UNIVERSITA DEGLI STUDI DI MILANO

DEPARTMENT OF ORGANIC AND INDUSTRIAL CHEMISTRY

DOCTORATE SCHOOL IN CHEMICAL SCIENCES AND TECHNOLOGIES

Curriculum

Chemical Science (XXIV Cycle)

SYNTHESIS OF IMMUNO-ACTIVE OLIGOSACCHARIDES AND GLYCOCONJUGATES AS ANTIGENS FOR VACCINE FORMULATION

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ABBREVIATIONS

Ac	Acetyl
Ac2O	Acetic anhydride
AcOH	Acetic acid
aq.	Acqueous
Arom.	Aromatic
b	Broad
Bn	Bezyl
BS(PEG)2	Bis-succinimidyl penta-ethylene glycol
Bz	Benzoly
CSA	Camphor-10-sulfonic acid
Cbz	Benzyloxycarbonil
CPS	Capsular polysaccharide
CRM_{197}	Cross-Reacting Material 197
d	Doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N-N' -dicyclohexilcarbodiimide
DCM	dicholormethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
Eq	Equivalent
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionisation
Fmoc	Fluorenylmethyloxycarbonyl
Hib	Haemophilus influenzae type B
HSA	Human serum albumin
IMH	Imidazole

J	Coupling constant
KHMDS	Potassium bis(trimethylsilyl)amide
KLH	Keyhole Limpet Hemocyanin
Lev	Levulinic
LPS	Lipopolysaccharides
MenA	N.mengingitisis type A
MALDI-TOF	Matrix-Assisted Laser Desorption/ Ionization Time of Flight
MS	Mass spectroscopy/molecular sieves
m/z	Mass to charge ratio
M.W.	Molecular weight
NAP	2-naphthylmethyl
NMR	Nuclear Magnetic Resonance
NOEsy	Nuclear Overhauser Effect Spectroscopy
PAMP	Pathogen-associated molecular patterns
Piv	Trimethlacetyl
PTSA	p-Toluenesulfonic acid
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Satd	saturated
t	Triplet
TBAI	Tetra-n-butylammonium iodide
TBAF	Tetra-n-butylammonium fluoride
TBDMS	tert-butyldimethylsilyl
TEAB	Triethylammonium bicarbonate
TDS	Thexyldimethylsilyl
Tf	Trifluoromethanesulfonic
THF	Tetrahydrofuran

Synthesis of analogues of fragments of the capsular polysaccharide from *Neisseria meningitidis* Type A

INTRODUCTION

Carbohydrates and Immunology

Together with proteins and nucleic acids, carbohydrates constitute one of the important classes of biomolecules. However, the carbohydrate compounds are the least exploited as potential therapeutic agents despite the crucial roles they play in numerous biological recognition processes (e.g., bacterial and viral infection, cancer metastasis and inflammatory reactions) [1]

All cells are coated with thick layers of complex carbohydrates known as the glycocalyx, in which the glycan components are present in many different glycoforms, such as glycoproteins, proteoglycans, glycolipids and glycophosphatidylinositol-linked proteins. (Figure 1) Therefore, the cell glycocalyx is involved in cell differentiation, recognition, adhesion and many other important events, including pathological developments. The exposure of carbohydrates on cells' surfaces enables them to interact with the immune system, acting as cell antigenic determinants. On the other hand, the dense surface distributions of often unique glycan structures on diverse pathogens and on malignant cells make carbohydrates attractive vaccine targets.

Despite the important roles of carbohydrates in biological process, use of carbohydrates onto inducing immunity is still a relatively new strategy,

nevertheless the seminal finding that the pneumococcal antigens targeted by the immune system are polysaccharides dates back to 1923.[2]

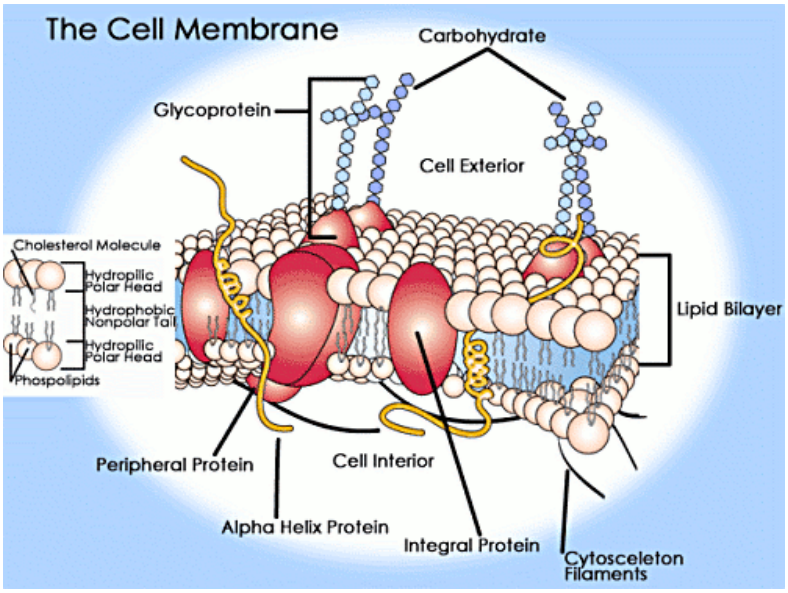

Figure 1. Cell membrane and carbohydrate

The advent of chemotherapeutics and antibiotics dampened enthusiasm for the development of carbohydrate vaccines, but the emergence of multidrug resistance phenomena and their constant increase roused renewed interest. In 1983 PneumoVax (Merck and Co.), the first polysaccharide vaccine, was commercially launched. This vaccine was made up of unconjugated capsular polysaccharide isolated from 14 serotypes of the Streptococcus pneumoniae bacterium, whereas the current version includes 23 out of approximately 90 known

serotypes. In healthy adults, this vaccine induces good protection against most of the infections caused by these pathogens.[3]

Immune System

The immune system is the body's defense against infectious organisms and other invaders. Through a series of steps called immune response, the immune system attacks organisms and substances that invade body systems and cause disease.

The immune system is made up of a network of cells, tissues, and organs that work together to protect the body (Figure 2)

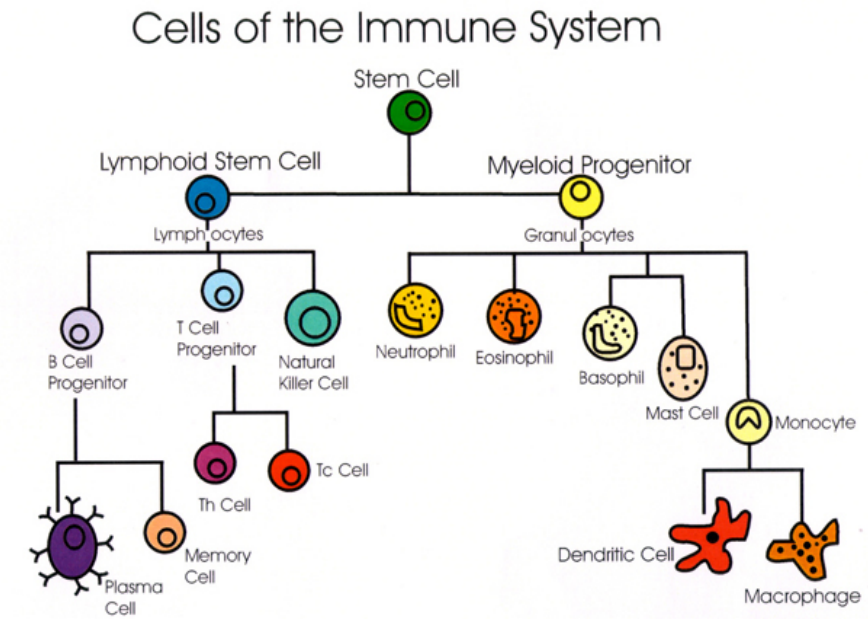

Figure 2. Cells of the immune system

Molecules which are perceived by the immune system as a foreign invader or simply as potentially dangerous for the host refer to antigen. The immune system responds to antigens by eliciting suitable immune response. More specifically, the integration of two distinct arms of the immune response, the innate and the adaptive (antigen-specific) responses, leads to protective immunity against pathogen exposure.

The innate immune is rapid and unspecific; it consists of soluble factors, such as complement proteins, and diverse cellular components including granulocytes (basophils, eosinophils and neutrophils), mast cells, macrophages, dendritic cells and natural killer cells; it functions as the first line of defense and responding to pathogen-associated molecular patterns (PAMPs). In contrast, the adaptive immune response is slower to develop, but manifests an increased antigenic specificity and memory. It consists of antibodies, B cells, and CD4+ and CD8+ T lymphocytes. Natural killer T cells and T cells are cytotoxic lymphocytes that straddle the interface of innate and adaptive immunity. The adaptive immune response is specific response such as production of antibodies against particular pathogen. [4]The adaptive response could occur during the lifetime of an individual as an adaptation to infection with the pathogen. An adaptive immune response confers lifelong protected immunity to reinfections with the same pathogen in many cases.

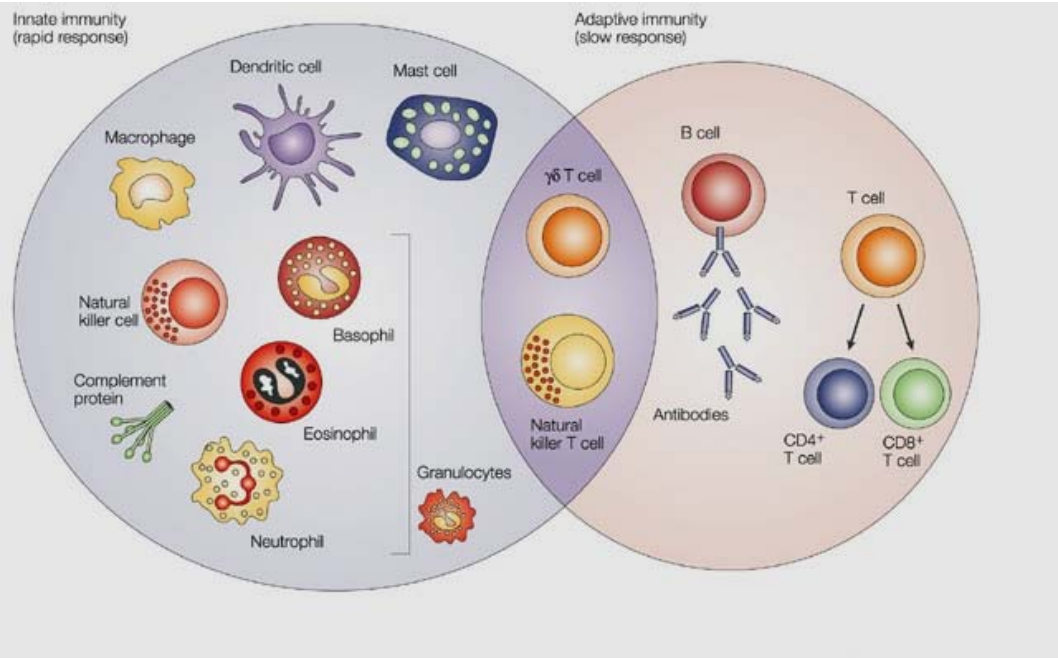

Figure 3 Innate immune response and adaptive immune response

Both innate immunity and adaptive immune response rely on the activities of the white blood cell or leukocytes. Innate immune responses mainly involve granulocytes and macrophages while adaptive ones largely depend upon lymphocytes which provide the lifelong immunity that can follow exposure to disease or vaccination. The innate and adaptive immune system together could afford an effective defense system. Many infections are handled successfully by the innate system and cause no disease; others that cannot be resolved by innate immunity trigger an adaptive immune response and are then overcome; followed by lasting immunological memory.

Immunology of Polysaccharide antigens from bacterium

As mentioned before, polysaccharides construct the major components on the bacterial surface. These can be in the form of capsules, glycoproteins or glycolipids. For examples, in Gram-negative bacteria, the lipopolysaccharide (LPS) covers up to 40% of the bacterial surface; the capsular polysaccharides (CPSs) are present in both Gram-negative bacteria such as *N. meningitidis, Haemophilus influenzae*, *E. coli* or *Salmonella typhi* and in Gram-positive such as *Streptococci* and *Staphylococci* (Figure 4).

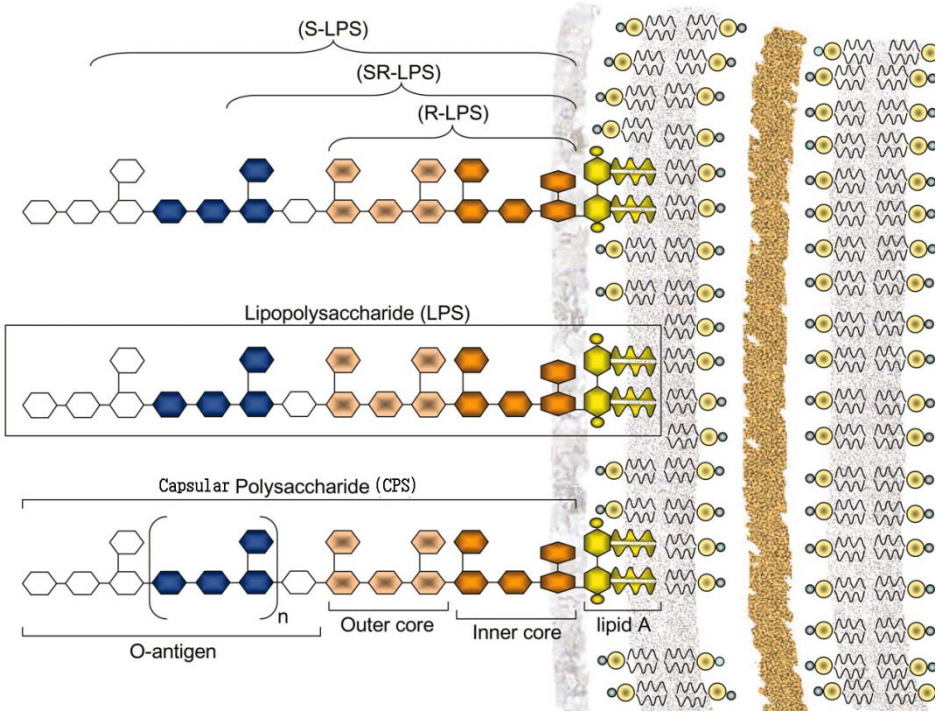

Figure 4. LPS and CPS in bacterial surface

It has been well established that the surface polysaccharides could invoke the immune response confering protection against the disease since 1920s.[5] The

introduction of antibiotics, however, put an effective stop for the next several decades to the development of vaccine based on either CPS or neoglycocojugates. Then in the 1970s, it was realized that the usage of antibiotics, although brought largely success, was not the ultimate solution to handle infection.

Advances in immunology with delineation of B and T lymphocyte responses, and the role of T cell for the immunological memory functions, as well as the structural elucidation of surface polysaccharide made possible the development of new, polysaccharide-based vaccines. Today, several vaccines based on either purified CPSs or on neoglycoconjugates are available. In spite of the increased knowledge in polysaccharide immunology, there are several problems that remain to be considered and solved:

1) Due to the structural differences in the surface polysaccharide and the serogrouping or serotyping system, the carbohydrate antigens exhibit large degree of antigenic variation. i.e. N.meningitidis with over 13 [6] serogroups and Streptococcus pneumoniae involves over 90 different serotypes.

2) Homology between carbohydrate structures present on bacterial surface and those of host cell membrane has to be taken into consideration. The mimicry of host-associated carbohydrate structures by bacterial polysaccharides could be a potential virulence and evasion factor[7].

3) Polysaccharide antigens are mostly poor immunogens due to their T cell independent (TI) nature. Children below 2 years of age and elderly respond poorly to polysaccharide antigens.[8]

Neisseria Meningitidis

Meningitis is an inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges. The inflammation may be caused by infection with viruses, bacteria, or other microorganisms, and less commonly by certain drugs. Meningitis can be life-threatening because of the inflammation's proximity to the brain and spinal cord; therefore the condition is classified as a medical emergency.[9] (Figure 5)

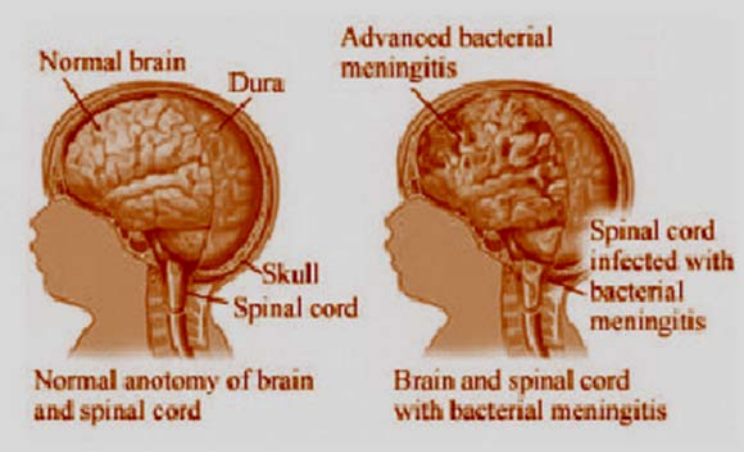

Figure 5. Meningitis

Bacterial meningitis has still a profound impact on public health. Worldwide, approximately 1,200,000 cases (with more than 170,000 deaths) of bacterial meningitis are recorded annually, with at least a 5-10% of case fatality in industrialized countries and a 20% in the developing world. In addition, serious sequelae such as brain damage, hearing loss or a learning disability affect up to one third of survivors.[10] Several different microorganisms can cause meningitis. Especially, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib) and *Neisseria meningitides* [11] are responsible for over 80% of all cases of bacterial meningitis.

Neisseria meningitidis is an encapsulated diplococcus bacterium. Its outer membrane is surrounded by a polysaccharide coat (Figure 6) which is essential for its pathogenicity, exerting a protective function against the host's immune defense. Thirteen different serogroups of the Gram-negative bacterium N. meningitidis have been defined so far, although about 90% of meningitis infections are due to serogroups A, B, C, Y, W135 and X.[12]

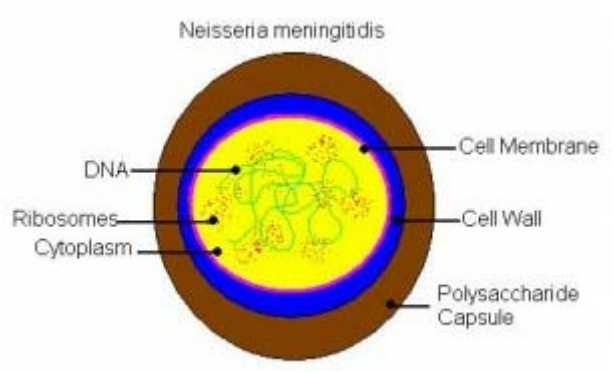

Figure 6. Structure of *Neisseria meningitides*

All these serotypes can cause epidemics, but their relative incidences are strictly dependent on geographic area. Serogroup B and C are responsible for the majority of cases of meningococcal disease in developed countries, with more than 50% of causes due to serogroup B. Serogroup A however takes the main responsiblility of meningitis epidemics and outbreaks in developing countries, predominantly throughout what is known as the African meningitis belt. (Figure 7) In the African meningitis belt countries, the estimated incidence for the 20 year period 1970 -1992 was about 800000 cases[13]

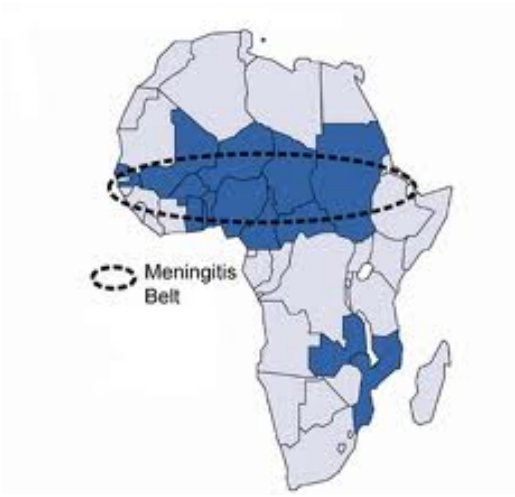

Figure 7. African meningitis belt

The resistance to this type of infection is mediated by the production of specific antibodies against the bacterial capsular polysaccharides (CPSs), suggesting that a vaccine composed of purified CPSs as antigenic material can be effective for protection against meningococcal disease.

Carbohydrate based vaccine towards N. meningitidis

Although polysaccharide-based vaccines were demonstrated highly effective in preventing disease in adults and older children, they possess several shortcomings. Owing to their T cell independent nature, polysaccharide antigens induce immune responses of relatively short duration, and no class switch from low affinity IgM to high affinity IgG antibodies occurs. Moreover, polysaccharides cause the maximum immune response after one dose, but do not induce immunological memory, i.e. they fail to induce a booster effect after revaccination. [14] As a consequence, plain polysaccharides do not mount a protective immune response in the immature immune system of infants and children under two years of age, and this holds true in elderly and immunocompromised patients.

However, polysaccharides can be converted into T cell dependent immunogens by chemical conjugation to carrier proteins (typically, CRM_{197}, tetanus or diphtheria toxoid, Protein D).[15] In this way, immunological memory is established, raising a strong, durable and protective immune response from early childhood. In 2005, a tetravalent meningococcal vaccine containing N. meningitidis serogroup A, C, Y and W-135 CPS conjugated individually to diphtheria toxoid was approved in the United States.[16] Recently, a second anti-meningococcal glycoconjugate vaccine, where meningococcal CPS oligosaccharides are covalently linked to CRM_{197}, has been licensed.[17]

Capsular polysaccharide from Neisseria Meningitidis type A

The structure of N. meningitidis serogroup A (Men A) consists of (1→6)-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units[18]. The presence of O-acetyl groups at the position C-3 is approximately 70% of the ManNAc residues. (Figure 8) The first effective anti-MenA vaccine was based on purified natural CPS and was licensed in the seventies in combination with other meningococcal CPSs.[19]

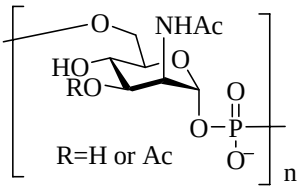

Figure 8. Structure of the repeating unit of the CPS of N.meningitidis A. Structure heterogerogeneity derives from 3-O acetylation (R = H or R = Ac)

An additional feature of MenA CPS is the chemical lability in water, mainly due to the inherent instability of the anomeric glycosyl phosphodiesters.[20] Phosphodiesters are normally stable but when they are present at the anomeric position of a carbohydrate residue, the linkage become much more labile due to the possibility of the electron pair on the ring oxygen displacing the phosphate or phosphodiester. (Figure 9)

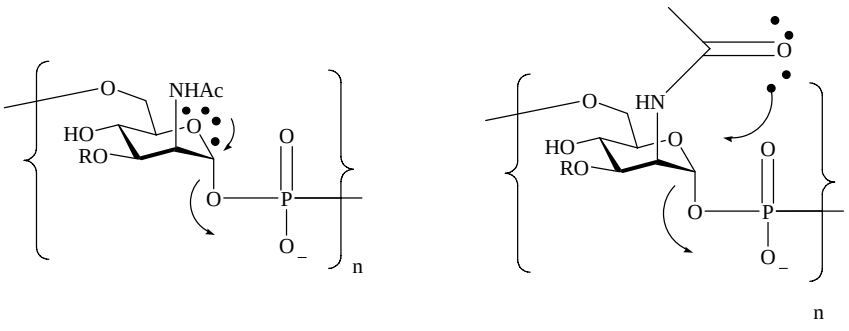

Figure 9 Mechanism for the phosphodiester cleavage

The chemical lability represents the main problem in the preparation and use of glycoconjugate vaccines against MenA, which are mainly intended for use in the "meningitis belt" countries. Thus, we became interested in the design and synthesis of novel and hydrolytically stable analogues of MenA CPS repeating unit. These could be incorporated into a saccharide chain in order to obtain oligomers endowed with enhanced shelf-life. On the other hand, their conjugation to a proper immunogenic protein carrier should elicit protective antibodies that will cross-react with the bacterial capsule.[21]

Previously, our group developed a series of "C-phosphonate" analogues of MenA CPS repeating unit. (*Figure 10*) The presumption is that the carbon-phosphorous bond is incapable of being hydrolyzed by the ordinary enzymes involved in the phosphonate cleavage. Accordingly, we recently reported on the synthesis of phosphonoester-linked oligomers of MenA CPS[22] and their conjugation to gold nanoparticles, in order to be displayed in multivalent fashion for a stronger interaction with the target protein.[23] ELISA competitive assays showed that both the monovalent, non-conjugated antigens and the corresponding gold glyconanoparticles are recognized by a human polyclonal anti-MenA serum, confirming that the replacement of the anomeric oxygen atom with a methylene group does not prevent the antibody recognition and binding.[24]

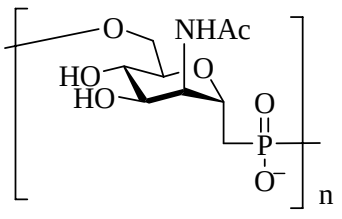

Figure 10. C-phosphonate analogue of MenA CPS

Similar with the C-phosphonate analogues strategy, another stabilization of glycosyl 1-O-phosphates can also be achieved by using carbasugar analogues, where a methylene group replaces the pyranose oxygen atom.[25] (*Figure 11*) This structural modification leads to the loss of the acetalic character of the phosphodiester, that it is expected to gain improved stability towards hydrolysis.[26]

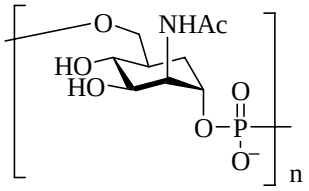

Figure 11. Carba sugar analogue of MenA CPS

In recent research of our group, carba-rhamnose was shown to be able to preserve the conformation and the biological activity -in terms of antibody affinity- of the natural rhamnopyranose when inserted into the Streptococcus pneumoniae type 19F CPS trisaccharide repeating unit.[27] Prompted by this result, we synthesized the monomer carba-N-acetylmannosamine-1-O-phosphate and compared its geometrical and conformational properties with the naturally occurring N-acetylmannosamine-1-O-phosphate, the MenA CPS repeating unit.[28] The results showed that they owned similar conformational behavior, so that the carba-N-acetylmannosamine-1-O-phosphate can be regarded to as a potential mimic of MenA CPS repeating unit and be used in the construction of carba oligomers endowed with enhanced stability in comparison with the native polymer.

Therefore, the first subject of the PhD thesis is the synthesis of carba analogues of MenA CPS project. In particular, the research will be focused on the synthesis of designed monomer, dimer and trimer carba analogues of MenA CPS; A suitable strategy to prepare this kind of derivatives in relatively large amount in lab will be described; Preliminary immunological evaluation of the target molecules has been carried out based by competitive ELISA Assay; In addition, glyconcojugation of the synthesized oligosaccharides with carrier proteins will be accomplished; Last but not the least, several attemps using H-phosphonate polycondensation reaction to achieve oligosaccharides with higher number of repeating units will also be described.

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CHEMSTRY PART

1 Target Molecules

The synthesis, preliminary biological evaluation and glycoconjugate of the carba analogues from Neisseria meningitides A capsular polysaccharide will be described in details in this part.[1]

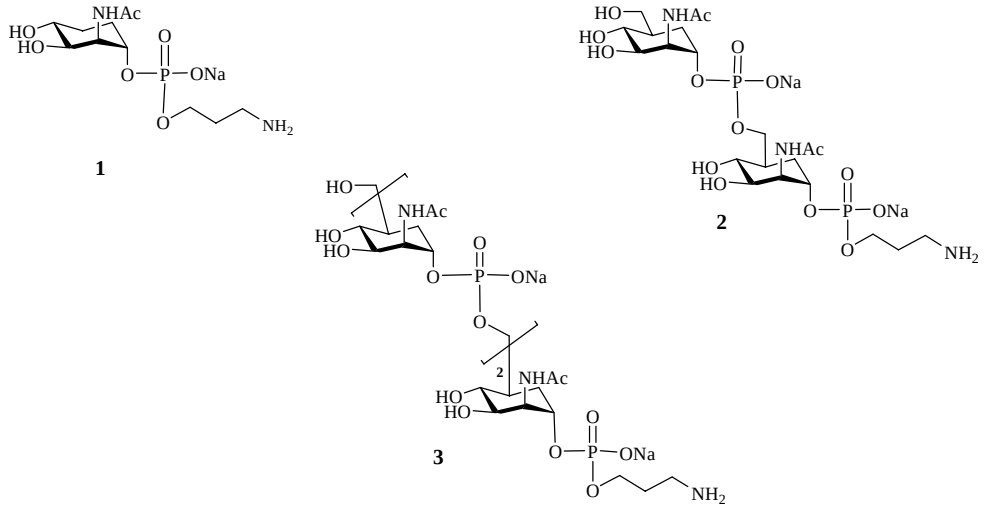
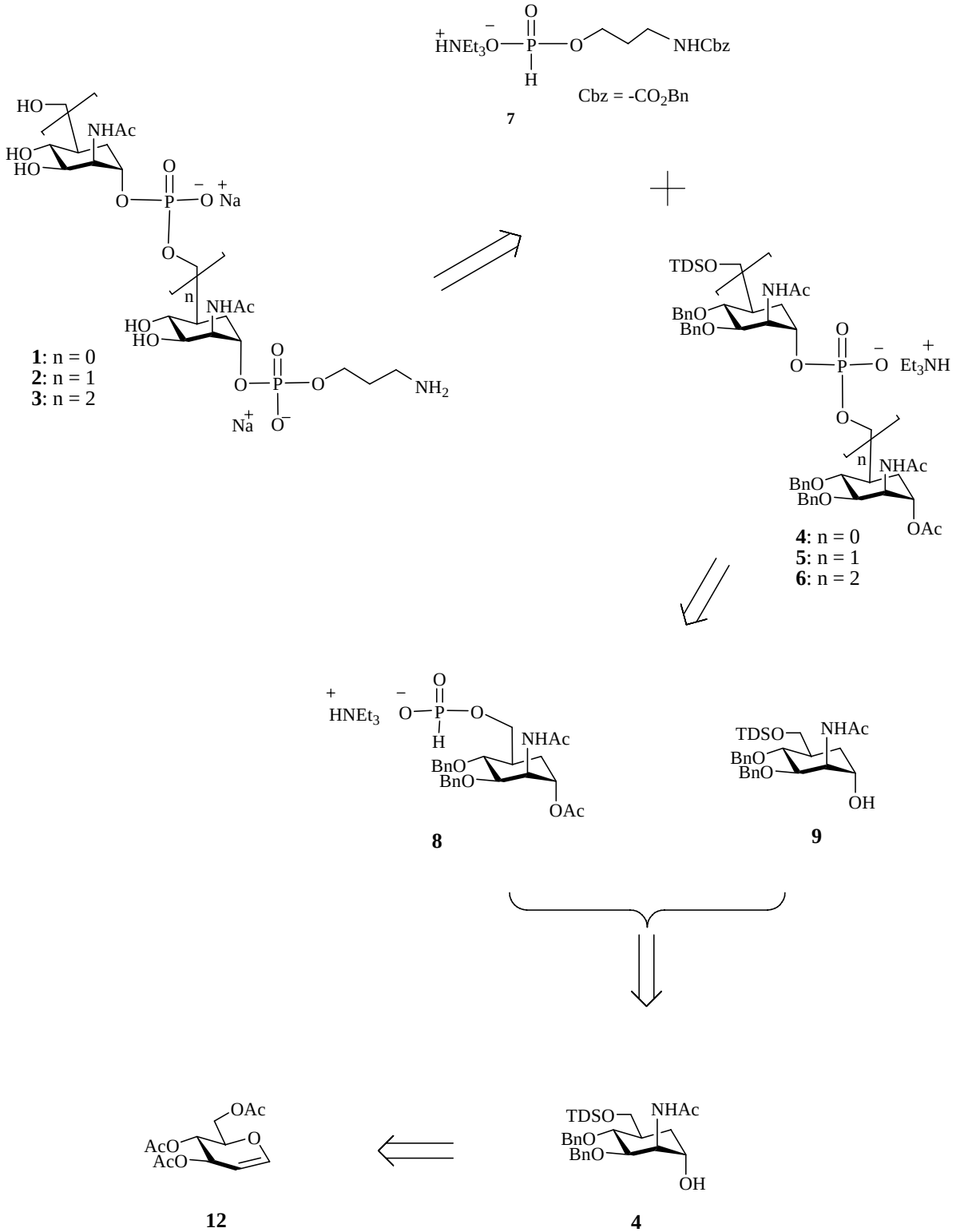


Figure 1. Target molecules

The designed target molecules **1**,**2** and **3** are all provided with a phosphodiester linked aminopropyl spacer at their reducing end to facilitate conjugation of the molecules to carrier protein (CRM_{197} and HSA) for further immunogenic studies (*Figure 1*).

Our synthetic route towards compounds**1-3** is outlined in *Scheme 1*, which is based on the H-phosphonate methodology [2] for the formation of the phosphodiester bridges. Therefore, both the introduction of the phosphodiester linked spacer at C-1 position of oligomers and also the elongation of oligomers are based on this method. **1**, **2** and **3** can be achieved from the assembly between phosphonate alcohol **7** and the intermediates **4-6**. Dimer **5** and trimer **6** can be afforded from monosaccharide building blocks **8** and **9**, which are in turn obtained from the key precursor **4**. The key intermediate **4** can be thus achieved

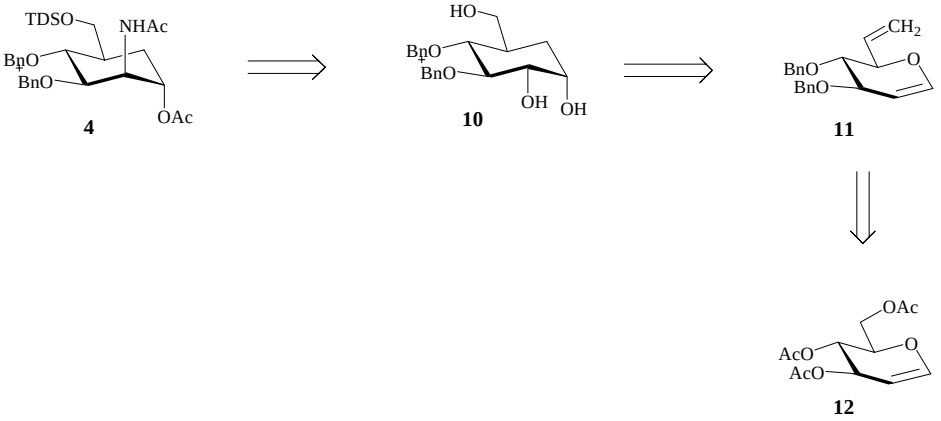
from commercially available compound **12**



Scheme 1. Retrosynthetic approach

2 Synthesis of key precursor 4

The first synthesis of carba sugar precursor **4** has been recently reported by our group [3]. The synthetic method however was proved to be not satisfied in large scale preparation. In this work, the first task is to synthesize the intermediate **4** on large scale. And also, based on the same synthetic route, an improved preparation of **4** has been developed which is more suitable for large scale synthesis.

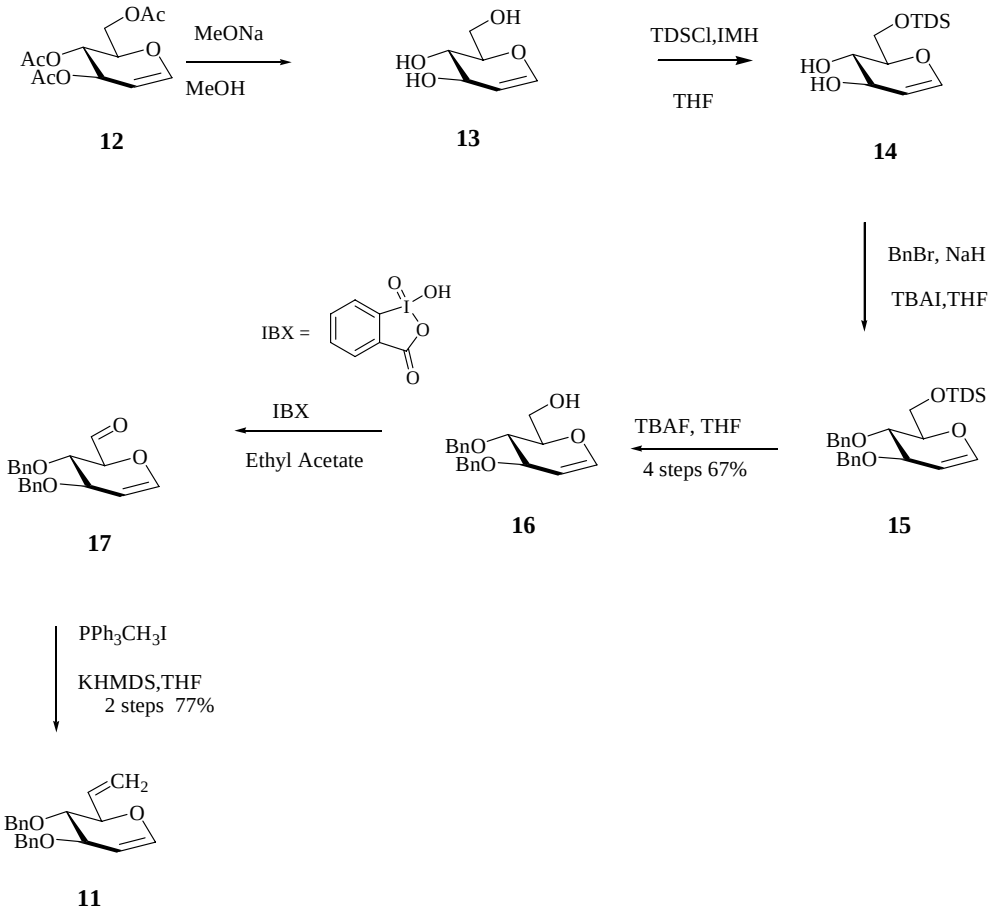


Scheme 2. Retrosynthectic analysis of **4**

The synthesis of precursor **4** will be divided into three parts: Coversion from commercially available glucal **12** to an alkene **11**; from **11** to a carbocyclic ring **10**; finally from **10** to compound **4**. (*Scheme 2*)

2.1 Synthesis of 11

Firstly, the alkene **11** was synthesized in six steps from the commercially available 2,3,4-tri-O-acetyl-glucal **12**. Treatment of **12** in typical Zemplén conditions by 1M solution of sodium methoxide in methanol afforded de-acetylated compound **13**. Regioselective 6-O-silylation of **13** was carried out with Chloro(dimethyl)thexylsilane (TDSCl), imidazole and tetrahydrofuran (THF), followed by benzylation of C-3 and C-4 hydroxyls with benzyl bromide, tetrabutylammonium iodide (TBAI), sodium hydride in THF, affording compound **15**. Removal of the silyl ether of **15** in the presence of 1M tetra-n-butylammonium fluoride (TBAF) in THF, led to corresponding intermediate **16** in 67% overall yield in four steps.

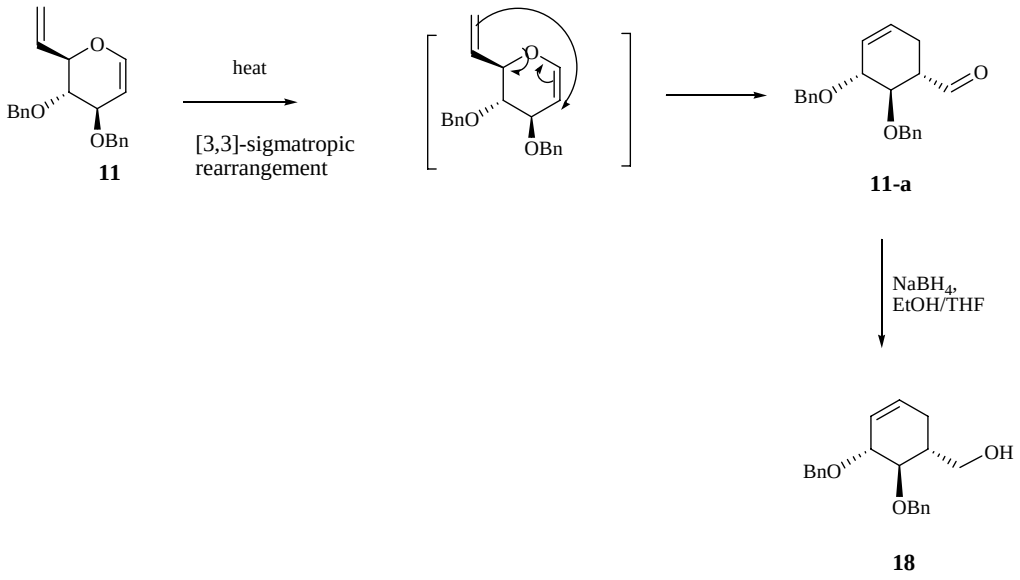


Scheme 3. Synthesis of **11**

Previously, the primary hydroxyl in **16** was oxidized by pyridiniumdichromate (PDC) to result an aldehyde; after olefination of the aldehyde in the presence of methyltriphenylphosphonium iodide and sodium amide, alkene **11** could be obtained in a modest yield of 40 over two steps %[4]. In our synthesis, 2-Iodoxybenzoic acid (IBX) was found to be a more efficient oxidant than PDC, the alcohol **16** could be converted completely to the desired aldehyde in the presence of IBX and Ethyl Acetate at reflux temperature. Next, potassium bis(trimethylsilyl)amide (KHMDS) was used as a base instead of the previously reported sodium amide during the Wittig reaction, furnishing **11** in a improved yield of 77% over two steps. The preparation of **11** could be scaled in multi gram scale. (Scheme **3**).

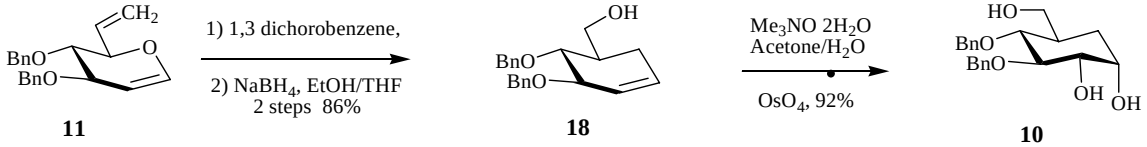
2.2 From Pyranose Ring to Carbocyclic Ring

Conversion of alkene **11** to a carbocyclic compound **18** was achieved by applying heat-mediated Claisen rearrangement[5]. Heating **11** in a sealed tube in *p*-dichlorobenzene at 240 ℃ could initiate the [3,3] sigmatropic rearrangement to form a new carbon-carbon bond, the resulting unstable intermediate **11-a** was then reduced with sodium borohydride in ethanol and THF suspension leading to **18** in 86% yield. (Scheme **4**)



Scheme 4. Reaction mechanism for compound **18** formation

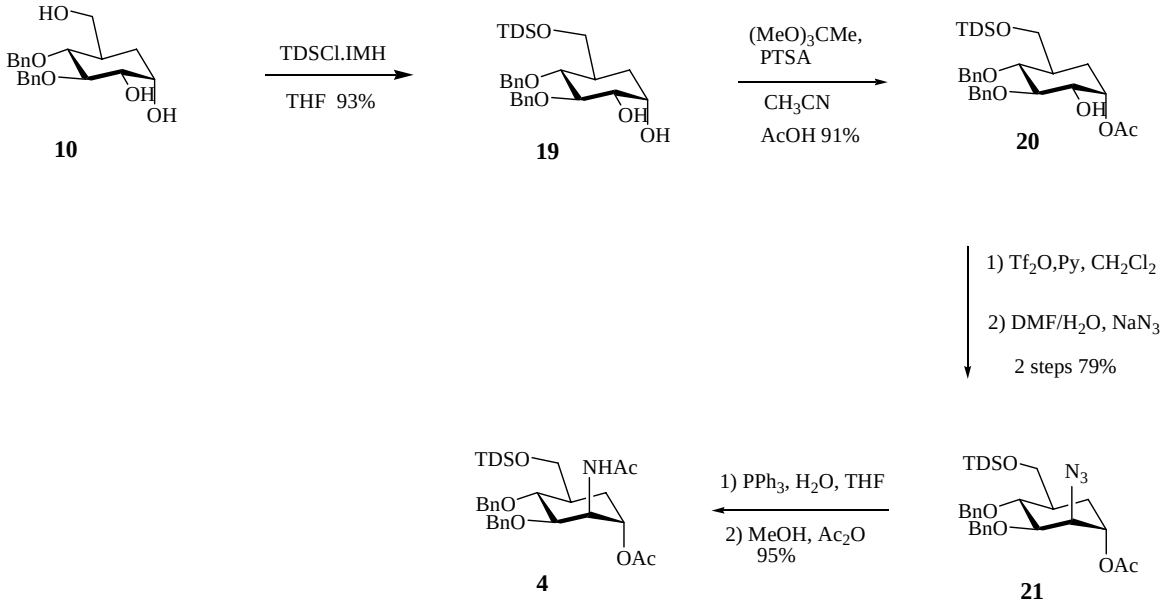
Stereoselective OsO_4-mediated syn-dihyroxylation of double bond in **18** produced less hindered α-face product **10** in 92% yield. The preparation of **10** could be scaled in multi gram scale. (Scheme **5**)



Scheme 5. Synthesis of **10**

2.3 Preparation of Intermediate 4

Then, the primary hydroxyl of triol **10** was selectively protected as TDS ether, leading to **19** in 93% yield. Since the C-1 axial hydroxyl of **19** has lost its anomeric character, intermediate **19** was able to undergo a regioselective acetylation at its C-1 position, which was carried out with trimethyl orthoacetate, and a catalytic amount of p-toluenesulfonic, followed by 80% HOAc, providing intermediate **20** in 91% yield.



Scheme 6. Synthesis of **4**

The C-2 hydroxyl group of **20** was then first activated as a triflate ester in the presence of triflic anhydride and pyridine, and the resulting intermediate was reacted with sodium azide instead of previously used tetrabutylammonium azide [3]to install the manno-congiguration, providing **21** in modest yield (50%). Interestingly, we found the reaction could be improved significantly by using a mixture of DMF and water. After several attemps (*table 1*), compound **21**could be afforded in 79% yield. We envisaged that trace amount of water could better

dissolve the sodium azide, which accelerate the SN2 reaction.

Entry	Solvent	NaN3 Equiv	Time	Temperature	Yield
1	DMF	15	12 hours	100℃	41%
2	DMF:H_2O=9:1	10	4 hours	75℃	72%
3	DMF:H_2O=4:1	5	6 hours	80℃	63%
4	DMF:H_2O=19:1	5	4 hours	70℃	74%
5	Acetone	5	12 hours	70℃	30%
4	DMF:H_2O=19:1	5	12 hours	40℃	79%

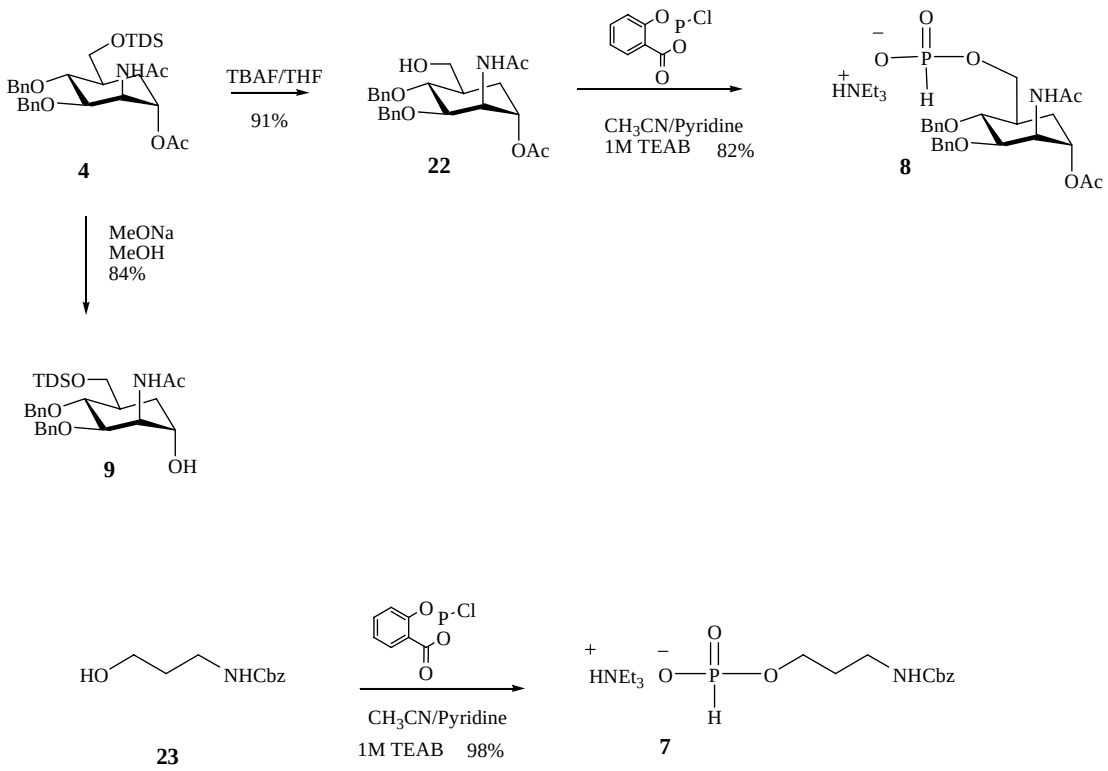
Table 1. Reaction conditions for preparing **21**

Eventually, reduction of the azide under Staudinger's condition with triphenylphosphine and water, followed by N-acetylation with acetic anhydride gave the precursor **4** in 95% yield.(Scheme **6**) Altogether, the key precursor **4** was obtained in fourteen steps on multi gram scale in 26 % overall yield, to be compared with 8% reported in our previous synthesis [3].

3 Synthesis of building blocks 8, 9 and spacer 7

After successfully accomplishing the preparation of key precursor **4**, our attention was thus focused on the synthesis of phosphonate coupling building blocks **8** and **9** and spacer **7**. Selective removal of protective groups of precursor **4** provided access either to **8** or to **9**. C-6 hydroxyl free intermediate **22** was afforded in the presence of TBAF in 91% yield. Treatment of **22** with a slight excess (1.2 equivalents) of 2-chloro-4H-1,3,2-benzodioxa phosphinin-4-one (commonly named salicylchlorophosphite) in a mixture of acetonitrile and pyridine resulted in an H-phosphonate intermediate. After work up and purification by flash chromatography, the product was recovered as its triethylammonium salt **8** in quantitative yield (82%) by washing a solution of the resulting compound in $CHCl_3$ with 1M aquesous solution of Triethylammonium bicarbonate (TEAB) buffer. Building block **9** was furnished by the deacetylation of precursor **4** under Zemplén conditions in 84% yield. (Scheme **7**)

Following a similar procedure, commercially available benzyl N-(3-hydroxypropyl) carbamate **23** was activated as an H-phosphonate intermediate **7** (98% yield) [6] in order to be inserted at the reducing end of the oligomers.



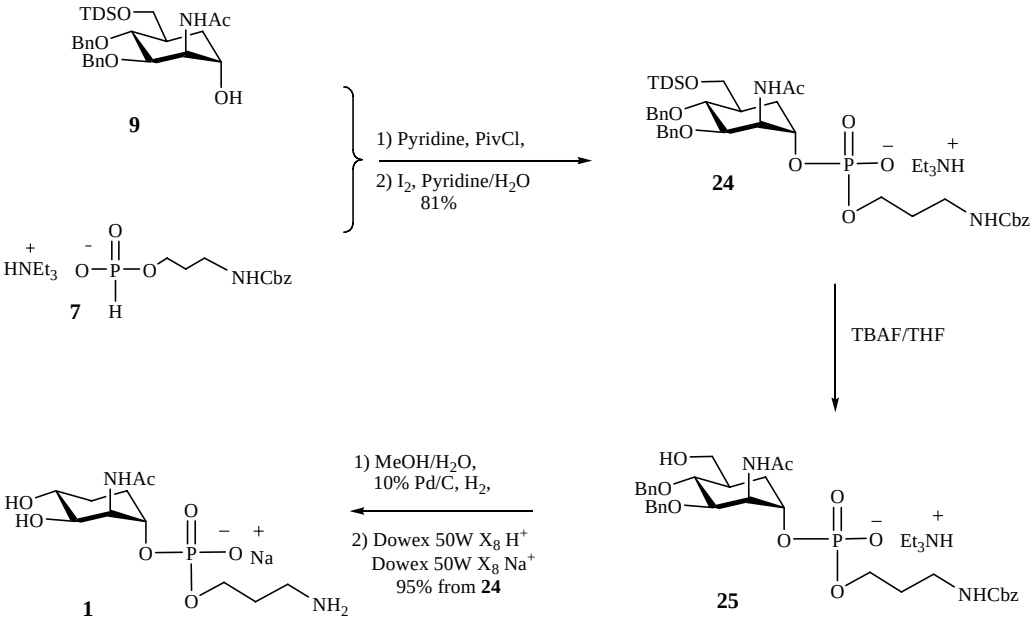
Scheme 7. Synthesis of building blocks **8**, **9** and spacer **7**.

4 Synthesis of Monomer 1

Having all the synthetic building blocks **7**, **8** and **9** in hands, the stage was set for the synthesis of target molecules. The first synthetic oligomer is the monomer carba-analogue **1**.

First, according to a standard H-phosphonate protocol [2], H-phosphonate alcohol **7** and compound **9** were condensed in the presence of trimethylacetyl (Pivaloyl) chloride in pyridine. The resulting H-phosphonate diester intermediate was oxidized *in situ* by iodine in a 19:1 mixture of pyridine and water to afford the glycosyl phosphodiester **24** in 81% yield.

Global removal of the protecting group on **24** was accomplished in two steps. First, the TDS ether of **24** was removed by treatment with TBAF in THF, yielding **25**. Thereafter, the remaining protecting group (benzyls and benzyloxycarbonyl) were cleaved by hydrogenolysis over 10% palladium on carbon in a methanol-water mixture. Final purification was completed by elution of a water solution of the deprotected fragment over a column filled with Dowex 50W X_8 resin (H+ form), followed by a second ion exchange on the same resin in Na^+ form. Lyophilization of the eluted compounds provided oligomer **1** with its sodium salt in 95% yield in two steps. (Scheme **8**).



scheme 8. Synthesis of monomer 1

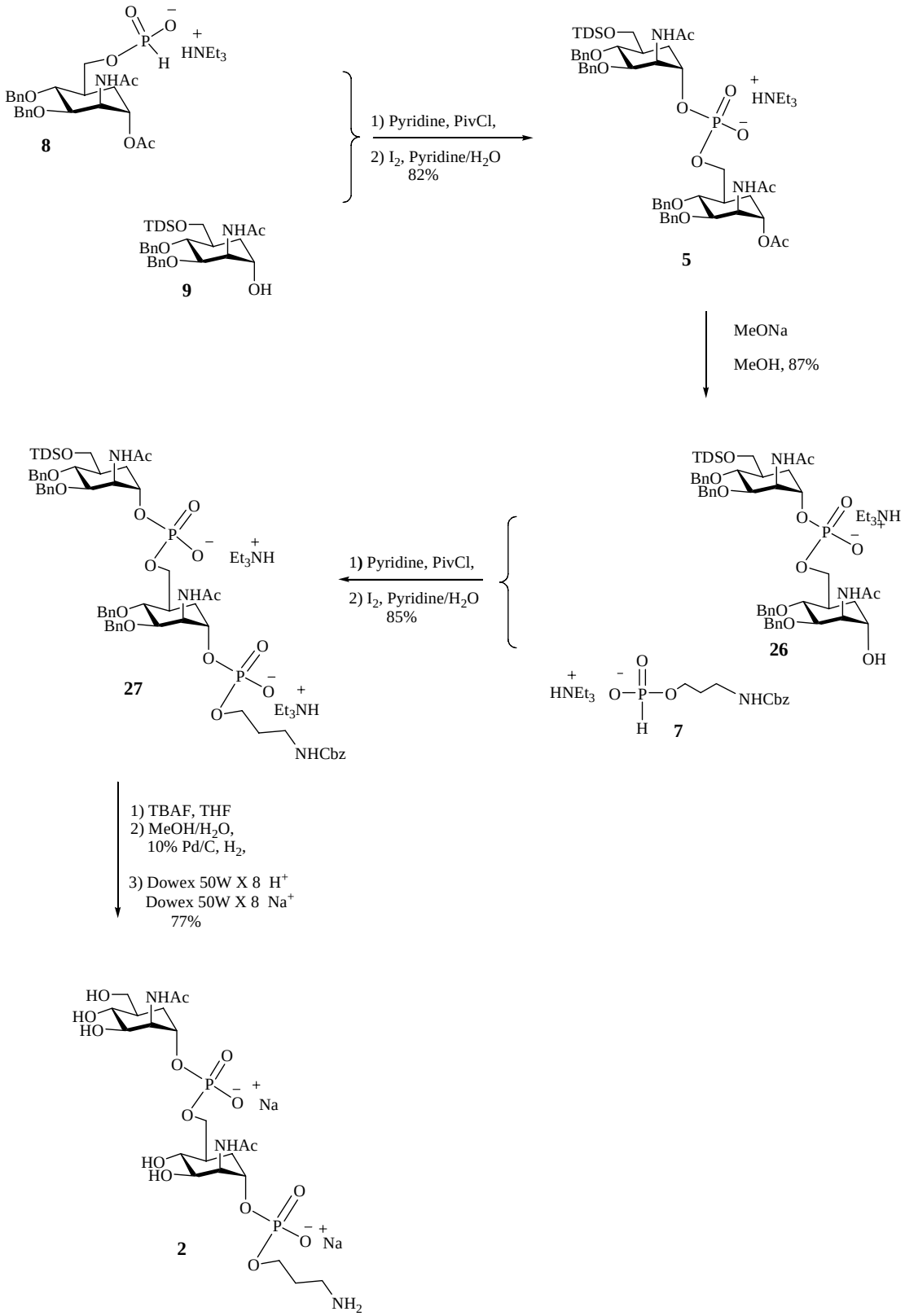
5 Synthesis of Dimer 2

Synthesis of carba analogue **2** started with building blocks **8** and **9**. First, protected dimer **5** was achieved via the phosphonate condensation between H-phosphonate **8** and **9**, followed by iodine oxidation in 82% yield. Next, **5** was deacetylated under Zemplén conditions, generating compound **26**. Then, the free hydroxyl of **26** was condensed with spacer H-phosphonate **7**, and after the iodine oxidation, spacer bearing **27** was obtained in 45% yield. Interestingly, we found the yield of phosphonate coupling reaction could be significantly improved up to 85% by increasing the amount (5 equivalents) of the H-phosphonate **7**. (Table **2**) We envisaged that it might be due to the relative low reactivity of free hydroxyl of **26**, which needed the presence of a large excess of H-phosphonte donor in the course of phosphonate condensation.

Entry	Equiv of 7	Temperature	Condensation Time	Yield
1	1.2 Equiv	25 ℃	45 min	45%
2	1.5 Equiv	35 ℃	120 min	42%
3	2 Equiv	25 ℃	45 min	50%
34	5 Equiv	25 ℃	45 min	85%

Table 2. Reaction conditions for synthesis of 27

After this, removal of the silyl group in TBAF and THF, followed by a palladium-mediated hydrogenolysis in methanol-water yielded the fully deprotected product. After the ion exchange purification with Dowex 50W X_8 resin (H^+ form and Na^+ form) and the lyophilization, carba oligomer **2** was obtained in 77% yield as sodium salt. (Scheme **9**)



Scheme 9. Synthesis of Dimer 2

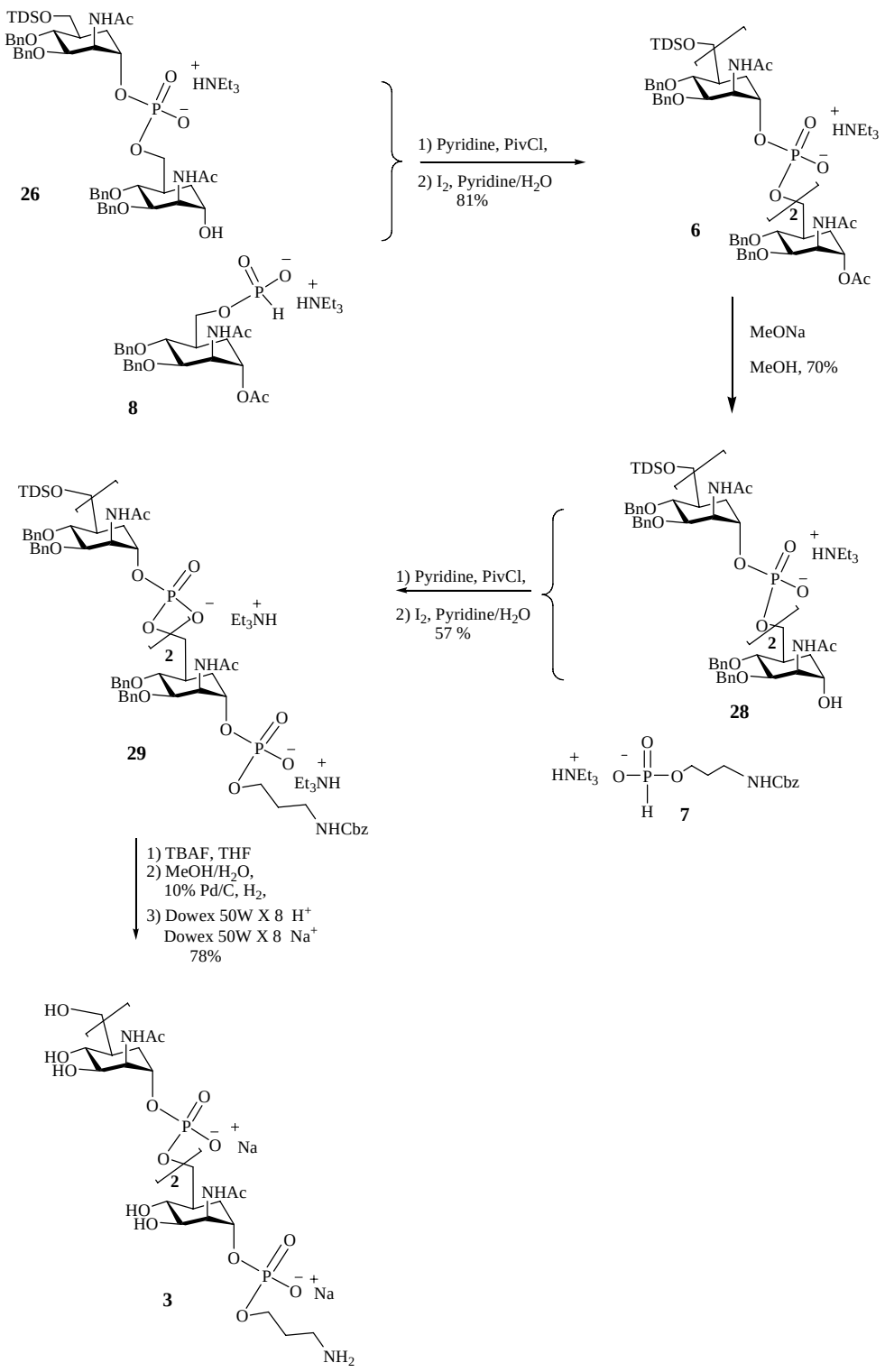
6 Synthesis of Trimer 3

For the preparation of trimer carba analogue **3**, first, similar phosphonate condensation reaction between **26** and H-phosphponate **8** and subsequent iodine oxidation afforded trimer intermediate **6** in 81% yield. The Zemplén deacetylation product **28** (from **6**, 70% yield) was coupled with spacer alcohol phosphonate **7**, and in situ oxidized, to be converted into the fully protected trimer precursor **23** in moderate yield of 57%. Increasing the amount of H-phosphonate **7** did not remarkably improve the reaction yield (Table **3**). Finally, after removal of all the protecting groups (TDS ether, benzyls and benzyloxycarbonyl), ion exchange purification (Dowex 50W X8 resin, H^+ form and Na^+ form) and the lyophilization, oligomer **3** was provided as sodium salt. (Scheme **10**)

Entry	Equiv of 7	Temperature	Condensation Time	Yield
1	1.5 Equiv	25 ℃	45 min	50%
2	2 Equiv	25 ℃	60min	52%
3	5 Equiv	25 ℃	45 min	57%

Table 3. Reaction conditions for synthesis of **29**

The identity and purity of the target compounds (**1**, **2** and **3**) was ascertained by 1H, 13C, and 31P NMR spectroscopic analyses, including two-dimensional techniques.



Scheme 10. Synthesis of Trimer **3**

7. Preliminary Biological Evaluation

Research work at Novartis Vaccine and Diagnostics Research Centre in Siena

In collaboration with Novartis Vaccines & Diagnostics group, (Vaccine Chemistry Department, Novartis Italy), the relative immunogenicities of oligomers **1**,**2** and **3** have been investigated. The abilities of increasing concentrations (from 0.5 x 10^{-7} Mm to 0.5 mM) of the newly synthesized *carba* oligomers **1-3** to inhibit the binding between the MenA CPS, coated onto plates, and a polyclonal anti-MenA serum were evaluated by competitive ELISA assay using native MenA CPS and native Men*A*$_{av}$DP15 (MenA oligosaccharide with average polymerization degree 15) as positive controls, and Laminarin as negative control.

The inhibition capacity of the synthetic compounds was determined in comparison with a native MenA oligosaccharide with average polymerization degree 3 (native Men*A*$_{av}$DP3). The results showed that the best inhibition was given by native MenA CPS and by native Men*A*$_{av}$DP15 (IC_{50} of 5.15 x 10^{-6} and 4.3 x 10^{-3} mM, respectively, and a 98% of inhibition of serum, Table **4** and Figure **2**). Only dimer **2** was able to induce a 90% of serum inhibition with an IC_{50} of 0.16 - 0.091 mM (Table 1). In contrast, compounds **1** and **3** were only poor competitors, with an inhibition around 30% of the antibody binding. Carba disaccharide **2** showed a similar trend to native Men*A*$_{av}$DP3 oligosaccharide, which reached 93% of serum inhibition with an IC_{50} of 4.3 x 10^{-2}mM.

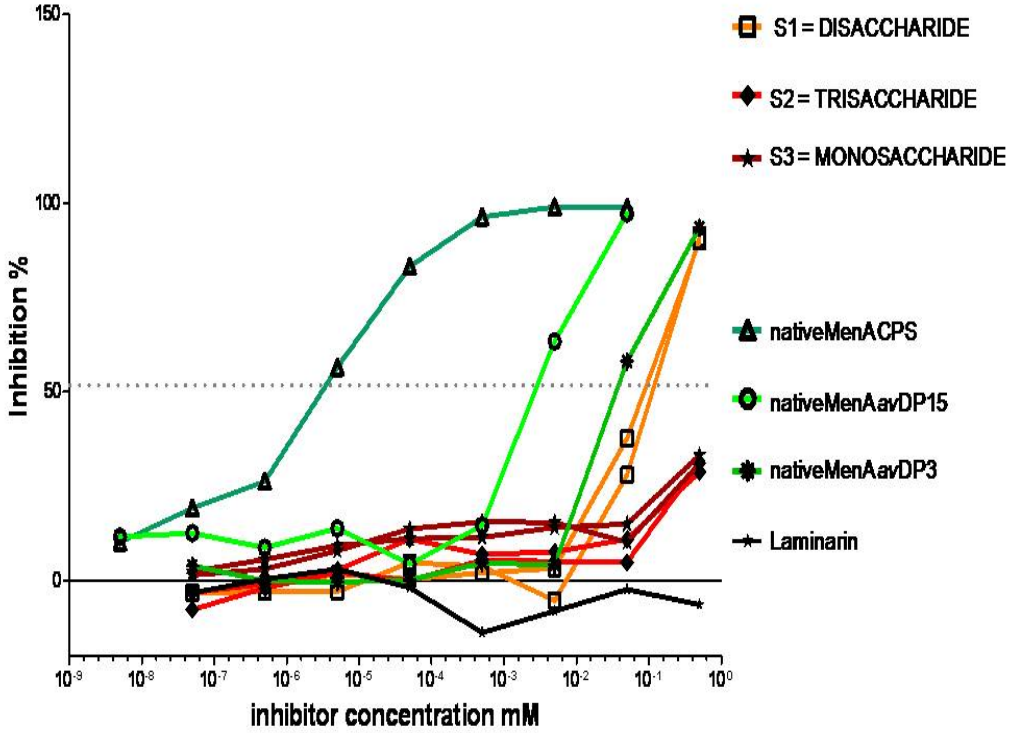


Figure 2. Competitive ELISA betweenthe native MenA CPS and oligosaccharide inhibitors for anti-MenA CPSpolyclonal serum. S1, S2 and S3 are the synthetic inhibitors compared with the native molecules MenA CPS, avDP15 and avDP3 oligosaccharides. The non correlated polysaccharide Laminarin was used as negative control.

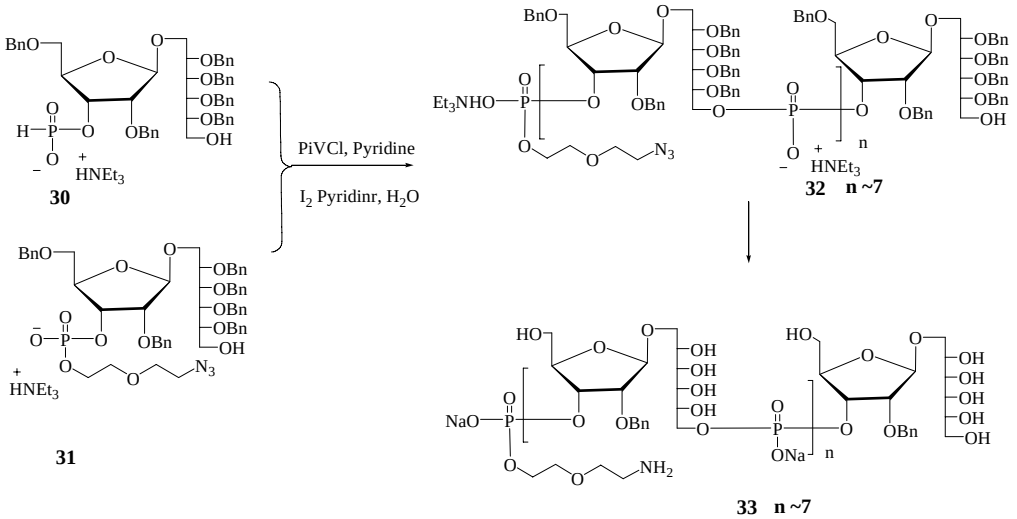
Meningococcal A antigens	1	2	3	Native MenA CPS	Native *av*DP15	Native *av*DP3
IC_{50}(mM)	----	0.16 - 0.091	---	5.15×10^{-6}	4.3×10^{-3}	4.3×10^{-2}

Table 4. Competitive ELISA IC_{50}Values (mM)

8. H-Phosphonate polycondensation

In this PhD work, attempts to achieve longer chain carba oligomers were also carried out. Although the traditional solution and solid-phase techniques seemed feasible to construct oligomers with controlled numbers of repeating units, these strategies still own several problem like time consuming, lack of atom economy and unreliability in large scale preparation. Recently, several research groups have reported the one-step polycondensation reaction based on H-phosphonate chemistry to achieve poly (glycosyl phosphates) compounds in high yield, short number of steps and high reaction rate.

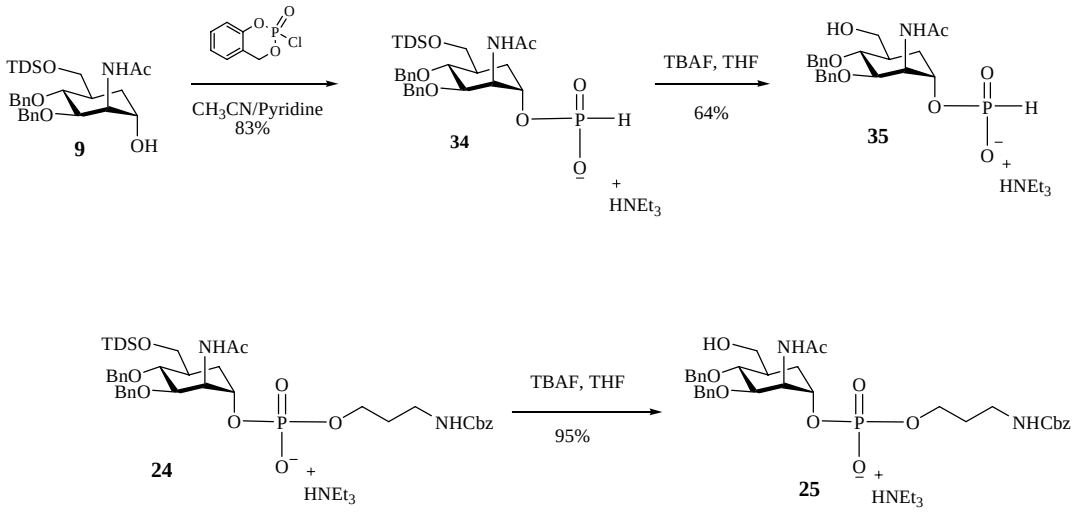
For example, in their preparation of the first synthetic carbohydrate based vaccine, Verez-Bencomo and coworkers used the H-phosphonate polycondensation method to obtain their target oligosaccharide. After phosphonate condensation, *in situ* oxidation and size exclusion chromatography purification, the desired oligomers with an average of eight repeating units was obtained in 80% yield [7].



Scheme 11 Example of H-Phosphonate polycondensation by Bencomo

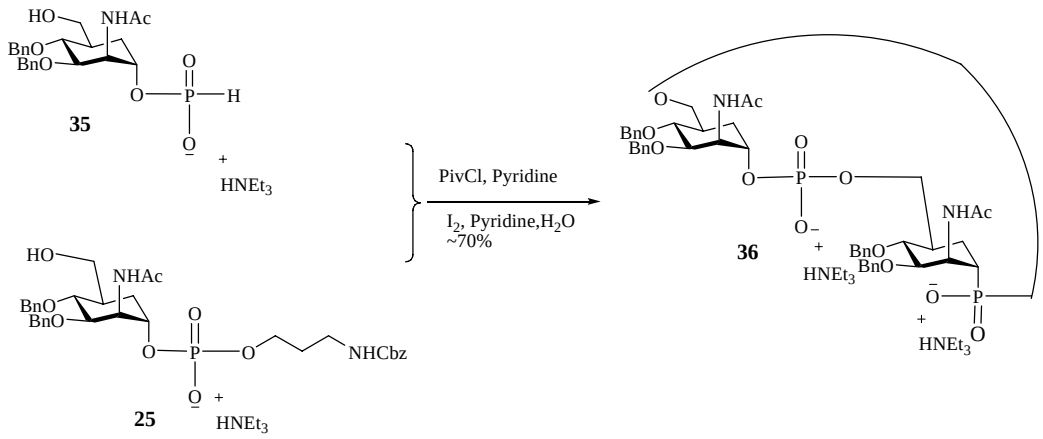
Besides this, some other groups have also published the use of this strategy to quickly construct oligomers with high number of repeating units. [8] The high efficiency and high reaction rate of the H-phosphonate condensation reaction prompted us to investigate the possibility of applying this method onto our carba sugar intermediates to achieve longer chain oligomers.

Therefore, following the same strategy described above for the synthesis of glycosyl phosphodiesters, monomer building blocks **35** and **25** were synthesized from intermediate **9** and **24**. Treatment of **9** with salicylchlorophosphite gave **34**, and removal of the sily ether from **34** delivered bifunctional H-phosphonate building block **35**. **25** was the precursor for the synthesis of monomer **1**. (Scheme **12**)



Scheme 12. Synthesis of monomer H-phosphonate building blocks

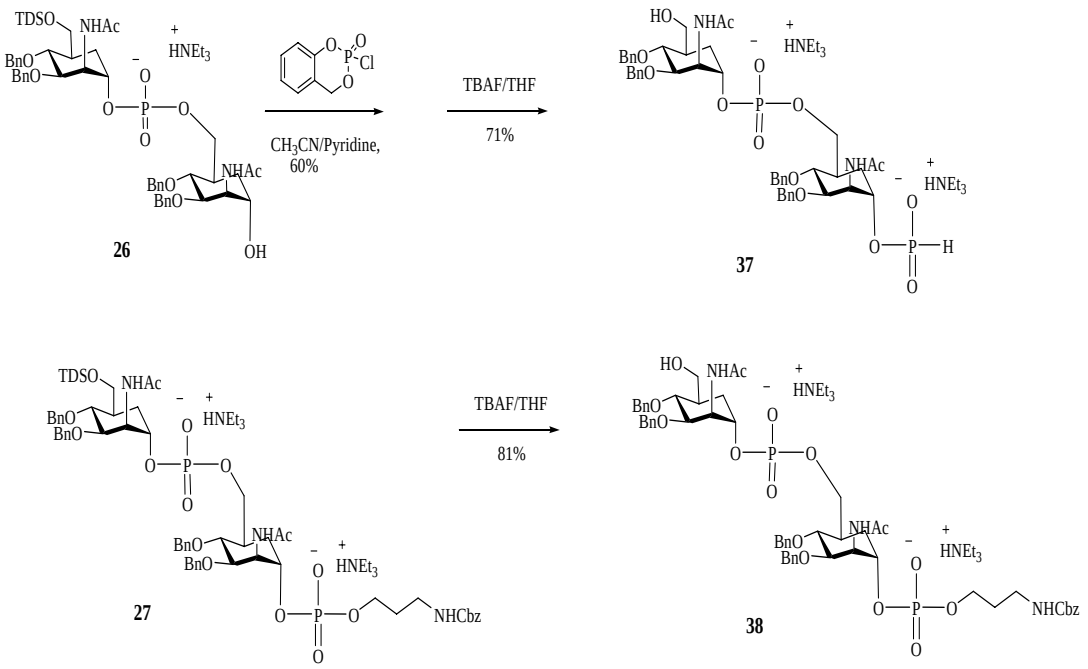
However, the H-phosphonate condensation between **35** and **25** by using typical published procedure, after the ESI analysis, was shown to only provide cyclic dimer **36** instead of desired linear poly(glycosyl phosphate)as main product. Using high concentration (1 M of **35** in pyridine) of reaction which was supposed to favor the H-phosphonate polycondensation did not change the result. (Scheme **13**)



Scheme 13 Test for polycondensation

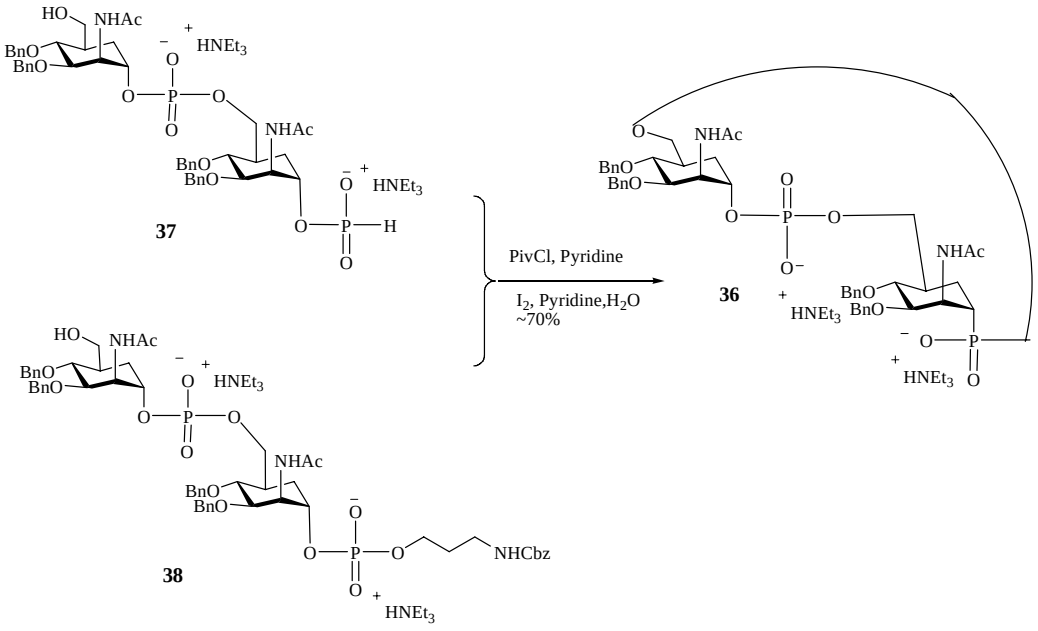
We envisaged that using dimer H-phosphonate building blocks might be helpful to avoid the generation of cyclic dimer as it should in principle increase the molecule's spacial distance to prevent the formation of the cyclic intermediates during the coupling course.

Therefore, as shown in scheme **14**, dimer H-phosphonate building block **37** was prepared from dimer precursor **26**, which alcohol **38** was obtained by desilylation of dimer **27**.



Scheme 14. Synthesis of dimer H-phosphonate building blocks and test for polycondensation

Then, as shown in the Scheme **15**, compounds **37** and **38**, were tested for polycondensation reaction. Disappointingly, the coupling between **37** and **38** still generated dimer cyclic product **36**.



Scheme 15. Test for polycondensation using dimer H-phosphonate building blocks

We thought the reason for the failure of H-phosphonate polycondensation might be due to the nature of carba sugar, which might be different from real olisaccharide at the molecular space. Although the real mechanism is still under the investigation, for the future work, due to the convenience of achieving oligosaccharides with higher number of repeating units, this method will be still explored.

In short, we investigated the possibilities of applying polyconsation reaction on carba sugar analoues, wishing to achieve more advanced oligomers in one pot reaction. However, till now we have not found a suitable way to achieve this target. The corresponding monomer and dimer H-phosphonate building blocks only generated cyclic dimer product.

9. Protein conjugation

Research work at Novartis Vaccine and Diagnostics Research Centre in Siena

9.1 NEO-glycoconjuates for biological assays

For the purpose of better the understanding immunogenical profile of the carba analogues of MenA CPS, and to investigate whether the carba oligomer glycoconjuates can compete with natural purified MenA CPS neo-glycoconjugates, a series of polysaccharide-protein glycoconjugates based on the carba analogues of MenA CPS fragments have been designed and synthesized.

First of all, two different immunogenic proteins: CRM_{197} (Cross-Reacting Material 197 [9]) and HSA (Human Serum Albumin) were chosen to be conjugated with the carba oligomers.

CRM_{197} first isolated in 1973, does not need chemical detoxification and could still maintain native three-dimensional structure during processing. CRM_{197} is commonly used as carrier protein in carbohydrate based vaccine, especially in routine childhood vaccines. [10] Currently licensed vaccine Menveo® (Novartis Vaccine,Switzerland) is a tetravalent meningococcal CRM_{197}-conjuate vaccine against meningococcal serogroup A, C, W-135 and Y. [11]

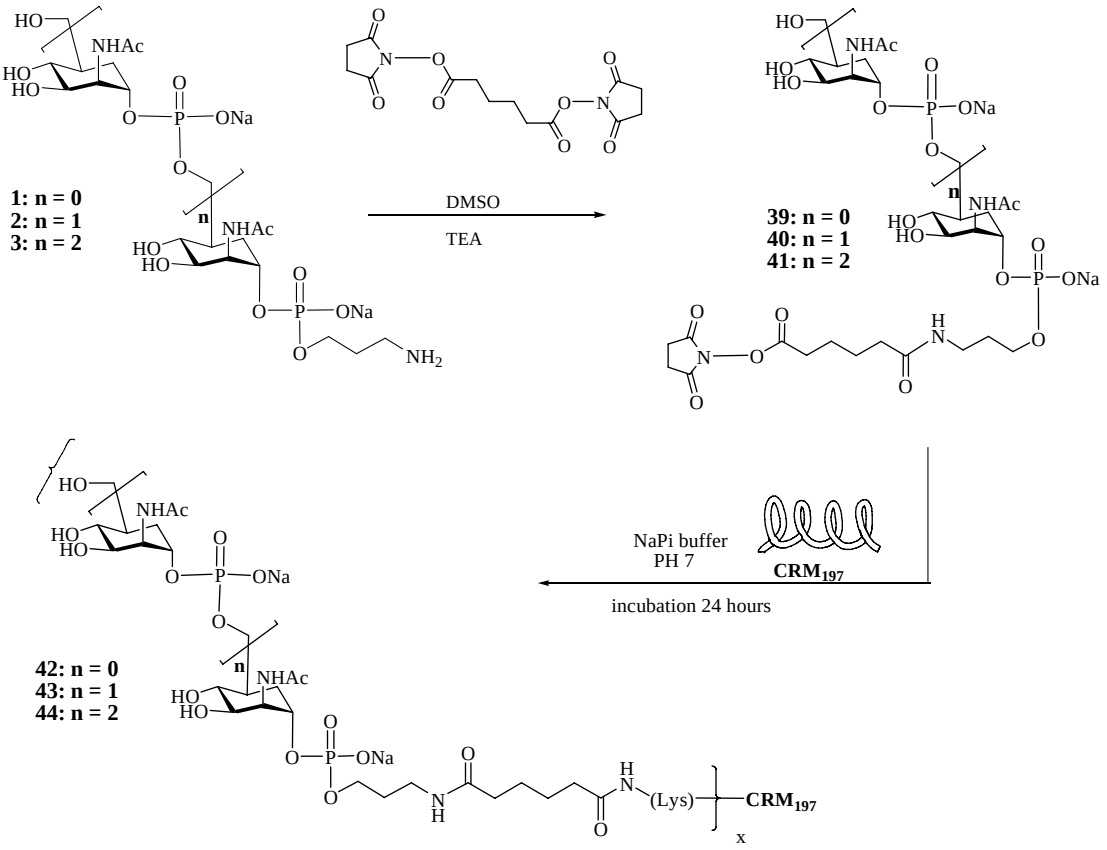
Human serum albumin is the most abundant protein in human blood plasma. It is produced in the liver. Albumin constitutes about half of the blood serum protein. It is also a commonly used carrier protein. [12]

In this research, neo-glycoconjugates obtained form carba oligomers conjugated with carrier protein CRM_{197} will be mainly used for mice immunization, while

those prepared from carba oligomers linked with HSA will be employed for further ELISA study. Lysine residues in CRM_{197} and HSA presented outwardly on the molecules can link with carba oligomers through suitable "linker molecule". Furthermore, to investigate whether the "linker molecule" influences the immunological activities, two chemically different spacer will be used as molecular bridge, namely an Disuccinimidyl adipate for CRM_{197} and $BS(PEG)_5$-based spacer for HSA.

9.2 Conjugation of carba oligomers to carrier protein CRM_{197}

First, using disuccinimidyl adipate linker chemistry (Scheme **16**), [13] the synthesized carba oligomers **1**, **2** and **3** were converted into activated monomer, dimer and trimer precursors **39**, **40** and **41** respectively in DMSO, in the presence of TEA and disuccinimidyl adipate (known as SIDEA). After the purification (precipitation and centrifugation for several times, lyophilization), the freshly prepared activated oligomers **39**, **40** and **41** were conjugated with CRM_{197} in sodium phosphate buffer with one or two days' incubation at room temperature.



Scheme 16. Synthesis of oligomers-CRM_{197} glycoconjugate **42**, **43** and **44**

The fundamental parameters of the CRM_{197} conjugation is shown in *Table 4*. The sugar-loading was determined by MALDI spectra, while the SDS-PAGE allowed to

confirm the occurrence of the conjugation. All the neo-glycoconjugates contained at least ten oligomers.

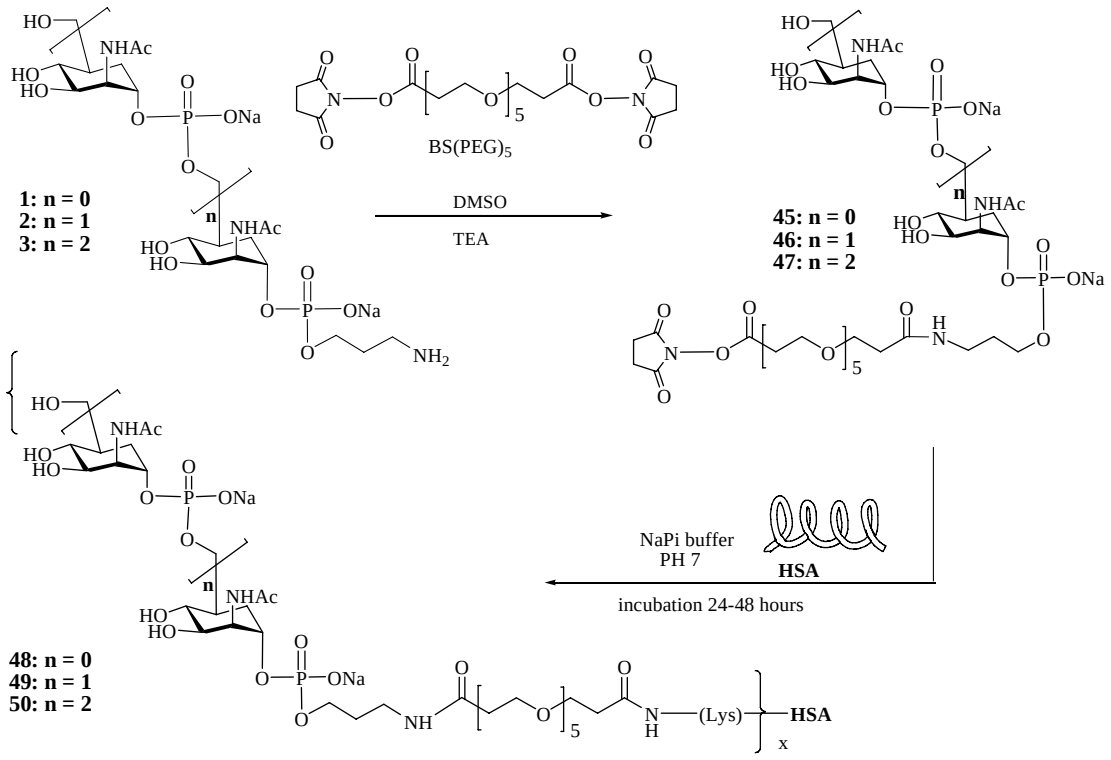
Product	Activated Ester/Protein	Loading (X)	MW	Protein Total	Sugar Concentration mg/ml	Protein Concentration mg/ml
42	40:1	18.5	67526	1.35	0.089	0.68
43	50:1	12.3	68252	0.88	0.063	0.44
44	50:1	10.5	69957	1.72	0.14	0.86

Table 4. Data of CRM_{197} neo-glycoconjugates

9.3 Conjugation of carba oligomers to carrier proteins HSA

For the conjugation with carrier protein HSA, a similar approach to prepare the neo-glycoconjugates was employed. Only the bis-succinimidyl penta-ethylene-glycol (called $BS(PEG)_5$) was used instead of SIDEA for the purpose of investigating the influence of "linker molecules".

Thus, the activated monomer, dimer trimer precursors **45**, **46** and **47** were prepared in DMSO, in the presence of TEA and $BS(PEG)_5$. After purification, the activated oligomers were conjugated with HSA in sodium phosphate buffer solution with one or two days' incubation, generating the neo-glycoconjugates **48**, **49** and **50**. (*Scheme 15, table 5*)



Scheme 17. Synthesis of oligomers-HSA glycoconjugate **48**, **49** and **50**

As shown in *table 5*, the HSA glycolconjugation are similar to those of CRM_{197} conjugation. The relatively lower oligomers loading on **50** (about eight sugar chains per protein molecule) might be mainly due to the steric hindrance of trimer carba analogue.

Product	Activated Ester/Protein	Loading X	MW	Protein Total	Sugar Concentration mg/ml	Protein Concentration mg/ml
48	40:1	14.6	74954	0.94	0.062	0.47
49	50:1	14.5	76899	1.62	0.125	0.81
50	50:1	7.9	73869	1.51	0.091	0.76

Table 5. Data of HSA neo-glycoconjugates

In short, the synthesized carba sugar compounds were all successfully conjugated with two kinds of carrier protein by two different linkers. The loading efficiency, as shown in Table **4** and Table **5**, is satisfatory and will be helpful for the future immunological test. The biological evaluation of these neoglycoconjugates is still on the course in Novartis laboratory.

Reference List

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CONCLUSION

Overall, we completed the synthesis and preliminary biological evaluation of the carba analogues of MenA CPS.

For the synthesis work, a reliable procedure for preparing all the needed building blocks compounds has been established. The key intermediate **4** could be synthesized in lab in multi gram scale, which facilitates the synthesis of these derivatives. Based on the H-phosphonate coupling reactions, the oligomer elongation and phosphonate diester-linked spacer introduction were successfully achieved in high yield. Also, the global deprotection and purification procedure proved to be efficient and are believed to be practical for preparing more complex derivatives.

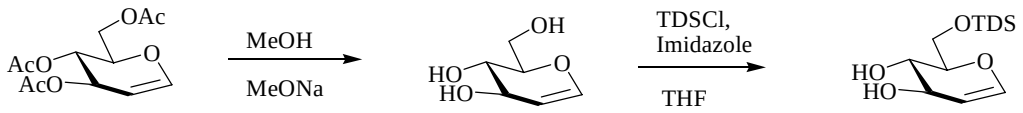
We have also carried out several trials employing H-phosphonate polycondensation reactions to achieve carba oligomers with higher number of repeating units. However, with current building blocks (monomer and dimer H-phosphonates) and method, we only produced the undesired cyclic dimer. Further work will be focused on this part to find a feasible way to realize the H-phosphonate polycondensation.

The synthesized carba sugar compounds 1-3 were successfully conjugated with two kinds of carrier protein (CRM_{197} and HSA) by two different linkage molecules, which will be useful for the future immunological test for these analogues. The biological evaluation of the neoglycoconjugates is still on the course.

EXPERIMENTAL PART

General: Reagents were obtained from commercial sources and used as purchased. All the organic solvents were purchased anhydrous and used without further purification. Unless otherwise noted, all reactions were carried out at room temperature in oven-dried glassware with magnetic stirring. Molecular sieves were flame-dried under high vacuum prior to use. Organic solutions were concentrated under diminished pressure with bath temperatures <40℃. NMR spectra were recorded on Bruker AC 300 and Bruker Avance 400 spectrometers at 298 K, unless otherwise reported. In ^{1}H NMR spectra, multiplicities are quoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). In ^{13}C NMR spectra, signals corresponding to aromatic carbons are omitted. Chemical shifts are reported on the d (ppm) scale and in ^{31}P spectra they are relative to H3P04. Peak assignments were based on analysis of 2D spectra (H,H-COSY and HSQC or HMQC spectra). HRMS spectra were recorded in the negative or positive modes on Jeol AX-505 and Bruker Daltonics APEX TMII (FT-ICR) instruments. TOF MS analysis was performed operating in positive ion mode (Bruker model Ultraflex MALDI-TOF mass spectrometer). Optical rotations were measured at room temperature with a Perkin-Elmer 241 polarimeter. TLC and HPTLC were carried out on Merck silica gel 60 F-254 plates (0.25 mm and 0.2 mm thickness, respectively), and spots were viewed by spraying with a solution containing H2S04 (31 mL), ammonium molybdate (21 g) and Ce(S04)2 (1 g) in 500 mL water, followed by heating at 110 8C for 5 mins. Column chromatography was performed by the flash procedure on Merck silica gel 60 (230-400 mesh). Solvents were dried by standard procedures.

6-O-thexydimethylsilyl D-glucal 14



12 **13** **14**

Synthetic Procedure:

Synthesis of 13:

3, 4, 6-Tri-O-Acetyl-D-Glucal **12** (25 g, 92 mmol) was dissolved in dry MeOH (150 mL) under nitrogen atmosphere, NaOMe (1.5 g, 27.7 mmol) was added, the mixture was stirred at room temperature for 2 h, then the reaction mixture was neutralized to PH 7 with IR-120 resin, (H^+ form, previously washed by MeOH), filtered, and concentrated, get a 16 g **13** as colorless oil.

Synthesis of 14:

To a solution of **13** (16 g, 92 mmol) and imidazole (14.6 g, 211 mmol) in THF (250 mL), was slowly added thexyldimethylsilyl chloride (35 mL, 164 mmol) at 20℃. After stirring at room temperature for 24 hours, satd $NaHCO_3$ (150 mL) was added to the reaction mixture, followed by extraction with CH_2Cl_2 (3 × 100 mL). The organic phases were dried (Na_2SO_4), filtered, and concentrated. The crude materials was purified by flash chromatography (40% EtOAc/Hexane), aforded **14** (22 g, 2 steps 83 %) as a clear oil.

Product characterization 13

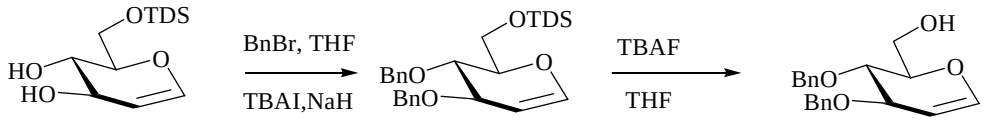
Formula	C6H1004
Molecular Wright	146.14
TLC conditions	Rf: 0.3, CH_2CL_2 : MeOH = 9 : 1

Product characterization 14

Formula	C14H2804Si
Molecular Wright	288.46
TLC conditions	Rf: 0.4, Hexane : ETOAc = 1 : 1

The spectroscopic data were in agreement with the reported data [1]

3,4-di-O-Benzyl-D-glucal 16



14 **15** **16**

Synthetic Procedure:

Synthesis of 15:

14 (22 g, 76 mmol) was dissolved in dry THF (150 mL). BnBr (19.5 mL, 164 mmol) was added at room temperature, then 60% NaH (6.11 g, 156 mmol) was added little by little, followed by TBAI (2.87 g, 8 mmol), the mixture was stirred under nitrogen atmosphere at room temperature for 2 hours. MeOH (100mL) was then added to quench the reaction, and another NH_4Cl solution (250 ml) was added, followed by extraction with CH_2Cl_2 (3 × 100 mL). The organic phases were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (5% EtOAc/Hexane), providing **15** (34 g, 95 %) as a clear oil.

Synthesis of 16:

15 (34 g, 73 mmol) was dissolved in dry THF (150 mL), TBAF (1M solution in THF + 5% H_2O, 87 mL, 87 mmol) was added at 0℃, the solution was then stirred under nitrogen at room temperature. After 2 hours, satd NH_4Cl solution (200 mL) was added and stirred for 10 min, CH_2Cl_2 (300 mL) was added for extraction, and the organic layer was washed with brine (200 mL), dried (Na_2SO_4), filtered, and concentrated. The crude material was purified by flash chromatography (40% EtOAc/Hexane), providing **16** (20.2 g, 85 %; 4 steps, 67 %) as a white solid.

Product characterization 15

Formula C28H4004Si

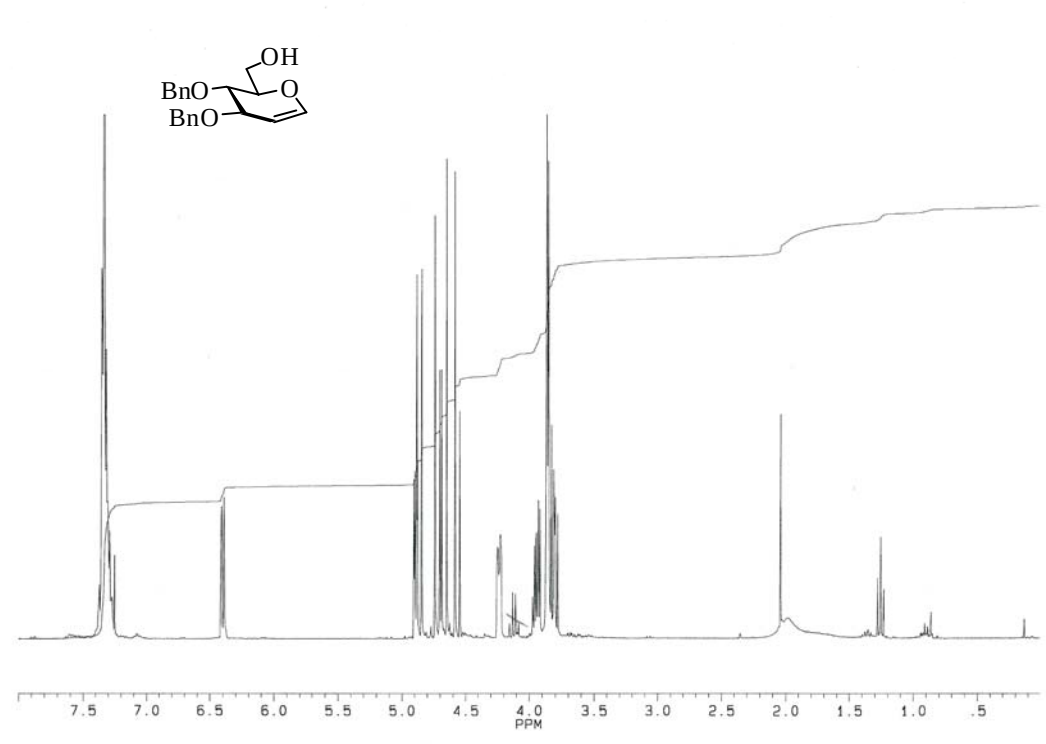
Molecular Wright	468.7
TLC conditions	Rf: 0.5, Hexane : EtOAc = 19 : 1

Product characterization 16

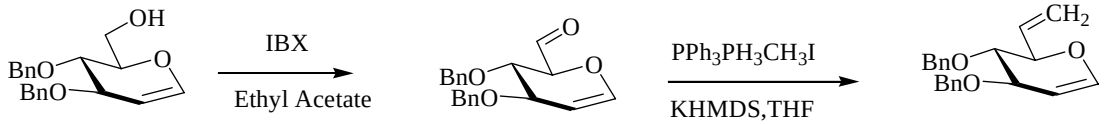
Formula	C20H2204
Molecular Wright	326.39
TLC conditions	Rf 0.35, Hexane : EtOAc = 3 : 1

The spectroscopic data were in agreement with the reported data

^{1}H NMR spectrum of compound **16**



1,5-Anhydro-di-O-benzyl-2,6,7-trideoxy-d-arabino-hept-1,6-dienitol, 11



16 **17** **11**

Synthetic Procedure:

Synthesis of 17

To a solution of **16** (12.3 g, 38 mmol) in dry EtOAc (400 mL), freshly prepared 2-iodoxybenzoic acid (89 g, 319 mmol, prepared from 2-iodobenzoic acid and Oxone [2]) was added, and the suspension was stirred under nitrogen at 75 ℃ for 4 hours. The mixture was then cooling, filtered over a celite pad and concentrated. The crude was co-vaporated with toluene (3 × 50 mL) to obtain the aldehyde intermediate (12.3 g, 99%). NMR analysis showed the complete conversion from **17** to the aldehyde.

Synthesis of 11

Freshly prepared PPh_3CH_3I (23.3 g, 57 mmol) was dissolved in dry THF (40 mL), the solution was cooling to -78 ℃, then KHMDS (1M solution in THF, 57 mL, 57 mmol) was added under nitrogen. The mixture was stirred at -78 ℃ for 30 minutes, then another 1 hour at 0 ℃. The solution of the aldehyde 17 (12.3 g, 38 mmol) in dry THF was added to the mixture at -78 ℃. The reaction mixture was then stirred at room temperature. After 3 hours, saturated aqueous solution of NH_4Cl (200 mL) was added and stirred for 10 minutes, the mixture was diluted with CH_2Cl_2 (300 mL) and the organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (40% Toluene/Hexane) providing **11** (9.4 g, 77 %) as a clear oil.

Product characterization 17

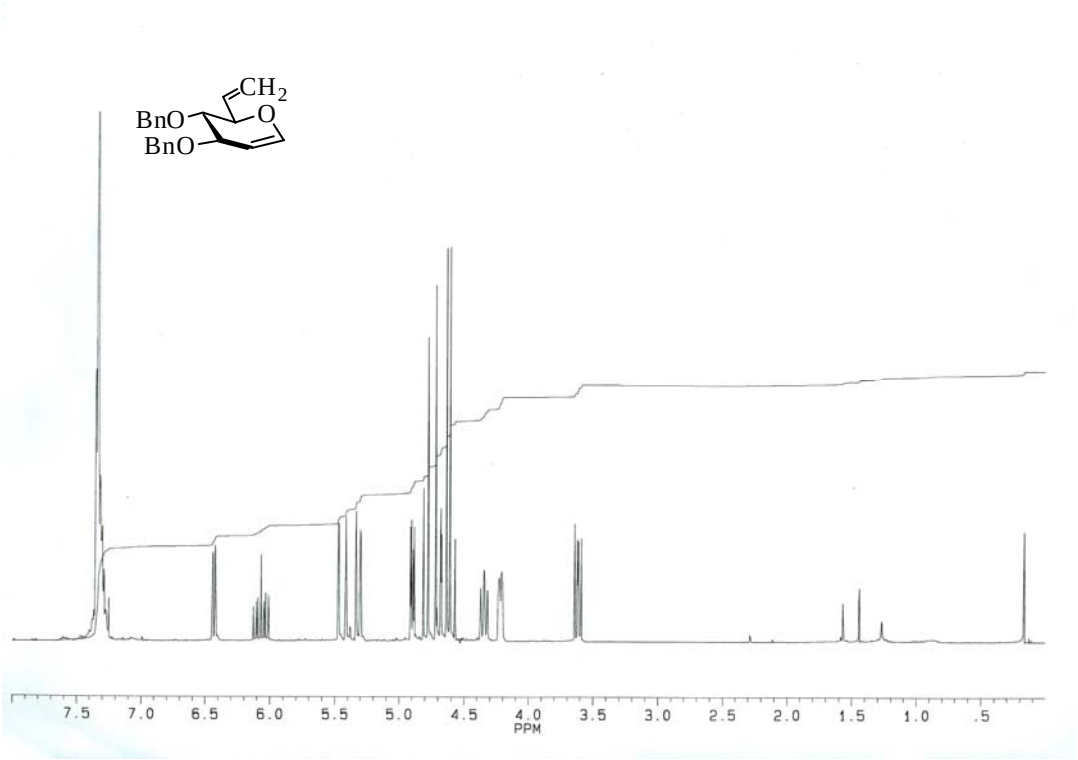
Formula	C20H2004
Molecular Wright	324.37
TLC conditions	Rf 0.5, Hexane : EtOAc = 3 : 1

Product characterization 11

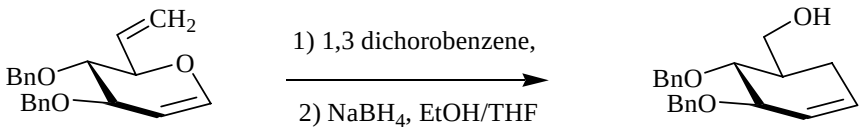
Formula	C21H2203
Molecular Wright	322.4
TLC conditions	Rf 0.5, Hexane : EtOAc = 9 : 1

The optical rotation and the spectroscopic characterization data were in agreement with the reported data [3]

^{1}H NMR spectrum of compound **11**



(3R,4R,5R)-3,4-dibenzyloxy-5-(hydroxymethyl) cyclohexene, 18



11 **18**

Synthetic Procedure:

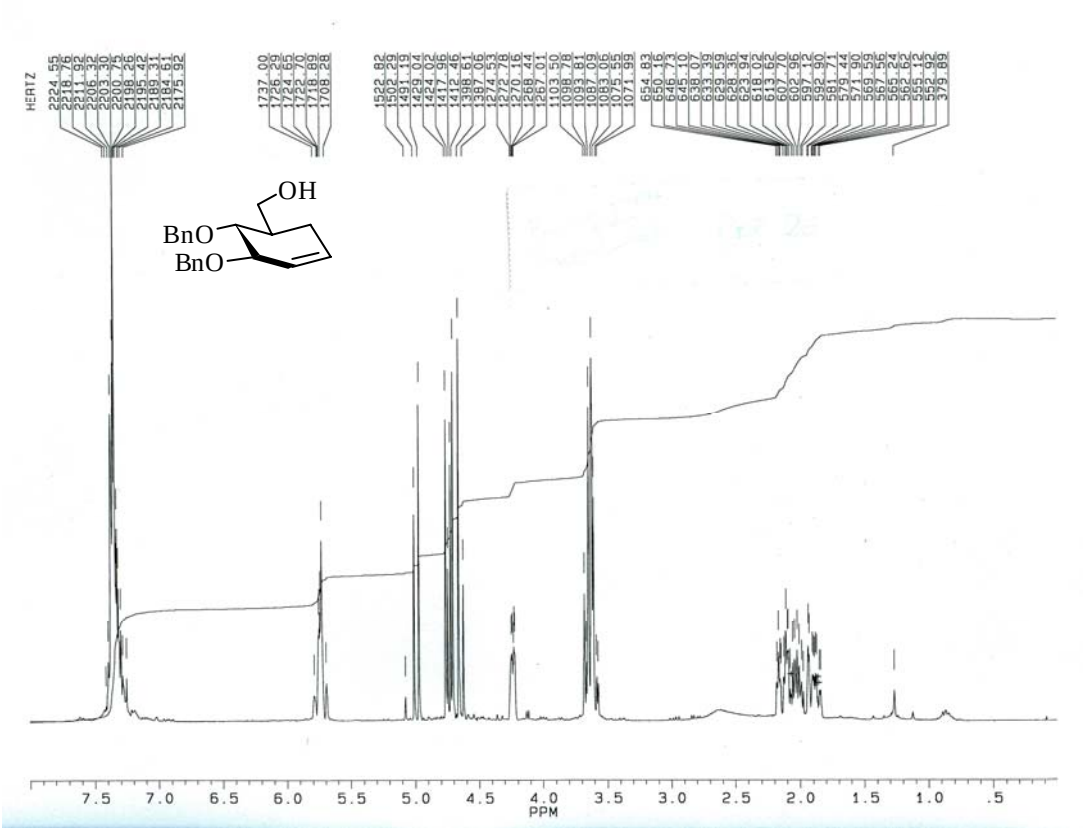
Compound **11** (7.5 g, 23.3 mol) was dissolved in 1,6-dichlorobenzene (25 mL) in a sealed tube and heated at 240 ℃ for 2 h. After cooling down, the mixture was slowly poured into a suspension of $NaBH_4$ (500 mg, 13 mmol) in THF (100mL) and EtOH (25 mL) and stirred for 15 minutes. Then water (200 mL) was added, and the mixture was extracted with CH_2Cl_2 (3×100 mL). The organic layer was washed with brine (200 mL), dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (30% EtOAc/Hexane), providing **13** (6.5 g, 86 %) as a clear oil.

Product characterization 18

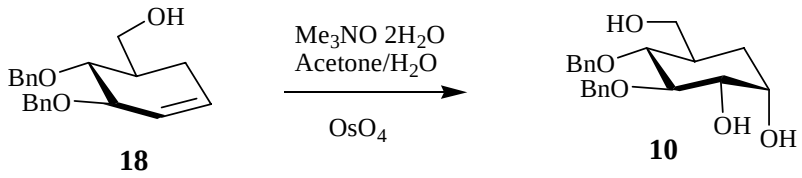
Formula	C21H2403
Molecular Wright	324.41
TLC conditions	Rf 0.4 Hexane : EtOAc = 2 : 1

The optical rotation and the spectroscopic characterization data were in agreement with the reported data [2]

^{1}H NMR spectrum of compound **18**



3,4-Di-O-benzyl-5a-carba-α-d-glucopyranose, 10



18 **10**

Synthetic procedure:

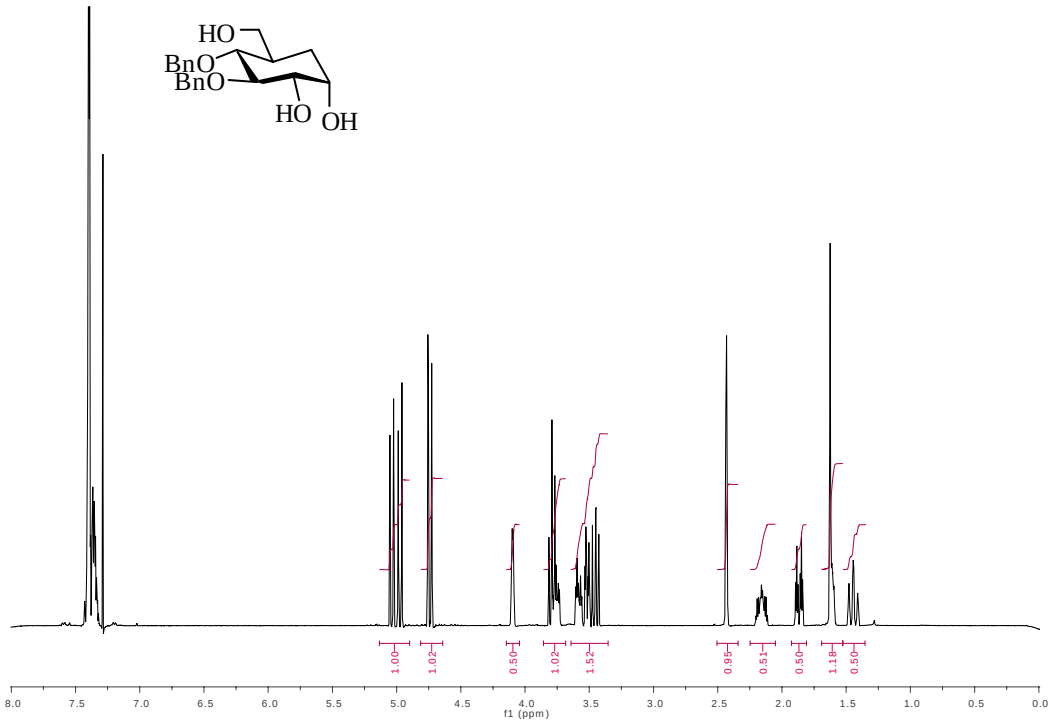
Compound **18** (6.5 g, 20.8 mmol) was dissolved in a mixture of acetone (75 mL) and H_2O (25 mL), OsO_4 (CAUTION!) solution (250 mg in 4.5 mL H_2O and 18 mL acetone) was added at room temperature, followed by Me_3NO (5.075 g, 46 mmol), and continued stirring for 48 h at room temperature. A saturated aqueous solution of $Na_2S_2O_3$ (50 mL) was added and stirred for 30 minutes to reduce the OsO_4, chloroform (300 mL) was added and the organic layer was washed with brine (200 mL), dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (5% MeOH/CH_2Cl_2), providing **10** (6.23 g, 86.3%) as a white solid.

Product characterization 10

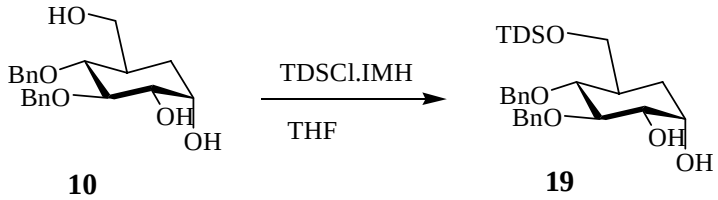
Formula	C21H2605
Molecular Wright	358.43
TLC conditions	Rf 0.2, CH_2Cl_2 : MeOH = 19 : 1

The optical rotation and the spectroscopic characterization data were in agreement with the reported data [4]

^{1}H NMR spectrum of compound **10**



3,4-di-*O*-benzyl-6-*O*-thexyldimethylsilyl-5a-carba-α-D-glucopyranose, 19



10 **19**

Synthetic Procedure

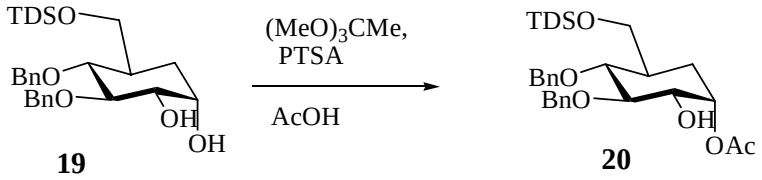
To a solution of **10** (8.1 g, 22.6 mmol) and imidazole (4.6 g, 68 mmol) in THF (200 mL), thexyldimethylsilyl chloride (9.8 mL, 52 mmol) was added dropwise at 15 ℃. The mixture was stirred at room temperature for 24 hours, then saturated aqueous solution of $NaHCO_3$ (100 mL) was added, followed by extraction with EtOAc (3 × 150 mL). The organic phases were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (30% EtOAc/Hexane), yielding **19** (10.5 g, 93 %) as a colorless oil.

Product characterization 19

Formula	C29H4405Si
Molecular Wright	500.74
TLC conditions	Rf 0.4, Hexane : EtOAc = 5 : 2

The optical rotation and the spectroscopic characterization data were in agreement with the reported data [3]

1-*O*-Acetyl-3,4-di-*O*-benzyl-6-*O*-thexyldimethylsilyl-5a-carba-α-D-glucopyranose, 20



Synthetic Procedure

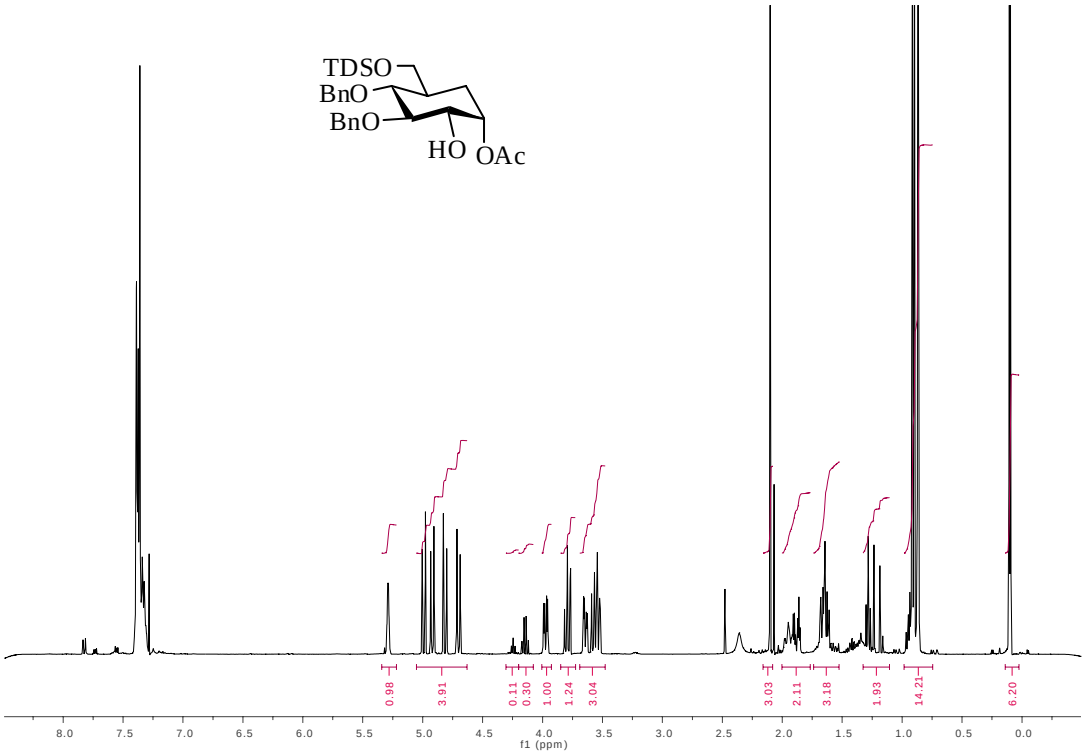
Compound **19** was successively dissolved in dry acetonitrile (150 mL) under nitrogen. Trimethyl orthoacetate (6.25 mL, 50 mmol) was added at room temperature, followed by a catalytic amount of p-toluenesulfonic acid. After 15 minutes, 80% aqueous solution of acetic acid (150 mL) was added and stirring was continued for 15 minutes. CH_2Cl_2 (200 mL) was added and the organic layer was washed with H_20 (200 mL) and saturated aqueous solution of $NaHCO_3$ (200 mL), dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (25% EtOAc/Hexane), providing **20** (10.45 g, 91%) as a clear oil.

Product characterization 20

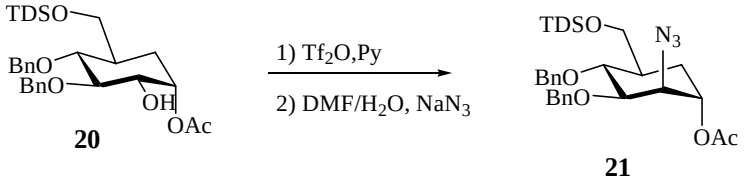
Formula	C31H4606Si
Molecular Wright	542.78
TLC conditions	Rf 0.25, Hexane : EtOAc = 3 : 1

The optical rotation and the spectroscopic characterization data were in agreement with the reported data [3]

^{1}H NMR spectrum of compound **20**



1-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose, 21



20 **21**

Synthetic Procedure

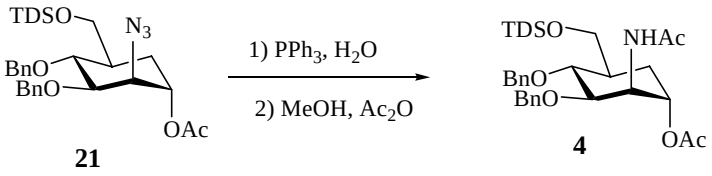
20 (10.3 g, 19 mmol) was dissolved in a mixture of CH_2Cl_2-Pyridine (5:1, 360 mL), then triflic anhydride (17 mL, 104 mmol) were added dropwise at -10 ℃. The mixture was stirred at 0℃ for 60 minutes. Then saturated aqueous solution of $NaHCO_3$ (150 mL) was added, the organic layer was washed with brine (200 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was then dissolved in a 19:1 mixture of DMF-H_2O (100 mL), and NaN_3 (6.2 g, 95 mmol) was added. The reaction mixture was stirred overnight at 40 ℃, then the solvent was evaporated and the crude was purified by flash chromatography (2% EtOAc/Toluene), giving **21** (8.5 g, 79%) as an oil.

Product characterization 21

Formula	C31H45N3O5Si
Molecular Wright	567.79
TLC conditions	Rf 0.5, Toluene : EtOAc = 24 : 1

The optical rotation and the spectroscopic characterization data were in agreement with the reported data [3]

2-Acetamido-1-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose ,4



21 **4**

Synthetic Procedure

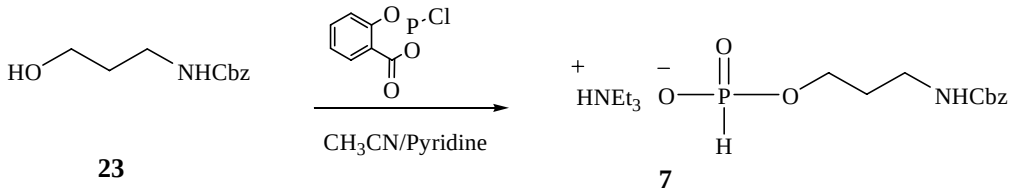
A mixture of **21** (9.1 g, 16 mmol) and PPh_3 (12.6 g, 48 mmol) in dry THF (250 mL) was stirred overnight at 60 ℃ under nitrogen atmosphere. After addition of water (40 mL), the reaction was stirred for 24 h at the same temperature, then the solvent was evaporated. The residue was dissolved in MeOH (200 mL) and acetic anhydride (30 mL, 320 mmol) was added. After 24 h the solvent was evaporated and the crude material was purified by flash chromatography (30% EtOAc/Hexane), providing **4** (8.86 g, 95%) as an oil.

Product characterization 4

Formula	C33H49NO6Si
Molecular Wright	583.83
TLC conditions	Rf 0.3, Hexane : EtOAc = 3 : 1

The optical rotation and the spectroscopic characterization data were in agreement with the reported data [3]

3-*N*-carbonbenzyloxy-propyl hydrogenphosphonate, triehtylammonium salt,7



23 **7**

Synthetic Procedure

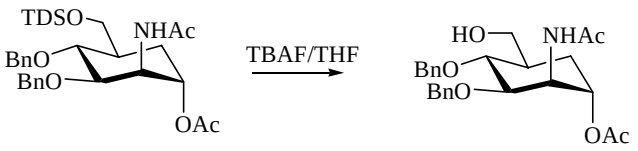
To a stirred solution of benzyl 3-hydroxypropylcarbamate **17** (13 mg, 0.06 mmol) in dry CH_3CN (0.3) mL and pyridine (0.1 mL), a 0.4 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin -4-one in dry CH_3CN was added (0.2 mL, 0.08 mmol) at room temperature. The mixture was stirred at room temperature under nitrogen atmosphere for 45 minutes. Then a 1:1 mixture of pyridine-H_2O (1mL) was added. TEAB (Triethylammonium bicarbonate buffer, 1M solution in H_2O, 0.5 mL) was then added to the mixture, then the mixture was diluted with $CHCl_3$ and concentrated without phase separation. The residue was purified by flash chromatography (5→10 % MeOH/CH_2Cl_2, 1% TEA), providing **7** (22 mg, 98%).

Product characterization 7

Formula	C11H15N05P
Molecular Wright	272.21
TLC conditions	Rf 0.25 CH_2Cl_2 : MeOH = 14 : 1

The spectroscopic characterization data was in agreement with the reported data [5]

2-Acetamido-1-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranose, 22



4 **22**

Synthetic Procedure

A stirred solution of **4** (1.05 g, 1.8 mmol) in dry THF (30 mL) was treated with TBAF (1M solution in THF + 5% H_2O, 2.2 mL, 2.2 mmol), the mixture was stirred at room temperature under nitrogen atmosphere for 2 hours. The mixture was then diluted with CH_2Cl_2 (50 mL), the organic layer was washed with saturated aqueous solution of NH_4Cl (50 mL), dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (5% MeOH/CH_2Cl_2), affording **22** (722 mg, 91%)

Product characterization 22

Formula	C25H31NO6
Molecular Wright	441.52
TLC conditions	Rf, 0.5, CH_2Cl_2 : MeOH = 19 : 1

$[\alpha]_D$ +32.3(c 1.0 in $CHCl_3$)

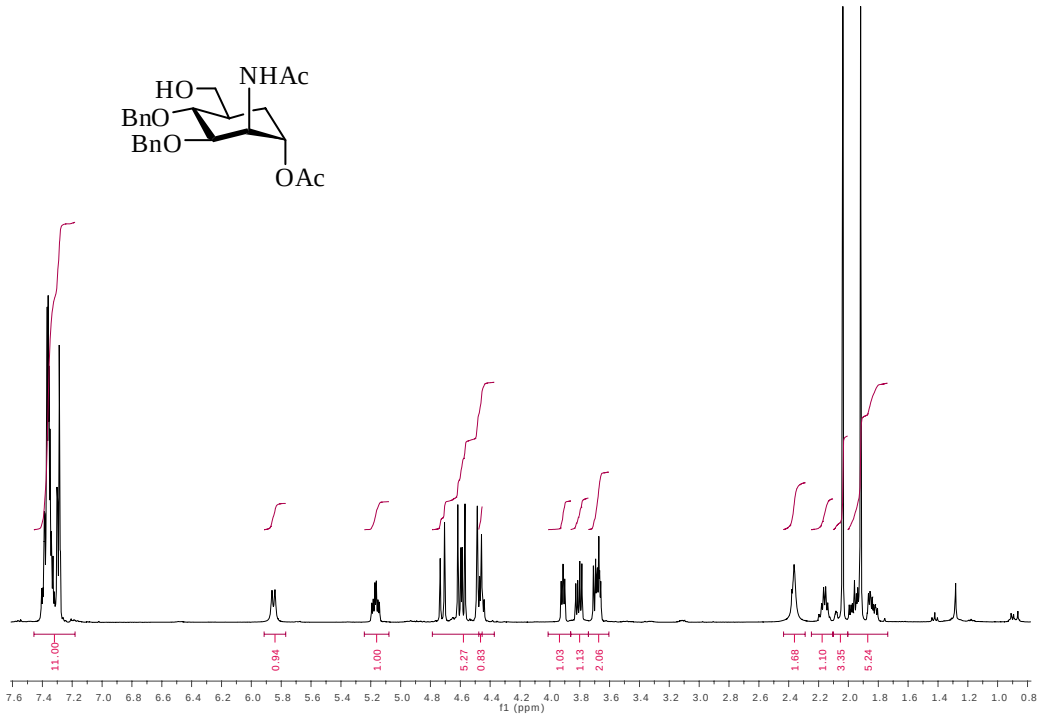
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.51-7.20 (m, 10 H, H_{Ar}); 6.20 (d, *J* = 8.5 Hz, 1 H, N*H*); 5.2-5.1 (m, 1 H, H-1); 4.80-4.73 (d, *J* = 11.4 Hz, 1H, CH*H*Ph); 4.62-4.58 (m, 2 H, C*H*$_2$Ph); 4.45 (m, 2 H, C*H*HPh, H-2); 3.98 (m, 1 H, H-3); 3.78 (m, 1 H, H-6); 3.60-3.82 (m, 2 H, H-6' , H-4,); 2.15 (m, 1 H, H-5); 2.03 (s, 3 H, OAc); 1.95 (s, 3 H, NHAc); 1.92 (m, 1 H, H-5a'); 1.85 (m, 1 H, H-5a);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 172.6 (2 *C*=O); 138.1-127.9 (12 Ph); 78.9 (C-3);

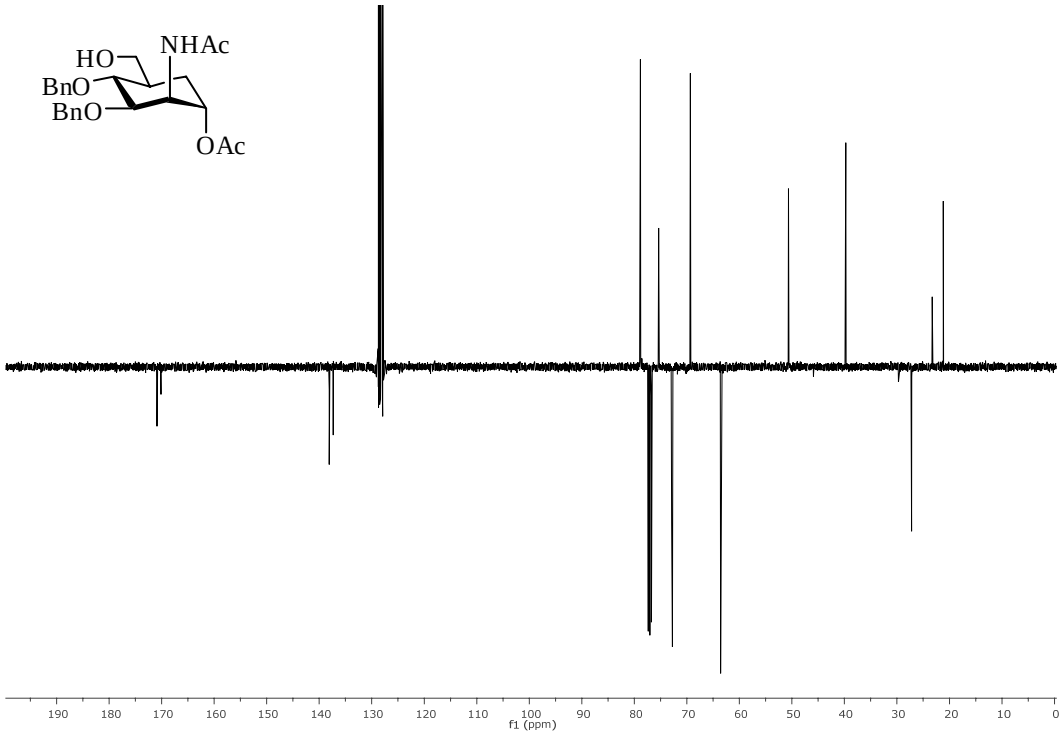
76.7 (C-4); 72.9 72.8 (2CH_2Ph); 68.3 (C-1); 63.6 (C-6); 50.7 (C-2); 39.8 (C-5); 29.7 (C-$_{thexyl}$); 27.2 (C-5a); 23.3 (NHAc); 21.1 (OAc);

ES HRMS cacld for $C_{25}H_{31}N_1O_6Na$ 464.20436, found 464.20417

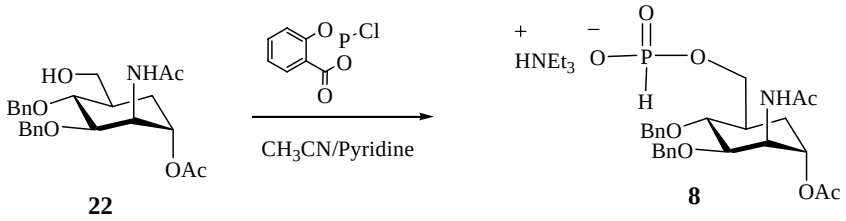
^{1}H NMR spectrum of compound **22**



^{13}C NMR spectrum of compound **22**



[6-O-(2-Acetamido-1-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl)] hydrogen phosphonate triehtylammonium salt, 8



Synthetic Procedure

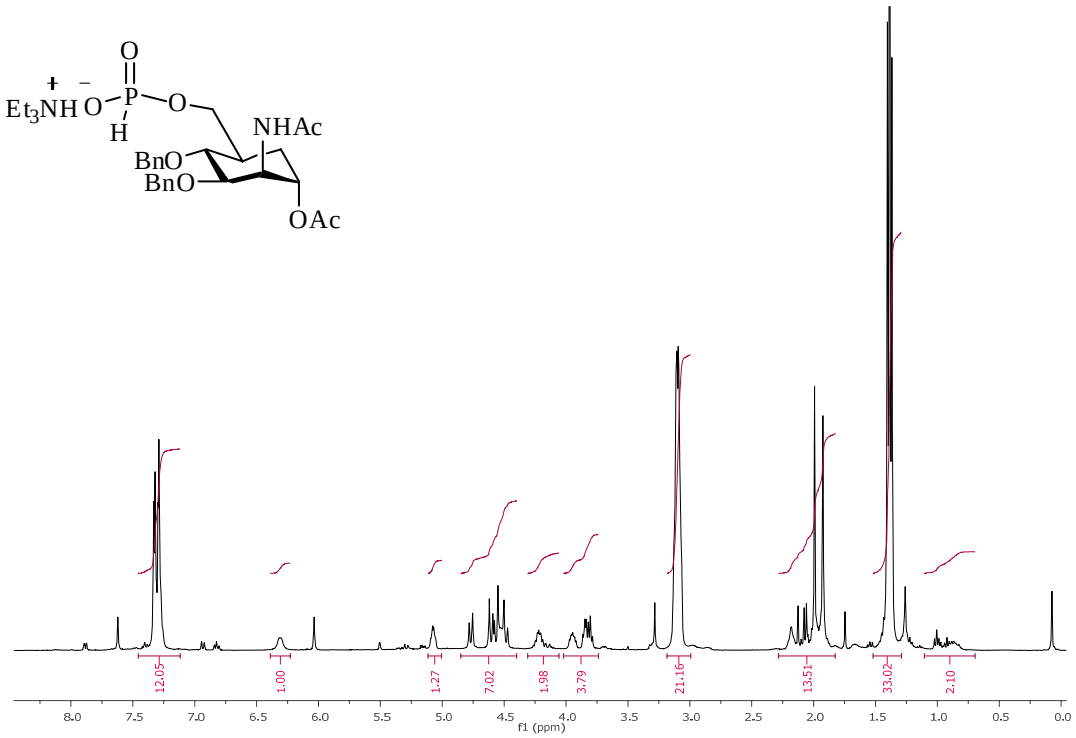
To a solution of **22** (70 mg, 0.16 mmol) in dry CH_3CN (2 mL) and pyridine (0.7 mL), 0.4 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one in dry CH_3CN (0.5 mL, 0.2 mmol) was added at room temperature. The mixture was stirred at room temperature under nitrogen atmosphere for 45 minutes. Then a 1:1 mixture of pyridine-H_2O (1:1, 1mL) solution was added and the mixture was diluted with $CHCl_3$ (20 mL). The organic layer was washed with H_2O (10 mL) and 1 M TEAB(10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (5% MeOH/CH_2Cl_2, 1% TEA), providing a clean oil, the oil was diluted with $CHCl_3$ (20 mL), then washed again by 0.5 M TEAB (10 mL), dried (Na_2SO_4), filtered, and concentrated get product **8** (80 mg, 82%) as a syrup.

Product characterization 8

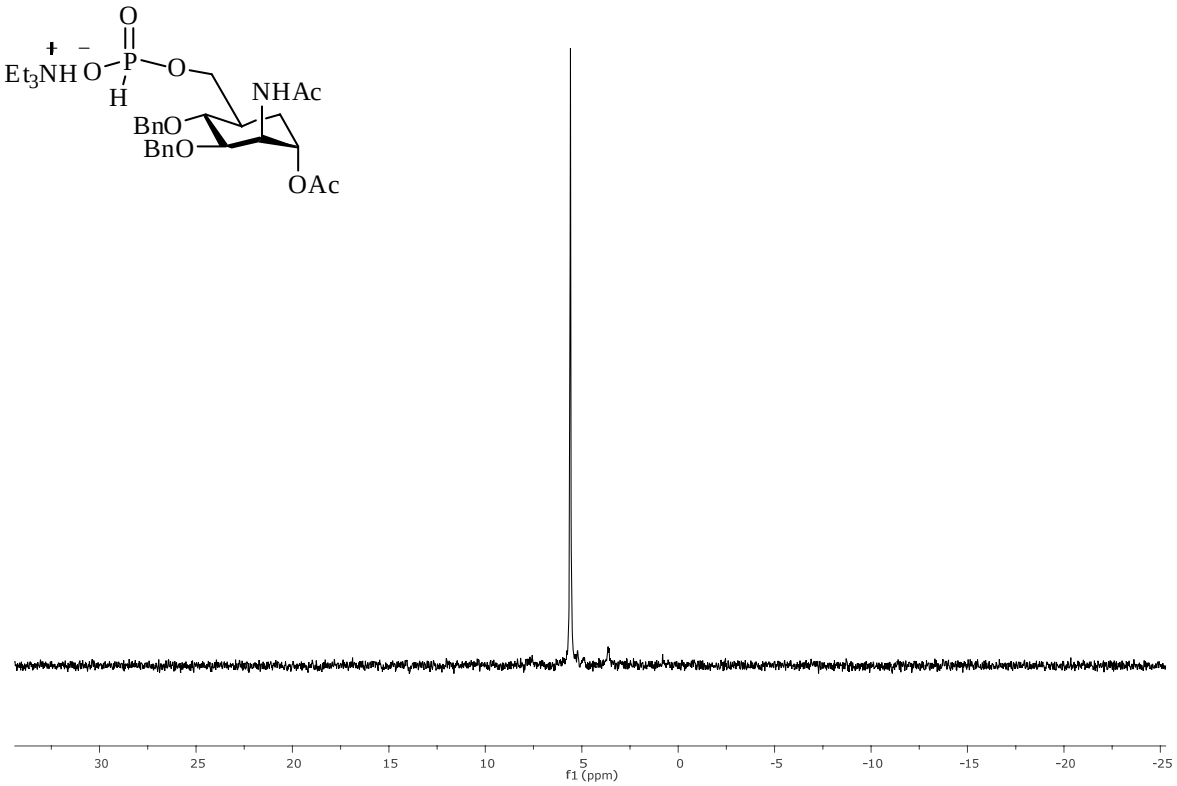
Formula	C25H31NO8P
Molecular Wright	504.49
TLC conditions	Rf 0.25 , CH_2Cl_2 : MeOH = 19 : 1

The formation of the H-phosphonate intermediate was ascertained by ^{1}H NMR analysis, which showed the diagnostic doublet at δ 6.03 ($J_{H,P}$ = 635 Hz), compound **8** was used directly in the following coupling steps without further characterization.

^{1}H NMR spectrum of compound **8**



^{31}P NMR spectrum of compound **8**



General Procedure A: Zemplén Deactylation

A solution of precursor **(4, 5 or 6)** in dry MeOH was treated with 0.08 M NaOMe in MeOH and stirred under nitrogen atmosphere. After reaction completion, the mixture was diluted with MeOH, neutralized with Amberlite IR 120 (H^+) resin, filtered, and concentrated. The crude was purified by flash chromatography, providing corresponding deacetylated product.

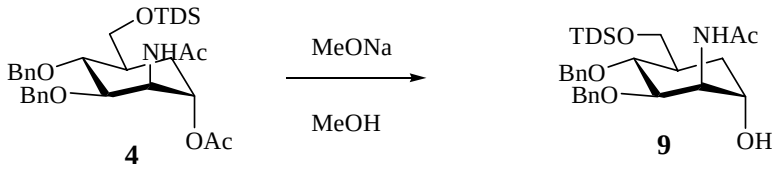
General Procedure B: Phosphonate Coupling and Oxidation.

The acceptor (9, 26 or 28) and the H-phosphonate (**7** or **8**) were coevaporated with pyridine for three times under high vacuum. The residue was then dissolved in the dry pyridine, and PivCl was added. The mixture was usually stirred under nitrogen atmosphere for 40 to 45 minutes. Then a freshly prepared solution of I_2 in a 19:1 mixture of pyridine- H_2O was added and the mixture was stirred for another 15 minutes. After this, $CHCl_3$ was added to dilute, and the organic layer was washed with 1 M aqueous solution of $Na_2S_2O_3$ and 0.5 M TEAB, dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatograph, providing the phosphodiester derivatives.

General Procedure C: Global Removal of Protection Group (Desilylation, Hydrogenation and Ion exchange)

The protected oligomer (**24**, **27**or **29**) was dissolved in dry THF and treated with a 1M solution of TBAF in THF under nitrogen atmosphere for 4 hours. The mixture was then diluted with $CHCl_3$,and the organic layer was washed with asaturated aqueous solution of NH_4Cl and 0.5 M TEAB, dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash chromatography, providing the correspondingdesilylated derivative. This intermediate was hydrogenolysed over Pd/C in a 1:1 mixture of MeOH and H_2O at room temperature for 48 h. The mixture was filtered over a Celite pad and the filtrate was concentrated. Then the residue was dissolved in H_2O and first eluted through a column filled with Dowex 50W-X8 resin (H^+ form), and then through a column filled with the same resin in Na^+ form. The eluate was concentrated and lyophilized to afford the target compound as sodium salt.

2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-a-D-mannopyranose, 9



Synthetic Procedure

Compound **4** (600mg, 1.02 mmol) was dissolved in MeOH (20 mL) and deactylated with 0.08 M NaOMe in MeOH (5mL) according to general procedure A. The crude was purified by flash chromatograph (5% MeOH/CH_2Cl_2), providing alcohol **9** (470 mg, 84%)

Product characterization 9

Formula	C31H47NO5Si
Molecular Wright	541.79
TLC conditions	Rf 0.5, CH_2Cl_2 : MeOH = 19 : 1

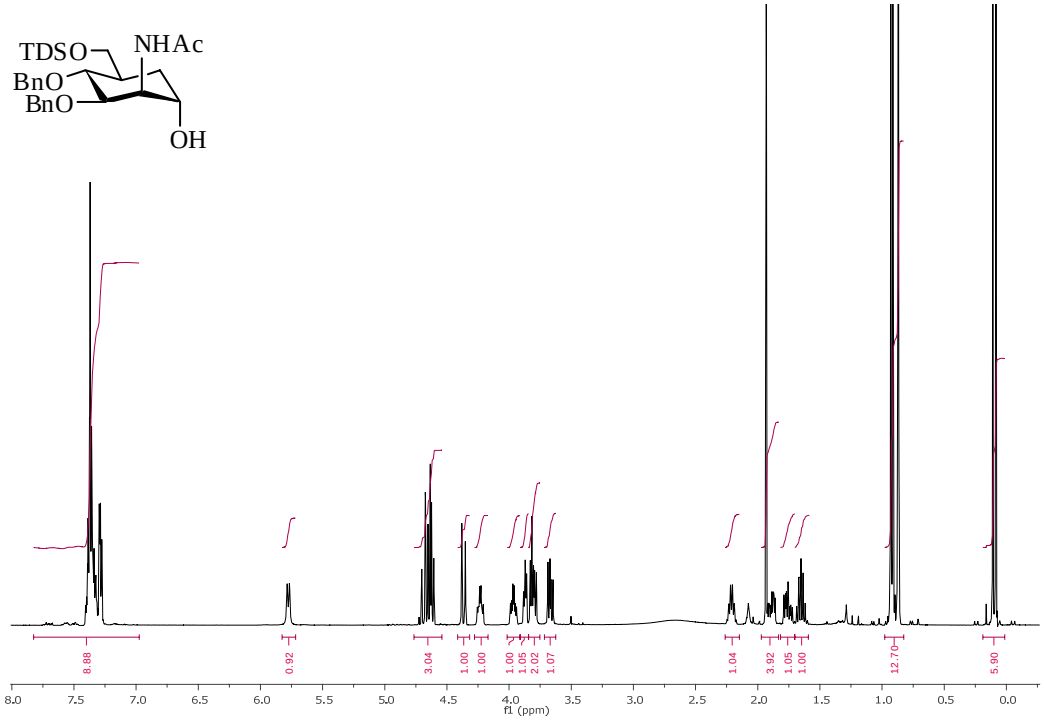
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30-7.43 (m, 10H, H_{Ar}); 5.78 (d, *J* = 7.8 Hz, 1H, NH); 4.73 (d, *J* = 11.6 Hz, 1H, CHHPh); 4.65-4.58 (AB system, 2 H, C*H*$_2$Ph); 4.43 (d, *J* = 11.5 Hz, 1 H, CH*H*Ph); 4.23 (m, 1 H, H-2,); 3.98 (m, 1 H, H-1); 3.88-3.77 (m, 3 H, H-3, H-6' , H-4,); 3.67 (dd, *J* = 5.6, 9.6 Hz, 1 H, H-6); 2.21 (d, *J* = 5.6 Hz, 1 H, H-5); 1.92 (s, 3 H, NHAc); 1.84 (m, 1 H, H-5a'); 1.79 (m, 1 H, H-5a); 1.66 (m, 1 H, C*H*$_{thexyl}$); 0.92 (d, *J* = 6.8 Hz, 6 H, 2 C*H*$_3$CH$_{thexyl}$); 0.88 (s, 6 H, 2 CH$_{3thexyl}$); 0.09, 0.12 (2 s, 6 H, 2 C*H*$_3$Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 172.6 (2 C=O); 139.4-128.4 (12 Ph); 79.5 (C-3); 74.5 (C-4); 72.4, 73.3(CH_2Ph); 68.2 (C-1); 63.5 (C-6); 54.1 (C-2); 39.8 (C-5); 34.5 (CH_{thexyl}); 30.3 (C-5a); 25.3 (C_{thexyl}); 23.4 (NHAc); 20.4 ($2CH_{3\text{-}thexyl}$); 18.7 ($2CH_3CH_{thexyl}$); -3.6, -3.5 (CH_3Si);

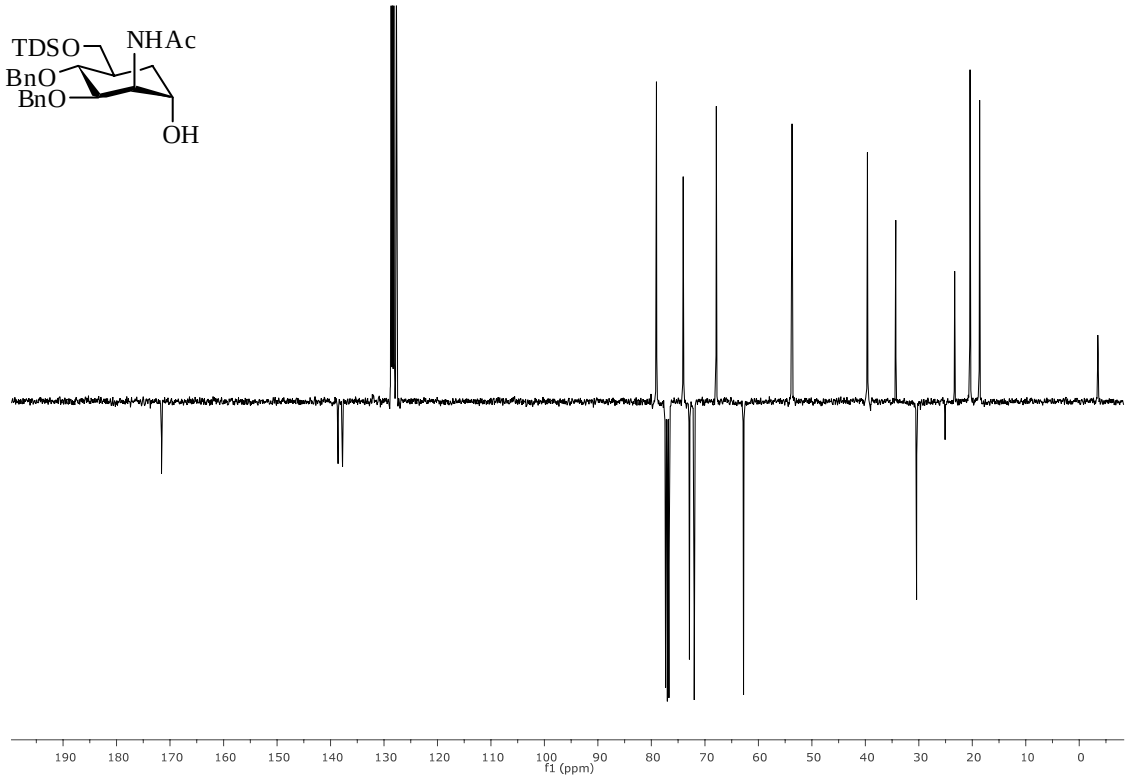
(Found: C, 68.00; H, 8.42; N, 2.35. Calc. for $C_{33}H_{49}NO_6Si$: C, 67.89; H, 8.46; N, 2.40);

ES HRMS cacld for $C_{31}H_{47}N_1O_5SiNa$ 564.31157, found 564.31148

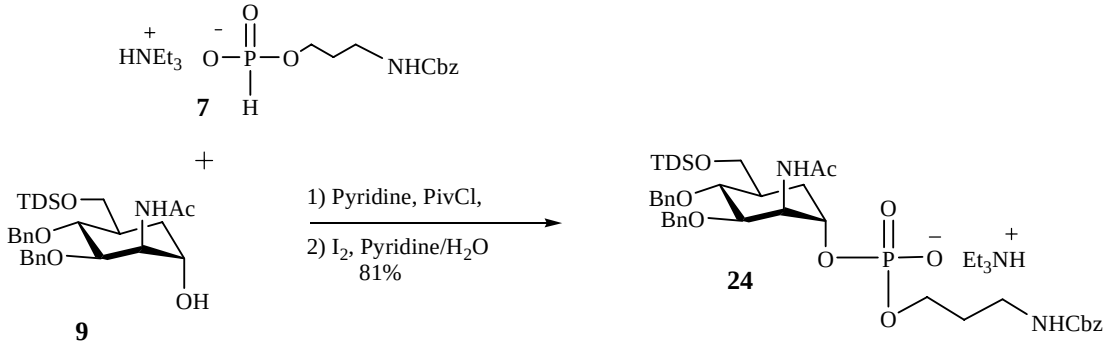
^{1}H NMR spectrum of compound **9**



^{13}C NMR spectrum of compound **9**



3-*N*-carbobenyloxy-propyl-*O*-(2-acetamido-3,4-di-*O*-benzyl-6-*O*-thexyldimethylsilyl-2-deoxy-5a-carba-α-D-mannopyranosyl) phosphate triethylammonium salt, 24



Synthetic procedure

Alcohol **9** (204 mg, 0.38 mmol) was condensed with H-phosphonate **7** (282 mg, 0.75 mmol) in pyridine (5 mL) in the presence of PivCl (0.1 mL, 0.79 mmol), and *in situ* oxidized by solution of I_2 (155 mg, 0.61 mmol) in pyridine-H_2O (4 mL) according to general procedure B. to generate phosphodiester. The crude was purified by flash chromatograph (5% MeOH/CH_2Cl_2, 1% TEA), yielding compound **19** (281 mg, 81%) as a colorless oil.

Product characterization 24

Formula	C42H60N2O10PSi
Molecular Wright	811.99
TLC conditions	Rf 0.2 , CH_2Cl_2 : MeOH = 19 : 1

$[\alpha]_D$ +14.7 (c 1.0 in $CHCl_3$)

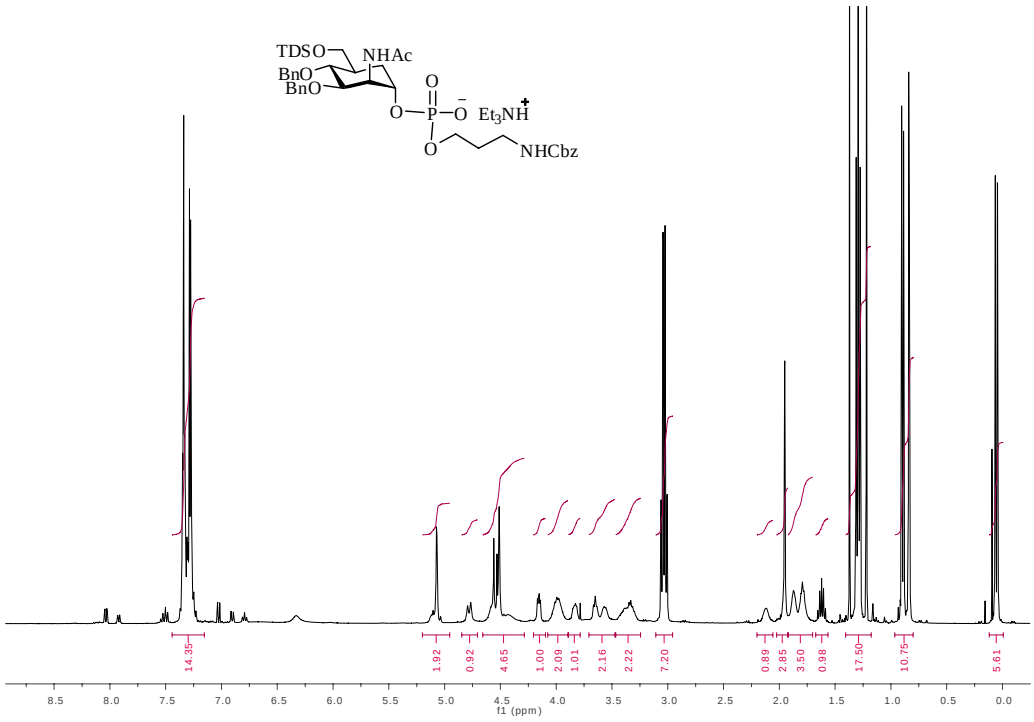
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40-7.23 (m, 15 H, H_{Ar}); 5.15-5.11 (m, 2 H, C*H*$_2$Ph); 4.78 (d, *J* = 11.4 Hz, 1 H, CH*H*Ph);. 4.63-4.34 (m, 5 H, H-1, H-2, C*H*$_2$Ph, CH*H*Ph); 4.17 (dd, *J* = 4.4, 6.4 Hz, 1 H, H-3); 4.07-3.91 (m, 2 H, OC*H*$_2$CH$_2$CH$_2$N); 3.83 (m, 1 H, H-6'); 3.65 (m, 1 H, H-4); 3.57 (m, 1 H, H-6); 3.45-3.27 (m, 2 H, OCH$_2$CH$_2$C*H*$_2$N); 3.03 (q, 6 H, 3 C*H*$_{2\text{-Et}}$); 2.11 (m, 1 H, H-5); 1.95 (s, 3 H, NHAc); 1.87 (m, 2 H, H-5a, H-5a'); 1.79 (bt, 2 H, OCH$_2$C*H*$_2$CH$_2$N); 1.62 (m, 1 H, C*H*$_{\text{texyl}}$); 1.29 (t, *J* = 7.3 Hz, 9 H, 3 C*H*$_{3\text{-Et}}$); 0.89 (d, *J* = 6.8 Hz, 6 H, 2 C*H*$_3$CH$_{\text{texyl}}$); 0.84, 0.83 (2 s, 6 H, 2 C*H*$_{3\text{-texyl}}$); 0.07, 0.07 (2s, 6H, 2 C*H*$_3$Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 167.1 181.8 (2*C*=O); 133.5-116.0 (18 Ph); 78.2 (C-3); 76.5 (C-4); 72.4, 72.5 (2 *C*H$_2$Ph); 70.4 (C-1); 66.3 (*C*H$_2$Ph); 62.7 (O*C*H$_2$CH$_2$CH$_2$N, C-6); 52.8 (C-2); 45.6 (CH$_{2\text{-Et}}$); 40.0 (C-5); 37.2 (OCH$_2$*C*H$_2$CH$_2$N); 34.3 (*C*H$_{\text{texyl}}$); 30.4 (OCH$_2$*C*H$_2$CH$_2$N); 29.6 (C-5a); 25.1 (C-$_{\text{thexyl}}$); 23.3 (NHAc); 20.4 (2*C*H$_{3\text{-thexyl}}$); 18.6 (2*C*H$_3$CH$_{\text{thexyl}}$); 8.5 (*C*H$_{3\text{-Et}}$); -3.5 -3.6 (2 *C*H$_3$Si);

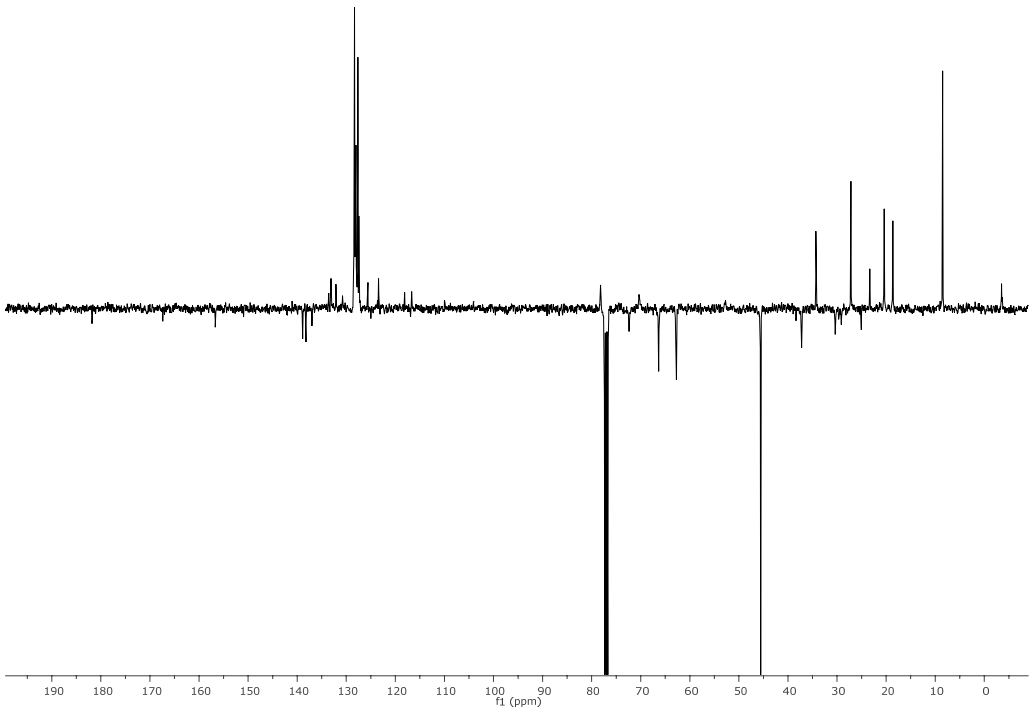
^{31}P NMR ($CDCl_3$, 162 MHz) δ 1.28.

ES HRMS cacld for $C_{42}H_{60}N_2O_{10}PSi$ 811.37603, found 811.37373

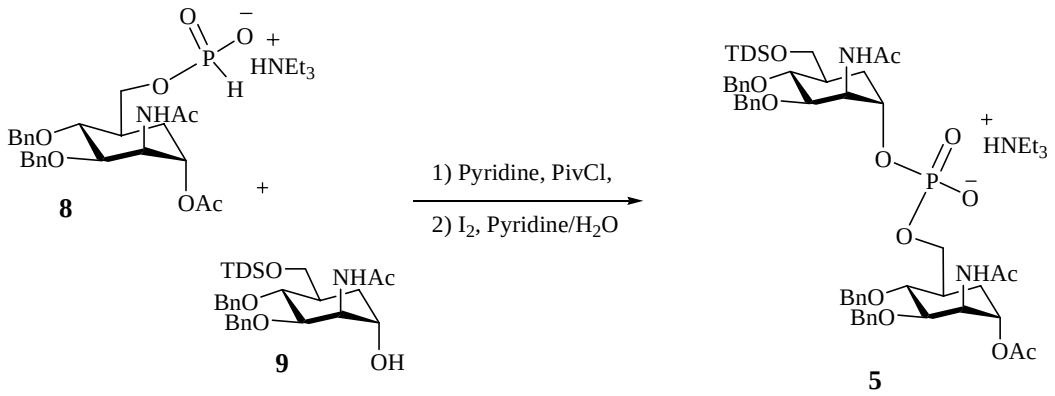
^{1}H NMR spectrum of compound **24**



^{13}C NMR spectrum of compound **24**



2-acetamido-1-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranoside 6-(2-acetamido-3,4-di-*O*-benzyl-6-*O*-thexyldimethylsilyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate), triethylammonium salt,5



Alcohol **9** (80 mg, 0.0725 mmol) was condensed with H-phosphonate **8** (48 mg, 0.079 mmol) in pyridine (2 mL) in the presence of PivCl (23 uL, 0.18, and *in situ* oxidized by solution of I_2 (60 mg, 0.24 mmol) in pyridine-H_2O (1.2 mL) according to general procedure B. to generate phosphodiester. The crude was purified by flash chromatograph (10% MeOH/CH_2Cl_2, 1% TEA), yielding compound **5** (100 mg, 82%) as a colorless oil.

Product characterization 5

Formula	C56H76N2O13PSi
Molecular Wright	1044.27
TLC conditions	Rf 0.25 , CH_2Cl_2 : MeOH = 9 : 1

$[\alpha]_D$ +17.3 (c 1.0 in $CHCl_3$)

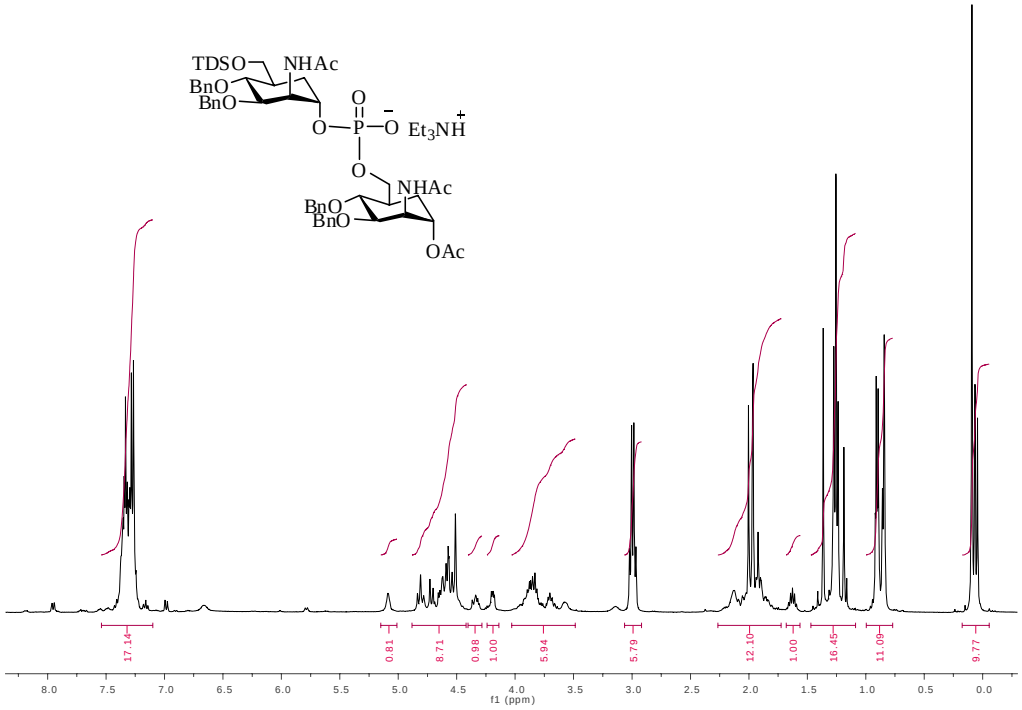
^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03-6.91 (m, 20 H, H_{Ar}); 5.15 (m, 1 H, H-1); 4.81 (m, 2 H, CH_2Ph); 4.73-4.52 (m, 8 H, H-2', H-2, 3 CH_2Ph); 4.42-4.30 (m, 1 H, H-6'$_{(e)}$); 4.31-4.20 (m, 1 H, H-3'); 4.03-3.82 (m, 5 H, H-1', H-3, H-6'$_{(a)}$; H-6$_{(e)}$; H-4); 3.81-3.60 (m, 1 H, H-4'); 3.58 (m, 1 H, H-6$_{(a)}$); 3.03 (q, 6 H, 3 CH_2-$_{Et}$); 2.21-2.03 (m, 2 H, H-5', H-5); 2.1 (s, 3 H, OAc); 1.95 (s, 6 H, 2NHAc); 1.9-1.8 (m, 2 H, H-5a'$_{(a)}$; H-5a'$_{(e)}$); 1.62 (m, 1 H, CH_{thexyl}); 1.29 (t, 11 H, 3 CH_{3-Et}, H-5a$_{(a)}$; H-5a$_{(e)}$); 0.89 (d, J = 6.8 Hz, 6 H, 2 CH_3CH_{thexyl}); 0.84, 0.83 (2 s, 6 H, 2 $CH_{3-thexyl}$); 0.07,0.06 (2 s, 6 H, 2 CH_3Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 170.5 170.3 170.0 (3 C=O); 138.9-122.9 (24 Ph); 79.1 78.4 (C-3', C-3); 75.8 75.2 (C-4', C-4); 74.2 72.3 72.8 72.0 (4 CH_2Ph); 70.3 67.5 (C-1', C-1); 65.2 62.7 (C-6',C-6); 52.0 49.6 (C-2', C-2); 45.3 (3 CH_{2-Et}); 39.7 38.3 (C-5', C-5); 34.3 (CH_{thexyl}); 29.7 (C-5a', C-5a); 25.1 (C-$_{thexyl}$); 23.5 23.2 (2NHAc); 21.2 (CH_3-C=O); 20.4 (2 $CH_{3-thexyl}$); 18.7 (2 CH_3CH_{thexyl}); 8.5 (3 CH_{3-Et}); -3.5, -3.6 (2 CH_3Si);

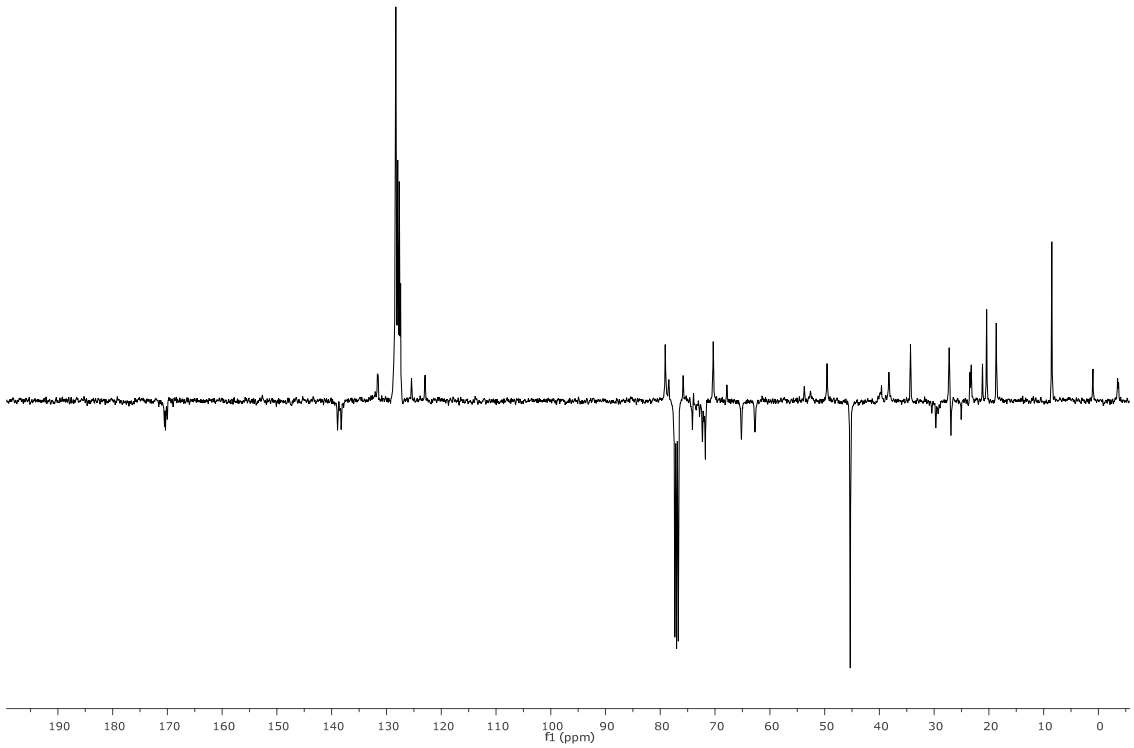
^{31}P NMR ($CDCl_3$, 162 MHz) δ 1.46.

ES HRMS cacld for $C_{56}H_{74}N_2O_{13}P$ Si 1043.48598, found 1043.48326

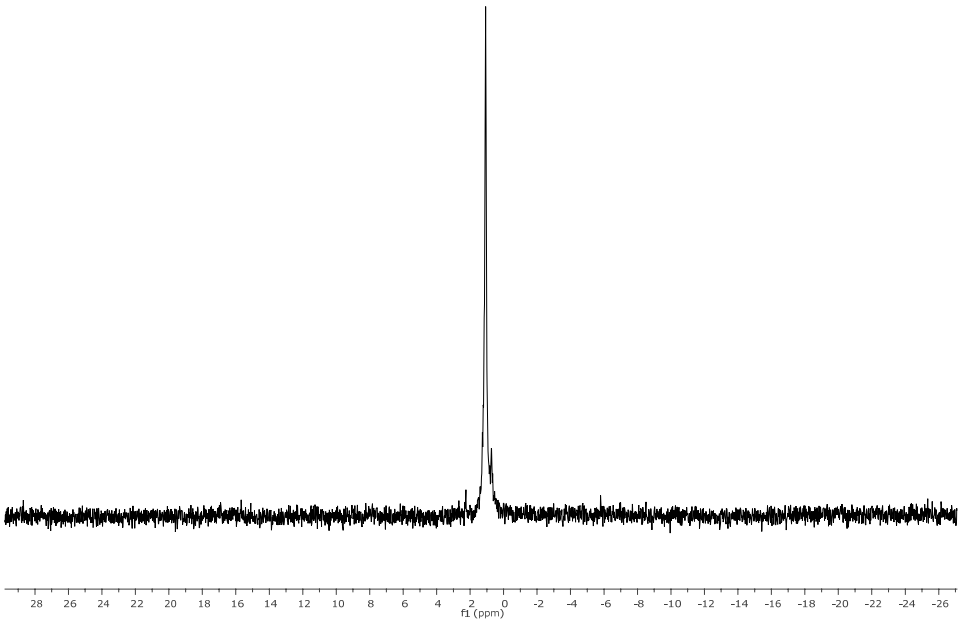
^{1}H NMR spectrum of compound **5**



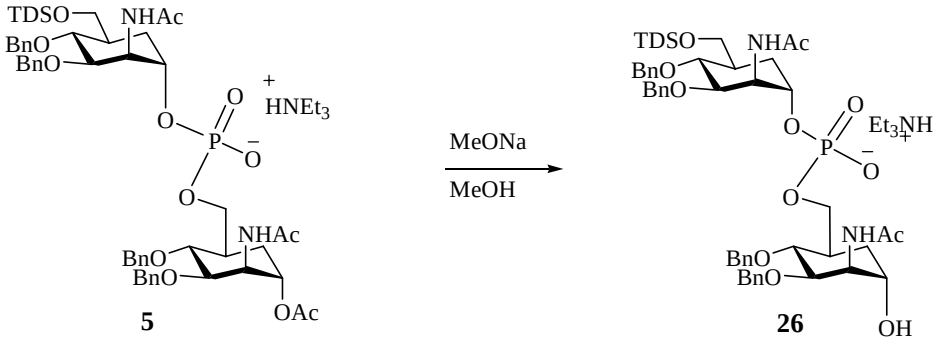
^{13}C NMR spectrum of compound **5**



^{31}P NMR spectrum of compound **5**



2-acetamido-3,4-di-*O*-benzyl-2-deoxy-5a-carba-α-D-mannopyranoside 6-(2-acetamido-3,4-di-*O*-benzyl-6-*O*-thexyldimethylsilyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate), triethylammonium salt, 26



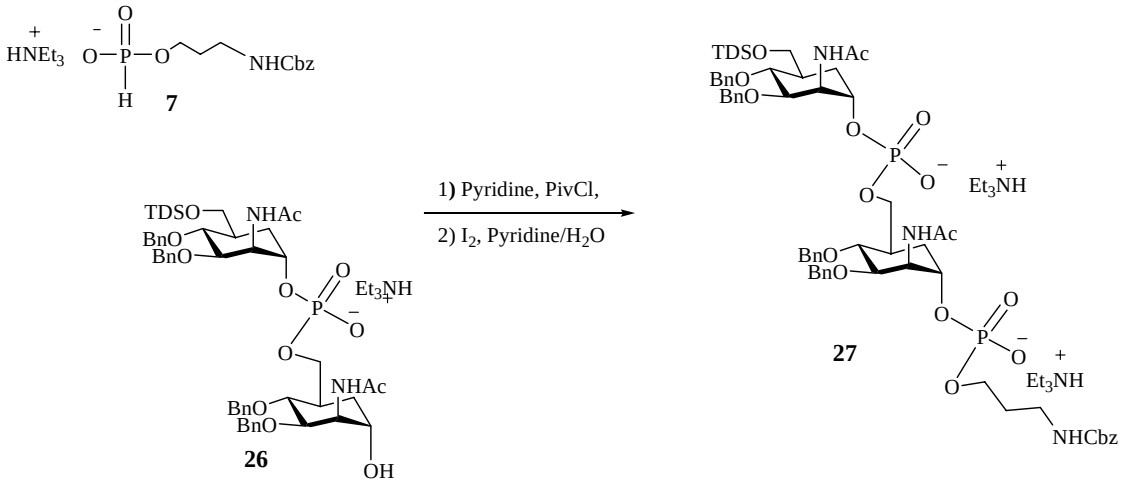
Synthetic procedure

Compound **5** (200 mg, 0.17 mmol) dissolved in MeOH (5 mL) and deactylated with 0.08 M NaOMe in MeOH (3.5mL) according to general procedure A. The crude was purified by flash chromatograph (20% MeOH/CH_2Cl_2, 1% TEA), providing corresponding deacetylated intermediate (166 mg, 87%).

Product characterization 26

Formula	C54H74N2O12PSi
Molecular Wright	1002.23
TLC conditions	Rf 0.3 , CH_2Cl_2 : MeOH = 4 : 1

3-(Benzyloxycarbonyl)aminopropyll-O-[2-acetamido-3,4-di-O-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-3,4-di-O-benzyl-2-deoxy-6- O-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl phosphate)], bis-triethylammonium salt, 27



Synthetic Procedure

26 (48 mg, 0.043 mmol) was condensed with H-phosphonate **7** (80 mg, 0.215 mmol) in pyridine (2 mL) in the presence of PivCl (13 uL, 0.1 mmol), and *in situ* oxidized by solution of I_2 (29 mg, 0.12 mmol) in pyridine-H_2O (2 mL) according to general procedure B. to generate phosphodiester. The crude was purified by flash chromatograph (20% MeOH/CH_2Cl_2, 1% TEA), yielding compound **27** (281 mg, 81%) as a colorless foam.

Product characterization 27

Formula	C65H87N3O17P2Si
Molecular Wright	1272.43
TLC conditions	Rf 0.35 , CH_2Cl_2 : MeOH = 4 : 1

$[\alpha]_D$ +7.9 (c 1.0 in $CHCl_3$)

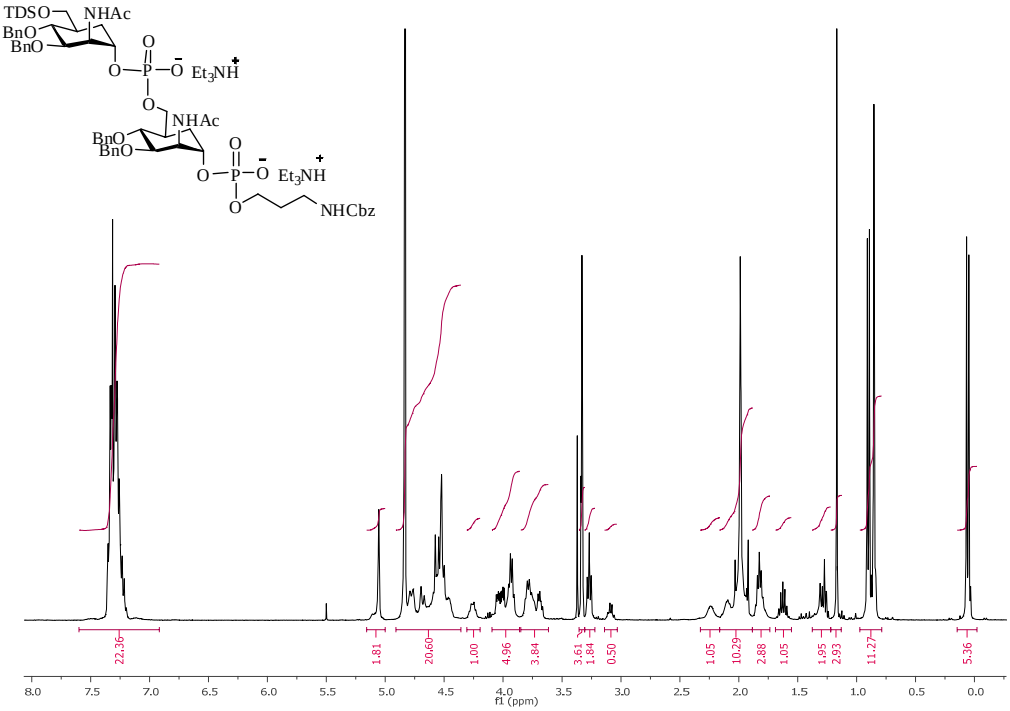
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35-7.21 (m, 25 H, H_{Ar}); 5.05 (s, 2 H, C*H*$_2$Ph); 4.78-4.57 (m, 8 H, 4 C*H*$_2$Ph); 4.55-4.49 (m, 4 H, H-1' , H-1, H-2' , H-2); 4.25 (m, 1 H, H-6'$_{(e)}$); 4.02-3.91 (m, 6 H, H-3' , H-3, H-6'$_{(a)}$; H-6$_{(e)}$; OC*H*$_2$CH$_2$); 3.90-3.69 (m, 3 H, H-4, H-4' , H-6$_{(a)}$); 3.25 (m, 2 H, CH_2C*H*$_2$N); 2.15 (m, 2H, H-5, H-5'); 2.02-1.98 (2 s, 6 H, 2 C*H*$_3$-C=O); 1.93-1.81 (m, 6 H, OCH$_2$C*H*$_2$, H-5a, H-5a'); 1.62 (m, 1 H, C*H*$_{thexyl}$); 0.92-0.90 (d, 6 H, 2C*H*$_3$CH$_{thexyl}$); 0.85 (2 s, 6H, 2C*H*$_{3-thexyl}$); 0.08-0.06 (2s, 6 H, 2C*H*$_3$Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 171.9 (3 C=O); 138.9-127.8 (30 Ph); 78.6 78.5 (C-3' , C-3); 75.5 (C-4' , C-4); 71.7 (4 *C*H$_2$Ph); 70.3 (C-1' , C-1); 65.9 (*C*H$_2$Ph); 65.5 63.1 (C-6' ,C-6); 62.8 (O*C*H$_2$CH$_2$); 52.0 51.0 (C-2' , C-2); 39.5 38.5 (C-5' , C-5); 37.3 (CH_2*C*H$_2$N); 34.2 (*C*H$_{thexyl}$); 30.5 (OCH$_2$*C*H$_2$); 29.3 28.6 (C-5a' , C-5a,); 25.0 (*C*-$_{thexyl}$); 21.8 21.4 (2 *C*H$_3$-C=O); 19.7 (2 *C*H$_{3-thexyl}$); 17.7 (2 *C*H$_3$CH$_{thexyl}$); -4.45 (2 *C*H$_3$Si);

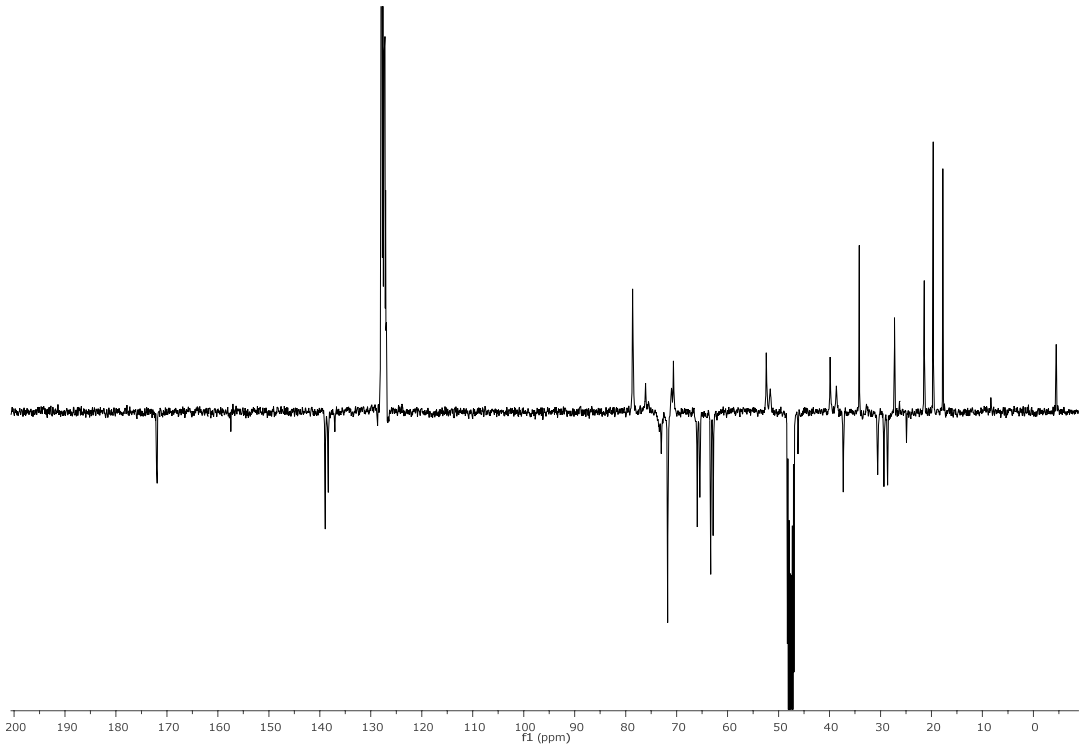
^{31}P NMR ($CDCl_3$, 162 MHz) δ 0.85, 0.33,.

ES HRMS cacld for $C_{65}H_{87}N_3O_{17}P_2SiNa$ 1295.51832, found 1295.52018

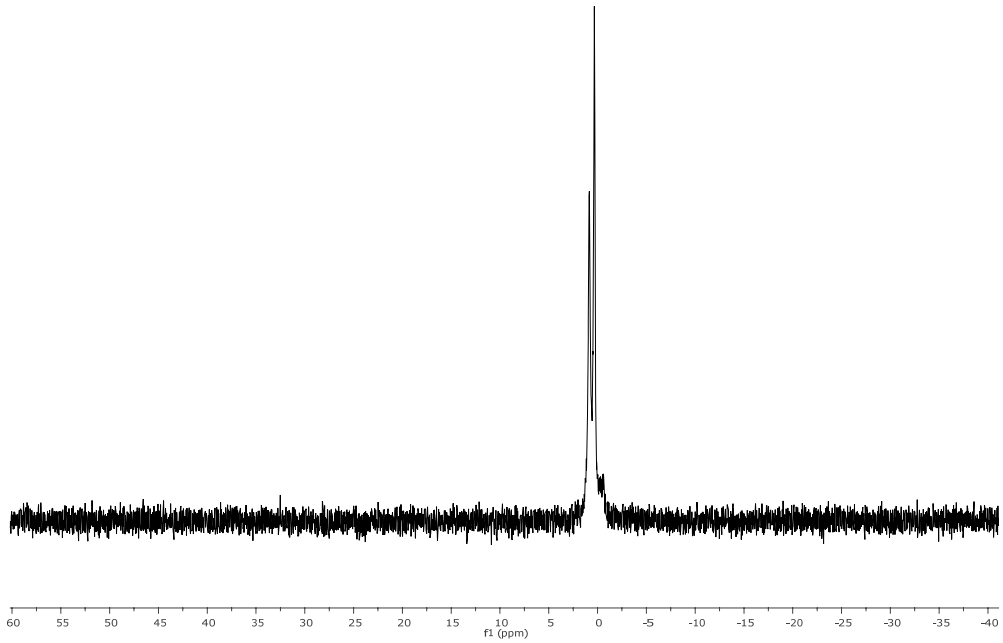
^{1}H NMR spectrum of compound **27**



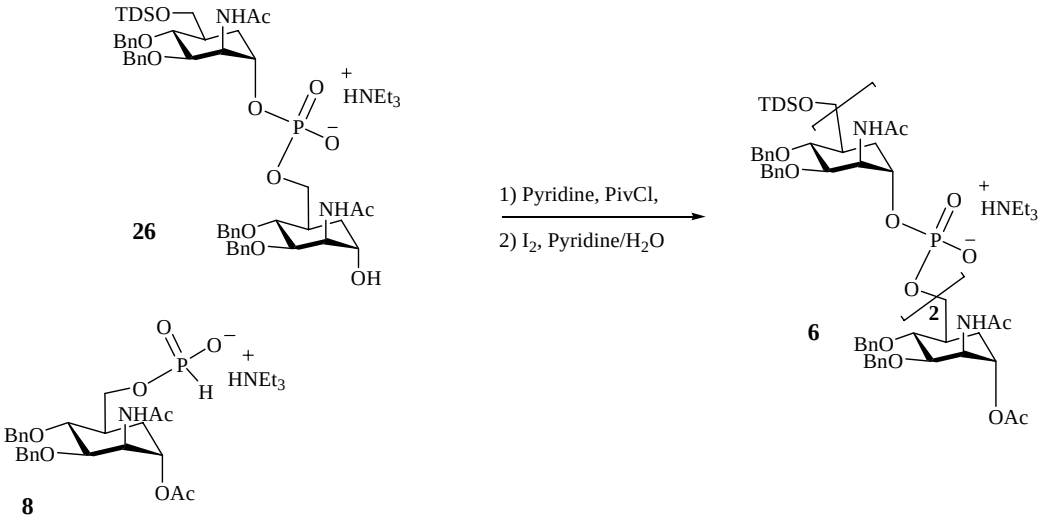
^{13}C NMR spectrum of compound **27**



^{31}P NMR spectrum of compound **27**



1-O-Acetyl-2-acetamido-3,4-di-O-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl 6-[2-acetamido-3,4-di-O-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl phosphate)], bis-triethylammonium salt (6)



Synthetic Procedure

26 (80 mg, 0.073 mmol) was condensed with H-phosphonate **8** (48 mg, 0.079 mmol) in pyridine (2 mL) in the presence of PivCl (23 uL, 0.18, and *in situ* oxidized by solution of I_2 (60 mg, 0.24 mmol) in pyridine-H_2O (1.2 mL) according to general procedure B. to generate phosphodiester. The crude was purified by flash chromatograph (15% MeOH/CH_2Cl_2, 1% TEA), yielding compound **6** (100 mg, 81%) as a colorless oil.

Product characterization 6

Formula	C79H103N3O20P2Si
Molecular Wright	1504.7
TLC conditions	Rf 0.3 , CH_2Cl_2 : MeOH = 6 : 1

$[\alpha]_D$ +12.4 (c 1.0 in $CHCl_3$)

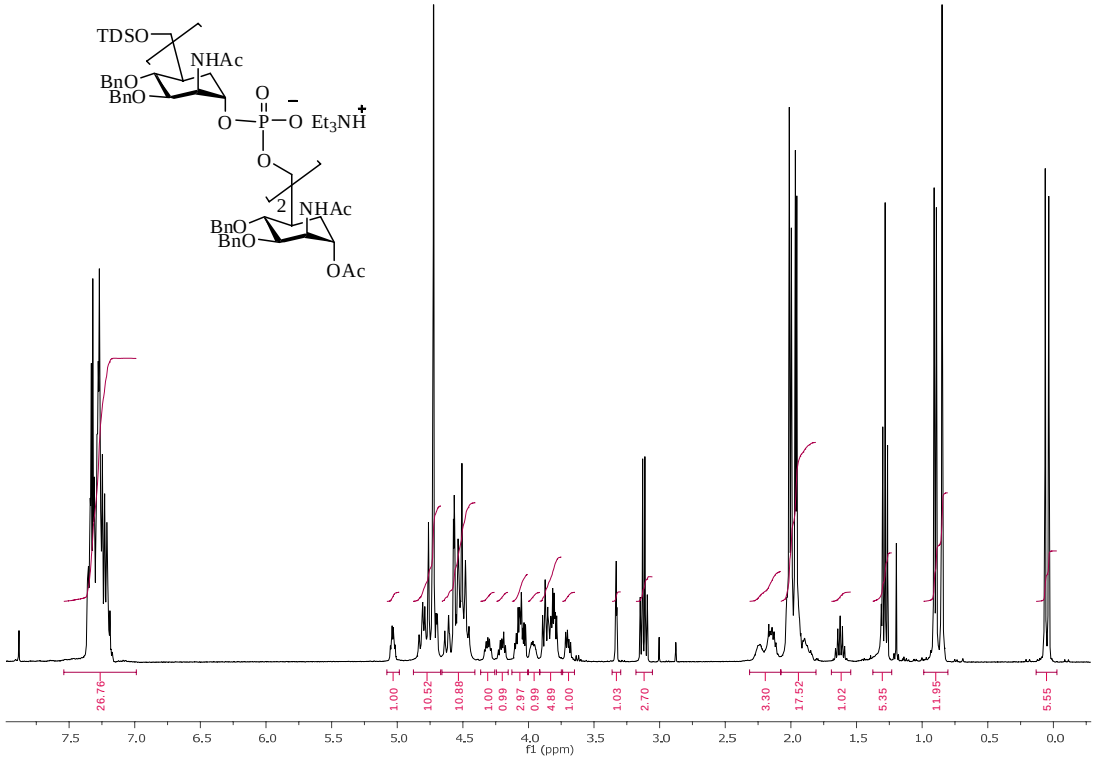
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37-7.21 (m, 30 H, H_{Ar}); 5.23 (m, 1 H, H-1); 4.82-4.69 (m, 7 H, H-2'', 6 OC*H*HPh); 4.64-4.45 (m, 10 H, H-2, 2', H-1', 1'', 6 OC*H*HPh); 4.32 (m, 1 H, H-6''$_{(e)}$); 4.22 (m, 1 H, H-6'$_{(e)}$); 4.10-4.02 (m, 2 H, H-3'', H-6'$_{(a)}$); 3.95 (m, 1 H, H-6''$_{(a)}$); 3.89-3.72 (m, 6 H, H-4, 4', 4'', H-3, H-3', H-6$_{(e)}$); 3.67 (m, 1 H, H-6$_{(a)}$); 3.15 (q, 12 H, 6 C$H_{2\text{-Et}}$); 2.17 (m, 3 H, H-5, 5', 5''); 2.01-1.95 (m, 18 H, 2H-5a', 5a'', 5a''', 4OAc,); 1.62 (m, 1 H, CH_{thexyl}); 1.31 (t, J = 7.6 Hz, 18 H, 6 C$H_{3\text{-Et}}$); 0.90 (d, 6H, 2 CH_3CH$_{\text{thexyl}}$); 0.85 (2 s, 6 H, 2 C$H_{3\text{-thexyl}}$); 0.08, 0.06 (2 s, 6 H, 2 CH_3Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 172.0 171.8 171.6 170.3 (4 C=O); 139.2-126.8 (36 Ph); 79.1 78.6 78.5 (C-3'', C-3', C-3); 75.8 (C-4'', C-4, C-4); 73.8 71.9 71.5 (6 *C*H$_2$Ph); 70.0 (C-1'', C-1', C-1); 65.4 65.3 63.4 (C-6'', C-6', C-6); 52.6 50.8 49.7 (C-2'', C-2',C-2); 40.2 38.6 38.2 (C-5'', C-5', C-5); 47.6 (6 CH$_{2\text{-Et}}$); 34.2 (*C*H$_{\text{thexyl}}$); 29.6 28.8 26.7 (C-5a'', C-5a', C-5a); 24.5 (C-$_{\text{thexyl}}$); 21.5 21.4 21.3 (4 *C*H$_3$-C=O);19.6 (2 *C*H$_{3\text{-thexyl}}$,); 17.8 (2 *C*H$_3$CH$_{\text{thexyl}}$); 7.9 (6 *C*H$_{3\text{-Et}}$); -4.0 (2 *C*H$_3$Si);

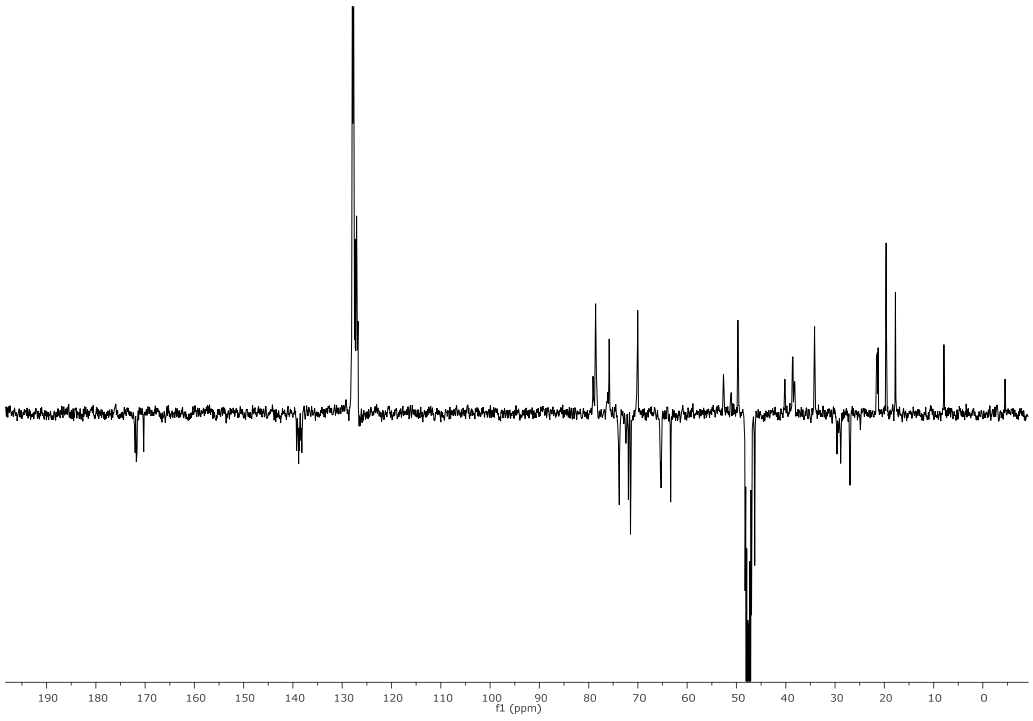
^{31}P NMR ($CDCl_3$, 162 MHz) δ 0.77, 0.18.

ES HRMS cacld for $C_{79}H_{104}N_3O_{20}P_2Si$ 1504.64632 found 1504.64788

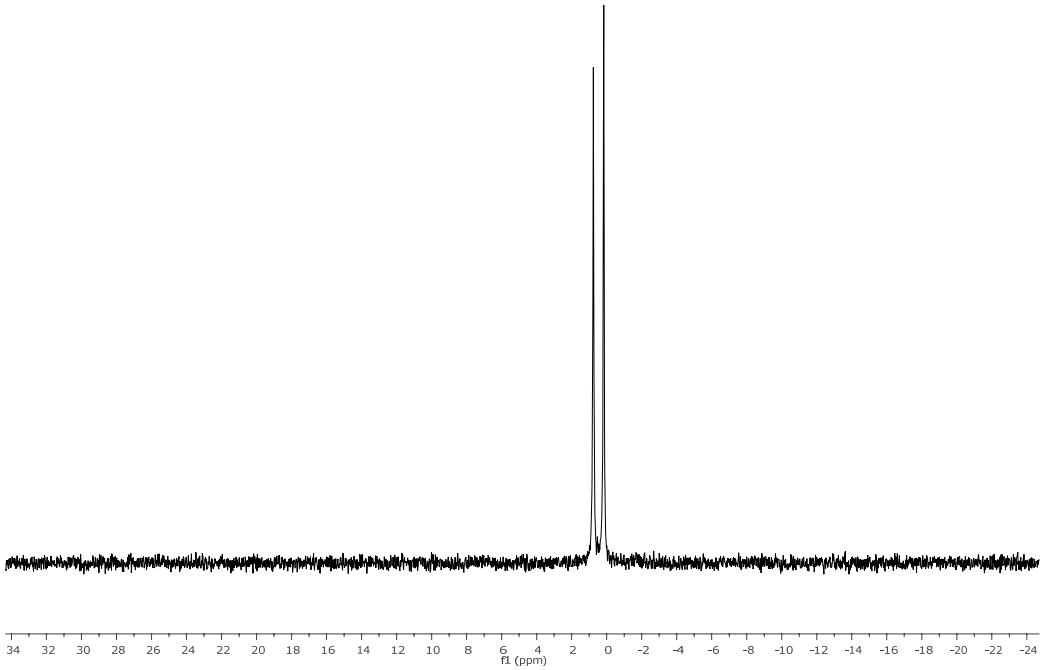
^{1}H NMR spectrum of compound **6**



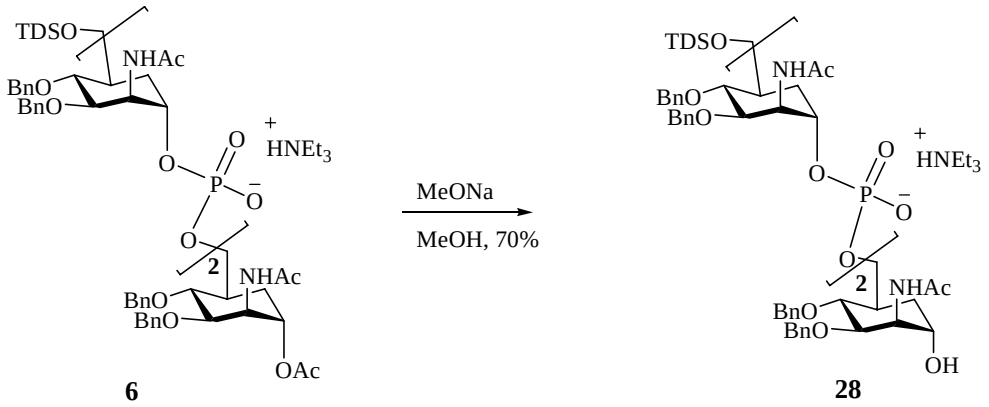
^{13}C NMR spectrum of compound **6**



^{31}P NMR spectrum of compound **6**



2-acetamido-3,4-di-O-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl 6-[2-acetamido-3,4-di-O-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl phosphate)], bis-triethylammonium salt, 28



6 **28**

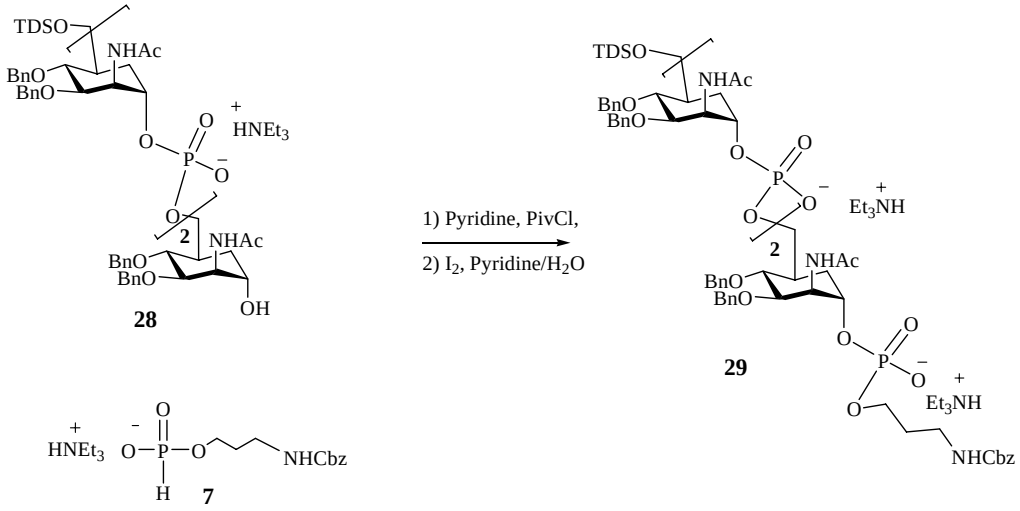
Synthetic Procedure

Compound **6** (100 mg, 0.059 mmol) dissolved in MeOH (5 mL) and deactylated with 0.08 M NaOMe in MeOH (3 mL) according to general procedure A. The crude was purified by flash chromatograph (25% MeOH/CH_2Cl_2, 1% TEA), providing corresponding deacetylated intermediate (68 mg, 70%).

Product characterization 6

Formula	C77H101N3O19P2Si
Molecular Wright	1461.63
TLC conditions	Rf 0.25 , CH_2Cl_2 : MeOH = 3 : 1

3-(Benzyloxycarbonyl)aminopropyll-O-{2-acetamido-3,4-di-O-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-[2-acetamido-3,4-di-O-benzyl-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-thexyldimethylsilyl-5a-carba-α-D-mannopyranosyl phosphate)]}, tris-triethylammonium salt, 29



Synthetic Procedure:

28 (68 mg, 0.041 mmol) was condensed with H-phosphonate **7** (90 mg, 0.24 mmol) in pyridine (2 mL) in the presence of PivCl (17 uL, 0.13 mmol), and *in situ* oxidized by solution of I_2(27 mg, 0.11 mmol) in pyridine-H_2O (2 mL) according to general procedure A. to generate phosphodiester. The crude was purified by flash chromatograph (35% MeOH/CH_2Cl_2, 1% TEA), yielding compound **29** (47 mg, 57%) as a colorless oil.

Product characterization 29

Formula	C88Hl14N4024P3Si
Molecular Wright	1732.87
TLC conditions	Rf 0.3 , CH_2Cl_2 : MeOH = 2: 1

$[\alpha]_D$ +13 (c 1.0 in $CHCl_3$)

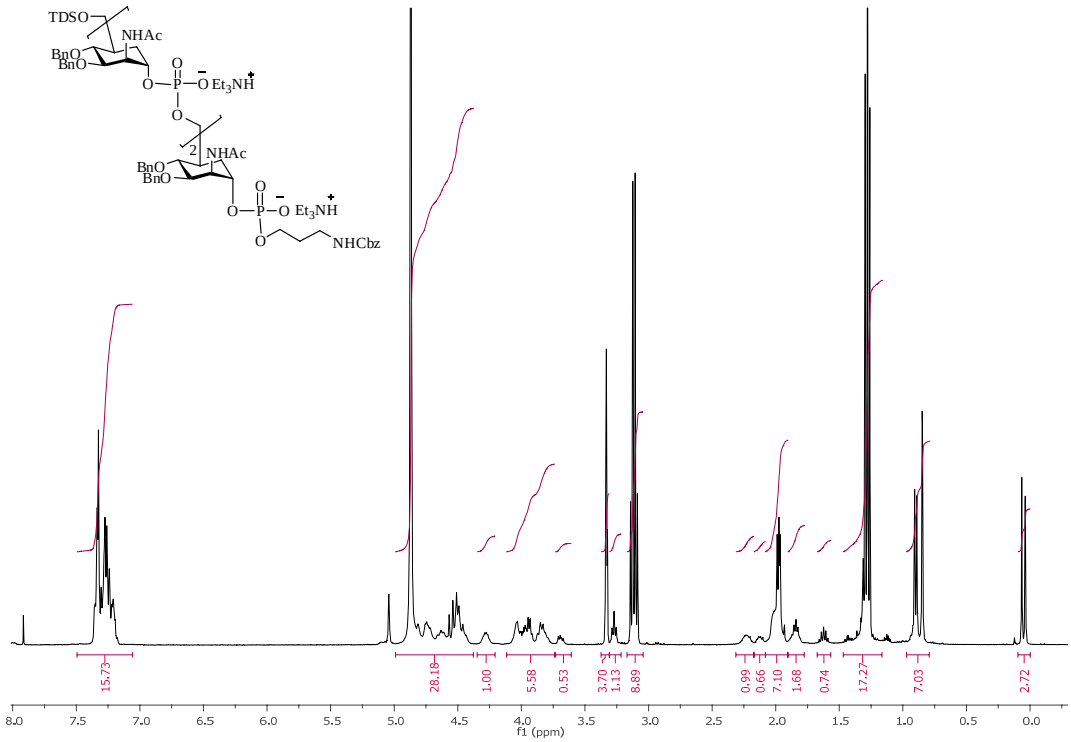
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35-7.15 (m, 35 H, H_{Ar}); 5.05 (m, 2 H, CH_2Ph); 4.83-4.44 (m, 18 H, H-1, 1' , 1' ' , H-2, 2' , 2' ' , 6 CH_2Ph); 4.28 (m, 2 H, OCH_2CH$_2$CH$_2$N); 4.06-3.91 (m, 7 H, H-3, 3' , 3' ' , H-6, 6' '); 3.88-3.79 (m, 4 H, H-4, 4' , 4' ' , H-6' $_{(e)}$); 3.71 (m, 1 H, H-6' $_{(a)}$); 3.28 (t, J = 6.7 Hz, 2 H, OCH$_2$CH$_2$CH_2N); 2.95 (q, J = 7.3 Hz, 18 H, 9 C$H_{2\text{-Et}}$); 2.31 (m, 3 H, H-5, 5' , 5' '); 2.21-1.98 (m, 13 H, H-5a, 5a' , 3 OAc); 1.82 (m, 2 H, OCH$_2$CH_2CH$_2$N); 1.65 (m, 1 H, CH_{thexyl}); 1.37 (m, 2 H, H-5a' ' $_{(a)}$; 5a' ' $_{(e)}$); 1.13 (t, J = 7.3 Hz, 27 H, 9 C$H_{3\text{-Et}}$); 0.92 (d, 6 H, 2 CH_3CH$_{\text{thexyl}}$); 0.85 (2 s, 6 H, 2 C$H_{3\text{-thexyl}}$); 0.08, 0.06 (2 s, 6 H, 2 CH_3Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) 172.0 171.7 171.6 (4 C=O); 139.2-127.0 (42 Ph); 79.2 79.0 78.5 (C-3' ' , C-3' , C-3); 76.5 (C-4' ' , C-4' , C-4); 71.4 (C-1' ' , C-1' , C-1); 71.9 (7 CH$_2$Ph); 65.8 (OCH$_2$CH$_2$CH$_2$N); 65.5 63.4 62.7 (C-6' ' , C-6' , C-6); 52.2 52.1 50.5 (C-2' ' , C-2' , C-2); 46.0 (9 CH$_{2\text{-Et}}$); 39.9 39.7 38.2 (C-5' ' , C-5' , C-5); 37.2 (OCH$_2$CH$_2$$CH_2$N); 34.2 ($CH_{\text{thexyl}}$); 30.5 (OCH$_2$$CH_2CH_2$N); 29.6 29.3 28.9 (C-5a' ' , C-5a' , C-5a); 25.1 (C-$_{\text{thexyl}}$); 21.5 21.4 21.4 (3 CH$_3$-C=O); 19.6 (2 CH$_{3\text{-thexyl}}$); 17.8 (2 CH$_3$CH$_{\text{thexyl}}$); 8.48 (9 CH$_{3\text{-Et}}$); -4.48 (2 CH$_3$Si);

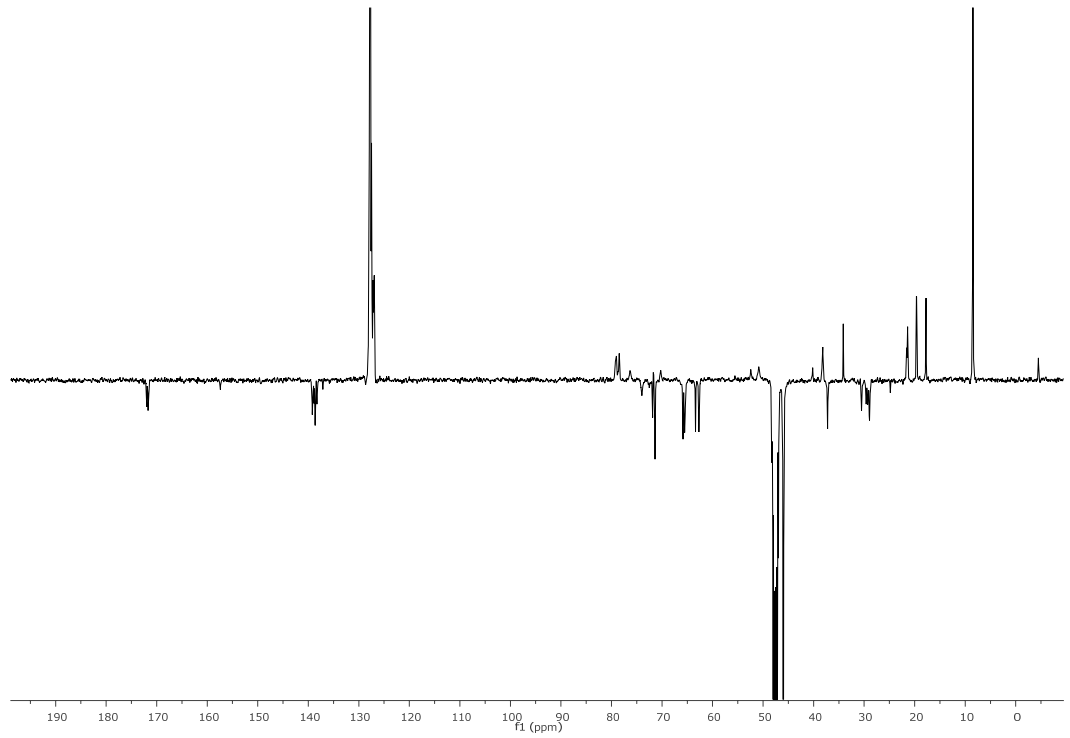
^{31}P NMR ($CDCl_3$, 162 MHz) δ 1.11, 0.68,0.5.

ES HRMS cacld for $C_{88}H_{114}N_4O_{24}P_3SiNa_2$ 1777.66060 found 1777.66337

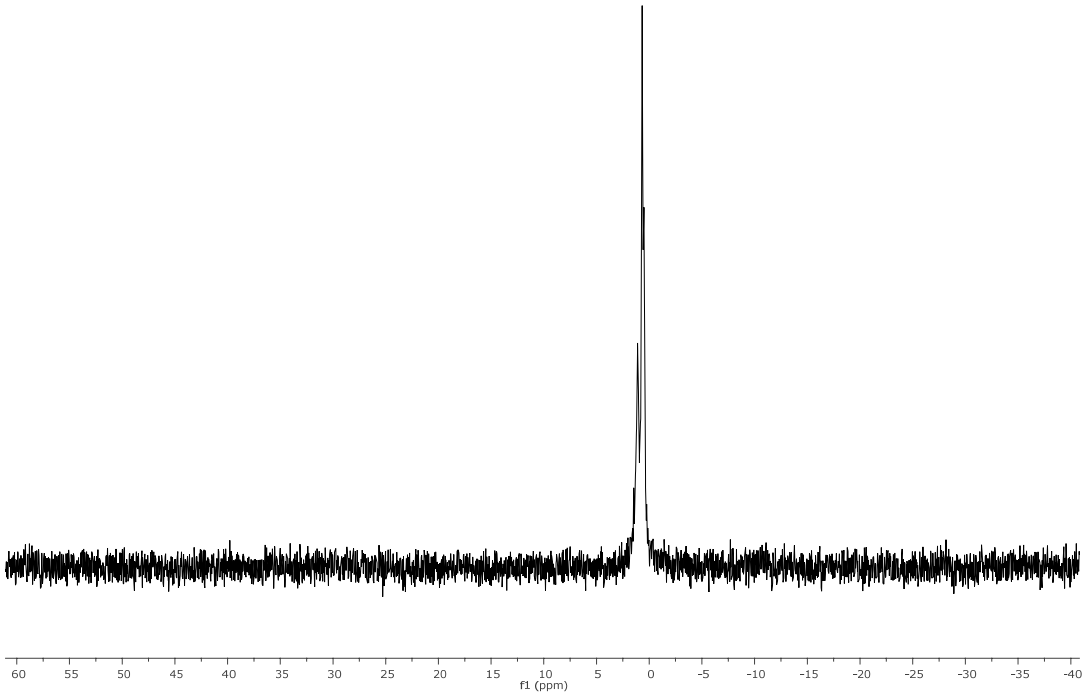
^{1}H NMR spectrum of compound **29**



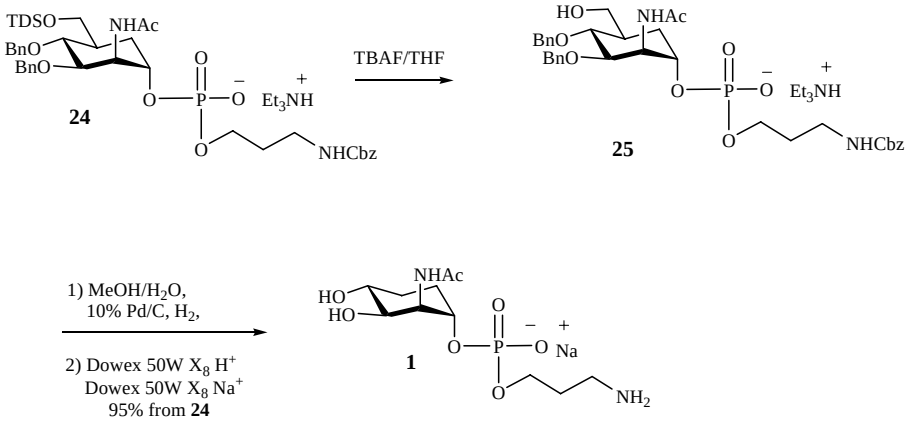
^{13}C NMR spectrum of compound **29**



^{31}P NMR spectrum of compound **29**



3-Aminopropyl 1-O-(2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosylphosphate), sodiumsalt, 1



Synthetic Procedure:

Compound **24** (36 mg, 0.039mmol) was dissolved in THF (2 mL) and treated with TBAF in THF (0.4 mL, 0.4 mmol) according to general procedure C. The crude residue was purified by flash chromatography (25%, MeOH/CH_2Cl_2, 1% TEA), providing the desilylated compound **25**.

This intermediate (30 mg) was hydrogenolysed over Pd/C (60 mg) in 50% MeOH/H_2O (4 mL) and subjected to ion exchange and lyophilization according to general procedure C, affording monomer **1** as a white solid (14 mg,95% over two steps):

Product characterization 1

Formula	C11H22N2O7P
Molecular Wright	325.28

$[\alpha]_D$ +7.6 (c 1.0 in H_2O)

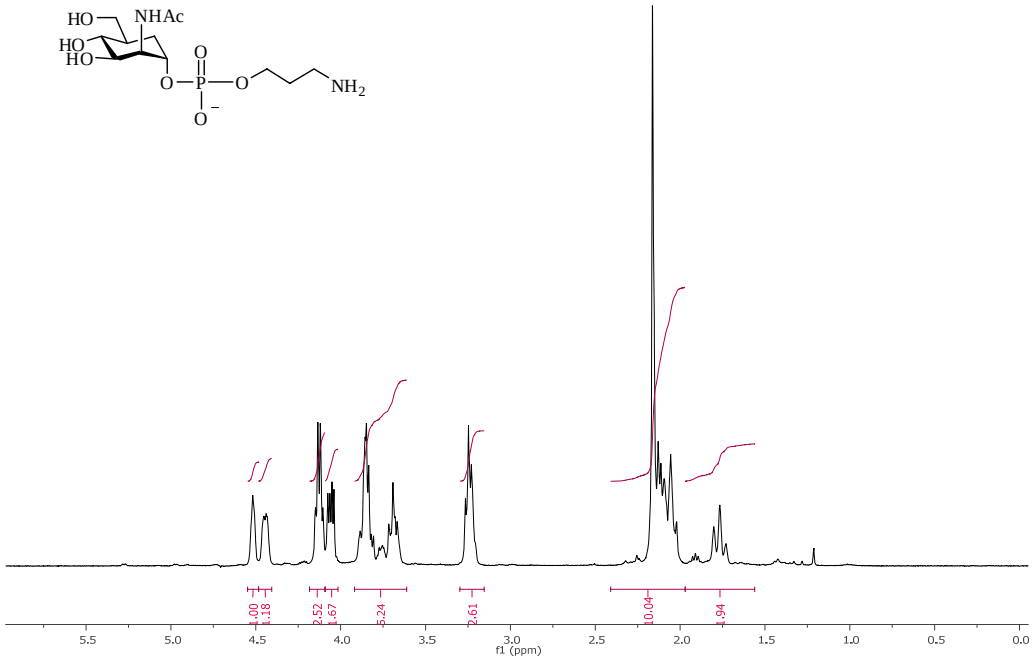
^{1}H NMR (D_2O, 400 MHz) δ 4.52 (bs, 1 H, H-2); 4.45 (bdd, 1 H, H-1); 4.26 (q, *J* = 6.1 Hz, 2 H, $OCH_2CH_2CH_2N$); 4.12 (dd, *J* = 4.8, 9.7 Hz, 1 H, H-3); 3.90-3.64 (m, 3 H, H-4, H-6, H-6'); 3.26-3.20 (m, 2 H, $OCH_2CH_2CH_2N$); 2.16 (s, 3 H, NHAc); 2.15-2.02 (m, 4 H, H-5, H-5a', $OCH_2CH_2CH_2N$); 1.77 (bt, 1 H, H-5a);

^{13}C NMR (D_2O, 100.6 MHz) δ 174.9 (*C*=O); 72.8 (C-1); 71.2 (C-3); 71.0 (C-4); 63.7 (OCH_2); 62.4 (C-6); 54.1 (C-2); 39.9 (C-5); 37.5 (CH_2N); 28.4 ($OCH_2CH_2CH_2N$); 28.3 (C-5a); 22.2 (OAc);

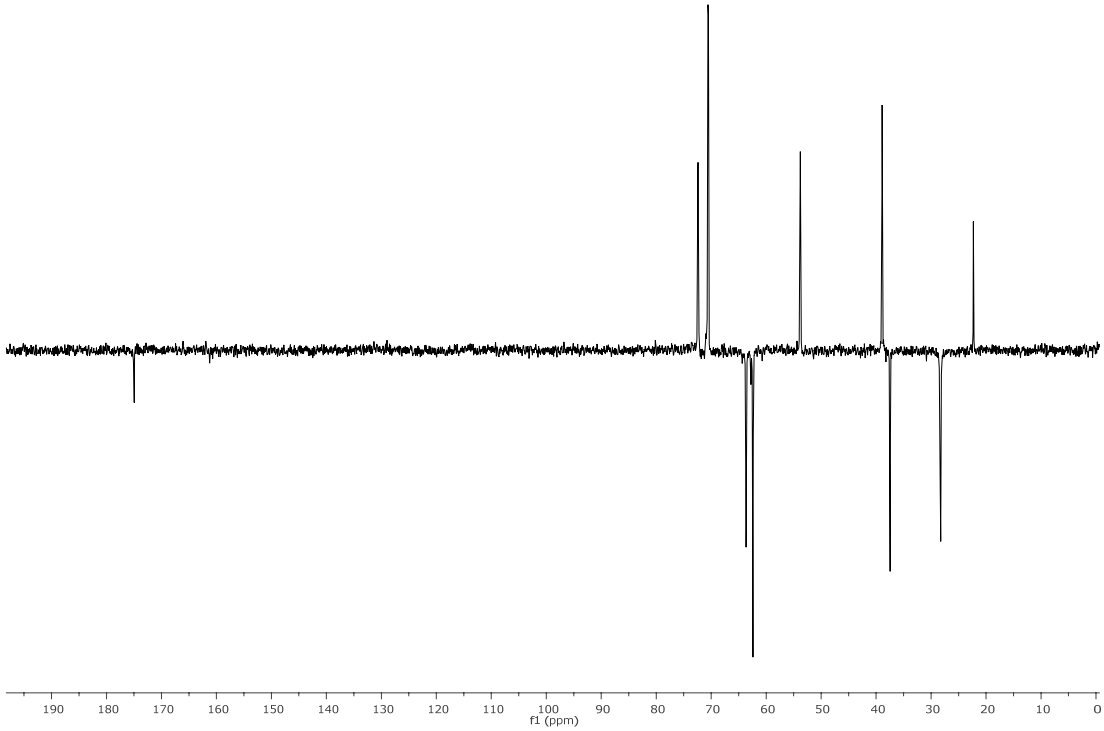
^{31}P NMR (D_2O, 162 MHz) δ 1.10.

ES HRMS cacld for $C_{12}H_{24}N_2O_8P$ 355.12758, found 355.12727

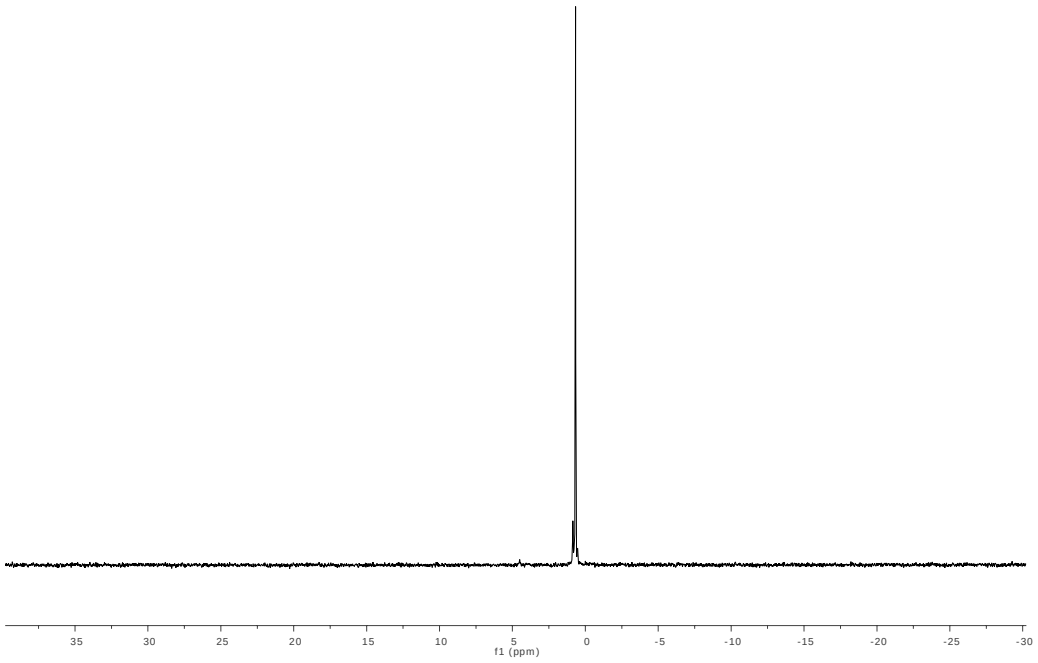
^{1}H NMR spectrum of compound **1**



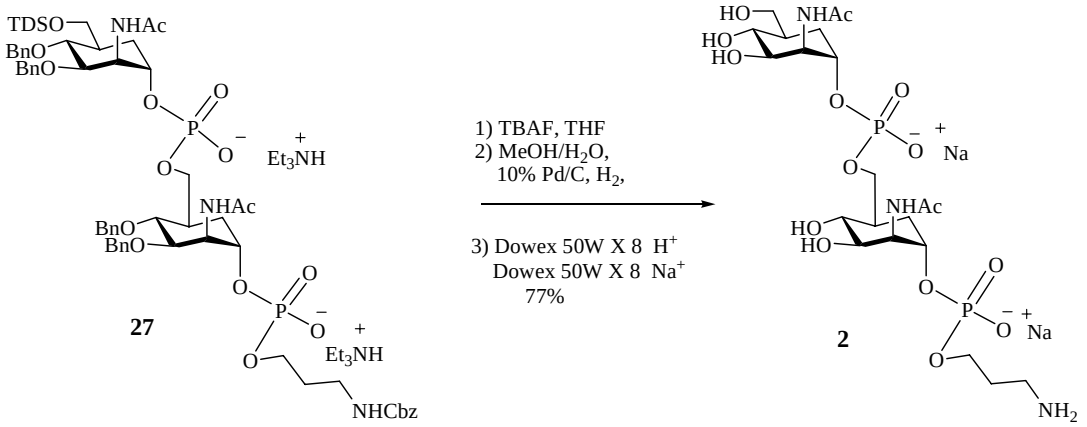
^{13}C NMR spectrum of compound **1**



^{31}P NMR spectrum of compound **1**



3-Aminopropyl 1-O-[2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate)], disodium salt, 2



Synthetic Procedure:

Compound **27** (53 mg, 0.040 mmol) was dissolved in THF (4 mL) and treated with TBAF in THF (0.60 mL, 0.60 mmol) according to general procedure B. The crude residue was purified by flash chromatography (30% MeOH/CH_2Cl_2, 1% TEA), providing the desilylated compound.

This intermediate (38 mg) was hydrogenolysed over Pd/C (80 mg) in 50% MeOH/H_2O (4 mL) and subjected to ion exchange and lyophilization according to general procedure B, affording dimer **2** as a white solid (21 mg, 77% over two steps)

Product characterization 2

Formula	C21H39N3O15P2
Molecular Wright	635.49

$[\alpha]_D$ +10.8 (c1.0 in H_2O).

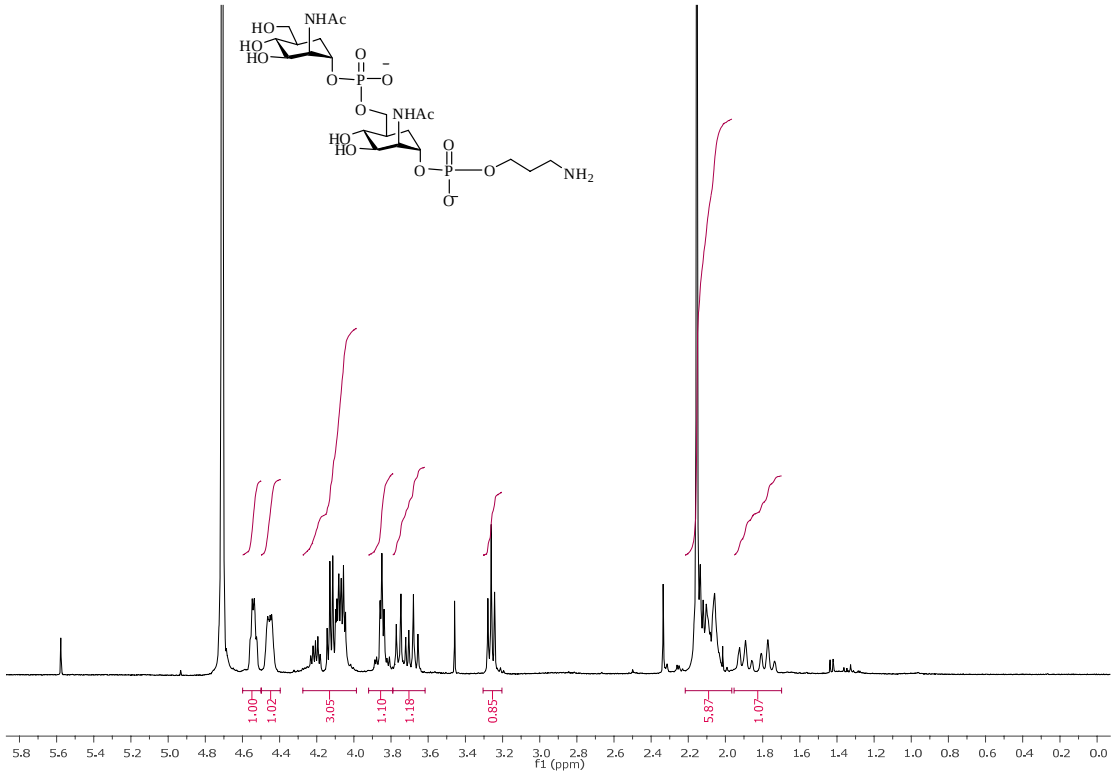
^{1}H NMR (D_2O, 400 MHz) δ 4.58-4.52 (m, 2 H, H-2, H-2'); 4.46-4.44 (m, 2 H, H-1, H-1'); 4.23-4.18 (m, 1 H, H-6'$_{(e)}$); 4.14-4.05 (m, 5 H, H-6$_{(e)}$; H-3, H-3', $OCH_2CH_2CH_2N$); 3.89-3.81 (m, 2 H, H-6$_{(a)}$; H-6'$_{(a)}$); 3.75 (t, *J* = 10 Hz, 1 H, H-4); 3.68 (t, *J* = 9.8 Hz, 1 H, H-4'); 3.26 (t, *J* = 7.2 Hz, 2 H, $OCH_2CH_2CH_2N$); 2.15 (s, 6 H, 2 NHAc); 2.17-2.05 (m, 6 H, H-5a$_{(e)}$; H-5a'$_{(e)}$; H-5, H-5', $OCH_2CH_2CH_2N$); 1.89 (bt, *J* = 12.8 Hz,1 H, H-5a$_{(a)}$); 1.77 (bt, 1 H, *J* = 14 Hz, H-5a'$_{(a)}$);

^{13}C NMR (D_2O, 100.6 MHz) δ 72.4 72.3 (C-1', C-1); 70.9 (C-4'); 70.5 (C-3', C-3); 69.5 (C-4); 66.0 (C-6); 63.6 ($OCH_2CH_2CH_2N$); 62.4 (C-6'); 53.8 (C-2', C-2); 38.9 (C-5'); 37.9 (C-5); 37.5 ($OCH_2CH_2CH_2N$); 28.3 ($OCH_2CH_2CH_2N$); 28.1 (C-5a', C-5a); 22.3 (2 NHAc)

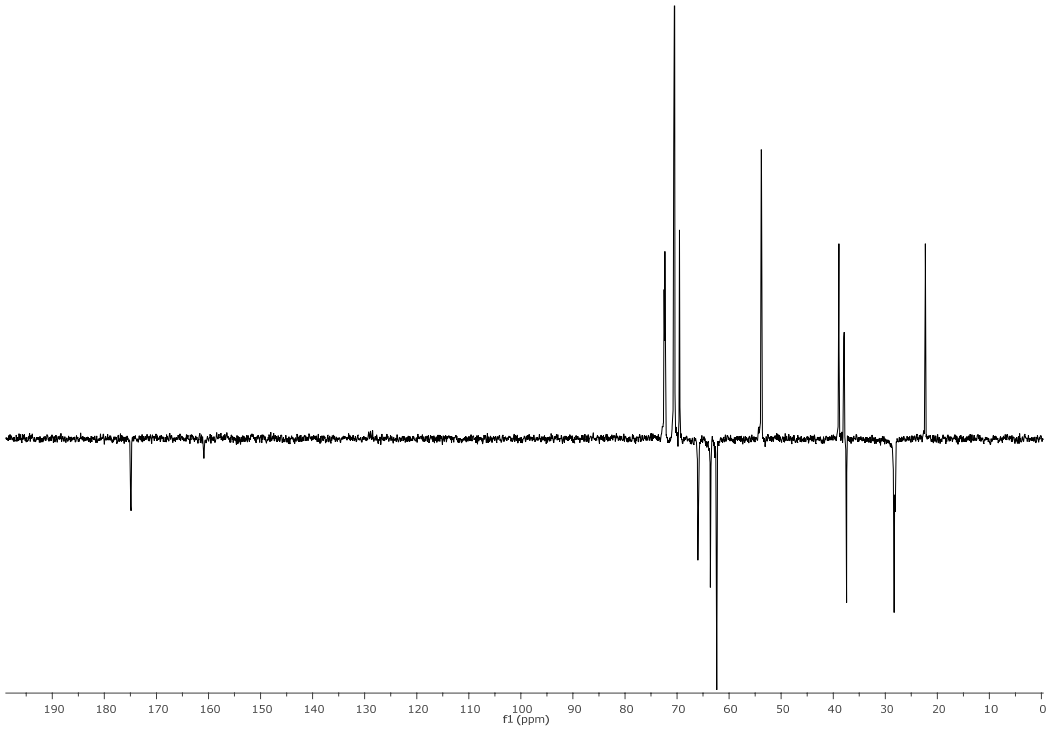
^{31}P NMR (D_2O, 162 MHz) δ 0.93, 0.61.

ES HRMS cacld for $C_{21}H_{40}N_3O_{15}P_2$ 636.19401 found 636.19368

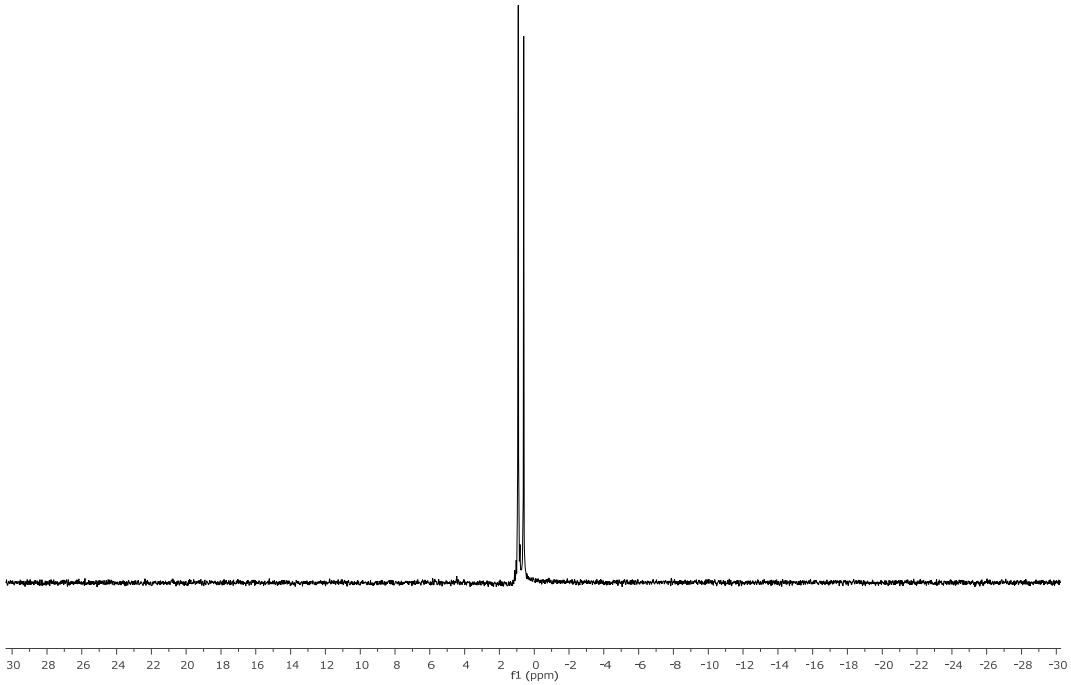
^{1}H NMR spectrum of compound **2**



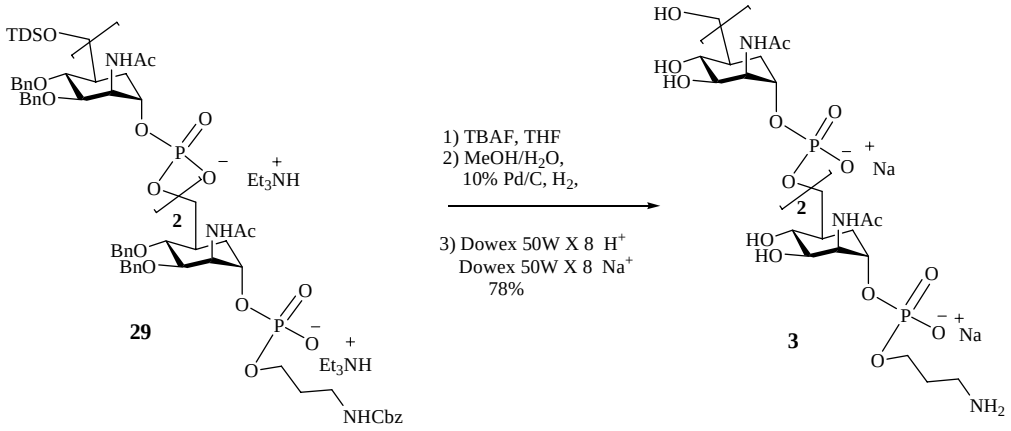
^{13}C NMR spectrum of compound **2**



^{31}P NMR spectrum of compound **2**



3-Aminopropyl 1-O-{2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-[2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate 6-(2-acetamido-2-deoxy-5a-carba-α-D-mannopyranosyl phosphate)]},trisodium salt,3



Synthetic Procedure:

Compound **29** (45 mg, 0.022 mmol) was dissolved in THF (5 mL) and treated with TBAF in THF (0.45 mL, 0.45 mmol) according to general procedure B. The crude was purified by flash chromatography (50% MeOH/CH_2Cl_2, 1% TEA), providing the desilylated compound.

This intermediate (32 mg) was hydrogenolysed over Pd/C (80 mg) in 50% MeOH/H_2O (4 mL) and subjected to ion exchange and lyophilization according to general procedure B, affording trimer **3** as a white solid (17 mg, 78% over two steps)

Product characterization 3

Formula	C30H54N4O22P3
Molecular Wright	915.69

$[\alpha]_D$ +8.2 (c 1.0 in H_2O).

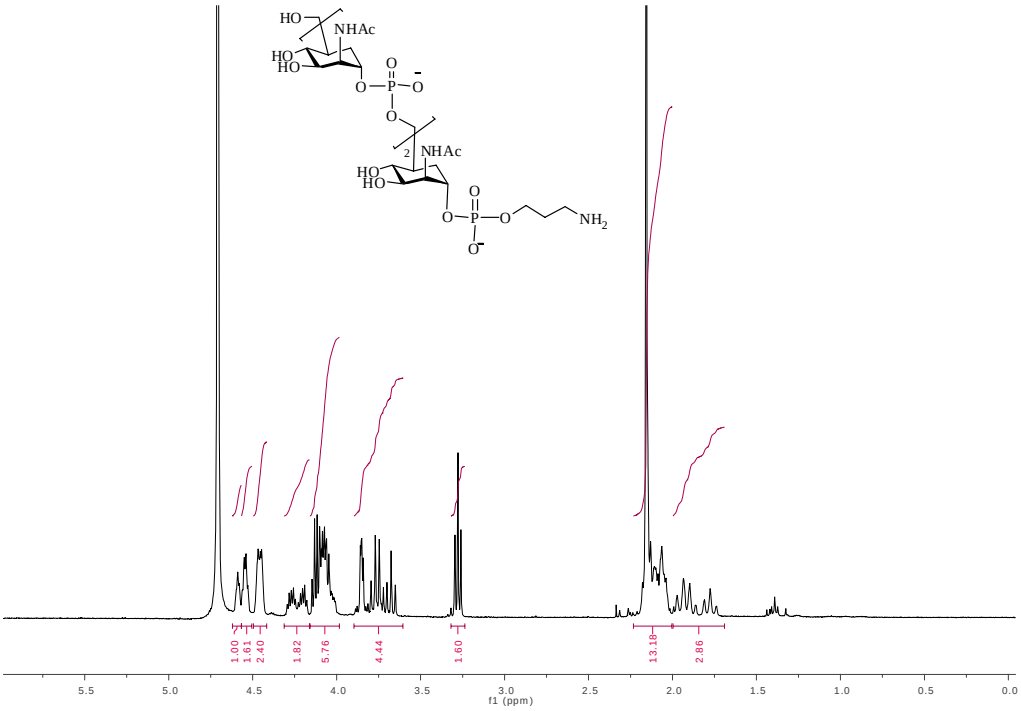
^{1}H NMR (D_2O, 400 MHz) δ 4.58-4.51 (m, 3 H, H-2, 2', 2''); 4.47-4.44 (m, 3 H, H-1, 1', 1''); 4.29-4.01 (m, 8 H, H-4, 4', H-6, 6', $OCH_2CH_2CH_2N$); 3.85 (m, 2 H, H-6''); 3.70-3.65 (m, 4 H, H-3, 3', 3'', H-4''); 3.29 (t, J = 7.2 Hz, 2 H, $OCH_2CH_2CH_2N$); 2.15 (s, 9 H, 3Ac); 2.10-2.04 (m, 5 H, H-5, 5', 5'', $OCH_2CH_2CH_2N$); 1.97-1.77 (m, 6 H, H-5a, 5a', 5a'');

^{13}C NMR (D_2O, 100.6 MHz) δ 174.9 (3 C=O); 72.3 (C-1'', C-1', C-1); 70.5 70.5 70.4 (C-4'', C-4', C-4); 69.5 69.4 69.3 (C-3'', C-3', C-3); 66.0 65.7 (C-6', C-6); 63.5 ($OCH_2CH_2CH_2N$); 62.3 (C-6''); 53.7 53.7 51.7 (C-2'', C-2', C-2); 38.9 38.9 37.8 (C-5'', C-5', C-5); 37.4 ($OCH_2CH_2CH_2N$); 28.2 ($OCH_2CH_2CH_2N$); 26.8, 27.5, 27.8 (C-5a'', C-5a', C-5a); 22.2 (3 CH_3-C=O);

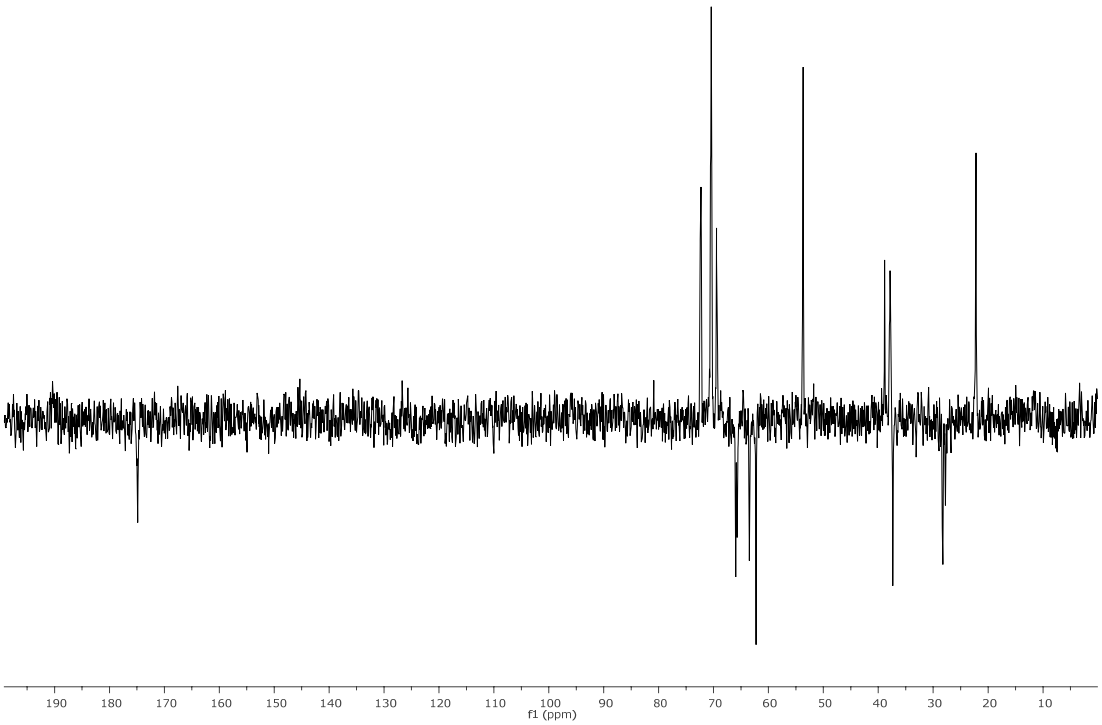
^{31}P NMR (D_2O, 162 MHz) δ 0.95, 0.86, 0.60.

ES HRMS cacld for $C_{30}H_{55}N_4O_{22}P_3Na$ 939.24240 found 939.24192

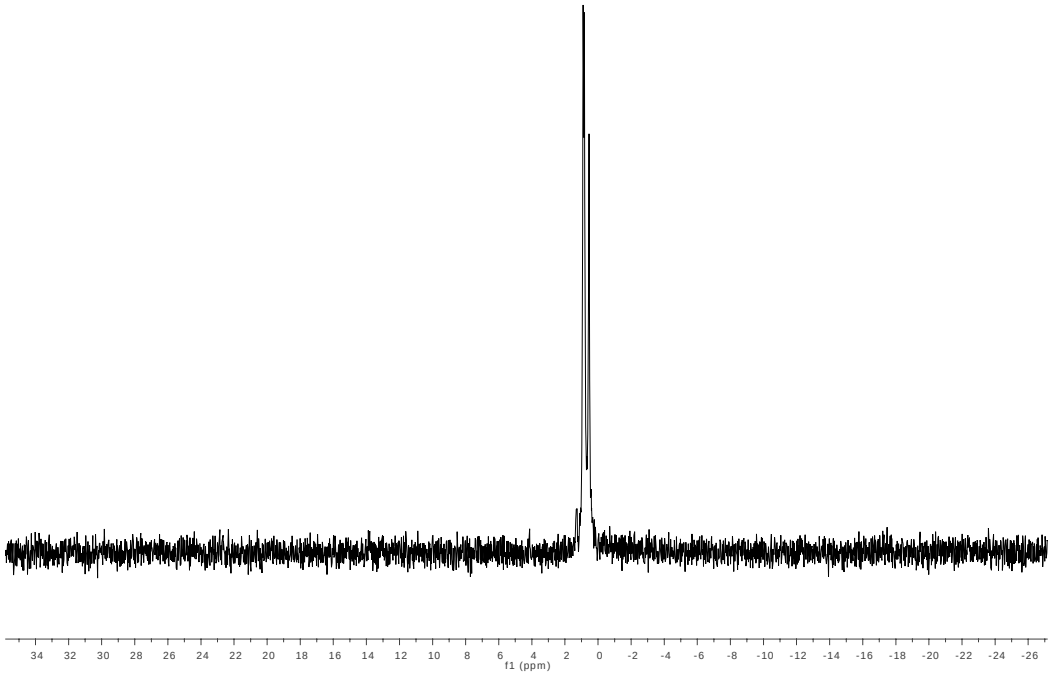
^{1}H NMR spectrum of compound **3** (T = 40 °C)



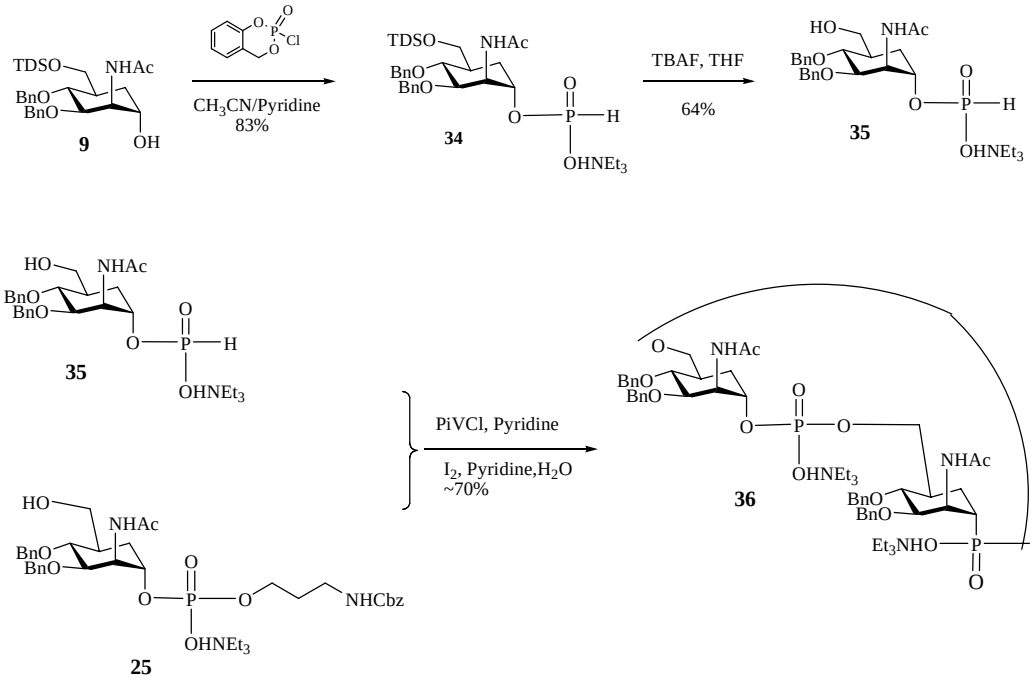
^{13}C NMR spectrum of compound **3**



^{31}P NMR spectrum of compound **3**



H-phosphonate Polycondensation (monomer)



Synthetic Procedure for 34 and 35 and polycondensation

To a solution of **9** (200 mg, 0.37 mmol) in dry CH_3CN (4 mL) and pyridine (1.4 mL), 0.4 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one in dry CH_3CN (1 mL, 0.4 mmol) was added at room temperature. The mixture was stirred at room temperature under nitrogen atmosphere for 45 minutes. Then a 1:1 mixture of pyridine-H_2O (1:1, 2 mL) solution was added and the mixture was diluted with $CHCl_3$ (30 mL). The organic layer was washed with H_2O (10 mL) and 1 M TEAB (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (5% MeOH/CH_2Cl_2, 1% TEA), providing a clean oil, the oil was diluted with $CHCl_3$ (20 mL), then washed again by 0.5 M TEAB (10 mL), dried (Na_2SO_4), filtered, and concentrated get product **34** (205 mg, 82%) as a syrup.

A stirred solution of **34** (205 mg, 0.29 mmol) in dry THF (10 mL) was treated with TBAF (1M solution in THF + 5% H_2O, 1 mL, 1 mmol), the mixture was stirred at room temperature under nitrogen atmosphere for 8 hours. The mixture was then

diluted with $CHCl_3$ (20 mL), the organic layer was washed with saturated aqueous solution of NH_4Cl (20 mL) and 1 M TEAB (20 mL), dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (10% MeOH/CH_2Cl_2), affording **35** (104 mg, 64%)

Polycondensation

The H-phosphonate compound **35** (104 mg, 0.18 mmol) and **25** (14 mg, 0.018 mmol) were dissolved in dry pyridine (1 mL) and the solvent evaporated under high vacuum. This procedure was repeated three times to remove traces of residual moisture. The dried compound was dissolved in a mixture of anhydrous pyridine and triethylamine (10:1, 200 uL), followed by dropwise addition of pivaloyl chloride (45 uL, 0.36 mmol). After 30 min, a fresh lot of pivaloyl chloride (30 uL, 0.24mmol) was added. The reaction mixture was stirred at room temperature for 3 hours, a freshly prepared iodine solution (440 uL, 70 mg, 0.28 mmol in pyridine-water, 95:5) was added, and stirring was continued for another 2 hours. Next the reaction was quenched by addition of CHCl3, and the organic layer washed with ice-cold solutions of $Na_2S_2O_3$ (1 M) and TEAB buffer, dried over Na2SO4, and concentrated under high vacuum. The crude was purified by flash chromatography (20% MeOH/CH_2Cl_2), the affording product was analyzed by ESI mass spectrum and the **36** (922 m/z) was indicated as main product (70%)

Product characterization 34

The formation of the H-phosphonate intermediates **34** was ascertained by ^{1}H NMR analysis, which showed the diagnostic doublet at δ 6.10 ($J_{H,P}$ = 649 Hz), compound **34** was used directly in the following steps without further characterization.

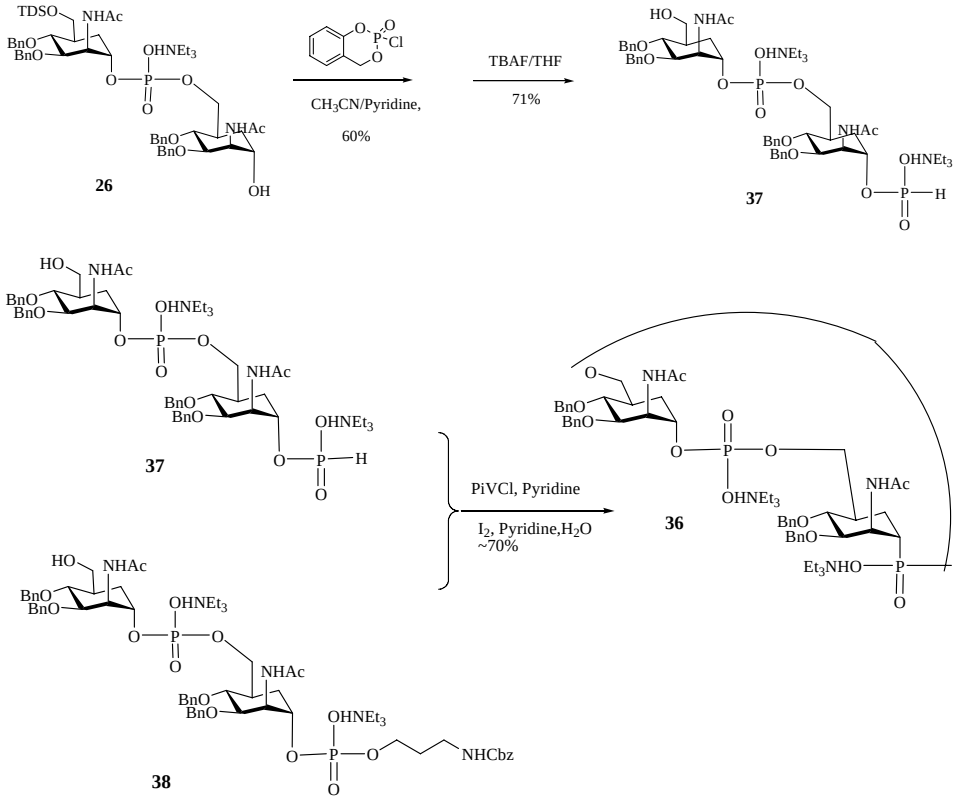
^{31}P NMR ($CDCl_3$, 162 MHz) δ 4.33

Product characterization 35

The formation of the H-phosphonate intermediates **35** was ascertained by ^{1}H NMR analysis, which showed the diagnostic doublet at δ 6.06 ($J_{H,P}$ = 645 Hz),compound **35** was used directly in the following steps without further characterization.

^{31}P NMR ($CDCl_3$, 162 MHz) δ 4.61

H-phosphonate Polycondensation (dimer)



Synthetic Procedure for 37 and polycondensation

To a solution of **26** (250 mg, 0.23 mmol) in dry CH_3CN (5 mL) and pyridine (1.7 mL), 0.4 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one in dry CH_3CN (0.6 mL, 0.24 mmol) was added at room temperature. The mixture was stirred at room temperature under nitrogen atmosphere for 45 minutes. Then a 1:1 mixture of pyridine-H_2O (1:1, 2 mL) solution was added and the mixture was diluted with $CHCl_3$ (30 mL). The organic layer was washed with H_2O (5 mL) and 1 M TEAB (10 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography (10% MeOH/CH_2Cl_2, 1% TEA), providing a clean oil, the oil was diluted with $CHCl_3$ (20 mL), then washed again by 0.5 M TEAB (10 mL), dried (Na_2SO_4), filtered, and concentrated get H-phosphonate product (174 mg, 60%) as a syrup.

A stirred solution of H-phosphonate product (174 mg, 0.14 mmol) in dry THF (10 mL) was treated with TBAF (1M solution in THF + 5% H_2O, 2 mL, 2 mmol), the mixture was stirred at room temperature under nitrogen atmosphere for 8 hours. The mixture was then diluted with $CHCl_3$ (25 mL), the organic layer was washed with saturated aqueous solution of NH_4Cl (20 mL) and 1 M TEAB (20 mL), dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (10% MeOH/CH_2Cl_2), affording **37** (110 mg, 71%)

Polycondensation

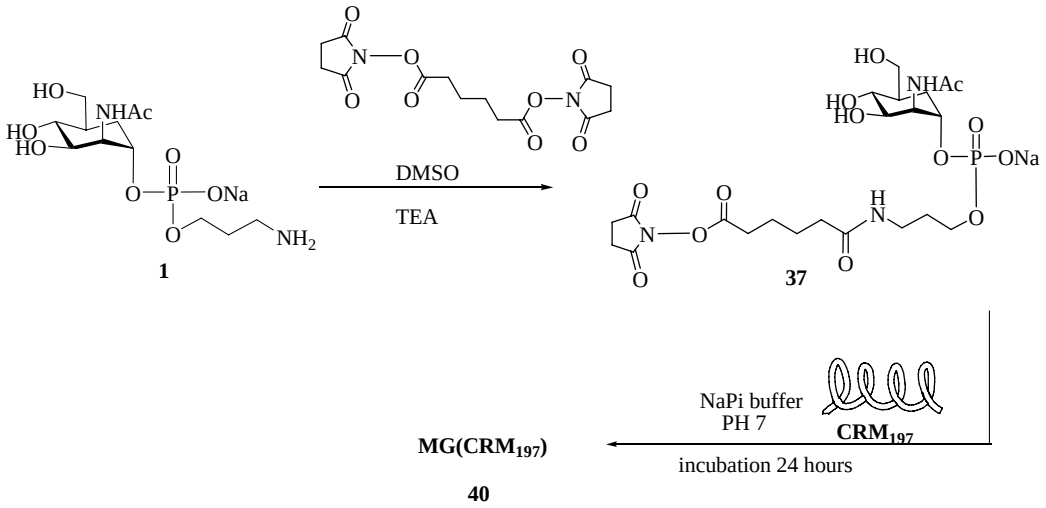
The dimer H-phosphonate compound **37** (100 mg, 0.89 mmol) and **38** (intermediate from preparation of **2**, 11 mg, 0.089 mmol) were dissolved in dry pyridine (1 mL) and the solvent evaporated under high vacuum. This procedure was repeated three times to remove traces of residual moisture. The dried compound was dissolved in a mixture of anhydrous pyridine and triethylamine (10:1, 150 uL), followed by dropwise addition of pivaloyl chloride (25 uL, 0.20 mmol). After 30 min, a fresh lot of pivaloyl chloride (12 uL, 0.1 mmol) was added. The reaction mixture was stirred at room temperature for 3 hours, a freshly prepared iodine solution (440 uL, 70 mg, 0.28 mmol in pyridine-water, 95:5) was added, and stirring was continued for another 2 hours. Next the reaction was quenched by addition of CHCl3, and the organic layer washed with ice-cold solutions of $Na_2S_2O_3$ (1 M) and TEAB buffer, dried over Na2SO4, and concentrated under high vacuum. The crude was purified by flash chromatography (25% MeOH/CH_2Cl_2), the affording product was analyzed by ESI mass spectrum and the **36** (922 m/z) was indicated as main product (70%)

Product characterization 37

The formation of the H-phosphonate intermediates **37** was ascertained by ^{1}H NMR analysis, which showed the diagnostic doublet at δ 6.22 ($J_{H,P}$ = 655 Hz), compound **37** was used directly in the following steps without further characterization.

^{31}P NMR ($CDCl_3$, 162 MHz) δ 2.99, 1.01

Monomer-Glycoconjugate (CRM_{197})



40

Synthetic Procedure

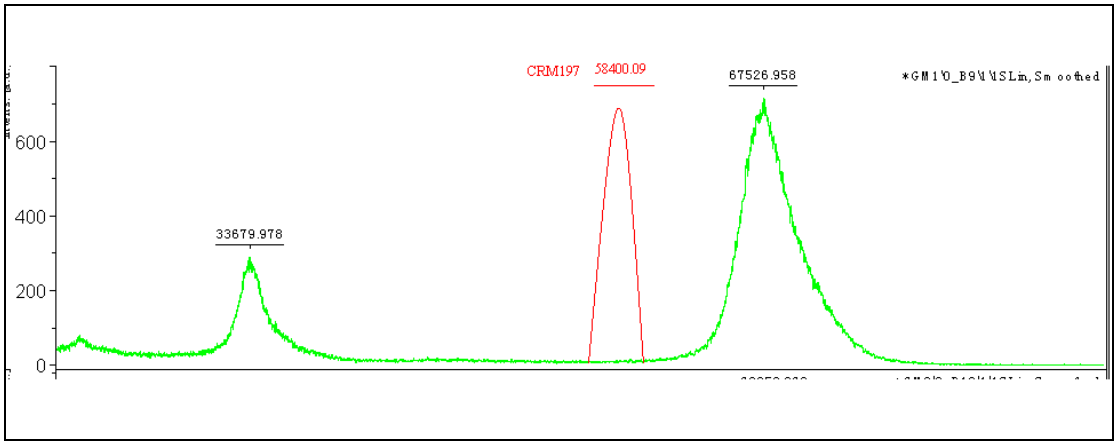
Monomer **1** (3 mg, 7.9 umol) was dissolved in a solution of DMSO (0.2 mL) and TEA (0.02 mL), then the solution was dropped into a mixture of SIDEA (10 eq) in DMSO (0.15 mL), the reaction was stirred for 2 hours. After purification from the excess of bi-functional linker by precipitation (Ethyl Acetate, 3 mL), washing (Ethyl Acetate, 7 × 1 ml) and lyophilization, the activated oligosaccharides **37** (1.5 mg) was obtained .

Compound **37** was reacted with 1.7 mg of protein (0.057 mL of 30 mg/mL of protein in NaPi 10 mM + 10% saccharosium, pH 7.2) in 100 uL of 100 mM NaPi buffer (pH 7.2): the conjugation stoimetry was about 100:1 sugar/protein (mol/mol). The mixture was incubated for 24 hours at room temperature, mixing very gently with a magnetic stirrer. The glycoconjugate was purified from the excess of unconjugated carbohydrate precipitation by (NH4)2S04.

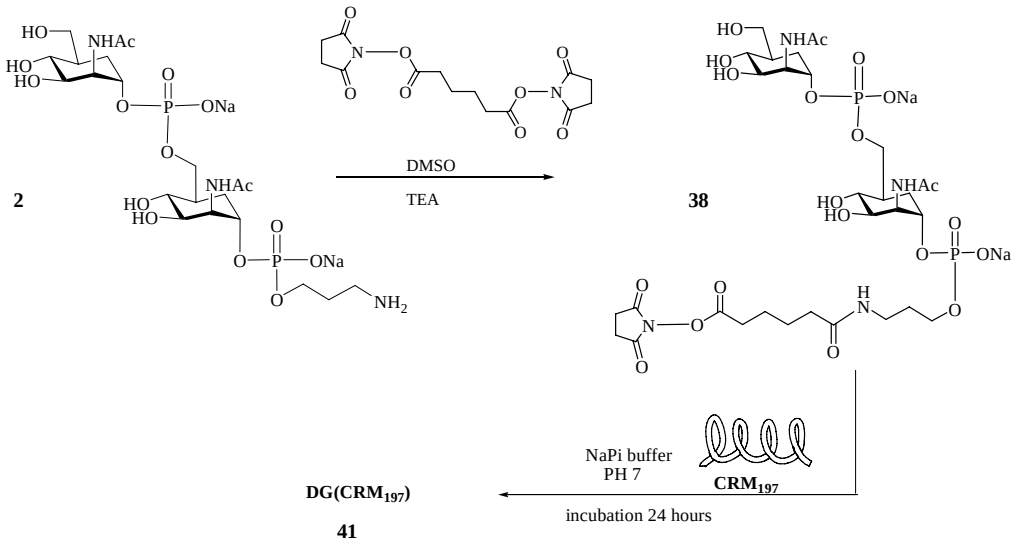
Product characterization 40

MALDI-TOF mass spectrum of **40** was recorded: the found value for the glycoconjugate **40** was 67526m/z, corresponding to approximately 19 oligosaccharide chains per CRM_{197} molecule (average m/z about 58400).

Product	Activated Ester/Protein	Loading (X)	MW	Protein Total	Sugar Concentration mg/ml	Protein Concentration mg/ml
40	40:1	18.5	67526	1.35	0.089	0.68



Dimer-Glycoconjugate (CRM_{197})



Synthetic Procedure

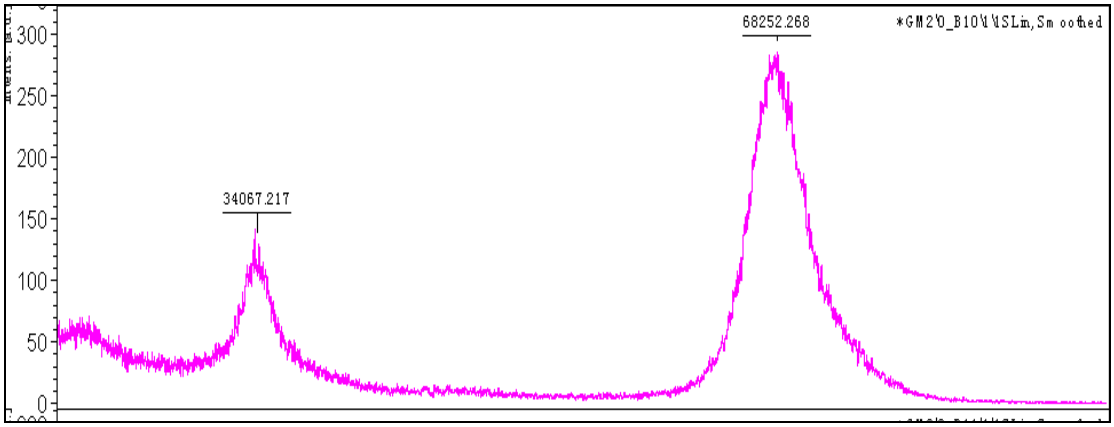
Dimer **2** (4 mg, 6.3 umol) was dissolved in a solution of DMSO (0.55 mL) and TEA (0.04 mL), then the solution was dropped into a mixture of SIDEA (10 eq) in DMSO (0.25 mL), the reaction was stirred for 2 hours. After purification from the excess of bi-functional linker by precipitation (Ethyl Acetate, 3 mL), washing (Ethyl Acetate, 10 × 1.5 ml) and lyophilization, the activated oligosaccharides **38** (4.1 mg) was obtained .

Compound **38** was reacted with 2 mg of protein (0.067 mL of 30 mg/mL of protein in NaPi 10 mM + 10% saccharosium, pH 7.2) in 100 uL of 100 mM NaPi buffer (pH 7.2): the conjugation stoimetry was about 100:1 sugar/protein (mol/mol). The mixture was incubated for 24 hours at room temperature, mixing very gently with a magnetic stirrer. The glycoconjugate was purified from the excess of unconjugated carbohydrate precipitation by (NH4)2S04.

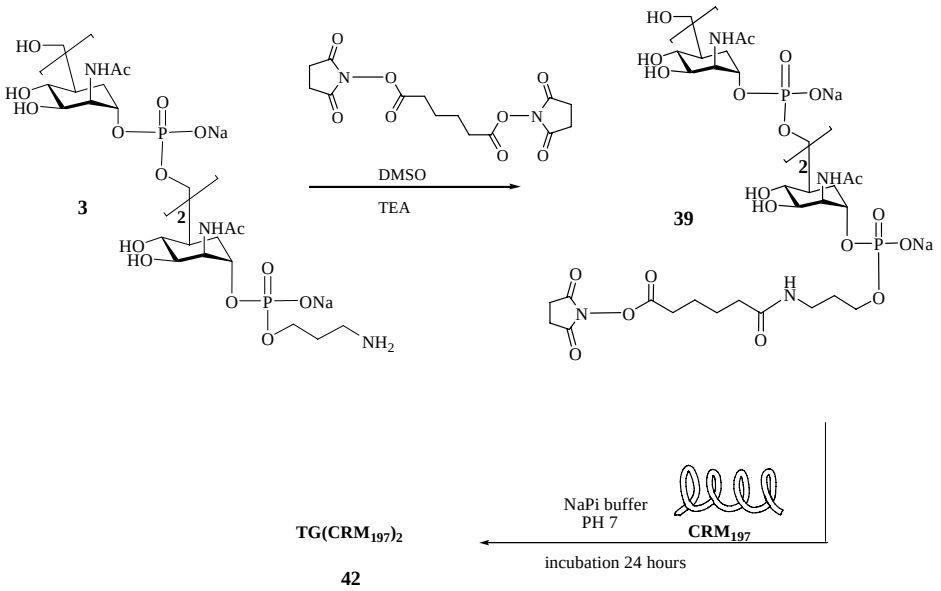
Product characterization 41

MALDI-TOF mass spectrum of **41** was recorded: the found value for the glycoconjugate **41** was 68252 m/z, corresponding to approximately 12 oligosaccharide chains per CRM_{197} molecule (average m/z about 58400).

Product	Activated Ester/Protein	Loading (X)	MW	Protein Total	Sugar Concentration mg/ml	Protein Concentration mg/ml
41	50:1	12.3	68252	0.88	0.063	0.44



Trimer-Glycoconjugate (CRM_{197})



Synthetic Procedure

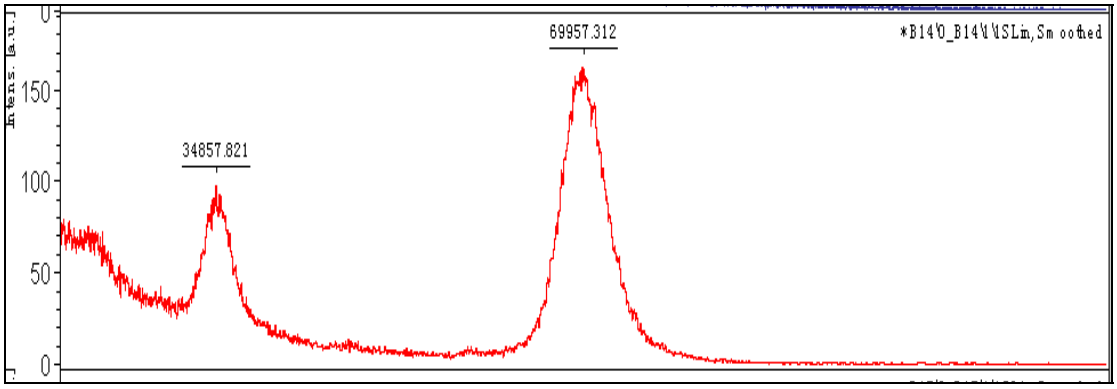
Trimer **3** (4 mg, 4.7 umol) was dissolved in a solution of DMSO (0.55 mL), TEA (0.04 mL) and trace amount of water (15 uL) then the solution was dropped into a mixture of SIDEA (15 eq) in DMSO (0.25 mL), the reaction was stirred for 2 hours. After purification from the excess of bi-functional linker by precipitation (Ethyl Acetate, 3 mL), washing (Ethyl Acetate, 10 × 1.5 ml) and lyophilization, the activated oligosaccharides **39** (4.0 mg) was obtained .

Compound **39** was reacted with 2 mg of protein (0.067 mL of 30 mg/mL of protein in NaPi 10 mM + 10% saccharosium, pH 7.2) in 100 uL of 100 mM NaPi buffer (pH 7.2): the conjugation stoimetry was about 100:1 sugar/protein (mol/mol). The mixture was incubated for 48 hours at room temperature, mixing very gently with a magnetic stirrer. The glycoconjugate was purified from the excess of unconjugated carbohydrate precipitation by (NH4)2S04.

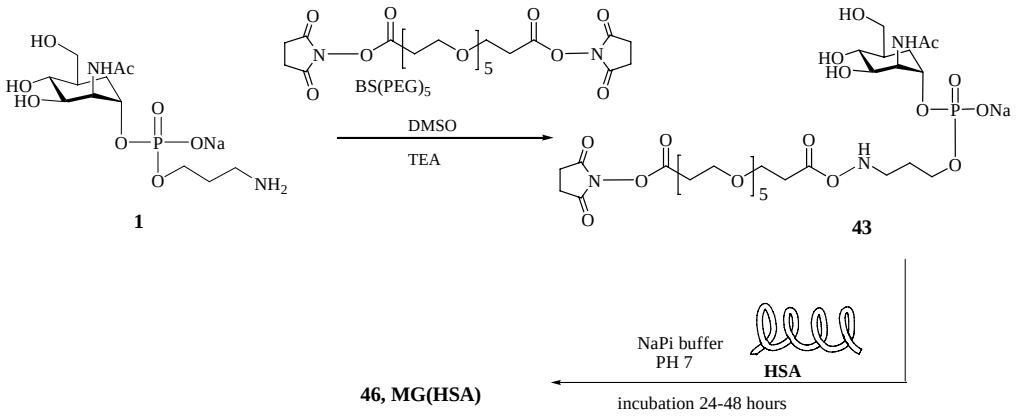
Product characterization 42

MALDI-TOF mass spectrum of **42** was recorded: the found value for the glycoconjugate **42** was 69957 m/z, corresponding to approximately 11 oligosaccharide chains per CRM_{197} molecule (average m/z about 58400).

Product	Activated Ester/Protein	Loading (X)	MW	Protein Total	Sugar Concentration mg/ml	Protein Concentration mg/ml
42	50:1	10.5	69957	1.72	0.14	0.86



Monomer-Glycoconjugate (HSA, 46)



Synthetic Procedure:

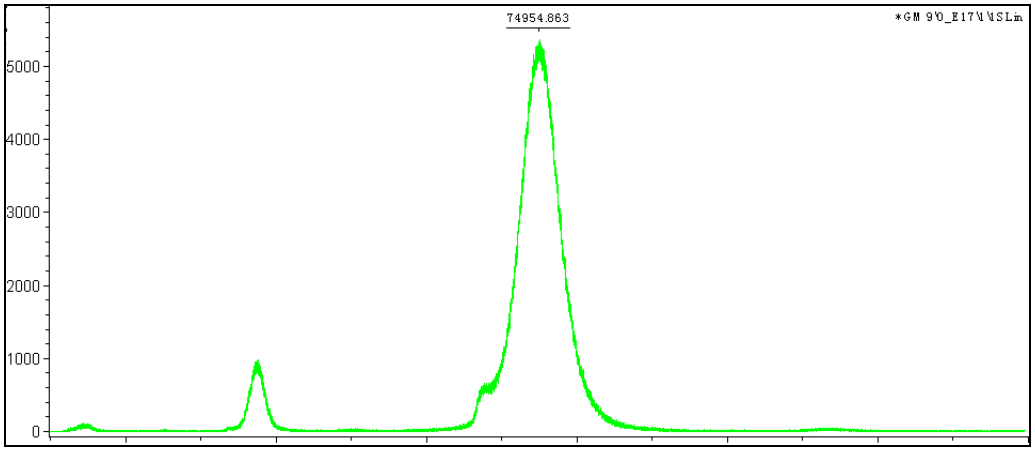
Monomer **1** (3 mg, 7.9 umol) was dissolved in a solution of DMSO (200 uL) and TEA (20 uL), then the solution was slowly dropped into a solution of BS(PEG)5 (10 eq) in DMSO. After 3 hours of stirring, the activated oligosaccharides was purified from the excess of bi-functional linker by precipitation (Ethyl Acetate, 5 mL), washing (Ethyl Acetate, 10 × 1 ml) and lyophilization, **43** (2.5 mg) was obtained.

Compound **43** was then reacted with HSA (1.5 mg, 50 uL) in 100 uM NaPi buffer 7.2 (20 uL), with a conjugation stoichiometry of 150:1 sugar/protein (mol/mol). After
incubation for 24 hours at room temperature, mixing very gently with a magnetic stirrer, the glycoconjugate was purified from the excess of unconjugated carbohydrate precipitation by (NH4)2SO4.

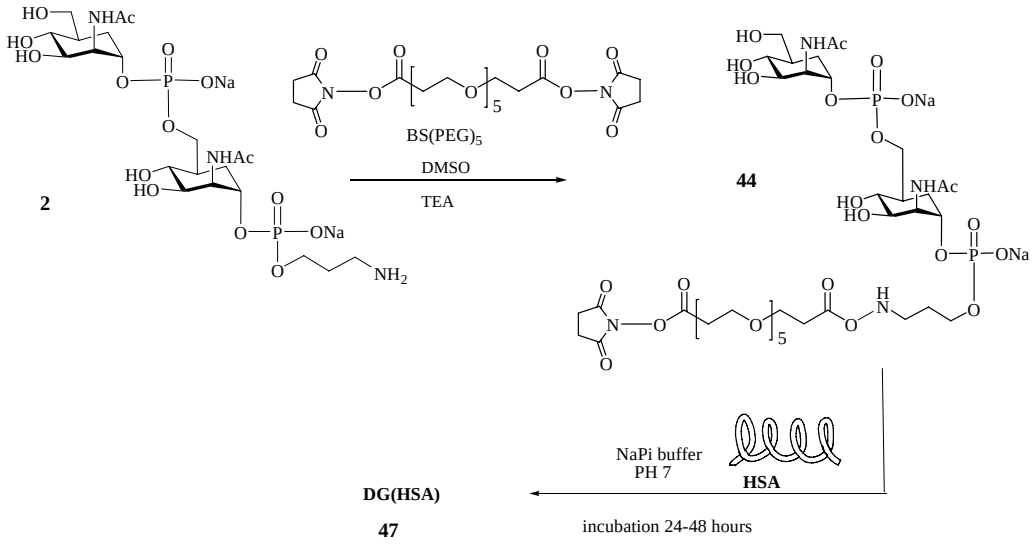
Product characterization 46

MALDI-TOF mass spectrum of **46** was recorded: the found value for the glycoconjugate **46** was 74954 m/z, corresponding to approximately 15 oligosaccharide chains per HSA molecule (average m/z about 66504).

Product	Activated Ester/Protein	Loading X	MW	Protein Total	Sugar Concentration mg/ml	Protein Concentration mg/ml
46	40:1	14.6	74954	0.94	0.062	0.47



Dimer-Glycoconjugate (HSA, 47)



Synthetic Procedure:

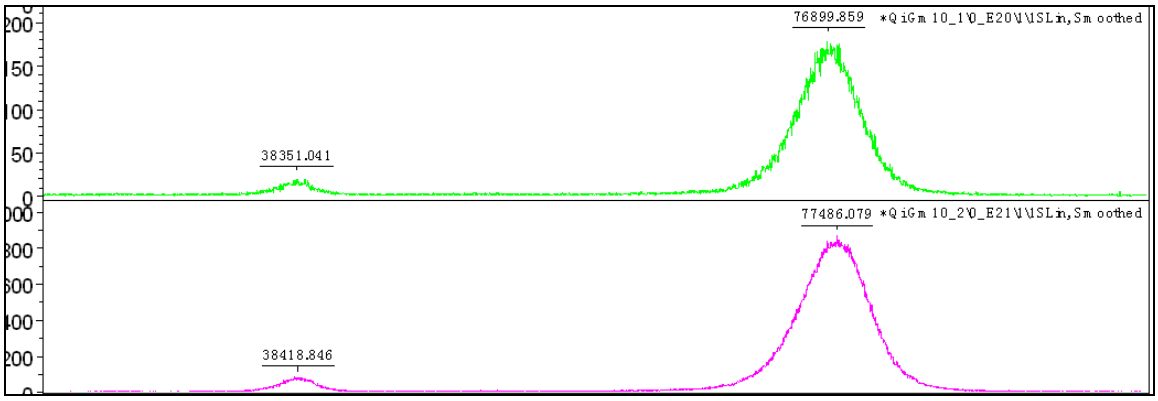
Dimer **2** (4 mg, 6.3 umol) was dissolved in a solution of DMSO (500 uL) and TEA (40 uL), then the solution was slowly dropped into a solution of BS(PEG)5 (20 eq) in DMSO. After 3 hours of stirring, the activated oligosaccharides was purified from the excess of bi-functional linker by precipitation (Ethyl Acetate, 5 mL), washing (Ethyl Acetate, 10 × 1 ml) and lyophilization, **44** (3.8 mg) was obtained.

Compound **44** was then reacted with HSA (2.2 mg, 50 uL) in 100 uM NaPi buffer 7.2 (20 uL), with a conjugation stoichiometry of 150:1 sugar/protein (mol/mol). After
incubation for 48 hours at room temperature, mixing very gently with a magnetic stirrer, the glycoconjugate was purified from the excess of unconjugated carbohydrate precipitation by (NH4)2SO4.

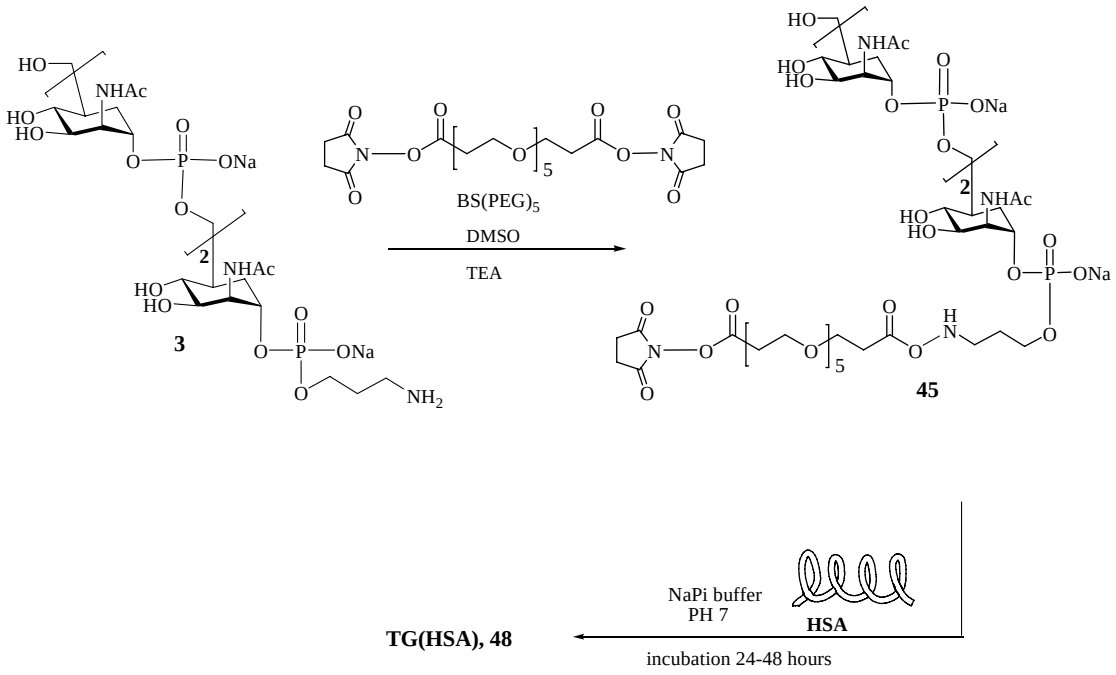
Product characterization 47

MALDI-TOF mass spectrum of **47** was recorded: the found value for the glycoconjugate **47** was 76899 m/z, corresponding to approximately 14 oligosaccharide chains per HSA molecule (average m/z about 66504).

Product	Activated Ester/Protein	Loading X	MW	Protein Total	Sugar Concentration mg/ml	Protein Concentration mg/ml
47	50:1	14.5	76899	1.62	0.125	0.81



Trimer-Glycoconjugate (HSA, 48)



Synthetic Procedure:

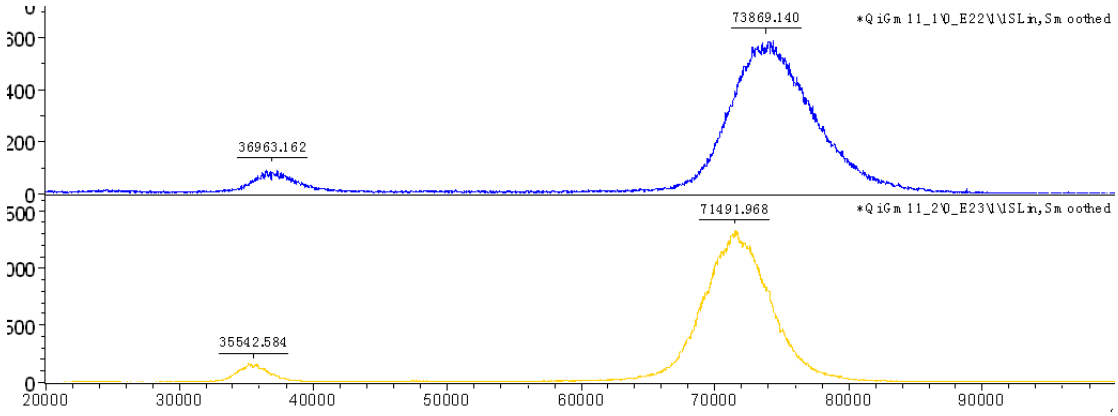
Trimer **3** (4 mg, 4.7 umol) was dissolved in a solution of DMSO (500 uL), TEA (40 uL) and water (20 uL) then the solution was slowly dropped into a solution of BS(PEG)5 (20 eq) in DMSO. After 3 hours of stirring, the activated oligosaccharides was purified from the excess of bi-functional linker by precipitation (Ethyl Acetate, 5 mL), washing (Ethyl Acetate, 10 × 2 ml) and lyophilization, **45** (3.1 mg) was obtained.

Compound **45** was then reacted with HSA (1.8 mg, 50 uL) in 100 uM NaPi buffer 7.2 (20 uL), with a conjugation stoichiometry of 150:1 sugar/protein (mol/mol). After
incubation for 48 hours at room temperature, mixing very gently with a magnetic stirrer, the glycoconjugate was purified from the excess of unconjugated carbohydrate precipitation by (NH4)2SO4.

Product characterization 48

MALDI-TOF mass spectrum of **48** was recorded: the found value for the glycoconjugate **48** was 73869 m/z, corresponding to approximately 8 oligosaccharide chains per HSA molecule (average m/z about 66504).

Product	Activated Ester/Protein	Loading X	MW	Protein Total	Sugar Concentration mg/ml	Protein Concentration mg/ml
48	50:1	7.9	73869	1.51	0.091	0.76



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Design and Synthesis of Glycosylphosphatidylinositol (GPI) Anchors of *Plasmodium falciparum*

INTRODUCTION

Innate immunity and TLRs

The innate immune system is an evolutionarily ancient system that detects the presence of microbial invaders and activates protective responses. It responds rapidly to pathogen associated molecular patterns (PAMP). Recognition of these PAMPs is mediated by sets of receptors, whose activation results in acute proinflammatory responses such as the production of a diverse set of cytokines and chemokines, direct local attack against the invading pathogen and initiation of responses that activate and regulate the adaptive component of the immune system.[1]

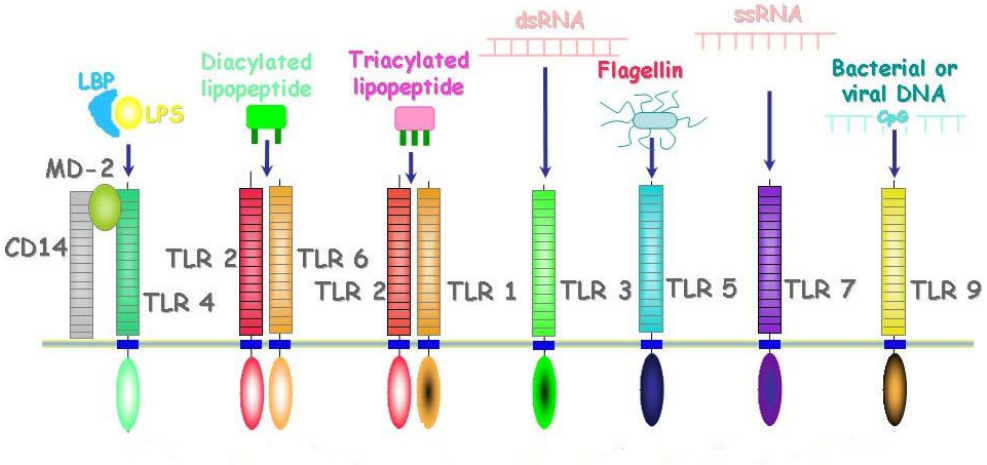


Figure 1 Activation of TLRs by microbial molecules

The discovery of Toll-like receptors (TLRs) has advanced the understanding of

early events in microbial recognition, subsequent host defense responses and the development of adaptive immune responses. Thirteen mammalian TLRs have been identified, each recognizing a discrete class of PAMP. For example, lipopolysaccharides (LPS) are recognized by TLR4, bacterial flagellin by TLR5, double-stranded RNA by TLR3, and bacterial DNA by TLR9. TLR2 recognizes structurally the broadest range of microbial compounds including lipoproteins, lipopeptides, peptidoglycan, lipoarabinomannans, lipomannans, lipoteichoic acids and glycosylphosphatidylinositol (GPI). The promiscuity of TLR2 has been attributed to its ability to form heterodimers with TLR1 and TLR6. [2] (Figure 1)

TLRs are single transmembrane glycoproteins having an extracellular domain, a transmembrane domain and an intracellular signaling domain that is homologous to the cytoplasmic tail of the interleukin (IL-1) receptor (TIR domain). The TLRs detect pathogens by ligand-induced dimerization of the ectodomains leading to dimerization of cytoplasmic TIR domains, which then serves as a docking site for specific adaptor proteins. These adaptor proteins recruit kinases to initiate the activation of Mitogen activated protein (MAP) kinases and transcription factors. This leads to the upregulation of hundreds of genes including those directly responsible for destroying the invading pathogen, alerting neighboring cells to the presence of danger, and initiating effective adaptive immune responses. [3]

TLRs as therapeutic targeting of innate immune response

Evidence is emerging that innate immune responses can be exploited for therapeutic purposes such as the development of adjuvants for vaccination and as immune modulators for the treatment of a wide range of diseases including asthma, rheumatoid arthritis, and cancer. [4] Toll-like receptors have many properties that make them attractive therapeutic targets such as overexpression in disease, knockout mice being resistant to disease, ligands exacerbating inflammation in disease models and genetic differences in TLRs or their signaling proteins correlate with risk of disease. [5] Several TLR agonists have been examined in clinical trials and for example the TLR4 agonist monophosphoryl lipid - A (MPL) has been shown to possess favorable properties as adjuvant for vaccination [6]

A concern of the use of TLR agonists is that they may over-activate the innate immune system leading to severe inflammation. [7] Furthermore, TLR agonists induce a wide range of responses someone which might be beneficial whereas others harmful. Thus, the design of safe immune modulators requires a detailed knowledge of structure-activity relationships to harness beneficial effects without causing toxicity. This information is difficult to obtain by using isolated compounds due to structural heterogeneity and possible contaminations with other inflammatory components. The synthesis and immunological evaluation of panels of well-defined TLR ligands can address this deficiency.

Malaria and GPI

Malaria is a mosquito-borne infectious disease of human and other animals caused by eukaryotic protist (a type of microorganism) of the genus plasmodium. The disease results from the multiplication of Plasmodium parasites within red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma or death. It is widespread in tropical and subtropical regions, including much of Sub-Saharan Africa, Asia and the Americas. [8] (Figure 2)

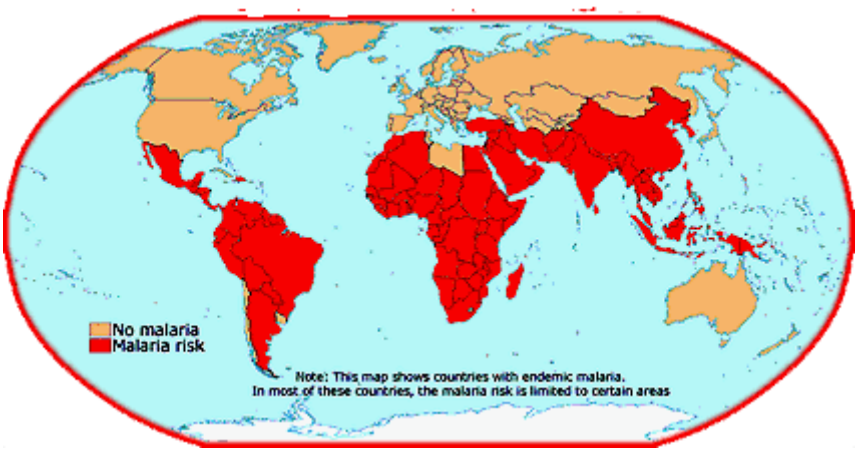


Figure 2 Countries with high risk of Malaria

A particular form of malaria, referred to as pregnancy associated malaria, is especially distressing because it intervenes at the very beginning of human life causing maternal anemia, poor pregnancy outcomes and death. A mosquito infects a person by taking a blood meal. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells (hepatocytes), where they multiply into merozoites, rupture the liver cells, and escape back into the bloodstream. Then, the merozoites infect red blood cells, where they develop into ring forms, trophozoites and schizonts which in turn produce further merozoites. Sexual forms (gametocytes) are also produced, which, if

taken up by a mosquito, will infect the insect and continue the life cycle. (Figure 3) Among all the Malaria, infections caused by plasmodium falciparum take account for about 90% of the deaths. [9]

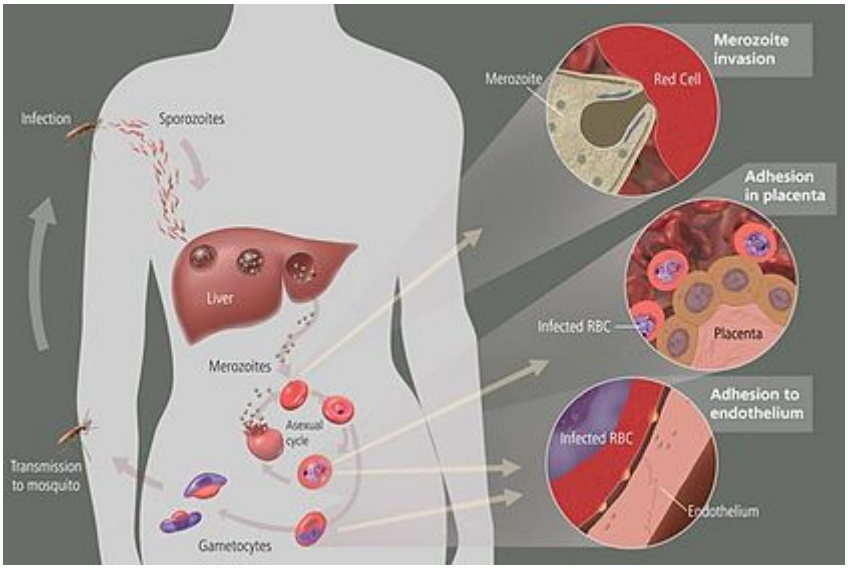


Figure 3. Malaria life cycle

During malaria infection, the host produces high levels of proinflammatory cytokines that have important roles in controlling parasite growth and govern the specificity and effectiveness of adaptive immunity. However, the proinflammatory mediators can be harmful when overproduced and population studies in malaria endemic areas and animal models indicate that excessive production of cytokines is associated with the pathology of malaria.[*9] Glycosylphosphatidylinositol (GPI) of P. falciparum is an important factor in the induction of proinflammatory responses and it has been shown to initiate such responses by interacting with TLR2. [10]

Innate Immune responses elicited by CPI anchors of Plasmodium falciparum

People in endemic areas have anti-GPI antibodies, which provide some protection against malaria symptoms. Research found that immunizations of mice with a synthetic glycan moiety of P. falciparum GPI could elicit anti-GPI antibodies, which prevent progression to cerebral malaria. [11] It has been suggested that inhibition of GPI-induced inflammatory responses will be a more effective approach for combating the pathology of malaria.[*10]

GPIs, which are expressed ubiquitously by eukaryotes, are primarily involved in anchoring proteins to the cell surface. [12] The basic structure of GPIs comprises a conserved ethanolamine phosphate-substituted oligosaccharide moiety, (EtN-P-6Man α(1-2)Man α(1-6)Man α(1-4)GlcN) linked to phosphatidylinositol.[13] GPIs expressed by different organisms differ in the nature and composition of the lipids as well as in having additional sugars and/or ethanolamine-phosphates attached to the conserved glycan. These variations confer broad structural diversity and may impart functional roles to GPIs. Characteristic features of parasite GPI include the presence of a saturated fatty-acyl moiety at the sn-1 position, an unsaturated fatty-acyl residue at *Sn*-2 of glycerol and a saturated fatty-acyl substituent at C-2 of inositol (Fig. 4).[14]

The innate immune receptor TLR2 can be activated with GPI derived from the parasite P. falciparum, which is the causative agent of malaria, without responding to self GPIs. Although it is known that human and P. falciparum GPIs differ in some structural aspects, [15] specific features that are important for TLR2 binding and inducing proinflammatory response have not been established. Such information, however, is critical for understanding host-parasite interactions and for developing cell-based screening assays for indentifying

inhibitors of GPI-initiated cytokines production.

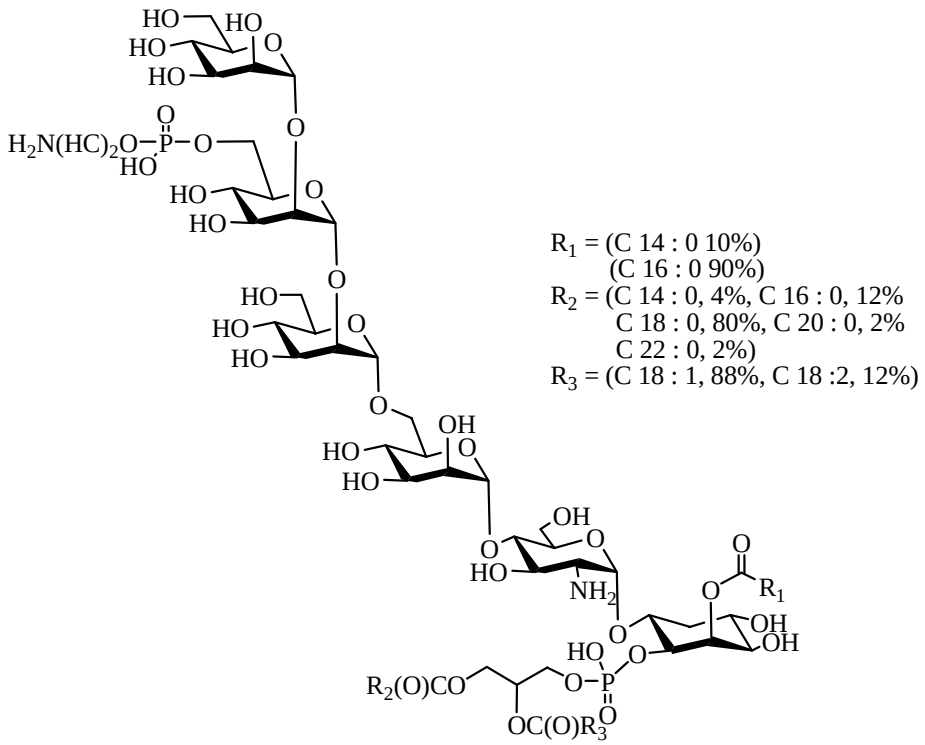


Figure 4 GPI anchors of Plasmodium falciparum

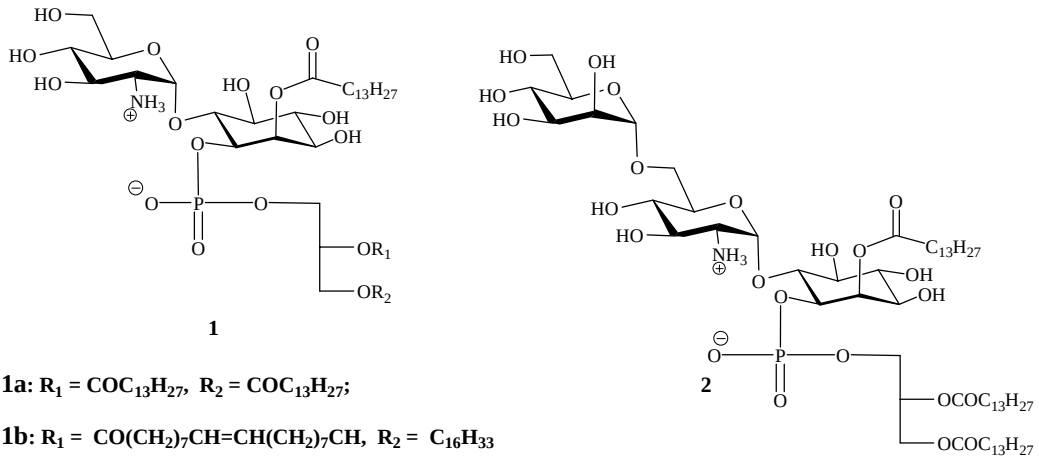
Chemical Synthesis and innate properties of Plasmodium falciparum GPI anchors

It has been proposed that inhibition of GPI-induced proinflammatory responses will provide an approach to combat the pathology of malaria [16]. Such a therapeutic strategy requires, however, a detailed knowledge of the structural features of GPI responsible for inducing proinflammatory responses.

Therefore, the purpose of this work is to develop a reliable synthetic methodology that give easy access to a wide range of GPIs derivatives for structure activity relationship studies, and furthermore, rely on possible biological studies onto the synthesized molecules to define the structural features of GPI which are critical for TLR-mediated inflammatory responses.

P. falciparum GPI displays considerable structural heterogeneity and differs considerably from human GPI (Fig. 4). In particular, human GPI contains mainly alkyl substituents, which are longer in chain length and have a higher degree of unsaturation than those of parasites. It has been found that cleavage of GPI into oligosaccharide and phosphate inositol lipid abolishes proinflammatory responses, indicating that both lipid and carbohydrate are critical for activity. [17] Furthermore, enzymatic remodeling of GPI suggests that a compound containing a core tri-mannoside represents the minimum structure for activity. [18]

Above all, for the purpose of establishing a reliable GPI synthesis strategy and preliminary structure activity relationship, three preliminary target molecules were designed (Figure 5), which represent : 1) different lipid chain saturation; 2) acyl vs. alkyl linked phosphonate lipids; 3) trisacchcaride and disaccharide.



1

1a: $R_1 = COC_{13}H_{27}$, $R_2 = COC_{13}H_{27}$;

1b: $R_1 = CO(CH_2)_7CH=CH(CH_2)_7CH$, $R_2 = C_{16}H_{33}$

2

Figure 5. Target molecules **1a**, **1b** and **2**

Consideration of convergent approach for the synthesis of GPI anchors

Several attractive strategies have been developed for the synthesis of GPI anchors. [19] In general, these approaches employ benzyl ethers as a permanent protecting group, and therefore cannot be used for the synthesis of compounds having unsaturated fatty acids. To address this problem, benzoyl esters have been used as permanent protecting group. However, their removal resulted in partial loss of fatty esters. [20] Guo and coworkers introduced a more attractive approach that employed p-methoxybenzyl (PMB) ethers for permanent protection.[21] PMB ethers can be removed by acid treatment or oxidation with DDQ and hence are compatible with the presence of alkenes. However, this approach did not use C-2 esters for controlling anomeric selectivities in glycosylations. Furthermore, PMB ethers are rather fragile in acid catalyzed glycosylation and can cause difficulties for the preparation of complex compounds.

The methyl naphtyl (NAP) ether has been recently introduced as novel protecting group in carbohydrate synthesis, and can be easily removed in high yield by oxidation with DDQ [22]. In previous projects of Boons' research group in University of Georgia, NAP ether has been used as a permanent protecting group, and could be removed as many as ten by using DDQ oxidation. [23] NAP ether is a more attractive permanent protecting group than the PMB ether because of its much higher acid stability [24]. Therefore, in this synthetic work towards GPI anchors, the NAP will be employed as a permanent protecting group.

Besides, Fmoc, Lev and TDS will be used as orthogonal protecting groups for parallel combinatorial synthesis of complex oligosaccharides. These protecting groups can be removed under mild conditions without affecting fatty acids of GPI final products. Furthermore, Lev and Fmoc will ensure neighboring group participation in glycosylations thereby controlling 1,2-trans selectivities, which will be convenient for the Building blocks assembly.

Above all, the synthesis work towards target molecules **1a**, **1b** and **2** will be described in the following chemistry section .

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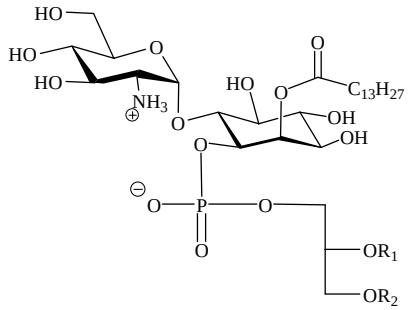
GPI-CHEMISTRY SECTION

1 Target Molecule and retrosynthetic analysis:

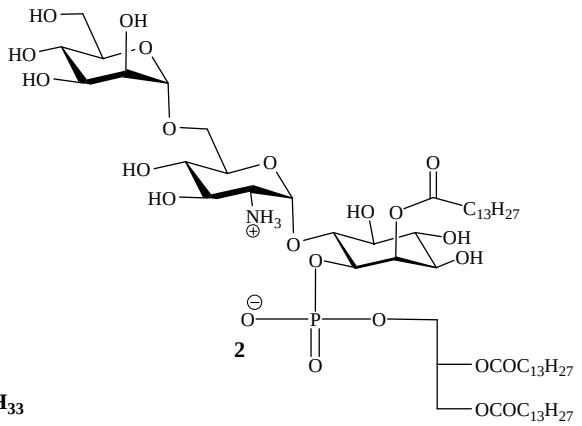
The synthesis work of the designed GPI target molecules: disaccharides **1a** and 1b and trisaccharide **2** (Figure 1) will be described in this part.



1

1a: $R_1 = COC_{13}H_{27}$, $R_2 = COC_{13}H_{27}$;

1b: $R_1 = CO(CH_2)_7CH=CH(CH_2)_7CH$, $R_2 = C_{16}H_{33}$



2

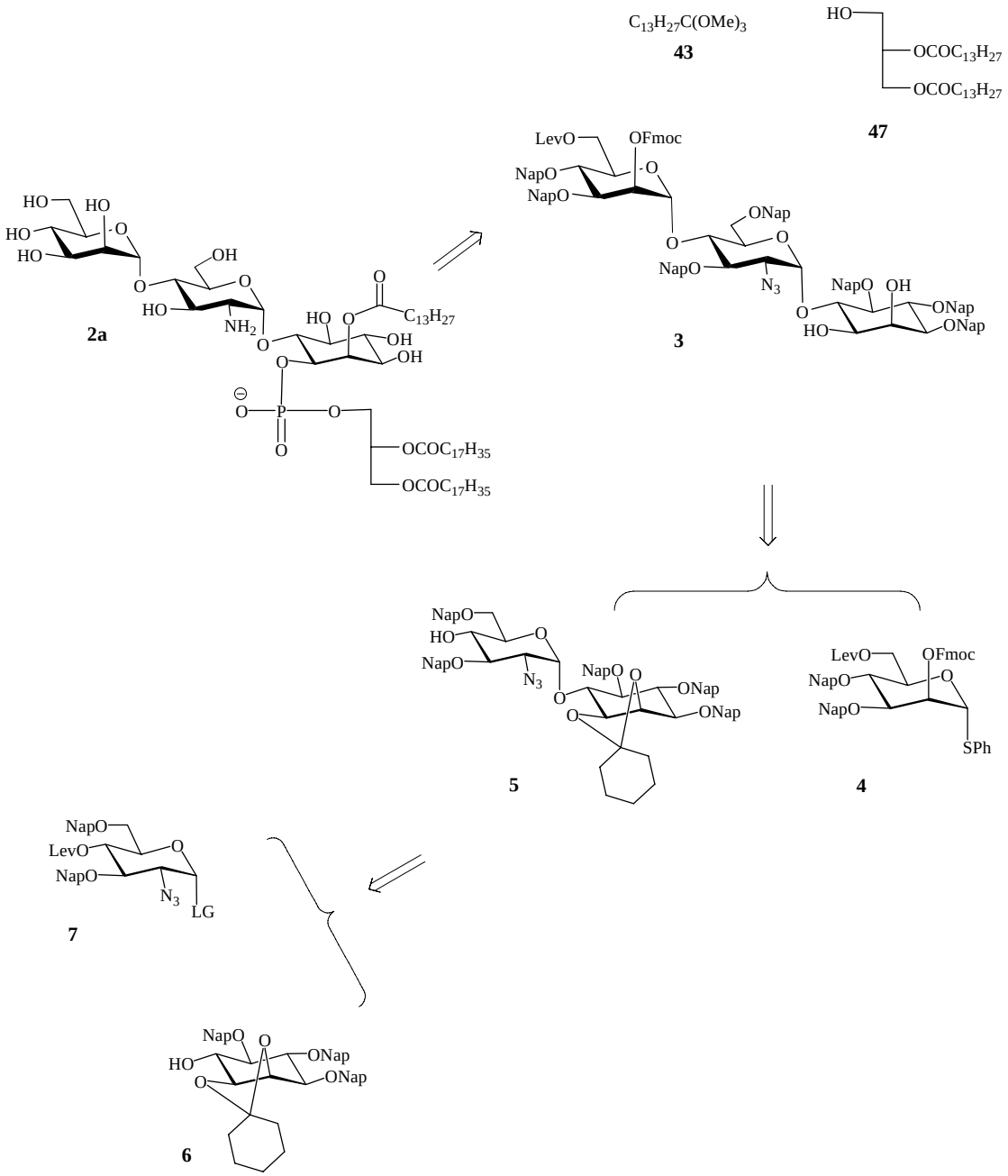
Figure 1. Target molecules

As discussed in the introduction part, the two disaccharides bear the same inositol acylation ($COC_{13}H_{17}$). However, for the purpose of research, they carry different phosphate lipid group (saturated lipid in **1a** and unsaturated lipid in **1b**). The trisaccharide **2** shares the same lipid group with disaccharide 1a, and has an extra mannose moiety at its C-6 position.

The retrosynthetic analysis of the target molecules is outlined in Scheme **1**. Several protecting groups will be employed in this project like NAP, Lev and Fmoc (see the introduction part).

Taki the trisaccharide **2** as an example, fatty acid acylation and phosphorylation onto the trisaccharide intermediate **3** could generate the fully protected precursor. Intermediate **3** could be obtained by the glycosylation

between pseudodisaccharide acceptor **5** and mannosyl donor **4**, while **5** could be in turn achieved from conjugation between inositol **6** and a suitable glucosamine donor **7**.



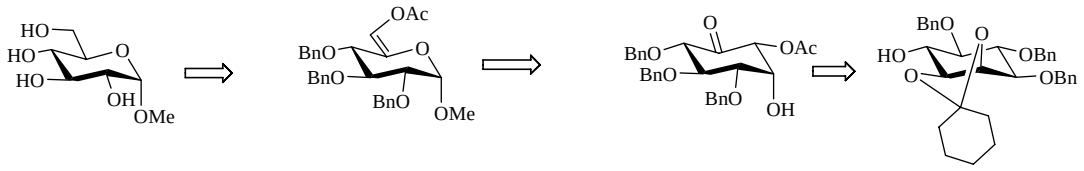
Scheme 1. Retrosynthetic approach

2. Building blocks synthesis

The first part of the work is the preparation of the needed building blocks, namely the inositol **6**, the gluocosamine donor **7** and the mannosyl donor **4**.

2.1 Synthesis of an optically pure myo-inositol derivative 6

Recently, Fraser-Reid group developed a novel methodology for the preparation of an optically pure myo-inositol acceptor starting from commercially available methyl glucoside [1] (Scheme **2**). The procedure relied on the discovery that enolesters could undergo the Ferrier reaction with excellent stereocontrol [2]. Among all the published GPI chemical synthesis work, this strategy represents the most efficient synthesis of myo-inositol. Therefore, our synthetic work towards this moiety was mainly carried out on the basis of this method.

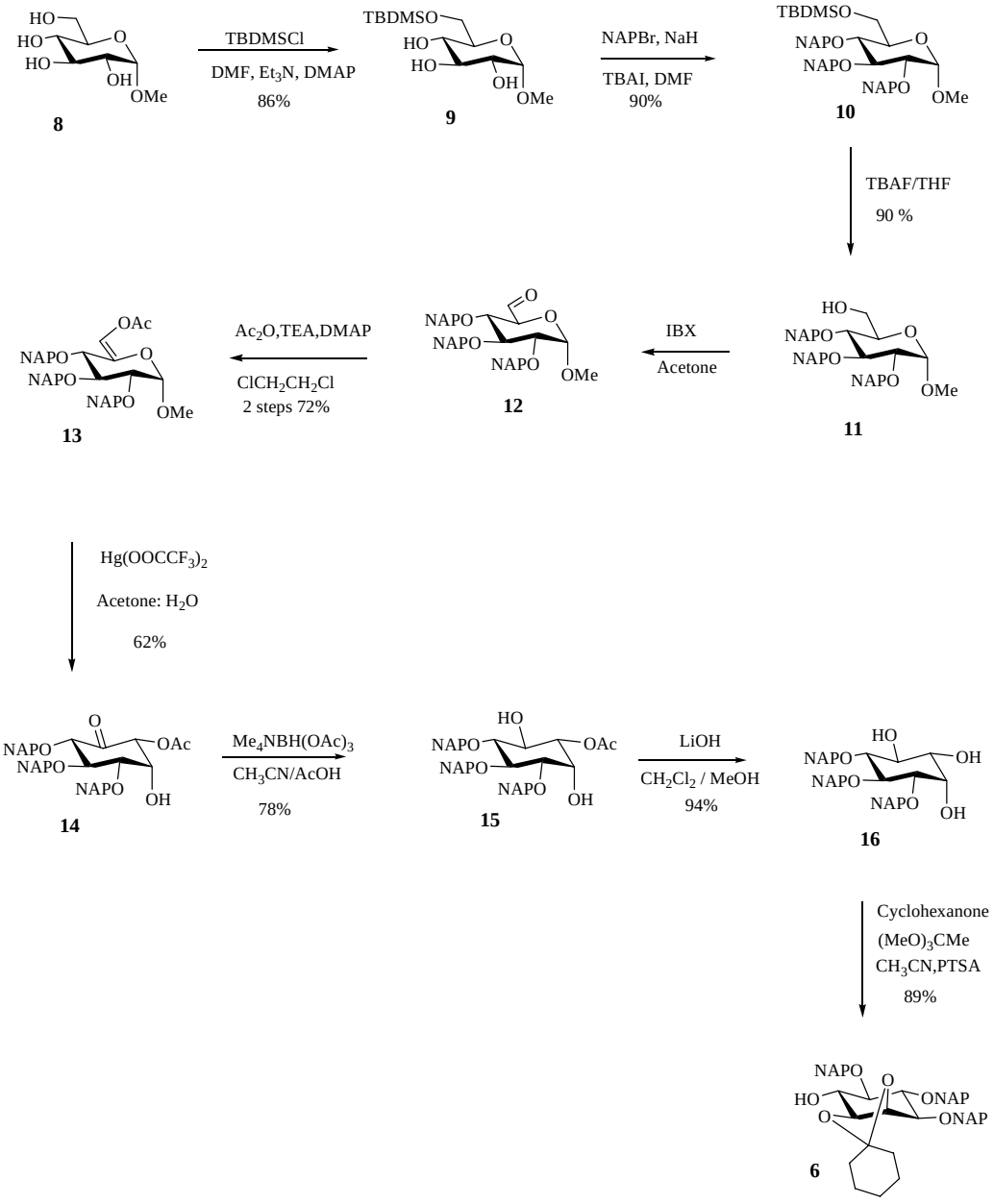


8 **b** **c** **d**

Scheme 2. Synthesis of myo-inositol derivative by Fraser-Reid group.

Thus, as shown in Scheme **3**, C-6 hydroxyl group in glucoside **8** was selectively protected in the presence of tert-butyldimethylsilyl chloride (TBDMSCl), TEA, DMAP and DMF, to afford compound **9** in 86% yield. Then the remaining C-2, C-3 and C-4 hydroxyl groups were all protected as NAP-ehters in condition of sodium hydride, 2-(naphthyl)methyl bromide (NAPBr), TBAI and DMF, to provide fully protected intermediate **10** in 90% yield. Removal of the C-6 silyl group using tetrabutylammonium fluoride delivered compound **11** in 90% yield. Then **11** was

converted into aldehyde **12** by using IBX oxidation, followed by enol acetylation to afford the enol acetate **13** in 2 steps in 72% yield. With higher oxidation efficiency and simpler work up (without column purification), the IBX oxidation proved to be more efficient than the previously published Moffat oxidation or Swern oxidation conditions [1].



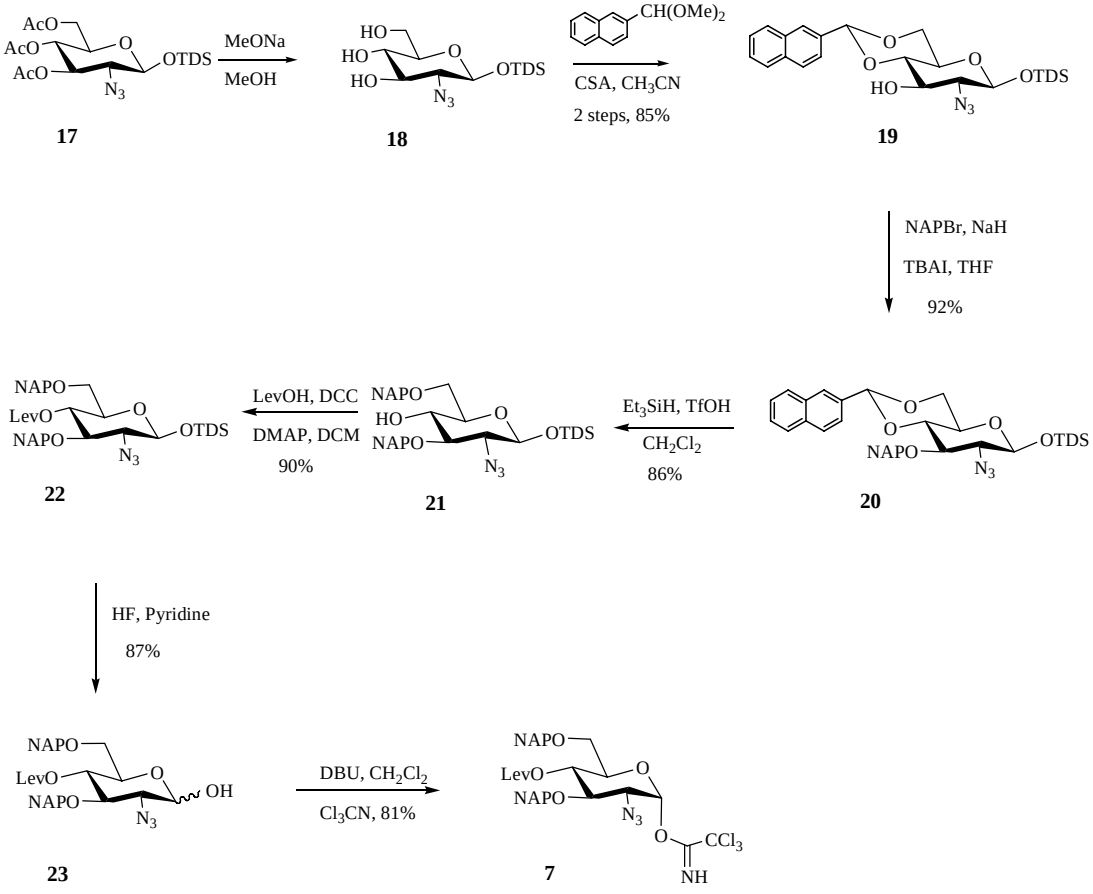
Scheme 3. Synthesis of **6**

Then, on treatment with mercury(II) trifluoroacetate, the enol acetate **13** underwent type II Ferrier rearrangement, and furnished cyclohexan-6-one **14** in 62% yield. The reduction of the ketone **14** into intermediate **15** was successfully accomplished by using tetramethylammonium triacetoxy borohydride in 78% yield. The stereoselectivity in this reduction is likely a result of the axial 2-position hydroxyl group coordinating to the reducing agent, leading to hydride delivery from the top face and therefore to the equatorial hydroxyl group at the 6-positon [3]. Removal of the acetyl protection of **15** afforded the triol **16** in 94% yield. Finally, 1,2-O-cyclohexylidenation of the triol **16** in the presence of cylcohexanone, catalytic amount PTSA and trimethyl orthoformate by using acetonitrile as a solvent furnished the required 6-OH D-myo-inositol derivative **6** in 89% of yield.

In short, synthesis of **6** from commercial starting material **8** was accomplished in nine steps in 20.2% yield in gram scale.

2.2 Synthesis of glucosamine building block 7 and 7a

For the synthesis of glucosamine building block **7**, as shown in Scheme **5**, first, treatment of commercially available **17** under typical Zemplén conditions by 1M solution of sodium methoxide in methanol afforded de-acetylated compound **18**. The methyl naphtyl acetal was then used to protect C-4 and C-6 hydroxyls by using 2-(dimethoxymethyl)naphthalene and a catalytic amount of Camphorsulfonic acid (CSA), to provide **19** in two steps in 85% yield. Alkylation of C-3 hydroxyl by using sodium hydride, NAPBr, TBAI and THF led to compound **20** in 92% yield. After this, the methyl naphtyl acetal was selectively opened in the presence of triethylsilane and TfOH to reveal the C-4 hydroxyl group, providing **21** in 86% yield.



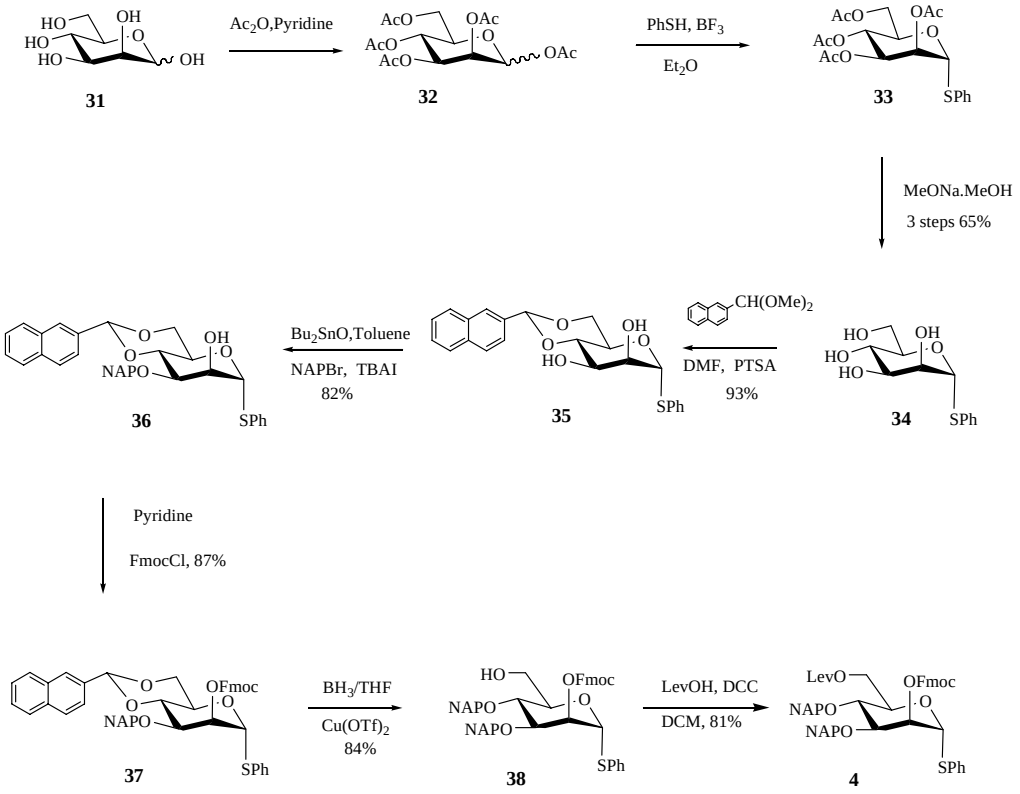
Scheme 5. Synthesis of glucosamine building block

Installation of a Lev group at the C-4 hydroxyl on intermediate **21** was accomplished with levulinic acid, dicyclohexylcarbodiimide (DCC) and DMAP, and generated **22** in yield of 90%. The Lev group will allow to delivery of the C-4 hydroxyl for the glycosylation with the mannosyl moiety in the synthesis of the trisaccharide. Then the anomeric silyl group of **22** was removed by using hydrogen fluoride in pyridine, to provide isomer **23** in 87% yield. Eventually, **23** was turned into glycosyl trichloroacetimidate **7** (81%) with trichloroacetonitrile, DBU in DCM.

Briefly, the preparation of building block 7 was achieved by seven steps in 42.6% yield in multigram scale.

2.3 Synthesis of mannose building block 4

Preparation of mannosyl donor **4** started with commercially available D-mannose **31**. After fully acetylation in pyridine and acetic anhydride; installation of the thiophenyl moiety at the anomeric position was achieved with thiophenol and boron trifluoride in ether; After Zemplén deacetylation with sodium methoxide and methanol, **34** was obtained in three steps in 65% yield [4]. C-4 and C-6 hydroxyls of compound **34** were then protected by 2-(naphthyl) methylene acetal with 2-(dimethoxymethyl)naphthalene and CSA in DMF, affording **35** in 93% yield. Then the C-3 hydroxyl was selectively alkylated by NAP ether applying a Tin-mediated method (Bu2SnO, NAPBr and TBAI) [5], leading intermediate **36** in 82% yield.



Scheme 6. Synthesis of mannose building block

Subsequently, C-2 hydroxy group of **36** was protected as Fmoc ether in pyridine with FmocCl (87%), to deliver product **37**. Then the methyl naphtyl acetal of **37** was selectively opened by using BH_3/THF solution and a catalytic amount of copper(II) triflate to reveal the C-6 hydroxy group [6], affording **38** in 86% yield. Then, C-6 hydroxyl of **38** was protected by Lev group with levulinic acid, DCC and DMAP, giving compound **4** in 81% yield.(Scheme **6**)

Thus, synthesis of mannosyl building block **4** was carried out in eight steps in 29.3% overall yield.

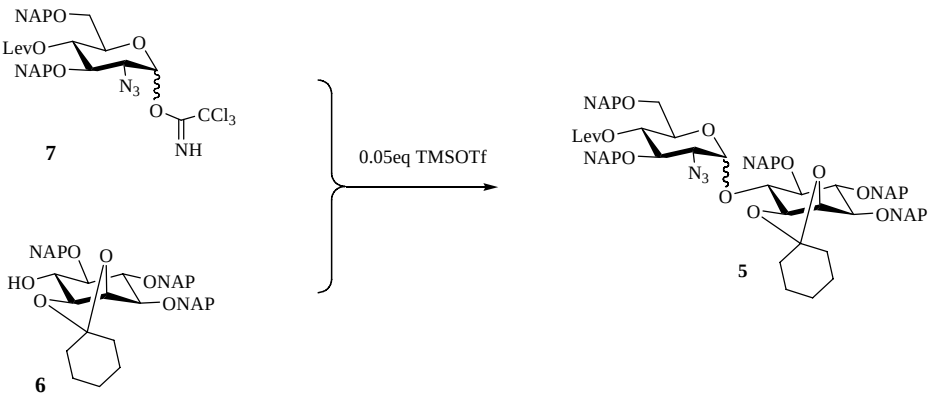
3. Building blocks assembling

With all the needed building blocks in hand, the next stage of the work is the oligosaccharides (disaccharide and trisaccharide) assembly. This part of the work included both the inositol **6** glycosylation with glucosamine donor **7** to construct disaccharide **5** and the mannose glycosylation reaction between disaccharide acceptor **40** and mannosyl donor **4** to achieve trisaccharide **41**.

3.1 Inositol glycosylation

3.1 Using glysosyl trichloroactimidate donor

For the synthesis of pseudodisaccharide precusor **5**, TMSOTf-promoted glycosylation between acceptor **6** and glycosyl trichloroacetimidate donor **7** was expected to produce mainly the α-anomer product [7]. Therefore, the reaction was carried out under typical glycosylation condition by using catalytic amount of TMSOTf (Scheme **7**).



Scheme 7. Inositol glycosylation using glysosyl trichloroactimidate donor

However, the result of the inositol glycosylation proved to be not satisfactory. Both the yield of reaction and the stereoselectivity of glycosylated product were quite low (*table 1*) . Using different solvent system and temperature did not help to improve the reaction.(Table 1) The main reason for this result is the relatively low reactivity of the C-6 hydroxyl of inositol [8]. Besides, the presence of C-2 azido group on glycosyl donor 7 increases the difficulty for glycosylation. Although there are many reports dealing with the inositol glycosylation, the use of traditional glycosylation methods to afford inositol pseudodisaccharide still seems to be quite difficult.[9]

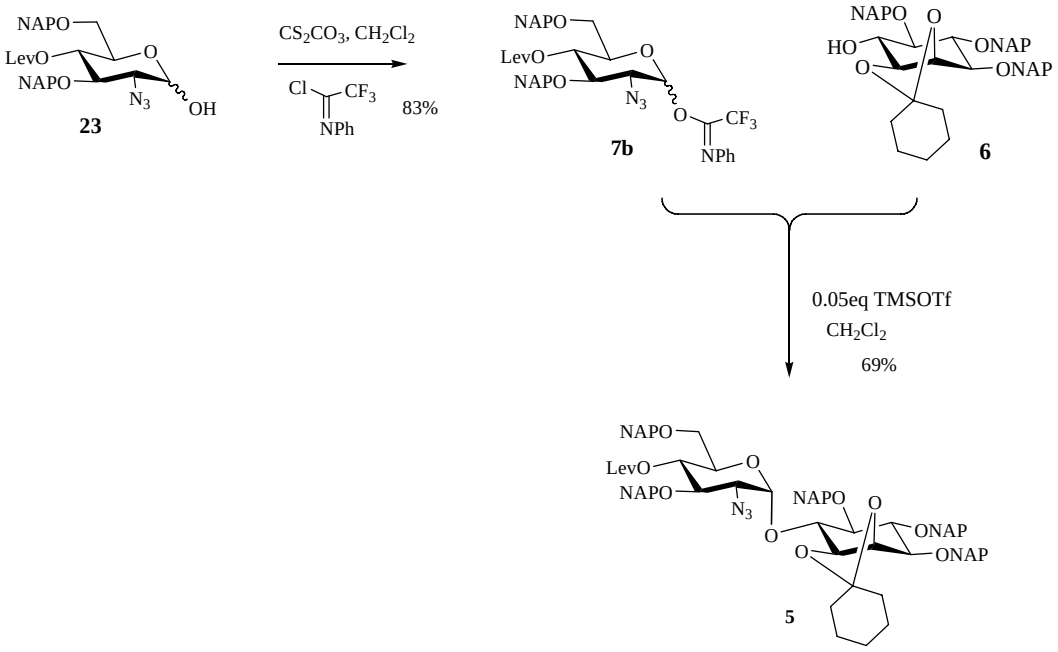
Entry	Solvent	Temp	Time	Yield	α : β
1	Et_20	-20 ℃	12 h	25%	1.5:1
1	Toluene	-40 ℃ to rt	12h	trace	
1	THF	-40 ℃ to rt	6 h	trace	
1	CH_2Cl_2	-40 ℃ to rt	6 h	30%	1:1

Table 1 Conditions for Inositol glycosylation using glysosyl trichloroactimidate donor

3.2 using glycosyl (N-phenyl)trifluoroacetimidates donor

The recently published reports which relied on glycosyl (N-phenyl)trifluoroacetimidates as glycosyl donors to construct efficiently α-glucosamine products have drawn our attention [10]. Encouraged by these results, we therefore decided to test the glycosyl (N-phenyl)trifluoroacetimidate donor onto our inositol glycosylation.

Thus, as shown in Scheme **8**, hemiacetal **23** was transformed to donor **7b** with cesium carbonate and 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride in DCM in 83% yield.



Scheme 8. Inositol glycosylation using glycosyl (N-phenyl)trifluoroacetimidates donor

Surprisingly, the glycosylation between **7b** and **6**, after several attemps (Table **2**), produced pseudodisaccharide precusor **5** in 69% yield with exclusive α-configuration at the newly formed glycosylation bond. This result, to the best

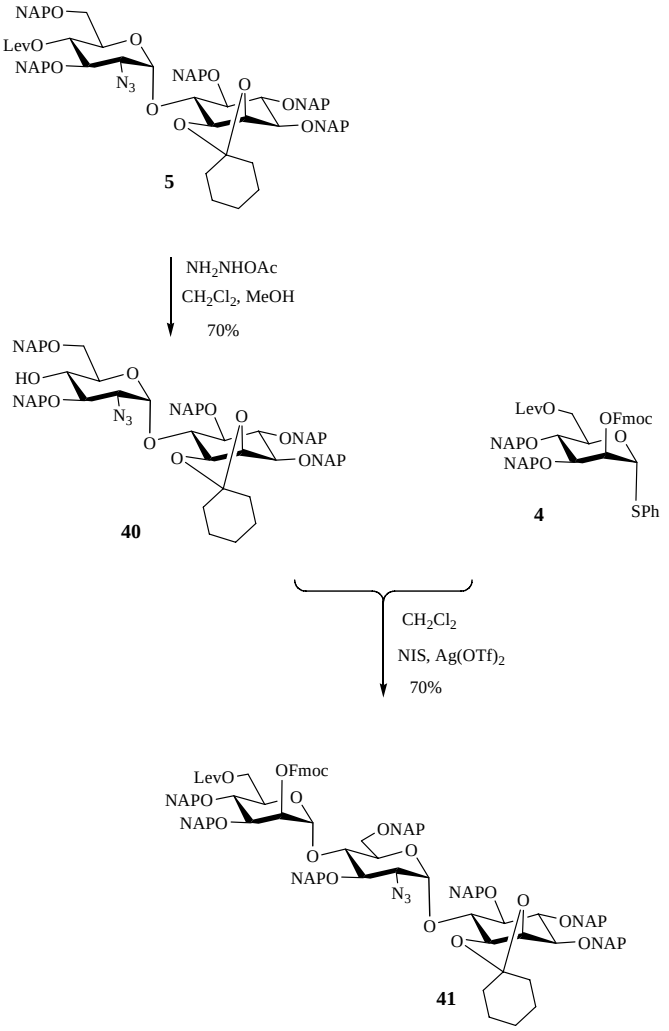
of our knowledge, represents the highest yield and best stereo-control ever described in GPI's inositol glycosylation. Although the mechanism has still not been clarified yet, this method seems a reliable strategy for the inositol glycosylation, and the reaction could be scaled up to five hundreds milligram scale with stable yield.

Entry	Solvent	Temp	Time	7a : 6	yield
1	CH_2Cl_2	-40 ℃	2 h	1.5 : 1	52%
1	CH_2Cl_2	-20 ℃	2 h	2 : 1	61%
1	CH_2Cl_2	0 ℃	1 h	1.2 : 1	64%
1	CH_2Cl_2	-10 ℃	1 h	1.5 : 1	69%

Table 2 Conditions for Inositol glycosylation using glycosyl (N-phenyl)trifluoroacetimidate donor

3.2 Mannose Installation

After the synthesis of pseudodisaccharide **5**, the next stage work is to assemble the α- mannose moiety onto C-4 hydroxyl group to establish trisaccharide. Thus, as shown in Scheme **9**, C-4 O-Lev group of disaccharide **5** was first removed by using hydrazine actetate, delivered **40** in 70% yield. Then the glycosylation between thiophenyl mannosyl donor **4** and **40** was carried out in the in DCM with NIS/silver triflate as promoter system to provide trisaccharide **41** in 70% yield. As C-2 fmoc of donor **4** performed neighboring group participating effect during the glycosylation, exclusively α-product was generated.



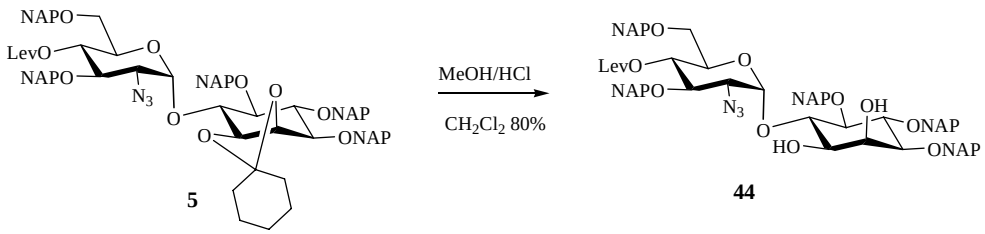
Scheme 9. Synthesis of trisaccharide

4. Lipid Introduction (disaccharide)

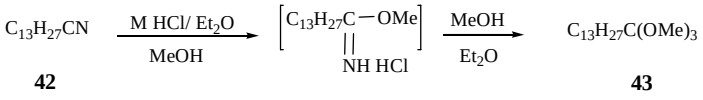
After the successful synthesis of the disaccharide and trisaccharide precursors, the next stage of the work was the lipid introduction. This part of the work included the selective acylation of inositol's C-2 axial hydroxyl and the phosphorylation of inositol's C-1 equatorial hydroxyl.

4.1 Selective Acylation

The selective acylation started with disaccharide **5**. First, cyclohexylidene moiety of **5** was removed using 0.5 M Hydogen chloride in methanol to give diol **44** in 80% yield. (Scheme **10**)



5 **44**

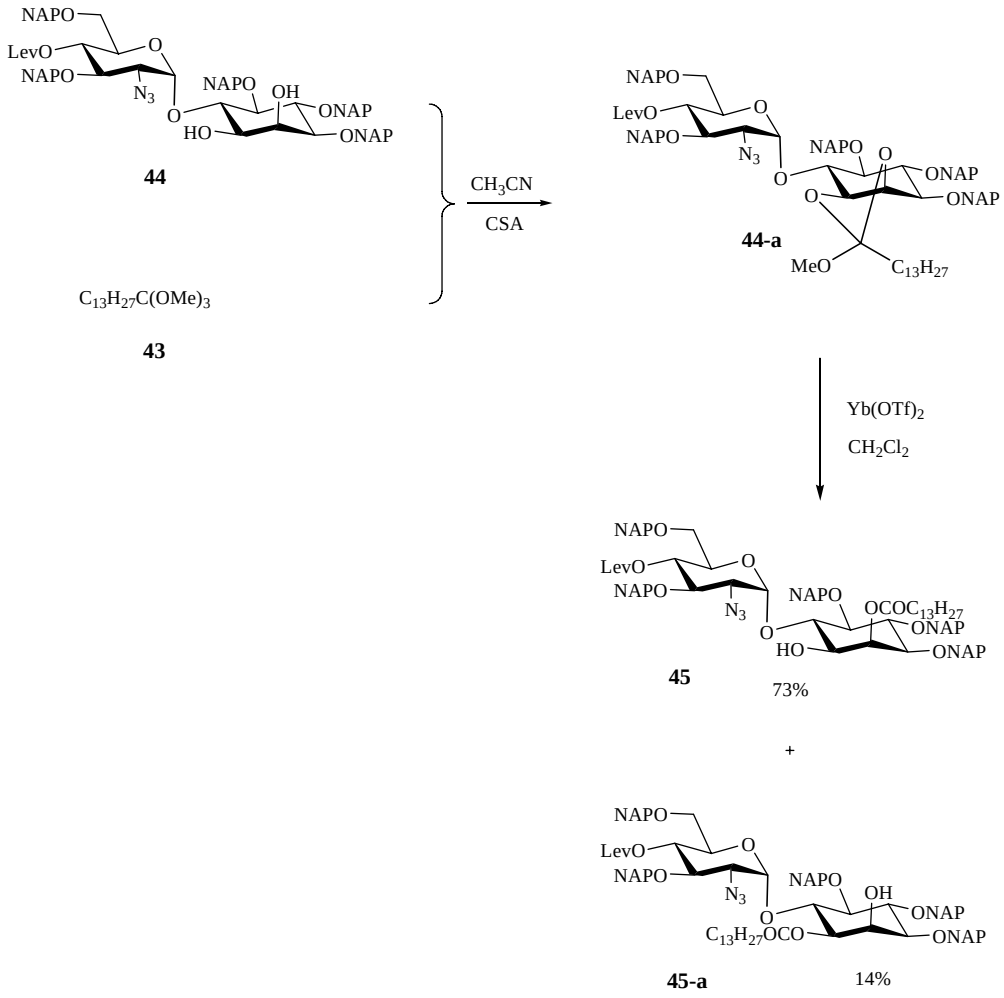


42 **43**

Scheme 10. Synthesis of diol **44** and trimethyl orthoester **43**

Studies by Frasier-Reid and coworker have shown that the axial C-2 position of the resulting inositol diol can be selectively acylated by treatment with alkyl trimethyl orthoester to generate a cyclic orthoester intermediate, which upon treatment with Ytterbium(III) triflate will regioselectively open to the axial C-2 position to give the C-2 acylated compound [11]. Thus, following their strategy, freshly prepared trimethyl orthopalmitate **43** [12] was mixed with diol **44** in acetonitrile in 40 ℃ with a catalytic amount of CSA to generate the

cyclic orthoester. Then, after simple work up (quenched with triethylamine and solvent evaporation), and treatment with 0.5 equiv of Ytterbium(III) triflate in DCM, the desired C-2 acylated compound **45** was obtained as a main product in 73% yield with traces C-1 acetylated byproduct (Scheme **11**).

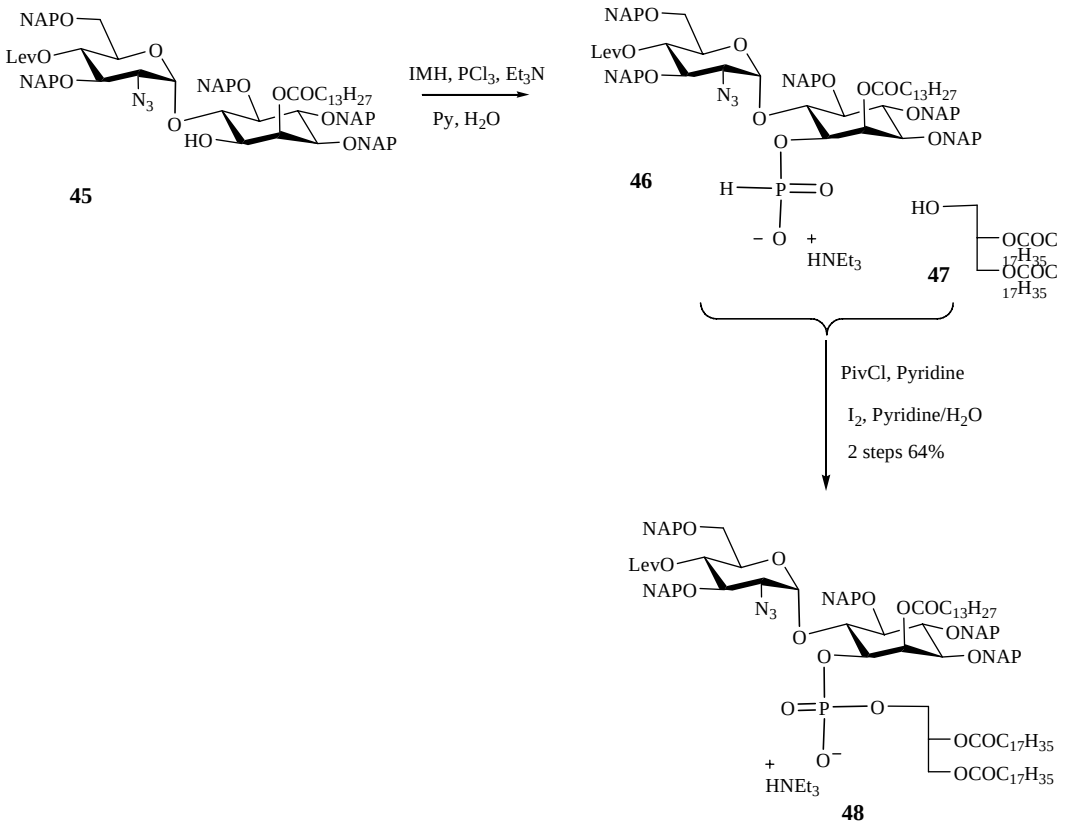


Scheme 11. Acetylation of Inostol

4.2 Phosphorylation (Lipid)

After the selective acylation of C-2, the last functionalizeation step is the installation of the phospholipid group at C-1 position. According to our previous experiences in the synthesis of phosphodiesters, we chose to rely on the H-phosphonate coupling strategy to achieve the C-1 phosphorylation.

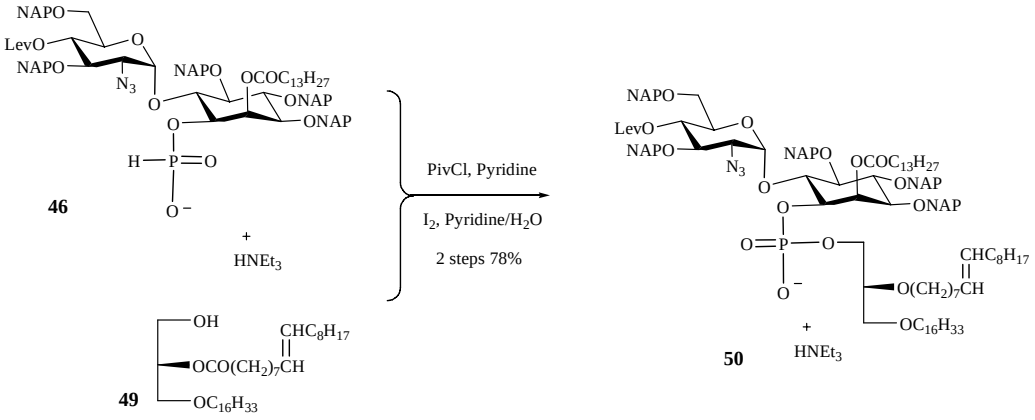
Therefore, for preparing the glycosyl H-phosphonate compound, a solution of **45** in acetonitrile was added dropwise to the phosphorus trichloride- imidazole - triethylamine mixture reagent to generate the H-phosphonate intermediate. After work up and purification by flash chromatography column, the sugar H-phosphonate was recovered as its triethylammonium salt **46**. [13]



Scheme 12. Synthesis of 48

Then, freshly prepared H-phosphonate **46** was coupled with alcohol **47** under typical reaction condition (using pivaloyl chloride as promoter, followed by *in situ* oxidation), providing protected disaccharide precursor **48** with saturated lipid chain over two steps in 64% yield. (Scheme **12**)

To prepare the protected precursor **50** containing unsaturated lipid chain, H-phosphonate **46** was coupled with alcohol **49**, which was synthesized as described in the literature [14]. After condensation and *in situ* oxidation, compound **50** was obtained in two steps in 78% yield over two steps.



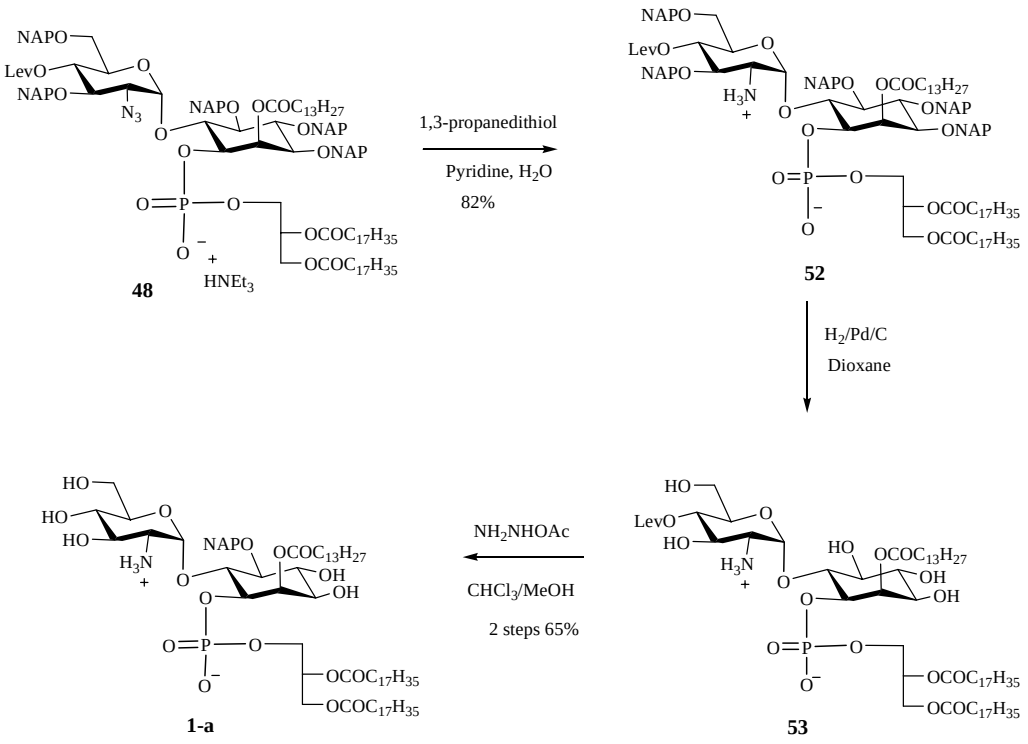
Scheme 12. Synthesis of **50**

5. Global Deprotection

The final part of the work is the removal of all the protecting groups, which involved azide-amine conversion, NAP ether removal and O-Lev and O-Fmoc cleavage.

5.1 Synthesis of 1-a

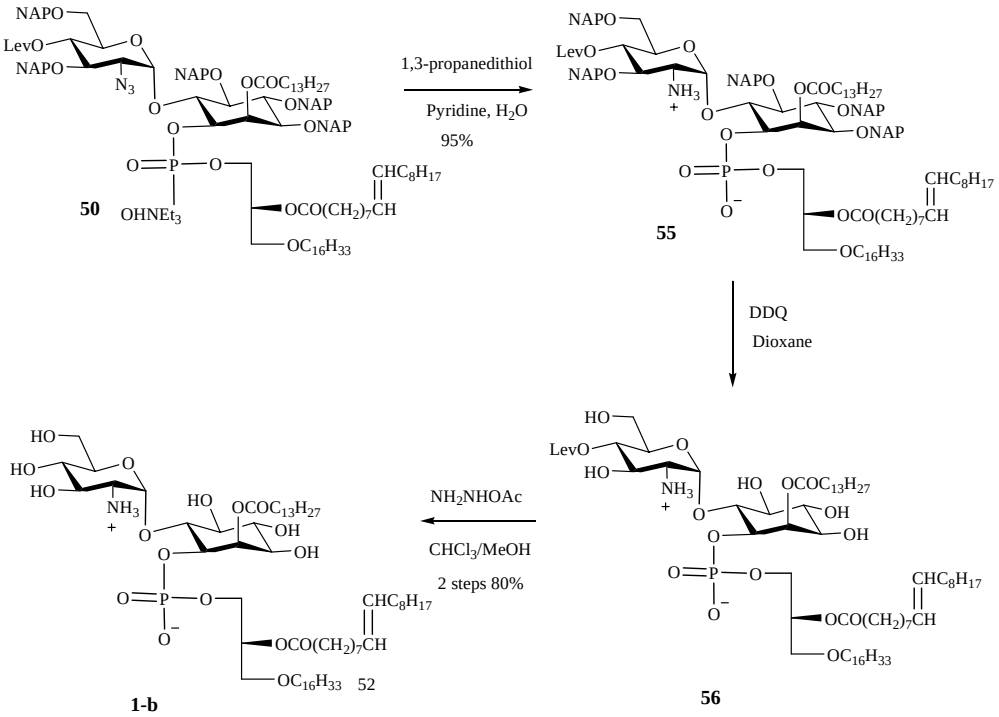
For the synthesis of 1-a, first, the azido group of **48** was transformed into amine by using 1,3-propanedithiol, pyridine and water in 82% yield, leading to **52**. Traditional Staudinger reduction and Zinc-mediated reduction only delivered the amine product in very low yield (<40%). Thereafter, the remaining NAP ether groups were cleaved by hydrogenolysis over 10% palladium on carbon in dioxane to generate **53** and the remaining O-Lev group of **53** was finally removed in the presence of hydrazine acetate in chloroform and methanol, affording **1-a** in 65% yield in two steps. (Scheme **13**)



Scheme 13. Synthesis of 1-a (Target molecular contains saturated lipid chain)

5.2 Synthesis of 1-b

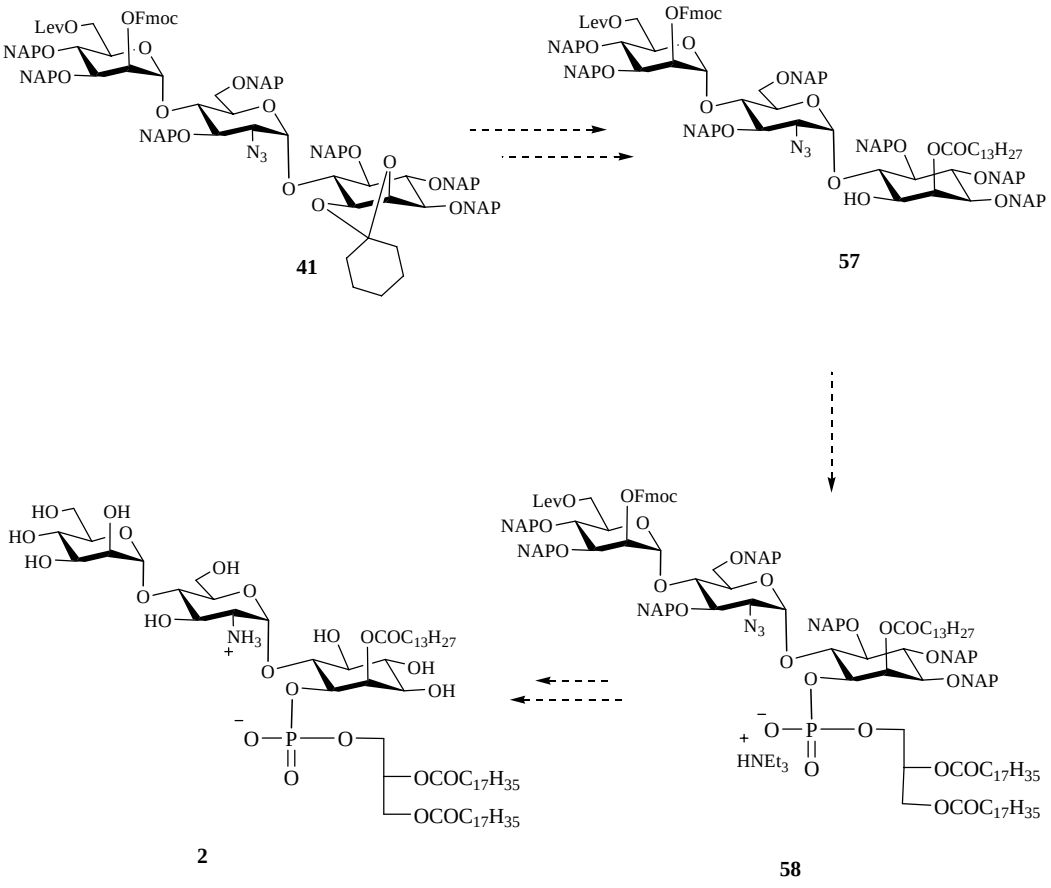
For disaccharide **1-b**, the azide reduction under the same condition described above provided **55** in 95% yield. As the precursor **55** contained unsaturated lipid group, the removal of NAP ether groups was carried out using DDQ oxidation, which did not influence the lipid chain [15]. The purification of the crude product resulting from DDQ oxidation relied on preparative TLC to remove most of the DDQ byproduct, followed by C18 reverse phase column, generating **56**. After the cleavage of the Lev group in **56** with hydrazine acetate, the target molecule **1-b** was obtained in 80% over two stpes. (Scheme **14**)



*Scheme **14**.* Synthesis of 1-b(Target molecular contains unsaturated lipid chain)

6. Synthesis of 2

The synthesis of trisaccharide **2** is still on the course, as outlined un Scheme 15, the selective introduction of the fatty acid at C-2 position on inositol moiety of trisaccharide **41** will give intermediate **57**; installation of phospholipid at C-1 position will generate protected precursor **58**. After the global deprotection steps (azide reduction, hydrogenation and Fmoc, Lev cleavage), the target molecule **2** will be obtained.



Scheme 15. Synthesis of **2** (Trisaccharide target molecular)

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CONCLUSION

In summary, the design and synthesis of GPI anchors of plasmodium-falciparum have been described.

The target molecules **1a** and **1b** and the protected trisaccharide precursor **41** were synthesized.

A convergent approach for preparing GPI molecules was established during the synthesis course. Several difficulties like selectively α-glycosylation on inositol moiety, selectively fatty acid acetylation at C-2 position of inositol and phosphonate lipid introduction have been addressed during the synthesis work.

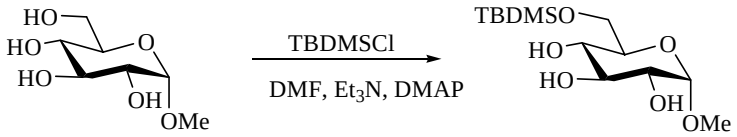
NAP ether was first employed as permanent protecting group as GPI synthesis, and was proved to be practical in synthesis work. Removal of NAP ether could be achieved by DDQ oxidation which does not affect the unsaturated phosphonate lipid moiety.

Synthesis of trisaccharide **2** and more advanced oligosaccharides will be carried out in the future following the similar strategies.

EXPERIMENTAL PART

General: Reagents were obtained from commercial sources and used as purchased. Dichloromethane was freshly distilled using standard procedures. Other organic solvents were purchased anhydrous and used without further purification. Unless otherwise noted, all reactions were carried out at room temperature in oven-dried glassware with magnetic stirring. Molecular sieves were flame-dried under high vacuum prior to use. Organic solutions were concentrated under diminished pressure with bath temperatures < 40 ℃. Flash column chromatography was carried out on silica gel G60 (Silicycle, 60-200 μm, 60Å). Thin-layer chromatography was carried out on Silica gel 60 F254 (EMD Chemicals Inc.) with detection by UV absorption (254 nm) were applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at 150℃ or by spraying with a solution of $(NH_4)6Mo7O_{24}.H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at 150℃. ^{1}H, ^{13}C NMR and ^{31}P spectra were recorded on a Varian Inova-400 (400/100 MHz) or a Varian Inova-500 (500/125 MHz), spectrometer equipped with Sun work stations. Multiplicities are quoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). Spectra were assigned using COSY, DEPT, and HSQC experiments. Mass spectra were recorded on an Applied Biosystems 4800 MALDI-TOF proteomics analyzer. The matrix used was 2,5-dihydroxybenzoic acid (DHB) as the internal standard.

6-O-tert-Butyldimethylsilyl-methyl-D-glucopyranoside, 9



8 **9**

Synthetic Procedure:

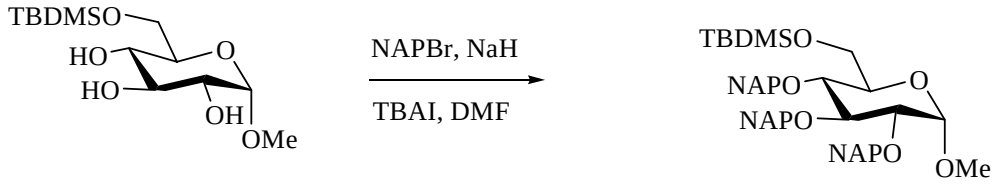
To a stirred solution of methyl-glucopyranoside **8** (10 g, 51 mmol), triethylamine (25 mL, 180 mmol), and DMAP (330 mg, 2.7 mmol) in DMF (100 mL) at 0 ℃ was added TBDMSCl (8.5 g, 56 mmol). The reaction was then allowed to proceed at room temperature for 12 h. Upon reaction completion, the mixture was poured over ice-water (100 mL) and extracted with CH_2Cl_2 (250 mL), and the organic mixture was washed with saturated NH_4Cl solution and brine (150 mL, each). The organic extract was then dried over Na_2SO_4 and stripped of solvent in vacuum, and the concentrated residue was purified by flash column chromatography (10% MeOH/ CH_2Cl_2) to afford **9** (14.98 g, 86%) as a white solid

Product characterization 8

Formula	C13H2806Si
Molecular Wright	308.44
TLC conditions	Rf: 0.35, CH_2Cl_2 : MeOH = 9 : 1

The spectroscopic data were in agreement with the reported data [1]

6-O-tert-Butyldimethylsilyl-2,3,4-tri-O-naphthylmethyl-methyl-D-glucopyranoside, 10



9 **10**

Synthetic Procedure

To a stirred solution of **9** (1.9 g, 6.15 mmol) in DMF (23 mL), NAPBr (5.45 g, 24.9 mmol) was added at room temperature. Then NaH (60%, 1 g, 24.96 mmol) and TBAI (227 mg, 0.6 mmol) were slowly added at 0℃. The reaction was then allowed to stir at room temperature for 2 h. Upon reaction completion, the mixture was quenched with methanol (5 mL) and diluted with ethyl acetate (30 mL). The organic mixture was washed with saturated NH_4Cl solution (20 mL) and brine (10 mL). The organic phases were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (5-10% EtOAc/Hexane), providing **10** (4.06 g, 90 %) as a clear oil.

Product characterization 10

Formula	C46H5206Si
Molecular Wright	728.99
TLC conditions	Rf: 0.5, Hexane : EtOAc = 9 : 1

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.87-7.23 (m,21 H, H_{Ar}); 5.21-4.80 (m, 6 H, 3 CH_2NAP); 4.58 (d, J = 12 Hz, 1 H, H-1); 4.20-4.02 (m, 1 H, H-2); 3.80-3.75 (m, 2 H, H-4, H-6); 3.70-3.54 (m, 3 H, H-6', H-3, H-5); 3.40 (m, 3 H, OMe);0.92 (s,9 H, 3 CH_3Si); 0.08 (s, 3 H, CH3Si); 0.07 (s, 3 H, CH3Si);

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 751.9, found: 751.4

2,3,4-tri-O-naphthylmethyl-methyl-D-glucopyranoside,11

10 **11**

Synthetic Procedure:

To a stirred solution of **10** (3.98 g, 5.46 mmol) in THF (50 mL), 1 M TBAF (6 mL, 6 mmol) was added at 0℃, and the reaction was then allowed to stir at room temperature for two hours. Upon reaction completion, CH_2Cl_2 (100 mL) was added, and washed with saturated NH_4Cl solution (40 mL). The organic phases were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (33% EtOAc/Hexane), providing **11** (3.01 g, 90 %) as white solid.

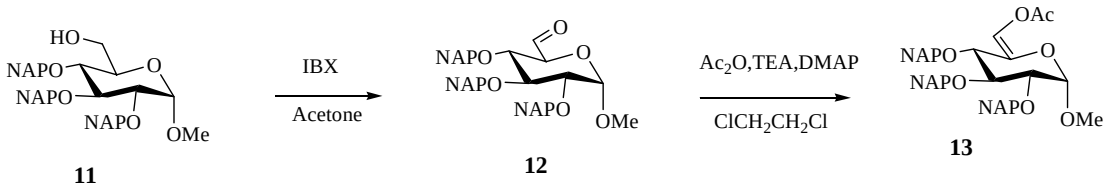
Product characterization 11

Formula	C40H3806
Molecular Wright	614.73
TLC conditions	Rf: 0.3, Hexane : EtOAc = 2 : 1

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85-7.21 (m,21 H, H_{Ar}); 5.22-4.75 (m, 6 H, 3 CH_2NAP); 4.60 (d, J = 12 Hz, 1 H, H-1); 4.20-4.02 (m, 1 H, H-2); 3.80-3.45 (m, 5 H, H-4, H-6, H-6', H-3, H-5); 3.40 (m, 3 H, OMe);

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 637.7, found: 637.6

Methyl 6-O-acetyl-2,3,4-tri-O-naphthylmethyl-a-D-glucohex-5-enopyranoside,13



11 **12** **13**

Synthetic Procedure:

From 11 to 12:

11 (3.5 g, 5.7 mmol) was dissolved in dry acetone (150 mL), freshly prepared IBX (4.9 g, 17 mmol) was added to the solution. The reaction mixture was allowed to stir at 50℃ for 12 hours. Upon reaction completion, the suspension was cool down to room temperature and filtrated through a Celite pad. The Celite pad was washed by CH_2Cl_2 (200 mL). The resulting solution was then concentrated and covapored with toluene for three times (50 mL * 3), affording crude of 12 4.2 g as a yellow oil.

From 12 to 13

To a solution of 12 (4.2 g) in $ClCH_2CH_2Cl$ (100 mL), triethylamine (2.8 mL, 20mmol), Acetic anhydride (1.9 mL, 20 mmol) were added, and the reaction mixture was stirred at 80℃ for 5 hours. After TLC analysis showed consumption of starting material, the mixture was cooled to room temperature and concentrated. The residue was purified by flash chromatography (20% EtOAc/Hexane), providing **11** (2.68 g, 72 %) as white wax.

Product characterization 12

Formula	C40H3606
Molecular Wright	612.71
TLC conditions	Rf: 0.25, Hexane : EtOAc = 3 : 1

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 635.7, found: 634.8

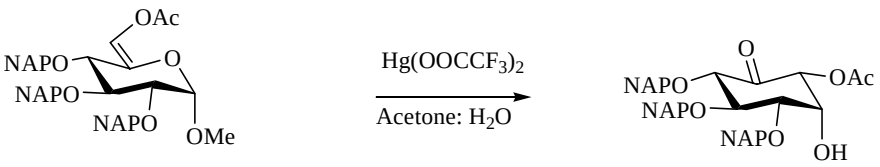
Product characterization 13

Formula	C42H3807
Molecular Wright	654.75
TLC conditions	Rf: 0.25, Hexane : EtOAc = 5 : 1

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85-7.21 (m,22 H, H_{Ar}, RC=CHOAc); 5.22-4.75 (m, 7 H, 3 CH_2NAP, H-1); 4.60 (d, J = 12 Hz, 1 H, H-1); 4.20-4.02 (m, 2 H, H-2, H-3); 3.80-3.45 (m, 1 H, H-4); 3.40 (m, 3 H, OMe); 2.27, (s, 3 H, OAc)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 677.7, found: 677.3

2-hydroxy-3,4,5-tri-O-naphthylmethyl-6-oxocyclohexyl acetate 8



13 **14**

Synthetic precedure:

To a solution of **13** (1.9 g, 2.907 mmol) in acetone (80 mL) and water (20 mL) was added mercury(II) trifluoroacetate (1.41g, 3.49 mmol). The mixture was sttired at room temperature for 1 hours. After TLC analysis indicating consumption of the **13**, the suspension was diluted with brine (50 mL) and stiired for another 1 hour. Then CH_2Cl_2 was added to dilute the mixture and the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (33% EtOAc/Hexane), providing **14** (1.27 g, 62 %) as white wax.

Product characterization 14

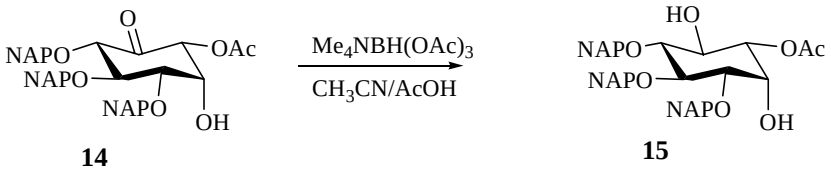
Formula	C41H3607
Molecular Wright	640.72
TLC conditions	Rf: 0.25, Hexane : EtOAc = 5 : 1

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.85-7.21 (m,21 H, H_{Ar}); 5.25 (m, 1 H, H-1), 5.20-4.66 (m, 6 H, 3 CH_2NAP); 4.38 (brs,1 H, H-2); 4.32-4.18 (m, 2 H, H-4, H-5); 3.85 (m, 1 H, H-3);); 2.63 (brs, 1 H, OH); 2.27, (s, 3 H, OAc)

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 167.1 181.8 ((COCH3), *C*=O); 133.5-121.0 (C-Ph); 83.2. (C-4); 82.8 (C-5); 80.5 (C-3); 78.8 (CH2Ph); 75.4 (C-1); 74.4 (2*C*H_2Ph); 70.1 (C-2) 23.3 (OAc)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 663.7, found: 663.6

1-O-Acetyl-3,4,5-tri-O-naphthylmethyl-D-myo-inositol 15



14 **15**

Synthetic Procedure:

A mixture of acetic acid (12 mL) and acetonitrile (12 mL) was cooled to -40℃ and to this was added tetramethylammonium triacetoxyborohydride (3.07 g, 11.7 mmol).After 10 mins, a solution of 14 (1.5 g , 2.34 mmol) in CH_2Cl_2 (15 mL) and CH_3CN (15 mL) were added to the mixture, at -40℃. Then the reaction mixture was stiired at room temperature for 3 hours. After TLC analysis indicating consumption of the **14**, the reaction was quenched by the addition of aqueous saturated Rochelle's Salt solution (15 mL). Then the CH_2Cl_2 was added and washed with brine, the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (60% EtOAc/Hexane), providing **15** (1.17 g, 78 %) as white wax.

Product characterization 15

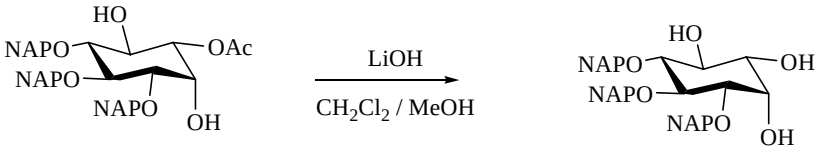
Formula	C41H3807
Molecular Wright	642.26
TLC conditions	Rf: 0.4, Hexane : EtOAc = 1 : 2

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82-7.17 (m,21 H, H_{Ar}); 5.23 (m, 1 H, H-1), 5.17-4.66 (m, 6 H, 3 CH_2NAP); 4.45-4.37 (m,1 H, H-2); 4.37-4.18 (m, 3 H, H-4, H-5, H-6); 3.85 (m, 1 H, H-3); 2.67 (brs, 1 H, OH); 2.27, (s, 3 H, OAc); 1.78 (brs,1 H OH)

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 167.1(COCH3); 133.3-121.3 (C-Ph); 83.4 (C-4); 82.5 (C-5); 81.5 (C-3); 79.2 (CH2Ph); 74.9 (C-1); 74.1 (2CH_2Ph); 70.6 (C-2), 69.3 (C-6); 23.3 (OAc)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 665.2, found: 665.8

3,4,5-tri-O-naphthylmethyl-D-myo-inositol, 16



15 **16**

Synthetic Procedure

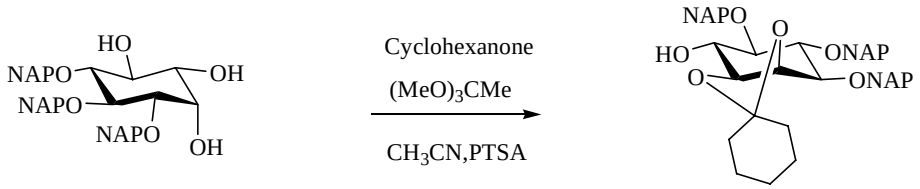
15 (1.15 g, 1.79 mmol) was dissolved in a mixture of CH_2Cl_2 (30 mL) and MeOH (30 mL). To this solution was added lithium hydroxide (42 mg, 1.76 mmol), and the mixture was stirred at room temperature for 1 hour, After TLC analysis indicating consumption of the starting material, the reaction was quenched by the addition of saturated NH_4Cl solution (5 mL). Then the reaction mixture was diluted by $CHCl_3$ (50 mL), and washed with brine, the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (5-10% MeOH/ CH_2Cl_2), providing **16** (1.01 g, 94 %) as white solid.

Product characterization 16

Formula	C39H3606
Molecular Wright	600.7
TLC conditions	Rf: 0.5, CH_2Cl_2 : MeOH = 19 : 1

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 623.7, found: 623.4

1,2-O-cylcohexylidine-3,4,5-tri-O-naphthylmethyl-D-myo-inositol, 16



16 **6**

Synthetic Procedure:

16 (2 g, 3.3 mmol), Trimethyl orthoformate (2 mL, 16.7 mmol) and PTSA (120 mg, 0.63 mmol) was disolved in CH_3CN and the reaction was stiired at room temperature for 10 mins. Then cyclohexanone (12 mL, 112 mmol) was added, and the mixture was stirred at room temperature for another 2 hours. After TLC anylysis showing consumption of the starting material, the reaction was quenched by the addition of saturated $NaHCO_3$ solution (50 mL). After extraction with CH_2Cl_2 (50 mL * 3), the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (20% EtOAc /Toluene), providing **6** (1.99 g, 89 %) as white solid.

Product characterization 6

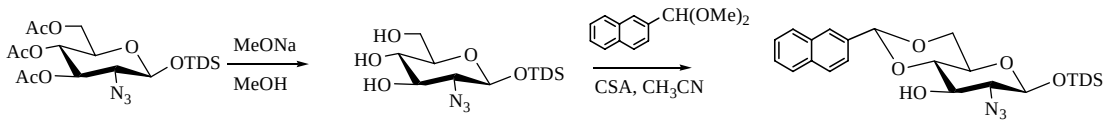
Formula	C45H4406
Molecular Wright	680.83
TLC conditions	Rf: 0.3, EtOAc : Toluene = 1 : 4

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80-7.05 (m,21 H, H_{Ar}); , 5.01-4.64 (m, 6 H, 3 CH_2NAP); 4.28-4.21 (m,1 H, H-2); 3.97-3.65 (m, 4 H, H-4, H-5, H-1, H-3); 3.29-3.23 (m, 1 H, H-6); 2.67 (brs, 1 H, C-6-OH);); 1.78-1.41 (m ,8 H, 4 CH2), 1.21 (m, CH2)

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 132.2-125.6 (C-Ph); 81.4 (C-6); 80.8 (C-4); 78.5 (C-5); 76.7 (C-3); 75.0 (2 CH2Ph); 74.7 (C-1); 74.4 (C-2), 74.0 ($\mathit{C}H_2$Ph); 39.3, 35.2, 31.1, 27.8, 24.7, 24.5 (6 CH2)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 703.3, found: 703.8

Thexyldimethylsilyl 2-azido-4,6-Naptylmethylene-β-D-glucopyranoside,19



17 **18** **19**

Synthetic procedure:

For 18

17 (1 g, 2.88 mmol) was dissolved in 1M MeONa/MeOH solution (50 mL) under nitrogen atmosphere, the mixture was stirred at room temperature for 2 h, then the reaction mixture was neutralized to PH 7 with IR-120 resin, (H^+ form, previously washed by MeOH), filtered, and concentrated, get a crude of **18** (740 mg) as colourless oil.

For 19:

Crude of **18** (740 mg) was dissolved in dry acetonirile (30 mL), then dimethoxymethyl naphthalene (600 mg, 2.97 mmol) was added to the solution, followed by CSA (40 mg, 0.2 mmol). The reaction mixture was stirred at room temperature for 12 hours. After TLC analysis showing the consumption of **18**, the reaction was quenched by adding saturated $NaHCO_3$ solution (30 mL). Then CH_2Cl_2 (30 mL * 2) was added for extraction. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (17% EtOAc /Hexane), providing **19** (1.31 g, 85 %) as white solid.

Product characterization 19

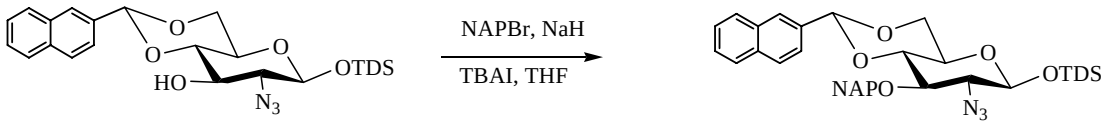
Formula	C25H35N3O5Si
Molecular Wright	485.65
TLC conditions	Rf: 0.3, EtOAc : Hexane = 1 : 5

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80-7.45 (m,7 H, H_{Ar}); 5.8 (s, 1 H, $H_{acetalic}$), 4.61-4.59 (d, 1 H, J = 9.6 Hz H-1); 4.34-4.31 (m,1 H, H-6a); 3.83-3.81 (m, 1 H, H-6b,); 3.58-3.53 (m, 2 H, H-4, H-3); 3.41-3.36 (m, 2 H, H-5, H-2); 1.72 (m ,1 H,CH_{thexyl}); 0.88 (s, 12 H, 4 $CH_{3thexyl}$); 0.09, 0.08 (2 s, 6 H, 2 CH_3Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 126.2-122.2 (C-Ph); 92.4 ($C_{acetalic}$); 89.8 (C-1); 75.5 (C-3); 65.4 (C-4); 61.2 (C-2);, 60.8 (C-6); 59.8 (C-5);) 20.4 ($2CH_{3thexyl}$); 18.7 ($2CH_3CH_{thexyl}$); -3.6, -3.5 (CH_3Si)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 508.8, found: 508.2

Thexyldimethylsilyl 2-azido-3-O-naphthylmethyl-4,6-Naptylmethylene-β-D-glucopyranoside, 20



19 **20**

Synthetic Procedure:

19 (2.3 g, 4.74 mmol) was dissolved in THF (150 mL). At 0℃, NAPBr (1570 mg , 7.1 mmol) was added to the mixture, followed by Sodium hydride (200 mg, 5 mmol) and TBAI (190 mg, 0.5 mmol). The reaction mixture was allowed to stir at room temperature for 2 hours. After TLC analysis showing the consumption of 19, the reaction was quenched by adding MeOH (20 mL) and saturated NH_4Cl sooltion (100 mL). CH_2Cl_2 (2 * 100 mL) was adding for extraction, and then the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (5% EtOAc/Hexane), providing **20** (2.72 g, 92 %) as colorless oil.

Product characterization 20

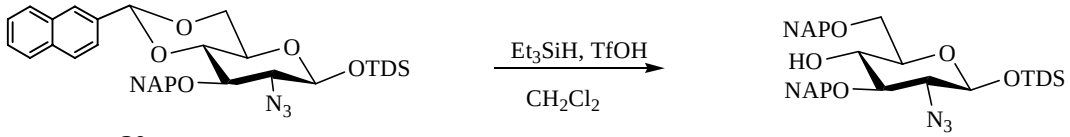
Formula	C36H43N3O5Si
Molecular Wright	625.83
TLC conditions	Rf: 0.4, EtOAc : Hexane = 1 : 9

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99-7.35 (m,14 H, H_{Ar}); 5.82 (s, 1 H, $H_{acetalic}$); 5.08 (m, 2 H, CH2Ph); 4.60-4.57 (d, 1 H, J = 10 Hz , H-1); 4.33-4.31 (m,1 H, H-6a); 3.79-3.77 (m, 2 H, H-6b, H-3); 3.72 (m, 1 H, H-4); 3.43 (m, 2 H, H-5, H-2); 1.74 (m ,1 H,CH_{thexyl}); 0.91 (s, 12 H, 4 $CH_{3thexyl}$); 0.09, 0.08 (2 s, 6 H, 2 CH_3Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 132.4-121.8 (C-Ph); 99.8 ($C_{acetalic}$); 95.8 (C-1); 85.3 (C-3); 78.4 (C-4); 71.8 (CH2Ph); 74.2 (C-2); 69.8 (C-6); 67.8 (C-5); 20.4 ($2CH_{3thexyl}$); 18.7 ($2CH_3CH_{thexyl}$); -3.6, -3.5 (CH_3Si)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 650.8, found: 650.3

Thexyldimethylsilyl 2-azido-3,6-O-di-naphthylmethyl-β-D-glucopyranoside, 21



20 **21**

Synthetic Procedure:

20 (1.2 g 1.92 mmol) was dissolved in CH_2Cl_2 (20 mL). Then the solution was cool down to -70℃, and triethylsilane (0.92 mL, 5.76 mmol) was slowly added to the reaction mixture, followed by TfOH (0.51 mL). The reaction mixture was then stirred at -40℃ for 30 minutes. The reaction was then cooled down to -70℃, and quenched by adding (10 mL), followed by triethylamine. The solvent was concentrated, and the resulting crude was purified by flash chromatography (20% EtOAc/Hexane), providing **21** (1.03 g, 86 %) as white solid.

Product characterization 21

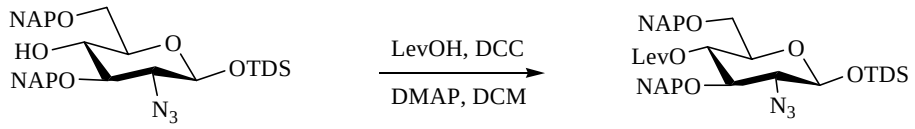
Formula	C36H45N3O5Si
Molecular Wright	627.85
TLC conditions	Rf: 0.25, EtOAc : Hexane = 1 : 4

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75-7.09 (m,14 H, H_{Ar}); 4.90-4.63 (m, 4 H, 2 CH2Ph); 4.54-4.51 (d, 1 H, J = 12 Hz); 4.33-4.31 (m,1 H, H-6a); 3.78-3.62 (m, 3 H, H-6a, H-6b, H-4); 3.42-3.25 (m, 3 H, H-5, H-2, H-3); 2.17 (s, 1 H, C-6-OH); 1.74 (m ,1 H,CH_{thexyl}); 0.90 (s, 12 H, 4 $CH_{3thexyl}$); 0.09, 0.08 (2 s, 6 H, 2 CH_3Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 131.0-124.8 (C-Ph); 100.3 (C-1); 86.2 (C-3); 78.4 (C-4); 74.7 (C-5); 73.2, 72.9 (CH2Ph); 72.3 (C-4); 71.6 (C-6); 69.8 (C-2); 23.2 ($2CH_{3thexyl}$); 18.4 ($2CH_3CH_{thexyl}$); -3.6, -3.5 (CH_3Si)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 650.8, found: 650.3

Thexyldimethylsilyl 2-azido-4- O-levulinoyl-3,6-O-di-naphthylmethyl-β-D-glucopyranoside, 22



21 **22**

Synthetic Procedure:

21 (1.1 g 1.75 mmol) was dissolved in CH_2Cl_2 (50 mL), LeVOH (305 mg, 2.66 mmol), DCC (630 mg, 3.12 mmol) and DMAP (25 mg, 0.35 mmol) were added. The reaction mixture was stirred at room temperature for 2 hours. The solvent was concentrated and the resulting crude was purified by flash chromatography (30% EtOAc /Hexane), providing **22** (1.15 g, 90 %) as colorless oil.

Product characterization 22

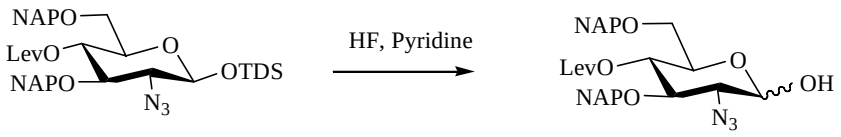
Formula	C41H51N3O7Si
Molecular Wright	725.95
TLC conditions	Rf: 0.3, EtOAc: Hexane = 1 : 2

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81-7.29 (m,14 H, H_{Ar}); 5.15-4.65 (m, 5 H, 2 CH2Ph, H-4); 4.56-4.53 (d, 1 H, J = 12 Hz); 3.78-3.63 (m,3 H, H-6a, H-6b, H-5); 3.40-3.22 (m, 2 H, H-3, H-2); 2.42-2.2 (m, 4 H, $CH2_{Lev}$); 1.98 (s, 2 H, OAc); 1.75 (m ,1 H,CH_{thexyl}); 0.92 (s, 12 H, 4 $CH_{3thexyl}$); 0.09, 0.08 (2 s, 6 H, 2 CH_3Si);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 166.7 (CO), 129.3-126.8 (C-Ph); 99.9 (C-1); 85.8 (C-3); 76.4, 75.8 (CH2Ph); 74.5 (C-5); 73.8 (C-4); 72.1 (C-6); 70.8 (C-2); 38.8 ($CH2_{LeV}$); 31.2 (OAc_{lev}); 28.7 ($CH2_{Lev}$); 24.5 ($2CH_{3thexyl}$); 18.3 ($2CH_3CH_{thexyl}$); -3.6, -3.5 (CH_3Si)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 748.9, found: 748.2

2-azido-4- O-levulinoyl-3,6-O-di-naphthylmethyl-D-glucopyranoside, 23



22 **23**

Synthetic procedure:

A solution of **22** (1.1 g, 1.52 mmol) in pyridine (10 mL) was slowly added in 40% HF/pyridine (20 mL) at -20 ℃, the mixture was allowed to stir at room temperature for 2-3 hours. After TLC analysis showing consumption of **22**, the reaction was quenched by saturated $NaHCO_3$ solution (50 mL). CH_2Cl_2 (30 mL *3) was used for extraction, and then the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (40% EtOAc /Hexane), providing **23** (770 mg, 87 %) as white solid.

Product characterization 23

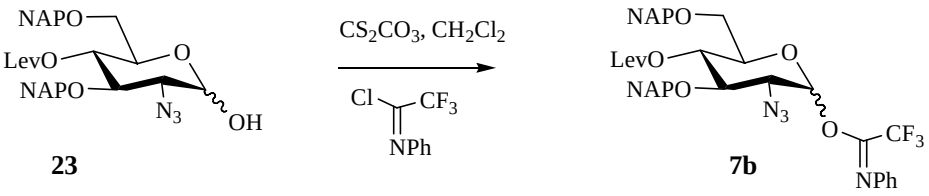
Formula	C41H51N3O7Si
Molecular Wright	725.95
TLC conditions	Rf: 0.4-0.5, EtOAc : Hexane = 1 : 1

^{1}H NMR ($CDCl_3$, 400 MHz) δ Spectra for β isomer 7.78-7.39 (m,14 H, H_{Ar}); 5.21-4.73 (m, 4 H, 2 CH2Ph,); 4.66 (m, H-4); 4.26-4.23 (m, 1 H, H-1β); 3.82-3.61 (m,3 H, H-6a, H-6b, H-5); 3.45-3.27 (m, 2 H, H-3, H-2); 2.37 (s, 1 H, C-1-OH)2.32-2.16 (m, 4 H, $CH2_{Lev}$); 2.01 (s, 3 H, OAc);

^{13}C NMR ($CDCl_3$, 100.6 MHz) δ Spectra for β isomer 170.7 (CO), 131.3-127.8 (C-Ph); 88.3, (C-1); 84.1 (C-3); 78.4, 77.3 (CH2Ph); 73.5 (C-5); 72.9 (C-4); 71.7 (C-6); 69.7 (C-2); 33.8 ($CH2_{LeV}$); 27.2 (OAc_{Lev}); 26.6 ($CH2_{Lev}$);

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 606.6, found: 606.2

2-azido-4-O-levulinoyl-3,6-O-di-naphthylmethyl-D-glucopyranoside *N*-Phenyltrifluoroacetimidate, 7 b



Synthetic procedure:

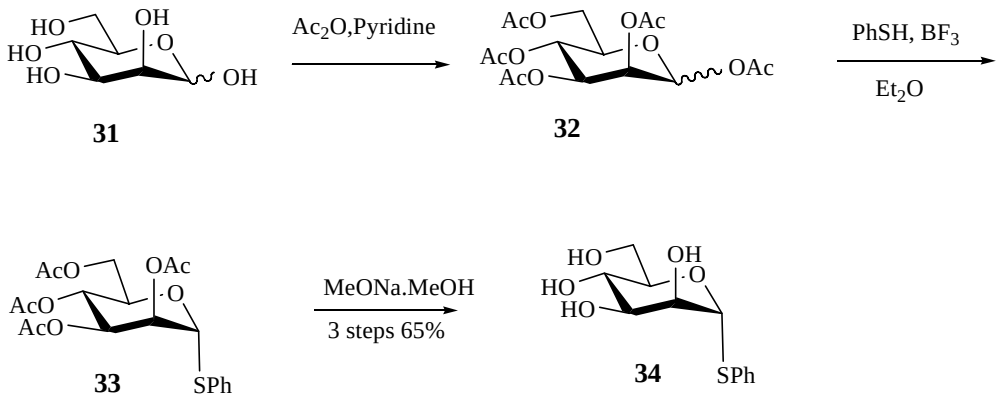
23 was disslolved in anhydrous CH_2Cl_2 (5 mL) and treated with F3CC(NPh)Cl (106 mg, 0.513 mmol) and Cs2CO3 (167 mg, 0.513 mmol). The mixture was vigorously stirred for 3 h, diluted with cyclohexane (5 mL), and decanted to afford a cloudy solution, which was concentrated under reduced pressure. The resulting crude product was purified with flash chromatography (20% EtOAc/Hexane, 1% TEA) to yield N-phenyltrifluoroimidate **7b**.

Product characterization 7b

Formula	C41H37F3N4O7
Molecular Wright	754.75
TLC conditions	Rf: 0.5, EtOAc : Hexane = 1 : 2

MALDI-MS (negative mode, DHB): $[M-H]^-$: m/z calcd.: 753.4, found: 754.0

S-Phenyl 1-thio-α-D-mannopyranoside, 34



Synthetic procedure:

For 32:

D-mannose **31** (3.4 g, 18.3 mmol) was dissolved in pyridine (50 mL), and acetic anhydride (30 mL) was added at room temperature. The reaction mixture was allowed to stirred at room temperature for 5 hours. After TLC showing consumption of **31**, CH_2Cl_2 (150 ml) was added to dilute the mixture, and extracted with H_20 (200mL), saturated $NaHCO_3$ (200 mL) and brine (100 mL). the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (17% EtOAc/Hexane), providing **32** (6.5 g, 91 %) as white solid.

For 33:

A solution of pentaacetate **32** (6.50 g, 16.7 mmol) dissolved in CH_2Cl_2 (100 mL) was charged with thiophenol (2.3 mL, 22.4 mmol) under an argon atmosphere. Boron trifluoride ether (3.1 mL, 24.2 mmol) was added over a period of 10 minutes. The reaction mixture was stirred for 14 hours at room temperature and then poured into an ice/water mixture, then neutralized with saturated $NaHCO_3$ solution. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (20% EtOAc/Hexane), providing **33** (5.74 g, 78 %) as colorless oil.

For 34

33 (5.74 g, 13.02 mmol) was dissolved in 1M MeONa/MeOH solution (50 mL) under nitrogen atmosphere, the mixture was stirred at room temperature for 2 h, then the reaction mixture was neutralized to PH 7 with IR-120 resin, (H^+ form, previously washed by MeOH), filtered, and concentrated. The crude product was purified with flash chromatography (20% MeOH/ CH_2Cl_2) to yield **34** (3.36 g, 95%, 3 steps 65%) as white solid.

Product characterization 32

Formula	C16H22O11
Molecular Wright	390.34
TLC conditions	Rf: 0.3, EtOAc : Hexane = 1: 5

Product characterization 33

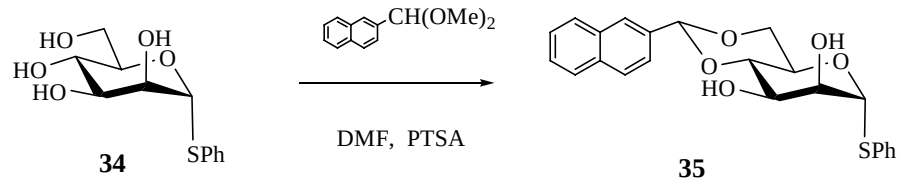
Formula	C20H24O9S
Molecular Wright	440.46
TLC conditions	Rf: 0.25, EtOAc : Hexane = 1: 4

Product characterization 34

Formula	C12H16O5S
Molecular Wright	272.32
TLC conditions	Rf: 0.4, MeOH : CH_2Cl_2 = 1: 4

The spectroscopic data were in agreement with the reported data [2]

S-Phenyl 4,6-Naptylmethylene-1-thio-α-D-mannopyranoside, 35



Synthetic procedure:

34 (1 g, 3.67 mmol) was dissolved in dry DMF (25 mL), then dimethoxymethyl naphthalene (917 mg, 4.41 mmol) was added to the solution, followed by PTSA (20 mg, 0.1 mmol). The reaction mixture was stirred at room temperature for 24 hours. After TLC analysis showing the consumption of **34**, the reaction was quenched by adding saturated $NaHCO_3$ solution (50 mL). Then CH_2Cl_2 (30 mL * 2) was added for extraction. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (5% MeOH/ CH_2Cl_2), providing **35** (1.4 g, 95.3 %) as white solid.

Product characterization 35

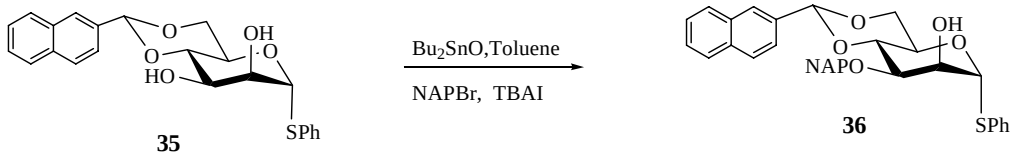
Formula	C23H2205S
Molecular Wright	410.48
TLC conditions	Rf: 0.5, MeOH : CH_2Cl_2 = 1: 19

^{1}H NMR (DMSO 400 MHz) δ 8.10-7.25 (m,12 H, H_{Ar}); 5.82 (s, 1 H, $H_{acetalic}$), 5.52 (d, 1 H, J = 1.2 Hz H-1); 4.25-4.15 (m, 2 H, H-5, H-6a); 4.11-4.02 (m, 2 H, H-2, H-4); 3.88-3.63 (m, 2 H, H-6b, H-3);

^{13}C NMR (DMSO 100.6 MHz) δ 135.1-122.6 (C-Ph); 102.3 ($C_{acetalic}$); 89.9 (C-1); 81.3 (C-4); 74.5 (C-2); 72.7 (C-3); 71.1 (C-6); 68.7 (C-5);

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 410.2, found: 433.3

S-Phenyl 3-O-Naptylmethyl-4,6-O-Naptylmethylene-l-thio-α-D-mannopyranoside, 36



Synthetic procedure:

n-Bu2SnO (486 mg, 1.65 mmol) was added to s solution of **35** (612 mg, 1.5 mmol) in Toluene (20 mL), and the resulting mixture was refluxed with removing water by using Dean Stark apparatus for 3 hours. The reaction mixture was then cooled down, and NAPBr (400 mg, 1.8 mmol) and TBAI (111 mg, 0.33 mmol) were adding. The reaction mixture was allowed to stir overnight at room temperature. Saturated NH_4Cl solution (40 mL) was used to quench the reaction. After extracted by CH_2Cl_2 (30 mL *2), the organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (33% EtOAc /Hexane), providing **36** (670 mg, 82.3 %) as white wax.

Product characterization 36

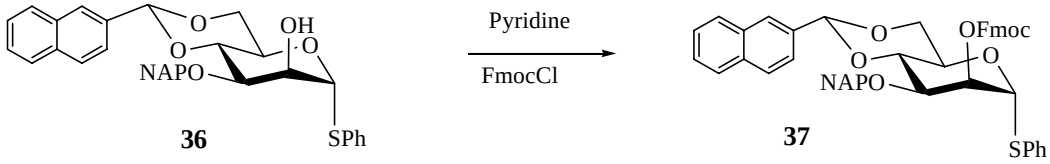
Formula	C34H3005S
Molecular Wright	550.66
TLC conditions	Rf: 0.5, EtOAc: Hexane = 1: 2

^{1}H NMR ($CDCl_3$ 400 MHz) δ 8.10-7.25 (m,19 H, H_{Ar}); 5.80 (s, 1 H, $H_{acetalic}$), 5.64 (d, 1 H, J = 1.2 Hz H-1); 5.10-4.82 (m, 2 H, CH2Ph); 4.45-4.21 (m, 4 H, H-5, H-4, H-2, H-6a); 4.17-4.04 (m, 2 H, H-3, H-6b); 3.02 (brs, 1 H, C-2-OH)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 136.4-126.2 (C-Ph); 104.1 ($C_{acetalic}$); 89.8 (C-1); 80.8 (C-4); 76.3 (C-3); 73.8 (CH2Ph); 72.1 (C-2); 72.7 (C-3); 70.5 (C-6); 66.2 (C-5);

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 560.6, found: 583.9

S-Phenyl 2-O-(9-fluorenylmethoxycarbonyl)-3-O-Naptylmethyl-4,6-O-Naptylmethylene-1-thio-α-D-mannopyranoside, 37



36 **37**

Synthetic Procedure:

36 (120 mg, 0.22 mmol) was dissolved in 5 mL pyridine, and 170 mg FomocCl (170 mg, 0.657 mmol) was added. The reaction mixture was allowed to stir at room temperature for 6 hours. After TLC analysis showing consumption of **36**, CH_2Cl_2 (10 mL) was added to dilute, and the solution was washed by saturated $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (17% Ether/Hexane), providing **37** (147 mg, 87 %) as colorless oil.

Product characterization 37

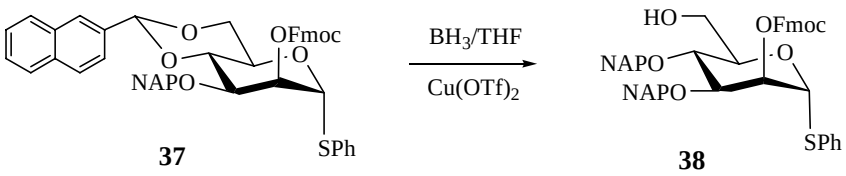
Formula	C49H4007S
Molecular Wright	772.9
TLC conditions	Rf: 0.25, Ether: Hexane = 1: 5

^{1}H NMR ($CDCl_3$ 400 MHz) δ 8.10-7.25 (m,27 H, H_{Ar}); 5.91 (s, 1 H, $H_{acetalic}$), 5.62 (d, 1 H, J = 1.2 Hz H-1); 5.53-5.51 (m, 1 H, H-2); 5.05-4.93 (m, 2 H, CH2Ph); 4.54-4.27 (6, 5 H, H-3, H-4, CH2Fomc, H-5, H-6a); 4.22-4.09 (m, 2 H, CH-fmoc, H-6b)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 135.4-120.2 (C-Ph); 104.2 ($C_{acetalic}$); 90.2 (C-1); 82.2 (C-4); 79.8 (C-2); 76.7 (C-3); 74.1 (CH2Ph); 73.2 (CH2-Fmoc); 71.2 (C-6); 67.6 (C-5); 50.2 (CH-Fmoc);

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 772.3, found: 795.2

S-Phenyl 2-O-(9-fluorenylmethoxycarbonyl)-3,4-O-di-Naptylmethyl-l-thio-α-D-mannopyranoside, 38



37 **38**

Synthetic procedure:

37 (112 mg, 0.145 mmol) was dissolved in 1M BH3/THF solution (0.73 mL, 0.73 mmol), the mixture was stirred for 10 minutes. At 0℃, Cu(OTf)2 (2.6 mg, 0.007 mmol) was added and the suspension was allowed to stir at room temperature for 3 hours. After TLC analysis showing consumption of **37**, the reaction was quenched by adding MeOH (1 mL) and pyridine (1 mL) at -30℃. After concentrated under reduced pressure, the resulting crude product was purified with flash chromatography (25% EtOAc/Hexane) to yield **38** (95 mg, 84%).

Product characterization 38

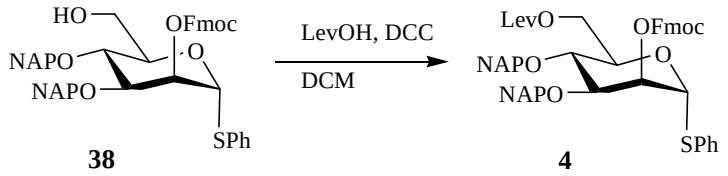
Formula	C49H4207S
Molecular Wright	774.92
TLC conditions	Rf: 0.2, EtOAc: Hexane = 1: 3

^{1}H NMR ($CDCl_3$ 400 MHz) δ 8.10-7.25 (m,27 H, H_{Ar}); 5.60 (d, 1 H, J = 1.6 Hz H-1); 5.56-5.53 (m, 1 H, H-2); 5.18-4.78 (m, 4 H, 2 CH2Ph); 4.51-3.91 (m, 8 H CH2Fomc, H-5, CH-Fmoc, H-4, H-3, H-6a, H-6b); 3.03 (brs, 1 H, C-6-OH)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 135.2-120.6 (C-Ph); 88.7 (C-1); 81.8 (C-4); 77.8 (CH2Ph); 76.9 (C-2); 76.7 (C-3); 75.8 (C-5); 74.5 (CH2Ph); 73.0 (CH2-Fmoc); 71.0 (C-6); 47.6 (CH-Fmoc);

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 774.3, found: 797.7

S-Phenyl 2-O-(9-fluorenylmethoxycarbonyl)-6-O-levulinoyl-3,4-O-di-Naptylmethyl-1-thio-α-D-mannopyranoside, 4



38 **4**

38 (90 mg 0.116 mmol) was dissolved in CH_2Cl_2 (50 mL), LeVOH (20 mg, 0.20 mmol), DCC (42 mg, 0.20 mmol) and DMAP (1 mg, 0.001 mmol) were added. The reaction mixture was stirred at room temperature for 2 hours. The solvent was concentrated and the resulting crude was purified by flash chromatography (30% EtOAc/Hexane), providing **4** (82 mg, 81 %) as colorless oil.

Product characterization 38

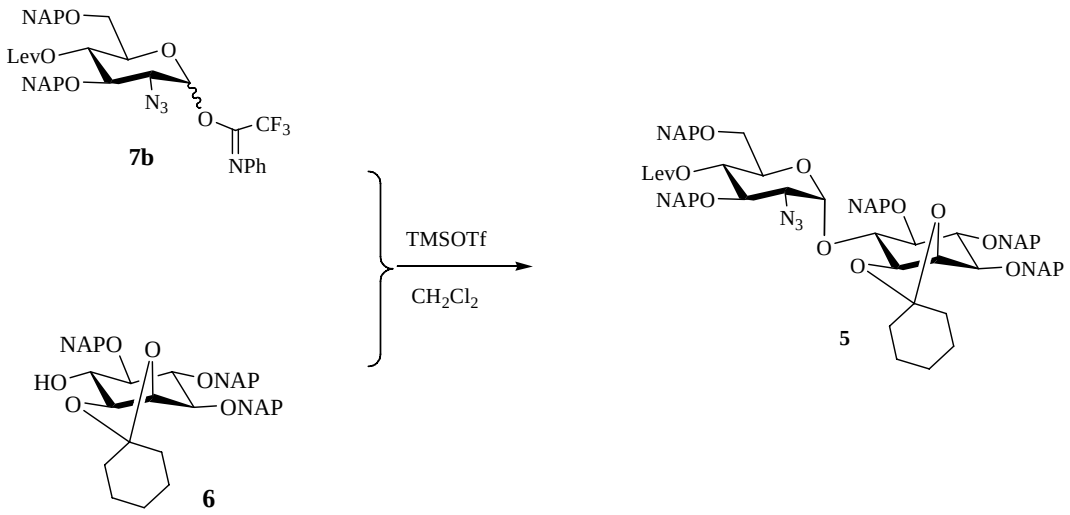
Formula	C54H4809S
Molecular Wright	873.02
TLC conditions	Rf: 0.3, EtOAc: Hexane = 3: 7

^{1}H NMR ($CDCl_3$ 400 MHz) δ 8.10-7.25 (m,27 H, H_{Ar}); 5.60 (d, 1 H, J = 1.6 Hz H-1); 5.56-5.53 (m, 1 H, H-2); 5.18-4.76 (m, 6 H, 2 CH2Ph, H-6); 4.51-4.03 (m, 6 H CH2Fomc, H-5, CH-Fmoc, H-4, H-3); 2.43-2.24 (m, 4 H, $CH2_{Lev}$); 1.98 (s, 2 H, OAc)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 135.3-122.4 (C-Ph); 88.9 (C-1); 85.4 (C-6); 82.3 (C-4); 77.8 (CH2Ph); 77.2 (C-2); 76.9 (C-3); 74.9 (C-5); 74.1 (CH2Ph); 73.0 (CH2-Fmoc); 47.6 (CH-Fmoc); 33.8 ($CH2_{LeV}$); 27.2 (OAc_{Lev}); 26.6 ($CH2_{Lev}$);

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 872.3, found: 895.7

1,2-O-cylcohexylidine-3,4,5-tri-O-naphthylmethyl-6-O-(2-azido-4-O-levulinoyl-3,6-O-di-naphthylmethyl-α-D-glucopyranosyl)-D-myo-inositol,5



Synthetic Procedure:

Myo-inositol **6** (96 mg, 0.14 mmol) and glycosyl donor **7b** (150 mg, 0.21 mmol) were dried together by covapored with anhydrous toluene (5 mL * 3) and left under vacuum for 4 h. Then molecular sieves (4 A, 10 mg) and anhydrous CH_2Cl_2 (3 mL) were added under Argon atmosphere. The suspension were stirred at room temperature for 1 hour, and then cooled down to -10℃, TMSOTf (1.76 uL, 0.01 mmol, diluted by 176 uL CH_2Cl_2) was added to the reaction mixture dropwisely, the reaction was allowed to stir at -10℃ for 1 hour. After TLC analysis showing consumption of donor **7b**, the reaction was quenched by TEA (1 mL). The solvent was concentrated and the resulting crude was purified by flash chromatography (7% EtOAc/toluene), providing **5** (119 mg, 81 %) as colorless oil.

Product characterization 5

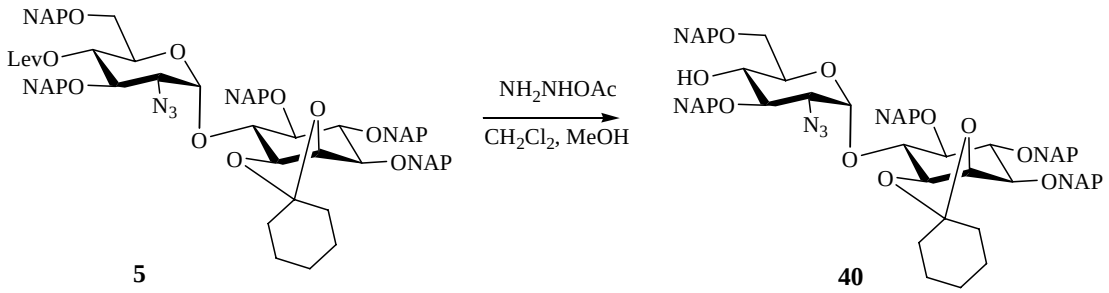
Formula	C78H75N3O12
Molecular Wright	1246.44
TLC conditions	Rf: 0.3, EtOAc: toluene = 1: 9

^{1}H NMR ($CDCl_3$ 500 MHz) δ 8.02-7.25 (m,35 H, H_{Ar}), 5.21-4.73 (m, 11 H, 5 CH_2NAP, H-1, α isomer was confirmed by delata value of HSQC, 168.12); 4.65-4.57 (m,1 H, H-4); 4.54 (m, 1 H, H-2'), 4.18-3.99 (m, 3 H, H-6' , H-1' , H-4'); 3.87 (m, 1 H, H-3'); 3.68-3.44 (m, 4 H, H-5' H-2, H-6a,CH H-6b); 3.40-3.21 (m ,2 H, H-5, H-3), 2.30-2.14 (m, 4 H, $CH2_{Lev}$); 1.91 (s, 3 H, OAc)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 137.0-117.6 (C-Ph); 102.4 (C-1); 85.6 (C-5'); 83.8 (C-3); 81.9 (C-4'); 81.5 (C-6'); 80.4 (C-1'); 79.7 (C-3'); 77.5 (CH2Ph); 77.1 (CH2Ph); 76.8, 76.2, 76.0 (CH2Ph); 74.7 (C-4); 74.1 (C-2'); 72.5 (C-5); 70.3 (C-6), 69.8 (C-2); 39.8-21.8 ($CH2L_{ev}$, OAc_{Lev}, Carbons of cyclohexylidene)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 1246.45, found: 1268.5

1,2-O-cylcohexylidine-3,4,5-tri-O-naphthylmethyl-6-O-(2-azido-3,6-O-di-naphthylmethyl-α-D-glucopyranosyl)-D-myo-inositol,40



5 **40**

Synthetic Procedure:

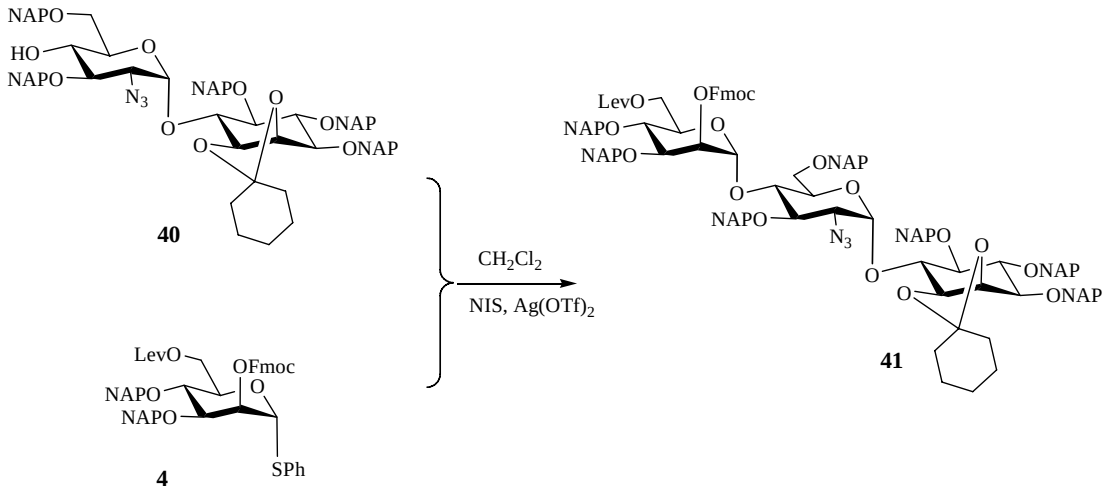
5 (75 mg, 0.06 mmol) was dissolved in a mixture of CH_2Cl_2 (5 mL) and MeOH (5 mL), NH2NHOAc (60 mg, 0.65 mmol) was added at room temperature, the mixture was stirred at room temperature for 1 hour. After TLC analysis showing consumption of **5**, the solvent was concentrated and the resulting crude was purified by flash chromatography (8% EtOAc/toluene), providing **40** (48 mg, 70 %) as colorless oil.

Product characterization 40

Formula	C73H69N3O10
Molecular Wright	1148.34
TLC conditions	Rf: 0.3, EtOAc: toluene = 1: 12

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 1148.3, found: 1172.0

1,2-O-cylcohexylidine-3,4,5-tri-O-naphthylmethyl-6-O-(2-azido-3,6-O-di-naphthylmethyl-4-O-(2-O-(9-fluorenylmethoxycarbonyl)-6-O-levulinoyl-3,4-O-di-Naptylmethyl-α-D-mannopyranossyl)-α-D-glucopyranosyl)-D-myo-inositol,41



Synthetic Procedure:

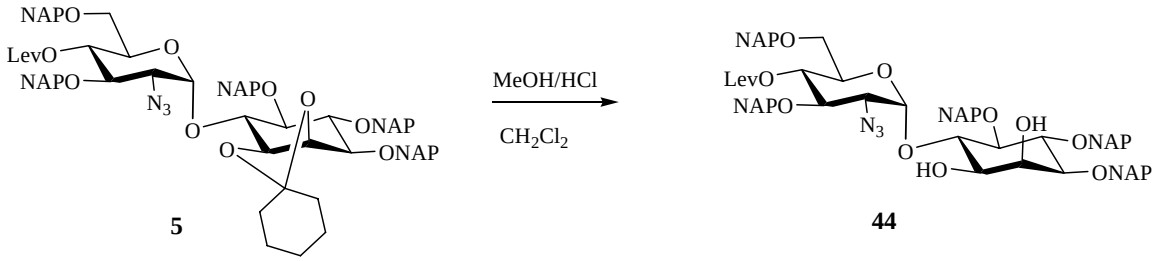
40 (44 mg, 0.038 mmol) and mannose donor **4** (42 mg, 0.048 mmol) were dried together by covapored with anhydrous toluene (2 mL * 3) and left under vacuum for 4 h. Then molecular sieves (4 A, 10 mg) and anhydrous CH_2Cl_2 (3 mL) were added under Argon atmosphere. The suspension were stirred at room temperature for 1 hour, and then cooled down to -20℃, NIS (17 mg, 0.076 mmol) and Ag(OTf)2 (19 mg, 0.076 mmol) were added to the reaction mixture, the reaction was allowed to stir at -20℃ for 1 hour and another 1 hour at room temperature. After TLC analysis showing consumption of donor **4**, the reaction was quenched by adding pyridine (1 mL). The solvent was concentrated and the resulting crude was purified by flash chromatography (5% acetone/toluene), providing **41** (51 mg, 70 %) as colorless oil.

Product characterization 40

Formula	C121H111N3019
Molecular Wright	1911.18
TLC conditions	Rf: 0.6, Acetone : toluene = 1: 9

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 1911.2, found: 1933.6

3,4,5-tri-O-naphthylmethyl-6-O-(2-azido-4-O-levulinoyl-3,6-O-di-naphthylmethyl-α-D-glucopyranosyl)-D-myo-inositol,44



5 44

Synthetic Procedure:

5 (136 mg, 0.11 mmol) was dissolevd CH_2Cl_2 5 mL, 0.5 M HCl/MeOH (5 mL) was added to the mixture, the reaction mixture was allowed to stir at 40℃ for 2 hours. After TLC analysis indicating consumption of **5**, the reaction was quenched by adding TEA (3 mL). The solvent was concentrated and the resulting crude was purified by flash chromatography (5% MeOH/CH_2Cl_2), providing **44** (101 mg, 80 %) as white wax.

Product characterization 44

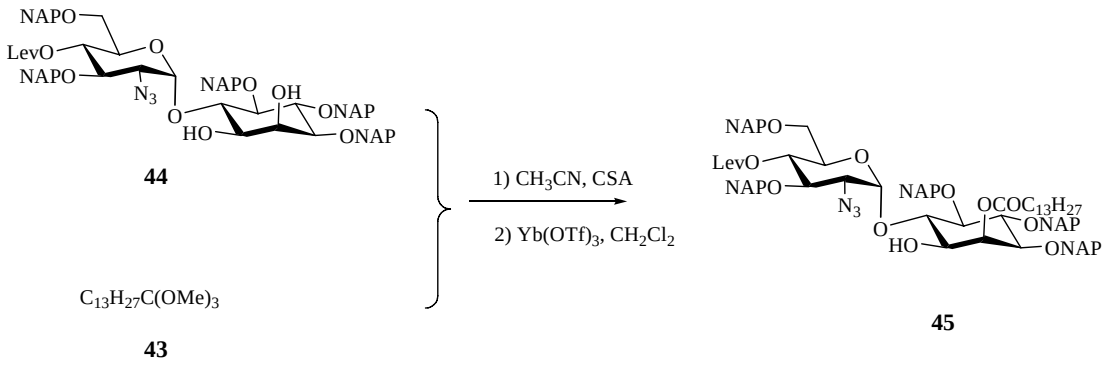
Formula	C72H67N3O12
Molecular Wright	1166.32
TLC conditions	Rf: 0.5, MeOH : CH_2Cl_2 = 1: 19

^{1}H NMR ($CDCl_3$ 500 MHz) δ 7.90-7.21 (m,35 H, H_{Ar}), 5.25-4.68 (m, 10 H, 5 CH_2NAP); 4.61 (s,1 H, H-1); 4.54 (m, 1 H, H-2'), 4.36 (s, 1 H, H-4); 4.21 (m, 1 H, H-2'); 4.15 (m, 1 H, H-6'); 3.78-3.57 (m ,7 H, H-1' , H-4' ,H-3' , H-6a, H-6b, H-5' , H-2); 3.48-3.40 (m, 2 H, H-5, H-3); 3.23 (2 brs, C-1' -OH, C-2' -OH); 2.44-2.22 (m, 4 H, $CH2_{Lev}$); 1.95 (s, 3 H, OAc)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 130.0-125.1 (C-Ph); 102.3 (C-1); 84.4 (C-6'); 83.9 (C-5'); 83.6 (C-2'); 81.8 (C-3); 80.9 (C-4'); 79.9-73.6 (CH2Ph); 75.6 (C-3'); 73.4 (C-1'); 71.5 (C-4); 70.3 (C-6); 67.6 (C-2); 61.2 (C-5); 38.5-32.3 ($CH2L_{ev}$, OAc_{Lev})

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 1266.3, found: 1299.8

3,4,5-tri-O-naphthylmethyl-2-O-tetradecanoyl-6-O-(2-azido-4-O-levulinoyl-3,6-O-di-naphthylmethyl-α-D-glucopyranosyl)-D-myo-inositol,44



Synthetic procedure:

44 (108 mg, 0.93 mmol) was dissolved in acetonitrile (5 mL), catalytic amount of CSA was added, the reaction mixture was stirred at 40℃ for 2 hours. After TLC analysis indicating consumption of **44**, the reaction was quenched by adding TEA (1 mL). The solvent was concentrated and covapored with toluene for three times (2 mL * 3) to get a yellow crude.

The crude intermediate was dissolved in anhydous CH_2Cl_2 (5 mL), and 5 mg of Yb(OTf)3 was added to the mixture. The reaction mixture was stirred at room temperature for 3 hours. After TLC analysis showing consumption of the starting materials, the reaction was quenched by TEA (1 mL). The solvent was concentrated and the resulting crude was purified by flash chromatography (17% ETOAc/toluene), providing **45** (101 mg, 73 %) as white wax.

Product characterization 45

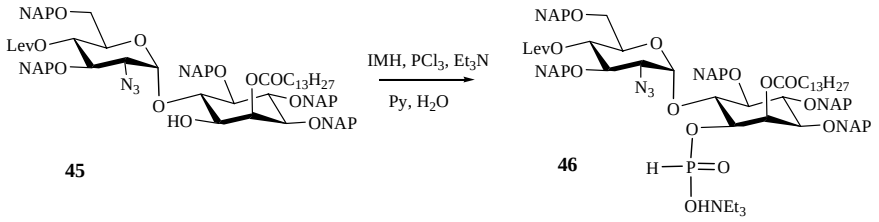
Formula	C86H93N3O13
Molecular Wright	1375.67
TLC conditions	Rf: 0.3, ETOAc : toluene = 1: 4

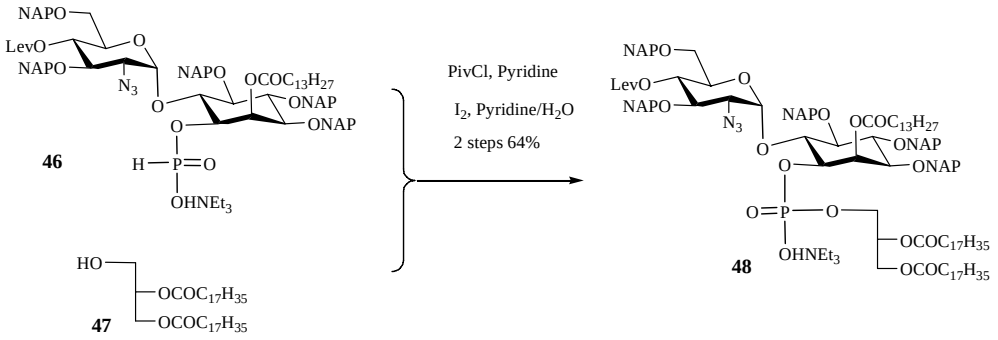
^{1}H NMR ($CDCl_3$ 500 MHz) δ 7.77-7.35 (m,35 H, H_{Ar}), 5.82 (d, 1 H, J = 1.0 Hz H-2'); 5.49-4.33 (m, 14 H, 5 CH_2NAP, H-1, H-4, H-6a, H-6b); 4.24-4.08 (m, 2 H, H-4' , H-5'), 3.83-3.70 (m, 4 H, H-3' , H-6' , H-1'); 3.66-3.45 (m, 3 H, H-2, H-5, H-3); 2.40-2.16 (m, 4 H, $CH2_{Lev}$); 2.02 (s, 3 H, OAc), 1.78-0.92(m, H of fatty acid)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 129.6-125.1 (C-Ph); 104.0 (C-1); 84.7 (C-3'); 83.5 (C-5'); 83.1 (C-4'); 82.8 (C-3); 81.5-75.0 (CH2Ph); 79.9 (C-6'); 74.1 (C-6); 73.2 (C-4); 71.5 (C-1');69.7 (C-2'); 67.2 (C-2); 61.0 (C-5); 38.2-15.5 ($CH2L_{ev}$, OAc_{Lev} , Carbons of fatty acid)

MALDI-MS (positive mode, DHB): $[M + Na]^+$: m/z calcd.: 1375.3, found: 1398.1

Triethylammonium (3,4,5-tri-O-naphthylmethyl-2-O-tetradecanoyl-6-O-(2-azido-4-O-levulinoyl-3,6-O-di-naphthylmethyl-α-D-glucopyranosyl)- 1-O-(1, 2-di-O-octadecanoyl-sn-glyceryl-phosphonato)-D-myo-inositol, 46





Synthetic procedure:

For 46

Imidazole (850 mg, 12.5 mmol) was co-evaporated with toluene (3 x 5 mL) and dried in vacum for 1 hour, and then dissolved in Toluene (10 mL). The solution was cooled to -10℃, followed by addition of PCl3 (235 mL, 2.7 mmol) in Toluene (3 mL) and Et3N (0.975 mL, 7 mmol). The reaction mixture was then allowed to stir for 30 min at 0℃. Meanwhile, **45** (200 mg, 0.17 mmol) was co-evaporated with Toluene (3 x 3 mL) and dried in vacuo and dissolved in a mixture of toluene (6 mL) and CH_2Cl_2 (6 mL). This solution was added dropwise to the reaction mixture over a period of 80 minutes. The mixture was then allowed to stir for further 40 minutes before being quenched by addition of water/pyridine (1:1, 15 mL). The aqueous layer was washed with $CHCl_3$ (2 * 30 mL) and the

combined organic layers were further washed with 1M TEAB buffer (10 mL) and dried over Na_2SO_4. Evaporation in vacuo gave the crude residue, which was subjected to flash column chromatography with Et3N-deactivated silica gel (10% MeOH/ CH_2Cl_2) to afford H-phosphonate **46** (217 mg ,81%) as a white solid.

For 48

46 (80 mg, 0.052 mmol) and alcohol 47 (132 mg,0.26 mmol) were co-evaporated with anhydrous pyridine (3 x 3 mL) and dried under high vacuum overnight. The mixture was dissolved in anhydrous pyridine (5 mL) at room temperature, and pivaloyl chloride (30.2 uL, 0.24 mmol) was added and the mixture was stirred at room temperature for 3 h. Iodine (72 mg, 0.29 mmol) in a mixture of pyridine/water (19:1, 1 mL) was added for oxidation. The reaction mixture was further stirred 0.5 h at room temperature, and diluted with $CHCl_3$ (25 mL), washed with saturated $Na_2S_2O_3$ solution (10 mL), 1M TEAB buffer (10 mL) and dried over Na_2SO_4. Evaporation under reduced pressure gave the crude residue, which was subjected to flash column with Et3N-deactivated silica gel ((12.5% MeOH/CH_2Cl_2) to give 48 (88 mg, 79%) as a white syrup.

Product characterization 46

Formula	C86H93N3O15P
Molecular Wright	1438.6
TLC conditions	Rf: 0.5, MeOH: CH_2Cl_2 = 1: 9

The formation of the H-phosphonate **45** was ascertained by ^1H NMR analysis, which showed the diagnostic doublet at δ 6.23 ($J_{H,P}$ = 653 Hz), compound **34** was used directly in the following steps without further characterization.

^{31}P NMR ($CDCl_3$, 162 MHz) δ 4.53

Product characterization 46

Formula	C125H167N3020P
Molecular Wright	2061.2
TLC conditions	Rf: 0.4, MeOH : CH_2Cl_2 = 1: 7

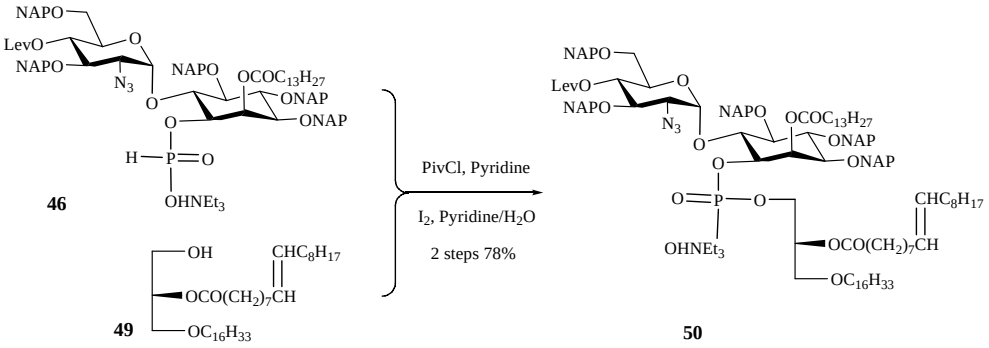
^{1}H NMR ($CDCl_3$ 500 MHz) δ 7.80-7.32 (m,35 H, H_{Ar}), 5.88 (d, 1 H, J = 1.0 Hz H-2'); 5.49-4.30 (m, 16 H, 5 CH_2NAP, CH-glyceryl H-1, H-4, H-6a, H-6b, H-1',); 4.24-4.02 (m, 2 H, H-4' , H-5'), 3.91-3.68 (m, 6 H, H-3' , H-6' , 2 CH2-O); 3.66-3.45 (m, 3 H, H-2, H-5, H-3); 2.98 (q, 6 H, 3 C$H_{2\text{-Et}}$), 2.38-1.98 (m, 13 H, $CH2_{Lev,}$, CH2Lipid, OAc), 1.81-1.10 (m, H of fatty acid, lipid , 3 C$H_{3\text{-Et}}$)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 132.0-125.1 (C-Ph); 108.7 (C-2'); 102.3 (C-1); 85.2 (C-3'); 81.2 (C-1'); 80.3 (C-4'); 79.8 (C-glyceryl); 79.1 (C-4); 78.2-72.1 (CH2Ph); 75.2 (C-5'); 73.8 (C-3);; 72.4 (C-6); 71.7 (C-6'); 70.4 (C-2); 68.0 (C-5); 63.0-61.8 (C-O on lipid); 47.1-11.0 ($CH2L_{ev}$, OAc , CH2CH3 NEt_3, Carbons of fatty acid, cabobons of lipid)

^{31}P NMR ($CDCl_3$, 162 MHz) δ 2.11

MALDI-MS (negative mode, DHB): $[M-H]^-$: m/z calcd.: 2061.3, found: 2061.0

Triethylammonium (3,4,5-tri-O-naphthylmethyl-2-O-tetradecanoyl-6-O-(2-azido-4-O-levulinoyl-3,6-O-di-naphthylmethyl-α-D-glucopyranosyl)- 1-O-(1-O-hexadecyl-sn-glycerol-phosphonato)-D-myo-inositol, 50



Synthetic procedure:

46 (80 mg, 0.052 mmol) and alcohol **49** (143 mg,0.26 mmol), which was prepared from reported method[3], were co-evaporated with anhydrous pyridine (3 x 3 mL) and dried under high vacuum overnight. The mixture was dissolved in anhydrous pyridine (5 mL) at room temperature, and pivaloyl chloride (45 uL, 0.36 mmol) was added and the mixture was stirred at room temperature for 2 hours. Iodine (72 mg, 0.29 mmol) in a mixture of pyridine/water (19:1, 1 mL) was added for oxidation. The reaction mixture was further stirred 0.5 h at room temperature, and diluted with $CHCl_3$ (25 mL), washed with saturated $Na_2S_2O_3$ solution (10 mL), 1M TEAB buffer (10 mL) and dried over Na_2SO_4. Evaporation under reduced pressure gave the crude residue, which was subjected to flash column with Et3N-deactivated silica gel ((10% MeOH/ CH_2Cl_2) to give 50 (108 mg, 95%) as a white syrup.

Product characterization 50

Formula	C122H163N3O18P
Molecular Wright	1989.2
TLC conditions	Rf: 0.5, MeOH: CH_2Cl_2 = 1: 9

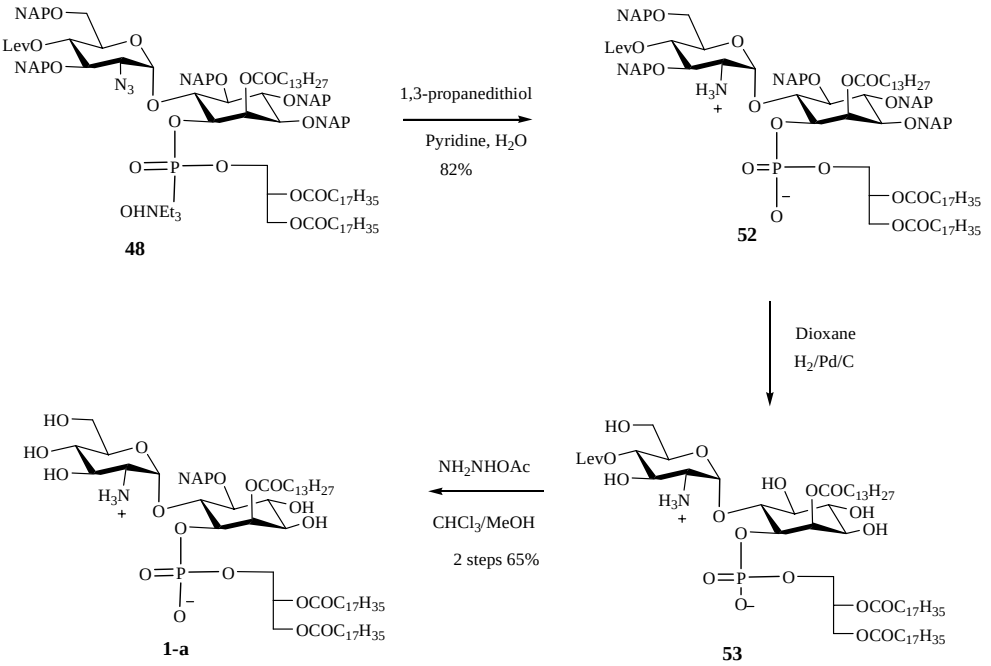
^{1}H NMR ($CDCl_3$ 500 MHz) δ 7.80-7.32 (m,35 H, H_{Ar}), 5.90 (s, H-2'); 5.57-4.11 (m, 20 H, 5 CH_2NAP, CH-glycery,1 H-1, H-4, H-6a, H-6b, H-1' CH=CH, H-4' , H-5'), 3.98-3.31 (m, 9 H, H-3' , H-6' , 2 CH2-O, H-2, H-5, H-3); 3.03(q, 6 H, 3 C$H_{2\text{-}NEt3}$), 2.31-0.98 (m, H of $CH2_{Lev.}$, CH2Lipid, OAc, fatty acid, lipid and C$H_{3\text{-}NEt3}$)

^{13}C NMR ($CDCl_3$ 100.6 MHz) δ 131.0-125.7 (C-Ph); 118.7 (CH=CH); 105.2 (C-2'); 102.2 (C-1); 88.7 (C-3'); 84.2 (C-1'); 83.1 (C-4'); 80.8 (C-4); 78.2-73.1 (CH2Ph); 77.2 (C-5'); 76.8 (CH-glyceryl); 76.5 (C-3); 74.9 (C-6'); 73.5 (C-6); 72.1 (C-2); 71.5 (C-O on lipid); 71.0 (C-5); 68.2 (C-O on lipid); 49.5-15.0 (Carbons of $CH2L_{ev}$, OAc , $CH2CH3_{NEt3}$, fatty acid, unsaturated lipid)

^{31}P NMR ($CDCl_3$, 162 MHz) δ 2.35

MALDI-MS (negative mode, DHB): $[M-H]^-$: m/z calcd.: 2017.5, found: 2017.6

6-O-(2-amino-2-deoxy-a-D-glucopyranosyl)-2-O-tetradecanoyl-1-O-(1, 2-di-O-octadecanoyl-sn-glyceryl-phosphonato)-D-myo-inositol, 1



Synthetic procedure:

48 (50 mg, 0.024 moml) was dissolved in a solution of pyridine and water (5 mL/1 mL). Then a 1,3-propanedithiol(50 uL, 0.5 mmol) and TEA (70 uL, 0.5 mmol) was added to the mixture and the reaction mixture was allowed to stir at room temperature for 2 hours. After the MALDI-TOF spectrum showed fully conversion of the amine, evaporation under reduced pressure gave the crude residue, which was subjected to flash column with (12.5 % MeOH/CH_2Cl_2) to give **52** (41 mg, 82%) as a colourless oil.

Intermediate **52** (41 mg, 0.02mmol) was hydrogenolysed over Pd/C in dioxane (5 mL) for 24 hours at 40℃. After the MALDI-TOF spectrum showed completely removal of NAP ether, the mixture was filtered over a Celite pad and the filtrate was concentrated. Then the residue was purified through a C-18 reverse column (water, MeOH and dioxane as eluent) to give purified **53** 18 mg.

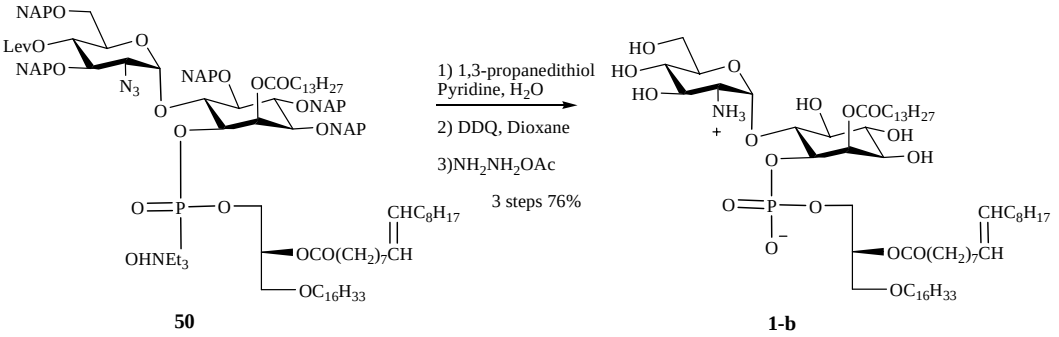
53 was then dissolved in a solution of $CHCl_3$ (2 mL) and MeOH (4 mL), and treated with hydrazine acetate (5 mg, 0.05 mmol), the reaction mixture was stirred at room temperature for 2 hours. After the MALDI-TOF spectrum indicated the comsuption of **53**, the reaction mixture was evaporated under reduced pressure, and the resulting crude was purified through a C-18 reverse column (water, MeOH and dioxane as eluent), further lyophilization gave the lipidated GPI anchor **1a** as a white solid (16 mg, 2 steps for 65%).

Product characterization 1a

^{1}H NMR ($CDCl_3$: CD_3OD : D_2O = 3 : 3 : 1 500 MHz) δ, 5.63 (m, 1 H, H-2'); 5.45-5.23 (m, 3 H, CH-glyceryl, H-1, H-1'); 4.89-3.45 (m, H of sugar, CH-O on lipid), 2.57-0.81 (m, H of fatty acid, lipid)

MALDI-MS (negative mode, DHB): calcd.for C65H123N018P [M-H]- m/z,.: 1236.85, found: 1236.75

6-O-(2-amino-2-deoxy-a-D-glucopyranosyl)-2-O-tetradecanoyl-1-O-(1-O-hexadecyl-sn-glycerol-phosphonato)-D-myo-inositol, 2



50 **1-b**

Synthetic procedure:

50 (50 mg, 0.025 moml) was dissolved in a solution of pyridine and water (5 mL/1 mL). Then a 1,3-propanedithiol(50 uL, 0.5 mmol) and TEA (70 uL, 0.5 mmol) was added to the mixture and the reaction mixture was allowed to stir at room temperature for 2 hours. After the MALDI-TOF spectrum showed fully conversion of the amine, evaporation under reduced pressure gave the crude residue, which was subjected to flash column with (12.5% MeOH/CH_2Cl_2) to give amine intermediate (47 mg, 95%) as a colourless oil.

Intermediate **52** (47 mg, 0.024 mmol) was dissolved in a mixture of $CHCl_3$ (5 mL) and MeOH (5 mL), and DDQ (0.18 mmol) was added, and the reaction mixture was stirred at 40℃ for 24 hours. The the solvents system was changed to dioxane (10 mL), and another 40 mg DDQ was added and the reaction mixtiure was allowed to stir at 40℃ for another 24 hours over. After the MALDI-TOF spectrum showed completely removal of NAP ether, the mixture was filtered over a Celite pad and the filtrate was concentrated. The residue was first purfied by a prepartive TLC to remove most of the DDQ (Eluent: 50% MeOH/CH_2Cl_2), then was purified through a C-18 reverse column (water, MeOH and dioxane as eluent) to give purified intermediate. The resulting intermediate was dissolved in Dioxane and treated with hydrazine acetate (5 mg), after MALDI-TOF spectrum indicated fully

removal of Lev, the residue was purified through a C-18 reverse column (water, MeOH and dioxane as eluent), further lyophilization gave the lipidated GPI anchor **1b** as a white solid (22 mg, 3 steps for 76%).

Product characterization 1b

^{1}H NMR (Dioxane-d6 500 MHz) δ, 5.60 (brs, 1 H, H-2'); 5.57-5.11 (m, 5 H, CH-glyceryl,CH=CH, H-1, H-1'); 4.98-3.08 (m, H of sugar, CH-O on lipid), 2.32-0.65 (m, H of fatty acid, lipid)

MALDI-MS (negative mode, DHB): calcd.for C63H119NO17P [M-H]- m/z,.: 1192.82, found: 1192.93

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