EPISODES IN CARBOHYDRATE CHEMISTRY:

FROM β-LINKED MANNOSIDES TO GLYCOCONJUGATES

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To my family

Preface

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Filip Ekholm

Abstract

Carbohydrates are one of the most abundant classes of biomolecules on earth. In the initial stages of research on carbohydrates much effort was focused on investigation and determination of the structural aspects and complex nature of individual monosaccharides. Later on, development of protective group strategies and methods for oligosaccharide synthesis became the main topics of research. Today, the methodologies developed early on are being utilized in the production of carbohydrates for biological screening events. This multidisciplinary approach has generated the new discipline of glycobiology which focuses on research related to the appearance and biological significance of carbohydrates. In more detail, studies in glycobiology have revealed the essential roles of carbohydrates in cell-cell interactions, biological recognition events, protein folding, cell growth and tumor cell metastasis. As a result of these studies, carbohydrate derived diagnostic and therapeutic agents are likely to be of growing interest in the future.

In this doctoral thesis, a journey through the fundamentals of carbohydrate synthesis is presented. The research conducted on this journey was neither limited to the study of any particular phenomena nor to the addressing of a single synthetic challenge. Instead, the focus was deliberately shifted from time to time in order to broaden the scope of the thesis, to continue the learning process and to explore new areas of carbohydrate research. Throughout the work, several previously reported synthetic protocols, especially procedures related to glycosylation reactions and protective group manipulations, were evaluated, modified and utilized or rejected. The synthetic molecules targeted within this thesis were either required for biological evaluations or utilized to study phenomena occuring in larger molecules. In addition, much effort was invested in the complete structural characterization of the synthesized compounds by a combination of NMR spectroscopic techniques and spectral simulations with the PERCH-software.

This thesis provides the basics of working with carbohydrate chemistry. In more detail, synthetic strategies and experimental procedures for many different reactions and guidelines for the NMR-spectroscopic characterization of oligosaccharides and glycoconjugates are provided. Therefore, the thesis should prove valuable to researchers starting their own journeys in the ever expanding field of carbohydrate chemistry.

Abstrakt

Kolhydrater tillhör en av de största grupperna av naturligt förekommande biomolekyler. Den tidiga forskningen inom kolhydratkemi fokuserades på bestämning av enskilda kolhydraters strukturer och undersökning av den komplexa naturen hos monosackarider. Senare blev utvecklingen av skyddsgruppsstrategier och metoder för oligosackaridsyntes de viktigaste forskningsgrenarna inom området. I dagens läge används de tidigare utvecklade metoderna vid framställning av kolhydrater för biologiska studier. Denna tvärvetenskapliga forskning har gett upphov till en ny forskningsgren som bär namnet glykobiologi. Forskningen inom glykobiologi är fokuserad på kolhydraters förekomst och biologiska funktioner. Studier i glykobiologi har bland annat avslöjat att kolhydrater spelar viktiga roller i cell-cell interaktioner, biologiska igenkänningsfenomen, proteininveckning, cellväxt och cancermetastas. På grund av dessa studier kommer sannolikt kolhydratbaserade diagnostiska och terapeutiska föreningar att vara av växande intresse i framtiden.

I denna doktorsavhandling kommer en utflykt genom den fundamentala kolhydratkemin att presenteras. Forskningen som utfördes under denna resa var inte begränsad till undersökning av specifika fenomen eller syntetiska utmaningar. Istället skiftades vikten för studierna från tid till tid för att utvidga avhandlingens omfattning, fortsätta inlärningsprocessen och undersöka nya områden inom kolhydratkemi. Under hela arbetet evaluerades, modifierades och användes en stor mängd tidigare rapporterade syntetiska protokoll, speciellt procedurer relaterade till glykosyleringsreaktioner och skyddsgruppsmanipuleringar. De syntetiska målmolekylerna inom arbetet var av två slag: molekyler för undersökning av fundamentala processer och molekyler för evaluering av deras biologiska tillämpningar. Dessutom lades mycket arbete ner på strukturell karakterisering av de syntetiska substanserna med hjälp av både NMR spektroskopiska tekniker och spektrala simuleringar med programmet PERCH.

Denna avhandling innehåller de fundamentala elementen för arbete inom kolhydratkemi eftersom syntetisk planering, experimentella procedurer och riktlinjer för NMR spektroskopisk analys av kolhydrater finns beskrivna. Därför torde avhandlingen vara av värde för forskare som startar egna resor inom det expanderande ämnesområdet kolhydratkemi.

List of Original Publications and Manuscripts

I **Synthesis of Divalent 2,2′-Linked Mannose Derivatives by Homodimerization.** F. S. Ekholm, M. Poláková, A. J. Pawłowicz, R. Leino, *Synthesis* **2009**, *4*, 567–576.

II **Synthesis of β-Linked Oligomannosides.** M. Poláková, M. U. Roslund, F. S. Ekholm, T. Saloranta, R. Leino, *Eur. J. Org. Chem.* **2009**, 870–888.

III **Fully Deprotected β-(1→2)-Linked Mannotetraose Forms a Contorted α-Helix in Solution: Convergent Synthesis and Conformational Characterization by NMR and DFT.** F. S. Ekholm, J. Sinkkonen, R. Leino, *New. J. Chem.* **2010**, *34*, 667–675.

IV **A Short Semi-Synthesis and Complete NMR Spectroscopic Characterization of the Naturally Occuring Lignan Glycoside Matairesinol Diglucoside.** F. S. Ekholm, P. Eklund, R. Leino, *Carbohydr. Res.* **2010**, *345*, 1963–1967.

V **Synthesis of a Small Library of Estradiol Based Glycosteroid Mimics Containing a Modified D-Ring.** F. S. Ekholm, G. Schneider, J. Wölfling, R. Leino, *Eur. J. Org. Chem.* **2011**, 1064–1077.

VI **An Approach to the Synthesis and Attachment of Scillabiose to Steroids.** F. S. Ekholm, G. Schneider, J. Wölfling, R. Leino, *Steroids* **2011**, *76*, 588–595.

VII **Stereo- and Regioselective Glycosylation of 4-Deoxy-ε-Rhodomycinone.** F. S. Ekholm, L. Lagerquist, R. Leino, *Carbohydr. Res.* **2011**, *346*, 858–862.

VIII **Studies on Norway Spruce Galactoglucomannans: Chemical Synthesis, Conformational Analysis, NMR Spectroscopic Characterization and Molecular Recognition of Model Compounds.** F. S. Ekholm, A. Ardá, P. Eklund, S. André, H.-J. Gabius, J. Jiménez-Barbero, R. Leino, *manuscript submitted*.

List of Related Publications

IX **Applying Biocatalysis to the Synthesis of Diastereomerically Enriched Cyanohydrin Mannosides.** A. Hietanen, F. S. Ekholm, R. Leino, L. T. Kanerva. *Eur. J. Org. Chem.* **2010**, 6974–6980.

X **Rapid, Simple and Efficient Deprotection of Benzyl/Benzylidene Protected Carbohydrates Utilizing Flow Chemistry.** F. S. Ekholm, I. Mándity, F. Fülöp, R. Leino, *Tetrahedron Lett.* **2011**, *52*, 1839–1841.

List of Abbreviations

Ac	acetyl
ACN	acetonitrile
AcOH	acetic acid
AgOTf	silver triflate
All	allyl
B3LYP	Becke three-parameter hybrid with Lee-Yang-Parr correlation functional
$BF_3 \cdot OEt_2$	boron trifluoride etherate
Bn	benzyl
BSP	1-phenylsulfinyl piperidine
Bz	benzoyl
COSY	correlation spectroscopy
CSA	camphorsulfonic acid
Cy	cyclohexyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DFT	density functional theory
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
Et	ethyl
Gal	galactose
Glc	glucose
HF	hydrogen fluoride
HMBC	heteronuclear multiple bond correlation
HPAEC	high pH anion exchange chromatography
HRMS	high resolution mass
HSQC	heteronuclear single-quantum coherence
J	scalar spin-spin coupling constant
Man	mannose
Me	methyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide

NMO	*N*-methylmorpholine oxide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
Ph	phenyl
PG	protective group
PTSA	*para*-toluenesulfonic acid
Rha	rhamnose
r.t.	room temperature
TBAB	tetrabutylammonium bromide
TBDMS	*tert*-butyldimethylsilyl
*t*Bu	*tert*-butyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TOCSY	total correlation spectroscopy
TTBP	2,4,6-tri-*tert*-butyl pyrimidine

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Introduction

1 Biological Applications of Carbohydrates

Carbohydrates, nucleotides, lipids and peptides constitute the major classes of biomolecules found in living organisms.[1] The division of molecules into these categories is not as straightforward as the categories may imply since many of the biomolecules exist in nature in mixed forms, *e.g.*, glycoproteins, glycolipids etc., containing structural elements from multiple categories. Research on carbohydrates has always received considerable attention as these molecules are present in large quantities in nature, display diverse structural variations and have many biological functions.

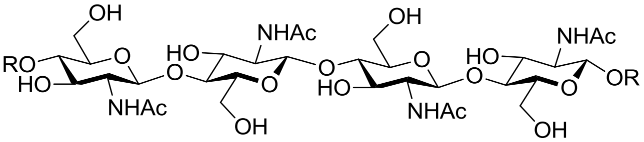


Figure 1. The chemical structure of chitin (repeating β-1,4-linked *N*-acetylglucosamine units).

Early on, already before the 1960s, carbohydrates were considered to be important structural molecules in biological membranes and surfaces as often exemplified by chitin,[2] a polysaccharide consisting of β-1,4-linked *N*-acetylglucosamine residues (Figure 1). Chitin is the main constituent of fungal cell walls and the exoskeleton of arthropods. In addition, carbohydrates were recognized as energy storage molecules, *e.g.*, glucose can be stored in the form of glycogen in animals and as starch in plants. In humans, glucose is released from glycogen during glycolysis thus providing ATP and pyruvate as substrates for the Krebs cycle.[3] While these features remain important today, many new and exciting applications for carbohydrates have emerged in recent years, simultaneously expanding the borders of glycoscience and generating the discipline of glycobiology.[4] Studies in glycobiology have revealed the vital roles of carbohydrates in cell-cell interactions,[5] biological recognition events,[6] protein folding,[7] cell growth[8] and tumor cell metastasis.[9] Since all these interactions are mediated at least partially by carbohydrates (often specific glycans or glycoconjugates), the potential utilization of carbohydrates as biomarkers for celltypes, disease and developmental states is a highly promising area of research.[10] In the next section, the general concept of carbohydrate biomarkers and their potential applications will be addressed and discussed. These areas are, according to the personal view of this author, among the most important research topics in modern glycobiology and accordingly relevant background information for glycoscience as a whole. Large numbers of carbohydrate derived compounds and materials are also utilized extensively in food technology[11] and the pharmaceutical industry.[12]

1.1 Carbohydrate Biomarkers

A carbohydrate biomarker is by definition a carbohydrate utilizable as an indicator for a biological state.[10] The most well documented example of carbohydrate biomarkers stems from the identification of the bloodtype determinants which are utilized in the ABO blood-system. In more detail, Watkins and James established the carbohydrate composition of these biomarkers in the 1950s (Figure 2) by studying the cell surface of red blood cells.[13] This knowledge together with information on the serological relationships of the bloodtype determinants has enabled the safe blood transfusion processes which save many lives each year.[14] This is but one example of the beneficial and health promoting effects that research on carbohydrates can provide for society. From the structures in Figure 2, it can be noticed that even small differences in the structure of biomarkers (one sugar residue or a single functional group) can have significant impact on their biological properties (blood clotting if blood from an incompatible bloodtype is utilized during blood transfusions).

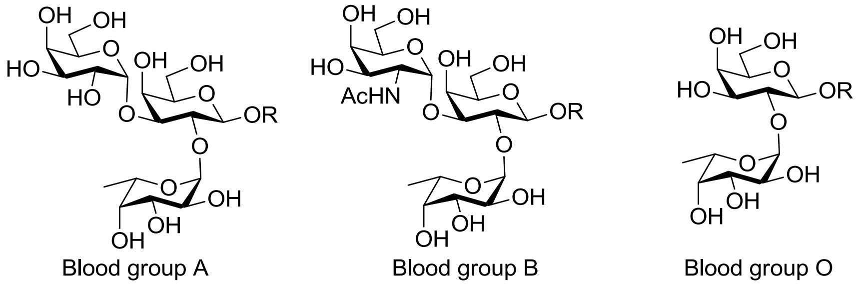
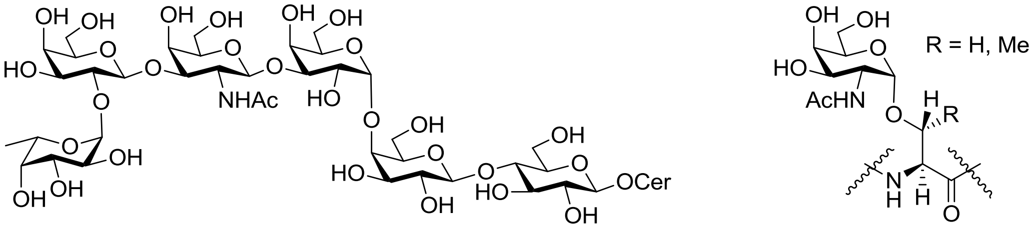


Figure 2. The carbohydrate biomarkers of the ABO blood-system.

While carbohydrates bear great potential as biomarkers for biological states, the identification of specific biomarkers was for a long time considered challenging due to the presence of thick layers of heterogenous mixtures of complex carbohydrates (in many glycoforms) on cell surfaces and in the extracellular matrix. In recent years, however, advancements in analytical tools (nuclear magnetic resonance spectroscopy, mass spectrometry and gene sequencing) and multidisciplinary approaches of large collaborative networks between scientists in different fields (chemistry, biochemistry, cellbiology, molecular biology etc.) have enabled the isolation and identification of carbohydrate biomarkers on an acceptable timescale. Once a biomarker has been identified, a large number of possibilities are generated towards deepening the understanding of biological events, monitoring changes in biological systems, and developing new and improved therapeutic and diagnostic agents.[10,15] Monitoring changes in carbohydrate (biomarker) composition and expression patterns at a cellular level is often done indirectly with selective carbohydrate binders, such as lectins (carbohydrate binding proteins), which recognize specific sugar residues.[16] In order to further underline and exemplify the importance of carbohydrate biomarkers, their use in the identification and monitoring of cancer onset and progression is provided below.



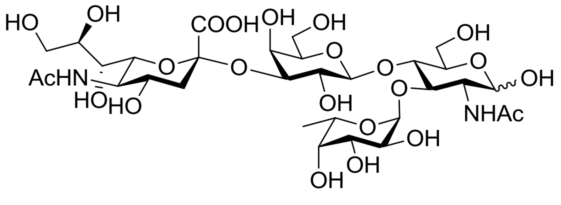
Globo H antigen

Tn antigen

Figure 3. Chemical structures of two common carbohydrate associated cancer antigens.

Cancer is the general term for a large group of diseases where celldivision is accelerated and uncontrolled thereby resulting in the formation and spreading of malignant tumors.[17] Transformation of a normal cell into a diseased cancer cell is often accompanied by alterations of genes which results in changes in biosynthetic pathways, protein functions etc.[17,18] Although many changes occur in cancerous cells, the discussion will herein be limited to the fate of glycans. Changes in the composition of glycans and their expression patterns, during the transformation of healthy cells to cancer cells, has been thoroughly studied over the years.[4a] As a result, several carbohydrates are currently considered as cancer associated antigens (CAAs).[19] CAAs present on the cell surface of cancerous cells can be either glycolipid- or glycoprotein based. Globo H, depicted in Figure 3, is a common CAA belonging to the glycolipid category and is often expressed on the cell surface of breast cancer cells.[20] The Tn antigen (Figure 3) on the other hand is a member of the glycoprotein category and overexpressed in malignant cells due to defects in the glycan biosynthetic pathway.[21] In fact, the occurrence of elevated amounts of the

Tn antigen has been identified in more than 90 % of all solid tumors.[10b] Consequently, the Tn antigen is an excellent biomarker for carcinomas and has found further use in the generation of anticancer vaccines.[22] Another common trend in the altered glycan expression of malignant cells is an increased amount of sialylated carbohydrates.[4a] Increased capping of *N*-glycans by α-(2→6)-linked sialyl acid residues[23] and overexpression of sialyl lewis^x (Figure 4)[24] are closely associated with carcinomas. The increase in sialylated oligosaccharides and glycans in cancerous cells is according to current understanding, a way for the cancer to escape/delay deadly encounters with the immunesystem.[4a] While many other changes in glycan compositions occur in malignant cells, such as the increased length and branching patterns of *N*-glycans and the aberrant glycosylation patterns of mucins, [4a] a more thorough discussion on these alterations is outside the scope of this thesis.



sLe^x

Figure 4. Chemical structure of sialyl lewis^x.

The utilization of carbohydrates as biomarkers for biological states has large potential which is also apparent from the limited examples provided above. In the first example on the ABO blood-system, carbohydrates on the cell surface of red blood cells were shown to be biomarkers for bloodtype. Furthermore, a minimal deviation in one functional group in these biomarkers was sufficient to alter the bloodtype of an individual. This is a clear indication on how sensitive biomarkers are in biological systems. In the second example, a change in the biological state of a cell (from normal to cancerous) was accompanied by several changes in carbohydrate expression patterns. This example provided undeniable evidence of the potential of carbohydrate biomarkers in the determination and monitoring of biological properties and states. Carbohydrate biomarkers are not limited to these examples as they are also widely utilized in studies on signal transduction pathways,[25] stem cell differentiation processes,[26] and invasion processes of infectious diseases.[27]

This far, the focus has been purely on the appearance of carbohydrates and their use as biomarkers in the monitoring of biological states. As also mentioned earlier, the identification of carbohydrate biomarkers provides several other opportunities related to the understanding of biological events and development of new and improved therapeutic agents.[12c,28] In the next section, the utilization of carbohydrate biomarkers in immunology and vaccine development will be briefly discussed.

1.2 Immunological Applications of Carbohydrates

In modern medicine, diseases and infections are commonly treated with pharmaceuticals. While much progress has undoubtedly been achieved by the use of pharmaceuticals, infectious diseases caused by microorganisms (viruses, bacteria, fungi and parasites) still represent a major global health concern due to the emergence of multidrug resistant strains and mutated forms of such species.[28a] The use of long-term protective therapies, such as vaccines, might offer a solution to these concerns and is generally considered by the World Health Organization (WHO) as the most cost-effective strategy for controlling infectious diseases.

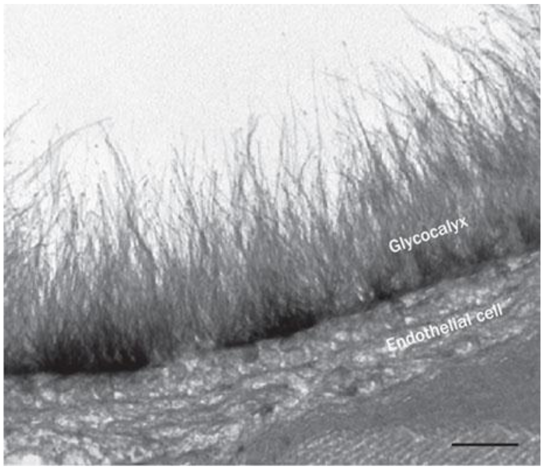


Figure 5. The cell surface glycocalyx of an endothelial cell; bar = 200 nm. Reprinted with permission from Wolters Kluwer Health.[29]

As mentioned on page 2, cells are coated with thick layers of heterogenous complex carbohydrates (Figure 5).[4a] These carbohydrates play essential roles in many molecular recognition events such as adhesion and invasion processes of microorganisms.[27] Such processes are often mediated by unique glycan structures found on the cell surfaces of pathogens (absent in the host). As a consequence, these structures are also utilizable as biomarkers for specific pathogens. While the term biomarker has been frequently used, such molecules will here be referred to as antigens. Antigens are molecules which are recognized as foreign or potentially dangerous by the immune system of the host. The host immune system responds to antigens by the production of antibodies which recognize them and mark them for disposal. The long-term protection of vaccines is in part due to the immunological memory which allows the immune system to respond rapidly to previously encountered antigens.[30]Alongside these principals, two general strategies for protective therapies against infectious diseases have arisen.[31]

The first strategy is based on generation of protective antibodies against the corresponding antigen(s). This information will be stored in the immunological memory in case of future encounters with the particular antigen(s).[30] The generation of antibodies can be achieved by a few deviating routes. The most common way of generating antibodies against a particular pathogen is to administer complete cells of heat killed or attenuated microorganims into humans.[31] This is a good way of generating antibodies for different cell surface structures present in the pathogen. The main drawback of this approach is the challenges/problems associated with the cultivation and storage of large quantities of potentially dangerous pathogens. In addition, the number of antigens present at cell surfaces of entire pathogens is overwhelming and can therefore result in allergic side reactions in humans.

The second approach is based on utilization of pure carbohydrate antigens or conjugates thereof.[32] These can be isolated from cell walls or synthesized by chemical means.[28a] Here, the focus will be on fully synthetic compounds. A synthetic approach offers several advantages over utilization of weakened or heat killed pathogens. First, synthetic carbohydrates are homogeneous compounds, devoid of biological contaminations, and can, in principle, be manufactured on large scale without any batch to batch variation. Second, the number of different antigens are by these routes limited to the synthetic compound and, therefore, allergic sidereactions can be avoided. There are, however, several challenges associated with the production of synthetic vaccines. The carbohydrate antigens have to be selected without risking cross-reactivity with carbohydrate fractions present in healthy cells in individuals. In addition, identification of the correct antigenic epitope in pathogens is a time consuming process with many stages and possibilities to fail. Nevertheless, solving these challenges is highly motivated as the final products are safer alternatives to the general vaccines with capabilities of saving millions of lives.

These are the two basic pathways available for raising antibodies against pathogens in order to achieve long-term protection from infectious diseases. As mentioned above, another strategy for dealing with pathogens exists and the basic principles of this strategy will be briefly addressed next.

Adhesion and invasion processes of microorganisms are often mediated by carbohydrates present at the cell surfaces of such species.[27] Instead of generating antibodies against pathogens, it is possible to design and produce specific carbohydrate binding molecules with high affinity for unique oligosaccharides present on the cell surfaces of microorganisms (absent in the host).[10a,16a] In recent years, much success has been achieved by the utilization of artificial lectins (carbohydrate binding proteins). These lectins can be divided into two main categories: boronic acid based and non-boronic acid based.[33] While natural lectins are selective in their recognition events, they cannot be applied in these applications due to cross-reactivity issues with healthy cells. The main concept of this approach relies on the high binding affinity between artificial binders and the unique glycan structures present on the cell surface of pathogens. Once binding is achieved, the functionalized binder can transport the pathogen away from the infection site or mark it for disposal by the immune system.

The carbohydrate based approaches briefly addressed above are frequently applied in the development of modern protective therapies for infectious diseases.[28] In order to convey the health promoting potential of these approaches, one additional example on the development of a synthetic carbohydrate vaccine against *Haemophilus influenza* will be provided next.

Haemophilus influenza (Hib) was the leading cause for bacterial meningititis in children in the USA before the 1990s.[28a] As a result, much effort was devoted to finding and producing a vaccine against *Haemophilus influenza*. After fourteen years of experimentation and optimization, a Hib vaccine with a success rate of 99.7 % in children was developed by Verez-Bencomo and Roy.[34] This vaccine is the first and only commercially available synthetic carbohydrate vaccine on the market and it is also a member of the Cuban vaccination program since 2004. The vaccine, the chemical structure of which is shown in Figure 6, is based upon a conjugated form of the repeating carbohydrate unit found in the cell wall of *Haemophilus influenza*.[35] Although not discussed previously, conjugation of carbohydrate antigens to carrier proteins is usually required in carbohydrate based vaccines. This is mainly because the generation of long-term immunological memory is difficult to achieve with oligosaccharides as such.[36] While the vaccine is available in developed countries, WHO estimates that Hib causes more than three million serious infections and over 350000 deaths in children in third world countries each year. The large number of infections and deaths still observed today is due to the high cost and low availability of the vaccine in developing countries.

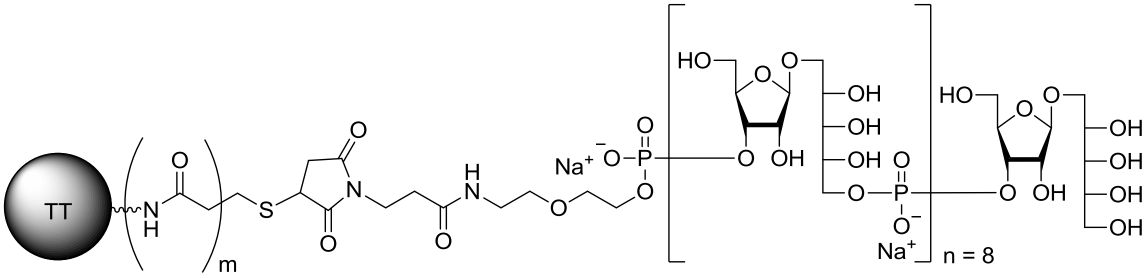


Figure 6. Chemical structure of the Hib vaccine developed by Verez-Bencomo and Roy.

This introduction on biological applications of carbohydrates is not intended as a thorough survey of all the possible applications of carbohydrates found in the literature as this would be redundant and space consuming to write. Instead, a focused and brief description of a few important areas were highlighted in order to provide an overall picture of the potential benefits studies on carbohydrates can provide for the general public and society. As mentioned previously, the identification of carbohydrate biomarkers is the cornerstone of biological studies on their nature and roles. While the initial identification of carbohydrate biomarkers can be performed by extracting material from cell surfaces and carefully analyzing the samples by mass spectrometry and NMR spectroscopy,[37] the small amounts obtainable by these routes are seldom sufficient to launch a deeper investigation on the biological profiles of single components. In addition, obtaining pure samples devoid of biological contaminations by fragmentation of cell walls and isolation processes may be challenging. As a consequence, chemical (and/or enzymatic) synthesis is usually relied on for production of larger amounts of single components for more demanding biological screening events.[28a,38] Chemical synthesis of carbohydrates is also the main topic of this thesis and, therefore, a brief introduction to carbohydrate chemistry will be provided next.

2 Carbohydrate Chemistry

The role of carbohydrates in biological applications is mostly studied by the use of pure synthetic oligosaccharides as these are devoid of biological contaminations and can be produced on large scale without any batch to batch variation. The synthesis of oligosaccharides is a laborious process as there are no universal reaction conditions or general applicable methodologies available for their construction. Instead, each oligosaccharide synthesis is an independent challenge which requires systematic research of trained synthetic chemists to solve.[39] Ole Hindsgaul has estimated that a trained carbohydrate chemist will spend on average seven weeks for each monosaccharide residue while synthesizing oligosaccharides.[40] The biological target molecules are seldom smaller than trisaccharides and, therefore, it will take on average more than five months for a carbohydrate chemist to prepare, by the traditional routes, one sample for biological screening events. In the next section, the main approaches used in the synthesis of carbohydrates will be briefly addressed. As in any synthesis, the starting materials and their structural features play an important role in the design of synthetic pathways and, hence, a short introduction to the structural properties of carbohydrates is provided first.

2.1 Structural Properties of Carbohydrates

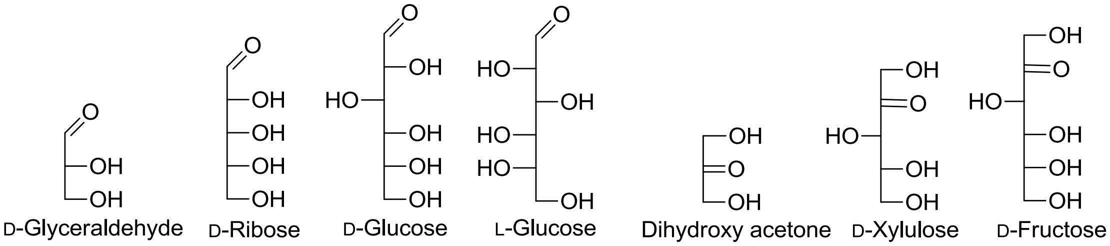
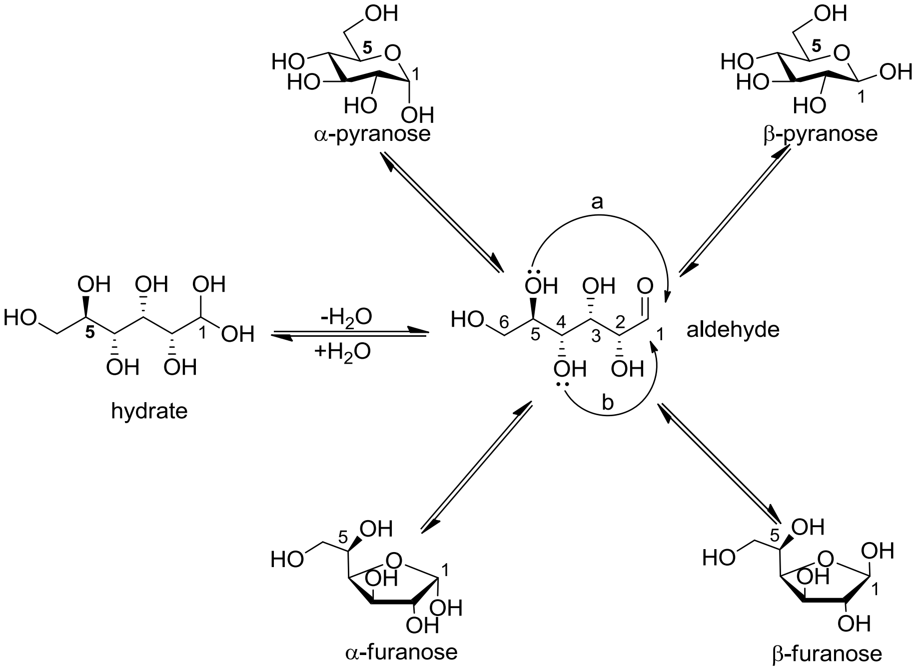


Figure 7. Fischer projections of the acyclic forms of some selected aldoses and ketoses.

Carbohydrates are generally divided into three separate categories (mono-, oligo- and polysaccharides) depending on the number of sugar residues present in the molecule. Monosaccharides are the simplest form of carbohydrates and this category contains molecules such as the aldoses and ketoses with three to nine carbon atoms. Even though these molecules are relatively small, a large number of structural variations are nevertheless possible due to the presence of multiple stereocenters (*R* or *S*) and the existence of two distinct enantiomers of each respective sugar (the D- and L-sugars).[41] The pioneering work on structural identification of monosaccharides was conducted by Emil Hermann Fischer starting from the early 1880s. The excellent work of Fischer, for which he was later awarded the Nobel Prize, provided the structural basis for carbohydrate chemistry in the form of accurate chemical structures of the D-aldoses.[42] Inversion of the stereochemistry of D-aldoses, according to the method of Rosanoff, generates the corresponding enantiomeric L-aldoses.[43] As mentioned previously, some monosaccharides appear in nature as ketoses. Ketoses are generated upon extension of dihydroxy acetone while aldoses are generated upon extension of glyceraldehyde.[41] Some selected examples of sugars from these two categories are depicted in Figure 7.



Scheme 1. Formation of hemiacetals and the effects of mutarotation displayed for D-glucose in water: Pathway (a) shows formation of pyranoses and pathway (b) the formation of furanoses.

While Fischer assumed that monosaccharides were acyclic, it is today known that they exist mostly in their ring-closed forms as either pyranoses or furanoses. This is due to the intramolecular attack of the hydroxyl groups at either C-4 or C-5 on the carbonyl carbon. Furthermore, the hemiacetals or hemiketals that result from this attack may possess either α- or β-anomeric configuration depending on which face of the carbonyl is attacked. The hemiacetals and hemiketals open spontaneously and equilibrate between the different ring-closed products and the acyclic forms due to mutarotation as shown in Scheme 1 for D-glucose in water.[44] The ratio between the different forms of a sugar present in solution is governed by the electronic and steric properties of the individual sugar and the nature of the solvent used.[45] In general, however, cyclic forms are favored over acyclic ones and pyranoses over furanoses due to the lower amount of ring strain in a six-membered ring. The complex structural features of monosaccharides are not limited to the occurrence of the forms shown in Scheme 1 as carbohydrates can adopt several different three dimensional shapes even within the individual cyclic forms. These structural arrangements are also termed conformations and the different conformations of carbohydrate pyranosides and furanosides will be briefly addressed below.[46]

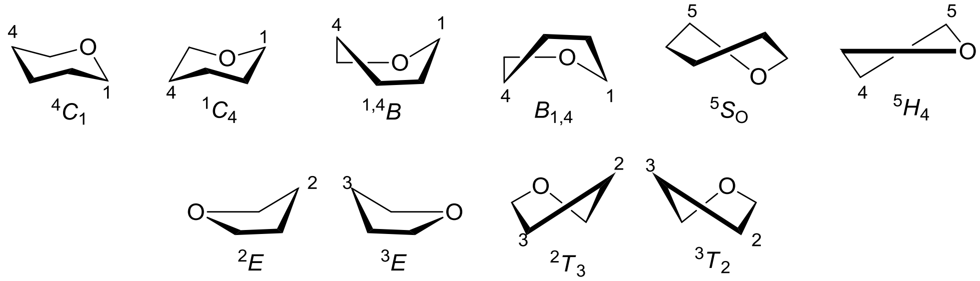
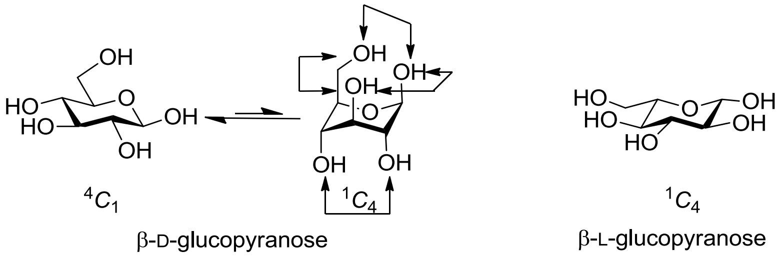


Figure 8. Representative conformations of pyranoses (top row) and furanoses (bottom row).

In pyranoses, four main ring shapes and a large number of variations of these are possible. To distinguish between pyranose conformations the following notation system is applied; *C* for chair, *B* for boat, *S* for skew and *H* for half-chair.[46a,47] The ring atoms that are above the plane of the sugar ring are given as superscripts before the notation and the ring atoms that are below the plane are given as subscripts after the notation. The pyranose ring can adopt a total of two chair, six boat, twelve half-chair and six skew conformations. Furanoses on the other hand, can adopt two main ring shapes which are designated as *E* for envelope and *T* for twist.[47,48] A total of ten envelope conformations and ten twist conformations are possible for furanoses. A few examples of different pyranose and furanose conformations are provided in Figure 8. While pyranoses mainly prefer the $^4C_1$ or $^1C_4$ conformations and furanoses the ^2E, E_2, ^3E or E_3 conformations, the high energy conformations of such structures may be important in biological interactions and recognition events.[49] The ring shapes adopted by free sugars in solution are governed by steric and electronic factors. Naturally, similar factors also contribute to the preferred conformation. The discussion on steric and electronic interactions and their effect on the conformation adopted by sugars will here be limited to a few examples on pyranosides.

Generally, substituents at different positions in pyranoses will tend to be placed in equatorial positions in order to reduce the amount of steric ring strain. If this is not possible for all of the individual substituents, then, large substituents will be placed equatorially in preference to small substituents. This is one of the main reasons for the common occurrence of the $^4C_1$ conformer in D-sugars and $^1C_4$ conformer in L-sugars as the largest substituent, the hydroxymethyl substituent at C-5, can obtain the favorable equatorial position in both cases.[50] These main steric factors are summarized in Scheme 2.



Scheme 2. The equilibrium between chair conformations in β-D-glucopyranose (left, arrows indicate unfavorable steric interactions), and the stable $^1C_4$ chair conformation of β-L-glucopyranose (right).

Another conformational feature of monosaccharides, especially aldohexoses existing in the pyranose ring, is the rotation around the C-5–C-6 bond which gives rise to three distinct low-energy conformations termed gg (gauche-gauche), gt (gauche-trans) and tg (trans-gauge). These conformers are determined by the O-5–C-5–C-6–O-6 torsion angle (–60° for gg, +60° for gt, 180° for tg) and the population equilibrium between them is heavily influenced by steric constraints.[51] For example, different conformers are favored for D-glucopyranose and D-galactopyranose due to the stereochemistry of the hydroxyl group at C-4. If the hydroxyl group is equatorial, as in D-glucopyranose, the gg and gt conformational states will be populated and if the hydroxyl group is axial, as in D-galactopyranose, the gt and tg conformational states will be populated (Figure 9).

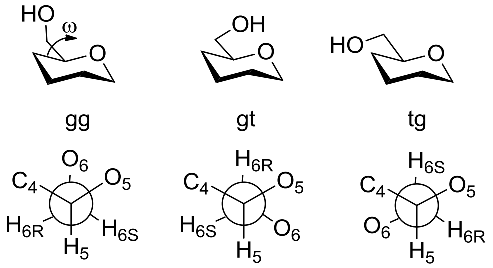
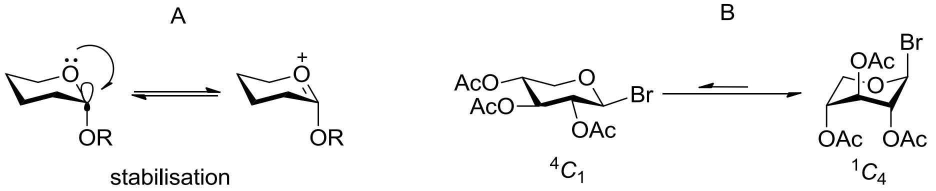


Figure 9. Possible low-energy conformations of the C-5–C-6 bond in pyranosides.

With the main steric interactions and their effects on the conformation of monosaccharides considered, the electronic interactions will be addressed below. The electronic interactions present in monosaccharides existing in the pyranose form are mainly limited to the anomeric effect. The anomeric effect, first described in the mid 20th century by Edwards and Lemieux,[52] is a partial delocalization of the lone pair electrons of the ring oxygen to the antibonding orbital at the anomeric centre. Alternatively, the anomeric effect can be described as a more favorable dipole-dipole interaction. Generally, the anomeric effect tends to place anomeric substituents in an axial position and has therefore the opposite effect as the steric interactions discussed earlier. The anomeric effect stabilizes the molecule and lowers its energy and the effect is greater when more electron withdrawing substituents are present at the anomeric position. One example of the net result of the anomeric effect is the existence of 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl bromide in the $^{1}C_{4}$ conformation although this places large steric constraints on the molecule as all substituents occupy axial positions (Scheme 3).[14]



Scheme 3. The partial delocalization of the lone pair electrons of the ring oxygen into the antibonding orbital at the anomeric center (left) and the conformational equilibrium of 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl bromide (right).

Controlling the ring shape, anomeric configuration and conformation of monosaccharides is essential when synthesizing carbohydrates for biological purposes as small variations in the structure of individual sugars can have severe consequences on the biological activity. This brief introduction on the structural properties of monosaccharides was given in order to provide a background to the challenges that may arise during the synthesis of oligosaccharides.

2.2 Synthesis of Carbohydrates

As mentioned in the introductory section, appropriate starting materials are the corner stone of all synthetic routes. Fortunately, carbohydrates are present in large quantities in nature. As a consequence, many monosaccharides, such as D-mannose, D-glucose and D-galactose, and some disaccharides, such as lactose, are available in large amounts at low cost. Nevertheless, it would be naive to think that all sugars would be cheap and readily available. For example, the price for ten grams of the common monosaccharide D-galactose is around 18 euro while the price for the rare enantiomer L-galactose is around 9000 euro for a similar amount.[53] In addition, some rare sugars cannot be purchased at all and need to be synthesized from other starting materials.[54]

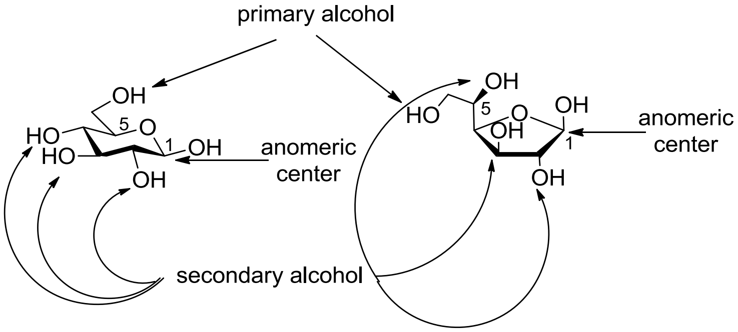
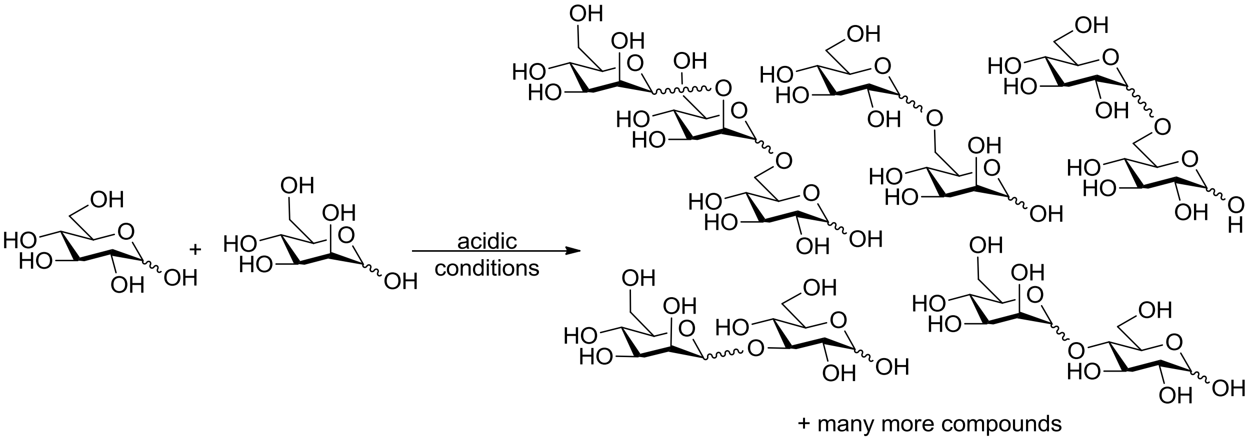


Figure 10. The different hydroxyl functionalities in pyranoses and furanoses, illustrated with β-D-glucopyranose (left) and β-D-glucofuranose (right).

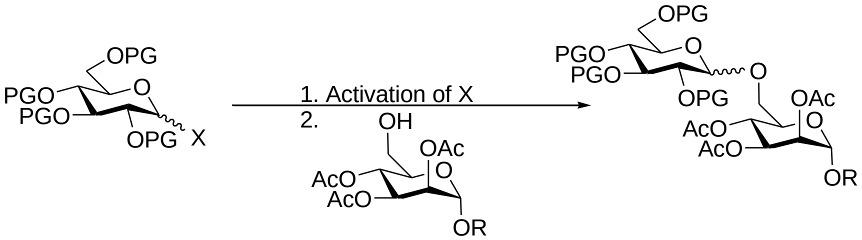
As depicted in Figure 10, three types of functionalities are generally found in carbohydrates [can also be more (*e.g.* aminosugars) or less (*e.g.* deoxysugars) depending on the structure]. These functionalities consist of the hemiacetal at the anomeric center, the secondary hydroxyl groups and the primary hydroxyl group. The reactivity of the anomeric center is different from the other hydroxyl groups and can therefore be selectively manipulated under acidic conditions. While the manipulation is selective, it can still give rise to two different anomers if reaction conditions are not appropriately optimized. Under basic conditions on the other hand, the primary hydroxyl group, being the least hindered one, is most reactive followed by the anomeric hydroxyl group and the secondary ones. While equatorial hydroxyl groups are, in general, more reactive than axial ones,[55] the reactivity pattern can easily be modified by other functionalities present in the molecule and the conformation adopted. Furthermore, at elevated temperatures the ring may flip, simultaneously inverting all its stereocenters. Therefore, the reactivities of the secondary hydroxyl groups are usually modified and distinguished by the use of protective groups. The protective group strategies for selective protection of individual hydroxyl groups and the basis for carbohydrate synthesis were developed in the 1970s.[56] With the basics of both the structural features and reactivity patterns of individual monosaccharides explained, the introduction will be finalized by addressing issues related to the synthesis of oligosaccharides.



Scheme 4. An example of a glycosylation of unprotected sugars under acidic conditions (not all of the possible compounds formed are shown).

Oligosaccharides are carbohydrates consisting of several sugar residues. As mentioned on page 9, biologically active carbohydrates usually contain at least three sugar units. The synthesis of such structures is important for the production of compounds for biological evaluations. There are many factors that require consideration when constructing oligosaccharides.[14,57] First, the glycosidic linkages should exist between specific positions and the anomeric configuration should be well defined. If two unprotected sugars were utilized as such in a glycosylation attempt, the end result would be detrimental. Not only would there be no control of anomeric configuration and regioselectivity, but the individual sugars could cross-react with each other and react more than once. This would lead to a complex mixture of many compounds with a fairly low chance for isolation of a single component for further screening events. One hypothetical example of such a reaction is shown in Scheme 4. From this example, the major challenges associated with synthesis of oligosaccharides are evident. In the coupling of two sugar units together, the regioselectivity, stereoselectivity (anomeric configuration) and the number of glycosylation reactions should be carefully controlled. This can be done by two major pathways involving either the use of enzymes or organic chemistry or both.[57,58]

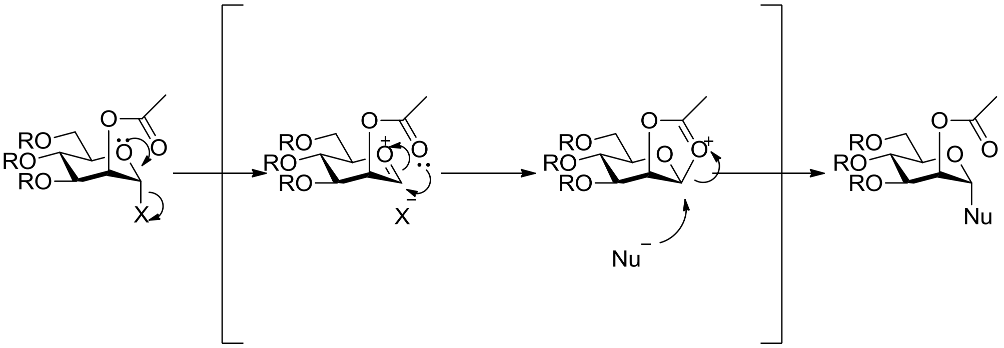
The enzymatic pathway, which employs glycosyltranferases (carbohydrate synthesizing enzymes) is appealing as naturally occurring unprotected monosaccharides can be utilized as donors and acceptors in glycosylation reactions. This approach circumvents the need for protective groups and minimizes the number of synthetic steps required to reach oligosaccharides. Enzymes are, however, simultaneously rather limited to specific substrates (and linkages) and can therefore not be considered as a general strategy for synthesis of carbohydrates. In addition, commercial enzymes for construction of challenging linkages, such as the β-(1→2)-mannosidic linkage, are not necessary available on the market. As in most areas of science, the methodologies used in enzymatic synthesis of carbohydrates are constantly evolving and enzymatic synthesis certainly has large potential in future applications related to this area.



Scheme 5. A typical glycosylation reaction. Activation of a glycosyl donor followed by nucleophilic displacement of the leaving group (X) by the incoming glycosyl acceptor, PG = protective group.

Chemical synthesis on the other hand, requires very different considerations than enzymatic synthesis. To accomplish the challenges related to control of stereoselectivity, regioselectivity and amount of glycosylation possibilities a number of chemical tools are available. The main strategy relies on the use of a fully protected glycosyl donor, containing an anomeric leaving group, and a selectively protected glycosyl acceptor, containing one free hydroxyl group.[57] In a typical glycosylation reaction, the leaving group in the glycosyl donor is first activated followed by a displacement reaction by the incoming nucleophile (glycosyl acceptor) as depicted in Scheme 5.[59] This approach solves the challenges related to regioselectivity and excludes the possibility of uncontrolled glycosylation events (which would lead to mixtures of compounds with different chain length). The anomeric configuration in the newly formed glycoside can be controlled by the use of participating protective groups or by careful control of reaction conditions.

Participating protective groups have been utilized extensively in carbohydrate chemistry to influence the stereoselective outcome of glycosylation reactions and in exploration of reaction mechanisms.[60] The most common example is the use of ester protective groups at C-2 to generate 1,2-*trans* glycosides (the reaction mechanism is shown in Scheme 6). This is the main reason why 1,2-*trans* glycosides are easy to synthesize and 1,2-*cis* glycosides more challenging.



Scheme 6. Neighbouring group participation exemplified by the 1,2-*trans* glycosylation of a 2-*O*-Ac-D-mannoside, R = protective group, X = leaving group, Nu = nucleophile.

Stereoselective 1,2-*cis* glycosylations on the other hand, are more difficult to perform. The first requirement in 1,2-*cis* glycosylations is the use of nonparticipating protective groups in key positions in the molecule (usually ether protective groups at C-2). In several cases, utilization of appropriate solvent mixtures has been applied to inflict high *cis* selectivities in glycosylation reactions.[61] Further possibilities include conformational control of the glycosyl donor by the use of protective groups. Such procedures have been utilized in 1,2-*cis* glycosylation reactions with good results.[62] In addition, indirect approaches based on intermolecular aglycon delivery (molecular tethering prior to glycosylation reactions)[63] and isomerization reactions of 1,2-*trans* glycosides have been found to be effective strategies for generating 1,2-*cis* linkages.[64]

This introduction to carbohydrate chemistry was not intended to cover all the progress which has been made in the field as this information is available in any textbook on the subject.[12c,14,15b] Instead, a brief introduction to structural features and synthetic aspects of carbohydrate chemistry was given. It is important to understand the structural features of carbohydrates when planning synthetic routes and, especially, when searching for biologically active compounds. In the section on structural properties of carbohydrates, their existence in several different forms (cyclic vs. acyclic) and conformations ($^{1}C_{4}$, $^{4}C_{1}$, ^{3}E, gg, gt etc.) were shown in order to highlight the complex nature of monosaccharides and sugars in general. Furthermore, the steric and electronic interactions which determine the preferred structure of an individual sugar were briefly addressed. In the second part of the introduction to carbohydrate chemistry, the functional groups and the reactivity patterns of unprotected sugars were discussed. In addition, a coarse view on the general approaches and considerations of oligosaccharide synthesis was provided. A more detailed discussion on glycosyl donors and acceptors, activation protocols and protective groups and their manipulations (especially, orthogonal protection strategies) was not provided as these issues will hopefully become clear throughout the episodes presented in this thesis.

3 Outline of the Thesis

This thesis is based on the personal explorations and adventures of the author in the field of carbohydrate chemistry during the years 2008–2011. The topics of the individual episodes summarized in this thesis are not related to each other and represent three larger entities where special interest was invested during the years.

In Episode 1, synthesis of β-(1→2)-linked mannosides and structurally related compounds resembeling cell wall structures of *Candida albicans* will be addressed.[65] The synthesis of these structures was conducted in order to produce compounds for immunological screening of their potential applications in the treatment of atopic allergies. In addition, structural features of such molecules were investigated by the use of NMR spectroscopic methods and molecular modelling in order to obtain valuable information on their three dimensional properties.

In Episode 2, the synthesis of a repeating trisaccharide unit and a less abundant tetrasaccharide fragment found in the galactoglucomannan-polysaccharide isolated from *Picea abies* is addressed. These molecules were synthesized in order to provide reference samples for analyzing the effect of individual fragments in similar applications as the polymer is used. In addition, acetyl-group migration phenomena reported in the polysaccharide were investigated and some comparative NMR spectroscopic studies were conducted by the use of small molecular model substrates.

In Episode 3, methods for the glycosylation of different aglycones are investigated and the synthesis of a limited amount of glycoconjugates with biological potential is described.[66] Most of the molecules have not yet been submitted for biological screening. Several of them are, however, close mimics of compounds with known biological activity (*e.g.*, anticancer activity of anthracyclines).

While not discussed in the Introduction, reliable synthesis of new molecules requires reliable analytical data on the prepared substrates. The author of this thesis has never been pleased with the low standard of NMR spectroscopic assignments often found acceptable in synthetic reports and, as a consequence, overwhelming in the literature. Therefore, throughout this work, special attention was focused on the NMR spectroscopic characterization (and spectral simulations) of the synthesized molecules and a short section on the spectral analysis of each class of compounds will be given at the end of each Episode.

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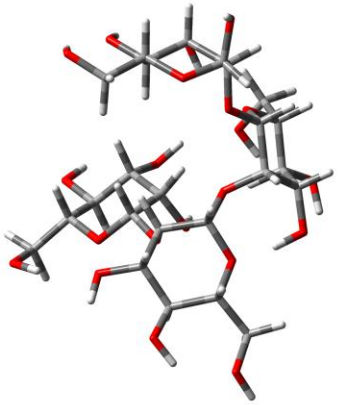
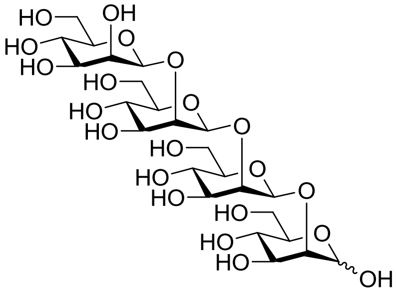
Episode 1: Synthesis of β-(1→2)-Linked Mannosides





β-(1→2)-Linked oligomannosides found in the cell wall of *Candida albicans* are promising structures for the development of *C. albicans* vaccines. Furthermore, these structures have been shown to possess interesting immunostimulating properties applicable to the treatment of atopic disorders such as allergic inflammation. Due to the biological applications of β-(1→2)-linked oligomannosides, considerable effort has in recent years been devoted to the synthesis of these compounds. In this chapter, synthetic challenges related to the synthesis of β-(1→2)-linked oligomannosides will be discussed and methods for overcoming these difficulties addressed. In addition, synthesis of divalent mannosides and structural details related to the three-dimensional nature of β-(1→2)-linked mannosides will be presented.

This chapter is based on the previously published papers: F. S. Ekholm, M. Poláková, A. J. Pawłowicz, R. Leino, **"Synthesis of Divalent 2,2′-Linked Mannose Derivatives by Homodimerization"**, *Synthesis* **2009**, *4*, 567–576; M. Poláková, M. U. Roslund, F. S. Ekholm, T. Saloranta, R. Leino, **"Synthesis of β-Linked Oligomannosides"**, *Eur. J. Org. Chem.* **2009**, 870–888; F. S. Ekholm, J. Sinkkonen, R. Leino, **"Fully Deprotected β-(1→2)-Linked Mannotetraose Forms a Contorted α-Helix in Solution: Convergent Synthesis and Conformational Characterization by NMR and DFT"**, *New. J. Chem.* **2010**, *34*, 667–675.

1 Introduction

The yeast *Candida albicans*, an opportunistic pathogen found in the mucous membrane of the gut, skin, gastrointestinal tract and mouth, is considered to be part of the normal human flora. In immunocompromised persons *C. albicans* can grow out of control, a condition termed candidiasis, thereby resulting in severe infections.[1] Through studies on the composition of *C. albicans'* cell wall, an epitope consisting of β-(1→2)-linked mannan isolated from the acid labile fraction of the phosphopeptidomannan has been identified as important for the inhibition of such infections (see Figure 1).[1c,2] To date, several biological studies have confirmed the protective role of β-(1→2)-linked mannans and furthermore implicated their involvement in the stimulation of TNF-α production from macrophages.[1c,3] Although the interest in *C. albicans* vaccines has been the main motivator behind most of the biological work conducted with these oligosaccharides, the interest in these molecules is here connected to their applications in the treatment of atopic allergies.[4] Atopic allergies are a growing health concern in the western world due to the decreased exposure to microbe loads in developed countries. The decreased exposure to microbes has altered the immune response of some humans to favor a Th2-pathway which is one of the characteristics of atopic allergies.[5] While a detailed description of the biology of atopic allergies is beyond the scope of this introduction and thesis, an optimal synthetic molecule would favor a Th1-pathway and thereby induce the production of Th1- and Treg-cytokines (such as IFN-γ, IL-12 and IL-18) and suppress the production of Th2-cytokines (IL-4, IL-5 and IL-13).[6] In general, biological studies of this type require large amounts of homogenous pure compound. The option of isolating material from natural sources can often be ruled out due to: 1) The heterogeneous nature of cell wall oligosaccharides; and, 2) The low quantities of compound available for extraction and purification. As a result, chemical methods must be sought for the production of analytically pure compounds in sufficient quantities.

There are several synthetic challenges associated with the construction of β-(1→2)-linked mannosides.[7] First, both thermodynamic and kinetic conditions favor the formation of the α-anomer. In addition, neighboring group participation (NGP) from ester protective groups at C-2 will lead to formation of the α-mannoside. Despite these challenges, several successful methodologies have been developed to overcome the obstacles.[7a,8] Eventhough the development of selective methodologies for the synthesis of complex carbohydrates is important, the full spectroscopic analysis of such compounds is of equal importance.

The cell wall composition of *C. albicans* has been the subject of several structural studies.[2] Special attention has been drawn to β-(1→2)-linked mannans not only due to the immunochemical activity shown by portions of these polymers but also because such structures have been predicted to display interesting conformational properties.[9] Delicate conformational studies by Bundle and coworkers on propyl-capped glycosides have revealed that these carbohydrates form contorted α-helical-like structures in solution.[9b] Studies on fully deprotected oligomannosides have, however, not been reported earlier.

In light of the extensive and elegant work previously conducted in this field, this chapter is devoted to the synthesis and NMR spectroscopic characterization of β-(1→2)-linked oligomannosides,[10] culminating in the synthesis and conformational study of a β-(1→2)-linked mannotetraose.[10c]

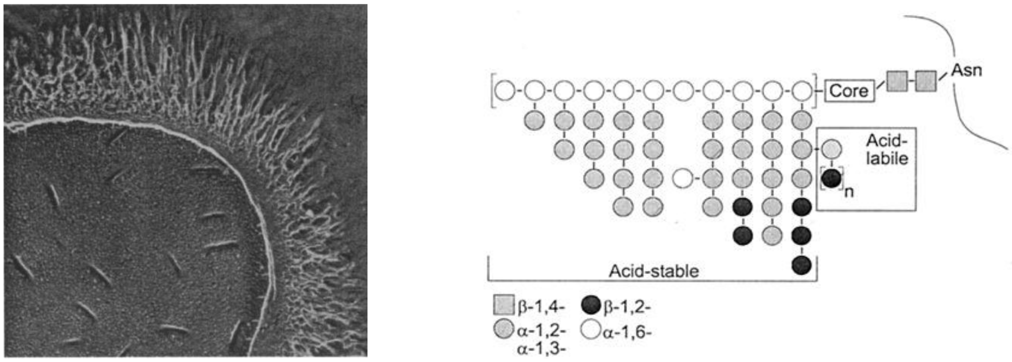


Figure 1. The cell wall of *C. albicans* (left) and the schematic structure of the phosphopeptidomannan (right). Reproduced with permission from the American Society for Microbiology.[1c]

2 Results and Discussion

2.1 Synthesis of β-(1→2)-Linked Oligomannosides

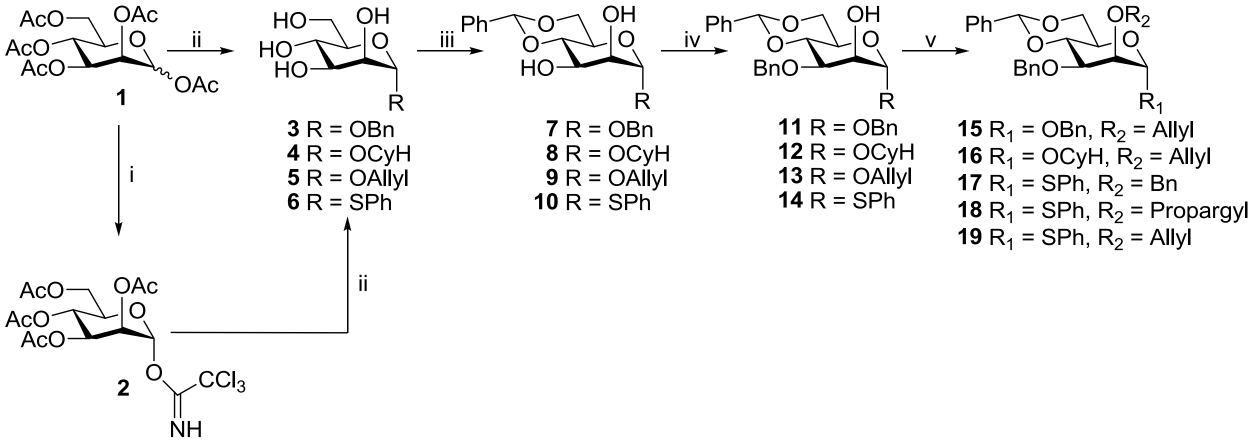
Considerable effort has been devoted to solving the challenges related to the chemical synthesis of β-(1→2)-linked oligomannosides due to their biological potential. As also mentioned in the introductory paragraph, synthesis of β-linked mannosides is challenging for various reasons. The greatest challenges are the thermodynamic and kinetic preference for formation of the α-anomer.[7] To date, three main pathways (and variations thereof) for constructing the β-mannosidic linkage have emerged.

The first method published by Hindsgaul in 1994 features molecular tethering of the acceptor and donor prior to the glycosylation reaction via intramolecular aglycon delivery.[8a] Although the β-selectivity was excellent as a result of the direct attack from the β-face of the ring, a significant drawback of the methodology was its low efficiency in the construction of β-(1→2)-mannosidic linkages,[11] which in turn was the linkage of interest in our studies.

The second approach, developed initially by Lichtenthaler and later modified by Bundle and coworkers[12] features the utilization of a glucopyranosyl trichloroacetimidate donor (ulosyl donor by Lichtenthaler). In this approach, the β-glucosidic linkage is constructed first, followed by epimerization at C-2 by an oxidation-reduction sequence to generate the β-linked mannoside. While this methodology is appealing, both the glycosylation and the epimerization yields have been shown to decline when moving towards larger oligosaccharides.[8b]

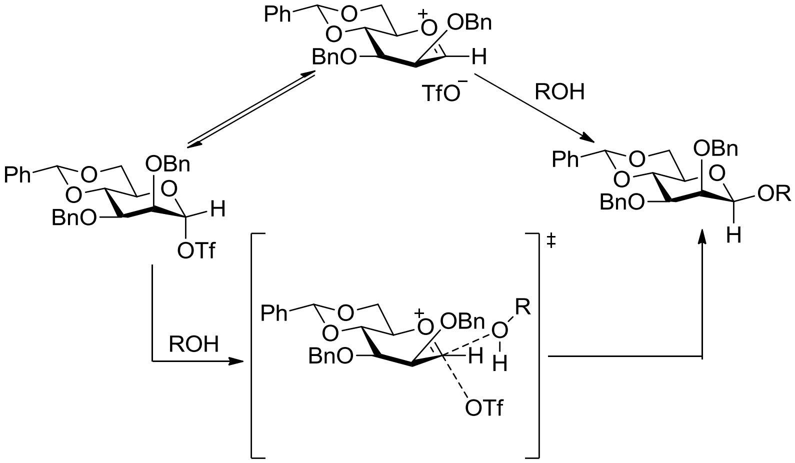
The third and final pathway to β-linked mannosides, developed by Crich and coworkers,[8e] features a highly selective direct coupling of two appropriately protected mannopyranoside building blocks. This protocol is based on the triflate activation of a 4,6-*O*-benzylidene protected mannopyranosyl sulfoxide donor in the presence of TTBP at –60 °C, followed by an S_N2-like reaction with a glycosyl acceptor. Crich and coworkers have shown the robustness of this methodology both in their synthesis of a cyclohexyl capped β-(1→2)-linked mannooctasaccharide and several oligomannosides in the β-(1→3)- and β-(1→4)-series.[8e,9a,13]

The Crich protocol was utilized here as a starting point for the synthesis of β-(1→2)-linked oligomannosides due to its robustness and high efficiency. Synthesis of the building blocks, following previously reported procedures,[10a,13b,14] commenced by the route shown in Scheme 1. In short, peracetylated mannose was subjected to $BF_3{\cdot}OEt_2$ promoted glycosylation with various acceptors and the corresponding glycosides were easily deacetylated under Zemplén conditions[15] thereby providing compounds **3**–**6**. When the glycosylation was tested with cyclohexanol the yield was only around 5 %. Therefore, the more reactive imidate donor **2**[16] was utilized in the glycosylation with cyclohexanol, thus providing cyclohexyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside in 69 % yield. The deprotected molecules **3**–**6** were converted into the 4,6-*O*-benzylidene protected substrates **7**–**10** with benzaldehyde dimethylacetal under acidic conditions.[17] The moderate yields of this reaction are due to the *cis* relationship of the 2- and 3-hydroxyl groups which decreases the yield of the desired product by formation of a dibenzylidene protected mannoside.[18] Substrates **7**–**10** were converted to acceptors **11**–**14** by selectively benzylating the equatorial 3-*OH*-group through alkylation of a 2,3-dibutylstannylene acetal intermediate with benzyl bromide.[19] The thio acceptors were converted into two different donors, bearing either a 2-*O*-benzyl or 2-*O*-propargyl protective group, for screening the effects of the protective group at C-2 on the β-selectivity of the glycosylation reaction. Furthermore, the allyl glycoside **13** and the compounds bearing a 2-*O*-allyl protective group (**15**, **16**, **19**) could potentially be applied in a cross coupling metathesis pathway for generation of divalent carbohydrates.[10a,20] With the building blocks synthesized, the attention was turned towards the preparation of oligosaccharides.



Scheme 1. i) 1. Hydrazine acetate, DMF, 55 °C, 2 h, 95 %; 2. DBU, CCl_3CN, CH_2Cl_2, r.t., 1.5 h, 72 %; ii) 1. $BF_3{\cdot}OEt_2$, corresponding alcohol (BnOH, CyOH, AllOH, SHPh), CH_2Cl_2, r.t., 24 h; 2. NaOMe, MeOH, rt, 3–24 h, **3** (75 %), **4** (63 %), **5** (73 %), **6** (90 %); iii) PTSA, $C_6H_5OCH(OMe)_2$, DMF, 60 °C, 200 mbar, 2 h, **7** (56 %), **8** (57 %), **9** (51 %), **10** (53 %); iv) 1. Bu_2SnO, toluene, 120 °C, 3 h; 2. TBAB, CsF, BnBr, 120 °C, 3 h, **11** (75 %), **12** (84 %), **13** (80 %), **14** (85 %); v) NaH, corresponding bromide, DMF, r.t., 1–3 h, **15** (96 %), **16** (95 %), **17** (96 %), **18** (94 %), **19** (95 %).

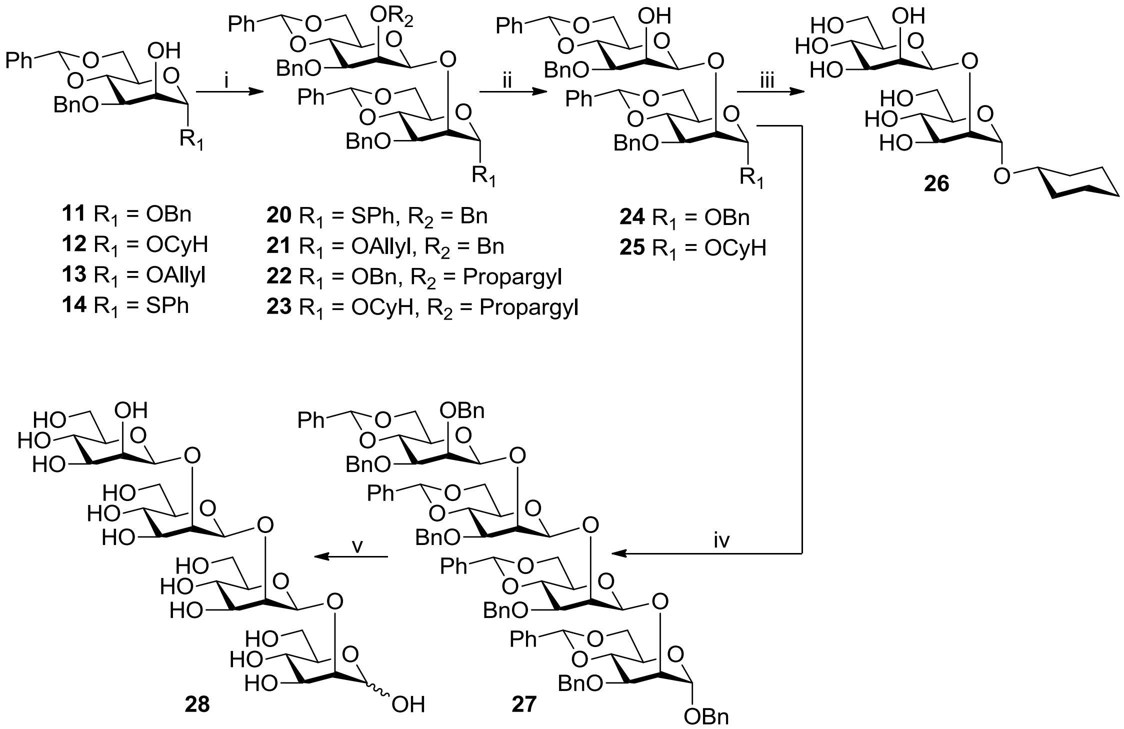
In the β-mannosylation protocol originally reported by Crich and utilized here for the current synthesis, the thioglycoside is activated with Tf_2O in the presence of TTBP and BSP in CH_2Cl_2 at –60 °C to yield an α-mannopyranosyl triflate.[8e] To the activated donor species is then added the corresponding acceptor at –78 °C. After 1.5 hours the reaction is essentially completed and the disaccharide isolated by conventional work-up procedures.[21] Mechanistic studies by Crich have revealed that the selectivity of the reactions most probably stems from a dissociative mechanism involving a transient contact ion pair intermediate which is in equilibrium with the α-mannopyranosyl triflate as depicted in Scheme 2.[22]



Scheme 2. The proposed β-mannosylation mechanism.

The initial glycosylation reaction was performed with donor **17** and acceptor **14** in order to produce a disaccharide donor for the tetrasaccharide synthesis. The reaction proceeded with a β:α-selectivity of 10:1 and disaccharide **20** could be isolated in up to 76 % yield. A similar selectivity (β:α 10:1) was observed when donor **17** was coupled with acceptor **13** thereby showing a clear connection between selectivity and the 2-OBn protective group. Next, donor **18**, bearing a 2-*O*-propargyl group, was evaluated in the glycosylation of acceptors **11** and **12**. Both glycosylations were found to proceed smoothly thus providing disaccharides **22** and **23** in high yields and excellent selectivities (β:α 20:1). Similar results were reported already in 2005 by Crich and coworkers. In their work, the effect was suggested to be a combination of the minimal steric bulk and disarming nature of the propargyl group.[13a] The propargyl group was next cleaved, in yields ranging from 80–85 %, by isomerization of the triple bond with ^{t}BuOK to give an allenyl ether followed by oxidative cleavage utilizing NMO/OsO_4 in acetone/water.[13b,23] The cyclohexyl disaccharide **25** was deprotected by hydrogenolysis (2.8 bar H_2-pressure) to give the cyclohexyl capped trisaccharide mimic **26** in 94 % yield.

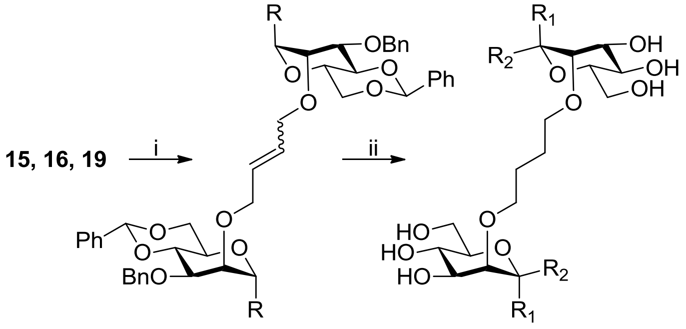
After experimenting on the synthesis of disaccharides and smaller building blocks, a route for the synthesis of a fully deprotected β-(1→2)-linked mannotetraose was developed.



Scheme 3. i) 1. Donor **17** or **18**, TTBP, BSP, Tf_2O, CH_2Cl_2, –60 °C, 0.5 h; 2. corresponding acceptor –78 °C, 3 h, **20** (78 %), **21** (72 %), **22** (74 %), **23** (72 %); ii) 1. tBuOK, THF, r.t., 5 h; 2. OsO_4, NMO, acetone/H_2O (4:1), rt, 3 h, **24** (83 %), **25** (85 %); iii) H_2, Pd/C, MeOH:EtOAc (10:1), 2.8 bar, r.t., 19 h, 98 %; iv) 1. **20**, TTBP, BSP, Tf_2O, CH_2Cl_2,–60 °C, 0.5 h; 2. **24** –78 °C, 3 h, 55 %; v) H_2, Pd/C, MeOH, 2.8 bar, r.t., 19 h, 75 %.

The synthesis of larger oligosaccharides can in general be considered as the ultimate proving ground for any glycosylation methodology. In the linear glycosylation protocol utilized by the research group of Crich, the selectivity of the individual glycosylation reactions was found to decline rapidly when progressing towards the tetrasaccharide stage (from β:α 9.9:1 for the trisaccharide to 3.9:1 for the tetrasaccharide).[8e] Despite the decrease in selectivity, a high efficiency was maintained throughout the entire synthetic pathway. Bundle and coworkers, on the other hand, noted in their linear synthesis of a hexasaccharide in the β-(1→2)-series that the efficiency was significantly reduced from 65 % at the trisaccharide stage to 48 % at the tetrasaccharide stage when utilizing glucose-based imidate donors.[8b] Based on these two approaches it seemed obvious that the linear approach may not be the best alternative for constructing larger oligosaccharides. In general, the convergent approach has several advantages over the linear one, the most important being a shorter synthetic route.[24] One previous study on convergent synthesis, featuring a disaccharide/disaccharide coupling reaction for the construction of a β-(1→2)-linked mannotetrasaccharide, has been reported by Mallet and coworkers.[8d] In their work, however, neither the selectivity in the crucial glycosylation reaction nor the NMR spectroscopic data of the compounds were reported. In addition, three different types of protective groups were utilized leading to a three step deprotection sequence in order to obtain the target tetrasaccharide. In the present work, a convergent strategy featuring disaccharide donor **20** and disaccharide acceptor **24**, containing only one type of protective groups, was utilized. The reaction between the two disaccharide building blocks proceeded smoothly following the standard activation protocol and tetrasaccharide **27** could, after work-up, be isolated in 55 % yield (β:α 10:1).[10c] In comparison with the earlier reported linear approach,[8e] the selectivity in the crucial glycosylation was more than two times higher when the convergent protocol was applied. The yield was only slightly lower than those reported earlier by Crich and coworkers[8e] (53 % yield, starting from the disaccharide acceptor and monosaccharide donor) but slightly higher than the yield reported by Bundle *et al.*[8b] (31 % yield, starting from the disaccharide acceptor and monosaccharide donor). In contrast to the convergent approach of Mallet,[8d] the deprotection of the β-(1→2)-linked mannotetrasaccharide was here achieved in one step by hydrogenolysis instead of the three step deprotection sequence required in their approach. The hydrogenolysis of **27** under 2.8 bar H_2–pressure in methanol proceeded smoothly to provide **28**. The yield was highly dependent on the deprotection conditions. When 2.5 weight equiv. of Pd/C (10 % Pd) was utilized with a substrate concentration of 10 mg/ml (in MeOH), a reduced tetrasaccharide containing mannitol at the reducing end was obtained in 40 % yield. The formation of this byproduct was verified by both HRMS (*m/z* 691.2281 $[M + Na]^+$) and NMR spectroscopy showing three anomeric signals in the ^{1}H-NMR spectrum at 4.89, 4.82 and 4.81 ppm and in the ^{13}C-NMR spectrum at 101.1, 100.5 and 99.7 ppm. Formation of this undesired side-product was strongly suppressed when two weight equiv. Pd/C was used and the concentration of **28** was 20 mg/ml (in MeOH), thereby increasing the yield of the desired product to 75 % (for purification details, see the experimental section).

With the tetrasaccharide fragment synthesized, the attention was next turned towards the synthesis of divalent carbohydrates.[10a] Multivalent carbohydrate structures are in general considered to be important due to their ability to target multiple receptors simultaneously which in many cases leads to an increase in the biological response.[25] Divalent molecules are the simplest form of multivalent compounds and, therefore, ideal systems to start the exploration of the effects of multivalency on the biological activity. In this work, cross-coupling metathesis (CCM)[26] featuring Grubbs 1st generation catalyst was utilized as a tool for the creation of divalent mannose derivatives. This is also apparent since many of the building blocks in Scheme 1 and 3 contain a terminal alkene.[10a] It should be noted that the applications of “Click” chemistry, especially the coupling between an alkyne and an azide,[27] were rather limited in the field of carbohydrates at the outset of these investigations. Later on, “Click” chemistry has been shown to be a simple, efficient and robust method for the preparation of multivalent carbohydrate assemblies.[28] The main limitations for the use of CCM in the construction of divalent carbohydrates have previously been the poor diastereoselectivities sometimes observed and the fact that, in contrast to ring-closing olefin metathesis, the CCM reaction is not driven by an increase in entropy.[29]



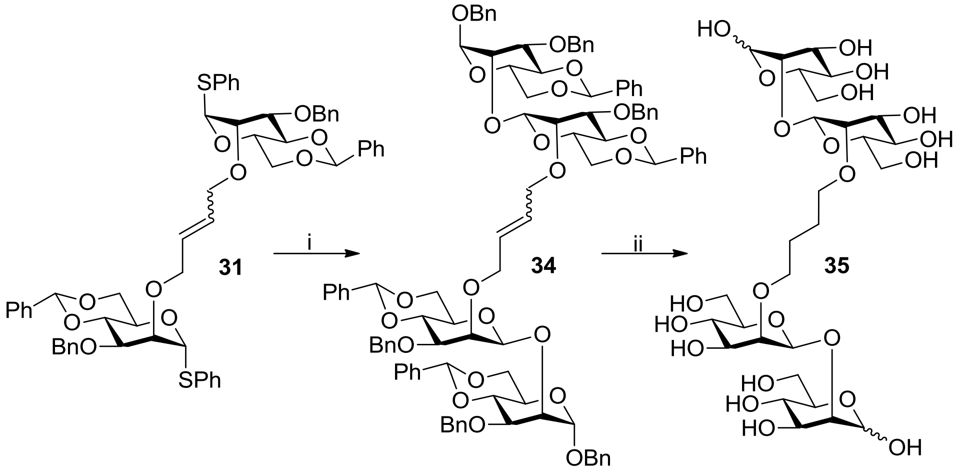
29 R = OBn
30 R = OCyc
31 R = SPh

32 R_1 = OH, R_2 = H or R_1 = H, R_2 = OH
33 R_1 = OCyc, R_2 = H

Scheme 4. i) Grubbs 1st generation catalyst, CH_2Cl_2, 40 °C, 6 h, **29** (95 %), **30** (75 %), **31** (74 %); ii) H_2, Pd/C, MeOH:EtOAc (9:1), 1.4 bar, r.t., 18 h, **32** (70 %), **33** (70 %).

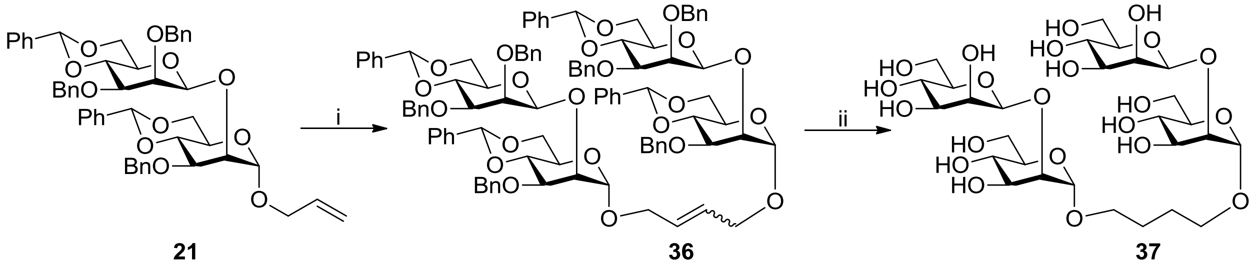
In order to evaluate cross coupling metathesis as a methodology for the preparation of divalent carbohydrates, the monosaccharide building blocks **15**, **16** and **19** bearing a terminal alkene moiety were screened in the reaction following experimental procedures reported previously by Roy and coworkers.[30] Homodimerizations of compounds **15**, **16** and **19** proceeded with excellent diastereoselectivites (*E*/*Z* ratios up to 20:1) to give the divalent compounds **29**–**31** in good to excellent yields after purification.[10a] In previous examples, *E*/*Z* ratios ranging from 1:1 to 6:1

have been observed in homodimerization reactions.[29a,31] Higher selectivities have been witnessed previously also when the two starting moieties were tethered together prior to the CCM reaction.[32] The high diastereoselectivites observed here may be due to the protective groups present in compounds **15**, **16** and **19**. Furthermore, electron rich substituents at the anomeric position influence the diastereoselective outcome of the reaction as the benzyl and thiophenyl mannosides gave higher selectivities than the cyclohexyl mannoside. The selectivity of the CCM reaction was not important in this study since the newly formed alkene functionality is hydrogenated in the hydrogenolyses reaction. For future modifications and development of CCM reactions, however, it may turn out to be useful information. Compounds **29** and **30** were deprotected by hydrogenolyses to give the divalent 2,2′-linked mannosides **32** and **33** in 70 % yield.



Scheme 5. i) 1. TTBP, BSP, Tf_2O, –60 °C, 0.5 h; 2. **11** –78 °C, 3 h, 70 %; ii) H_2, Pd/C, MeOH:EtOAc (9:1), 1.4 bar, r.t., 18 h, 50 %.

With the divalent monosaccharides prepared, the study on β-linked mannosides was extended and finalized by the synthesis of divalent disaccharides. For this purpose, divalent donor **31** and allyl glycoside **21** were utilized. The divalent donor **31** was activated as previously described[8e] and coupled with acceptor **11** to give the divalent disaccharide **34**, after work-up, in 70 % yield as a single ββ-anomer. To our knowledge, this was the first time when several leaving groups were activated simultaneously in the synthesis of β-linked mannosides. Due to the high efficiency in the double glycosylation reaction, similar strategies might be applied also to the synthesis of other structures containing interconnected carbohydrates attached at multiple locations in the aglycone. Compound **35** could after hydrogenolyses be isolated in 50 % yield as a mixture containing an 8:2:1 ratio of αα:αβ:ββ-anomers.[10a]



Scheme 6. i) Grubbs 1st generation catalyst, CH_2Cl_2, 40 °C, 6 h, 65 %; ii) H_2, Pd/C, MeOH:EtOAc (9:1), 1.2 bar, r.t., 18 h, 95 %.

Synthesis of the divalent 1,1′-linked disaccharide **37** commenced by the route shown in Scheme 6. The homodimerization of **21** gave a slightly lower yield than the monosaccharides, most probably due to the significant size difference which adds a factor of steric constrain. Also the *E*/*Z* value of 5:1 in the reaction was poor compared to the *E*/*Z* values in the homodimerization of monosaccharides **15**, **16** and **19**. Hydrogenolyses of **36** proceeded smoothly to afford **37** as a colorless oil in 95 % yield.

Overall, a variety of β-linked mannosides including a trisaccharide mimic, a fully deprotected mannotetraose and divalent mono- and disaccharides were prepared and subjected for biological screening of their immunostimulatory effects. While the biological data is not addressed here in more detail, it should be mentioned that some deviances from earlier reported TNF-α values were found in the cytokine stimulation assay.[1a–c] When synthesizing oligosaccharides of this type, it is important to ensure the integrity of the synthesized compounds. This is usually achieved with a number of different methods including NMR spectroscopy, HRMS and optical rotation. The NMR spectroscopic characterization of oligosaccharides can sometimes be complicated due to overlapping signals in the ^{1}H-NMR spectra. The β-linked mannopyranosides add a few more challenges than the average oligosaccharides and, therefore, the next section will be devoted to the NMR spectroscopic characterization of these substrates.

2.2 NMR Spectroscopic Characterization

It would be redundant to discuss the NMR spectroscopic characterization of all synthesized compounds here and, therefore, the NMR spectroscopic characterization will be exemplified by the complete characterization of compounds **27** and **28** (Figure 2). All other molecules were assigned by the use of similar NMR spectroscopic techniques.

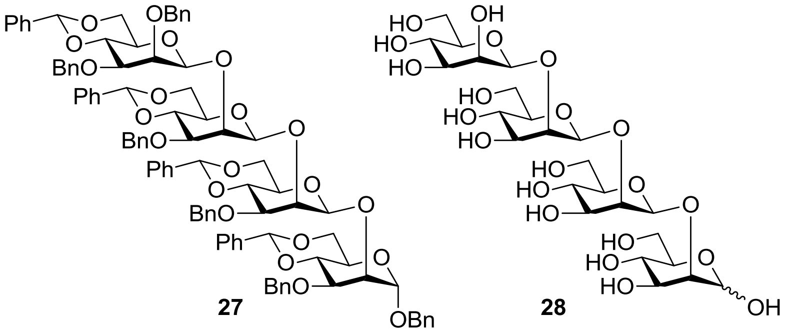


Figure 2. The chemical structures of compounds **27** and **28**.

During the convergent synthesis of **27** it was important to verify the linkages in the tetrasaccharide formed. In general, the H-1–H-2 coupling constants are utilized in determination of α:β-ratios of carbohydrates. In the case of mannopyranosides, however, these values differ insignificantly (difference ~ 1 Hz) and cannot be used as a reliable measure. In the literature, the chemical shifts of H-3 and H-5 protons in β-linked mannopyranosides have been reported to appear at a considerably lower frequency than in the corresponding α-linked mannopyranosides.[8e,10b,33] Furthermore, the C-5 carbons have been suggested to appear at a slightly higher frequency in the case of β-linked mannopyranosides as compared to α-linked mannopyranosides. In the ^{1}H-NMR spectrum of **27** (Figure 3), chemical shifts for the three H-5$_{\beta}$ protons were observed at low frequencies (3.42 ppm for H-5″, 3.31 ppm for H-5′ and 3.30 ppm for H-5‴) in comparison to the chemical shift of H-5$_{\alpha}$ (3.86 ppm for H-5) thereby suggesting that the β-anomer had indeed been formed in the disaccharide/disaccharide coupling reaction. A similar difference was witnessed in the ^{13}C-NMR spectrum (Figure 4) where the three C-5$_{\beta}$ carbons (67.9 ppm for C-5″, 67.8 ppm for C-5′, 67.5 ppm for C-5‴) appear at slightly higher frequency than the C-5$_{\alpha}$ (64.2 ppm for C-5).

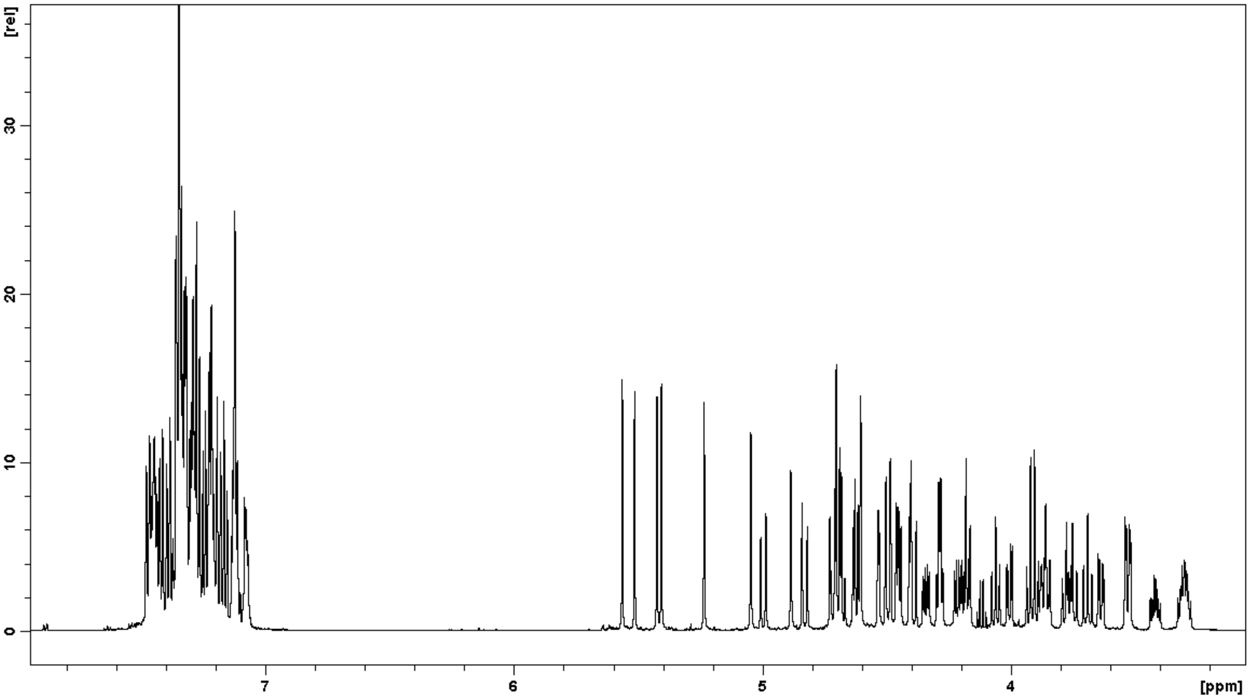


Figure 3. The region between 8.00–3.00 ppm in the ^{1}H-NMR spectrum of **27**.

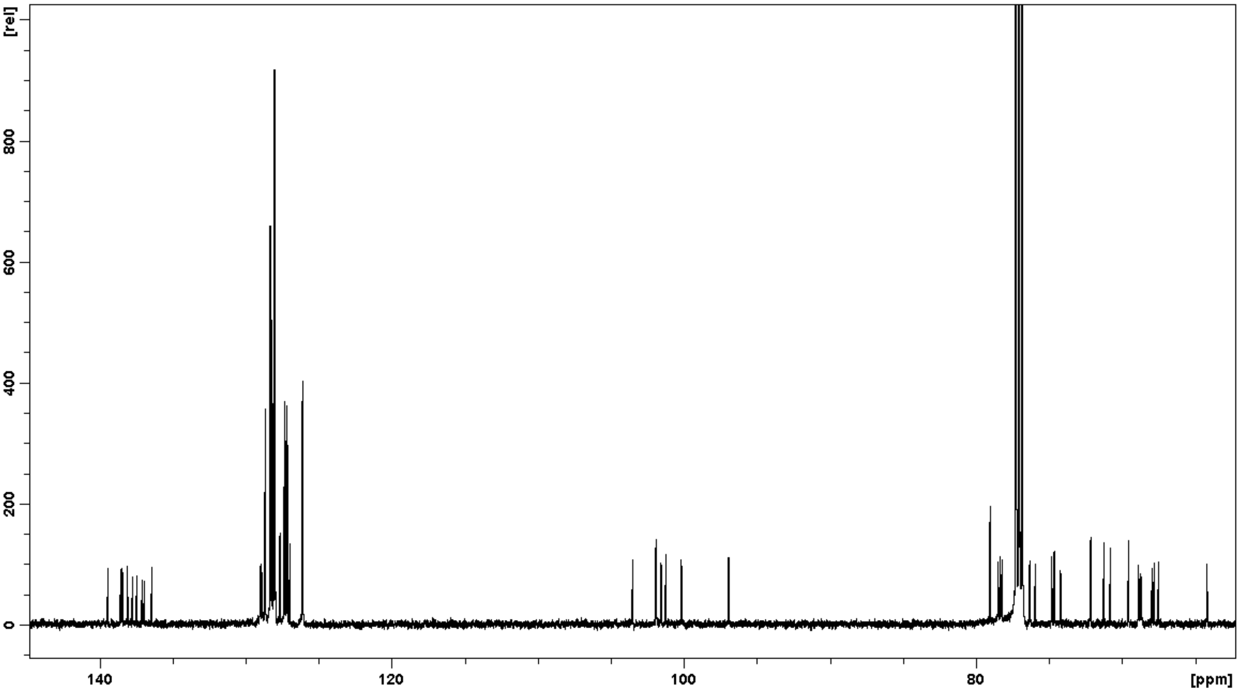


Figure 4. The region between 145.0–60.0 ppm in the ^{13}C-NMR spectrum of **27**.

Furthermore, an up-field shift of the three H-3$_{\beta}$ protons (3.64 ppm for H-3′, 3.53 ppm for H-3‴ and H-3″) was observed further indicating a different linkage than the one existing in the first residue (4.01 ppm for H-3). As mentioned earlier, these results are in accordance with the general view on the chemical shifts of α- and β-mannopyranosides.[8e,10b,33] In general, the full spectral characterization of complex oligosaccharides is one of the most demanding tasks in carbohydrate chemistry.[34] For this purpose, 1D-TOCSY (1D-TOtal Correlation SpectroscopY) proved to be a valuable tool.[35] Well separated signals corresponding to the different ring units were targeted by selective excitation with a spinlock time of 500 ms being suitable for assuring magnetization spreading to the entire ring-system. The 1D-TOCSY spectra of the different residues of **27** are shown in Figure 5.

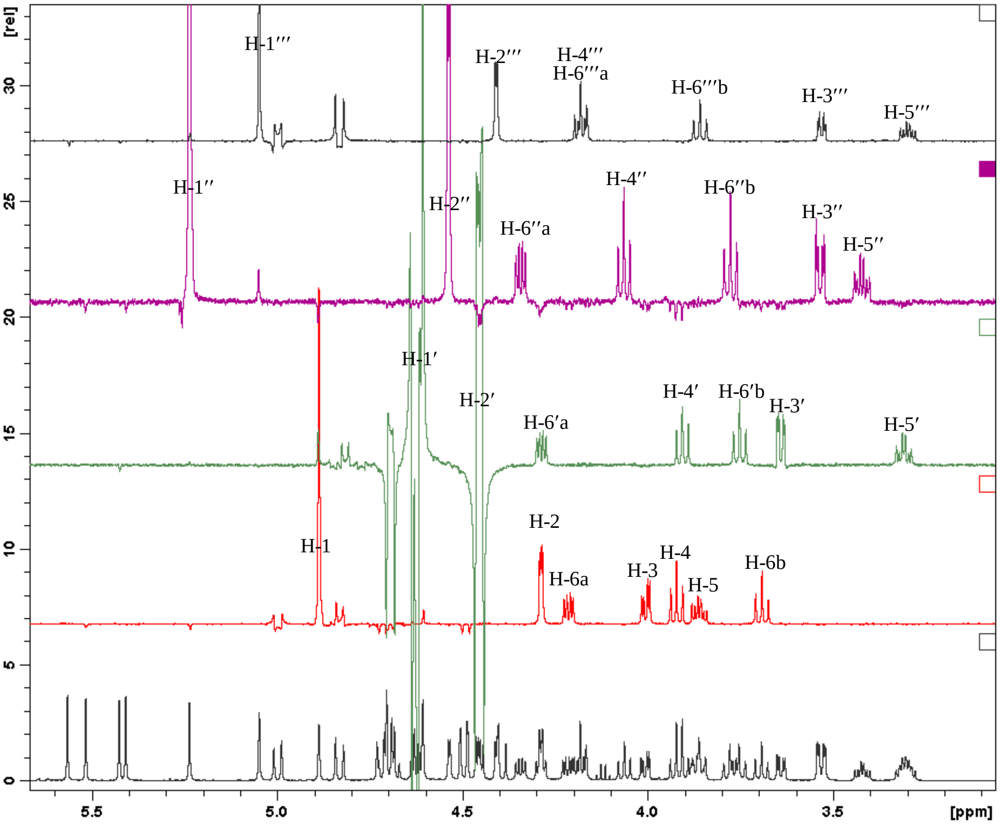


Figure 5. 1D-TOCSY spectra of the different residues and the entire ^{1}H-NMR spectrum of the carbohydrate region of **27**.

As also seen from Figure 5, the use of 1D-TOCSY reduces the spectral complexity to a monosaccharide level. The individual 1D-TOCSY spectra are easier to interpret as the amount of overlapping signals is reduced. In order to obtain accurate coupling constants, the spectral simulation software PERCH (Peak ResearCH) was utilized.[36] The coupling constants obtained (although not reliable as such) by simulation of the ^{1}H-NMR spectrum clearly indicated a difference between the H-1–H-2 coupling constants for the α-linked residue ($J_{1,2}$ = 1.6 Hz for H-1) and the β-linked ones ($J_{1'',2''}$ = 0.3 Hz for H-1″, $J_{1''',2'''}$ = 0.8 Hz for H-1‴ and $J_{1',2'}$ = 0.7 Hz for H-1′). Ultimately, the linkages were proven by the use of coupled HSQC showing large differences in the $J_{\mathrm{C,H}}$ coupling contants ($J_{\mathrm{C\text{-}1''',H\text{-}1'''}}$ = 158.3 Hz for C-1‴, $J_{\mathrm{C\text{-}1'',H\text{-}1''}}$ = 158.6 Hz for C-1″, $J_{\mathrm{C\text{-}1',H\text{-}1'}}$ = 156.0 Hz for C-1′ and $J_{\mathrm{C\text{-}1,H\text{-}1}}$ = 167.8 Hz for C-1).[37] The order of the residues and positions of protecting groups were determined by HMBC correlations by conventional methods.

In order to fully assign the ^{1}H-NMR spectrum of **28**, a similar set of NMR spectroscopic methods was applied. The NMR spectrum of a tetrasaccharide alone is in many cases problematic to solve. Here, due to mutarotation, an α:β ratio of 7:2 for **28** was observed thereby further complicating the assignment. Again, 1D-TOCSY proved to be a powerful tool and was utilized to overcome the complications.[35] Compound **28** gives rise to well separated signals corresponding to the H-1 and H-2 protons on the different residues of both anomers. These signals were also targeted using 1D-TOCSY. The separation was found to be highly influenced by the NMR-solvent used (MeOD vs. D_2O). In MeOD, an enhanced separation of the crucial signals was observed. From the biological standpoint, however, D_2O is of more interest and, accordingly, NMR spectroscopic data was recorded in both solvents. The importance of an improved signal separation is significant and thus the following discussion is based on the NMR spectra recorded in MeOD. By narrowing the excitation range in 1D-TOCSY, even signals separated by only 6 Hz could be selectively excitated to give the ^{1}H-NMR spectrum of a single residue. With the eight individual 1D-TOCSY spectra recorded, one for each of the eight residues present in the ^{1}H-NMR spectrum, the attention was turned towards determination of the order of residues. For this purpose, the ^{13}C chemical shifts of the reducing end anomers were chosen as a starting point. These signals appear at a considerably lower frequency (94.1 ppm for C-1α and 95.5 for C-1β) when compared with the other anomeric carbons (between 103.3–101.1 ppm). In HMBC, the long range coupling between H-2-C-1′ was clearly visible. The inverse coupling between H-1′-C-2 was also observed. By iteration of this approach, the different monomers were determined in order starting from the reducing end residue. H-5‴ was shown to give a signal at a higher frequency in both anomers

(3.36 ppm for H-5‴α and 3.39 for H-5‴β) than the remaining of the H-5$_β$ protons (between 3.25–3.20 ppm). This also indicates that the residues were assigned correctly. In D_2O this effect does not occur and therefore all the H-5$_β$ protons appear at a similar chemical shift. Furthermore, the 2D-NOESY spectrum showed correlations between H-1$^{n+'}$ and H-2^{n} (where n= 0, ′, ″) which were in agreement with the assignment based on the HMBC spectrum. Determination of the order of residues of the α-anomer of **28** by 2D-NOESY is depicted with red arrows in Figure 6.

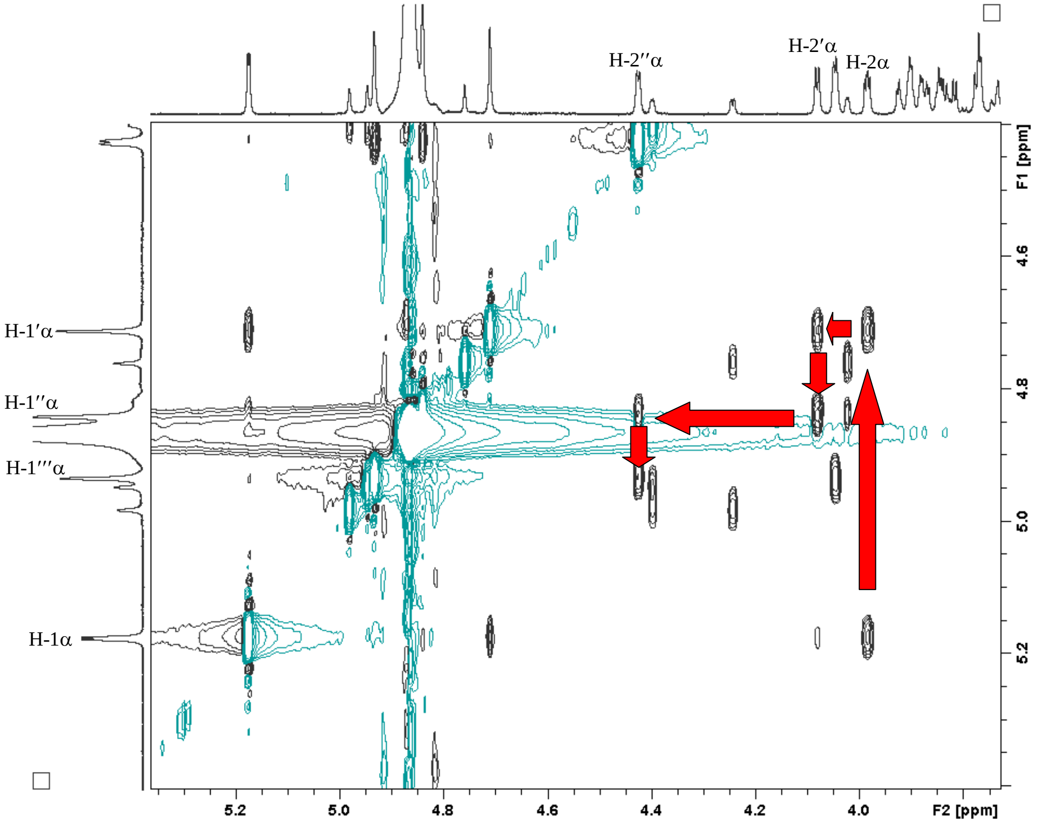


Figure 6. Determination of the order of residues in the α-anomer of **28** with 2D-NOESY.

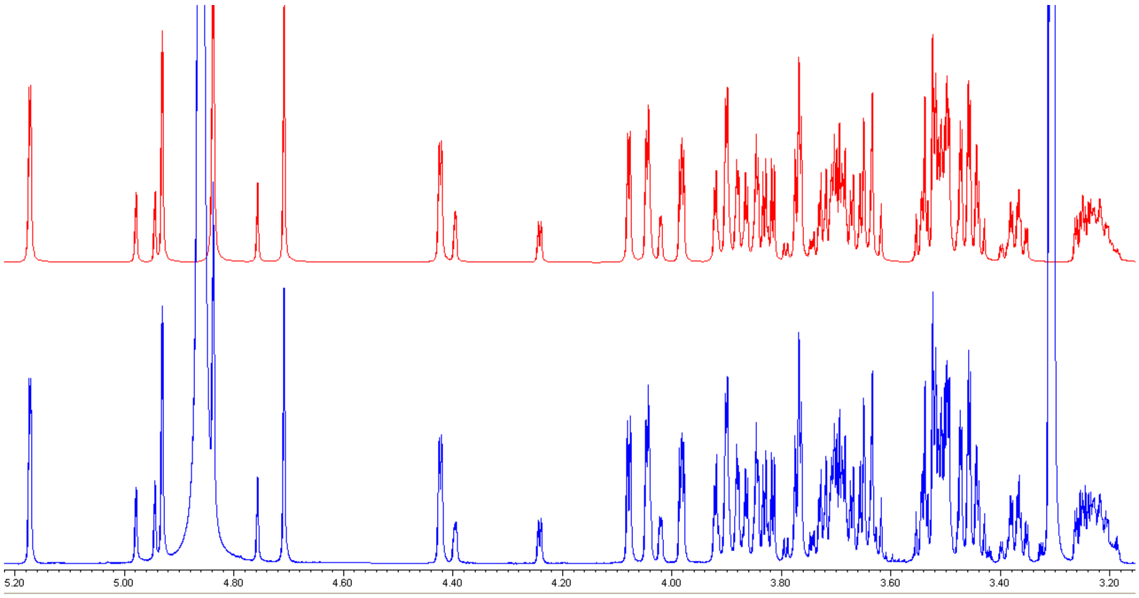


Figure 7. Spectral simulation of the ^{1}H-NMR spectrum of **28** in MeOD (5.2–3.1 ppm region) with Perch NMR software: simulated spectrum (above), observed spectrum (below).

The information gained by 2D-NOESY will be further discussed below when assessing the conformation of this molecule. With the information received from these standard NMR spectroscopic techniques (^{1}H, ^{13}C, DQF-COSY, HSQC, HMBC, 1D-TOCSY and 2D-NOESY), the ^{1}H-NMR spectrum was simulated with PERCH.[10c] In order to simplify the matter, the individual 1D-TOCSY spectra were first simulated followed by simulation of the complete spectrum containing both anomers. The result of the simulation is shown in Figure 7. With the NMR spectroscopic characterization completed, several NOESY correlations between non-contiguous residues were observed in the 2D-NOESY spectrum of **28**. Intrigued by these findings a more detailed conformational analysis of the fully deprotected β-(1→2)-linked mannotetraose was performed.

2.3 Conformational Analysis

The β-(1→2)-linked oligomannosides were predicted to form interesting three dimensional structures more than 30 years ago.[9c] The earlier work by Bundle and coworkers on a β-(1→2)-linked mannopentasaccharide revealed these assumptions to be correct.[9b] NOE-contacts between non-contiguous residues were witnessed suggesting that a contorted α-helical-like structure is formed. These studies were conducted with propyl-capped glycosides. Further evidence of their contorted nature was reported by Crich[9a] based on a crystal structure of a fully protected tetrasaccharide. In the present study, the conformational analysis was performed with the unprotected tetrasaccharide **28**. In contrast to the previous studies where β-linked glycosides have been used, the discussion below is based on the information of the dominating α-anomer of the hemiacetal.

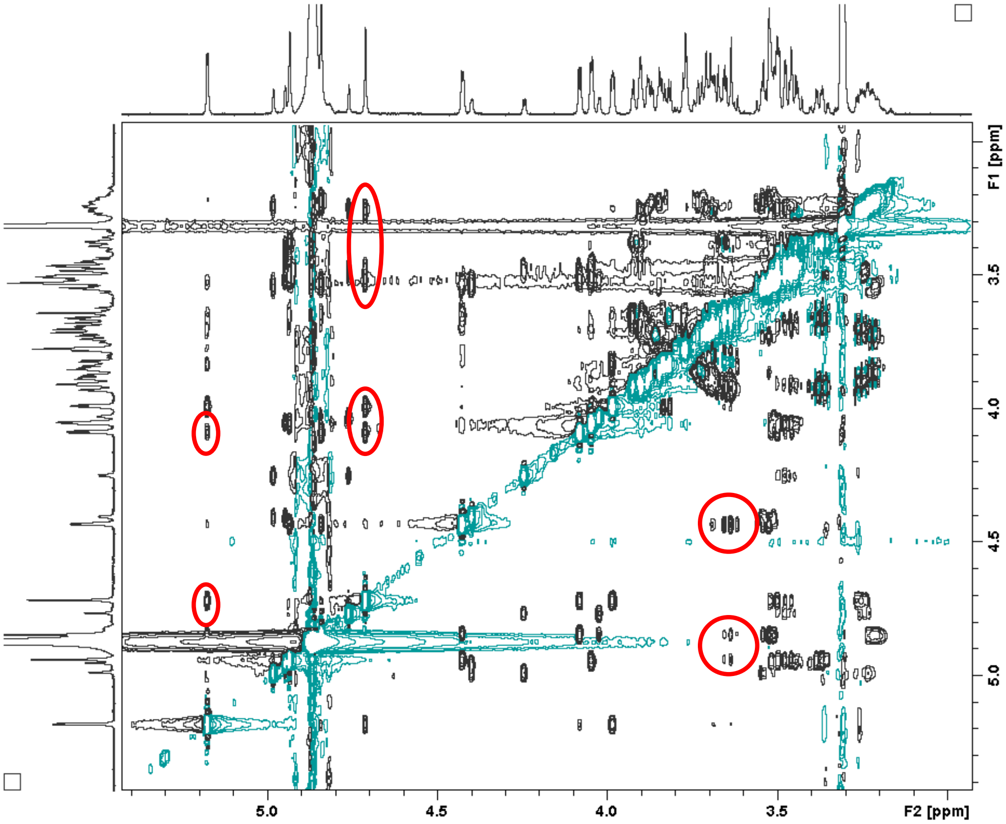


Figure 8. 2D-NOESY spectrum of **28** in MeOD displaying the correlations between protons close in space. The most important correlations are highlighted by a red circle.

In the ^{1}H-NMR spectrum of **28** recorded in MeOD, the key signal for this conformational study was well separated (H-4, dd at 3.63 ppm). This was not the case in D_2O. From the 2D-NOESY spectrum the correlations also witnessed by Bundle and coworkers were confirmed.[9b] The most important correlations between the non-contiguous residues are H-4–H-2″, H-4–H-1″ and H-4–H-1‴ (Figures 8 and 9). The H-4–H-2″ correlation was stronger than the H-4–H-1″ and H-4–H-1‴ correlations as also seen in Figure 8. This information confirms the contorted structure predicted several years ago and witnessed by Bundle[9b] and Crich.[9a] A correlation between H-1–

H-1′ and H-1–H-2′ was observed here for the first time. The lack of this correlation in the study of Bundle and coworkers may have been partly due to the use of propyl-capped β-linked mannoglycosides. As mentioned earlier, 2D-NOESY correlations between H-$1^{n+\prime}$ and H-2^{n} (where n= 0, ′, ″) were witnessed thereby suggesting steric interactions to place the residues in a particular order. In addition, correlations between H-1–H-2, H-1–H-3 and H-1–H-5 of the β-linked residues along with the accurate coupling constants obtained by PERCH suggest the different residues to exist in the $^{4}C_{1}$ chair conformation. To further investigate and verify the conformation suggested by NMR, computational methods were utilized to model the preferred structure.

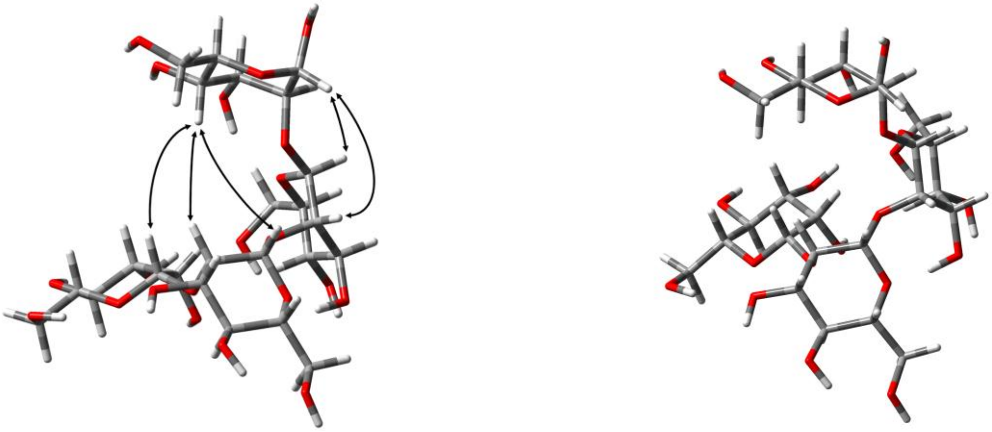


Figure 9. The B3LYP/6-31G(d,p) optimized structure of the α-anomer showing the most important NOESY correlations on the left and the α-helical nature of **28** on the right.

The initial structure was first sketched by rapid modeling using molecular mechanics with Chem3D Pro 11.0 software. The geometry of the obtained structure was further optimized by DFT method B3LYP using 6-31G(d,p) as a basis set. This method has been found to give good results in a reasonable time for fairly large molecules.[38] Since the potential energy surface for relatively flexible molecules may consist of several minima, the initial optimization did not find the structure corresponding to all of the NMR spectroscopic (especially NOE) results. Therefore, the torsion angles were modified in order to obtain a starting structure in agreement with the witnessed NOE correlations. When this structure was optimized, a stationary point was found which was in full agreement with the experimental results. Frequency calculation was performed in order to prove that the stationary point found was a minimum energy point. The optimized structure is presented in Figure 9. In the optimized structure, the distances between the protons for the key NOE connectivities were found to be 3.19 Å (H-4-H-2′′), 4.13 Å (H-4-H-1′′) and 3.65 Å (H-4-H-1′′′). These values were in good agreement with the cross-peak intensities in the NOESY spectrum. All mannose moieties were found to exist in the $^4C_1$ conformation as expected. The optimized results also show the contorted α-helical nature of the molecule, although, a tetrasaccharide does not seem to be sufficient in length for forming a complete helix (Figure 9).

As seen from the models shown in Figure 9, the glycosidic linkages appear to be hidden in the middle of the contorted α-helical-like structure creating a hydrophobic interior. The hydroxyl groups are oriented outwards further creating a hydrophilic exterior. A similar pattern was also suggested in the previous studies.[9b] The nature of these molecules are but one example of the large diversity of three dimensional structures often found in naturally occurring carbohydrates. The creation of models by molecular modeling together with the complete NMR spectroscopic characterization of these compounds provide important reference material for others working with similar substrates and contribute to the overall understanding of the biological nature of such molecules.

3 Conclusions

A variety of β-(1→2)-inspired mannopyranoside derivatives were prepared by chemical synthesis. Based on the presented findings, a convergent route to larger oligomannosides may be a suitable alternative to the earlier reported linear approaches. In addition, several divalent molecules were prepared by utilization of highly diastereoselective CCM reactions. The synthesized molecules were prepared for biological purposes in order to evaluate their potential as *C. albicans* vaccines and as agents for the treatment of atopic allergies. The results from the immunostimulatory studies show slight deviation from the previously published literature and will be reported in detail elsewhere.

Furthermore, much effort was invested in the complete NMR spectroscopic characterization of these compounds and as a result the first fully assigned ^{1}H- and ^{13}C-NMR spectra of the fully deprotected β-(1→2)-linked mannotetraose could be reported. This information should prove valuable not only to chemists synthesizing compounds but also to scientists working with the isolation of oligosaccharides from cell walls. From the 2D-NOESY spectra of **28**, interesting cross-peaks were found revealing the three dimensional α-helical nature of these oligomannosides. It should, however, be noted that a tetrasaccharide is not sufficient in length to complete a helix-like structure. Overall, a complete study encompassing chemical synthesis, NMR spectroscopic characterization and conformational analysis of β-(1→2)-inspired mannopyranosides was accomplished and presented in this Episode.

4 Experimental Section

Reaction solvents were dried and distilled prior to use when necessary. All reactions containing moisture- or air-sensitive reagents were carried out under argon atmosphere. All chemicals were purchased from commercial sources, mainly the chemical vendors Acros and Aldrich.

The NMR spectra were recorded with Bruker Avance NMR spectrometers operating at 500.13 MHz (^1H: 500.13 MHz, ^{13}C: 125.77 MHz) or 600.13 MHz (^1H: 600.13 MHz, ^{13}C: 150.90 MHz). The probe temperature during the experiments was kept at 25 °C unless indicated otherwise. All products were fully characterized by utilization of ^1H, ^{13}C and TOCSY 1D-NMR techniques and DQF-COSY, NOESY, HSQC (both coupled and decoupled) and HMBC 2D-NMR techniques by using pulse sequences provided by the manufacturer. For more details on the pulse sequences utilized during the conformational analysis see reference [10c]. Chemical shifts are expressed on the δ scale (in ppm) using TMS (tetramethylsilane), residual chloroform, acetone, H_2O or methanol as internal standards. Coupling constants are given in Hz and provided only once when first encountered. Coupling patterns are given as *s* (singlet), *d* (doublet), *t* (triplet) etc. When signals appeared at similar chemical shifts in structures **27** and **28** additional decimals were used to determine the order of the signals. The computational analysis of the ^1H-NMR spectra of all compounds, except **35**, was achieved by utilization of the PERCH NMR software with starting values and spectral parameters obtained from the various NMR techniques used.[36]

HRMS were recorded using Bruker Micro Q-TOF with ESI (electrospray ionization) or a Fison ZabSpecOaTOF with EI (electron impact) operated in positive mode. Optical rotations were measured at 23 °C with a Perkin Elmer polarimeter equipped with a Na-lamp (589 nm). TLC was performed on aluminium sheets precoated with silica gel 60 F_{254} (Merck). Preparative TLC was performed on aluminium sheets precoated with silica gel 60 F_{254} (0.5 cm, Merck). Flash chromatography was carried out on silica gel 60 (0.040–0.060 mm, Merck). Spots were visualized by UV followed by charring with 1:10 H_2SO_4/MeOH and heating. Compound **28** was purified by High-pH anion exchange chromatography (HPAEC) with a Dionex DX600 system equipped with an amperometric detector. Compounds **32**, **33** and **35** were purified by HPLC utilizing an Agilent 1100 series liquid chromatographic system.

The initial structure for modeling was drawn with ChemBioDraw Ultra 11.0 and modelled by Chem3D Pro 11.0 using a MM2 molecular mechanic method. All higher quality calculations were done by Gaussian 03 software using density functional method B3LYP as a hybrid functional and 6-31G(d,p) as basis set.[39] The results were examined by GaussView 4.1.2 program.

4.1 General Experimental Procedures

General procedure for glycosylation of 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose. To a solution containing 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose (1 equiv.) and 4 Å MS in CH_2Cl_2 (3 ml/1 mmol) was added the corresponding alcohol (1.5–4 equiv.) and the resulting mixture was stirred for 0.5 h. The mixture was then cooled with an ice bath and $BF_3{\cdot}OEt_2$ (5–8 equiv.) was added dropwise. The resulting mixture was stirred for 0.5 h, brought to r.t. and stirring was continued for 20 h. The reaction mixture was diluted with CH_2Cl_2 (8 ml/1 mmol starting material) and poured into ice-cold H_2O (16 ml/1 mmol starting material) with stirring. The organic phase was separated and washed with sat. $NaHCO_3$ (8 ml/1 mmol starting material), H_2O (8 ml/1 mmol starting material) and brine (8 ml/1 mmol starting material). The organic phase was dried over anhydrous $MgSO_4$ or Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 4:1) to give the corresponding glycoside.

General procedure for synthesis of acetylated imidate donors. To a solution containing peracetylated mannose (1 equiv.) in dry DMF (15 ml/1 g of starting material) was added hydrazine acetat (1.1 equiv.). The resulting mixture was stirred for 2 h at 55 °C, cooled to r.t., diluted with EtOAc (30 ml/1 g), washed with sat. $NaHCO_3$ (20 ml/1 g), filtered through celite whereafter the organic phase was separated. The organic phase was then washed with H_2O (2 × 20 ml/ 1 g), brine (20 ml/1 g), dried over anhydrous $MgSO_4$, filtered and concentrated to give the free hemiacetal. The hemiacetal was then dissolved in dry CH_2Cl_2 (13 ml/1 g) and DBU (12 mol%) and trichloroacetonitrile (1.2 equiv.) were added at 0 °C. The reaction mixture was stirred for 1.5 h, brought to r.t., diluted with CH_2Cl_2 (13 ml/1 g) and washed with brine (20 ml/1 g). The organic phase was dried over Na_2SO_4, filtered and concentrated to give the crude product. The crude product was purified through a plug of silica (hexane:EtOAc:Et_3N 2:1:0.01) to give the corresponding imidate donor.

General procedure for deacetylation. To a solution containing the acetylated glycoside (1 equiv.) in dry MeOH or dry MeOH:THF mixture (2:1) (1 ml/100 mg substrate) was added NaOMe (2 equiv.) and the resulting mixture was stirred for 3–20 h at r.t., neutralized with DOWEX 50 H^+-form, filtered and concentrated. The crude product was purified by column chromatography ($CH_2Cl_2 \rightarrow CH_2Cl_2$:MeOH, 5:1) to give the corresponding deacetylated glycoside.

General procedure for synthesis of 4,6-*O*-benzylidene-α-D-mannopyranosides. To a solution of the corresponding deacetylated glycoside (1 equiv.) in DMF (1 ml/40 mg starting material) was added PTSA (10 mol%) and benzaldehyde dimethyl acetal (1 equiv.). The resulting mixture was stirred at 60 °C and 200 mbar for 2 h and concentrated. To the concentrated residue was added H_2O (10 ml/500 mg) and formation of a white solid was received. The solid was filtered off and washed with H_2O (10 ml/ 500 mg) and Et_2O (3 ml/1 g) to obtain the crude product. The crude product was either used as such or in some cases further purified by column chromatography ($CH_2Cl_2 \rightarrow CH_2Cl_2$:MeOH, 5:1) to give the corresponding 4,6-*O*-benzylidene-α-D-mannopyranosides.

General procedure for the selective benzylation of the equatorial 3-OH group. To a solution of the corresponding 4,6-*O*-benzylidene-α-D-mannopyranoside (1 equiv.) in toluene (4 ml/100 mg starting material) was added Bu_2SnO (1 equiv.) and the resulting mixture was refluxed for 3 h at 120 °C. The reaction mixture was then cooled to r.t. and TBAB (1.05 equiv.), CsF (1.02 equiv.) and BnBr (1.04 equiv.) were added and the mixture was again refluxed for 3 h, cooled to r.t., diluted with EtOAc (4 ml/100 mg) and sat. $NaHCO_3$ (3 ml/100 mg) and filtered through celite. The organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 3 ml/ 100 mg). The combined organic phase was washed with H_2O (4 ml/100 mg) and brine (3 ml/100 mg), dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 4:1) to give the corresponding 3-*O*-benzyl derivatives.

General procedure for alkylation of the 2-OH group. To a solution containing the corresponding 3-*O*-benzyl derivative (1 equiv.) in DMF (2 ml/100 mg starting material) was added NaH (1.5–1.9 equiv.) at 0 °C. The reaction mixture was stirred for 15 min, then brought to r.t., stirred for 10 min then the corresponding bromide (1.5 equiv.) was added. The resulting mixture was stirred for 1–3 h, quenched with MeOH (0.4 ml/1 mmol starting material), diluted with CH_2Cl_2 (4 ml/100 mg) and washed with sat. $NaHCO_3$ (3 ml/100 mg). The organic phase was separated and the aqueous phase was washed with CH_2Cl_2 (3 × 3 ml/100 mg). The combined organic phase was washed with brine (3 ml/100 mg), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 8:1) to give the fully protected substrates **15**–**19**.

General procedure for β-mannosylation. To a solution containing the corresponding donor (1 equiv.) in dry CH_2Cl_2 (4 ml/ 0.5 mmol of donor) was added at – 60 °C (acetone + dry ice) BSP (1.2 equiv.), TTBP (1.5 equiv.) and Tf_2O (1.3 equiv.). The resulting mixture was stirred for 0.5 h followed by cooling to –78 °C and addition of the corresponding acceptor (1.15 equiv.), dissolved in CH_2Cl_2 (3 ml/0.5 mmol of acceptor), over a timeperiod of 15 min. The reaction mixture was stirred for 2 h and quenched by addition of triethyl phosphite (3 equiv.). The reaction mixture was stirred for 1 h at –78 °C, brought to r.t., diluted with CH_2Cl_2 (3 ml/100 mg) and washed with sat. $NaHCO_3$-solution (3 ml/100 mg). The water phase was separated and extracted with CH_2Cl_2 (3 × 3 ml/100 mg). The combined organic phase was washed with brine (3 ml/100 mg), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 6:1→4:1→3:2) to give the glycosylated product.

General procedure for propargyl group deprotection. To a solution of the corresponding 2-*O*-propargylated compound (1 equiv.) in dry THF (2 ml/100 mg of starting material) was added ^{t}BuOK (1.2 equiv.). The resulting mixture was stirred at r.t. for 5 h, diluted with CH_2Cl_2 (3 ml/ 100 mg) and washed with H_2O (2 ml/100 mg). The organic phase was dried and concentrated and the crude product was utilized as such in the following reaction. Successful isomerization was confirmed by NMR spectroscopy at this stage. The isomerized 2-*O*-allenyl sugar (1 equiv.) was dissolved in acetone/water (4:1) and treated with a catalytic amount of OsO_4 followed by NMO (2 equiv.). The reaction mixture was stirred for 3 h, diluted with (3 ml/100 mg) and washed with H_2O (2 ml/100 mg). The water phase was extracted with CH_2Cl_2 (2×3 ml/100 mg) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 4:1→3:2) to give the depropargylated product.

General procedure for hydrogenolysis. To a solution of the corresponding protected mannoside (1 equiv.) in MeOH or MeOH:EtOAc (10:1, 1 ml/10 mg of starting material) was added Pd/C (10 % Pd, 2.5 equiv. by mass). The reaction mixture was stirred in an autoclave under H_2 (1.4–2.8 bar) overnight, filtered through celite and concentrated to give the deprotected substrates.

General procedure for homodimerization reactions. To a solution containing the terminal olefin (1 equiv.) in CH_2Cl_2 (1 ml/100 mg starting material) was added Grubbs 1st generation catalyst (10 mol%). The reaction mixture was refluxed for 6 h, cooled to r.t. and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 8:1→1:2) to give brownish oils. The resulting oils were diluted with CH_2Cl_2 (5 ml/100 mg) and stirred for 19 h with activated carbon (0.1 equiv. by mass), filtered through celite and finally through a plug of silica to give the corresponding divalent molecules.

4.2 Analytical Data on Selected Substrates[40]

2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose trichloroacetimidat (2). Synthesized from **1** (1.67 g, 4.78 mmol) according to the general procedure for synthesis of acetylated imidate donors to give the title compound as a yellowish oil (2.00 g, 72 %). TLC: R_f= 0.58 (hexane:EtOAc 1:1). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 8.79 (s, 1 H, N*H*), 6.29 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 5.48 (dd, 1 H, $J_{2,3}$ = 3.2 Hz, H-2), 5.41 (dd, 1 H, $J_{3,4}$ = 10.1 Hz, H-3), 5.40 (dd, 1 H, $J_{4,5}$ = 10.0 Hz, H-4), 4.28 (dd, 1 H, $J_{6b,5}$ = 5.0, $J_{6b,6a}$ = –12.4 Hz, H-6b), 4.19 (ddd, 1 H, $J_{5,6a}$ = 2.4 Hz, H-5), 4.17 (dd, 1 H, H-6a), 2.20, 2.10, 2.07, 2.01 (each s, each 3 H, $OCOCH_3$).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 170.6, 169.8, 169.7, 169.6 (O*C*OCH_3), 159.8 (*C*Cl_3), 94.5 (C-1), 71.2 (C-5), 68.8 (C-3), 67.8 (C-2), 65.4 (C-4), 62.0 (C-6), 20.8, 20.7, 20.7, 20.6 (OCO*C*H_3).
HRMS: *m/z* calcd. for $C_{16}H_{20}Cl_3NO_{10}Na$ $[M+Na]^+$ 514.0045; found 514.0029.

Benzyl α-D-mannopyranosid (3). Synthesized from **1** (4.7 g, 12.7 mmol) according to the general procedure for glycosylation of 1,2,3,4,6-penta-*O*-Ac-D-mannopyranose to give the acetylated benzyl glycoside as a colorless oil (4.4 g, 79 %). This product (0.7 g, 1.6 mmol) was deprotected according to the general procedure for deacetylation to give the title compound as a white solid (0.4 g, 91 %). TLC: R_f = 0.44 (CH_2Cl_2:MeOH 4:1). $[\alpha]_D^{23}$ +85.9° (c 1.0, MeOH); ^{1}H NMR (600.13 MHz, CD_3OD, 25 °C): δ 7.29–7.14 (m, 5 H, arom. *H*), 4.83 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.75 and 4.51 (each d, each 1 H, *J* = –11.8 Hz, 1-C*H*$_2$Ph), 3.84 (dd, 1 H, $J_{6a,5}$ = 2.2, J_{6a6b} = –11.8 Hz, H-6a), 3.82 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 3.72 (dd, 1 H, $J_{3,4}$ = 9.3 Hz, H-3), 3.71 (dd, 1 H, $J_{6b,5}$ = 6.0 Hz, H-6b), 3.62 (dd, 1 H, $J_{4,5}$ = 9.9 Hz, H-4), 3.59 (ddd, 1 H, H-5).
^{13}C NMR (150.90 MHz, CD_3OD, 25 °C): δ 139.1, 129.4, 129.2, 128.8 (arom. *C*), 100.7 (C-1), 75.0 (C-5), 72.7 (C-3), 72.3 (C-2), 69.9 (1-*C*H_2Ph), 68.7 (C-4), 63.0 (C-6).
HRMS: *m/z* calcd. for $C_{13}H_{18}O_6Na$ $[M+Na]^+$ 293.0996; found 293.0999.

Cyclohexyl α-D-mannopyranoside (4). Synthesized from **2** (0.84 g, 1.7 mmol) according to the general procedure for glycosylation of 1,2,3,4,6-penta-*O*-Ac-D-mannopyranose to give the acetylated cyclohexyl glycoside as a colorless oil (0.5 g, 69 %). This product (0.41 g, 0.96 mmol) was deprotected according to the general procedure for deacetylation to give the title compound as a white foam (0.23 g, 91 %). TLC: R_f = 0.42 (CH_2Cl_2:MeOH 4:1). $[\alpha]_D^{23}$ + 93.5° (c 1.0, MeOH). ^{1}H NMR (600.13 MHz, CD_3OD, 25 °C): δ 4.89 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1), 3.80 (dd, 1 H, $J_{6a,5}$ = 2.2, $J_{6a,6b}$ = –11.8 Hz, H-6a), 3.73 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 3.70 (dd, 1 H, $J_{6b,5}$ = 5.6 Hz, H-6b), 3.70 (dd, 1 H, $J_{3,4}$ = 9.2 Hz, H-3), 3.70 – 3.62 (m, 1 H, OC*H*C_5H_{10}), 3.60 (ddd, 1 H, $J_{5,4}$ = 9.3 Hz, H-5), 3.60 (dd, 1 H, H-4), 1.93 – 1.70 (m, 4 H, OCHC_5*H*$_{10}$), 1.58 – 1.2 (m, 6 H, OCHC_5*H*$_{10}$).
^{13}C NMR (150.90 MHz, CD_3OD, 25 °C): δ 99.4 (C-1), 75.8 (O*C*HC_5H_{10}), 74.7 (C-5), 72.8 (C-2), 72.7 (C-3), 68.8 (C-4), 63.0 (C-6), 34.5, 32.4, 26.9, 25.1, 24.9 (OCH*C*$_5H_{10}$).
HRMS: *m/z* calcd. for $C_{12}H_{22}O_6Na$ $[M+Na]^+$ 285.1314; found 285.1321.

Allyl α-D-mannopyranoside (5). Synthesized from **1** (1.0 g, 2.6 mmol) according to the general procedure for glycosylation of 1,2,3,4,6-penta-*O*-Ac-D-mannopyranose to give the acetylated allyl glycoside as a colorless oil (0.6 g, 69 %). This product (3.4 g, 8.7 mmol) was deprotected according to the general procedure for deacetylation to give the title compound as a colorless oil (1.9 g, quant.). TLC: R_f = 0.25 (CH_2Cl_2:MeOH 5:1). $[\alpha]_D^{23}$ +73.6° (c 1.0, MeOH). ^{1}H NMR (600.13 MHz, CD_3OD, 25 °C): δ 5.93 (dddd, 1 H, $J_{CHCH2,CH2a}$ = 5.1, $J_{CHCH2,CH2b}$ = 6.0, $J_{CHCH2,CHCH2cis}$ = 10.5, $J_{CHCH2,CHCH2trans}$ = 17.2 Hz, 1-$OCH_2CH{=}CH_2$), 5.29 (dddd, 1 H, $J_{CH2trans,CH2b}$ = –1.6, $J_{CH2trans,CH2a}$ = –1.8, $J_{CH2trans,CH2cis}$ = –1.9 Hz, 1-$OCH_2CH{=}CH_{2trans}$), 5.16 (dddd, 1 H, $J_{CH2cis,CH2b}$ = –1.3, $J_{CH2cis,CH2a}$ = –1.4 Hz, 1-$OCH_2CH{=}CH_{cis}$), 4.79 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.21 (dddd, 1 H, $J_{CH2a,CH2b}$ = –13.1 Hz, 1-$OCH_{2a}CH{=}CH_2$), 4.00 (dddd, 1 H, 1-$OCH_{2b}CH{=}CH_2$), 3.83 (dd, 1 H, $J_{6a,5}$ = 2.3, $J_{6a,6b}$ = –11.8 Hz, H-6a), 3.80 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 3.70 (dd, 1 H, $J_{6b,5}$ = 6.0 Hz, H-6b), 3.70 (dd, 1 H, $J_{3,4}$ = 9.5 Hz, H-3), 3.60 (dd, 1 H, $J_{4,5}$ = 9.9 Hz, H-4), 3.53 (ddd, 1 H, H-5).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 135.5 (1-$OCH_2CH{=}CH_2$), 117.3 (1-$OCH_2CH{=}CH_2$), 100.8 (C-1), 74.8 (C-5), 72.7 (C-3), 72.2 (C-2), 68.9 (1-$OCH_2CH{=}CH_2$), 68.7 (C-4), 63.0 (C-6).

HRMS: *m/z* calcd. for $C_9H_{16}O_6Na$ $[M+Na]^+$ 243.0839; found 243.0836.

Phenyl 1-thio-α-D-mannopyranoside (6). Synthesized from **1** (11.17 g, 28.6 mmol) according to the general procedure for glycosylation of 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose to give the thio glycoside as a yellowish oil (11.9 g, 95 %). This product (11.9 g, 27.1 mmol) was deprotected according to the general procedure for deacetylation to give the title compound as a colorless oil (7.1 g, 96 %). TLC: R_f = 0.29 (CH_2Cl_2:MeOH 5:1). ^{1}H NMR (600.13 MHz, CD_3OD, 25 °C): δ 7.53–7.25 (m, 5 H, arom. *H*), 5.42 (dd, 1 H, $J_{1,2}$ = 1.6 Hz, H-1), 4.08 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, H-2), 4.03 (ddd, 1 H, $J_{5,6a}$ = 2.4, $J_{5,6b}$ = 5.6, $J_{5,4}$ = 9.8 Hz, H-5), 3.81 (dd, 1 H, $J_{6a,6b}$ = –12.0 Hz, H-6a), 3.76 (dd, 1 H, H-6b), 3.72 (dd, 1 H, $J_{4,3}$ = 9.5 Hz, H-4), 3.68 (dd, 1 H, H-3).

^{13}C NMR (150.90 MHz, CD_3OD, 25 °C): δ 136.0, 133.0, 130.1, 128.6 (arom. *C*), 90.6 (C-1), 75.7 (C-5), 73.8 (C-2), 73.2 (C-3), 68.8 (C-4), 62.7 (C-6).

HRMS: *m/z* calcd. for $C_{12}H_{16}O_5SNa$ $[M+Na]^+$ 295.0611; found 295.0603; *m/z* calcd. for $C_{12}H_{16}O_9SK$ $[M+K]^+$ 311.0350; found 311.0348.

Benzyl 4,6-*O*-benzylidene-α-D-mannopyranoside (7). Synthesized from **3** (0.4 g, 1.4 mmol) according to the general procedure for synthesis of 4,6-*O*-benzylidene-α-D-mannopyranosides to give the title compound as a white solid (0.3 g, 56 %). TLC: R_f = 0.06 ($CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.50–7.32 (m, 10 H, arom. *H*), 5.58 (s, 1 H, C*H*Ph), 4.98 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.75 and 4.54 (each d, each 1 H, *J* = –11.9 Hz, 1-CH_2Ph), 4.27 (dd, 1 H, $J_{6a,5}$ = 5.0, $J_{6a,6b}$ = –10.4 Hz, H-6a), 4.15 (ddd, 1 H, $J_{3,3\text{-}OH}$ = 0.4, $J_{3,2}$ = 3.5, $J_{3,4}$ = 9.6 Hz, H-3), 4.11 (ddd, 1 H, $J_{2,2\text{-}OH}$ = 1.2 Hz, H-2), 3.95 (dd, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.90 (ddd, 1 H, $J_{5,6b}$ = 10.3 Hz, H-5), 3.84 (dd, 1 H, H-6b), 2.59 (d, 1 H, 3-O*H*), 2.56 (d, 1 H, 2-O*H*).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 137.2, 136.9, 129.3, 128.6, 128.4, 128.0, 126.2 (arom. *C*), 102.3 (*C*HPh), 99.3 (C-1), 78.9 (C-4), 71.0 (C-2), 69.5 (1-*C*H_2Ph), 68.8 (C-6), 68.7 (C-3), 63.2 (C-5).

HRMS: *m/z* calcd. for $C_{20}H_{22}O_6Na$ $[M+Na]^+$ 381.1309; found 381.1316.

Cyclohexyl 4,6-*O*-benzylidene-α-D-mannopyranoside (8). Synthesized from **4** (70 mg, 0.3 mmol) according to the general procedure for synthesis of 4,6-*O*-benzylidene-α-D-mannopyranosides to give the title compound as a colorless oil (57 mg, 57 %). TLC: R_f = 0.06 ($CHCl_3$). $[\alpha]_D^{23}$ + 70.5° (c 1.0, CH_2Cl_2). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.51–7.36 (m, 5 H, arom. *H*), 5.58 (s, 1 H, C*H*Ph), 5.03 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.27 (dd, 1 H, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –10.4 Hz, H-6a), 4.13 (ddd, 1 H, $J_{3,3\text{-}OH}$ = 3.4, $J_{3,2}$ = 3.5, $J_{3,4}$ = 9.5 Hz, H-3), 4.02 (ddd, 1 H, $J_{2,2\text{-}OH}$ = 2.4 Hz, H-2), 3.93 (dd, 1 H, $J_{4,5}$ = 8.9 Hz, H-4), 3.92 (ddd, 1 H, $J_{5,6b}$ = 10.1 Hz, H-5), 3.82 (dd, 1 H, H-6b), 3.67–3.58 (m, 1 H, OC*H*C_5H_{10}), 2.57 (d, 1 H, 2-O*H*), 2.54 (d, 1 H, 3-O*H*), 1.95–1.80 (m, 2 H, $OCHC_5H_{10}$), 1.78–1.68 (m, 2 H, $OCHC_5H_{10}$), 1.56–1.18 (m, 6 H, $OCHC_5H_{10}$).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 137.3, 129.3, 128.4, 126.2 (arom. *C*), 102.2 (*C*HPh), 98.0 (C-1), 79.2 (C-4), 75.3 (O*C*HC_5H_{10}), 71.6 (C-2), 68.9 (C-6), 68.8 (C-3), 63.0 (C-5), 33.4, 31.3, 25.6, 24.0, 23.8 ($OCHC_5H_{10}$).

HRMS: *m/z* calcd. for $C_{19}H_{26}O_6Na$ $[M+Na]^+$ 373.1622; found 373.1632.

Allyl 4,6-*O*-benzylidene-α-D-mannopyranoside (39). Synthesized from **5** (0.4 g, 1.8 mmol) according to the general procedure for synthesis of 4,6-*O*-benzylidene-α-D-mannopyranosides to give the title compound as a white solid (0.4 g, 70 %). TLC: R_f = 0.04 ($CHCl_3$). $[\alpha]_D^{23}$ +71.4° (c

1.0, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.50–7.37 (m, 5 H, arom. *H*), 5.91 (dddd, 1 H, $J_{CHCH2,CH2a}$ = 5.1, $J_{CHCH2,CH2b}$ = 6.1, $J_{CHCH2,CHCH2cis}$ = 10.5, $J_{CHCH2,CHCH2trans}$ = 17.2 Hz, 1-$OCH_2CH{=}CH_2$), 5.58 (s, 1 H, C*H*Ph), 5.31 (dddd, 1 H, $J_{CH2trans,CH2b}$ = –1.5, $J_{CH2trans,CH2cis}$ = –1.6, $J_{CH2trans,CH2a}$ = –1.7 Hz, 1-$OCH_2CH{=}CH_{2trans}$), 5.23 (dddd, 1 H, $J_{CH2cis,CH2b}$ = –1.2, $J_{CH2cis,CH2a}$ = –1.3 Hz, 1-$OCH_2CH{=}CH_{2cis}$), 4.93 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.28 (dd, 1 H, $J_{6a,5}$ = 5.0, $J_{6a,6b}$ = –10.3 Hz, H-6a), 4.21 (dddd, 1 H, $J_{CH2a,CH2b}$ = –12.9 Hz, 1-$OCH_{2a}CH{=}CH_2$), 4.12 (dd, 1 H, $J_{3,2}$ = 3.5, $J_{3,4}$ = 9.7 Hz, H-3), 4.08 (dd, 1 H, H-2), 4.02 (dddd, 1 H, 1-$OCH_{2b}CH{=}CH_2$), 3.94 (dd, 1 H, $J_{4,5}$ = 9.4 Hz, H-4), 3.87 (ddd, 1 H, $J_{5,6b}$ = 10.3 Hz, H-5), 3.83 (dd, 1 H, H-6b).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 137.2 (arom. *C*), 133.4 (1-$OCH_2CH{=}CH_2$), 129.3, 128.4, 126.2 (arom. *C*), 117.8 (1-$OCH_2CH{=}CH_2$), 102.3 (*C*HPh), 99.3 (C-1), 78.9 (C-4), 71.0 (C-2), 68.8 (C-6), 68.7 (C-3), 68.3 (1-$OCH_2CH{=}CH_2$), 63.1 (C-5).

HRMS: *m/z* calcd. for $C_{16}H_{20}O_6Na$ $[M+Na]^+$ 331.1152; found 331.1164.

Benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (11). Synthesized from **7** (0.64 g, 1.8 mmol) according to the general procedure for selective benzylation of the equatorial 3-OH group to give the title compound as a colorless oil (0.65 g, 75 %). TLC: R_f = 0.55 (hexane:EtOAc 1:1). $[\alpha]_D^{23}$ +55.9° (c 1.0, CH_2Cl_2). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.32–7.28 (m, 15 H, arom. *H*), 5.63 (s, 1 H, C*H*Ph), 4.97 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.86 and 4.72 (each d, each 1 H, *J* = –11.8 Hz, CH_2Ph_a), 4.71 and 4.52 (each d, each 1 H, *J* = –11.8 Hz, CH_2Ph_b), 4.27 (dd, 1 H, $J_{6a,5}$ = 4.9, $J_{6a,6b}$ = –10.3 Hz, H-6a), 4.12 (dd, 1 H, $J_{4,5}$ = 9.5, $J_{4,3}$ = 9.6 Hz, H-4), 4.11 (ddd, 1 H, $J_{2,2\text{-}OH}$ = 1.4, $J_{2,3}$ = 3.5 Hz, H-2), 3.98 (dd, 1 H, H-3), 3.91 (ddd, 1 H, $J_{5,6b}$ = 10.5 Hz, H-5), 3.87 (dd, 1 H, H-6b), 2.65 (d, 1 H, 2-O*H*).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 138.0, 137.5, 136.9, 128.9, 128.5, 128.2, 128.1, 127.9, 127.8, 126.0 (arom. *C*), 101.6 (*C*HPh), 99.2 (C-1), 78.9 (C-4), 75.7 (C-3), 73.1 (CH_2Ph_a), 70.1 (C-2), 69.4 (CH_2Ph_b), 68.9 (C-6), 63.5 (C-5).

HRMS: *m/z* calcd. for $C_{27}H_{28}O_6Na$ $[M+Na]^+$ 471.1778; found 471.1765.

Cyclohexyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (12). Synthesized from **8** (0.13 g, 0.4 mmol) according to the general procedure for selective benzylation of the equatorial 3-OH group to give the title compound as a colorless oil (0.14 g, 84 %). TLC: R_f = 0.64 i hexane:EtOAc 1:1. $[\alpha]_D^{23}$ +49.5° (c 1.0, CH_2Cl_2). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ

7.52–7.30 (m, 10 H, arom. *H*), 5.62 (s, 1 H, C*H*Ph), 5.03 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.88 and 4.73 (each d, each 1 H, *J* = –11.7 Hz, 3-CH_2Ph), 4.27 (dd, 1 H, $J_{6a,5}$ = 4.9, $J_{6a,6b}$ = –10.4 Hz, H-6a), 4.10 (dd, 1 H, $J_{4,3}$ = 9.6, $J_{4,5}$ = 9.7 Hz, H-4), 4.04 (ddd, 1 H, $J_{2,2\text{-O}H}$ = 1.4, $J_{2,3}$ = 3.4 Hz, H-2), 3.97 (dd, 1 H, H-3), 3.93 (ddd, 1 H, $J_{5,6b}$ = 10.4 Hz, H-5), 3.85 (dd, 1 H, H-6b), 3.65–3.55 (m, 1 H, OC*H*C$_5$H$_{10}$), 2.63 (d, 1 H, 2-O*H*), 1.86–1.71 (m, 4 H, OCHC$_5H_{10}$), 1.58–1.23 (m, 6 H, OCHC$_5H_{10}$).

^{13}C NMR (150.90 MHz, CDCl$_3$, 25 °C): δ 138.1, 137.6, 128.9, 128.5, 128.2, 127.9, 127.8, 126.0 (arom. *C*), 101.5 (*C*HPh), 97.8 (C-1), 79.1 (C-4), 75.8 (C-3), 75.2 (O*C*HC$_5$H$_{10}$), 73.1 (3-*C*H$_2$Ph), 70.6 (C-2), 68.9 (C-6), 63.3 (C-5), 33.4, 31.3, 25.6, 24.1, 23.8 (OCHC_5H$_{10}$).

HRMS: *m/z* calcd. for $C_{26}H_{32}O_6$ [M]$^+$ 440.2198; found 440.2199.

Allyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (13). Synthesized from **9** (0.15 g, 0.5 mmol) according to the general procedure for selective benzylation of the equatorial 3-OH group to give the title compound as a colorless oil (0.15 g, 80 %). TLC: R_f = 0.65 (hexane:EtOAc 1:1). $[\alpha]_D^{23}$ +52.8° (c 1.0, CHCl$_3$). ^{1}H NMR (600.13 MHz, CDCl$_3$, 25 °C): δ 7.51 – 7.29 (m, 10 H, Ph), 5.90 (dddd, 1 H, $J_{\mathrm{C}H\mathrm{CH2,C}H\mathrm{2a}}$ = 5.1, $J_{\mathrm{C}H\mathrm{CH2,C}H\mathrm{2b}}$ = 6.2, $J_{\mathrm{C}H\mathrm{CH2,CHC}H\mathrm{2cis}}$ = 10.4, $J_{\mathrm{C}H\mathrm{CH2,CHC}H\mathrm{2trans}}$ = 17.3 Hz, 1-OCH$_2$C*H*=CH$_2$), 5.62 (s, 1 H, C*H*Ph), 5.31 (dddd, 1 H, $J_{\mathrm{C}H\mathrm{2trans,C}H\mathrm{2b}}$ = –1.0, $J_{\mathrm{C}H\mathrm{2trans,C}H\mathrm{2a}}$ = –1.3, $J_{\mathrm{C}H\mathrm{2trans,C}H\mathrm{2cis}}$ = –1.6 Hz, 1-OCH$_2$CH=C$H_{2\mathrm{trans}}$), 5.22 (dddd, 1 H, $J_{\mathrm{C}H\mathrm{2cis,C}H\mathrm{2b}}$ = –1.0, $J_{\mathrm{C}H\mathrm{2cis,C}H\mathrm{2a}}$ = –1.5 Hz, 1-OCH$_2$CH=C$H_{2\mathrm{cis}}$), 4.93 (d, 1 H, $J_{1,2}$ = 1.4 Hz, H-1), 4.87 and 4.73 (each d, each 1 H, *J* = –11.8 Hz, 3-CH_2Ph), 4.27 (dd, 1 H, $J_{6a,5}$ = 5.1, $J_{6a,6b}$ = –10.5 Hz, H-6a), 4.19 (dddd, 1 H, $J_{\mathrm{C}H\mathrm{2a,C}H\mathrm{2b}}$ = –12.9 Hz, 1-OC$H_{2\mathrm{a}}$CH=CH$_2$), 4.11 (dd, 1 H, $J_{4,5}$ = 9.4, $J_{4,3}$ = 9.6 Hz, H-4), 4.09 (ddd, 1 H, $J_{2,2\text{-O}H}$ = 1.4, $J_{2,3}$ = 3.5 Hz, H-2), 4.00 (dddd, 1 H, 1-OC$H_{2\mathrm{b}}$CH=CH$_2$), 3.96 (dd, 1 H, H-3), 3.87 (ddd, 1 H, $J_{5,6b}$ = 10.3 Hz, H-5), 3.86 (dd, 1 H, H-6b), 2.64 (d, 1 H, 2-O*H*).

^{13}C NMR (150.90 MHz, CDCl$_3$, 25 °C): δ 138.0, 137.5 (arom. *C*), 133.5 (1-OCH$_2$*C*H=CH$_2$), 128.9, 128.5, 128.2, 127.9, 127.8, 126.1 (arom. *C*), 117.9 (1-OCH$_2$CH=*C*H$_2$), 101.6 (*C*HPh), 99.1 (C-1), 78.9 (C-4), 75.7 (C-3), 73.1 (3-*C*H$_2$Ph), 70.0 (C-2), 68.9 (C-6), 68.2 (1-O*C*H$_2$CH=CH$_2$), 63.4 (C-5).

HRMS: *m/z* calcd. for $C_{23}H_{26}O_6$ [M]$^+$ 398.1729; found 398.1733.

Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (14). Synthesized from **10** (2.6 g, 7.2 mmol) according to the general procedure for selective benzylation of the equatorial 3-OH group to give the title compound as a colorless oil (2.9 g, 85 %). TLC: $R_f = 0.65$ (hexane:EtOAc 1:1). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.52–7.28 (m, 15 H, arom. *H*), 5.63 (s, 1 H, C*H*Ph), 5.60 (d, 1 H, $J_{1,2}$ = 1.2 Hz, H-1), 4.90 and 4.75 (each d, each 1 H, *J* = –11.8 Hz, 3-CH_2Ph), 4.34 (ddd, 1 H, $J_{5,6a}$ = 4.9, $J_{5,4}$ = 9.6, $J_{5,6b}$ = 10.2 Hz, H-5), 4.29 (ddd, 1 H, $J_{2,2\text{-O}H}$ = 1.4, $J_{2,3}$ = 3.4 Hz, H-2), 4.21 (dd, 1 H, $J_{6a,6b}$ = –10.4 Hz, H-6a), 4.18 (dd, 1 H, $J_{4,3}$ = 9.6 Hz, H-4), 3.97 (dd, 1 H, H-3), 3.86 (dd, 1 H, H-6b), 2.82 (d, 1 H, 2-O*H*).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 137.7, 137.4, 133.3, 131.8, 129.2, 129.0, 128.6, 128.3, 128.1, 127.9, 127.7, 126.1 (arom. *C*), 101.6 (*C*HPh), 87.8 (C-1), 79.0 (C-4), 75.7 (C-3), 73.2 (3-*C*H_2Ph), 71.4 (C-2), 68.5 (C-6), 64.6 (C-5).

HRMS: *m/z* calcd. for $C_{26}H_{26}O_5SNa$ $[M+Na]^+$ 473.1393; found 473.1403.

Benzyl 2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (15). Synthesized from **11** (0.18 g, 0.4 mmol) according to the general procedure for alkylation of the 2-OH group to give the title compound as a white solid (0.21 g, quant.). TLC: $R_f = 0.84$ (hexane:EtOAc 1:1). $[\alpha]_D^{23}$ +66.6º (c 1.0, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.50–7.27 (m, 15 H, arom. *H*), 5.90 (dddd, 1 H, $J_{CHCH2,CH2a}$ = 6.0, $J_{CHCH2,CH2b}$ = 6.2, $J_{CHCH2,CHCH2cis}$ = 10.3, $J_{CHCH2,CHCH2trans}$ = 17.2 Hz, 2-OCH_2C*H*=CH_2), 5.63 (s, 1 H, C*H*Ph), 5.26 (dddd, 1 H, $J_{CH2trans,CH2b}$ = –1.5, $J_{CH2trans,CH2a}$ = –1.5, $J_{CH2trans,CH2cis}$ = –1.7 Hz, 1-OCH_2CH=CH_{2trans}), 5.17 (dddd, 1 H, $J_{CH2cis,CH2b}$ = –1.1, $J_{CH2cis,CH2a}$ = –1.2 Hz, 1-OCH_2CH=CH_{2cis}), 4.90 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.87 and 4.70 (each d, each 1 H, *J* = –12.1 Hz, C$H_2$$Ph_a$), 4.72 and 4.49 (each d, each 1 H, *J* = –11.9 Hz, C$H_2$$Ph_b$), 4.25 (dddd, 1 H, $J_{CH2a,CH2b}$ = –12.9 Hz, 1-OCH_{2a}CH=CH_2), 4.24 (dd, 1 H, $J_{6a,5}$ = 4.7, $J_{6a,6b}$ = –10.2 Hz, H-6a), 4.21 (dd, 1 H, $J_{4,5}$ = 9.2, $J_{4,3}$ = 10.0 Hz, H-4), 4.15 (dddd, 1 H, 1-OCH_{2b}CH=CH_2), 4.01 (dd, 1 H, $J_{3,2}$ = 3.2 Hz, H-3), 3.88 (dd, 1 H, $J_{6b,5}$ = 10.4 Hz, H-6b), 3.85 (ddd, 1 H, H-5), 3.82 (dd, 1 H, H-2).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 138.7, 137.7, 137.0 (arom. *C*), 134.8 (2-OCH_2*C*H=CH_2), 128.8, 128.5, 128.3, 128.2, 128.0, 127.9, 127.5, 126.0 (arom. *C*), 117.8 (2-OCH_2CH=*C*H_2), 101.4 (*C*HPh), 98.9 (C-1), 79.2 (C-4), 76.6 (C-2), 76.3 (C-3), 73.3 (*C*$H_2$$Ph_a$), 73.1 (2-O*C*$H_2$CH=$CH_2$), 69.3 (*C*$H_2$$Ph_b$), 68.8 (C-6), 64.4 (C-5).

HRMS: *m/z* calcd. for $C_{30}H_{32}O_6Na$ $[M+Na]^+$ 511.2091; found 511.2103.

Cyclohexyl 2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (16). Synthesized from **12** (20 mg, 0.4 mmol) according to the general procedure for alkylation of the 2-OH group to give the title compound as a colorless oil (22 mg, quant.). TLC: R_f = 0.82 (hexane:EtOAc 1:1). $[\alpha]_D^{23}$ +48.3° (c 0.1, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.51–7.27 (m, 10 H, arom. *H*), 5.94 (dddd, 1 H, $J_{CHCH2,CH2a}$ = 5.5, $J_{CHCH2,CH2b}$ = 6.3, $J_{CHCH2,CHCH2cis}$ = 10.3, $J_{CHCH2,CHCH2trans}$ = 17.2 Hz, 2-$OCH_2CH=CH_2$), 5.63 (s, 1 H, C*H*Ph), 5.29 (dddd, 1 H, $J_{CH2trans,CH2b}$ = –1.6, $J_{CH2trans,CH2a}$ = –1.6, $J_{CH2trans,CH2cis}$ = –1.7 Hz, 2-$OCH_2CH=CH_{2trans}$), 5.20 (dddd, 1 H, $J_{CH2cis,CH2b}$ = –1.1, $J_{CH2cis,CH2a}$ = –1.3 Hz, 2-$OCH_2CH=CH_{2cis}$), 4.95 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.88 and 4.71 (each d, each 1 H, *J* = –12.1 Hz, 3-CH_2Ph), 4.30 (dddd, 1 H, $J_{CH2a,CH2b}$ = –13.0 Hz, 2-$OCH_{2a}CH=CH_2$), 4.24 (dd, 1 H, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –10.2 Hz, H-6a), 4.18 (dd, 1 H, $J_{4,5}$ = 9.2, $J_{4,3}$ = 10.0 Hz, H-4), 4.17 (dddd, 1 H, 2-$OCH_{2b}CH=CH_2$), 3.99 (dd, 1 H, $J_{3,2}$ = 3.2 Hz, H-3), 3.87 (ddd, 1 H, $J_{5,6b}$ = 10.5 Hz, H-5), 3.85 (dd, 1 H, H-6b), 3.74 (dd, 1 H, H-2), 3.61–3.53 (m, 1 H, $OCHC_5H_{10}$), 1.89–1.65 (m, 4 H, $OCHC_5H_{10}$), 1.58–1.20 (m, 6 H, $OCHC_5H_{10}$).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 138.8, 137.7 (arom. *C*), 135.0 (2-$OCH_2CH=CH_2$), 128.8, 128.3, 128.2, 127.5, 127.4, 126.0 (arom. *C*), 117.6 (2-$OCH_2CH=CH_2$), 101.3 (*C*HPh), 97.5 (C-1), 79.4 (C-4), 78.2 (C-2), 76.5 (C-3), 75.1 ($OCHC_5H_{10}$), 73.2 (3-CH_2Ph), 73.1 (2-$OCH_2CH=CH_2$), 68.9 (C-6), 64.2 (C-5), 33.3, 31.3, 25.6, 24.0, 23.8 ($OCHC_5H_{10}$).

HRMS: *m/z* calcd. for $C_{29}H_{38}O_6$ $[M+H]^+$ 481.2585; found 481.2574; *m/z* calcd. for $C_{29}H_{37}O_6Na$ $[M+Na]^+$ 503.2404; found 503.2386; *m/z* calcd. for $C_{29}H_{37}O_6K$ $[M+K]^+$ 519.2143; found 519.2120.

Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-propargyl-1-thio-α-D-mannopyranoside (18). Synthesized from **14** (55 mg, 0.1 mmol) according to the general procedure for alkylation of the 2-OH group to give the title compound as a colorless oil (60 mg, 98 %). TLC: R_f = 0.86 (hexane:EtOAc 1:1). $[\alpha]_D^{23}$ +143.9° (c 1.0, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.52–7.28 (m, 15 H, arom. *H*), 5.63 (s, 1 H, C*H*Ph), 5.62 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.88 and 4.76 (each d, each 1 H, *J* = –12.1 Hz, 3-CH_2Ph), 4.43 (dd, 1 H, $J_{CH2a,CH}$ = 2.3, $J_{CH2a,CH2b}$ = –16.2 Hz, 2-$OCH_{2a}C\equiv CH$), 4.41 (dd, 1 H, $J_{CH2b,CH}$ = 2.4 Hz, 2-$OCH_{2b}C\equiv CH$),4.29 (ddd, 1 H, $J_{5,6a}$ = 4.8, $J_{5,4}$ = 9.4, $J_{5,6b}$ = 10.2 Hz, H-5), 4.27 (dd, 1 H, $J_{2,3}$ = 3.2 Hz, H-2), 4.23 (dd, 1 H, $J_{4,3}$ = 10.0 Hz, H-4), 4.22 (dd, 1 H, $J_{6a,6b}$ = –10.3 Hz, H-6a), 4.00 (dd, 1 H, H-3), 3.87 (dd, 1 H, H-6b), 2.44 (dd, 1 H, 2-$OCH_2C\equiv CH$).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 138.2, 137.5, 131.6, 129.2, 128.4, 128.2, 127.7, 126.1 (arom. *C*), 101.5 (*C*HPh), 87.4 (C-1), 79.5 (2-OCH_2C≡CH), 79.2 (C-4), 77.7 (C-2), 76.1 (C-3), 75.2 (2-OCH_2C≡*C*H), 73.3 (3-*C*H_2Ph), 68.5 (C-6), 65.3 (C-5), 58.8 (2-O*C*H_2C≡CH).

HRMS: *m/z* calcd. for $C_{29}H_{28}O_5SNa$ $[M+Na]^+$ 511.1550; found 511.1562.

Phenyl 2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (19). Synthesized from **14** (0.16 g, 0.35 mmol) according to the general procedure for alkylation of the 2-OH group to give the title compound as a colorless oil (0.18 g, quant.). TLC: R_f = 0.93 (hexane:EtOAc 1:1). $[\alpha]_D^{23}$ +145.2° (c 1.0, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.52–7.27 (m, 15 H, arom. *H*), 5.92 (dddd, 1 H, $J_{CHCH2,CH2a}$ = 5.8, $J_{CHCH2,CH2b}$ = 5.9, $J_{CHCH2,CHCH2cis}$ = 10.3, $J_{CHCH2,CHCH2trans}$ = 17.2 Hz, 2-$OCH_2CH=CH_2$), 5.64 (s, 1 H, C*H*Ph), 5.53 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 5.29 (dddd, 1 H, $J_{CH2trans,CH2b}$ = –1.5, $J_{CH2trans,CH2a}$ = –1.5, $J_{CH2trans,CH2cis}$ = –1.7 Hz, 2-$OCH_2CH=CH_{2trans}$), 5.20 (dddd, 1 H, $J_{CH2cis,CH2b}$ = –1.2, $J_{CH2cis,CH2a}$ = –1.2 Hz, 2-$OCH_2CH=CH_{2cis}$), 4.88 and 4.73 (each d, each 1 H, *J* = –12.2 Hz, 3-CH_2Ph), 4.29 (ddd, 1 H, $J_{5,6a}$ = 4.8, $J_{5,4}$ = 9.5, $J_{5,6b}$ = 10.0 Hz, H-5), 4.26 (dd, 1 H, $J_{4,3}$ = 9.9 Hz, H-4), 4.22 (dd, 1 H, $J_{6a,6b}$ = –10.3 Hz, H-6a), 4.20 (dddd, 1 H, $J_{CH2a,CH2b}$ = –12.2 Hz, 2-$OCH_{2a}CH=CH_2$), 4.18 (dddd, 1 H, 2-$OCH_{2b}CH=CH_2$), 3.99 (dd, 1 H, $J_{2,3}$ = 3.2 Hz, H-2), 3.96 (dd, 1 H, H-3), 3.88 (dd, 1 H, H-6b).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 138.4, 137.6 (arom. *C*), 134.5 (2-$OCH_2CH=CH_2$), 131.5, 129.1, 128.9, 128.4, 128.2, 127.7, 127.6, 126.1 (arom. *C*), 118.0 (2-$OCH_2CH=CH_2$), 101.5 (*C*HPh), 87.3 (C-1), 79.1 (C-4), 78.1 (C-2), 76.1 (C-3), 73.2 (3-*C*H_2Ph), 72.6 (2-O*C*$H_2CH=CH_2$), 68.5 (C-6), 65.4 (C-5).

HRMS: *m/z* calcd. for $C_{29}H_{30}O_5SNa$ $[M+Na]^+$ 513.1706; found 513.1740; *m/z* calcd. for $C_{29}H_{30}O_5SK$ $[M+K]^+$ 529.1446; found 529.1478.

Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (20). Synthesized from **17** (40 mg, 0.07 mmol) and **14** (1.15 equiv.) according to the general procedure for β-mannosylation to give the title compound as a white foam (47 mg, 76 %). $[\alpha]_D^{23}$ = –3.5 (c 0.2, CH_2Cl_2). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): *δ* = 7.51–7.25 (m, 30 H, arom. *H*), 5.60 (s, 1 H, C*H*Ph), 5.51 (s, 1 H, C*H*′Ph), 5.49 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 5.04 and 4.96 (each d, each 1 H, *J* = –12.3 Hz, 3′-CH_2Ph), 4.80

and 4.76 (each d, each 1 H, *J* = –12.1 Hz, 3-CH_2Ph), 4.69 and 4.61 (each d, each 1 H, *J* = –12.5 Hz, 2′-CH_2Ph), 4.62 (d, 1 H, $J_{1',2'}$ = 0.9 Hz, H-1′), 4.51 (dd, 1 H, $J_{2,3}$ = 3.2 Hz, H-2), 4.33 (ddd, 1 H, $J_{5,6a}$ = 4.8, $J_{5,4}$ = 9.5, $J_{5,6b}$ = 10.2 Hz, H-5), 4.25 (dd, 1 H, $J_{4',5'}$ = 9.3, $J_{4',3'}$ = 9.9 Hz, H-4′), 4.25 (dd, 1 H, $J_{6'a,5'}$ = 4.8, $J_{6'a,6'b}$ = –10.4 Hz, H-6′a), 4.23 (dd, 1 H, $J_{6a,6b}$ = –10.2 Hz, H-6a), 4.17 (dd, 1 H, $J_{4,3}$ = 10.0 Hz, H-4), 3.98 (dd, 1 H, H-3), 3.97 (dd, 1 H, $J_{2',3'}$ = 3.2 Hz, 2′-H), 3.87 (dd, 1 H, $J_{6'b,5'}$ = 10.1 Hz, H-6′b), 3.79 (dd, 1 H, H-6b), 3.59 (dd, 1 H, H-3′), 3.31 (ddd, 1 H, H-5′) ppm.
^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): *δ* = 138.5–126 (arom. C), 101.7 (*C*′HPh), 101.4 (*C*HPh), 99.8 (C-1′), 86.4 (C-1), 78.7 (C-4), 78.4 (C-4′), 77.5 (C-3′), 76.2 (C-2), 76.0 (C-2′), 74.6 (3′-*C*H_2Ph), 74.3 (C-3), 72.3 (2′-*C*H_2Ph), 71.5 (3-*C*H_2Ph), 68.6 (C-6), 68.5 (C-6′), 67.8 (C-5′), 65.4 (C-5) ppm.
HRMS: *m/z* calcd. for $C_{55}H_{51}O_{10}S$ $[M–H]^-$ 903.3203; found 903.3810.

Benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-propargyl-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (22). Synthesized from **18** (0.32 g, 0.66 mmol) and **11** (1.15 equiv.) according to the general procedure for β-mannosylation to give the title compound as a white foam (0.44 g, 82 %). $[\alpha]_D^{23}$ = –37.0 (c 1.0, CH_2Cl_2). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): *δ* = 7.51–7.28 (m, 25 H, arom. *H*), 5.60 (s, 1 H, C*H*Ph), 5.57 (s, 1 H, C*H*′Ph), 4.96 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1), 4.80 (s, 2 H, 3′-CH_2Ph), 4.73 and 4.50 (each d, each 1 H, *J* = –11.9 Hz, 1-CH_2Ph), 4.72 (s, 2 H, 3-CH_2Ph), 4.70 (dd, 1 H, $J_{CH2a,CH}$ = 2.4, $J_{CH2a,CH2b}$ = –16.1 Hz, 2′-OCH_{2a}C≡CH), 4.63 (d, 1 H, $J_{1',2'}$ = 0.8 Hz, H-1′), 4.61 (dd, $J_{CH2b,CH}$ = 2.4 Hz, 2′-OCH_{2b}C≡CH), 4.27 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 4.24 (dd, 1 H, $J_{6'a,5'}$ = 4.8, $J_{6'a,6'b}$ = –10.4 Hz, H-6′a), 4.24 (dd, 1 H, $J_{2',3'}$ = 3.1 Hz, H-2′), 4.23 (dd, 1 H, $J_{6a,5}$ = 4.9, $J_{6a,6b}$ = –10.2 Hz, H-6a), 4.16 (dd, 1 H, $J_{4',5'}$ = 9.4, $J_{4',3'}$ = 9.9 Hz, H-4′), 4.12 (dd, 1 H, $J_{4,5}$ = 9.5, $J_{4,3}$ = 10.0 Hz, H-4), 4.02 (dd, 1 H, H-3), 3.87 (ddd, 1 H, $J_{5,6b}$ = 10.4 Hz, H-5), 3.84 (dd, 1 H, $J_{6'b,5'}$ = 10.1 Hz, H-6′b), 3.82 (dd, 1 H, 6b-H), 3.60 (dd, 1 H, H-3′), 3.26 (ddd, 1 H, H-5′), 2.44 (dd, 1 H, 2′-OCH_2C≡C*H*) ppm.
^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): *δ* = 138.7–126.0 (arom. *C*), 101.6 (*C*HPh), 101.4 (*C*′ HPh), 100.2 (C-1′), 97.3 (C-1), 80.4 (2′-OCH_2*C*≡CH), 78.6 (C-4), 78.3 (C-4′), 76.9 (C-3′) , 75.0 (2′-OCH_2C≡*C*), 74.5 (C-2′), 74.4 (C-2), 74.4 (C-3), 72.2 (3′-*C*H_2Ph), 71.7 (3-*C*H_2Ph), 69.4 (1-*C*H_2Ph), 68.9 (C-6), 68.5 (C-6′), 67.8 (C-5′), 64.4 (C-5), 59.8 (2′-O*C*H_2C≡CH) ppm.
HRMS: *m/z* calcd. for $C_{50}H_{50}O_{11}$ $[M]^+$ 826.3353; found 826.3325.

Cyclohexyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-propargyl-β-D-mannopyranosyl)-α-D-mannopyranoside (23). Synthesized from **18** (87 mg, 0.18 mmol) and **12** (1.15 equiv.) according to the genereal procedure for β-mannosylation to give the title compound as white foam (0.1 g, 72 %). TLC: R_f = 0.82 (hexane:EtOAc 1:1). $[\alpha]_D^{23}$ –39.0° (c 1.0, CH_2Cl_2). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 7.50–7.25 (m, 20 H, arom. *H*), 5.60 (s, 1 H, C*H*Ph), 5.58 (s, 1 H, C*H*′Ph), 5.00 (d, 1 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.82 (s, 2 H, 3-CH_2Ph), 4.73 (s, 2 H, 3′-CH_2Ph), 4.72 (dd, 1 H, $J_{CH2a,CH}$ = 2.3, $J_{CH2a,CH2b}$ = –16.1 Hz, 2′-OCH_{2a}C≡CH), 4.68 (d, 1 H, $J_{1',2'}$ = 0.7 Hz, H-1′), 4.62 (dd, 1 H, $J_{CH2b,CH}$ = 2.4 Hz, 2′-OCH_{2b}C≡CH), 4.27 (dd, 1 H, $J_{6'a,5'}$= 4.8, $J_{6'a,6'b}$= –10.4 Hz, H-6′a), 4.26 (dd, 1 H, $J_{2',3'}$ = 3.1 Hz, H-2′), 4.24 (dd, 1 H, $J_{6a,5}$ = 4.7, $J_{6a,6b}$ = –10.2 Hz, H-6a), 4.18 (dd, 1 H, $J_{2,3}$ = 3.3 Hz, H-2), 4.17 (dd, 1 H, $J_{4',5'}$ = 9.3, $J_{4',3'}$ = 9.9 Hz, H-4′), 4.10 (dd, 1 H, $J_{4,5}$ = 9.5, $J_{4,3}$ = 10.0 Hz, H-4), 3.99 (dd, 1 H, H-3), 3.89 (ddd, 1 H, $J_{5,6b}$ = 10.4 Hz, H-5), 3.86 (dd, 1 H, $J_{6'b,5'}$ = 10.0 Hz, H-6′b), 3.80 (dd, 1 H, H-6b), 3.63 (dd, 1 H, H-3′), 3.61–3.54 (m, 1 H, OC*H*C_5H_{10}), 3.32 (ddd, 1 H, H-5′), 2.50 (dd, 1 H, 2′-OCH_2C≡C*H*), 1.86–1.18 (m, 10 H, OCHC_5H_{10}).

^{13}C NMR (150.90 MHz, $CDCl_3$, 25 °C): δ 138.9, 138.2, 137.6, 137.5, 129.1, 129.0, 128.5, 128.3, 128.2, 127.8, 127.5, 127.6, 126.0, 125.9 (arom. *C*), 101.4 (*C*HPh, *C*′HPh), 100.4 (C-1′), 96.1 (C-1), 81.0 (2′-OCH_2*C*≡CH), 79.0 (C-4), 78.2 (C-2), 76.9 (C-3′), 75.7 (O*C*HC_5H_{10}), 75.4 (C-2′), 75.0 (2′-OCH_2C≡*C*H), 74.8 (C-4′), 74.5 (C-3), 72.2 (3-*C*H_2Ph), 71.6 (3-*C*′H_2Ph), 69.0 (C-6′), 68.7 (C-6), 67.8 (C-5′), 64.3 (C-5), 59.8 (2′-O*C*H_2C≡CH), 33.3, 31.3, 25.6, 23.8, 23.5 (OCHC_5H_{10}).

HRMS: *m/z* calcd. for $C_{49}H_{54}O_{11}$ $[M]^+$ 818.3666; found 818.3730.

Benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (24). Synthesized from **22** (0.4 g, 0.5 mmol) according to the general procedure for propargyl group deprotection to give the title compound as a colorless oil (0.3 g, 80 %). $[\alpha]_D^{23}$ = –15.0 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.49–7.25 (m, 25 H, arom. *H*), 5.56 (s, 1 H, C*H*Ph), 5.47 (s, 1 H, C*H*′Ph), 4.90 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1), 4.82 and 4.75 (each d, each 1 H, *J* = –12.3 Hz, 3-CH_2Ph), 4.80 and 4.75 (each d, each 1 H, *J* = –11.8 Hz, 3′-CH_2Ph), 4.71 and 4.49 (each d, each 1 H, *J* = –11.9 Hz, 1-CH_2Ph), 4.67 (d, 1

H, $J_{1',2'}$ = 1.3 Hz, H-1′), 4.43 (dd, 1 H, $J_{2,3}$ = 3.5 Hz, H-2), 4.24 (dd, 1 H, $J_{4',3'}$ = 9.1, $J_{4',5'}$ = 9.6 Hz, H-4′), 4.21 (dd, 1 H, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –10.3 Hz, H-6a), 4.21 (dd, 1 H, $J_{6'a,5'}$ = 4.9, $J_{6'a,6'b}$ = –10.4 Hz, H-6′a), 4.14 (dd, 1 H, $J_{4,5}$ = 9.4, $J_{4,3}$ = 9.9 Hz, H-4), 4.14 (dd, 1 H, $J_{2',3'}$ = 3.7 Hz, H-2′), 4.04 (dd, 1 H, H-3), 3.85 (ddd, 1 H, $J_{5,6b}$ = 10.4 Hz, H-5), 3.78 (dd, 1 H, H-6b), 3.73 (dd, 1 H, $J_{6'b,5'}$ = 10.0 Hz, H-6′b), 3.64 (dd, 1 H, H-3′), 3.34 (ddd, 1 H, H-5′), 3.22 (s, 1 H, 2′-O*H*) ppm.

^{13}C NMR (150.90 MHz, CDCl$_3$, 25 °C): δ = 138.7–126.0 (arom. *C*), 101.4 (*C*HPh), 101.3 (*C*′HPh), 98.2 (C-1′), 97.9 (C-1), 78.8 (C-4), 78.4 (C-4′), 76.0 (C-3′), 74.2 (C-3), 72.8 (C-2), 72.6 (3′-*C*H$_2$Ph), 72.4 (3-*C*H$_2$Ph), 69.4 (C-2′), 69.1 (1-*C*H$_2$Ph), 68.4, (C-6), 68.3 (C-6′), 66.8 (C-5′), 63.9 (C-5) ppm.

HRMS: *m/z* calcd. for $C_{47}H_{48}O_{11}$ [M]$^+$ 788.3197; found 788.3211.

Cyclohexyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-α-D-mannopyranoside (25). Synthesized from **23** (90 mg, 0.1 mmol) according to the general procedure for propargyl group deprotection to give the title compound as a white foam (75 mg, 85 %). TLC: R_f = 0.63 (hexane:EtOAc 1:1). $[\alpha]_D^{23}$ –15.1° (c 0.1, CHCl$_3$).

^{1}H NMR (600.13 MHz, CDCl$_3$, 25 °C): δ 7.50–7.28 (m, 20 H, arom. *H*), 5.55 (s, 1 H, C*H*Ph), 5.48 (s, 1 H, C*H*′Ph), 4.95 (d, 1 H, $J_{1,2}$ =1.7 Hz, H-1), 4.83 and 4.76 (each d, each 1 H, *J* = –12.3 Hz, 3-C*H*$_2$Ph), 4.81 and 4.76 (each d, each 1 H, *J* = –11.7 Hz, 3′-C*H*$_2$Ph), 4.74 (d, 1 H, $J_{1',2'}$ = 1.3 Hz, H-1′), 4.34 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 4.27 (dd, 1 H, $J_{4',3'}$ = 9.0, $J_{4',5'}$ = 9.5 Hz, H-4′), 4.26 (dd, 1 H, $J_{6'a,5'}$ = 4.9, $J_{6'a,6'b}$ = –10.3 Hz, H-6′a), 4.21 (dd, 1 H, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –10.3 Hz, H-6a), 4.16 (ddd, 1 H, $J_{2',2'\text{-}OH}$ = 3.1, $J_{2',3'}$ = 3.8 Hz, H-2′), 4.11 (dd, 1 H, $J_{4,5}$ = 9.2, $J_{4,3}$ = 9.9 Hz, H-4), 4.02 (dd, 1 H, H-3), 3.87 (ddd, 1 H, $J_{5,6b}$ = 10.4 Hz, H-5), 3.76 (dd, 1 H, H-6b), 3.76 (dd, 1 H, $J_{6'b,5'}$ = 10.0 Hz, H-6′b), 3.67 (dd, 1 H, H-3′), 3.60–3.54 (m, 1 H, OC*H*C$_5$H$_{10}$), 3.40 (ddd, 1 H, H-5′), 3.22 (d, 1 H, 2′-OH), 1.80–1.65 (m, 4 H, OCHC$_5$*H*$_{10}$), 1.55–1.15 (m, 6 H, OCHC$_5$*H*$_{10}$).

^{13}C NMR (150.90 MHz, CDCl$_3$, 25 °C): δ 139.4, 138.4, 137.6, 137.3, 128.9, 128.8, 128.4, 128.3, 128.2, 127.9, 127.8, 127.6, 126.0 (arom. *C*), 101.4 (*C*HPh, *C*′HPh), 98.3 (C-1′), 96.7 (C-1), 79.0 (C-4), 78.5 (C-4′), 76.3 (C-3′), 75.5 (O*C*HC$_5$H$_{10}$), 74.8 (C-3), 73.9 (C-2), 72.6 (3-*C*H$_2$Ph, 3′-*C*H$_2$Ph), 68.8 (C-2′), 68.7 (C-6′, C-6), 66.9 (C-5′), 64.0 (C-5), 33.3, 31.3, 25.5, 24.0 (OCH*C*$_5$H$_{10}$).

HRMS: *m/z* calcd. for $C_{46}H_{52}O_{11}Na$ [M+Na]$^+$ 803.3402; found 803.3380; *m/z* calcd. for $C_{46}H_{52}O_{11}K$ [M+K]$^+$ 819.3141; found 819.3105.

Cyclohexyl 2-*O*-(β-D-mannopyranosyl)-α-D-mannopyranoside (26). Synthesized from **25** (22 mg, 0.03 mmol) according to the general procedure for hydrogenolyses to give the title compound as a white solid (12 mg, quant.). $[\alpha]_D^{23}$ +0.5° (c 1.0, MeOH). ^1H NMR (600.13 MHz, CD_3OD, 25 °C): δ 5.04 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1), 4.66 (d, 1 H, $J_{1',2'}$ = 1.0 Hz, H-1′), 3.98 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 3.90 (dd, 1 H, $J_{2',3'}$ = 3.2 Hz, H-2′), 3.87 (dd, 1 H, $J_{6'a,5'}$ = 2.3, $J_{6'a,6'b}$ = –12.0 Hz, H-6′a), 3.80 (dd, 1 H, $J_{6a,5}$ = 2.4, $J_{6a,6b}$ = –11.8 Hz, H-6a), 3.73 (dd, 1 H, $J_{3,4}$ = 9.6 Hz, H-3), 3.70 (dd, 1 H, $J_{6b,5}$ = 5.8 Hz, H-6b), 3.68 (dd, 1 H, $J_{6'b,5'}$ = 6.4 Hz, H-6′b), 3.70–3.65 (m, 1 H, $OC\mathit{H}C_5H_{10}$), 3.63 (dd, 1 H, $J_{4,5}$ = 9.8 Hz, H-4), 3.60 (ddd, 1 H, H-5), 3.54 (dd, 1 H, $J_{4',3'}$ = 9.4, $J_{4',5'}$ = 9.7 Hz, H-4′), 3.47 (dd, 1 H, H-3′), 3.24 (ddd, 1 H, H-5′), 1.98–1.82 (m, 2 H, $OCHC_5\mathit{H}_{10}$), 1.80–1.70 (m, 2 H, $OCHC_5\mathit{H}_{10}$), 1.55–1.20 (m, 6 H, $OCHC_5\mathit{H}_{10}$).

^{13}C NMR (150.90 MHz, CD_3OD, 25 °C): δ 100.0 (C-1′), 97.1 (C-1), 79.2 (C-2), 78.6 (C-5′), 76.4 ($O\mathit{C}HC_5H_{10}$), 75.1 (C-3′), 74.8 (C-5), 72.6 (C-2′), 72.1 (C-3), 69.1 (C-4), 68.5 (C-4′), 62.9 (C-6′), 62.7 (C-6), 34.5, 32.6, 26.8, 25.2, 25.0 ($OCH\mathit{C}_5H_{10}$).

HRMS: *m/z* calcd. for $C_{18}H_{32}O_{11}Na$ $[M+Na]^+$ 447.1837; found 447.1843.

Benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (27). The synthesis commenced by following the general procedure for β-mannosylation starting from donor **20** (76 mg, 0.09 mmol) and acceptor **24** (78 mg, 0.1 mmol). The purification of **27** was accomplished by preparative TLC (hexane:EtOAc 3:2). The spots containing the product were scraped off the plate and dissolved in EtOAc (50 ml). After 2 h of stirring the mixture was filtered and concentrated to give the title compound as a colorless oil (73 mg, β:α 10:1, 55 %). TLC: R_f = 0.40 (hexane:EtOAc 3:2). $[\alpha]_D$ = –25.4 (c 0.5, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ = 7.48–7.07 (m, 50 H, arom. *H*), 5.57 (s, 1 H, $C\mathit{H}'''Ph$), 5.52 (s, 1 H, $C\mathit{H}Ph$), 5.43 (s, 1 H, $C\mathit{H}'Ph$), 5.41 (s, 1 H, $C\mathit{H}''Ph$), 5.24 (d, 1 H, $J_{1'',2''}$ = 0.3 Hz, H-1″), 5.05 (d, 1 H, $J_{1''',2'''}$ = 0.8 Hz, H-1‴), 5.00 and 4.83 (each d, each 1 H, *J* = –12.5 Hz, $2'''-C\mathit{H}_2Ph$), 4.89 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1), 4.72 and 4.495 (each d, each 1 H, *J* = –11.9 Hz, $1-C\mathit{H}_2Ph$), 4.71 and 4.68 (each d, each 1 H, *J* = –12.4 Hz, $3-C\mathit{H}_2Ph$), 4.69 and 4.62 (each d, each 1 H, *J* = –12.3 Hz, $3'-C\mathit{H}_2Ph$), 4.63 and

4.45 (each d, each 1 H, J = –12.0 Hz, 3″-CH_2Ph), 4.496 and 4.39 (each d, each 1 H, J = –11.8 Hz, 3‴-CH_2Ph), 4.61 (d, 1 H, $J_{1',2'}$ = 0.7 Hz, H-1′), 4.53 (dd, 1 H, $J_{2'',3''}$ = 3.1 Hz, H-2″), 4.45 (dd, 1 H, $J_{2',3'}$ = 3.2 Hz, H-2′), 4.41 (dd, 1 H, $J_{2''',3'''}$ = 3.2 Hz, H-2‴), 4.34 (dd, 1 H, $J_{6''a,\ 5''}$ = 4.7, $J_{6''a,6''b}$ = –10.2 Hz, H-6″a), 4.29 (dd, 1 H, $J_{2,3}$ = 3.5 Hz, H-2), 4.29 (dd, 1 H, $J_{6'a,5'}$ = 4.7, $J_{6'a,6'b}$ = –10.3 Hz, H-6′a), 4.22 (dd, 1 H, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –10.3 Hz, H-6a), 4.182 (dd, 1 H, $J_{4''',5'''}$ = 9.3, $J_{4''',3'''}$ = 9.8 Hz, H-4‴), 4.177 (dd, 1 H, $J_{6'''a,5'''}$ = 4.7, $J_{6'''a,6'''b}$ = –10.3 Hz, H-6‴a), 4.06 (dd, 1 H, $J_{4'',5''}$ = 9.5, $J_{4'',3''}$ = 9.6 Hz, H-4″), 4.01 (dd, 1 H, $J_{3,4}$ = 10.0 Hz, H-3), 3.92 (dd, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.91 (dd, 1 H, $J_{4',5'}$ = 9.4, $J_{4',3'}$ = 9.9 Hz, H-4′), 3.864 (ddd, 1 H, $J_{5,6b}$ = 10.3 Hz, H-5), 3.862 (dd, 1 H, $J_{6'''b,5'''}$ = 10.3 Hz, H-6‴b), 3.78 (dd, 1 H, $J_{6''b,5''}$ = 10.0 Hz, H-6″b), 3.75 (dd, 1 H, $J_{6'b,5'}$ = 10.1 Hz, H-6′b), 3.69 (dd, 1 H, H-6b), 3.64 (dd, 1 H, H-3′), 3.53 (dd, 1 H, H-3‴), 3.53 (dd, 1 H, H-3″), 3.42 (ddd, 1 H, H-5″), 3.31 (ddd, 1 H, H-5′), 3.30 (ddd, 1 H, H-5‴) ppm.

^{13}C NMR (150.90 MHz, CDCl$_3$, 25 °C): δ = 139.4–126.1 (arom. *C*), 103.5 (C-1‴, $J_{C\text{-}1''',H\text{-}1'''}$ = 158.3 Hz), 101.91 (*C*HPh), 101.88 (*C*′HPh), 101.87 (C-1″, $J_{C\text{-}1'',H\text{-}1''}$ = 158.6 Hz), 101.5 (*C*″HPh), 101.2 (*C*‴HPh), 100.2 (C-1′, $J_{C\text{-}1',H\text{-}1'}$ = 156.0 Hz), 96.9 (C-1, $J_{C\text{-}1,H\text{-}1}$ = 167.8 Hz), 79.0 (C-3‴, C-4), 78.5 (C-4′), 78.3 (C-4‴), 78.2 (C-4″), 76.9 (C-3″), 76.34 (C-3′), 76.27 (C-2″), 75.9 (C-2‴), 74.8 (C-2), 74.63 (2‴-*C*H$_2$Ph), 74.60 (C-3), 74.2 (C-2′), 72.2 (3-*C*H$_2$Ph), 72.1 (3‴-*C*H$_2$Ph), 71.2 (3′-*C*H$_2$Ph), 70.8 (3″-*C*H$_2$Ph), 69.5 (1-*C*H$_2$Ph), 68.87 (C-6″), 68.85 (C-6), 68.72 (C-6‴), 68.66 (C-6′), 67.9 (C-5″), 67.8 (C-5′), 67.5 (C-5‴), 64.2 (C-5) ppm.

HRMS: *m/z* calcd. for $C_{94}H_{95}O_{21}Na$ $[M + Na]^+$ 1582.6264; found 1582.6207; *m/z* calcd. for $C_{94}H_{95}O_{21}K$ $[M + K]^+$ 1598.6003; found 1598.5932.

β-D-Mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-β-D-mannopyranosyl-(1→2)-D-mannopyranose (28). The synthesis of **28** commenced by the general procedure for hydrogenolysis (H_2-pressure 2.8 bar, 2 weight equiv. Pd/C) starting from **27** (0.1 g, 0.064 mmol). The crude product received after the work-up, was purified by HPAEC (CarboPac PA 1 column; 9 × 250 mm) with the gradient elution, 50 mM NaOH over 0-10 min, followed by a linear gradient from 50 to 150 mM NaOH over 10-30 min, and finally a linear gradient from 0 to 200 mM NaAc in 150 mM NaOH over 30-60 min. The product was collected and the fractions were neutralized by addition of 1 M aqueous acetic acid. The sample was desalted using a column of successive cation and anion exchange resins (Bio-Rad AG 50W-8X Resin 200-400 mesh, hydrogen form and Bio-Rad AG 1-X8 Resin 200-400 mesh, acetate form) and concentrated to give the title compound (32 mg, β:α 7:2, 75 %). Data for the α-anomer. ^{1}H NMR (600.13 MHz, CD_3OD, 25 °C): δ = 5.17 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1), 4.93 (d, 1 H, $J_{1''',2'''}$ = 0.9 Hz, H-1‴), 4.84 (d, 1 H, $J_{1'',2''}$ = 0.8 Hz, H-1″), 4.71 (d, 1 H, $J_{1',2'}$ = 0.7 Hz, H-1′), 4.42 (dd, 1 H, $J_{2'',3''}$ = 3.1 Hz, H-2″), 4.08 (dd, 1 H, $J_{2',3'}$ = 2.8 Hz, H-2′), 4.04 (dd, 1 H, $J_{2''',3'''}$ = 3.1 Hz, H-2‴), 3.98 (dd, 1 H, $J_{2,3}$ = 3.1 Hz, H-2), 3.91 (dd, 1 H, $J_{6'''a,5'''}$ = 2.2, $J_{6'''a,6'''b}$ = –12.2 Hz, H-6‴a), 3.89 (dd, 1 H, $J_{6'a,5'}$ = 2.3, $J_{6'a,6'b}$ = –12.0 Hz, H-6′a), 3.85 (dd, 1 H, $J_{6''a,5''}$ = 2.5, $J_{6''a,6''b}$ = –11.7 Hz, H-6″a), 3.82 (dd, 1 H, $J_{3,4}$ = 9.6 Hz, H-3), 3.78 (dd, 1 H, $J_{6a,5}$ = 4.1, $J_{6a,6b}$ = –12.0 Hz, H-6a), 3.76 (dd, 1 H, $J_{6b,5}$ = 2.2 Hz, H-6b), 3.71 (dd, 1 H, $J_{6''b,5''}$ = 5.8 Hz, H-6″b), 3.70 (ddd, 1 H, $J_{5,4}$ = 9.7 Hz, H-5), 3.69 (dd, 1 H, $J_{6'b,5'}$ = 5.8 Hz, H-6′b), 3.65 (dd, 1 H, $J_{6'''b,5'''}$ = 7.3 Hz, H-6‴b), 3.63 (dd, 1 H, H-4), 3.53 (dd, 1 H, $J_{4'',3''}$ = 9.5, $J_{4'',5''}$ = 9.7 Hz, H-4″), 3.52 (dd, 1 H, H-3″), 3.51 (dd, 1 H, $J_{3',4'}$ = 9.7 Hz, H-3′), 3.50 (dd, 1 H, $J_{3''',4'''}$ = 9.5 Hz, H-3‴), 3.46 (dd, 1 H, $J_{4''',5'''}$ = 9.2 Hz, H-4‴), 3.45 (dd, 1 H, $J_{4',5'}$ = 9.3 Hz, H-4′), 3.37 (ddd, 1 H, H-5‴), 3.25 (ddd, 1 H, H-5′), 3.21 (ddd, 1 H, H-5″) ppm.

^{13}C NMR (150.90 MHz, CD_3OD, 25 °C): δ = 103.3 (C-1″), 102.3 (C-1‴), 101.1 (C-1′), 94.1 (C-1), 81.8 (C-2′), 81.5 (C-2), 79.1 (C-2″), 78.3 (C-5′, C-5″), 78.1 (C-5‴), 75.2 (C-3‴), 74.3 (C-3″), 74.2 (C-5), 74.1 (C-3′), 72.1 (C-2‴), 71.2 (C-3), 69.1 (C-4, C-4‴), 69.0 (C-4′), 68.6 (C-4″), 63.5 (C-6‴), 62.5 (C-6′, C-6″), 62.3 (C-6) ppm.

Data for the β-anomer. ^{1}H NMR (600.13 MHz, CD_3OD, 25 °C): δ = 4.98 (d, 1 H, $J_{1'',2''}$ = 0.8 Hz, H-1″), 4.94 (d, 1 H, $J_{1''',2'''}$ = 1.0 Hz, H-1‴), 4.84 (d, 1 H, $J_{1,2}$ = 0.8 Hz, H-1), 4.76 (d, 1 H, $J_{1',2'}$ = 0.7 Hz, H-1′), 4.40 (dd, 1 H, $J_{2'',3''}$ = 2.3 Hz, H-2″), 4.24 (d, 1 H, $J_{2',3'}$ = 3.2 Hz, H-2′), 4.04 (dd, 1 H, $J_{2''',3'''}$ = 2.6 Hz, H-2‴), 4.02 (dd, 1 H, $J_{2,3}$ = 2.4 Hz, H-2), 3.91 (dd, 1 H, $J_{6'''a,5'''}$ = 2.4, $J_{6'''a,6'''b}$ = –12.3 Hz, H-6‴a), 3.89 (dd, 1 H, $J_{6'a,5'}$ = 2.3, $J_{6'a,6'b}$ = –11.8 Hz, H-6′a), 3.86 (dd, 1 H, $J_{6''a,5''}$ = 2.5, $J_{6''a,6''b}$ = –11.9 Hz, H-6″a), 3.83 (dd, 1 H, $J_{6a,5}$ = 2.1, $J_{6a,6b}$ = –12.1 Hz, H-6a), 3.73 (dd, 1 H, $J_{6b,5}$ = 5.3 Hz, H-6b), 3.72 (dd, 1 H, $J_{6''b,5''}$ = 5.9 Hz, H-6″b), 3.68 (dd, 1 H, $J_{6'b,5'}$ = 6.2 Hz, H-6′b), 3.65 (dd, 1 H, $J_{6'''b,5'''}$ = 7.4 Hz, H-6‴b), 3.54 (dd, 1 H, $J_{3'',4''}$ = 9.5 Hz, H-3″), 3.53 (dd, 1 H, $J_{4'',5''}$ = 9.5 Hz, H-4″), 3.52 (dd, 1 H, $J_{4,3}$ = 9.7, $J_{4,5}$ = 9.7 Hz, H-4), 3.50 (dd, 1 H, H-3), 3.50 (dd, 1 H, $J_{3''',4'''}$ = 9.0 Hz, H-3‴), 3.48 (dd, 1 H, $J_{3',4'}$ = 9.7 Hz, H-3′), 3.45 (dd, 1 H, $J_{4',5'}$ = 9.7 Hz, H-4′), 3.44 (dd, 1 H, $J_{4''',5'''}$ = 8.9 Hz, H-4‴), 3.39 (ddd, 1 H, H-5‴), 3.24 (ddd, 1 H, H-5′), 3.23 (ddd, 1 H, H-5″), 3.20 (ddd, 1 H, H-5) ppm.

^{13}C NMR (150.90 MHz, CD_3OD, 25 °C): δ = 103.3 (C-1″), 103.0 (C-1′), 102.3 (C-1‴), 95.5 (C-1), 82.3 (C-2), 81.5 (C-2′), 79.1 (C-2″), 78.4 and 78.3 (C-5, C-5′, C-5″), 78.0 (C-5‴), 75.2 (C-3‴), 74.3 (C-3′, C-3″), 74.2 (C-3), 72.1 (C-2‴), 69.1 (C-4′, C-4‴), 68.8 (C-4), 68.6 (C-4″), 63.6 (C-6‴), 62.6 (C-6, C-6′, C-6″) ppm.

HRMS: *m/z* calcd. for $C_{24}H_{24}O_{21}Na$ $[M + Na]^+$ 689.2111; found 689.2131.

1,4-Bis(benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-α-D-mannopyranosyl)but-2-ene (29). Synthesized from **15** (0.13 g, 0.26 mmol) according to the general procedure for homodimerization reactions to give the title compound as a white foam (0.12 g, 95 %, *E*/*Z* 20:1). TLC: *Rf* = 0.81 (hexane:EtOAc 1:1). ^{1}H NMR of the *E*-isomer (600 MHz, $CDCl_3$, 25 °C): δ = 7.50–7.23 (m, 30 H, arom. *H*), 5.79–5.78 (m, 2 H, $OCH_2CH{=}CHCH_2O$), 5.61 (s, 2 H, C*H*Ph), 4.89 (d, 2 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.85 and 4.68 (each d, each 2 H, *J* = –12.1 Hz, CH_2Ph_a), 4.70 and 4.47 (each d, each 2 H, *J* = –11.9 Hz, CH_2Ph_b), 4.26–4.24 (m, 2 H, $OCH_{2a}CH{=}CHCH_{2a}O$), 4.22 (dd, 2 H, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –10.2 Hz, H-6a), 4.19 (dd, 2 H, $J_{4,5}$ = 9.3, $J_{4,3}$ = 10.0 Hz, H-4), 4.13–4.10 (m, 2 H, $OCH_{2b}CH{=}CHCH_{2b}O$), 4.00 (dd, 2 H, $J_{3,2}$ = 3.2 Hz, H-3), 3.86 (dd, 2 H, $J_{6b,5}$ = 10.5 Hz, H-6b), 3.84 (ddd, 2 H, H-5), 3.78 (dd, 2 H, H-2).

^{13}C NMR of the *E*-isomer (150.90 MHz, $CDCl_3$, 25 °C): δ = 138.5–126.1 (arom. *C*), 129.8 ($OCH_2CH{=}CHCH_2O$), 101.7 (*C*HPh), 99.1 (C-1), 79.5 (C-4), 77.0 (C-2), 76.5 (C-3), 73.6 (CH_2Ph_a), 72.5 ($OCH_2CH{=}CHCH_2O$), 69.5 (CH_2Ph_b), 69.0 (C-6), 64.7 (C-5).

HRMS: *m/z* calcd. for $C_{58}H_{60}O_{12}Na$ $[M + Na]^+$ 971.3977; found 971.3988; *m/z* calcd. for $C_{58}H_{60}O_{12}K$ $[M + K]^+$ 987.3716; found 987.3726.

1,4-Bis(cyclohexyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-α-D-mannopyranosyl)but-2-ene (30). Synthesized from **16** (20 mg, 0.04 mmol) according to the general procedure for homodimerization reactions to give the title compound as a white foam (15 mg, 75 %, *E*/*Z* 10:1). TLC: *Rf* = 0.35 (hexane:EtOAc 1:1). ^{1}H NMR of the *E*-isomer (600 MHz, $CDCl_3$, 25 °C): δ = 7.50–7.23 (m, 20 H, arom. *H*), 5.86–5.84 (m, 2 H, $OCH_2CH{=}CHCH_2O$), 5.62 (s, 2 H, C*H*Ph), 4.94 (d, 2 H, $J_{1,2}$ = 1.7 Hz, H-1), 4.87 and 4.70 (each d, each 2 H, *J* = –12.2 Hz, 3-CH_2Ph), 4.33–4.30 (m, 2 H, $OCH_{2a}CH{=}CHCH_{2a}O$), 4.23 (dd, 2 H, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –10.1 Hz, H-6a), 4.17 (dd, 2 H, $J_{4,5}$ = 9.3, $J_{4,3}$ = 10.0 Hz, H-4), 4.18–4.15 (m, 2 H, $OCH_{2b}CH{=}CHCH_{2b}O$), 3.98 (dd, 2 H, $J_{3,2}$

= 3.2 Hz, H-3), 3.87 (ddd, 2 H, $J_{5,6b}$ = 10.4 Hz, H-5), 3.84 (dd, 2 H, H-6b), 3.72 (dd, 2 H, H-2) 3.60–3.54 (m, 2 H, OC*H*C_5H_{10}), 1.85–1.19 (m, 20 H, $OCHC_5$*H_{10}*).

^{13}C NMR of the *E*-isomer (150.90 MHz, $CDCl_3$, 25 °C): δ = 138.8–126.0 (arom. *C*), 129.9 (OCH_2*C*H=*C*HCH_2O), 101.3 (*C*HPh), 97.5 (C-1), 79.4 (C-4), 77.7 (C-2), 76.6 (C-3), 75.2 (O*C*HC_5H_{10}), 73.3 (3-*C*H_2Ph), 72.2 (O*C*H_2CH=CH*C*H_2O), 68.9 (C-6), 64.2 (C-5), 33.3, 31.4, 31.0, 24.1, 23.8 (OCH*C*$_5H_{10}$).

HRMS: *m/z* calcd. for $C_{56}H_{68}O_{12}Na$ $[M + Na]^+$ 955.4603; found 955.4607; *m/z* calcd. for $C_{56}H_{68}O_{12}K$ $[M + K]^+$ 971.4342; found 971.4340.

1,4-Bis(phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-2-*O*-α-D-mannopyranosyl)but-2-ene (31). Synthesized from **19** (0.13 g, 0.28 mmol) according to the general procedure for homodimerization reactions to give the title compound as a white foam (0.10 g, 75 %, *E*/*Z* 20:1). TLC: *Rf* = 0.89 (hexane:EtOAc 1:1). ^{1}H NMR of the *E*-isomer (600 MHz, $CDCl_3$, 25 °C): δ = 7.51–7.27 (m, 30 H, arom. *H*), 5.83–5.82 (m, 2 H, OCH_2C*H*=C*H*CH_2O), 5.61 (s, 2 H, C*H*Ph), 5.51 (d, 2 H, $J_{1,2}$ = 1.3 Hz, H-1), 4.87 and 4.70 (each d, each 2 H, *J* = –12.1 Hz, 3-C*H_2*Ph), 4.28 (ddd, 2 H, $J_{5,6a}$ = 5.1, $J_{5,4}$ = 9.4, $J_{5,6b}$ = 10.2 Hz, H-5), 4.24 (dd, 2 H, $J_{4,3}$ = 9.7 Hz, H-4), 4.20 (dd, 2 H, $J_{6a,6b}$ = –10.4 Hz, H-6a), 4.20–4.12 (m, 4 H, OC*H_2*CH=CHC*H_2*O), 3.95 (dd, 2 H, $J_{2,3}$ = 3.1 Hz, H-2), 3.94 (dd, 2 H, H-3), 3.86 (dd, 2 H, H-6b).

^{13}C NMR of the *E*-isomer (150.90 MHz, $CDCl_3$, 25 °C): δ = 137.6–126.1 (arom. *C*), 129.6 (OCH_2*C*H=*C*HCH_2O), 101.5 (*C*HPh), 87.3 (C-1), 79.1 (C-4), 78.7 (C-2), 76.2 (C-3), 73.2 (3-*C*H_2Ph), 71.6 (O*C*H_2CH=CH*C*H_2O), 68.5 (C-6), 65.3 (C-5).

HRMS: *m/z* calcd. for $C_{56}H_{56}O_{12}Na$ $[M + Na]^+$ 975.3207; found 975.3219; *m/z* calcd. for $C_{56}H_{56}O_{12}K$ $[M + K]^+$ 991.2946; found 991.2943.

1,4-Bis(2-*O*-D-mannopyranose)butane (32). Synthesized from **29** (16 mg, 0.023 mmol) according ot the general procedure for hydrogenolyses to give the title compound as a colorless oil (7 mg, 70 %, αα:αβ:ββ 5:1:1). ^{1}H NMR of the αα-anomer (600 MHz, CD_3OD, 25 °C): δ = 5.18 (d, 2 H, $J_{1,2}$ = 1.7 Hz, H-1), 3.81 (dd, 2 H, $J_{6a,5}$ = 2.4, $J_{6a,6b}$ = –11.7 Hz, H-6a), 3.79 (dd, 2 H, $J_{3,2}$ = 3.5, $J_{3,4}$ = 9.6 Hz, H-3), 3.70 (ddd, 2 H, $J_{5,6b}$ = 5.9, $J_{5,4}$ = 9.7 Hz, H-5), 3.68 (dd, 2 H, H-6b), 3.64–3.59 (m, 4 H, OC*H_2*CH_2CH_2C*H_2*O), 3.58 (dd, 2 H, H-4), 3.50 (dd, 2 H, H-2), 1.70–1.66 (m, 4 H, OCH_2C*H_2*C*H_2*CH_2O).

^{13}C NMR of the αα-anomer (150.90 MHz, CD_3OD, 25 °C): δ = 93.2 (C-1), 81.3 (C-2), 74.2 (C-5), 72.4 (O*C*$H_2CH_2CH_2$*C*H_2O, C-3), 69.3 (C-4), 63.2 (C-6), 27.7 (OCH_2*C*H_2*C*H_2CH_2O).
HRMS: *m/z* calcd. for $C_{16}H_{30}O_{12}Na$ $[M + Na]^+$ 437.1629; found 437.1608.

1,4-Bis(cyclohexyl 2-*O*-D-mannopyranosyl)butane (32). Synthesized from **30** (38 mg, 0.04 mmol) according ot the general procedure for hydrogenolyses to give the title compound as a colorless oil (17 mg, 70 %). ^{1}H NMR (600 MHz, CD_3OD, 25 °C): δ = 5.01 (d, 2 H, $J_{1,2}$ = 1.7 Hz, H-1), 3.81 (dd, 2 H, $J_{6a,5}$ = 2.3, $J_{6a,6b}$ = –11.7 Hz, H-6a), 3.74 (dd, 2 H, $J_{3,2}$ = 3.5, $J_{3,4}$ = 9.4 Hz, H-3), 3.70–3.65 (m, 2 H, OC*H*C_5H_{10}), 3.66 (dd, 2 H, $J_{6b,5}$ = 5.9 Hz, H-6b), 3.64–3.58 (m, 4 H, O$CH_2$$CH_2CH_2CH_2$O), 3.57 (ddd, 2 H, $J_{5,4}$ = 9.6 Hz, H-5), 3.56 (dd, 2 H, H-4), 3.46 (dd, 2 H, H-2), 1.95–1.70 (m, 8 H, $OCHC_5H_{10}$), 1.70–1.60 (m, 4 H, $OCH_2CH_2CH_2CH_2O$), 1.56–1.00 (m, 12 H, $OCHC_5H_{10}$).
^{13}C NMR (150.90 MHz, CD_3OD, 25 °C): δ = 96.9 (C-1), 81.1 (C-2), 76.1 (O*C*HC_5H_{10}), 74.9 (C-5), 72.6 (C-3), 72.3 (O*C*$H_2CH_2CH_2$*C*H_2O), 69.3 (C-4), 63.2 (C-6), 34.5, 32.6 ($OCHC_5H_{10}$), 27.6 (OCH_2*C*H_2*C*H_2CH_2O), 26.9, 25.2, 25.0 ($OCHC_5H_{10}$).
HRMS: *m/z* calcd. for $C_{28}H_{50}O_{12}Na$ $[M + Na]^+$ 601.3194; found 601.3194.

1,4-Bis[benzyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosyl-(2→1)-3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-β-D-mannopyranosyl]but-2-ene (34). Synthesized from **19** (74 mg, 0.8 mmol) and **11** (2.3 equiv.) according to the general procedure for β-mannosylation to give the title compound as a colorless oil (88 mg, 70 %, β:α 10:1). TLC: *Rf* = 0.10 (hexane-EtOAc 1:1). ^{1}H NMR of the *E*-isomer (600 MHz, $CDCl_3$, 25 °C): δ = 7.50–7.21 (m, 50 H, arom. *H*), 6.02–5.96 (m, 2 H, $OCH_2CH{=}CHCH_2O$), 5.57 (s, 2 H, C*H*Ph_a), 5.50 (s, 2 H, C*H*Ph_b), 4.89 (d, 2 H, $J_{1,2}$ = 1.6 Hz, H-1), 4.76 and 4.71 (each d, each 2 H, *J* = –10.5 Hz, CH_2Ph_a), 4.72 (s, 4 H, CH_2Ph_b), 4.68 and 4.46 (each d, each 2 H, *J* = –12.0 Hz, CH_2Ph_c), 4.53–4.49 (m, 2 H, $OCH_{2a}CH{=}CHCH_{2a}O$), 4.25 (d, 2 H, $J_{1',2'}$ = 0.7 Hz, H-1′), 4.34–4.30 (m, 2 H, $OCH_{2b}CH{=}CHCH_{2b}O$), 4.20 (dd, 2 H, $J_{2,3}$ = 3.3 Hz, H-2), 4.19 (dd, 2 H, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = –10.1 Hz, H-6a), 4.18 (dd, 2 H, $J_{6'a,5'}$ = 4.9, $J_{6'a,6'b}$ = –10.2 Hz, H-6′a), 4.15 (dd, 2 H, $J_{4',5'}$ = 9.3, $J_{4',3'}$ = 9.9 Hz, H-4′), 4.12 (dd, 2 H, $J_{4,5}$ = 9.3, $J_{4,3}$ = 10.0 Hz, H-4), 3.99 (dd, 2 H, H-3), 3.86 (dd, 2 H, $J_{2',3'}$ = 3.2 Hz, H-2′), 3.84 (ddd, 2 H, $J_{5,6b}$ = 10.3 Hz, H-5), 3.79 (dd, 2 H, H-6b), 3.78 (ddd, 2 H, $J_{6'b,5'}$ = 10.1 Hz, H-6′b), 3.53 (dd, 2 H, H-3′), 3.21 (ddd, 2 H, H-5′).

^{13}C NMR of the *E*-isomer (150.90 MHz, CDCl$_3$, 25 °C): δ = 138.8–126.0 (arom. *C*), 130.2 (OCH$_2$*C*H=*C*HCH$_2$O), 101.5 (*C*HPh$_a$), 101.3 (*C*HPh$_b$), 100.7 (C-1′, $^1J_{C,H}$ = 155.2 Hz), 97.7 (C-1, $^1J_{C,H}$ = 168.5 Hz), 78.6 (C-4), 78.4 (C-4′), 77.2 (C-3′), 77.1 (C-2′), 75.2 (C-2), 74.3 (C-3), 73.6 (O*C*H$_2$CH=CH*C*H$_2$O), 72.2 (*C*H$_2$Ph$_a$), 71.6 (*C*H$_2$Ph$_b$), 69.3 (*C*H$_2$Ph$_c$), 68.8 (C-6, C-6′), 68.5 (C-5′), 67.6 (C-5).

HRMS: *m/z* calcd. for $C_{98}H_{100}O_{22}Na$ [M + Na]$^+$ 1651.6598; found 1651.6665.

1,4-Bis[2-*O*-β-D-mannopyranosyl-(1→2)-D-mannopyranose]butane (35). Synthesized from **34** (20 mg, 0.01 mmol) according to the general procedure for hydrogenolyses to give the title compound as a colorless oil (5 mg, 50 %, αα:αβ:ββ 8:2:1). ^{1}H NMR of the αα-anomer (600 MHz, CD$_3$OD, 25 °C): δ = 5.18 (d, 2 H, $J_{1,2}$ = 1.8 Hz, H-1), 4.64 (d, 2 H, $J_{1',2'}$ = 0.8 Hz, H-1′), 3.94 (dd, 2 H, $J_{2,3}$ = 3.2 Hz, H-2), 3.91–3.86 (m, 2 H, OCH_{2a}CH$_2$CH$_2$CH_{2a}O), 3.87 (dd, 2 H, $J_{6'a,5'}$ = 2.3, $J_{6'a,6'b}$ = –12.0 Hz, H-6′a), 3.82–3.80 (m, 2 H, H-6a), 3.78 (dd, 2 H, $J_{3,4}$ = 9.6 Hz, H-3), 3.77–3.73 (m, 2 H, OCH_{2b}CH$_2$CH$_2$CH_{2b}O), 3.73–3.67 (m, 8 H, H-2′, H-3′, H-6′b, H-6b), 3.65 (dd, 2 H, $J_{4,5}$ = 9.5 Hz, H-4), 3.53 (dd, 2 H, $J_{4'5'}$ = 8.8, $J_{4',3'}$ = 9.5 Hz, H-4′), 3.50–3.45 (m, 2 H, H-5), 3.21 (ddd, 2 H, $J_{5',6'b}$ = 5.8 Hz, H-5′), 1.80–1.60 (m, 4 H, OCH$_2$CH_2CH_2CH$_2$O).

^{13}C NMR of the αα-anomer (150.90 MHz, CD$_3$OD, 25 °C): δ = 101.4 (C-1′), 93.7 (C-1), 81.0 (C-2′), 80.6 (C-2), 78.8 (C-5′), 75.3 (C-5), 75.1 (O*C*H$_2$CH$_2$CH$_2$*C*H$_2$O), 74.3 (C-3′), 71.6 (C-3), 69.5 (C-4), 68.8 (C-4′), 62.9 (C-6, C-6′), 27.6 (OCH$_2$*C*H$_2$*C*H$_2$CH$_2$O).

HRMS: *m/z* calcd. for $C_{28}H_{59}O_{22}Na$ [M + Na]$^+$ 761.2686; found 761.2688.

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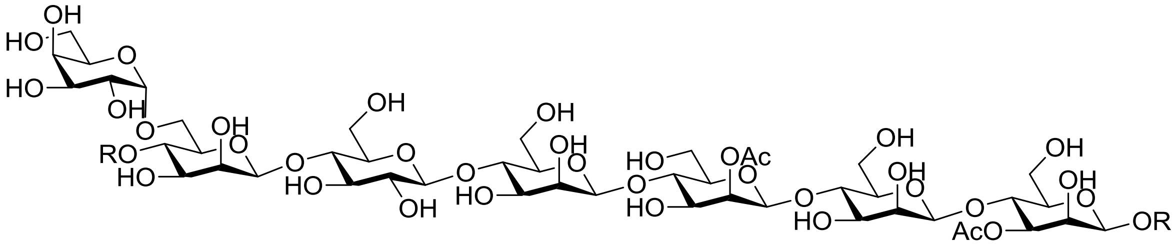
Episode 2: Synthesis of Galactoglucomannan Core Tri/Tetrasaccharides



Galactoglucomannan (GGM), a heteropolymer consisting of mainly mannose, glucose and galactose, is the most abundant hemicellulose in Norway spruce (*Picea abies*). Apart from the large amounts present in *P. abies*, GGM has also been found in the cell wall of flax seed, tobacco plants and kiwifruit. Although many applications of the GGM polymer have been developed, for example in pulp and paper manufacturing, no attempts to synthesize and study the individual fragments of this oligosaccharide have been made previously. In this chapter, the synthesis of one of the core trisaccharides and a less abundant tetrasaccharide fragment of GGM is discussed. In addition, some preliminary comparative NMR spectroscopic studies on the individual fragments and the GGM polymer will be presented along with other studies related to this polymer.

This chapter is based on unpublished results: F. S. Ekholm, A. Ardá, P. Eklund, S. André, H.-J. Gabius, J. Jiménez-Barbero, R. Leino, **"Studies on Norway Spruce Galactoglucomannans: Chemical Synthesis, Conformational Analysis, NMR Spectroscopic Characterization and Molecular Recognition of Model Compounds"**, *manuscript submitted*.

1 Introduction

Galactoglucomannan (GGM) is the most abundant hemicellulose in Norway spruce (*Picea abies*).[1] GGM has also been found in several other plants and fruits including clubmosses, horsetails, whisk fern, kiwifruit and tobacco plants.[2] The utilization of water soluble polysaccharides, in particular GGM, in mechanical pulping and papermaking has traditionally been of great importance.[3] Recently, methods for the large scale isolation of AcGGM from *Picea abies* were developed simultaneously sparking new interest in these molecules. By use of these methods, polysaccharide material can today be isolated on kg-scale thus providing sufficient amounts of material for screening and extension of the applications of these compounds.[1a,4] As a result, a deeper understanding of the roles of GGM in pulp and paper manufacturing has been achieved along with new applications of GGM in immunology.[5] GGM has also been shown to be a good substitute to the widely utilized gum arabic (arabinogalactan) as an emulsifier of hydrophobic beverages.[1a]

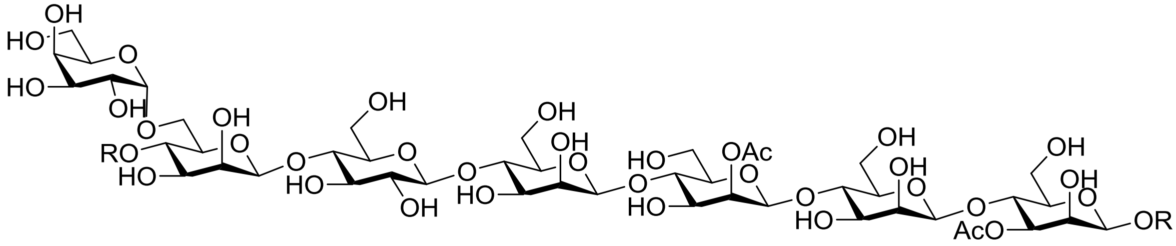


Figure 1. The proposed chemical structure of AcGGM from *Picea abies*.

Structural elucidation of water soluble AcGGM from *P. abies* has been the focus of several studies.[6] It has been suggested that the main chain consists of randomly distributed β-(1→4)-linked mannopyranosyl and β-(1→4)-linked glucopyranosyl units. Furthermore, the existence of α-(1→6)-linked galactopyranosyl residues has been confirmed in several studies.[6a,7] In all studies, the galactopyranosyl units were found to be connected to mannopyranoside residues in the linear backbone of GGM. These monosaccharides have been suggested to appear in a Man:Glc:Gal ratio of 4:1:0.1. This ratio is highly influenced by the isolation conditions.[8] In addition, the acetyl group pattern of *P. abies* AcGGM, bearing an acetylation degree of 0.3, has been analyzed and it is today widely accepted that acetyl groups are mainly present at the *O*-2 and *O*-3 of the mannopyranosyl units.[1a,6a]

While AcGGM can be isolated on large scale, several ambiguities concerning the structure and acetylation pattern of AcGGM remain. Furthermore, determination of the structure and size has been extremely difficult due to the heterogenous nature of the native material. This is also reflected in the wide range of molar masses (between 20–78 kDa) suggested for these polysaccharides.[1a,4] In addition, acetyl group migration has been proposed to occur even from the *O*-2 and *O*-3 to the *O*-6 position in the mannopyranosyl residues.[1a] Investigation of such phenomena is difficult to perform on the heterogenous polysaccharide samples available. Therefore, it was decided to synthesize and utilize small molecular models instead. In this Episode, the synthesis of one of the trisaccharide repeating units of AcGGM, a tetrasaccharide fragment containing an α-(1→6)-linked galactopyranosyl residue and some model substrates for the acetyl group migration study are presented. In addition, some comparative NMR spectroscopic data on the native polysaccharide and the synthetic model substrates will be discussed.

2 Results and Discussion

2.1 Synthesis of AcGGM Tri- and Tetrasaccharide Units

As mentioned in the introduction, the applications of AcGGM have in recent years rapidly expanded from the traditional roles in pulp and papermaking processes to applications in the food and medicinal industries.[1a,3,5] From a biochemical standpoint, the roles of the individual fragments of the GGM-polysaccharide would be interesting to study as such especially in immunological applications. This has not been possible previously due to the heterogenous nature of the native samples. In the present work, the synthesis of fragments found in AcGGM was planned in order to provide material for such studies (Figure 2).

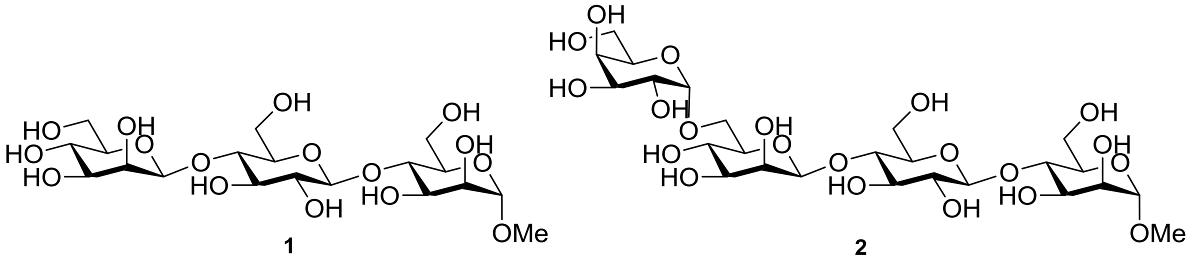
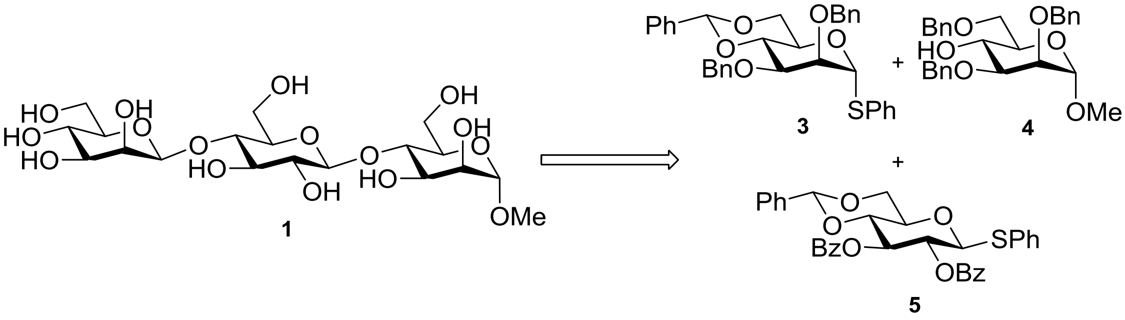
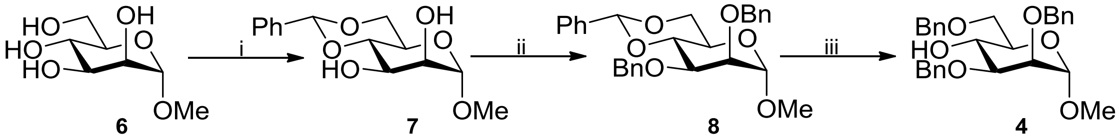


Figure 2. The synthetic targets representing one of the trisaccharide repeating units of GGM (**1**) (left), and a less abundant tetrasaccharide fragment (**2**) (right).



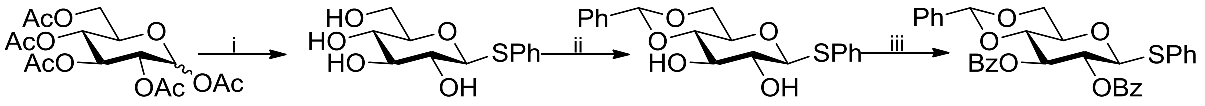
Scheme 1. Retrosynthetic analysis of trisaccharide **1**.

In the case of AcGGM, the individual fragments contain challenging glycosidic linkages, such as the β-mannopyranosyl and α-galactopyranosyl linkages, which for a long time were considered difficult to construct by chemical methods. The synthetic study towards these fragments is currently ongoing and, therefore, at the time of the writing, synthesis of the acetylated trisaccharide fragment representing the second repeating unit (Figure 1) has not yet been completed. For the initial attempt on the synthesis of trisaccharide **1**, a linear strategy was deviced starting from the three monosaccharide building blocks **3**, **4** and **5** (Scheme 1).



Scheme 2. Synthesis of acceptor **4**: i) PTSA, $C_6H_5OCH(OMe)_2$, 2 h, 60 °C, 200 mbar, 60 %; ii) BnBr, NaH, DMF, 3 h, r.t., 88 %; iii) TFA, Et_3SiH, CH_2Cl_2, 2 h, r.t., 50 %.

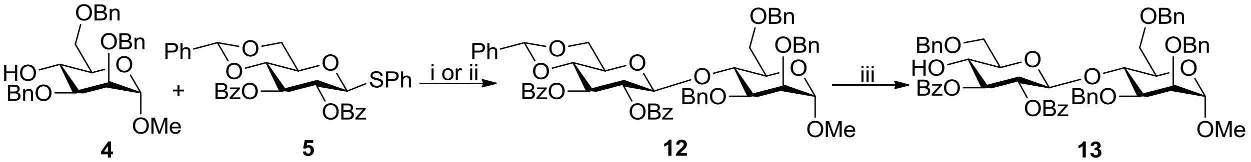
The synthesis of donor **3** was discussed in the previous Episode and a similar preparative route was also used in the present synthesis.[9] Acceptor **4** was prepared by the route shown in Scheme 2. The commercially available methyl mannopyranoside **6** was converted into **7** utilizing one equivalent of benzaldehyde dimethylacetal under slightly acidic conditions according to the procedures described by McGowan in 1982.[10] The remaining hydroxyl groups were benzylated by the standard protocol using NaH and BnBr. Next, the benzylidene acetal was partially cleaved by the reductive methodology of Fraser-Reid, DeNinno and coworkers to give the 4-OH/6-OBn containing acceptor **4** in moderate yield.[11] A similar efficiency was also witnessed previously in cases where benzylidene protected mannopyranosides were partially cleaved under these conditions.[12] With building blocks **3** and **4** at hand, the attention was turned towards preparation of building block **5**.



9 **10** **11** **5**

Scheme 3. Synthesis of donor **5**: i) 1. SHPh, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 24 h, r.t., 96 %; 2. NaOMe, MeOH:THF 3:1, 24 h, r.t., 95 %; ii) PTSA, $C_6H_5OCH(OMe)_2$, DMF, 60 °C, 200 mbar, 88 %; iii) BzCl, pyridine, 5 h, r.t., 90 %.

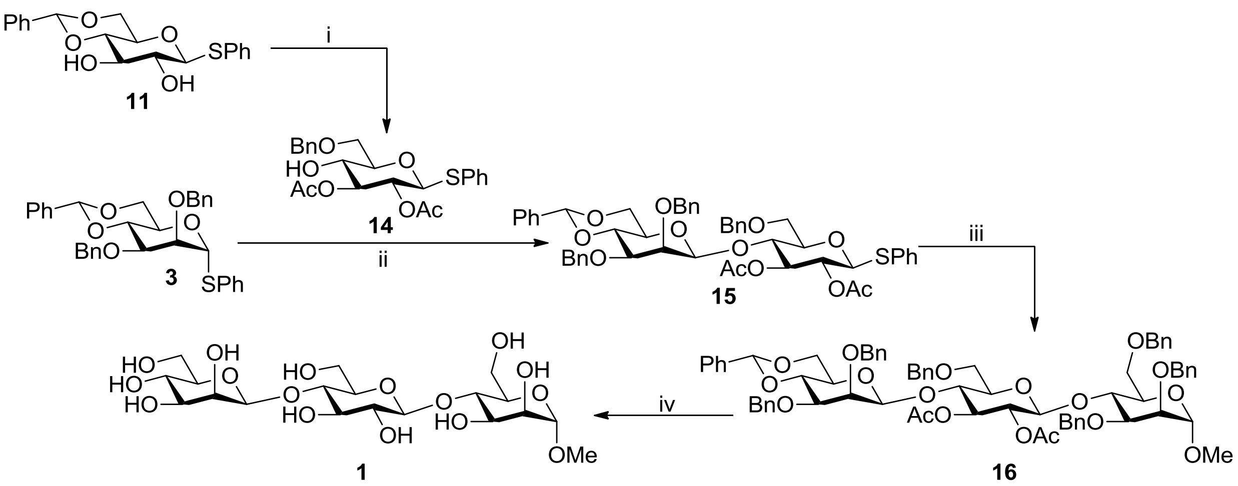
Donor **5** was synthesized according to the synthetic route shown in Scheme 3. Peracetylated D-glucopyranose was subjected to a $BF_3{\cdot}OEt_2$ promoted glycosylation followed by deacetylation under Zemplén conditions.[13] The benzylidene protected thioglycoside **11** was synthesized according to earlier described procedures.[10] As expected, the yields were significantly higher in the benzylidene formation with the glucopyranoside in comparison to those of the mannopyranosides. This is mainly due to the *trans*-relationship of the 2- and 3-hydroxyl groups in the glucose moiety which significantly reduces the formation of the dibenzylidene side product formed with mannopyranosides. The remaining hydroxyl groups were next benzoylated by standard methods using BzCl in pyridine. With the building blocks prepared the focus was shifted towards assembly of the trisaccharide **1**.



4 **5** **12** **13**

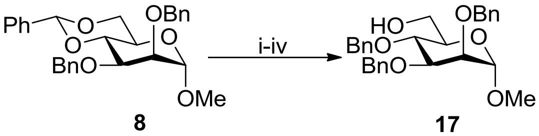
Scheme 4. Synthesis of disaccharide **12**: i) NIS (1.2 eq.), TMSOTf (0.12 eq.), CH_2Cl_2, 1.5 h, – 25 °C, 47 %; ii) NIS (1.2 eq.), TMSOTf (0.06 eq.), CH_2Cl_2, 1.5 h, – 25 °C, 76 %; iii) TFA, Et_3SiH, CH_2Cl_2, r.t., 1–5 h, <15 %.

Following a linear pathway, donor **5** was first coupled with acceptor **4** by the conventional promoter/activator system featuring NIS and TMSOTf (Scheme 4).[14] Several difficulties were encountered already during the first attempts of this glycosylation. In our initial attempt, following the standard activation protocol featuring 0.12 equivalents of TMSOTf, a moderate yield of 47 % was obtained due to formation of several side products. When the amount of TMSOTf was lowered to 0.06 equivalents, the yield of the desired disaccharide **12** increased. The main difficulty encountered during the synthesis was the highly crystalline nature of the benzoylated glucose donor **5** and disaccharide **12**. This resulted in extremely difficult purification procedures since the products crystallized in the column while performing column chromatography, despite being highly soluble in the solvent system as such. In addition, crystallization of disaccharide **12** in the presence of donor **5** was found to be impossible due to the small scale of the synthesis. Furthermore, the partial reductive cleavage following the protocol described by Fraser-Reid and coworkers resulted in a poor yield of the desired disaccharide **13**.[11] As a result of these trials, the synthetic strategy required revision. Since the selective ring-opening of the benzylidene acetal in disaccharide **12** turned out to be difficult, it was decided to change the synthetic route from linear to convergent. The convergent route would add two potential benefits to the synthetic pathway. The β-mannopyranosyl linkage could be constructed first followed by convergent coupling of a disaccharide to acceptor **4**. In addition, the selective ring-opening of the benzylidene acetal could now be performed on the glucose building block instead of the disaccharide. In order to avoid the problematic crystalline nature displayed by dibenzoylated glucoside, the benzoyl groups were simultaneously replaced by acetyl groups. The synthesis of trisaccharide **1** is shown in Scheme 5. The synthesis of donor **3** was described in Episode 1 and was available on large scale for the present synthetic route. Acceptor **14** was synthesized from **11** by acetylation of the free hydroxyl group followed by selective ring opening of the benzylidene acetal by use of the methods described previously. This time, the reaction yield was excellent.[11] The large variation in the efficiency of the methodology described by Fraser-Reid *et al.* was found to be highly dependent on the stereochemistry of the individual sugars. Similar results have been reported in the literature previously.[12] Next, donor **3** was activated according to the β-mannosylation protocol described by Crich and coworkers and coupled with acceptor **14**.[15] The glycosylation reaction proceeded with moderate efficiency and high selectivity (β:α 10:1). The yield of this individual glycosylation reaction could probably have been increased by addition of 1-octene to the reaction mixture following the activation of donor **3**. 1-Octene has been shown to be a mild reagent which traps the thioadducts formed as side products during the activation and conversion of donor **3** to the activated glycosyl triflate.[16] This useful information was, however, not known to the author of this thesis at the time when the synthesis was conducted. With donor **15** synthesized, it was coupled with acceptor **4** under the optimized reaction conditions (0.06 equivalents of TMSOTf) from our previous synthetic attempt displayed in Scheme 4. The convergent glycosylation proceeded smoothly to give the protected trisaccharide **16** in 77 % isolated yield. A two step deprotection sequence featuring deacetylation[13b] followed by hydrogenolyses gave the trisaccharide **1** in 84 % overall yield.



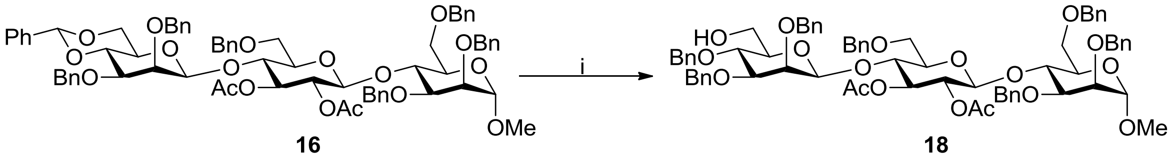
Scheme 5. Synthesis of GGM core trisaccharide **1**: i) 1. Ac_2O, pyridine, r.t., 5 h, 95 %; 2. Et_3SiH, TFA, 0 °C→r.t., 3 h, 85 %; ii) 1. BSP, TTBP, Tf_2O, CH_2Cl_2, –60 °C, 0.5 h: 2. **14**, –78 °C, 2 h, 55 %; iii) **4**, NIS, TMSOTf, CH_2Cl_2, –40 °C, 1.5 h, 77 %; iv) 1. NaOMe, MeOH:THF 1:1, r.t., 19 h, 84 %; 2. Pd/C, H_2 (2.8bar), MeOH, r.t, 19 h, quant.

With the trisaccharide repeating unit synthesized, the focus was shifted towards assembly of the less abundant tetrasaccharide fragment **2**. Trisaccharide **16** was chosen as a suitable starting material for the synthesis of tetrasaccharide **2**. Once again, selective ring-opening of the benzylidene acetal was required as a key transformation in preparation of the trisaccharide acceptor. This time, however, the 6-OH/4-OBn substrate was targeted as the galactopyranosyl residue in **2** is α-(1→6)-linked to the sugar backbone. For this purpose, several procedures have been described in the literature. Some of the utilized procedures feature reagents including $PhBCl_2/Et_3SiH$,[17] $LiAlH_4/AlCl_3$,[18] $TMSOTf/BH_3{\cdot}THF$[19] and $Cu(OTf)_2/BH_3{\cdot}THF$.[20] Instead of evaluating these procedures on trisaccharide **16**, the appropriately protected monosaccharide **8** was utilized in the initial screening of reaction conditions. The results of this initial screening of methodologies are summarized in Scheme 6. In our hands, several of the previously reported procedures did not work with the efficiencies reported in the literature.[17–19] In our initial trials, both the $PhBCl_2/Et_3SiH$[17] and $LiAlH_4/AlCl_3$[18] protocols were quickly ruled out from further use due to poor efficiencies. Considering the moderate efficiency displayed by the $TMSOTf/BH_3{\cdot}THF$ protocol,[19] a continued investigation into borane based protocols was started. The selectivity in the partial cleavage of benzylidene acetals is generally a combination of steric and electronic factors. It has been suggested that 6-OBn derivatives are formed when borane is activated by Lewis acid thereby coordinating to the more nucelophilic 6-OH group. If borane is not activated under the reaction conditions, the Lewis acid is the most electrophilic species in the mixture and coordinates to the 6-OH group of the sugar thus providing the 4-OBn derivative as a product.[21] Consequently, in the present study, it was important to investigate different Lewis acids under similar conditions. Recently Shie published a procedure using $Cu(OTf)_2$ as a Lewis acid in the selective ring-opening of benzylidene acetals.[20] Initial attempts utilizing this protocol resulted in complete cleavage of the benzylidene acetal. The solvent was identified as the major obstacle in this reaction and after switching from THF to CH_2Cl_2, monosaccharide **18** could be isolated in high yield. While this procedure appeared to be functioning well in the case of monosaccharides, only a few reports on the applications of similar protocols to oligosaccharides have been described previously.



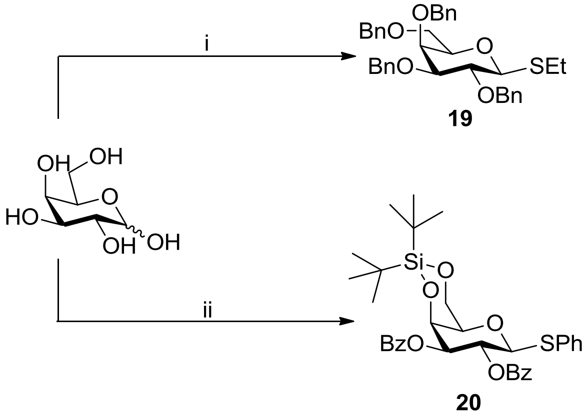
8 **17**

Scheme 6. Screening of methodologies for the selective ring-opening of benzylidene acetals: i) $PhBCl_2$, Et_3SiH, –78 °C, CH_2Cl_2, 2–4 h, 35 %; ii) $LiAlH_4$, $AlCl_3$, CH_2Cl_2: Et_2O 4:1, r.t. →50 °C, 1 h, 35 %; iii) TMSOTf, $BH_3{\cdot}THF$, CH_2Cl_2, r.t., o.n., 50 %; iv) $Cu(OTf)_2$, $BH_3{\cdot}THF$, CH_2Cl_2, r.t., o.n., 82 %.



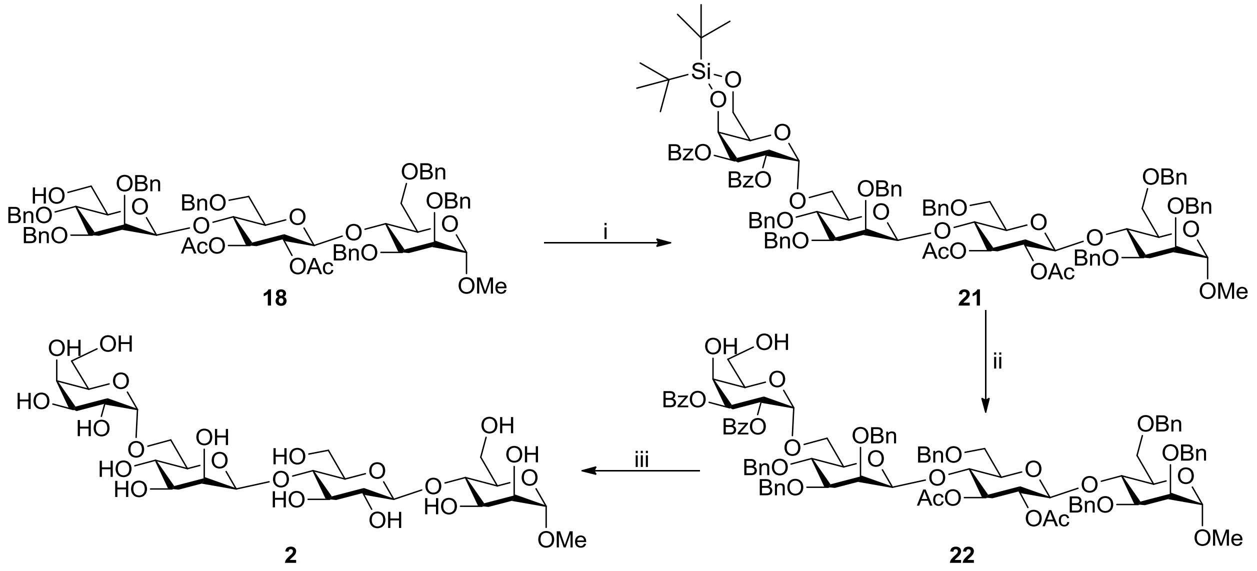
Scheme 7. Synthesis of the trisaccharide acceptor **18**: i) $Cu(OTf)_2$, BH_3·THF, CH_2Cl_2, r.t., o.n., 85 %.

Fortunately, applying similar conditions to the protected trisaccharide **16** gave the trisaccharide acceptor **18** in excellent yield. This was, to our knowledge, one of the first times where a similar procedure has been applied to an oligosaccharide. The high efficiency and selectivity observed under these conditions are encouraging for future applications of this methodology.



Scheme 8. Synthesis of donors **19** and **20**: i) 1. Ac_2O, NaOAc, reflux, 2 h, 50 % (pure β); 2. SHEt, BF_3·OEt_2, CH_2Cl_2, 0 °C, 4 h, 95 %; 3. NaOMe, MeOH:THF 4:3, r.t., o.n., quant.; 4. BnBr, NaH, DMF, r.t., 20 h, 80 %; ii) 1. Ac_2O, NaOAc, reflux, 2 h, 50 % (pure β); 2. SHPh, BF_3·OEt_2, CH_2Cl_2, 0 °C, 4 h, 95 %; 3. NaOMe, MeOH:THF 1:1, r.t., o.n., quant; 4. a) Di-TBS$(OTf)_2$, pyridine, r.t., 3 h; b) BzCl, r.t., 2 h, 57 %.

With the trisaccharide acceptor prepared, the challenge concerning the α-galactosylation remained. For this purpose, donors **19** and **20** were prepared according to literature procedures by the routes shown in Scheme 8.[22] Both of these donors have in recent years been applied in α-galactosylation of various acceptors and were therefore also screened in the present study. Donor **19** has previously been shown to give high α-selectivities and efficiencies in ethereal solvents such as Et_2O.[23] As a result, these conditions were first screened in the α-galactosylation of acceptor **18** featuring the NIS/TMSOTf promoter system.[14] Unfortunately, moderate to low selectivity (α:β 4:1) was achieved under these conditions and separation of the two diastereomers by column chromatography turned out to be challenging. While several variations in solvent proportions (Et_2O:CH_2Cl_2) and activation/promoter systems were tried, no improvements on the selectivity of the glycosylation could be achieved. Therefore, another methodology, featuring $CuBr_2$, TBAB, AgOTf in dichloroethane and DMF, reported previously by Mukherjee and coworkers was screened.[24] This methodology was successfully applied to the α-galactosylation of a tetrasaccharide acceptor in their work. In the present study the protocol failed to yield the desired product.



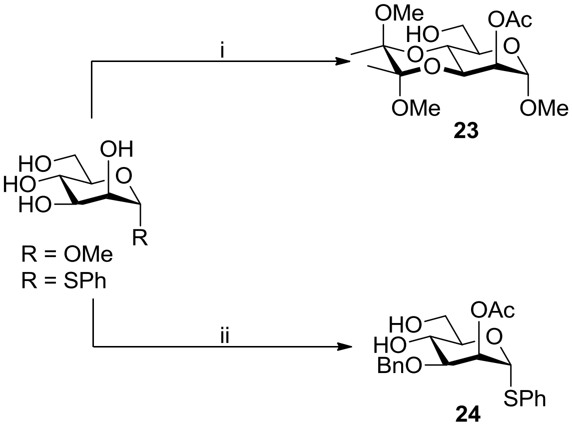
Scheme 9. Synthesis of tetrasaccharide **2**: i) **20**, NIS, TfOH (or TMSOTf), CH_2Cl_2, 0 °C, 1.5 h, 68 %; ii) HF-pyridine, THF, 0 °C → r.t., 6 h, 68 %; iii) 1. NaOMe, MeOH:THF 1:1, r.t., o.n., 96 %; 2. Pd/C, H_2 (2.8 bar), MeOH, r.t., o.n., 96 %.

As a result, our hope was placed on donor **20**. This donor has previously been applied to the α-selective galactosylation of several oligosaccharides and various aglycones by Ando and coworkers.[22b-c,25] In the present study, the methodology was applied to the α-selective galactosylation of **18** providing tetrasaccharide **21** in good yield as shown in Scheme 9. While the yield was good and the selectivity excellent, the formation of a second tetrasaccharide was also observed. Since the tetrasaccharide byproduct appeared to be α-linked (based on $J_{H\text{-}1,H\text{-}2}$),[26] it was most probably an orthoester of the product. With the efficiency already in the desired range, further attempts to optimize the experimental conditions were not made. The reason for the high α-selectivity in the glycosylation reaction is puzzling since ester protective groups are present at both the *O*-2 and *O*-3 in donor **20**. The only possible explanation for the high α-selectivity may, at least in part, be due to the steric and electronic properties caused by the 4,6-silylene acetal. For synthesis of the deprotected tetrasaccharide **2**, the protective groups present in **21** had to be removed. Since silicon has great affinity for fluorine, the silylene acetal was first removed with HF-pyridine complex in 68 % yield.[27] The ester protective groups were next cleaved under Zemplén conditions with NaOMe in a MeOH:THF solvent mixture, followed by hydrogenolyses of the benzyl groups in 92 % overall yield over two steps.

With one of the core trisaccharide repeating units (**1**) and a less abundant tetrasaccharide unit (**2**) synthesized, the attention was turned to the investigation of other phenomena occurring during the isolation of GGM.

2.2 Acetyl Group Migration in Mannopyranosyl Units of GGM

As mentioned in the introduction, the acetylation degree of GGM is ~0.3 slightly varying depending on the methods utilized in the isolation process.[1a,6] The acetyl groups are mainly located at the *O*-2 and *O*-3 positions of the mannopyranosyl residues. It has been proposed that acetyl groups can migrate either directly from *O*-2 to *O*-6 or from *O*-3 to *O*-6 in the mannopyranosyl units.[1a] Migration between *O*-2 and *O*-3 is known to take place in substrates containing acetyl protective groups and will not be discussed in more detail in this context. The migration from *O*-2 and *O*-3 to *O*-6 is unique as it would require the acetyl group to “leap” across the molecule in space. This phenomenon is difficult to study on the native polysaccharide due to severe overlap of signals in the NMR spectra. As a consequence, small molecular models were synthesized in order to study these phenomena in more detail. It is known that mannopyranosides mainly exist in the energetically favoured $^4C_1$-conformation. Based on this assumption a few different acetylated mannopyranosides were synthesized (Scheme 10). The synthesis of these compounds followed standard protecting group manipulations and will not be addressed in more detail here. In our initial study, the acetyl group migration possibilities from *O*-2 to *O*-6 in $^4C_1$-mannopyranosides were evaluated. Compound **23** was chosen as a suitable model substrate for studying acetyl group migration from *O*-2 to *O*-6 as it is locked in the $^4C_1$-conformation by the diacetal protective group between positions 3 and 4. No migration was observed when this substrate was subjected to the conditions utilized during the isolation of GGM (75 °C, D_2O:DMSO 1:1). One possible reason for this result could be the low degree of flexibility in this molecule and hence mannoside **24** was synthesized. While **24** has a much higher degree of flexibility, migration did not take place in this substrate either.



Scheme 10. Synthesis of model substrates **23** and **24**: i) 1. Butanedione, trimethyl orthoformate, CSA (camphorsulfonic acid), MeOH, reflux, 24 h, 79 %; 2. TBDMSCl, imidazole, DMF, 0 °C → r.t., 24 h, 90 %; 3. Ac_2O, pyridine, DCM, 48 h, 92 %; 4. 70 % HF-pyridine, THF, 0 °C → r.t., 24 h, 93 %; ii) 1. PTSA, $C_6H_5OCH(OMe)_2$, DMF, 60 °C, 200 mbar, 2 h, 53 %; 2. a) Bu_2SnO, toluene, 120 °C, 3 h; b) TBAB, CsF, BnBr, 120 °C, 3 h, 85 %; 3. Ac_2O, pyridine, r.t., 3 h, 93 %; 4. 80 % AcOH, 70 °C, 1.5 h, 87 %.

Based on these results, it seems highly unlikely that migration would occur in the GGM-polysaccharide from *O*-2 to *O*-6 as has been suggested previously.[1a] More model substrates are needed in order to investigate the acetyl group migration from *O*-3 to *O*-6. In order to investigate the overall possibility of acetyl group migration, appropriately protected high energy conformations, such as those depicted in Figure 4, should be synthesized and studied under similar conditions. Such an approach should further be combined with molecular modeling in order to create appropriate models for investigating these phenomena in detail. The synthesis of appropriately protected mannosides for further studies on the acetyl migration behavior of GGM is currently under planning. The acetylated trisaccharide fragment (in preparation) representing the second repeating unit in the GGM backbone might prove to be an additional substrate utilizable in the study on acetyl group migration/cleavage patterns of GGM.

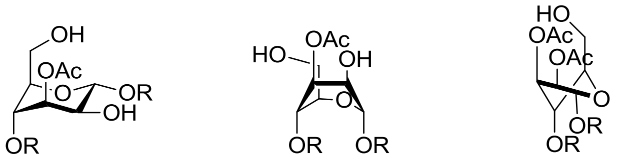
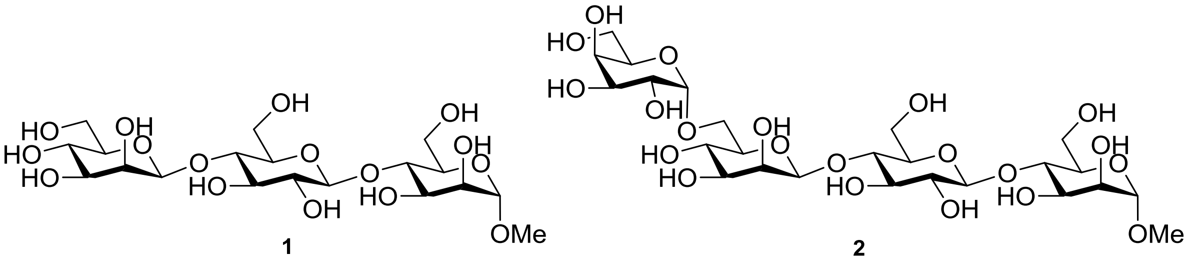


Figure 4. High energy conformations of mannopyranosides where acetyl group migration could be possible; from the left to right $^{1}C_{4}$, $B_{1,4}$, $^{5}H_{4}$.

2.3 NMR Spectroscopic Comparison of the Synthetic Model Substrates vs. Native GGM



1

2

Figure 5. The synthetic targets representing one of the trisaccharide repeating units of GGM (left) and a less abundant tetrasaccharide fragment (right).

In order to compare the NMR data of the synthetic structures and native GGM, the complete spectral characterization of both ^{13}C- and ^{1}H-NMR spectra of **1** and **2** was desirable. In the previous chapter the complete characterization of a β-(1→2)-linked mannotetraose was described and a similar set of NMR experiments was utilized also for the current spectral characterization. The NMR spectra were recorded at 308 K (in D_2O) in order to shift the water peak, as it was otherwise overlapping with anomeric protons, and in order to increase the solubility of the polysaccharide sample. The spectral characterization of oligosaccharides was discussed in detail in the previous chapter and the discussion will be less thorough here. In short, the ^{1}H-NMR spectrum of **1** gives rise to three well separated anomeric protons at 4.76 (d, H-1), 4.74 (d, H-1″) and 4.52 (d, H-1′) ppm. These signals were also targeted by selective excitation utilizing the 1D-TOCSY experiment[28] and a spinlock time of 300 ms. The results of these experiment are visualized in Figure 6. With the help of COSY, HSQC (coupled and decoupled) and HMBC both the ^{1}H- and ^{13}C-NMR spectra could be completely solved.[29]

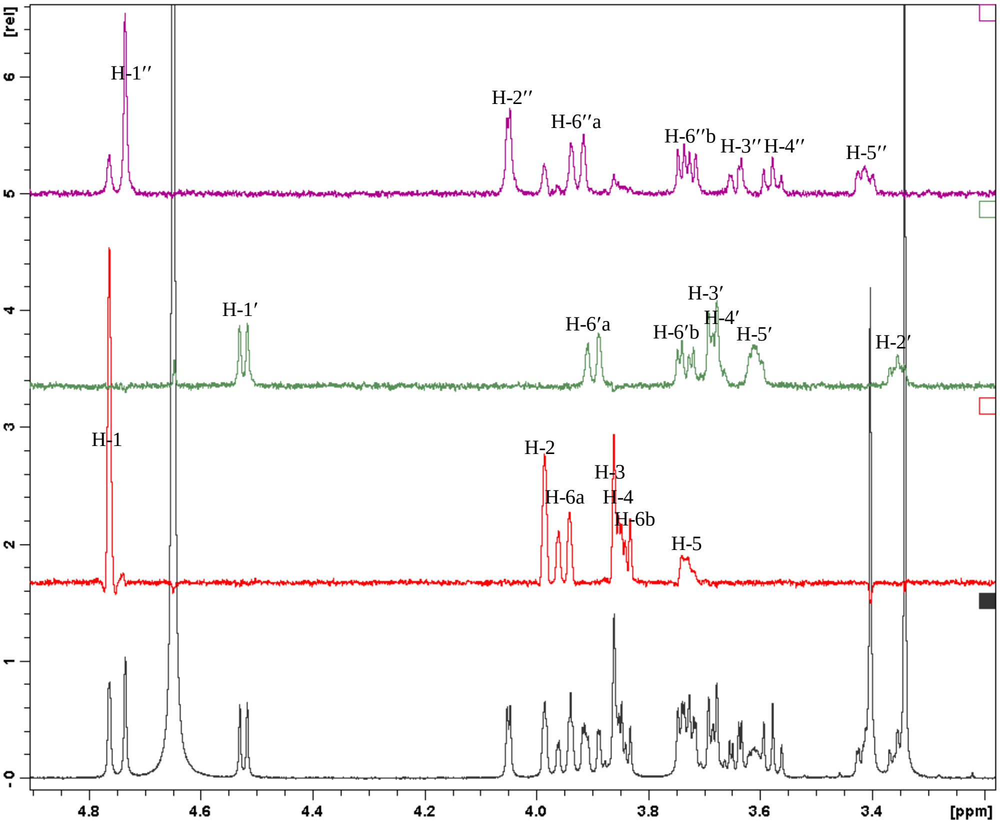


Figure 6. 1D-TOCSY spectra of the different residues in **1**: from the top, Man*p*″, Glc*p*′, Man*p* and the entire ^{1}H-NMR spectrum of **1**.

Characterization of **2** followed a similar strategy. Well separated signals from each of the sugar residues in the ^{1}H-NMR spectrum were targeted by selective excitation using the 1D-TOCSY experiment (Figure 7).[28] Utilization of standard NMR experiments [COSY, HSQC (both coupled and decoupled) and HMBC] allowed the complete assignment of both the ^{1}H- and ^{13}C-NMR spectra of **2**. The ^{1}H-NMR spectra of **1** and **2** were simulated with the PERCH software in order to obtain reliable coupling constants for the individual protons. With the NMR spectra of model substrates **1** and **2** solved, the attention was focused on comparing the chemical shifts with those of native GGM. The proton and carbon chemical shifts of **1**, **2** and literature values of the chemical shifts of GGM[6a,8a,30] are summarized in Table 1. The coupling constants and patterns observed in the ^{1}H-NMR spectrum and the $J_{C,H}$ coupling constants for the anomeric centers are provided in the experimental section for **1** and **2**.

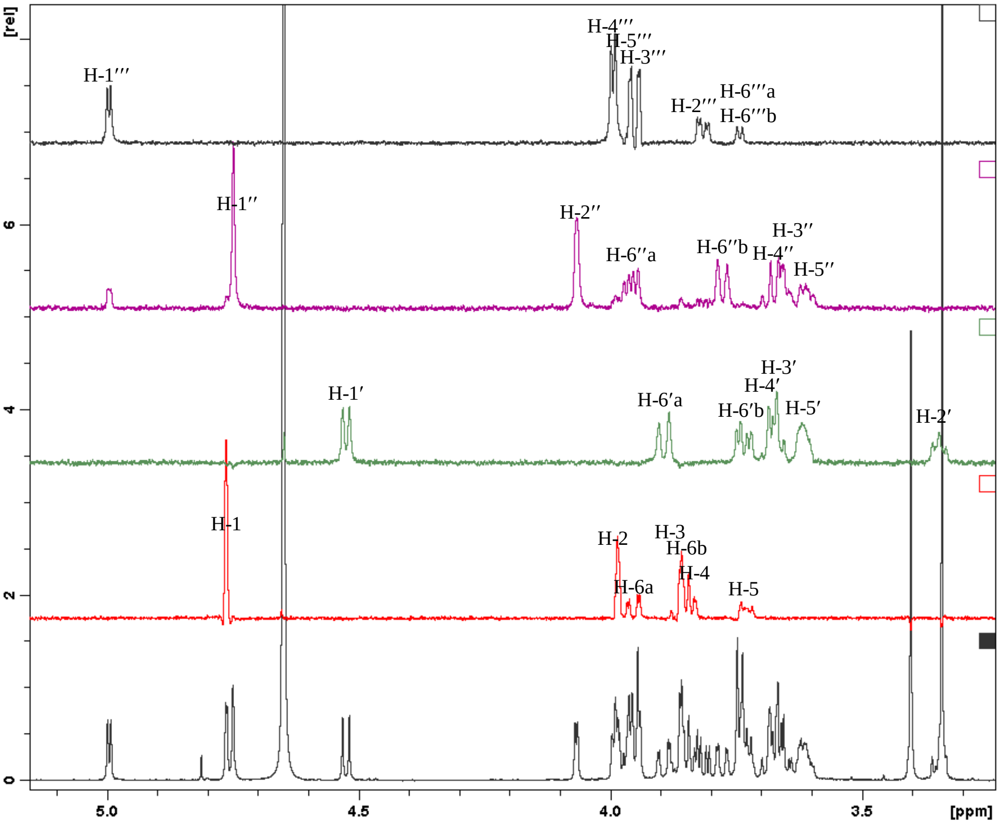


Figure 7. 1D-TOCSY spectra of the different residues in **2**: from the top, Gal*p*‴, Man*p*″, Glc*p*′, Man*p* and the entire ^{1}H-NMR spectrum of **2**.

As seen from Table 1, many of the chemical shifts for the individual signals in **1** and **2** correlate relatively well with the values reported in the literature for native GGM.[6a,8a,30] There are certain signals where significant chemical shift differences have been reported in the literature. One of the main signals where large deviations have been reported is the H-2 of the β-(1→4)-linked mannopyranoside residue. This signal has been reported to appear in native GGM at 3.08 or 4.08 ppm.[6a,8a,30] In both model substrates **1** and **2** the chemical shift for H-2 was found to be close to 4.08 ppm simultaneously indicating these literature values to be correct. In fact, all other signals in this residue are located at similar frequencies in both the model substrates and native GGM thus suggesting an error or typo in the literature value given by Hannuksela and coworkers for the chemical shift of H-2 in native GGM.[6a]

Sugar	**H-1** **C-1**	**H-2** **C-2**	**H-3** **C-3**	**H-4** **C-4**	**H-5** **C-5**	**H-6a, b** **C-6**
Native GGM						
β-Man*p*-(1→	4.75 101.2	4.08 (or 3.08)[6a] 71.12	3.66 72.50	3.58 77.63	3.45 76.1	3.95, 3.735 61.9
→4)-β-Glc*p*-(1→	4.53 103.6	3.364 73.87	3.702 74.98	3.63 79.519	3.78 77.5	-
α-Gal*p*-(1→	5.038 99.84	3.83 69.46	3.958 70.31	4.01 70.26	3.92 72.3	3.77 62.29
→6)-β-Man*p*-(1→	4.79 -	-	-	-	3.70 -	4.03–3.94, 3.84–3.79 -
Model substrate 1						
β-Man*p*-(1→	4.73 100.6	4.05 71.1	3.65 73.4	3.58 67.3	3.41 77.0	3.93, 3.73 61.5
→4)-β-Glc*p*-(1→	4.52 103.0	3.35 73.5	3.69 74.6	3.68 79.2	3.61 75.3	3.90, 3.73 60.8
→4)-α-Man*p*	4.76 101.2	3.99 70.1	3.86 69.8	3.86 77.2	3.73 71.8	3.95, 3.84 60.9
Model substrate 2						
→6)-β-Man*p*-(1→	4.75 100.8	4.07 71.1	3.65 73.5	3.68 67.2	3.61 75.1	3.96, 3.78 66.8
→4)-β-Glc*p*-(1→	4.53 103.0	3.35 73.5	3.69 74.7	3.67 79.6	3.62 75.1	3.89, 3.73 60.8
→4)-α-Man*p*	4.76 101.2	3.98 70.1	3.86 69.9	3.85 77.3	3.73 71.8	3.95, 3.84 60.9
α-Gal*p*-(1→	5.00 98.9	3.81 69.1	3.95 71.6	3.99 69.9	3.95 70.0	3.74, 3.74 61.8

Table 1. The chemical shifts of native GGM and model substrates **1** and **2** are expressed on the δ-scale (in ppm) utilizing CD_3OD/D_2O as internal standards. The chemical shifts for the GGM polysaccharide are literature values.[6a,8a,30]

The chemical shifts for the H-6a, H-6b and C-6 of the β-(1→4)-linked glucopyranoside residues have not been reported in the literature for native GGM but were found to differ less than 0.2 ppm in both model substrates and could, therefore, be assumed to be similar in the polysaccharide structure. The H-5 and C-5 chemical shifts in the β-(1→4)-linked glucopyranoside residues of native GGM appeared at higher frequency than in model substrates **1** and **2**. As the values are almost exactly similar for all other signals in this residue, there might be some uncertainties in the chemical shifts reported for GGM in the literature. Suprisingly, there was little data available on the β-(1→4)-linked mannopyranoside residue bearing an α-(1→6)-linked galactopyranosyl unit. These chemical shifts were easily identified in our model substrates and will complement the data available in the literature. Interestingly, the H-5 proton is shifted towards higher frequency and C-5 carbon towards lower frequency when the β-(1→4)-linked mannopyranosyl unit is substituted by an α-(1→6)-linked galactopyranosyl residue. A further notable difference in chemical shifts of the α-(1→6)-galactosylated mannopyranosyl unit is the large difference for the C-6 carbon which is located at 66.8 ppm in the substituted mannopyranosyl residue vs. 61.5 ppm in the unsubstituted one. This will provide a good reference point for future determinations of the amount of 6-substituted β-(1→4)-linked mannopyranosyl units in the GGM backbone.

While slight deviations were found in the NMR spectroscopic characterization of GGM as compared to our model substrates, most signals were found to be in good agreement with the previously suggested spectral data. These results suggest that it is indeed possible to study the composition of polysaccharides by utilization of small molecular models of limited size. To further evaluate and continue the study on the GGM polymer, several other small molecular fragments will need to be synthesized and analyzed. Most important among these fragments will be the synthesis and characterization of the second repeating unit bearing acetyl groups along with a complete conformational study on all of the fragments.

3 Conclusions

In this chapter, recent studies related to the nature of GGM found in *P. abies* were described. In the first part of the chapter the synthesis of some model compounds of the GGM backbone were discussed. One of the trisaccharide repeating units of GGM was synthesized along with a less abundant tetrasaccharide fragment. The synthesis of the tetrasaccharide required a thorough investigation of methodologies for selective ring-opening of benzylidene acetals[11,12,17,18,19,20] as well as methodologies for α-selective galactosylation[23,24] of oligosaccharides.

In the second part of the chapter, a brief investigation of the previously suggested acetyl group migration patterns of GGM were analyzed by utilization of synthetic monosaccharide models. No migration was found to occur between the *O*-2 and *O*-6 in the mannopyranoside unit when unflexible and flexible models existing in the $^4C_1$-conformation were utilized. Since the mannosides generally exist in this low energy conformation also in polysaccharides, the acetyl group migration seems highly unlikely to occur in the polysaccharide material. A continued study on these phenoma would require the synthesis of partially acetylated (*O*-2, *O*-3) mannopyranosides existing in high energy conformations. In these substrates the *O*-6 of the mannopyranoside is closer to the *O*-2 and *O*-3 and, therefore, the acetyl group transfer might be more favoured.

In the third part of the chapter, NMR spectroscopic characterization of the synthetic structures were described along with a short comparison of chemical shifts between the model substrates and those reported in the literature for native GGM.[6a,8a,30] Although many of the chemical shifts were found to be in good agreement, several of the uncertainties found in the literature values of GGM could be pointed out with the help of the fragments synthesized. Further information gained with small molecular models involved the significant downfield shift (6 ppm) of the C-6 in the β-(1→4)-linked mannopyranoside residue when bearing an α-(1→6)-galactopyranosyl unit vs. the β-(1→4)-linked mannopyranoside without any substituents. In addition, several chemical shifts not present in the literature were found such as those for the (→6)-β-Man*p*-(1→)-fragment. Based on the results reported here, it appears possible to study the fine chemical structure of large polysaccharides by use of small molecular model substrates. Encouraged by these findings, we aim to synthesize the remaining repeating unit of GGM, the partially acetylated mannotriose, and continue the NMR spectroscopic comparison of model substrates vs. native GGM. The individual fragments will also be submitted for evaluation of their immunological properties.

4 Experimental Section

Reaction solvents were dried and distilled prior to use when necessary. All reactions containing moisture- or air-sensitive reagents were carried out under argon atmosphere. The NMR spectra were recorded with Bruker Avance NMR spectrometer operating at 600.13 MHz (^{1}H: 600.13

MHz, ^{13}C: 150.90 MHz). The probe temperature during the experiments was kept at 25 °C unless indicated otherwise. All products were fully characterized by utilization of the following 1D-techniques: ^{1}H, ^{13}C and TOCSY and the following 2D-techniques; DQF-COSY, NOESY, HSQC and HMBC by using pulse sequences provided by the manufacturer. Chemical shifts are expressed on the δ scale (in ppm) using TMS (tetramethylsilane), residual chloroform or methanol as internal standards. Coupling constants are given in Hz and provided only once, when first encountered. Coupling patterns are given as *s* (singlet), *d* (doublet), *t* (triplet) etc. The computational analysis of the ^{1}H-NMR of all compounds was achieved by utilization of the PERCH NMR software with starting values and spectral parameters obtained from the various NMR techniques used.[29] HRMS were recorded using Bruker Micro Q-TOF with ESI (electrospray ionization) operated in positive mode. Optical rotations were measured at 23 °C, unless otherwise stated, with a Perkin Elmer polarimeter equipped with a Na-lamp (589 nm). TLC was performed on aluminium sheets precoated with silica gel 60 F254 (Merck). Flash chromatography was carried out on silica gel 60 (0.040-0.060 mm, Merck). Spots were visualized by UV followed by charring with 1:4 H_2SO_4/MeOH and heating.

4.1 General Experimental Procedures

General procedure for glycosylation of peracetylated sugars. To a solution containing peracetylated sugar (1 equiv.) and 4 Å MS in CH_2Cl_2 (3 ml/1 mmol) was added the corresponding alcohol (1.5–4 equiv.) and the resulting mixture was stirred for 0.5 h. The mixture was then cooled with an ice bath and $BF_3{\cdot}OEt_2$ (5–8 equiv.) was added dropwise. The resulting mixture was stirred for 0.5 h, brought to r.t. and stirring was continued for 20 h. The reaction mixture was diluted with CH_2Cl_2 (8 ml/ 1 mmol starting material) and poured into ice-cold H_2O (16 ml/1 mmol starting material) with stirring. The organic phase was separated and washed with sat. $NaHCO_3$ (8 ml/1 mmol starting material), H_2O (8 ml/1 mmol starting material) and brine (8 ml/1 mmol starting material). The organic phase was dried over anhydrous $MgSO_4$ or Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 4:1) to give the corresponding glycoside.

General procedure for deacetylation. To a solution containing the acetylated glycoside (1 equiv.) in dry MeOH or dry MeOH:THF mixture (2:1) (1 ml/100 mg substrate) was added NaOMe (2 equiv.) and the resulting mixture was stirred for 3–20 h at r.t., neutralized with DOWEX 50 H^+-form, filtered and concentrated. The crude product was purified by column chromatography (CH_2Cl_2→CH_2Cl_2:MeOH, 5:1) to give the corresponding deacetylated glycoside.

General procedure for synthesis of 4,6-*O*-benzylidene acetals. To a solution of the corresponding deacetylated glycoside (1 equiv.) in DMF (1 ml/40 mg starting material) was added PTSA (10 mol%) and benzaldehyde dimethyl acetal (1 equiv.). The resulting mixture was stirred at 60 °C and 200 mbar for 2 h and concentrated. To the concentrated residue was added H_2O (10 ml/500 mg) and formation of a white solid was received. The solid was filtered off and washed with H_2O (10 ml/500 mg) and Et_2O (3 ml/1 g) to obtain the crude product. The crude product was either used as such or in some cases further purified by column chromatography (CH_2Cl_2→CH_2Cl_2:MeOH, 5:1) to give the corresponding 4,6-*O*-benzylidene-protected-glycosides.

General procedure for the selective benzylation of the equatorial 3-OH-group. To a solution of the corresponding 4,6-*O*-benzylidene-α-D-mannopyranoside (1 equiv.) in toluene (4 ml/100 mg starting material) was added Bu_2SnO (1 equiv.) and the resulting mixture was refluxed at 120 °C for 3 h. The reaction mixture was the cooled to r.t. and TBAB (1.05 equiv.), CsF (1.02 equiv.) and BnBr (1.04 equiv.) were added and the mixture was again refluxed for 3 h, cooled to r.t., diluted with EtOAc (4 ml/100 mg) and sat. $NaHCO_3$ (3 ml/100 mg) and filtered through celite. The organic phase was separated and the aqueous phase was extracted with EtOAc (3 × 3 ml/100 mg). The combined organic phase was washed with H_2O (4 ml/100 mg) and brine (3 ml/100 mg), dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 4:1) to give the corresponding 3-*O*-benzyl derivatives.

General procedure for alkylation of free hydroxyl groups. To a solution containing the partially protected glycoside (1 equiv.) in DMF (2 ml/100 mg starting material) was added NaH (1.5–1.9 equiv./free *OH*-group) at 0 °C. The reaction mixture was stirred for 15 min, then brought to r.t., stirred for 10 min then the corresponding bromide (1.5 equiv./free *OH*-group) was added. The resulting mixture was stirred for 1–20 h, quenched with MeOH (0.4 ml/ mmol starting material), diluted with CH_2Cl_2 (4 ml/100 mg) and washed with sat. $NaHCO_3$ (3 ml/100 mg). The organic phase was separated and the aqueous phase was washed with CH_2Cl_2 (3 × 3 ml/100 mg). The combined organic phase was washed with brine (3 ml/100 mg), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 8:1).

General procedure for silylation of 6-OH group. To a solution containing the corresponding glycoside (1 equiv.) in DMF (30 ml/1 g of starting material) was added imidazole (2 equiv.) and the reaction mixture was cooled to 0 °C. TBDMSiCl (1.1 equiv.) was added and the reaction was brought to r.t. and stirring was continued for 24 h. The solution was concentrated, diluted with EtOAc (100 ml/3 g) and washed with H_2O (80 ml/3 g). The water phase was then extracted with EtOAc (3 × 50 ml/3 g) and the combined organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography (hexane:EtOAc 3:1) to give the protected glycoside.

General procedure for ring-opening of benzylidene acetal to give the 4-OH/6-OBn substrate. To a solution containing the corresponding 4,6-*O*-benzylidene acetal (1 equiv.) in CH_2Cl_2 (6 ml/0.5 g of starting material) was added Et_3SiH (5 equiv.), followed by dropwise addition of TFA (5 equiv.) at 0 °C. The resulting mixture was brought to r.t., stirred for 2 h, diluted with EtOAc (20 ml/0.5 g), washed with sat. $NaHCO_3$ (20 ml/0.5 g) and brine (20 ml/0.5 g), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 3:2) to give the corresponding acceptor.

General procedure for ring-opening of benzylidene acetal to give the 4-OBn/6-OH substrate. To a solution containing the corresponding 4,6-*O*-benzylidene acetal (1 equiv.) in dry DCM (3 ml/0.3 g of starting material) was added pre activated 4 Å molecular sieves and borane/tetrahydrofuran complex in THF (5 equiv.). The resulting mixture was stirred for 10 minutes and $Cu(OTf)_2$ (0.07 equiv.) was added and stirring was continued for 3 hours. The reaction mixture was then cooled to 0 °C and the reaction was quenched by the addition of Et_3N

and MeOH. The resulting mixture was concenctrated to give the crude product which was purified by column chromatography (hexane:EtOAc 3:2→1:1) to give the corresponding acceptor.

General procedure for insertion of 3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl) protective group. To a solution of methyl α-D-mannopyranoside (1 equiv.) and CSA (10 mol%) in MeOH (10 ml/1 g) was added trimethylorthoformate (4.2 equiv.) and butane-2,3-dione (1.6 equiv.). The resulting mixture was refluxed for 19 h and cooled to r.t. The reaction was quenched with Et_3N and concentrated to give the crude product which was purified by column chromatography (Et_2O) to give the protected substrate.

General procedure for esterification of/insertion of 4,6-*O*-di-tertbutyl silylene protective group. To a solution containing the partially protected glycoside in pyridine (3 ml/1 g of starting material) was added either Ac_2O (3 ml/1 g of starting material), BzCl (1.6 ml/1 g of starting material) or $DTBS(OTf)_2$ (0.4 ml/0.2 g glycoside). The resulting mixture was stirred for 1–5 h, cooled to 0 °C, quenched with MeOH (3 ml/1 g of starting material), brought to r.t., diluted with CH_2Cl_2 (50 ml/2 g) and washed with sat. $NaHCO_3$ (50 ml/2 g). In the case of $DTBS(OTf)_2$, the remaining hydroxyl groups were simultaneously converted to esters by the addition of BzCl according to the procedures above and the work up was conducted accordingly. The organic phase was separated and washed with H_2O (3 × 50 ml/3 g), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography chromatography (hexane:EtOAc 6:1).

General procedure for β-mannosylation. To a solution containing the corresponding donor (1 equiv.) in dry DCM (4 ml/0.5 mmol of donor) was added at – 60 °C (acetone + dry ice) BSP (1.2 equiv.), TTBP (1.5 equiv.) and Tf_2O (1.3 equiv.). The resulting mixture was stirred for 0.5 h followed by cooling to –78 °C and addition of the corresponding acceptor (1.15 equiv.), dissolved in DCM (3 ml/0.5 mmol of acceptor), over a timeperiod of 15 min. The reaction mixture was stirred for 2 h and quenched by addition of triethylphosphite (3 equiv.). The reaction mixture was stirred for 1 h at –78 °C, brought to r.t., diluted with CH_2Cl_2 (3 ml/100 mg) and washed with sat. $NaHCO_3$-solution (3 ml/100 mg). The water phase was separated and extracted with CH_2Cl_2 (3 × 3 ml/100 mg). The combined organic phase was washed with brine (3 ml/100 mg), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 6:1→4:1→3:2) to give the glycosylated product.

General procedure for glycosylation utilizing NIS/TMSOTf. To a solution containing the corresponding donor (1.2 equiv.) and the corresponding acceptor (1 equiv.) in dry DCM (2.5 ml/100 mg of starting material) was added pre-activated 4 Å molecular sieves and the reaction mixture was cooled to –40 °C. To the solution was added NIS (1.2 equiv.) and TMSOTf (0.06 equiv.) and the resulting mixture was stirred for 1.5 hours. The reaction was quenched by the addition of sat. $NaHCO_3$-solution, brought to rt, diluted with CH_2Cl_2 (30 ml/400 mg of substrate) and washed with sat. $NaHCO_3$-solution (20 ml/400 mg of substrate). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 3:2) to give the corresponding glycoside.

General procedure for removal of silyl protective groups. To a solution containing the silyl-protected glycoside (1 equiv.) in dry THF (3 ml/0.2 g starting material) at 0 °C was added HF-pyridine complex (18 μl/0.03 mmol of starting material) and the resulting mixture was brought to r.t. and stirred for 19 h. The reaction mixture was diluted with CH_2Cl_2 (30 ml/0.5 g) and quenched by addition of sat. $NaHCO_3$-solution. The organic phase was washed with brine (20 ml/0.5 g), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc 1:1) to give the deprotected glycoside.

General procedure for hydrogenolysis. To a solution of the corresponding protected mannoside (1 equiv.) in MeOH or MeOH-EtOAc (10:1, 1 ml/10 mg of starting material) was added Pd/C (10 % Pd, 2.5 equiv. by mass). The reaction mixture was stirred in an autoclave under H_2 (1.4–2.8 bar) overnight, filtered through celite and concentrated to give the deprotected substrates.

4.2 Analytical Data on Selected Substrates

The analytical data will here be limited to the substrates prepared while synthesizing the GGM model oligosaccharides.[31]

Thiophenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranoside (15). Synthesized from donor **3** (116 mg, 0.24 mmol) and **14** (110 mg, 0.25 mmol, 1.15 equiv.) according to the general procedure for β-mannosylation to give the title compound as a white foam (100 mg, 53 %). TLC: R_f = 0.54 (hexane:EtOAc 3:2). $[\alpha]_D$ = –46.2 (c 0.2, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.51–7.24 (m, 25 H, arom. *H*), 5.55 (s, 1 H, C*H*′Ph), 5.23 (dd, 1 H, $J_{3,2}$ = 9.3, $J_{3,4}$ = 9.4 Hz, H-3), 4.92 (dd, 1 H, $J_{2,1}$ = 10.1 Hz, H-2), 4.78 and 4.67 (each d, each 1 H, *J* = –12.1 Hz, 2′-CH_2Ph), 4.71 and 4.57 (each d, each 1 H, *J* = –12.4 Hz, 3′-CH_2Ph), 4.68 (d, 1 H, H-1), 4.57 and 4.38 (each d, each 1 H, *J* = –11.9 Hz, 6-CH_2Ph), 4.38 (d, 1 H, $J_{1',2'}$ = 1.0 Hz, H-1′), 4.26 (dd, 1 H, $J_{6'a,5'}$ = 4.8, $J_{6'a,6'b}$ = –10.2 Hz, H-6′a), 4.07 (dd, 1 H, $J_{4',5'}$ = 9.3, $J_{4',3'}$ = 9.8 Hz, H-4′), 3.89 (dd, 1 H, $J_{4,5}$ = 9.9 Hz, H-4), 3.78 (dd, 1 H, $J_{6'b,5'}$ = 10.0 Hz, H-6′b), 3.68 (dd, 1 H, $J_{6a,5}$ = 1.9, $J_{6a,6b}$ = –11.2 Hz, H-6a), 3.65 (dd, 1 H, $J_{2',3'}$ = 3.1 Hz, H-2′), 3.52 (dd, 1 H, $J_{6b,5}$ = 3.5 Hz, H-6b), 3.46 (ddd, 1 H, H-5), 3.40 (dd, 1 H, H-3′), 3.13 (ddd, 1 H, H-5′), 2.08 (s, 3 H, 2-OCOCH_3), 2.08 (s, 3 H, 3-OCOCH_3).

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 170.0 (3-O*C*OCH_3), 169.5 (2-O*C*OCH_3), 138.6–126.0 (arom. *C*), 101.9 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ = 157.4 Hz), 101.4 (*C*′HPh), 85.6 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ = 157.1 Hz), 78.9 (C-5), 78.6 (C-4′), 78.0 (C-3′), 76.4 (C-2′), 75.3 (C-4), 74.6 (2′-*C*H_2Ph), 74.2 (C-3), 73.6 (6-*C*H_2Ph), 72.4 (3′-*C*H_2Ph), 70.2 (C-2), 68.6 (C-6′), 68.3 (C-6), 67.4 (C-5′), 20.9 (3-OCO*C*H_3), 20.8 (2-OCO*C*H_3).

HRMS: *m/z* calcd. for $C_{50}H_{52}O_{12}SNa$ $[M + Na]^+$ 899.3077; found 899.3080.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (16). Synthesized from donor **15** (81 mg, 0.09 mmol, 1.2 equiv.) and acceptor **4** (36 mg, 0.08 mmol) according to the general procedure for glycosylation utilizing NIS/TMSOTf to give the title compound as a white foam (73 mg, 77 %). TLC: R_f = 0.39 (hexane:EtOAc 3:2). $[\alpha]_D$ = –11.0 (c 0.1, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.47–7.13 (m, 35 H, arom. *H*), 5.54 (s, 1 H, C*H*′′Ph), 5.06 (dd, 1 H, $J_{3',4'}$ = 9.3, $J_{3',2'}$ = 9.7 Hz, H-3′), 4.86 (dd, 1 H, $J_{2',1'}$ = 8.1 Hz, H-2′), 4.77 and 4.63 (each d, each 1 H, *J* = –12.1 Hz, 2′′-CH_2Ph), 4.77 and 4.51 (each d, each 1 H, *J* = –11.9 Hz, 6-CH_2Ph), 4.76 and 4.57 (each d, each 1 H, *J* = –12.5 Hz, 3-CH_2Ph), 4.73 (d, 1 H, $J_{1,2}$ = 1.9 Hz, H-1), 4.72 and 4.67 (each d, each 1 H, *J* = –12.4 Hz, 2-CH_2Ph), 4.65 and 4.51 (each d, each 1

H, $J = –12.4$ Hz, 3″-CH_2Ph), 4.65 (d, 1 H, H-1′), 4.41 and 4.20 (each d, each 1 H, $J = –12.0$ Hz, 6′-CH_2Ph), 4.33 (s, 1 H, H-1″), 4.25 (dd, 1 H, $J_{6''a,5''} = 4.9$, $J_{6''a,6''b} = –10.3$ Hz, H-6″a), 4.24 (dd, 1 H, $J_{4,3} = 9.0$, $J_{4,5} = 9.7$ Hz, H-4), 4.05 (dd, 1 H, $J_{4'',5''} = 9.3$, $J_{4'',3''} = 9.8$ Hz, H-4″), 3.87 (dd,1 H, $J_{4',5'} = 9.8$ Hz, H-4′), 3.83 (dd, 1 H, $J_{3,2} = 3.2$ Hz, H-3), 3.79 (dd, 1 H, $J_{6a,5} = 4.6$, $J_{6a,6b} = –11.0$ Hz, H-6a), 3.77 (dd, 1 H, $J_{6''b,5''} = 10.0$ Hz, H-6″b), 3.74 (dd, 1 H, H-2), 3.69 (dd, 1 H, $J_{6b,5} = 1.9$ Hz, H-6b), 3.66 (ddd, 1 H, H-5), 3.59 (d, 1 H, $J_{2'',3''} = 3.1$ Hz, H-2″), 3.40 (dd, 1 H, $J_{6'a,5'} = 2.1$, $J_{6'a,6'b} = –11.3$ Hz, H-6′a), 3.33 (dd, 1 H, H-3″), 3.31 (s, 3 H, OCH_3), 3.28 (dd, 1 H, $J_{6'b,5'} = 3.2$ Hz, H-6′b), 3.11 (ddd, 1 H, H-5′), 3.09 (dd, 1 H, H-5″), 1.99 (s, 3 H, 3′-OCOCH_3), 1.93 (s, 3 H, 2′-OCOCH_3).

^{13}C NMR (150.9 MHz, CDCl$_3$): δ 170.0 (3′-O*C*OCH$_3$), 169.7 (2′-O*C*OCH$_3$), 139.3–126.0 (arom. *C*), 102.1 (C-1″), 101.3 (*C*″HPh), 100.6 (C-1′), 99.2 (C-1), 78.5 (C-4″), 78.4 (C-3), 77.9 (C-3″), 76.3 (C-2″), 75.8 (C-4′), 75.3 (C-4), 75.2 (C-2), 74.6 (2″-*C*H$_2$Ph), 74.4 (C-5′), 73.6 (6-*C*H$_2$Ph), 73.4 (6′-*C*H$_2$Ph), 73.4 (C-3′), 73.0 (2-*C*H$_2$Ph), 72.5 (C-2′), 72.3 (3″-*C*H$_2$Ph, 3-*C*H$_2$Ph), 71.4 (C-5), 68.6 (C-6″, C-6), 68.0 (C-6′), 67.4 (C-5″), 54.8 (O*C*H$_3$), 21.0 (3′-OCO*C*H$_3$), 20.7 (2′-OCO*C*H$_3$).

HRMS: *m/z* calcd. for $C_{72}H_{78}O_{18}Na$ $[M + Na]^+$ 1253.5086; found 1253.5080.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside. Synthesized from **16** (33 according to the general procedure for deacetylation (column purification solvent: hexane:EtOAc 1:1) to give the title compound as a white foam (26 mg, 84 %). TLC: $R_f = 0.40$ (hexane:EtOAc 1:1). $[\alpha]_D = +6.8$ (c 1.0, CHCl$_3$). ^{1}H NMR (600.13 MHz, CDCl$_3$): δ 7.48–7.12 (m, 35 H, arom. *H*), 5.58 (s, 1 H, C*H*Ph), 4.76 and 4.56 (each d, each 1 H, $J = –12.2$ Hz, 3″-CH_2Ph), 4.75 and 4.70 (each d, each 1 H, $J = –12.2$ Hz, 2″-CH_2Ph), 4.75 and 4.61 (each d, each 1 H, $J = –12.3$ Hz, 3-CH_2Ph), 4.73 and 4.63 (each d, each 1 H, $J = –12.3$ Hz, 6-CH_2Ph), 4.72 (d, 1 H, $J_{1,2} = 1.9$ Hz, H-1), 4.71 and 4.65 (each d, each 1 H, $J = –12.4$ Hz, 2-CH_2Ph), 4.59 (d, 1 H, $J_{1',2'} = 7.9$ Hz, H-1′), 4.44 and 4.09 (each d, each 1 H, $J = –12.1$ Hz, 6′-CH_2Ph), 4.36 (dd, 1 H, $J_{4,3} = 9.2$, $J_{4,5} = 9.6$ Hz, H-4), 4.28 (dd, 1 H, $J_{6''a,5''} = 5.0$, $J_{6''a,6''b} = –10.4$ Hz, H-6″a), 4.26 (s, 1 H, H-1″), 4.15 (dd, 1H, $J_{4'',5''} = 9.0$, $J_{4'',3''} = 9.9$ Hz, H-4″), 3.96 (dd, 1 H, $J_{6a,5} = 4.2$, $J_{6a,6b} = –11.5$ Hz, H-6a), 3.90 (dd, 1 H, $J_{3,2} = 3.1$ Hz, H-3), 3.88 (dd, 1H, $J_{6''b,5''} = 10.1$ Hz, H-6″b), 3.78 (dd, 1 H, $J_{6b,5} = 2.5$

Hz, H-6b), 3.77 (ddd, 1 H, H-5), 3.73 (dd, 1 H, H-2), 3.54 (dd, 1 H, $J_{4',3'}$ = 8.7, $J_{4',5'}$ = 9.6 Hz, H-4′), 3.52 (dd, 1 H, $J_{3',2'}$ = 9.3 Hz, H-3′), 3.48 (d, 1 H, $J_{2'',3''}$ = 3.0 Hz, H-2″), 3.39 (dd, 1 H, H-3″), 3.38 (dd, 1 H, H-2′), 3.29 (s, 3 H, OC*H*$_3$), 3.29 (ddd, 1 H, H-5″), 3.19 (dd, 1 H, $J_{6'a,5'}$ = 2.0, $J_{6'a,6'b}$ = –10.8 Hz, H-6′a), 3.11 (dd, 1 H, $J_{6'b,5'}$ = 3.4 Hz, H-6′b), 3.11 (dd, 1 H, H-5′).

^{13}C NMR (150.9 MHz, CDCl$_3$): δ 139.0–126.0 (arom. *C*), 103.4 (C-1′), 102.7 (C-1″), 101.4 (*C*HPh), 99.1 (C-1), 81.3 (C-4′), 79.1 C-3), 78.4 (C-4″), 78.3 (C-3″), 75.7 (C-2″), 75.4 (C-4), 74.9 (C-2), 74.8 (2″-O*C*H$_2$Ph), 74.4 (C-3′), 74.1 (C-2′), 73.8 (C-5′), 73.4 (6-O*C*H$_2$Ph), 73.3 (6′-O*C*H$_2$Ph), 73.0 (3″-O*C*H$_2$Ph), 72.8(2-O*C*H$_2$Ph), 71.9 (3-O*C*H$_2$Ph), 70.9 (C-5), 69.2 (C-6), 68.0 (C-6″), 67.9 (C-6′), 67.4 (C-5″), 54.8 (O*C*H$_3$).

HRMS: *m/z* calcd. for $C_{68}H_{74}O_{16}Na$ $[M + Na]^+$ 1169.4875; found 1169.4862.

Methyl β-D-mannopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-mannopyranoside (1). Synthesized from Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (26 mg, 0.02 mmol) according to the general procedure for hydrogenolysis thereby providing **1** as a colorless oil (11 mg, quant.). $[\alpha]_D$ = +11.1 (c 1.0, MeOH). ^{1}H NMR (600.13 MHz, D$_2$O, 35 °C): δ 4.76 (d,1 H, $J_{1,2}$ = 1.8 Hz, H-1), 4.74 (d 1 H, $J_{1'',2''}$ = 1.9 Hz, H-1″), 4.52 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.05 (dd, 1 H, $J_{2'',3''}$ = 3.3 Hz, H-2″), 3.99 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2), 3.95 (dd, 1 H, $J_{6a,5}$ = 2.3, $J_{6a,6b}$ = –12.3 Hz, H-6a), 3.93 (dd, 1 H, $J_{6''a,5''}$ = 2.3, $J_{6''a,6''b}$ = –12.3 Hz, H-6″a),), 3.90 (dd, 1 H, $J_{6'a,5'}$ = 2.2, $J_{6'a,6'b}$ = –12.4 Hz, H-6′a), 3.86 (dd, 1 H, $J_{3,4}$ = 9.2 Hz, H-3), 3.85 (dd, 1 H, $J_{4,5}$ = 9.7 Hz, H-4), 3.85 (dd, 1 H, $J_{6b,5}$ = 5.5 Hz, H-6b), 3.73 (dd, 1 H, $J_{6'b,5'}$ = 5.2 Hz, H-6′b), 3.73 (dd, 1 H, $J_{6''b,5''}$ = 6.4 Hz, H-6″b), 3.73 (ddd, 1 H, H-5), 3.69 (dd, 1 H, $J_{3',4'}$ = 8.8, $J_{3',2'}$ = 9.7 Hz, H-3′), 3.68 (dd, 1 H, $J_{4',5'}$ = 9.7 Hz, H-4′), 3.64 (dd, 1 H, $J_{3'',4''}$ = 9.7 Hz, H-3″), 3.61 (ddd, 1 H, H-5′), 3.58 (dd, 1 H, $J_{4'',5''}$ = 9.8 Hz, H-4″), 3.41 (ddd, 1 H, H-5″), 3.40 (s, 3 H, OC*H*$_3$), 3.35 (dd, 1 H, H-2′).

^{13}C NMR (150.9 MHz, D$_2$O, 35 °C): δ 103.0 (C-1′, $^1J_{C\text{-}1',H\text{-}1'}$ = 163.5 Hz), 101.2 (C-1, $^1J_{C\text{-}1,H\text{-}1}$ = 173.4 Hz), 100.6 (C-1″, $^1J_{C\text{-}1'',H\text{-}1''}$ = 160.8 Hz), 79.2 (C-4′), 77.2 (C-4), 77.0 (C-5″), 75.3 (C-5′), 74.6 (C-3′), 73.5 (C-2′), 73.4 (C-3″), 71.8 (C-5), 71.1 (C-2″), 70.1 (C-2), 69.8 (C-3), 67.3 (C-4″), 61.5 (C-6″), 60.9 (C-6), 60.8 (C-6′), 55.4 (O*C*H$_3$).

HRMS: *m/z* calcd. for $C_{19}H_{34}O_{16}Na$ $[M + Na]^+$ 541.1745; found 541.1744.

Methyl 2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (18). Synthesized from **16** (70 mg, 0.06 mmol) according to the general procedure for ring-opening of benzylidene acetal to give the 4-OBn/6-OH substrate thereby providing the title compound as a colorless oil (60 mg, 85 %). TLC: R_f = 0.52 (hexane:EtOAc 1:1). $[\alpha]_D$ = –11.5 (c 0.1, CHCl$_3$). ^{1}H NMR (600.13 MHz, CDCl$_3$): δ 7.37–7.15 (m, 35 H, arom. *H*), 5.08 (dd, 1 H, $J_{3',4'}$ = 9.3, $J_{3',2'}$ = 9.7 Hz, H-3′), 4.88 (dd, 1 H, $J_{2',1'}$ = 8.0 Hz, H-2′), 4.88 and 4.57 (each d, each 1 H, *J* = –11.6 Hz, 3-C*H*$_2$Ph), 4.77 and 4.51 (each d, each 1 H, *J* = –11.9 Hz, 6-C*H*$_2$Ph), 4.76 and 4.62 (each d, each 1 H, *J* = –12.5 Hz, 2″-C*H*$_2$Ph), 4.76 and 4.56 (each d, each 1 H, *J* = –11.1 Hz, 4″-C*H*$_2$Ph), 4.72 (d, 1 H, $J_{1,2}$ = 1.9 Hz, H-1), 4.70 and 4.66 (each d, each 1 H, *J* = –12.4 Hz, 2-C*H*$_2$Ph), 4.64 (d, 1 H, H-1′), 4.42 and 4.38 (each d, each 1 H, *J* = –11.8 Hz, 3″-C*H*$_2$Ph), 4.40 and 4.29 (each d, each 1 H, *J* = –12.1 Hz, 6′-C*H*$_2$Ph), 4.38 (s, 1 H, H-1″), 4.24 (dd, 1 H, $J_{4,5}$ = 9.0, $J_{4,3}$ = 10.0 Hz, H-4), 3.86 (dd, 1 H, $J_{4',5'}$ = 9.8 Hz, H-4′), 3.82 (dd, 1 H, $J_{6a,5}$ = 4.3, $J_{6a,6b}$ = –11.0 Hz, H-6a), 3.82 (dd, 1 H, $J_{6''a,5''}$ = 2.9, $J_{6''a,6''b}$ = –9.9 Hz, H-6″a), 3.79 (dd, 1 H, $J_{3,2}$ = 3.6 Hz, H-3), 3.74 (dd, 1 H, $J_{4'',3''}$ = 9.3, $J_{4'',5''}$ = 9.5 Hz, H-4″), 3.72 (dd, 1 H, H-2), 3.68 (dd, 1 H, $J_{6b,5}$ = 1.8 Hz, H-6b), 3.66 (ddd, 1 H, H-5), 3.64 (dd, 1 H, $J_{6''b,5''}$ = 5.8 Hz, H-6″b), 3.61 (d, 1 H, $J_{2'',3''}$ = 2.9 Hz, H-2″), 3.51 (dd, 1 H, $J_{6'a,5'}$ = 2.1, $J_{6'a,6'b}$ = –11.4 Hz, H-6′a), 3.34 (dd, 1 H, $J_{6'b,5'}$ = 3.5 Hz, H-6′b), 3.31 (s, 3 H, OC*H*$_3$), 3.31 (dd, 1 H, H-3″), 3.23 (ddd, 1 H, H-5′), 3.19 (ddd, 1 H, H-5″), 2.00 (s, 3 H, 3′-OCOC*H*$_3$), 1.93 (s, 3 H, 2′-OCOC*H*$_3$).

^{13}C NMR (150.9 MHz, CDCl$_3$): δ 170.4 (3′-O*C*OCH$_3$), 169.7 (2′-O*C*OCH$_3$), 139.3–127.1 (arom. *C*), 100.8 (C-1″), 100.7 (C-1′), 99.4 (C-1), 82.3 (C-3″), 78.5 (C-3), 76.0 (C-5″), 75.4 (C-4, C-2), 75.3 (3-O*C*H$_2$Ph), 75.2 (C-4′), 74.9 (C-4″), 74.8 (C-5′), 74.4 (C-2″), 74.0 (2″-O*C*H$_2$Ph), 73.8 (6-O*C*H$_2$Ph), 73.6 (6′-O*C*H$_2$Ph), 73.5 (C-3′), 72.9 (2-O*C*H$_2$Ph), 72.6 (C-2′, 4″-O*C*H$_2$Ph), 71.6 (C-5), 71.5 (3″-O*C*H$_2$Ph), 68.7 (C-6), 68.5 (C-6′), 62.8 (C-6″), 55.0 (O*C*H$_3$), 21.0 (3′-OCO*C*H$_3$), 20.9 (2′-OCO*C*H$_3$).

HRMS: *m/z* calcd. for $C_{72}H_{80}O_{18}Na$ $[M + Na]^+$ 1255.5242; found 1255.5249.

Methyl 2,3-di-*O*-benzoyl-4,6-*O*-di-tertbutylsilylene-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (21). Synthesized from acceptor **18** (21 mg, 0.017

mmol) and donor **20** (13 mg, 0.020 mmol, 1.2 equiv.) according to the general procedure for glycosylation with NIS/TMSOTf thus providing the title compound as a colorless oil (20 mg, 69 %). TLC: R_f = 0.52 (hexane:EtOAc 3:2). $[\alpha]_D$ = +24.9 (c 1.0, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.00–7.10 (m, 45 H, arom. *H*), 5.73 (dd, 1 H, $J_{2''',1'''}$ = 3.7, $J_{2''',3'''}$ = 10.6 Hz, H-2‴), 5.54 (dd, 1 H, $J_{3''',4'''}$ = 3.3 Hz, H-3‴), 5.28 (d, 1 H, H-1‴), 5.06 (dd, 1 H, $J_{3',4'}$ = 9.3, $J_{3',2'}$ = 9.7 Hz, H-3′), 4.96 and 4.66 (each d, each 1 H, *J* = –11.9 Hz, 2″-CH_2Ph), 4.85 (dd, 1 H, $J_{2',1'}$ = 8.0 Hz, H-2′), 4.77 and 4.56 (each d, each 1 H, *J* = –12.4 Hz, 3-CH_2Ph), 4.74 and 4.48 (each d, each 1 H, *J* = –11.9 Hz, 6-CH_2Ph), 4.72 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 4.71 and 4.65 (each d, each 1 H, *J* = –12.1 Hz, 2-CH_2Ph), 4.67 and 4.50 (each d, each 1 H, *J* = –11.8 Hz, 4″-CH_2Ph), 4.67 (dd, 1 H, $J_{4''',5'''}$ = 1.0 Hz, H-4‴), 4.62 (d, 1 H, H-1′), 4.37 and 4.25 (each d, each 1 H, *J* = –12.1 Hz, 6′-CH_2Ph), 4.35 and 4.30 (each d, each 1 H, *J* = –11.8 Hz, 3″-CH_2Ph), 4.25 (d, 1 H, $J_{1'',2''}$ = 0.5 Hz, H-1″), 4.22 (dd, 1 H, $J_{4,3}$ = 8.9, $J_{4,5}$ = 9.6 Hz, H-4), 4.09 (dd, 1 H, $J_{6'''a,5'''}$ = 1.6, $J_{6'''a,6'''b}$ = –12.9 Hz, H-6‴a), 3.89 (dd, 1 H, $J_{6''a,5''}$ = 1.9, $J_{6''a,6''b}$ = –11.7 Hz, H-6″a), 3.86 (dd, 1 H, $J_{4'',3''}$ = 9.1, $J_{4'',5''}$ = 9.5 Hz, H-4″), 3.85 (dd, 1 H, $J_{6'''b,5'''}$ = 1.4 Hz, H-6‴b), 3.83 (ddd, 1 H, H-5‴), 3.82 (dd 1 H, $J_{3,2}$ = 3.1 Hz, H-3), 3.81 (dd, 1 H, $J_{6''b,5''}$ = 4.3 Hz, H-6″b), 3.78 (dd, 1 H, $J_{6a,5}$ = 4.6, $J_{6a,6b}$ = –10.9 Hz, H-6a), 3.74 (dd, 1 H, $J_{4',5'}$ = 9.7 Hz, H-4′), 3.72 (dd, 1 H, H-2), 3.69 (dd, 1 H, $J_{6b,5}$ = 1.9 Hz, H-6b), 3.66 (ddd, 1 H, H-5), 3.57 (dd, 1 H, $J_{2'',3''}$ = 3.0 Hz, H-2″), 3.42 (dd, 1 H, $J_{6'a,5'}$ = 2.0, $J_{6'a,6'b}$ = –11.7 Hz, H-6′a), 3.31 (s, 3 H, OCH_3), 3.30 (dd, 1 H, $J_{6'b,5'}$ = 4.2 Hz, H-6′b), 3.23 (dd, 1 H, H-3″), 3.18 (ddd, 1 H, H-5″), 3.14 (ddd, 1 H, H-5′), 2.10 (s, 3 H, 3′-OCOCH_3), 1.93 (s, 3 H, 2′-OCOCH_3), 1.10 and 0.92 (each s, each 9 H, C(CH_3)$_3$).

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 170.4 (3′-OCOCH$_3$), 169.5 (2′-OCOCH$_3$), 166.1 (2‴-OCOPh), 165.8 (3‴-OCOPh), 139.3–126.9 (arom. *C*), 101.6 (C-1″), 100.7 (C-1′), 99.2 (C-1), 98.0 (C-1‴), 82.2 (C-3″), 78.4 (C-3), 75.8 (C-4′), 75.4 (C-2), 75.3 (C-4, C-5″), 74.6 (2″-OCH$_2$Ph, C-5′), 74.2 (C-4″), 73.9 (C-2″), 73.6 (4″-OCH$_2$Ph), 73.5 (6-OCH$_2$Ph), 73.4 (6′-OCH$_2$Ph), 73.1 (C-3″), 72.8 (2-OCH$_2$Ph), 72.4 (C-2′, 3-OCH$_2$Ph), 71.4 (C-5), 71.1 (C-4‴), 71.0 (3″-OCH$_2$Ph, C-3‴), 68.9 (C-6″), 68.8 (C-2‴), 68.6 (C-6), 68.3 (C-6′), 67.0 (C-5‴), 66.8 (C-6‴), 54.8 (OCH$_3$), 27.5 and 27.3 (C(CH$_3$)$_3$), 23.1 and 20.8 (C(CH$_3$)$_3$), 20.8 (3′-OCOCH$_3$), 20.7 (2′-OCOCH$_3$).

HRMS: *m/z* calcd. for $C_{100}H_{114}O_{25}SiNa$ $[M + Na]^+$ 1765.7316; found 1765.7266.

Methyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (22). Synthesized from **21** (50 mg, 0.03 mmol) according to the general procedure for removal of silyl protective groups to give the title compound as a colorless oil (31 mg, 68 %). TLC: R_f = 0.26 (hexane:EtOAc 1:2). $[\alpha]_D$ = +20.8 (c 0.8, $\mathrm{CHCl_3}$). ^{1}H NMR (600.13 MHz, $\mathrm{CDCl_3}$): δ 8.02–7.18 (m, 45 H, arom. *H*), 5.72 (dd, $J_{2''',1'''}$ = 3.8, $J_{2''',3'''}$ = 10.5 Hz, H-2‴), 5.71 (d, 1 H, H-1‴), 5.65 (dd, 1 H, $J_{3''',4'''}$ = 3.2 Hz, H-3‴), 5.12 (dd, 1 H, $J_{3',4'}$ = 9.5, $J_{3',2'}$ = 9.8 Hz, H-3′), 5.09 (dd, 1 H, $J_{2',1'}$ = 8.2 Hz, H-2′), 4.81 and 4.51 (each d, each 1 H, J = –11.1 Hz, 4″-CH_2Ph), 4.75 and 4.52 (each d, each 1 H, J = –11.9 Hz, 6-CH_2Ph), 4.73 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 4.73 and 4.56 (each d, each 1 H, J = –12.5 Hz, 3-CH_2Ph), 4.71 (d, 1 H, H-1′), 4.70 and 4.47 (each d, each 1 H, J = –12.1 Hz, 2″-CH_2Ph), 4.69 and 4.65 (each d, each 1 H, J = –12.4 Hz, 2-CH_2Ph), 4.38 and 4.22 (each d, each 1 H, J = –12.1 Hz, 6′-CH_2Ph), 4.34 and 4.30 (each d, each 1 H, J = –11.8 Hz, 3″-CH_2Ph), 4.29 (dd, 1 H, $J_{4''',5'''}$ = 1.2 Hz, H-4‴), 4.25 (s, 1 H, H-1″), 4.21 (dd, 1 H, $J_{4,3}$ = 8.8, $J_{4,5}$ = 9.8 Hz, H-4), 4.19 (dd, 1 H, $J_{4',5'}$ = 9.7 Hz, H-4′), 4.10 (ddd, 1 H, $J_{5''',6'''\mathrm{b}}$ = 3.1, $J_{5''',6'''\mathrm{a}}$ = 6.3 Hz, H-5‴), 4.05 (dd, 1 H, $J_{6''\mathrm{a},5''}$ = 1.7, $J_{6''\mathrm{a},6''\mathrm{b}}$ = –12.5 Hz, H-6″a), 3.89 (dd, 1 H, $J_{6'''\mathrm{a},6'''\mathrm{b}}$ = –12.4 Hz, H-6‴a), 3.83 (dd, 1 H, $J_{3,2}$ = 3.2 Hz, H-3), 3.80 (dd, 1 H, $J_{6\mathrm{a},5}$ = 4.6, $J_{6\mathrm{a},6\mathrm{b}}$ = –11.1 Hz, H-6a), 3.71 (dd, 1 H, H-2), 3.70 (dd, 1 H, H-6‴b), 3.69 (dd, 1 H, $J_{6\mathrm{b},5}$ = 1.8 Hz, H-6b), 3.66 (ddd, 1 H, H-5), 3.64 (dd, 1 H, $J_{4'',3''}$ = 9.1, $J_{4'',5''}$ = 9.5 Hz, H-4″), 3.62 (dd, 1 H, $J_{6''\mathrm{b},5''}$ = 6.1 Hz, H-6″b), 3.55 (d, 1 H, $J_{2'',3''}$ = 3.0 Hz, H-2″), 3.38 (dd, 1 H, $J_{6'\mathrm{a},5'}$ = 2.4, $J_{6'\mathrm{a},6'\mathrm{b}}$ = –11.0 Hz, H-6′a), 3.33 (dd, 1 H, $J_{6'\mathrm{b},5'}$ = 2.9 Hz, H-6′b), 3.32 (s, 3 H, OCH_3), 3.31 (ddd, 1 H, H-5″), 3.23 (dd, 1 H, H-3″), 3.19 (ddd, 1 H, H-5′), 2.08 (s, 3 H, 3′-OCOCH_3), 2.00 (s, 3 H, 2′-OCOCH_3).

^{13}C NMR (150.9 MHz, $\mathrm{CDCl_3}$): δ 170.9 (3′-OCOCH$_3$), 170.0 (2′-OCOCH$_3$), 166.0 (3‴-OCOPh), 165.8 (2‴-OCOPh), 139.3–126.7 (arom. C), 101.0 (C-1″, $^1J_{\mathrm{C}\text{-}1'',\mathrm{H}\text{-}1''}$ = 155.8 Hz, C-1′, $^1J_{\mathrm{C}\text{-}1',\mathrm{H}\text{-}1'}$ = 162.1 Hz), 99.1 (C-1, $^1J_{\mathrm{C}\text{-}1,\mathrm{H}\text{-}1}$ = 168.0 Hz), 97.6 (C-1‴, $^1J_{\mathrm{C}\text{-}1''',\mathrm{H}\text{-}1'''}$ = 173.5 Hz), 82.1 (C-3″), 78.4 (C-3), 76.5 (C-5″), 75.7 (C-4), 74.8 (4″-OCH$_2$Ph), 74.3 (C-4″, C-5′), 73.9 (C-4), 73.6 (C-2″, 2″-OCH$_2$Ph, 6-OCH$_2$Ph), 73.4 (6′-OCH$_2$Ph), 72.8 (C-3′), 72.7 (2-OCH$_2$Ph), 72.3 (C-2′), 72.0 (3-OCH$_2$Ph), 71.3 (C-5, C-3‴), 71.1 (C-5‴), 71.0 (3″-OCH$_2$Ph), 69.8 (C-4‴), 69.3 (C-2‴), 69.0 (C-6″), 68.7 (C-6), 67.7 (C-6′), 63.2 (C-6‴), 54.8 (OCH$_3$), 20.9 (3′-OCOCH$_3$), 20.8 (2′-OCOCH$_3$).

HRMS: *m/z* calcd. for $C_{92}H_{98}O_{25}Na$ $[M + Na]^+$ 1625.6295; found 1625.6266.

Methyl α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside. Synthesized from **22** (33 mg, 0.02 mmol) according to the general procedure for deacetylation to give the title compound as a white foam (26 mg, quant.). TLC: R_f = 0.38 (MeOH:DCM 1:10). ^{1}H NMR (600.13 MHz, CD_3OD + $CDCl_3$): δ 7.38–7.18 (m, 35 H, arom. *H*), 4.89 (d, 1 H, $J_{1''',2'''}$ = 4.4 Hz, H-1‴), 4.88 and 4.60 (each d, each 1 H, *J* = –11.9 Hz, 2-CH_2Ph), 4.78 and 4.59 (each d, each 1 H, *J* = –11.7 Hz, 3-CH_2Ph), 4.76 and 4.69 (each d, each 1 H, *J* = –12.2 Hz, 2″-CH_2Ph), 4.75 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 4.73 and 4.60 (each d, each 1 H, *J* = –12.0 Hz, 6-CH_2Ph), 4.69 and 4.66 (each d, each 1 H, *J* = –12.2 Hz, 4″-CH_2Ph), 4.50 (d, 1 H, $J_{1',2'}$ = 7.8 Hz, H-1′), 4.46 (s, 2 H, 3″-CH_2Ph), 4.44 and 4.23 (each d, each 1 H, *J* = –12.1 Hz, 6′-CH_2Ph), 4.34 (d, 1 H, $J_{1'',2''}$ = 0.7 Hz H-1″), 4.28 (dd, 1 H, $J_{4,3}$ = 8.8, $J_{4,5}$ = 9.6 Hz, H-4), 4.01 (dd, 1 H, $J_{6a,5}$ = 4.9, $J_{6a,6b}$ = –11.4 Hz, H-6a), 3.95 (dd, 1 H, $J_{4''',5'''}$ = 1.2, $J_{4''',3'''}$ = 3.3 Hz, H-4‴), 3.91 (dd, 1 H, $J_{6b,5}$ = 2.1 Hz, H-6b), 3.89 (dd, 1 H, $J_{3,2}$ = 3.3 Hz, H-3), 3.86 (dd, 1 H, $J_{3''',2'''}$ = 9.9 Hz, H-3‴), 3.81 (ddd, 1 H, H-5), 3.80 (dd, 1 H, $J_{6''a,5''}$ = 2.7, $J_{6''a,6''b}$ = –11.6 Hz, H-6″a), 3.80 (ddd, 1 H, $J_{5''',6'''b}$ = 5.5, $J_{5''',6'''a}$ = 6.3 Hz, H-5‴), 3.77 (dd, 1 H, H-2), 3.75 (dd, 1 H, $J_{6'''a,6'''b}$ = –11.1 Hz, H-6‴a), 3.74 (dd, 1 H, H-6‴b), 3.74 (dd, 1 H, $J_{6''b,5''}$ = 8.2 Hz, H-6″b), 3.74 (dd, 1 H, H-2‴), 3.68 (dd, 1 H, $J_{4'',3''}$ = 9.2, $J_{4'',5''}$ = 9.8 Hz, H-4″), 3.64 (dd, 1 H, $J_{2'',3''}$ = 3.0 Hz, H-2″), 3.52 (ddd, 1 H, H-5″), 3.51 (dd, 1 H, $J_{4',3'}$ = 8.5, $J_{4',5'}$ = 9.9 Hz, H-4′), 3.48 (dd, 1 H, $J_{3',2'}$ = 9.4 Hz, H-3′), 3.41 (dd, 1 H, H-3″), 3.37 (dd, 1 H, $J_{6'a,5'}$ = 2.2, $J_{6'a,6'b}$ = –11.1 Hz, H-6′a), 3.35 (s, 3 H, OCH_3), 3.29 (dd, 1 H, H-2′), 3.29 (dd, 1 H, $J_{6'b,5'}$ = 3.7 Hz, H-6′b), 3.18 (ddd, 1 H, H-5′).

^{13}C NMR (150.9 MHz, CD_3OD + $CDCl_3$): 139.5–127.5 (arom. *C*), 103.6 (C-1′), 102.4 (C-1″), 99.6 (C-1‴), 99.6 (C-1), 82.7 (C-3″), 82.0 (C-4′), 78.8 (C-3), 76.0 (C-4), 75.7 (C-3′, C-2), 75.5 (2-O*C*H_2Ph), 75.1 (C-4″), 74.7(2″-O*C*H_2Ph), 74.3 (C-2″, C-5″), 74.2 (C-5′), 74.1 (C-2′), 73.9 (6′-O*C*H_2Ph), 73.7 (6-O*C*H_2Ph), 73.2 (4″-O*C*H_2Ph), 72.6 (3-O*C*H_2Ph), 72.4 (3″-O*C*H_2Ph), 71.8 (C-5), 71.1 (C-5‴), 70.3 (C-4‴, C-3‴), 69.8 (C-2‴), 69.7 (C-6), 68.7 (C-6′), 67.2 (C-6″), 62.1 (C-6‴), 55.1 (O*C*H_3).

HRMS: *m/z* calcd. for $C_{74}H_{86}O_{21}Na$ $[M + Na]^+$ 1333.5559; found 1333.5548.

Methyl α-D-galactopyranosyl-(1→6)-β-D-mannopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-α-D-mannopyranoside (2). Synthesized from Methyl α-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-mannopyranoside (24 mg, 0.02 mmol) according to the general procedure for hydrogenolysis thereby providing **2** as a colorless oil (12 mg, quant.). $[\alpha]_D$ = +31.8 (c 1.0, MeOH). ^{1}H NMR (600.13 MHz, D_2O, 35 °C): δ 5.00 (d, 1 H, $J_{1''',2'''}$ = 3.9 Hz, H-1‴), 4.76 (d,1 H, $J_{1,2}$ = 1.8 Hz, H-1), 4.75 (d, 1 H, $J_{1'',2''}$ = 0.9 Hz, H-1″), 4.52 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.07 (dd, 1 H, $J_{2'',3''}$ = 3.3 Hz, H-2″), 3.99 (dd, 1 H, $J_{4''',3'''}$ = 1.3, $J_{4''',5'''}$ = 3.8 Hz, H-4‴), 3.98 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 3.96 (dd, 1 H, $J_{6''a,5''}$ = 6.7, $J_{6''a,6''b}$ = –10.6 Hz, H-6″a), 3.95 (dd, 1 H, $J_{6a,5}$ = 2.7, $J_{6a,6b}$ = –12.6 Hz, H-6a), 3.95 (dd, 1 H, $J_{5''',6'''a}$ = 3.0, $J_{5''',6'''b}$ = 8.8 Hz, H-5‴), 3.95 (dd, 1 H, $J_{3''',2'''}$ = 10.2 Hz, H-3‴), 3.89 (dd, 1 H, $J_{6'a,5'}$ = 2.2, $J_{6'a,6'b}$ = –12.5 Hz, H-6′a), 3.86 (dd, 1 H, $J_{3,4}$ = 9.6 Hz, H-3), 3.85 (dd, 1 H, $J_{6b,5}$ = 5.3 Hz, H-6b), 3.85 (dd, 1 H, $J_{4,5}$ = 9.8 Hz, H-4), 3.81 (dd, 1 H, H-2‴), 3.78 (dd, 1 H, $J_{6''b,5''}$ = 2.1 Hz, H-6″b), 3.74 (dd, 1 H, $J_{6'''a,6'''b}$ = –11.1 Hz, H-6‴a), 3.74 (dd, 1 H, H-6‴b), 3.73 (dd, 1 H, $J_{6'b,5'}$ = 5.1 Hz, H-6′b), 3.73 (ddd, 1 H, H-5), 3.68 (dd, 1 H, $J_{4',3'}$ = 9.5, $J_{4',5'}$ = 9.8 Hz, H-4′), 3.68 (dd, 1 H, $J_{4'',3''}$ = 9.7, $J_{4'',5''}$ = 9.9 Hz, H-4″), 3.67 (dd, 1 H, $J_{3',2'}$ = 10.0 Hz, H-3′), 3.65 (dd, 1 H, H-3″), 3.62 (ddd, 1 H, H-5′), 3.61 (ddd, 1 H, H-5″), 3.40 (s, 3 H, OCH_3), 3.34 (dd, 1 H, H-2′).

^{13}C NMR (150.9 MHz, D_2O, 35 °C): 103.0 (C-1′, $^1J_{C-1',H-1'}$ = 163.3 Hz), 101.2 (C-1, $^1J_{C-1,H-1}$ = 172.5 Hz), 100.8 (C-1″, $^1J_{C-1'',H-1''}$ = 159.4 Hz), 98.9 (C-1‴, $^1J_{C-1''',H-1'''}$ = 170.6 Hz), 79.6 (C-4′), 77.3 (C-4), 75.1 (C-5′, C-5″), 74.7 (C-3′), 73.5 (C-2′, C-3″), 71.8 (C-5), 71.6 (C-3‴), 71.1 (C-2″), 70.1 (C-2), 70.0 (C-5‴), 69.9 (C-4‴, C-3), 69.1 (C-2‴), 67.2 (C-4″), 66.8 (C-6″), 61.8 (C-6‴), 60.9 (C-6), 60.8 (C-6′), 55.4 (OCH_3).

HRMS: *m/z* calcd. for $C_{25}H_{44}O_{21}Na$ $[M + Na]^+$ 703.2273; found 703.2273.

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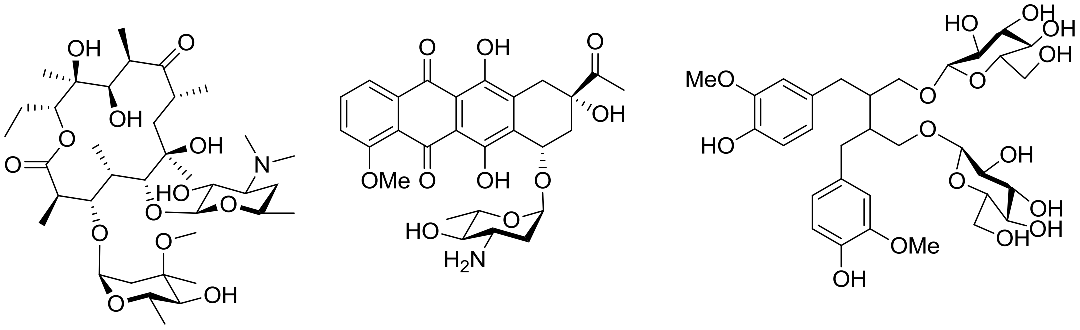
Episode 3: Synthesis of Glycoconjugates



Glycoconjugate is a general term for a large group of structures containing a glycone permanently linked to another chemical species (some examples are shown above). There has always been much interest in the synthesis of these compounds and their mimics due to the wide occurrence of such compounds in nature and their biological applications. This chapter focuses on the synthesis of several biologically relevant classes of glycoconjugates including saponins, lignan glycosides and anthracyclines. In addition, some key guidelines for the NMR spectroscopic characterization of glycoconjugates will be provided.

This chapter is based on the previously published papers: F. S. Ekholm, P. Eklund, R. Leino, **"A Short Semi-Synthesis and Complete NMR-Spectroscopic Characterization of the Naturally Occurring Lignan Glycoside Matairesinol 4,4′-di-O-β-D-glucoside"**, *Carbohydr. Res.* **2010**, *345*, 1963–1967; F. S. Ekholm, G. Schneider, J. Wölfling, R. Leino, **"Synthesis of a Small Library of Estradiol-Based Glycosteroid Mimics Containing a Modified D-Ring"**, *Eur. J. Org. Chem.* **2011**, 1064–1077; F. S. Ekholm, G. Schneider, J. Wölfling, R. Leino, **"An Approach to the Synthesis and Attachment of Scillabiose to Steroids"**, *Steroids* **2011**, *76*, 588–595; F. S. Ekholm, L. Lagerquist, R. Leino, **"Stereo- and Regioselective Glycosylation of 4-Deoxy-ε-Rhodomycinone"**, *Carbohydr. Res.* **2011**, *346*, 858–862.

1 Introduction

Glycoconjugate is a general term encompassing many classes of compounds including glycoproteins, glycopeptides and glycolipids.[1] More precisely, glycoconjugates consist of a glycone part permanently attached to an aglycone core. In this Episode, both the term glycoconjugate and the discussion will be limited mainly to aglycones consisting of lignans, tetracyclines and steroids.[2] These classes of compounds are widely distributed and common in nature.[2,3] In addition to their wide occurrence, many of the glycoconjugates isolated from plant and animal species possess interesting biological properties.[2,3,4] Classical examples of such properties include the cytotoxic activities of anthracyclines and the physiological and pharmacological effects of cardenolides and saponins.[5] The overall biological activity of these compounds are usually connected to the nature of the molecule as a whole (containing both the glycone and the aglycone).[6] The isolation and synthesis of glycoconjugates (and mimics thereof) has always been an important topic in carbohydrate chemistry due to the biological significance of such compounds. The isolation of intact glycoconjugates from their natural sources is often difficult due to the small quantities of material present in the biological matrix and the unstable nature of the glycosides towards acidic extractions. Chemical synthesis is, therefore, usually the preferred choice for large scale preparation of glycoconjugates after the initial identification of a new glycoconjugate with biological potential. Accordingly, one of the main goals of this research field is the synthesis of complex compounds for biological evaluations and to simultaneously increase the understanding on their biological interactions, functions and properties.

The synthesis of glycoconjugates can be achieved by two slightly different pathways. The first route to glycoconjugates, and a long-time provider of new chemical reactions, is total synthesis.[7] This approach offers several benefits as the construction of partially protected aglycones can be achieved from readily available starting materials. As a result, difficult glycosylation reactions may be performed at early stages in the synthetic route simultaneously eliminating the difficulties associated with regioselective glycosylations of unprotected aglycones. On the other hand, it is often expensive and time-consuming to perform the tedious synthetic transformations usually required for total synthesis of naturally occurring aglycones.

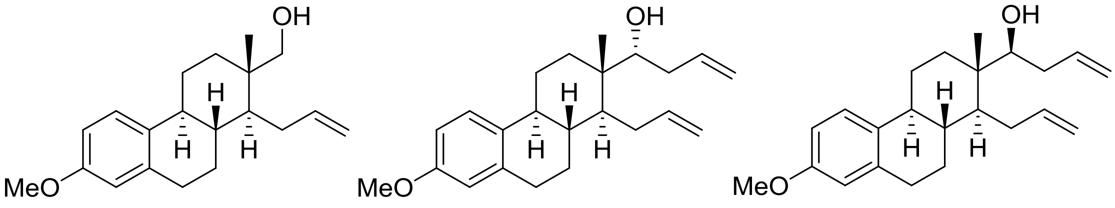
The second route to glycoconjugates involves the regio- and stereoselective glycosylation of isolated aglycones (either partially protected or unprotected) in cases where large amounts of aglycone are accessible.[8] If the actual aglycone can be isolated on large scale, such procedure is often to be preferred over total synthesis routes despite that the aglycone can not be modified with similar freedom. There are many challenges associated with the glycosylation of aglycones. First, aglycones may contain several sites of glycosylation of which only a few might be glycosylated in the naturally occuring product. In addition, aglycones usually contain many other functional groups which may increase the steric hindrance near the glycosylation site. When combining steric hindrance with the low reactivity of secondary alcohols (often the glycosylation sites in aglycones), problems associated with the efficiency of glycosylation reactions may arise.

In this Episode, methods for the glycosylation of aglycones are addressed and discussed, culminating in the synthesis of various glycoconjugates including glycosteroids, saponin mimics, lignan glycosides and glycosylated anthracyclines.[8a,9] In addition, potential biological applications and some key-guidelines concerning the NMR spectroscopic characterization of these glycoconjugates will be presented.

2 Results and Discussion

2.1 Synthesis of Estradiol-Based Glycosteroid Mimics

Glycosteroids are a class of naturally occurring compounds consisting of a lipophilic steroid part and a hydrophilic carbohydrate moiety. Recent advances in glycobiology have revealed that these glycoconjugates play important biological roles ranging from prevention of bacterial infections to manipulation of immune responses.[10] In addition, it has been reported previously that amphiphilic glycosteroids may enhance the transport of polar molecules across cellular membranes.[1a] Steroids are also known to be more active in certain areas of the human body, *e.g.*, estradiol activities have been reported not only in the female reproductive system but also in locations ranging from the lungs and brain to the bones.[11] Combining these two principles would potentially provide molecules capable of increasing the transport of polar molecules across membranes at partially selective locations in the body. Such molecules could also be considered as primitive delivery systems. In this thesis, the interest in glycosteroids is partially connected to these applications. The most important part of a delivery system would naturally be the steroid backbone. In this work, an estradiol based steroid mimic was selected as a suitable backbone structure. To diminish or remove the apparent side effects caused by estradiol itself, modifications to the steroid backbone were planned. It is well known that the hydroxyl group at C-3 is important for the pharmacological activity of steroids.[12] In order to prevent these undesirable pharmacological side-effects, this functionality should be permanently blocked when aiming at transport molecules. Glycosylation of this hydroxyl group cannot be considered as permanent protection since glycosides are generally cleaved under physiological conditions.



1 **2** **3**

Figure 1. Chemical structures of the steroid acceptors **1**–**3**.

The steroid acceptors **1**–**3** used in the present study were constructed from the common precursor 16,17-*seco*-3-methoxyestra-1,3,5(10),16-tetraene-13α-carbaldehyde. This precursor can be prepared from estrone 3-methyl ether in four steps by Grob fragmentation, as the key transformation, as reported previously by Schneider and coworkers.[13] Conveniently, this procedure allows the protection of the hydroxyl group at C-3 as a methyl ether during the early stages of the synthesis. Steroid acceptor **1** was synthesized from this precursor in almost quantitative yield by reduction with $NaBH_4$ in ethanol.[14] Compounds **2** and **3** can be synthesized as a separable mixture from the common precursor by metal-mediated allylation.[15] Additional modifications to the steroid backbone were desirable in order to minimize the possible pharmacological side-effects. The D-ring of the steroid was chosen as the site for further modifications which is obvious when examining the structures of the steroid acceptors in Figure 1. In several previous reports, large variations in the steroid D-ring were tolerated while retaining the biological role and mode of action of steroids.[16] Consequently, in the present study, it was interesting to evaluate the effects of acyclic versus cyclic D-rings. In addition, the D-ring hydroxyl group in steroids **1**–**3** was subjected to further modifications by glycosylation. Modification by glycosylation should influence the solubility of these molecules and the overall selectivity of the system as also mentioned in the introduction. Glycosylation of steroids is challenging due to the low reactivity of secondary alcohols.[6] As a result, several earlier reported procedures on steroid glycosylation suffer from moderate yields.[17] In the present study, the methyl group (C-18) close to the glycosylation center increases the steric hindrance, thereby making highly selective and efficient glycosylation reactions an even greater challenge. Of the steroid acceptors investigated here, compound **1** contains a primary hydroxyl group in contrast to the secondary hydroxyl groups in acceptors **2** and **3**. It was accordingly selected as the model substrate for initial glycosylation screening (Figure 1).

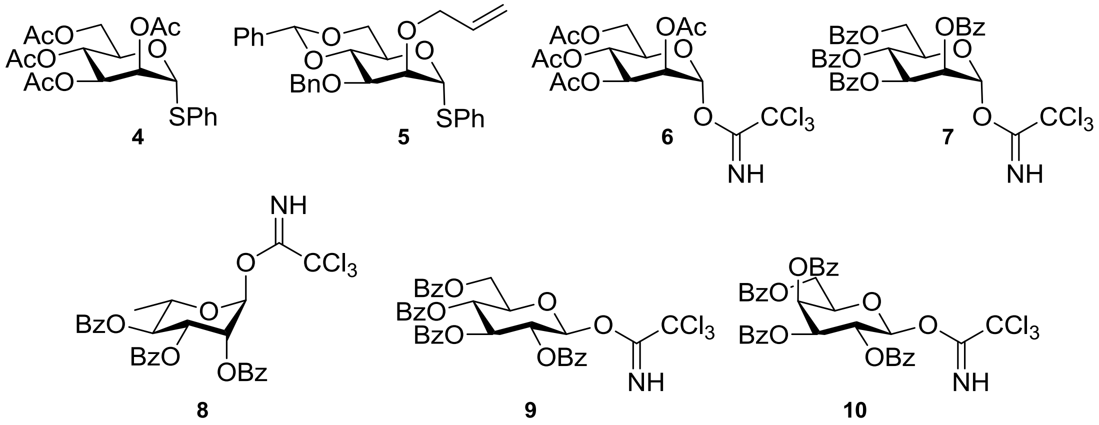
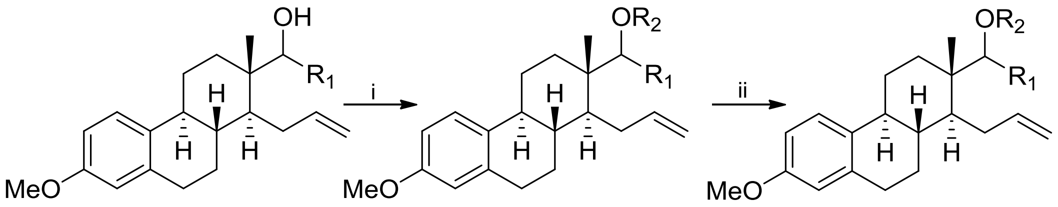


Figure 2. Chemical structures of the carbohydrate donors **4**–**10**.

Several glycosyl donors have been investigated earlier for glycosylation of steroids.[2b,6] In light of our previous experience with thio-donors,[18] these were selected as a starting point for the initial screening of glycosyl donors. Given that all the acceptors **1**–**3** contain alkene functionalities, the commonly employed NIS/TfOH promoter system was not feasible for activation of the thio-donors.[19] Instead, the attention was turned to the BSP/TTBP/Tf_2O activation protocol developed by Crich and coworkers[20] (utilized earlier at our laboratory as shown in Episode 1) for activation of glycosyl donors **4** and **5**. In both cases, however, moderate yields (~50%) were observed. Furthermore, in glycosylation with **5**, the selectivity was fairly low (β/α, 4:1). In addition to the moderate efficiency and poor selectivity of this protocol, the major drawback of this approach is the need for a four-fold molar excess of the promoter over the substrate. Consequently, the thio-donors were abandoned and the search for a different glycosylation strategy was initiated.

Another class of frequently utilized glycosyl donors are based on imidates, commonly trichloracetimidates (TCA).[21] In several previous studies, acetylated TCA-donors have been used for the glycosylation of steroids.[6] In Episode 1, $BF_3{\cdot}OEt_2$ was found as the best promoter for TCA-donor in the glycosylation of cyclohexanol.[18b] With steroids sharing the lipophilic features of cyclohexanol, this promoter was intitially tested in the glycosylation of **1** with donor **6**.

Unfortunately, in the case of **1**, the use of $BF_3{\cdot}OEt_2$ resulted in decomposition of starting materials and failed to provide the desired target molecule. Next, trimethylsilyl trifluoromethanesulfonate (TMSOTf) was tested as a promoter. TMSOTf has been successfully applied as a promoter in glycosylation reactions with imidate donors previously.[2b,6,22] Under similar reaction conditions, with TMSOTf as a promoter, the glycosylation gave a mixture of three components which were isolated and identified by NMR spectroscopic analysis as the target compound (33%), the acetylated acceptor (33 %) and a mixture of partially deacetylated glycosteroids (33 %).[23] In order to circumvent these problems and to retain the beneficial properties of neighboring group participation displayed by ester groups at C-2, the acetyl protecting groups were exchanged to the slightly bulkier benzoyl groups.



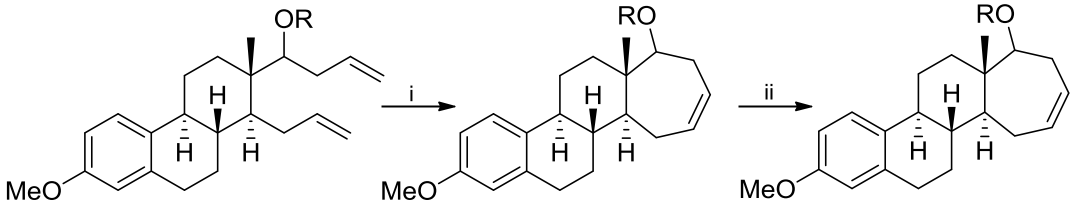
R_1 = H, allyl

11 R_1 = H, R_2 = OBz-Man
12 R_1 = Allyl, R_2 = OBz-Man
13 R_1 = H, R_2 = OBz-Rha
14 R_1 = Allyl, R_2 = OBz-Rha
15 R_1 = H, R_2 = OBz-Glc
16 R_1 = Allyl, R_2 = OBz-Glc
17 R_1 = H, R_2 = OBz-Gal
18 R_1 = Allyl, R_2 = OBz-Gal

19 R_1 = H, R_2 = Man
20 R_1 = Allyl, R_2 = Man
21 R_1 = H, R_2 = Rha
22 R_1 = Allyl, R_2 = Rha
23 R_1 = H, R_2 = Glc
24 R_1 = Allyl, R_2 = Glc
25 R_1 = H, R_2 = Gal
26 R_1 = Allyl, R_2 = Gal

Scheme 1. Reaction conditions: i) TMSOTf, corresponding acceptor (**1**–**3**), corresponding donor (**4**–**10**), –20 °C, CH_2Cl_2, 1.5–2 h, **11** (91 %), **12** (90 %), **13** (92 %), **14** (88 %), **15** (not determined due to purification difficulties), **16** (77 %), **17** (not determined due to purification difficulties), **18** (79 %); ii) NaOMe, MeOH/THF, 1:1, r.t., 3–20 h, **19** (90 %), **20** (quant.), **21** (88 %), **22** (quant.), **23** (85 % over two steps), **24** (77 %), **25** (85 % over two steps), **26** (91 %).[24]

Under unoptimized reaction conditions with 0.2 equiv. of TMSOTf as promoter in dichloromethane at –20 °C, the reaction between **1** and **7** proceeded in 91% yield. Applying similar reaction conditions to the sterically more demanding acceptor **3** did not result in any significant decrease in yield. In comparison to the activation strategy utilized with thio-donors (4 equiv. of promoters), this glycosylation protocol is much more benign and more efficient. After this successful first reaction, donors **8**–**10** were prepared according to slightly modified literature procedures (Figure 2).[25] The glycosylation reactions were then performed by applying the same protocol as in the initial screening with the results summarized in Scheme 1. Interestingly, donors **9** and **10** gave slightly lower yields than donors **7** and **8**. This is most likely due to the less intrusive arrangement of the substituents at C-1 and C-2 which, in the case of **7** and **8**, occupy an axial–axial relationship. Furthermore, glycosylation of the more sterically hindered acceptors **2** and **3** with **9** and **10** resulted in lower yield, probably due to the equatorial 2-OBz group which apparently increases the steric bulk near the reacting center. Bearing only ester protective groups, the glycosteroids could easily be deprotected under Zemplén conditions with NaOMe in MeOH/THF.[26] Tetrahydrofuran was required as co-solvent due to the amphiphilic character of the molecules in the deprotected state. All deprotections proceeded in good to excellent yields (77–99 %). Due to the amphiphilicity of the final products (R_f values 0.4–0.7 in MeOH/CH_2Cl_2, 1:5), they were also amenable to purification by column chromatography thus enabling a simple method for removing the methyl benzoate formed during the final deprotection step.



R = benzoylated sugar

27 R = OBz-Man
28 R = OBz-Rha
29 R = OBz-Glc
30 R = OBz-Gal

31 R = Man
32 R = Rha
33 R = Glc
34 R = Gal

Scheme 2. Reaction conditions: i) Grubbs 2nd generation catalyst (10 mol%), reflux 6 h, then r.t. overnight, CH_2Cl_2, **27** (97 %), **28** (quant.), **29** (72 %), **30** (75 %); ii) NaOMe, MeOH/THF, 1:1, r.t., 3–20 h, **31** (98 %), **32** (quant.), **33** (82 %), **34** (90 %).[27]

With the glycosteroids **12**, **14**, **16**, and **18** each possessing two terminal double bonds at hand, their application in ring-closing olefin metathesis (RCM) was investigated next.[28] RCM is an excellent method for creating medium-sized rings as exemplified by the large number of papers dealing with this subject.[29] Since the reaction produces two molecules (substrate and ethylene) from one and is driven by an increase in entropy, the initial reactions were performed at room temperature using Grubbs 2nd generation catalyst. At this temperature the reaction failed to yield the desired product and, consequently, the mixture was refluxed in CH_2Cl_2 for six hours and allowed to stand overnight. These conditions proved successful and the products were isolated in up to 97% yields (Scheme 2). With the manno- and rhamnosylated steroids the yields were nearly quantitative, whereas in the case of gluco- and galactosteroids the yields were slightly lower (~77%). The difference in yields is possibly due to the equatorial benzoyl group at C-2 in the latter two substrates which increases the steric bulk near the reacting center. As a logical extension, an evaluation of the possibility for a RCM-glycosylation sequence was performed. Unfortunately, glycosylation of the more sterically hindered ring-closed products decreased the yield significantly and this approach was abandoned already during the early stages of this work. In addition, RCM reactions directly on the debenzoylated substrate would have been interesting to study. Such an approach would, however, require a non-conventional metathesis catalyst instead of the commercially available Grubbs catalyst and was therefore not investigated further in the present study. The ring-closed products were instead smoothly deprotected under conditions described previously thereby providing the glycosteroids **31**–**34**.

To summarize, several glycosyl donors were investigated for the glycosylation of steroids. In this work, benzoylated TCA-donors proved superior. The benzoylated TCA-donors prepared were, as shown above, utilized in the synthesis of a small library of amphiphilic glycosteroids. The amphiphilic character of the molecules depends on both the number of hydroxyl groups in the sugars and the acyclic vs. cyclic structure of the D-ring. As a logical next step, the procedures developed for the synthesis of glycosteroids in the current section were evaluated in the synthesis of more challenging targets. In the following sections, the glycosylation of several relevant aglycones are described based heavily on the procedures developed during this initial screening of glycosyl donors.

2.2 Coupling of Scillabiose to Model Steroid

Scillabiose (4-*O*-(β-D-glucopyranosyl)-α-L-rhamnopyranoside) is a frequently occuring disaccharide glycone in natural steroids.[30] In addition to the presence of scillabiose in steroids, a closely related glycon containing glucuronic acid instead of glucose has been found in molecules isolated from *Acrosiphonia centralis, Ulva lactuca* and *Klebsiella*.[31] Two well known saponins bearing the scillabiose glycone (Figure 3) are hellebrin, a potent cytotoxic compound isolated from *Helleborus niger*, and transvaalin, a constituent of herbal medicines isolated from *Urginea* *sanguinea*.[30a,30d,32] In addition to the biological properties mentioned above, hellebrin displays T-cell suppressive effects which can be applied in immunoregulation. Transvaalin in turn, has been utilized for the purification of blood, removal of abdominal pain and as abortifacient.[30d,32,33] Due to the occurrence of scillabiose and closely related compounds in several naturally occurring biomolecules and pharmaceutically active species, we became interested in developing a synthetic route for the attachment of scillabiose to steroids. In general, a convergent route is usually preferred as it minimizes the amount of synthetic steps with the incorporated aglycone simultaneously decreasing the risk of altering sensitive functionalities in the aglycone.

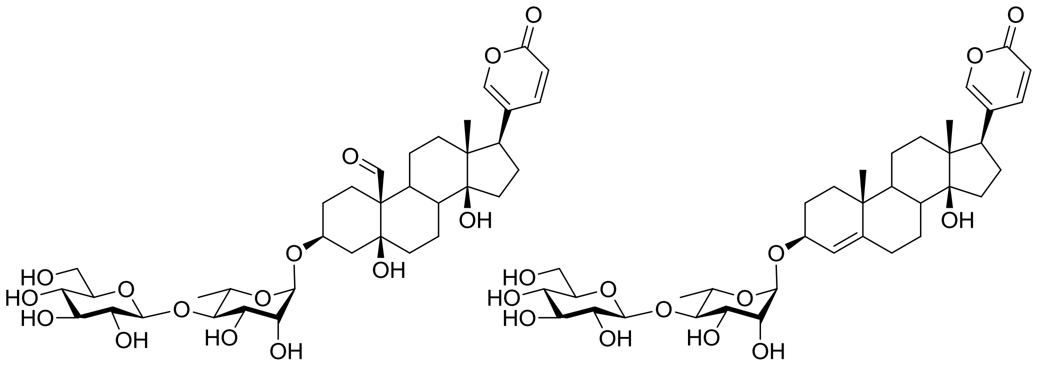
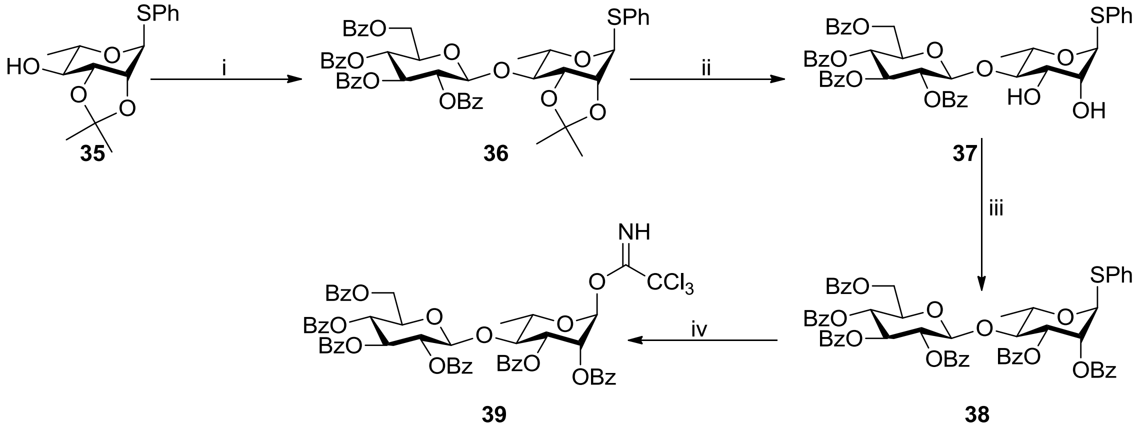


Figure 3. Natural products containing the scillabiose glycon: hellebrin (left) and transvaalin (right).

Synthesis of saponins has received significant attention in recent years.[34] As mentioned in the previous section, the utilization of benzoylated imidate donors in the glycosylation of steroids was superior in terms of efficiency and minimal waste generation.[9a,22] Additional advantages of imidate donors are the simple and rapid methods applied for their preparation.[21a] Therefore, they were also implemented in the current synthetic approach. While the synthesis of the methyl glycoside of scillabiose has been published earlier,[35] glycosylations utilizing scillabiose based donors are scarce in the literature and, apparently, only one report concerning the subject has emerged to date.[36] In that work, the thio donor utilized resulted in an α:β mixture of the product.

Overall, in the synthesis of glycosylated biomolecules, minimization of the number of synthetic steps incorporating the aglycone is preferred. This is mainly to ensure that reactive centers in the aglycone moiety remain unaltered. Therefore, wherever possible, glycosylations by convergent routes are preferred over linear ones.[2a,37] Furthermore, protective groups should essentially be removable in one step and under mild conditions in order to avoid multiple deprotection sequences which may affect sensitive functional groups. Based on these requirements an approach for the preparation of a scillabiose glycosyl donor (containing protective groups removable in a single step) was deviced.

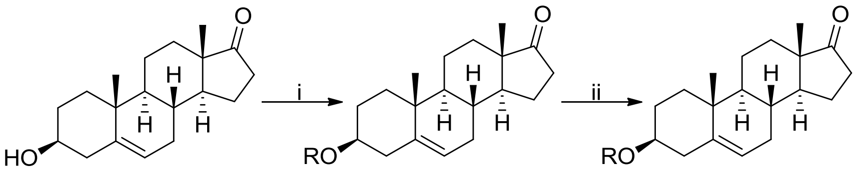


Scheme 3. Synthesis of donors **38** and **39**: i) **9** (see previous section), TMSOTf, CH_2Cl_2, 1.5–2 h, 85%; ii) 80% AcOH, 80 °C, 5 h, 96%; iii) BzCl, pyridine, 4 h, 89%; iv) 1. NBS, Acetone:H_2O 10:1, 1 h, 92%; 2. DBU, Cl_3CCN, CH_2Cl_2, 0 °C, 1.5 h, 77%.

The synthesis of donors **38** and **39** is illustrated in Scheme 3. The present strategy was based on the preparation of a thiophenyl rhamnoside acceptor. The thiophenyl functionality has been shown to be stable under various reaction conditions and, furthermore, it can be selectively activated in the presence of other functionalities and under mild conditions, thereby making it an excellent anomeric protective group.[38] In order to minimize the protective group manipulations needed, the *cis* C-2 and C-3 hydroxyl groups were protected in one step as an isopropylidene acetal.[39] Conveniently, the hydroxyl group at C-4 remains untouched thus providing acceptor **35**. In the previous synthesis by Bebault and Dutton, the formation of this isopropylidene acetal was followed by an acetylation/deacetylation sequence at C-4.[35] These two steps are, however, not necessary for preparation of acceptor **35** as also shown in a more recent study.[40] In short, the synthesis of acceptor **35** commenced by slightly modified literature procedures involving $BF_3 \cdot OEt_2$ promoted glycosylation of peracetylated L-rhamnose with thiophenol,[41] followed by deacetylation under Zemplén conditions[26] and, as the final step, formation of the 2,3-*O*-isopropylidene acetal in 61% overall yield. Glycosylation of acceptor **35** with donor **9** (see page

111) using TMSOTf as promoter provided the disaccharide **38** in 85% isolated yield.[2a,9a,22] While compound **38** could, in principle, be used as a glycosyl donor, a similar donor was shown to yield a mixture of products with poor selectivity in previous work.[36] In addition, removal of the 2,3-*O*-isopropylidene acetal requires harsh conditions which may affect sensitive functionalities in biomolecules.[42] Furthermore, in contrast to ester protective groups at C-2, the isopropylidene acetal does not enhance the α-selectivity in the glycosylation. For enhancing the beneficial properties, in particular neighboring group participation displayed by ester protective groups, the isopropylidene acetal was cleaved under acidic conditions (80 % AcOH) at 80 °C providing **37** in 96% yield. The newly formed hydroxyl groups were benzoylated thus providing thiodonor **38** in 89% yield. As mentioned above, the best results in glycosidation of steroid alcohols were obtained by TMSOTf promoted activation of an imidate donor.[9a] Consequently, in the present study, the scillabiose thiodonor was converted to an imidate donor. The thiophenyl group was activated with NBS, hydrolyzed,[43] and the newly formed hemiacetal converted to the corresponding imidate with DBU and trichloroacetonitrile[21a] thus providing donor **39** in 71% isolated yield over two steps as shown in Scheme 3.

With donors **38** and **39** synthesized, the focus was shifted to the glycosylation study. 3β-Hydroxy-androst-5-en-17-one (**40**) was selected as a model substrate for the glycosylation reaction. This steroid carries identical stereochemistry (at C-3, C-8, C-9, C-13 and C-14) to the aglycones of transvaalin and hellebrin and was therefore considered to be a suitable model.[30a,30d] In order to explore the suitability of TMSOTf-promoted glycosylation with imidate donors in the present study, the monosaccharide donors **8** and **9** (see page 111) were first evaluated. With both of these donors the glycosylations proceeded smoothly and the unprotected glycosteroids **44** and **45** were after deprotection under Zemplén conditions[26] isolated in high yields (77–89% over two steps). Next, the possibility of utilizing donor **38** in the glycosidation was explored. The use of this donor would be desirable as it shortens the reaction sequence by two steps. Due to the presence of an alkene functionality in steroid **40**, the commonly employed activation procedure featuring NIS/TfOH was yet again avoided.[38] Instead, activation of the thioglycoside using the methodology of Crich was employed (for a more detailed discussion see Episode 1).[44] A moderate conversion (~50%) was obtained by the use of this protocol. In order to evaluate and compare the reactivity of scillabiose donors **38** and **39**, the imidate donor was tested next. When the glycosylation reaction was performed with donor **39**, following the standard activation protocol, saponin **43** could be isolated in 88% yield. One step debenzoylation under Zemplén conditions provided the deprotected glycosteroid **46** in 90% yield. THF was required as co-solvent in the deprotection due to the amphiphilic nature of the deprotected molecule. Column purification of the final compound was once again possible and enabled a simple way of removing the methyl benzoate formed during the deprotection.



40

41 R = OBz-Rha
42 R = OBz-Glc
43 R = OBz-Glc-Rha

44 R = Rha
45 R = Glc
46 R = Glc-Rha

Scheme 4. Glycosylation of model steroid **40**: i) Donor **8**, **9** or **39**, TMSOTf, –20 °C, 1.5–2 h, CH_2Cl_2, **41** (not determined due to purification difficulties), **42** (not determined due to purification difficulties), **43** (88 %); ii) NaOMe, MeOH:THF 1:1, 3–20 h, **44** (89 % over two steps), **45** (78 over two steps), **46** (90 %).

To summarize, a viable approach to the synthesis and attachment of scillabiose to steroids was developed. This convergent approach may also be applied to the synthesis of other naturally occurring saponins sharing this glycone.[30,31] Due to the potent cytotoxic activity of hellebrin the compounds prepared in this section were also screened for cytotoxic activity, being however, not toxic.[45] After demonstrating the efficiency of benzoylated TCA-donors in the glycosylation of steroids, the study was continued by investigating their applicability to other aglycones of biological interest. In the next section, the applicability of the benzoylated TCA-donors for the regioselective glycosylation of the anthracycline 4-deoxy-ε-rhodomycinone is described.

2.3 Stereo- and Regioselective Glycosylation of 4-Deoxy-ε-rhodomycinone

Anthracyclines are compounds consisting of a sugar residue attached to a tetracyclic aglycone core. Anthracyclines, such as doxorubicin and daunorubicin (Figure 4), possess many biologically and clinically important properties and have found widespread use in the treatment of leukemia, breast carcinomas and other solid tumors.[46] While bearing large potential, the undesired side-effects (such as myelosuppression, gastrointestinal disorders and cumulative cardiotoxicity) have limited the applications of anthracyclines.[47] In order to reduce the number of side effects associated with anthracyclines, a vast number of such compounds have been prepared simultaneously providing advances in glycosylation methodologies, isolation processes and medical properties.[48] By these routes, both naturally occurring and synthetically modified anthracyclines have been produced.[49] Suprisingly, several of the glycosylation methodologies developed suffer from poor selectivites, low efficiencies and long reaction times.[50] Therefore, it was of interest to screen the imidate donors prepared earlier (see page 111) in the glycosylation of anthracyclines. It should be noted, that the ester protective groups at C-2 (in the donors described here) influence the stereoselectivities of the glycosylation reactions. In many of the earlier reports where poor selectivities were observed, 2-deoxy sugar derivatives of anthracyclines were targeted. Nevertheless, circumventing the stereoselectivity issues and generating a method with increased efficiency and shorter reaction time would still be appealing for the industrial production of similar and related pharmaceuticals.

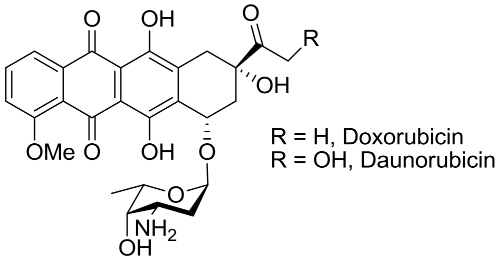
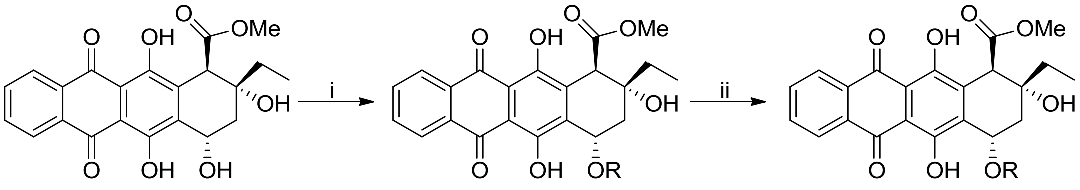


Figure 4. Chemical structures of the two well known anthracyclines doxorubicin and daunorubicin.

4-Deoxy-ε-rhodomycinone (**47**) was selected as a suitable model substrate for evaluation of benzoylated TCA-donors.[51] 4-Deoxy-ε-rhodomycinone resembles rhodomycinone which is an intermediate in the biosynthetic pathway leading to doxorubicin.[52] Suprisingly, imidate donors have not been applied to the glycosylation of tetracyclines as frequently as bromo-, chloro-, thio- and glycal donors.[50b,53] Nevertheless, they have been applied extensively in the glycosylation of other biomolecules as also discussed in previous sections of this Episode.[2a,9a] The regioselective glycosylation of **47** could, in principle, be considered challenging due to the presence of four free hydroxyl groups in the aglycone structure. It is, however, much less challenging since two of them are aromatic and one is sterically hindered by an alkyl group. In the present work, benzoylated imidate donors were considered to be better than acetylated imidate donors due to the larger size of the benzoyl groups in comparison to acetyl groups. This could, potentially, enhance the regioselectivity of the glycosylation. As mentioned previously, the ester protective groups at C-2 in the donors increase the overall stereoselectivity of the glycosidation by neighboring group participation.[54] In an initial experiment, TMSOTf and $BF_3{\cdot}OEt_2$ were screened as promoters. The weaker Lewis acid TMSOTf proved to be superior and was thus utilized as a promoter in the remaining experiments. Following the standard activation protocol by the inverse glycosylation approach using only 0.2 equivalents of TMSOTf as a promoter and 1.1 equivalents of donor **9** (Figure 2), 7-*O*-glucosylated 4-deoxy-ε-rhodomycinone was isolated in 96 % yield. Intrigued by these initial results, imidate donors **7**, **8**, and **10** (Figure 2) were next evaluated in the glycosylation reaction. The reactions proceeded in 93–98 % conversions (based on NMR) and the results are shown in Scheme 5. The products were simply filtered after the glycosylation reaction and utilized as such in the final deprotection step. The regioselectivities of the products were easily determined from the HMBC correlation between H-7 and C-1′. The stereoselectivities of the reactions were determined based on the H-1′–H-2′ coupling constants and compared to literature values.[55] Deprotection under Zemplén conditions proceeded smoothly to give the desired compounds in high yields.[26] It was noticed that upon addition of sodium methoxide the solution turned purple and upon neutralization back to red thus providing a simple way of monitoring the conditions in the final deprotection. Owing to the amphiphilic character of the deprotected molecules, the compounds could be purified by column chromatography thereby providing a simple method for removing the methyl benzoate formed as a side product in the final deprotection.

Based on a literature survey, this is the first example of benzoylated imidate donors being applied to the glycosylation of anthracyclines and based on the successfully prepared set of anthracyclines presented here, such donors may find further use in the glycosylations of similar compounds. Furthermore, the methodology described herein utilizes only 1.1 equiv. of donor and 0.2 equiv. of promoter as compared to other procedures where 2–3 equiv. of both donor and promoter have been applied thus making it more environmentally benign compared with the earlier reported methods.



47

48 R = OBz-Glc
49 R = OBz-Man
50 R = OBz-Rha
51 R = OBz-Gal

52 R = Glc
53 R = Man
54 R = Rha
55 R = Gal

Scheme 5. i) 1. TMSOTf, –40 °C, CH_2Cl_2, 2. corresponding donor, 2 h, **48** (96 %), **49** (not determined), **50** (not determined), **51** (not determined); ii) NaOMe, MeOH/THF, 1:1, r.t., 3–20 h, **52** (97%), **53** (86 % over two steps), **54** (90 % over two steps), **55** (92 % over two steps).

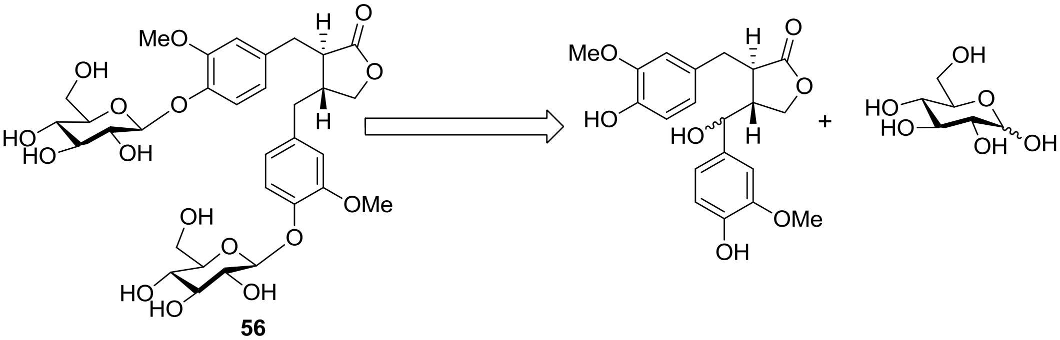
To summarize, benzoylated imidate donors were found to be suitable alternatives, in comparison with other carbohydrate donors, for glycosylation of anthracyclines. The method described herein and utilized in the presented synthesis of glycosylated anthracyclines should prove valuable to others working on similar compounds due to its high efficiency and operational simplicity. The synthesized compounds will in future work be screened for biological activity.

With benzoylated TCA-donors performing well in the glycosylation of several of the above mentioned aglycones, it was desirable and logical to incorporate the use of these donors also in the synthesis of naturally occurring compounds, not only natural product mimics. The naturally occurring lignan glycoside MDG (matairesinol diglucoside) was chosen as suitable target and the semi-synthesis of this compound will be presented next.

2.4 Semi-Synthesis of the Naturally Occurring Lignan Glycoside Matairesinol Diglucoside

Lignans, defined as two cinnamic acid residues connected through a β–β-linkage,[56] are a widespread class of natural products present in plants, trees, and fruits.[57] Isolated lignans possess biologically interesting properties including antioxidative, antiestrogenic, and anticarcinogenic activities.[58] Due to their wide occurrence and biological relevance these structures have also been the target of several total synthesis studies.[59] The previous studies have, however, solely focused on the aglycones although many of the lignans exist in nature as glycosides. The neglectance of lignan glycosides may be due to the early isolation processes where strong acids were used for extraction. The acidic conditions resulted in the cleavage of glycosidic bonds and, therefore, only the deglycosylated aglycones were isolated.[60] In recent years, advanced isolation processes have been developed correlating with an increased number of reports on lignan glycosides.[61] While the structures of many lignan glycosides isolated from natural sources have been elucidated, reports dealing with their synthesis are scarce.[62] Surprisingly, lignan glycosides have not been prepared previously by semi-synthetic approaches. A synthetic route to these compounds is important for a number of reasons: 1) As mentioned previously, isolation of intact lignan glycosides from natural sources is difficult; 2) Natural sources seldom provide sufficient amounts of material for biological evaluation; and 3) By chemical synthesis a diversity of structural analogues may be prepared for exploration and comparison of the biological properties of both natural and unnatural lignans and lignan glycosides.

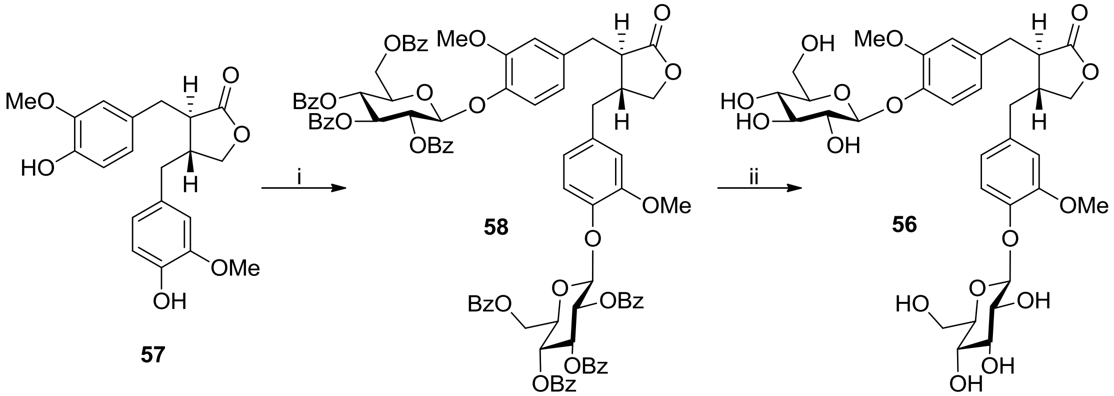
In the present study, (8*R*,8′*R*)-matairesinol 4,4′-di-*O*-β-D-glucopyranoside (MDG) found in the stems of *Trachelospermum asiaticum var. intermedium*[63] was selected as the target molecule to be prepared by semi-synthesis. A simple retrosynthetic analysis suggests the construction of this compound from glucose and hydroxymatairesinol (HMR) (Scheme 6). Such semi-synthetic approach is appealing with D-glucose being the most abundant biomolecule on earth, whereas (7*R*,8*R*,8′*R*)/(7*R*,8*R*,8′*S*)-hydroxymatairesinol (HMR) mixture, the major isomer being easily convertible to (*R*,*R*)-matairesinol (MR), is readily available on multi-ton scale from pulp mill side streams.[64] As mentioned in the introductory part of this Episode, tedious total synthetic strategies should be avoided whenever the aglycone can be isolated on large scale from natural sources. Therefore, we set out to synthesize MDG by the route shown in Scheme 7.



Scheme 6. Retrosynthetic analysis of matairesinol diglucoside.

Phenolic compounds, such as the aglycon of **56**, are often considered as difficult glycosylation targets due to the electron-withdrawing properties of the aromatic ring. Furthermore, steric hindrance from other substituents on the aromatic ring contributes to the difficulties associated with aromatic *O*-glycosylation. It has been shown previously that electron-rich aromatic aglycons are preferably glycosylated under acidic conditions with anomeric acetates or trichloroacetimidates (TCA).[65] Since the TCA-donors[66] are in general more reactive than the anomeric acetates, they were selected as glycosyl donors also in the present study. In the literature, mainly acetylated TCA-donors have been utilized. In our hands, as also mentioned on page 112, the acetyl group at C-2 is occasionally activated and transferred to the acceptor molecule under the employed reaction conditions thereby decreasing the yield of the desired product. In glycosylation of steroids, these side reactions were avoided by replacing the acetyl groups with benzoyl groups simultaneously retaining the beneficial properties of neighboring group participation displayed by ester protective groups at C-2.

The benzoylated glucose TCA-donor was prepared according to the methods decribed earlier. HMR on the other hand, is found in exceptionally large concentrations (>10 % of dry weight) in the knots of Norway spruce (*Picea abies*). In a typical isolation procedure the knots are dried, ground and extracted with acetone-water to obtain HMR.[64a, 64c] For large scale isolation, the HMR–K-acetate adduct can be utilized as described by Freudenberg.[67] Eklund, Sjöholm and coworkers have previously described the conversion of HMR to MR in nearly quantitative yield in one step by hydrogenation or metal hydride reduction.[68] In the present study, the latter procedure was utilized for preparation of MR (**57**).



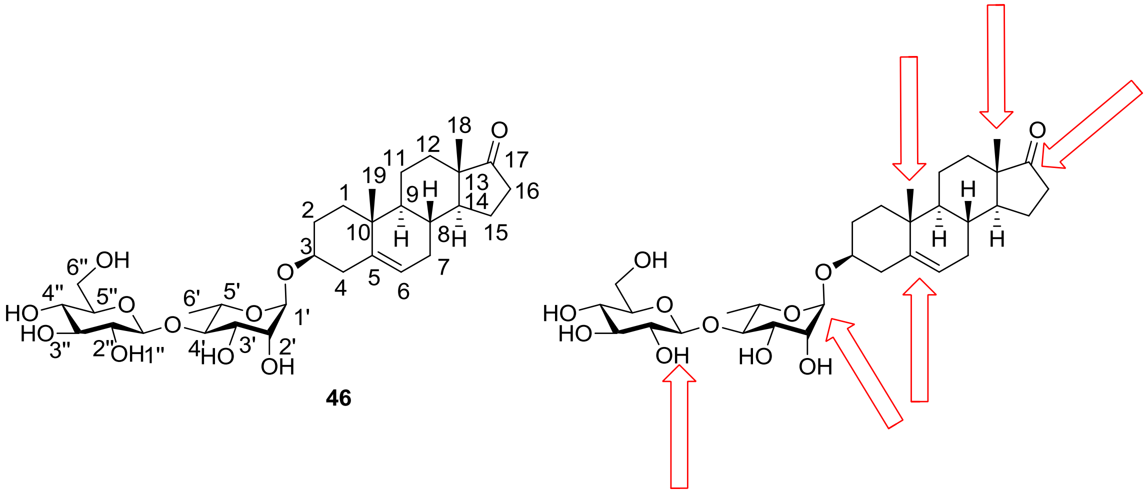
Scheme 7. Reactions conditions for synthesis of MDG; i) **9** (see Figure 2, page 111), $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C, 1.5–2 h, 69 %; ii) MeOH:THF:H_2O:Et_3N (5:6:1:2), 55 °C, 100 h, 67 %.

With the starting materials at hand, the focus was shifted to the glycosylation reaction. Generally, TCA-donors can be activated by a catalytic amount of Lewis acid.[66] In many cases, TMSOTf has been successfully applied as the promoter in glycosylation reactions and was accordingly screened also for the glycosylation of MR.[65,69] Here, the use of TMSOTf as promoter resulted in an *O*/*C*-linked mixture of diglucosylated matairesinol in moderate conversion (50 %). Changing the reaction temperature (–50–0 °C) or the amount of TMSOTf (0.2–1 equiv) did not improve the glycosylation selectivity and efficiency. The promoter was therefore switched to the stronger Lewis acid $BF_3{\cdot}OEt_2$ which has previously been used with success in similar reactions.[65,70] With $BF_3{\cdot}OEt_2$ as the promoter, the reaction proceeded smoothly under unoptimized reaction conditions to give the desired compound in 69 % yield after purification by column chromatography. For deprotection of benzoyl and ester protective groups in general, NaOMe in MeOH (Zemplén conditions) is the most commonly employed procedure.[26] In the present study, deprotection under these conditions gave a mixture of products probably due to presence of the lactone functionality or the slightly acidic α-proton in the vicinity of this center. Accordingly, a milder deprotection method was desired. In the literature, Et_3N has been utilized for sensitive substrates as a milder alternative in solvent mixtures containing $MeOH{:}H_2O{:}Et_3N$ (5:1:1).[71] An initial screening reaction at ambient temperature proved unsuccessful using this solvent combination. By adjusting the proportions of the mixture to $MeOH{:}THF{:}H_2O{:}Et_3N$ (5:6:1:2) and heating the reaction mixture at 55 °C for four days the conversion was found to be complete as indicated by TLC ($MeOH{:}CH_2Cl_2$ 1:3, $R_f = 0.26$). After purification of the crude mixture by column chromatography, the product was isolated in 67 % yield. The specific rotation, measured here in MeOH ($[\alpha]_D$ –19.8°), was in good agreement with the value reported by Nishibe and coworkers for the isolated native compound ($[\alpha]_D$ –24.0° in EtOH).[63]

Interestingly, the complete NMR spectroscopic characterization of MDG has not been reported previously. Furthermore, for various reasons, many of the aglycones are difficult to fully assign which is generally also visible in the literature as many of the individual signals in the ^1H-NMR spectra are reported in regions without their respective coupling patterns. The next section will provide some basic guidelines to the complete characterization of the different classes of compounds discussed in this Episode.

2.5 NMR Spectroscopic Characterization of Glycoconjugates

Accurate NMR data is important for a number of reasons. It is commonly utilized to ensure the identity of a synthetic or isolated compound but can also be used in the determination of three dimensional structures (as shown in Episode 1) and biological properties. The NMR spectroscopic characterization of glycoconjugates can be challenging. Therefore, this section is devoted to the NMR spectroscopic characterization of the compounds discussed in this Episode. The characterization flow will here be exemplified by the complete NMR spectroscopic characterization of glycosteroid **46** and lignan glycoside **56**. All other compounds synthesized were characterized utilizing the same principles and techniques.



46

Figure 6. Numbering of saponin **46** (left); and the starting points for the NMR spectroscopic characterization with red arrows (right).

As shown in Figure 6, several signals were selected as starting points for the NMR spectroscopic characterization of glycosteroid **46.** The glycone part was first characterized starting with the well separated anomeric protons of rhamnose (d at 4.82 ppm) and glucose (d at 4.58 ppm). Since some of the signals from these two sugars were overlapping in the ^{1}H-NMR spectrum (especially the region between 3.90–3.65 ppm), the anomeric signals where targeted by selective excitation (400 ms spinlock time) utilizing the 1D-TOCSY experiment.[72] By use of this measurement, these two spin-systems could be easily separated thus providing a convenient way of identifying the signals corresponding to each sugar respectively. The 1D-TOCSY spectra are shown in Figure 7. By the use of COSY and HSQC (Figures 8 and 9), all signals from the glycone part could next be identified in both the ^{1}H- and ^{13}C-NMR spectra.

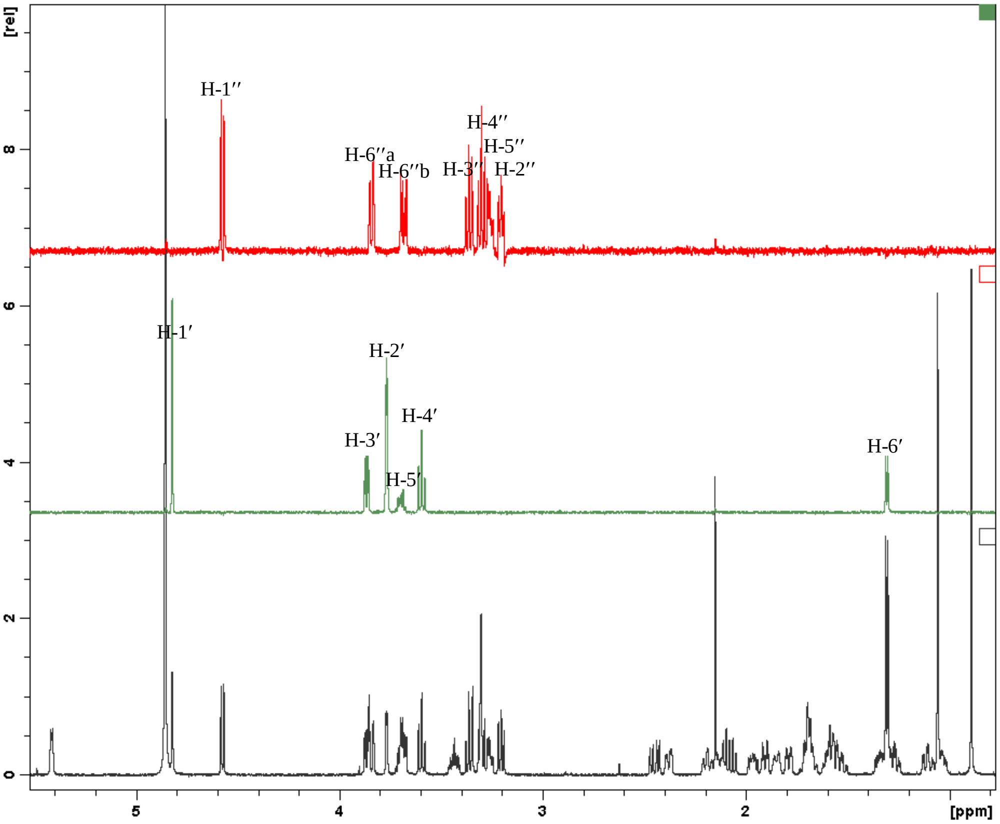


Figure 7. 1D-TOCSY spectra of the different sugar residues in **46**: from the top, Glc*p*″, Rha*p*′ and the entire ^{1}H-NMR spectrum.

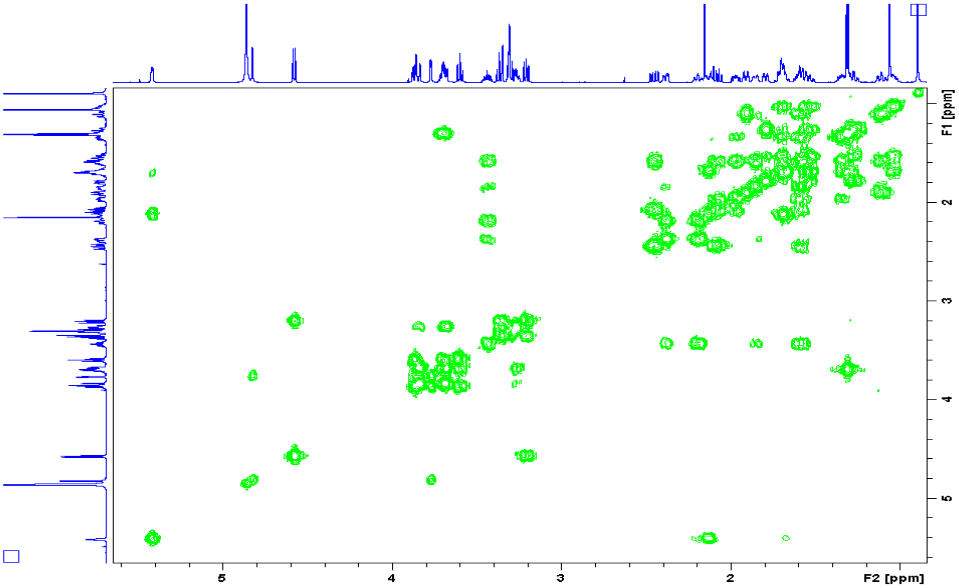


Figure 8. COSY spectrum (showing proton *J*-coupled to eachother) of compound **46**.

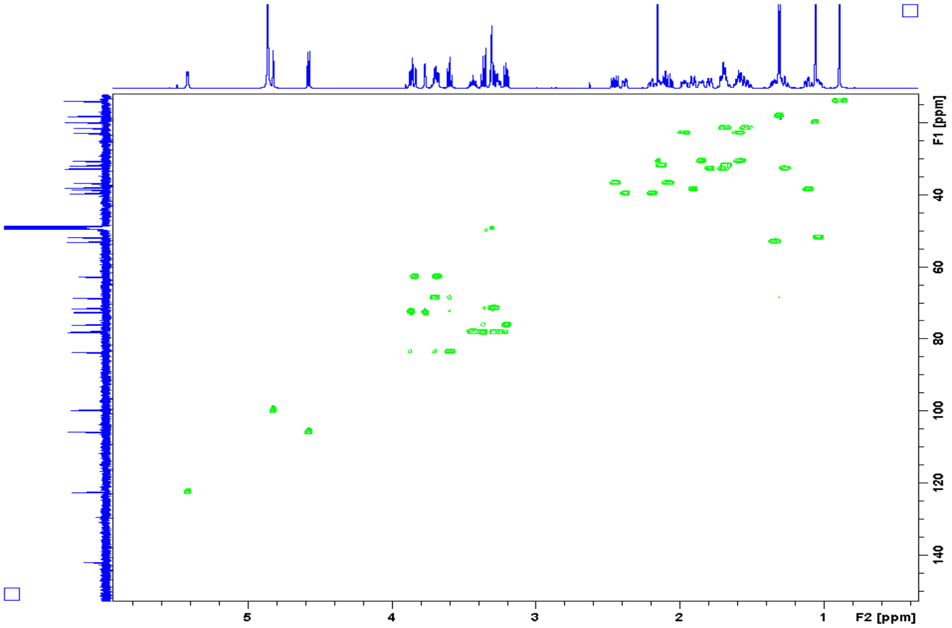


Figure 9. HSQC spectrum (showing proton–carbon correlations) of compound **46**.

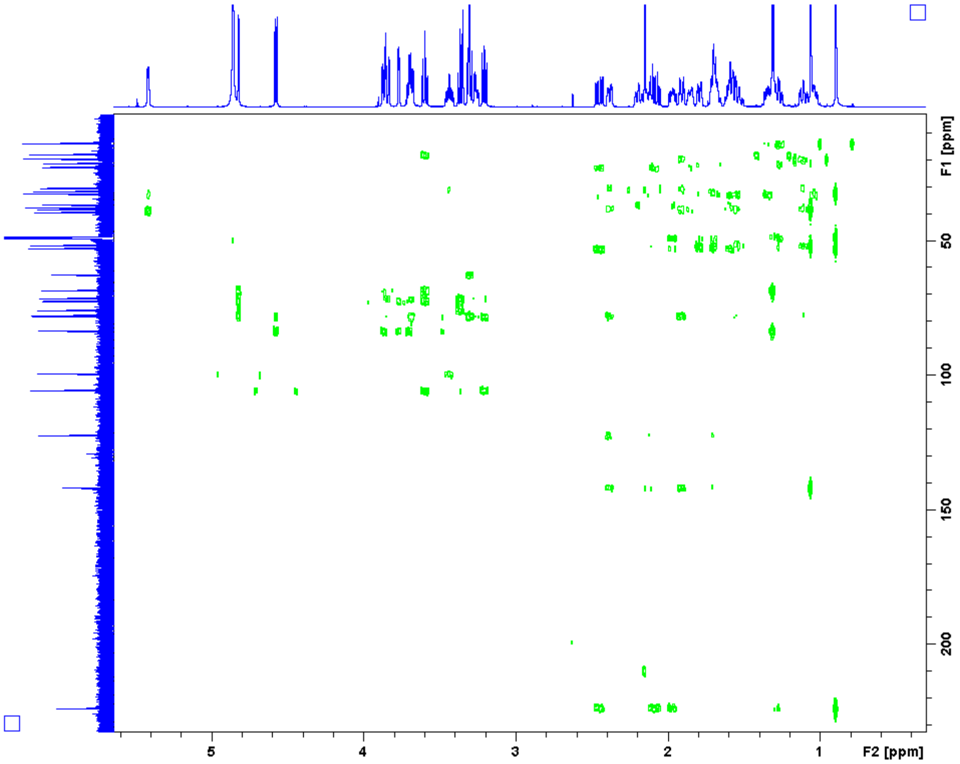


Figure 10. HMBC spectrum (showing proton–carbon correlations over multiple bonds) of compound **46**.

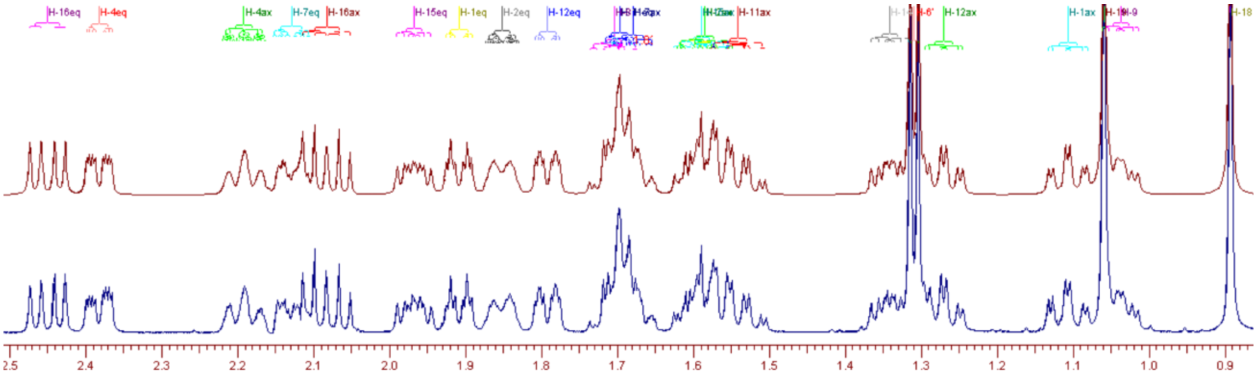
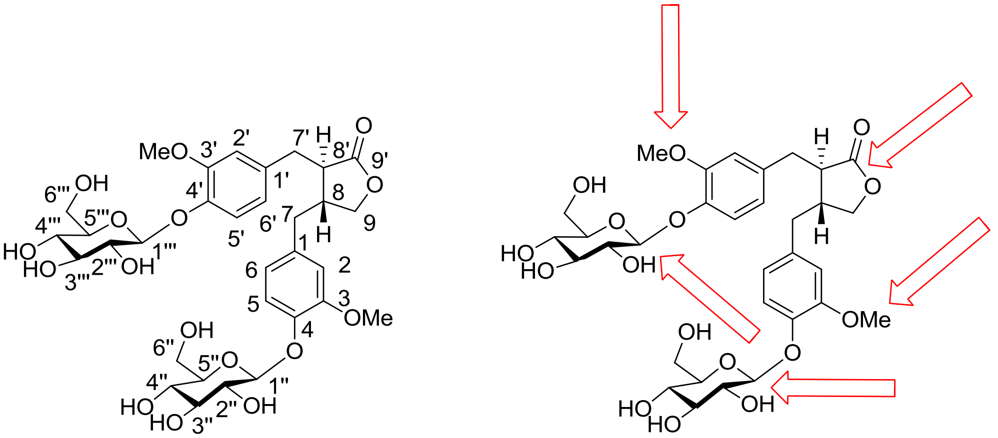


Figure 11. Spectral simulation of the 2.52–0.85 ppm region of the ^{1}H NMR spectrum of **46** using PERCH NMR software: simulated spectrum (above), observed spectrum (below).

From the HMBC spectrum (Figure 10), the correlation between H-1′ and C-3 (77.9 ppm) and H-3 (3.44 ppm) and C-1′ (99.6 ppm) was clearly visible thus providing a starting point for the characterization of the aglycone. By use of COSY, both of the H-2 and H-4 protons could be identified. The corresponding signals in the ^{13}C-NMR spectra were easily determined by the use of HSQC. Next, the spectral characterization was continued from the well separated signals of H-6 (ddd at 5.42 ppm), C-6 (122.4 ppm) and C-5 (141.9 ppm). From the HMBC correlation of C-5, H-19 (s at 1.06 ppm) and H-1eq (ddd at 1.91 ppm) could be identified. By use of HSQC the corresponding carbon signals were solved. From the COSY spectra the correlations between H-6 and both of the H-7 protons were visible. Furthermore, the HMBC correlation between H-6 and C-10 was used to completely solve the A-ring of the steroid. The HMBC correlation of H-19–C-9 and H-6–C-8 enabled the complete characterization of the B-ring. For solving the C- and D-rings, the carbonyl carbon of C-17 (223.8 ppm) and the methyl group connected to C-13 were chosen as starting points. From the HMBC correlations of C-17 both of the H-15 protons could be found. From the HMBC correlations of H-18 it was possible to identify C-12, C-13 and C-14. By use of COSY and HSQC the C- and D-rings could now be completely solved. While assignations of this type can be considered straightforward or routine, obtaining accurate coupling constants in these systems is extremely difficult. This is mainly due to the severe overlap of signals in the steroid region. As decribed in Episode 1, the complete assignation of a β-1,2-linked mannotetraose by use of the NMR simulation software PERCH was performed in earlier work.[73] Herein, this program was utilized to completely assign the proton spectra of the compounds prepared, exemplified by the high field region of the ^{1}H-NMR spectrum of **46** shown in Figure 11.



56

Figure 12. Numbering of MDG (**56**) (left); and the starting points for the NMR spectroscopic characterization with red arrows (right).

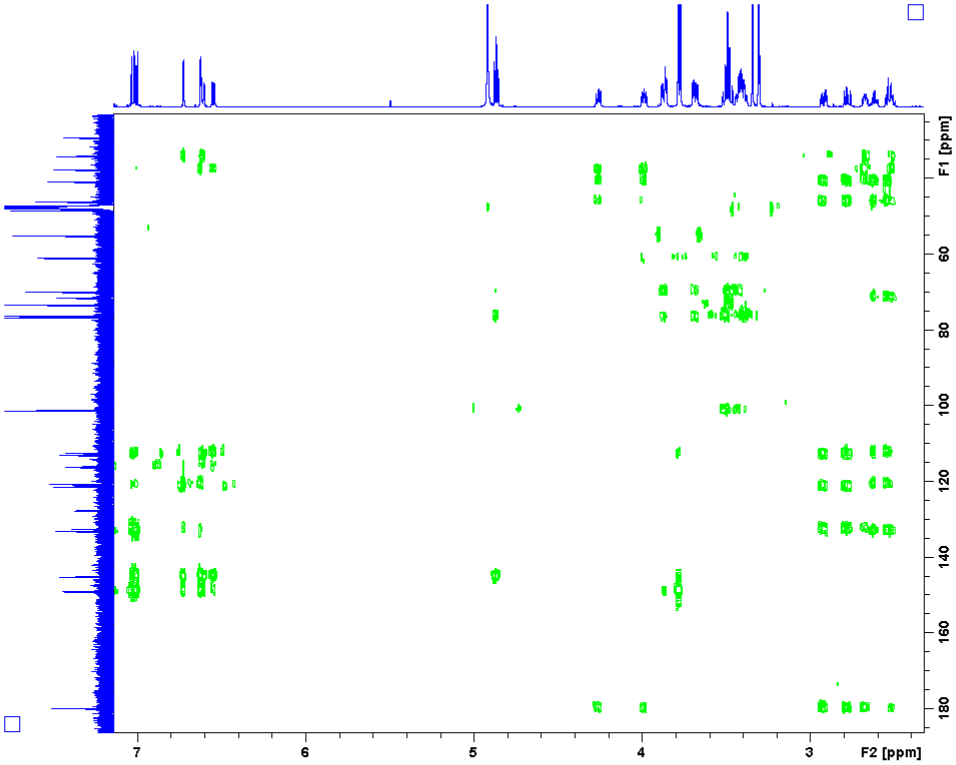


Figure 13. HMBC spectrum (showing proton–carbon correlations over multiple bonds) of compound **56**.

The NMR spectroscopic data reported in the literature on the naturally occurring lignan glycoside MDG[63] was not convincing. Therefore, the necessary NMR spectroscopic experiments were conducted with the synthesized structure **56**. The NMR spectra of MDG were recorded at 20 °C in order to shift the water signal which was otherwise overlapping with the anomeric protons. The ^{13}C-NMR signal of C-9′ (181.4 ppm) was selected as a starting point for the structural elucidation. The correlations between C-9′–H-7′a, C-9′–H-7′b, C-9′–H-8, C-9′–H-9a and C-9′–H-9b were clearly visible by HMBC (Figure 13). By use of COSY and HSQC both the ^{1}H- and ^{13}C-NMR chemical shifts of these signals, as well as the signals for H-8′ and C-8′, could be determined. Assignation of the aromatic rings was based on the HMBC correlation between H-7a (2.62 ppm) and H-7b (2.53 ppm) to C-6 (122.2 ppm) and C-2 (114.0 ppm).

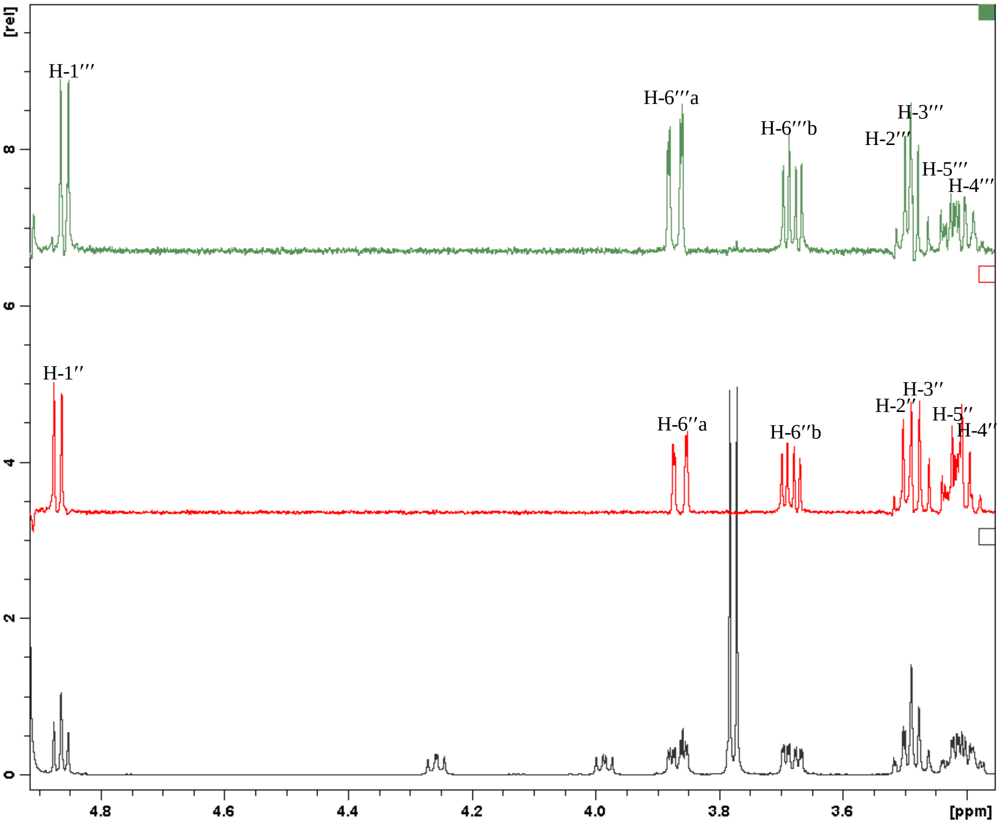


Figure 14. 1D-TOCSY spectra of the different sugar residues and the entire ^{1}H-NMR spectrum of the carbohydrate region of **56**.

The remaining signals from the aromatic ring were then easily assigned by HMBC and a similar methodology was also applied for resolving the signals of the second aromatic ring. The positions of the glucose moieties were determined by HMBC based on the correlation between C-4 (146.6 ppm) and H-1″ (4.87 ppm). While the assignation of these signals can be considered fairly straightforward, the assignation of the carbohydrate moieties of MDG turned out to be extremely difficult due to severe signal overlap. For resolving the carbohydrate part, 1D-TOCSY (TOtal Correlation SpectroscopY) was again utilized.[72] The only separable signals were those from the anomeric protons (4.87 and 4.86 ppm) and even their separation was marginal as shown in Figure 14. As a result, a narrow 1D-TOCSY excitation range was required. Here, such a range was achieved by prolonging the pulse and reducing the pulse power.

While the 1D-TOCSY NMR spectra significantly enhanced the complete assignation, accurrate and reliable coupling constants could not be extracted from the spectral data alone. Therefore, the spectral simulation software PERCH was utilized to acquire accurate chemical shifts and coupling patterns for the individual signals.[73]

As discussed above, utilization of basic NMR spectroscopic techniques (^{1}H, ^{13}C, 1D-TOCSY, COSY, HSQC, HMBC and NOESY) in combination with spectral simulations led to the complete assignation of all glycoconjugates synthesized in the present chapter. The most important experiments were in most cases high resolution HMBC and 1D-TOCSY which significantly simplified the characterization of the ^{1}H- and ^{13}C-NMR spectra.

3 Conclusions

To summarize, glycoconjugates are structures consisting of a carbohydrate moiety permanently linked to another chemical species. These structures are widely distributed in nature and often associated with various biological applications and functions. In this Episode, methods for the glycosylation of several different aglycones, including steroids, tetracyclines and lignans, were addressed and discussed. The initial screening of glycosyl donors revealed that benzoylated imidate donors are optimal for the glycosylation of aglycones. These donors were next applied to the synthesis of a limited library of glycosteroids. Evaluation of the synthesized molecules in pharmaceutical delivery systems has not been investigated yet.

In the second part of the Episode, a method for the attachment of scillabiose to steroids was developed. Since the saponins synthesized resembled the structure of hellebrin (a potent cytotoxic compound) they were also tested in similar applications but were found to be inactive.[45]

In the third part, benzoylated TCA-donors were applied in the regioselective glycosylation of 4-deoxy-ε-rhodomycinone with excellent results. The environmentally benign approach utilized in the synthesis of these structures could be valuable for industrial synthesis of similar compounds due to the operational simplicity, short reaction time and minimal waste generation of the protocol. The deprotected anthracyclines will in future work be screened for cytotoxicity.

In the last part dealing with synthesis of glycoconjugates, the naturally occurring lignan glycoside MDG was synthesized utilizing the cheap and renewable starting materials HMR and D-glucose. While not discussed in the present Episode, a few other lignan glycosides were also prepared to ensure the reliability of the methodology.[8a] Both lignans and lignan glycosides are currently of interest in the food industry due to their wide occurrence and health promoting effects.

In addition to the synthesis, some key-guidelines to solving NMR spectroscopic challenges associated with glycoconjugates were given and exemplified by the complete characterization of structures **46** and **56**.

4 Experimental Section

Reaction solvents were dried and distilled prior to use when necessary. All reactions containing moisture- or air-sensitive reagents were carried out under argon atmosphere. The NMR spectra were recorded with Bruker Avance NMR spectrometer operating at 600.13 MHz (^{1}H: 600.13 MHz, ^{13}C: 150.90 MHz). The probe temperature during the experiments was kept at 25 °C unless indicated otherwise. All products were fully characterized by utilization of the following 1D-techniques: ^{1}H, ^{13}C and TOCSY and the following 2D-techniques; DQF-COSY, NOESY, HSQC and HMBC by using pulse sequences provided by the manufacturer. Chemical shifts are expressed on the δ scale (in ppm) using TMS (tetramethylsilane), residual chloroform or methanol as internal standards. Coupling constants are given in Hz and provided only once, when first encountered. Coupling patterns are given as *s* (singlet), *d* (doublet), *t* (triplet) etc. The following indexes are used to clarify between protons on the same carbon: eq = equatorial, ax = axial, t = *trans* and c = *cis*. The computational analysis of all compounds was achieved by utilization of the PERCH NMR software with starting values and spectral parameters obtained from the various NMR techniques used.[73] HRMS were recorded using Bruker Micro Q-TOF with ESI (electrospray ionization) operated in positive mode. Optical rotations were measured at 23 °C, unless otherwise stated, with a Perkin Elmer polarimeter equipped with a Na-lamp (589 nm).

TLC was performed on aluminium sheets precoated with silica gel 60 F_{254} (Merck). Flash chromatography was carried out on silica gel 60 (0.040–0.060 mm, Merck). Spots were visualized by UV followed by charring with 1:4 H_2SO_4/MeOH and heating.

4.1 General Experimental Procedures

General procedure for glycosylation of steroids and tetracyclines. To a solution containing the corresponding acceptor (1 equiv) and pre-activated 4 Å MS in dry CH_2Cl_2 (1.6 ml/0.1 mmol substrate) was added TMSOTf (0.2 equiv.) at –20 °C. The reaction mixture was stirred for 10 minutes and the corresponding donor (1.3 equiv) dissolved in dry CH_2Cl_2 (1.7 ml/0.1 mmol substrate) was added dropwise to the solution. The resulting mixture was stirred for 1.5–2 h, brought to r.t., diluted with CH_2Cl_2 (20 ml) and washed with sat. $NaHCO_3$-solution (20 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc, 4:1) to give the corresponding protected glycoconjugate.

General procedure for deprotection for steroids and anthracyclines. To a solution containing the protected glycosteroid (1 equiv) in dry MeOH or dry MeOH:THF mixture (2:1) (6.3 ml/0.1 mmol substrate) was added NaOMe (2 equiv) and the resulting mixture was stirred for 3–20 h at r.t., neutralized with DOWEX 50 H^+-form, filtered and concentrated. The crude product was purified by column chromatography ($CH_2Cl_2 \rightarrow CH_2Cl_2$:MeOH 5:1 for all compounds except **21**, **22**, **32** which were purified with EtOAc) to give the deprotected glycoconjugate.

General procedure for ring-closing metathesis. To a solution containing the corresponding glycosteroid bearing two terminal alkenes (1 equiv) in dry CH_2Cl_2 (1.7 ml/0.1 mmol substrate) was added Grubbs 2nd generation catalyst (10 mol%) and the resulting mixture was refluxed for 6 h at 40 °C, brought to r.t., stirred overnight and concentrated. The crude product was purified by column chromatography (hexane:EtOAc, 4:1) to give the corresponding ring-closed glycosteroid.

General procedure for removal of isopropylidene acetal. A solution containing the corresponding protected substrate (1 equiv.) in 80 % AcOH (17 ml/1 g) was stirred at 80 °C for 5 h, brought to r.t. and concentrated. The crude product was purified by column chromatography with hexane-EtOAc 2:3 as eluent to give the deprotected substrate.

General procedure for benzoylation of hydroxyl groups. The substrate bearing hydroxyl groups was dissolved in a mixture of pyridine (15 ml/1 g) and BzCl (7.5 ml/1 g) and allowed to stir until TLC indicated complete disappearance of starting material. The reaction was quenched after 4 h with MeOH, concentrated, dissolved in CH_2Cl_2 (40 ml) and washed with water (2 × 20 ml) and brine (20 ml). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography with hexane:EtOAc (1:0 → 4:1) as eluent to give the benzoylated compound.

General procedure for converting thio-glycoside into an imidate donor. To a solution containing thioglycoside (1 equiv.) in acetone:H_2O (10:1, 28 ml/ 1 g) was added NBS (1.5 equiv.) at 0 °C. The reaction was brought to r.t. and stirring was continued for 1 h. This process was repeated twice after which TLC indicated the reaction to be complete. The reaction was quenched with sat. $NaHCO_3$-solution (15 ml), diluted with CH_2Cl_2 (30 ml) and washed with brine (20 ml). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography with hexane:EtOAc 4:1 as eluent to give the corresponding hemiacetal. The hemiacetal (1 equiv.) was dissolved in CH_2Cl_2 (20 ml/1 g) and DBU (0.1 equiv.) and Cl_3CCN (2.4 equiv.) was added at 0 °C. The reaction mixture was stirred for 1.5 h, brought to r.t., diluted with CH_2Cl_2 (30 ml) and washed with brine (20 ml). The organic phase was dried with anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography with hexane:EtOAc:Et_3N 3:1:0.01 as eluent to give the corresponding imidate donor.

General procedure for glycosylation of lignans. To a solution containing lignan (1 equiv.), glycosyl donor (2.6 equiv.) and pre-activated 4 Å MS in dry CH_2Cl_2 (14 ml/1 g) was added $BF_3{\cdot}OEt_2$ (0.5 equiv.) at 0 °C. The resulting mixture was stirred for 1.5–2 h, brought to r.t., diluted with CH_2Cl_2 (20 ml) and washed with sat. $NaHCO_3$-solution (20 ml). The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc, 1:1) to give the lignan glycoside.

General procedure for deprotection of lignan glycosides. The protected lignan glycoside (1 equiv.) was dissolved in a mixture containing MeOH:THF:H_2O:Et_3N (5:6:1:2, 20 ml/1 g) and the solution was stirred at 55 °C for 100 h and concentrated. The crude product was purified by column chromatography (MeOH:CH_2Cl_2 1:3) to give the deprotected lignan glycoside.

4.2 Analytical Data on Selected Substrates

13,17-seco-13α-methyl-1′-(2″,3″,4″,6″-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-3-methoxyestra-1,3,5(10),16-tetraene (11). Synthesized from **1** (51 mg, 0.17 mmol) and **7** (176 mg, 0.24 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **11** as a white foam (135 mg, 91 %). R_f = 0.65 (hexane:EtOAc 1:1); $[\alpha]_D$ –11.3° (c 0.3, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.12–7.25 (m, 20 H, arom. *H*), 7.26 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.75 (dd, 1 H, $J_{2,4}$ = 2.8 Hz, H-2), 6.64 (d, 1 H, H-4), 6.08 (dd, 1 H, $J_{4'',3''}$ = 10.1, $J_{4'',5''}$ = 10.2 Hz, H-4″), 5.93 (dddd, 1 H, $J_{16,15a}$ = 6.2, $J_{16,15b}$ = 6.4, $J_{16,17c}$ = 10.3, $J_{16,17t}$ = 17.2 Hz, H-16), 5.92 (dd, 1 H, $J_{3'',2''}$ = 3.4 Hz, H-3″), 5.77 (dd, 1 H, $J_{2'',1''}$ = 1.8 Hz, H-2″), 5.10 (dddd, 1 H, $J_{17t,17c}$ = –1.4, $J_{17t,15b}$ = –1.6, $J_{17t,15a}$ = –1.9 Hz, H-17t), 5.09 (d, 1 H, H-1′), 5.02 (dddd, 1 H, $J_{17c,15a}$ = –1.6, $J_{17c,15b}$ = –1.9 Hz, H-17c), 4.71 (dd, 1 H, $J_{6''a,5''}$ = 2.6, $J_{6''a,6''b}$ = –12.2 Hz, H-6″a), 4.50 (dd, 1 H, $J_{6''b,5''}$ = 5.0 Hz, H-6″b), 4.44 (ddd, 1 H, H-5″), 3.78 (s, 3 H, 3-OCH_3), 3.76 and 3.25 (each d, each 1 H, *J* = –9.4 Hz, H-1′), 2.87 (ddd, 1 H, $J_{6a,7eq}$ = 2.7, $J_{6a,7ax}$ = 5.5, $J_{6a,6b}$ = –13.6 Hz, H-6a), 2.85 (ddd, 1 H, $J_{6b,7eq}$ = 5.8, $J_{6b,7ax}$ = 12.4 Hz, H-6b), 2.36 (ddd, 1 H, $J_{9,11eq}$ = 3.4, $J_{9,8}$ = 10.4, $J_{9,11ax}$ = 12.2 Hz, H-9), 2.35 (dddd, 1 H, $J_{11eq,12eq}$ = 2.9, $J_{11eq,12ax}$ = 3.8, $J_{11eq,11ax}$ = –12.7 Hz, H-11eq), 2.32 (ddddd, 1 H, $J_{15a,14}$ = 3.0, $J_{15a,15b}$ = –16.6 Hz, H-15a), 2.16 (dddd, 1 H, $J_{7eq,8}$ = 2.5, $J_{7eq,7ax}$ = –12.8 Hz, H-7eq), 2.08 (ddddd, 1 H, $J_{15b,14}$ = 5.6 Hz, H-15b), 1.81 (ddd, 1 H, $J_{12ax,12eq}$ = –12.8, $J_{12ax,11ax}$ = 13.9 Hz, H-12ax), 1.76 (ddd, 1 H, $J_{12eq,11ax}$ = 3.3 Hz, H-12eq), 1.53 (ddd, 1 H, $J_{14,8}$ = 11.1 Hz, H-14), 1.46 (dddd, 1 H, H-11ax), 1.43 (dddd, 1 H, $J_{8,7ax}$ = 11.9 Hz, H-8), 1.38 (dddd, 1 H, H-7ax), 0.91 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.1 (6″-O*C*OPh), 165.5 (3″-O*C*OPh, 4″-O*C*OPh, 2″-O*C*OPh), 157.5 (C-3), 140.0 (C-16), 137.9 (C-5), 133.5–128.3 (arom. *C*), 132.7 (C-10), 126.6 (C-1), 114.5 (C-17), 113.4 (C-4), 111.7 (C-2), 98.4 (C-1″), 77.4 (C-1′), 70.5 (C-2″), 70.2 (C-3″),

69.0 (C-5″), 67.0 (C-4″), 63.0 (C-6″), 55.2 (3-O*C*H$_3$), 44.6 (C-14), 43.3 (C-9), 41.6 (C-8), 38.2 (C-13), 36.6 (C-12), 32.7 (C-15), 30.6 (C-6), 27.5 (C-7), 26.4 (C-11), 16.2 (C-18) ppm.
HRMS: *m/z* calcd. for $C_{54}H_{54}O_{11}Na$ $[M + Na]^+$ 901.3564; found 901.3546.

(1′*S*)-13,17-seco-13α-(but-3′-en-1′-(2″,3″,4″,6″-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-1′-yl)-3-methoxyestra-1,3,5(10),16-tetraene (12). Synthesized from **3** (87 mg, 0.255 mmol) and **7** (265 mg, 0.358 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **12** as a white foam (211 mg, 90 %). R_f = 0.83 (hexane:EtOAc 1:1); $[\alpha]_D$ –19.1° (c 0.5, CHCl$_3$). ^{1}H NMR (600.13 MHz, CDCl$_3$): δ 8.10–7.25 (m, 20 H, arom. *H*), 7.26 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.75 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.63 (d, 1 H, H-4), 6.18 (dd, 1 H, $J_{4'',5''}$ = 10.0, $J_{4'',3''}$ = 10.2 Hz, H-4″), 6.01 (dddd, 1 H, $J_{16,15a}$ = 6.4, $J_{16,15b}$ = 6.9, $J_{16,17c}$ = 10.2, $J_{16,17t}$ = 17.1 Hz, H-16), 5.99 (dddd, 1 H, $J_{3',2'a}$ = 5.7, $J_{3',2'b}$ = 8.4, $J_{3',4'c}$ = 10.1, $J_{3',4't}$ = 17.0 Hz, H-3′), 5.92 (dd, 1 H, $J_{3'',2''}$ = 3.1 Hz, H-3″), 5.81 (dd, 1 H, $J_{2'',1''}$ = 1.9 Hz, H-2″), 5.27 (dddd, 1 H, $J_{4't,2'b}$ = –1.1, $J_{4't,2'a}$ = –1.4, $J_{4't,4'c}$ = –1.8 Hz, H-4′t), 5.22 (d, 1 H, H-1″), 5.16 (dddd, 1 H, $J_{17t,17c}$ = –1.4, $J_{17t,15b}$ = –1.6, $J_{17t,15a}$ = –2.1 Hz, H-17t), 5.14 (dddd, 1 H, $J_{4'c,2'b}$ = –0.7, $J_{4'c,2'a}$ = –1.3 Hz, H-4′c), 5.02 (dddd, 1 H, $J_{17c,15b}$ = –1.2, $J_{17c,15a}$ = –2.1 Hz, H-17c), 4.72 (dd, 1 H, $J_{6''a,5''}$ = 2.5, $J_{6''a,6''b}$ = –12.1 Hz, H-6″a), 4.55 (ddd, 1 H, $J_{5'',6''b}$ = 3.6 Hz, H-5″), 4.44 (dd, 1 H, H-6″b), 3.81 (dd, 1 H, $J_{1',2'a}$ = 2.5, $J_{1',2'b}$ = 8.2 Hz, H-1′), 3.79 (s, 3 H, 3-OC*H*$_3$), 2.85 (ddd, 1 H, $J_{6a,7eq}$ = 2.3, $J_{6a,7ax}$ = 7.4, $J_{6a,6b}$ = –12.3 Hz, H-6a), 2.85 (ddd, 1 H, $J_{6b,7eq}$ = 5.4, $J_{6b,7ax}$ = 9.6 Hz, H-6b), 2.59 (ddddd, 1 H, $J_{15a,14}$ = 2.8, $J_{15a,15b}$ = –16.4 Hz, H-15a), 2.55 (ddddd, 1 H, $J_{2'a,2'b}$ = –15.0 Hz, H-2′a), 2.45 (ddddd, 1 H, H-2′b), 2.36 (dddd, 1 H, $J_{11eq,9}$ = 3.3, $J_{11eq,12ax}$ = 3.3, $J_{11eq,12eq}$ = 4.7, $J_{11eq,11ax}$ = –13.0 Hz, H-11eq), 2.32 (ddd, 1 H, $J_{9,8}$ = 10.7, $J_{9,11ax}$ = 12.3 Hz, H-9), 2.27 (ddddd, 1 H, $J_{15b,14}$ = 4.3 Hz, H-15b), 2.25 (dddd, 1 H, $J_{7eq,8}$ = 2.1, $J_{7eq,7ax}$ = –12.9 Hz, H-7eq), 1.86 (ddd, 1 H, $J_{12ax,12eq}$ = –11.4, $J_{12ax,11ax}$ = 13.9 Hz, H-12ax), 1.77 (ddd, 1 H, $J_{12eq,11ax}$ = 3.3 Hz, H-12eq), 1.61 (ddd, 1 H, $J_{14,8}$ = 12.5 Hz, H-14), 1.51 (dddd, 1 H, $J_{8,7ax}$ = 11.1 Hz, H-8), 1.43 (dddd, 1 H, H-11ax), 1.34 (dddd, 1 H, H-7ax), 0.99 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CDCl$_3$): δ 166.1 (6″-O*C*OPh), 165.5 (3″-O*C*OPh, 4″-O*C*OPh), 165.3 (2″-O*C*OPh), 157.5 (C-3), 139.2 (C-16), 138.0 (C-5), 136.3 (C-3′), 133.4–128.3 (arom. *C*), 132.5 (C-10), 126.6 (C-1), 118.2 (C-4′), 115.3 (C-17), 113.4 (C-4), 111.7 (C-2), 98.7 (C-1″), 87.7 (C-1′), 71.0 (C-2″), 70.1 (C-3″), 69.9 (C-5″), 66.8 (C-4″), 62.7 (C-6′), 55.2 (3-O*C*H$_3$), 43.6 (C-14), 43.4 (C-9), 42.1 (C-13), 41.3 (C-8), 35.6 (C-2′), 33.6 (C-12), 33.0 (C-15), 30.5 (C-6), 27.7 (C-7), 26.3 (C-11), 18.5 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{57}H_{58}O_{11}Na$ [M + Na]$^+$ 941.3877; found 941.3864.

13,17-seco-13α-methyl-1′-(2″,3″,4″-tri-*O*-benzoyl-α-L-rhamnopyranosyloxy)-3-methoxyestra-1,3,5(10),16-tetraene (13). Synthesized from **1** (72 mg, 0.24 mmol) and **8** (208 mg, 0.34 mmol) according to the glycosylation of steroids and tetracyclines **13** as a white foam (168 mg, 92 %). R_f = 0.75 (hexane:EtOAc 1:1); $[\alpha]_D$ +96.7° (c 0.12, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.13–7.23 (m, 15 H, arom. *H*), 7.26 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 6.74 (dd, 1 H, $J_{2,4}$ = 2.8 Hz, H-2), 6.65 (d, 1 H, H-4), 5.94 (dddd, 1 H, $J_{16,15a}$ = 6.3, $J_{16,15b}$ = 6.9, $J_{16,17c}$ = 10.1, $J_{16,17t}$ = 17.1 Hz, H-16), 5.85 (dd, 1 H, $J_{3'',2''}$ = 3.5, $J_{3'',4''}$ = 10.1 Hz, H-3″), 5.70 (dd, 1 H, $J_{2'',1''}$ = 1.8 Hz, H-2″), 5.70 (dd, 1 H, $J_{4'',5''}$ = 9.7 Hz, H-4″), 5.08 (dddd, 1 H, $J_{17t,17c}$ = –1.6, $J_{17t,15a}$ = –1.7, $J_{17t,15b}$ = –1.8 Hz, H-17t), 5.00 (dddd, 1 H, $J_{17c,15a}$ = –1.5, $J_{17c,15b}$ = –1.5 Hz, H-17c), 4.97 (d, 1 H, H-1″), 4.20 (dq, 1 H, $J_{5'',6''}$ = 6.3 Hz, H-5″), 3.78 (s, 3 H, 3-OC*H*$_3$), 3.56 and 3.41 (each d, each 1 H, *J* = –9.5 Hz, H-1′), 2.88 (ddd, 1 H, $J_{6a,7eq}$ = 2.5, $J_{6a,7ax}$ = 5.0, $J_{6a,6b}$ = –17.3 Hz, H-6a), 2.86 (ddd, 1 H, $J_{6b,7eq}$ = 5.9, $J_{6b,7ax}$ = 13.0 Hz, H-6b), 2.41 (ddd, 1 H, $J_{9,11eq}$ = 4.2, $J_{9,11ax}$ = 11.6, $J_{9,8}$ = 11.9 Hz, H-9), 2.36 (dddd, 1 H, $J_{11eq,12eq}$ = 3.1, $J_{11eq,12ax}$ = 3.7, $J_{11eq,11ax}$ = –12.9 Hz, H-11eq), 2.31 (ddddd, 1 H, $J_{15b,14}$ = 3.7, $J_{15b,15a}$ = –15.6 Hz, H-15b), 2.19 (dddd, 1 H, $J_{7eq,8}$ = 3.6, $J_{7eq,7ax}$ = –14.4 Hz, H-7eq), 2.12 (ddddd, 1 H, $J_{15a,14}$ = 4.6 Hz, H-15a), 1.90 (ddd, 1 H, $J_{12ax,12eq}$ = –13.1, $J_{12ax,11ax}$ = 13.7 Hz, H-12ax), 1.70 (ddd, 1 H, $J_{12eq,11ax}$ = 3.3 Hz, H-12eq), 1.62 (ddd, 1 H, $J_{14,8}$ = 11.0 Hz, H-14), 1.49 (dddd, 1 H, H-11ax), 1.44 (dddd, 1 H, $J_{8,7ax}$ = 12.6 Hz, H-8), 1.42 (dddd, 1 H, H-7ax), 1.40 (d, 3 H, H-6″), 0.92 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 165.8 (4″-O*C*OPh), 165.5 (2″-O*C*OPh, 3″-O*C*OPh), 157.5 (C-3), 140.0 (C-16), 138.0 (C-5), 133.4–128.3 (arom. *C*), 132.9 (C-10), 126.4 (C-1), 114.4 (C-17), 113.4 (C-4), 111.6 (C-2), 98.1 (C-1″), 76.8 (C-1′), 71.9 (C-4″), 70.8 (C-2″), 70.2 (C-3″), 66.8 (C-5″), 55.2 (3-O*C*H$_3$), 44.5 (C-14), 43.3 (C-9), 41.4 (C-8), 38.3 (C-13), 36.5 (C-12), 32.8 (C-15), 30.5 (C-6), 27.5 (C-7), 26.3 (C-11), 17.7 (C-6″), 16.6 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{47}H_{50}O_9Na$ [M + Na]$^+$ 781.3352; found 781.3336; *m/z* calcd. for $C_{47}H_{54}O_9N$ [M + NH_4]$^+$ 776.3799; found 776.3777.

(1′*S*)-13,17-seco-13α-(but-3′-en-1′-(2″,3″,4″-tri-*O*-benzoyl-α-L-rhamnopyranosyloxy)-1′-yl)-3-methoxyestra-1,3,5(10),16-tetraene (14). Synthesized from **3** (122 mg, 0.358 mmol) and **8** (311 mg, 0.508 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **14** as a white foam (251 mg, 88 %). R_f = 0.83 (hexane:EtOAc 1:1); $[\alpha]_D$ +116.3° (c 0.16, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.10–7.22 (m, 15 H, arom. *H*), 7.26 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 6.73 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.63 (d, 1 H, H-4), 6.07 (dddd, 1 H, $J_{3',2'a}$ = 6.4, $J_{3',2'b}$ = 7.2, $J_{3',4'c}$ = 10.2, $J_{3',4't}$ = 17.1 Hz, H-3′), 5.93 (dddd, 1 H, $J_{16,15b}$ = 5.5, $J_{16,15a}$ = 7.5, $J_{16,17c}$ = 10.1, $J_{16,17t}$ = 16.8 Hz, H-16), 5.83 (dd, 1 H, $J_{3'',2''}$ = 3.2, $J_{3'',4''}$ = 10.1 Hz, H-3″), 5.75 (dd, 1 H, $J_{2'',1''}$ = 1.8 Hz, H-2″), 5.68 (dd, 1 H, $J_{4'',5''}$ = 9.8 Hz, H-4″), 5.15 (dddd, 1 H, $J_{4't,2'a}$ = –1.4, $J_{4't,2'b}$ = –1.5, $J_{4't,4'c}$ = –2.0 Hz, H-4′t), 5.08 (dddd, 1 H, $J_{4'c,2'b}$ = –1.0, $J_{4'c,2'a}$ = –1.4, H-4′c), 5.06 (d, 1 H, H-1″), 5.05(dddd, 1 H, $J_{17t,17c}$ = –1.4, $J_{17t,15a}$ = –1.8, $J_{17t,15b}$ = –2.1 Hz, H-17t), 4.85 (dddd, 1 H, $J_{17c,15a}$ = –1.7, $J_{17c,15b}$ = –1.8 Hz, H-17c), 4.34 (dq, 1 H, $J_{5'',6''}$ = 6.2 Hz, H-5″), 3.78 (s, 3 H, 3-OCH_3), 3.69 (dd, 1 H, $J_{1',2'a}$ = 2.8, $J_{1',2'b}$ = 7.5 Hz, H-1′), 2.88 (ddd, 1 H, $J_{6a,7eq}$ = 1.0, $J_{6a,7ax}$ = 5.0, $J_{6a,6b}$ = –16.5 Hz, H-6a), 2.85 (ddd, 1 H, $J_{6b,7eq}$ = 6.3, $J_{6b,7ax}$ = 13.8 Hz, H-6b), 2.55 (ddddd, 1 H, $J_{2'a,2'b}$ = –15.7 Hz, H-2′a), 2.48 (ddddd, 1 H, $J_{15a,14}$ = 2.8, $J_{15a,15b}$ = –16.5 Hz, H-15a), 2.43 (ddddd, 1 H, H-2′b), 2.42 (ddd, 1 H, $J_{11eq,9}$ = 3.6, $J_{9,8}$ = 11.3, $J_{9,11ax}$ = 11.8 Hz, H-9), 2.36 (dddd, 1 H, $J_{11eq,12eq}$ = 3.1, $J_{11eq,12ax}$ = 3.8, $J_{11eq,11ax}$ = –12.9 Hz, H-11eq), 2.19 (dddd, 1 H, $J_{7eq,8}$ = 2.7, $J_{7eq,7ax}$ = –12.3 Hz, H-7eq), 2.13 (ddddd, 1 H, $J_{15b,14}$ = 5.5 Hz, H-15b), 1.91 (ddd, 1 H, $J_{12ax,12eq}$ = –13.2, $J_{12ax,11ax}$ = 13.8 Hz, H-12ax), 1.73 (ddd, 1 H, $J_{14,8}$ = 10.5 Hz, H-14), 1.63 (ddd, 1 H, $J_{12eq,11ax}$ = 3.8 Hz, H-12eq), 1.51 (dddd, 1 H, $J_{7ax,8}$ = 10.8 Hz, H-7ax), 1.46 (dddd, 1 H, H-8), 1.42 (dddd, 1 H, H-11ax), 1.35 (d, 1 H, H-6″), 0.95 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.0 (4″-O*C*OPh), 165.7 (3″-O*C*OPh), 165.6 (2″-O*C*OPh), 157.6 (C-3), 140.0 (C-16), 138.2 (C-5), 137.5 (C-3′), 133.5–128.4 (arom. *C*), 132.9 (C-10), 126.7 (C-1), 116.2 (C-4′), 115.1 (C-17), 113.5 (C-4), 111.8 (C-2), 100.1 (C-1″), 88.7 (C-1′), 72.1 (C-4″), 71.0 (C-2″), 70.3 (C-3″), 67.3 (C-5″), 55.4 (3-O*C*H_3), 43.7 (C-14), 43.2 (C-9), 42.7 (C-13), 42.4 (C-8), 35.8 (C-2′), 33.2 (C-15), 32.9 (C-12), 30.7 (C-6), 27.7 (C-7), 26.5 (C-11), 18.0 (C-18), 17.8 (C-6″) ppm.

HRMS: *m/z* calcd. for $C_{50}H_{54}O_9Na$ $[M + Na]^+$ 821.3666; found 821.3644; *m/z* calcd. for $C_{50}H_{58}O_9N$ $[M + NH_4]^+$ 816.4112; found 816.4092.

(1′*S*)-13,17-seco-13α-(but-3′-en-1′-(2″,3″,4″,6″-tetra-*O*-benzoyl-β-D-glucopyranosyloxy)-1′-yl)-3-methoxyestra-1,3,5(10),16-tetraene (16). Synthesized from **3** (75 mg, 0.220 mmol) and **9** (228 mg, 0.308 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **16** as a white foam (153 mg, 77 %). R_f = 0.70 (hexane:EtOAc 1:1); $[\alpha]_D$ +28.0° (c 0.2, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.06–7.15 (m, 20 H, arom. *H*), 7.00 (d, 1 H, $J_{1,2}$ = 8.7 Hz, H-1), 6.64 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.50 (d, 1 H, H-4), 6.06 (dddd, 1 H, $J_{3',2'a}$ = 5.2, $J_{3',2'b}$ = 8.4, $J_{3',4'c}$ = 10.1, $J_{3',4't}$ = 17.0 Hz, H-3′), 5.90 (dd, 1 H, $J_{3'',4''}$ = 9.5, $J_{3'',2''}$ = 9.9 Hz, H-3″), 5.86 (dddd, 1 H, $J_{16,15b}$ = 5.4, $J_{16,15a}$ = 7.4, $J_{16,17c}$ = 10.2, $J_{16,17t}$ = 17.1 Hz, H-16), 5.67 (dd, 1 H, $J_{4'',5''}$ = 9.9 Hz, H-4″), 5.60 (dd, 1 H, $J_{2'',1''}$ = 7.8 Hz, H-2″), 5.13 (dddd, 1 H, $J_{17c,15b}$ = –1.5, $J_{17c,17t}$ = –1.6, $J_{17c,15a}$ = –1.9 Hz, H-17c), 5.08 (dddd, 1 H, $J_{17t,15a}$ = –1.7, $J_{17c,15b}$ = –2.2 Hz, H-17t), 4.96 (d, 1 H, H-1″), 4.95 (dddd, 1 H, $J_{4't,2'a}$ = –1.3, $J_{4't,4'c}$ = –2.0, $J_{4't,2'b}$ = –2.2 Hz, H-4′t), 4.84 (dddd, 1 H, $J_{4'c,2'b}$ = –1.3, $J_{4'c,2'a}$ = –1.3 Hz, H-4′c), 4.68 (dd, 1 H, $J_{6''a,5''}$ = 3.1, $J_{6''a,6''b}$ = –12.0 Hz, H-6″a), 4.47 (dd, 1 H, $J_{6''b,5''}$ = 5.2 Hz, H-6″b), 4.08 (ddd, 1 H, H-5″), 3.75 (s, 3 H, 3-OC*H*$_3$), 3.63 (dd, 1 H, $J_{1',2'a}$ = 2.8, $J_{1',2'b}$ = 7.4 Hz, H-1′), 2.59 (ddd, 1 H, $J_{6b,7eq}$ = 6.0, $J_{6b,7ax}$ = 11.9, $J_{6a,6b}$ = –16.5 Hz, H-6b), 2.51 (ddd, 1 H, $J_{6a,7eq}$ = 1.8, $J_{6a,7ax}$ = 5.4, H-6a), 2.41 (ddddd, 1 H, $J_{2'a,2'b}$ = –15.5 Hz, H-2′a), 2.40 (ddddd, 1 H, H-2′b), 2.22 (ddddd, 1 H, $J_{15a,14}$ = 2.3, $J_{15a,15b}$ = –16.6 Hz, H-15a), 2.02 (dddd, 1 H, $J_{11eq,9}$ = 3.3, $J_{11eq,12eq}$ = 3.4, $J_{11eq,12ax}$ = 3.9, $J_{11eq,11ax}$ = –12.8 Hz, H-11eq), 1.87 (ddddd, 1 H, $J_{15b,14}$ = 6.1 Hz, H-15b), 1.75 (dddd, 1 H, $J_{7eq,8}$ = 2.5, $J_{7eq,7ax}$ = –12.9 Hz, H-7eq), 1.60 (ddd, 1 H, $J_{12ax,12eq}$ = –13.3, $J_{12ax,11ax}$ = 13.5 Hz, H-12ax), 1.48 (ddd, 1 H, $J_{12eq,11ax}$ = 3.2 Hz, H-12eq), 1.38 (ddd, 1 H, $J_{9,8}$ = 10.5, $J_{9,11ax}$ = 12.1 Hz, H-9), 1.33 (ddd, 1 H, $J_{14,8}$ = 10.7 Hz, H-14), 1.13 (dddd, 1 H, H-11ax), 1.11 (dddd, 1 H, $J_{8,7ax}$ = 11.8 Hz, H-8), 0.73 (s, 3 H, H-18), 0.44 (dddd, 1 H, H-7ax) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.1 (6″-O*C*OPh), 165.9 (3″-O*C*OPh), 165.2 (4″-O*C*OPh), 165.0 (2″-O*C*OPh), 157.1 (C-3), 140.4 (C-16), 137.7 (C-3′), 137.5 (C-5), 133.4–128.3 (arom. *C*), 132.4 (C-10), 126.6 (C-1), 115.0 (C-17, C-4′), 113.0 (C-4), 111.3 (C-2), 101.7 (C-1″), 84.9 (C-1′), 72.9 (C-3″), 72.3 (C-2″), 71.9 (C-5″), 69.9 (C-4″), 63.1 (C-6″), 55.1 (3-O*C*H$_3$), 42.6 (C-14), 42.3 (C-13), 42.1 (C-8, C-9), 35.9 (C-2′), 33.3 (C-15), 31.5 (C-12), 30.2 (C-6), 26.7 (C-7), 26.1 (C-11), 17.3 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{57}H_{58}O_{11}Na$ $[M + Na]^+$ 941.3871; found 941.3866; *m/z* calcd. for $C_{57}H_{58}O_{11}K$ $[M + K]^+$ 957.3611; found 957.3595.

(1′*R*)-13,17-seco-13α-(but-3′-en-1′-(2″,3″,4″,6″-tetra-*O*-benzoyl-β-D-galactopyranosyloxy)-1′-yl)-3-methoxyestra-1,3,5(10),16-tetraene (18). Synthesized from **2** (81 mg, 0.237 mmol) and **10** (246 mg, 0.330 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **18** as a white foam (173 mg, 79 %). R_f = 0.18 (hexane:EtOAc 4:1); $[\alpha]_D$ +69.3° (c 0.4, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.09–7.23 (m, 20 H, arom. *H*), 7.20 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.72 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.61 (d, 1 H, H-4), 5.95 (dd, 1 H, $J_{4'',5''}$ = 1.1, $J_{4'',3''}$ = 3.6 Hz, H-4″), 5.88 (dddd, 1 H, $J_{3',2'a}$ = 6.3, $J_{3',2'b}$ = 8.0, $J_{3',4'c}$ = 10.0, $J_{3',4't}$ = 17.4 Hz, H-3′), 5.80 (dddd, 1 H, $J_{16,15b}$ = 6.1, $J_{16,15a}$ = 7.3, $J_{16,17c}$ = 10.0, $J_{16,17t}$ = 16.8 Hz, H-16), 5.76 (dd, 1 H, $J_{2'',1''}$ = 7.8, $J_{2'',3''}$ = 10.4 Hz, H-2″), 5.60 (dd, 1 H, H-3″), 5.04 (d, 1 H, H-1″),4.95 (dddd, 1 H, $J_{4'c,2'b}$ = –0.6, $J_{4'c,2'a}$ = –0.8, $J_{4'c,4't}$ = –1.0 Hz, H-4′c), 4.94 (dddd, 1 H, $J_{4't,2'b}$ = –1.1, $J_{4't,2'a}$ = –1.8 Hz, H-4′t), 4.94 (dddd, 1 H, $J_{17t,15a}$ = –0.8, $J_{17t,17c}$ = –1.6, $J_{17t,15b}$ = –2.3 Hz, H-17t), 4.91 (dddd, 1 H, $J_{17c,15a}$ = –0.7, $J_{17c,15b}$ = –1.0 Hz, H-17c), 4.64 (dd, 1 H, $J_{6''a,5''}$ = 7.0, $J_{6''a,6''b}$ = –11.4 Hz, H-6″a), 4.43 (dd, 1 H, $J_{5'',6''b}$ = 6.0 Hz, H-6″b), 4.26 (ddd, 1 H, H-5″), 3.91 (dd, 1 H, $J_{1',2'a}$ = 2.3, $J_{1',2'b}$ = 8.7 Hz, H-1′), 3.78 (s, 3 H, 3-OC*H*$_3$), 2.82 (ddd, 1 H, $J_{6a,7eq}$ = 2.1, $J_{6a,7ax}$ = 7.6, $J_{6a,6b}$ = –12.1 Hz, H-6a), 2.82 (ddd, 1 H, $J_{6b,7eq}$ = 5.6, $J_{6b,7ax}$ = 9.5 Hz, H-6b), 2.41 (ddddd, 1 H, $J_{2'a,2'b}$ = –14.5 Hz, H-2′a), 2.21 (dddd, 1 H, $J_{11eq,12eq}$ = 2.4, $J_{11eq,12ax}$ = 4.0, $J_{11eq,9}$ = 5.8 , $J_{11eq,11ax}$ = –13.4 Hz, H-11eq), 2.20 (ddddd, 1 H, $J_{15a,14}$ = 3.1, $J_{15a,15b}$ = –15.8 Hz, H-15a), 2.17 (ddd, 1 H, $J_{9,11ax}$ = 11.4, $J_{9,8}$ = 11.5 Hz, H-9), 2.14 (ddddd, 1 H, H-2′b), 2.12 (dddd, 1 H, $J_{7eq,8}$ = 2.2, $J_{7eq,7ax}$ = –12.2 Hz, H-7eq), 1.91 (ddddd, 1 H, $J_{15b,14}$ = 5.2 Hz, H-15b), 1.79 (ddd, 1 H, $J_{12eq,11ax}$ = 3.8, $J_{12eq,12ax}$ = –13.2 Hz, H-12eq), 1.43 (dddd, 1 H, $J_{8,14}$ = 9.8, $J_{8,7ax}$ = 11.5 Hz, H-8), 1.37 (dddd, 1 H, $J_{11ax,12ax}$ = 13.0 Hz, H-11ax), 1.36 (ddd, 1 H, H-12ax), 1.29 (ddd, 1 H, H-14), 1.26 (dddd, 1 H, H-7ax), 1.06 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.1 (6″-O*C*OPh), 165.7 (3″-O*C*OPh), 165.6 (4″-O*C*OPh), 165.1 (2″-O*C*OPh), 157.4 (C-3), 139.9 (C-16), 137.9 (C-5), 136.6 (C-3′), 133.5–128.3 (arom. *C*), 132.8 (C-10), 126.5 (C-1), 116.7 (C-4′), 114.6 (C-17), 113.4 (C-4), 111.6 (C-2), 100.3 (C-1″), 83.4 (C-1′), 71.8 (C-3″), 71.1 (C-5″), 70.3 (C-2″), 68.4 (C-4″), 62.3 (C-6″), 55.2 (3-O*C*H$_3$), 45.5 (C-14), 43.6 (C-9), 42.2 (C-8), 41.6 (C-13), 34.5 (C-2′), 32.8 (C-15), 31.0 (C-12), 30.5 (C-6), 27.7 (C-7), 25.9 (C-11), 17.6 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{57}H_{58}O_{11}Na$ $[M + Na]^+$ 941.3877; found 941.3872.

13,17-seco-13α-methyl-1′-(α-D-mannopyranosyloxy)-3-methoxyestra-1,3,5(10),16-tetraene (19). Synthesized from **11** (128 mg, 0.145 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **19** as a white solid (60 mg, 90 %).R_f = 0.48 (MeOH:CH_2Cl_2 1:5); [α]$_D$ +115.6° (c 0.1, MeOH). ^{1}H NMR (600.13 MHz, CD_3OD): δ 7.17 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.66 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.58 (d, 1 H, H-4), 5.90 (dddd, 1 H, $J_{16,15a}$ = 6.1, $J_{16,15b}$ = 7.1, $J_{16,17c}$ = 10.2, $J_{16,17t}$ = 17.1 Hz, H-16), 5.04 (dddd, 1 H, $J_{17t,17c}$ = –1.5, $J_{17t,15b}$ = –1.7, $J_{17t,15a}$ = –2.1 Hz, H-17t), 4.94 (dddd, 1 H, $J_{17c,15b}$ = –1.6, $J_{17c,15a}$ = –1.7 Hz, H-17c), 4.72 (d, 1 H, $J_{1'',2''}$ = 1.7 Hz, H-1″), 3.84 (dd, 1 H, $J_{2'',3''}$ = 3.3 Hz, H-2″), 3.82 (dd, 1 H, $J_{6''a,5''}$ = 2.4, $J_{6''a,6''b}$ = –11.6 Hz, H-6″a), 3.73 (s, 3 H, 3-OCH_3), 3.72 (dd, 1 H, $J_{6''b,5''}$ = 5.5 Hz, H-6″b), 3.71 (dd, 1 H, $J_{3'',4''}$ = 9.4 Hz, H-3″), 3.71 and 3.11 (each d, each 1 H, *J* = –9.5 Hz, H-1′), 3.63 (dd, 1 H, $J_{4'',5''}$ = 9.9 Hz, H-4″), 3.57 (ddd, 1 H, H-5″), 2.80 (ddd, 1 H, $J_{6a,7eq}$ = 2.3, $J_{6a,7ax}$ = 6.3, $J_{6a,6b}$ = –12.2 Hz, H-6a), 2.79 (ddd, 1 H, $J_{6b,7eq}$ = 5.9, $J_{6b,7ax}$ = 11.5 Hz, H-6b), 2.39 (ddddd, 1 H, $J_{15a,14}$ = 3.2, $J_{15a,15b}$ = –16.2 Hz, H-15a), 2.28 (dddd, 1 H, $J_{11eq,12eq}$ = 3.0, $J_{11eq,9}$ = 3.6, $J_{11eq,12ax}$ = 3.9, $J_{11eq,11ax}$ = –13.1 Hz, H-11eq), 2.24 (ddd, 1 H, $J_{9,8}$ = 10.6, $J_{9,11ax}$ = 12.1 Hz, H-9), 2.19 (dddd, 1 H, $J_{7eq,8}$ = 2.5, $J_{7eq,7ax}$ = –13.1 Hz, H-7eq), 2.08 (ddddd, 1 H, $J_{15b,14}$ = 5.4 Hz, H-15b), 1.73 (ddd, 1 H, $J_{12ax,12eq}$ = –13.2, $J_{12ax,11ax}$ = 13.9 Hz, H-12ax), 1.62 (ddd, 1 H, $J_{12eq,11ax}$ = 3.4 Hz, H-12eq), 1.47 (ddd, 1 H, $J_{14,8}$ = 11.2 Hz, H-14), 1.38 (dddd, 1 H, $J_{8,7ax}$ = 11.6 Hz, H-8), 1.37 (dddd, 1H, H-11ax), 1.25 (dddd, 1 H, H-7ax), 0.88 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CD_3OD): δ 158.9 (C-3), 141.4 (C-16), 138.9 (C-5), 133.8 (C-10), 127.4 (C-1), 114.9 (C-17), 114.2 (C-4), 112.6 (C-2), 102.5 (C-1″), 78.0 (C-1′), 74.7 (C-5″), 72.8 (C-3″), 72.2 (C-2″), 68.5 (C-4″), 62.9 (C-6″), 55.5 (3-OCH_3), 46.4 (C-14), 45.0 (C-9), 43.0 (C-8), 39.3 (C-13), 37.9 (C-12), 33.9 (C-15), 31.5 (C-6), 28.9 (C-7), 27.7 (C-11), 17.0 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{26}H_{38}O_7Na$ [M + Na]$^+$ 485.2503; found 485.2503; *m/z* calcd. for $C_{26}H_{42}O_7N$ [M + NH_4]$^+$ 480.2961; found 480.2952.

(1′*S*)-13,17-seco-13α-(but-3′-en-1′-(α-D-mannopyranosyloxy)-1′-yl)-3-methoxyestra-1,3,5(10),16-tetraene (20). Synthesized from **12** (59 mg, 0.064 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **20** as a white solid (33 mg, quant.). R_f = 0.52 (MeOH:CH_2Cl_2 1:5). [α]$_D$ +71.5° (c 0.2, MeOH).^{1}H NMR (600.13 MHz,

CD_3OD): δ 7.16 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.65 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.58 (d, 1 H, H-4), 6.02 (dddd, 1 H, $J_{16,15a}$ = 6.3, $J_{16,15b}$ = 7.3, $J_{16,17c}$ = 10.2, $J_{16,17t}$ = 17.1 Hz, H-16), 5.95 (dddd, 1 H, $J_{3',2'a}$ = 5.7, $J_{3',2'b}$ = 8.3, $J_{3',4'c}$ = 10.2, $J_{3',4't}$ = 17.1 Hz, H-3′), 5.14 (dddd, 1 H, $J_{4't,2'a}$ = –1.3, $J_{4't,2'b}$ = –1.5, $J_{4't,4'c}$ = –1.6 Hz, H-4′t), 5.08 (dddd, 1 H, $J_{4'c,2'b}$ = –0.2, $J_{4'c,2'a}$ = –1.9, H-4′c), 5.05 (dddd, 1 H, $J_{17t,17c}$ = –1.4, $J_{17t,15b}$ = –1.6, $J_{17t,15a}$ = –2.1 Hz, H-17t), 4.95 (dddd, 1 H, $J_{17c,15b}$ = –1.4, $J_{17c,15a}$ = –1.9 Hz, H-17c), 4.89 (d, 1 H, $J_{1'',2''}$ = 1.9 Hz, H-1″), 3.93 (dd, 1 H, $J_{2'',3''}$ = 3.3 Hz, H-2″), 3.78 (dd, 1 H, $J_{6''b,5''}$ = 4.2, $J_{6''b,6''a}$ = –14.0 Hz, H-6″b), 3.77 (dd, 1 H, $J_{6''a,5''}$ = 2.2, H-6″a), 3.74 (dd, 1 H, $J_{4'',5''}$ = 9.7, $J_{4'',3''}$ = 9.9 Hz, H-4″), 3.72 (s, 3 H, 3-OCH_3), 3.72 (dd, 1 H, $J_{1',2'a}$ = 2.0, $J_{1',2'b}$ = 7.9 Hz, H-1′), 3.71 (dd, 1 H, H-3″), 3.71 (ddd, 1 H, H-5″), 2.78 (ddd, 1 H, $J_{6a,7eq}$ = 1.9, $J_{6a,7ax}$ = 6.2, $J_{6a,6b}$ = –12.3 Hz, H-6a), 2.78 (ddd, 1 H, $J_{6b,7eq}$ = 6.2, $J_{6b,7ax}$ = 11.5 Hz, H-6b), 2.57 (ddddd, 1 H, $J_{15a,14}$ = 3.7, $J_{15a,15b}$ = –16.0 Hz, H-15a), 2.55 (ddddd, 1 H, $J_{2'a,2'b}$ = –14.8 Hz, H-2′a), 2.27 (ddddd, 1 H, H-2′b), 2.27 (dddd, 1 H, $J_{11eq,12eq}$ = 3.1, $J_{11eq,9}$ = 3.7, $J_{11eq,12ax}$ = 3.9, $J_{11eq,11ax}$ = –13.2 Hz, H-11eq), 2.24 (dddd, 1 H, $J_{7eq,8}$ = 2.4, $J_{7eq,7ax}$ = –12.7 Hz, H-7eq), 2.20 (ddd, 1 H, $J_{9,8}$ = 10.7, $J_{9,11ax}$ = 12.1 Hz, H-9), 2.18 (ddddd, 1 H, $J_{15b,14}$ = 4.8 Hz, H-15b), 1.77 (ddd, 1 H, $J_{12ax,12eq}$ = –13.2, $J_{12ax,11ax}$ = 13.8 Hz, H-12ax), 1.72 (ddd, 1 H, $J_{14,8}$ = 10.9 Hz, H-14), 1.51 (ddd, 1 H, $J_{12eq,11ax}$ = 3.1 Hz, H-12eq), 1.40 (dddd, 1 H, $J_{8,7ax}$ = 11.4 Hz, H-8), 1.32 (dddd, 1 H, H-11ax), 1.25 (dddd, 1 H, H-7ax), 0.93 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CD_3OD): δ 158.9 (C-3), 141.3 (C-16), 139.0 (C-5), 138.7 (C-3′), 133.7 (C-10), 127.4 (C-1), 117.3 (C-4′), 115.1 (C-17), 114.2 (C-4), 112.6 (C-2), 102.8 (C-1″), 87.5 (C-1′), 75.2 (C-5″), 72.7 (C-2″, C-3″), 68.1 (C-4″), 62.5 (C-6′), 55.5 (3-OCH_3), 44.7 (C-9), 44.5 (C-14), 43.2 (C-13), 43.1 (C-8), 37.0 (C-15), 34.8 (C-12), 34.4 (C-2′), 31.5 (C-6), 28.9 (C-7), 27.6 (C-11), 19.2 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{29}H_{42}O_7Na$ $[M + Na]^+$ 525.2828; found 525.2813; *m/z* calcd. for $C_{29}H_{46}O_7N$ $[M + NH_4]^+$ 520.3274; found 520.3272.

13,17-seco-13α-methyl-1′-(α-L-rhamnopyranosyloxy)-3-methoxyestra-1,3,5(10),16-tetraene (21). Synthesized from **13** (164 mg, 0.216 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **21** as a white solid (85 mg, 88 %). R_f = 0.21 (EtOAc); $[\alpha]_D$ +13.8° (c 0.10, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.18 (d, 1 H, $J_{1,2}$ = 8.7 Hz, H-1), 6.70 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.60 (d, 1 H, H-4), 5.82 (dddd, 1 H, $J_{16,15b}$ =

6.5, $J_{16,15a}$ = 6.7, $J_{16,17c}$ = 10.2, $J_{16,17t}$ = 17.1 Hz, H-16), 4.99 (dddd, 1 H, $J_{17t,17c}$ = –1.5, $J_{17t,15b}$ = –1.5, $J_{17t,15a}$ = –2.0 Hz, H-17t), 4.93 (dddd, 1 H, $J_{17c,15b}$ = –1.2, $J_{17c,15a}$ = –1.9 Hz, H-17c), 4.70 (d, 1 H, $J_{1'',2''}$ = 1.6 Hz, H-1″), 3.94 (dd, 1 H, $J_{2'',3''}$ = 3.3 Hz, H-2″), 3.79 (dd, 1 H, $J_{3'',4''}$ = 9.3 Hz, H-3″), 3.76 (s, 3 H, 3-OC*H*$_3$), 3.66 (dq, 1 H, $J_{5'',6''}$ = 6.2, $J_{5'',4''}$ = 9.4 Hz, H-5″), 3.51 (dd, 1 H, H-4″), 3.37 and 3.24 (each d, each 1 H, *J* = –9.5 Hz, H-1′), 2.82 (ddd, 1 H, $J_{6a,7eq}$ = 2.3, $J_{6a,7ax}$ = 5.2, $J_{6a,6b}$ = –17.1 Hz, H-6a), 2.80 (ddd, 1 H, $J_{6b,7eq}$ = 6.4, $J_{6b,7ax}$ = 12.2 Hz, H-6b), 2.25 (ddd, 1 H, $J_{9,11eq}$ = 4.2, $J_{9,11ax}$ = 10.9, $J_{9,8}$ = 12.0 Hz, H-9), 2.23 (dddd, 1 H, $J_{11eq,12eq}$ = 2.7, $J_{11eq,12ax}$ = 3.9, $J_{11eq,11ax}$ = –11.8 Hz, H-11eq), 2.20 (ddddd, 1 H, $J_{15a,14}$ = 5.1, $J_{15a,15b}$= –15.8 Hz, H-15a), 2.12 (dddd, 1 H, $J_{7eq,8}$ = 2.4, $J_{7eq,7ax}$ = –12.8 Hz, H-7eq), 2.00 (ddddd, 1 H, $J_{15b,14}$ = 3.4 Hz, H-15a), 1.73 (ddd, 1 H, $J_{12ax,12eq}$ = –12.6, $J_{12ax,11ax}$ = 14.1 Hz, H-12ax), 1.49 (ddd, 1 H, $J_{12eq,11ax}$ = 3.4 Hz, H-12eq), 1.46 (ddd, 1 H, $J_{14,8}$ = 10.7 Hz, H-14), 1.39 (dddd, 1 H, H-11ax), 1.35 (dddd, 1 H, $J_{8,7ax}$ = 11.1 Hz, H-8), 1.34 (d, 3 H, H-6″), 1.29 (dddd, 1 H, H-7ax), 0.77 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 157.4 (C-3), 140.2 (C-16), 137.9 (C-5), 132.8 (C-10), 126.4 (C-1), 114.1 (C-17), 113.4 (C-4), 111.6 (C-2), 99.9 (C-1″), 75.8 (C-1′), 73.0 (C-4″), 72.1 (C-3″), 71.1 (C-2″), 68.0 (C-5″), 55.2 (3-O*C*H$_3$), 44.2 (C-14), 43.4 (C-9), 41.3 (C-8), 38.1 (C-13), 36.5 (C-12), 32.7 (C-15), 30.5 (C-6), 27.6 (C-7), 26.2 (C-11), 17.6 (C-6″), 16.5 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{26}H_{38}O_6Na$ [M + Na]$^+$ 496.2566; found 496.2546.

(1′*S*)-13,17-seco-13α-(but-3′-en-1′-(α-L-rhamnopyranosyloxy)-1′-yl)-3-methoxyestra-1,3,5(10),16-tetraene (22). Synthesized from **14** (59 mg, 0.064 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **22** as a white solid (33 mg, quant.). R_f = 0.4 (EtOAc). $[\alpha]_D$ +20.5° (c 0.07, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CHCl_3$): δ 7.18 (d, 1 H, $J_{1,2}$ = 8.7 Hz, H-1), 6.70 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.60 (d, 1 H, H-4), 6.00 (dddd, 1 H, $J_{3',2'a}$ = 6.7, $J_{3',2'b}$ = 6.8, $J_{3',4'c}$ = 10.2, $J_{3',4't}$ = 17.2 Hz, H-3′), 5.83 (dddd, 1 H, $J_{16,15b}$ = 5.8, $J_{16,15a}$ = 7.0, $J_{16,17c}$ = 10.2, $J_{16,17t}$ = 17.0 Hz, H-16), 5.05 (dddd, 1 H, $J_{4't,4'c}$ = –1.6, $J_{4't,2'a}$ = –1.7, $J_{4't,2'b}$ = –1.7 Hz, H-4′t), 5.02 (dddd, 1 H, $J_{17t,17c}$ = –1.4, $J_{17t,15a}$ = –1.9, $J_{17t,15b}$ = –2.0 Hz, H-17t), 5.00 (dddd, 1 H, $J_{4'c,2'b}$ = –1.1, $J_{4'c,2'a}$ = –1.9, H-4′c), 4.98 (dddd, 1 H, $J_{17c,15b}$ = –1.7, $J_{17c,15a}$ = –1.7 Hz, H-17c), 4.76 (d, 1 H, $J_{1'',2''}$ = 1.7 Hz, H-1″), 3.98 (dd, 1 H, $J_{2'',3''}$ = 3.3 Hz, H-2″), 3.79 (dq, 1 H, $J_{5'',6''}$ = 6.2, $J_{5'',4''}$ = 9.4 Hz, H-5″), 3.76 (s, 3 H, 3-OC*H*$_3$), 3.74 (dd, 1 H, $J_{3'',4''}$ = 9.3 Hz, H-3″), 3.50 (dd, 1 H, $J_{1',2'a}$ = 3.4, $J_{1',2'b}$ = 6.7 Hz, H-1′), 3.47 (dd, 1 H, H-4″), 2.81 (ddd, 1 H, $J_{6a,7eq}$ = 1.5,

$J_{6a,7ax}$ = 5.4, $J_{6a,6b}$ = –13.9 Hz, H-6a), 2.80 (ddd, 1 H, $J_{6b,7eq}$ = 6.5, $J_{6b,7ax}$ = 12.3 Hz, H-6b), 2.45 (ddddd, 1 H, $J_{2'a,2'b}$ = –15.4 Hz, H-2′a), 2.35 (ddddd, 1 H, $J_{15a,14}$ = 2.7, $J_{15a,15b}$ = –16.4 Hz, H-15a), 2.28 (ddddd, 1 H, H-2′b), 2.23 (dddd, 1 H, $J_{11eq,12eq}$ = 2.9, $J_{11eq,9}$ = 3.7, $J_{11eq,12ax}$ = 3.9, $J_{11eq,11ax}$ = –13.1 Hz, H-11eq), 2.21 (ddd, 1 H, $J_{9,8}$ = 10.2, $J_{9,11ax}$ = 12.1 Hz, H-9), 2.13 (dddd, 1 H, $J_{7eq,8}$ = 2.7, $J_{7eq,7ax}$ = –12.8 Hz, H-7eq), 1.99 (ddddd, 1 H, $J_{15b,14}$ = 6.2 Hz, H-15b), 1.66 (ddd, 1 H, $J_{12ax,12eq}$ = –12.8, $J_{12ax,11ax}$ = 14.1 Hz, H-12ax), 1.50 (ddd, 1 H, $J_{12eq,11ax}$ = 3.3 Hz, H-12eq), 1.50 (ddd, 1 H, $J_{14,8}$ = 11.0 Hz, H-14), 1.37 (dddd, 1 H, $J_{8,7ax}$ = 12.3 Hz, H-8), 1.33 (dddd, 1 H, H-11ax), 1.29 (dddd, 1 H, H-7ax), 1.27 (d, 3 H, H-6″), 0.82 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CHCl$_3$): δ 157.4 (C-3), 139.9 (C-16), 137.9 (C-5), 137.5 (C-3′), 132.5 (C-10), 126.6 (C-1), 115.7 (C-4′), 114.8 (C-17), 113.3 (C-4), 111.7 (C-2), 102.2 (C-1″), 87.9 (C-1′), 73.3 (C-4″), 72.0 (C-3″), 71.1 (C-2″), 68.2 (C-5″), 55.2 (3-O*C*H$_3$), 43.7 (C-14), 43.1 (C-9), 42.5 (C-13), 42.3 (C-8), 35.7 (C-2′), 32.9 (C-15), 32.6 (C-12), 30.6 (C-6), 27.7 (C-7), 26.4 (C-11), 17.4 (C-6″), 17.3 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{29}H_{42}O_6Na$ [M + Na]$^+$ 509.2879; found 509.2855.

13,17-seco-13α-methyl-1′-(β-D-glucopyranosyloxy)-3-methoxyestra-1,3,5(10),16-tetraene (23). Synthesized from **1** (44 mg, 0.146 mmol) and **9** (152 mg, 0.205 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing an impure mixture (126 mg, 97 %). The deprotection step was performed according to the general procedure for deprotection of glycosteroids and anthracyclines starting with the mixture (85 mg, 0.096 mmol) and providing **23** as a white solid (39 mg, 88 %). R_f = 0.62 (MeOH:CH$_2$Cl$_2$ 1:5); $[\alpha]_D$ +29.4° (c 2.5, MeOH). ^{1}H NMR (600.13 MHz, CD$_3$OD): δ 7.16 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.65 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.57 (d, 1 H, H-4), 5.97 (dddd, 1 H, $J_{16,15a}$ = 6.3, $J_{16,15b}$ = 7.2, $J_{16,17c}$ = 10.3, $J_{16,17t}$ = 17.1 Hz, H-16), 5.04 (dddd, 1 H, $J_{17t,17c}$ = –1.5, $J_{17t,15b}$ = –2.0, $J_{17t,15a}$ = –2.2 Hz, H-17t), 4.92 (dddd, 1 H, $J_{17c,15b}$ = –1.6, $J_{17c,15a}$ = –1.8 Hz, H-17c), 4.24 (d, 1 H, $J_{1'',2''}$ = 7.8 Hz, H-1″), 3.88 (dd, 1 H, $J_{6''a,5''}$ = 2.2, $J_{6''a,6''b}$ = –12.0 Hz, H-6″a), 3.72 (s, 3 H, 3-OC*H*$_3$), 3.70 and 3.40 (each d, each 1 H, *J* = –9.6 Hz, H-1′), 3.69 (dd, 1 H, $J_{6''b,5''}$ = 5.6 Hz, H-6″b), 3.36 (dd, 1 H, $J_{3'',4''}$ = 9.0, $J_{3'',2''}$ = 9.2 Hz, H-3″), 3.30 (dd, 1 H, $J_{4'',5''}$ = 9.6 Hz, H-4″), 3.26 (ddd, 1 H, H-5″), 3.22 (dd, 1 H, H-2″), 2.78 (ddd, 1 H, $J_{6a,7eq}$ = 1.9, $J_{6a,7ax}$ = 6.1, $J_{6a,6b}$ = –11.9 Hz, H-6a), 2.78 (ddd, 1 H, $J_{6b,7eq}$ = 6.6, $J_{6b,7ax}$ = 11.5 Hz, H-6b), 2.35 (ddddd, 1 H, $J_{15a,14}$ = 3.3, $J_{15a,15b}$ = –16.0 Hz, H-15a), 2.26

(dddd, 1 H, $J_{11eq,12eq}$ = 3.8, $J_{11eq,9}$ = 3.8, $J_{11eq,12ax}$ = 3.9, $J_{11eq,11ax}$ = –13.3 Hz, H-11eq), 2.25 (ddd, 1 H, $J_{9,8}$ = 10.7, $J_{9,11ax}$ = 12.0 Hz, H-9), 2.19 (dddd, 1 H, $J_{7eq,8}$ = 2.2, $J_{7eq,7ax}$ = –13.0 Hz, H-7eq), 2.05 (ddddd, 1 H, $J_{15b,14}$ = 5.6 Hz, H-15b), 1.85 (ddd, 1 H, $J_{12ax,11ax}$ = 13.3, $J_{12ax,12eq}$ = –13.6 Hz, H-12ax), 1.63 (ddd, 1 H, $J_{14,8}$ = 11.5 Hz, H-14), 1.60 (ddd, 1 H, $J_{12eq,11ax}$ = 3.4 Hz, H-12eq), 1.36 (dddd, 1 H, $J_{8,7ax}$ = 11.2 Hz, H-8), 1.35 (dddd, 1H, H-11ax), 1.25 (dddd, 1 H, H-7ax), 0.84 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CD_3OD): δ 158.9 (C-3), 142.0 (C-16), 139.0 (C-5), 134.0 (C-10), 127.4 (C-1), 114.6 (C-17), 114.3 (C-4), 112.6 (C-2), 104.9 (C-1″), 78.9 (C-1′), 78.2 (C-3″), 77.9 (C-5″), 75.3 (C-2″), 71.7 (C-4″), 62.8 (C-6″), 55.5 (3-O*C*H_3), 45.4 (C-14), 44.9 (C-9), 43.2 (C-8), 39.5 (C-13), 37.6 (C-12), 34.2 (C-15), 31.5 (C-6), 28.9 (C-7), 27.7 (C-11), 17.0 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{26}H_{38}O_7Na$ $[M + Na]^+$ 485.2515; found 485.2500.

(1′*S*)-13,17-seco-13α-(but-3′-en-1′-(β-D-glucopyranosyloxy)-1′-yl)-3-methoxyestra-1,3,5(10),16-tetraene (24). Synthesized from **15** (60 mg, 0.065 mmol) according to the general procedure for deprotection glycosteroids and anthracyclines providing **24** as a white solid (25 mg, 77 %). R_f = 0.43 (MeOH:CH_2Cl_2 1:5). $[\alpha]_D$ +24.0° (c 1.67, MeOH).^{1}H NMR (600.13 MHz, CD_3OD): δ 7.17 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.65 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.57 (d, 1 H, H-4), 6.18 (dddd, 1 H, $J_{3',2'b}$ = 6.2, $J_{3',2'a}$ = 7.6, $J_{3',4'c}$ = 10.1, $J_{3',4't}$ = 17.2 Hz, H-3′), 6.05 (dddd, 1 H, $J_{16,15b}$ = 6.1, $J_{16,15a}$ = 6.5, $J_{16,17c}$ = 10.2, $J_{16,17t}$ = 17.2 Hz, H-16), 5.09 (dddd, 1 H, $J_{17t,17c}$ = –1.6, $J_{17t,15b}$ = –1.8, $J_{17t,15a}$ = –2.1 Hz, H-17t), 5.00 (dddd, 1 H, $J_{4't,2'b}$ = –1.4, $J_{4't,2'a}$ = –1.5, $J_{4't,4'c}$ = –2.3 Hz, H-4′t), 4.98 (dddd, 1 H, $J_{17c,15b}$ = –1.4, $J_{17c,15a}$ = –2.1 Hz, H-17c), 4.93 (dddd, 1 H, $J_{4'c,2'b}$ = –0.9, $J_{4'c,2'a}$ = –1.2, H-4′c), 4.41 (d, 1 H, $J_{1'',2''}$ = 8.0 Hz, H-1″), 3.85 (dd, 1 H, $J_{6''a,5''}$ = 2.5, $J_{6''a,6''b}$ = –11.7 Hz, H-6″a), 3.72 (s, 3 H, 3-OC*H*$_3$), 3.69 (dd, 1 H, $J_{1',2'a}$ = 3.7, $J_{1',2'b}$ = 6.5 Hz, H-1′), 3.68 (dd, 1 H, $J_{6'b,5''}$ = 5.6, H-6″b), 3.33 (dd, 1 H, $J_{3'',4''}$ = 9.3, $J_{3'',2''}$ = 9.5 Hz, H-3″), 3.30 (dd, 1 H, $J_{4'',5''}$ = 9.1, Hz, H-4″), 3.22 (dd, 1 H, H-2″), 3.20 (ddd, 1 H, H-5″), 2.78 (ddd, 1 H, $J_{6a,7eq}$ = 1.8, $J_{6a,7ax}$ = 5.9, $J_{6a,6b}$ = –13.2 Hz, H-6a), 2.77 (ddd, 1 H, $J_{6b,7eq}$ = 6.3, $J_{6b,7ax}$ = 11.5 Hz, H-6b), 2.47 (ddddd, 1 H, $J_{15a,14}$ = 2.1, $J_{15a,15b}$ = –16.7 Hz, H-15a), 2.46 (ddddd, 1 H, $J_{20a,20b}$ = –14.6 Hz, H-2′a), 2.40 (ddddd, 1 H, H-2′b), 2.29 (ddd, 1 H, $J_{9,11eq}$ = 3.5, $J_{9,8}$ = 11.2, $J_{9,11ax}$ = 11.5 Hz, H-9), 2.27 (dddd, 1 H, $J_{11eq,12eq}$ = 3.5, $J_{11eq,12ax}$ = 3.8, $J_{11eq,11ax}$ = –12.7 Hz, H-11eq), 2.18 (dddd, 1 H, $J_{7eq,8}$ = 2.5, $J_{7eq,7ax}$ = –12.6 Hz, H-7eq), 2.05 (ddddd, 1 H, $J_{15b,14}$ = 6.2 Hz, H-15b), 2.02 (ddd, 1 H, $J_{14,8}$ =

10.6 Hz, H-14), 1.96 (ddd, 1 H, $J_{12ax,12eq}$ = –13.3, $J_{12ax,11ax}$ = 13.4 Hz, H-12ax), 1.55 (ddd, 1 H, $J_{12eq,11ax}$ = 3.3 Hz, H-12eq), 1.36 (dddd, 1 H, $J_{8,7ax}$ = 11.1 Hz, H-8), 1.30 (dddd, 1 H, H-7ax), 1.29 (dddd, 1 H, H-11ax), 0.87 (s, 3 H, H-18) ppm.
^{13}C NMR (150.9 MHz, CD_3OD): δ 158.9 (C-3), 142.0 (C-16), 140.2 (C-3′), 139.0 (C-5), 134.0 (C-10), 127.6 (C-1), 115.2 (C-17), 114.8 (C-4′), 114.2 (C-4), 112.6 (C-2), 105.2 (C-1″), 86.0 (C-1′), 78.3 (C-3″), 77.6 (C-5″), 75.8 (C-2″), 71.9 (C-4″), 63.0 (C-6″), 55.5 (3-O*C*H_3), 44.2 (C-9, C-8), 43.6 (C-13), 43.3 (C-14), 42.9 (C-13), 37.3 (C-2′), 34.5 (C-15), 33.2 (C-12), 31.7 (C-6), 29.1 (C-7), 27.9 (C-11), 18.2 (C-18) ppm.
HRMS: *m/z* calcd. for $C_{29}H_{42}O_7Na$ $[M + Na]^+$ 525.2828; found 525.2821.

13,17-seco-13α-methyl-1′-(β-D-galactopyranosyloxy)-3-methoxyestra-1,3,5(10),16-tetraene (25). Synthesized from **1** (19 mg, 0.063 mmol) and **10** (65 mg, 0.088 mmol) according to the general procedure for glycosylation of steroids and tetracyclines. A pure product was, however, not obtained and thus the deprotection step was carried out according to the general procedure for deprotection of glycosteroids and anthracyclines providing **25** as a white solid (30 mg, 85 % over two steps). R_f = 0.58 (MeOH:CH_2Cl_2 1:5); $[\alpha]_D$ +38.0° (c 0.87, MeOH). ^{1}H NMR (600.13 MHz, CD_3OD): δ 7.17 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.66 (dd, 1 H, $J_{2,4}$ = 2.6 Hz, H-2), 6.58 (d, 1 H, H-4), 5.98 (dddd, 1 H, $J_{16,15a}$ = 6.0, $J_{16,15b}$ = 7.4, $J_{16,17c}$ = 10.2, $J_{16,17t}$ = 17.1 Hz, H-16), 5.03 (dddd, 1 H, $J_{17t,17c}$ = –1.2, $J_{17t,15b}$ = –1.5, $J_{17t,15a}$ = –2.3 Hz, H-17t), 4.91 (dddd, 1 H, $J_{17c,15b}$ = –1.5, $J_{17c,15a}$ = –1.9 Hz, H-17c), 4.19 (d, 1 H, $J_{1'',2''}$ = 7.7 Hz, H-1″), 3.63 (dd, 1 H, $J_{4'',5''}$ = 1.3, $J_{4'',3''}$ = 3.5 Hz, H-4″), 3.79 (dd, 1 H, $J_{6''a,5''}$ = 6.0, $J_{6''a,6''b}$ = –11.6 Hz, H-6″a), 3.73 (dd, 1 H, $J_{6''b,5''}$ = 4.8 Hz, H-6″b), 3.73 (s, 3 H, 3-OC*H*$_3$), 3.69 and 3.42 (each d, each 1 H, *J* = –9.5 Hz, H-1′), 3.55 (dd, 1 H, $J_{2'',3''}$ = 9.8 Hz, H-2″), 3.49 (ddd, 1 H, H-5″), 3.46 (dd, 1 H, H-3″), 2.79 (ddd, 1 H, $J_{6a,7eq}$ = 1.8, $J_{6a,7ax}$ = 5.6, $J_{6a,6b}$ = –11.5 Hz, H-6a), 2.79 (ddd, 1 H, $J_{6b,7eq}$ = 6.5, $J_{6b,7ax}$ = 12.1 Hz, H-6b), 2.35 (ddddd, 1 H, $J_{15a,14}$ = 3.4, $J_{15a,15b}$ = –16.0 Hz, H-15a), 2.27 (dddd, 1 H, $J_{11eq,12eq}$ = 3.3, $J_{11eq,9}$ = 3.7, $J_{11eq,12ax}$ = 3.9, $J_{11eq,11ax}$ = –13.0 Hz, H-11eq), 2.26 (ddd, 1 H, $J_{9,8}$ = 10.9, $J_{9,11ax}$ = 11.9 Hz, H-9), 2.21 (dddd, 1 H, $J_{7eq,8}$ = 2.3, $J_{7eq,7ax}$ = –13.0 Hz, H-7eq), 2.06 (ddddd, 1 H, $J_{15b,14}$ = 5.2 Hz, H-15b), 1.87 (ddd, 1 H, $J_{12ax,12eq}$ = –13.3, $J_{12ax,11ax}$ = 13.7 Hz, H-12ax), 1.65 (ddd, 1 H, $J_{14,8}$ = 11.3 Hz, H-14), 1.60 (ddd, 1 H, $J_{12eq,11ax}$ = 3.4 Hz, H-12eq), 1.37 (dddd, 1 H, $J_{8,7ax}$ = 10.8 Hz, H-8), 1.36 (dddd, 1H, H-11ax), 1.27 (dddd, 1 H, H-7ax), 0.84 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CD$_3$OD): δ 158.9 (C-3), 142.1 (C-16), 139.0 (C-5), 134.0 (C-10), 127.4 (C-1), 114.5 (C-17), 114.3 (C-4), 112.6 (C-2), 105.5 (C-1″), 78.9 (C-1′), 76.6 (C-5″), 75.1 (C-3″), 72.7 (C-2″), 70.4 (C-4″), 62.5 (C-6″), 55.5 (3-O*C*H$_3$), 45.3 (C-14), 44.9 (C-9), 43.2 (C-8), 39.5 (C-13), 37.7 (C-12), 34.2 (C-15), 31.6 (C-6), 29.0 (C-7), 27.8 (C-11), 17.1 (C-18) ppm.
HRMS: *m/z* calcd. for C$_{26}$H$_{38}$O$_7$Na [M + Na]$^+$ 485.2515; found 485.2497.

(1′*R*)-13,17-seco-13α-(but-3′-en-1′-(β-D-galactopyranosyloxy)-1′-yl)-3-methoxyestra-1,3,5(10),16-tetraene (26). Synthesized from **18** (40 mg, 0.044 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **26** as a colorless oil (20 mg, 91 %). R_f = 0.66 (MeOH:CH$_2$Cl$_2$ 1:5). [α]$_D$ +41.8° (c 1.3, MeOH).^{1}H NMR (600.13 MHz, CD$_3$OD): δ 7.16 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.65 (dd, 1 H, $J_{2,4}$ = 2.5 Hz, H-2), 6.57 (d, 1 H, H-4), 6.17 (dddd, 1 H, $J_{3',2'b}$ = 7.0, $J_{3',2'a}$ = 7.1, $J_{3',4'c}$ = 10.2, $J_{3',4't}$ = 17.0 Hz, H-3′), 5.90 (dddd, 1 H, $J_{16,15b}$ = 6.3, $J_{16,15a}$ = 7.0, $J_{16,17c}$ = 10.1, $J_{16,17t}$ = 17.0 Hz, H-16), 5.09 (dddd, 1 H, $J_{4't,2'a}$ = –1.5, $J_{4't,2'b}$ = –1.5, $J_{4't,4'c}$ = –2.1 Hz, H-4′t), 5.03 (dddd, 1 H, $J_{17t,15b}$ = –1.7, $J_{17t,17c}$ = –1.8, $J_{17t,15a}$ = –1.8 Hz, H-17t), 5.00 (dddd, 1 H, $J_{4'c,2'b}$ = –0.9, $J_{4'c,2'a}$ = –0.9, H-4′c), 4.96 (dddd, 1 H, $J_{17c,15b}$ = –1.6, $J_{17c,15a}$ = –1.6 Hz, H-17c), 4.42 (d, 1 H, $J_{1'',2''}$ = 7.6 Hz, H-1″), 3.91 (dd, 1 H, $J_{1',2'a}$ = 2.8, $J_{1',2'b}$ = 8.2 Hz, H-1′), 3.83 (dd, 1 H, $J_{4'',5''}$ = 1.1, $J_{4'',3''}$ = 3.5 Hz, H-4″), 3.74 (dd, 1 H, $J_{6''a,5''}$ = 6.6, $J_{6''a,6''b}$ = –11.1 Hz, H-6″a), 3.72 (s, 3 H, 3-OC*H*$_3$), 3.71 (dd, 1 H, $J_{6''b, 5''}$ = 5.8 Hz, H-6″b), 3.50 (dd, 1 H, $J_{2'',3''}$ = 9.7 Hz, H-2″), 3.45 (dd, 1 H, H-3″), 3.41 (ddd, 1 H, H-5″), 2.80 (ddd, 1 H, $J_{6a,7eq}$ = 2.6, $J_{6a,7ax}$ = 5.2, $J_{6a,6b}$ = –13.9 Hz, H-6a), 2.78 (ddd, 1 H, $J_{6b,7eq}$ = 6.3, $J_{6b,7ax}$ = 11.5 Hz, H-6b), 2.51 (ddddd, 1 H, $J_{2'a,2'b}$ = –14.7 Hz, H-2′a), 2.41 (ddddd, 1 H, H-2′b), 2.34 (ddddd, 1 H, $J_{15a,14}$ = 2.7, $J_{15a,15b}$ = –16.2 Hz, H-15a), 2.23 (dddd, 1 H, $J_{11eq,12eq}$ = 3.1, $J_{11eq,12ax}$ = 3.8, $J_{11eq,9}$ = 4.6, $J_{11eq,11ax}$ = –12.8 Hz, H-11eq), 2.19 (ddd, 1 H, $J_{9,8}$ = 10.6, $J_{9,11ax}$ = 14.7 Hz, H-9), 2.18 (dddd, 1 H, $J_{7eq,8}$ = 2.4, $J_{7eq,7ax}$ = –15.4 Hz, H-7eq), 2.00 (ddddd, 1 H, $J_{15b,14}$ = 5.0 Hz, H-15b), 1.82 (ddd, 1 H, $J_{12eq,11ax}$ = 3.4, $J_{12eq,12ax}$ = –13.4 Hz, H-12eq), 1.57 (ddd, 1 H, $J_{12ax,11ax}$ = 13.0 Hz, H-12ax), 1.41 (dddd, 1 H, $J_{8,14}$ = 11.0, $J_{8,7ax}$ = 11.7 Hz, H-8), 1.40 (ddd, 1 H, H-14), 1.30 (dddd, 1 H, H-11ax), 1.26 (dddd, 1 H, H-7ax), 1.04 (s, 3 H, H-18) ppm.
^{13}C NMR (150.9 MHz, CD$_3$OD): δ 158.9 (C-3), 141.5 (C-16), 139.1 (C-3′), 138.9 (C-5), 133.9 (C-10), 127.4 (C-1), 116.5 (C-4′), 115.0 (C-17), 114.3 (C-4), 112.6 (C-2), 104.1 (C-1″), 84.8 (C-1′), 76.4 (C-5″), 75.2 (C-3″), 73.2 (C-2″), 70.2 (C-4″), 62.3 (C-6″), 55.5 (3-O*C*H$_3$), 46.7 (C-14),

45.0 (C-9), 43.9 (C-8), 42.9 (C-13), 35.9 (C-2′), 34.0 (C-15), 32.6 (C-12), 31.5 (C-6), 29.1 (C-7), 27.4 (C-11), 18.6 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{29}H_{42}O_7Na$ $[M + Na]^+$ 525.2828; found 525.2828.

3-Methoxy-D-dihomo-estra-17bβ-(2′,3′,4′,6′-tetra-*O*-benzoyl-α-D-mannopyranosyloxy)-1,3,5(10),16-tetraene (27). Synthesized from **12** (132 mg, 0.143 mmol) according to the general procedure for ring-closing metathesis providing **27** as a yellowish foam (124 mg, 97 %). R_f = 0.33 (hexane:EtOAc 4:1); $[\alpha]_D$ –30.0° (c 0.15, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.12–7.26 (m, 20 H, arom. *H*), 7.16 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.73 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.65 (d, 1 H, H-4), 6.08 (dd, 1 H, $J_{4',5'}$ = 10.1, $J_{4',3'}$ = 10.2 Hz, H-4′), 5.91 (dddd, 1 H, $J_{16,17a2}$ = –1.9, $J_{16,15b}$ = 4.2, $J_{16,15a}$ = 8.5, $J_{16,17}$ = 10.0 Hz, H-16), 5.91 (dd, 1 H, $J_{3',2'}$ = 3.2 Hz, H-3′), 5.63 (dddd, 1 H, $J_{17,17a1}$ = 3.1, $J_{17,15a}$ = –5.8, $J_{17,17a2}$ = 7.5 Hz, H-17), 5.59 (dd, 1 H, $J_{2',1'}$ = 1.6 Hz, H-2′), 4.75 (dd, 1 H, $J_{6'a,5'}$ = 2.2, $J_{6'a,6'b}$ = –12.0 Hz, H-6′a), 4.61 (ddd, 1 H, $J_{5',6'b}$ = 5.6 Hz, H-5′), 4.49 (dd, 1 H, H-6′b), 3.78 (s, 3 H, 3-OC*H*$_3$), 3.45 (dd, 1 H, $J_{17b,17a2}$ = 2.1, $J_{17b,17a1}$ = 10.2 Hz, H-17b), 2.84 (ddd, 1 H, $J_{6a,7eq}$ = 2.9, $J_{6a,7ax}$ = 4.1, $J_{6a,6b}$ = –15.4 Hz, H-6a), 2.83 (ddd, 1 H, $J_{6b,7eq}$ = 7.3, $J_{6b,7ax}$ = 12.3 Hz, H-6b), 2.68 (dddd, 1 H, $J_{17a1,15b}$ = 2.5, $J_{17a1,17a2}$ = –15.8 Hz, H-17a1), 2.33 (dddd, 1 H, H-17a2), 2.26 (dddd, 1 H, $J_{11eq,12ax}$ = 3.2, $J_{11eq,9}$ = 3.7, $J_{11eq,12eq}$ = 3.7, $J_{11eq,11ax}$ = –13.7 Hz, H-11eq), 2.25 (ddd, 1 H, $J_{12eq,11ax}$ = 3.3, $J_{12eq,12ax}$ = –12.1 Hz, H-12eq), 2.25 (dddd, 1 H, $J_{15a,14}$ = 1.0, $J_{15a,15b}$ = –16.0 Hz, H-15a), 2.21 (dddd, 1 H, $J_{7eq,8}$ = 2.5, $J_{7eq,7ax}$ = –13.8 Hz, H-7eq), 2.10 (ddd, 1 H, $J_{9,8}$ = 11.0, $J_{9,11ax}$ = 11.7 Hz, H-9), 2.09 (dddd, 1 H, $J_{15b,14}$ = 10.4, H-15b), 1.53 (dddd, 1 H, $J_{11ax,12ax}$ = 13.8 Hz, H-11ax), 1.42 (ddd, 1 H, H-12ax), 1.26 (dddd, 1 H, $J_{7ax,8}$ = 9.0 Hz, H-7ax), 1.25 (dddd, 1 H, $J_{8,14}$ = 9.0 Hz, H-8), 1.22 (ddd, 1 H, H-14), 1.18 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.1 (6′-O*C*OPh), 166.0 (4′-O*C*OPh), 165.6 (3′-O*C*OPh, 2′-O*C*OPh), 157.5 (C-3), 138.1 (C-5), 134.6 (C-16), 133.5–128.3 (arom. *C*), 133.2 (C-10), 126.1 (C-1), 125.8 (C-17), 113.2 (C-4), 111.4 (C-2), 95.2 (C-1′), 85.1 (C-17b), 71.7 (C-2′), 70.2 (C-5′), 70.1 (C-3′), 67.0 (C-4′), 63.4 (C-6′), 55.2 (3-O*C*H$_3$), 49.0 (C-14), 43.1 (C-9), 42.7 (C-13), 39.3 (C-8), 38.5 (C-12), 30.2 (C-6), 28.1 (C-17a), 27.8 (C-7), 26.1 (C-11), 25.9 (C-15), 12.8 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{55}H_{54}O_{11}Na$ $[M + Na]^+$ 913.3564; found 913.3550.

3-methoxy-D-dihomo-estra-17bβ-(2′,3′,4′-tri-*O*-benzoyl-α-L-rhamnopyranosyloxy)-1,3,5(10),16-tetraene (28). Synthesized from **14** (164 mg, 0.205 mmol) according to the general procedure for ring-closing metathesis providing **28** as a white foam (159 mg, quant.). R_f = 0.29 (hexane:EtOAc 4:1); $[\alpha]_D$ +76.3° (c 0.15, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$): δ 8.13–7.23 (m, 15 H, arom. *H*), 7.24 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 6.74 (dd, 1 H, $J_{2,4}$ = 2.6 Hz, H-2), 6.64 (d, 1 H, H-4), 5.91 (dddd, 1 H, $J_{16,17a1}$ = –1.9, $J_{16,15b}$ = 4.4, $J_{16,15a}$ = 8.2, $J_{16,17}$ = 10.5 Hz, H-16), 5.83 (dd, 1 H, $J_{3',2'}$ = 3.5, $J_{3',4'}$ = 10.0 Hz, H-3′), 5.72 (dd, 1 H, $J_{4',5'}$ = 10.0 Hz, H-4′), 5.68 (dd, 1 H, $J_{2',1'}$ = 1.6 Hz, H-2′), 5.63 (dddd, 1 H, $J_{17,17a1}$ = 3.0, $J_{17,15b}$ = –4.7, $J_{17,17a2}$ = 7.7 Hz, H-17), 5.12 (d, 1 H, H-1′), 4.33 (dq, 1 H, $J_{5',6'}$ = 6.3 Hz, H-5′), 3.77 (s, 3 H, 3-OCH_3), 3.35 (dd, 1 H, $J_{17b,17a2}$ = 2.7, $J_{17b,17a1}$ = 10.6 Hz, H-17b), 2.86 (ddd, 1 H, $J_{6a,7eq}$ = 3.5, $J_{6a,7ax}$ = 5.0, $J_{6a,6b}$ = –16.1 Hz, H-6a), 2.83 (ddd, 1 H, $J_{6b,7eq}$ = 6.6, $J_{6b,7ax}$ = 12.1 Hz, H-6b), 2.77 (dddddd, 1 H, $J_{17a1,15a}$ = 0.9, $J_{17a1,15b}$ = 5.3, $J_{17a1,17a2}$ = –15.6 Hz, H-17a1), 2.47 (ddd, 1 H, H-17a2), 2.37 (dddd, 1 H, $J_{11eq,12eq}$ = 3.0, $J_{11eq,12ax}$ = 3.8, $J_{11eq,9}$ = 4.2, $J_{11eq,11ax}$ = –13.1 Hz, H-11eq), 2.29 (ddd, 1 H, $J_{9,8}$ = 8.8, $J_{9,11ax}$ = 12.9 Hz, H-9), 2.27 (ddd, 1 H, $J_{12eq,11ax}$ = 3.4, $J_{12eq,12ax}$ = –13.0 Hz, H-12eq), 2.28 (ddd, 1 H**,** $J_{15a,14}$ = 1.0, $J_{15a,15b}$ = –16.0 Hz, H-15a), 2.23 (dddd, 1 H, $J_{7eq,8}$ = 1.8, $J_{7eq,7ax}$ = –12.7 Hz, H-7eq), 2.08 (ddddd, 1 H, $J_{15b,14}$ = 5.6 Hz, H-15b), 1.54 (dddd, 1 H, $J_{11ax,12ax}$ = 13.2 Hz, H-11ax), 1.47 (ddd, 1 H, H-12ax), 1.37 (d, 1 H, H-6′), 1.32 (dddd, 1 H, $J_{7ax,8}$ = 14.1 Hz, H-7ax), 1.28 (dddd, 1 H, $J_{8,14}$ = 12.3 Hz, H-8), 1.26 (ddd, 1 H, H-14), 1.16 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 165.8 (4′-O*C*OPh), 165.7 (2′-O*C*OPh), 165.6 (3′-O*C*OPh), 157.5 (C-3), 138.1 (C-5), 134.0 (C-16), 134.0–128.3 (arom. *C*), 133.2 (C-10), 126.1 (C-1, C-17), 113.3 (C-4), 111.4 (C-2), 100.2 (C-1′), 90.6 (C-17b), 71.9 (C-4′), 71.1 (C-2′), 70.2 (C-3′), 67.1 (C-5′), 55.2 (3-O*C*H_3), 48.8 (C-14), 43.4 (C-9), 43.2 (C-13), 39.6 (C-8), 37.9 (C-12), 31.5 (C-17a), 30.3 (C-6), 27.9 (C-7), 26.3 (C-15), 26.0 (C-11), 17.6 (C-6′), 12.7 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{48}H_{50}O_9Na$ $[M + Na]^+$ 793.3353; found 793.3331; *m/z* calcd. for $C_{48}H_{54}O_9N$ $[M + NH_4]^+$ 788.3799; found 788.3768.

3-Methoxy-D-dihomo-estra-17bβ-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glucopyranosyloxy)-1,3,5(10),16-tetraene (29). Synthesized from **16** (98 mg, 0.107 mmol) according to the general procedure for ring-closing metathesis providing **29** as a white foam (69 mg, 72 %). R_f = 0.29 (hexane:EtOAc 4:1); $[\alpha]_D$ +8.5° (c 0.15, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$): δ 8.05–7.28

(m, 20 H, arom. *H*), 6.87 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.67 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.58 (d, 1 H, H-4), 5.92 (dd, 1 H, $J_{3',4'}$ = 9.5, $J_{3',2'}$ = 9.9 Hz, H-3′), 5.79 (dddd, 1 H, $J_{16,17a1}$ = –2.5, $J_{16,15b}$ = 4.5, $J_{16,15a}$ = 8.9, $J_{16,17}$ = 9.9 Hz, H-16), 5.61 (t, 1 H, $J_{4',5'}$ = 9.9 Hz, H-4′), 5.59 (dd, 1 H, $J_{2',1'}$ = 7.9 Hz, H-2′), 5.31 (dddd, 1 H, $J_{17,15b}$ = –2.6, $J_{17,17a1}$ = 3.8, $J_{17,17a2}$ = 8.4 Hz, H-17), 4.92 (d, 1 H, H-1′), 4.66 (dd, 1 H, $J_{6'a,5'}$ = 3.1, $J_{6'a,6'b}$ = –12.0 Hz, H-6′a), 4.50 (dd, 1 H, $J_{6'b,5'}$ = –6.5 Hz, H-6′b), 4.16 (ddd, 1 H, H-5′), 3.75 (s, 3 H, 3-OCH_3), 3.63 (dd, 1 H, $J_{17b,17a2}$ = 2.8, $J_{17b,17a1}$ = 11.1 Hz, H-17b), 2.78 (ddd, 1 H, $J_{6a,7eq}$ = 3.3, $J_{6a,7ax}$ = 5.7, $J_{6a,6b}$ = –16.9 Hz, H-6a), 2.74 (ddd, 1 H, $J_{6b,7eq}$ = 6.2, $J_{6b,7ax}$ = 10.9 Hz, H-6b), 2.67 (ddddd, 1 H, $J_{17a1,15b}$ = 2.5, $J_{17a1,17a2}$ = –15.7 Hz, H-17a1), 2.56 (ddd, 1 H, H-17a2), 2.13 (dddd, 1 H, $J_{7eq,8}$ = 3.2, $J_{7eq,7ax}$ = –13.2 Hz, H-7eq), 2.13 (ddd, 1 H, $J_{15a,14}$ = 0.1, $J_{15a,15b}$ = –15.7 Hz, H-15a), 2.02 (ddd, 1 H, $J_{9,11eq}$ = 4.1, $J_{9,8}$ = 10.6, $J_{9,11ax}$ = 11.5 Hz, H-9), 1.91 (ddddd, 1 H, $J_{15b,14}$ = 10.1 Hz, H-15b), 1.90 (ddd, 1 H, $J_{12eq,11eq}$ = 3.3, $J_{12eq,11ax}$ = 3.3, $J_{12ax,12eq}$ = –13.4 Hz, H-12eq), 1.50 (dddd, 1 H, $J_{11eq,12ax}$ = 4.0, $J_{11eq,11ax}$ = –13.9 Hz, H-11eq), 1.22 (dddd, 1 H, $J_{7a,8}$ = 10.5 Hz, H-7ax), 1.06 (ddd, 1 H, $J_{14,8}$ = 11.1 Hz, H-14), 1.06 (dddd, 1 H, H-8), 1.05 (ddd, 1 H, $J_{12ax,11ax}$ = 13.3 Hz, H-12ax), 0.90 (dddd, 1 H, H-11ax), 0.82 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CDCl$_3$): δ 166.1 (6′-O*C*OPh), 165.9 (3′-O*C*OPh), 165.3 (4′-O*C*OPh), 165.1 (2′-O*C*OPh), 157.5 (C-3), 138.1 (C-5), 133.8 (C-16), 133.5–128.3 (arom. *C*), 133.3 (C-10), 126.8 (C-17), 125.7 (C-1), 113.1 (C-4), 111.4 (C-2), 103.6 (C-1′), 90.5 (C-17b), 72.9 (C-3′), 72.2 (C-2′), 72.1 (C-5′), 70.1 (C-4′), 63.4 (C-6′), 55.2 (3-OCH_3), 48.8 (C-14), 43.5 (C-13), 43.0 (C-9), 39.4 (C-8), 37.8 (C-12), 31.9 (C-17a), 30.2 (C-6), 27.9 (C-7), 25.9 (C-15), 25.0 (C-11), 12.0 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{55}H_{54}O_{11}Na$ [M + Na]$^+$ 913.3558; found 913.3545; *m/z* calcd. for $C_{55}H_{54}O_{11}K$ [M + K]$^+$ 929.3298; found 929.3284.

3-Methoxy-D-dihomo-estra-17bα-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-galactopyranosyloxy)-1,3,5(10),16-tetraene (30). Synthesized from **18** (89 mg, 0.097 mmol) according to the general procedure for ring-closing metathesis providing **30** as a colorless oil (65 mg, 75 %). R_f = 0.33 (Hexane:EtOAc 4:1); $[\alpha]_D$ +32.2° (c 0.27, CHCl$_3$). ^{1}H NMR (600.13 MHz, CDCl$_3$): δ 8.11–7.20 (m, 20 H, arom. *H*), 7.21 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.72 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.62 (d, 1 H, H-4), 5.99 (dd, 1 H, $J_{4',5'}$ = 1.2, $J_{4',3'}$ = 3.6 Hz, H-4′), 5.79 (dd, 1 H, $J_{2',1'}$ = 7.9, $J_{2',3'}$ = 10.4 Hz, H-2′), 5.64 (dd, 1 H, H-3′), 5.50 (dddd, 1 H, $J_{16,17a2}$ = –1.3, $J_{16,15b}$ = 2.3, $J_{16,15a}$ = 8.0, $J_{16,17}$ = 10.8

Hz, H-16), 5.31 (dddd, 1 H, $J_{17,15a}$ = –2.5, $J_{17,17a1}$ = 5.2, $J_{17,17a2}$ = 5.8 Hz, H-17), 4.81 (d, 1 H, H-1′), 4.68 (dd, 1 H, $J_{6'a,5'}$ = 6.8, $J_{6'a,6'b}$ = –11.3 Hz, H-6′a), 4.43 (dd, 1 H, $J_{6'b,5'}$ = –6.6 Hz, H-6′b), 4.29 (ddd, 1 H, H-5′), 3.77 (s, 3 H, 3-OCH_3), 3.63 (dd, 1 H, $J_{17b,17a2}$ = 1.6, $J_{17b,17a1}$ = 8.5 Hz, H-17b), 2.80 (ddd, 1 H, $J_{6a,7eq}$ = 3.6, $J_{6a,7ax}$ = 5.7, $J_{6a,6b}$ = –11.3 Hz, H-6a), 2.77 (ddd, 1 H, $J_{6b,7eq}$ = 6.2, $J_{6b,7ax}$ = 12.4 Hz, H-6b), 2.40 (dddd, 1 H, $J_{17a1,15b}$ = 3.4, $J_{17a1,17a2}$ = –17.9 Hz, H-17a1), 2.27 (dddd, 1 H, $J_{11eq,12eq}$ = 2.9, $J_{11eq,12ax}$ = 3.9, $J_{11eq,9}$ = 4.0, $J_{11eq,11ax}$ = –13.0 Hz, H-11eq), 2.23 (ddd, 1 H, $J_{9,8}$ = 10.5, $J_{9,11ax}$ = 12.4 Hz, H-9), 2.17 (dddd, 1 H, H-17a2), 2.15 (dddd, 1 H, $J_{15a,14}$ = 0.2, $J_{15a,15b}$ = –17.5 Hz, H-15a), 2.03 (dddd, 1 H, $J_{7eq,8}$ = 2.8, $J_{7eq,7ax}$ = –12.2 Hz, H-7eq), 1.83 (ddd, 1 H, $J_{12ax,11ax}$ = 13.8, $J_{12ax,12eq}$ = –13.3 Hz, H-12ax), 1.79 (dddd, 1 H, H-15b), 1.82 (ddd, 1 H, $J_{12eq,11ax}$ = 3.7 Hz, H-12eq), 1.53 (ddd, 1 H, $J_{14,8}$ = 11.1 Hz, H-14), 1.47 (dddd, 1 H, H-11ax), 1.22 (dddd, 1 H, $J_{8,7a}$ = 10.4 Hz, H-8), 1.15 (dddd, 1 H, H-7ax), 0.97 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.0 (6′-O*C*OPh), 165.7 (4′-O*C*OPh), 165.6 (3′-O*C*OPh), 165.1 (2′-O*C*OPh), 157.4 (C-3), 138.1 (C-5), 133.6 (C-10), 133.5–128.3 (arom. *C*), 132.3 (C-16), 126.5 (C-1), 125.8 (C-17), 113.2 (C-4), 111.4 (C-2), 99.3 (C-1′), 84.7 (C-17b), 71.8 (C-3′), 71.3 (C-5′), 69.9 (C-2′), 68.3 (C-4′), 62.0 (C-6′), 55.2 (3-OCH_3), 44.0 (C-14), 43.3 (C-9), 40.7 (C-13), 39.0 (C-8), 33.2 (C-12), 30.2 (C-6), 27.8 (C-17a), 27.1 (C-7), 26.3 (C-15), 26.0 (C-11), 17.0 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{55}H_{54}O_{11}Na$ $[M + Na]^+$ 913.3564; found 913.3552; m*/z* calcd. for $C_{55}H_{54}O_{11}K$ $[M + K]^+$ 929.3303; found 929.3301.

3-Methoxy-D-dihomo-estra-17bβ-(α-D-mannopyranosyloxy)-1,3,5(10),16-tetraene (31). Synthesized from **27** (119 mg, 0.134 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **31** as a white solid (53 mg, 98 %). R_f = 0.52 (MeOH:CH_2Cl_2 1:5). ^{1}H NMR (600.13 MHz, CD_3OD): δ 7.17 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.68 (dd, 1 H, $J_{2,4}$ = 2.6 Hz, H-2), 6.60 (d, 1 H, H-4), 5.89 (dddd, 1 H, $J_{16,17a2}$ = –2.1, $J_{16,15b}$ = 4.2, $J_{16,15a}$ = 8.3, $J_{16,17}$ = 9.8 Hz, H-16), 5.60 (dddd, 1 H, $J_{17,15a}$ = –2.7, $J_{17,17a1}$ = 5.1, $J_{17,17a2}$ = 7.7 Hz, H-17), 4.87 (d, 1 H, $J_{1',2'}$ = 1.6 Hz, H-1′), 3.83 (dd, 1 H, $J_{6'a,5'}$ = 2.4, $J_{6'a,6'b}$ = –12.0 Hz, H-6′a), 3.75 (dd, 1 H, $J_{6'b,5'}$ = 5.1 Hz, H-6′b), 3.75 (s, 3 H, 3-OCH_3), 3.74 (dd, 1 H, $J_{2',3'}$ = 3.3 Hz, H-2′), 3.73 (ddd, 1 H, $J_{5',4'}$ = 9.8 Hz, H-5′), 3.72 (dd, 1 H, $J_{3',4'}$ = 9.4 Hz, H-3′), 3.68 (dd, 1 H, H-4′), 3.37 (dd, 1 H, $J_{17b,17a2}$ = 1.9, $J_{17b,17a1}$ = 9.9 Hz, H-17b), 2.83 (ddd, 1 H, $J_{6a,7eq}$ = 3.1, $J_{6a,7ax}$ = 5.6, $J_{6a,6b}$ = –16.4 Hz, H-6a), 2.80 (ddd, 1 H, $J_{6b,7eq}$ = 6.0, $J_{6b,7ax}$ = 11.7 Hz, H-6b), 2.53 (dddd, 1 H, $J_{17a1,15b}$ =

2.8, $J_{17a1,17a2}$ = –15.7 Hz, H-17a1), 2.30 (dddd, 1 H, $J_{11eq,12eq}$ = 2.8, $J_{11eq,12ax}$ = 3.5, $J_{11eq,9}$ = 3.6, $J_{11eq,11ax}$ = –15.1 Hz, H-11eq), 2.28 (dddd, 1 H, H-17a2), 2.23 (dddd, 1 H, $J_{15a,14}$ = 1.3, $J_{15a,15b}$ = –15.5 Hz, H-15a), 2.12 (dddd, 1 H, $J_{7eq,8}$ = 2.7, $J_{7eq,7ax}$ = –13.3 Hz, H-7eq), 2.06 (ddd, 1 H, $J_{15a,14}$ = 10.5Hz, H-15b), 1.45 (dddd, 1 H, $J_{11ax,12ax}$ = 13.8 Hz, H-11ax), 1.43 (ddd, 1 H, H-12ax), 1.30 (ddd, 1 H, $J_{14,8}$ = 10.3 Hz, H-14), 1.27 (dddd, 1 H, $J_{7ax,8}$ = 9.7 Hz, H-7ax), 1.22 (dddd, 1 H, H-8), 1.05 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CD_3OD): δ 158.7 (C-3), 138.9 (C-5), 135.3 (C-16), 134.3 (C-10), 126.8 (C-17), 126.7 (C-1), 113.9 (C-4), 112.3 (C-2), 98.2 (C-1′), 83.7 (C-17b), 75.2 (C-2′), 72.8 (C-5′), 72.5 (C-3′), 68.2 (C-4′), 62.7 (C-6′), 55.5 (3-O*C*H_3), 50.2 (C-14), 44.4 (C-9), 43.6 (C-13), 40.6 (C-8), 39.6 (C-12), 31.1 (C-6), 28.8 (C-7), 28.2 (C-17a), 27.0 (C-11), 26.7 (C-15), 13.3 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{27}H_{38}O_7Na$ $[M + Na]^+$ 498.2515; found 498.2504; *m/z* calcd. for $C_{27}H_{38}O_7K$ $[M + K]^+$ 513.2255; found 513.2238.

3-Methoxy-D-dihomo-estra-17bβ-(α-L-rhamnopyranosyloxy)-1,3,5(10),16-tetraene (32). Synthesized from **28** (155 mg, 0.200 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **32** as a white solid (71 mg, quant.). R_f = 0.17 (EtOAc); $[\alpha]_D$ –32.5° (c 0.08, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.15 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.68 (dd, 1 H, $J_{2,4}$ = 2.4 Hz, H-2), 6.61 (d, 1 H, H-4), 5.83 (dddd, 1 H, $J_{16,17a1}$ = –2.1, $J_{16,15b}$ = 4.3, $J_{16,15a}$ = 8.3, $J_{16,17}$ = 10.4 Hz, H-16), 5.58 (dddd, 1 H, $J_{17,17a1}$ = 2.6, $J_{17,15b}$ = –4.4, $J_{17,17a2}$ = 7.7 Hz, H-17), 4.84 (d, 1 H, $J_{1',2'}$ = 1.6 Hz, H-1′), 4.00 (dd, 1 H, $J_{2',3'}$ = 3.3 Hz, H-2′), 3.81 (dq, 1 H, $J_{5',6'}$ = 6.3, $J_{5',4'}$ = 9.0 Hz, H-5′), 3.78 (dd, 1 H, $J_{3',4'}$ = 9.6 Hz, H-3′), 3.75 (s, 3 H, 3-OC*H_3*), 3.52 (dd, 1 H, H-4′), 3.21 (dd, 1 H, $J_{17b,17a2}$ = 3.0, $J_{17b,17a1}$ = 10.4 Hz, H-17b), 2.81 (ddd, 1 H, $J_{6a,7eq}$ = 4.5, $J_{6a,7ax}$ = 5.6, $J_{6a,6b}$ = –16.4 Hz, H-6a), 2.78 (ddd, 1 H, $J_{6b,7eq}$ = 6.5, $J_{6b,7ax}$ = 12.8 Hz, H-6b), 2.60 (ddddd, 1 H, $J_{17a1,17a2}$ = –15.3 Hz, H-17a1), 2.38 (ddd, 1 H, H-17a2), 2.22 (dddd, 1 H, $J_{11eq,12ax}$ = 3.2, $J_{11eq,12eq}$ = 4.3, $J_{11eq,9}$ = 4.3, $J_{11eq,11ax}$ = –12.6 Hz, H-11eq), 2.21 (ddd, 1 H, $J_{9,8}$ = 8.7, $J_{9,11ax}$ = 14.0 Hz, H-9), 2.19 (ddd, 1 H, $J_{15a,14}$ = 1.0, $J_{15a,15b}$ = –15.6 Hz, H-15a), 2.16 (dddd, 1 H, $J_{7eq,8}$ = 1.8, $J_{7eq,7ax}$ = –11.7 Hz, H-7eq), 2.00 (ddd, 1 H, $J_{12eq,11ax}$ = 3.8, $J_{12eq,12ax}$ = –12.9 Hz, H-12eq), 1.95 (ddddd, 1 H, $J_{15b,14}$ = 8.5, H-15b), 1.43 (dddd, 1 H, $J_{11ax,12ax}$ = 11.3 Hz, H-11ax), 1.34 (d, 1 H, H-6′), 1.32 (ddd, 1 H, H-12ax), 1.26 (dddd, 1 H, $J_{7ax,8}$ = 11.3 Hz, H-7ax), 1.18 (ddd, 1 H, $J_{14,8}$ = 10.0 Hz, H-14), 1.18 (dddd, 1 H, H-8), 0.96 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 157.5 (C-3), 138.1 (C-5), 133.6 (C-16), 133.3 (C-10), 126.3 (C-17), 125.9 (C-1), 113.2 (C-4), 111.4 (C-2), 102.8 (C-1′), 89.8 (C-17b), 73.1 (C-4′), 72.0 (C-3′), 71.2 (C-2′), 68.4 (C-5′), 55.2 (3-OCH_3), 48.8 (C-14), 43.4 (C-13), 43.2 (C-9), 39.6 (C-8), 38.1 (C-12), 31.4 (C-17a), 30.2 (C-6), 27.9 (C-7), 26.2 (C-15), 25.9 (C-11), 17.5 (C-6′), 12.6 (C-18) ppm.
HRMS: *m/z* calcd. for $C_{27}H_{38}O_6Na$ $[M + Na]^+$ 481.2566; found 481.2552.

3-Methoxy-D-dihomo-estra-17bβ-(β-D-glucopyranosyloxy)-1,3,5(10),16-tetraene (33). Synthesized from **29** (66 mg, 0.075 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **33**as a white solid (29 mg, 82 %). R_f = 0.66 (MeOH:CH_2Cl_2 1:5). ^{1}H NMR (600.13 MHz, CD_3OD): δ ^{1}H NMR (600.13 MHz, CD_3OD): δ 7.15 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.66 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.59 (d, 1 H, H-4), 5.84 (dddd, 1 H, $J_{16,17a1}$ = –4.6, $J_{16,15b}$ = 2.8, $J_{16,15a}$ = 8.7, $J_{16,17}$ = 10.3 Hz, H-16), 5.66 (dddd, 1 H, $J_{17,17a1}$ = 2.2, $J_{17,15b}$ = –4.5, $J_{17,17a2}$ = 8.6 Hz, H-17), 4.36 (d, 1 H, $J_{1',2'}$ = 7.7 Hz, H-1′), 3.85 (dd, 1 H, $J_{6'a,5'}$ = 2.4, $J_{6'a,6'b}$ = –11.8 Hz, H-6′a), 3.73 (s, 3 H, 3-OCH_3), 3.69 (dd, 1 H, $J_{6'b,5'}$ = 5.5 Hz, H-6′b), 3.34 (dd, 1 H, $J_{3',4'}$ = 8.5, $J_{3',2'}$ = 9.5 Hz, H-3′), 3.30 (dd, 1 H, $J_{4',5'}$ = 10.0, H-4′), 3.25 (dd, 1 H, $J_{17b,17a2}$ = 2.7, $J_{17b,17a1}$ = 11.0 Hz, H-17b), 3.25 (ddd, 1 H, H-5′), 3.20 (dd, 1 H, Hz, H-2′), 2.82 (ddd, 1 H, $J_{6a,7eq}$ = 2.1, $J_{6a,7ax}$ = 5.8, $J_{6a,6b}$ = –16.2 Hz, H-6a), 2.78 (ddd, 1 H, $J_{6b,7eq}$ = 5.6, $J_{6b,7ax}$ = 11.3 Hz, H-6b), 2.63 (ddddd, 1 H, $J_{17a1,15b}$ = 2.3, $J_{17a1,17a2}$ = –15.4 Hz, H-17a1), 2.59 (ddd, 1 H, H-17a2), 2.50 (ddd, 1 H, $J_{12eq,11eq}$ = 2.6, $J_{12eq,11ax}$ = 4.3, $J_{12eq,12ax}$ = –13.7 Hz, H-12eq), 2.25 (dddd, 1 H, $J_{11eq,12ax}$ = 3.6, $J_{11eq,9}$ = 3.7, $J_{11eq,11ax}$ = –12.8 Hz, H-11eq), 2.22 (dddd, 1 H, $J_{7eq,8}$ = 3.2, $J_{7eq,7ax}$ = –13.0 Hz, H-7eq), 2.22 (ddd, 1 H, $J_{9,8}$ = 11.6, $J_{9,11ax}$ = 12.5 Hz, H-9), 2.21 (ddd, 1 H, $J_{15a,14}$ = 0.6, $J_{15a,15b}$ = –15.7 Hz, H-15a), 2.03 (ddddd, 1 H, $J_{15a,14}$ = 10.2 Hz, H-15b), 1.38 (dddd, 1 H, $J_{11ax,12ax}$ = 12.5 Hz, H-11ax), 1.33 (ddd, 1 H, H-12ax), 1.27 (dddd, 1 H, $J_{7ax,8}$ = 9.6 Hz, H-7ax), 1.16 (ddd, 1 H, $J_{14,8}$ = 10.9 Hz, H-14), 1.16 (dddd, 1 H, H-8), 1.06 (s, 3 H, H-18) ppm.
^{13}C NMR (150.9 MHz, $CDCl_3$): δ 159.0 (C-3), 139.1(C-5), 134.7(C-16), 134.6 (-10), 128.6 (C-17), 126.8 (C-1), 114.1 (C-4), 112.4 (C-2), 106.7 (C-1′), 89.9 (C-17b), 78.3 (C-3′), 77.7 (C-5′), 75.9 (C-2′), 71.7 (C-4′), 62.8 (C-6′), 55.5 (3-OCH_3), 50.5 (C-14), 44.9 (C-13), 44.7 (C-9), 39.0 (C-8), 33.2 (C-17a), 31.3 (C-6), 29.3 (C-7), 29.3 (C-7), 27.0 (C-11, C-15), 12.7 (C-18) ppm.
HRMS: *m/z* calcd. for $C_{27}H_{38}O_7Na$ $[M + Na]^+$ 497.2515; found 497.2500.

3-Methoxy-D-dihomo-estra-17bα-(β-D-galactopyranosyloxy)-1,3,5(10),16-tetraene (34). Synthesized from **30** (63 mg, 0.071 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **34** as a white solid (27 mg, 90 %). R_f = 0.33 (hexane:EtOAc 4:1); $[\alpha]_D$ +27.0° (c 0.10, MeOH). ^{1}H NMR (600.13 MHz, CD_3OD): δ 7.16 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 6.66 (dd, 1 H, $J_{2,4}$ = 2.7 Hz, H-2), 6.58 (d, 1 H, H-4), 5.73 (dddd, 1 H, $J_{16,17a2}$ = –1.3, $J_{16,15b}$ = 2.9, $J_{16,15a}$ = 7.9, $J_{16,17}$ = 10.8 Hz, H-16), 5.59 (dddd, 1 H, $J_{17,15b}$ = –3.1, $J_{17,17a1}$ = 3.8, $J_{17,17a2}$ = 6.6 Hz, H-17), 4.21 (d, 1 H, $J_{1',2'}$ = 7.7 Hz, H-1′), 3.85 (dd, 1 H, $J_{4',5'}$ = 1.0, $J_{4',3'}$ = 3.4 Hz, H-4′), 3.75 (dd, 1 H, $J_{6'a,5'}$ = 6.1, $J_{6'a,6'b}$ = –11.2 Hz, H-6′a), 3.73 (s, 3 H, 3-OCH_3), 3.72 (dd, 1 H, $J_{6'b,5'}$ = –6.4 Hz, H-6′b), 3.64 (dd, 1 H, $J_{17b,17a2}$ = 1.6, $J_{17b,17a1}$ = 9.1 Hz, H-17b), 3.50 (dd, 1 H, $J_{2',3'}$ = 9.7 Hz, H-2′), 3.47 (dd, 1 H, H-3′), 3.46 (ddd, 1 H, H-5′), 2.81 (ddd, 1 H, $J_{6a,7eq}$ = 4.6, $J_{6a,7ax}$ = 5.0, $J_{6a,6b}$ = –16.3 Hz, H-6a), 2.78 (ddd, 1 H, $J_{6b,7eq}$ = 5.7, $J_{6b,7ax}$ = 13.1Hz, H-6b), 2.61 (dddd, 1 H, $J_{17a1,15b}$ = 3.1, $J_{17a1,17a2}$ = –17.1 Hz, H-17a1), 2.31 (ddd, 1 H, $J_{15a,14}$ = 1.1, $J_{15a,15b}$ = –16.5 Hz, H-15a), 2.29 (dddd, 1 H, $J_{11eq,12eq}$ = 3.4, $J_{11eq,9}$ = 3.7, $J_{11eq,12ax}$ = 3.8, $J_{11eq,11ax}$ = –12.8 Hz, H-11eq), 2.26 (ddddd, 1 H, $J_{17a1,15b}$ = 2.3 Hz, H-17a2), 2.22 (ddd, 1 H, $J_{9,8}$ = 10.1, $J_{9,11ax}$ = 12.1 Hz, H-9), 2.17 (dddd, 1 H, $J_{7eq,8}$ = 2.9, $J_{7eq,7ax}$ = –12.8 Hz, H-7eq), 1.96 (ddddd, 1 H, H-15b), 1.86 (ddd, 1 H, $J_{12ax,11ax}$ = 13.5, $J_{12ax,12eq}$ = –13.5 Hz, H-12ax), 1.68 (ddd, 1 H, $J_{12eq,11ax}$ = 3.5 Hz, H-12eq), 1.66 (ddd, 1 H, $J_{14,8}$ = 12.0 Hz, H-14), 1.44 (dddd, 1 H, H-11ax), 1.26 (dddd, 1 H, $J_{8,7a}$ = 9.6 Hz, H-8), 1.22 (dddd, 1 H, H-7ax), 1.04 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 159.0 (C-3), 139.0 (C-5), 134.6 (C-10), 133.0 (C-16), 128.2 (C-17), 127.0 (C-1), 114.1 (C-4), 112.5 (C-2), 103.1 (C-1′), 86.2 (C-17b), 76.5 (C-5′), 75.2 (C-3′), 72.6 (C-2′), 70.3 (C-4′), 62.3 (C-6′), 55.6 (3-OCH_3), 46.5 (C-14), 45.1 (C-9), 41.7 (C-13), 40.6 (C-8), 34.0 (C-12), 31.3 (C-6), 29.5 (C-17a), 28.6 (C-7), 27.4 (C-11), 27.3 (C-15), 17.7 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{27}H_{38}O_7Na$ $[M + Na]^+$ 497.2515; found 497.2510.

Phenyl 2,3-*O*-isopropylidene-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-1-thio-α-L-rhamnopyranoside (36). Synthesized from **35** (91 mg, 0.3 mmol) and **9** (295 mg, 0.4 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **36** as a white foam (290 mg, 85 %). R_f = 0.41 (hexane:EtOAc 2:1); $[\alpha]_D$ –73.3 (c 0.2, $CHCl_3$). ^{1}H NMR

(600.13 MHz, $CDCl_3$): δ 8.04–7.23 (m, 20 H, arom. *H*), 5.94 (dd, 1 H, $J_{3',4'}$ = 9.5, $J_{3',2'}$ = 9.8 Hz, H-3′), 5.66 (dd, 1 H, $J_{4',5'}$ = 10.0 Hz, H-4′), 5.66 (d, 1 H, $J_{1,2}$ = 0.9 Hz, H-1), 5.52 (dd, 1 H, $J_{2',1'}$ = 8.0 Hz, H-2′), 5.34 (d, 1 H, H-1′), 4.66 (dd, 1 H, $J_{6'a,5'}$ = 3.1, $J_{6'a,6'b}$ = –12.1 Hz, H-6′a), 4.48 (dd, 1 H, $J_{6'b,5'}$ = 5.7 Hz, H-6′b), 4.20 (dd, 1 H, $J_{2,3}$ = 5.6 Hz, H-2), 4.15 (ddd, 1 H, H-5′), 4.04 (dq, $J_{5,6}$ = 6.2, $J_{5,4}$ = 10.0 Hz, H-5), 4.03 (dd, 1 H, $J_{3,4}$ = 7.5 Hz, H-3), 3.67 (dd, 1 H, H-4), 1.48 and 1.26 (each s, each 3 H, $O_2C(C\mathit{H}_3)_2$), 1.22 (d, 3 H, H-6) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.1 (6′-O*C*OPh), 165.8 (3′-O*C*OPh), 165.3 (2′-O*C*OPh, 4′-O*C*OPh), 133.5–127.7 (arom. *C*), 109.5 ($O_2\mathit{C}(CH_3)_2$), 100.5 (C-1′), 83.6 (C-1), 81.0 (C-4), 77.7 (C-3), 76.6 (C-2), 73.1 (C-3′), 72.3 (C-5′), 72.1 (C.2′), 69.9 (C-4′), 65.5 (C-5), 63.2 (C-6′), 27.9 and 26.3 ($O_2C(\mathit{C}H_3)_2$), 17.3 (C-6) ppm.

HRMS: *m/z* calcd. for $C_{49}H_{46}O_{13}SNa$ $[M + Na]^+$ 897.2557; found 897.2554.

Phenyl 4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-1-thio-α-L-rhamnopyranoside (37). Synthesized from **36** (300 mg, 0.34 mmol) according to the general procedure for removal of isopropylidene acetal to give **37** as a colorless oil (210 mg, 95 %). R_f = 0.28 (hexane:EtOAc 1:1); $[\alpha]_D$ –125.0 (c 0.1, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$): δ 8.04–7.20 (m, 20 H, arom. *H*), 5.95 (dd, 1 H, $J_{3',4'}$ = 9.5, $J_{3',2'}$ = 9.8 Hz, H-3′), 5.69 (dd, 1 H, $J_{4',5'}$ = 9.9 Hz, H-4′), 5.56 (dd, 1 H, $J_{2',1'}$ = 7.9 Hz, H-2′), 5.42 (d, 1 H, $J_{1,2}$ = 1.4 Hz, H-1), 5.29 (d, 1 H, H-1′), 4.70 (dd, 1 H, $J_{6'a,5'}$ = 3.1, $J_{6'a,6'b}$ = –12.1 Hz, H-6′a), 4.46 (dd, 1 H, $J_{6'b,5'}$ = 5.3 Hz, H-6′b), 4.18 (ddd, 1 H, H-5′), 4.18 (dq, $J_{5,6}$ = 6.2, $J_{5,4}$ = 9.6 Hz, H-5), 4.06 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 3.81 (dd, 1 H, $J_{3,4}$ = 9.3 Hz, H-3), 3.74 (dd, 1 H, H-4), 1.26 (d, 3 H, H-6) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.2 (6′-O*C*OPh), 165.9 (3′-O*C*OPh), 165.4 (4′-O*C*OPh), 165.3 (2′-O*C*OPh), 133.9–127.5 (arom. *C*), 101.0 (C-1′), 87.3 (C-1), 81.4 (C-4), 73.1 (C-3′), 72.6 (C-2), 72.4 (C.2′), 72.3 (C-5′), 71.4 (C-3), 69.7 (C-4′), 67.5 (C-5), 62.9 (C-6′), 17.5 (C-6) ppm.

HRMS: *m/z* calcd. for $C_{46}H_{42}O_{13}SNa$ $[M + Na]^+$ 857.2544; found 857.2215.

Phenyl 2,3-di-*O*-benzoyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-1-thio-α-L-rhamnopyranoside (38). Synthesized from **37** (207 mg, 0.25 mmol) according to the general procedure for benzoylation of hydroxyl groups to give **38** as a slightly yellowish foam (230 mg, 89 %). R_f = 0.60 (hexane:EtOAc 1:1); $[\alpha]_D$ –35.0 (c 0.2, $CHCl_3$). ^1H NMR (600.13 MHz, $CDCl_3$):

δ 8.07–7.05 (m, 30 H, arom. *H*), 5.84 (dd, 1 H, $J_{2,1}$ = 1.7, $J_{2,3}$ = 3.3 Hz, H-2), 5.80 (dd, 1 H, $J_{3',4'}$ = 9.6, $J_{3',2'}$ = 9.9 Hz, H-3′), 5.67 (dd, 1 H, $J_{4',5'}$ = 9.9 Hz, H-4′), 5.55 (dd, 1 H, $J_{2',1'}$ = 7.9 Hz, H-2′), 5.55 (d, 1 H, H-1), 5.55 (dd, 1 H, $J_{3,4}$ = 9.7 Hz, H-3), 5.15 (d, 1 H, H-1′), 4.80 (dd, 1 H, $J_{6'a,5'}$ = 3.2, $J_{6'a,6'b}$ = –12.1 Hz, H-6′a), 4.48 (dd, 1 H, $J_{6'b,5'}$ = 5.3 Hz, H-6′b), 4.48 (dq, $J_{5,6}$ = 6.2, $J_{5,4}$ = 9.4 Hz, H-5), 4.25 (ddd, 1 H, H-5′), 4.14 (dd, 1 H, H-4), 1.48 (d, 3 H, H-6) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.1 (6′-O*C*OPh), 165.7 (3′-O*C*OPh), 165.2 (4′-O*C*OPh), 165.1 (2′-O*C*OPh, 2-O*C*OPh), 164.8 (3-O*C*OPh), 137.8–125.3 (arom. *C*), 101.3 (C-1′), 85.7 (C-1), 77.6 (C-4), 72.8 (C-3′), 72.5 (C-3), 72.0 (C-5′, C-2), 71.8 (C-2′), 69-7 (C-4′), 68.5 (C-5), 62.9 (C-6′), 17.9 (C-6) ppm.

HRMS: *m/z* calcd. for $C_{60}H_{50}O_{15}SNa$ $[M + Na]^+$ 1065.2768; found 1065.2763.

2,3-Di-*O*-benzoyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-α-L-rhamnopyranose trichloroacetimidate (39). Synthesized from **38** (157 mg, 0.15 mmol) according to the general procedure for converting thio-glycoside into an imidate donor to give 2,3-di-*O*-benzoyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-α-L-rhamnopyranose as a white foam (131 mg, 92 %). The synthesis was continued from 2,3-di-*O*-benzoyl-4-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glycopyranosyl)-α-L-rhamnopyranose (97 mg, 0.10 mmol) according to the general procedure for converting thio-glycoside into an imidate donor to give the title compound as a white foam (86 mg, 77 %). R_f = 0.64 (hexane:EtOAc 1:1); $[\alpha]_D$ +25.2 (c 0.2, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.71 (s, 1 H, OCN*H*CCl_3), 8.06–7.05 (m, 30 H, arom. *H*), 6.33 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 5.76 (dd, 1 H, $J_{2,3}$ = 3.4 Hz, H-2), 5.75 (dd, 1 H, $J_{3',4'}$ = 9.5, $J_{3',2'}$ = 9.9 Hz, H-3′), 5.62 (dd, 1 H, $J_{4',5'}$ = 9.9 Hz, H-4′), 5.52 (dd, 1 H, $J_{3,4}$ = 9.6 Hz, H-3), 5.49 (dd, 1 H, $J_{2',1'}$ = 7.9 Hz, H-2′), 5.12 (d, 1 H, H-1′), 4.78 (dd, 1 H, $J_{6'a,5'}$ = 3.2, $J_{6'a,6'b}$ = –12.0 Hz, H-6′a), 4.45 (dd, 1 H, $J_{6'b,5'}$ = 5.4 Hz, H-6′b), 4.21 (ddd, 1 H, H-5′), 4.18 (dq, $J_{5,6}$ = 6.2, $J_{5,4}$ = 9.6 Hz, H-5), 4.12 (dd, 1 H, H-4), 1.49 (d, 3 H, H-6) ppm.

HRMS: *m/z* calcd. for $C_{56}H_{46}O_{16}Cl_3Na$ $[M + Na]^+$ 1116.1780; found 1116.1755.

3β-(2′,3′-di-*O*-benzoyl-4′-(2″,3″,4″,6″-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-L-rhamnopyranosyloxy)-androst-5-en-17-one (43). Synthesized from **40** (17 mg, 0.06 mmol) and **39** (82 mg, 0.07 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **43** as a white foam (82 mg, 88 %). R_f = 0.48 (hexane:EtOAc 1:1); $[\alpha]_D$ +15.0 (c 0.2, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 8.06–7.07 (m, 30 H, arom. *H*), 5.74 (dd, 1 H, $J_{3'',4''}$ = 9.7, $J_{3'',2''}$ = 9.8 Hz, H-3″), 5.63 (dd, 1 H, $J_{4'',5''}$ = 9.9 Hz, H-4″), 5.49 (dd, 1 H, $J_{3',2'}$ = 3.4, $J_{3',4'}$ = 9.7 Hz, H-3′), 5.49 (dd, 1 H, $J_{2',1'}$ = 1.5 Hz, H-2′), 5.49 (dd, 1 H, $J_{2'',1''}$ = 7.9 Hz, H-2″), 5.34 (ddd, 1 H, $J_{6,4ax}$ = –1.8, $J_{6,7ax}$ = 2.1, $J_{6,7eq}$ = 5.2 Hz, H-6), 5.08 (d, 1 H, H-1″), 5.01 (d, 1 H, H-1′), 4.78 (dd, 1 H, $J_{6''a,5''}$ = 3.3, $J_{6''a,6''b}$ = –12.0 Hz, H-6″a), 4.46 (dd, 1 H, $J_{6''b,5''}$ = 5.3 Hz, H-6″b), 4.21 (ddd, 1 H, H-5″), 4.06 (dq, $J_{5',6'}$ = 6.2, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.49 (dddd, 1 H, $J_{3,2eq}$ = 4.5, $J_{3,4eq}$ = 4.8, $J_{3,4ax}$ = 11.3, $J_{3,2ax}$ = 11.5 Hz, H-3), 2.46 (ddd, 1 H, $J_{16eq,15eq}$ = 1.0, $J_{16eq,15ax}$ = 8.9, $J_{16eq,16ax}$ = –19.3 Hz, H-16eq), 2.35 (ddd, 1 H, $J_{4eq,2ax}$ = –2.3, $J_{4eq,4ax}$ = –13.3 Hz, H-4eq), 2.27 (dddddd, 1 H, $J_{4ax,2eq}$ = –1.1, $J_{4ax,7eq}$ = 2.7, $J_{4ax,7ax}$ = 3.3 Hz, H-4ax), 2.10 (dddd, 1 H, $J_{7eq,8}$ = 5.0, $J_{7eq,7ax}$ = –16.8 Hz, H-7eq), 2.08 (ddd, 1 H, $J_{16ax,15eq}$ = 8.9, $J_{16ax,15ax}$ = 9.3 Hz, H-16ax), 1.94 (dddd, 1 H, $J_{15eq,14}$ = 5.7, $J_{15eq,15ax}$ = –12.4 Hz, H-15eq), 1.93 (dddd, 1 H, $J_{2eq,1ax}$ = 3.9, $J_{2eq,2ax}$ = –12.2 Hz, H-2eq), 1.88 (ddd, 1 H, $J_{1eq,2eq}$ = 3.3, $J_{1eq,2ax}$ = 3.7, $J_{1eq,1ax}$ = –13.8 Hz, H-1eq), 1.86 (ddd, 1 H, $J_{12eq,11eq}$ = 2.7, $J_{12eq,11ax}$ = 4.3, $J_{12eq,12ax}$ = –13.1 Hz, H-12eq), 1.69 (dddd, $J_{11eq,12ax}$ = 4.1, $J_{11eq,9}$ = 4.5, $J_{11eq,11ax}$ = –13.8 Hz, H-11eq), 1.67 (ddddd, 1 H, $J_{2ax,1ax}$ = 13.4 Hz, H-2ax), 1.66 (dddd, 1 H, $J_{8,7ax}$ = 10.2, $J_{8,14}$ = 10.8, $J_{8,9}$ = 11.0 Hz, H-8), 1.62 (dddd, 1 H, H-7ax), 1.54 (dddd, 1 H, $J_{15ax,14}$ = 12.9 Hz, H-15ax), 1.50 (dddd, 1 H, $J_{11ax,9}$ = 11.0, $J_{11ax,12ax}$ = 13.4 Hz, H-11ax), 1.42 (d, 1 H, H-6′), 1.29 (ddd, 1 H, H-12ax), 1.28 (ddd, 1 H, H-14), 1.08 (ddd, 1 H, H-1ax), 1.05 (s, 3 H, H-19), 1.00 (ddd, 1 H, H-9), 0.89 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$): δ 221.2 (C-17), 166.1 (6″-O*C*OPh), 165.7 (3″-O*C*OPh), 165.4 (2′-O*C*OPh), 165.2 (4″-O*C*OPh, 2″-O*C*OPh), 164.8 (3′-OCOPh), 140.7 (C-5), 133.5–128.1 (arom. *C*), 121.1 (C-6), 101.4 (C-1″), 95.9 (C-1′), 77.9 (C-3), 77.8 (C-4′), 72.8 (C-3″), 72.3 (C-3′), 71.9 (C-5″), 71.8 (C-2″), 71.2 (C-2′), 69.8 (C-4″), 67.0 (C-5′), 63.0 (C-6″), 51.8 (C-14), 50.2 (C-9), 47.6 (C-13), 38.5 (C-4), 37.2 (C-1), 36.9 (C-10), 35.9 (C-16), 31.5 (C-8), 31.4 (C-12), 30.8 (C-7), 29.3 (C-2), 21.9 (C-15), 20.3 (C-11), 19.4 (C-19), 18.0 (C-6′), 13.5 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{73}H_{72}O_{17}Na$ $[M + Na]^+$ 1243.4667; found 1243.4642.

3β-(α-L-rhamnopyranosyloxy)-androst-5-en-17-one (44). Synthesized from **40** (32 mg, 0.12 mmol) and **8** (100 mg, 0.16 mmol) according to the general procedure for glycosylation of steroids and tetracyclines, however a pure product was not obtained and thus the deprotection step was done according to the general procedure for deprotection of glycosteroids and anthracyclines providing **10** as a white solid (40 mg, 89 % over two steps). R_f = 0.23 (EtOAc). ^{1}H NMR (600.13 MHz, CD_3OD and $CDCl_3$): δ 5.39 (ddd, 1 H, $J_{6,4ax}$ = –1.9, $J_{6,7ax}$ = 2.0, $J_{6,7eq}$ = 5.2 Hz, H-6), 4.88 (d, 1 H, $J_{1',2'}$ = 1.7 Hz, H-1′), 3.82 (dd, 1 H, $J_{2',3'}$ = 3.4 Hz, H-2′), 3.71 (dd, 1 H, $J_{3',4'}$ = 9.4 Hz, H-3′), 3.69 (dq, 1 H, $J_{5',6'}$ = 6.2, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.50 (dddd, 1 H, $J_{3,2eq}$ = 4.4, $J_{3,4eq}$ = 4.8, $J_{3,2ax}$ = 11.4, $J_{3,4ax}$ = 11.4 Hz, H-3), 3.39 (dd, 1 H, H-4′), 2.48 (ddd, 1 H, $J_{16eq,15eq}$ = 0.4, $J_{16eq,15ax}$ = 8.8, $J_{16eq,16ax}$ = –19.5 Hz, H-16eq), 2.38 (ddd, 1 H, $J_{4eq,2ax}$ = –2.1, $J_{4eq,4ax}$ = –13.1 Hz, H-4eq), 2.20 (ddddd, 1 H, $J_{4ax,7ax}$ = 3.2, $J_{4ax,7eq}$ = 3.2 Hz, H-4ax), 2.13 (dddd, 1 H, $J_{7eq,8}$ = 5.4, $J_{7eq,7ax}$ = –17.4 Hz, H-7eq), 2.11 (ddd, 1 H, $J_{16ax,15eq}$ = 8.9, $J_{16ax,15ax}$ = 9.3 Hz, H-16ax), 1.98 (dddd, 1 H, $J_{15eq,14}$ = 5.8, $J_{15eq,15ax}$ = –12.5 Hz, H-15eq), 1.88 (ddd, 1 H, $J_{1eq,2eq}$ = 3.3, $J_{1eq,2ax}$ = 3.7, $J_{1eq,1ax}$ = –13.5 Hz, H-1eq), 1.87 (ddd, 1 H, $J_{2eq,1ax}$ = 3.8, $J_{2eq,2ax}$ = –12.6 Hz, H-2eq), 1.84 (ddd, 1 H, $J_{12eq,11eq}$ = 2.8, $J_{12eq,11ax}$ = 4.3, $J_{12eq,12ax}$ = –13.5 Hz, H-12eq), 1.70 (dddd, $J_{11eq,12ax}$ = 4.2, $J_{11eq,9}$ = 5.3, $J_{11eq,11ax}$ = –13.7 Hz, H-11eq), 1.69 (dddd, 1 H, $J_{8,7ax}$ = 10.3, $J_{8,9}$ = 10.9, $J_{8,14}$ = 11.2 Hz, H-8), 1.68 (ddd, 1 H, H-7ax), 1.59 (ddddd, 1 H, $J_{2ax,1ax}$ = 14.0 Hz, H-2ax), 1.58 (dddd, 1 H, $J_{15ax,14}$ = 12.7 Hz, H-15ax), 1.51 (dddd, 1 H, $J_{11ax,9}$ = 12.3, $J_{11ax,12ax}$ = 13.8 Hz, H-11ax), 1.32 (ddd, 1 H, H-14), 1.28 (d, 1 H, H-6′), 1.29 (ddd, 1 H, H-12ax), 1.11 (ddd, 1 H, H-1ax), 1.05 (s, 3 H, H-19), 1.03 (ddd, 1 H, H-9), 0.91 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CD_3OD and $CDCl_3$): δ 222.8 (C-17), 140.4 (C-5), 121.0 (C-6), 97.9 (C-1′), 76.2 (C-3), 72.8 (C-4′), 71.2 (C-3′), 72.1 (C-2′), 68.1 (C-5′), 51.6 (C-14), 50.1 (C-9), 47.6 (C-13), 38.2 (C-4), 37.1 (C-1), 36.7 (C-10), 35.7 (C-16), 31.3 (C-8), 31.2 (C-12), 30.6 (C-7), 29.1 (C-2), 21.7 (C-15), 20.1 (C-11), 19.1 (C-19), 17.1 (C-6′), 12.3 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{25}H_{38}O_6Na$ $[M + Na]^+$ 457.2566; found 457.2549.

3β-(β-D-glucopyranosyloxy)-androst-5-en-17-one (45). Synthesized from **40** (37 mg, 0.13 mmol) and **9** (133 mg, 0.18 mmol) according to the general procedure for glycosylation of steroids and tetracyclines, however a pure product was not obtained and thus the deprotection step was done according to the general procedure for deprotection of glycosteroids and anthracyclines providing **45** as a white powder (41 mg, 78 % over two steps). R_f = 0.60 ($MeOH:CH_2Cl_2$ 1:5). ^{1}H NMR (600.13 MHz, CD_3OD and $CDCl_3$): δ 5.42 (ddd, 1 H, $J_{6,4ax}$ = –1.6, $J_{6,7ax}$ = 2.5, $J_{6,7eq}$ = 5.3 Hz, H-6), 4.40 (d, 1 H, $J_{1',2'}$ = 7.8 Hz, H-1′), 3.86 (dd, 1 H, $J_{6'a,5'}$ = 2.6,

$J_{6'a,6'b}$ = –11.9 Hz, H-6′a), 3.71 (dd, 1 H, $J_{6'b,5'}$ = 5.5 Hz, H-6′b), 3.62 (dddd, 1 H, $J_{3,2eq}$ = 4.4, $J_{3,4eq}$ = 4.7, $J_{3,4ax}$ = 11.3, $J_{3,2ax}$ = 11.6 Hz, H-3), 3.40 (dd, 1 H, $J_{3',4'}$ = 8.9, $J_{3',2'}$ = 9.3 Hz, H-3′), 3.35 (dd, 1 H, $J_{4',5'}$ = 9.8 Hz, H-4′), 3.29 (ddd, 1 H, H-5′), 3.21 (dd, 1 H, H-2′), 2.48 (ddd, 1 H, $J_{16eq,15eq}$ = 1.1, $J_{16eq,15ax}$ = 8.9, $J_{16eq,16ax}$ = –19.6 Hz, H-16eq), 2.46 (ddd, 1 H, $J_{4eq,2ax}$ = –2.4, $J_{4eq,4ax}$ = –13.2 Hz, H-4eq), 2.30 (dddddd, 1 H, $J_{4ax,2eq}$ = –2.4, $J_{4ax,7ax}$ = 2.6, $J_{4ax,7eq}$ = 2.7 Hz, H-4ax), 2.14 (dddd, 1 H, $J_{7eq,8}$ = 5.0, $J_{7eq,7ax}$ = –17.3 Hz, H-7eq), 2.11 (ddd, 1 H, $J_{16ax,15eq}$ = 8.9, $J_{16ax,15ax}$ = 9.4 Hz, H-16ax), 1.99 (dddd, 1 H, $J_{15eq,14}$ = 5.9, $J_{15eq,15ax}$ = –12.4 Hz, H-15eq), 1.96 (ddddd, $J_{2eq,1eq}$ = 2.4, $J_{2eq,1ax}$ = 3.8, $J_{2eq,2ax}$ = –12.9 Hz, H-2eq), 1.90 (ddd, 1 H, $J_{1eq,2ax}$ = 3.6, $J_{1eq,1ax}$ = –13.4 Hz, H-1eq), 1.82 (ddd, 1H, $J_{12eq,11eq}$ = 2.6, $J_{12eq,11ax}$ = 4.2, $J_{12eq,12ax}$ = –12.8 Hz, H-12eq), 1.71 (dddd, 1 H, $J_{8,7ax}$ = 10.9, $J_{8,14}$ = 10.9, $J_{8,9}$ = 11.1 Hz, H-8), 1.70 (dddd, $J_{11eq,12ax}$ = 4.1, $J_{11eq,9}$ = 4.8, $J_{11eq,11ax}$ = –13.9 Hz, H-11eq), 1.68 (dddd, 1 H, H-7ax), 1.64 (ddddd, 1 H, $J_{2ax,1ax}$ = 13.9 Hz, H-2ax), 1.60 (dddd, 1 H, $J_{15ax,14}$ = 12.8 Hz, H-15ax), 1.53 (dddd, 1 H, $J_{11ax,12ax}$ = 12.5, $J_{11ax,9}$ = 13.0 Hz, H-11ax), 1.34 (ddd, 1 H, H-14), 1.29 (ddd, 1 H, H-12ax), 1.11 (ddd, 1 H, H-1ax), 1.07 (s, 3 H, H-19), 1.03 (ddd, 1 H, H-9), 0.91 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CD_3OD and $CDCl_3$): δ 222.4 (C-17), 140.2 (C-5), 120.5 (C-6), 100.7 (C-1′), 78.1 (C-3), 76.1 (C-3′), 75.8 (C-5′), 73.1 (C-2′), 69.8 (C-4′), 61.1 (C-6′), 51.2 (C-14), 49.8 (C-9), 47.1 (C-13), 38.0 (C-4), 36.7 (C-1), 36.2 (C-10), 35.2 (C-16), 30.9 (C-8), 30.8 (C-12), 30.2 (C-7), 28.9 (C-2), 21.2 (C-15), 19.7 (C-11), 18.4 (C-19), 12.6 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{25}H_{38}O_7Na$ $[M + Na]^+$ 473.2515; found 473.2513.

3β-(4′-(β-D-glucopyranosyl)-α-L-rhamnopyranosyloxy)-androst-5-en-17-one (46). Synthesized from **43** (55 mg, 0.05 mmol) according to the general procedure for deprotection of steroids and anthracyclines providing **46** as a white solid (24 mg, 90 %). R_f = 0.35 (MeOH:CH_2Cl_2 1:5); $[\alpha]_D$ –32.1 (c 1.0, MeOH). ^{1}H NMR (600.13 MHz, CD_3OD): δ 5.42 (ddd, 1 H, $J_{6,4ax}$ = –1.8, $J_{6,7ax}$ = 2.2, $J_{6,7eq}$ = 5.2 Hz, H-6), 4.82 (d, 1 H, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.58 (d, 1 H, $J_{1'',2''}$ = 7.8 Hz, H-1″), 3.87 (dd, 1 H, $J_{3',2'}$ = 3.3, $J_{3',4'}$ = 9.7 Hz, H-3′), 4.84 (dd, 1 H, $J_{6''a,5''}$ = 2.4, $J_{6''a,6''b}$ = –11.9 Hz, H-6″a), 3.77 (dd, 1 H, H-2′), 3.70 (dq, $J_{5',6'}$ = 6.4, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.69 (dd, 1 H, $J_{6''b,5''}$ = 5.3 Hz, H-6″b), 3.60 (dd, 1 H, H-4′), 3.44 (dddd, 1 H, $J_{3,2eq}$ = 4.4, $J_{3,4eq}$ = 4.7, $J_{3,2ax}$ = 11.2, $J_{3,4ax}$ = 11.6 Hz, H-3), 3.36 (dd, 1 H, $J_{3'',2''}$ = 9.2, $J_{3'',4''}$ = 9.6 Hz, H-3″), 3.30 (dd, 1 H, $J_{4'',5''}$ = 9.0 Hz, H-4″), 3.26 (ddd, 1 H, H-5″), 3.21 (dd, 1 H, H-2″), 2.45 (ddd, 1 H, $J_{16eq,15eq}$ =

0.4, $J_{16eq,15ax}$ = 8.9, $J_{16eq,16ax}$ = –19.5 Hz, H-16eq), 2.38 (ddd, 1 H, $J_{4eq,2ax}$ = –2.3, $J_{4eq,4ax}$ = –13.1 Hz, H-4eq), 2.19 (dddddd, 1 H, $J_{4ax,2eq}$ = –2.2, , $J_{4ax,7eq}$ = 2.9, $J_{4ax,7ax}$ = 3.2 Hz, H-4ax), 2.13 (dddd, 1 H, $J_{7eq,8}$ = 4.7, $J_{7eq,7ax}$ = –16.6 Hz, H-7eq), 2.08 (ddd, 1 H, $J_{16ax,15eq}$ = 9.0, $J_{16ax,15ax}$ = 9.4 Hz, H-16ax), 1.97 (dddd, 1 H, $J_{15eq,14}$ = 5.8, $J_{15eq,15ax}$ = –12.5 Hz, H-15eq), 1.91 (ddd, 1 H, $J_{1eq,2eq}$ = 3.4, $J_{1eq,2ax}$ = 3.8, $J_{1eq,1ax}$ = –13.4 Hz, H-1eq), 1.85 (dddd, 1 H, $J_{2eq,1ax}$ = 3.8, $J_{2eq,2ax}$ = –12.7 Hz, H-2eq), 1.79 (ddd, 1 H, $J_{12eq,11eq}$ = 2.6, $J_{12eq,11ax}$ = 4.2, $J_{12eq,12ax}$ = –12.7 Hz, H-12eq), 1.70 (dddd, 1 H, $J_{8,7ax}$ = 10.5, $J_{8,14}$ = 11.1, $J_{8,9}$ = 11.2 Hz, H-8), 1.70 (dddd, $J_{11eq,12ax}$ = 4.3, $J_{11eq,9}$ = 5.0, $J_{11eq,11ax}$ = –13.4 Hz, H-11eq), 1.68 (dddd, 1 H, H-7ax), 1.59 (dddd, 1 H, $J_{15ax,14}$ = 12.7 Hz, H-15ax), 1.58 (ddddd, 1 H, $J_{2ax,1ax}$ = 13.9 Hz, H-2ax), 1.54 (dddd, 1 H, $J_{11ax,9}$ = 12.6, $J_{11ax,12ax}$ = 13.5 Hz, H-11ax), 1.34 (ddd, 1 H, H-14), 1.31 (d, 1 H, H-6′), 1.27 (ddd, 1 H, H-12ax), 1.11 (ddd, 1 H, H-1ax), 1.06 (s, 3 H, H-19), 1.04 (ddd, 1 H, H-9), 0.89 (s, 3 H, H-18) ppm.

^{13}C NMR (150.9 MHz, CD_3OD): δ 223.8 (C-17), 141.9 (C-5), 122.4 (C-6), 105.7 (C-1″), 99.6 (C-1′), 83.6 (C-4′), 78.2 (C-3″), 78.0 (C-5″), 77.9 (C-3), 76.1 (C-2″), 72.6 (C-2′), 72.4 (C-3′), 71.5 (C-4″), 68.5 (C-5′), 62.7 (C-6″), 53.0 (C-14), 51.8 (C-9), 48.8 (C-13), 39.5 (C-4), 38.5 (C-1), 38.0 (C-10), 36.7 (C-16), 32.8 (C-8), 32.7 (C-12), 31.9 (C-7), 30.6 (C-2), 22.8 (C-15), 21.5 (C-11), 19.8 (C-19), 18.1 (C-6′), 13.9 (C-18) ppm.

HRMS: *m/z* calcd. for $C_{31}H_{48}O_{11}Na$ $[M + Na]^+$ 619.3094; found 619.3080.

7-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-glucopyranosyl)-4-deoxy-ε-rhodomycinone (48). Synthesized from **47** (29 mg, 0.07 mmol) and **9** (57 mg, 0.077 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **48** as a red solid (67 mg, 96 %). R_f = 0.96 (MeOH:CH_2Cl_2 1:10); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 13.41 (s, 1 H, 6-O*H*), 13.18 (s, 1 H, 11-O*H*), 8.29–6.90 (m, 24 H, arom. *H*), 6.02 (dd, 1 H, $J_{3',4'}$ = 9.5, $J_{3',2'}$ = 10.0 Hz, H-3′), 5.72 (dd, 1 H, $J_{4',5'}$ = 10.0 Hz, H-4′), 5.56 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.45 (dd, 1 H, H-2′), 5.34 (dd, 1 H, $J_{7,8a}$ = 1.8, $J_{7,8b}$ = 4.2 Hz, H-7), 4.76 (dd, 1 H, $J_{6'a,5'}$ = 3.1, $J_{6'a,6'b}$ = −12.1 Hz, H-6′a), 4.58 (dd, 1 H, $J_{6'b,5'}$ = 5.0 Hz, H-6′b), 4.32 (d, 1 H, $J_{10,8a}$ = −1.2 Hz, H-10), 4.30 (ddd, 1 H, H-5′), 3.67 (s, 3 H, 10-COOCH_3), 2.55 (ddd, 1 H, $J_{8a,8b}$ = −15.0 Hz, H-8a), 2.20 (dd, 1 H, H-8b), 1.73 (dq, 1 H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −13.9 Hz, H-13a), 1.45 (dq, 1 H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.10 (dd, 1 H, H-14).

^{13}C NMR (150.90 MHz, CDCl$_3$): δ 186.7 (C-5), 186.2 (C-12), 171.7 (10-*C*OOCH$_3$), 166.3 (6′-O*C*OPh), 165.8 (3′-O*C*OPh), 165.3 (4′-O*C*OPh), 165.2 (2′-O*C*OPh), 156.3 (C-6), 156.1 (C-11), 135.2–128.1 (arom. *C*), 111.7 (C-11a), 111.2 (C-5a), 103.1 (C-1′), 72.5 (C-5′), 72.4 (C-3′), 72.1 (C-2′), 71.8 (C-7), 70.8 (C-9), 69.8 (C-4′), 63.0 (C-6′), 52.4 (10-COO*C*H$_3$), 51.4 (C-10), 34.7 (C-8), 32.8 (C-13), 6.8 (C-14).

HRMS: *m/z* calcd. for $C_{56}H_{46}O_{17}Na_1$ [M+Na]$^+$ 1013.2633; found 1013.2668.

7-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-α-D-mannopyranosyl)-4-deoxy-ε-rhodomycinone (49). Synthesized from **47** (31 mg, 0.074 mmol) and **7** (61 mg, 0.081 mmol) according to the general procedure for glycosylation of steroids and anthracyclines providing **49** as an orange solid (containing 10 % of hydrolyzed donor) (107 mg). R_f = 0.58 (hexane:EtOAc 1:1) . ^{1}H NMR (600.13 MHz, CDCl$_3$): δ 13.80 (s, 1 H, 6-O*H*), 13.40 (s, 1 H, 11-O*H*), 8.40–7.21 (m, 24 H, arom. *H*), 6.23 (dd, 1 H, $J_{4',3'}$ = 10.1, $J_{4',5'}$ = 10.2 Hz, H-4′), 5.82 (dd, 1 H, $J_{3',2'}$ = 3.2 Hz, H-3′), 5.64 (d, 1 H, $J_{1',2'}$ = 1.9 Hz, H-1′), 5.64 (dd, 1 H, $J_{7,8a}$ = 1.7, $J_{7,8b}$ = 3.9 Hz, H-7), 5.61 (dd, 1 H, H-2′), 4.96 (ddd, 1 H, $J_{5',6'a}$ = 2.5, $J_{5',6'b}$ = 3.6 Hz, H-5′), 4.83 (dd, 1 H, $J_{6'a,6'b}$ = –12.2 Hz, H-6′a), 4.58 (dd, 1 H, H-6′b), 4.48 (d, 1 H, $J_{10,8a}$ = –1.1 Hz, H-10), 3.74 (s, 3 H, 10-COOC*H*$_3$), 2.53 (ddd, 1 H, $J_{8a,8b}$ = –15.4 Hz, H-8a), 2.20 (dd, 1 H, H-8b), 1.86 (dq, 1 H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = –13.9 Hz, H-13a), 1.57 (dq, 1 H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.21 (dd, 1 H, H-14).

^{13}C NMR (150.90 MHz, CDCl$_3$): δ 187.1 (C-5), 187.0 (C-12), 171.7 (10-*C*OOCH$_3$), 166.4 (6′-O*C*OPh), 165.7 (2′-O*C*OPh), 165.6 (4′-O*C*OPh, 3′-O*C*OPh), 157.0 (C-6), 156.3 (C-11), 136.4–129.1 (arom. *C*), 112.5 (C-11a), 111.9 (C-5a), 95.1 (C-1′), 71.1 (C-2′), 71.0 (C-9), 70.2 (C-5′), 70.0 (C-3′), 67.7 (C-7), 66.6 (C-4′), 62.9 (C-6′), 52.6 (10-COO*C*H$_3$), 52.1 (C-10), 32.7 (C-13), 29.8 (C-8), 6.9 (C-14).

HRMS: *m/z* calcd. for $C_{56}H_{46}O_{17}Na_1$ [M+Na]$^+$ 1013.2633; found 1013.2626.

7-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-α-L-rhamnopyranosyl)-4-deoxy-ε-rhodomycinone (50). Synthesized from **47** (25 mg, 0.06 mmol) and **8** (41 mg, 0.067 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **50** as a orange solid (containing 10 % of hydrolyzed donor) (68 mg). R_f = 0.74 (hexane:EtOAc 1:1) . ^{1}H NMR (600.13 MHz, CDCl$_3$): δ 13.53 (s, 1 H, 6-O*H*), 13.37 (s, 1 H, 11-O*H*), 8.36–7.17 (m, 19 H, arom. *H*), 5.82

(dd, 1 H, $J_{2',1'}$ = 1.9, $J_{2',3'}$ = 3.4 Hz, H-2′), 5.73 (dd, 1 H, $J_{4',5'}$ = 9.8, $J_{4',3'}$ = 10.1 Hz, H-4′), 5.65 (d, 1 H, H-1′), 5.64 (dd, 1 H, H-3′), 5.40 (dd, 1 H, $J_{7,8a}$ = 1.7, $J_{7,8b}$ = 4.4 Hz, H-7), 4.45 (dq, 1 H, $J_{5',6'}$ = 6.2 Hz, H-5′), 4.40 (d, 1 H, $J_{10,8a}$ = –1.2 Hz, H-10), 3.75 (s, 3 H, 10-COOC*H*$_3$), 2.50 (ddd, 1 H, $J_{8a,8b}$ = –15.0 Hz, H-8a), 2.36 (dd, 1 H, H-8b), 1.92 (dq, 1 H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = –14.0 Hz, H-13a), 1.54 (dq, 1 H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.45 (d, 1 H, H-6′), 1.20 (dd, 1 H, H-14).

^{13}C NMR (150.90 MHz, $CDCl_3$): δ 187.1 (C-12), 186.7 (C-5), 171.5 (10-*C*OOCH$_3$), 165.9 (4′-O*C*OPh), 165.4 (2′-O*C*OPh, 3′-O*C*OPh), 156.8 (C-6), 156.1 (C-11), 134.6–127.2 (arom. *C*), 112.3 (C-11a), 111.9 (C-5a), 101.5 (C-1′), 72.8 (C-7), 71.6 (C-4′), 71.1 (C-9), 70.6 (C-2′), 70.0 (C-3′), 68.1 (C-5′), 52.6 (10-COO*C*H$_3$), 52.0 (C-10), 33.8 (C-8), 32.4 (C-13), 17.7 (C-6′), 6.9 (C-14).

HRMS: *m/z* calcd. for $C_{49}H_{42}O_{15}Na_1$ $[M+Na]^+$ 893.2421; found 893.2400.

7-*O*-(2′,3′,4′,6′-tetra-*O*-benzoyl-β-D-galactopyranosyl)-4-deoxy-ε-rhodomycinone (51). Synthesized from **47** (41 mg, 0.10 mmol) and **10** (82 mg, 0.11 mmol) according to the general procedure for glycosylation of steroids and tetracyclines providing **51** as a red solid (containing 10 % hydrolyzed donor) (136 mg). R_f = 0.94 (MeOH:CH_2Cl_2 1:10); ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 13.42 (s, 1 H, 6-O*H*), 13.20 (s, 1 H, 11-O*H*), 8.32–6.90 (m, 24 H, arom. *H*), 6.06 (dd, 1 H, $J_{4',5'}$ = 1.4, $J_{4',3'}$ = 3.5 Hz, H-4′), 5.77 (dd, 1 H, $J_{3',2'}$ = 10.5 Hz, H-3′), 5.68 (dd, 1 H, $J_{2',1'}$ = 8.0 Hz, H-2′), 5.55 (d, 1 H, H-1′), 5.39 (dd, 1 H, $J_{7,8a}$ = 1.8, $J_{7,8b}$ = 4.2 Hz, H-7), 4.78 (dd, 1 H, $J_{6'a,5'}$ = 6.5, $J_{6'a,6'b}$ = –11.2 Hz, H-6′a), 4.50 (ddd, 1 H, $J_{5',6'b}$ = 6.6 Hz, H-5′), 4.49 (dd, 1 H, H-6′b), 4.37 (d, $J_{10,8a}$ = 1.2 Hz, 1 H, H-10), 3.69 (s, 3 H, 10-COOC*H*$_3$), 2.61 (ddd, 1 H, $J_{8a,8b}$ = –14.9 Hz, H-8a), 2.26 (dd, 1 H, H-8b), 1.75 (dq, 1 H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = –14.0 Hz, H-13a), 1.55 (dq, 1 H, $J_{13b,14}$ = 7.4 Hz, H-13b), 1.20 (dd, 1 H, H-14).

^{13}C NMR (150.90 MHz, $CDCl_3$): δ 186.5 (C-5), 186.0 (C-12), 171.5 (10-*C*OOCH$_3$), 166.0 (6′-O*C*OPh), 165.6 (4′-O*C*OPh), 165.4 (3′-O*C*OPh), 165.1 (2′-O*C*OPh), 156.1 (C-6), 155.9 (C-11), 135.2–126.6 (arom. *C*), 111.5 (C-11a), 111.0 (C-5a), 103.3 (C-1′), 71.7 (C-7), 71.6 (C-5′), 71.0 (C-9), 70.7 (C-3′), 69.8 (C-2′), 68.1 (C-4′), 62.0 (C-6′), 52.3 (10-COO*C*H$_3$), 51.0 (C-10), 34.8 (C-8), 32.9 (C-13), 6.7 (C-14).

HRMS: *m/z* calcd. for 1013.2633; found 1013.2564.

7-*O*-(β-D-glucopyranosyl)-4-deoxy-ε-rhodomycinone (52). Synthesized from **48** (91 mg, 0.09 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **52** as a red solid (51 mg, 97 %). R_f = 0.14 (MeOH:CH_2Cl_2 1:10). ^{1}H NMR (600.13 MHz, CD_3OD+$CDCl_3$): δ 8.20–7.75 (m, 4 H, arom. *H*), 5.29 (dd, 1 H, $J_{7,8a}$ = 1.7, $J_{7,8b}$ = 4.5 Hz, H-7), 4.91 (d, 1 H, $J_{1',2'}$ = 7.8 Hz, H-1′), 4.24 (d, 1 H, $J_{10,8a}$ = –1.2 Hz, H-10), 3.93 (dd, 1 H, $J_{6'a,5'}$ = 2.2, $J_{6'a,6'b}$ = –11.8 Hz, H-6′a), 3.79 (dd, 1 H, $J_{6'b,5'}$ = 5.1 Hz, H-6′b), 3.70 (s, 3 H, 10-COOC*H*$_3$), 3.47 (dd, 1 H, $J_{3',4'}$ = 8.9, $J_{3',2'}$ = 9.3 Hz, H-3′), 3.43 (ddd, 1 H, $J_{5',4'}$ = 9.8 Hz, H-5′), 3.39 (dd, 1 H, H-4′), 3.22 (dd, 1 H, H-2′), 2.66 (ddd, 1 H, $J_{8a,8b}$ = –15.1 Hz, H-8a), 2.22 (dd, 1 H, H-8b), 1.71 (dq, 1 H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = –14.0 Hz, H-13a), 1.57 (dq, 1 H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.15 (dd, 1 H, H-14).

^{13}C NMR (150.90 MHz, CD_3OD+$CDCl_3$): δ 188.1 (C-5), 187.9 (C-12), 172.8 (10-*C*OOCH$_3$), 157.8 (C-6), 156.8 (C-11), 137.6–127.9 (arom. *C*), 112.9 (C-11a), 112.6 (C-5a), 106.2 (C-1′), 78.1 (C-5′), 77.7 (C-3′), 75.5 (C-2′), 72.3 (C-7), 71.9 (C-9), 71.3 (C-4′), 62.5 (C-6′), 52.8 (10-COO*C*H$_3$), 52.0 (C-10), 36.2 (C-8), 34.1 (C-13), 7.2 (C-14).

HRMS: *m/z* calcd. for $C_{28}H_{30}O_{13}Na_1$ $[M+Na]^+$ 597.1584; found 597.1588.

7-*O*-(α-D-mannopyranosyl)-4-deoxy-ε-rhodomycinone (53). Synthesized from **49** (107 mg (containing impurites), 0.1 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **53** as a red solid (37 mg, 86 % (over two steps)). R_f = 0.16 (MeOH:CH_2Cl_2 1:10) . ^{1}H NMR (600.13 MHz, CD_3OD+$CDCl_3$): δ 8.37–7.85 (m, 4 H, arom. *H*), 5.53 (dd, 1 H, $J_{7,8a}$ = 1.8, $J_{7,8b}$ = 3.9 Hz, H-7), 5.28 (d, 1 H, $J_{1',2'}$ = 1.7 Hz, H-1′), 4.34 (d, 1 H, $J_{10,8a}$ = –1.1 Hz, H-10), 4.14 (ddd, 1 H, $J_{5',6'a}$ = 2.9, $J_{5',6'b}$ = 3.3, $J_{5',4'}$ = 10.0 Hz, H-5′), 3.94 (dd, 1 H, $J_{6'a,6'b}$ = –12.1 Hz, H-6′a), 3.93 (dd, 1 H, H-6′b), 3.82 (dd, 1 H, $J_{4',3'}$ = 9.5, Hz, H-4′), 3.81 (dd, 1 H, $J_{2',3'}$ = 3.3 Hz, H-2′), 3.72 (s, 3 H, 10-COOC*H*$_3$), 3.68 (dd, 1 H, H-3′), 2.48 (ddd, 1 H, $J_{8a,8b}$ = –15.3 Hz, H-8a), 2.07 (dd, 1 H, H-8b), 1.79 (dq, 1 H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = –14.1 Hz, H-13a), 1.54 (dq, 1 H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.15 (dd, 1 H, H-14).

^{13}C NMR (150.90 MHz, CD_3OD+$CDCl_3$): δ 186.7 (C-5), 186.4 (C-12), 171.3 (10-*C*OOCH$_3$), 156.1 (C-6), 155.3 (C-11), 135.1–126.6 (arom. *C*), 111.8 (C-11a), 111.3 (C-5a), 97.0 (C-1′), 73.4 (C-5′), 70.8 (C-3′), 70.7 (C-2′), 70.5 (C-9), 66.3 (C-4′), 65.3 (C-7), 60.9 (C-6′), 51.9 (10-COO*C*H$_3$), 51.6 (C-10), 32.2 (C-13), 29.1 (C-8), 6.0 (C-14).

HRMS: *m/z* calcd. for $C_{28}H_{30}O_{13}Na_1$ $[M+Na]^+$ 597.1584; found 597.1588.

7-*O*-(L-rhamnopyranosyl)-4-deoxy-ε-rhodomycinone (54). Synthesized from **50** (65 mg (containing impurites), 0.074 mmol) according to the general procedure for deprotection glycosteroids and anthracyclines providing **54** as a red solid (30 mg, 90 % over two steps). R_f = 0.45 (EtOAc) . ^{1}H NMR (600.13 MHz, $CD_3OD+CDCl_3$): δ 8.30–7.82 (m, 4 H, arom. *H*), 5.38 (d, 1 H, $J_{1',2'}$ = 1.9 Hz, H-1′), 5.25 (dd, 1 H, $J_{7,8a}$ = 1.7, $J_{7,8b}$ = 4.5 Hz, H-7), 4.26 (d, 1 H, $J_{10,8a}$ = −1.3 Hz, H-10), 3.95 (dd, 1 H, $J_{2',3'}$ = 3.3 Hz, H-2′), 3.88 (dq, 1 H, $J_{5',6'}$ = 6.2, $J_{5',4'}$ = 9.5 Hz, H-5′), 3.73 (s, 3 H, 10-COOCH_3), 3.55 (dd, 1 H, $J_{3',4'}$ = 9.5 Hz, H-3′), 3.48 (dd, 1 H, H-4′), 2.40 (ddd, 1 H, $J_{8a,8b}$ = −15.0 Hz, H-8a), 2.16 (dd, 1 H, H-8b), 1.84 (dq, 1 H, $J_{13a,14}$ = 7.3, $J_{13a,13b}$ = −14.0 Hz, H-13a), 1.50 (dq, 1 H, $J_{13b,14}$ = 7.3 Hz, H-13b), 1.38 (d, 1 H, H-6′), 1.15 (dd, 1 H, H-14).

^{13}C NMR (150.90 MHz, $CD_3OD+CDCl_3$): δ 186.5 (C-5), 186.4 (C-12), 171.3 (10-*C*OOCH$_3$), 156.2 (C-6), 155.4 (C-11), 134.9–126.6 (arom. *C*), 111.4 (C-11a), 111.2 (C-5a), 104.1 (C-1′), 72.2 (C-4′), 71.4 (C-7), 70.8 (C-3′), 70.6 (C-9), 70.3 (C-2′), 69.3 (C-5′), 51.9 (10-COO*C*H$_3$), 51.4 (C-10), 33.6 (C-8), 32.0 (C-13), 16.7 (C-6′), 6.1 (C-14).

HRMS: *m/z* calcd. for $C_{28}H_{30}O_{12}Na_1$ $[M+Na]^+$ 581.1635; found 581.1624.

7-*O*-(β-D-galactopyranosyl)-4-deoxy-ε-rhodomycinone (55). Synthesized from **51** (136 mg, 0.1 mmol) according to the general procedure for deprotection of glycosteroids and anthracyclines providing **55** as a red solid (54 mg, 92% yield over two steps). R_f = 0.17 (MeOH:CH_2Cl_2 1:10). ^{1}H NMR (600.13 MHz, $CD_3OD+CDCl_3$): δ 8.33–7.86 (m, 4 H, arom. *H*), 5.30 (dd, 1 H, $J_{7,8a}$ = 1.9, $J_{7,8b}$ = 4.3 Hz, H-7), 4.83 (d, 1 H, $J_{1',2'}$ = 7.7 Hz, H-1′), 4.28 (s, 1 H, H-10), 3.94 (dd, 1 H, $J_{4',5'}$ = 1.4, $J_{4',3'}$ = 3.2 Hz, H-4′), 3.85 (dd, 1 H, $J_{6'a\text{-}5'}$ = 5.8, $J_{6'a\text{-}6'b}$ = −11.7 Hz, H-6′a), 3.84 (dd, 1 H, $J_{6'b\text{-}5'}$ = 6.3 Hz, H-6′b), 3.72 (s, 3 H, 10-COOCH_3), 3.67 (ddd, 1 H, H-5′), 3.62 (dd, 1 H, $J_{3',2'}$ = 9.5 Hz, H-3′), 3.56 (dd, 1 H, H-2′), 2.69 (ddd, 1 H, $J_{8a,8b}$ = −15.0 Hz, H-8a), 2.21 (dd, 1 H, H-8b), 1.75 (dq, 1 H, $J_{13a,14}$ = 7.1, $J_{13a,13b}$ = −14.0 Hz, H-13a), 1.53 (dq, 1 H, $J_{13b,14}$ = 7.6 Hz, H-13b), 1.14 (dd, 1 H, H-14).

^{13}C NMR (150.90 MHz, $CD_3OD+CDCl_3$): δ 186.5 (C-5, C-12), 171.3 (10-*C*OOCH$_3$), 156.1 (C-6), 155.3 (C-11), 134.6–126.5 (arom. *C*), 111.4 (C-11a), 111.2 (C-5a), 105.1 (C-1′), 74.8 (C-5′),

72.8 (C-3′), 71.4 (C-7), 71.1 (C-2′), 70.1 (C-9), 68.2 (C-4′), 60.6 (C-6′), 51.7 (10-COO*C*H_3), 50.3 (C-10), 34.0 (C-8), 32.5 (C-13), 5.9 (C-14).
HRMS: *m/z* calcd. for $C_{28}H_{30}O_{13}Na_1$ $[M+Na]^+$ 597.1584; found 597.1577.

(*8R,8′R*)-Matairesinol 4,4′-di-*O*-β-D-glucopyranoside (56). Synthesized from **58** (73 mg, 0.048 mmol) according to the general procedure on the deprotection of lignan glycosides and providing the title compound as a white foam (22 mg, 67 %). R_f = 0.26 (MeOH:CH_2Cl_2 1:3); $[\alpha]_D^{30}$ –19.8° (c 1.00, MeOH). ^{1}H NMR (600.13 MHz, CD_3OD, 20 °C): δ 7.03 (d, 1 H, $J_{5',6'}$ = 8.2 Hz, H-5′), 7.00 (d, 1 H, $J_{5,6}$ = 8.2 Hz, H-5), 6.72 (d, 1 H, $J_{2',6'}$ = 2.0 Hz, H-2′), 6.62 (d, 1 H, $J_{2,6}$ = 2.0 Hz, H-2), 6.61 (dd, 1 H, H-6′), 6.55 (dd, 1 H, H-6), 4.87 (d, 1 H, $J_{1'',2''}$ = 7.8 Hz, H-1″), 4.86 (d, 1 H, $J_{1''',2'''}$ = 7.7 Hz, H-1‴), 4.26 (dd, 1 H, $J_{9a,8}$ = 7.4, $J_{9a,9b}$ = –9.1 Hz, H-9a), 3.99 (dd, 1 H, $J_{9b,8}$ = 6.9 Hz, H-9b), 3.87 (dd, 1 H, $J_{6'''a,5'''}$ = 2.3, $J_{6'''a,6'''b}$ = –12.1 Hz, H-6‴a), 3.86 (dd, 1 H, $J_{6''a,5''}$ = 2.2, $J_{6''a,6''b}$ = –12.0 Hz, H-6″a), 3.78 and 3.77 (each s, each 3 H, 3-OC*H*$_3$ and 3′-OC*H*$_3$), 3.68 (dd, 1 H, $J_{6''b,5''}$ = 5.6 Hz, H-6″b), 3.68 (dd, 1 H, $J_{6'''b,5'''}$ = 5.7 Hz, H-6‴b), 3.50 (dd, 1 H, $J_{2'',3''}$ = 9.4 Hz, H-2″), 3.50 (dd, 1 H, $J_{2''',3'''}$ = 9.4 Hz, H-2‴), 3.48 (dd, 1 H, $J_{3''',4'''}$ = 9.0 Hz, H-3‴), 3.48 (dd, 1 H, $J_{3'',4''}$ = 9.1 Hz, H-3″), 3.43 (ddd, 1 H, $J_{5''',4'''}$ = 9.9 Hz, H-5‴), 3.42 (ddd, 1 H, $J_{5'',4''}$ = 9.8 Hz, H-5″), 3.40 (dd, 1 H, H-4″), 3.39 (dd, 1 H, H-4‴), 2.92 (dd, 1 H, $J_{7'a,8'}$ = 4.9, $J_{7'a,7'b}$ = –13.9 Hz, H-7′a), 2.92 (dd, 1 H, $J_{7'b,8'}$ = 8.2 Hz, H-7′b), 2.67 (ddd, 1 H, $J_{8',8}$ = 7.4 Hz, H-8′), 2.62 (dd, 1 H, $J_{7a,8}$ = 7.4, $J_{7a,7b}$ = –13.8 Hz, H-7a), 2.53 (dd, 1 H, $J_{7b,8}$ = 7.9 Hz, H-7b), 2.52 (ddddd, 1 H, H-8) ppm.
^{13}C NMR (150.9 MHz, CD_3OD, 20 °C): δ 181.4 (C-9′), 150.5 (C-3′, C-3), 146.8 (C-4′), 146.6 (C-4), 134.6 (C-1), 134.1 (C-1′), 122.9 (C-6′), 122.2 (C-6), 117.7 (C-5), 117.6 (C-5′), 114.5 (C-2′), 114.0 (C-2), 102.8 (C-1″, C-1‴), 78.1 (C-5″, C-5‴), 77.8 (C-3″, C-3‴), 74.9 (C-2″, C-2‴), 73.0 (C-9), 71.4 and 71.3 (C-4″, C-4‴), 62.5 (C-6″, C-6‴), 56.6 (3-O*C*H_3,3′-O*C*H_3), 47.7 (C-8′), 42.3 (C-8), 39.1 (C-7), 35.6 (C-7′) ppm.
HRMS: *m/z* calcd. for $C_{32}H_{42}O_{16}Na$ $[M+Na]^+$ 705.2371; found 705.2331.

(*8R,8′R*)-Matairesinol 4,4′-di-2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (58). Synthesized from **57** (28 mg, 0.079 mmol) and **9** (152 mg, 2.6 equiv.) according to the general procedure for glycosylation of lignans to give the title compound as a white solid (83 mg, 69 %). R_f = 0.17

(hexane:EtOAc 1:1); $[\alpha]_D^{23}$ +0.0° (c 0.15, $CHCl_3$). ^{1}H NMR (600.13 MHz, $CDCl_3$, 25 °C): δ 8.01–7.27 (m, 40 H, arom. *H*), 7.13 (d, 1 H, $J_{5',6'}$ = 8.1 Hz, H-5′), 7.08 (d, 1 H, $J_{5,6}$ = 8.1 Hz, H-5), 6.55 (d, 1 H, $J_{2',6'}$ = 2.0 Hz, H-2′), 6.49 (dd, 1 H, H-6′), 6.30 (d, 1 H, $J_{2,6}$ = 2.0 Hz, H-2), 6.29 (dd, 1 H, H-6), 6.00 (dd, 1 H, $J_{3'',4''}$ = 9.5, $J_{3'',2''}$ = 9.7 Hz, H-3″), 5.99 (dd, 1 H, $J_{3''',4'''}$ = 9.5, $J_{3''',2'''}$ = 9.7 Hz, H-3‴), 5.79 (dd, 1 H, $J_{2''',1'''}$ = 7.8 Hz, H-2‴), 5.79 (dd, 1 H, $J_{2'',1''}$ = 7.8 Hz, H-2″), 5.74 (dd, 1 H, $J_{4''',5'''}$ = 9.9 Hz, H-4‴), 5.73 (dd, 1 H, $J_{4'',5''}$ = 9.9 Hz, H-4″), 5.22 (d, 1 H, H-1‴), 5.20 (d, 1 H, H-1″), 4.65 (dd, 1 H, $J_{6'''a,5'''}$ = 3.2, $J_{6'''a,6'''b}$ = –12.1 Hz, H-6‴a), 4.64 (dd, 1 H, $J_{6''a,5''}$ = 3.1, $J_{6''a,6''b}$ = –12.1 Hz, H-6″a), 4.54 (dd, 1 H, $J_{6''b,5''}$ = 5.9 Hz, H-6″b), 4.52 (dd, 1 H, $J_{6'''b,5'''}$ = 5.9 Hz, H-6‴b), 4.23 (ddd, 1 H, H-5″), 4.22 (ddd, 1 H, H-5‴), 4.00 (dd, 1 H, $J_{9a,8}$ = 7.4, $J_{9a,9b}$ = –9.2 Hz, H-9a), 3.75 (dd, 1 H, $J_{9b,8}$ = 7.8 Hz, H-9b), 3.30 (s, 3 H, 3′-OC*H*$_3$), 3.27 (s, 3 H, 3-OC*H*$_3$), 2.91 (dd, 1 H, $J_{7'a,8'}$ = 5.1, $J_{7'a,7'b}$ = –14.1 Hz, H-7′a), 2.81 (dd, 1 H, $J_{7'b,8'}$ = 7.3 Hz, H-7′b), 2.50 (dd, 1 H, $J_{7a,8}$ = 6.2, $J_{7a,7b}$ = –14.1 Hz, H-7a), 2.48 (ddd, 1 H, $J_{8',8}$ = 7.8 Hz, H-8′), 2.36 (ddddd, 1 H, $J_{8,7b}$ = 9.0 Hz, H-8), 2.35 (dd, 1 H, H-7b) ppm.

^{13}C NMR (150.9 MHz, $CDCl_3$, 25 °C): δ 178.3 (C-9′), 166.0 (6″-O*C*OPh, 6‴-O*C*OPh), 165.8 (3″-O*C*OPh, 3‴-O*C*OPh), 165.2 (2″-O*C*OPh, 2‴-O*C*OPh), 165.1 (4″-O*C*OPh, 4‴-O*C*OPh), 150.9 (C-3′, C-3), 145.0 (C-4′), 144.9 (C-4), 134.5 (C-1, C-1′), 133.5–128.3 (arom. *C*), 121.1 (C-6′), 121.0 (C-5), 120.8 (C-5′), 120.3 (C-6), 113.2 (C-2′), 112.7 (C-2), 101.6 (C-1‴), 101.5 (C-1″), 72.6 (C-3″, C-3‴), 72.4 (C-5″, C-5‴), 71.7 (C-2″, C-2‴), 71.0 (C-9), 69.7 (C-4″, C-4‴), 63.1 (C-6″, C-6‴), 55.4 (3-O*C*H$_3$,3′-O*C*H$_3$), 46.4 (C-8′), 41.0 (C-8), 38.3 (C-7), 34.7 (C-7′) ppm.
HRMS: *m/z* calcd. for $C_{88}H_{74}O_{24}Na$ $[M+Na]^+$ 1537.4468; found 1537.4425.

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Summary and Conclusions

Carbohydrates are one of the major classes of biomolecules found in nature. In the biological introduction of this thesis, several relevant areas where carbohydrates are utilized today were highlighted. Special focus was placed on the use of carbohydrates as biomarkers (*e.g.*, in the ABO-bloodsystem) and in immunological applications (*e.g.*, carbohydrate based vaccines) although carbohydrates are widely applied also in many other areas. Due to the biological importance of carbohydrates, the limited amount of complex carbohydrates available in nature and the problems associated with their isolation, the synthesis of such compounds is important in order to provide pure oligosaccharides or conjugates thereof (devoid of biological contaminations) for biological evaluations. In the introduction to carbohydrate chemistry, structural and conformational properties of monosaccharides were described. Furthermore, a brief introduction to the reactivities of individual monosaccharides and the basics of oligosaccharide synthesis were provided. With these prerequisites, a short journey through several areas related to carbohydrate synthesis was started.

In Episode 1, synthesis of β-(1→2)-linked mannopyranosides and various analogues was described. The synthesis culminated in the preparation of a β-(1→2)-linked mannotetraose, a cyclohexyl capped trisaccharide mimic and several 1,1′- or 2,2′-linked divalent mannosides (Figure 1). For preparation of divalent oligosaccharides, the Nobel prize winning procedures of Robert Grubbs featuring cross coupling metathesis with ruthenium based catalysts was utilized. In the β-selective mannosylations, the protocol originally developed by David Crich was applied. In the synthesis of the tetrasaccharide, however, the protocol was further modified and a convergent approach was found to be more suitable than the previously reported linear ones. Although much elegant work has undoubtedly been done in the field of β-linked mannosylations by talented scientists, it is the personal view of this author that there is still need for a more benign glycosylation methodology. This view is based on the facts concerning the reduced efficiency in the synthesis of larger oligosaccharides in the protocol developed by Bundle and the amounts of waste (4.3 equivalents of promoters/donor) formed when utilizing the protocol developed by Crich.

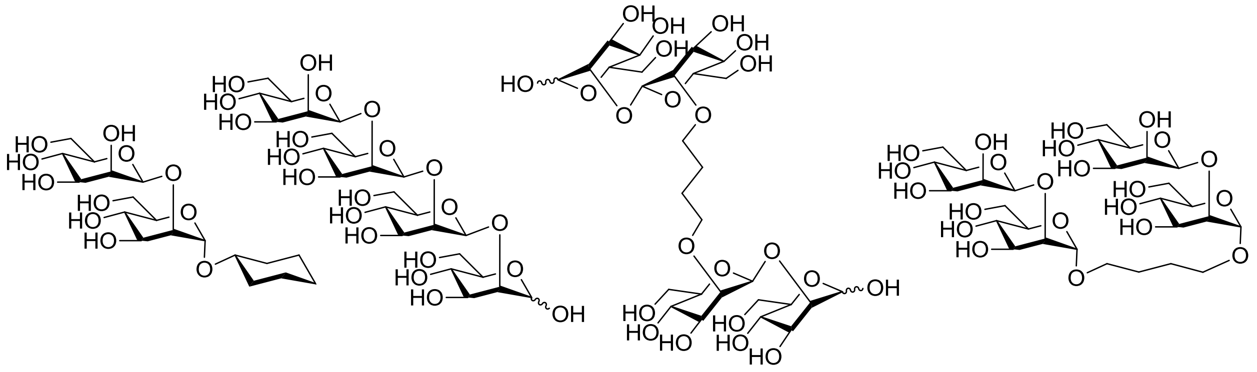

Figure 1. Chemical structures of some of the synthesized compounds from Episode 1.

In addition to the synthesis of molecules for biological screening of their immunostimulatory effects, the three dimensional structure of β-(1→2)-linked oligomannosides was investigated in detail by the use of NMR spectroscopic techniques, spectral simulation and molecular modeling. By these methods the first fully characterized ^{1}H- and ^{13}C-NMR spectra for the β-(1→2)-linked mannotetraose found in the cell wall of *C. albicans* could be reported (Figure 2).

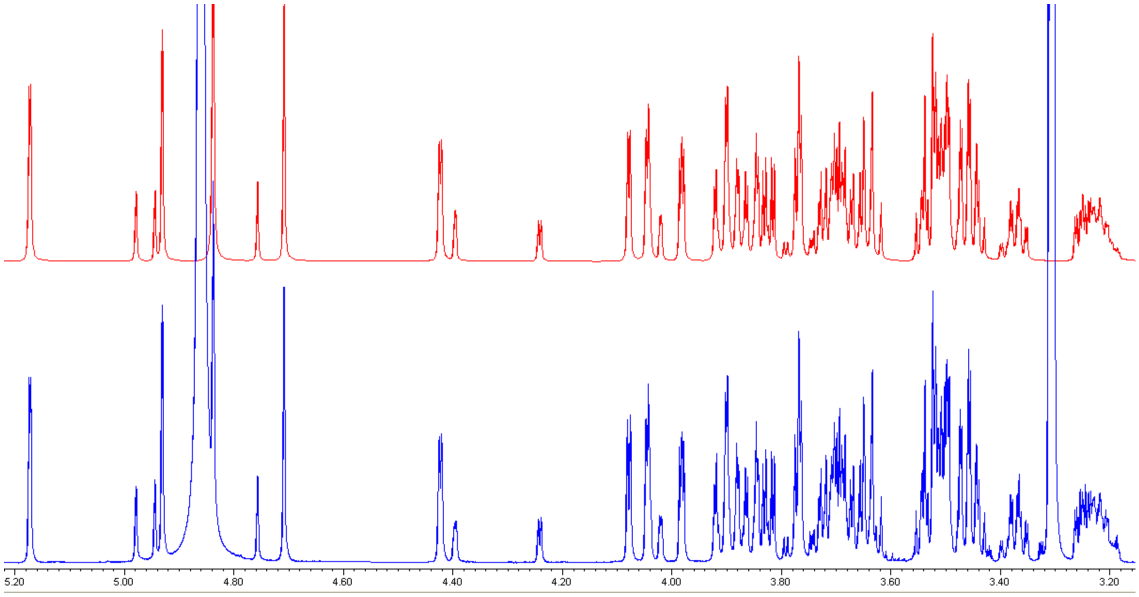

Figure 2. Spectral simulation of the ^{1}H-NMR spectrum of β-(1→2)-linked mannotetraose in MeOD (5.2–3.1 ppm region) with the PERCH NMR software: simulated spectrum (above), observed spectrum (below).

In Episode 2, the synthesis of a repeating trisaccharide unit and a less abundant tetrasaccharide fragment found in the galactoglucomannan-polysaccharide isolated from *Picea abies* was decribed (Figure 3). The synthetic strategy was built around the selective ring-opening of benzylidene acetals in order to achieve acceptors for subsequent glycosylation reactions. Several procedures for the partial reduction of benzylidenes and α-selective galactosylations were screened before optimal reaction conditions were found. The chemical shifts of the synthesized compounds were compared to literature chemical shifts reported for the galactoglucomannan polysaccharide. While some deviances were obvious, most signals were found to be in good correlation with the values reported for the polymer. In addition, several chemical shifts not reported for the polysaccharide were found for the small molecular models and can be used as reference values when interpreting spectra of GGM in the future. In addition to these studies, small molecular models were synthesized in order to investigate whether the acetyl group "leap" from *O*-2/*O*-3 directly to *O*-6 would be possible as suggested previously in the literature for AcGGM. Based on our initial findings, such a leap is not possible for mannosides existing in the $^4C_1$-conformation which is the preferred low energy conformation of naturally existing mannopyranoses. As mentioned in Episode 2, the studies related to this project is currently on-going and the synthesis of the second trisaccharide repeating unit of the GGM-backbone is currently under planning along with some additional experiments related to the acetyl-group "leap".

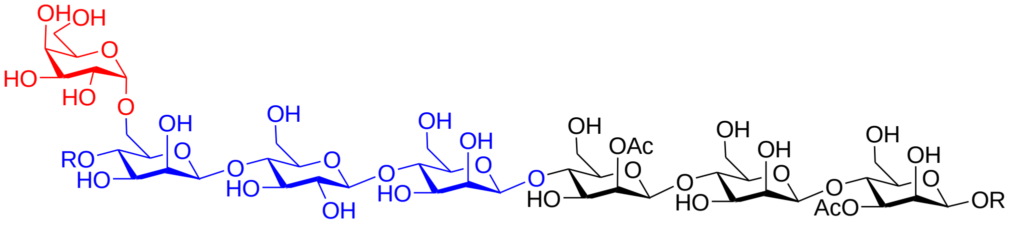

Figure 3. The proposed chemical structure for GGM, the synthesis of the blue and blue-red fragments was discussed in Episode 2.

In Episode 3, methods for the glycosylation of different types of aglycones were investigated. An initial screening of glycosyl donors revealed benzoylated imidate donors to be most suitable for the glycosylation of steroid aglycones.

In the first part of the Episode, benzoylated imidate donors were applied to the synthesis of a small library of glycosteroids with excellent efficiencies. These molecules were synthesized in order to evaluate their potential applications in drug delivery purposes. Biological screening events of the glycosteroid backbones containing permanently attached active pharmaceuticals have, however, not been conducted yet. These are potential applications of the molecules which may be explored in the future.

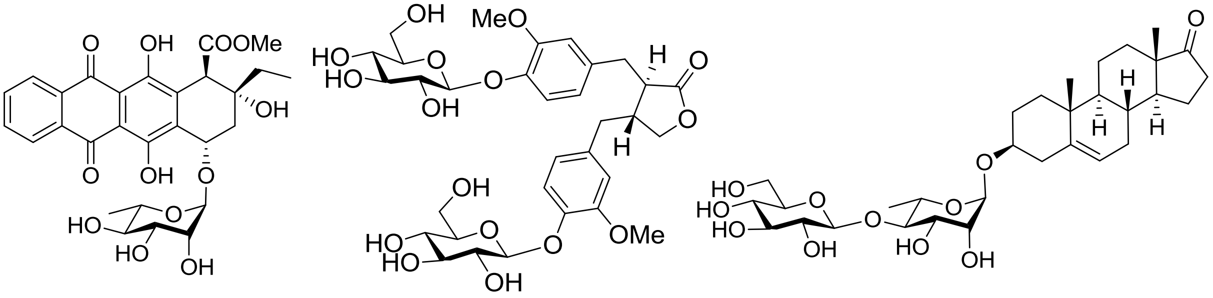

Figure 4. Chemical structures of some selected substrates from Episode 3.

In the second part of Episode 3, a high yielding route for the coupling of the naturally occuring glycon scillabiose to steroids was developed. The synthesis was based on a convergent approach which minimized the synthetic steps with the incorporated aglycone in order to assure that sensitive functionalities remain unaltered in this moiety. The coupling of scillabiose was exemplified with the synthesis of a saponin resembling the structure of the naturally occuring cytotoxic compound hellebrin. In future work, it would be interesting to apply the procedure developed in Episode 3 to the synthesis of one of the naturally occuring saponins containing this glycone.

In the third part of Episode 3, benzoylated TCA-donors were further applied in the regioselective glycosylation of 4-deoxy-ε-rhodomycinone. The environmentally benign approach, utilized in the synthesis of these structures could be valuable for industrial synthesis of similar compounds due to the operational simplicity, short reaction time and minimal waste generation of the protocol. The synthesized compounds resemble the structures of existing anticancer drugs and will in future work be screened in similar applications.

In the last part of Episode 3, a convenient semi-synthetic approach for the synthesis of MDG was developed starting from the two readily available starting materials HMR and D-glucose. This was, to our knowledge, one of the first synthetic protocols for the glycosylation of lignans and the first semi-synthesis reported for MDG. In the future, MDG will be screened for biological activity and the glycoyslation protocol, developed in the synthesis of MDG, extended to the synthesis of other naturally occuring lignan glycosides.

To conclude, a wide range of carbohydrate donors, activation protocols and protective groups were screened in this work. The synthetic protocols utilized in the different reaction sequences for glycosylation reaction, insertion and cleavage of protective group etc. can be found in the experimental sections of each Episode. Altogether, several of the commonly employed carbohydrate donors, such as thio- and imidate donors, sugar halides and peracetylated sugars, and protective groups, such as acetyl, benzoyl, allyl, propargyl, benzyl, methyl, tertbutyldimethylsilyl, benzylidene, isopropylidene, ditertbutylsilylene, 2,3-dimethoxybutan-2,3-diyl etc., were utilized at one point or another during the synthetic pathways. In addition, both simple 1,2-*trans* glycosides and difficult 1,2-*cis* glycosides were synthesized during the work described in Episodes 1–3. Apart from the synthesis of target compounds, much effort was invested in the accurate characterization of the molecules by the use of a wide range of one- and two-dimensional NMR techniques in combination with spectral simulation and even molecular modeling. As a result, some basic guidelines to the characterization of oligosaccharides and several different classes of glycoconjugates are reported in each of the three Episodes. Taken together, this thesis is written for researchers starting their work in carbohydrate chemistry and contains the crucial basics for both the synthetic and NMR spectroscopic knowledge needed to succeed.

Epilogue and Future Perspectives

While the basic strategies developed in the 1970s in carbohydrate synthesis are still widely utilized today, new technological and methodological improvements such as automated solution- and solid-phase synthetic protocols have emerged in recent times. These improvements enable the rapid synthesis of oligosaccharides, simultaneously extending the knowledge on their biological roles and sparking new interest in the fields of carbohydrate research. As a result, the field of carbohydrate science is flourishing now more than ever and achieving the reputation that it deserves. It will be of topical interest to follow the scientific breakthroughs that undoubtedly lie ahead in the near future.

APPENDIX

List of Conference Contributions and Presentations

1. **Cross Coupling Metathesis as a Tool for Synthesizing Bivalent Oligosaccharides.** F. S. Ekholm, R. Leino, *3rd ERA-Chemistry "Flash" Conference, Carbohydrates at the Interfaces of Biology, Medicine and Materials Science*, March 9–13, 2008, Killarney, Irland. (Poster presentation)

2. **Synthesis of 2,2'-Linked Mannose Derivatives.** F. S. Ekholm, R. Leino, 5th Finnish Glycoscience Meeting, Glycoscience Graduate School Annual Seminar, September 23–24, 2008, Tvärminne Zoological Station, Finland. (Oral presentation)

3. **Applications of Cross Coupling Metathesis to the Synthesis of Divalent Mannosides.** F. S. Ekholm, R. Leino, COST Chemistry Action D 40 Workshop, Innovation-III 2009, May 26–28, 2009, Åbo, Finland. (Poster presentation)

4. **Synthesis and Conformational Study of a β-(1,2)-Linked Mannotetrasaccharide.** F. S. Ekholm, J.Sinkkonen, R. Leino, XI Spring Meeting of Synthetic Chemistry, June 8–9, 2009, Helsinki, Finland. (Oral presentation)

5. **Utilization of Cross Coupling Metathesis in the Synthesis of Divalent Mannosides.** F. S. Ekholm, M. Poláková, R. Leino, 15th European Carbohydrate Symposium, July 19–24, 2009, Vienna, Austria. (Poster presentation)

6. **Synthesis and Structural Characterization of a β-(1,2)-Linked Mannotetrasaccharide.** F. S. Ekholm, R. Leino, Glycoscience Graduate School Annual Seminar, September 1–3, 2009, Seili, Finland. (Oral presentation)

7. **Glycosylation of Biomolecules.** F. S. Ekholm, R. Leino, Glycoscience Graduate School Annual Seminar, September 22–24, 2010, Hyytiälä, Finland. (Oral presentation)

8. **Semi-Synthesis and NMR Spectroscopic Characterization of the Naturally Occuring Lignan Glycoside MDG.** F. S. Ekholm, P. Eklund, R. Leino, XII Spring Meeting of the Division of Synthetic Chemistry, June 7–8, 2011, Jyväskylä, Finland. (Poster presentation)

9. **Synthesis and NMR Spectroscopic Characterization of a Wide Range of Glycoconjugates.** F. S. Ekholm, R. Leino, 16th European Carbohydrate Symposium, July 3–7, 2011, Sorrento, Italy. (Oral presentation)

10. **Synthesis and NMR Spectroscopic Characterization of Oligosaccharides and Glycoconjugates.** F. S. Ekholm, R. Leino, 17th European Symposium on Organic Chemistry, July 10–15, 2011, Hersonissos, Greece. (Poster presentation)

11. **NMR Spectroscopic Characterization of Organic Molecules – A Brief Introduction.** F. S. Ekholm, R. Leino, Glycoscience Graduate School Annual Seminar, September 28–30, 2011, Konnevesi, Finland. (Oral presentation)