Università degli Studi di Milano
Facoltà di Scienze e Tecnologie
Dipartimento di Chimica

Synthesis of *Neisseria meningitidis* serogroup A *carba* analogues as hydrolytically stable antigens for antimeningococcal glycoconjugate vaccines

PhD thesis of

Ludovic AUBERGER

R11505 - Cycle XXXI

July 2016-2019

Tutor: Prof. Luigi Lay

Co-tutor: Dr. Laura Polito

with the collaboration of Dr. Jeroen Codée and Dr. Roberto Adamo

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"Vous savez, moi je ne crois pas qu'il y ait de bonne ou de mauvaise situation. Moi, si je devais résumer ma vie aujourd'hui avec vous, je dirais que c'est d'abord des rencontres. Des gens qui m'ont tendu la main, peut-être à un moment où je ne pouvais pas, où j'étais seul chez moi. Et c'est assez curieux de se dire que les hasards, les rencontres forgent une destinée… Parce que quand on a le goût de la chose, quand on a le goût de la chose bien faite, le beau geste, parfois on ne trouve pas l'interlocuteur en face je dirais, le miroir qui vous aide à avancer. Alors ça n'est pas mon cas, comme je disais là, puisque moi au contraire, j'ai pu : et je dis merci à la vie, je lui dis merci, je chante la vie, je danse la vie… je ne suis qu'amour ! Et finalement, quand beaucoup de gens aujourd'hui me disent « Mais comment fais-tu pour avoir cette humanité ? », et bien je leur réponds très simplement, je leur dis que c'est ce goût de l'amour ce goût donc qui m'a poussé aujourd'hui à entreprendre une construction mécanique, mais demain qui sait ? Peut-être simplement à me mettre au service de la communauté, à faire le don, le don de soi…"

Edouard Baer, *Asteric & Obélix : mission Cléopatre*, 2007.

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Abbreviations and acronyms

Abs	Antibodies
Ac	Acetyl
ACN	Acetonitrile
APC	Antigen-presenting cell
BAIB	[bis(acetoxy)iodo]benzene
B cell	Blood lymphocyte
BCG	Bacillus Calmette–Guérin
BCR	B cell receptor
BSA	Bovin serum albumin
Bn	Benzyl
$CD4^+$ T/T_H	T Helper cell
$CD8^+$ $T/CTL/T_C$	Cytotoxic T cell
Cbz	Carboxybenzyl (carbamate protective group)
CDC	Center for Disease Control and prevention
CPS	Capsular Polysaccharide
CRM_{197}	Cross Reactive Material 197
CSO	(1S)-(+)-(10-camphorsulfonyl)-oxaziridine
Da	Dalton
DC	Dendritic cell
DCI	4,5-Dicyanoimidazole
(*o,m,p*)-DCB	(*ortho*, *meta*, *para*)-Dichlorobenzene
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DFT	Density functional theory
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminium hydride
DMAP	4-Dimethylaminopyridine
DIPEA	*N*,*N*-Diisopropylethylamine

DMF	Dimethylformamide
DMP	Dess-Martin periodiniane
DMTr	Dimethoxytrityl
DNA	Deoxyribonucleic acid
DP	Degree of Polymerization
DT	Diphteria toxoid
ELISA	Enzyme linked immunosorbent assays
ESI	Electrospray Ionization
EVD	Ebola virus disease
F_c	Fragment crystallizable
FDA	Food & Drug Administration
GAVI	Global Alliance for Vaccines and Immunization
GSK	GlaxoSmithKline Sarl
HA	Haemagglutinin
HBV	Hepatite B virus
HIV	Human immunodeficiency viruses
IBX	2-Iodoxybenzoic acid
IFN	Interferon
Ig	Immunoglobin
IL	Interleukin
ITN	Innovative Training Network
KHMDS	Potassium bis (trimethylsilyl)amide
KLH	Keyhole limphet hemocyanin
LC/MS	Liquid chromatography/mass spectrometry
LPS	Lipopolysaccharide
MALDI-TOF	Matrix-assisted laser desorption ionization - Time of flight
MBC	Memory B cell
MenA	*Neisseria Meningitidis* serogroup A
MHC	Major histocompatibility complex
MVA	Modified vaccinia virus
MVP	Meningitis Vaccine Project
NA	Neuraminidase

NHAc	N-acetamide
NMR	Nuclear magnetic resonance
NPs	Nanoparticles
OMPC	Outer membrane protein C of *N. meningitidis*
PAHO	Pan American Health Organisation
PAMPs	Pathogen-associated molecular patterns
PATH	Partnership for advance technologies in healthcare service
PDT	1,3-propanedithiol
Ph	Phenyl
PHiD	Protein D
Piv	Pivaloyl
pKa	acid dissociation constant
PMB	Paramethoxybenzyl
PMP	Paramethoxyphenyl
PRR	Pattern-recognition receptors
PPTS	Pyridinium *p*-toluenesulfonate
PS	Polysaccharide
Py/Pyr	Pyridine
(m)RNA	(messenger) Ribonucleic acid
rSBA	rabbit serum bactericidal assay
RT	Room temperature
RV	Reverse vaccinology
S_N2	Nucleophile substitution type 2
TACA	Tumor associated carbohydrate antigen
TB	Tuberculosis
TBAI	Tetrabutylammonium iodide
TBDMS/TBS	*tert*-butyldimethylsilyl
TBSOTf	*tert*-butyldimethylsilyl trifluoromethanesulfonate
tBu	*tert*-butyl
TCA	Trichloroacetic acid
T cell	Thymus lymphocyte
TCR	T cell receptor

TD	T-dependent
TDS	dimethylthexylsilyl
TEA	Triethylamine
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
Tf	Triflate
THF	Tetrahydrofuran
TI	T-independent
TLC	Thin layer chromatography
TLR	Toll-like receptor
TMANO	Trimethylamine *N*-oxide
T_{reg}	regulatory T cell
TT	Tetanus toxoid
UNICEF	United Nations International Children's Emergency Fund
VLP	Virus-like particle
WHO	World Health Organization
ZP	Zwitterionic polysaccharide

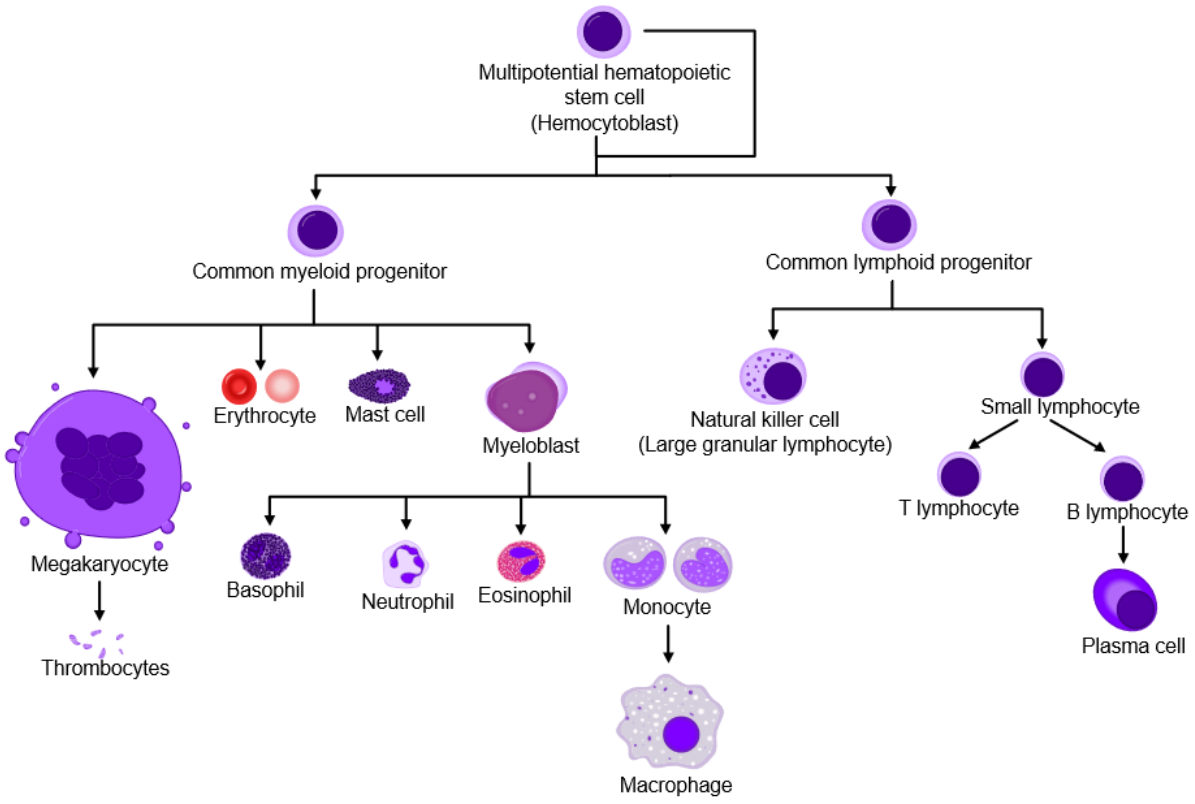
I- Carbohydrates and Immunology

1) Introduction (glycocalyx, cells of the immune system, innate/adaptive IR)

Carbohydrates are ubiquitous in nature. These complex polymers might be literally considered, with proteins and nucleic acids, as the building blocks of life. The glycocalyx is the symbol of this pervasiveness. Cell coat of every cells, the glycocalyx is essentially constituted of different glycoforms such as *N*-linked and *O*-linked glycoproteins, glycolipids and glycophosphatidylinositol-linked proteins. This carbohydrate pericellular matrix, first time empirically identified by Luft[1] using electron microscopy, provides a physical protection to the external membrane of a cell, among its many functions. We can distinguish two types of glycocalyx depending on their structure. On one hand, the unorganized and gelatinous form of glycocalyx is called slime layer: the glycoprotein molecules are loosely associated with the cell wall. On the other hand, the structurally well-organized form of glycocalyx is called capsule. Capsules are essentially constituted of polysaccharides (to a lesser extent proteins) tightly packed to the cell wall by a covalent bond.[2] Bacteria that possess a capsule are called encapsulated. This surrounding shell hides the bacteria from the innate immune system by shielding the recognition of other antigens (mainly proteins) expressed on their surface. The phagocytosis is the primary mechanism activated by the immune system, when a foreign intruder penetrates the host. The capsule provides the bacterium the capacity to evade this first mechanism of defense.[3] Else, the capsule contains water and so, also prevents the bacterium from desiccation helping its survival in the highly rough conditions rules by the mammalian immune system.[4] The innate immune response is rapid (active within minutes after the infection) and antigen-aspecific. All foreign invaders that does not express self-antigen of the host are targeted and attacked. The innate immune response is therefore the first line of defense (if we leave the skin apart) in all primitive multicellular organisms.[5] This defense consists of soluble factors, such as complement proteins, and diverse cellular components including granulocytes (basophils, eosinophils and neutrophils), mast cells, macrophages, dendritic cells (DC) and natural killer cells. The glycocalyx is the theater of cell-cell interactions. It offers a cell-cell interface, a surface able to adhere to other cell surfaces. This adhesion permits a cell-cell recognition, crucial to induce the appropriate immune response, highly required for instance against the stealth ability of encapsulated bacteria. Thus bacteria cells, among others, follow a huge process of identification when they penetrate inside the organism. All antigens - an acronym standing for antibody generator - are intercepted by antigen-presenting cells. Among the different components, antigen-presenting cells (APC) - cells that can present a complexed form of the antigen on their surface - are vital for an effective immune response. DC are playing a crucial role as APCs in the global immune response. DC surface is plenty of pattern-recognition receptors (PRRs, introduced in 1989),[6] including the recently discovered Toll-like receptor family (TLRs), that can recognize a huge variety of pathogen-associated molecular patterns (PAMPs)

on the surface of bacteria.[7] PAMPs activation leads to the secretion of cytokines. Cytokines are co-stimulatory molecules (~5-20kDa) involved in cell signaling, triggering the maturation process of APCs. Like DCs, cytokines are a bridge between the innate immune response and the adaptive immune response. The innate response indeed creates the pro-inflammatory context (production of cytokines and chemokines) required for a proper activation of the antigen-specific adaptive immune response.[8]

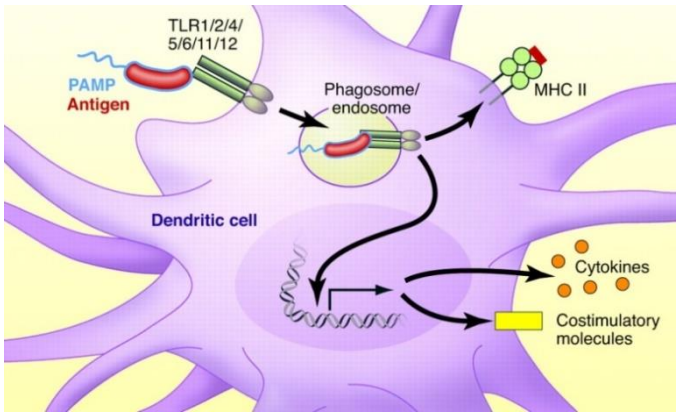
The adaptive immune response is slow but highly specific. It is mediated by B and T lymphocytes. T and B cells are a type of white blood cells and distinct lymphocyte subtypes. The antigen is presented to B cells binding the B cell receptors (BCRs) present on their surface. When properly activated by T-cell-dependent immunogens (T-independent and T-dependent immune response will be explained in chapters I-2 and I-3), B cells differentiate into plasma cells or memory B cells (MBCs). T cells are also divided in many subtypes according to their specificity. Among them we can find: T helper cells (T_H cells or also known as $CD4^+$ T cells) which assists the maturation of B cells;[9] cytotoxic T cells (T_C cells also known as $CD8^+$ T cells) designed to kill virus-infected cells and tumor cells;[10] regulatory T cells (Treg are part of the $CD4^+$ T cells population) maintain the immune homeostasis[11] and many memory T cells subtypes.[12]



scheme 1: Chart showing the development of different blood cells from hematopoietic stem cells to mature cells (cellular immune system on the left, humoral immune system on the right)[13]

According to the basic mechanisms of sensing microbial infections, activated DCs migrate to the draining lymph nodes where they encounter native T cells. The crucial event of the T cells activation-

differentiation process is the immunological synapse, initiated by formation of a ternary complex major histocompatibility complex (MHC)-antigen-T cell receptor (TCR) on T cell surface, followed by further specific interactions.[14] The antigen presentation, turnover step of this mechanism, is enabled by the MHC molecules. MHC is the general name given to the highly polymorphic glycoproteins encoded by MHC class I and MHC class II genes, which are involved in the antigens' presentation to T cells.[5]



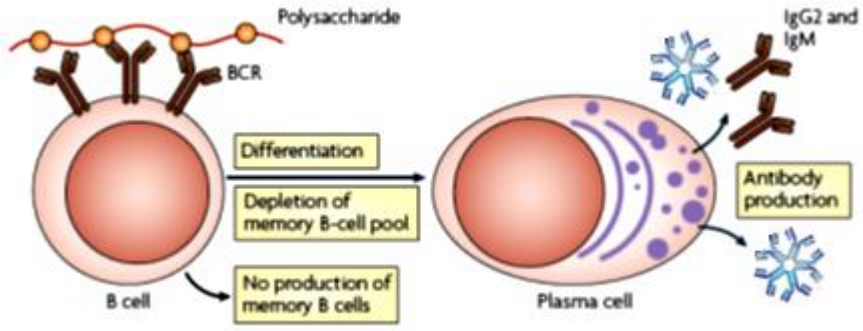
scheme 2: Cell-extrinsic recognition of pathogens. Bacteria detected by DCs through TLRs are internalized into the phagosome where bacterial antigens are processed for presentation on MHC class II. Bacterial antigens (red) and PAMPs (blue) are present in the same phagosome, which indicates to the DC their common origin. TLR-mediated recognition of bacterial PAMPs promotes the selection of bacterial antigens for optimal presentation on MHC class II. TLR signaling also leads to the induction of costimulatory molecules and cytokines necessary for activation and differentiation of T lymphocytes [8]

Now that we have set up the immunological scene, you will successively find in this thesis the explained mechanisms on the different relationships between the pairs: carbohydrate antigens - immune system, protein antigens - immune system and glycoconjugate antigens - immune system. In a second chapter, a closer look to the correlation between the immune system and vaccines will be provided. This will include overviews on how vaccines have been designed since today; the role of the added adjuvants in the vaccine recipe; the crucial role of the conjugation between the carbohydrate moiety and the associated protein in the synthesis of glycoconjugate vaccines. Then the chapter 3 will be dedicated to *Neisseria Meningitidis*, a group of bacteria responsible for an epidemic mainly in Africa and in Asia. The answers brought to prevent it will be therefore presented. Finally, the fourth chapter will detail out the chemical synthesis of the glycoconjugate vaccine against the *Neisseria Meningitidis* type A bacterium. The fifth and last chapter will be an insight of all the experimental data gathered over these three years.

2) Polysaccharide antigen immune response (T cell independent IR)

Omnipresent, carbohydrates constitute the entire shell of encapsulated bacteria like the one of *Neisseria Meningitidis*. Because of their polymeric nature and their structural similarities with common glycolipids and glycoproteins of human metabolism, polysaccharide (PS) antigens are poorly

immunogenic. Hence, the immunological response of children aged below 2 years and elderly against these plain saccharides is very weak or inexistent. Indeed, according to the nature of the antigen, two different immune responses can arise: T-cell dependent (TD) or T-cell independent (TI). While proteins and peptides usually induce a TD immune response, polysaccharide antigens induce a TI immune response. Accurately, TI antigens are again classified into TI type 1 antigens and TI type 2 antigens according to their interaction with B cells.[15] On one hand, TI type 1 are polyclonal B-cell activators able to induce mitosis on B cells and so, able to induce the production of memory B cells despite the non-assistance of T cells.[16-17] Typically, lipopolysaccharides (LPS) belong to the TI type 1 antigens group.[18] On the other hand, TI type 2 antigens, mainly represented by PS antigens, are not able to bind MHC class II required to recruit T cells. PS directly bind mature B cells by cross-coupling BCRs.[15] This mechanism results in a fast differentiation of naïve B cells into short-lived plasma B cells, able to secrete low affinity antibodies. Antibodies, also called immunoglobin (Ig), are Y-shaped proteins secreted by plasma lymphocytes (types of differentiated B cells). However, the antibodies IgG2 (subclass of IgG with extremely low binding affinity to Fc [fragment crystallizable] protein receptors of phagocytic cells) and IgM (larger antibodies with a very high avidity) secreted by the short-plasma B cells activate by TI type 2 antigens are highly unspecific.[19] Additionally, the non-production of memory B cells, essential for generating an accelerated and highly effective immune response in the presence of re-infection, reduce considerably the efficiency of this defense and even preclude a long-term protection against the bacterial infection.

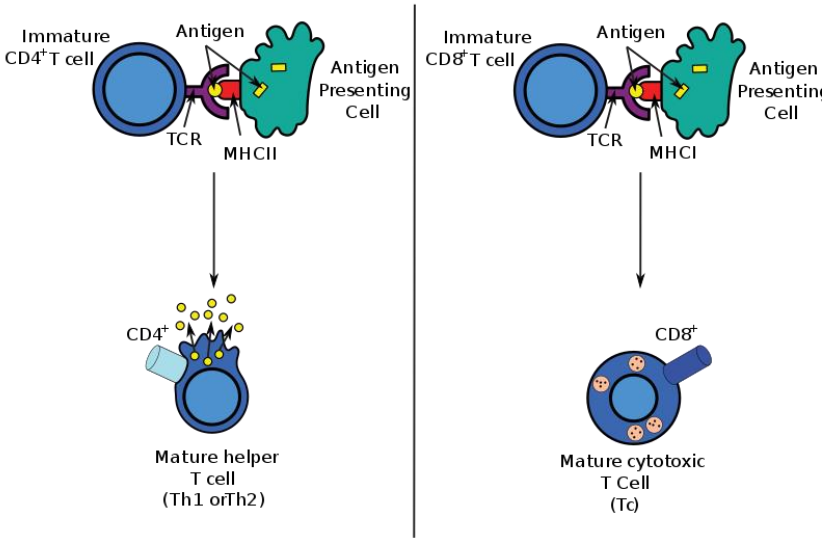


scheme 3: Mechanism of the TI immune response induced by PS antigens. The PS directly binds the B cell by a cross-coupling to the BCRs. The naïve B cell matures into a short-lived plasma B cell able to secrete low affinity IgG2 (Y shape) and IgM (star shape) antibodies

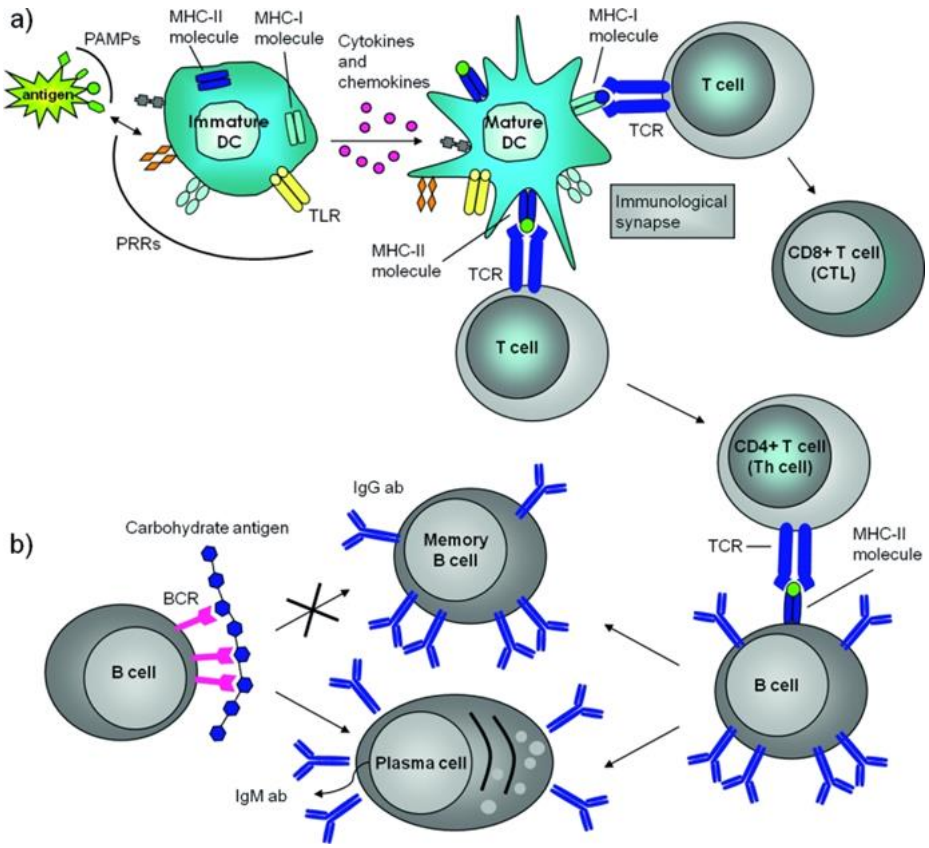
3) Protein antigen immune response (T cell dependent IR)

In contrast with the TI immune response, the TD immune response typically raised by protein antigens involves the intervention of T-lymphocytes and specifically T-helper cells (T_H or $CD4^+$ T cells). With respect to TI response, B cell activation to TD antigens takes longer time but antibodies generated have a higher affinity and an improved functionality. The TD mechanism implies the interaction of PAMPs to PRRs on the surface of immature DCs. The stimulation of the PRRs brings the necessary co-inflammatory context and thus the secretion of cytokines, regrouping smaller proteins like chemokines

(chemotactic cytokines)/interferons (IFNs)/interleukins (ILs) or lymphokines, leading DCs to evolve from immature to mature APCs. The antigen is then internalized into DC and processed in the lysosomal compartments, driving to the formation of smaller peptide fragments. The latter bind the MHC molecule and the resulting complex is shuttled to the DC surface and exposed to TCR giving the so-called immunological synapse: MHC-antigen-TCR ternary complex. In case the antigen binds MHC class II molecule (antigens derived from exogenous microorganisms, such as bacteria), T cells priming induces differentiation into T_H cells, responsible for humoral immunity, mainly characterized by the secretion of neutralizing antibodies. On the other hand, processed antigens derived from endogenous pathogens (such as viruses) are exposed to TCR by MHC-class I molecules, leading T lymphocytes to differentiate into cytotoxic T cells (CTL or T_C also called $CD8^+$ T cells or killer T cells), triggering the cellular immune response. As mentioned above, humoral immunity, by producing antigen-specific antibodies, ensures the elimination of the invading pathogen via different mechanisms including pathogen and toxin neutralization, classical complement activation, and opsonin promotion of phagocytosis and pathogen elimination. On the other end, CTLs aim to induce the apoptosis of the target cell via secretion of cytotoxins.[20] In particular, T_H cells activate B lymphocytes via an immunological synapse involving TCR-antigen-MHC II molecule (scheme 4) priming their maturation and differentiation process into short-lived plasma blasts, for immediate protection, and long-lived plasma cells and memory B cells for long-term protections. Long-lived plasma cells are class-switched and isotype-switched to secrete IgG-type immunoglobulins (IgG1 and IgG3 with a very high affinity to Fc receptor on phagocytic cells). Memory B cells instead ensure long-term protection of the host, as they are able to remain independently in the bone marrow for decades and even to regenerate themselves.[21]



Scheme 4: Mechanism of differentiation of T cells

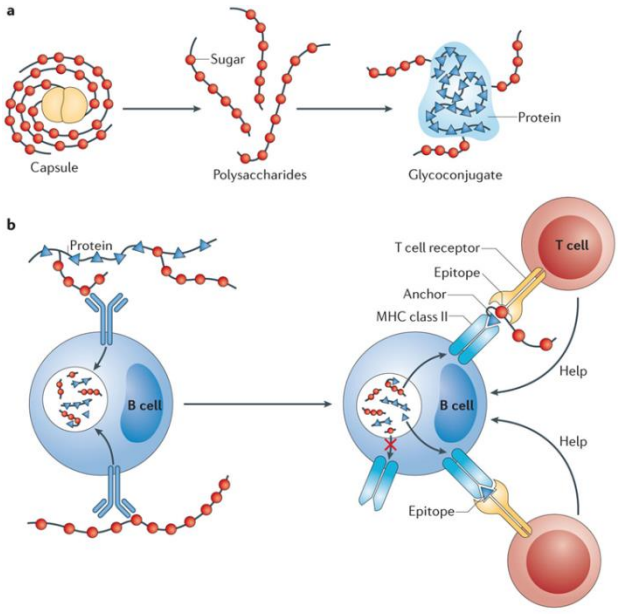


scheme 5: Diagrammatic representation of the Cell-mediated Immune Response with a **a)** TD immunogen and **b)** TI-2 immunogen[22]

4) Glycoconjugate antigen immune response

So far, we have reported the mechanism of immune response to a TI type 1 or 2 antigens and the mechanism of immune response to a TD antigen. Given their poor immunogenicity, it was crucial to find out a way to enhance the immune response against TI type 2 antigens like PSs. To this end, Avery and Goebel reported in 1931 the increased effectiveness of the immune response raised by a glycoconjugate antigen. Indeed, the poor immunogenicity of PSs can be palliated by chemical conjugation to a carrier protein.[23] The precise mechanism of activation of the immune system by a glycoconjugate antigen is still under debate. It was first theorized the peptide only-presentation, spawn of the glycoconjugate, to TCRs and its recognition by T cells.[24] This model is based on studies of haptens conjugated to carrier proteins, highlighting the so-called "carrier effect" dated from the seventies. The "carrier effect" can be described by an interaction between two classes of cells: the one producing helper lymphocytes and the other one providing antibody secretors. Mitchison underlined the "intrinsic" potency of the carrier and thus the ability of various hapten-protein conjugates, prepared from the same hapten covalently bound to different carrier proteins, to elicit an anti-hapten response.[25-26] Proteolytic

enzymes digest the protein component and chop it into peptides, which bind the MHC-II to be presented on the surface of APC to T_H cells. The TD-immune response occurs, T_H cells assist on the production of the desired anti PS immune response. Highly avid immunoglobulin IgM class-are converted into PS-specific IgG. In accordance to the mechanism explained in the previous paragraph, highly efficacious PS-specific antibodies are produced in a TD fashion targeting the carbohydrate portion of the glycoconjugate that directly cross-primes the B cells by BCRs.[27] Substantially, this mechanism was based on the non-binding of the carbohydrate antigen to the MHC-II. However, the strong covalent bond of the protein with the carbohydrate moiety is rarely cleavable in the endosome of the B lymphocytes and nothing has proved so far that the carbohydrate antigen is not recognized by the T cells.[28] Therefore, a novel mechanism was proposed by Kasper and coworkers, suggesting that not only peptide but also glycopeptide fragments are produced following uptake and processing by APCs. Accordingly, a specific binding does occur between glycopeptide antigens and MHC-II, giving rise to a new T cell clone, labeled T_{carbs} which are able to induces the desired TD-immune response against the carbohydrate portion of the processed glycopeptide.[28-31]



scheme 6:

a. schematic representation of a glycoconjugate antigen

b. immune response mechanism over glycoconjugate antigens: the PS moiety binds B cells by BCRs in a direct cross-coupling. The covalently attached peptide is digested by enzymes in the endosome and cleaved into peptides. Peptides activate the complex MHC-II and afford the presentation of the glycoconjugate antigen (covalent protein linker rarely cleaved) to T cells with the complex B cell-MHC class II-peptide-protein spacer-carbohydrate-TCR-T cell. T cell are activated and T_H are elicited to induce a TD-immune response. These T_H cells thus recognizing and binding the carbohydrate antigen are called T_{carbs}. The second complex drawn just below represents the "traditional" activation of T_H cells by the only presentation of the peptide: complex B cell-MHC class II-protein-TCR-T cell[32]

Before detailing out the History and the design of vaccines, a brief mention should be devoted to Zwitterionic polysaccharides (ZPs). While most of bacterial PS are neutral or either negatively charged, ZPs are carbohydrates containing both positive and negative charges within a single repeating unit (zwitterionic unit). They were expected to act as PS-antigen but, surprisingly, the zwitterionic motif allows ZPs to bind MHCII.[33] From this point of view, ZPs behave as TD antigens. However, much more investigations are needed to clarify the peculiar immunological profile of ZPs and to prove they are able

to induce a TD-immune response by rising $CD4^+$ T cells and eliciting the essential memory response.[27, 34]

Next, the chapter II will be dedicated to the story of vaccines and the new strategies employed for their design. Especially it will be interesting to point out the optimizations brought to the formulation of vaccines like adjuvants, multivalence or nanoparticles.

II- Global formulation of vaccines

1) A brief history of vaccines

To introduce the MenA glycoconjugate vaccine, a word must be written first on the early stages of vaccination. In 1979, smallpox was eradicated thanks to a global campaign of vaccination. Vaccines were first formulated empirically according to the following "3i" procedure: isolation of the antigen, inactivation of the pathogen and injection of the immunogenic fragment. Basically, a wide range of vaccines currently on the market is still answering to this 3-step process (Varicella zoster virus, Rotavirus, Seasonal Influenza virus…). Despite the lack of data on how vaccines actually act, their efficiency is no more contestable as statistics of load cases talk straight: tetanus (Clostridium tetani bacterium),[35] diphteria (Corynebacterium diphtheriae bacterium),[36] pertussis (Bordetella pertussis bacterium),[37] tuberculosis (Mycobacterium tuberculosis bacteria),[38] poliomyelitis (Poliovirus)[39-40] etc. Even if these diseases are not considered eradicated as smallpox is,[41] vaccines have induced a crucial effect of herd immunity, able to almost reach the eradication state on the targeted diseases. However, the regular rise of new pathogen agents urges researchers to find new strategies to increase the potency of vaccines. Thanks to emerging technologies the following parameters are aimed: better determination and delivery of antigens, development of highly specific adjuvants, conjugation methods, methods of production and higher safety of vaccines[42] (figure 1).

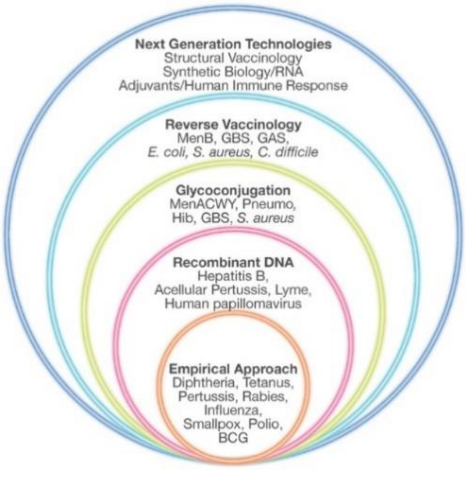


figure 1: from empirism to a rational design of vaccines [43]

First of all, mother of vaccination, the variolation was a (very) empirical method carried out in China and then in Europe over centuries to prevent from smallpox. The smallpox vaccine came out centuries after. Based on the works of Edward Jenner (1749-1823) and Louis Pasteur (1822-1895), it was made from an attenuated modified vaccinia virus (MVA).

From the XIX° century to the early XX° century, vaccines were developed according to the "3i" approach described above. Yet, the traditional approach *in vitro* culture, growth and inactivation, does not suit numerous pathogens like, for instance, hepatitis B virus (HBV). Instead, in the 70's Maurice Hilleman introduced a new vaccine formulation against the HBV. Named viral subunit vaccine or "split" vaccines, the antigenicity and the immunogenicity of this new generation of vaccines are based on

fractions of the virus, defined as virus-like particles (VLPs), extracted out of the plasma of infected patients. After purification and inactivation, VLPs were used as immunogens for the formulation of an anti-HBV vaccine.[44] This modern method is also an alternative used for the formulation of a trivalent vaccine against the seasonal flu, based on two proteins isolated from three strains of the virus, the haemagglutinin (HA) and the neuraminidase (NA).[45]

Noticeably being a very risky approach due to the manipulation of such living organisms, subunit vaccines were therefore improved by arising DNA technologies. Safer, recombinant DNA vaccines lay on cloning the gene responsible of encoding the antigen surface of HBV. Therefore, it results in the first formulation of a vaccine without an *in vitro* culture thanks to the formation of synthetic VLPs endowed with a comparable antigenic character.[46] Unfortunately, the immunized host runs the risk of an integration of DNA plasmid into its genome. Thus, *de novo* DNA vaccines are (m)RNA-based vaccines, safer from this point of view and very promising when used with virus particle to increase drastically its antigenicity. The mechanism of action of this kind of vaccines, however, still requires further explorations, such as a proper definition of the role of miRNA as intercellular messenger.[47]

Besides, up-to-date advances on DNA sequencing pioneered in the 90's led to the discovery of a wide range of new antigenic structures, thus a new strategy for vaccine formulation came up, called reverse vaccinology (RV).[48] For instance, the first vaccine developed according to RV was addressed to *Neisseria Meningitidis B* because of the polysialic structure of the MenB CPS, a motif omnipresent on mammalian cells and therefore tolerated by the immune system as self-immunogen. Homologous to natural human glycoproteins, the DNA sequencing allowed the identification of heterogenous epitopes specific to the encapsulated bacterium.[49] Over the last decade, the RV has been improved from the study of the genome to the study of the immune response. Indeed, the RV 2.0 is based on the study of B lymphocytes and the antibodies they are producing to neutralize the pathogenic antigen.[50] Hence, an experimental and computational cartography of epitopes is constantly expanding to optimize vaccine antigens.[51]

The current state-of-the-art is related to the vectorization of antigens. This approach particularly suits as a solution to the prevention of viral diseases against which no vaccine is available or badly non-efficient (HIV, TB, Malaria…). The global idea lays on heterologous genomic fragments (like nucleic acids) leading to the expression of proteins inside DCs able to induce a suitable immune response following the infection caused by the same antigen. Viruses are generally defective and able to carry only one cycle of cellular replication.[52] The vaccine against the Ebola virus disease (EVD), for instance, is rationally designed according to this approach. Indeed, here the immunogen is the replication-defective recombinant chimpanzee adenovirus type3-vectored Ebola virus vaccine which encodes wild-type glycoprotein antigens and proved to provide an efficient protection against the disease in nonhuman primate model. The antigen-vectored based vaccine is currently in phase 1 clinical evaluation.[53]

Likewise, a viral vector-based vaccine-type is addressed to the prevention of human immunodeficiency viruses (HIV)[54] or also to the prevention of tuberculosis (TB)[55] in order to improve the historical live attenuated bacteria-based vaccine bacille Calmette-Guérin (BCG) which provides only a short-term protection.

Now that we have briefly overviewed the evolution of vaccine design[56] over a broad spectrum of pathogens, the same study will be applied on encapsulated bacteria like *Neisseria Meningitidis*. Indeed, an identical evolution specific to polysaccharide vaccines, from empiricism to rational design, can be observed.

2) The rational design of glycoconjugate vaccines

Diverse pathogens expose on their surface a dense, often highly conserved array of glycan structures that exert a protective function against the host's immune defense and are essential for their pathogenicity. Typical examples are lipopolysaccharides (LPS) of Gram-negative bacteria, the polysaccharide coat (capsular polysaccharides, CPS) of encapsulated bacteria, and glycoproteins/glycolipids specifically expressed on the surface of viruses and parasites. All these glycoforms are capable of interacting with the immune system as epitopes inducing the production of carbohydrate-specific antibodies. Despite their poor immunogenicity (cf. chapter I-2), they therefore represent attractive targets for vaccine design. Hence, the first generation of vaccines against encapsulated bacteria was based on polysaccharide vaccines. Based on the heterogeneous structure of their CPS, the specific saccharide antigen of each serogroup is extracted, purified and eventually injected in the patient to induce an immune response against the bacterium, considering the serogroup CPS targeted immunogenic at least in adults.[57] These multi-serotype polysaccharide-based vaccines have been used for decades for both *Streptococcus pneumoniae* and *Neisseria Meningitidis*. However, as detailed above, saccharides are TI type 2 antigens, unable to stimulate the protein complex MHC-class 2, and so unable to raise memory B cells.[17] Nevertheless, they provide an efficient short-term protection in adults. For instance, the 23-valent saccharide-based vaccine against the pneumococcal disease revealed an efficient 90% coverage rate.[58] Plain polysaccharide-based vaccines have been finally proven too much limited particularly because of their inefficiency in children aged below 2 years and elderly (older than 65 years), which are the population most exposed and affected by infectious diseases and need higher coverage.[59-60] Last but not the least, the lack of memory response inhibits any chance to induce herd immunity.

To palliate these severe drawbacks, glycoconjugate vaccines came up as a new generation of saccharide-based vaccines, able to elicit a long-term memory immune response and efficient in young children the.

The first licensed conjugate vaccine was based on purified *Haemophilus influenzae* type b (Hib) PS linked to CRM_{197} (cross-reactive material), a nontoxic mutant of diphtheria toxin.[61-62] As detailed above (cf. chapter I-4), unlike free PS (TI type 2 antigens), glycoconjugates behave as TD antigen. This phenomenon, known since 1929 and first mentioned by Avery and Goebel[63] has led to the formulation of glycoconjugate vaccines that rapidly proved their efficacy in terms of prevention of the population from the targeted pathogen and in terms of effective herd immunity.[64] Therefore the research and development of glycoconjugate-based vaccines has been greatly encouraged, despite the historical hurdles[65] and the contemporary opposition to vaccine policy.[66] A famous historical fact about vaccination is the unfortunate infection of Winston Churchill by *Hepatitis B Virus* blood-contaminated needles during a massive vaccination campaign against the Yellow fever in 1942.[67]

To increase the potential of glycoconjugate vaccines, the following parameters have been defined as key to influence the immune response induced by the glycoconjugate antigen: the nature of the spacer and the protein carrier, the glycan/protein ratio, the chain length of the glycan, the conjugation strategy (random conjugation *vs* site specific conjugation) and the chemistry employed for the conjugation.[68]

First, the choice of the antigen depends on the pathogen. For instance, concerning bacteria, the O-antigen of LPS or the capsular polysaccharides of encapsulated bacteria are extracted from the cell, purified and employed for protein conjugation. Directly correlated to the antigenicity of the glycoconjugate vaccine is the size of the saccharide conjugated to the protein. Indeed, a minimum size is required from the PS fragment to raise functional, carbohydrate-specific Abs. Likewise, since the aim is a specific immune response against the polysaccharide, the optimal carbohydrate density (carbohydrate/protein ratio) must be determined, such as to avoid the carrier-induced-epitope suppression. This phenomenon is caused by pre-existing immunity to a carrier protein that may suppress the immune response to a hapten or saccharide linked to the same carrier, thus impairing the hapten or (poly)saccharide immune responses. On the other hand, what is displayed from MHC-II complex are peptide fragments generated by processing of the protein into APCs. Therefore, an unbalanced ratio with an excess of PS can interfere with this display and hinder the following activation of T cells.[69] Likewise, conformation, shape, charges, etc. impact on the presentation of the epitope and so on the immunogenicity of the glycoconjugate.[70]

Furthermore, the choice of the protein carrier, second component of the glycoconjugate, is equally crucial. The following immunogenic carrier proteins can be used for glycoconjugate vaccine design: diphteria toxoid (DT), nontoxic cross-reactive material of diphteria toxin (CRM_{197}), tetanus toxoid (TT), keyhole limphet hemocyanin (KLH), the outer membrane protein C of *N. meningitidis* (OMPC), bovin serum albumin (BSA) and more recently protein D (PHiD) derived from non-typeable *H. influenzae*. This hapten-carrier association, aimed to increase the immunogenicity of the PS antigen, is still deeply studied to raise the most carbohydrate-specific antibodies acquired during natural infection[71] (table 1).

Carrier Protein	Description
Diphtheria toxoid (DT)	The formaldehyde-treated nontoxic form of diphtheria toxin is derived from certain strains of Corynebacterium diphtheria. DT is a single polypeptide chain with a molecular weight (MW) of 62 kDa and was used as a carrier protein in the first licenced glycoconjugate vaccine, ProHibit® against Hib. To date, only a single licensed vaccine (Menactra®) uses DT as a carrier protein.
Tetanus toxoid (TT)	The formaldehyde-treated nontoxic form of tetanus toxin is isolated from Clostridium tetani. TT has two polypeptide chains (53 and 107 kDa) linked by a disulphide bond to form a 150 kDa polypeptide. It has 106 amino groups, 10 sulfhydryl groups, 81 tyrosine residues, and 14 histidine groups and is currently used as a carrier protein in a number of licenced glycoconjugate vaccines against Hib, *N. meningitidis* and *S. pneumoniae*.
Diphtheria toxin derivative (CRM197)	An enzymatically inactive and nontoxic mutant of diphtheria toxin. CRM197 has a single point mutation (Gly52 → Glu52) with a MW of 63 kDa, possesses 40 lysine and 14 histidine residues. It is a widely used carrier protein in licenced glycoconjugate vaccines.
Outer membrane protein (OMP)	A complex formed from five major classes (46, 41, 38, 33 and 28 kDa) of outer membrane proteins derived from *Neisseria meningitidis* serotype B1 and is used in the PedaxHib® vaccine against Hib.
Protein-D (PHiD)	A nonlipidated cell surface protein (42 kDa) derived from non-typeable (unencapsulated) Haemophilus influenzae comprised of 346 amino acids. PHiD is used in Synflorix® vaccine against *S. pneumoniae*.
Keyhole limpet hemocyanin (KLH)	An extremely large, multi-subunit, oxygen-carrying metalloprotein (4.5×105 to 1.3×107 kDa) derived from the keyhole limpet Megathura crenulata (a marine mollusc). KLH carries chelated copper of non-heme origin. With a MW of 5×106, KLH contains over 2000 amines from lysine residues, above 700 sulfhydryls from cysteine residues, and more than 1900 tyrosine residues. It is used as a carrier molecule in preclinical studies but has not been included in licensed vaccines due to problems in defining its structure, poor solubility and toxicity caused by trace metals.
Bovine serum albumin (BSA)	BSA is single polypeptide chain (67 kDa) derived from cattle and is less immunogenic than KLH. BSA has 59 amine groups of which 30-35 are primary amines that are reactive during conjugation process. Moreover, one free cysteine sulfhydryl group, 19 tyrosine phenolate residues, and 17 histidine imidazole groups are also present. The many carboxyl groups in BSA give a net negative charge to it. It is used as a carrier protein in preclinical studies.

table 1: Common carrier proteins in glycoconjugate vaccine development[72]

In many cases, the selected carrier protein is covalently linked to the carbohydrate thanks to a spacer. In this way, site-selective chemical strategies are applied to the carrier to improve the conjugation. Furthermore, protein site-selective modifications proved to bring great improvements regarding the stability, the homogeneity and the flexibility of glycoconjugates.[73-74]

The optimal PS-protein carrier association resides also in the choice of the spacer, that will covalently bind the two entities. The optimal conformation of the glycoconjugate should expose at best the antigenic epitopes, leading to the best binding affinities with antibodies. In this way, the synthetic protein spacer should respect the space conformation of the whole glycoconjugate complex. Hence the length of the spacer might be the foremost solution to ease the steric hindrance faced in the case of a massive carbohydrate antigen. The structural puzzle lays on the flexibility of the complex, dictated by the length of the protein spacer.[75] Synthetic spacers are classified as either homobifunctional (same functional group at both ends) or heterobifunctional (different and orthogonal functional group at each end). The choice of one over another may rely on the chemistry used for the conjugation. With an homobifunctional linker, both terminal ends have the same reactivity, while in a heterobifunctional spacer the two terminal functional groups react under well different conditions.

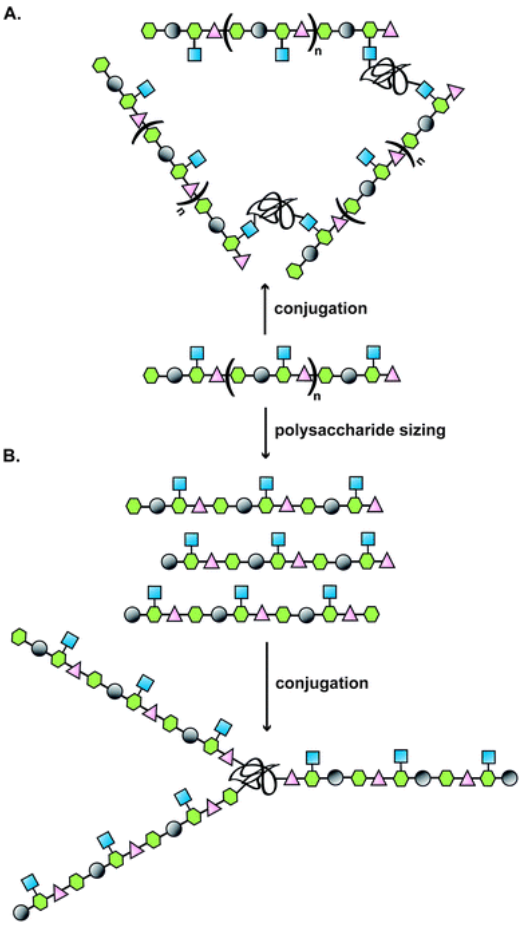


figure 2: classical approach to the design of a carbohydrate-based vaccine using polysaccharides extracted from biological sources; (A) direct conjugation to carrier protein; (B) polysaccharide sizing followed by end terminal conjugation of generated oligosaccharides [70]

Two general methods of conjugation are known: single point attachment or cross-linked lattice method. Earlier in the 80's, Roy and coworkers pioneered a single point attachment highly efficient strategy for the coupling of oligosaccharides to protein by reductive amination, where the anomeric carbon of the reducing carbohydrate residue reacts with the lysine (amino group of the side chain) of the carrier protein.[76] More modern lattice-type conjugates are highly immunogenic compared to neoglycoconjugates and the multiplicity of the conjugation methods has undoubtedly led to an equal diversity of efficient vaccines. Indeed, since the first covalent conjugation carried out by Avery by diazotization,[77] many new coupling methods have arisen, involving various functional groups and therefore providing a wide library of procedures. Not exhaustively listed out: the reductive amination (reaction of an amine with an alcohol softly oxidized to the corresponding aldehyde), the cardodiimide-mediated condensation (condensation of a carbodiimide with a cyano-ether), the cyanylation (condensation of an amine with a cyano-ether), the thioalkylation or the thioether method (reaction of a thiol with a bromo-*N*-alkylacetamide), the active ester method (condensation of an amine with a derivative of a *N*-hydroxysuccinimidyl ester), the thiol-maleimide addition (Michael addition of a thiol group on a maleimide moiety), and finally the Huisgen azide-alkyne [3+2] cycloaddition (click reaction of an alkyne moiety with an azide functional group); represent a good sample of nowadays' coupling methods.[72]

Despite their great success, glycoconjugate vaccines might encounter some practical pitfalls. Indeed, the challenging isolation and purification of the PS from the natural source is exacerbated by the poor amount of material available. Moreover, the heterogeneity of naturally occurring glycans makes the conjugation also tricky. Finally, the low batch-to-batch consistency of glycoconjugates encouraged the development of glycoconjugate vaccines based on fully synthetic carbohydrate antigens. Synthetic carbohydrate antigens conjugated to protein carriers portray the third generation of polysaccharide vaccines. Besides a higher reproducibility of the biological properties and a better safety profile than the natural carbohydrate antigen, the fully synthetic generation allows for the introduction of specific

chemical modifications, giving the access to structural analogues. The next chapter illustrates the advantage of using carbohydrate analogues for the formulation of more efficient anti-*Neisseria Meningitidis* A glycoconjugate vaccines.

3) Role of adjuvants, methods of production and safety of vaccines

Despite all the efforts provided to optimize their antigenicity, non-living vaccines are often poorly immunogenic and may require a help to provide the adequate protection. In this regard, immunostimulatory molecules known as adjuvants (from the Latin word *adiuvare* meaning 'help') can be the key of a more effective vaccine. Adjuvants are nowadays rationally designed with the aim to satisfy definite parameters (**BOX 2**).[78] According to their structure, properly designed adjuvants are able either to enhance the innate immune response or the adaptive immune response. This structure-function relationship has been considerably improved by the recent discovery of Toll-like Receptors (TLRs) displayed on the surface of APCs, that interact directly with some adjuvants.[79-80]

BOX 2

Why adjuvants are included in vaccines

1. Lower antigen dose in vaccine.
2. Increase breadth of response – heterologous activity.
3. Enable complex combination vaccines, overcome antigenic competition.
4. Overcome limited immune response in some populations – such as the elderly, young children, chronic diseases and immunocompromised.
5. Increase effector T cell response and antibody titers.
6. Induce protective responses more rapidly.
7. Extend the duration of response by enhancing memory B and T cell responses.

To date, only a handful of adjuvants are licensed. Indeed, the adjuvants need to meet strict requirements (**BOX1**),[81] most of the time not fulfilled. Alum is the first adjuvant used in human vaccines. Discovered in the 20's by Glenny and coworkers,[82] Alum consists in insoluble aluminum salts. Paradoxically, despite its extensive use, the mechanism of action of Alum remains an unsolved puzzle, even after 90 years. Without detailing the state of the art that is still very fuzzy,[83] Alum adjuvants have been identified as potent immunostimulants in vaccines.[84-86] The safety of which has also

Box 1. Required properties of an adjuvant

- Is non-toxic or has a negligible toxicity at the dose range for effective adjuvanticity
- Stimulates a strong humoral and/or T cell immune response
- Provides good immunological memory or long-term immunity
- Does not induce autoimmunity
- Is non-mutagenic, carcinogenic or teratogenic
- Is non-pyrogenic
- Is stable under broad ranges of storage time, temperature, and pH

been dissected and secured.[87] In some glycoconjugate vaccines, specific adjuvants can be covalently linked as immunoadjuvants and replace the immunogenic protein;[70] a strategy particularly employed for the formulation of TACAs (Tumor Associated Carbohydrate Antigen)-based vaccines.[88]

In conclusion, since the discovery of TLRs, the design of new adjuvants helps in eliciting the appropriate sustained antibody response which also represents a valuable strategy to overcome age-related hypo-responsiveness to vaccination.[43]

Although the efficiency of vaccines is now unquestionable, however there is still a profound lack of information about the mechanisms of interaction of the immunogens present in human vaccines with the mammalian immune system. As a consequence, the safety of vaccines relies on a continuous monitoring of the ones on the market, according to a clinical phase III that basically never stops. Moreover, the modern formulation of vaccines for tomorrow takes into consideration the hurdles pointed out yesterday.[89] For instance, the need of a single formulation of a multivalent vaccine to avoid repetitive injections (Arthus reaction) of different serotypes for the same bacterium, has been reported early in the 80's.[58] Also, modern formulations of synthetic glycoconjugates vaccines are directed towards automated synthesis, which is safer, more reproducible and more suitable to an industrial development.[90-91]

Following these lines about the evolution of vaccine design over the decades, an obvious conclusion can be drawn: the more we know about the human immune response the best we can tackle new pathogens. Once the pathogen is identified and the epitope well-defined, optimizations can be brought to the formulation and to the immunological properties of the vaccine.

III- *Neisseria Meningitidis* serotype A and its vaccines

1) The epidemiology of MenA

Neisseria Meningitidis is an anaerobic encapsulated Gram-negative diplococcus, only hosted by mankind. In its asymptomatic form (carrier state) it colonizes the nasopharynx. MenA structure is constituted of an inner membrane and an outer membrane composed by peptidoglycans. The capsule itself is essentially constituted of PS that stand as the antigen and so, the source of virulence of the pathogen (cf. chapter I-2). Among the 13 serotypes identified so far, six (A, B, C, W, X and Y) are mostly responsible of the death cases according to group B (51%), C (24%) A (18%), Y (2%), W (1%) and 3% was not identified. All of them can cause epidemics, serotype A is the main accountable for epidemic meningococcal infection in Africa and Asia.[92] The bacterium spreads by respiratory or throat secretions. Cases of meningitis are typically sporadic, and outbreaks, by the same isolate, occur in close communities over a short period of time. Nevertheless, meningitis is a severe disease with a high fatality rate (up to 50% when untreated) and high frequency (more than 10%) of severe sequelae.[93] Early antibiotic intake can treat the infection and improve the outcomes. However, the increased occurrence of antimicrobial resistance has promoted the vaccination to become the most cost-effective strategy of protection from infectious pathogen, according to the WHO.[94] However, to each serotype is associated a different capsule, thus a different carbohydrate antigen. Such a highly heterogeneous array of glycan structures makes it impossible the formulation of a global 13-valent Meningitis vaccine, therefore only oligovalent antimeningococcal glycoconjugate vaccines, including the CPS of the most virulent serogroups, have been licensed so far.

Year	Event
1996	250,000 cases of meningitis are reported in Africa, with 20,000 deaths—the largest outbreak ever recorded
2000	The WHO and national ministries of health in Africa and the eastern Mediterranean endorse a plan to introduce a conjugate vaccine and eliminate meningitis epidemics in Africa
2001	The Gates Foundation awards $70 million to PATH and the WHO for the development of meningitis vaccines for Africa; discussions are held with African public health officials, setting a cost ceiling for a dose of the vaccine at fifty cents
2002	With no pharmaceutical companies interested in developing the vaccine, MVP explores alternative strategies
2003	The Center for Biologics Evaluation and Research initiates the transfer of technology to the Serum Institute of India
2004	PATH enters into a long-term sublicense and supply agreement with the Serum Institute to develop, test, and produce clinical and commercial lots of MenAfriVac at a target price of forty cents per dose
2005	Standard operating procedures are finalized for enhanced meningitis surveillance in the twenty-five countries in the meningitis belt
2006	The Serum Institute completes construction of a new building where the vaccine will be produced; the Phase I clinical trial shows that the vaccine is safe and effective
2007	The Phase II trial reports that the vaccine is safe and highly effective; additional Phase II and Phase II/III trials are launched
2008	A Phase II infant trial is launched in Ghana; the meningitis investment case is approved by the GAVI Alliance's board, and $84 million is released ($55 million for epidemic response and $29 million to support the vaccine's introduction in Burkina Faso, Mali, and Niger)
2009	The Serum Institute applies for a license to sell the vaccine in India; MVP applies for approval from the WHO
2010	The WHO prequalifies the vaccine; on December 6, a mass vaccination campaign is launched in Burkina Faso; one week later, vaccination campaigns begin in Mali and Niger; more than 19.5 million doses are given before the end of the year

table 1: Timeline of the Meningitis Vaccine Project MVP[95]

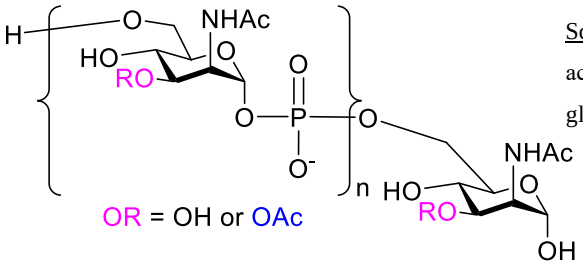
For instance, concerning the serogroup B which accounts by itself alone for half of the total *Neisseria Meningitidis* caseloads, the licensed multicomponent meningococcal serogroup B vaccine (4CMenB; Bexsero®) was developed from reverse vaccinology (cf. chapter II-1).[96] More recently, a new anti-MenB vaccine based on two recombinant lipidated factor H binding protein (Trumenba®, Pfizer) has been licensed by FDA (Food & Drug Administration) and approved for use in EU countries in 2017.[97] The first tetravalent A, C, W and Y glycoconjugate vaccine was manufactured in India as a polysaccharide-diphteria toxoid conjugate and licensed in 2005. Then a second tetravalent vaccine conjugated to CRM_{197} protein carrier was licensed in 2010. In addition, three other tetravalent meningococcal A, C, W and Y conjugate vaccines have been commercialized since today and will be detailed out hereafter.

In 2001 the philanthropic foundation Bill & Melinda Gates (Seattle, USA), awarded a 70 million dollars grant to a partnership with WHO-PATH (World Health Organisation-The Partnership for Advanced Technologies in Healthcare services) for the Meningitis Vaccine Project (MVP),[98] to identify and formulate the future monovalent glycoconjugate vaccine MenAfriVac[99] against MenA. The timeline of the MVP is shown in table 1.[95] The result of this endeavor has been the so-called MenAfriVac, a monovalent conjugate of the MenA CPS with the Tetanus Toxoid as immunogenic protein (PsA-TT). The glycoconjugate vaccine PsA-TT was widely administered in the sub-Saharan region of Africa called Meningitis belt to control the epidemics of MenA. This massive vaccination campaign started in 2010,[100-101] on people aged from 1 to 29 years old, expecting the rise of a phenomenon of herd immunity afterwards. The vaccine is amazingly cheap, around US$ 0.60 per dose while other meningococcal vaccine prices range from US$ 2.50 to US$ 117.00 per dose (indicative prices according to the data provided by UNICEF, PAHO and CDC). After careful follow-up[102] and computational modeling to improve the immunization strategy on the long-term,[103-104] the results today are clear-cut: the caseload of MenA has plummeted to zero in 16 countries that used MenAfriVac.[105] Only decreasing cases of carriage remain and will require more time to be treated, which will also promote *a posteriori* the phenomenon of serogroup replacement, already anticipated in 2011.[96, 106] Nevertheless, cases of MenA are still detected in developing countries, in Asia and Minor Asia.

2) Instability of the MenA CPS structure: introduction of analogues

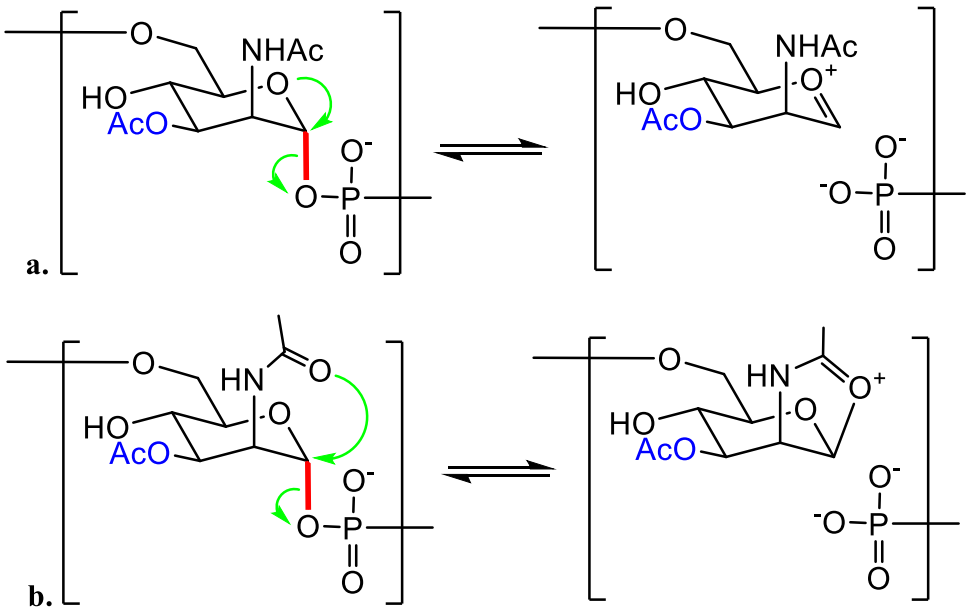
Since 2010, the MenAfriVac has greatly protected population from *Neisseria meningitidis* A in epidemic zones. The vaccine is made out from the extraction and purification of the antigenic PS that constitutes the capsule of the bacterium subsequently covalently linked to the immunogenic protein carrier CRM_{197}. The formulation of MenAfriVac followed the traditional procedure: hydrolysis of the PS to oligosaccharide fragments, then reductive amination of the reducing terminus to afford the

corresponding amino oligosaccharide which is finally coupled to the protein to get the desired conjugate.[107] However, the structure of the natural MenA CPS, composed of *N*-acetylmannosamine repeating units interconnected by α-(1-->6) phosphodiester bridges (scheme 1), suffers from chemical lability in water. Only one anti MenA vaccine in liquid formulation (Menactra®) has been licensed by Sanofi Pasteur and is currently on the market. Menactra® is a tetravalent MenACYW vaccine with a one-year shelf life when stored at 2-8°C and cannot be frozen.[108] Globally, the intrinsic instability of the MenA CPS highly shortens the shelf life of the vaccine that must be rigorously stored at low temperature.



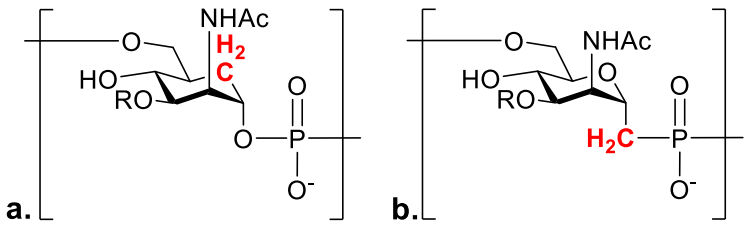
Scheme 1: Structure of the natural MenA CPS, oligomer of *N*-acetylmannosamine repeating unit interconnected by an α-(1-->6) glycosidic phosphodiester bridge.

A solution to address the chemical lability of MenA CPS is the formulation of the glycoconjugate vaccine in lyophilized form, which renders it far more stable. Indeed, in most cases, anti MenA vaccines are finalized with a freeze-drying process. For instance, the quadrivalent MenACYW licensed by GSK, named Menveo®, is constituted of liquid MenCYW and lyophilized MenA strain, and it has a life expectancy of 8 hours at 25°C once the MenA conjugate is reconstituted. The tetravalent MenACYW Menomune®, formulated by Sanofi Pasteur out of a lyophilization process, can be maintained at 2-8°C over 3 years or up to 6 weeks at a temperature of 60°C.[108] However, the hurdles encountered in their tedious administration[109] urged the development of a fully liquid formulation leading to the production and distribution of the vaccine in classical syringes, thus greatly facilitating the administration. To this end, the mechanisms of degradation of the natural MenA CPS must be explained. Two mechanisms contribute to the cleavage of the α-glycosyl phosphodiester bond, either thanks to the delocalization of the lone electron pair from the endocyclic oxygen to the anomeric carbon C_1 (scheme 2.**a**) or either by the assistance of the axially oriented acetamide at the position C_2 (scheme 2.**b**). The attack of the oxygen of the acetamide consequently leads to the formation of a thermodynamically stable oxazoline.



Scheme 2: Mechanisms of cleavage of the α-(1-->6) glycosyl phosphodiester bridge. **a**) the endocyclic oxygen promotes the cleavage of the C1-O1 bond due to the acetalic character of native MenA CPS **b**) the cleavage of the same bond is facilitated by the assistance of the axially oriented -NHAc group

Hence, to break the acetalic character of the natural MenA CPS, we can bring a specific chemical modification to obtain a fully synthetic carbohydrate antigen (cf. II-2). Two main approaches can be envisaged to improve the stability in water of the glycosyl phosphodiester linkages: we can either replace the oxygen of the pyranose ring with a methylene group affording the *carba*-analogues (scheme 3.**a**) or replace the anomeric oxygen of the phosphodiester again with a methylene group to obtain the *C*-phosphonate analogues (scheme 3.**b**).



Scheme 3: **a**) *Carba* analogue of the natural MenA CPS, **b**) Phosphono analogue of the natural MenA CPS, -OR= -OH or -OAc

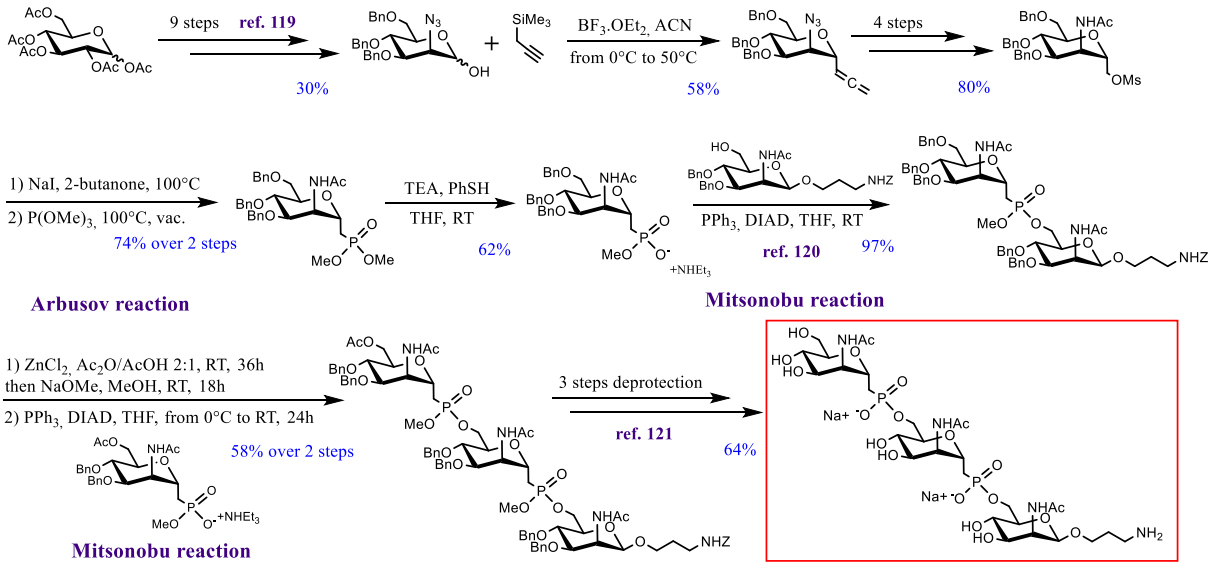
The synthesis of the first "pseudo-sugar" was published by McCasland and coworkers[110-112] in the 1960's. Concerning MenA, the first glycoconjugate using synthetic oligosaccharides with a natural structure (up to the trimer) was published by Pozsgay and coworkers.[113] Obviously, as explained above,

such structures are chemically labile in water. Meanwhile, the development of a *carba*-sugar was being undertaken, as described by Odón Arjona and co-workers.[114] Finally, the first MenA *carba* analogue was synthesized by Lay and co-workers.[115] Besides, *C*-phosphonates have been proposed as metabolically stable analogues of natural *O*-phosphates[116-117] and their first synthesis was also proposed by Lay and co-workers.[118]

3) MenA analogues: State of the Art and Immunogenicity

Regarding both MenA analogues, the state of the art has been the total synthesis of CRM_{197}-*carba* and phosphono glycoconjugates, up to the trimer.

The formulation of the MenA phosphono analogues was described up to the dimer and found hydrolytically stable. Torres-Sanchez *et al*. reported the formation of the MenA *C*-phosphonate feature via Arbuzov reaction and its elongation up to the trimer via a Mitsonobu reaction, ready for protein conjugation[119-121] (scheme 4).



Scheme 4: total synthesis of the [Aminopropyl (2-acetamido-2-deoxy-α-D-mannopyranosidyl) *C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonyl] *C*-(2-acetamido-2-deoxy-α-D-mannopyranosyl)methanephosphonate disodium salt, *C*-phosphonate trimer (red squared)

Soon after, the group of Oscarson also described a similar synthesis of the *C*-phosphono analogue up to the tetramer.[122] More importantly, the competitive ELISA assay of the synthetic phosphono analogues showed that a human polyclonal anti-MenA serum can recognize the phosphonoester-bridged fragments.[121] Also, the modeling study with a DFT approach of the MenA phosphonate fragment

exposed the suitable $^4C_1$ conformation displayed by both the analogue and the natural MenA structure. Hence the methylene group does not induce any significant modification of the conformation and comforts the potential of the *C*-phosphonate as an antigen for the formulation of a MenA glycoconjugate vaccine.[123] More recently, Fallarini *et al.* reported the conjugation of small fragments of mono and disaccharides of MenA *C*-phosphonates to gold nanoparticles (Au-NPs). The purpose was to boost the immunogenicity of the glycan analogue by exploiting their multivalent presentation on the Au-NP surface. Indeed, the work reported by Fallarini *et al.* demonstrated the ability of small size, fully synthetic, non-immunogenic saccharides to induce immune cell responses when they are conjugated to Au-NPs.[124] Synthetic MenA *C*-phosphono analogues (from the monomer up to trimer) were also conjugated to the carrier protein HSA (Human Serum Albumin) using a bifunctional squarate linker (figure 1). The competitive ELISA assay revealed a recognition of the synthesized *C*-phosphonates by the polyclonal anti-MenA serum, and proved this analogue is able to mimic the natural MenA CPS.[125]

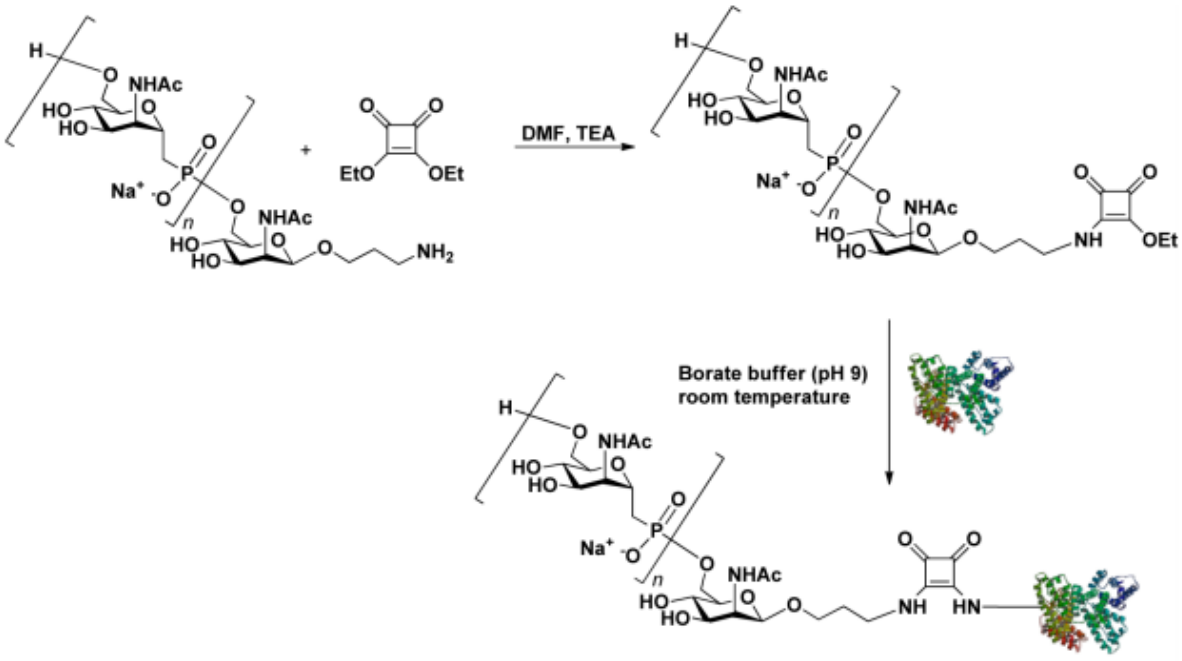
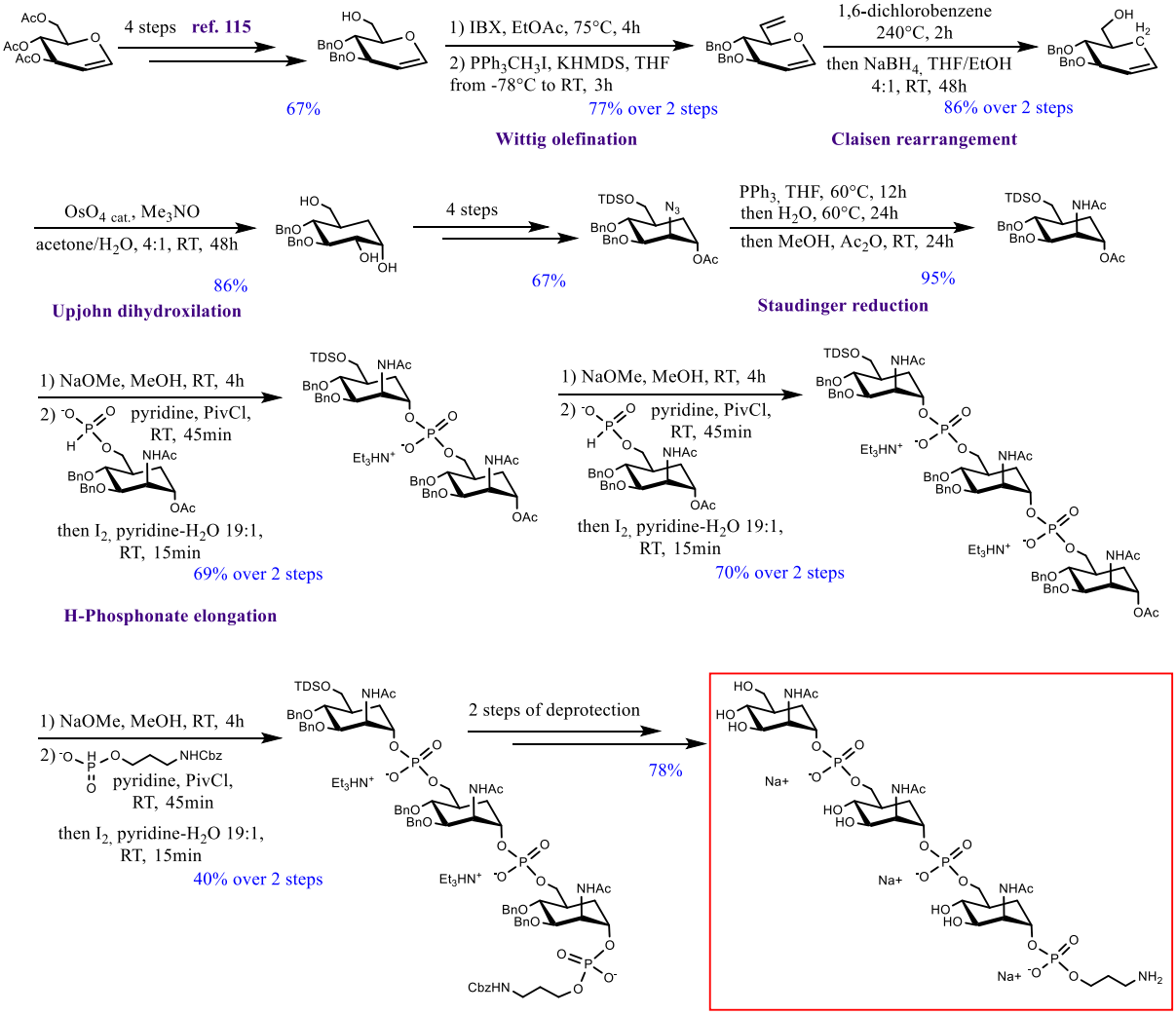


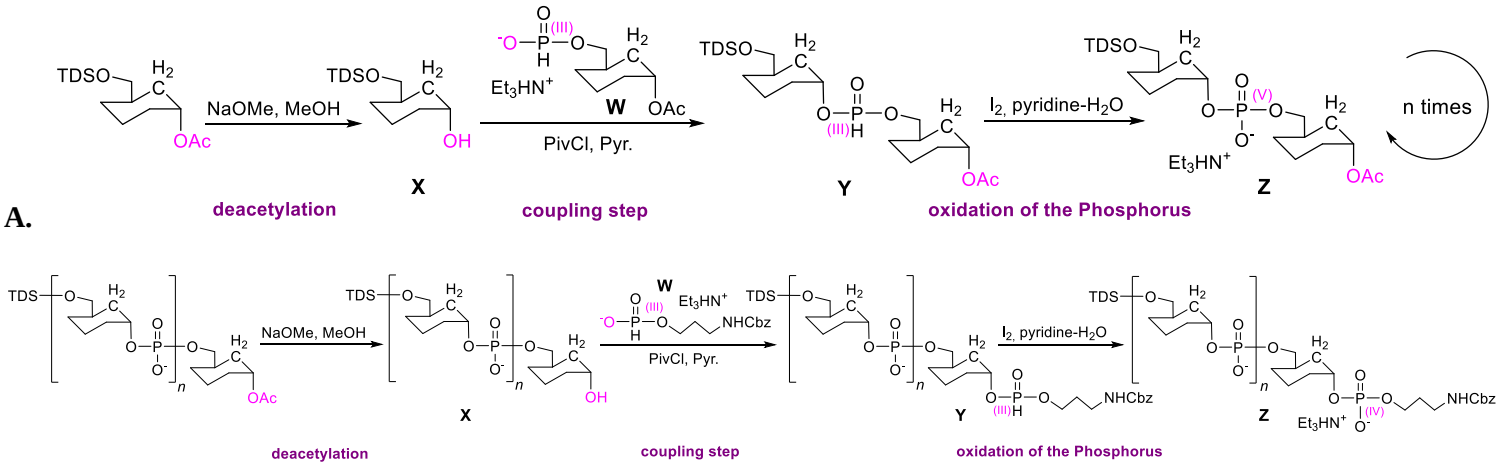
figure 1: Conjugation of *C*-phophonate oligomers to HSA using an homobifunctionnal squarate linker (*n* from 0 up to 3)[125]

On the other hand, the MenA *carba* analogues, main focus of the experimental work reported in this thesis, have been deeply investigated. The current published state of the art is the total synthesis of oligomers of *carba N*-acetylmannosamine α-(1-->6) phosphodiester up to the trimer by Gao *et al.*.[115] The route taken involved especially a sigmatropic Claisen rearrangement to design the *carba* moiety and an elongation to the trimer carried out thanks to the hydrogen-phosphonate method.



Scheme 5: total synthesis of the 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, MenA *carba* trimer (red squared)[115]

Briefly, the glycosyl hydrogenphosphonate approach, P(III) chemistry, consists of the coupling of the H-phosphonate derivative **W** with alcohol **X** in the presence of a condensing agent (here, Pivaloyl chloride) to produce the H-phosphonic diester intermediate **Y**, further oxidized (here with I_2) into the aimed glycosyl phosphodiester **Z** (scheme 6). **A.** represents the elongation step (reproducible *n* times) and **B.** represents the termination step.[126]



scheme 6: representation of the oligomerization via the H-phosphonate approach

All synthetic *carba* analogues were conjugated to the immunogenic protein CRM_{197}. Only the dimer showed a weak binding affinity towards the anti-MenA polyclonal antibody in competitive ELISA assays. The obtained glycoconjugates were hydrolytically stable but poorly immunogenic. In particular, conjugates of DP1 and DP2 were not immunogenic at all, while the DP3 glycoconjugate was able to raise MenA polysaccharide-specific IgG titers and to induce an IgM-IgG class switching.[127] Despite the good antigenicity and the immunogenicity of the *carba* DP3, the immune response induced was far less potent than the one induced by the natural CPS. However, the results of the biological assays showed an increased IgM and IgG levels and bactericidal titers as the chain length of the glycoconjugate is elongated (table 2). Moreover, the modeling study with a DFT approach of the MenA *carba* fragment showed up the conformations displayed by both the natural MenA and its *carba* analogue at the O_5-C_1-α-O_1-P glycosidic bond. The lowest energy geometries of the different molecules have been calculated using a combination of quantum mechanical techniques and molecular dynamics simulations. The predicted results were compared and validated using NMR experiments. The results indicated that the more stable glycomimetics may adopt the conformation adopted by the natural monomer, although they display a wider flexibility around the torsional angle. The natural MenA CPS displays the usual exo-syn conformation, while the *carba* analogue displays both exo and non exo conformations (about 1:1). Nevertheless, the energy barrier between them is very low and so we can assume that the *carba* antigen can easily adopt the right conformation to be recognized by the Abs.[128]

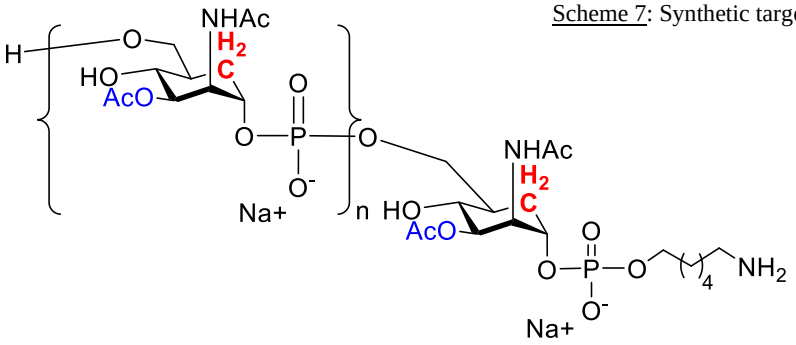
Glycoconjugates	activated ester/protein (mol/mol)[a]	saccharide conjugation (mol/mol)[b]	MW	loading efficiency %[a]	rSBA titers
PBS					<16
monomer *carba* MenA-CRM	40:1	18,5	67526	46	128
dimer *carba* MenA-CRM	50:1	12,3	68252	25	<16
trimer *carba* MenA-CRM	50:1	10,5	69957	21	512
monomer *carba* MenA-HSA	40:1	14,8	75382	37	
dimer *carba* MenA-HSA	50:1	14,5	76899	29	
trimer *carba* MenA-HSA	50:1	7,9	73869	16	
native MenA DP6-CRM	25:1	8,1		32	2048
native MenA DP15-CRM	25:1	5,2		20	4096

table 2: characteristics of the synthesized glycoconjugates and rSBA titers of sera from immunized mice. [a]NHS ester:protein molar ratio used in the conjugation reaction. [b]Sugar:protein ratio determined by MALDI-TOF MS for the *carba*-CRM conjugates[127]

To improve the immunogenicity of the glycoconjugate *carba* DP3, the structure must be elongated at least until DP8, a size that was found to be the minimal chain length to be recognized by MenA antiserum, and that seems reasonable to be attained through synthetic chemistry. Such elongation has effectively been carried out and the preliminary biological results confirm the correlation between glycan length and immunogenicity. Indeed, the *carba*MenA DP8 was able to compete with the natural CPS for binding of an anti-MenA functional monoclonal Ab. Besides, the sugar:protein molar ratio used in the conjugation reaction was closely identical (~10.5mol/mol) to the one optimized during the previous *carba*MenA DP3 biological study (table 2).[129]

Taken together, these results show that the *carba*MenA DP8 is a very promising lead antigen for the development of a hydrolytically stable anti MenA glycoconjugate vaccine. However, the remaining poor immunogenicity of the DP8 conjugate was ascribed to a second, crucial parameter, mentioned by Berry and coworkers. The natural MenA homopolymer is partially *O*-acetylated with a rate of 70 to 95% at the position 3, and this acetylation pattern is considered key to induce a robust immune response with the production of protective antibodies.[130] Since the *carba* MenA oligomers previously synthesized are not *O*-acetylated, a further step forward in terms of immunoactivity is expected by the synthesis of the *carba* MenA DP8 reproducing the major acetylation pattern occurring in the natural MenA CPS.

To conclude, our prime synthetic target is a 3-OAc-*N*-acetylmannosamine-α-(1-->6)-phosphodiester oligomer up to the octamer. The terminal end will be ended by a hexylamine linker (scheme 7) that will be chemically conjugated to the CRM_{197} protein carrier for immunological evaluation.



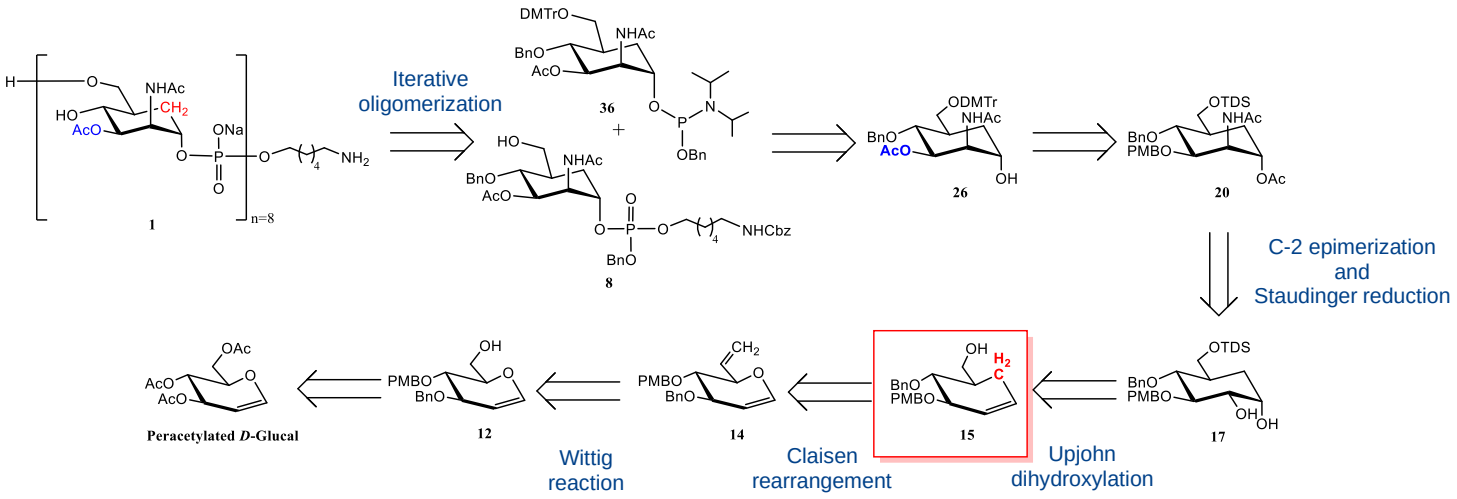
Scheme 7: Synthetic target 3-OAc-*N*-acetylmannosamine-α-(1-->6)-phosphodiester, n up to 7.

IV- Results and Discussion

1) Retrosynthetic approach

The present PhD project is mainly focused on the synthesis of *carba* oligomers fragments of MenA CPS as structural analogues of this bacterial polysaccharide, endowed with improved stability in water, due to the loss of the acetalic character of the natural CPS, without dampening the biological/immunological activity. In particular, we have identified and optimized a reliable synthetic route towards the octamer of 3-*O*-acetylated *carba* analogue of *N*-acetyl mannosamine **1**.

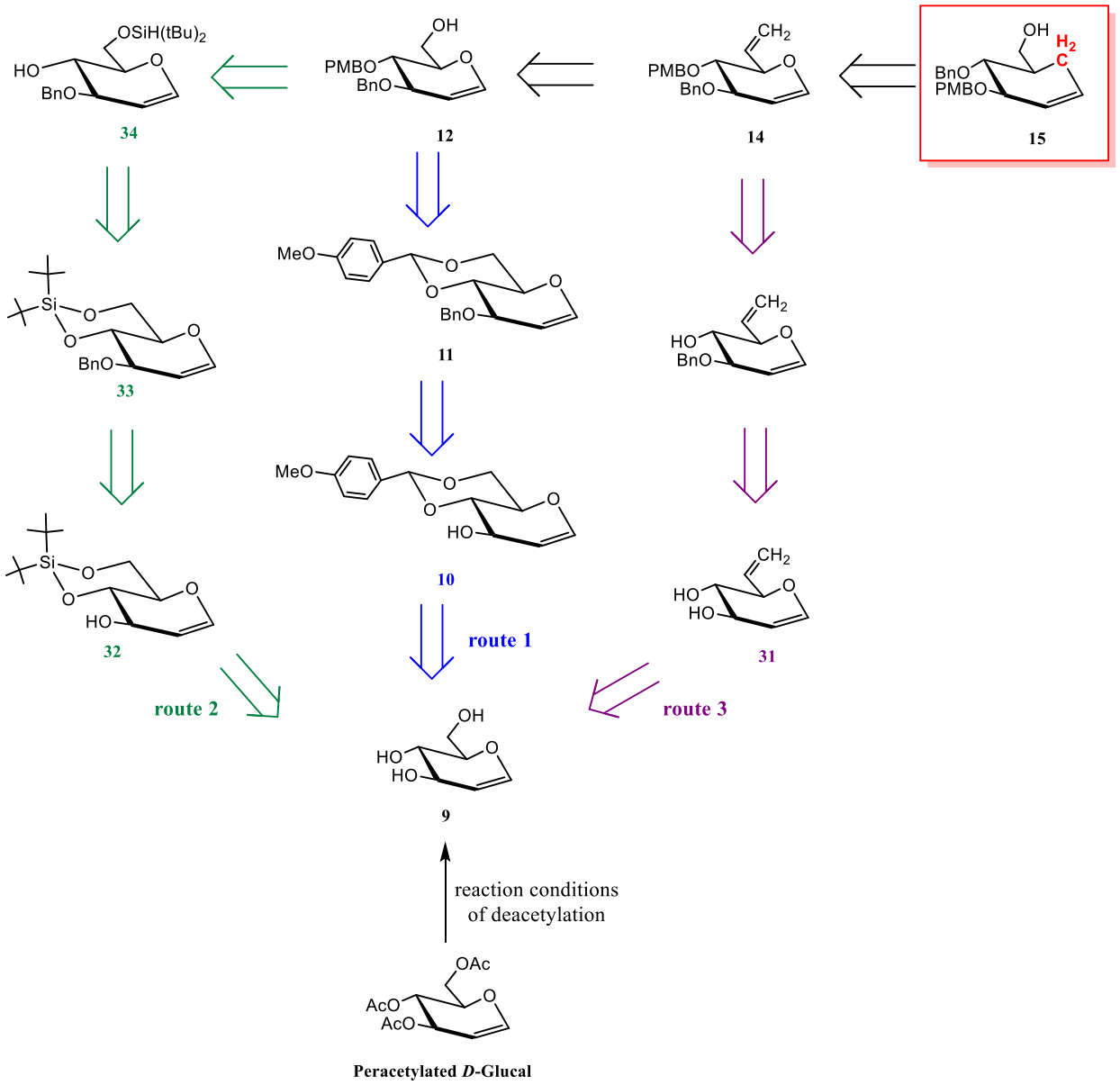
To obtain the final target **1**, the oligomers were successively assembled thanks to the phosphoramidite approach. Monomer **8** and phosphoramidite **36** were both obtained from the key building block **26**, which gathers all the required features described earlier: the 3-*O*-acetyl group to boost up the immunogenicity and the *carba N*-acetylmannosamine structure. To oversee how the antithetic analysis was achieved, we need to enlighten how these two key features could be afforded. Compared to the synthesis carried out by Q. Gao and J. Enotarpi to get the previous *carba* oligomers, we had the new challenge to differentiate the 3-OH and the 4-OH to be able to selectively acetylate at 3-OH afterwards. We proposed here to use the paramethoxybenzyloxy- (PMBO-) protecting group, orthogonally cleavable in oxidant conditions. Also, the solution brought to get the *carba* cyclohexene **15** was a copycat of the Claisen rearrangement our lab had been experienced, atom-economy reaction and turnover step of our route. Nevertheless, we always kept in my mind this process inverts the position 3-OH and 4-OH of our starting material so the PMB- had to be inserted at the position 4-OH before the sigmatropic rearrangement. To design the mannosamine body structure, a double bond at position 1 and 2-OH would fit as a retron. It would allow the manipulation of a material with less stereochemical centers in the first instance, like compound **15**, and would be functionalized then by a 1,2-dihydroxylation. Therefore, the commercial and cheap peracetylated D-glucal was proposed as a suitable retron of the cyclohexene **15** (scheme 1).



scheme 1: Retrosynthetic scheme of the 3-*O*-acetylated phosphodiester-linked *carba* oligomers up to the octamer

2) Road to intermediate 15

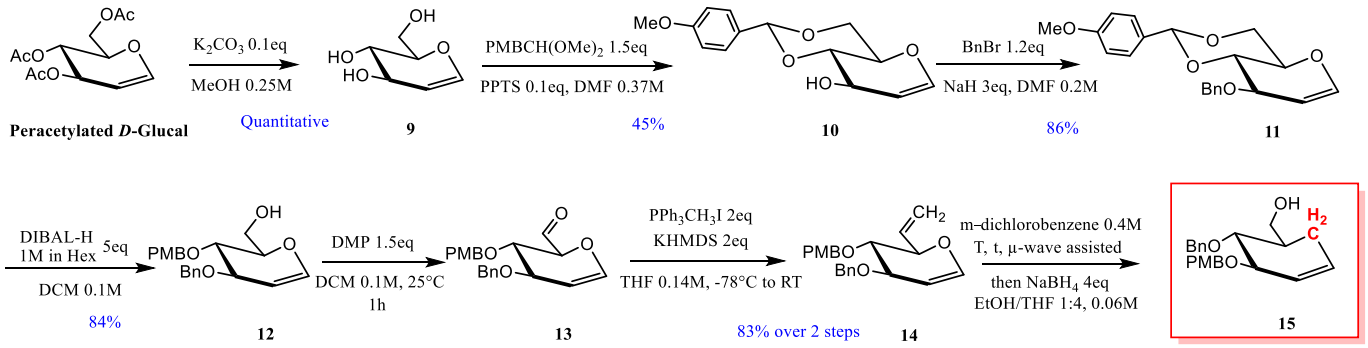
Before going ahead with the straight synthesis from the commercial D-glucal, we need to emphasize the inversion of the positions 3-OH and 4-OH occurring during the Claisen rearrangement. In this way the precursor starting material of this [3,3]-sigmatropic reaction must present the orthogonal protecting group -PMB at 4-OH that will be available at the position 3-OH after the rearrangement.



scheme 2: alternative routes in order to get the Claisen product **15** from the commercial peracetylated D-glucal

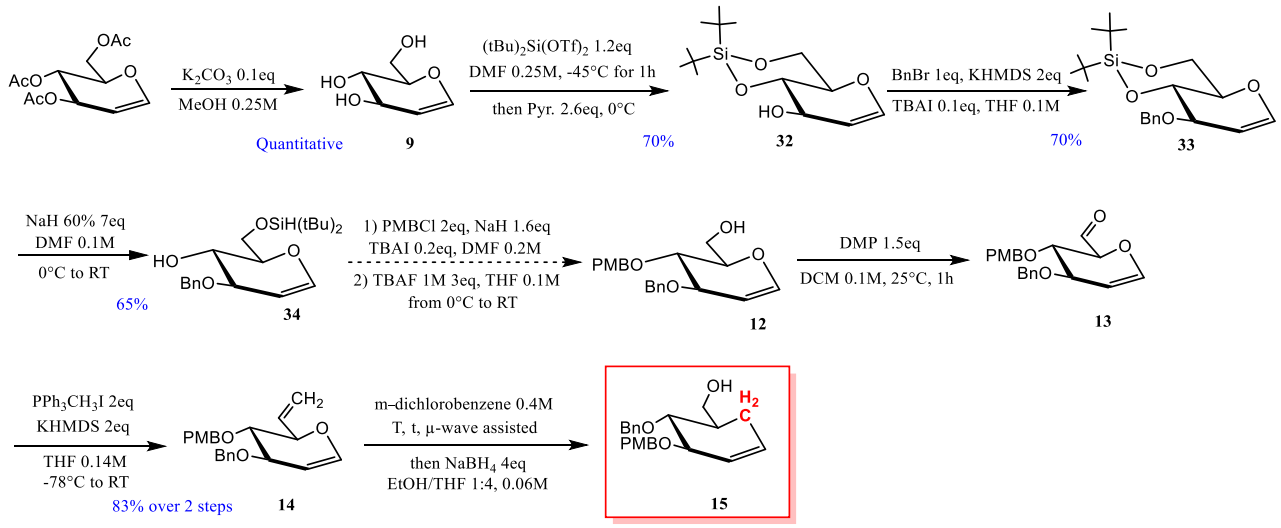
Anyway, our routes started with the quantitative deacetylation of the peracetylated D-glucal. Route 1, the synthesis of the 4,6-*O*-parametoxybenzilidene-D-glucal **10** was optimized from 5% yield to a final yield of 27% by keeping a very careful control on the evaporation of methanol generated *in situ* and on the pH, which was fixed at 6-6.5 with a catalytic amount of PPTS. At pH 7 the reaction barely worked and at pH lower than 6 the compound underwent degradation. Indeed, the *p*-methoxybenzylidene acetal requires acidic conditions that are not compatible with the very acid sensitive D-glucal. An exceptional yield of 45% was reached only once. This first protection eased the subsequent protection of the position 3 with a benzyl group in 86% yield. Next, the benzylidene acetal was selectively opened with DIBAL-

H in 84% yield to afford compound **12**. A soft oxidation of the primary alcohol with Dess-Martin periodinane followed by Wittig olefination afforded compound **14** in 83% yield over two steps. Compound **15** was produced after a Claisen reaction in thermal conditions that will be detailed out afterwards.



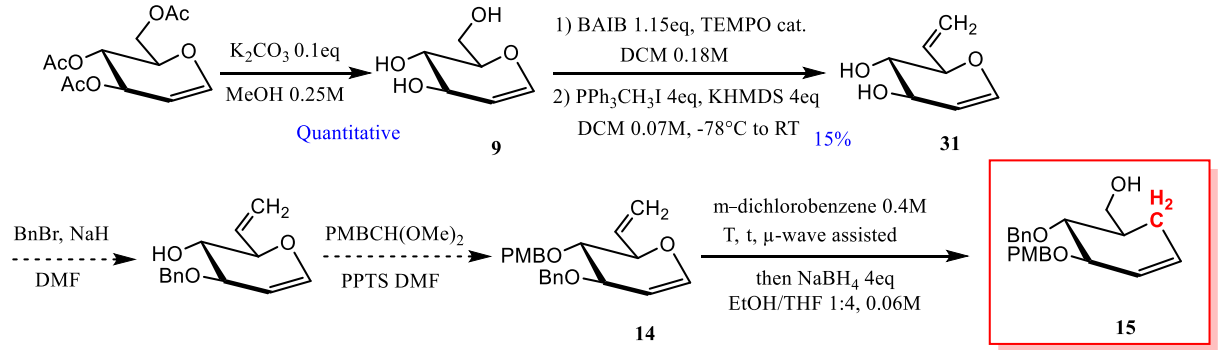
scheme 3: **route 1** to get key compound **15** through a 4,6-paramethoxybenzilidene approach

In route 2, as an alternative to avoid the 4,6-paramethoxybenzilidene protection step, we proposed to consider the same strategy of 4-OH/6-OH protection in basic conditions to preserve the integrity of our D-glucal, with a silylidene group. Formation of compound **32** was nicely carried out in 70% yield.[131-132] The following benzyl protection was accomplished in slightly different conditions from our designed route. We had to select a non-nucleophilic base to protect the 4,6-silylidene group. To facilitate the benzyl protection, we also added a catalytic amount of TBAI that afforded **33** in 70% yield.[133] Then we were able to selectively open the sylilidene with an excess of NaH (7eq.) to allow the formation of compound **34** in 65% yield.[131] The idea was next to carry out the selective protection of the 4-OH with the orthogonal PMB- protecting group, followed by a desilylation at the 6-OH without intermediate purification step, to get compound **12**, common to our first route. Despite the very good feedback, the ultimately tried route 2 could not be achieved by lack of time but remains a rational alternative to our total synthesis.



scheme 4: alternative **route 2** to get compound **15**

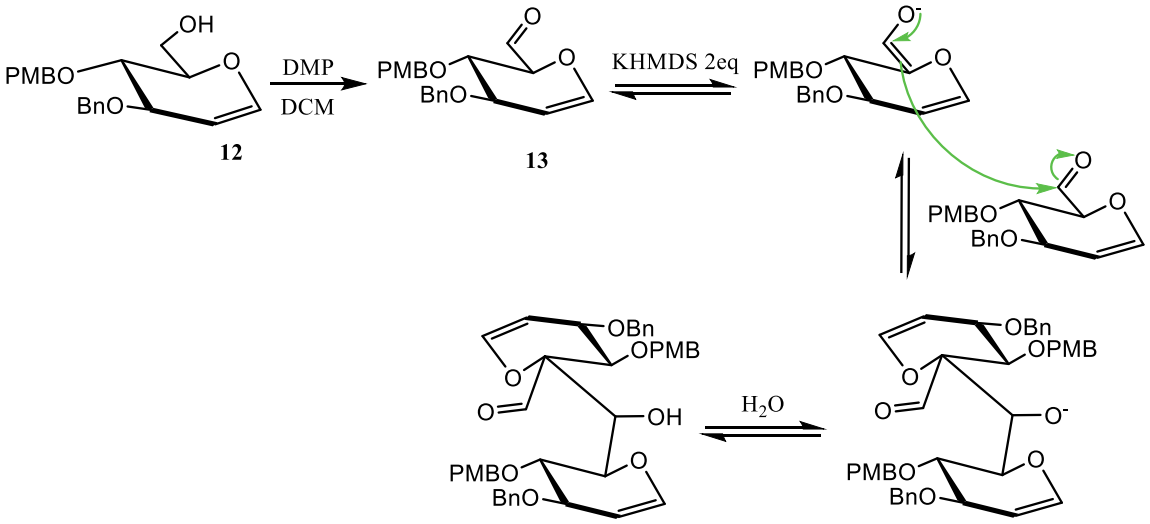
For the same reason, route 3 was drawn as an alternative to route 1. We proposed a one-pot selective oxidation/olefination of the primary 6-OH alcohol using the oxidizing agent [bis(acetoxy)iodo]benzene (BAIB) with a catalytic amount of (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) and the ylide $Ph_3P^+CH_2^-$ to carry out a selective one-pot oxidation/olefination over the 6-OH of our D-glucal, affording the formation of compound **31**.[134] Unfortunately, we witnessed a complex mixture, including the desired compound in 15% yield, that forced us to drop this route 3. The idea was then to benzylate selectively the more reactive allylic alcohol and protect the 4-OH with the same orthogonal protective group (-OPMB) to obtain the alkene **14**.



scheme 5: alternative **route 3** to get compound **15**

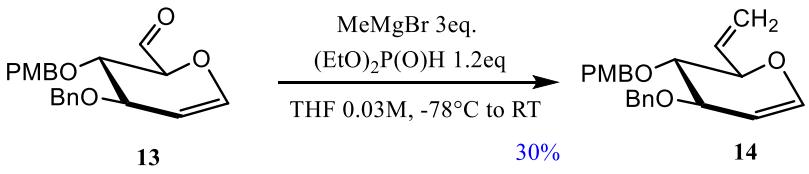
Else, a hurdle was met during the Wittig reaction to get the alkene **14** (scheme 3). We noticed how important was the previous work-up to clean the aldehyde **13** off the oxidant agent involved. In fact, the Dess Marin Periodinane and the acetic acid co-formed, highly threat for stability of the glucal. Moreover,

as the aldehyde was very unstable, these two steps of oxidation/olefination had to be carried out in a row to be efficient. Indeed, the aldehyde **13** had to be involved in the olefination as soon as possible after its formation. Even more crucial for the ylide, achieved from the reaction of the Wittig salt with the KHMDS base at very low temperature, we can actually assume that a trivial manipulation of the *in situ* ylide was responsible of a competitive aldol reaction over **13** (self-condensation). This result was supported by ^{1}H NMR and LC/MS analysis.



scheme 6: possible mechanism of the self-condensation of compound **13**

As an alternative to avoid the use of an unstable ylide to get the alkene **14**, we proposed to replace it by the Grignard reagent methylmagnesiumbromide (MeMgBr) in the presence of diethyl phosphite. However, 30% yield after 3 assays were definitely not enough to make this option for the olefination step reliable and beneficial.[135]

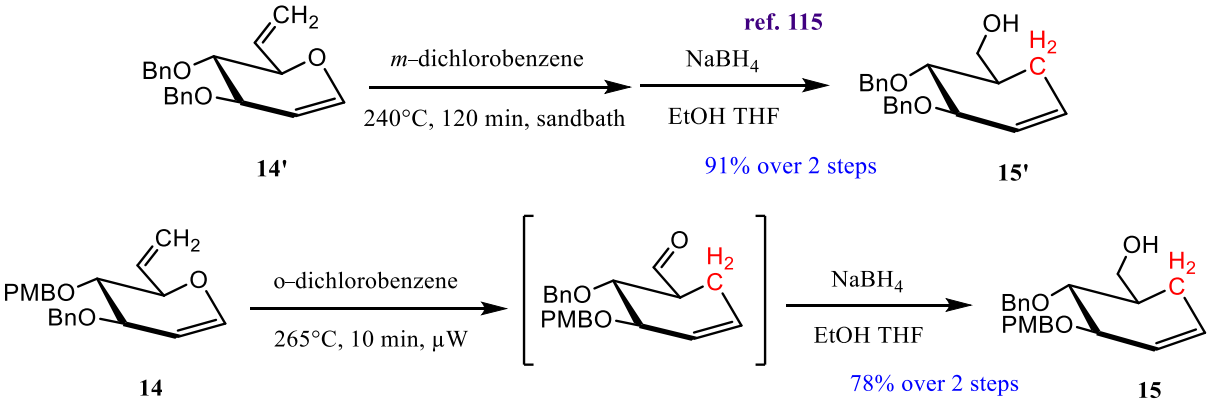


scheme 7: Grignard alternative to the Wittig olefination

3) The Claisen rearrangement

Turnover reaction of all our routes, the atom economy [3,3] sigmatropic Claisen rearrangement, was optimized reaching high temperature by means of a sand bath. By matter of reproducibility and

scale-up, we explored the use of microwave-assisted chemical synthesis and we found more reliable and efficient conditions to lay on bigger scales. Under these conditions, we obtained alcohol **15** in 78% yield over two steps, including the reduction of the aldehyde. We set up a scope of the Claisen rearrangement reaction to find the best conditions. The following parameters have been optimized: organic solvent involved (*o*-dichlorobenzene or *m*-dichlorobenzene), reaction time and reaction temperature. In addition, the reaction was carried out either in sand bath or under microwaves. Please note that our benchmark was the full conversion in 86% yield in thermal sand bath conditions on the material **14'** synthesized by Qi Gao,[115] characterized by two BnO- at positions 3 and 4.



scheme 8: one-pot Claisen rearrangement/reduction

Assays carried out with the sand bath in *m*-DCB			
Alkene **14** n≤1mmol	Time	Temperature	Yield
	1.5h, 2h, 2.5h, 3h	240°C	10 - 37%
	2.5h	250°C	23%
	2h	240°C	31%

table 1: scope of the Claisen rearrangement in sand bath, over 6 reactions

Assays carried out under µ-waves in ***m*-DCB**			
Alkene **14** n≤1mmol	Time	Temperature	Yield
	2h	230°C	40%
	2h, 2.5h, 3h	240°C	20 - 31%
	1h	250°C	23 - 58%
	30 min	260°C	44%
	10 min	**265°C**	**78%**
	7.5 min	275°C	25%
	7.5 min	280°C	31 - 66%
	4 min	285°C	40%

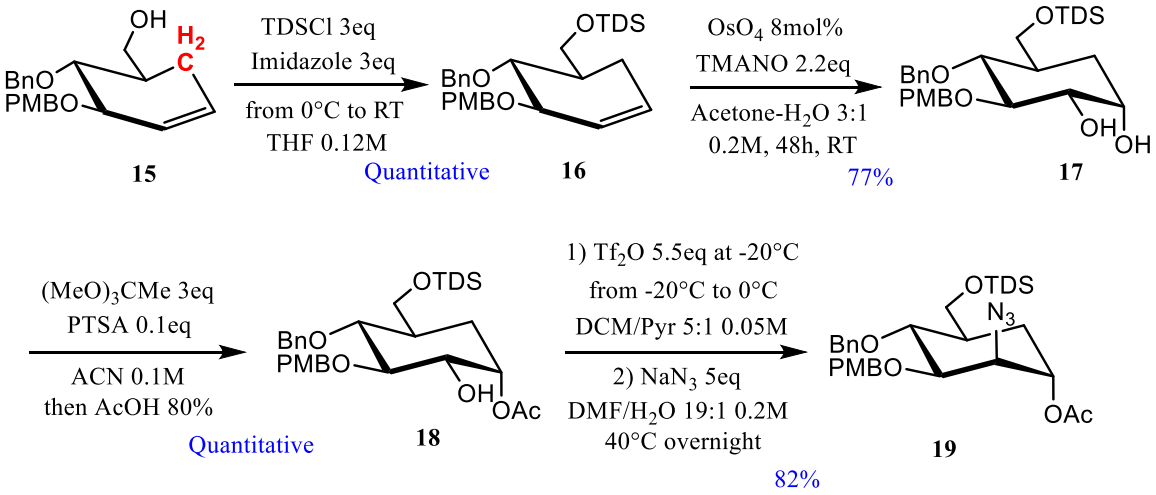
Assays carried out under µ-waves in ***o*-DCB**			
Alkene **14** n≤1mmol	Time	Temperature	Yield
	30 min	240°C	degradation
	20 min	230°C	45%
	20 min	220°c	35%
	20 min	215°C	42%
	20 min	210°C	55%
	35 min	**205°C**	**62%**
	30 min	205°C	60%
	30 min	200°C	42%
	20 min	200°C	52%

table 2: scope of the microwave-assisted Claisen rearrangement in *m*-DCB or *o*-DCB, over 10 reactions for each solvent

Throughout this study we can first point out that no treadline can be drawn. To be noted, all these experiments were carried under normal atmosphere. A single try was attempted under inert atmosphere, we were able to notice how cleaner the reaction was on TLC, without relevant improvement of the yield. Besides we underlined it was preferable to drive the reaction at lower temperature, recovering some starting material afterwards (for instance 1h at 250°C yielded up to 58% in *m*-DCB), than aiming at a full conversion at higher temperature (4 min at 285°C yielded 40% in *m*-DCB), which put also in competition the thermal degradation of the sugar. The best yield reached for the microwave-assisted Claisen rearrangement to get the *carba* structure **15** was up to 78% over 2 steps, at 265°C for 10min in *m*-DCB, according to tables 1 and 2.

4) Road to building block 26

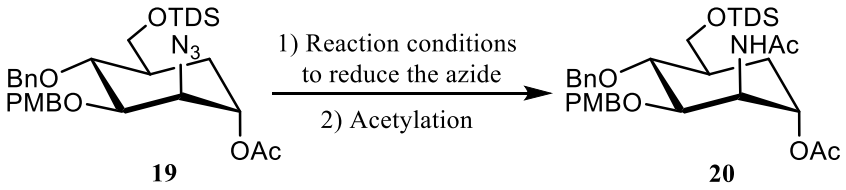
The position 6-OH of the post-Claisen product **15** was quantitatively protected with a dimethylthexylsilyl group (TDS-) and the glucal C-C double bond was syn-dihydroxylated according to Upjohn conditions, providing **17** in 77% yield. The two hydroxyl groups were differentiated by regioselective acetylation of the axial pseudo anomeric hydroxyl at C-1, using an orthoester intermediate and allowing the formation of the intermediate **18**, quantitatively. The position 2-OH was thus available to be converted into a good leaving group (-OTriflate) and substituted via a S_N2 nucleophilic displacement by NaN_3, affording **19** in 82% yield, the first mannose-like moiety of our route.



scheme 9: route followed to get the first *carba* mannose-like structure **19** from compound **15**

Then, the reduction of the azide **19** into the desired amine was tried according to four different conditions, detailed here below (table 3). Van Der Es[136] reported the procedure **c.** with PMe_3 (4eq) then acetylation in DCM affording the acetamide **20** in 75% yield on our substrate. Else, procedure **d.**

proposed a one-pot reduction/acetylation with activated zinc in a mixture of THF/Ac_2O/AcOH affording the acetamide **20** in 65% yield. Also, we tried to reduce the azide with propanedithiol[137] followed by acetylation in pyridine (procedure **e**), affording acetamide **20** in 6% yield. The classical Staudinger reduction **b.** with recrystallized PPh_3 (3eq), followed by acetylation in methanol, afforded the desired compound **20** in 64% yield. We were subsequently able to optimize the yield up to 75% by applying slight modifications over the initial Staudinger reaction. It is interesting to note the sporadic co-formation of an oxazole in these conditions, obtained after an aza-Wittig from the phosphazene intermediate formed first and a ring distortion to allow the reaction with the neighboring 1-OAc. However, a catalytic amount of pyridine (0.3eq) prevented this undesired cyclisation and our optimized procedure of reduction followed by acetylation **a.** in pyridine led to the formation of acetamide **20** in 75% yield.

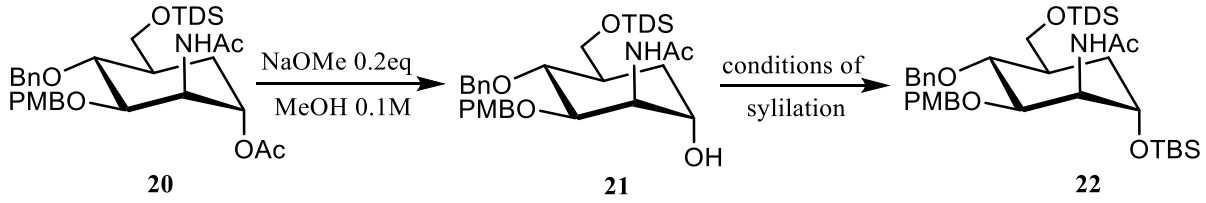


scheme 10: two-step reduction of the azide/acetylation

Assays	Reaction conditions to reduce the azide	Reaction conditions of acetylation	Yields
a.	PPh_3 recrys. 2.5eq, Py. 0.3eq in THF/H_2O (85:15) 0.1M, 60°C	Ac_2O in Py. 0.1M	**75%**
b.	PPh_3 recrys. 2.5eq in THF/H_2O (85:15) 0.1M, 60°C	Ac_2O in MeOH 0.1M	64%
c.	PMe_3 1M 3.8eq, Py. 0.3eq in dioxane/H_2O 85:15, 30-60min	Ac_2O in Py. 0.1M	75%
d.	Zn activated 3eq in THF/Ac_2O/AcOH (3:2:1) 0.1M		65%
e.	PDT 5eq, DIPEA 5eq in MeOH 0.1M, 25°C	Ac_2O, DMAP 0.1eq in Py. 0.1M	6%

table 3: reaction conditions carried out to obtain compound **20**

The acetamide **20** was then deacetylated with sodium methoxide at the C_1 position and resulting free 1-OH was directly protected using the hindered *tert*-butyldimethylsilyl (TBDMS-) protecting group. First, the protection was carried out with TBDMSCl (2.5eq), imidazole (2.5eq) in THF (0.1M) (0°C to RT) but, presumably, conditions **b.** were too mild and no reaction occurred, leading to the partial recovery of intermediate **21**. According to the procedure **c.** of Z. Guo and coworkers,[138] with TBSOTf as a silylating agent and 2,6-lutidine as a base, compound **22** was obtained in an unsatisfactory 16% yield. Assay **d.** was achieved using tert-Butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and 2,6-lutidine as a base, starting at -78°C then warmed up slowly till RT overnight, driving to compound **22** in 18% yield. Finally, compound **22** was successfully achieved in 91% yield according to procedure **a.** of J. D. C. Codée and coworkers using a mixture of TBSOTf/pyridine as a base and warming up the reaction at 70°C (table 4).[139] Therefore, it appeared that imidazole and 2,6-lutidine were bases not strong enough to deprotonate 1-OH (pKa Imidazole/lutidine/pyridine: 6.95/6.60/5.23) and pyridine as solvent and base was required. Heating at 70°C and a catalytic amount of DMAP were crucial to provide energy to the system.



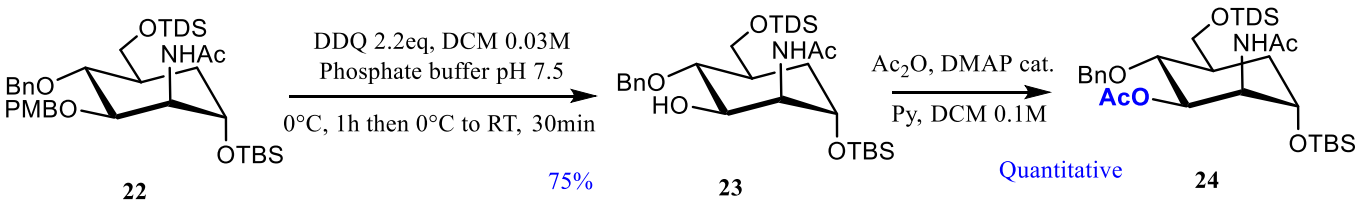
20 **21** **22**

scheme 11: two-step 1-OH deacetylation/silylation

Assays	Reaction conditions of silylation	Yields over two steps
a.	TBSOTf 2eq in Py. 0.2M, 0°C then 70°C overnight	**91%**
b.	TBDMSCl 2.5eq, Imidazole 2.5eq in THF 0.1M from 0°C to RT	64%
c.	TBSOTf 2eq, 2,6-lutidine 2.5eq in DCM 0.2M from 0°C to RT	16%
d.	TBSOTf 2eq, 2,6-lutidine 2.5eq in DCM 0.2M from -78°C to RT	18%

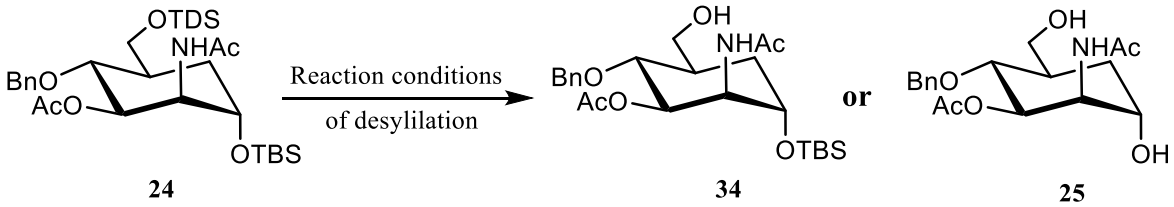
table 4: reaction conditions carried out to obtain compound **22**

The *p*-methoxybenzyl group was then removed under oxidative conditions (DDQ) in 75% yield. The resulting alcohol **23** was quantitavely acetylated at the 3-OH.



scheme 12: selective acetylation of the 3-OH to get compound **24**

Compound **24** was deprotected at the positions 1-OH and 6-OH. Using any source of fluoride anion, the TDS- protecting group on the primary 6-OH was properly removed. Nonetheless, the TBS protecting group on the 1-OH could not be cleaved with TBAF 1M in THF leading to the 1-OTBS protected *carba* sugar **34** in quantitative yield. The full deprotection of both silyl ethers was well accomplished (procedure **b.**) in 60% yield using 80eq. of 30% HF/Py solution in THF, starting at 0°C and leaving the mixture warming up until RT overnight. Controlling the same reaction over 3h at 0°C with 80 equivalents of the same solution of HF raised the yield up to 89% (procedure **c.**).



scheme 13: desilylation of 1-OH and 6-OH

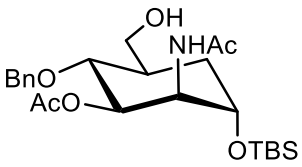
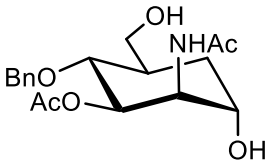
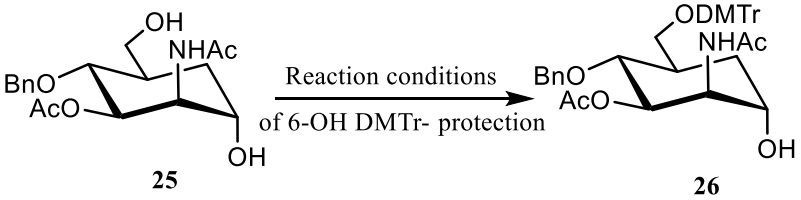
Assays	Reaction conditions of desilylation	Yields	Product obtained
a.	TBAF 1M 4eq in THF 0.1M from 0°C to RT	Quantitative	 **34**
b.	HF/Py. 30% 80eq in THF 0.1M from 0°C to RT, overnight	60%	 **25**
c.	HF/Py. 30% 80eq in THF 0.1M, 0°C, 3h	**89%**	

table 5: reaction conditions carried out to obtain compound **25**

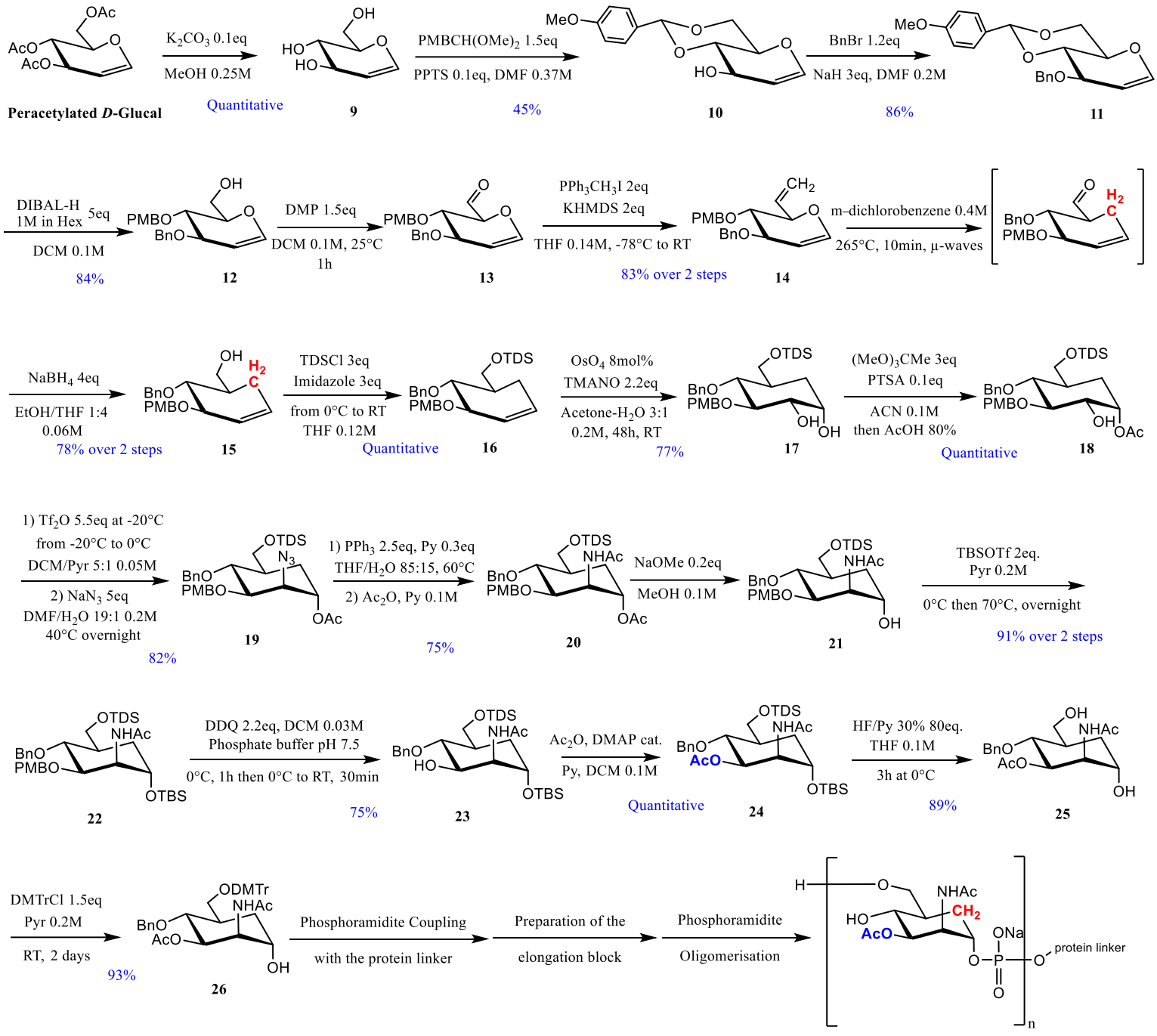
Finally, the 6-OH of compound **25** was protected with the very labile dimethoxytrityl group to obtain, according to procedure **a.**, the building block **26** in 74% yield. Carrying this last reaction slightly differently with pyridine as solvent over 2 days improved the yield up to 93% (procedure **b.**).



scheme 14: regioselective protection of the primary 6-OH with the dimethoxytrityl protecting group

Assays	Reaction conditions of DMTr- protection	Yields
a.	DMTrCl 1.5eq, Py. 1.5eq in THF 0.1M	74%
b.	DMTrCl 1.5eq in Py. 0.2M at RT over 2 days	**93%**

table 6: reaction conditions carried out to obtain compound **26**

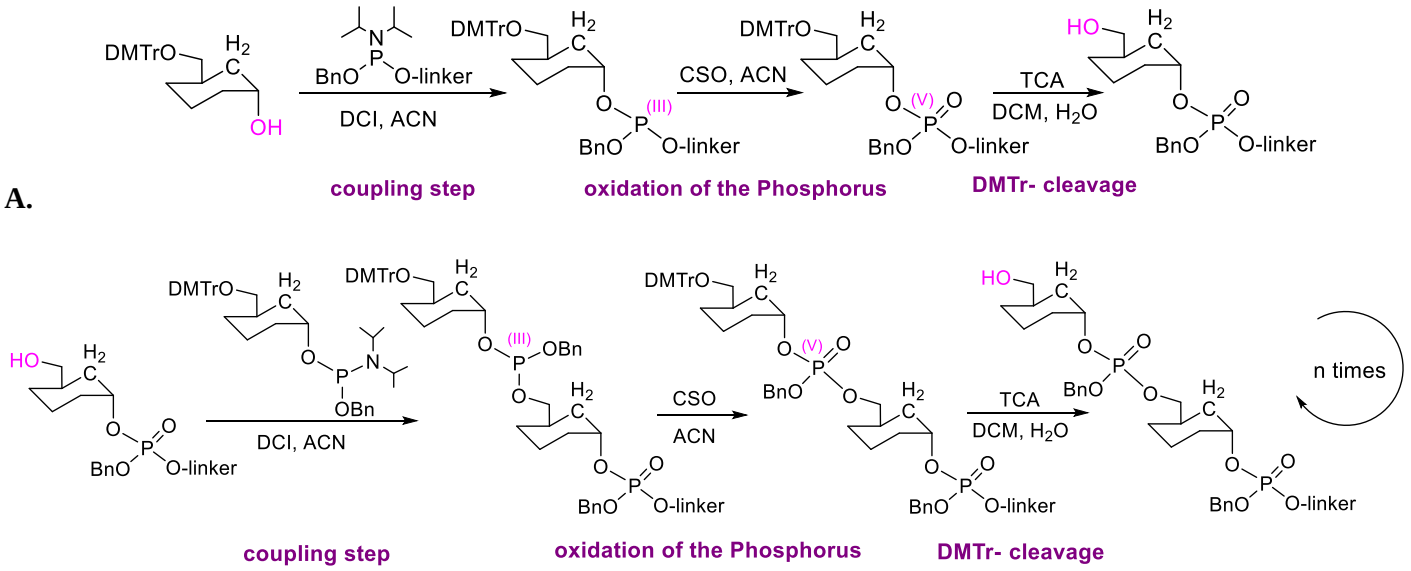


scheme 15: optimized route for the synthesis of the 3-*O*-acetylated mannosamine *carba* building block **26**

5) Iterative oligomerization by phosphoramidite approach

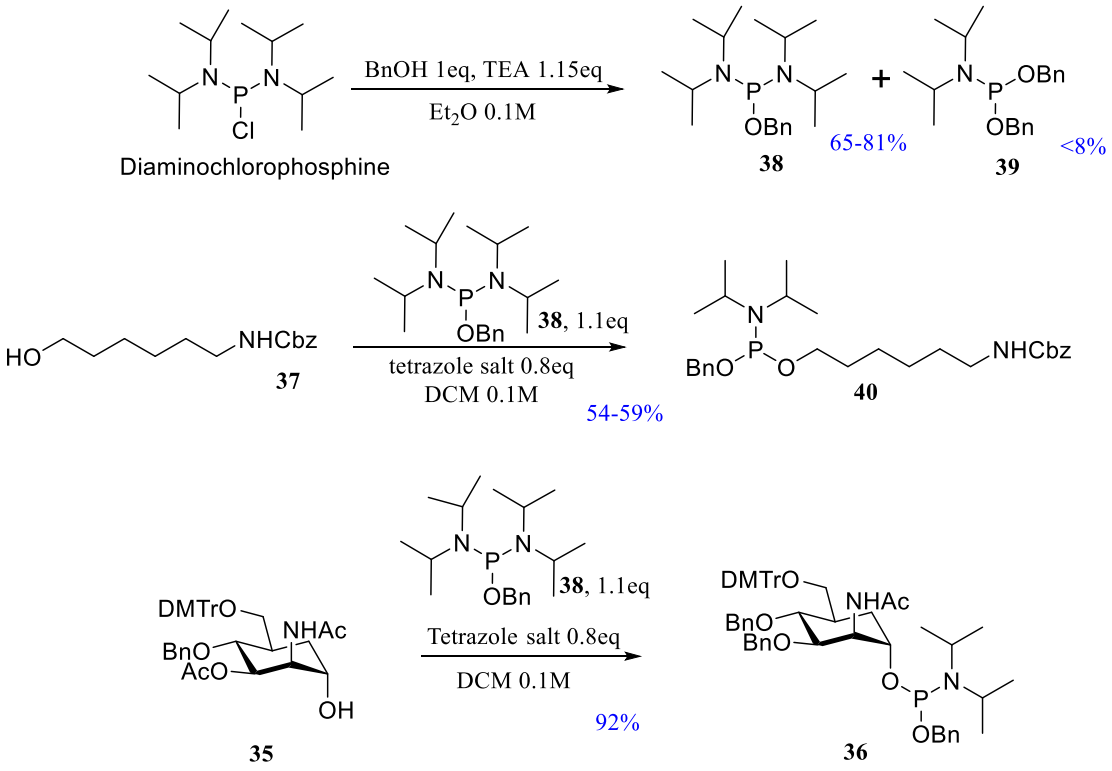
To perform the iterative oligomerization, we decided to rely on the phosphoramidite approach. In the context of the Marie Curie ITN Glycovax, the oligomerization was undertaken at the university of Leiden, with the active collaboration of **Jacopo Enotarpi** and **Thijs Voskuilen** at the university of Leiden, Netherlands.

The phosphoramidite method, P(III) chemistry, is the following: **3.A.** preparation of the downstream residue, carrying the linker; **3.B.** oligomerization from the terminal residue up to the desired oligomers[126] (scheme 16).



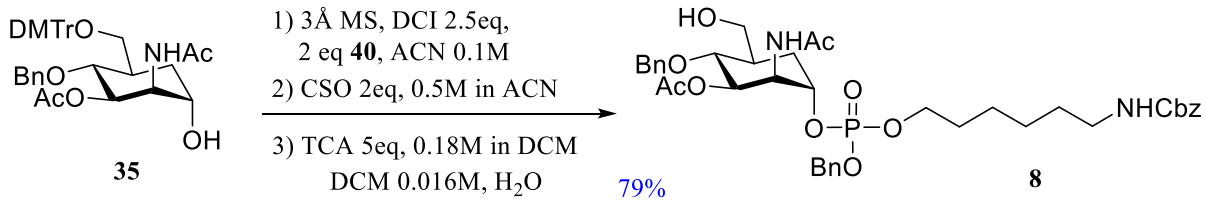
scheme 16: representation of the oligomerization via the phosphoramidite approach

To do so, we needed to prepare the required phosphoramidite blocks: the phosphoramidite containing the carbamate spacer **40** and the phosphoramidite elongation building block **36**. For the synthesis of the phosphoramidite terminal end **40**, we started from the cheap and commercial diaminochlorophosphine. By a nucleophile substitution, the benzyl-*N*,*N*,*N'*,*N'*-tetraisopropylphosphorodiamidite **38** was formed in 81% yield. Please note that leaving the reaction going too long might induce the formation of the double substituted dibenzyl *N*,*N*-diisopropylphosphoramidite **39**. The condensation of the amino spacer **37** to **38** with tetrazole salt led to the first phosphoramidite moiety **40**, terminal end, in 59% yield. The key phosphoramidite elongation block **36** was equally made from the condensation of our key *carba* monosaccharide **35** with the phosphorodiamidite **38** in 92% yield.



scheme 17: preparation of the iterative elongation *via* the phosphoramidite approach

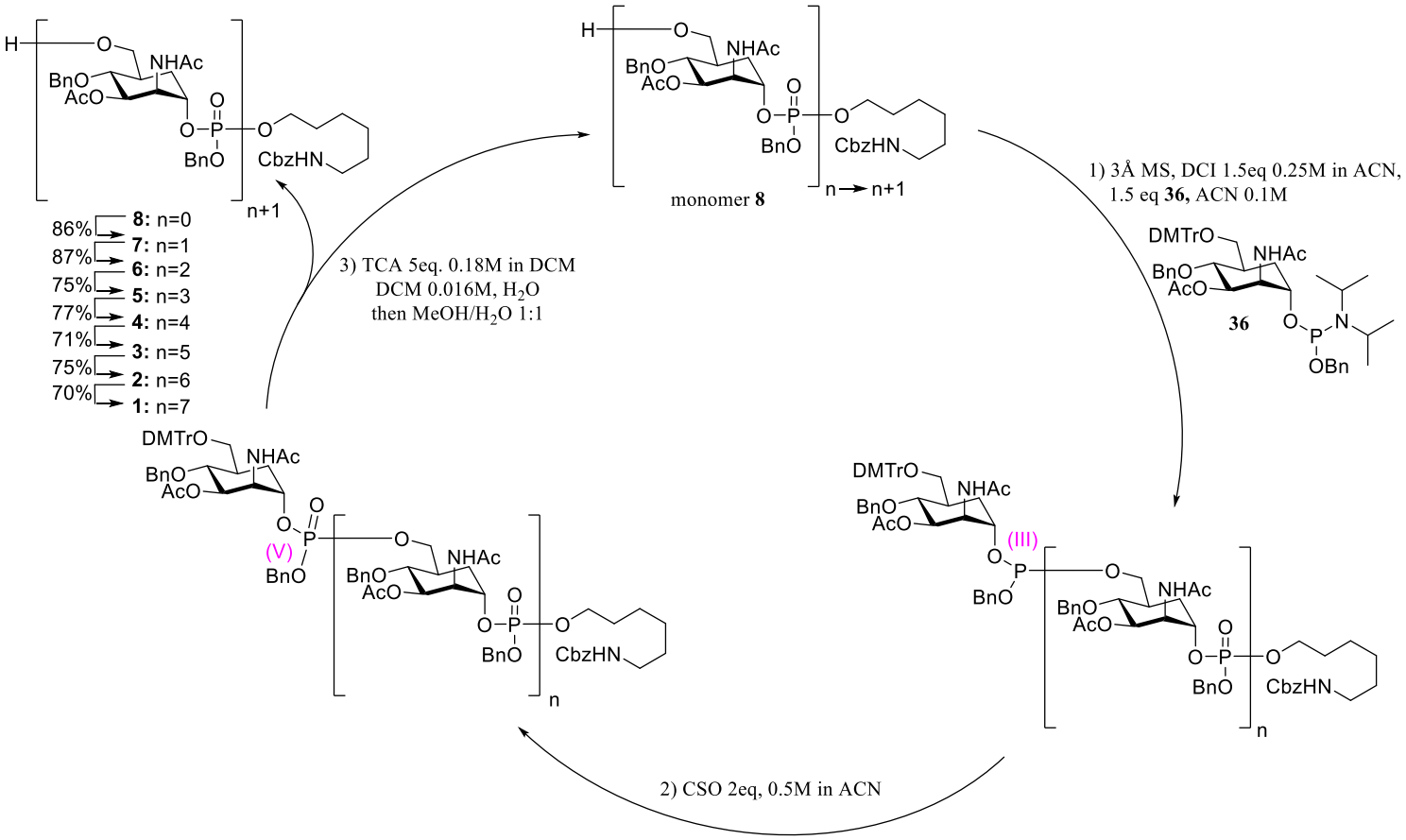
Basically, the phosphoramidite **40** is activated by 4,5-dicyanoimidazole (DCI) in acetonitrile in a couple of hours, to turn the phosphodiester into the phosphotriester. The phosphite triester with a phosphorus (III) is however unstable in acidic conditions. After completion of the coupling, we oxidized the phosphorus (III) to a stable phosphorus (V) in a couple of minutes with (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO). Finally, the acid labile DMTr- protecting group was cleaved by a solution of trichloroacetic acid (TCA) in order to get the downstream residue **8** in 79% yield.



scheme 18: preparation of the phosphoramidite terminal end **8**

The iterative oligomerization may start, from the monomer **8** up to the octamer **1**. The method lays on the same chain reactions:1) activation of the phosphoramidite elongation building block **36** with DCI

and coupling to the 6-OH of our previous *n*-mer, 2) oxidation with CSO and 3) cleavage of the DMTr-protecting group in acid conditions to repeat further elongations (scheme 19).



scheme 19: Phosphoramidite approach: synthesis of the required building blocks

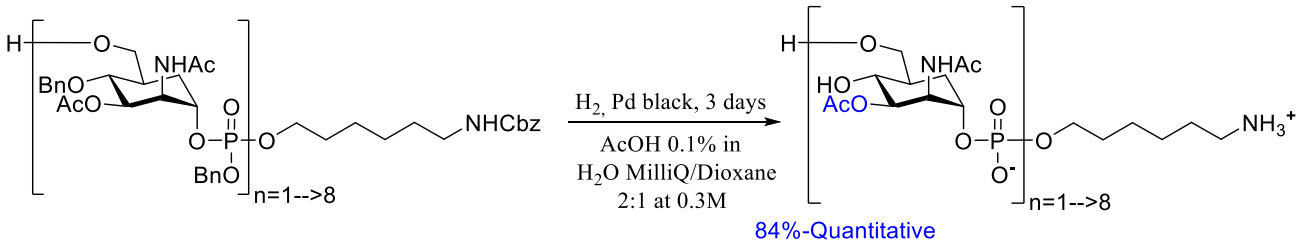
A comment might be added about the use of 4,5-dicyanoimidazole (DCI) instead of the traditional 1*H*-tetrazole or a derivative and the use of (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) as oxidant instead of the traditional molecular iodine. Since the coupling step is carried out in acetonitrile, DCI is more soluble and also less acidic than any tetrazole derivative. The use of DCI then secures the stability of the very acid-sensitive DMTr- protecting group (table 7). Besides, the oxidation was performed with CSO, able to oxidize phosphite triesters to phosphate triesters in less than 30 seconds in organic solvent and dry conditions. On the contrary, I_2 needs aqueous conditions and peroxides tend to be unstable and so, too risky. Extra equivalents of TCA were sometimes necessary to completely cleave the DMTr-protecting group.

Activator	pKa	Solubility in Acetonitrile
5-Ethylthio-1H-Tetrazole	4.3	0.75M
5-Benzylthio-1H-Tetrazole	4.1	0.44M
4,5-Dicyanoimidazole	5.2	1.2M
Acetic Acid	4.8	N/A

table 7: physical properties of phosphoramidite activators

The iterative oligomerization was eventually carried out smoothly up to the octamer with a gradual fall of yield due to the progressive growth of the chain length and the bulkiness. The final oligomers, after purification by size exclusion, were deprotected by hydrogenolysis to remove the remaining benzyl groups. Initially using a catalytic amount of acetic acid to clean the palladium off the free amine, we could also observe the migration of the acetyl group from 3-OH to 4-OH, and even to 6-OH for the upstream residue. However, with a precise measure of 0.1% AcOH and a lyophilization of the deprotected oligomers, we were able to deprotect our *n*-mers without any additional hurdles in 84% to a quantitative yield (scheme 20). The *n*-mers were also not turned into their sodium form that actually eases the acetyl migration.

The deviation of the integrals in the H-NMR spectrum with the protons of the octamer may suggest the presence of shorter fragments. The reason for this is that the Sephadex LH-20, used to purify the fragments during the elongation, is limited on big fragments. Unfortunately, no further purification with size exclusion HW40 was possible because this facilitated the migration of the acetyl. Nevertheless, there can be concluded that octamer **1** was successfully synthesized, result supported by the exact mass with MALDI mass spectrometry and the experimental data of the assembly of the oligomers.



scheme 20: Hydrogenolysis of our *carba n*-mers **8** (monomer) to **1** (octamer)

The last steps are currently in progress: conjugation of our synthetic oligomers to the CRM_{197} protein carrier according to the protocols of GlaxoSmithKline (GSK) Vaccines, followed by test of the antigenicity by competitive ELISA and mice immunization with the neo-glycoconjugates.

6) Conclusions and perspectives: conjugation and immunochemistry

Succinctly, we were able to synthesize the 3-*O*-acetylated mannosamine *carba* building block **26** over 19 steps in 5.6% overall yield. Next, we were able to isolate each MenA 3-OAc-*carba* oligomer according to their length: from the deprotected MenA 3-OAc-*carba* dimer (57.1%, 3 steps), trimer (50.8%, 4 steps), tetramer (41.2%, 5 steps), hexamer (24.2%, 7 steps) up to the 1-*O*-octa-(2-Acetamido-2-deoxy-3-*O*-acetyl-7-*carba*-α-D-mannopyranosyl-1-*O*-phosphoryl)-6-hexyl-amine, the desired MenA 3-OAc-*carba* octamer, in 11.7% yield over 9 steps, considering the best yield obtained for each step.

As mentioned above, future work on this project implies the conjugation of our synthetic oligomers to the CRM_{197} protein carrier, followed by a test of the antigenicity by competitive ELISA and a mice immunization with the neo-glycoconjugates. In the context of the Marie Curie ITN Glycovax, this biological evaluation will be undertaken at GSK Vaccines laboratories, Siena, with the active collaboration of **Evita Balducci** and **Filippo Carboni**. The serum will be collected and an infection challenge will be set up to test out the functional activity against the natural MenA CPS bacterium of the anti-*carba* MenA antibodies raised in mice (figure 1). The immunological profile of the resulting *neo*-glycoconjugates will be carefully investigated, with the purpose to highlight the effect of the carbohydrate chain length and the 3-OAcetylation on the immunoactivity.

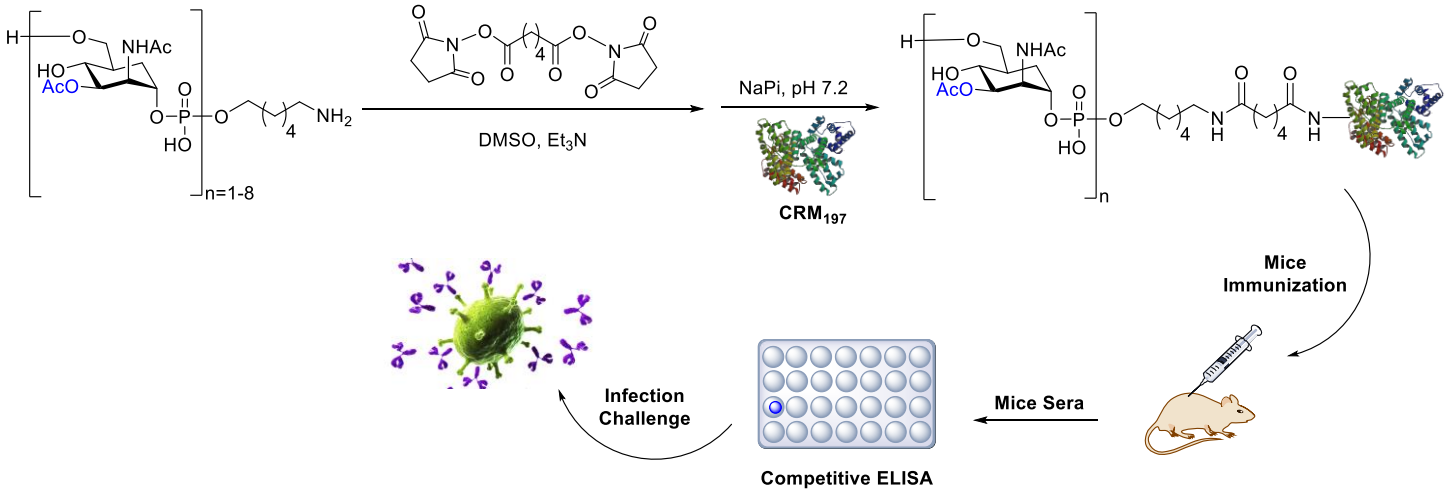


figure 1: Conjugation, biological and immunological evaluation carried out on length-selected *carba* oligomers

GSK is currently filing a patent to protect the intellectual property of the overall *carba* project.

V- Experimental Part

General experimental methods

Thin Layer Chromatography

Milan UMIL university: Thin Layer Chromatography (TLC)were performed on glass support Merck $60F_{254}$ (0.25mm thickness) and High-Performance Thin Layer Chromatography (HPTLC) were performed on Kiesgel $60F_{254}$.

Reactions were always followed by TLC analysis. Compounds were visualized, when appropriate by UV light (254nm), and always by spraying with:

- molybdic solution (21g of $(NH_4)_6Mo_7O_{24}$, 1g of $Ce(SO_4)_2$, 31mL of H_2SO_4 98%, 970mL H_2O)
- sulfuric acid (50mL of H_2SO_4 98%, 450mL of MeOH, 450mL H_2O)
- ninhydrin (2.7g of 2,2-dihydroxyindane-1,3-dione, 27mL of AcOH, 900mL of EtOH)

with detection by charring at 300°C.

Leiden UL university: TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F_{245}).

Compounds were visualized by UV absorption (245 nm) and by spraying with:

- a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 25 g/l and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ 10 g/l, in 10% aqueous H_2SO_4
- sulfuric solution (20% H_2SO_4 in ethanol)

with detection by charring at +/- 140°C.

Some unsaturated compounds were visualized by spraying with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water.

Flash Chromatography

Milan UMIL university: according to Still procedure,[140] compounds were purified by flash chromatography, using Silica gel (SiO_2, high-purity Merck grade, pore size 60Å, 230-400 mesh particle size from Sigma-Aldrich. Otherwise it was also used a flash purification system, Biotage SP1. Normal phase Biotage SNAP cartbridges (sizes from 3g to 340g, standard 50µm silica) were used to purify the compounds.

Leiden UL university: Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm).

NMR Analysis

Milan UMIL university: NMR spectra were recorded on a Bruker AMX 400 instrument (400 and 100.6MHz for ^{1}H and ^{13}C respectively).

Leiden UL university: ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded with a Bruker AV 400 (400, 101 and 162MHz respectively), a Bruker AV 500 (500 and 202MHz respectively) or a Bruker DMX 600 (600 and 151MHz respectively).

All NMR were run at room temperature (298K), unless stated otherwise.

The samples were prepared using deuterated solvents ($CDCl_3$, D_2O, CD_3OD, CD_3CN and acetone-d_6 from Sigma-Aldrich). Chemical shifts (δ) are reported in ppm and the coupling constants (*J*) in Hz. Chemical shifts were referenced to the residual proton in the solvent (*i.e.* the $CHCl_3$, 0.01% in 99.99% $CDCl_3$), according to Gottlieb and Nudelman.[141] Multiplicities are abbreviated as: br (broad or Brownian), s (singlet), d (doublet), t (triplet), q (quadruplet), hept (heptet), m (multiplet) or combinaison thereof. ^{1}H-NMR were recorded for all the synthesized products. In the case of unknown structure, the characterization is reported by ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR. Bidimensional experiments (COSY, TOCSY, HSQC and HMBC) were used to better assign peaks to the structure. Carbon atoms and protons on each compound were identified by numbers in accordance to figure 1. C-7 methylene protons were labelled with letters “a” and “b”.

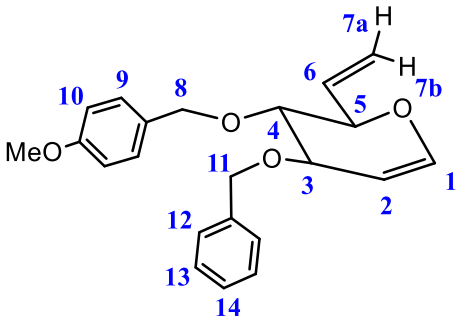


figure 1

Mass Analysis

Milan UMIL university: low resolution mass analyses were recorded in negative or positive mode on a Thermo Finnigan LCQ Advantage equipped with an ESI source. High resolution mass analyses were recorded on a Waters Micromass Q-Tof micro equipped with a LockSpray ESI source or on a Bruker Daltonics ICR-FTMS APEX II at C.I.G.A, University of Milan.

Leiden UL university: High resolution mass spectra were recorded by direct injection (2µl of a 2µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5kV, sheath gas flow 10, capillary temperature 250°C) with resolution R=60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

Anhydrous environment

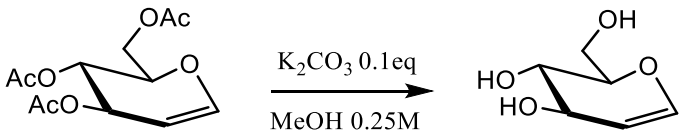
Unless stated otherwise, all the reactions were conducted under extra dry nitrogen atmosphere. When stirred overnight, reactions were put under anhydrous atmosphere by an Argon-balloon.

Solvents

Milan UMIL university: unless stated otherwise, all the reactions were performed using dry solvents. DCM, Et_2O, MeOH, pyridine, toluene, DMF, CH_3CN and THF over molecular sieves were purchased from Sigma-Aldrich and used without further purifications.

Leiden UL university: all the reactions were performed using house made dry solvents. DCM, Et_2O, MeOH, pyridine, toluene, CH_3CN and THF high quality 97+% purity grade corresponding solvents were purchased from Sigma-Aldrich and left on molecular sieves 4Å, overnight. Commercial DMF was used, as it stood, without further purification. Commercial EtOAc and Toluene underwent an additional distillation to remove the extra mineral oil and water impurities present in each.

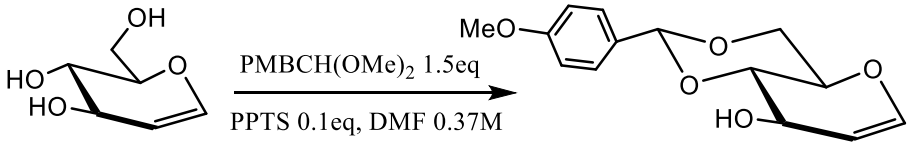
Work carried out in Milan



Peracetylated *D*-Glucal Quantitative **9**

D-Glucal (9)

To a mixture of 3,4,6-tri-*O*-acetyl-D-glucal (10.0g, 36.7mmol) was added K_2CO_3 (508mg, 3.67mmol) in $MeOH_{dry}$ (150mL) and then stirred under N_2 at room temperature. After 1 hour the reaction was completed and quenched with acetic acid to reach a pH of 7. The solvent was evaporated under reduce pressure and the crude product of D-glucal **9**, a transparent oil, was directly involved in the next step.



9 45% **10**

4,6-*O*-(4-Methoxybenzylidene)-D-glucal (10)

To the crude compound **9** in dry DMF (100mL) were added anisaldehyde dimethyl acetal (9.40mL, 55.1mmol) and then pyridine *p*-toluenesulfonate (922mg, 3.67mmol) under N_2. The reaction was carried at 25-30°C under vacuum (180mbar) for 2.5-3 hours, on a rotavapor. The DMF was then evaporated under reduced pressure and the crude product was extracted by 100 mL of DCM. The organic layer was washed successively by 50 mL NH_4Cl, 50 mL of distilled water and 50 mL of a brine solution. Finally, the gathered aqueous layers was extracted by 50 mL DCM. The mixture was then dried over Na_2SO_4 and evaporated under reduced pressure to obtain 4,6-O-(4-Methoxybenzylidene)-D-glucal **10** as a white powder with a yield of 45%, average yield of 25%.

δ ^{1}H (400 MHz; CDCl3)

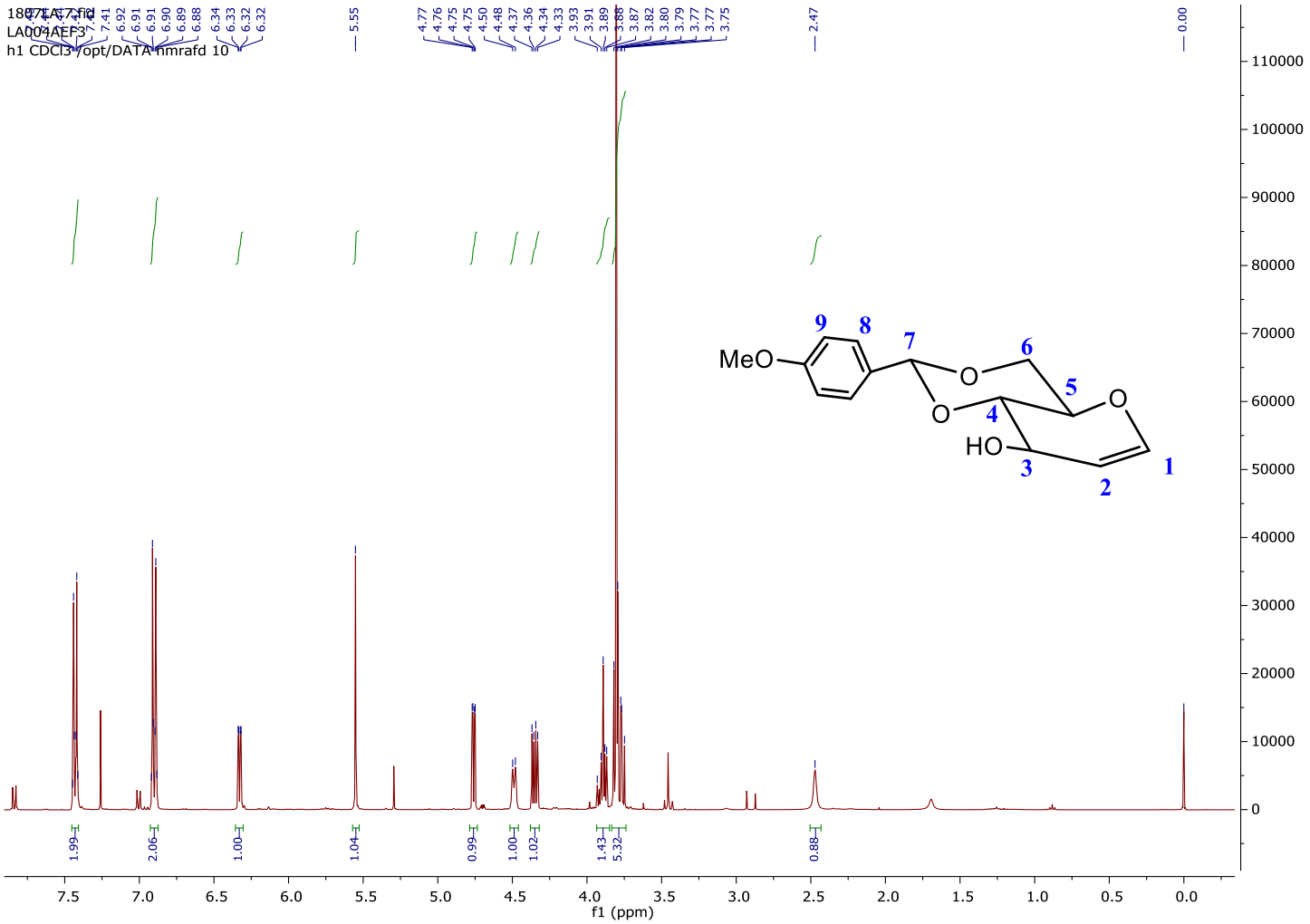
7.43 (2H, td, J 8.6, J 4.7, 8-H), 6.90 (2H, dt, J 8.8, J 4.9, 9-H), 6.33 (1H, ddd, J 6.1, J 1.6, J 0.4, 1-H), 5.55 (1H, s, 7-H), 4.76 (1H, dd, J 6.1, J 2.0, 2-H), 4.49 (1H, br d, J 7.3, 3-H), 4.35 (1H, dd, J 10.3, J 5.0, 5-H), 3.93-3.87 (1H, m, 6-H), 3.83-3.79 (1H, m, 6-H), 3.80 (3H, s, -OMe), 3.77-3.75 (1H, m, 4-H), 2.47 (1H, s, -OH).

δ ^{13}C (100 MHz; CDCl3)

159.4 (11-C), 143.3 (1-C), 128.6 (8-C), 126.7 (9-C), 112.8 (10-C), 102.7 (2-C), 100.9 (7-C), 79.8 (4-C), 68.9 (5-C), 67.6 (6-C), 65.7 (3-C), 54.4 (OMe).

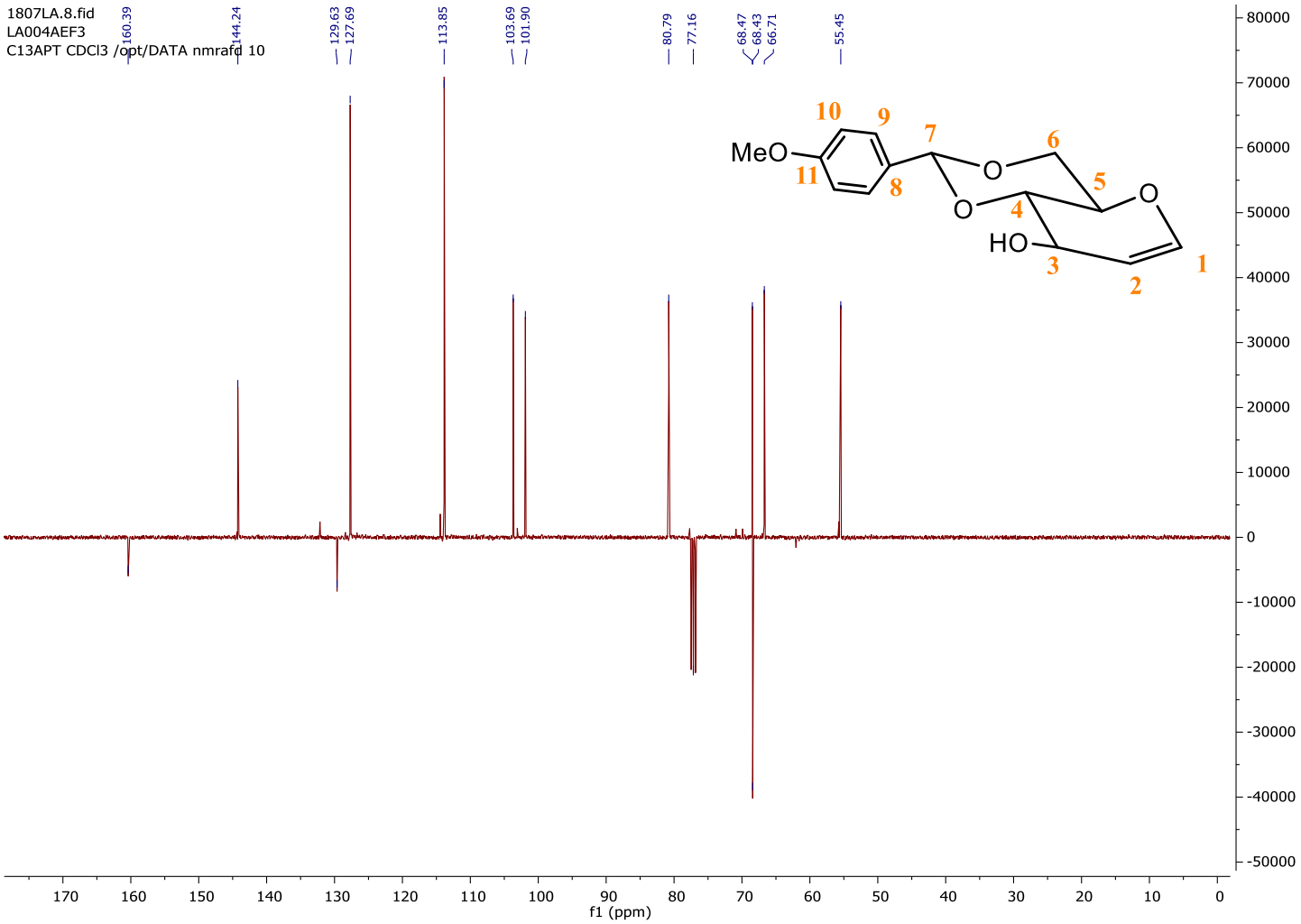
Exact mass TOF MS ESI+ $[C_{14}H_{16}O_5 + Na]^+$ required 287.10, found 287.09

<u>4,6-*O*-(4-Methoxybenzylidene)-D-glucal **(10)**</u>



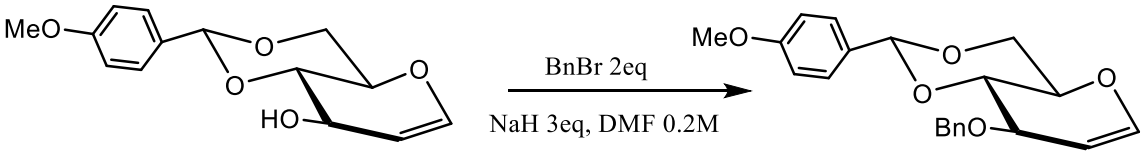
δ ^{1}H (400 MHz; CDCl3)

7.43 (2H, td, J 8.6, J 4.7, 8-H), 6.90 (2H, dt, J 8.8, J 4.9, 9-H), 6.33 (1H, ddd, J 6.1, J 1.6, J 0.4, 1-H), 5.55 (1H, s, 7-H), 4.76 (1H, dd, J 6.1, J 2.0, 2-H), 4.49 (1H, br d, J 7.3, 3-H), 4.35 (1H, dd, J 10.3, J 5.0, 5-H), 3.93-3.87 (1H, m, 6-H), 3.83-3.79 (1H, m, 6-H), 3.80 (3H, s, -OMe), 3.77-3.75 (1H, m, 4-H), 2.47 (1H, s, -OH).



δ ^{13}C (100 MHz; CDCl3)

159.4 (11-C), 143.3 (1-C), 128.6 (8-C), 126.7 (9-C), 112.8 (10-C), 102.7 (2-C), 100.9 (7-C), 79.8 (4-C), 68.9 (5-C), 67.6 (6-C), 65.7 (3-C), 54.4 (OMe).



10 86% **11**

3-*O*-Benzyloxy-4,6-*O*-(4-Methoxybenzylidene)-D-glucal (11)

To a solution of **10** (16.05g, 60.7mmol) in DMF (350mL) at 0°C was added portionwise Sodium Hydride 60% in mineral oil (7.29g, 182mmol). NaH can be previously washed off its mineral oil with n-Hexane dry 3 times. After 30 minutes stirring at the same temperature, the ice bath was removed. Benzyl Bromide was added (14.4mL, 121mmol) and the reaction was stirred overnight, while the temperature was warming up to room temperature. The mixture was then quenched by methanol (20mL) and the DMF was evaporated under reduced pressure. The organic phase was extracted by 100 mL of EtOAc and then the organic layer was washed with NH_4Cl, $NaHCO_3$ and brine (50mL each). The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (EtOAc/Hexane=3:7) to afford 3-O-Benzyloxy-4,6-O-(4-Methoxybenzylidene)-D-glucal **11** (18.43g, 86%) as a white powder.

δ ^{1}H (400 MHz; CDCl3)

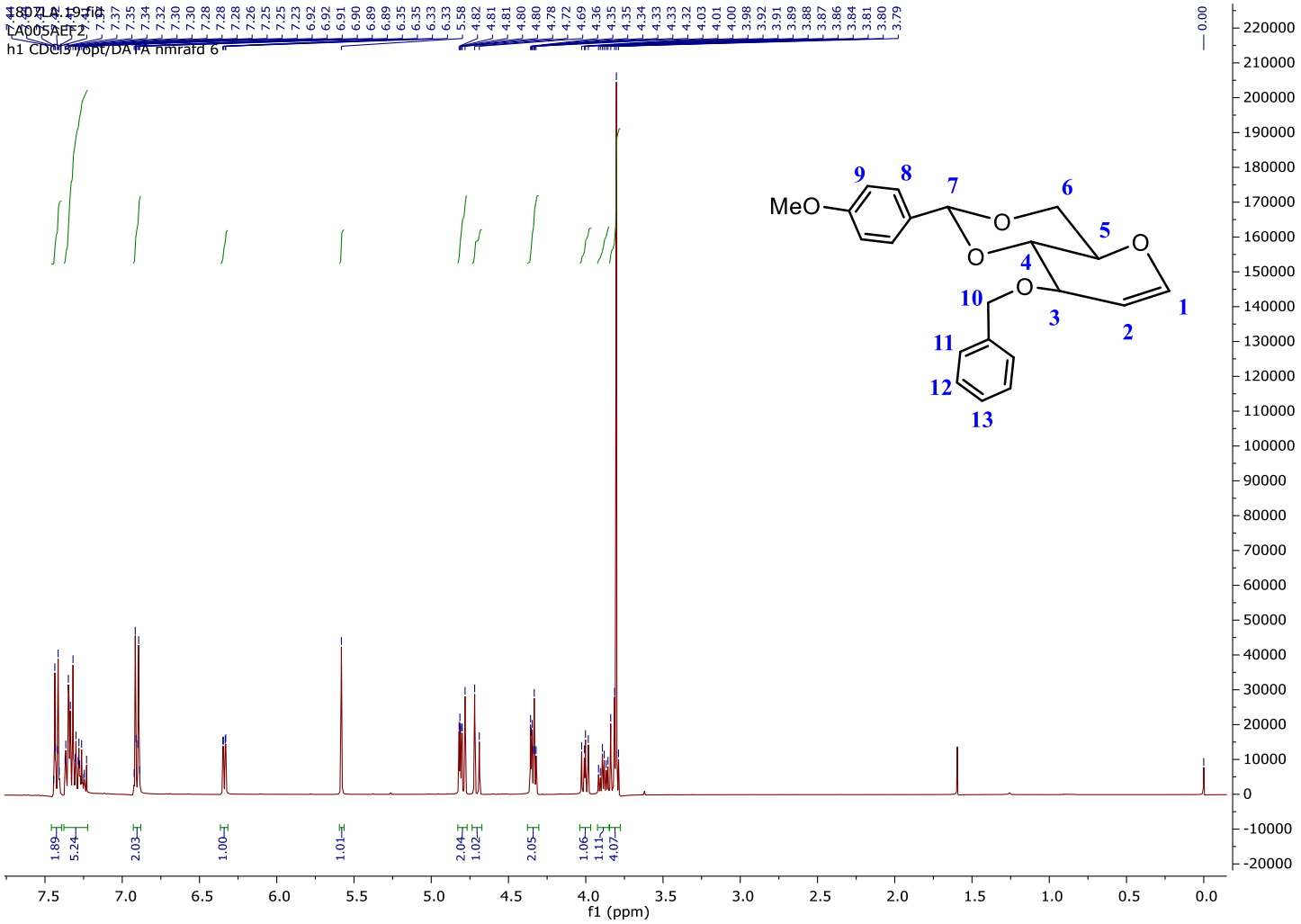
7.42 (2H, dt, J 8.5, J 4.6, 8-H), 7.37-7.23 (7H, m, H_{arom}), 6.90 (2H, dt, J 8.9, J 4.9, 9-H), 6.34 (1H, dd, J 6.2, J 1.4, 1-H), 5.58 (1 H, s, 7-H), 4.81 (1H, dd, J 6.17, J 2.06, 2-H), 4.79 (1H, d, J 12.1, 10-H CH_2 Ph), 4.70 (1H, d, J 12.1, 10-H CH_2 Ph), 4.36–4.32 (2H, m, 3-H, 6a-H), 4.00 (1H, dd, J 9.8, J 7.4, 6b-H), 3.88 (1H, td, J 10.1, J 4.7, 5-H), 3.81 (1H, t, J 10.1, 4-H), 3.80 (3H, s, -OMe).

δ ^{13}C (100 MHz; CDCl3)

160.2 (11-C), 144.5 (1-C), 138.6 (13-C), 129.9 (8-C), 129.9-127.2 (C_{arom} 9, 14, 15, 16-C), 113.7 (10-C), 102.4 (2-C), 101.3 (7-C), 80.1 (5-C), 73.2 (4-C), 72.1 (6-C), 68.8 (3-C), 68.4 (12-C), 55.4 (-OMe).

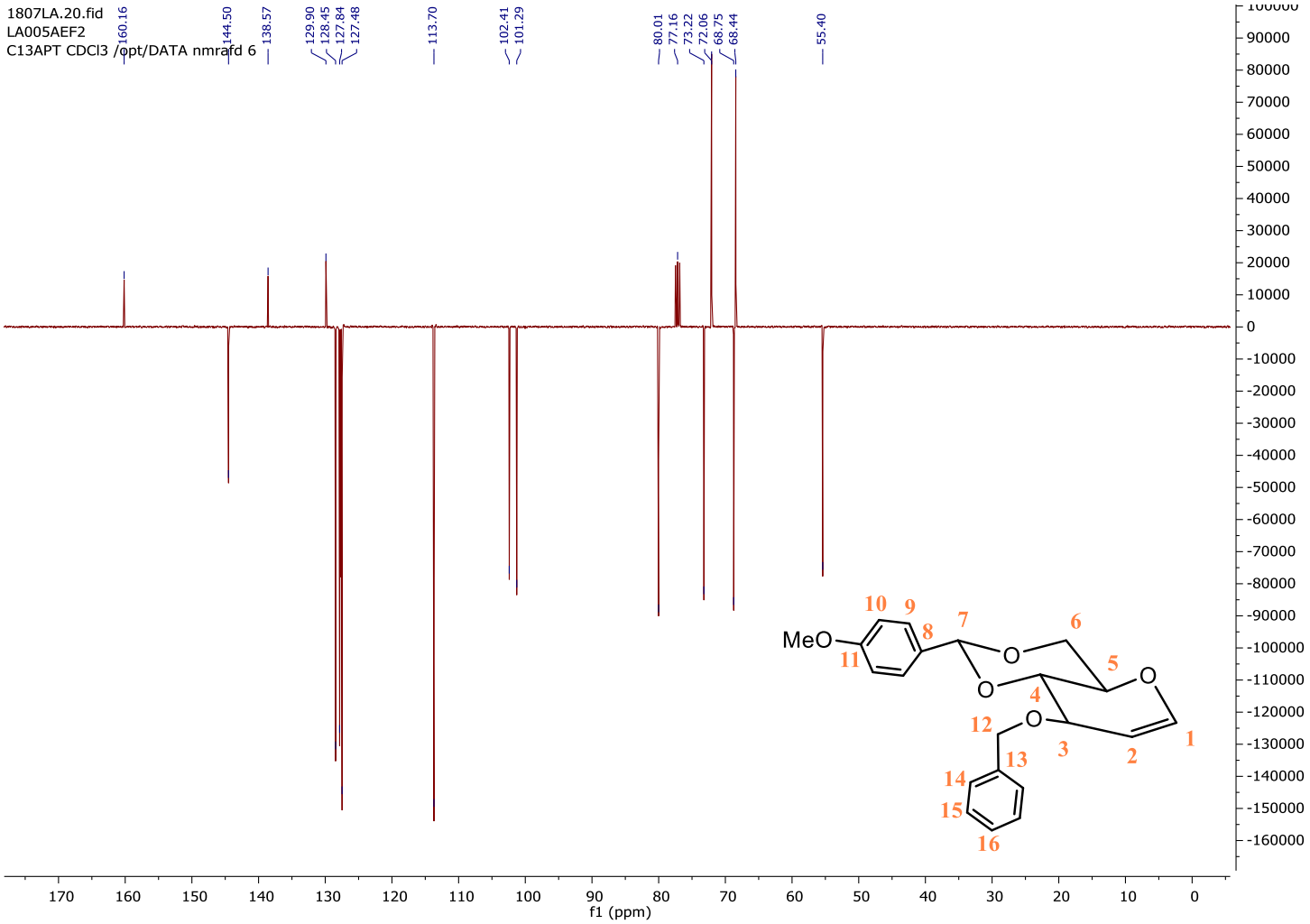
Exact mass TOF MS ESI+ $[C_{21}H_{22}O_5 + Na]^+$ required 377.15, found 377.14

3-*O*-Benzyloxy-4,6-*O*-(4-Methoxybenzylidene)-D-glucal **(11)**



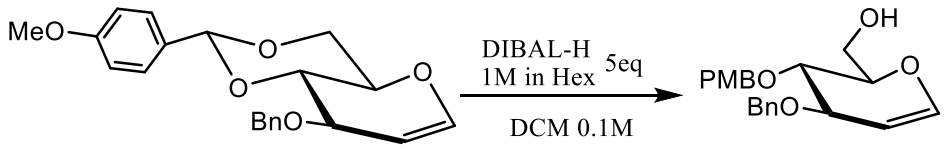
δ ^{1}H (400 MHz; CDCl3)

7.42 (2H, dt, J 8.5, J 4.6, 8-H), 7.37-7.23 (7H, m, H_{arom}), 6.90 (2H, dt, J 8.9, J 4.9, 9-H), 6.34 (1H, dd, J 6.2, J 1.4, 1-H), 5.58 (1 H, s, 7-H), 4.81 (1H, dd, J 6.17, J 2.06, 2-H), 4.79 (1H, d, J 12.1, 10-H CH_2 Ph), 4.70 (1H, d, J 12.1, 10-H CH_2 Ph), 4.36–4.32 (2H, m, 3-H, 6a-H), 4.00 (1H, dd, J 9.8, J 7.4, 6b-H), 3.88 (1H, td, J 10.1, J 4.7, 5-H), 3.81 (1H, t, J 10.1, 4-H), 3.80 (3H, s, -OMe).



δ ^{13}C (100 MHz; CDCl3)

160.2 (11-C), 144.5 (1-C), 138.6 (13-C), 129.9 (8-C), 129.9-127.2 (C_{arom} 9, 14, 15, 16-C), 113.7 (10-C), 102.4 (2-C), 101.3 (7-C), 80.1 (5-C), 73.2 (4-C), 72.1 (6-C), 68.8 (3-C), 68.4 (12-C), 55.4 (-OMe).



11 84% **12**

3-*O*-Benzyloxy-4-*O*-(4-Methoxybenzyloxy)-D-glucal (12)

The glucal **11** (780mg, 2.20mmol) was dissolved in DCM (20mL), cooled at 0°C and stirred for 20 minutes at RT. DIBAL-H 1M in hexane (11.0mL, 11.0mmol) was then added dropwise at 0°C. The mixture was stirred for 2h at 0°C. The reaction was quenched by a solution of potassium sodium tartrate tetrahydrate commonly named Rochelle salt in distilled water (1.5g tartrate in 7.5mL water) for 20 minutes. The mixture was then extracted by DCM (30mL) and the organic layer was washed by distilled water twice and brine (40mL each). The aqueous layers were finally extracted with DCM (20mL). The organic phases were grouped and dried on Na_2SO_4. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/Hexane=1:3) to afford 3-O-Benzyloxy-4-O-(4-Methoxybenzyloxy)-D-glucal **12** as a white solid yielding 84%

δ ^{1}H (400 MHz; CDCl3)

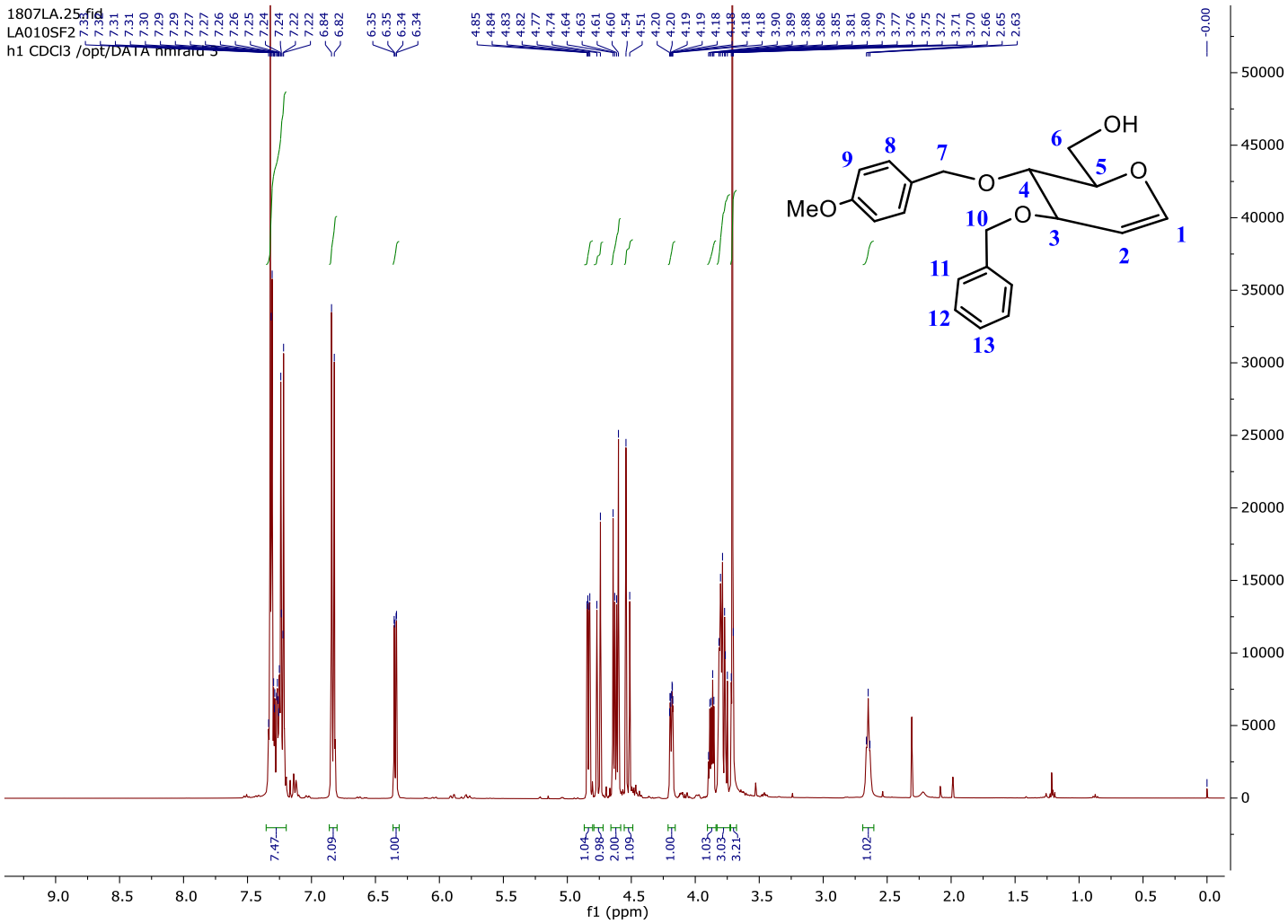
7.34-7.20 (7H, m, H_{arom}), 6.83 (2H, dt, J 8.7, J 4.8, 9-H), 6.34 (1H, dd, J 6.1, J 1.2, 1-H), 4.82 (1H, dd, J 6.1, J 2.6, 2-H), 4.75 (1H, d, J 11.1, 10-H CH_2 Ph), 4.63 (1H, d, J 11.1, 10-H CH_2 Ph), 4.61 (1H, d, J 11.8, 7-H CH_2 Ph(4-OMe)), 4.52 (1H, d, J 11.8, 7-H CH_2 Ph(4-OMe)), 4.19 (1H, ddd, J 6.3, J 2.4, J 2.3, 3-H), 3.87 (1H, dt, J 8.8, J 4.2, 5-H), 3.81-3.79 (2H, m, 6-H), 3.77 (1H, dd, J 8.7, J 6.3 , 4-H), 3.71 (3 H, s, -OMe), 2.65 (1H, s, -OH).

δ ^{13}C (100 MHz; CDCl3)

159.2 (11-C), 144.4 (1-C), 138.1 (13-C), 130.1 (8-C), 129.7-127.6 (C_{arom} 9, 14, 15, 16-C), 113.7 (10-C), 100.1 (2-C), 77.5 (5-C), 75.6 (3-C), 74.1 (4-C), 73.3 (12-C), 70.4 (7-C), 61.4 (6-C), 55.1 (-OMe).

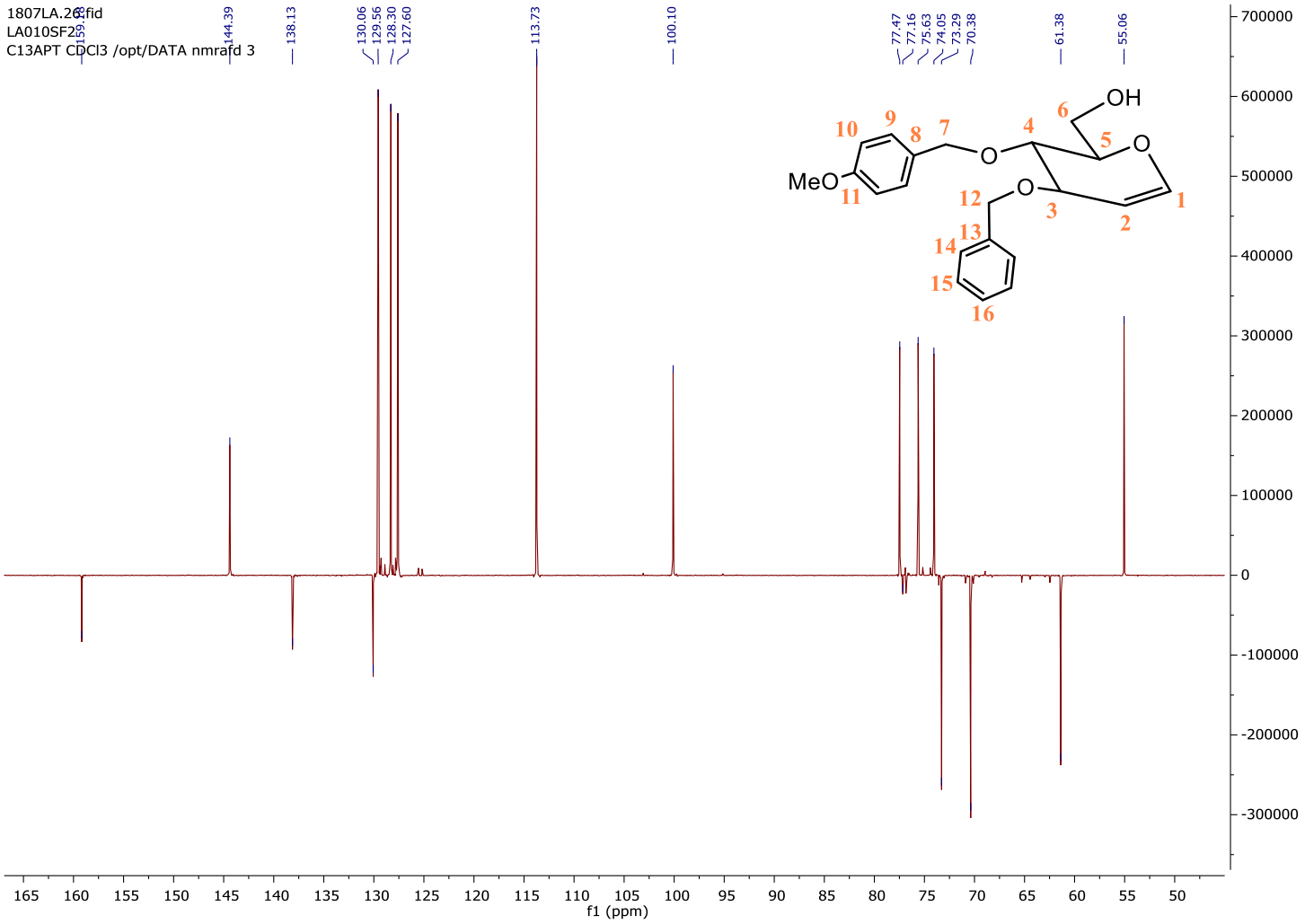
Exact mass TOF MS ESI+ $[C_{21}H_{24}O_5 + Na]^+$ required 379.16, found 379.15

3-*O*-Benzyloxy-4-*O*-(4-Methoxybenzyloxy)-5-(hydroxymethyl)-D-glucal **(12)**



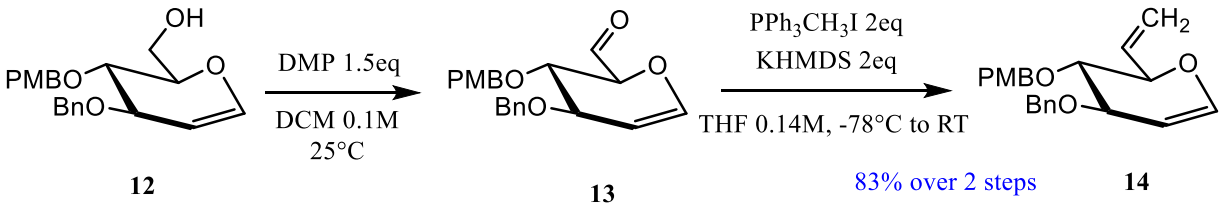
δ ^{1}H (400 MHz; CDCl3)

7.34-7.20 (7H, m, H_{arom}), 6.83 (2H, dt, J 8.7, J 4.8, 9-H), 6.34 (1H, dd, J 6.1, J 1.2, 1-H), 4.82 (1H, dd, J 6.1, J 2.6, 2-H), 4.75 (1H, d, J 11.1, 10-H CH_2 Ph), 4.63 (1H, d, J 11.1, 10-H CH_2 Ph), 4.61 (1H, d, J 11.8, 7-H CH_2 Ph(4-OMe)), 4.52 (1H, d, J 11.8, 7-H CH_2 Ph(4-OMe)), 4.19 (1H, ddd, J 6.3, J 2.4, J 2.3, 3-H), 3.87 (1H, dt, J 8.8, J 4.2, 5-H), 3.81-3.79 (2H, m, 6-H), 3.77 (1H, dd, J 8.7, J 6.3 , 4-H), 3.71 (3 H, s, -OMe), 2.65 (1H, s, -OH).



δ ^{13}C (100 MHz; CDCl3)

159.2 (11-C), 144.4 (1-C), 138.1 (13-C), 130.1 (8-C), 129.7-127.6 (C_{arom} 9, 14, 15, 16-C), 113.7 (10-C), 100.1 (2-C), 77.5 (5-C), 75.6 (3-C), 74.1 (4-C), 73.3 (12-C), 70.4 (7-C), 61.4 (6-C), 55.1 (-OMe).



12 **13** 83% over 2 steps **14**

1,5-Anhydro-3-*O*-benzyloxy-4-*O*-(4-methoxybenzyloxy)-2,6,7-trideoxy-D-arabino-hept-1,6-dienitol (14)

To a solution of the previous alcohol **12** (650mg, 1.82mmol) in DCM dry (6.1mL) was added DMP (926mg, 2.18mmol). The mixture was then stirred at room temperature (25°C) for 1 hour. `

Meanwhile, the ylide was prepared with fresh PPh_3CH_3I (1.48g, 3.65mmol) in THF dry (12.0mL) at -78°C and stirred for 25 minutes. KHMDS (7.3mL, 3.65mmol, 0.5M in Toluene) was then added dropwise at -78°C. The mixture was sequentially stirred at -78°C for 20 min, at 0°C for 50 min and finally at -78°C for 30 min to form the ylide.

Besides the oxidation reaction was quenched by a solution of $Na_2S_2O_3$ (30mL) and $NaHCO_3$ (30mL) for 10 min. Then the aldehyde was worked up with DCM (3*40mL), dried over Na_2SO_4 and the DCM was evaporated under reduced pressure.

The aldehyde in THF dry (11.0mL) was then added dropwise to the ylide at -78°C. The reaction was stirred overnight. The mixture was worked up with NH_4Cl (20mL) and DCM (50mL). Then the organic layer was again extracted with DCM (2*30mL), washed by NaCl (80mL) and dried over Na_2SO_4. The residue was purified by flash chromatography (nHexane/EtOAc=7:3) to afford the alkene as a yellow oil with a yield of 83% over 2 steps.

δ ^{1}H (400 MHz; CDCl3)

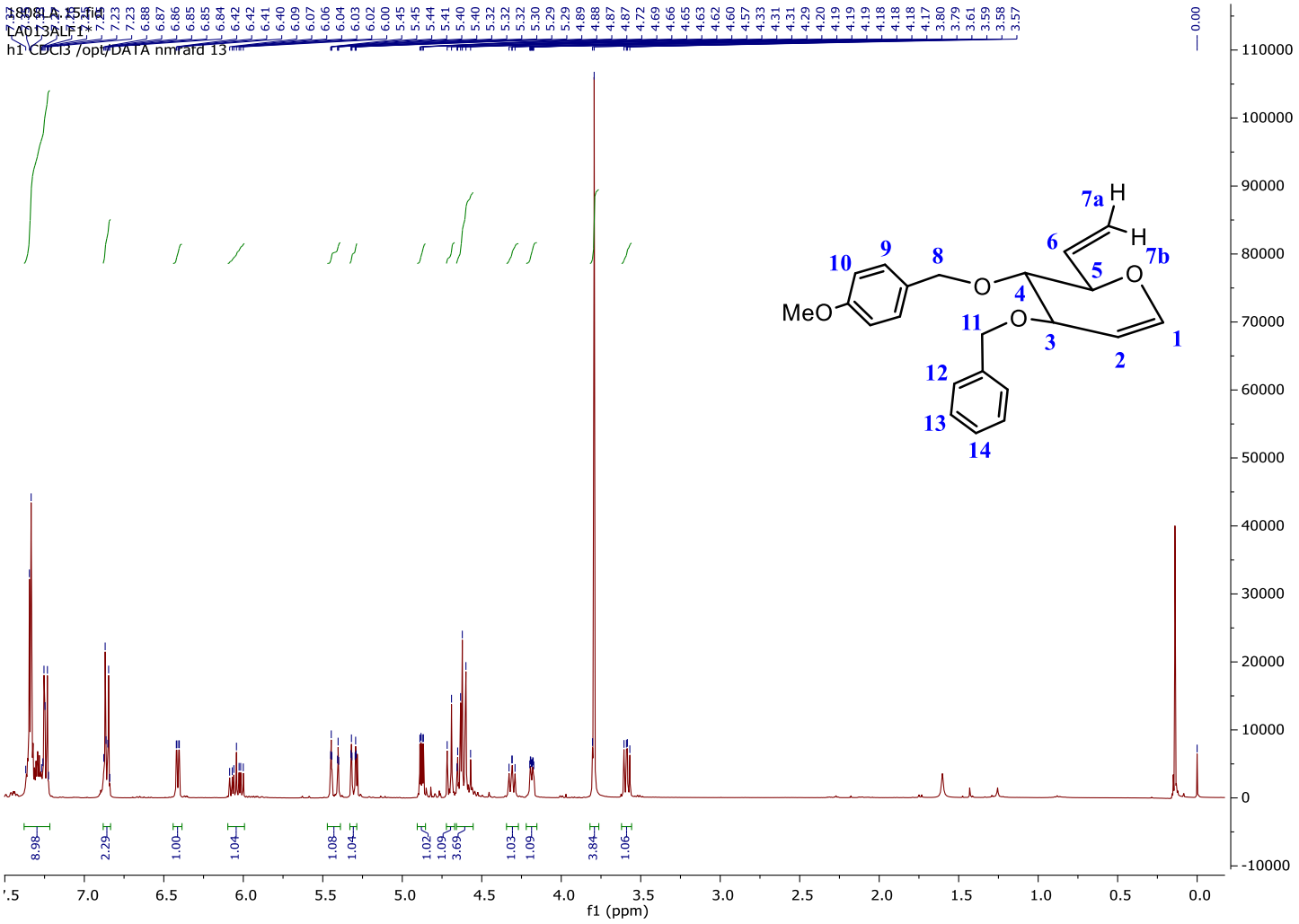
7.37-7.27 (4H, m, H_{arom}), 7.24 (2H, dt, J 8.6, J 5.5, 9-H), 6.86 (2H, td, J 8.7, J 5.5, 10-H), 6.41 (1H, dd, J 6.1, J 1.3, 1-H), 6.04 (1H, ddd, J 17.2, J 10.6, J 6.6, 6-H), 5.43 (1H, dt, J 2.9, J 17.3, 7b-H), 5.31 (1H, dt, J 2.6, J 10.6, 7a-H), 4.88 (1H, dd, J 6.2, J 2.7, 2-H), 4.70 (1H, d, J 10.9, 11-H, CH_2 Ph), 4.64 (1H, d, J 11.7, 8-H, CH_2 Ph(4-OMe)), 4.62 (1H, d, J 10.9, 11-H CH_2 Ph), 4.58 (1H, d, J 11.7, 8-H CH_2 Ph(4-OMe)), 4.31 (1H, dd, J 7.1, J 8.0, 5-H), 4.19 (1H, ddd, J 6.2, J 2.5, J 1.5, 3-H), 3.79 (3H, s, -OMe), 3.59 (1H, dd, J 8.6, J 6.2, 4-H).

δ ^{13}C (100 MHz; CDCl3)

159.4 (12-C), 144.6 (1-C), 138.5 (14-C), 134.5 (6-C), 130.3 (9-C), 129.8-127.8 (C_{arom} 10, 15, 16, 17-C), 118.4 (7-C), 113.9 (11-C), 100.5 (2-C), 78.2 (5-C), 78.0 (4-C), 75.5 (3-C), 73.6 (8-C), 70.8 (13-C), 55.4 (-OMe).

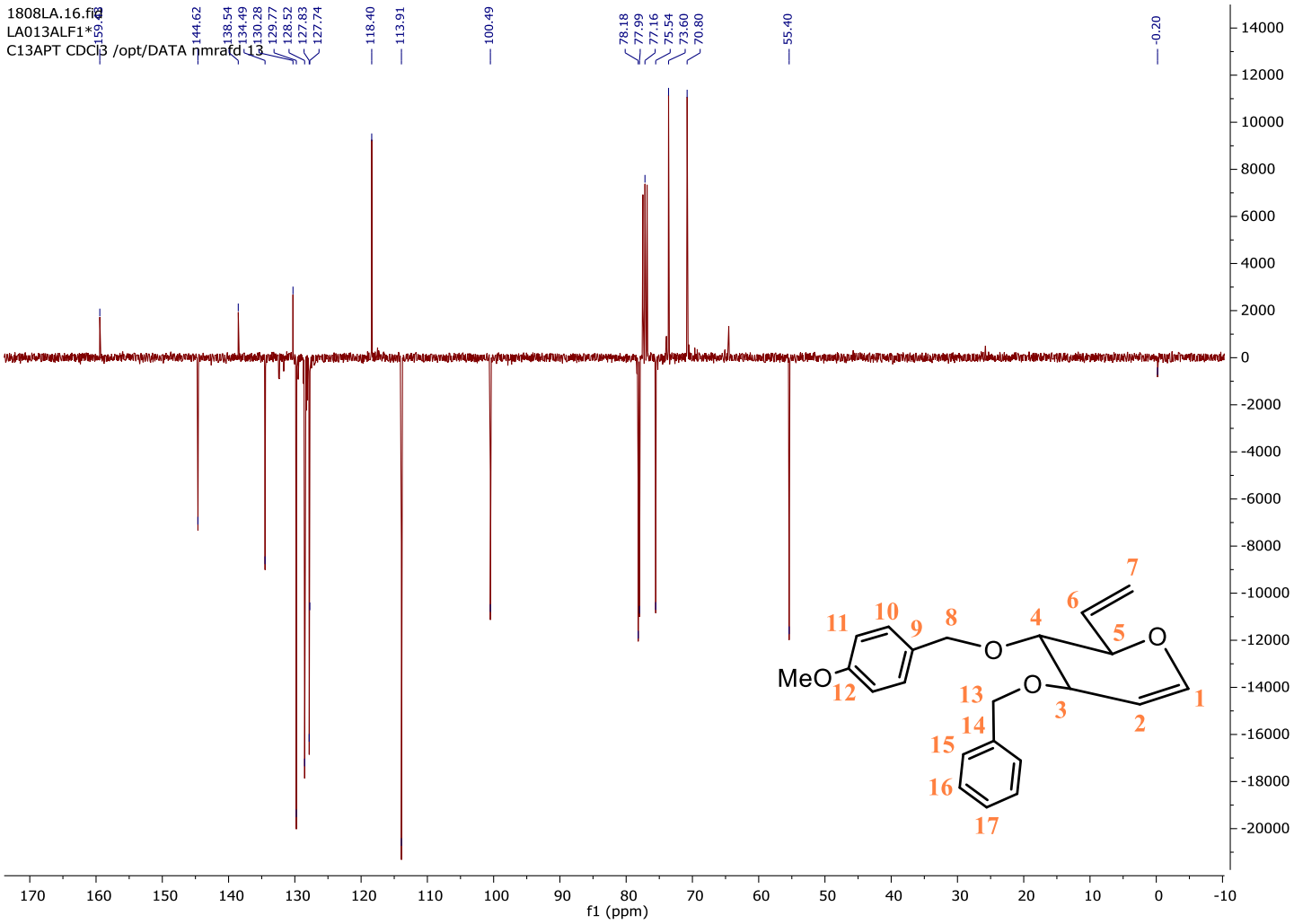
Exact mass TOF MS ESI+ $[C_{22}H_{24}O_4 + Na]^+$ required 375.17, found 375.16

1,5-Anhydro-3-*O*-Benzyloxy-4-*O*-(4-Methoxybenzyloxy)-2,6,7-trideoxy-D-arabino-hept-1,6-dienitol **(14)**



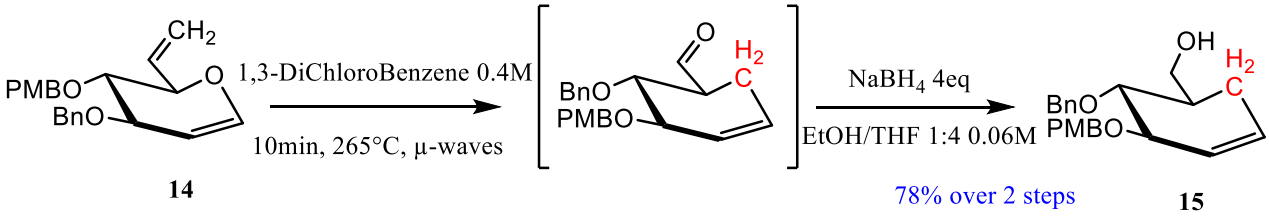
δ ^{1}H (400 MHz; CDCl3)

7.37-7.27 (4H, m, H_{arom}), 7.24 (2H, dt, J 8.6, J 5.5, 9-H), 6.86 (2H, td, J 8.7, J 5.5, 10-H), 6.41 (1H, dd, J 6.1, J 1.3, 1-H), 6.04 (1H, ddd, J 17.2, J 10.6, J 6.6, 6-H), 5.43 (1H, dt, J 2.9, J 17.3, 7b-H), 5.31 (1H, dt, J 2.6, J 10.6, 7a-H), 4.88 (1H, dd, J 6.2, J 2.7, 2-H), 4.70 (1H, d, J 10.9, 11-H, CH_2 Ph), 4.64 (1H, d, J 11.7, 8-H, CH_2 Ph(4-OMe)), 4.62 (1H, d, J 10.9, 11-H CH_2 Ph), 4.58 (1H, d, J 11.7, 8-H CH_2 Ph(4-OMe)), 4.31 (1H, dd, J 7.1, J 8.0, 5-H), 4.19 (1H, ddd, J 6.2, J 2.5, J 1.5, 3-H), 3.79 (3H, s, -OMe), 3.59 (1H, dd, J 8.6, J 6.2, 4-H).



δ ^{13}C (100 MHz; CDCl3)

159.4 (12-C), 144.6 (1-C), 138.5 (14-C), 134.5 (6-C), 130.3 (9-C), 129.8-127.8 (C_{arom} 10, 15, 16, 17-C), 118.4 (7-C), 113.9 (11-C), 100.5 (2-C), 78.2 (5-C), 78.0 (4-C), 75.5 (3-C), 73.6 (8-C), 70.8 (13-C), 55.4 (-OMe).



14

78% over 2 steps

15

(3R,4R,5R)-4-*O*-Benzyloxy-3-*O*-(4-methoxybenzyloxy)-5-(hydroxymethyl)cyclohexene (15)

The alkene **14** (200mg, 0.57mmol) was dissolved in m-DCB (1.43mL, 0.4M) at RT in a micro-wave vial previously flushed with Nitrogen. The Claisen rearrangement was then carried out under micro-waves at 265°C for 10 min. After consumption of the starting material, the yellow solution of reactive aldehyde was immediately poured in a mixture of $NaBH_4$ (86mg, 2.27mmol) in THF/EtOH (10mL, 4:1) and stirred for 1h at RT (monospot on the TLC, orange solution). The reaction was quenched with distilled water (10mL). The aqueous phase was increased by 10 mL of distilled water and extracted with DCM (3*20mL). Finally, the organic layers were dried over Na_2SO_4. The residue was purified by flash chromatography (nHexane/EtOAc=8:2) to afford the alcohol **15** as a colorless oil with a yield of 78% over 2 steps.

δ ^{1}H (400 MHz; CDCl3)

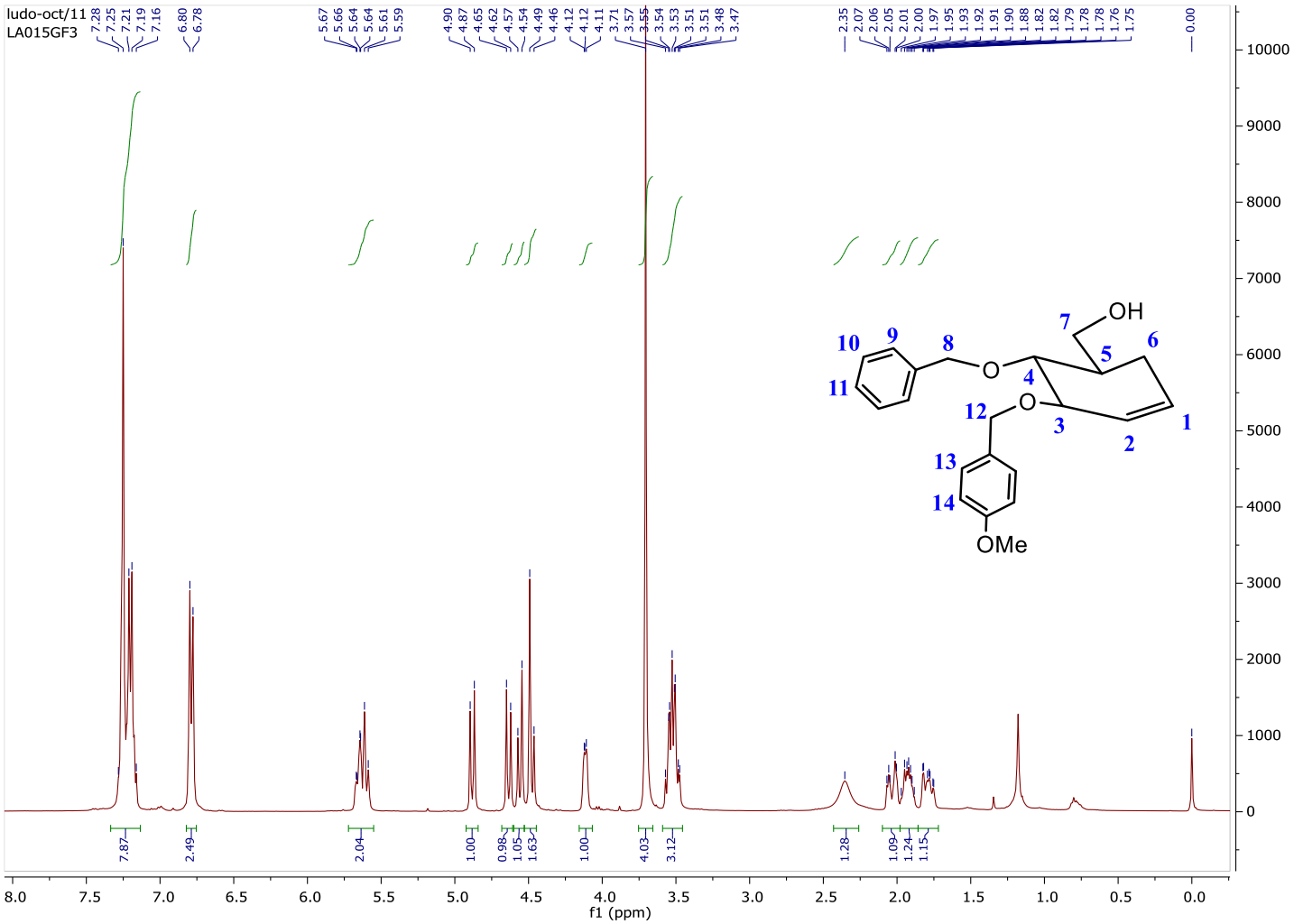
7.28-7.16 (7H, m, H_{arom}), 6.79 (2H, br d, J 8.3, 14-H), 5.67-5.64 (1H, m, 1-H), 5.64-5.59 (1H, m, 2-H), 4.88 (1H, d, J 11.3, 8-H CH_2 Ph), 4.64 (1H, d, J 11.3, 8-H CH_2 Ph), 4.56 (1H, d, J 11.2, 12-H CH_2 Ph(4-OMe)), 4.48 (1H, d, J 11.7, 12-H CH_2 Ph(4-OMe)), 4.12 (1H, br d, 4-H), 3.71 (3H, s, -OMe), 3.57-3.47 (3H, m, 3-H, 6-H), 2.35 (1H, s, -OH), 2.07-2.00 (1H, m, 7-H), 1.97-1.88 (1H, m, 5-H), 1.82-1.75 (1H, m, 7-H).δ ^{13}C (100 MHz; CDCl3)

δ ^{13}C (100 MHz; CDCl3)

159.4 (17-C), 138.5 (9-C), 132.1 (14-C), 130.5-128.0 (C_{arom} 10, 11, 12, 15-C), 127.7 (1-C), 126.1 (2-C), 114.0 (16-C), 82.3 (3-C), 80.9 (4-C), 74.4 (8-C), 71.1 (13-C), 65.9 (6-C), 55.4 (-OMe), 40.7 (5-C), 28.1 (7-C).

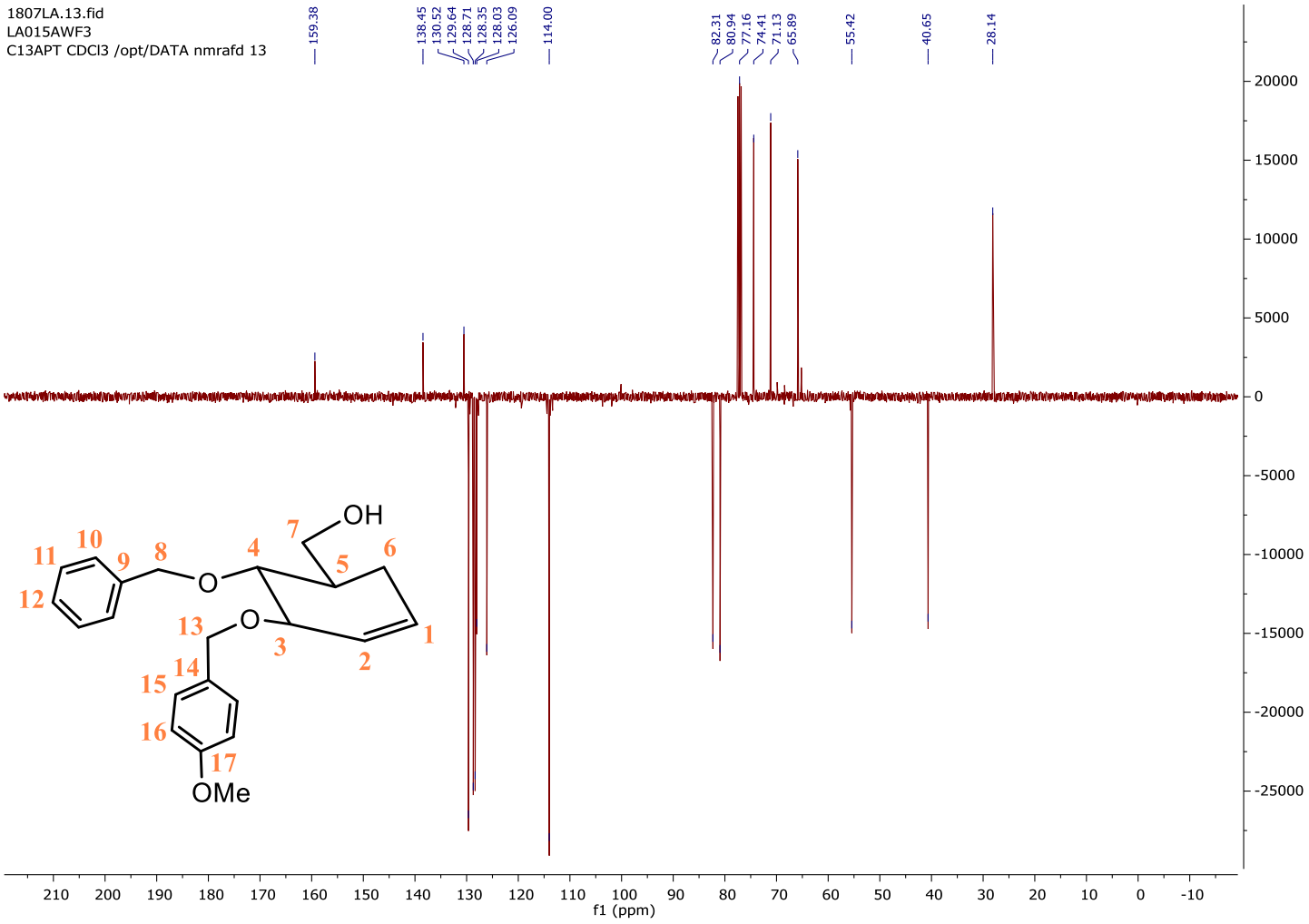
Exact mass TOF MS ESI+ $[C_{22}H_{26}O_4 + Na]^+$ required 377.18, found 377.17

4-*O*-Benzyloxy-3-*O*-(4-Methoxybenzyloxy)-5-(hydroxymethyl)cyclohexene **(15)**



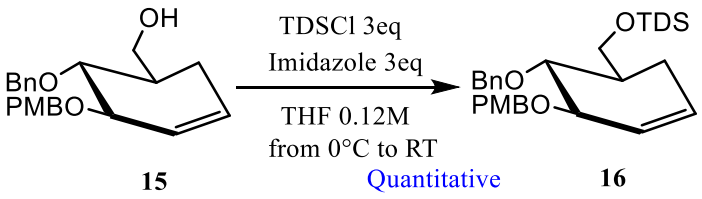
δ ^{1}H (400 MHz; CDCl3)

7.28-7.16 (7H, m, H_{arom}), 6.79 (2H, br d, J 8.3, 14-H), 5.67-5.64 (1H, m, 1-H), 5.64-5.59 (1H, m, 2-H), 4.88 (1H, d, J 11.3, 8-H CH_2 Ph), 4.64 (1H, d, J 11.3, 8-H CH_2 Ph), 4.56 (1H, d, J 11.2, 12-H CH_2 Ph(4-OMe)), 4.48 (1H, d, J 11.7, 12-H CH_2 Ph(4-OMe)), 4.12 (1H, br d, 4-H), 3.71 (3H, s, -OMe), 3.57-3.47 (3H, m, 3-H, 6-H), 2.35 (1H, s, -OH), 2.07-2.00 (1H, m, 7-H), 1.97-1.88 (1H, m, 5-H), 1.82-1.75 (1H, m, 7-H).



δ ^{13}C (100 MHz; CDCl3)

159.4 (17-C), 138.5 (9-C), 132.1 (14-C), 130.5-128.0 (C_{arom} 10, 11, 12, 15-C), 127.7 (1-C), 126.1 (2-C), 114.0 (16-C), 82.3 (3-C), 80.9 (4-C), 74.4 (8-C), 71.1 (13-C), 65.9 (6-C), 55.4 (-OMe), 40.7 (5-C), 28.1 (7-C).



15 Quantitative **16**

4-*O*-Benzyl-3-*O*-(4-methoxybenzyloxy)-*6*-*O*-thexyldimethylsilyl-5-methylcyclohexene (16)

The alcohol **15** (715mg, 2.02mmol) was dissolved in dry THF (17mL) at RT. Imidazole (125mg, 1.83mmol) was added and the mixture was stirred at RT for 5 min and then at 0°C for 10 min. ThexylDimethylSilylChloride (1.19mL, 6.05mmol) was then added dropwise to pay attention to the formation of a white precipitate. Thus, the ice bath was removed at the first precipitation and TDSCl remaining was added slowly to the mixture, left warming up to RT and stirring overnight. The reaction was monitored by TLC (Pent/AcOEt 3:1). The organic phase was extracted by EtOAc and then washed with distilled water (5 times). The residue was purified by flash chromatography (nHex/AcOEt 95:5) to allow the formation of compound **16** as a yellow oil with a quantitative yield.

δ ^{1}H (400 MHz; CDCl3)

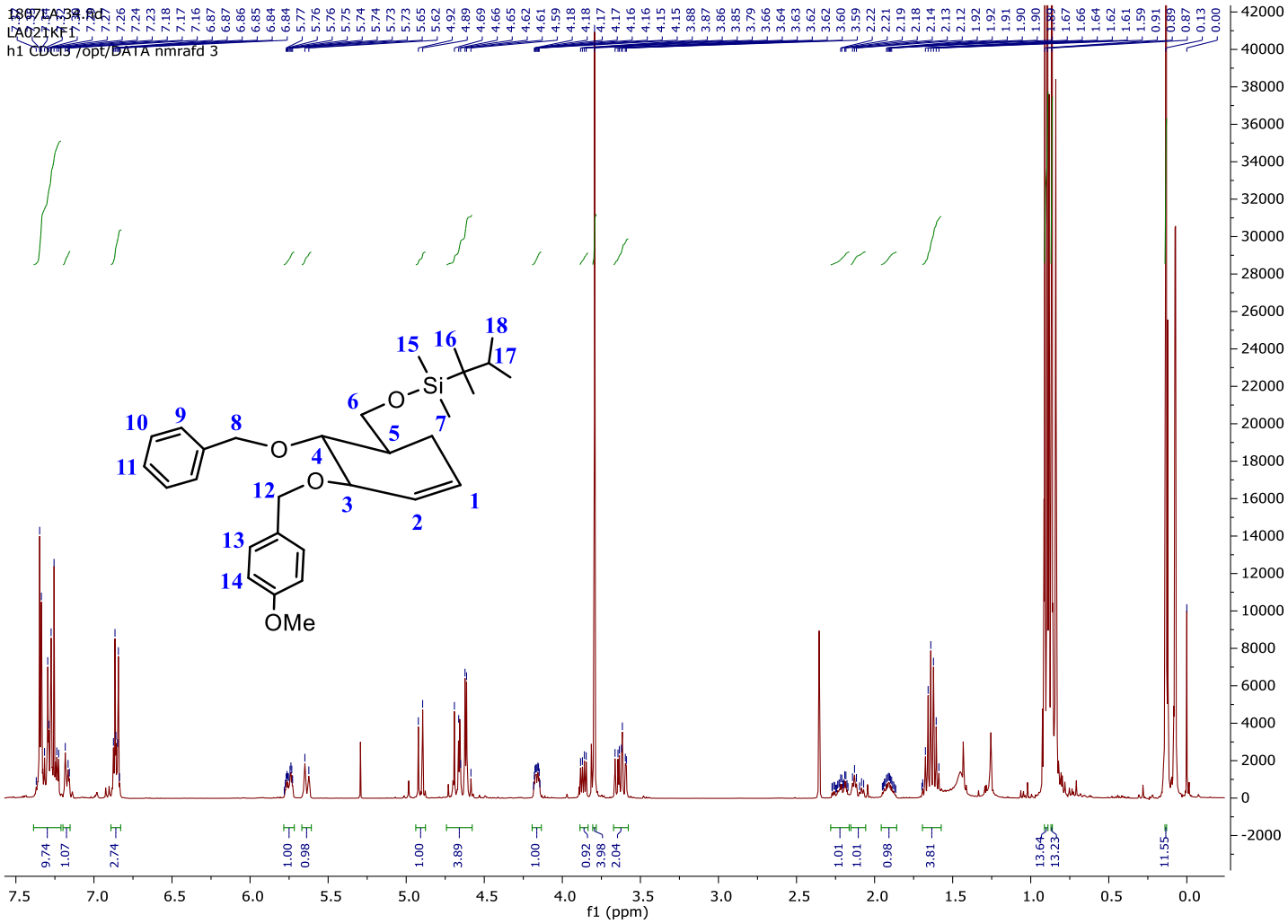
7.37-7.16 (7H, m, H_{arom}), 6.88-6.84 (2H, m, 14-H), 5.75 (1H, ddq, J 9.0, J 4.3, J 2.4, 1-H), 5.64 (1H, br d, 2-H), 4.91 (1H, d, J 11.0, 8-H CH_2 Ph), 4.68 (1H, d, J 11.0, 8-H CH_2 Ph), 4.64 (1H, d, J 11.3, 12-H CH_2 Ph(4-OMe)), 4.60 (1H, d, J 11.3, 12-H CH_2 Ph(4-OMe)), 4.16 (1H, ddq, J 7.1, J 3.6, J 1.8, 3-H), 3.86 (1H, dd, J 9.8, J 4.8, 6-H), 3.79 (3H, s, -OMe), 3.64 (1H, dd, J 10.0, J 6.6, 4-H), 3.63-3.58 (1H, m, 6-H), 2.28-2.16 (1H, m, 7-H), 2.10 (1H, dt, J 18.4, J 5.3, 7-H), 1.91 (1H, ttd, J 10.5, J 5.1, J 2.7, 5-H), 1.64 (1H, hept, J 6.9, 17-H), 0.90 (6H, d, J 6.9, 18-H), 0.87 (6H, s, 16-H), 0.13 (6H, s, 15-H).

δ ^{13}C (100 MHz; CDCl3)

159.3 (14-C), 139.3 (9-C), 133.8 (17-C), 131.0-128.0 (C_{arom} 10, 11, 12, 15-C), 127.6 (1-C), 126.3 (2-C), 113.9 (16-C), 81.5 (3-C), 79.7 (4-C), 74.7 (8-C), 71.5 (13-C), 62.6 (6-C), 55.4 (-OMe), 41.4 (5-C), 34.3 (21-C), 28.7 (7-C), 25.3 (19-C), 20.5-20.3 (20-C), 18.8-18.7 (22-C), -3.27- -3.46 (18-C).

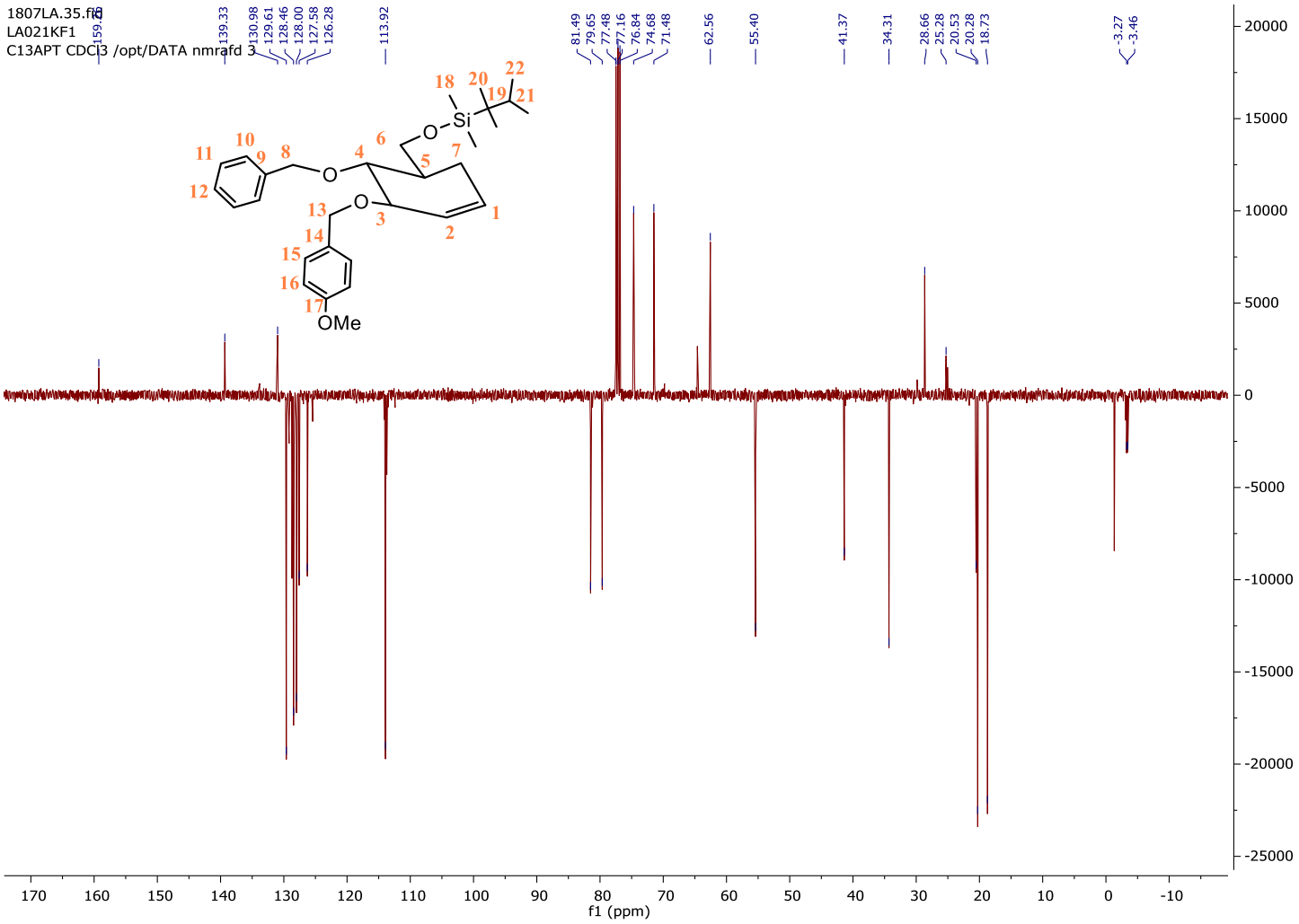
Exact mass TOF MS ESI+ $[C_{30}H_{44}O_4Si + Na]^+$ required 519.30, found 519.29

4-*O*-Benzyloxy-3-*O*-(4-Methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-cyclohexene **(16)**



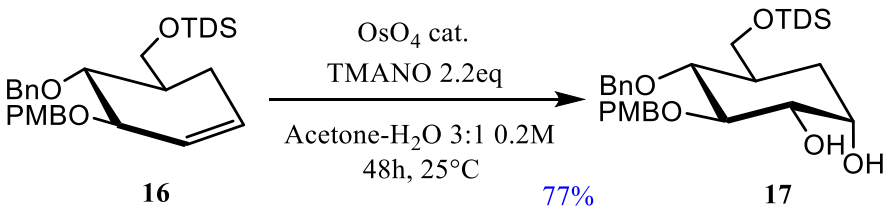
δ ^{1}H (400 MHz; CDCl3)

7.37-7.16 (7H, m, H_{arom}), 6.88-6.84 (2H, m, 14-H), 5.75 (1H, ddq, J 9.0, J 4.3, J 2.4, 1-H), 5.64 (1H, br d, 2-H), 4.91 (1H, d, J 11.0, 8-H CH_2 Ph), 4.68 (1H, d, J 11.0, 8-H CH_2 Ph), 4.64 (1H, d, J 11.3, 12-H CH_2 Ph(4-OMe)), 4.60 (1H, d, J 11.3, 12-H CH_2 Ph(4-OMe)), 4.16 (1H, ddq, J 7.1, J 3.6, J 1.8, 3-H), 3.86 (1H, dd, J 9.8, J 4.8, 6-H), 3.79 (3H, s, -OMe), 3.64 (1H, dd, J 10.0, J 6.6, 4-H), 3.63-3.58 (1H, m, 6-H), 2.28-2.16 (1H, m, 7-H), 2.10 (1H, dt, J 18.4, J 5.3, 7-H), 1.91 (1H, ttd, J 10.5, J 5.1, J 2.7, 5-H), 1.64 (1H, hept, J 6.9, 17-H), 0.90 (6H, d, J 6.9, 18-H), 0.87 (6H, s, 16-H), 0.13 (6H, s, 15-H).



δ ^{13}C (100 MHz; CDCl3)

159.3 (14-C), 139.3 (9-C), 133.8 (17-C), 131.0-128.0 (C_{arom} 10, 11, 12, 15-C), 127.6 (1-C), 126.3 (2-C), 113.9 (16-C), 81.5 (3-C), 79.7 (4-C), 74.7 (8-C), 71.5 (13-C), 62.6 (6-C), 55.4 (-OMe), 41.4 (5-C), 34.3 (21-C), 28.7 (7-C), 25.3 (19-C), 20.5-20.3 (20-C), 18.8-18.7 (22-C), -3.27- -3.46 (18-C).



16 **17**

4-*O*-Benzyl-3-*O*-(4-methoxybenzyloxy)-*6*-*O*-thexyldimethylsilyl-5a-carba-α-D-glucopyranose (17)

Compound **16** (230mg, 0.46mmol) was dissolved in a mixture of acetone (1.69mL) and water (562µL). A solution of OsO_4 (537µL based on a preparation of 250mg OsO_4 in 4.5mL H_2O and 18mL acetone) and TMANO (116mg, 1.02mmol) were added at RT. The reaction was carried out at 25°C for 48h. A saturated aqueous solution of $Na_2S_2O_3$ (2mL) was the added and the mixture was stirred at RT to reduce the OsO_4. The organic phase was extracted by $CHCl_3$ (15mL), washed by brine (10mL) and finally dried over Na_2SO_4. The crude product was purified by flash chromatography (nHex/AcOEt, 8.2) to afford the formation of the diol **17** as a colorless oil with a yield of 77%.

δ ^{1}H (400 MHz; CDCl3)

7.37-7.15 (7H, m, H_{arom}), 6.87 (2H, br d, J 8.7, 14-H), 4.90 (1H, d, J 12, 8-H CH_2 Ph), 4.88 (1H, d, J 8, 12-H CH_2 Ph(4-OMe)), 4.69 (1H, d, J 10.9, 8-H CH_2 Ph), 4.61 (1H, d, J 11.1, 12-H CH_2 Ph(4-OMe)), 4.05 (1H, br d, J 2.7, 1-H), 3.96 (1H, dd, J 10.0, J 3.3, 6-H), 3.78 (3H, s, -OMe), 3.71 (1H, t, J 9.4, 3-H), 3.48 (2H, t, J 10.0, 6-H, 4-H), 3.43 (1H, dd, J 2.3, J 9.4, 2-H), 2.64 (1H, s, -OH), 2.58 (1H, s, -OH), 2.09-2.03 (1H, m, 5-H), 1.77 (1H, dt, J 14.5, J 3.6, 7-H), 1.62 (1H, hept, J 6.9, 17-H), 1.59-1.52 (1H, m, 7-H), 0.88 (6H, d, J 6.9, 18-H), 0.85 (6H, d, d 1.2, 16-H), 0.07 (6H, s, 15-H).

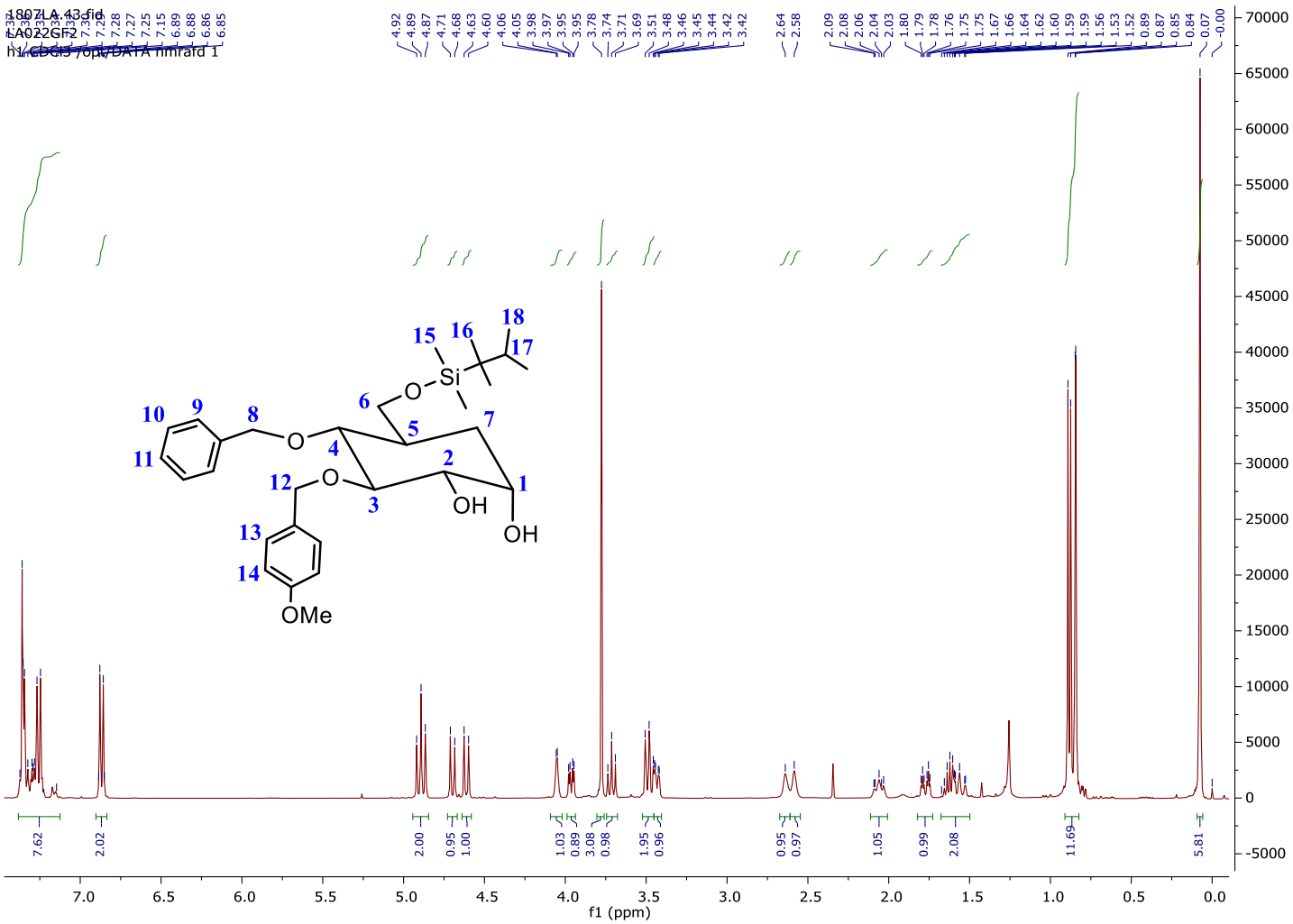
δ ^{13}C (100 MHz; CDCl3)

159.5 (14-C), 138.9 (9-C), 130.9 (17-C), 129.7-127.7 (C_{arom} 10, 11, 12, 15-C), 114.2 (16-C), 83.4 (3-C), 81.0 (4-C), 75.1 (13-C), 74.9 (8-C), 74.6 (2-C), 68.5 (1-C), 62.1 (6-C), 55.3 (-OMe), 38.9 (5-C), 34.3 (21-C), 30.4 (7-C), 25.2 (19-C), 20.5-20.4 (20-C), 18.8-18.7 (22-C), -3.35- -3.56 (18-C).

Exact mass TOF MS ESI+ $[C_{30}H_{46}O_6Si + Na]^+$ required 553.31, found 553.30

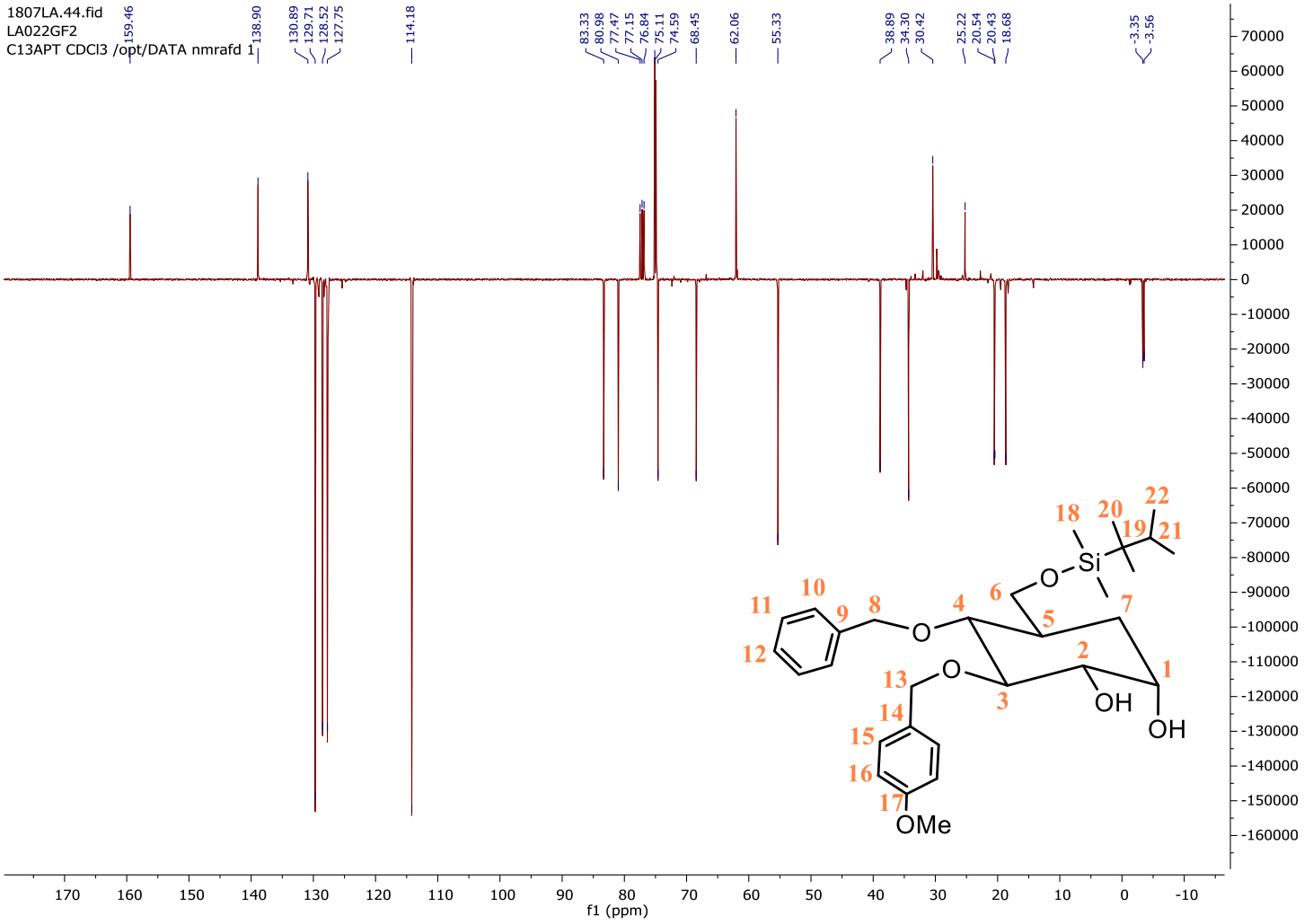
4-*O*-Benzyloxy-3-*O*-(4-Methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-glucopyranose

(17)



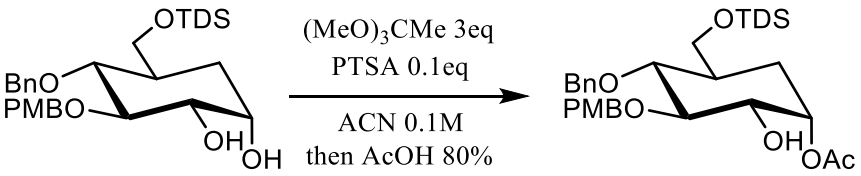
δ ^{1}H (400 MHz; CDCl3)

7.37-7.15 (7H, m, H_{arom}), 6.87 (2H, br d, J 8.7, 14-H), 4.90 (1H, d, J 12, 8-H CH_2 Ph), 4.88 (1H, d, J 8, 12-H CH_2 Ph(4-OMe)), 4.69 (1H, d, J 10.9, 8-H CH_2 Ph), 4.61 (1H, d, J 11.1, 12-H CH_2 Ph(4-OMe)), 4.05 (1H, br d, J 2.7, 1-H), 3.96 (1H, dd, J 10.0, J 3.3, 6-H), 3.78 (3H, s, -OMe), 3.71 (1H, t, J 9.4, 3-H), 3.48 (2H, t, J 10.0, 6-H, 4-H), 3.43 (1H, dd, J 2.3, J 9.4, 2-H), 2.64 (1H, s, -OH), 2.58 (1H, s, -OH), 2.09-2.03 (1H, m, 5-H), 1.77 (1H, dt, J 14.5, J 3.6, 7-H), 1.62 (1H, hept, J 6.9, 17-H), 1.59-1.52 (1H, m, 7-H), 0.88 (6H, d, J 6.9, 18-H), 0.85 (6H, d, d 1.2, 16-H), 0.07 (6H, s, 15-H).



δ ^{13}C (100 MHz; CDCl3)

159.5 (14-C), 138.9 (9-C), 130.9 (17-C), 129.7-127.7 (C_{arom} 10, 11, 12, 15-C), 114.2 (16-C), 83.4 (3-C), 81.0 (4-C), 75.1 (13-C), 74.9 (8-C), 74.6 (2-C), 68.5 (1-C), 62.1 (6-C), 55.3 (-OMe), 38.9 (5-C), 34.3 (21-C), 30.4 (7-C), 25.2 (19-C), 20.5-20.4 (20-C), 18.8-18.7 (22-C), -3.35- -3.56 (18-C).



17 Quantitative **18**

1-*O*-Acetyl-4-*O*-benzyl-3-*O*-(4-methoxybenzyloxy)-*6*-*O*-thexyldimethylsilyl-5a-carba-α-D-glucopyranose (18)

Compound **17** (155mg, 0.29mmol) was dissolved in acetonitrile (2.9mL) at room temperature, under nitrogen. Trimethyl orthoacetate (115µL, 0.88mmol) and PTSA (5mg, 0.03mmol) were successively added to the mixture which was then stirred for 60min at room temperature under nitrogen. After completion of the reaction, a solution of AcOH 80% (2.32mL AcOH + 0.58mL H_2O) was added. The following reaction of acetylation was fully ended in 60min. The organic phase was extracted with DCM (5mL) then washed by water (5mL) and $NaHCO_3$ (5mL) and finally dried over Na_2SO_4. The residue was purified by flash chromatography (nHex/AcOEt) to afford the compound **18** selectively acetylated on the pseudo anomeric position as an uncolored oil in a quantitative yield.

δ ^{1}H (400 MHz; CDCl3)

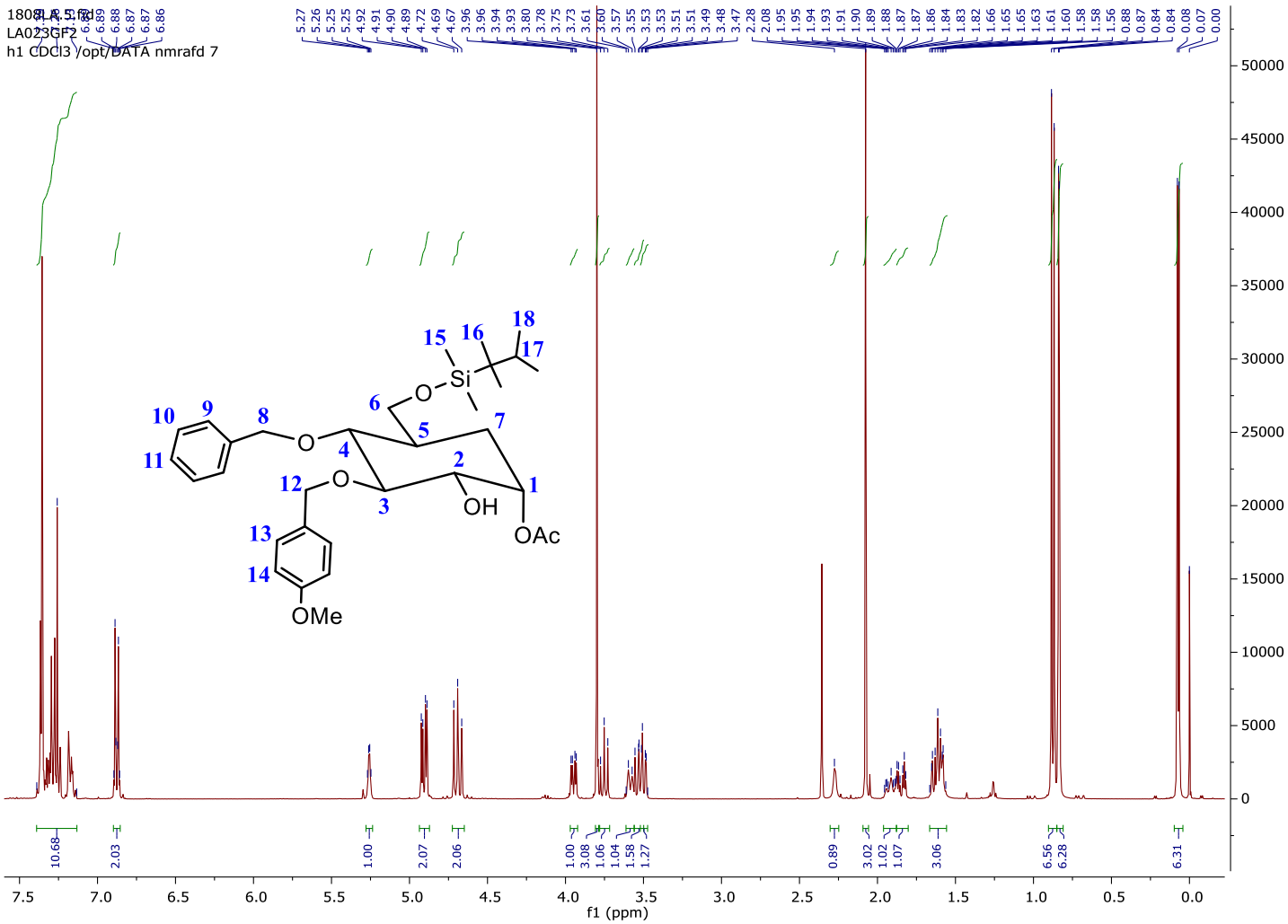
7.39-7.13 (7H, m, H_{arom}), 6.87 (2H, dt, J 8.7, J 5.0, 14-H), 5.26 (1H, dd, J 5.7, J 3.0, 1-H), 4.91 (1H, d, J 10.6, 8-H CH_2 Ph), 4.90 (1H, d, J 10.9, 12-H CH_2 Ph(4-OMe)), 4.70 (1H, d, J 10.0, 8-H CH_2 Ph), 4.68 (1H, d, J 10.5, 12-H CH_2 Ph(4-OMe)), 3.95 (1H, dd, J 10.0, J 3.5, 6-H), 3.80 (3H, s, -OMe), 3.75 (1H, t, J 9.3, 3-H), 3.58 (1H, br d, J 9.6, 2-H), 3.53 (1H, dd, J 9.1, J 10.1, 4-H), 3.50 (1H, dd, J 9.8, J 2.4, 6-H), 2.28 (1H, s, -OH), 2.08 (3H, s, -OAc), 1.95-1.88 (1H, m, 5-H), 1.85 (1H, dt, J 14.8, J 7.6, 7-H), 1.61 (1H, dt, J 13.8, J 6.9, 7-H), 1.61 (1H, hept, J 6.9, 17-H), 0.88 (6H, d, J 6.8, 18-H), 0.84 (6H, d, J 1.7, 16-H), 0.07 (6H, d, J 4.4, 15-H).

δ ^{13}C (100 MHz; CDCl3)

170.9 (C(O), -OAc), 159.5 (14-C), 138.7 (9-C), 130.8 (17-C), 129.8-127.9 (C_{arom} 10, 11, 12, 15-C), 114.8 (16-C), 84.0 (3-C), 80.5 (4-C), 75.4 (13-C), 75.3 (8-C), 73.4 (2-C), 71.8 (1-C), 61.8 (6-C), 55.4 (-OMe), 39.6 (5-C), 34.3 (21-C), 28.8 (7-C), 25.3 (19-C), 21.4 (CH_3, -OAc), 20.5-20.4 (20-C), 18.8-18.7 (22-C), -3.28- -3.53 (18-C).

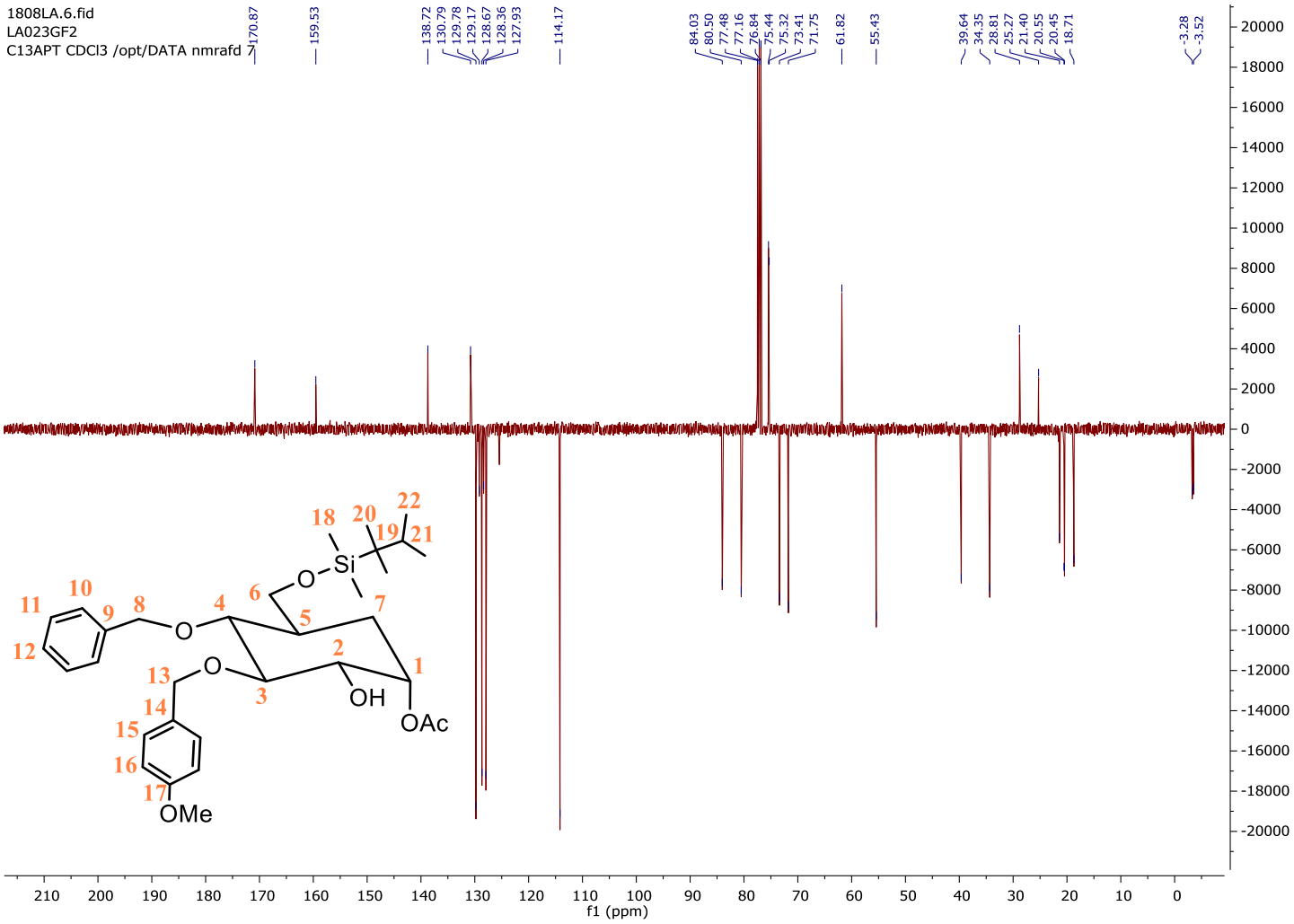
Exact mass TOF MS ESI+ $[C_{32}H_{48}O_7Si + Na]^+$ required 595.32, found 595.31

1-*O*-Acetyl-4-*O*-Benzyloxy-3-*O*-(4-Methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-glucopyranose **(18)**



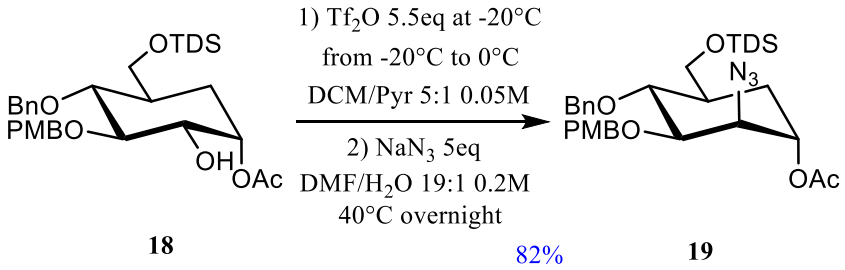
δ ^{1}H (400 MHz; CDCl3)

7.39-7.13 (7H, m, H_{arom}), 6.87 (2H, dt, J 8.7, J 5.0, 14-H), 5.26 (1H, dd, J 5.7, J 3.0, 1-H), 4.91 (1H, d, J 10.6, 8-H CH_2 Ph), 4.90 (1H, d, J 10.9, 12-H CH_2 Ph(4-OMe)), 4.70 (1H, d, J 10.0, 8-H CH_2 Ph), 4.68 (1H, d, J 10.5, 12-H CH_2 Ph(4-OMe)), 3.95 (1H, dd, J 10.0, J 3.5, 6-H), 3.80 (3H, s, -OMe), 3.75 (1H, t, J 9.3, 3-H), 3.58 (1H, br d, J 9.6, 2-H), 3.53 (1H, dd, J 9.1, J 10.1, 4-H), 3.50 (1H, dd, J 9.8, J 2.4, 6-H), 2.28 (1H, s, -OH), 2.08 (3H, s, -OAc), 1.95-1.88 (1H, m, 5-H), 1.85 (1H, dt, J 14.8, J 7.6, 7-H), 1.61 (1H, dt, J 13.8, J 6.9, 7-H), 1.61 (1H, hept, J 6.9, 17-H), 0.88 (6H, d, J 6.8, 18-H), 0.84 (6H, d, J 1.7, 16-H), 0.07 (6H, d, J 4.4, 15-H).



δ ^{13}C (100 MHz; CDCl3)

170.9 (C(O), -OAc), 159.5 (14-C), 138.7 (9-C), 130.8 (17-C), 129.8-127.9 (C_{arom} 10, 11, 12, 15-C), 114.8 (16-C), 84.0 (3-C), 80.5 (4-C), 75.4 (13-C), 75.3 (8-C), 73.4 (2-C), 71.8 (1-C), 61.8 (6-C), 55.4 (-OMe), 39.6 (5-C), 34.3 (21-C), 28.8 (7-C), 25.3 (19-C), 21.4 (CH_3, -OAc), 20.5-20.4 (20-C), 18.8-18.7 (22-C), -3.28- -3.53 (18-C).



18 82% **19**

1-*O*-Acetyl-2-azido-4-*O*-benzyloxy-3-*O*-(4-methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (19)

Compound **18** (220mg, 0.38mmol) was dissolved in a mixture of DCM/Pyridine (5:1, 0.05M) and stirred for 10min at -10°C under nitrogen. Triflate anhydride (355µL, 2.11mmols) was added dropwise at -10°C. The mixture was sequentially stirred for 30 min to slowly reach 0°C and another 30 min at 0°C. After completion of the reaction, the organic phase was washed with $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and the crude afforded was directly involved in the next step after coevaporation with toluene (3 times). Next, the dry crude was dissolved in DMF/H_2O (19:1, 0.2M) at 40°C. Sodium azide (125mg, 1.92mmols) and 15-crown-5 (15.2µL, 0.08mmol) were added at room temperature and the reaction was processed overnight at 40°C. After the complete disappearance of the triflate intermediate, the solvent was evaporated and the residue was finally purified by flash chromatography (nHex/EtOAc) to allow the formation of the azide **19** with a yield of 82% as an uncolored oil.

δ ^{1}H (400 MHz; CDCl3)

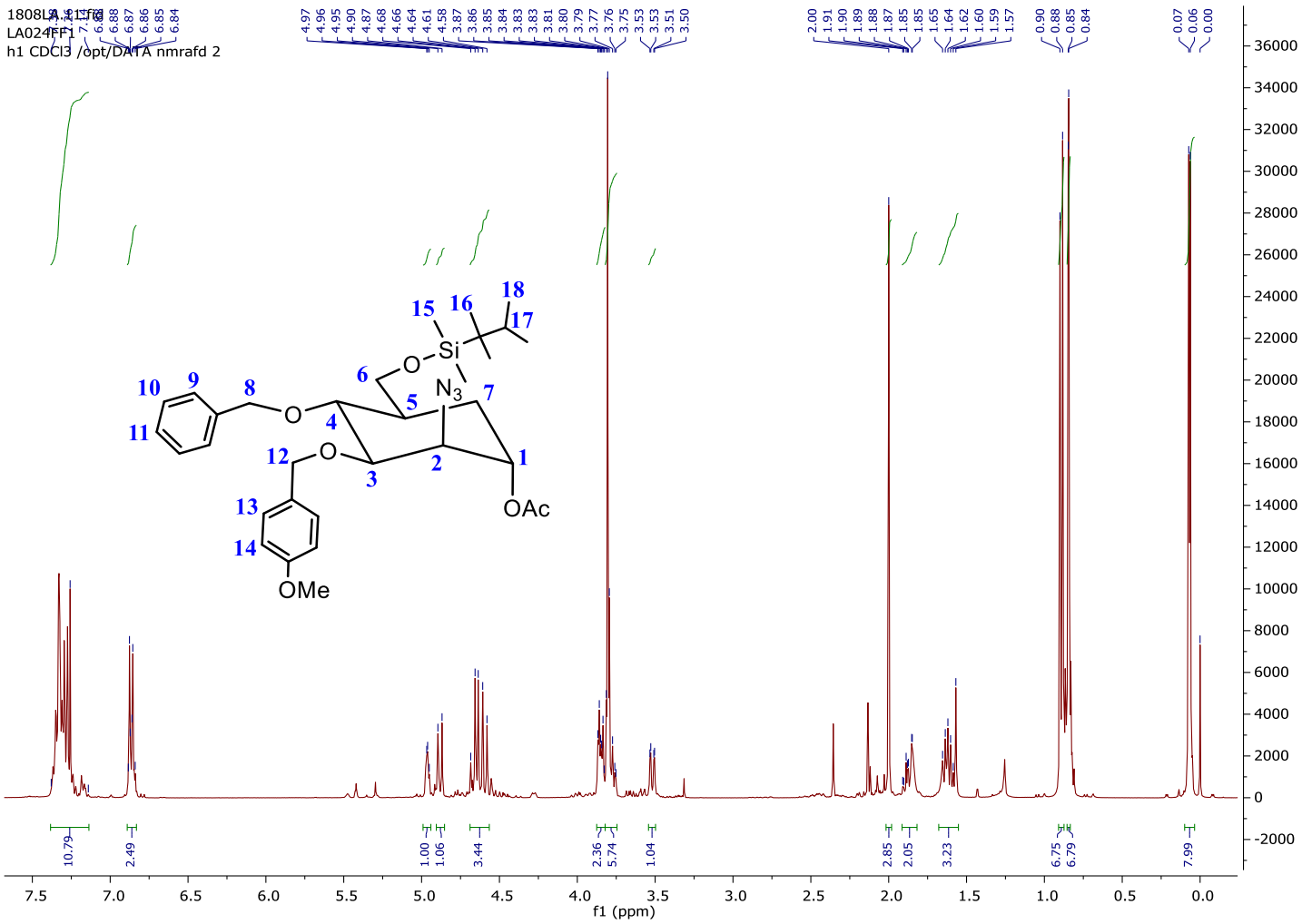
7.38-7.14 (7H, m, H_{arom}), 6.86 (2H, dt, J 8.6, J 4.9, 14-H), 4.98-4.94 (1H, m, 1-H), 4.88 (1H, d, J 10.7, 8-H CH_2 Ph), 4.66 (1H, d, J 19.1, 12-H CH_2 Ph(4-OMe)), 4.63 (1H, d, J 19.5, 12-H CH_2 Ph(4-OMe)), 4.59 (1H, d, J 10.9, 8-H CH_2 Ph), 3.87-3.84 (1H, m, 2-H), 3.84 (1H, dd, J 6.3, J 2.7, 6-H), 3.80 (3H, s, -OMe), 3.82-3.75 (2H, m, 4-H, 3-H), 3.52 (1H, dd, J 9.9, J 2.1, 6-H), 2.00 (3H, s, -OAc), 1.91-1.82 (2H, m, 5-H, 7-H), 1.65-1.57 (2H, m, 7-H, 17-H), 0.89 (6H, d, J 6.9, 18-H), 0.85 (6H, d, J 1.2, 16-H), 0.07 (6H, d, J 4.1, 15-H).

δ ^{13}C (100 MHz; CDCl3)

169.8 (C(O), -OAc), 159.6 (14-C), 138.9 (9-C), 130.2 (17-C), 129.8-127.8 (C_{arom} 10, 11, 12, 15-C), 114.0 (16-C), 81.1 (4-C), 77.0 (3-C), 75.4 (8-C), 72.9 (13-C), 70.6 (1-C), 62.2 (6-C), 61.4 (2-C), 55.4 (-OMe), 39.8 (5-C), 34.4 (21-C), 27.1 (7-C), 25.3 (19-C), 21.2 (CH_3, -OAc), 20.6-20.5 (20-C), 18.8-18.7 (22-C), -3.35- -3.52 (18-C).

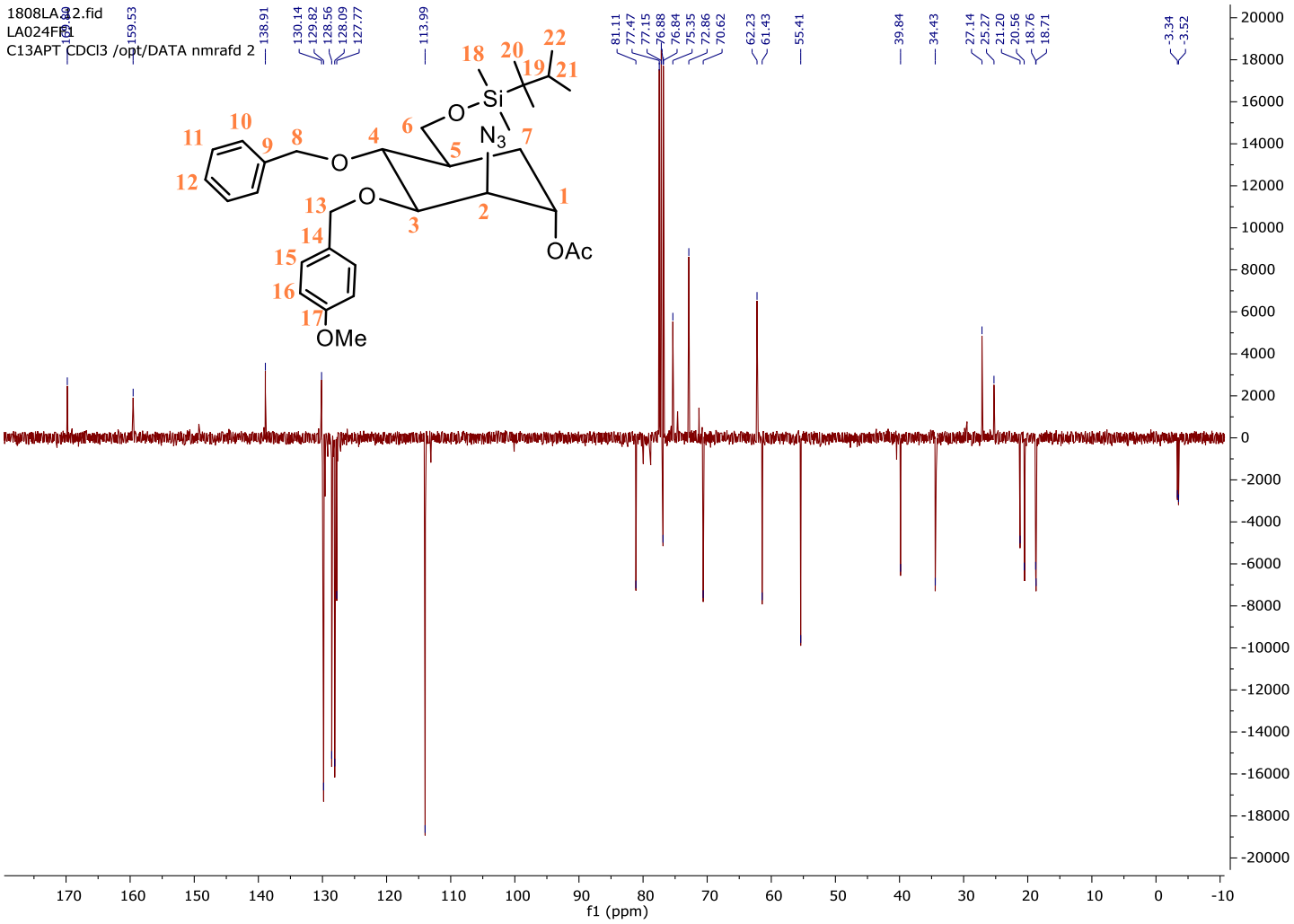
Exact mass TOF MS ESI+ $[C_{32}H_{47}N_3O_6Si + Na]^+$ required 620.32, found 620.31

1-*O*-Acetyl-2-azido-4-*O*-Benzyloxy-3-*O*-(4-Methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose **(19)**



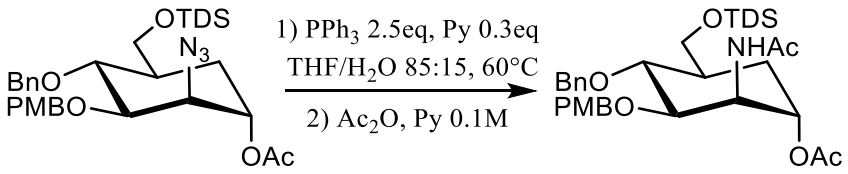
δ ^{1}H (400 MHz; CDCl3)

7.38-7.14 (7H, m, H_{arom}), 6.86 (2H, dt, J 8.6, J 4.9, 14-H), 4.98-4.94 (1H, m, 1-H), 4.88 (1H, d, J 10.7, 8-H CH_2 Ph), 4.66 (1H, d, J 19.1, 12-H CH_2 Ph(4-OMe)), 4.63 (1H, d, J 19.5, 12-H CH_2 Ph(4-OMe)), 4.59 (1H, d, J 10.9, 8-H CH_2 Ph), 3.87-3.84 (1H, m, 2-H), 3.84 (1H, dd, J 6.3, J 2.7, 6-H), 3.80 (3H, s, -OMe), 3.82-3.75 (2H, m, 4-H, 3-H), 3.52 (1H, dd, J 9.9, J 2.1, 6-H), 2.00 (3H, s, -OAc), 1.91-1.82 (2H, m, 5-H, 7-H), 1.65-1.57 (2H, m, 7-H, 17-H), 0.89 (6H, d, J 6.9, 18-H), 0.85 (6H, d, J 1.2, 16-H), 0.07 (6H, d, J 4.1, 15-H).



δ ^{13}C (100 MHz; CDCl3)

169.8 (C(O), -OAc), 159.6 (14-C), 138.9 (9-C), 130.2 (17-C), 129.8-127.8 (C_{arom} 10, 11, 12, 15-C), 114.0 (16-C), 81.1 (4-C), 77.0 (3-C), 75.4 (8-C), 72.9 (13-C), 70.6 (1-C), 62.2 (6-C), 61.4 (2-C), 55.4 (-OMe), 39.8 (5-C), 34.4 (21-C), 27.1 (7-C), 25.3 (19-C), 21.2 (CH_3, -OAc), 20.6-20.5 (20-C), 18.8-18.7 (22-C), -3.35- -3.52 (18-C).



19 75% **20**

1-*O*-Acetyl-2-acetamide-4-*O*-benzyloxy-3-*O*-(4-methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (20)

A mixture of **19** (334mg, 0.56mmol), PPh_3 (366mg, 1.40mmols) and a catalytic amount of Pyridine (13.6µL, 0.17mmol) in THF/H_2O (85:15, 0.14M) was stirred at 60°C for 24h. After disappearance of the starting material, the generated amine was dried off the solvent and then dissolved in Pyridine (5.6mL). Acetic anhydride (1.06mL, 11.2mmols) was added and the solution was again stirred 24h. The crude material was purified by flash chromatography (nHex/AcOEt), providing the acetamide **20** as a yellow oil in 75% yield.

δ ^{1}H (400 MHz; CDCl3)

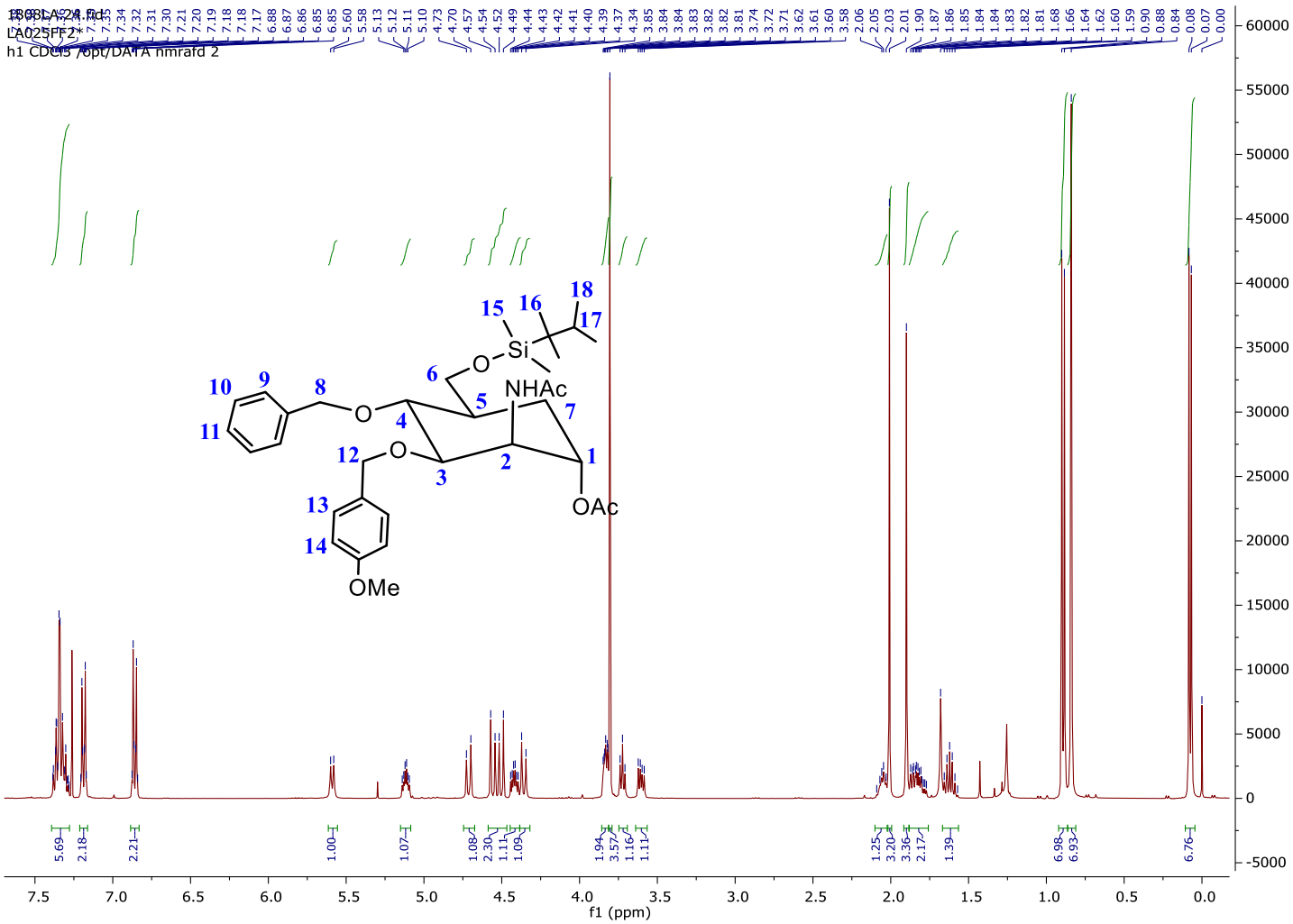
7.39-7.28 (5H, m, H_{arom}), 7.19 (2H, dt, J 9.4, J 4.6, 13-H), 6.86 (2H, dt, J 9.4, J 4.8, 14-H), 5.59 (1H, d, J 8.1, NHAc), 5.12 (1H, td, J 7.2, J 3.9, 1-H), 4.71 (1H, d, J 11.3, 8-H CH_2 Ph), 4.56 (1H, d, J 11.3, 8-H CH_2 Ph), 4.50 (1H, d, J 11.2, 12-H CH_2 Ph(4-OMe)), 4.42 (1H, td, J 7.7, J 4.1, 2-H), 4.36 (1H, d, J 11.2, 12-H CH_2 Ph(4-OMe)), 3.84 (1H, dd, J 2.4, J 4.0, 3-H), 3.85-3.82 (1H, m, 6-H), 3.80 (3H, s, -OMe), 3.72 (1H, t, J 6.3, 4-H), 3.60 (1H, dd, J 9.9, J 5.5, 6-H), 2.09-2.02 (1H, m, 5-H), 2.01 (3H, s, -OAc), 1.90 (3H, s, -NHAc), 1.82 (2H, tdd, J 14.2, J 7.4, J 4.6, 7-H), 1.66-1.57 (1H, hept, J 6.9, 17-H), 0.89 (6H, d, J 6.9, 18-H), 0.84 (6H, s, 16-H), 0.08 (6H, d, J 6.2, 15-H).

δ ^{13}C (100 MHz; CDCl3)

170.7 (C(O), -NHAc), 170.1 (C(O), -OAc), 159.6 (14-C), 138.6 (9-C), 130.0 (15-C), 129.9 (17-C), 128.6-127.8 (C_{arom} 10, 11, 12-C), 114.1 (16-C), 78.7 (3-C), 74.4 (4-C), 73.6 (8-C), 71.9 (13-C), 69.6 (1-C), 62.5 (6-C), 55.4 (-OMe), 50.6 (2-C), 39.9 (5-C), 34.4 (21-C), 27.1 (7-C), 25.2 (19-C), 23.5 (CH3, -NHAc) , 21.3 (CH3, -OAc), 20.5 (20-C), 18.8 (22-C), -3.37- -3.48 (18-C).

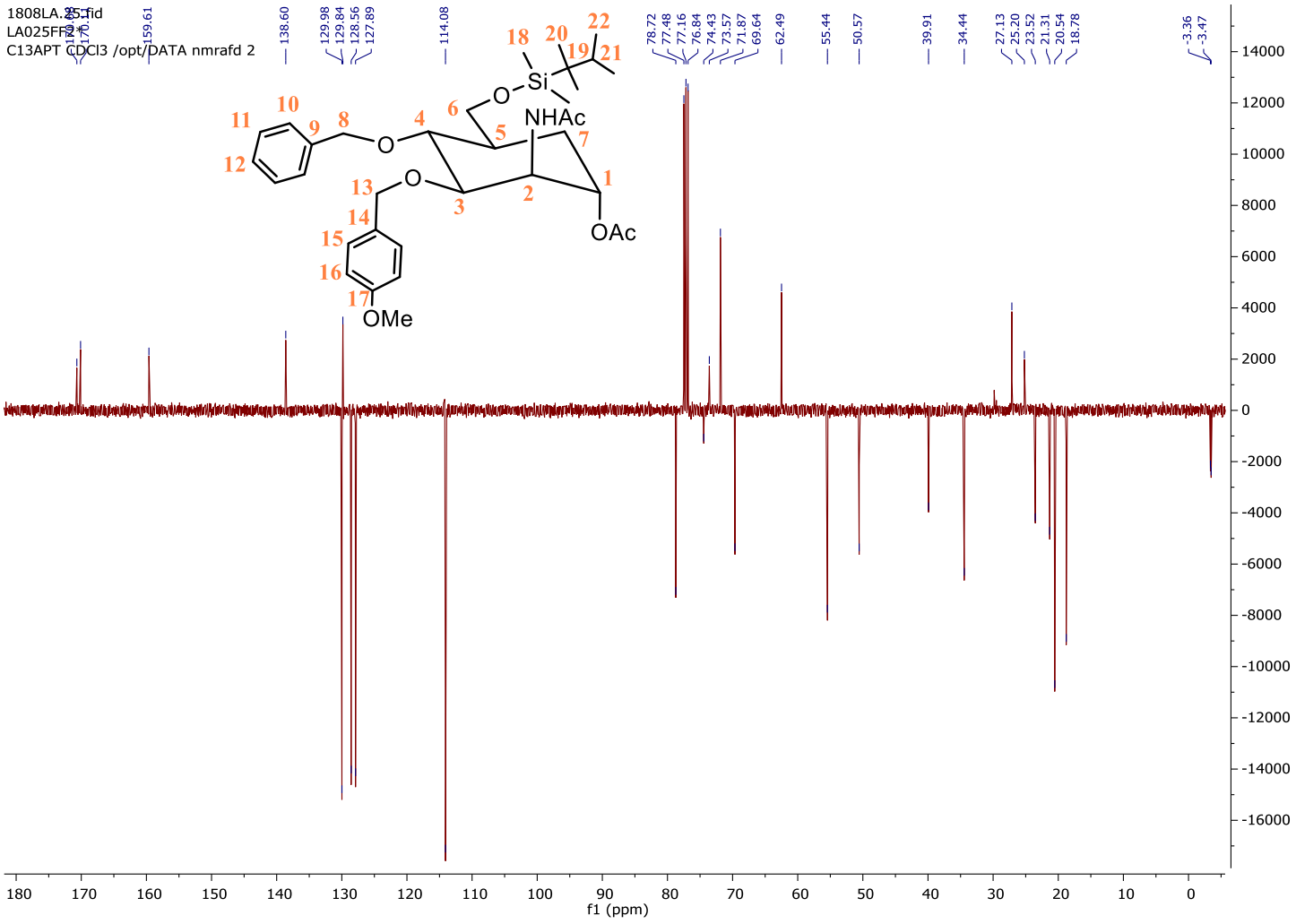
Exact mass TOF MS ESI+ $[C_{34}H_{51}NO_7Si + Na]^+$ required 636.34, found 636.33

1-*O*-Acetyl-2-acetamide-4-*O*-Benzyloxy-3-*O*-(4-Methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose **(20)**



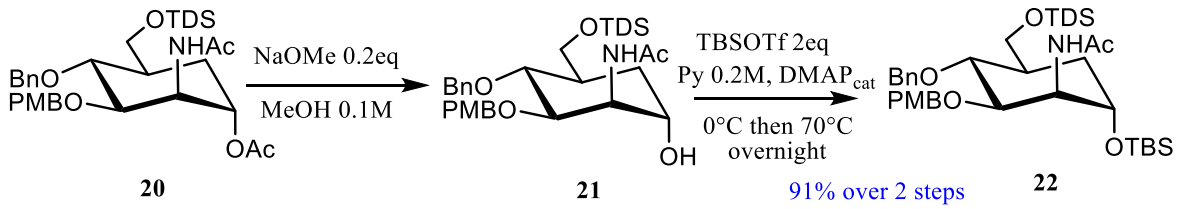
δ ^{1}H (400 MHz; CDCl3)

7.39-7.28 (5H, m, H_{arom}), 7.19 (2H, dt, J 9.4, J 4.6, 13-H), 6.86 (2H, dt, J 9.4, J 4.8, 14-H), 5.59 (1H, d, J 8.1, NHAc), 5.12 (1H, td, J 7.2, J 3.9, 1-H), 4.71 (1H, d, J 11.3, 8-H CH_2 Ph), 4.56 (1H, d, J 11.3, 8-H CH_2 Ph), 4.50 (1H, d, J 11.2, 12-H CH_2 Ph(4-OMe)), 4.42 (1H, td, J 7.7, J 4.1, 2-H), 4.36 (1H, d, J 11.2, 12-H CH_2 Ph(4-OMe)), 3.84 (1H, dd, J 2.4, J 4.0, 3-H), 3.85-3.82 (1H, m, 6-H), 3.80 (3H, s, -OMe), 3.72 (1H, t, J 6.3, 4-H), 3.60 (1H, dd, J 9.9, J 5.5, 6-H), 2.09-2.02 (1H, m, 5-H), 2.01 (3H, s, -OAc), 1.90 (3H, s, -NHAc), 1.82 (2H, tdd, J 14.2, J 7.4, J 4.6, 7-H), 1.66-1.57 (1H, hept, J 6.9, 17-H), 0.89 (6H, d, J 6.9, 18-H), 0.84 (6H, s, 16-H), 0.08 (6H, d, J 6.2, 15-H).



δ ^{13}C (100 MHz; CDCl3)

170.7 (C(O), -NHAc), 170.1 (C(O), -OAc), 159.6 (14-C), 138.6 (9-C), 130.0 (15-C), 129.9 (17-C), 128.6-127.8 (C_{arom} 10, 11, 12-C), 114.1 (16-C), 78.7 (3-C), 74.4 (4-C), 73.6 (8-C), 71.9 (13-C), 69.6 (1-C), 62.5 (6-C), 55.4 (-OMe), 50.6 (2-C), 39.9 (5-C), 34.4 (21-C), 27.1 (7-C), 25.2 (19-C), 23.5 (CH3, -NHAc) , 21.3 (CH3, -OAc), 20.5 (20-C), 18.8 (22-C), -3.37- -3.48 (18-C).



1-*O*-terbutylsylil-2-acetamide-4-*O*-benzyl-2-deoxy-3-*O*-(4-methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (22)

Compound **20** (582mg, 0.95mmol) was dissolved in MeOH (9.5mL). To the mixture was added NaOMe (11mg, 0.2mmol). The reaction was stirred for 3h at RT. Amberlite H^+ ion exchange resin was added until neutral pH was reached. The suspension was filtered and concentrated *in vacuo*. The crude was coevaporated 3 times with Toluene.

Under a flow of N_2 gas, the flask was charged with a solution of **21** (0.95 mmol) in pyridine (4.75mL). At 0°C, added was a catalytic amount of DMAP (23mg, 0.19mmol) followed by TBSOTf (437µL, 1.9mmols) in a dropwise fashion. The mixture was heated to 70°C and stirred overnight. After its completion, the reaction was cooled to RT, quenched with MeOH and the mixture was diluted with chloroform. The mixture was washed with 10% aq. $CuSO_4$ solution (2x), H_2O and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (nHex/EtOAc) furnished the title compound **22** as an orange oil in 91% yield over 2 steps.

δ ^{1}H (400 MHz; CDCl3)

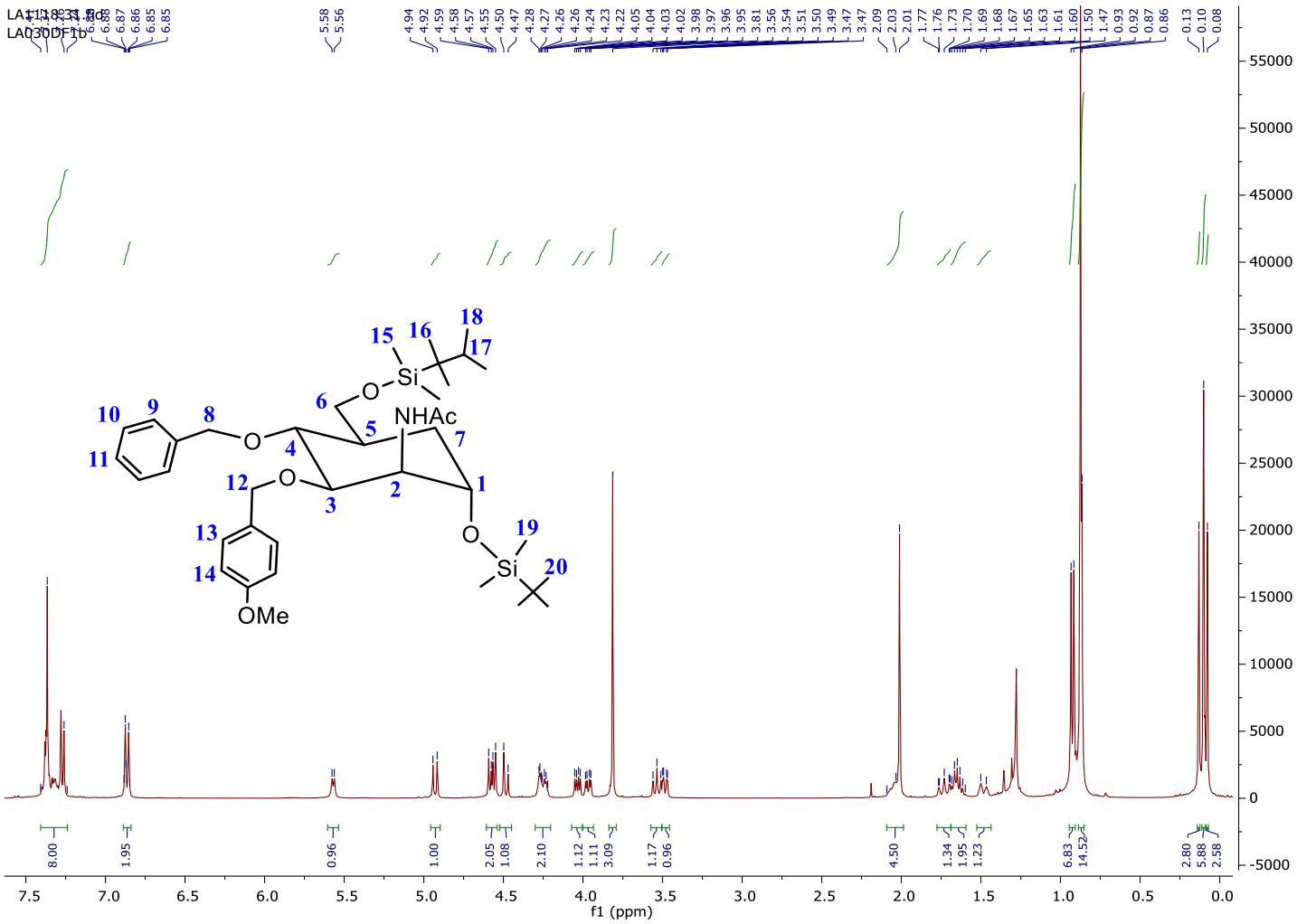
7.41-7.24 (5H, m, H_{arom}), 7.19 (2H, dt, J 9.5, J 4.6, 13-H), 6.86 (2H, dt, J 9.4, J 4.8, 14-H), 5.57 (1H, d, J 5.7, NHAc), 4.93 (1H, d, J 10.6, 8-H CH_2 Ph), 4.58 (1H, d, J 10.5, 8-H CH_2 Ph), 4.56 (1H, d, J 11.1, 12-H CH_2 Ph(4-OMe)), 4.48 (1H, d, J 11.1, 12-H CH_2 Ph(4-OMe)), 4.27 (1H, dd, J 5.2, J 2.3, 2-H), 4.25-4.21 (1H, m, 1-H), 4.03 (1H, dd, J 9.6, J 4.5, 3-H), 3.97 (1H, dd, J 9.7, J 3.6, 6-H), 3.81 (3H, s, -OMe), 3.54 (1H, t, J 9.9, 4-H), 3.48 (1H, dd, J 9.7, J 2.2, 6-H), 2.09-2.02 (1H, m, 5-H), 2.01 (3H, s, -NHAc), 1.78-1.69 (1H, m, 7-H), 1.69-1.59 (1H, m, 17-H), 1.52-1.45 (1H, m, 7-H), 0.93 (6H, d, J 6.9, 18-H), 0.87 (6H, s, 16-H), 0.86 (6H, s, 20-H), 0.84 (6H, s, 16-H), 0.12 (6H, d, J 12.0, 19-H), 0.09 (6H, d, J 9.4, 15-H).

δ ^{13}C (100 MHz; CDCl3)

170.7 (C(O), -NHAc), 159.5 (14-C), 139.1 (9-C), 130.2 (17-C), 130.0 (15-C), 128.6-127.7 (C_{arom} 10, 11, 12-C), 114.0 (16-C), 78.5 (3-C), 77.6 (4-C), 75.5 (8-C), 71.4 (13-C), 67.7 (2-C), 62.6 (6-C), 55.4 (-OMe), 53.4 (1-C), 38.6 (5-C), 34.6 (21-C), 30.4 (7-C), 25.9 (25-C) , 25.2 (19-C), 23.6 (CH_3 -NHAc), 20.7-20.6 (20-C), 18.9-18.8 (22-C), 18.0 (24-C), -3.37- -3.58 (18-C), -4.82- -4.92 (23-C).

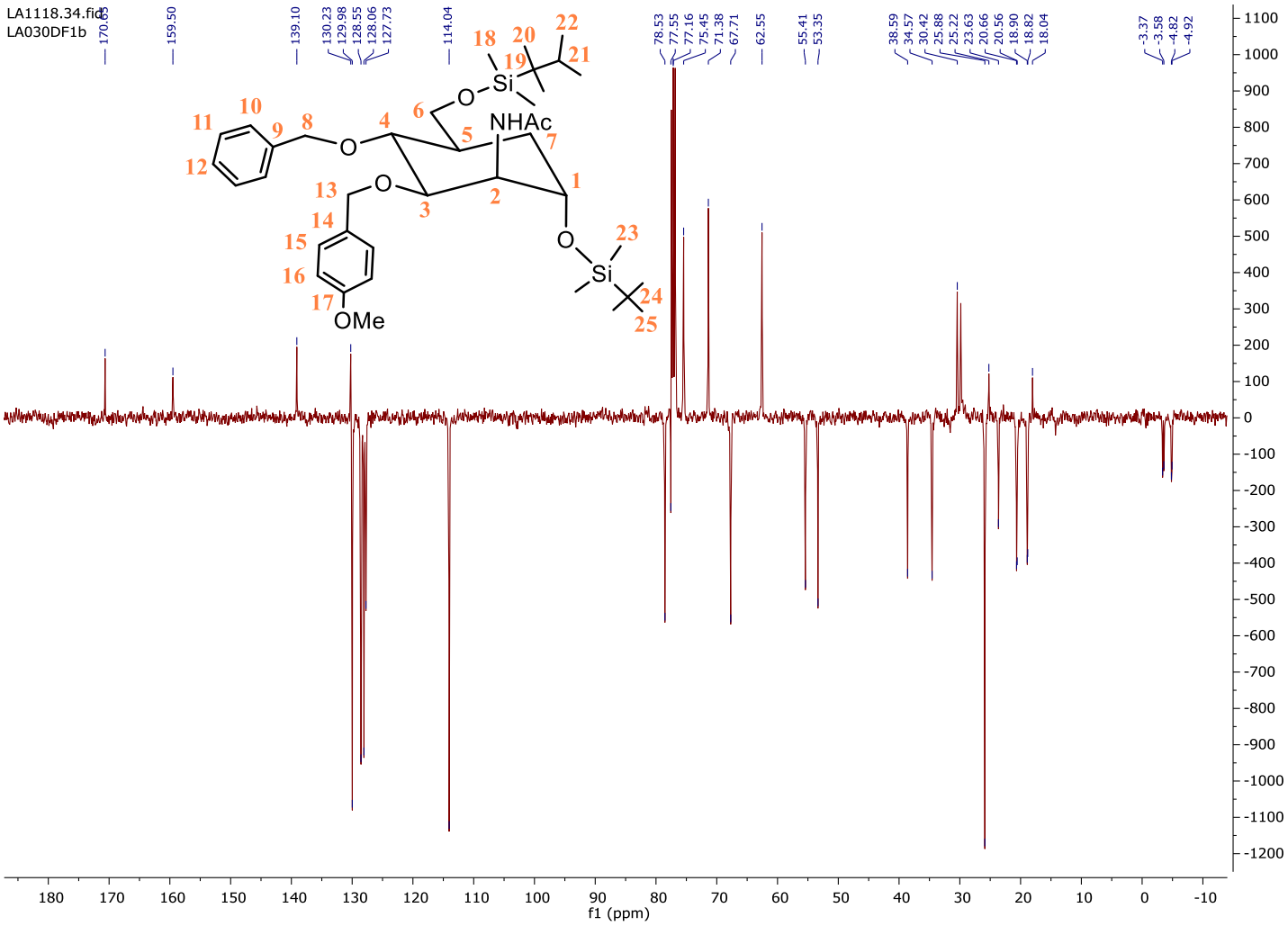
Exact mass TOF MS ESI+ $[C_{38}H_{63}NO_6Si_2 + Na]^+$ required 708.42, found 708.41

1-*O*-tertbutylsylil-2-acetamide-4-*O*-Benzyloxy-3-*O*-(4-Methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose **(22)**



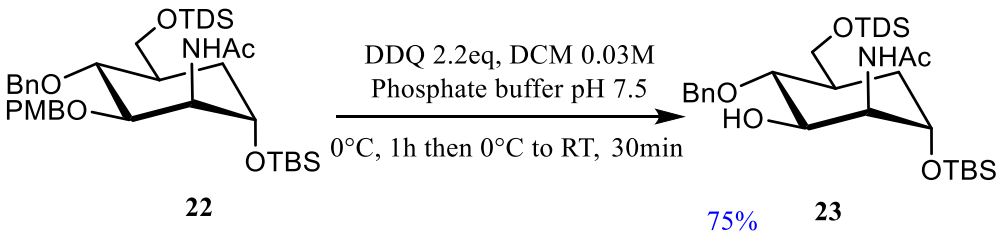
δ ^{1}H (400 MHz; CDCl3)

7.41-7.24 (5H, m, H_{arom}), 7.19 (2H, dt, J 9.5, J 4.6, 13-H), 6.86 (2H, dt, J 9.4, J 4.8, 14-H), 5.57 (1H, d, J 5.7, NHAc), 4.93 (1H, d, J 10.6, 8-H CH_2 Ph), 4.58 (1H, d, J 10.5, 8-H CH_2 Ph), 4.56 (1H, d, J 11.1, 12-H CH_2 Ph(4-OMe)), 4.48 (1H, d, J 11.1, 12-H CH_2 Ph(4-OMe)), 4.27 (1H, dd, J 5.2, J 2.3, 2-H), 4.25-4.21 (1H, m, 1-H), 4.03 (1H, dd, J 9.6, J 4.5, 3-H), 3.97 (1H, dd, J 9.7, J 3.6, 6-H), 3.81 (3H, s, -OMe), 3.54 (1H, t, J 9.9, 4-H), 3.48 (1H, dd, J 9.7, J 2.2, 6-H), 2.09-2.02 (1H, m, 5-H), 2.01 (3H, s, -NHAc), 1.78-1.69 (1H, m, 7-H), 1.69-1.59 (1H, m, 17-H), 1.52-1.45 (1H, m, 7-H), 0.93 (6H, d, J 6.9, 18-H), 0.87 (6H, s, 16-H), 0.86 (6H, s, 20-H), 0.84 (6H, s, 16-H), 0.12 (6H, d, J 12.0, 19-H), 0.09 (6H, d, J 9.4, 15-H).



δ ^{13}C (100 MHz; CDCl3)

170.7 (C(O), -NHAc), 159.5 (14-C), 139.1 (9-C), 130.2 (17-C), 130.0 (15-C), 128.6-127.7 (C_{arom} 10, 11, 12-C), 114.0 (16-C), 78.5 (3-C), 77.6 (4-C), 75.5 (8-C), 71.4 (13-C), 67.7 (2-C), 62.6 (6-C), 55.4 (-OMe), 53.4 (1-C), 38.6 (5-C), 34.6 (21-C), 30.4 (7-C), 25.9 (25-C) , 25.2 (19-C), 23.6 (CH_3 -NHAc), 20.7-20.6 (20-C), 18.9-18.8 (22-C), 18.0 (24-C), -3.37- -3.58 (18-C), -4.82- -4.92 (23-C).



22 75% **23**

1-*O*-tertbutylsylil-2-acetamide-4-*O*-benzyloxy-3-*O*-(4-methoxybenzyloxy)-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (23)

To a cooled (0°C) solution of **22** (71mg, 0.10mmol) in DCM (3.4mL) a freshly prepared phosphate buffer (362µL, pH 7.5, 10mM) was added. Freshly prepared DDQ (50.0mg, 0.22mmol) was added over 1h in small portions, after which the mixture was allowed to warm up to RT and was stirred for 30min. The mixture was diluted with $NaHCO_3$ and the aqueous layer was extracted with DCM twice. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (nHex/EtOAc) afforded the compound **23** as an orange solid yielding 75%.

Dan Van Der Es, Thesis, 2016, Universiteit Leiden, pp160.

δ ^{1}H (400 MHz; CDCl3)

7.41-7.27 (5H, m, H_{arom}), 5.52 (1H, d, J 5.4, NHAc), 4.73 (2H, s, 8-H CH_2 Ph), 4.26 (1H, br d, J 2.7, 1-H), 4.16 (1H, dt, J 9.0, J 3.8, 3-H), 4.06 (1H, dd, J 9.0, J 4.5, 2-H), 3.94 (1H, dd, J 9.9, J 3.7, 6-H), 3.53 (1H, dd, J 10.0, J 2.1, 6-H), 3.46 (1H, t, J 9.5, 4-H), 2.73 (1H, s, -OH), 2.10-2.03 (1H, m, 5-H), 2.00 (3H, s, -NHAc), 1.81-1.69 (1H, m, 7-H), 1.69-1.59 (1H, m, 14-H), 1.51 (1H, dt, J 13.7, J 3.2, 7-H), 0.93 (6H, d, J 6.9, 15-H), 0.88 (6H, s, 17-H), 0.87 (6H, s, 13-H), 0.14-0.04 (12H, m, 16-H, 12-H).

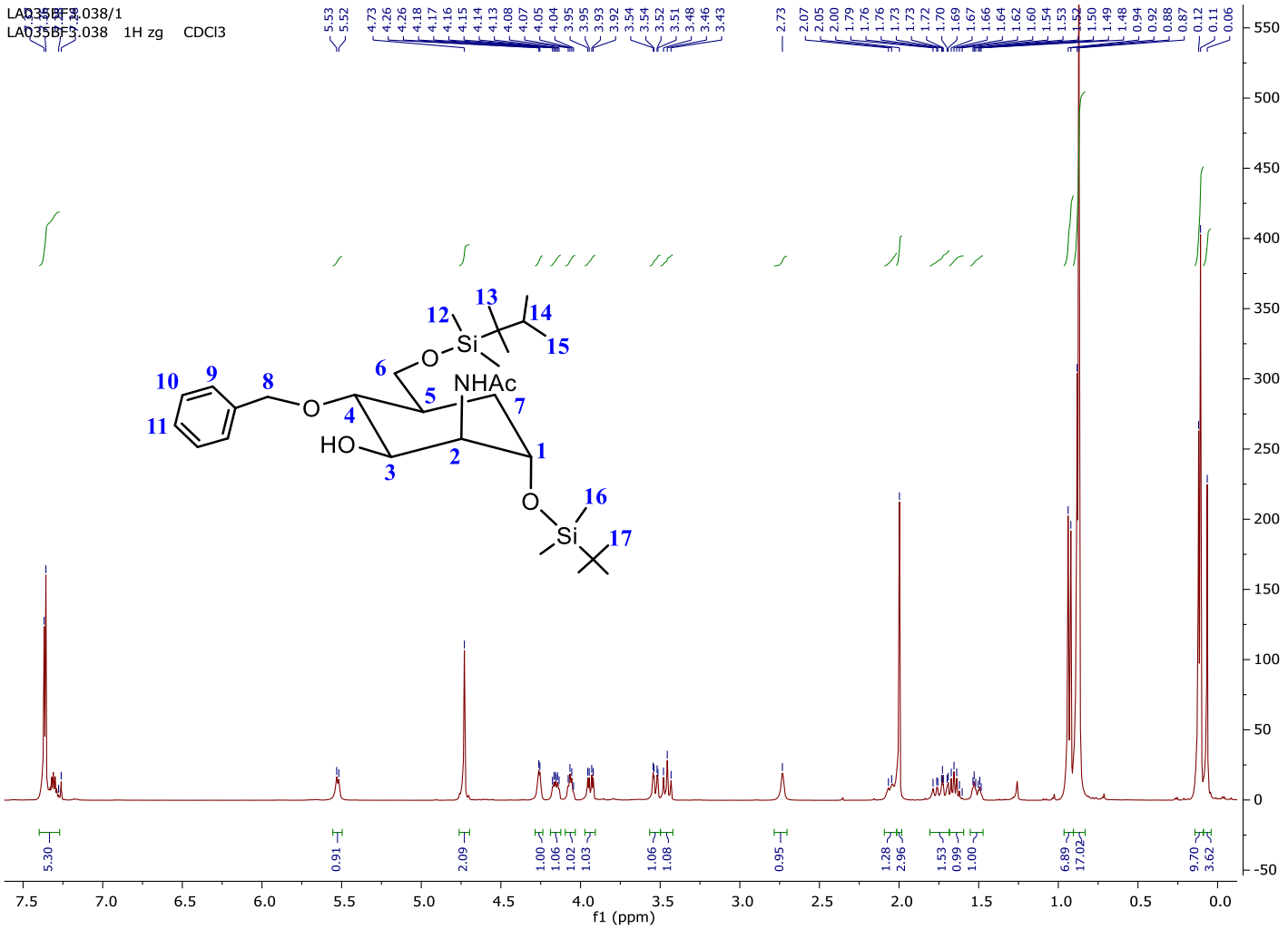
δ ^{13}C (100 MHz; CDCl3)

170.1 (C(O), -NHAc), 138.8 (9-C), 128.7-127.7 (C_{arom} 10, 11, 12-C), 79.6 (4-C), 74.8 (8-C), 70.7 (3-C), 67.6 (1-C), 62.9 (6-C), 56.5 (2-C), 38.5 (5-C), 34.6 (16-C), 31.2 (7-C), 25.9 (20-C), 25.3 (14-C), 23.6 (CH3, -NHAc), 20.7-20.6 (15-C), 18.9-18.8 (17-C), 18.0 (19-C), -3.37- -3.53 (13-C), -4.80- -4.90 (18-C).

Exact mass TOF MS ESI+ $[C_{30}H_{55}NO_5Si_2 + H]^+$ required 566.36, found 566.37

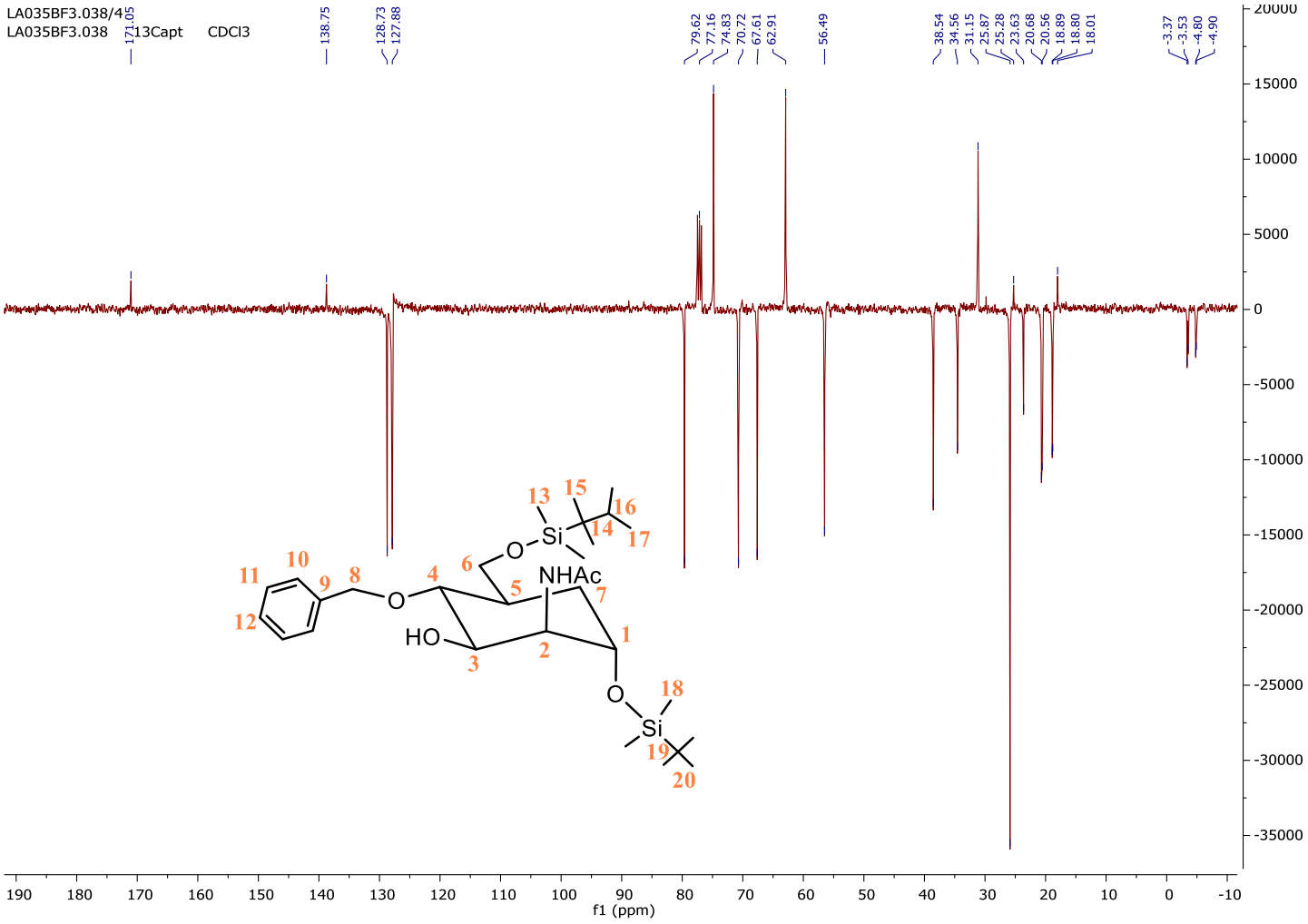
Exact mass TOF MS ESI+ $[C_{30}H_{55}NO_5Si_2 + Na]^+$ required 588.36, found 588.35

1-*O*-tertbutylsylil-2-acetamide-4-*O*-Benzyloxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose **(23)**



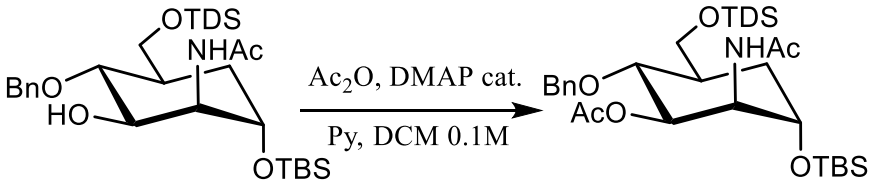
δ ^{1}H (400 MHz; CDCl3)

7.41-7.27 (5H, m, H_{arom}), 5.52 (1H, d, J 5.4, NHAc), 4.73 (2H, s, 8-H CH_2 Ph), 4.26 (1H, br d, J 2.7, 1-H), 4.16 (1H, dt, J 9.0, J 3.8, 3-H), 4.06 (1H, dd, J 9.0, J 4.5, 2-H), 3.94 (1H, dd, J 9.9, J 3.7, 6-H), 3.53 (1H, dd, J 10.0, J 2.1, 6-H), 3.46 (1H, t, J 9.5, 4-H), 2.73 (1H, s, -OH), 2.10-2.03 (1H, m, 5-H), 2.00 (3H, s, -NHAc), 1.81-1.69 (1H, m, 7-H), 1.69-1.59 (1H, m, 14-H), 1.51 (1H, dt, J 13.7, J 3.2, 7-H), 0.93 (6H, d, J 6.9, 15-H), 0.88 (6H, s, 17-H), 0.87 (6H, s, 13-H), 0.14-0.04 (12H, m, 16-H, 12-H).



δ ^{13}C (100 MHz; CDCl3)

170.1 (C(O), -NHAc), 138.8 (9-C), 128.7-127.7 (C_{arom} 10, 11, 12-C), 79.6 (4-C), 74.8 (8-C), 70.7 (3-C), 67.6 (1-C), 62.9 (6-C), 56.5 (2-C), 38.5 (5-C), 34.6 (16-C), 31.2 (7-C), 25.9 (20-C), 25.3 (14-C), 23.6 (CH3, -NHAc), 20.7-20.6 (15-C), 18.9-18.8 (17-C), 18.0 (19-C), -3.37- -3.53 (13-C), -4.80- -4.90 (18-C).



23 Quantitative **24**

1-*O*-tertbutylsylil-2-acetamide-4-*O*-benzyloxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (24)

Compound **23** (180mg, 0.32mmol) was dissolved in dry DCM (3.2mL) at RT under nitrogen. Pyridine (257µL, 3.18mmols), acetic anhydride (601µL, 6.36mmols) and a catalytic amount of DMAP (7.8mg, 0.06mmol) were successively added and the mixture was stirred until the reaction was over. The solution was quenched with MeOH and then concentrated under reduced pressure. Purification by flash chromatography (nHex/EtOAc) allowed the formation of compound **24** as a yellow oil in a quantitative yield.

δ ^{1}H (400 MHz; CDCl3)

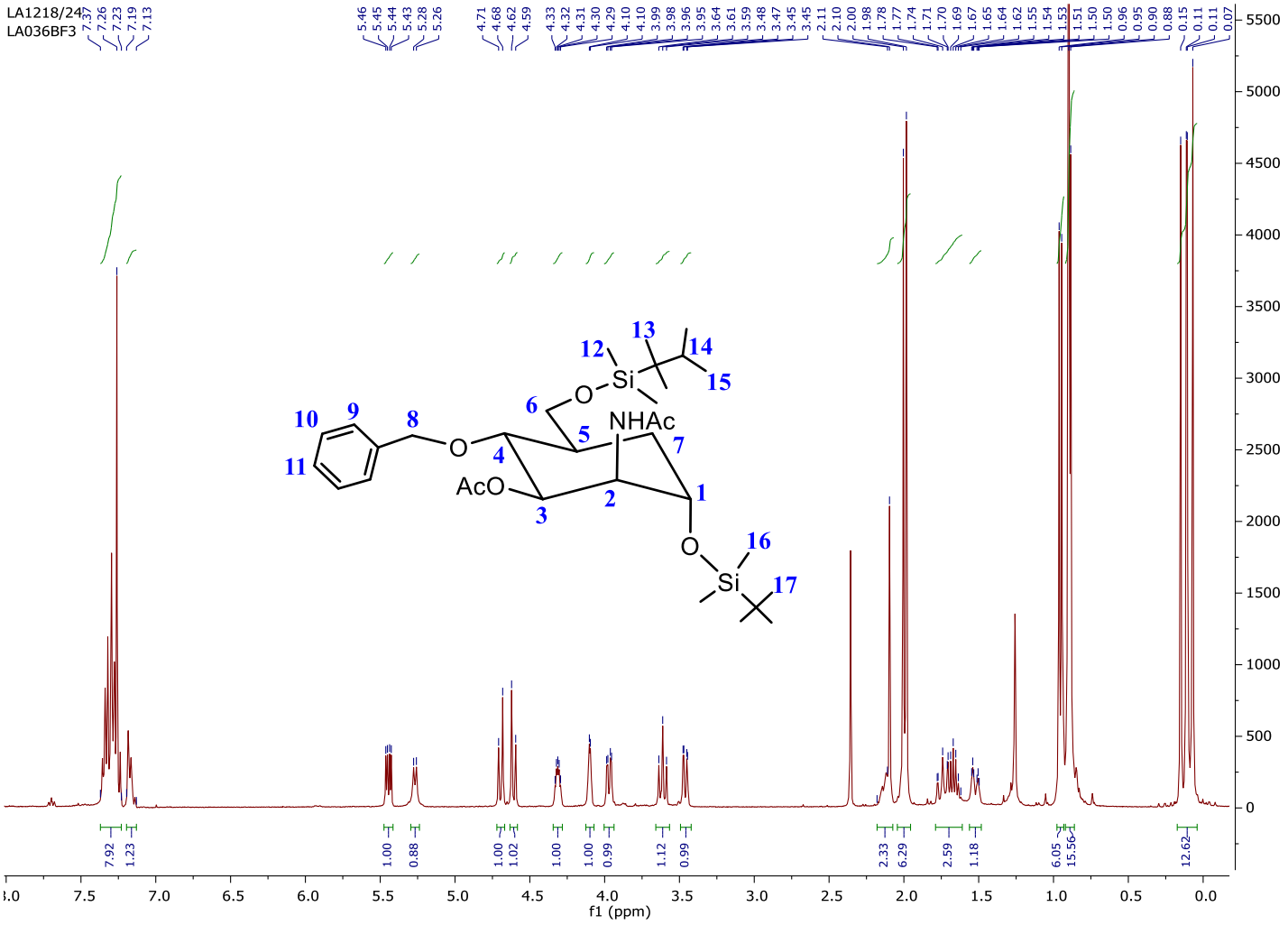
7.37-7.13 (5H, m, H_{arom}), 5.44 (1H, dd, J 10.3, J 4.5, 3-H), 5.27 (1H, d, J 7.4, NHAc), 4.70 (2H, d, J 10.9, 8-H CH_2 Ph), 4.61 (1H, d, J 10.9, 8-H CH_2 Ph), 4.31 (1H, dt, J 7.3, J 3.8, 2-H), 4.10 (1H, br d, J 2.7, 1-H), 3.97 (1H, dd, J 9.8, J 3.2, 6-H), 3.61 (1H, t, J 10.3, 4-H), 3.46 (1H, dd, J 9.8, J 2.0, 6-H), 2.18-2.11 (1H, m, 5-H), 2.00 (3H, s, -NHAc), 1.98 (3H, s, -OAc), 1.79-1.70 (1H, m, 7-H), 1.70-1.61 (1H, m, 14-H), 1.52 (1H, dt, J 14.3, J 2.8, 7-H), 0.95 (6H, d, J 6.9, 15-H), 0.90 (6H, s, 17-H), 0.88 (6H, s, 13-H), 0.13 (6H, d, J 15.1, 16-H), 0.09 (6H, d, J 14.8, 12-H).

δ ^{13}C (100 MHz; CDCl3)

170.0 (C(O), -NHAc), 169.8 (C(O), -OAc), 138.7 (9-C), 128.6-127.6 (C_{arom} 10, 11, 12-C), 76.2 (4-C), 75.1 (8-C), 73.2 (3-C), 68.1 (1-C), 62.3 (6-C), 54.0 (2-C), 38.7 (5-C), 34.6 (16-C), 30.6 (7-C), 25.8 (20-C), 25.3 (14-C), 23.6 (CH3, -NHAc), 21.2 (CH3, -OAc), 20.7-20.6 (15-C), 19.0-18.9 (17-C), 18.1 (19-C), -3.41- -3.62 (13-C), -4.90- -4.99 (18-C).

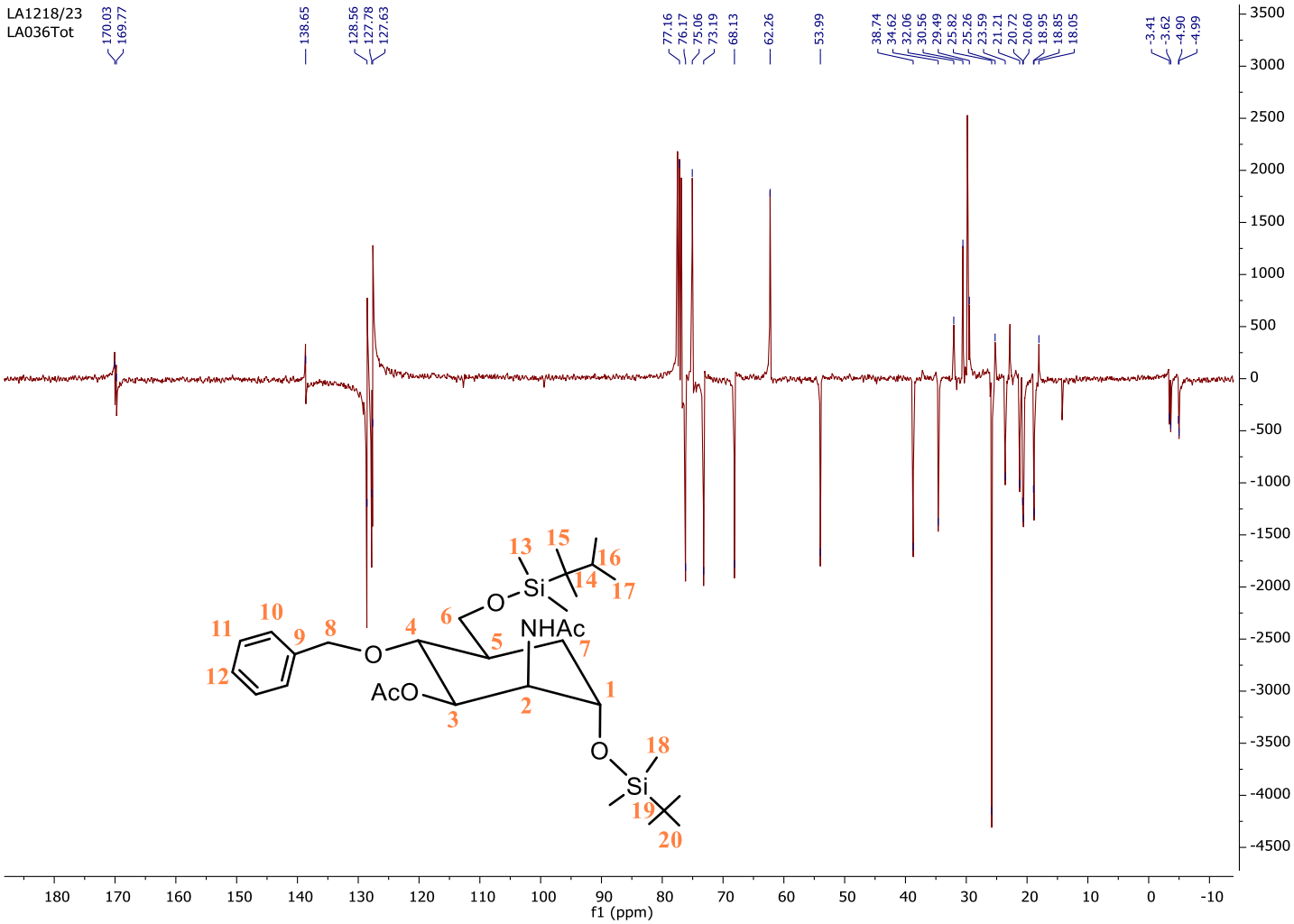
Exact mass TOF MS ESI+ $[C_{32}H_{57}NO_5Si_2 + Na]^+$ required 630.67, found 630.36

1-*O*-tertbutylsylil-2-acetamide-3-*O*-acetyl-4-*O*-Benzyloxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose **(24)**



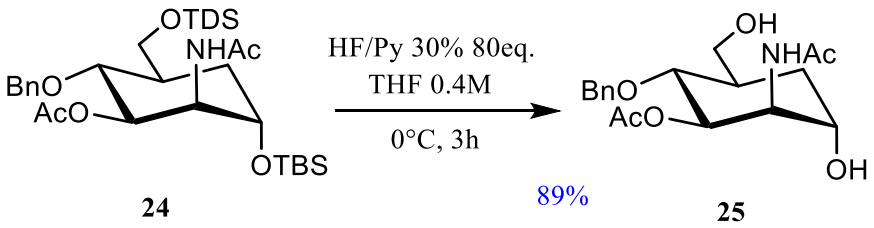
δ ^{1}H (400 MHz; CDCl3)

7.37-7.13 (5H, m, H_{arom}), 5.44 (1H, dd, J 10.3, J 4.5, 3-H), 5.27 (1H, d, J 7.4, NHAc), 4.70 (2H, d, J 10.9, 8-H CH_2 Ph), 4.61 (1H, d, J 10.9, 8-H CH_2 Ph), 4.31 (1H, dt, J 7.3, J 3.8, 2-H), 4.10 (1H, br d, J 2.7, 1-H), 3.97 (1H, dd, J 9.8, J 3.2, 6-H), 3.61 (1H, t, J 10.3, 4-H), 3.46 (1H, dd, J 9.8, J 2.0, 6-H), 2.18-2.11 (1H, m, 5-H), 2.00 (3H, s, -NHAc), 1.98 (3H, s, -OAc), 1.79-1.70 (1H, m, 7-H), 1.70-1.61 (1H, m, 14-H), 1.52 (1H, dt, J 14.3, J 2.8, 7-H), 0.95 (6H, d, J 6.9, 15-H), 0.90 (6H, s, 17-H), 0.88 (6H, s, 13-H), 0.13 (6H, d, J 15.1, 16-H), 0.09 (6H, d, J 14.8, 12-H).



δ ^{13}C (100 MHz; CDCl3)

170.0 (C(O), -NHAc), 169.8 (C(O), -OAc), 138.7 (9-C), 128.6-127.6 (C_{arom} 10, 11, 12-C), 76.2 (4-C), 75.1 (8-C), 73.2 (3-C), 68.1 (1-C), 62.3 (6-C), 54.0 (2-C), 38.7 (5-C), 34.6 (16-C), 30.6 (7-C), 25.8 (20-C), 25.3 (14-C), 23.6 (CH3, -NHAc), 21.2 (CH3, -OAc), 20.7-20.6 (15-C), 19.0-18.9 (17-C), 18.1 (19-C), -3.41- -3.62 (13-C), -4.90- -4.99 (18-C).



24 **25**

2-acetamide-4-*O*-benzyloxy--5a-carba-α-D-mannopyranose (25)

Compound **24** (2.14g, 3.4mmol) was dissolved in dry THF (9.0mL) at 0°C. A solution of HF/Py 70-30% (6.95mL, 267mmol) was added dropwise and the reaction was left stirring 3h at 0°C. The reaction was diluted with EtOAc and the organic layer was successively washed with HCL 1M, $NaHCO_3$ and brine, all one time. The combined aqueous fractions were extracted seven times with a mixture of isopropanol/chloroform (1:4). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude compound **25** afforded was filtrated on silica to provide a white solid in 89% yield.

δ ^{1}H (400 MHz; CD_3OD)

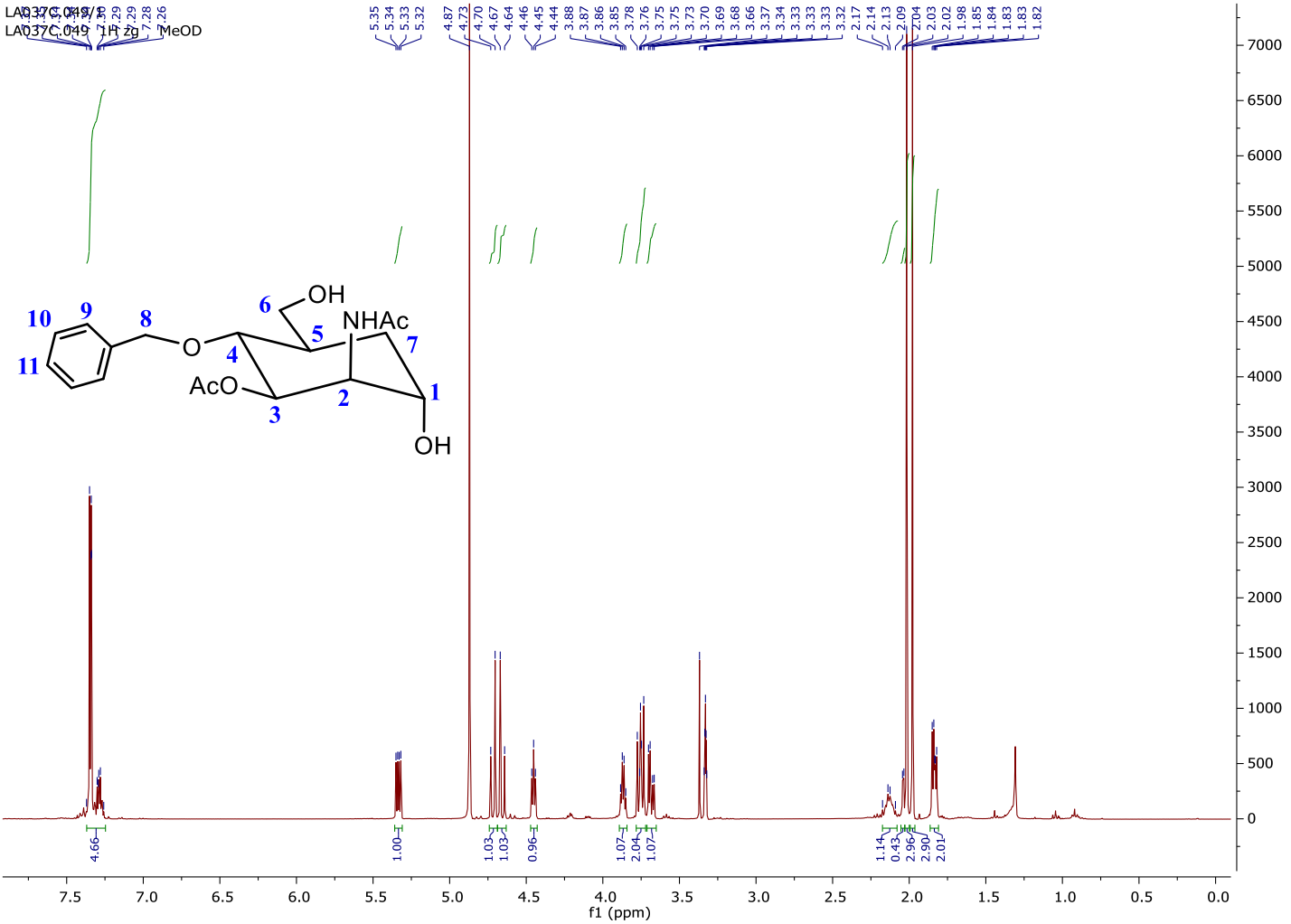
7.37-7.26 (5H, m, H_{arom}), 5.33 (1H, dd, J 8.4, J 4.4, 3-H), 4.72 (2H, d, J 11.4, 8-H CH_2 Ph), 4.66 (1H, d, J 11.4, 8-H CH_2 Ph), 4.45 (1H, t, J 4.8, 2-H), 4.10 (1H, br d, J 2.7, 1-H), 3.87 (1H, q, J 4.5, 1-H), 3.78-3.73 (2H, m, 4-H, 6-H), 3.68 (1H, dd, J 10.6, J 4.2, 6-H), 2.17-2.09 (1H, m, 5-H), 2.04 (1H, s, -OH), 2.03 (1H, s -OH), 2.02 (3H, s, -NHAc), 1.98 (3H, s, -OAc), 1.83 (2H, dd, J 7.8, J 3.8, 7-H).

δ ^{13}C (100 MHz; CDCl3)

173.6 (C(O), -NHAc), 172.0 (C(O), -OAc), 140.0 (9-C), 129.3-128.6 (C_{arom} 10, 11, 12-C), 77.2 (4-C), 74.9 (8-C), 74.7 (3-C), 68.2 (1-C), 63.1 (6-C), 54.0 (2-C), 40.7 (5-C), 30.9 (7-C), 22.5 (CH3, -NHAc), 21.1 (CH3, -OAc).

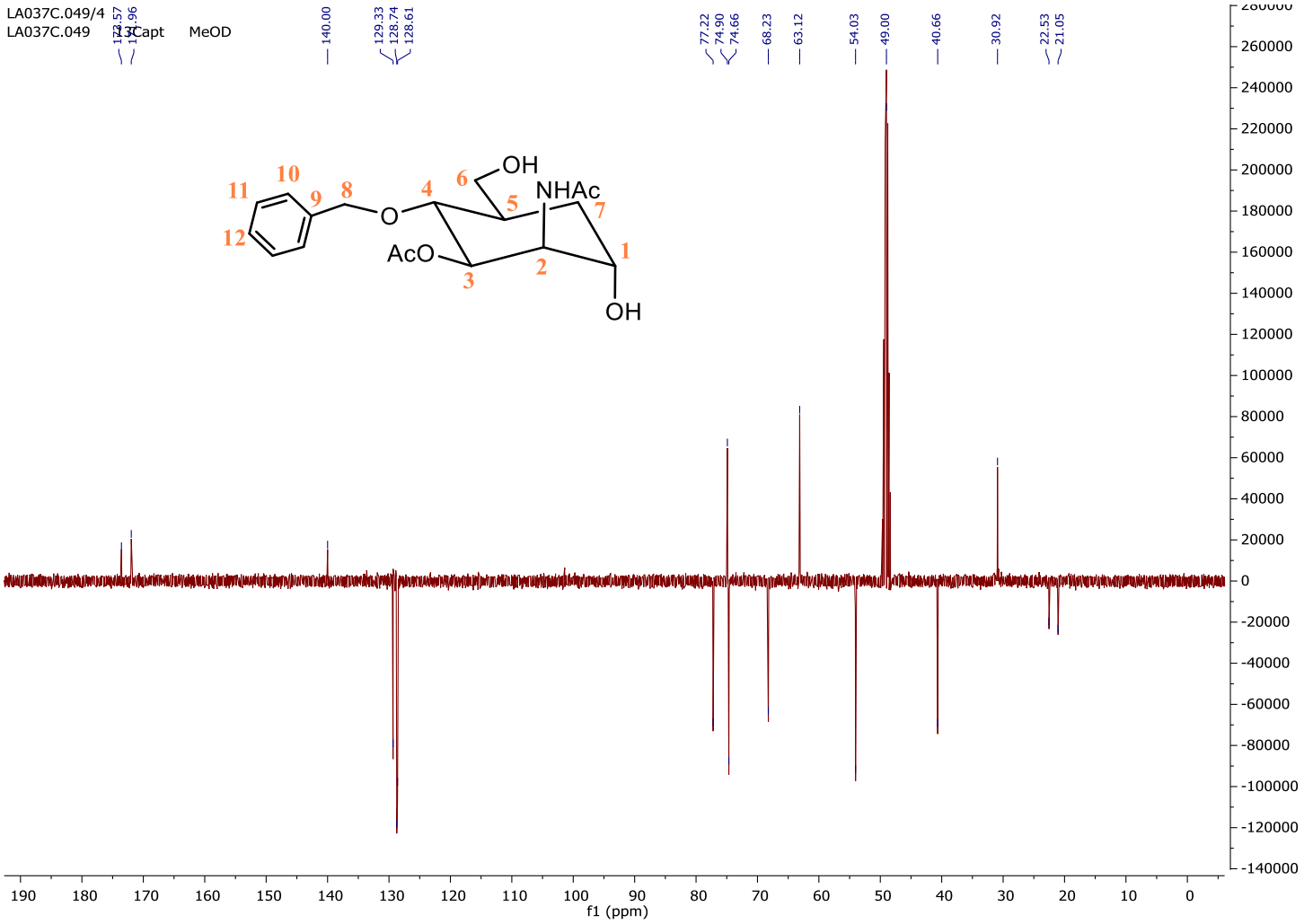
Exact mass TOF MS ESI+ $[C_{18}H_{25}NO_6 + Na]^+$ required 374.17, found 374.16

2-acetamide-3-*O*-acetyl-4-*O*-Benzyloxy-5a-carba-α-D-mannopyranose **(25)**



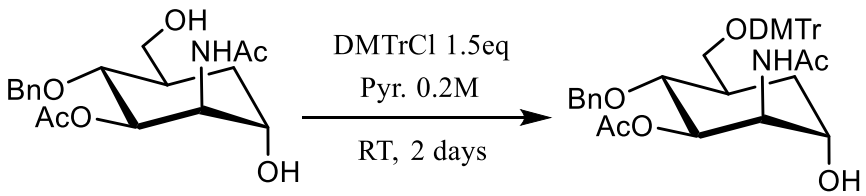
δ ^{1}H (400 MHz; CD_3OD)

7.37-7.26 (5H, m, H_{arom}), 5.33 (1H, dd, J 8.4, J 4.4, 3-H), 4.72 (2H, d, J 11.4, 8-H CH_2 Ph), 4.66 (1H, d, J 11.4, 8-H CH_2 Ph), 4.45 (1H, t, J 4.8, 2-H), 4.10 (1H, br d, J 2.7, 1-H), 3.87 (1H, q, J 4.5, 1-H), 3.78-3.73 (2H, m, 4-H, 6-H), 3.68 (1H, dd, J 10.6, J 4.2, 6-H), 2.17-2.09 (1H, m, 5-H), 2.04 (1H, s, -OH), 2.03 (1H, s -OH), 2.02 (3H, s, -NHAc), 1.98 (3H, s, -OAc), 1.83 (2H, dd, J 7.8, J 3.8, 7-H).



δ ^{13}C (100 MHz; CDCl3)

173.6 (C(O), -NHAc), 172.0 (C(O), -OAc), 140.0 (9-C), 129.3-128.6 (C_{arom} 10, 11, 12-C), 77.2 (4-C), 74.9 (8-C), 74.7 (3-C), 68.2 (1-C), 63.1 (6-C), 54.0 (2-C), 40.7 (5-C), 30.9 (7-C), 22.5 (CH3, -NHAc), 21.1 (CH3, -OAc).



25 93% **26**

2-acetamide-3-*O*-acetyl-4-*O*-benzyloxy-5a-carba-6-*O*-dimethoxytrityl-α-D-mannopyranose (26)

Compound **25** (2.32g, 6.6mmol) was dissolved in dry pyridine (33mL) under nitrogen at RT. DMTrCl (3.35g, 9.9mmol) was added and the mixture was then stirred for 2 days at RT. The mixture was diluted in DCM. The organic layer was washed once with $CuSO_4$ and then with a mixture of brine/$NaHCO_3$ (1:1). The combined aqueous layers were extracted four times with isopropanol/chloroform mixture (1/4). The combined organic layers were dried over $NaSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (2:8 pentane/EtOAc +2% Et_3N to 8:2 EtOAc/ EtOH) to obtain a white foam in 93% yield.

δ ^{1}H (400 MHz; CD_3OD)

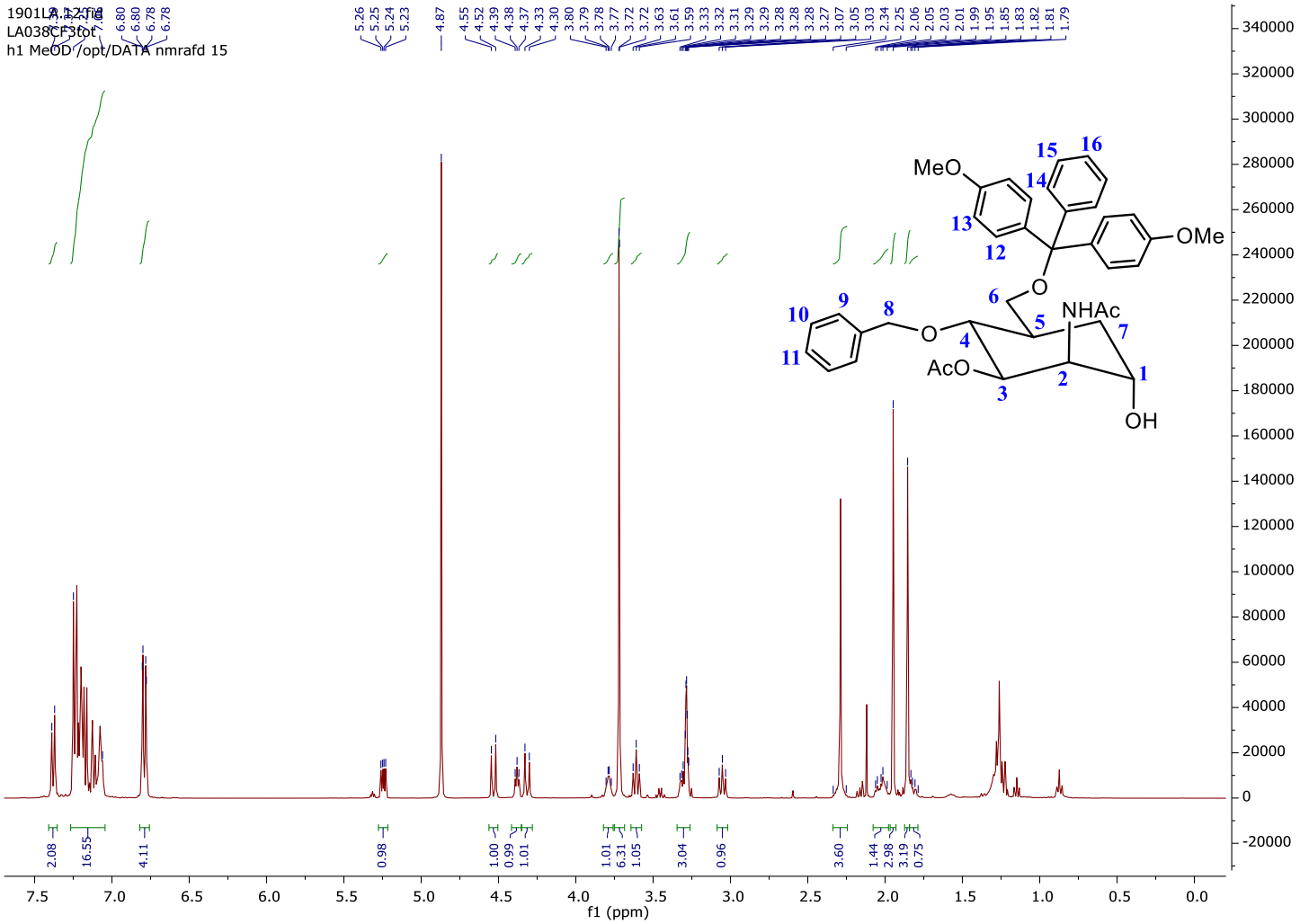
7.40-7.05 (14H, m, H_{arom}), 6.79 (4H, dd, J 8.9, J 1.7, 13-H), 5.24 (1H, dd, J 7.9, J 4.3, 3-H), 4.53 (1H, d, J 11.3, 8-H CH_2 Ph), 4.38 (1H, t, J 4.8, 2-H), 4.31 (1H, d, J 11.3, 8-H CH_2 Ph), 3.79 (1H, q, J 5.2, 1-H), 3.72 (3H, s, -OMe), 3.72 (3H, s, -OMe), 3.61 (1H, t, J 8.1, 4-H), 3.34-3.26 (1H, m, 6-H), 3.05 (1H, t, J 8.3, 6-H), 2.34-2.24 (1H, m, 5-H), 2.08-1.98 (1H, m, 7-H), 1.95 (3H, s, -NHAc), 1.86 (3H, s, -OAc), 1.85-1.79 (1H, m, 7-H).

δ ^{13}C (100 MHz; CD_3OD)

173.6 (C(O), -NHAc), 172.0 (C(O), -OAc), 160.0 (17-C), 146.7 (9-C), 137.6 (14-C), 137.5 (14-C), 137.3 (9-C), 131.4 (18-C), 129.9-126.3 (C_{arom} 10, 11, 12, 15, 19, 20, 21-C), 114.0 (16-C), 87.2 (13-C), 77.3 (4-C), 74.5 (3-C), 74.4 (8-C), 68.0 (1-C), 65.0 (6-C), 55.7 (-OMe), 54.1 (2-C), 39.2 (5-C), 31.9 (7-C), 22.5 (CH3, -NHAc), 21.1 (CH3, -OAc).

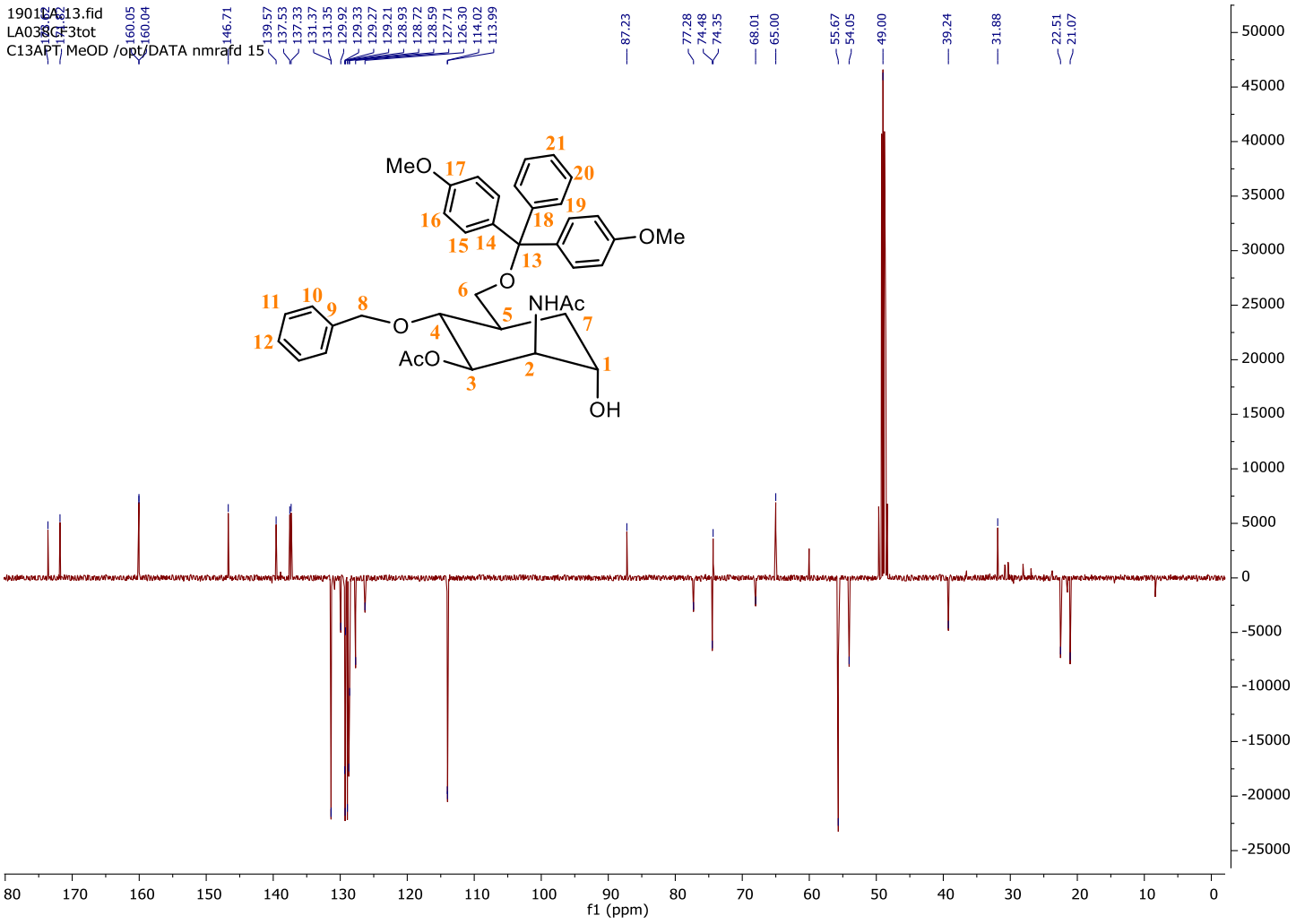
Exact mass TOF MS ESI+ $[C_{39}H_{43}NO_8 + Na]^+$ required 676.30, found 676.29

2-acetamide-3-*O*-acetyl-4-*O*-benzyloxy-5a-carba-6-O-dimethoxytrityl-α-D-mannopyranose **(26)**



δ ^{1}H (400 MHz; CD_3OD)

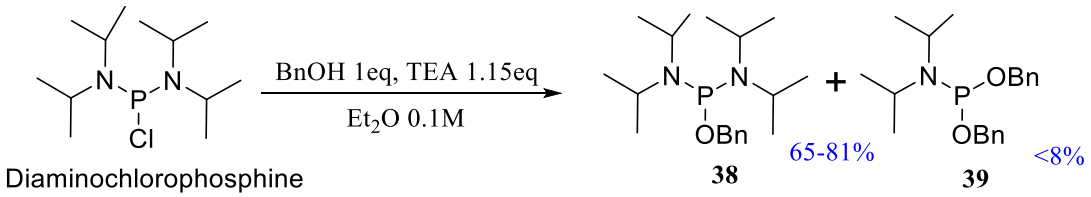
7.40-7.05 (14H, m, H_{arom}), 6.79 (4H, dd, J 8.9, J 1.7, 13-H), 5.24 (1H, dd, J 7.9, J 4.3, 3-H), 4.53 (1H, d, J 11.3, 8-H CH_2 Ph), 4.38 (1H, t, J 4.8, 2-H), 4.31 (1H, d, J 11.3, 8-H CH_2 Ph), 3.79 (1H, q, J 5.2, 1-H), 3.72 (3H, s, -OMe), 3.72 (3H, s, -OMe), 3.61 (1H, t, J 8.1, 4-H), 3.34-3.26 (1H, m, 6-H), 3.05 (1H, t, J 8.3, 6-H), 2.34-2.24 (1H, m, 5-H), 2.08-1.98 (1H, m, 7-H), 1.95 (3H, s, -NHAc), 1.86 (3H, s, -OAc), 1.85-1.79 (1H, m, 7-H).



δ ^{13}C (100 MHz; CD_3OD)

173.6 (C(O), -NHAc), 172.0 (C(O), -OAc), 160.0 (17-C), 146.7 (9-C), 137.6 (14-C), 137.5 (14-C), 137.3 (9-C), 131.4 (18-C), 129.9-126.3 (C_{arom} 10, 11, 12, 15, 19, 20, 21-C), 114.0 (16-C), 87.2 (13-C), 77.3 (4-C), 74.5 (3-C), 74.4 (8-C), 68.0 (1-C), 65.0 (6-C), 55.7 (-OMe), 54.1 (2-C), 39.2 (5-C), 31.9 (7-C), 22.5 (CH3, -NHAc), 21.1 (CH3, -OAc).

Work achieved in Leiden



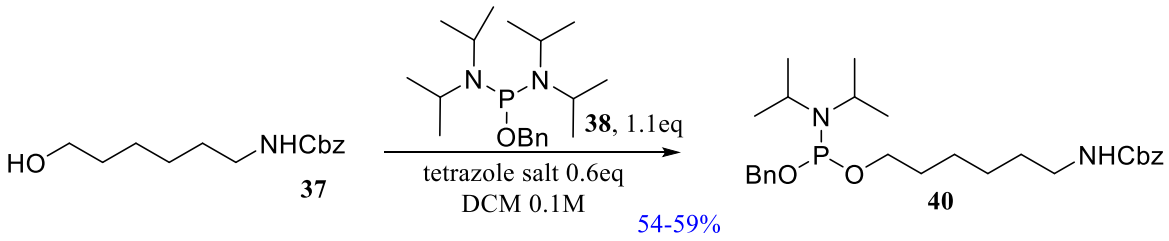
1-(benzyloxy)-*N*,*N*,*N'*,*N'*-bis(diisopropyldiamidephosphinite) (38)

Bis(diisopropylamino)chlorophosphine (0.8g, 3.0mmol) was suspended in dry Et_2O (17 mL) and activated 3Å molecular sieves were added. The benzylalcohol (0.32mL, 3mmol) was co-evaporated three times with toluene and dissolved in dry Et_2O (13.6mL). The benzylalcohol solution was added to the phosphine at 0°C and stirred for 3 hours. The reaction was filtrated over celite and concentrated *in vacuo*. The product was purified by column chromatography (96:4 hexane/Et_3N) to obtain a mixture of mono- **38** and di-benzylated **39** phosphinite as a colorless oil (0.74g, 65%).

^{1}H NMR (400MHz, Acetonitrile-d_3) δ 7.58-6.88 (5H, m), 4.62 (2H, d, J 7.7Hz), 3.70-3.38 (4H, m), 1.17 (24H, d, J 6.8Hz).

^{31}P NMR (162MHz, CD_3CN) δ 147.61, 123.07.

The spectroscopic data were in agreement with the reported data in our lab.



Benzyl-6-([*N*,*N*-diisopropylamino]-2-benzyloxy-phosphoramidite)-hexyl-1-carbamate (40)

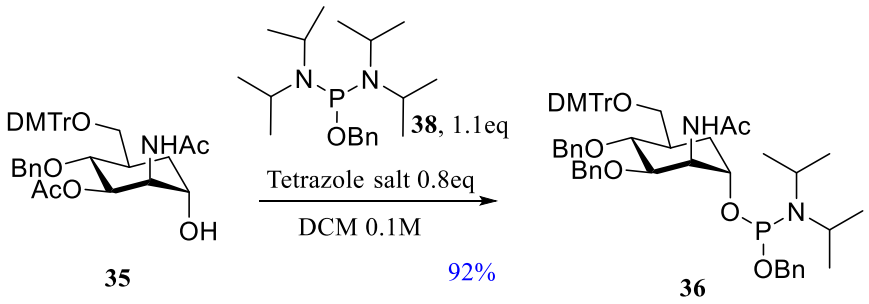
Benzyl(6-hydroxyhexyl) carbamate **37** (0.51g, 2.05mmol) was co-evaporated three times with toluene and dissolved in DCM (20mL). Activated 3Å molsieves, and diisopropylammonium tetrazol-2-ide (0.28g, 1.64mmol) were added to the reaction and was stirred for 30 min. The previously prepared benzyl phosphinite **38** (11.3mL, 2.25mmol) was added and the reaction was stirred for 4 hours. The reaction was quenched with H_2O and diluted with DCM. The organic layer was washed with Brine/$NaHCO_3$ (1:1) mixture and the aqueous layer was extracted three times with DCM. The combined organic layers were dried over $NaSO_4$ and concentrated *in vacuo*. The product was purified by column chromatography (8:2 to 7:3 + 4% Et_3N pentane/EtOAc) to obtain the phosphoramidite **40** as a colorless oil (0.95g, 59%).

^{1}H NMR (400MHz, Acetonitrile-d_3) δ 7.61-7.13 (10H, m), 5.60 (1H, s), 5.03 (2H, s), 4.74-4.50 (2H, s), 3.93-3.49 (2H, m), 3.16-2.99 (2H, m), 1.68-1.50 (2H, m), 1.52-1.39 (2H, m), 1.41-1.21 (4H, m), 1.26-1.06 (12H, m).

^{13}C NMR (101MHz, CD_3CN) δ 129.3, 129.2, 128.7, 128.2, 127.9, 66.5, 65.7, 64.0, 43.6, 41.4, 31.8, 30.5, 27.0, 26.3, 24.8, 1.5, 1.3, 1.0.

^{31}P NMR (162 MHz, CD_3CN) δ 146.46.

The spectroscopic data were in agreement with the reported data in our lab.



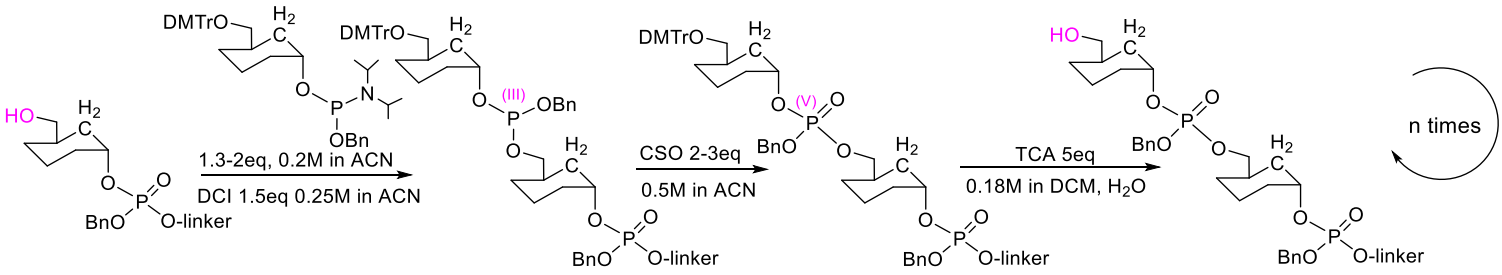
1-*O*-((*N*,*N*-Diisopropylamino)-*O*-2-benzyl-phosphoramidite))-2-acetamido-3-acetyl-4-*O*-benzyl-2-deoxy-6-*O*-(bis(4-methoxyphenyl)(phenyl))-5a-carba-α-D-mannopyranose (36)

Alcohol **35** (1.98g, 3.04 mmol) was co-evaporated three times with ACN. To the alcohol were added activated 3Å molecular sieves and diisopropylammonium tetrazol-2-ide (0.42g, 2.43mmol) and the mixture was stirred for 30 min. To the solution was added the previously prepared phosphinite **38** (16.7mL, 3.34mmol, 0.2M) and the reaction was stirred or 3 hours. To observe the full conversion of the starting material **35**, phophinite **38** (3.04mL, 0.61mmol, 0.2M) might be added and the reaction stirred for 1 extra hour. The reaction was quenched with H_2O and diluted with DCM. The organic layer was washed Brine/$NaHCO_{3(aq)}$ mixture and the aqueous layer was extracted three times with DCM. The combined organic layers were dried $NaSO_4$ and concentrated *in vacuo.* The product was purified with column chromatography (8:2 to 3:7 + 4% Et_3N pentane/EtOAc) to obtain the phosphoramidite elongation block **36** (2.62g, 92%).

^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.69-7.15 (16H, m), 7.12-6.97 (2H, m), 6.93-6.68 (4H, m), 6.42 (1H, t, J 8.9 Hz), 5.35-5.08 (1H, m), 4.85-4.67 (2H, m), 4.59-4.39 (2H, m), 4.27 (2H, t, J 12.0), 4.16-4.01 (1H, m), 3.88-3.54 (9H, m), 3.43-3.25 (1H, m), 3.03 (1H, t, J 8.7, J 7.0), 2.37-2.10 (1H, m), 2.03-1.85 (8H, m), 1.40-0.98 (12H, m).

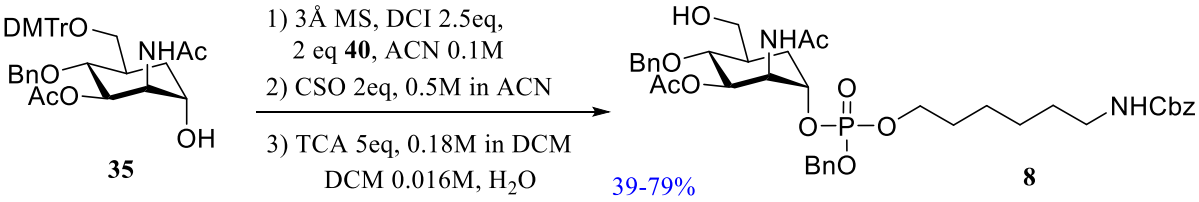
^{13}C NMR (101 MHz, CD_3CN) δ 170.9, 159.5, 146.4, 137.1, 137.0, 131.0, 129.2, 128.7, 127.9, 127.7, 113.9, 113.9, 86.5, 77.0, 74.5, 74.4, 70.8, 65.8, 64.4, 64.2, 55.8, 53.1, 44.0, 38.8, 24.93, 23.02, 21.25.

^{31}P NMR (162 MHz, CD_3CN) δ 147.41, 146.62.



General procedure for phosphoramedite coupling, oxidation and de-tritylation on the scale 0.9-0.07 mmol

Starting alcohol was co-evaporated with ACN for three times. To the alcohol were added 3Å molecular sieves and DCI (0.25M in ACN, 1.5eq). The solution was stirred for 15-30 min, followed by the addition of the phosphoramidite reagent (0.2M in ACN, 1.3-2.0eq). The reaction was stirred until the total conversion of starting alcohol (1-2 hours). Subsequently CSO (0.5M in ACN, 2-3eq) was added to the reaction and stirred for 15 min. The mixture was diluted with DCM and washed with a 1:1 solution of $NaHCO_3$/brine. The water layer was extracted four times with DCM. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. To the crude was added TCA (0.18M in DCM, 5eq) and stirred for 1 hour. The reaction was quenched with MeOH and stirred for 15 min. The reaction was washed with a 1:1 solution of $NaHCO_3$/brine and the water layer was extracted four times with DCM. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo.* The product was purified by column chromatography (acetone/DCM or MeOH/DCM) and/or size exclusion chromatography (sephadex LH-20, MeOH/DCM 1:1). Recovered DMTr-protected phosphodiester was dissolved in TCA (0,18M in DMC, 5eq) and was stirred for 1 hour. The reaction was quenched with MeOH and stirred for 15 min. The reaction was washed with a 1:1 solution $NaHCO_3$/brine and the water layer was four times extracted with DCM. The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo*. The product was purified by column chromatography (acetone/DCM or MeOH/DCM) and/or size exclusion (sephadex LH-20, MeOH/DCM 1:1).



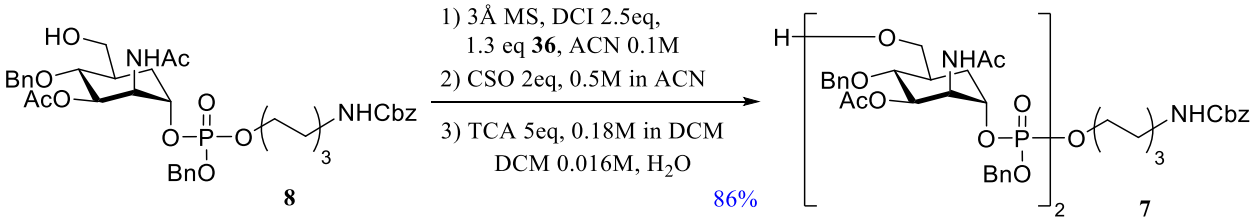
1-*O*-((2-Acetamido-3-*O*-acetyl-4-*O*-benzyl-2-deoxy-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)2-benzyl)-6-hexyl-benzyl-carbamate (8)

Alcohol **35** (0.59g, 0.90mmol) was coupled to the previously prepared phosphoramidite terminal end **40** (5.93mL, 1.18mmol, 0.2M in ACN), oxidized and de-tritylated according to the general procedure described above. The crude was purified by column chromatography (DCM/ acetone, 8:2 to 2:8) to obtain monomer **8** (0.54g, 79%).

^{1}H NMR (400MHz, Acetonitrile-d_3) δ 7.47-7.21 (15H, m, H_{arom}), 6.62 (1H, m, NH), 5.87-5.70 (1H, m, NH), 5.18-4.93 (5H, m, H-3, $CH_{2\ benzyl}$), 4.82-4.57 (2H, m, $CH_{2\ benzyl}$), 4.52 (2H, m, H-1, H-2), 4.14-3.93 (2H, m, $CH_{2\ spacer}$), 3.73 (1H, m, H-4), 3.69-3.50 (2H, m, H-6), 3.05 (3H, m, $CH_{2\ spacer,}$ OH), 2.04-1.79 (3H, m, 2x$CH_{3\ acetyl}$, H-5, H-7), 1.61 (2H, m, $CH_{2\ spacer}$), 1.43 (2H, m, $CH_{2\ spacer}$), 1.35-1.25 (4H, m, 2x$CH_{2\ spacer}$).

^{13}C NMR (101MHz, CD_3CN) δ 171.2($C_{\ acetyl}$), 170.9$C_{\ acetyl}$), 129.5($CH_{\ benzyl}$), 129.4($CH_{\ benzyl}$), 129.2($CH_{\ benzyl}$), 128.*($CH_{\ benzyl}$), 128.8($CH_{\ benzyl}$), 128.7($CH_{\ benzyl}$), 128.7($CH_{\ benzyl}$), 128.6($CH_{\ benzyl}$), 128.5($CH_{\ benzyl}$), 76.17(C-4), 75.1(C-1), 74.5($CH_{2benzyl}$), 73.8(C-3), 70.0($CH_{2benzyl}$), 68.8($CH_{2spacer}$), 66.5($CH_{2benzyl}$), 62.2(C-6), 51.7(C-2), 41.3($CH_{2\ spacer}$), 40.2(C-5), 30.7($CH_{2\ spacer}$), 30.3($CH_{2\ spacer}$), 29.4 (C-7), 26.7($CH_{2\ spacer}$), 25.67($CH_{2\ spacer}$), 23.0($CH_{3\ acetyl}$), 21.2($CH_{3\ acetyl}$).

^{31}P NMR (162 MHz, CD_3CN) δ -0.42, -0.55.



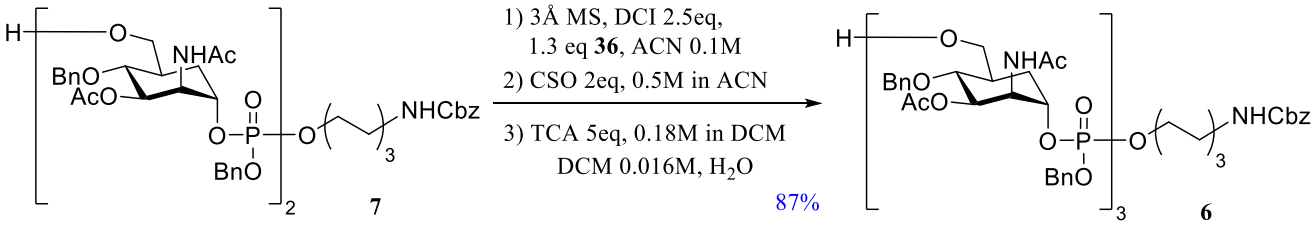
1-di-*O*-((2-Acetamido-3-*O*-acetyl-4-di-*O*-benzyl-2-deoxy-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)2-benzyl)-6-hexyl-benzyl-carbamate (7)

Monomer **8** (0.54g, 0.71mmol) was coupled to the phosphoramidite elongation block **36** (4.60mL, 0.92mmol, 0.2M), oxidized and de-tritylated according to the general procedure. The crude was purified by size exclusion (sephadex LH-20, MeOH/DCM 1:1) and column chromatography (DCM/acetone 5:5 to 0:10) to obtain dimer **7** (0.77g, 86%).

^{1}H NMR (400MHz, Acetonitrile-d_3) δ 7.53-7.17 (25H, m, H_{arom}), 7.15-6.96 (1H, m, NH), 6.77-6.65 (1H, m, NH), 5.78 (1H, s, NH), 5.22-4.94 (8H, m, 2xH-3, 2x$CH_{2\ benzyl}$), 4.75-4.43 (8H, m, 2xH-1, 2xH-2, 2x$CH_{2\ benzyl}$), 4.36-4.18 (1H, m, H-6), 4.08-3.86 (3H, m, H-6, $CH_{2\ spacer}$), 3.84-3.68 (2H, m, 2xH-4), 3.68-3.44 (2H, m, H-6), 3.10-2.99 (2H, m, $CH_{2\ spacer}$), 2.04-1.80 (22H, m, 4x$CH_{3\ acetyl}$, 2xH-5, 2xH-7), 1.68-1.55 (2H, m, $CH_{2\ spacer}$), 1.46-1.38 (2H, m, $CH_{2\ spacer}$), 1.36-1.19 (4H, m, 2x$CH_{2\ spacer}$).

^{13}C NMR (126MHz, CD_3CN) δ 171.0 ($C_{\ acetyl}$), 170.4 ($C_{\ acetyl}$), 170.2 ($C_{\ acetyl}$), 170.1 ($C_{\ acetyl}$), 139.0 ($CH_{2\ benzyl}$), 128.7 ($CH_{2\ benzyl}$), 128.7 ($CH_{2\ benzyl}$), 128.7 ($CH_{2\ benzyl}$), 128.6 ($CH_{2\ benzyl}$), 128.6 ($CH_{2\ benzyl}$), 128.5($CH_{2\ benzyl}$), 128.4 ($CH_{2\ benzyl}$), 28.0 ($CH_{2\ benzyl}$), 127.9 ($CH_{2\ benzyl}$), 127.8 ($CH_{2\ benzyl}$), 127.6 ($CH_{2\ benzyl}$), 75.5 (C-4), 74.3 (C-1), 74.1 ($CH_{2\ benzyl}$), 73.9 ($CH_{2\ benzyl}$), 73.2 (C-3), 73.0 (C-3), 69.4 ($CH_{2\ benzyl}$), 69.2 ($CH_{2\ benzyl}$), 68.04 ($CH_{2\ spacer}$), 67.2 (C-6), 65.7 ($CH_{2\ benzyl}$), 61.4 (C-6), 51.1 (C-2), 40.5 ($CH_{2\ spacer}$), 39.2 (C-5), 29.8 ($CH_{2\ spacer}$), 29.4 ($CH_{2\ spacer}$), 28.5 (C-7), 25.9 ($CH_{2\ spacer}$), 24.9 ($CH_{2\ spacer}$), 22.2 ($CH_{3\ acetyl}$), 22.2 ($CH_{3\ acetyl}$), 20.4 ($CH_{3\ acetyl}$), 20.33 ($CH_{3\ acetyl}$).

^{31}P NMR (162 MHz, CD_3CN) δ 0.01, -0.52, -0.57, -0.62, -0.68, -0.73.



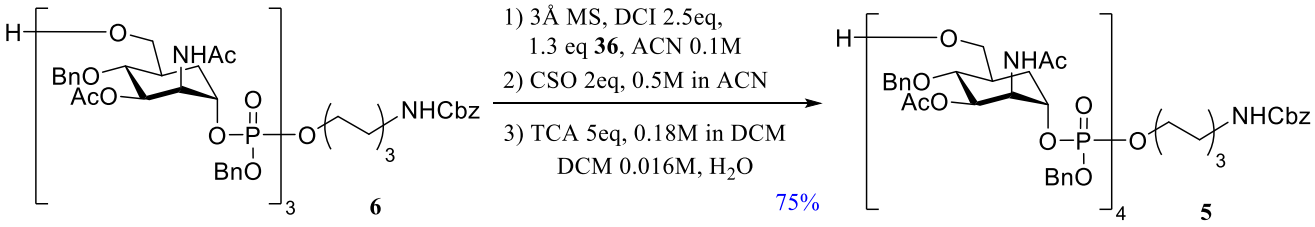
1-tri-*O*-((2-Acetamido-3-*O*-acetyl-4-di-*O*-benzyl-2-deoxy-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)2-benzyl)-6-hexyl-benzyl-carbamate (6)

Dimer **7** (0.55g, 0.43mmol) was coupled to the phosphoramidite elongation block **36** (2.80mL, 0.56 mmol, 0.2M), oxidized and de-tritylated according to the general procedure. The crude was purified by size exclusion (sephadex LH-20, MeOH/DCM 1:1) to obtain trimer **6** (0.66g, 87%).

^{1}H NMR (500MHz, Acetonitrile-d_3) δ 7.50-7.20 (35H, m, H_{arom}), 7.17-7.08 (1H, m, NH), 6.80-6.69 (2H, m, NH), 5.89-5.79 (1H, m, NH), 5.22-4.98 (11H, m, 3xH-3, 4x$CH_{2\ benzyl}$), 4.76-4.44 (12H, m, 3xH-1, 3xH-2, 3x$CH_{2\ benzyl}$), 4.36-4.22 (2H, m, 2xH-6), 4.13-3.88 (4H, m, 2xH-6, $CH_{2\ spacer}$), 3.86-3.70 (3H, m, 3xH-4), 3.68-3.51 (2H, m, H-6), 3.11-3.02 (2H, m, $CH_{2\ spacer}$), 2.12-1.78 (27H, m, 6x$CH_{3\ acetyl}$, 3xH-7, 3xH-5), 1.60 (2H, m, $CH_{2\ spacer}$), 1.42 (2H, m, $CH_{2\ spacer}$), 1.37-1.19 (4H, m, 2x$CH_{2\ spacer}$).

^{13}C NMR (126MHz, CD_3CN) δ 172.0 ($C_{\ acetyl}$), 171.7 ($C_{\ acetyl}$), 171.3 ($C_{\ acetyl}$), 171.1 ($C_{\ acetyl}$), 171.0 ($C_{\ acetyl}$), 170.9 ($C_{\ acetyl}$), 139.6 ($C_{\ benzyl}$), 137.1 ($C_{\ benzyl}$), 129.6 ($CH_{\ benzyl}$), 129.6 ($CH_{\ benzyl}$), 129.5 ($CH_{\ benzyl}$), 129.5 ($CH_{\ benzyl}$), 129.4 ($CH_{\ benzyl}$), 129.4 ($CH_{\ benzyl}$), 129.3 ($CH_{\ benzyl}$), 129.2 ($CH_{\ benzyl}$), 129.1 ($CH_{\ benzyl}$), 129.1 ($CH_{\ benzyl}$), 128.9 ($CH_{\ benzyl}$), 128.9 ($CH_{\ benzyl}$), 128.8 ($CH_{\ benzyl}$), 128.7 ($CH_{\ benzyl}$), 128.7 ($CH_{\ benzyl}$), 128.6 ($CH_{\ benzyl}$), 128.5 ($CH_{\ benzyl}$), 128.5 ($CH_{\ benzyl}$), 76.4 (C-4), 75.7 (C-1), 74.8 ($CH_{2\ benzyl}$), 73.8 (C-3), 70.3 ($CH_{2\ benzyl}$), 70.1 ($CH_{2\ benzyl}$), 68.3 ($CH_{2\ spacer}$), 68.1 (C-6), 66.5 ($CH_{2\ benzyl}$), 62.3 (C-6), 51.5 (C-2), 41.4 ($CH_{2\ spacer}$), 40.01 (C-5), 30.8 ($CH_{2\ spacer}$), 30.4 ($CH_{2\ spacer}$), 29.3 (C-7), 26.8 ($CH_{2\ spacer}$), 25.7 ($CH_{2\ spacer}$), 23.2 ($CH_{3\ acetyl}$), 23.1 ($CH_{\ 3acetyl}$), 21.2 ($CH_{3\ acetyl}$).

^{31}P NMR (202 MHz, CD_3CN) δ 0.07, -0.07, -0.54, -0.61, -0.65, -0.69, -0.71.



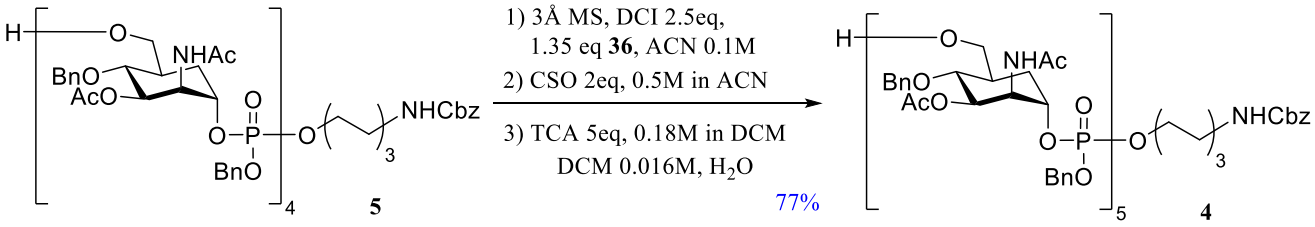
1-tetra-*O*-((2-Acetamido-3-*O*-acetyl-4-di-*O*-benzyl-2-deoxy-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)2-benzyl)-6-hexyl-benzyl-carbamate (5)

Trimer **6** (0.51g, 0.29 mmol) was coupled to the phosphoramidite elongation block **36** (1.86mL, 0.37mmol, 0.2M), oxidized and de-tritylated according to the general procedure. The crude was purified by size exclusion (sephadex LH-20, MeOH/DCM 1:1) and column chromatography (DCM/ acetone, 6:4 to 9:1) to obtain tetramer **5** (0.48g, 75%).

^{1}H NMR (500MHz, Acetonitrile-d_3) δ 7.61-7.20 (45H, m, H_{arom}), 7.14 (2H, s, NH), 7.07-7.00 (1H, m, NH), 6.76 (1H, m, NH), 5.92-5.73 (1H, m, NH), 5.20-4.96 (14H, m, 4xH-3, 5x$CH_{2\ benzyl}$), 4.76-4.47 (16H, m, 4xH-1, 4xH-2, 4x$CH_{2\ benzyl}$), 4.38-4.21 (3H, m, 3xH-6), 4.10-3.88 (5H, m, 3xH-6, $CH_{2\ spacer}$), 3.89-3.70 (4H, m, 4xH-4), 3.69-3.53 (2H, m, H-6), 3.09-3.04 (2H, m, $CH_{2\ spacer}$), 2.08-1.79 (36H, m, 8x$CH_{3\ acetyl}$, 4xH-7, 4xH-5), 1.62 (2H, dt, J 14.7, J 7.4Hz, 2H, $CH_{2\ spacer}$), 1.49-1.39 (2H, m, $CH_{2\ spacer}$), 1.36-1.23 (4H, m, 2x $CH_{2\ spacer}$).

^{13}C NMR (126MHz, CD_3CN) δ 171.1 ($C_{\ acetyl}$), 171.0 ($C_{\ acetyl}$), 170.4 ($C_{\ acetyl}$), 170.4 ($C_{\ acetyl}$), 170.2 ($C_{\ acetyl}$), 170.2 ($C_{\ acetyl}$), 170.1 ($C_{\ acetyl}$), 170.1 ($C_{\ acetyl}$), 170.1 ($C_{\ acetyl}$), 138.8 ($C_{\ benzyl}$), 136.3 ($C_{\ benzyl}$), 128.76 ($CH_{\ benzyl}$), 128.1 ($CH_{\ benzyl}$), 128.7 ($CH_{\ benzyl}$), 128.6 ($CH_{\ benzyl}$), 128.5 ($CH_{\ benzyl}$), 128.5 ($CH_{\ benzyl}$), 128.4 ($CH_{\ benzyl}$), 128.3 ($CH_{\ benzyl}$), 128.2 ($CH_{\ benzyl}$), 128.1 ($CH_{\ benzyl}$), 128.0 ($CH_{\ benzyl}$), 127.9 ($CH_{\ benzyl}$), 127.8 ($CH_{\ benzyl}$), 127.8 ($CH_{\ benzyl}$), 127.7 ($CH_{\ benzyl}$), 127.6 ($CH_{\ benzyl}$), 75.5 (C-4), 74.8 (C-1), 73.9 ($CH_{2\ benzyl}$), 72.9 (C-3), 69.5 ($CH_{2\ benzyl}$), 69.2 ($CH_{2\ benzyl}$), 68.0 ($CH_{2\ spacer}$), 67.1 (C-6), 65.7 ($CH_{2\ benzyl}$), 61.4 (C-6), 50.7 (C-2), 40.5 ($CH_{2\ spacer}$), 39.2 (C-5), 29.9 ($CH_{2\ spacer}$), 29.4 ($CH_{2\ spacer}$), 28.4 (C-7), 25.9 ($CH_{2\ spacer}$), 24.9 ($CH_{2\ spacer}$), 22.3 ($CH_{3\ acetyl}$), 22.2 ($CH_{3\ acetyl}$), 22.2 ($CH_{3\ acetyl}$), 20.4 ($CH_{3\ acetyl}$), 20.4 ($CH_{3\ acetyl}$).

^{31}P NMR (202 MHz, CD_3CN) δ -1.28, -1.33, -1.36, -1.40, -1.47, -1.55, -1.71, -1.97, -2.05, -2.09, -2.11, -2.14, -2.24, -2.27, -2.30, -2.32, -2.34, -2.37.



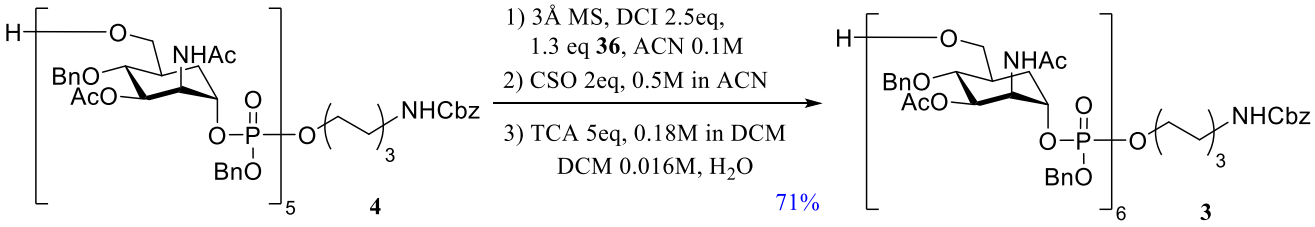
1-penta-*O*-((2-Acetamido-3-*O*-acetyl-4-di-*O*-benzyl-2-deoxy-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)2-benzyl)-6-hexyl-benzyl-carbamate (4)

Tetramer **5** (0.47g, 0.21mmol) was coupled to the phosphoramidite elongation block **36** (1.35mL, 0.27mmol, 0.2M), oxidized and de-tritylated according to the general procedure. The crude was purified by column chromatography (DCM/ MeOH, 97.5:2.5 to 88.5:12.5) and size exclusion (sephadex LH-20, MeOH/DCM 1:1) to obtain pentamer **4** (0.45 g, 77%)

^{1}H NMR (500MHz, Acetonitrile-d_3) δ 7.51-7.22 (55H, m, H_{arom}), 7.15 (1H, s, NH), 6.79-6.65 (1H, m, NH), 5.82 (1H, s, NH), 5.21-4.98 (17H, m, 5xH-3, 2x$CH_{2\ benzyl}$), 4.75-4.44 (20H, m, 5xH-1, 5xH-2, 5x$CH_{2\ benzyl}$), 4.42-4.20 (4H, m, H-6), 4.10-3.88 (6H, m, 4xH-6, $CH_{2\ spacer}$), 3.87-3.72 (4H, m, H-4), 3.70-3.55 (2H, m, H-6), 3.08-3.05 (2H, m, $CH_{2\ spacer}$), 2.17-1.70 (45H, m, 10x$CH_{3\ acetyl}$, 5x H-7, 5xH-5), 1.69-1.53 (2H, m, $CH_{2\ spacer}$), 1.49-1.37 (2H, m, $CH_{2\ spacer}$), 1.38-1.22 (4H, m, 2x$CH_{2\ spacer}$).

^{13}C NMR (126MHz, CD_3CN) δ 171.1 (C_{acetyl}), 171.0 (C_{acetyl}), 170.4 (C_{acetyl}), 170.2 (C_{acetyl}), 170.2 (C_{acetyl}), 170.1 (C_{acetyl}), 170.1 (C_{acetyl}), 138.8 (C_{benzyl}), 136.3 (C_{benzyl}), 128.8 (CH_{benzyl}), 128.7 (CH_{benzyl}), 128.5 (CH_{benzyl}), 128.4 (CH_{benzyl}), 128.4 (CH_{benzyl}), 128.3 (CH_{benzyl}), 128.2 (CH_{benzyl}), 128.1 (CH_{benzyl}), 128.0 (CH_{benzyl}), 127.8 (CH_{benzyl}), 127.8 (CH_{benzyl}), 127.7 (CH_{benzyl}), 127.6 (CH_{benzyl}), 74.9 (C-4), 73.9 ($CH_{2\ benzyl}$), 73.0 (C-2), 69.5 ($CH_{2\ benzyl}$), 69.2 ($CH_{2\ benzyl}$), 68.1 ($CH_{2\ spacer}$), 67.2 (C-6), 65.7 ($CH_{2\ benzyl}$), 61.4 (C-6), 50.7 (C-2), 40.5 ($CH_{2\ spacer}$), 39.2 (C-5), 29.9 ($CH_{2\ spacer}$), 29.5 ($CH_{2\ spacer}$), 28.4 (C-7), 25.9 ($CH_{2\ spacer}$), 24.9 ($CH_{2\ spacer}$), 22.3 ($CH_{3\ acetyl}$), 22.3 ($CH_{3\ acetyl}$), 22.2 ($CH_{3\ acetyl}$), 20.4 ($CH_{3\ acetyl}$), 20.4 ($CH_{3\ acetyl}$), 20.3 ($CH_{3\ acetyl}$).

^{31}P NMR (202 MHz, CD_3CN) δ -1.30, -1.32, -1.35, -1.38, -1.40, -1.47, -1.54, -1.96, -2.05, -2.11, -2.14, -2.24, -2.27, -2.30, -2.32, -2.35.



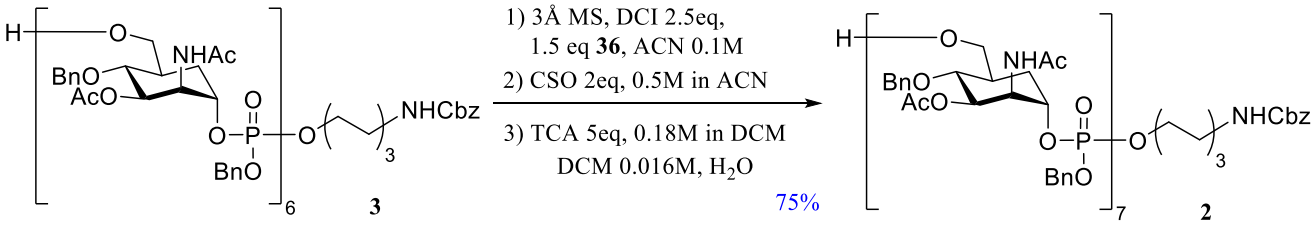
1-hexa-*O*-((2-Acetamido-3-*O*-acetyl-4-di-*O*-benzyl-2-deoxy-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)2-benzyl)-6-hexyl-benzyl-carbamate (3)

Pentamer **4** (0.43g, 0.15mmol) was coupled to the phosphoramidite elongation block **36** (1.0mL, 0.2mmol, 0.2M), oxidized and de-tritylation according to the general procedure. The crude was purified by column chromatography (DCM/MeOH, 97.5:2.5 to 90:10) and size exclusion (sephadex LH-20, MeOH/DCM 1:1) to obtain hexamer **3** (0.36g, 71%).

^{1}H NMR (500MHz, Acetonitrile-d_3) δ 7.49-7.20 (65H, m, H_{arom}), 7.16 (1H, m, NH), 6.80-6.69 (1H, m, NH), 5.89-5.79 (1H, m, NH), 5.21-5.00 (20H, m, 6xH-3, 7x$CH_{2\ benzyl}$), 4.75-4.35 (24H, m, 6xH-1, 6xH-2, 6x$CH_{2\ benzyl}$), 4.38-4.24 (5H, m, 5xH-6), 4.08-3.90 (7H, m, 5xH-6, $CH_{2\ spacer}$), 3.91-3.69 (6H, m, 6xH-4), 3.71-3.54 (2H, m, H-6), 3.12-2.99 (2H, m, $CH_{2\ spacer}$), 2.09-1.72 (60H, m, 12x$CH_{3\ acetyl}$, 6xH-7, 6xH-5), 1.69-1.54 (2H, m, $CH_{2\ spacer}$), 1.48-1.37 (2H, m, $CH_{2\ spacer}$), 1.36-1.22 (4H, m, 2x $CH_{2\ spacer}$).

^{13}C NMR (126MHz, CD_3CN) δ 172.0 (C_{acetyl}), 178.0 (C_{acetyl}), 172.0 (C_{acetyl}), 171.9 (C_{acetyl}), 171.1 (C_{acetyl}), 171.0 (C_{acetyl}), 170.9 (C_{acetyl}), 139.7 (C_{benzyl}), 137.1 (C_{benzyl}), 129.6 (CH_{benzyl}), 129.6 (CH_{benzyl}), 129.56 (CH_{benzyl}), 129.5 (CH_{benzyl}), 129.2 (CH_{benzyl}), 129.2 (CH_{benzyl}), 128.9 (CH_{benzyl}), 128.8 (CH_{benzyl}), 128.7 (CH_{benzyl}), 128.7 (CH_{benzyl}), 128.6 (CH_{benzyl}), 128.5 (CH_{benzyl}), 128.5 (CH_{benzyl}), 75.7 (C-4), 74.8 ($CH_{2\ benzyl}$), 73.8 (C-1), 70.3 ($CH_{2\ benzyl}$), 70.0 ($CH_{2\ benzyl}$), 68.9 ($CH_{2\ spacer}$), 68.1 (C-6), 66.5 ($CH_{2\ benzyl}$), 62.2 (C-6), 51.8 (C-2), 41.3 ($CH_{2\ spacer}$), 40.0 (C-5), 30.8 ($CH_{2\ spacer}$), 30.3 ($CH_{2\ spacer}$), 29.3 (C-7), 26.8 ($CH_{2\ spacer}$), 25.7 ($CH_{2\ spacer}$), 23.2 ($CH_{3\ acetyl}$), 23.0 ($CH_{3\ acetyl}$), 21.3 ($CH_{3\ acetyl}$), 21.2 ($CH_{3\ acetyl}$).

^{31}P NMR (202 MHz, CD_3CN) δ 0.15, 0.11, 0.08, 0.05, -0.03, -0.09, -0.51, -0.60, -0.66, -0.69, -0.80, -0.82, -0.86, -0.90, -0.93.



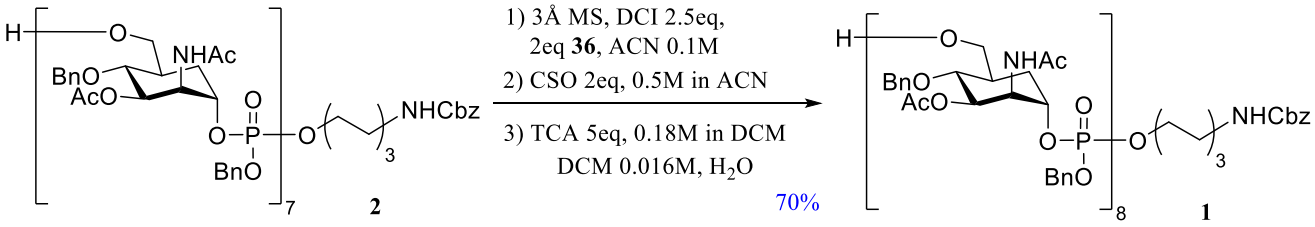
1-hepta-*O*-((2-Acetamido-3-*O*-acetyl-4-di-*O*-benzyl-2-deoxy-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)2-benzyl)-6-hexyl-benzyl-carbamate (2)

Hexamer **3** (0.32g, 0.10mmol) was coupled to the phosphoramidite elongation block **36** (0.73 mL, 0.15mmol, 0.2M), oxidized and de-tritylated according to the general procedure. The crude was purified by size exclusion (sephadex LH-20, MeOH/DCM 1:1) and column chromatography (DCM/ MeOH, 10:0 to 8:2) to obtain heptamer **2** (0.28g, 75%)

^{1}H NMR (500MHz, Acetonitrile-d_3) δ 7.55-7.21 (75H, m, CH_{arom}), 7.18-7.10 (1H, m, NH), 6.81-6.71 (1H, m, NH), 5.87-5.74 (1H, m, NH), 5.23-4.97 (23H, m, 7xH-3, 8x$CH_{2\ benzyl}$), 4.77-4.47 (28H, m, 7xH-1, 7xH-2, 7x$CH_{2\ benzyl}$), 4.31 (6H, m, 6xH-6), 3.97 (8H, m, 6xH-6, $CH_{2\ spacer}$), 3.89-3.74 (7H, m, 7xH-4), 3.72-3.56 (2H, m, H-6), 3.10-3.04 (2H, m, $CH_{2\ spacer}$), 2.15-1.70 (63H, m, 14x$CH_{3\ acetyl}$, 7xH-7, 7xH-5), 1.62 (2H, m, $CH_{2\ spacer}$), 1.44 (2H, m, $CH_{2\ spacer}$), 1.38-1.24 (4H, m, 2x $CH_{2\ spacer}$).

^{13}C NMR (126MHz, CD_3CN) δ 171.1 ($C_{\ acetyl}$), 171.0 ($C_{\ acetyl}$), 170.4 ($C_{\ acetyl}$), 170.2 ($C_{\ acetyl}$), 170.1 ($C_{\ acetyl}$), 170.1 ($C_{\ acetyl}$), 138.8 ($C_{\ benzyl}$), 136.3 ($C_{\ benzyl}$), 128.8 ($CH_{\ benzyl}$), 128.7 ($CH_{\ benzyl}$), 128.7 ($CH_{\ benzyl}$), 128.6 ($CH_{\ benzyl}$), 128.5 ($CH_{\ benzyl}$), 128.5 ($CH_{\ benzyl}$), 128.3 ($CH_{\ benzyl}$), 128.2 ($CH_{\ benzyl}$), 128.1 ($CH_{\ benzyl}$), 128.0 ($CH_{\ benzyl}$), 127.8 ($CH_{\ benzyl}$), 127.8 ($CH_{\ benzyl}$), 127.7 ($CH_{\ benzyl}$), 74.9 (C-4), 74.3 ($CH_{2\ benzyl}$), 73.0 (C-1), 69.49 ($CH_{2\ benzyl}$), 69.2 ($CH_{2\ benzyl}$), 68.0 ($CH_{2\ spacer}$), 67.2 (C-6), 65.4 ($CH_{2\ benzyl}$), 61.4 (C-6), 51.0 (C-2), 40.5 ($CH_{2\ spacer}$), 37.4 (C-5), 29.9 ($CH_{2\ spacer}$), 29.4 ($CH_{2\ spacer}$), 28.4 (C-7), 25.9 ($CH_{2\ spacer}$), 24.9 ($CH_{2\ spacer}$), 22.3 ($CH_{3\ acetyl}$), 22.18 ($CH_{3\ acetyl}$), 20.4 ($CH_{3\ acetyl}$), 20.4 ($CH_{3\ acetyl}$).

^{31}P NMR (202 MHz, CD_3CN) δ -1.31, -1.34, -1.38, -1.40, -1.49, -1.55, -1.96, -1.98, -2.05, -2.11, -2.14, -2.25, -2.27, -2.31, -2.35, -2.38.



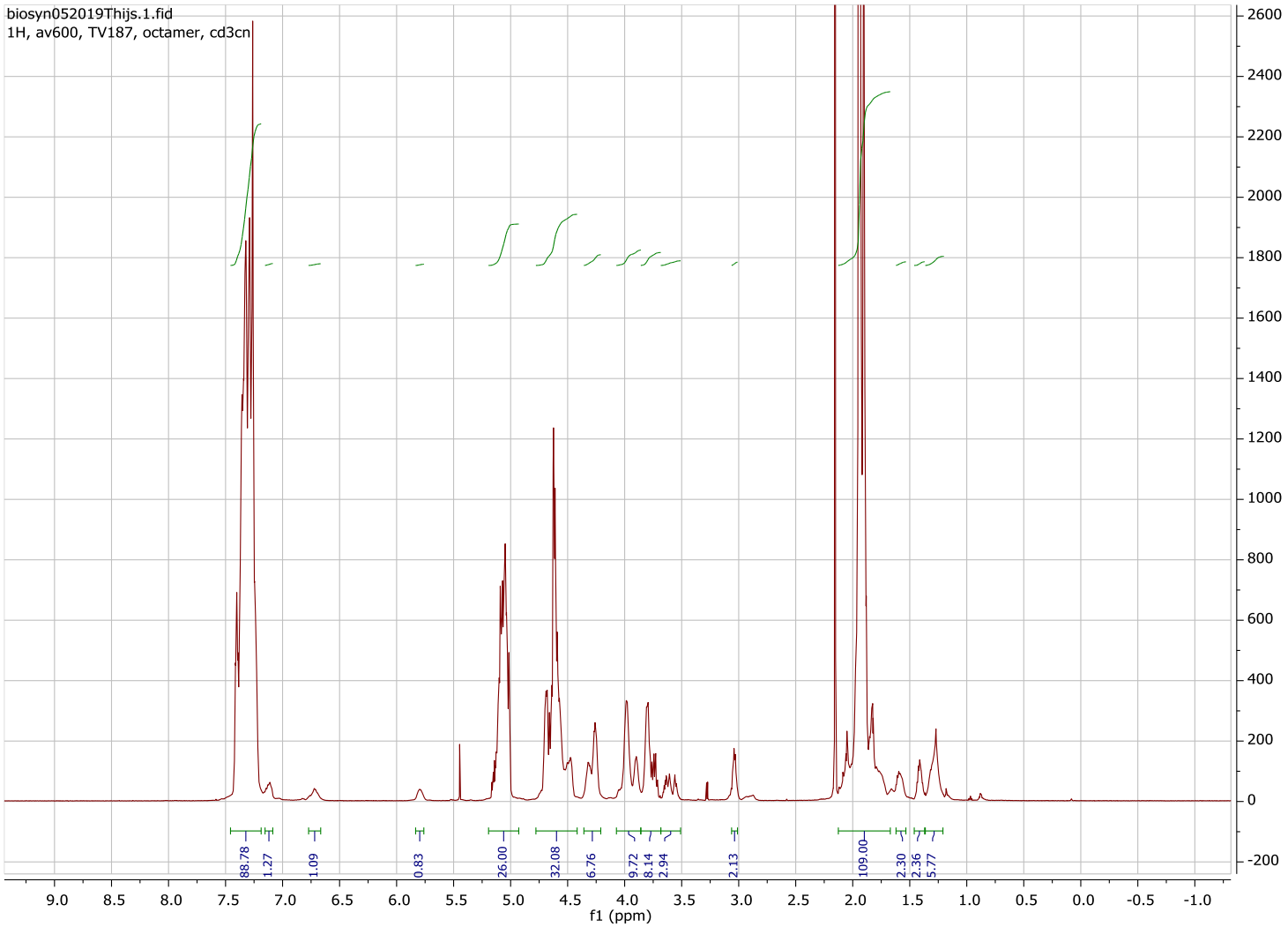
1-octa-*O*-((2-Acetamido-3-*O*-acetyl-4-di-*O*-benzyl-2-deoxy-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)2-benzyl)-6-hexyl-benzyl-carbamate (1)

Heptamer **3** (0.26g, 0.07mmol) was coupled to the phosphoramidite elongation block **36** (0.70mL, 0.14mmol, 0.2M), oxidized and de-tritylated according to the general procedure. The crude was purified by size exclusion (sephadex LH-20, MeOH/DCM 1:1) and column chromatography (DCM/ MeOH, 10:0 to 8:2) to obtain octamer **1** (0.21g, 0.05mmol, 70%).

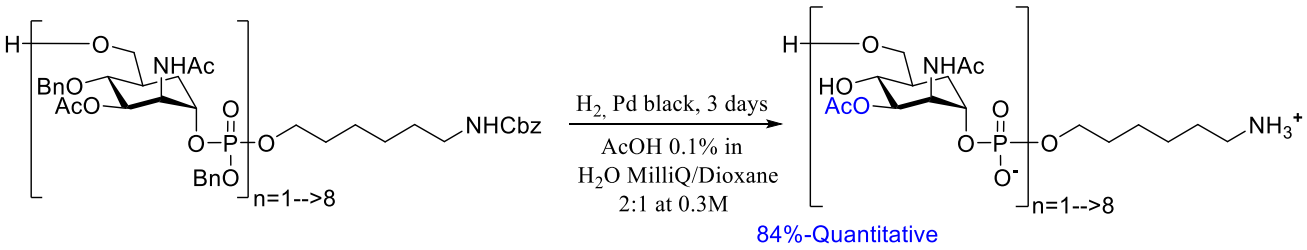
^{1}H NMR (600MHz, Acetonitrile-d_3) δ 7.48-7.19 (85H, m, H_{arom}), 7.12 (1H, m, NH), 6.72 (1H, m, NH), 5.80 (1H, m, NH), 5.41-4.92 (26H, m, 8xH-3, 9x$CH_{2\ benzyl}$), 4.75-4.45 (24H, m, 8xH-1, 8xH-2, 8x$CH_{2\ benzyl}$), 4.38-4.16 (7H, m, H-6), 4.07-3.87 (9H, m, 7xH-6, $CH_{2\ spacer}$), 3.87-3.52 (8H, m, 8Xh-4), 3.68-3.50 (2H, m, H-6), 3.10-2.98 (2H, m, $CH_{2\ spacer}$), 2.10-1.79 (72H, m, 16x$CH_{3\ acetyl}$, 8xH-7, 8xH-5), 1.67-1.52 (2H, m, $CH_{2\ spacer}$), 1.48-1.38 (2H, m, $CH_{2\ spacer}$), 1.35-1.19 (4H, m, 2x $CH_{2\ spacer}$).

^{13}C NMR (151MHz, CD_3CN) δ 172.0 (C_{acetyl}), 171.9 (C_{acetyl}), 171.3 (C_{acetyl}), 171.1 (C_{acetyl}), 171.1 (C_{acetyl}), 171.0 (C_{acetyl}), 139.7 (C_{benzyl}), 137.2 (C_{benzyl}), 129.7 (CH_{benzyl}), 129.6 (CH_{benzyl}), 129.5 (CH_{benzyl}), 129.5 (CH_{benzyl}), 129.4 (CH_{benzyl}), 129.3 (CH_{benzyl}), 129.0 (CH_{benzyl}), 129.0 (CH_{benzyl}), 128.9 (CH_{benzyl}), 128.7 (CH_{benzyl}), 128.6 (CH_{benzyl}), 128.6 (CH_{benzyl}), 128.5 (CH_{benzyl}), 75.8 (C-4), 75.2 ($CH_{2\ benzyl}$), 73.9 (C-1), 70.38 ($CH_{2\ benzyl}$), 70.1 ($CH_{2\ benzyl}$), 69.0 ($CH_{2\ spacer}$), 68.1 (C-6), 66.6 ($CH_{2\ benzyl}$), 62.3 (C-6), 51.8 ($CH_{2\ spacer}$), 38.3 (C-5), 30.8 ($CH_{2\ spacer}$), 30.3 ($CH_{2\ spacer}$), 29.3 (C-7), 26.8 ($CH_{2\ spacer}$), 25.7 ($CH_{2\ spacer}$), 23.2 ($CH_{3\ acetyl}$), 23.21 ($CH_{3\ acetyl}$), 23.1 ($CH_{3\ acetyl}$), 21.3 ($CH_{3\ acetyl}$), 21.3 ($CH_{3\ acetyl}$), 21.2 ($CH_{3\ acetyl}$).

^{31}P NMR (162 MHz, CD_3CN) δ 0.10, -0.52, -0.60, -0.67, -0.80, -0.82.

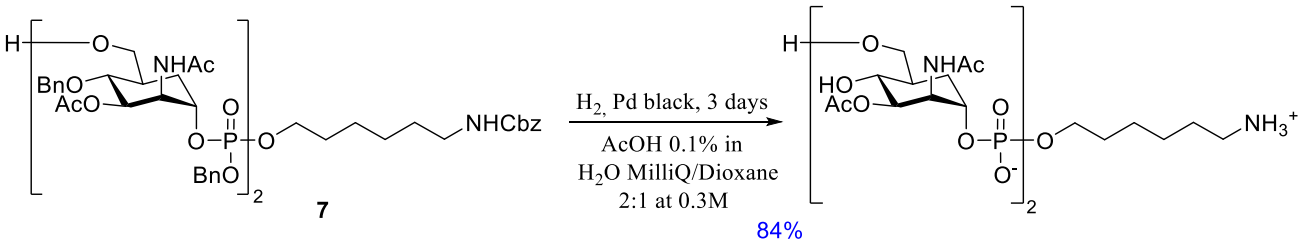


^{1}H NMR (600MHz, Acetonitrile-d_3) δ 7.48-7.19 (85H, m, H_{arom}), 7.12 (1H, m, NH), 6.72 (1H, m, NH), 5.80 (1H, m, NH), 5.41-4.92 (26H, m, 8xH-3, 9x$CH_{2\ benzyl}$), 4.75-4.45 (24H, m, 8xH-1, 8xH-2, 8x$CH_{2\ benzyl}$), 4.38-4.16 (7H, m, H-6), 4.07-3.87 (9H, m, 7xH-6, $CH_{2\ spacer}$), 3.87-3.52 (8H, m, 8Xh-4), 3.68-3.50 (2H, m, H-6), 3.10-2.98 (2H, m, $CH_{2\ spacer}$), 2.10-1.79 (72H, m, 16x$CH_{3\ acetyl}$, 8xH-7, 8xH-5), 1.67-1.52 (2H, m, $CH_{2\ spacer}$), 1.48-1.38 (2H, m, $CH_{2\ spacer}$), 1.35-1.19 (4H, m, 2x $CH_{2\ spacer}$).



General procedure of the benzyl groups deprotection by hydrogenolysis

Starting alcohol was dissolved in AcOH in miliQ (0.1mL in 100mL, 2mL/10µmol). Dioxane was added till starting alcohol was completely dissolved. The mixture was purged with argon, followed by the addition of catalytic amount of Pd black. The reaction was purged with argon. The mixture was purged with H_2 and stirred for three days under H_2 atmosphere. The reaction was filtrated over Whatman filters and concentrated *in vacuo*. ^{1}H- and ^{31}P-NMR confirmed the full de-protection of the benzyls. The product was purified by a Octadecylsilane C-18 column and lyophilized.



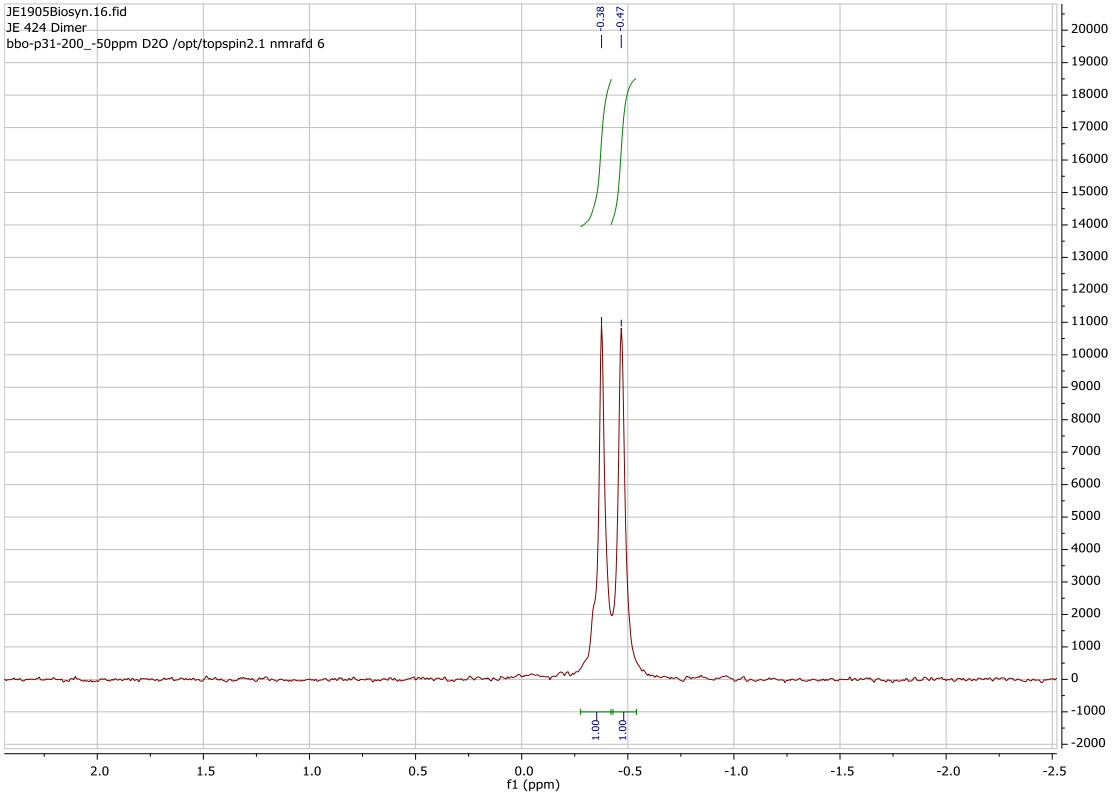
1-*O*-di-(2-Acetamido-2-deoxy-3-*O*-acetyl-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)-6-hexyl- amine

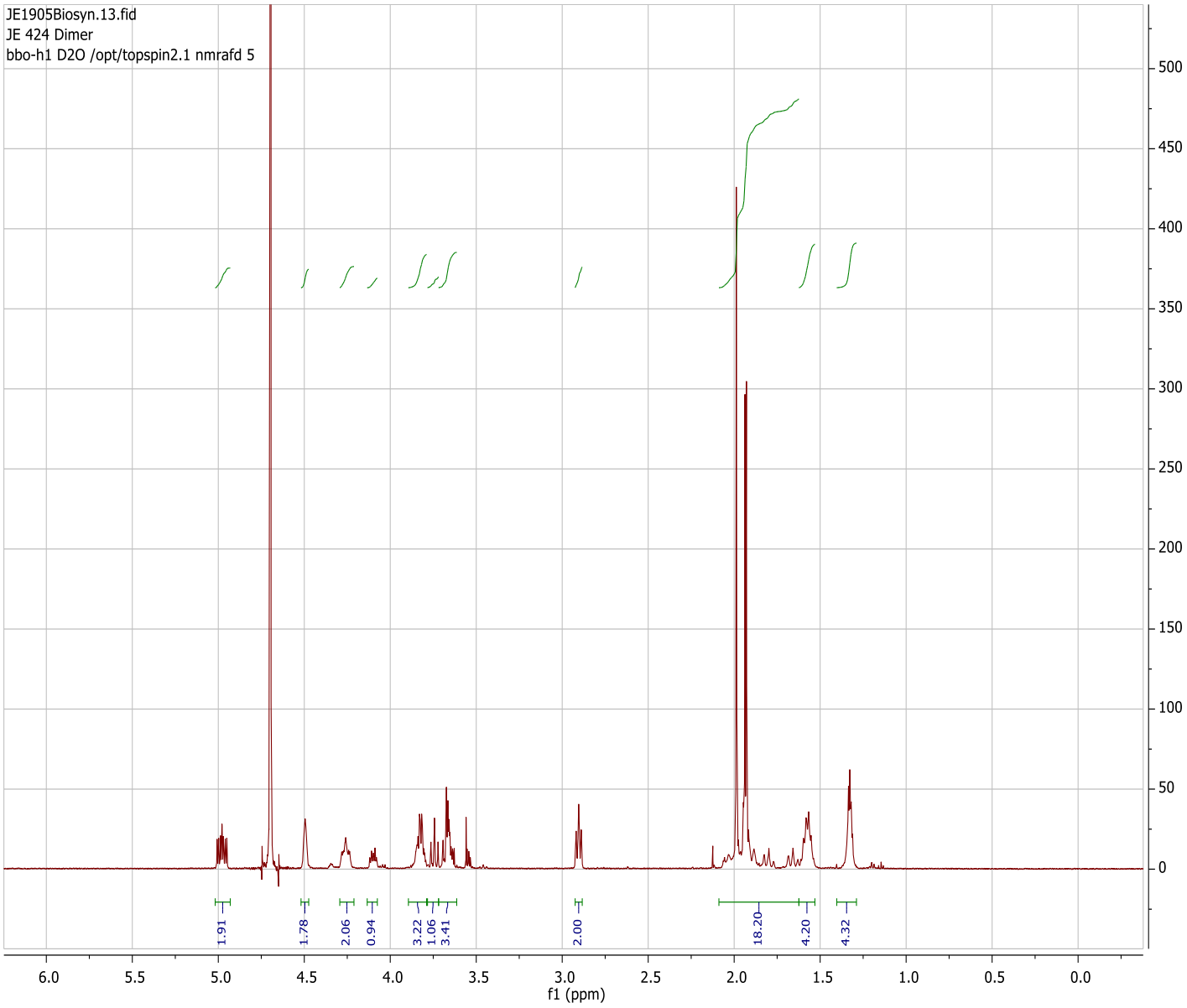
Dimer **7** (23.0mg, 18.0µmol) was deprotected according to the general procedure to obtain the corresponding deprotected dimer (11.6mg, 84%).

^{1}H NMR (500MHz, Deuterium Oxide) δ 5.07-4.89 (2H, m, 2xH-3), 4.61-4.40 (2H, m, 2xH-2), 4.30-4.20 (2H, m, 2xH-1), 4.15-4.04 (1H, m, H-6), 3.87-3.79 (3H, m, H-6, $CH_{2\ spacer}$), 3.78-3.71 (1H, m, H-4), 3.68-3.62 (3H, m, 2xH-4, H-6), 2.89 (2H, t, J 7.5 Hz, $CH_{2\ spacer}$), 2.07-1.73 (18H, m, 4x$CH_{3\ acetyl}$, 2xH-5, 2xH7), 1.59-1.50 (4H, m, 2x$CH_{2spcaer}$), 1.31 (4H, t, J 3.7 Hz, 2x$CH_{2\ spacer}$).

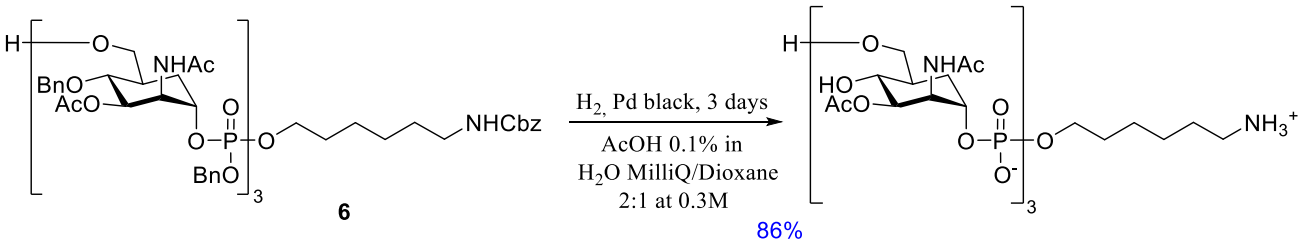
^{13}C NMR (126MHz, D_2O) δ 174.5 ($CH_{\ acetyl}$), 173.4 ($CH_{\ acetyl}$), 73.8 (C-3), 71.9 (C-1), 67.5 (C-4), 66.5 (C-4), 66.4 ($CH_{2\ spacer}$), 65.3 (C-6), 61.7 (C-6), 50.9 (C-2), 39.4 ($CH_{2\ spacer}$), 38.7 (C-5), 37.7 (C-5), 29.42 ($CH_{2\ spacer}$), 28.1 (C-7), 26.6 ($CH_{2\ spacer}$), 25.1 ($CH_{2\ spacer}$), 24.4 ($CH_{2\ spacer}$), 21.8 ($CH_{3\ acetyl}$), 20.4 ($CH_{3\ acetyl}$).

^{31}P NMR (202 MHz, D_2O) δ -0.44, -0.56.





^{1}H NMR (500MHz, Deuterium Oxide) δ 5.07-4.89 (2H, m, 2xH-3), 4.61-4.40 (2H, m, 2xH-2), 4.30-4.20 (2H, m, 2xH-1), 4.15-4.04 (1H, m, H-6), 3.87-3.79 (3H, m, H-6, $CH_{2\ spacer}$), 3.78-3.71 (1H, m, H-4), 3.68-3.62 (3H, m, 2xH-4, H-6), 2.89 (2H, t, J 7.5 Hz, $CH_{2\ spacer}$), 2.07-1.73 (18H, m, 4x$CH_{3\ acetyl}$, 2xH-5, 2xH7), 1.59-1.50 (4H, m, 2x$CH_{2spcaer}$), 1.31 (4H, t, J 3.7 Hz, 2x$CH_{2\ spacer}$).



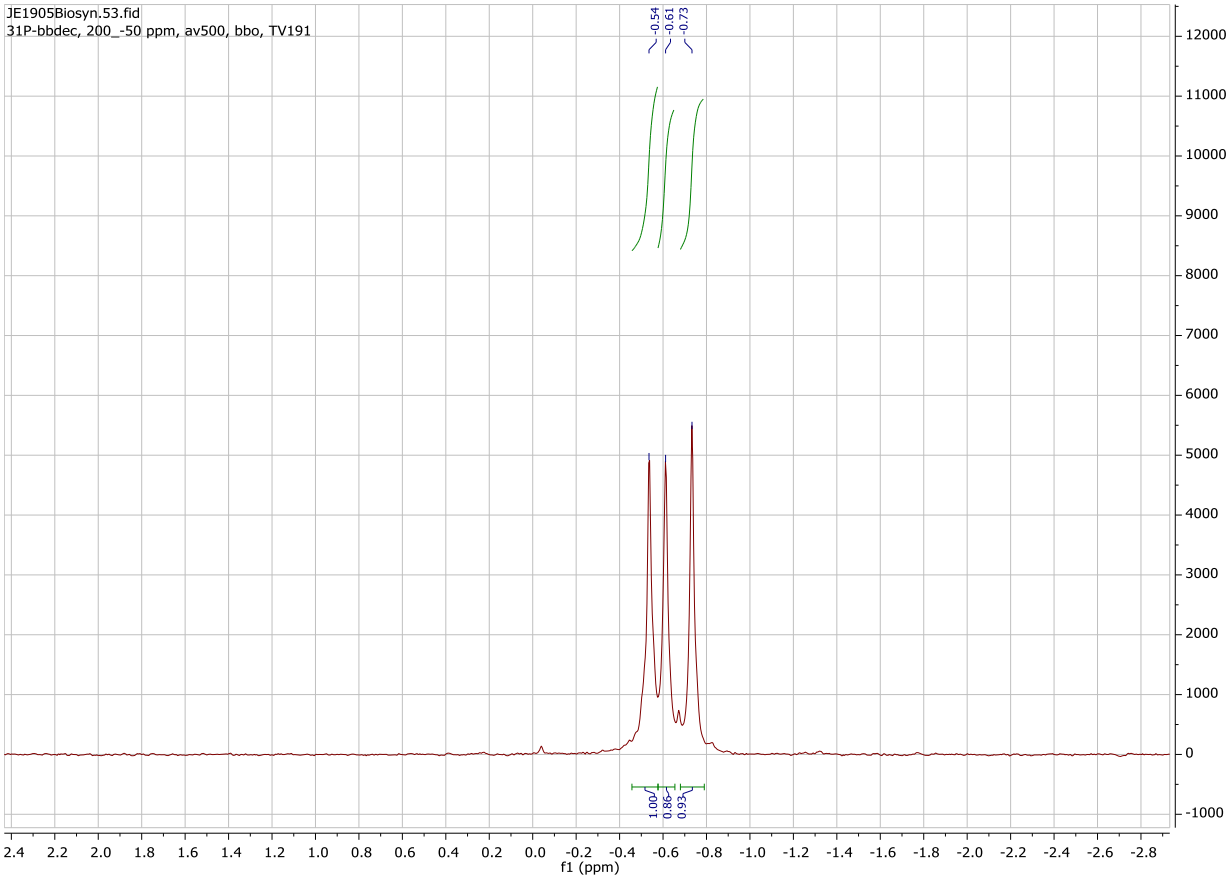
1-*O*-tri-(2-Acetamido-2-deoxy-3-*O*-acetyl-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)-6-hexyl- amine

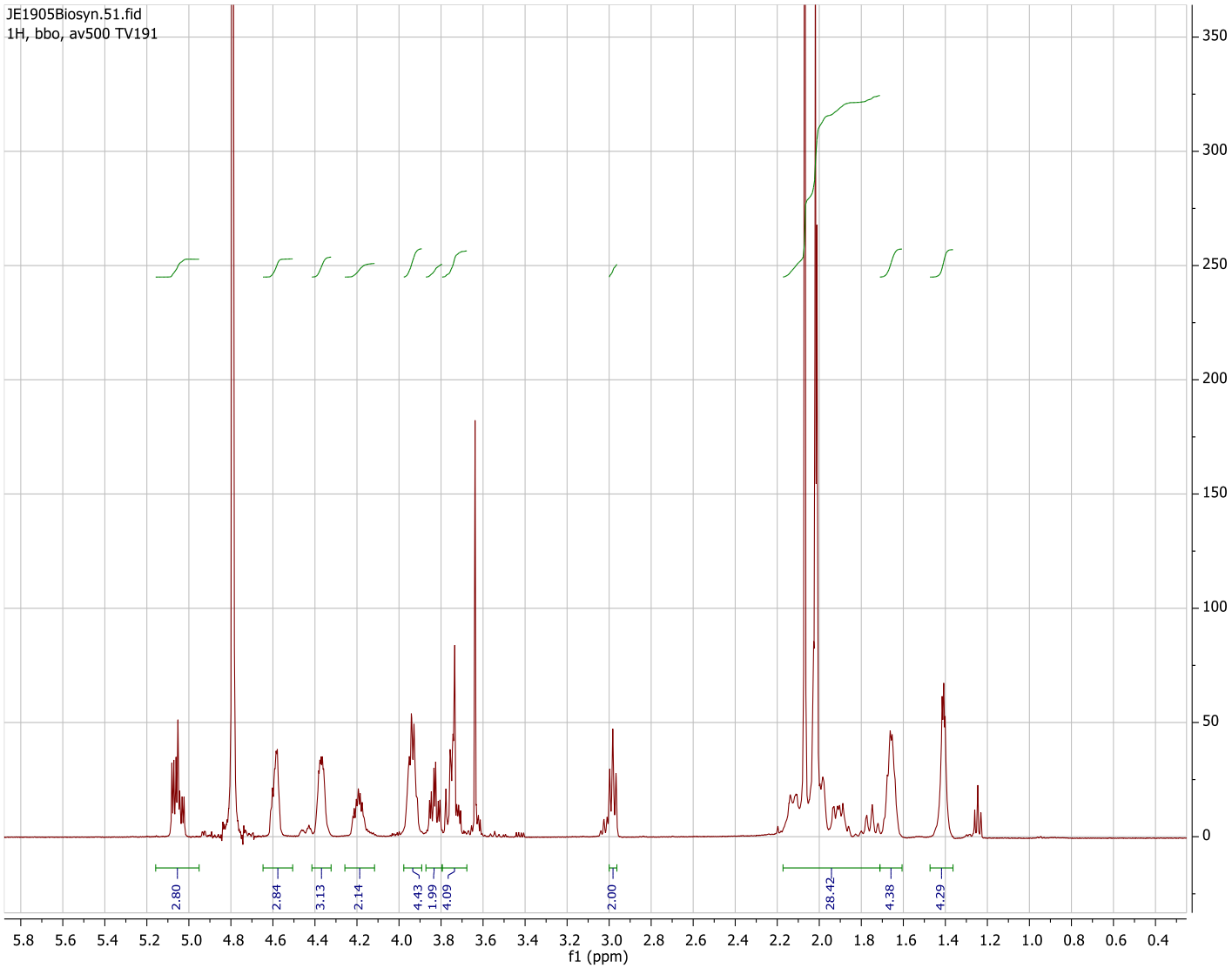
Trimer **6** (29.0mg, 16.0µmol) was deprotected according to the general procedure to obtain the corresponding deprotected trimer (18.0mg, 86%).

^{1}H NMR (500MHz, Deuterium Oxide) δ 5.08-5.01 (3H, m), 4.62-4.53 (3H, m), 4.40-4.34 (3H, m), 4.23-4.16 (2H, m), 3.99-3.88 (4H, m), 3.87-3.79 (2H, m), 3.78-3.69 (3H,m), 2.97 (2H, d, J 7.6 Hz), 2.19-1.87 (27H, m), 1.69-1.60 (4H,m), 1.45-1.37 (4H, m).

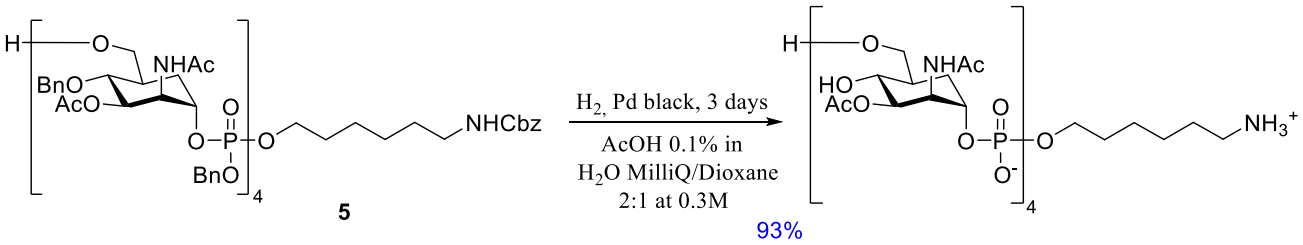
^{13}C NMR (126MHz, D_2O) δ 174.5, 173.4, 73.8, 72.1, 67.5, 66.6, 66.5, 61.7, 51.0, 39.4, 38.8, 37.7, 29.4, 28.1, 26.6, 25.1, 24.4, 21.9, 20.4.

^{31}P NMR (202 MHz, D_2O) δ -0.54, -0.61, -0.73.





^{1}H NMR (500MHz, Deuterium Oxide) δ 5.08-5.01 (3H, m), 4.62-4.53 (3H, m), 4.40-4.34 (3H, m), 4.23-4.16 (2H, m), 3.99-3.88 (4H, m), 3.87-3.79 (2H, m), 3.78-3.69 (3H,m), 2.97 (2H, d, J 7.6 Hz), 2.19-1.87 (27H, m), 1.69-1.60 (4H,m), 1.45-1.37 (4H, m).



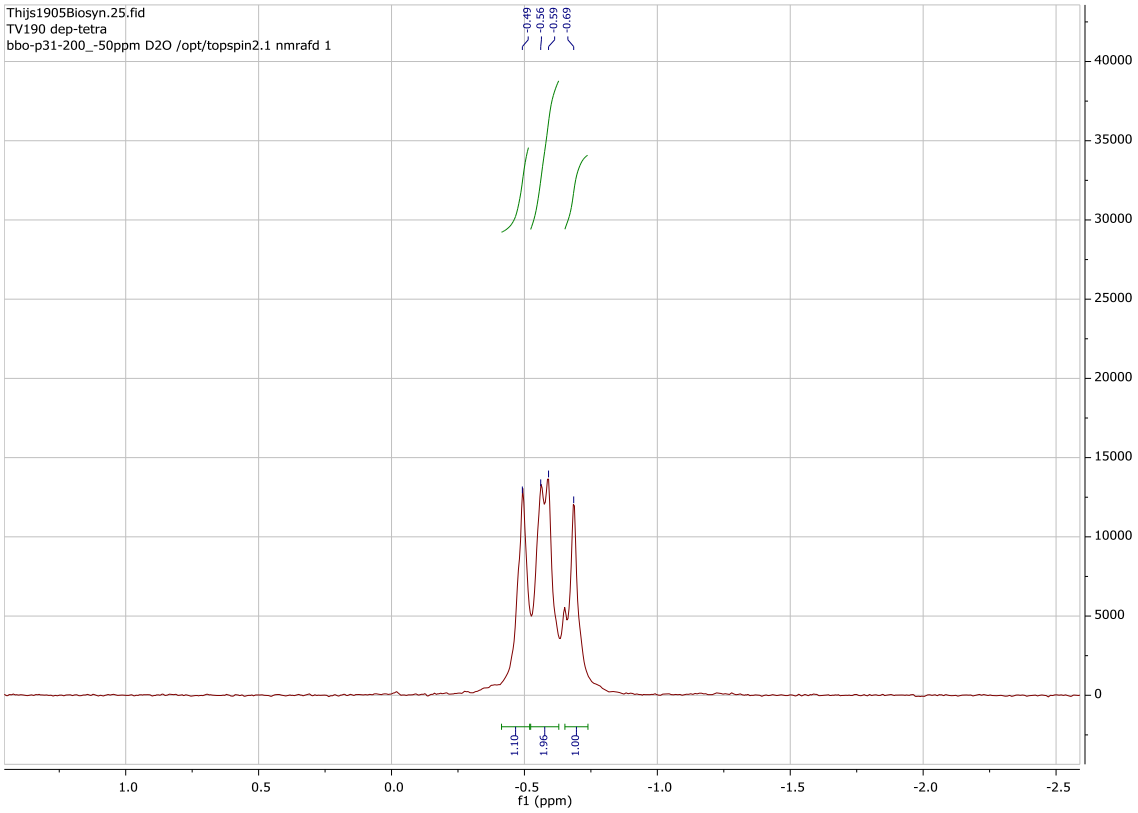
1-*O*-tetra-(2-Acetamido-2-deoxy-3-*O*-acetyl-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)-6-hexyl- amine

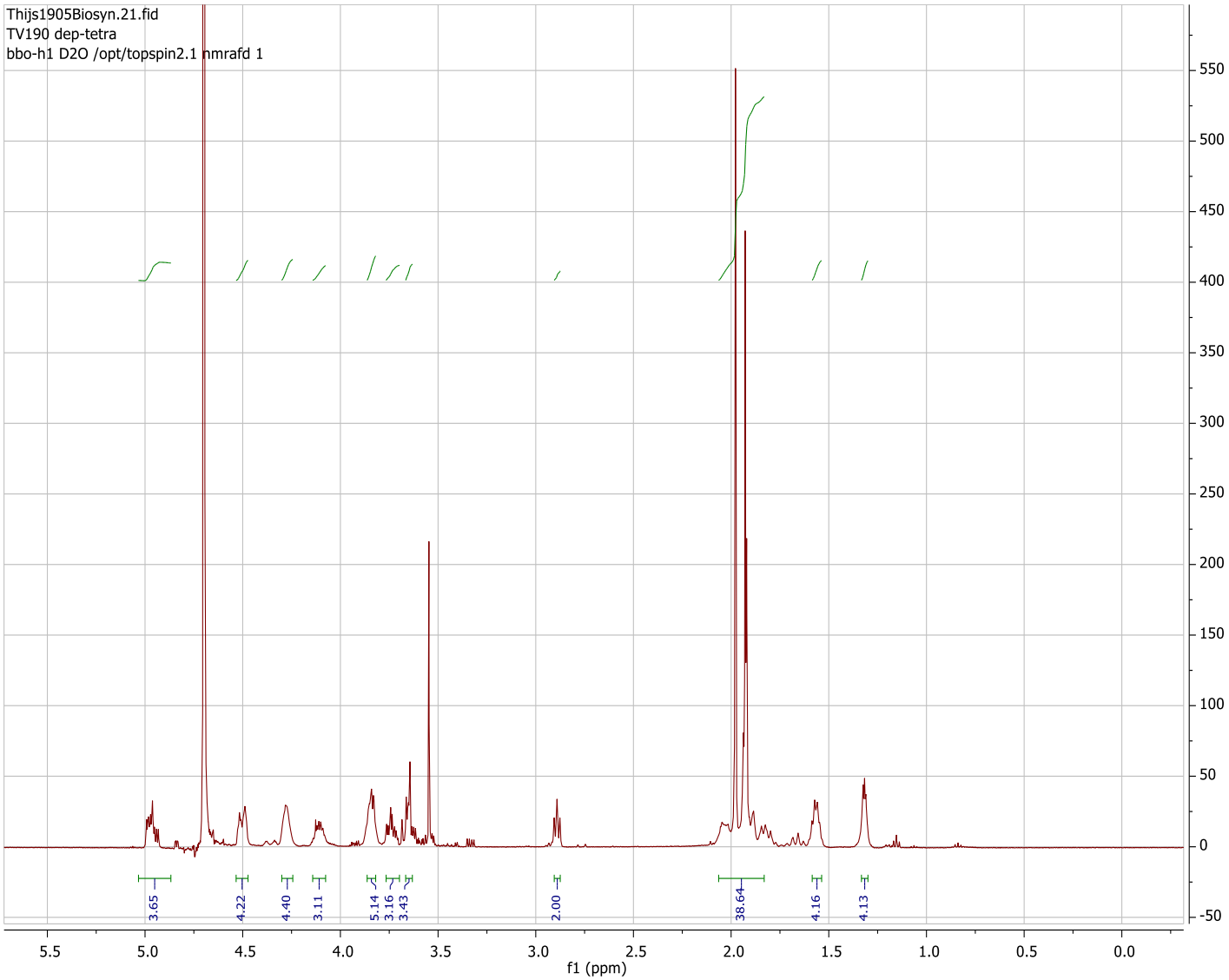
Tetramer **5** (26.0mg, 11.1µmol) was deprotected according to the general procedure to obtain the corresponding deprotected tetramer (15.8mg, 93%).

^{1}H NMR (500MHz, Deuterium Oxide) δ 5.02-4.89 (4H, m, 4xH-3), 4.52-4.45 (4H, m, 4xH-2), 4.32-4.24 (4H, m, 4xH-1), 4.17-4.04 (3H, m, 3xH6), 3.89-3.79 (5H, m, 3xH-6, $CH_{2\ spacer}$), 3.79-3.71 (3H, m, 3xH-4), 3.71-3.60 (3H, m, H-4, H-6), 2.88 (2H, t, J 7.5 Hz, $CH_{2\ spacer}$), 2.09-1.72 (36H, m, 8xCH_3, 4xH-7, 4xH-5), 1.59-1.55 (4H, m, 2x$CH_{2\ spacer}$), 1.33-1.30 (4H, m, 2x$CH_{2\ spacer}$).

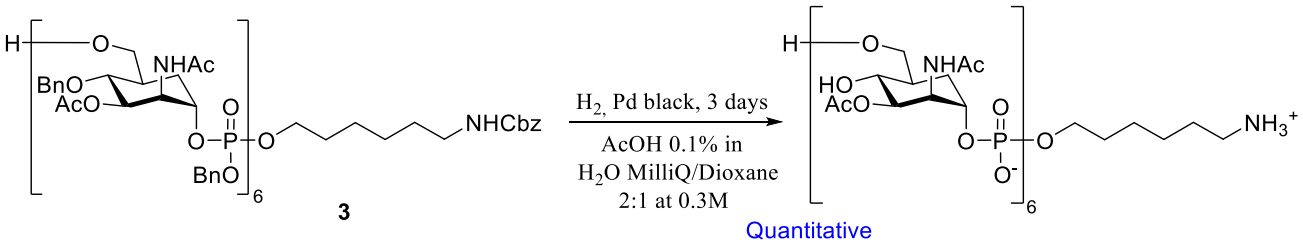
^{13}C NMR (126MHz, D_2O) δ 174.5 ($C_{\ acetyl}$), 173.4 ($C_{\ acetyl}$), 73.9 (C-3), 72.2 (C-1), 67.5 (C-4), 66.6 ($CH_{2\ spacer}$), 66.5 (C-4), 65.4 (C-6), 61.7 (C-6), 51.0 (C-2), 39.5 ($CH_{2\ spacer}$), 38.8 (C-5), 37.7 (C-5), 29.5 ($CH_{2\ spacer}$), 28.06 (C-7), 26.6 ($CH_{2\ spacer}$), 25.1 ($CH_{2\ spacer}$), 24.5 ($CH_{2\ spacer}$), 21.9 ($CH_{3\ acetyl}$), 20.5 ($CH_{3\ acetyl}$).

^{31}P NMR (202 MHz, D_2O) δ -0.43, -0.47, -0.58., -0.69





^{1}H NMR (500MHz, Deuterium Oxide) δ 5.02-4.89 (4H, m, 4xH-3), 4.52-4.45 (4H, m, 4xH-2), 4.32-4.24 (4H, m, 4xH-1), 4.17-4.04 (3H, m, 3xH6), 3.89-3.79 (5H, m, 3xH-6, $CH_{2\ spacer}$), 3.79-3.71 (3H, m, 3xH-4), 3.71-3.60 (3H, m, H-4, H-6), 2.88 (2H, t, J 7.5 Hz, $CH_{2\ spacer}$), 2.09-1.72 (36H, m, 8xCH_3, 4xH-7, 4xH-5), 1.59-1.55 (4H, m, 2x$CH_{2\ spacer}$), 1.33-1.30 (4H, m, 2x$CH_{2\ spacer}$).



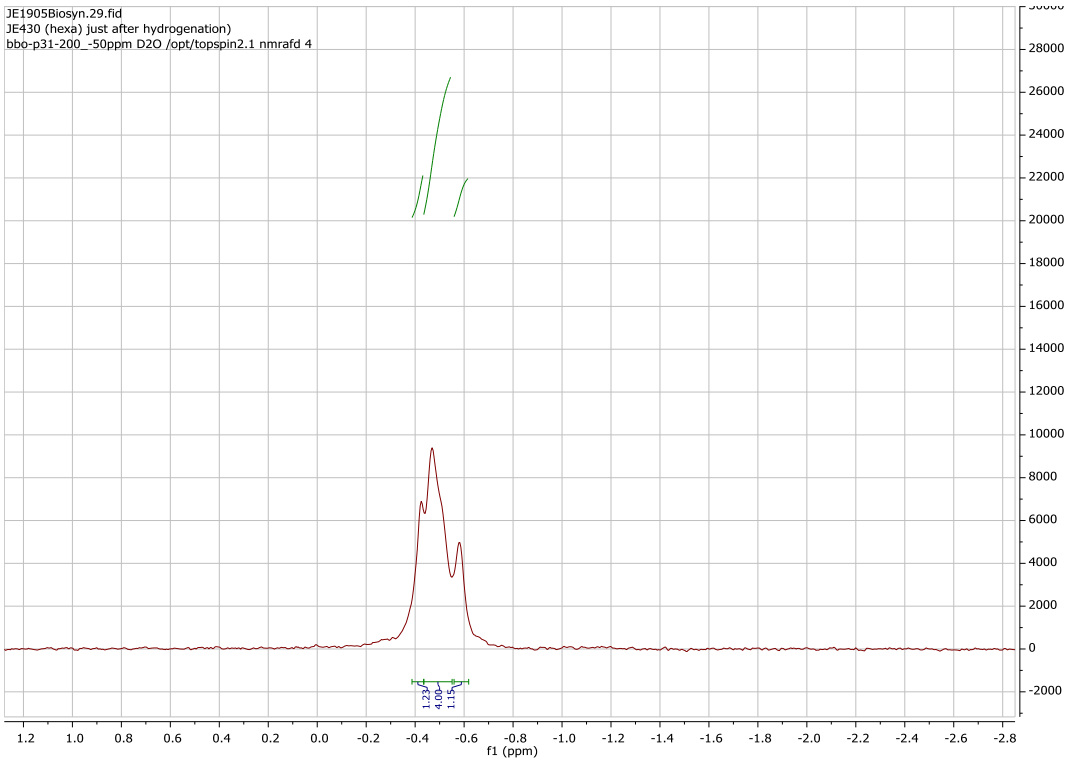
1-*O*-hexa-(2-Acetamido-2-deoxy-3-*O*-acetyl-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)-6-hexyl- amine

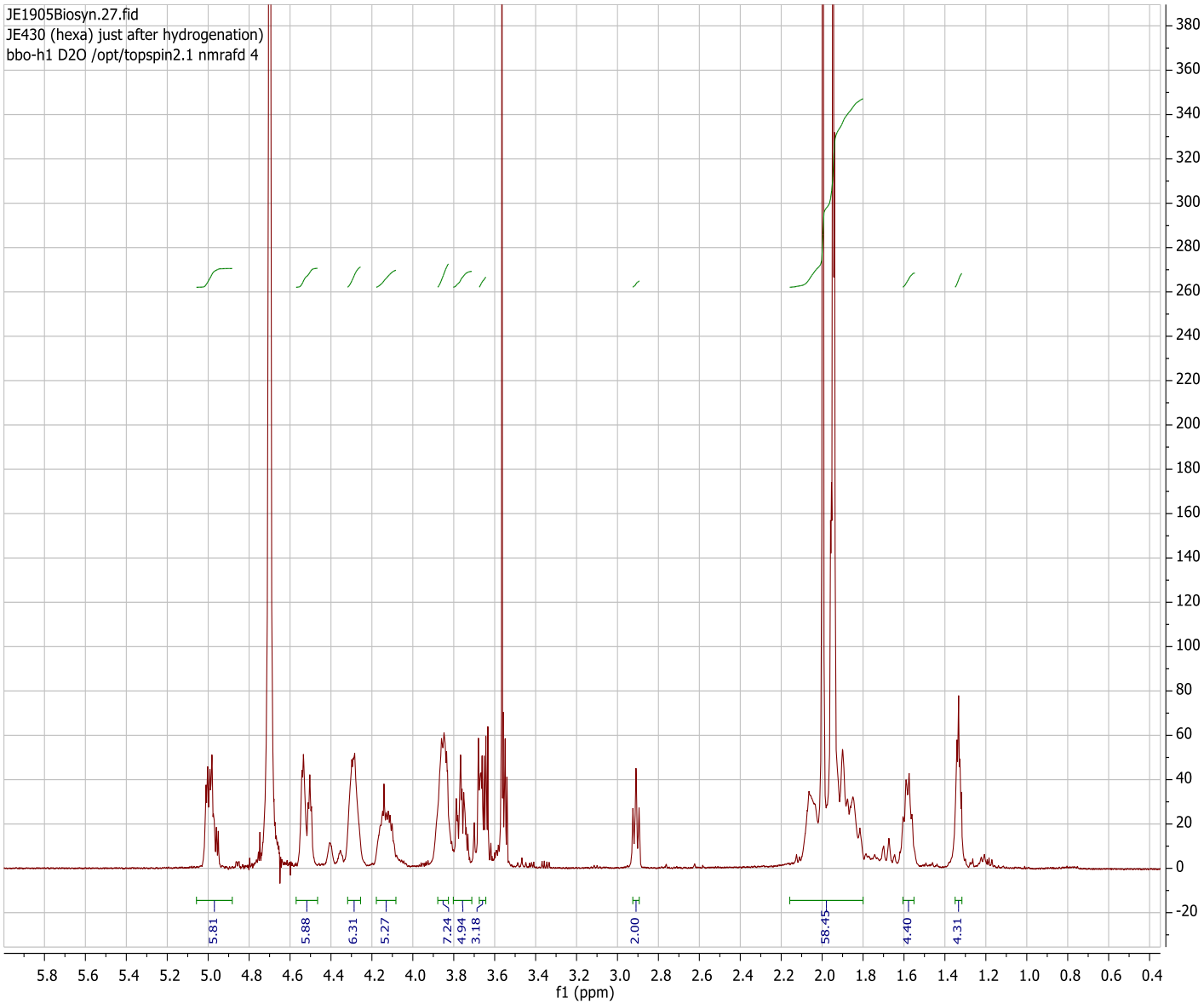
Hexamer **3** (9.0mg, 2.9µmol) was deprotected according to the general procedure to obtain the corresponding deprotected hexamer (6 .0mg, Quantitative).

^{1}H NMR (500MHz, Deuterium Oxide) δ 5.06-4.92 (6H, m, 6xH-3), 4.56-4.45 (6H, m, H-2), 4.34-4.25 (6H, m, H-1), 4.17-4.06 (5H, m, 5xH-6), 3.93-3.82 (7H, m, 5xH-6, $CH_{2\ spacer}$), 3.80-3.70 (5H, m, 5xH-4), 3.68-3.63 (3H, m, H-4, H-6), 2.91 (2H, t, J 7.5 Hz, $CH_{2\ spacer}$), 2.14-1.74 (54H, m, 12x$CH_{3\ acetyl}$, 6xH-5, 6xH-7), 1.61-1.54 (4H, m, 2x$CH_{2spacer}$), 1.39-1.28 (4H, m, 2x$CH_{2\ spacer}$).

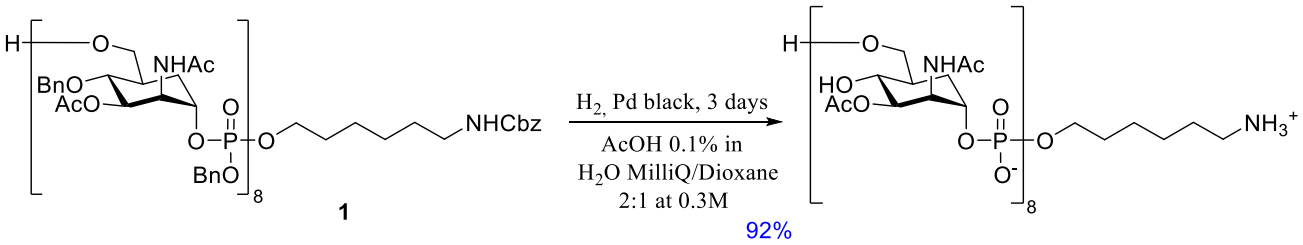
^{13}C NMR (126MHz, D_2O) δ 174.5 ($C_{\ acetyl}$), 173.4 ($C_{\ acetyl}$), 73.9 (C-3), 72.2 (C-1), 67.6 (C-4), 66.8 ($CH_{2\ spacer}$), 66.6 (C-4), 65.3 (C-6), 50.9 (C-1), 39.5 ($CH_{2spacer}$), 37.7 (C-5), 29.4 ($CH_{2spacer}$), 28.1 (C-7), 26.6 ($CH_{2\ spacer}$), 25.2 ($CH_{2\ spacer}$), 24.5 ($CH_{2\ spacer}$), 21.9 ($CH_{3\ acetyl}$), 20.5 ($CH_{3\ acetyl}$).

^{31}P NMR (202 MHz, D_2O) δ -0.43, -0.47, -0.58.





^{1}H NMR (500MHz, Deuterium Oxide) δ 5.06-4.92 (6H, m, 6xH-3), 4.56-4.45 (6H, m, H-2), 4.34-4.25 (6H, m, H-1), 4.17-4.06 (5H, m, 5xH-6), 3.93-3.82 (7H, m, 5xH-6, $CH_{2\ spacer}$), 3.80-3.70 (5H, m, 5xH-4), 3.68-3.63 (3H, m, H-4, H-6), 2.91 (2H, t, J 7.5 Hz, $CH_{2\ spacer}$), 2.14-1.74 (54H, m, 12x$CH_{3\ acetyl}$, 6xH-5, 6xH-7), 1.61-1.54 (4H, m, 2x$CH_{2spacer}$), 1.39-1.28 (4H, m, 2x$CH_{2\ spacer}$).



1-*O*-octa-(2-Acetamido-2-deoxy-3-*O*-acetyl-7-carba-α-D-mannopyranosyl-1-*O*-phosphoryl)-6-hexyl- amine

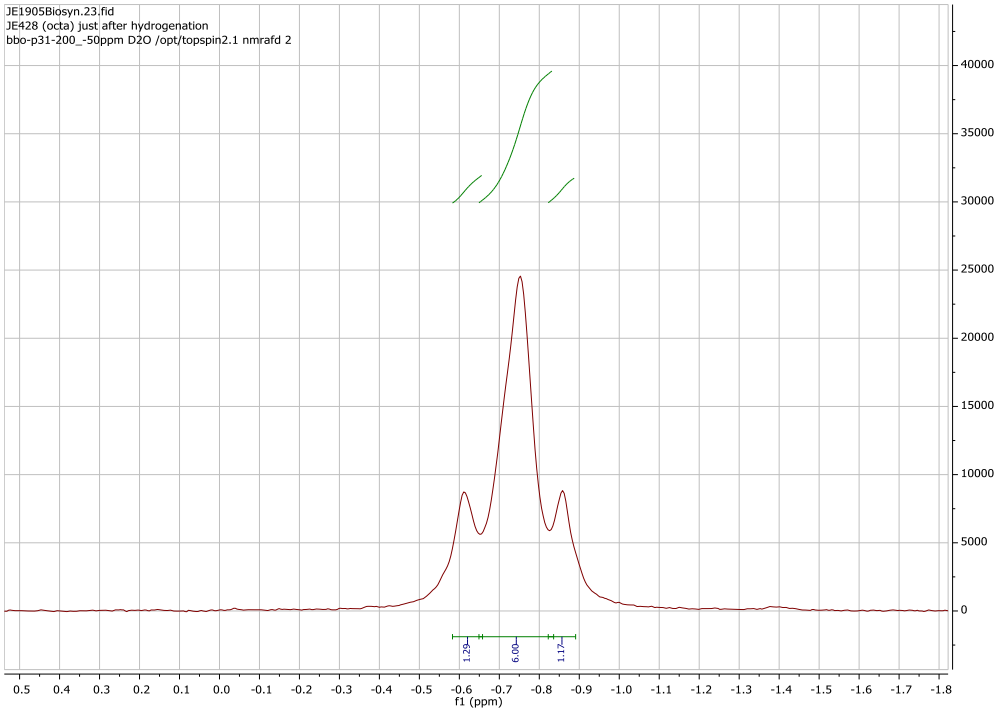
Octamer **1** (6.70mg, 2.70µmol) was deprotected according to the general procedure to obtain the corresponding deprotected octamer (6.10mg, 92%).

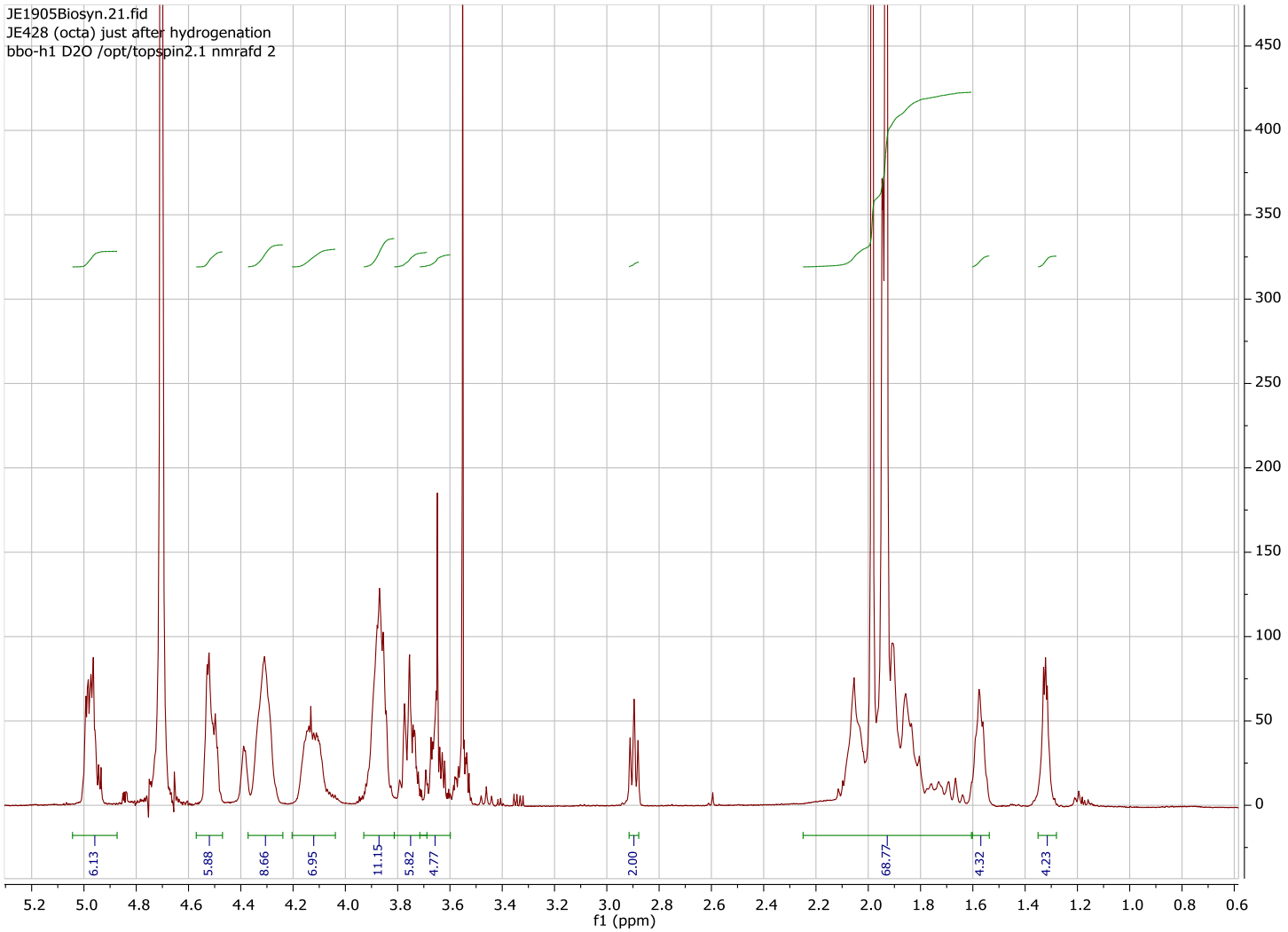
^{1}H NMR (500MHz, Deuterium Oxide) δ 5.15-4.99 (8H, m, 8xH-3), 4.65-4.54 (8H, m, 8xH-2), 4.49-4.34 (8H, m, 8xH-1), 4.28-4.16 (7H, m, 7xH-6), 3.99-3.91 (9H, m, 7xH-6, $CH_{2\ spacer}$), 3.90-3.79 (1H, m, 6xH-4), 3.79-3.69 (1H, m, 2xH-4, H-6), 2.98 (2H, t, J 7.6Hz, $CH_{2\ spacer}$), 2.14 (8H, m, H-5), 2.09-2.05 (24H, m, 8x$CH_{3\ acetyl}$), 2.04-2.00 (32H, m, H-7, 8x$CH_{3\ acetyl}$), 1.96-1.89 (8H, m, H-7), 1.71-1.62 (4H, m, 2x$CH_{2\ spacer}$), 1.46-1.35 (4H, m, 2x$CH_{2\ spacer}$).

^{13}C NMR (126MHz, D_2O) δ 174.5 ($CH_{\ acetyl}$), 173.4 ($CH_{\ acetyl}$), 73.7 (C-3), 72.4 (C-1), 66.8 ($CH_{2\ scpacer}$), 66.5 (C-4), 65.6 (C-6), 61.7 (C-6), 50.9 (C-2), 39.4 ($CH_{2\ spacer}$), 37.6 (C-5), 29.4($CH_{2\ spacer}$), 28.1 (C-7), 26.6 ($CH_{2\ spacer}$), 25.1 ($CH_{2\ spacer}$), 24.5 ($CH_{2\ spacer}$), 21.9 ($CH_{3\ acetyl}$), 20.5 ($CH_{3\ acetyl}$).

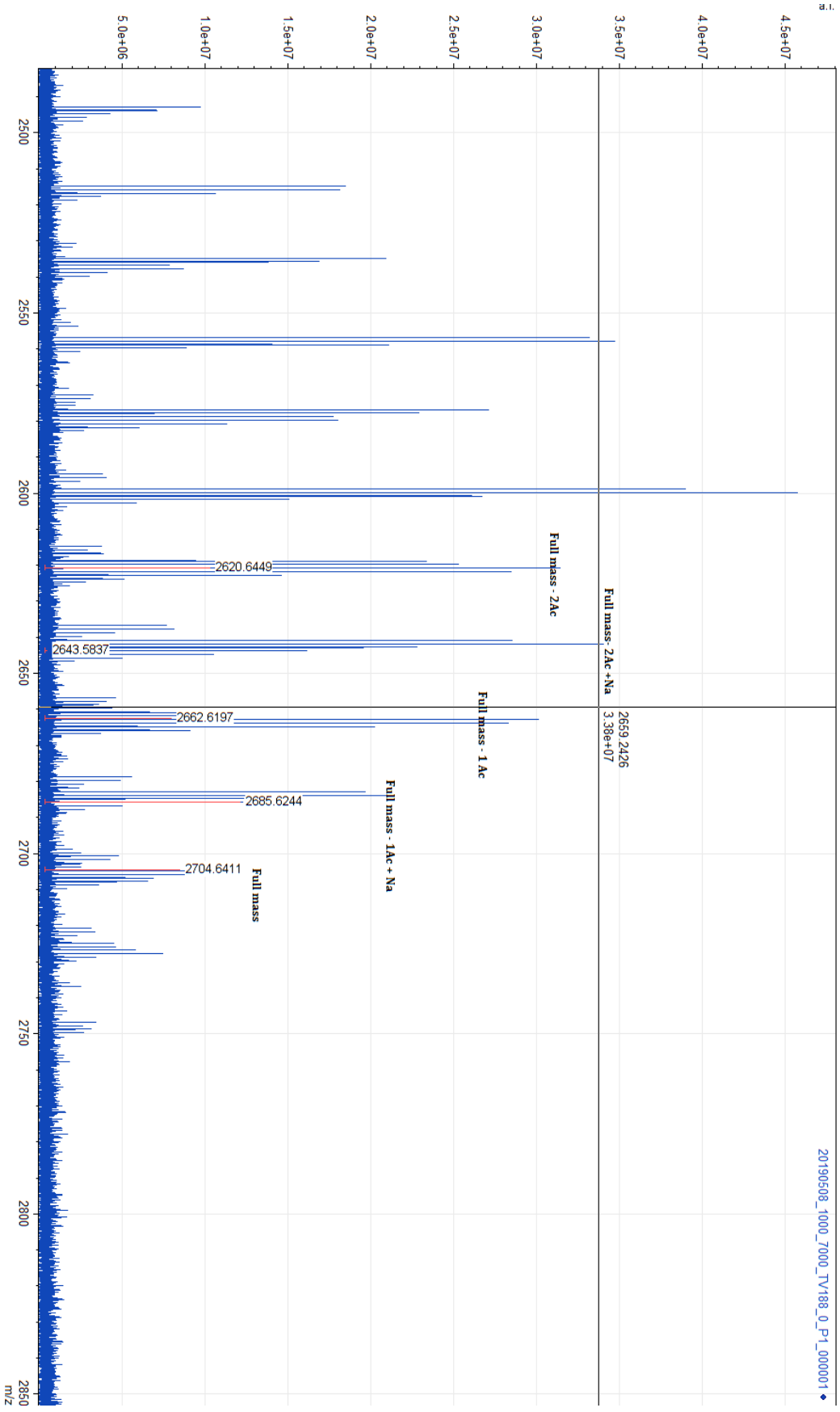
^{31}P NMR (202 MHz, D_2O) δ 0.85, 0.72, 0.62.

[MALDI]: $[C_{94}H_{159}N_9O_{65}P_8+H]^+$ required 2704.09, founded 2704.64





^{1}H NMR (500MHz, Deuterium Oxide) δ 5.15-4.99 (8H, m, 8xH-3), 4.65-4.54 (8H, m, 8xH-2), 4.49-4.34 (8H, m, 8xH-1), 4.28-4.16 (7H, m, 7xH-6), 3.99-3.91 (9H, m, 7xH-6, $CH_{2\ spacer}$), 3.90-3.79 (1H, m, 6xH-4), 3.79-3.69 (1H, m, 2xH-4, H-6), 2.98 (2H, t, J 7.6Hz, $CH_{2\ spacer}$), 2.14 (8H, m, H-5), 2.09-2.05 (24H, m, 8x$CH_{3\ acetyl}$), 2.04-2.00 (32H, m, H-7, 8x$CH_{3\ acetyl}$), 1.96-1.89 (8H, m, H-7), 1.71-1.62 (4H, m, 2x$CH_{2\ spacer}$), 1.46-1.35 (4H, m, 2x$CH_{2\ spacer}$).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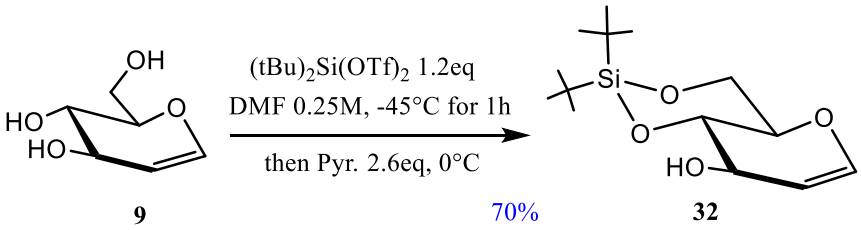


9 70% **32**

4,6-*O*-(di*tert*butylsilylidene)-D-glucal (32)

D-glucal (1.0g, 3.67mmol) was dissolved in dry DMF (15mL) and cooled to -45°C/-50°C, *tert*-$Bu_2Si(OTf)_2$ (1.44mL, 4.40mmol) was slowly added. The solution was stirred at -45°C for 1h and then anhydrous pyridine (772µL, 9.55mmol) was added. The mixture was warmed to 0°C and a saturated solution of $NaHCO_3$ was added until pH=6. The mixture was diluted with Et_2O and washed with water (3times). The organic layer was dried over Na_2SO_4, filtered, and the solvent removed under vacuum. The resulted residue was purified by flash chromatography (nHex/EtOAc) to give **32** (732mg, 70%) as a white solid.

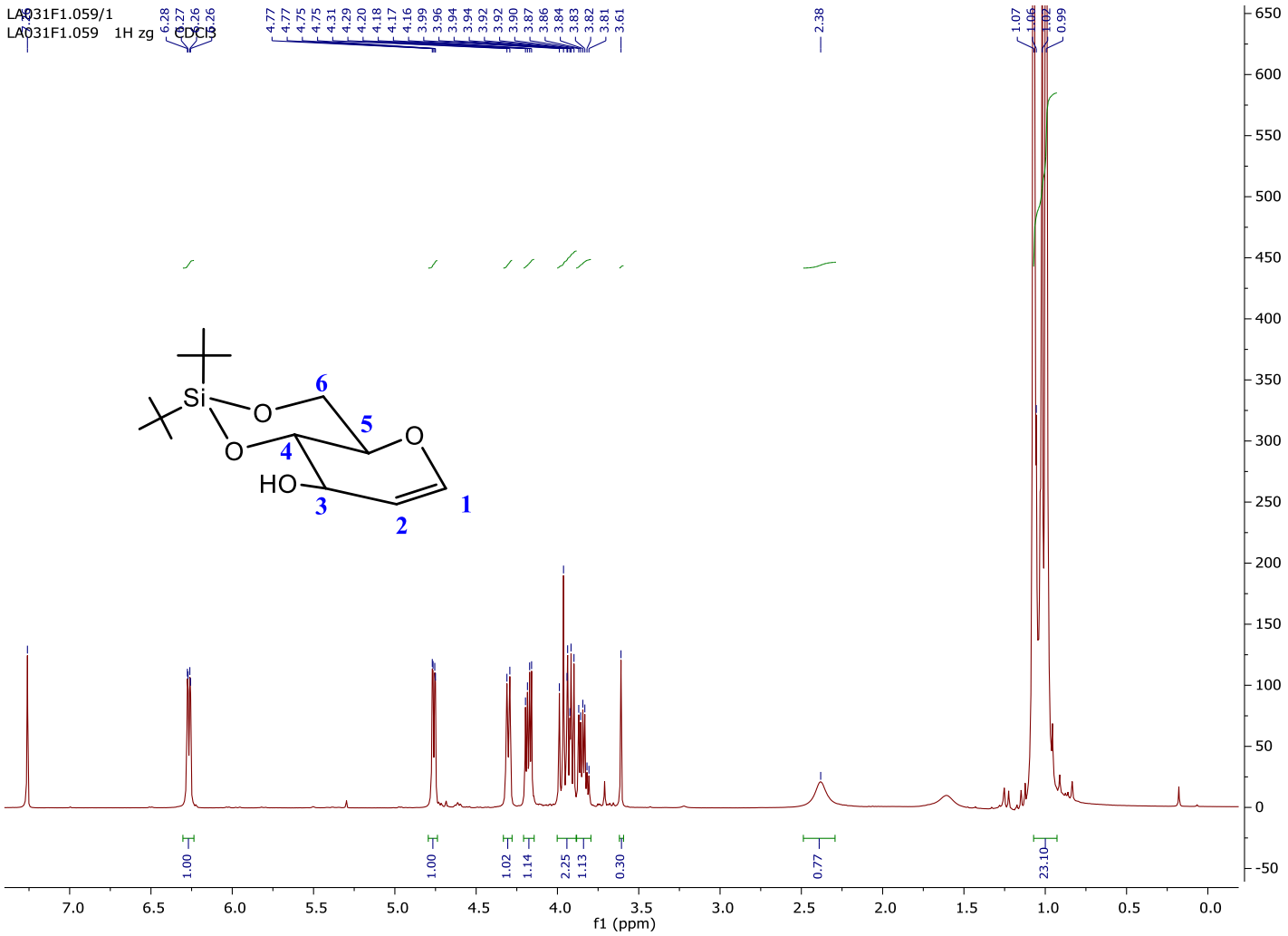
δ ^{1}H (400 MHz; CDCl3)

6.27 (1H, dd, J 6.0, J 1.6, 1-H), 4.76 (1H, dd, J 6.6, J 1.7, 2-H), 4.30 (1H, d, J 7.2, 3-H), 4.18 (1H, dd, J 10.2, J 4.8, 6-H), 4.00-3.89 (2H, m, 6-H, 4-H), 3.84 (1H, dt, J 10.2, J 4.8, 5-H), 2.38 (1H, br s, -OH), 1.10-0.97 (18H, m, SitBuCH$_3$).

δ ^{13}C (100 MHz; CDCl3)

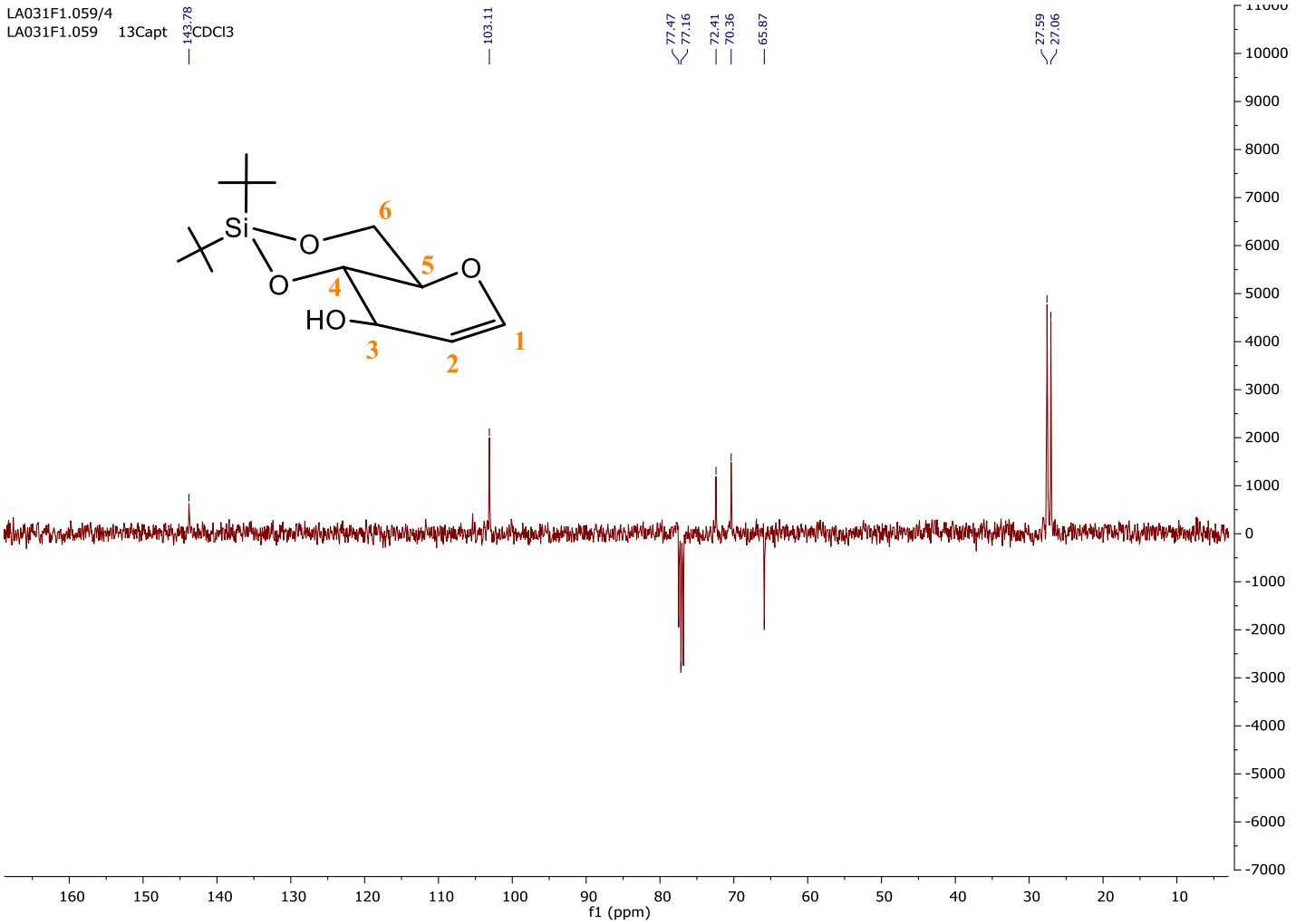
143.8 (1-C), 103.1 (2-C), 77.5 (4-C), 72.4 (5-C), 70.4 (3-C), 65.9 (6-C), 28.0-27.1 (SitBuCH$_3$).

4,6-*O*-(di*tert*butylsilylidene)-D-glucal **(32)**



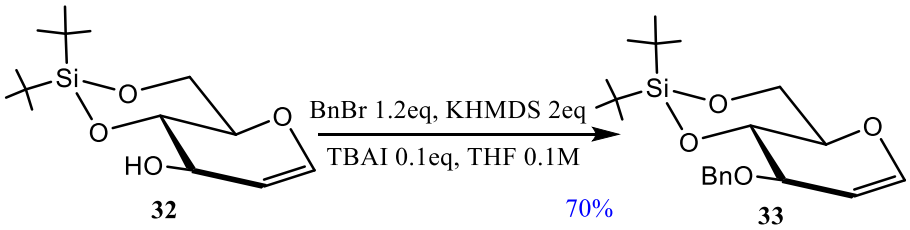
δ ^{1}H (400 MHz; CDCl3)

6.27 (1H, dd, J 6.0, J 1.6, 1-H), 4.76 (1H, dd, J 6.6, J 1.7, 2-H), 4.30 (1H, d, J 7.2, 3-H), 4.18 (1H, dd, J 10.2, J 4.8, 6-H), 4.00-3.89 (2H, m, 6-H, 4-H), 3.84 (1H, dt, J 10.2, J 4.8, 5-H), 2.38 (1H, br s, -OH), 1.10-0.97 (18H, m, SitBuCH$_3$).



δ ^{13}C (100 MHz; CDCl3)

143.8 (1-C), 103.1 (2-C), 77.5 (4-C), 72.4 (5-C), 70.4 (3-C), 65.9 (6-C), 28.0-27.1 ($SitBuCH_3$).



32 **33**

3-*O*-Benzyloxy-4,6-*O*-(di*tert*butylsilylidene)-D-glucal (33)

KHDMS (7.68mL, 0.5M in Toluene, 3.84mmol) was added to a solution of the alcohol **32** (550mg, 1.92mmol) in dry THF (18mL) at -78°C over the course of 30 minutes. Benzyl bromide (279µL, 2.30mmol) and TBAI (75mg, 0.2mmol) were then added at -78°C and the resulting mixture was warmed to 25°C and stirred until completion. The reaction mixture was poured into brine, washed with NH_4Cl and extracted 3 times with EtOAc. The combined organic layers were then washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resultant residue was purified by flash chromatography (nHex/EtOAc) to give the benzyl protected compound **33** (506mg, 70% yield) as a white solid.

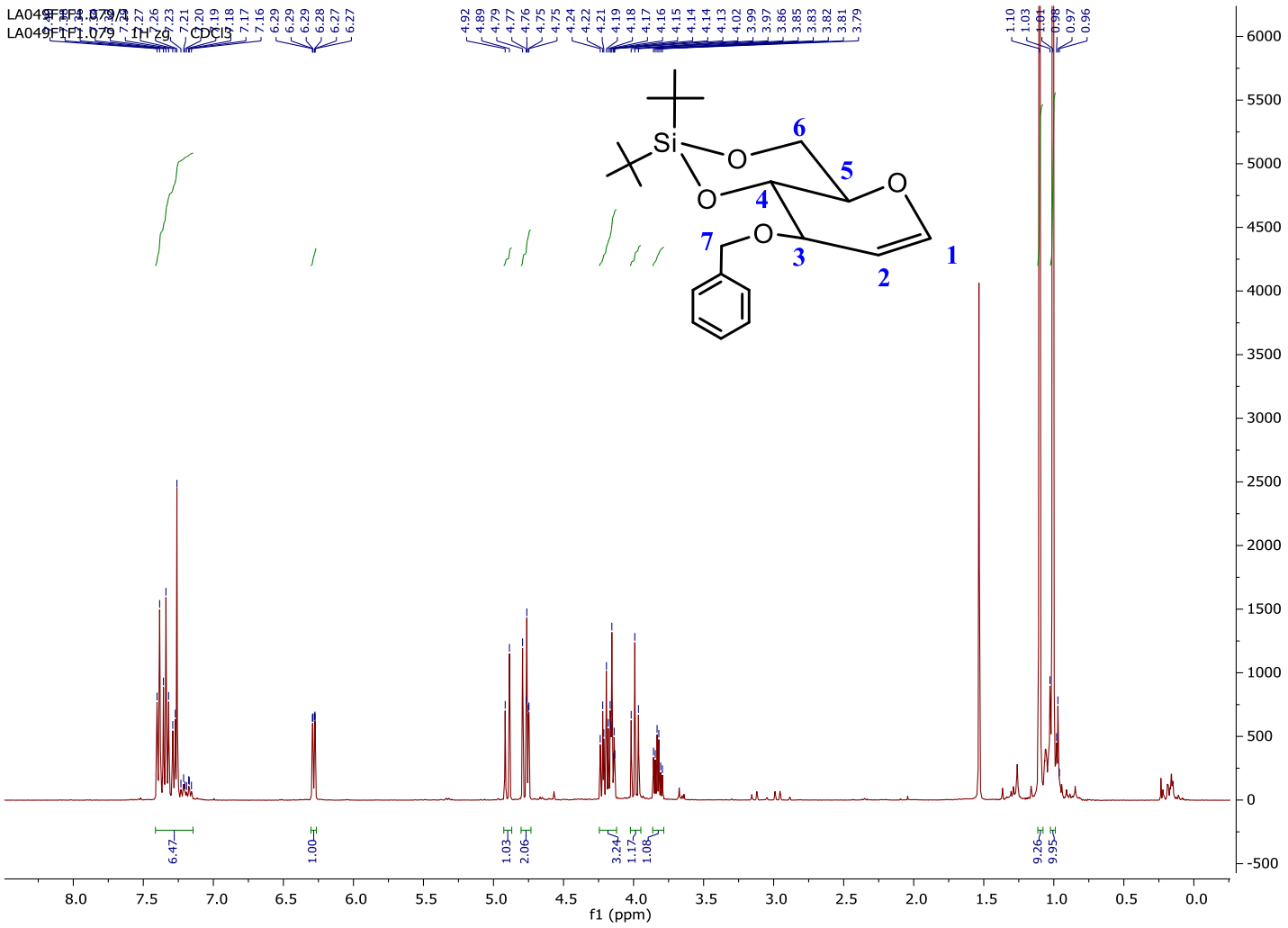
δ ^{1}H (400 MHz; CD_3OD)

7.42 – 7.15 (5H, m, H_{arom}), 6.30 – 6.27 (1H, m, 1-H), 4.90 (1H, d, J 12.2, 7-H), 4.78 (1H, d, J 12.1, 7-H), 4.76 (1H, dd, J 6.05, J 1.97, 1H, 2-H), 4.25 – 4.13 (3H, m, 3-H, 4-H, 6-H), 3.99 (1H, t, J 10.4, 1H, 6-H), 3.83 (1H, td, J 10.3, J 5.0, 5-H), 1.10 (s, 6H, SitBuCH_3), 1.01 (s, 6H, SitBuCH_3).

δ ^{13}C (100 MHz; CD_3OD)

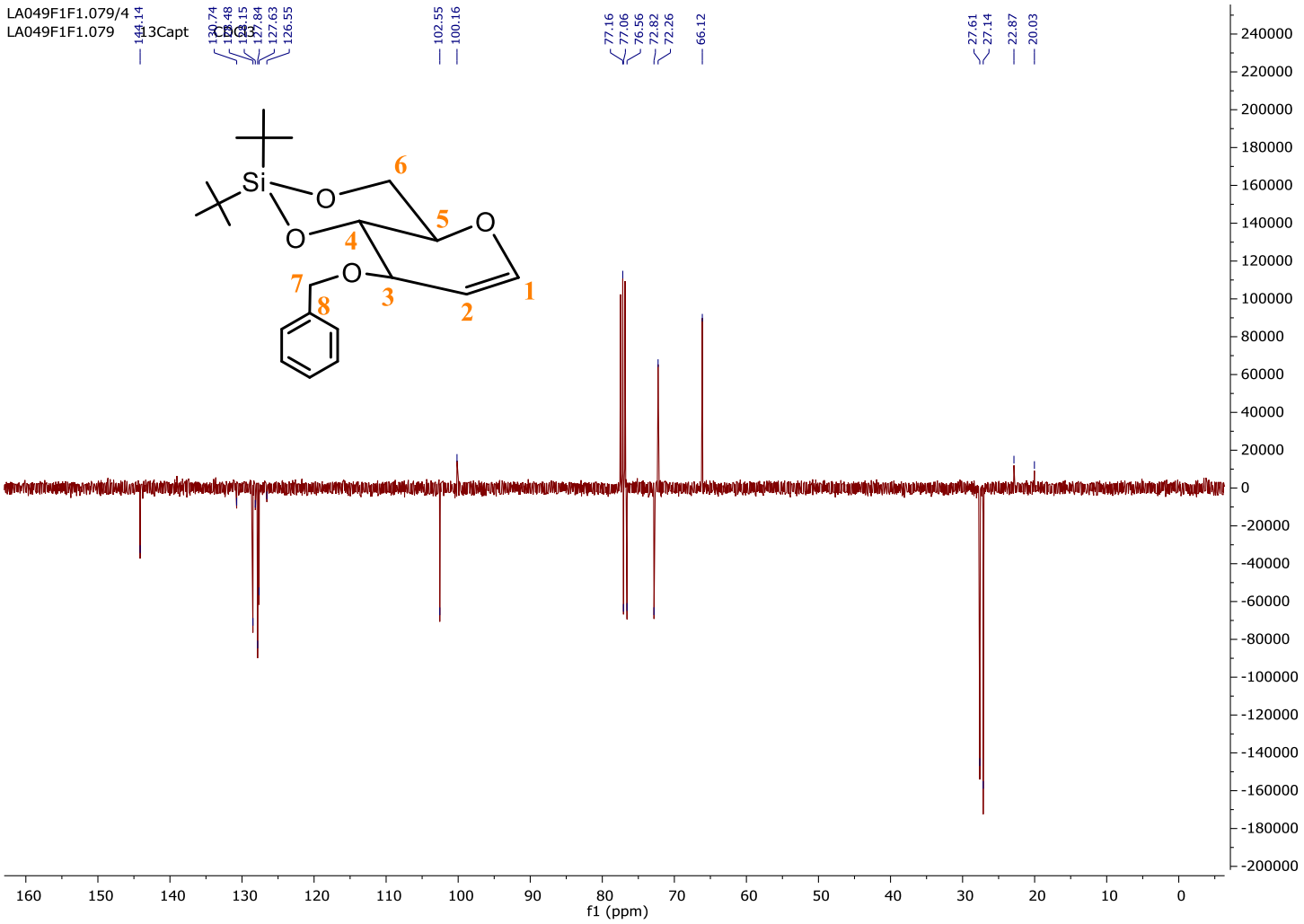
144.1 (1-C), 128.5-127.6 (H_{arom}), 102.6 (2-C), 100.2 (8-C), 77.1 (3-C), 76.6 (4-C), 72.8 (5-C), 72.3 (7-C), 66.1 (6-C), 27.6-27.1 (SitBuCH_3), 22.9-20.0 (SitBuC)

3-*O*-Benzyloxy-4,6-*O*-(di*tert*butylsilylidene)-D-glucal **(33)**



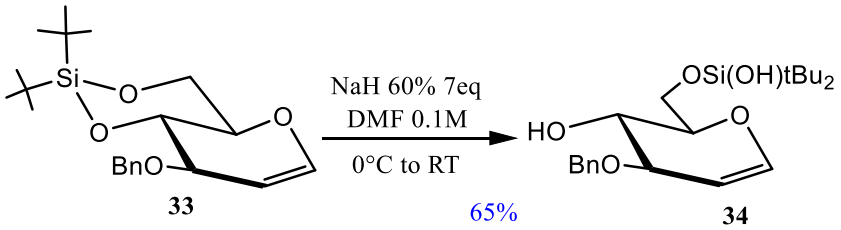
δ ^{1}H (400 MHz; CD_3OD)

7.42 – 7.15 (5H, m, H_{arom}), 6.30 – 6.27 (1H, m, 1-H), 4.90 (1H, d, J 12.2, 7-H), 4.78 (1H, d, J 12.1, 7-H), 4.76 (1H, dd, J 6.05, J 1.97, 1H, 2-H), 4.25 – 4.13 (3H, m, 3-H, 4-H, 6-H), 3.99 (1H, t, J 10.4, 1H, 6-H), 3.83 (1H, td, J 10.3, J 5.0, 5-H), 1.10 (s, 6H, SitBuCH_3), 1.01 (s, 6H, SitBuCH_3).



δ ^{13}C (100 MHz; CD_3OD)

144.1 (1-C), 128.5-127.6 (H_{arom}), 102.6 (2-C), 100.2 (8-C), 77.1 (3-C), 76.6 (4-C), 72.8 (5-C), 72.3 (7-C), 66.1 (6-C), 27.6-27.1 ($SitBuCH_3$), 22.9-20.0 (SitBuC)



33 **34**

3-*O*-Benzyloxy-6-*O*-(di*tert*butyl(hydroxy)silyl-D-glucal (34)

Compound **33** (104mg, 0.28mmol) was dissolved in dry DMF (2.75mL) at 0°C. NaH (77.1mg, 1.93mmol, 60% in mineral oil) was added. The reaction was stirred for 45 min and allowed to warm up to room temperature. Once completed and controlled by TLC, the reaction was quenched with methanol. The mixture was separated from DMF with Et_2O (3 times). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The crude residue was purified by flash chromatography (nHex/EtOAc) to afford compound **34** (70mg, 65% yield) as a transparent oil.

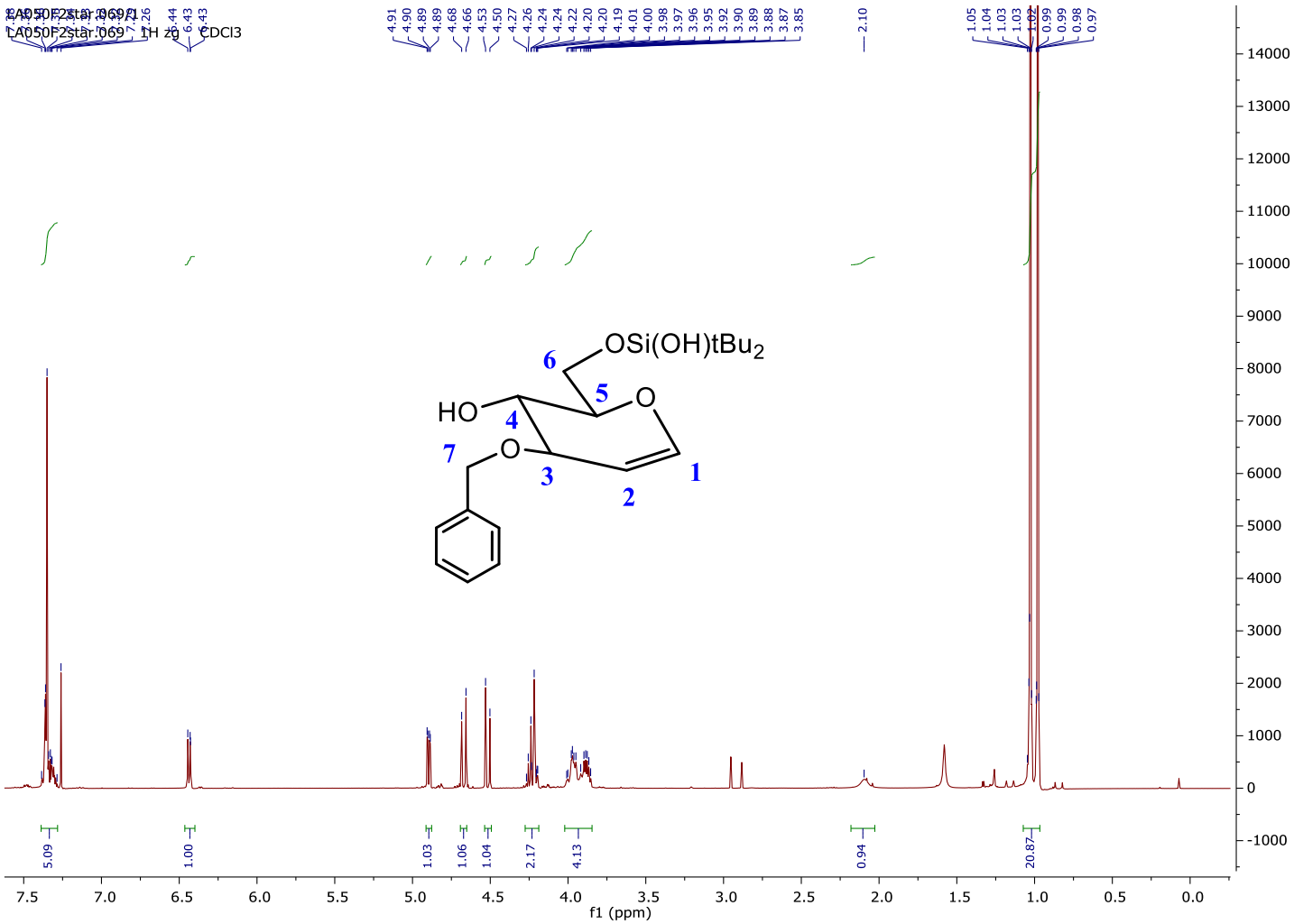
δ ^{1}H (400 MHz; CDCl3)

7.39-7.28 (5H, m, H_{arom}), 6.45-6.42 (1H, m, 1-H), 4.90 (1H, dd, J 6.1, J 2.0, 2-H), 4.67 (1H, d, J 11.2, 7-H), 4.52 (1H, d, J 11.2, 7-H),4.28-4.19 (2H, m, 3-H, 4-H), 4.02-3.85 (3H, m, 6-H, 5-H), 2.10 (1H, s, SiOH), 1.06-0.96 (18H, m, SitBuCH_3).

δ ^{13}C (100 MHz; CDCl3)

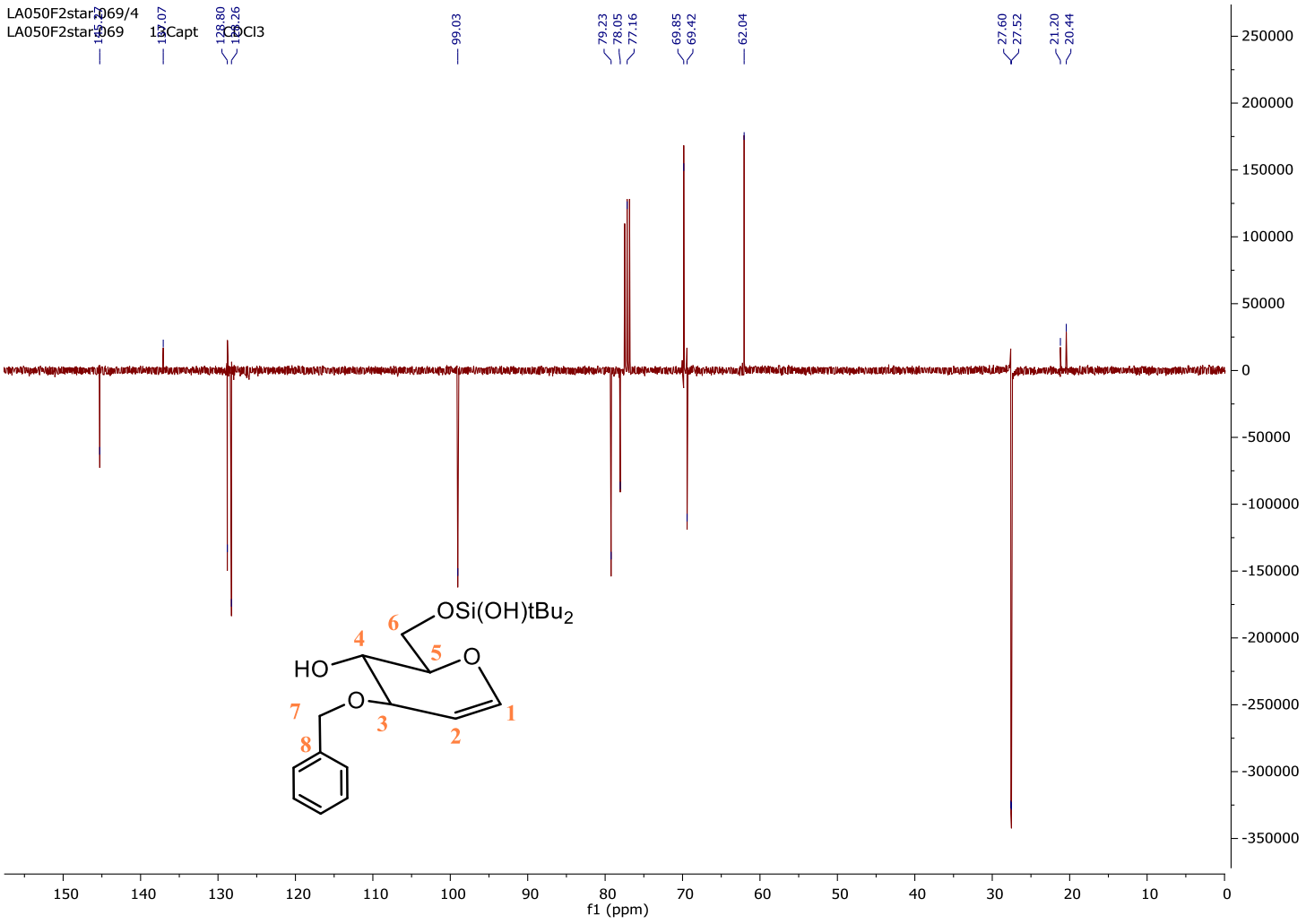
145.3 (1-C), 137.1 (8-C), 128.8-128.3 (C_{arom}), 99.0 (2-C), 79.2 (4-C), 78.1 (5-C), 69.9-69.4 (7-C), 62.0 (6-C), 27.6-27.5 (SitBuCH_3), 21.2-20.4 (SitBuC).

3-*O*-Benzyloxy-6-*O*-(di*tert*butyl(hydroxy)silyl-D-glucal **(34)**



δ ^{1}H (400 MHz; CDCl3)

7.39-7.28 (5H, m, H_{arom}), 6.45-6.42 (1H, m, 1-H), 4.90 (1H, dd, J 6.1, J 2.0, 2-H), 4.67 (1H, d, J 11.2, 7-H), 4.52 (1H, d, J 11.2, 7-H),4.28-4.19 (2H, m, 3-H, 4-H), 4.02-3.85 (3H, m, 6-H, 5-H), 2.10 (1H, s, SiOH), 1.06-0.96 (18H, m, SitBuCH_3).



δ ^{13}C (100 MHz; CDCl3)

145.3 (1-C), 137.1 (8-C), 128.8-128.3 (C_{arom}), 99.0 (2-C), 79.2 (4-C), 78.1 (5-C), 69.9-69.4 (7-C), 62.0 (6-C), 27.6-27.5 (SitBuCH_3), 21.2-20.4 (SitBuC).

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Abstract

Synthesis of *Neisseria meningitidis* serogroup A *carba* analogues as hydrolytically stable antigens for antimeningococcal glycoconjugate vaccines

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The Gram-negative encapsulated bacterium *Neisseria meningitidis* type A (MenA) is a major cause of meningitis in developing countries, especially in the sub-Saharan region of Africa [1]. The development and manufacture of an efficient glycoconjugate vaccine against MenA is largely hampered by the poor stability in water of the natural capsular polysaccharide (CPS)[2], composed of (1→6)-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units, with acetyl substituents. As a consequence, most of MenA glycoconjugates currently available have been licensed as lyophilisates. The availability of MenA polysaccharide mimics resistant to hydrolysis, however, is highly attractive for the development of a more stable glycoconjugate vaccine in liquid formulation. To this end, we envisaged that the replacement of the endocyclic ring oxygen with a methylene group to get a carbocyclic analogue will lead to the loss of the acetalic character of the phosphodiester and consequently to the enhancement of its chemical stability [3], [4]. Furthermore, the 3-*O*-acetylation aims to strengthen the immunological profile of our *neo*-glycoconjugates, structurally designed even closer to the natural CPS MenA oligomer, partially acetylated at the position 3-OH with a rate of 75-95% [5].

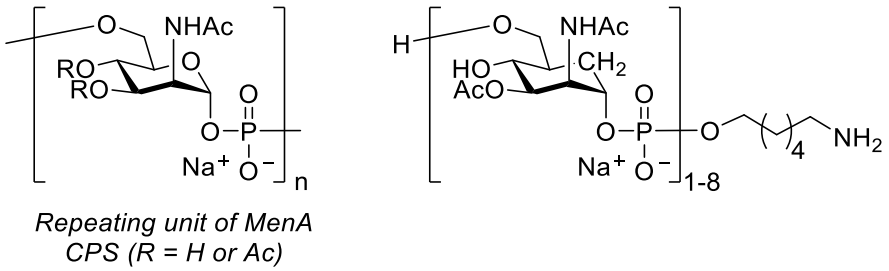

Figure 1. Structures of MenA CPS repeating unit and target carba-oligomers.

Thus, we describe our synthetic approaches to 3-*O*-acetylated phosphodiester-linked *carba* oligomers of *N*-acetyl mannosamine (the repeating unit of MenA CPS, fig. 1) containing up to 8 repeating units. The increased stability of the synthetic *carba* oligomers was first confirmed by an accelerated stability study, then selected fragments were conjugated to CRM_{197} (a

diphtheria toxin mutant) as a protein carrier. Finally, the immunological profile of the resulting *neo*-glycoconjugates will be carefully investigated, with the purpose to highlight the effect of the carbohydrate chain length and of the 3-*O*-acetylation on the immunoactivity [6].

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