), 3.74 (d, J = 4.8 Hz, 4 H, 2-CH_2), 3.90 – 3.92 (m, 4 H, CH_2Ar, 5-CH_2), 4.03 (B part of AB system, J_{AB} = 13.1 Hz, 2 H, CH_2Ar), 4.06 – 4.09 (m, 4 H, 4-H, 2-H), 7.45– 7.47, 7.60 – 7.63 (2 m, 4 H each, Ar) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 27.2, 28.2 (2 q, CH_3), 49.9 (d, C-5), 52.1 (t, CH_2Ar), 60.8 (d, C-3), 63.5 (t, 5-CH_2), 64.6 (t, 2-CH_2), 68.9 (d, C-2), 72.6 (d, C-4), 75.8 (s, C-6), 128.2, 130.3 (2 d, Ar), 138.5, 141.4 (2 s, Ar) ppm.

IR (ATR): ν = 3360-3290 (OH, NH), 2970-2880 (C-H), 1470 (C=C), 1230 (C-O), 1090 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{32}H_{48}N_2O_8 \cdot H^+$: calcd.: 589.3463
found: 589.3478

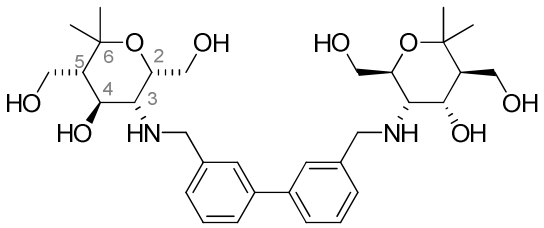
$C_{32}H_{48}N_2O_8 \cdot Na^+$: calcd.: 611.3303
found: 611.3294

Elemental Analysis: $C_{32}H_{48}N_2O_8$ (588.7) calcd. (%): C 65.28, H 8.22, N 4.76
found. (%): C 64.63, H 8.04, N 4.76

Divalent amine (**96**)

JNS171

According to **GP-6**, aminopyran **3** (80 mg, 0.39 mmol) was dissolved in MeOH (6.8 mL), and 3,3'-biphenyldicarbaldehyde (37 mg, 0.18 mmol) was added followed by $MgSO_4$ (114 mg, 0.95 mmol). The reaction mixture was stirred for 17 h. After filtration and cooling to 0 °C, $NaBH_4$ (15 mg, 0.39 mmol) was added. After stirring at r.t. for 3 h, the reaction was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (3x 10 mL), washed with brine, dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ 7N NH_3 in MeOH 20:1) affording **96** (72 mg, 70%) as a colorless solid.



96

Chemical Formula: $C_{32}H_{48}N_2O_8$

Molecular Weight: 588.7 g·mol^{-1}

Melting point: 85 °C

Optical rotation: $[\alpha]_D^{22}$ = +35.4 (c = 1.04, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.30, 1.41 (2 s, 6 H each, CH_3), 1.65 (q, $J \approx 3.9$ Hz, 2 H, 5-H), 2.73 – 2.77 (m, 2 H, 3-H), 3.63 (dd, J = 3.1, 11.6 Hz, 2 H, 5-CH_2), 3.70, 3.73 (AB part

of ABX system, J_{AB} = 11.7 Hz, J_{AX} = 4.9 Hz, J_{BX} = 5.1 Hz, 2 H each, 2-CH_2), 3.83 – 3.90 (m, 4 H, CH_2Ar, 5-CH_2), 3.98 (B part of AB system, J_{AB} = 13.1 Hz, 2 H, CH_2Ar), 4.03 – 4.08 (m, 4 H, 2-H, 4-H), 7.34 (d, J = 7.6 Hz, 2 H, Ar), 7.41 (t, J = 7.6 Hz, 2 H, Ar), 7.55 (d, J = 7.6 Hz, 2 H, Ar), 7.66 (s, 1 H, Ar) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 27.2, 28.3 (2 q, CH_3), 49.5 (d, C-5), 52.8 (t, CH_2Ar), 60.7 (d, C-3), 63.7 (t, 5-CH_2), 64.4 (t, 2-CH_2), 69.6 (d, C-2), 73.1 (d, C-4), 75.7 (s, C-6), 127.0, 128.3, 128.6, 130.1 (4 d, Ar), 141.2, 142.6 (2 s , Ar) ppm.

IR (ATR): ν = 3370-3300 (OH, NH), 2970-2850 (C-H), 1470 (C=C), 1230 (C-O), 1090 (C-O-C) cm^{-1}.

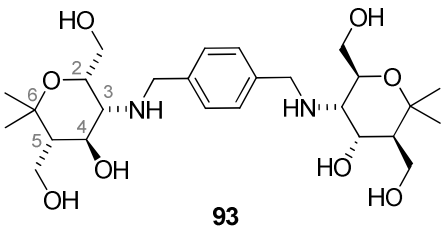
HRMS (pos. ESI-TOF): $C_{32}H_{48}N_2O_8 \cdot H^+$: calcd.: 589.3483
found: 589.3540

Elemental Analysis: $C_{32}H_{48}N_2O_8$ (588.7): calcd. (%): C 65.28, H 8.22, N 4.76
found. (%): C 65.31, H 8.32, N 4.75

Divalent amine (**93**)

JNS138

According to **GP-6**, aminopyran **3** (100 mg, 0.48 mmol) was dissolved in MeOH (8.5 mL), and terephthalaldehyde **92** (32 mg, 0.24 mmol) was added followed by $MgSO_4$ (143 mg, 1.19 mmol). The reaction mixture was stirred for 24 h. After filtration and cooling to 0 °C, $NaBH_4$ (20 mg, 0.52 mmol) was added. After stirring at r.t. for 3 h, the reaction was quenched with H_2O (10 mL) and extracted with CH_2Cl_2 (3x 10 mL), washed with brine, dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH 6:1 to 4:1) affording **93** (60 mg, 50%) as a colorless solid.



93

Chemical Formula: $C_{26}H_{44}N_2O_8$

Molecular Weight: 512.6 g·mol^{-1}

Melting range: 143 – 146 °C

Optical rotation: $[\alpha]_D^{22}$ = +22.5 (c = 1.32, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.32, 1.41 (2 s, 6 H each, CH_3), 1.64 (m_c, 2 H, 5-H), 2.70 – 2.72 (m, 2 H, 3-H), 3.61 (dd, *J* = 3.1, 11.5 Hz, 2 H, 5-CH_2), 3.66, 3.69 (AB part of ABX system, J_{AB} = 11.7 Hz, J_{AX} = J_{BX} = 5.1 Hz, 2 H each, 2-CH_2), 3.76 (A part of AB system, J_{AB} = 13.0 Hz, 2 H, CH_2Ar), 3.86 (dd, *J* = 4.5, 11.5 Hz, 2 H, 5-CH_2), 3.90 (B part of AB system, J_{AB} = 13.0 Hz, 2 H, CH_2Ar), 4.00 (t, *J* = 3.9 Hz, 2 H, 4-H), 4.04 (dt, *J* = 2.9, 5.1 Hz, 2 H, 2-H), 7.34 (s, 4 H, Ar) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 27.2, 28.3 (2 q, CH_3), 49.4 (d, C-5), 52.5 (t, CH_2Ar), 60.7 (d, C-3), 63.6 (t, 5-CH_2), 64.3 (t, 2-CH_2), 69.5 (d, C-2), 73.0 (d, C-4), 75.7 (s, C-6), 129.8 (d, Ar), 139.6 (s, Ar) ppm.

IR (ATR): ν = 3350 (OH, NH), 2980-2830 (C-H), 1470 (C=C), 1215 (C-O), 1090 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{26}H_{44}N_2O_8 \cdot Na^+$: calcd.: 535.2990
found: 535.3017

Elemental Analysis: $C_{26}H_{44}N_2O_8$ (512.6): calcd. (%): C 60.92, H 8.65, N 5.46
found. (%): C 58.56, H 8.43, N 5.09

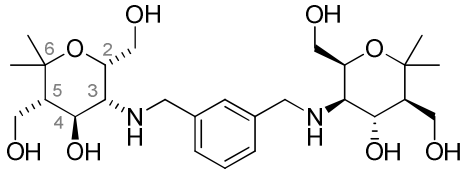
A better elemental analysis could not be obtained.

Divalent amine (**91**)

JNS144

According to **GP-6**, aminopyran **3** (200 mg, 0.97 mmol) was dissolved in MeOH (17 mL), and isophthalaldehyde **90** (60 mg, 0.44 mmol) was added followed by $MgSO_4$ (285 mg, 2.37 mmol). The reaction mixture was stirred for 21 h. After filtration and cooling to 0 °C, $NaBH_4$ (42 mg, 1.11 mmol) was added. After stirring at r.t. for 6.5 h, the reaction was

quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (3x 20 mL), washed with brine, dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH 6:1 to 4:1) affording **91** (62 mg, 28%) as a colorless solid.



91

Chemical Formula: $C_{26}H_{44}N_2O_8$

Molecular Weight: 512.6 g·mol^{-1}

Melting point: 154 °C

Optical rotation: $[\alpha]_D^{22}$ = +39.2 (c = 0.52, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.33, 1.42 (2 s, 6 H each, CH_3), 1.64 – 1.67 (m, 2 H, 5-H), 2.76 (t, *J* = 3.2 Hz, 2 H, 3-H), 3.63 (dd, *J* = 3.0, 11.4, Hz, 2 H, 5-CH_2), 3.70 (d, *J* = 4.8 Hz, 4 H, 2-CH_2), 3.83 (A part of AB system, J_{AB} = 13.0 Hz, 2 H, CH_2Ar), 3.89 (dd, *J* = 4.3, 11.4 Hz, 2 H, 5-CH_2), 3.98 (B part of AB system, J_{AB} = 13.0 Hz, 2 H, CH_2Ar), 4.05 – 4.07 (m, 4 H, 2-H, 4-H), 7.37 – 7.27 (m, 4 H, Ar) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 27.2, 28.2 (2 q, CH_3), 49.3 (d, C-5), 52.4 (t, CH_2Ar), 60.7 (d, C-3), 63.5 (t, 5-CH_2), 64.5 (t, 2-CH_2), 68.9 (d, C-2), 72.6 (d, C-4), 75.7 (s, C-6), 127.3, 128.3, 129.7 (3 d, Ar), 139.4 (s, Ar) ppm.

IR (ATR): ν = 3350 (OH, NH), 2975-2840 (C-H), 1475 (C=C), 1160 (C-O), 1095 (C-O-C) cm^{-1}.

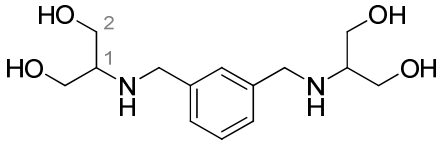
HRMS (pos. ESI-TOF): $C_{26}H_{44}N_2O_8 \cdot H^+$: calcd.: 513.3170
found: 513.3211

Elemental Analysis: $C_{26}H_{44}N_2O_8$ (512.6): calcd. (%): C 60.92, H 8.65, N 5.46
found. (%): C 60.94, H 8.69, N 5.41

Divalent amine (**98**)

JNS161

According to **GP-6**, serinol **4** (200 mg, 2.20 mmol) was dissolved in MeOH (34 mL), and isophthalaldehyde **90** (134 mg, 1.00 mmol) was added followed by $MgSO_4$ (645 mg, 5.36 mmol). The reaction mixture was stirred for 21 h. After filtration and cooling to 0 °C, $NaBH_4$ (83.2 mg, 2.20 mmol) was added. After stirring at r.t. for 3 h, the reaction mixture was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (3x 40 mL), washed with brine, dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1) affording **98** (72 mg, 25%) as a colorless oil.



98

Chemical Formula: $C_{14}H_{24}N_2O_4$

Molecular Weight: 284.3 g·mol^{-1}

^{1}H-NMR (500 MHz, CD_3OD): δ = 2.76 (quint., $J \approx 5.6$ Hz, 2 H, 1-H), 3.56, 3.64 (AB part of ABX system, J_{AB} = 11.1 Hz, J_{AX} = 5.5 Hz, J_{BX} = 5.8 Hz, 4 H each, 2-H), 3.86 (s, 4 H, CH_2Ar), 7.25 – 7.33 (m, 3 H, Ar), 7.39 (s, 1 H, Ar) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 52.1 (t, CH_2Ar), 61.2 (d, C-1), 62.4 (t, C-2), 128.4, 129.6, 129.7 (3 d, Ar), 141.1 (s, Ar) ppm.

IR (ATR): ν = 3325 (OH, NH), 2955-2855 (C-H), 14650 (C=C), 1155 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{14}H_{24}N_2O_4 \cdot H^+$: calcd.: 285.1809
found: 285.1801

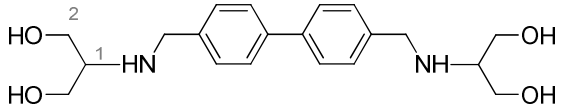
$C_{14}H_{24}N_2O_4 \cdot Na^+$: calcd.: 307.1628
found: 307.1618

Elemental Analysis: $C_{14}H_{24}N_2O_4$ (284.3) calcd. (%): C 59.13, H 8.51, N 9.85
found. (%): C 59.19, H 8.76, N 9.79

Divalent amine (**97**)

JNS142

According to **GP-6**, serinol **4** (200 mg, 2.20 mmol) was dissolved in MeOH (38 mL), and biphenyl-4,4'-dicarbaldehyde (220 mg, 1.04 mmol) was added followed by $MgSO_4$ (645 mg, 5.36 mmol). The reaction mixture was stirred for 3 h. After filtration and cooling to 0 °C, $NaBH_4$ (89 mg, 2.35 mmol) was added. After stirring at r.t. for 2 h, the reaction was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (3x 40 mL), washed with brine, dried and concentrated. The crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1) affording **97** (123 mg, 33%) as a colorless oil.



97

Chemical Formula: $C_{20}H_{28}N_2O_4$

Molecular Weight: 360.4 g·mol^{-1}

^{1}H-NMR (500 MHz, DMF-d_7): δ = 2.69 – 2.78 (m, 2 H, 1-H), 3.55, 3.62 (AB part of ABX system, J_{AB} = 10.7 Hz, J_{AX} = J_{BX} = 5.5 Hz, 4 H each, 2-H), 3.90 (s, 4 H, CH_2Ar), 4.49 (bs, 4 H, OH), 7.49, 7.66 (2 d, J = 8.0 Hz, 4 H each, Ar) ppm.

^{13}C-NMR (125 MHz, DMF-d_7): δ = 51.2 (t, CH_2Ar), 61.2 (d, C-1), 62.0 (t, C-2), 126.7, 128.8 (2 d, Ar), 139.2, 141.0 (2 s, Ar) ppm.

IR (ATR): ν = 3365-3280 (OH, NH), 2970-2850 (C-H), 1450 (C=C), 1120 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{20}H_{28}N_2O_4 \cdot H^+$: calcd.: 361.2122
found: 361.2135

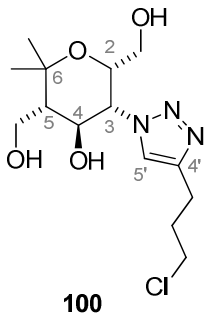
Elemental Analysis: $C_{20}H_{28}N_2O_4$ (360.4) calcd. (%): C 66.64, H 7.83, N 7.77
found. (%): C 66.57, H 7.79, N 7.77

6.2.4. Copper(I)-catalyzed azide alkyne cycloaddition

(2*S*,3*R*,4*S*,5*S*)-3-{[4-(3-Chloropropyl)-1*H*-1',2',3'-triazol-1'-yl]-4-hydroxy-6,6-dimethyltetrahydro-2*H*-pyran-2,5-diyl}dimethanol (**100**)

JNS222

According to **GP-9**, to a solution of azidopyran **10** (24 mg, 0.10 mmol) in dioxane (0.3 mL) was added Cu/C (11 mg, 5.20 μmol), Et_3N (17 μL, 0.13 mmol) and 5-chloro-1-pentyne (12.8 mg, 13 μL, 0.13 mmol). The reaction mixture was stirred at 60 °C for 21 h. The mixture was filtrated through a pad of Celite® and washed with MeOH. Solvents were removed in vacuo affording **100** (35 mg, quant.) as a pale yellow oil.



100

Chemical Formula: $C_{14}H_{24}ClN_3O_4$

Molecular Weight: 333.8 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = +55.9 (c =1.32, MeOH)

^{1}H-NMR (700MHz, CD_3OD): δ = 1.29, 1.39 (2 s, 3 H each, CH_3), 2.06 (td, $J \approx 6.2$, 12.2 Hz, 1H, 5-H), 2.10 – 2.17 (m, 2 H, CH_2), 2.86 – 2.91 (m, 2 H, CH_2), 2.96, 3.04 (AB part of ABX system, J_{AB} = 11.5 Hz, J_{AX} = 4.5 Hz, J_{BX} = 7.5 Hz, 1 H each, 2-CH_2), 3.60 (t, J = 6.5 Hz, 2 H, CH_2), 3.69 (dd, J = 6.3, 11.3 Hz, 1 H, 5-CH_2), 3.89 (dd, J = 6.1, 11.3 Hz, 1 H, 5-CH_2), 4.24 – 4.31 (m, 2 H, 2-H, 4-H), 4.70 (dd, J = 5.2, 7.0 Hz, 1 H, 3-H), 7.79 (s, 1 H, 5'-H) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 23.47 (t, CH_2), 23.50, 26.7 (2 q, CH_3), 33.3 (t, CH_2), 44.8 (t, CH_2), 49.4 (d, C-5), 61.6 (t, 2-CH_2), 62.3 (t, 5-CH_2), 70.3 (d, C-3), 72.0 (t, C-2), 73.1 (t, C-4), 77.5 (s, C-6), 123.8 (d, C-5'), 147.4 (s, C-4') ppm.

IR (ATR): ν = 3310 (OH), 2960-2850 (C-H), 1440, 1370 (C=C, =C-N), 1050 (C-O-C) cm^{-1}.

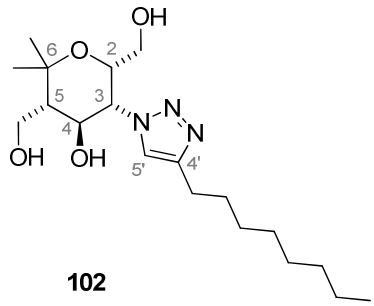
HRMS (pos. ESI-TOF): $C_{14}H_{24}ClN_3O_4 \cdot Na^+$: calcd.: 356.1342
found: 356.1353

Elemental Analysis: $C_{14}H_{24}ClN_3O_4$ (333.8) calcd. (%): C 50.37, H 7.25, N 12.59
found. (%): C 49.92, H 6.97, N 12.12

(2*S*,3*R*,4*S*,5*S*)-[4-Hydroxy-6,6-dimethyl-3-(4-octyl-1*H*-1,2,3-triazol-1-yl)tetrahydro-2*H*-pyran-2,5-diyl]dimethanol (**102**)

JNS272

According to **GP-9**, to a solution of azidopyran **10** (50 mg, 0.22 mmol) in dioxane (0.6 mL) was added Cu/C (23 mg, 11 µmol), Et_3N (34 µL, 0.26 mmol) and 1-decyne (36 mg, 46 µL, 0.26 mmol). The reaction mixture was stirred at 60 °C for 22 h. The mixture was filtrated through a pad of Celite® and washed with MeOH. Solvents were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1) affording a mixture of **102** and **10** (52 mg, 0.140 mmol, 65%) as colorless oil.
Ratio calculated according to signals in the ^{1}H-NMR spectrum (**102**/**10** 1.0:2.2, 33% product).



102

Chemical Formula: $C_{19}H_{35}N_3O_4$
Molecular Weight: 369.5 g·mol^{-1}

Data obtained from the mixture.
^{1}H-NMR (500MHz, CD_3OD): δ = 0.90 (t, *J* = 7.0 Hz, 3 H, 8''-H), 1.29 (s, 3H, CH_3), 1.36 – 1.31 (m, 10 H, CH_2), 1.38 (s, 3 H, CH_3), 1.68 (m_c, 2 H, CH_2), 2.05 (m_c, 1 H, 5-H), 2.70 (t, *J* = 7.6 Hz, 2 H, CH_2), 2.93, 3.04 (AB part of ABX system, J_{AX} = 4.7 Hz, J_{BX} = 7.6 Hz, J_{AB} = 11.5 Hz, 1 H each, 2-CH_2), 3.68 (m_c, 1 H, 5-CH_2), 3.89 (dd, *J* = 6.1, 11.3 Hz, 1 H, 5-CH_2), 4.24 – 4.30 (m, 2 H, 2-H, 4-H), 4.69 (dd, *J* = 5.1, 7.0 Hz, 1H, 3-H), 7.72 (s, 1 H, 5'-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 14.4, 23.5 (2 q, CH_3), 26.3 (t, CH_2), 26.7 (q, CH_3), 27.0, 30.2, 30.3, 30.4, 30.6, 33.0 (6 t, CH_2), 48.4 (d, C-5), 61.6 (t, 2-CH_2), 62.2 (t, 5-CH_2), 70.2 (d, C-3), 72.1 (d, C-2), 73.1 (d, C-4), 77.5 (s, C-6), 123.3 (d, C-5'), 124.8 (s, C-4') ppm.

IR (ATR): ν = 3370 (OH), 2960 (=C-H), 2930-2855 (C-H), 1640, 1460 (C=C), 1260 (C-O), 1125, 1090 (C-O-C) cm^{-1}.

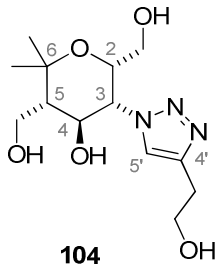
HRMS (pos. ESI-TOF): $C_{19}H_{35}N_3O_4 \cdot H^+$: calcd.: 370.2700
found: 370.2697

$C_{19}H_{35}N_3O_4 \cdot Na^+$: calcd.: 392.2520
found: 392.2527

(2*S*,3*R*,4*S*,5*S*)-{4-Hydroxy-3-[4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl]-6,6-dimethyl tetrahydro-2*H*-pyran-2,5-diyl}dimethanol (**104**)

JNS273

According to **GP-9**, to a solution of azidopyran **10** (40 mg, 0.17 mmol) in dioxane (0.6 mL) was added Cu/C (37 mg, 17 μmol), Et_3N (70 μL, 0.52 mmol) and 3-butin-1-ol (28 mg, 31 μL, 0.40 mmol). The reaction mixture was stirred at 60 °C for 20 h. The mixture was filtrated through a pad of Celite® and washed with MeOH. Solvents were removed in vacuo and the crude was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1 to 6:1) affording **104** (47 mg, 0.156 mmol, 90%) as a colorless solid.



104

Chemical Formula: $C_{13}H_{23}N_3O_5$

Molecular Weight: 301.3 $g \cdot mol^{-1}$

Melting point: 156 – 157 °C

Optical rotation: $[\alpha]_D^{22}$ = + 13.7 (c = 0.975, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.28, 1.39 (2 s, 3 H each, CH_3), 2.06 (td, $J \approx 6.2$, 12.2 Hz, 1 H, 5-H), 2.92 (t, J = 6.6 Hz, 2 H, CH_2), 2.98, 3.06 (AB part of ABX system, J_{AB} = 11.5 Hz, J_{AX} = 4.9 Hz, J_{BX} = 7.5 Hz, 1 H each, 2-CH_2), 3.69 (dd, J = 6.4, 11.3 Hz, 1 H, 5-CH_2), 3.82 (t, J = 6.6 Hz, 2 H, CH_2), 3.89 (dd, J = 6.0, 11.3 Hz, 1 H, 5-CH_2), 4.24 – 4.30 (m, 2 H, 2-H, 4-H), 4.70 (dd, J = 5.2, 7.0 Hz, 1 H, 3-H), 7.80 (s, 1H, 5'-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 23.5, 26.7 (2 q, CH_3), 29.9 (t, CH_2), 49.5 (d, C-5), 61.6 (t, 2-CH_2), 62.1 (t, CH_2), 62.3 (t, 5-CH_2), 70.2 (d, C-3), 71.9 (d, C-2), 73.2 (d, C-4), 77.5 (s, C-6), 124.2 (d, C-5'), 146.0 (s, C-4') ppm.

IR (ATR): ν = 3360 (OH), 3160 (=C-H), 2960-2850 (C-H), 1560, 1370, 1320 (C=C), 1060, 1030 (C-O-C) cm^{-1}.

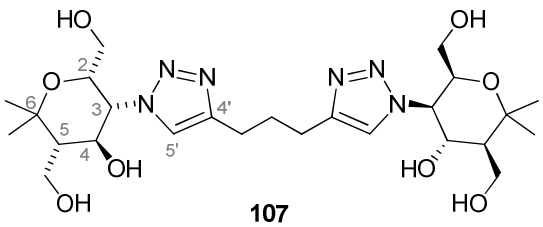
HRMS (pos. ESI-TOF): $C_{13}H_{23}N_3O_5 \cdot Na^+$: calcd.: 324.1530
found: 324.1555

Elemental Analysis: $C_{13}H_{23}N_3O_5$ (301.3) calcd. (%): C 51.82, H 7.69, N 13.94
found. (%): C 51.96, H 7.72, N 13.36

Divalent triazole (**107**)

JNSAG010

According to **GP-7**, azide **10** (100 mg, 0.432 mmol), TBTA (46.2 mg, 0.087 mmol), CuI (16.6 mg, 0.087 mmol), Et_3N (0.73 mL, 0.087 mmol) and 1,6-heptadiyne (18.4 mg, 22.9 µL, 0.198 mmol) were dissolved in MeCN (20 mL). The mixture was stirred at r.t. overnight and worked up as **GP-7**. The crude product was purified by flash column chromatography (silica gel, DCM, DCM/MeOH 95:5, 90:1) affording **106** (71 mg, 65%) as a colorless solid. Monosubstituted product **107** was also isolated (9 mg, 8%) as a colorless solid.



107

Chemical Formula: $C_{25}H_{42}N_6O_8$

Molecular Weight: 554.6 g·mol^{-1}

Melting point: 91 °C

Optical rotation: $[\alpha]_D^{22}$ = + 0.19 (c = 0.5, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.29, 1.39 (2 s, 6 H each, CH_3), 2.05 (m, 4 H, 5-H, CH_2), 2.77 (t, J = 7.5 Hz, 4 H, CH_2), 2.95, 3.05 (AB part of ABX system, J_{AB} = 11.4 Hz, J_{AX} = 4.7 Hz, J_{BX} = 7.5 Hz, 2 H each, 2-CH_2), 3.69 (dd, J = 6.5, 11.3 Hz, 2 H, 5-CH_2), 3.89 (dd, J = 6.0, 11.3 Hz, 2 H, 5-CH_2), 4.23 – 4.32 (m, 4 H, 2-H, 4-H), 4.67 – 4.70 (m, 2 H, 3-H), 7.78 (s, 2 H, 5'-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 22.0 (q, CH_3), 24.3 (t, CH_2), 25.4 (q, CH_3), 28.9 (t, CH_2), 48.4 (d, C-5), 60.3 (t, 2-CH_2), 61.0 (t, 5-CH_2), 68.9 (d, C-3), 70.7 (d, C-2), 71.8 (d, C-4), 76.2 (s, C-6), 122.4 (d, C-5'), 146.9 (s, C-4') ppm.

IR (ATR): ν = 3366 (OH), 2970-2925 (C-H), 1450 (C=C), 1230-1030 (C-O-C) cm^{-1}.

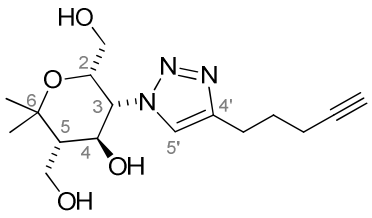
HRMS (pos. ESI-TOF): $C_{25}H_{42}N_6O_8 \cdot Na^+$: calcd.: 577.2956
found: 577.2954

$C_{25}H_{42}N_6O_8 \cdot K^+$: calcd.: 593.2696
found: 593.2691

Elemental Analysis: $C_{25}H_{42}N_6O_8$ (554.6) calcd. (%): C 54.14, H 7.63, N 15.15
found. (%): C 47.36, H 7.01, N 12.42

A better elemental analysis could not be obtained.

Monovalent triazole (**107**)



107

Chemical Formula: $C_{16}H_{25}N_3O_4$

Molecular Weight: 323.34 g·mol^{-1}

^{1}H-NMR (400 MHz, CD_3OD): δ= 1.29, 1.39 (2 s, 3 H each, CH_3), 1.83 - 1.92 (m, 2 H, CH_2), 2.05 (td, $J \approx$ 6.2, 12.2 Hz, 1 H, 5-H), 2.20 – 2.28 (m, 3 H, CH_2, ≡CH), 2.84 (t, J = 7.6 Hz, 2 H, CH_2), 2.94, 3.05 (AB part of ABX system, J_{AB} = 11.5 Hz, J_{AX} = 4.8 Hz, J_{BX} = 7.5 Hz, 1 H each, 2-CH_2), 3.69 (dd, J = 6.5, 11.3 Hz, 1 H, 5-CH_2), 3.89 (dd, J = 6.0, 11.3 Hz, 1 H, 5-CH_2), 4.24 – 4.29 (m, 2 H, 4-H, 2-H), 4.69 (dd, J = 5.1, 7.0 Hz, 1 H, 3-H), 7.77 (s, 1 H, 5'-H) ppm.

IR (ATR): ν = 3400 (OH), 2980-2935 (C-H), 2110 (C≡C), 1220-1040 (C-O-C) cm^{-1}.

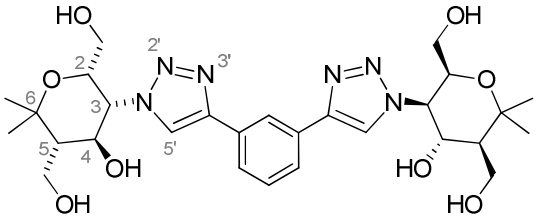
HRMS (pos. ESI-TOF): $C_{16}H_{25}N_3O_4 \cdot H^+$: calcd.: 324.1918
found: 324.1925

$C_{16}H_{25}N_3O_4 \cdot Na^+$: calcd.: 346.1737
found: 346.1748

Divalent triazole (**111**)

JNSAG012

According to **GP-7**, azide **10** (181 mg, 0.783 mmol), TBTA (83.1 mg, 0.157 mmol), CuI (29.8 mg, 0.157 mmol), Et_3N (21.8 µL, 0.157 mmol) and 1,3-diethynylbenzene (42.8 mg, 54.1 µL, 0.339 mmol) were dissolved in MeCN (30 mL). The fluorescent yellow mixture was stirred at r.t. overnight and worked up as in **GP-7**. The crude product was purified by flash column chromatography (silica gel, DCM, DCM/MeOH 95:5, 90:1) affording **111** (105 mg, 53%) as a colorless solid. Monosubstituted product **112** was also isolated (7 mg, 4%) as a colorless solid.



111

Chemical Formula: $C_{28}H_{40}N_6O_8$

Molecular Weight: 588.7 g·mol^{-1}

Melting point: 171 °C

Optical rotation: $[\alpha]_D^{22}$ = + 0.34 (c = 0.5, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ= 1.33, 1.41 (2 s, 6 H each, CH_3), 2.11 (td, *J* = 6.3, 12.0 Hz, 2 H, 5-H), 3.08, 3.15 (AB part of ABX system, J_{AB} = 11.4 Hz, J_{AX} = 5.0 Hz, J_{BX} = 7.4 Hz, 2 H each, 2-CH_2), 3.72 (dd, *J* = 6.3, 11.3 Hz, 2 H, 5-CH_2), 3.93 (dd, *J* = 6.3, 11.3 Hz, 2 H, 5-CH_2), 4.33 (dt, *J* ≈ 5.1, 7.2, Hz, 2 H, 2-H), 4.41 (dd, *J* = 7.2, 12.0, Hz, 2 H, 4-H), 4.81 (dd, *J* = 5.3, 7.1 Hz, 2 H, 3-H), 7.52 (t, *J* = 7.8 Hz, 1 H, Ar), 7.85 (d, *J* = 7.8 Hz, 2 H, Ar), 8.32 (bs, 1 H, Ar), 8.40 (s, 2 H, 5'-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ= 23.5, 26.7 (2 q, CH_3), 49.7 (d, C-5), 61.7 (t, 2-CH_2), 62.3 (t, 5-CH_2), 70.6 (d, C-3), 71.9 (d, C-2), 73.1 (d, C-4), 77.6 (s, C-6), 123.0 (d, C-5'), 124.0, 126.4, 130.7 (3 d, Ar), 132.4 (s, Ar), 148.1 (s, C-4') ppm.

IR (ATR): ν = 3285 (OH), 2970-2925 (C-H), 1620 (C=C), 1230-1030 (C-O-C) cm^{-1}.

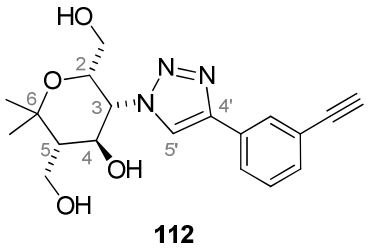
HRMS (pos. ESI-TOF): $C_{28}H_{40}N_6O_8 \cdot Na^+$: calcd.: 611.2800
found: 611.2777

$C_{28}H_{40}N_6O_8 \cdot K^+$: calcd.: 627.2539
found: 627.2511

Elemental Analysis: $C_{28}H_{40}N_6O_8$ (588.7): calcd. (%): C 57.13, H 6.85, N 14.28
found. (%): C 47.40, H 6.43, N 11.57

A better elemental analysis could not be obtained.

Monovalent triazole (**112**)



112

Chemical Formula: $C_{19}H_{23}N_3O_4$

Molecular Weight: 357.4 g·mol^{-1}

^{1}H-NMR (400 MHz, CD_3OD): δ= 1.33, 1.41 (2 s, 3 H each, CH_3), 2.09 (td, $J \approx 6.2$, 12.1 Hz, 1 H, 5-H), 3.05, 3.12 (AB part of ABX system, J_{AB} = 11.4 Hz, J_{AX} = 5.1 Hz, J_{BX} = 7.3 Hz, 1 H each, 2-CH_2), 3.54 (s, 1 H, ≡CH), 3.71 (dd, J = 6.4, 11.3 Hz, 1 H, 5-CH_2), 3.92 (dd, J = 6.0, 11.3 Hz, 1 H, 5-CH_2), 4.32 (m_c, 1 H, 2-H), 4.39 (dd, J = 7.1, 12.1, Hz, 1 H, 4-H), 4.78 (dd, J = 5.2, 7.1 Hz, 1 H, 3-H), 7.41 – 7.46 (m, 2 H, Ar), 7.85 – 7.88, 7.97 – 7.98 (2 m, 1 H each, Ar), 8.38 (s, 1 H, 5'-H) ppm.

IR (ATR): ν = 3400 (OH), 3235 (≡C-H), 2960-2880 (C-H), 2110 (C≡C), 1240-1040 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{19}H_{23}N_3O_4 \cdot Na^+$: calcd.: 380.1581
found: 381.1654

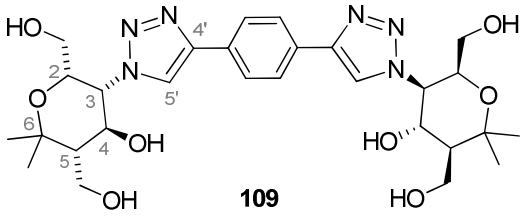
$C_{19}H_{23}N_3O_4 \cdot K^+$: calcd.: 396.1320
found: 397.1397

Divalent triazole (**109**)

JNSAG013

According to **GP-7**, azide **10** (100 mg, 0.432 mmol), TBTA (45.9 mg, 0.086 mmol), CuI (16.5 mg, 0.086 mmol), Et_3N (12 µL, 0.087 mmol) and 1,4-diethynylbenzene (24.8 mg, 0.197 mmol) were dissolved in MeCN (20 mL). The orange colored mixture was stirred at r.t. for 136 h and worked up as in **GP-7**. The crude product was purified by flash column

chromatography (silica gel, DCM, DCM/MeOH 95:5, 90:1) affording **109** (74 mg, 64%) as a yellow solid.



109

Chemical Formula: $C_{28}H_{40}N_6O_8$

Molecular Weight: 588.7 g·mol^{-1}

Melting point: 131 °C

Optical rotation: $[\alpha]_D^{22}$ = + 0.41 (c = 0.5, MeOH)

^{1}H-NMR (400 MHz, CD_3OD): δ = 1.33, 1.41 (2 s, 6 H each, CH_3), 2.10 (dt, $J \approx$ 6.2, 12.2 Hz, 2 H, 5-H), 3.08, 3.14 (AB part of ABX system, J_{AB} = 11.4 Hz, J_{AX} = 5.1 Hz, J_{BX} = 7.3 Hz, 2 H each, 2-CH_2), 3.72 (dd, J = 6.3, 11.3 Hz, 2 H, 5-CH_2), 3.93 (dd, J = 6.1, 11.3 Hz, 2 H, 5-CH_2), 4.33 (m_c, 2 H, 2-H), 4.41 (dd, J = 7.1, 12.0 Hz, 2 H, 4-H), 4.80 (dd, J = 5.3, 7.1 Hz, 2 H, 3-H), 7.94 (s, 4 H, Ar), 8.36 (s, 2 H, 5'-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 22.2, 25.4 (2 q, CH_3), 48.4 (d, C-5), 60.4 (t, 2-CH_2), 61.0 (t, 5-CH_2), 69.3 (d, C-3), 70.6 (d, C-2), 71.8 (d, C-4), 76.3 (s, C-6), 122.8 (d, C-5'), 127.3 (d, Ar), 131.6 (s, Ar), 148.0 (s, C-4') ppm.

IR (ATR): ν = 3335 (OH), 2930 (C-H), 1620 (C=C), 1230-1030 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{28}H_{40}N_6O_8 \cdot H^+$: calcd.: 589.2980
found: 589.2972

$C_{28}H_{40}N_6O_8 \cdot Na^+$: calcd.: 611.2800
found: 611.2797

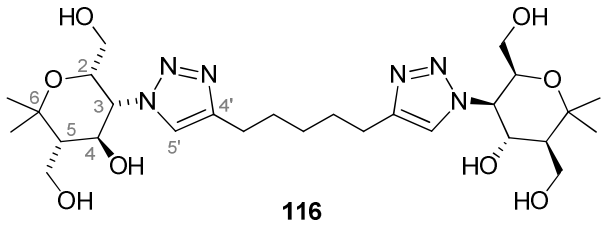
Elemental Analysis: $C_{28}H_{40}N_6O_8$ (588.7): calcd. (%): C 57.13, H 6.85, N 14.28
found. (%): C 50.33, H 6.63, N 12.04

A better elemental analysis could not be obtained.

Divalent triazole (**116**)

JNS230

According to **GP-9**, to a solution of azidopyran **10** (70 mg, 0.303 mmol) in dioxane (0.8 mL) was added Cu/C (28 mg, 13 µmol), Et_3N (50 µL, 0.30 mmol) and alkyne **115** (14.2 mg, 19.8 µL, 0.132 mmol). The reaction mixture was stirred at 60 °C for 24 h. Additional Cu/C (28 mg, 13 µmol) and Et_3N (50 µL, 0.30 mmol) were added and the reaction was stirred at 60 °C for another 24 h. The mixture was filtrated through a pad of Celite® and washed with MeOH. Solvents were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1 to 4:1) affording **116** (71 mg, 91%) as a pale yellow oil.



116

Chemical Formula: $C_{27}H_{46}N_6O_8$

Molecular Weight: 582.7 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = +25.9 (c =1.28, MeOH)

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.28, 1.39 (2 s, 6 H each, CH_3), 1.43 (dd, *J* = 7.9, 15.3 Hz, 4 H, CH_2), 1.75 – 1.68 (m, 4 H, CH_2), 2.05 (td, *J* ≈ 6.2, 12.2 Hz, 2 H, 5-H), 2.71 (t, *J* = 7.5 Hz, 4 H, CH_2), 2.93, 3.04 (AB part of ABX system, J_{AB} = 11.5 Hz, J_{AX} = 4.7 Hz, J_{BX} = 7.5 Hz, 2 H each, 2-CH_2), 3.70 (dd, *J* = 6.3, 11.4 Hz, 2 H, 5-CH_2), 3.89 (dd, *J* = 6.0, 11.4 Hz, 2 H, 5-CH_2), 4.24 – 4.32 (m, 4 H, 2-H, 4-H), 4.69 (dd, *J* = 5.2, 6.9 Hz, 2 H, 3-H), 7.73 (s, 2 H, 5'-H) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 23.5 (q, CH_3), 26.1 (t, CH_2), 26.7 (q, CH_3), 29.5, 30.2 (2 t, CH_2), 49.6 (d, C-5), 61.6 (t, 2-CH_2), 62.3 (t, 5-CH_2), 70.2 (d, C-3), 72.0 (d, C-2), 73.1 (d, C-4), 77.5 (s, C-6), 123.4 (d, C-5'), 148.8 (s, C-4') ppm.

IR (ATR): ν = 3450 (OH), 2980-2855 (C-H), 1645 (C=C), 1225-1030 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{27}H_{46}N_6O_8 \cdot Na^+$: calcd.: 605.3269
found: 605.3270

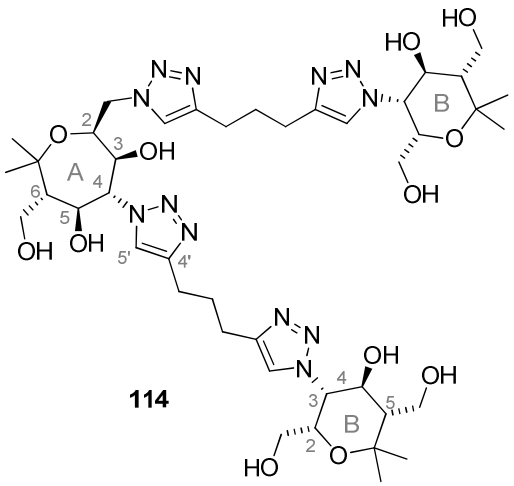
Elemental Analysis: $C_{27}H_{46}N_6O_8$ (582.7) calcd. (%): C 55.65, H 7.96, N 14.42
found. (%): C 52.15, H 7.82, N 12.83

A better elemental analysis could not be obtained.

Divalent triazole (**114**)

JNS302

According to **GP-9**, to a solution of azidopyran **10** (31 mg, 0.133 mmol) in dioxane (1 mL) was added Cu/C (23 mg, 10.6 µmol), Et_3N (20 µL, 0.133 mmol) and alkyne **113** (25 mg, 53 µmol). The reaction mixture was stirred at 60 °C for 72 h. The mixture was filtrated through a pad of Celite® and washed with MeOH. The solvents were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1 to 4:1) affording **114** (12 mg, 24%) as a yellow solid.



114

Chemical Formula: $C_{42}H_{68}N_{12}O_{12}$

Molecular Weight: 933.1 g·mol^{-1}

Melting point: 235 °C

Optical rotation: $[\alpha]_D^{22}$ = -20.3 (c = 0.09, MeOH)

An assignment of the signals to one of the two B-rings and to the four triazole units is not possible.

^{1}H-NMR (700 MHz, CD_3OD): δ = 0.54, 1.27 (2 s, 3 H each, CH_3^A), 1.29, 1.39 (2 s, 6 H each, CH_3^B), 1.93 (m_c, 1 H, 6-H^A), 2.02 – 2.10 (m, 6 H, CH_2, 5-H^B), 2.75 – 2.78 (m, 8 H, CH_2), 2.93 – 2.98, 3.03 – 3.06 (2 m, 2 H each, 2-CH_2^B), 3.54 – 3.61 (m, 2 H, 6-CH_2^A), 3.70 (dd, *J* = 6.4, 11.3 Hz, 2 H, 5-CH_2^B), 3.89 (dd, *J* = 6.1, 11.3 Hz, 2 H, 5-CH_2^B), 4.23 – 4.28 (m, 2 H, 4-H^B, 2-H^B), 4.28 – 4.32 (m, 2 H, 5-H^A, 3-H^A), 4.43 – 4.46 (m, 2 H, 2-CH_2^A, 3-H^A), 4.52 (B part of ABX system, J_{AB} = 14.3 Hz, J_{BX} = 3.1, 1 H, 2-CH_2^A), 4.60 (dd, *J* = 7.4, 9.5 Hz, 1 H, 4-H^A), 4.68 – 4.72 (m, 2 H, 3-H^B), 7.77 (s, 1 H, 5'-H), 7.77 (bs, 2 H, 5'-H), 7.79 (s, 1 H, 5'-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 20.1, 23.5 (2 q, CH_3), 25.47, 25.51, 25.52, 25.6 (4 t, CH_2), 26.7, 26.8 (2 q, CH_3), 30.1, 30.5 (2 t, CH_2), 30.9, 31.4 (2 q, CH_3), 49.4 (d, C-5^B), 53.2 (t, 2-CH_2^A), 53.3 (d, C-2^A), 60.3 (d, C-6^A), 61.7 (t, 2-CH_2^B), 62.3 (t, 5-CH_2^B), 63.5 (t, 6-CH_2^A), 69.8 (d, C-3^B), 70.3 (d, C-2^B), 72.0 (d, C-4^B), 73.0 (d, C-5^A), 74.3 (d, C-3^A), 75.1 (d, C-4^A), 77.5, 77.9 (2 s, C-6^B, C-7^A), 123.65, 123.73, 125.3, 125.4 (4 d, C-5'), 148.21, 148.28, 148.29, 148.31 (4 s, C-4') ppm.

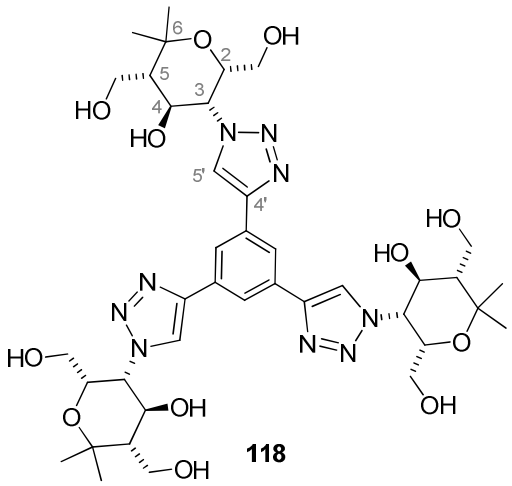
IR (ATR): ν = 3370 (OH), 2925 (C-H), 1595 (C=C), 1060 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{42}H_{68}N_{12}O_{12}{\cdot}Na^+$: calcd.: 955.4966
found: 955.5003

Trivalent triazole (**118**)

JNSW8

According to **GP-9**, to a solution of azidopyran **10** (49 mg, 195 µmol) in dioxane (1.0 mL) was added Cu/C (183 mg, 86 µmol), Et_3N (50 µL, 361 µmol) and 1,3,5-triethynylbenzene (8 mg, 54 µmol). The reaction mixture was stirred at 60 °C for 21 h. Cu/C (183 mg, 86 µmol), Et_3N (50 µL, 361 µmol) and dioxane (1 mL) were additionally added. After stirring for 2 d, the mixture was filtrated through a pad of Celite® and washed with MeOH. The solvents were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 4:1) affording **118** (31 mg, 69%) as a light yellow solid.



118

Chemical Formula: $C_{39}H_{57}N_9O_{12}$

Molecular Weight: 843.9 g·mol^{-1}

Melting point: 195 – 197 °C

Optical rotation: $[\alpha]_D^{22}$ = + 101.0 (c = 0.63 MeOH)

^{1}H NMR (500 MHz, CD_3OD): δ = 1.35, 1.42 (2 s, 9 H each, CH_3), 2.12 (td, *J* = 6.2, 12.1 Hz, 3 H, 5-H), 3.12, 3.18 (AB part of ABX system, J_{AB} = 11.5 Hz, J_{AX} = 5.3 Hz, J_{BX} = 7.2 Hz, 3 H each, 2-CH_2), 3.74, 3.97 (2 dd, *J* = 6.2, 11.4 Hz, 3 H each, 5-CH_2), 4.35 (dt, *J* = 5.3, 7.2, Hz, 3 H, 2-H), 4.46 (dd, *J* = 7.2, 12.1 Hz, 3 H, 4-H), 4.84 (dd, *J* = 5.3, 7.2 Hz, 3 H, 3-H), 8.21 (s, 3 H, Ar), 8.41 (s, 3 H, 5'-H) ppm.

^{13}C NMR (CD_3OD, 126 MHz): δ = 23.6, 26.7 (2 q, CH_3), 49.7 (d, C-5), 61.7 (t, 2-CH_2), 62.4 (t, 5-CH_2), 70.7 (d, C-3), 71.9 (d, C-2), 73.2 (d, C-4), 77.6 (s, C-6), 123.2 (d, C-5'), 123.3 (d, Ar), 133.0 (s, Ar), 147.7 (s, C-4') ppm.

IR (ATR): ν = 3365 (OH), 2930 (C-H), 1600 (C=C), 1230 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{39}H_{57}N_9O_{12}\cdot H^+$: calcd.: 844.4206
found: 844.4212

$C_{39}H_{57}N_9O_{12}\cdot Na^+$: calcd.: 866.4025
found: 866.4034

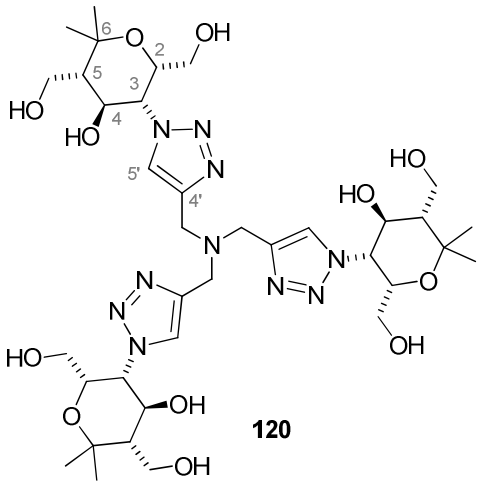
Elemental Analysis: $C_{39}H_{57}N_9O_{12}$ (843.9) calcd. (%): C 55.50, H 6.81, N 14.94
found. (%): C 50.15, H 7.40, N 13.28

A better elemental analysis could not be obtained.

Trivalent triazole (**120**)

JNSW9

According to **GP-9**, to a solution of azidopyran **10** (70 mg, 303 µmol) in dioxane (1.4 mL) was added Cu/C (481 mg, 227 µmol), Et_3N (140 µL, 1.01 mmol) and alkyne **119** (9.9 mg, 10.7 µL, 75.7 µmol). The reaction mixture was stirred at 60 °C for 21 h. The mixture was filtrated through a pad of Celite® and washed with MeOH. The solvents were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 4:1) affording **120** (37 mg, 60%) as a light pink solid.



120

Chemical Formula: $C_{36}H_{60}N_{10}O_{12}$

Molecular Weight: 824.9 g·mol^{-1}

Melting range: 125 – 131 °C

Optical rotation: $[\alpha]_D^{22}$ = +69.5 (c = 0.71 MeOH)

^{1}H NMR (700 MHz, CD_3OD): δ = 1.30, 1.40 (2 s, 9 H each, CH_3), 2.07 (td, *J* = 6.2, 12.1 Hz, 3 H, 5-H), 2.99, 3.05 (AB part of ABX system, J_{AB} = 11.4 Hz, J_{AX} = 5.1 Hz, J_{BX} = 7.2 Hz, 3 H each, 2-CH_2), 3.70 (dd, *J* = 6.2, 11.3 Hz, 3 H, 5-CH_2), 3.78 (s, 6 H, NCH_2), 3.90 (dd, *J* = 6.2, 11.3 Hz, 3 H, 5-CH_2), 4.28 (dt, *J* = 5.1, 7.2 Hz, 3 H, 2-H), 4.34 (dd, *J* = 6.9, 12.1 Hz, 3 H, 4-H), 4.75 (dd, *J* = 5.1, 6.9 Hz, 3 H, 3-H), 8.03 (s, 3 H, 5'-H) ppm.

^{13}C NMR (176 MHz, CD_3OD): δ = 23.6, 26.8 (2 q, CH_3), 48.5 (t, NCH_2), 49.6 (d, C-5), 61.7 (t, 2-CH_2), 62.3 (t, 5-CH_2), 70.4 (d, C-3), 71.9 (d, C-2), 73.1 (d, C-4), 77.5 (s, C-6), 126.0 (d, C-5'), 145.0 (s, C-4') ppm.

IR (ATR): ν = 3385 (OH), 2970-2935 (C-H), 1650 (C=C), 1155 (C-O), 1230 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{36}H_{60}N_{10}O_{12}\cdot H^+$: calcd.: 825.4471
found: 825.4471

$C_{36}H_{60}N_{10}O_{12}\cdot Na^+$: calcd.: 847.4296
found: 847.4303

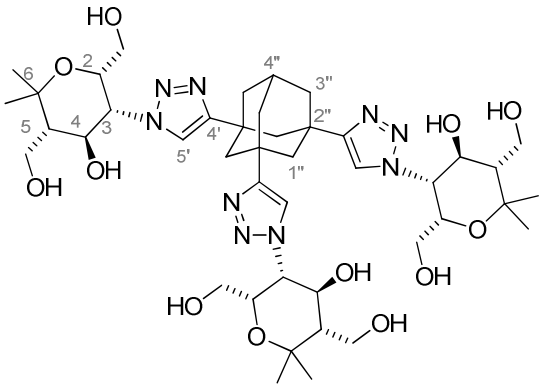
Elemental Analysis:

$C_{36}H_{60}N_{10}O_{12}$ (824.9) + 4 H_2O calcd. (%): C 48.20, H 7.64, N 15.62
found. (%): C 48.43, H 7.59, N 15.32

Trivalent triazole (**122**)

JNS313

According to **GP-8**, to a suspension of azidopyran **10** (80 mg, 0.346 mmol) in toluene (0.2 mL) was added CuI (19 mg, 0.101 mmol), the alkyne **121** (16 mg, 76 μmol) followed by DIPEA (290 μL, 1.70 mmol) and HOAc (97.3 μL, 1.70 mmol). The reaction mixture was stirred at r.t. for 16 h. The solution was quenched with 7N NH_3 in MeOH and the resulting mixture was filtered through a short silica gel column and washed with a mixture of CH_2Cl_2/7N NH_3 in MeOH 10:1. After removing the solvents in vacuo, the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 9:1 to 4:1) affording **122** (20 mg, 29%) as a yellow solid.



122

Chemical Formula: $C_{43}H_{67}N_9O_{12}$

Molecular Weight: 902.0 g·mol^{-1}

Melting point: 291 °C

Optical rotation: $[\alpha]_D^{22}$ = +22.7 (c = 0.45, MeOH)

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.29, 1.39 (2 s, 9 H each, CH_3), 2.05 (td, *J* = 6.3, 12.3 Hz, 3 H, 5-H), 2.10 (d, *J* = 2.1 Hz, 6 H, 3''-H), 2.23, 2.26 (AB system, J_{AB} = 12.3 Hz, 3 H each, 1''-H), 2.44 – 2.47 (m, 1 H, 4''-H), 2.93, 3.03 (AB part of ABX system, J_{AB} = 11.5, J_{AX} = 4.7 Hz, J_{BX} = 7.6 Hz, 3 H each, 2-CH_2), 3.69, 3.89 (2 dd, *J* = 6.3, 11.3 Hz, 3 H each, 5-CH_2), 4.24 – 4.28 (m, 3 H, 2-H), 4.31 (dd, *J* = 7.0, 12.3 Hz, 3 H, 4-H), 4.71 (dd, *J* = 5.2, 7.0 Hz, 3 H, 3-H), 7.80 (s, 3 H, 5'-H) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 23.5, 26.7 (2 q, CH_3), 30.6 (d, C-4''), 35.3 (s, C-2''), 41.9 (t, C-3''), 47.5 (t, C-1''), 49.7 (d, C-5), 61.7 (t, 2-CH_2), 62.3 (t, 5-CH_2), 70.3 (d, C-3), 72.1 (d, C-2), 73.0 (d, C-4), 77.5 (s, C-6), 121.6 (d, C-5'), 157.1 (s, C-4') ppm.

IR (ATR): ν = 3400 (OH), 2975 (C-H), 1640 (C=C), 1160 (C-O), 1230 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{43}H_{67}N_9O_{12}$·Na^+: calcd.: 924.4801
found: 924.4824

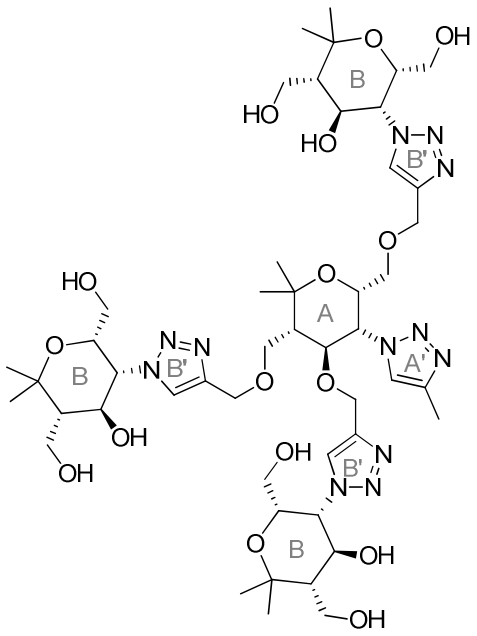
Elemental Analysis:

$C_{43}H_{67}N_9O_{12}$ (902.0) + 4 H_2O calcd. (%): C 53.02, H 7.76, N 12.94
found. (%): C 53.46, H 7.23, N 11.78

Trivalent triazole (**126**)

JNS269

According to **GP-9**, to a solution of azidopyran (24 mg, 0.104 mmol) in dioxane (1 mL) was added Cu/C (165 mg, 77.7 µmol), Et_3N (50 µL, 0.337 mmol) and alkyne **125** (10 mg, 0.26 mmol). The reaction mixture was stirred at 60 °C for 17 h, then filtrated through a pad of Celite® and washed with MeOH. Solvents were removed under reduced pressure and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 4:1) affording **126** (22 mg, 79 %) as a yellow solid.



126

Chemical Formula:	$C_{48}H_{78}N_{12}O_{16}$
Molecular Weight:	1079.2 g·mol^{-1}
Melting point:	220 °C
Optical rotation:	$[\alpha]_D^{22}$ = -34.2 (c = 0.58, MeOH)

An assignment of the signals to two of the three B-rings and to the four triazole units is not possible.

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.28 – 1.29 (m, 12 H, CH_3), 1.35 (s, 3 H, CH_3), 1.38 – 1.40 (m, 9 H, CH_3), 2.04 – 2.09 (m, 3 H, 5-H^B), 2.22 (ddd, *J* = 3.6, 7.7, 11.5 Hz, 1 H, 5-H^A), 2.31 (s, 3 H, CH_3), 2.90 – 2.92 (m, 1 H, 2-CH_2^A), 2.91 – 2.94, 2.94 – 2.97, 2.98 – 3.01 (3 m, 2 H each, 2-CH_2^B), 3.17 (m_c, 1 H, 2-CH_2^A), 3.61 (m_c, 1 H, 5-CH_2^A), 3.67 – 3.74 (m, 5 H, 5-

$CH_2^{A,B}$), 3.87 – 3.95 (m, 2 H, 5-CH_2^B), 4.12 (dd, *J* = 6.1, 11.5 Hz, 1 H, 4-H^A), 4.24 – 4.29 (m, 7 H, 2-H^B, 4-H^B, CH_2), 4.36 (bs, 2 H, CH_2), 4.40 (dd, *J* = 6.2, 11.3 Hz, 1 H, 2-H^A), 4.45 (d, *J* = 12.5 Hz, 1 H, CH_2), 4.57 (bs, 2 H, CH_2), 4.70 – 4.75 (m, 3 H, 3-H^B), 4.79 – 4.81 (m, 1 H, 3-H^A), 7.66, 7.74, 7.87, 8.00 (4 s, 1 H each, 5'-$H^{A,B}$) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 10.8, 23.5, 23.6, 24.4, 26.66, 26.68, 26.75, 27.3 (8 q, CH_3), 47.2, 49.1, 49.3, 49.4 (4 d, C-$5^{A,B}$), 61.57, 61.59, 61.64 (3 t, 2-CH_2^B), 62.28, 62.38, 62.42 (3 t, 5-CH_2^B), 64.5, 64.76, 64.84 (3 t, CH_2), 68.4 (d, C-3^A), 68.9 (t, 5-CH_2^A), 69.3 (t, 2-CH_2^A), 69.5 (d, C-2^A), 70.3, 70.5, 70.6 (3 d, C-3^B), 71.86, 71.89, 71.96 (3 d, C-2^B), 73.1, 73.2, 73.34 (3 d, C-4^B), 77.49, 77.51, 77.53 (3 s, C-6^B) 78.2 (s, C-6^A), 79.3 (d, C-4^A), 124.2, 125.2, 154.4, 125.6 (4 d, C-$5'^{A,B}$), 144.4, 145.1, 145.2, 145.4 (4 s, C-$4'^{A,B}$) ppm.

IR (ATR): ν = 3385 (OH), 2970-2850 (C-H), 1650, 1450, 1370 (C=C), 1050 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{48}H_{78}N_{12}O_{16}{\cdot}Na^+$: calcd.: 1102.1924
found: 1102.5881

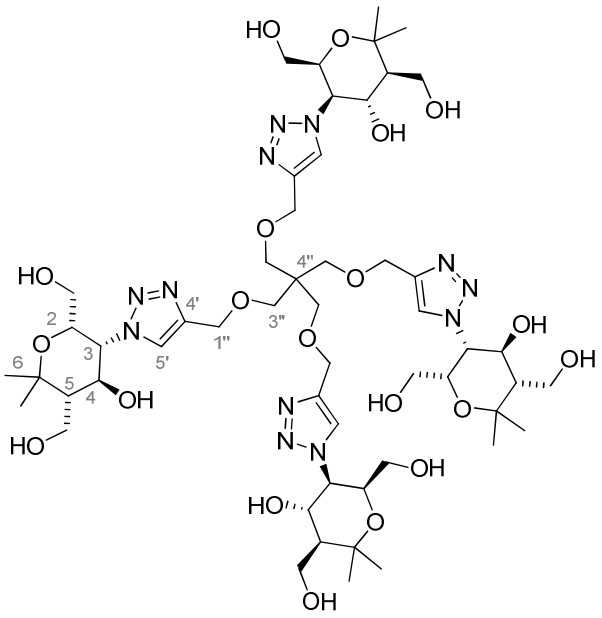
Elemental Analysis:

$C_{48}H_{78}N_{12}O_{16}$ (1079.2) + 3 H_2O calcd. (%): C 50.87, H 7.74, N 14.83
found. (%): C 51.61, H 7.42, N 14.21

Tetravalent triazole (**128**)

JNS279

According to **GP-9**, to a solution of azidopyran **10** (31 mg, 0.13 mmol) in dioxane (1.5 mL) was added Cu/C (235 mg, 0.111 mmol), Et_3N (70 μL, 0.50 mmol) and alkyne **127** (8 mg, 28 μmol). The reaction mixture was stirred at 60 °C for 19 h. The mixture was filtrated through a pad of Celite® and washed with MeOH. The solvents were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 4:1) affording **128** (32 mg, 95%) as a yellow solid.



128

Chemical Formula: $C_{53}H_{88}N_{12}O_{20}$

Molecular Weight: 1213.4 g·mol^{-1}

Melting range: 122 – 125 °C

Optical rotation: $[\alpha]_D^{22}$ = +10.5 (c = 0.12, MeOH)

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.29, 1.39 (2 s, 12 H each, CH_3), 2.07 (td, *J* = 6.3, 12.0 Hz, 4 H, 5-H), 2.95, 3.03 (AB part of ABX system, J_{AB} = 11.5 Hz, J_{AX} = 4.8 Hz, J_{BX} = 7.5 Hz, 4 H each, 2-CH_2), 3.41 (s, 8 H, 3''-H), 3.70, 3.90 (2 dd, *J* = 6.3, 11.3 Hz, 4 H each, 5-CH_2), 4.28 (dt, *J* ≈ 5.0, 7.5 Hz, 4 H, 2-H), 4.36 (dd, *J* = 7.1, 12.0 Hz, 4 H, 4-H), 4.52, 4.55 (AB system, J_{AB} = 12.7 Hz, 8 H, 1''-H), 4.76 (dd, *J* = 5.2, 7.1 Hz, 4 H, 3-H), 7.97 (s, 4 H, 5'-H) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 23.6, 26.7 (2 q, CH_3), 43.5 (s, C-4''), 49.7 (d, C-5), 61.6 (t, 2-CH_2), 62.3 (t, 5-CH_2), 65.2 (t, C-1''), 69.9 (t, C-3''), 70.3 (d, C-3), 72.0 (d, C-2), 73.0 (d, C-4), 77.7 (s, C-6), 125.6 (d, C-5'), 145.8 (s, C-4') ppm.

IR (ATR): ν = 3375 (OH), 2965-2875 (CH), 1650 (C=C), 1230-1055 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{53}H_{88}N_{12}O_{20}$·Na^+: calcd.: 1235.6125
found: 1235.6102

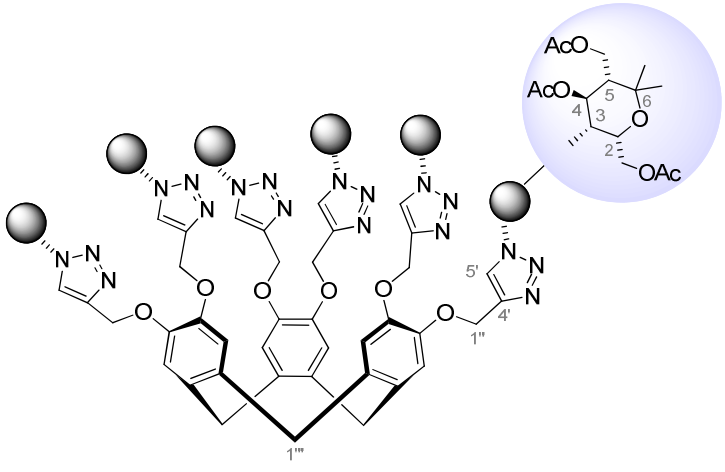
Elemental Analysis: $C_{53}H_{88}N_{12}O_{20}$ (1213.4) calcd. (%): C 52.46, H 7.31, N 13.85
found. (%): C 50.31, H 5.99, N 11.81

A better elemental analysis could not be obtained.

Hexavalent triazole (**139**)

JNS255

According to **GP-9**, to a solution of azidopyran **25** (50 mg, 0.14 mmol) in dioxane (0.8 mL) was added Cu/C (42 mg, 20 µmol), Et_3N (60 µL, 0.42 mmol) and alkyne **138** (6.6 mg, 11 µmol). The reaction mixture was stirred at 60 °C for 3 d, then was filtrated through a pad of Celite® and washed with MeOH. The solvents were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1) affording **139** (18 mg, 60 %) as a yellow solid.



139

Chemical Formula: $C_{129}H_{168}N_{18}O_{48}$

Molecular Weight: 2738.8 g·mol^{-1}

Melting range: 278 – 283 °C

Optical rotation: $[\alpha]_D^{22}$ = +23.2 (c = 0.18, MeOH)

A splitting of several signals was observed**.**

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.30, 1.32 (2 s, 9 H each, CH_3), 1.379, 1.384 (2 s, 9 H each, CH_3), 1.55, 1.81 (2 s, 9 H each, $COCH_3$), 1.89, 1.92 (2 s, 9 H each, $COCH_3$), 1.97, 1.99 (2 s, 18 H, $COCH_3$), 2.49 (qd, *J* = 5.4, 11.9 Hz, 6 H, 5-H), 3.55 (d, *J* = 13.4 Hz, 3 H, 1’’’-H),

3.65 (d, *J* = 6.1 Hz, 12 H, 2-CH_2), 4.01 – 4.07, 4.19 – 4.27 (2 m, 6 H each, 5-CH_2), 4.52 – 4.63 (m, 6 H, 2-H), 4.68 (d, *J* = 13.4 Hz, 3 H, 1’’’-H), 5.04 (m_c, 6H, 3-H), 5.17 – 5.30 (m, 12 H, 1’’-H), 5.56 – 5.65 (m, 6 H, 4-H), 7.16, 7.19 (2 s, 3 H each, Ar), 8.12, 8.13 (2 s, 3 H each, 5’-H) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 20.5, 20.7, 21.0 (3 q, $COCH_3$), 24.0, 26.2 (2 q, CH_3), 45.1 (d, C-5), 62.5 (t, 5-CH_2), 63.3 (t, C-1’’’), 63.4 (t, 2-CH_2), 64.1 (t, C-1’’), 66.7 (d, C-3), 69.0 (d, C-2), 73.2 (d, C-4), 77.8 (s, C-6), 119.2, 120.0 (2 d, Ar), 125.9 (d, C-5’), 145.2, 145.3, 145.5, 145.7 (4 s, Ar), 148.4 (s, C-4’), 171.5, 171.9, 172.2 (3 s, C=O) ppm.

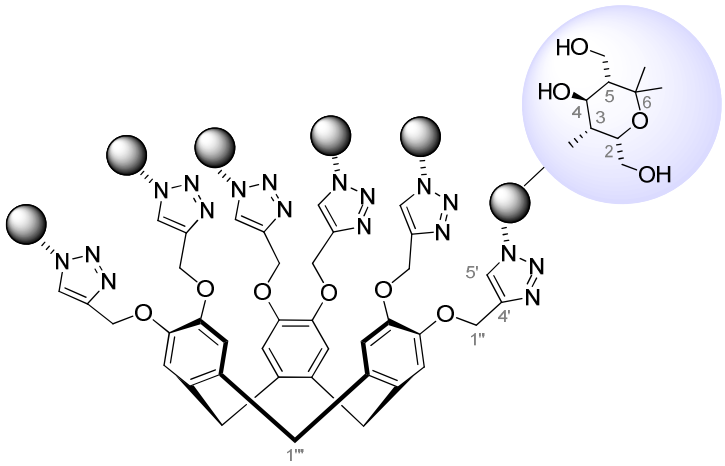
IR (ATR): ν = 2975-2920 (C-H), 1740 (C=O), 1510, 1450 (C=C), 1230, 1090, 1040 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $[2738.8 \cdot 2Na]^{2+}$: calcd.: 1392.0537
found: 1392.0553

Hexavalent triazole (**140**)

JNS266

According to **GP-8**, to a solution of azidopyran **10** (44 mg, 0.19 mmol) in dioxane (1.1 mL) was added Cu/C (58 mg, 9.1 μmol), Et_3N (80 μL, 15 μmol) and alkyne **138** (9 mg, 15 μmol). Cu/C (58 mg, 9.1 μmol), Et_3N (80 μL, 15 μmol) were additionally added. After stirring for 2 d, the mixture was filtrated through a pad of Celite® and washed with MeOH. The solvents were removed under reduced pressure and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 4:1) affording **140** (20 mg, 67 %) as a yellow solid.



140

Chemical Formula: $C_{93}H_{132}N_{18}O_{30}$

Molecular Weight: 1982.15 g·mol^{-1}

Melting range: 137 – 140 °C

Optical rotation: $[\alpha]_D^{22}$ = +18.1 (c =0.85, MeOH)

A splitting of several signals was observed.

^{1}H-NMR (700 MHz, CD_3OD): δ = 1.19, 1.23 (2 s, 6 H each, CH_3), 1.29 (bs, 12 H, CH_3), 1.35, 1.36 (2 s, 6 H each, CH_3), 1.99 – 2.07 (m, 6 H, 5-H), 2.87 – 2.93, 2.96 – 3.01 (2 m, 6 H each, 2-CH_2), 3.63 – 3.70 (m, 6 H, 5-CH_2), 3.74 – 3.79 (m, 2 H, 1’’’-H), 3.82 – 3.89 (m, 6 H, 5-CH_2), 4.10 – 4.17 (m, 2 H, 1’’’-H), 4.21 – 4.24, 4.25 – 4.29 (2 m, 6 H each, 4-H, 2-H), 4.30 – 4.34 (m, 2 H, 1’’’-H), 4.67 – 4.74 (m, 6 H, 3-H), 5.12 – 5.19 (m, 12 H, 1’’-H), 7.15 – 7.20 (m, 6 H, Ar), 7.99 (s, 6 H, 5’-H) ppm.

^{13}C-NMR (175 MHz, CD_3OD): δ = 23.6, 26.8, 30.6, 30.7, 30.8, 33.1 (6 q, CH_3), 49.5 (d, C-5), 61.6 (t, 2-CH_2), 62.1 (t, 5-CH_2), 62.6 (t, C-1’’’), 64.0 (t, C-1’’), 70.3 (d, C-3), 72.0 (d, C-2), 73.1 (d, C-4), 77.5 (s, C-6), 118.5, 126.2, 127.7, 130.0, 135.1 (5 d, C-5’, Ar), 144.9 (s, Ar), 148.3 (s, C-4’) ppm.

IR (ATR): ν = 3370 (OH), 2960-2850 (C-H), 1510, 1455 (C=C), 1260, 1055 (C-O) cm^{-1}.

HRMS (pos. ESI-TOF): $[1982.15 \cdot 2Na]^{2+}$: calcd.: 1013.9586
found: 1013.9587

$1982.15 \cdot Na^+$: calcd.: 2004.9279

found: 2004.9269

Elemental Analysis: $C_{129}H_{168}N_{18}O_{48}$ (2738.8) calcd. (%): C 56.35, H 6.71, N 12.72
found. (%): C 50.51, H 5.06, N 9.17

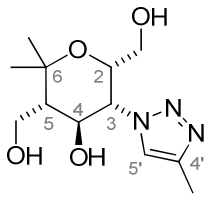
A better elemental analysis could not be obtained.

6.2.5. Metal-free 1,2,3,-triazole formation

(2*S*,3*R*,4*S*,5*S*)-[4-Hydroxy-6,6-dimethyl-3-(4-methyl-1*H*-1',2',3'-triazol-1'-yl)tetrahydro-2*H*-pyran-2,5-diyl]dimethanol (**123**)

JNS232

According to **GP-10**, to a cooled solution of amine (58 mg, 0.28 mmol) in EtOH (3.3 mL) was added KOAc (583 mg, 0.85 mmol) and the resulting solution was stirred for 10 min at 0 °C. Hydrazone **143** (109 mg, 0.37 mmol) was dissolved in MeCN (2.2 mL) and added dropwise to the reaction mixture. Stirring was continued at r.t. for 21 h. All volatiles were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1) affording **123** (78 mg, quant.) as a colorless solid.



123

Chemical Formula: $C_{12}H_{21}N_3O_4$

Molecular Weight: 271.3 g·mol^{-1}

Melting point: 176 °C

Optical rotation: $[\alpha]_D^{22}$ = +2.68 (c = 0.77, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.28, 1.39 (2 s, 3 H each, CH_3), 2.05 (td, $J \approx 6.2$, 12.3 Hz, 1 H, 5-H), 2.33 (s, 3 H, CH_3), 2.95, 3.05 (AB part of ABX system, J_{AB} = 11.5 Hz, J_{AX} = 4.8 Hz, J_{BX} = 7.5 Hz, 1 H each, 2-CH_2), 3.69 (dd, J = 6.4, 11.3 Hz, 1 H, 5-CH_2), 3.89 (dd, J = 6.0,

11.3 Hz, 1 H, 5-CH_2), 4.22 – 4.28 (m, 2 H, 2-H, 4-H), 4.67 (dd, *J* = 5.1, 7.0 Hz, 1 H, 3-H), 7.72 (s, 1 H, 5'-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 10.5, 23.5, 26.7 (3 q, CH_3), 49.6 (d, C-5), 61.6 (t, 2-CH_2), 62.3 (t, 5-CH_2), 70.2 (d, C-3), 71.9 (d, C-2), 73.1 (d, C-4), 77.5 (s, C-6), 123.8 (d, C-5'), 143.9 (s, C-4') ppm.

IR (ATR): ν = 3370 (OH), 3150 (=C-H), 2975-2885 (C-H), 1560, 1440, 1390 (C=C), 1055 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{12}H_{21}N_3O_4 \cdot H^+$: calcd.: 272.1605
found: 272.1604

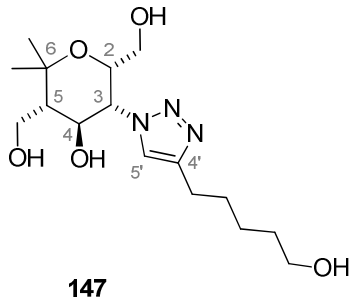
$C_{12}H_{21}N_3O_4 \cdot Na^+$: calcd.: 294.1424
found: 294.1435

Elemental Analysis: $C_{12}H_{21}N_3O_4$ (271.3) calcd. (%): C 53.12, H 7.80, N 15.49
found. (%): C 53.27, H 7.80, N 15.52

(2*S*,3*R*,4*S*,5*S*)-{4-Hydroxy-3-[4-(5-hydroxypentyl)-1*H*-1',2',3'-triazol-1-yl]-6,6-dimethyltetrahydro-2*H*-pyran-2,5-diyl}dimethanol (**147**)

JNSU23

According to **GP-10**, to a cooled solution of amine **3** (26 mg, 0.13 mmol) in EtOH (1.5 mL) was added KOAc (37 mg, 0.38 mmol) and the resulting solution was stirred for 10 min at 0 °C. Tosylhydrazone **146** (60 mg, 0.16 mmol) was dissolved in MeCN (1.2 mL) and added dropwise to the reaction mixture. Stirring was continued at room temperature for 26 h. All volatiles were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 95:5 to 9:1) affording **147** (28 mg, 65%) as a yellow oil.



147

Chemical Formula: $C_{16}H_{29}N_3O_5$

Molecular Weight: 343.4 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = + 27.3 (c = 1.29, MeOH)

^{1}H NMR (700 MHz, CD_3OD): δ = 1.29, 1.39 (2 s, 3 H each, CH_3), 1.40 – 1.45 (m, 2 H, CH_2), 1.54 – 1.60 (m, 2 H, CH_2), 1.71 (quint., *J* = 7.6 Hz, 2 H, CH_2), 2.05 (td, *J* ≈ 6.2, 12.2 Hz, 1 H, 5-H), 2.73 (t, *J* = 7.6 Hz, 2 H, CH_2), 2.93, 3.04 (AB part of ABX system, J_{AB} = 11.5 Hz, J_{AX} = 4.8 Hz, J_{BX} = 7.6 HZ, 1 H each, 2-CH_2), 3.55 (t, *J* = 6.5 Hz, 2 H, CH_2), 3.69 (dd, *J* = 6.4, 11.3 Hz, 1 H, 5-CH_2), 3.89 (dd, *J* = 6.0, 11.3 Hz, 1 H, 5-CH_2), 4.23 – 4.31 (m, 2 H, 2-H, 4-H), 4.69 (dd, *J* = 5.1, 7.0 Hz, 1 H, 3-H), 7.74 (s, 1 H, 5'-H) ppm.

^{13}C NMR (175 MHz, CD_3OD): δ = 23.5 (q, CH_3), 26.3, 26.4 (2 t, CH_2), 26.7 (q, CH_3), 30.4, 33.3 (2 t, CH_2), 48.5 (d, C-5), 61.6 (t, 2-CH_2), 62.3 (t, 5-CH_2), 62.8 (t, CH_2), 70.2 (d, C-3), 72.0 (d, C-2), 73.1 (d, C-4), 77.5 (s, C-6), 123.4 (d, C-5'), 148.8 (s, C-4') ppm.

IR (ATR): ν = 3355 (OH), 2930-2860 (C-H), 1550 (C=C), 1170-1010 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{16}H_{29}N_3O_5 \cdot Na^+$: calcd.: 366.1994
found: 366.2001

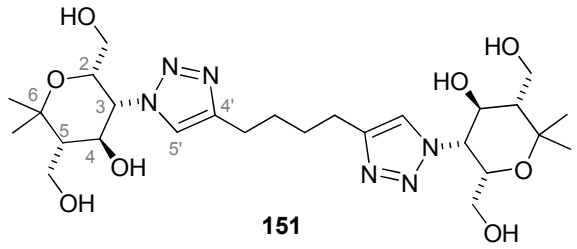
Elemental Analysis: $C_{16}H_{29}N_3O_5$ (343.4) calcd. (%): C 55.96, H 8.51, N 12.24
found. (%): C 54.44, H 8.48, N 10.72

A better elemental analysis could not be obtained.

Divalent triazole (**151**)

JNS308

According to **GP-10**, to a cooled solution of amine **3** (100 mg, 0.487 mmol) in EtOH (5.7 mL) was added KOAc (147 mg, 1.49 mmol) and the resulting solution was stirred for 10 min at 0 °C. Hydrazone **150** (116 mg, 0.187 mmol) was dissolved in MeCN (3.8 mL) and added dropwise to the colorless solution. The reaction mixture turned bright yellow after 10 min. Stirring was continued at r.t. for 20 h. All volatiles were removed in vacuo and the crude product was purified by flash column chromatography (silica gel, CH_2Cl_2/ MeOH, 9:1 to 6:1) affording **151** (80 mg, 75%) as a colorless solid.



151

Chemical Formula: $C_{26}H_{44}N_6O_8$

Molecular Weight: 568.6 g·mol^{-1}

Melting point: 184 °C

Optical rotation: $[\alpha]_D^{22}$ = +33.2 (c = 1.18, MeOH)

^{1}H-NMR (500 MHz, CD_3OD): δ = 1.29, 1.39 (2 s, 6 H each, CH_3), 1.69 – 1.76 (m, 4 H, CH_2), 2.05 (td, J ≈ 6.2, 12.2 Hz, 2 H, 5-H), 2.75 (t, J = 6.4 Hz, 4 H, CH_2), 2.92, 3.03 (AB part of ABX system, J_{AB} = 11.4 Hz, J_{AX} = 4.7 Hz, J_{BX} = 7.5 Hz, 2 H each, 2-CH_2), 3.67 – 3.72 (m, 2 H, 5-CH_2), 3.89 (dd, J = 6.0, 11.3 Hz, 2 H, 5-CH_2), 4.23 – 4.30 (m, 4 H, 2-H, 4-H), 4.68 (dd, J = 5.2, 7.0 Hz, 2 H, 3-H), 7.74 (s, 2 H, 5'-H) ppm.

^{13}C-NMR (125 MHz, CD_3OD): δ = 23.5 (q, CH_3). 25.9 (t, CH_2), 26.7 (q, CH_3), 29.9 (t, CH_2), 49.6 (d, C-5), 61.6 (t, 2-CH_2), 62.3 (t, 5-CH_2), 70.2 (d, C-3), 72.0 (d, C-2), 73.0 (d, C-4), 77.5 (s, C-6), 123.5 (d, C-5'), 148.6 (s, C-4') ppm

IR (ATR): ν = 3370 (OH), 2930 (C-H), 1680 (C=C), 1045 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{26}H_{44}N_6O_8 \cdot H^+$: calcd.: 569.3293

found: 569.3301

$C_{26}H_{44}N_6O_8 \cdot Na^+$: calcd.: 591.3107
found: 591.3128

Elemental Analysis: $C_{26}H_{44}N_6O_8$ (568.6) calcd. (%): C 54.91, H 7.80, N 14.78
found. (%): C 51.37, H 7.63, N 10.68

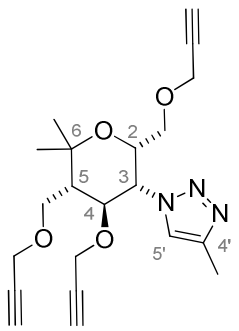
A better elemental analysis could not be obtained.

6.2.6. Miscellaneous subsequent reactions

(2*S*,3*R*,4*S*,5*S*)-1-{-6,6-Dimethyl-4-(prop-2-ynyloxy)-2,5-bis[(prop-2-ynyloxy)methyl]-tetrahydro-2*H*-pyran-3-yl}-4-methyl-1*H*-1',2',3'-triazole (**125**)

JNS268

According to **GP-1**, to a cooled solution of triazole **123** (72 mg, 0.26 mmol) in anhydrous DMF (1 mL) was added NaH (25 mg, 0.11 mmol) under argon atmosphere. The resulting suspension was stirred at r.t. for 1 h. After cooling to 0 °C, propargyl bromide **124** (1.51 g, 1.2 mL, 12.7 mmol) was added dropwise and the reaction mixture was stirred for 22 h. MeOH (2 mL) was added and the mixture was stirred for 30 min. After removing all volatiles in vacuo, the resulting mixture was dissolved with H_2O (5 mL) and extracted with EtOAc (3 x 10mL). The combined organic phases were dried with Na_2SO_4 and the solvent was removed in vacuo. The crude was purified by flash column chromatography (silica gel, hexanes/ EtOAc, 5:1 to 2:1) affording **125** (72 mg, 71%) as an orange oil.



125

Chemical Formula: $C_{21}H_{27}N_3O_4$

Molecular Weight: 385.5 g·mol^{-1}

Optical rotation: $[\alpha]_D^{22}$ = -4.90 (c = 0.59, MeOH)

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.33, 1.36 (2 s, 3 H each, CH_3), 2.14 (ddd, *J* = 3.8, 7.4, 11.4 Hz, 1 H, 5-H), 2.25 (t, *J* = 2.4 Hz, 1 H, ≡CH), 2.37 – 2.33 (m, 4 H, CH_3, ≡CH), 2.41 (t, *J* = 2.4 Hz, 1 H, ≡CH), 2.98, 3.11 (AB part of ABX system, J_{AB} = 10.0 Hz, J_{AX} = 6.0 Hz, J_{BX} = 6.5 Hz, 1 H each, 2-CH_2), 3.62 – 3.69 (m, 2 H, 5-CH_2) 3.85 (dd, *J* = 2.4, 15.7 Hz, 1 H, CH_2C≡), 3.91, 3.94 (AB part of ABX system, J_{AB} = 15.7 Hz, J_{AX} = J_{BX} = 2.4 Hz, 1 H each, CH_2C≡), 4.02 – 4.14 (m, 3 H, 4-H, CH_2C≡), 4.30 (dt, *J* ≈ 4.4, 6.2 Hz, 1 H, 2-H), 4.84 (dd, *J* = 4.4, 5.4 Hz, 1 H, 3-H), 7.48 (s, 1 H, 5'-H) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 11.0, 24.2, 27.2 (3 q, CH_3), 45.7 (d, C-5), 58.0, 58.4, 58.6 (3 t, CH_2C≡), 66.5 (d, C-3), 67.6 (t, 5-CH_2), 68.2 (t, 2-CH_2), 68.8 (d, C-2), 74.6, 74.7, 74.9 (3 d, ≡CH), 76.9 (s, C-6), 77.9 (d, C-4), 78.9, 79.1, 79.5 (3 s, C≡C), 121.4 (d, C-5'), 143.5 (s, C-4') ppm.

IR (ATR): ν = 3290 (≡C-H), 3150-3060 (=C-H), 2960-2840 (C-H), 2115 (C≡C), 1550, 1440, 1390, 1360 (C=C), 1080, 1030 (C-O-C) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{21}H_{27}N_3O_4{\cdot}Na^+$: calcd.: 408.1894
found: 408.1904

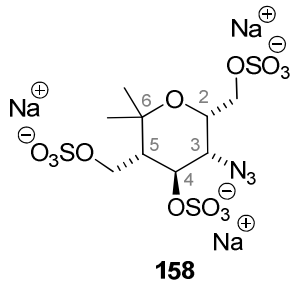
Elemental Analysis: $C_{21}H_{27}N_3O_4$ (385.5): calcd. (%): C 65.44, H 7.06, N 10.90
found. (%): C 65.84, H 7.06, N 10.26

6.2.7. Polysulfation

Azide (**158**)

JNS221

According to **GP-11**, polyol **13** (19 mg, 0.082 mmol), SO_3·DMF (47%, 240 mg, 0.739 mmol) and DMF-d_7 (0.8 mL) were stirred overnight. After ^{1}H-NMR control, additional SO_3·DMF (120 mg) was added and the reaction mixture was stirred for 2 d. According to Method A, NaOH 1 M was added dropwise until pH 12 was reached. The solvents were removed in vacuo and the crude product was purified by dialysis (tube width: 10-16 mm, molecular weight cut off: 100-500 Da). The product was filtrated through a syringe filter affording **158** (5 mg, 11%) as a colorless solid.



158

Chemical Formula:	$C_9H_{14}N_3Na_3O_{13}S_3$
Molecular Weight:	537.4 g·mol^{-1}
Melting point:	203 °C (decomposition)
Optical rotation:	$[\alpha]_D^{22}$ = +13.5 (c = 0.08, H_2O)

^{1}H-NMR (700 MHz, D_2O): δ = 1.28, 1.50 (2 s, 3 H each, CH_3), 2.41 (dt, *J* = 4.4, 7.9 Hz, 1 H, 5-H), 4.03 – 4.06 (m, 1 H, 3-H), 4.11 (dd, *J* = 7.4, 10.7 Hz, 1 H, 2-CH_2), 4.23 (dd, *J* = 4.9, 10.7 Hz, 1 H, 2-CH_2), 4.30 (d, *J* = 7.9 Hz, 2 H, 5-CH_2), 4.46 (ddd, *J* = 2.5, 4.9, 7.4 Hz, 1 H, 2-H), 4.86 – 4.87 (m, 1 H, 4-H) ppm.

^{13}C-NMR (175 MHz, D_2O): δ = 25.6, 25.7 (2 q, CH_3), 42.6 (d, C-5), 59.7 (d, C-3), 66.5 (d, C-2), 66.7 (t, 5-CH_2), 67.6 (t, 2-CH_2), 74.2 (d, C-4), 75.3 (s, C-6) ppm.

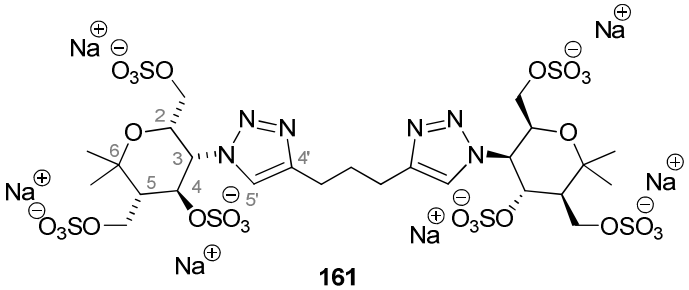
IR (ATR): ν = 2905 (C-H), 2110 (N_3), 1240 (C-O), 1130 (C-O-C, $SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $C_9H_{14}N_3Na_3O_{13}S_3$·$Na^+$: calcd.: 559.9274
found: 559.9418

Divalent triazole (**161**)

JNS217

According to **GP-11**, polyol **106** (15 mg, 0.027 mmol), SO_3·DMF (97%, 75 mg, 0.49 mmol) and DMF-d_7 (0.6 mL) were stirred overnight. After ^{1}H-NMR control, additional SO_3·DMF (60 mg) was added and the reaction mixture was stirred for 1 d. According to Method A, NaOH 1 M was added dropwise until pH 12 was reached. The solvents were removed in vacuo and the crude product was purified by dialysis (tube width: 10-16 mm, molecular weight cut off: 100-500 Da). The product was filtrated through a syringe filter affording **161** (15 mg, 48%) as a colorless solid.



161

Chemical Formula: $C_{25}H_{36}N_6Na_6O_{26}S_6$

Molecular Weight: 1166.9 g·mol^{-1}

Melting point: 267 °C (decomposition)

Optical rotation: $[\alpha]_D^{22}$ = +65.2 (c = 0.1, H_2O)

^{1}H-NMR (700 MHz, D_2O): δ = 1.46, 1.51 (2 s, 6 H each, CH_3), 2.04 – 2.12 (m, 2 H, CH_2), 2.66 – 2.74 (m, 2 H, 5-H), 2.78 (t, *J* = 6.7 Hz, 4 H, CH_2), 3.51 (dd, *J* = 7.7, 11.0 Hz, 2 H, 2-CH_2), 3.65 (dd, *J* = 4.8, 11.0 Hz, 2 H, 2-CH_2), 4.20 (m_c, 2 H, 5-CH_2), 4.41 (dd, *J* = 4.4, 10.8 Hz, 2 H, 5-CH_2), 4.74 – 4.77 (m, 2 H, 2-H), 4.97 (dd, *J* = 6.5, 12.1 Hz, 2 H, 4-H), 5.16 (m_c, 2 H, 3-H), 7.92 (s, 2 H, 5'-H) ppm.

^{13}C-NMR (175 MHz, D_2O): δ = 22.2 (q, CH_3), 23.7 (t, CH_2), 26.0 (q, CH_3), 28.0 (t, CH_2), 44.1 (d, C-5), 65.6 (d, C-3), 65.8 (t, 2-CH_2), 66.6 (t, 5-CH_2), 68.1 (d, C-2), 77.3 (d, C-4), 77.9 (s, C-6), 124.5 (d, C-5'), 151.2 (s, C-4') ppm.

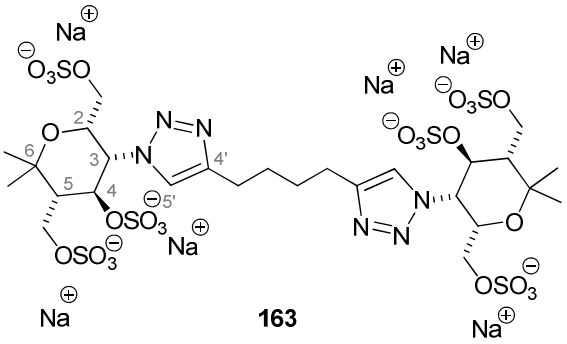
IR (ATR): ν = 2950-2850 (C-H), 1460 (C=C), 1220 (C-O), 1130 (C-O-C, $SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{25}H_{36}N_6Na_6O_{26}S_6$·$Na^+$: calcd.: 1188.9281
found: 1188.9303

Divalent triazole (**163**)

JNS310

According to **GP-11**, polyol **151** (30 mg, 0.053 mmol), SO_3·DMF (97%, 145 mg, 0.949 mmol) and DMF-d_7 (0.8 mL) were stirred overnight. After ^{1}H-NMR control, the reaction mixture was stirred for 3 d and each day a new portion of SO_3·DMF (145 mg) was added. According to Method B, NaOH 0.5 M was added dropwise until pH 8 was reached. The reaction mixture was filtrated through an ion exchange DOWEX® Na^+ column. The solvents were removed in vacuo and the crude product was purified by dialysis (tube width: 10-16 mm, molecular weight cut off: 100-500 Da). The product was filtrated through a syringe filter affording **163** (54 mg, 87%) as a colorless solid.



163

Chemical Formula:	$C_{26}H_{38}N_6Na_6O_{26}S_6$
Molecular Weight:	1180.9 g·mol^{-1}
Melting point:	269 °C (decomposition)
Optical rotation:	$[\alpha]_D^{22}$ = +25.1 (c = 0.06, H_2O)

^{1}H-NMR (700 MHz, D_2O): δ = 1.47, 1.52 (2 s, 6 H each, CH_3), 1.73 (bs, 4 H, CH_2), 2.68 – 2.76 (m, 2 H, 5-H), 2.79 (bs, 4 H, CH_2), 3.51 (dd, *J* = 7.7, 10.8 Hz, 2 H, 2-CH_2), 3.65 (dd, *J* = 4.8, 10.8 Hz, 2 H, 2-CH_2), 4.21 (m_c, 2 H, 5-CH_2), 4.41 (dd, *J* = 4.6, 10.9 Hz, 2 H, 5-CH_2), 4.75 – 4.77 (m, 2 H, 2-H), 4.99 (dd, *J* = 6.3, 12.1 Hz, 2 H, 4-H), 5.14 – 5.19 (m, 2 H, 3-H), 7.90 (s, 2 H, 5'-H), ppm.

^{13}C-NMR (175 MHz, D_2O): δ = 22.3 (q, CH_3), 24.1 (t, CH_2), 26.0 (q, CH_3), 27.9 (t, CH_2), 44.1 (d, C-5), 65.4 (d, C-3), 65.5 (t, 2-CH_2), 66.6 (t, 5-CH_2), 68.1 (d, C-2), 77.2 (d, C-4), 77.9 (s, C-6), 124.3 (d, C-5'), 148.7 (s, C-4') ppm.

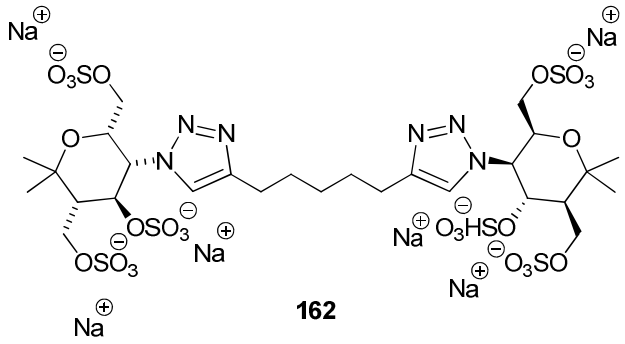
IR (ATR): ν = 2970-2850 (C-H), 1440 (C=C), 1225 (C-O), 1200 ($SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{26}H_{38}N_6Na_6O_{26}S_6 \cdot Na^+$: calcd.: 1202.9438
found: 1202.9407

Divalent triazole (**162**)

JNS254

According to **GP-11**, polyol **116** (15 mg, 0.026 mmol), SO_3·DMF (47%, 151 mg, 0.463 mmol) and DMF-d_7 (0.7 mL) were stirred overnight. After ^{1}H-NMR control, additional SO_3·DMF (151 mg) was added and the reaction mixture was stirred for 1 d. According to Method A, NaOH 1 M was added dropwise until pH 12 was reached. The solvents were removed in vacuo and the crude product was purified twice by dialysis (tube width: 10-16 mm, molecular weight cut off: 100-500 Da). The product was filtrated through a syringe filter affording **162** (12 mg, 39%) as a colorless solid.



162

Chemical Formula: $C_{27}H_{40}N_6Na_6O_{26}S_6$

Molecular Weight: 1195.0 g·mol^{-1}

Melting point: 265 °C (decomposition)

Optical rotation: $[\alpha]_D^{22}$ = +30.5 (c = 0.08, H_2O)

^{1}H-NMR (700 MHz, D_2O): δ = 1.40, 1.47 (2 s, 6 H each, CH_3), 1.63 – 1.76 (m, 4 H, CH_2), 2.62 – 2.67 (m, 2 H, 5-H), 2.67– 2.70 (m, 6 H, CH_2), 3.41 – 3.51 (m, 2 H, 2-CH_2), 3.59 (B part of ABX system, J_{AB} = 10.8 Hz, J_{BX} = 4.9 Hz, 2 H, 2-CH_2), 4.11 – 4.22 (m, 2 H, 5-CH_2), 4.36 (dd, J = 4.5, 10.7 Hz, 2 H, 5-CH_2), 4.67 – 4.73 (m, 2 H, 2-H), 4.93 (dd, J = 6.6, 12.1 Hz, 2 H, 4-H), 5.07 – 5.14 (m, 2 H, 3-H), 7.84 (s, 2 H, 5'-H) ppm.

^{13}C-NMR (175 MHz, D_2O): δ = 22.2 (q, CH_3), 24.4 (t, CH_2), 26.0 (q, CH_3), 27.7, 28.3 (2 t, CH_2), 44.1 (d, C-5), 65.5 (d, C-3), 65.7 (t, 2-CH_2), 66.6 (t, 5-CH_2), 68.1 (d, C-2), 77.2 (d, C-4), 77.9 (s, C-6), 124.0 (d, C-5')* ppm; *C-5' attributed by HMQC; C-4' could not be detected.

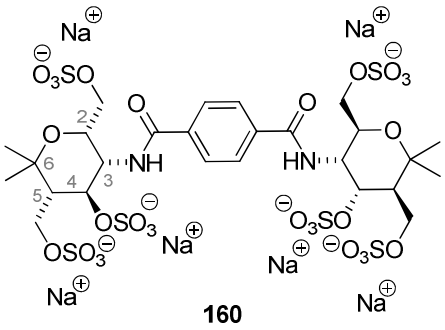
IR (ATR): ν = 2910 (C-H), 1460 (C=C), 1205 ($SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{27}H_{40}N_6Na_6O_{26}S_6 \cdot Na^+$: calcd.: 1216.9600
found: 1216.9607

Divalent amide (**160**)

JNS174

According to **GP-11**, polyol **54** (15 mg, 0.028 mmol), SO_3·DMF (97%, 77 mg, 0.50 mmol) and DMF-d_7 (0.6 mL) were stirred overnight. According to Method A, NaOH 1 M was added dropwise until pH 10 was reached. The solvents were removed in vacuo and the crude product was purified twice by dialysis (tube width: 10-16 mm, molecular weight cut off: 100-500 Da) affording **160** (19 mg, 60%) as a colorless solid.



160

Chemical Formula: $C_{26}H_{34}N_2Na_6O_{28}S_6$

Molecular Weight: 1152.9 g·mol^{-1}

Melting point: 275 °C (decomposition)

Optical rotation: $[\alpha]_D^{22}$ = +33.7 (c = 0.08, H_2O)

^{1}H-NMR (700 MHz, D_2O): δ = 1.36, 1.52 (2 s, 6 H each, CH_3), 2.53 (td, $J \approx 7.6$, 14.3 Hz, 1 H, 5-H), 4.09, 4.19 (AB part of ABX system, J_{AB} = 10.9 Hz, J_{AX} = 4.3 Hz, J_{BX} = 7.5 Hz, 2 H each, 2-CH_2), 4.25, 4.32 (AB part of ABX system, J_{AB} = 10.5 Hz, J_{AX} = 6.3 Hz, J_{BX} = 7.9 Hz,

2 H each, 5-CH_2), 4.53 – 4.57 (m, 2 H, 3-H), 4.57 – 4.62 (m, 2 H, 2-H), 4.69 – 4.76 (m, 2 H, 4-H), 7.88 (s, 4 H, Ar) ppm.

^{13}C-NMR (175 MHz, D_2O): δ = 24.8, 26.4 (2 q, CH_3), 44.2 (d, C-5), 52.3 (d, C-2), 67.3 (t, 2-CH_2), 67.6 (t, 5-CH_2), 67.7 (d, C-3), 76.2 (d, C-4), 76.6 (s, C-6), 128.7 (d, Ar) ppm; signals for C=O and Ar singlet could not be detected.

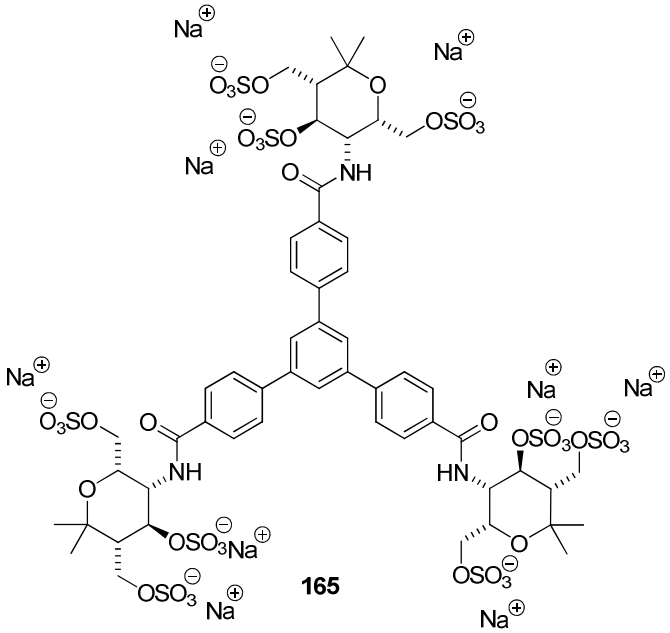
IR (ATR): ν = 3480 (NH), 2985 (C-H), 1535 (C=C), 1635 (C=O), 1255 (C-O), 1130 ($SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{26}H_{34}N_2Na_6O_{28}S_6 \cdot Na^+$: calcd.: 1174.8906
found: 1174.8819

Trivalent triazole (**165**)

JNS297

According to **GP-11**, polyol **70** (32 mg, 0.032 mmol), SO_3·DMF (97%, 136 mg, 0.864 mmol) and DMF-d_7 (0.7 mL) were stirred overnight. After ^{1}H-NMR control, the reaction mixture was stirred for 2 d and each day a new portion of SO_3·DMF (136 mg) was added. According to Method B, NaOH 0.5 M was added dropwise until pH 9 was reached. The reaction mixture was filtrated through an ion exchange DOWEX® Na^+ column. The solvents were removed removed in vacuo and the crude product was purified by dialysis (tube width: 10-16 mm, molecular weight cut off: 500-1000 Da). The final product was filtrated through a syringe filter affording **165** (51 mg, 84%) as a colorless solid.



165

Chemical Formula: $C_{54}H_{60}N_3Na_9O_{42}S_9$

Molecular Weight: 1918.5 g·mol^{-1}

Melting point: 260 °C (decomposition)

Optical rotation: $[\alpha]_D^{22}$ = +23.4 (c = 0.5, H_2O)

^{1}H-NMR (700 MHz, D_2O): δ = 1.41, 1.55 (2 s, 9 H each, CH_3), 2.56 (m_c, 3 H, 5-H), 4.14 (A part of ABX system, J_{AB} = 10.2 Hz, J_{AX} = 7.8 Hz, 3 H, 2-CH_2), 4.23 – 4.26 (m, 3 H, 2-CH_2), 4.27 – 4.30 (m, 3 H, 5-CH_2), 4.36 – 4.38 (m, 3 H, 5-CH_2), 4.61 (m_c, 3 H, 2-H), 4.62 – 4.66 (m, 3 H, 3-H), 8.00 (s, 12 H, Ar), 8.17 (s, 3 H, Ar) ppm; the signal of 4-H could not be detected (overlapping with D_2O peak ≈ 4.77 ppm).

^{13}C-NMR (175 MHz, D_2O): δ = 24.2, 26.0 (2 q, CH_3), 43.9 (d, C-5), 52.0 (d, C-2), 67.1 (t, 5-CH_2), 67.4 (t, 2-CH_2), 67.5 (d, C-3), 76.3 (d, C-4), 76.4 (s, C-6), 125.8, 127.4, 128.2 (3 d, Ar), 132.9, 141.3, 143.7 (3 s, Ar), 171.0 (s, C=O) ppm.

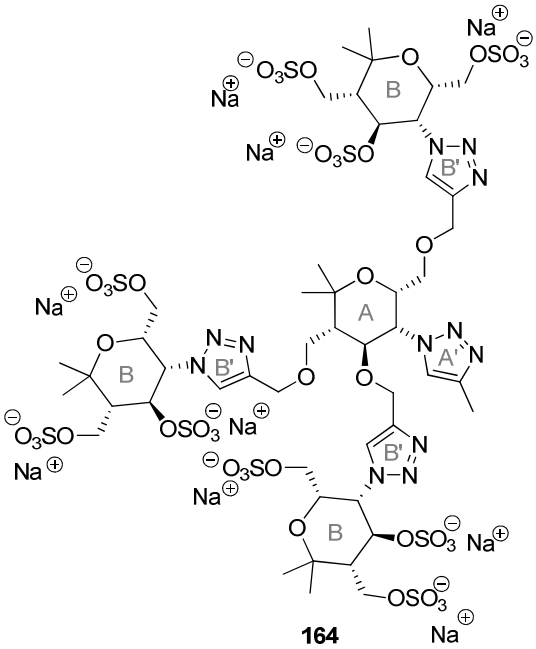
IR (ATR): ν = 3460 (NH), 2960 (C-H), 1535 (C=C), 1635 (C=O), 1220 (C-O), 1130 ($SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $[C_{54}H_{60}N_3Na_9O_{42}S_9 \cdot 2Na]^{2+}$: calcd.: 981.9495
found: 981.9458

Trivalent triazole (**164**)

JNS303

According to **GP-11**, polyol **126** (16 mg, 0.015 mmol), SO_3·DMF (97%, 64 mg, 0.40 mmol) and DMF-d_7 (0.7 mL) were stirred overnight. After ^{1}H-NMR control, the reaction mixture was stirred for 5 d and each day a new portion of SO_3·DMF (64 mg) was added. According to Method B, NaOH 0.5 M was added dropwise until pH 9 was reached. The reaction mixture was then filtrated through an ion exchange DOWEX® Na^+ column. The solvents were removed in vacuo and the crude product was purified by dialysis (tube width: 10-16 mm, molecular weight cut off: 500-1000 Da). The product was filtrated through a syringe filter affording **164** (30 mg, quant.) as a colorless solid.



164

Chemical Formula:	$C_{48}H_{69}N_{12}Na_9O_{43}S_9$
Molecular Weight:	1997.6 g·mol^{-1}
Melting point:	290 °C (decomposition)
Optical rotation:	$[\alpha]_D^{22}$ = +10.7 (c =0.09, H_2O)

An assignment of the signals to two of the three B-rings and to the four triazole units is not possible.

^{1}H-NMR (700 MHz, D_2O): δ = 1.38, 1.48, 1.55, 1.56, 1.57 (5 s, 3 H each, CH_3), 1.61 (s, 6 H, CH_3), 1.62 (s, 3 H, CH_3), 2.42 – 2.48 (m, 1 H, 5-H^A), 2.50 (s, 3 H, CH_3), 2.77 – 2.84 (m, 3 H, 5-H^B), 3.11 (dd, *J* = 7.6, 10.6 Hz, 1 H, 2-CH_2^A), 3.24 (dd, *J* = 4.4, 10.6 Hz, 1 H, 2-CH_2^A), 3.62 – 3.64, 3.66 – 3.67, 3.73 – 3.75 (3 m, 2 H each, 2-CH_2^B), 3.76 – 3.78 (m, 1 H, 5-CH_2^A) 3.88

(m_c, 1 H, 5-CH_2^A), 4.29 – 4.32 (m, 4 H, 5-CH_2^B), 4.47– 4.52 (m, 4 H, 2-H, 2-H^B), 4.55 – 4.66 (m, 4 H, CH_2), 4.83 – 4.89 (m, 4 H, 4-H, 4-H^B), 5.01 – 5.10 (m, 2 H, CH_2), 5.10 – 5.13, 5.23 – 5.26, 5.26– 5.30, 5.30 – 5.33 (4 m, 1 H each, 3-$H^{A,B}$), 7.83, 7.96, 8.13, 8.26 (4 s, 1 H each, 5'-$H^{A,B}$) ppm.

^{13}C-NMR (175 MHz, D_2O): δ = 9.7, 9.8, 22.27, 22.37, 22.42, 26.0, 26.1, 26.2 (8 q, CH_3), 44.0, 44.2, 44.3, 45.0 (4 d, C-$5^{A,B}$), 63.0, 63.2, 63.4 (3 t, CH_2), 65.7 (d, C-3^A), 66.6, 66.7 (2 t, 5-CH_2^A, 2-CH_2^A), 68.0 (d, C-2 ^{A}), 77.4 (d, C-4 ^{A}), 78.0 (s, C-6 ^{A}), 126.5 (d, C-5'$^{A\text{ or }B}$) ppm; C-4' of A and B and further signals from B-rings could not be assigned.

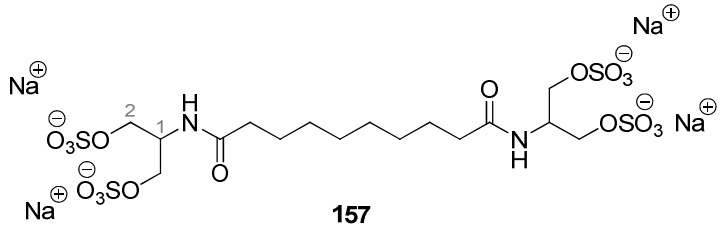
IR (ATR): ν = 2960-2855 (C-H), 1465 (C=C), 1250 (C-O), 1130 (C-O-C, $SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $[C_{48}H_{69}N_{12}Na_9O_{43}S_9 \cdot 2Na]^{2+}$: calcd.: 1020.9963
found: 1021.0006

Divalent amide (**157**)

JNS168

According to **GP-11**, polyol **56** (20 mg, 0.057 mmol), SO_3·DMF (106 mg, 0.689 mmol) and DMF-d_7 (0.6 mL) were stirred overnight. According to Method A, NaOH 1 M was added dropwise until pH 12 was reached. The solvents were removed in vacuo and the crude product was purified by dialysis (tube width: 10-16 mm, molecular weight cut off: 100-500 Da) affording **157** (34 mg, 79%) as a colorless solid.



157

Chemical Formula: $C_{16}H_{28}N_2Na_4O_{18}S_4$
Molecular Weight: 756.6 g·mol^{-1}
Melting range: 200 – 205 °C (decomposition)

^{1}H-NMR (500 MHz, D_2O): δ = 1.36 (bs, 8 H, CH_2), 1.61 – 1.69 (m, 4 H, CH_2), 2.34 (t, J = 7.4 Hz, 4 H, CH_2), 4.17, 4.20 (AB part of ABX system, J_{AB} = 9.0 Hz, J_{AX} = 3.6 Hz, J_{BX} = 4.3 Hz, 4 H each, 2-H), 4.47 (m_c, 2 H, 1-H) ppm.

^{13}C-NMR (125 MHz, D_2O): δ = 25.6, 28.4, 28.5, 36.1, (4 t, CH_2), 48.2 (d, C-1), 66.9 (t, C-2), 177.8 (s, C=O) ppm.

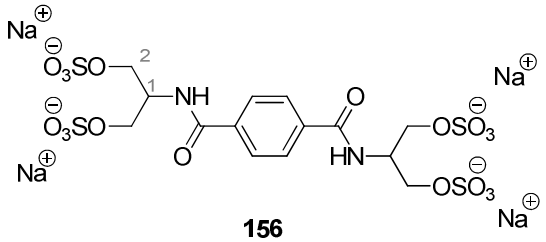
IR (ATR): ν = 3385 (NH), 2980 (C-H), 1740 (C=O), 1255 (C-O), 1130 ($SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{16}H_{28}N_2Na_4O_{18}S_4 \cdot Na^+$: calcd.: 778.9703
found: 778.9682

Divalent amide (**156**)

JNS159

According to **GP-11**, polyol **54** (20 mg, 0.064 mmol), SO_3·DMF (118 mg, 0.770 mmol) and DMF-d_7 (0.6 mL) were stirred overnight. After ^{1}H-NMR control, the reaction mixture was stirred for 4 d and each day a new portion of SO_3·DMF (118 mg) was added. According to Method A, NaOH 1 M was added dropwise until pH 12 was reached. The solvents were removed in vacuo and the crude product was purified by dialysis (tube width: 10-16 mm, molecular weight cut off: 100-500 Da) affording **156** (32 mg, 69%) as a colorless solid.



156

Chemical Formula: $C_{14}H_{16}N_2Na_4O_{18}S_4$

Molecular Weight: 720.5 g·mol^{-1}

Melting point: 215 – 217 °C (decomposition)

^{1}H-NMR (500 MHz, D_2O): δ = 4.20, 4.23 (AB part of ABX system, J_{AB} = 9.7 Hz, J_{AX} = 4.1 Hz, J_{BX} = 5.0 Hz, 4 H each, 2-H), 4.58 – 4.63 (m, 2 H, 1-H), 7.81 (s, 4 H, Ar) ppm.

^{13}C-NMR (125 MHz, D_2O): δ = 49.0 (d, C-1), 66.7 (t, C-2), 127.9 (d, Ar), 136.9 (s, Ar), 170.6 (s, C=O) ppm.

IR (ATR): ν = 3375 (NH), 2985 (C-H), 1535 (C=C), 1645 (C=O), 1290 (C-O), 1130 ($SO_3^-Na^+$) cm^{-1}.

HRMS (pos. ESI-TOF): $C_{14}H_{16}N_2Na_4O_{18}S_4 \cdot Na^+$: calcd.: 742.8758
found: 742.8763

6.3.Surface plasmon resonance (SPR) assay

Experiments were performed on a BIACORE X instrument (GE Healthcare, Freiburg, Germany) at 25 °C using a sensor chip (sensor chip SA, GE Healthcare).

The running buffer during the assay consisted of 20 mM HEPES pH 7.4, with 150 mM NaCl and 1 mM $CaCl_2$.

6.3.1. Assay protocol

Selectins (L-, P- or E-) Fc chimeras (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany) were coupled to Protein A gold particles (AuNP) (diam. 15 nm, Biotrend Chemikalien GmbH, Cologne, Germany) and led over a sensor chip surface with two flow cells (Fc1 and Fc2). On Fc2 was immobilized the selectin ligand composed of sulfated Tyrosine and tetrasaccharide sialyl-Lewis-X presented in a multivalent fashion on a polyacrylamide backbone (sTyr/sLe^X-PAA). On Fc1 (reference lane) was immobilized N-acetyllactosamine (LacNac-PAA) as background control. The signal from Fc1 was automatically subtracted from Fc2 during each measurement.

To evaluate selectin binding of potential inhibitors, before loading over the sensor chip, each sample was incubated with inhibitor (protein A gold particles coated with the respective selectin) for 18 min at r.t. at the desired final inhibitor concentrations.

The samples (35 µL) were injected over the reference lane and over the sTyr/SLe^x-PAA lane at a flow rate of 20 µL/min. Each cycle consisted of aprox. 1 min waiting period for monitoring baseline stability, a 105 sec period of association phase and 180 sec dissociation phase. Regeneration of the surface was done by injecting 4 M $MgCl_2$ at a flow rate of 100 µL/min for 60 sec.

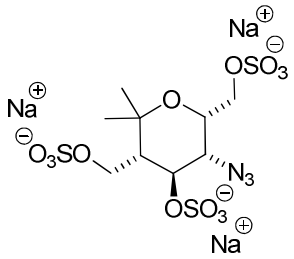
Measurements without inhibitor served as 100% binding references and were performed before and after each assay series to control baseline deviations. Each concentration was measured in duplicates.

6.3.2. Data evaluation

Reference lane data were subtracted from sTyr/sLe^x-PAA lane data. Responses of the sample injections were extracted between report points set at the start of the injection (0 sec) and at the end of the dissociation phase (285 sec). Each point represents the mean value of 2 measurements.

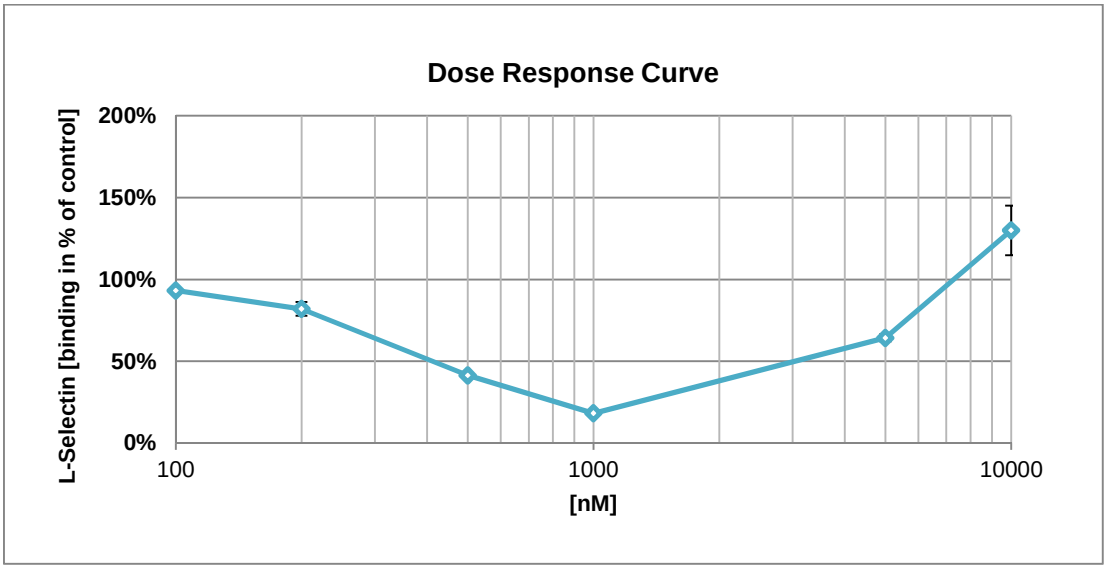
The mean value of the first and last 100% values were plotted against total number of data points. A linear regression between both points was set and using the resulting formula, 100% values were calculated for each data point. To obtain the relative binding value all mean results were divided by the respective 100% value. The relative binding was plotted against the corresponding inhibitor concentration and the IC_{50} was determined manually.

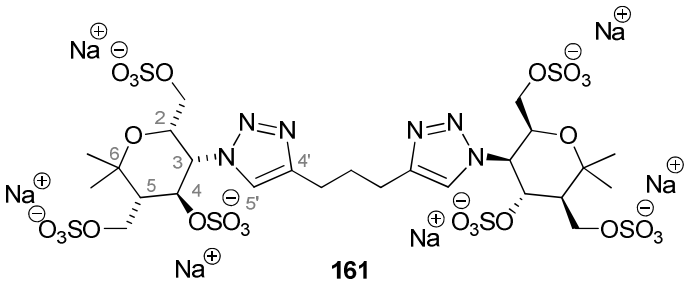
6.3.3. Experimental data



158

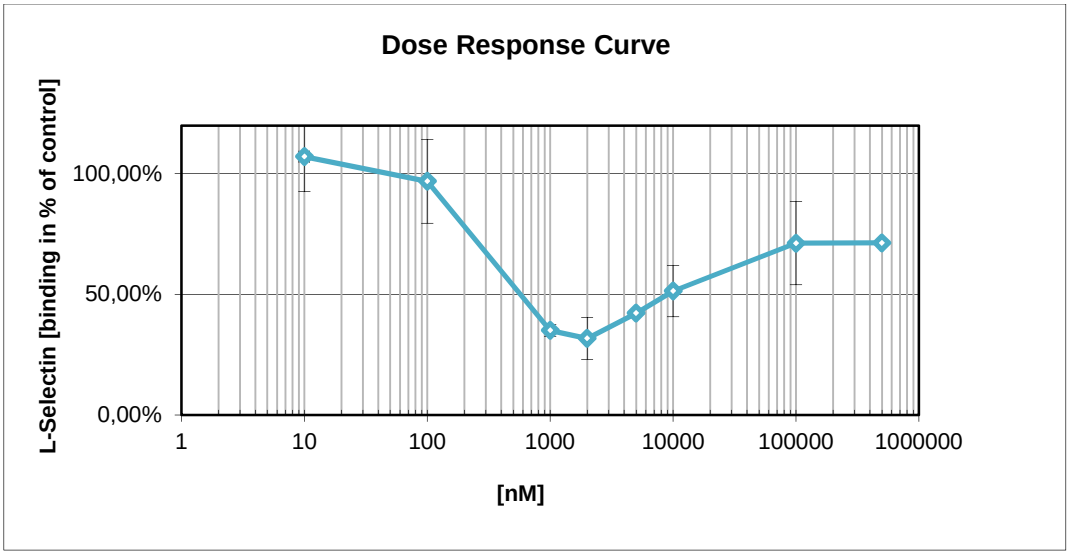
Baseline Corrected Values		Inhibitor Concentration [nM]	Error/ Diviation
100%			
2	1,30	10000	0,20
3	0,64	5000	0,03
4	0,18	1000	0,00
5	0,41	500	0,01
6	0,82	200	0,06
7	0,93	100	0,00

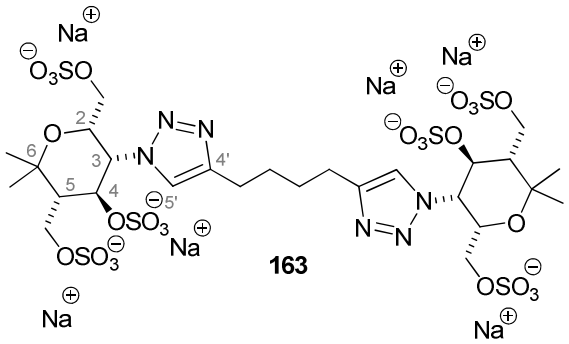




161

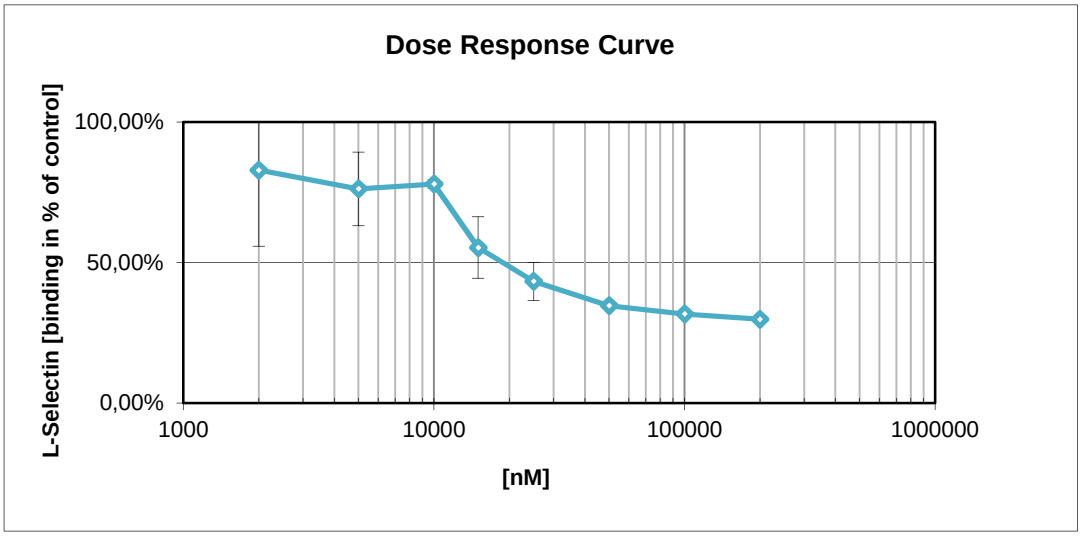
Baseline Corrected Values		Inhibitor Concentration [nM]	Error/ Diviation
100%			
2	0,71	500000	0,01
3	0,71	100000	0,17
4	0,51	10000	0,11
5	0,42	5000	0,00
6	0,32	2000	0,09
7	0,35	1000	0,02
8	0,97	100	0,17
11	1,07	10	0,15

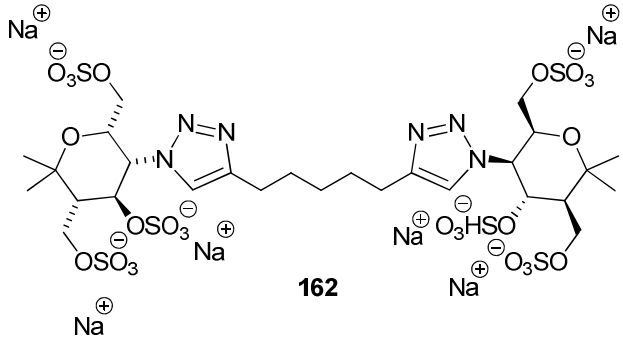




163

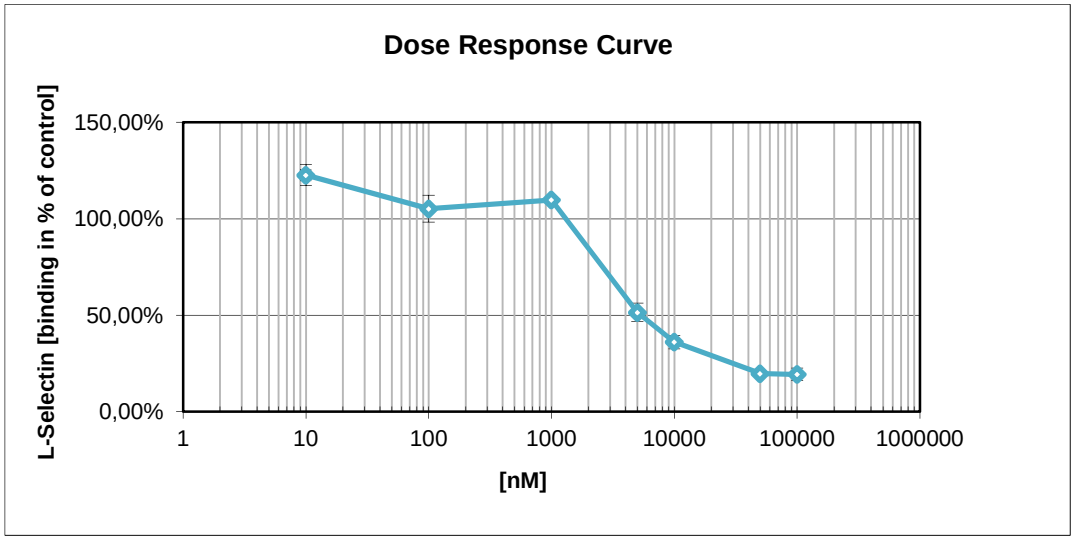
Baseline Corrected Values		Inhibitor Concentration [nM]	Error/ Diviation
100%			
2	0,30	200000	0,01
3	0,32	100000	0,01
4	0,35	50000	0,00
5	0,43	25000	0,07
6	0,55	15000	0,11
7	0,78	10000	0,02
8	0,76	5000	0,13
9	0,83	2000	0,27

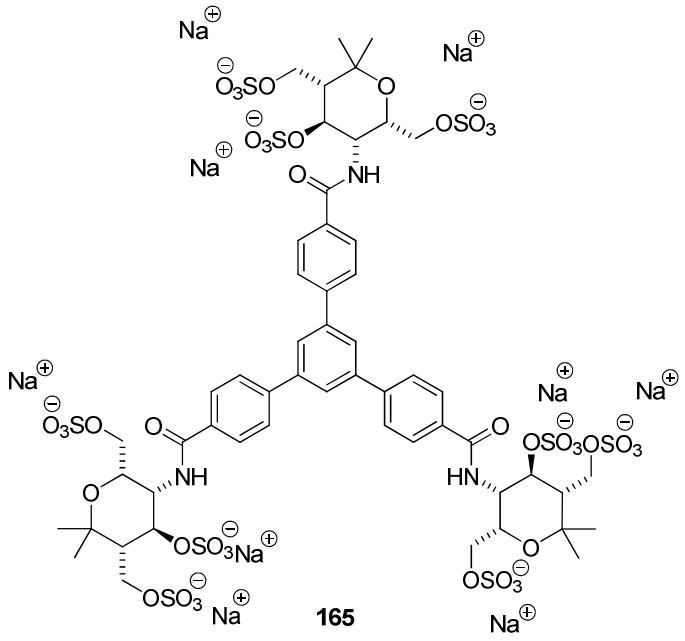




162

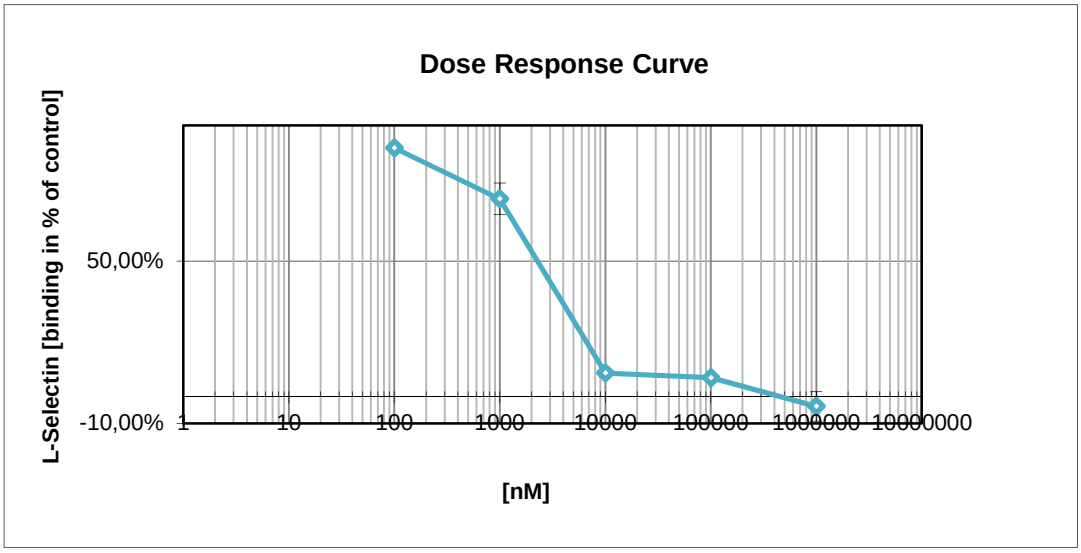
Baseline Corrected Values		Inhibitor Concentration [nM]	Error/ Diviation
100%			
2	0,19	100000	0,03
3	0,20	50000	0,00
4	0,36	10000	0,04
5	0,51	5000	0,05
6	1,10	1000	0,02
7	1,05	100	0,07
8	1,23	10	0,05

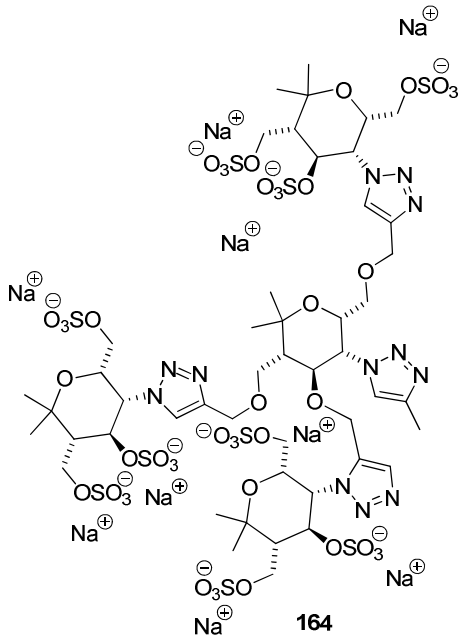




165

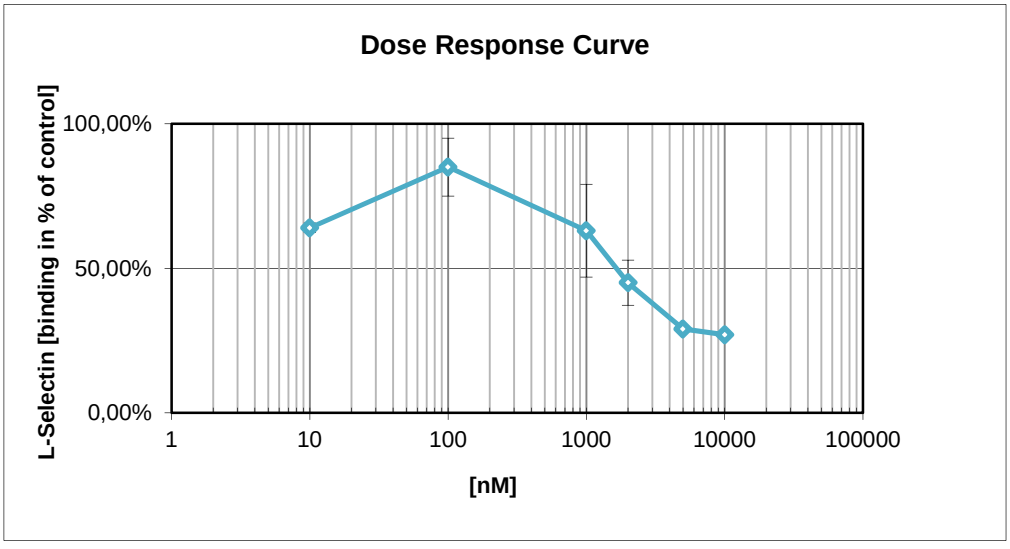
Baseline Corrected Values		Inhibitor Concentration [nM]	Error/ Diviation
100%			
2	-0,04	1000000	0,05
3	0,07	100000	0,00
4	0,09	10000	0,02
5	0,73	1000	0,06
6	0,92	100	0,00

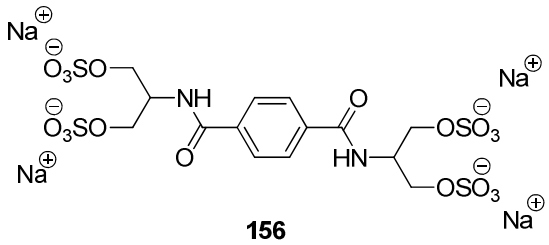




164

Baseline Corrected Values		Inhibitor Concentration [nM]	Error/ Diviation
100%			
2	0,27	10000	0,05
3	0,29	5000	0,02
4	0,45	2000	0,10
5	0,63	1000	0,02
6	0,85	100	0,16
7	0,64	10	0,08





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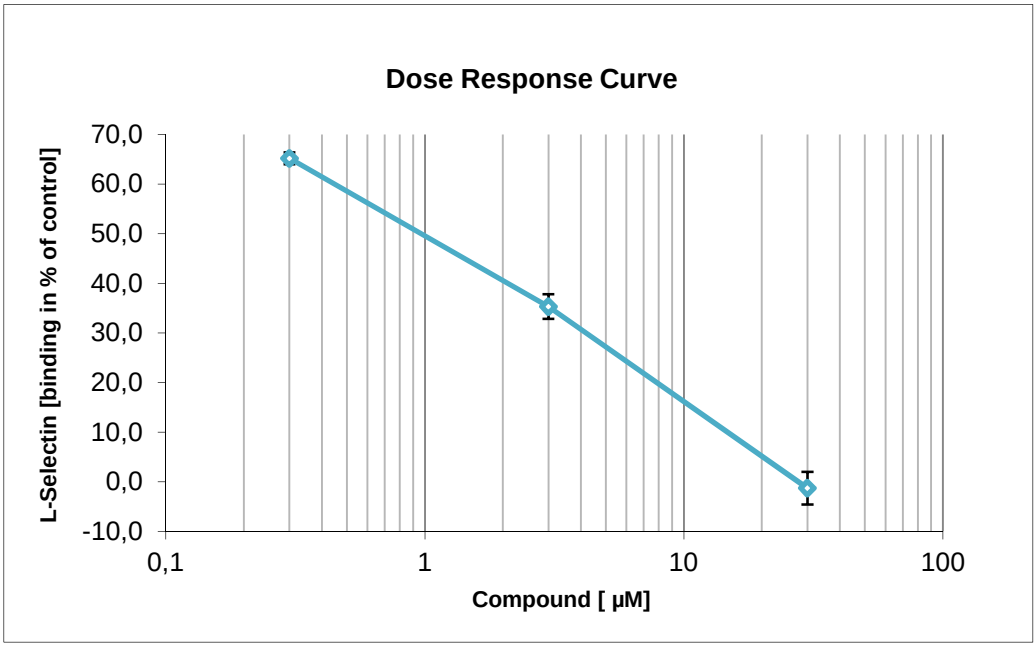
Baseline Corrected Values		Inhibitor Concentration [nM]	Error/ Diviation
100%			
2	0,01	30000	0,03
3	0,35	3000	0,02
4	0,65	300	0,01



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