12-11-2016

Development of an HPLC-based oligosaccharide synthesizer

Salvatore Pistorio
University of Missouri-St. Louis

Development of an HPLC-based oligosaccharide synthesizer

By

SALVATORE G. PISTORIO

Master of Science (Chemistry), University of Missouri-St. Louis, August 2014

Master of Science (Chemistry and Pharmaceutical Technologies), Universita' degli Studi di Catania (Italy), May 2011

A Dissertation

Submitted to the Graduate School of the

UNIVERSITY OF MISSOURI – ST. LOUIS

in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

December 2016

Dissertation Committee

Prof. Alexei V. Demchenko, Ph.D. (Chair)

Prof. Eike B. Bauer, Ph.D.

Prof. Bruce C. Hamper, Ph.D.

Prof. Keith J. Stine, Ph.D.

ABSTRACT

Development of an HPLC-based oligosaccharide synthesizer

Salvatore G. Pistorio
Doctor of Philosophy, University of Missouri – St. Louis
Prof. Alexei V. Demchenko, Advisor

Carbohydrates are the most abundant molecules on Earth. They are involved in a wide range of fundamental biological processes: anti-inflammation, immune response, joint lubrication, cell growth, antigenic determination. Carbohydrates are also held responsible for many damaging cellular processes, such as bacterial and viral infections, development of tumors, etc.. Therefore, the development of effective methods for the synthesis of complex carbohydrates has become a critical area of glycosciences. One challenge that stands out is the stereocontrol in the synthesis of glycosidic bonds, the linkage that serves as the only means to connect simple monosaccharides into complex oligomeric networks. Beyond this, the lack of a simple automated platform, similar to that utilized in the preparation of oligonucleotides and oligopeptides, significantly hampers access to oligosaccharides. The work presented herein addresses both of these limitations of oligosaccharides synthesis: stereocontrol and the lack of automation. The stereocontrolled formation of very challenging beta-mannosidic linkages has been achieved using a new reaction called Hydrogen-bond-mediated Aglycone Delivery (HAD). Automation in Oligosaccharide solid phase synthesis has been accomplished using an *Agilent*^{TM} HPLC, model 1260 Infinity, that was adapted to the polymer supported synthesis of oligosaccharides. The utility of the HAD reaction and the HPLC-based automated technology has been demonstrated by the synthesis of different oligosaccharide sequences in high stereoselectivities and yields.

ACKNOWLEDGEMENTS

I cannot start the acknowledgments without expressing my special thanks to my mentor Professor Alexei V. Demchenko, he has been an incredible Boss and a true friend. Alexei is someone you never forget once you meet him. I was terrified at the first time when I meet Alexei, a tall Russian/Italian/American professor, but after only 5 minutes of talking, you realize that Alexei is the funniest adviser and one of the smartest people on the planet. I really would like to thank him for thousand reasons, unfortunately I have limited space and I will list just some: thank you for allowing me join the big family of "Glycoworld", I am very proud to have been a part of your research group. Thank you for supporting and encouraging my research in all 4 ½ years. Thank you for allowing me to grow as a research scientist. Thank you for the all cowboy hat pictures that you took when I made mistakes. Thank you for all the international and regional conferences that you allowed me to go to. Thank you for the Figaro-video. Thank you for all Hokkaido Chinese restaurant lunches that you paid for and you did not pay for. Thank you for all the celebrations that we has after submitting grant proposals. Alexei, you are an incredible adviser and an amazing person, working for you has been an incredible honor for me, and I will be forever grateful because you are the reason of my success. No matter what happens in the future, I will always be a member of the "Glycoworld" family.

I am grateful to all professors in my dissertation committee including Prof. Eike B. Bauer, Prof. Bruce C. Hamper and Prof. Keith J. Stine for their help, comments and support. I also thank Dr. Jagodige P. Yasomanee for her help and for training me during my first year at UMSL. A special thanks go to Dr. Rensheng Luo for his assistance with NMR spectroscopy, Prof. Rudolf Winter and Mr. Joe Kramer for their help with mass spectrometry. I thank all the past and present members of the

Glycoworld family, Xiao (extremely knowledgeable in just about everything, you are the second smartest man on this planet, after your graduation the lab was not the same anymore), Swati (thanks for all of your help with HPLC, you are the best), big Scott (should I call you professor? Thanks for all your advices) and small Scott (now called new Xiao, thanks for the 1 g of glucosamine), Michael (aka Micheluzzo, you always gave me the priority on the rotary evaporator), First (you are the kindest man on the planet, and a very good friend), Matteo (you know too much, write a book and give me the profits), Mithila and Ting (who is the tallest between you two?). Special thanks to Prof. Cristina De Meo from Southern Illinois University, Edwardsville (SIUE) and 100% Sicilian. Thank you for pushing me to come to the USA, you will be always a part of my family. After all, it is time to thank my beautiful wife and a future PhD in Biochemistry. She has always been there for me, she is my best friend, I love her and thank her for all her advice and support (now it is time to get the PhD). I also thank all my Italian friends for providing friendship despite they are in the other face of the globe. A special thanks go to my mom, dad, and brother. My parents have sacrificed their lives for me and my brother to give us a better education and a better life, I miss you all very much and I will never thank you enough for all you have done for me, Grazie.

LIST OF ABBREVIATIONS

Å.....Angstrom

Ac.....Acetyl

Bn.....Benzyl

br……Broad

Bz.....Benzoyl

BF_3-Et_2O.....Boron trifluoride etherate

$Cu(OTf)_2$.....Copper trifluoromethanesulfonate

d.....Doublet

1,2-DCE.....1,2-Dichloroethane

DCM .....Methylene chloride

dd.....Doublet of doublets

DMF.....*N*,*N*-Dimethylformamide

DMTST.....Dimethyl(methylthio)sulfonium trifluoromethanesulfonate

Et.....Ethyl

EtOAc..... Ethyl acetate

Et_2O..... Diethyl ether

Glc..... Glucose

h..... Hour(s)

HPLC……..High Performance Liquid Chromatography

HR-FAB MS.....High Resolution Fast Atom Bombardment Mass Spectroscopy

Hz .....Hertz

m..... Multiplet

Man..... Mannose

min..... Minute(s)

m/z ..... Mass to charge ratio

Me ..... Methyl

MeOTf..... Methyl trifluoromethanesulfonate

MeCN ..... Acetonitrile

MeOH ..... Methanol

MS ..... Molecular sieves

NaOH ..... Sodium hydroxide

NaOMe ..... Sodium methoxide

NIS ..... *N*-Iodosuccinimide

NMR ..... Nuclear magnetic resonance

Ph ..... Phenyl

Phth…….…… Phthalimido

Pic……... Picolinyl

Pico…..…….Picoloyl

ppm ..... Parts per million

R_f ..... Retention factor

rt ..... Room temperature

s ..... Singlet

SEt..... *S*-Ethyl

SBox ..... *S*-Benzoxazolyl

SPh..... *S*-Phenyl

t ..... Triplet

TBDMS ..... *tert*-Butyldimethylsilyl

TBAF ..... Tetra-*n*-butyl ammonium fluoride

TFA ..... Trifluoroacetic acid

TfOH ..... Trifluoromethanesulfonic (triflic) acid

TLC ..... Thin layer chromatography

TMS……..……Trimethylsilyl

TMSOTf ..... Trimethylsilyl trifluoromethanesulfonate

TABLE OF CONTENTS

CHAPTER 1

Automated chemical synthesis of oligosaccharides and glycoconjugates

CHAPTER 2

Hydrogen bond-mediated aglycone delivery: focus on β-Mannosylation

CHAPTER 3

Hydrogen bond-mediated aglycone delivery: synthesis of the N-linked glycoprotein core pentasaccharide.

CHAPTER 4

HPLC-assisted automated oligosaccharide synthesis: the implementation of the autosampler as a mode of the reagent delivery

CHAPTER 5

LIST OF FIGURES

CHAPTER 5

LIST OF SCHEMES

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 5

CHAPTER 1

Automated chemical synthesis of oligosaccharides and glycoconjugates

S. G. Pistorio, K. J. Stine, A. V. Demchenko. Automated chemical synthesis of oligosaccharides and glycoconjugates. In "Carbohydrate Chemistry: State-of-the-art and challenges for drug development," L. Cipolla Ed., Imperial College Press, London, **2015**, 247-276

1.1 General introduction

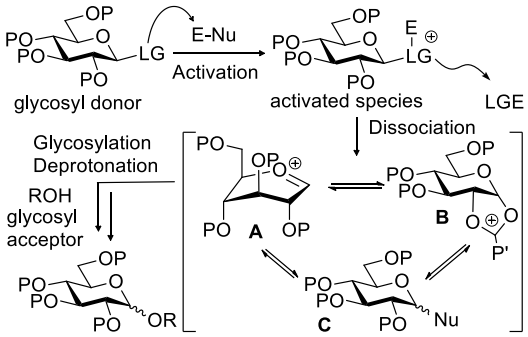
It has been long known that carbohydrates are involved in a wide range of fundamental biological processes and are often called "essential molecules of life."[1] Indeed, our life begins with fertilization, which takes place via a selective carbohydrate-protein recognition.[2] In addition, carbohydrates help us maintain a healthy lifestyle via their active involvement in anti-inflammation, immune response, joint lubrication, cell growth, antigenic determination, etc.[3] The explosive growth of glycosciences in the recent years also brought the understanding of the roles of sugars as "molecules of death" due to their sizable contribution to harmful processes. Bacterial, parasitic, and viral infections; development and growth of tumors; metastasis; tissue rejection; septic shock; congenital disorders are only few to mention.[4] Elucidating the roles of carbohydrates in pathogenesis of cancer, AIDS, pneumonia, septicemia, diabetes, hepatitis, and malaria has been particularly stimulating for major efforts in the field of modern glycosciences.[5]

It is already appreciated that both chemical and enzymatic syntheses[6] could lead to natural oligosaccharides of glycoconjugates. These compounds are typically needed for studying of their composition,[7] conformation,[8] interaction with other molecules,[9] and biological roles.[10] Isolation from natural sources offers another viable means to obtain sugars. Only the synthetic approach, however, can provide unnatural glycomimetics that are often of interest due to their therapeutic[4, 11] and/or diagnostic potential.[12] Manufacturers have shown an interest in producing carbohydrate-based pharmaceuticals; however, even with significant progress, controlled chemical synthesis of complex carbohydrates remains difficult. Resultantly, the entire area of glycosciences remains somewhat underdeveloped, whereas other major classes of natural biopolymers, peptides and polynucleotides, can be studied by advanced

methods and their synthesis can be automated using commercially available synthesizers. Low accessibility and high cost of complex carbohydrates hinders their large-scale development, and examples such as heparin analogs,[13] oligosaccharide antibiotics,[14] oligosaccharide and glycoconjugate-based vaccines,[15] etc.,[5b, c, 16] are still rare.

Practically all complex carbohydrates have an oligomeric sequence wherein monosaccharide residues are linked via O-glycosidic linkages. This linkage is obtained by a glycosylation reaction that typically involves the nucleophilic displacement of a leaving group (LG, Scheme 1.1) on the glycosyl donor by a hydroxyl group of the acceptor (ROH) in the presence of an electrophilic promoter or activator.[17] The remaining functional groups of both components are temporarily masked with protecting groups (P). A detailed mechanism has not yet been elucidated, although certain conventions and the involvement of the key reaction intermediates (**A-C**, Scheme 1.1) have been established.[18] In spite of significant recent progress, chemical glycosylation remains challenging due to the requirement to achieve complete stereocontrol and to suppress side reactions.[17]

Scheme 1.1 Chemical glycosylation



The single-step glycosylation is only one challenge researchers working on oligosaccharide synthesis face: often additional protecting and/or leaving group

modifications between each glycosylation step are required. Because these manipulations become increasingly inefficient with larger oligosaccharides, many advanced strategies focus on minimizing the intermediate reaction steps.[19] A few relevant examples include Fraser-Reid's armed-disarmed approach,[20] Nicolaou's selective activation,[21] Danishefsky's glycal-based assembly,[22] Kahne's one-pot synthesis,[23] Roy's[24] and Boons'[25] active-latent concept, Ogawa's orthogonal strategy,[26] Huang's preactivation concept,[27] etc.[28]

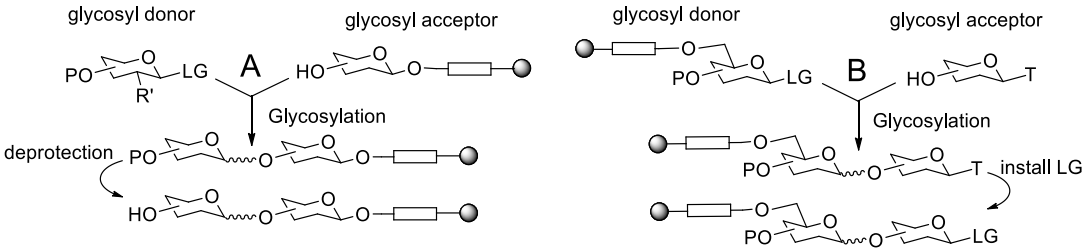
In light of recent progress made in the areas of glycobiology and glycomics the discovery of efficient methods and technologies for the synthesis of complex carbohydrates is critical because reliable access to these compounds is becoming truly essential for research, development and application in therapeutics and diagnostics. This Chapter will discuss recent development of novel technologies for automation of oligosaccharide synthesis both on solid phase and in solution. Following numerous attempts to streamline oligosaccharide synthesis using predictive or computational reactivity assessment performed by the Fraser-Reid, Ley, and Wong groups in the 1990's, the first example of the truly automated synthesis is credited to Seeberger and co-workers, who reported the first automated synthesizer in 2001. In this application, they used a peptide synthesizer ABI 433 that after a few modifications was optimized for the synthesis of oligosaccharides.[29] From that moment, an automated approach to oligosaccharide synthesis became possible, and a new era in carbohydrate research has been plotted. Another important milestone is the commercialization of this automated platform, Glyconeer 2.1 that is now available via GlycoUniverse. It should be mentioned that Activotec has also developed a peptide synthesizer-based oligosaccharide synthesizer that is now commercially available.

1.2 Automation of the polymer-supported synthesis

Solid-phase synthesis using insoluble polymer supports[30] has been widely used in the preparation of many organic molecules[30-31] including oligopeptides[32] and oligonucleotides.[33] The early example of solid phase peptide synthesis by Merrifield[34] was followed by oligosaccharide synthesis by Fréchet and Schuerch that emerged shortly thereafter.[35] Since the early attempts, the polymer-supported oligosaccharide synthesis has become a viable means for the rapid synthesis of oligosaccharide sequences without the necessity of purifying (and characterizing) the intermediates.[36] Another important advantage of oligosaccharide synthesis on solid support is the ease of excess reagent removal (usually can be achieved by filtration and rinsing).

There are two main strategies for solid phase saccharide synthesis that differ in the type of the attachment (Scheme 1.2). In strategy A, the glycosyl acceptor unit is bound to the solid support either at the anomeric position or other suitable hydroxyl. In this case, an excess of the glycosyl donor and promoter are in the solution. In approach B, the glycosyl donor unit, linked to the solid support via a suitable hydroxyl group, is reacted with the solution phase acceptor. Two-directional techniques, combining approaches A and B are also known.[37] To improve the operational simplicity of the oligosaccharide assembly Seeberger developed an automated approach, which is based on strategy A: using a solid phase acceptor and a liquid phase donor.[29a] Since this approach is already well-documented in the literature, including a number of review articles,[16, 29b, 38] herein only a brief historical perspective with a few representative examples and milestones will be discussed.

Scheme 1.2. Glycosylation on polymer support

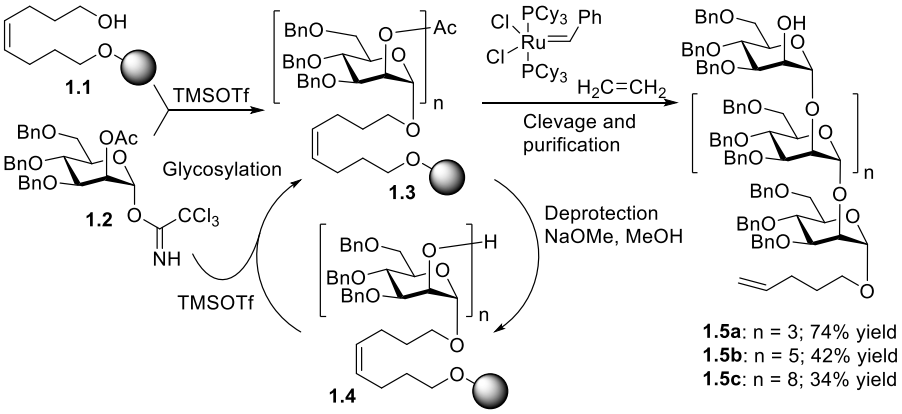


1.2.1 Peptide synthesizer-based automation of polymer-supported synthesis

The automation was initially accomplished by using a peptide synthesizer that had been modified to perform oligosaccharide assembly at low temperature.[29a] Merrifield resin was chosen as the solid support, for its good swelling properties in organic solvents commonly used in glycosylation. The design of the linker between the sugar and the solid support is of the key importance. In this application, the polymer support was equipped with an olefin-type linker due to its high stability in both basic and acidic media. Thus, the octenediol-functionalized resin **1.1** was glycosylated with trichloroacetimidate donor **1.2** (10-fold excess) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf, Scheme 1.3). The use of acetyl group at C-2 of glycosyl donor **1.2** was chosen to control the anomeric stereoselectivity for the formation of oligosaccharides **1.3**, and to be selectively deprotected with NaOMe in methanol/dichloromethane to obtain the next generation glycosyl acceptor **1.4**. To reduce the formation of side products and truncated sequences, both glycosylation and deprotection steps were performed twice. This cycle of glycosylation-washing-deprotection was repeated to obtain the desired linear oligosaccharide sequence. After that, the linker was removed using Grubbs' catalyst to afford penta-, hepta- and decasaccharides **1.5a-c** as pentenyl glycosides. For instance, using the automated approach, heptasaccharide **1.5b** was obtained in 24 h in

42% over-all yield, whereas the manual synthesis was much more laborious (14 days) and less efficient (9% over-all yield).[39]

Scheme 1.3. First automated oligosaccharide synthesis

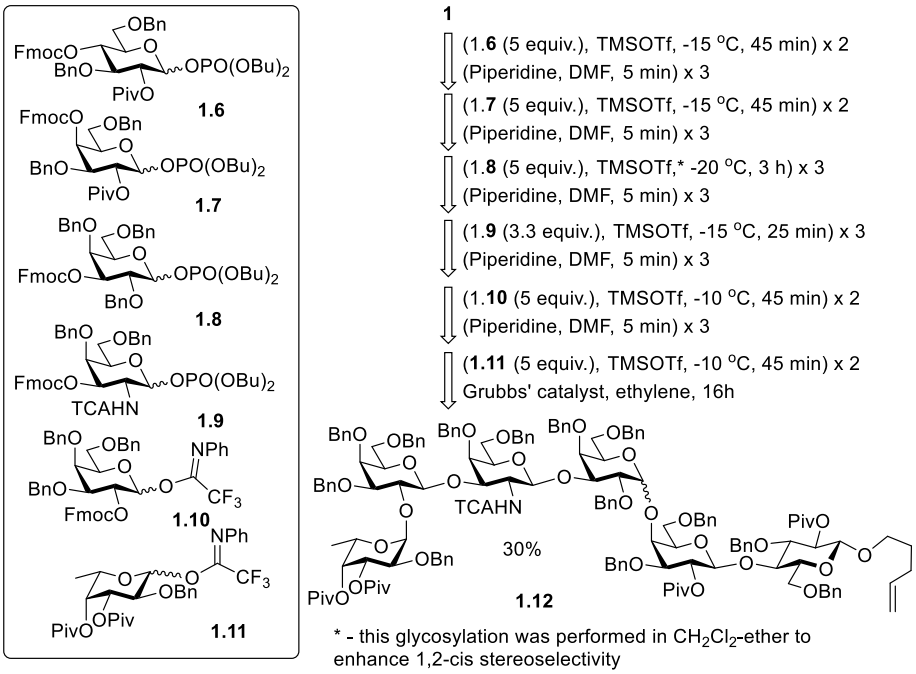


Working on expanding this promising technology, Seeberger and co-workers, approached the synthesis of oligosaccharides containing other important residues and linkages including aminosugars,[40] sialic acids,[41] furanosides,[42] 1,2-cis glycosides,[43] glycopeptides,[44] and branched oligosaccharide sequences.[29a] Codee et al. reported the synthesis of β-mannosides[45] using essentially the same automation platform. The acquired knowledge was applied to a very effective synthesis of Globo-H hexasaccharide. This oligosaccharide is expressed on surfaces of different types of cancer cells, and this represents a key target for the development of anti-cancer vaccines and therapeutics.[15c, 16, 46] The appreciation of the biological significance of Globo-H stimulated the interest in the synthetic community and the antigen has been synthesized by different methods,[27c, 37b, 43, 47]

This effort culminated in the automated synthesis by Seeberger, which was the first attempt to conquer the challenge on 1,2-cis glycosidic bond formation using automated approach.[43] Careful refinement of reaction conditions allowed 1,2-cis galactosylation in dichloromethane-ether and Globo-H sequence was assembled as

depicted in Scheme 1.4. First, glycosyl phosphate donor **1.6** was linked to the resin **1.1** via glycosylation using TMSOTf (repeated once) as the promoter, followed by deprotection of the Fmoc substituent with piperidine (repeated twice) to provide polymer-bound acceptor. In this synthesis, Fmoc group was chosen as a protecting group, for its high stability in acid and easy cleavage in presence of mildly basic amines. In addition, after cleavage Fmoc provides dibenzofluorene adduct that can be used to monitor the efficiency of deprotection via quantitative colorimetric assay.[48] The general synthetic protocol consists of repetitive cycles of glycosylation using either glycosyl phosphate (**1.6-1.9**) or glycosyl N-phenyl trifluoroacetimidate donors (**1.10** and **1.11**) followed by the deprotection with piperidine. The final product **1.12** was obtained under an atmosphere of ethylene in the presence of Grubbs' catalyst[49] in an overall yield of 30%.

Scheme 1.4. Automated synthesis of Globo H hexasaccharide



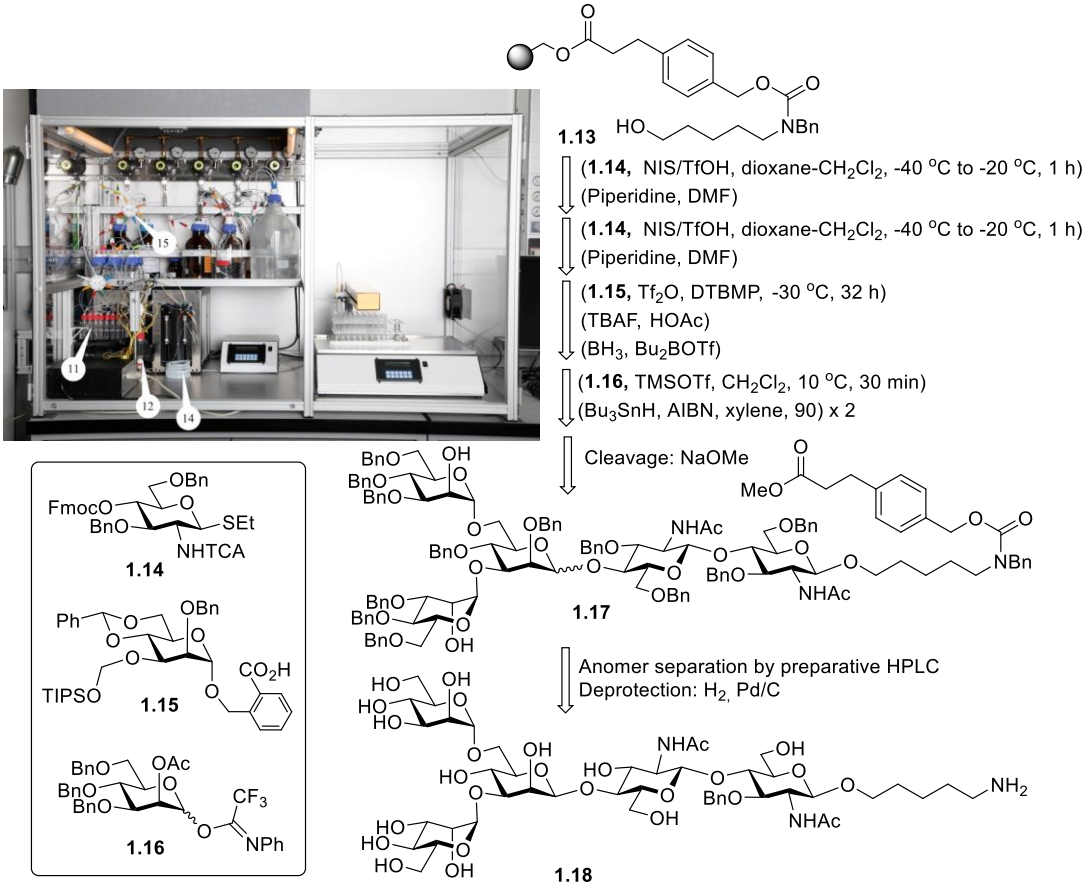
1.2.2 Dedicated oligosaccharide synthesizer for the automation of polymer-supported synthesis

More recently, Seeberger and co-workers introduced "the first fully automated solid-phase oligosaccharides synthesizer".[50] The new automated synthesizer was developed by combining the following equipment: syringe pumps, solenoid valves, a cryostat with the temperature controlling system capable of maintaining the reaction temperature between -50 ^{o}C and 90 ^{o}C, and a fraction collector (Scheme 1.5). The reaction vessel is equipped with the porous glass filter that allows for delivering the inert gas to maintain anhydrous reaction conditions. The gas is also used for rapid removal of the solution phase reagents and solvents. The glycosylation and deprotection protocol can be set using a computer program. This allows for the full automation of reactions, temperature control, cleavage, and the collection of the released oligosaccharide product.

To illustrate the versatility of this new synthesizer Seeberger and co-workers performed the synthesis of a few oligosaccharides, included a high mannose type branched glucan **1.18** as depicted in Scheme 1.5. To ensure the feasibility of the complete automation, a new type of linker **1.13** stable under the most common glycosylation and protecting group removal conditions was introduced. The synthesis of pentasaccharide **1.18** began with the assembly of the chitobiose portion using two glycosylation-deprotection cycles with glycosyl donor **1.14**. A challenging β-mannosidic linkage was then obtained with donor **1.15**, equipped with 2-(hydroxycarbonyl)benzyl leaving group developed by Kim.[51] Subsequently, the treatment with TBAF and selective opening of benzylidene acetal gave 3,6-diol. The latter was then subjected to bis-mannosylation using donor **1.16** to obtain the final branched sequence. Cleavage from the solid support was affected in the presence of MeONa to afford precursor **1.17** (α/β = 1/3). The undesired α-linked anomer formed

as the by-product during the β-mannosylation step was separated off by preparative HPLC, and the resulting diastereomerically pure compound was deprotected by hydrogenation to afford the target pentasaccharide **1.18** in 3.5% yield overall.

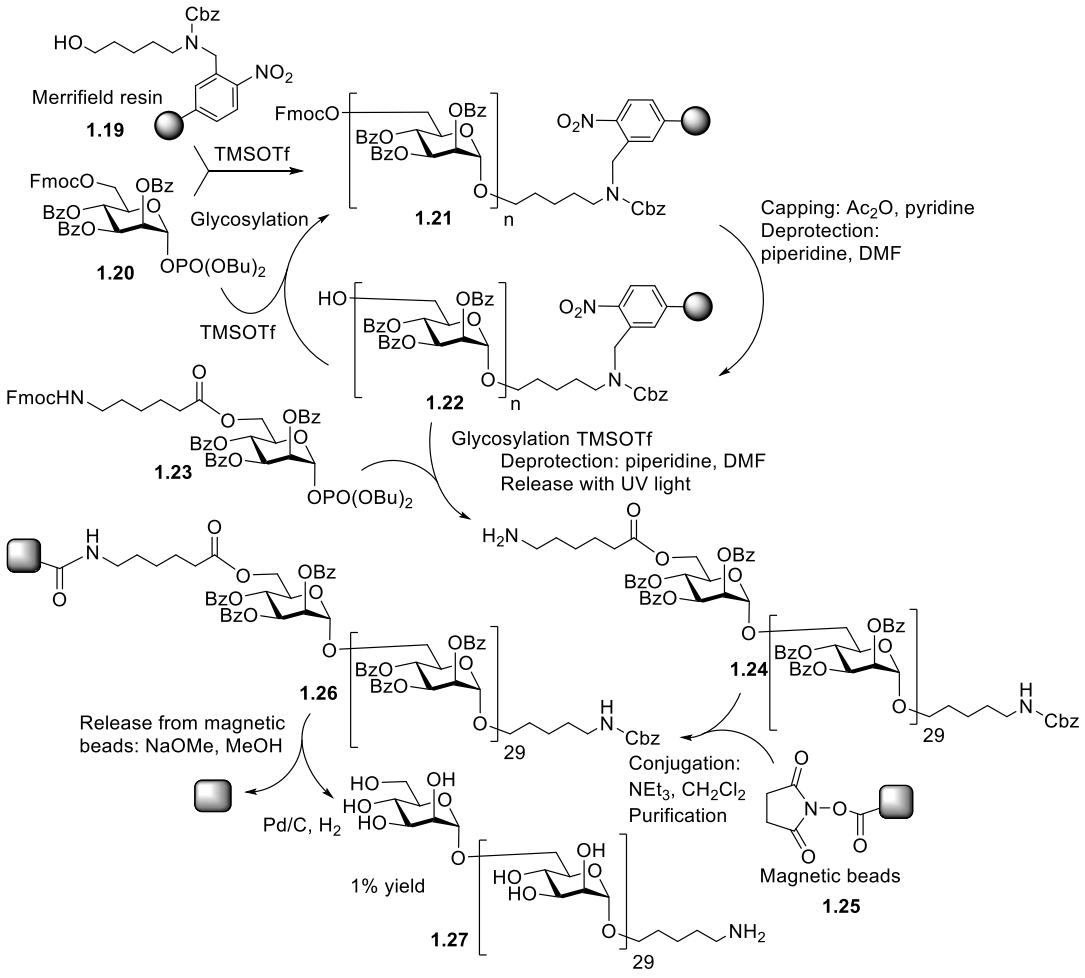
Scheme 1.5. Automated synthesis of branched oligomannan 1.18 using second generation synthesizer



Very recently, making use of essentially the same technology, Seeberger and co-workers obtained a α-(1→6)-linked linear oligosaccharide sequence containing 30 mannose residues.[52] To undergo this impressive synthesis, a modified Merrifield resin **1.19** equipped with the photocleavable *p*-nitrophenyl linker was repeatedly glycosylated with phosphate donor **1.20** in the presence of TMSOTf (Scheme 1.6). The capping of the unreacted hydroxyls in **1.21** was affected by acetylation with Ac_2O in the presence of pyridine. The deprotection of 6-*O*-Fmoc substituent was then

affected with piperidine to afford glycosyl acceptor of the next generation **1.22**. Benzoyl protecting groups were used at other positions to ensure high stability, stereoselectivity, and yields throughout the synthesis. Upon execution of 29 glycosylation cycles with the interim capping-deprotection steps, the resulting 29-mer was then glycosylated with donor **1.23**. The latter is equipped with the spacer moiety to allow for a very effective cap-and-tag purification technique.[53] Thus, upon release from the polymer support using UV light, the resulting 30-mer **1.24** was conjugated to magnetic beads via the amino group of the terminal mannose unit equipped with an ε-aminocaproic ester spacer.

Scheme 1.6. Automated synthesis of the linear 30-mer oligomannan using second generation synthesizer.



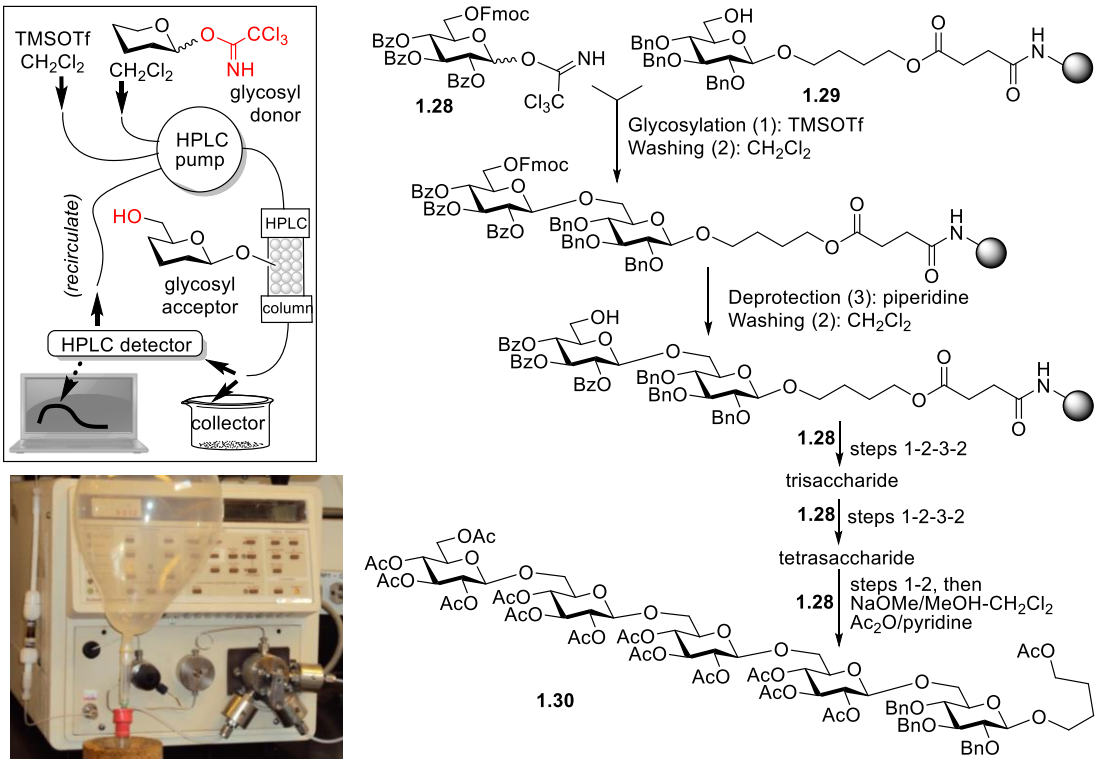
This approach allowed for magnetic separation of the tagged oligosaccharide **1.26** followed by simple washing with dichloromethane and methanol. Subsequent release from the magnetic beads and concomitant debenzoylation with sodium methoxide in methanol followed by hydrogenation to remove Cbz group led to the fully uprotected 30-mer manno-oligosaccharide **1.27**. Resultantly, the longest oligosaccharide sequence ever made by chemical synthesis was obtained in a total yield of 1%, which accounts for 96% average yield per synthetic step.

1.2.3 HPLC-based automation of polymer-supported synthesis

Demchenko, Stine and their co-workers developed a new experimental set-up based on an unmodified HPLC instrument. In brief, an Omnifit chromatography column was packed with the pre-swelled polymer resin TentaGel-NH_2. The column was then connected to the HPLC system consisting of a reciprocating pump containing three chambers, a variable UV range detector, and a computer with standard HPLC-operating software installed (Scheme 1.7).[54] The column was packed with the glycosyl acceptor loaded on resin, purged with the solvent and then two separate solutions containing glycosyl donor and promoter were delivered concomitantly. After a relatively short reaction time, typically 30-60 min, the system was purged (washed) with solvent. At this time, the resin is loaded with the disaccharide derivative, and the oligosaccharide elongation can be continued via alternating deprotection-glycosylation steps. Although the versatility of the HPLC-assisted method is still to be demonstrated, the synthesis of linear pentasaccharide **1.30** has already been accomplished (Scheme 1.7). For the synthesis of this molecule, TentaGel-NH_2 resin loaded glycosyl acceptor **1.29** was packed into the Omnifit column (alternatively, the loading could be performed using the HPLC-based set up). The elongation of the glucan sequence was then performed using glycosyl donor **1.28**

equipped with 2-*O*-benzoyl group to ensure the stereoselectivity control and 6-*O*-Fmoc as the selectively removable temporary substituent. Two reagent bottles, one with a solution of donor **1.28** and one with a solution of promoter (TMSOTf) were then used to deliver the solutions to the column via dedicated pumps. After 1 h, the two pumps were stopped and the system was purged with dichloromethane using a separate pump. Subsequently, a solution of piperidine in DMF was delivered to perform Fmoc-deprotection with a typical reaction time of 5 min, after which the system was purged with dichloromethane. The cycle (glycosylation-washing-deprotection-washing) was repeated until the desired oligosaccharide was assembled. The latter was then cleaved off from the polymer support by using a recirculating solution of NaOMe in methanol-dichloromethane. Resultantly, pentasaccharide **1.30** was isolated in 62% yield with an important reduction in time with respect to the same pentasaccharide obtained using a manual approach (7 hours vs. 7 days).

Scheme 1.7. HPLC-assisted automated oligosaccharide synthesis



It was also demonstrated that all steps of the HPLC-assisted synthesis could be monitored using a standard HPLC detection system set to record changes in the UV absorbance of the solution eluting off the column.[54] A solution of reagents can be recirculated to reduce the amount of reagents and to ensure complete conversion at each reaction step. This experimental set up offers the following advantages in comparison to that of conventional (manual) oligosaccharide synthesis on polymer supports: faster reaction times, real-time reaction monitoring using an HPLC detection system, and all steps and sequences can be automated using the standard HPLC-managing computer software. Novel *O*-benzoxazolyl (OBox) imidates were found promising glycosyl donors for HPLC-based applications.[55]

1.3 Other platforms for the automated synthesis

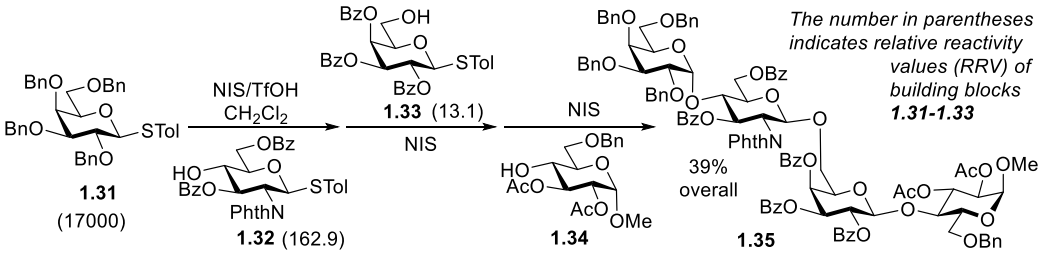
In spite of remarkable progress, the solid phase technique still suffers from significant limitations: large reagent excess, limited use of molecular sieves, large volume of waste solvent, cumbersome analysis of intermediates, lower stereoselectivity, loss and poisoning of resin, reagent trapping, etc. In addition, the necessity to carefully select (match) reaction components, linkers, resins, activators, solvents, temperature, etc. have also become apparent and required thorough refinement. This section will discuss major efforts to automate the synthesis of oligosaccharides either in solution with or without using soluble tags or with the use of alternative solid supports.

1.3.1 Automation of the one-pot oligosaccharide synthesis in solution

Oligosaccharide synthesis using the principle of chemoselectivity introduced by Fraser-Reid has been used in a variety of ways and found broad application in synthesis.[20, 56] With the discovery of multiple reactivity levels ranging from the

superdisarmed to the superarmed building blocks and systems,[28c, 57] the versatility of the chemoselective approach to oligosaccharide synthesis was enhanced. Wong and co-workers devised a mathematical approach, assigning relative reactivity values (RRVs) to a wide library of building blocks that were then used for oligosaccharide assembly in one-pot.[58] The determination of RRVs was made in standardized reaction conditions, tolyl thioglycoside donors in the presence of an NIS/TfOH promoter system. The cumulative reactivity data was then compiled into a predictive computer program called Optimer.[58] Following these studies, a well-rounded technology for one-pot oligosaccharide synthesis based on RRVs emerged. A relevant example is shown in Scheme 1.8 (synthesis of **1.35**) wherein sequential activation of building blocks **1.31**, **1.32**, and **1.33** was based on their relative reactivity, which was found to be 17000/162.8/13.1, respectively.[58] In this context, the reactivity difference between similarly protected sugars of different series has to be also taken into consideration. For example, the reactivity ratio between per-benzylated S-(*p*-methylphenyl) glycosides of L-fuco, D-galacto, and D-gluco series was found to be 27.1/6.4/1 respectively.[58] This approach has been used to obtain various oligosaccharides and glycoconjugates of biological significance and medicinal relevance.[59] Similar reactivity scales have also been devised by Fraser-Reid,[60] Ley,[61] and others.[56]

Scheme 1.8. Programmable oligosaccharide synthesis assisted by the predictive computer program Optimer.

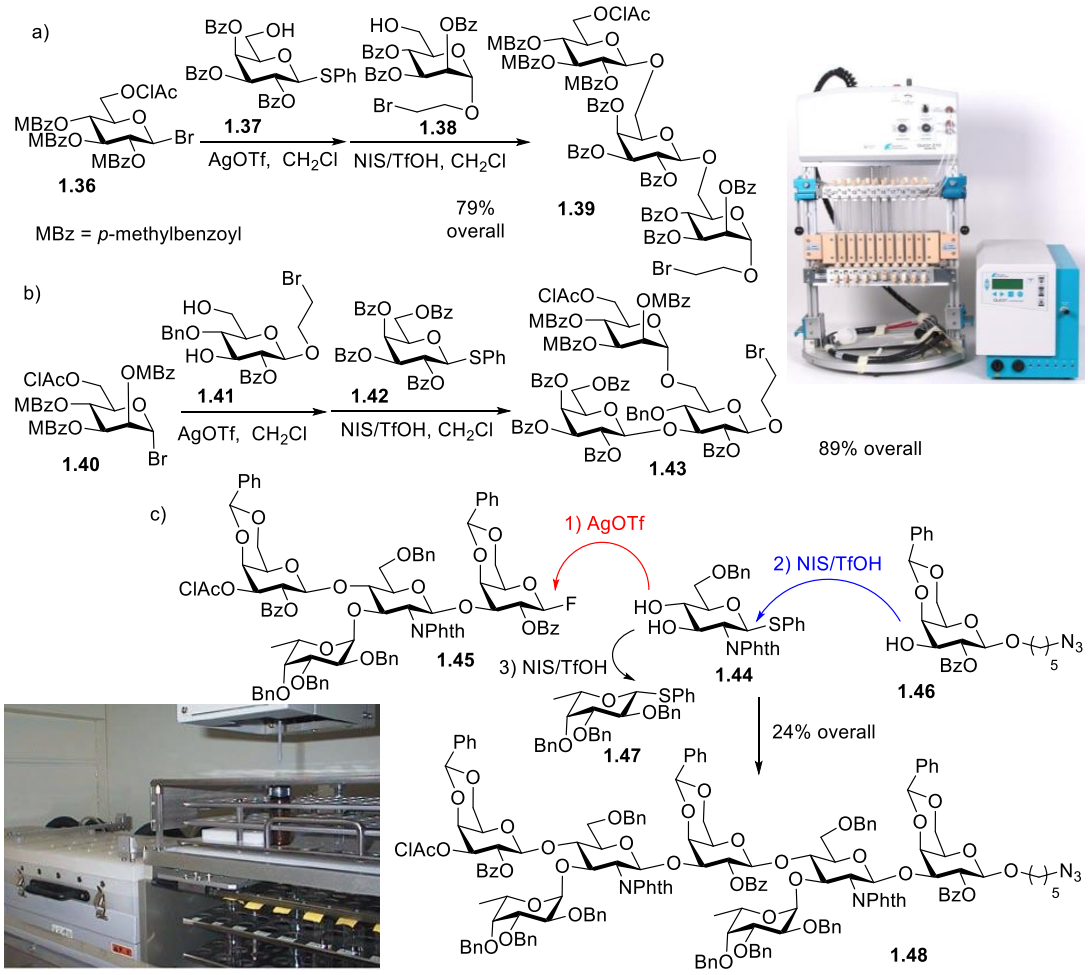


Takahashi and co-workers probed a number of automation platforms for the solution-based one-pot oligosaccharide synthesis.[62] In a majority of applications, selective activation of different leaving groups, another common approach to expeditious oligosaccharide synthesis,[19, 28b] was executed. For instance, a parallel synthesis instrument Quest 210 by Argonaut Technologies, was applied to the automated one-pot synthesis of linear and branched oligosaccharides shown in Scheme 1.9a and 1.9b.[63] Thus, for the synthesis of trisaccharide **1.39**, glycosyl bromide donor **1.36** was selectively activated for reaction with thioglycoside acceptor **1.37** in the presence of AgOTf as promoter. The anomeric thiophenyl leaving group of the disaccharide intermediate was then directly activated by the addition of NIS/TfOH and acceptor **1.38** to provide linear trisaccharide **1.39** in 79% yield over two steps. Scheme 1.9b illustrates the formation of the branched trisaccharide **1.43**, which was obtained by two-step sequential glycosylation of diol **1.41**. First, the primary hydroxyl of acceptor **1.41** was glycosylated with bromide **1.40** in the presence of AgOTf. Subsequent addition of thiophenyl donor **1.42** and NIS/TfOH promoter system resulted in the glycosylation of the remaining secondary hydroxyl to afford trisaccharide **1.43** in 89% yield over two steps.

In an effort to obtain more complex oligosaccharide sequences, Takahashi adapted another instrument, L-COS by Moritex, which was used to make a dimeric Le^x **1.48** and its analogues. Using this instrument, Takahashi et al. achieved automation of stirring, temperature control, and rate of reagent addition for each glycosylation and deprotection step shown in Scheme 1.9c.[62, 64] The synthesis began by the coupling of fluoride donor **1.45** with diol acceptor **1.44** in the presence of AgOTf as the promoter. This reactions proceeded regioselectively at the C-4 position, and the resulting pentasaccharide equipped with the anomeric thiophenyl leaving

group was used as the glycosyl donor for the coupling with glycosyl acceptor **1.46**, which was added along with NIS/TfOH to affect the activation. Finally, the remaining 3'-OH of the resulting hexasaccharide intermediate was fucosylated by the addition of donor **1.47** and NIS/TfOH. Resultantly, the desired heptasaccharide **1.48** was obtained in eight hours in 24% yield (average 79% yield per step). The automated synthesizer could be combined with Combi Flash automated column chromatograph, which was used for purification of the resulting oligosaccharides.

Scheme 1.9 Automation of one-pot synthesis using parallel synthesizers Quest-210 (a and b) and Moritex L-cos (c).



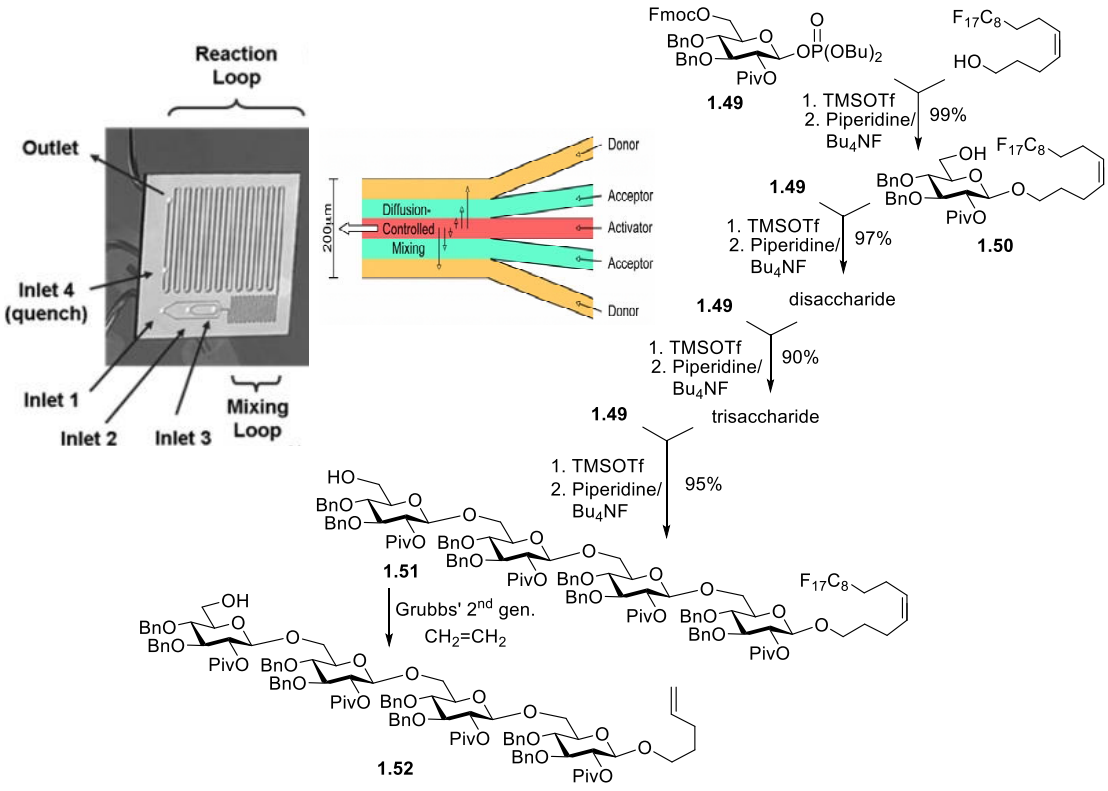
1.3.2 Automation of the fluorous tag-supported synthesis

Fluorous tag-supported synthesis has emerged as a new and attractive strategy for obtaining oligosaccharides with good prospects for automation. Apparently, fluorous protecting groups (groups incorporating a fluorinated alkyl chain) allow for the separation of fluorinated species (typically glycosyl acceptors) from non-fluorinated species (glycosyl donors) by simple partitioning between perfluorohexanes and methanol (or toluene).[65]

Solution-based microreactors, developed in the late 1990's, have been adapted to fluorous tag-supported synthesis of oligosaccharides by Seeberger and co-workers.[66] A five-port silicon microfluidic reactor was utilized for the synthesis of homotetramer **1.52**. For this purpose, glycosyl phosphate donor **1.48** was first glycosidated with a fluorous linker **1.49** in the presence of TMSOTf.[66] To accomplish the glycosylation reaction in a microfluidic device, three separate solutions in dichloromethane were prepared: glycosyl acceptor, phosphate donor, and the activator (TMSOTf). Using three syringe pumps, the solutions of acceptor, donor, and promoter were connected via inlets 1, 2 and 3, respectively (Scheme 1.10). By diffusion-controlled sampling, each solution is then delivered into the mixing zone and the concentration of each component is controlled by the concentration inside the syringe and by the flow rate of each stream. After mixing, the reaction then takes place inside the reaction zone. The reaction can be monitored using different types of instruments connected at the outlet of the microreactor, including UV-vis, IR, or mass spectrometer. The glycosylation step was then followed by the removal of the Fmoc group with piperidine and TBAF (used to remove 6-O-TMS formed as a result of competing silylation) to afford fluorous glycosyl acceptor **1.50**. The latter was then

glycosylated with glycosyl donor **1.48** and the deprotection-glycosylation sequence was repeated until the desired tetrasaccharide **1.51** had been assembled.

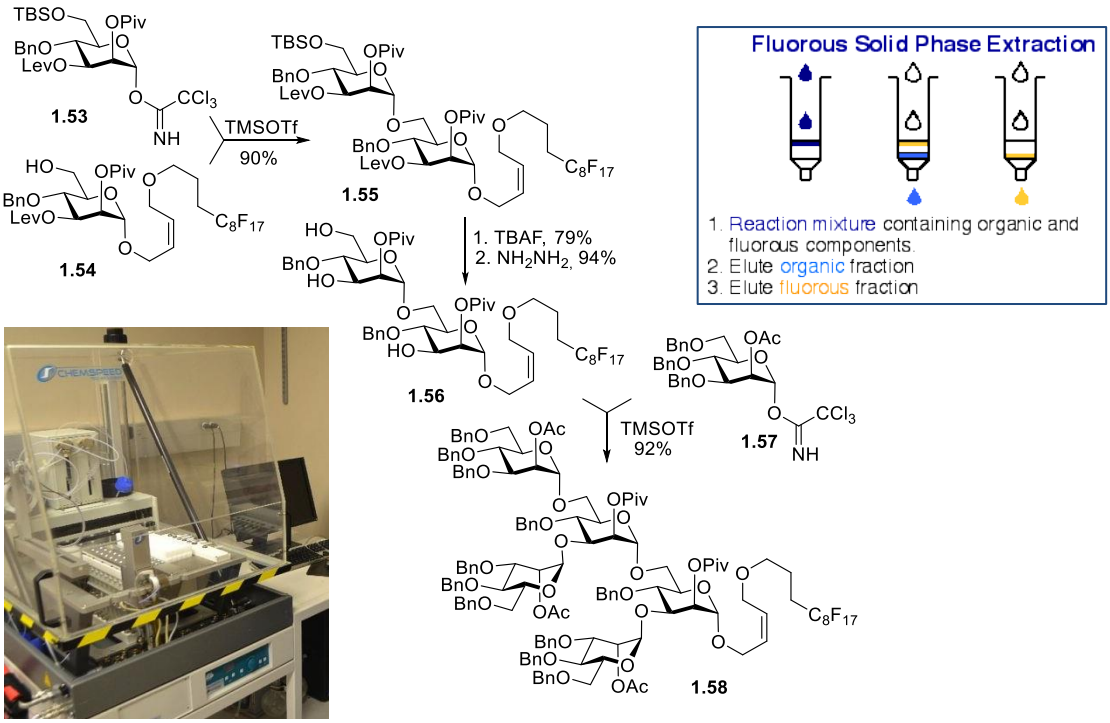
Scheme 1.10 Fluorous tag-supported synthesis in the microreactor.



The tetrasaccharide was then cleaved from the fluorous support by the treatment with second-generation Grubbs' catalyst to provide tetrasaccharide **1.52**. The reaction times for glycosylations were 20 sec for the formation of the disaccharide, and 60 sec each for the tri- and tetrasaccharides. The yields for the reactions after purification were 97, 90, and 95% for di-, tri-, and tetrasaccharides, respectively. The benefits of using microfluidic reactors in oligosaccharide synthesis include ease of scale up, the ability to readily optimize reaction conditions and control the reaction outcome by varying the flow rate and amounts of reagents, improved safety, a greater control of the reaction temperature, the compatibility with various analytical techniques and automation platforms. Inherent microfluidic system

drawback include: possible longer times if a low flow-rate is required and the incompatibility with solids.

Scheme 1.11 Pohl automated synthesis using fluorous tag approach.



Jaipuri and Pohl also used the fluorous tag-assisted glycosylation strategy for developing their own automation platform using the fluorous solid-phase extraction protocol (Scheme 1.11).[67] Their approach involved the use of a fluorous tag attached glycosidically to glycosyl acceptor **1.54**, which was glycosylated using excess of trichloroacetimidate donor **1.53** in the presence of TMSOTf. The resultant disaccharide **1.55** was separated using an automated three-step fluorous solid phase extraction protocol. First, the mixture containing organic and fluorous components are loaded on a separation column. All fluorine-free molecules elute off the column using 20% solution of water in methanol. After that, fluorous molecules that are retained on the column can be released using a fluorophilic wash, such as methanol or THF. This procedure can be automated using commercially available equipment that is able to

create a positive pressure or vacuum. Subsequently, disaccharide **1.55** was subjected to the two-step deprotection of TBS (with TBAF) and Lev groups (with hydrazine) to afford triol **1.56**. The latter was then glycosylated with excess trichloroacetimidate donor **1.57** to afford pentasaccharide **1.58** in a high yield of 92%.

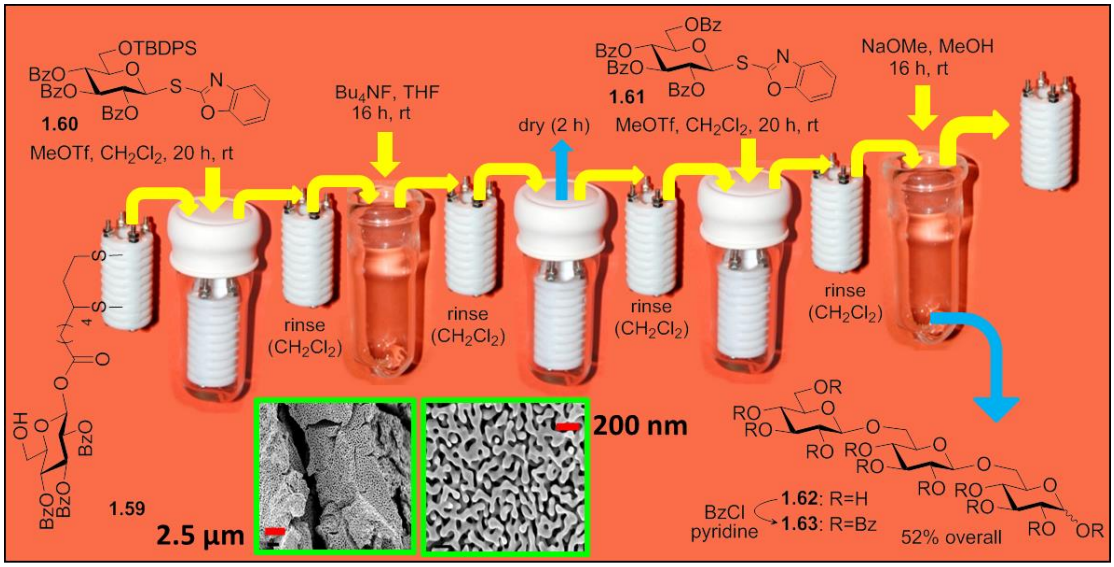
1.3.3 STICS: Surface-Tethered Iterative Carbohydrate Synthesis

In attempt to address major drawbacks of the polymer-supported synthesis, predominantly swelling, reagent trapping, and poisoning, Demchenko and Stine introduced the Surface-Tethered Iterative Carbohydrate Synthesis (STICS), a novel approach to oligosaccharide synthesis using nanoporous gold (NPG) as a solid support.[68] NPG is a high-surface area, sponge-like nanomaterial. It can be utilized as a set of NPG plates (8 x 8 x 0.2 mm) assembled in a stacked Teflon mini reactor as shown in Scheme 1.12. The glycosyl acceptor can be anchored to NPG using thiolated linkers and the oligosaccharide assembly is accomplished via alternating glycosylation, washing, deprotection and drying steps as depicted in Scheme 1.12. Thus, 6-*O*-TBDPS-protected S-benzoxazolyl (SBox) glycosyl donor **1.60** was coupled to the lipoic acid anchored acceptor **1.59** in the presence of MeOTf. Then, after a quick rinse, the tethered disaccharide intermediate was treated with Bu_4NF to deprotect the silyl group to afford the second-generation glycosyl acceptor. The latter was dried and reacted with SBox donor **1.61**, and the resulting trisaccharide was released by the treatment with NaOMe in MeOH. Finally, for the purification and characterization purpose, the trisaccharide was benzoylated to afford compound **1.63** in 52% overall. The pictures in Scheme 1.12 are SEM images of NPG plates with two different bar scales (2.5 μm and 200 nm).

Over-all, the STICS approach offers a promising new platform for oligosaccharide synthesis. NPG surface only requires wetting; upon reaction, the

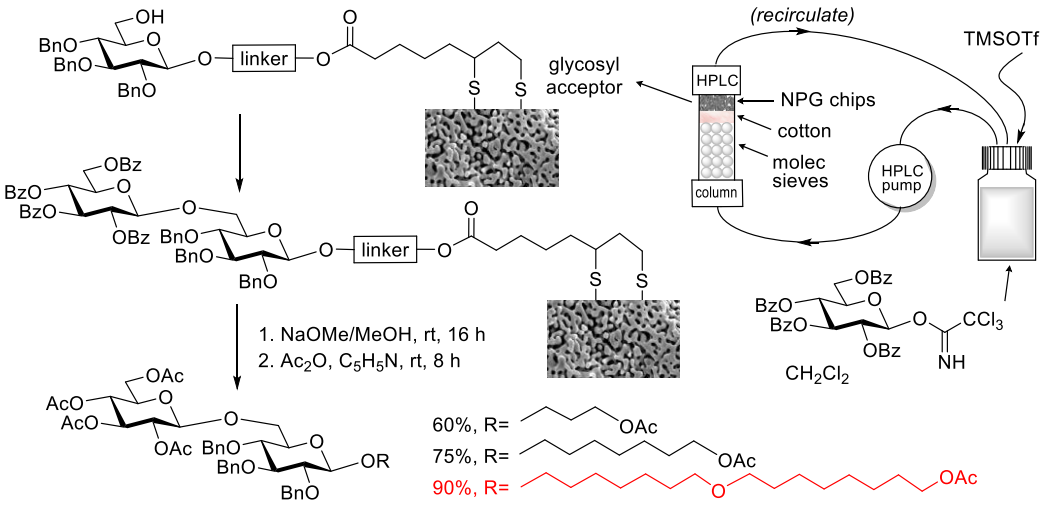
access reagent is simply rinsed off; reagent trapping/resin poisoning is not an issue with NPG because it does not trap any reagent and can be regenerated to bare surface by electrochemical methods if necessary, and gold is fully recoverable. The limitations of the STICS approach are similar to other standard drawbacks of the polymer-supported synthesis and include: difficulty of monitoring the reaction and analyzing intermediates, longer reaction times than those for reactions in solution and the requirement to use large (5-10 fold) excess of reagents.

Scheme 1.12 STICS: Surface-Tethered Iterative Carbohydrate Synthesis



To address some of these drawbacks, the subsequent study made use of the HPLC-assisted automated technology for oligosaccharide synthesis.[69] The HPLC pump was used to force the circulation of the donor inside the column and the NPG chips placed in the Omnifit column (Scheme 1.13). Also investigated was the role of the spacer in the lipoic acid anchoring systems. Using this strategy in combination with a long chain spacer between the acceptor and lipoic acid anchor it was possible to increase the disaccharide yield from 60% to 90%.[69]

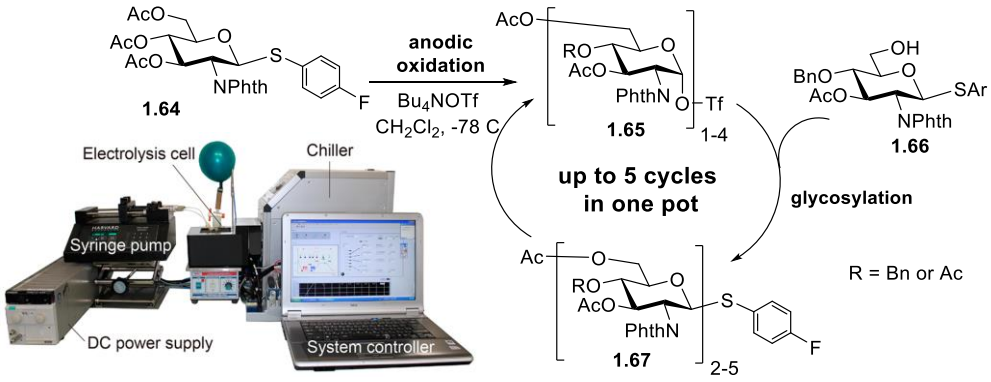
Scheme 1.13 HPLC-assisted surface-tethered iterative carbohydrate synthesis



1.3.4 Solution phase-based automation using electrochemical activation of thioglycosides

Electrochemical activation of calcogenoglycosides has been known for years.[70] Very recently, Nokami and co-workers introduced an automated oligosaccharide synthesis based on the electrochemical activation of thioglycosides via the corresponding glycosyl triflates.[71] The automated synthesizer was specifically developed for this application, and the synthesis of a series of β-(1→6)-linked glycans consisting of multiple N-acetylglucosamine units has been accomplished. As depicted in Scheme 1.14, aryl thioglycoside donor **1.64** was preactivated via the corresponding glycosyl triflate **1.65** by electrochemical oxidation at -80 ^{o}C and 1.0 F/mol at 1.73 Volt for 40 min. The temperature was then increased to -50-60 ^{o}C and glycosyl acceptor **1.66** equipped with the S-aryl leaving group is added resulting in the formation of disaccharide **1.67** in 30 min. Essentially the same two-step preactivation-glycosylation sequence could be repeated multiple times to afford the desired pentasaccharide **1.68** in 9-15% overall yield (62-68%, average yield per cycle) in 10 h.[71]

Scheme 1.14 Automation of the electrochemical activation of thioglycosides in solution.



1.4 Conclusions

To keep pace with the exploding area of glycobiology, it is critical to make complex carbohydrates more accessible to the general chemical, biochemical and industrial audience. This lofty goal can be achieved only by the development of reliable, stereoselective and expeditious methods for glycoside synthesis and oligosaccharide assembly that are applicable to both laboratory and industrial-scale preparations. Traditional chemical assembly of oligosaccharides in solution involves multiple protecting group and/or leaving group manipulations and requires tedious purification between each glycosylation steps. Expeditious strategies for oligosaccharide assembly in solution require specialist knowledge of all aspects of carbohydrate chemistry and fine-tuning of the reaction conditions and reactivity levels. Manual polymer-supported synthesis helps to streamline the synthesis and purification, and the automated platform developed by Seeberger introduces an idea of operational simplicity. Yet, it requires a sophisticated and expensive synthesizer.

Recent developments of automation with the use of inexpensive laboratory equipment including syringe pumps, HPLC components, microreactors, and parallel synthesizers offer a promise of simple automation using commonly available and relatively inexpensive equipment. The modular character of many new wave

synthesizers allows for endless opportunities to implement attachments, reagent delivery modes, detecting systems, accessories, and software packages. Some automation platforms allow for real-time reaction monitoring, which, in turn, helps reduce the reaction time and the amount of reagents needed. Further development of existing and new platform for the automated synthesis remains a significant area of research. While most automated platforms remain underdeveloped, solution-based manual (standard) synthesis will still remain as an important (major) tool to obtain complex oligosaccharides or particularly challenging sequences.

1.5 References

1. Stick, R. V.; Williams, S. J., *Carbohydrates: the essential molecules of life*. Second ed.; Elsevier: Amsterdam - Boston - Heidelberg, 2009.

2. Benoff, S., Carbohydrates and fertilization: an overview. *Molecular Human Reproduction* 1997, *3*, 599-637.

3. Varki, A.; Cummings, R. D.; Esko, J. D.; Freeze, H. H.; Bertozzi, C. R.; Stanley, P.; Hart, G. W.; Etzler, M. E., *Essentials of Glycobiology*. Second ed.; CSH Laboratory Press: New York, 2009.

4. Cipolla, L.; Arajo, A. C.; Bini, D.; Gabrielli, L.; Russo, L.; Shaikh, N., Discovery and design of carbohydrate-based therapeutics. *Expert Opin. Drug Discovery* 2010, *5*, 721-737.

5. (a) Witczak, Z. J., Carbohydrates as new and old targets for future drug design. In *Carbohydrates in Drug Design*, Witczak, Z. J.; Nieforth, K. A., Eds. Marcel Dekker, Inc.: New York, 1997; pp 1-37; (b) Wong, C. H., *Carbohydrate-Based Drug Discovery*. Wiley-VCH: Weinheim, 2003; (c) Klyosov, A. A.; Witczak, Z. J.; Platt, D., *Carbohydrate Drug Design*. ACS: Washington, 2006; Vol. 932.

6. Muthana, S.; Cao, H.; Chen, X., Recent progress in chemical and chemoenzymatic synthesis of carbohydrates. *Curr. Opin. Chem. Biol.* 2009, *13*, 573-581.

7. Duus, J. O.; Gotfredsen, C. H.; Bock, K., Carbohydrate structural determination by NMR spectroscopy: Modern methods and limitations. *Chem. Rev.* 2000, *100* (12), 4589.

8. Wormald, M. R.; Petrescu, A. J.; Pao, Y. L.; Glithero, A.; Elliott, T.; Dwek, R. A., Conformational studies of oligosaccharides and glycopeptides: complementarity of NMR, X-ray crystallography, and molecular modeling. *Chem. Rev.* 2002, *102* (2), 371-386.

9. (a) Seah, H.; Basu, A., Carbohydrate-carbohydrate interactions. In *Encyclopedia of Chemical Biology*, Begley, T., Ed. John Wiley & Sons: 2008; (b) Jin, S.; Cheng, Y.; Reid, S.; Li, M.; Wang, B., Carbohydrate recognition by boronolectins, small molecules, and lectins. *Med. Res. Rev.* 2010, *30* (2), 171-257.

10. Varki, A., Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* 1993, *3* (2), 97-130.

11. Cheng, Y.; Li, M.; Wang, S.; Peng, H.; Reid, S.; Ni, N.; Fang, H.; Xu, W.; Wang, B., Carbohydrate biomarkers for future disease detection and treatment. *Sci. China Chem.* 2010, *53* (1), 3-20.

12. (a) Dube, D. H.; Bertozzi, C. R., Glycans in cancer and inflammation-potential for therapeutics and diagnostics. *Nature Rev.* 2005, *4*, 477-488; (b) Murrey, H. E.; Hsieh-Wilson, L. C., The chemical neurobiology of carbohydrates. *Chem. Rev.* 2008, *108*, 1708-1731; (c) Chaubard, J. L.; Krishnamurthy, C.; Yi, W.; Smith, D. F.; Hsieh-Wilson, L. C., Chemoenzymatic probes for detecting and imaging fucose-alpha(1-2)-galactose glycan biomarkers. *J. Am. Chem. Soc.* 2012, *134* (10), 4489-4492.

13. (a) Poletti, L.; Lay, L., Chemical contributions to understanding heparin activity: synthesis of related sulfated oligosaccharides. *Eur. J. Org. Chem.* 2003, 2999-3024; (b) Linhardt, R. J.; Toida, T., Role of glycosaminoglycans in cellular communication. *Acc. Chem. Res.* 2004, *37*, 431-438.

14. (a) Kotra, L. P.; Mobashery, S., A renaissance of interest in aminoglycoside antibiotics. *Curr. Org. Chem.* 2001, *5* (2), 193-205; (b) Ito, Y.; Manabe, S., Other glycoconjugates: Synthesis of enediyne antibiotic oligosaccharides. In *Glycoscience: Chemistry and Chemical Biology*, Fraser-Reid, B.; Tatsuta, K.; Thiem, J., Eds. Springer: Berlin - Heidelberg - New York, 2001; Vol. 3, pp 2441-2470.

15. (a) Lucas, A. H.; Reason, D. C., Polysaccharide vaccines as probes of antibody repertoires in man. *Immunol. Rev.* 1999, *171*, 89-104; (b) Kuberan, B.; Linhardt, R. J., Carbohydrate based vaccines. *Curr. Org. Chem.* 2000, *4*, 653-677; (c) Danishefsky, S. J.; Allen, J. R., From the laboratory to the clinic: a retrospective on fully synthetic carbohydrate-based anticancer vaccines. *Angew. Chem. Int. Ed.* 2000, *39*, 836-863; (d) Pozsgay, V., Oligosaccharide-protein conjugates as vaccine candidates against bacteria. *Adv. Carbohydr. Chem. Biochem.* 2001, *56*, 153-199; (e) Galonic, D. P.; Gin, D. Y., Chemical glycosylation in the synthesis of glycoconjugate antitumour vaccines. *Nature* 2007, *446* (7139), 1000-1007.

16. Seeberger, P. H.; Werz, D. B., Synthesis and medical applications of oligosaccharides. *Nature* 2007, *446* (7139), 1046-1051.

17. Demchenko, A. V., *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*. Wiley-VCH: Weinheim, Germany, 2008.

18. (a) Lemieux, R. U., Some implications in carbohydrate chemistry of theories relating to the mechanisms of replacement reactions. *Adv. Carbohydr. Chem.*

Biochem. 1954, *9*, 1-57 and references therein; (b) Capon, B., Mechanism in carbohydrate chemistry. *Chem. Rev.* 1969, *69*, 407-496; (c) Gervay, J.; Nguyen, T. N.; Hadd, M. J., Mechanistic studies on the stereoselective formation of glycosyl iodides: first characterization of glycosyl iodides. *Carbohydr. Res.* 1997, *300* (2), 119-125; (d) Nukada, T.; Berces, A.; Zgierski, M. Z.; Whitfield, D. M., Exploring the mechanism of neighboring group assisted glycosylation reactions. *J. Am. Chem. Soc.* 1998, *120*, 13291-13295; (e) Nguyen, H. M.; Chen, Y. N.; Duron, S. G.; Gin, D. Y., Sulfide-mediated dehydrative glycosylation. *J. Am. Chem. Soc.* 2001, *123*, 8766-8772; (f) Ayala, L.; Lucero, C. G.; Romero, J. A. C.; Tabacco, S. A.; Woerpel, K. A., Stereochemistry of Nucleophilic Substitution Reactions Depending upon Substituent: Evidence for Electrostatic Stabilization of Pseudoaxial Conformers of Oxocarbenium Ions by Heteroatom Substitution. *J. Am. Chem. Soc.* 2003, *125*, 15521-15528; (g) Crich, D.; Chandrasekera, N. S., Mechanism of 4,6-O-benzylidene-directed b-mannosylation as determined by a-deuterium kinetic isotope effects. *Angew. Chem. Int. Ed.* 2004, *43*, 5386-5389 and references therein; (h) Boebel, T. A.; Gin, D. Y., Probing the mechanism of sulfoxide-catalyzed hemiacetal activation in dehydrative glycosylation. *J. Org. Chem.* 2005, *70*, 5818-5826; (i) Li, Z.; Gildersleeve, J., Mechanistic studies and methods to prevent aglycon transfer of thioglycosides. *J. Am. Chem. Soc.* 2006, *128*, 11612-11619; (j) Jensen, H. H.; Bols, M., Stereoelectronic substituent effects. *Acc. Chem. Res.* 2006, *39*, 259-265; (k) Whitfield, D. M., Computational studies of the role of glycopyranosyl oxacarbenium ions in glycobiology and glycochemistry. *Adv. Carbohydr. Chem. Biochem.* 2009, *62*, 83-159; (l) Mydock, L. K.; Demchenko, A. V., Mechanism of chemical O-glycosylation: from early studies to recent discoveries. *Org. Biomol. Chem.* 2010, *8*, 497-510; (m) Beaver, M. G.; Woerpel, K. A., Erosion of stereochemical control with increasing

nucleophilicity: O-glycosylation at the diffusion limit. *J. Org. Chem.* 2010, *75*, 1107-1118; (n) Crich, D., Mechanism of a chemical glycosylation reaction. *Acc. Chem. Res.* 2010, *43*, 1144-1153; (o) Pedersen, C. M.; Marinescu, L. G.; Bols, M., Glycosyl donors in ‘‘unusual’’ conformations – influence on reactivity and selectivity. *C. R. Chimie* 2010, *14*, 17-43; (p) Nokami, T.; Shibuya, A.; Manabe, S.; Ito, Y.; Yoshida, J., a- and b-Glycosyl sulfonium ions: generation and reactivity. *Chem. Eur. J.* 2009, *15*, 2252-2255; (q) Huang, M.; Retailleau, P.; Bohe, L.; Crich, D., Cation clock permits distinction between the mechanisms of a- and b-O- and b-C-glycosylation in the mannopyranose series: evidence for the existence of a mannopyranosyl oxocarbenium ion. *J. Am. Chem. Soc.* 2012, *134*, 14746-14749; (r) Huang, M.; Garrett, G. E.; Birlirakis, N.; Bohe, L.; Pratt, D. A.; Crich, D., Dissecting the mechanisms of a class of chemical glycosylation using primary ^{13}C kinetic isotope effects. *Nature: Chemistry* 2012, *4*, 663-667; (s) Crich, D., Methodology Development and Physical Organic Chemistry: A Powerful Combination for the Advancement of Glycochemistry. *J. Org. Chem.* 2011, *76*, 9193-9209; (t) Kaeothip, S.; Yasomanee, J. P.; Demchenko, A. V., Glycosidation of thioglycosides in the presence of bromine: mechanism, reactivity, and stereoselectivity. *J. Org. Chem.* 2012, *77*, 291-299; (u) Kononov, L. O.; Malysheva, N. N.; Orlova, A. V.; Zinin, A. I.; Laptinskaya, T. V.; Kononova, E. G.; Kolotyrkina, N. G., Concentration dependence of glycosylation outcome: a clue to reproducibility and understanding the reasons behind. *Eur. J. Org. Chem.* 2012, 1926-1934; (v) Whitfield, D. M., Plausible transition states for glycosylation reactions. *Carbohydr. Res.* 2012, *356*, 180-190; (w) Whitfield, D. M., Complications of modeling glycosylation reactions: can the anomeric conformation of a donor determine the glycopyranosyl oxacarbenium ring conformation? *Carbohydr. Res.* 2012, *356*, 191-195; (x) Ranade, S. C.; Demchenko,

A. V., Mechanism of chemical glycosylation: focus on the mode of activation and departure of anomeric leaving groups. *J. Carbohydr. Chem.* 2013, *32*, 1-43.

19. Smoot, J. T.; Demchenko, A. V., Oligosaccharide synthesis: from conventional methods to modern expeditious strategies. *Adv. Carbohydr. Chem. Biochem.* 2009, *62*, 161-250.

20. (a) Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B., "Armed" and "disarmed" n-pentenyl glycosides in saccharide couplings leading to oligosaccharides. *J. Am. Chem. Soc.* 1988, *110*, 5583-5584; (b) Fraser-Reid, B.; Udodong, U. E.; Wu, Z. F.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R., n-Pentenyl glycosides in organic chemistry: a contemporary example of serendipity. *Synlett* 1992, (12), 927-942 and references therein.

21. Nicolaou, K. C.; Ueno, H., Oligosaccharide synthesis from glycosyl fluorides and sulfides. In *Preparative Carbohydrate Chemistry*, Hanessian, S., Ed. Marcel Dekker, Inc.: New York, 1997; pp 313-338.

22. Williams, L. J.; Garbaccio, R. M.; Danishefsky, S. J., Iterative assembly of glycals and glycal derivatives: the synthesis of glycosylated natural products and complex oligosaccharides. In *Carbohydrates in Chemistry and Biology*, Ernst, B.; Hart, G. W.; Sinay, P., Eds. Wiley-VCH: Weinheim, New York, 2000; Vol. 1, pp 61-92.

23. Kahne, D.; Walker, S.; Cheng, Y.; van Engen, D., Glycosylation of unreactive substrates. *J. Am. Chem. Soc.* 1989, *111*, 6881-6882.

24. Roy, R.; Andersson, F. O.; Letellier, M., "Active" and "latent" thioglycosyl donors in oligosaccharide synthesis. Application to the synthesis of a-sialosides. *Tetrahedron Lett.* 1992, *33* (41), 6053-6056.

25. Boons, G. J.; Isles, S., Vinyl glycosides in oligosaccharide synthesis. Part 1: A new latent-active glycosylation strategy. *Tetrahedron Lett.* 1994, *35*, 3593-3596.

26. (a) Kanie, O.; Ito, Y.; Ogawa, T., Orthogonal glycosylation strategy in oligosaccharide synthesis. *J. Am. Chem. Soc.* 1994, *116*, 12073-12074; (b) Kanie, O., Orthogonal strategy in oligosaccharide synthesis. In *Carbohydrates in Chemistry and Biology*, Ernst, B.; Hart, G. W.; Sinay, P., Eds. Wiley-VCH: Weinheim, New York, 2000; Vol. 1, pp 407-426.

27. (a) Huang, L.; Wang, Z.; Huang, X., One-pot oligosaccharide synthesis: reactivity tuning by post-synthetic modification of aglycone. *Chem. Commun.* 2004, 1960-1961; (b) Huang, X.; Huang, L.; Wang, H.; Ye, X. S., Iterative one-pot synthesis of oligosaccharides. *Angew. Chem. Int. Ed.* 2004, *43*, 5221-5224; (c) Wang, Z.; Zhou, L.; Ei-Boubbou, K.; Ye, X. S.; Huang, X., Multi-component one-pot synthesis of the tumor-associated carbohydrate antigen Globo-H based on preactivation of thioglycosyl donors. *J. Org. Chem.* 2007, *72*, 6409-6420.

28. (a) Parameswar, A. R.; Demchenko, A. V., One-pot oligosaccharide synthesis. In *Progress in the synthesis of complex carbohydrate chains of plant and microbial polysaccharides*, Nifantiev, N. E., Ed. Transworld Res. Network: Kerala, 2009; pp 463-488; (b) Kaeothip, S.; Demchenko, A. V., Expeditious oligosaccharide synthesis via selective and orthogonal activation. *Carbohydr. Res.* 2011, *346*, 1371-1388; (c) Premathilake, H. D.; Demchenko, A. V., Superarmed and superdisarmed building blocks in expeditious oligosaccharide synthesis. In *Topics in Current Chemistry: Reactivity Tuning in Oligosaccharide Assembly*, Fraser-Reid, B.; Lopez, J. C., Eds. Springer-Verlag: Berlin-Heidelberg, 2011; Vol. 301, pp 189-221.

29. (a) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H., Automated solid-phase synthesis of oligosaccharides. *Science* 2001, *291* (5508), 1523-1527; (b) Seeberger, P.

H., Automated oligosaccharide synthesis. *Chem. Soc. Rev.* 2008, *37*, 19-28; (c) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H., Development of an automated oligosaccharide synthesizer. *Adv. Carbohydr. Chem. Biochem.* 2003, *58*, 35-54.

30. (a) Fruchtel, J. S.; Jung, G., Organic chemistry on solid supports. *Angew. Chem. Int. Ed. Engl.* 1996, *35*, 17-42; (b) Winter, M., Supports for solid-phase organic synthesis. In *Combinatorial peptide and nonpeptide libraries: a handbook*, Jung, G., Ed. VCH: Wienheim, New York, Basel, Cambridge, Tjokyo, 1996; pp 465-510.

31. (a) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D., Solid-phase organic reactions: a review of the recent literature. *Tetrahedron* 1996, *52* (13), 4527-4554; (b) Brown, R. C. D., Recent developments in solid-phase organic synthesis. *J. Chem. Soc., Perkin Trans. 1* 1998, 3293-3320.

32. Merrifield, B., The role of the support in solid phase peptide synthesis *Br. Polym. J.* 1984, *16*, 173-178.

33. Toy, P. H.; Lam, Y., *Solid-Phase Organic Synthesis*. John Wiley & Sons, Inc. : Hoboken, 2012.

34. Merrifield, R. B., Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* 1963, *85*, 2149-2154.

35. Frechet, J. M.; Schuerch, C., Solid-phase synthesis of oligosaccharides. I. Preparation of the solid support. Poly[p-(1-propen-3-ol-1-yl)styrene]. *J. Am. Chem. Soc.* 1971, *93*, 492-496.

36. (a) Schmidt, R. R.; Jonke, S.; Liu, K., New Aspects of Glycoside Bond Formation: Solid-Phase Oligosaccharide Synthesis In *ACS Symp. Ser. (Frontiers in Modern Carbohydrate Chemistry)* Demchenko, A. V., Ed. Oxford Univ. Press: 2007; Vol. 960, pp 209-237; (b) Seeberger, P. H., Solid phase oligosaccharide synthesis

(Reprinted from Glycochemistry: Principles, synthesis, and applications, pg 1- 32, 2001). *J. Carbohydr. Chem.* 2002, *21* (7-9), 613-643; (c) Seeberger, P. H.; Haase, W. C., Solid-phase oligosaccharide synthesis and combinatorial carbohydrate libraries. *Chem. Rev.* 2000, *100* (12), 4349-4393; (d) Tanaka, K.; Fukase, K., Oligosaccharide synthesis on solid, soluble polymer, and tag supports. In *Solid-Phase Organic Synthesis*, Toy, P. H.; Lam, Y., Eds. John Wiley & Sons, Inc. : Hoboken, 2012; pp 489-530.

37. (a) Zhu, T.; Boons, G. J., A two-directional approach for the solid-phase synthesis of trisaccharide libraries. *Angew. Chem. Int. Ed.* 1998, *37* (13/14), 1898-1900; (b) Zhu, T.; Boons, G. J., A two-directional and highly convergent approach for the synthesis of the tumor-associated antigen Globo-H. *Angew. Chem., Int. Ed. Engl.* 1999, *38* (23), 3495-3497.

38. (a) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H., Development of an automated oligosaccharide synthesizer. 2003; Vol. 58, pp 35-54; (b) Seeberger, P. H., Automated carbohydrate synthesis to drive chemical glycomics. *Chem. Commun.* 2003, 115-1121; (c) Seeberger, P. H.; Werz, D. B., Automated synthesis of oligosacchraides as a basis for drug discovery. *Nature Rev.* 2005, *4*, 751-763; (d) Lepenies, B.; Yin, J.; Seeberger, P. H., Applications of synthetic carbohydrates to chemical biology. *Current Opinion in Chemical Biology* 2010, *14* (3), 404-411.

39. Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H., Solid-Phase Oligosaccharide Synthesis: Preparation of Complex Structures Using a Novel Linker and Different Glycosylating Agents. *Org. Lett.* 1999, *1*, 1811-1814.

40. (a) Palmacci, E. R.; Plante, O. J.; Hewitt, M. C.; Seeberger, P. H., Automated Synthesis of Oligosaccharides. *Helvetica Chimica Acta* 2003, *86* (12), 3975-3990; (b) Melean, L. G.; Love, K. R.; Seeberger, P. H., Toward the automated solid-phase

synthesis of oligoglucosamines: Systematic evaluation of glycosyl phosphate and glycosyl trichloroacetimidate building blocks. *Carbohydrate Research* 2002, *337* (21-23), 1893-1916; (c) Kanemitsu, T.; Seeberger, P. H., Use of Olefin Cross-Metathesis to Release Azide-Containing Sugars from Solid Support. *Organic Letters* 2003, *5* (24), 4541-4544; (d) Eller, S.; Collot, M.; Yin, J.; Hahm, H. S.; Seeberger, P. H., Automated solid-phase synthesis of chondroitin sulfate glycosaminoglycans. *Angew. Chem. Int. Ed.* 2013, *52* (22), 5858-5861.

41. Esposito, D.; Hurevich, M.; Castagner, B.; Wang, C. C.; Seeberger, P. H., Automated synthesis of sialylated oligosaccharides. *Beilstein Journal of Organic Chemistry* 2012, *8*, 1601-1609.

42. Kandasamy, J.; Hurevich, M.; Seeberger, P. H., Automated solid phase synthesis of oligoarabinofuranosides. *Chem. Commun.* 2013, *49* (40), 4453-4455.

43. Werz, D. B.; Castagner, B.; Seeberger, P. H., Automated synthesis of the tumor-associated carbohydrate antigens Gb-3 and Globo-H: incorporation of a-galactosidic linkages. *J. Am. Chem. Soc.* 2007, *129* (10), 2770-2771.

44. Hurevich, M.; Seeberger, P. H., Automated glycopeptide assembly by combined solid-phase peptide and oligosaccharide synthesis. *Chem. Commun.* 2014, *50*, 1851-1853.

45. (a) Codée, J. D. C.; Kröck, L.; Castagner, B.; Seeberger, P. H., Automated solid-phase synthesis of protected oligosaccharides containing β-mannosidic linkages. *Chemistry - A European Journal* 2008, *14* (13), 3987-3994; (b) Walvoort, M. T. C.; van den Elst, H.; Plante, O. J.; Krock, L.; Seeberger, P. H.; Overkleeft, H. S.; van der Marel, G. A.; Codee, J. D. C., Automated Solid-Phase Synthesis of b-Mannuronic Acid Alginates. *Angew. Chem. Int. Ed.* 2012, *51*, 4393-4396.

46. (a) Hakomori, S., Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. *Ann. Rev. Biochem.* 1981, *50*, 733-764; (b) Hakomori, S., Tumor-Associated Carbohydrate Antigens. *Annu. Rev. Immunol.* 1984, *2*, 103-126; (c) Hakomori, S., Cancer-associated glycosphingolipid antigens: their structure, organization and function. *Acta Anat.* 1998, *161*, 79-90; (d) Hakomori, S., Structure and function of glycosphingolipids and sphingolipids: recollections and future trends. *Biochim. Biophys. Acta* 2008, *1780*, 325-346; (e) Guo, Z.; Wang, Q., Recent development in carbohydrate-based cancer vaccines. *Curr. Opinion in Chem. Biology* 2009, *13*, 608-617.

47. (a) Allen, J. R.; Allen, J. G.; Zhang, X. F.; Williams, L. J.; Zatorski, A.; Ragupathi, G.; Livingston, P. O.; Danishefsky, S. J., A second generation synthesis of the MBr1(Globo-H) breast tumor antigen: new application of the n-pentenyl glycoside method for achieving complex carbohydrate protein linkages. *Chem. Eur. J.* 2000, *6* (8), 1366-1375; (b) Bosse, F.; Marcaurelle, L. A.; Seeberger, P. H., Linear synthesis of the tumor-associated carbohydrate antigens Globo-H, SSEA-3, and Gb3. *J. Org. Chem.* 2002, *67*, 6659-6670; (c) Jeon, I.; Iyer, K.; Danishefsky, S. J., A practical total synthesis of Globo-H for use in anticancer vaccines. *J. Org. Chem.* 2009, *74*, 8452-8455.

48. Love, K. R.; Seeberger, P. H., Automated Solid-Phase Synthesis of Protected Tumor-Associated Antigen and Blood Group Determinant Oligosaccharides. *Angewandte Chemie - International Edition* 2004, *43* (5), 602-605.

49. Grubbs, R. H.; Miller, S. J.; Fu, G. C., Ring-closing metathesis and related processes in organic synthesis. *Acc. Chem. Res.* 1995, *28*, 446-452.

50. Krock, L.; Esposito, D.; Castagner, B.; Wang, C.-C.; Bindschadler, P.; Seeberger, P. H., Streamlined access to conjugation-ready glycans by automated synthesis. *Chem. Sci.* 2012, *3*, 1617-1622.

51. Kim, K. S.; Kim, J. H.; Lee, Y. J.; Lee, Y. J.; Park, J., 2-(Hydroxycarbonyl)benzyl glycosides: a novel type of glycosyl donors for highly efficient b-mannopyranosylation and oligosaccharide synthesis by latent-active glycosylation. *J. Am. Chem. Soc.* 2001, *123*, 8477-8481.

52. Calin, O.; Eller, S.; Seeberger, P. H., Automated polysaccharide synthesis: Assembly of a 30mer mannoside. *Angew. Chem. Int. Ed.* 2013, *52* (22), 5862-5865.

53. (a) Egusa, K.; Kusumoto, S.; Fukase, K., Solid-Phase Synthesis of a Phytoalexin Elicitor Pentasaccharide Using a 4-Azido-3-chlorobenzyl Group as the Key for Temporary Protection and Catch-and-Release Purification. *Eur. J. Org. Chem.* 2003, *2003*, 3435-3445; (b) Hanashima, S.; Manabe, S.; Ito, Y., Divergent synthesis of sialylated glycan chains: combined use of polymer support, resin capture-release, and chemoenzymatic strategies. *Angew. Chem. Int. Ed.* 2005, *44*, 4218-4228; (c) Ando, H.; Manabe, S.; Nakahara, Y.; Ito, Y., Tag-reporter strategy for facile oligosaccharide synthesis on polymer support. *J. Am. Chem. Soc.* 2001, *123*, 3848-3849; (d) Wu, B.; Hua, Z.; Warren, J. D.; Ranganathan, K.; Wan, Q.; Chen, G.; Tan, Z.; Chen, J.; Endo, A.; Danishefsky, S. J., Synthesis of the fucosylated biantennary N-glycan of erythropoietin *Tetrahedron Lett.* 2006, *47*, 5577-5579.

54. Vijaya Ganesh, N.; Fujikawa, K.; Tan, Y. H.; Stine, K. J.; Demchenko, A. V., HPLC-assisted automated oligosaccharide synthesis. *Org. Lett.* 2012, *14*, 3036-3039.

55. Nigudkar, S. S.; Parameswar, A. R.; Pornsuriyasak, P.; Stine, K. J.; Demchenko, A. V., O-Benzoxazolyl imidates as versatile glycosyl donors for chemical glycosylation. *Org. Biomol. Chem.* 2013, *11* (24), 4068-4076.

56. Fraser-Reid, B.; Lopez, J. C., *Reactivity Tuning in Oligosaccharide Assembly*. Springer-Verlag: Berlin-Heidelberg, 2011; Vol. 301.

57. (a) Jensen, H. H.; Pedersen, C. M.; Bols, M., Going to extremes: "super" armed glycosyl donors in glycosylation chemistry. *Chem. Eur. J.* 2007, *13*, 7576-7582; (b) Pedersen, C. M.; Nordstrom, L. U.; Bols, M., "Super armed" glycosyl donors: conformational arming of thioglycosides by silylation. *J. Am. Chem. Soc.* 2007, *129*, 9222-9235; (c) Mydock, L. K.; Demchenko, A. V., Superarming the S-benzoxazolyl glycosyl donors by simple 2-O-benzoyl-3,4,6-tri-O-benzyl protection. *Org. Lett.* 2008, *10*, 2103-2106; (d) Premathilake, H. D.; Mydock, L. K.; Demchenko, A. V., Superarming common glycosyl donors by simple 2-O-benzoyl-3,4,6-tri-O-benzyl protection. *J. Org. Chem.* 2010, *75*, 1095-1100.

58. Zhang, Z.; Ollmann, I. R.; Ye, X. S.; Wischnat, R.; Baasov, T.; Wong, C. H., Programmable one-pot oligosaccharide synthesis. *J. Am. Chem. Soc.* 1999, *121*, 734-753.

59. (a) Hsu, C. H.; Hung, S. C.; Wu, C. Y.; Wong, C. H., Toward automated oligosaccharide synthesis. *Angew. Chem. Int. Ed.* 2011, *50*, 11872-11923; (b) Huang, T. Y.; Zulueta, M. M. L.; Hung, S. C., One-pot strategies for the synthesis of the tetrasaccharide linkage region of proteoglycans. *Org. Lett.* 2011, *13*, 1506-1509; (c) Huang, Y. L.; Hung, J. T.; Cheung, S. K.; Lee, H. Y.; Chu, K. C.; Li, S. T.; Lin, Y. C.; Ren, C. T.; Cheng, T. J.; Hsu, T. L.; Yu, A. L.; Wu, C. Y.; Wong, C. H., Carbohydrate-based vaccines with a glycolipid adjuvant for breast cancer. *Proc. Nat. Acad. Sci.* 2013, *110* (7), 2517-22.

60. Wilson, B. G.; Fraser-Reid, B., n-Pentenyl glycoside based methodology for determining the relative reactivities of variously protected pairs of glycosides. *J. Org. Chem.* 1995, *60*, 317-320.

61. (a) Grice, P.; Ley, S. V.; Pietruszka, J.; Priepke, H. W. M.; Walther, E. P. E., Tuning the reactivity of glycosides: efficient one-pot oligosaccharide synthesis. *Synlett* 1995, 781-784; (b) Baeschlin, D. K.; Chaperon, A. R.; Charbonneau, V.; Green, L. G.; Ley, S. V.; Lucking, U.; Walther, E., Rapid assembly of oligosaccharides: Total synthesis of a glycosylphosphatidylinositol anchor of trypanosoma brucei. *Angew. Chem. Int. Edit.* 1998, *37* (24), 3423-3428; (c) Douglas, N. L.; Ley, S. V.; Lucking, U.; Warriner, S. L., Tuning glycoside reactivity: new tool for efficient oligosaccharides synthesis. *J. Chem. Soc., Perkin Trans. 1* 1998, 51-65.

62. Tanaka, H.; Yamada, H.; Takahashi, T., Rapid Synthesis of Oligosaccharides Based on One-Pot Glycosylation. *Trends Glycosci. Glycotechnol.* 2007, *19*, 183-193.

63. Takahashi, T.; Adachi, M.; Matsuda, A.; Doi, T., Combinatorial synthesis of trisaccharides via solution-phase one-pot glycosylation. *Tetrahedron Lett.* 2000, *41*, 2599-2603.

64. Tanaka, H.; Matoba, N.; Tsukamoto, H.; Takimoto, H.; Yamada, H.; Takahashi, T., Automated Parallel Synthesis of a Protected Oligosaccharide Library Based upon the Structure of Dimeric Lewis X by One-Pot Sequential Glycosylation HirAutomated Parallel Synthesis of a Protected Oligosaccharide Library. *Synlett* 2005, 0824–08282.

65. Miura, T.; Goto, K.; Waragai, H.; Matsumoto, H.; Hirose, Y.; Ohmae, M.; Ishida, H.; Satoh, A.; Inazu, T., Rapid oligosaccharide synthesis using a fluorous protective group. *J. Org. Chem.* 2004, *69*, 5348-5353.

66. Carrel, F. R.; Geyer, K.; Codée, J. D. C.; Seeberger, P. H., Oligosaccharide synthesis in microreactors. *Org. Lett.* 2007, *9*, 2285-2288.

67. Jaipuri, F. A.; Pohl, N. L., Toward solution-phase automated iterative synthesis: Fluorous-tag assisted solution-phase synthesis of linear and branched mannose oligomers. *Org. Biomol. Chem.* 2008, *6*, 2686-2691.

68. Pornsuriyasak, P.; Ranade, S. C.; Li, A.; Parlato, M. C.; Sims, C. R.; Shulga, O. V.; Stine, K. J.; Demchenko, A. V., STICS: surface-tethered iterative carbohydrate synthesis. *Chem. Commun.* 2009, 1834-1836.

69. Ganesh, N. V.; Fujikawa, K.; Tan, Y. H.; Nigudkar, S. S.; Stine, K. J.; Demchenko, A. V., Surface-tethered iterative carbohydrate synthesis: a spacer study. *J. Org. Chem.* 2013, *78* (14), 6849-57.

70. (a) Amatore, C.; Jutand, A.; Mallet, J. M.; Meyer, G.; Sinay, P., Electrochemical glycosylation using phenyl S-glycosides. *J. Chem. Soc., Chem. Commun.* 1990, 718-719; (b) Balavoine, G.; Berteina, S.; Gref, A.; Fischer, J. C.; Lubineau, A., Thio glycosides as potential glycosyl donors in electrochemical glycosidation reactions. Part 1. Their preparation and reactivity toward simple alcohols. *J. Carbohydr. Chem.* 1995, *14*, 1217-1236; (c) Yamago, S.; Kokubo, K.; Yoshida, J.-i., O-Glycosidation of Telluroglycoside by Electrochemical Oxidation. *Chem. Lett.* 1997, *26* (2), 111-112; (d) Yamago, S.; Kokubo, K.; Hara, O.; Masuda, S.; Yoshida, J., Electrochemistry of chalcogenoglycosides. Rational design of iterative glycosylation based on reactivity control of glycosyl donors and acceptors by oxidation potentials. *J. Org. Chem.* 2002, *67*, 8584-8592; (e) France, R. R.; Compton, R. G.; Davis, B. G.; Fairbanks, A. J.; Rees, N. V.; Wadhawan, J. D., Selective electrochemical glycosylation by reactivity tuning. *Org. Biomol. Chem.* 2004, *2* (15), 2195-2202; (f) Nokami, T.; Shibuya, A.; Tsuyama, H.; Suga, S.; Bowers, A. A.; Crich, D.; Yoshida, J., Electrochemical generation of glycosyl triflate pools. *J. Am. Chem. Soc.* 2007, *129*, 10922-10928.

71. Nokami, T.; Hayashi, R.; Saigusa, Y.; Shimizu, A.; Liu, C.-Y.; Mong, K.-K. T.; Yoshida, J.-i., Automated Solution-Phase Synthesis of Oligosaccharides via Iterative Electrochemical Assembly of Thioglycosides. *Org. Lett.* 2013, *15*, 4520-4523.

CHAPTER 2

Hydrogen bond-mediated aglycone delivery: focus on β-mannosylation

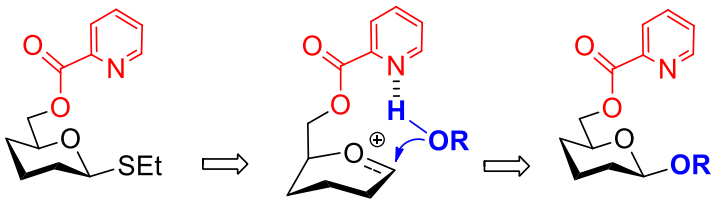
2.1 General Introduction

S. G. Pistorio, J. P. Yasomanee, A. V. Demchenko. Hydrogen bond-mediated aglycone delivery: focus on β-mannosylation. *Org. Lett.*, **2014**, *16*, 716-719

The vast majority of complex carbohydrates consists of monosaccharide residues connected via *O*-glycosidic linkages.[1] Uncontrolled chemical *O*-glycosylations often lead to low yields and/or mixtures of anomers. The goal of stereocontrolling glycosylation has been a major inspiration and driving force of progress in the field. The synthesis of 1,2-*cis* glycosides, which cannot be assisted by conventional neighboring acyl group participation,[2] is more challenging. Many factors affect the stereoselectivity of glycosylation, but none can guarantee complete 1,2-*cis* stereoselectivity.[3] Therefore, all individual factors and combinations thereof are typically considered when glycosylations are attempted.

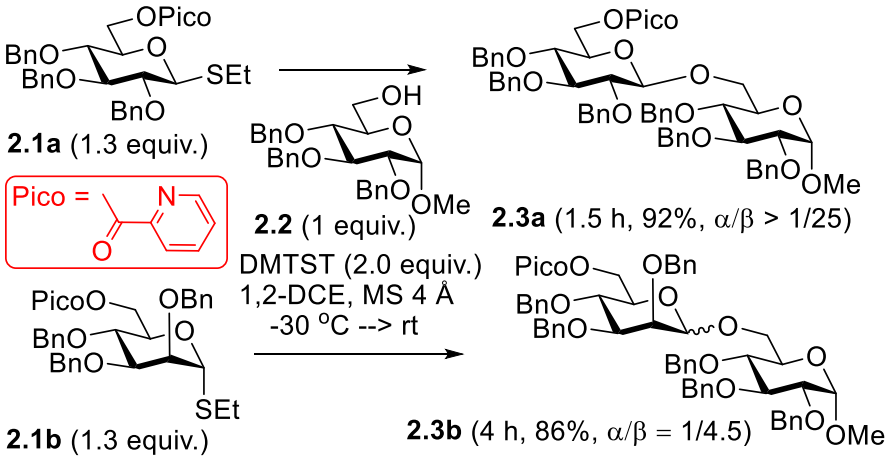
Amongst a variety of unconventional protecting groups that have been introduced in recent years to control the stereoselectivity of glycosylations,[4] the neighboring 2-*O*-picolinyl group formally participates in glycosylation and provides 1,2-*trans* products stereoselectively as a result of the *anti* attack by the glycosyl acceptor.[5] Remarkably, when placed at remote positions (C-3, C-4, and C-6), picolinyl and similar picoloyl substituents also provide high selectivity, but act via a different mode. All glycosylations proceed with stereoselectivity consistent with *syn* attack, because the remote picolinyl moiety acts as an H-bond acceptor for the incoming nucleophile (Scheme 2.1).[6] Therefore, since this remote protecting group assistance is not directly correlated with the orientation of the substituent at C-2, this approach should in principle be suitable for the assisted synthesis of either 1,2-*cis* or 1,2-*trans*-linked glycosides.

Scheme 2.1. 6-*O*-Picoloyl-assisted β-D-glycosylation



For instance, as illustrated in Scheme 2.2, glycosidation of the 6-picoloyl (Pico) glucosyl donor **2.1a** with acceptor **2.2** in the presence of dimethyl(methylthio)sulfonium triflate (DMTST)[7] afforded disaccharide **2.3a** in 92% yield and complete β-selectivity.[6] A similar DMTST-promoted glycosylation with mannosyl donor **2.1b** was significantly less stereoselective (α/β = 1/4.5) and afforded the corresponding disaccharide **2.3b** in 86% yield.[6]

Scheme 2.2. Synthesis of β-glucosides vs. β-mannosides



Further screening of the reaction conditions showed that the NIS/TfOH promoter system provides a better environment for β-mannosylation with donor **2.1b**. Under these reaction conditions, disaccharide **2.3b** was obtained in 87% yield and enhanced β-selectivity (α/β = 1/9.5, Table 2.1, entry 1).[6] The synthesis of β-mannosides has been regarded as one of the greatest challenges of glycochemistry.[8]

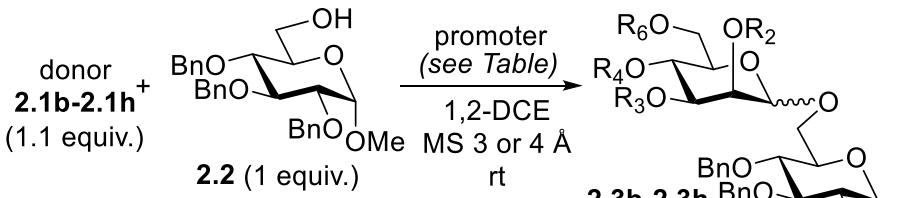
Some promising methods have been established by Crich[9] and others,[10] but these are typically limited to specific types of glycosyl donor and require extreme reaction conditions or indirect methods[11] to ensure that reactions proceed highly stereoselectively. Building upon promising preliminary results, herein we present our systematic study of H-bond mediated aglycone delivery reaction as applied to β-mannosylation.

2.2 Results and Discussion

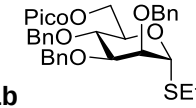
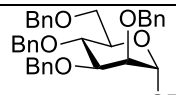
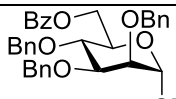
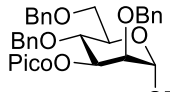
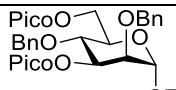
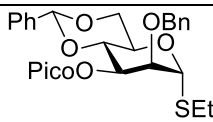
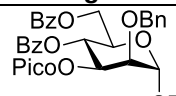
As the starting comparison point, known per-*O*-benzylated glycosyl donor **2.1c**[12] was coupled with glycosyl acceptor **2.2** in the presence of DMTST or NIS/TfOH under high dilution reaction conditions that became the standard for *O*-picoloylated glycosyl donors: 5.0 mM concentration in 1,2-dichloroethane. Previously, we conducted all reactions at -30 → 42 ^{o}C.[6] Herein we determined no significant temperature dependence and conducted all reactions at rt. Thus, a glycosylation reaction between donor **2.1c** and acceptor **2.2**[13] gave the corresponding disaccharide **2.3c**[14] in good yield, but no selectivity was observed in the case of either promoter (Table 2.1, entry 2). Similarly, glycosidation of 6-*O*-benzoyl donor **2.1d**[15] provided disaccharide **2.3d** with no stereoselection (entry 3). Since D-mannose has two remote substituents projecting above the pyranose ring (O-3 and O-6) we were curious to compare 6-*O*-picoloyl donor **2.1b** with its 3-*O*-picoloyl counterpart **2.1e**. Encouragingly, donor **2.1e** gave excellent yields (89-92%) and high β-stereoselectivity (α/β = 1/7-8, entry 4). Further screening of protecting groups included the 3,6-di-*O*-picoloyl donor **2.1f**, the 4,6-*O*-benzylidene donor **2.1g**, and the 4,6-di-*O*-benzoyl donor **2.1h**. Among this 3-*O*-picoloyl donor series, the best results

were achieved with donor **2.1h**, which afforded the corresponding disaccharide **2.3h** in good yield and with high stereoselectivity (α/β > 1/12, entry 7).

Table 2.1. Comparative investigation of mannosyl donors 2.1b-2.1h



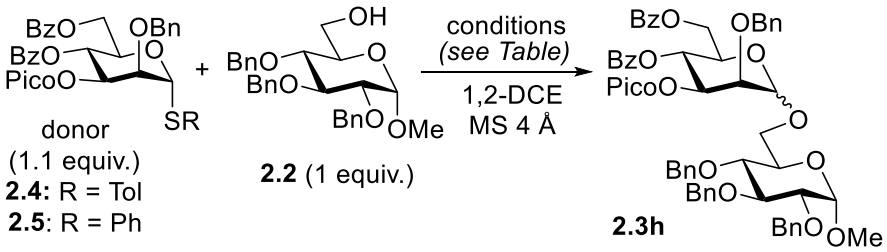
b: $R_2 = R_3 = R_4 = Bn$; $R_6 = Pico$
c: $R_2 = R_3 = R_4 = R_6 = Bn$
d: $R_2 = R_3 = R_4 = Bn$; $R_6 = Bz$
e: $R_2 = R_4 = R_6 = Bn$; $R_3 = Pico$
f: $R_2 = R_4 = Bn$; $R_3 = R_6 = Pico$
g: $R_2 = Bn$; $R_3 = Pico$; R_4, R_6 = >Ph
h: $R_2 = Bn$; $R_3 = Pico$; $R_4 = R_6 = Bz$

entry	donor	promoter, time	product (yield, α/β ratio)
1[6]	 **2.1b**	DMTST, 4 h NIS/TfOH, 2.5 h	**2.3b** (86%, 1/4.5) **2.3b** (87%, 1/9.5)
2	 **2.1c**	DMTST, 72 h NIS/TfOH, 72 h	**2.3c** (65%, 1/1.4) **2.3c** (67%, 1/1.0)
3	 **2.1d**	DMTST, 24 h NIS/TfOH, 24 h	**2.3d** (68%, 1/1.0) **2.3d** (70%, 1/1.0)
4	 **2.1e**	DMTST, 30 min NIS/TfOH, 20 min	**2.3e** (92%, 1/7.0) **2.3e** (89%, 1/8.0)
5	 **2.1f**	DMTST, 50 min NIS/TfOH, 3 h	**2.3f** (72%, 1/10.0) **2.3f** (86%, 1/8.7)
6	 **2.1g**	DMTST, 40 min NIS/TfOH, 40 min	**2.3g** (73%, 1/5.7) **2.3g** (73%, 1/6.5)
7	 **2.1h**	DMTST, 1.5 h NIS/TfOH, 1 h	**2.3h** (86%, 1/12.1) **2.3h** (72%, 1/12.3)

Having identified the most promising glycosyl donor **2.1h** of the ethyl thioglycoside series, we conducted further studies with similarly protected *p*-tolyl and

phenyl thioglycoside series, **2.4** and **2.5**, respectively. In addition to studying the effect of the anomeric leaving groups, this in-depth study involved screening promoters (DMTST and NIS/TfOH), concentration (1-50 mM), and temperature (rt and -30 ^{o}C). Although all experiments summarized in Table 2.2 proceeded with high selectivity ($\alpha/\beta > 1/5$), the most beneficial conditions for β-mannosylation of acceptor **2.2** include: glycosyl donor **2.5** (5 mM in 1,2-dichloromethane) activated with DMTST at rt. Resultantly, disaccharide **2.3h** was obtained in excellent yield and stereoselectivity (91%, $\alpha/\beta = 1/18.5$, entry 5).

Table 2.2. Investigation of *S*-tolyl 2.4 and *S*-phenyl 2.5 glycosyl donors



entry	donor (conc.)	conditions	time	yield, α/β ratio of **2.3h**
1	**2.4** (5 mM)	DMTST, rt	2 h 50 min	89%, 1/9.5
2	**2.4** (5 mM)	NIS/TfOH, rt	12 h	86%, 1/9.5
3	**2.5** (50 mM)	DMTST, rt	7 h	73%, 1/5.0
4	**2.5** (50 mM)	NIS/TfOH, rt	1h 10 min	97%, 1/8.8
5	**2.5** (5 mM)	DMTST, rt	2 h 50 min	91%, 1/18.5
6	**2.5** (5 mM)	NIS/TfOH, rt	12 h	96%, 1/7.2
7	**2.5** (5 mM)	DMTST, -30 ^{o}C	5 h	87%, 1/17.6
8	**2.5** (1 mM)	DMTST, rt	2 h	80%, 1/14.0

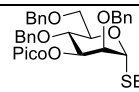
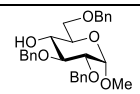
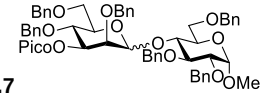
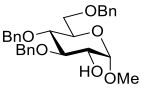
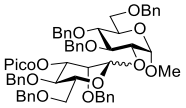
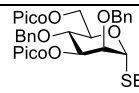
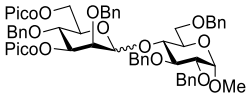
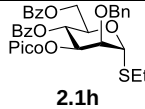
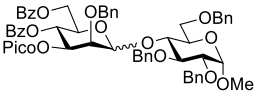
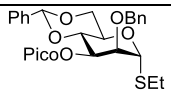
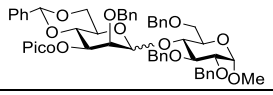
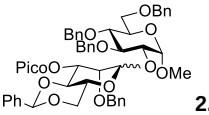
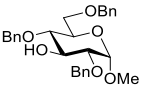
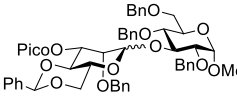
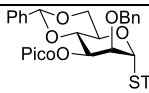
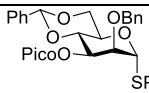
Following this, we decided to investigate whether these mannosyl donors would also be suitable for stereoselective couplings with the secondary glycosyl acceptors **3.6**, **2.8**, and **2.14**. Concerning glycosyl donors of the ethyl thioglycoside series, the best results for glycosylation of primary glycosyl acceptor **2.2** were obtained with **2.1e**, **2.1f**, and **2.1h** (see Table 2.1). Unfortunately, when applied to glycosylations of secondary acceptors **6** and **2.8**,[19] none of these donors performed up to our expectations (Table 2.3, entries 1-4). Although the corresponding disaccharides **2.7** and **2.9-2.11** were obtained in respectable yields of 72-87%, stereoselectivity was much lower (α/β = 1/2.9-5.2, Table 2.3) in comparison to that achieved with the primary acceptor **2.2**.

In contrast, the glycosyl donor **2.1g**, which was not very effective with the primary acceptor **2.2**, showed respectable results in coupling reactions with the secondary glycosyl acceptors. Thus, coupling of the donor **2.1g** with the 4-OH acceptor **2.6** afforded disaccharide **2.12** in good yields 71-83% and commendable β-stereoselectivity (α/β ~ 1/10, entry 5). Similar results were obtained in glycosylations of the acceptors **2.8** and **2.14**, which led to the formation of disaccharides **2.13** and **2.15**, respectively, in 71-88% yield with high stereoselectivity (α/β = 1/6-10, entries 6 and 7). Similarly protected *S*-tolyl **2.16** and *S*-phenyl **2.17** donors provided disaccharide **2.12** with even higher yields (80-96%) albeit with lower stereoselectivity (α/β = 1/5.4-8, entries 8 and 9) in comparison to that obtained with their *S*-ethyl counterpart **2.1g**.

Having investigated the synthesis of both primary and secondary β-mannosides, we felt well equipped to evaluate an oligosaccharide synthesis, to probe the protecting/leaving group combinations and conditions. For this purpose, we

obtained disaccharide **2.3h** (91%, α/β = 1/18.5) using best conditions for primary glycosyl accep-

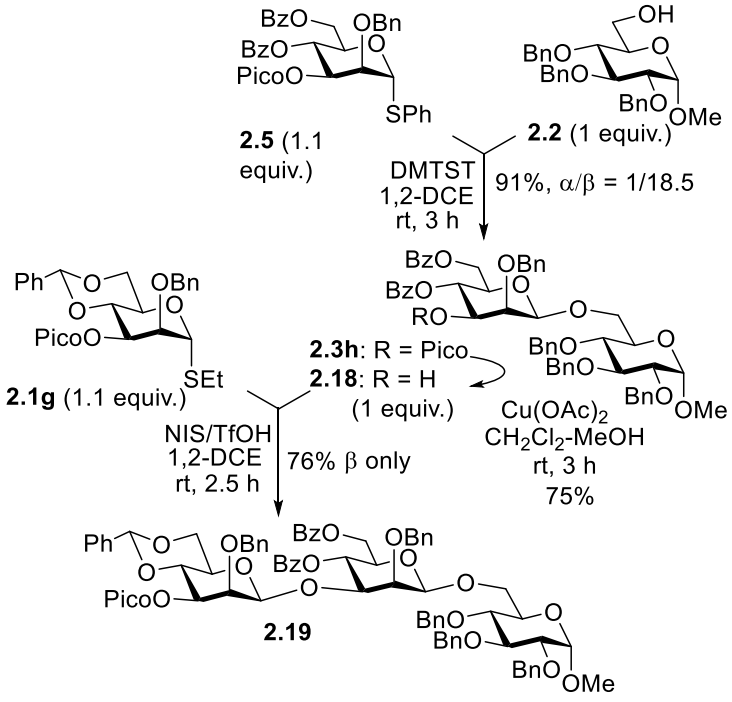
Table 2.3. Glycosylation of secondary glycosyl acceptors 2.6, 2.8, and 2.14

entry	donor	acceptor	promoter, time	product	yield, α/β ratio
1	 **2.1e**	 **2.6**	NIS/TfOH, 30 min	 **2.7**	80%, 1/2.9
2	**2.1e**	 **2.8**	NIS/TfOH, 30 min	 **2.9**	78%, 1/4.9
3	 **2.1f**	**2.6**	DMTST, 2 h NIS/TfOH, 3 h	 **2.10**	73%, 1/2.9 87%, 1/2.9
4	 **2.1h**	**2.6**	DMTST, 1.5 h NIS/TfOH, 1.5 h	 **2.11**	86%, 1/5.2 72%, 1/3.5
5	 **2.1g**	**2.6**	DMTST, 40 min NIS/TfOH, 2 h	**2.12** 	71%, 1/10.0 83%, 1/9.8
6	**2.1g**	**2.8**	DMTST, 1.5 h	 **2.13**	78%, 1/7.3
7	**2.1g**	 **2.14**	DMTST, 2 h NIS/TfOH, 2 h	 **2.15**	71%, 1/6.0 88%, 1/10
8	 **2.16**	**2.6**	DMTST, 24 h NIS/TfOH, 2 h[b]	**2.12**	80%, 1/5.4 96%, 1/8.0
9	 **2.17**	**2.6**	DMTST, 2.5 h NIS/TfOH, 12 h[c]	**2.12**	86%, 1/5.5 89%, 1/7.4

[a] Unless noted otherwise, performed under standard conditions: 5 mM concentration of donor, 1,2-dichloroethane (10 mL), rt; [b] Performed at 50 mM concentration of donor, no reaction at 5 mM; [c] Performed at 50 mM concentration of donor, lower stereoselectivity obtained at 5 mM

tor **2.2**: *S*-phenyl donor **2.5** activated with DMTST at rt. The β-linked disaccharide was separated and subjected to selective removal of 3'-*O*-picoloyl group, which was selectively affected in the presence of copper(II) acetate to give the 3'-OH derivative **2.18** (Scheme 2.3). The latter was glycosylated with donor **2.1g**, which was found the most suitable for glycosylation of secondary hydroxyls. This coupling was promoted in the presence of NIS and TfOH and resulted in the formation of trisaccharide **2.19** in 76% yield and, to our delight, with complete β-stereoselectivity (α/β > 1/25).

Scheme 2.3. The synthesis of trisaccharide 2.19



2.3 Conclusions

We have discovered that a remote 3-*O*-picoloyl group can effectively mediate β-mannosylation reactions with high facial *syn* selectivity for attack of the glycosyl acceptor. The applicability of this approach was demonstrated for the synthesis of an

oligosaccharide containing both primary and secondary β-mannosidic linkages. Further application of this new stereoselective glycosylation reaction to other targets and systems is currently underway in our laboratory.

2.4 Experimental

2.4.1 General methods

Column chromatography was performed on silica gel 60 (70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F254. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 and $ClCH_2CH_2Cl$ (1,2-DCE) were distilled from CaH_2 directly prior to application. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Molecular sieves (3 Å or 4 Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. Optical rotations were measured at 'Jasco P-1020' polarimeter. Unless noted otherwise, ^1H n.m.r. spectra were recorded in $CDCl_3$ at 300 MHz, ^{13}C n.m.r. spectra were recorded in $CDCl_3$ at 75 or 150 MHz. Two-dimensional heteronuclear *J*-resolved spectra (HETERO2D) were recorded in $CDCl_3$ at 600 MHz.

2.4.2 Synthesis of Glycosyl S-Et donors series

Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-picolinyl-1-thio-β-D-glucopyranoside (2.1a). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[6]

Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.1b). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[6]

Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-mannopyranoside (2.1c). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[12]

Ethyl 6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (2.1d). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[15]

Ethyl 2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.1e). Picolinic acid (136 mg, 1.11 mmol), *N*,*N'*-dicyclohexylcarbodiimide (DCC, 343 mg, 1.66 mmol), and 4-dimethylaminopyridine (DMAP, 2.0 mg, 0.17 mmol) were added to a solution of ethyl 2,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside[16] (**2.S1**, 350 mg, 0.856 mmol) in CH_2Cl_2 (12 mL), and the resulting mixture was stirred under argon for 25 min at rt. The reaction mixture was diluted with CH_2Cl_2 (~50 mL), the solid was filtered off, and the filtrate was washed with cold water (10 mL), sat. aq. $NaHCO_3$ (10 mL), and water (2 x 10 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound as an amorphous powder in 90% yield (462 mg, 0.277 mmol). Analytical data for **2.1e**: R_f = 0.52 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{22}$ +66.9 (c = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 1.33 (t,

3H, J = 7.4 Hz, SCH_2CH_3), 2.69 (q, 2H, SCH_2CH_3), 3.78 (dd, $J_{5,6a}$ = 10.9 Hz, H-6a), 3.93 (dd, $J_{5,6b}$ = 10.9 Hz, H-6b), 4.17 (dd, 1H, $J_{2,3}$ = 1.8 Hz, H-2), 4.34-4.44 (m, 2H, H-4, 5), 4.54-4.86 (m, 6H, 3 x CH_2Ph), 5.51 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 5.54 (d, 1H, $J_{2,3}$ = 3.3 Hz, H-3), 7.12-7.53 (m, 17H, aromatic), 7.82-8.05 (m, 1H, aromatic), 8.04 (d, 1H, J = 7.8 Hz, aromatic), 8.82-8.83 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 14.9, 25.2, 68.9, 71.7, 72.3, 73.4, 73.5, 74.8, 75.6, 81.7, 125.2, 126.8, 127.5, 127.6, 127.7 (x2), 127.8 (x2), 127.9 (x3), 128.0 (x2), 128.2 (x3), 128.3 (x2), 136.8, 137.7, 138.1, 138.2, 147.8, 150.0, 164.2 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{35}H_{37}O_6NS$ 600.2420, found 600.2428

Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.1g). Picolinic acid (168 mg, 1.36 mmol), DCC (0.31 g, 1.5 mmol), and DMAP (25 mg, 0.2 mmol) were added to a solution of ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside[17] (**2.S2**, 0.47 g, 1.0 mmol) in CH_2Cl_2 (15 mL) and the resulting mixture was stirred under argon for 40 min at rt. The reaction mixture was diluted with CH_2Cl_2 (~100 mL) and the solid was filtered off. The filtrate was washed with cold water (10 mL), sat. aq. $NaHCO_3$ (10 mL), and water (2 x 10 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound as an amorphous powder in 90% yield (450 mg, 0.88 mmol). Analytical data for **2.1g**: R_f = 0.55 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{23}$ +17.7 (c = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 1.31 (t, 3H, J = 7.4 Hz, SCH_2CH_3), 2.66 (q, 2H, SCH_2CH_3), 3.96 (t, 1H, $J_{2,3}$ = 10.2 Hz, H-2), 4.22-4.32 (m, 2H, H-6a, 6b), 4.39-4.56 (m, 2H, H-4, 5), 4.70 (dd, 2H, ^2J = 12.0 Hz, CH_2Ph), 5.44 (s, 1H, H-1), 5.65 (dd, 1H $J_{3,4}$ = 10.1 Hz, H-3), 5.65

(s, 1H, >C*H*Ph), 7.18-7.47 (m, 11H, aromatic), 7.76-7.81 (m, 1H, aromatic), 8.08 (m, 1H, *J* = 7.7 Hz, aromatic), 8.79 (m, 1H, *J* = 4.2 Hz, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 14.9, 25.4, 53.6, 64.6, 68.6, 72.1, 73.1, 76.4, 83.0, 101.8, 125.5, 126.3 (x2), 127.0, 128.0, 128.1 (x3) 128.2 (x3), 128.4 (x3), 129.0, 136.9, 137.3, 137.4 ppm; HR FAB MS [M+H$^+$] calcd for $C_{28}H_{30}NO_6S$ 508.1763, found 508.1786.

Ethyl 2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.1f). Copper(II) trifluoromethanesulfate (8.0 mg, 0.02 mmol) was added to a solution of α-**2.1g** (100 mg, 0.2 mmol) in 1M BH_3-THF (1.0 mL) and the resulting mixture was stirred under argon for 2 h at rt. The reaction mixture was cooled to 0 ºC and quenched with triethylamine (0.5 mL) until pH = 7. MeOH (1.5 mL) was added dropwise and the volatiles were removed *in vacuo*. The residue was purified by column chromatography on silica gel (acetone-toluene gradient elution) to give ethyl 2,4-di-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (**2.S3**) as white amorphous solid in 77% yield (78 mg, 0.15 mmol). Analytical data for **2.S3**: R_f = 0.54 (acetone/toluene, 1/9, v/v); $[\alpha]_D^{22}$ +168.3 (c = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 1.27 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 2.58 (q, 2H, SCH_2CH_3), 3.56-3.70 (m, 4H, H-2, 4, 5, 6a), 3.81-3.86 (m, 2H, H-4, 6b), 4.50 (dd, 2H, ^2J = 11.7 Hz, CH_2Ph), 4.62 (dd, 2H, ^2J = 11.2 Hz, CH_2Ph), 5.23 (s, 1H, H-1), 7.28-7.40 (m, 11H, aromatic), 7.81 (d, 1H, *J* = 7.9 Hz, aromatic), 7.93-7.95 (m, 1H, aromatic), 8.70 (d, 1H, *J* = 5.5 Hz, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 14.8, 25.1, 62.2, 71.6, 72.3, 72.5, 74.9, 79.9, 81.2, 127.8, 127,9 (x4), 128.1 (x4), 128.2, 128.5 (x3), 128.7 (x4), 137.5, 138.9 ppm; HR FAB MS [M+H$^+$] calcd for $C_{28}H_{32}NO_6S$ 510.1950, found 510.1972.

Picolinic acid (60 mg, 0.494 mmol), 3-(ethyliminomethyleneamino)-*N*,*N*-dimethylpropan-1-amine (EDC, 0.176 g, 0.6 mmol), and DMAP (9.2 mg, 0.076 mmol) were added to a solution of **2.S3** (0.13 g, 0.25 mmol) in CH_2Cl_2 (16 mL) and the resulting mixture was stirred under argon for 40 min at rt. The reaction mixture was diluted with CH_2Cl_2 (~50 mL) and was washed with cold water (5 mL), sat. aq. $NaHCO_3$ (5 mL), and water (5 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound white amorphous solid in 85% yield (0.135 g, 0.22 mmol). Analytical data for **2.1g**: R_f = 0.25 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{23}$ +38.4 (c = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 1.24 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 2.64 (q, 2H, SCH_2CH_3), 4.17 (dd, 1H, $J_{2,3}$ = 3.1 Hz, H-2), 4.38 (t, 1H, $J_{3,4}$ = 9.2 Hz, H-4), 4.53-4.47 (m, 1H, H-5), 4.56-4.89 (m, 3H, H-6a, 6b, $^1/_2$ CH_2Ph), 4.87 (d, 1H, *J* = 11 Hz, $^1/_2$ CH_2Ph), 5.44 (d, 1H, $J_{1,2}$ = 5.4 Hz, H-1), 5.54 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 7.10-8.06 (m, 16H, aromatic), 8.75-8.79 (m, 2H, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 14.9, 25.2, 64.3, 70.0, 72.3, 73.1, 74.8, 75.8, 81.7, 125.2, 125.3, 126.8, 127.0, 127.72 127.75 (x2), 127.8 (x2), 128.1 (x2), 128.2 (x2), 128.3 (x2), 136.8, 136.9, 137.6, 137.7, 147.7, 147.8, 150, 150.1, 164.3, 164.6 ppm; HR FAB MS [M+H$^+$] calcd for $C_{34}H_{35}N_2O_7S$ 615.2165, found 615.2164.

Ethyl 4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.1h). Water (400 μL) and trifluoroacetic acid (TFA)/CH_2Cl_2 (1.8 mL, 1/9, v/v) were added to a stirring mixture of **2.1g** (1.8 g, 3.5 mmol) in CH_2Cl_2 (40 mL) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was neutralized with Et_3N (~4

mL), and diluted with CH_2Cl_2 (~200 mL) and washed with cold water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and cold water (3 x 20 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol-CH_2Cl_2 gradient elution) to afford ethyl 2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (**2.S4**) as a white amorphous solid in 87% yield (1.29 g, 3.18 mmol). Analytical data for **2.S4**: R_f = 0.47 (methanol/CH_2Cl_2, 1/9, v/v); $[\square]_D^{22}$ +148.9 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 1.28 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 2.63 (q, 2H, SCH_2CH_3), 2.90 (t, 1H, *J* = 12.4 Hz, OH), 3.93-3.96 (m, 2H, H-6a, 6b), 4.09-4.18 (m, 3H, H-2, 5, OH), 4.42-4.50 (m, 1H, H-4), 4.60 (dd, 2H, ^2J = 12.4 Hz, CH_2Ph), 5.27 (dd, 1H, $J_{3,4}$ = 10.0 Hz, H-3), 5.39 (s, 1H, H-1), 7.08-7.82 (m, 7H, aromatic), 7.99 (d, 1H, *J* = 7.9 Hz, aromatic), 8.75 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 14.9, 25.4, 62.7, 66.3, 72.7, 72.8, 82.1, 125.5, 125.7, 127.3, 127.9, 128.2 (x2), 128.4 (x2), 129.2, 137.2, 137.8, 147.7, 149.9, 164.9 ppm; HR FAB MS [M+H$^+$] calcd for $C_{21}H_{26}NO_6S$ 420.1481, found 420.1475.

Benzoyl chloride (0.41 mL, 4.8 mmol) and DMAP (14 mg, 0.12 mmol) were added to a solution of **2.S4** (0.50 g, 1.2 mmol) in anhydrous pyridine (5.0 mL) and the resulting mixture was stirred under argon for 15 min at 0 °C. After that, the reaction mixture was allowed to warm to rt and stirred for 12 h. The reaction mixture was then cooled to 0 ˚C, quenched with MeOH (~0.5 mL), and concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (~50 mL) and washed with H_2O (10 mL), sat. aq. $NaHCO_3$ (2 x 10 mL), and H_2O (2 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone-toluene gradient elution) to afford the title compound white amorphous solid in 93%

yield (0.70 g, 1.11 mmol). Analytical data for **2.1h**: R_f = 0.76 (acetone-toluene, 2/3, v/v); $[\alpha]_D^{22}$ +97.4 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 1.29 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 2.67 (q, 2H, SCH_2CH_3), 4.29 (dd, 1H, $J_{2,3}$ = 1.7 Hz, H-2), 4.55-4.73 (m, 4H, H-6a, 6b, CH_2Ph), 4.81 (m, 1H, H-5), 5.58 (s, 1H, H-1), 5.82 (dd, 1H, $J_{3,4}$ = 10.0 Hz, H-3), 6.21 (t, 1H, $J_{3,4}$ = 10.0 Hz, H- 4), 7.13-7.48 (m, 12H, aromatic), 7.55-7.62 (m, 1H, aromatic), 797-8.09 (m, 5H, aromatic), 8.64 (d, 1H, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 51.6, 63.0, 67.3, 68.8, 72.2, 72.6, 81.6, 124.9, 127.3, 127.4, 127.8 (x2), 127.9 (x3), 128.1 (x2), 128.6 (x2), 128.7, 129.1, 129.3, 129.4, 129.5, 132.5, 132.6, 133.0, 136.6, 137.2, 137.3, 146.7, 149.7, 163.5, 165.1, 165.7 ppm; HR FAB MS [M+H$^+$] calcd for $C_{35}H_{34}NO_8S$ 628.2005, found 628.2008.

2.4.3 Synthesis of Glycosyl S-Tolyl donor series

***p*-Tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.16).** Picolinic acid (470 mg, 3,84 mmol), DCC (0.91 mg, 4.42 mmol), and DMAP (72 mg, 0.59 mmol) were added to a solution of *p*-tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside[18] (**2.S5**, 1.4 g, 2.95 mmol) in CH_2Cl_2 (45 mL) and the resulting mixture was stirred under argon for 40 min at rt. The reaction mixture was diluted with CH_2Cl_2 (~200 mL) and the solid was filtered off. The filtrate was washed with cold water (15 mL), sat. aq. $NaHCO_3$ (15 mL), and water (2 x 15 mL), dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound as white amorphous solid in 93% yield (1.55 g, 2.72 mmol). Analytical data for **2.16**: R_f = 0.46 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{23}$ +93.3 (c = 1.0, $CHCl_3$); ^{1}H

n.m.r. (300 MHz): δ, 2.39 (s, 3H, CH_3), 3.94 (m, 1H, $J_{5,6a}$ = 9.5 Hz, H-6a), 4.30 (m, 1H, $J_{5,6b}$ = 4.1 Hz, H-6b), 4.77 (dd, 1H, $J_{1,2}$ = 1.3 Hz, H-2), 4.50-4.61 (m, 3H, H-4, 5, $^1/_2$ C*H*$_2$Ph), 4.72 (d, 1H, ^2J = 12.1 Hz, $^1/_2$ C*H*$_2$Ph), 5.55 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1), 5.64-5.69 (m, 2H, >C*H*Ph, H-3), 7.14-7.27 (m, 5H, aromatic), 7.28-7.50 (m, 11H, aromatic), 7.82-7.87 (m, 1H, aromatic), 8.10-8.13 (m, 1H, aromatic), 8.82-8.84 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 21.2, 65.4, 68.5, 71.8, 72.9, 76.1, 86.77, 101.8, 125.5, 126.2 (x2), 126.9, 127.8, 128.1 (x2), 128.2 (x2), 128.3 (x2), 128.9, 129.6, 130.0 (x2), 132.5 (x2) 136.8, 137.1 (x2), 137.2, 138.1, 147.7, 150.0, 164.3 ppm; HR FAB MS [M+H$^+$] calcd for $C_{33}H_{32}NO_6S$ 570.1950, found 570.1957.

***p*-Tolyl 4,6-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.4).** Water (100 μL) and TFA/CH_2Cl_2 (1/9, v/v, 0.8 mL) were added to a mixture of **2.16** (0.5 g, 0.87 mmol) in CH_2Cl_2 (11 mL) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was neutralized with Et_3N (~1 mL), diluted with CH_2Cl_2 (50 mL) and washed with cold water (5 mL), sat. aq. $NaHCO_3$ (5 mL), and cold water (2 x 5 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol-CH_2Cl_2 gradient elution) to afford *p*-tolyl 2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (**2.S6**) as a white amorphous solid in 93% yield (0.39 g, 0.81 mmol). Analytical data for **2.S6**: R_f = 0.37 (methanol/CH_2Cl_2, 1/9, v/v); $[\alpha]_D^{23}$ +47.5 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 2.25 (s, 3H, CH_3), 3.00 (m, 1H, OH), 3.33 (m, 1H, OH), 3.75-3.95 (m, 2H, H-6a, 6b), 4.18-4.55 (m, 5H, H-2, 4, 5, C*H*$_2$Ph), 5.17 (dd, 1H, $J_{3,4}$ = 9.9 Hz, H-3), 5.43 (s, 1H, H-1), 6.09-7.39 (m, 10H, aromatic), 7.70 (m, 1H, *J* = 7.7 Hz,

aromatic), 7.92 (d, 1H, *J* = 7.7 Hz, aromatic), 8.62 (d, 1H, *J* = 3.8 Hz aromatic), ppm; ^{13}C n.m.r. (75 MHz): δ, 21.2, 62.3, 65.4, 72.3, 73.5, 76.3, 86.0, 125.6, 127.3, 127.7, 128.0 (x3) 128.2 (x3), 130.1, 132.5 (x3), 137.3, 137.4, 137.9, 147.4, 149.4, 164.4 ppm; HR FAB MS [M+H$^+$] calcd for $C_{26}H_{28}NO_6S$ 482.1623, found 482.1636.

Benzoyl chloride (0.20 mL, 2.43 mmol) and DMAP (10 mg, 0.081 mmol) were added to a solution of **2.S6** (0.39 g, 0.81 mmol) in anhydrous pyridine (5.0 mL) and the resulting mixture was stirred under argon for 15 min at 0 °C. After that, the reaction mixture was allowed to warm to rt and stirred for 12 h. The reaction mixture was then cooled to 0 ˚C, quenched with dry MeOH (0.5 mL), and concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (~50 mL) and washed successively with H_2O (10 mL), sat. aq. $NaHCO_3$ (2 x 10 mL), H_2O (2 x 10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone-toluene gradient elution) to afford the title compound as a white amorphous solid in 87% yield (0.53 g, 0.77 mmol). Analytical data for **2.4**: R_f = 0.77 (acetone-toluene, 2/3, v/v); $[\alpha]_D^{23}$ +97.3 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 2.32 (s, 3H, SPhC*H*$_3$), 4.40-4.41 (m, 1H, H-4), 4.51-4.64 (m, 3H, H-6a, 6b, $^1/_2$ C*H*$_2$Ph), 4.75 (d, 1H, ^2J= 12.1 Hz, $^1/_2$ C*H*$_2$Ph), 4.91 (m, 1H, H-5), 5.68 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1), 5.76 (dd, 1H, $J_{3,4}$ = 10 Hz, H-3), 6.09 (t, 1H, $J_{4,3}$ = 10 Hz, H-4), 7.04 (d, 2H, *J* = 7.9 Hz, aromatic), 7.15-7.59 (m, 12H, aromatic), 7.75-7.78 (m, 2H, aromatic), 7.99-8.08 (m, 7H, aromatic), 8.76 (d, 1H, *J* = 3.9 Hz, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 21.2, 63.6, 67.5, 69.7, 72.6, 72.8, 85.7, 125.5, 127.1, 127.8 (x2), 127.9 (x2), 128.3 (x2), 128.4 (x2), 129.0, 129.4, 129.6, 129.8 (x2), 129.9 (x2), 130.0 (x2), 120.1, 132.3 (x2), 132.9, 133.9, 133.4, 137.1, 137.3, 138.1, 147.0,

150.2, 163.7, 165.5, 166.3 ppm; HR FAB MS [M+H$^+$] calcd for $C_{40}H_{36}NO_8S$ 690.2162, found 690.2169.

2.4.4 Synthesis of Glycosyl S-Phenyl donor series

Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.17). Picolinic acid (168 mg, 1.36 mmol), DCC (0.31 g, 1.5 mmol), and DMAP (25 mg, 0.20 mmol) were added to a solution of phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside[19] (**2.S7**, 0.47 g, 1.0 mmol) in CH_2Cl_2 (15 mL) and the resulting mixture was stirred under argon for 40 min at rt. The reaction mixture was diluted with CH_2Cl_2 (~100 mL) and the solid was filtered off. The filtrate was washed with cold water (10 mL), sat. aq. $NaHCO_3$ (10 mL), and water (2 x 10 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound a white amorphous solid in 89% yield (0.50 g, 0.88 mmol). Analytical data for **2.17**: R_f = 0.54 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{22}$ +66.3 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.95 (m, 1H, $J_{5,6a}$ = 9.9 Hz, H-6a), 4.30 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, H-6b), 4.38 (dd, 1H, $J_{2,3}$ = 3.4 Hz, H-2), 4.49-4.62 (m, 3H, H-4, 5, $^1/_2$ C*H*$_2$Ph), 4.73 (d, 1H, ^2J = 12.0 Hz, $^1/_2$ C*H*$_2$Ph), 5.62 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1), 5.66 (m, 2H, H-3, >C*H*Ph), 7.14-7.53 (m, 16H, aromatic), 7.84-7.85 (m, 1H, aromatic), 8.10-8.13 (m, 1H, aromatic), 8.82-8.84 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 65.5, 68.5, 71.8, 73.1, 76.2, 77.3, 86.5, 101.8, 125.5, 126.3 (x2), 126.9, 127.8, 127.9, 128.1 (x2), 128.2 (x2), 128.3 (x2), 129.9, 129.2 (x2), 131.8 (x2), 133.5, 136.8, 127.1, 137.2, 147.7, 150.0, 164.3 ppm; HR FAB MS [M+H$^+$] calcd for $C_{32}H_{30}NO_6S$ 556.1794, found 556.1782.

Phenyl 4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (2.5). Water (100 μL) and TFA/CH_2Cl_2 (1.0 mL, 1/9, v/v) were added to a mixture of **2.17** (0.48 g, 0.84 mmol) in CH_2Cl_2 (10 mL) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was neutralized with Et_3N (~1 mL), diluted with CH_2Cl_2 (~50 mL) and washed with cold water (5 mL), sat. aq. $NaHCO_3$ (5 mL), and cold water (2 x 5 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol-CH_2Cl_2 gradient elution) to afford phenyl 2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (**2.S8**) as a white amorphous solid in 94% yield (0.31 g, 0.82 mmol). Analytical data for **2.S8**: R_f = 0.37 (methanol/CH_2Cl_2, 1/9, v/v); $[\alpha]_D^{22}$ +22.3 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 2.99 (s, 1H, OH), 3.95-3.99 (m, 2H, H-6a, 6b), 4.28-4.34 (m, 2H, H-2, 5), 4.47-4.69 (m, 4H, H-4, OH, C*H*$_2$Ph), 5.30 (dd, 1H, $J_{3,4}$ = 9.7 Hz, H-3), 5.61 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1), 7.06-7.53 (m, 11H, aromatic), 7.84-7.87 (m, 1H, aromatic), 8.03-8.05 (m, 1H, aromatic), 8.75 (d, 1H, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 62.3, 65.7, 72.4, 73.5, 76.4, 76.5, 85.7, 125.5, 127.2, 127.7, 127.8, 128.0 (x2), 128.2 (x2), 129.1 (x2), 131.9 (x2), 133.8, 137.2, 137.4, 147.4, 149.5, 164.6 ppm; HR FAB MS [M+H$^+$] calcd for $C_{25}H_{26}NO_6S$ 468.1481, found 468.1476.

Benzoyl chloride (0.43 mL, 4.95 mmol) and DMAP (26 mg, 0.22 mmol) were added to a solution of **2.S8** (0.41 g, 1.10 mmol) in anhydrous pyridine (5.0 mL) and the resulting mixture was stirred for 15 min at 0 ºC. The reaction mixture was allowed to warm to rt and stirred for 24 h. After that, the reaction mixture was cooled to 0 ˚C, quenched with dry MeOH (0.5 mL), and concentrated *in vacuo*. The residue was diluted with CH_2Cl_2 (~50 mL) and washed with H_2O (5 mL), saturated aq. $NaHCO_3$ (2 x 5 mL), and H_2O (2 x

5 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone-toluene gradient elution) to afford the title compound as white amorphous solid in 92% yield (0.68 g, 1.00 mmol). Analytical data for **2.5**: R_f = 0.6 (acetone/toluene, 2/3, v/v); $[\alpha]_D^{23}$ +47.6 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 4.42 (m, 1H, H-2), 4.55-4.65 (m, 3H, H-6a, 6b, $^1/_2$ C*H*$_2$Ph), 4.77 (d, 1H, ^2J= 12.0 Hz, $^1/_2$ C*H*$_2$Ph), 4.90 (m, 1H, H-5), 5.72-5.76 (m, 2H, H-1, 3), 6.09 (dd, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 7.18-7.56 (m, 16H, aromatic), 7.75-7.79 (m, 1H, aromatic), 8.08-8.11 (m, 1H, aromatic), 8.08-8.11 (m, 1H, aromatic) 8.75 (d, 1H, aromatic) ppm; ^{13}C n.m.r. (75 MHz): δ, 52.0, 63.5, 67.5, 69.8, 72.7, 72.8, 85.4, 125.5, 127.1, 127.7, 127.8 (x2), 127.9 (x2), 128.3 (x2), 128.4 (x2), 128.5, 129.0, 129.2 (x2), 129.6, 129.7, 129.8, 130.1, 131.6 (x2), 132.9, 133.0, 133.3, 133.4, 137.1, 137.2, 147.0, 150.2, 163.7, 165.5, 166.2 ppm; HR-FAB MS [M+H$^+$] calcd for $C_{39}H_{34}NO_8S$ 676.2005, found 676.2009.

2.4.5 Synthesis of Disaccharides

Metod A. A general procedure for glycosylation in the presence of DMTST. A mixture of a glycosyl donor (0.50 mmol), glycosyl acceptor (0.45 mmol), and freshly activated molecular sieves (4 Å, 200 mg) in $(ClCH_2)_2$ (1.0 mL or 10 mL) was stirred under argon for 1 h at rt. DMTST[7] (0.10 mmol) was added and the resulting mixture was stirred at rt for the time specified in Tables 1-3 of the manuscript. *Alternative procedure involved stirring at -30 ºC as indicated in Tables.* Upon completion, the solid was filtered off, and the residue was washed sucessively with CH_2Cl_2. The combined filtrate (~30-40 mL) was washed with 20% aq. $NaHCO_3$ (10 mL) and water (3 x 10 mL). The organic

phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution). Anomeric ratios (or anomeric purity) were determined by comparison of the integral intensities of relevant signals in ^{1}H n.m.r. spectra.

Metod B. A general procedure for glycosylation in the presence of NIS/TfOH. A mixture of a glycosyl donor (0.50 mmol), glycosyl acceptor (0.45 mmol), and freshly activated molecular sieves (4 Å, 200 mg) in $(ClCH_2)_2$ (1.0 mL or 10 mL) was stirred under argon for 1 h at rt. NIS (0.10 mmol) and TfOH (0.02 mmol) were added and the resulting mixture stirred at rt for the time specified in Tables 1-3 of the manuscript. *Alternative procedure involved stirring at -30 ºC as indicated in Tables.* Upon completion, the solid was filtered off, and the residue was washed sucessively with CH_2Cl_2. The combined filtrate (~30-40 mL) was washed with Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution). Anomeric ratios (or anomeric purity) were determined by comparison of the integral intensities of relevant signals in ^{1}H n.m.r. spectra.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-α-D-glucopyranoside (2.3c). The title compound was obtained by Method A or B from donor **2.1c** and acceptor **2.2** as a clear syrup in 65% (α/β = 1/1.4, 5.0 mM) or 67% yield (α/β =

1/1, 5.0 mM), respectively. Analytical data for **2.3c** was in accordance with that reported previously.[14]

Methyl 6-*O*-(6-*O*-benzoyl-2,3,4-tri-*O*-benzyl-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.3d). The title compound was obtained by Method A or B from donor **2.1d** and acceptor **2.2** as a clear syrup in 68% (α/β = 1/1, 5.0 mM) or 70% yield (α/β = 1/1, 5.0 mM), respectively. Analytical data for α-**2.3d**: R_f = 0.66 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{23}$ +20.8 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.33 (s, 3H, OCH_3), 3.35-3.57 (m, 3H, H-2, 4, 6a), 3.65-3.88 (m, 2H, H-5, 5'), 3.99-4.07 (m, 2H, H-2', 3), 4.15 (m, 1H, H-6b), 4.25 (dd, 1H, $J_{4',5'}$ = 9.5, H-4'), 4.39 (s, 1H, H-1'), 4.53-4.77 (m, 14H, H-1, 6a', 6b', $5^1/_2$ CH_2Ph), 5.03 (d, 1H, ^2J = 10.9 Hz, $^1/_2$ CH_2Ph), 5.16 (dd, 1H, $J_{3',4'}$ = 9.5 Hz, H-3'), 7.06-8.05 (m, 33H, aromatic), 8.72-8.84 (m, 2H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.5, 63.5, 67.4, 68.5, 69.7, 72.1, 73.7, 73.8, 74.8, 75.0, 75.2, 76.1, 77.7, 79.1, 80.1, 98.6, 100.5 ($^1J_{C1,H1}$ = 175.2 Hz, $^1J_{C1',H1'}$ = 168.7 Hz) 128.1, 129.7, 129.8, 130.0, 130.2 (x2), 130.4 (x2), 130.5, 130.6 (x2), 130.7 (x2), 130.8 (x2), 130.84 (x2), 130.87 (x2), 131.0 (x4), 131.84 (x2), 131.8, 132.3 (x2), 132.3 (x2), 132.4 (x2), 135.4, 135.9, 139.8, 140.3, 140.8, 140.9, 141.9, 149.5, 152.8, 166.2, 167.9, 168.7 ppm; HR FAB MS [M+Na^+] calcd for $C_{62}H_{64}NaO_{12}$ 1023.4295, found 1023.4291.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (2.3e). The title compound was obtained by Method A or B from donor **2.1e** and acceptor **2.2** as a clear syrup in 92% (α/β = 1/7, 5.0 mM) or 89% yield (α/β = 1/8, 5.0 mM), respectively. Analytical data for β-**2.3e**: R_f = 0.39 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{20}$ -24.1 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ,

3.32 (s, 3H, OCH_3), 3.35-3.50 (m, 4H, H-2, 4, 5, 5'), 3.76-3.74 (m, 3H, H-3, 6a', 6b'), 3.94 (d, 1H, $J_{2,3}$ = 4.0 Hz, H-2), 3.97-4.15 (m, 3H, H-4', 6a, 6b), 4.25 (s, 1H, H-1'), 4.51-4.61 (m, 6H, 3 x CH_2Ph), 4.64-4.74 (m, 2H, H-1, $^1/_2$ CH_2Ph), 4.78-4.87 (m, 3H, $1^1/_2$ x CH_2Ph), 4.99 (d, 1H, ^2J = 10.9 Hz, $^1/_2$ CH_2Ph), 5.05 (dd, 1H, $J_{3',4'}$ = 9.8 Hz, H-3'), 6.97-7.37 (m, 30H, aromatic), 7.44-7.49 (m, 1H, aromatic), 7.77 (m, 1H, *J* = 1.6 Hz, aromatic), 7.93 (d, 1H, *J* = 7.8 Hz, aromatic), 8.78 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.2, 68.6, 69.6, 70.1, 73.4, 73.5, 73.7, 74.5, 74.8, 75.0, 75.1, 75.9, 75.9, 77.4, 80.0, 82.4, 82.4, 97.9, 101.3 ($^1J_{C1,H1}$ = 161.7 Hz, $^1J_{C1',H1'}$ = 155.6=5 Hz), 125.4, 127.1, 127.6, 127.7, 127.8 128.0 (x2) 128.1 (x2), 128.1 (x2), 128.2 (x2), 128.2 (x2), 128.3 (x3), 128.4 (x2), 128.5 (x4), 128.5 (x2), 128.6 (x2), 128.7 (x2), 137.0, 138.1, 138.2, 138.3, 138.4, 138.5, 138.9, 147.8, 150.2 (x2), 164.2 ppm; HR FAB MS [M+Na^+] calcd for $C_{61}H_{63}NNaO_{12}$ 1024.4242, found 1024.4342.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (2.3f). The title compound was obtained by Method A or B from donor **2.1f** and acceptor **2.2** as a clear syrup in 72% (α/β = 1/10, 5.0 mM) or 86% yield (α/β = 1/8.7, 5.0 mM), respectively. Analytical data for **2.3f** R_f = 0.3 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{20}$ -1.76 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.28 (s, 3H, OCH_3), 3.37-3.49 (m, 3H, H-2, 4, 6a), 3.65-3.82 (m, 2H, H-5, 5'), 3.94-4.00 (m, 2H, H-2', 3), 4.09-4.12 (m, 1H, H-6b), 4.20 (dd, 1H, $J_{4',5'}$ = 9.5 Hz, H-4'), 4.34 (s, 1H, H-1'), 4.48-4.86 (m, 12H, H-1, 6a', 6b', $4^1/_2$ CH_2Ph), 4.98 (d, 1H, ^2J = 10.9 Hz, $^1/_2$ CH_2Ph), 5.11 (dd, 1H, $J_{2',3'}$ = 3.1 Hz, H-3'), 7.12-7.50 (m, 27H, aromatic), 7.46-7.50 (m, 1H, aromatic), 7.77-7.82 (m, 1H, aromatic), 7.97-8.00 (m, 1H, aromatic), 8.71-8.80 (m, 2H,

aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ 55.1, 64.9, 68.5, 69.9, 73.3, 7.38, 73.4, 74.3, 74.7, 74.9, 75.0, 75.8, 77.2, 77.6, 79.9, 82.2, 97.8, 101.2 ($^{1}J_{C1,H1}$ = 171.6 Hz, $^{1}J_{C1',H1'}$ = 155.6 Hz), 125.3, 125.4, 126.8, 127.1, 127.5, 127.7, 127.8, 128.0 (x2), 128.1 (x7), 128.2 (x4), 128.4 (x4), 128.5 (x4), 128.6, 136.9, 137.0, 137.6, 138.1 (x2), 138.2, 138.8, 147.6, 147.9, 150.0, 150.1, 164.7, 164.7 ppm; HR FAB MS [M+Na^{+}] calcd for $C_{60}H_{60}N_2NaO_{13}$ 1039.3993, found 1039.3943.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-*O*-benzyl-4,6-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)- α-D-glucopyranoside (2.3g). The title compound was obtained by Method A or B from donor **2.1g** and acceptor **2.2** as a clear syrup in 73% (α/β = 1/5.7, 5 mM) or 73% yield (α/β = 1/6.5, 5 mM) respectively. Analytical data for **2.3g**: R_f = 0.45 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{24}$ -7.3 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.32 (s, 3H, OCH_3), 3.39-3.51 (m, 4H, H-2, 4, 5', 6a), 3.75 (m, 1H, H-5), 3.88-4.11 (m, 4H, H-2', 3, 6a', 6b), 4.25-4.29 (m, 2H, H-1', 6b'), 4.33 (dd, 1H, $J_{4',5'}$ = 9.5 Hz, H-4'), 4.52-4.65 (m, 4H, H-1, $1^1/_2$ CH_2Ph), 4.75-4.86 (m, 4H, 2 x CH_2Ph), 5.00 (d, 1H, ^{2}J = 10.9 Hz, $^1/_2$ CH_2Ph), 5.15 (dd, 1H, $J_{2',3'}$ = 3.2 Hz, H-3'), 5.54 (s, 1H, >C*H*Ph), 7.04-7.07 (m, 3H, aromatic), 7.19-7.47 (m, 18H, aromatic), 7.77 (m, 1H, aromatic), 7.80-8.02 (m, 1H, aromatic), 8.78-8.79 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.3, 64.4, 67.5, 68.5, 69.1, 69.8, 72.6, 73.0, 73.5, 73.7, 74.9, 75.0, 75.6, 75.6, 75.9, 77.9, 80.0, 82.1, 97.8, 101.6 ($^{1}J_{C1,H1}$ = 168.1 Hz, $^{1}J_{C1',H1'}$ = 157.3 Hz), 102.1, 125.6, 126.2, 127.3, 127.9 (x2), 128.1 (x2), 128.2, 128.3 (x2), 128.4 (x2), 128.6 (x3), 128.8 (x2), 129.8, 130.1, 133.0, 133.1, 136.9, 137.1, 137.8, 138.1, 138.2, 138.3, 138.8, 147.6, 150.1, 164.0, 165.7, 166.2 ppm; HR FAB MS [M+H^{+}] calcd for $C_{54}H_{56}NO_{12}$ 910.3803, found 910.3790.

Methyl 6-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.3h). The title compound was obtained by Method A or B from donor **2.1h**, **2.4** or **2.5** and acceptor **2.2** as a clear syrup in 86% (α/β = 1/12.1, 5 mM), 89% (α/β = 1/9.5, 5 mM), 91% (α/β = 1/18.5, 5 mM), 72% (α/β = 1/7.2, 5 mM), 86% (α/β = 1/7.2, 5 mM), or 96% (α/β = 1/7.2, 5 mM). Additional experiments have been done using the donor **2.5** and acceptor **2.2** as shown in Table 3.2. Analytical data for **2.3h**: R_f = 0.5 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{20}$ -18.8 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.29 (s, 3H, OCH_3), 3.36-3.53 (m, 3H, H-2, 4, 6a), 3.73-3.80 (m, 1H, H-5), 3.88-3.92 (m, 1H, H-5'), 3.96-4.01 (m, 2H, H-2', 3), 4.10-4.14 (m, 1H, H-6b), 4.38 (s, 1H, H-1'), 4.49-4.64 (m, 6H, H-1, 6a', 6b', $1^1/_2$ CH_2Ph), 4.73-4.87 (m, 4H, 2 x CH_2Ph), 4.98 (d, 1H, ^2J = 10.8 Hz, $^1/_2$ CH_2Ph), 5.24 (dd, 1H, $J_{3',4'}$ = 10.1 Hz, H-3'), 5.88 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 7.00-7.05 (m, 3H, aromatic), 7.19-7.45 (m, 24H, aromatic), 7.69-7.72 (m, 1H, aromatic), 7.83-7.93 (m, 5H, aromatic), 8.69 (d, 1H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ 55.5, 63.5, 67.4, 68.5, 69.7, 72.1, 73.7 (x2), 73.8, 74.8, 75.1, 75.2, 76.1, 77.7, 79.1, 80.2, 98.6, 100.5 ($^1J_{C1,H1}$ = 168.6 Hz, $^1J_{C1',H1'}$ = 156.5 Hz), 125.6, 127.2, 127.3, 127.5, 127.6 (x2), 127.9 (x4), 128.2 (x6), 128.3 (x2), 128.4 (x2), 128.5 (x4), 128. 9 (x2), 129.3, 129.8 (x4), 129.9, 132.9, 122.4, 137.3, 137.8, 138.3, 138.5, 139.5, 147.0, 150.3, 163.7, 165.4, 166.2 ppm; HR FAB MS [M+Na^+] calcd for $C_{61}H_{59}NNaO_{14}$ 1052.3833, found 1052.3943.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (2.7). The title compound was obtained by

Method B from donor **2.1e** and acceptor **2.6**[13b] as a clear syrup in 80% yield (α/β = 1/2.9, 5 mM). Analytical data for **2.7**: R_f = 0.38 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{24}$ -47.9 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.32-3.33 (m, 1H, H-5'), 3.38 (s, 3H, OCH_3), 3.48 (dd, 1H, $J_{2,3}$ = 3.7 Hz, H-2), 3.54-3.72 (m, 5H, H-5, 6a, 6b, 6a', 6b'), 3.88-4.01 (m, 3H, H-2', 3, 4), 4.16 (dd, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 4.42-4.79 (m, 13H, H-1, 1', 5 $^1/_2$ CH_2Ph), 4.99 (dd, 1H, H-3'), 5.20 (d, 1H, ^2J = 11.3 Hz, $^1/_2$ CH_2Ph), 7.03-7.38 (m, 31H, aromatic), 7.74-7.80 (m, 1H, aromatic), 7.90-7.93 (m, 1H, aromatic), 8.79-8.81 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.4, 68.7, 69.3, 69.9, 73.3, 73.7, 73.8 (x2), 74.8, 74.9, 75.3, 76.0, 76.2, 79.2, 80.5, 98.5, 100.7, ($^1J_{C1,H1}$ = 166.1 Hz, $^1J_{C1',H1'}$ = 154.1 Hz), 125.3, 127.0, 127.2, 127.3, 127.4, 127.5 (x2), 127.6, 127.7, 127.8 (x2), 127.9 (x4), 128.2 (x8), 128.3 (x2), 128.4 (x4), 128.3 (x2), 128.5 (x2), 128.6, 137.0, 137.7, 138.3, 138.5, 138.6, 138.9, 139.8, 147.8, 150.2, 164.2 ppm; HR FAB MS [M+Na^+] calcd for $C_{61}H_{63}NNaO_{12}$ 1024.4248, found 1024.4342.

Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (2.9). The title compound was obtained by Method B from donor **2.1e** and acceptor **2.8** as a clear syrup in 80% yield (α/β = 1/2.9, 5 mM). Analytical data for **2.9**: R_f = 0.39 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{24}$ +2.5 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ 3.36 (s, 3H, OCH_3), 3.42-3.46 (m, 1H, H-5), 3.62-3.84 (m, 7H, H-2, 3, 4, 6a, 6b, 6a', 6b'), 3.97 (m, 1H, $J_{4,5}$ = 9.7 Hz, H-5), 4.05 (d, $J_{2',3'}$ = 3.1 Hz, H-2'), 4.21 (t, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 4.47-4.4.91 (m, 13H, H-1', 6 x CH_2Ph), 4.96 (s, 1H, Hz, H-1), 5.04 (dd, 1H, $J_{3',4'}$ = 9.8 Hz, H-3'), 7.04-7.35 (m, 30H, aromatic), 7.45-7.49 (m, 1H, aromatic),7.78 (m, 1H, aromatic), 7.96 (d, 1H, *J* = 7.8 Hz, aromatic),

8.79 (d, 1H, *J* = 3.9 Hz, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.3, 55.4, 68.6, 68.7, 69.2, 70.3, 70.5, 73.1, 73.2, 73.8, 74.4, 75.1 (x2), 75.2, 75.5, 75.8, 76.1, 76.9, 77.6, 78.4, 79.0, 82.1, 83.4, 99.5, 100.0 ($^1J_{C1,H1}$ = 165.7 Hz, $^1J_{C1',H1'}$ = 152.5 Hz), 102.2, 125.3, 127.4, 127.6, 127.7, 127.8 (x3), 127.9, 128.0 (x2), 128.1 (x3), 128.2, 128.3, 128.4, 128.5 (x4), 128.6, 128.9, 136.9 (x2), 138.1, 138.2, 138.3, 138.4, 138.5, 138.6, 138.8, 147.9, 150.2 (x2), 164.1 ppm; HR FAB MS [M+Na$^+$] calcd for $C_{61}H_{63}NNaO_{12}$ 1024.4248 found 1024.4342.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (2.10). The title compound was obtained by Method A or Method B from donor **2.1f** and acceptor **2.6** as a clear syrup in 73% yield (α/β 1/2.9, 5 mM) or 87% yield (α/β = 1/2.9, 5 mM), respectively. Analytical data for **2.10**: R_f = 0.3 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{25}$ -10.7 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.35 (s, 3H, OCH_3), 3.39-3.44 (m, 1H, H-5'), 3.47 (dd, 1H, $J_{2,3}$ = 3.8 Hz, H-2), 3.60-3.68 (m, 3H, H-5, 6a, 6b), 3.86 (dd, 1H, $J_{3,4}$ = 8.9 Hz, H-3), 3.92 (m, 1H, H-4), 3.99 (d, 1H, $J_{2',3'}$ = 2.9 Hz, H-2'), 4.20 (dd, 1H, $J_{4',5'}$ = 9.6 Hz, H-4'), 4.41-4.23 (m, 3H, H-1', CH_2Ph), 4.45-4.78 (m, 10H, H-1, 6a', 6b', 3$^1/_2$ CH_2Ph), 4.99 (dd, 1H, $J_{3',4'}$ = 9.7 Hz, H-3'), 5.13 (d, 1H, ^2J = 11.2 Hz, $^1/_2$ CH_2Ph), 7.04-7-49 (m, 28H, aromatic), 7.75-7.84 (m, 2H, aromatic), 7.96 (d, 1H, *J* = 7.8 Hz, aromatic), 8.69-8.71 (m, 1H, aromatic), 8.77-8.78 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.5, 64.3, 68.6, 69.8, 72.7, 73.3, 73.7, 73.8, 74.8, 74.9, 75.2, 75.3, 77.7, 78.0, 79.0, 80.4, 98.6, 100.8 ($^1J_{C1,H1}$ = 166.6 Hz, $^1J_{C1',H1'}$ = 157.5 Hz), 125.4, 125.6, 136.7, 127.2 (x2), 127.4 (x2), 127.6, 127.8, 127.9, 128.0 (x2), 128.1 (x4), 128.2 (x4), 128.3 (x4), 128.4 (x2), 128.5 (x2), 128.8 (x2), 136.9, 137.1, 137.8,

138.4, 138.7, 139.5, 147.7, 147.8, 150.1, 150.3, 164.3, 164.4 ppm HR FAB MS [M+Na^+] calcd for $C_{60}H_{60}N_2NaO_{13}$ 1039.3993, found 1039.4087.

Methyl 4-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (2.11). The title compound was obtained by Method A or B from donor **2.1h** and acceptor **2.6** as a clear syrup in 86% yield (α/β = 1/5.2, 50 mM) or 72% (α/β = 1/3.5, 5 mM), respectively. Analytical data for **2.11**: R_f = 0.29 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{25}$ -29.4 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.36 (s, 3H, OCH_3), 3.45-3.52 (m, 2H, H-2, 5'), 3.65-3.70 (m, 3H, H-5, 6a, 6b), 3.88 (dd, 1H, $J_{3,4}$ = 6.3 Hz, H-3), 3.95-3.98 (m, 2H, H-2', 4), 4.14 (dd, 1H, $J_{6a',6b'}$ = 12.0 Hz, H-6a'), 4.31 (dd, 1H, H-6b'), 4.39 (m, 1H, $^1/_2$ CH_2Ph), 4.53-4.78 (m, 8H, H-1, 1', 3 x CH_2Ph), 5.00 (d, 1H, $J_{2',3'}$ = 3.1 Hz, H-3'), 5.10 (d, 1H, $^1/_2$ CH_2Ph), 5.84 (dd, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 7.07-7.47 (m, 27H, aromatic), 7.67-7.72 (m, 1H, aromatic), 7.73-7.79 (m, 6H, aromatic), 8.69 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.3, 64.2, 67.9, 68.7, 70.0, 72.4, 73.5, 74.4, 74.7, 74.8, 74.9, 75.9, 80.0, 82.3, 97.9, 101.4 ($^1J_{C1,H1}$ = 168.1 Hz, $^1J_{C1',H1'}$ = 157.3 Hz), 125.7, 127.2, 127.7, 127.8, 128.1 (x2), 128.2 (x2), 128.3 (x5), 128.4 (x7), 128.5 (x2), 128.6 (x2), 128.6 (x2), 128.7 (x2), 129.2, 129.8 (x2), 129.8 (x2), 133.1, 133.5, 137.2, 138.1, 138.2, 138.3, 138.9, 147.0, 150.3, 163.8, 165.6, 166.3 ppm. HR-FAB MS [M+Na^+] calcd for $C_{61}H_{59}NNaO_{14}$ 1052.3833, found 1052.3943.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (2.12). The title compound was obtained by Method A or Method B from donor **2.1g**, **2.16**, or **2.17** and acceptor **2.6** as a clear syrup

in 71% (α/β = 1/10, 5 mM), 80% (α/β = 1/5.4, 5 mM), 86% (α/β = 1/5.5, 5 mM), 83% (α/β = 1/9.8, 5 mM), 96% (α/β = 1/8, 5 mM), or 89% (α/β = 1/7.4, 50 mM). Analytical data for **2.12** R_f = 0.41 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{25}$ -25.1 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.10-3.11 (m, 1H, H-5'), 3.38 (s, 1H, OCH_3), 3.47-3.53 (m, 2H, H-2, 6a'), 3.59-3.64 (m, 3H, H-5, 6a, 6b), 3.83 (dd, 1H, $J_{3,4}$ = 9.0 Hz, H-3), 3.92-3.98 (m, 2H, H-2', 4), 4.02-4.07 (m, 1H, H-6b'), 4.19 (dd, 1H, $J_{4',5'}$ = 9.8 Hz, H-4'), 4.40 (d, 1H, ^2J = 12.0 Hz, $^1/_2$ CH_2Ph), 4.52-4.81 (m, 8H, H-1, 1', 3 x CH_2Ph), 4.97 (dd, 1H, $J_{3',4'}$ = 10.4 Hz, H-3'), 5.04 (d, 1H, ^2J = 10.9 Hz, $^1/_2$ CH_2Ph), 5.46 (s, 1H, >C*H*Ph), 7.07-7.09 (m, 23H, aromatic), 7.77 (m, 1H, aromatic), 8.01 (d, 1H, *J* =7.9 Hz, aromatic), 8.82 (d, 1H, *J* =3.8 Hz, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.4, 68.0, 69.2, 70.1, 73.5, 73.9, 74.9, 75.0, 75.3, 75.5, 75.7, 76.7, 78.2, 79.0, 80.5, 82.9, 85.0, 98.5, 102.6 ($^1J_{C1,H1}$ = 166.0 Hz, $^1J_{C1',H1'}$ = 155.0 Hz), 126.9, 127.7 (x2), 127.9 (x6), 128.1 (x2), 128.2 (x2), 129.4 (x3), 128.5 (x6), 128.6 (x2), 129.2, 129.9, 134.6, 138.0, 138.1, 138.4, 138.6, 138.7 (x2), 138.8, 139.7, ppm. HR- FAB MS [M+H^+] calcd for $C_{54}H_{56}NO_{12}$ 910.3803, found 910.3790.

Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (2.13) The title compound was obtained by Method A from donor **2.1g** and acceptor **2.8** as a clear syrup in 78% yield (α/β = 1/7.3, 5 mM). Analytical data for **2.13**: R_f = 0.53 (acetone/toluene, 1/4, v/v); $[\alpha]_D^{25}$ -8.8 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.41 (s, 3H, OCH_3), 3.36-3.44 (m, 1H, H-5'), 3.64-3.79 (m, 5H, H-2, 3, 4, 5, 6a), 3.86-4.06 (m, 2H, H-6a',6b), 4.06 (d, 1H, $J_{2',3'}$ = 3.2 Hz, H-2'), 4.24-4.37 (m, 2H, H-4', 6b), 4.68-4.56 (m, 3H, H-1', CH_2Ph,), 4.63 (d, 1H, ^2J = 12.1 Hz ,

$^{1}/_{2}$ C*H*$_2$Ph), 4.69 (d, 1H, ^{2}J = 12.1 Hz , $^{1}/_{2}$ C*H*$_2$Ph) 4.79-4.93 (m, 5H, H-1, 2 x C*H*$_2$Ph), 5.12 (dd, 1H, $J_{3',4'}$ = 10.3 Hz, H-3'), 5.54 (s, 1H, >C*H*Ph), 7.08-7.48 (m, 25H, aromatic), 7.76-7.82 (m, 1H, aromatic), 7.76-7.82 (m, 1H, aromatic), 8.04-8.07 (d, 1H, *J* = 7.8 Hz, aromatic), 8.79 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.4, 67.6, 68.6, 67.6, 68.6, 70.4, 73.7, 73.8, 75.1, 75.2, 75.6, 78.5, 78.7, 82.2, 99.9, 101.8 ($^{1}J_{C1,H1}$ = 167.0 Hz, $^{1}J_{C1',H1'}$ = 160.0 Hz), 102.7, 125.6, 126.3 (x2), 126.6, 127.1, 127.7, 127.93 (x3), 128.1 (x2), 128.2 (x3), 128.3 (x2), 128.4 (x2), 128.5, 128.6 (x4), 128,7 (x2), 120.9 (x2), 129.1, 129.2, 137.0, 137.3, 138.1, 138.1, 138.4, 147.9, 150.2, 164.2 ppm. HR FAB MS [M+H$^+$] calcd for $C_{54}H_{56}NO_{12}$ 910.3803, found 910.3790.

Methyl 2,4,6-tri-*O*-benzyl-3-*O*-(2-*O*-benzyl-4,6-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside (2.15). The title compound was obtained by Method A or B from donor **2.1g** and acceptor **2.14** as a clear syrup in 71% (α/β = 1/6.0, 5 mM) or 88% yield (α/β = 1/10, 5 mM), respectively. Analytical data for **2.15:** R_f = 0.52 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{25}$ +23.7 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.35 (s, 3H, OCH_3), 3.39-3.80 (m, 6H, H-2, 4, 5, 5', 6a, 6a'), 4.19-4.39 (m, 5H, H-2', 3, 4', 6b, 6b'), 4.54 (d, 1H, ^{2}J = 12.1 Hz, $^{1}/_{2}$ C*H*$_2$Ph), 4.60-4.74 (m, 5H, H-1, 2 x C*H*$_2$Ph), 4.84 (d, 1H, ^{2}J = 12.0 Hz, $^{1}/_{2}$ C*H*$_2$Ph), 5.15 (d, 1H, ^{2}J = 9.3 Hz, $^{1}/_{2}$ C*H*$_2$Ph), 5.27 (dd, 1H, $J_{3',4'}$ = 10.4 Hz, H-3'), 5.59 (s, 1H, >C*H*Ph), 7.07-7.13 (m, 3H, aromatic), 7.21-7.53 (m, 23H, aromatic), 7.81-7.84 (m, 1H, aromatic), 8.09 (d, 1H, *J* = 7.86 Hz aromatic), 8.84 (d, 1H, *J* = 4.11 Hz aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 55.3, 67.3, 68.5, 68.8, 69.9, 73.3, 73.7, 73.9, 75.0, 75.5, 75.9, 76.1, 76.5, 80.0, 80.2, 97.5, 101.8 ($^{1}J_{C1,H1}$ = 166.9 Hz, $^{1}J_{C1',H1'}$ = 161.0 Hz), 102.1, 125.6, 126.3 (x2), 127.1, 127.7, 127.8, 127.9, 128.2 (x4),

128.3 (x4), 128.5 (x2), 128.6 (x5), 128.8 (x2), 129.0 (x2), 129.1, 137.0, 137.4, 137.6, 138.0, 138.3, 138.7, 147.8, 150.2, 164.3 ppm. HR FAB MS [$M+H^+$] calcd for $C_{54}H_{56}NO_{12}$ 910.3803, found 910.3790.

2.4.6 Synthesis of trisaccharide 2.19

Methyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (2.19). $Cu(OAc)_2$ (9 mg, 0.05 mmol), was added to a solution of disaccharide **2.3h** (39 mg, 0.039 mmol) in CH_2Cl_2 (2.0 mL) and MeOH (0.1 mL) and the resulting mixture was stirred under argon for 3 h at rt. The reaction mixture was quenched with sat. aq. NH_4Cl (~1.0 mL), the solid was filtered off, and rinsed successively with CH_2Cl_2. The combined filtrate (~10 mL) was washed with 1M aq. HCl (2 x 3 mL), and water (3 x 3 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**2.18**) as an amorphous powder in 75% yield (27 mg, 0.029 mmol). Analytical data for **2.18:** R_f = 0.7 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{21}$ +0.22 (*c* = 1.0, $CHCl_3$): ^{1}H n.m.r. (300 MHz): δ, 2.61 (d, 1H, *J* = 10.6 Hz, OH), 3.35 (s, 3H, OCH_3), 3.45 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.51 (dd, 1H, $J_{2,3}$ = 3.5 Hz, H-2), 3.60-3.64 (m, 1H, H-6a), 3.78-3.89 (m, 4H, H-2', 3', 5, 5'), 4.04 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.20-4.24 (m, 1H, H-6b), 4.48-5.08 (m, 12H, H-1, 1', 6a', 6b', 4 x CH_2Ph), 5.43 (dd, 1H, $J_{4',5'}$ = 9.1 Hz, H-4'), 7.27-7.57 (m, 26H, aromatic), 7.97-8.01 (m, 4H, aromatic) ppm; ^{13}C n.m.r. (75

MHz): δ, 55.2, 64.2, 68.9, 71.3, 71.8, 72.4, 73.5, 74.8, 75.0, 76.1 77.9, 80.2, 82.3, 97.9, 102.2, 127.9, 128.0, 128.1, 128.2 (x7), 128.3 (x4), 1288.4 (x2), 128.5 (x2), 128.6 (x3), 128.6 (x5), 129.6, 129.8 (x2), 129.9, 130.0 (x2), 133.1, 133.5, 138.1, 138.3, 138.4, 138.8, 166.2, 166.3 ppm; HR FAB MS [M+Na^+] calcd for $C_{55}H_{56}NaO_{13}$ 947.3619 found, 947.3605.

The title compound was obtained by Method B from donor **2.1g** and acceptor **2.18** as clear syrup in 76% yield α/β > 1/25). Analytical data for **2.19** R_f = 0.5 (ethyl acetate/ hexane, 2/3, v/v); $[\alpha]_D^{23}$ -7.5 (*c* = 1.0, $CHCl_3$); ^{1}H n.m.r. (300 MHz): δ, 3.07 (m, 1H, H-5), 3.30 (s, 3H, OCH_3), 3.34-3.61 (m, 4H, H-2, 4, 6a, 6a'), 3.83-3.90 (m, 4H, H-2', 2", 5, 5'), 3.97-4.04 (m, 5H, H-3, 3', 4", 6b, 6b"), 4.08 (s, 1H, H-1'), 4.32-4.38 (m, 2H, H-1", $^1/_2$ C*H*$_2$Ph), 4.49-4.57 (m, 3H, H-1, 6a', 6b'), 4.62 (d, 1H, ^2J = 12.0 Hz, $^1/_2$ C*H*$_2$Ph), 4.74-4.82 (m, 3H, 1$^1/_2$ C*H*$_2$Ph), 4.88 (d, 1H, ^2J = 10.3 Hz, $^1/_2$ C*H*$_2$Ph), 4.93-5.00 (m, 2H, H-3", $^1/_2$ C*H*$_2$Ph), 5.41 (s, 1H, >C*H*Ph), 5.68 (dd, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 6.90-7.08 (m, 5H, aromatic), 7.21-7.52 (m, 32H, aromatic), 7.70-7.76 (m, 1H, aromatic), 7.90-7.97 (m, 5H, aromatic), 8.74-8.76 (m, 1H, aromatic) ppm; ^{13}C n.m.r. (150 MHz): δ, 64.2, 67.4, 68.3, 68.4, 69.0, 69.6, 71.5, 72.5, 72.8, 73.3, 73.5, 74.7, 74.8, 75.2, 75.4, 75.5, 75.7, 77.8, 79.8, 82.0, 97.7, 97.8, 101.5 ($^1J_{C1,H1}$ = 170.3 Hz, $^1J_{C1',H1'}$ = 163.7 Hz, $^1J_{C1'',H1''}$ = 157.0), 101.9, 125.4, 126.1 (x2), 126.8, 127.2, 127.6, 127.7, 127.8 (x2), 127.9 (x5), 128.0 (x2), 128.1 (x3), 128.2 (x5), 128.3, 128.4 (x5), 128.5 (x2), 128.6 (x2), 128.7 (x2), 129.0, 129.6 (x2), 129.7, 129.9 (x2), 130.0, 132.8, 132.9, 136.7, 137.0, 137.6, 137.9, 138.0, 138.2, 138.6, 147.4, 149.9, 163.8, 165.6, 166.2 ppm. HR FAB MS [M+Na^+] calcd for $C_{81}H_{79}O_{19}NNa$ 1392.5144. found 1392.5131.

2.5 References

1. (a) Zhu, X.; Schmidt, R. R., New principles for glycoside-bond formation. *Angew. Chem. Int. Ed.* **2009,** *48,* 1900-1934; (b) Demchenko, A. V., *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*. Wiley-VCH: Weinheim, Germany, 2008.

2. Goodman, L., Neighboring-group participation in sugars. *Adv. Carbohydr. Chem. Biochem.* **1967,** *22*, 109-175.

3. Demchenko, A. V., 1,2-cis O-Glycosylation: methods, strategies, principles. *Curr. Org. Chem.* **2003,** *7* (1), 35-79.

4. (a) Manabe, S.; Ito, Y., Optimizing glycosylation reaction selectivities by protecting group manipulation. *Curr. Bioact. Comp.* **2009,** *4*, 258-281; (b) Fraser-Reid, B.; Jayaprakash, K. N.; López, J. C.; Gómez, A. M.; Uriel, C., Protecting Groups in Carbohydrate Chemistry Profoundly Influence All Selectivities in Glycosyl Couplings. In *ACS Symp. Ser. (Frontiers in Modern Carbohydrate Chemistry)* Demchenko, A. V., Ed. Oxford Univ. Press: 2007; Vol. 960, pp 91-117; (c) Guo, J.; Ye, X. S., Protecting groups in carbohydrate chemistry: influence on stereoselectivity of glycosylations. *Molecules* **2010,** *15,* 7235-7265.

5. (a) Smoot, J. T.; Demchenko, A. V., How the arming participating moieties can broaden the scope of chemoselective oligosaccharide synthesis by allowing the inverse armed-disarmed approach *J. Org. Chem.* **2008,** *73*, 8838-8850; (b) Smoot, J. T.; Pornsuriyasak, P.; Demchenko, A. V., Development of an arming participating group for

stereoselective glycosylation and chemoselective oligosaccharide synthesis. *Angew. Chem. Int. Ed.* **2005,** *44*, 7123-7126.

6. Yasomanee, J. P.; Demchenko, A. V., The effect of remote picolinyl and picoloyl substituents on the stereoselectivity of chemical glycosylation. *J. Am. Chem. Soc.* **2012,** *134*, 20097-20102.

7. Ravenscroft, M.; Roberts, R. M. G.; Tillett, J. G., The reaction of some cyclic and open-chain disulphides with methyl trifluoromethanesulphonate. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1569-1972.

8. (a) Gridley, J. J.; Osborn, H. M. I., Recent advances in the construction of b-D-mannose and b-D-mannosamine linkages. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1471-1491; (b) Crich, D., Chemistry of glycosyl triflates: Synthesis of b-mannopyranosides (Reprinted from Glycochemistry: Principles, Synthesis, and Applications, pg 53-75, 2001). *J. Carbohydr. Chem.* **2002,** *21* (7-9), 667-690.

9. (a) Crich, D., Methodology Development and Physical Organic Chemistry: A Powerful Combination for the Advancement of Glycochemistry. *J. Org. Chem.* **2011,** *76*, 9193-9209; (b) Crich, D., Mechanism of a chemical glycosylation reaction. *Acc. Chem. Res.* **2010,** *43*, 1144-1153; (c) Crich, D.; Sharma, I., Is Donor−Acceptor Hydrogen Bonding Necessary for 4,6-O-Benzylidene-directed β-Mannopyranosylation? Stereoselective Synthesis of β-C-Mannopyranosides and α-C-Glucopyranosides. *Org. Lett.* **2008,** *10*, 4731-4734; (d) Crich, D.; Wu, B.; Jayalath, P., Convergent synthesis of a b-(1--3)-mannohexaose. *J. Org. Chem.* **2007,** *72*, 6806-6815; (e) Crich, D.; Banerjee, A.;

Yao, Q., Direct chemical synthesis of the b-D-mannans: the b-(1-2) and b-(1-4) series. *J. Am. Chem. Soc.* **2004,** *126*, 14930-14934; (f) Crich, D.; Sun, S., Direct formation of b-mannopyranosides and other hindered glycosides from thioglycosides. *J. Am. Chem. Soc.* **1998,** *120*, 435-436; (g) Crich, D.; Sun, S., Formation of b-mannopyranosides of primary alcohols using the sulfoxide method. *J. Org. Chem.* **1996,** *61*, 4506-4507.

10. (a) Baek, J. Y.; Choi, T. J.; Jeon, H. B.; Kim, K. S., A highly reactive and stereoselective b-mannosylation system: mannosyl 4-pentenoate/PhSeOTf. *Angew. Chem. Int. Ed.* **2006,** *45*, 7436-7440; (b) El Ashry, E. S. H.; Rashed, N.; Ibrahim, E. S. I., Strategies of synthetic methodologies for constructing b-mannosidic linkage. *Curr. Org. Synth.* **2005,** *2* (2), 175-213; (c) De Meo, C.; Kamat, M. N.; Demchenko, A. V., Remote participation-assisted synthesis of b-mannosides. *Eur. J. Org. Chem.* **2005**, 706-711.

11. (a) Fairbanks, A. J., Intramolecular aglycon delivery (IAD): The solution to 1,2-cis stereocontrol for oligosaccharide synthesis? *Synlett* **2003,** (13), 1945-1958; (b) Ishiwata, A.; Lee, Y. J.; Ito, Y., Recent advances in stereoselective glycosylation through intramolecular aglycon delivery. *Org. Biomol. Chem.* **2010,** *8*, 3596-3608; (c) Barresi, F.; Hindsgaul, O., Synthesis of b-mannosides by intramolecular aglycon delivery. *J. Am. Chem. Soc.* **1991,** *113*, 9376-9377.

12. Wang, C.; Sanders, B.; Baker, D. C., Synthesis of a glycodendrimer incorporating multiple mannosides on a glucoside core. *Can. J. Chem.* **2011,** *89* (8), 959-963.

13. (a) Kuester, J. M.; Dyong, I., Partially benzylated carbohydrates, 2. Synthesis of all methyl mono-, di-, and tri-O-benzyl-a-D-glucopyranosides. *Justus Liebigs Ann. Chem.*

1975, (12), 2179-2189; (b) Ranade, S. C.; Kaeothip, S.; Demchenko, A. V., Glycosyl alkoxythioimidates as complementary building blocks for chemical glycosylation. *Org. Lett.* **2010,** *12*, 5628-5631.

14. Chang, G. X.; Lowary, T. L., A glycosylation protocol based on activation of glycosyl 2-pyridyl sulfones with samarium triflate. *Org. Lett.* **2000,** *2* (11), 1505-1508.

15. Douglas, N. L.; Ley, S. V.; Lucking, U.; Warriner, S. L., Tuning glycoside reactivity: new tool for efficient oligosaccharides synthesis. *J. Chem. Soc., Perkin Trans. 1* **1998**, 51-65.

16. Lemanski, G.; Ziegler, T., Prearranged glycosides. Part 12. Intramolecular mannosylation of glucose derivatives via prearranged glycosides. *Helv. Chim. Acta* **2000,** *83*, 2655-2675.

17. Cherif, S.; Clavel, J.-M.; Monneret, C., SYNTHESIS OF THE TETRASACCHARIDE Glc α (1→3) Man α (1→2) Man α (1→2) Man α (OMe) AS INHIBITOR OF CALNEXIN BINDING TO GlcMan 9GlcNAc 2 a. *J. Carbohydr. Chem.* **2002,** *21* (1-2), 123-130.

18. Tam, P.-H.; Lowary, T. L., Synthesis of deoxy and methoxy analogs of octyl α-d-mannopyranosyl-(1→6)-α-d-mannopyranoside as probes for mycobacterial lipoarabinomannan biosynthesis. *Carbohydr. Res.* **2007,** *342* (12–13), 1741-1772.

19. Ali, A.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C., The methylsulfonylethoxymethyl (Msem) as a hydroxyl protecting group in oligosaccharide synthesis. *Tetrahedron* **2010,** *66* (32), 6121-6132.

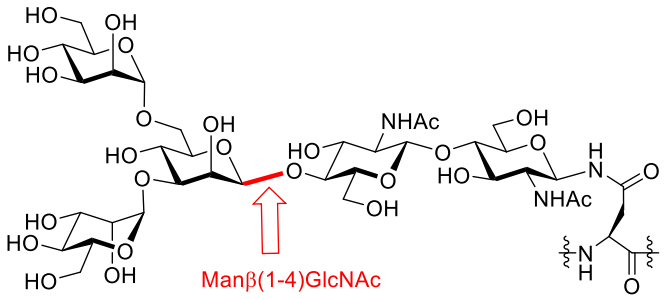
CHAPTER 3

Hydrogen bond-mediated aglycone delivery: synthesis of the *N*-linked glycoprotein core pentasaccharide.

3.1. Introduction

The glycoproteins equipped with *N*-glycans play diverse roles in a wide range of fundamental biological processes.[1-3] *N*-Glycans and post-translational oligosaccharides are covalently attached to proteins at asparagine (Asn) residues by an *N*-glycosidic bond. *N*-Glycans are also found on the surface of a variety of pathogens and are involved in mediation of the pathogenesis of cancers,[4] AIDS,[5] Alzheimer's disease,[6] etc.[7-8] All *N*-glycans are classified into three general classes: high-mannose, hybrid, and complex,[9] but all share the common core sequence, Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAcβ1→Asn (**Scheme 3.1**). The construction of the core structure as the key intermediate towards a diverse library of *N*-glycans has been a vibrant area of research. Chemical and chemoenzymatic syntheses of the *N*-glycan core pentasaccharide have been reported by Ogawa,[10] Danishefsky,[11-12] Seeberger,[13-14] Boons,[3] Wang,[15] and Ito,[16-18] amongst others. Nevertheless, the construction of these glycan sequences remains a notable challenge, which slows biomedical studies related to understanding the roles of glycoproteins and creation of *N*-glycan-derived pharmaceuticals.[19]

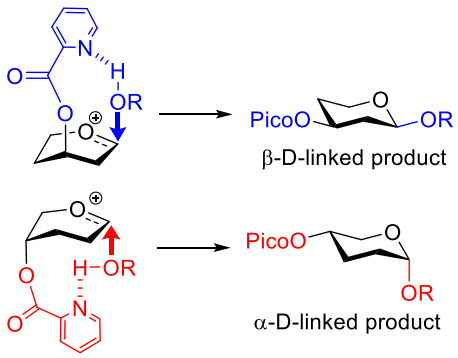
Scheme 3.1. The core pentasaccharide sequence of all *N*-glycans with the most challenging Manβ(1→4)GlcNAc linkage highlighted



One of the challenges of the chemical synthesis of *N*-glycans is the introduction of the Manβ(1→4)GlcNAc unit present at the branching point of the core sequence.[20-

[21] A strong anomeric effect in mannosides, and the impossibility of using the neighboring group participation to aid in the synthesis of the β-linkage in the manno series, make this bond one of the most difficult steps in the synthesis.[21] Some promising methods have been established by Crich[22-28] and others,[29-31] the approach developed by us (see Chapter 2) also allows us to achieve excellent stereocontrol in β-mannosylation.[32] Our approach relies on the use of picoloyl (Pico) protecting groups at remote positions capable of controlling the stereoselectivity via the H-bond-mediated Aglycone Delivery (HAD).[33-35] According to this approach, the hydroxyl of the glycosyl acceptor forms the hydrogen bond with the picoloyl nitrogen of the glycosyl donor. As a result, the nucleophile is delivered from the same (*syn*) face of the sugar ring in respect to the picoloyl group (**Scheme 3.2)**. For compounds of the D-manno series, excellent stereocontrol could be achieved with picoloyl groups at C-3 and/or C-6 positions. A series of β-mannosides have been obtained with high stereoselectivity and yields. Hence, we were curious to investigate whether this approach would be suitable for the synthesis of the core pentasaccharide sequence of *N*-glycans.

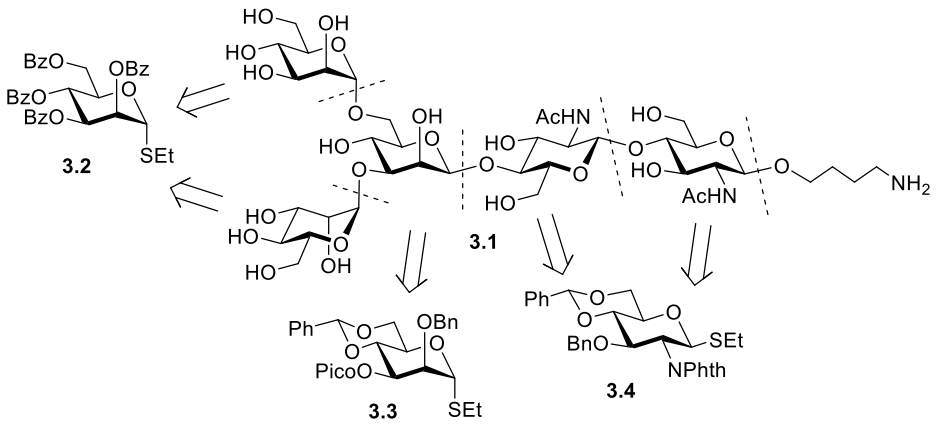
Scheme 3.2. H-bond-mediated Aglycone Delivery (HAD) assisted by the remote picoloyl substituents



3.2. Results and Discussion

Described herein is our approach to the synthesis of the *N*-linked core pentasaccharide **3.1**, using the HAD reaction for controlling the formation of the β-mannosidic linkage. For this synthesis, three building blocks (**3.2-3.4**) were designed in accordance of the retrosynthetic analysis of the final target depicted in **Scheme 3.3**. Among these, building block **3.4**[36] was chosen for introducing the two glucosamine units at the reducing end of the sequence. Building block **3.3** equipped with the picoloyl protecting group at position C-3, the key intermediate of the entire synthesis, was selected to carry out the HAD-assisted synthesis of the Manβ(1→4)GlcNAc linkage. Finally, building block **3.2**[37] was selected for the introduction of the two terminal α-mannosyl residues. 4-Azidobutyl spacer was selected to allow for a selective conjugation or surface immobilization of the final product because the azido group can be easily transformed into a versatile amino group.

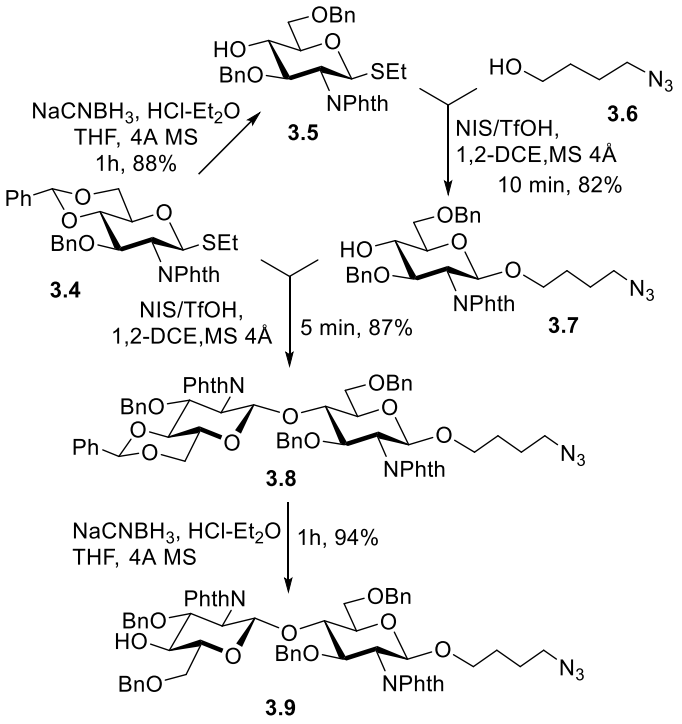
Scheme 3.3. Retrosynthetic analysis of pentasaccharide 3.1 from building blocks 3.2-3.4.



In accordance with the retrosynthetic analysis, the assembly of the target pentasaccharide **3.1** started with the universal precursor **3.4** that was used to introduce both glucosamine units. First, compound **3.4** was converted into glycosyl donor **3.5** that

was reacted with 4-azidobutanol **3.6** to give the functionalized monosaccharide **3.7** in 82% yield. Second, glycosylation of acceptor **3.7** with donor **3.4** led to the formation of disaccharide **3.8** in 87% yield. The subsequent reductive cleave of the benzylidene acetal afforded compound **3.9** in 94% yield. Disaccharide **3.9** was then used as a glycosyl acceptor for β-mannosylation.

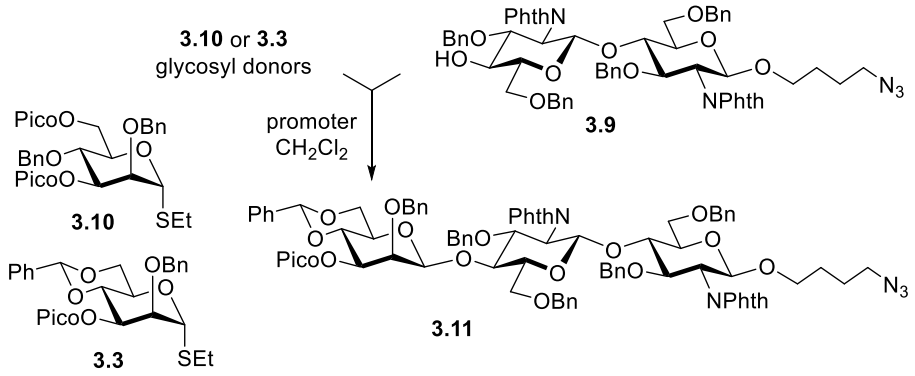
Scheme 3.4. Synthesis of disaccharide acceptor 3.9



The next synthetic step, the introduction of the β-linked mannose unit, represents the key step in the entire synthesis of the N-glycan pentasaccharide. Originally we were planning to use 3-6-O-dipicolylated ethylthio glycosyl donor **3.10** for the glycosylation of acceptor **3.9**, however all attempts were unsuccessful (Table 3.1). All attempts to achieve the glycosylation product including the amount of the donor, type and amount of the promoter have failed (entries 1-5). Hence, we decided to investigate 3-*O*-picoloylated ethylthio glycosyl donor **3.3** for the introduction of the β-

linked mannose unit. Initially, our attempts were unsuccessful, and conventional reaction conditions for the HAD reaction did not work (entries 6 and 7).[32]

Table 3.1. Optimization of the synthesis of trisaccharide 3.11



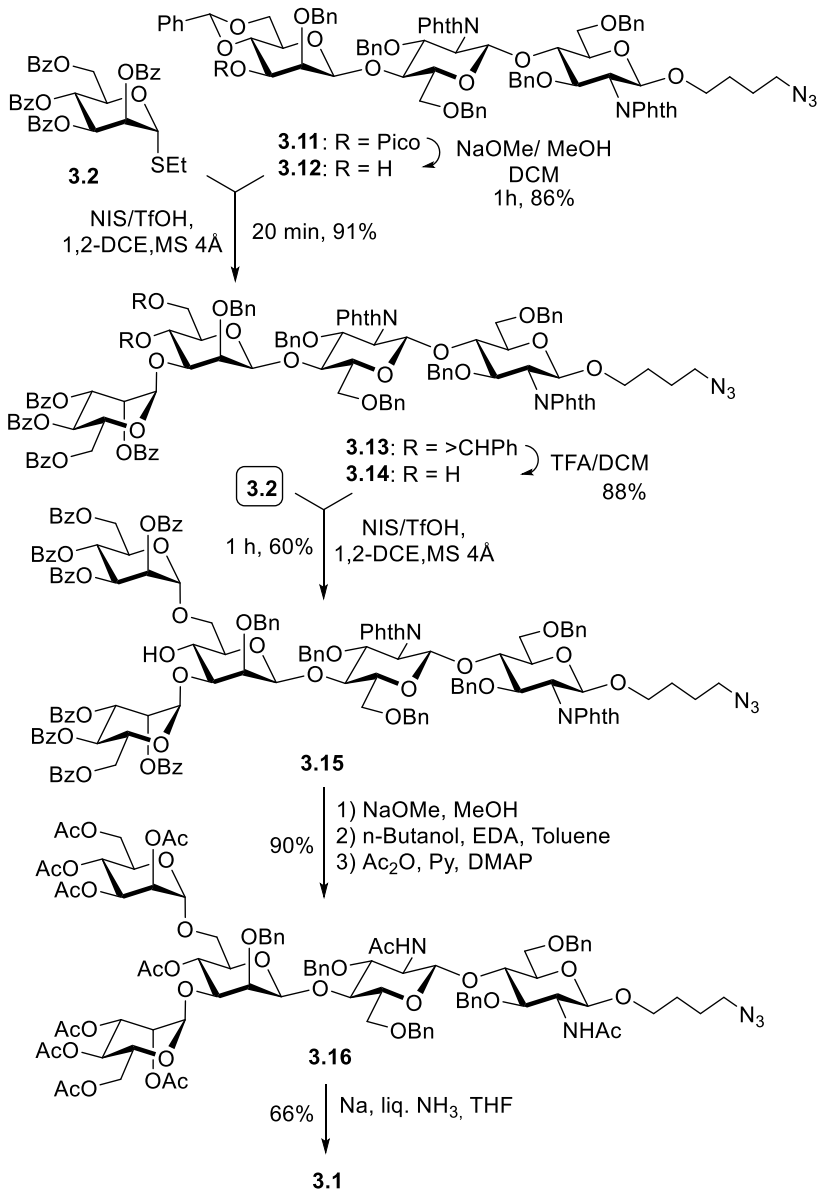
entry	donor (equiv.)	promoter (equiv.)	Product (yield, α/β ratio)
1	**3.10** (1.1)	NIS (2.0), TfOH (0.2)	no reaction
2	**3.10** (1.3)	NIS (2.0), TfOH (0.2)	no reaction
3	**3.10** (3.0)	NIS (3.0), TfOH (0.3)	no reaction
4	**3.10** (1.3)	DMTST (2.0)	no reaction
5	**3.10** (1.3)	MeOTf (4.5)	no reaction
6	**3.3** (1.1)	NIS (2.0), TfOH (0.2)	no reaction
7	**3.3** (1.1)	DMTST (2.0)	no reaction
8	**3.3** (1.1)	NIS (6.0), TfOH (0.3)	**3.11** (50%, α/β = 1/11.2)
9	**3.3** (1.3)	NIS (6.0), TfOH (0.3)	**3.11** (56%, α/β = 1/10.0)
10	**3.3** (1.6)	NIS (4.0), TfOH (0.3)	**3.11** (61%, α/β = 1/13.1)
11	**3.3** (3.0)	NIS (3.0), TfOH (0.3)	**3.11** (92%, α/β = 1/12.0)

Success then emerged when we decided "to push" the reaction and added large access of NIS. Under these conditions the coupling of donor **3.3** and acceptor **3.9** produced trisaccharide **3.11** in 50% yield (α/β =1/11.2, entry 8). Encouraged by this progress, we continued the optimization of the reaction conditions and discovered that

using 3 equiv. of donor **3.3** and 3 equiv. of NIS in respect to the donor (9 equiv. in respect to the acceptor) are necessary to bring the reaction to completion. As a result, trisaccharide **3.11** was obtained in a high yield of 92% and commendable stereoselectivity (α/β = 1/12, entry 10). It should be noted that this reaction was performed at the ambient temperature, which offers an important experimental advantage over other known methods for β-mannosylation that require super-low temperatures.[21, 27, 38-39]

With trisaccharide **3.11** in hand, we continued the synthesis of the target pentasaccharide core depicted in **Scheme 3.4**. 3”-*O*-Picoloyl group in **3.11** was removed with NaOMe in MeOH-DCM. The resulting trisaccharide acceptor **3.12** was glycosylated with mannosyl donor **3.2** in the presence of NIS/TfOH to afford tetrasaccaharide **3.13** in 91% yield. The latter was subjected to acid hydrolysis of 4”,6”-*O*-benzylidene acetal. The resulting tetrasaccharide acceptor **3.14**, obtained in 88% yield, was coupled with mannosyl donor **3.2** to afford the protected *N*-linked core pentasaccharide **3.15** in 60% yield. With compound **3.15** in hand, we began the global deprotection shown in **Scheme 3.4**. Benzoyl protecting groups were removed by the treatment with NaOMe/MeOH. The resulting crude product was then subjected to the treatment with 1,2-ethylenediamine in *n*-butanol-toluene to remove the phthaloyl groups. Finally, the crude deprotected product was per-acetylated with acetic anhydride in the presence of pyridine to afford compound **3.16** in 90% yield over the three steps. The latter was subjected to the Birch conditions (Na/liq. NH_3) that affected the global deprotection by removing benzyls, acetates, and reducing the azide group. The fully deprotected N-glycan pentasaccharide core **3.1** was isolated in 66% yield.

Scheme 3.5. The final assembly and deprotection to obtain the *N*-glycan pentasaccharide core 3.1



3.3. Conclusions

In summary, this Chapter discussed the application of the H-bond-mediated aglycone delivery (HAD) method on the synthesis of the *N*-linked glycan core pentasaccharide, which represents a number of synthetic challenges to chemists. The synthesis of the final oligosaccharide sequences was accomplished with a high overall yield step. High diastereoselectivity for β-mannosylation was achieved at room temperature.

3.4. Experimental

3.4.1. General Methods

Column chromatography was performed on silica gel 60 (70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F254. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 and $ClCH_2CH_2Cl$ (1,2-DCE) were distilled from CaH_2 directly prior to application. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Molecular sieves (3 Å or 4 Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. Optical rotations were measured at 'Jasco P-1020' polarimeter. Unless noted otherwise, ^{1}H n.m.r. spectra were recorded in $CDCl_3$ at 300 MHz, ^{13}C n.m.r. spectra were recorded in $CDCl_3$ at 75 or 150 MHz. Two-dimensional heteronuclear *J*-resolved spectra (HETERO2D) were recorded in $CDCl_3$ at 600 MHz.

3.4.2. Synthesis of Glycosyl Donors

Ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (3.2). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[37]

Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (3.3). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[32]

Ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3.4). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[36]

Ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3.5). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[36]

Ethyl 2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-1-thio-α-D-mannopyranoside (3.10). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[32]

3.4.3. Assembly of pentasaccharide 3.14.

4-Azidobutyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3.7). A mixture of **3.5** (0.36 g, 0.66 mmol), 4-azidobutanol[40] (**3.6**, 0.09 g, 0.80 mmol), and freshly activated molecular sieves (4 Å, 0.90 g) in CH_2Cl_2 (15 mL) was stirred under argon for 1 h at rt. *N*-Iodosuccinimide (NIS, 0.30 g, 1.30 mmol) and TfOH (12 μL, 0.13 mmol) were added, and the resulting mixture was stirred for 10 min at rt. After that, the solids were filtered off through a pad of Celite and washed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound (0.322 g, 82%) as a colorless foam. Analytical data for **3.7**: R_f = 0.46 (ethyl acetate / hexanes, 1/1.5, v/v); $[\alpha]_D^{20}$ +58.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.23-1.46 (m, 4H, 2 x CH_2), 2.92 (d, 1H, *J* = 2.4 Hz, OH), 3.01 (t, 2H, *J* = 6.8 Hz, NCH_2), 3.35-3.39 (m, 1H, OCH_2^a), 3.63 (m, 1H, H-6a), 3.73-3.84 (m, 4H, H-4, 5, 6b, OCH_2^b), 4.09-4.24 (m, 2H, H-2, 3), 4.52 (d, 1H, ^2J = 12.2 Hz, ½ CH_2Ph), 4.46 (d, 2H, ^2J = 8.7 Hz, CH_2Ph), 4.73 (d, 1H, ^2J = 12.0 Hz, ½ CH_2Ph), 5.12 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 6.92-6.96 (m, 3H, aromatic), 7.03-7.05 (m, 2H, aromatic), 7.24-7.35 (m, 5H, aromatic), 7.61-7.85 (m,

4H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 27.9, 29.1, 53.5, 57.9, 71.3, 73.4, 76.0, 76.4, 76.9, 77.2, 81.2, 100.9, 130.1 (x2), 130.4 (x2),130.5 (x4), 130.6 (x2), 130.8 (x4), 131.1 (x2), 131.2 (x2), 140.2, 140.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{32}H_{34}N_4O_7Na$ 609.2339, found 609.2309.

4-Azidobutyl *O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.8). A mixture of **3.4** (0.59 g, 1.10 mmol), **3.7** (0.50 g, 0.80 mmol), and freshly activated molecular sieves (4 Å, 1.50 g) in CH_2Cl_2 (24 mL) was stirred under argon for 1 h at rt. *N*-Iodosuccinimide (NIS, 0.50 g, 2.20 mmol) and TfOH (20 μL, 0.20 mmol) were added, and the resulting mixture was stirred for 5 min at rt. After that, the solids were filtered off through a pad of Celite and washed successively with CH_2Cl_2. The combined filtrate (~200 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound (1.00 g, 87%) as a colorless foam. Analytical data for **3.8**: R_f = 0.68 (ethyl acetate / hexanes, 1/1 v/v); $[\alpha]_D^{20}$ +58.2 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.38-1.44 (m, 4H, 2 x CH_2), 3.01-3.05 (m, 2H, *J* = 6.8 Hz, NCH_2), 3.31-3.63 (m, 6H, H-4, 5, 5', 6a, 6a', OCH_2^a), 3.71-3.81 (m, 2H, H-4', OCH_2^b), 4.13-4.32 (m, 5H, H-2, 2', 3, 6b, 6b'), 4.44-4.57 (m, 5H, H-3', 2 x C*H*$_2$Ph), 4.85 (dd, 2H, ^2J= 8.5 Hz, C*H*$_2$Ph), 5.00 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 5.43 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1'), 5.57 (s, 1H, C*H*Ph), 6.94-7.13 (m, 10H, aromatic), 7.33-7.57 (m, 10H, aromatic), 7.70-8.10 (m, 8H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 14.3, 21.1, 25.3, 26.4, 50.9, 55.7, 56.6, 60.4, 65.8, 68.5, 72.8, 74.6, 83.2, 97.8, 98.1, 101.3, 123.3, 136.1, 126.2 (x3), 127.2, 127.4 (x3), 127.5, 127.6, 127.9

(x3), 128.1 (x10), 128.2 (x3), 128.3 (x10), 129.1, 131.6, 133.9, 137.5, 138.0, 138.3, 138.6 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{57}N_5O_{13}Na$ 1078.3851, found 1078.3838.

4-Azidobutyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.9). Sodium cyanoborohydride ($NaCNBH_3$, 0.80 g, 12.9 mmol) was added to a solution of **3.8** (1.00 g, 0.96 mmol) and molecular sieves (3 Å, 3 g) in dry THF (30 mL) at rt. A solution of HCl in diethyl ether (details, ~6.4 mL) was added dropwise under vigorous stirring until gas evolution ceased. The resulting mixture was stirred for 1 h at. After that, the solids were filtered off through a pad of Celite and washed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with water (10 mL), $NaHCO_3$ (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound (0.94 g, 94%) as a white amorphous solid. Analytical data for **3.9**: R_f = 0.43 (ethyl acetate / hexanes, 1/1.5 v/v); $[\alpha]_D^{20}$ +29.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ, 1.35-1.41 (m, 4H, 2 x CH_2), 3.01 (t, 2H, J = 6.8 Hz, NCH_2), 3.17 (d, 1H, J = 1.2 Hz, OCH_2^a), 3.32-3.34 (m, 2H, H-4, 6a), 3.43-3.46 (m, 2H, H-5', 6b') 3.33-3.59 (m, 2H, H-6a', 6b), 3.71-3.74 (m, 2H, H-5, OCH_2^b), 3.85 (dd, 1H, $J_{4,5}$ = 4.8 Hz, H-4'), 4.12-4.21 (m, 3H, H-2, 2', 3), 4.28 (dd, 1H, $J_{3,4}$ = 5.0 Hz, H-3'), 4.49-4.59 (m, 6H, 3 x CH_2Ph), 4.82 (dd, 2H, ^2J = 7.3 Hz, CH_2Ph), 4.97 (d, 1H, $J_{1,2}$ = 4.7 Hz, H-1), 5.33 (d, 1H, $J_{1',2'}$ = 4.9 Hz, H-1'), 6.87-7.06 (m, 10H, aromatic), 7.33-7.39 (m, 10H, aromatic), 7.55-7.80 (m, 8H, aromatic) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ, 25.7, 26.7, 51.2, 56.0, 56.5, 68.5, 68.8, 71.3, 73.0, 73.2, 74.1, 74.6, 74.7, 74.9, 75.8, 76.1, 78.7, 97.4, 98.4, 123.5, 124.0, 127.3,

127.7 (x3), 127.8, 128.1 (x3), 128.1 (x3), 128.2 (x6), 128.3 (x3), 128.5 (x3), 128.6 (x3), 128.9 (x4), 132.0, 132.2, 134.3, 134.4, 138.7 (x2), 139.0, 168.8, 168.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{59}N_5O_{13}Na$ 1080.4007, found 1080.4017.

4-Azidobutyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.11). A mixture of **3.3** (0.452 g, 0.90 mmol), **3.9** (0.313 g, 0.30 mmol), and freshly activated molecular sieves (4 Å, 1.50 g) in 1,2-dichloroethane (90 mL) was stirred under argon for 1 h at rt. *N*-Iodosuccinimide (NIS, 0.60 g, 2.60 mmol) and TfOH (230 μL, 0.26 mmol) were added, and the resulting mixture was stirred for 10 min at rt. After that, the solids were filtered off through a pad of Celite and washed successively with CH_2Cl_2. The combined filtrate (~200 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone-toluene) to afford the title compound (0.425 g, 95%) as a white amorphous solid. Analytical data for **3.11**: R_f = 0.34 (acetone / toluene, 1/10, v/v); $[\alpha]_D^{20}$ -18.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.12-1.21 (m, 4H, 2 x CH_2), 2.85 (t, 2H, J = 6.8 Hz, NCH_2), 3.08-3.21 (m, 4H, H-5', 6a, 6a', OCH_2^a), 3.28-3.44 (m, 4H, H-5'', 6a'', 6b, 6b''), 3.52.-3.58 (m, 2H, H-6b', OCH_2^b) 3.97-4.14 (m, 10H, H-1'', 2, 2', 2'', 3, 3', 4, 4', 4'', 5), 4.14-4.56 (m, 6H, 3 x CH_2Ph), 4.65-4.82 (m, 5H, H-1, 2 x CH_2Ph) 4.99 (d, 1H, $J_{2'',3''}$ = 3.1 Hz, H-3''), 5.17 (d, 1H, $J_{1',2'}$ = 9.0 Hz, H-1'), 5.33 (s, 1H, C*H*Ph), 6.65-7.01 (m, 14H, aromatic), 7.14-7.66 (m, 26H, aromatic), 7.89 (d, 1H, aromatic), 8.71 (d, 1H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 25.4, 26.5, 51.0, 55.8, 56.7, 67.3, 67.9, 68.4, 68.5, 68.6, 72.8, 73.5, 73.9,

74.5, 74.7 (x2), 75.7, 75.8, 76.0, 76.6, 76.9, 77.2, 77.4, 79.2, 97.2, 98.3, 101.1, 101.7, 123.3, 123.8, 125.6 (x2), 126.3 (x2), 127.0 (x2), 127.1 (x2), 127.3 (x2), 127.4 (x2), 127.5 (x2), 127.7 (x2), 127.8 (x2), 127.0 (x5), 128.2 (x3), 128.3 (x2), 128.4 (x10), 128.5 (x2), 128.8 (x2), 129.1, 131.8, 133.8, 134.04, 137.0, 137.4, 137.9, 138.2, 138.6, 138.8, 139.0, 147.7, 150.2, 164.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{86}H_{82}N_6O_{19}Na$ 1525.5532, found 1525.5530.

4-Azidobutyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.12). A 1 M solution of NaOMe in MeOH (2.0 mL) was added to a solution of trisaccharide **3.11** (0.480, 0.319 mmol) in CH_2Cl_2 (2.0 mL) and MeOH (18 mL) and the resulting mixture was stirred for 1 h at rt. DOWEX (H^+) was added until pH = 6, the resin was filtered off, and rinsed successively with CH_2Cl_2 (5 x 5.0 mL) and MeOH (5 x 5.0 mL). The combined filtrate (~70 mL) was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound as a white amorphous solid in 86% yield (380 mg, 0.272 mmol). Analytical data for **3.12**: R_f = 0.55 (ethyl acetate / hexane gradient elution, 1/1, v/v); $[\alpha]_D^{20}$ +0.64 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.20-1.44 (m, 4H, 2 x CH_2), 2.33 (d, 1H, OH), 2.97 (t, 2H, CH_2), 3.10-3.33 (m, 4H), 3.39-3.70 (m, 8H) 4.06-4.24 (m, 7H), 4.36-4.68 (m, 9H), 4.83-5.00 (m, 4H), 5.27 (d, 1H, $J_{1',2'}$ = 3.2 Hz, H-1'), 5.40 (s, 1H, C*H*Ph), 6.76-6.98 (m, 11H, aromatic), 7.23-7.43 (m, 19H, aromatic), 7.64-7.68 (m, 8H, aromatic), ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.4, 26.5, 50.9 (x2), 55.8, 56.6, 66.9, 67.9, 68.3, 68.5, 68.6, 71.0, 72.7, 73.5, 74.4, 74.6, 74.7 (x3), 75.9, 76.8, 79.0, 79.2, 79.5, 97.1, 98.2, 102.0 (x2), 123.2, 126.4 (x3), 127.0, 127.1, 127.4 (x5), 127.6 (x5),

127.9 (x5), 128.0 (5), 128.1 (x5), 128.3 (x6), 128.6 (x3), 128.7 (x3), 129.1, 131.7, 133.7, 137.3, 137.8, 138.3, 138.5, 138.7, 138.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{80}H_{79}N_5O_{18}Na$ 1420.5318, found 1420.5320.

4-Azidobutyl *O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→4)-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.13). A mixture of **3.2** (0.524 g, 0.81 mmol), trisaccharide **3.12** (0.880 g, 0.62 mmol), and freshly activated molecular sieves (4 Å, 1.50 g) in 1,2-dichloromethane (40 mL) was stirred under argon for 1 h at rt. *N*-Iodosuccinimide (NIS, 0.364 g, 1.60 mmol) and TfOH (14 μL, 0.16 mmol) were added, and the resulting mixture was stirred for 20 min at rt. After that, the solids were filtered off through a pad of Celite and washed successively with CH_2Cl_2. The combined filtrate (~200 mL) was washed with sat. aq. Na_2SO_4 (20 mL) and water (3 x 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone-toluene gradient elution) to afford the title compound (1.10 g, 91%) as a white amorphous solid. Analytical data for **3.13**: R_f = 0.60 (acetone / toluene, 1/10, v/v); $[\alpha]_D^{21}$ -3.84 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.30-1.51 (m, 4H, 2 x CH_2), 3.08 (t, 2H, J = 6.8 Hz, NCH_2), 3.14 (m, 1H), 3.34-3.37 (m, 3H), 3.39-3.60 (m, 4H) 3.73-3.76 (m, 2H), 3.87 (m, 1H), 3.95 (m, 1H), 4.16-4.76 (m, 19H), 4.91-5.10 (m, 4H), 5.39 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1') 5.54-5.57 (m, 2H), 5.95 (m, 1H), 6.03-6.13 (m, 2H), 7.01-7.29 (m, 11H, aromatic), 7.30-8.08 (m, 47H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 25.4 (x2), 26.5 (x2), 51.0 (x2), 55.9, 56.7, 62.6, 63.0, 65.5, 66.8, 67.3, 67.8, 68.6, 69.6, 71.1, 70.6, 72.9, 73.5, 74.6, 74.7, 74.8, 75.5, 75.7, 76.3, 78.3, 78.4, 79.3 (x2), 82.9, 97.4, 98.2, 99.0, 101.1, 123.3,

125.4, 126.0, 127.0, 127.2 (x5), 127.5 (x2), 127.6 (x2), 128.0 (x2), 128.1 (x5), 128.2 (x4), 128.4 (x4), 128.6 (x8), 128.7 (x7), 128.8, 129.0, 129.1 (x2), 129.2 (x4), 129.3, 129.5, 129.9 (x4), 130.0 (x4), 133.3, 133.4, 133.6, 133.7, 134.6, 135.9, 137.9 (x2), 138.0, 138.6, 138.7, 138.8, 165.1, 165.5, 165.6 (x2), 165.8, 166.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{114}H_{105}N_5O_{27}Na$ 1998.6895, found 1998.6915.

4-Azidobutyl *O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.14). Water (350 μL) and trifluoroacetic acid (TFA, 3.5 mL) were added to a stirring mixture of trisaccharide **3.13** (1.80 g, 0.60 mmol) in CH_2Cl_2 (35 mL) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was neutralized with Et_3N (~4 mL), diluted with CH_2Cl_2 (~200 mL), and washed with cold water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and cold water (3 x 20 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford the title compound as a white amorphous solid in 88% yield (0.98 g, 0.52 mmol). Analytical data for **3.14**: R_f = 0.33 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{21}$ -7.20 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.20-1.34 (m, 4H, 2 x CH_2), 2.96 (t, 2H, *J* = 6.8 Hz, NCH_2), 3.10 (m, 1H), 3.24-3.33 (m, 3H), 3.40-3.46 (m, 3H) 3.51-3.54 (m, 2H), 3.64-3.69 (m, 3H), 3.81-3.82 (m, 1H,) 4.04-4.45 (m, 12H), 4.49-4.58 (m, 5H), 4.73-4.94 (m, 4H), 5.09-5.13 (d, 2H, *J* = 12 Hz), 5.29 (d, 1H, *J* = 7.4 Hz) 5.38 (s, 1H), 5.73 (s, 1H), 5.90 (dd, 1H, *J* = 2.8 Hz), 6.08 (dd, 1H, *J* = 9.9 Hz), 6.74-6.76 (m, 3H, aromatic), 6.88-6.97 (m, 8H, aromatic), 7.16-7.67 (m, 33H, aromatic), 7.82-7.84 (m, 5H, aromatic), 7.98-8.01 (m, 4H, aromatic) ppm; ^{13}C NMR (75 MHz,

$CDCl_3$): δ, 50.8, 55.7, 56.5, 62.5, 62.8, 66.6, 67.1, 67.7, 68.2, 68.4, 69.5, 69.9, 70.4, 70.5, 72.7, 73.3, 74.4, 74.5 (x2), 74.6, 75.5, 76.1, 78.3, 78.4, 82.7, 97.2, 98.1, 98.8, 100.9, 123.1, 123.7, 126.9, 127.0 (x2), 127.1, 127.2 (x2), 127.3 (x3), 127.4 (x2), 127.5 (x2), 127.6, 127.8 (x2), 127.9 (x2), 128.0 (x4), 128.1 (x2), 128.2 (x3), 128.3 (x2), 128.4 (x6), 128.5 (x2), 128.6 (x4), 128.9, 129.0, 129.2, 129.6, 129.7 (x2), 129.8 (x4), 129.9 (x2), 131.4, 131.7, 133.2, 133.3, 133.4, 133.5, 133.6, 133.9, 134.1, 137.7, 138.4, 138.5, 138.6, 138.7, 165.4 (x2), 165.6, 166.1, 167.6 (x2), 168.6 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{107}H_{101}N_5O_{27}Na$ 1910.6582, found 1910.6584.

4-Azidobutyl di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3,1→6)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3.15). A mixture of **3.2** (0.365 g, 0.570 mmol), tetrasaccharide **3.14** (0.980 g, 0.519 mmol), and freshly activated molecular sieves (4 Å, 1.50 g) in 1,2-dichloromethane (70 mL) was stirred under argon for 1 h at rt. *N*-Iodosuccinimide (NIS, 0.256 g, 1.14 mmol) and TfOH (10 μL, 0.114 mmol) were added, and the resulting mixture was stirred for 10 min at rt. After that, the solids were filtered off through a pad of Celite and washed successively with CH_2Cl_2. The combined filtrate (~200 mL) was washed with sat. aq. Na_2SO_4 (20 mL) and water (3 x 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford the title compound (0.740 g, 60%) as a white amorphous solid. Analytical data for **3.15**: R_f = 0.51 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{21}$ -1.41 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.23-1.49 (m, 4H, 2 x CH_2), 2.96 (t, 2H, *J* = 6.8 Hz, NCH_2), 3.26-3.47 (m, 8H), 3.61-3.70 (m, 2H), 3.80-3.91 (m, 2H) 4.22-4.51 (m,

10H), 4.53-4.67 (m, 4H), 4.84-4.95 (m, 4H,) 5.13-5.37 (m, 4H), 5.69 (dd, 1H, *J* = 3 Hz), 5.82-5.88 (m, 2H), 5.99-6.02 (m, 3H), 6.62-6.64 (m, 2H, aromatic), 6.73-6.75 (m, 2H, aromatic), 67.22-7.62 (m, 32H, aromatic), 7.73-7.75 (m, 14H, aromatic), 7.80-8.06 (m, 19H, aromatic), ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 14.0, 20.9, 25.1 (x2), 26.2 (x2), 50.6 (x2), 55.5, 56.3, 60.2, 62.4, 62.5, 66.5, 66.7, 67.3, 67.8, 68.0, 68.2, 68.7, 69.3, 69.8, 69.9, 70.0, 70.3, 72.4, 73.0, 74.2 (x2), 73.0, 74.2 (x2), 74.3, 74.4, 74.7,79.3, 82.8, 97.0, 97.8, 97.9, 99.0, 101, 122.9 (x2), 127.3 (x4), 127.5 (x4), 127.6 (x8), 127.8 (x4), 127.9 (x4), 128.0 (x4), 128.1 (x4), 128.2 (x4), 128.3 (x4), 128.4 (x3), 128.7 (x3), 128.8, 128.9, 129.1 (x3), 129.6 (x4), 129.7 (x4), 131.3 (x2), 131.6 (x2), 133.2 (x2), 133.3 (x2), 133.6 (x2), 137.7 (x2), 138.2 (x2), 138.4 (x2), 138.5 (x4), 164.7 (x2), 165.0 (x2), 165.1 (x2), 165.2 (x2), 165.3 (x2), 165.4 (x2), 165.9 (x2), 166.0 (x2), 167.2, 167.4, 168.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{141}H_{127}N_5O_{36}Na$ 2488.8158, found 2488.8135.

3.4.4. Global deprotection

4-Azidobutyl di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3,1→6)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-*O*-benzyl-β-D-glucopyranoside (3.16). A 1 M solution of NaOMe in MeOH (4.0 mL) was added to a solution of **3.15** (0.460 mg, 0.186 mmol) in CH_2Cl_2 (3.8 mL) and MeOH (38 mL) and the resulting mixture was kept for 12 h at rt. DOWEX (H^+) was added until pH = 6, the resin was filtered off and washed with MeOH (7 x 10 mL). The combined filtrate (~ 120 mL) was concentrated under the reduced pressure. The crude residue was dissolved in toluene (6.1 mL) and *n*-BuOH (12 mL), 1,2-ethylenediamine (3.7 mL) was added and the resulting mixture was stirred for 12 h at 90 ºC. The volatiles were removed *in vacuo* and the residue was co-evaporated with toluene (2 x 10 mL). The crude residue

was dissolved in pyridine (10 mL), Ac_2O (5.0 mL) was added and the resulting mixture was kept for 12 h at rt. Methanol (~ 10 mL) was added, the volatiles were evaporated *in vacuo*, and the residue was co-evaporated with toluene (2 x 10 mL). The residue was purified by column chromatography on silica gel (acetone-toluene) to afford compound **3.16** (0.309 g, 90%) as a white amorphous solid. Analytical data for **3.16**: R_f = 0.44 (acetone / toluene, 4/6, v/v); $[\alpha]_D^{21}$ -2.42 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.50-1.56 (m, 4H, 2 x CH_2), 1.75-2.14 (m, 33H, 11 x $COCH_3$), 3.19-3.42 (m, 7H), 3.52-3.86 (m, 13H), 3.94-4.04 (m, 5H) 4.19 (dd, 1H, *J* = 8.2 Hz), 4.32 (dd, 1H, *J* = 15 Hz), 4.40-4.60 (m, 6H), 4.64-4.69 (m, 4H), 4.72-4.97 (m, 3H), 5.10 (m 1H), 5.16-5.30 (m, 6H), 6.40 (d, 1H), 7.13-7.38 (m, 25H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 20.8 (x4), 20.9 (x2), 21.0 (x2), 23.3, 23.6, 25.7, 26.8, 51.3, 55.5, 62.4, 65.6, 66.1, 68.1, 68.5 (x2), 68.6, 68.9, 69.1, 69.4, 69.8, 72.3, 73.0, 73.5, 73.7, 73.9, 74.1, 74.5, 74.7, 74.9, 78.6, 79.6, 97.34, 99.0, 100.1, 100.2, 100.5, 127.3 (x3), 127.5, 127.7 (x3), 127.9, 128.0, 128.1 (x3), 128.2 (x3), 128.3 (x3), 128.4 (x3), 128.5 (x7), 128.6 (x3), 128.8 (x3), 137.9, 138.1, 138.3, 138.7, 138.8, 169.6, 169.7, 169.9, 170.1, 170.2 (x2), 170.4 (x2), 170.5, 170.7, 171.0 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{91}H_{113}N_5O_{35}Na$ 1858.7135, found 1858.7153.

4-Aminobutyl di-*O*-(α-D-mannopyranosyl)-(1→3,1→6)-*O*-(β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (3.1). A three-necked flask equipped with a Dewar-type condenser was cooled to -78 ºC and charged with predried (Na) liquid ammonia (150 mL) and anhydrous THF (15 mL). Sodium (0.250 g, 0.01 mmol) was added and the resulting mixture was stirred for 1 h at -78 ºC. A suspension of pentasaccharide **3.16** (0.300 g, 0.19 mmol) in THF (15 mL) was added and the resulting

mixture was stirred for 6 h at -78 ºC. After that, NH_4Cl (0.30 g) was added and the resulting mixture was allowed to warm to rt. The volatiles were removed by passing a stream of nitrogen (16 h). The residue was dissolved in water, purified by passing through a short column of Sephadex G-10, and lyophilized to provide the title compound (0.105 g, 66%) as a white amorphous solid. Analytical data for **3.1**: R_f = 0.55 (methanol / dichloromethane, 3/7, v/v); $[\alpha]_D^{21}$ +0.46 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.52-1.56 (m, 4H, 2 x CH_2), 1.94, 1.98 (2 s, 6H, 2 x OCH_3), 2.97 (t, 2H, CH_2), 3.41-3.87 (m, 40H), 3.96 (m, 1H), 4.15 (m, 1H), 4.38 (d, 1H), 4.50 (d, 1H), 4.81 (d, 1H), 5.08 (d, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 22.4, 23.0, 22.4, 23.1 (x2), 25.6, 30.7, 40.6, 56.5, 61.4 (x2), 63.0, 63.2, 67.3, 67.9, 68.9, 69.1, 70.1, 71.2, 71.3, 72.0 (x2), 72.4, 73.4, 74.4, 75.1, 76.3, 76.4, 81.2 (x2), 82.5, 92.1, 101.1, 102.0, 102.9 (x2), 104.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{38}H_{67}N_3O_{26}Na$ 1004.3910, found 1004.3929.

3.5. References

1. Moremen, K. W.; Tiemeyer, M.; Nairn, A. V., Vertebrate protein glycosylation: diversity, synthesis and function. *Nat. Rev. Mol Cell. Biol.* **2012,** *13* (7), 448-462.

2. Helenius, A.; Aebi, M., Intracellular functions of N-linked glycans. *Science* **2001,** *291*, 2364-2369.

3. Wang, Z.; Chinoy, Z. S.; Ambre, S. G.; Peng, W.; McBride, R.; de Vries, R. P.; Glushka, J.; Paulson, J. C.; Boons, G. J., A general strategy for the chemoenzymatic synthesis of asymmetrically branched N-glycans. *Science* **2013,** *341* (6144), 379-383.

4. Walczak, M. A.; Hayashida, J.; Danishefsky, S. J., Building biologics by chemical synthesis: practical preparation of di- and triantennary N-linked glycoconjugates. *J. Am. Chem. Soc.* **2013,** *135* (12), 4700-4703.

5. Aussedat, B.; Vohra, Y.; Park, P. K.; Fernandez-Tejada, A.; Alam, S. M.; Dennison, S. M.; Jaeger, F. H.; Anasti, K.; Stewart, S.; Blinn, J. H.; Liao, H. X.; Sodroski, J. G.; Haynes, B. F.; Danishefsky, S. J., Chemical synthesis of highly congested gp120 V1V2 N-glycopeptide antigens for potential HIV-1-directed vaccines. *J. Am. Chem. Soc.* **2013,** *135* (35), 13113-13120.

6. Futakawa, S.; Nara, K.; Miyajima, M.; Kuno, A.; Ito, H.; Kaji, H.; Shirotani, K.; Honda, T.; Tohyama, Y.; Hoshi, K.; Hanzawa, Y.; Kitazume, S.; Imamaki, R.; Furukawa, K.; Tasaki, K.; Arai, H.; Yuasa, T.; Abe, M.; Arai, H.; Narimatsu, H., A unique N-glycan on human transferrin in CSF: a possible biomarker for iNPH. *Neurobiol. Aging* **2012,** *33* (8), 1807-1815.

7. Feizi, T., Glycobiology of aids. In *Carbohydrates in Chemistry and Biology*, Ernst, B.; Hart, G. W.; Sinay, P., Eds. Wiley-VCH: Weinheim, New York, 2000; Vol. 2, pp 851-863.

8. Coss, K. P.; Byrne, J. C.; Coman, D. J.; Adamczyk, B.; Abrahams, J. L.; Saldova, R.; Brown, A. Y.; Walsh, O.; Hendroff, U.; Carolan, C.; Rudd, P. M.; Treacy, E. P., IgG N-glycans as potential biomarkers for determining galactose tolerance in Classical Galactosaemia. *Molec. Genet. Metab.* **2012,** *105* (2), 212-220.

9. Dwek, R. A., Glycobiology: toward understanding the function of sugars. *Chem. Rev.* **1996,** *96*, 683-720.

10. Ogawa, T.; Sugimoto, M.; Kitajima, T.; Sadozai, K. K.; Nukada, T., Total synthesis of a nudecasaccharide: a typical carbohydrate sequence for the complex type of glycan chains of a glycoprotein. *Tetrahedron Lett.* **1986,** *27* (47), 5739-5742.

11. Wu, B.; Hua, Z.; Warren, J. D.; Ranganathan, K.; Wan, Q.; Chen, G.; Tan, Z.; Chen, J.; Endo, A.; Danishefsky, S. J., Synthesis of the fucosylated biantennary N-glycan of erythropoietin *Tetrahedron Lett.* **2006,** *47*, 5577-5579.

12. Geng, X.; Dudkin, V. Y.; Mandal, M.; Danishefsky, S. J., *Angew. Chem. Int. Ed.* **2004,** *43,* 2562-2565.

13. Ratner, D. M.; Plante, O. J.; Seeberger, P. H., A Linear synthesis of branched high-mannose oligosaccharides from the HIV-1 viral surface envelope glycoprotein gp120. *Eur. J. Org. Chem.* **2002,** *2002* (5), 826-833.

14. Ratner, D. M.; Swanson, E. R.; Seeberger, P. H., Automated Synthesis of a Protected N-Linked Glycoprotein Core Pentasaccharide. *Org. Lett.* **2003,** *5*, 4717-4720.

15. Li, L.; Liu, Y.; Ma, C.; Qu, J.; Calderon, A. D.; Wu, B.; Wei, N.; Wang, X.; Guo, Y.; Xiao, Z.; Song, J.; Sugiarto, G.; Li, Y.; Yu, H.; Chen, X.; Wang, P. G., Efficient chemoenzymatic synthesis of an N-glycan isomer library. *Chem. Sci.* **2015,** *6*, 5652–5661.

16. Ito, Y.; Ohnishi, Y., Oligosaccharides: Synthesis: Stereoselective synthesis of b-manno glycosides. In *Glycoscience: Chemistry and Chemical Biology*, Fraser-Reid, B.; Tatsuta, K.; Thiem, J., Eds. Springer: Berlin - Heidelberg - New York, 2001; Vol. 2, pp 1589-1620.

17. Matsuo, I.; Kashiwagi, T.; Totani, K.; Ito, Y., First chemical synthesis of triglucosylated tetradecasaccharide (Glc3Man9GlcNAc2), a common precursor of asparagine-linked oligosaccharides. *Tetrahedron Lett.* **2005,** *46*, 4197-4200.

18. Matsuo, I.; Totani, K.; Tatami, A.; Ito, Y., Comprehensive synthesis of ER related high-mannose-type sugar chains by convergent strategy. *Tetrahedron* **2006,** *62*, 8262-8277.

19. Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A., Antibody Domain

Exchange Is an Immunological Solution to Carbohydrate Cluster Recognition. *Science* **2003,** *300* (5628), 2065-2071.

20. Gridley, J. J.; Osborn, H. M. I., Recent advances in the construction of b-D-mannose and b-D-mannosamine linkages. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1471-1491.

21. Crich, D., Chemistry of glycosyl triflates: Synthesis of b-mannopyranosides (Reprinted from Glycochemistry: Principles, Synthesis, and Applications, pg 53-75, 2001). *J. Carbohydr. Chem.* **2002,** *21* (7-9), 667-690.

22. Crich, D., Methodology Development and Physical Organic Chemistry: A Powerful Combination for the Advancement of Glycochemistry. *J. Org. Chem.* **2011,** *76*, 9193-9209.

23. Crich, D., Mechanism of a chemical glycosylation reaction. *Acc. Chem. Res.* **2010,** *43*, 1144-1153.

24. Crich, D.; Sharma, I., Is Donor−Acceptor Hydrogen Bonding Necessary for 4,6-O-Benzylidene-directed β-Mannopyranosylation? Stereoselective Synthesis of β-C-Mannopyranosides and α-C-Glucopyranosides. *Org. Lett.* **2008,** *10*, 4731-4734.

25. Crich, D.; Wu, B.; Jayalath, P., Convergent synthesis of a b-(1--3)-mannohexaose. *J. Org. Chem.* **2007,** *72*, 6806-6815.

26. Crich, D.; Banerjee, A.; Yao, Q., Direct chemical synthesis of the b-D-mannans: the b-(1-2) and b-(1-4) series. *J. Am. Chem. Soc.* **2004,** *126*, 14930-14934.

27. Crich, D.; Sun, S., Direct formation of b-mannopyranosides and other hindered glycosides from thioglycosides. *J. Am. Chem. Soc.* **1998,** *120*, 435-436.

28. Crich, D.; Sun, S., Formation of b-mannopyranosides of primary alcohols using the sulfoxide method. *J. Org. Chem.* **1996,** *61*, 4506-4507.

29. Baek, J. Y.; Choi, T. J.; Jeon, H. B.; Kim, K. S., A highly reactive and stereoselective b-mannosylation system: mannosyl 4-pentenoate/PhSeOTf. *Angew. Chem. Int. Ed.* **2006,** *45*, 7436-7440.

30. El Ashry, E. S. H.; Rashed, N.; Ibrahim, E. S. I., Strategies of synthetic methodologies for constructing b-mannosidic linkage. *Curr. Org. Synth.* **2005,** *2* (2), 175-213.

31. De Meo, C.; Kamat, M. N.; Demchenko, A. V., Remote participation-assisted synthesis of b-mannosides. *Eur. J. Org. Chem.* **2005**, 706-711.

32. Pistorio, S. G.; Yasomanee, J. P.; Demchenko, A. V., Hydrogen bond-mediated aglycone delivery: focus on β-mannosylation. *Org. Lett.* **2014,** *16*, 716-719.

33. Yasomanee, J. P.; Demchenko, A. V., The effect of remote picolinyl and picoloyl substituents on the stereoselectivity of chemical glycosylation. *J. Am. Chem. Soc.* **2012,** *134*, 20097-20102.

34. Yasomanee, J. P.; Demchenko, A. V., Hydrogen bond-mediated aglycone delivery: the synthesis of linear and branched α-glucans. *Angew. Chem. Int. Ed.* **2014,** *53*, 10453–10456.

35. Yasomanee, J. P.; Demchenko, A. V., Hydrogen bond-mediated aglycone delivery (HAD): a highly stereoselective synthesis of 1,2-cis α-D-glucosides from common glycosyl donors in the presence of bromine. *Chem. Eur. J.* **2015,** *21*, 6572-6581.

36. Nagorny, P.; Fasching, B.; Li, X.; Chen, G.; Aussedat, B.; Danishefsky, S. J., Toward Fully Synthetic Homogeneous β-Human Follicle-Stimulating Hormone (β-hFSH) with a Biantennary N-Linked Dodecasaccharide. Synthesis of β-hFSH with Chitobiose Units at the Natural Linkage Sites. *J. Am. Chem. Soc.* **2009,** *131*, 5792-5799.

37. Sail, D.; Kovac, P., Benzoylated ethyl 1-thioglycosides: direct preparation from per-O-benzoylated sugars. *Carbohydr. Res.* **2012,** *357*, 47-52.

38. Crich, D.; Jayalath, P.; Hutton, T. K., Enhanced diastereoselectivity in B-mannopyranosylation through the use of sterically minimal propargyl ether protecting groups. *J. Org. Chem.* **2006,** *71*, 3064-3070.

39. Adero, P. O.; Furukawa, T.; Huang, M.; Mukherjee, D.; Retailleau, P.; Bohe, L.; Crich, D., Cation Clock Reactions for the Determination of Relative Reaction Kinetics in Glycosylation Reactions: Applications to Gluco- and Mannopyranosyl Sulfoxide and Trichloroacetimidate Type Donors. *J. Am. Chem. Soc.* **2015,** *137* (32), 10336-10345.

40. Alewood, P. F.; Benn, M.; Reinfried, R., Cyclizations of Azidoformates to Tetrahydro-1,3-oxazin-2-ones and Oxazolidin-2-ones. *Can. J. Chem.* **1974,** *52*, 4083-4089.

CHAPTER 4

HPLC-assisted automated oligosaccharide synthesis: the implementation of the autosampler as a mode of the reagent delivery

S. G. Pistorio, S. S Nigudkar, K. J. Stine, A. V. Demchenko. HPLC-assisted automated oligosaccharide synthesis: the implementation of the autosampler as a mode of the reagent delivery. *The Journal of Organic Chemistry* **2016,** *81,* 8796-8805

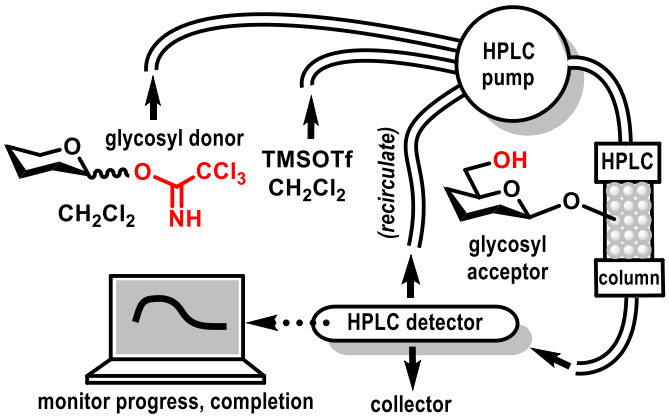
4.1 Introduction

Glycans are oligomeric carbohydrates wherein monomers are connected via the glycosidic linkage. This linkage is obtained by a glycosylation reaction, which remains challenging to synthetic chemists due to the requirement to achieve high stereocontrol[1] and yields by suppressing side reactions.[2] Beyond that, glycan synthesis may require further manipulations between each glycosylation step. Due to significant advances, the chemical synthesis of many glycans can now be streamlined by using expeditious strategies.[3] Solid-phase synthesis,[4] which eliminates the need for purifying intermediates and simplifies the removal of excess reagents, has been widely used in the preparation of peptides[5] and oligonucleotides.[6] Since 1971, solid-phase synthesis has been used for the preparation of oligosaccharides;[7] and in 2001 Seeberger et al. reported the first automated oligosaccharide synthesis using a modified peptide synthesizer.[8] In 2012, Seeberger reported "the first fully automated solid-phase oligosaccharide synthesizer," initially in its experimental form;[9] and in 2013 it was marketed as Glyconeer 2.1. Approaches by Wong,[10] Takahashi,[11] Chen,[12] Pohl,[13] Wang,[14] and Nokami[15] are based on the automation of chemical, enzymatic, or chemoenzymatic syntheses in solution with or without using tags.[16]

In light of recent progress made in the areas of glycobiology[17] and glycomics[18] "*widely applicable methods to generate both large and small quantities of glycans are needed*."[19] Oligosaccharides can be obtained by isolation/release from natural sources, or prepared enzymatically and/or chemically. All three approaches are viable, each offering certain advantages, but none can significantly outperform the others. Oligosaccharide synthesis in solution requires a significant deal of know-how. The automated platform for solid-phase synthesis developed by Seeberger introduces an idea of operational simplicity and highlights that the development of accessible

methods for glycan production is essential for further innovations and practical applications in all areas of glycosciences.

Scheme 4.1. The original set-up for HPLC-assisted synthesis.[20]



The development of the automated synthesizer in our labs began with the introduction of the Surface-Tethered Iterative Carbohydrate Synthesis (STICS).[21] The basis for this concept is a surface-functionalized stack of nanoporous gold plates that simplifies the transfer of the gold surface-bound molecules between reaction vessels. At the end of the synthesis, the resulting glycan can be either cleaved-off for further processing or deprotected directly on the gold surface to be used for recognition studies or immunoassay development.[22] The STICS concept was developed with robotic arm automation in mind. However, we discovered that standard HPLC equipment would offer a more accessible platform for automation. This approach was discovered with nanoporous gold,[23] but we have also investigated more traditional polymer supports. Using the acceptor-bound approach,[24] preloaded Tentagel® resin was packed in the Omnifit® column and integrated into the HPLC system (Scheme 4.1).[20] All steps were automated using a three-headed HPLC pump and the reagent consumption was monitored using a standard UV detector. Reagents were recirculated, but still 10 equiv. of trichloroacetimidate donors were used for each

glycosylation.[20] More recently, Pentelute (and co-workers) investigated the HPLC-assisted synthesis of peptides.[25] Other exciting developments in the area of high throughput and automated syntheses have been paticularly inspiring to our own research endeavors.[26]

4.2 Results and Discussion

Presented herein is the development of a broadly useful technology for simple, scalable, and transformative automation of solid-phase synthesis that does not rely on specialized equipment. Broadly available and used in most labs, the setup of the HPLC equipment requires no investment. This platform allows for real-time UV detector monitoring of all steps including glycosylation, which, in turn, helps reduce the reaction time and the amount of reagents and solvents needed. The use of a computer interface and standard HPLC liquid handling equipment and software will allow recording a successful automated sequence as a computer program that can then be reproduced by both specialists and non-specialists with a “press of a button”. While this approach has a potential to revolutionize the way the automation is conducted, solid-phase synthesis suffers from many inherent limitations. Practically every aspect of solid-phase synthesis needs to be refined. Along with the introduction of the autosampler for the reagent delivery, this article is also dedicated to the refinement of some basic aspects of this methodology. Our new basic set-up is using standard Agilent 1260 Infinity series HPLC system equipped with a quad pump, a UV detector and a autosampler.

4.2.1 Selection of resins, spacers and linkers

Our preliminary work on the HPLC-assisted synthesis was solely based on Tentagel resin.[20] Previously, we compared Tentagel vs. Merrifield resins using manual approach, but saw no significant difference in efficiency and yields.[24] A recent comparative study by Seeberger et al. determined that the Merrifield resin gives the best efficiency in application to their automation platform.[27] To gain a better understanding of how loading, swelling, mechanical robustness, size, and other factors may affect the HPLC-assisted synthesis we performed a side-by-side comparison study of Merrifield, Wang,[28] and JandaJel,[29] all of which have been found to be excellent supports for oligosaccharide synthesis and have loading capacities up to 1.0 mmol g^{-1}. Although identifying the best support for universal application might be simply impossible, in a series of comparative experiments we identified JandaJel as the most suitable resin for HPLC-mediated synthesis in terms of loading, reaction times and yields.

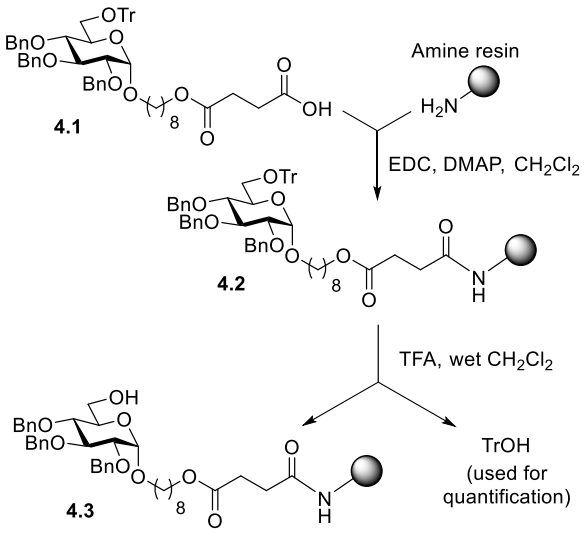
It has become common knowledge that the type of the spacer and/or linker between the acceptor and the polymer support may be of critical importance.[30] Factors to consider are the chemical composition, stability towards various experimental conditions, and selective (mild) conditions for its cleavage. In our preliminary study, we were using a C4 spacer in combination with succinoyl linker that worked well, and the cleavage was reliably achieved using a small amount (~2 mL) of a recirculating 0.1 M solution of NaOMe in MeOH-CH_2Cl_2. With the general anticipation that extension of the spacer length could move the glycosyl acceptor further out into solution and enhance the efficiency of the reaction with the solution-based glycosyl donor we performed a comparative study. In our study of glycosylations using nanoporous gold, we obtained better yields with the acceptor equipped with longer

C8-O-C8 spacer than those of acceptors with shorter C4 or C8 spacers.[23] With the use of polymer beads we report that while the C8-O-C8 spacer helps to enhance the yields obtained with the C4 spacer, it practically offers no advantage over the more synthetically accessible C8 spacer. Hence, all syntheses described in the article used the C8 spacer.

4.2.2 Loading practices and quantification

The resin loading capacity is important, but over-crowding of the reactive sites may prevent further elongation, particularly in case of sterically demanding and branched oligosaccharides. During our exploratory study with JandaGel and Tentagel resins, it was observed that the desired loading capacities could be achieved much faster using HPLC-based reagent delivery rather than the manual loading in a flask. Nevertheless, large-scale resin preloading (2-10 g) for this study was performed by the manual approach using the flask and the shaker as depicted in Scheme 4.2. Building block **4.1** was coupled with amine JandaJel resin in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP). The loading can be confirmed by weighing the unloaded versus loaded resin, as well as cleaving and quantifying of the loaded acceptor if so desired. The preloaded JandaJel resin **4.2** was then subjected to detritylation with 10% trifluoroacetic acid in wet CH_2Cl_2. The detritylation results in the formation of glycosyl acceptor **4.3**, but is also releases triphenylcarbinol (TrOH), which could be used for the initial quantification of the loading by its isolation by evaporation and weighing. Quantification of TrOH is the key step for determining of the loading capacity of the resin.

Scheme 4.2. The synthesis of the solid-phase-bound acceptor 4.3.



4.2.3 Glycosylation: reagent delivery, recirculation, monitoring, and synthetic methods

Glycosylation is a complex multi-step process, and reactions on solid supports bring additional hurdles related to the mismatch between highly reactive solution-based vs. unreactive solid-phase-based reactants. This mismatch is typically addressed by using a large excess (5-10 equiv.) of the solution-based reactant, most commonly the donor, and repeating the reaction 2-3 times to ensure that all solid-supported acceptor is consumed.[7e] Automation offers some operational simplicity to oligosaccharide synthesis, but the entire concept may suffer from the inherited drawbacks of conventional methods.

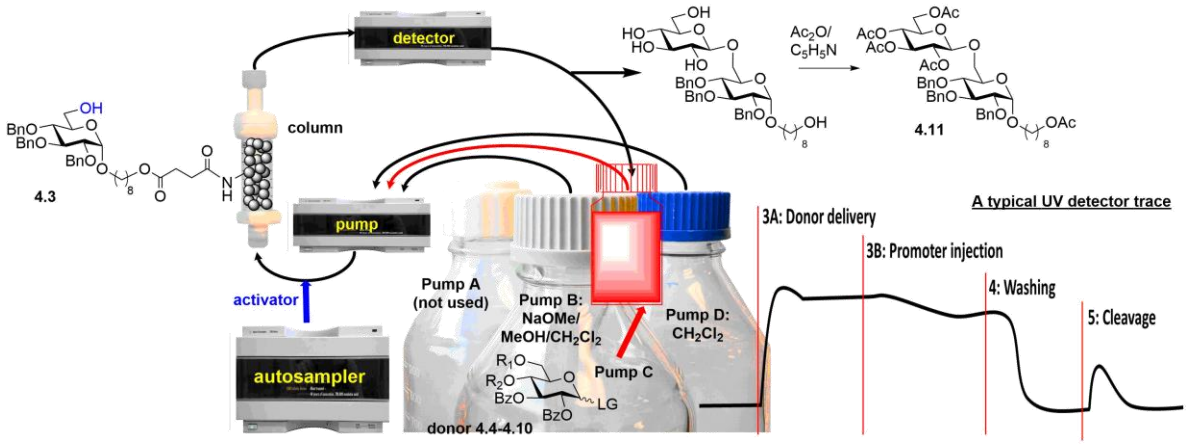
Our experience with HPLC-assisted reactions is still limited, but we already established the protocol for separate delivery of solutions of glycosyl donor and promoter using HPLC pumps.[20] The primary focus of the earlier study was to determine ranges of the variables, beginning with reagent ratios, concentration, velocity, and pressure. The reaction efficiency is likely to improve with increased speed of the reagent delivery. However, this may have potential downfalls if not

properly addressed. If the reagents are delivered too fast, the internal column pressure may increase to a point where the resin beads collapse or fracture.[4b] We have not observed this at our operating velocity of 0.5-2.0 mL/min (1-12 bar).

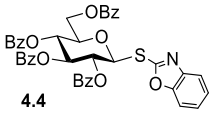
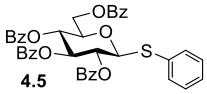
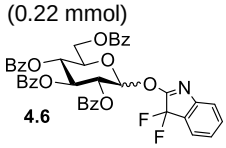
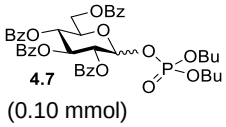
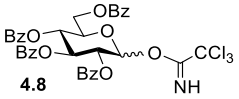
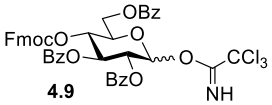
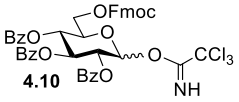
The initial reagent delivery via HPLC pump offered a notable limitation of our platform in comparison to Seeberger's automated synthesizer that has 32 intake lines.[9] In principle, essentially the same capability can be achieved with the HPLC setup by splitting of the pump intake lines with eight-way split valves. However, as further steps towards complete automation, we envisaged the use of a standard HPLC autosampler. Autosamplers are abundant, cheap, easily fit into the HPLC-automation concept. This approach opens access to hundreds of intake/delivery lines. This approach allows us to liberate other pump intake lines for the delivery of solvent for reactions, washing, and deprotection because only one line is now used for the donor delivery and recirculation. It should be mentioned that the recirculation has already been previously optimized with the purpose of addressing the main drawback of all solid-phase syntheses: the requirement for a large excess of solution-based reagents.

The outline of the automation set-up, program sequence, and the key results for basic glycosylation reactions are depicted in Scheme 4.3. JandaJel resin (50 mg) functionalized with glycosyl acceptor **4.3** (0.022 mmol) was packed in an Omnifit® glass chromatography column. The column was connected to the standard Agilent Infinity 1260 HPLC system and the automation sequence was programmed as follows. Pump D was programmed to deliver CH_2Cl_2 at a flow rate of 1.0 mL/min. After discarding the first ~5 mL of the eluate (washing, step 1, Scheme 4.3) the system was switched to the recirculation mode and 2 mL of CH_2Cl_2 was recirculated for 30 min at a flow rate of 1.0 mL/min (swelling, step 2). After that, pump C was programmed to deliver

Scheme 4.3. Refinement of the glycosylation-cleavage sequence for the synthesis of disaccharide 4.11.



Step	Operation	Mode	Flow rate	Total volume	Time
1	Washing of the resin with CH_2Cl_2 (50 mg, 0.022 mmol)	Pump D	1.0 mL/min	5.0 mL	5 min
2	Swelling resin with CH_2Cl_2	Pump D	1.0 mL/min	2.0 mL (recirculation)	30 min
3A	Glycosylation, delivery of the donor (**4.4-4.10**, 4.4 equiv., 0.10 mmol or 10 equiv., 0.22 mmol, see the table below) in CH_2Cl_2	Pump C	0.5 mL/min	2.0 mL (recirculation)	10 min
3B	Glycosylation, injection of the promoter (see the table below) at 10, 12, 14 min time points	Autosampler		3 x 100 µL	60-90 min
4	Washing with CH_2Cl_2	Pump D	1.0 mL/min	10 mL	10 min
5	Cleavage with 0.1 N NaOMe/MeOH/ CH_2Cl_2 to release the disaccharide	Pump B	1.0 mL/min	10 mL (recirculation)	20 min

Entry	Donor	Promoter	Yield of **4.11**
1	 **4.4** (0.10 mmol)	AgOTf	50%
2	 **4.5** (0.22 mmol)	NIS/TfOH	57%
3	 **4.6** (0.10 mmol)	TMSOTf	73%
4	 **4.7** (0.10 mmol)	TMSOTf	75%
5	 **4.8** (0.10 mmol)	TMSOTf	85%
6	 **4.9** (0.10 mmol)	TMSOTf	89%
7	 **4.10** (0.10 mmol) (0.22 mmol)	TMSOTf	76% 95%

a solution of the glycosyl donor (0.10 mmol) in CH_2Cl_2 (2 mL) at 0.5 mL/min and the system was left recirculating for 10 min (step 3A). Beginning from this stage the synthesis was monitored using the integrated UV detector set at 254 nm. A typical trace is shown in Scheme 4.3.

The integrated autosampler was then programmed to inject a solution of promoter (40 μL) in CH_2Cl_2 (3 injections of 100 μL) at 10, 12, and 14 min after the initial delivery of the donor (step 3B). The system was left recirculating for 60-90 min, and the reaction was monitored by the UV detector in real-time. When the detector trace reaches the plateau, no change in the absorbance of the recirculating solution, the reaction is stopped. In principle, low-efficiency reactions can be supplemented with fresh reagents/reactants at this time. After a typical reaction time of 60-90 min, the system was switched to pump D and washed with CH_2Cl_2 (1.0 mL/min flow rate) to remove excess reagents (step 4). The eluate from the washing step (~10 mL) total is discarded. Again, this step was monitored by the UV detector, and the washing was typically stopped after ~10 min when the detector trace reached the base line corresponding to pure CH_2Cl_2.

To affect the product cleavage from the solid support, pump B was then programmed to deliver a solution of NaOMe/CH_2Cl_2/MeOH (0.04/1/1, v/v/v) at a flow rate of 1.0 mL/min for 10 min (step 5). This step was also monitored by the UV detector. Typically, the cleavage is completed at this stage and the use of the detector monitoring is discontinued. The resulting mixture was recirculated for an additional ~10 min. The eluate was collected, neutralized, concentrated under the reduced pressure and the residue was acetylated with Ac_2O in pyridine to afford disaccharide **4.11**. The purification of **4.11**

was achieved by conventional column chromatography and its identity was proven by traditional spectral methods.

Our initial study of the HPLC-assisted synthesis[20] was exclusively based on trichloroacetimidates[31] as glycosyl donors. In an attempt to broaden the scope of this methodology, we performed a comparative study of other common and novel leaving groups. Thioglycosides are generally much less reactive than O-imidates and hence considered less desirable for polymer-supported synthesis. With some prior success of using thioglycosides in glycosylations using polymer[24] and nanoporous supports[21] we investigated the S-benzoxazolyl (SBox) donor **4.4**[32] and the S-phenyl glycosyl donor **4.5**[33] in the HPLC-automated reactions. Glycosylation of SBox donor **4.4** with resin-bound acceptor **4.3** was performed in the presence of AgOTf. Following the general programming described above, disaccharide **4.11** was obtained in a good yield of 50% (Scheme 4.3, entry 1). A similar result was achieved with SPh donor **4.5**, wherein NIS/TfOH promoted reaction afforded disaccharide **4.11** in 57% yield (entry 2). While the outcome of these reactions could be improved by injecting additional quantities of reagents, we chose to explore other classes of glycosyl donors.

Recently, we developed a new class of glycosyl donors, O-benzoxazolyl (OBox) imidates, which were also tested in the HPLC-based applications, but could not outperform traditional trichloroacetimidates.[34] We also introduced 3,3-difluoro-3*H*indol-2-yl (OFox) imidates,[35] which showed a very high reactivity and allowed us to obtain impressive results in the HPLC-based application. Thus, glycosylation of OFox donor **4.6** with resin-bound acceptor **4.3** was performed in the presence of TMSOTf. Following the general programming, disaccharide **4.11** was obtained in a good yield of 73% (entry 3). A

very similar outcome was obtained with phosphate donor **4.7**, a glycosylation approach frequently used in Seeberger's automation method.[36] The phosphate donor **4.7** also provided a very impressive result in our HPLC-based platform wherein TMSOTf-promoted activation led to disaccharide **4.11** in 75% yield (entry 4). Nevertheless, the most consistent result and the highest yield was obtained with trichloroacetimidate **4.8**.[37] TMSOTf-promoted activation led to disaccharide **4.11** in an excellent yield of 85% using only 4.4 equiv of the donor (entry 5). In order to expand this procedure to selectively protected imidates we investigated donors **4.9** and **4.10**[20] containing a selectively removable Fmoc protecting group at C-4 and C-6, respectively. TMSOTf promoted glycosylations afforded disaccharide **4.11** in 89 and 76% yields, respectively. The latter yield could be increased to 95% by using 10 equiv of donor **4.10**.

4.2.4 Fmoc deprotection and reiteration for the synthesis of oligosaccharides

Having optimized conditions for glycosylation we decided to undertake the synthesis of two linear oligosaccharides **4.12** and **4.14**. General programming outline is presented in Scheme 4.4. For the synthesis of trisaccharide **4.12** we selected glycosyl donor **4.10** equipped with the selectively removable Fmoc group at C-6. Previously, we have shown that Fmoc can be removed using mild reagents (piperidine/DMF, 2-5 min or TEA/CH_2Cl_2 10-20 min using a HPLC set-up) and also provides a very straightforward and informative mode for monitoring the deprotection step and quantification of the glycosylation.[20] To gain better yields and minimize side reactions, we decided to use a larger excess of donor **4.10** (10 equiv). After washing and swelling of the resin containing the acceptor **4.3** (0.022 mmol, pump D, steps 1 and 2, Scheme 4.4), pump C was

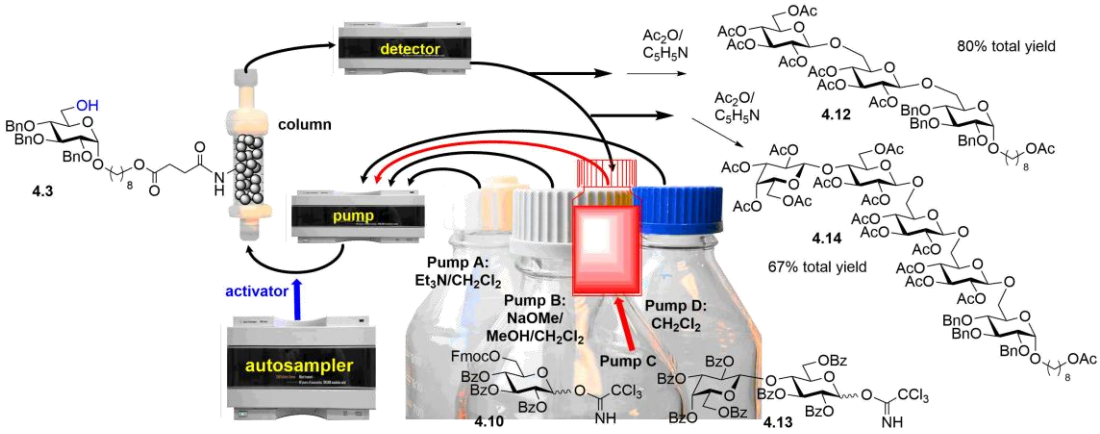
programmed to deliver donor **4.10** (0.22 mmol) in CH_2Cl_2 (2 mL total volume) at 0.5 mL/min, which was then recirculated for 10 min. Again, all automated sequence steps have been monitored with the UV detector. The autosampler was programmed to deliver a solution of promoter in CH_2Cl_2 (3 injections of 100 μL each) and the resulting reaction mixture was recirculated for 60-90 min. When the UV-monitoring showed no change in absorbance of the eluate passing through the detector, the system was washed with CH_2Cl_2 (pump D, 1.0 mL/min rate flow for 10 min).

A capping step in the synthetic cycle is important because it prevents the accumulation of shorter oligosaccharides due to incomplete reactions. Capping can be as simple as acetylation with Ac_2O in pyridine,[38] or by using benzoyl isocyanate in CH_2Cl_2, a procedure developed by Schmidt.[39] It should be mentioned that due to high yields achieved in glycosylations of reactive primary hydroxyls with trichloroacetimidates, capping was found unnecessary. To affect the deprotection of the Fmoc group, pump A was programmed to deliver a solution of triethylamine/CH_2Cl_2 (1/1, v/v 1.0 mL/min flow rate).

The release of the dibenzofulvene-triethylamine adduct was monitored by using the UV detector set at 312 nm. Upon reaching the base line indicating that dibenzofulvene-triethylamine is no longer produced (20 min/20 mL total volume for step 5), the pump D was engaged for washing (step 6, 10 min). The resulting solid-phase bound disaccharide acceptor was glycosylated with donor **4.10** following essentially the same programming sequence as that for the first cycle. Upon completion of the glycosylation and washing (steps 7 and 8) pump B was engaged to deliver a solution of NaOMe/CH_2Cl_2/MeOH (0.04/1/1, v/v/v) at the flow rate of 1.0 mL/min for 10 min to

remove the resulting trisaccharide (step 13). The eluate was collected, neutralized, concentrated and the residue was acetylated with Ac_2O in pyridine to afford trisaccharide **4.12** in 80% yield.

Scheme 4.4. Automation of glycosylation-deprotection-cleavage sequences for the synthesis of oligosaccharides 4.12 and 4.14.



Step	Operation	Mode	Flow rate	Total volume	Time
1	Washing of the resin with CH_2Cl_2 (50 mg, 0.022 mmol)	Pump D	1.0 mL/min	5.0 mL	5 min
2	Swelling resin with CH_2Cl_2	Pump D	1.0 mL/min	2.0 mL (recirculation)	30 min
3	Glycosylation, delivery of **4.10** (10 equiv, 0.22 mmol) in CH_2Cl_2 Injection of TMSOTf of 10, 12 and 14 min	Pump C Autosampler	0.5 mL/min	2.0 mL (recirculation) 3 x 100 µL	60-90 min
4	Washing with CH_2Cl_2	Pump D	1.0 mL/min	10 mL	10 min
5	Fmoc deprotection, delivery of Et_3N/CH_2Cl_2	Pump A	1.0 mL/min	20 mL	20 min
6	Washing with CH_2Cl_2	Pump D	1.0 mL/min	10 mL	10 min
7	Glycosylation, delivery of **4.10** (10 equiv, 0.22 mmol) in CH_2Cl_2 Injection of TMSOTf at 10, 12, and 14 min	Pump C Autosampler	0.5 mL/min	2.0 mL (recirculation) 3 x 100 µL	60-90 min
8	Washing with CH_2Cl_2	Pump D	1.0 mL/min	10 mL	10 min
	*Synthesis of **4.12** go to step 13, Synthesis of **4.14** continue to step 9*				
9	Fmoc deprotection, delivery of Et_3N/CH_2Cl_2	Pump A	1.0 mL/min	20 mL	20 min
10	Washing with CH_2Cl_2	Pump D	1.0 mL/min	10 mL	10 min
11	Glycosylation, delivery of **4.13** (10 equiv 0.22 mmol) in CH_2Cl_2 Injection of TMSOTf at 10, 12, 14 min time points	Pump C Autosampler	0.5 mL/min	2.0 mL (recirculation) 3 x 100 µL	60-90 min
12	Washing with CH_2Cl_2	Pump D	1.0 mL/min	10 mL	10 min
13	Cleavage with 0.1 N NaOMe/MeOH/CH_2Cl_2 to release the oligosaccharide	Pump B	1.0 mL/min	10 mL recirculation)	20 min

To investigate whether the trisaccharide sequence achieved during the synthesis of **4.12** could be extended further we explored a possibility for the chain elongation. For this purpose, we repeated the same steps 1-8 as those described for the synthesis of **4.12**. It should be noted that in this case a completely automated sequence was reproduced simply by using the same program as previously. The solid phase bound trisaccharide intermediated was subjected to Fmoc deprotection (step 9) and washing (step 10). The subsequent glycosylation step was performed using lactosyl donor **4.13**[40] with the main aim of determining the scope of using larger building blocks (step 11). The glycosylation with disaccharide donor **4.13** was performed following essentially the same programming sequence as that for other glycosylations described in this article. Upon completion of the glycosylation and washing (steps 11 and 12), pump B was engaged to deliver a solution of NaOMe/CH_2Cl_2/MeOH (0.04/1/1, v/v/v) at the flow rate of 1.0 mL/min for 10 min followed by recirculation for additional 10 min to remove the resulting pentasaccharide (step 13). The eluate was collected, neutralized, concentrated and the residue was acetylated with Ac_2O in pyridine to afford pentasaccharide **4.14** in 67% yield over-all.

4.3 Conclusions

In conclusion, we optimized the synthetic and operational strategies for HPLC-based automation, and have created a generally useful tool for accelerating glycan synthesis. This automated technology offering a transformative, semi-automated approach to synthesis. Automated HPLC-based synthesis introduces rather sophisticated yet affordable in-situ monitoring and reagent recirculation concepts. This basic approach provided a solid basis for the implementation of a standard autosampler system for the

fully automated delivery of reagents. Further optimization of HPLC technology and its application using different resin, spacers, linkers is currently underway. Our efforts are also focusing on developing efficient protocols for the synthesis of branched heterooligosaccharides as well as using the autosampler for delivering all sugar building blocks and deprotecting reagents necessary for the synthesis.

4.4 Experimental

4.4.1 General methods

The reactions were performed using commercial reagents and ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 ^{o}C. CH_2Cl_2 and $ClCH_2CH_2Cl$ were distilled from CaH_2 directly prior to application. Pyridine and acetonitrile were dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Molecular sieves (4Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. DOWEX MONOSPHERE 650C (H^+) was washed three times with MeOH and stored under MeOH. Optical rotations were measured using a polarimeter. ^{1}H NMR spectra were recorded at 300 or 600 MHz , ^{13}C NMR spectra were recorded at 75 MHz or 150 MHz. The ^{1}H chemical shifts are referenced to the signal of residual $CHCl_3$ (δ_H = 7.24 ppm). The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.23 ppm). HRMS

determinations were performed with the use of a mass spectrometer with FAB ionization and ion-trap detection. Agilent 1260 infinity HPLC System and an Agilent 1260 Variable Wavelength UV-vis Detector were used to assemble the automated synthesizer.

4.4.2 Synthesis of Glycosyl Acceptor 4.3

8-(*tert*-Butyldiphenylsilyloxy)oct-1-yl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (4.17). A mixture of ethyl 2,3,4-tri-*O*-benzyl-1-thio-6-*O*-triphenylmethyl-α-D-glucopyranoside (**4.15**,[41] 3.0 g, 4 mmol), 8-(*tert*-butyldiphenylsilyloxy)octan-1-ol (**4.16**,[42] 1.3 g, 3.3 mmol), and freshly activated molecular sieves (4 Å, 3.0 g) in diethyl ether (100 mL) was stirred under argon for 1 h at rt. *N*-Iodosuccinimide (NIS, 1.8 g, 8.0 mmol) and TfOH (71 μL, 0.8 mmol) were added, and the resulting mixture was stirred for 20 min at rt. After that, the solids were filtered off and washed successively with CH_2Cl_2. The combined filtrate (~200 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford compound **4.17** (2.27 g, 65%) as a colorless foam. Analytical data for **4.17**: R_f = 0.62 (ethyl acetate / hexanes, 1/4, v/v); $[\alpha]_D^{25}$ +25.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.89 (s, 9H, $C(CH_3)_3$), 1.12-1.20 (m, 8H, 4 x CH_2), 1.38, 1.50 (2 m, 4H, 2 x CH_2), 3.03 (dd, 1H, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6a), 3.31-3.32 (m, 2H, H-6b, OCH_2^a), 3.42-3.50 (m, 4H, H-2, 4, CH_2), 3.56 (m, 1H, OCH_2^b), 3.68 (m, 1H, H-5), 3.82 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 4.13 (d, 1H, ^2J = 10.4 Hz, ½ CH_2Ph), 4.52-4.72 (m, 5H, H-1, 2 x CH_2Ph), 4.81 (d, 1H, ^2J = 10.6 Hz, ½ CH_2Ph), 6.69-7.52 (m, 40H, aromatic) ppm; ^{13}C NMR (75 MHz,

$CDCl_3$): δ, 19.4, 25.9 (x3), 26.4, 29.5 (x2), 29.6 (x4), 30.2, 32.7 (x2), 63.8, 64.2, 70.5, 73.3, 75.2, 76.1, 78.5, 80.6, 82.5, 86.4, 96.7, 126.9 (x2), 127.1, 127.7 (x6), 127.8 (x4), 127.9 (x3), 128.1, 128.3 (x2), 128.4, 128.6 (x3), 128.9 (x4), 129.0 (x3), 129.6 (x3), 134.3, 135.7 (x6), 138.1, 138.7, 139.0, 144.1, 144.7 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{70}H_{78}O_7SiNa$ 1081.5415, found 1081.5435.

8-Hydroxyoct-1-yl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (4.18). Tetrabutylammonium fluoride (TBAF, 1.72 mL, 1.995 mmol) was added to a solution of **4.17** (2.0 g, 1.995 mmol) in THF (14 mL) and the resulting mixture was stirred under argon for 3 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~150 mL), washed with water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and water (20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford compound **4.18** (1.68 g, 95%) as a colorless foam. Analytical data for **4.18**: R_f = 0.45 (ethyl acetate / hexanes, 1/2, v/v); $[\alpha]_D^{26}$ +19.7 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.21-1.29 (m, 8H, 4 x CH_2), 1.43, 1.61 (2 m, 4H, 2 x CH_2), 3.17 (dd, 1H, $J_{5,6a}$ = 3.0 Hz, $J_{6a,6b}$ = 9.8 Hz, H-6a), 3.45-3.61 (m, 6H, H-2, 4, 6b, OCH_2^a, OCH_2), 3.66 (m, 1H, OCH_2^b), 3.83 (m, 1H, H-5), 3,96 (dd, 1H, $J_{3,4}$ = 10.2 Hz, H-3), 4.27 (d, 1H, ^2J = 10.4 Hz, ½ CH_2Ph), 4.67 (d, 1H, ^2J = 10.3 Hz, ½ CH_2Ph), 4.69 (d, 1H, ^2J = 12.1 Hz, ½ CH_2Ph), 4.76-4.86 (m, 3H, H-1, CH_2Ph), 4.95 (d, 1H, ^2J = 10.6 Hz, ½ CH_2Ph), 6.83-7.46 (m, 30H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 25.8, 26.3, 29.4, 29.5, 29.6, 32.9, 62.8, 63.1, 68.1, 70.5, 73.2, 75.2, 76.0, 78.4, 80.6, 82.4, 86.4, 96.7, 127.0 (x3) 127.7, 127.8, 127.9 (x6), 128.0 (x3), 128.2 (x2) 128.3 (x5), 128.4 (x4), 128.9

(x6), 138.0, 138.6, 138.9, 144.1 (x2) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{54}H_{60}O_7Na$ 843.4237, found 843.4257.

8-(3-Carboxypropanoyloxy)oct-1-yl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (4.1). Succinic anhydride (0.440 g, 4.39 mmol) and 4-dimethylaminopyridine (DMAP, 0.053 g, 0.438 mmol) were added to a solution of compound**4.18** (1.3 g, 1.464 mmol) in pyridine (5.0 mL) and the resulting mixture was stirred under argon for 16 h at 65 °C. After that, the volatiles were removed under the reduced pressure and the residue was co-evaporated with toluene (3 x 10 mL) and purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford the title compound (1.30 g, 97%) as a colorless foam. Analytical data for **4.1**: R_f = 0.25 (ethyl acetate / hexanes, 1/1, v/v); $[\alpha]_D^{27}$ +21.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.28-1.37 (m, 8H, CH_2), 1.54-1.66 (m, 4H, 2 x CH_2), 2.55-2.63 (m, 4H, 2 x CH_2), 3.17 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 9.8 Hz, H-6a), 3.44-3.47 (m, 2H, H-6b, OCH_2^a), 3.53-3.61 (m, 2H, H-2, 4), 3.69-3.72 (m, 1H, OCH_2^b), 3.82 (m, 1H, H-5), 3.95 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 4.03 (t, 2H, J = 6.6 Hz, CH_2), 4.25 (d, 1H, ^2J = 10.3 Hz, ½ CH_2Ph), 4.66 (d, 1H, ^2J = 10.4 Hz, ½ CH_2Ph), 4.68 (d, 1H, ^2J = 12.1 Hz, ½ CH_2Ph), 4.75-4.84 (m, 4H, H-1, 1½ CH_2Ph) 4.93 (d, 1H, ^2J = 10.7 Hz, ½ CH_2Ph), 6.83-7.44 (m, 30H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 25.1, 26.1, 28.5, 28.7, 28.9, 29.1, 29.3, 20.4, 62.7, 65.0, 67.9, 70.3, 73.1, 75.1, 75.9, 78.3, 80.4, 82.2, 86.3, 95.5, 126.9 (x3), 127.7 (x2), 127.8 (x6), 127.9 (x2), 128.1 (x3), 128.2 (x3), 128.3 (x5), 128.4, 128.8 (x5), 137.8, 138.4, 138.6, 138.8, 144.0 (x3), 144.5, 172.2 ppm; HR-FAB $[M+Na]^+$ calcd for $C_{58}H_{64}NaO_{10}$ 943.4397, found 943.4371.

Resin-bound acceptor 4.3. JandaJel™ amine resin (1% cross-linked polystyrene, 500 mg, 0.25 mmol) was added to a solution of **4.1** (253 mg, 0.275 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 105.4 mg, 0.55 mmol), and DMAP (30 mg, 0.25 mmol) in CH_2Cl_2 (5.0 mL) and the resulting suspension was agitated under argon for 18 h at rt. When the Kaiser test[43] showed the negative result, the resin was filtered off, washed with CH_2Cl_2 (3 x 20 mL), methanol (3 x 20 mL) and CH_2Cl_2 (3 x 20 mL), and dried *in vacuo* for 4 h. The resulting resin **4.2** was swelled in CH_2Cl_2 (10 mL) for 60 min at rt. A 10% solution of TFA in wet CH_2Cl_2 (5.0 mL) was added dropwise and the resulting suspension was agitated for 3 h at rt. The resin was filtered off, washed with CH_2Cl_2 (3 x 20 mL), methanol (3 x 20 mL) and CH_2Cl_2 (3 x 20 mL), and dried *in vacuo* for 6 h to afford the title compound. The loading (0.44 mmol/g) was determined by the quantification of TrOH forming as a result of the treatment with TFA.

4.4.3 Synthesis of Glycosyl Donors

Benzoxazolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (4.4). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[32]

Phenyl 2,3,4,6-Tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (4.5). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[44]

3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (4.6). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[35]

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranoside bis(butyl)phosphate (4.7). A mixture of **4.5** (700 mg, 1.0 mmol), dibutyl phosphate (0.58 mL, 3.0 mmol), and freshly activated molecular sieves (4 Å, 1.5 g) in CH_2Cl_2 (15 mL) was stirred under argon for 1 h at rt. After that, NIS (265 mg, 1.2 mmol) and TfOH (10 μL, 0.12 mmol) were added and the resulting mixture was stirred for 18 h at rt. The solid was then filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~40 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound (1.30 g, 97%) as a clear syrup. Analytical data for **4.7**: R_f = 0.24 (ethyl acetate / hexanes, 1/1, v/v); $[\alpha]_D^{26}$ +33.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.64, 0.79 (2 t, 6H, 2 x CH_3), 0.90 (2 m, 4H, 2 x CH_2), 1.18-1.69 (m, 4H, 2 x CH_2), 3.73 (m, 2H, OCH_2^a), 3.99 (m, 2H, OCH_2^b), 4.28 (m, 1H, H-5), 4.46 (dd, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.64 (dd, 1H, $J_{5,6b}$ = 2.5 Hz, H-6b), 5.60-5.75 (m, 3H, H-1, 2, 4), 5.91 (dd, 1H, $J_{3,4}$ = 10.5 Hz, H-3), 7.15-7.80 (m, 20H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 13.4, 13.5, 18.2, 18.5, 31.7, 31.8, 32.0, 62.6, 67.0, 68.0, 68.1, 68.2, 69.0, 71,7, 71.8, 72.5, 73.0, 96.6, 128.3 (x4), 128.5 (x4), 128.6, 128.7, 129.4, 129.8 (x5), 129.9 (x4), 133.3, 133.4, 133.5, 133.6 ppm. HR-FAB $[M+Na]^+$ calcd for $C_{42}H_{45}O_{13}PNa$ 811.2495, found 811.2505.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate (4.8). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[37, 45]

2,3,6-Tri-*O*-benzoyl-4-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranosyl trichloroacetimidate (4.9). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[20]

2,3,4-Tri-*O*-benzoyl-6-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranosyl trichloroacetimidate (4.10). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[20]

***O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate (4.13).** The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[40]

4.4.4 HPLC-Mediated Synthesis of Oligosaccharides

A general procedure for glycosylation and cleavage. Functionalized JandaJel resin **4.3** (50 mg, 0.022 mmol) was packed in an Omnifit™ glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH_2Cl_2

at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL, step 1). The system was then switched to the recirculation mode and the delivery of CH_2Cl_2 continued for 30 min at 1.0 mL/min (swelling, step 2). After that, pump D was stopped and pump C was programmed to deliver a solution of glycosyl donor (**4.4 - 4.10**, 0.10 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 3). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of the promoter in CH_2Cl_2 (3 x 100 µL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 4). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in CH_3OH/CH_2Cl_2 (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min (step 5). Pump B was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H^+) resin. The resin was filtered off, washed successively with CH_2Cl_2 and CH_3OH, and the combined filtrate was concentrated *in vacuo* to afford the crude residue that was subjected to subsequent acetylation.

A general procedure for acetylation of released disaccharide. A crude residue was redissolved in pyridine (2.0 mL), Ac_2O (73 µL, 0.771 mmol) was added dropwise and the resulting mixture was stirred for 16 h at rt. The reaction mixture was quenched with CH_3OH (~1.0 mL) and the volatiles were removed under the reduced pressure. The

residue was diluted with CH_2Cl_2 (20 mL) and washed with 1 N HCl (2 x 10 mL), water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and water (2 x 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford disaccharide **4.11**.

8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (4.11). The title compound was synthesized from glycosyl donors **4.4-4.10** and glycosyl acceptor **4.3** in 50-89% yield. Analytical data for **4.11**: R_f = 0.57 (ethyl acetate / hexanes, 1/1, v/v); $[\alpha]_D^{27}$ + 8.90 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.22-1.29 (m, 8H, 4 x CH_2), 1.50-1.58 (m, 4H, 2 x CH_2), 1.91-2.07 (5 s, 15H, 5 x $COCH_3$), 3.32-3.50 (m, 3H, H-2, 4, OCH_2^a), 3.55-3.75 (m, 4H, ½ OCH_2^a, H-5, H-6b, H-5'), 3.95 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 3.98-4.23 (m, 5H, H-6a, 6'a, 6'b, OCH_2^b), 4.47 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1'), 4.50 (dd, 1H, ^2J = 9.8 Hz, ½ CH_2Ph), 4.60 (d, 1H, ^2J = 12.0 Hz, ½ CH_2Ph), 4.68 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.74 (d, 1H, ^2J = 12.0 Hz, ½ CH_2Ph), 4.76 (d, 1H, ^2J = 10.9 Hz, ½ CH_2Ph), 4.83 (d, 1H, ^2J = 10.7 Hz, ½ CH_2Ph), 4.96 (d,1H, ^2J = 11.0 Hz, ½ CH_2Ph), 5.02 (dd, 1H, $J_{2',3'}$ = 9.2 Hz, H-2'), 5.05 (dd, 1H, $J_{4',5'}$ = 9.6 Hz, H-4'), 5.15 (dd, 1H, $J_{3',4'}$ = 9.4 Hz, H-3'), 7.22-7.31 (m, 15H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 20.8 (x2), 20.9 (x2), 21.3, 26.1, 26.3, 28.7, 29.4, 29.5, 29.6, 57.2, 62.1, 64.8, 68.2, 68.3, 68.5, 68.6, 69.6, 71.4, 71.9, 73.0, 73.2, 73.3, 75.1, 75.8, 80.2, 96.8, 100.8, 101.8, 127.7, 128.0 (x2), 128.1, 128.2, 128.3 (x2), 128.5 (x2), 128.6 (x2), 128.7 (x2), 138.3, 138.4, 139.0, 169.2, 169.5, 170.5, 170.8, 171.4 ppm; HR-FAB $[M+Na]^+$ calcd for $C_{51}H_{66}O_{17}Na$ 973.4198, found 973.4175.

8-Acetyloxyoct-1-yl-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside (4.12). Functionalized JandaJel resin **4.3** (50 mg, 0.022 mmol) was packed in an Omnifit^{TM} glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL, step 1). The system was then switched to the recirculation mode and the delivery of CH_2Cl_2 continued for 30 min at 1.0 mL/min (swelling, step 2). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **4.10** (188 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 3). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μL, 0.44 mmol) in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 4). After that, pump D was stopped and pump A was programmed to deliver a solution of TEA/CH_2Cl_2 (1/1, v/v) for 20 min at 1.0 mL/min (step 5). This step was monitored by the integrated UV detector (λ_{max} = 312 nm). After that, pump A was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 6). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **4.10** (188 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 7). This step was monitored by the

integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μL, 0.44 mmol) in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 8). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in CH_3OH/CH_2Cl_2 (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min (step 9). Pump B was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H^+) resin. The resin was filtered off, washed successively with CH_2Cl_2 and CH_3OH, and the combined filtrate was concentrated *in vacuo* to afford the crude residue that was subjected to subsequent acetylation in accordance with the general procedure, as described for the synthesis of compound **4.11**. The crude residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford trisaccharide **4.12** in 80% yield. Analytical data for **4.12**: R_f = 0.44 (ethyl acetate / hexanes, 1/1, v/v); $[\alpha]_D^{27}$ +6.34 (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 1.22-1.28 (m, 8H, 4 x CH_2), 1.58-159 (m, 4H, 2 x CH_2), 1.95-2.06 (8 s, 24H, 8 x $COCH_3$), 3.31 (m, 1H, OCH_2^a), 3.45-3.49 (m, 2H, H-2, 4), 3.56-3.63 (m, 4H, H-5', 5", 6'a, OCH_2^b), 3.74-3.70 (m, 2H, H-5, 6a), 3.81 (d, 1H, $J_{6'a,6'b}$ = 10.5 Hz, H-6'b), 3.96 (dd, 1H, $J_{3,4}$ = 9.1 Hz, H-3), 3.93-4.08 (m, 4H, H-6"a, 6b, CH_2), 4.22 (dd, 1H, $J_{5",6"b}$ = 4.4 Hz, $J_{6"a,6"b}$ = 12.3 Hz, H-6"b), 4.46-4.49 (m, 3H, H-1', 1", ½ CH_2Ph), 4.60 (d, 1H, ^2J = 12.1 Hz, ½ CH_2Ph), 4.68 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1), 4.75 (dd, 2H, ^2J = 13.7 Hz, CH_2Ph), 4.86-4.82 (m, 2H, H-4', ½ CH_2Ph), 4.90-5.01 (m,

4H, H-2', 2", 4", ½ C*H*$_2$Ph), 5.08-5.12 (m, 2H, H-3', 3"), 7.23-7.32 (m, 15H, aromatic) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, 20.7, 20.8, 20.9 (x2), 20.92, 21.2, 25.9, 26.4, 28.7, 29.4, 29.5, 29.6, 61.9, 64.7, 68.0, 68.1, 63.3, 68.4, 69.2, 69.7, 71.2, 71.5, 72.1, 72.8, 73.1, 73.2, 73.4, 75.1, 75.8, 77.6, 80.2, 82.0, 96.9, 100.5, 100.9, 127.7, 128.0 (x3), 128.1 (x3), 128.2 (x2), 128.4, 128.5 (x2), 128.6 (x2), 128.7, 128.8 (x2), 138.3, 138.4, 139.0, 169.2, 169.3, 169.5, 169.7, 170.3, 170.4, 170.8, 171.4 ppm; HR-FAB $[M+Na]^+$ calcd for $C_{63}H_{82}O_{25}Na$ 1261.5025, found 1261.5071.

8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (4.14). Functionalized JandaJel resin **4.3** (50 mg, 0.022 mmol) was packed in an Omnifit^{TM} glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL, step 1). The system was then switched to the recirculation mode and the delivery of CH_2Cl_2 continued for 30 min at 1.0 mL/min (swelling, step 2). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **4.10** (188 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 3). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μL, 0.44 mmol) in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was

programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 4). After that, pump D was stopped and pump A was programmed to deliver a solution of TEA/CH_2Cl_2 (1/1, v/v) for 20 min at 1.0 mL/min (step 5). This step was monitored by the integrated UV detector (λ_{max} = 312 nm). After that, pump A was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 6). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **4.10** (188 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 7). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μL, 0.44 mmol) in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 8). After that, pump D was stopped and pump A was programmed to deliver a solution of TEA/CH_2Cl_2 (1/1, v/v) for 20 min at 1.0 mL/min (step 9). This step was monitored by the integrated UV detector (λ_{max} = 312 nm). After that, pump A was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 10). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **4.13** (266 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 11). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (81 μL, 0.44 mmol) in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min

and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 12). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in CH_3OH/CH_2Cl_2 (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min (step 13). Pump B was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H^+) resin. The resin was filtered off, washed successively with CH_2Cl_2 and CH_3OH, and the combined filtrate was concentrated *in vacuo* to afford the crude residue that was subjected to subsequent acetylation in accordance with the general procedure, as described for the synthesis of compound **4.11**. The crude residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford pentasaccharide **4.14** in 67% yield. Analytical data for **4.14**: R_f = 0.26 (ethyl acetate / hexanes, 1/1, v/v); $[\alpha]_D^{27}$ -1.94 (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ, 1.26-1.30 (m, 8H, 4 x CH_2), 1.55-159 (m, 4H, 2 x CH_2), 3.30 (m, 1H, OCH_2^a), 3.44-3.46 (m, 2H, H-2, 4), 3.53-3.63 (m, 6H, H-5', 5", 5"', 6'a, 6"a, OCH_2^b), 3.68-3.73 (m, 2H, H-6a, 6"b), 3.77-3.86 (m, 4H, H-5, 5"", 6""a, 6'b), 3.94 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 4.03-4.10 (m, 7H, H-2", 2"", 6b, 6"b, 6""b, CH_2), 4.42-4.52 (m, 6H, H-1', 1", 1"', 1"", 6"'b, ½ CH_2Ph), 4.59 (d, 1H, ^2J = 12.0 Hz, ½ CH_2Ph), 4.69 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1), 4.73 (dd, 2H, ^2J = 11.6 Hz, CH_2Ph), 4.82-7.97 (m, 7H, H-2', 2"', 4', 4", 4"', ½ CH_2Ph), 4.99 (dd, 1H, $J_{3"",4""}$ = 8.4 Hz, H-3''''), 5.06-5.16 (m, 4H, H-3', 3", 3"', ½ CH_2Ph), 5.31 (d, 1H, $J_{4"",5""}$ = 2.1 Hz, H-4""), 7.24-7.32 (m, 15H, aromatic) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ, 20.6 (x2), 20.7 (x3), 20.8, 20.9 (x2),

21.1, 26.0, 26.2, 28.7, 29.4, 29.5, 29.8 (x2), 62.0, 64.4 (x2), 66.6, 68.0, 68.1, 69.0, 69.1, 69.2 (x2), 69.7, 70.7 (x2), 71.0, 71.2, 71.4 (x2), 71.5, 72.8, 72.9, 73.0, 73.1 (x2), 73.2, 75.0, 75.7, 76.2, 77.5, 80.1, 81.9, 96.7, 100.5, 100.7, 100.9, 101.1, 127.6, 127.9 (x7), 128.0, 128.1 (x3), 128.4 (x3), 128.5 (x3), 128.6 (x3), 138.3, 138.4, 138.9, 169.1, 169.2, 169.3, 169.5, 169.6, 169.8, 170.2, 170.3, 170.4 (x3), 171.3 ppm; HR-FAB $[M+Na]^+$ calcd for $C_{87}H_{114}O_{41}Na$ 1837.6716, found 1837.6703.

4.5 References

1. (a) Mydock, L. K.; Demchenko, A. V., Mechanism of chemical O-glycosylation: from early studies to recent discoveries. *Org. Biomol. Chem.* **2010,** *8*, 497-510; (b) Crich, D., Mechanism of a chemical glycosylation reaction. *Acc. Chem. Res.* **2010,** *43*, 1144-1153.

2. Christensen, H. M.; Oscarson, S.; Jensen, H. H., Common side reactions of the glycosyl donor in chemical glycosylation. *Carbohydr Res* **2015,** *408*, 51-95.

3. Smoot, J. T.; Demchenko, A. V., Oligosaccharide synthesis: from conventional methods to modern expeditious strategies. *Adv. Carbohydr. Chem. Biochem.* **2009,** *62*, 161-250.

4. (a) Fruchtel, J. S.; Jung, G., Organic chemistry on solid supports. *Angew. Chem. Int. Ed. Engl.* **1996,** *35*, 17-42; (b) Winter, M., Supports for solid-phase organic synthesis. In *Combinatorial peptide and nonpeptide libraries: a handbook*, Jung, G., Ed. VCH: Wienheim, New York, Basel, Cambridge, Tjokyo, 1996; pp 465-510.

5. (a) Merrifield, B., The role of the support in solid phase peptide synthesis *Br. Polym. J.* **1984,** *16*, 173-178; (b) Krishnamurthy, V. R.; Dougherty, A.; Kamat, M.; Song,

X.; Cummings, R. D.; Chaikof, E. L., Synthesis of an Fmoc-threonine bearing core-2 glycan: a building block for PSGL-1 via Fmoc-assisted solid-phase peptide synthesis. *Carbohydr. Res.* **2010,** *345,* 1541-1547.

6. Toy, P. H.; Lam, Y., *Solid-Phase Organic Synthesis*. John Wiley & Sons, Inc. : Hoboken, 2012.

7. (a) Frechet, J. M.; Schuerch, C., Solid-phase synthesis of oligosaccharides. I. Preparation of the solid support. Poly[p-(1-propen-3-ol-1-yl)styrene]. *J. Am. Chem. Soc.* **1971,** *93,* 492-496; (b) Schmidt, R. R.; Jonke, S.; Liu, K., New Aspects of Glycoside Bond Formation: Solid-Phase Oligosaccharide Synthesis In *ACS Symp. Ser. (Frontiers in Modern Carbohydrate Chemistry)* Demchenko, A. V., Ed. Oxford Univ. Press: 2007; Vol. 960, pp 209-237; (c) Seeberger, P. H., Solid phase oligosaccharide synthesis (Reprinted from Glycochemistry: Principles, synthesis, and applications, pg 1- 32, 2001). *J. Carbohydr. Chem.* **2002,** *21* (7-9), 613-643; (d) Seeberger, P. H.; Haase, W. C., Solid-phase oligosaccharide synthesis and combinatorial carbohydrate libraries. *Chem. Rev.* **2000,** *100* (12), 4349-4393; (e) Tanaka, K.; Fukase, K., Oligosaccharide synthesis on solid, soluble polymer, and tag supports. In *Solid-Phase Organic Synthesis*, Toy, P. H.; Lam, Y., Eds. John Wiley & Sons, Inc. : Hoboken, 2012; pp 489-530.

8. (a) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H., Automated solid-phase synthesis of oligosaccharides. *Science* **2001,** *291* (5508), 1523-1527; (b) Seeberger, P. H., Automated oligosaccharide synthesis. *Chem. Soc. Rev.* **2008,** *37,* 19-28; (c) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H., Development of an automated oligosaccharide synthesizer. *Adv. Carbohydr. Chem. Biochem.* **2003,** *58,* 35-54; (d) Seeberger, P. H., The logic of automated glycan assembly. *Acc. Chem. Res.* **2015,** *48* (5), 1450-1463.

9. Krock, L.; Esposito, D.; Castagner, B.; Wang, C.-C.; Bindschadler, P.; Seeberger, P. H., Streamlined access to conjugation-ready glycans by automated synthesis. *Chem. Sci.* **2012,** *3*, 1617-1622.

10. (a) Sears, P.; Wong, C. H., Toward automated synthesis of oligosaccharides and glycoproteins. *Science* **2001,** *291* (5512), 2344-2350; (b) Hsu, C. H.; Hung, S. C.; Wu, C. Y.; Wong, C. H., Toward automated oligosaccharide synthesis. *Angew. Chem. Int. Ed.* **2011,** *50*, 11872-11923.

11. (a) Tanaka, H.; Matoba, N.; Tsukamoto, H.; Takimoto, H.; Yamada, H.; Takahashi, T., Automated Parallel Synthesis of a Protected Oligosaccharide Library Based upon the Structure of Dimeric Lewis X by One-Pot Sequential Glycosylation *Synlett* **2005**, 824-828; (b) Machida, K.; Hirose, Y.; Fuse, S.; Sugawara, T.; Takahashi, T., Development and application of a solution-phase automated synthesizer, 'ChemKonzert'. *Chem. Pharm. Bull.* **2010,** *58*, 87-93.

12. (a) Sugiarto, G.; Lau, K.; Qu, J.; Li, Y.; Lim, S.; Mu, S.; Ames, J. B.; Fisher, A. J.; Chen, X., A sialyltransferase mutant with decreased donor hydrolysis and reduced sialidase activities for directly sialylating Lewis(x). *ACS Chem. Biol.* **2012,** *7*, 1232-1240; (b) Chen, Y.; Thon, V.; Li, Y.; Yu, H.; Ding, L.; Lau, K.; Qu, J.; Hie, L.; Chen, X., One-pot three-enzyme synthesis of UDP-GlcNAc derivatives. *Chem. Commun.* **2011,** *47*, 10815-10817; (c) Muthana, M. M.; Qu, J.; Li, Y.; Zhang, L.; Yu, H.; Ding, L.; Malekan, H.; Chen, X., Efficient one-pot multienzyme synthesis of UDP-sugars using a promiscuous UDP-sugar pyrophosphorylase from Bifidobacterium longum (BLUSP). *Chem. Commun.* **2012,** *48*, 2728-2730.

13. (a) Jaipuri, F. A.; Pohl, N. L., Toward solution-phase automated iterative synthesis: Fluorous-tag assisted solution-phase synthesis of linear and branched mannose oligomers. *Org. Biomol. Chem.* **2008,** *6*, 2686-2691; (b) Song, E.-H.; Osanya, A. O.; Petersen, C. A.; Pohl, N. L. B., Synthesis of Multivalent Tuberculosis and Leishmania-Associated Capping Carbohydrates Reveals Structure-Dependent Responses Allowing Immune Evasion. *J. Am. Chem. Soc.* **2010,** *132*, 11428–11430; (c) Liu, L.; Pohl, N. L. B., A Fluorous Phosphate Protecting Group with Applications to Carbohydrate Synthesis. *Org. Lett.* **2011,** *13*, 1824–1827; (d) Tang, S. L.; Pohl, N. L., Automated Solution-Phase Synthesis of beta-1,4-Mannuronate and beta-1,4-Mannan. *Org. Lett.* **2015,** *17* (11), 2642-5.

14. (a) Chen, C.; Zhang, Y.; Xue, M.; Liu, X. W.; Li, Y.; Chen, X.; Wang, P. G.; Wang, F.; Cao, H., Sequential one-pot multienzyme (OPME) synthesis of lacto-N-neotetraose and its sialyl and fucosyl derivatives. *Chem. Commun.* **2015,** *51* (36), 7689-7692; (b) Li, L.; Liu, Y.; Ma, C.; Qu, J.; Calderon, A. D.; Wu, B.; Wei, N.; Wang, X.; Guo, Y.; Xiao, Z.; Song, J.; Sugiarto, G.; Li, Y.; Yu, H.; Chen, X.; Wang, P. G., Efficient chemoenzymatic synthesis of an N-glycan isomer library. *Chem. Sci.* **2015**, Advance Article DOI: 10.1039/C5SC02025E

15. (a) Nokami, T.; Hayashi, R.; Saigusa, Y.; Shimizu, A.; Liu, C.-Y.; Mong, K.-K. T.; Yoshida, J.-i., Automated Solution-Phase Synthesis of Oligosaccharides via Iterative Electrochemical Assembly of Thioglycosides. *Org. Lett.* **2013,** *15*, 4520-4523; (b) Nokami, T.; Isoda, Y.; Sasaki, N.; Takaiso, A.; Hayase, S.; Itoh, T.; Hayashi, R.; Shimizu, A.; Yoshida, J., Automated electrochemical assembly of the protected potential

TMG-chitotriomycin precursor based on rational optimization of the carbohydrate building block. *Org. Lett.* **2015,** *17* (6), 1525-8.

16. Pistorio, S. G.; Stine, K. J.; Demchenko, A. V., Automated chemical synthesis of oligosaccharides and glycoconjugates. In *Carbohydrate Chemistry: State-of-the-art and challenges for drug development*, Cipolla, L., Ed. Imperial College Press: London, 2015; pp 247-276.

17. (a) Varki, A.; Cummings, R. D.; Esko, J. D.; Freeze, H. H.; Bertozzi, C. R.; Stanley, P.; Hart, G. W.; Etzler, M. E., *Essentials of Glycobiology*. Second ed.; CSH Laboratory Press: New York, 2009; (b) DeMarco, M. L.; Woods, R. J., Structural glycobiology: A game of snakes and ladders. *Glycobiology* **2008,** *18*, 426-440; (c) Bertozzi, C. R.; Kiessling, L. L., Chemical glycobiology. *Science* **2001,** *291*, 2357-2364; (d) Dwek, R. A., Glycobiology: toward understanding the function of sugars. *Chem. Rev.* **1996,** *96*, 683-720.

18. Cummings, R. D.; Pierce, J. M., The challenge and promise of glycomics. *Chem. Biol.* **2014,** *21*, 1-15.

19. *Transforming Glycoscience: A Roadmap for the Future.* *http://dels.nas.edu/Report/Transforming-Glycoscience-Roadmap/13446;* 2012.

20. Vijaya Ganesh, N.; Fujikawa, K.; Tan, Y. H.; Stine, K. J.; Demchenko, A. V., HPLC-assisted automated oligosaccharide synthesis. *Org. Lett.* **2012,** *14*, 3036-3039.

21. Pornsuriyasak, P.; Ranade, S. C.; Li, A.; Parlato, M. C.; Sims, C. R.; Shulga, O. V.; Stine, K. J.; Demchenko, A. V., STICS: surface-tethered iterative carbohydrate synthesis. *Chem. Commun.* **2009**, 1834-1836.

22. Stine, K. J., Glycans in Mesoporous and Nanoporous Materials. In *Carbohydrate Nanotechnology*, Stine, K. J., Ed. Wiley: Hoboken, NJ, 2016.

23. (a) Vijaya Ganesh, N.; Fujikawa, K.; Tan, Y. H.; Nigudkar, S. S.; Stine, K. J.; Demchenko, A. V., Surface-tethered iterative carbohydrate synthesis: a spacer study. *J. Org. Chem.* **2013,** *78* (14), 6849-6857; (b) Ganesh, N. V.; Fujikawa, K.; Tan, Y. H.; Nigudkar, S. S.; Stine, K. J.; Demchenko, A. V., Surface-tethered iterative carbohydrate synthesis: a spacer study. *J. Org. Chem.* **2013,** *78* (14), 6849-57.

24. Parlato, M. C.; Kamat, M. N.; Wang, H.; Stine, K. J.; Demchenko, A. V., Application of glycosyl thioimidates in solid-phase oligosaccharide synthesis. *J. Org. Chem.* **2008,** *73*, 1716-1725.

25. Simon, M. D.; Heider, P. L.; Adamo, A.; Vinogradov, A. A.; Mong, S. K.; Li, X.; Berger, T.; Policarpo, R. L.; Zhang, C.; Zou, Y.; Liao, X.; Spokoyny, A. M.; Jensen, K. F.; Pentelute, B. L., Rapid flow-based peptide synthesis. *Chembiochem : a European journal of chemical biology* **2014,** *15* (5), 713-720.

26. (a) Li, J.; Ballmer, S. G.; Gillis, E. P.; Fujii, S.; Schmidt, M. J.; Palazzolo, A. M. E.; Lehmann, J. W.; Morehouse, G. F.; Burke, M. D., Synthesis of many different types of organic small molecules. *Science* **2015,** *347*, 1221-1226; (b) Santanilla, A. B.; Regalado, E. L.; Pereira, T.; Shevlin, M.; Bateman, K.; Campeau, L.-C.; Schneeweis, J.; Berritt, S.; Shi, Z.-C.; Nantermet, P.; Liu, Y.; Helmy, R.; Welch, C. J.; Vachal, P.; Davies, I. W.; Cernak, T.; Dreher, S. D., Nanomole-scale high-throughput chemistry for the synthesis of complex molecules. *Science* **2015,** *347*, 49-53.

27. Collot, M.; Eller, S.; Weishaupt, M.; Seeberger, P. H., Glycosylation efficiencies on different solid supports using a hydrogenolysis-labile linker. *Beilstein J. Org. Chem.* **2013,** *9*, 97-105.

28. Wang, S. S., p-Alkoxybenzyl alcohol resin and p-alkoxybenzyloxycarbonylhydrazide resin for solid phase synthesis of protected peptide fragments. *J. Am. Chem. Soc.* **1973,** *95*, 1328-1333.

29. Nguyen, S. H.; Trotta, A. H.; Cao, J.; Straub, T. J.; Bennett, C. S., Direct O-glycosidation of resin bound thioglycosides. *Org. Biomol. Chem.* **2012**, 2373-2376.

30. (a) James, I. W., Linkers for Solid Phase Organic Synthesis. *Tetrahedron* **1999,** *55*, 4855-4946; (b) Guillier, F.; Orain, D.; Bradley, M., Linkers and Cleavage Strategies in Solid-Phase Organic Synthesis and Combinatorial Chemistry. *Chem. Rev.* **2000,** *100*, 2091-2157; (c) Brase, S.; Dahmen, S., Linkers for solid-phase synthesis. In *Handbook of Combinatorial Chemistry*, 2002; Vol. 1, pp 59-169.

31. (a) Schmidt, R. R.; Jung, K. H., Trichloroacetimidates. In *Carbohydrates in Chemistry and Biology*, Ernst, B.; Hart, G. W.; Sinay, P., Eds. Wiley-VCH: Weinheim, New York, 2000; Vol. 1, pp 5-59; (b) Schmidt, R. R.; Kinzy, W., Anomeric-oxygen activation for glycoside synthesis: the trichloroacetimidate method. *Adv. Carbohydr. Chem. Biochem.* **1994,** *50*, 21-123; (c) Schmidt, R. R.; Michel, J., Facile synthesis of a- and b-O-glycosyl imidates; Preparation of glycosides and disaccharides. *Angew. Chem., Int. Ed. Engl.* **1980,** *19* (9), 731-732.

32. Kamat, M. N.; Rath, N. P.; Demchenko, A. V., Versatile synthesis and mechanism of activation of S-benzoxazolyl glycosides. *J. Org. Chem.* **2007,** *72*, 6938-6946.

33. Ferrier, R. J.; Furneaux, R. H., 1,2-trans-1-Thioglycosides. In *Methods in Carbohydrate Chemistry*, Whistler, R. L.; BeMiller, J. N., Eds. Academic Press: New York - London, 1980; Vol. 8, pp 251-253.

34. Nigudkar, S. S.; Parameswar, A. R.; Pornsuriyasak, P.; Stine, K. J.; Demchenko, A. V., O-Benzoxazolyl imidates as versatile glycosyl donors for chemical glycosylation. *Org. Biomol. Chem.* **2013,** *11* (24), 4068-4076.

35. Nigudkar, S. S.; Stine, K. J.; Demchenko, A. V., Renenerative glycosylation under nucleophilic catalysis. *J. Am. Chem. Soc.* **2014,** *136*, 921-923.

36. Plante, O. J.; Andrade, R. B.; Seeberger, P. H., Synthesis and use of glycosyl phosphates as glycosyl donors. *Org. Lett.* **1999,** *1* (2), 211-214.

37. Colonna, B.; Harding, V. D.; Nepogodiev, S. A.; Raymo, F. M.; Spencer, N.; Stoddart, J. F., *Chem. Eur. J.* **1998,** *4*, 1244-1254.

38. Carrel, F. R.; Seeberger, P. H., Cap-and-Tag Solid Phase Oligosaccharide Synthesis. *J. Org. Chem.* **2008,** *73*, 2058-2065.

39. Wu, X.; Schmidt, R. R., Solid-phase synthesis of complex oligosaccharides using a novel capping reagent. *J. Org. Chem.* **2004,** *69*, 1853-1857.

40. Sandbhor, M. S.; Soya, N.; Albohy, A.; Zheng, R. B.; Cartmell, J.; Bundle, D. R.; Klassen, J. S.; Cairo, C. W., Substrate Recognition of the Membrane-Associated Sialidase NEU3 Requires a Hydrophobic Aglycone. *Biochemistry* **2011,** *50*, 6753-6762.

41. Ottosson, H., Synthesis of p-trifluoroacetamidophenyl 3,6-di-O-{2-O-[α-D-mannopyranosyl 6-(disodium phosphate)]-α-D-mannopyranosyl}-α-D-mannopyranoside. *Carbohydr. Res.* **1990,** *197*, 101-107.

42. Clausen, M. H.; Madsen, R., *Carbohydr. Res.* **2004,** *339*, 2159-2169.

43. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I., Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. *Anal. Biochem.* **1970,** *34*, 595-598.

44. Dinkelaar, J.; de Jong, A. R.; van Meer, R.; Somers, M.; Lodder, G.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A., Stereodirecting Effect of the Pyranosyl C-5 Substituent in Glycosylation Reactions. *J. Org. Chem.* **2009,** *74*, 4982-4991.

45. Verduyn, R.; Douwes, M.; van der Klein, P. A. M.; Mösinger, E. M.; van der Marel, G. A.; van Boom, J. H., *Tetrahedron* **1993,** *49*, 7301-7316.

CHAPTER 5

Automated HPLC-assisted synthesis of protected *N*-linked Glycoprotein core pentasaccharide

5.1 Introduction

Oligosaccharides play important roles in various biological processes. Therefore, the general interest in these compounds has rapidly increased in the recent years.[1] Unlike proteins and nucleic acids, which can be obtained using automated methods such as peptide synthesizer and polymerase chain reaction (PCR),[2] glycans are most commonly isolated from nature.[3] The isolation from natural sources has limitations, the quantities that can be obtained from biological systems are often small, the purity is low, and structure modification is difficult or impractical.

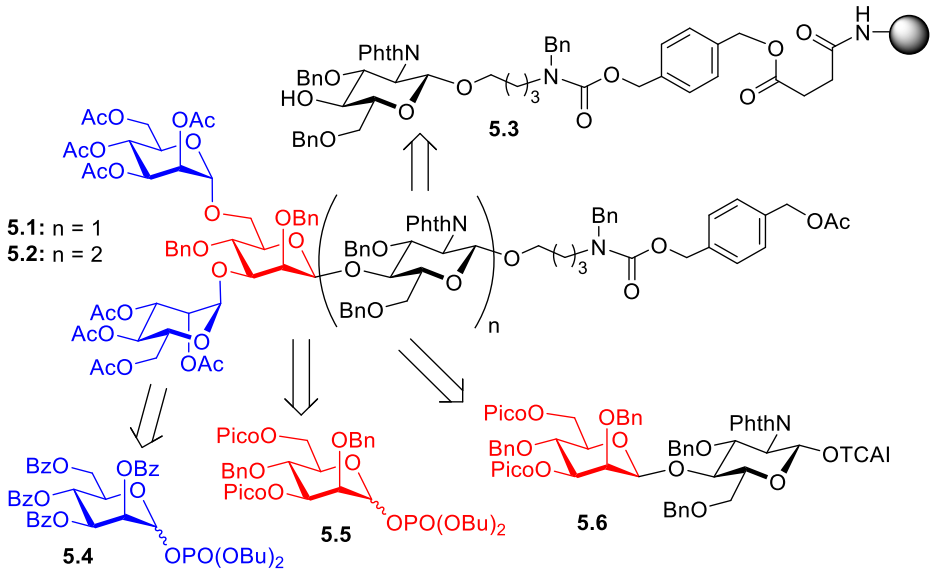
The traditional solution phase synthesis is an important alternative method to obtain oligosaccharides in high quantity and allow to incorporate structure modifications in the final product. Recently, successful methods for the solid-phase oligosaccharide synthesis[4] have been developed by several research groups.[5] This approach exhibits important advantages over the traditional solution phase synthesis, such as: 1) sample purification process, it is easier to remove excess reactants or byproducts from the product; 2) no need to purify the reaction intermediates. Seeberger and co-workers have been the first to perform the synthesis of many oligosaccharide sequences using an automated oligosaccharide synthesizer.[4e, 6] These advances have clearly demonstrated that solid phase synthesis can be an important alternative for the obtaining of complex glycans. Described herein is an automated solid phase synthesis approach for *N*-linked core oligosaccharides using an HPLC-based synthesizer developed in our laboratory.[5e]

5.2 Results and Discussion

In accordance with the structural analysis, our strategic plan to assemble the *N*-glycan core tetrasaccharide **5.1** and pentasaccharide **5.2** involved building blocks **5.3-5.6**

depicted in Figure 5.1. The tetra- and the pentasaccharide targets contain two synthetic challenges: the presence of branching and the Manβ(1→4)GlcNAc linkage. The stereoselective formation of the *β*-mannosidic linkage is difficult due to the strong anomeric effect and the steric effect of the axial substituent at the C2 position, both favoring the formation of α-mannosides. A well-known strategy for the synthesis of *β*-mannosidic linkage has been developed by Crich et. al.[7] Our laboratory also reported *β*-stereoselective mannosylation using 3- and/or 6-O-picoloyl thiomannosyl donor via the H-bond-mediated Aglycone Delivery (HAD).[8] Excellent *β*-stereoselectivity achieved in preliminary mannosylations (Chapter 2) and orthogonal conditions for the removal of picoloyl groups stimulated our interest in studying 3,6-di-O-picoloyl protected donor **5.5** for the formation of the key Manβ(1→4)GlcNAc bond in solid phase synthesis. As a back-up plan**,** we also decided to explore an alternative approach wherein the Manβ(1-4)GlcNAc linkage was presynthesized in solution and the resulting disaccharide building block **5.6** was then used for the indirect introduction of the β-mannosidic unit on solid support.

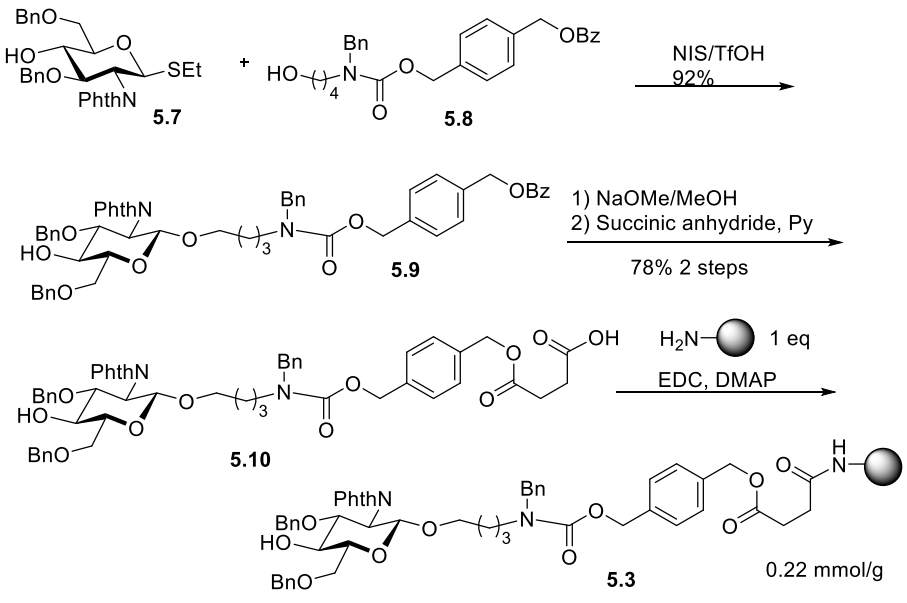
Figure 5.1. Retrosynthetic analysis of *N*-linked tetrasaccharide and pentasaccharide targets 5.1 and 5.2.



5.2.1 The synthesis of the key monosaccharide building blocks 5.3-5.5

Having identified the building blocks necessary for the synthesis we prepared polymer-supported building block **5.3** from known glucosamine derivative **5.7**.[9] The latter was coupled with spacer **5.8** in the presence of NIS/TfOH to afford glucosamine-spacer intermediate **5.9** in 92% yield. Next, benzoyl deprotection followed by the treatment with succinic anhydride in pyridine allowed us to obtain building block **5.10.** The latter was coupled with the amine of JandaJel resin in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) to afford building block **5.3**. The loading capacity of JandaJel functionalized acceptor **5.3** was determined to be 0.22 mmol/g by cleaving off and quantifying the loaded acceptor.

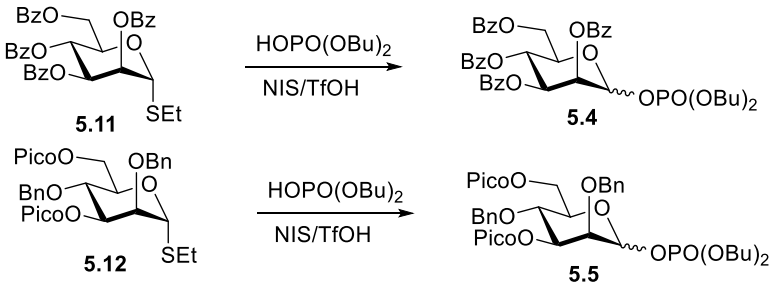
Scheme 5.1. Synthesis of the support-bound acceptor 5.3



Mannosyl phosphate donors **5.4** and **5.5** were formed by simple conversion of the respective known thioglycosides **5.11** and **5.12** by reaction with

dibutylphosphate in the presence of NIS/TfOH. It should be noted that highly reactive glycosyl phosphates were found to be more suitable glycosyl donors for the polymer-supported synthesis than their more stable thioglycoside counterparts.[10]

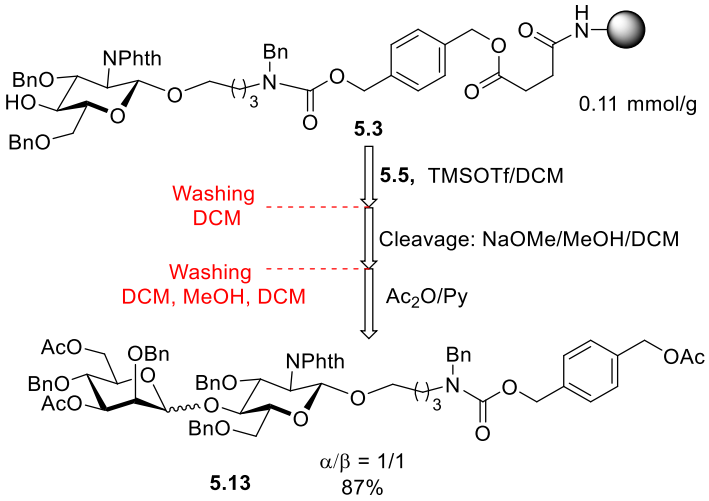
Scheme 5.2. Synthesis of mannosyl donors 5.4 and 5.5



5.2.2. The synthesis of Manβ(1-4)GlcNAc linkage on solid phase and in solution.

With the necessary building blocks **5.3-5.5** in hand, we investigated the applicability of the HAD assisted β-mannosylation using the HPLC synthesizer.[5e] JandaJel resin functionalized with glycosyl acceptor **5.3** (50 mg, 0.011 mmol) was packed in Omnifit™ glass chromatography column. The column was integrated into the standard Agilent Infinity 1260 HPLC system and the automated assembly was programmed as shown in Scheme **5.3**. To affect the initial washing and swelling of the resin containing acceptor **5.3** (0.011 mmol), Pump D was programmed to delivery 35 mL of dichloromethane (steps 1 and 2). After that, Pump C was programmed to deliver donor 5.5 (0.11 mmol) in CH_2Cl_2 (2.0 mL total volume) at 0.5 mL/min, which was then recirculated for 10 min and the delivery of the donor has been monitored with the UV detector. The autosampler was programmed to deliver a solution of promoter in CH_2Cl_2 (3 injections of 100 μL each) and the resulting reaction mixture was recirculated for 90 min (steps 3A and 3B). When the UV-monitoring showed no change in absorbance of the eluate passing through the detector, the system was washed with CH_2Cl_2 (Pump D, 1.0 mL/min rate flow for 10 min, step 4).

Scheme 5.3. HPLC-assisted synthesis of disaccharide 5.13



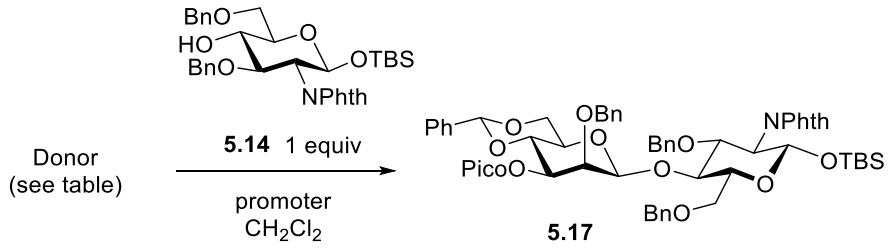
Step	Operation	Mode	Flow rate	Total volume	Time
1	Washing of the resin with CH_2Cl_2 (50 mg, 0.011 mmol)	Pump D	1.0 mL/min	5.0 mL	5 min
2	Swelling resin with CH_2Cl_2	Pump D	1.0 mL/min	2.0 mL (recirculation)	30 min
3A	Glycosylation, delivery of the donor (**5.5**, 10 equiv, 0.11 mmol,) in CH_2Cl_2	Pump C	0.5 mL/min	2.0 mL (recirculation)	10 min
3B	Glycosylation, injection of the promoter) at 10, 12, 14 min time points	Autosampler		3 x 100 µL	60-90 min
4	Washing with CH_2Cl_2	Pump D	1.0 mL/min	10 mL	10 min
5	Cleavage with 0.1 N NaOMe/ MeOH/CH_2Cl_2 to release the disaccharide	Pump B	1.0 mL/min	10 mL (recirculation)	20 min

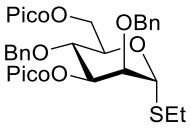
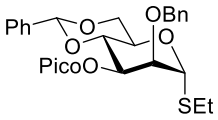
The resulting solid-phase bound disaccharide was then cleaved off from the solid support by passing a recirculating solution of NaOMe in CH_2Cl_2/MeOH (0.04/1/1, v/v/v, Pump B) at the flow rate of 1.0 mL/min for 10 min (step 5). The eluate was collected, neutralized, concentrated and the residue was acetylated with Ac_2O in pyridine to afford disaccharide **5.13** in 86% yield. Unfortunately, no stereoselectivity (α/β = 1.0/1.0) was obtained. This result indicates that no HAD effect took place during the glycosylation step 3 with donor **5.5**. Perhaps the reason for the poor stereocontrol is the continuous flow of the donor in the system

that disfavors the formation of a stable hydrogen bond between the donor and acceptor necessary for the HAD.

With this failure to introduce the β-mannosyl linkage directly, we abandoned the idea of obtaining the tetrasaccharide **5.2** and decided to focus on the synthesis of pentasaccharide **5.1** instead. For this purpose we turned our attention to the synthesis disaccharide building block **5.6**. The preassembled building block **5.6** should allow for the introduction of this difficult linkage in the target molecule. In our previous studies we showed that 3,6-di-O-picoloylated mannosyl donor **5.12** provides excellent stereoselectivity in *β*-mannosylation of primary and secondary acceptors.[8] Unfortunately, all attempts to use donor **5.12** with acceptor **5.14** failed, and no formation of the disaccharide product has been observed (Table 5.1, entries 1-4). A possible explanation of this result can be that the presence of two disarming protecting groups like picoloyl on 3-C and 6-C position affect the reactivity of this donor making it impossible to obtain the desired disaccharide. Next, we decided to investigate the use of a more reactive donor **5.16.** Still, to ensure the completeness of the reaction we had to use excess of donor and promoter as shown in Table 5.1 (entry 5). Under these conditions we were able to obtain disaccharide **5.17** in excellent yield of 95% and commendable stereoselectivity (α/β = 1/6.0).

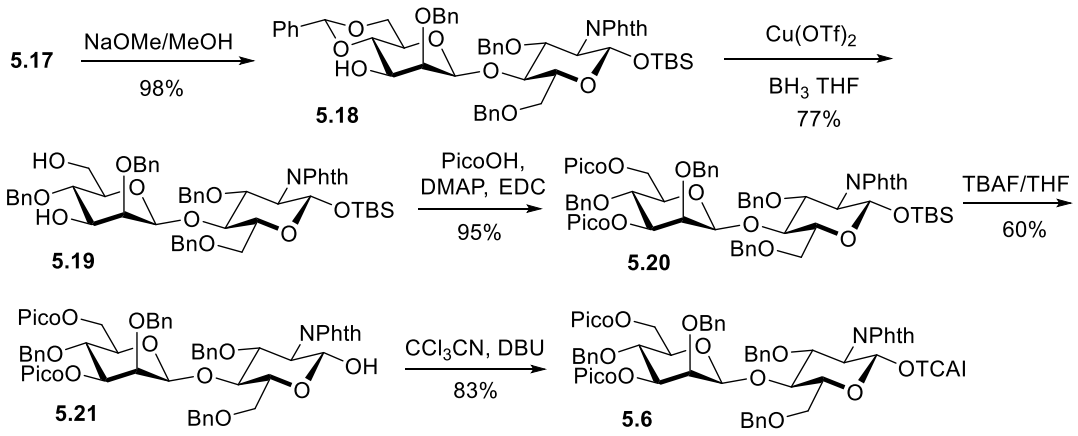
Table 5.1. Investigation of β-mannosylation in solution



Entry	Donor (equiv.)	Promoter (equiv.)	Yield, % (α/β ratio)
1	 **5.12** (1.2)	NIS/TfOH (2.4/0.24)	N.R.
2	**5.12** (1.4)	NIS/TfOH (2.8/0.28)	N.R.
3	**5.12** (1.5)	NIS/TMSOTf (3.0/0.30)	N.R.
4	**5.12** (3.0)	NIS/TfOH (6.0/0.60)	N.R.
5	 **5.16** (2.0)	NIS/TfOH (4.0/0.40)	**5.17** (95, 1/6.0)

The intermediate **5.17** was then employed in the synthesis of the key building block **5.6**. This was accomplished via a series of sequential transformations depicted in Scheme 5.4. Picoloyl deprotection was affected using a 1 M solution of NaOMe in MeOH to afford compound **5.18**. The reductive opening of the benzylidene acetal afforded disaccharide **5.19** in 77% yield. The later was 3,6-di-picoloylated to give **5.20** in 95% yield. Compound **5.20** was then treated with TBAF to liberate the hemiacetal in 60%. Finally, compound **5.21** was converted into imidate donor **5.6** in 83% yield by reaction with trichloroacetonitrile in the presence of DBU.

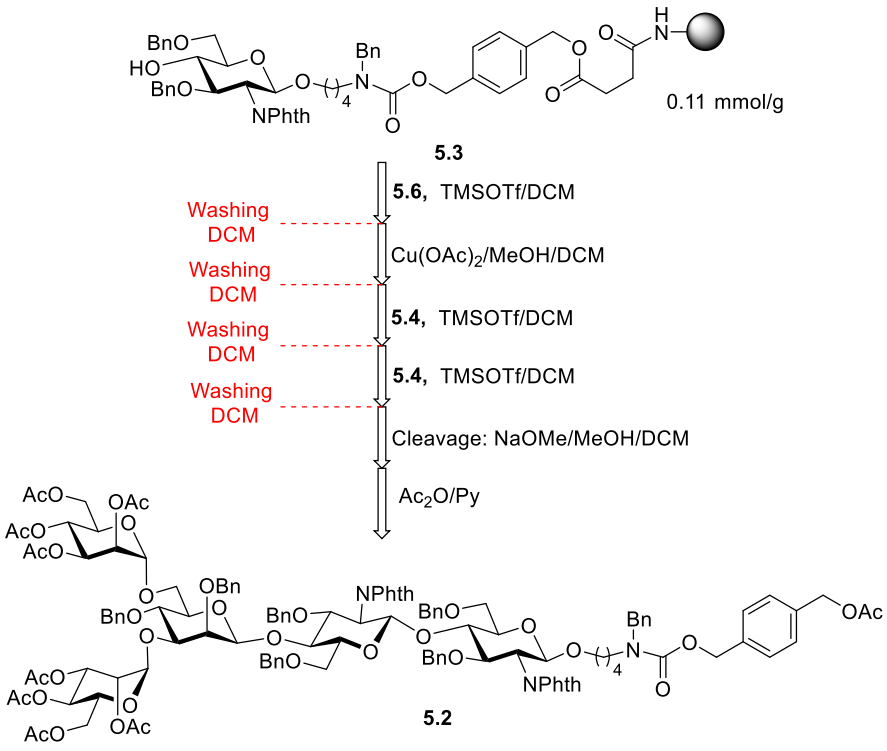
Scheme 5.4. Synthesis of disaccharide donor 5.6



5.2.3 HPLC-assisted assembly of *N*-glycan core pentasaccharide 5.2

With the desired building blocks **5.3**, **5.5** and **5.6** in hand we proceeded to the automated synthesis of the *N*-glycan core pentasaccharide. The automated assembly followed typical cycles of glycosylation and deprotection separated by washing as used in our pervious automated oligosaccharide synthesis (Chapter 3). Glycosylation with disaccharide **5.6** and acceptor **5.3** seemed to be the most challenging step, and to ensure a complete conversion 10 equiv of donor **5.6** has been used during this step. Simultaneous deprotection of picoloyl groups from position C3 and C6 was than performed using same conditions used for tetrasaccharide synthesis. The final branching was introduced by glycosylation with mannosyl donor **5.4**. This reaction was repeated two times to ensure complete glycosylation of both hydroxyls of the trisaccharide acceptor linked to the solid support. Finally, the resin was treated with a solution of NaOMe in CH_2Cl_2 and MeOH (0.04/1/1, v/v/v) at the flow rate of 1.0 mL/min for 10 min to release the resulting pentasaccharide from the resin. The eluate was collected, neutralized, concentrated and the residue was acetylated with Ac_2O in pyridine to afford pentasaccharide **5.2** in 31% yield.

Scheme 5.5. Assembly of *N*-glycan core pentasaccharide 5.2 on HPLC



5.3 Conclusions

In conclusion, we accomplished the synthesis of the N-glycan core pentasaccharide in a total of 8 h using an HPLC-assisted semiautomated approach. Unfortunately, the use of the HAD method was much less efficient in solid supported glycosylations. This shows a limitation of this promising method and offers venues for further explorations. The target synthesis was successfully accomplished using a block approach according to which the challenging Manβ(1→4)GlcNAc bond was preformed in solution and then introduced using the disaccharide building block. This was our first attempt to apply the HPLC-baser synthesizer to the synthesis of branched oligosaccharides. The overall assembly stage was swift, 8 h total assembly time, and efficient (31% over all yield).

5.4 Experimental

Column chromatography was performed on silica gel 60 (70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F254. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 and $ClCH_2CH_2Cl$ (1,2-DCE) were distilled from CaH_2 directly prior to application. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3 Å). Molecular sieves (3 Å or 4 Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. Optical rotations were measured at 'Jasco P-1020' polarimeter. Unless noted otherwise, ^1H n.m.r. spectra were recorded in $CDCl_3$ at 300 MHz, ^{13}C n.m.r. spectra were recorded in $CDCl_3$ at 75 or 150 MHz. Two-dimensional heteronuclear *J*-resolved spectra (HETERO2D) were recorded in $CDCl_3$ at 600 MHz.

5.4.1 Synthesis glycosyl acceptor 5.3

***N*-(*p*-Benzoyloxymethyl)benzyloxycarbonyl-4-benzylaminobutyl 3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5.9).** A mixture of **5.7** (0.50 g, 0.94 mmol), *N*-(*p*-benzoyloxymethyl)benzyloxycarbonyl-4-benzylaminobutanol (**5.8**, 0.45 g, 1.13 mmol), and freshly activated molecular sieves (4 Å, 1.5 g) in CH_2Cl_2 (40 mL) was stirred under argon for 1 h at rt. *N*-Iodosuccinimide (NIS, 0.42 g, 1.88 mmol) and TfOH (17 μL, 0.18 mmol) were added, and the resulting mixture was stirred for 10 min at rt. After that, the solids were filtered off through a pad of Celite and washed successively with CH_2Cl_2. The combined filtrate (~150 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with

$MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound (0.75 g, 92%) as a colorless syrup. Analytical data for **5.9**: R_f = 0.48 (ethyl acetate / hexanes, 1/1.5, v/v); $[\alpha]_D^{21}$ +13.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.21-1.33 (m, 4H, 2 x CH_2), 2.95-3.04 (m, 2H, ½ NCH_2, OH), 3.25-3.31 (m, 1H, H-5), 3.60-3.64 (m, 1H, ½ NCH_2), 3.62-3.78 (m, 3H, H-4, 6a, 6b), 4.06-4.28 (m, 4H, OCH_2, H-2, 3), 4.48-4.63 (m, 3H, 1 ½ C*H*$_2$Ph), 4.72 (d, 1H, ^2J = 12.2 Hz, ½ C*H*$_2$Ph), 5.03-5.07 (m, 3H, H-1, C*H*$_2$Ph), 5.33 (s, 2H, C*H*$_2$Ph), 6.91-7.61 (m, 26H, aromatic), 8.04-8.06 (d, 2H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 27.0, 55.5, 66.6, 74.0 (x2), 78.8, 98.5, 107.5 (x2), 127.5, 128.0 (x4), 128.1 (x8), 128.2 (x4), 128.3 (x4), 128.5 (x4), 128.6 (x4), 128.7 (x8), 129.9 (x4), 130.5, 133.2 (x2), 137.7, 138.0 ppm; HR-FAB MS $[M+Na]^+$ calcd 941.3625 for $C_{55}H_{54}N_2O_{11}Na$, found 941.3635.

N-(p-Succinoyloxymethyl)benzyloxycarbonyl-4-benzylaminobutyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5.10) A 1 M solution of NaOMe in MeOH (2.0 mL) was added to a solution of **5.9** (0.60 g, 0.69 mmol) in MeOH (25 mL) and CH_2Cl_2 (5.0 mL) and the resulting mixture was stirred for 1 h at rt. DOWEX (H^+) was added until pH = 6, the resin was filtered off, and rinsed successively with CH_2Cl_2 (5 x 5.0 mL) and MeOH (5 x 5.0 mL). The combined filtrate (~80 mL) was concentrated and dried *in vacuo* for 6 h. The crude residue was dissolved in pyridine (20 mL), succinic anhydride (0.11 g, 1.10 mmol) was added. and the resulting mixture was stirred under argon for 18 h at 65 °C. After that, the volatiles were removed under the reduced pressure, the residue was co-evaporated with toluene (3 x 10 mL), and purified by column chromatography on silica gel

(methanol – dichloromethane) to afford the title compound (0.52 g, 78%) as a colorless foam. Analytical data for **5.10**: R_f = 0.41 (methanol/ dichloromethane, 1/10, v/v); $[\alpha]_D^{21}$ +16.7 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 1.19-1.31 (m, 4H, 2x CH_2), 2,26 (s, 4H, 2x CH_2), 2.95-3.04 (m, 2H, ½ NCH_2, OH), 3.40-3.43 (m, 1H, H-5), 3.58-3.64 (m, 1H, ½ NCH_2), 3.72-3.77 (m, 3H, H-4, 6a, 6b), 4.07-4.23 (m, OCH_2, H-2, 3), 4.49-4.63 (m, 1 ½ C*H*$_2$Ph),4.73 (d, 1H, ^2J = 12.2 Hz , ½ C*H*$_2$Ph), 5.01-5.10 (m, 5H, 2 x C*H*$_2$Ph, H-1), 6.90-7.74 (m, 26h, aromatic), 8.59-8.60 (d, 2H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 24.9, 25.4, 27.4, 28.9, 55.5 (x2), 65.5, 66.4, 70.4, 73.9, 74.2, 74.5, 75.0, 82.3, 65.5, 66.4, 70.4, 73.9, 75.0, 78.9, 98.5, 127.6 (x4), 128.0 (x4), 128.1 (x4), 128.3 (x5), 128.4, 128.7 (x8), 133.1, 137.7, 148.0, 157.3, 172.2 ppm; HR-FAB MS $[M+Na]^+$ calcd 937.3426 for $C_{52}H_{54}N_2O_{13}Na$, found 937.3514.

Resin-bound acceptor (5.3) JandaJel^{TM} amine resin (1% cross-linked polystyrene, 400 mg, 0.20 mmol) was added to a solution of **5.12** (270 mg, 0.29 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 169 mg, 0.88 mmol), and DMAP (43 mg, 0.35 mmol) in CH_2Cl_2 (10.0 mL) and the resulting suspension was agitated under argon for 18 h at rt. After that, the resin was filtered off, washed with CH_2Cl_2 (3 x 20 mL), methanol (3 x 20 mL) and CH_2Cl_2 (3 x 20 mL). The combined filtrate was concentrated and dried *in vacuo* for 4 h. The loading (0.22 mmol/g) of the acceptoron the solid phase support was determined by cleaving off the linker from 0.50 g of the resin.

5.4.2 Synthesis of Glycosyl Donors 5.4 and 5.5

Ethyl 2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-1-thio-α-D-mannopyranoside (5.12). The title compound was synthesized according to the reported procedure and its analytical data was essentially the same as reported previously.[8]

2,4-Di-*O*-benzyl-3,6-di-*O*-picoloyl-α-D-mannopyranosyl dibutyl phosphate (5.5). A mixture of **5.7** (490 mg, 0.79 mmol), dibutyl phosphate (0.43 mL, 2.39 mmol), and freshly activated molecular sieves (4 Å, 1.5 g) in CH_2Cl_2 (10 mL) was stirred under argon for 1 h at rt. After that, NIS (227 mg, 1.03 mmol) and TfOH (9 µL, 0.10 mmol) were added and the resulting mixture was stirred for 1 h at rt. The solid was filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~40 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound (0.61 g, 99%) as a clear syrup. Analytical data for **5.4**: R_f = 0.29 (ethyl acetate / hexanes, 1/1.5, v/v); $[\alpha]_D^{21}$ +20.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.84-0.92 (2 m, 6H, 2 x CH_3), 1.34-1.41 (2 m, 4H, 2 x CH_2), 1.53-1.65(2 m, 4H, 2 x CH_2), 3.89-4.12 (m, 5H, H-2, 2 x CH_2), 4.22-4.25 (m, 1H, H-5), 4.38 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 4.55-4.70 (m, 5H, 1 ½ CH_2Ph, H-6a, 6b), 4.82 (d, 1H, ^2J = 10.8 Hz, ½ CH_2Ph), 5.57 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 5.80 (d, 1H, $J_{1,2}$ = 6.4 Hz, H-1), 7.09-8.03 (5 m, 16H, aromatic), 8.03-8.78 (m, 2H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 13.6 (x2), 18.6 (x2), 29.6, 32.2, 63.7, 68.0 (x2), 71.7, 72.0, 73.0, 74.5, 75.0, 75.3, 95.2, 125.3, 125.4, 126.9, 127.1, 127.8 (x4), 128.2 (x2), 128.3 (x4), 136.9, 137.0, 137.3, 147.5, 147.6, 150.0 (x2), 164.2, 164.5, 178.0 ppm; HR-FAB MS $[M+Na]^+$ calcd 785.2815 for $C_{40}H_{47}O_{11}NPNa$, found 785.2830.

Ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside (5.11). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[10]

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl dibutyl phosphate (5.4). A mixture of **5.11** (1.30 g, 2.0 mmol), dibutyl phosphate (1.18 mL, 6.0 mmol), and freshly activated molecular sieves (4 Å, 3 g) in CH_2Cl_2 (25 mL) was stirred under argon for 20 min at rt. After that, NIS (572 mg, 2.6 mmol) and TfOH (23μL, 0.26 mmol) were added and the resulting mixture was stirred for 18 h at rt. The solid was then filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~40 mL) was washed with sat. aq. Na_2SO_4 (10 mL) and water (3 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford the title compound (1.5 g, 95%) as a clear syrup. Analytical data for **5.4**: R_f = 0.33 (ethyl acetate / hexanes, 3/7, v/v); [α] D^{21} -49.2(c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 0.87-0.89 (2 m, 6H, 2 x CH_3), 1.35-1.44 (2 m, 4H, 2 x CH_2), 1.64-1.70 (2 m, 4H, 2 x CH_2), 4.07-4.19 (2 m, 4H, 2 x OCH_2), 4.47 (dd, 1H, *J* = 3.93 Hz, H6a) 4.60-4.68 (m, 2H, H-5, 6a), 5.75 (d, 1H, *J* = 2.2 Hz, H-1), 5.84-5.89 (m, 1H, H-2), 5.71 (d, 1H, *J* = 3.21 Hz, H-3), 6.18 (dd, 1H, *J* = 10.1 Hz, H-4), 7.21-7.57 (m, 12H, aromatic), 7.78-8.08 (m, 8H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 13.5 (x2), 13.6 (x2), 18.6, 32.1, 32.2, 62.2, 66.0, 68.2, 68.3, 69.3, 69.7, 70.5, 94.9, 128.3 (x3), 128.4 (x3), 128.6 (x3), 128.7 (x3), 128.8, 129.7 (x3), 129.8 (x3), 133.1, 133.3, 133.5, 133.6, 164.9, 165.2, 165.3, 165.7 ppm; HR-FAB MS $[M+Na]^+$ calcd 811.2451for $C_{42}H_{45}O_{13}PNa$, found 811.2493.

5.4.3 Synthesis of disaccharide Glycosyl Donors 5.17

tert-Butyldimethylsilyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5.14). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[11]

Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (5.16). The synthesis of the title compound was performed in accordance with the reported procedure and its analytical data was in accordance with that previously described.[8]

tert-Butyldimethylsilyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D glucopyranoside (5.17). A mixture of **5.15** (1.76 g, 3.48 mmol), **5.14** (1.5 g, 2.48 mmol), and freshly activated molecular sieves (4 Å, 4.5 g) in CH_2Cl_2 (550 mL) was stirred under argon for 20 min at rt. After that, NIS (2.26 g, 10 mmol) and TfOH (187 µL, 1.2 mmol) were added and the resulting mixture was stirred for 6 h at rt. The solid was then filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~800 mL) was washed with sat. aq. Na_2SO_4 (50 mL) and water (3 x 50 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford the title compound (3.0 g, 95%) as a clear syrup. Analytical data for **5.17**: R_f = 0.32 (ethyl acetate / dichloromethane, 1/19, v/v); $[\alpha]\ D^{21}$ -70.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.28 (s, 3H, CH_3), -0.27 (s, 3H, CH_3), 0.46 (s, 9H, $C(CH_3)_3$), 3.10-3.15 (m, 1H, H-5'), 3.34-3.42 (m, 2H, H-5, 6a), 3.50-3.54 (m, 2H, H-1', 6b), 3.89-4.15 (m, 4H, H-2, 3, 4, 6a'), 4.29 (d, 1H, ^2J = 12.3

Hz , ½ C*H*$_2$Ph), 4.40 (d, 1H, ^2J = 12.1 Hz , ½ C*H*$_2$Ph), 4.46-4.73 (m, 4H, 2 x C*H*$_2$Ph), 4.97 (dd, 1H, *J* = 3.21 Hz, H-3'), 5.16 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 5.31 (s, 1H, C*H*Ph), 6.68-7.61 (m, 26H, aromatic), 7.82-7.84 (m, 1H, aromatic), 8.59-8.60 (m, 1H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 4.01, 5.01, 17.7, 25.5 (x3), 58.0, 67.4, 68.7 (x2), 73.7, 73.9, 74.5, 74.9, 75.6, 75.8, 76.5, 76.8, 79.5, 93.6, 101.6, 101.7, 125.6, 126.3 (x3), 127.1 (x2), 127.7, 127.9 (x6), 128.0 (x3), 128.1, 128.4 (x9), 128.7 (x3), 129.1, 137.0, 137.4, 138.0, 138.1, 139.0, 147.7, 150.2, 164.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{64}N_2O_{13}NaSi$ 1071.4074, found 1071.4064.

tert-Butyldimethylsilyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5.18). A 1 M solution of NaOMe in MeOH (3.0 mL) was added to a solution of **5.17** (2.37 g, 2.26 mmol) in CH_2Cl_2 (10 mL) and MeOH (20 mL) and the resulting mixture was stirred for 1 h at rt. DOWEX (H^+) was added until pH = 6, the resin was filtered off, and rinsed successively with CH_2Cl_2 (5 x 10 mL) and MeOH (5 x 10 mL). The combined filtrate (~140 mL) was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound as a white amorphous solid in 98% yield (2.1 g, 2.2 mmol). Analytical data for **5.18**: R_f = 0.73 (ethyl acetate / hexane gradient elution, 1/1, v/v); $[\alpha]_D^{21}$ +11.5 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.24 (s, 3H, CH_3), -0.08 (s, 3H, CH_3), 0.51 (s, 9H, $C(CH_3)_3$), 2.20 (d, 1H, *J* = 8.52 Hz, OH), 3.03-3.08 (m, 1H, H-5'), 3.49-3.65 (m, 6H, H-6a, 6b, 2', 3', 4', 5), 3.90-4.16 (m, 5H, H-2, 3, 4, 6a', 6b'), 4.30 (d, 1H, ^2J = 12 Hz , ½ C*H*$_2$Ph), 4.38 (d, 1H, ^2J = 12 Hz , ½ C*H*$_2$Ph), 4.51-4.62 (m, 2H, C*H*$_2$Ph), 4.72 (d, 1H, ^2J = 12 Hz , ½ C*H*$_2$Ph), 4.89 (d, 1H, ^2J = 12 Hz , ½ C*H*$_2$Ph), 5.19 (d, 1H, *J* = 9 Hz, H-1), 5.30 (s, 1H, C*H*Ph), 6.72-6.82 (m, 5H,

aromatic), 7.15-7.33 (m, 14H, aromatic), 5.52-7.53 (m, 5H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 4.08, 5.34, 17.7, 25.4 (x3), 57.9, 67.0, 68.6, 68.7, 71.0, 73.8, 74.5, 75.8, 79.0, 79.3, 79.8, 93.6, 102.0, 102.2, 126.4 (x3), 127.1, 127.8 (x3), 127.9 (x3), 128.0 (x19), 128.1, 128.3 (x3), 128.6 (x3), 128.7 (x3), 129.1, 137.3, 137.8, 138.2, 138.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{54}H_{61}NO_{12}NaSi$ 966.3860, found 966.3871.

tert-Butyldimethylsilyl *O*-(2,4-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5.19). Copper (II) trifluoromethanesulfonate (80 mg, 0.22 mmol) was added to a solution of **5.18** (2.1 g, 2.2 mmol) in a 1 M soln. of BH_3 in THF (11 mL) and the resulting mixture was stirred under argon for 1 h at rt. The reaction mixture was cooled to 0 ºC and quenched with triethylamine (~1 mL) until pH = 7 and the volatiles were removed *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give **5.19** as white amorphous solid in 77% yield (1.62 g, 1.71 mmol). Analytical data for **5.19**: R*f* =0.4 (ethyl acetate-hexane, 1/1, v/v); $[\alpha]_D^{21}$ +18.5(c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.22 (s, 3H, CH_3), -0.07 (s, 3H, CH_3), 0.55 (s, 9H, $C(CH_3)_3$), 189 (dd, 1H, *J* = 6.87 Hz, OH), 2.19 (dd, 1H, *J* = 9.36 Hz, OH), 3.06-3.09 (m, 1H, H-5'), 3.33-3.65 (m, 9H, H-5. 6a, 6b, 1', 2', 3', 4', 6a', 6b'), 3.92 (dd, 1H, *J* = 9.0 Hz, H-4), 4.02 (d, 1H, *J* = 8.0 Hz, H-2), 4.18 (dd, 1H, *J* = 9.67 Hz, H-3), 4.33 (d, 1H, ^2J = 12 Hz , ½ CH_2Ph), 4.40-4.60 (m, 4H, 2x CH_2Ph), 4.75 (dd, 2H, ^2J = 12.0 Hz, CH_2Ph), 4.92 (d, 1H, ^2J = 12.0 Hz , ½ CH_2Ph), 5.23 (d, 1H, *J* = 9.0 Hz, H-1), 6.77-6.84 (m, 5H, aromatic), 7.13-7.25 (m, 15H, aromatic), 7.53-7.56 (m, 4H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$); δ, 4.7, 5.2, 17.7, 25.5 (x2), 58.3, 62.5, 68.7, 73.8, 74.4, 74.5, 74.9, 75.0, 75.3, 77.0, 78.4, 79.2, 93.6, 101.5,

107.5, 127.3 (x2), 127.5 (x2), 128.0 (x6), 128.1 (x6), 128.6 (x4), 128.7 (x4), 128.8 (x4), 137.9, 138.3, 138.4, 138.5 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{54}H_{63}NO_{12}NaSi$ 968.4016, found 968.4003.

tert-Butyldimethylsilyl *O*-(2,4-di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5.20)

Picolinic acid (2.08 g, 16.9 mmol), 3-(ethyliminomethyleneamino)-*N*,*N*-dimethylpropan-1-amine (EDC, 3.23 g, 1.69 mmol), and DMAP (237 mg, 1.94 mmol) were added to a solution of **5.18** (1.22 g, 1.67 mmol) in CH_2Cl_2 (70 mL) and the resulting mixture was stirred under argon for 20 min at rt. The reaction mixture was diluted with CH_2Cl_2 (~100 mL) and was washed with cold water (10 mL), sat. aq. $NaHCO_3$ (10 mL), and water (10 mL). The organic phase was separated, dried with magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound white amorphous solid in 95% yield (1.67 g, 1.44 mmol). Analytical data for **5.20**: R*f* = 0.25 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{21}$ -36.5 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, -0.13, 0.02 (2 s, 6H, 2 x CH_3), 0.64 (s, 9H, $C(CH_3)_3$), 3.51-3.72 (m, 4H, H-5, 5', 6a, 6b), 4.09-4.29 (m, 5H, H-2, 3, 4, 4', 6b'), 4.41 (dd, 1H, *J* = 6 Hz, H-6a'), 4.48-4.89 (m, 10H, 4 x C*H*$_2$Ph, H-1', 2'), 5.16 (dd, 1H, *J* = 6.0 Hz, H-3'), 5.29 (d, 1H, *J* = 9.0 Hz, H-1), 6.62-6.77 (m, 5H, aromatic), 7.09-7.80 (m, 23H, aromatic), 7.96-8.01 (m, 2H, aromatic), 8.61-8.63 (m, 1H, aromatic), 8.78-82 (m, 1H , aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$); 4.0, 5.3, 17.7, 25.5 (x3), 58.1, 64.5, 68.6, 73.3, 73.4, 73.7, 74.5, 74.8, 74.9, 75.0, 75.7, 76.7, 80.1, 93.6, 101.3, 107.5, 125.4, 125.7, 126.9 (x2), 127.2, 127.7, 127.7 (x2), 127.9, (x4), 128.0 (x5), 128.2 (x8), 128.3 (x2), 128.5 (x2), 128.7 (x2), 137.1, 137.6, 137.7, 137.9, 138.4, 138.9, 147.7, 147.8,

149.9, 150.3, 164.3, 164.7 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{66}H_{69}N3O_{14}NaSi$ 1178.4447 found 1178.4457

***O*-(2,4-Di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose (5.21)** A 1 M soln. of tetrabutylammonium fluoride (TBAF, 4.7 mmol) in THF (1.36 mL) was added to a solution of compound **5.20** (1.65 g, 1.4 mmol) in dry THF (45 mL) and the resulting mixture was stirred for 2 h at rt. The volatiles were removed under the reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford the title compound that was contaminated with a tetrabutyl ammonium salt. To simplify the separation, the entire mixture was dissolved in pyridine (10 mL), acetic anhydride (5 mL) was added and the resulting mixture was stirred for 18 h at rt. The reaction mixture was quenched with CH_3OH (~1.0 mL) and the volatiles were removed under the reduced pressure. The residue was diluted with CH_2Cl_2 (10 mL) and washed with 1 N HCl (2 x 5 mL), water (10 mL), sat. aq. $NaHCO_3$ (10 mL), and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford the acetylated intermediate. The latter was dissolved in DMF (10 mL), hydrazine acetate (80 mg) was added and the resulting mixture was stirred for 1h. The reaction mixture was concentrated by continuous flashing with a stream of air. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound as a white amorphous solid in 60% yield (850 mg, 0.84 mmol). Analytical data for **5.20**: R*f* = 0.42 (ethyl acetate/hexane, 1/1.5, v/v); $[\alpha]_D^{22}$ +28.6 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ, 3.52-3.71 (m, 4Hm H-

5, 6a, 6b, 5'), 3.98-4.17 (m, 4H, H-2, 4, 4', 6a'), 4.27 (dd, 1H, *J* = 9.0 Hz, H-3), 4.41-4.72 (m, 9H, 3x C*H*$_2$Ph, H-1', 2, 6b'), 4.85 (dd, 2H, ^2J = 12.0 Hz, C*H*$_2$Ph), 5.04 (dd, 1H, *J* = 3.0 Hz, H-3'), 5.28 (d, 1H, *J* = 9.0 Hz, H-1), 6.60-6.75 (m, 4H, aromatic), 7.07-7.99 (m, 26H, aromatic), 8.62-8.94 (m, 1H, aromatic) 8.78-8.79 (m, 1H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$); 57.3, 64.0, 68.3, 72.7, 73.0, 74.3, 74.4, 74.6, 74.7, 75.6, 79.5, 92.7, 100.8, 123.1, 125.1, 125.4, 126.6 (x2), 127.0 (x2), 127.4 (x6), 127.5 (x3), 127.8 (x6), 129.9 (x5), 128.1 (x2), 128.5 (x2), 131.4 (x2), 133.5, 136.8, 137.1, 137.3, 137.4, 138.0, 138.5, 147.2, 147.3, 149.6, 150.0, 162.5, 163.9, 164.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{55}N_3O_{14}Na$ 10643581, found 1064.3549

***O*-(2,4-Di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (5.6).** CCl_3CN (80 μL, 4.0 mmol) and DBU (3.0 μL, 0.02 mmol) were added to a solution of **5.21** (200 mg, 0.2 mmol) in CH_2Cl_2 (5.0 mL) and the resulting mixture was stirred for 2 h at rt. After that, the volatiles were removed *in vacuo* and the residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to give the title compound in 83% yield (192 mg, 0.165 mmol) as a white amorphous solid. Analytical data for **5.6**: R_f = 0.45 (ethyl acetate/hexane, 6/4, v/v); $[\alpha]_D^{22}$ +33.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ, 3.54-3.67 (m, 1H, H-5'), 3.68-3.82 (m, 3H, H-4, 2',4'), 4.29-4.89 (m, 11H, 3 x C*H*$_2$Ph, H-1', 2, 3, 6a', 6b') 4.86 (dd, 2H, ^2J = 12 Hz, C*H*$_2$Ph), 5.05 (dd, 1H, *J* = 9 Hz, H-3'), 6.37 (d, 1H, *J* = 9 Hz, H-1), 6.60-6.76 (m, 5H, aromatic), 7.09-8.03 (m, 26H, aromatic), 8.51 (s, 1H, NH), 8.64-8.66 (d, 1H, aromatic), 8.78-8.80 (d, 1H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ, 14.2, 21.0, 54.5, 60.3, 64.2, 67.9, 72.9, 73.1, 73.5, 74.6, 74.7, 74.8, 75.5, 75.6, 79.1, 90.3,

94.0, 100.8, 123.2, 125.2, 125.6, 126.7,126.8, 127.1, 127.5 (x2), 127.6 (x2), 127.8 (x2), 128.8 (x3), 127.9 (x3), 128.0 (x4), 128.1 (x2), 128.3 (x3), 128.6 (x2), 131.3, 133.7, 136.9, 137.3, 137.4, 137.5, 138.1, 138.5, 147.4, 147.5, 149.8, 150.1, 160.8, 164.1, 164.4 ppm; HR-FAB MS $[M+Na]^+$ calcd 1207.2678 for $C_{62}H_{55}Cl_3N_4O_{14}Na$, found 1207.2658

5.4.4 HPLC-mediated synthesis of disaccharide and pentasaccharide

A general procedure for glycosylation and cleavage. Functionalized JandaJel resin 5.3 (50 mg, 0.011 mmol) was packed in an Omnifit^{TM} glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL,). The system was then switched to the recirculation mode and the delivery of CH_2Cl_2 continued for 30 min at 1.0 mL/min (swelling). After that, pump D was stopped and pump C was programmed to deliver a solution of glycosyl donor (5.5 - 5.4 - 5.6 0.11 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min. This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of the promoter in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in CH_3OH/CH_2Cl_2 (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min. Pump B was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H^+) resin.

The resin was filtered off, washed successively with CH_2Cl_2 and CH_3OH, and the combined filtrate was concentrated in vacuo to afford the crude residue that was subjected to subsequent acetylation.

A general procedure for acetylation of released oligosaccharide. A crude residue was redissolved in pyridine (2.0 mL), Ac_2O (73 μL, 0.771 mmol) was added dropwise and the resulting mixture was stirred for 16 h at rt. The reaction mixture was quenched with CH_3OH (~1.0 mL) and the volatiles were removed under the reduced pressure. The residue was diluted with CH_2Cl_2 (20 mL) and washed with 1 N HCl (2 x 10 mL), water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and water (2 x 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford oligosaccharide**.**

***N*-(*p*-Acetoxymethyl)benzyloxycarbonyl-4-benzylaminobutyl *O*-(4,6-di-*O*-acetyl-2,3-*O*-benzyl-α/β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D glucopyranoside (5.13).** Functionalized JandaJel resin **5.3** (50 mg, 0.011 mmol) was packed in an Omnifit™ glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL, step 1). The system was then switched to the recirculation mode and the delivery of CH_2Cl_2 continued for 30 min at 1.0 mL/min (swelling, step 2). After that, pump D was stopped and pump C was programmed to deliver a solution of donor **5.4** (83 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 3). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (20 μL, 0.11 mmol) in CH_2Cl_2 (3 x

100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 4). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in CH_3OH/CH_2Cl_2 (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min (step 5). Pump B was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H^+) resin. The resin was filtered off, washed successively with CH_2Cl_2 and CH_3OH, and the combined filtrate was concentrated in vacuo to afford the crude residue that was subjected to subsequent acetylation in accordance with the general procedure, as described for the synthesis of compound 5.13. The crude residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford tetrasaccharide **5.13** in 86% yield. Analytical data for **5.13**: R_f = 0.52 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{22}$ + 1.6 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 1.29-1.55 (m, 4H, CH_2), 1.89-2.07 (m, 9H, 3 x CH_3), 2.90-3.12 (m, 2H, CH_2), 3.20-40 (m, 2H), 3.5-4.10 (m, 7H), 4.12-4.59 (m, 11H, 4 x CH_2Ph), 5.05-5.12 (m, 6H), 6.87-7.34 (m, 28H, aromatic), 7.58-7.82 (m, 5, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.7, 26.7, 51.2, 56.0, 56.5, 68.5, 68.8, 71.3, 73.0, 73.2, 74.1, 74.6, 74.7, 74.9, 75.8, 76.1, 78.7, 97.4, 98.4, 123.5, 124.0, 127.3, 127.7 (x3), 127.8, 128.1 (x3), 128.1 (x3), 128.2 (x6), 128.3 (x3), 128.5 (x3), 128.6 (x3), 128.9 (x4), 132.0, 132.2, 134.3, 134.4, 138.7 (x2), 139.0, 168.8, 168.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{74}H_{78}N_2O_{18}Na$ 1305.5148, found 1305.5157.

***N*-(*p*-Acetoxymethyl)benzyloxycarbonyl-4-benzylaminobutyl di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3,1→6)-*O*-(2-*O*-benzyl-α/β-D mannopyranosyl)-(1→4)-*O*-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5.2).** Functionalized JandaJel resin **5.3** (50 mg, 0.011 mmol) was packed in an Omnifit^{TM} glass chromatography column and the latter was integrated into the HPLC system. Pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 5 min (5 mL, step 1). The system was then switched to the recirculation mode and the delivery of CH_2Cl_2 continued for 30 min at 1.0 mL/min (swelling, step 2). After that, pump D was stopped and pump C was programmed to deliver a solution of donor 5.5 (128 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 3). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (20 μL, 0.11 mmol) in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 4). After that, pump D was stopped and pump A was programmed to deliver a solution of $Cu(AcO)_2$/MeOH/CH_2Cl_2 (50mg/1/10, g/v/v) for 20 min at 1.0 mL/min (step 5). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). After that, pump A was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 6). After that, pump D was stopped and pump C was programmed to deliver a solution of donor 5.6 (87 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 7). This step was

monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (20 μL, 0.11 mmol) in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 8). After that, pump D was stopped and pump C was programmed to deliver a solution of donor 5.6 (87 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at a flow rate of 0.5 mL/min (step 11). This step was monitored by the integrated UV detector (λ_{max} = 254 nm). The integrated autosampler was programmed to inject a solution of TMSOTf (20 μL, 0.11 mmol) in CH_2Cl_2 (3 x 100 μL) at 10, 12, and 14 min and the resulting mixture (~2.3 mL) was recirculated for 60-90 min until the UV detector recorded no change in absorbance of the eluate. After that, pump C was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min, and the eluate was discarded after washing for 10 min (10 mL, step 12). After that, pump D was stopped and pump B was programmed to deliver a 0.1 M solution of NaOMe in CH_3OH/CH_2Cl_2 (10 mL, 0.04/1/1, v/v/v) that was recirculated at 1.0 mL/min for 20 min (step 13). Pump B was stopped and pump D was programmed to deliver CH_2Cl_2 at 1.0 mL/min for 10 min, and the combined eluate was neutralized with Dowex (H^+) resin. The resin was filtered off, washed successively with CH_2Cl_2 and CH_3OH, and the combined filtrate was concentrated in vacuo to afford the crude residue that was subjected to subsequent acetylation in accordance with the general procedure, as described for the synthesis of compound **5.2**. The crude residue was purified by column chromatography on silica gel (ethyl acetate – hexane gradient elution) to afford pentasaccharide **5.2** in 31% yield. Analytical data for **5.2**: R_f = 0.6 (ethyl

acetate/hexane, 6/4, v/v); $[\alpha]_D^{22}$ +40.7 (c = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): 1.19-1.24 (m, 4H, 2 x CH_2), 1.79-2.12 (m, 27H, 9 x CH_3), 2.90-2.99 (m, 2H), 3.24-3.37 (m, 5H), 3.44-3.87 (m, 13H), 4.07-4.20 (m, 10H), 4.37-4.63 (m, 8H), 4.71-4.89 (m, 6H), 5.00-5.16 (m, 6H), 5.20-5.35 (m, 7H), 6.69-6.93 (m, 12H, aromatic), 7.17-7.54 (m, 36H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$); δ, 14.3, 20.6, 20.7 (x3), 20.8 (x5), 20.9**,** 21.1 (x2), 26.5, 31.0, 55.7, 56.4, 60.4, 62.4, 62.5, 62.4, 62.5, 65.8, 66.0 (x3), 66.1, 66.7, 66.8, 68.5, 68.9, 69.0, 62.2, 69.5 (x2), 72.7, 73.4, 74.1, 74.4, 74.5, 74.6, 74.8, 75.0, 75.1 (x2), 76.7, 77.8, 78.6, 82.1, 97.4, 97.5, 98.1, 99.7, 101.1, 123.2, 123.5, 126.1, 127.1 (x2), 127.2 (x2), 127.3, 127.4 (x2), 127.5 (x4), 127.6, 127.7 (x3), 127.8 (x3), 127.9 (x3), 128.0 (x10), 128.2 (x2), 128.3 (x3), 128.4 (x6), 128.5 (x4), 128.7, 131.5, 131.9, 133.7, 134.0, 135.6, 136.9 (x2), 137.8, 137.9, 138.0, 138.5, 138.7 (x2), 167.5, 168.4, 169.6, 169.7, 169.8, 170.0, 170.5, 170.7, 170.9 ppm; HR-FAB MS $[M+Na]^+$ calcd 2352.8520 for $C_{126}H_{135}N_3O_{40}Na$ found 2352.8545.

5.5 References

1. (a) Varki, A., Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* 1993, *3* (2), 97-130; (b) Dwek, R. A., Glycobiology: toward understanding the function of sugars. *Chem. Rev.* 1996, *96*, 683-720; (c) Ernst, B.; Hart, G. W.; Sinaý, P., Frontmatter. In *Carbohydrates in Chemistry and Biology*, Wiley-VCH Verlag GmbH: 2008; pp I-LXII.

2. Winter, M., Supports for solid-phase organic synthesis. In *Combinatorial peptide and nonpeptide libraries: a handbook*, Jung, G., Ed. VCH: Wienheim, New York, Basel, Cambridge, Tjokyo, 1996; pp 465-510.

3. Smoot, J. T.; Demchenko, A. V., Oligosaccharide synthesis: from conventional methods to modern expeditious strategies. *Adv. Carbohydr. Chem. Biochem.* 2009, *62*, 161-250.

4. (a) Frechet, J. M.; Schuerch, C., Solid-phase synthesis of oligosaccharides. I. Preparation of the solid support. Poly[p-(1-propen-3-ol-1-yl)styrene]. *J. Am. Chem. Soc.* 1971, *93*, 492-496; (b) Schmidt, R. R.; Jonke, S.; Liu, K., New Aspects of Glycoside Bond Formation: Solid-Phase Oligosaccharide Synthesis In *ACS Symp. Ser. (Frontiers in Modern Carbohydrate Chemistry)* Demchenko, A. V., Ed. Oxford Univ. Press: 2007; Vol. 960, pp 209-237; (c) Seeberger, P. H., Solid phase oligosaccharide synthesis (Reprinted from Glycochemistry: Principles, synthesis, and applications, pg 1- 32, 2001). *J. Carbohydr. Chem.* 2002, *21* (7-9), 613-643; (d) Tanaka, K.; Fukase, K., Oligosaccharide synthesis on solid, soluble polymer, and tag supports. In *Solid-Phase Organic Synthesis*, Toy, P. H.; Lam, Y., Eds. John Wiley & Sons, Inc. : Hoboken, 2012; pp 489-530; (e) Palmacci, E. R.; Plante, O. J.; Hewitt, M. C.; Seeberger, P. H., Automated Synthesis of Oligosaccharides. *Helv. Chim. Acta* 2003, *86* (12), 3975-3990.

5. (a) Codée, J. D. C.; Kröck, L.; Castagner, B.; Seeberger, P. H., Automated solid-phase synthesis of protected oligosaccharides containing β-mannosidic linkages. *Chem. Eur. J.* 2008, *14* (13), 3987-3994; (b) Sears, P.; Wong, C. H., Toward automated synthesis of oligosaccharides and glycoproteins. *Science* 2001, *291* (5512), 2344-2350; (c) Tanaka, H.; Matoba, N.; Tsukamoto, H.; Takimoto, H.; Yamada, H.; Takahashi, T., Automated Parallel Synthesis of a Protected Oligosaccharide Library Based upon the Structure of Dimeric Lewis X by One-Pot Sequential Glycosylation *Synlett* 2005, 824-828; (d) Machida, K.; Hirose, Y.; Fuse, S.; Sugawara, T.; Takahashi, T., Development and application of a solution-phase automated synthesizer, 'ChemKonzert'. *Chem. Pharm. Bull.* 2010, *58*, 87-93; (e) Pistorio, S. G.; Nigudkar, S. S.; Stine, K. J.; Demchenko, A. V., HPLC-assisted automated

oligosaccharide synthesis: the implementation of the autosampler as a mode of the reagent delivery. *The Journal of Organic Chemistry* 2016.

6. (a) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H., Automated solid-phase synthesis of oligosaccharides. *Science* 2001, *291* (5508), 1523-1527; (b) Palmacci, E. R.; Plante, O. J.; Seeberger, P. H., Oligosaccharide synthesis in solution and on solid support with glycosyl phosphates. *Eur. J. Org. Chem.* 2002, 595-606; (c) Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H., Oligosaccharide synthesis with glycosyl phosphate and dithiophosphate triesters as glycosylating agents. *J. Am. Chem. Soc.* 2001, *123*, 9545-9554; (d) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H., Development of an automated oligosaccharide synthesizer. *Adv. Carbohydr. Chem. Biochem.* 2003, *58*, 35-54.

7. (a) Crich, D., Methodology Development and Physical Organic Chemistry: A Powerful Combination for the Advancement of Glycochemistry. *J. Org. Chem.* 2011, *76*, 9193-9209; (b) Crich, D., Mechanism of a chemical glycosylation reaction. *Acc. Chem. Res.* 2010, *43*, 1144-1153.

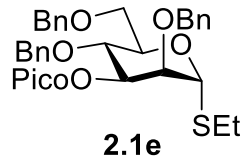
8. Pistorio, S. G.; Yasomanee, J. P.; Demchenko, A. V., Hydrogen bond-mediated aglycone delivery: focus on β-mannosylation. *Org. Lett.* 2014, *16*, 716-719.

9. Nagorny, P.; Fasching, B.; Li, X.; Chen, G.; Aussedat, B.; Danishefsky, S. J., Toward Fully Synthetic Homogeneous β-Human Follicle-Stimulating Hormone (β-hFSH) with a Biantennary N-Linked Dodecasaccharide. Synthesis of β-hFSH with Chitobiose Units at the Natural Linkage Sites. *J. Am. Chem. Soc.* 2009, *131*, 5792-5799.

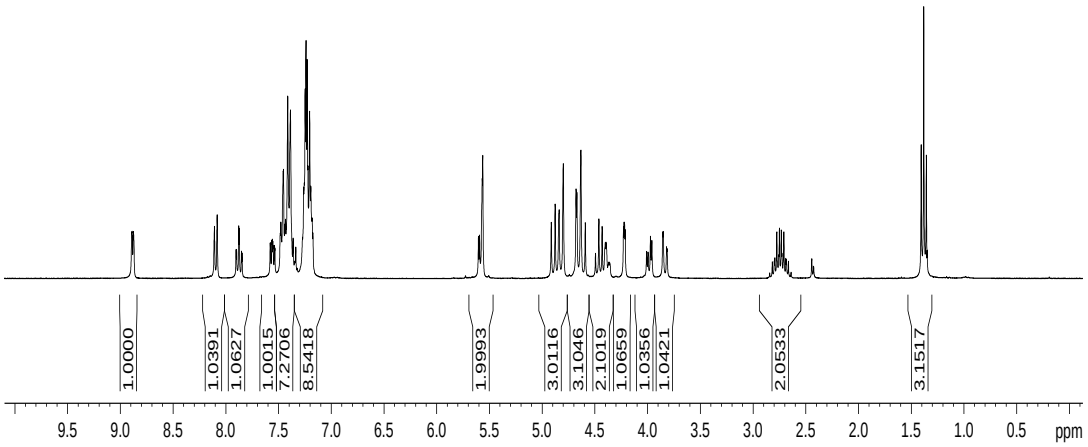
10. Sail, D.; Kováč, P., Benzoylated ethyl 1-thioglycosides: direct preparation from per-O-benzoylated sugars. *Carbohydrate Research* 2012, *357*, 47-52.

11. Chang, R.; Moquist, P.; Finney, N. S., Chemical synthesis of UDP-4-O-methyl-GlcNAc, a potential chain terminator of chitin synthesis. *Carbohydrate Research* 2004, *339* (8), 1531-1536.

APPENDIX

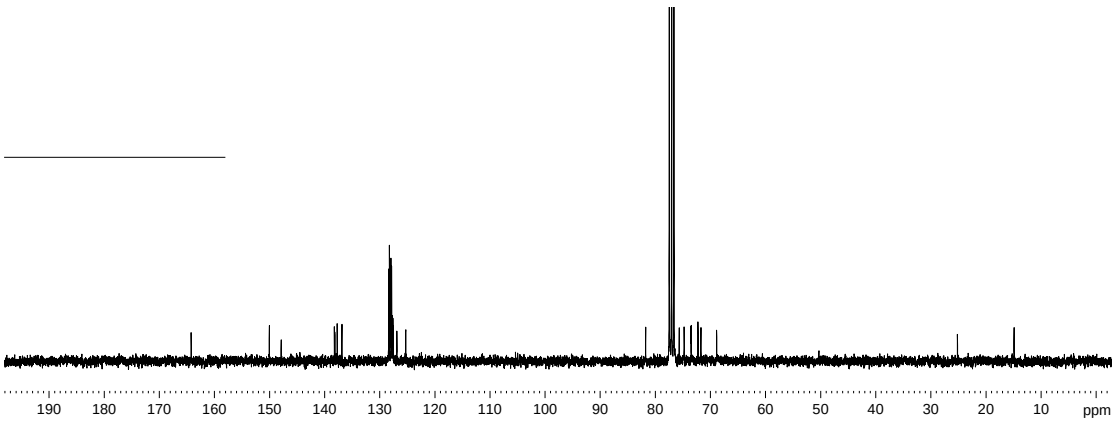


2.1e



$CDCl_3$ 300 MHz

Figure A-1: ^{1}H NMR spectrum of Ethyl 2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (**2.1e**)



$CDCl_3$ 75 MHz

Figure A-2: ^{13}C NMR spectrum of Ethyl 2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (**2.1e**)

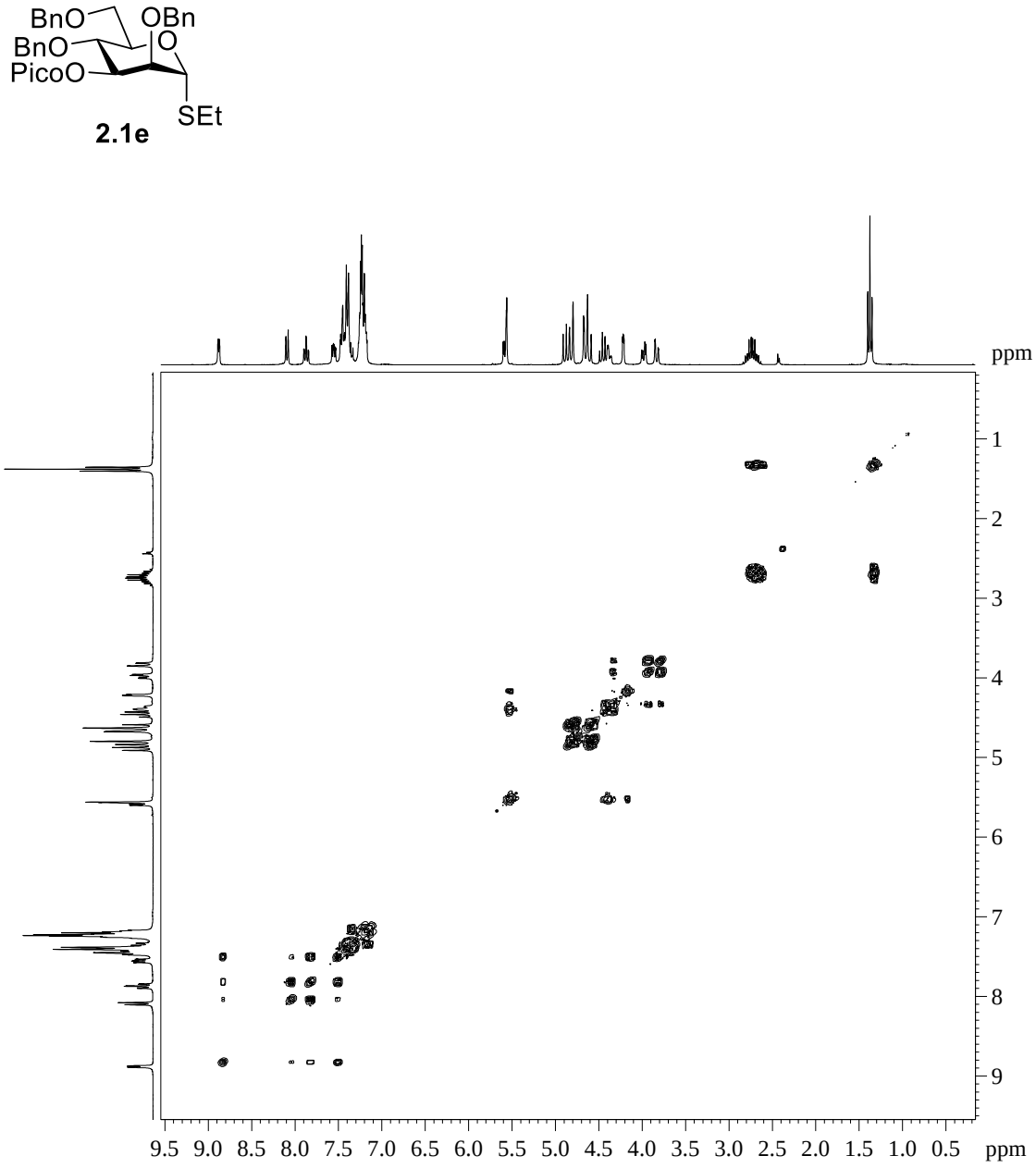
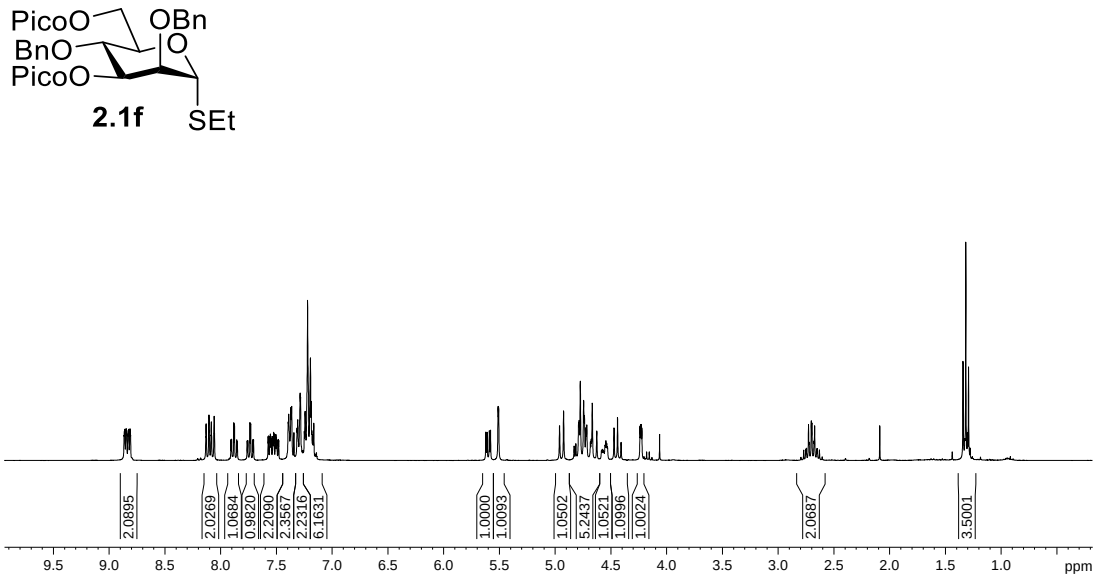
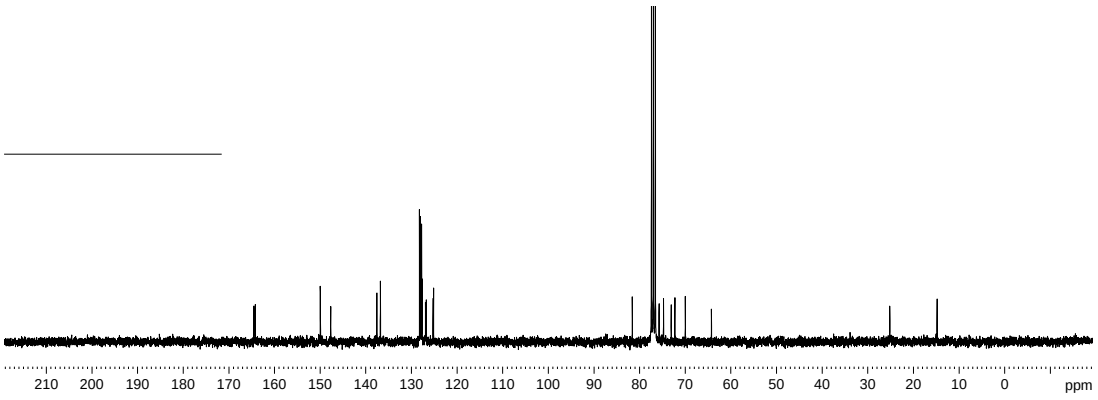


Figure A-3: 2-D NMR COSY spectrum of Ethyl 2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside (**2.1e**)



$CDCl_3$ 300 MHz

Figure A-4: ^{1}H NMR spectrum of Ethyl 2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1f)**



$CDCl_3$ 75 MHz

Figure A-5: ^{13}C NMR spectrum of Ethyl 2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1f)**

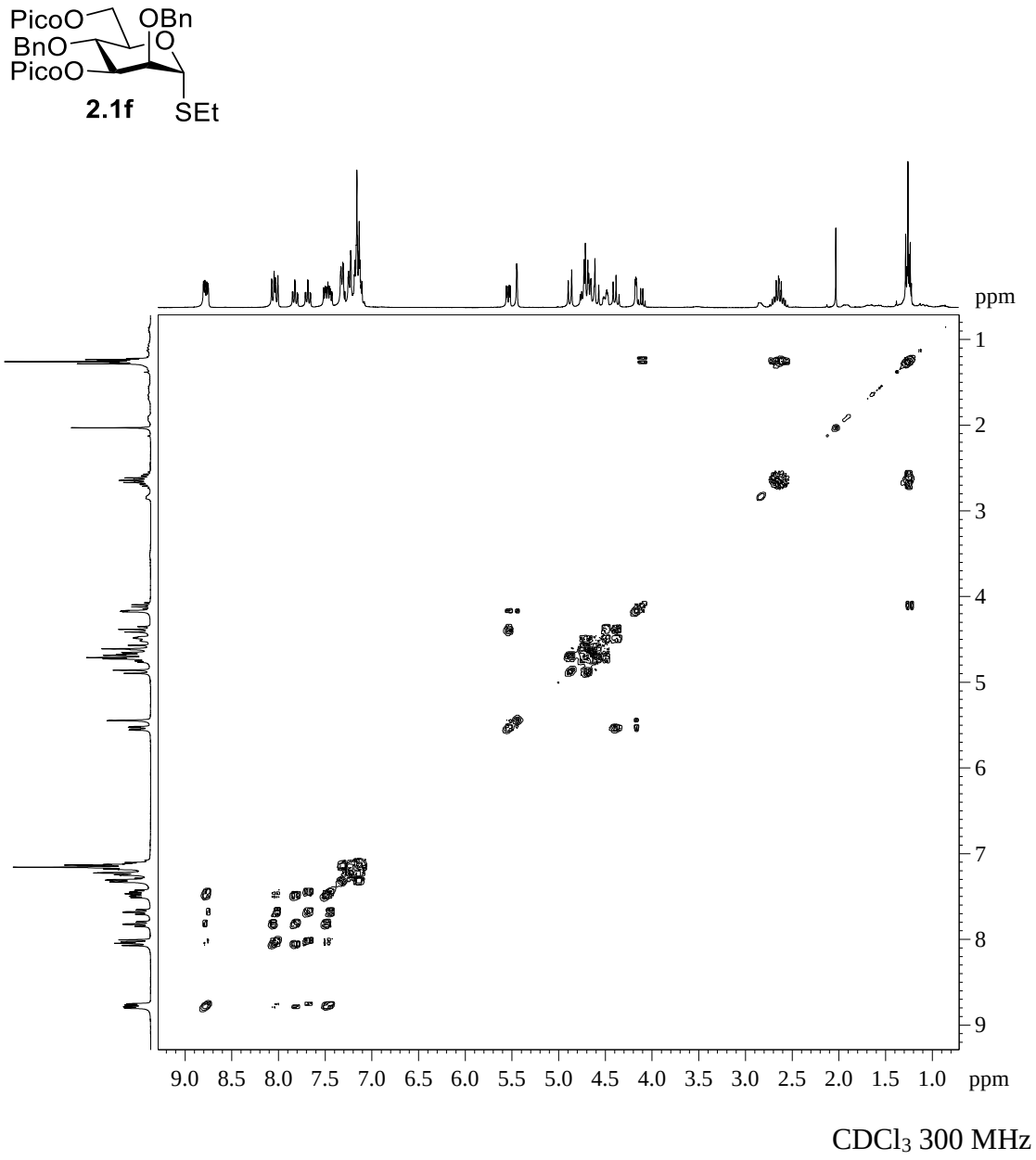
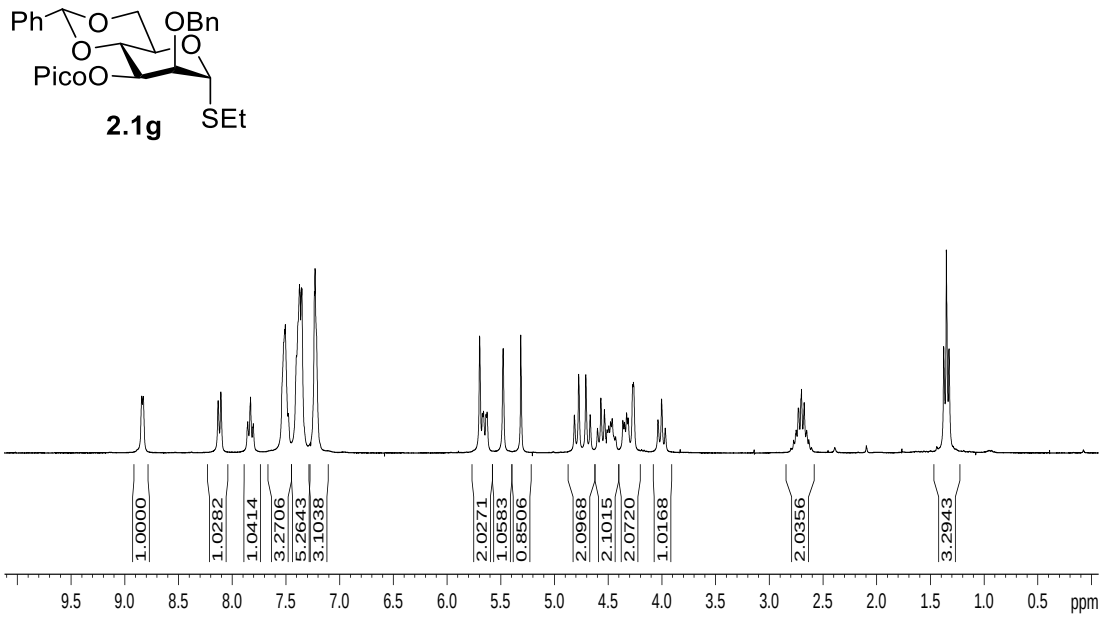
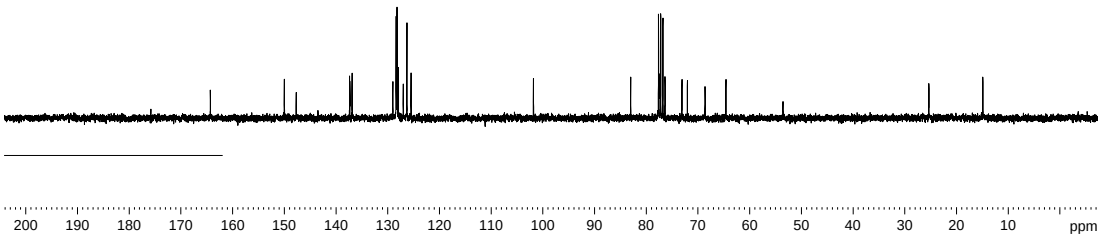


Figure A-6: 2-D NMR COSY spectrum of Ethyl 2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1f)**



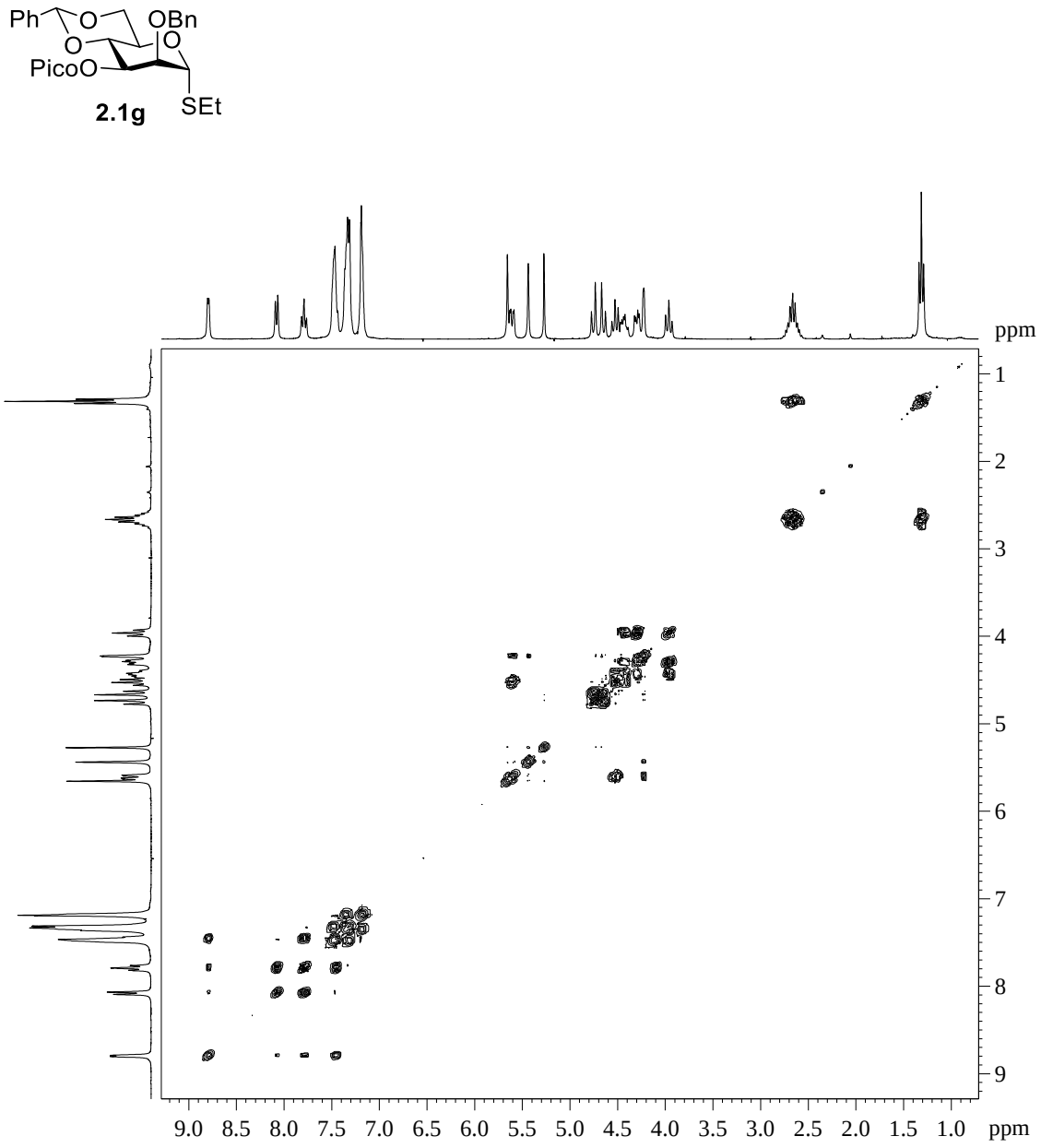
$CDCl_3$ 300 MHz

Figure A-7: ^{1}H NMR spectrum of Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1g)**



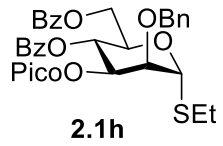
$CDCl_3$ 75 MHz

Figure A-8: ^{13}C NMR spectrum Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1g)**

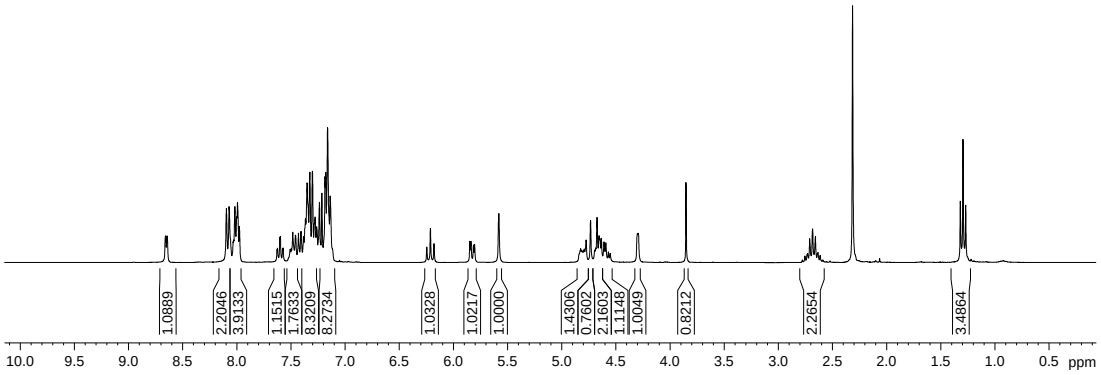


$CDCl_3$ 300 MHz

Figure A-9: 2-D NMR COSY spectrum Ethyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1g)**

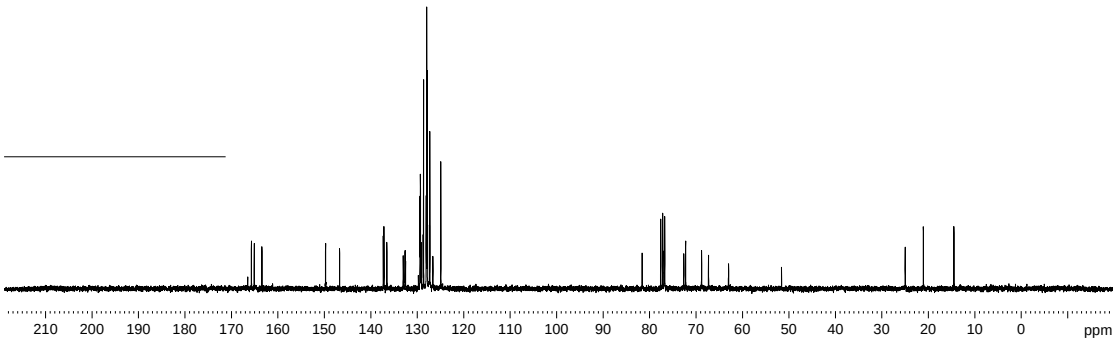


2.1h



$CDCl_3$ 300 MHz

Figure A-10: ^{1}H NMR spectrum Ethyl 4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1h).**



$CDCl_3$ 75 MHz

Figure A-11: ^{13}C NMR spectrum Ethyl 4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1h).**

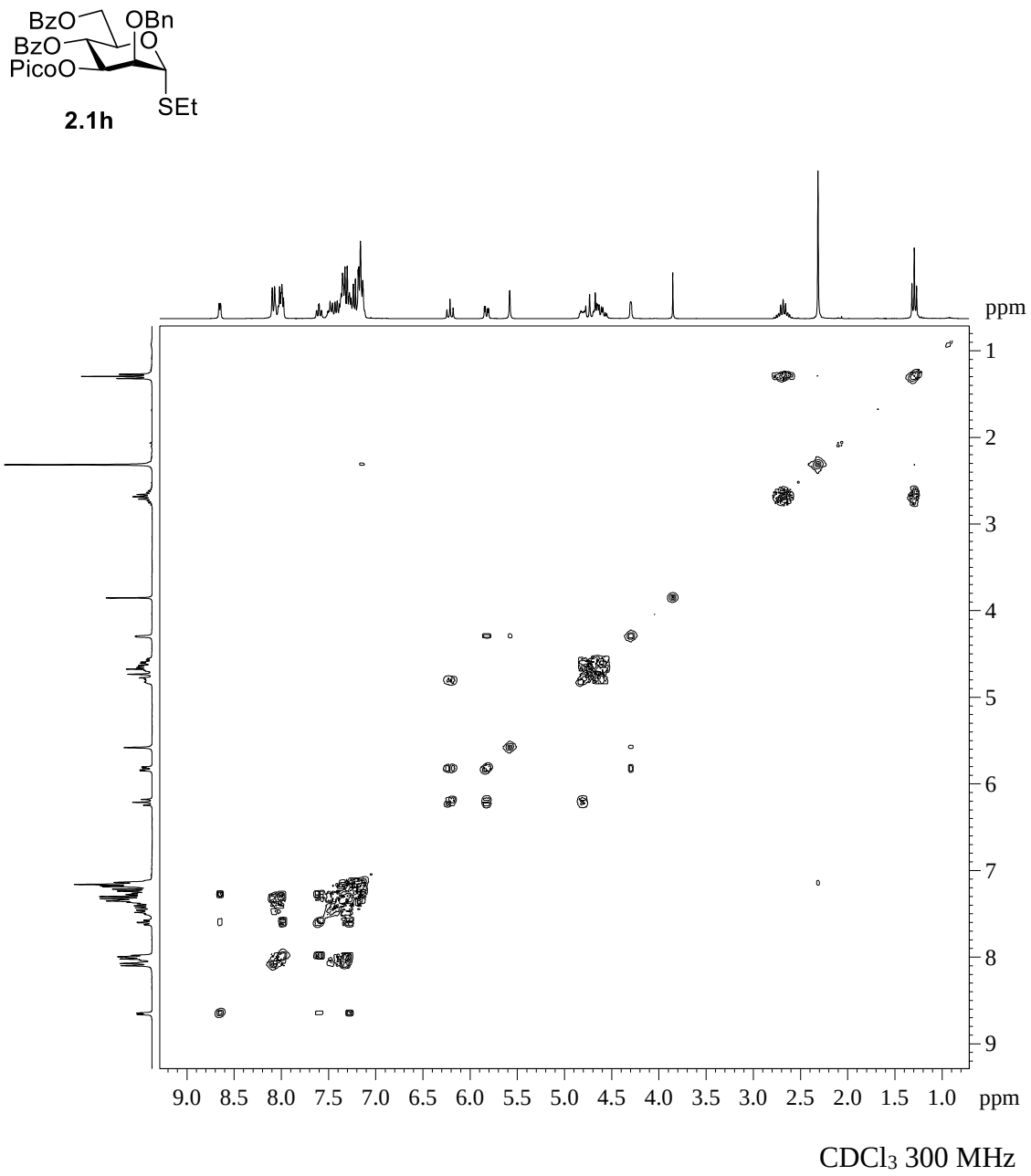
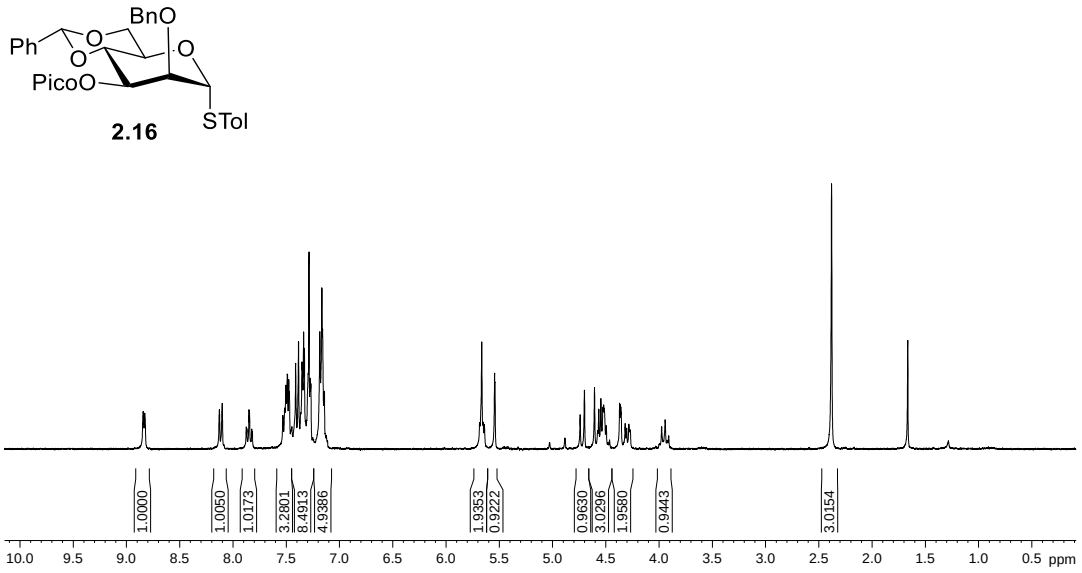
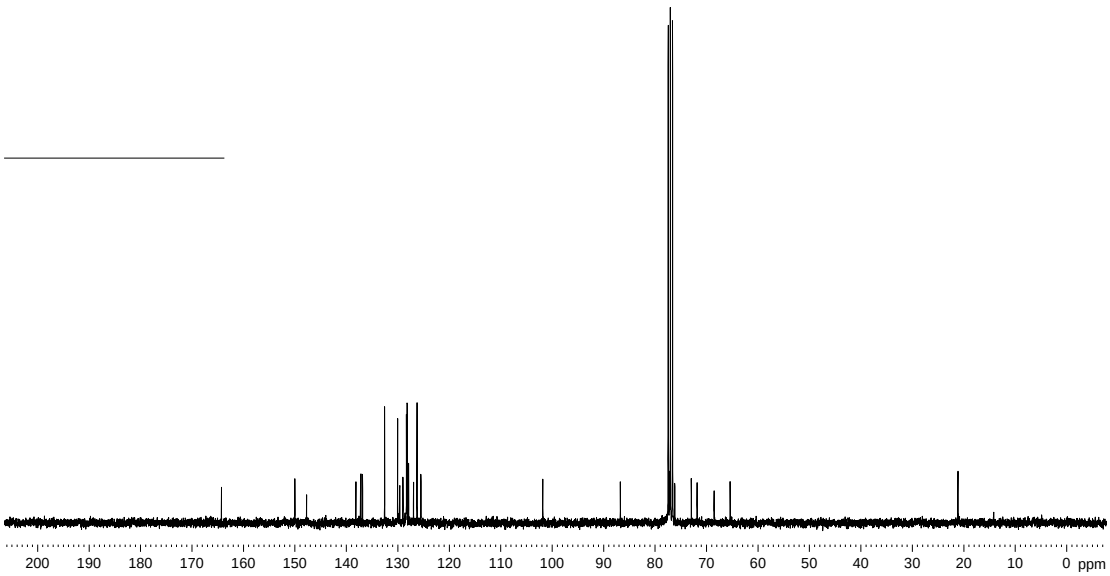


Figure A-12: 2-D NMR COSY spectrum of Ethyl 4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.1h).**



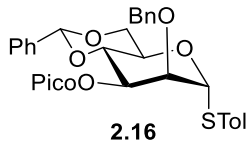
$CDCl_3$ 300 MHz

Figure A-13: ^{1}H NMR spectrum of *p*-Tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.16)**

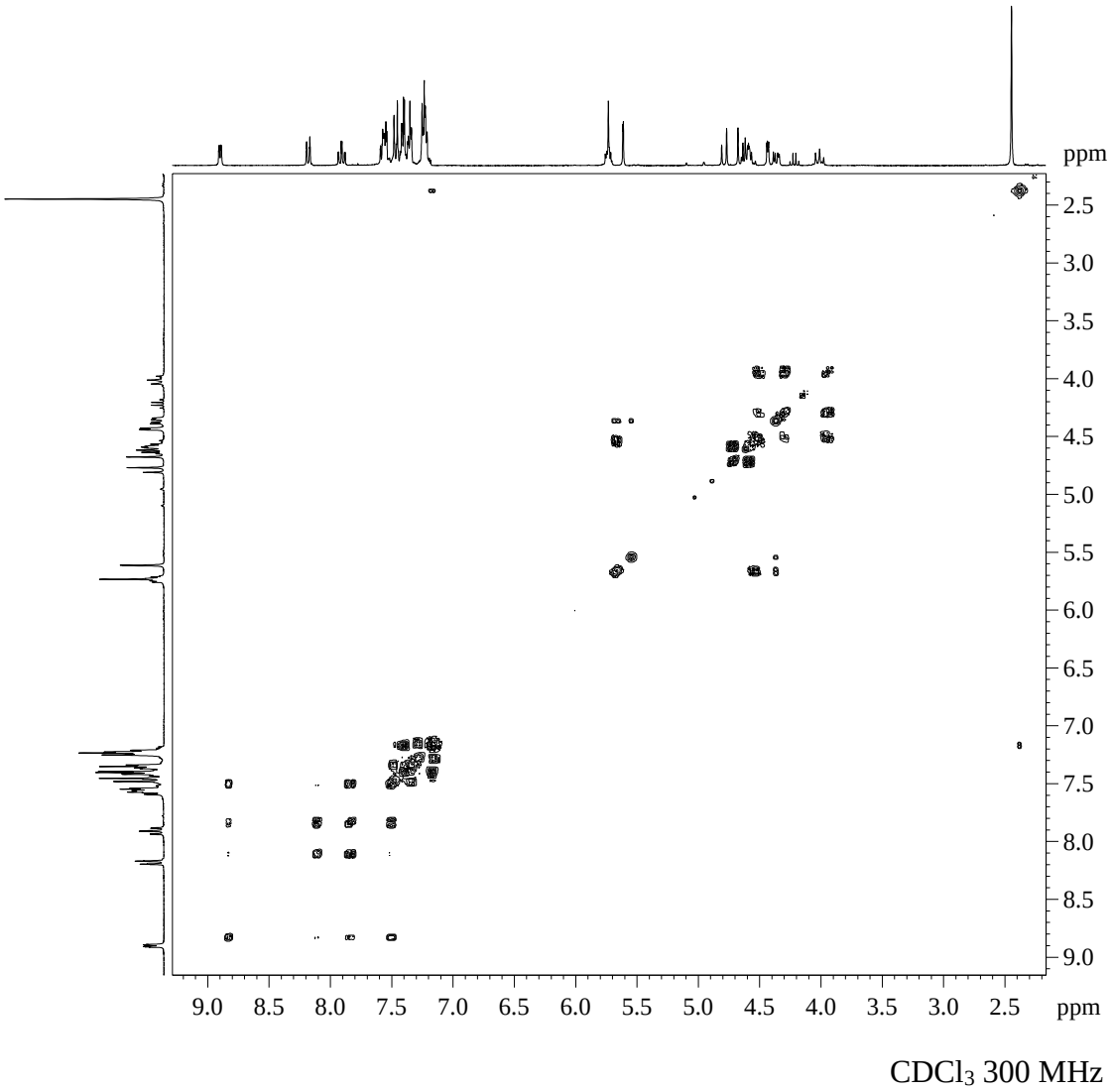


$CDCl_3$ 75 MHz

Figure A-14: ^{13}C NMR spectrum of *p*-Tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.16)**

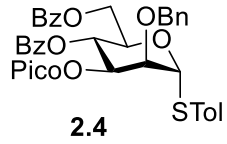


2.16

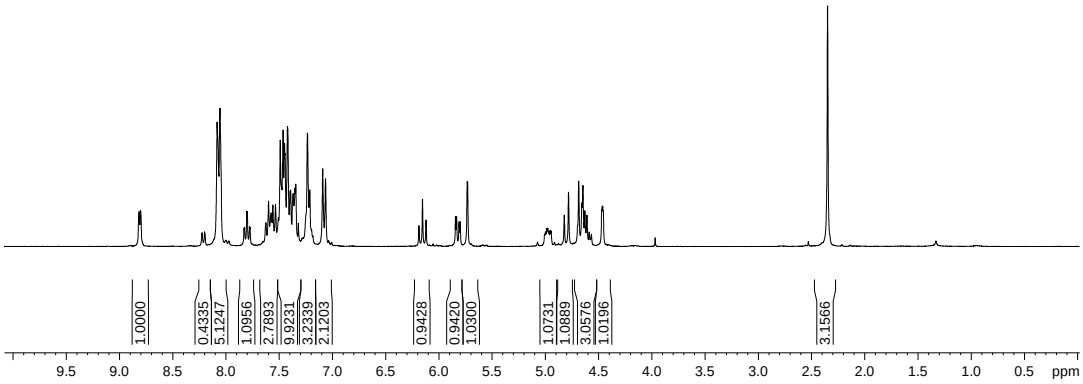


$CDCl_3$ 300 MHz

Figure A-15: 2-D NMR COSY spectrum of *p*-Tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.16)**

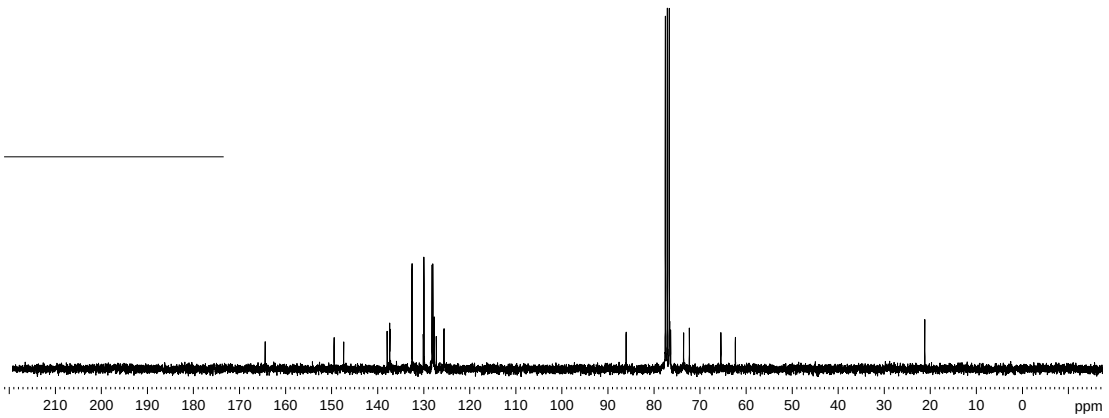


2.4



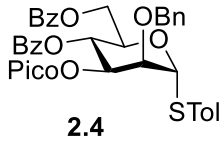
$CDCl_3$ 300 MHz

Figure A-16: ^{1}H NMR spectrum of *p*-Tolyl 4,6-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.4)**

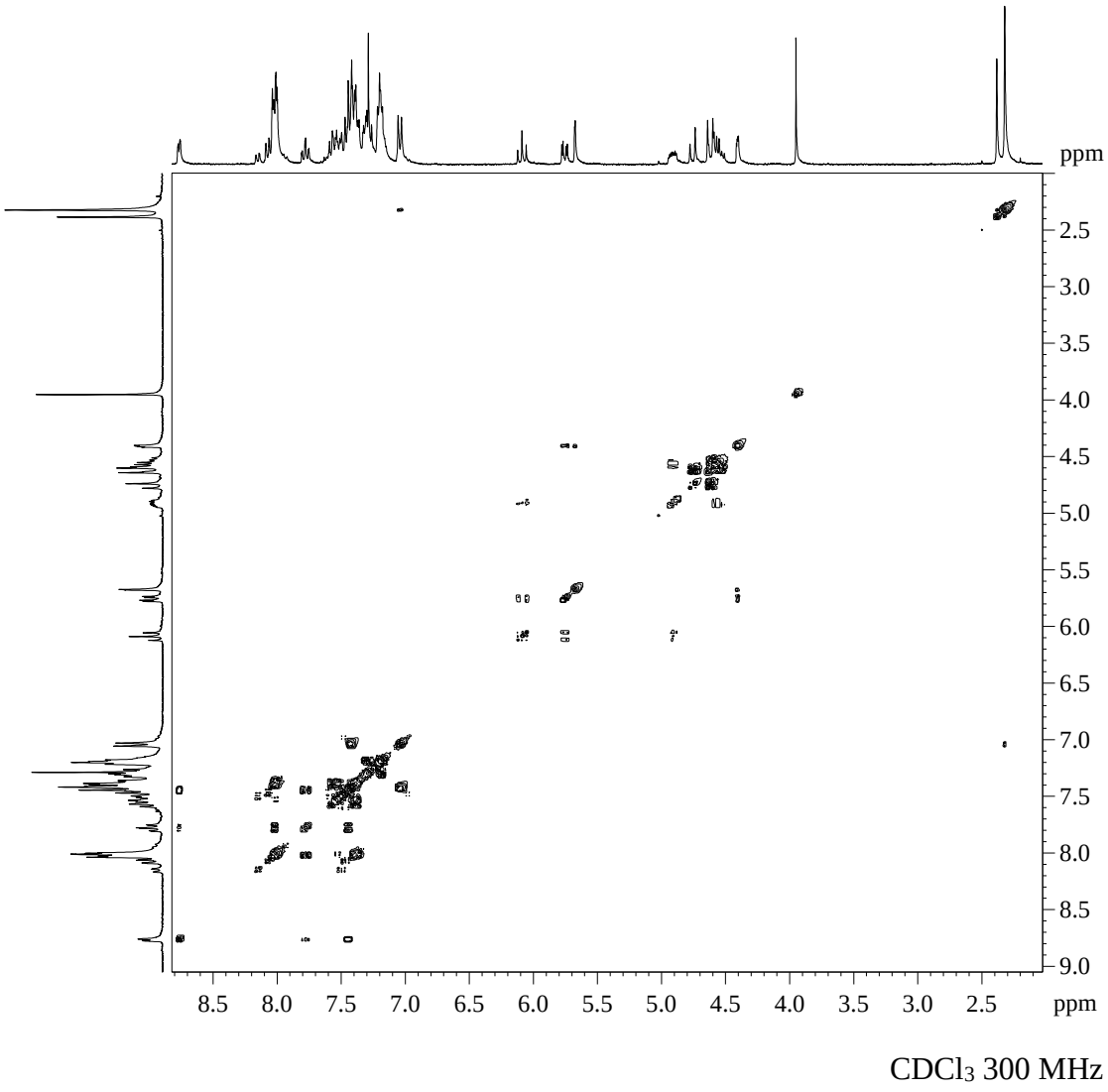


$CDCl_3$ 75 MHz

Figure A-17: ^{13}C NMR spectrum of *p*-Tolyl 4,6-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.4)**

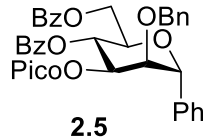


2.4

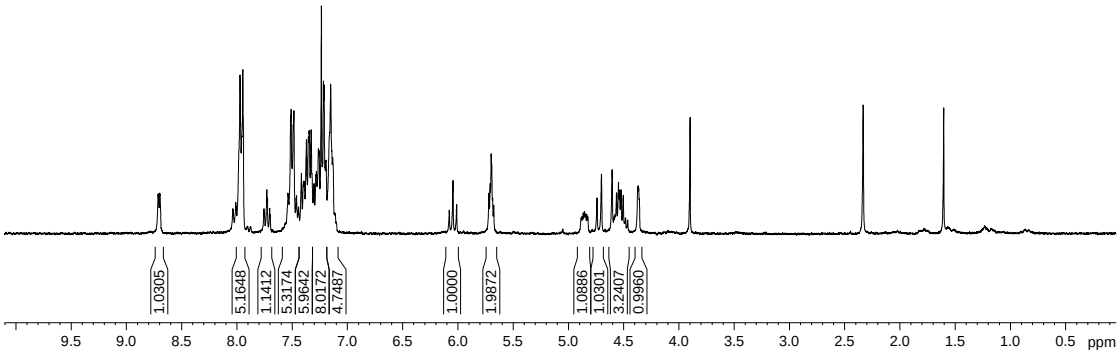


$CDCl_3$ 300 MHz

Figure A-18: 2-D NMR COSY spectrum of *p*-Tolyl 4,6-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.4)**

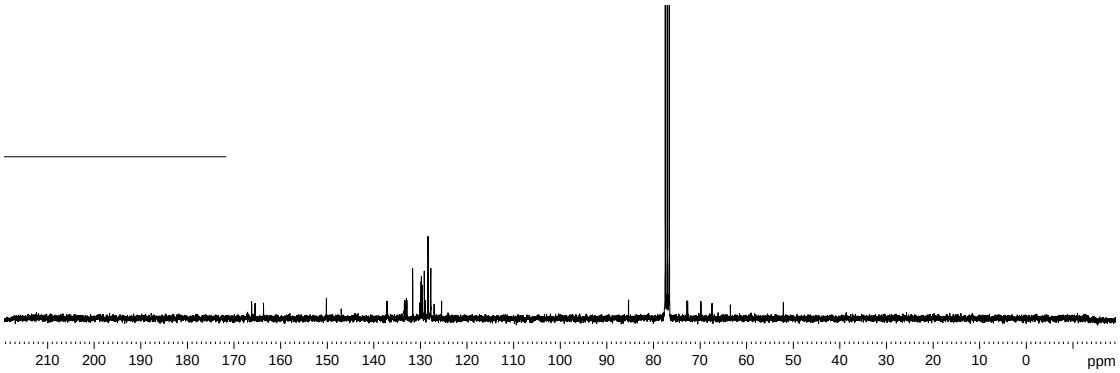


2.5



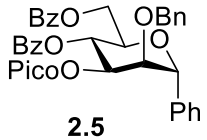
$CDCl_3$ 300 MHz

Figure A-19: ^{1}H NMR spectrum of Phenyl 4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.5).**

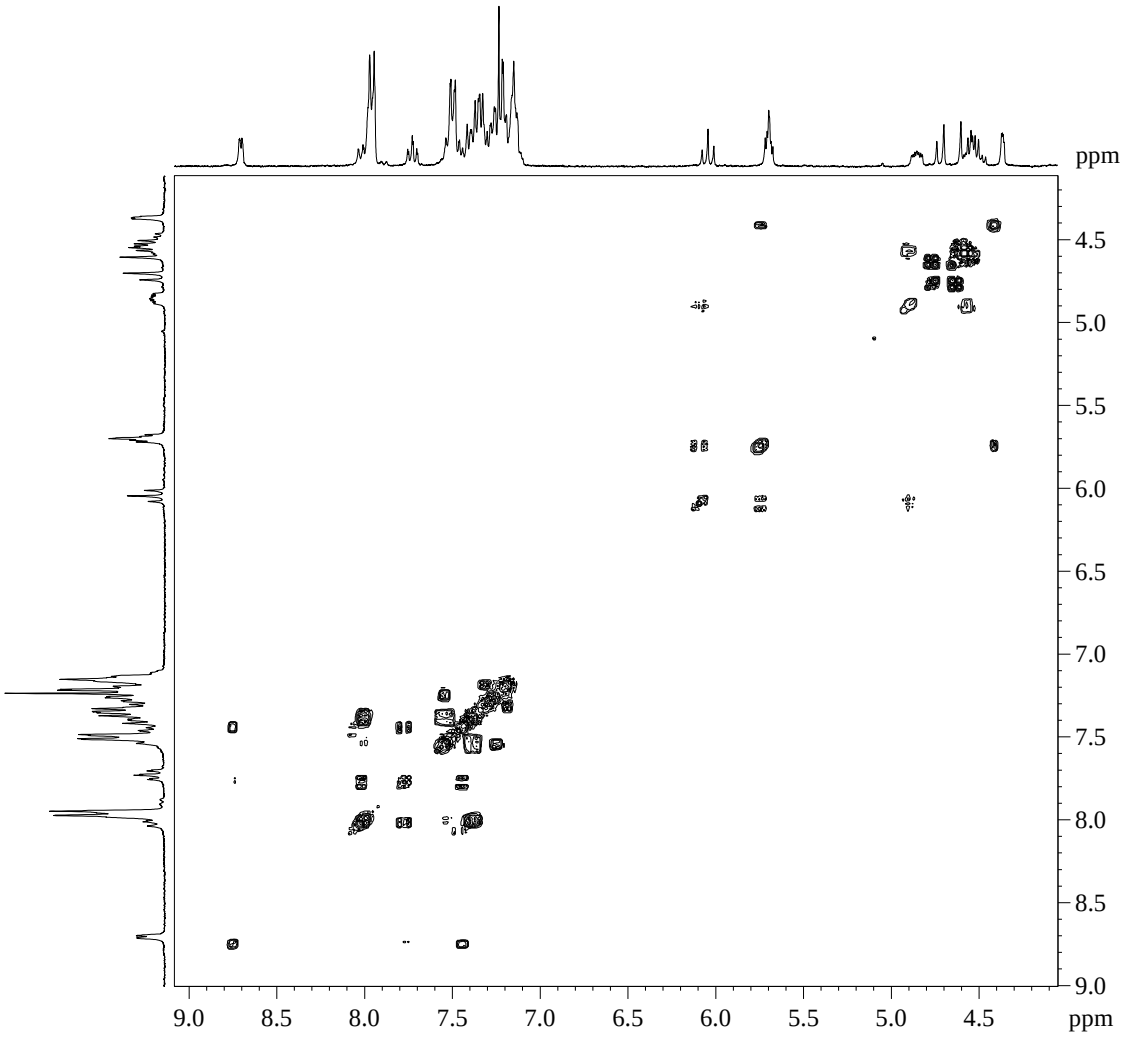


$CDCl_3$ 75 MHz

Figure A-20: ^{13}C NMR spectrum of Phenyl 4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.5).**

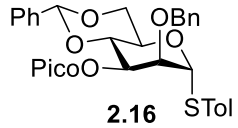


2.5

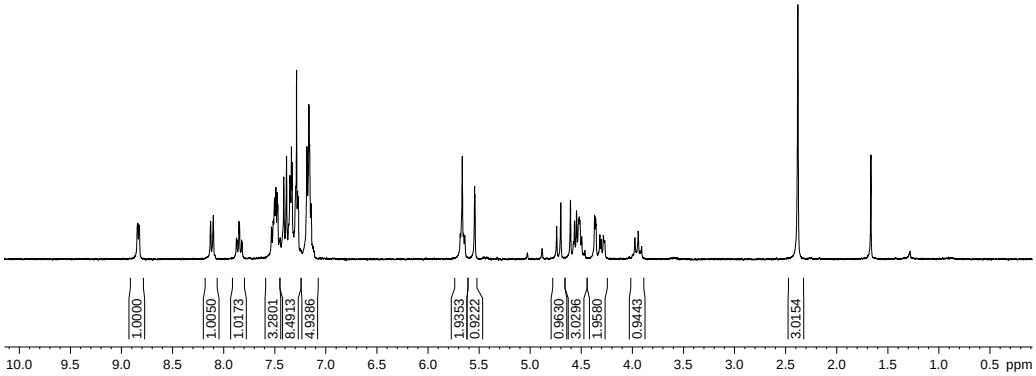


$CDCl_3$ 300 MHz

Figure A-21: 2-D NMR COSY spectrum of Phenyl 4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.5).**

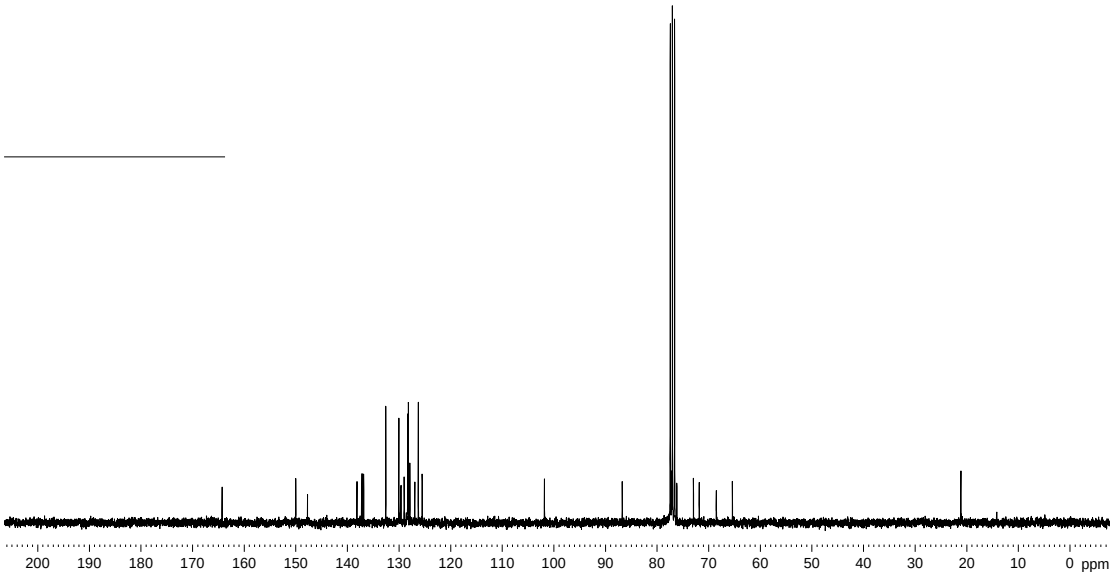


2.16



$CDCl_3$ 300 MHz

Figure A-22: ^{1}H NMR spectrum of *p*-Tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.16)**



$CDCl_3$ 75 MHz

Figure A-23: ^{13}C NMR spectrum of *p*-Tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.16)**

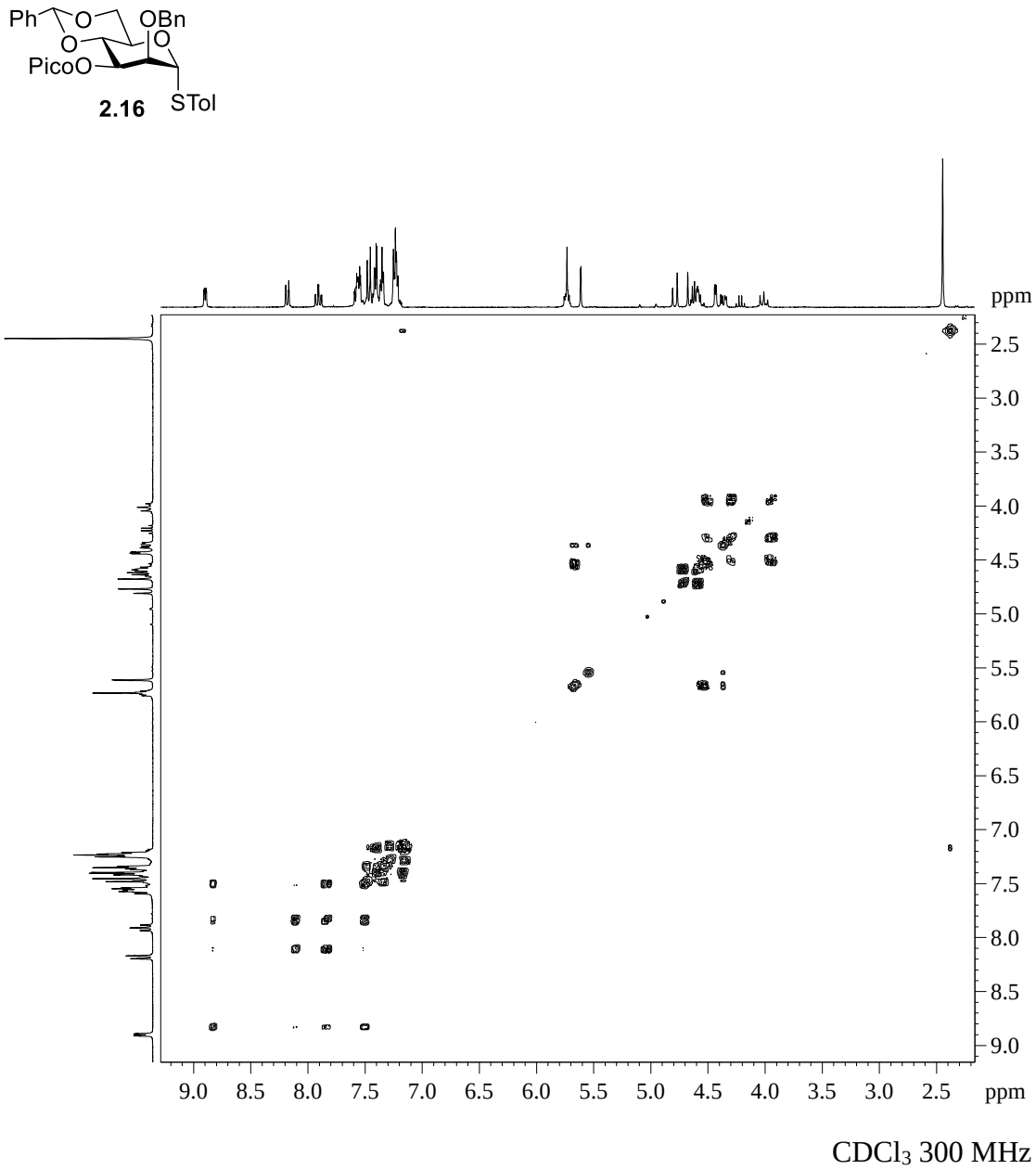
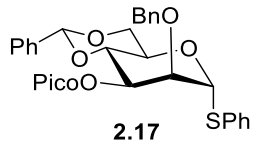
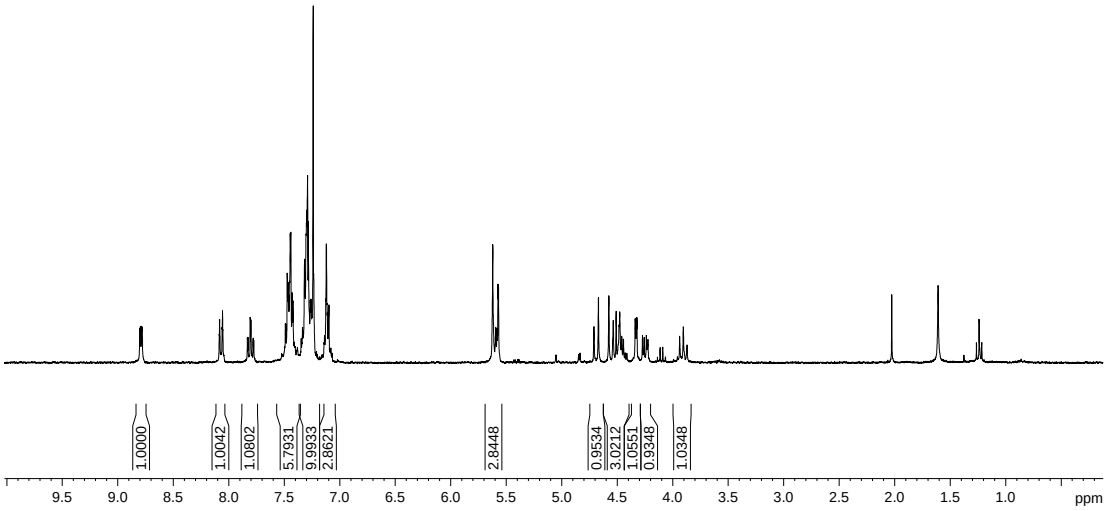


Figure A-24: 2-D NMR COSY spectrum of *p*-Tolyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.16)**

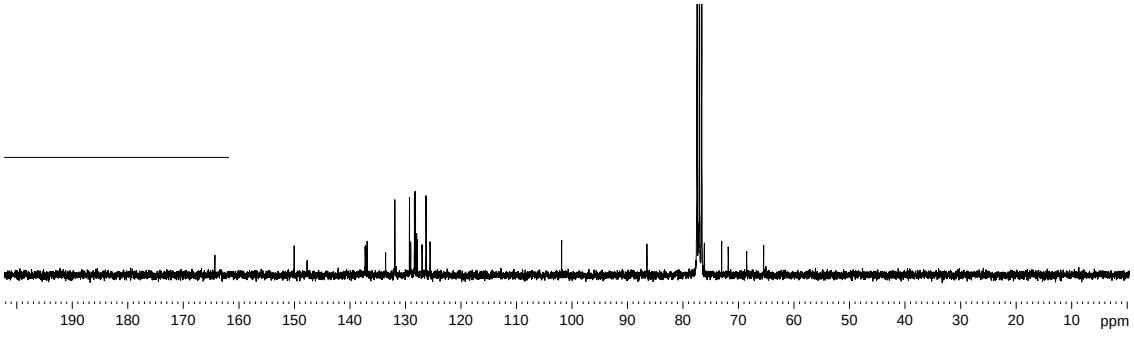


2.17



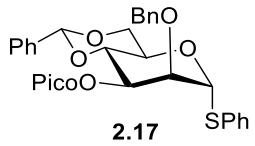
$CDCl_3$ 300 MHz

Figure A-25: ^{1}H NMR spectrum of Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.17)**

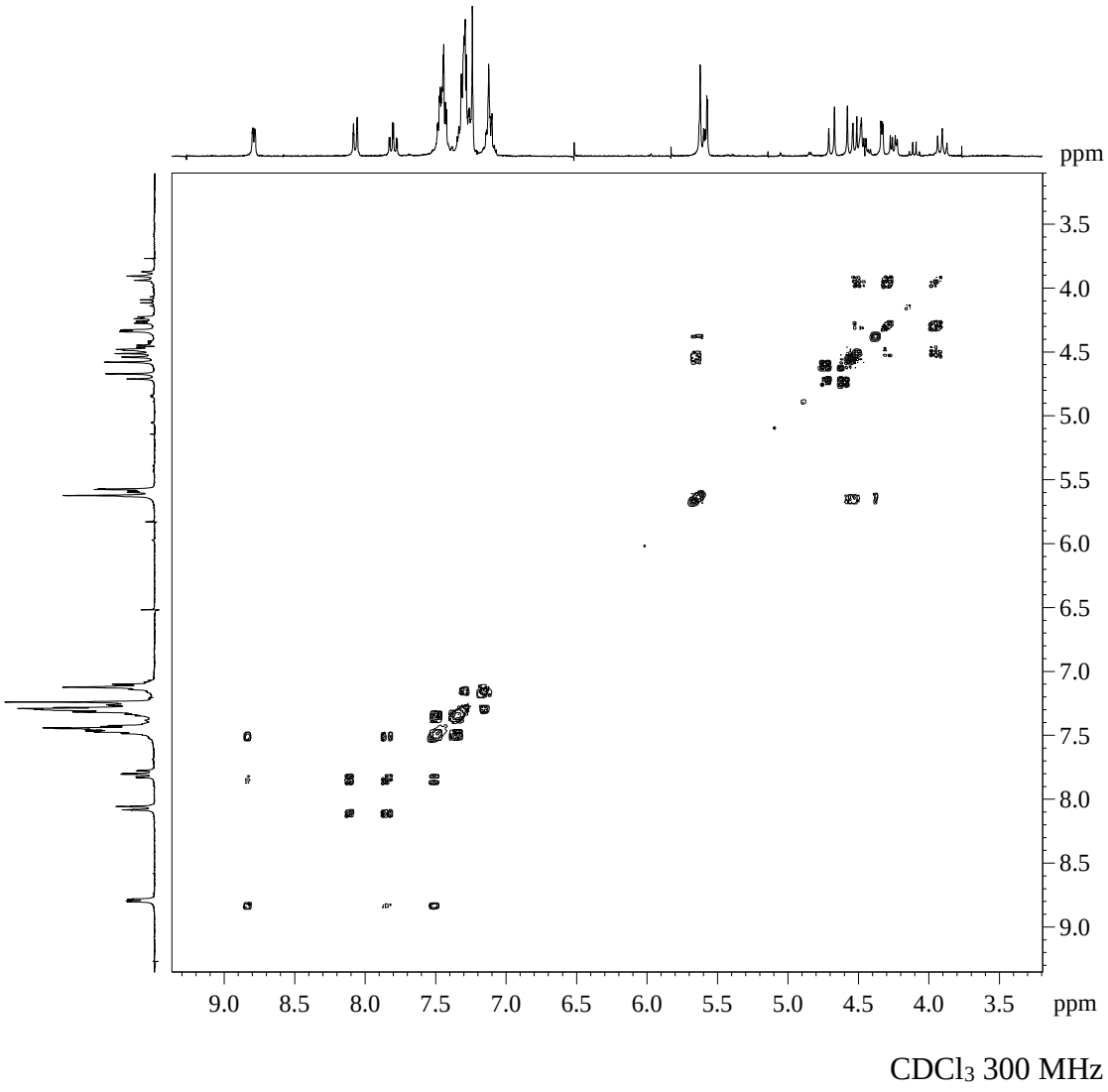


$CDCl_3$ 75 MHz

Figure A-26: ^{13}C NMR spectrum of Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.17)**

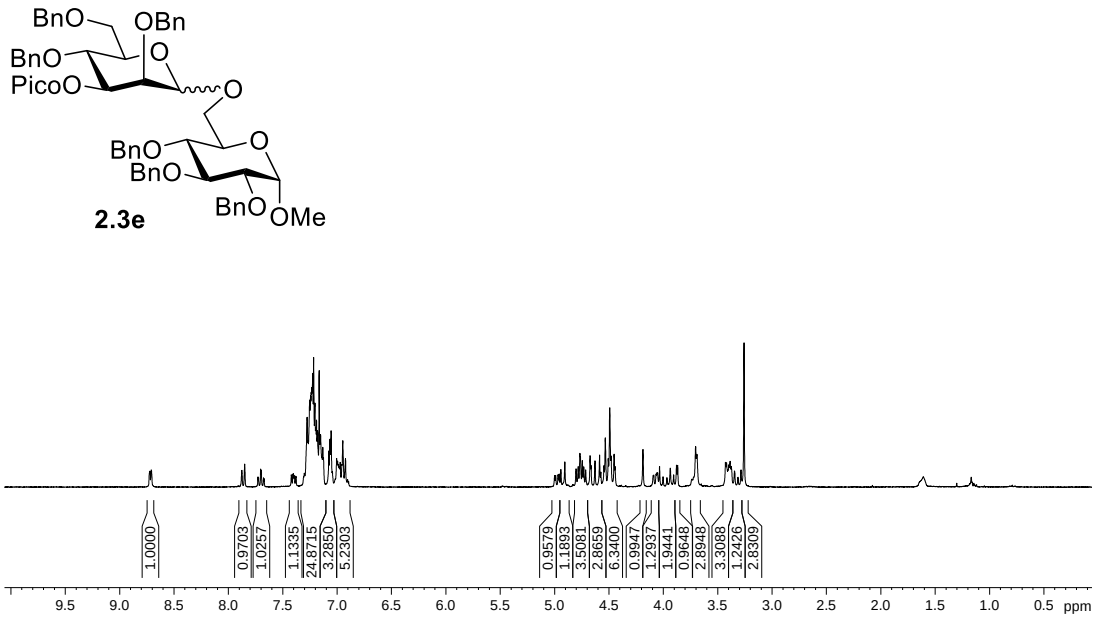


2.17



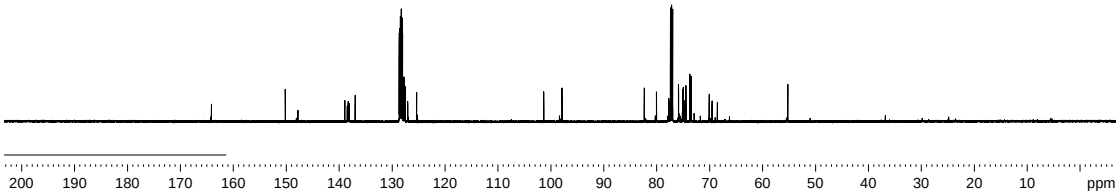
$CDCl_3$ 300 MHz

Figure A-27: 2-D NMR COSY spectrum of Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-1-thio-α-D-mannopyranoside **(2.17)**



$CDCl_3$ 300 MHz

Figure A-28: ^{1}H NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.3e).**



$CDCl_3$ 150 MHz

Figure A-29: ^{13}C NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.3e).**

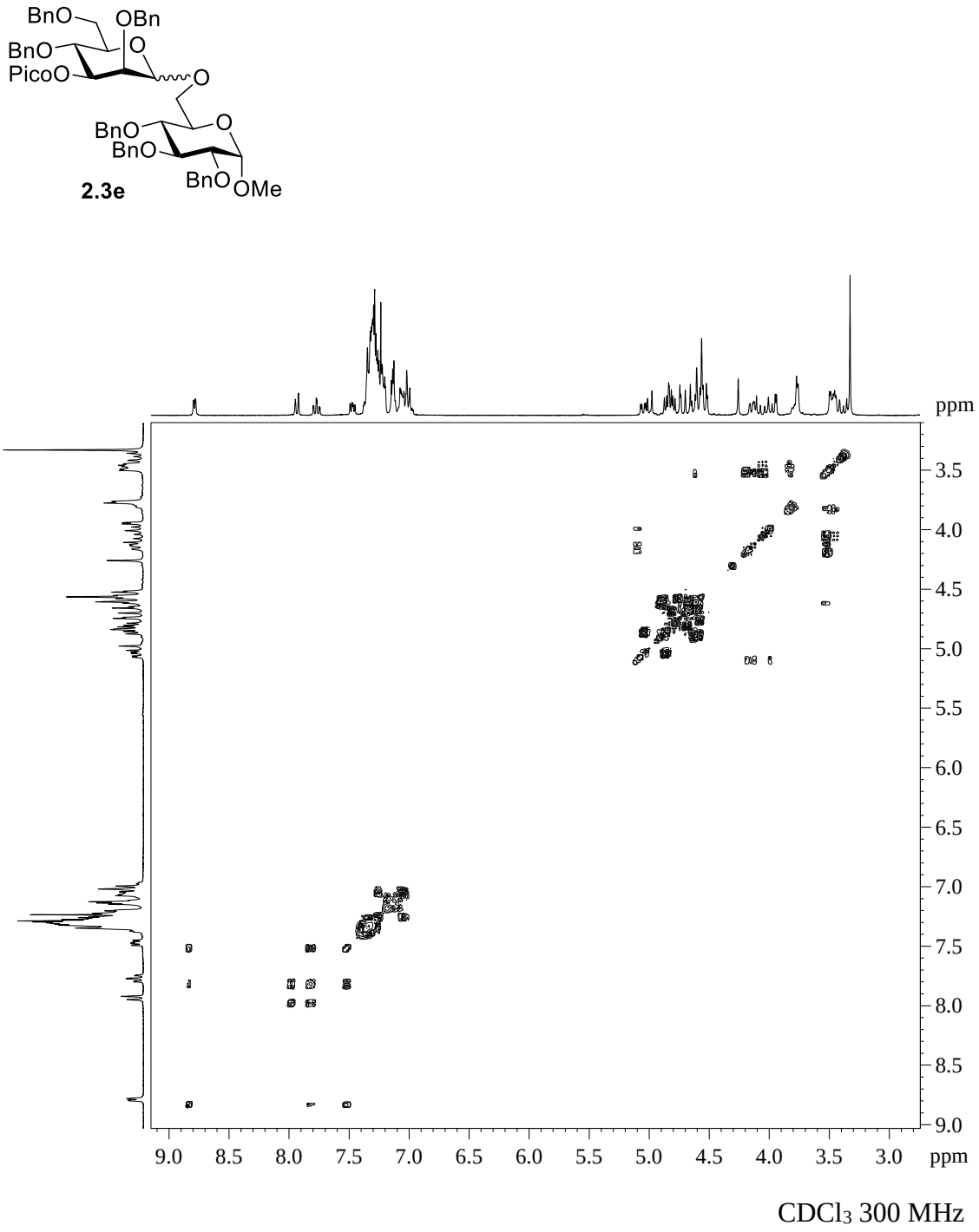
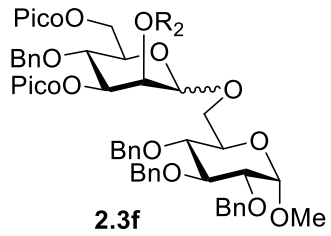
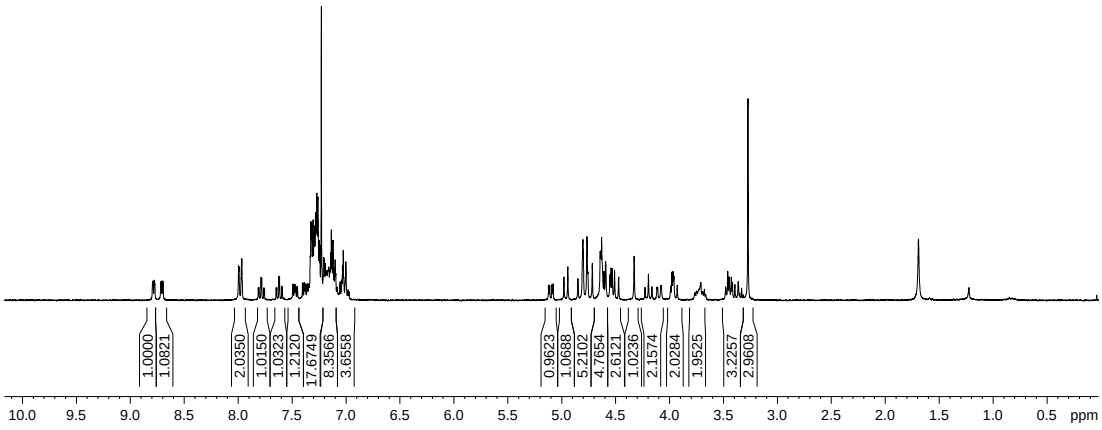


Figure A-30: 2-D NMR COSY spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.3e).**

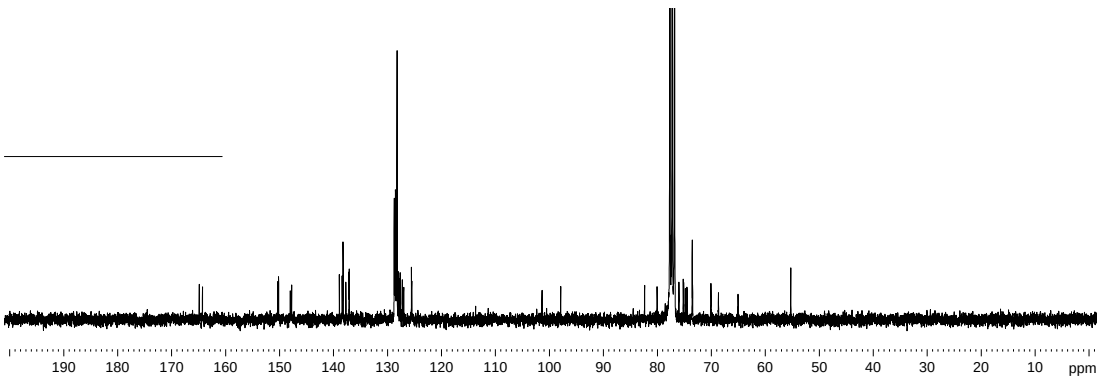


2.3f



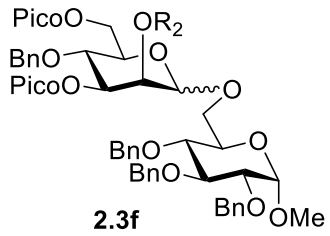
$CDCl_3$ 300 MHz

Figure A-31: ^{1}H NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.3f).**



$CDCl_3$ 150 MHz

Figure A-32: ^{13}C NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.3f).**



2.3f

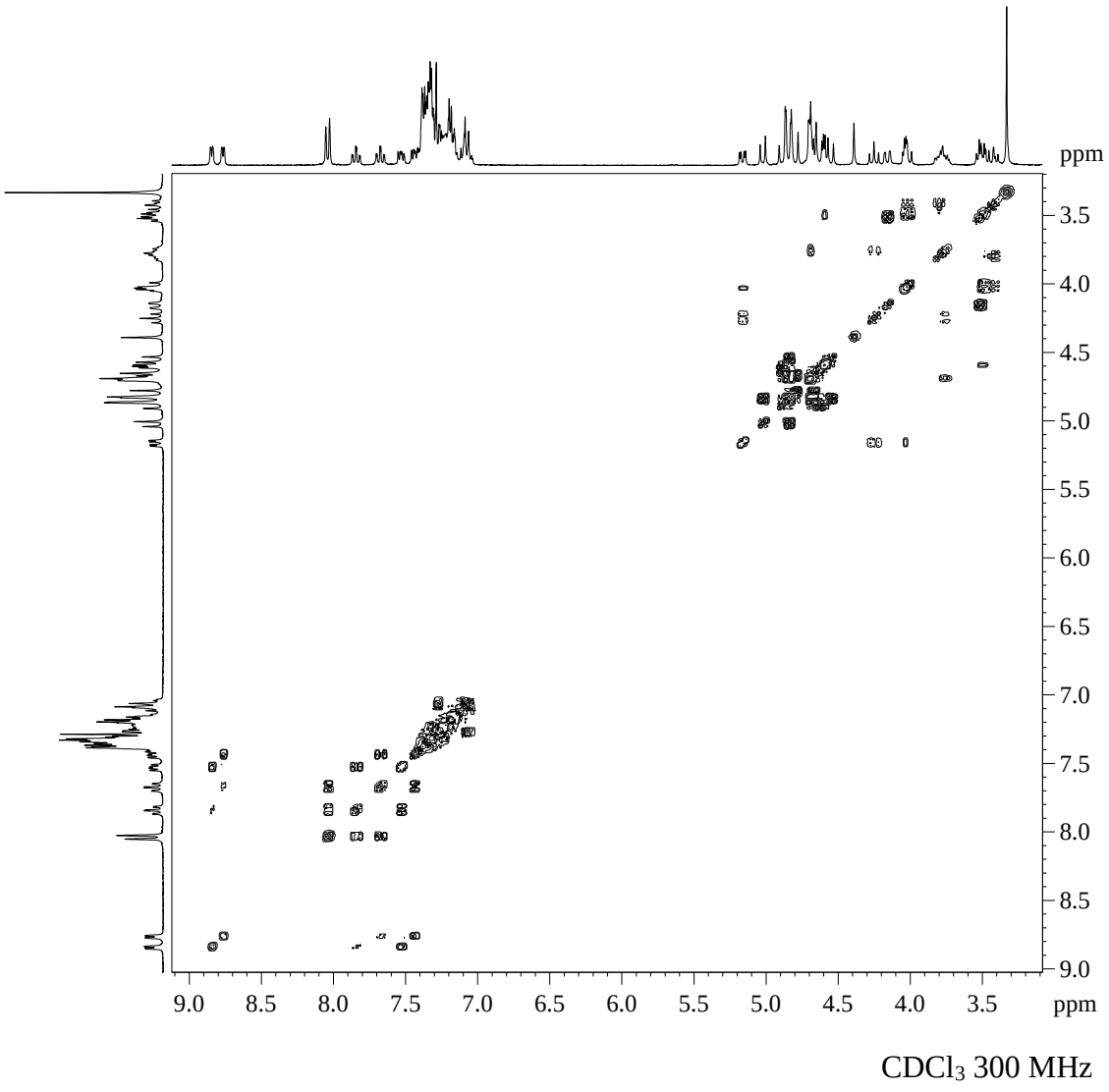
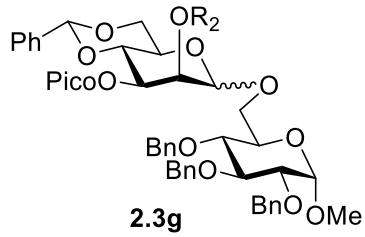
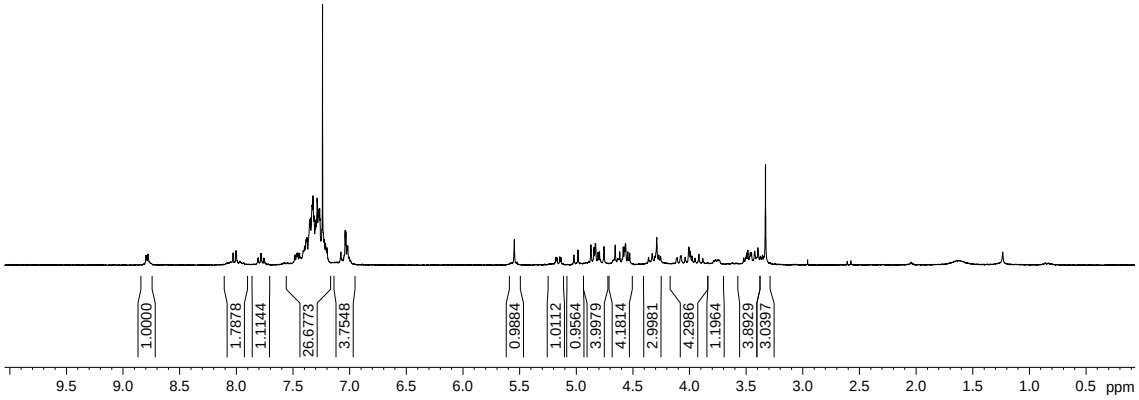


Figure A-33: 2-D NMR COSY spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.3f).**

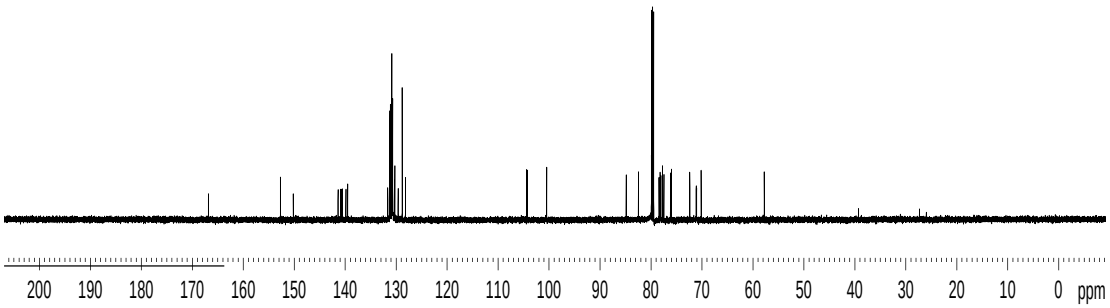


2.3g



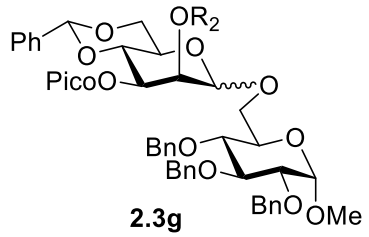
$CDCl_3$ 300 MHz

Figure A-34: ^{1}H NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-*O*-benzyl-4,6-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)- α-D-glucopyranoside **(2.3g).**

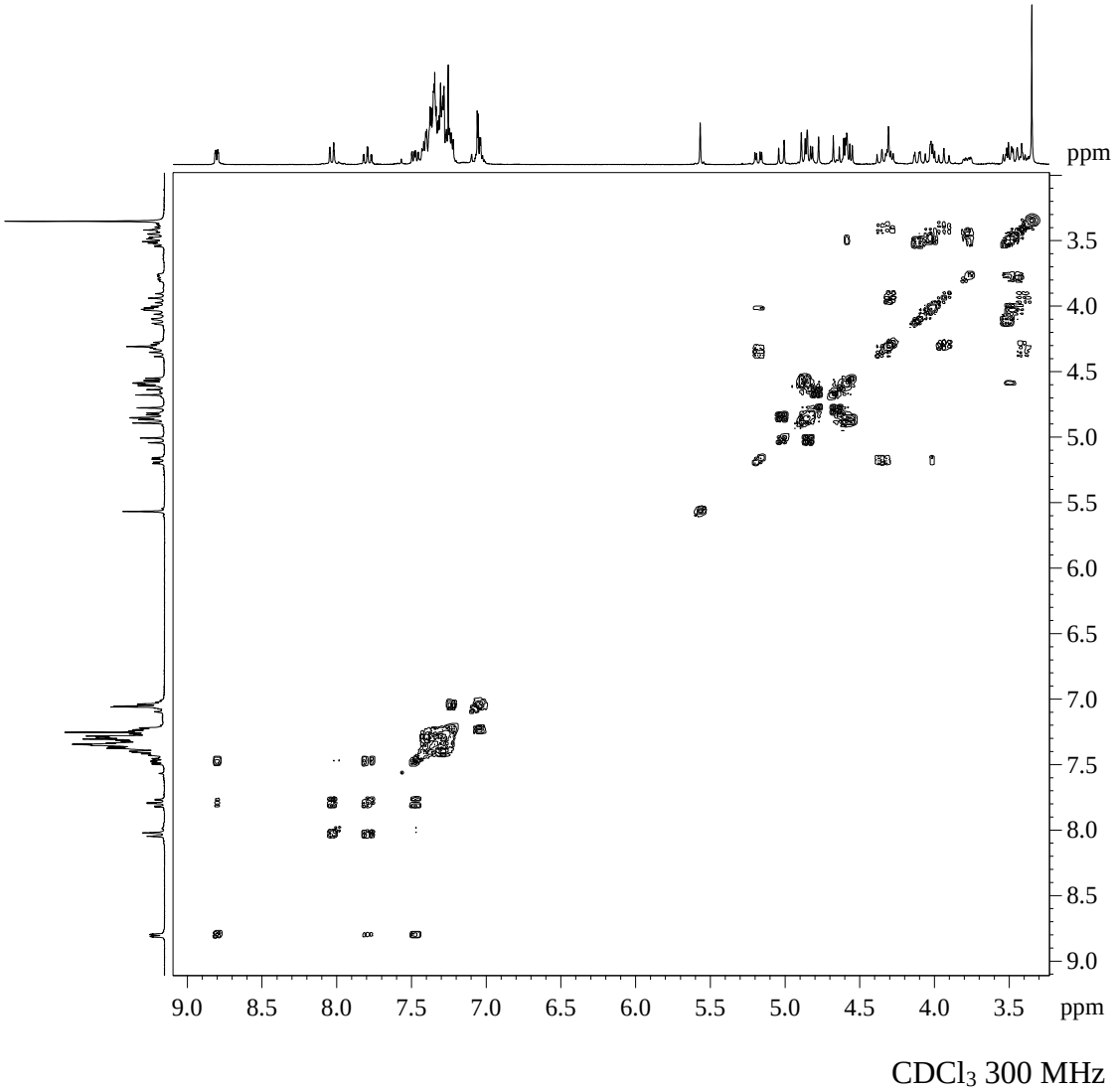


$CDCl_3$ 150 MHz

Figure A-35: ^{13}C NMR spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-*O*-benzyl-4,6-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)- α-D-glucopyranoside **(2.3g).**

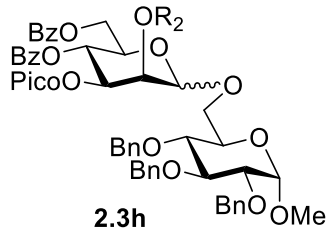


2.3g

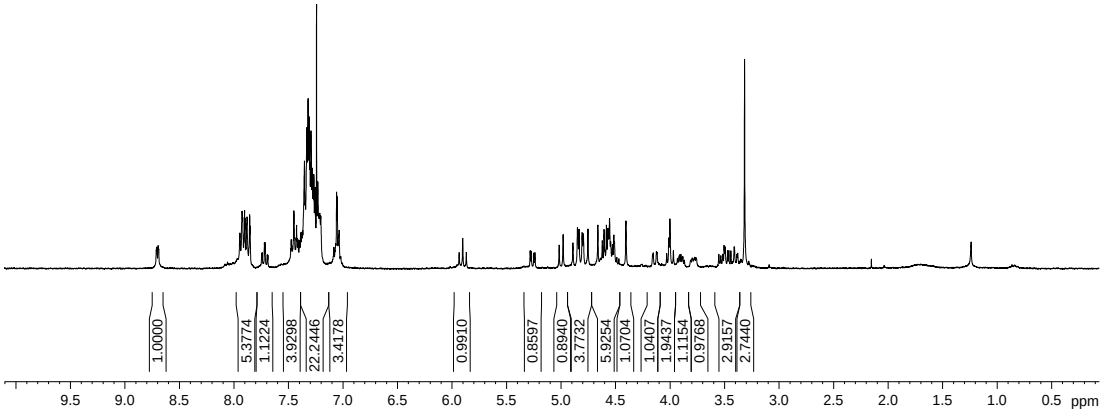


$CDCl_3$ 300 MHz

Figure A-36: 2-D NMR COSY spectrum of Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-*O*-benzyl-4,6-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)- α-D-glucopyranoside **(2.3g).**

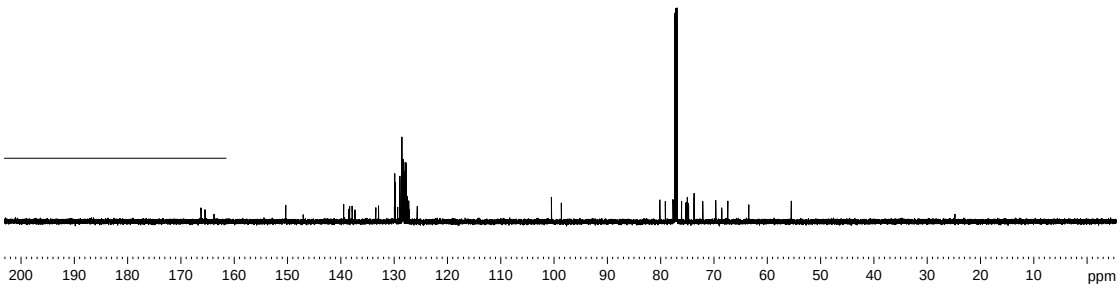


2.3h



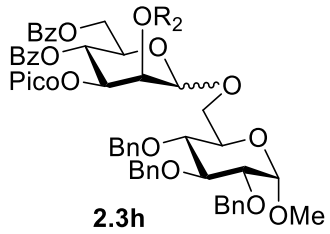
$CDCl_3$ 300 MHz

Figure A-37: ^{1}H NMR spectrum of Methyl 6-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(2.3h).**



$CDCl_3$ 150 MHz

Figure A-38: ^{13}C NMR spectrum of Methyl 6-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(2.3h).**



2.3h

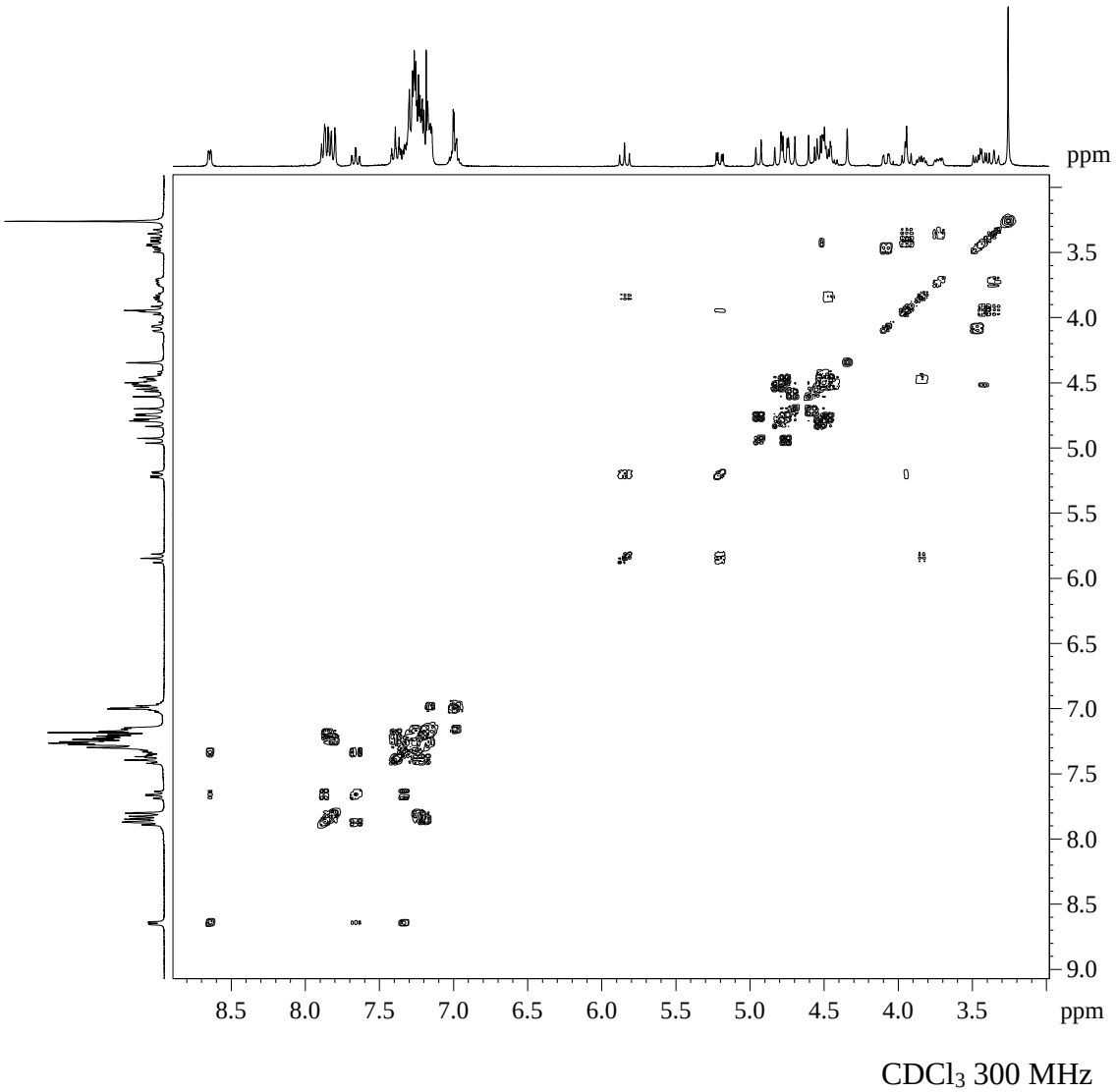
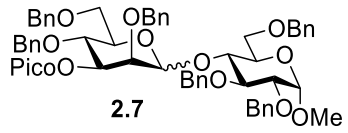
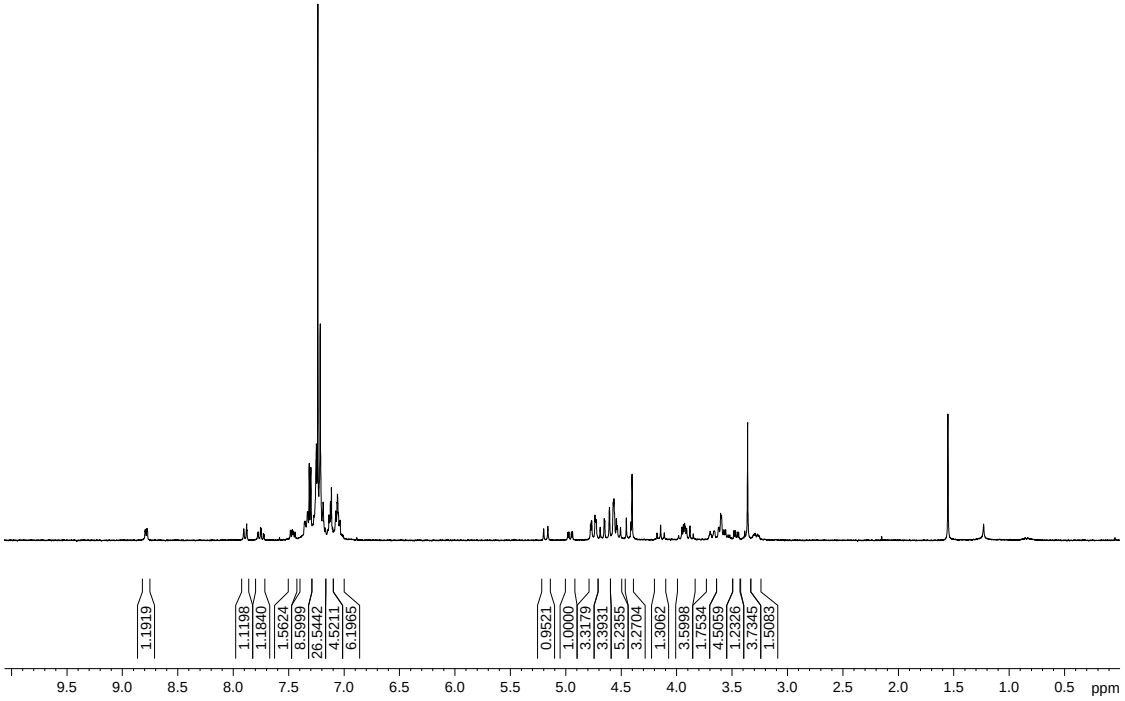


Figure A-36: 2-D NMR COSY spectrum of Methyl 6-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(2.3h).**

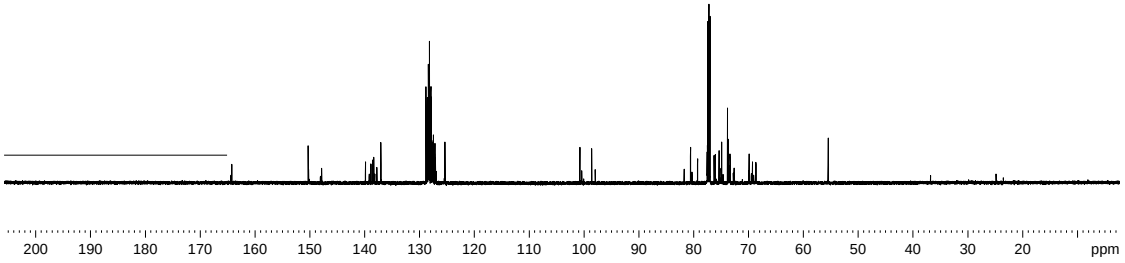


2.7



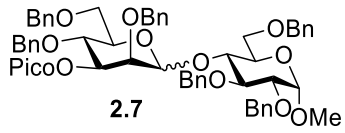
$CDCl_3$ 300 MHz

Figure A-37: ^{1}H NMR spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.7).**



$CDCl_3$ 150 MHz

Figure A-38: ^{13}C NMR spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.7).**



2.7

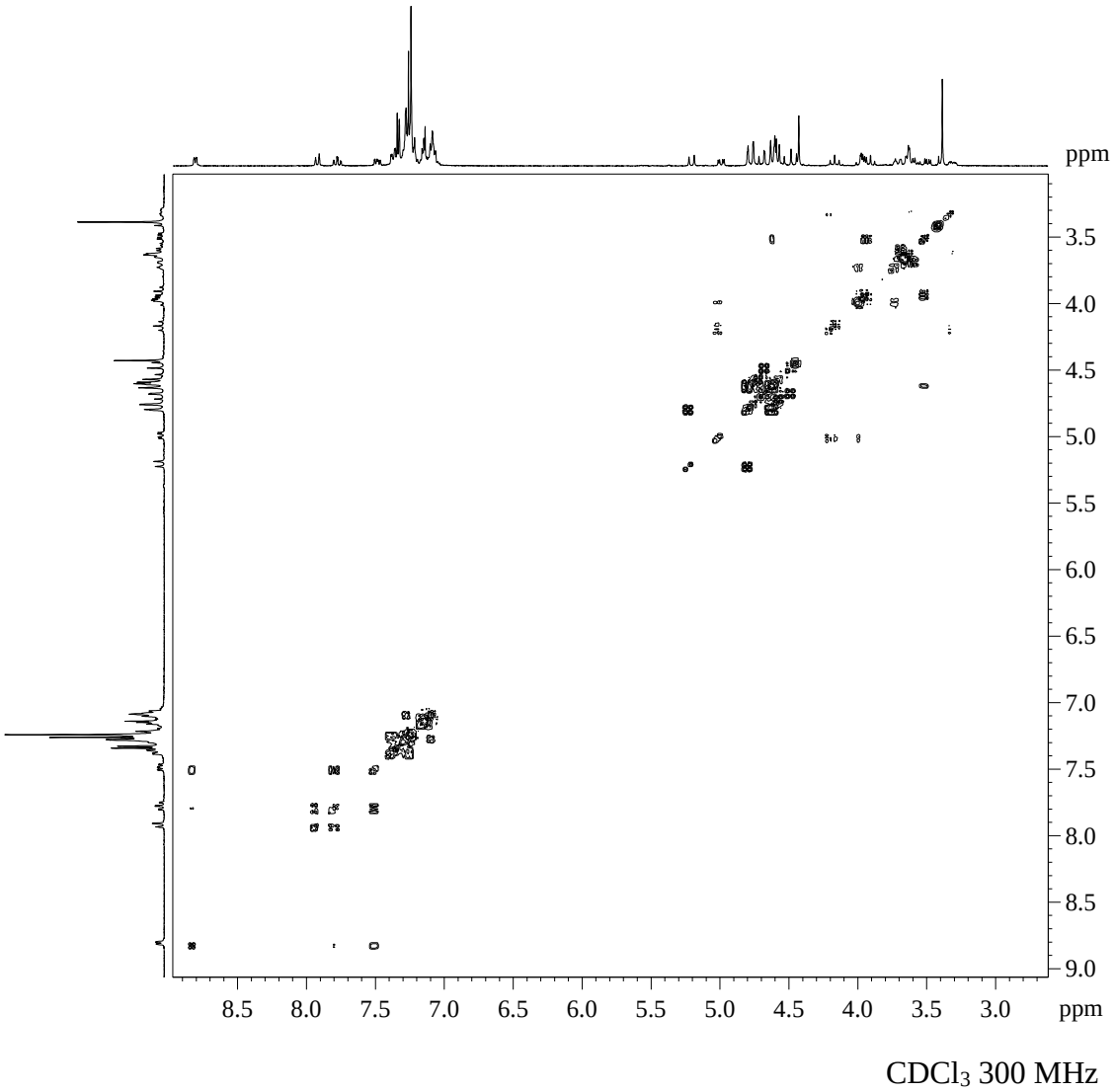
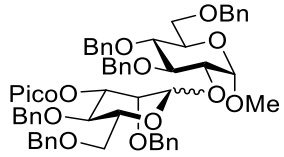
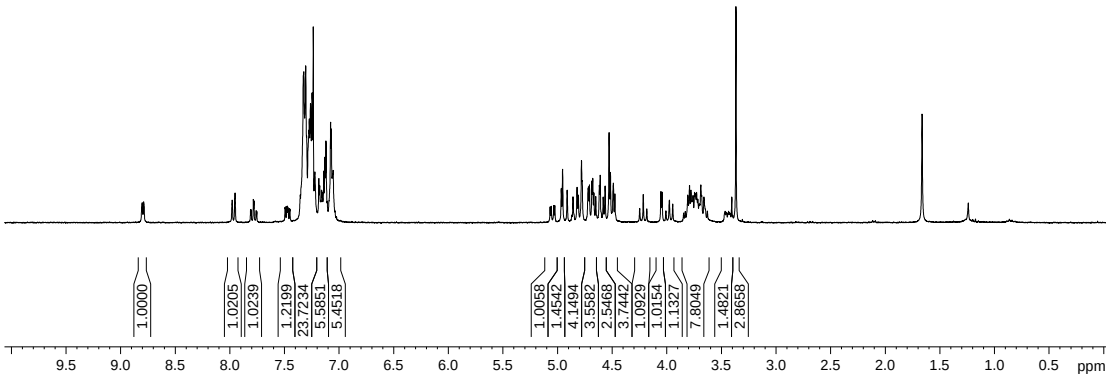


Figure A-39: 2-D NMR COSY spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.7).**

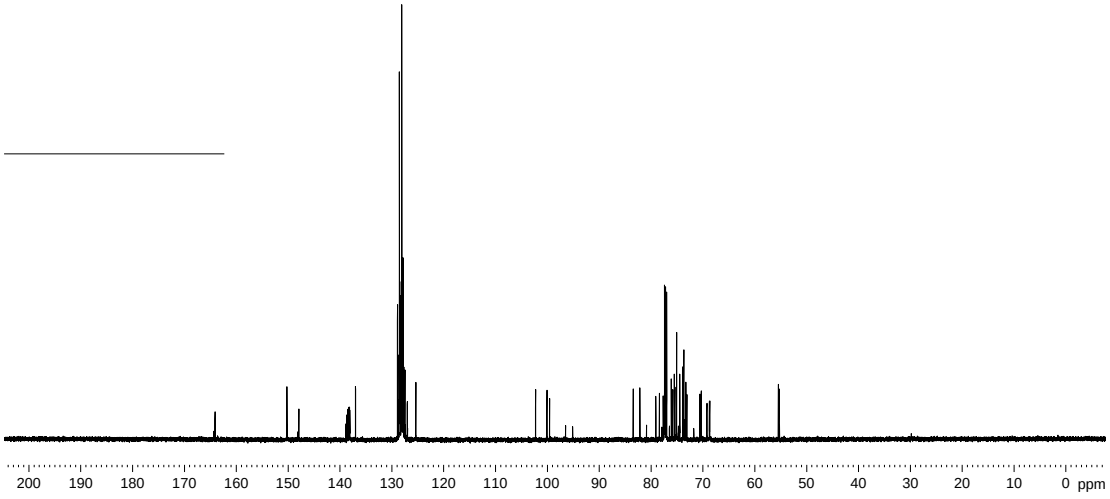


2.9



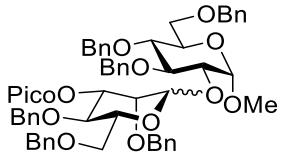
$CDCl_3$ 300 MHz

Figure A-40: ^{1}H NMR spectrum of Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.9).**

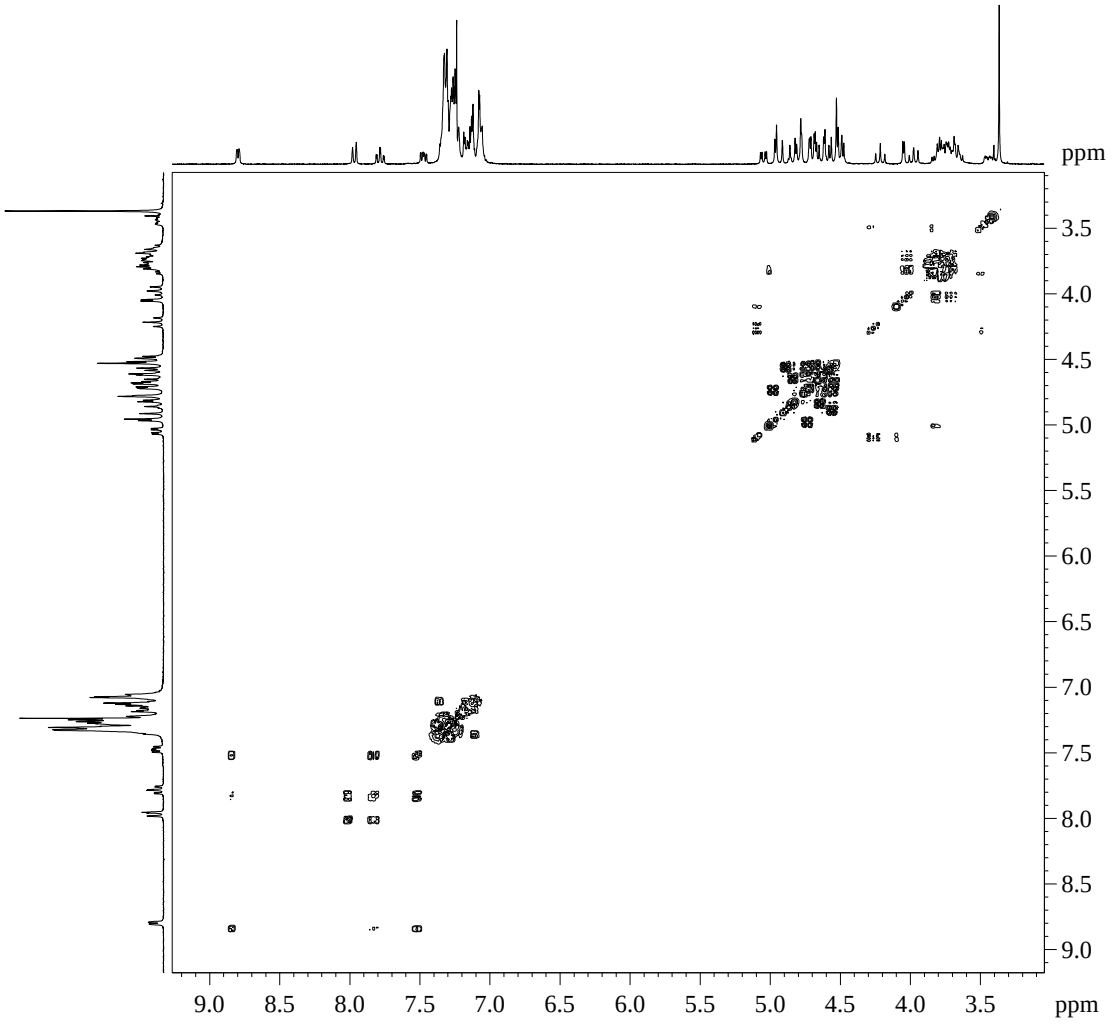


$CDCl_3$ 150 MHz

Figure A-41: ^{13}C NMR spectrum of Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.9).**

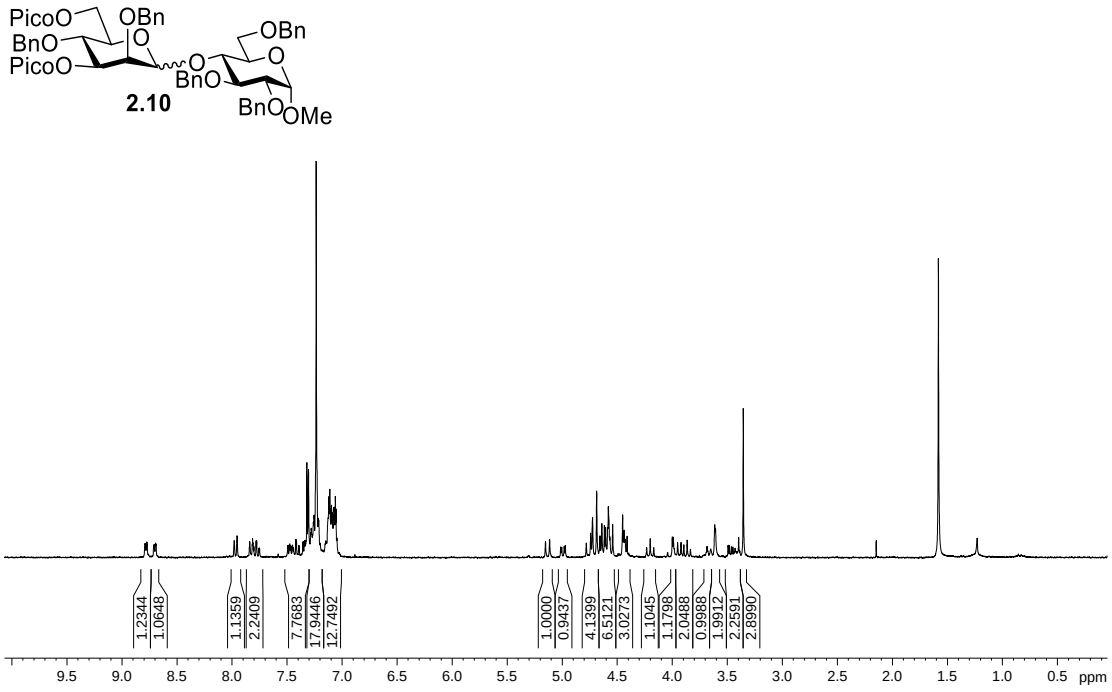


2.9



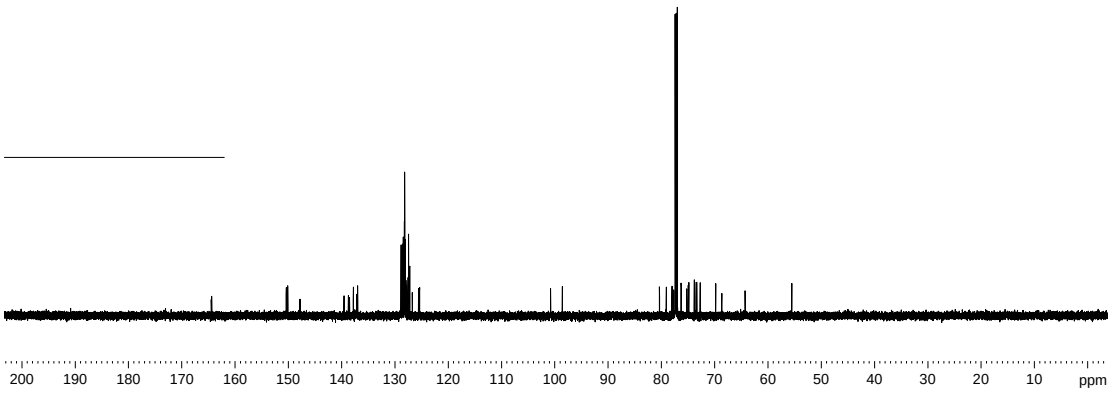
$CDCl_3$ 300 MHz

Figure A-42: 2-D NMR COSY spectrum of Methyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,4,6-tri-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.9).**



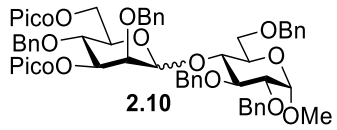
$CDCl_3$ 300 MHz

Figure A-43: ^{1}H NMR spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.10).**



$CDCl_3$ 150 MHz

Figure A-43: ^{13}C NMR spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.10).**



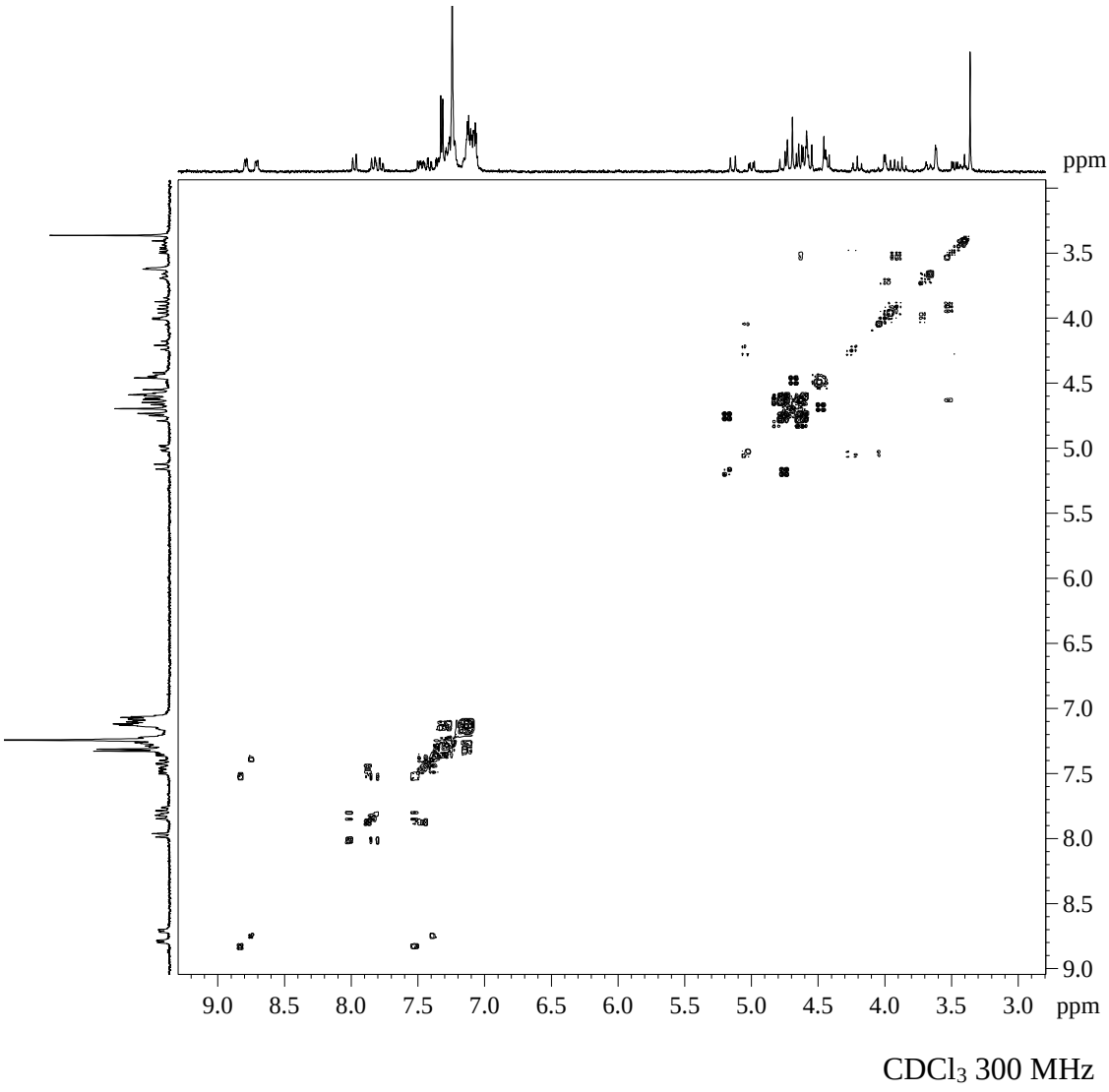
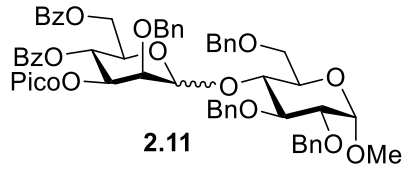
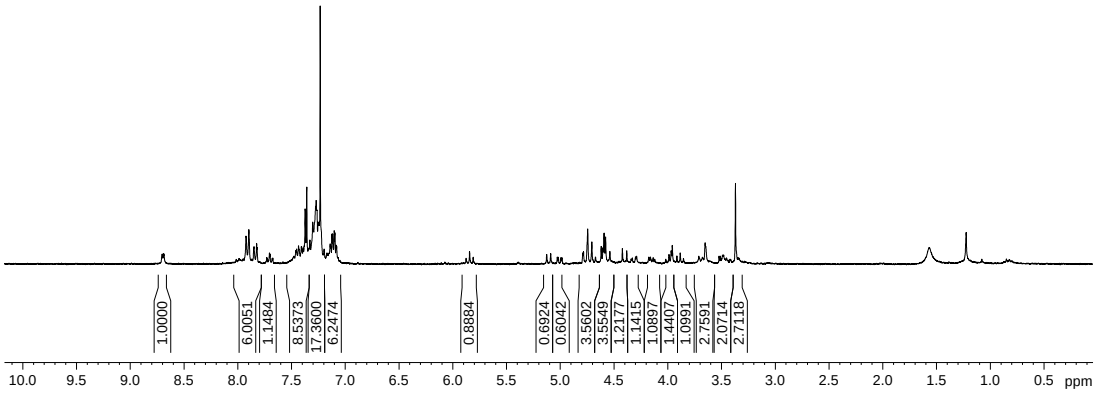


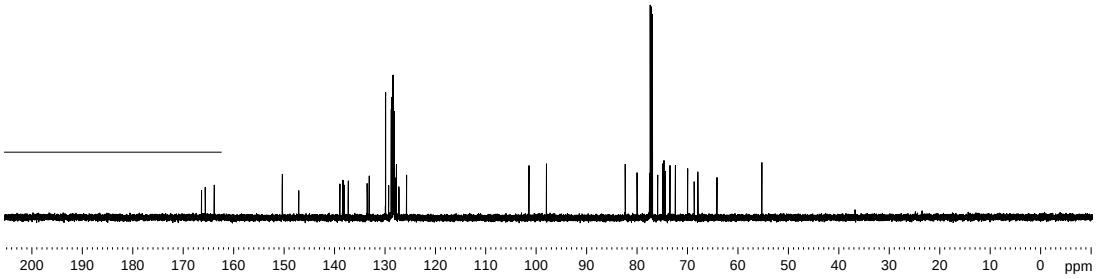
Figure A-44: 2-D NMR COSY spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,4-di-*O*-benzyl-3,6-di-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.10).**





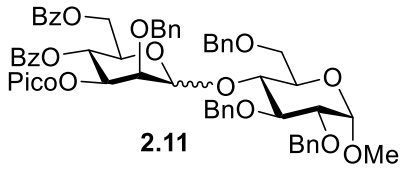
$CDCl_3$ 300 MHz

Figure A-45: ^{1}H NMR spectrum of Methyl 4-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(2.11).**

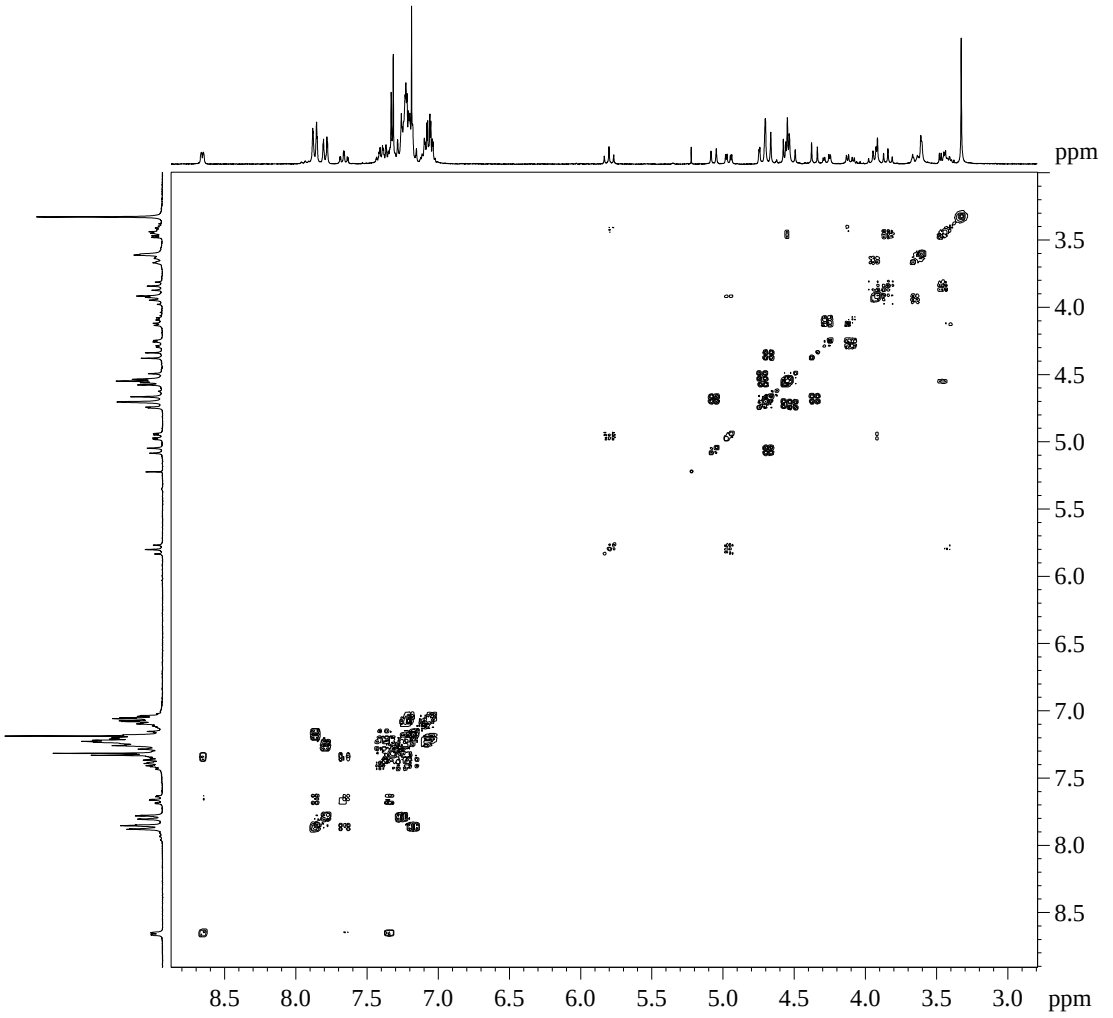


$CDCl_3$ 150 MHz

Figure A-46: ^{13}C NMR spectrum of Methyl 4-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(2.11).**

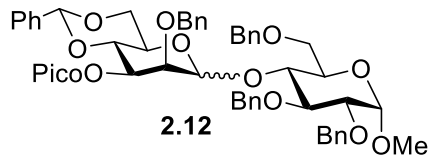


2.11

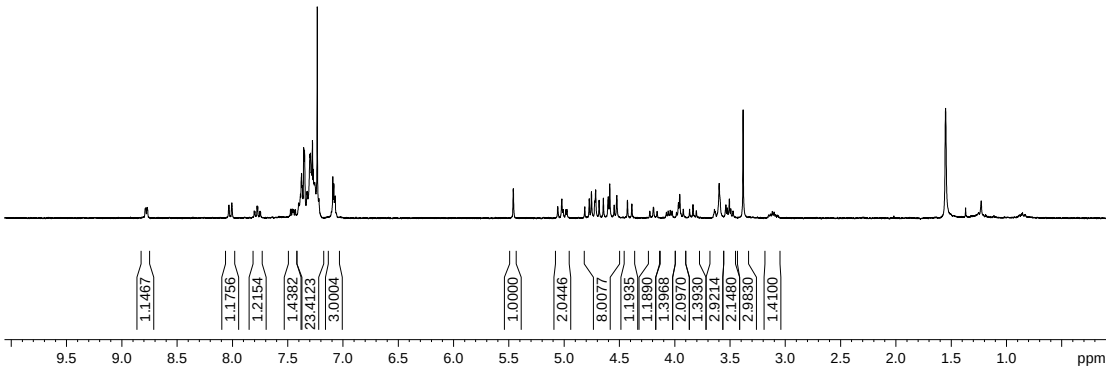


$CDCl_3$ 300 MHz

Figure A-47: 2-D NMR COSY spectrum of Methyl 4-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-3-*O*-picoloyl-α/β-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **(2.11).**

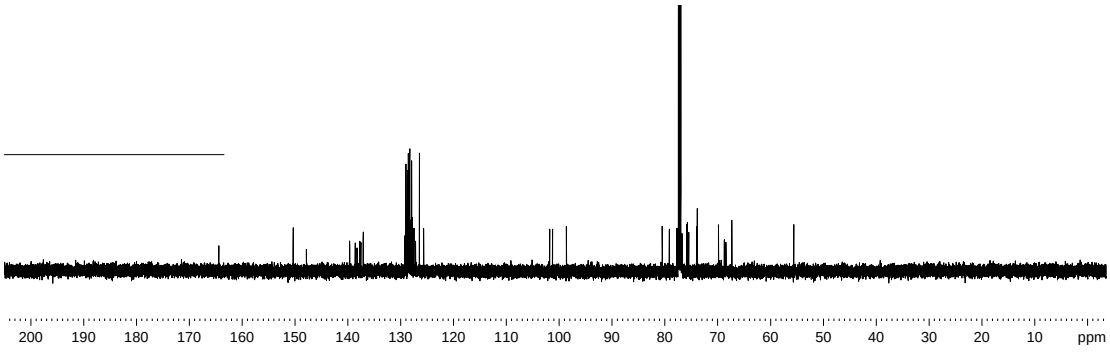


2.12



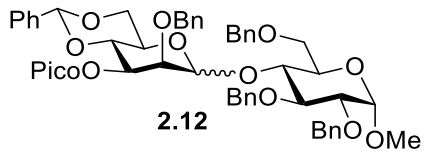
$CDCl_3$ 300 MHz

Figure A-48: ^{1}H NMR spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.12).**

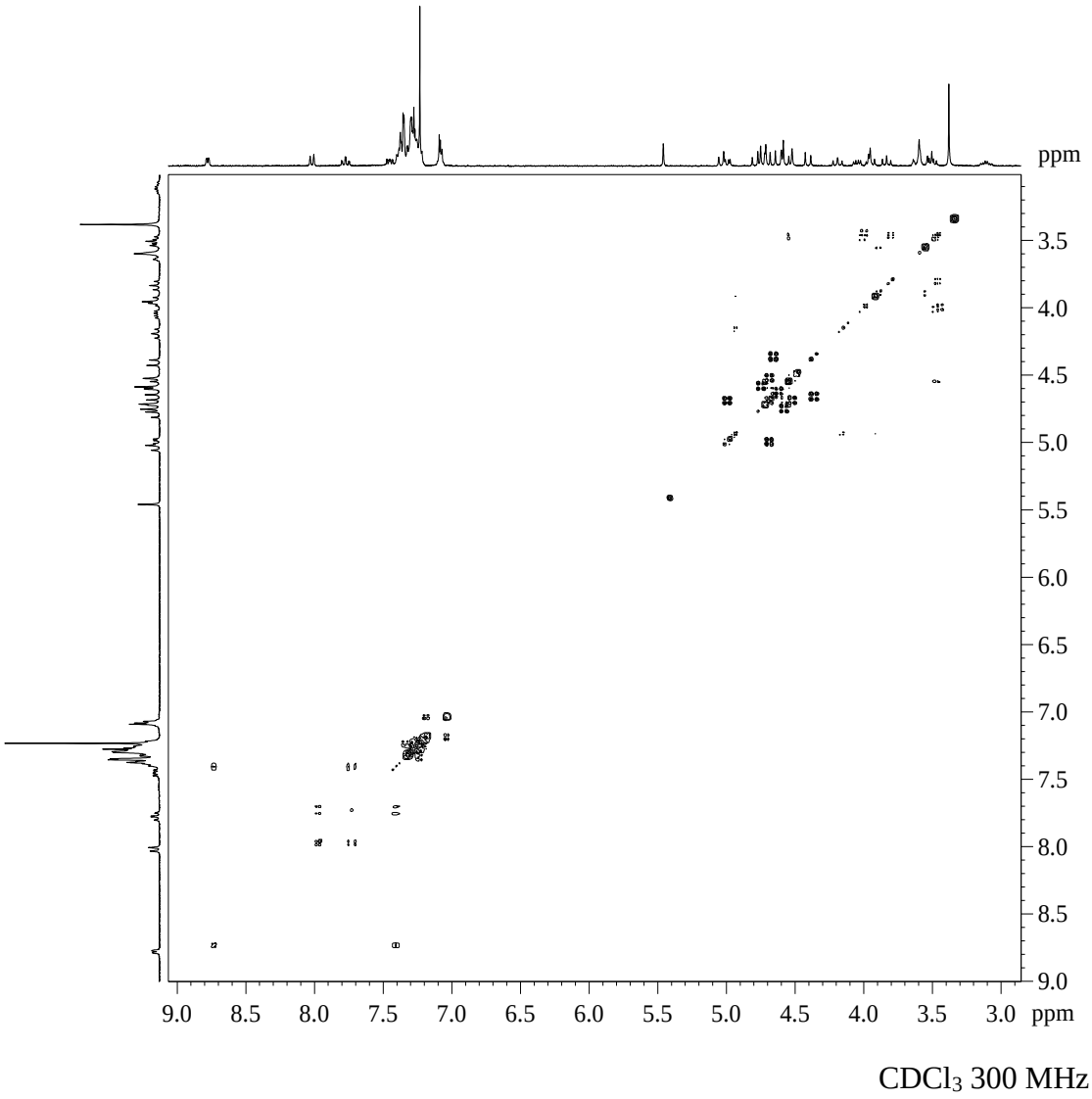


$CDCl_3$ 150 MHz

Figure A-49: ^{13}C NMR spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.12).**

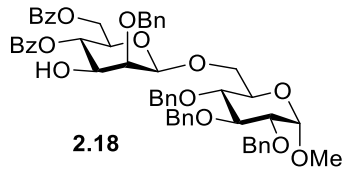


2.12

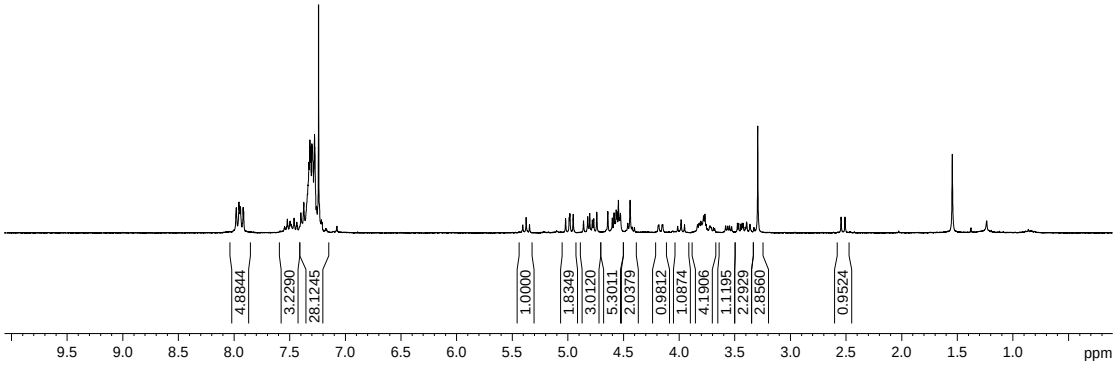


$CDCl_3$ 300 MHz

Figure A-50: 2-D NMR COSY spectrum of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-α/β-D-mannopyranosyl)-α-D-glucopyranoside **(2.12).**

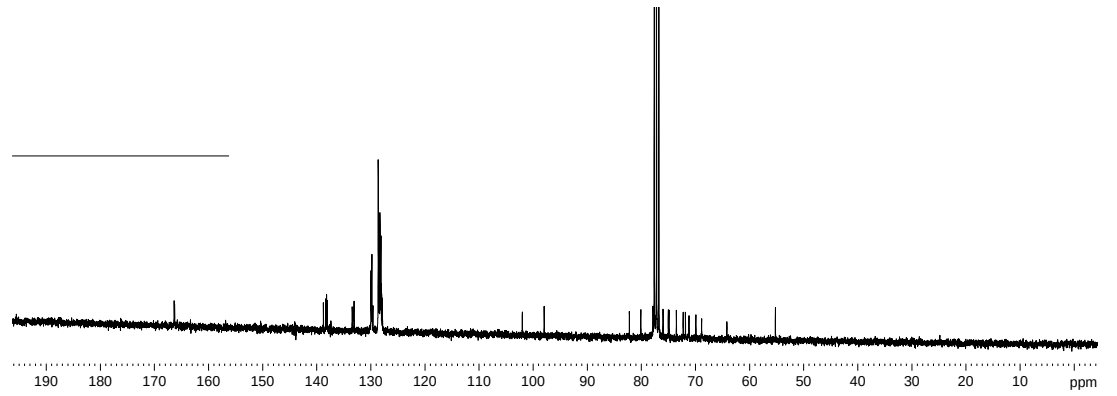


2.18



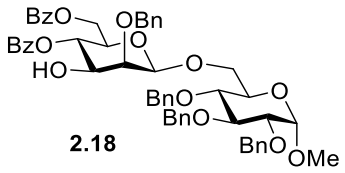
$CDCl_3$ 300 MHz

Figure A-51: ^{1}H NMR spectrum of methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**2.18**)

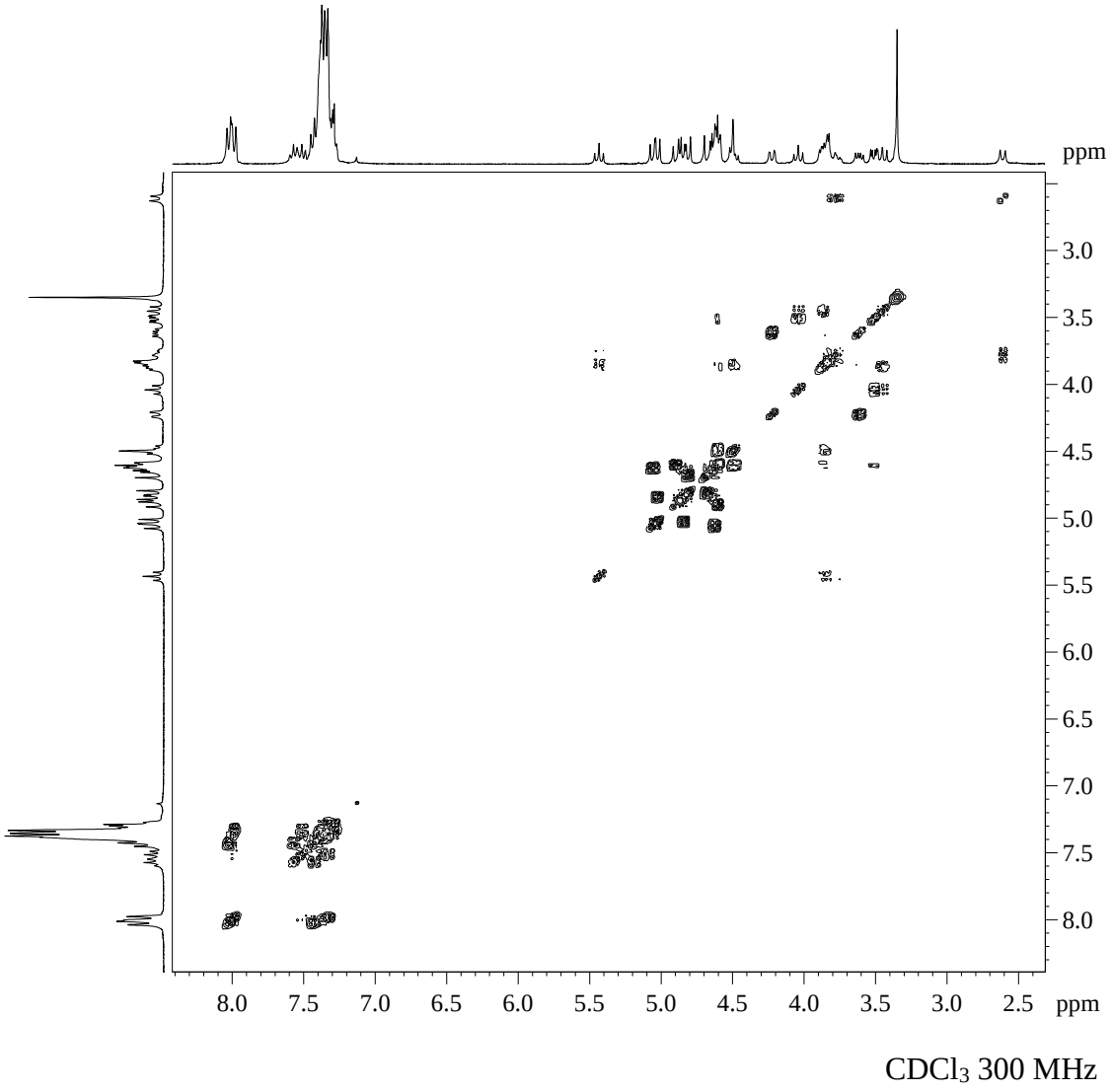


$CDCl_3$ 75 MHz

Figure A-52: ^{13}C NMR spectrum of methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**2.18**)

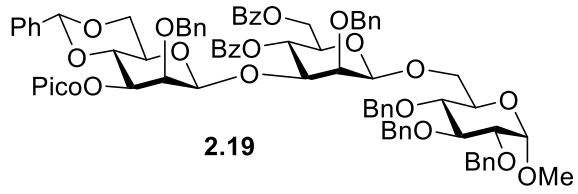


2.18

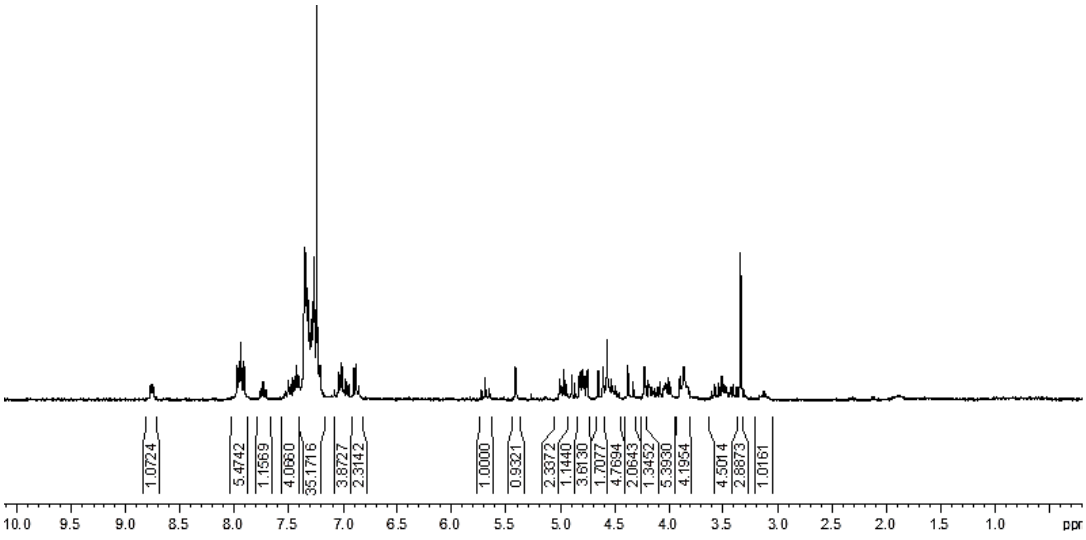


$CDCl_3$ 300 MHz

Figure A-53: 2-D NMR COSY spectrum of methyl 2,3,4-tri-*O*-benzyl-6-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranosyl)-α-D-glucopyranoside (**2.18**)

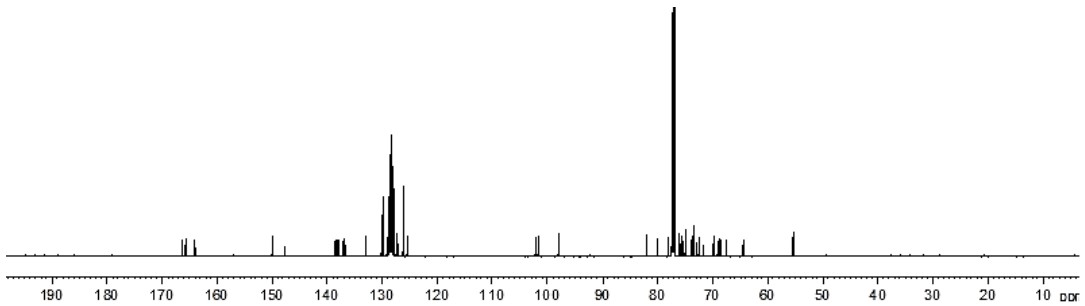


2.19



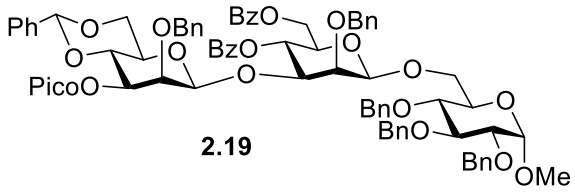
$CDCl_3$ 300 MHz

Figure A-54: ^{1}H NMR spectrum of Methyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(2.19).**

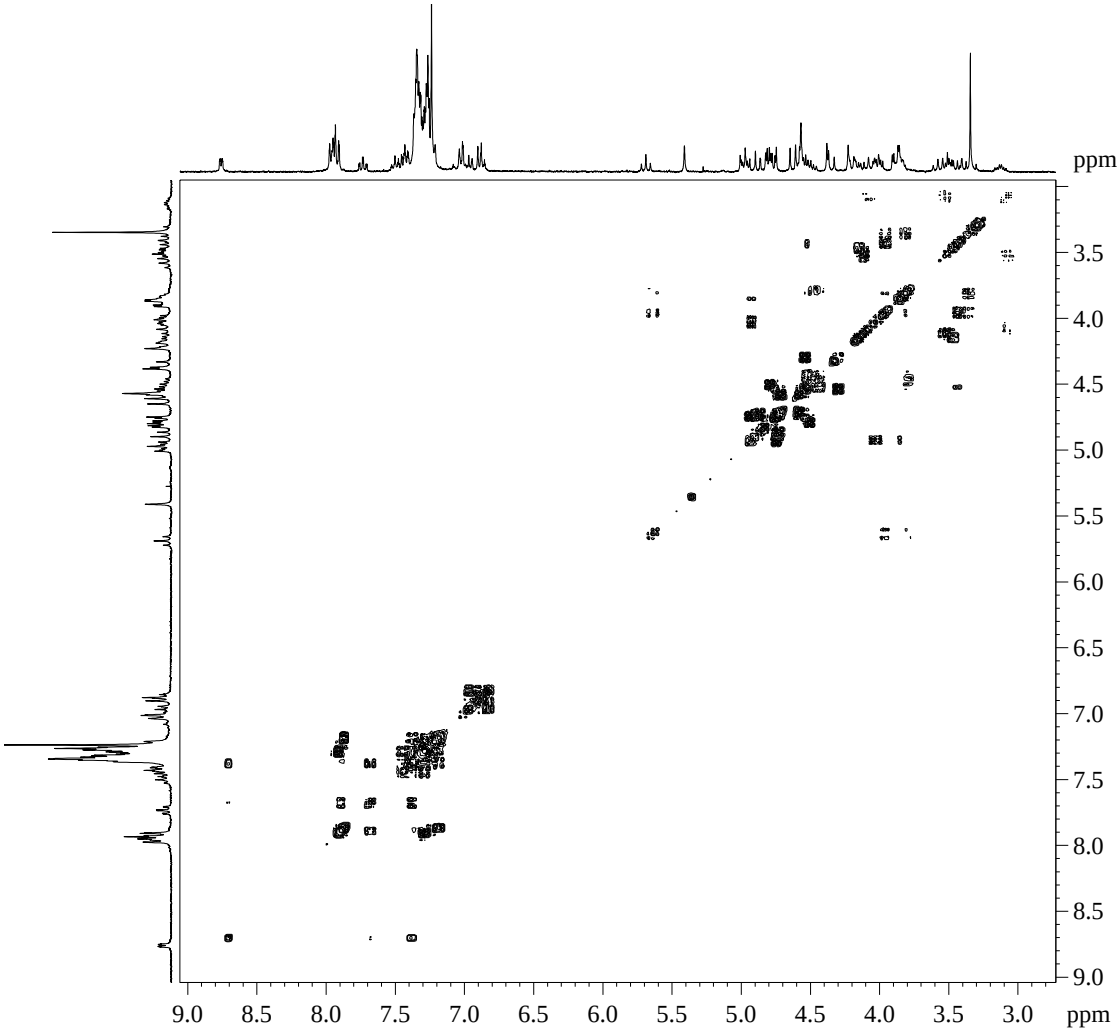


$CDCl_3$ 150 MHz

Figure A-55: ^{13}C NMR spectrum of Methyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(2.19).**

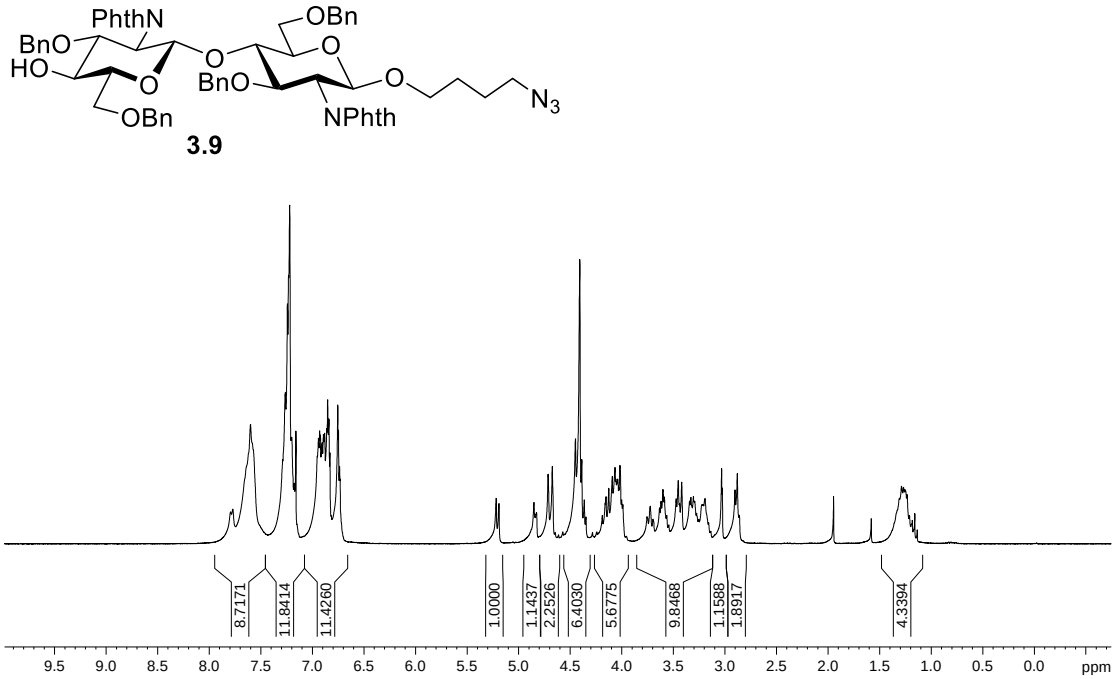


2.19



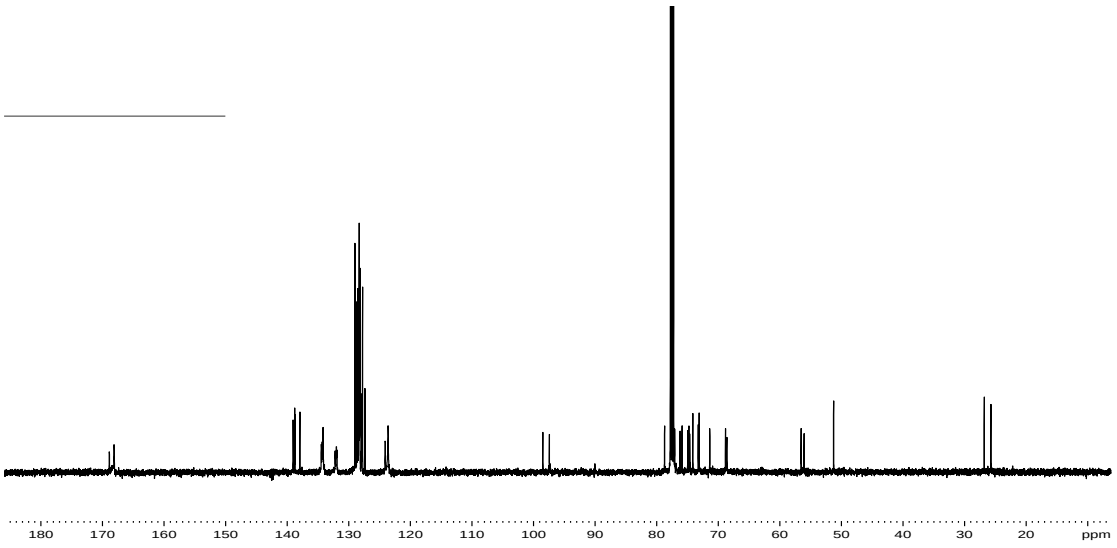
$CDCl_3$ 300 MHz

Figure A-56: 2-D NMR COSY spectrum of Methyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→3)-*O*-(4,6-di-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(2.19).**



$CDCl_3$ 300 MHz

Figure A-57: ^{1}H NMR spectrum of 4-Azidobutyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.9).**



$CDCl_3$ 150 MHz

Figure A-58: ^{13}C NMR spectrum of 4-Azidobutyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.9).**

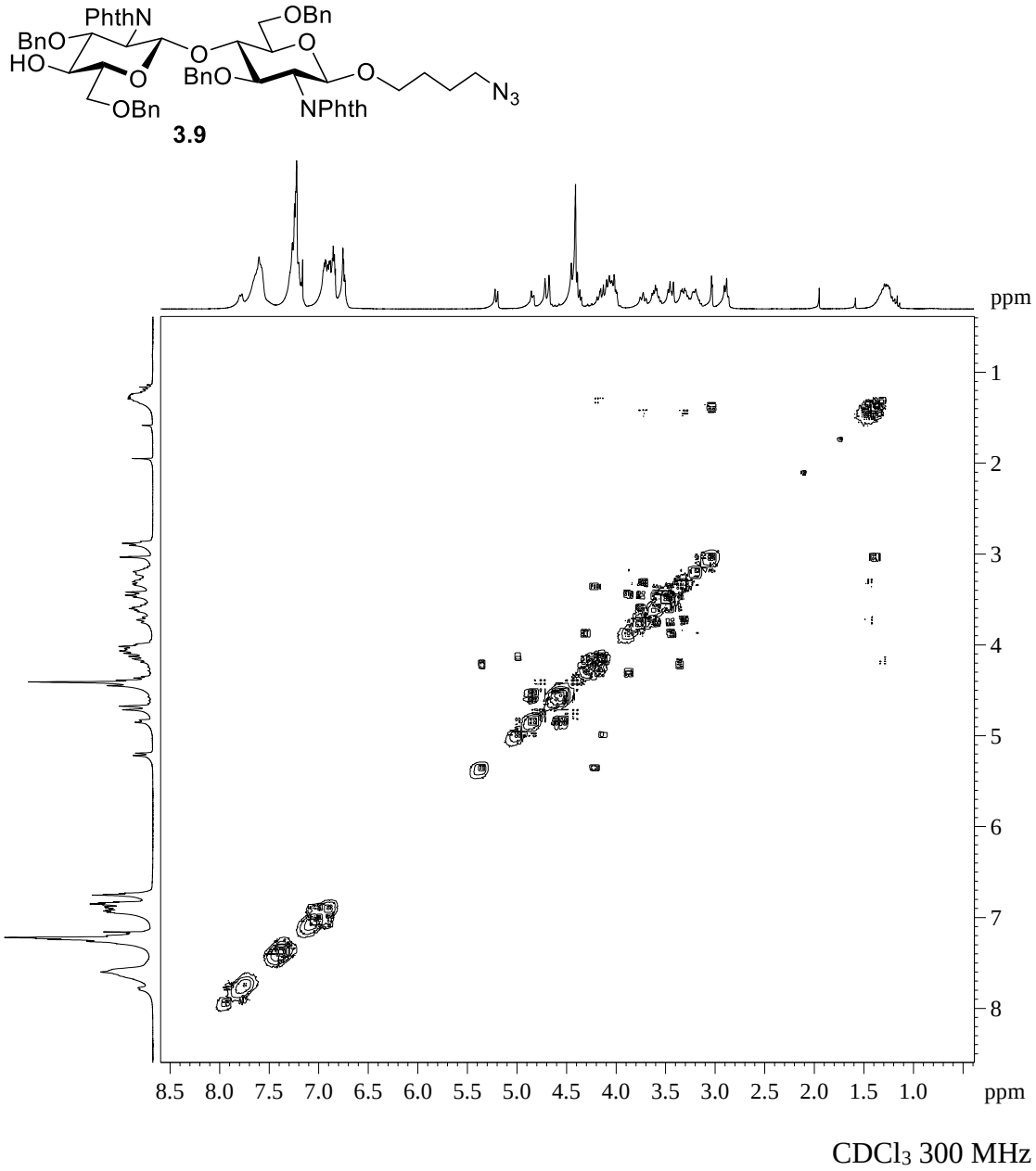
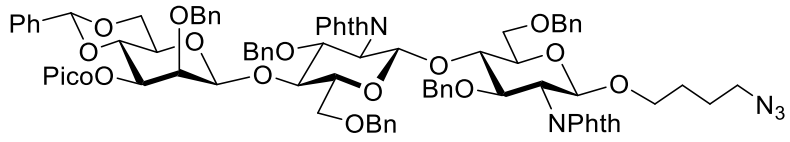
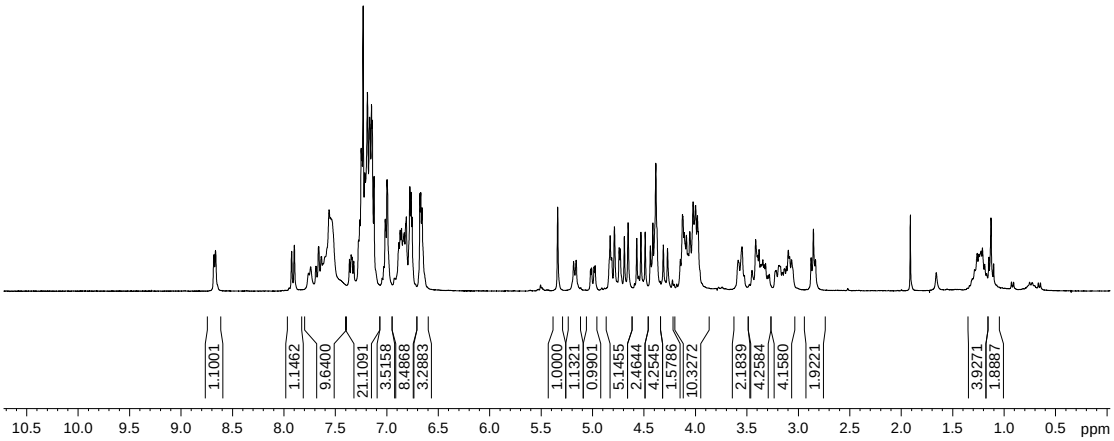


Figure A-59: 2-D NMR COSY spectrum of 4-Azidobutyl *O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.9).**

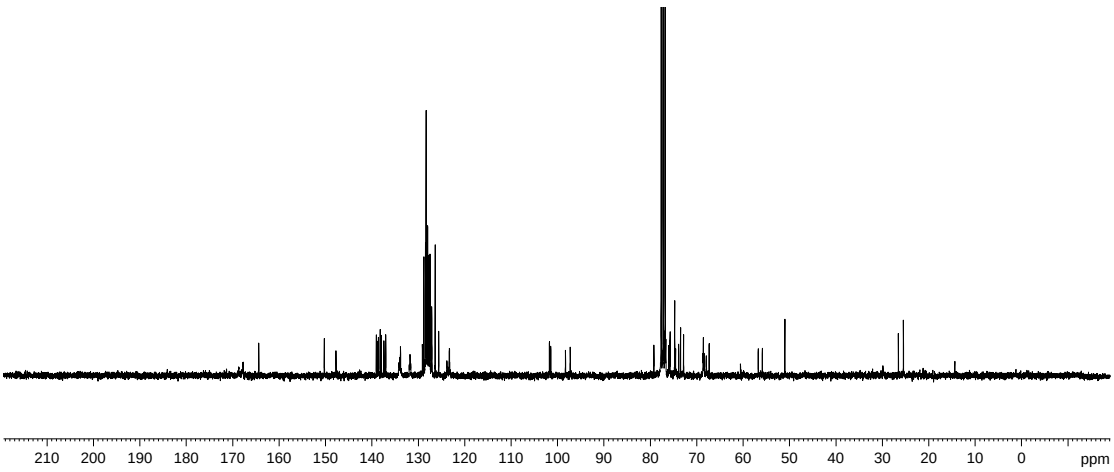


3.11



$CDCl_3$ 300 MHz

Figure A-60: ^{1}H NMR spectrum of 4-Azidobutyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.11).**



$CDCl_3$ 150 MHz

Figure A-61: ^{13}C NMR spectrum of 4-Azidobutyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.11).**

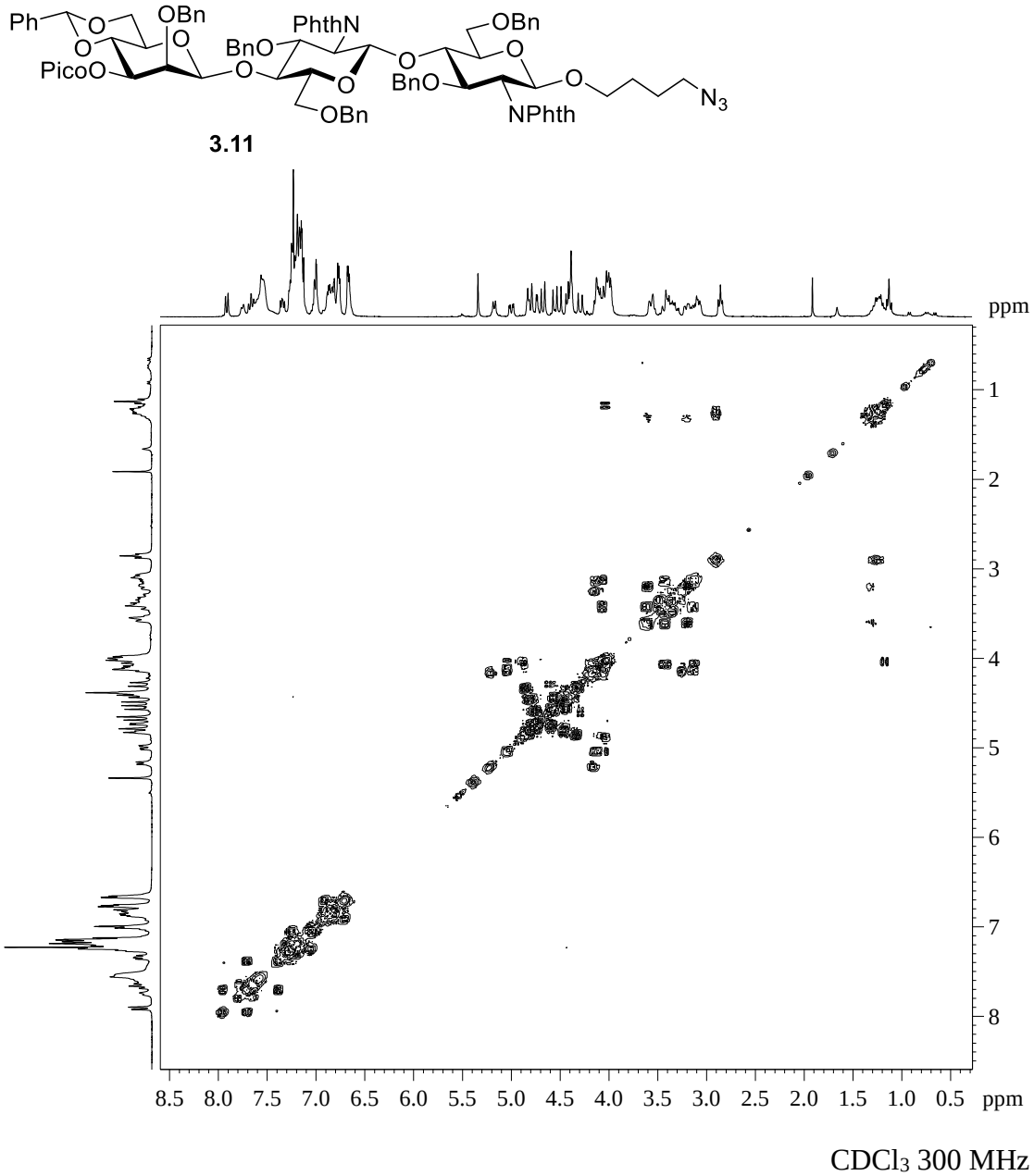
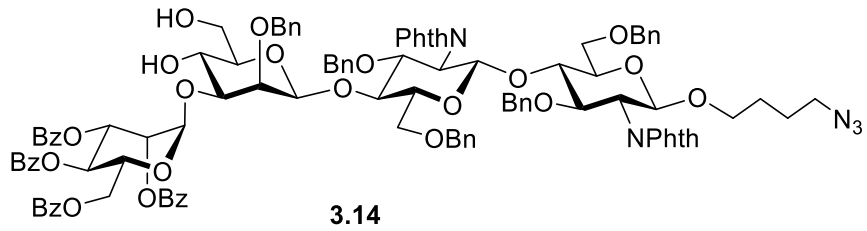
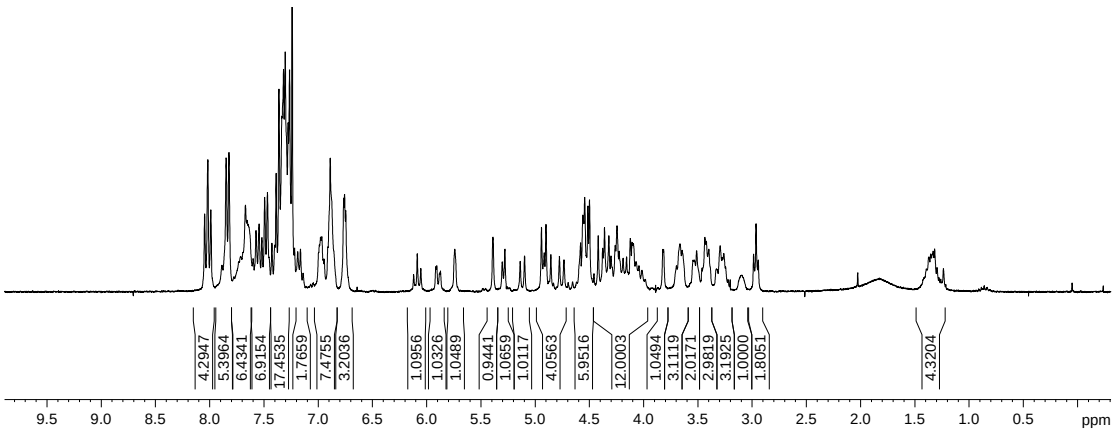


Figure A-62: 2-D NMR COSY spectrum of 4-Azidobutyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.11).**

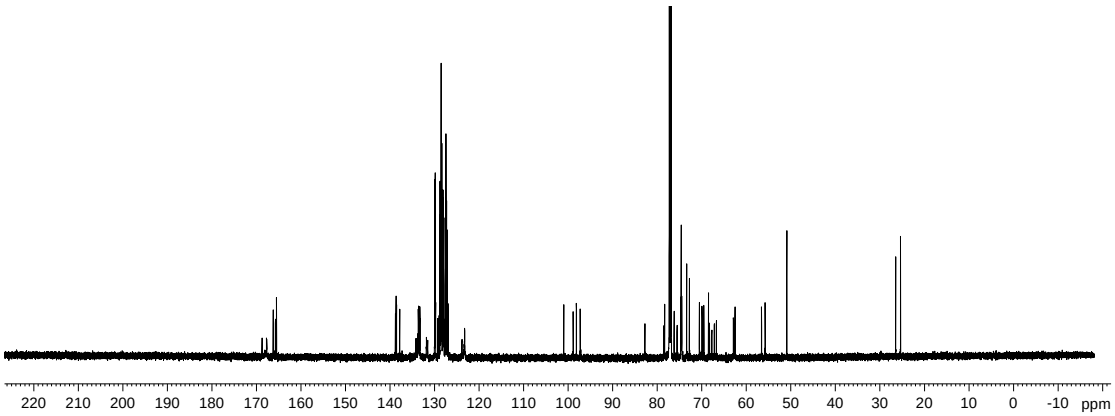


3.14



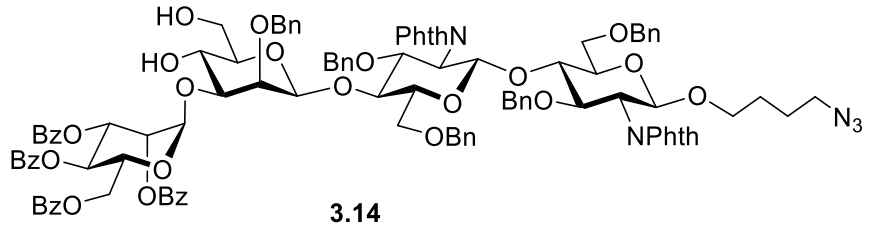
$CDCl_3$ 300 MHz

Figure A-63: ^{1}H NMR spectrum of 4-Azidobutyl *O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.14).**

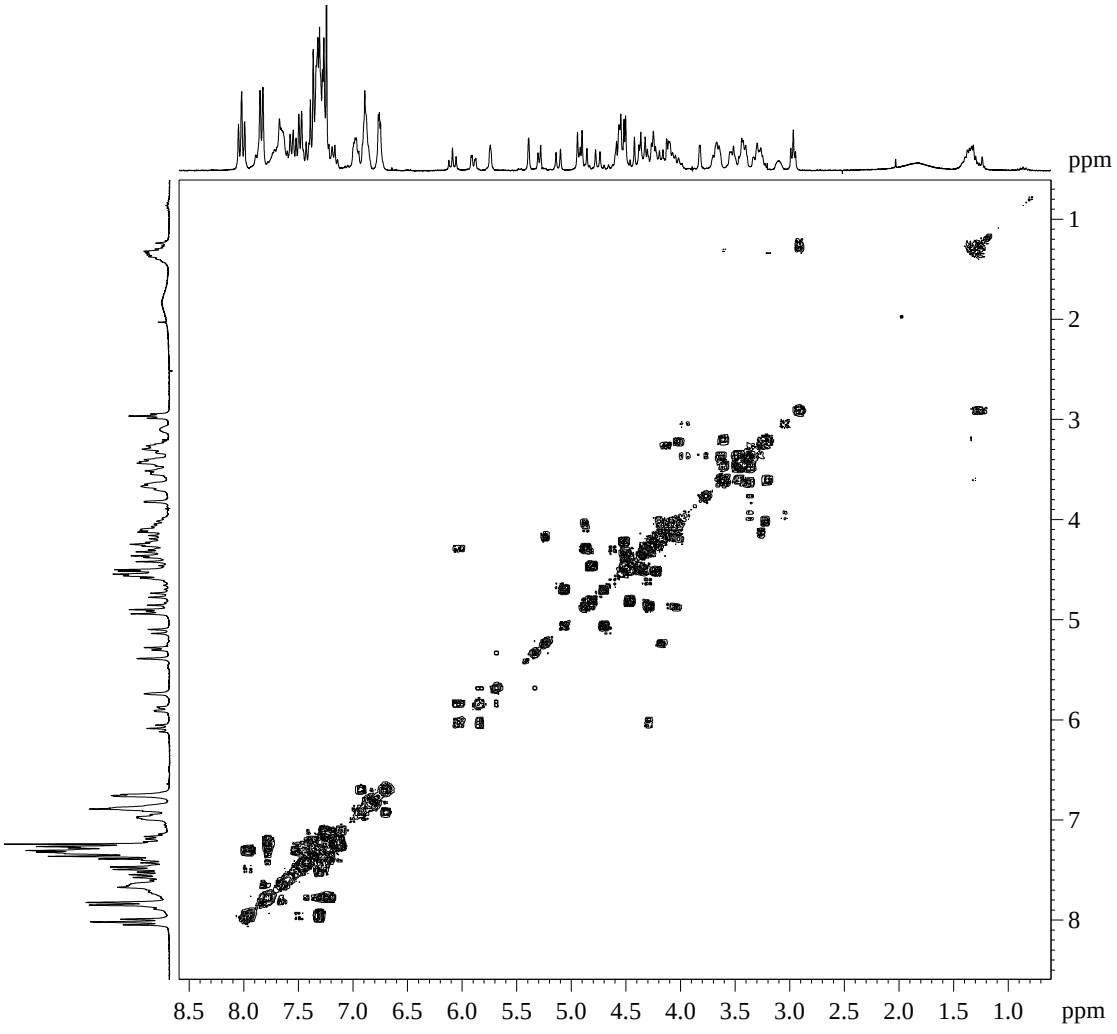


$CDCl_3$ 150 MHz

Figure A-64: ^{13}C NMR spectrum of 4-Azidobutyl *O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.14).**



3.14



$CDCl_3$ 300 MHz

Figure A-65: 2-D NMR COSY spectrum of 4-Azidobutyl *O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.14).**

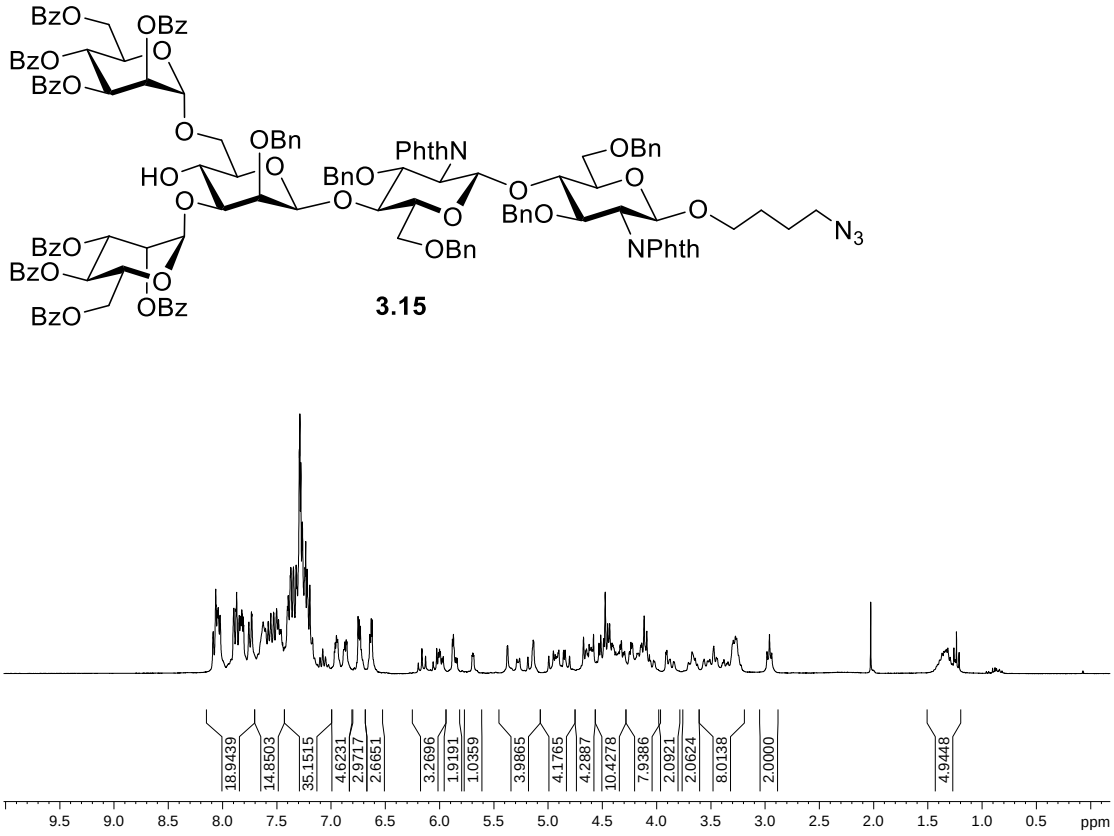


Figure A-66: ^{1}H NMR spectrum of 4-Azidobutyl di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3,1→6)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.15).**

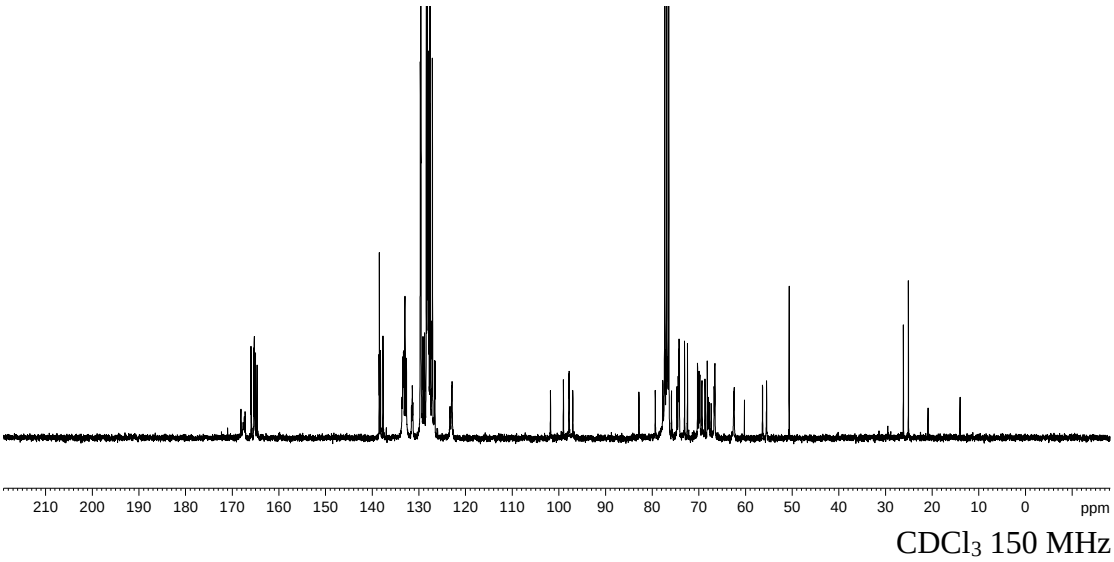


Figure A-67: ^{13}C NMR spectrum of 4-Azidobutyl di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3,1→6)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.15).**

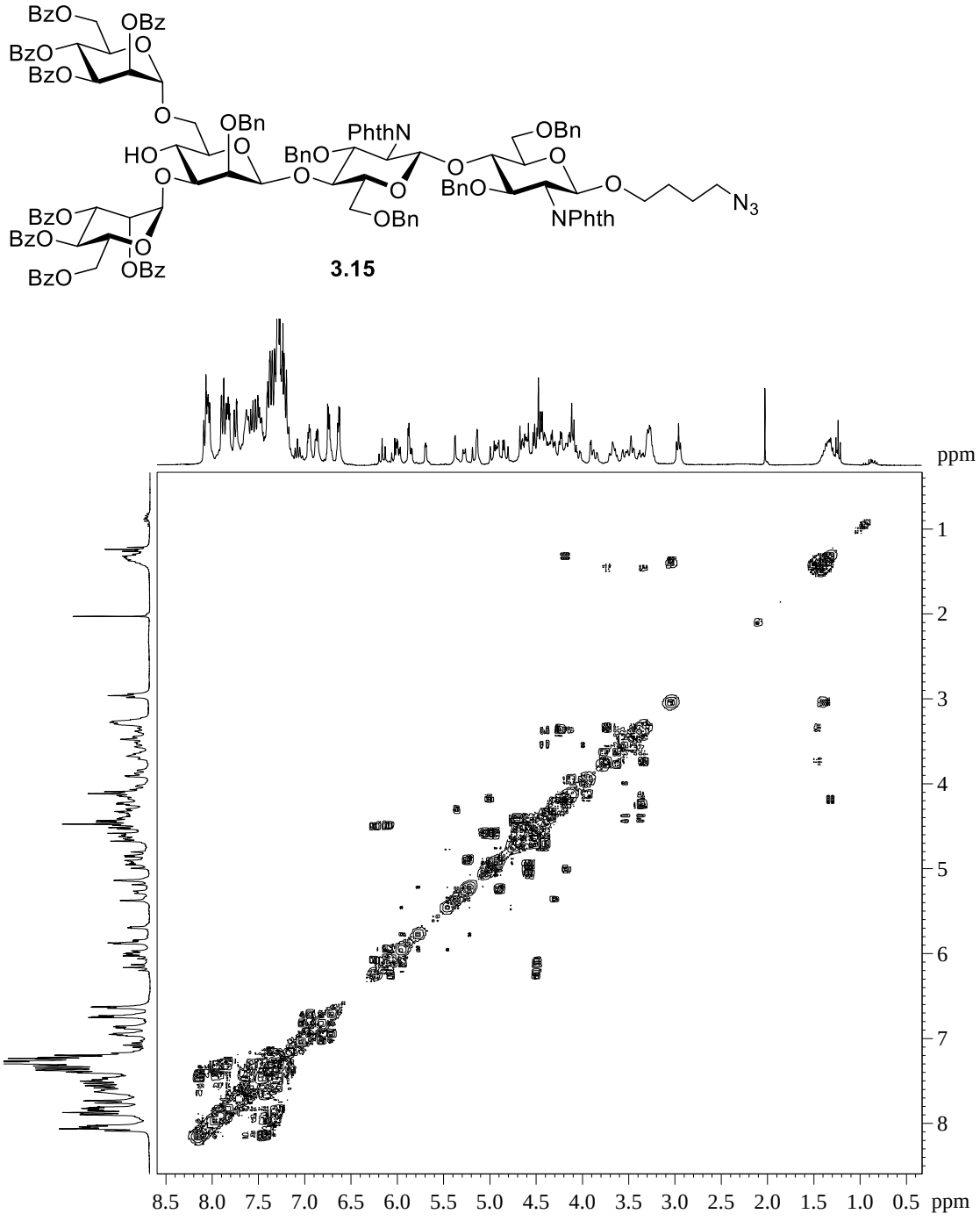
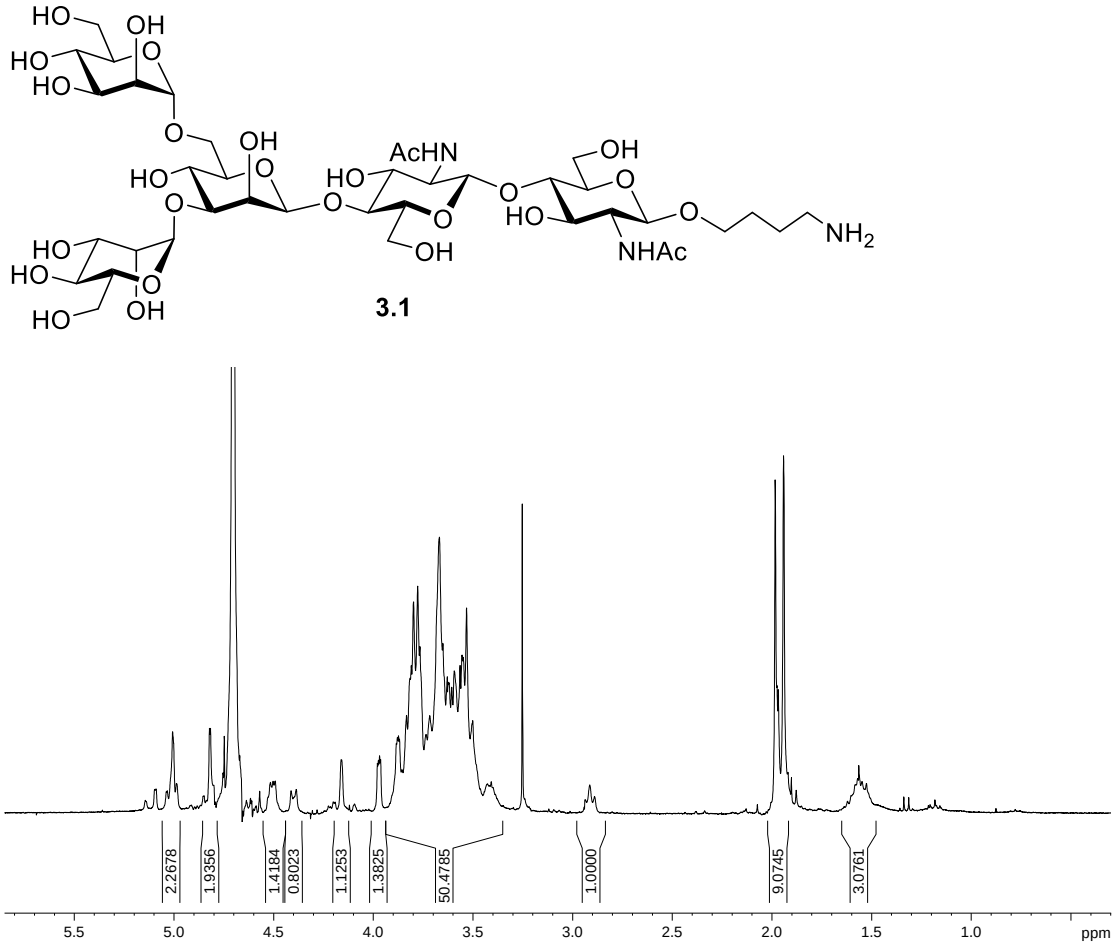
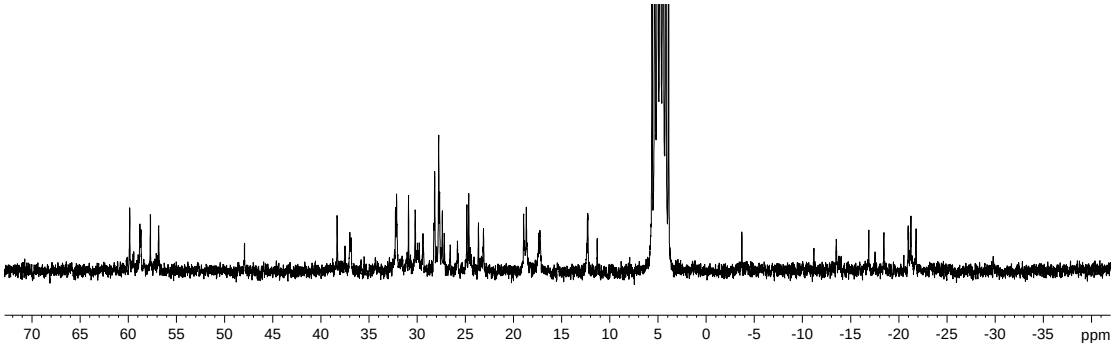


Figure A-68: 2-D NMR COSY spectrum of 4-Azidobutyl di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-(1→3,1→6)-*O*-(2-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(3.15).**



$CDCl_3$ 300 MHz

Figure A-69: ^{1}H NMR spectrum of 4-Aminobutyl di-*O*-(α-D-mannopyranosyl)-(1→3,1→6)-*O*-(β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside **(3.1).**



$CDCl_3$ 150 MHz

Figure A-70: ^{13}C NMR spectrum of 4-Aminobutyl di-*O*-(α-D-mannopyranosyl)-(1→3,1→6)-*O*-(β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside **(3.1).**

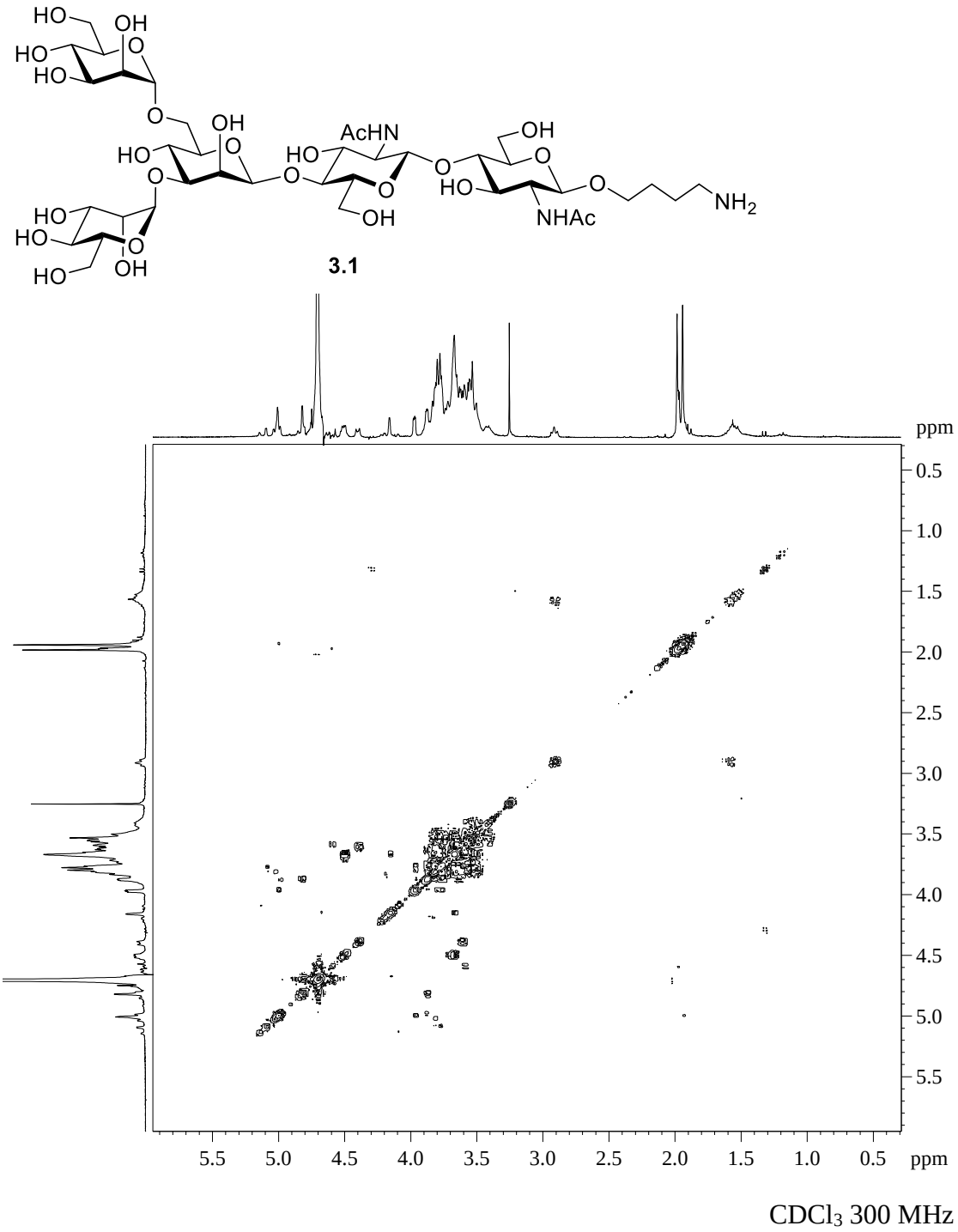
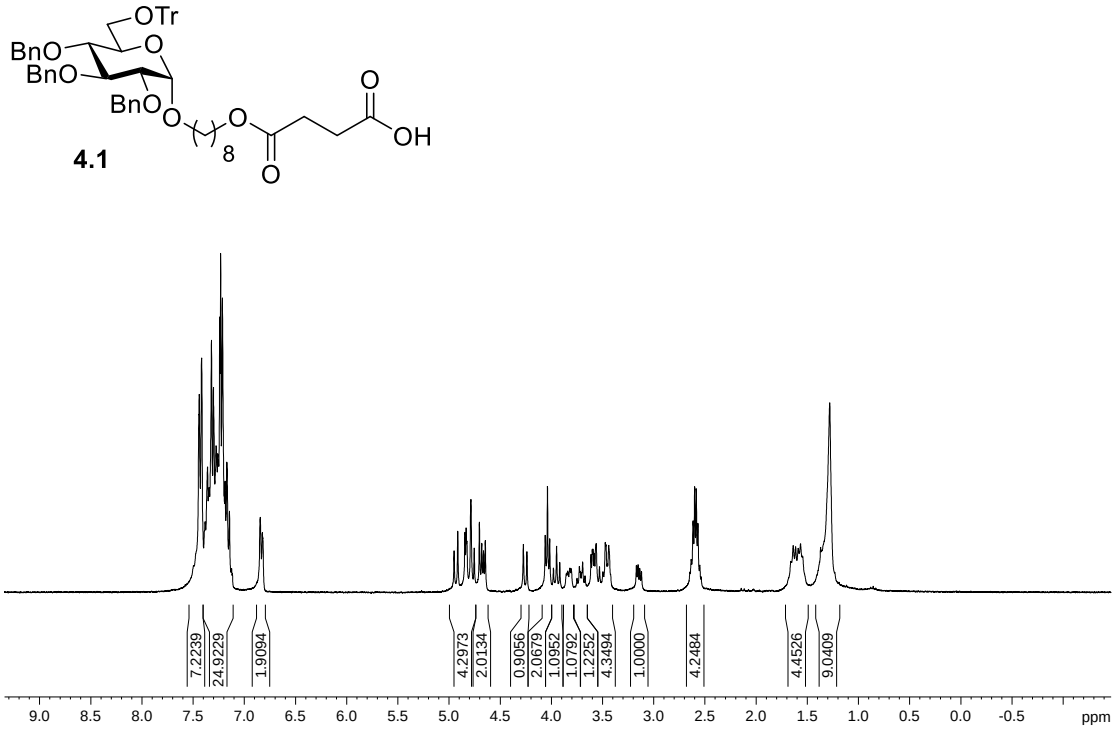
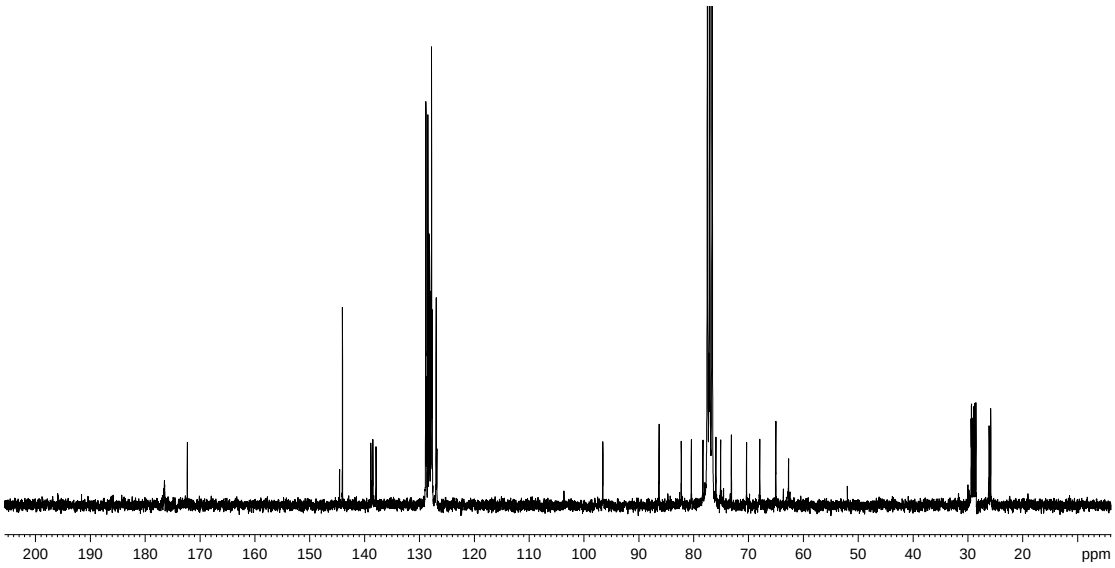


Figure A-71: 2-D NMR COSY spectrum of 4-Aminobutyl di-*O*-(α-D-mannopyranosyl)-(1→3,1→6)-*O*-(β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside **(3.1).**



$CDCl_3$ 300 MHz

Figure A-72: ^{1}H NMR spectrum of 8-(3-Carboxypropanoyloxy)oct-1-yl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside **(4.1).**



$CDCl_3$ 150 MHz

Figure A-73: ^{13}C NMR spectrum of 8-(3-Carboxypropanoyloxy)oct-1-yl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside **(4.1).**

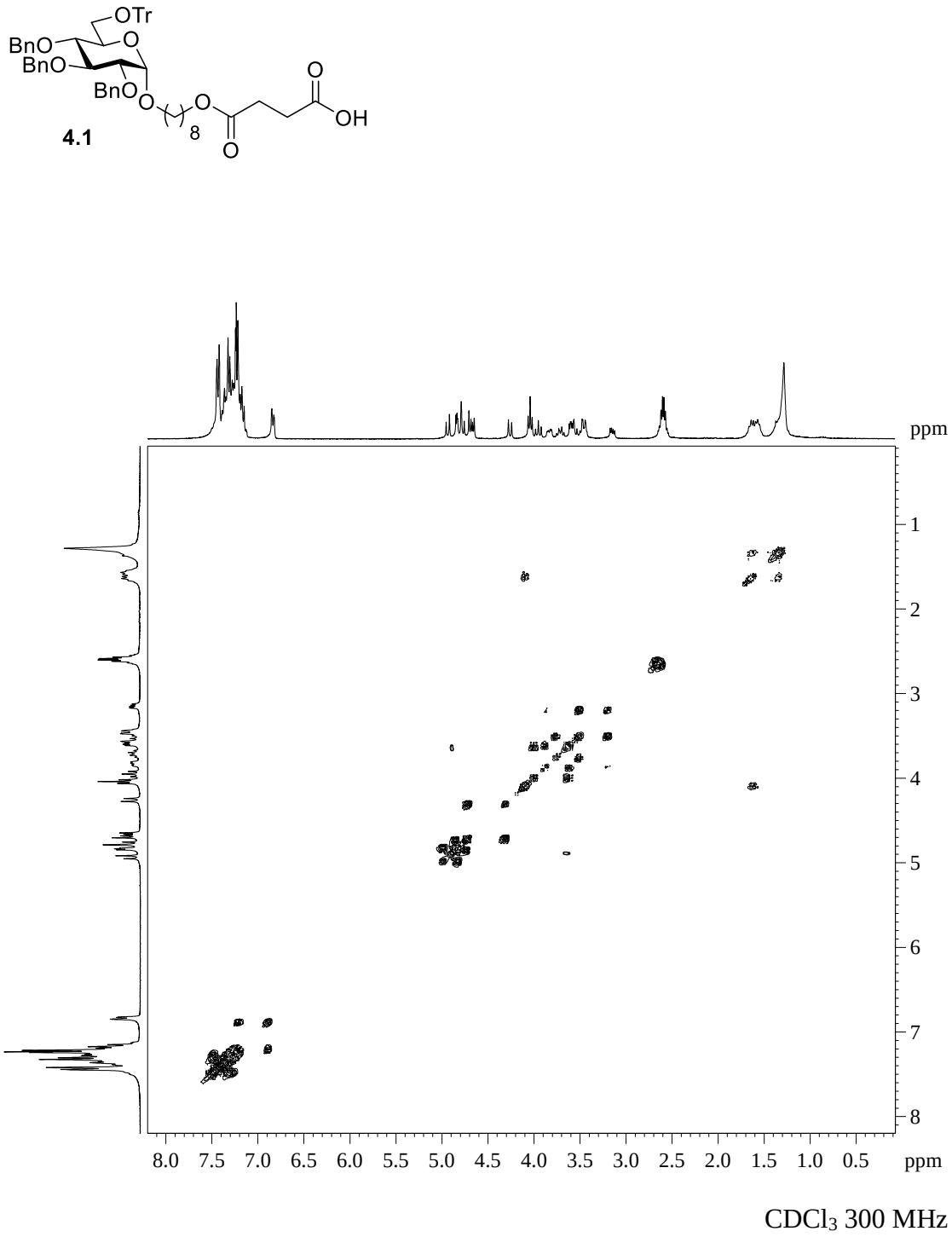
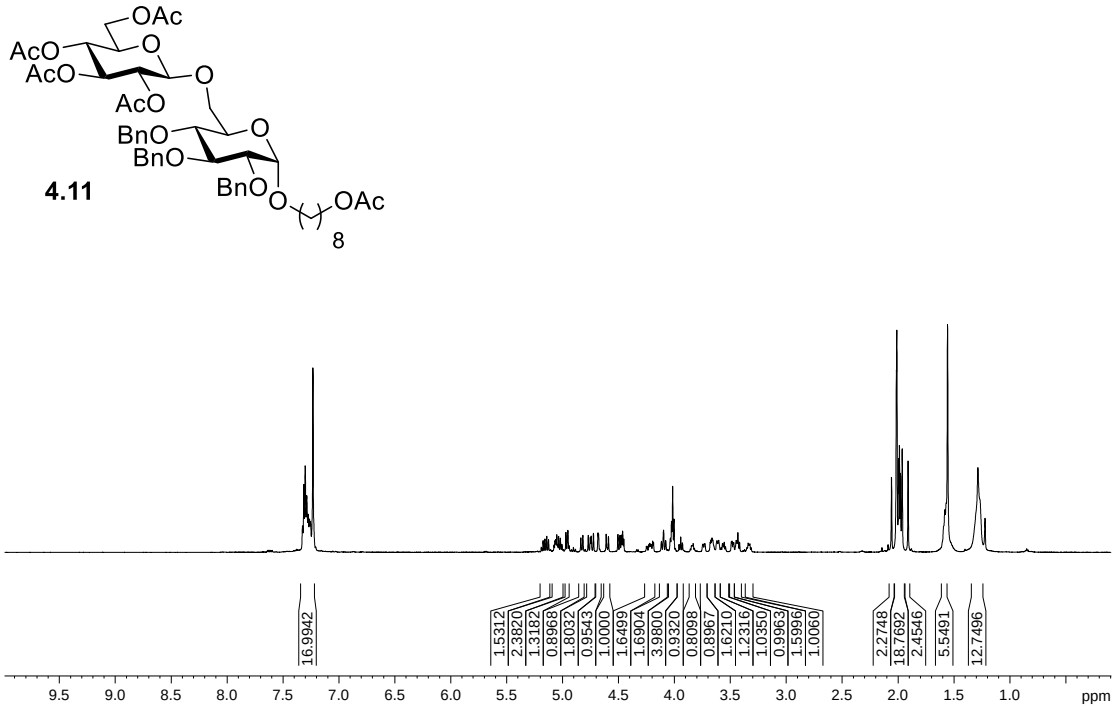
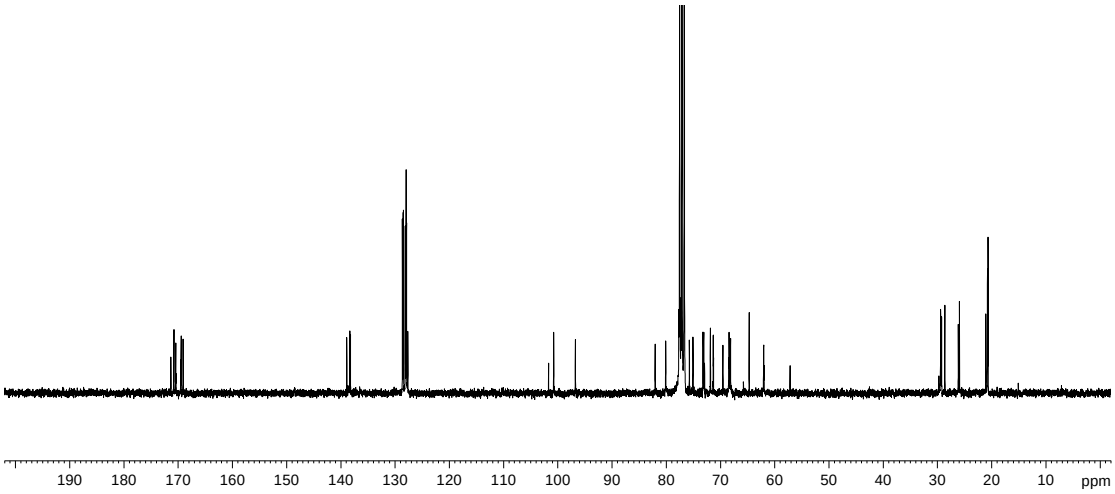


Figure A-74: 2-D NMR COSY spectrum of 8-(3-Carboxypropanoyloxy)oct-1-yl 2,3,4-tri-*O*-benzyl-6-*O*-triphenylmethyl-α-D-glucopyranoside **(4.1).**



$CDCl_3$ 300 MHz

Figure A-75: ^{1}H NMR spectrum of 8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(4.11).**



$CDCl_3$ 150 MHz

Figure A-76: ^{13}C NMR spectrum of 8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(4.11).**

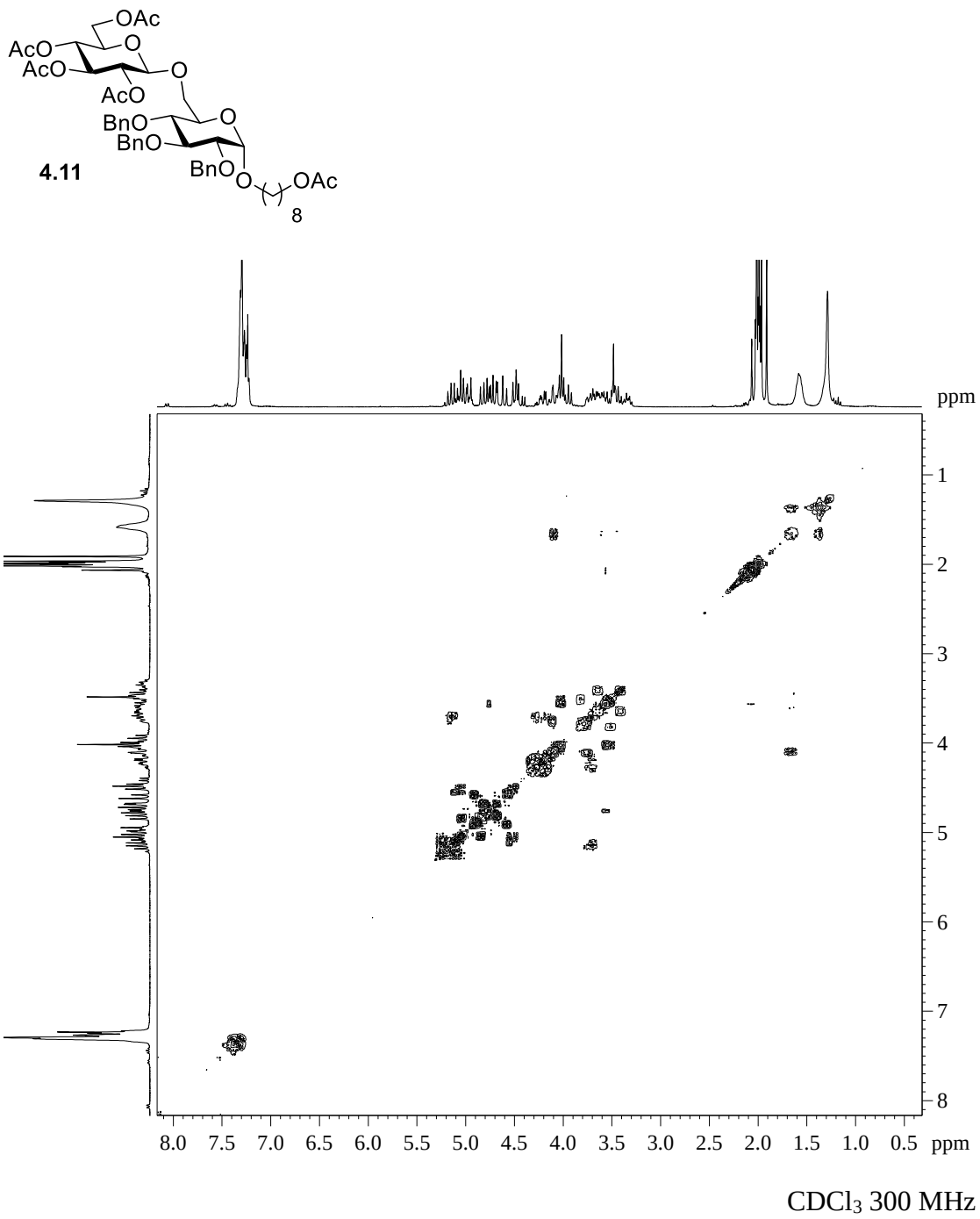
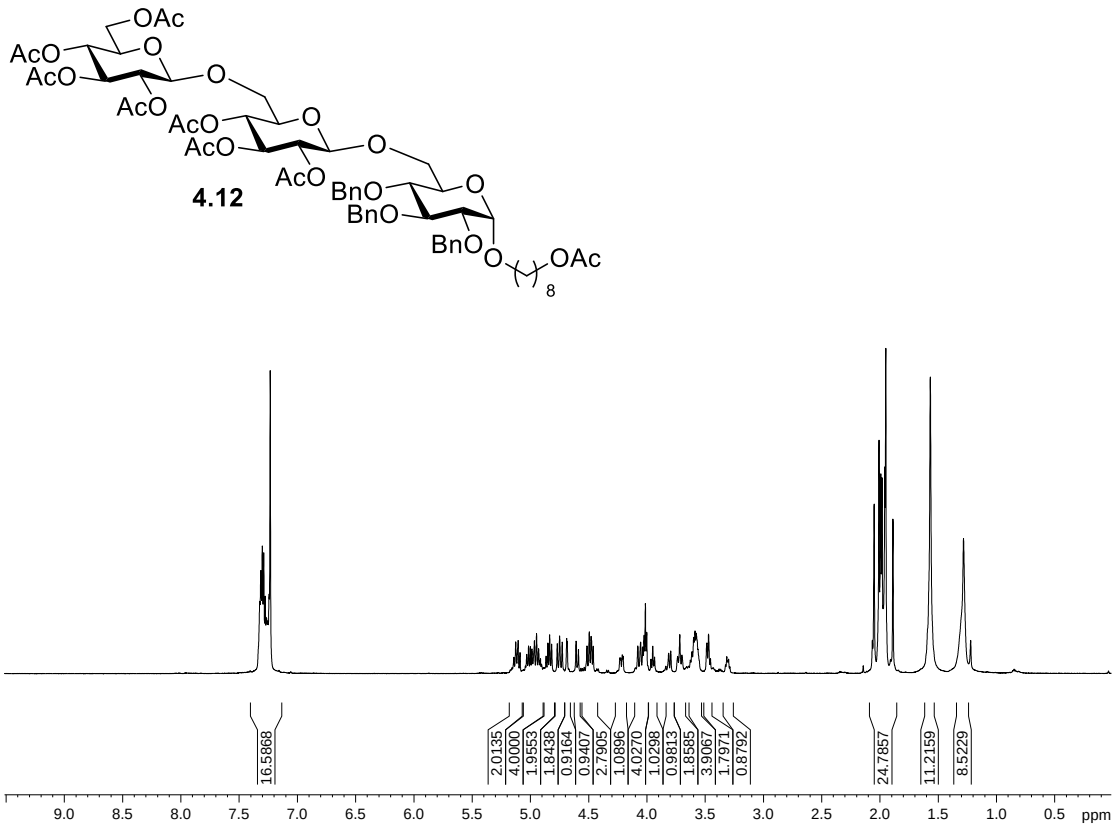
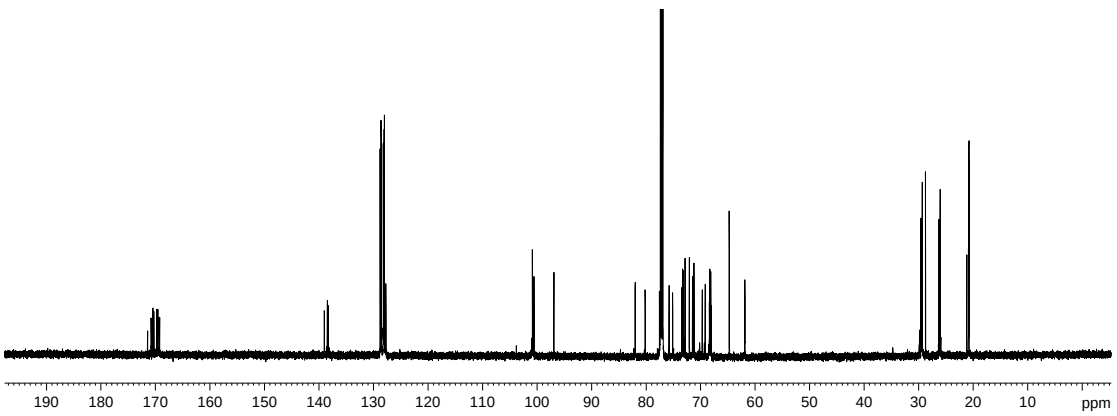


Figure A-77: 2-D NMR COSY spectrum of 8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(4.11).**



$CDCl_3$ 300 MHz

Figure A-78: ^{1}H NMR spectrum of 8-Acetyloxyoct-1-yl-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside **(4.12).**



$CDCl_3$ 150 MHz

Figure A-79: ^{13}C NMR spectrum of 8-Acetyloxyoct-1-yl-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside **(4.12).**

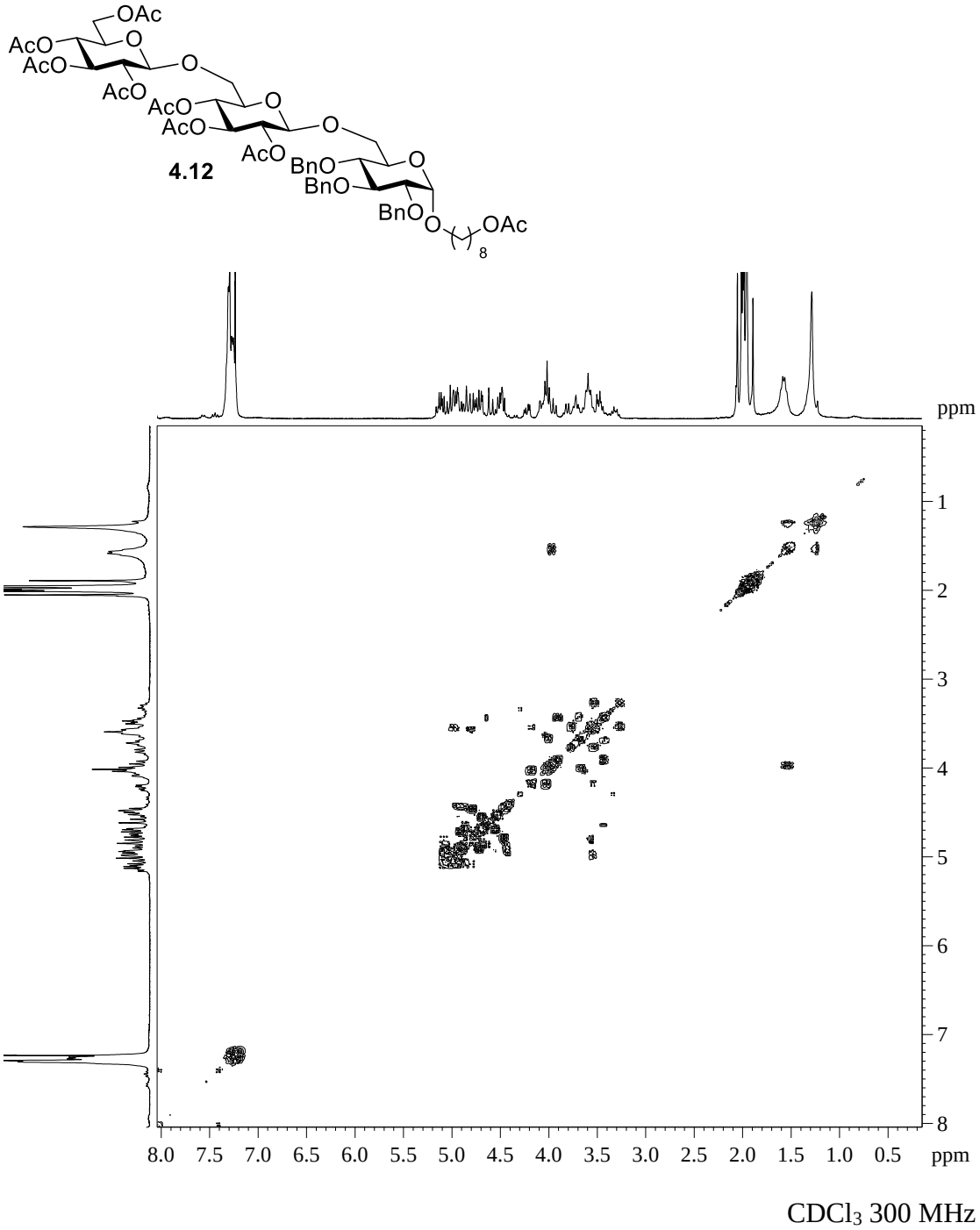


Figure A-80: 2-D NMR COSY spectrum of 8-Acetyloxyoct-1-yl-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-glucopyranoside **(4.12).**

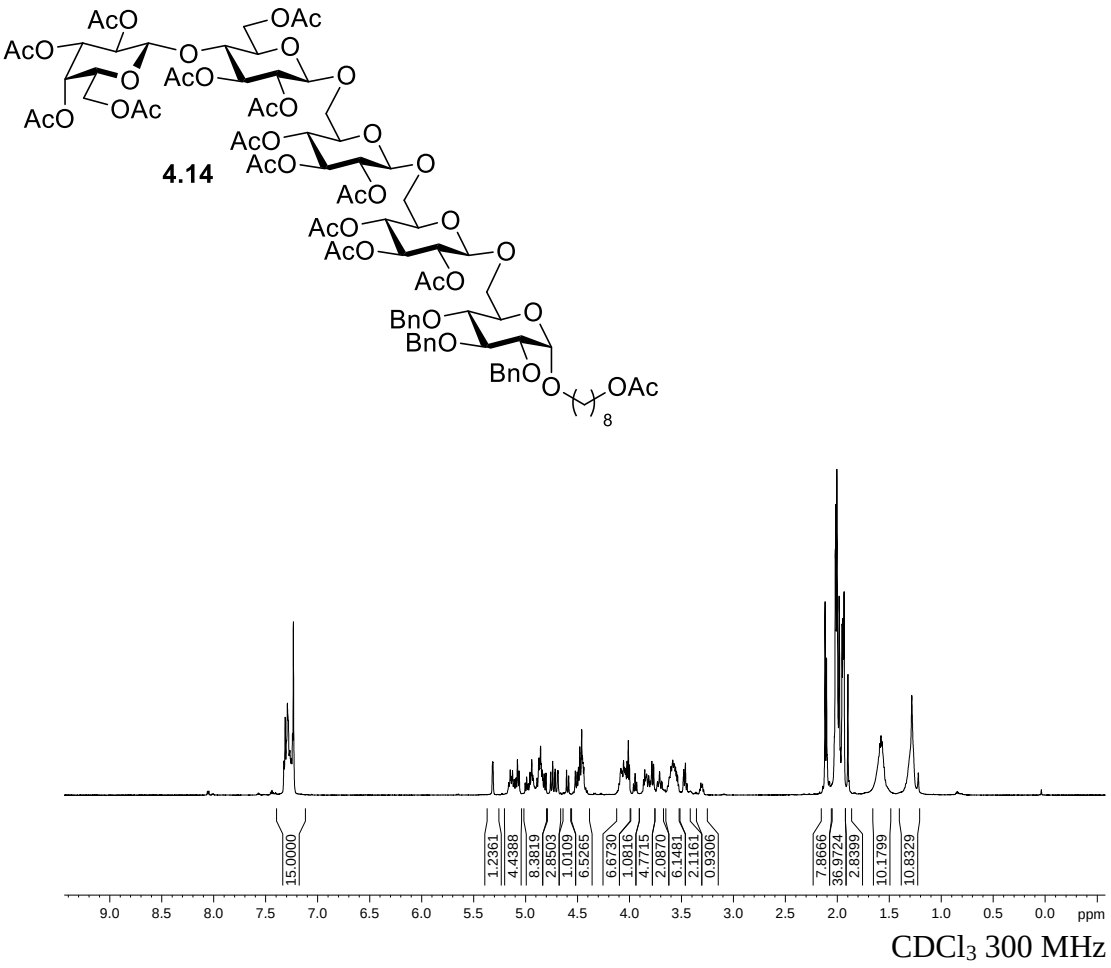


Figure A-81: ^{1}H NMR spectrum of 8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(4.14).**

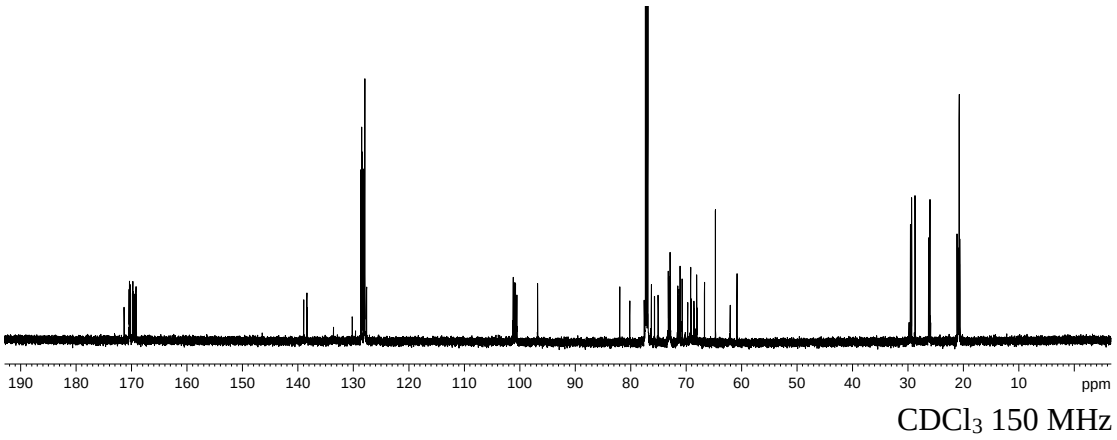


Figure A-82: ^{13}C NMR spectrum of 8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(4.14).**

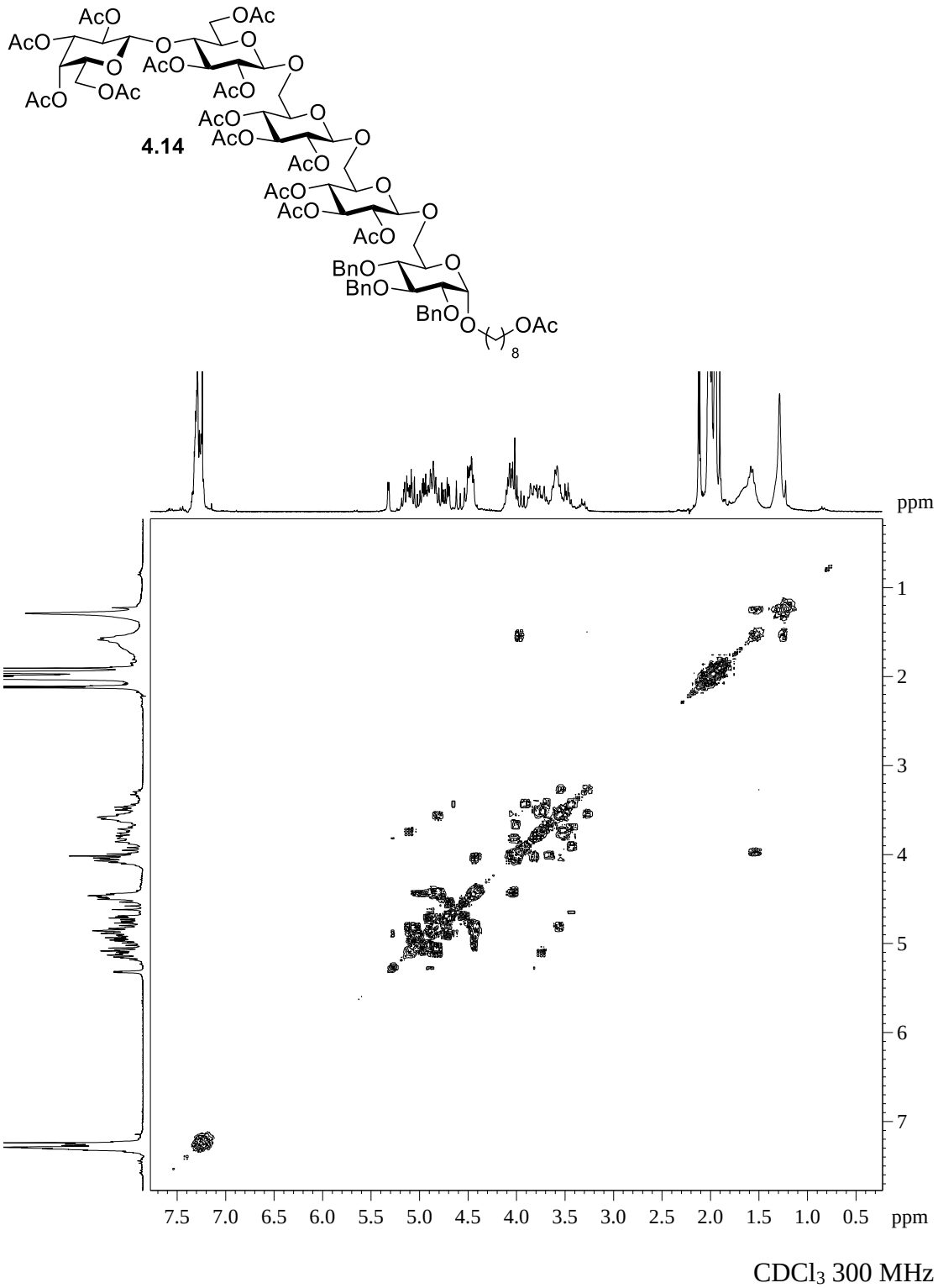
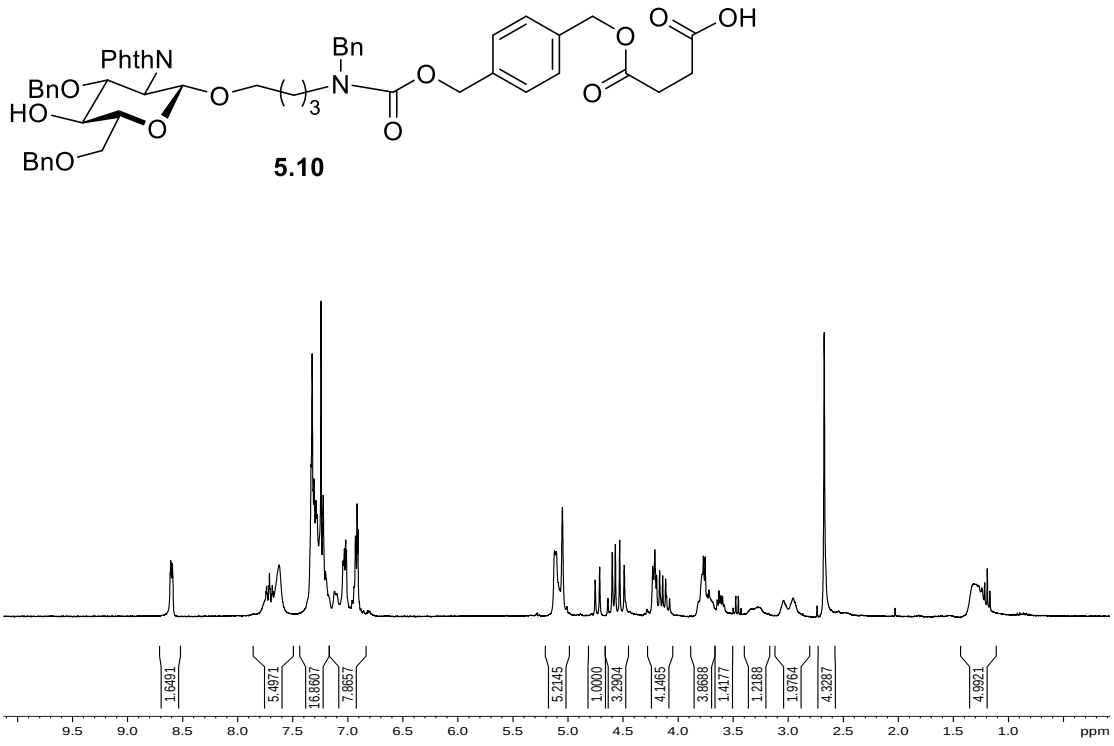
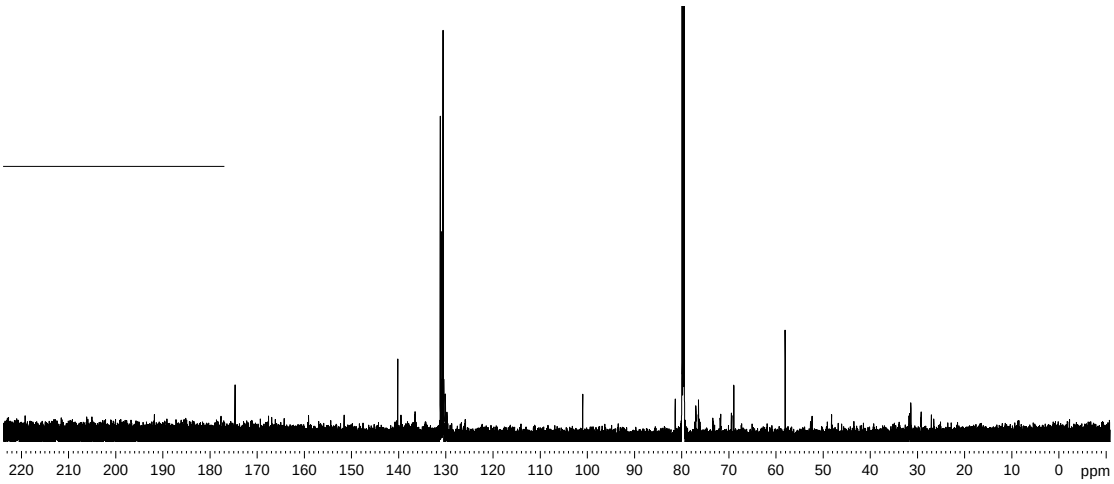


Figure A-83: 2-D NMR COSY spectrum of 8-Acetyloxyoct-1-yl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **(4.14).**



$CDCl_3$ 300 MHz

Figure A-84: ^{1}H NMR spectrum of N-(p-Succinoyloxymethyl)benzyloxycarbonyl-4-benzylaminobutyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(5.10)**



$CDCl_3$ 150 MHz

Figure A-85: ^{13}C NMR spectrum of N-(p-Succinoyloxymethyl)benzyloxycarbonyl-4-benzylaminobutyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(5.10)**

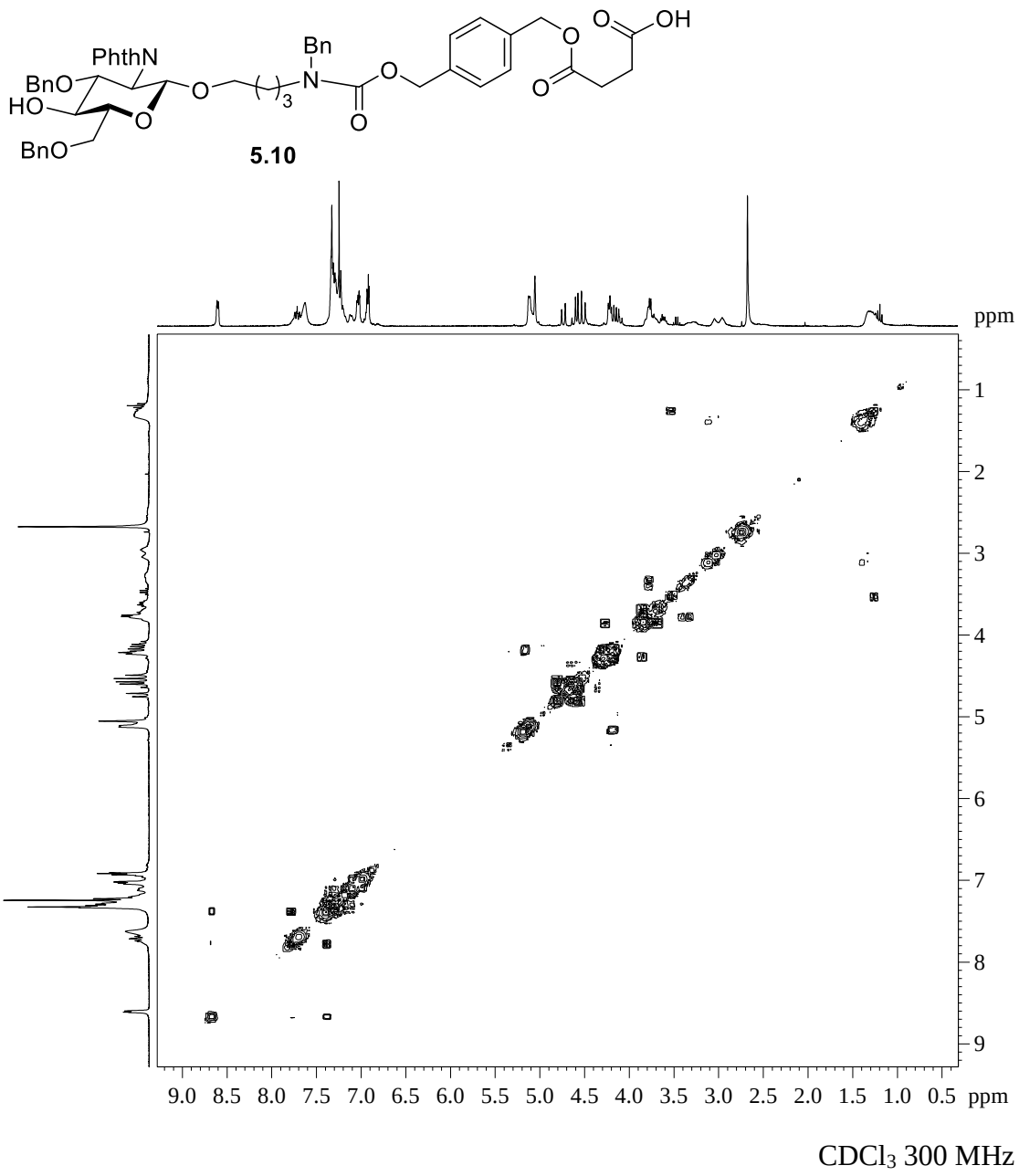
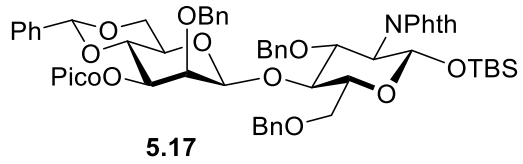
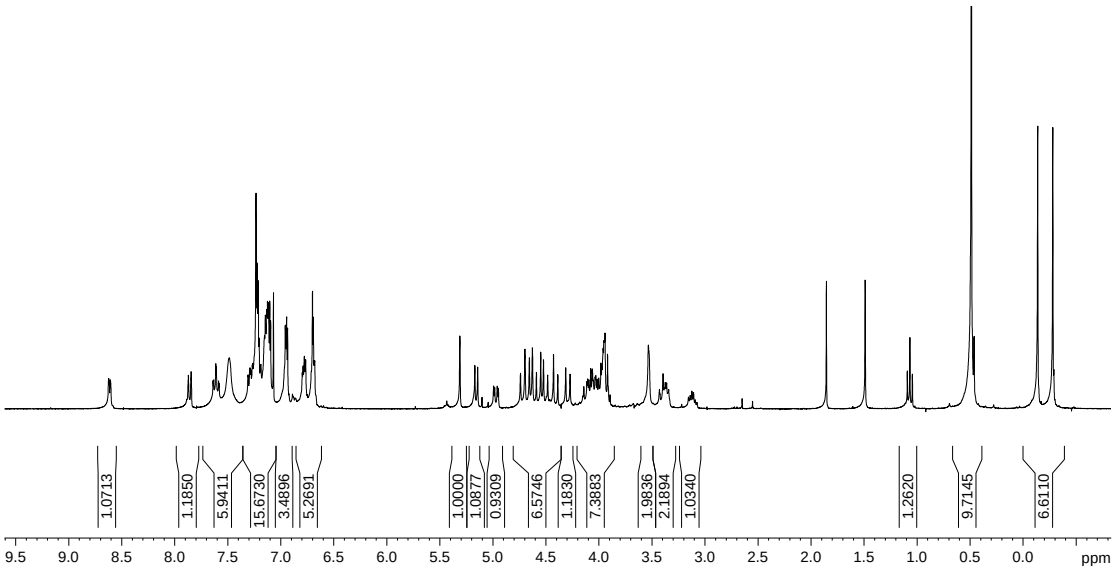


Figure A-86: 2-D NMR COSY spectrum of N-(p-Succinoyloxymethyl) benzyloxycarbonyl-4-benzylaminobutyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(5.10)**

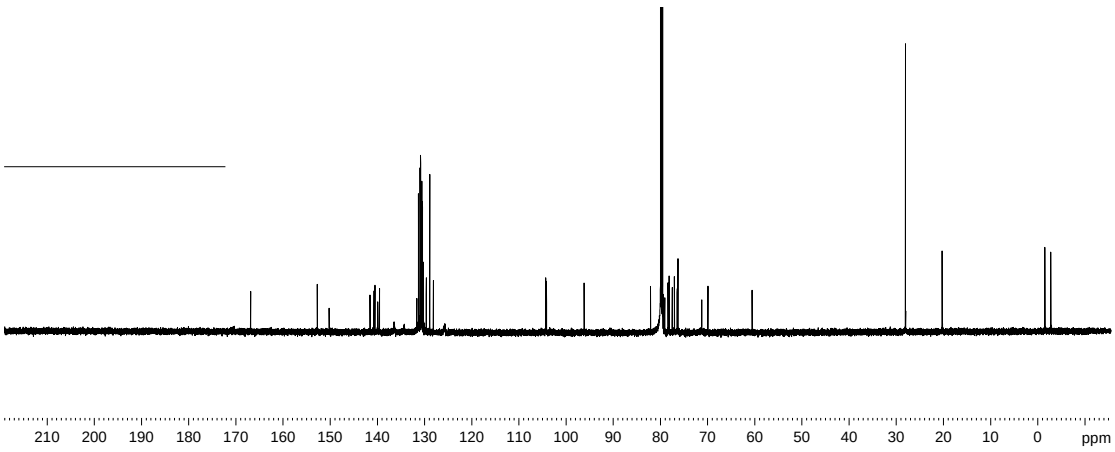


5.17



$CDCl_3$ 300 MHz

Figure A-87: ^{1}H NMR spectrum of tert-Butyldimethylsilyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D
glucopyranoside **(5.17).**



$CDCl_3$ 150 MHz

Figure A-88: ^{13}C NMR spectrum of tert-Butyldimethylsilyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D
glucopyranoside **(5.17).**

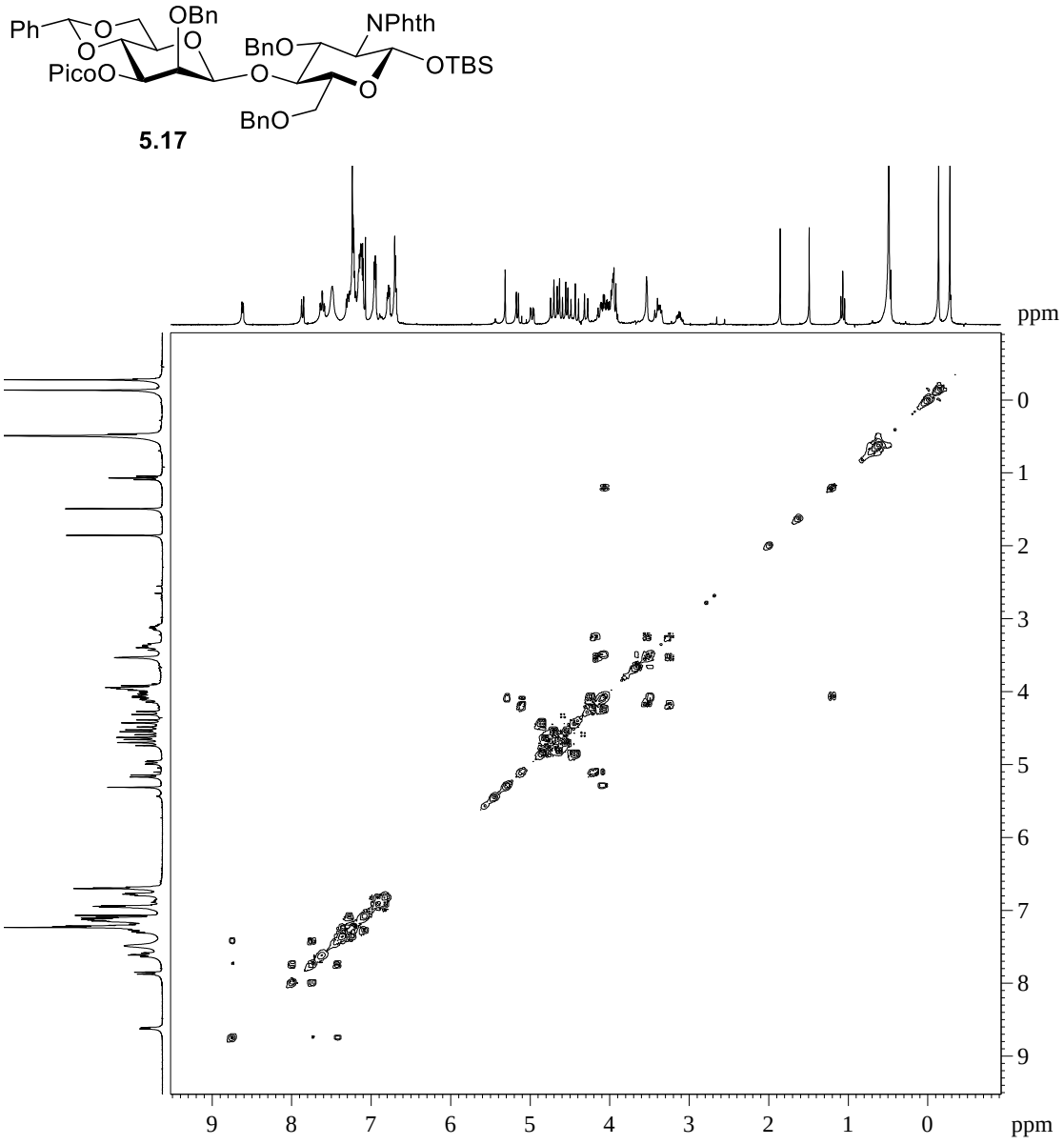
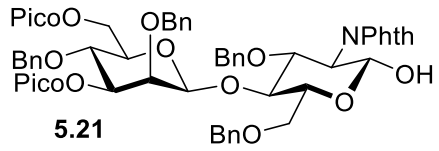
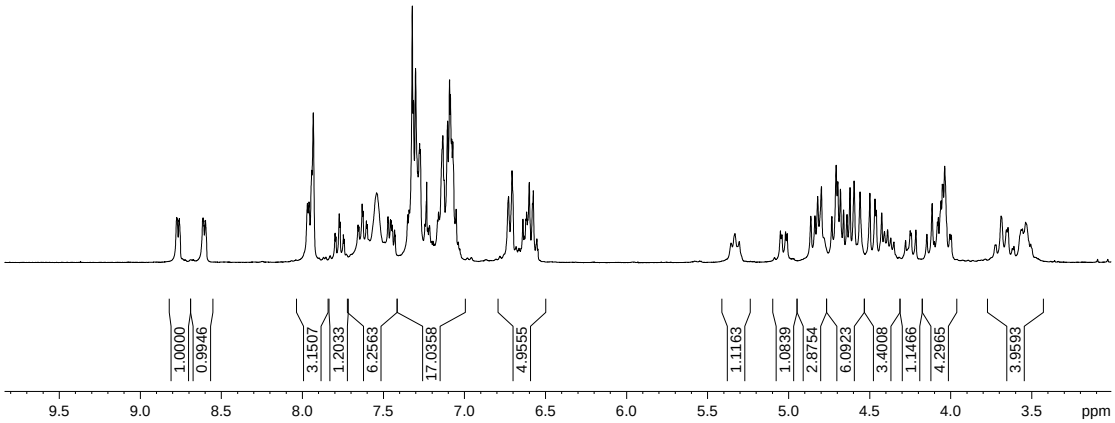


Figure A-89: 2-D NMR COSY spectrum of tert-Butyldimethylsilyl *O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D
glucopyranoside **(5.17).**

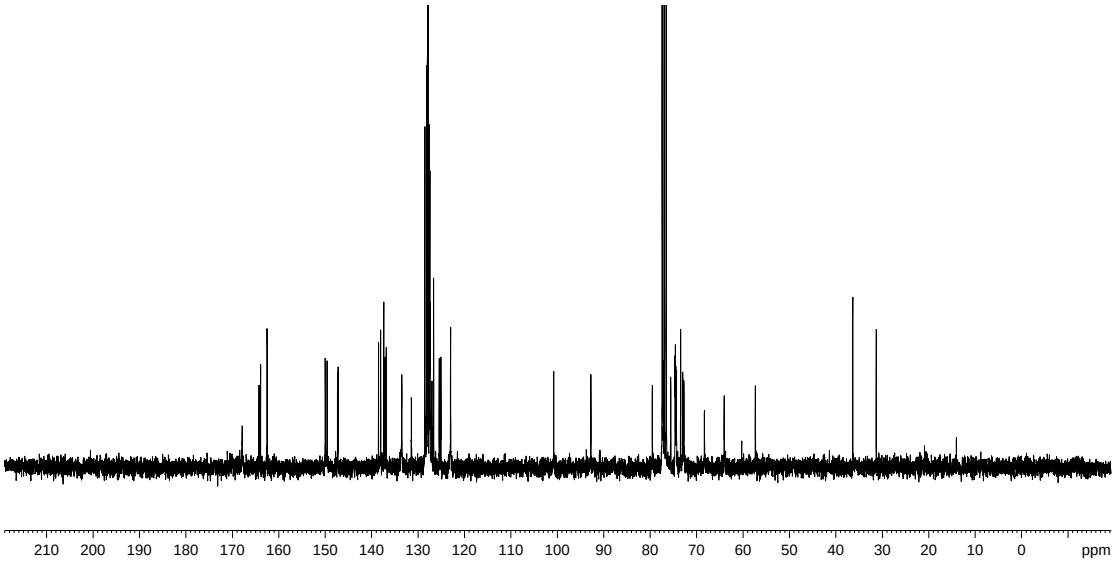


5.21



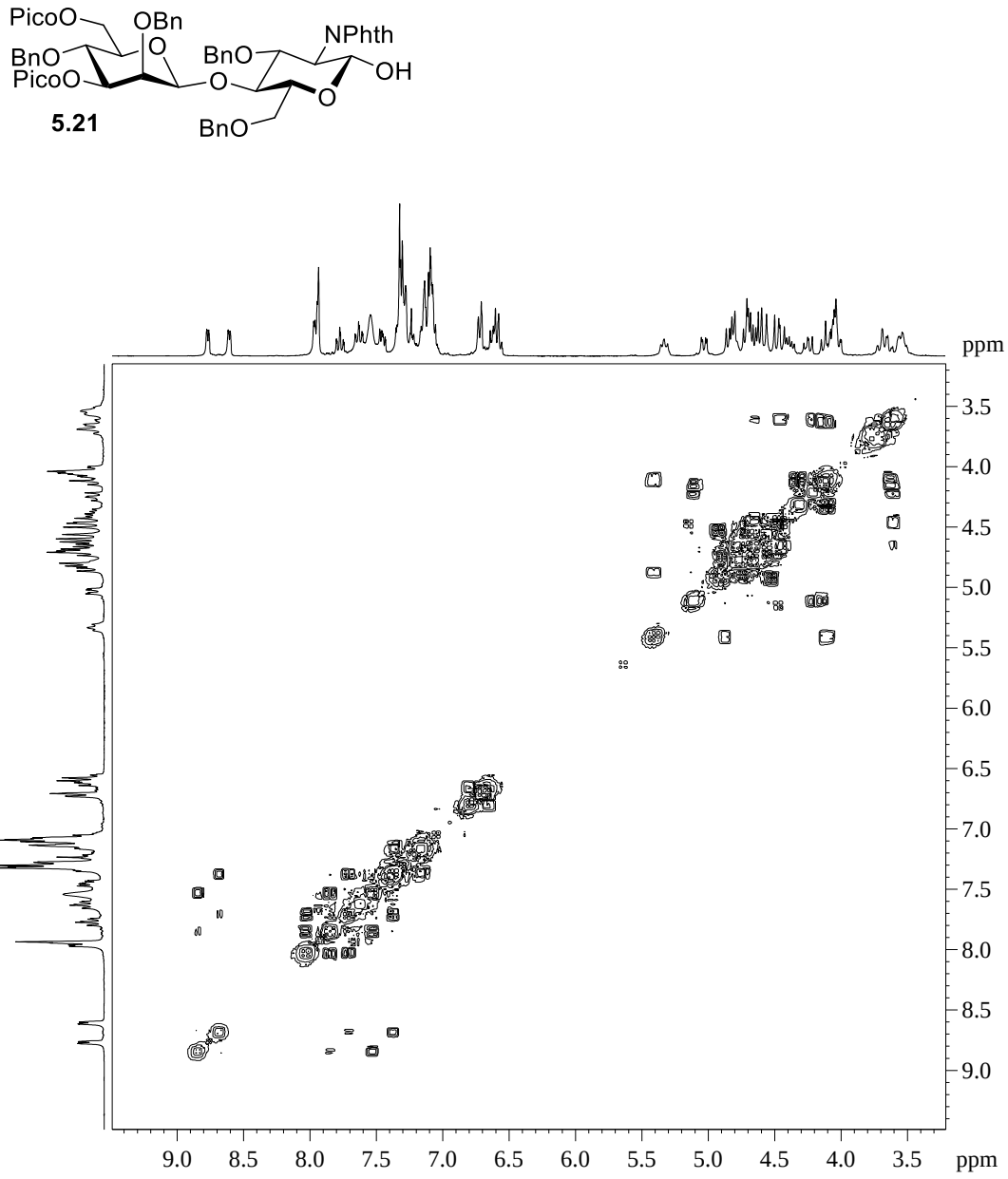
$CDCl_3$ 300 MHz

Figure A-90: ^{1}H NMR spectrum of *O*-(2,4-Di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose **(5.21)**



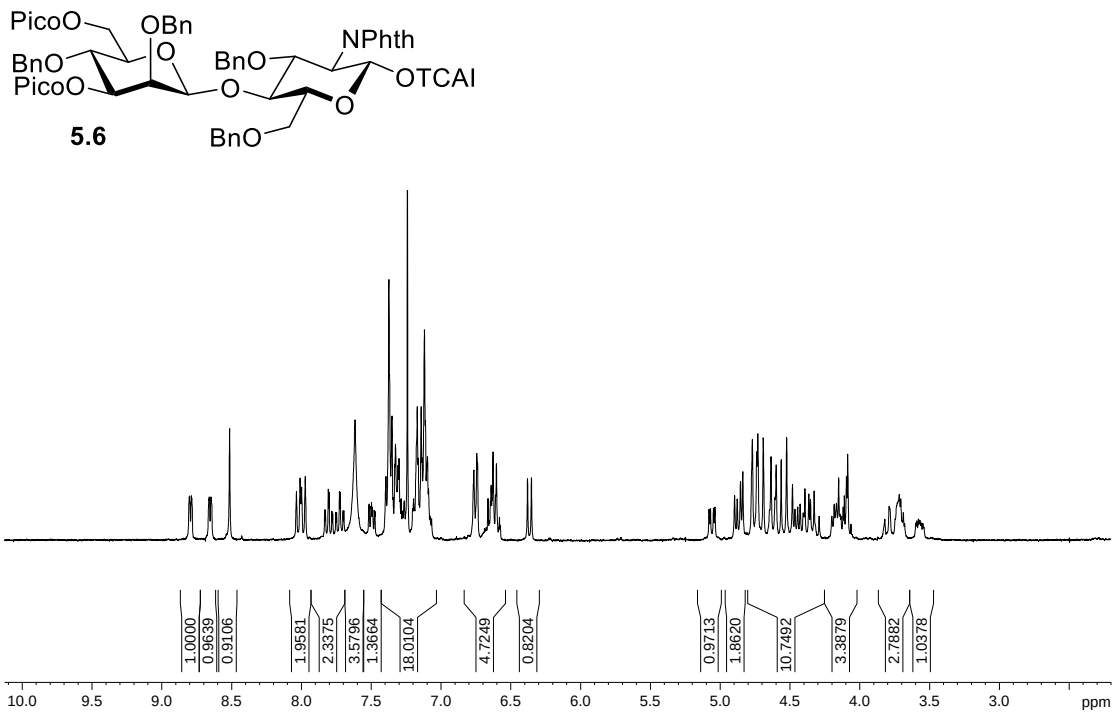
$CDCl_3$ 150 MHz

Figure A-91: ^{13}C NMR spectrum of *O*-(2,4-Di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose **(5.21)**



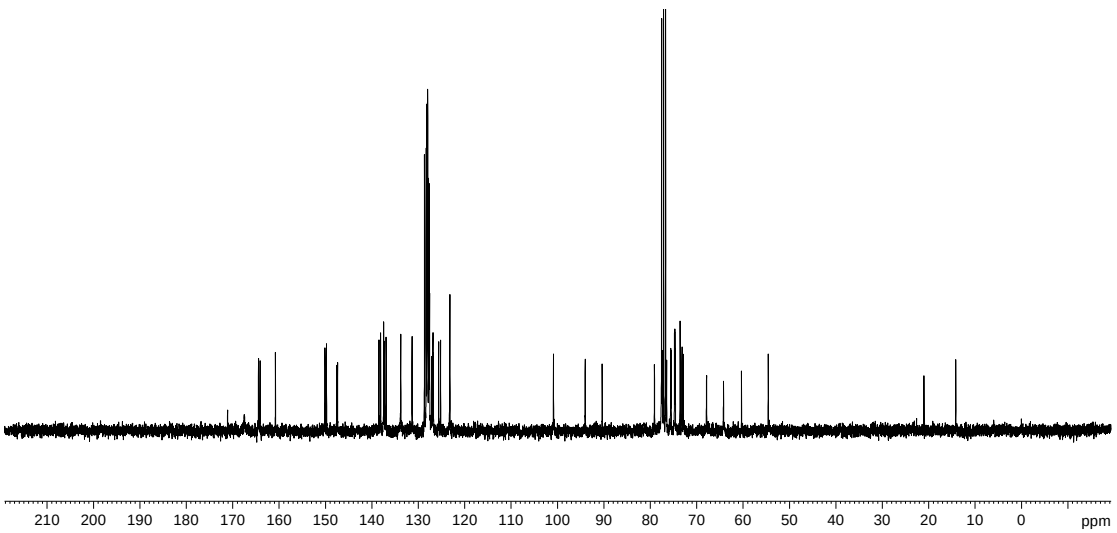
$CDCl_3$ 300 MHz

Figure A-92: 2-D NMR COSY spectrum of *O*-(2,4-Di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose **(5.21)**



$CDCl_3$ 300 MHz

Figure A-93: ^{1}H NMR spectrum of *O*-(2,4-Di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate **(5.6).**



$CDCl_3$ 150 MHz

Figure A-94: ^{13}C NMR spectrum of *O*-(2,4-Di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate **(5.6).**

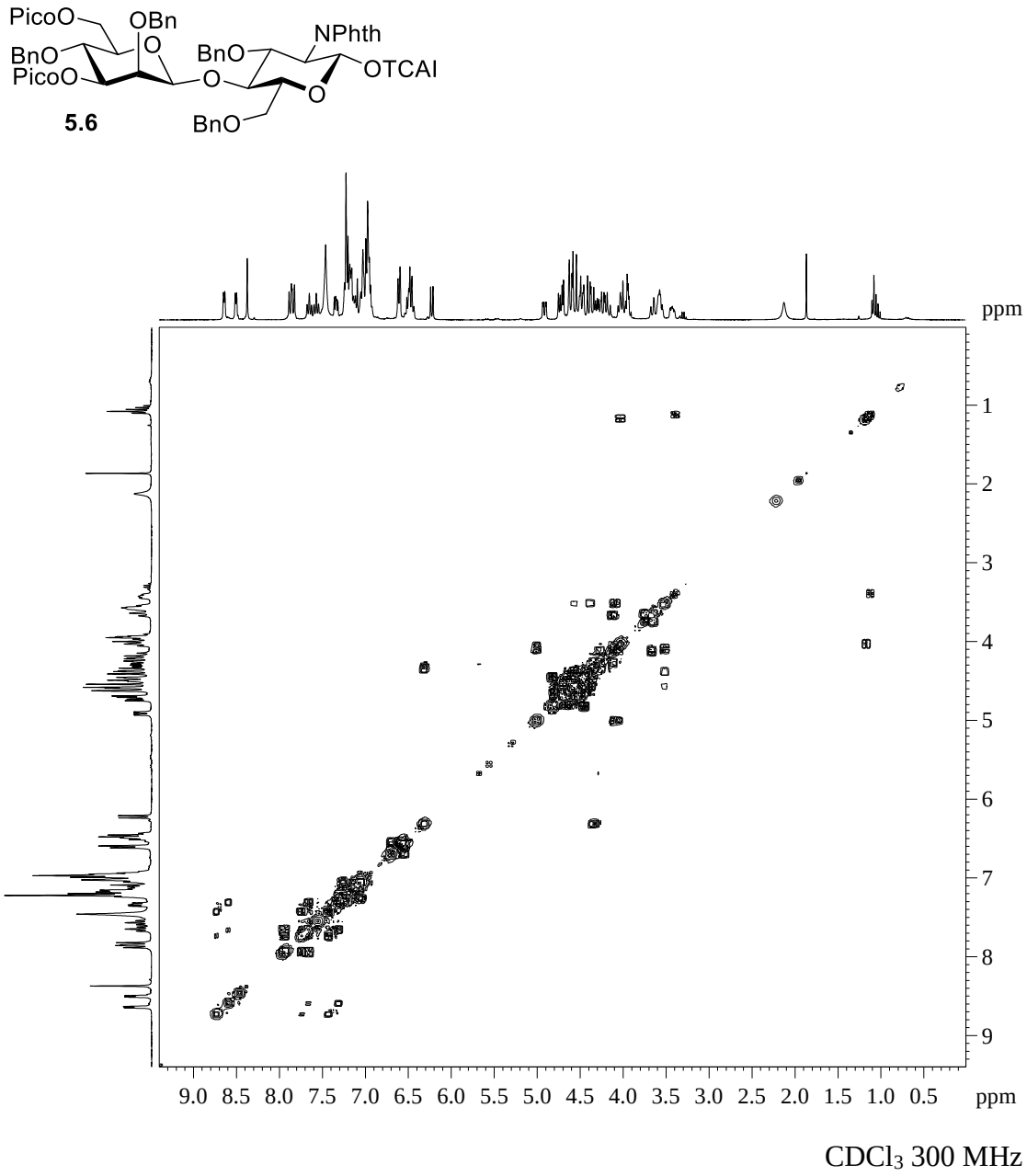
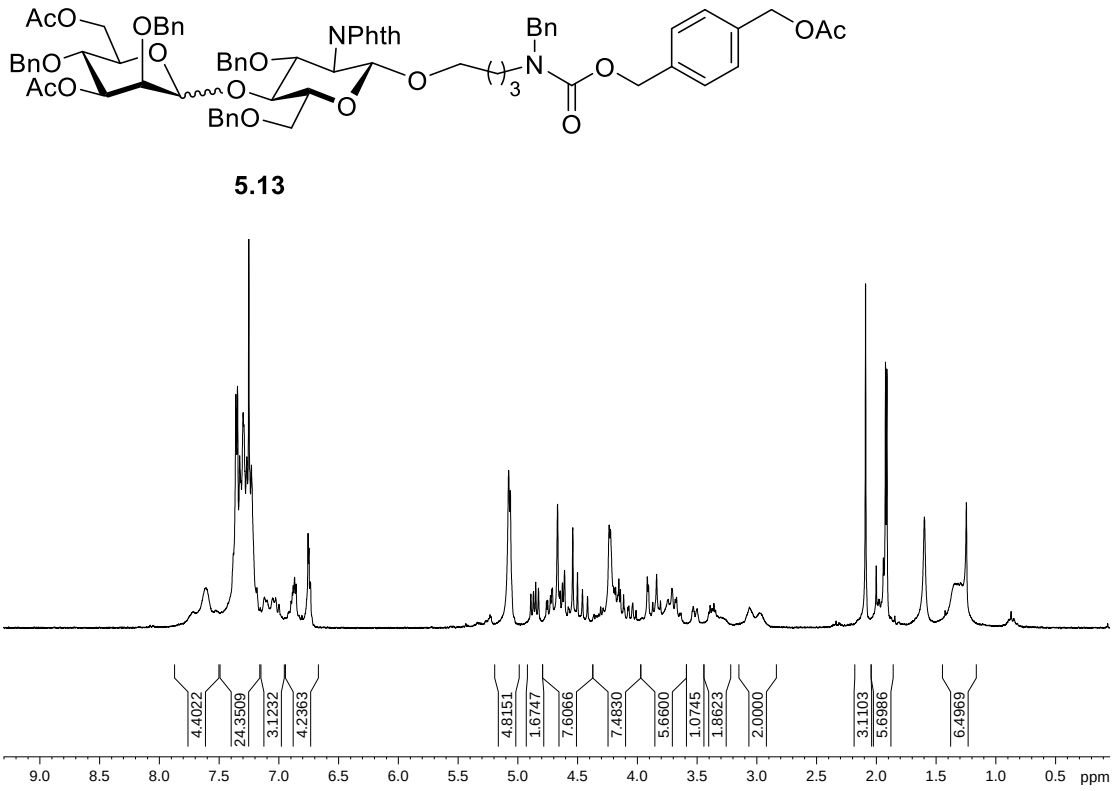


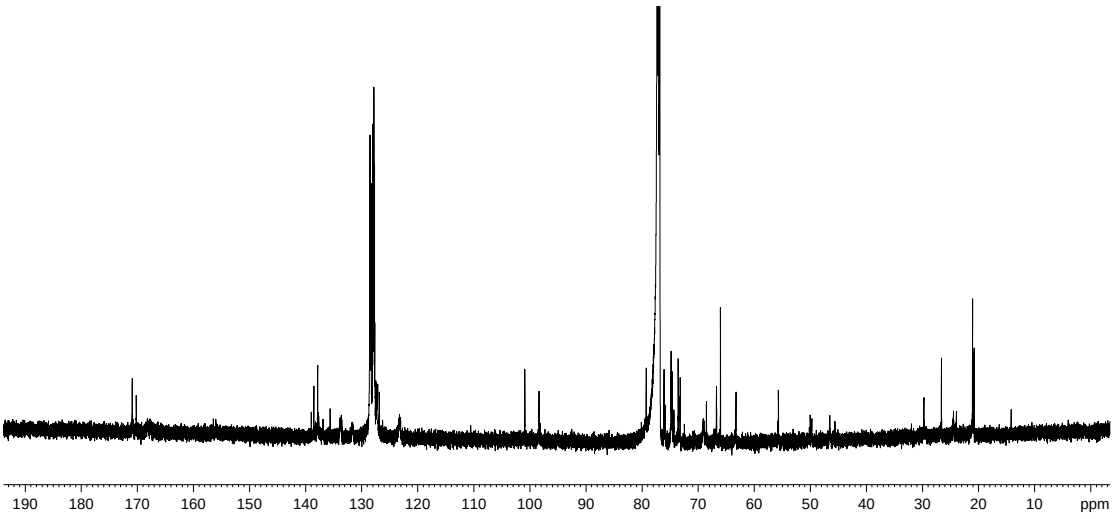
Figure A-95: 2-D NMR COSY spectrum of *O*-(2,4-Di-*O*-benzyl-3,6-*O*-picoloyl-β-D-mannopyranosyl)-(1→4)-3,6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate **(5.6).**



5.13

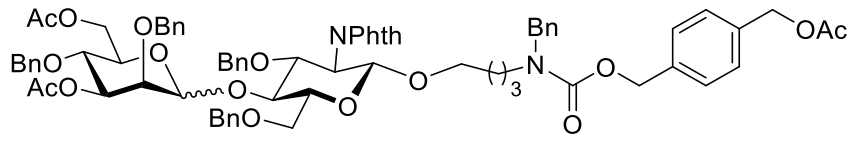
$CDCl_3$ 300 MHz

Figure A-96: ^{1}H NMR spectrum of N-(p-Acetoxymethyl)benzyloxycarbonyl-4-benzylaminobutyl O-(4,6-di-O-acetyl- 2,3-O-benzyl-α/β-D-mannopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D glucopyranoside **(5.13).**

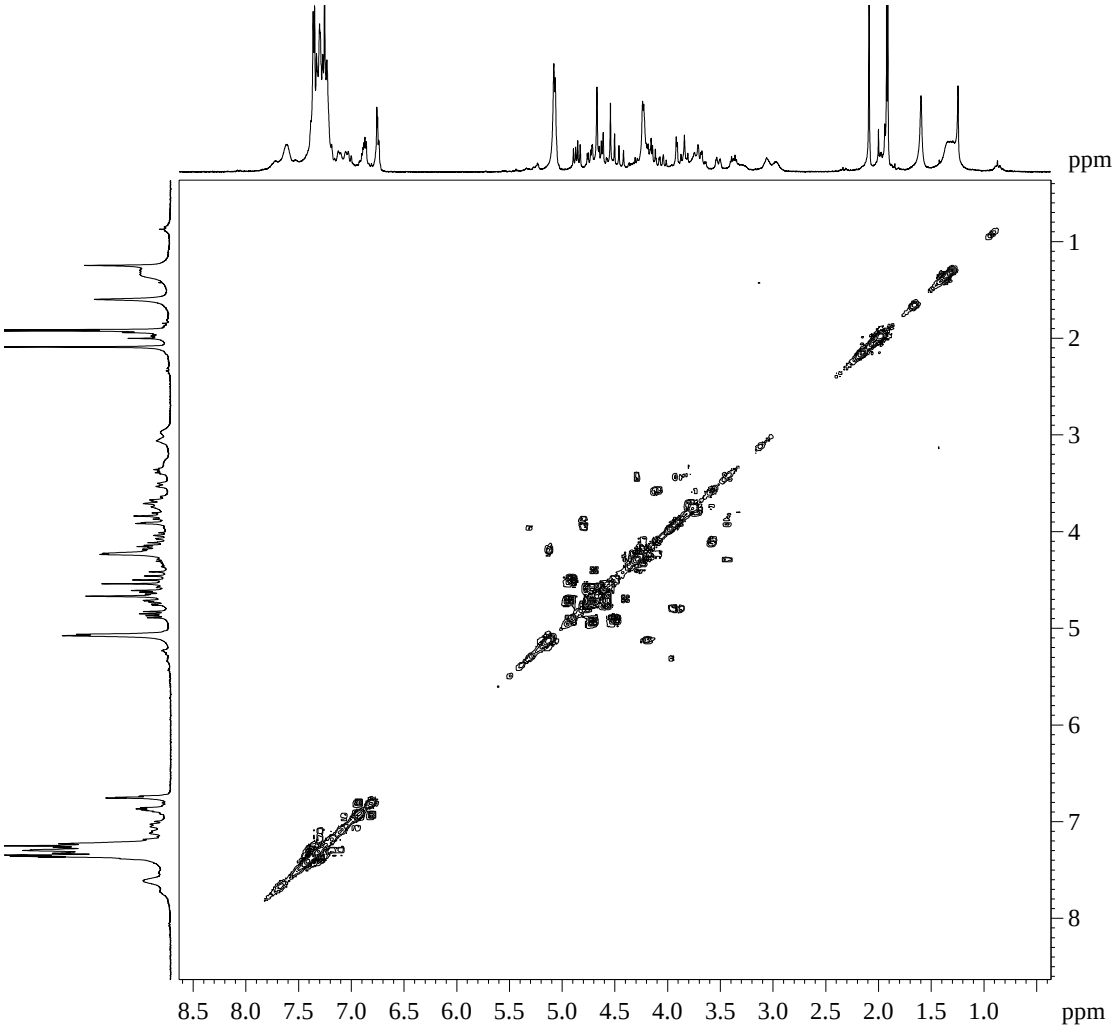


$CDCl_3$ 150 MHz

Figure A-97: ^{13}C NMR spectrum of N-(p-Acetoxymethyl)benzyloxycarbonyl-4-benzylaminobutyl O-(4,6-di-O-acetyl- 2,3-O-benzyl-α/β-D-mannopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D glucopyranoside **(5.13).**

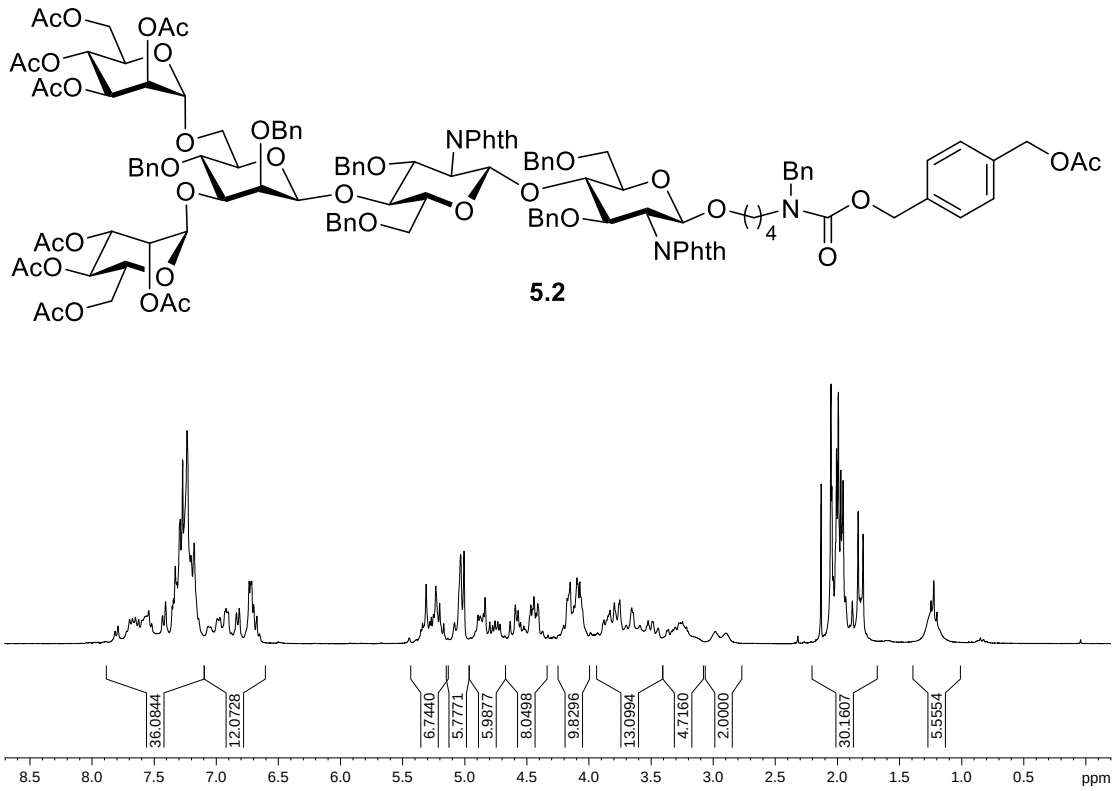


5.13



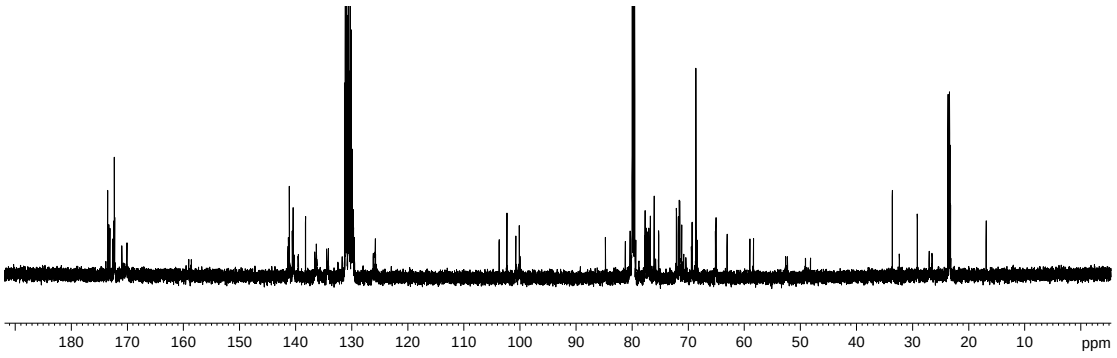
$CDCl_3$ 300 MHz

Figure A-98: 2-D NMR COSY spectrum of N-(p-Acetoxymethyl)benzyloxycarbonyl-4-benzylaminobutyl O-(4,6-di-O-acetyl- 2,3-O-benzyl-α/β-D-mannopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D glucopyranoside **(5.13).**



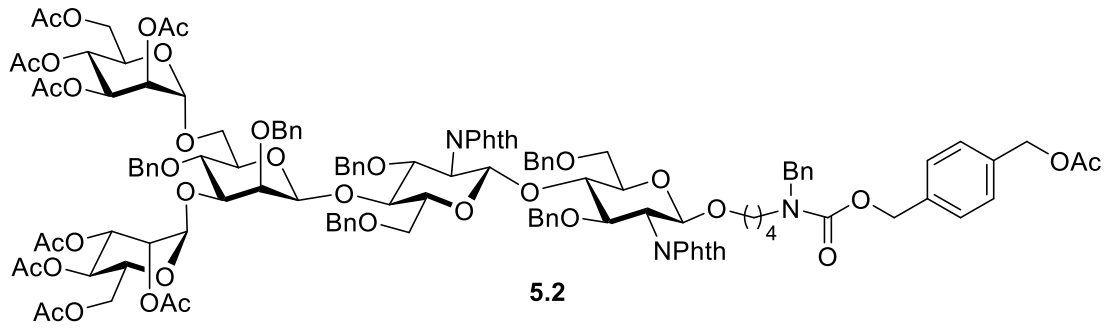
$CDCl_3$ 300 MHz

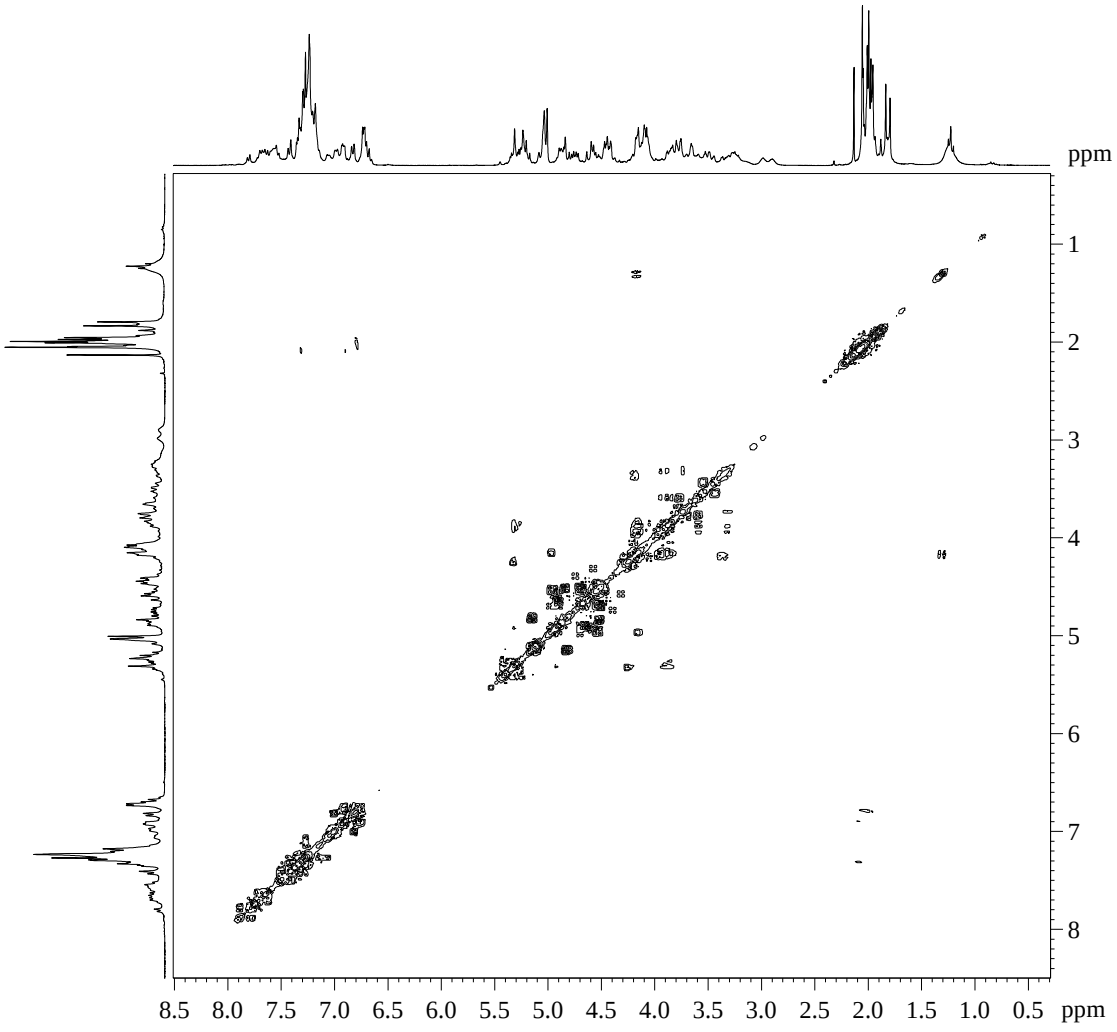
Figure A-99: ^{1}H NMR spectrum of N-(p-Acetoxymethyl)benzyloxycarbonyl-4-benzylaminobutyl di-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→3,1→6)-O-(2-O-benzyl-α/β-D mannopyranosyl)-(1→4)-O-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(5.2**).



$CDCl_3$ 150 MHz

Figure A-100: ^{13}C NMR spectrum of N-(p-Acetoxymethyl)benzyloxycarbonyl-4-benzylaminobutyl di-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→3,1→6)-O-(2-O-benzyl-α/β-D mannopyranosyl)-(1→4)-O-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(5.2**).





$CDCl_3$ 300 MHz

Figure A-101: 2-D NMR COSY spectrum of N-(p-Acetoxymethyl)benzyloxycarbonyl-4-benzylaminobutyl di-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-(1→3,1→6)-O-(2-O-benzyl-α/β-D mannopyranosyl)-(1→4)-O-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(5.2**).