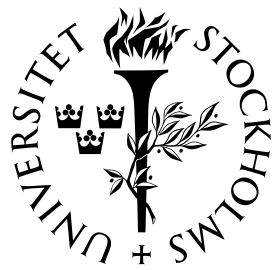
Synthesis of oligosaccharides related to the capsular polysaccharide of *Neisseria meningitidis* serotype A

by

Peter Teodorović



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Abstract

In order to find suitable stable vaccine candidates against *Neisseria meningitidis* group A, several structures related to the capsular polysaccharide have been synthesised. The first part of the thesis describes the synthesis of C-phosphonate analogues starting from glucose. The key step is a Mitsunobu coupling of a methyl C-phosphonate monomer to the 6-hydroxyl group of a 2-acetamido mannose derivative. Contained within this work is a description of an improved synthesis of 2-azido-2-deoxy-D-mannopyranose. The second part outlines the synthesis of structural elements present in the native capsular polysaccharide of *Neisseria meningitidis* serotype A including different acetylation and phosphorylation patterns. The final chapter describes an improved synthesis of the Lewis b hexasaccharide needed for purification of and interaction studies with the *Helicobacter pylori* adhesin BabA.

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List of Papers

This thesis is based on the following papers, which will be referred to by their Roman numerals **I-IV**.

I. Improved Synthesis of 1,3,4,6-tetra-*O*-acetyl-2-Azido-2-deoxy-α-D-mannopyranose
Peter Teodorović, Rikard Slättegård and Stefan Oscarson
Submitted for publication in Carbohydr. Res.

II. Synthesis of C-Phosphonate Analogues of *Neisseria meningitidis* group A Capsular Polysaccharide Structures
Peter Teodorović and Stefan Oscarson
Manuscript

III. Synthesis of Structures Corresponding to the Capsular Polysaccharide of *Neisseria meningitidis* Group A
Rikard Slättegård, Peter Teodorović, Henok Hadgu Kinfe, Neil Ravenscroft, David W. Gammon and Stefan Oscarson
Submitted for publication in Org. Biomol. Chem.

IV. Synthesis of the Lewis b hexasaccharide and HSA-conjugates thereof
Martina Lahmann, Linnea Bülow, Peter Teodorović, Helena Gybäck and Stefan Oscarson
Glycoconj. J. 21 (2004) 251-256.

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Abbreviations

AgOTf	silver trifluoromethanesulfonate (silver triflate)
BuLi	butyl lithium
CPS	capsular polysaccharide
DBU	1,8-diaza-7-bicyclo[5.4.0]undecene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DIAD	diisopropyl azodicarboxylate
DPPA	diphenylphosphonic azide
EDA	ethylenediamine
Fuc	fucose
Gal	galactose
Glc	glucose
Man	mannose
mCPBA	3-chloroperoxybenzoic acid
NIS	*N*-iodosuccinimide
PivCl	pivaloyl chloride
TBAF	tetra *n*-butylammonium fluoride
TEA	triethylamine
Tf_2O	trifluoromethanesulfonic anhydride (triflic anhydride)
TMS	trimethylsilyl
TREAT-HF	triethylamine tris(hydrogen fluoride)
Z	benzyloxycarbonyl

1. General introduction

1.1 The bacterium and the disease

Neisseria meningitidis serogroup A is a bacterium that causes epidemic outbreaks of meningitis, mainly in parts of Africa south of the Sahara.[1] Meningitis is an infection of the meninges, the thin lining that surrounds the brain and spinal cord. Several kinds of bacteria can cause meningitis, and *N. meningitidis* is one of the most important, others being *Streptococcus pneumoniae* and *Haemophilus influenzae* type b. There are several subgroups of *N. meningitidis*, which are differentiated by the structure of the capsular polysaccharide that surrounds the bacterium. A capsular polysaccharide is a protective extracellular polysaccharide that surrounds parts of, or the whole of, certain bacteria. The presence of a capsular polysaccharide is often associated with virulence. Twelve serogroups of *N. meningitidis* have been identified, of which groups A, B, C, Y and W135 are known to cause epidemics. The serogroups differ in pathogenicity and in immunogenicity. The nasopharynx is a common reservoir for asymptomatic carriage; the bacteria are carried by between 10 to 25% of the population. In most cases, the disease is acquired through exposure to asymptomatic carriers, and then, sometimes, for reasons not fully understood, the bacteria overwhelm the body's defences and the infection spreads through the bloodstream to the brain. In Africa, there were about 800,000 cases of the disease between 1970 and 1992. This combined with the devastating effects of the disease make finding a well working vaccine to control the disease a matter of urgency. Even with antibiotic treatment, the mortality is still 10%, compared to 60-80% without treatment, and the disease also causes severe and permanent neurological defects in survivors. The need for an effective vaccine is further underlined by the increasing antibiotic resistance that is being observed worldwide.

1.2 Vaccines

In 1798, Jenner experimentally proved that injection of pus from cowpox gave immunity towards the related virus that causes smallpox, and in doing so coined the term vaccine, derived from the Latin name for cow, vacca. Although Jenner's work was rejected by the Royal Society of Medicine, as unconvincing and incomplete, the method was soon widely accepted and used on a large scale. The first vaccination against smallpox in Sweden was carried out in 1801 and 1816 it was compulsory to vaccinate all children.[2] Vaccinations against smallpox have now been carried out worldwide, and in 1980 the World Health Organisation (WHO) declared that the disease was eradicated, with the last reported case in 1977 in Somalia.[3] This demonstrates the power of vaccines; a disease that in the 18th century killed 60 million people (of which 90% were children) has now been totally eliminated. The hope is that a similar result can be achieved with meningitis caused by *N. meningitidis* serogroup A, but this will require the development of a good vaccine and a large effort, similar to that of the WHO in the 1960's and 70's for the elimination of smallpox. In principle, vaccinations are a much more cost efficient way of controlling a disease than other therapies, and the cost is a crucial factor, especially in the developing world.[4]

1.3 The immune system

Polysaccharides and other large molecules can cause an immune response even though they are not proteins. They are referred to as T-cell independent antigens, which means, that they can give an immune response without the involvement of T-cells (thymus-derived cells). This response lacks several important properties that characterise the T-cell dependent immune response, such as immunological memory, class-switch from IgM to IgG, and affinity maturation. These antigens trigger the B-cells (bone-marrow-derived cells) to proliferate and form antibodies without the help of T-cells. Upon initiation of an immune response, all antibodies are of the IgM-class, but after a couple of days, antibodies of other types start to form, mainly IgG. The main functional difference between the two types is that the IgM antibodies generally bind less tightly and with less specificity than the IgG antibodies.
When the immune system encounters a T-dependent antigen for the first time, in a primary immune response, memory cells are formed, so when the antigen is seen again a faster and stronger immune response ensues (a secondary response). One way to stimulate a secondary response is by booster vaccinations, i.e. immunising more than once.

1.4 Glycoconjugate vaccines

A significant limitation with the current, purely carbohydrate based vaccines against *N. meningitidis* is that the immune system of small children, under around two years of age does not respond. Unfortunately, this is the age group at greatest risk for bacterial meningitis. Even in older children and adults, these vaccines induce only short term immunity, which has usually dissipated within two years of vaccination. Another important limitation is that those vaccines do not prevent the asymptomatic carriage and hence allow the bacteria to maintain in the population allowing a base for new epidemics. If, on the other hand, the carbohydrate portion is connected to a carrier protein, forming a glycoconjugate, different parts of the immune system are triggered and this results in the creation of memory cells. Furthermore, this type of response is capable of giving protection to young children.[5] Glycoconjugate vaccines, as the name implies, are so called because they comprise a polysaccharide antigen linked to a protein. The polysaccharide part is usually a functionalised bacterial CPS, but it can also be synthetically derived portion or derivative thereof. Synthetic carbohydrate structures have a number of potential advantages over those based on carbohydrates from natural sources.[6] Firstly, naturally derived carbohydrates are heterogeneous mixtures and may include small amounts of natural impurities and contaminants. By contrast, synthetic carbohydrates can be produced as homogeneous single compounds in a controlled manner, with little or no batch-to-batch variability. Another advantage of synthetic structures is that they can be made to include functional groups for derivatisation or modifications of the carbohydrate moiety that are difficult or impossible to perform on the native material. The carrier protein is also an important factor in the modulation of the immunogenicity. Various carriers have been used for conjugation and the best results have been achieved using detoxified versions of strongly immunogenic proteins like diphtheria and tetanus toxins.

Against *N. meningitidis* group C, a working glycoconjugate vaccine has recently been developed.[7] In the case of *N. meningitidis* group Y and W135 the physical nature of the capsular polysaccharide is not a limitation for the development of glycoconjugate vaccines, however for *N. meningitidis* group B, it is difficult or even impossible to use the capsular polysaccharide as an antigen, because it consists of carbohydrate structures which are found in humans. Using the capsular polysaccharide from *N. meningitidis* group A as an antigen is also problematic because of the inherent instability of this structure[8] (*vide infra*).

1.5 Native structure and problems with stability

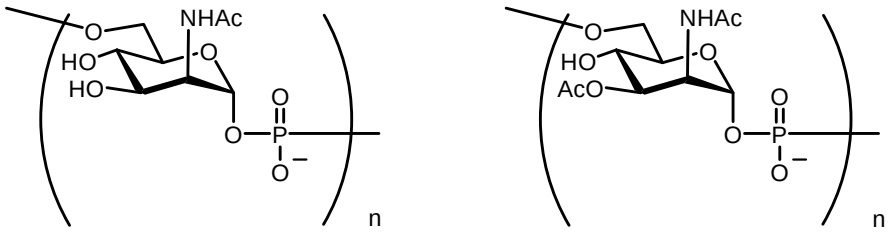


Figure 1.1 The repeating unit of *N. meningitidis* serogroup A capsular polysaccharide

The capsular polysaccharide of *N. meningitidis* serogroup A is composed of phosphodiester α-(1→6) linked *N*-acetylmannosamines (Fig. 1.1).[9,10] In the native structure, approximately 70-90 % is 3-*O*-acetylated with a small amount of 4-*O*-acetylated material also found, presumably forming as a result of acetyl migration.

Usually the easiest and most cost-efficient way to make a glycoconjugate vaccine is to use the native capsular polysaccharide structure of the bacterium and conjugate it to a suitable carrier protein. However, the anomeric diester linkage of the *N. meningitidis* CPS render vaccines prepared from it unstable (Fig. 1.2).

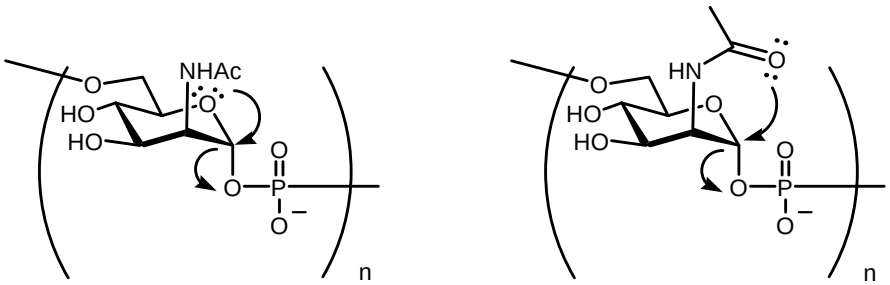


Figure 1.2 Mechanism for phosphodiester cleavage

Phosphodiesters are normally quite stable, but when they are present at the anomeric position of a carbohydrate residue, the linkage becomes much more labile due to the possibility of the electron pair on the ring oxygen displacing the phosphate or phosphodiester. This feature has been exploited in the use of anomeric phosphates as donors in glycosylation reactions in which the phosphate or phosphonate is activated with a catalytic amount of acid.[11] In the

case of *N. meningitidis* capsular polysaccharide there is a possible additional effect from the participating acetamido group at C-2 (Fig. 1.2).

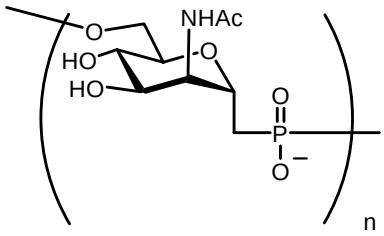


Figure 1.3 C-phosphonate analogue

Our approach to increasing the stability of the substrate (and thus to making a potential stabilised vaccine candidate) has been to replace the anomeric phosphodiester linkage with a C-phosphonate linkage (Fig. 1.3). Thus the first part of this work describes the synthesis of the dimer and trimer C-phosphonate analogues.
The second part of the thesis describes the synthesis of structures related to the native *N. meningitidis* capsular polysaccharide (with the phosphodiester intact) for binding studies and stability tests. Recent research has shown that the size of the antigen in conjugate vaccines required to induce an immune response can be much smaller than previously believed. For evaluation of the size needed for protection in the *N. meningitidis* serogroup A case, we have synthesised the repeating structure with a varying number of monomers. Some disaccharides and four trisaccharides (3,4-OH or 3,4-*O*-acetylated) both containing and lacking a terminal phosphate have been synthesised. These will be tested in immunisation studies after conjugation to a carrier protein. If larger structures are required, the synthesis is designed so that further elongation is possible using the same precursors and methodology developed for this described synthesis.

1.6 C-Phosphonic acid and phosphonates as isosteres for phosphates and phosphodiesters

Phosphonates and phosphodiesters have previously been investigated as potential metabolic regulators. The presumption is that the carbon-phosphorous bond is incapable of being hydrolysed by the ordinary enzymes involved in the phosphate cleavage.[12] Such functional group transformations are common in drug design and synthesis. Several examples exists where a natural R-O-R'

motif is replaced by R-CH_2-R' in attempts to make metabolically stable drugs or enzyme inhibitors.[13] The CF_2-group is considered a better replacement for oxygen than the CH_2-group because the lone pair electrons on the fluorine atoms mimic those of the oxygen atom. However, in our case such a substitution was considered to complex as a synthetic target; CF_2-isosteres of anomeric phosphates have been made, using radical chemistry on anomeric alkenes, but the method of formation strongly favours the β-product and gives much lower yields on pyranoses than on furanoses.[14]

In terms of length and angle constraints, the phosphonate linkage is very similar to that of the phosphodiester; the C-O-P-O distance is slightly shorter, about 0.8%, than the distance C-C-P-O, so the term isosteric is clearly reasonable for these systems.[12] Another consideration in using carbon substituted analogues of phosphates/ phosphodiesters is the change in pK_a of the molecule. The acidity of the molecule would be expected to decrease with the addition of the extra electron donating alkyl group, which could result in a different dissociation state for the analogue compared to the natural compound.

Probably the only way to really see if these structures are possible vaccine candidates is to synthesise them and test whether antibodies raised against them crossreact with the native capsular polysaccharide and give protection.

2. Synthesis of stable C-phosphonate analogues corresponding to *N. meningitidis* serogroup A capsular polysaccharide structure. (Papers I and II)

Paper II describes the synthesis of C-phosphonate analogues of the natural polymeric *N. meningitidis* group A capsular polysaccharide structure. The target molecules (Fig. 2.1) are equipped with a spacer to facilitate conjugation of the molecules to a carrier protein for immunogenic studies.

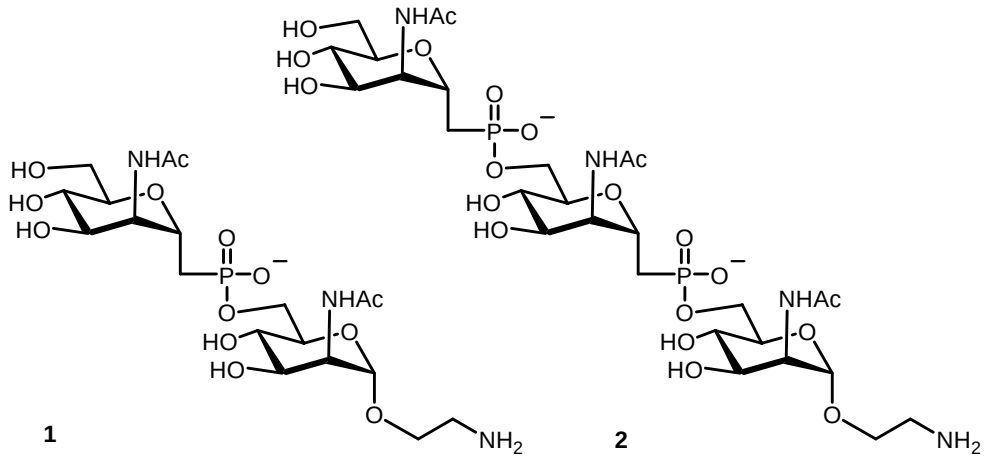
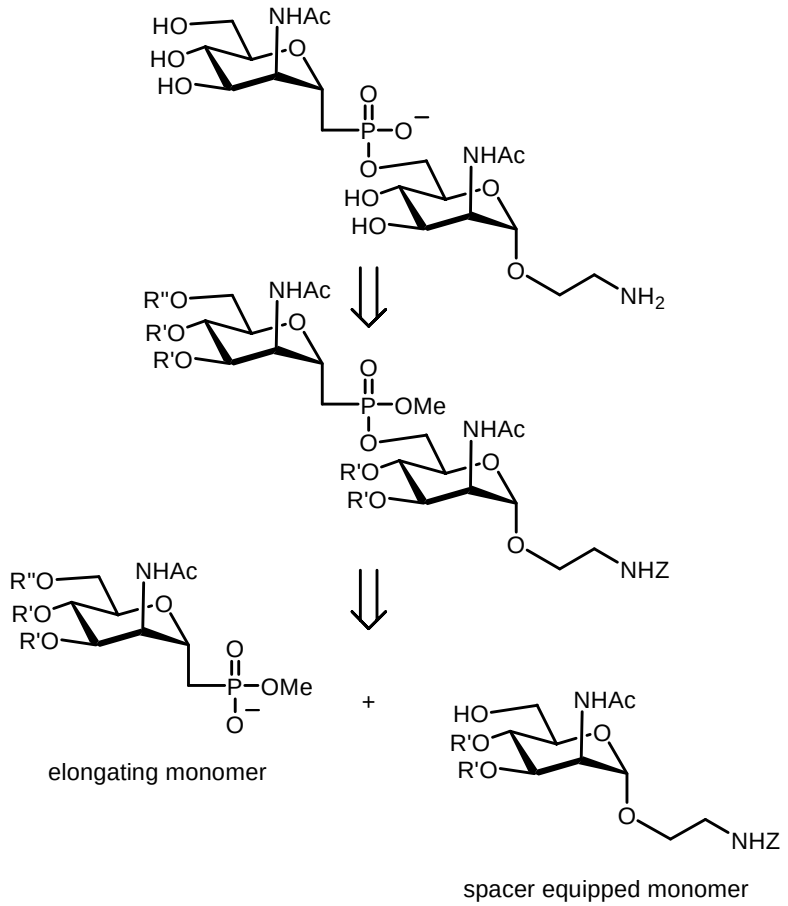


Figure 2.1 Target molecules **1** and **2**



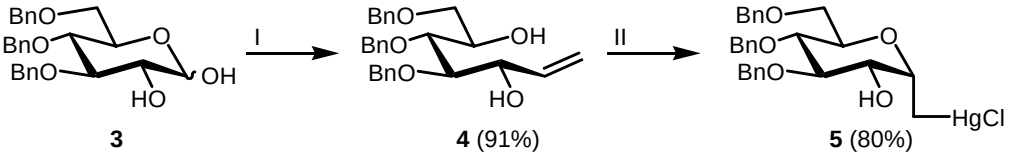
Scheme 2.2 Retrosynthetic analysis of the target disaccharide

The retrosynthetic analysis, outlined in Scheme 2.2, shows that the plan was to elongate a spacer equipped monomer in a linear fashion with an elongating monomer. The protecting group strategy thus needs to allow for elongation at the 6-position.

2.1 Synthesis of the C-phosphonate building block

One attractive possibility for making the C-phosphonate building block is to use a one step Horner-Emmons/Michael reaction. Such a reaction has been reported by Borodkin *et al*. for the stereoselective preparation of α-D-mannosyl methane phosphonate derivatives.[15] They obtained predominantly the α-anomer when 2,3,4,6-tetra-*O*-benzyl-mannopyranose was used and mainly β with 2,3:4,6-di-*O*-isopropylidene-mannose.
In our case it was considered too expensive to start from a mannosamine precursor (at this time of work our improved method for preparation of azido-mannose (Scheme 2.7) was not yet developed), so we tried the reaction on

2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose with the idea of a later inversion at C-2 to get to the mannosamine derivative. However, this reaction only resulted in anomerisation of the starting material. Although there may have been possibilities to modify conditions and/or the protecting group pattern to make this shortcut work, we chose instead a longer, but more reliable route to the elongating monomer.

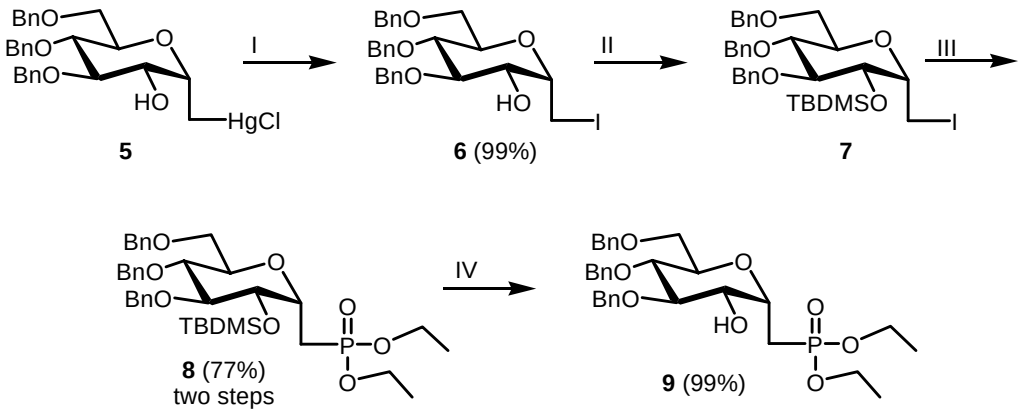


3 **4** (91%) **5** (80%)

(I) BuLi/Methyl triphenylphosphonium bromide (II) $Hg(OAc)_2$, KCl

Scheme 2.3 Wittig reaction and mercurycyclisation

Compound **3**[16] as an anomeric mixture (readily accessible from glucose, without the need for chromatography) was carbon elongated via a Wittig reaction[17] (Scheme 2.3) with methyl bromotriphenylphosphine to give the alkene **4** in 91% yield. This alkene has previously been synthesised from D-arabinose and divinylzinc.[18] Cyclisation of compound **4** with mercury acetate[18] afforded exclusively the α-linked C-glycoside. Interestingly, the corresponding tetra-*O*-benzyl protected alkene yields an α/β mixture. The mercury acetate compound was readily converted, by adding KCl, to the corresponding mercury chloride derivative **5** in 80% yield.



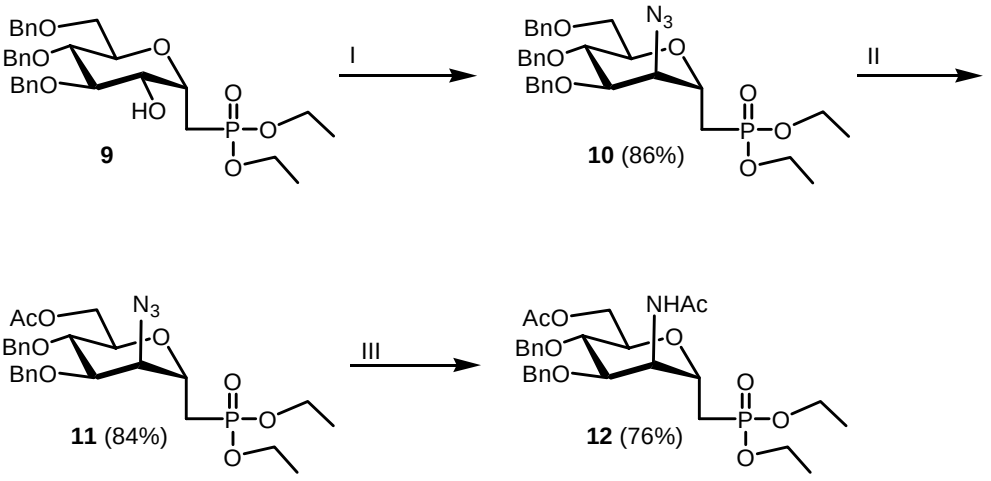
5 **6** (99%) **7**

8 (77%) two steps **9** (99%)

(I) I_2 (II) TBDMSCl/Pyridine/imidazole (III) $P(OEt)_3$ (IV) TBAF

Scheme 2.4 Introduction of phosphorous via the iodo-derivative **6**

The mercury chloride was subsequently displaced with iodine to give **6** quantitatively. (Scheme 2.4) The 2-hydroxyl group of **6** was then protected as a silyl ether, prior to the introduction of the phosphorous, to prevent formation of a cyclic phosphonate.[19] This allowed the C-phosphonate to be formed by treatment of the primary iodide **7** with triethyl phosphite to give **8** in 77% overall yield over two steps. The silyl ether was subsequently cleaved off using tetrabutylammonium fluoride (TBAF)[20], to give **9** in 99% yield.

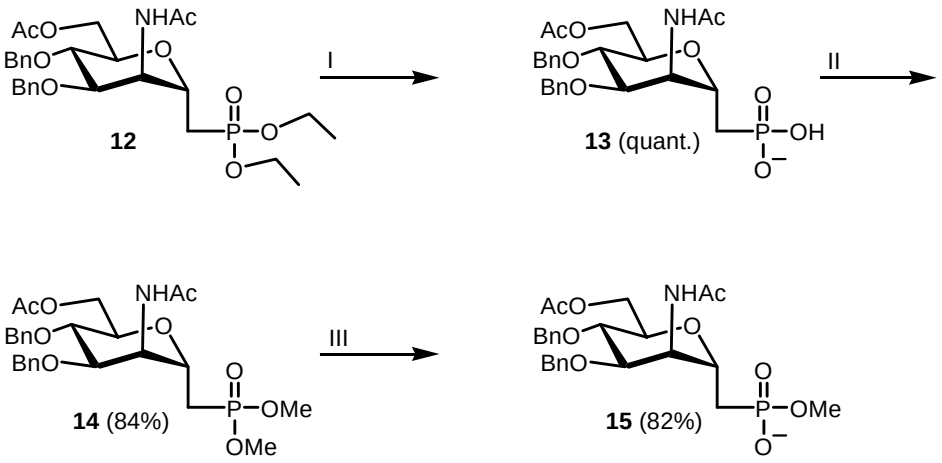


(I) DIAD, Ph_3P, DPPA (II) $HOAc/Ac_2O$, H_2SO_4 (III) 1. $NaBH_4$, $NiCl_2(H_2O)_6$ 2. Ac_2O

Scheme 2.5 Inversion to 2-azido mannose, introduction of a temporary protecting group in position 6 and conversion of the azide to an acetamido group.

The axial acetamido group was introduced by a displacement reaction with azide followed by reduction and acetylation. (Scheme 2.5) Several attempts using various conditions were made to displace the easily formed triflate of **9** with azide ion, but the yields were disappointing (30%). However, by using Mitsunobu conditions[21,22] and diphenylphosphoryl azide (DPPA) as the azide source, a high yield (86%) of the azido derivative **10** was obtained. To introduce an orthogonal protecting group at *O*-6 the primary benzyl ether was replaced with an acetate ester giving **11** in 84% yield using standard acetolysis conditions. The reaction worked better when a mixture of acetic anhydride and acetic acid (1/1) was used together with sulphuric acid. When acetic anhydride alone was used, a lower selectivity for the primary benzyl ether was observed. The azido moiety of **11** was reduced with sodium borohydride in the presence of nickel chloride hexahydrate,[23] and the resulting amine was acetylated with acetic anhydride to give the acetamido derivative **12** in 76% yield.

The ethyl phosphonate esters were subsequently converted to the methyl esters, (Scheme 2.6) for two reasons; firstly, it was impossible to selectively remove one of the ethyl groups and secondly the conditions to remove the ethyl groups are relatively harsh (bromotrimethyl silane), which would certainly also cleave the inter-glycosidic linkage. By comparison, the methyl esters can be cleaved under much milder conditions (a weak base and a good nucleophile) which is also the reason why they were not introduced earlier in the synthesis.

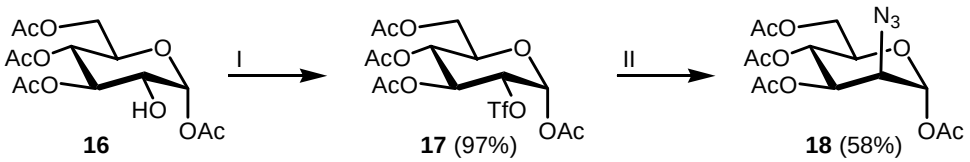


(I) TMSBr (II) trimethylorthoacetat/HOAc (III) PhSH/TEA

Scheme 2.6 Conversion to monomethyl phosphonate

Accordingly, the ethyl groups of **12** were removed quantitatively by treatment with bromotrimethyl silane (TMSBr)[17], and the phosphonic acid **13** formed was converted into the corresponding dimethyl phosphonate[24] **14** in 84% yield using acetic acid and trimethyl orthoacetate (Scheme 2.6). This reaction had to be carefully monitored because deacetylation is a competing side-reaction with prolonged reaction times. Treatment of the dimethyl phosphonate with triethylamine (TEA) in presence of phenyl mercaptan efficiently afforded the monomethyl phosphonate[25] **15** in 82% yield.

2.2 Preparation of the spacer equipped acceptor (Paper I)



(I) Tf_2O, pyridine (II) NaN_3

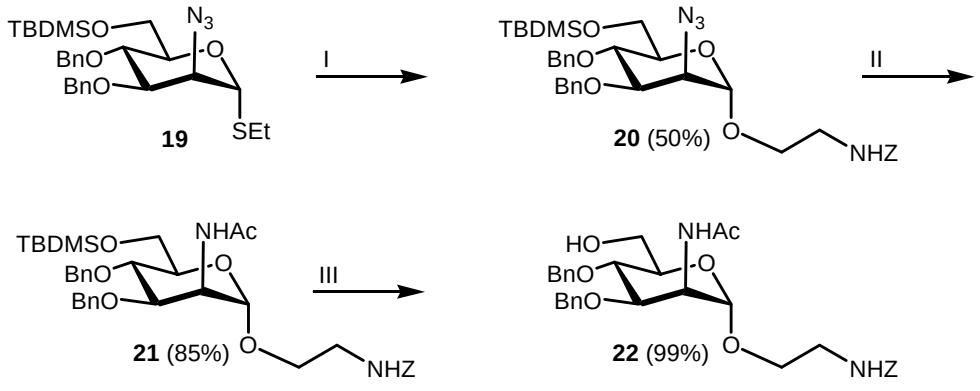
Scheme 2.7 Synthetic route to **18**

The target acceptor **22** (Scheme 2.8) was a spacer equipped *N*-acetyl mannos amine derivative containing a free 6-hydroxyl group and *N*-acetyl mannosamine would be an ideal precursor for the synthesis. However, although this compound is a common residue in bacterial polysaccharides,[26,27] there is no cheap natural source of it. For this reason it is most often synthesised from other precursors.

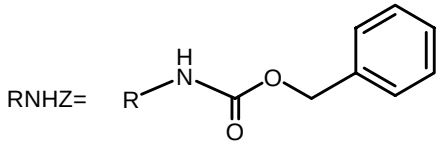
Despite the fact that 2-azido-mannose is a compound of great importance as a precursor to a variety of mannosamine derivatives, to date there has been no simple and/or high yielding way of synthesising these compounds, either by azidonitration[28] of a glucal or by azide displacement of a selectively protected 2-OH-glucose derivative.[29] The azidonitration method is quite low yielding in case of mannose, and the displacement reaction is most often performed on rather elaborate derivatives. An obvious and readily available precursor is 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose **16** which is synthesised from D-glucose in a one pot reaction without the need for chromatography.[30]
In an attempt to synthesise **18**, triflation of compound **16** using triflic anhydride to give **17**, followed by NaN_3 treatment was tried, and according to TLC and MALDI-TOF-MS the 2-azido product was apparently formed in high yield. However, during work-up, the product started to decompose decreasing the isolated yield to only about 20%. Similar problems and even lower yields have been reported by other groups.[31] The corresponding reaction from 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose, however, gives the 2-azido glucose compound in 86% yield without problems with decomposition during work-up. We tested numerous work-up procedures without significantly improving the yield. The solution proved to be an anhydrous work-up. Thus, most of the DMF was removed under reduced pressure and the residue was transferred to a dried column containing a slurry of pre-dried silica gel in dry toluene. The remaining DMF was first eluted with toluene and then the product was eluted using a

gradient of increasing ethyl acetate in toluene. After chromatography the product was no longer sensitive to water.
A reaction with $TMSN_3$ and TBAF is another method that has been used to make 2-azide glucose from the mentioned Man-2-OH precursor. [33,34] These conditions were also tried on compound **16**, but after initial formation of the 2-azido mannose product some decomposition occurred, and the yield was not as good as for the method described above.
The methodology outlined in Scheme 2.7 represents a most attractive route to mannosamine derivatives from a simple precursor. The starting material is very easy to synthesise on a large scale in a one-pot synthesis from glucose,[30] and the intermediate 2-*O*-triflate was obtained using standard conditions in 97% yield.[32] The isolated yield from the inversion is, as mentioned, highly dependent on how water-free the workup is. When using standard dried glassware and chemicals together with preheated silica for the chromatography, we were able to raise the isolated yield to a reproducible 58%.

With the 2-azido-mannose derivative **18** in hand, the next step was to convert it to the corresponding ethyl thioglycoside by treatment with $EtSH/BF_3OEt_2$. The thioglycoside is a more stable derivative than its precursor and also a better glycosyl donor. The acetate esters were removed and the primary position was regioselectively silylated followed by benzylation of the remaining free hydroxyl groups to give **19**. (Scheme 2.8)



(I) NIS, AgOTf, $HO(CH_2)_2NHZ$ (II) 1. $NaBH_4$, $NiCl_2(H_2O)_6$ 2. Ac_2O (III) TBAF



Scheme 2.8 Synthesis of the acceptor

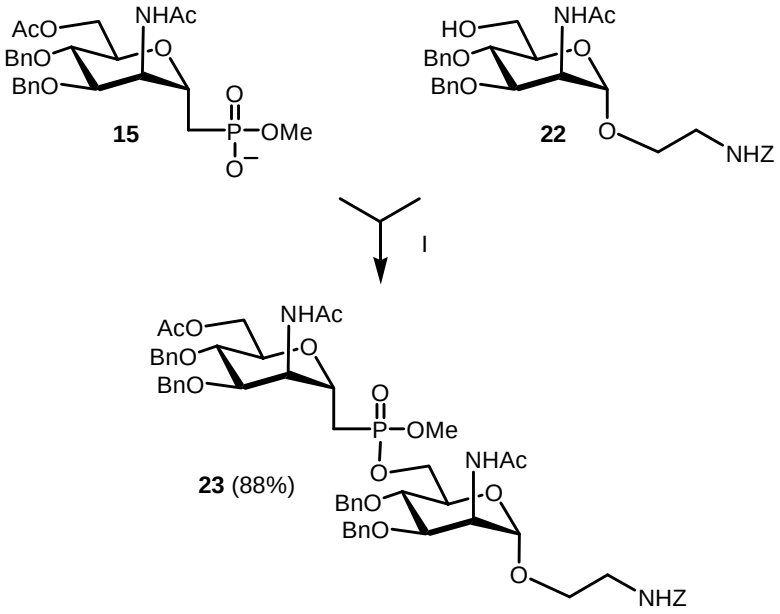
NIS/AgOTf-promoted coupling of **19** to the spacer (Z-protected ethanolamine) gave a high yield of the spacer glycoside but with low stereoselectivity (α/β=1). This was a bit surprising, since normally, high α/β ratios are obtained in couplings with mannosyl donors even without participating protecting groups. However, Pozsgay *et al*. experienced the same problems with low stereo selectivity in a similar glycosidation.[35]
Usually it is difficult to obtain high selectivity for the β-mannoside in a glycosylation reaction. Crich *et al*. explained an enhanced β-selectivity as being due to the low steric bulk of the *O*-2 protecting group.[36] Crich has also shown that the use of an electron-withdrawing group in position two enhanced the β-selectivity.[37] Since azide is both a strongly electron-withdrawing group and a group with a low steric bulk, this might explain the low α/β ratio of our coupling. These results are in accordance with the ones obtained by van Boom *et al*.[38], who got high β-selectivity in couplings with 2-azido mannose donors. However, if the benzyl ethers are replaced with acetate esters (coupling to form **34** in chapter 3), the outcome of the reaction is very different with only traces of β-glycoside and an 86% isolated yield of the α-product.
Since the stereoselectivity in the coupling to the spacer was so much better with the acetylated azido mannose donor, this was tried out as precursor for the synthesis, but an unpredicted benzylation of the nitrogen in the spacer in the following step made us abandon this approach and accept the lower yield of the α-derivative (50%) in the coupling of **19**.
The azido group of **20** was reduced using sodiumborohydride and a catalytical amount of nickel chloride hexahydrate[23], and the resulting amine was acetylated with acetic anhydride to give **21** in 85% yield over two steps. Finally, the silyl ether was removed with TBAF[20] to give the acceptor **22** in 99% yield.

2.3 Methods to form phosphonic acid esters

There are several ways to form phosphonic acid esters of more complex alcohols including the use of peptide-coupling reagents, e.g., *N*,*N*'-dicyclohexyl carbodiimide (DCC) and Mitsunobu conditions. The limitation with the Mitsunobu reaction is that it inverts the configuration, which means that on a secondary hydroxyl groups the epimer has to be used as a starting material, and this can sometimes generate problems in the synthesis. Furthermore, even though inversions of secondary hydroxyls are possible using Mitsunobu conditions, it is a quite sterically demanding reaction to carry out with the relatively large reactive triphenylphosphine complex.
The advantage in using the monoester as a starting material is that the products are non-charged, which simplifies purification. The drawback is that

diastereoisomers are formed, which makes characterisation more difficult. However, very few of the published methods have been used for the synthesis of oligosaccharides with C-phosphonate intersaccharide linkages. To our knowledge, there is only one publication describing synthesis of such structures.[39]

At first we tried to couple the phosphonic acid **13** (Scheme 2.6) directly to the primary alcohol on the monosaccharide **22** with very poor results. Instead, the mono methyl phosphonate **15** was tried in the coupling to **22**. Several coupling reagents were tested including, DCC, 2,4,6-triisopropylbenzenesulfonyl chloride (TIPS-chloride) and (2,4,6-trimethylphenyl) (3-nitro-1,2,4-triazol-1-yl) sulfone (MSNT), but all resulted in low yields caused either by problems of low reactivity or by the formation of pyrophosphonate as the major product, (according to MALDI-TOF-MS). The best isolated yield of **23** was 20%, which was achieved with 5 equiv. of DCC in pyridine.



(I) tris chlorotriphenylphosphine, DIAD, TEA

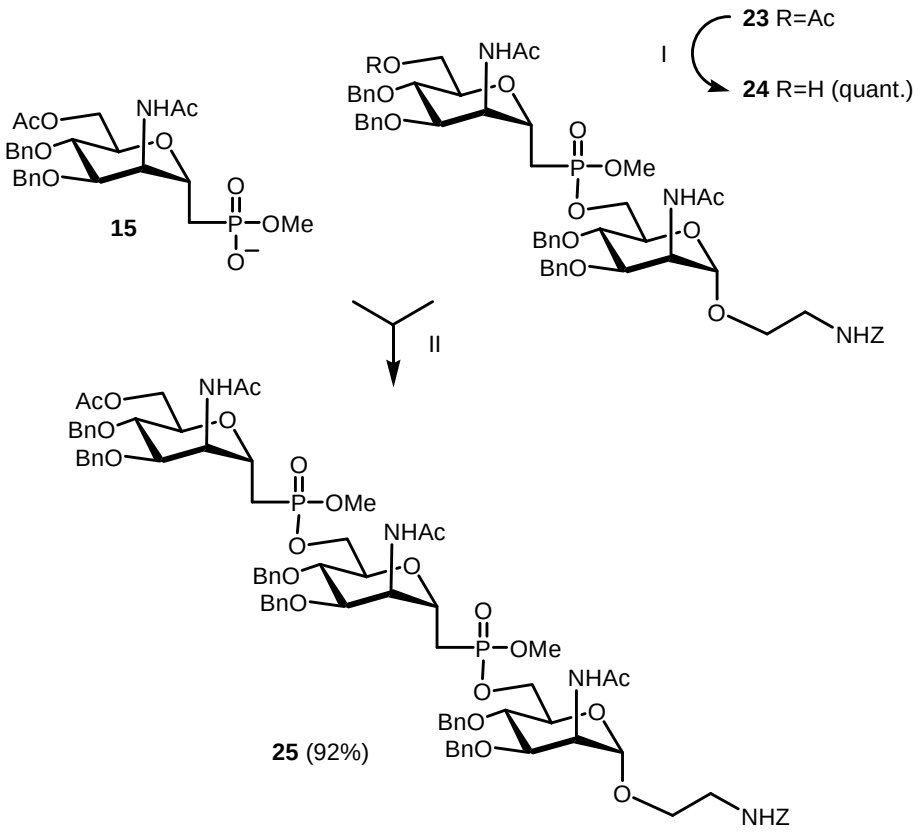
Scheme 2.9 Formation of the dimer

As mentioned, another possibility is to use Mitsunobu conditions, (Scheme 2.9) and this proved to be successful in our case. Using standard Mitsunobu conditions (triphenylphosphine, DIAD),[21] the yield of **23** increased considerably to almost 50%, and by using tris chlorotriphenylphosphine instead of

triphenylphosphine, and having triethylamine present as described by Campbell *et al.*[40,41] the yield increased to 88% and the reaction time was shortened considerably.

One of the major differences with the Mitsunobu reaction as compared to the other coupling conditions is that activation takes place on the alcohol, which prevents the formation of a pyrophosphonate. A new problem could be formation of the ether dimer of the acceptor, but no traces of such a compound were ever detected by MALDI-TOF-MS.

A problem that has to be considered in these reactions is the low nucleophilicity of the monoester compared to other charged nucleophiles. Thus the presence of other nucleophiles must be avoided. We have observed reacetylation when acetate ions have been present and 6-chlorination, where the source of chloride ions has been acidic chloroform from a previous LH-20 column.



(I) KOH, MeOH (II) tris chlorotriphenylphosphine, DIAD, TEA

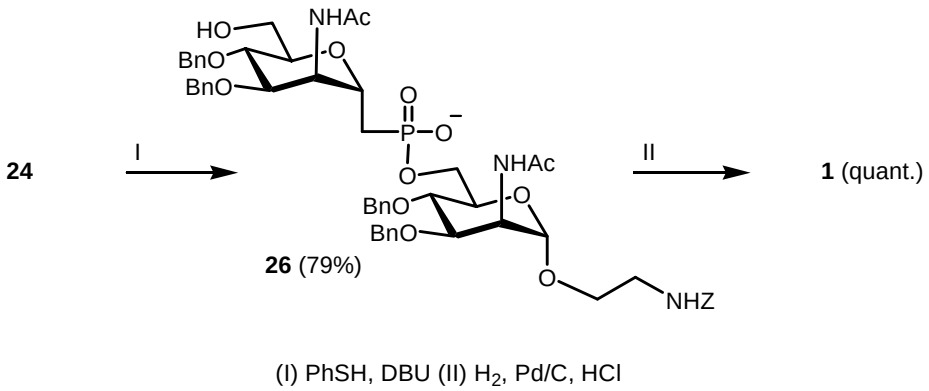
Scheme 2.10 Formation of the trimer

To form a new acceptor for the formation of a trimer, (Scheme 2.10) the acetate ester on **23** was cleaved using KOH in methanol prior to the elongation. Using

the same conditions as for coupling to the dimer, the trimer **25** was formed in an excellent 92% yield.

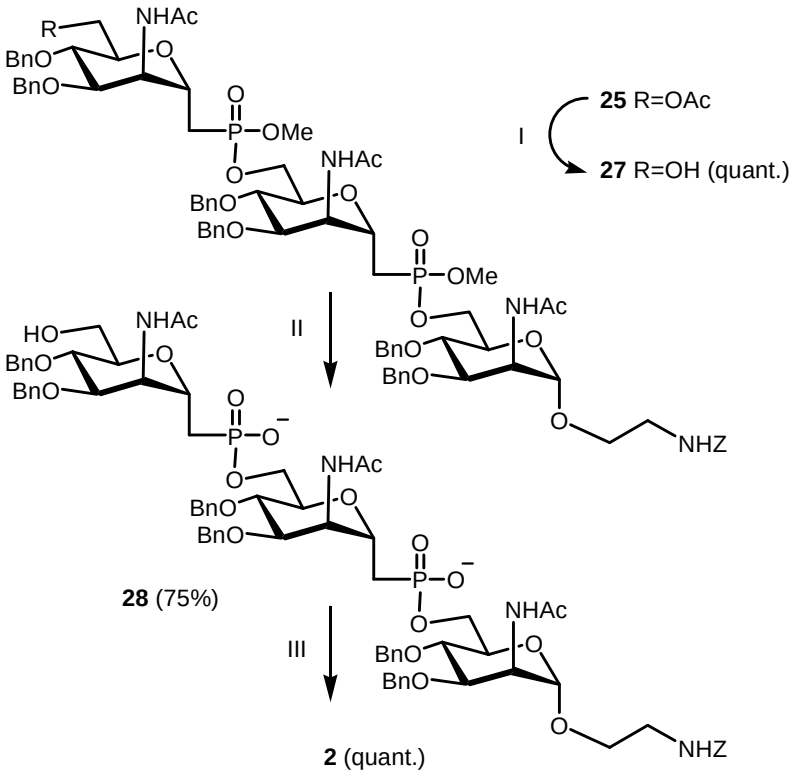
To investigate whether a polycondensation approach could be used as an efficient one-pot synthesis of larger oligomers, a test reaction was carried out with the monosaccharide acceptor **22**, an excess of the deacetylated **15** elongating monomer and the terminating donor **15**. MALDI-TOF-MS showed a clean formation of an oligomeric mixture from tetramers up to heptamers all containing the spacer residue with very little or no decomposition in between the oligomers. Judging from these preliminary results this approach looks most promising as a quick and efficient way to vaccine candidates.

2.4 Deprotection



Scheme 2.11 Deprotection of the dimer

The methyl group on the phosphonate diester **24** was removed (Scheme 2.11) using 1,8-diaza-7-bicyclo[5.4.0]undecene (DBU), which was faster than using TEA and phenyl mercaptan, and gave **26** in 79% yield. The benzyl ethers were then cleaved together with the benzyloxycarbonyl using hydrogenolysis with palladium on activated charcoal to give the target structure **1** quantitatively. To get the reaction to go to completion, $HCl_{(aq)}$ had to be added so that the resulting amine in the spacer was present as its hydrochloride salt and does not poison the palladium catalyst.



(I) KOH, MeOH (II) PhSH, DBU (III) H_2, Pd/C, HCl

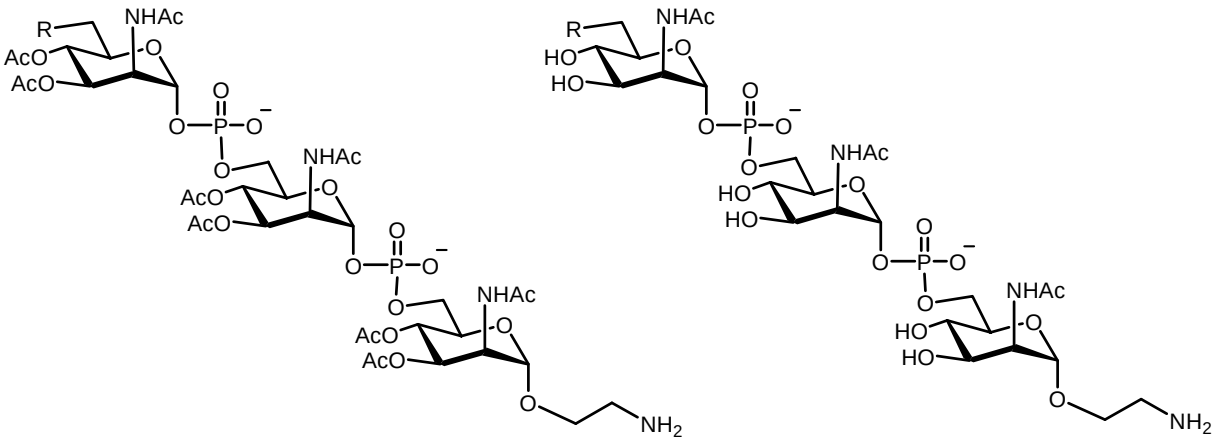
Scheme 2.12 Deprotection of the trimer

Deprotection of the trimer derivative (Scheme 2.12) followed the same procedure as the deprotection of the dimer derivative. Firstly the acetate ester was cleaved quantitatively to give **27** and subsequently the methyl esters were hydrolysed to give **28** in 75% yield. The benzyl ethers were then removed together with the *Z*-group to give the target structure **2** quantitatively.

In conclusion, an efficient synthesis of a trimer of a stable C-phosphonate analogue of the repeating unit of *N. meningitidis* Group A capsular polysaccharide has been investigated. The methodology allows introduction of a terminal phosphate and continued synthesis of larger oligomers as well as polycondensation, variations that might be needed to achieve the wanted immunological properties of the glycoconjugate vaccine.

3. Synthesis of structures corresponding to the capsular polysaccharide of *Neisseria meningitidis* group A (Paper III)

As mentioned in the introductory chapter, there is still very little known about the immunodominant motifs of the *N. meningitidis* serogroup A CPS. A number of well-defined synthetic analogues of the native polysaccharide have now been synthesised (Fig. 3.1) to be subsequently conjugated to a carrier protein and used in immunisation studies to investigate various issues: whether a terminal phosphate is beneficial or not; what size of the carbohydrate structure is required to give protection; and the effects of acetate groups. Another aim was to design a pathway that would allow the synthesis of stabilized analogues. Stabilisation by peracetylation and the use of various electron-withdrawing acetamido substitutes was considered.



29 R = phosphate
30 R = OH

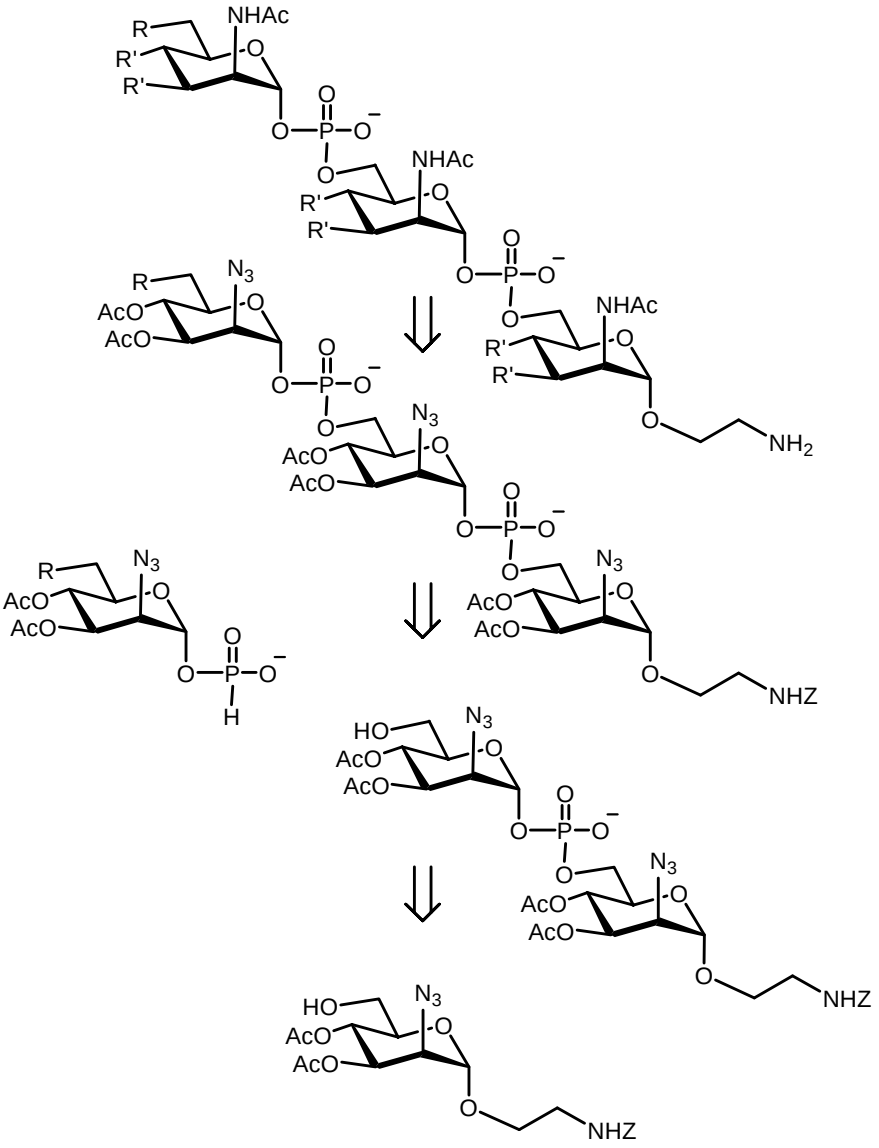
31 R = phosphate
32 R = OH

Figure 3.1 Target molecules **29-32**

Chernyak, Utkina and Oscarson[42] designed a pathway using 2-azido-mannose derivatives to stabilise the anomeric phosphodiester linkage during synthesis with the phosphodiester linkage being formed utilising H-phosphonate chemistry. They targeted the native structure with 3-*O*-acetyl groups in the molecule and used a benzyl ether as the 4-hydroxyl protecting group. This methodology was then employed by Pozsgay *et al.*[35] in their synthesis of a non-acetylated trimer target structure with 3,4-di-*O*-benzyl protection. In the present approach acetate esters are used, both as protecting groups but also because they

are present in the target structures, to stabilise the structures and also to be able to evaluate their immunological importance.

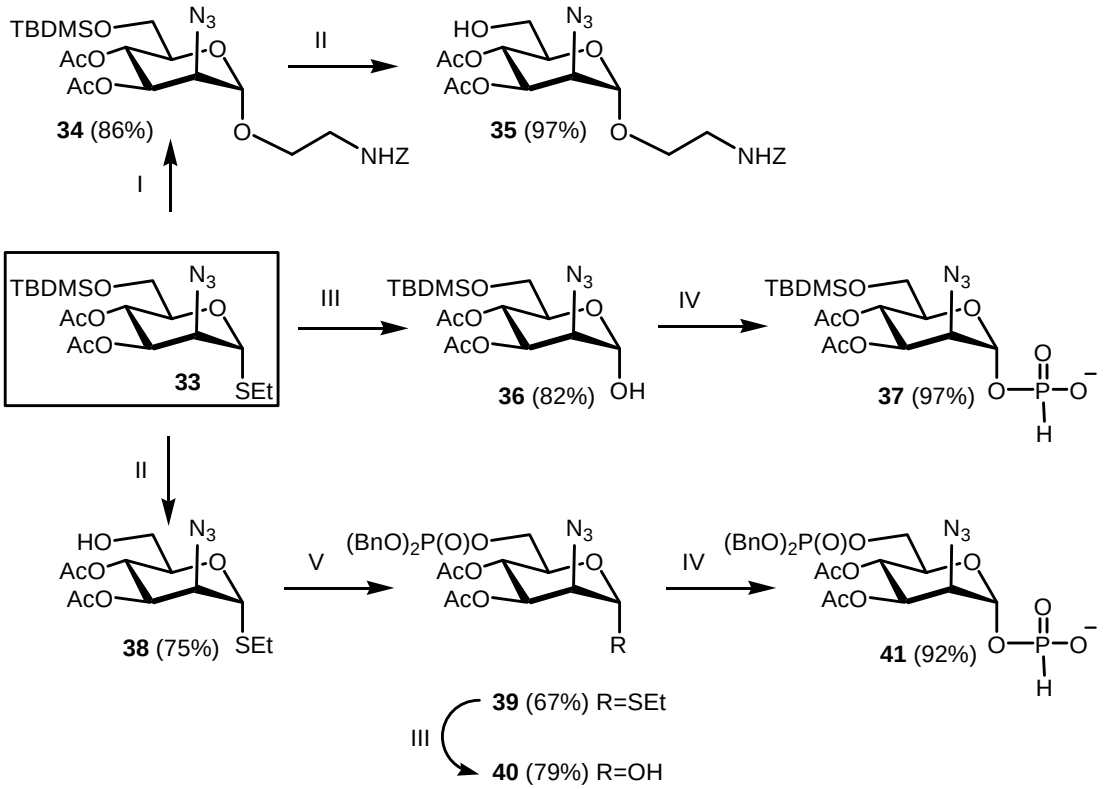
Although the synthetic pathway allows for the synthesis of larger oligosaccharides, four trisaccharides were chosen as primary target molecules. (Fig. 3.1)



Scheme 3.2 Retrosynthetic analysis

To have the option to obtain target molecules both with and without acetate esters, the protecting group strategy was based on the use of acetate esters as permanent protecting groups (Scheme 3.2). In order to stabilise the anomeric phosphodiester linkage, the azido functionality was used as a precursor for the acetamido group as far as possible in the synthesis.[35,42,43] The electron-withdrawing properties of the azido group and its lack of a participating effect strongly stabilise the linkage as compared to the native acetamido group. After initial problems with the use of a dimethoxytrityl ether as a temporary protecting group for the *O*-6, a TBDMS ether was selected instead. The cleavage of the TBDMS ethers could be a problem, bearing in mind the high risk of acetyl migration to position 6 and the cleavage of the labile phosphodiesterlinkage. However, TREAT-HF, although slow, especially when having an azide in position two, cleanly removed the TBDMS ethers without such problems. TBAF, which was successfully used for deprotection of TBDMS-ethers in the previous C-phosphonate synthesis, could not be used due to acetyl migration.

3.1 Synthesis of the building blocks



(I) $HO(CH_2)_2NHZ$, NIS, AgOTf (II) TREAT-HF (III) NIS, AgOTf, CH_2Cl_2/H_2O (IV) PCl_3, imidazole, TEA
(V) 1. dibenzyl diisopropylphosphoramidate, tetrazole 2. *m*CPBA

Scheme 3.3 Synthesis of donors and acceptor

For the synthesis, three monosaccharide building blocks were needed (Scheme 3.3); a spacer-equipped monomer 6-OH acceptor **35**, an α-H-phosphonate elongating monomer with temporary 6-OH protection **37** and a terminating α-H-phosphonate monomer phosphorylated in the primary position **41**. To achieve the target molecules **29** and **31** with a terminal phosphate, a phosphate-containing donor had to be used, because attempts to phosphorylate the trisaccharide failed. The reason for the interest in the derivatives containing a terminal phosphate is that the bacterial capsular polysaccharide naturally decomposes and leaves such a residue.

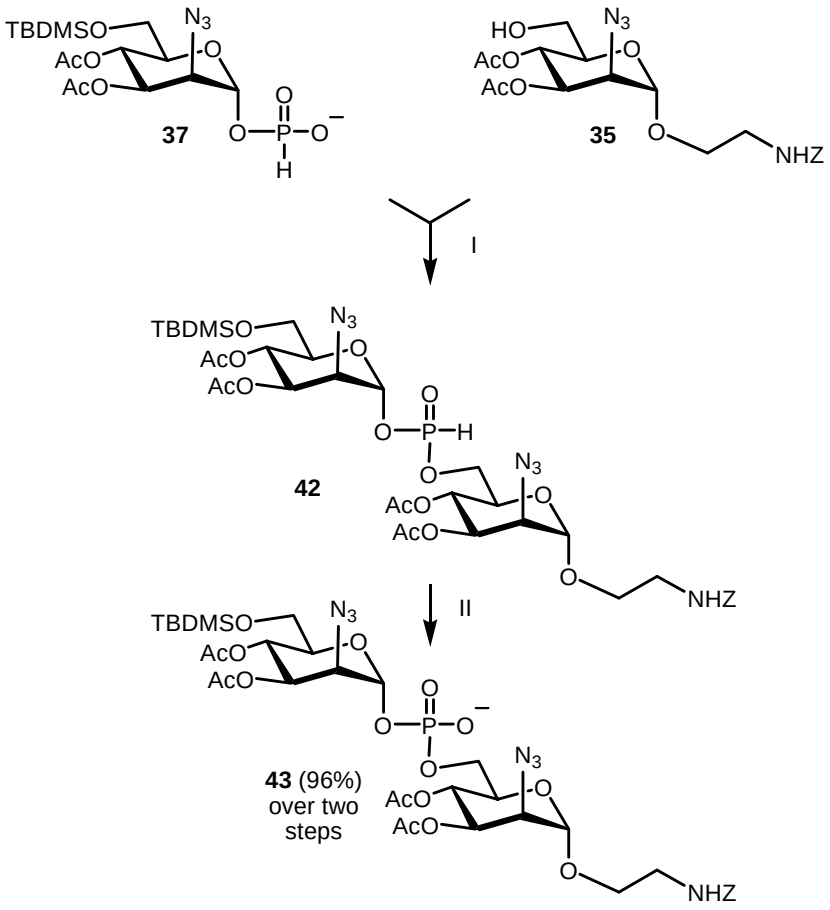
To simplify the synthesis, all three units were synthesised from the same precursor **33**, which was obtained in four steps from **18** as described for compound **19** (Scheme 2.8), except for the last step which was an acetylation

instead of a benzylation. The donor **33** was coupled using NIS as promoter to a benzyloxycarbonyl-protected (Z-protected) ethanolamine spacer to give **34** in 86% yield (Scheme 3.3) and as discussed in chapter two a very high α-selectivity was observed in this coupling. The TBDMS-ether was then removed using TREAT-HF to give the acceptor **35** in 97% yield.

In order to synthesise donor **37**, the thioglycoside was hydrolysed using NIS as promoter and water at -20°C and the desired α-anomer **36** was obtained exclusively in 82% yield. If the reaction was carried out at room temperature, a high percentage of the β-anomer was formed. The α-hemiacetal **36** could then be phosphonylated without anomerisation using PCl_3/ imidazole to give donor **37** in 97% yield.

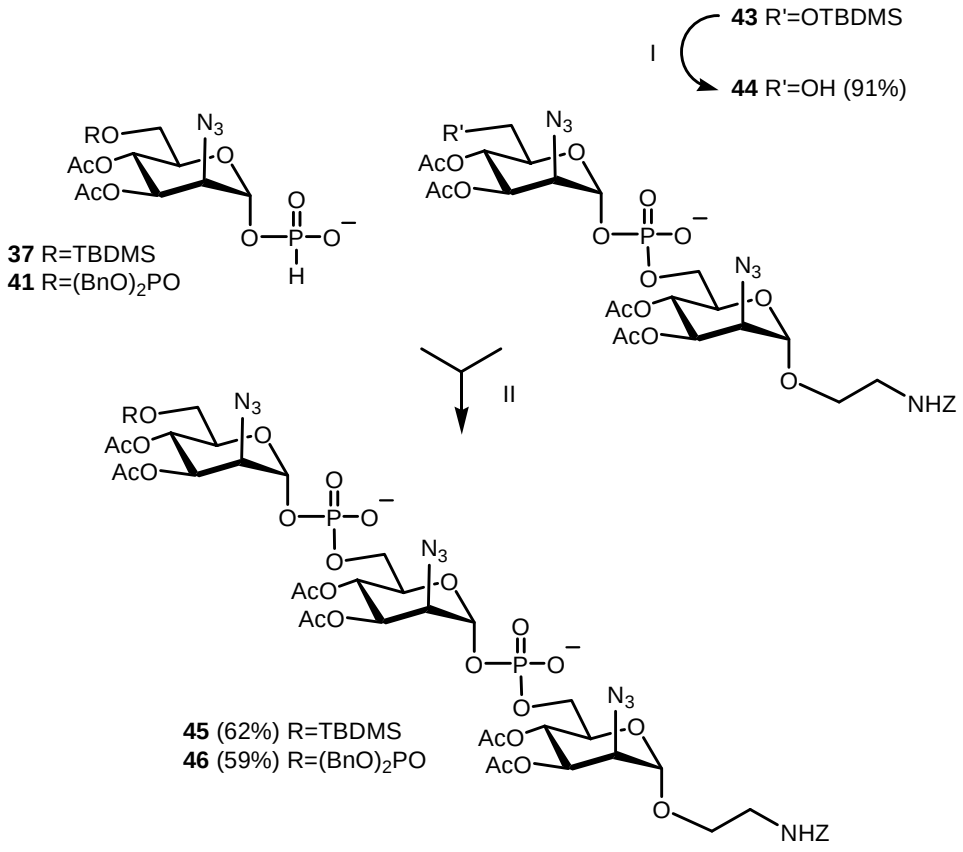
To synthesise donor **41**, the TBDMS-ether was cleaved to give **38** in 75% yield by treatment with TREAT-HF and the hydroxyl group was then phosphorylated using dibenzyl diisopropylphosphoramidate followed by *m*CPBA to yield **39** with a dibenzyl ester protected phosphate in position 6 (67%). The ethyl thioglycoside was hydrolysed in the same way as for **36** (79% yield) and the obtained α-hemiacetal **40** which was then phosphonylated to provide the H-phosphonate donor **41** in 92% yield.

3.2 Formation of di- and trisaccharides



Scheme 3.4 Formation of the disaccharide **43**

The formation of the phosphodiester linkages (Scheme 3.4) was carried out using standard H-phosphonate chemistry, [44] where the H-phosphonate **37** and the acceptor **35** were coupled using pivaloyl chloride (PivCl) as promoter. The resulting H-phosphonate diester **42** was oxidised using iodine/ pyridine in water and the disaccharide **43** was obtained in very high yield (96%).

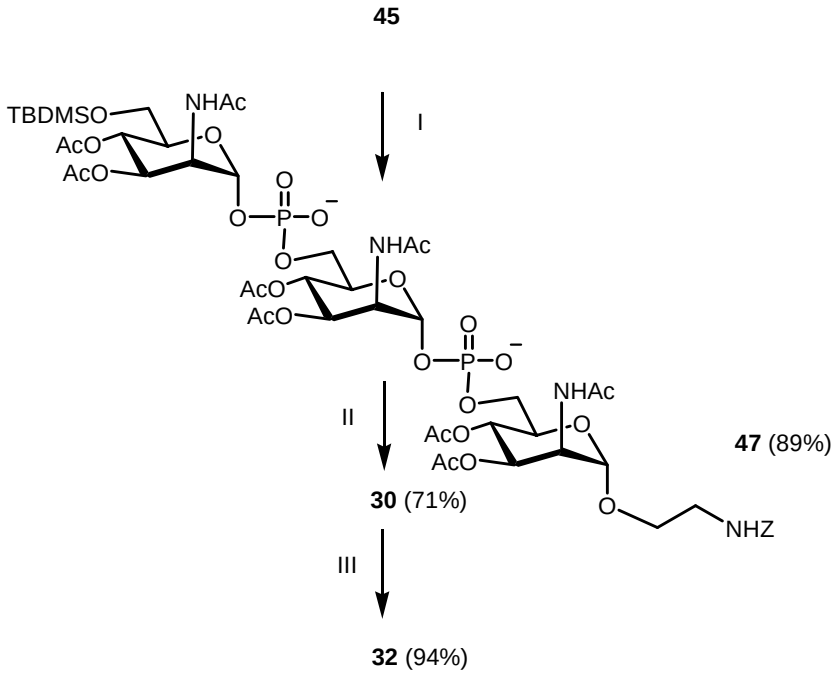


Scheme 3.5 Formation of the trisaccharides **45** and **46**

To obtain a new acceptor, (Scheme 3.5) the TBDMS-ether on the disaccharide **43** was removed in 91% yield, once more using TREAT-HF. Subsequent coupling, again using donor **37** together with **44** and the same conditions as for the formation of the disaccharide, afforded the trisaccharide **45** in 62%. To obtain trisaccharide **46** donor **41** was coupled to acceptor **44** in 59% yield.
As always in the synthesis of higher oligomers of structures containing inter-glycosidic anomeric phosphodiester linkages, the yield in the diester formation decrease due to decomposition of the diesters already present in the acceptor.[35]

The azido groups in both derivatives **45** and **46** were reduced to amines without affecting the Z-protected amine, using sodiumborohydride and a catalytical amount of nickel chloride hexahydrate and the resulting amines were acetylated with acetic anhydride to afford compounds **47** and **48** in 89% and 64% respectively.

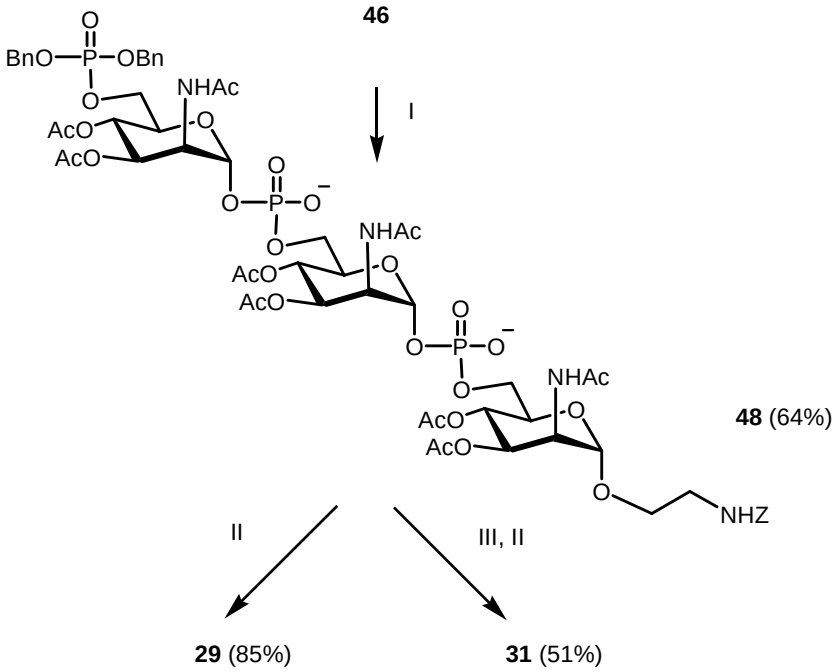
3.3 Deprotection



(I) 1. $NaBH_4$, $NiCl_2(H_2O)_6$ 2. Ac_2O (II) 1. H_2, Pd/C, 2. TREAT-HF (III) NaOMe/MeOH

Scheme 3.6 Deprotection to give target molecules **30** and **32**

These two trisaccharides were deprotected in slightly different ways. To achieve the target molecules **30** and **32** (Scheme 3.6) the *Z*-group of **47** was first cleaved off using catalytic hydrogenolysis (83%). To avoid problems with hydrolysis of the phosphodiester linkages basic ion exchange resin was added to the reaction mixture. The TBDMS-ether was then removed, again using TREAT-HF, to yield target structure **30** in 85% yield. To obtain the second target structure **32**, compound **30** was simply deacetylated using NaOMe in MeOH (94%).



(I) 1. $NaBH_4/NiCl_2(H_2O)_6$ 2. Ac_2O (II) H_2, Pd/C (III) NaOMe/MeOH

Scheme 3.7 Deprotection to give target molecules **29** and **31**

To obtain target structure **29**, (Scheme 3.7) the Z-group was removed from derivative **48** in the same way as in the deprotection of compound **47** in 85% yield. In order to obtain compound **31** the acetate esters were this time removed (80%) prior to hydrogenolysis (64%), since the final deacetylation was not successful, which is not unusual with free amines present in the molecule.

In conclusion, a straightforward synthesis of four trisaccarides related to the capsular polysaccharide of *Neisseria meningitidis* has been achieved starting from a common precursor. The strategy allows synthesis of larger structures and includes target structures with a terminal phosphate.

4. An improved synthesis of the Lewis b hexasaccharide (Paper IV)

4.1 Introduction

Helicobacter pylori is a bacterium that is the main cause of peptic ulcers in humans.[45] To be able to infect the stomach lining the bacteria binds to receptors on the epithelial cell surfaces of the host. Several receptors for the bacteria are known, but the most important one is considered to be the Lewis b blood group antigen. The bacterial adhesin[46] involved in this interaction is identified as a membrane protein called Bab A (Blood group antigen binding Adhesin). For further the understanding of this carbohydrate-protein interaction there is a need for pure Bab A protein, which can be obtained using affinity chromatography where Lewis b structures are bound to the solid phase. To produce such a column a larger amount of Lewis b hexasaccharide (Fig. 4.1) was needed and thus a synthetic method for making Lewis b hexasaccharide in multigram scale.
A large scale synthesis of the Lewis b tetrasaccharide has been published,[48] but larger structures with higher binding constants to the adhesin were needed to get good separation in the affinity chromatography. The hexasaccharide will also be a valuable tool in a number of interaction studies with both the purified Bab A and the whole bacteria.
A synthetic method has been developed which overcome the scale up problems from our previous synthesis of the hexasaccharide.[49] The main drawback of the earlier synthesis was the lability of a used tetrasaccharide building block donor, α-L-Fuc-(1→2)-β-D-Gal-(1→3)-[α-L-Fuc-(1→4)]- β-D-GlcNPhth→SEt, which eliminated ethyl mercaptan during glycosylation and also upon storing. Furthermore, the deprotection was not particularly high yielding.

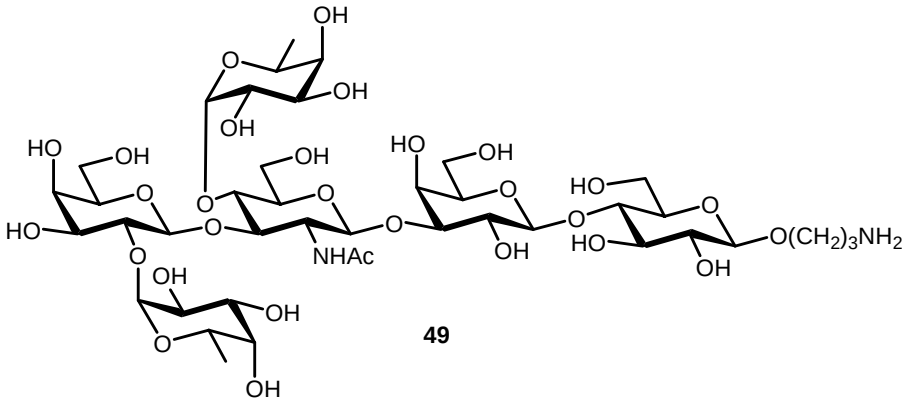
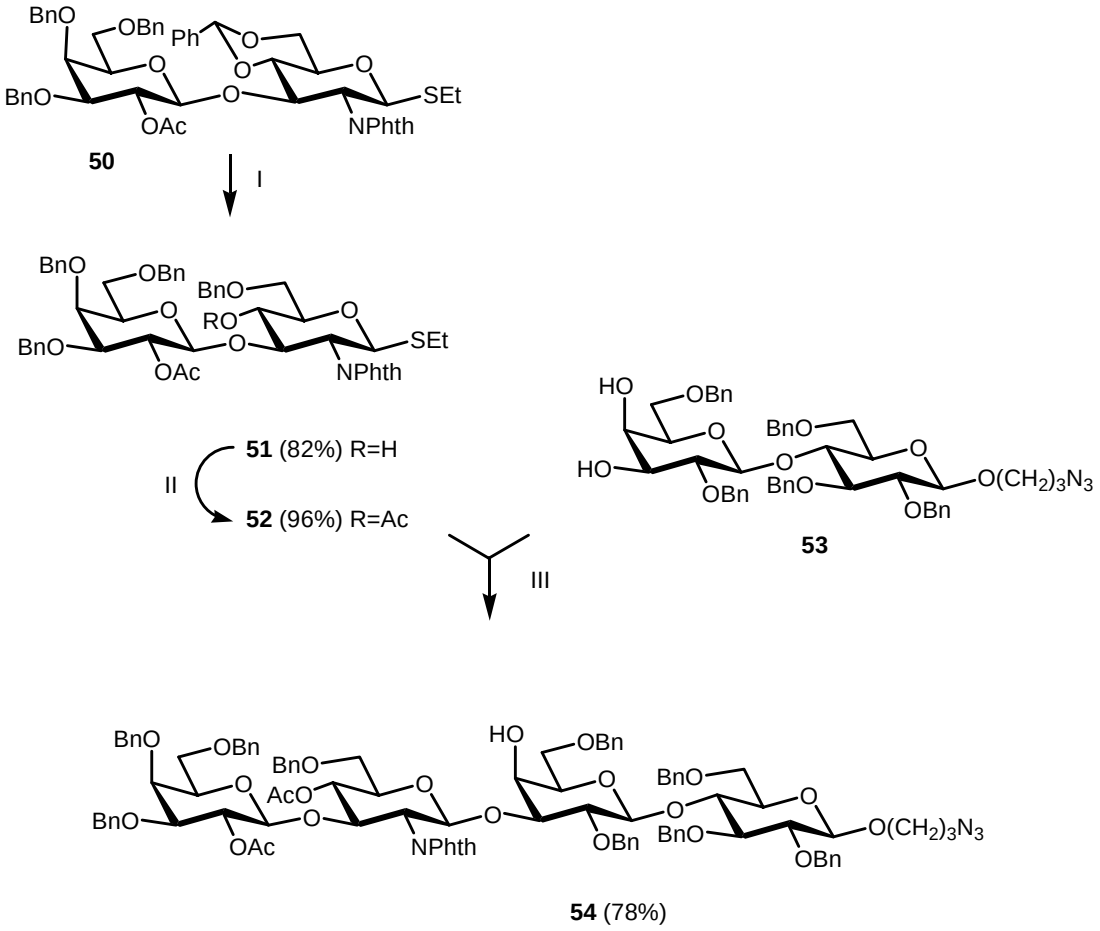


Figure 4.1 The target hexasaccharide

The target molecule is equipped with an aminopropyl spacer to facilitate conjugation to the solid phase.

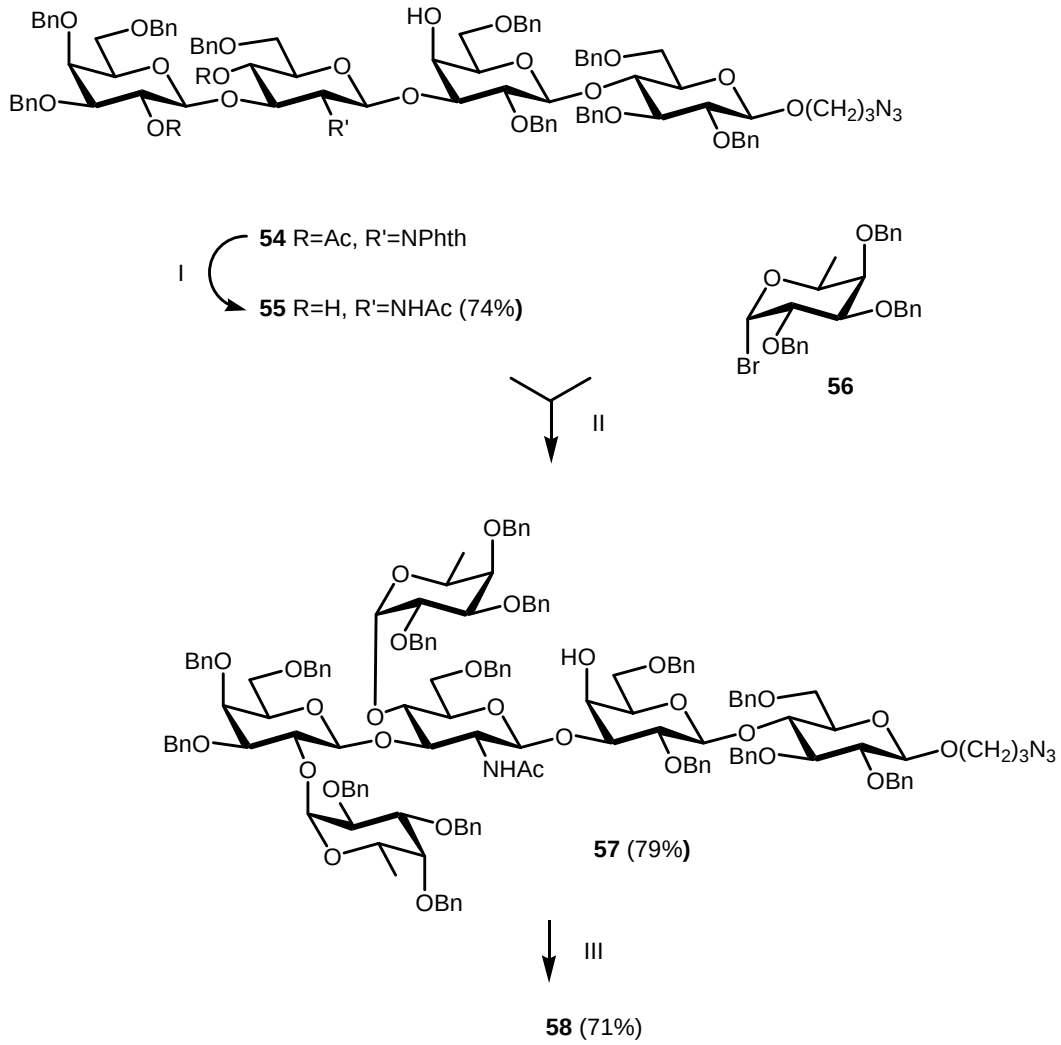
4.2 Formation of tetra- and hexasaccharide and deprotection

To avoid the previously mentioned problems, a new synthetic pathway was designed in which a more easily available and stable disaccharide donor was used, β-D-Gal-(1→3)-β-D-GlcNPhth→SEt **50**, and the introduction of of the fucose residues was postponed until the last step before deprotection.
Attempts to couple the known lactose 3,4-diol acceptor **53** with this disaccharide donor **50** resulted in high yields of tetrasaccharide but with low regioselectivity. This problem was solved by performing the glycosylation with a donor not containing a benzylidene acetal **52** (Scheme 4.2).



Scheme 4.2 Formation of the tetrasaccharide **54**

The benzylidene acetal of **50**[50] was reductively opened using $NaCNBH_3$ and HCl in diethyl ether to give **51** with a benzyl ether at position 6 in 82% yield and the resulting free 4-hydroxyl group was temporarily protected as an acetate ester in 96% yield. A NIS-promoted coupling with this donor now gave excellent selectivity for the 3-position in the acceptor **53**[50] and the tetrasaccharide **54** was obtained in 78%. The phthalimido group was now converted to the native acetamido function by ethylendiamine treatment followed by acetylation with acetic anhydride. Subsequently, the acetate esters remaining after the ethylendiamine treatment were removed using NaOMe in MeOH to allow the introduction of the two fucose units. (Scheme 4.3)



Scheme 4.3 Formation of the hexasaccharide **57** and deprotection to **58**

Once more we relied on the anticipated low reactivity of the axial Gal 4-OH, to perform a regioselective difucosylation with **56** of the triol acceptor **55**. Using halide-assisted conditions only very minor amount of the heptasaccharide were detected (by MALDI-TOF-MS) and the desired protected Lewis b hexasaccharide **57** could be isolated in 79% yield.

Although the pathway was designed to include a last simple deprotection step, catalytic hydrogenolysis of **57** to remove benzyl ethers and reduce the azido group, major efforts were necessary to find high-yielding conditions.

The addition of one equivalent of HCl to the reaction mixture was finally found to give a quick and clean deprotection and the target structure **58** was obtained in 71% yield. Since fucosyl glycosides are known to be acid labile we tried

mildly acidic conditions (acetic acid) first, but the prolonged reaction time were a bigger threat to the fucosyl linkages than using stronger acid for a shorter time.

In conclusion, an improved synthesis of the Lewis b hexasaccaride has been developed that overcomes the previous lability and deprotection problems. The overall yield was improved and the pathway now allows large-scale synthesis.

Acknowledgements

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Andy, Mia, Ricky and Rolle (for interesting football analyses)

Simon for all the help and fun

My other friends outside the chemistry world

My family

Malin and Alva ♡

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