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„Synthesis of Phosphocholine-substituted Carbohydrate Ligands“

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Special thanks I give to my parents who supported me throughout my studies and encouraged me to pursue my goals determinedly.

Abbreviations

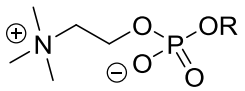
APT	Attached Proton Test
aq.	aqueous
BSA	Bovine Serum Albumin
CAM	Cerium Ammonium Molybdate
cat.	catalytic
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIPEA	*N,N*-Diisopropylethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	Dimethylformamide
ELISA	Enzyme-linked Immunosorbent Assay
GlcNAc	*N*-Acetylglucosamine
HILIC	Hydrophilic Interaction Chromatography
HMBC	Heteronuclear Multiple Bond Correlation
HPTLC	High Performance Thin Layer Chromatography
HR-MS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
LC-MS	Liquid Chromatography Mass Spectrometry
MALDI-TOF-MS	Matrix-assisted Laser Desorption / Ionization
m-CPBA	*meta*-Chloroperoxybenzoic Acid
MPLC	Medium Pressure Liquid Chromatography
MS	Molecular Sieves
PE	Phosphoethanolamine
TBAF	Tetrabutylammonium Fluoride
TBDPS	*tert*-Butyldiphenylsilyl
THF	Tetrahydrofuran
TLR	Toll-like Receptor
TMS	Trimethylsilyl
Troc	2,2,2-Trichloroethoxycarbonyl

Content

1. Introduction

Glycans as parts of cell surface and secreted glycoproteins and glycolipids are known to play an important role in the interaction between cells[1]. It has been reported that ovarian cancer cells are able to escape attacks of the immune system using at least four different glycoprotein-dependent molecular mechanisms[2]. Worm parasites manage to modulate the immune system of their hosts in a way that allows them to survive in these organisms by suppressing inflammatory responses to the parasite infection. The contribution of glycans to this effect is subject of current research and their potential use as drugs against autoimmune diseases such as multiple sclerosis and arthritis is discussed[3]. This gives an idea of their importance in general.

Glycoconjugate structures of a broad variety of parasites such as protozoa[4], bacteria[5] and nematodes[6-7] have been elucidated. Special attention is paid to glycan structures bearing phosphocholine (PC) substituents that are suspected to be of particular importance for these organisms concerning the survival in their hosts[8]. PC substituents have been found in the lipopolysaccharide of Haemophilus influenzae[9], in a glycopeptide from Penicillium charlesii[10], in *N*-glycans from Ascaris suum[6-7] and other parasitic organisms[11]. PC substitution is not restricted to a particular position of a particular monosaccharide, but in nematodes PC substitution on position 6 of *N*-acetylglucosamine (GlcNAc) that is β-(1→3) connected to mannose has been found to be a common epitope[8].



R = Carbohydrate moiety

Scheme 1: PC substitution on carbohydrates

The interaction of several host proteins with PC-substituted glycans has been studied: Streptococcus pneumoniae and Haemophilus influenzae use their PC-substituted glycans to ensure adherence to nasopharynx cells by interaction with

the platelet-activating factor receptor (a G-protein-coupled receptor). C-reactive protein is a part of the immune system and binds to PC as well as to its non-methylated counterpart phosphoethanolamine (PE), although the binding constant is dependent on the chemical environment of the PC or PE moiety. Toll-like receptors (TLR), components of the innate immune system, are one target protein family of ES-62, a secreted PC-substituted glycoprotein from Acanthocheilonema viteae. Interaction of this protein with a TLR results in reduced mast cell degranulation. Furthermore the ability of murine myeloma proteins to bind to PC is used widely to identify PC-containing compounds in immune assays[1, 8, 11-13].

Isolation of these zwitterionic compounds out of biological sources requires sophisticated sequences of homogenisation, extraction, multiple steps of enzymatic and/or chemical digestion and fractionation by chromatographic methods (size exclusion, normal phase, reversed phase, ion exchange and others). The so obtained glycans are then analyzed mostly by mass spectrometry, often as mixtures[7, 14]. Whereas the use of mixtures for mass spectrometry analysis is reasonable, other analytic techniques (e. g. NMR spectroscopy and protein binding studies) require pure substances for satisfactory results. The desired level of purity can be achieved by chemical synthesis: Although the establishment of a proper synthetic route can be challenging, issues concerning the separation and purification of highly polar compounds (such as zwitterionic phosphodiesters) that inevitably appear when using biological sources can be circumvented largely if a suitable protecting group strategy is applied. In case that larger amounts of the product are needed, upscaling is possible in principle, regardless of the natural abundance of the compound in biological samples.

Interactions of synthetic carbohydrates with proteins can be examined via glycoarrays that allow covalent or non-covalent immobilization of carbohydrates on surfaces. Covalent immobilization can be realized by attachment of a linker (spacer) bearing a suitable functional group (e. g. amines, azides, alcohols) to the carbohydrate in the course of the synthesis and subsequent coupling to the functionalized surface. Frequently used techniques for glycoarray readout are based on fluorescence detection and ELISA[15]. The biochemical principle of the

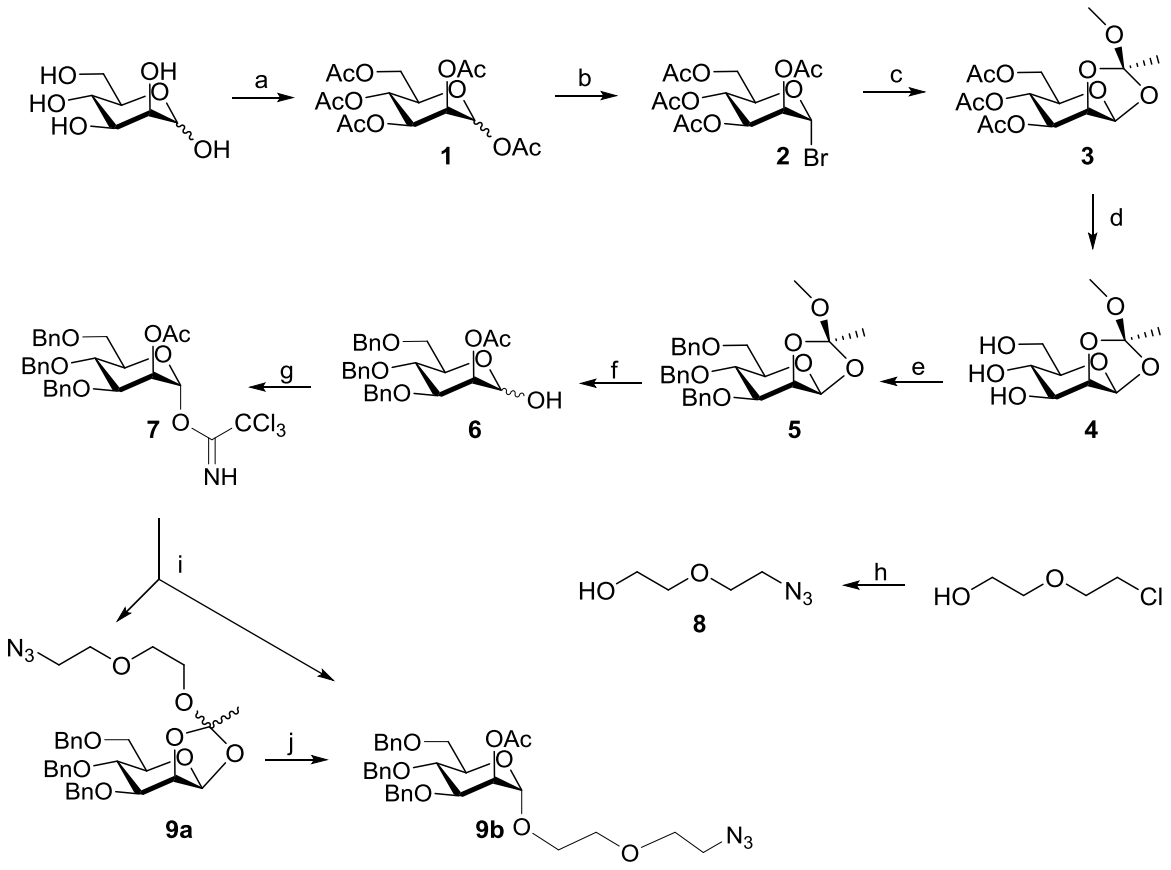
latter is also applied in the Western Blot[16] method that includes separation of proteins (via size/charge) prior to detection. To use this technique for studies of carbohydrate-protein interaction, neoglycoconjugates must be prepared by coupling the carbohydrate to a carrier protein like bovine serum albumin (BSA)[17].

2. Aim

The aim of this thesis was to synthesize a PC-substituted disaccharide that resembles glycan parts of parasitic nematodes. Starting from commercially available D-mannose a synthetic route leading to 2-acetamido-2-deoxy-6-*O*-phosphocholine-β-D-glucopyranosyl-(1→2)-[2-(2-aminoethoxy)ethyl] α-D-mannopyranoside should be established, including two glycosylation reactions and attachment of a PC moiety on position 6 of the GlcNAc part. Special attention should be paid to the reaction sequence that led to formation of the phosphodiester, as the zwitterionic product was expected to give rise to purification problems due to its highly polar character. The mannose part of the target disaccharide was equipped with a linker bearing a functional group that allowed preparation of neoglycoconjugates (for immunoblotting) as well as immobilization on functionalized surfaces for glycoarray-based protein binding studies. To show the impact of PC substitution on carbohydrate-protein interaction in these biological tests the non-PC-substituted counterparts should also be synthesized and serve as reference compounds.

3. Results and Discussion

3.1. Synthetic Route



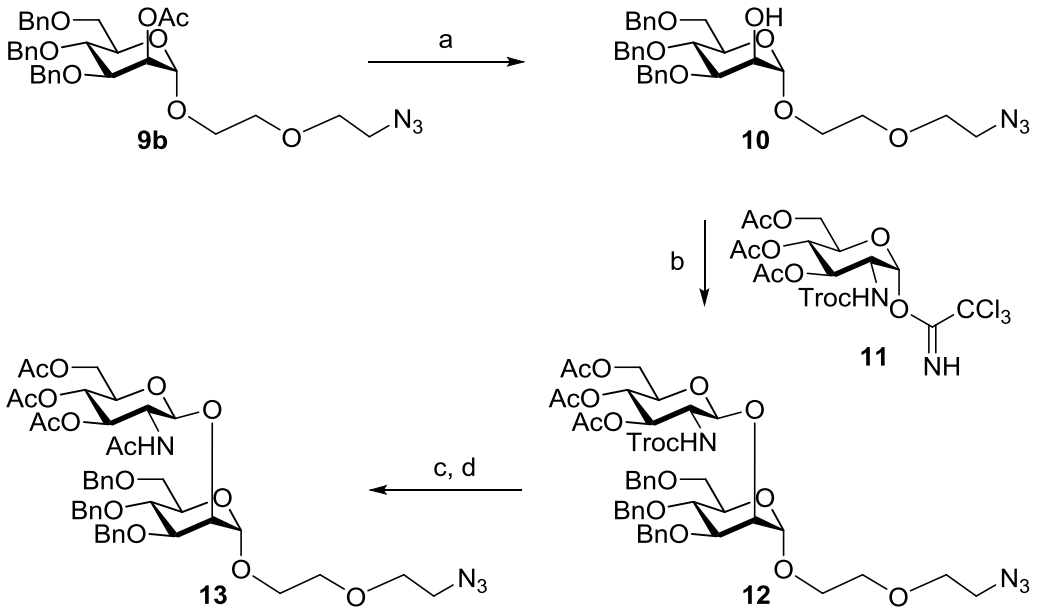
Scheme 2: a) Ac_2O, I_2, 140 min; b) HBr, AcOH, 24 h; c) MeOH, Bu_4NBr, collidine, DCM, reflux, 4.5 h, 36% (three steps from D-mannose); d) K_2CO_3, MeOH, 16 h; e) NaH, BnBr, DMF, 16 h; f) AcOH, H_2O, 2 h, 90% (three steps from **3**); g) Cl_3CCN, DBU, DCM, 40 min, 97%; h) NaN_3, DMF, 80 °C, 8 h, 66%; i) MS 4 Å, **8**, TMSOTf, DCM, 30 min; j) MS 4 Å, TMSOTf, DCM, 25 min, 67% (two steps from **7**)

The synthesis of the phosphodiester-substituted disaccharide started with commercially available D-mannose which was converted into pentaacetate **1**[18]. For the first glycosylation reaction a donor with orthogonal protecting groups on position 2 and positions 3, 4 and 6 was necessary. This pattern was achieved via orthoester **5**[19].

Therefore pentaacetate **1** was treated with hydrogen bromide to give bromide **2** that was converted into orthoester **3** by treatment with methanol and base.

Recrystallization gave **3** in 36% yield over three steps from D-mannose. The acetyl groups on position 3, 4 and 6 were then cleaved off by treatment with potassium carbonate. Triol **4** was converted into the 3,4,6-tribenzyl ether **5** with sodium hydride and benzyl bromide. Orthoester **5** could be opened conveniently with acetic acid (60% in water)[20] to give the free hydroxyl group on the anomeric center in compound **6** (90% yield over three steps from **3**, anomeric ratio 7:1). The donor for the first glycosylation was prepared from this anomeric alcohol by treatment with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the desired trichloroacetimidate **7**[21]. Acceptor **8** (holding the azide group that was crucial for the biological tests) was obtained by treatment of 2-(2-chloroethoxy)ethanol with sodium azide[22].

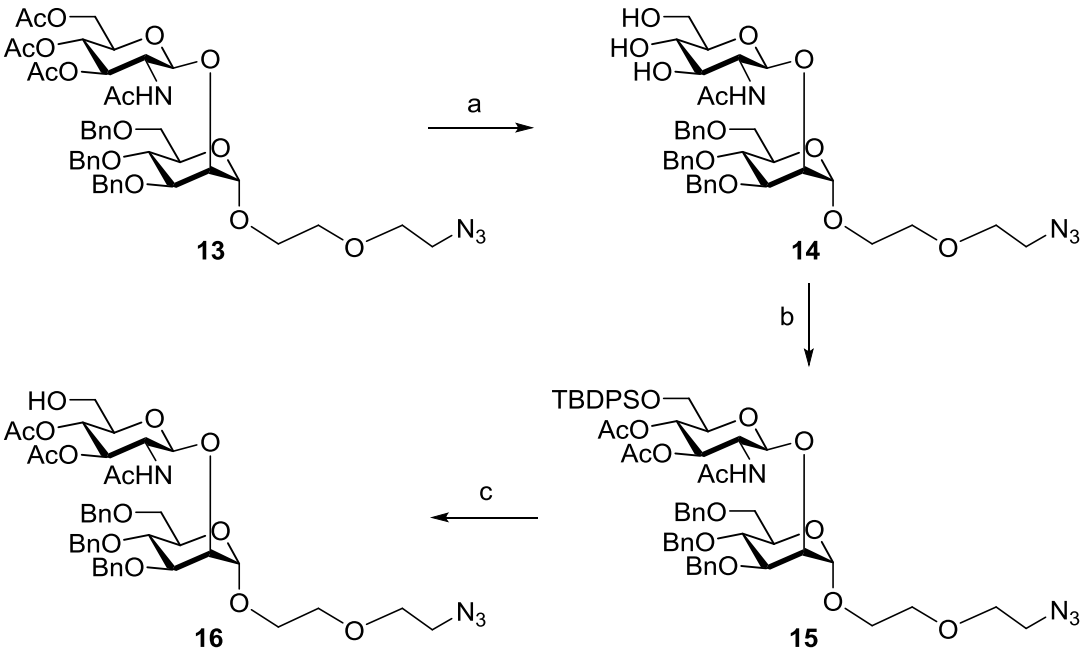
In the following glycosylation[21] using trimethylsilyl triflate as promotor a mixture of the desired glycoside **9b** and the corresponding orthoester **9a** was obtained. The mixture was then treated again with trimethylsilyl triflate to induce rearrangement which resulted in pure glycoside **9b** (67% yield in total for glycosylation and rearrangement).



Scheme 3: a) K_2CO_3, MeOH, 17 h, 98%; b) MS 4 Å, TMSOTf, 45 min, 38% (calculated); c) LiOH, H_2O, THF, reflux, 5.5 h; d) Ac_2O, DMAP, pyridine, 1 d, 75%

Deacetylation with potassium carbonate gave **10**, the acceptor for the next glycosylation. The trichloroacetimidate donor **11** for this reaction was provided by the archive[23]. The following glycosylation reaction was carried out in a similar manner as the first one but gave the desired disaccharide **12** in inferior (38%; calculated) yield. To avoid the use of HPLC at this stage it was accepted that the product contained a large amount of unreacted acceptor because it could be separated easily by column chromatography after the following steps.

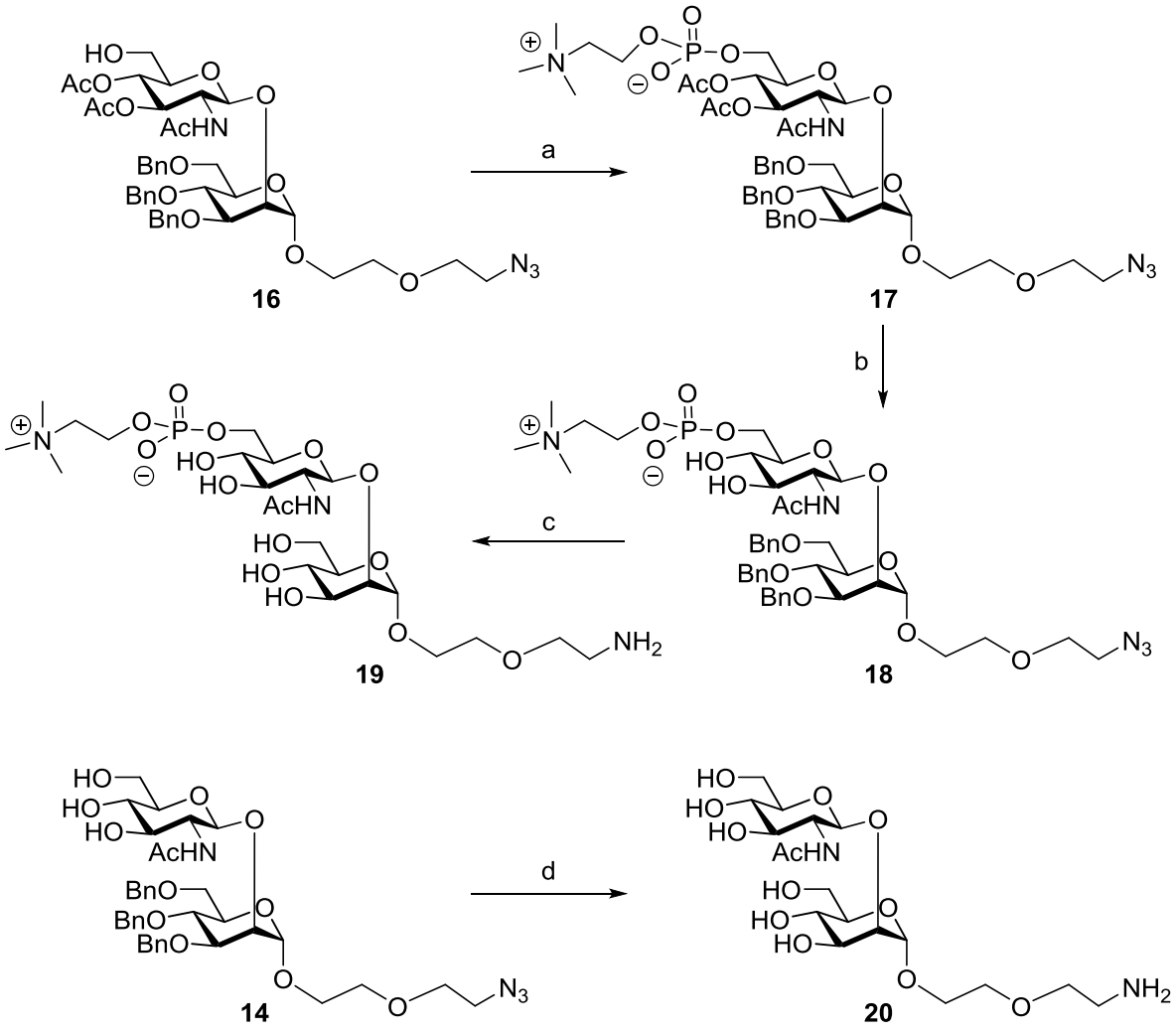
To exchange the 2,2,2-trichloroethoxycarbonyl (troc) group by an acetyl group, disaccharide **12** was treated with aqueous lithium hydroxide solution[24] followed by 4-(dimethylamino)pyridine (DMAP) catalyzed acetylation[25] of the free amino and all free hydroxyl groups to give the *N*-acetylglucosamine derivative **13**. At this stage the disaccharide could be purified by column chromatography and the unreacted acceptor could be recycled in its acetylated form (**9b**).



Scheme 4: a) K_2CO_3, MeOH, 17 h, 85%; b) TBDPSCl, DIPEA, 3 d, then Ac_2O, DMAP, pyridine, 2 h, 80%; c) TBAF, AcOH, DMF, 1 d, 62%

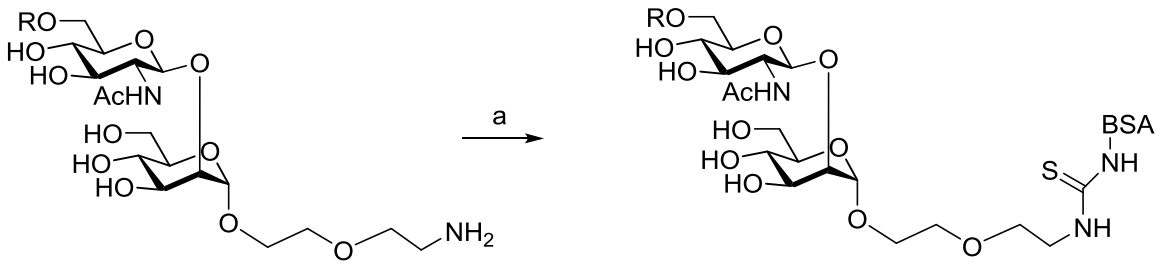
For the preparation of the phosphodiester a protected disaccharide with a free hydroxyl group on position 6' was necessary. Selective deprotection did not seem to be appropriate for the substrate and therefore regioselectivity was introduced

by a sterically demanding silyl protecting group: *O*-Acetyl groups were cleaved off from **13** as mentioned before to give **14** that was not only the substrate for the protection of the primary alcohol but was also used for the preparation of the reference compound for the biological tests. The primary alcohol function on **14** was then selectively protected as *tert*-butyldiphenylsilyl (TBDPS) ether and the two remaining hydroxyl groups were protected with acetyl groups directly afterwards to give **15** in 80% yield. The desired protected disaccharide with a free primary alcohol function (**16**) was obtained by treatment of **15** with tetrabutylammonium fluoride (TBAF).



Scheme 5: a) 2-Cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite, 1H-tetrazole, 30 min, then 1H-tetrazole, choline tosylate, 1 h, then MeOH, *m*-CPBA, 40 min, then Et_3N, 3 h, 44%; b) K_2CO_3, MeOH, 4 h, 94%; c) H_2 (1 bar), Pd/C (10%), THF/H_2O (1:1), AcOH, 2 d, 64%; d) H_2 (50 bar), Pd/C (10%), THF/H_2O (1:1), AcOH, H-Cube®, two runs, 59%

To introduce the PC moiety a method using 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite[26] was applied successfully. It included intermediary formation of a carbohydrate phosphormonoamidite that was directly converted into the corresponding phosphite with choline tosylate. Oxidation with *meta*-chloroperoxybenzoic acid (*m*-CPBA) followed by cleavage of the protecting group on phosphor gave the desired phosphodiester **17** in 44% yield. The acetyl groups were cleaved off as mentioned before to give **18** which was the starting material for the final deprotection and azide reduction with hydrogen over palladium/charcoal that gave the final phosphodiester **19**. This step was also carried out with disaccharide **14** and yielded the reference compound **20**.



19 R = Phosphocholine
20 R = H

21 R = Phosphocholine
22 R = H

Scheme 6: a) $CSCl_2$, $NaHCO_3$, $H_2O/CHCl_3$, 100-180 min, then BSA, 3-4 d

Small amounts of the unprotected amines **19** and **20** were converted into the corresponding isothiocyanates that were coupled to bovine serum albumin (BSA) through ε-nitrogens of its lysine residues[27] to give the BSA conjugates **21** and **22**. The ligand to protein ratio (mol/mol) was determined by MALDI-TOF-MS to be 3.4 and 7.2 respectively.

The individual steps are discussed in more detail in the following chapters.

3.2. Preparation of the donor and acceptor for the first glycosylation reaction

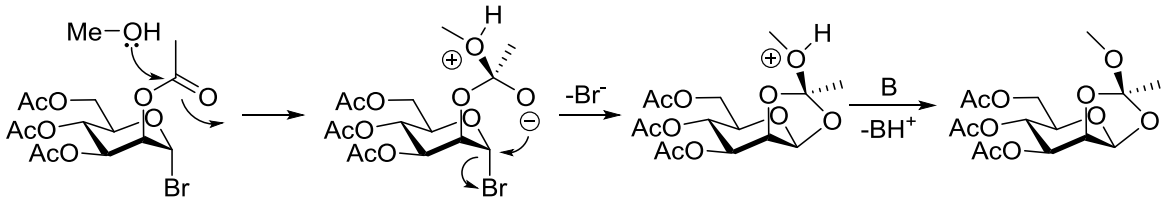
The first subgoal of this work was the preparation of a mannosyl donor that should be coupled to a linker with an azide group. In the subsequent glycosylation the mannosyl moiety should act as acceptor. This reaction demanded a protecting group pattern that allowed distinction between position 2 and 3, 4, 6 on the mannose part. This regioselectivity was defined early in the synthesis with a fully protected orthoester[19].

Preparation of the mentioned orthoester started with D-mannose that needed to be converted into the corresponding pentaacetate **1**. A widely used procedure for acylations includes the use of pyridine and DMAP[25]. An alternative is the use of a Lewis acid catalyst like iodine that allows for fast acylations (especially with mannose) in combination with acetic anhydride as acyl donor without need for an extra solvent[18]. Although the reaction itself is fast, it happened that the reaction was not initiated at room temperature during the first approach. Initiation is easily observable due to the significant color change of the mixture from colorless to dark brown. The pentaacetate was obtained in quantitative yield as anomeric mixture (ratio 5:3).

The pentaacetate was then converted into anomeric bromide **2** by treatment with hydrogen bromide in acetic acid. The reaction conditions favored formation of the thermodynamically more stable product with an axially attached bromine (α anomer). This is contrary to general steric considerations on six membered rings, where equatorial substitution is usually preferred, especially for large substituents. The preference of electron-withdrawing substituents such as halides for the axial position is caused by the ring oxygen and is called anomeric effect[28]. The α anomer was obtained exclusively and was used for the next step without further purification.

Formation of orthoester **3** was achieved by addition of methanol to **2** under elimination of hydrogen bromide (trapped by collidine)[29]. At this stage the product

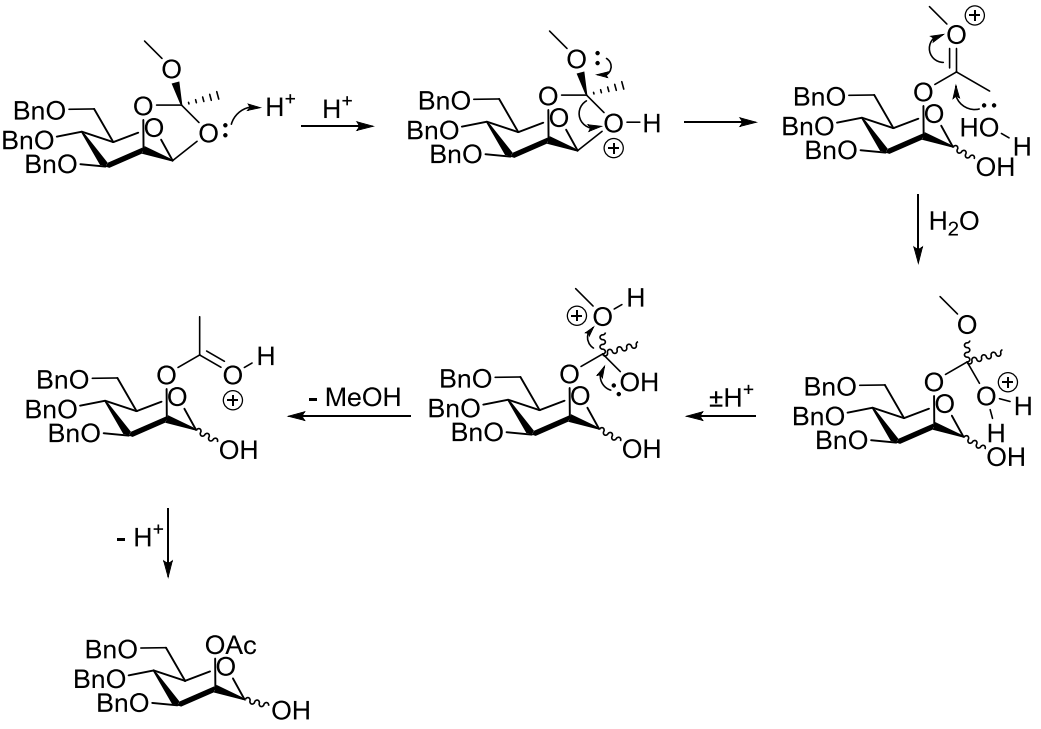
was recrystallized (first purification step in the synthesis) and obtained in 36% yield over three steps from D-mannose. The diastereomeric ratio (exo and endo configuration is possible) was determined by NMR to be 95:5. According to previously published literature the major isomer was identified as the exo isomer[19, 30]. The proposed mechanism for the orthoester formation is shown in Scheme 7.



Scheme 7: Orthoester formation

The orthoester is stable to bases and therefore cleavage of the acetate groups on position 3, 4 and 6 was possible under modified Zemplén conditions (potassium carbonate in methanol) to give triol **4**. The following benzylation was carried out with benzyl bromide and sodium hydride and yielded the fully protected orthoester **5**.

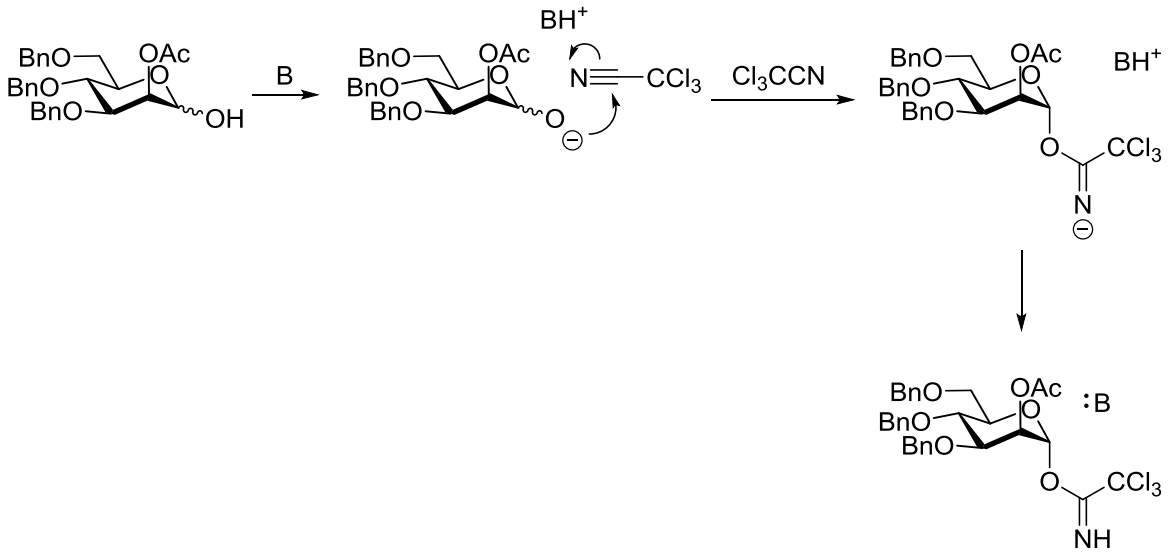
For the preparation of the mannosyl donor an anomeric alcohol was necessary. A common synthetic method for preparation of these compounds uses hydrazine acetate that cleaves acetate groups on C-1 selectively[31]. Using this approach the anomeric alcohol could be achieved in three steps (opening of the orthoester, acetylation and selective deprotection[21]). A more convenient route is based on selective opening of the orthoester which spared two steps and avoided the use of toxic reagents. The selective opening was carried out in aqueous acetic acid (60%) and the anomeric alcohol **6** (anomeric ratio 7:1) was obtained in 90% yield over three steps from **3**[20]. The proposed mechanism is shown in Scheme 8.



Scheme 8: Orthoester opening

The next task was to prepare the anomeric alcohol for connection to a linker through a glycosidic bond. Several approaches to synthetic glycosidic bond formation have been reported, most of which rely on activation of a glycosyl moiety that is coupled to a nucleophilic “acceptor” (accepting the glycosyl moiety) in a separate reaction. The activation consists in the introduction of a good leaving group, such as halides (Koenigs-Knorr method)[32], phosphites[33], thioethers[34] and trichloroacetimidates[35]. The product of this activation is called glycosyl donor.

The anomeric alcohol was converted into the corresponding trichloroacetimidate using the standard procedure[21] with trichloroacetonitrile and DBU. Donor **7** was obtained with high (95:5) anomeric selectivity in 97% yield. The proposed mechanism is shown in Scheme 9.



Scheme 9: Trichloroacetimidate donor formation

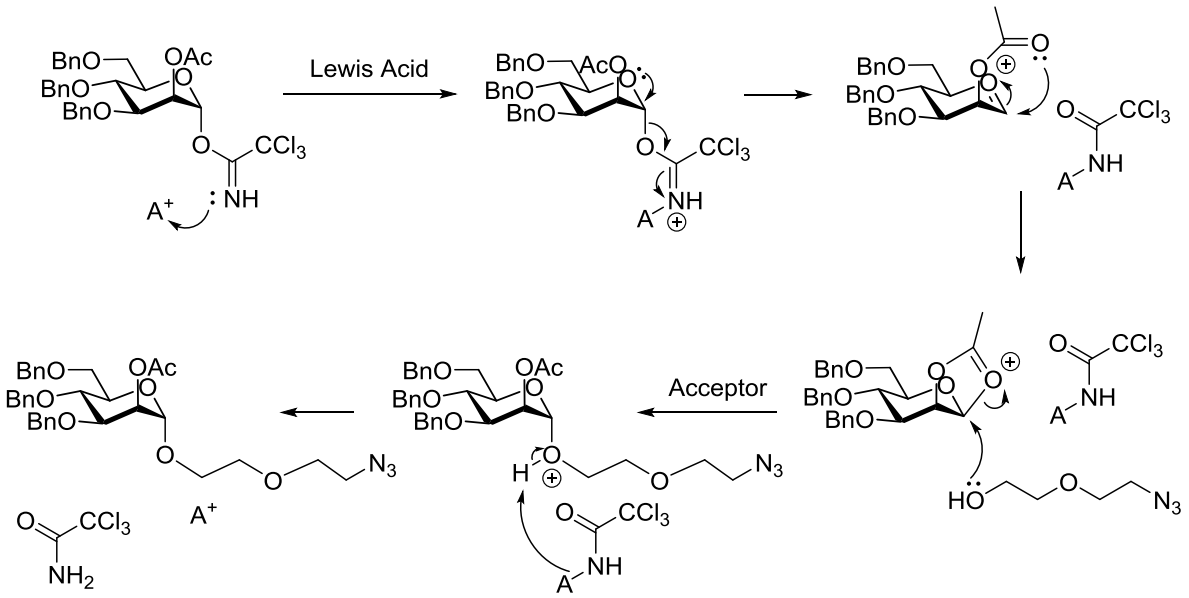
Acceptor **8** was obtained by treatment of 2-(2-chloroethoxy)ethanol with sodium azide[22] in an S_N2 type reaction in 66% yield. The azide was not isolated for safety reasons. Instead it was obtained, stored and used as a solution in DMF.

3.3. First glycosylation reaction

Trichloroacetimidates react directly with carboxylic and phosphoric acids to the corresponding esters without need for an additional catalyst. The same is true for thioacetic and hydrazoic acid (to give the corresponding *S*-glycoside and azide respectively), whereas reactions with less acidic nucleophiles such as alcohols (including carbohydrates), thiols and aromatic heterocycles require an acid catalyst ("promoter"; boron trifluoride diethyl etherate and trimethylsilyl triflate are widely used). The driving force of all these reactions is the irreversible formation of trichloroacetamide[35].

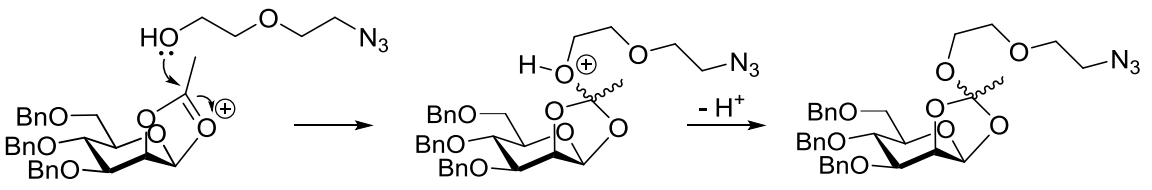
The stereochemical outcome of the glycosylation (i. e. the quantitative distribution of α and β anomer) is a result of the stereochemistry of the donor (α/β), potential neighboring group effects and reaction conditions (favoring the kinetic or thermodynamic product)[35]. In the present case the donor has α configuration and

there is a participating neighboring group on C-2. Trimethylsilyl triflate was chosen as a promoter to force the reaction to go through an S_N1 mechanism that should lead to α configuration in the product. The proposed mechanism is shown in Scheme 10.



Scheme 10: First glycosylation reaction (neighboring group effect)

It was observed that the desired α glycoside **9b** was not the major product under the chosen conditions. Instead a mixture of the glycoside and orthoester **9a** was obtained (this outcome had been reported before[36]). The proposed mechanism for the orthoester formation is shown in Scheme 11.



Scheme 11: Unexpected orthoester formation

The orthoester was identified by characteristic shifts in the NMR spectra: The carbonyl carbon in the glycoside resonated at 169.58 ppm, whereas the "orthoester carbon" resonated at 123.79 ppm in the ^{13}C spectrum ($CDCl_3$, 150

MHz). In the proton spectrum ($CDCl_3$, 600 MHz) the methyl protons of the acetate group in the glycoside resonated at 2.14 ppm, whereas the “orthoester methyl protons” did so at 1.75 ppm (which is a strong indicator for exo configuration[30]). Formation of only one diastereomer of the orthoester was observed.

The orthoester-glycoside mixture was subjected to rearrangement using trimethylsilyl triflate and pure glycoside **9b** was obtained in 67% yield (glycosylation and rearrangement combined).

3.4. Preparation of the disaccharide

Although the second glycosylation in this synthesis seemed to be similar to the first one, much more efforts had to be put into this reaction to achieve disaccharide **12** in acceptable yield. As in the first glycosylation a trichloroacetimidate donor was coupled to an alcohol (**10**; obtained by deacetylation of **9b** with potassium carbonate in methanol as before) using trimethylsilyl triflate as acid catalyst.

The troc-protected glucosamine derivative **11** seemed to be a suitable donor for the glycosylation because of the potential neighboring group participation of the troc group (although this effect can be overruled by others[37]) that should lead to good α/β selectivity in an S_N1 type reaction, favoring the desired β anomer. If the reaction followed an S_N2 type mechanism (making neighboring group participation impossible), the outcome would be a β glycoside as well due to the α configuration of the donor. Indeed α/β selectivity was not a severe issue, the anomeric ratio was determined to be 5.5:1 (β:α) for the first approach.

Although the donor was used in excess (two equivalents) in all approaches the acceptor was never consumed completely (in contrast to the donor that was always consumed completely). This circumstance gave rise to difficulties with the purification: Disaccharide and unreacted acceptor showed very similar retention behavior on silica (eluent: hexane / ethyl acetate (1:1)), which required the use of

normal phase HPLC. This approach yielded the disaccharide in 32%, which did not seem satisfactory if taking into account the required amounts of solvents and time (numerous runs would have been necessary to obtain the needed amount of the compound).

Therefore the (column) chromatography eluent was changed to toluene / ethyl acetate (3:2) for the second approach. Separation of disaccharide and unreacted acceptor was still not possible with this system, thus it was decided to acetylate (standard procedure using acetic anhydride and DMAP in pyridine[25]) the latter to decrease its polarity and therefore enable separation with column chromatography. This worked as expected, but proton NMR (Figure 1) showed the presence of an impurity of 6% (mol/mol) that was not conspicuous before:

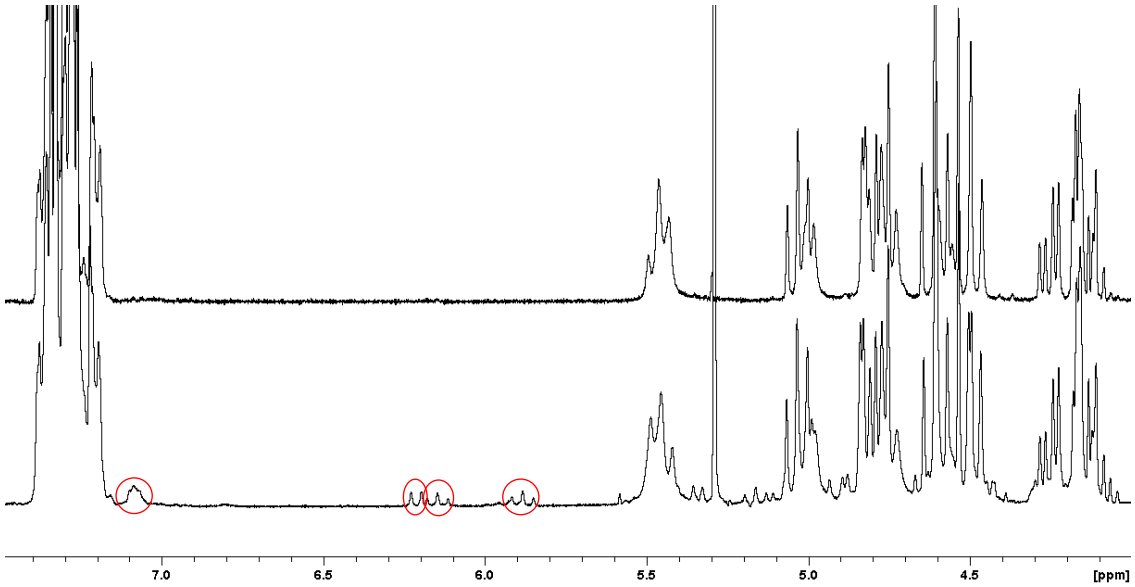


Figure 1: Unknown impurity in disaccharide **12** (bottom; pure disaccharide on top); $CDCl_3$, 300 MHz

To identify the compound the mixture was separated by HPLC as before and the minor compound was obtained in sufficient purity.

Proton NMR of the unknown compound showed that there was an “impurity in the impurity” of 20% (mol/mol) and six distinguishable acetyl groups on the compound. If hydrolyzed donor had reacted with intact donor, there were only

three distinguishable acetyl groups and no aromatic signals. In fact the spectrum showed signals in the aromatic region in the intensity of nearly 15 protons, which meant that there must be one mannosyl and two glucosyl moieties (moreover there were two distinguishable signals for troc carbamate carbons in the ^{13}C spectrum) in the compound, which was confirmed by LC-MS. The measured and calculated isotope distributions of this trisaccharide that mainly arise from the presence of six chlorine atoms are shown in Figure 2 and Figure 3 respectively.

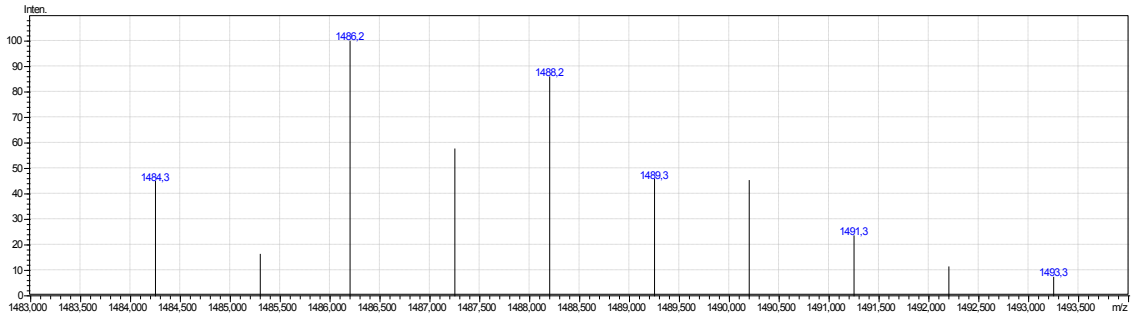


Figure 2: Measured isotope distribution of the trisaccharide side product (negative mode)

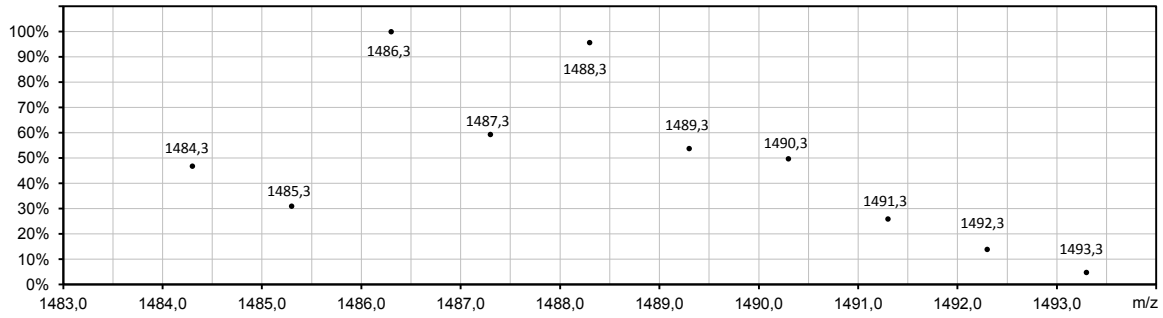
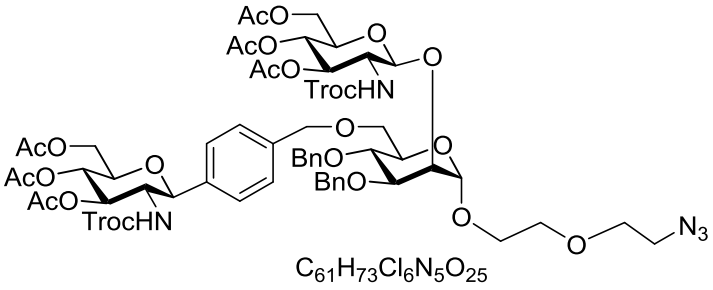


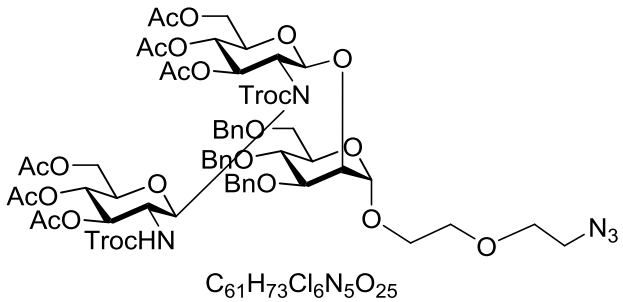
Figure 3: Calculated isotope distribution of the trisaccharide side product ([M-H]$^-$)

A trisaccharide consisting of the used components cannot be connected exclusively through *O*-glycosidic bonds, but must also have one *N*- or *C*-glycosidic bond. This was confirmed by the ^{13}C spectrum that showed only two signals around 100 ppm (which is the typical shift region of anomeric carbons of *O*-glycosides), but one additional signal at 81.24 ppm ($CDCl_3$, 150 MHz). Two proposed structures are shown in Scheme 12. They should be distinguishable from each other via proton NMR by count of the N*H* and *Ph* signals easily in principle. Unfortunately these signals overlapped and could not be assigned with

sufficient certainty (also due to the mentioned impurity). However, the shifts of the "third" anomeric carbon and proton (6.21 ppm; $CDCl_3$, 600 MHz)[38-40] as well as the presence of an HMBC cross peak (Figure 4) between the latter and one carbamate carbon are indicators for an *N*-glycosidic bond in this side product.



$C_{61}H_{73}Cl_6N_5O_{25}$
1488,97



$C_{61}H_{73}Cl_6N_5O_{25}$
1488,97

Scheme 12: Possible structures of the trisaccharide side product

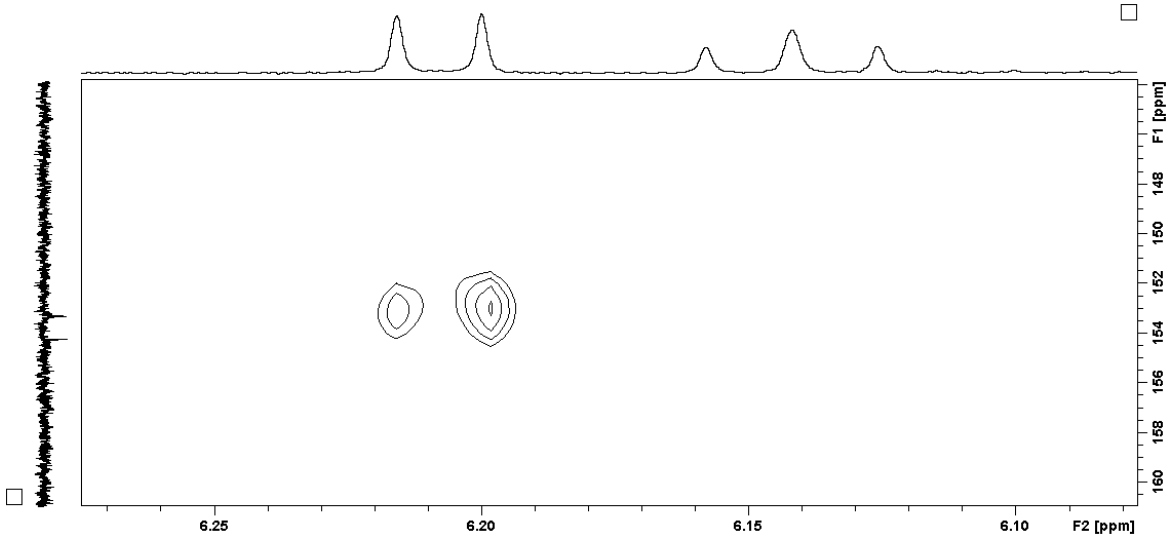


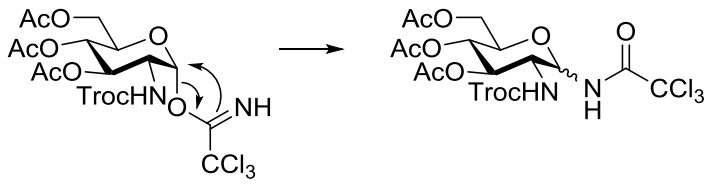
Figure 4: ^{13}C-^{1}H HMBC cross peak of one carbamate carbon to the "third" anomeric proton; $CDCl_3$, 600 MHz (^{1}H), 125 MHz (^{13}C)

Multiple attempts had been undertaken to alter the reaction conditions (temperature: -13°C to room temperature; amount of catalyst: 0.05 to 0.20 equivalents) to oppress formation of the trisaccharide and to improve the yield of the disaccharide. Mixtures of toluene and ethyl acetate as well as toluene and methanol were used as eluents for column chromatography in various mixing ratios but none of these measures led to pure disaccharide in satisfying yield. Finally the breakthrough was the use of a mixture of dichloromethane (DCM) and ethyl acetate (4:1, 1% of triethyl amine) as an eluent that enabled separation of the trisaccharide from the disaccharide using column chromatography. Separation of the unreacted acceptor was not possible with this eluent and would have required an additional column chromatography run (promising eluent: DCM / acetone (9:1)) that was not carried out due to the fact that one stage later the acetylated acceptor could be separated conveniently from the mixture. The yield of the desired disaccharide **12** was determined to be 38% by NMR (without taking into account mixed fractions that could be collected and subjected to column chromatography again).

To proceed with the synthesis the mixture of disaccharide and unreacted acceptor was treated with aqueous lithium hydroxide solution to cleave off the troc group. The deprotected intermediate was then directly acetylated with acetic anhydride and DMAP in pyridine to give **13**. At this stage the unreacted acceptor (now acetylated) could be separated by column chromatography without problems. These steps were also carried out with pure disaccharide **12** (from the archive), which yielded **13** in 75%.

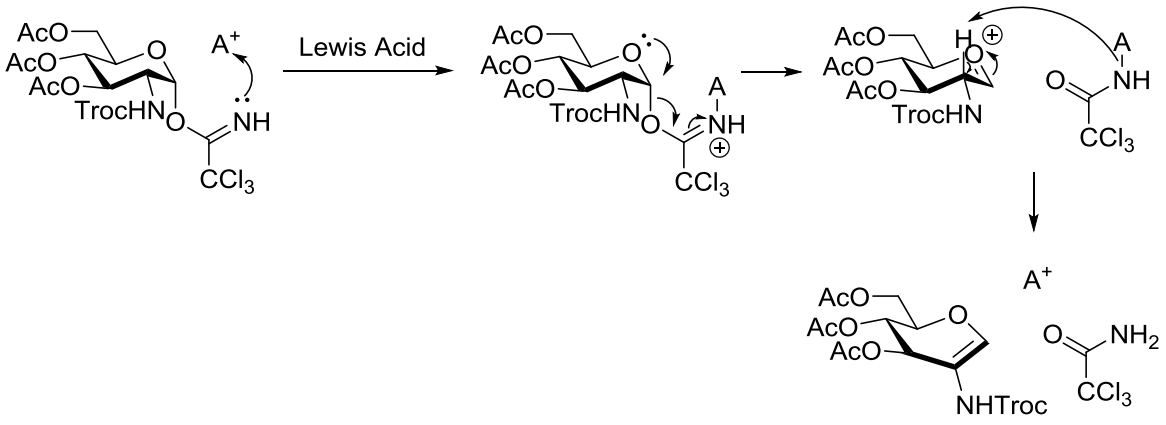
After the issues with obtaining **12** (respectively **13**) in desired purity had been solved, the rather modest yield of the glycosylation still had to be explained. As mentioned before the acceptor was never consumed completely although the donor was used in excess. Since the donor was always consumed completely there must have been a side reaction that converted the donor into a species that was no more capable of undergoing coupling with the acceptor under the chosen conditions. It is known that trichloroacetimidates can undergo rearrangement to the corresponding amides (that cannot act as donors) at very high temperatures

(Chapman rearrangement)[41-43]. The proposed mechanism is shown in Scheme 13.



Scheme 13: Chapman rearrangement

These conditions are not used for glycosylation reactions, but Lewis acids are and it has been reported that they are able to catalyze the rearrangement under typical glycosylation conditions[44], which can even be used deliberately for the synthesis of glycosyl amines[45]. Nevertheless formation of this rearrangement product was not observed. Another mechanism that could lead to significant decrease of yield is hydrolysis of the donor, although the corresponding product was identified in only one approach in small amounts. Instead a different species of decomposed donor was found: The elimination product (glycal). The proposed mechanism for its formation is shown in Scheme 14 (see experimental part for NMR data).



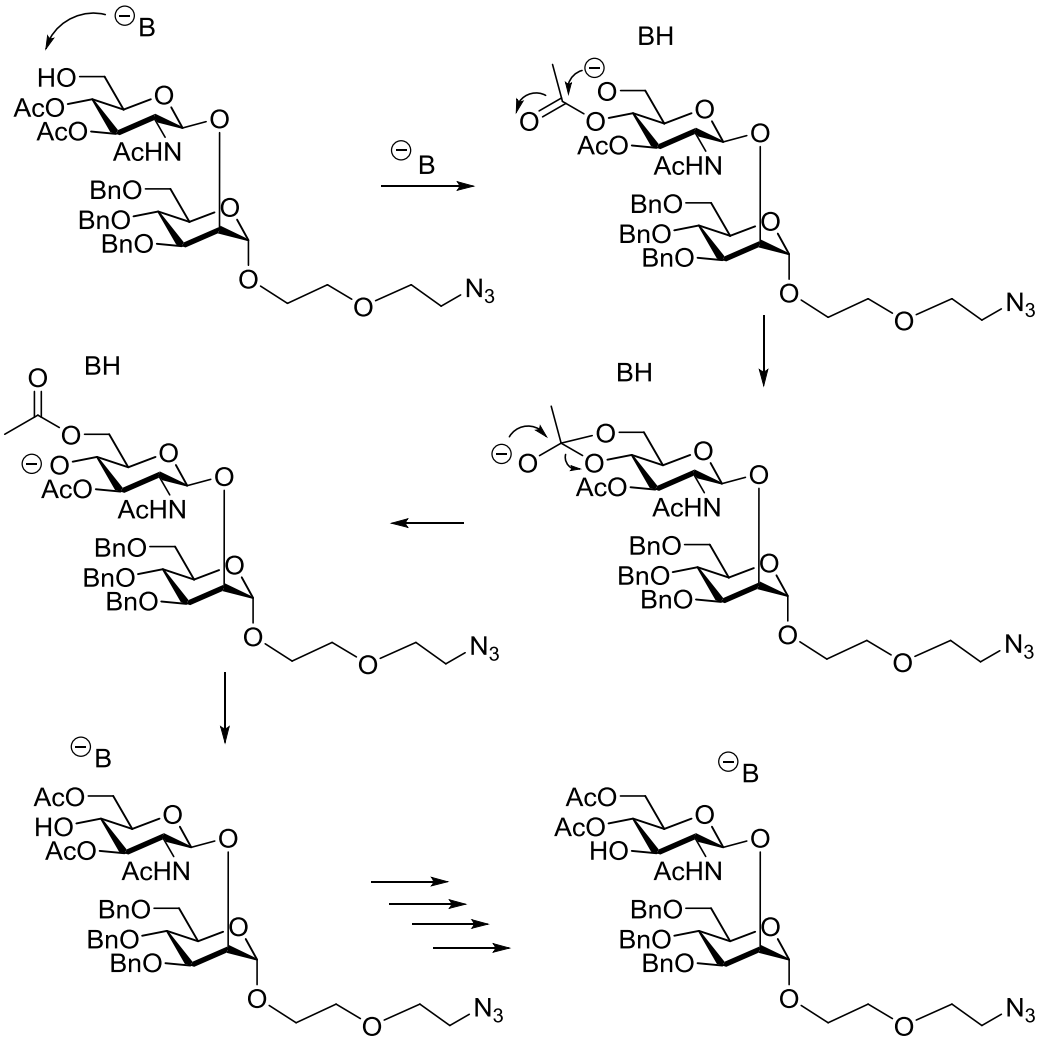
Scheme 14: Glycal formation

It can be concluded that there is room for improvement concerning the second glycosylation reaction. If economic use of the donor is not required, larger excess may lead to better yields, whereas substitution by a different type of donor (and/or alteration of the catalyst) would be the more elegant way to achieve them.

3.5. Preparation of the primary alcohol

The introduction of the phosphodiester moiety required primary alcohol **16** that was prepared from **13** in multiple steps. Deacetylation as before led to **14** which was selectively protected as TBDPS ether using the corresponding chloride[46]. This reaction was carried out several times under various conditions (alteration of solvents, temperature, amount and type of base; even other silicon based protecting groups were used, namely *tert*-butyldimethylsilyl and triisopropylsilyl ethers), but all small (up to 20 mg) scale approaches suffered from low (maximum 43%, including the subsequent acetylation) yield. This was due to low conversion of the triol and could not be overruled by addition of more reagent. At a larger scale (up to 184 mg) the yield improved to 80% (including the acetylation) using *N,N*-diisopropylethylamine (DIPEA) as a base[47]. The diol was then directly acetylated as before to **15**.

Desilylation was carried out with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF)[48]. Acetic acid was added to prevent acyl migration that is known to proceed quite fast in carbohydrates, especially under basic conditions[49]. Whereas the use of low amounts of acetic acid (i. e. equimolar in respect of TBAF) led to isolation of solely migration products (two migration steps are possible in this compound), the use of larger amounts (up to eight equivalents in respect of TBAF) led to an acceptable yield of 62% (not taking into account the mixed fractions from chromatography) for the desired primary alcohol **16**. Mixed fractions and migration products were collected and used for deacetylation to give **14**. The proposed mechanism for the acyl migration is shown in Scheme 15.



Scheme 15: Acyl migration

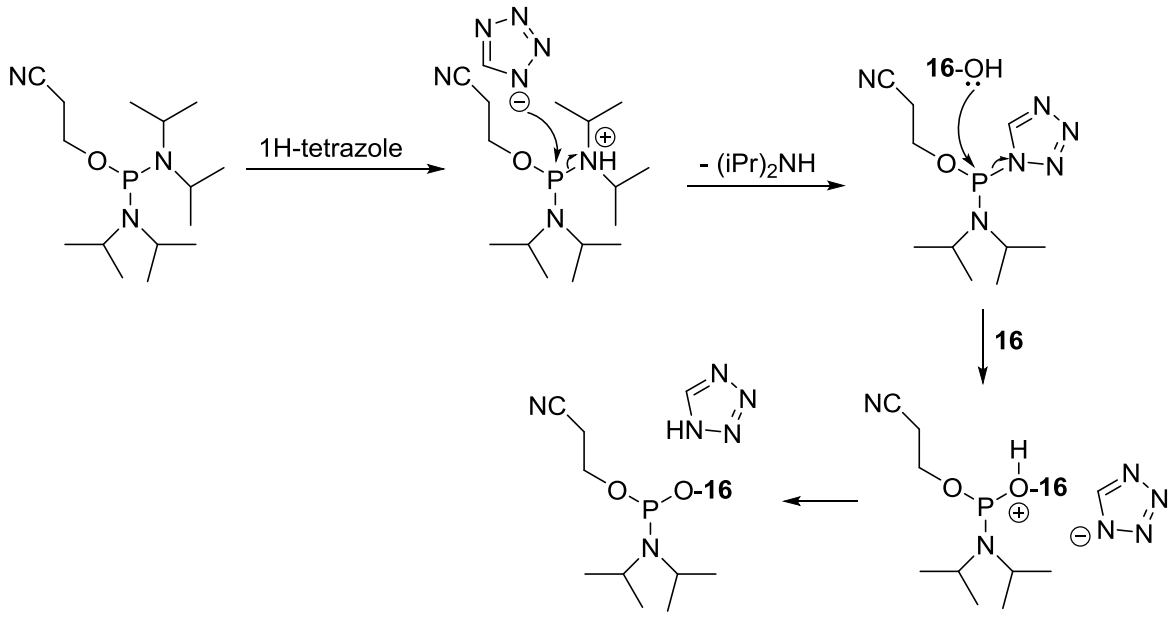
3.6. Introduction of the phosphodiester moiety

Several methods have been described for the introduction of PC moieties in carbohydrates, namely via 2-bromoethyl phosphoryl dichloride[50], 2-chloro-2-oxo-1,3,2-dioxaphospholane[51], phosphoryl chloride and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite[26].

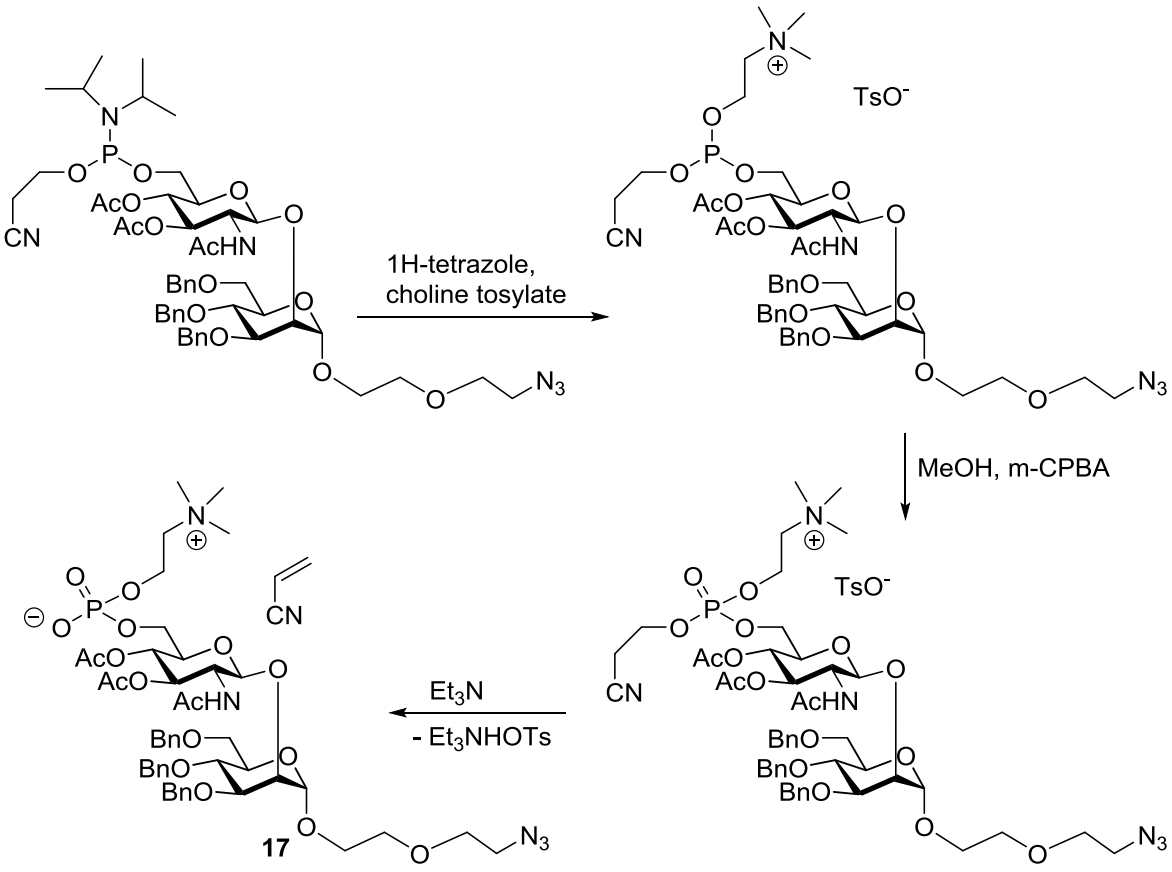
The first approach in this work used the phosphoryl chloride method that seemed tempting due to its apparently easy chemistry (substitution of the first chlorine by the carbohydrate oxygen, substitution of the second chlorine by the choline

oxygen and hydrolysis of the remaining P-Cl bond). The reaction was carried out in a small scale (2 mg of the primary alcohol) and did not yield the desired phosphodiester. Instead the starting material and the acyl migration product was obtained (due to basic conditions in the first substep). This approach was not further pursued.

The phosphorodiamidite method was applied successfully and included four steps that were carried out in a one pot sequence[26]. The individual steps and the proposed mechanism of the key step are shown in Scheme 16 and Scheme 17.



Scheme 16: Formation of the intermediary phosphoramidite



Scheme 17: Formation of phosphodiester **17**

The crude product was purified using column chromatography with a very polar eluent (containing DCM, methanol, water and ethyl acetate). Phosphodiester **17** was obtained in 44% yield. The ^{31}P-^{1}H HMBC spectrum is shown in Figure 5.

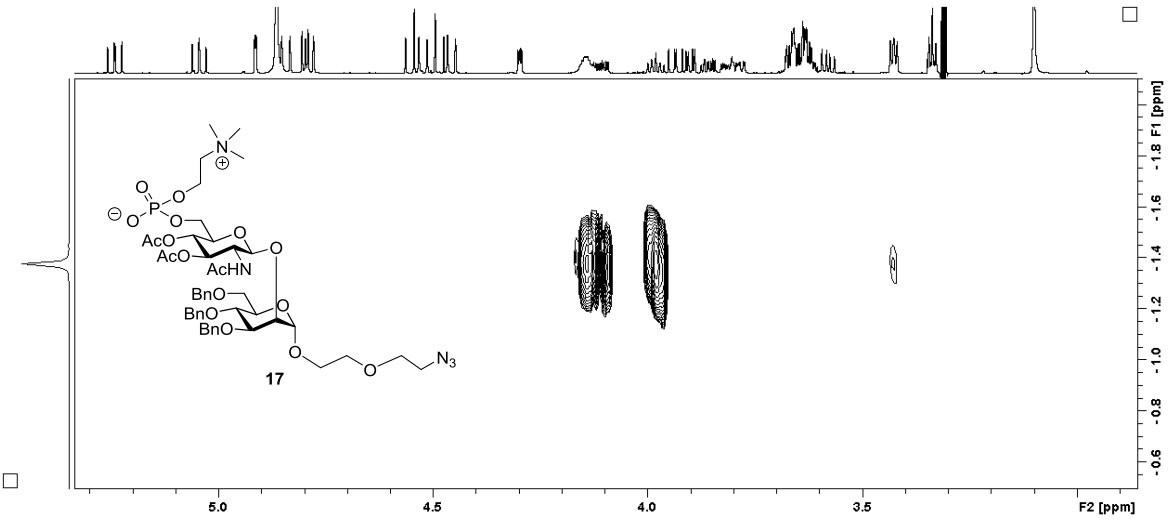


Figure 5: ^{31}P-^{1}H HMBC spectrum of phosphodiester **17**; MeOD, 600 MHz (^{1}H), 243 MHz (^{31}P)

The proton-decoupled ^{31}P-NMR spectrum showed one signal at -1.38 ppm and no signs for the presence of two (phosphorus) diastereomers were found. Long range couplings of phosphorus with the 6' protons of the disaccharide (4.11 and 3.98 ppm) and to both methylene groups of choline (4.14 and 3.43 ppm) were observed. Furthermore weak long range couplings with 5'-H and the choline methyl protons were found.

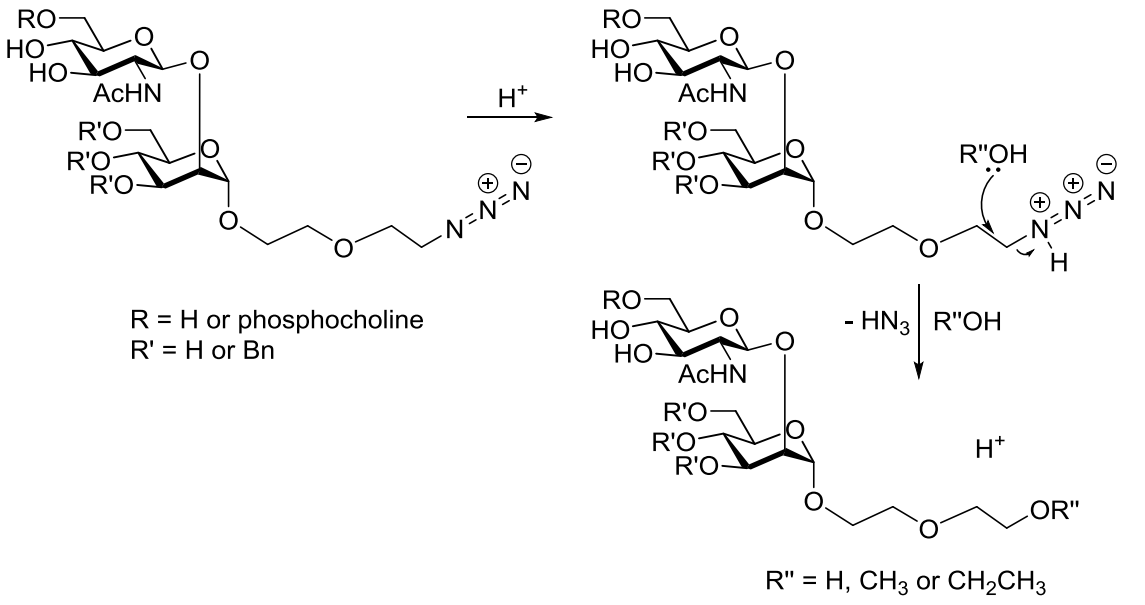
3.7. Deprotection

Cleavage of the *O*-acetyl groups was carried out as before to give **18**. The final deprotection (including the reduction of the azide) was done by hydrogenation (using 10% palladium on charcoal as a catalyst and ambient pressure of hydrogen, except for the last approach). In the first approach methanol was used as a solvent and 1% acetic acid was added to prevent catalyst poisoning by the formed amine. These conditions led to formation of mainly two products: The *m/z* value (determined by LC-MS) of the major product was determined to be 28 units larger than expected for the desired product. The HSQC spectrum of the mixture showed the presence of an additional methyl signal. This outcome of azide hydrogenations in methanol had been reported previously and was explained by reaction of the newly formed amine with formaldehyde (that was present in the solvent in traces)[52]. The imine intermediate (whose formation is catalyzed by acid) was then reduced to the methylated amine which can undergo another cycle of reductive amination. The *m/z* value of the minor product was determined to be 15 units larger than expected for the desired product.

The azide reduction was the first event that occurred in this hydrogenation (i. e. the benzyl ethers needed more time to be cleaved), therefore shortening the reaction time would not have led to success. Thus the solvent was changed to ethanol (containing 1% of acetic acid) in the second approach. The reaction was very slow and was aborted after four days. LC-MS analysis of the mixture showed intermediates (with partly cleaved benzyl ethers) with *m/z* values that were 28 units larger than desired again. Furthermore there were minor intermediates with

m/z values that were 29 units larger than desired. It was noticed that these intermediates were 14 units larger compared to those of the methanol approach and that this difference was the same as between methanol and ethanol.

To avoid the use of methanol and ethanol various other solvents were tested (not only on the phosphodiester but also on the non-phosphorylated disaccharide **14**): The use of pure acetic acid resulted in decomposition of the compound, whereas the use of water (containing 1% of acetic acid) led to an extremely slow reaction. A mixture of water and THF (1:1, 1% of acetic acid) finally led to formation of the desired product. Additionally the crude mixture contained a side product that was one unit larger than the desired product. At this stage it was obvious that solvent molecules must be directly involved in its formation because its mass was dependent on the applied solvent (water, methanol and ethanol caused mass increases of 1, 15 and 29 units). The observed *m/z* values could be explained by nucleophilic substitution of the (protonated) azide by water, methanol or ethanol, resulting in the corresponding primary alcohol, methyl ether or ethyl ether respectively. Nevertheless the obtained amounts of these side products were very small, too small to be recognized in the NMR spectra (which could have given the final evidence for the structures). The proposed mechanism of their formation is shown in Scheme 18.



Scheme 18: Azide substitution

To separate these compounds from the desired amine the crude mixture was purified by adsorption/desorption on an ion exchange resin which gave amine **19** in 64% yield. Proton and carbon (APT) NMR spectra are shown in Figure 6 and Figure 7 (see experimental part for signal assignment).

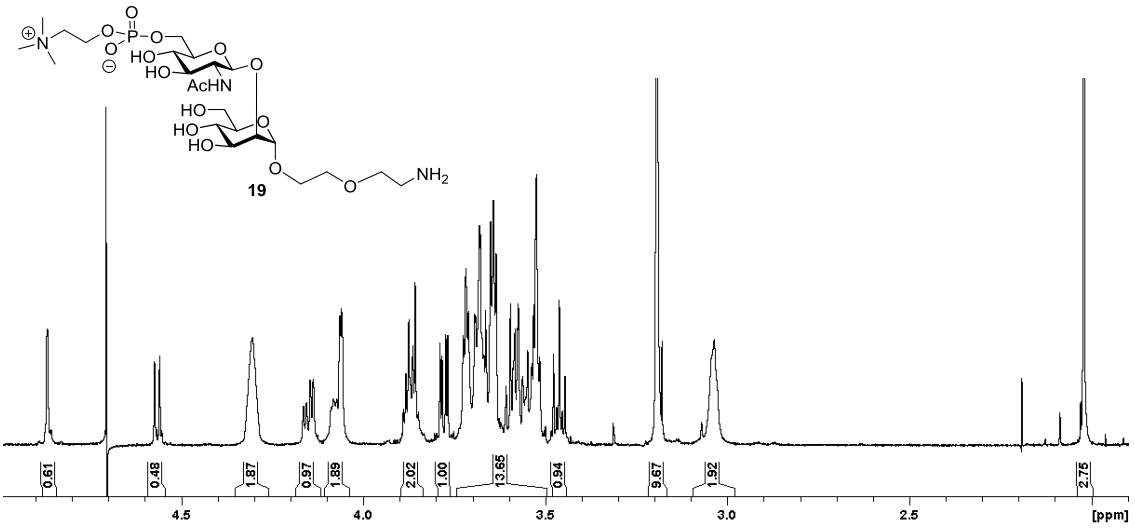


Figure 6: Final phosphodiester **19**; D_2O, 600 MHz

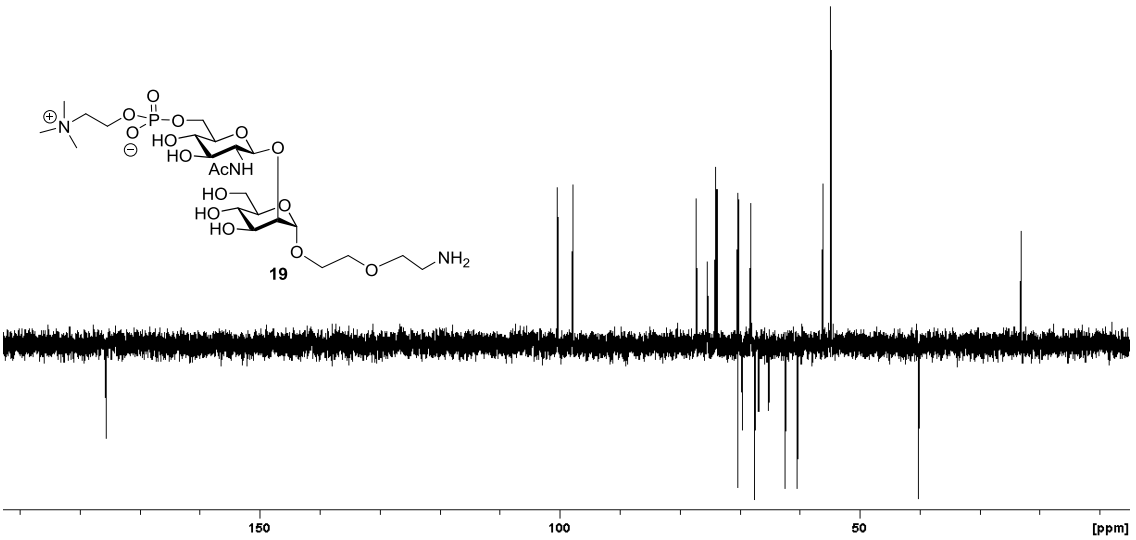


Figure 7: Final phosphodiester **19**; D_2O, 150 MHz

One batch of the amine contained a significant amount of another side product with an *m/z* value that was 72 (the mass of THF) units too large. It was not separated by the ion exchange process and was clearly visible in the proton NMR spectrum and in the LC-MS chromatogram (Figure 8 and Figure 9 respectively).

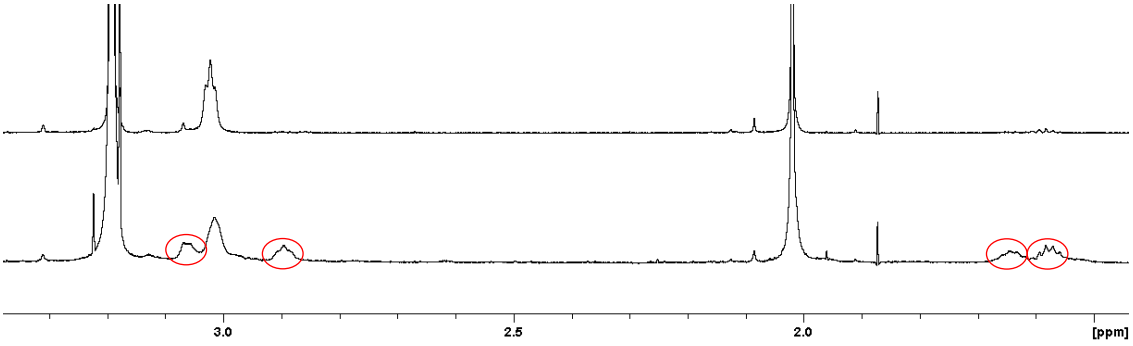


Figure 8: Side product in amine **19** (bottom), pure amine (top); D_2O, 600 MHz

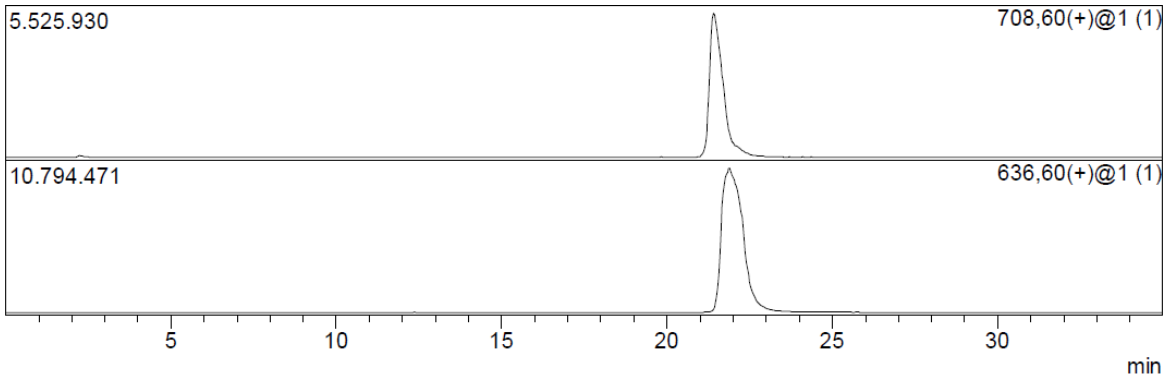
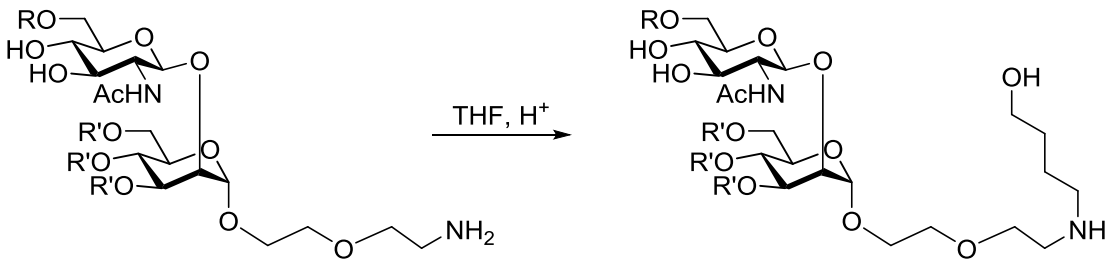


Figure 9: Extracted ion chromatograms of a mixture of the desired primary amine (bottom) and the side product with Δ(*m/z*)=72 (top)

The side product was identified as the reaction product of the amine with protonated THF, which explained the additional (methylene) signals. The proposed reaction is shown in Scheme 19.



R = H or phosphocholine
R' = H or Bn

Scheme 19: Reaction of the amine with THF under acidic conditions

To avoid this side reaction the amount of acetic acid was lowered to 0.1% in one batch. This resulted in a very slow reaction and was therefore not further pursued. Instead the reaction was carried out in THF and water (1:1, 1% acetic acid) again and the hydrogen pressure was increased to 50 bar using an H-Cube®. This fast method yielded pure reference compound **20** in 59% (loss of product due to the ion exchange process that is actually not obligatory when using the H-Cube®).

3.8. Conjugation to BSA

The unprotected amine **19** was converted into the corresponding isothiocyanate using thiophosgene in a biphasic system. The isothiocyanate was then directly coupled to BSA[27]. Unreacted small molecules were separated by dialysis and conjugate **21** was obtained (ligand to protein ratio (mol/mol) 3.4; determined by MALDI-TOF-MS). The BSA conjugate of the reference compound **22** was prepared in the same way from **20** (ligand to protein ratio (mol/mol) 7.2).

4. Experimental Part

4.1. General

All purchased chemicals were used without further purification unless stated otherwise. Solvents were dried over activated 4 Å (DCM, DMF, pyridine) or 3 Å (acetonitrile) molecular sieves. Dry MeOH (Merck) was purchased. All reactions that were carried out in dry solvents were carried out under argon atmosphere. Molecular sieves 4 Å (acid washed) were used as a powder (for reactions).

Cation exchange resin Dowex 50 H^+ was regenerated by consecutive washing with HCl (3 M), water and dry MeOH. Aqueous solutions of salts were saturated unless stated otherwise. Concentration of organic solutions was performed under reduced pressure <40 °C.

Thin layer chromatography was performed on precoated glass plates (Merck Silica gel 60 F_{254}) and on HPTLC plates with 2.5 cm concentration zone (Merck). Spots were detected by dipping reagent (*p*-anisaldehyde, sulfuric acid, acetic acid, ethanol). For column chromatography silica gel (0.040-0.063 mm) was used. For MPLC silica gel (0.040-0.063 mm) in glass columns or prefilled columns (Biotage; Si 12+ M, Si 25+ M) were used.

LC-MS for reaction monitoring was carried out on a system with two gradient pumps, degasser, column oven and auto injector. HILIC and C_4 columns were used for separation. The chromatograms were obtained by Alltech 3300 ELSD and Shimadzu LCMS-2020 instruments.

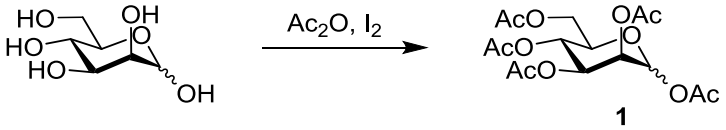
Optical rotations were measured with a Perkin Elmer 243 B (compound **9b**) or Anton Paar MCP 100 (all other compounds) polarimeter. $[\alpha]_D^T$ values are given in units of 10^{-1}*deg*cm^2*g^{-1}. Concentrations are given in g / 100 mL.

NMR spectra were recorded with a Bruker Avance III 600 (600.22 MHz for ^{1}H, 150.93 MHz for ^{13}C, 242.96 MHz for ^{31}P) or Bruker DPX 300 instrument using

standard Bruker NMR software. ^{1}H spectra were referenced to 0.00 (tetramethyl silane in $CDCl_3$), 3.31 (solvent residual peak, MeOD) and 0.00 (D_2O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) ppm unless stated otherwise. ^{13}C spectra were referenced to 77.16 ($CDCl_3$), 49.00 (MeOD) and 67.40 (D_2O, external calibration to 1,4-dioxane) ppm. ^{31}P spectra in MeOD and D_2O were referenced to 0.00 ppm (*ortho*-phosphoric acid in MeOD and D_2O respectively) by external calibration. Chemical shifts are given in ppm, whereas coupling constants are given in Hz.

HR-MS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument. MALDI-TOF-MS data were obtained on a Bruker Autoflex Speed (equipped with a 1000 Hz Smartbeam™-II laser) instrument using 2,5-dihydroxy acetophenone as matrix.

4.2. 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (3)



1

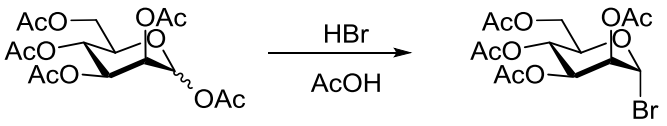
$C_6H_{12}O_6$
180,16

$C_{16}H_{22}O_{11}$
390,34

D-Mannose (5.00 g, 27.8 mmol) was suspended in 25 mL Ac_2O and I_2 (0.13 g, 0.5 mmol) was added under stirring at room temperature. After 140 min of stirring the mixture (clear dark brown solution) was diluted with 30 mL of DCM and the mixture was washed with 2x25 mL of $Na_2S_2O_3$ solution (aq., 5%).

The organic layer was washed with 3x25 mL $NaHCO_3$ (aq. solution) and 1x25 mL brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. There was still Ac_2O/AcOH in the product afterwards, therefore it was washed

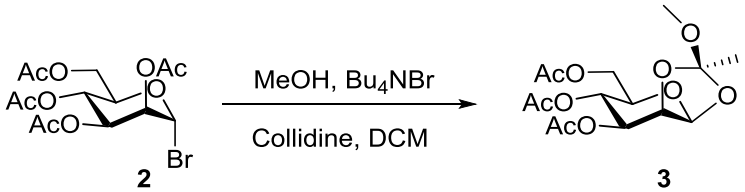
again with 2x30 mL $NaHCO_3$ solution until no more gas development was observable. The organic layer was dried again and the solvent was removed under reduced pressure. The product (pale yellow highly viscous liquid) was used for the next step without further purification.



1
$C_{16}H_{22}O_{11}$
390,34

2
$C_{14}H_{19}BrO_9$
411,20

The peracetylated carbohydrate **1** (10.22 g, 26.18 mmol) was dissolved in 20 mL of AcOH and then cooled down to 0°C. HBr (33% in AcOH, 23 mL) was added under stirring. The mixture was allowed to warm up slowly to room temperature within 24 h. The mixture was poured into 100 mL of ice water and 100 mL of DCM were added. The organic layer was washed with 2x100 mL $NaHCO_3$ (aq. solution), dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a highly viscous brown liquid that was used for the next step without further purification.



2
$C_{14}H_{19}BrO_9$
411,20

3
$C_{15}H_{22}O_{10}$
362,33

Bromide **2** (9.64 g, 23.65 mmol) was dissolved in 50 mL of dry DCM under stirring and Bu_4NBr (3.78 g, 11.72 mmol), MeOH (9.5 mL, 7.51 g, 234.45 mmol) and collidine (6.2 mL, 5.68 g, 46.89 mmol) were added. The mixture was heated to reflux for 4.5 h and washed with 150 mL water, 150 mL $NaHCO_3$ (aq. Solution) and 150 mL water subsequently. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to give a brown highly viscous liquid which was recrystallized from 75 mL of Et_2O (product washed with cold MeOH) to give white needles. Diastereomeric ratio (determined by proton NMR): 95:5.

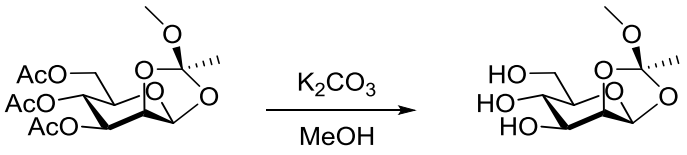
Yield: 3.61 g (36% over three steps from D-mannose).

TLC: R_f=0.46 (TLC; hexane / EtOAc (1:1))

^{1}H-NMR (major isomer, 300 MHz, $CDCl_3$): δ 1.75 (s, 3H, C*H*$_3$ from orthoester), 2.06 (s, 3H, OC(O)C*H*$_3$), 2.08 (s, 3H, OC(O)C*H*$_3$), 2.13 (s, 3H, OC(O)C*H*$_3$), 3.28 (s, 3H, OC*H*$_3$), 3.68 (m, 1H, 5-*H*), 4.11-4.28 (stack, 2H, 6a-*H*, 6b-*H*), 4.62 (apparent t, 1H, J=3.2, 2-*H*), 5.15 (dd, 1H, $J_{3,4}$=9.7, $J_{2,3}$=4.0, 3-*H*), 5.30 (apparent t, 1H, J=9.7, 4-*H*), 5.50 (d, 1H, $J_{1,2}$=2.5, 1-*H*)

The NMR data is in accordance with previously published literature[19].

4.3. 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranose (6)



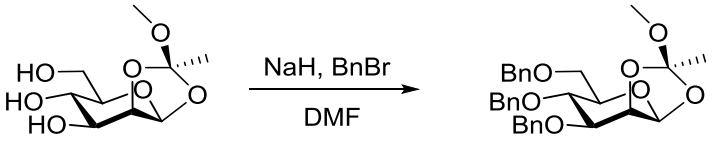
3

$C_{15}H_{22}O_{10}$
362,33

4

$C_9H_{16}O_7$
236,22

Orthoester **3** (3.47 g, 9.58 mmol) was dissolved in 40 mL of dry MeOH and K_2CO_3 (0.104 g, 0.75 mmol) was added as a solid and the mixture was stirred for 16 h at room temperature. The solvent was removed under reduced pressure to give a brown/grey solid. The product was used for the next step without further purification.



4

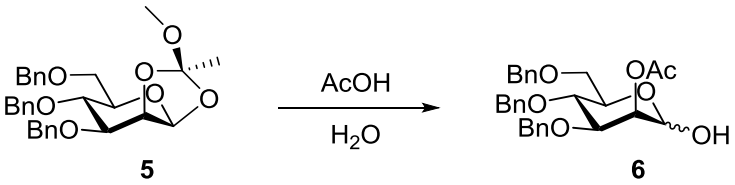
$C_9H_{16}O_7$
236,22

5

$C_{30}H_{34}O_7$
506,59

The deprotected orthoester **4** (2.26 g, 9.58 mmol) was dissolved in 30 mL of dry DMF. The solution was cooled down to 0°C and NaH (60% suspension in mineral oil, 3.52 g, 88.0 mmol NaH) was added under stirring. After 15 min of stirring BnBr (7.8 mL, 11.23 g, 66.0 mmol) was added dropwise.

The mixture was allowed to warm up to room temperature slowly within 16 h. 5 mL of methanol were added and the solution was stirred for another 2 h. The solution was diluted with Et_2O, washed with NH_4Cl (aq. solution) and brine and was dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was used for the next step without further purification.



5 $C_{30}H_{34}O_7$ 506,60

6 $C_{29}H_{32}O_7$ 492,57

The benzyl ether protected orthoester **5** (4.85 g, 9.58 mmol) was dissolved in 200 mL of 60% AcOH (aq.). The mixture was stirred at room temperature for 2 h. The solvents were removed under reduced pressure. The residue was dissolved in DCM and washed with $NaHCO_3$ (aq. solution). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified using column chromatography (hexane / ethyl acetate (2:1)) to give a colorless syrup. Traces of water were removed by coevaporation with toluene under reduced pressure. Anomeric ratio (determined by proton NMR): 7:1.

Yield: 4.25 g (90% over three steps from **3**).

TLC: R_f=0.32 (major anomer; TLC; hexane / EtOAc (2:1))
R_f=0.16 (minor anomer; TLC; hexane / EtOAc (2:1))

^{1}H-NMR (major anomer, 300 MHz, $CDCl_3$): δ 2.14 (s, 3H, OC(O)C*H*$_3$), 3.62-3.77 (stack, 3H, 4-*H*, 6a-*H*, 6b-*H*), 3.99-4.11 (stack, 2H, 3-*H*, 5-*H*), 4.41-4.55 (stack, 3H, OC*H*$_2$Ph), 4.60 (d, 1H, J=12.2, OC*H*$_2$Ph), 4.69 (d, 1H, J=11.2, OC*H*$_2$Ph), 4.85 (d, 1H, J=10.8, OC*H*$_2$Ph), 5.20 (m, 1H, 1-*H*), 5.36 (m, 1H, 2-*H*), 7.10-7.37 (stack, 15H, *Ph*)

The NMR data is in accordance with previously published literature[20].

4.4. 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α/β-D-mannopyranosyl trichloroacetimidate (7)

6

$C_{29}H_{32}O_7$
492,57

7

$C_{31}H_{32}Cl_3NO_7$
636,95

Anomeric alcohol **6** (2.08 g, 4.22 mmol) was dissolved in 8 mL of dry DCM and the solution was cooled down to 0°C. Trichloroacetonitrile (2.11 mL, 3.05 g, 21.11 mmol) and DBU (158 µL, 161 mg, 1.06 mmol) were added subsequently under stirring. The mixture was stirred for 40 min and then the solvent was removed under reduced pressure. The residue was dissolved in hexane / EtOAc (1.6:1; 0.1% Et_3N) and filtered through silica gel. The solvent of the filtrate was removed under reduced pressure to give a slightly yellow syrup which was used for the next step without further purification. Anomeric ratio (determined by proton NMR): 95:5.

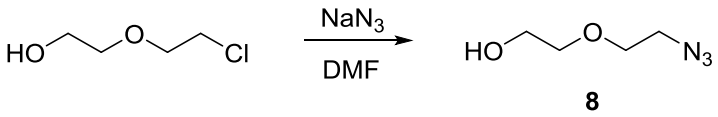
Yield: 2.61 g (97%)

TLC: R_f=0.70 (TLC, hexane / EtOAc (2:1))

^{1}H-NMR (major isomer, 300 MHz, $CDCl_3$): δ 2.19 (s, 3H, OC(O)C*H*$_3$), 3.71 (dd, 1H, $J_{6a,6b}$=11.1, $J_{5,6a}$=1.4, 6a-*H*), 3.84 (dd, 1H, $J_{6a,6b}$=11.1, $J_{5,6b}$=3.3, 6b-*H*), 3.94-4.06 (stack, 3H, 3-*H*, 4-*H*, 5-*H*), 4.47-4.77 (stack, 5H, OC*H*$_2$Ph), 4.87 (d, 1H, J=10.7, OC*H*$_2$Ph), 5.49 (apparent t, 1H, J=2.1, 2-*H*), 6.30 (d, 1H, $J_{1,2}$=2.0, 1-*H*), 7.15-7.37 (stack, 15H, *Ph*), 8.67 (s, 1H, N*H*)

The NMR data is in accordance with previously published literature[21].

4.5. 2-(2-Azidoethoxy)ethanol (8)



8

$C_4H_9ClO_2$
124,56

$C_4H_9N_3O_2$
131,14

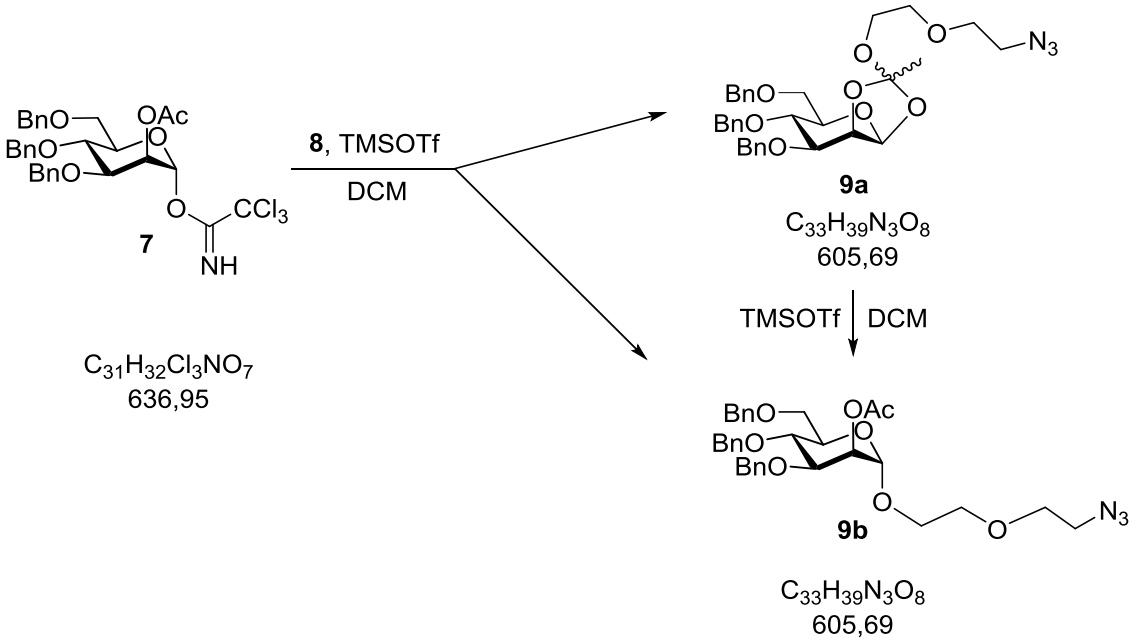
2-(2-Chloroethoxy)ethanol (4.22 mL, 4.98 g, 40.0 mmol) was dissolved in 100 mL of DMF and NaN_3 (2.86 g, 44.0 mmol) was added under stirring. The mixture was stirred for 8 h at 80°C. 300 mL of water were added and the solution was split into 3 portions (for safety reasons). Each portion was extracted with 3x30 mL EtOAc (except for the 2nd portion which was - by mistake - only extracted once). The aqueous layer of portion 2 was extracted again twice with EtOAc and these organic layers were added to portion 3.

The combined organic layers were dried over Na_2SO_4 and the solution was concentrated under reduced pressure to give a colorless solution of the azide in DMF. Note: TLC staining with anisaldehyde and CAM did not work satisfactorily. The product concentration was calculated by ^{1}H-NMR integration for each portion.

Yield: Portion 1: 3.40 g, 24.1% azide (0.815 g)
Portion 2: 3.93 g, 23.7% azide (0.932 g)
Portion 3: 7.24 g, 23.7% azide (1.716 g)
Total: 3.46 g azide (66%)

^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.28 (apparent t, 1H, J=6.1, O*H*), 3.42 (apparent t, 2H, J=4.9, N_3-C*H*$_2$), 3.63 (m, 2H, OC*H*$_2$CH$_2$OH), 3.71 (m, 2H, N_3-CH$_2$C*H*$_2$), 3.76 (m, 2H, OH-C*H*$_2$)

4.6. 2-(2-Azidoethoxy)ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (9b)



A solution of donor **7** (2.61 g, 4.09 mmol) and acceptor **8** (24% in DMF, 3.1 mL, 0.75 g, 5.72 mmol) in 8 mL of dry DCM was added to MS 4 Å (2000 mg). The mixture was stirred for 60 min at room temperature. The mixture was cooled down to 0°C and TMSOTf (0.1 M solution in dry DCM, 1.1 mL, 0.11 mmol) was added dropwise. The cooling bath was removed immediately afterwards and stirring was continued for 30 min. Et_3N (50 µL, 37 mg, 0.36 mmol) was added and after additional 5 min of stirring the mixture was filtered through celite. The solvent was removed under reduced pressure. The intermediate was purified using MPLC (hexane / EtOAc (2.3:1)) and a mixture of orthoester and glycoside was obtained.

The mixture was dissolved in 20 mL of dry DCM and added to MS 4 Å (900 mg). The mixture was stirred for 30 min at room temperature and then cooled down to 0°C. TMSOTf (0.1 M solution in dry DCM, 1.71 mL, 0.17 mmol) was added dropwise and the cooling bath was removed immediately. After 25 min of stirring Et_3N (50 µL, 37 mg, 0.36 mmol) was added. The mixture was stirred for another 5 min and then it was filtered through celite. The product (colorless syrup) was used

for the next step without further purification.

Yield: 1.66 g (67%)

TLC: R_f=0.32 (glycoside; TLC; hexane / EtOAc (2:1))
R_f=0.28 (orthoester intermediate; TLC; hexane / EtOAc (2:1))

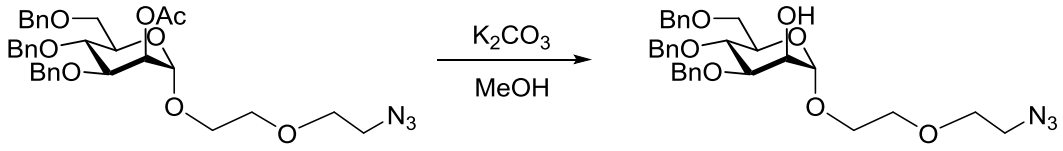
^{1}H-NMR (600 MHz, $CDCl_3$): δ 2.14 (s, 3H, OC(O)CH_3), 3.35 (m, 2H, N_3-CH_2), 3.59-3.66 (stack, 5H, 4H of OCH_2, 6a-*H*), 3.71 (apparent dd, 1H, J=10.5, J=1.8, OCH_2), 3.77-3.85 (stack, 3H, OCH_2, 5-*H*, 6b-*H*), 3.88 (apparent t, 1H, J=9.5, 4-*H*), 4.00 (dd, 1H, $J_{3,4}$=9.2, $J_{2,3}$=3.6, 3-*H*), 4.48 (d, 1H, J=10.8, OCH_2Ph), 4.50-4.54 (stack, 2H, OCH_2Ph), 4.67 (d, 1H, J=11.8, OCH_2Ph), 4.70 (d, 1H, J=11.2, OCH_2Ph), 4.85 (d, 1H, J=10.7, OCH_2Ph), 4.89 (d, 1H, $J_{1,2}$=1.9, 1-*H*), 5.40 (dd, 1H, $J_{1,2}$=1.8, $J_{2,3}$=3.3, 2-*H*), 7.15-7.17 (stack, 2H, *Ph*), 7.24-7.36 (stack, 13H, *Ph*)

^{13}C-NMR (150 MHz, $CDCl_3$): δ 20.26 (CH_3, OC(O)*C*H_3), 49.82 (CH_2, N_3-*C*), 66.04 (CH_2, 6-*C*), 67.88 (CH, 2-*C*), 68.12 (CH_2, O*C*H_2), 69.24 (CH_2, O*C*H_2), 69.27 (CH_2, O*C*H_2), 70.64 (CH, 5-*C*), 70.94 (CH_2, O*C*H_2Ph), 72.61 (CH_2, O*C*H_2Ph), 73.51 (CH, 4-*C*), 74.30 (CH_2, O*C*H_2Ph), 77.27 (CH, 3-*C*), 97.14 (CH, 1-*C*), 126.73 (CH, *Ph*), 126.75 (CH, *Ph*), 126.83 (CH, *Ph*), 126.93 (CH, *Ph*), 127.00 (CH, *Ph*), 127.19 (CH, *Ph*), 127.46 (CH, *Ph*), 127.51 (CH, *Ph*), 137.20 (quart. C, ipso *Ph*), 137.41 (quart. C, ipso *Ph*), 137.58 (quart. C, ipso *Ph*), 169.58 (quart. C, *C*=O)

HR-MS: *m/z* calculated for $[M+NH_4]^+$: 623.3075; found: 623.3085

Optical Rotation: $[\alpha]_D^{21}$ +26.0 (c 1.1, $CHCl_3$)

4.7. 2-(2-Azidoethoxy)ethyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (10)



9b

$C_{33}H_{39}N_3O_8$
605,69

10

$C_{31}H_{37}N_3O_7$
563,65

Glycoside **9b** (722 mg, 1.19 mmol) was dissolved in 5 mL of dry MeOH and K_2CO_3 (10 mg, 0.07 mmol) was added under stirring. After 17 h of stirring the mixture was neutralized to pH 7 by addition of Amberlite IR120 (H^+). The resin was filtered off and the solvent was removed from the filtrate under reduced pressure to give a colorless syrup.

Yield: 656 mg (98%)

TLC: R_f=0.28 (TLC; hexane / EtOAc (1:1))

^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.30-3.38 (m, 2H, N_3-C*H*$_2$), 3.59-3.67 (stack, 5H, 4H of OC*H*$_2$, 6a-*H*), 3.68-3.76 (stack, 2H, OC*H*$_2$), 3.78-3.86 (stack, 3H, 4-*H*, 5-*H*, 6b-*H*), 3.91 (dd, 2H, $J_{2,3}$=3.3, $J_{3,4}$=8.6, 3-*H*), 4.08 (dd, 1H, $J_{1,2}$=1.7, $J_{2,3}$=3.3, 2-*H*), 4.49-4.55 (stack, 2H, OC*H*$_2$Ph), 4.61-4.72 (stack, 3H, OC*H*$_2$Ph), 4.82 (d, 1H, J=11.1, OC*H*$_2$Ph), 4.94 (d, 1H, $J_{1,2}$=1.6, 1-*H*), 7.16-7.19 (stack, 2H, *Ph*), 7.24-7.37 (stack, 13H, *Ph*)

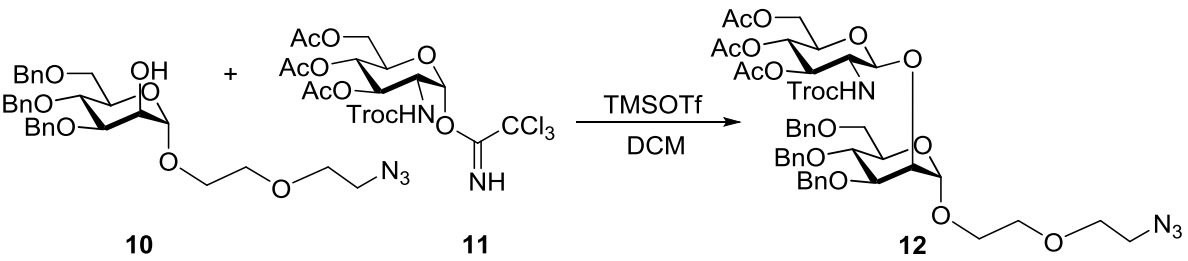
^{13}C-NMR (150 MHz, $CDCl_3$): δ 50.79 (CH_2, N_3-*C*), 66.82 (CH_2, 6-*C*), 68.43 (CH, 2-*C*), 69.20 (CH_2, O*C*H$_2$), 70.17 (CH_2, O*C*H$_2$), 70.25 (CH_2, O*C*H$_2$), 71.33 (CH, 5-*C*), 72.10 (CH_2, O*C*H$_2$Ph), 73.62 (CH_2, O*C*H$_2$Ph), 74.49 (CH, 4-*C*), 75.24 (CH_2, O*C*H$_2$Ph), 80.26 (CH, 3-*C*), 99.53 (CH, 1-*C*), 127.69 (CH, *Ph*), 127.79 (CH, *Ph*), 127.95 (CH, *Ph*), 127.97 (CH, *Ph*), 128.01 (CH, *Ph*), 128.05 (CH, *Ph*), 128.45

(CH, *Ph*), 128.48 (CH, *Ph*), 128.64 (CH, *Ph*), 138.14 (quart. C, ipso *Ph*), 138.43 (quart. C, ipso *Ph*), 138.49 (quart. C, ipso *Ph*)

HR-MS: *m/z* calculated for $[M+Na]^+$: 586.2524; found: 586.2535

Optical Rotation: $[\alpha]_D^{20}$ +47.5 (c 1.0, $CHCl_3$)

4.8. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroethoxycarbonylamino-β-D-glucopyranosyl-(1→2)-[2-(2-azidoethoxy)ethyl] 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (12)

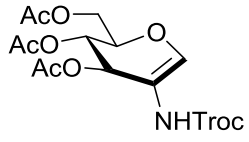


10 $C_{31}H_{37}N_3O_7$ 563,65

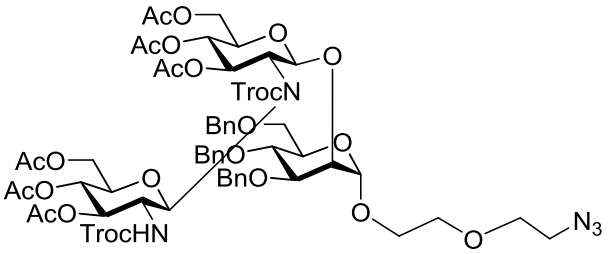
11 $C_{17}H_{20}Cl_6N_2O_{10}$ 625,05

12 $C_{46}H_{55}Cl_3N_4O_{16}$ 1026,31

Side products:



Glycal
$C_{15}H_{18}Cl_3NO_9$
462,66



Trisaccharide
$C_{61}H_{73}Cl_6N_5O_{25}$
1488,97

A solution of donor **11** (1.34 g, 2.14 mmol) and acceptor **10** (603 mg, 1.07 mmol) in 11 mL of dry DCM was added to MS 4 Å (1.5 g). The mixture was stirred for 75 min at room temperature. TMSOTf (0.1 M solution in dry DCM, 0.53 mL, 0.05 mmol) was added dropwise and the mixture was stirred for 45 min. Et_3N (15 µL,

11 mg, 0.11 mmol) was added and after additional 5 min of stirring the mixture was filtered through celite. The solvent was removed under reduced pressure. The crude product was subjected to column chromatography (DCM / EtOAc (4:1), 1% Et_3N, 150 fold amount of silica) which delivered a mixture of disaccharide and unreacted acceptor (765 mg in total).
The ratio was determined by comparison of the proton NMR integrals (51% (w/w) disaccharide). The mixture (colorless syrup) was used for the next step without further purification.

Yield (calculated): 390 mg (38%)

TLC: R_f=0.28 (disaccharide; TLC; DCM / EtOAc (4:1), 1% Et_3N)
R_f=0.48 (trisaccharide side product; TLC; DCM / EtOAc (4:1), 1% Et_3N)
R_f=0.44 (disaccharide; TLC; hexane / EtOAc (1:1))
R_f=0.63 (glycal side product; TLC; hexane / EtOAc (1:1))

^{1}H-NMR (glycal side product, 300 MHz, $CDCl_3$): δ 2.10 (s, 9H, 3xOC(O)C*H*$_3$), 4.22 (m, 1H, 6a-*H*), 4.33-4.47 (stack, 2H, 5-*H*, 6b-*H*), 4.74 (s, 2H, Cl_3CCH_2), 5.23 (apparent t, 1H, J=4.9, 4-*H*), 5.44 (d, 1H, $J_{3,4}$=4.0, 3-*H*), 6.18 (s, 1H, N*H*), 7.05 (s, 1H, 1-*H*)

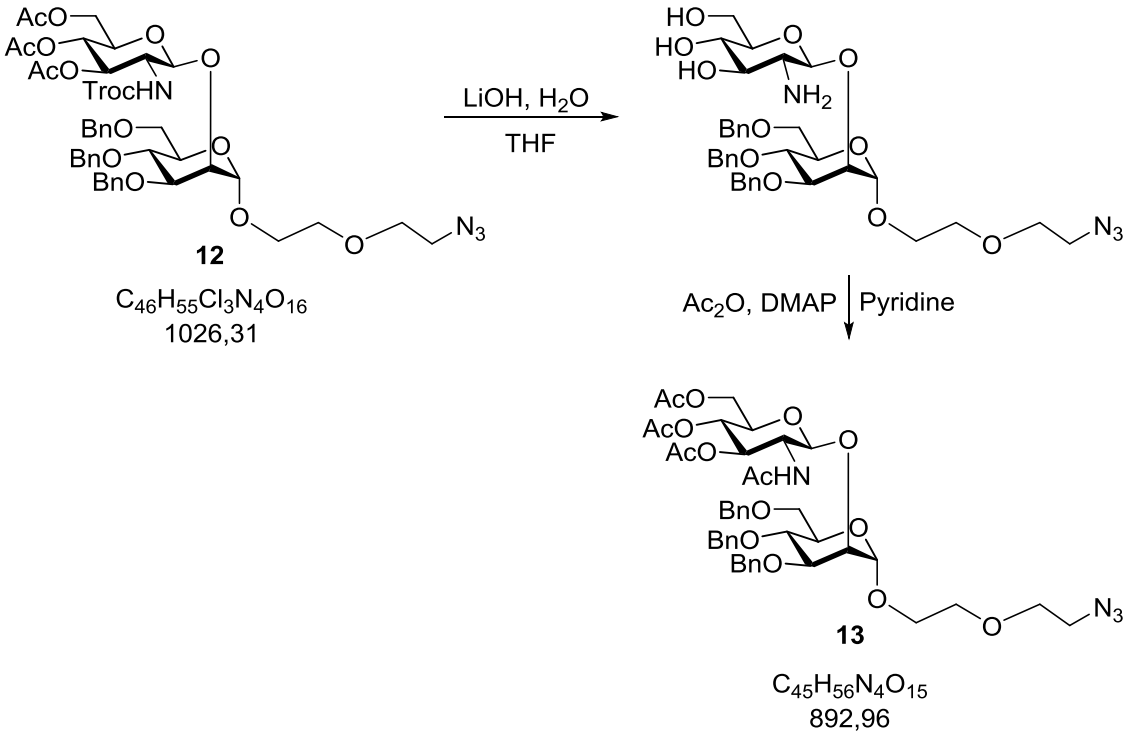
^{1}H-NMR (trisaccharide side product, 600 MHz, $CDCl_3$): δ 1.79 (s, 3H, OC(O)C*H*$_3$), 1.99 (s, 3H, OC(O)C*H*$_3$), 2.006 (s, 3H, OC(O)C*H*$_3$), 2.011 (s, 3H, OC(O)C*H*$_3$), 2.02 (s, 3H, OC(O)C*H*$_3$), 2.13 (s, 3H, OC(O)C*H*$_3$), 3.27-3.41 (stack, 3H, N_3-C*H*$_2$, 2''-*H*), 3.48-3.54 (stack, 2H, 4-*H*, 6a-*H*), 3.55-3.76 (stack, 8H, 3xOC*H*$_2$, 2'-*H*, 5'-*H*), 3.79-3.83 (m, 1H, 6b-*H*), 3.90-3.96 (stack, 3H, 5-*H*, 5''-*H*, 3-*H*), 4.12 (dd, 1H, $J_{6'a,6'b}$=12.4, $J_{5',6'a}$=2.2, 6'a-*H*), 4.18 (dd, 1H, $J_{6''a,6''b}$=12.4, $J_{5'',6''a}$=4.0, 6''a-*H*), 4.21-4.28 (stack, 2H, 6'b-*H*, 6''b-*H*), 4.30 (dd, 1H, $J_{1,2}$=1.5, $J_{2,3}$=3.5, 2-*H*), 4.39-4.48 (stack, 3H, 2H of OC*H*$_2$Ph, 1H of Cl_3CCH_2), 4.49-4.54 (stack, 2H of OC*H*$_2$Ph), 4.64 (d, 1H, J=11.8, Cl_3CCH_2), 4.82 (d, 1H, J=11.9, Cl_3CCH_2), 4.87 (br, 1H, 1-*H*), 4.91 (d, 1H, J=12.1, OC*H*$_2$Ph), 4.99-5.02 (stack, 2H, 1H of OC*H*$_2$Ph, 1H of Cl_3CCH_2), 5.07 (apparent t, 1H, J=9.8, 4''-*H*), 5.16 (apparent t, 1H, J=9.8, 4'-*H*), 5.34 (d, 1H, $J_{1',2'}$=8.5, 1'-*H*), 5.88 (apparent t, 1H, J=9.9, 3'-*H*),

6.14 (apparent t, 1H, J=9.6, 3''-*H*), 6.21 (d, 1H, $J_{1'',2''}$=9.5, 1''-*H*), 7.05-7.10 and 7.20-7.29 and 7.34-7.39 (stack, 16H, *Ph*, N*H*)

^{13}C-NMR (trisaccharide side product, 150 MHz, $CDCl_3$): δ 20.73 (CH_3, br, OC(O)*C*H_3), 20.79 (CH_3, OC(O)*C*H_3), 20.90 (CH_3, OC(O)*C*H_3), 20.92 (CH_3, OC(O)*C*H_3), 20.93 (CH_3, OC(O)*C*H_3), 50.68 (CH_2, N_3-*C*), 55.29 (CH, 2''-*C*), 57.26 (CH, 2'-*C*), 62.08 (CH_2, 6''-*C*), 62.34 (CH_2, 6'-*C*), 67.70 (CH_2, 6-*C*), 68.62 (CH, 4'-*C*), 69.07 (CH, 4''-*C*), 69.86 (CH_2, 2xO*C*H_2), 70.34 (CH_2, O*C*H_2), 70.53 (CH, 3'-*C*), 70.99 (CH, 3''-*C*), 71.33 (CH_2, O*C*H_2Ph), 72.00 (CH, 5-*C* or 5''-*C*), 72.72 (CH, 5'-*C*), 73.44 (CH_2, O*C*H_2Ph), 73.73 (CH, 5-*C* or 5''-*C*), 74.72 (Cl_3C*C*H_2), 75.48 (CH_2, O*C*H_2Ph), 75.50 (CH, 4-*C*), 76.35 (CH, 2-*C*), 76.69 (CH_2, Cl_3C*C*H_2), 78.30 (CH, 3-*C*), 81.24 (CH, 1''-*C*), 99.27 (CH, 1-*C*), 100.88 (CH, 1'-*C*), 127.46 (CH, *Ph*), 127.58 (CH, *Ph*), 127.78 (CH, *Ph*), 127.98 (CH, *Ph*), 128.21 (CH, *Ph*), 128.49 (CH, br, *Ph*), 128.57 (CH, br, *Ph*), 137.87 (quart. C, ipso *Ph*), 138.07 (quart. C, ipso *Ph*), 138.16 (quart. C, ipso *Ph*), 153.26 (quart. C, N*C*(O)O), 154.18 (quart. C, N*C*(O)O), 169.34 (quart. C, O*C*(O)CH_3), 169.77 (quart. C, O*C*(O)CH_3), 169.99 (quart. C, O*C*(O)CH_3), 170.70 (quart. C, O*C*(O)CH_3), 170.81 (quart. C, O*C*(O)CH_3), 170.84 (quart. C, O*C*(O)CH_3)

Note: The ^{13}C shift value of 3'-C was obtained from the HSQC spectrum. As mentioned in Results and Discussion, the structure of the trissacharide could not be confirmed certainly due to an impurity, signal overlap and moderate signal-to-noise ratio in the ^{13}C spectrum.

4.9. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-(2-azidoethoxy)ethyl] 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (13)



12

$C_{46}H_{55}Cl_3N_4O_{16}$

1026,31

13

$C_{45}H_{56}N_4O_{15}$

892,96

Disaccharide **12** (402 mg, 0.39 mmol) was dissolved in 6 mL of THF and 3 mL of 1 M LiOH in water were added under stirring. The mixture was heated to reflux for 5.5 h. The mixture was cooled down to room temperature, diluted with MeOH and neutralized by addition of 1 M HCl. The solvents were removed carefully under reduced pressure and water was coevaporated with toluene.

DMAP (2 mg, 0.02 mmol) was added to the residue and the mixture was dissolved in 6 mL of dry pyridine. Ac_2O (300 µL, 324 mg, 3.17 mmol) was added under stirring. The same amounts of Ac_2O were added after 2.5 and 7 h of stirring and the mixture was stirred overnight. Next day MeOH (5 mL) was added and the mixture was stirred for 3 h. The solvents were removed under reduced pressure, the residue was suspended in DCM and washed with water (and extracted again with DCM). The combined organic layers were dried over Na_2SO_4 and the solvent

was removed under reduced pressure. The crude product was purified using MPLC (hexane / EtOAc (2:5)) to give an amorphous solid.

Yield: 262 mg (75%)

TLC: R_f=0.52 (HPTLC; EtOAc)

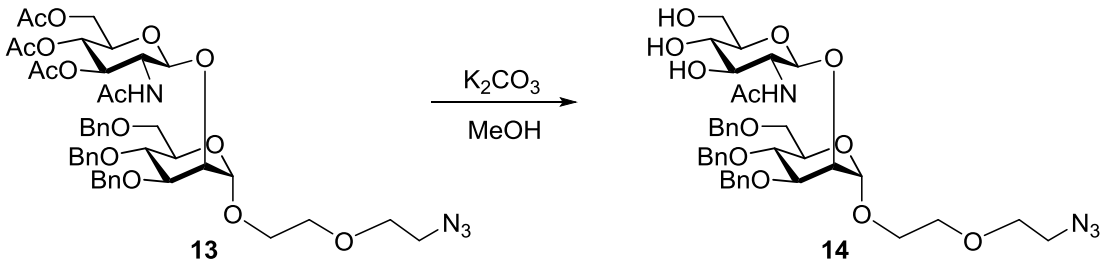
^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.78 (s, 3H, NHC(O)C*H*$_3$), 2.00 (s, 3H, OC(O)C*H*$_3$), 2.02 (s, 3H, OC(O)C*H*$_3$), 2.03 (s, 3H, OC(O)C*H*$_3$), 3.38 (m, 2H, N_3-C*H*$_2$), 3.45 (m, 1H, 2'-*H*), 3.60-3.67 (stack, 6H, 6a-*H*, 5H of 3xOC*H*$_2$), 3.70-3.77 (stack, 3H, 5-*H*, 5'-*H*, 1H of OC*H*$_2$), 3.80 (m, 1H, 6b-*H*), 3.91 (apparent t, 1H, J=8.7, 4-*H*), 3.96 (dd, 1H, $J_{2,3}$=3.1, $J_{3,4}$=8.5, 3-*H*), 4.16 (dd, 1H, $J_{6'a,6'b}$=12.2, $J_{5',6'a}$=2.4, 6'a-*H*), 4.18 (apparent t, 1H, J=2.9, 2-*H*), 4.25 (dd, 1H, $J_{6'a,6'b}$=12.0, $J_{5',6'b}$=5.2, 6'b-*H*), 4.47-4.59 (m, 4H, OC*H*$_2$Ph), 4.77 (d, 1H, J=11.3, OC*H*$_2$Ph), 4.80 (d, 1H, $J_{1,2}$=2.6, 1-*H*), 4.86 (d, 1H, J=11.0, OC*H*$_2$Ph), 5.02 (dd, 1H, $J_{4',5'}$=9.9, $J_{3',4'}$=9.2, 4'-*H*), 5.09 (d, 1H, $J_{1',2'}$=8.4, 1'-*H*), 5.50 (d, 1H, $J_{2',NH}$=7.65, N*H*), 5.62 (dd, 1H, $J_{2'3'}$=10.5, $J_{3',4'}$=9.3, 3'-*H*), 7.16-7.39 (stack, 15H, *Ph*)

^{13}C-NMR (150 MHz, $CDCl_3$): δ 20.82 (CH_3, OC(O)*C*H$_3$), 20.86 (CH_3, 2xOC(O)*C*H$_3$), 23.36 (CH_3, NHC(O)*C*H$_3$), 50.74 (CH_2, N_3-*C*), 56.09 (CH, 2'-*C*), 62.66 (CH_2, 6'-*C*), 66.92 (CH_2, 6-*C*), 69.35 (CH, 4'-*C*), 69.55 (CH_2, O*C*H$_2$), 70.20 (CH_2, O*C*H$_2$), 70.30 (CH_2, O*C*H$_2$), 71.68 (CH_2, O*C*H$_2$Ph), 71.70 (CH, 3'-*C*), 71.97 and 72.14 (CH, 5-*C*, 5'-*C*), 73.40 (CH_2, O*C*H$_2$Ph), 73.89 (CH, 2-*C*), 74.74 (CH, 4-*C*), 74.91 (CH_2, O*C*H$_2$Ph), 78.09 (CH, 3-*C*), 97.77 (CH, 1-*C*), 98.30 (CH, 1'-*C*), 127.69 (CH, *Ph*), 127.79 (CH, *Ph*), 127.81 (CH, *Ph*), 127.82 (CH, *Ph*), 128.11 (CH, *Ph*), 128.38 (CH, *Ph*), 128.45 (CH, *Ph*), 128.49 (CH, *Ph*), 128.54 (CH, *Ph*), 138.42 (quart. C, ipso *Ph*), 138.61 (quart. C, br, ipso *Ph*), 169.75 (quart. C, *C*=O), 170.43 (quart. C, *C*=O), 170.81 (quart. C, *C*=O), 171.19 (quart. C, *C*=O)

HR-MS: *m/z* calculated for $[M+K]^+$: 931.3374; found: 931.3385

Optical Rotation: $[\alpha]_D^{20}$ +10.7 (c 0.7, $CHCl_3$)

4.10. 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-(2-azidoethoxy)ethyl] 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (14)



Acetylated disaccharide **13** (251 mg, 0.28 mmol) was dissolved in 2 mL of dry MeOH and K_2CO_3 (16 mg, 0.11 mmol) was added under stirring. After 17 h of stirring the mixture was diluted with MeOH and Amberlite IR120 was added until the mixture was at pH 7. The resin was filtered off and the solvent was removed from the filtrate under reduced pressure. The residue was purified using MPLC (DCM / MeOH (9:1)) to give a colorless syrup.

Yield: 184 mg (85%)

TLC: R_f=0.28 (TLC; DCM / MeOH (9:1))

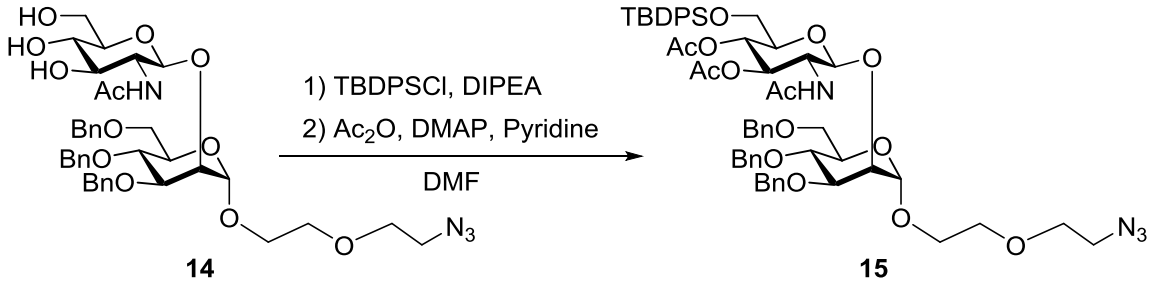
^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.82 (s, 3H, NHC(O)C*H*$_3$), 3.29 (m, 1H, 2'-*H*), 3.34 (m, 2H, N_3-C*H*$_2$), 3.44 (m, 1H, 5'-*H*), 3.57-3.68 (stack, 7H, 4'-*H*, 6a-*H*, 5H of 3xOC*H*$_2$), 3.72-3.82 (stack, 4H, 1H of OC*H*$_2$, 5-*H*, 3'-*H*, 6b-*H*), 3.84 (dd, 1H, $J_{6'a,6'b}$=11.9, $J_{5',6'a}$=5.0, 6'a-*H*), 3.94 (dd, 1H, $J_{6'a,6'b}$=11.9, $J_{5',6'b}$ =3.8, 6'b-*H*), 3.99 (dd, 1H, $J_{3,4}$=9.2, $J_{2,3}$=3.1, 3-*H*), 4.04 (apparent t, 1H, J=9.3, 4-*H*), 4.19 (apparent t, 1H, J=2.5, 2-*H*), 4.42 (d, 1H, J=11.9, OC*H*$_2$Ph), 4.47 (d, 1H, J=11.2, OC*H*$_2$Ph), 4.50 (d, 1H, $J_{1',2'}$=8.3, 1'-*H*), 4.53 (d, 1H, J=11.8, OC*H*$_2$Ph), 4.67 (d, 1H, J=11.5, OC*H*$_2$Ph), 4.72 (d, 1H, J=11.6, OC*H*$_2$Ph), 4.89 (d, 1H, J=10.8, OC*H*$_2$Ph), 4.91 (d, 1H, $J_{1,2}$=2.0, 1-*H*), 7.18-7.40 (stack, 15H, *Ph*)

^{13}C-NMR (150 MHz, $CDCl_3$): δ 23.54 (CH_3, NHC(O)*C*H_3), 50.78 (CH_2, N_3-*C*), 59.46 (CH, 2'-*C*), 62.96 (CH_2, 6'-*C*), 67.34 (CH_2, 6-*C*), 68.89 (CH_2, O*C*H_2), 70.35 (CH_2, *OC*H_2), 71.56 (CH, 4'-*C*), 71.78 (CH, 5-*C*), 72.27 (CH_2, O*C*H_2Ph), 73.46 (CH, 2-*C*), 73.50 (CH_2, O*C*H_2Ph), 73.67 (CH, 3'-*C*), 74.34 (CH, 4-*C*), 75.33 (CH_2, O*C*H_2Ph), 75.98 (CH, 5'-*C*), 78.25 (CH, 3-*C*), 97.48 (CH, 1-*C*), 99.19 (CH, 1'-*C*), 127.80 (CH, *Ph*), 127.89 (CH, *Ph*), 128.06 (CH, *Ph*), 128.09 (CH, *Ph*), 128.13 (CH, *Ph*), 128.34 (CH, *Ph*), 128.55 (CH, *Ph*), 128.61 (CH, *Ph*), 128.63 (CH, *Ph*), 137.92 (quart. C, ipso *Ph*), 138.04 (quart. C, ipso *Ph*), 138.51 (quart. C, ipso *Ph*), 172.84 (quart. C, *C*=O)

HR-MS: *m/z* calculated for $[M+Na]^+$: 789.3317; found: 789.3326

Optical Rotation: $[\alpha]_D^{20}$ -7.4 (c 0.9, $CHCl_3$)

4.11. 2-Acetamido-3,4-di-*O*-acetyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-(2-azidoethoxy)ethyl] 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (15)



14

$C_{39}H_{50}N_4O_{12}$
766,85

15

$C_{59}H_{72}N_4O_{14}Si$
1089,32

Disaccharide **14** (184 mg, 0.24 mmol) was dissolved in 0.75 mL of dry DMF and DIPEA (210 µL, 155 mg, 1.20 mmol) and TBDPSCl (98 µL, 86 mg, 0.31 mmol) were added subsequently under stirring at room temperature. Pyridine (300 µL), Ac_2O (91 µL, 98 mg, 0.96 mmol) and DMAP (cat. amount) were added subsequently after 3 d of stirring. MeOH (1 mL) was added after 2 h of stirring and

the mixture was stirred for another hour. The solvents were removed under reduced pressure and the residue was purified using MPLC (hexane / EtOAc (2:5)) to give an amorphous solid.

Yield: 208 mg (80%)

TLC: R_f=0.70 (HPTLC; EtOAc)

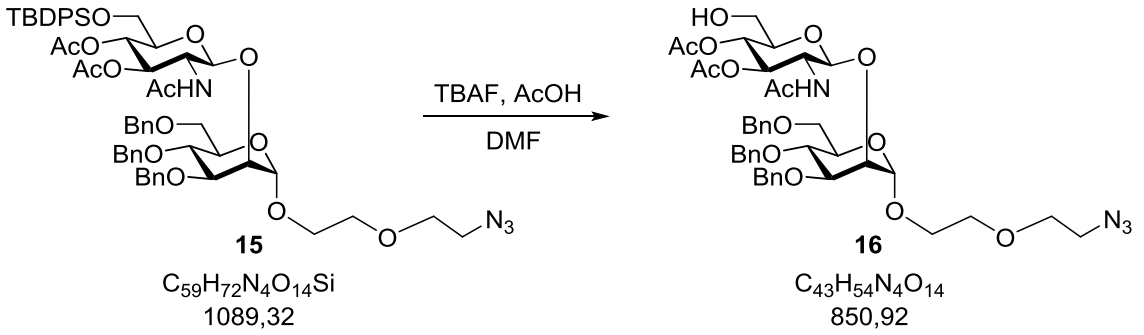
^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.03 (s, 9H, 3xC*H*$_3$), 1.79 (stack, 6H, NHC(O)C*H*$_3$ and OC(O)C*H*$_3$), 1.99 (s, 3H, OC(O)C*H*$_3$), 3.37 (m, 2H, N_3-C*H*$_2$), 3.51 (m, 1H, 2'-*H*), 3.57-3.74 (stack, 9H, 3xOC*H*$_2$, 5'-*H*, 6a-*H*, 6'a-*H*), 3.75-3.85 (stack, 3H, 5-*H*, 6b-*H*, 6'b-*H*), 3.89 (apparent t, 1H, J=8.6, 4-*H*), 3.98 (dd, 1H, $J_{2,3}$=3.0, $J_{3,4}$=8.3, 3-*H*), 4.26 (apparent t, 1H, J=2.9, 2-*H*), 4.44-4.56 (stack, 4H, OC*H*$_2$Ph), 4.81-4.85 (stack, 2H, OC*H*$_2$Ph), 4.86 (d, 1H, $J_{1,2}$=2.7, 1-*H*), 4.94 (apparent t, 1H, J=9.6, 4'-*H*), 5.07 (d, 1H, $J_{1',2'}$=8.4, 1'-*H*), 5.53-5.63 (stack, 2H, N*H* and 3'-*H*), 7.18-7.42 (stack, 21H, *Ph*), 7.60-7.66 (stack, 4H, *Ph*)

^{13}C-NMR (150 MHz, $CDCl_3$): δ 19.31 (quart. C, $(CH_3)_3$*C*Si), 20.72 (CH_3, OC(O)*C*H_3), 20.90 (CH_3, OC(O)*C*H_3), 23.37 (NHC(O)*C*H_3), 26.91 (CH_3, SiC(*C*H_3)$_3$), 50.73 (CH_2, N_3-*C*), 55.96 (CH, 2'-*C*), 63.60 (CH_2, 6'-*C*), 67.06 (CH_2, 6-*C*), 69.58 (CH_2, O*C*H_2), 69.76 (CH, 4'-*C*), 70.17 (CH_2, O*C*H_2), 70.35 (CH_2, O*C*H_2), 71.38 (CH_2, O*C*H_2Ph), 72.07 and 72.18 (CH, 5-*C*, 3'-*C*), 73.39 (CH_2, O*C*H_2Ph), 73.43 (CH, 2-*C*), 74.69 (CH_2, O*C*H_2Ph), 74.79 (CH, 4-*C*), 75.10 (CH, 5'-*C*), 77.81 (CH, 3-*C*), 97.96 (CH, 1-*C*), 98.23 (CH, 1'-*C*), 127.74 (CH, br, *Ph*), 127.79 (CH, *Ph*), 127.87 (CH, *Ph*), 127.90 (CH, *Ph*), 128.12 (CH, *Ph*), 128.42 (CH, *Ph*), 128.46 (CH, *Ph*), 128.52 (CH, *Ph*), 128.53 (CH, *Ph*), 129.86 (CH, *Ph*), 129.90 (CH, *Ph*), 133.20 (quart. C, ipso *Ph*), 133.36 (quart. C, ipso *Ph*), 135.70 (CH, *Ph*), 135.74 (CH, *Ph*), 138.32 (quart. C, ipso *Ph*), 138.57 (quart. C, br, ipso *Ph*), 169.68 (quart. C, *C*=O), 170.58 (quart. C, *C*=O), 171.13 (quart. C, *C*=O)

HR-MS: *m/z* calculated for $[M+K]^+$: 1127.4446; found: 1127.4439

Optical Rotation: $[\alpha]_D^{20}$ +13.1 (c 1.0, $CHCl_3$)

4.12. 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-(2-azidoethoxy)ethyl] 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (16)



15
$C_{59}H_{72}N_4O_{14}Si$
1089,32

16
$C_{43}H_{54}N_4O_{14}$
850,92

Silylated disaccharide **15** (79 mg, 0.073 mmol) was dissolved in 2 mL of DMF and cooled to 0°C in a teflon vessel. AcOH (1018 µL of a 1 M solution in DMF, 1.02 mmol) and TBAF (124 µL of a 1 M solution in THF, 0.124 mmol) were added subsequently under stirring and the mixture was slowly warmed up to room temperature under stirring overnight. Next day the solvents were removed from the crude product under reduced pressure. The residue was dissolved in EtOAc and filtered through silica to remove TBAF and TBAAc. The solvent was removed from the filtrate under reduced pressure and the crude product was purified using MPLC (100% EtOAc) to give a colorless syrup.

Yield: 38.6 mg (62%)

TLC: R_f=0.32 (TLC; EtOAc)

^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.79 (s, 3H, NHC(O)C*H*$_3$), 2.01 (s, 3H, OC(O)C*H*$_3$), 2.03 (s, 3H, OC(O)C*H*$_3$), 3.38 (m, 2H, N_3-C*H*$_2$), 3.50 (m, 1H, 2'-*H*), 3.55 (m, 1H, 5'-*H*), 3.56-3.73 (stack, 9H, 3xOC*H*$_2$, 6'a-*H*, 6'b-*H*, 6a-*H*), 3.75 (m, 1H, 5-*H*), 3.80 (m, 1H, 6b-*H*), 3.90 (apparent t, 1H, J=8.8, 4-*H*), 3.96 (dd, 1H, $J_{3,4}$=8.4, $J_{2,3}$=3.0, 3-*H*), 4.13 (apparent t, 1H, J=2.9, 2-*H*), 4.48 (d, 1H, J=11.8, OC*H*$_2$Ph), 4.50 (d, 1H, J=11.2, OC*H*$_2$Ph), 4.54 (d, 1H, J=11.8, OC*H*$_2$Ph), 4.64 (d, 1H, J=11.6, OC*H*$_2$Ph), 4.75 (d, 1H, J=11.5, OC*H*$_2$Ph), 4.80 (d, 1H, $J_{1,2}$=2.6, 1-*H*), 4.85 (d, 1H,

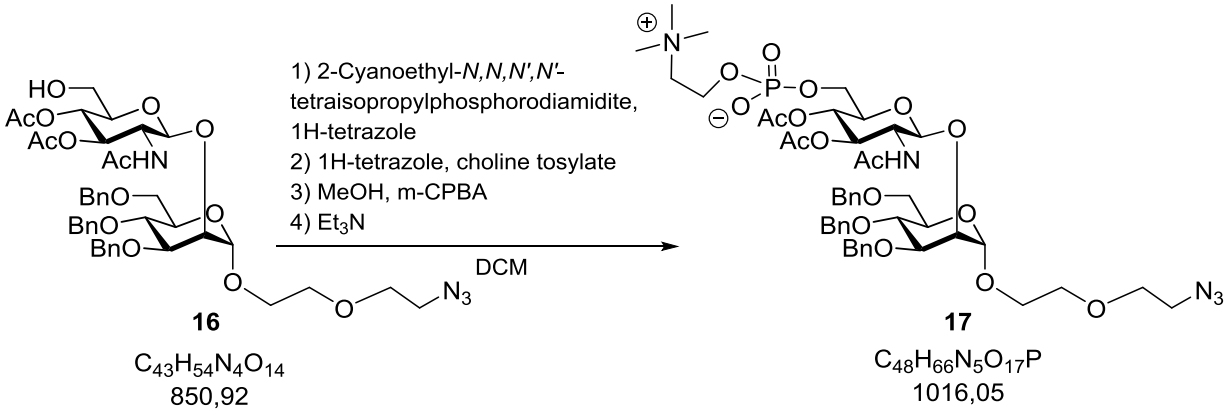
J=10.9, OC*H*$_2$Ph), 4.98 (apparent t, 1H, J=9.5, 4'-*H*), 5.07 (d, 1H, $J_{1',2'}$=8.2, 1'-*H*), 5.56-5.62 (stack, 2H, N*H* and 3'-*H*), 7.16-7.41 (stack, 15H, *Ph*)

^{13}C-NMR (150 MHz, $CDCl_3$): δ 20.84 (CH_3, OC(O)*C*H$_3$), 20.88 (CH_3, OC(O)*C*H$_3$), 23.34 (CH_3, NHC(O)*C*H$_3$), 50.72 (CH_2, N$_3$-*C*), 55.95 (CH, 2'-*C*), 61.74 (CH_2, 6'-*C*), 66.97 (CH_2, 6-*C*), 69.45 (CH, 4'-*C*), 69.52 (CH_2, O*C*H$_2$), 70.17 (CH_2, O*C*H$_2$), 70.30 (CH_2, O*C*H$_2$), 71.67 (CH, 3'-*C*), 72.08 (CH_2, O*C*H$_2$Ph), 72.18 (CH, 5-*C*), 73.37 (CH_2, O*C*H$_2$Ph), 74.26 (CH, 5'-*C*), 74.46 (CH, 2-*C*), 74.83 (CH_2, O*C*H$_2$Ph), 74.86 (CH, 4-*C*), 78.19 (CH, 3-*C*), 97.90 (CH, 1-*C*), 98.60 (CH, 1'-*C*), 127.72 (CH, *Ph*), 127.81 (CH, *Ph*), 127.89 (CH, *Ph*), 128.12 (CH, br, *Ph*), 128.50 (CH, *Ph*), 128.54 (CH, br, *Ph*), 138.41 (quart. C, ipso *Ph*), 138.50 (quart. C, ipso *Ph*), 138.52 (quart. C, ipso *Ph*), 170.26 (quart. C, *C*=O), 170.51 (quart. C, *C*=O), 171.12 (quart. C, *C*=O)

HR-MS: *m/z* calculated for $[M+K]^+$: 889.3268; found: 889.3269

Optical Rotation: $[\alpha]_D^{20}$ +11.8 (c 1.1, $CHCl_3$)

4.13. 2-Acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-phosphocholine-β-D-glucopyranosyl-(1→2)-[2-(2-azidoethoxy)ethyl] 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (17)



2-Cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphorodiamidite (34 µL, 33 mg, 0.108 mmol) was added to a suspension of disaccharide **16** (36.8 mg, 0.043 mmol) and molecular sieves 4 Å (80 mg) in 1.1 mL of dry DCM and stirred for 25 min at room temperature. 1H-Tetrazole (0.45 M solution in dry acetonitrile, 106 µL, 0.048 mmol) was added under stirring. Dried choline tosylate (obtained by repeated coevaporation of water with toluene under reduced pressure, high vacuum; 72 mg, 0.260 mmol) was added after 30 min of stirring. The mixture was stirred for 10 min and a second portion of 1H-tetrazole (0.45 M solution, 460 µL, 0.207 mmol) was added. After 1 h of stirring *m*-CPBA (base washed; 36 mg, 0.208 mmol) was added at 0°C and the mixture was warmed to room temperature. After 40 min the mixture was again cooled down to 0°C and Et_3N (300 µL, 2.16 mmol) was added under stirring. The mixture was then stored at +4°C.

Next day the mixture was stirred at room temperature for 3 h. The mixture was filtered over celite and the solvent was removed from the filtrate under reduced pressure. The crude product was purified using MPLC (manual gradient: DCM / MeOH / H_2O / EtOAc (12:5:1:5 to 12:5:1:0) to give a colorless hygroscopic solid.

Yield: 19.4 mg (44%)

TLC: R_f=0.15 (HPTLC; DCM / MeOH / H_2O / EtOAc (12:5:1:5))

^{1}H-NMR (600 MHz, MeOD): δ 1.88 (s, 3H, NHC(O)C*H*$_3$), 1.98 (s, 3H, OC(O)C*H*$_3$), 2.05 (s, 3H, OC(O)C*H*$_3$), 3.10 (s, 9H, N(C*H*$_3$)$_3$), 3.34 (m, 2H, N_3-C*H*$_2$), 3.43 (m, 2H, $(CH_3)_3$NC*H*$_2$), 3.58 (apparent dd, 1H, J=10.7, J=6.5, OC*H*$_2$), 3.60-3.70 (stack, 7H, 5H of OC*H*$_2$, 4-*H*, 6a-*H*), 3.79 (m, 1H, 5-*H*), 3.82 (m, 1H, 5'-*H*), 3.86 (m, 1H, 6b-*H*), 3.90 (dd, 1H, $J_{3,4}$=9.3, $J_{2,3}$=3.3, 3-*H*), 3.94 (dd, 1H, $J_{2',3'}$=10.7, $J_{1',2'}$=8.6, 2'-*H*), 3.98 (m, 1H, 6'a-*H*), 4.11 (ddd, 1H, $J_{6'a,6'b}$=11.4, J=5.9, J=2.8, 6'b-*H*), 4.14 (m, 2H, OP(O)OC*H*$_2$), 4.30 (dd, 1H, $J_{2,3}$=3.3, $J_{1,2}$=2.1, 2-*H*), 4.46 (d, 1H, J=11.0, OC*H*$_2$Ph), 4.49 (d, 1H, J=12.0, OC*H*$_2$Ph), 4.52 (d, 1H, J=11.7, OC*H*$_2$Ph), 4.55 (d, 1H, J=12.1, OC*H*$_2$Ph), 4.79 (d, 1H, J=10.7, OC*H*$_2$Ph), 4.80 (d, 1H, $J_{1',2'}$=8.5, 1'-*H*), 4.84 (d, 1H, J=11.6, OC*H*$_2$Ph), 4.91 (d, 1H, $J_{1,2}$=1.9, 1-*H*), 5.05 (apparent t, 1H, J=9.7, 4'-*H*), 5.24 (dd, 1H, $J_{2',3'}$=10.7, $J_{3',4'}$=9.3, 3'-*H*), 7.17-7.20 (stack, 2H, *Ph*),

7.25-7.37 (stack, 11H, *Ph*), 7.44-7.47 (stack, 2H, *Ph*)

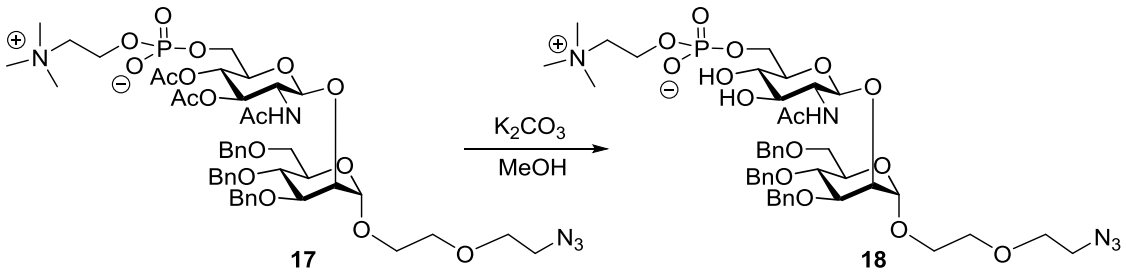
^{13}C-NMR (150 MHz, MeOD): δ 20.61 (CH_3, OC(O)*C*H_3), 20.77 (CH_3, OC(O)*C*H_3), 23.09 (CH_3, NHC(O)*C*H_3), 51.76 (CH_2, N_3-*C*), 54.68 (CH_3, apparent t, $J_{C,P}$=3.6, N(*C*H_3)$_3$), 55.38 (CH, 2'-*C*), 60.37 (CH_2, apparent d, $J_{C,P}$=5.6, OP(O)O*C*H_2), 65.59 (CH_2, apparent d, $J_{C,P}$=5.5, 6'-*C*), 67.43 (CH_2, apparent q, $J_{C,P}$=3.3, (CH_3)$_3$N*C*H_2), 68.34 (CH_2, 6-*C*), 70.75 (CH, 4'-*C*), 71.06 (CH_2, O*C*H_2), 71.11 (CH_2, O*C*H_2), 71.18 (CH_2, O*C*H_2), 71.68 (CH_2, O*C*H_2Ph), 72.61 (CH, 5-*C*), 74.17 (CH, 3'-*C*), 74.23 (CH_2, O*C*H_2Ph), 74.38 (CH, apparent d, $J_{C,P}$=8.0, 5'-*C*), 74.81 (CH, 2-*C*), 75.72 (CH_2, O*C*H_2Ph), 75.99 (CH, 4-*C*), 79.22 (CH, 3-*C*). 98.85 (CH, 1-*C*), 100.77 (CH, 1'-*C*), 128.61 (CH, *Ph*), 128.74 (CH, *Ph*), 128.99 (CH, *Ph*), 129.12 (CH, *Ph*), 129.29 (CH, *Ph*), 129.43 (CH, *Ph*), 129.49 (CH, *Ph*), 129.65 (CH, *Ph*), 139.55 (quart. C, ipso *Ph*), 139.88 (quart. C, br, ipso *Ph*), 171.50 (quart. C, *C*=O), 171.90 (quart. C, *C*=O), 173.81 (quart. C, *C*=O)

^{31}P-NMR (243 MHz, MeOD): δ *-1.38*

HR-MS: *m/z* calculated for $[M+K]^+$: 1054.3823; found: 1054.3836

Optical Rotation: $[\alpha]_D^{20}$ -4.4 (c 0.8, MeOH)

4.14. 2-Acetamido-2-deoxy-6-*O*-phosphocholine-β-D-glucopyranosyl-(1→2)-[2-(2-azidoethoxy)ethyl] 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (18)



Phosphodiester **17** (12.4 mg, 0.012 mmol) was dissolved in 0.7 mL of dry MeOH and a cat. amount of K_2CO_3 was added under stirring. After 4 h the mixture was diluted with MeOH and pH was set to 6 by addition of Dowex 50 (H^+). The resin was filtered off and the solvent was removed from the filtrate under reduced pressure to give a hygroscopic solid.

Yield: 10.73 mg (94%)

TLC: R_f=0.39 (HPTLC; DCM / MeOH / H_2O (12:5:1))

^{1}H-NMR (600 MHz, MeOD): δ 1.97 (s, 3H, NHC(O)CH_3), 3.09 (s, 9H, N(CH_3)$_3$), 3.33 (m, 2H, N_3-CH_2), 3.36-3.44 (stack, 3H, $(CH_3)_3$NCH_2, 5'-*H*), 3.49-3.55 (stack, 2H, 3'-*H*, 4'-*H*), 3.59-3.72 (stack, 9H, 3xOCH_2, 4-*H*, 6a-*H*, 2'-*H*), 3.80 (ddd, 1H, J=9.9, J=6.8, J=1.8, 5-*H*), 3.84-3.88 (m, 1H, 6b-*H*), 3.90 (dd, 1H, $J_{3,4}$=9.4, $J_{2,3}$=3.3, 3-*H*), 4.11-4.21 (stack, 3H, OP(O)OCH_2 and 6'a-*H*), 4.24 (dd, 1H, $J_{2,3}$=3.3, $J_{1,2}$=2.0, 2-*H*), 4.26 (m, 1H, 6'b-*H*), 4.46-4.58 (stack, 5H, 4H of OCH_2Ph, 1'-*H*), 4.79-4.84 (stack, 2H, OCH_2Ph), 4.90 (d, 1H, $J_{1,2}$=1.8, 1-*H*), 7.19-7.22 (stack, 2H, *Ph*), 7.24-7.37 (stack, 11H, *Ph*), 7.44-7.47 (stack, 2H, *Ph*)

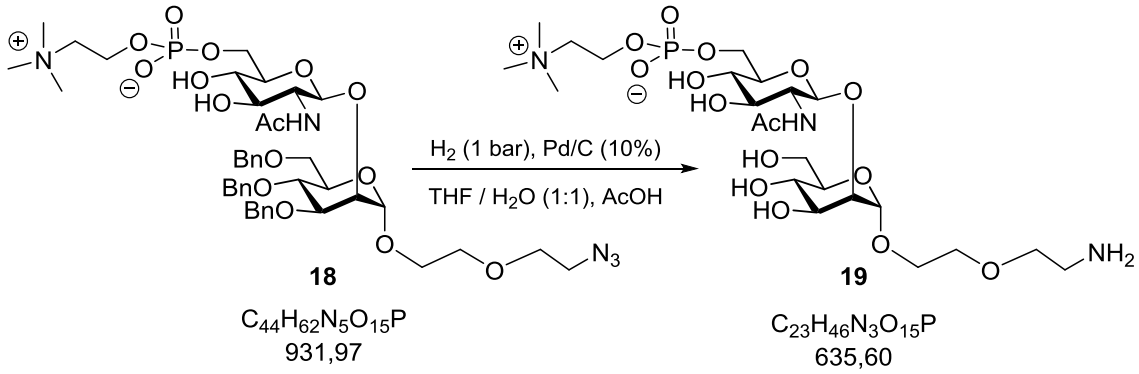
^{13}C-NMR (150 MHz, MeOD): δ 23.40 (CH_3, NHC(O)CH_3), 51.75 (CH_2, N_3-*C*), 54.78 (CH_3, m, N(CH_3)$_3$), 57.34 (CH, 2'-*C*), 60.49 (CH_2, apparent d, $J_{C,P}$=5.0, OP(O)OCH_2), 65.63 (CH_2, apparent d, $J_{C,P}$=5.5, 6'-*C*), 67.34 (CH_2, m, $(CH_3)_3$NCH_2), 68.37 (CH_2, 6-*C*), 71.12 (CH_2, OCH_2), 71.19 (CH_2, OCH_2), 71.28 (CH_2, OCH_2), 71.40 (CH, 4'-*C*), 71.89 (CH_2, OCH_2Ph), 72.67 (CH, 5-*C*), 74.23 (CH_2, OCH_2Ph), 75.04 and 75.07 (CH, 2-*C*, 3'-*C*), 75.72 (CH_2, OCH_2Ph), 76.22 (CH, 4-*C*), 77.08 (CH, apparent d, $J_{C,P}$=7.2, 5'-*C*), 79.54 (CH, 3-*C*), 99.05 (CH, 1-*C*), 101.68 (CH, 1'-*C*), 128.63 (CH, *Ph*), 128.77 (CH, *Ph*), 128.92 (CH, *Ph*), 129.14 (CH, *Ph*), 129.33 (CH, *Ph*), 129.44 (CH, *Ph*), 129.56 (CH, *Ph*), 129.61 (CH, *Ph*), 139.55 (quart. C, ipso *Ph*), 139.90 (quart. C, ipso *Ph*), 174.03 (quart. C, *C*=O)

^{31}P-NMR (243 MHz, MeOD): δ -0.64

HR-MS: *m/z* calculated for $[M+Na]^+$: 954.3872; found: 954.3871

Optical Rotation: $[\alpha]_D^{20}$ -13.2 (c 0.6, MeOH)

4.15. 2-Acetamido-2-deoxy-6-*O*-phosphocholine-β-D-glucopyranosyl-(1→2)-[2-(2-aminoethoxy)ethyl] α-D-mannopyranoside (19)



Pd/C (10%, 2.1 mg) and phosphodiester **18** (6.3 mg, 6.8 µmol) were suspended in 0.75 mL of THF / H_2O (1:1, 1% acetic acid). H_2 was bubbled through the mixture using a balloon under stirring. After 2 days under H_2 atmosphere (1 bar) the mixture was filtered through paper and the solvents were removed from the filtrate under reduced pressure (repeated coevaporation of acetic acid with heptane). The residue was dissolved in water and the solution was passed 5 times over Dowex 50 (H^+, 400 mg) to remove the byproduct (primary alcohol instead of amine). The resin was washed with water and the product was eluted with 2.5% NH_3 (aq.). The solvent was removed under reduced pressure and the amine was obtained as hygroscopic solid.

Yield: 2.73 mg (64%)

^{1}H-NMR (600 MHz, D_2O): δ 2.02 (s, 3H, NHC(O)C*H*$_3$), 3.04 (m, 2H, H_2N-C*H*$_2$), 3.19 (s, 9H, N(C*H*$_3$)$_3$), 3.46 (m, 1H, 4-*H*), 3.51-3.74 (stack, 13H, 5H of OC*H*$_2$, 5-*H*, 6a-*H*, 2'-*H*, 3'-*H*, 4'-*H*, 5'-*H*, $(CH_3)_3$NC*H*$_2$), 3.78 (dd, 1H, $J_{3,4}$=9.6, $J_{2,3}$=3.4, 3-*H*),

3.84-3.90 (stack, 2H, 1H of OCH_2, 6b-*H*), 4.04-4.10 (stack, 2H, 2-*H*, 6'a-*H*), 4.15 (dd, 1H, $J_{6'a,6'b}$=10.8, $J_{5',6'b}$=5.0, 6'b-*H*), 4.30 (m, 2H, OP(O)OCH_2), 4.57 (d, 1H, $J_{1',2'}$=8.5, 1'-*H*), 4.87 (d, 1H, $J_{1,2}$=1.2, 1-*H*)

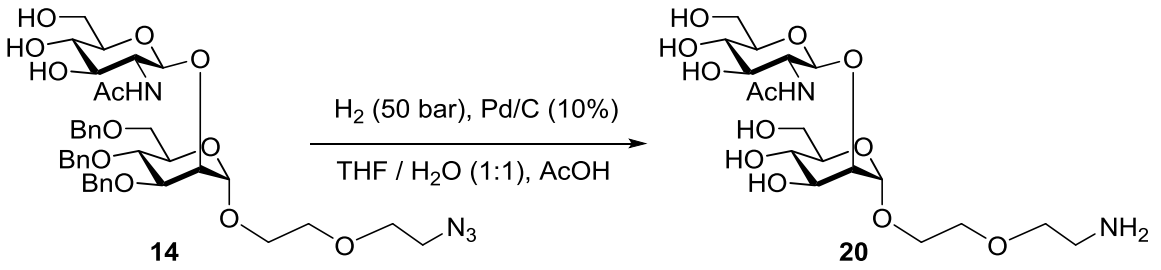
^{13}C-NMR (150 MHz, D_2O): δ 23.13 (CH_3, NHC(O)*C*H_3), 40.14 (CH_2, H_2N-*C*H_2), 54.81 (CH_3, apparent t, $J_{C,P}$=3.5, N(*C*H_3)$_3$), 56.17 (CH, 2'-*C*), 60.38 (CH_2, d, $J_{C,P}$=5.6, OP(O)O*C*H_2), 62.38 (CH_2, 6-*C*), 65.19 (CH_2, d, $J_{C,P}$=4.4, 6'-*C*), 66.83 (CH_2, m, (CH_3)$_3$N*C*H_2), 67.51 (CH_2, O*C*H_2), 68.13 (CH, 4-*C*), 69.14 (CH_2, O*C*H_2), 70.23 (CH, 4'-*C*), 70.30 (CH_2, O*C*H_2), 70.37 (CH, 3-*C*), 73.79 (CH, 5-*C*), 73.99 (CH, 3'-*C*), 75.38 (CH, d, $J_{C,P}$=8.0, 5'-*C*), 77.18 (CH, 2-*C*), 97.83 (CH, 1-*C*), 100.32 (CH, 1'-*C*), 175.56 (quart. C, *C*=O)

^{31}P-NMR (243 MHz, D_2O): δ -0.11

HR-MS: *m/z* calculated for $[M+H]^+$: 636.2740; found: 636.2739

Optical Rotation: $[\alpha]_D^{20}$ -2.3 (c 0.5, H_2O)

4.16. 2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-(2-aminoethoxy)ethyl] α-D-mannopyranoside (20)



14
$C_{39}H_{50}N_4O_{12}$
766,85

20
$C_{18}H_{34}N_2O_{12}$
470,47

Disaccharide **14** (4.7 mg, 6.1 µmol) was dissolved in 5 mL of THF / H_2O (1:1, 1% AcOH) and the solution was passed through an H-Cube® (Pd/C 10%, 0.2 mL/min,

50 bar H_2) twice. The solvents were removed under reduced pressure (repeated coevaporation of acetic acid with heptane). The residue was dissolved in water and the solution was passed over Dowex 50 ion exchange resin (H^+, 500 mg). The resin was washed with water and the product was eluted with 2.5% NH_3 (aq.). The solvent was removed under reduced pressure and the amine was obtained as hygroscopic solid.

Yield: 1.7 mg (59%)

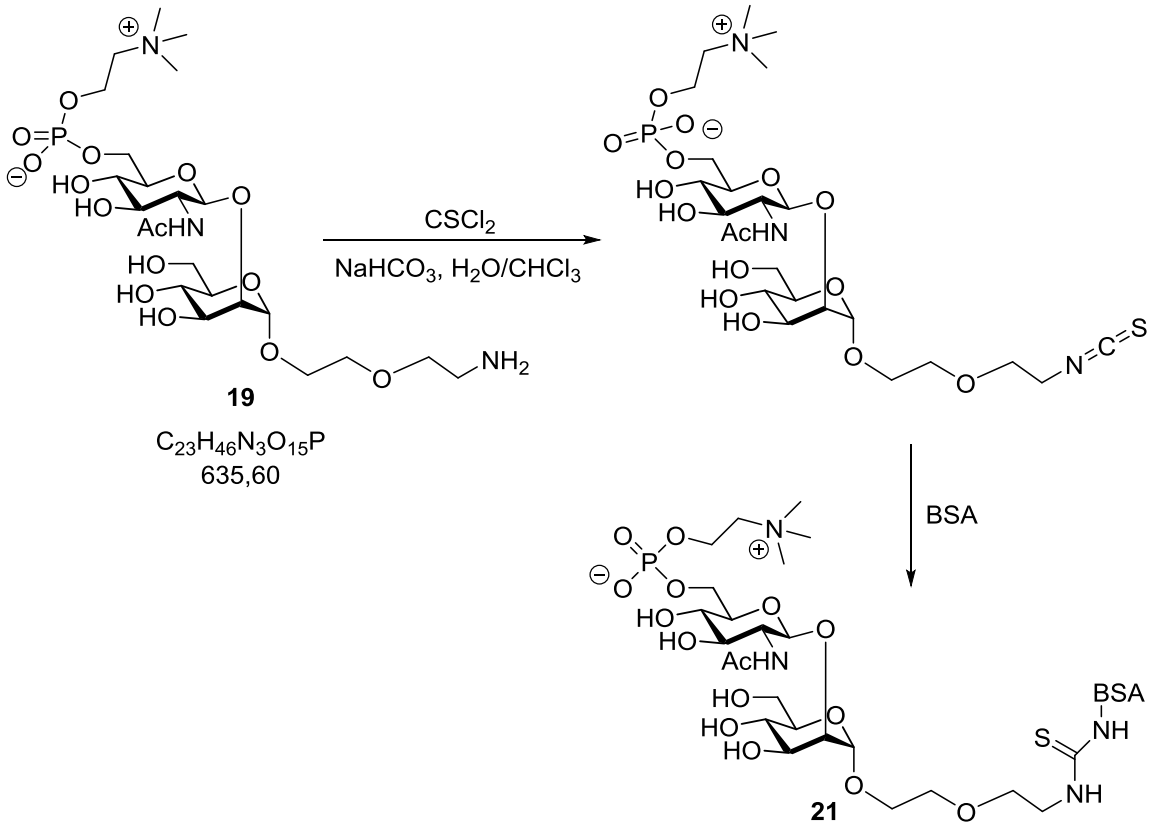
^{1}H-NMR (600 MHz, D_2O): δ 2.02 (s, 3H, NHC(O)C*H*$_3$), 2.99 (apparent t, 2H, J=5.0, H_2N-C*H*$_2$), 3.37-3.75 (stack, 13H, 5H of OC*H*$_2$, 4-*H*, 5-*H*, 6a-*H*, 2'-*H*, 3'-*H*, 4'-*H*, 5'-*H*, 6'a-*H*), 3.80 (dd, 1H, $J_{3,4}$=9.7, $J_{2,3}$=3.4, 3-*H*), 3.83-3.91 (stack, 3H, 1H of OC*H*$_2$, 6b-*H*, 6'b-*H*), 4.06 (m, 1H, 2-*H*), 4.53 (d, 1H, $J_{1',2'}$=8.3, 1'-*H*), 4.86 (d, 1H, $J_{1,2}$=1.5, 1-*H*)

^{13}C-NMR (150 MHz, D_2O): δ 23.15 (CH_3, NHC(O)*C*H$_3$), 40.32 (CH_2, H_2N-*C*H$_2$), 56.22 (CH, 2'-*C*), 61.50 (CH_2, 6'-*C*), 62.41 (CH_2, 6-*C*), 67.54 (CH_2, O*C*H$_2$), 68.13 (CH, 4-*C*), 70.28 (CH_2, O*C*H$_2$), 70.42 (CH, 3-*C*), 70.78 (CH, 4'-*C*), 73.80 (CH, 5-*C*), 74.18 (CH, 3'-*C*), 76.72 (CH, 5'-*C*), 77.12 (CH, 2-*C*), 97.97 (CH, 1-*C*), 100.36 (CH, 1'-*C*), 175.62 (quart. C, *C*=O)

HR-MS: *m/z* calculated for $[M+K]^+$: 509.1743; found: 509.1752

Optical Rotation: $[\alpha]_D^{20}$ -1.5 (c 0.8, H_2O)

4.17. BSA conjugate of the phosphodiester (21)



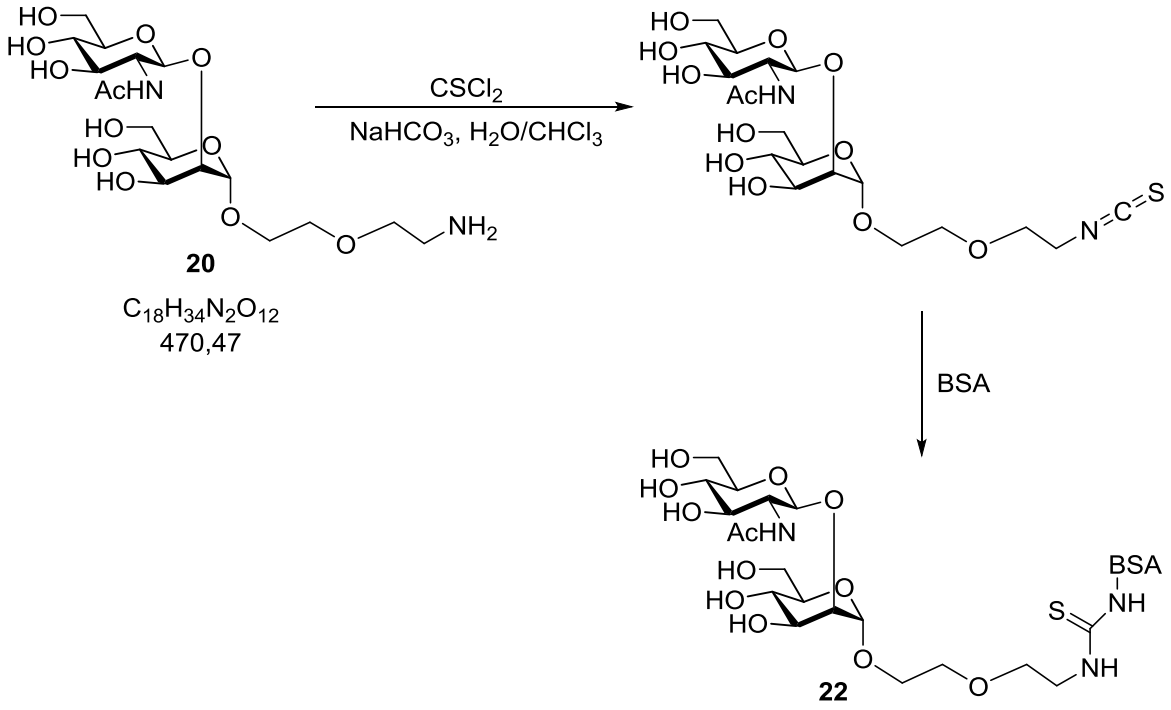
$C_{23}H_{46}N_3O_{15}P$
635,60

Phosphodiester **19** (2.1 mg, 3.3 µmol) was dissolved in 2 mL of 0.1 M $NaHCO_3$ (aq.) and thiophosgene (4 µL, 6 mg, 52 µmol, dissolved in 2 mL of $CHCl_3$) was added. The mixture was vigorously stirred for 3 h. Remaining thiophosgene was removed by repeated extraction with $CHCl_3$. Traces of $CHCl_3$ in the aqueous layer were removed by stirring under a stream of air. BSA (2.0 mg, 0.030 µmol, dissolved in 2 mL 0.1 M $NaHCO_3$, 0.3 M NaCl) was added and the mixture was stirred for 3 days. The solution was dialyzed against water (2x1.5 L) for 4 days and lyophilized.

Yield: 2.3 mg

MALDI-TOF-MS showed a mass difference (measured from the maxima) of 2327 Da to “naked” BSA, which means that in average 3.4 eq. of the carbohydrate were bound to the protein.

4.18. BSA conjugate of the reference compound (22)



20

$C_{18}H_{34}N_2O_{12}$
470,47

22

Disaccharide **14** (2.6 mg, 5.5 µmol) was dissolved in 2 mL of 0.1 M $NaHCO_3$ and thiophosgene (4 µL, 6 mg, 52 µmol, dissolved in 2 mL of $CHCl_3$) was added. The mixture was vigorously stirred for 100 min. Remaining thiophosgene was removed by repeated extraction with $CHCl_3$. Traces of $CHCl_3$ in the aqueous layer were removed by stirring under a stream of air. BSA (2.1 mg, 0.032 µmol, dissolved in 2 mL 0.1 M $NaHCO_3$, 0.3 M NaCl) was added and the mixture was stirred for 3 days. The solution was dialyzed against water (2x1.5 L) for 3 days and lyophilized.

Yield: 4.1 mg

MALDI-TOF-MS showed a mass difference (measured from the maxima) of 3696 Da to “naked” BSA, which means that in average 7.2 eq. of the carbohydrate were bound to the protein.

5. Conclusion and Outlook

A synthetic route to 2-acetamido-2-deoxy-6-*O*-phosphocholine-β-D-glucopyranosyl-(1→2)-[2-(2-aminoethoxy)ethyl] α-D-mannopyranoside was established, in which occurring issues (formation of side products, purification) could be solved or minimized to an acceptable level. The target compound was obtained in sufficient amount not only for characterization via NMR spectroscopy, mass spectrometry and optical rotation, but also for the preparation of BSA conjugates that were passed to the group of Iain B. H. Wilson to perform carbohydrate-protein-interaction studies based on Western Blot and glycoarray analysis. The non-PC-substituted counterparts were also provided and should serve as reference compounds. The planning of further steps is dependent on the results of these biological tests. If additional tests (potentially with a variety of proteins) are contemplated and larger amounts of the substances are required, upscaling is possible basically with the established synthetic route.

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Abstract

Glycans as parts of glycoproteins and glycolipids are known to play a major role in cell-to-cell communication. Carbohydrate structures that are produced by parasitic organisms like certain fungi, bacteria and nematodes and bear phosphocholine moieties are of special interest, as they have been found to contribute to the capability of these organisms to modulate the immune system of their hosts. Studies of these glycan-protein interactions require substances of high purity that are difficult to obtain out of biological sources due to purification issues. Thus the aim of this thesis was to prepare 2-acetamido-2-deoxy-6-*O*-phosphocholine-β-D-glucopyranosyl-(1→2)-[2-(2-aminoethoxy)ethyl] α-D-mannopyranoside synthetically. The target compound is equipped with a linker that can be bound covalently to other molecules through its amine functionality. This can be used not only for the preparation of conjugates with bovine serum albumin (for immunoblots), but also for immobilization on glycoarrays.

The developed synthetic route (including two glycosylation reactions, multiple protecting group operations and the formation of a phosphodiester moiety) has proved to be suitable for the preparation of the target compound starting from D-mannose. The obtained amount has been sufficient for chemical characterization via NMR spectroscopy, mass spectrometry and optical rotation. Furthermore conjugates with BSA have been prepared and passed to the group of Iain B. H. Wilson that will perform carbohydrate-protein-interaction studies based on Western Blot and glycoarray analysis. The non-PC-substituted counterparts have also been prepared and will serve as reference compounds.

Kurzfassung

Glykane als Bestandteile von Glykoproteinen und Glykolipiden spielen eine wichtige Rolle in der Zell-Zell-Kommunikation. Kohlenhydratstrukturen, die von parasitären Organismen wie bestimmten Pilzen, Bakterien und Nematoden produziert werden und Phosphocholin-Substituenten tragen, sind hierbei von besonderem wissenschaftlichen Interesse, da sie mit der Fähigkeit der genannten Parasiten, das Immunsystem ihrer Wirte zu beeinflussen, in Verbindung gebracht werden. Um die Wechselwirkungen dieser Glykane mit Proteinen zu untersuchen, werden sie in hoher Reinheit benötigt, die bei Gewinnung aus biologischen Quellen aufgrund der Schwierigkeiten, die mit der Aufreinigung komplexer Mischungen von polaren Substanzen verbunden sind, nicht immer gewährleistet werden kann. Ziel dieser Arbeit ist es daher gewesen, 2-Acetamido-2-Deoxy-6-*O*-Phosphocholin-β-D-Glucopyranosyl-(1→2)-[2-(2-Aminoethoxy)ethyl] α-D-Mannopyranosid synthetisch herzustellen. Die Zielverbindung enthält einen Linker, der über die enthaltene Aminofunktion kovalent an andere Moleküle gebunden werden kann. Auf diese Weise können sowohl Konjugate mit Rinderserumalbumin (für Immunoblots) hergestellt werden, als auch die Kohlenhydratstruktur auf Glycoarrays immobilisiert werden.

Die im Zuge dieser Arbeit entwickelte, von D-Mannose ausgehende Syntheseroute, die zwei Glykosylierungsreaktionen, mehrere Schutzgruppenoperationen sowie die Bildung einer Phosphodiestergruppe beinhaltet, hat die Herstellung der Zielverbindung in ausreichender Menge ermöglicht, sodass eine chemische Charakterisierung mittels Kernresonanzspektroskopie, Massenspektrometrie und optischer Drehung möglich gewesen ist. Darüber hinaus sind Rinderserumalbumin-Konjugate hergestellt worden, die an die Arbeitsgruppe von Iain B. H. Wilson weitergeleitet worden sind, die damit Kohlenhydrat-Protein-Wechselwirkungsstudien auf Western-Blot- und Glycoarray-Basis durchführen wird. Als Vergleichssubstanzen dienen dabei die jeweiligen Verbindungen ohne Phosphocholin-Substituenten, die ebenfalls im Zuge dieser Arbeit hergestellt worden sind.