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Chemical Synthesis of Staphylococcal Oligosaccharides

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CHEMICAL SYNTHESIS OF STAPHYLOCOCCAL OLIGOSACCHARIDES

By

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ABSTRACT

CHEMICAL SYNTHESIS OF STAPHYLOCOCCAL

OLIGOSACCHARIDES

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Prof. Alexei V. Demchenko, Advisor

The Gram positive, cluster forming bacteria *Staphylococcus aureus* belongs to the family of opportunistic pathogens that may cause blood stream infections when the integrity of skin is broken and the immune system can no longer fight the infection. *S. aureus* has become one of the most frequent causes of infections in newborns, surgical patients, trauma and burn patients, patients receiving an implant, and dialysis patients with high mortality rates. As a matter of fact, *S. aureus* has become one of the largest public health and economic impacts amongst all bacterial infectious diseases worldwide. This situation is further complicated by the rapid increase in anti-microbial drug resistance. Since the *S. aureus* bacterial cell is surrounded by a polysaccharide capsule, preventive vaccination based on polysaccharide or saccharide-protein conjugates is a suitable tool against the bacterial invasion. Serotyping has revealed that the majority of *S. aureus* strains express either capsular polysaccharides type 5 (CP5) or type 8 (CP8).

Previous studies at Pfizer Inc. involved the preparation of conjugates of the purified native CPs derived from fermentation with protein carriers. Characterization of the activated products derived from purified native CPs by spectroscopic methods proved to be difficult owing to their large size. It was also noted that over activation and side reactions taking place during the conjugation process could lead to a loss of the epitopes required to achieve high immunogenicity of CP conjugate vaccines. This, in turn, can lead to the failure of the vaccine candidates in clinical trials.

This thesis details the total syntheses of oligosaccharides structurally related to the repeating units of capsular polysaccharides *S. aureus* type 5 and 8. Our targets including two disaccharides, two trisaccharides and a hexasaccharide that will be studied by our collaborators at Pfizer Inc. to understand chemical activation for conjugation to the experimental carrier proteins. The improved understanding of the conjugation process will, in turn, lead to a more controlled, predictable, and reproducible outcome of polysaccharide conjugations. The additional potential outcome of this synthetic study is the development of alternative treatments to fight *S. aureus* infections.

This dissertation is dedicated to my beloved grandmother Malinee Chansomboon,
with love and respect.

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LIST OF ABBREVIATIONS

Å	Angstrom
Ac	Acetyl
AcOH	Acetic acid
ACS	American Chemical Society
AcSH	Thioacetic acid
Ag ₂ O	Silver (I) oxide
AgOTf	Silver(I) trifluoromethanesulfonate
BAIB	(Diacetoxy)iodobenzene
BF ₃ -OEt ₂	Boron trifluoride etherate
Bn	Benzyl
BnBr	Benzyl bromide
br	Broad
Br ₂	Bromine
BSP	1-Benzene sulfinyl piperidine
Bu ₃ SnH	Tributyltin(IV) hydride
Bu ₂ SnO	Dibutyltin(IV) oxide
Bz	Benzoyl
CaH ₂	Calcium hydride
CAN	Ceric ammonium nitrate
CBz	Carboxybenzyl
CCl ₃ CN	Trichloroacetonitrile
CDCl ₃	Deuterated chloroform

CD ₃ OD	Deuterated methanol
CDI	Carbonyldiimidazole
CDT	Carbonylditriazole
CH ₂ Cl ₂	Dichloromethane
CH ₃ I	Iodomethane
ClCH ₂ CH ₂ Cl	1,2-Dichloroethane
(COCl) ₂	Oxalyl chloride
CP	Capsular polysaccharide
CRM 197	Cross reactive material 197
CrO ₃	Chromium trioxide
CSA	Camphorsulfonic acid
CsCO ₃	Cesium carbonate
d	Doublet
Da	Dalton
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-Dicyclohexylcarbodiimide
dd	Doublet of doublets
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	N,N-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMT	Dimethoxytoluene
DMSO	Dimethylsulfoxide

DTBS	Di- <i>tert</i> -butylsilylidene
EDC	1-Ethyl-3-(3-(dimethylamino)propyl)-carbodiimide
Et	Ethyl
ETA	<i>Pseudomonas aeruginosa</i> endotoxin A
Et ₂ O	Diethylether
EtOAc.....	Ethyl acetate
EtOH.....	Ethanol
FucNAc	N-Acetyl-fucosamine
GalNAcA	N-Acetyl galactosamine uronic acid
GlcA	Glucuronic acid
GlcNAc	N-Acetyl glucosamine
h	Hour(s)
H ₂	Hydrogen
HCl	Hydrogen chloride
HF	Hydrogen fluoride
H ₂ O ₂	Hydrogen peroxide
HR-ESI MS	High Resolution Electrospray Ionization mass spectrometry
HR-FAB MS	High Resolution Fast Atom Bombardment mass spectrometry
Hz	Hertz
k	Kilo
K ₂ CO ₃	Potassium carbonate
KOH	Potassium hydroxide
KSBox	Potassium thiobenzoxazolate

LevOH	Levulinic acid
M	Molar
m	Multiplet
Man	Mannose
ManNAcA	N-Acetyl mannosamine uronic acid
Me	Methyl
MeCN	Acetonitrile
MeOH	Methanol
MeOTf	Methyl trifluoromethanesulfonate
MgSO ₄	Magnesium sulfate
min	Minute(s)
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MS	Molecular sieves
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
MurNAc	N-Acetyl muramic acid
MW	Molecular weight
<i>m/z</i>	Mass to charge ratio
N ₃	Azido
Na	Sodium
NaBH ₄	Sodium borohydride
Na ₂ B ₄ O ₇	Sodium tetraborate
NAc	Acetamido
NaCl	Sodium chloride

NaClO	Sodium hypochlorite
NaClO ₂	Sodium chlorite
NaCNBH ₃	Sodium cyanoborohydride
NaH	Sodium hydride
NaHCO ₃	Sodium bicarbonate
NaN ₃	Sodium azide
NaOAc	Sodium acetate
NaOH	Sodium hydroxide
NaOMe	Sodium methoxide
Na ₂ S ₂ O ₃	Sodium thiosulfate
NBS	N-Bromosuccinimide
H ₂ NNH ₂ AcOH	Hydrazine acetate
H ₂ NNH ₂ H ₂ O	Hydrazine hydrate
NIS	N-Iodosuccinimide
NMR	Nuclear magnetic resonance
NPhth	Phthalimido
Pd/C	Palladium on carbon
PdCl ₂	Palladium(II) chloride
Pd(OH) ₂ /C	Palladium hydroxide on carbon
Pent	4-Penten-1-yl
Ph	Phenyl
Ph ₂ SO	Diphenyl sulfoxide
PMB	<i>p</i> -Methoxybenzyl

PMP	<i>p</i> -Methoxyphenyl
ppm	Parts per million
<i>p</i> -TsOH	<i>p</i> -Toluenesulfonic acid
Py	Pyridine
rEPA.....	<i>Pseudomonas aeruginosa</i> recombinant exoprotein A
R _f	Retention factor
rt	Room temperature
s	Singlet
SAR	Structure-activity relationship
SBox	S-benzoxazolyl
SEt	S-Ethyl
t	Triplet
TBAF	Tetra- <i>n</i> -butyl ammonium fluoride
TBAN ₃	Tetra- <i>n</i> -butyl ammonium azide
TBAI	Tetra- <i>n</i> -butyl ammonium iodide
TBDMS	<i>tert</i> -Butyldimethylsilyl
TBS	<i>tert</i> -Butyldiphenylsilyl
TBSOTf	<i>tert</i> -Butyldimethylsilyl trifluoromethanesulfonate
<i>t</i> -BuOH	<i>tert</i> -Butanol
TDS	Dimethylhexylsilyl
TEA	Triethylamine
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TFA	Trifluoroacetic acid

Tf ₂ O	Trifluoromethanesulfonic (triflic) anhydride
TfOH	Trifluoromethanesulfonic (triflic) acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TTBP	2,4,6-Tri- <i>tert</i> -butylpyrimidine
UDP	Uridine diphosphate
Zn	Zinc
ZnCl ₂	Zinc chloride

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CHAPTER 1

Staphylococcus aureus capsular
polysaccharides: a structural and
synthetic prospective

1.1 Introduction

An opportunistic gram-positive bacteria *Staphylococcus aureus* normally colonizes on the human skin.¹ It can cause blood stream infections when the immune system gets weakened and can no longer fight the infection. *S. aureus* is one of the most frequent causes of infections in newborns, surgical patients, and immunocompromised patients with high mortality rates. As a matter of fact, *S. aureus* has become one of the largest public health and economic impacts amongst all bacterial infectious diseases worldwide. This situation is further complicated by the rapid increase in anti-microbial drug resistance. One of the greatest concerns is antibiotic (methicillin)-resistant strains (MRSA), which have limited treatment options.² The development of new antibiotics is not sustainable due to the continuous increase in antibiotic-resistance of MRSA. Passive or active immunizations of patients may be promising alternatives to the prophylactics of infections caused by MRSAs. In particular, immunotherapies based on the conjugates of capsular polysaccharides of *S. aureus* are promising targets.³

1.2 Biosynthesis of *S. aureus* capsular polysaccharides

Encapsulation of pathogenic bacteria allows for evasion of host immune systems.⁴ The capsule is an extracellular component that can extend hundreds of microns from the cellular surface and is predominantly made up of long chains of capsular polysaccharides (CPs). This results in the masking of surface proteins, the typical antigens that trigger an adaptive immune response in a host. Many bacterial species have been shown to produce CPs, and a single species can produce numerous, structurally distinct CPs, forming a basis for serotyping isolates. There have been 13 distinct *S. aureus* serotypes identified so

far with type 5 (CP5) and type 8 (CP8) being the most prevalent in clinical isolates.⁵ Expression of both CP5 and CP8 was shown to be important for *S. aureus* to evade opsonophagocytic killing, and furthermore MRSA and Methicillin-sensitive *Staphylococcus aureus* (MSSA) strains were killed by anti-CP antibodies. While CPs are poorly immunogenic both in the young and elderly, CP-protein conjugates have been shown to elicit robust, T-cell dependent immune responses across all age groups. Several vaccines against *S. aureus* containing CP5- and CP8-protein conjugates have been developed as clinical candidates. Thus, the study of *S. aureus* CPs has been an active area of research for academic and industrial scientists.

The biosyntheses of both CP5 and CP8 have been recently reviewed by Grundling⁶ and are proposed to follow the typical Gram positive Wzy-dependent mechanism. In accordance with this mechanism, membrane-linked trisaccharide repeating units are assembled in a non-processive polymerization. However, literature reports of experimental evidence for the entire biosynthetic pathway is incomplete. Production of both CP5 and CP8 requires 16 genes. Of these genes, 12 were reported to code for enzymes with high sequence homology between CP5 and CP8 and were proposed to play common roles in production of the CPSs. This leaves 4 genes that were proposed to produce proteins playing serotype-specific biosynthetic functions.

The common precursor for all three of the requisite monosaccharide building blocks of both CP5 and CP8 has been proposed to be UDP-D-GlcNAc.⁶ Conversion to UDP-D-ManNAcA requires epimerization of the C-2 position and oxidation of the primary hydroxyl at C-6. These steps have been reported to be catalyzed by the epimerase CapP and the dehydrogenase CapO, respectively. Conversion of UDP-D-

GlcNAc to UDP-D-FucNAc requires epimerization of C-4 and reduction of the C-6 primary alcohol. These transformations have been proposed to occur by the action of dehydrogenase CapD and the reductase CapN. UDP-L-FucNAc has been reported to be derived from UDP-D-GlcNAc by the sequential action of bifunctional dehydrogenase epimerase CapE, reductase CapF, and epimerase CapG. CP5 requires O-acetylation of the C-3 hydroxyl of UDP-L-FucNAc, and this has been proposed to be accomplished by the action of Cap5H. Likewise, Cap8J is thought to acetylate the C-4 hydroxyl of UDP-D-ManNAcA for production of CP8.

Assembly of the CPs is thought to begin with transfer of UDP-D-FucNAc to membrane-bound undecaprenyl phosphate by glycotransferase CapM in the cytoplasm. Then UDP-L-FucNAc and UDP-D-ManNAcA are transferred respectively by CapM and Cap5I (for CP5) or Cap8H (for CP8) to generate a membrane-bound trisaccharide repeat unit. Cap K has been proposed to translocate membrane-bound repeat unit to the outer leaflet of the membrane. Trisaccharide repeating units are then proposed to be assembled by the action of either Cap5J (for CP5) or Cap8I (for CP8), with CapA and CapB believed to be regulators of the polysaccharide chain length. The completed polysaccharides were shown to be transferred from the undecaprenyl-linked intermediate to the C-6 hydroxyl of MurNAc in peptidoglycan by LytR-CpsA-Psr (LCP) enzymes.⁷

1.3 Characterization of *Staphylococcus aureus* capsular polysaccharides

In 1982, Karakawa and Vann have developed the system to classify *S. aureus* capsular polysaccharides based on the immunological specificity.⁸ Based on the typing studies, 11 different serotypes have been identified⁹ and nowadays 13 serotypes have

been described.¹⁰ According to the colony morphology, these capsular polysaccharides can be divided into two main groups. CPs serotype 1 and 2 that belong to the first main group are classified as mucoid-type capsules due to heavily encapsulated capsules and mucoid colonies. CPs of the second main group, serotypes 3-11, are classified as microcapsules because of thin layer capsules and non-mucoid colonies.¹¹

1.3.1 Capsular polysaccharide Type 1

The polysaccharide capsule of type 1 prototype strain (strain D) was found to have the following trisaccharide repeating unit: $\rightarrow 4$)- α -2-acetamido-2-deoxy-D-galactopyranosyl uronic acid-(1 \rightarrow 4)- α -2-acetamido-2-deoxy-D-galactopyranosyl uronic acid-(1 \rightarrow 3)- α -2-acetamido-2-deoxy-D-fucopyranosyl(1 \rightarrow).^{8, 12} The strain M *S. aureus* capsular polysaccharide was initially isolated by Smith in 1962.¹³ The character of a capsulate *S. aureus* was reported by Scott in 1969.¹³ In 1974, Liao et.al. have analyzed the chemical components of polysaccharide from *Staphylococcus aureus* M strain.¹⁴ They have found the components of surface antigen which comprises Taurine, D-galactosamine uronic acid, and D-fucosamine. After that, the ratio of the chemical composition was reported by Liao et.al. in 1977 which found to be hexasaccharide comprises N-acetyl-D-galactosamine uronic acid, N-acetyl-D-fucosamine and taurine in molar ratios of 4:2:1.¹⁵ Furthermore, the isolated disaccharide was characterized as N-acetyl-D-galactosamine uronic acid-(1 \rightarrow 3)-N-acetyl-D-fucosamine. Taurine was found to be connected with N-acetyl-D-galactosamine uronic acid by forming amide bond with carboxylic group. The complete chemical structure of strain M was reported by Murthy et.al. in 1983.¹⁶ The M strain polysaccharide comprises repeating unit of $\rightarrow 4$)-O-2-

acetamido-2-deoxy- α -D-galactopyranosyl uronic acid-(1 \rightarrow 4)-O-2-acetamido-2-deoxy- α -D-galactopyranosyl uronic acid-(1 \rightarrow 3)-O-2-acetamido-2-deoxy- α -D-fucopyranosyl(1 \rightarrow). Taurine is linked to every fourth 2-acetamido-2-deoxy-D-galactopyranosyl uronic acid residue by amide bond. However, taurine residue was not found in strain D but it could have some variations of taurine expression in strain M which depend on the culture condition.¹⁷

Table 1.1. The serotypes and chemical structures of *S. aureus* CPs.

Type	Strain	Final chemical structure of the repeating unit	Established	Year
1	D	\rightarrow 4)- α -D-GalNAcA-(1 \rightarrow 4)- α -D-GalNAcA-	Karakawa	1982
	M	(1 \rightarrow 3)- α -D-FucNAc(1 \rightarrow \rightarrow 4)- α -D-GalNAcA-(1 \rightarrow 4)- α -D-GalNAcA- (1 \rightarrow 3)- α -D-FucNAc(1 \rightarrow *) *Taurine is linked to every fourth α -D-GalNAcA by amide bond with carboxylic acid group	Murthy	1983
2	Smith	GlcNAcA β -(1 \rightarrow 4)-2-(N-acetylalanyl)amino-2-deoxy-D-GlcA	Hanessian	1964
	K-93M	GlcNAcA β -(1 \rightarrow 4)-2-(N-acetylalanyl)amino-2-deoxy-D-GlcA	Karakawa	1971
3	Mardi	unknown	NA	NA
4	T 7007	D-ManNAcA-(1 \rightarrow 3)-D-FucNAc	Wu	1971
		ManNAcA-(1 \rightarrow 3)-FucNAc	Karakawa	1974
5	Reynold	\rightarrow 4)- β -D-ManNAcA-(1 \rightarrow 4)- α -L-FucNAc(3-OAc)-(1 \rightarrow 3)- α -D-FucNAc(1 \rightarrow	Jones	2005
6	C	unknown	NA	NA
7	207	unknown	NA	NA
8	Becker	\rightarrow 3)- β -D-ManNAcA(4-OAc)-(1 \rightarrow 3)- α -L-FucNAc-(1 \rightarrow 3)- α -D-FucNAc(1 \rightarrow	Jones	2005
9	91	unknown	NA	NA
10	537	unknown	NA	NA
11	191	unknown	NA	NA
12	NA	unknown	NA	NA
13	NA	unknown	NA	NA

1.3.2 Capsular polysaccharide Type 2

The capsular polysaccharide of type 2 prototype strain (11127-var) was reported by Karakawa et. al. in 1982.⁸ It is similar to the polysaccharide of strain K-93M, which was reported previously in 1972 by the same group.²⁰ It was also found to be the same as the polysaccharide from strain Smith which was reported in 1962.²¹ The strain Smith was found to contain an aminosugar by Perkin in 1963.²² The complete chemical structure of type 2 capsular polysaccharide was reported by Hanessian in 1964.²³ This staphylococcal polysaccharide has been shown to consist of equimolar portion of 2-acetamido-2-deoxy-D-glucuronic acid and 2-[(N-acetylalanyl)amino]-2-deoxy-D-glucuronic acid linked by β -(1 \rightarrow 4) linkages.

1.3.3 Capsular polysaccharide Type 3

The type 3 capsular polysaccharide was identified by Karakawa in 1982⁹ by immunological method using prototype strain Mardi which was described in 1979.²⁴ The chemical structure of type 3 polysaccharide antigen has not yet been established.²⁵

1.3.4 Capsular polysaccharide Type 4

The capsular polysaccharide type 4 (prototype strain: 7007)⁹ was reported in 1974 by Karakawa et.al.²⁶ It was shown to contain the following sequence: 2-acetamido-2-deoxymannopyranosyl uronic acid-(1 \rightarrow 3)-O-2-acetamido-2-deoxy-fucopyranose. It also has been found to be cross reactive with strain T described by Wu et. al. in 1971.²⁷ That is because both polysaccharides contain similar residues, N-acetylmannosamine uronic acid

and N-acetylfucosamine. Nevertheless, no complete chemical structures have yet been reported.

1.3.5 Capsular polysaccharide Type 5

The type 5 capsular polysaccharide was isolated from strain Reynold by Fournier in 1987.²⁸ Initially, its chemical structure was described by Van in 1988.²⁹ It was found to consist of repeating units containing two molecules of N-acetylfucosamine and one molecule of N-acetylmannosamine uronic acid. The complete preliminary chemical structure of type 5 capsular polysaccharide was reported by Moreau et. al. in 1990.³⁰ It was initially assumed that the polysaccharide has the following repeating unit: $\rightarrow 4$)-2-acetamido-3-O-acetyl-2-deoxyl- β -D-mannopyranosyl uronic acid-(1 \rightarrow 4)-2-acetamido-2-deoxy- α -L-fucopyranosyl-(1 \rightarrow 3)-2-acetamido-2-deoxy- β -D-fucopyranosyl-(1 \rightarrow . In 2005, Jones has reported the revised chemical structure of capsular polysaccharide type 5. The revised structure was described as follows: $\rightarrow 4$)-2-acetamido-2-deoxyl- β -D-mannopyranosyl uronic acid-(1 \rightarrow 4)-2-acetamido-3-O-acetyl-2-deoxy- α -L-fucopyranosyl-(1 \rightarrow 3)-2-acetamido-2-deoxy- β -D-fucopyranosyl-(1 \rightarrow .³¹ It should be noted that high immunological cross-reactivity between type 4 and type 5 has been reported.³²

1.3.6 Capsular polysaccharide Type 6

The capsular polysaccharide type 6 prototype strain (strain C) was reported in 1982.⁹ However, there no structural study of this polysaccharide has been reported yet.

The type 6 capsule has been found to be cross-reactive with anti-type 8 monoclonal antibodies prepared by Nelles et. al.³²

1.3.7 Capsular polysaccharide Type 7

The type 7 capsular polysaccharide prototype strain (strain 207) was described in 1982,⁹ although no structural study of this capsular polysaccharide has yet emerged.

1.3.8 Capsular polysaccharide Type 8

The capsular polysaccharide type 8 was isolated from the prototype strain Becker in 1984 by Fournier.³³ Its repeating unit was originally thought to contain O-acetyl groups, N-acetylfucosamine and an aminouronic acid, possibly N-acetylgalactosamine uronic acid. In 1988, a preliminary study of its chemical structure showing the same composition as that of type 5 repeating unit was reported. It was noted, however, that both substitution and the anomeric configuration of the N-acetylfucosamine residues are different.²⁹ The revised chemical structure of capsular polysaccharide type 8 was established by Jones in 2005 as follows: $\rightarrow 3$)-2-acetamido-4-O-acetyl-2-deoxyl- β -D-mannopyranosyl uronic acid-(1 \rightarrow 3)-2-acetamido-2-deoxy- α -L-fucopyranosyl-(1 \rightarrow 3)-2-acetamido-2-deoxy- α -D-fucopyranosyl-(1 \rightarrow).³¹

1.3.9 Capsular polysaccharide Types 9 – 13

In 1985, Sompolinsky et. al. reported three other types of *S. aureus* capsular polysaccharide which are type 9 (prototype 91), type 10 (prototype 537) and type 11

(prototype 191).¹⁰ After that, serotypes 12 and 13 have been described.¹² However, no further characterization of these serotypes is yet available.

1.4 The chemical syntheses of *Staphylococcus aureus* related oligosaccharides

The native *S. aureus* CPs acquired by bacterial fermentation have been conjugated to carrier protein such as, *Pseudomonas aeruginosa* recombinant exoprotein A (rEPA), *Pseudomonas aeruginosa* endotoxin A (ETA) or non-toxic variant of diphtheria toxin (CRM 197).³⁴⁻³⁸ The resulting conjugate vaccines can provide the different immune response due to the variation of the ratio between polysaccharides and carrier proteins. Moreover, the characterization of the activated products derived from purified native polysaccharides by spectroscopic methods proved to be very difficult owing to their large size (MW > 50 kDa).³⁸ The over-activation and side reactions taking place during the conjugation process could lead to a loss of the epitopes required for immunogenicity of CPs conjugate vaccines.³⁹

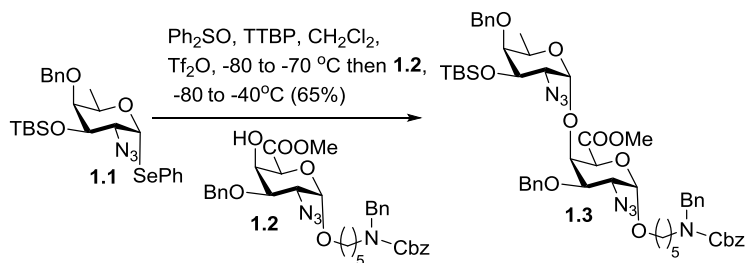
The chemical synthesis could be used to overcome these problems. It would allow us to modify chemical structures. For instance, the introduction of spacer molecules would reduce the steric hindrance between polysaccharides and carrier protein and facilitate the conjugation process.⁴⁰ Furthermore, the synthetic compounds can be used for structure-activity relationship (SAR) study, and the determination of required minimal epitope for immune response. Also, the synthetic compounds will allow us to understand the sites of activation, reactivity profile of different functional groups involved in the conjugation reactions, and to learn controlling side reactions.

There are three serotypes of *S. aureus* capsular polysaccharide which are type 1, 5 and 8 have been acquired by chemical synthesis thus far.^{39, 41-45}

1.4.1 The synthesis of *Staphylococcus aureus* capsular polysaccharide type 1 (CP1)

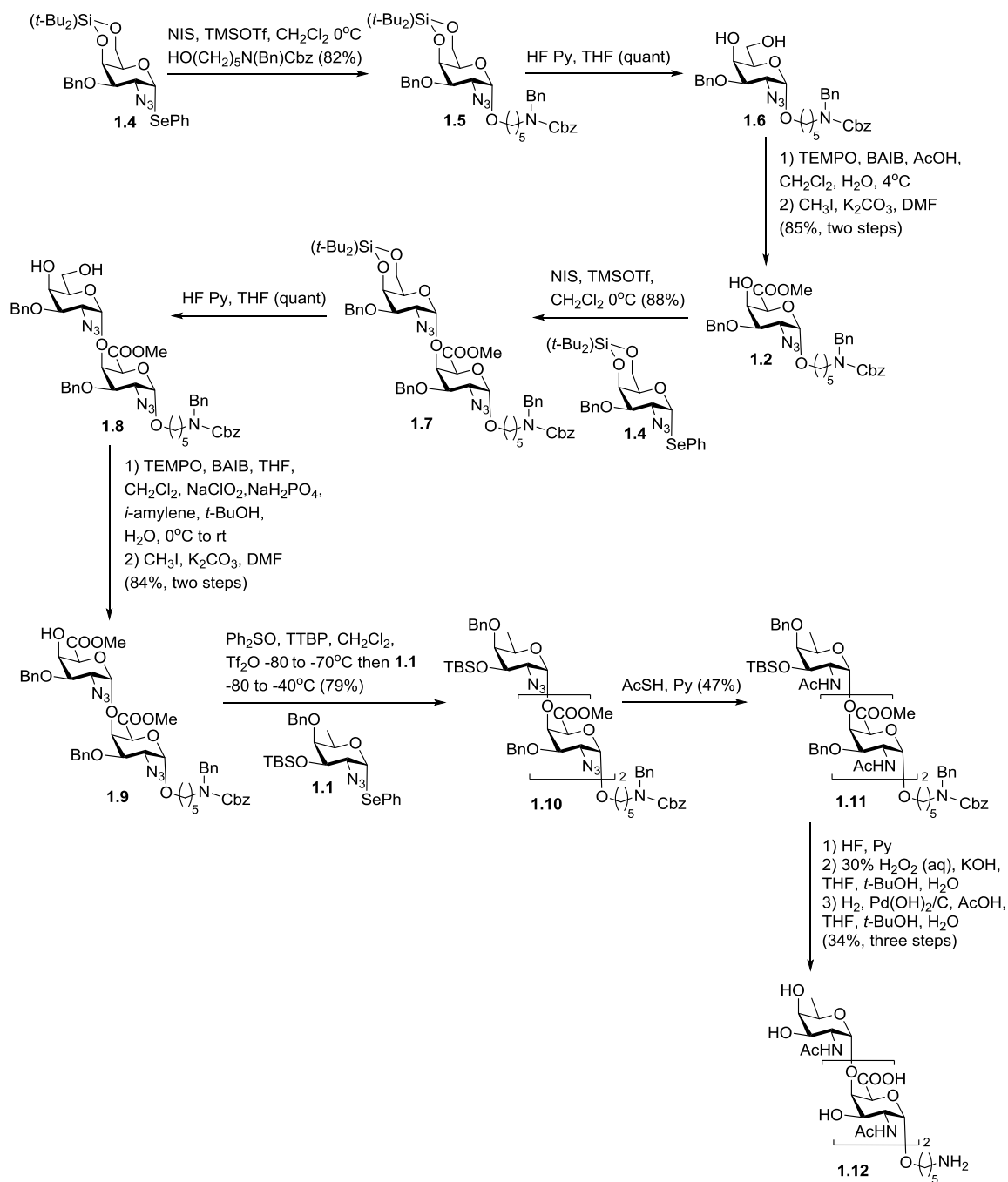
The chemical synthesis of *S. aureus* type 1 strain M disaccharide was recently reported by the Codee group.⁴⁵ In 2017, they presented the synthesis of protected disaccharide **1.3** (Scheme 1.1). The synthesis involved glycosylation between D-fucosyl donor **1.1** bearing the selenophenyl leaving group at the anomeric center and D-galactosamine uronate acceptor **1.2** using donor preactivation strategy with diphenyl sulfoxide (Ph₂SO), 2,4,6-tri-*tert*-butylpyrimidine (TTBP) and Tf₂O in CH₂Cl₂ to yield disaccharide **1.3** with α -linkage exclusively.

Scheme 1.1. Synthesis of fully protected CP1 disaccharide 1.3.



Subsequently, the same group has reported the chemical synthesis of a trisaccharide representing the *S. aureus* strain M capsular polysaccharide repeating unit.⁴⁴ As shown in Scheme 1.2, the synthesis started from the glycosylation of di-*tert*-butylsilylidene (DTBS)-functionalized galactopyranosyl donor **1.4** and the N-substituted 5-aminopentanol derivative in the presence of NIS and TMSOTf as the promoter system in CH₂Cl₂. As a result, glycoside **1.5** was obtained with complete α -selectivity.

Scheme 1.2. Synthesis of trisaccharide repeating unit CP1 1.12.



After that, silyl ether of **1.5** was removed with hydrogen fluoride in pyridine (HF-Py) in THF to provide 4,6-diol **1.6**. The latter was then subjected to selective oxidation of the primary hydroxyl with TEMPO and BAIB in AcOH and wet CH₂Cl₂. Subsequent

esterification was affected with iodomethane (CH_3I) in the presence of potassium carbonate (K_2CO_3) in DMF to afford glycosyl acceptor **1.2**. The latter was glycosylated with galactosyl donor **1.4** in the presence of NIS and TMSOTf in CH_2Cl_2 to form 1,2-cis-linked disaccharide **1.7**. Then, selective silyl ether removal of **1.7** was achieved by the treatment with HF-Py in THF to obtain diol **1.8**. The 6'-OH of **1.8** was then selectively oxidized using the Pinnick oxidation sequence (TEMPO/BAIB) to obtain carboxylic acid which was esterified with CH_3I in the presence of K_2CO_3 in DMF to provide disaccharide **1.9**. After that, 4'-OH was glycosylated with D-fucosamine donor **1.1** preactivated with Ph_2SO , TTBP and Tf_2O in CH_2Cl_2 to afford trisaccharide **1.10**. The functionalization of trisaccharide was initiated by the reaction with thioacetic acid (AcSH) to form triacetamido derivative **1.11**. Then, the TBS ether was removed with HF-Py, followed by saponification of the methyl ester with potassium hydroxide and hydrogen peroxide in THF and wet *tert*-butanol to yield dicarboxylated trisaccharide. Finally, all benzylic substituents were removed by hydrogenation using $\text{Pd}(\text{OH})_2/\text{C}$ in AcOH, THF and wet *t*-BuOH to afford the target compound **1.12**.

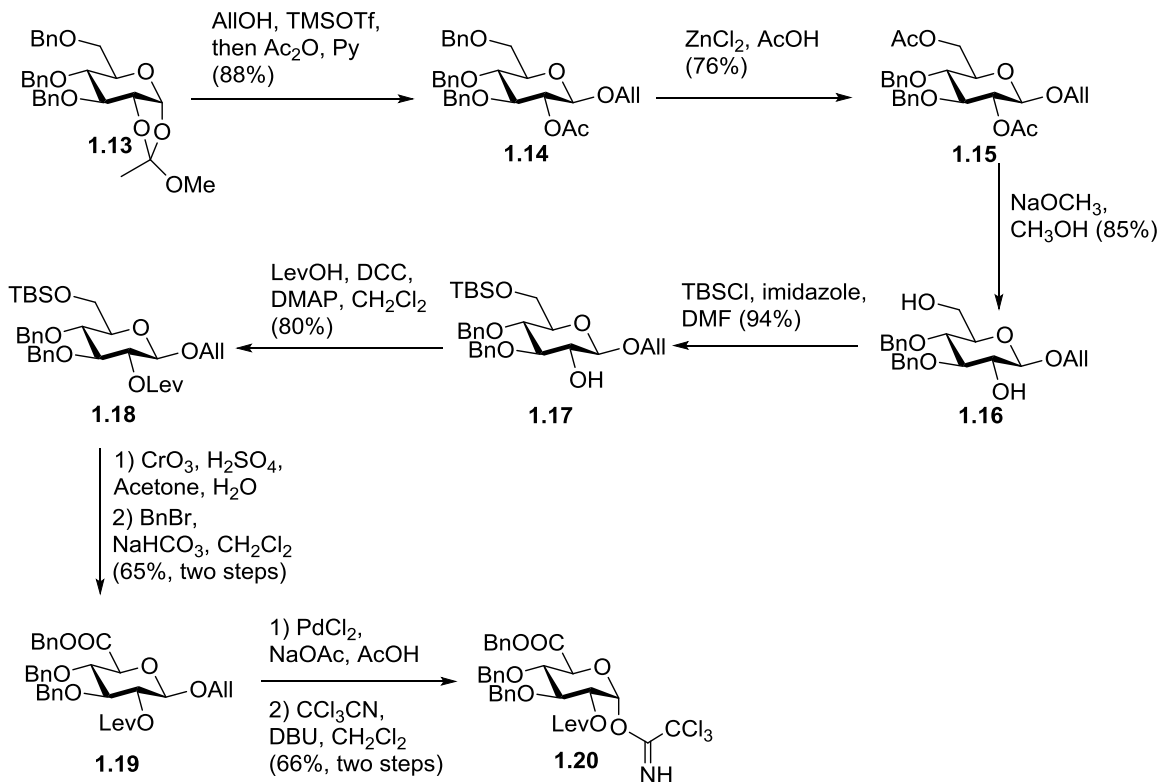
1.4.2 The synthesis of *Staphylococcus aureus* capsular polysaccharide type 5 (CP5)

The first chemical synthesis of the repeating unit of *S. aureus* type 5 capsular polysaccharide (CP5) was achieved by Adamo in 2012.⁴³ They decided to synthesize CP5 trisaccharide repeating unit containing aminopropyl spacer at the reducing end which can be conjugated to carrier protein. The following key steps were employed in their synthetic strategy. They pursued the C-2' epimerization strategy according to which a β -glucosyl residue in introduced followed by epimerization to β -mannosyl residue. Then,

disaccharide product was glycosylated with D-fucosamine acceptor to construct the trisaccharide repeating unit.

The D-glucuronic acid donor was prepared by glycosylation of allyl alcohol with orthoester **1.13** using TMSOTf as a promoter (Scheme 1.3). This reaction was accompanied with concomitant partial deacetylation. Therefore, the crude reaction mixture was acetylated to afford allyl 2-O-acetyl derivative **1.14**. Then, acetolysis of the 6-O-benzyl ether was conducted in the presence of ZnCl₂ in acetic acid to acquire 6-O-acetyl compound **1.15**. The removal of acetyl groups was affected with sodium methoxide in methanol gave diol compound **1.16**, followed by selective protection of the primary alcohol of **1.16** with *t*-butyldimethylsilyl chloride and imidazole to obtain compound **1.17**. Afterward, 2-OH was esterified with levulinic acid in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to obtain compound **1.18**. Then, one-pot desilylation and oxidation at C-6 was performed by Jones oxidation, followed by benzyl ester formation at C-6 using benzyl bromide (BnBr) and sodium bicarbonate (NaHCO₃) to afford benzyl carboxylate derivative **1.19**. In order to obtain trichloroacetimidate donor **1.20**, selective deallylation was performed with palladium chloride (PdCl₂), followed by trichloroacetimidate introduction with trichloroacetonitrile in the presence of and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

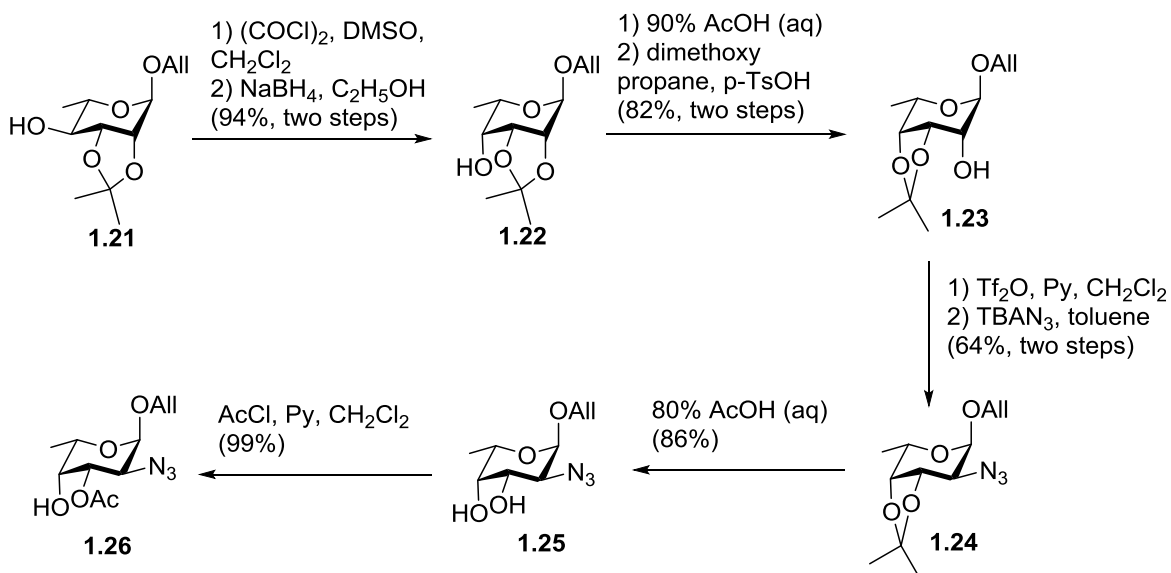
Scheme 1.3. Synthesis of D-glucosyl donor 1.20.



The L-fucosamine acceptor was prepared by sequential di-epimerization of a L-rhamnose precursor at C-4 and C-2. Thus, allyl 2,3-O-isopropylidene- α -L-rhamnopyranoside **1.21** was epimerized at C-4 via Swern oxidation followed by the reduction with sodium borohydride (NaBH_4) to give L-talose derivative **1.22** (Scheme 1.4). Then, the 2,3-O-isopropylidene group of **1.22** was cleaved with aqueous acetic acid, followed by the 3,4-O-isopropylidene introduction using dimethoxypropane in the presence *p*-toluenesulfonic acid (*p*-TsOH) to afford compound **1.23**. The latter was 2-O-sulfonated with trifluoromethanesulfonic anhydride (Tf_2O) in the presence of pyridine, followed by epimerization in the presence of freshly prepared tetrabutylammonium azide to yield 2-azido-L-fucoside **1.24**. At last, the isopropylidene group was removed by acidic hydrolysis to yield diol compound **1.25**, followed by regioselective acetylation at

C-3 with acetyl chloride in the presence of pyridine in dichloromethane to obtain L-fucosamine acceptor **1.26**.

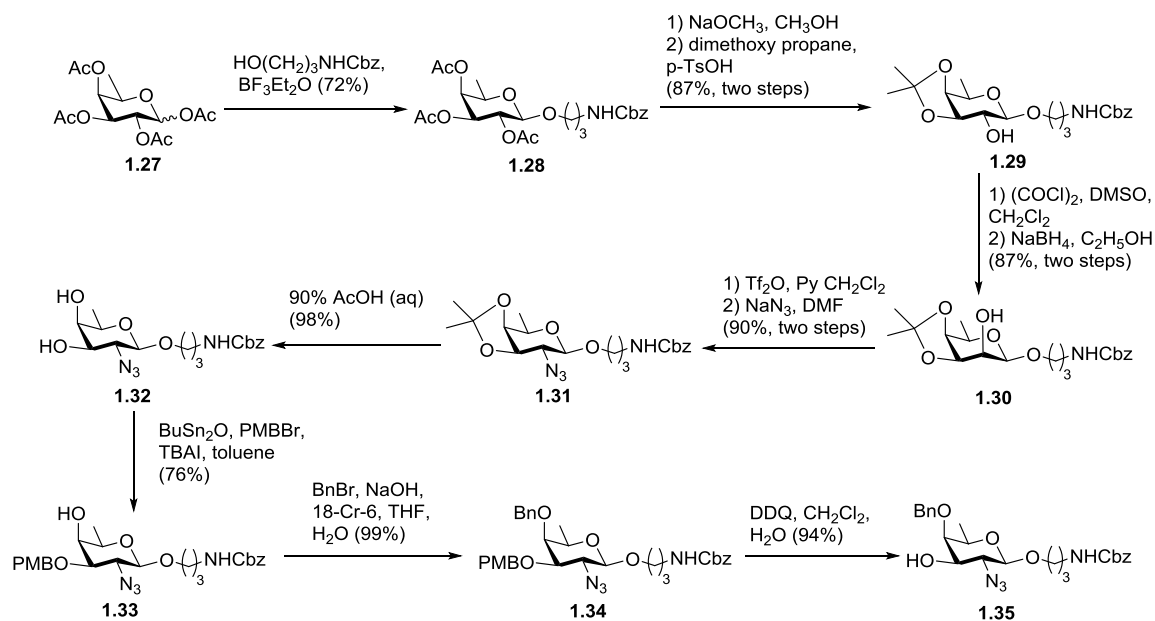
Scheme 1.4. Synthesis of L-fucosyl acceptor 1.26.



The preparation of D-fucosamine acceptor **1.35** began from glycosylation of benzyl N-(3-hydroxypropyl)carbamate with tetra-O-acetyl-D-fucopyranose **1.27** in the presence of boron trifluoride diethyl etherate ($\text{BF}_3\text{-Et}_2\text{O}$) to yield compound **1.28** (Scheme 1.5). After that, deacetylation with NaOMe , followed by isopropylidene introduction at C-3 and C-4 hydroxyl groups in the presence of dimethoxypropane and *p*-toluenesulfonic acid produced compound **1.29**. The hydroxyl group of **1.29** was then subjected to the Swern oxidation followed by the reduction with NaBH_4 to yield talose derivative **1.30**. Then, 2-O-triflate was introduced, followed by epimerization with sodium azide (NaN_3) in dimethyl formamide (DMF) to provide 2-azido D-fucoside **1.31**. The latter was subjected to isopropylidene removal with 90% aq. acetic acid to give diol **1.32**. The temporary selective *p*-methoxybenzyl ether (PMB) introduction at 3-OH of **1.32** was affected by dibutyl tin (IV) oxide (Bu_2SnO), *p*-methoxy benzyl bromide

(PMBBr) and tetrabutylammonium iodide (TBAI) in toluene to give compound **1.33**. Then, C-4 of **1.33** was benzylated with BnBr in the presence of NaOH and crown ether in THF to yield compound **1.34**. Finally, the PMB group removal was achieved by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in wet CH_2Cl_2 to acquire the desired D-fucosamine acceptor **1.35**.

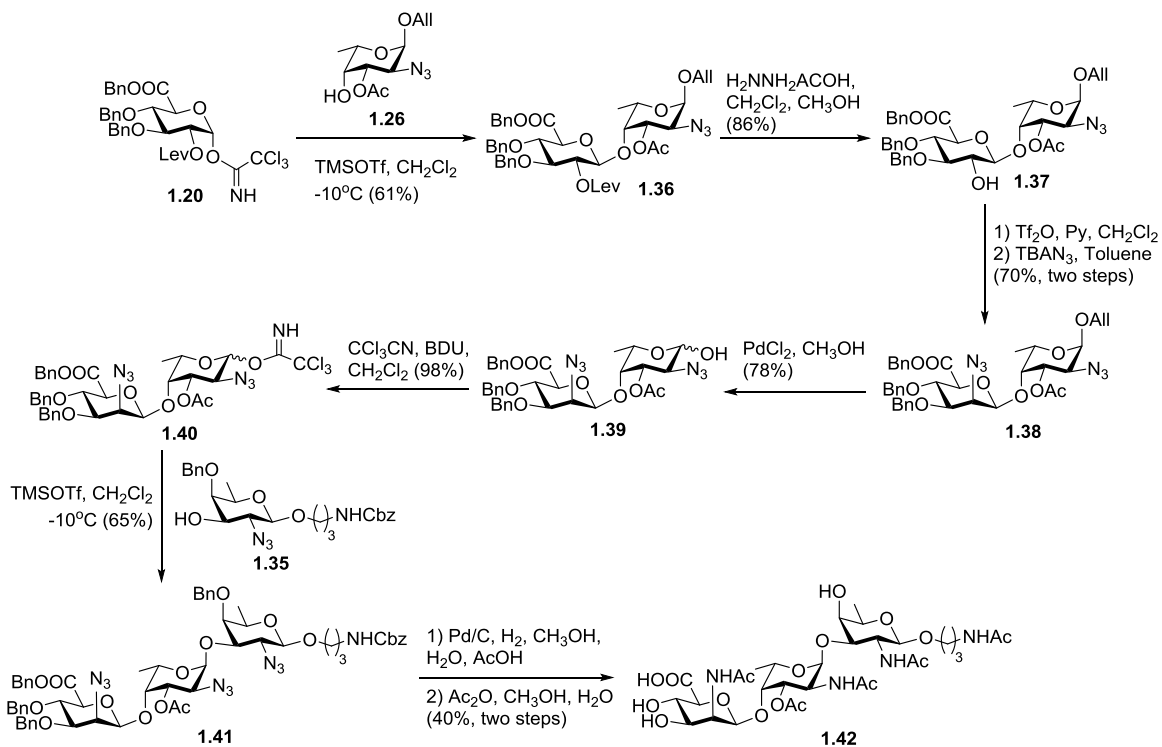
Scheme 1.5. Synthesis of D-fucosyl acceptor **1.35**.



As shown in Scheme 1.6, the desired sequence was achieved with glucuronate **1.20**, L-fucosamine **1.26**, and D-fucose **1.35** building blocks. Thus, in order to obtain disaccharide **1.36**, trichloroacetimidate **1.20** was glycosidated with fucosyl acceptor **1.26** in the presence of TMSOTf in CH_2Cl_2 at $-10\text{ }^\circ\text{C}$. Then, the 2-O-levulinoyl group of disaccharide **1.36** was selectively removed with hydrazine acetate ($\text{H}_2\text{NNH}_2\text{-AcOH}$) to yield 2-OH derivative **1.37**. The inversion of configuration at C-2' was achieved by sulfonation with Tf_2O followed by the nucleophilic substitution with tetrabutylammonium azide to obtain β -manno-configured disaccharide **1.38**. The

anomeric allyl group of disaccharide **1.38** was removed with PdCl₂, and the trichloroacetimidoyl leaving group was introduced with trichloroacetonitrile in the presence of DBU to yield trichloroacetimidate **1.40** as a mixture of α,β -anomers. The glycosidation of disaccharide donor **1.40** with D-fucosyl acceptor **1.35** was performed in the presence of TMSOTf in CH₂Cl₂ at -10 °C to yield trisaccharide **1.41** as a mixture of α,β -anomers. The desired α -linked trisaccharide was separated and subjected to hydrogenation with a 10% Pd/C cartridge to reduce the azide groups and remove benzylic protecting groups. Subsequent N-acetylation with acetic anhydride in MeOH afforded the target compound **1.42**.

Scheme 1.6. Synthesis of CP5 trisaccharide **1.42**.

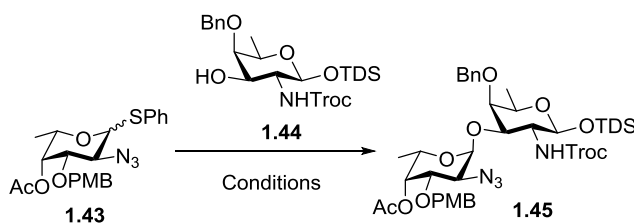


Another chemical synthesis of *S. aureus* type 5 trisaccharide repeating unit was reported by Boons in 2015.⁴² They decided to apply a [1 + 2] strategy to synthesize the

trisaccharide repeating unit that was equipped with the aminopentyl spacer at the reducing end. In according to their design, L-fucosamine donor was first coupled with D-fucosamine acceptor and the β -mannosidic linkage was introduced last using direct mannosylation with a S-phenyl D-Man ρ N $_3$ donor via the intermediacy of the α -anomeric triflate at low temperature.

The trisaccharide synthesis was initiated with the glycosylation of L-fucosamine donor **1.43** and D-fucosamine acceptor **1.44** using various reaction conditions to form disaccharide **1.45** (Table 1.1). It was found that the glycosylation in the presence of 1-benzene sulfinyl piperidine (BSP) and Tf $_2$ O with TTBP as a base in CH $_2$ Cl $_2$ at -60 °C afforded only α -fucosidic linkage, albeit with a low yield. A similar result was achieved with diphenylsulfoxide (DSP) and Tf $_2$ O, which is more powerful system. Glycosylations in the presence of NIS and TMSOTf or silver trifluoromethanesulfonate (AgOTf) at -60 °C produced disaccharide **1.45** in a much higher yield, albeit lower stereoselectivity.

Table 1.2. Glycosylation optimization of 1.43 and 1.44.



promoter/conditions	yield	β/α
BSP, Tf $_2$ O, TTBP, CH $_2$ Cl $_2$, -60 °C	30%	α
DPS, Tf $_2$ O, TTBP, CH $_2$ Cl $_2$, -60 °C	30%	α
NIS, TMSOTf, CH $_2$ Cl $_2$, -60 °C	73%	1/4
NIS, AgOTf, CH $_2$ Cl $_2$, -60 °C	70%	1/4
NIS, TMSOTf, CH $_2$ Cl $_2$ /Et $_2$ O (4/1), -60 °C	72%	1/4

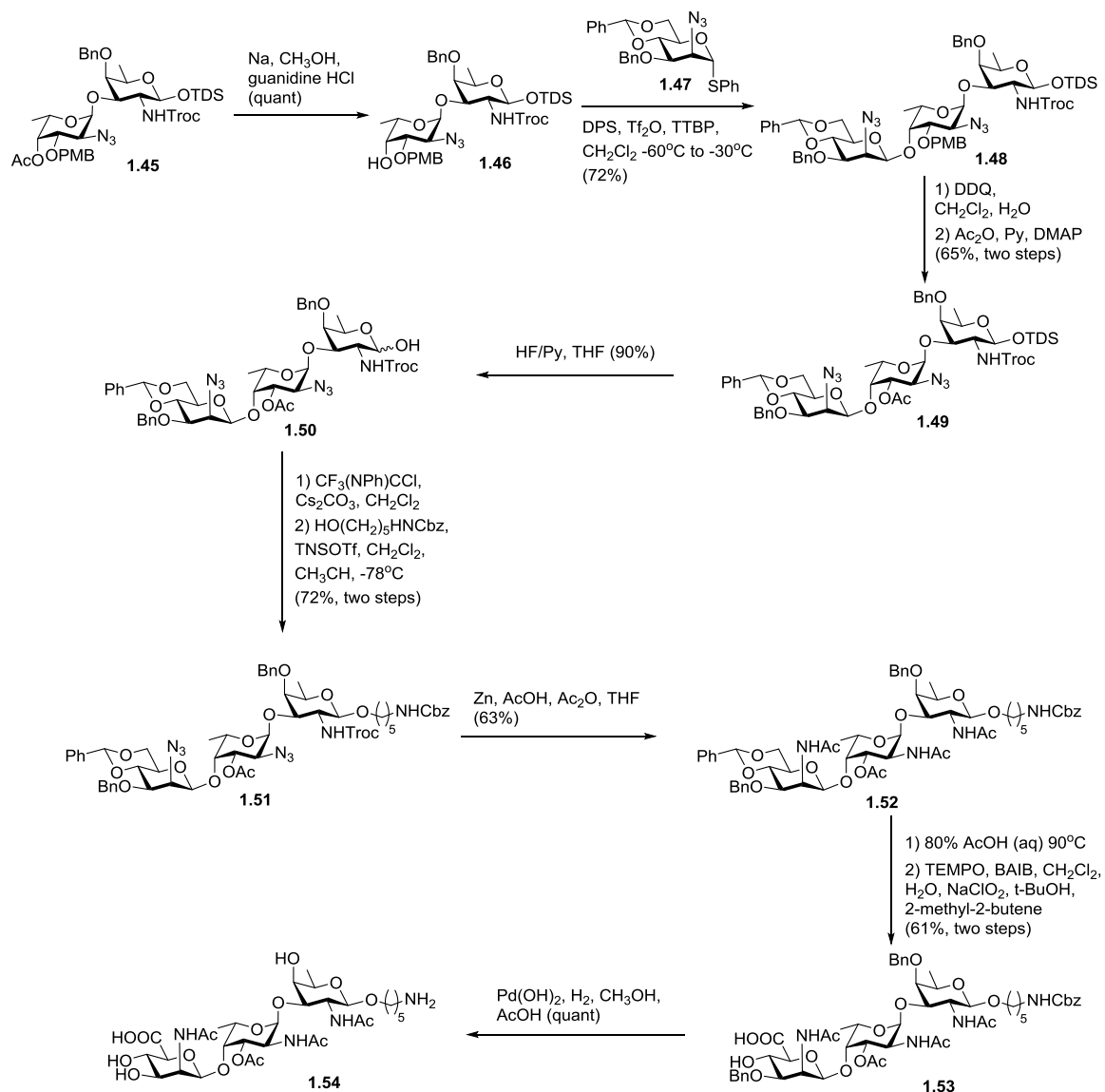
The disaccharide **1.45** was then deacetylated in the presence of NaOMe in MeOH to acquire disaccharide acceptor **1.46**. (Scheme 1.7) Next, glycosidation of D-

mannosamine donor **1.47** with disaccharide acceptor **1.46** using DSP and Tf₂O as the promoter system gave trisaccharide **1.48** with exclusive β-manno stereoselectivity. The functionalization of trisaccharide **1.48** was initiated by the PMB ether removal in the presence of DDQ, followed by acetylation with Ac₂O in the presence of 4-dimethylamino pyridine (DMAP) in pyridine to give trisaccharide **1.49**. Then, selective removal of the anomeric TDS group in the presence of hydrogen fluoride – pyridine complex afforded hemiacetal **1.50**. The latter was converted to N-phenyl trifluoroacetimidate with 2,2,2-trifluoro-N-phenylacetamidoyl chloride in the presence of cesium carbonate (CsCO₃) in CH₂Cl₂. The glycosidation of the imidate donor with 5-(benzyloxycarbonyl)aminopentanol was achieved in the presence of TMSOTf as the promoter in CH₂Cl₂/ CH₃CN at -78 °C to obtain trisaccharide **1.51**. The latter was subjected to the concomitant Troc group removal and the azide group reduction with Zn, AcOH and Ac₂O in THF to yield triacetamido derivative **1.52**. Then, benzylidene group of **1.52** was cleaved with 80% aq. AcOH at 90 °C, and the resulting diol was selectively oxidized at the primary position using a modified Huang's one-pot procedure involving (2,2,6,6-tetramethyl-piperidin-1-yl)oxyl (TEMPO)/NaOCl-NaClO₂ to yield carboxylic acid **1.53**. Finally, the hydrogenation in the presence of palladium hydroxide (PdOH)₂ to remove benzyl ethers and benzyloxycarbamate gave the target compound **1.54**.

In 2016, our group developed another approach for the synthesis of *S. aureus* type 5 trisaccharide repeating unit bearing capping methyl groups at the points of propagation of the polysaccharide sequences.³⁹ In order to acquire the β-mannosidic linkage, glucosylation was performed, followed by the inversion of the stereocenter at C-2' post-

glycosylationally. Disaccharide was then glycosylated to form trisaccharide subunit. The detailed description of this synthesis is presented in Chapter 3.

Scheme 1.7. Synthesis of CP5 trisaccharide 1.54.



In 2017, Codee reported the synthesis of *S. aureus* type 5 trisaccharide repeating unit equipped with the same spacer as that in the study by Boons.⁴⁵ The synthesis of the trisaccharide repeating unit was initiated by the glycosidation of selenoglycoside donor 1.55 with aminopentanol in the presence of Ph_2SO , TTBP and Tf_2O in CH_2Cl_2 and Et_2O .

the L-fucosyl donor **1.58** in the presence of Ph₂SO, TTBP, Tf₂O in CH₂Cl₂ to provide disaccharide **1.59** as a single anomer. After that, all silyl ethers of disaccharide **1.59** were removed with tetrabutylammonium fluoride (TBAF) in THF, followed by selective benzylation at 3'-OH with Taylor's diphenylborinate catalyst **1.60**, benzoyl chloride, N,N-diisopropylethylamine (DIPEA) in CH₃CN to afford disaccharide **1.61**. The glycosylation of disaccharide acceptor **1.61** with mannosyl donor **1.62** was performed with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) as a promoter in CH₂Cl₂ to form trisaccharide **1.63** with exclusive β-manno stereoselectivity. The ester groups of trisaccharide **1.63** were removed by the treatment with potassium hydroxide (KOH) and hydrogen peroxide (H₂O₂) in water and THF to provide trisaccharide **1.64**. The C-3' hydroxyl group of **1.64** was acetylated with Ac₂O in pyridine, followed by the treatment with thioacetic acid (AcSH) in pyridine to yield the triacetamide derivative **1.65**. The final compound **1.54** was obtained by hydrogenation of **1.65** with palladium hydroxide on carbon (Pd(OH)₂/C).

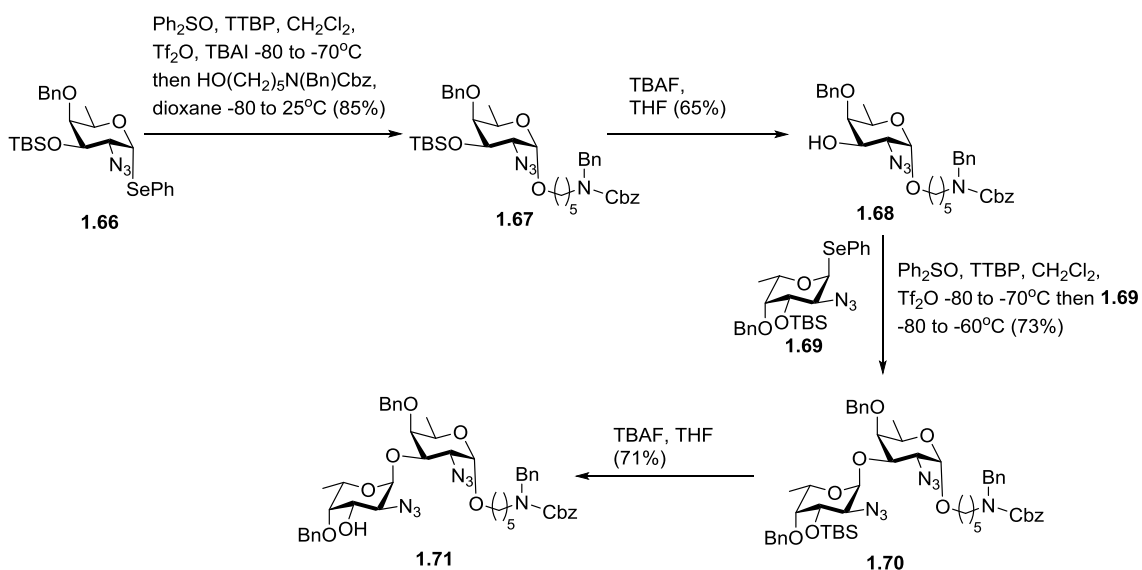
1.4.3 The chemical synthesis of *Staphylococcus aureus* capsular polysaccharide type 8 (CP8)

The first chemical synthesis of the repeating unit of capsular polysaccharide *S. aureus* type 8 (CP8) was reported by our group in 2015.⁴¹ The synthesized trisaccharide subunit contains methyl group as capping groups at the propagating point of the polysaccharide sequence. The synthetic strategy was similar to CP5 synthesis described previously, which was the β-glycosylation with neighboring group participation, followed

by epimerization to yield β -mannosidic linkage. The detailed description of this synthesis is presented in Chapter 2.

In 2017, the Codee group has also reported the chemical synthesis of CP8 derived disaccharide containing L-fucosamine, D-fucosamine and the aminopentyl spacer. The synthesis of the disaccharide target began with the glycosidation of D-fucosamine donor **1.66** with aminopentanol acceptor in the presence of Ph_2SO , TTBP and Tf_2O in CH_2Cl_2 and 1,4-dioxane. This reaction proceeding via the intermediacy of the anomeric triflate afforded spacer-containing fucosamine derivative **1.67** as a mixture of anomers ($\alpha/\beta = 7/1$, Scheme 1.9). After that, the TBS group of **1.67** was removed selectively with TBAF in THF to yield D-fucosyl acceptor **1.68**. The glycosylation of the latter with L-fucosamine donor **1.69** was performed in the presence of Ph_2SO , TTBP and Tf_2O in CH_2Cl_2 to acquire disaccharide **1.70** as a mixture of diastereomers ($\alpha/\beta = 7/1$). Finally, the silyl ether of **1.70** was removed with TBAF in THF to obtain disaccharide **1.71**.

Scheme 1.9. Synthesis of the CP8-derived disaccharide **1.71**.



1.5 Conclusions and Outlook

The *S. aureus* bacterium is surrounded by capsular polysaccharides. These capsular polysaccharides are important in the pathogenesis of staphylococcal infection. The majority strains express capsular polysaccharides type 5 (CP5) or 8 (CP8). There are 11 serotypes of capsular polysaccharides that have been identified, and up to 13 serotypes have been described. Some effort has been made to establish chemical structures of CPs and the structures of repeating units of CP1, CP2, CP5 and CP8 have been fully elucidated. In addition, the chemical structure of CP4 has been partially established. The chemical syntheses of CP1, CP5 and CP8 derived oligosaccharides have been successfully achieved by a number of groups. The chemical syntheses provide analogs or structural motifs of native CPs, which are of high demand for a number of purposes. Chemically synthesized molecules can help to confirm the elucidated structures of native CPs. Synthetic analogues are useful for establishing the structure-activity relationship or could be used to determine the minimal epitope required for prophylactic immune response. Also, the synthetic small molecule models based on the repeat unit can be used for the study of activation and protein conjugation process. This would offer a means to fully characterize the structure of activated CP, identify and quantify the activation site and understand the side reaction occurring during activation process.

1.6 References

1. Ala'Aldeen, D. A. A.; Hiramatsu, K., *Staphylococcus Aureus: Molecular and Clinical Aspects*. Horwood Publishing Limited Published by Elsevier Ltd.: 2004.
2. Lowy , F. D., Staphylococcus aureus Infections. *N. Engl. J. Med.* **1998**, *339*, 520-532.
3. Fridkin , S. K.; Hageman , J. C.; Morrison , M.; Sanza , L. T.; Como-Sabetti , K.; Jernigan , J. A.; Harriman , K.; Harrison , L. H.; Lynfield , R.; Farley , M. M., Methicillin-Resistant Staphylococcus aureus Disease in Three Communities. *N. Engl. J. Med.* **2005**, *352*, 1436-1444.
4. Daum, R. S.; Spellberg, B., Progress Toward a Staphylococcus aureus Vaccine. *Clin. Infect. Dis.* **2012**, *54*, 560-567.
5. Jedrzejewski, M. J., Pneumococcal virulence factors: structure and function. *Microbiol. Mol. Biol. Rev.* **2001**, *65*, 187-207.
6. Nanra, J. S.; Buitrago, S. M.; Crawford, S.; Ng, J.; Fink, P. S.; Hawkins, J.; Scully, I. L.; McNeil, L. K.; Aste-Amézaga, J. M.; Cooper, D.; Jansen, K. U.; Anderson, A. S., Capsular polysaccharides are an important immune evasion mechanism for Staphylococcus aureus. *Hum. Vaccines Immunother* **2013**, *9*, 480-487.
7. Gründling, A., Cell Wall Assembly and Physiology. In *Staphylococcus: Genetics and Physiology*, Somerville, G. A., Ed. Caister Academic Press: Norfolk, UK, 2016; pp 133-170.
8. Chan, Y. G.; Kim, H. K.; Schneewind, O.; Missiakas, D., The capsular polysaccharide of Staphylococcus aureus is attached to peptidoglycan by the LytR-CpsA-Psr (LCP) family of enzymes. *J. Biol. Chem.* **2014**, *289*, 15680-15690.

9. Karakawa, W. W.; Kane, J. A., Capsular polysaccharides of *Staphylococcus aureus*. In *Seminars in infectious disease. Vol. IV. Bacterial vaccines*, Wienstein, L.; Field, B. N., Eds. Thieme-Stratton Inc. Stuttgart, Germany; Georg Thieme Verlag: New York, USA, 1982; Vol. 4, pp 285-293.
10. Sompolinsky, D.; Samra, Z.; Karakawa, W. W.; Vann, W. F.; Schneerson, R.; Malik, Z., Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. *J. Clin. Microbiol.* **1985**, *22*, 828-834.
11. O'Riordan, K.; Lee, J. C., *Staphylococcus aureus* Capsular Polysaccharides. *Clin. Microbiol. Rev.* **2004**, *17*, 218-234.
12. von Eiff, C.; Taylor, K. L.; Mellmann, A.; Fattom, A. I.; Friedrich, A. W.; Peters, G.; Becker, K., Distribution of capsular and surface polysaccharide serotypes of *Staphylococcus aureus*. *Diagn. Microbiol. Infect. Dis.* **2007**, *58*, 297-302.
13. Lee, C. Y., Capsule Production. In *Staphylococcus aureus Infection and Disease*, Honeyman, A. L.; Friedman, H.; Bandinelli, M., Eds. Kluwer Academic/Plenum Publishers: New York, 2001.
14. Karakawa, W. W.; Young, D. A.; Kane, J. A., Structural analysis of the cellular constituents of a fresh clinical isolate of *Staphylococcus aureus*, and their role in the interaction between the organisms and polymorphonuclear leukocytes in the presence of serum factors. *Infect. Immun.* **1978**, *21*, 496-505.
15. Scott, A. C., A capsulate *Staphylococcus aureus*. *J. Med. Microbiol.* **1969**, *2*, 253-260.

16. Liao, D.-F.; Melly, M. A.; Hash, J. H., Surface Polysaccharide from *Staphylococcus aureus* M That Contains Taurine, d-Aminogalacturonic Acid, and d-Fucosamine. *J. Bacteriol.* **1974**, *119*, 913-922.
17. Liao, D. F.; Hash, J. H., Structural analysis of the surface polysaccharide of *Staphylococcus aureus* M. *J. Bacteriol.* **1977**, *131*, 194-200.
18. Murthy, S. V. K. N.; Ann Melly, M.; Harris, T. M.; Hellerqvist, C. G.; Hash, J. H., The repeating sequence of the capsular polysaccharide of *Staphylococcus aureus* M. *Carbohydr. Res.* **1983**, *117*, 113-123.
19. Giehl, T. J.; Qoronfleh, M. W.; Wilkinson, B. J., Transport, Nutritional and Metabolic Studies of Taurine in *Staphylococci*. *Microbiology* **1987**, *133* (4), 849-856.
20. Karakawa, W. W.; Kane, J. A., Characterization of the Surface Antigens of *Staphylococcus Aureus*, Strain K-93M. *J. Immunol.* **1972**, *108*, 1199-1208.
21. Morse, S. I., Isolation and properties of a surface antigen of *Staphylococcus aureus*. *J. Exp. Med* **1962**, *115*, 295-311.
22. Perkins, H. R., A polymer containing glucose and aminohexuronic acid isolated from the cell walls of *micrococcus lysodeikticus*. *Biochem. J.* **1963**, *86*, 475-483.
23. Hanessian, S.; Haskell, T. H., Structural Studies on *Staphylococcal* Polysaccharide Antigen. *J. Biol. Chem.* **1964**, *239*, 2758-2764.
24. Karakawa, W. W.; Young, D. A., Immunochemical study of diverse surface antigens of a *Staphylococcus aureus* isolate from an osteomyelitis patient and their role in *in vitro* phagocytosis. *J. Clin. Microbiol.* **1979**, *9*, 399-408.

25. Fournier, J.-M., Capsular Polysaccharides of *Staphylococcus aureus*. In *Pathogenesis of Wound and Biomaterial-Associated Infections*, Wadström, T.; Eliasson, I.; Holder, I.; Ljungh, Å., Eds. Springer London: London, 1990; pp 533-544.
26. Karakawa, W. W.; Kane, J. A.; Smith, M. R., Isolation of an Acidic Surface Antigen from a Conventional Strain of *Staphylococcus aureus*. *Infect. Immun.* **1974**, *9*, 511-518.
27. Wu, T. C. M.; Park, J. T., Chemical Characterization of a New Surface Antigenic Polysaccharide from a Mutant of *Staphylococcus aureus*. *J. Bacteriol.* **1971**, *108*, 874-884.
28. Fournier, J. M.; Hannon, K.; Moreau, M.; Karakawa, W. W.; Vann, W. F., Isolation of type 5 capsular polysaccharide from *Staphylococcus aureus*. *Annales de l'Institut Pasteur. Microbiology* **1987**, *138*, 561-567.
29. Vann, W. F.; Moreau, M.; Sutton, A.; Byrd, R. A.; Karakawa, W. W., Structure and immunochemistry of *Staphylococcus aureus* capsular polysaccharides. *UCLA symp Mol Cell Biol* **1988**, *64*, 187-198.
30. Moreau, M.; Richards, J. C.; Fournier, J.-M.; Byrd, R. A.; Karakawa, W. W.; Vann, W. F., Structure of the type 5 capsular polysaccharide of *Staphylococcus aureus*. *Carbohydr. Res.* **1990**, *201*, 285-297.
31. Jones, C., Revised structures for the capsular polysaccharides from *Staphylococcus aureus* Types 5 and 8, components of novel glycoconjugate vaccines. *Carbohydr. Res.* **2005**, *340*, 1097-1106.

32. Nelles, M. J.; Niswander, C. A.; Karakawa, W. W.; Vann, W. F.; Arbeit, R. D., Reactivity of type-specific monoclonal antibodies with *Staphylococcus aureus* clinical isolates and purified capsular polysaccharide. *Infect. Immun.* **1985**, *49*, 14-18.
33. Fournier, J. M.; Vann, W. F.; Karakawa, W. W., Purification and characterization of *Staphylococcus aureus* type 8 capsular polysaccharide. *Infect. Immun.* **1984**, *45*, 87-93.
34. Fattom, A.; Schneerson, R.; Szu, S. C.; Vann, W. F.; Shiloach, J.; Karakawa, W. W.; Robbins, J. B., Synthesis and immunologic properties in mice of vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides conjugated to *Pseudomonas aeruginosa* exotoxin A. *Infect. Immun.* **1990**, *58*, 2367-2374.
35. Fattom, A.; Schneerson, R.; Watson, D. C.; Karakawa, W. W.; Fitzgerald, D.; Pastan, I.; Li, X.; Shiloach, J.; Bryla, D. A.; Robbins, J. B., Laboratory and clinical evaluation of conjugate vaccines composed of *Staphylococcus aureus* type 5 and type 8 capsular polysaccharides bound to *Pseudomonas aeruginosa* recombinant exoprotein A. *Infect. Immun.* **1993**, *61*, 1023-1032.
36. Fattom, A. I.; Horwith, G.; Fuller, S.; Propst, M.; Naso, R., Development of StaphVAX™, a polysaccharide conjugate vaccine against *S. aureus* infection: from the lab bench to phase III clinical trials. *Vaccine* **2004**, *22*, 880-887.
37. Nissen, M.; Marshall, H.; Richmond, P.; Shakib, S.; Jiang, Q.; Cooper, D.; Rill, D.; Baber, J.; Eiden, J.; Gruber, W.; Jansen, K. U.; Emini, E. A.; Anderson, A. S.; Zito, E. T.; Girgenti, D., A randomized phase I study of the safety and immunogenicity of three ascending dose levels of a 3-antigen *Staphylococcus aureus* vaccine (SA3Ag) in healthy adults. *Vaccine* **2015**, *33*, 1846-1854.

38. Freese, S. J.; Anderson, A.; Pavliak, V.; Jansen, K. U.; Scully, I. L.; Scott, T. D.; Nanra, J. S.; Prasad, A. K.; Green, B. A. Compositions and methods for preparing Staphylococcus aureus serotype 5 and 8 capsular polysaccharide conjugate immunogenic compositions. US9125951B2, 2015.
39. Yasomane, J. P.; Visansirikul, S.; Pornsuriyasak, P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V., The synthesis of the repeating unit of capsular polysaccharide Staphylococcus aureus type 5 to study carbonylditriazole-mediated activation for conjugation of native CP5. *J. Org. Chem.* **2016**, *81*, 5981-5987.
40. Stefanetti, G.; Rondini, S.; Lanzilao, L.; Saul, A.; MacLennan, C. A.; Micoli, F., Impact of conjugation chemistry on the immunogenicity of S. Typhimurium conjugate vaccines. *Vaccine* **2014**, *32*, 6122-6129.
41. Visansirikul, S.; Yasomane, J. P.; Pornsuriyasak, P.; Kamat, M. N.; Podvalnyy, N. M.; Gobble, C. P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V., A Concise Synthesis of the Repeating Unit of Capsular Polysaccharide Staphylococcus aureus Type 8. *Org. Lett.* **2015**, *17*, 2382-2384.
42. Gagarinov, I. A.; Fang, T.; Liu, L.; Srivastava, A. D.; Boons, G. J., Synthesis of Staphylococcus aureus Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization. *Org. Lett.* **2015**, *17*, 928-931.
43. Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R., Synthesis of Staphylococcus aureus type 5 capsular polysaccharide repeating unit using novel L-FucNAc and D-FucNAc synthons and immunochemical evaluation. *Bioorg. Med. Chem.* **2012**, *20*, 6403-6415.

44. Hagen, B.; van Dijk, J. H. M.; Zhang, Q.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C., Synthesis of the Staphylococcus aureus Strain M Capsular Polysaccharide Repeating Unit. *Org. Lett.* **2017**, *19*, 2514-2517.
45. Hagen, B.; Ali, S.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C., Mapping the Reactivity and Selectivity of 2-Azidofucosyl Donors for the Assembly of N-Acetylfucosamine-Containing Bacterial Oligosaccharides. *J. Org. Chem.* **2017**, *82*, 848-868.

CHAPTER 2

A concise synthesis of the repeating unit of capsular polysaccharide *Staphylococcus aureus* type 8

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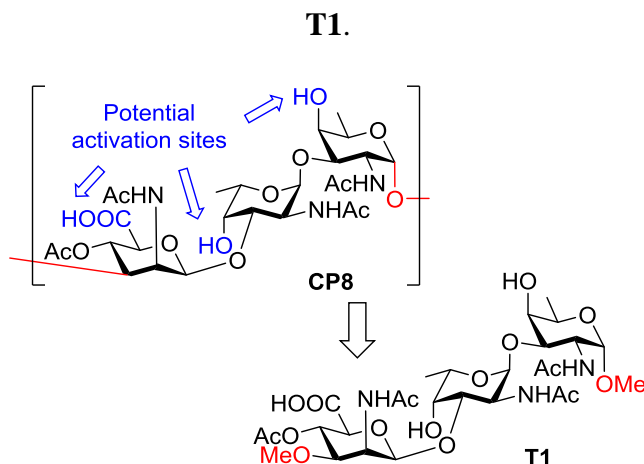
2.1 Introduction

The Gram positive, cluster forming bacteria *Staphylococcus aureus* normally colonizes in the human nose and skin.¹ It belongs to the family of opportunistic pathogens that may cause blood stream infections when the integrity of skin is broken and the immune system can no longer fight the infection. *S. aureus* has become one of the most frequent causes of infections in surgical patients, trauma and burn patients, patients receiving an implant, newborns, dialysis patients with high mortality rates. Immunocompromised individuals and patients with prosthetic devices or long-term intravascular catheters are particularly vulnerable.² The *S. aureus* bacterium is surrounded by a polysaccharide capsule, preventive vaccination based on polysaccharide or saccharide-protein conjugates is a suitable tool against the bacterial invasion.³

Serotyping has revealed that the majority of *S. aureus* strains express either capsular polysaccharide type 5 (CP5) or type 8 (CP8). The structures of CP5 and CP8 have been established⁴ and chemical syntheses of the repeating unit of CP5 have been reported.⁵⁻⁶ Herein, we report the first synthesis of trisaccharide repeating unit of CP8. The CP8 repeating unit consists of a trisaccharide, but it is an unusual sequence of three uncommon monosaccharide residues (Figure 2.1). The trisaccharide bears a terminal ManNAcA (D-mannosamine uronic acid) residue, which is 4-O-acetylated. ManNAcA is then β -(1,2-cis) glycosidically linked to the L-FucNAc (L-fucosamine) unit via 3-OH. L-FucNAc is in turn linked via the 1,2-cis-glycosidic bond to the C-3 of D-FucNAc. In the natural polysaccharide sequence, D-FucNAc is then linked to another D-ManNAcA via α -(1-3) linkage, etc. The synthetic target **T1** was designed as a tool compound to study activation and conjugation of full length CP8 derived from fermentation of *S. aureus* as

previously described.⁷ Hence, in order to preserve the conjugation sites the synthetic trisaccharide **T1** (Scheme 2.1) needs to be equipped with capping groups (methyl) at the points of propagation of the polysaccharide sequence.

Scheme 2.1. *S. aureus* type 8: polysaccharide CP8 and the synthetic target



From a synthetic perspective, one could anticipate a number of challenges and hurdles. For instance, the introduction of 1,2-cis glycosidic linkages⁸ often proceeds with low selectivity, and our synthetic target **T1** has three such linkages. In addition, all three monosaccharide components of the repeat unit are uncommon and hence one could anticipate the necessity to perform the total synthesis of each monosaccharide. Our initial goal was to perform the direct synthesis of the β -mannosidic linkage,⁹ an approach that was successfully used for other targets containing ManNAcA.^{6,10} However, the direct mannosylation failed presumably due to a very low reactivity profile for the benzylidene-protected mannosamine donors. After extended experimentation with thioglycoside and S-benzoxazolyl (SBox) donors, we abandoned the mannosylation route and chose to proceed via a glucosylation route instead, followed by inversion of the stereocenter at C-2 post-glycosylation.

2.2 Results and Discussion

According to this revised plan, the 2-OH of known thioglycoside **2.1**¹¹ was protected as levulinoyl (Lev) ester by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP)-mediated coupling with LevOH to give **2.2** in 98% (Scheme 2.2). Reaction of **2.2** with bromine followed by the introduction of S-benzoxazolyl (SBox) leaving group with KSBox¹² in the presence of 18-crown-6 allowed glucosyl donor **2.3** in 67% yield over two steps.

The synthesis of L-fucosyl acceptor for the introduction of the middle unit in the trisaccharide sequence was originated from known L-fucal **2.4**¹³ as follows. Azidonitration of **2.4** with sodium azide in the presence of ceric ammonium nitrate (CAN) in MeCN at -15 °C followed by the reaction with sodium 4-penten-1-oxide gave β -pentenyl glycoside **2.5b** in 65% yield over two steps. Diol **2.5b** was then protected as 3,4-O-benzylidene acetal using dimethoxytoluene (DMT) in the presence of camphorsulfonic acid (CSA) in MeCN to afford compound **2.6** in 87% yield. The reductive benzylidene ring opening in **2.6** with NaCNBH₃ in 2 M HCl in diethyl ether afforded L-fucosyl acceptor **2.7** in 94% yield.

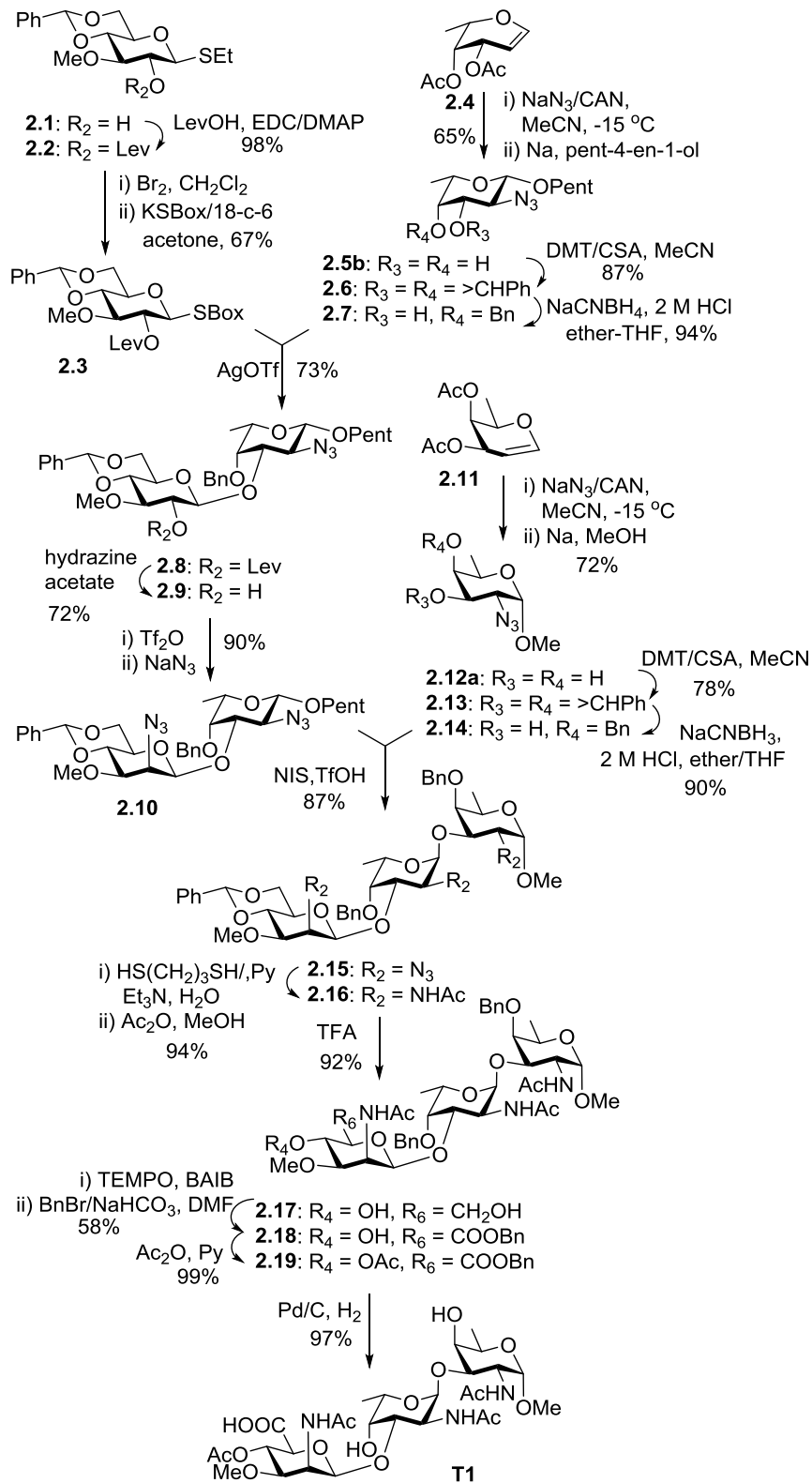
With glucosyl donor **2.3** and fucosyl acceptor **2.7** in hand, we conducted selective activation of the SBox leaving group in the donor in the presence of O-pentenyl anomeric group in the acceptor. This was affected in the presence of silver trifluoromethanesulfonate (AgOTf) in 1,2-dichloroethane. As a result, disaccharide **2.8** was isolated in 73% yield. In this context, thioglycoside donor **2.2** could also be activated over O-pentenyl acceptor **2.7**. This activation requires MeOTf,¹⁴ a relatively mild activator, which in this particular application gave modest results due to a highly

disarmed reactivity profile of donor **2.2**. Selective levulinoyl group removal in **2.8** with hydrazine acetate in methanol and dichloromethane (1/20, v/v) gave disaccharide **2.9** in 72% yield. The latter was then subjected to sequential treatment with Tf_2O and sodium azide to obtain the C-2' epimerized¹⁵ D-mannosyl disaccharide **2.10** in 90% yield over two steps.

The synthesis of D-fucosyl acceptor for the introduction of the reducing end unit in the trisaccharide sequence was initiated from D-galactose via known D-fucal **2.11**¹⁶⁻¹⁷ as follows. Azidonitration of **2.11** with NaN_3 and CAN followed by methyl glycoside formation with NaOMe gave α -fucoside **2.12a** in 72% yield over two steps. Benzylidene acetal protection of diol **2.12a** with DMT in the presence of CSA gave 3,4-O-benzylidene fucoside **2.13** in 78% yield. The reductive benzylidene ring opening in **2.13** with NaCNBH_3 in 2 M HCl in diethyl ether and THF afforded the desired D-fucosyl acceptor **2.14** in 90% yield. The latter was glycosylated with disaccharide donor **2.10**. O-Pentenyl leaving group was then activated with N-iodosuccinimide (NIS) and TfOH to afford trisaccharide **2.15** with exclusive 1,2-cis stereoselectivity in 87% yield.

With the backbone sequence constructed, and after thorough experimentation, we settled on the following sequence for the conversion of **2.15** into the target trisaccharide **T1**. First, the three azide groups were reduced with propane-1,3-dithiol in the presence of triethylamine¹⁸ (TEA) in wet pyridine followed by acetylation with Ac_2O in methanol to obtain trisaccharide **2.16** in 94% yield over 2 steps. Second, the benzylidene acetal removal was performed with trifluoroacetic acid (TFA) in wet CH_2Cl_2 to acquire diol trisaccharide **2.17** in 92% yield.

Scheme 2.2. Synthesis of CP8 repeating unit T1.



The oxidation of the primary alcohol in **2.17** was effected with (2,2,6,6-tetramethyl-piperidin-1-yl)oxyl (TEMPO) and (diacetoxyiodo)benzene (BAIB) in wet CH_2Cl_2 .¹⁹ The resulting carboxyl group was esterified with BnBr in the presence of NaHCO_3 in DMF to afford trisaccharide **2.18** in 58% yield over two steps. The 4-OH of the mannosamine subunit was then acetylated with Ac_2O in pyridine to afford trisaccharide **2.19** in 99% yield. Finally, all benzylic protecting groups in **2.19** were removed by hydrogenation in the presence of palladium on charcoal in wet ethanol to acquire the final compound **T1** in 97% yield.

2.3 Conclusions and Outlook

We report the synthesis of a trisaccharide repeating unit of *S. aureus* CP8 bearing terminal methyl groups to preserve the activation sites. Characterization of the activation products derived from natural polysaccharides is very difficult owing to their large molecular size (MW = 100-2000 kDa). The study of small model compound **T1** based on the structure of the repeat unit offers a means to fully characterize the structure of the activated polysaccharides, quantify the level of activation and begin to understand side reactions occurring after polysaccharide activation for conjugation. Over-activation and side reactions can lead to a loss of the epitopes required for immunogenicity of polysaccharide conjugate vaccines, leading to product failure. The improved understanding of the conjugation process will, in turn, lead to a more controlled, predictable, and reproducible outcome of polysaccharide conjugations.

2.4 Experimental

2.4.1 General Methods

The reactions were performed using commercial reagents (Aldrich or Acros) and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F₂₅₄ (EM Science). 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. CH₂Cl₂ and ClCH₂CH₂Cl were distilled from CaH₂ directly prior to application. Molecular sieves (3Å), 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. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to application. Optical rotations were measured at 'Jasco P-1020' polarimeter. ¹H NMR spectra were recorded at 300 MHz (Bruker Avance) or 500 MHz (Bruker ARX-500) or 600 MHz (Agilent), ¹³C-NMR spectra were recorded at 75 MHz (Bruker Avance) or 125 MHz (Bruker ARX-500) or 150 MHz (Agilent). The ¹H chemical shifts are referenced to the signal of the residual CHCl₃ ($\delta_{\text{H}} = 7.27$ ppm) for solutions in CDCl₃ or the signal of the residual CH₃OH ($\delta_{\text{H}} = 3.31$ ppm) for solutions in CD₃OD. The ¹³C chemical shifts are referenced to the central signal of CDCl₃ ($\delta_{\text{C}} = 77.23$ ppm) for solutions in CDCl₃ or the central signal of CD₃OD ($\delta_{\text{C}} = 49.24$ ppm) for solutions in CD₃OD. HRMS determinations were made with the use of JEOL MStation (JMS-700) mass spectrometer.

2.4.2 Preparation of monosaccharide building blocks 2.3, 2.7, and 2.14.

Ethyl 4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl-1-thio- β -D-glucopyranoside (2.2). Levulinic acid (1.08 g, 9.25 mmol), EDC (2.73 g, 14.24 mmol) and DMAP (174 mg, 1.42 mmol) were added to a stirring solution of ethyl 4,6-*O*-benzylidene-3-*O*-methyl-1-thio- β -D-glucopyranoside¹¹ (**2.1**, 2.41 g, 7.38 mmol) in CH₂Cl₂ (60 mL) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was diluted with CH₂Cl₂ (~200 mL) and washed with sat. aq. NaHCO₃ (50 mL) and water (2 x 50 mL). The organic phase was separated, dried over MgSO₄, 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 (3.07 g, 98%). Analytical data for **2.2**: R_f = 0.30 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{20}$ 28.5 (c = 1, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ , 1.25 (t, 3H, CH₂CH₃), 2.19 (s, 3H, COCH₃), 2.60-2.78 (m, 4H, COCH₂CH₂CO), 2.82 (q, 2H, CH₂CH₃), 3.45-3.53 (m, 2H, H-3, 5), 3.57 (s, 3H, OCH₃), 3.65 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.76 (dd, 1H, $J_{6a,6b}$ = 10.3 Hz, H-6a), 4.35 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, H-6b), 4.47 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 4.97 (dd, 1H, $J_{2,3}$ = 8.4 Hz, H-2), 5.54 (s, 1H, CHPh), 7.35-7.48 (m, 5H, aromatic) ppm; ¹³C NMR (150 MHz, CDCl₃): δ , 15.0, 24.1, 28.2, 30.0, 38.0, 60.9, 68.7, 70.8, 71.8, 81.3, 82.1, 84.3, 101.4, 126.2 (x 2), 128.4 (x 2), 129.2, 137.3, 171.7, 206.4 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₁H₂₈NaO₇S 447.1453, found 447.1450.

Benzoxazolyl 4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl-1-thio- β -D-glucopyranoside (2.3). A solution of **2.2** (1.75 g, 4.13 mmol) and activated molecular sieves (3Å, 2.1 g) in CH₂Cl₂ (60 mL) was stirred under argon for 1 h at rt. The mixture

was cooled to 0 °C, freshly prepared solution of Br₂ in CH₂Cl₂ (40 mL, 1/165, v/v) was added, and the resulting mixture was stirred for 15 min at 0 °C. After that, the solid was filtered off, rinsed successively with CH₂Cl₂ and the combined filtrate (~200 mL) was concentrated under reduced pressure at rt and dried *in vacuo* for 2 h. The crude residue was dissolved in dry acetone (20 mL), K₂S₂O₈¹² (2.35 g, 12.4 mmol) and 18-crown-6 (0.334 g, 1.24 mmol) were added and the reaction mixture was stirred under argon for 1 h at rt. After that, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ (~250 mL), washed with 1% aq. NaOH (50 mL) and water (3x 50 mL). The organic phase was separated, over MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane graduate elution) to afford the title compound in 67% yield (1.43 g, 2.79 mmol) as a white amorphous solid. Analytical data for **2.3**: R_f = 0.52 (ethyl acetate/hexane, 1/1, v/v); [α]_D²¹ 16.6 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 2.17 (s, 3H, COCH₃), 2.57-2.66 (m, 2H, COCH₂), 2.69-2.87 (m, 2H, COCH₂), 3.61 (s, 3H, OCH₃), 3.63-3.83 (m, 4H, H-3, 4, 5, 6a), 4.41 (m, 1H, J_{5,6a} = 3.9 Hz, J_{6a,6b} = 8.6 Hz, H-6b), 5.21 (dd, 1H, J_{2,3} = 8.4 Hz, H-2), 5.60 (s, 1H, CHPh), 5.73 (d, 1H, J_{1,2} = 10.4 Hz, H-1), 7.28-7.53 (m, 8H, aromatic), 7.68 (m, 1H, aromatic); ¹³C NMR (75 MHz, CDCl₃): δ, 28.0, 29.9, 37.9, 61.1, 68.5, 71.1, 71.5, 81.0, 82.0, 84.2, 101.5, 110.3, 119.1, 124.7, 124.7, 126.2, 128.4, 128.5, 129.2, 137.1, 141.7, 152.1, 161.3, 171.7, 206.1 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₆H₂₇NNaO₈S 536.1355, found 536.1357.

4-Pentenyl 2-azido-2,6-dideoxy-α-galactopyranoside (2.5a) and 4-Pentenyl 2-azido-2,6-dideoxy-β-L-galactopyranoside (2.5b). Ceric ammonium nitrate (16.4 g, 29.9

mmol) and sodium azide (1.46 g, 22.4 mmol) were added to a solution of 3,4-di-*O*-acetyl-1,5-anhydro-*L*-*lyxo*-hex-1-enitol¹³ (**2.4**, 2.0 g, 9.3 mmol) in anhydrous acetonitrile (50 mL) and the resulting mixture was stirred for 3 h at -15 °C. After that, the volatiles were evaporated under reduced pressure at rt. The residue was dissolved in a mixture of diethyl ether and ethyl acetate (1/1, v/v, ~120 mL) and washed with water (30 mL). The organic phase was separated and the aqueous layer was extracted with a mixture of diethyl ether and ethyl acetate (1/1, v/v, 3 x 50 mL). The combined organic phase was dried with MgSO₄ and concentrated *in vacuo* and the residue was dried *in vacuo* for 3 h. The crude residue was dissolved in 4-penten-1-ol (10.0 mL), cooled to 0 °C, sodium (240 mg, 10.4 mmol) was added portionwise, and the resulting mixture was stirred for 2 h at 0 °C. After that, Dowex (H⁺) was added till pH ~ 7, the resin was filtered off, and washed successively with MeOH. The combined filtrate (~80 mL) was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane graduate elution) to yield **2.5a** (1.11 g, 46%) and **2.5b** (445 mg, 19%) as a pale-yellow foam. Analytical data for **2.5a**: R_f = 0.51 (ethyl acetate/hexane, 7/3, v/v); [α]_D³² -118.0 (c = 1, CH₃OH); ¹H NMR (500 MHz, CD₃OD): δ, 1.23 (d, 3H, J_{5,6} = 6.6 Hz, C-6), 1.71-1.75 (m, 2H, CH₂), 2.18-2.23 (m, 2H, CH₂), 3.36 (dd, 1H, J_{2,3} = 10.8 Hz, H-2), 3.46-3.49 (m, 1H, CH₂^a), 3.70-3.75 (m, 2H, H-4, CH₂^b), 3.97-4.02 (m, 2H, H-3, 5), 4.84 (d, 1H, J_{1,2} = 3.6 Hz, H-1), 4.98-5.08 (m, 2H, CH=CH₂), 5.80-5.90 (m, 1H, CH=CH₂) ppm; ¹³C NMR (125 MHz, CD₃OD): δ, 14.8, 28.1, 29.6, 59.2, 65.9, 66.5, 67.3, 71.6, 98.0, 113.5, 137.5 ppm; HR-FAB MS [M+H]⁺ calcd for C₁₁H₂₀O₄N₃ 258.1454, found 258.1444.

Analytical data for **2.5b**: R_f = 0.47 (ethyl acetate/hexane, 7/3, v/v); [α]_D²⁶ -7.86 (c = 1, CH₃OH); ¹H NMR (600 MHz, CD₃OD): δ, 1.26 (d, 3H, J_{5,6} = 6.5 Hz, C-6), 1.68-1.73

(m, 2H, CH₂), 2.16-2.19 (m, 2H, CH₂), 3.37-3.41 (m, 2H, H-2, 3), 3.52-3.60 (m, 3H, H-4, 5, CH₂^b), 3.88-3.92 (m, 1H, CH₂^a), 4.23 (d, 1H, $J_{1,2} = 7.3$ Hz, H-1), 4.95-5.05 (m, 2H, CH=CH₂), 5.80-5.85 (m, 1H, CH=CH₂) ¹³C NMR (150 MHz, CD₃OD): δ, 16.8, 30.3, 31.4, 65.8, 70.1, 72.1, 72.5, 73.6, 103.7, 115.5, 139.5 ppm; HR-FAB MS [M+Na]⁺ calcd for C₁₁H₁₉O₄NaN₃ 280.1273, found 280.1277.

4-Pentenyl 2-azido-3,4-O-benzylidene-2,6-dideoxy-β-L-galactopyranoside (2.6).

Dimethoxytoluene (1.9 mL, 12.6 mmol) and camphorsulfonic acid (74 mg, 0.32 mmol) we added to a solution of **2.5b** (1.62 g, 6.3 mmol) in acetonitrile (20 mL) and the resulting mixture was stirred under argon for 1 h at rt. After that, triethylamine (~0.3 mL) was added and the volatiles were removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~200 mL) and washed with water (40 mL), sat. aq. NaHCO₃ (40 mL), and water (2 x 40 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid (1.89 g, 87%). Analytical data for **2.6**: $R_f = 0.58$ (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{21} 85.2$ (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.49 (d, 3H, C-6), 1.71-1.78 (m, 2H, CH₂), 2.13-2.21 (m, 2H, CH₂), 3.38-3.43 (m, 1H, H-2), 3.51-3.56 (m, 1H, OCH₂^a), 3.85-3.97 (m, 2H, H-5, OCH₂^b), 3.98-4.06 (m, 2H, C-3, 4), 4.24 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1), 4.95-5.10 (m, 2H, CH=CH₂), 5.77 (m, 1H, CH=CH₂), 5.94 (s, 1H, CHPh), 7.39-7.41 (m, 3H, aromatic), 7.42-7.54 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.6, 28.7, 30.1, 65.5, 68.8, 69.3, 76.8, 78.0, 101.8, 104.5, 115.1, 126.6 (x 2), 128.5 (x 2), 129.5, 137.1, 138.0 ppm; HR-FAB MS [M+Na]⁺ calcd for C₁₈H₂₃O₄NaN₃ 368.1586, found 368.1582.

4-Pentenyl 2-azido-4-O-benzyl-2,6-dideoxy- β -L-galactopyranoside (2.7). A solution of compound **2.6** (400 mg, 1.16 mmol) and activated molecular sieves (3Å, 800 mg) in THF (10 mL) was stirred under argon for 1 h at rt. NaCNBH₃ (876 mg, 13.9 mmol) was added in one portion, 2M HCl in diethyl ether (17 mL, 17.4 mmol) was added dropwise, and the resulting mixture was kept for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH₂Cl₂. The combined filtrate (~100 mL) was washed with water (20 mL), sat. aq. NaHCO₃ (20 mL), and water (2 x 20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a clear syrup (376 mg, 94%). Analytical data for **2.7**: R_f = 0.48 (ethyl acetate/hexane, 3/7, v/v); [α]_D²¹ 48.7 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.22 (d, 3H, C-6), 1.65 (m, 2H, CH₂), 2.17 (m, 2H, CH₂), 2.55 (d, 1H, J_{3,OH} = 7.0 Hz, OH), 3.40-3.60 (m, 5H, H-2, 3, 4, 5, OCH₂^a), 3.94-4.00 (m, 1H, OCH₂^b), 4.21 (d, 1H, J_{1,2} = 7.6 Hz, H-1), 4.79 (dd, 2H, ²J = 11.6 Hz, CH₂Ph), 4.87-4.99 (m, 2H, CH=CH₂), 5.85 (m, 1H, CH=CH₂), 7.30-7.50 (m, 5H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 16.9, 28.8, 30.1, 64.7, 69.3, 70.8, 72.9, 75.8, 78.4, 102.3, 115.0, 128.0, 128.2 (x 2), 128.5 (x 2), 138.0, 138.1 ppm; HR-FAB MS [M+Na]⁺ calcd for C₁₈H₂₅O₄NaN₃ 370.1743, found 370.1750.

Methyl 2-azido-2,6-dideoxy- α -D-galactopyranoside (2.12a) and Methyl 2-azido-2,6-dideoxy- β -D-galactopyranoside (2.12b). Ceric ammonium nitrate (7.72 g, 14.08 mmol) and sodium azide (686 mg, 10.61 mmol) were added to a solution of 3,4-di-O-acetyl-1,5-

anhydro-D-*lyxo*-hex-1-enitol¹⁶⁻¹⁷ (**2.11**, 942 mg, 4.4 mmol) in anhydrous acetonitrile (30 mL) and the resulting mixture was stirred for 3 h at -15 °C. After that, the volatiles were removed under the reduced pressure at rt. The residue was dissolved in a mixture of diethyl ether and ethyl acetate (1/1, v/v, 120 mL) and washed with water (30 mL). The organic phase was separated and the aqueous layer was extracted with a mixture of diethyl ether and ethyl acetate (1/1, v/v, 3 x 50 mL). The combined organic extract was dried with MgSO₄, concentrated *in vacuo*, and the residue was dried *in vacuo* for 3 h. The crude residue was dissolved in methanol (~10 mL), sodium (194 mg, 8.42 mmol) was added portionwise, and the resulting mixture was kept for 30 min at 0 °C. After that, the reaction mixture was neutralized with Dowex (H⁺), the resin was filtered off, rinsed successively with MeOH, and the combined filtrate (~60 mL) was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane graduate elution) to yield **2.12a** (328 mg, 37%) and **2.12b** (315 mg, 35%) as white amorphous solids. Analytical data for **2.12a**: R_f = 0.48 (ethyl acetate/hexane, 7/3, v/v); [α]_D³⁰ 146.0 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.27 (d, 3H, C-6), 3.39 (s, 3H, OCH₃), 3.48-3.53 (m, 2H, J_{2,3} = 10.4 Hz, H-2, OH), 3.78 (br. s, 1H, H-4), 3.85 (br. s, 1H, OH), 3.94 (m, 1H, H-5), 3.98 (m, 1H, H-3), 4.77 (d, 1H, J_{1,2} = 3.4 Hz, H-1) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.2, 55.5, 60.2, 65.8, 69.0, 72.1, 99.1 ppm; HR-FAB MS [M+Na]⁺ calcd for C₇H₁₃NaO₄N₃ 226.0804, found 226.0810. Analytical data for **2.12b**: R_f = 0.46 (ethyl acetate/hexane, 7/3, v/v); [α]_D³⁰ 27.3 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.32 (d, 3H, C-6), 3.40-3.59 (m, 4H, H-2, 3, 5, OH), 3.54 (s, 3H, OCH₃), 3.68-3.72 (m, 1H, H-4), 3.88 (d, 1H, J_{3,OH} = 6.2 Hz, OH), 4.13 (d, 1H, J_{1,2} = 7.4

Hz, H-1) ppm; ^{13}C NMR (75 MHz, CDCl_3): δ , 16.3, 57.2, 65.8, 70.7, 71.1, 72.8, 103.3 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_7\text{H}_{13}\text{NaO}_4\text{N}_3$ 226.0804, found 226.0819.

Methyl 2-azido-3,4-O-benzylidene-2,6-deoxy- α -D-galactopyranoside (2.13).

Dimethoxytoluene (0.45 mL, 2.96 mmol) and camphorsulfonic acid (17 mg, 0.07 mmol) were added to a solution of compound **2.12a** (300 mg, 1.48 mmol) in acetonitrile (20 mL) and the resulting mixture was stirred under argon for 1 h at rt. After that, triethylamine (~0.3 mL) was added and the volatiles were removed *in vacuo*. The residue was diluted with CH_2Cl_2 (~200 mL) and washed with water (40 mL), sat. aq. NaHCO_3 (40 mL), and water (2 x 40 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 yield the title compound as a clear syrup (336 mg, 78%). Analytical data for **2.13**: R_f = 0.61 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{26}$ 225.9 (c = 1, CHCl_3); ^1H NMR (600 MHz, CDCl_3): δ , 1.47 (d, 3H, C-6), 3.41 (dd, 1H, $J_{2,3}$ = 8.4 Hz, H-2), 3.45 (s, 3H, OCH_3), 4.17 (dd, 1H, $J_{4,5}$ = 2.5 Hz, H-4), 4.19-4.22 (m, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 4.51 (dd, 1H, $J_{3,4}$ = 5.6 Hz, H-3), 4.73 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 6.00 (s, 1H, CHPh), 7.40-7.55 (m, 5H, aromatic) ppm; ^{13}C NMR (150 MHz, CDCl_3): δ , 18.9, 58.2, 64.3, 65.8, 76.2, 80.7, 101.5, 106.6, 128.9 (x 2), 131.1 (x 2), 131.9, 140.2 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{14}\text{H}_{17}\text{O}_4\text{NaN}_3$ 314.1117, found 314.1119.

Methyl 2-azido-4-O-benzyl-2,6-dideoxy- α -D-galactopyranoside (2.14). A solution of compound **2.13** (114 mg, 0.33 mmol) and activated molecular sieves (3Å, 500 mg) in THF (10 mL) was stirred under argon for 1 h at rt. NaCNBH_3 (328 mg, 5.2 mmol) was

added in one portion, then 2M HCl in diethyl ether (2.6 mL, 5.2 mmol) was added dropwise and the resulting mixture was stirred under argon for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH₂Cl₂. The combined filtrate (~100 mL) was washed with water (20 mL), sat. aq. NaHCO₃ (20 mL), and water (2 x 20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid (103 mg, 90%). Analytical data for **2.14**: R_f = 0.53 (ethyl acetate/hexane, 2/3, v/v); [α]_D²⁶ 226.1 (c = 1, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ, 1.29 (d, 3H, C-6), 2.16 (dd, 1H, J_{3,OH} = 6.2 Hz, OH), 3.39 (s, 3H, OCH₃), 3.49 (dd, 1H, J_{2,3} = 10.6 Hz, H-2), 3.65 (d, 1H, J_{4,5} = 3.1 Hz, H-4), 3.94 (dd, 1H, J_{5,6} = 6.7 Hz, H-5), 4.03 (dd, 1H, J_{3,4} = 3.2 Hz, H-3), 4.75 (dd, 2H, ²J = 11.5 Hz, CH₂Ph), 4.76 (d, 1H, J_{1,2} = 3.5 Hz, H-1), 7.31-7.37 (m, 5H, aromatic), ppm; ¹³C NMR (150 MHz, CDCl₃): δ, 19.4, 58.1, 63.9, 68.9, 71.7, 78.8, 82.7, 101.7, 130.8 (x 2), 130.9, 131.3 (x 2), 140.4 ppm; HR-FAB MS [M+Na]⁺ calcd for C₁₄H₁₉O₄NaN₃ 316.1273, found 316.1271.

2.4.3 Synthesis and modification of oligosaccharides

4-Pentenyl O-(4,6-O-benzylidene-2-O-levulinoyl-3-O-methyl-β-D-glucopyranosyl)-(1→3)-2-azido-4-O-benzyl-2,6-dideoxy-β-L-galactopyranoside (2.8). A mixture of donor **2.3** (1.76 g, 3.43 mmol), acceptor **2.7** (1.08 g, 3.12 mmol), and freshly activated molecular sieves (3Å, 6.0 g) in ClCH₂CH₂Cl (70 mL) was stirred under argon for 2 h at rt. Freshly conditioned AgOTf (1.6 g, 6.24 mmol) was added and the resulting mixture was stirred under argon for 1 h at rt. After that, the solids were filtered-off and rinsed

successively with CH_2Cl_2 . The combined filtrate (~150 mL) was washed with sat. aq. NaHCO_3 (40 mL) and water (2 x 40 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 as a white foam (1.29 g, 73%). The yield calculation is based on acceptor **2.7** recovered (218 mg, 0.63 mmol). Analytical data for **2.8**: $R_f = 0.50$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{20} -104.2$ (c = 1, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.18 (d, 3H, $J_{5,6} = 6.3$ Hz, C-6), 1.76 (m, 2H, CH_2), 2.11 (s, 3H, COCH_3), 2.16 (m, 2H, CH_2), 2.50-2.66 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CO}$), 3.47-3.56 (m, 4H, H-3', 5, 5', OCH_2^a), 3.58 (s, 3H, OCH_3), 3.65-3.92 (m, 6H, H-2, 3, 4, 4', 6a', OCH_2^b), 4.17 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1), 4.45 (dd, 1H, $J_{5',6b'} = 4.9$ Hz, $J_{6a',6b'} = 10.5$ Hz, H-6b'), 4.75 (dd, 2H, $^2J = 11.6$ Hz, CH_2Ph), 4.86 (d, 1H, $J_{1',2'} = 7.4$ Hz, H-1'), 4.97-5.07 (m, 3H, H-2', $\text{CH}=\text{CH}_2$), 5.59 (s, 1H, CHPh), 5.84 (m, 1H, $\text{CH}=\text{CH}_2$), 7.25-7.60 (m, 10H, aromatic) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ , 16.8, 27.9, 28.9, 30.0, 30.2, 37.8, 60.5, 63.4, 66.5, 68.8, 69.5, 70.5, 73.9, 74.9, 75.6, 80.6, 80.9, 81.2, 100.3, 101.4, 102.4, 115.0, 126.2 (x 2), 127.7, 128.2 (x 2), 128.3 (x 2), 128.4 (x 2), 128.6, 129.2, 138.4, 138.6, 171.4, 206.5 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{37}\text{H}_{47}\text{NaO}_{11}\text{N}_3$ 732.3108, found 732.3104.

4-Pentenyl O-(4,6-O-benzylidene-3-O-methyl- β -D-glucopyranosyl)-(1 \rightarrow 3)-2-azido-4-O-benzyl-2,6-dideoxy- β -L-galactopyranoside (2.9). Hydrazine acetate (214 mg, 2.37 mmol) was added to a solution of disaccharide **2.8** (874 mg, 1.23 mmol) in CH_2Cl_2 /methanol (40 mL, 20/1, v/v) and the resulting mixture was stirred for 3 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~100 mL) and washed with sat.

aq. NaHCO₃ (20 mL) and water (2 x 20 mL). The organic phase was separated, dried with MgSO₄, 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 foam (528 mg, 72%). Analytical data for **2.9**: R_f = 0.58 (ethyl acetate/hexane, 2/3, v/v); [α]_D²⁰ -94.4 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.19 (d, 3H, J_{5,6} = 6.4 Hz, C-6), 1.73 (m, 2H, CH₂), 2.15 (m, 2H, CH₂), 2.97 (d, 1H, J_{2,OH} = 2.6 Hz, OH), 3.40-3.51 (m, 4H, H-3', 5, 5', OCH₂^a), 3.58-3.65 (m, 4H, H-2', 3, 4, 4'), 3.68 (s, 3H, OCH₃), 3.79 (dd, 1H, J_{2,3} = 7.8 Hz, H-2), 3.82 (dd, 1H, J_{5',6a'} = 4.9 Hz, J_{6a',6b'} = 10.5 Hz, H-6a'), 3.93 (m, 1H, OCH₂^b), 4.22 (d, 1H, J_{1,2} = 7.8 Hz, H-1), 4.37 (dd, 1H, J_{5',6b'} = 4.9 Hz, H-6b'), 4.64 (d, 1H, J_{1',2'} = 7.8 Hz, H-1'), 4.90 (dd, 2H, ²J = 11.7 Hz, CH₂Ph), 5.01-5.10 (m, 2H, CH=CH₂), 5.57 (s, 1H, CHPh), 5.80-5.90 (m, 1H, CH=CH₂), 7.26-7.50 (m, 10H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.9, 28.9, 30.3, 61.2, 62.1, 67.0, 68.8, 69.5, 70.6, 72.9, 74.9, 75.8, 81.4, 82.4, 101.3, 101.5, 102.3, 115.0, 126.2 (x 2), 127.9, 128.4, 128.5 (x 4), 128.6 (x 2), 129.3, 137.3, 138.3, 138.4 ppm; HR-FAB MS [M+Na]⁺ calcd for C₃₂H₄₁NaO₉N₃ 634.2770, found 634.2755.

4-Pentenyl ***O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-β-L-galactopyranoside**

(2.10). Pyridine (0.5 mL) was added to a solution of disaccharide **2.9** (150 mg, 0.23 mmol) in CH₂Cl₂ (5.0 mL) and the resulting mixture was cooled to 0 °C. Trifluoromethanesulfonic anhydride (100 μL, 0.59 mmol) was added dropwise and the reaction mixture was kept for 3 h at 0 °C. After that, the reaction was quenched with ice-cold water (~5 mL), diluted with CH₂Cl₂ (~100 mL), and washed with cold sat. aq.

NaHCO₃ (20 mL) and cold water (2 x 20 mL). The organic phase was separated, dried over MgSO₄, and concentrated *in vacuo*. The crude residue was dissolved in DMF (2.0 mL), NaN₃ (96 mg, 1.47 mmol) was added, and the resulting suspension was stirred for 16 h at 60 °C. Upon completion, ethyl acetate (~50 mL) and water (~50 mL) were added. Organic layer was separated and washed with sat. aq. NaCl (2 x 15 mL). The combined aqueous phase was extracted with ethyl acetate (3 x 30 mL). The combined organic extract was dried with MgSO₄ 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 foam (135 mg, 90% over 2 steps). Analytical data for **2.10**: R_f = 0.3 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]_D^{20}$ -90.9 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.36 (d, 3H, $J_{5,6}$ = 6.4 Hz, C-6), 1.75 (m, 2H, CH₂), 2.16 (m, 2H, CH₂), 3.22 (m, 1H, H-5'), 3.29 (dd, 1H, $J_{3',4'}$ = 3.7 Hz, H-3'), 3.47-3.62 (m, 4H, $J_{2',3'}$ = 9.6 Hz, H-2', 4, 5, OCH₂^a), 3.53 (s, 3H, OCH₃), 3.66-3.77 (m, 2H, H-2, 3), 3.85-3.99 (m, 3H, H-4', 6a', OCH₂^b), 4.21 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1), 4.29 (dd, 1H, $J_{5',6b'}$ = 4.9 Hz, $J_{6a',6b'}$ = 10.5 Hz, H-6b'), 4.45 (s, 1H, H-1'), 4.77 (dd, 2H, ² J = 12.2 Hz, CH₂Ph), 4.95-5.07 (m, 2H, CH=CH₂), 5.55 (s, 1H, CHPh), 5.83 (m, 1H, CH=CH₂), 7.33-7.48 (m, 10H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 17.2, 28.7, 30.1, 59.1, 62.2, 67.6, 68.3, 69.4, 70.4, 75.3, 75.7, 77.1, 77.2, 78.3, 78.7, 97.0, 101.7, 102.3, 115.0, 126.1 (x 2), 128.2, 128.4 (x 2), 128.5 (x 2), 128.6 (x 2), 129.2, 137.2, 138.2, 138.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for C₃₂H₄₀NaO₈N₆ 659.2805, found 659.2810.

Methyl O-(2-azido-4,6-O-benzylidene-2-deoxy-3-O-methyl-β-D-mannopyranosyl)-(1→3)-O-(2-azido-4-O-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-azido-4-

O-benzyl-2,6-dideoxy- α -D-galactopyranoside (2.15). A mixture of donor **2.10** (336 g, 0.53 mmol), acceptor **2.14** (186 mg, 0.63 mmol), and freshly activated molecular sieves (4Å, 1.0 g) in $\text{ClCH}_2\text{CH}_2\text{Cl}$ (20 mL) was stirred under argon for 2 h at rt. The resulting mixture was cooled to 0 °C, *N*-iodosuccinimide (237 mg, 1.05 mmol) and trifluoromethanesulfonic acid (17 mg, 0.11 mmol) were added, and the resulting mixture was stirred under argon for 2 h at 0 °C. After that, the solids were filtered-off and rinsed successively with CH_2Cl_2 . The combined filtrate (~100 mL) was washed with sat. aq. NaHCO_3 (40 mL) and water (2 x 40 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 as a white amorphous solid (282 mg, 87%). The yield calculation is based on acceptor **2.14** recovered (73 mg, 0.25 mmol). Analytical data for **2.15**: $R_f = 0.47$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{26} -86.6$ (c = 1, CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3): δ , 1.25 (2d, 6H, C-6, 6'), 3.25 (m, 1H, H-5''), 3.38 (dd, 1H, $J_{3'',4''} = 9.6$ Hz, H-3''), 3.40, 3.55 (2s, 6H, 2 x OCH_3), 3.58 (dd, 1H, $J_{4,5} = 1.0$ Hz, H-4), 3.64 (dd, 1H, $J_{4',5'} = 1.0$ Hz, H-4'), 3.72 (dd, 1H, $J_{2'',3''} = 3.2$ Hz, H-2''), 3.75 (dd, 1H, $J_{2',3'} = 10.7$ Hz, H-2'), 3.81 (dd, 1H, $J_{5'',6a''} = 4.8$ Hz, $J_{6a'',6b''} = 9.6$ Hz, H-6a''), 3.91-3.99 (m, 4H, H-2, 4'', 5, 5'), 4.07 (dd, 1H, $J_{3,4} = 2.4$ Hz, H-3), 4.15 (dd, 1H, $J_{5'',6b''} = 4.9$ Hz, H-6b''), 4.22 (dd, 1H, $J_{3',4'} = 2.7$ Hz, H-3'), 4.61 (br. s, 1H, H-1''), 4.74 (dd, 2H, $^2J = 14.8$ Hz, CH_2Ph), 4.76 (dd, 2H, $^2J = 11.3$ Hz, CH_2Ph), 4.83-4.86 (m, 2H, $J_{1,2} = 3.5$ Hz, H-1), 5.26 (d, 1H, $J_{1',2'} = 3.5$ Hz, H-1'), 5.54 (s, 1H, CHPh), 7.31-7.51 (m, 15H, aromatic) ppm; $^{13}\text{C NMR}$ (150 MHz, CDCl_3): δ , 16.8, 16.9, 55.5, 58.5, 59.3, 60.8, 62.6, 66.8, 67.6, 67.8, 68.4, 74.9, 75.4, 76.0, 76.8, 77.0, 78.4, 78.9, 79.8, 98.1, 99.1, 99.9, 101.7, 126.2 (x 2), 127.9 (x 2), 128.0, 128.3, 128.4 (x 2), 128.5 (x 2),

128.6 (x 2), 128.7 (x 2), 129.2, 137.3, 138.0, 138.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{41}H_{49}NaO_{11}N_9$ 866.3449, found 866.3440.

Methyl ***O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (2.16).**

Water (9.0 mL), triethylamine (2.0 mL, 14.2 mmol) and 1,3-propanedithiol (20 mL, 19.2 mmol) were added to a solution of trisaccharide **2.15** (270 mg, 0.32 mmol) in pyridine (35.0 mL) and the resulting mixture was kept for 6 h at rt. After that, the reaction mixture was concentrated and dried *in vacuo*. The crude residue was dissolved in MeOH (3.0 mL), Ac_2O (0.4 mL, 3.84 mmol) was added, and the resulting suspension was stirred under argon for 16 h at rt. Upon completion, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white foam (268 mg, 94% over 2 steps). Analytical data for **2.16**: $R_f = 0.77$ (methanol/dichloromethane, 1/4, v/v); $[\alpha]_D^{26} -20.1$ (c = 1, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$): δ , 1.19 (d, 3H, $J_{5,6} = 6.5$ Hz, C-6), 1.29 (d, 3H, $J_{5',6'} = 6.5$ Hz, C-6'), 1.95, 1.99, 2.10 (3s, 9H, 3 x $COCH_3$), 3.27 (m, 1H, H-5''), 3.36 (s, 3H, OCH_3), 3.41 (dd, 1H, $J_{2'',3''} = 4.6$ Hz, H-3''), 3.46-3.48 (m, 2H, H-4, 4'), 3.49 (s, 3H, OCH_3), 3.59 (dd, 1H, $J_{3'',4''} = 9.7$ Hz, H-4''), 3.63 (dd, 1H, $J_{2,3} = 10.7$ Hz, $J_{3,4} = 2.3$ Hz, H-3), 3.68 (dd, 1H, $J_{6a'',6b''} = 10.3$ Hz, H-6a''), 3.82 (m, 1H, C-5), 3.86 (dd, 1H, $J_{2',3'} = 10.8$ Hz, $J_{3',4'} = 2.5$ Hz, C-3'), 3.93 (m, 1H, C-5'), 4.20 (dd, 1H, $J_{5'',6b''} = 4.9$ Hz, H-6b''), , 4.54 (d, 1H, $J_{1'',2''} = 1.6$ Hz, H-1''), 4.61 (dd, 1H, $J_{1',2'} = 3.7$ Hz, H-2'), 4.62 (dd, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.66 (dd, 2H, $^2J =$

11.4 Hz, CH_2Ph) 4.73 (dd, 1H, H-2''), 4.75-4.83 (m, 4H, $^2J = 11.7$ Hz, H-1', 2, CH_2Ph), 5.52 (s, 1H, $CHPh$), 5.74 (d, 1H, $J_{2,NH} = 9.6$ Hz, 2-NH), 5.90 (d, 1H, $J_{2'',NH} = 8.6$ Hz, 2''-NH), 6.03 (d, 1H, $J_{2',NH} = 9.6$ Hz, 2'-NH), 7.25-7.51 (m, 15H, aromatic) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ , 16.9, 17.1, 23.4, 23.8, 24.2, 47.8, 48.9, 50.6, 55.3, 58.5, 67.0, 67.1, 67.8, 68.8, 75.2, 75.5, 76.4, 77.4, 78.7, 78.8, 79.3, 80.1, 98.8, 99.1, 100.6, 102.0, 126.3 (x 2), 127.3 (x 2), 127.7, 128.0, 128.1 (x 2), 128.4 (x 2), 128.5 (x 2), 128.7 (x 2), 129.3, 170.9, 171.2, 172.0 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{47}H_{61}NaO_{14}N_3$ 914.4051, found 914.4041.

Methyl *O*-(2-acetamido-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (2.17). Trifluoroacetic acid (2.0 mL) was added to a solution of trisaccharide **2.16** (203.0 mg, 0.2 mmol) in CH_2Cl_2 (20.0 mL) and water (50 μ L) and the resulting mixture was stirred for 16 h at rt. After that, the reaction mixture was neutralized with triethylamine (~5.0 mL) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (169 mg, 92%). Analytical data for **2.17**: $R_f = 0.54$ (methanol/ CH_2Cl_2 , 1/4, v/v); $[\alpha]_D^{26} -13.9$ (c = 1, $CHCl_3$); 1H NMR (600 MHz, CD_3OD): δ , 1.21-1.24 (2d, 6H, C-6,6'), 1.92 (2s, 6H, 2 x $COCH_3$), 2.04 (s, H, $COCH_3$), 3.16 (dd, 1H, $J_{5'',6a''} = 6.6$ Hz, $J_{5'',6b''} = 3.1$ Hz, H-5''), 3.23 (dd, 1H, $J_{2'',3''} = 4.2$ Hz, H-3''), 3.30, 3.34, 3.42 (3s, 9H, 3 x OCH_3), 3.53, (dd, 1H, $J_{3'',4''} = 9.7$ Hz, H-4''), 3.62 (dd, 1H, H-4), 3.67 (dd, 1H, $J_{6a'',6b''} = 11.4$ Hz, H-6a''), 3.78 (dd, 1H, H-6b''), 3.81 (dd, 1H, H-4'), 3.95-4.03 (m, 4H, H-3, 3', 5, 5'), 4.41

(dd, 1H, , $J_{2',3'} = 11.3$ Hz, H-2'), 4.53 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.62 (dd, 1H, $J_{2,3} = 11.1$ Hz, H-2), 4.71 (dd, 1H, H-2''), 4.73 (d, 1H, $J_{1'',2''} = 2.7$ Hz, H-1''), 4.75 (dd, 2H, $^2J = 11.0$ Hz, CH_2Ph), 4.86 (dd, 2H, $^2J = 11.7$ Hz, CH_2Ph), 5.02 (d, 1H, $J_{1',2'} = 3.7$ Hz, H-1'), 7.25-7.47 (m, 10H, aromatic) ppm; ^{13}C NMR (150 MHz, CD_3OD): δ , 17.2, 17.3, 22.8, 23.0, 23.6, 49.3, 49.8, 50.7, 50.8, 55.7, 58.0, 61.5, 66.8, 68.2, 68.9, 75.4, 76.4, 76.6, 78.1, 78.2, 78.6, 81.0, 83.7, 99.8, 100.4, 100.7, 128.7, 128.9, 129.0 (x 2), 129.3 (x 2), 129.5 (x 2), 129.6 (x 2), 140.4, 173.7, 173.8, 173.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{40}H_{57}NaO_{14}N_3$ 826.3738, found 826.3706.

Methyl *O*-(benzyl 2-acetamido-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl uronate)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (2.18). Water (0.5 mL), 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, 0.7 mg, 0.01 mmol) and diacetoxy iodobenzene (16.1 mg, 0.05 mmol) were added to a solution of trisaccharide **2.17** (18 mg, 0.02 mmol) in CH_2Cl_2 (1.0 mL) and the resulting mixture was stirred for 4 h at rt. After that, the reaction mixture was passed through a pad of silica gel, concentrated *in vacuo*, and dried. The crude residue was dissolved in DMF (2.0 mL), benzyl bromide (0.01 mL, 0.15 mmol) and $NaHCO_3$ (7.6 mg, 0.09 mmol) were added, and the resulting suspension was stirred under argon for 16 h at rt. After that, the reaction was the reaction was quenched with ice-cold water (~5 mL) and extracted with ethyl acetate (3 x 10 mL). The organic extract was washed with brine (2 x 10 mL). The organic extract was dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound

as a white amorphous solid (10.1 mg, 58% over 2 steps). Analytical data for **2.18**: $R_f = 0.71$ (methanol/dichloromethane, 1/4, v/v); $[\alpha]_D^{27} -27.3$ ($c = 1$, CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3): δ , 1.18 (d, 3H, $J_{5',6'} = 6.5$ Hz, H-6'), 1.30 (d, 3H, $J_{5,6} = 6.5$ Hz, H-6), 1.96, 1.97, 2.04 (3s, 9H, 3 x COCH_3), 3.09 (d, 1H, $J_{4'',\text{OH}} = 5.8$ Hz, 4''-OH), 3.20 (dd, 1H, $J_{2'',3''} = 4.1$ Hz, H-3''), 3.37 (s, 3H, OCH_3), 3.42 (dd, 1H, H-4'), 3.46 (dd, 1H, H-4) 3.47 (s, 3H, OCH_3), 3.71-3.75 (m, 2H, H-3', 5'), 3.80 (d, 1H, $J_{4'',5''} = 9.2$ Hz, H-5''), 3.81-3.87 (m, 2H, $J_{3'',4''} = 8.9$ Hz, H-3, 4''), 3.92 (dd, 1H, H-5), 4.58 (d, 1H, $J_{1'',2''} = 1.8$ Hz H-1''), 4.59 (dd, 1H, $J_{2',3'} = 10.2$ Hz, H-2'), 4.63 (dd, 2H, $^2J = 11.4$ Hz, CH_2Ph), 4.65 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.67 (dd, 1H, H-2''), 4.75 (br. s., 2H, CH_2Ph), 4.77 (dd, 1H, $J_{2,3} = 10.3$ Hz, H-2), 4.85 (d, 1H, $J_{1',2'} = 3.7$ Hz, H-1'), 5.33 (dd, 2H, $^2J = 12.0$ Hz, CH_2Ph), 5.77 (d, 1H, $J_{2,\text{NH}} = 9.7$ Hz, 2-NH), 6.09 (d, 1H, $J_{2'',\text{NH}} = 8.4$ Hz, 2''-NH), 6.19 (d, 1H, $J_{2',\text{NH}} = 9.5$ Hz, 2'-NH), 7.21-7.45 (m, 15H, aromatic) ppm; $^{13}\text{C NMR}$ (150 MHz, CDCl_3): δ , 16.9, 17.3, 23.3, 23.7, 23.8, 47.9, 48.9, 49.0, 55.3, 58.2, 67.2, 67.6, 67.9, 68.1, 74.9, 75.2, 75.3, 76.8, 77.3, 78.7, 80.6, 81.4, 98.1, 99.2, 100.7, 127.5 (x 2), 127.6, 127.7, 128.1 (x 2), 128.4 (x 2), 128.6 (x 2), 128.8, 128.9 (x 2), 129.2 (x2), 135.2, 138.5, 138.9, 168.7, 170.7, 171.9, 172.0 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{47}\text{H}_{61}\text{NaO}_{15}\text{N}_3$ 930.4000, found 930.4014.

Methyl *O*-(benzyl 2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl- β -D-mannopyranosyluronate)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (2.19). Ac_2O (0.3 mL) was added to a solution of trisaccharide **2.18** (10 mg, 0.01 mmol) in pyridine (2.0 mL) and the resulting mixture was stirred under argon for 16 h. After that, the reaction was quenched with MeOH (~3.0 mL), the volatiles were removed *in vacuo*, and the

residue was co-evaporated with toluene (3 x 3 mL) The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (12 mg, 99%). Analytical data for **2.19**: $R_f = 0.61$ (methanol/dichloromethane, 3/17, v/v); $[\alpha]_D^{25} -27.9$ (c = 1, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.14-1.21 (2d, 6H, H-6, 6'), 1.73, 1.90, 1.92, 1.97 (4s, 12H, 4 x CH_3CO), 3.18 (s, 3H, OCH_3), 3.27 (dd, 1H, H-3''), 3.28 (s, 3H, OCH_3), 3.43 (dd, 1H, H-4), 3.65 (dd, 1H, H-4'), 3.82-3.87 (m, 3H, H-3, 5, 5'), 4.03 (d, 1H, $J_{4'',5''} = 4.7$ Hz, H-5''), 4.32 (dd, 1H, $J_{3',4'} = 9.4$ Hz, H-3'), 4.56-4.69 (m, 3H, H-1, 2', 2''), 4.67 (dd, 2H, $^2J = 12.0$ Hz, CH_2Ph), 4.87 (dd, 1H, $J_{1,2} = 3.6$ Hz, H-2), 4.89 (dd, 2H, $^2J = 12.1$ Hz, CH_2Ph), 4.95-4.98 (2d, 2H, H-1', 1''), 5.01 (br. s., 2H, CH_2Ph), 5.35 (dd, 1H, $J_{3'',4''} = 5.7$ Hz, H-4''), 5.67 (d, 1H, $J_{2,\text{NH}} = 9.7$ Hz, 2-NH), 6.28 (d, 1H, $J_{2'',\text{NH}} = 9.3$ Hz, 2''-NH), 6.47 (d, 1H, $J_{2',\text{NH}} = 9.3$ Hz, 2'-NH), 7.22-7.38 (m, 15H, aromatic) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ , 17.2, 17.5, 21.1, 23.1, 23.7, 23.8, 46.2, 47.9, 49.0, 55.3, 58.9, 67.3, 67.5, 67.7, 68.1, 71.7, 74.4, 74.6, 75.5, 76.6, 76.8, 77.2, 77.4, 79.6, 93.2, 99.3, 100.3, 127.3 (x 2), 127.5, 127.6 (x 2), 128.4 (x 2), 128.5 (x 2), 128.7, 128.8 (x 2), 129.0 (x 2), 135.1, 138.4, 139.2, 167.8, 170.0, 170.6, 171.1, 172.0 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{49}\text{H}_{63}\text{NaO}_{16}\text{N}_3$ 972.4106, found 972.4089.

Methyl *O*-(2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl- β -D-mannopyranosyluronic acid)-(1 \rightarrow 3)-*O*-(2-acetamido-2,6-dideoxy- α -L-fucopyranosyl)-(1 \rightarrow 3)-2-acetamido-2,6-dideoxy- α -D-galactopyranoside (T1). 10% Pd on activated charcoal (200 mg) was added to a solution of trisaccharide **2.19** (50 mg, 0.044 mmol) in 90% aq. ethanol (5.0 mL) and the resulting suspension was vigorously stirred under H_2 atmosphere for 24 h at

rt. After that, the solids were filtered off and rinsed successively with methanol. The combined filtrate (~35 mL) was concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (dichloromethane - methanol gradient elution) to afford the title compound as a white amorphous solid (35 mg, 98% yield). Analytical data for **1**: $R_f = 0.18$ (methanol/dichloromethane, 3/7, v/v); $[\alpha]_D^{27} -40.0$ (c = 1, CH₃OH); ¹H NMR (300 MHz, CD₃OD): δ , 1.21-1.24 (2d, 6H, H-6, 6'), 1.91, 2.00, 2.04, 2.05 (4s, 12H, 4 x CH₃CO), 3.33 (s, 3H, OCH₃), 3.36 (s, 3H, OCH₃), 3.51 (dd, 1H, $J_{3'',4''} = 9.8$ Hz, H-3''), 3.64 (dd, 1H, H-4), 3.70 (d, 1H, H-5''), 3.83 (dd, 1H, $J_{3,4} = 2.9$ Hz, H-3), 3.88 (dd, 1H $J_{3',4'} = 2.7$ Hz, H-4'), 3.92 (dd, 1H, $J_{5,6} = 6.6$ Hz, H-5), 4.02 (dd, 1H, $J_{5',6'} = 6.5$ Hz, H-5'), 4.07 (dd, 1H, $J_{2',3'} = 11.2$ Hz, H-3'), 4.27 (dd, 1H, $J_{1',2'} = 3.9$ Hz, H-2'), 4.46 (dd, 1H, $J_{2,3} = 11.0$ Hz, H-2), 4.53 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.69 (d, 1H, $J_{2'',3''} = 3.9$ Hz, H-2''), 4.81 (s, 1H, H-1''), 4.96 (d, 1H, H-1'), 5.12 (dd, 1H, $J_{4'',5''} = 10.1$ Hz, H-4'') ppm; ¹³C NMR (150 MHz, CD₃OD): δ , 16.8, 17.0, 21.1, 22.8, 22.9, 23.5, 49.3, 50.0, 50.7, 55.7, 57.7, 67.5, 67.8, 69.1, 70.3, 72.8, 74.6, 75.8, 76.5, 81.0, 96.3, 100.4, 100.7, 171.9, 173.4, 173.8, 174.7, 175.9 ppm; HR-FAB MS $[M-H+2Na]^+$ calcd for C₂₈H₄₄O₁₆N₃Na₂ 724.2517, found 724.2524.

2.5 References

1. Ala'Aldeen, D. A. A.; Hiramatsu, K., *Staphylococcus Aureus: Molecular and Clinical Aspects*. Horwood Publishing Limited Published by Elsevier Ltd.: 2004.
2. del Rio, A.; Cervera, C.; Moreno, A.; Moreillon, P.; Miró, J. M., Patients at Risk of Complications of Staphylococcus aureus Bloodstream Infection *Clin. Infect. Dis.* **2009**, *48 (Suppl. 4)*, S246-S253.

3. Jansen, K. U.; Girgenti, D. Q.; Scully, I. L.; Anderson, A. S., Vaccine review: "Staphylococcus aureus vaccines: problems and prospects". *Vaccine* **2013**, *31*, 2723-2730.
4. Jones, C., Revised structures for the capsular polysaccharides from *Staphylococcus aureus* types 5 and 8, components of novel glycoconjugate vaccines. *Carbohydr. Res.* **2005**, *340*, 1097-1106.
5. Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R., Synthesis of Staphylococcus aureus type 5 capsular polysaccharide repeating unit using novel L-FucNAc and D-FucNAc synthons and immunochemical evaluation. *Bioorg. Med. Chem.* **2012**, *20*, 6403-6415.
6. Gagarinov, I. A.; Fang, T.; Liu, L.; Srivastava, A. D.; Boons, G. J., Synthesis of Staphylococcus aureus Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization. *Org. Lett.* **2015**, *17*, 928-931.
7. Freese, S. J.; Anderson, A.; Pavliak, V.; Jansen, K. U.; Dodge, I. L.; Scott, T. D.; Nanra, J. S.; Prasad, K.; Green, B. A. Compositions And Methods For Preparing Staphylococcus Aureus Serotype 5 And 8 Capsular Polysaccharide Conjugate Immunogenic Compositions. WO 2011/041003 A2, 2011.
8. Nigudkar, S. S.; Demchenko, A. V., Stereocontrolled 1,2-cis glycosylation as the driving force of progress in synthetic carbohydrate chemistry. *Chem. Sci.* **2015**, *6*, 2687-2704.
9. 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*, 667-690.

10. Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A., Equatorial Anomeric Triflates from Mannuronic Acid Esters. *J. Am. Chem. Soc.* **2009**, *131*, 12080-12081.
11. Petitou, M.; Imberty, A.; Duchaussoy, P.; Driguez, P.-A.; Ceccato, M.-L.; Gourvenec, F.; Sizun, P.; Herault, J.-P.; Perez, S.; Herbert, J.-M., Experimental proof for the structure of a thrombin-inhibiting heparin molecule. *Chem. Eur. J.* **2001**, *7*, 858-873.
12. 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.
13. Alhassan, A.-B.; McCutcheon, D. C.; Zeller, M.; Norris, P., Azidonitration of Di-O-acetyl-L-fucal: X-Ray Crystal Structures of Intermediate Azidodeoxysugars and of the Bacterial Aminosugar N-Acetyl-L-fucosamine. *J. Carbohydr. Chem.* **2012**, *31*, 371-383.
14. Demchenko, A. V.; De Meo, C., semi-Orthogonality of O-pentenyl and S-ethyl glycosides: application for the oligosaccharide synthesis. *Tetrahedron Lett.* **2002**, *43*, 8819-8822.
15. Hale, K. J.; Hough, L.; Manaviazar, S.; Calabrese, A., An update of the rules for pyranoside sulfonate displacement. *Org. Lett.* **2014**, *16*, 4838-4841.
16. Illarionov, P. A.; Torgov, V. I.; Hancock, I. I.; Shibaev, V. N., A novel synthesis of N-acetyl- α -D-fucosamine 1-phosphate and uridine 5'-diphospho-N-acetyl- α -D-fucosamine. *Russ. Chem. Bull.* **2001**, *50*, 1303-1308.
17. Adinolfi, M.; Iadonisi, A.; Ravida, A.; Schiattarella, M., Efficient and direct synthesis of saccharidic 1,2-ethylidenes, orthoesters, and glycals from peracetylated

sugars via the in situ generation of glycosyl iodides with I₂/Et₃SiH. *Tetrahedron Lett.* **2003**, *44*, 7863-7866.

18. Bayley, H.; Standring, D. N.; Knowles, J. R., Propane-1,3-dithiol: a selective reagent for the efficient reduction of alkyl and aryl azides to amines. *Tetrahedron Lett.* **1978**, *19*, 3633-3634.

19. Zulueta, M. M.; Lin, S. Y.; Lin, Y. T.; Huang, C. J.; Wang, C. C.; Ku, C. C.; Shi, Z.; Chyan, C. L.; Irene, D.; Lim, L. H.; Tsai, T. I.; Hu, Y. P.; Arco, S. D.; Wong, C. H.; Hung, S. C., α -Glycosylation by D-glucosamine-derived donors: synthesis of heparosan and heparin analogues that interact with mycobacterial heparin-binding hemagglutinin. *J. Am. Chem. Soc.* **2012**, *134*, 8988-8995.

CHAPTER 3

The synthesis of the repeating unit of capsular polysaccharide *Staphylococcus aureus* type 5 to study chemical activation and conjugation of native CP5

Yasomanee, J. P.; Visansirikul, S.; Pornsuriyasak, P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V., The synthesis of the repeating unit of capsular polysaccharide *Staphylococcus aureus* type 5 to study carbonylditriazole-mediated activation for conjugation of native CP5. *J. Org. Chem.* **2016**, *81*, 5981-5987.

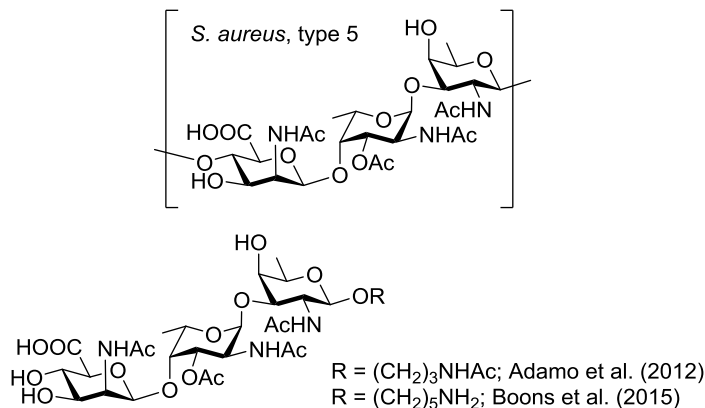
3.1 Introduction

*Staphylococcus aureus*¹ is one of the most frequent causes of infections in newborns, surgical patients, and immunocompromised individuals.² The majority of *S. aureus* strains express either capsular polysaccharide type 5 (CP5) or type 8 (CP8), which coat the cell for the purpose of immune evasion and define its serotype.³ These polysaccharides are comprised of many repeats of a unique arrangement of monosaccharides (the repeat unit) specific to a given bacterial serotype. The chemical composition of both types has been established⁴ and chemical syntheses of trisaccharides related to CP5⁵⁻⁶ and CP8 have been reported.⁷ The trisaccharide repeating unit of CP5 consists of the terminal D-mannosamine uronic acid (ManNAcA) that is β -(1 \rightarrow 4)-linked to 2,3-di-N,O-acetylated L-fucosamine (3Ac-L-FucNAc, Figure 3.1). The latter is α -(1 \rightarrow 3)-linked to D-fucosamine (D-FucNAc). In the bacterial polysaccharide, D-FucNAc is then linked to another D-ManNAcA via β -(1 \rightarrow 4) linkage, etc.⁴ Though this is a natural product, chemical syntheses of CP5 have been achieved by targeting a trisaccharide repeating unit equipped with the conjugation amenable spacer group at the reducing end (Scheme 3.1).^{5,6} Each building block required total synthesis from common sugars as the monosaccharide components are not available commercially.

Conjugates of the purified native *S. aureus* capsular polysaccharides (CPs) derived from fermentation with two different protein carriers have been tested in human clinical trials.⁸⁻⁹ Fattom, *et al.* reported on the preparation of conjugates of CP5 and CP8 with *Pseudomonas aeruginosa* endotoxin A (ETA).¹⁰ Freese *et al.* reported the preparation of conjugates of CP5 and CP8 with cross-reacting material 197 (CRM₁₉₇).¹¹ Covalent bond formation between native CPs and protein carriers requires chemically

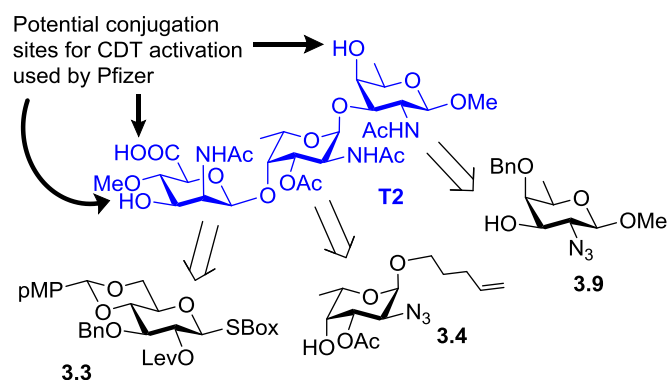
mediated activation of the CPs, and in some instances the protein carrier. The reagents used to activate the polysaccharides in the aforementioned CP5 and CP8 conjugates included 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), carbonyldiimidazole (CDI), and carbonylditriazole (CDT).

Scheme 3.1. *S. aureus* type 5 and previously synthesized trisaccharides.



Characterization of activation products derived from purified native CPs by spectroscopic methods is very difficult owing to their large size (MW > 50 kDa). Over activation and side reactions can lead to a loss of the epitopes required for immunogenicity of CP conjugate vaccines leading to product failure. The study of small model compounds based on the structure of the repeat unit offers a means to fully characterize the structure of the activated CP, identify and quantify sites of activation, and understand side reactions occurring after CP activation. Reported herein is the synthesis of the trisaccharide **T2** that on one hand mimics the repeat unit of CP5, and on the other hand preserves the potential conjugation sites as in the native polysaccharide. For this purpose, trisaccharide **T2** will be equipped with capping methyl groups at the points of propagation of the polysaccharide sequence, C-1 and C-4” (Scheme 3.2).

Scheme 3.2. Retrosynthetic analysis of *S. aureus* type 5 for the CDT activation studies.



It should be noted that although the direct introduction of the β -manno linkage was proven feasible by Boons et al.,⁶ it was rather ineffective in our hands. We relate this disappointing result to a fairly low reactivity profile of 4,6-acetalated mannosyl donors tested and 3-*O*-acetylated L-fucosyl acceptor **3.4**. Instead, β -glycosylation with *S*-benzoxazolyl (SBox) glycosyl donor **3.3** followed by the C-2 epimerization, an approach executed in our previous synthesis of CP8, was adopted instead.⁷

The SBox leaving group¹²⁻¹³ gave us far superior results in activation over *O*-pentenyl moiety of acceptor **3.4** than S*Et*. The activation of the S*Et* group over the *O*-pentenyl moiety is also feasible, but it requires the use of MeOTf as promoter,¹⁴ which in this case was ineffective due to the disarming protecting group pattern of the donor. Protecting groups in building blocks **3.3**, **3.4** and **3.9** were selected to ensure both selective deprotection for site-specific functionalization and the survival of the base-labile *O*-acetyl group that needs to be retained throughout the entire synthesis. The levulinoyl group was selected at C-2 of donor **3.3** for two reasons: first, to assist in β -glucosylation, and second, to give access to selective deprotection followed by epimerization at the disaccharide level. *p*-Methoxybenzylidene was found suitable for

gaining the ready access to the 4''-O-methylation position via the reductive opening at the trisaccharide level. The resulting 6''-*O*-*p*-methoxybenzyl group would then provide straightforward access to 6''-OH to be oxidized at the later stage of the synthesis. Finally, benzyl and azide protecting groups were found suitable for temporary protection of the remaining *O*- and *N*-positions because their removal would not affect 3'-*O*-acetyl group.

3.2 Results and Discussion

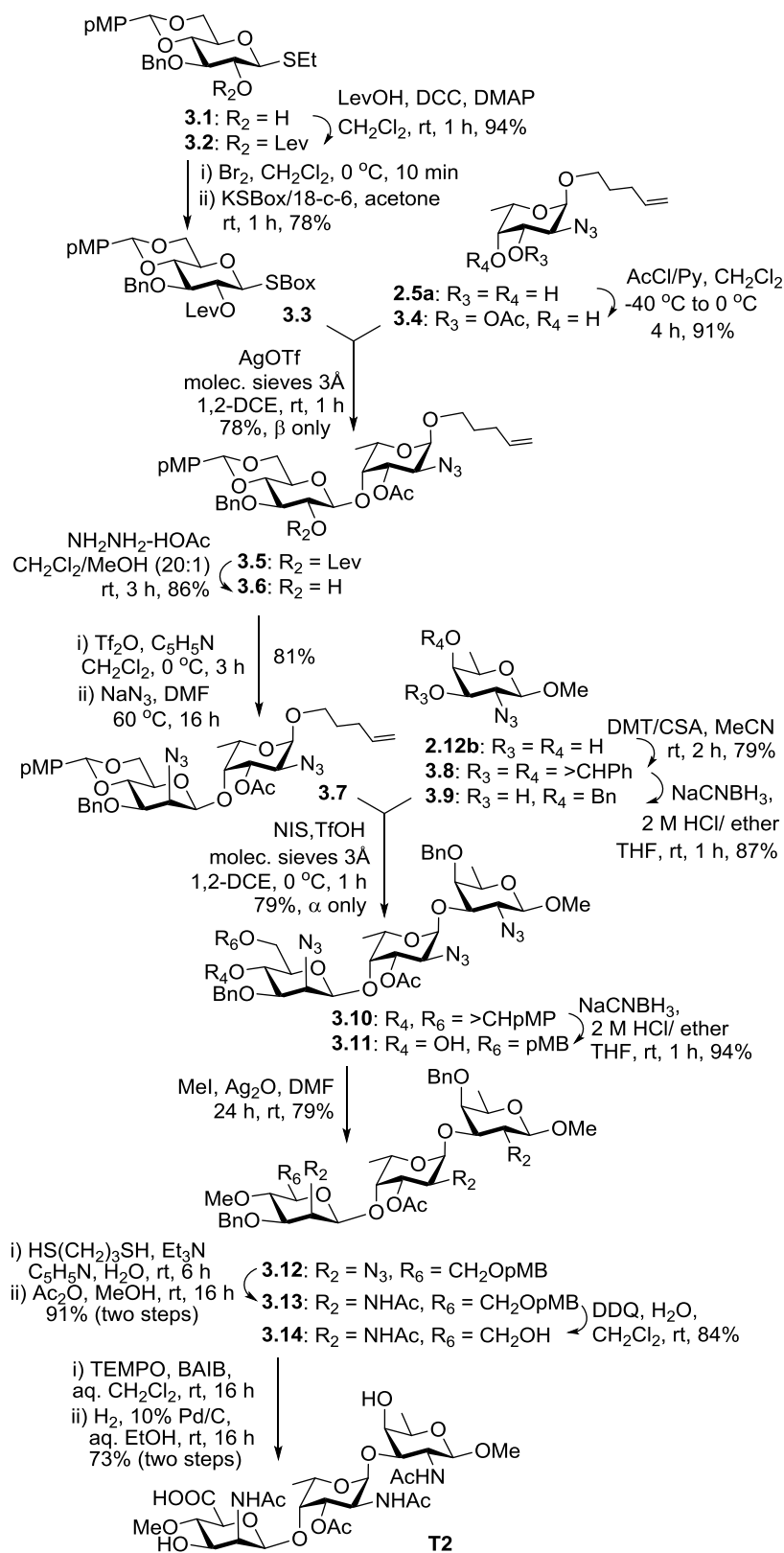
In accordance with our strategic plan the synthesis of glucosyl donor **3.3** began from known thioglycoside **3.1**.¹⁵ (Scheme 3.3) Accordingly, the 2-hydroxyl derivative **3.1** was reacted with levulinic acid (LevOH), dicyclohexycarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to afford 2-levulinoyl derivative **3.2** in 94% yield (Scheme 3.3). Reaction of thioglycoside **3.2** with bromine followed by the introduction of the SBox leaving group with KSBox^{12,16} in the presence of 18-crown-6 in acetone yielded the first key building block, glycosyl donor **3.3** in 78% yield over two steps. The synthesis of L-fucosyl acceptor **3.4** was originated from the previously described *O*-pentenyl glycoside **2.5a**.⁷ Regioselective acetylation of the equatorial 3-hydroxyl in 3,4-diol **2.5a** with acetyl chloride in the presence of pyridine in CH₂Cl₂ at 0 °C gave the desired L-fucosyl acceptor **3.4** in 91% yield. Selective activation of the SBox leaving group in glycosyl donor **2** over the *O*-pentenyl anomeric moiety of glycosyl acceptor **3** was achieved in the presence of silver trifluoromethanesulfonate (AgOTf). This glycosylation afforded the desired β-linked disaccharide **3.5** in 78% yield.

Disaccharide **3.5** was then subjected to a three-step synthetic sequence to epimerize the C-2' stereocenter of the non-reducing end monosaccharide in order to

create the desired D-manno configuration. As previously refined for the synthesis of CP8 (Chapter 2), 2'-*O*-levulinoyl group in **3.5** was removed with $\text{NH}_2\text{NH}_2\text{-AcOH}$ in methanol and dichloromethane (1/20, v/v) to afford intermediate **3.6** in 86% yield. Sulfonation of 2'-OH in compound **3.6** was affected with triflic anhydride in the presence of pyridine in dichloromethane at 0 °C. The resulting 2'-*O*-triflate was directly subjected to the nucleophilic displacement by reaction with sodium azide in DMF at 60 °C. As a result, disaccharide glycosyl donor **3.7** was isolated in 81% yield over two steps. The synthesis of the D-fucosyl acceptor **3.9** was originated from the previously described methyl fucoside **2.12b**.⁷ The 3,4-diol in compound **2.12b** was first protected as the benzylidene acetal upon treatment with dimethoxytoluene (DMT) in the presence of catalytic amount of camphorsulfonic acid (CSA) to afford compound **3.8** in 79% yield. Regioselective benzylidene ring opening in **3.8** was conducted with NaCNBH_3 in the presence of 2M HCl in diethyl ether and THF and led to the desired D-fucosyl acceptor **3.9** in 87% yield. Glycosylation of acceptor **3.9** with disaccharide donor **3.7** involved activation of the *O*-pentenyl leaving group with *N*-iodosuccinimide (NIS) and TfOH in the presence of molecular sieves (4 Å) in 1,2-dichloroethane at 0 °C. This approach allowed us to obtain the desired trisaccharide **3.10** as a pure α -anomer in 79% yield.

With the key trisaccharide intermediate **3.10** in hand, we then endeavored to identify a series of protecting and functional group modifications to obtain the target trisaccharide **T2**. After thorough experimentation, the following reaction sequence was developed with the main goal to avoid a spontaneous C-2'',6'' lactamization that was encountered when the carboxyl (or carboxylate) and amine functional groups were present concomitantly.

Scheme 3.3. Synthesis of CP5 repeating unit T2.



First, the 3'',4''-*p*-methoxybenzylidene acetal in **3.10** was regioselectively opened with NaCNBH₃ in the presence of 2 M HCl in diethyl ether and THF. As a result, 6''-*p*-methoxybenzyl protected trisaccharide **3.11** was obtained in 94% yield. The 4''-OH group in **3.11** was then methylated with MeI in the presence of silver(I) oxide in CH₂Cl₂ to yield compound **3.12** in 79% yield. The neutral reaction conditions for the methylation step were essential for the survivability of the 3'-*O*-acetyl group.

After that, all three azide groups in trisaccharide **3.12** were reduced with propane-1,3-dithiol and trimethylamine in wet pyridine.¹⁷ The resulting triamine was acetylated with Ac₂O in methanol to afford trisaccharide **3.13** in 91% yield over two steps. The 6''-*O*-PMB protecting group in **3.13** was then removed with DDQ in wet CH₂Cl₂ to give trisaccharide **3.14** in 84% yield. The primary alcohol in **3.14** was oxidized to the carboxylic acid with (2,2,6,6-tetramethyl-piperidin-1-yl)oxyl (TEMPO) and (diacetoxyiodo)benzene (BAIB) in wet dichloromethane¹⁸ and the remaining 3''- and 4-*O*-benzyl groups were removed by hydrogenation in the presence of 10% palladium on charcoal in wet ethanol to obtain the target trisaccharide **T2** in 73% yield over two steps.

3.3 Conclusions and Outlook

As a result of the elaborated synthetic strategy, we accomplished an efficient synthesis of CP5, trisaccharide **T2**. The developed strategy allowed assembly of the key trisaccharide intermediate **3.10** from building blocks **3.3**, **3.4** and **3.9** with an overall 43% yield. It should be noted that this yield presents a notable improvement over the assembly stage of the synthesis reported by Adamo and co-workers (28%) employing a similar concept of the indirect ManNAc introduction via β -glycosylation-epimerization. Our

assembly was at least as efficient as the assembly stage reported by Boons and co-workers, who employed a direct mannosylation approach (total yield for the assembly stage 42%). The protecting group strategy for selective functionalization employed in our synthesis allowed for an efficient modification of the key intermediate **3.10** into the target compound **T2**. This sequence was accomplished in an overall 41% yield, which surpasses the yields of the deprotection-functionalization sequences achieved by Adamo (26%) and Boons (21%). This synthesis provided tool compounds useful for understanding chemical activation of native CP5 and the propensity for side reactions. This research represents a novel approach to the synthesis of conjugation amenable repeating units designed to increasing process and product understanding of conjugate vaccines.

3.4 Experimental

3.4.1 General Methods

The reactions were performed using commercial reagents and the 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 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. CH₂Cl₂ and ClCH₂CH₂Cl were distilled from CaH₂ directly prior to application. Molecular sieves (3Å), 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. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to application. Optical rotations were measured at polarimeter. ¹H NMR spectra

were recorded at 300 MHz or 600 MHz, ^{13}C -NMR spectra were recorded at 75 MHz or 150 MHz. The ^1H chemical shifts are referenced to the signal of the residual CHCl_3 ($\delta_{\text{H}} = 7.24$ ppm) for solutions in CDCl_3 or the signal of the residual CH_3OH ($\delta_{\text{H}} = 4.78$ ppm) for solutions in CD_3OD . The ^{13}C chemical shifts are referenced to the central signal of CDCl_3 ($\delta_{\text{C}} = 77.23$ ppm) for solutions in CDCl_3 or the central signal of CD_3OD ($\delta_{\text{C}} = 49.24$ ppm) for solutions in CD_3OD . HRMS determinations were made with the use of JEOL MStation (JMS-700) mass spectrometer.

3.4.2 Preparation of monosaccharide building blocks 3.3, 3.4 and 3.9.

Ethyl 3-*O*-benzyl-2-*O*-levulinoyl-4,6-*O*-(*p*-methoxybenzylidene)-1-thio- β -D-glucopyranoside (3.2). Levulinic acid (1.10 g, 9.23 mmol), DCC (1.77 g, 8.58 mmol) and DMAP (0.11 g, 0.92 mmol) were added to a stirring solution of ethyl 3-*O*-benzyl-4,6-*O*-(*p*-methoxybenzylidene)-1-thio- β -D-glucopyranoside¹⁵ (**3.1**, 2.0 g, 4.62 mmol) in CH_2Cl_2 (60 mL) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~200 mL) and washed with sat. aq. NaHCO_3 (50 mL) and water (2 x 50 mL). The organic phase was separated, dried over 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 as a white amorphous solid in 94% yield (2.31 g, 4.35 mmol). Analytical data for **3.2**: $R_f = 0.43$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_{\text{D}}^{24} -46.1$ ($c = 1$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): δ , 1.24 (t, 3H, $J = 7.5$ Hz, CH_2CH_3), 2.15 (s, 3H, COCH_3), 2.45-2.55 (m, 2H, COCH_2), 2.55-2.80 (m, 4H, SCH_2CH_3 , COCH_2), 3.46 (m, 1H, H-5), 3.65-3.75 (m, 3H, H-3, 4, 6a), 3.79 (s, 3H, OCH_3), 4.32 (dd, 1H, $J_{5,6b} = 4.9$ Hz, $J_{6a,6b} = 10.4$ Hz, H-6b), 4.44

(d, 1H, $J_{1,2} = 10.1$ Hz, H-1), 4.78 (dd, 2H, $^2J = 12.0$ Hz, CH_2Ph), 5.04 (dd, 1H, $J_{2,3} = 8.9$ Hz, H-2), 5.52 (s, 1H, $>CHPh$), 6.80-7.50 (m, 9H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ , 14.9, 24.0, 28.0, 29.9, 37.9, 55.3, 68.5, 70.7, 71.7, 74.3, 79.6, 81.4, 84.2, 101.2, 113.7 (x 2), 127.4 (x 2), 127.7, 128.0 (x 2), 128.3 (x 2), 129.7, 138.3, 160.1, 171.5, 206.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{28}H_{34}NaO_8S$ 553.1872, found 553.1875.

Benzoxazolyl 3-O-benzyl-2-O-levulinoyl-4,6-O-(p-methoxybenzylidene)-1-thio- β -D-glucopyranoside (3.3). A mixture of **3.2** (2.30 g, 4.34 mmol) and activated molecular sieves (3Å, 2.1 g) in CH_2Cl_2 (70 mL) was stirred under argon for 1 h. Freshly prepared solution of Br_2 in CH_2Cl_2 (45 mL, 1/165, v/v) was then added and the reaction mixture was kept for 10 min at rt. After that, the solid was filtered-off and the filtrate was concentrated under reduced pressure at rt and then dried *in vacuo* for 2 h. The crude residue was then dissolved in dry acetone (30 mL), KSBox¹⁹ (1.64 g, 8.67 mmol) and 18-crown-6 (0.23 g, 0.87 mmol) were added and the resulting mixture was stirred for 1 h under argon at rt. After that, the reaction mixture was concentrated under reduced pressure. The residue was redissolved in CH_2Cl_2 (250 mL), washed with 1% aq. NaOH (50 mL) and water (3x 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane graduate elution) to afford the title compound in 78% yield (2.10 g, 3.39 mmol) as a white foam. Analytical data for **3.3**: $R_f = 0.46$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{24} +54.3$ (c = 1, $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$): δ , 2.11 (s, 3H, $COCH_3$), 2.35-2.58 (m, 2H, $COCH_2$), 2.60-2.80 (m, 2H, $COCH_2$), 3.60-3.90 (m, 7H, H-3, 4, 5, 6a, OCH_3), 4.37 (dd, 1H, $J_{5,6a} = 3.8$ Hz, $J_{6a,6b} = 9.4$ Hz, H-6b), 4.81 (dd, 2H, $^2J =$

11.9 Hz, CH_2Ph), 5.24 (dd, 1H, $J_{2,3} = 8.2$ Hz, H-2), 5.55 (s, 1H, $>CHPh$), 5.67 (d, 1H, $J_{1,2} = 10.4$ Hz, H-1), 7.80-7.70 (m, 13H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.0, 30.0, 37.9, 55.5, 68.5, 71.1, 71.5, 74.6, 79.5, 81.2, 84.3, 101.5, 110.3, 113.8 (x 2), 119.1, 124.7 (x 2), 127.5 (x 2), 127.9, 128.2 (x 2), 128.5 (x 2), 129.7, 138.1, 141.7, 152.1, 160.3, 161.4, 171.7, 206.0 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{33}H_{33}NaNO_9S$ 642.1774, found 642.1781.

4-Pentenyl 3-O-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (3.4). 4-Pentenyl 2-azido-2-deoxy- α -L-fucopyranoside⁷ (**2.5a**, 1.50 g, 5.83 mmol) in pyridine (23 mL) was stirred under argon at -40 °C. A solution of acetyl chloride (0.43 ml, 6.07 mmol) in dry toluene (6.3 mL) was then added dropwise and the resulting mixture was stirred at -40 °C for 30 min. Then, the temperature was gradually increased to 0 °C and the reaction mixture was stirred for additional 4 h. The reaction mixture was diluted with CH_2Cl_2 (250 mL) and the organic layer was washed with water (50 mL), saturated aq. $NaHCO_3$ (50 mL), and water (2 x 50 mL). The organic phase was separated, dried and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - dichloromethane gradient elution) to yield the title compound as a white amorphous solid in 91% yield (1.59 g, 5.31 mmol). Analytical data for **3.4**: $R_f = 0.51$ (acetone/dichloromethane, 1/9, v/v); $[\alpha]_D^{30} -134.3$ (c = 1, $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$): δ , 1.20 (d, 3H, CH_3), 1.63-1.72 (m, 2H, CH_2), 2.07-2.15 (m, 2H, CH_2), 2.12 (s, 3H, $COCH_3$), 2.53 (s, 1H, OH), 3.39-3.46 (m, 1H, CH_2), 3.57 (dd, 1H, $J_{2,3} = 11.1$ Hz, H-2), 3.62-3.70 (m, 1H, CH_2), 3.88 (br. s, 1H, H-4), 3.97-4.04 (dd, 1H, $J_{5,6} = 6.6$ Hz, H-5), 4.86 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.90-5.02 (m, 2H, $CH=CH_2$), 5.22 (dd, 1H, $J_{3,4} = 3.0$ Hz, H-

3), 5.69-5.78 (m, 1H, $CH=CH_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ , 16.0, 21.0, 28.6, 30.2, 57.1, 65.7, 67.8, 70.0, 71.2, 98.2, 115.1, 137.9, 170.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{13}H_{21}O_5NaN_3$ 322.1379, found 322.1375.

Methyl 2-azido-3,4-O-benzylidene-2,6-dideoxy- β -D-galactopyranoside (3.8).

Dimethoxytoluene (1.38 mL, 9.15 mmol) and camphorsulfonic acid (58 mg, 0.031 mmol) were added to a solution of methyl 2-azido-2-deoxy- β -D-fucopyranoside⁷ (**2.12b**, 0.62 g, 3.05 mmol) in THF (20 mL) and the resulting mixture was stirred under argon for 1 h at rt. After that, triethylamine (~0.3 mL) was added and the volatiles were removed *in vacuo*. The residue was diluted with CH_2Cl_2 (~200 mL) and washed with water (40 mL), sat. aq. $NaHCO_3$ (40 mL), and water (2 x 40 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 yield the title compound as a white amorphous solid in 79% yield (0.70 g, 2.41 mmol). Analytical data for **3.8**: R_f = 0.46 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{27}$ +44.2 (c = 1, $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$): δ , 1.46 (d, 3H, $J_{5,6}$ = 6.6 Hz, C-6), 3.37 (dd, 1H, $J_{2,3}$ = 8.2 Hz, H-2), 3.53 (s, 3H, OCH_3), 3.85 (m, 1H, H-5), 3.96 (dd, 1H, $J_{4,5}$ = 5.7 Hz, H-4), 4.02 (dd, 1H, $J_{3,4}$ = 2.1 Hz, H-3), 4.12 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 5.91 (s, 1H, $>CHPh$), 7.25-7.62 (m, 5H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ , 16.5, 56.8, 65.5, 68.8, 76.8, 78.0, 102.5, 104.5, 126.6 (x 2), 128.5 (x 2), 129.5, 137.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{14}H_{17}O_4NaN_3$ 314.1117, found 314.1123.

Methyl 2-azido-4-O-benzyl-2,6-dideoxy- β -D-galactopyranoside (3.9). A mixture of **3.8** (0.70 g, 2.41 mmol) and activated molecular sieves (3Å, 800 mg) in THF (20 mL) was stirred under argon for 1 h at rt. NaCNBH₃ (1.20 g, 19.3 mmol) was added followed by a dropwise addition of 2M HCl in diethyl ether (9.6 mL, 19.3 mmol) and the resulting mixture was stirred under argon for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH₂Cl₂. The combined filtrate (~100 mL) was washed with water (20 mL), sat. aq. NaHCO₃ (20 mL), and water (2 x 20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid in 87% (0.62 g, 2.10 mmol). Analytical data for **3.9**: R_f = 0.39 (ethyl acetate/hexane, 2/3, v/v); [α]_D³⁰ +75.0 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.33 (d, 3H, *J*_{5,6} = 6.5 Hz, C-6), 2.17 (m, 1H, OH), 3.40-3.60 (m, 7H, H-2, 3, 4, 5, OCH₃), 4.12 (d, 1H, *J*_{1,2} = 7.8 Hz, H-1), 4.77 (dd, 2H, ²*J* = 11.6 Hz, CH₂Ph), 7.20-7.45 (m, 5H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 17.1, 57.1, 64.8, 71.0, 73.2, 76.2, 78.7, 103.2, 114.2, 128.3 (x 2), 128.8 (x 2), 138.1 ppm; HR-FAB MS [M+Na]⁺ calcd for C₁₄H₁₉O₄NaN₃ 316.1273, found 316.1274.

3.4.3 Assembly of trisaccharide 3.10 from building blocks 3.3, 3.4 and 3.9.

4-Pentenyl O-(3-O-benzyl-2-O-levulinoyl-4,6-O-*p*-methoxybenzylidene- β -D-glucopyranosyl)-(1 \rightarrow 4)-3-O-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (3.5).

A mixture donor **3.3** (2.0 g, 3.23 mmol), glycosyl acceptor **3.4** (0.74 g, 2.48 mmol), and freshly activated molecular sieves (3Å, 2.8 g) in ClCH₂CH₂Cl (60 mL) was stirred under argon for 2 h. Freshly conditioned AgOTf (1.66 g, 6.46 mmol) was added and the

resulting mixture was stirred for 1 h at rt. The reaction mixture was then diluted with CH_2Cl_2 , the solid was filtered-off, and the residue was rinsed successively with CH_2Cl_2 . The combined filtrate (~200 mL) was washed with sat. aq. NaHCO_3 (40 mL) and water (2 x 40 mL). The organic phase was separated, dried over 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 as a white foam in 78% yield (1.48 g, 1.93 mmol). Analytical data for **3.5**: $R_f = 0.47$ (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{24} - 110.2$ (c = 1, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.18 (d, 3H, $J_{5,6} = 6.6$ Hz, C-6), 1.70 (m, 2H, CH_2), 1.95- 2.20 (m, 8H, 2 x COCH_3 , CH_2), 2.35-2.90 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CO}$), 3.25-3.50 (m, 2H, H-5', OCH_2^a), 3.55-3.80 (m, 5H, H-2, 3', 4', 6a', OCH_2^b), 3.82 (s, 3H, OCH_3), 3.95 (m, 1H, H-5), 4.06 (d, 1H, $J_{4,5} = 3.0$ Hz, H-4), 4.29 (dd, 1H, $J_{5',6b'} = 4.9$ Hz, $J_{6a',6b'} = 10.2$ Hz, H-6b'), 4.40 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1'), 4.78 (dd, 2H, $^2J = 12.1$ Hz, CH_2Ph), 4.81 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.90-5.05 (m, 4H, H-2', 3, $\text{CH}=\text{CH}_2$), 5.11 (dd, 1H, $J_{3,4} = 8.6$ Hz, H-3), 5.52 (s, 1H, $>\text{CHPh}$), 5.80 (m, 1H, $\text{CH}=\text{CH}_2$), 6.75-7.50 (m, 9H, aromatic) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ , 16.2, 21.4, 28.1, 28.7, 30.0, 30.4, 38.2, 55.5, 57.1, 65.5, 66.5, 67.8, 68.6, 70.6, 73.4, 74.2, 76.1, 78.3, 81.7, 98.0, 101.5, 102.2, 113.8 (x2), 115.3, 127.5 (x2), 127.8, 128.1 (x 2), 128.4 (x 2), 129.6, 138.1, 138.4, 160.3, 170.6, 171.5, 206.4 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{39}\text{H}_{49}\text{NaO}_{13}\text{N}_3$ 790.3163, found 790.3167.

4-Pentenyl O-(3-O-benzyl-4,6-O-p-methoxybenzylidene- β -D-glucopyranosyl)-(1 \rightarrow 4)-3-O-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (3.6). Hydrazine acetate (0.34 g, 3.78 mmol) was added to a stirred solution of **3.5** (1.45 g, 1.89 mmol) in

CH₂Cl₂/methanol (20/1, v/v, 21 mL) and the resulting mixture was stirred for 16 h at rt. After that, the reaction mixture was diluted with CH₂Cl₂ (~200 mL) and washed with sat. aq. NaHCO₃ (40 mL) and water (2 x 40 mL). The organic phase was separated, dried over MgSO₄, 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 foam in 86% yield (1.09 g, 1.62 mmol). Analytical data for **3.6**: R_f = 0.54 (ethyl acetate/hexane, 1/1, v/v); [α]_D²⁵ -124.4 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.39 (d, 3H, J_{5,6} = 6.6 Hz, C-6), 1.55-1.85 (m, 2H, CH₂), 2.07 (s, 3H, COCH₃), 2.08-2.20 (m, 2H, CH₂), 3.34 (m, 1H, H-5'), 3.44 (m, 2H, OCH₂^a), 3.55-3.75 (m, 6H, H-2, 2', 3', 4', 6a', OCH₂^b), 3.78 (s, 3H, OCH₃), 4.01 (m, 1H, H-5), 4.10 (d, 1H, J_{4,5} = 2.9 Hz, H-4), 4.25 (dd, 1H, J_{5',6b'} = 4.7 Hz, J_{6a',6b'} = 9.9 Hz, H-6b'), 4.32 (d, 1H, J_{1',2'} = 6.9 Hz, H-1'), 4.85 (dd, 2H, ²J = 11.6 Hz, CH₂Ph), 4.88 (d, 1H, J_{1,2} = 3.5 Hz, H-1), 4.93-5.10 (m, 3H, H-3, CH=CH₂), 5.49 (s, 1H, >CHPh), 5.78 (m, 1H, CH=CH₂), 6.80-7.50 (m, 9H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.1, 21.4, 28.7, 30.4, 55.7, 57.3, 65.9, 66.5, 67.9, 68.7, 70.6, 74.6, 74.9, 76.8, 80.7, 81.4, 98.0, 101.4, 104.6, 113.8 (x 2), 115.3, 127.5 (x 2), 127.9, 128.1 (x 2), 128.5 (x 2), 129.7, 138.0, 138.6, 160.2, 170.5 ppm; HR-FAB MS [M+Na]⁺ calcd for C₃₄H₄₃NaO₁₁N₃ 692.2795, found 692.2802.

4-Pentenyl O-(2-azido-3-O-benzyl-2-deoxy-4,6-O-p-methoxybenzylidene-β-D-mannopyranosyl)-(1→4)-3-O-acetyl-2-azido-2,6-dideoxy-α-L-galactopyranoside (3.7).

A solution of **3.6** (1.07 g, 1.60 mmol) in CH₂Cl₂ (40 mL) and pyridine (4 mL) was cooled to 0 °C. Trifluoromethanesulfonic anhydride (0.67 mL, 3.99 mmol) was slowly added and the resulting mixture was stirred for 4 h at 0 °C. After that, the reaction was

quenched with ice-cold water (~10 mL). The mixture was diluted with CH₂Cl₂ (~150 mL) and washed with cold sat. aq. NaHCO₃ (20 mL) and cold water (2 x 20 mL), the organic phase was separated, dried over MgSO₄, and concentrated *in vacuo*. The crude residue was dissolved in DMF (5 mL), NaN₃ (0.52 g, 7.99 mmol) was added and the resulting suspension was stirred for 3 h at 60 °C. After that, ethyl acetate (~100 mL) and water (~20 mL) were added. Organic phase was separated and washed with brine (2 x 20 mL). Combined aqueous phase was extracted with ethyl acetate (3 x 60 mL). The combined organic phase was dried over MgSO₄ 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 foam in 81% (0.91 g, 1.29 mmol). Analytical data for **10**: R_f = 0.59 (ethyl acetate/hexane, 2/3, v/v); [α]_D²⁵ -187.8 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.19 (d, 3H, J_{5,6} = 6.6 Hz, C-6), 1.70 (m, 2H, CH₂), 2.01-2.20 (m, 5H, COCH₃, CH₂), 3.22 (m, 1H, H-5'), 3.45 (m, 1H, CH₂^a), 3.58-3.68 (m, 2H, H-3', CH₂^b), 3.70-3.85 (m, 5H, H-2, 6a', OCH₃), 3.95 (dd, 1H, J_{4',5'} = 9.4 Hz, H-4'), 3.99 (m, 1H, H-5), 4.06 (d, 1H, J_{2',3'} = 3.5 Hz, H-2'), 4.16 (d, 1H, J_{4,5} = 3.1 Hz, H-4), 4.23 (dd, 1H, J_{5',6a'} = 4.8 Hz, J_{6a',6b'} = 10.2 Hz, H-6b'), 4.50 (s, 1H, H-1'), 4.77 (dd, 2H, ²J = 12.4 Hz, CH₂Ph), 4.85 (d, 1H, J_{1,2} = 3.2 Hz, H-1), 4.92-5.10 (m, 3H, H-3, CH=CH₂), 5.51 (s, 1H, >CHPh), 5.78 (m, 1H, CH=CH₂), 6.80-7.50 (m, 9H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 16.5, 21.4, 28.6, 30.3, 55.4, 57.0, 62.8, 65.3, 67.6, 67.9, 68.3, 70.2, 73.0, 76.0, 76.5, 78.4, 98.0, 101.5, 101.7, 113.7 (x 2), 115.2, 127.4 (x 2), 127.8 (x 2), 128.1, 128.6 (x 2), 129.7, 137.8, 138.0, 160.2, 170.7 ppm; HR-FAB MS [M+Na]⁺ calcd for C₃₄H₄₂NaO₁₀N₆ 717.2860, found 717.2869.

Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-*p*-methoxybenzylidene- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.10**). A mixture donor **3.7** (0.90 g, 1.30 mmol), acceptor **3.9** (0.35 g, 1.18 mmol), and freshly activated molecular sieves (3Å, 800 mg) in ClCH₂CH₂Cl (30 mL) was stirred under argon for 2 h. The reaction mixture was cooled to 0 °C, NIS (0.58 g, 2.59 mmol) and TfOH (23 μ L, 0.26 mmol) were added and the resulting mixture was stirred for 1 h at 0 °C. After that, the solid was filtered-off and rinsed successively with CH₂Cl₂. The combined filtrate (~50 mL) was washed with 10% aq. Na₂S₂O₃ (15 mL) and water (2 x 15 mL). The organic phase was separated, dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone - dichloromethane gradient elution) to afford the title compound as a white foam in 79% yield (0.85 g, 0.94 mmol). Analytical data for **3.10**: R_f = 0.49 (ethyl acetate/hexane, 1/1, v/v); [α]_D²⁸ -130.7 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.13 (d, 3H, $J_{5',6'}$ = 6.5 Hz, C-6'), 1.32 (d, 3H, $J_{5,6}$ = 6.4 Hz, C-6), 2.14 (s, 3H, COCH₃), 3.23 (m, 1H, H-5''), 3.37-3.58 (m, 6H, H-3, 4, 5, OCH₃), 3.65 (dd, 1H, $J_{3'',4''}$ = 9.6 Hz, H-3''), 3.74-3.91 (m, 7H, H-1, 2', 5', 6a''), OCH₃), 3.97 (dd, 1H, $J_{4'',5''}$ = 9.2 Hz, H-4''), 4.06 (d, 1H, $J_{2'',3''}$ = 3.7 Hz, H-2''), 4.01-4.18 (m, 2H, H-1, 4'), 4.25 (dd, 1H, $J_{5',6b'}$ = 4.8 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6b'), 4.50 (s, 1H, H-1''), 4.65-4.78 (m, 2H, CH₂Ph), 4.82-4.93 (m, 2H, CH₂Ph), 5.03 (dd, 1H, $J_{2',3'}$ = 11.3 Hz, $J_{3',4'}$ = 3.1 Hz, H-3'), 5.31 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1'), 5.54 (s, 1H, >CHPh), 6.80-7.50 (m, 14H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 16.6, 17.2, 21.4, 55.4, 56.8, 57.2, 62.8, 63.8, 66.1, 67.6, 68.3, 69.8, 70.9, 73.0, 75.1, 75.9, 76.4, 78.1, 78.4, 79.1, 99.4, 101.5, 101.8, 103.6, 113.7 (x 2), 127.5 (x 2), 127.9 (x 3), 128.0 (x 2), 128.1, 128.5 (x 2),

128.7 (x 2), 129.6, 137.8, 138.1, 160.2, 170.6 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{43}H_{51}O_{13}N_9Na$ 924.3504, found 924.3512.

3.4.4 Final functionalization of 3.10 into target trisaccharide T2.

Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.11**). A mixture of **3.10** (0.81 g, 0.90 mmol) and activated molecular sieves (3Å, 1.50 g) in THF (32 mL) was stirred under argon for 1 h at rt. NaCNBH₃ (0.45 g, 7.19 mmol) was added followed by a dropwise addition of 2M HCl in diethyl ether (2.7 mL, 5.4 mmol) and the resulting mixture was stirred under argon for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH₂Cl₂. The combined filtrate (~200 mL) was washed with water (20 mL), sat. aq. NaHCO₃ (20 mL), and water (2 x 20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid. Analytical data for **3.11**: R_f = 0.56 (acetone/dichloromethane, 1/19, v/v); $[\alpha]_D^{31}$ -149.6 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 1.04 (d, 3H, $J_{5',6'}$ = 6.5 Hz, C-6'), 1.24 (d, 3H, $J_{5,6}$ = 6.3 Hz, C-6), 2.03 (s, 3H, COCH₃), 2.68 (d, 1H, J = 2.1 Hz, 4''-OH), 3.19 (m, 1H, H-5''), 3.28 (dd, 1H, $J_{3'',4''}$ = 9.1 Hz, H-3''), 3.33-3.49 (m, 6H, H-3, 4, 5, OCH₃), 3.58 (m, 2H, H-6a'', 6b''), 3.70 (s, 3H, OCH₃), 3.71-3.82 (m, 4H, H-2, 2', 4'', 5'), 3.91 (d, 1H, $J_{2'',3''}$ = 3.5 Hz, H-2''), 4.02-4.10 (m, 2H, H-1, 4'), 4.34-4.41 (m, 3H, H-1'', CH₂Ph), 4.55-4.70 (m, 3H, 1/2 CH₂Ph), 4.82 (d, 1H, ² J = 11.6 Hz, 1/2 CH₂Ph), 4.99 (dd, 1H, $J_{2',3'}$ = 11.3 Hz, $J_{3',4'}$ = 3.1 Hz, H-3'), 5.22

(d, 1H, $J_{1',2'} = 3.7$ Hz, H-1'), 6.65-7.50 (m, 14H, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl_3): δ 16.6, 17.1, 21.2, 55.4, 56.9, 57.1, 60.7, 63.9, 66.3, 68.2, 69.5, 69.8, 70.9, 72.1, 73.4, 74.9, 75.1, 75.3, 78.1, 79.1, 79.8, 99.4, 100.8, 103.6, 114.0 (x2), 127.9, 128.0 (x4), 128.3, 128.5 (x2), 128.8 (x2), 129.6 (x2), 129.7, 137.6, 138.1, 159.5, 170.8 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{43}\text{H}_{53}\text{O}_{13}\text{N}_9\text{Na}$ 926.3661, found 926.3668.

Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-4-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (3.12). Silver oxide (1.95 g, 8.41 mmol) was added to a stirring solution of **3.11** (0.76 g, 0.84 mmol) and MeI (0.79 mL, 12.6 mmol) in DMF (5 mL) and the resulting mixture was stirred for 24 h at rt. After that, the solid was filtered-off and rinsed successively with CH_2Cl_2 . The combined filtrate (~150 mL) and washed with sat. aq. NaHCO_3 (20 mL) and water (2 x 20 mL). The organic phase was separated, dried over 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 as a white amorphous solid in 79% yield (0.61 g, 0.66 mmol). Analytical data for **3.12**: $R_f = 0.62$ (acetone/hexane, 1/1, v/v); $[\alpha]_{\text{D}}^{28} -79.2$ ($c = 1$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): δ , 1.11 (d, 3H, $J_{5',6'} = 6.5$ Hz, C-6'), 1.31 (d, 3H, $J_{5,6} = 6.4$ Hz, C-6), 2.13 (s, 3H, COCH_3), 3.17 (m, 1H, H-5''), 3.37-3.58 (m, 16H, H-3, 3'', 4, 4'', 5, 2 x OCH_3), 3.60-3.65 (m, 2H, H-6a'', 6b''), 3.78 (s, 3H, OCH_3), 3.80-3.92 (m, 3H, H-2, 2', 5'), 3.98 (d, 1H, $J_{2'',3''} = 3.5$ Hz, H-2''), 4.09-4.18 (m, 2H, H-1, 4'), 4.40 (s, 1H, H-1''), 4.47 (dd, 2H, $^2J = 11.5$ Hz, CH_2Ph), 4.65-4.74 (m, 3H, $1/2 \text{CH}_2\text{Ph}$), 4.90 (d, 1H, $^2J = 11.6$ Hz, $1/2 \text{CH}_2\text{Ph}$), 5.07 (dd, 1H, $J_{2',3'} = 11.3$ Hz, $J_{3',4'} = 3.1$ Hz, H-3'), 5.30

(d, 1H, $J_{1,2'} = 3.6$ Hz, H-1'), 6.75-7.50 (m, 14H, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl_3): δ , 16.7, 17.2, 21.1, 31.0, 55.4, 56.9, 57.1, 61.1, 61.4, 63.9, 66.3, 68.7, 69.4, 70.9, 72.1, 73.1, 75.1 (x 2), 75.8, 78.1, 79.1, 80.5, 99.5, 100.8, 103.6, 113.9 (x 2), 127.9 (x 3), 128.1 (x 3), 128.6 (x 2), 128.7 (x 2), 129.6 (x 2), 130.0, 137.8, 138.1, 159.4, 171.1 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{44}\text{H}_{55}\text{O}_{13}\text{N}_9\text{Na}$ 940.3817, found 940.3822.

Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-4-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (3.13). Water (5.0 mL), triethylamine (4.0 mL), and 1,3-propanedithiol (3.95 mL, 39.2 mmol) were added to a solution of trisaccharide **3.12** (0.60 g, 0.65 mmol) in pyridine (15.0 mL) and the resulting mixture was stirred for 3 h at rt. After that, the reaction mixture was concentrated and dried *in vacuo*. The crude residue was dissolved in MeOH (5.0 mL), Ac_2O (0.74 mL, 0.78 mmol) was added, and the resulting suspension was stirred under argon for 16 h at rt. The reaction mixture was then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white foam in 91% (0.57 g, 0.59 mmol). Analytical data for **3.13**: $R_f = 0.52$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_{\text{D}}^{28} -92.3$ (c = 1, CHCl_3); ^1H NMR (300 MHz, CDCl_3): δ , 1.10 (d, 3H, $J_{5',6'} = 6.4$ Hz, C-6'), 1.31 (d, 3H, $J_{5,6} = 6.3$ Hz, C-6), 1.92, 2.00, 2.04, 2.16 (4s, 12H, 4 x COCH_3), 3.17 (m, 1H, H-5''), 3.30-3.83 (m, 16H, H-3, 3'', 4, 4'', 5, 6a'', 6b'', 3 x OCH_3), 3.85-4.05 (m, 2H, H-4', 5'), 4.28-4.60 (m, 7H, H-1, 1', 2, 2', 1 $\frac{1}{2}$ CH_2Ph), 4.70-5.00 (m, 6H, H-1'', 2'', 3', 1 $\frac{1}{2}$ CH_2Ph), 6.10 (d, 1H, $J_{2',\text{NH}} = 9.3$ Hz, 2'-

NH), 6.84-6.88 (m, 2H, aromatic), 6.92 (d, 1H, $J_{2'',\text{NH}} = 9.3$ Hz, 2''-NH), 7.05 (d, 1H, $J_{2,\text{NH}} = 9.3$ Hz, 2-NH), 7.18-7.50 (m, 12H, aromatic) ppm; ^{13}C NMR (75 MHz, CDCl_3): δ , 16.5, 17.2, 20.9, 21.1, 23.3, 23.4, 23.6, 46.2, 49.7, 51.5, 55.4 (x2), 56.0, 61.0, 67.0, 67.9, 70.7, 70.9, 71.2, 73.2, 74.7, 75.1, 75.6, 78.3, 80.9, 99.7, 100.0, 101.7, 113.9 (x 2), 127.4 (x 2), 127.6, 127.7, 128.2 (x 2), 128.4 (x 2), 128.5 (x 2), 129.4, 129.9 (x 2), 138.3, 138.5, 159.5, 171.2 (x 2), 172.0, 172.5 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{50}\text{H}_{67}\text{O}_{16}\text{N}_3\text{Na}$ 988.4419, found 988.4432.

Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (3.14). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 0.27 g, 1.18 mmol) was added to a mixture of **3.13** (0.57 g, 0.59 mmol) in CH_2Cl_2 (10.0 mL) and water (0.5 mL) and the resulting mixture was stirred for 6 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~150 mL) and washed with water (2 x 20 mL), the organic phase was separated, dried over MgSO_4 , and the reaction mixture was concentrated *in vacuo*. Then the residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid in 84% yield (0.42 g, 0.50). Analytical data for **3.14**: $R_f = 0.36$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_{\text{D}}^{26} - 90.9$ ($c = 1$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): δ , 1.10 (d, 3H, $J_{5',6'} = 6.4$ Hz, C-6'), 1.40 (d, 3H, $J_{5,6} = 6.5$ Hz, C-6), 1.94, 2.03, 2.10, 2.11 (4s, 12H, 4 x COCH_3), 3.10-3.25 (m, 2H, H-4'', 5''), 3.38-3.60 (m, 8H, H-3'', 4, 2 x OCH_3), 3.61-4.00 (m, 6H, H-3, 4', 5, 5', 6a'', 6b''), 4.25-4.40 (m, 2H, H-1, 2), 4.43-4.58 (m, 2H, H-1'', $\frac{1}{2}$ CH_2Ph), 4.67 (dd,

1H, $J_{1',2'} = 3.5$ Hz, $J_{2',3'} = 11.4$ Hz, H-2'), 4.70-5.00 (m, 6H, H-1', 2'', 3', 1 ½ CH₂Ph), 5.67 (d, 1H, $J_{2,\text{NH}} = 9.3$ Hz, 2-NH), 6.29 (d, 1H, $J_{2',\text{NH}} = 9.3$ Hz, 2'-NH), 6.39 (d, 1H, $J_{2'',\text{NH}} = 9.3$ Hz, 2''-NH), 7.20-7.50 (m, 10H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 16.5, 17.3, 21.2, 23.3, 23.6 (x 2), 46.7, 49.9, 51.6, 55.9 (x 2), 61.1 (x 2), 61.8, 67.4, 70.8, 71.2, 71.6, 74.6, 75.8, 77.4, 78.2, 80.7, 99.7, 100.1, 101.4, 127.5 (x 2), 127.7, 128.1 (x 2), 128.5 (x 2), 128.6 (x 4), 138.2, 138.4, 171.4, 171.7, 171.8 ppm; HR-FAB MS [M+Na]⁺ calcd for C₄₂H₅₉O₁₅N₃Na 868.3844, found 868.3831.

Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-mannopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-β-D-galactopyranoside (T2). Water (4.0 mL), 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, 11 mg, 0.07 mmol) and diacetoxy iodobenzene (BAIB, 295 mg, 0.92 mmol) were added to a solution of trisaccharide **3.14** (310 mg, 0.37 mmol) in CH₂Cl₂ (4.0 mL) and the resulting mixture was stirred for 16 h at rt. After that, the reaction mixture was passed through a pad of silica gel, concentrated *in vacuo*, and dried. The crude residue was dissolved in 90% aq. ethanol (5.0 mL), 10% Pd on activated charcoal (200 mg) was added and the resulting suspension was vigorously stirred under H₂ atmosphere for 16 h at rt. After that, the solids were filtered off and rinsed successively with methanol. The combined filtrate (~35 mL) was concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (dichloromethane - methanol gradient elution) to afford the title compound as a white amorphous solid in 73% yield (144 mg, 0.21 mmol). Analytical data for **T2**: $R_f = 0.43$ (methanol/dichloromethane, 1/1, v/v); $[\alpha]_D^{25} -75.6$ (c = 0.6, CH₃OH); ¹H NMR (300

MHz, CD₃OD): δ , 1.19 (d, 3H, $J_{5',6'} = 6.5$ Hz, C-6'), 1.29 (d, 3H, $J_{5,6} = 6.3$ Hz, C-6), 1.88, 1.93, 2.08, 2.11 (4s, 12H, 4 x COCH₃), 3.27 (dd, 1H, $J_{4'',5''} = 9.7$ Hz, H-4''), 3.42, 3.48 (2s, 6H, 2 x OCH₃), 3.45-3.52 (m, 3H, H-5'', 6a'', 6b''), 3.57-3.68 (m, 4H, H-3, 3', 4, 5), 4.00-4.08 (m, 2H, H-2, 4'), 4.12 (m, 1H, H-5'), 4.20 (dd, 1H, $J_{1,2} = 8.4$ Hz, H-1), 4.40 (dd, 1H, $J_{1',2'} = 8.4$ Hz, H-1'), 4.52 (s, 1H, H-1''), 4.56 (d, 1H, $J_{2'',3''} = 4.0$ Hz, H-2''), 4.82- 4.90 (m, 1H, H-1'), 4.98 (dd, 1H, $J_{3',4'} = 2.7$ Hz, H-3') ppm; ¹³C NMR (150 MHz, CD₃OD): δ , 16.9 (x 2), 21.5, 23.1, 23.3, 52.6, 54.7, 57.1 (x 2), 60.8 (x 2), 67.6, 71.1, 71.9, 72.2, 73.8, 78.9, 79.1, 79.6, 81.7, 100.8, 102.2, 103.9, 173.2, 173.4, 173.7, 174.8, 177.1 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₈H₄₅O₁₆N₃Na 702.2698, found 702.2695.

3.5 References

1. Ala'Aldeen, D. A. A.; Hiramatsu, K., *Staphylococcus Aureus: Molecular and Clinical Aspects*. Horwood Publishing Limited Published by Elsevier Ltd.: 2004.
2. del Rio, A.; Cervera, C.; Moreno, A.; Moreillon, P.; Miró, J. M., Patients at Risk of Complications of Staphylococcus aureus Bloodstream Infection *Clin. Infect. Dis.* **2009**, *48 (Suppl. 4)*, S246-S253.
3. Nanra, J. S.; Buitrago, S. M.; Crawford, S.; Ng, J.; Fink, P. S.; Hawkins, J.; Scully, I. L.; McNeil, L. K.; Aste-Amézaga, J. M.; Cooper, D.; Jansen, K. U.; Anderson, A. S., Capsular polysaccharides are an important immune evasion mechanism for Staphylococcus aureus. *Human Vaccines & Immunotherapeutics* **2014**, *9 (3)*, 480-487.
4. Jones, C., Revised structures for the capsular polysaccharides from *Staphylococcus aureus* types 5 and 8, components of novel glycoconjugate vaccines. *Carbohydr. Res.* **2005**, *340*, 1097-1106.

5. Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R., Synthesis of Staphylococcus aureus type 5 capsular polysaccharide repeating unit using novel L-FucNAc and D-FucNAc synthons and immunochemical evaluation. *Bioorg. Med. Chem.* **2012**, *20*, 6403-6415.
6. Gagarinov, I. A.; Fang, T.; Liu, L.; Srivastava, A. D.; Boons, G. J., Synthesis of Staphylococcus aureus Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization. *Org. Lett.* **2015**, *17*, 928-931.
7. Visansirikul, S.; Yasomanee, J. P.; Pornsuriyasak, P.; Kamat, M. N.; Podvalnyy, N. M.; Gobble, C. P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V., A Concise Synthesis of the Repeating Unit of Capsular Polysaccharide Staphylococcus aureus Type 8. *Org. Lett.* **2015**, *17*, 2382-2384.
8. Fattom, A. I.; Horwith, G.; Fuller, S.; Propst, M.; Naso, R., Development of StaphVAX™, a polysaccharide conjugate vaccine against S. aureus infection: from the lab bench to phase III clinical trials. *Vaccine* **2004**, *22*, 880-887.
9. Nissen, M.; Marshall, H.; Richmond, P.; Shakib, S.; Jiang, Q.; Cooper, D.; Rill, D.; Baber, J.; Eiden, J.; Gruber, W.; Jansen, K. U.; Emini, E. A.; Anderson, A. S.; Zito, E. T.; Girgenti, D., A randomized phase I study of the safety and immunogenicity of three ascending dose levels of a 3-antigen Staphylococcus aureus vaccine (SA3Ag) in healthy adults. *Vaccine* **2015**, *33*, 1846-1854.
10. Fattom, A.; Schneerson, R.; Szu, S. C.; Vann, W. F.; Shiloach, J.; Karakawa, W. W.; Robbins, J. B., Synthesis and immunologic properties in mice of vaccines composed of Staphylococcus aureus type 5 and type 8 capsular polysaccharides conjugated to Pseudomonas aeruginosa exotoxin A. *Infect. Immun.* **1990**, *58*, 2367-2374.

11. Freese, S. J.; Anderson, A.; Pavliak, V.; Jansen, K. U.; Scully, I. L.; Scott, T. D.; Nanra, J. S.; Prasad, A. K.; Green, B. A. Compositions and methods for preparing *Staphylococcus aureus* serotype 5 and 8 capsular polysaccharide conjugate immunogenic compositions. US9125951B2, 2015.
12. Demchenko, A. V.; Malysheva, N. N.; De Meo, C., S-Benzoxazolyl (SBox) glycosides as novel, versatile glycosyl donors for stereoselective 1,2-cis glycosylation. *Org. Lett.* **2003**, *5*, 455-458.
13. Kamat, M. N.; De Meo, C.; Demchenko, A. V., S-Benzoxazolyl as a stable protecting moiety and a potent anomeric leaving group in oligosaccharide synthesis. *J. Org. Chem.* **2007**, *72*, 6947-6955.
14. Demchenko, A. V.; De Meo, C., semi-Orthogonality of O-pentenyl and S-ethyl glycosides: application for the oligosaccharide synthesis. *Tetrahedron Lett.* **2002**, *43*, 8819-8822.
15. Nilsson, M.; Svahn, C.-M.; Westman, J., Synthesis of the methyl glycosides of a tri- and a tetra-saccharide related to heparin and heparan sulphate. *Carbohydr. Res.* **1993**, *246*, 161-172.
16. Demchenko, A. V.; Kamat, M. N.; De Meo, C., S-Benzoxazolyl (SBox) glycosides in oligosaccharide synthesis: novel glycosylation approach to the synthesis of b-D-glucosides, b-D-galactosides, and a-D-mannosides. *Synlett* **2003**, 1287-1290.
17. Bayley, H.; Standring, D. N.; Knowles, J. R., Propane-1,3-dithiol: a selective reagent for the efficient reduction of alkyl and aryl azides to amines. *Tetrahedron Lett.* **1978**, *19*, 3633-3634.

18. Zulueta, M. M.; Lin, S. Y.; Lin, Y. T.; Huang, C. J.; Wang, C. C.; Ku, C. C.; Shi, Z.; Chyan, C. L.; Irene, D.; Lim, L. H.; Tsai, T. I.; Hu, Y. P.; Arco, S. D.; Wong, C. H.; Hung, S. C., α -Glycosylation by D-glucosamine-derived donors: synthesis of heparosan and heparin analogues that interact with mycobacterial heparin-binding hemagglutinin. *J. Am. Chem. Soc.* **2012**, *134*, 8988-8995.
19. Kamat, M. N.; Demchenko, A. V., Revisiting the armed-disarmed concept rationale: chemoselective activation of the S-benzoxazolyl glycosides in oligosaccharide synthesis. *Org. Lett.* **2005**, *7*, 3215-3218.

CHAPTER 4

**The synthesis of connecting
disaccharides between repeating units
of capsular polysaccharides**

***Staphylococcus aureus* type 5 and 8**

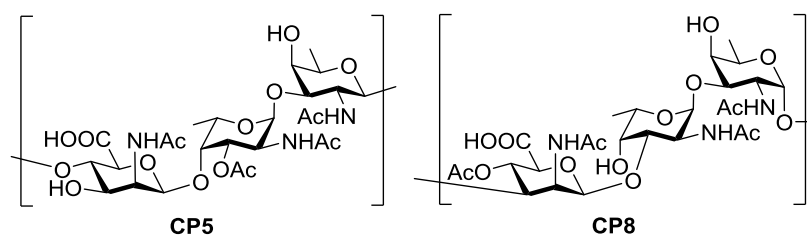
4.1 Introduction

Staphylococcus aureus (*S. aureus*), a Gram-positive bacterium, is a major opportunistic pathogen known to cause infections in newborns and patients with chronic conditions such as diabetes and cancer.¹ The emerging antibiotic resistance of the bacteria has seriously affected the global health system. Methicillin-resistant *S. aureus* (MRSA) strain is an example of a “superbug”, which shows resistance to several antibiotic drugs.² Due to the continuous occurrences of drug resistance incidents, the development of new antibiotics is becoming less effective. Prophylaxis with active and passive immunization of patients has been emerging as a promising alternative to control and prevent infections caused by MRSA.³ In particular, immunotherapies based on capsular polysaccharides of *S. aureus* have been proposed as promising targets for the development of glycoconjugate vaccines.⁴

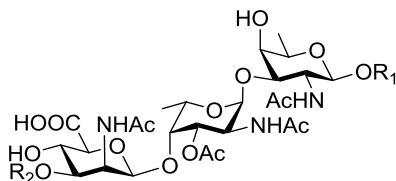
Capsular polysaccharides cover the outer part of *S. aureus* of which 13 serotypes have been described so far.⁵ A majority of *S. aureus* strains express capsular polysaccharide serotype 5 (CP5) and serotype 8 (CP8).⁶ Polysaccharides of these two serotypes have different sequences composed of the serotype-specific repeating units of trisaccharides. The chemical composition of these repeating units has been identified.⁷⁻⁹ The trisaccharide repeating unit of CP5 consists of the terminal 2-*N*-acetyl-D-mannosamine uronic acid (D-ManNAcA) residue, which is β -(1 \rightarrow 4)-glycosidically linked to 2,3-di-*N,O*-acetyl-L-fucosamine (3Ac-L-FucNAc). In turn, the latter is connected to 2-*N*-acetyl-D-fucosamine (D-FucNAc) residue by a α -(1 \rightarrow 3)-linkage. In the bacterial polysaccharide sequence, the D-FucNAc unit is then connected to another repeating unit via a β -(1 \rightarrow 4) linkage to D-ManNAcA (Scheme 4.1). The CP8

trisaccharide repeating unit contains 2,4-di-N,O-acetyl-D-mannosamine uronic acid (4Ac-D-ManNAcA) residue, which is β -(1 \rightarrow 3)-glycosidically linked to 2-N-acetyl-L-fucosamine (L-FucNAc). In turn, the latter is connected a D-FucNAc residue by a α -(1 \rightarrow 3)-linkage. In the bacterial polysaccharide sequence, the D-FucNAc unit is then connected to another repeating unit via an α -(1 \rightarrow 3)-linkage to D-ManNAcA (Scheme 4.1).

Scheme 4.1. Chemical structures of the trisaccharide repeating units of CP5 and CP8.



The chemical syntheses of both serotype-specific trisaccharide repeating units have been accomplished by several research groups. The CP5 trisaccharide synthesis has been achieved by Adamo in 2012,¹⁰ Boons in 2015,¹¹ our group in 2016,¹² and Codee in 2017.¹³ All approaches except ours targeted the trisaccharide repeating unit equipped with the conjugation-amenable spacer group at the anomeric center of the reducing end (Scheme 4.2). The structure accomplished by Adamo included an aminopropyl spacer at the anomeric center,¹⁰ whereas both Boons and Codee installed an aminopentyl linker.^{11,13} Our target trisaccharide was designed to study its conjugation to the CRM₁₉₇ protein that takes place away from the terminus.¹⁴ Therefore methyl groups were introduced in our trisaccharide target at the points of propagation of the polysaccharide sequence (Scheme 4.2).

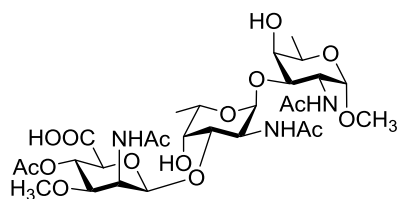
Scheme 4.2. Previously synthesized CP5 trisaccharides.¹⁰⁻¹³

$R_1 = (\text{CH}_2)_3\text{NHAc}$, $R_2 = \text{H}$; Adamo et al. (2012)

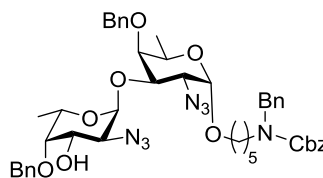
$R_1 = (\text{CH}_2)_5\text{NH}_2$, $R_2 = \text{H}$; Boons et al. (2015), Codee et al. (2017)

$R_1 = \text{CH}_3$, $R_2 = \text{CH}_3$; Demchenko et al. (2016)

The CP8 trisaccharide synthesis has been achieved by our group in 2015¹⁵ and a partial sequence was reported by Codee in 2017¹³ (Scheme 4.3). Our target also included methyl groups at the points of propagation of the polysaccharide sequence.

Scheme 4.3. Previously synthesized CP8 oligosaccharides.^{13, 15}

Demchenko et al. (2015)

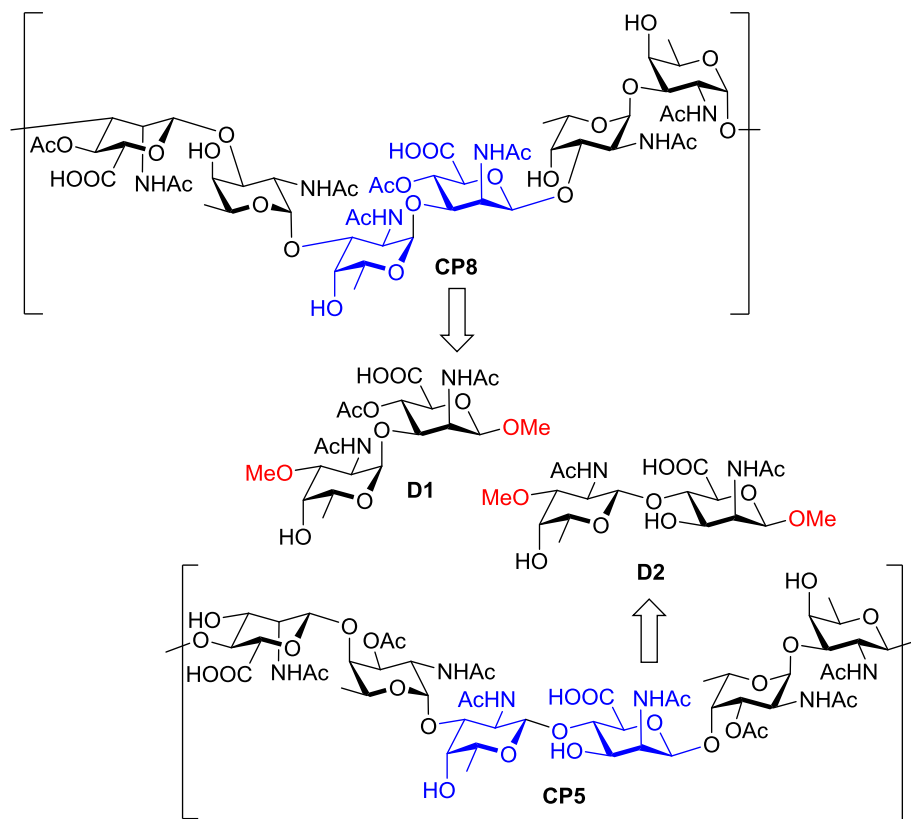


Codee et al. (2017)

To gain a better understanding of the conjugation chemistry taking place at the interface of the trisaccharide repeating units we turned our attention to the synthesis of disaccharides **D1** and **D2** representing the connection between the repeating units of CP8 and CP5, respectively (Scheme 4.4). Based on the structures of the disaccharide subunits, the CP8-derived disaccharide **D1** consists of a D-FucNAc unit linked to 4-O-Ac-D-ManNAcA via an α -(1 \rightarrow 3)-glycosidic linkage. The CP5-derived disaccharide **D2** contains the same monosaccharide components, but they are connected via a β -(1 \rightarrow 4)-glycosidic linkage and lack an *O*-acetyl group. This Chapter describes the synthesis of

two disaccharides corresponding to the connection point of two repeating units of *S. aureus* CP5 and CP8. As in our previous syntheses of trisaccharide repeating units, the potential propagation positions will be blocked with methyl groups.

Scheme 4.4. Structures of disaccharides D1 and D2 derived from the respective CPs.



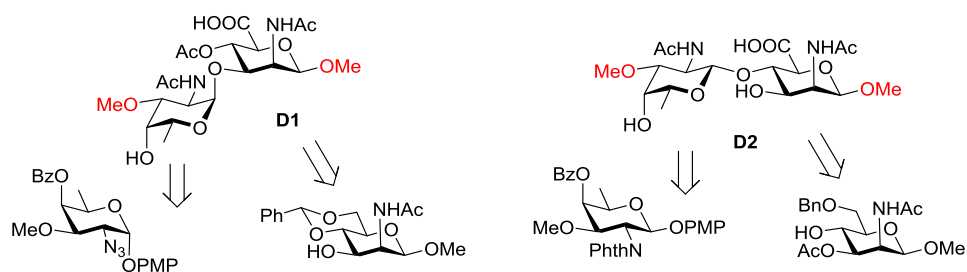
4.2 Results and Discussion

4.2.1 Retrosynthetic analysis of disaccharides D1 and D2

Although the disaccharide targets contain the same monosaccharide components, the retrosynthetic considerations to access each disaccharide differ. For instance, to control the stereoselectivity of α -glycosylation *en route* to **D1**, it is necessary to use a combination of the azido group at C-2 and a benzoyl group at C-4 of the D-fucosamine donor. Conversely, the use of 2-*N*-phthalimido group protection of the D-fucosamine

donor should be sufficient to ensure β -stereoselectivity for the synthesis of **D2** due to the neighboring group participation. Mannosyl acceptors for the synthesis of **D1** and **D2** will also differ to reflect the differential connection and substitution pattern. For the synthesis of **D1**, we chose a 4,6-*O*-benzylidene ManNAc derivative and for **D2** we decided to employ a 6-OBn protecting group that can provide a straightforward access to the primary hydroxyl group later in the synthesis (Scheme 4.5). For the synthesis of **D1** and **D2** disaccharides, we decided to synthesize D-fucosyl donor containing *p*-methoxyphenol (PMP) as the protecting group at the anomeric center which can be removed selectively by ceric ammonium nitrate (CAN)¹⁶ to install a suitable anomeric leaving group later in the synthesis. For the D-mannosamine acceptor synthesis, we decided to adapt the *O*-trichloroacetimidoyl protecting group migration approach developed by van der Marel¹⁷ for the one-pot intramolecular epimerization at C-2 to switch from the *D*-*gluco* to the *D*-*manno* configuration.

Scheme 4.5. Retrosynthetic considerations.

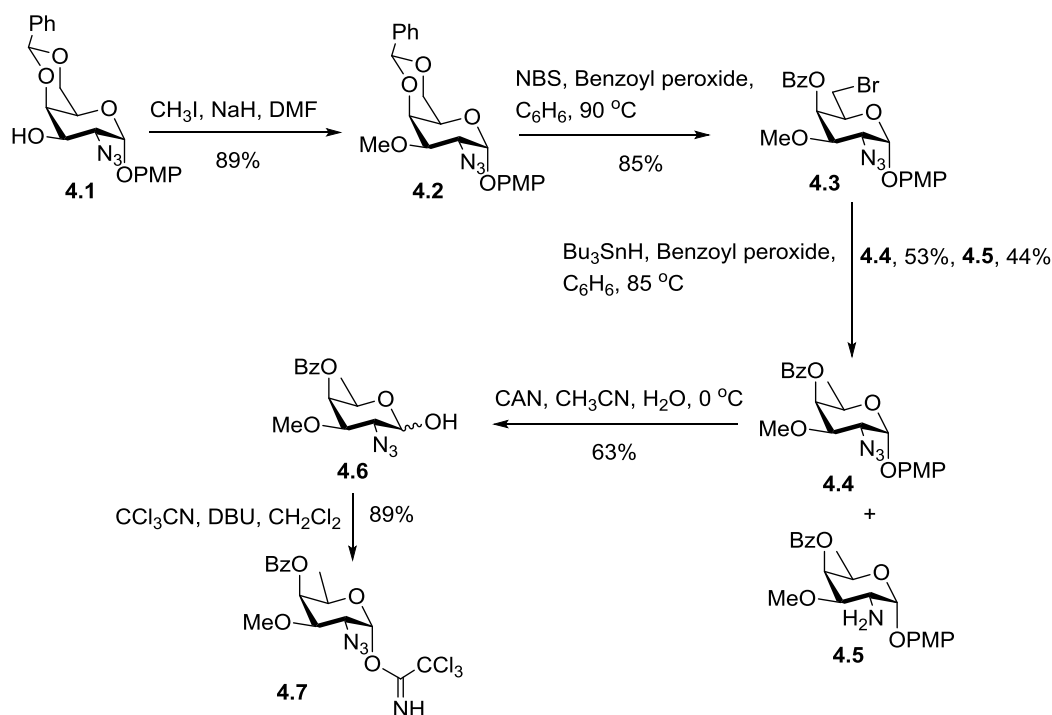


4.2.2 Synthesis of D-fucosyl donors (4.7 and 4.14)

The synthesis of D-fucosamine donor required for the assembly of **D1** was originated from known galactosamine **4.1**¹⁸ obtained from D-galactopyranose in eight steps. The methylation of compound **4.1** with methyl iodide in the presence of sodium

hydride yielded the methylated compound **4.2** in 89% yield (Scheme 4.6). Then, the 4,6-*O*-benzylidene group of **4.2** was oxidatively opened by performing the Hanessian–Hullar reaction.¹⁹ This reaction proceeded smoothly in the presence of *N*-bromosuccinimide (NBS) and benzoyl peroxide in benzene at 85 °C to afford the kinetic product²⁰ **4.3** in 85% yield.

Scheme 4.6. Synthesis of fucosyl donor **4.7**.

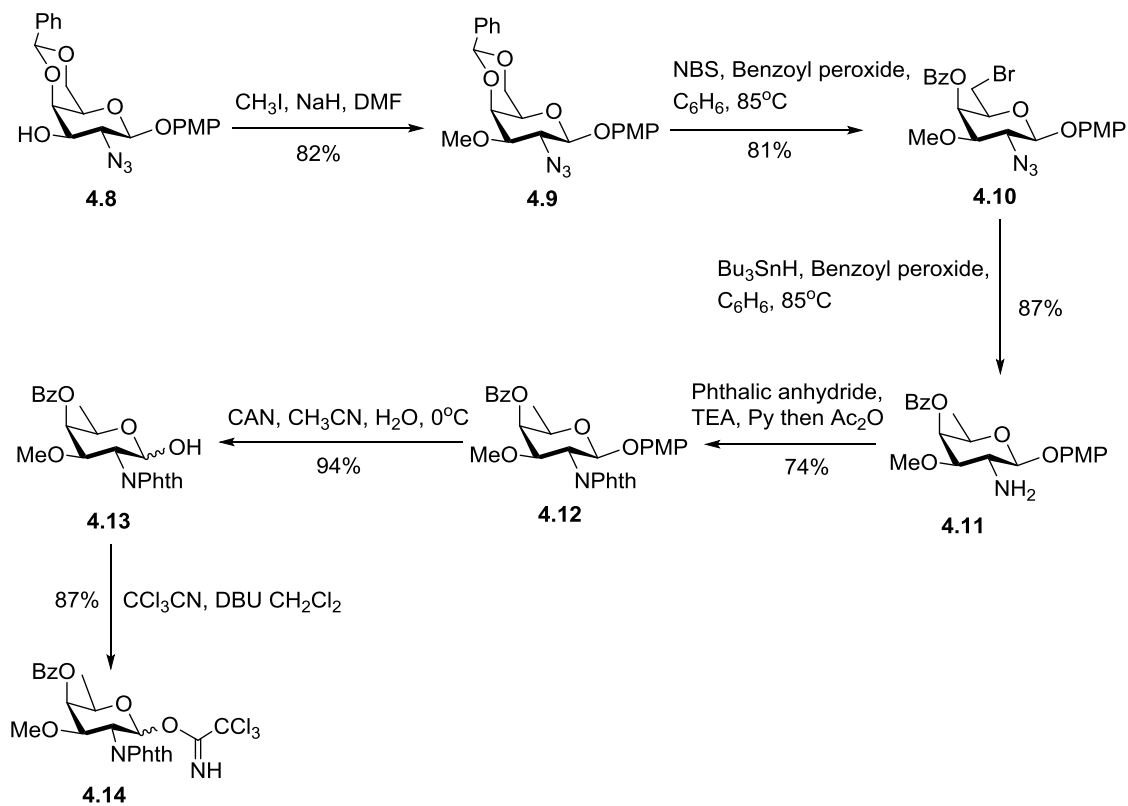


After that, compound **4.3** was reduced with tributyl tin(IV) hydride (Bu_3SnH) in the presence of benzoyl peroxide in benzene at $85\text{ }^\circ\text{C}$. Controlling the chemoselectivity of debromination versus the reduction of the azide group was proven challenging and as a result a mixture of 2-azido (**4.4**, 53% yield) and 2-amino (**4.5**, 44% yield) compounds was obtained. The compounds could easily be separated by column chromatography and the synthesis was continued with compound **4.4** isolated in 53% yield. *p*-Methoxyphenyl

(PMP) at the anomeric center was removed using ceric ammonium nitrate (CAN) to afford hemiacetal **4.6** in 63% yield. The latter was treated with trichloroacetonitrile in the presence of DBU to afford fucosyl donor **4.7** in 89% yield.

For the synthesis of D-fucosamine donor required for the assembly of **D2**, a known galactosamine derivative **4.8**²¹ was methylated with CH₃I in the presence of NaH to afford compound **4.9** in 82% yield (Scheme 4.7). The latter was subjected to the Hanessian–Hullar reaction¹⁹ in the presence of NBS and benzoyl peroxide in benzene at 85 °C to yield 6-bromo derivative **4.10** in 81% yield. Subsequent debromination and azide reduction were performed concomitantly in the presence of excess Bu₃SnH and benzoyl peroxide in benzene at 85 °C to give mannosamine **4.11** in 87% yield.

Scheme 4.7. Synthesis of D-fucosyl donor 4.13.



The amine group of **4.11** was then protected with phthalic anhydride in the presence of TEA. Subsequent acetylation with Ac₂O in the presence of pyridine afforded compound **4.12** in 74% yield. After that, the PMP group was selectively removed with CAN in wet CH₃CN to afford hemiacetal **4.13** in 94% yield. The latter was then reacted with trichloroacetonitrile in the presence of DBU to afford fucosyl donor **4.14** in 87% yield.

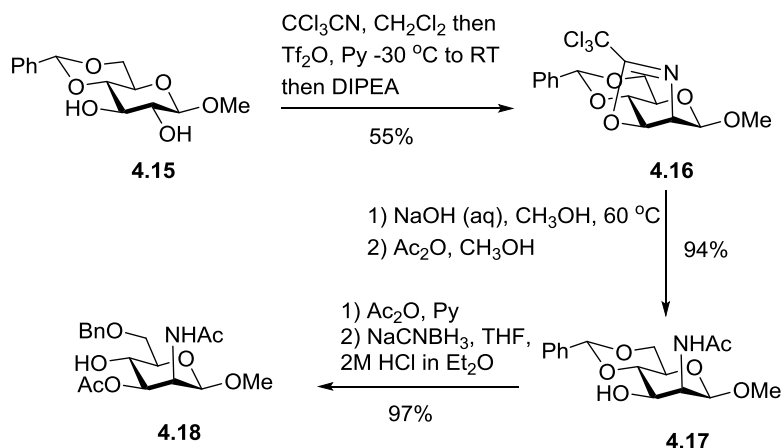
4.2.3 Synthesis of D-mannosyl acceptors (**4.17** and **4.18**)

The synthesis of D-mannosamine derivatives **4.17** and **4.18** was initiated by the selective introduction of the trichloroacetimidoyl group at C-3 of compound **4.15**.²² As depicted in Scheme 4.8, this transformation was accomplished with trichloroacetonitrile (CCl₃CN) in the presence of DBU in CH₂Cl₂, followed by the triflylation at 2-OH with triflic anhydride in the presence of pyridine at -30 °C. After that, *N,N*-diisopropylethylamine (DIPEA) was added to enhance the stereocenter inversion at C-2 to form 2,3-*N,O*-oxazoline intermediate **4.16** in 55% yield over three steps. The oxazoline ring was opened by the treatment with an aqueous sodium hydroxide in methanol at 60 °C. Subsequent *N*-acetylation with acetic anhydride (Ac₂O) in methanol afforded compound **4.17** in 94% yield over two steps. Compound **4.17** will be used as the glycosyl acceptor for the synthesis of disaccharide **D1**.

In addition, compound **4.17** was subjected to C-3 acetylation in the presence of Ac₂O in pyridine, followed by selective benzylidene ring opening with sodium cyanoborohydride (NaCNBH₃) in 2 M hydrogen chloride in diethyl ether in THF to

afford compound **4.18** in 97% yield over two steps. The latter compound will be used as the glycosyl acceptor for the synthesis of disaccharide **D2**.

Scheme 4.8. Synthesis of D-mannosyl acceptors **4.17** and **4.18**.

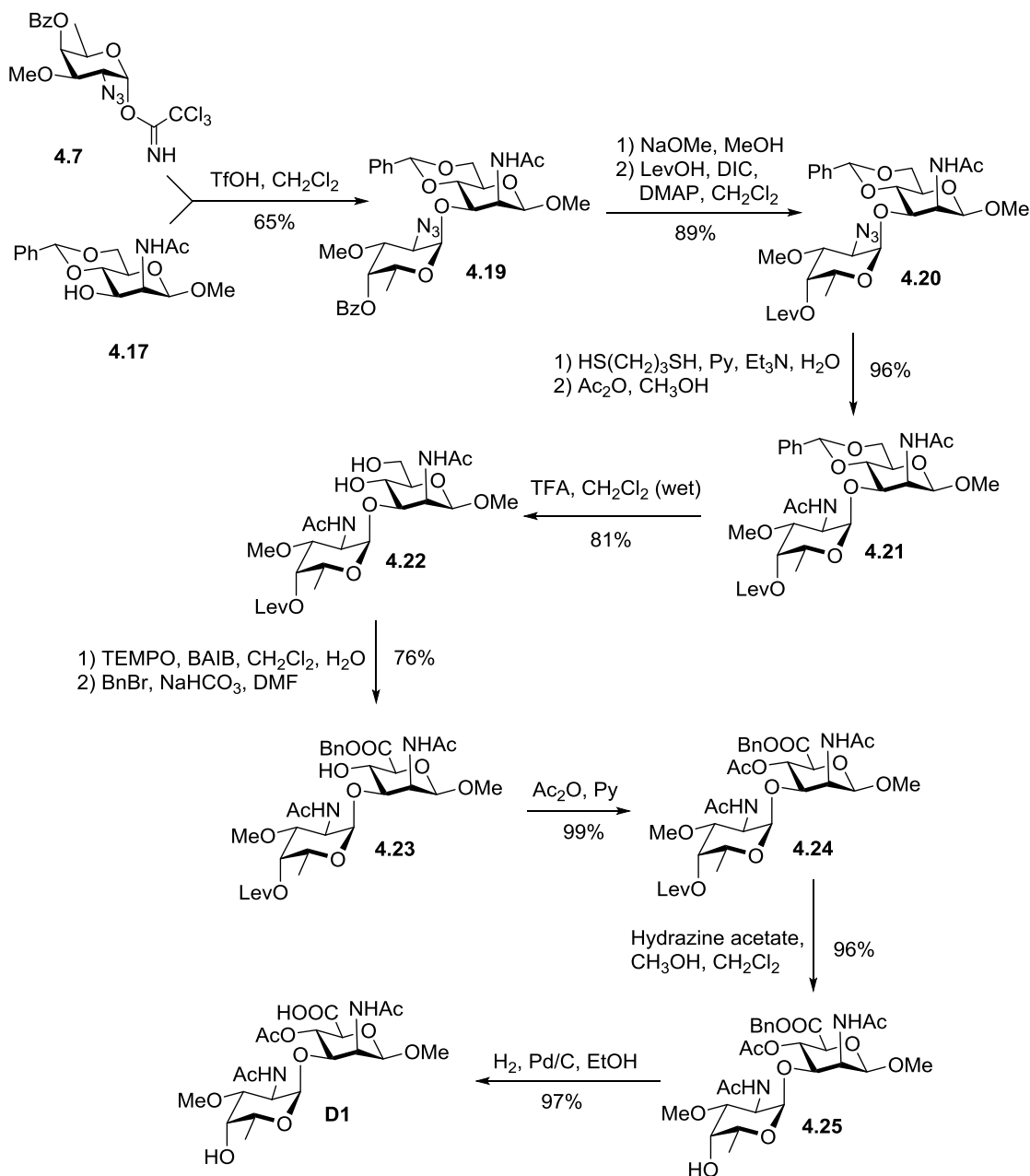


4.2.4 The assembly and functionalization of CP8 disaccharide (**D1**)

With fucosyl donor **4.7** and mannosyl acceptor **4.17** in hand, we performed the glycosylation reaction in the presence of trifluoromethanesulfonic acid (TfOH) as the promoter in CH_2Cl_2 to obtain disaccharide **4.19** in 65% yield with exclusive α -stereoselectivity (Scheme 4.9). Debenzoylation with sodium methoxide (NaOMe) followed by the treatment with levulinic acid (LevOH) in the presence of N,N' -diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP) afforded disaccharide **4.20** in 89% yield over two steps. Then, the azide group in **4.20** was reduced with 1,3-propanedithiol in the presence of triethylamine (TEA) in wet pyridine. This was followed by acetylation with Ac_2O in methanol to give disaccharide **4.21** in 96% yield over two steps. The latter was then subjected to the treatment with trifluoroacetic acid (TFA) in wet CH_2Cl_2 to acquire diol **4.22** in 81% yield. After that, the primary hydroxyl

was selectively oxidized with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and (diacetoxyiodo)benzene (BAIB) in wet CH_2Cl_2 .

Scheme 4.9. Assembly and functionalization of CP8 disaccharide D1.



The resulting carboxyl group was protected as a benzyl carboxylate by reaction with benzyl bromide (BnBr) in the presence of NaHCO_3 in DMF to afford disaccharide **4.23** in

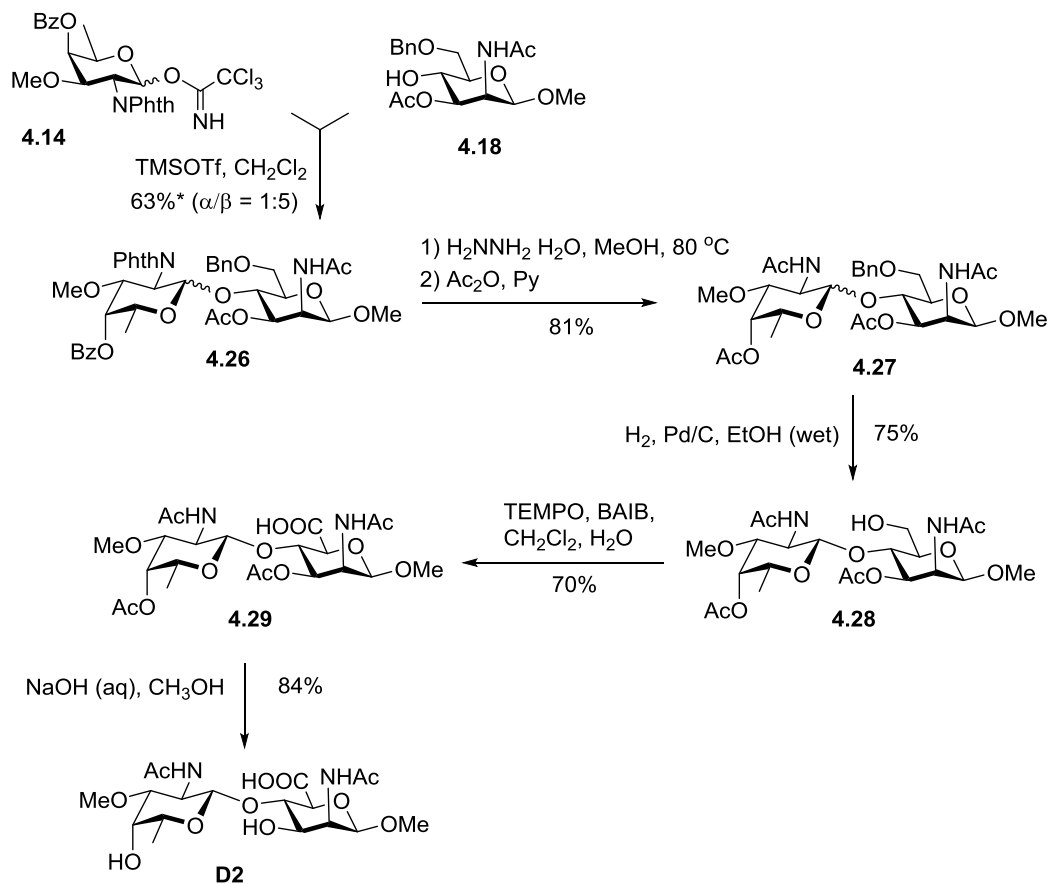
76% yield over two steps. Then, the 4-OH group of compound **4.23** was acetylated with Ac₂O in pyridine to give disaccharide **4.24** in 99% yield. Selective removal of the levulinoyl ester in **4.24** was accomplished in the presence of hydrazine acetate in methanol-CH₂Cl₂ in 96% yield. Finally, the benzyl ester was removed by hydrogenation with 10% palladium on charcoal in wet ethanol to afford the target disaccharide **D1** in 97% yield.

4.2.5 The assembly and functionalization of CP5 disaccharide (**D2**)

The assembly of CP5 disaccharide **D2** began with the glycosylation between fucosyl donor **4.14** and mannosyl acceptor **4.18**. This reaction was performed in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the promoter in CH₂Cl₂ at 0 °C to afford disaccharide **4.26** in 65% yield (Scheme 4.10). Although the 2-phthalimido group of fucosyl donor **4.14** had been installed to provide high β -stereoselectivity of the glycosylation via neighboring group participation, a substantial amount of the α -anomer of **4.26** was also obtained ($\alpha/\beta = 1/5$). This somewhat unexpected result could be rationalized by a double stereodifferentiation effect²³ that might be taking place due to the 4-OBz group of fucosyl donor **4.14** and the bulky phthalimido group of the glycosyl acceptor. The phthalimide group in α,β -**4.26** was removed in the presence of hydrazine hydrate in methanol at 80 °C. It was observed that all other ester groups were also removed. Therefore, the resulting compound was globally acetylated to afford disaccharide α,β -**4.27** in 81% yield over two steps. Then, the benzyl ether at C-6 of compound **4.27** was removed by hydrogenation in the presence of 10% palladium on charcoal in wet ethanol. At this stage, the α,β -anomers were separated by

column chromatography to afford pure β -linked disaccharide **4.28** in 75% yield. The primary hydroxyl group of **4.28** was oxidized with TEMPO and BAIB in wet CH_2Cl_2 to acquire carboxylic acid **4.29** in 70% yield. Finally, O-acetates were removed with NaOH in aqueous methanol to afford the target disaccharide **D2** in 84% yield (Scheme 4.10).

Scheme 4.10. Assembly and functionalization of CP5 disaccharide D2.



4.3 Conclusions and Outlook

The synthesis of disaccharide connecting units of *S. aureus* CP5 and CP8 with methyl groups at the point of propagation of the polysaccharide sequence have been reported. These compounds will be used to study their conjugation to the carrier protein conjugation to understand the sites of activation for conjugation and side reactions that

can occur during the conjugation process. With better understanding of the activation and conjugation process, it will be possible to improve, control, predict and reliably reproduce the outcome of conjugation of large bacterial polysaccharides. In turn, these studies are expected to benefit the area of anti-staphylococcal vaccine development in general.

4.4 Experimental

4.4.1 General Methods

The reactions were performed using commercial reagents (Aldrich or Acros), and the ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F₂₅₄ (EM Science). 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. CH₂Cl₂ and ClCH₂CH₂Cl were distilled from CaH₂ directly prior to application. 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 on 'Jasco P-2000' polarimeter. ¹H NMR spectra were recorded at 300 MHz (Bruker Avance) or 600 MHz (Agilent), ¹³C NMR spectra were recorded at 75 MHz (Bruker Avance) or 150 MHz (Agilent). The ¹H chemical shifts are referenced to the signal of the residual CHCl₃ (δ_H = 7.27 ppm) for solutions in CDCl₃ or the signal of the residual CH₃OH (δ_H = 3.31 ppm) for solutions in CD₃OD. The ¹³C chemical shifts are referenced to the central signal of CDCl₃ (δ_C = 77.23 ppm) for solutions in CDCl₃ or the

central signal of CD₃OD ($\delta_C = 49.24$ ppm) for solutions in CD₃OD. HRMS determinations were made with the use of JEOL MStation (JMS-700) mass spectrometer.

4.4.2 Synthesis of D-fucosyl donors 4.7 and 4.14

4-Methoxyphenyl 2-azido-4,6-O-benzylidene-2-deoxy-3-O-methyl- α -D-galactopyranoside (4.2). Methyl iodide (1.75 g, 12.32 mmol) and sodium hydride (739 mg, 18.48 mmol) were added to a stirring solution of 4-methoxyphenyl-2-azido-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (**4.1**,¹⁸ 2.46 g, 6.16 mmol) in DMF (20 mL) at 0 °C. The reaction mixture was allowed to reach rt. After 1 h, the reaction mixture was poured into ice-water and the resulting mixture was stirred for 15 min. The aqueous phase was extracted with EtOAc/Et₂O (1/1, v/v, 3 x 100 mL). The organic extracts were combined, dried over MgSO₄, 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 (2.26 g, 89%). Analytical data for **4.2**: $R_f = 0.54$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{22} 175.3$ (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 3.60, 3.78 (2 s, 6H, 2 x OCH₃), 3.85 (br. s, 1H, H-5) 3.96-4.06 (m, 2H, $J_{2,3} = 10.7$ Hz, , $J_{3,4} = 2.3$ Hz, H-2, 3), 4.08 (dd, 1H, $J_{5,6a} = 1.6$ Hz, $J_{6a,6b} = 12.6$ Hz, H-6a), 4.28 (dd, 1H, $J_{5,6b} = 1.4$ Hz, H-6b), 4.49 (dd, 1H, $J_{4,5} = 2.1$ Hz, H-4), 5.59 (d, 1H, $J_{1,2} = 3.0$ Hz, H-1), 5.62 (s, 1H, >CHPh), 6.83 (d, 2H, $J = 9.1$ Hz, PMP), 7.05 (d, 2H, $J = 9.1$ Hz, PMP), 7.36-7.38 (m, 3H, aromatic), 7.52-7.55 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 55.8, 56.9, 58.6, 63.6, 69.5, 72.3, 76.0, 98.1, 101.2, 114.8 (x 2), 117.7 (x 2), 126.4 (x 2), 128.4 (x 2), 129.2, 137.6, 150.7, 155.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for C₂₁H₂₃N₃O₆ 436.1484, found 436.1476.

4-Methoxyphenyl 2-azido-4-O-benzoyl-6-bromo-2,6-dideoxy-3-O-methyl- α -D-galactopyranoside (4.3). N-Bromosuccinimide (419 mg, 2.36 mmol) and benzoyl peroxide (29 mg, 0.12 mmol) were added to a solution of **4.2** (974 mg, 2.36 mmol) in benzene (20 mL) and the resulting mixture was heated at reflux for 0.5 h. After that, the volatiles were removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~150 mL) and washed with water (20 mL), 10% aq. Na₂S₂O₃ (20 mL), and water (20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white foam (990 mg, 85%). Analytical data for **4.3**: R_f = 0.64 (ethyl acetate/hexane, 2/3, v/v); [α]_D²³ 174.8 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 3.39-3.42 (m, 2H, H-6a, 6b), 3.57 (s, 3H, OCH₃), 3.78 (dd, 1H, J_{2,3} = 10.7 Hz, H-2), 3.80 (s, 3H, OCH₃), 4.06 (dd, 1H, J_{3,4} = 2.2 Hz, H-3), 4.39-4.44 (m, 1H, H-5), 5.51 (d, 1H, J_{1,2} = 3.5 Hz, H-1), 5.98 (dd, 1H, J_{4,5} = 2.1 Hz, H-4), 6.86 (d, 2H, J = 9.1 Hz, PMP), 7.11 (d, 2H, J = 9.1 Hz, PMP), 7.47-7.52 (m, 2H, aromatic), 7.59-7.65 (m, 1H, aromatic), 8.07-8.10 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 29.2, 55.8, 57.8, 59.3, 67.1, 70.6, 76.5, 98.6, 114.9 (x 2), 118.6 (x 2), 128.8 (x 2), 129.3, 130.1 (x 2), 133.8, 150.8, 155.8, 165.6 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₁H₂₂BrN₃O₆Na 514.0590, found 514.0571.

4-Methoxyphenyl 2-azido-4-O-benzoyl-2,6-dideoxy-3-O-methyl- α -D-galactopyranoside (4.4) and 4-methoxyphenyl 2-amino-4-O-benzoyl-2,6-dideoxy-3-O-methyl- α -D-galactopyranoside (4.5). Tributyl tin hydride (2.46 mL, 9.14 mmol) and

benzoyl peroxide (123 mg, 0.38 mmol) were added to a solution of **4.3** (3.75 g, 7.62 mmol) in benzene (50 mL) and the resulting mixture was heated at reflux for 3 h. After that, the volatiles were removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~300 mL) and washed with water (60 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - toluene gradient elution) to yield individual compounds **4.4** (1.66 g, 53%) as a syrup and **4.5** (1.31 g, 44%) as a clear syrup. Analytical data for **4.4**: $R_f = 0.62$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{22}$ 151.9 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.21 (d, 3H, H-6), 3.55 (s, 3H, OCH₃), 3.79-3.85 (m, 4H, $J_{2,3} = 10.7$ Hz, OCH₃, H-2), 4.06 (dd, 1H, $J_{3,4} = 2.5$ Hz, H-3), 4.32 (m, 1H, $J_{5,6} = 6.5$ Hz, H-5), 5.50 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 5.71 (dd, 1H, $J_{4,5} = 2.5$ Hz, H-4), 6.85 (d, 2H, $J = 9.0$ Hz, PMP), 7.05 (d, 2H, $J = 9.1$ Hz, PMP), 7.46-7.51 (m, 2H, aromatic), 7.58-7.64 (m, 1H, aromatic), 8.08-8.10 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 16.6, 55.8, 57.5, 59.3, 66.3, 69.5, 76.6, 98.3, 114.9 (x 2), 118.2 (x 2), 128.7 (x 2), 129.7, 130.1 (x 2), 133.6, 150.9, 155.5, 166.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for C₂₁H₂₃O₆N₃ 436.1485, found 436.1480. Analytical data for **4.5**: $R_f = 0.57$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{23}$ 133.0 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.18 (d, 3H, H-6), 3.28 (dd, 1H, $J_{2,3} = 10.3$ Hz, H-2), 3.47 (s, 3H, OCH₃), 3.56 (dd, 1H, $J_{3,4} = 2.2$ Hz, H-3), 3.75 (s, 3H, OCH₃), 4.26 (m, 1H, $J_{5,6} = 6.5$ Hz, H-5), 5.52 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1), 5.61 (dd, 1H, $J_{4,5} = 2.1$ Hz, H-4), 6.82 (d, 2H, $J = 9.1$ Hz, PMP), 7.04 (d, 2H, $J = 9.1$ Hz, PMP), 7.41-7.47 (m, 2H, aromatic), 7.53-7.58 (m, 1H, aromatic), 8.09-8.12 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 16.7, 51.3, 55.6, 57.4, 66.3, 69.0, 80.3, 99.8, 114.6 (x 2),

118.1 (x 2), 128.5 (x 2), 129.8, 129.9 (x 2), 133.3, 151.1 (x 2), 166.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{21}H_{25}O_6N_3Na$ 410.1580, found 410.1578.

2-Azido-4-O-benzoyl-2,6-dideoxy-3-O-methyl-D-galctopyranose (4.6). A solution of CAN (3.80 g, 6.89 mmol) in water (40 mL) was added dropwise to a stirring solution of **4.4** (1.14 g, 2.76 mmol) in CH_3CN (20 mL) at 0 °C. The reaction mixture was then allowed to reach rt. After 4 h, the volatiles were removed *in vacuo*. The residue was diluted with CH_2Cl_2 (~150 mL) and washed with water (2 x 30 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 yield the title compound as a white amorphous solid (532 mg, 63%). Analytical data for **4.6**: R_f = 0.50 (**α -4.6**), R_f = 0.38 (**β -4.6**, ethyl acetate/hexane, 2/3, v/v); 1H NMR (300 MHz, $CDCl_3$): δ , 1.19 (d, 3H, H-6 α), 1.26 (d, 3H, H-6 β), 3.30 (dd, 1H, $J_{3\beta,4\beta}$ = 3.33 Hz, H-3 β), 3.47 (m, 4H, OCH₃, OH α), 3.49 (s, 3H, OCH₃), 3.62 (dd, 1H, $J_{2\beta,3\beta}$ = 10.29 Hz, H-2 β), 3.74-3.84 (m, 2H, $J_{2\alpha,3\alpha}$ = 10.56 Hz, $J_{5\beta,6\beta}$ = 6.57 Hz, H-2 α , 5 β), 3.87 (dd, 1H, $J_{3\alpha,4\alpha}$ = 3.15 Hz, H-3 α), 4.09 (d, 1H, OH β), 4.37 (m, 1H, $J_{5\alpha,6\alpha}$ = 6.57 Hz, H-5 α), 4.62 (dd, 1H, $J_{1\beta,2\beta}$ = 13.08 Hz, H-1 β), 5.37 (dd, 1H, $J_{1\alpha,2\alpha}$ = 2.73 Hz, H-1 α), 5.53 (dd, 1H, $J_{4\beta,5\beta}$ = 2.79 Hz, H-4 β), 5.62 (dd, 1H, $J_{4\alpha,5\alpha}$ = 2.19 Hz, H-4 α), 7.44-7.57 (m, 4H, aromatic), 7.57-7.62 (m, 2H, aromatic), 8.08-8.09 (m, 4H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) **α -4.6**: δ , 16.6, 57.5, 60.0, 65.5, 69.6, 77.1, 92.5, 128.7 (x 2), 129.7, 130.1 (x 2), 133.6, 166.3 ppm; **β -4.6**: δ , 16.7, 58.0, 64.0, 68.5, 70.0, 80.9, 96.3, 128.7 (x 2), 129.5, 130.2 (x 2), 133.7, 166.3 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{14}H_{18}O_5N_3$ 308.1246, found 308.1268.

2-Azido-4-O-benzoyl-2,6-dideoxy-3-O-methyl- α -D-galactopyranosyl

trichloroacetimidate (4.7) To the solution of **4.6** (339 mg, 1.10 mmol) in dry CH_2Cl_2 (10 mL), trichloroacetonitrile (1.59 g, 11.03 mmol) and DBU (84 mg, 0.55 mmol) were added at rt and the resulting mixture was stirred for 30 min under argon. After that, the reaction was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white foam (440 mg, 89%). Analytical data for **4.7**: $R_f = 0.71$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{22}$ 126.2 (c = 1, CHCl_3); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.24 (d, 3H, H-6), 3.53 (s, 3H, OCH_3), 3.91-4.01 (m, 2H, $J_{3,4} = 2.8$ Hz, H-2, 3), 4.32 (m, 1H, $J_{5,6} = 6.5$ Hz, H-5), 5.71 (dd, 1H, $J_{4,5} = 1.2$ Hz, H-4), 6.44 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1), 7.47-7.57 (m, 2H, aromatic), 7.59-7.65 (m, 1H, aromatic), 8.08-8.12 (m, 2H, aromatic) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ , 16.6, 57.5, 58.8, 68.3, 70.0, 76.9, 91.2, 95.4, 128.8 (x 2), 129.5, 130.1 (x 2), 133.7, 161.0, 166.1 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{16}\text{H}_{17}\text{O}_5\text{N}_4\text{Cl}_3\text{Na}$ 473.0162, found 473.0126.

4-Methoxyphenyl**2-azido-4,6-O-benzylidene-2-deoxy-3-O-methyl- β -D-**

galactopyranoside (4.9). Methyl iodide (1.41 g, 9.91 mmol) and sodium hydride (595 mg, 14.88 mmol) were added to a stirring solution of 4-methoxyphenyl-2-azido-4,6-O-benzylidene-2-deoxy- β -D-galactopyranoside²¹ (**4.8**, 1.98 g, 4.96 mmol) in DMF (20 mL) at 0 °C. The reaction was allowed to reach rt. After 1 h, the reaction was poured into ice water and was stirred for 15 min. Then, the aqueous phase was extracted with EtOAc/Et₂O (1/1, v/v, 3 x 150 mL). The organic phase was collected, dried over 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 as a white amorphous solid (1.69 g, 82%). Analytical data for **4.9**: $R_f = 0.48$ (ethyl acetate/ hexane, 7/3, v/v); $[\alpha]_D^{23}$ 18.7 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 3.20-3.24 (dd, 1H, $J_{3,4} = 3.48$ Hz, H-3), 3.46 (br. s, 1H, H-5), 3.48, 3.74 (2 s, 6H, 2 x OCH₃), 3.98-4.06 (m, 2H, $J_{2,3} = 10.4$ Hz, $J_{5,6a} = 1.6$ Hz, $J_{6a,6b} = 12.5$ Hz, H-2, H-6a), 4.30 (dd, 1H, H-4), 4.32-4.36 (dd, 1H, $J_{5,6b} = 1.6$ Hz, H-6b), 4.71 (d, 1H, $J_{1,2} = 8.13$ Hz, H-1), 5.56 (s, 1H, CHPh), 6.78 (d, 2H, $J = 9.09$ Hz, PMP), 7.04 (d, 2H, $J = 9.09$ Hz, PMP), 7.31-7.34 (m, 3H, aromatic), 7.49-7.52 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 55.8, 57.5, 61.8, 66.8, 69.3, 71.6, 80.0, 101.4, 102.1, 114.6 (x 2), 119.2 (x 2), 126.6 (x 2), 128.4 (x 2), 129.3, 137.6, 151.2, 155.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for C₂₁H₂₃N₃O₆ 436.1484, found 436.1510.

4-methoxyphenyl 2-azido-4-O-benzoyl-6-bromo-2,6-dideoxy-3-O-methyl- β -D-galactopyranoside (4.10) N-bromosuccinimide (1.24 g, 6.97 mmol) and benzoyl peroxide (113 mg, 0.35 mmol) were added to a solution of **4.9** (2.88 g, 6.97 mmol) in benzene (70 mL) and the resulting mixture was refluxed for 2 h. After that, solvent was removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~300 mL) and washed with water (50 mL), 10% aq. Na₂S₂O₃ (50 mL), and water (50 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a clear syrup (2.78 g, 81%). Analytical data for **4.10**: $R_f = 0.62$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{23}$ 76.0 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ ,

3.32 (dd, 1H, $J_{3,4} = 2.6$ Hz, H-3), 3.47-3.51 (m, 5H, OCH₃, H-6a, 6-b), 3.88-3.98 (m, 2H, $J_{2,3} = 10.3$ Hz, H-2, 5), 4.79 (d, 1H, $J_{1,2} = 8.19$ Hz, H-1), 5.84 (dd, 1H, $J_{4,5} = 2.6$ Hz, H-4), 6.86 (d, 2H, $J = 9.1$ Hz, PMP), 7.13 (d, 2H, $J = 9.1$ Hz, PMP), 7.47-7.52 (m, 2H, aromatic), 7.60-7.66 (m, 1H, aromatic), 8.10-8.14 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 29.2, 55.8, 58.4, 62.4, 66.1, 74.5, 80.5, 102.0, 114.8 (x 2), 118.8 (x 2), 128.8 (x 2), 129.1, 130.3 (x 2), 133.9, 151.2, 156.0, 165.7 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₁H₂₂BrN₃O₆Na 514.0590, found 514.0572.

4-Methoxyphenyl **2-amino-4-O-benzoyl-2,6-dideoxy-3-O-methyl-β-D-galactopyranoside (4.11)**. Tributyl tin hydride (2.5 g, 8.59 mmol) and benzoyl peroxide (46 mg, 0.14 mmol) were added to a solution of **4.10** (1.41 g, 2.86 mmol) in benzene (20 mL) and the resulting mixture was heated at reflux for 16 h. After that, the volatiles were removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~ 200 mL) and washed with water (40 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a clear syrup (965 mg, 87%). Analytical data for **4.11**: $R_f = 0.5$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{23} -12.6$ (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.31 (d, 3H, C-6), 3.30 (dd, 1H, $J_{3,4} = 3.1$ Hz, H-3), 3.39-3.50 (m, 4H, $J_{2,3} = 10.2$ Hz, OCH₃, H-2), 3.78 (s, 3H, OCH₃), 3.88 (m, 1H, $J_{5,6} = 6.5$ Hz, H-5), 4.75 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1), 5.56 (dd, 1H, $J_{4,5} = 0.9$ Hz, H-4), 6.82 (d, 2H, $J = 9.1$ Hz, PMP), 7.04 (d, 2H, $J = 9.1$ Hz, PMP), 7.43-7.48 (m, 2H, aromatic), 7.55-7.58 (m, 1H, aromatic), 8.12-8.15 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 17.0, 52.8, 55.8, 57.8, 67.9, 70.0, 82.5, 103.9, 114.6 (x 2), 118.8 (x 2), 128.6 (x 2), 129.7,

130.2 (x 2), 133.4, 151.6, 155.5, 166.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{21}H_{25}O_6NNa$ 410.1580, found 410.1578.

4-Methoxyphenyl **4-O-benzoyl-2,6-dideoxy-3-O-methyl-2-phthalimido- β -D-**

galactopyranoside (4.12). To a solution of **4.11** (545 mg, 1.41 mmol) in pyridine (10 mL), phthalic anhydride (217 mg, 1.46 mmol) and triethylamine (0.20 mL, 1.41 mmol) were added at rt. The reaction was stirred under argon for 3h. After that, acetic anhydride was added (0.50 mL, 5.30 mmol). The resulting solution was stirred under argon overnight. Then, the solvent was removed *in vacuo*. The residue was diluted with CH_2Cl_2 (~120 mL) and 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 - hexane gradient elution) to yield the title compound as a white amorphous solid (538 mg, 74%). Analytical data for **4.12**: $R_f = 0.70$ (ethyl acetate/hexane, 7/3, v/v); $[\alpha]_D^{23}$ 135.0 (c = 1, $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$): δ , 1.35 (d, 3H, C-6), 3.32 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 4.08 (m, 1H, $J_{5,6} = 6.4$ Hz, H-5), 4.41 (dd, 1H, $J_{3,4} = 3.2$ Hz, H-3), 4.74 (dd, 1H, $J_{3,4} = 11.3$ Hz, H-2), 5.70-5.73 (m, 2H, $J_{1,2} = 8.5$ Hz, H-1, 4), 6.73 (d, 2H, $J = 9.1$ Hz, PMP), 6.88 (d, 2H, $J = 9.1$ Hz, PMP), 7.48-7.53 (m, 2H, aromatic), 7.57-7.58 (m, 1H, aromatic), 7.60-7.62 (m, 2H, aromatic), 7.81-7.92 (m, 2H, aromatic), 8.13-8.26 (m, 2H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ , 16.8, 52.8, 55.5, 57.5, 68.6, 69.9, 76.1, 98.0, 114.4 (x 2), 118.5 (x 2), 123.2, 123.7, 128.6 (x 2), 129.5, 130.1 (x 2), 131.5, 131.7, 133.4, 134.2, 134.3, 151.0, 155.4, 166.3, 167.8, 168.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{29}H_{27}O_8NaN$ 540.1634, found 540.1629.

4-O-Benzoyl-2,6-dideoxy-3-O-methyl-2-phthalimido-D-galactopyranose (4.13). A solution of CAN (1.43 g, 2.60 mmol) in water (10 mL) was added dropwise to a stirring solution of **4.12** (538 mg, 1.04 mmol) in CH₃CN (5 mL) at 0°C. The reaction was allowed to reach rt. After 4 h, the solvent was removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~80 mL) and washed with water (2 x 20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid (403 mg, 94%). Analytical data for **4.23**: R_f = 0.55 (ethyl acetate/hexane, 3/7, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.24 (d, 3H, H-6α), 1.30 (d, 3H, H-6β), 3.29 (s, 3H, OCH₃), 3.33 (s, 3H, OCH₃), 3.99 (m, 1H, J_{5β,6β} = 6.4 Hz, H-5β), 4.14 (d, 1H, J_{1β,2β} = 8.6 Hz, H-1β), 4.36 (d, 1H, J_{1α,2α} = 3.7 Hz, H-1α), 4.41 (m, 3H, OHα, OHβ, H-3 β), 4.52 (m, 1H, J_{5α,6α} = 6.9 Hz, H-5α), 4.75 (dd, 1H, J_{3α,4α} = 3.2 Hz, H-3α), 5.37 (dd, 1H, J_{2β,3β} = 8.4 Hz, H-2β), 5.46 (dd, 1H, J_{2α,3α} = 11.8 Hz, H-2α), 5.65 (dd, 1H, J_{4β,5β} = 1.4 Hz, H-4β), 5.73 (dd, 1H, J_{4α,5α} = 2.3 Hz, H-4α), 7.41-7.49 (m, 4H, aromatic), 7.53-7.57 (m, 2H, aromatic), 7.67-7.71 (m, 4H, aromatic), 7.84-7.87 (m, 4H, aromatic), 8.12-8.16 (m, 4H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃) **α-4.13**: 16.8, 52.6, 56.8, 65.5, 69.5, 71.7, 93.0, 123.4, 123.8, 128.6 (x 2), 129.7, 130.1, 130.2 (x 2), 131.8, 133.4 (x 2), 134.4, 166.5, 168.7, 169.0 δ, ppm; **β-4.13**: δ, 16.9, 54.9, 57.5, 68.7, 70.1, 75.9, 93.5, 123.6, 128.6 (x 2), 129.6, 130.1, 130.2 (x 2), 131.8 (x 2), 133.5, 134.3 (x 2), 166.5 (x 3) ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₂H₂₁O₇NaN 434.1216, found 434.1222.

4-O-Benzoyl-2,6-dideoxy-3-O-methyl-2-phthalimido-D-galactopyranosyl

trichloroacetimidate (4.14). To the solution of **4.13** (812 mg, 1.97 mmol) in dry CH₂Cl₂ (20 mL), trichloroacetonitrile (1.98 mL, 19.74 mmol) and DBU (0.15 mL, 0.50 mmol) were added at rt. The reaction was stirred for 30 min under argon. After that, it was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid (958 mg, 87%). Analytical data for **4.14**: R_f **α-4.14** = 0.64, **β-4.14** = 0.52 (ethyl acetate/hexane, 1/1, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.29 (d, 3H, H-6α), 1.36 (d, 3H, H-6β), 3.23, 3.41 (2s, 6H, 2 x OCH₃), 4.18 (m, 1H, J_{5β,6β} = 6.4 Hz, H-5β), 4.46-4.53 (m, 2H, J_{5α,6α} = 6.5 Hz, H-3β, 5α), 4.75 (dd, 1H, J_{2β,3β} = 11.2 Hz, H-2β), 4.84 (dd, 1H, J_{2α,3α} = 11.7 Hz, H-2α), 5.26 (dd, 1H, J_{3α,4α} = 3.1 Hz, H-3α), 5.73 (dd, 1H, J_{4β,5β} = 3.1 Hz, H-4β), 5.83 (dd, 1H, J_{4α,5α} = 2.8 Hz, H-4α), 6.47 (m, 2H, J_{1α,2α} = 3.1 Hz, J_{1β,2β} = 8.9 Hz, H-1α, 1β), 7.47-7.53 (m, 4H, aromatic), 7.57-7.63 (m, 2H, aromatic), 7.68-7.73 (m, 4H, aromatic), 7.80-7.84 (m, 4H, aromatic), 8.13-8.19 (m, 4H, aromatic), 8.58 (s, 1H, NH), 8.62 (s, 1H, NH) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.7, 16.8, 51.5, 52, 56.6, 57.8, 68.5, 68.6, 68.8, 71.2, 71.6, 76.0, 90.6, 91.0, 94.7, 95.8, 123.4, 123.5, 123.7, 128.8, 129.5, 130.1, 130.2, 133.6, 134.5, 161.3, 161.5, 163.9, 166.3, 166.5, 168.1, 175.0 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₄H₂₁O₇Na N₂Cl₃ 577.0312, found 577.0313.

4.4.3 Synthesis of D-mannosyl acceptors 4.17 and 4.18

Methyl 2-amino-4,6-O-benzylidene-2-deoxy-2,3-N,O-(2,2,2-trichloroethylylidene)-β-D-mannopyranoside (4.16). DBU (0.15 mL, 0.98 mmol) and trichloroacetonitrile (1.17 mL, 11.7 mmol) were added to a solution of methyl 4,6-O-benzylidene-β-D-

glucopyranoside²² (**4.15**, 2.76 g, 9.78 mmol) in dry CH₂Cl₂ (120 mL) and the resulting mixture was stirred under argon for 5 h at rt. After that, the reaction mixture was cooled to -30 °C, pyridine (3.94 mL, 48.9 mmol) and Tf₂O (1.96 mL, 11.7 mmol) were added, and the resulting mixture was stirred under argon for 2 h at -30 °C. The reaction mixture was then allowed to warm to rt, DIPEA (17.0 mL, 97.8 mmol) was added, and the resulting mixture was stirred under argon for 16 h at rt. After that, the volatiles were removed *in vacuo*, the residue was diluted with CH₂Cl₂ (~300 mL) and washed with water (2 x 40 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid (2.20 g, 55%). Analytical data for **4.16**: R_f = 0.50 (ethyl acetate/hexane, 2/3, v/v); [α]_D²² -76.5 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 3.46 (s, 3H, OCH₃), 3.64 (m, 1 H, J_{5,6a} = 10.1 Hz, J_{5,6b} = 4.4 Hz, H-5), 3.72 (dd, 1H, J_{6a,6b} = 10.0 Hz, H-6a), 4.38 (dd, 1H, H-6b), 4.56-5.65 (m, 2H, H-2, 4), 4.92 (d, 1H, J_{1,2} = 3.5 Hz, H-1), 5.02 (dd, 1H, J_{2,3} = 7.4 Hz, J_{3,4} = 9.8 Hz, H-3), 5.61 (s, 1H, CHPh), 7.34-7.42 (m, 3H, aromatic), 7.46-7.55 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 56.1, 64.5, 67.4, 70.3, 76.9, 82.6, 86.5, 97.5, 101.8, 126.4 (x 2), 128.5 (x 2), 129.5, 136.9, 164.7 ppm; HR-FAB MS: [M+Na]⁺ calcd for C₁₆H₁₆Cl₃NO₅Na, 429.9992; found, 430.0003.

Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-mannopyranoside (4.17).

A 1 M aq. soln. of NaOH (10 mL) was added dropwise to a solution of **4.16** (63 mg, 0.16 mmol) in MeOH (10 mL) and the resulting mixture was refluxed for 12 h. The reaction mixture was then allowed to cool to rt, Amberlite IR120 resin was added until pH = 7.

The resin was filtered off, rinsed successively with MeOH (7 x 5 mL), and the combined filtrate was concentrated *in vacuo*. The residue was dissolved in MeOH (10 mL), Ac₂O (0.5 mL, 5.30 mmol) was added, and the 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 (methanol - dichloromethane gradient elution) to yield the title compound as a white amorphous solid (50 mg, 94%). Analytical data for **4.17**: $R_f = 0.50$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{22} -42.0$ (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 2.11 (s, 3H, COCH₃), 3.46 (m, 1H, $J_{5,6a} = 10.2$ Hz, $J_{5,6b} = 4.9$ Hz, H-5), 3.52 (s, 3H, OCH₃), 3.70 (dd, 1H, $J_{4,5} = 9.4$ Hz, H-4), 3.80 (dd, 1H, $J_{6a,6b} = 10.2$ Hz, H-6a), 4.00 (dd, 1H, $J_{3,4} = 9.5$ Hz, H-3), 4.34 (dd, 1 H, H-6b), 4.52 (br dd, 1H, $J_{2,3} = 4.0$ Hz, H-2), 4.64 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 5.57 (s, 1H, CHPh), 5.98 (br. d, 1H, $J_{2,NH} = 6.0$ Hz, NH), 7.30-7.40 (m, 3H, aromatic), 7.44-7.53 (m, 2H, aromatic); ¹³C NMR (75 MHz, CDCl₃): δ , 23.3, 54.9, 57.3, 67.4, 68.6, 71.6, 79.7, 100.6, 102.3, 126.4 (x 2), 128.4 (x 2), 129.3, 137.1, 173.8 ppm; HR-FAB MS: $[M+Na]^+$ calcd for C₁₆H₂₁NO₆Na, 346.1267; found, 346.1254.

Methyl 2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy- β -D-mannopyranoside (4.18).

Ac₂O (4.0 mL, 42.40 mmol) was added to a solution of **4.17** (1.00 g, 3.09 mmol) in pyridine (20 mL) and the resulting mixture was stirred under argon for 3 h at rt. After that, methanol (20 mL) was added and the resulting mixture was concentrated *in vacuo*. The residue was dissolved in THF (40.0 mL), activated molecular sieves (3 Å, 1.50 g) were added and the resulting mixture was stirred under argon for 1 h at rt. NaCNBH₃ (2.68 g, 42.6 mmol) was added followed by a dropwise addition of a 2M solution of HCl

in diethyl ether (21.3 mL, 42.6 mmol). The resulting mixture was stirred under argon for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH₂Cl₂. The combined filtrate (~150 mL) was washed with water (30 mL), sat. aq. NaHCO₃ (30mL), and water (30 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to yield the title compound as a white foam (1.10 g, 97%). Analytical data for **4.18**: $R_f = 0.39$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{22} -55.3$ (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 1.98 (s, 3 H, COCH₃), 2.04 (s, 3 H, COCH₃), 3.24 (d, 1H, $J_{6,OH} = 3.72$ Hz, OH), 3.45-3.50 (m, 4 H, OCH₃, H-5), 3.75-3.83 (m, 6H, OCH₃, H-4, 6a, 6b), 4.52 (d, 1H, $J_{1,2} = 1.3$ Hz, H-1), 4.56-4.65 (m, 3H, $J_{2,3} = 3.9$ Hz, CH₂Ph, H-2), 4.75 (dd, 1H, $J_{3,4} = 9.8$ Hz, H-3), 5.80 (br. d, 1 H, $J_{2,NH} = 8.6$ Hz, NH), 7.28-7.36 (m, 5H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 21.2, 23.4, 50.5, 57.1, 66.5, 69.7, 73.8, 74.9, 75.3, 100.2, 127.8 (x 2), 128.1, 128.7 (x 2), 137.9, 171.0, 171.2 ppm; HR-FAB MS: $[M+Na]^+$ calcd for C₁₈H₂₅NO₇Na, 390.1529; found, 390.1550.

4.4.4 Assembly and functionalization of CP8 disaccharide D1

Methyl O-(2-azido-4-O-benzoyl-2,6-dideoxy-3-O-methyl-α-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-O-benzylidene-β-D-mannopyranoside (4.19). A mixture of donor **4.7** (596 mg, 1.32 mmol), acceptor **4.17** (470 mg, 1.45 mmol), and freshly activated molecular sieves (4 Å, 800 mg) in CH₂Cl₂ (20 mL) was stirred under argon for 2 h at rt. The resulting mixture was cooled to 0 °C, triflic acid (40 mg, 0.26 mmol) was added and the resulting mixture was allowed to warm to rt and stirred for 2 h.

After that, the solids were filtered-off and rinsed successively with CH₂Cl₂. The combined filtrate (~150 mL) was washed with water (30 mL), sat. aq. NaHCO₃ (30 mL) and water (30 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (528 mg, 65%). Analytical data for **4.19**: R_f = 0.66 (methanol/dichloromethane, 1/9, v/v); [α]_D²³ 103.7 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.21 (d, 3H, H-6'), 2.11 (s, 3H, COCH₃), 3.47-3.50 (m, 5H, J_{5,6b} = 4.8 Hz, H-2', 5, OCH₃), 3.54 (s, 3H, OCH₃), 3.81-3.90 (m, J_{3',4'} = 2.4 Hz, H-3', 4, 6a), 4.08 (dd, 1H, J_{3,4} = 10.4 Hz, H-3), 4.38 (dd, 1H, J_{6a,6b} = 10.4 Hz, H-6b), 4.2-4.69 (m, 2H, J_{1,2} = 1.71 Hz, J_{5',6'} = 6.5 Hz, H-1, 5'), 4.70 (dd, 1H, J_{2,3} = 4.4 Hz, H-2), 5.40 (d, 1H, J_{1',2'} = 3.4 Hz, H-1'), 5.65 (s, 1H, CHPh), 5.69 (dd, 1H, J_{4',5'} = 2.3 Hz, H-4'), 5.74 (d, 1H, J_{2,NH} = 9.3 Hz, NH), 7.37-7.57 (m, 8H, aromatic), 8.06-8.11 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.5, 23.7, 52.6, 57.4 (x 2), 59.0, 66.1, 67.1, 68.6, 70.0, 73.3, 75.2, 80.0, 99.3, 101.4, 101.8, 126.1 (x 2), 128.5 (x 2), 128.5 (x 2), 129.2, 129.8, 130.0 (x 2), 133.3, 137.1, 166.1, 171.1 ppm; HR-FAB MS [M+Na]⁺ calcd for C₃₀H₃₆NaO₁₀N₄ 635.2329, found 635.2302.

Methyl O-(2-azido-2,6-dideoxy-4-O-levulinoyl-3-O-methyl-α-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-O-benzylidene-β-D-mannopyranoside (4.20). A 1 M solution of sodium methoxide in methanol (2.0 mL) was added to a solution of disaccharide **4.19** (321 mg, 0.52 mmol) in methanol (10.0 mL) and the resulting mixture was stirred under argon at rt for 16 h. After that, Amberlite IR120 resin was added until

pH = 7. Then, the resin was filtered-off, rinsed successively with methanol (7 x 5 mL), and the combined filtrate was concentrated *in vacuo* and dried. The crude residue was dissolved in dry CH₂Cl₂ (20 mL), levulinic acid (80 mg, 0.69 mmol), DIC (0.16 mL, 1.05 mmol) and DMAP (14 mg, 0.11 mmol) were added, and the resulting mixture was stirred under argon for 24 h at rt. After that, the reaction mixture was diluted with CH₂Cl₂ (~100 mL) and washed with water (20 mL) sat. aq. NaHCO₃ (20 mL) and water (20 mL). The organic phase was separated, dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (281 mg, 89%). Analytical data for **4.20**: R_f = 0.63 (methanol/dichloromethane, 1/9, v/v); [α]_D²² 71.8 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.15 (d, 3H, H-6'), 2.05, 2.16 (2 s, 6H, 2 x COCH₃), 2.58-2.77 (m, 4H, 2 x CH₂), 3.27 (dd, 1H, J_{2',3'} = 10.9 Hz, H-2'), 3.38 (s, 3H, OCH₃), 3.41 (dd, 1H, J_{5,6a} = 4.7 Hz, J_{5,6b} = 5.1 Hz, H-5), 3.51 (s, 3H, OCH₃), 3.70 (dd, 1H, J_{2',3'} = 10.9 Hz, J_{3',4'} = 3.1 Hz, H-3'), 3.76 (m, 2H, J_{4,5} = 9.5 Hz, J_{6a,6b} = 10.41 Hz, H-4, 6a), 4.02 (dd, 1H, J_{3,4} = 9.9 Hz, H-3), 4.34 (dd, 1H, H-6b), 4.48 (m, 1H, J_{5',6'} = 6.5 Hz, H-5'), 4.57 (d, 1H, J_{1,2} = 1.7 Hz, H-1), 6.64 (dd, J_{2,3} = 4.5 Hz, 1H, H-2), 5.31 (d, 1H, J_{1',2'} = 3.5 Hz, H-1'), 5.40 (dd, 1H, J_{4',5'} = 2.3 Hz, H-4'), 5.61 (s, 1H, CHPh), 5.74 (d, 1H, J_{2,NH} = 9.3 Hz, NH), 7.34-7.37 (m, 3H, aromatic), 7.45-7.48 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.4, 23.7, 28.0, 30.0, 38.1, 52.5, 57.3, 57.4, 58.8, 65.9, 67.1, 68.5, 69.7, 73.2, 74.9, 80.0, 99.2, 101.4, 101.7, 126.0 (x 2), 128.4 (x 2), 129.2, 137.1, 171.0, 172.4, 206.5 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₈H₃₈NaO₁₁N₄ 629.2435, found 629.2445.

Methyl ***O*-(2-acetamido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-2-deoxy-4,6-*O*-benzylidene- β -D-mannopyranoside (4.21).** Water (1.5 mL), triethylamine (1.0 mL, 7.1 mmol) and 1,3-propanedithiol (0.71 mL, 7.0 mmol) were added to a solution of disaccharide **4.20** (212 mg, 0.35 mmol) in pyridine (35.0 mL) and the resulting mixture was stirred for 24 h at rt. After that, the reaction mixture was concentrated *in vacuo* and dried. The crude residue was dissolved in MeOH (5.0 mL), Ac₂O (0.4 mL, 4.20 mmol) was added, and the resulting suspension was stirred under argon for 16 h at rt. Upon completion, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (210 mg, 96%). Analytical data for **4.21**: R_f = 0.54 (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{22}$ 11.4 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.09 (d, 3H, H-6'), 1.43, 2.03, 2.11 (3s, 9H, 3 x COCH₃), 2.57 (m, 2H, CH₂), 2.67 (m, 2H, CH₂), 3.19 (s, 3H, OCH₃), 3.31-3.39 (m, 2H, H-3', 5), 3.43 (s, 3H, OCH₃), 3.75-3.88 (m, 3H, 3, 4, 6a), 4.23-4.39 (m, 3H, $J_{5',6'} = 6.5$ Hz, H-2', 5', 6b), 4.50 (d, 1H, $J_{1,2} = 1.4$ Hz, H-1), 4.62 (dd, 1H, $J_{2,3} = 6.7$ Hz, H-2), 4.95 (dd, 1H, $J_{1',2'} = 3.4$ Hz, H-1'), 5.29 (dd, 1H, $J_{4',5'} = 2.0$ Hz, H-4'), 5.56 (s, 1H, CHPh), 6.10 (d, $J_{2',NH} = 9.7$ Hz, 2'-NH), 6.28 (d, $J_{2,NH} = 9.7$ Hz, 2-NH), 7.23-7.36 (m, 5H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 16.4, 22.7, 23.5, 28.1, 29.9, 38.1, 48.6, 52.9, 57.1, 57.3, 66.2, 67.2, 68.5, 69.0, 76.2, 76.6, 78.7, 100.8, 101.5, 102.3, 126.1 (x 2), 128.6 (x 2), 129.6, 136.8, 170.3, 171.2, 172.4, 206.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for C₃₀H₄₂NaO₁₂N₂ 645.2636, found 645.2618.

Methyl ***O*-(2-acetamido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-2-deoxy- β -D-mannopyranoside (4.22).**

Trifluoroacetic acid (20 mL) was added to a mixture of disaccharide **4.21** (1.15 g, 1.85 mmol) in CH₂Cl₂ (80.0 mL) and water (5 mL), and the resulting mixture was stirred for 2 h at rt. After that, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (798 mg, 81%). Analytical data for **4.22**: R_f = 0.74 (methanol/CH₂Cl₂, 3/7, v/v); $[\alpha]_D^{22}$ 42.6 (c = 1, CH₃OH); ¹H NMR (300 MHz, CD₃OD): δ , 1.11 (d, 3H, H-6'), 1.97, 2.01, 2.18 (3s, 9H, 3 x COCH₃), 2.63 (m, 2H, CH₂), 2.80 (m, 2H, CH₂), 3.23 (dd, 1H, H-5), 3.29, 3.47 (2s, 6H, 2 x OCH₃), 3.50 (dd, 1H, $J_{3',4'} = 3.1$ Hz, H-3'), 3.66-3.69 (m, 2H, H-3, 4), 3.86 (m, 2H, H-6a, 6b), 4.16 (dd, 1H, $J_{2',3'} = 11.3$ Hz, H-2'), 4.47-4.52 (m, 2H, $J_{5',6'} = 6.5$ Hz, H-2, 5'), 4.55 (d, 1H, $J_{1,2} = 1.4$ Hz, H-1), 5.09 (d, 1H, $J_{1',2'} = 3.6$ Hz, H-1), 5.35 (dd, 1H, $J_{4',5'} = 2.3$ Hz, H-4') ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 16.9, 22.9, 29.1, 29.9, 38.9, 50.9, 54.4, 57.1, 57.4, 61.7, 66.9, 68.1, 70.7, 77.0, 78.4, 80.7, 101.4, 102.0, 173.9, 174.2, 209.6 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₃H₃₈NaO₁₂N₂ 557.2322, found 557.2335.

Methyl ***O*-(2-acetamido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-(benzyl 2-acetamido-2-deoxy- β -D-mannopyranosid)uronate (4.23).** Water (25 mL), 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, 226 mg, 1.44 mmol) and diacetoxy iodobenzene (BAIB, 4.64 mg, 14.4 mmol) were added to a solution of disaccharide **4.22** (771 mg, 1.44 mmol) in CH₂Cl₂ (50.0 mL) and the resulting mixture was stirred for 16 h at rt. After that, the reaction mixture was

concentrated *in vacuo*, passed through a pad of silica gel. The eluate was concentrated *in vacuo* and dried. The crude residue was dissolved in DMF (20 mL), benzyl bromide (1.03 mL, 8.64 mmol) and NaHCO₃ (1.21 g, 14.4 mmol) were added, and the resulting mixture was stirred under argon for 16 h at rt. After that, the reaction mixture was mixed with ice-cold water (~30 mL) and the resulting mixture was extracted with ethyl acetate (3 x 100 mL). The combined organic extract was washed with brine (2 x 30 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (700 mg, 76%). Analytical data for **4.23**: $R_f = 0.38$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{22} 45.3$ (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.17 (d, 3H, H-6'), 1.97, 2.04, 2.19 (3s, 9H, 3 x COCH₃), 2.65 (m, 2H, CH₂), 2.75 (m, 2H, CH₂), 3.33 (s, 3H, OCH₃), 3.37 (d, 1H, $J_{3',4'} = 2.5$ Hz, H-3'), 3.47 (s, 3H, OCH₃), 3.75 (dd, 1H, $J_{3,4} = 7.8$ Hz, H-3), 3.85-3.96 (m, 2H, H-4, 5), 4.08 (dd, 1H, $J_{2',3'} = 10.8$ Hz, H-2'), 4.44 (m, 1H, $J_{5',6'} = 6.5$ Hz, H-5'), 4.52-4.58 (m, 2H, $J_{2,3} = 3.7$ Hz, H-1, 2), 5.27 (s, 2H, CH₂Ph), 5.37 (dd, 1H, $J_{4',5'} = 1.9$ Hz, H-4'), 5.42 (d, 1H, $J_{1',2'} = 3.6$ Hz, H-1'), 5.87 (d, $J_{2',NH} = 9.6$ Hz, 2'-NH), 6.02 (d, $J_{2,NH} = 6.8$ Hz, 2-NH), 7.38-7.43 (m, 5H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ , 16.6, 23.6, 23.7, 28.1, 30.0, 38.2, 50.0, 51.4, 57.1, 57.5, 65.9, 67.7, 68.6, 69.1, 75.0, 76.2, 77.4, 78.7, 99.9, 101.0, 128.5 (x 2), 128.8 (x 2), 135.0, 169.7, 171.0, 171.5, 172.6, 206.7 ppm; HR-FAB MS $[M+Na]^+$ calcd for C₃₀H₄₂NaO₁₃N₂ 661.2584, found 661.2590.

Methyl ***O*-(2-acetamido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-(benzyl** **2-acetamido-4-*O*-acetyl-2-deoxy- β -D-**

mannopyranosid)uronate (4.24). Ac₂O (3.0 mL) was added to a solution of disaccharide **4.23** (498 mg, 0.72 mmol) in pyridine (10.0 mL) and the resulting mixture was stirred under argon for 16 h. After that, the reaction was quenched with MeOH (~10.0 mL), the volatiles were removed *in vacuo*, and the residue was co-evaporated with toluene (3 x 5 mL). The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (490 mg, 99%). Analytical data for **4.24**: R_f = 0.40 (methanol/dichloromethane, 1/9, v/v); [α]_D²³ 44.0 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.15 (d, 3H, H-6'), 1.83, 1.97, 2.04, 2.16 (4s, 12H, 4 x COCH₃), 2.58-2.82 (m, 4H, 2 x CH₂), 3.23-3.27 (m, 4H, J_{3',4'} = 2.9 Hz, OCH₃, H-3'), 3.43 (s, 3H, OCH₃), 3.83 (dd, 1H, J_{3,4} = 8.0 Hz, H-3), 4.03 (d, 1H, J_{4,5} = 7.6 Hz, H-5), 4.28 (ddd, 1H, J_{2',3'} = 9.2 Hz, H-2'), 4.46-4.98 (m, 2H, J_{2,3} = 3.8 Hz, J_{5',6'} = 6.5 Hz, H-2, 5'), 4.59 (d, 1H, J_{1,2} = 2.0 Hz, H-1), 4.90 (d, 1H J_{1',2'} = 3.7 Hz, H-1'), 5.10 (dd, 2H, ²J = 14.0 Hz, CH₂Ph), 5.27-5.35 (m, 2H, H-4, 4'), 5.69 (d, J_{2',NH} = 9.0 Hz, 2'-NH), 6.21 (d, J_{2,NH} = 9.2 Hz, 2-NH), 7.35-7.42 (m, 5H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.6, 20.7, 23.3, 23.6, 28.1, 29.9, 38.2, 48.6, 50.5, 57.3, 57.8, 66.3, 68.0, 68.8, 69.0, 72.3, 75.6, 76.1, 77.2, 100.4, 128.9 (x4), 129.1 134.6, 168.2, 170.4, 170.7, 171.1, 172.5, 206.7 ppm; HR-FAB MS [M+Na]⁺ calcd for C₃₂H₄₄NaO₁₄N₂ 703.2690, found 703.2701.

Methyl O-(2-acetamido-2,6-dideoxy-3-O-methyl-α-D-galactopyranosyl)-(1→3)-(benzyl 2-acetamido-4-O-acetyl-2-deoxy-β-D-mannopyranosid)uronate (4.25).

Hydrazine acetate (15 mg, 0.17 mmol) was added to a solution of disaccharide **4.24** (56 mg, 0.09 mmol) in CH₂Cl₂/methanol (10.5 mL, 20/1, v/v) and the resulting mixture was

stirred for 4 h at rt. After that, the reaction mixture was diluted with CH₂Cl₂ (~50 mL) and washed with sat. aq. NaHCO₃ (10 mL) and water (10 mL). The organic phase was separated, dried with MgSO₄, 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 foam (48 mg, 96%). Analytical data for **4.25**: R_f = 0.33 (methanol/dichloromethane, 1/9, v/v); [α]_D²² 32.5 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.29 (d, 3H, H-6'), 1.80, 1.95, 2.02 (3s, 9H, 3 x COCH₃), 3.13 (dd, 1H, J_{3',4'} = 2.9 Hz, H-3'), 3.33, 3.41 (2s, 6H, 2 x OCH₃), 3.82 (dd, 1H, J_{3,4} = 8.3 Hz, H-3), 3.91 (dd, J_{4',5'} = 1.8 Hz, 1H, H-4'), 4.02 (d, 1H, H-5), 4.31-4.41 (m, 2H, J_{2',3'} = 10.9 Hz, J_{5',6'} = 6.5 Hz, H-2', 5'), 4.47 (ddd, 1H, J_{2,3} = 3.9 Hz, H-2), 4.57 (d, 1H, J_{1,2} = 2.1 Hz, H-1), 4.80 (d, 1H, J_{1',2'} = 3.8 Hz, H-1'), 5.13 (br. s, 2H, CH₂Ph), 5.26 (dd, 1H, J_{4,5} = 7.8 Hz, H-4), 5.78 (d, J_{2',NH} = 9.0 Hz, 2'-NH), 6.29 (d, J_{2,NH} = 8.7 Hz, 2-NH), 7.29-7.41 (m, 5H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.6, 20.7, 23.3, 23.5, 47.6, 50.6, 56.9, 57.7, 66.9, 67.5, 67.9, 68.9, 72.2, 75.3, 77.2, 78.3, 100.5, 128.8 (x 4), 129.0, 134.6, 168.1, 170.4, 170.5, 171.0 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₇H₃₈NaO₁₂N₂ 605.2322, found 605.2311.

Methyl O-(2-acetamido-2,6-dideoxy-3-O-methyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4-O-acetyl-2-deoxy-β-D-mannopyranosid)uronic acid (D1). 10% Pd on activated charcoal (100 mg) was added to a solution of disaccharide **4.25** (48 mg, 0.082 mmol) in 90% aq. ethanol (10 mL) and the resulting suspension was stirred vigorously under H₂ atmosphere for 24 h at rt. After that, the solids were filtered off and rinsed successively with methanol. The combined filtrate (~50 mL) was concentrated *in vacuo*.

The residue was purified by column chromatography on silica gel (dichloromethane - methanol gradient elution) to afford the title compound as a white amorphous solid (39 mg, 97% yield). Analytical data for **D1**: $R_f = 0.21$ (methanol/dichloromethane, 3/7, v/v); $[\alpha]_D^{23} 64.0$ ($c = 1$, CH₃OH); ¹H NMR (300 MHz, CD₃OD): δ , 1.24 (d, 3H, H-6'), 1.92, 2.00, 2.05 (3s, 9H, 3 x COCH₃), 3.33-3.37 (m, 4H, $J_{3',4'} = 2.9$ Hz, OCH₃, H-3'), 3.47 (1s, 3H, OCH₃), 3.70 (d, 1H, H-5), 3.89 (dd, 1H, $J_{4',5'} = 2.3$ Hz, H-4'), 3.96 (dd, 1H, $J_{2,3} = 4.1$ Hz, $J_{3,4} = 10.0$ Hz, H-3), 4.14 (ddd, 1H, $J_{2',3'} = 11.7$ Hz, H-2'), 4.44-4.50 (m, 2H, $J_{5',6'} = 6.6$ Hz, H-2, 5'), 4.64 (d, 1H, $J_{1,2} = 1.1$ Hz, H-1), 4.91 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 5.14 (dd, 1H, $J_{4,5} = 10.1$ Hz, H-4), 7.86 (d, $J_{2',NH} = 8.4$ Hz, 2'-NH), 6.29 (d, $J_{2,NH} = 9.7$ Hz, 2-NH) ppm; ¹³C NMR (75 MHz, CD₃OD): δ , 16.9, 21.2, 22.9, 23.0, 49.9, 54.3, 56.5, 57.3, 67.9, 68.4, 71.8, 75.9, 76.9, 78.2, 100.2, 101.3, 171.6, 173.8, 173.9, 174.1 ppm; HR-FAB MS $[M+2Na]^+$ calcd for C₂₀H₃₂O₁₂N₂Na₂ 537.1672, found 537.1668.

4.4.5 Assembly and functionalization of CP5 disaccharide **D2**

Methyl *O*-(4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl-2-phthalimido- β -D-galactopyranosyl)-(1 \rightarrow 4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy- β -D-mannopyranoside (**4.26**). A mixture of donor **4.14** (163 mg, 0.29 mmol), acceptor **4.18** (140 mg, 0.38 mmol), and freshly activated molecular sieves (4Å, 400 mg) in CH₂Cl₂ (10 mL) was stirred under argon for 2 h at rt. TMSOTf (11 μ L, 0.06 mmol) was added at 0 °C and the resulting mixture was allowed to reach rt and stirred under argon for 4 h. After that, the solids were filtered-off and rinsed successively with CH₂Cl₂. The combined filtrate (~100 mL) was washed with water (20 mL), sat. aq. NaHCO₃ (20 mL) and water (20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in*

vacuo. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (121 mg, 63%, $\alpha/\beta = 1/5$). The yield calculation is based on acceptor **4.18** recovered (61 mg, 0.17 mmol). Analytical data for **4.26**: $R_f = 0.58$ (methanol/dichloromethane, 1/9, v/v); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.26 (d, 3H, H-6'), 1.96, 2.05 (2s, 6H, 2 x COCH_3), 3.25, 3.40 (2s, 6H, 2 x OCH_3), 3.45-3.50 (m, 2H, H-5, 6a), 3.70-3.81 (m, 1H, H-6b), 3.84 (m, 1H, $J_{5',6'} = 6.4$ Hz, H-5'), 3.95 (m, 2H, $^2J = 12.1$ Hz, $\frac{1}{2}$ CH_2Ph , H-4), 4.12 (d, 1H, $^2J = 12.1$ Hz, $\frac{1}{2}$ CH_2Ph), 4.26 (dd, 1H, $J_{3',4'} = 3.2$ Hz, H-3'), 4.39 (d, 1H, $J_{1,2} = 2.4$ Hz, H-1), 4.48 (d, 1H, $J_{2',3'} = 9.5$ Hz, H-2'), 4.60 (dd, 1H, $J_{2,3} = 4.1$ Hz, H-2), 5.04 (dd, 1H, $J_{3,4} = 9.5$ Hz, H-3), 5.32 (d, 1H, $J_{1',2'} = 8.5$ Hz, H-1'), 5.59-5.63 (m, 2H, NH, H-4'), 7.07-8.16 (m, 14H, aromatic) ppm; $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ , 16.8, 21.2, 23.6, 50.6, 53.4, 57.0, 57.6, 68.4, 68.5, 69.9, 72.6, 73.1, 73.2, 74.9, 76.1, 98.3, 100.1, 127.1 (x 4), 127.6 (x 2), 128.4 (x 4), 128.7 (x 2), 129.6, 130.2 (x 2), 133.5, 134.3, 138.4, 166.4, 168.0, 169.2, 170.0, 170.8 ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{40}\text{H}_{44}\text{O}_{13}\text{N}_2\text{Na}$ 783.2741, found 783.2701.

Methyl *O*-(2-acetamido-4-*O*-acetyl-2,6-dideoxy-3-*O*-methyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy- β -D-mannopyranoside (4.27).

Hydrazine hydrate (0.19 mL, 3.96 mmol) was added to a stirring solution of disaccharide **4.26** (118 mg, 0.13 mmol) in MeOH (10 mL). The reaction was stirred at 80 °C for 24 h. After that, the solution was concentrated *in vacuo*, and dried. Then, the crude residue was dissolved in pyridine (5.0 mL), Ac_2O (0.5 mL, 5.30 mmol) was added, and the resulting mixture was stirred under argon for 16 h at rt. After that, MeOH (5 mL) was added and

the volatiles were removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~100 mL), washed with water (20 mL), sat. aq. NaHCO₃ (20 mL) and water (20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (65 mg, 81%). Analytical data for **4.27**: R_f = 0.51 (methanol/dichloromethane, 1/9, v/v); ¹H NMR (300 MHz, CD₃OD): δ, 1.11 (d, 3H, H-6'), 1.94, 1.95, 1.99, 2.01 (4 s, 12H, 4 x COCH₃), 3.33, 3.46 (2 s, 6H, 2 x OCH₃), 3.53-3.63 (2H, H-3', 5), 3.65 (m, 1H, J_{5',6'} = 6.4 Hz, H-5'), 3.78-3.92 (m, 3H, H-4, 6a, 6b), 4.53-4.65 (m, 6H, J_{2,3} = 4.4 Hz, CH₂Ph, H-1, 1', 2, 2'), 4.98 (dd, 1H, J_{3,4} = 9.4 Hz, H-3), 5.30 (dd, 1H, J_{4',5'} = 1.6 Hz, H-4'), 7.30-7.46 (m, 14H, aromatic) ppm; ¹³C NMR (75 MHz, CD₃OD): δ, 17.0, 20.8, 21.4, 22.6, 23.4, 51.9, 54.1, 57.3, 58.1, 69.6, 70.1, 70.3, 73.8, 74.4, 74.8, 76.0, 79.8, 101.3, 102.0, 129.3, 129.5 (x 2), 129.8 (x 2), 139.3, 171.8, 172.4, 173.8, 174.1 ppm; HR-FAB MS [M+Na]⁺ calcd for C₂₉H₄₂O₁₂N₂Na 633.2636, found 633.2622.

Methyl O-(2-acetamido-4-O-acetyl-2,6-dideoxy-3-O-methyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3-O-acetyl-2-deoxy-β-D-mannopyranoside (4.28). 10% Pd on activated charcoal (100 mg) was added to a solution of disaccharide **4.27** (64 mg, 0.10 mmol) in 90% aq. ethanol (10 mL) and the resulting mixture was stirred vigorously under H₂ atmosphere for 24 h at rt. After that, the solids were filtered-off, rinsed successively with methanol (7 x 5), and the combined filtrate (~50 mL) was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (dichloromethane - methanol gradient elution) to afford the title compound as a white amorphous solid (41

mg, 75% yield). Analytical data for **4.28**: $R_f = 0.24$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{22}$ 14.4 ($c = 1$, CHCl_3); $^1\text{H NMR}$ (300 MHz, CD_3OD): δ , 1.11 (d, 3H, H-6'), 1.98, 1.99, 2.00, 2.11 (4s, 12H, 4 x COCH_3), 3.32 (s, 3H, OCH_3), 3.40-3.49 (m, 5H, OCH_3 , H-3', 5), 3.68-3.78 (m, 2H, $J_{2',3'} = 9.5$ Hz, $J_{5',6'} = 6.4$ Hz, H-2', 5'), 3.81-3.92 (m, 3H, H-4, 6a, 6b), 4.52-4.55 (m, 2H, $J_{1',2'} = 8.3$ Hz, H-1', 2), 4.64 (d, 1H, $J_{1,2} = 1.4$ Hz, H-1), 4.97 (dd, 1H, $J_{3',4'} = 4.3$ Hz, H-3'), 5.28 (dd, 1H, $J_{4',5'} = 2.8$ Hz, H-4') ppm; $^{13}\text{C NMR}$ (75 MHz, CD_3OD): δ , 17.0, 20.8, 21.4, 22.5, 23.2, 52.1, 53.7, 57.2, 58.1, 61.4, 69.6, 70.3, 73.8, 75.1, 76.7, 80.3, 101.1, 102.8, 171.8, 172.5, 174.0 (x 2) ppm; HR-FAB MS $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{22}\text{H}_{36}\text{O}_{12}\text{N}_2\text{Na}$ 543.2166, found 543.2180.

Methyl *O*-(2-acetamido-4-*O*-acetyl-2,6-dideoxy-3-*O*-methyl- β -D-fucopyranosyl)-(1 \rightarrow 4)-(2-acetamido-3-*O*-acetyl-2-deoxy- β -D-mannopyranosid)uronic acid (4.29).

Water (5.0 mL), TEMPO (21 mg, 0.14 mmol), and BAIB (271 mg, 0.84 mmol) were added to a solution of disaccharide **4.28** (175 mg, 0.34 mmol) in CH_2Cl_2 (10.0 mL) and the resulting mixture was stirred for 16 h at rt. After that, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (dichloromethane - methanol gradient elution) to afford the title compound as a white amorphous solid in (125 mg, 70%). Analytical data for **4.29**: $R_f = 0.44$ (methanol/dichloromethane, 3/7, v/v); $[\alpha]_D^{22}$ -12.9 ($c = 1$, CH_3OH); $^1\text{H NMR}$ (300 MHz, CD_3OD): δ , 1.08 (d, 3H, H-6'), 1.95, 1.97, 2.01, 2.07 (4s, 12H, 4 x COCH_3), 3.23-3.41 (m, 7H, $J_{3',4'} = 3.2$ Hz, 2 x OCH_3 , H-3'), 3.63-3.76 (m, 3H, $J_{2',3'} = 7.8$ Hz, $J_{5',6'} = 6.4$ Hz, H-2', 5, 5'), 3.81 (dd, 1H, $J_{4,5} = 9.6$ Hz, H-4), 4.43-4.51 (m, 2H, $J_{1',2'} = 8.6$ Hz, $J_{2,3} = 4.2$ Hz, H-1', 2), 4.91 (d, 1H, $J_{1,2} = 1.2$ Hz, H-1), 4.86 (dd, 1H $J_{3,4} = 9.6$ Hz, H-3), 5.22 (dd,

1H, $J_{4',5'} = 2.9$ Hz, H-4') ppm; ^{13}C NMR (75 MHz, CD_3OD): δ , 17.0, 20.8, 21.3, 22.6, 23.5, 52.1, 53.1, 57.3, 58.2, 69.8, 70.6, 74.0, 76.6, 79.3, 81.2, 101.1, 102.5, 172.2, 172.5, 174.1 (x 2), 174.5 ppm; HR-FAB MS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{22}\text{H}_{34}\text{N}_2\text{O}_{13}\text{Na}$, 557.1959; found, 557.1954.

Methyl *O*-(2-acetamido-2,6-dideoxy-3-*O*-methyl- β -D-fucopyranosyl)-(1 \rightarrow 4)-(2-acetamido-2-deoxy- β -D-mannopyranosid)uronic acid (D2). NaOH (4.0 mL, 1M aq.) was added dropwise to the solution of **4.29** (125 mg, 0.23 mmol) in MeOH (4 mL) and the mixture was stirred overnight at rt. After that, Amberlite IR120 resin was added to the solution until pH was 7. Then, the solid was filtered-off and the solution was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to yield the title compound as white amorphous solid (89 mg, 84%). Analytical data for **D2**: $R_f = 0.15$ (methanol/dichloromethane, 3/7, v/v); $[\alpha]_{\text{D}}^{22} -36.1$ (c = 1, CH_3OH); ^1H NMR (300 MHz, CD_3OD): δ , 1.28 (d, 3H, H-6'), 2.02 (br. s, 6H, 2 x COCH_3), 3.21 (dd, 1H, $J_{3',4'} = 3.0$ Hz, H-3'), 3.38, 3.43 (2s, 6H, 2 x OCH_3), 3.59-3.72 (m, 4H, $J_{5',6'} = 6.4$ Hz, H-1, 2, 4, 5'), 3.85 (dd, 1H, $J_{4',5'} = 2.7$ Hz, H-4'), 3.91 (dd, 1H, $J_{2',3'} = 10.8$ Hz, H-2'), 4.41 (d, 1H, $J_{1',2'} = 8.5$ Hz, H-1'), 4.49-4.52 (m, 2H, H-3, 5) ppm; ^{13}C NMR (75 MHz, CD_3OD): δ , 16.9, 22.9, 23.5, 52.2, 53.4, 57.2, 57.6, 68.0, 72.3, 72.4, 79.0, 80.3, 82.8, 101.9, 102.7, 174.4 (x 2), 176.6 ppm; HR-FAB MS: $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{18}\text{H}_{30}\text{N}_2\text{O}_{11}\text{Na}$, 473.1747; found, 473.1744.

4.5 References

1. del Rio, A.; Cervera, C.; Moreno, A.; Moreillon, P.; Miró, J. M., Patients at Risk of Complications of Staphylococcus aureus Bloodstream Infection *Clin. Infect. Dis.* **2009**, *48 (Suppl. 4)*, S246-S253.
2. Chambers, H. F.; Deleo, F. R., Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nat. Rev. Microbiol.* **2009**, *7*, 629-641.
3. Fattom, A. I.; Horwith, G.; Fuller, S.; Propst, M.; Naso, R., Development of StaphVAX™, a polysaccharide conjugate vaccine against S. aureus infection: from the lab bench to phase III clinical trials. *Vaccine* **2004**, *22*, 880-887.
4. Jansen, K. U.; Girgenti, D. Q.; Scully, I. L.; Anderson, A. S., Vaccine review: "Staphylococcus aureus vaccines: problems and prospects". *Vaccine* **2013**, *31*, 2723-2730.
5. von Eiff, C.; Taylor, K. L.; Mellmann, A.; Fattom, A. I.; Friedrich, A. W.; Peters, G.; Becker, K., Distribution of capsular and surface polysaccharide serotypes of Staphylococcus aureus. *Diagn. Microbiol. Infect. Dis.* **2007**, *58*, 297-302.
6. O'Riordan, K.; Lee, J. C., Staphylococcus aureus capsular polysaccharides. *Clin. Microbiol. Rev.* **2004**, *17*, 218-234.
7. Jones, C., Revised structures for the capsular polysaccharides from Staphylococcus aureus Types 5 and 8, components of novel glycoconjugate vaccines. *Carbohydr. Res.* **2005**, *340*, 1097-1106.
8. Vann, W. F.; Moreau, M.; Sutton, A.; Byrd, R. A.; Karakawa, W. W., Structure and immunochemistry of Staphylococcus aureus capsular polysaccharides. *UCLA symp. Mol. Cell. Biol.* **1988**, *64*, 187-198.

9. Moreau, M.; Richards, J. C.; Fournier, J.-M.; Byrd, R. A.; Karakawa, W. W.; Vann, W. F., Structure of the type 5 capsular polysaccharide of *Staphylococcus aureus*. *Carbohydr. Res.* **1990**, *201*, 285-297.
10. Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R., Synthesis of *Staphylococcus aureus* type 5 capsular polysaccharide repeating unit using novel L-FucNAc and D-FucNAc synthons and immunochemical evaluation. *Bioorg. Med. Chem.* **2012**, *20*, 6403-6415.
11. Gagarinov, I. A.; Fang, T.; Liu, L.; Srivastava, A. D.; Boons, G. J., Synthesis of *Staphylococcus aureus* Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization. *Org. Lett.* **2015**, *17*, 928-931.
12. Yasomanee, J. P.; Visansirikul, S.; Pornsuriyasak, P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V., The synthesis of the repeating unit of capsular polysaccharide *Staphylococcus aureus* type 5 to study carbonylditriazole-mediated activation for conjugation of native CP5. *J. Org. Chem.* **2016**, *81*, 5981-5987.
13. Hagen, B.; Ali, S.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C., Mapping the Reactivity and Selectivity of 2-Azidofucosyl Donors for the Assembly of N-Acetylfucosamine-Containing Bacterial Oligosaccharides. *J. Org. Chem.* **2017**, *82*, 848-868.
14. Freese, S. J.; Anderson, A.; Pavliak, V.; Jansen, K. U.; Scully, I. L.; Scott, T. D.; Nanra, J. S.; Prasad, A. K.; Green, B. A. Compositions and methods for preparing *Staphylococcus aureus* serotype 5 and 8 capsular polysaccharide conjugate immunogenic compositions. US9125951B2, 2015.

15. Visansirikul, S.; Yasomanee, J. P.; Pornsuriyasak, P.; Kamat, M. N.; Podvalnyy, N. M.; Gobble, C. P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V., A Concise Synthesis of the Repeating Unit of Capsular Polysaccharide *Staphylococcus aureus* Type 8. *Org. Lett.* **2015**, *17*, 2382-2384.
16. Zhang, Z.; Magnusson, G., Conversion of p-methoxyphenyl glycosides into the corresponding glycosyl chlorides and bromides, and into thiophenyl glycosides. *Carbohydr. Res.* **1996**, *295*, 41-55.
17. Christina, A. E.; Blas Ferrando, V. M.; de Bordes, F.; Spruit, W. A.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A., Multigram-scale synthesis of an orthogonally protected 2-acetamido-4-amino-2,4,6-trideoxy-d-galactose (AAT) building block. *Carbohydr. Res.* **2012**, *356*, 282-287.
18. Mukherjee, C.; Misra, A. K., Synthesis of a unique trisaccharide having an acetal linkage between open-chain and cyclic sugar found in the cell wall of *Proteus*. *Tetrahedron: Asymmetry* **2008**, *19*, 2746-2751.
19. Hullar, T. L.; Siskin, S. B., The facile bromination by N-bromosuccinimide of benzylidene acetals of carbohydrates. Application to the synthesis of 2,6-imino carbohydrates (substituted 2,5-oxazabicyclo[2.2.2]octanes). *J. Org. Chem.* **1970**, *35*, 225-228.
20. Crich, D.; Yao, Q.; Bowers, A. A., On the regioselectivity of the Hanessian–Hullar reaction in 4,6-O-benzylidene protected galactopyranosides. *Carbohydr. Res.* **2006**, *341*, 1748-1752.

21. Yang, S.; Wang, A. p.; Zhang, G.; Di, X.; Zhao, Z.; Lei, P., An investigation of construction of chondroitin sulfate E (CS-E) repeating unit. *Tetrahedron* **2016**, *72*, 5659-5670.
22. Evans, M. E., Methyl 4,6-O-benzylidene- α - and - β -d-glucosides. *Carbohydr. Res.* **1972**, *21*, 473-475.
23. Spijker, N. M.; van Boeckel, C. A. A., Double Stereodifferentiation in Carbohydrate Coupling Reactions: The Mismatched Interaction of Donor and Acceptor as an Unprecedented Factor Governing the α/β Ratio of Glycoside Formation. *Angew. Chem. Int. Ed.* **1991**, *30*, 180-183.

CHAPTER 5

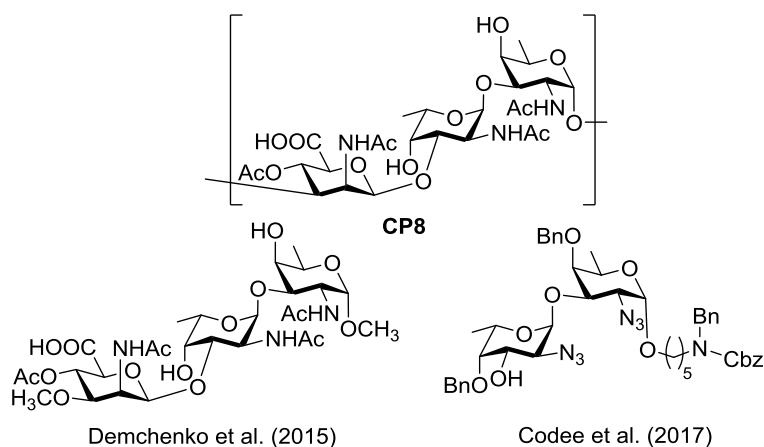
**The synthesis of the hexasaccharide
subunit of the capsular polysaccharide
Staphylococcus aureus Type 8**

5.1 Introduction

Staphylococcus aureus (*S. aureus*), Gram positive normal flora bacteria, is one of the most common opportunistic pathogens. It is usually found in human mucous membrane and skin.¹ When the integrity of skin is broken and the immune system is weakened, it may cause bloodstream infection which leads to a variety of potentially serious infections. Recently, one of the greatest concerns, methicillin-resistant *S. aureus* (MRSA), a drug-resistant strain, has emerged. The treatment options for the MRSA infections have been restricted due to the increasing drug resistance rates.² The alternative treatments such as immunotherapies or prophylactic vaccines might be the solutions of these issues. Since the outer part of the *S. aureus* is surrounded by capsular polysaccharides, the preventive vaccination based on the capsular polysaccharide conjugates may become a promising approach to protect against *S. aureus* infections.³

A majority of *S. aureus* strains express either capsular polysaccharide type 5 (CP5) or type 8 (CP8).⁴ The chemical structures of both types have been established⁵ and the chemical syntheses of oligosaccharide derivatives of each serotype have been reported.⁶⁻¹⁰ The structure of the CP8 capsular polysaccharide consists of a trisaccharide repeating unit composed of rather unique building blocks. The terminal 2,4-di-N,O-acetyl-D-mannosamine uronic acid (4-Ac-D-ManNAcA) residue is β -(1 \rightarrow 3)-glycosidically linked to 2-N-acetyl-L-fucosamine (L-FucNAc). In turn, the L-FucNAc is connected a D-FucNAc residue via a α -(1 \rightarrow 3)-linkage. In the bacterial polysaccharide capsule sequence, the D-FucNAc unit is then connected to another repeating unit via an α -(1 \rightarrow 3)-linkage to D-ManNAcA (Scheme 5.1).

Scheme 5.1. Native CP8 and previously synthesized CP8-derived oligosaccharide fragments.

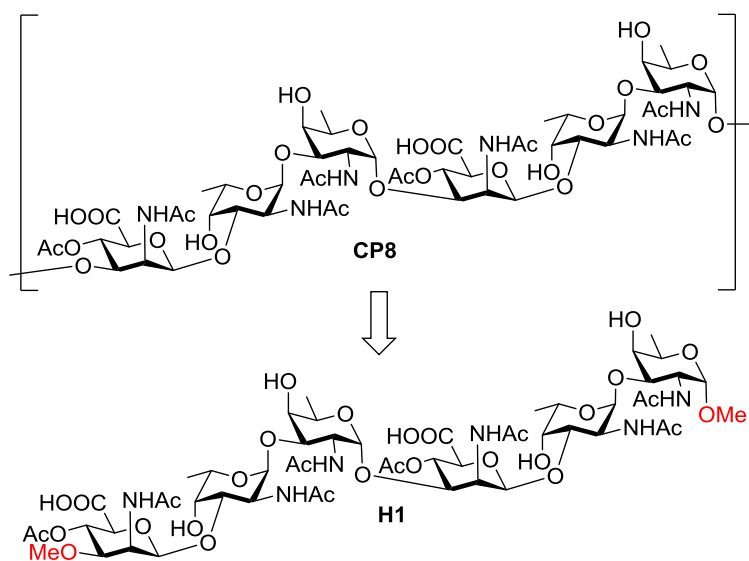


The chemical synthesis of CP8 repeating unit has been achieved by our group in 2015⁸ and a partial sequence has been reported by Codee in 2017¹⁰ (Scheme 5.1). Our synthetic CP8 trisaccharide was equipped with methyl groups at the propagation termini. This model was designed as a tool to study the activation and conjugation of the full length CP8 polysaccharide obtained by fermentation of *S. aureus*. To enhance our understanding of the conjugation process, we decided to synthesize a hexasaccharide comprising two sequential repeating units of CP8 (**H1**, Scheme 5.2). It is expected that the study of this larger polysaccharide and its conjugates will be beneficial for eliciting serotype-specific antibodies offering a window of opportunity for developing novel synthetic vaccines. To preserve the structural features and restrict the number of possible conjugation sites, the hexasaccharide will be capped with the terminal methyl groups.

The synthesis of the CP8 hexasaccharide represents several synthetic challenges. For instance, the introduction of 1,2-cis glycosidic linkages¹¹ often proceeds with low selectivity, and our synthetic target **H1** contains six such linkages. Moreover, multiple

azido groups are present in the molecule, which may require the development of suitable reaction conditions for their concomitant reduction.¹² In addition, access to multiple ManNAcA residues via the oxidation of the respective multiple sites may require different reaction conditions than the previously used, mild secondary oxidant (diacetoxyiodo)benzene (BAIB) in combination with (2,2,6,6-tetramethyl-piperidin-1-yl)oxyl (TEMPO).¹³

Scheme 5.2. Structure of hexasaccharide H1 derived from the two consecutive repeating units of CP8.



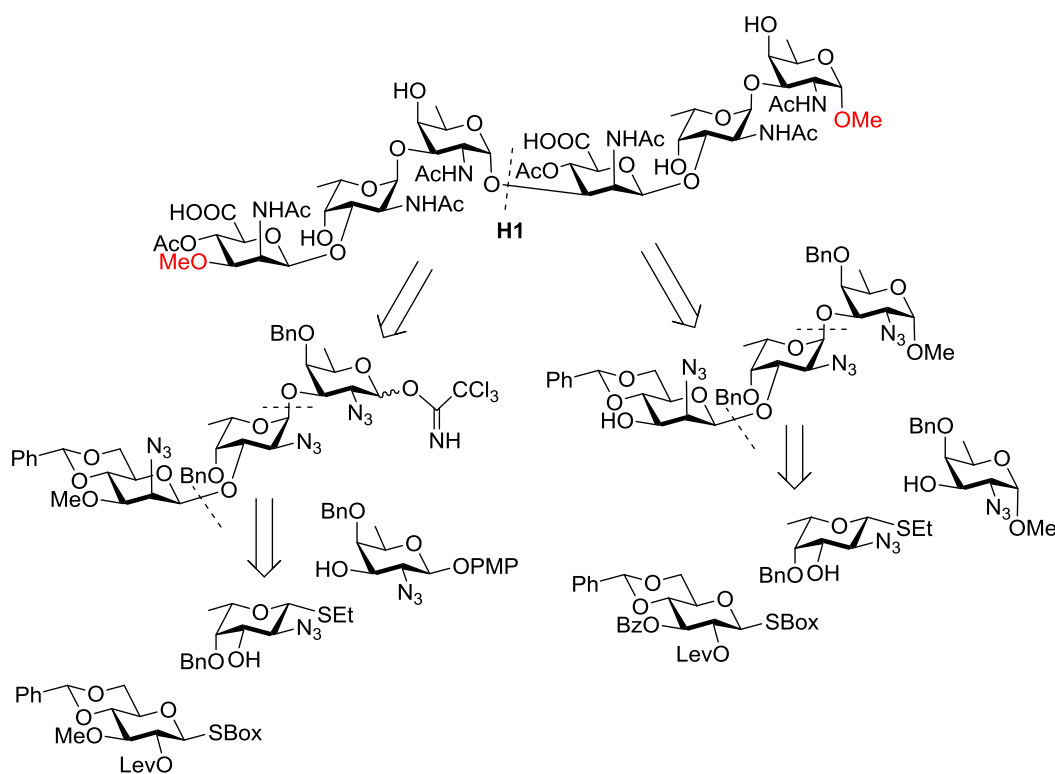
5.2 Results and Discussion

5.2.1 Retrosynthetic analysis of hexasaccharide H1

In order to assemble the CP8 hexasaccharide subunit, we decided to utilize a [3 + 3] glycosylation strategy using a trisaccharide donor and a trisaccharide acceptor as depicted in Scheme 5.3. We anticipated that this approach would allow us to apply similar synthetic considerations to those used in our previous syntheses of staphylococcal

trisaccharides. First, to obtain β -mannosidic linkages we chose to proceed via glucosylation using neighboring group participation to ensure the β -linkage, followed by epimerization of the stereocenter at C-2 to obtain the mannosamine residue. Second, the *S*-benzoxazolyl (SBox) leaving group of the glucosyl donor should allowed us to selectively activate the SBox leaving group in the presence of ethylthio glycoside by using silver(I) trifluoromethanesulfonate (AgOTf) as the promoter. Third, we chose to employ *p*-methoxyphenyl (PMP) as a temporary anomeric protecting group¹⁴ that can be converted to the trichloroacetimidoyl leaving group for subsequent glycosylation.

Scheme 5.3. Structure and retrosynthesis of CP8 hexasaccharide H1.

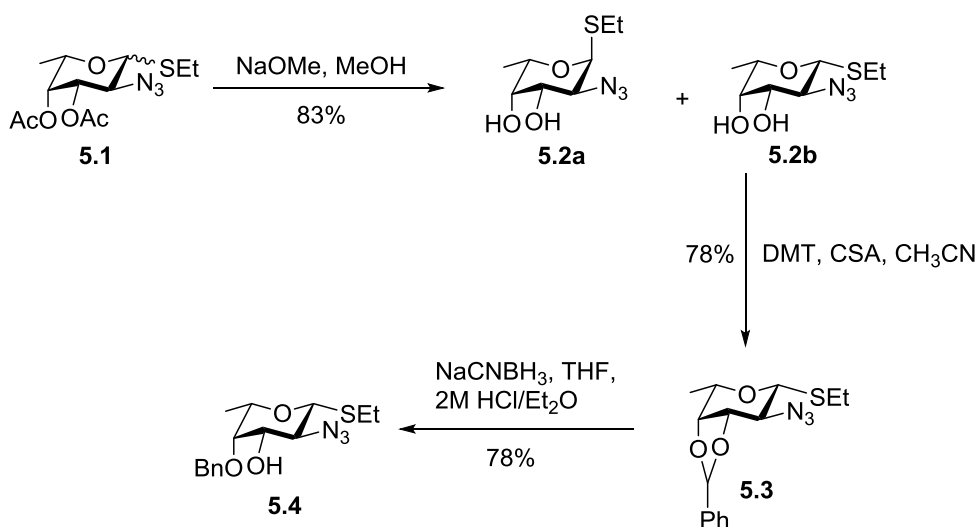


5.2.2 Synthesis of L-fucosyl acceptor 5.4

The synthesis of L-fucosyl acceptor for the introduction at the middle part of each trisaccharide was prepared from a known thioglycoside **5.1**¹⁵ as depicted in Scheme 5.4.

Deacetylation of compound **5.1** was affected with sodium methoxide (NaOMe) in methanol (MeOH) to obtain compounds **5.2a** and **5.2b** in 83% yield. The β -anomer **5.2b** was subjected to the benzylidene introduction with dimethoxytoluene (DMT) and camphorsulfonic acid (CSA) in acetonitrile (CH₃CN) to yield compound **5.3** in 78% yield. The latter was then reacted with sodium cyanoborohydride (NaCNBH₃) in 2 M hydrogen chloride solution in diethyl ether (2M HCl/Et₂O) in THF to afford the fucosyl acceptor **5.4** in 78% yield.

Scheme 5.4. Synthesis of L-fucosyl acceptor 5.4.

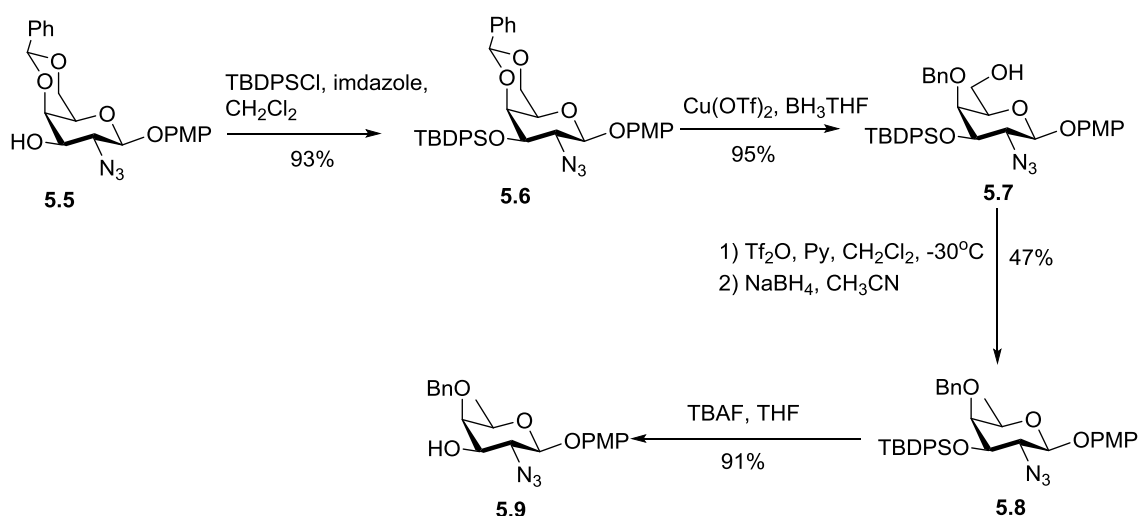


5.2.3 Synthesis of D-fucosyl acceptor 5.9

The synthesis of the D-fucosyl acceptor for the introduction of the reducing end unit in the trisaccharide donor was initiated from D-galactose derivative **5.5**¹⁶ as depicted in Scheme 5.5. The introduction of the *tert*-Butyldiphenylsilyl (TBDPS) group was performed with TBDPSCl in the presence of imidazole in CH₂Cl₂ to acquire compound **5.6** in 93% yield. The latter was reacted with copper(II) trifluoromethanesulfonate (Cu(OTf)₂) and a borane-tetrahydrofuran complex solution in THF to regioselectively

open benzylidene ring to obtain **5.7** in 95% yield. The primary hydroxyl group of **5.7** was reacted with trifluoromethanesulfonic anhydride (Tf₂O) in the presence of pyridine in CH₂Cl₂ at -30 °C, followed by the substitution of triflate by a hydride with sodium borohydride to give D-fucosamine **5.8** in 47% yield. After that, the silyl ether was removed with tetrabutylammonium fluoride (TBAF) in THF to afford D-fucosyl acceptor **5.9** in 91% yield.

Scheme 5.5. Synthesis of D-fucosyl acceptor **5.9**.

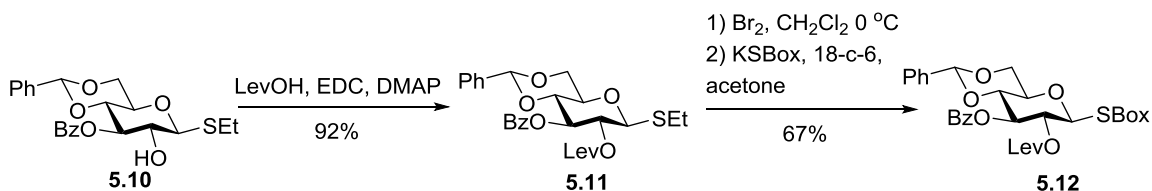


5.2.4 Synthesis of D-glucosyl donor **5.12**

The synthesis of glycosyl donor started from the known glucosyl derivative **5.10**¹⁷ as depicted in Scheme 5.6. Compound **5.10** was subjected to the introduction of the levulinoyl group with levulinic acid (LevOH) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) to yield compound **5.11** in 92% yield. The latter thioglycoside was converted to glucosyl bromide with bromine in dichloromethane at 0 °C, followed by the introduction of the S-

benzoxazolyl (SBox) leaving group with KSBBox¹⁸ in the presence of 18-crown-6 (18-c-6) in acetone to give thioimidate donor **5.12** in 67% yield over two steps.

Scheme 5.6. Synthesis of D-glucosyl donor **5.12**.

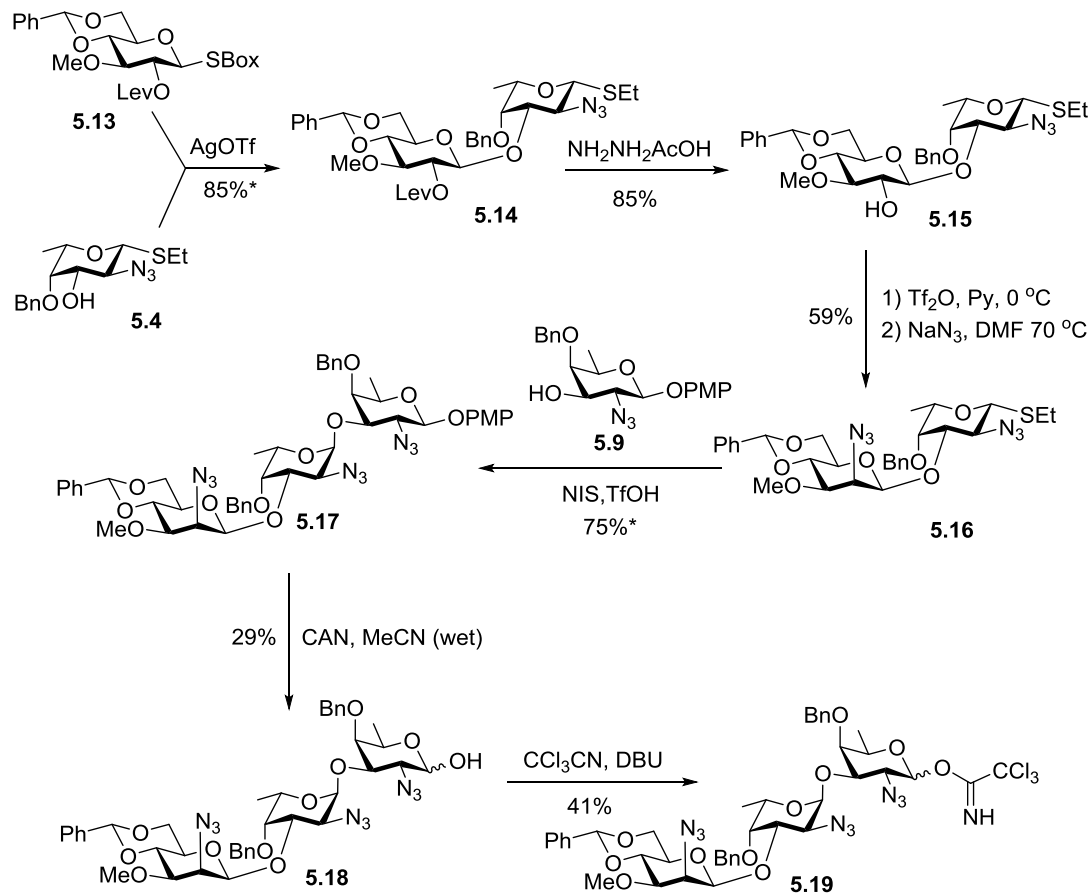


5.2.5 Synthesis of trisaccharide donor **5.19**

With all building blocks in hand, we conducted the selective activation of the SBox leaving group in the presence of the anomeric SEt group in the acceptor. Thus, glucosyl donor **5.13**⁸ was glycosylated with fucosyl acceptor **5.4** in the presence of AgOTf in CH₂Cl₂ to acquire disaccharide **5.14** in 85% yield (Scheme 5.7). The latter was reacted with hydrazine acetate in CH₂Cl₂ and MeOH to affect the selective removal of the levulinoyl ester to give disaccharide **5.15** in 85% yield. After that, disaccharide **5.15** was epimerized into the *manno*-configuration by a two-step procedure involving sulfonation at C-2' with Tf₂O in the presence of pyridine in CH₂Cl₂ at 0 °C, followed by epimerization by sodium azide (NaN₃) in DMF at 70 °C to yield D-mannosyl disaccharide **5.16** in 59% yield over two steps. After that, glycosylation between disaccharide donor **5.16** and D-fucosyl acceptor **5.9** was performed in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) in CH₂Cl₂ at 0 °C to afford trisaccharide **5.17** in 75% yield. Selective removal of the anomeric PMP group with cerium ammonium nitrate (CAN) in wet CH₃CN gave hemiacetal **5.18** in 29% yield. The reaction yield of PMP removal was low due to the low reactivity of the PMP group

at the reducing end of trisaccharide **5.17**. Then, the 1-OH group of trisaccharide **5.18** was reacted with trichloroacetonitrile (CCl_3CN) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 to obtain trisaccharide donor **5.19** in 41% yield.

Scheme 5.7. Synthesis of trisaccharide donor 5.19.

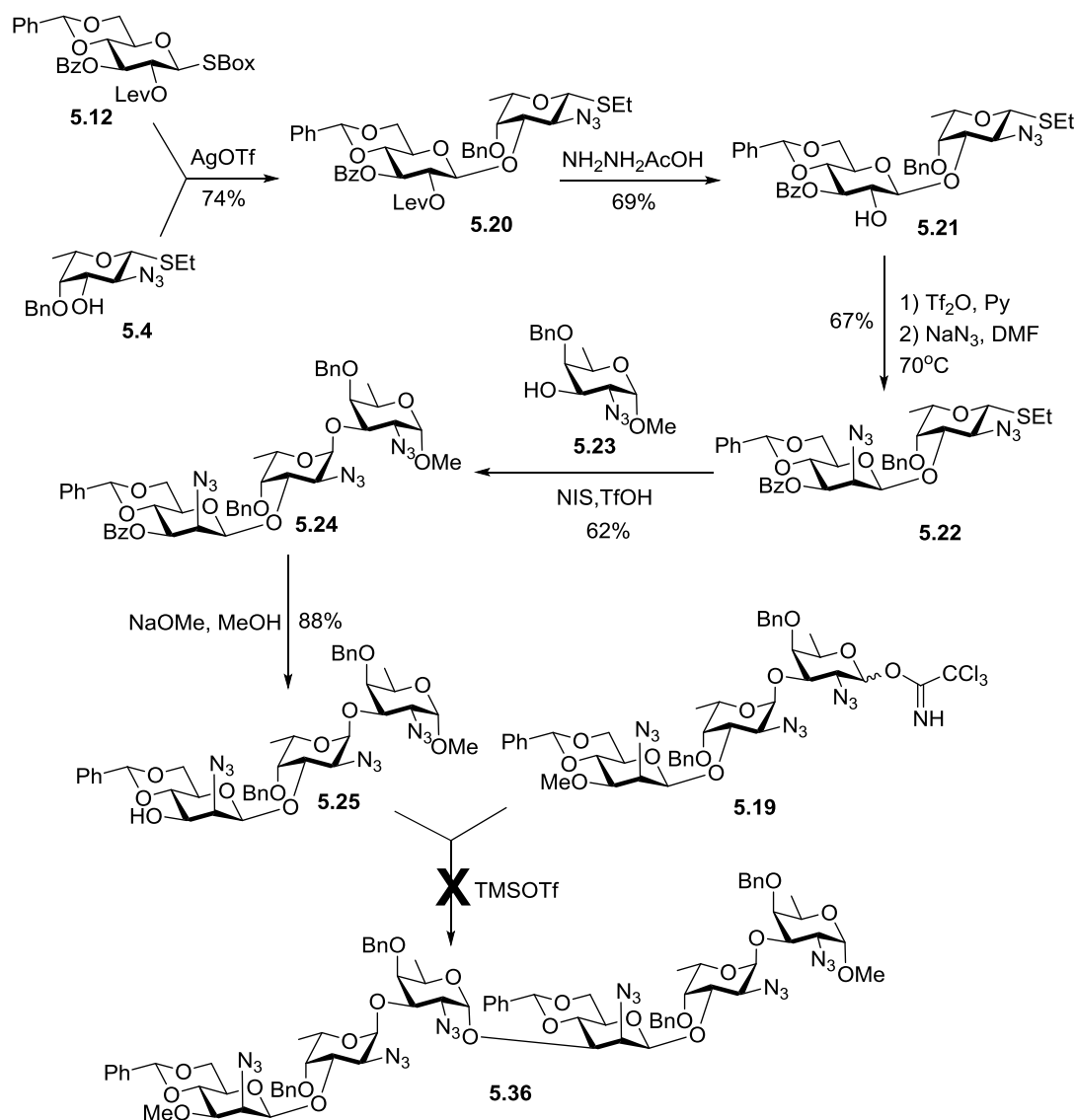


5.2.6 Synthesis of trisaccharide acceptor 5.25

The assembly of the trisaccharide acceptor was initiated by the glycosylation of the D-glucosyl donor **5.12** and the L-fucosyl acceptor **5.4** by selective activation of SBox in the presence of SET with AgOTf to obtain disaccharide **5.20** in 74% yield (Scheme 5.8). Then, the C-2' of disaccharide **5.20** was converted into the D-manno isomer via

sequential selective removal of the levulinoyl group with hydrazine acetate in CH_2Cl_2 to yield 2'-OH disaccharide **5.21** in 69% yield, sulfonation with Tf_2O , and epimerization with NaN_3 ¹⁹ to give disaccharide **5.22** in 67% yield over two steps. The latter was subjected to glycosylation with the D-fucosyl acceptor **5.23** in the presence of NIS and TfOH in CH_2Cl_2 at 0 °C to afford trisaccharide **5.24** in 62% yield with exclusive α -selectivity. Debenzoylation at C-3'' of trisaccharide **5.24** with NaOMe in MeOH provided the trisaccharide acceptor **5.25** in 88% yield.

Scheme 5.8. Synthesis of trisaccharide acceptor 5.25 and the [3 + 3] glycosylation.



5.2.7 The [3 + 3] glycosylation

With the trisaccharide donor **5.19** and trisaccharide acceptor **5.25** in hand, we attempted to perform glycosylation in the presence of TMSOTf as a promoter in CH₂Cl₂ at 0 °C (Scheme 5.8). However, the desired hexasaccharide was not observed, and only the trisaccharide acceptor **5.25** was recovered from the reaction. We hypothesized that this failure could have been due to the substantial steric bulk of the trisaccharide donor. To test the viability of this hypothesis, we decided to synthesize D-fucosyl donor **5.31**, which could be used for a model glycosylation.

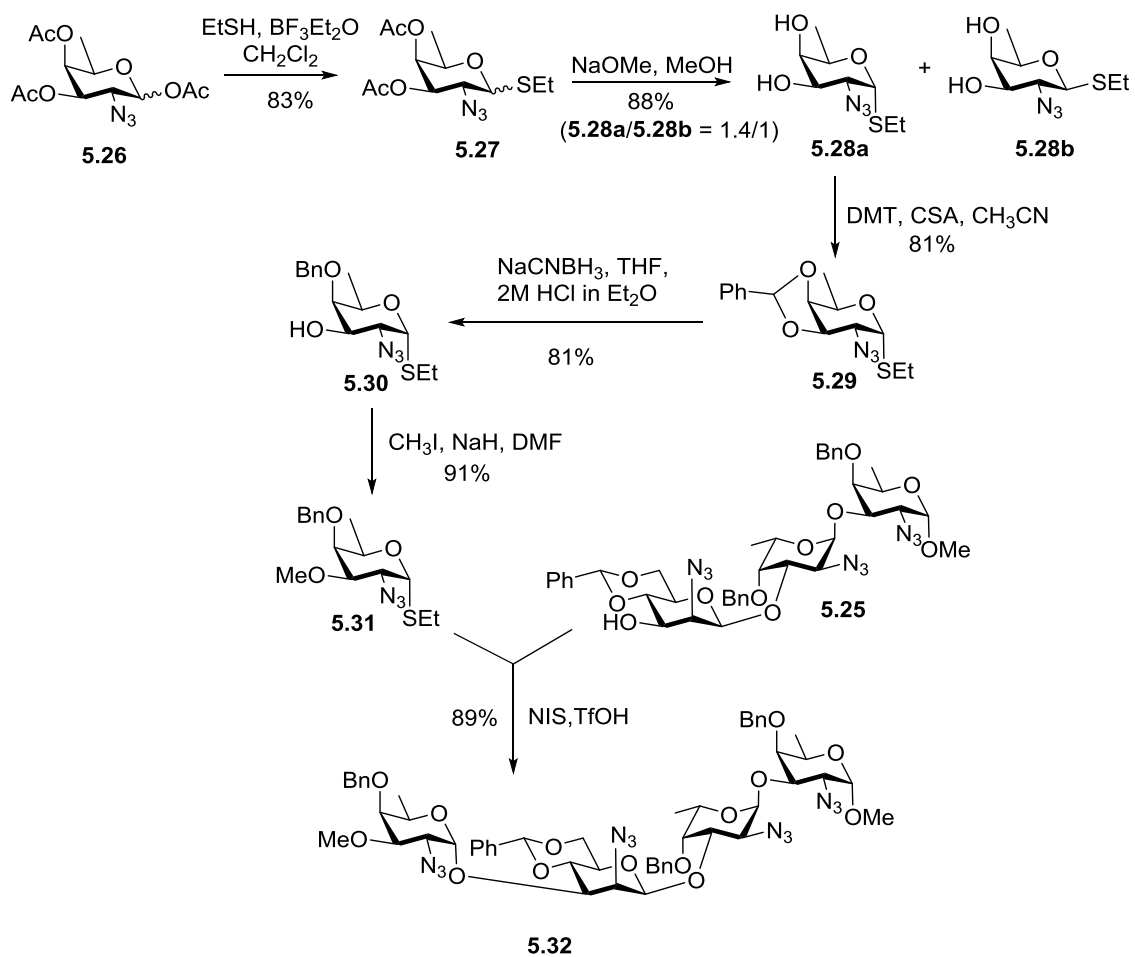
5.2.8 Model glycosylation of D-fucosyl donor **5.31** and trisaccharide acceptor **5.25**

The synthesis of the D-fucosyl donors **5.31** started from the known fucosyl compound **5.26**⁷ as depicted in Scheme 5.9. First, the thioethyl leaving group was introduced using EtSH in the presence of BF₃-Et₂O in CH₂Cl₂ to afford thioglycoside **5.27** in 83% yield. Deacetylation of compound **5.27** was performed in the presence of NaOMe in MeOH to yield diols **5.28a** and **5.28b** (**5.28a/5.28b** = 1.4/1) in 88% yield. The isolated α -isomer **5.28a** was subjected to benzylidene acetal introduction at C-3,4 with DMT in the presence of CSA in CH₃CN to give compound **5.29** in 81% yield. Regioselective benzylidene ring opening was achieved by the treatment with NaCNBH₃ in a HCl (2M in Et₂O) in THF to acquire 3-OH intermediate **5.30** in 81% yield. The latter was methylated with CH₃I in the presence of NaH in DMF to afford D-fucosyl donor **5.31** in 91% yield.

With D-fucosyl donor **5.31** in hand, we conducted the glycosylation between the D-fucosyl donor **5.31** and the trisaccharide acceptor **5.25** in the presence of NIS and

TfOH as the promoter system. As the result, tetrasaccharide **5.32** was smoothly produced in 89% yield with exclusive α -selectivity (Scheme 5.9).

Scheme 5.9. Synthesis of D-fucosyl donor 5.31 and model glycosylation of D-fucosyl donor 5.31 and trisaccharide acceptor 5.25.

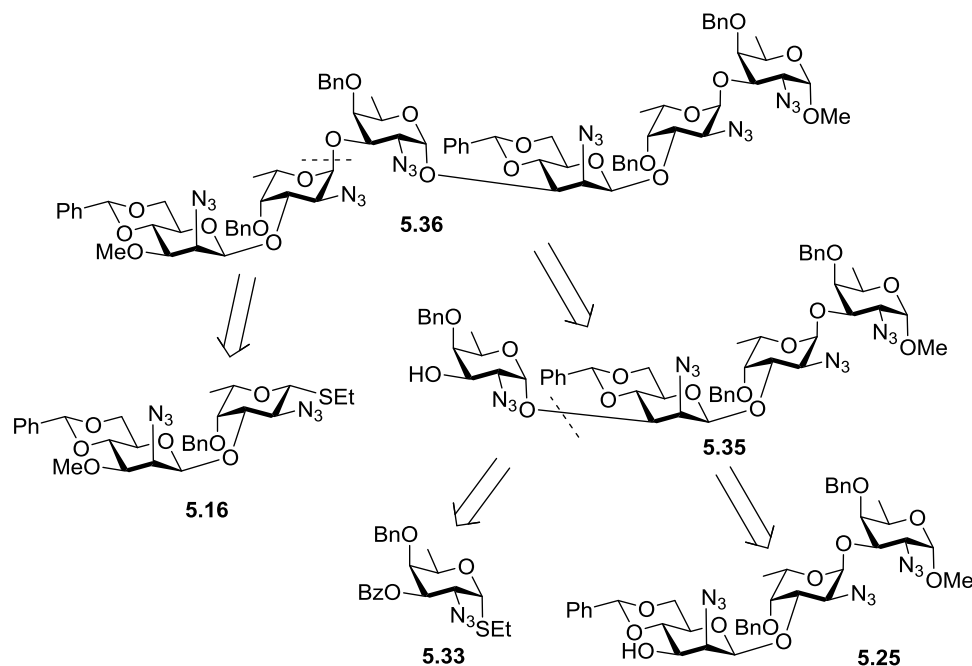


5.2.9 The revised retrosynthetic analysis of a key hexasaccharide intermediate 5.36

Based on the success in preparing tetrasaccharide **5.32**, we decided to revise our synthetic approach by utilizing the [2 + 1 + 3] strategy to obtain hexasaccharide **5.36** as depicted in Scheme 5.10. This strategy should allow us to use disaccharide **5.14** and

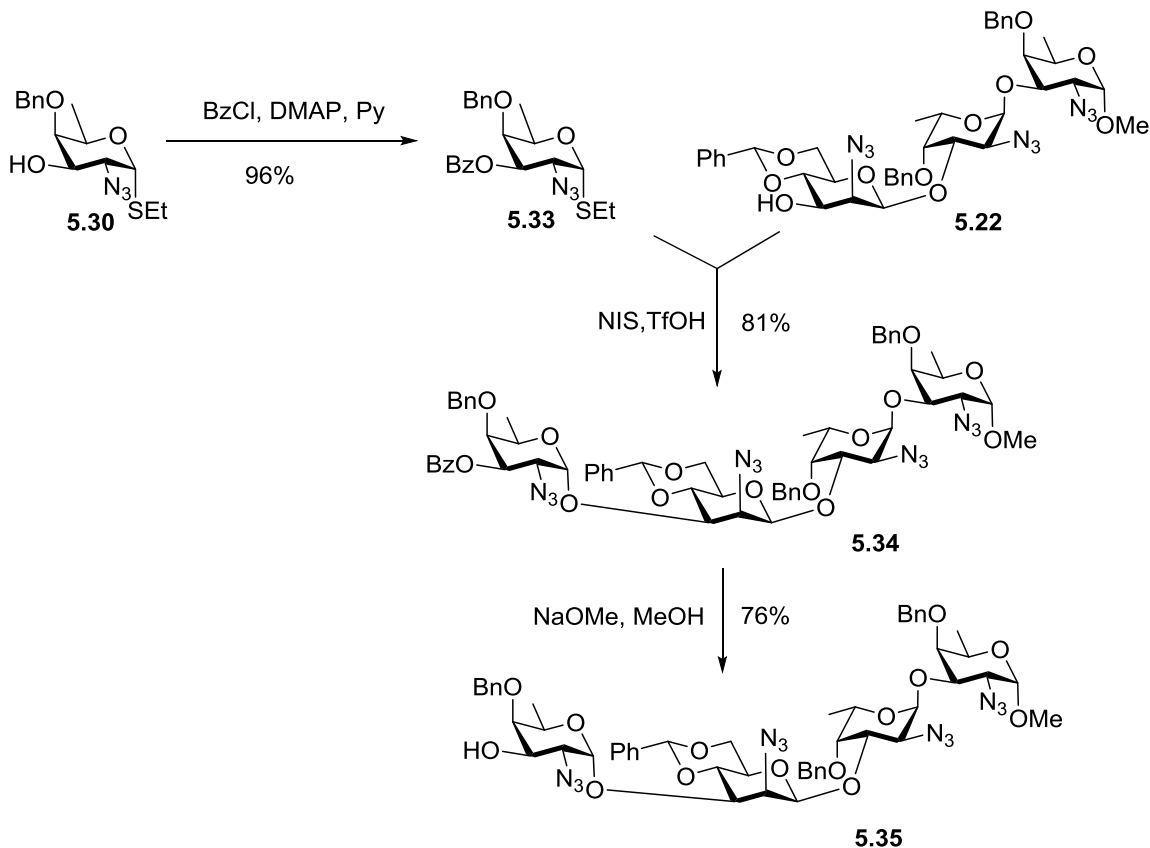
trisaccharide acceptor **5.25** that have been synthesized previously. However, D-fucosyl donor **5.33**, which connects these two subunits, will have to be prepared.

Scheme 5.10. The revised retrosynthesis of a key hexasaccharide intermediate 5.36.



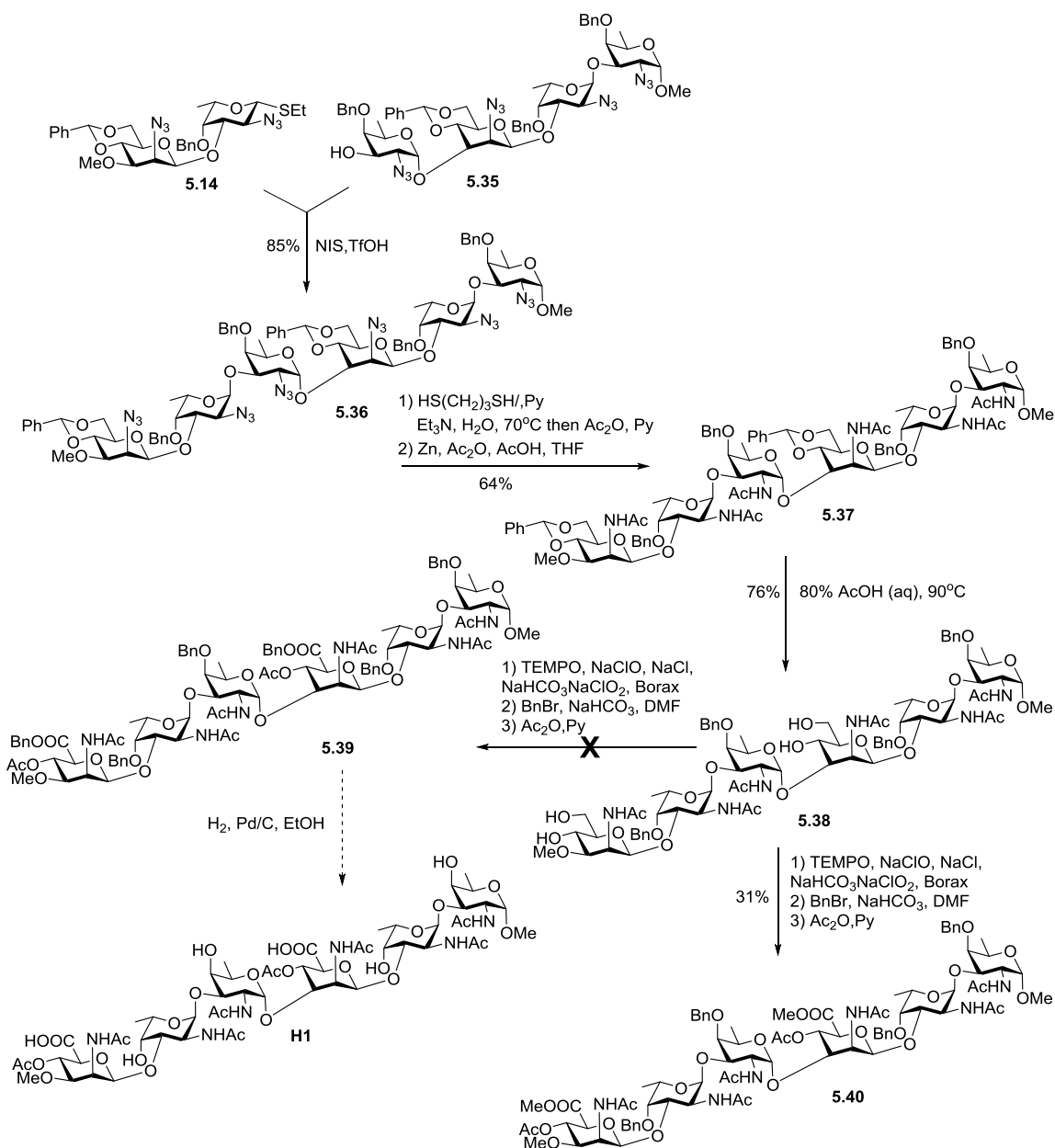
5.2.10 Synthesis of D-fucosyl donors 5.33 and tetrasaccharide acceptor 5.35

The D-fucosyl donor **5.33** used in the synthesis of tetrasaccharide acceptor **5.35** was obtained by benzylation of compound **5.30** with BzCl in the presence of DMAP in pyridine to afford D-fucosyl donor **5.33** in 96% yield (Scheme 5.11). To obtain tetrasaccharide acceptor **5.34**, the glycosylation of D-fucosyl donor **5.29** and trisaccharide acceptor **5.22** was conducted in the presence of NIS and TfOH as the promoter system in CH_2Cl_2 at $0\text{ }^\circ\text{C}$ (Scheme 5.11). As a result, tetrasaccharide **5.34** was isolated in 81% yield exclusively as the α -linked anomer. After that, the 3''-OBz group was removed in the presence of NaOMe in MeOH to give the tetrasaccharide acceptor **5.35** in 76% yield.

Scheme 5.11. Synthesis of D-fucosyl donor 5.33 and tetrasaccharide acceptor 5.35.**5.2.11 Assembly and functionalization to hexasaccharide H1**

The [2 + 4] glycosylation of disaccharide donor **5.14** and tetrasaccharide acceptor **5.35** was affected by NIS and TfOH in CH_2Cl_2 at 0 °C to afford the desired hexasaccharide **5.36** in 85% yield (Scheme 5.12). After that, the azido groups of hexasaccharide **5.36** were reduced by 1,3-propanedithiol and triethylamine (TEA) in wet pyridine at 70 °C, followed by *N*-acetylation in the presence of acetic anhydride (Ac_2O) in pyridine. After that, an additional one pot reduction-*N*-acetylation with zinc dust in acetic acid and acetic anhydride was performed to ensure the complete reduction of all azide groups. As a result, hexasaccharide **5.37** was obtained in 64% yield.

Scheme 5.12. Assembly and functionalization to CP8 hexasaccharide H1.



Then, benzylidene removal was affected by acetic acid in water at 90 °C to acquire tetraol **5.38** in 76% yield. To obtain the fully protected hexasaccharide **5.39**, the primary hydroxyl groups of compound **5.38** were selectively oxidized by TEMPO and BAIB in wet CH₂Cl₂.²⁰ However, the reaction processed slowly, and no dicarboxylic acid

derivative was observed due to the low reactivity of the secondary oxidizing reagent. To overcome this issue, we decided to perform the oxidation with a modified method²¹ involving the reaction with TEMPO in the presence of sodium chlorite (NaClO₂), sodium hypochlorite (NaClO), brine solution (NaCl), saturated sodium bicarbonate solution (NaHCO₃) and a borax buffer pH 9 in CH₃CN to obtain the dicarboxylic acid derivative. The latter was then converted into the benzyl dicarboxylate with benzyl bromide (BnBr) in the presence of NaH in DMF and the secondary hydroxyl groups were acetylated with Ac₂O in pyridine. Unexpectedly, the mass spectrometry and NMR experiments indicated that methyl ester derivative **5.40** was formed instead of benzyl ester hexasaccharide **5.39** in 31% yield over three steps. The transesterification might have occurred during the methanol quenching after the acetylation step. However, the hexasaccharide **5.40** should be useful for chemical activation study of CP8 and the structure-activity relationship study to determine the required minimal epitope for immune response.

5.3 Conclusions and Outlook

We successfully assembled in the assembly of the CP8 hexasaccharide bearing methyl groups as the capping groups. The original [3 + 3] glycosylation strategy was unsuccessful due to the repulsive interactions between the reaction counterparts. However, we modified the synthetic plan as a [1 + 2 + 3] glycosylation strategy that has been utilized. We succeeded in synthesizing the key intermediate hexasaccharide sequence **5.36** which was subjected to structure modification to obtain CP8 hexasaccharide **H1**. However, during the functionalization we unexpectedly obtained the methyl ester hexasaccharide **5.40**, probably due to the transesterification that have

occurred during the methanol quench step. Nevertheless, we believe that methyl ester CP8 hexasaccharide **5.40** as well as the tetrasaccharide derivative **5.32** will be useful compounds to study protein conjugation. In turn, this will lead to a better understanding of the activation process of native polysaccharides and will ultimately allow us to control, predict and reliably reproduce polysaccharide conjugations.

5.4 Experimental

5.4.1 General Methods

The reactions were performed using commercial reagents (Aldrich or Acros) and ACS grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F₂₅₄ (EM Science). 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. CH₂Cl₂ and ClCH₂CH₂Cl were distilled from CaH₂ directly prior to application. 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. The borax buffer solution (pH 9) was prepared with 0.05 M sodium tetraborate (Na₂B₄O₇) solution (85.6 mL) and 0.1 M hydrochloric acid (HCl) solution (14.4 mL). Optical rotations were measured at 'Jasco P-2000' polarimeter. ¹H NMR spectra were recorded at 300 MHz (Bruker Avance) or 600 MHz (Agilent), ¹³C-NMR spectra were recorded at 75 MHz (Bruker Avance) or 150 MHz (Agilent). The ¹H chemical shifts are referenced to the signal of the residual CHCl₃ (δ_H = 7.27 ppm) for solutions in CDCl₃ or the signal of the

residual CH₃OH ($\delta_{\text{H}} = 3.31$ ppm) for solutions in CD₃OD. The ¹³C chemical shifts are referenced to the central signal of CDCl₃ ($\delta_{\text{C}} = 77.23$ ppm) for solutions in CDCl₃ or the central signal of CD₃OD ($\delta_{\text{C}} = 49.15$ ppm) for solutions in CD₃OD. HRMS determinations were made with the use of JEOL MStation (JMS-700) mass spectrometer.

5.4.2 Synthesis of L-fucosyl acceptor 5.4

Ethyl 2-azido-2,6-dideoxy-1-thio- α -L-galactopyranoside (5.2a) and ethyl 2-azido-2,6-dideoxy-1-thio- β -L-galactopyranoside (5.2b). A 1M solution of NaOMe in MeOH (4.0 mL) was added to a stirring solution of ethyl 3,4-di-*O*-acetyl-2-azido-2,6-dideoxy-1-thio-L-galactopyranoside¹⁵ (**5.1**, 4.98 g, 15.7 mmol) in methanol (50 mL) and the resulting mixture was stirred under argon for 2 h at rt. After that, Amberlite IR120 H+ resin was added to the solution until pH = 7. Then, the resin was filtered-off, rinsed successively with MeOH (7 x 5 mL), and the combined filtrate was concentrated *in vacuo*. The residue was separated by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield **5.2a** (1.61 g, 40%) and **5.2b** (1.73 g, 43%) as a white amorphous solid. Analytical data for **5.2a**: $R_f = 0.31$ (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ , 1.28-1.33 (m, 6H, CH₂CH₃, H-6), 2.54 (q, 2H, CH₂CH₃), 3.80-3.84 (m, 2H, H-3, 4), 4.00 (dd, 1H, $J_{2,3} = 9.8$ Hz, H-2), 4.32 (m, 1H, H-5), 5.41 (d, 1H, $J_{1,2} = 5.6$ Hz, H-1) ppm; **5.2b**: $R_f = 0.26$ (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ , 1.30-1.36 (m, 6H, CH₂CH₃, H-6), 2.71 (q, 2H, CH₂CH₃), 3.42-3.56 (m, 2H, $J_{2,3} = 9.5$ Hz, $J_{3,4} = 3.1$ Hz, H-2, 3), 3.59 (m, 1H, H-5), 3.75 (dd, 1H, H-4), 4.26 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1) ppm.

Ethyl 2-azido-3,4-O-benzylidene-2,6-dideoxy-1-thio-β-L-galactopyranoside (5.3).

Dimethoxytoluene (2.4 mL, 15.78 mmol) and camphorsulfonic acid (92 mg, 0.39 mmol) were added to a solution of **5.2b** (1.73 g, 7.89 mmol) in acetonitrile (30 mL) and the resulting mixture was stirred under argon for 1 h at rt. After that, triethylamine (~0.3 mL) was added and the volatiles were removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~200 mL) and washed with water (30 mL), sat. aq. NaHCO₃ (30 mL), and water (30 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid (1.90 g, 78%). Analytical data for **5.3**: $R_f = 0.64$ (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.28 (t, 3H, CH₂CH₃), 1.49 (d, 3H, H-6), 2.66 (q, 2H, CH₂CH₃), 3.39 (dd, 1H, $J_{2,3} = 7.4$ Hz, H-2), 3.88 (m, 1H, $J_{5,6} = 6.6$ Hz, H-5), 4.09 (dd, 1H, $J_{4,5} = 2.3$ Hz, H-4), 4.17 (dd, 1H, $J_{3,4} = 5.6$ Hz, H-3), 4.25 (d, 1H, $J_{1,2} = 10.6$ Hz, H-1), 6.00 (s, 1H, CHPh), 7.39-7.42 (m, 3H, aromatic), 7.50-7.54 (m, 2H, aromatic) ppm.

Ethyl 2-azido-4-O-benzyl-2,6-dideoxy-1-thio-β-L-galactopyranoside (5.4). A solution of compound **5.3** (1.21 g, 3.78 mmol) and activated molecular sieves (3 Å, 1.50 g) in THF (30 mL) was stirred under argon for 1 h at rt. NaCNBH₃ (3.17 g, 50.27 mmol) was added in one portion, 2M HCl in diethyl ether (25.1 mL, 50.27 mmol) was added dropwise, and the resulting mixture was kept for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH₂Cl₂. The combined filtrate (~150 mL) was washed with water (30 mL), sat. aq. NaHCO₃ (30 mL), and water (30 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was

purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a clear syrup (957 mg, 78%). Analytical data for **5.4**: $R_f = 0.59$ (ethyl acetate/hexane, 2/3, v/v); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.28-1.36 (CH_2CH_3 , H-6), 2.29 (d, 1H, $J_{3,\text{OH}} = 8.1$ Hz, OH), 2.70 (q, 2H, CH_2CH_3), 3.46-3.62 (m, 4H, H-2, 3, 4, 5), 4.22 (d, 1H, $J_{1,2} = 9.6$ Hz, H-1), 4.66 (dd, 1H, $^2J = 11.6$ Hz, $\frac{1}{2}$ CH_2Ph), 4.83 (dd, 1H, $^2J = 11.6$ Hz, $\frac{1}{2}$ CH_2Ph), 7.32-7.39 (m, 5H, aromatic) ppm.

5.4.3 Synthesis of D-fucosyl acceptor 5.9

4-Methoxyphenyl 2-azido-4,6-O-benzylidene-3-O-tert-butylidiphenylsilyl-2-deoxy- β -D-galactopyranoside (5.6). *tert*-Butyldiphenylchlorosilane (1.56 mL, 6.01 mmol) and imidazole (819 mg, 12.03 mmol) were added to a stirring solution of 4-methoxyphenyl-2-azido-4,6-*O*-benzylidene-2-deoxy- β -D-galactopyranoside¹⁶ (**5.5**, 1.60 g, 4.01 mmol) in CH_2Cl_2 (50 mL). The resulting mixture was stirred under argon for 1 h at rt. After that, the volatiles were removed *in vacuo*. The residue was diluted with CH_2Cl_2 (~200 mL) and washed with water (30 mL), sat. aq. NaHCO_3 (30 mL), and water (30 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 yield the title compound as a white amorphous solid (2.37 g, 93%). Analytical data for **5.6**: $R_f = 0.67$ (ethyl acetate/hexane, 2/3, v/v); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.10 (s, 9H, $\text{C}(\text{CH}_3)_3$), 3.16 (m, 1H, H-5), 3.53 (dd, 1H, $J_{4,5} = 3.2$ Hz, H-4), 3.61 (dd, 1H, $J_{3,4} = 3.5$ Hz, H-3), 3.77-3.82 (m, 4H, OCH_3 , H-6a), 4.09 (dd, 1H, $J_{2,3} = 10.1$ Hz, H-2), 4.20 (m, 1H, H-6b), 4.63 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1), 5.22 (s, 1H, CHPh), 6.79 (d, 2H, $J =$

9.1 Hz, PMP), 7.04 (d, 2H, $J = 9.0$ Hz, PMP), 7.29-7.34 (m, 2H, aromatic), 7.40-7.46 (m, 7H, aromatic), 7.53-7.65 (m, 2H, aromatic), 7.74-7.86 (m, 4H, aromatic) ppm.

4-Methoxyphenyl 2-azido-4-O-benzyl-3-O-tert-butylidiphenylsilyl-2-deoxy- β -D-galactopyranoside (5.7). Copper(II) trifluoromethanesulfonate (145 mg, 0.40 mmol) was added to a stirring solution of compound **5.6** (2.37 g, 3.72 mmol) in a 1.0 M borane-tetrahydrofuran complex solution in THF (18.6 mL, 18.60 mmol) and the resulting mixture was stirred under argon for 4 h at rt. After that, triethylamine (~3.0 mL) and MeOH were added at 0 °C. Then, the volatiles were removed *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid (2.26 g, 95%). Analytical data for **5.7**: $R_f = 0.51$ (ethyl acetate/hexane, 2/3, v/v); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.11 (s, 9H, $\text{C}(\text{CH}_3)_3$), 3.30-3.38 (m, 3H, H-5, 6a), 3.44 (dd, 1H, $J_{4,5} = 2.7$ Hz, H-4), 3.63-3.71 (m, 5H, OCH_3 , H-3, 6a), 4.02 (dd, 1H, $J_{2,3} = 10.2$ Hz, H-2), 4.35 (d, 1H, $^2J = 11.5$ Hz, $\frac{1}{2}$ CH_2Ph), 4.55 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.88 (d, 1H, $^2J = 11.5$ Hz, $\frac{1}{2}$ CH_2Ph), 6.72 (d, 2H, $J = 9.1$ Hz, PMP), 6.88 (d, 2H, $J = 9.09$ Hz, PMP), 7.17-7.21 (m, 2H, aromatic), 7.34-7.43 (m, 9H, aromatic), 7.72-7.76 (m, 4H, aromatic) ppm.

4-Methoxyphenyl 2-azido-4-O-benzyl-3-O-tert-butylidiphenylsilyl-2,6-dideoxy- β -D-galactopyranoside (5.8). Pyridine (5.0 mL) was added to a solution of compound **5.7** (2.26 g, 3.53 mmol) in CH_2Cl_2 (20.0 mL) and the resulting mixture was cooled to -78 °C. Trifluoromethanesulfonic anhydride (0.71 mL, 4.24 mmol) was added dropwise and the reaction mixture was allowed to reach 0 °C in 30 mins. After that, the reaction was

quenched with ice-cold water (~5 mL), diluted with CH₂Cl₂ (~200 mL), and washed with 1 N HCl solution (50 mL). The organic phase was separated, dried over MgSO₄, and concentrated *in vacuo*. The crude residue was dissolved in CH₃CN (20.0 mL), NaBH₄ (401 mg, 10.59 mmol) was added, and the resulting suspension was stirred for 48 h at rt. Upon completion, CH₂Cl₂ (~200 mL) was added. Organic layer was washed with water (50 mL), 1 N aq. HCl solution (50 mL) and water (50 mL). The organic extract was dried with MgSO₄ 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 foam (1.03 g, 47%). Analytical data for **5.8**: R_f = 0.75 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.11 (s, 9H, C(CH₃)₃), 3.30-3.38 (m, 3H, H-5, 6a), 3.44 (dd, 1H, J_{4,5} = 2.7 Hz, H-4), 3.63-3.71 (m, 5H, OCH₃, H-3, 6a), 4.02 (dd, 1H, J_{2,3} = 10.2 Hz, H-2), 4.35 (d, 1H, ²J = 11.5 Hz, ½ CH₂Ph), 4.55 (d, 1H, J_{1,2} = 8.0 Hz, H-1), 4.88 (d, 1H, ²J = 11.5 Hz, ½ CH₂Ph), 6.72 (d, 2H, J = 9.1 Hz, PMP), 6.88 (d, 2H, J = 9.1 Hz, PMP), 7.17-7.21 (m, 2H, aromatic), 7.34-7.43 (m, 9H, aromatic), 7.72-7.76 (m, 4H, aromatic) ppm.

4-Methoxyphenyl 2-azido-4-O-benzyl-2,6-dideoxy-β-D-galactopyranoside (5.9). 1 M tetrabutylammonium fluoride solution in THF (2.0 mL, 1.99 mmol) was added to a solution of compound **5.8** (1.03 g, 1.66 mmol) in THF (20.0 mL) and the resulting mixture was stirred under argon for 3 h at rt. After that, the organic phase was removed *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 foam (581 mg, 91%). Analytical data for **5.9**: R_f = 0.47 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz,

CDCl₃): δ , 1.15-1.17 (m, 12H, C(CH₃)₃, H-6), 3.25 (dd, 1H, $J_{4,5} = 2.6$ Hz, H-4), 3.39 (m, 1H, H-5), 3.70 (dd, 1H, $J_{3,4} = 2.7$ Hz, H-3), 3.78 (s, 3H, OCH₃), 4.03 (dd, 1H, $J_{2,3} = 10.3$ Hz, H-2), 4.43 (d, 1H, $^2J = 11.4$ Hz, $\frac{1}{2}$ CH₂Ph), 4.55 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.94 (d, 1H, $^2J = 11.4$ Hz, $\frac{1}{2}$ CH₂Ph), 6.76 (d, 2H, $J = 9.0$ Hz, PMP), 6.96 (d, 2H, $J = 9.0$ Hz, PMP), 7.29-7.34 (m, 3H, aromatic), 7.36-7.49 (m, 8H, aromatic), 7.72-7.77 (m, 4H, aromatic) ppm.

5.4.4 Synthesis of D-fucosyl donors 5.31 and 5.33

Ethyl 3,4-di-*O*-acetyl-2-azido-2,6-dideoxy-1-thio-D-galactopyranoside (5.27). Ethane thiol (1.1 mL, 8.70 mmol) and BF₃-Et₂O (4.2 mL, 33.45 mmol) were added to a stirring solution of 1,3,4-tri-*O*-acetyl-2-azido-2,6-dideoxy-D-galactopyranose⁷ (**5.26**, 2.11 g, 6.69 mmol) in CH₂Cl₂ (40 mL) at 0 °C. The reaction was stirred under argon and allowed to reach rt. After 3 h, the reaction was diluted with CH₂Cl₂ (~200 mL) and washed with water (30 mL), sat. aq. NaHCO₃ (30 mL) and water (30 mL). The organic phase was separated, dried over MgSO₄, 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 (1.76 g, 83%). Analytical data for **5.27**: $R_f = 0.72$ (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ , 1.14 (d, 3H, H-6 α), 1.20 (d, 3H, H-6 β), 1.28-1.36 (2t, 6H, 2 x CH₂CH₃), 2.04, 2.05, 2.17, 2.18 (4s, 12H, 4 x COCH₃), 2.58 (q, 2H, CH₂CH₃), 2.77 (q, 2H, CH₂CH₃), 3.60 (dd, 1H, $J_{2\beta,3\beta} = 10.3$ Hz, H-2 β), 3.72 (m, 1H, $J_{5\beta,6\beta} = 6.5$ Hz, H-5 β), 4.19 (dd, 1H, $J_{2\alpha,3\alpha} = 11.0$ Hz, H-2 α), 4.34 (d, 1H, $J_{1\beta,2\beta} = 10.1$ Hz, H-1 β), 4.47 (m, 1H, $J_{5\alpha,6\alpha} = 6.5$ Hz, H-5 α), 4.85 (dd, 1H, $J_{3\beta,4\beta} = 3.3$ Hz, H-3 β), 5.10 (dd, $J_{3\alpha,4\alpha} = 3.3$ Hz, 1H, H-3 α), 5.22 (dd, 1H, $J_{4\beta,5\beta} = 0.8$ Hz, H-4 β),

5.27 (dd, 1H, $J_{4\alpha,5\alpha} = 1.1$ Hz, H-4 α), 5.44 (d, 1H, $J_{1\alpha,2\alpha} = 5.6$ Hz, H-1 α) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{12}H_{19}NaN_3O_5S$ 340.0943, found 340.0934.

Ethyl 2-azido-2,6-dideoxy-1-thio- α -D-galactopyranoside (5.28a) and ethyl 2-azido-2,6-dideoxy-1-thio- β -D-galactopyranoside (5.28b). A 1M solution of NaOMe in MeOH (3.0 mL) was added to a stirring solution of thiol compound **5.27** (6.38 g, 20.10 mmol) in methanol (60 mL) and the resulting mixture was stirred under argon for 4 h at rt. After that, Amberlite IR120 resin was added to the solution until pH was 7. Then, the solid was filtered-off and the solution was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield **5.28a** (2.45 g, 52%) and **5.28b** (1.70 g, 36%) as a white amorphous solid. **5.28a**: $R_f = 0.52$ (ethyl acetate/hexane, 7/3, v/v); 1H NMR (300 MHz, $CDCl_3$): δ , 1.28-1.33 (m, 6H, CH_2CH_3 , H-6), 2.27 (d, 1H, $J_{OH,4} = 3.7$ Hz, 4-OH), 2.51-2.70 (m, 3H, CH_2CH_3 , 3-OH), 3.79-3.85 (m, 2H, H-3, 4), 4.00 (m, 1H, $J_{2,3} = 9.9$ Hz, H-2), 4.32 (m, 1H, H-5), 5.41 (d, 1H, $J_{1,2} = 5.6$ Hz, H-1) ppm; **5.28b**: $R_f = 0.39$ (ethyl acetate/hexane, 7/3, v/v); 1H NMR (300 MHz, $CDCl_3$): δ , 1.30-1.35 (m, 6H, CH_2CH_3 , H-6), 2.68-2.83 (m, 3H, CH_2CH_3 , 4-OH), 3.21 (d, 1H, $J_{OH,3} = 7.0$ Hz, 3-OH), 3.46-3.56 (m, 2H, $J_{2,3} = 9.3$ Hz, $J_{3,4} = 2.3$ Hz, H-2, 3), 3.61 (m, 1H, H-5), 3.76 (dd, 1H, H-4), 4.26 (d, 1H, $J_{1,2} = 9.6$ Hz, H-1) ppm;

Ethyl 2-azido-3,4-O-benzylidene-2,6-dideoxy-1-thio- α -D-galactopyranoside (5.29). Dimethoxytolulene (0.76 mL, 5.04 mmol) and camphorsulfonic acid (29 mg, 0.13 mmol) we added to a solution of **5.28a** (552 mg, 2.52 mmol) in acetonitrile (15 mL) and the resulting mixture was stirred under argon for 2 h at rt. After that, triethylamine (~0.3 mL)

was added and the volatiles were removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~120 mL) and washed with water (10 mL), sat. aq. NaHCO₃ (10 mL), and water (20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a colorless syrup (657 mg, 81%). Analytical data for **5.29**: R_f = 0.69 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.29 (t, 3H, CH₂CH₃), 1.44 (d, 3H, H-6), 2.56 (m, 2H, CH₂CH₃), 3.87 (dd, 1H, J_{2,3} = 8.3 Hz, H-2), 4.13 (dd, 1H, J_{4,5} = 2.6 Hz, H-4), 4.34 (dd, 1H, J_{3,4} = 5.7 Hz, H-3), 4.53 (m, 1H, J_{5,6} = 6.7 Hz, H-5), 5.27 (d, 1H, J_{1,2} = 5.3 Hz, H-1), 5.97 (s, 1H, CHPh), 7.40-7.44 (m, 3H, aromatic), 7.52 (m, 2H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 15.0, 16.4, 25.0, 62.4, 64.2, 74.7, 78.1, 82.3, 104.1, 126.6 (x 2), 128.7 (x 2), 129.6, 137.6 ppm; HR-FAB MS [M+Na]⁺ calcd for C₁₅H₁₉O₃NaSN₃ 344.1045, found 344.1053.

Ethyl 2-azido-4-O-benzyl-2,6-deoxy-1-thio- α -D-galactopyranoside (5.30). A solution of compound **5.29** (657 mg, 2.04 mmol) and activated molecular sieves (3Å, 800 mg) in THF (10 mL) was stirred under argon for 1 h at rt. NaCNBH₃ (1.71 g, 27.19 mmol) was added in one portion, 2M HCl in diethyl ether (13.3 mL, 27.19 mmol) was added dropwise, and the resulting mixture was kept for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH₂Cl₂. The combined filtrate (~120 mL) was washed with water (20 mL), sat. aq. NaHCO₃ (20 mL), and water (20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a clear syrup (536 mg, 81%). Analytical data for **5.30**: R_f =

0.69 (ethyl acetate/hexane, 2/3, v/v); ^1H NMR (300 MHz, CDCl_3): δ , 1.29-1.32 (m, 6H, CH_2CH_3 , H-6), 2.15 (d, 1H, $J_{\text{OH},3} = 8.6$ Hz, 4-OH), 2.52 (m, 2H, CH_2CH_3), 3.65 (dd, 1H, $J_{4,5} = 1.0$ Hz, H-4), 3.82 (m, 1H, $J_{3,4} = 3.3$ Hz, H-3), 3.98 (dd, 1H, $J_{2,3} = 10.4$ Hz, H-2), 4.28 (m, 1H, H-5), 4.68 (d, 1H, $^2J = 11.5$ Hz, $\frac{1}{2}$ CH_2Ph), 4.81 (d, 1H, $^2J = 11.4$ Hz, $\frac{1}{2}$ CH_2Ph), 5.39 (d, 1H, $J_{1,2} = 5.5$ Hz, H-1), 7.33-7.38 (m, 5H, aromatic) ppm.

Ethyl 2-azido-4-O-benzyl-2,6-dideoxy-3-O-methyl-1-thio- α -D-galactopyranoside

(5.31). Methyl iodide (246 mg, 1.73 mmol) and sodium hydride (104 mg, 2.61 mmol) were added to a stirring solution of compound **5.30** (280 mg, 0.87 mmol) in DMF (5.0 ml) at 0°C . The reaction was allowed to reach at rt. After 2 h, the reaction was poured into iced water and was stirred for 15 mins. Then, the aqueous phase was extracted with EtOAc/Et₂O (1:1) (3 x 50 mL). The organic phase was collected, dried over 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 as a white amorphous solid (226 mg, 91%). Analytical data for **5.30**: $R_f = 0.70$ (ethyl acetate/hexane, 2/3, v/v); ^1H NMR (300 MHz, CDCl_3): δ , 1.19 (d, 3H, H-6), 1.26 (t, 3H, CH_2CH_3), 2.51 (m, 2H, CH_2CH_3), 3.47-3.52 (m, 4H, $J_{3,4} = 2.8$ Hz, OCH_3 , H-3), 3.71 (dd, 1H, $J_{4,5} = 1.7$ Hz, H-4), 4.16-4.29 (m, 2H, $J_{2,3} = 10.6$ Hz, $J_{5,6} = 6.5$ Hz, H-2, 5), 4.62 (d, 1H, $^2J = 11.5$ Hz, $\frac{1}{2}$ CH_2Ph), 4.93 (d, 1H, $^2J = 11.5$ Hz, $\frac{1}{2}$ CH_2Ph), 5.37 (d, 1H, $J_{1,2} = 5.6$ Hz, H-1), 7.28-7.40 (m, 5H, aromatic) ppm.

Ethyl 2-azido-3-O-benzoyl-4-O-benzyl-2,6-dideoxy-1-thio- α -D-galactopyranoside

(5.33). Benzoyl chloride (0.29 mL, 2.49 mmol) and DMAP (41 mg, 0.33 mmol) we

added to a solution of compound **5.30** (536 mg, 1.66 mmol) in acetonitrile (10 mL) and the resulting mixture was stirred under argon for 4 h at rt. After that, the volatiles were removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~120 mL) and washed with water (20 mL), sat. aq. NaHCO₃ (20 mL), and water (20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a colorless syrup (682 mg, 96%). Analytical data for **5.30**: R_f = 0.73 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.22 (d, 3H, H-6), 1.29 (t, 3H, CH₂CH₃), 2.53 (m, 2H, CH₂CH₃), 3.97 (dd, 1H, J_{4,5} = 2.3 Hz, H-4), 4.39 (m, 1H, J_{5,6} = 6.5 Hz, H-5), 4.51-4.60 (m, 2H, J_{2,3} = 11.0 Hz, ½ CH₂Ph, H-2), 4.65 (d, 1H, ²J = 11.3 Hz, ½ CH₂Ph), 5.28 (dd, 1H, J_{3,4} = 2.9 Hz, H-3), 5.50 (d, 1H, J_{1,2} = 5.6 Hz, H-1), 7.25-7.26 (m, 5H, aromatic), 7.46-7.51 (m, 2H, aromatic), 7.59-7.64 (m, 1H, aromatic), 8.07-8.10 (m, 2H, aromatic) ppm.

5.4.5 Synthesis of D-glucosyl donor 5.12

Ethyl 3-O-benzoyl-4,6-O-benzylidene-2-O-levulinyl-1-thio-β-D-glucopyranoside (5.11). Levulinic acid (972 mg, 8.37 mmol), EDC (2.47 g, 12.86 mmol) and DMAP (159 mg, 1.29 mmol) were added to a stirring solution of ethyl 4,6-O-benzylidene-3-O-benzoyl-1-thio-β-D-glucopyranoside¹⁷ (**5.10**, 2.68 g, 6.43 mmol) in CH₂Cl₂ (80 mL) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was diluted with CH₂Cl₂ (~300 mL) and washed with water (50 mL) sat. aq. NaHCO₃ (50 mL) and water (50 mL). The organic phase was separated, dried over MgSO₄, 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 (3.05 g, 92%). Analytical data for **5.11**: $R_f = 0.47$ (ethyl acetate/hexane, 2/3, v/v); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.26 (t, 3H, CH_2CH_3), 2.02 (s, 3H, COCH_3), 2.46-2.54 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CO}$), 2.72 (q, 2H, CH_2CH_3), 3.65 (m, 1H, $J_{5,6b} = 4.8$ Hz, H-5), 3.78-3.87 (m, 2H, H-4, 6a), 4.38 (m, 1H, $J_{6a,6b} = 10.41$ Hz, H-6b), 4.65 (d, 1H, $J_{1,2} = 10.1$ Hz, H-1), 5.20 (dd, 1H, $J_{2,3} = 9.5$ Hz, H-2), 5.51 (s, 1H, CHPh), 5.58 (dd, 1H, $J_{3,4} = 9.4$ Hz, H-3), 7.28-7.31 (m, 3H, aromatic), 7.37-7.45 (m, 4H, aromatic), 7.52-7.58 (m, 1H, aromatic) 7.99-8.02 (m, 2H, aromatic) ppm.

Benzoxazolyl **3-O-benzoyl-4,6-O-benzylidene-2-O-levulinoyl-1-thio- β -D-glucopyranoside (5.12)**. A solution of **5.11** (5.70 g, 11.08 mmol) and activated molecular sieves (3\AA , 5.54 g) in CH_2Cl_2 (166 mL) was stirred under argon for 1 h at rt. The mixture was cooled to $0\text{ }^\circ\text{C}$, freshly prepared solution of Br_2 in CH_2Cl_2 (106.4 mL, 1/165, v/v) was added, and the resulting mixture was stirred for 30 min at $0\text{ }^\circ\text{C}$. After that, the solid was filtered off, rinsed successively with CH_2Cl_2 and the combined filtrate (~300 mL) was concentrated under reduced pressure at rt and dried *in vacuo* for 2 h. The crude residue was dissolved in dry acetone (100 mL), KSBox^{18} (6.29 g, 33.23 mmol) and 18-crown-6 (878 mg, 3.32 mmol) were added and the reaction mixture was stirred under argon for 3 h at rt. After that, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (~400 mL), washed with 1% aq. NaOH (60 mL) and water (3x 60 mL). The organic phase was separated, over MgSO_4 , and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane graduate elution) to afford the title compound as a white amorphous solid (4.47 g, 67 %).

Analytical data for **5.12**: $R_f = 0.41$ (ethyl acetate/hexane, 2/3, v/v); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.97 (s, 3H, COCH_3), 2.43-2.50 (m, 2H, COCH_2), 2.55-2.63 (m, 2H, COCH_2), 3.79-3.97 (m, 3H, 4, 5, 6a), 4.42 (m, 1H, $J_{5,6a} = 3.1$ Hz, $J_{6a,6b} = 8.3$ Hz, H-6b), 5.42 (dd, 1H, $J_{2,3} = 9.2$ Hz, H-2), 5.54 (s, 1H, CHPh), 5.72 (dd, 1H, $J_{3,4} = 9.0$ Hz, H-3), 5.85 (d, 1H, $J_{1,2} = 10.4$ Hz, H-1), 7.29-7.41 (m, 10H, aromatic), 7.44 (m, 1H, aromatic), 7.47-7.50 (m, 1H, aromatic), 7.98-8.06 (m, 2H, aromatic) ppm.

5.4.6 Synthesis of trisaccharide donor **5.19**

Ethyl *O*-(4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl- β -D-glucopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio- β -L-galactopyranoside (5.14**).** A mixture of donor **5.13** (1.44 g, 2.81 mmol), acceptor **5.4** (700 mg, 2.16 mmol), and freshly activated molecular sieves (3Å, 3.0 g) in CH_2Cl_2 (50 mL) was stirred under argon for 2 h at rt. Freshly conditioned AgOTf (1.11 g, 4.32 mmol) was added and the resulting mixture was stirred under argon for 2 h at rt. After that, the solids were filtered-off and rinsed successively with CH_2Cl_2 . The combined filtrate (~200 mL) was washed with water (40 mL), sat. aq. NaHCO_3 (40 mL) and water (40 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 as a white foam (831 mg, 85%). The yield calculation is based on acceptor **5.4** recovered (254 mg, 0.73 mmol). Analytical data for **5.14**: $R_f = 0.32$ (ethyl acetate/hexane, 2/3, v/v); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.19 (d, 3H, H-6), 1.24 (t, 3H, CH_2CH_3), 2.09 (s, 3H, COCH_3), 2.61-2.77 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CO}$, CH_2CH_3), 3.51-3.56 (m, 3H, $J_{5,6} = 6.3$ Hz, H-3', 5, 5'), 3.58 (s, 3H, OCH_3), 3.61-3.65 (m, 2H, H-3, 4), 3.74-3.89 (m, 3H, H-

2, 4', 6a'), 4.20 (d, 1H, $J_{1,2} = 9.4$ Hz, H-1), 4.40 (m, 1H, H-6b'), 4.50 (dd, 1H, $^2J = 11.6$ Hz, $\frac{1}{2}$ CH₂Ph), 4.84 (d, 1H, $J_{1',2'} = 7.5$ Hz, H-1'), 4.88 (dd, 1H, $^2J = 11.6$ Hz, $\frac{1}{2}$ CH₂Ph), 5.01 (dd, 1H, $J_{2',3'} = 8.7$ Hz, H-2'), 5.58 (s, 1H, CHPh), 7.27-7.51 (m, 10H, aromatic) ppm.

Ethyl O-(4,6-O-benzylidene-3-O-methyl-β-D-glucopyranosyl)-(1→3)-2-azido-4-O-benzyl-2,6-dideoxy-1-thio-β-L-galactopyranoside (5.15). Hydrazine acetate (185 mg, 2.06 mmol) was added to a solution of disaccharide **5.14** (940 mg, 1.37 mmol) in CH₂Cl₂/MeOH (32 mL, 20/1, v/v) and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was diluted with CH₂Cl₂ (~200 mL) and washed with water (40 mL), sat. aq. NaHCO₃ (40 mL) and water (40 mL). The organic phase was separated, dried with MgSO₄, 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 foam (681 mg, 85%). Analytical data for **5.15**: $R_f = 0.51$ (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.18 (d, 3H, H-6), 1.25 (t, 3H, CH₂CH₃), 2.70 (m, 2H, CH₂CH₃), 3.38-3.48 (m, 3H, $J_{5,6} = 6.4$ Hz, $J_{5',6b'} = 4.9$ Hz, H-3', 5, 5'), 3.53 (dd, 1H, $J_{2',3'} = 8.7$ Hz, H-2'), 3.60-3.70 (m, 3H, OCH₃, H-4, 4'), 3.77-3.85 (m, 3H, $J_{6a',6b'} = 10.4$ Hz, H-2, 3, 6a'), 4.18 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1), 4.33 (d, 1H, H-6b'), 4.62 (d, 1H, $J_{1',2'} = 7.6$ Hz, H-1'), 4.72 (dd, 1H, $^2J = 11.7$ Hz, $\frac{1}{2}$ CH₂Ph), 4.94 (dd, 1H, $^2J = 11.8$ Hz, $\frac{1}{2}$ CH₂Ph), 5.55 (s, 1H, CHPh), 7.25-7.48 (m, 10H, aromatic) ppm.

Ethyl O-(2-azido-4,6-O-benzylidene-2-deoxy-3-O-methyl-β-D-mannopyranosyl)-(1→3)-2-azido-4-O-benzyl-2,6-dideoxy-1-thio-β-L-galactopyranoside (5.16). Pyridine

(3.0 mL) was added to a solution of disaccharide **5.15** (590 mg, 1.00 mmol) in CH₂Cl₂ (25.0 mL) and the resulting mixture was cooled to 0 °C. Trifluoromethanesulfonic anhydride (0.42 mL, 2.50 mmol) was added dropwise and the reaction mixture was kept for 4 h at 0 °C. After that, the reaction was quenched with ice-cold water (~30 mL), diluted with CH₂Cl₂ (~150 mL), and washed with cold sat. aq. NaHCO₃ (30 mL) and cold water (30 mL). The organic phase was separated, dried over MgSO₄, and concentrated *in vacuo*. The crude residue was dissolved in DMF (5.0 mL), NaN₃ (390 mg, 6.00 mmol) was added, and the resulting suspension was stirred for 16 h at 60 °C. Upon completion, ethyl acetate (~150 mL) and water (~150 mL) were added. Organic layer was separated and washed with sat. aq. NaCl (2 x 30 mL). The combined aqueous phase was extracted with ethyl acetate (3 x 50 mL). The combined organic extract was dried with MgSO₄ 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 foam (363 mg, 59%). Analytical data for **5.16**: R_f = 0.49 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.28 (t, 3H, CH₂CH₃), 1.35 (d, 3H, H-6), 2.72 (m, 2H, CH₂CH₃), 3.20 (m, 1H, J_{5',6a'} = 4.7 Hz, J_{5',6b'} = 4.9 Hz, H-5'), 3.30 (dd, 1H, J_{3',4'} = 9.6 Hz, H-3'), 3.51-3.62 (m, 5H, J_{2',3'} = 3.7 Hz, J_{5,6} = 6.4 Hz, OCH₃, H-2', 5), 3.65 (dd, 1H, J_{4,5} = 1.9 Hz, H-4), 3.73-3.96 (m, 4H, J_{6a',6b'} = 10.5 Hz, H-2, 3, 4', 6a'), 4.22 (d, 1H, J_{1,2} = 9.4 Hz, H-1), 4.28 (d, 1H, H-6b'), 4.50 (d, 1H, J_{1',2'} = 1.0 Hz, H-1'), 4.65 (dd, 1H, ²J = 12.1 Hz, ½ CH₂Ph), 4.84 (dd, 1H, ²J = 12.1 Hz, ½ CH₂Ph), 5.55 (s, 1H, CHPh), 7.35-7.48 (m, 10H, aromatic) ppm.

4-Methoxyphenyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.17**). A mixture of disaccharide donor **5.16** (280 mg, 0.46 mmol), acceptor **5.9** (229 mg, 0.59 mmol), and freshly activated molecular sieves (4Å, 500 mg) in CH₂Cl₂ (20 mL) was stirred under argon for 2 h at rt. The resulting mixture was cooled to 0 °C, *N*-iodosuccinimide (206 mg, 0.914 mmol) and trifluoromethanesulfonic acid (14 mg, 0.09 mmol) were added, and the resulting mixture was stirred under argon for 2 h at 0 °C. After that, the solids were filtered-off and rinsed successively with CH₂Cl₂. The combined filtrate (~120 mL) was washed with water (30 mL), 10% aq. Na₂S₂O₃ (30 mL) and water (30 mL). The organic phase was separated, dried with MgSO₄, 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 foam (230 mg, 75%). The yield calculation is based on acceptor **5.9** recovered (103 mg, 0.27 mmol). Analytical data for **5.17**: R_f = 0.27 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ , 1.24 (d, 3H, H-6), 1.36 (d, 3H, H-6'), 3.26 (m, 1H, $J_{5'',6a''} = 4.8$ Hz, $J_{5'',6b''} = 4.9$ Hz, H-5''), 3.40 (dd, 1H, $J_{3'',4''} = 9.6$ Hz, H-3''), 3.50 (dd, 1H, $J_{4',5'} = 3.2$ Hz, H-4'), 3.58 (m, 4H, OCH₃, H-4), 3.60-3.68 (m, 3H, $J_{5',6'} = 6.3$ Hz, H-2', 3, 5'), 3.75-3.78 (m, 5H, $J_{2'',3''} = 3.6$ Hz, OCH₃, H-2''), 3.81-3.89 (m, 2H, $J_{5,6} = 6.5$ Hz, H-5, 6a''), 3.95 (dd, 1H, $J_{4'',5''} = 9.5$ Hz, H-4''), 4.14-4.21 (m, 3H, H-2, 3', 6b''), 6.62 (d, 1H, H-1''), 4.70-4.79 (m, 4H, 3 x ½ CH₂Ph, H-1), 4.84 (d, 1H, ²J = 11.5 Hz, ½ CH₂Ph), 5.35 (d, 1H, $J_{1',2'} = 3.66$ Hz, H-1'), 5.58 (s, 1H, CHPh), 6.81 (d, 2H, $J = 9.1$ Hz, PMP), 7.04 (d, 2H, $J = 9.1$ Hz, PMP), 7.35-7.51 (m, 15H, aromatic) ppm.

***O*-(2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy-D-galactopyranose (5.18).** A solution of CAN (337 mg, 0.62 mmol) in water (10 mL) was added dropwise to a stirring solution of trisaccharide **5.17** (230 mg, 0.25 mmol) in CH₃CN (5 mL) at 0°C. The reaction was allowed to reach rt. After 2 h, the solvent was removed *in vacuo*. The residue was diluted with CH₂Cl₂ (~100 mL) and washed with water (20 mL). The organic phase was separated, dried with MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white amorphous solid (59 mg, 29%). Analytical data for **5.18**: R_f = 0.29 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ , 1.22-1.29 (m, 12H, H-6 α , 6 β , 6' α , 6' β), 3.24-3.31 (m, 2H, H-5'' α , 5'' β), 3.37-3.68 (m, 14H, 2 x OCH₃, H-2 α , 3'' α , 3'' β , 4 α , 4 β , 4' α , 4' β , 5' β), 3.72-3.75 (m, 2H, H-2'' α , 2'' β), 3.78-3.86 (m, 4H, H-2 β , 5' α , 6a'' α , 6a'' β), 3.91-3.98 (m, 4H, H-2' β , 4'' α , 4'' β , 5 α), 4.07-4.25 (m, 6H, H-3 α , 3 β , 3' α , 3' β , 5 β , 6b'' α , 6b'' β), 4.48 (d, 1H, $J_{1\beta,2\beta}$ = 7.8 Hz, H-1 β), 4.60-4.61 (m, 2H, H-1'' α , 1'' β), 4.65-4.86 (m, 8H, 4 x CH₂Ph), 5.25 (d, 1H, $J_{1'\alpha,2'\alpha}$ = 3.6 Hz, H-1' α), 5.29 (d, 1H, $J_{1\alpha,2\alpha}$ = 3.7 Hz, H-1 α), 5.34 (d, 1H, $J_{1'\beta,2'\beta}$ = 3.1 Hz, H-1' β), 5.55 (s, 2H, 2 x CHPh), 7.30-7.50 (m, 30H, aromatic) ppm.

***O*-(2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl trichloroacetimidate (5.19).** To the solution of **5.16** (39 mg, 0.05 mmol) in dry CH₂Cl₂ (5 mL), trichloroacetonitrile (0.05 mL, 0.47 mmol) and DBU (5.0 μ L, 0.03 mmol) were added at rt. The reaction was stirred for 2 h under

argon. After that, the reaction mixture was concentrated *in vacuo*, passed through a pad of silica gel, concentrated *in vacuo*, and dried. The crude residue (19 mg, 41%) was used for the next step.

5.4.7 Synthesis of trisaccharide acceptor 5.25

Ethyl *O*-(3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-levulinoyl- β -D-glucopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio- β -L-galactopyranoside (5.20). A mixture of donor **5.12** (1.51 g, 2.50 mmol), acceptor **5.4** (622 mg, 1.92 mmol), and freshly activated molecular sieves (3Å, 3.0 g) in CH₂Cl₂ (70 mL) was stirred under argon for 2 h at rt. Freshly conditioned AgOTf (1.28 g, 5.00 mmol) was added and the resulting mixture was stirred under argon for 5 h at rt. After that, the solids were filtered-off and rinsed successively with CH₂Cl₂. The combined filtrate (~200 mL) was washed with water (40 mL), sat. aq. NaHCO₃ (40 mL) and water (40 mL). The organic phase was separated, dried with MgSO₄, 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 foam (647 g, 74%). The yield calculation is based on acceptor **5.4** recovered (254 mg, 0.79 mmol). Analytical data for **5.20**: R_f = 0.49 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ , 1.20 (d, 3H, H-6), 1.29 (t, 3H, CH₂CH₃), 1.95 (s, 3H, COCH₃), 2.33 (m, 2H, COCH₂), 2.42 (m, 2H, COCH₂), 2.73 (m, 2H, CH₂CH₃), 3.51 (m, 1H, $J_{5,6}$ = 6.4 Hz, H-5), 3.68-3.76 (m, 2H, H-4, 6a'), 3.79-3.87 (m, 2H, H-2, 3), 3.90-4.00 (m, 2H, H-4, 6b'), 4.21 (d, 1H, $J_{1,2}$ = 9.7 Hz, H-1), 4.46 (dd, 1H, $J_{5',6a'}$ = 5.0 Hz, H-5'), 4.51 (dd, 1H, 2J = 11.4 Hz, $\frac{1}{2}$ CH₂Ph), 4.87 (dd, 1H, 2J = 11.6 Hz, $\frac{1}{2}$ CH₂Ph), 5.00 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1'), 5.24 (dd, 1H, $J_{2',3'}$ = 9.0 Hz, H-2'), 5.54 (s, 1H, $\frac{1}{2}$

CH_2Ph), 5.60 (dd, 1H, $J_{3',4'} = 9.3$ Hz, H-3'), 7.28-7.43 (m, 12H, aromatic), 7.52-7.56 (m, 1H, aromatic), 8.00-8.03 (m, 2H, aromatic) ppm.

Ethyl *O*-(3-*O*-benzoyl-4,6-*O*-benzylidene- β -D-glucopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio- β -L-galactopyranoside (5.21). Hydrazine acetate (150 mg, 1.67 mmol) was added to a solution of disaccharide **5.20** (647 mg, 0.83 mmol) in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (21 mL, 20/1, v/v) and the resulting mixture was stirred for 3 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~200 mL) and washed with water (40 mL), sat. aq. NaHCO_3 (40 mL) and water (40 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 as a white foam (390 mg, 69%). Analytical data for **5.21**: $R_f = 0.60$ (ethyl acetate/toluene, 1/4, v/v); ^1H NMR (300 MHz, CDCl_3): δ , 1.21 (d, 3H, H-6), 1.28 (t, 3H, CH_2CH_3), 2.72 (m, 2H, CH_2CH_3), 2.96 (d, 1H, $J_{\text{OH},2'} = 3.6$ Hz, 2'-OH), 3.49 (m, 1H, $J_{5,6} = 6.4$ Hz, H-5), 3.57 (m, 1H, $J_{5',6a'} = 5.0$ Hz, $J_{5',6b'} = 5.0$ Hz, H-5'), 3.69 (dd, 1H, $J_{4,5} = 2.3$ Hz, H-4), 3.76-3.92 (m, 4H, $J_{2',3'} = 9.4$ Hz, $J_{6a',6b'} = 10.6$ Hz, H-2', 3, 4', 6a'), 4.21 (d, 1H, $J_{1,2} = 9.7$ Hz, H-1), 4.40 (dd, 1H, H-6b'), 4.71 (dd, 1H, $^2J = 11.7$ Hz, CHHPh), 4.76 (d, 1H, $J_{1',2'} = 7.7$ Hz, H-1'), 4.93 (dd, 1H, $^2J = 11.7$ Hz, CHHPh), 5.42 (dd, 1H, $J_{3',4'} = 9.3$ Hz, H-3'), 5.56 (s, 1H, CHPh), 7.26-7.47 (m, 12H, aromatic), 7.55-7.58 (m, 1H, aromatic), 8.06-8.08 (m, 2H, aromatic) ppm.

Ethyl *O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio- β -L-galactopyranoside (5.22). Pyridine

(2.0 mL) was added to a solution of disaccharide **5.20** (390 mg, 0.58 mmol) in CH₂Cl₂ (15.0 mL) and the resulting mixture was cooled to 0 °C. Trifluoromethanesulfonic anhydride (0.24 mL, 1.44 mmol) was added dropwise and the reaction mixture was kept for 4 h at 0 °C. After that, the reaction was quenched with ice-cold water (~20 mL), diluted with CH₂Cl₂ (~150 mL), and washed with cold sat. aq. NaHCO₃ (30 mL) and cold water (30 mL). The organic phase was separated, dried over MgSO₄, and concentrated *in vacuo*. The crude residue was dissolved in DMF (5.0 mL), NaN₃ (224 mg, 3.45 mmol) was added, and the resulting suspension was stirred for 16 h at 60 °C. Upon completion, ethyl acetate (~150 mL) and water (~150 mL) were added. Organic layer was separated and washed with sat. aq. NaCl (2 x 30 mL). The combined aqueous phase was extracted with ethyl acetate (3 x 50 mL). The combined organic extract was dried with MgSO₄ 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 foam (270 mg, 67%). Analytical data for **5.22**: $R_f = 0.55$ (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.29 (m, 6H, CH₂CH₃, H-6), 2.74 (m, 2H, CH₂CH₃), 3.38 (m, 1H, $J_{5',6a'} = 4.9$ Hz, $J_{5',6b'} = 4.89$ Hz, H-5'), 3.61 (m, 1H, H-5), 3.67 (dd, 1H, $J_{4,5} = 2.1$ Hz, H-4), 3.75-3.86 (m, 3H, $J_{2',3'} = 3.8$ Hz, H-2, 2', 3), 3.90 (dd, 1H, $J_{6a',6b'} = 10.4$ Hz, H-6a'), 4.11 (dd, 1H, $J_{4',5'} = 9.7$ Hz, H-4'), 4.23 (d, 1H, $J_{1,2} = 9.2$ Hz, H-1), 4.34 (dd, 1H, H-6b'), 4.70-4.81 (m, 3H, $J_{1',2'} = 1.1$ Hz, CH₂Ph, H-1'), 5.21 (dd, 1H, $J_{3',4'} = 10.1$ Hz, H-3'), 5.57 (s, 1H, CHPh), 7.28-7.50 (m, 12H, aromatic), 7.58-7.61 (m, 1H, aromatic), 8.09-8.12 (m, 2H, aromatic) ppm.

Methyl *O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (5.24). A mixture of donor **5.22** (183 mg, 0.26 mmol), acceptor **5.23** (100 mg, 0.34 mmol), and freshly activated molecular sieves (4Å, 400 mg) in CH₂Cl₂ (20 mL) was stirred under argon for 2 h at rt. The resulting mixture was cooled to 0 °C, *N*-iodosuccinimide (117 mg, 0.52 mmol) and trifluoromethanesulfonic acid (8 mg, 0.05 mmol) were added, and the resulting mixture was stirred under argon for 2 h at 0 °C. After that, the solids were filtered-off and rinsed successively with CH₂Cl₂. The combined filtrate (~120 mL) was washed with water (30 mL), 10% aq. Na₂S₂O₃ (30 mL) and water (30 mL). The organic phase was separated, dried with MgSO₄, 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 foam (150 mg, 62%). The yield calculation is based on acceptor **5.23** recovered (103 mg, 0.27 mmol). Analytical data for **5.24**: R_f = 0.65 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ , 1.23 (2d, 6H, H-6, 6'), 3.42-3.51 (m, 5H, OCH₃, H-5', 5''), 3.60 (dd, 1H, $J_{4,5}$ = 1.7 Hz, H-4), 3.67 (dd, 1H, $J_{4',5'}$ = 1.9 Hz, H-4'), 3.76 (dd, 1H, $J_{2',3'}$ = 10.7 Hz, H-2'), 3.86-4.02 (m, 4H, H-2, 2'', 5, 6a''), 4.07-4.27 (m, 4H, H-3, 3', 4'', 6b''), 4.67-4.89 (m, 6H, 2 x CH₂Ph, H-1, 1''), 5.26-5.31 (m, 2H, $J_{1',2'}$ = 3.6 Hz, H-1', 3''), 5.59 (s, 1H, CHPh), 7.30-7.41 (m, 17H, aromatic), 7.59-7.61 (m, 1H, aromatic), 8.11-8.14 (m, 2H, aromatic) ppm.

Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-

dideoxy- α -D-galactopyranoside (5.25). 1M NaOMe in MeOH (1.0 mL) was added to a stirring solution of thiol compound **5.24** (214 mg, 0.23 mmol) in methanol (10.0 mL) and the resulting mixture was stirred under argon for 1 h at rt. After that, Amberlite IR120 resin was added to the solution until pH = 7. The resin was filtered-off, rinsed successively with MeOH (7 x 5 mL), and the combined filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white foam (167 mg, 88%). Analytical data for **5.25**: R_f = 0.47 (ethyl acetate/hexane, 2/3, v/v); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ , 1.23 (2d, 6H, H-6, 6'), 3.22 (m, 1H, $J_{5'',6b''} = 4.9$ Hz, H-5''), 3.41 (s, 3H, OCH₃), 3.58 (dd, 1H, $J_{4,5} = 1.9$ Hz, H-4), 3.65 (dd, 1H, $J_{4',5'} = 2.0$ Hz, H-4'), 3.75-3.83 (m, 5H, $J_{2',3'} = 10.7$ Hz, H-2', 2'', 3'', 4'', 6a''), 3.91-4.00 (m, 3H, H-2, 5, 5'), 4.06 (dd, 1H, $J_{3,4} = 2.7$ Hz, H-3), 4.12 (dd, 1H, $J_{6a'',6b''} = 10.7$ Hz, H-6b''), 4.22 (dd, 1H, $J_{3',4'} = 2.8$ Hz, H-3'), 4.66-4.88 (m, 6H, $J_{1,2} = 3.5$ Hz, 2 x CH₂Ph, H-1, 1''), 5.26 (d, 1H, $J_{1',2'} = 3.7$ Hz, H-1'), 5.53 (s, 1H, CHPh), 7.30-7.50 (m, 15H, aromatic) ppm.

5.4.8 Synthesis of tetrasaccharide 5.32

Methyl *O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (5.32). A mixture of donor **5.31** (35 mg, 0.10 mmol), trisaccharide acceptor **5.25** (66 mg, 0.08 mmol), and freshly activated molecular sieves (4Å, 100 mg) in CH₂Cl₂ (10 mL) was stirred under argon for 2 h at rt. The resulting mixture was cooled to 0 °C, *N*-iodosuccinimide (47 mg, 0.21 mmol) and

trifluoromethanesulfonic acid (3 mg, 0.02 mmol) were added, and the resulting mixture was stirred under argon for 3 h at 0 °C. After that, the solids were filtered-off and rinsed successively with CH₂Cl₂. The combined filtrate (~100 mL) was washed with water (20 mL), 10% aq. Na₂S₂O₃ (20 mL) and water (20 mL). The organic phase was separated, dried with MgSO₄, 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 (79 mg, 89%). Analytical data for **5.32**: R_f = 0.51 (ethyl acetate/hexane, 2/3, v/v); ¹H NMR (300 MHz, CDCl₃): δ, 1.18 (d, 3H, H-6'''), 1.24 (2d, 6H, H-6, 6'), 3.16 (m, 1H, J_{5'',6a''} = 4.9 Hz, J_{5'',6b''} = 4.83 Hz, H-5''), 3.42 (s, 3H, OCH₃), 3.58 (s, 3H, OCH₃), 3.58 (dd, 1H, J_{4,5} = 2.13 Hz, H-4), 3.64-3.70 (m, 2H, H-2''', 4'), 3.74-3.94 (m, 8H, J_{2',3'} = 10.7 Hz, J_{6a'',6b''} = 10.6 Hz, H-2, 2'', 3'', 3''', 4'', 5, 5', 6a''), 3.96-4.10 (m, 3H, J_{5''',6'''} = 6.5 Hz, H-3, 4', 5'''), 4.13 (dd, 1H, H-6b''), 4.23 (dd, 1H, J_{3',4'} = 2.8 Hz, H-3'), 4.58 (dd, 1H, H-1''), 4.63-4.79 (m, 4H, 4 x ½ CH₂Ph), 4.83-4.87 (m, 2H, ½ CH₂Ph, H-1), 4.92 (d, 1H, ²J = 11.5 Hz, ½ CH₂Ph), 5.26 (2d, 2H, J_{1'',2'''} = 3.4 Hz, H-1', 1''') 5.60 (s, 1H, CHPh), 7.30-7.53 (m, 20H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.9, 17.0, 17.1, 55.5, 57.7, 58.4, 59.0, 60.8, 64.8, 66.8, 67.5, 67.7, 67.8, 68.3, 74.9, 75.0, 75.1, 75.5, 75.7, 76.5, 76.7, 77.0, 77.4, 78.4, 79.8, 97.5, 99.1, 100.0, 100.9, 101.5, 126.0 (x 2), 127.9 (x 2), 128.1 (x 2), 128.4 (x 4), 128.5 (x 2), 128.6 (x 5), 128.7 (x 2), 129.1, 137.1, 137.8, 138.1, 138.3 ppm.

5.4.9 Synthesis of hexasaccharide 5.40

Methyl O-(2-azido-3-O-benzoyl-4-O-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-O-(2-azido-3-O-benzoyl-4,6-O-benzylidene-2-deoxy-β-D-mannopyranosyl)-

(1→3)-O-(2-azido-4-O-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1→3)-2-azido-4-O-benzyl-2,6-dideoxy- α -D-galactopyranoside (5.31). A mixture of donor **5.33** (59 mg, 0.14 mmol), trisaccharide acceptor **5.25** (76 mg, 0.09 mmol), and freshly activated molecular sieves (4Å, 200 mg) in CH₂Cl₂ (5 mL) was stirred under argon for 1 h at rt. The resulting mixture was cooled to 0 °C, *N*-iodosuccinimide (62 mg, 0.27 mmol) and trifluoromethanesulfonic acid (4.0 μ L, 0.04 mmol) were added, and the resulting mixture was stirred under argon for 2 h at 0 °C. After that, the solids were filtered-off and rinsed successively with CH₂Cl₂. The combined filtrate (~100 mL) was washed with water (20 mL), 10% aq. Na₂S₂O₃ (20 mL) and water (20 mL). The organic phase was separated, dried with MgSO₄, 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 foam (89 mg, 81%). Analytical data for **5.34**: R_f = 0.44 (ethyl acetate/hexane, 2/3, v/v); [α]_D²³ -4.4 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ , 1.17 (d, 3H, H-6''), 1.23 (2d, 6H, H-6, 6'), 3.25 (m, 1H, $J_{5'',6a''}$ = 5.2 Hz, $J_{5'',6b''}$ = 4.7 Hz, H-5''), 3.41 (s, 3H, OCH₃), 3.58 (dd, 1H, H-4), 3.65 (dd, 1H, H-4'), 3.74-3.99 (m, 8H, $J_{2''',3''}$ = 11.3 Hz, H-2, 2', 2'', 2''', 4'', 5, 5', 6a''), 4.05-4.08 (m, 2H, H-3, 4''), 4.11 (dd, 1H, $J_{6a'',6b''}$ = 10.7 Hz, H-6b''), 4.21-4.25 (m, 2H, H-3', 5'''), 4.51 (d, 1H, ²*J* = 11.3 Hz, ½ CH₂Ph), 4.57-4.62 (m, 2H, ½ CH₂Ph, H-1''), 4.65-4.86 (m, 5H, 4 x CHHPh, H-1), 5.25 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1'), 5.37 (d, 1H, $J_{1''',2''}$ = 3.7 Hz, H-1'''), 5.60 (s, 1H, CHPh), 5.61 (dd, 1H, $J_{3''',4''}$ = 2.8 Hz, H-3'''), 7.32-7.39 (m, 20H, aromatic), 7.43-7.48 (m, 2H, aromatic), 7.57-7.60 (m, 1H, aromatic), 8.08 -8.10 (m, 2H, aromatic) ppm; ¹³C NMR: δ , 16.9 (x 2), 17.0, 29.9, 55.6, 57.7, 58.5, 60.9, 64.7, 66.8, 67.4, 67.6, 67.9, 68.4, 71.4, 75.2, 75.5, 75.8 (x 2), 75.9, 76.7, 77.0, 77.4, 79.8, 98.0, 99.1, 100.0, 100.8, 101.6, 126.1 (x 2),

127.9 (x 2), 128.1, 128.2, 128.4 (x 6), 128.5 (x 3), 128.6 (x 6), 128.8 (x 4), 130.1, 137.2, 137.6, 137.9, 138.4, 165.9 ppm; HR-ESI MS $[M+K]^+$ calcd for $C_{60}H_{66}N_{12}O_{15}K$ 1233.4402, found 1233.4364.

Methyl *O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.35**). A 1M solution of NaOMe in MeOH (0.5 mL) was added to a stirring solution of thiol compound **5.34** (104 mg, 0.09 mmol) in methanol (5.0 mL) and the resulting mixture was stirred under argon for 2 h at rt. After that, Amberlite IR120 resin was added to the solution until pH = 7. The resin was filtered-off, rinsed successively with MeOH (7 x 5 mL), and the combined filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to yield the title compound as a white foam (72 mg, 76%). Analytical data for **5.35**: R_f = 0.39 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{22}$ -4.4 (c = 1, $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$): δ , 1.19-1.27 (3d, 9H, H-6, 6', 6''), 2.12 (d, $J_{OH,3'''} = 8.2$ Hz, 1H, 3'''-OH), 3.22 (m, 1H, $J_{5'',6a''} = 4.7$ Hz, $J_{5'',6b''} = 4.8$ Hz, H-5''), 3.38 (dd, 1H, H-2'''), 3.41 (s, 3H, OCH_3), 3.59 (dd, 1H, H-4), 3.65 (dd, 1H, H-4'), 3.71 (dd, 1H, H-4''), 3.76-3.84 (m, 3H, $J_{2'',3''} = 9.6$ Hz, H-2', 2'', 6a''), 3.85 (dd, 1H, $J_{3'',4''} = 3.8$ Hz, H-3''), 3.91-4.11 (m, 5H, H-2, 3, 4'', 5, 5'), 4.12 (m, 2H, H-3''', 6b''), 4.23 (m, 2H, H-3', 5'''), 4.61-4.77 (m, 6H, 5 x $\frac{1}{2}$ CH_2Ph , H-1''), 4.84-4.87 (m, 2H, $\frac{1}{2}$ CH_2Ph , H-1), 5.26-5.30 (2d, 2H, H-1', 1'''), 5.58 (s, 1H, $CHPh$), 7.26-7.50 (m, 20H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ , 16.9, 17.0, 17.1, 29.9, 55.6, 58.5, 60.6, 60.9, 64.8, 66.8, 67.6 (x 2), 67.9,

68.3, 68.4, 75.0, 75.5, 75.8, 76.4, 76.6, 77.0, 77.4, 79.8, 79.9, 97.7, 99.1, 100.0, 100.7, 101.6, 126.1 (x 2), 127.9 (x 2), 128.1, 128.4 (x 2), 128.5 (x 6), 128.6 (x 2), 128.8 (x 2), 128.9 (x 2), 129.2, 137.2, 137.9 (x 2), 138.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{53}H_{62}O_{14}NaN_{12}$ 1113.4406, found 1113.4390.

Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (5.36). A mixture of disaccharide donor **5.14** (180 mg, 0.29 mmol), acceptor **5.35** (72 mg, 0.07 mmol), and freshly activated molecular sieves (4Å, 200 mg) in $ClCH_2CH_2Cl$ (10 mL) was stirred under argon for 2 h at rt. The resulting mixture was cooled to 0 °C, *N*-iodosuccinimide (132 mg, 0.59 mmol) and trifluoromethanesulfonic acid (5.0 μ L, 0.06 mmol) were added, and the resulting mixture was stirred under argon for 4 h at 0 °C. After that, the solids were filtered-off and rinsed successively with CH_2Cl_2 . The combined filtrate (~150 mL) was washed with water (20 mL), 10% aq. $Na_2S_2O_3$ (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 - hexane gradient elution) to afford the title compound as a white amorphous solid (92 mg, 85%). Analytical data for **5.36**: R_f = 0.29 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{23}$ -65.9 (c = 1, $CHCl_3$); 1H NMR (300 MHz, $CDCl_3$): δ , 1.22-1.29 (m, 12H, H-6, 6', 6'', 6'''), 3.23-3.30 (m, 2H, H-5'', 5'''''), 3.37 (dd, 1H, H-3'''''), 3.42 (s, 3H, OCH_3), 3.55-3.60 (m, 5H, H-4, 4'''), 3.62-3.67 (m, 2H, H-4', 4'''),

3.70-4.01 (m, 11H, $J_{2''''',3'''''} = 3.69$ Hz, H-2, 2', 2'', 2''', 2''''', 2''''', 3'', 4''''', 5', 5''''', 6a''''', 6b'''''), 4.07-4.29 (m, 10H, H-3, 3', 3'', 3''''', 4'', 4''''', 5, 5''''', 6a'', 6b''), 4.61-4.88 (m, 11H, 4 x CH_2Ph , H-1, 1'', 1'''''), 5.27 (d, 1H, $J_{1',2'} = 3.5$ Hz, H-1'), 5.31 (d, 1H, $J_{1''',2'''} = 3.6$ Hz, H-1'''), 5.34 (d, 1H, $J_{1''''',2'''''} = 3.5$ Hz, H-1'''''), 5.55 (s, 1H, $CHPh$), 5.60 (s, 1H, $CHPh$), 7.34-7.51 (m, 30H, aromatic) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ , 16.9, 17.0 (x 2), 17.1, 55.5, 58.4 (x 2), 59.3, 59.8, 60.8, 62.5, 64.7, 66.8, 67.5, 67.6, 67.7 (x 2), 67.8, 68.3, 68.4, 74.8, 75.0, 75.5, 75.6, 75.7, 75.9, 76.5, 77.4, 78.3, 78.8, 79.7, 97.6, 98.0, 99.1, 99.8, 100.0, 100.4, 101.7, 126.0 (x 2), 126.2 (x 2), 127.9 (x 2), 128.0 (x 2), 128.1(x 2), 128.4 (x 10), 128.5 (x 6), 128.6 (x 4), 128.7 (x 4), 129.2 (x 2), 127.1, 137.3, 137.8, 138.0, 138.2, 138.3 ppm; HR-ESI MS $[M+K]^+$ calcd for $C_{80}H_{92}N_{18}O_{21}K$ 1679.6316, found 1679.6307.

Methyl ***O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (5.37).** Water (2 mL), triethylamine (1.0 mL, 7.1 mmol) and 1,3-propanedithiol (0.63 mL, 6.29 mmol) were added to a solution of hexasaccharide **5.33** (86 mg, 0.05 mmol) in pyridine (10.0 mL) and the resulting mixture was kept for 24 h at 60 °C. After that, the reaction mixture was concentrated and dried *in vacuo*. The crude residue was dissolved in pyridine (5.0 mL), Ac_2O (0.12 mL, 1.26 mmol) was added, and the resulting suspension was stirred under argon for 16 h at rt. Then, the reaction mixture

was concentrated *in vacuo* and dried. The residue crude was dissolved in THF (5 mL), zinc dust (100 mg), acetic acid (2 mL) and acetic anhydride (2 mL) were added. The reaction was stirred for 6 hr. After that, the solid was filtered-off and rinsed successively with CH₂Cl₂. The solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (58 mg, 64%). Analytical data for **5.37**: R_f = 0.29 (methanol/dichloromethane, 1/9, v/v); [α]_D²² -22.2 (c = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ, 1.15-1.32 (m, 12H, H-6, 6', 6'', 6'''), 1.94, 1.97, 1.98 (3s, 9H, 3 x COCH₃), 2.04 (s, 6H, 2 x COCH₃), 2.15 (s, 3H, COCH₃), 3.16 (m, 1H, J_{5''''',6a'''''} = 5.0 Hz, J_{5''''',6b'''''} = 4.7 Hz, H-5'''''), 3.35-3.40 (m, 5H, J_{5'',6b''} = 4.6 Hz, OCH₃, H-3''''', 5''), 3.47 (s, 3H, OCH₃), 3.52-3.73 (m, 9H, H-3'', 4, 4', 4'', 4''', 4''''', 6a'', 6a'''), 3.75-3.85 (m, 4H, H-3'', 3''''', 5', 5'''''), 3.89-4.00 (m, 3H, H-3, 3', 5) 4.04 (dd, 1H, J_{6a''''',6b'''''} = 10.32 Hz, H-6b'''''), 4.16 (dd, 1H, J_{6a'',6b''} = 10.0 Hz, H-6b''), 4.42-4.89 (m, 21H, 4 x CH₂Ph, H-1, 1'', 1''', 1''''', 1''''''', 2, 2', 2'', 2''', 2''''', 2''''''', 5'''), 5.49 (s, 1H, CHPh), 5.54 (s, 1H, CHPh), 5.80 (d, 1H, J = 9.6 Hz, NH), 5.98-5.06 (m, 3H, 3 x NH), 6.20 (d, 1H, J = 9.6 Hz, NH), 6.27 (d, 1H, J = 8.8 Hz, NH), 7.26-7.49 (m, 30H, aromatic) ppm; ¹³C NMR (75 MHz, CDCl₃): δ, 16.9 (x 2), 17.0, 17.1, 22.4, 23.3 (x 2), 23.8, 24.0, 24.1, 47.7, 47.8, 49.0, 49.4, 50.5, 55.3, 58.4, 66.7, 67.0, 67.1, 67.7, 67.8, 68.4, 68.7, 74.8, 75.2, 75.4, 75.5, 76.3, 76.4, 77.0, 77.4, 78.6 (x 2), 79.5, 80.4 (x 2), 98.1, 99.1, 99.8, 100.4, 101.6, 101.9, 102.6, 126.3 (x 6), 127.2 (x 2), 127.3 (x 2), 127.7, 127.8, 128.0 (x 2), 128.1 (x 2), 128.2 (x 2), 128.4 (x 6), 128.5 (x 2), 128.7 (x 6), 128.8 (x 2), 129.2, 130.0, 136.7, 137.3, 138.4, 138.7 (x 2), 138.8, 171.0, 171.4 (x 2), 171.5, 172.0, 172.1 ppm; HR-ESI MS [M+Na]⁺ calcd for C₉₂H₁₁₆N₆O₂₇Na 1759.7786, found 1759.7833.

Methyl *O*-(2-acetamido-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-3-*O*-benzoyl-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (5.38). Hexasaccharide **5.37** (50 mg, 0.03 mmol) was dissolved in acetic acid (4.0 mL) and water (1.0 mL) and the resulting mixture was stirred for 16 h at 90 °C. After that, the reaction mixture was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous solid (34 mg, 76%). Analytical data for **5.38**: $R_f = 0.57$ (methanol/CH₂Cl₂, 3/7, v/v); $[\alpha]_D^{22} -33.4$ (c = 1, CH₃OH); ¹H NMR (300 MHz, CD₃OD): δ , 1.13-1.24 (m, 12H, H-6, 6', 6'', 6'''), 1.92 (s, 6H, 2 x COCH₃), 1.94 (s, 6H, 2 x COCH₃), 2.04 (s, 6H, 2 x COCH₃), 3.13-3.18 (m, 2H, H-5'', 5'''), 3.22 (dd, 1H, $J_{2'',3''} = 4.2$ Hz, $J_{3'',4''} = 9.6$ Hz, H-3''), 3.32-3.34 (m, 4H, OCH₃), 3.42 (s, 3H, OCH₃), 3.50 (dd, 1H, H-4'''), 3.61-3.82 (m, 9H), 3.91-4.03 (m, 7H), 4.34-4.44 (m, 3H), 4.50-4.55 (m, 3H), 4.58-4.68 (m, 3H), 4.71-4.81 (m, 2H), 4.81 (d, 1H), 4.87 (d, 1H), 4.89-4.90 (m, 4H), 5.00-5.01 (m, 2H), 5.03-5.07 (m, 2H) 7.25-7.47 (m, 20H, aromatic) ppm; ¹³C NMR (75 MHz, CD₃OD): δ , 17.2, 17.3, 17.4, 17.5, 22.8, 22.9, 23.0, 23.3, 23.5 (x 2), 49.9, 50.7, 51.1, 54.6, 55.7, 58.0, 61.3, 61.4, 66.7, 67.5, 68.2, 68.8, 68.9, 69.0, 74.7, 75.3, 76.3 (x 2), 76.4, 76.6, 77.6, 78.0, 78.2, 78.4, 78.6, 80.9, 81.6, 83.7, 99.2, 99.7, 100.0, 100.4, 100.6, 102.7, 128.7, 128.8, 128.9 (x 2), 129.0 (x 2), 129.1 (x 2), 129.3 (x 4), 129.5 (x 4), 129.6 (x 6), 129.7 (x 2), 140.2, 140.4 (x 3), 173.7 (x 2), 173.8,

173.9 (x3) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{78}H_{108}NaO_{27}N_6$ 1583.7160, found 1583.7136.

Methyl *O*-(methyl-2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl- β -D-mannopyranosyluronate)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(methyl-2-acetamido-4-*O*-acetyl-3-*O*-benzoyl-2-deoxy- β -D-mannopyranosyluronate)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (5.40). Borax buffer (1 mL) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, 3.0 mg, 0.02 mmol) were added to a solution of hexasaccharide **5.38** (32 mg, 0.021 mmol) in acetonitrile (1 mL). After that, a solution of sodium chlorite ($NaClO_2$, 9 mg, 0.09 mmol) in water 1 mL was added dropwise at 0 °C. Then, the solution of bleach (0.6 mL), saturated sodium bicarbonate (0.13 mL) and brine (0.27 mL) was added dropwise. The resulting mixture was stirred for 24 h at rt. After that, the reaction mixture was concentrated *in vacuo*, and dried. The crude residue was dissolved in DMF (3 mL), benzyl bromide (0.05 mL, 0.42 mmol) and $NaHCO_3$ (21 mg, 0.25 mmol) were added. The resulting suspension was stirred under argon for 16 h at rt. After that, the reaction was the solvent was removed *in vacuo*. The crude residue was dissolved in pyridine (3.0 mL), Ac_2O (1.0 mL, 10.60 mmol) was added, and the resulting mixture was stirred under argon for 24 hr at rt. Then, MeOH (~5 mL) was added and the volatiles were removed *in vacuo*. The residue was purified by column chromatography on silica gel (methanol - dichloromethane gradient elution) to afford the title compound as a white amorphous

solid (11 mg, 31%). Analytical data for **5.40**: $R_f = 0.54$ (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{22} -17.6$ (c = 1, CHCl_3); $^1\text{H NMR}$ (600 MHz, CDCl_3): δ , 1.25-1.29 (m, 12H, H-6, 6', 6'', 6'''), 1.79, 1.95, 1.99 (3s, 9H, 3 x COCH_3), 2.02 (br. s, 6H, 2 x COCH_3), 2.06, 2.10, 2.11 (3s, 9H, 3 x COCH_3), 3.34, 3.36 (2s, 6H, 2 x OCH_3), 3.43 (dd, 1H), 3.46 (dd, 1H), 3.55 (dd, 1H), 3.58 (s, 3H, OCH_3), 3.65 (dd, 1H), 3.73-3.80 (m, 5H), 3.84 (dd, 1H), 3.54 (s, 3H, OCH_3), 3.87-3.89 (m, 4H), 4.04 (dd, 1H), 4.38 (dd, 1H), 4.44 (d, 1H), 4.57-4.64 (m, 7H), 4.68-4.77 (m, 4H), 4.81-4.86 (m, 5H), 4.89-4.91 (m, 1H), 4.97 (d, 1H), 5.04 (d, 1H), 5.22 (dd, 1H), 5.44 (dd, 1H), 5.66-5.69 (m, 2H, 2 x NH), 5.98 (d, 1H, NH), 6.08 (d, 1H, NH), 6.15 (d, 1H, NH), 6.42 (d, 1H, NH), 7.26-7.38 (m, 20H, aromatic) ppm; $^{13}\text{C NMR}$ (150 MHz, CDCl_3): δ , 17.0, 17.1, 17.2, 17.5, 21.0, 21.2, 22.9, 23.2, 23.4, 23.5, 23.6, 23.8, 29.9 (x 2), 46.0, 47.8 (x 2), 48.6, 48.8, 52.6 (x 2), 53.3, 55.3, 58.9, 67.1, 67.5, 67.7, 68.2, 68.4, 69.0, 71.4, 72.4, 74.4, 74.6 (x 2), 74.7, 75.3 (x 3), 76.1, 76.7, 79.3, 79.6, 80.5, 98.3, 99.2 (x 2), 99.4, 100.5, 100.7, 127.3 (x 2), 127.4 (x 2), 127.6, 127.7, 127.8 (x 2), 127.9 (x 2), 128.0, 128.4 (x 2), 128.5 (x 4), 128.6 (x 3), 138.2, 138.4, 138.8, 139.1, 167.2, 168.4, 170.1, 170.6, 170.8, 171.2 (x 2), 171.6, 171.9, 172.7 ppm; HR-ESI MS $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{84}\text{H}_{112}\text{N}_6\text{O}_{31}$ 1700.7372, found 1700.7274.

5.5 References

1. Ala'Aldeen, D. A. A.; Hiramatsu, K., *Staphylococcus Aureus: Molecular and Clinical Aspects*. Horwood Publishing Limited Published by Elsevier Ltd.: 2004.
2. Chambers, H. F.; Deleo, F. R., Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat. Rev. Microbiol.* **2009**, *7*, 629-641.

3. Jansen, K. U.; Girgenti, D. Q.; Scully, I. L.; Anderson, A. S., Vaccine review: "Staphylococcus aureus vaccines: problems and prospects". *Vaccine* **2013**, *31*, 2723-2730.
4. O'Riordan, K.; Lee, J. C., Staphylococcus aureus Capsular Polysaccharides. *Clin. Microbiol. Rev.* **2004**, *17* (1), 218-234.
5. Jones, C., Revised structures for the capsular polysaccharides from Staphylococcus aureus Types 5 and 8, components of novel glycoconjugate vaccines. *Carbohydr. Res.* **2005**, *340*, 1097-1106.
6. Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R., Synthesis of Staphylococcus aureus type 5 capsular polysaccharide repeating unit using novel L-FucNAc and D-FucNAc synthons and immunochemical evaluation. *Bioorg. Med. Chem.* **2012**, *20*, 6403-6415.
7. Gagarinov, I. A.; Fang, T.; Liu, L.; Srivastava, A. D.; Boons, G. J., Synthesis of Staphylococcus aureus Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization. *Org. Lett.* **2015**, *17*, 928-931.
8. Visansirikul, S.; Yasomanee, J. P.; Pornsuriyasak, P.; Kamat, M. N.; Podvalnyy, N. M.; Gobble, C. P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V., A Concise Synthesis of the Repeating Unit of Capsular Polysaccharide Staphylococcus aureus Type 8. *Org. Lett.* **2015**, *17*, 2382-2384.
9. Yasomanee, J. P.; Visansirikul, S.; Pornsuriyasak, P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V., The synthesis of the repeating unit of capsular polysaccharide Staphylococcus aureus type 5 to study chemical activation for conjugation of native CP5. *J. Org. Chem.* **2016**, *81*, 5981-5987.

10. Hagen, B.; Ali, S.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C., Mapping the Reactivity and Selectivity of 2-Azidofucosyl Donors for the Assembly of N-Acetylfucosamine-Containing Bacterial Oligosaccharides. *J. Org. Chem.* **2017**, *82*, 848-868.
11. Nigudkar, S. S.; Demchenko, A. V., Stereocontrolled 1,2-cis glycosylation as the driving force of progress in synthetic carbohydrate chemistry. *Chem. Sci.* **2015**, *6*, 2687-2704.
12. Liu, L.; Zha, J.; DiGiandomenico, A.; McAllister, D.; Stover, C. K.; Wang, Q.; Boons, G.-J., Synthetic Enterobacterial Common Antigen (ECA) for the Development of a Universal Immunotherapy for Drug-Resistant Enterobacteriaceae. *Angew. Chem. Int. Ed.* **2015**, *54*, 10953-10957.
13. Hagen, B.; van Dijk, J. H. M.; Zhang, Q.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C., Synthesis of the Staphylococcus aureus Strain M Capsular Polysaccharide Repeating Unit. *Org. Lett.* **2017**, *19*, 2514-2517.
14. Zhang, Z.; Magnusson, G., Conversion of p-methoxyphenyl glycosides into the corresponding glycosyl chlorides and bromides, and into thiophenyl glycosides. *Carbohydr. Res.* **1996**, *295*, 41-55.
15. Qin, C.; Schumann, B.; Zou, X.; Pereira, C. L.; Tian, G.; Hu, J.; Seeberger, P. H.; Yin, J., Total Synthesis of a Densely Functionalized Plesiomonas shigelloides Serotype 51 Aminoglycoside Trisaccharide Antigen. *J. Am. Chem. Soc.* **2018**, *140* (8), 3120-3127.
16. Yang, S.; Wang, A. p.; Zhang, G.; Di, X.; Zhao, Z.; Lei, P., An investigation of construction of chondroitin sulfate E (CS-E) repeating unit. *Tetrahedron* **2016**, *72*, 5659-5670.

17. Osborn, H. M. I.; Brome, V. A.; Harwood, L. M.; Suthers, W. G., Regioselective C-3-O-acylation and O-methylation of 4,6-O-benzylidene- β -d-gluco- and galactopyranosides displaying a range of anomeric substituents. *Carbohydr. Res.* **2001**, *332*, 157-166.
18. 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.
19. Hale, K. J.; Hough, L.; Manaviazar, S.; Calabrese, A., An update of the rules for pyranoside sulfonate displacement. *Org. Lett.* **2014**, *16*, 4838-4841.
20. Zulueta, M. M.; Lin, S. Y.; Lin, Y. T.; Huang, C. J.; Wang, C. C.; Ku, C. C.; Shi, Z.; Chyan, C. L.; Irene, D.; Lim, L. H.; Tsai, T. I.; Hu, Y. P.; Arco, S. D.; Wong, C. H.; Hung, S. C., α -Glycosylation by D-glucosamine-derived donors: synthesis of heparosan and heparin analogues that interact with mycobacterial heparin-binding hemagglutinin. *J. Am. Chem. Soc.* **2012**, *134*, 8988-8995.
21. Prakash, I.; Tanielyan, S. K.; Augustine, R. L.; Furlong, K. E.; Scherm, R. C.; Jackson, H. E. Bromine free TEMPO based catalyst system for oxidation of primary and secondary alcohols using NaOCl as an oxidant. US6825384B1, 2004.

APPENDIX

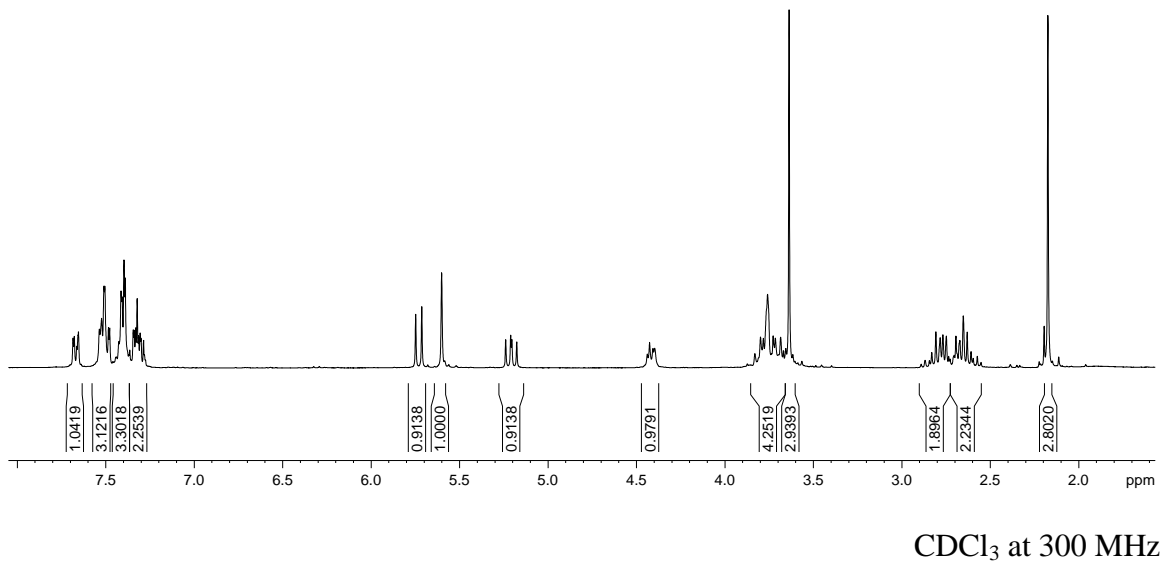
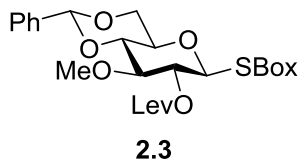


Figure A-1: ¹H NMR spectrum of Benzoxazolyl 4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl-1-thio-β-D-glucopyranoside (**2.3**)

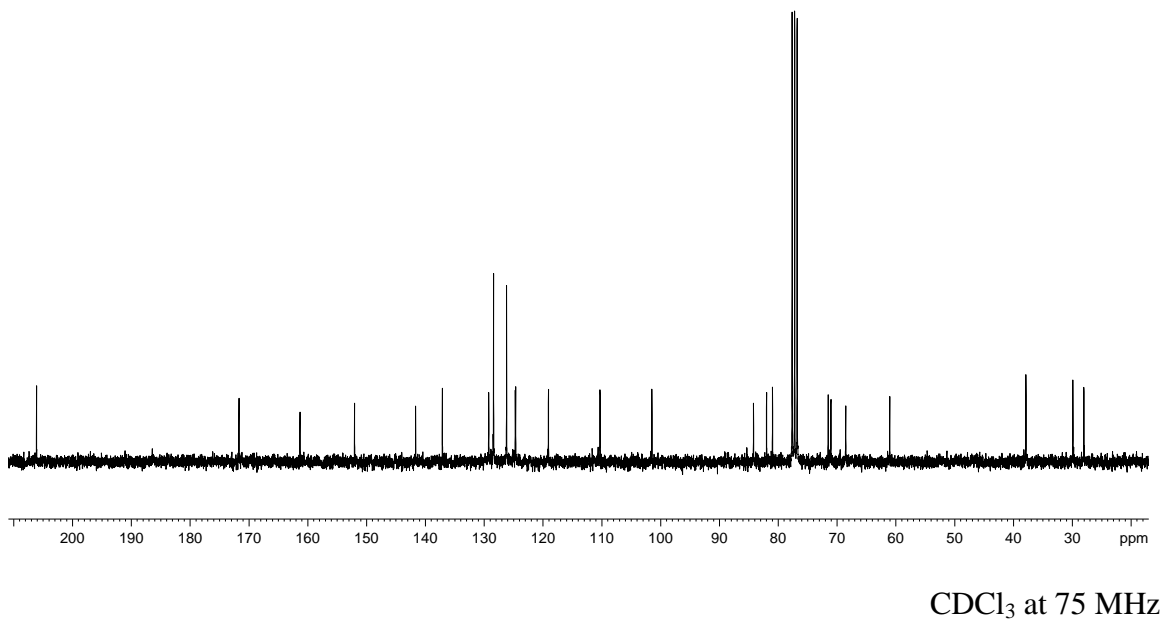


Figure A-2: ¹³C NMR spectrum of Benzoxazolyl 4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl-1-thio-β-D-glucopyranoside (**2.3**)

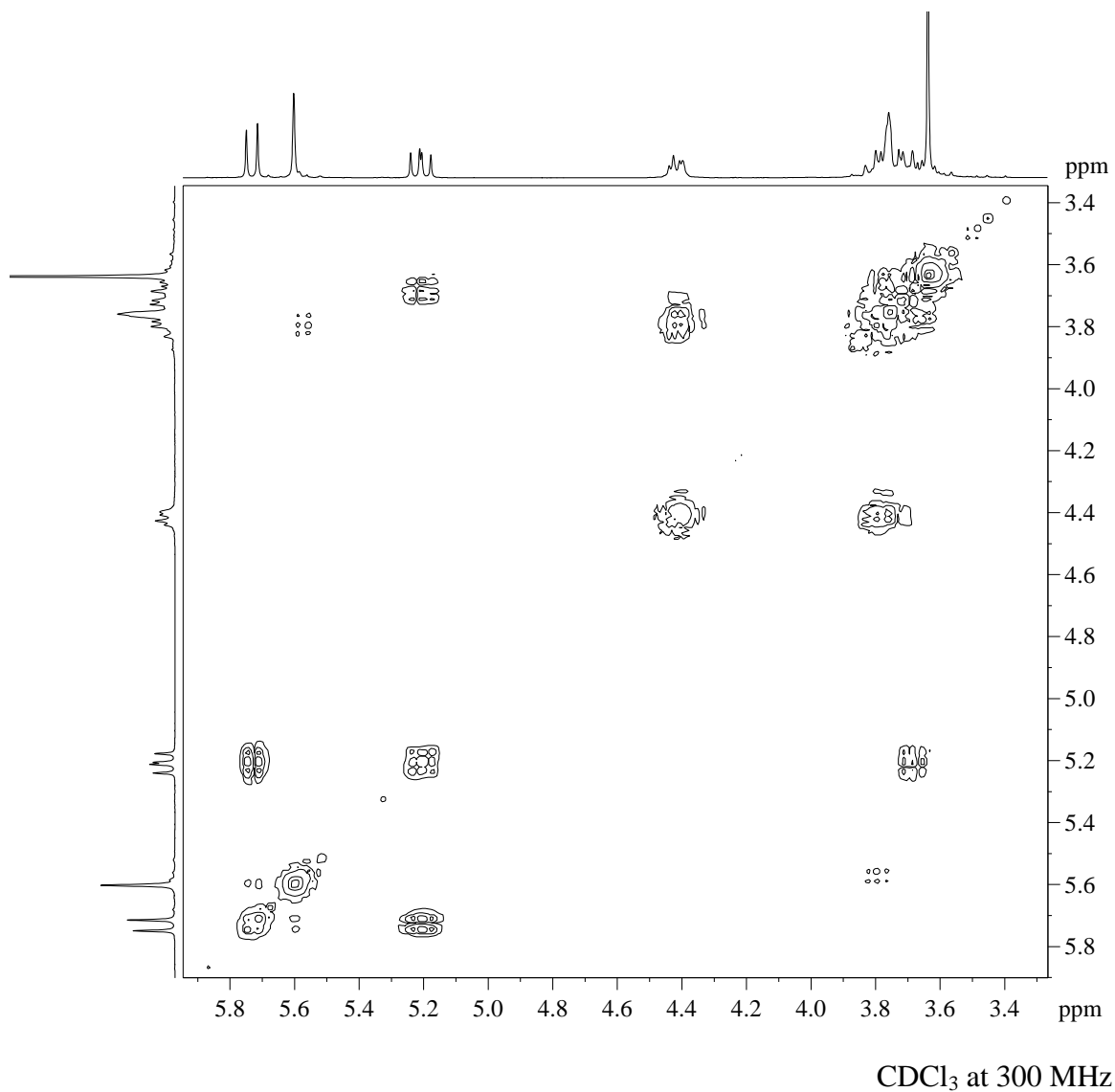
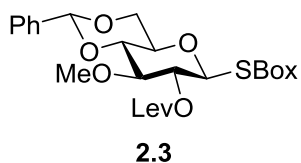
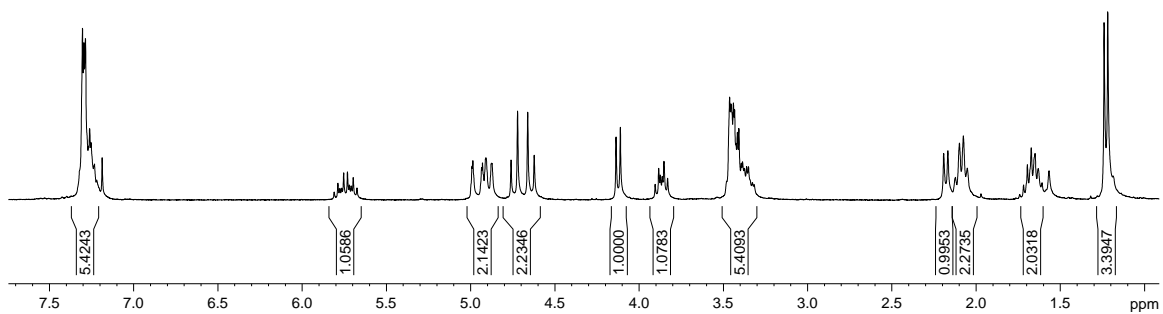
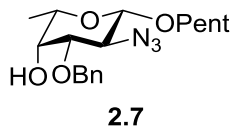
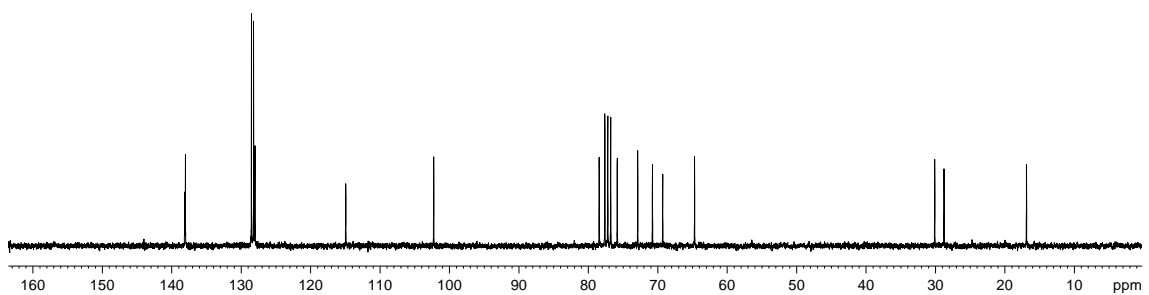


Figure A-3: 2-D NMR COSY spectrum of Benzoxazolyl 4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl-1-thio- β -D-glucopyranoside (**2.3**)



CDCl₃ at 300 MHz

Figure A-4: ¹H NMR spectrum of 4-Pentenyl 2-azido-4-*O*-benzyl-2,6-dideoxy-β-L-galactopyranoside (**2.7**)



CDCl₃ at 75 MHz

Figure A-5: ¹³C NMR spectrum of 4-Pentenyl 2-azido-4-*O*-benzyl-2,6-dideoxy-β-L-galactopyranoside (**2.7**)

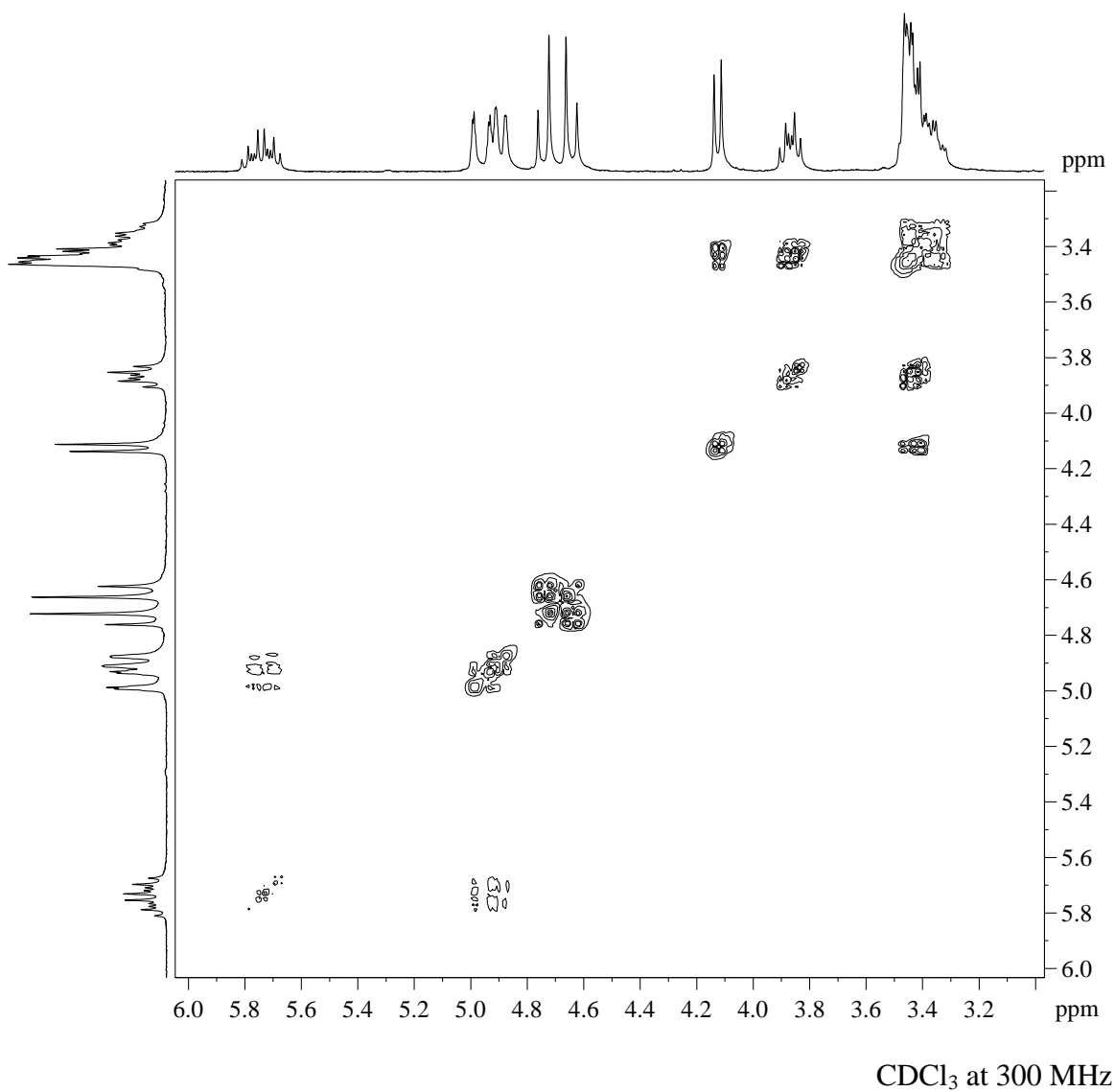
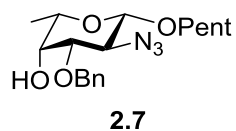


Figure A-6: 2-D NMR COSY spectrum of 4-Pentenyl 2-azido-4-*O*-benzyl-2,6-dideoxy- β -L-galactopyranoside (**2.7**)

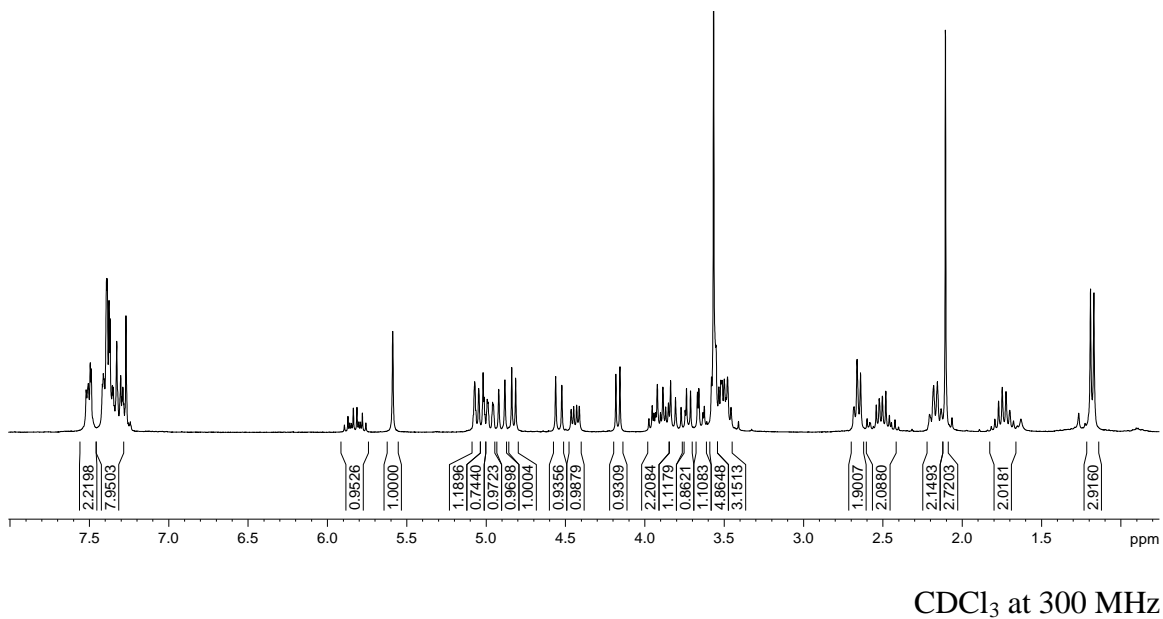
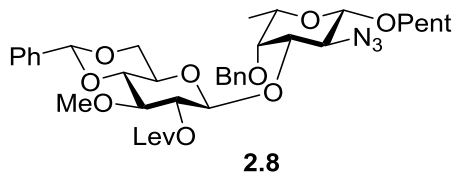


Figure A-7: ¹H NMR spectrum of 4-Pentenyl *O*-(4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl-β-D-glucopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-β-L-galactopyranoside (**2.8**)

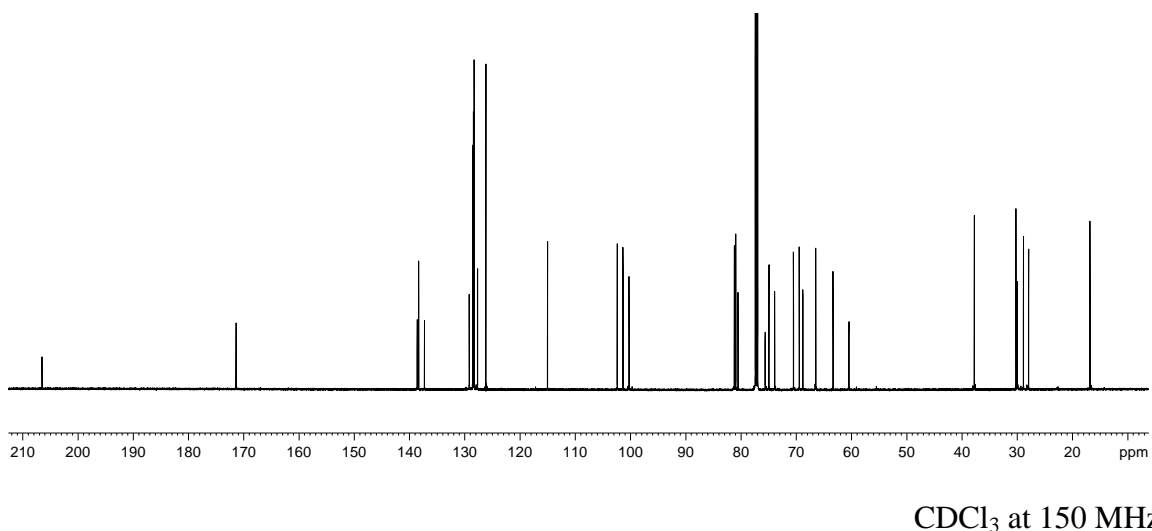


Figure A-8: ¹³C NMR spectrum of 4-Pentenyl *O*-(4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl-β-D-glucopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-β-L-galactopyranoside (**2.8**)

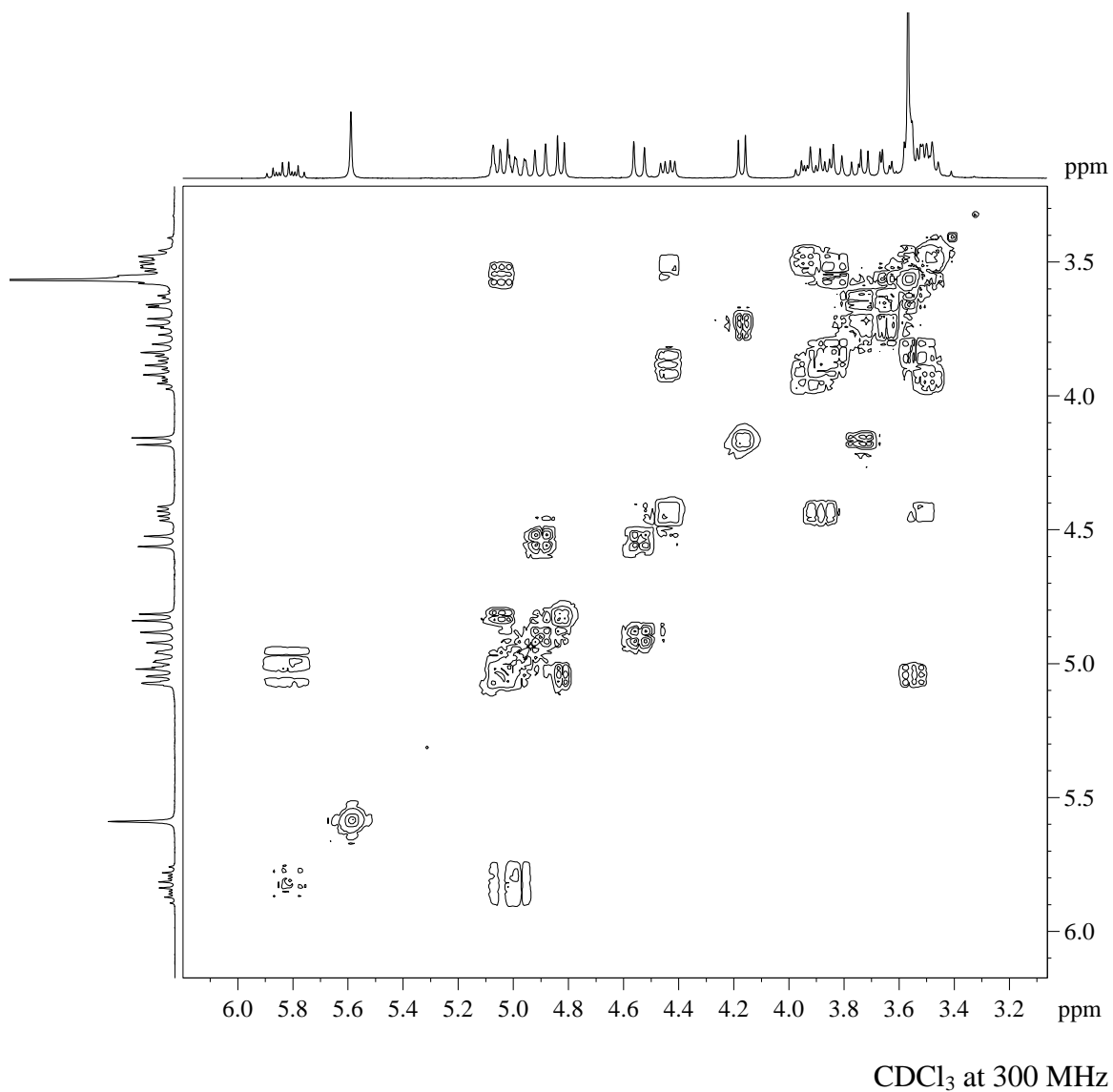
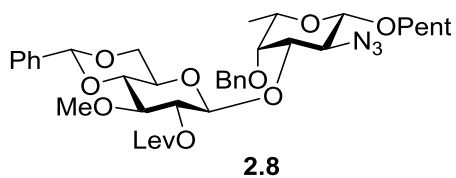


Figure A-9: 2-D NMR COSY spectrum of 4-Pentenyl *O*-(4,6-*O*-benzylidene-2-*O*-levulinoyl-3-*O*-methyl- β -D-glucopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -L-galactopyranoside (**2.8**)

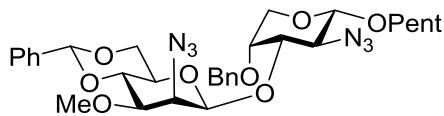
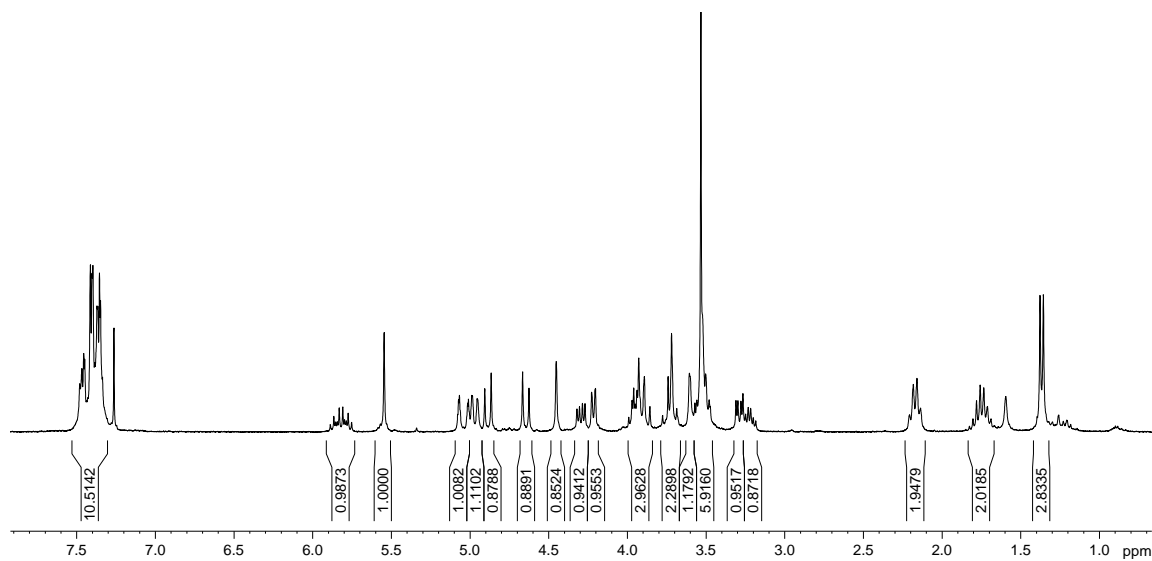
**2.10**CDCl₃ at 300 MHz

Figure A-10: ¹H NMR spectrum of 4-Pentenyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-β-L-galactopyranoside (**2.10**)

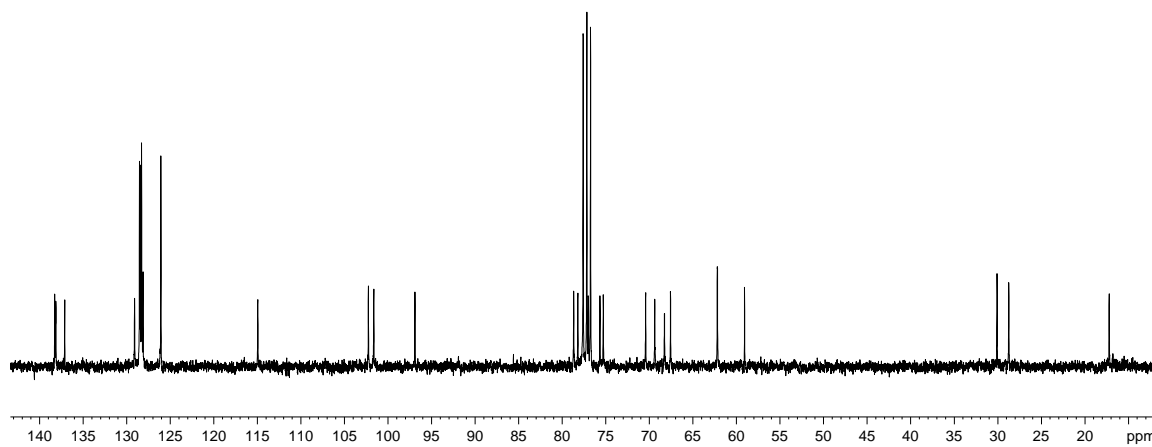
CDCl₃ at 75 MHz

Figure A-11: ¹³C NMR spectrum of 4-Pentenyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-β-L-galactopyranoside (**2.10**)

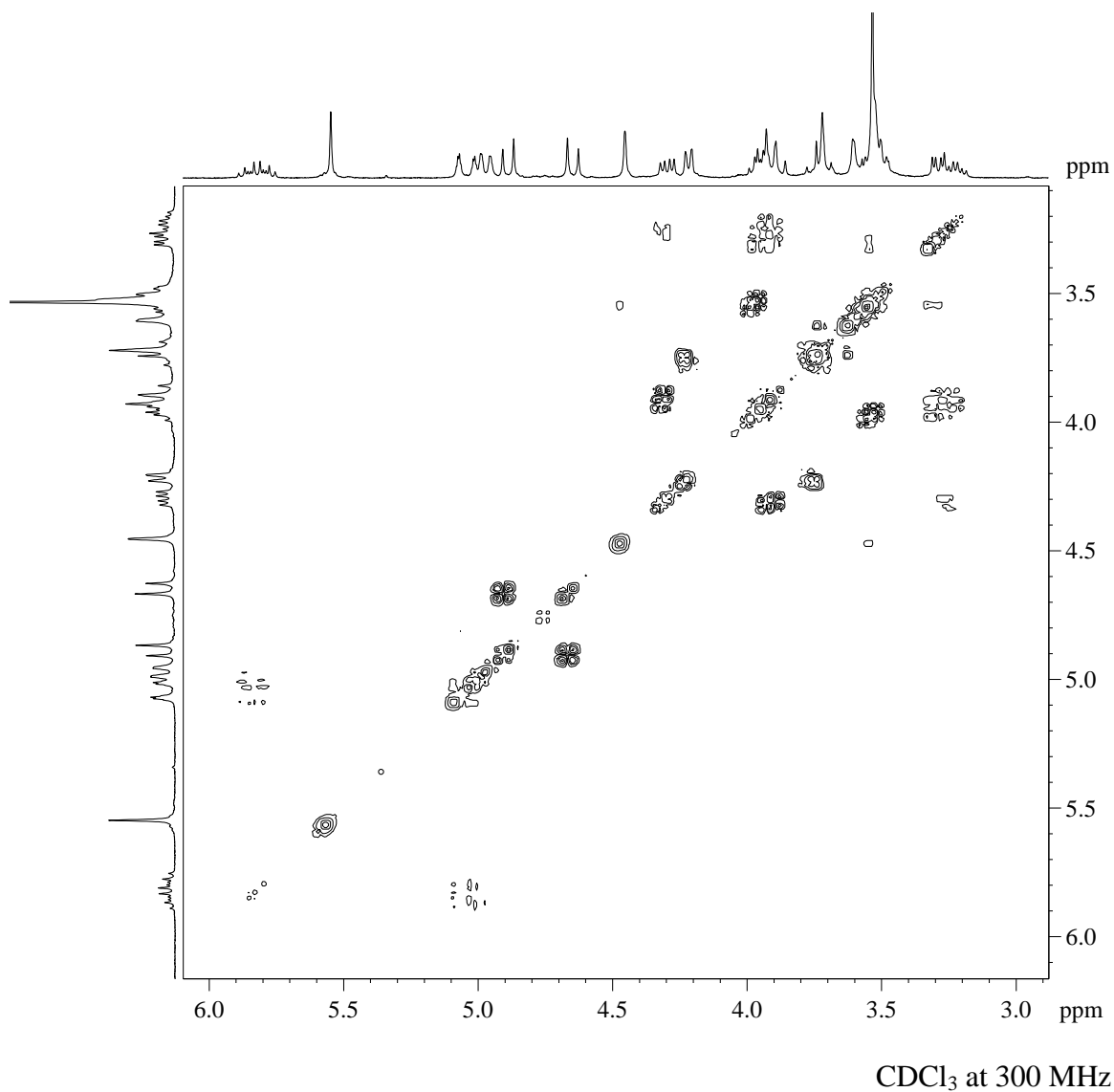
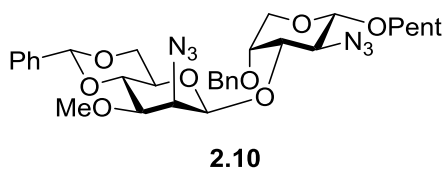
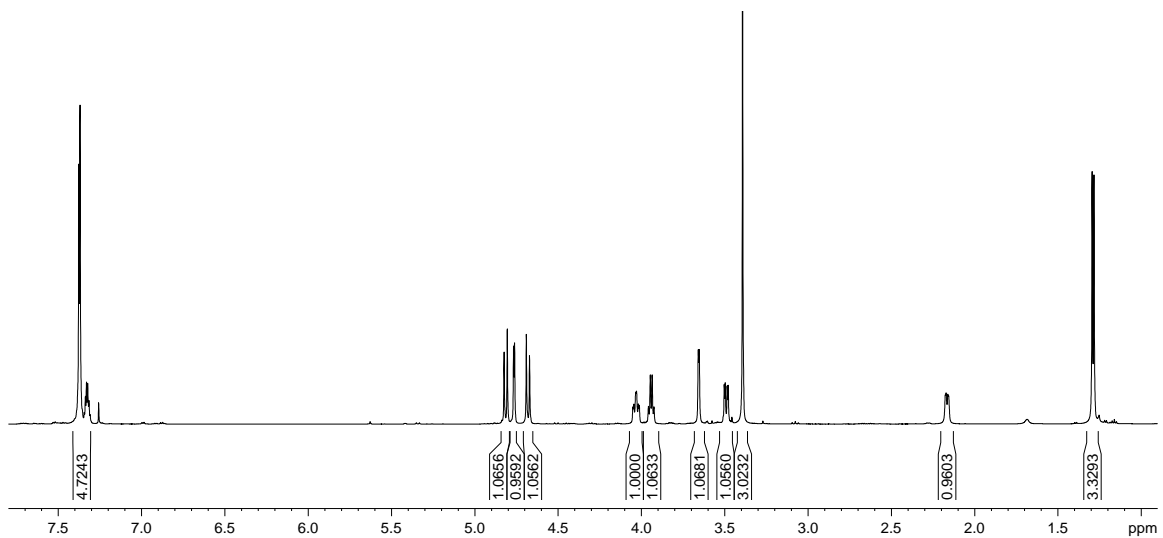
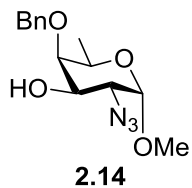
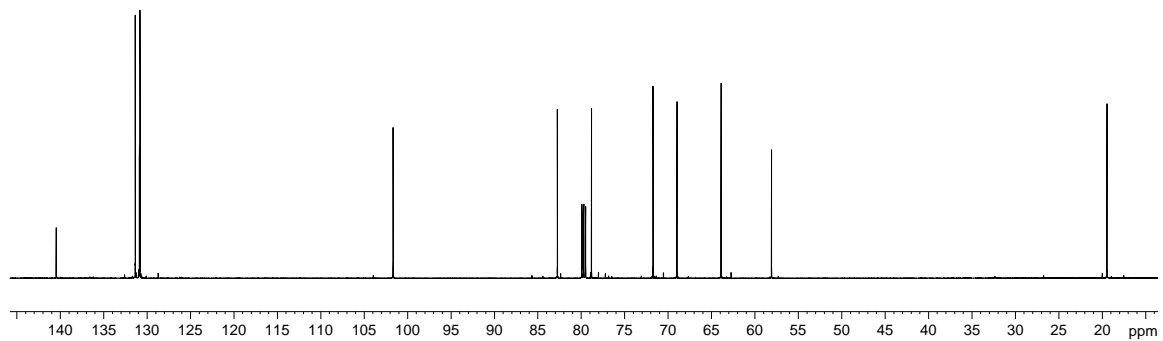


Figure A-12: 2-D NMR COSY spectrum of 4-Pentenyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -L-galactopyranoside (**2.10**)



CDCl₃ at 600 MHz

Figure A-13: ¹H NMR spectrum of Methyl 2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.14**)



CDCl₃ at 150 MHz

Figure A-14: ¹³C NMR spectrum of Methyl 2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.14**)

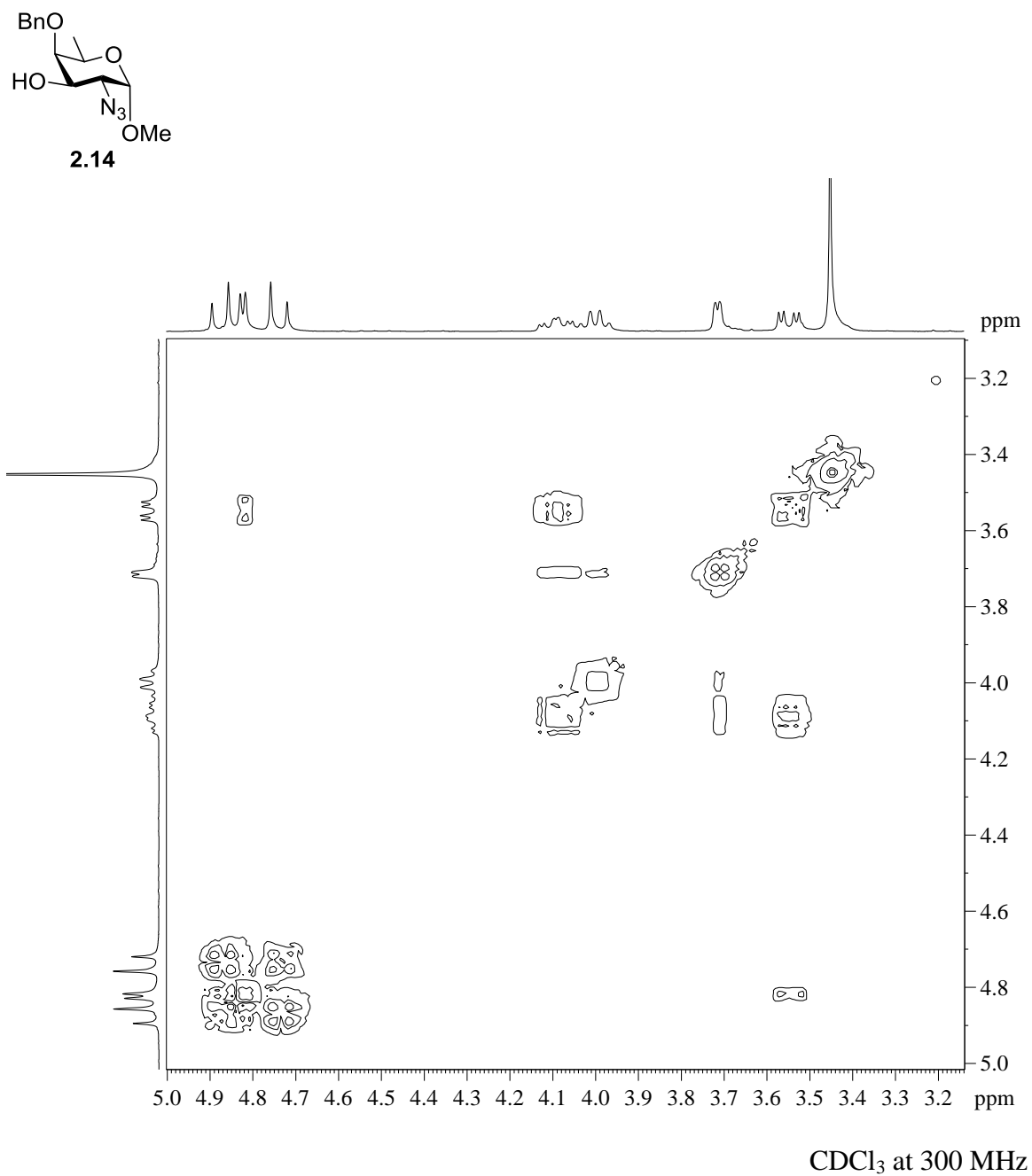


Figure A-15: 2-D NMR COSY spectrum of Methyl 2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.14**)

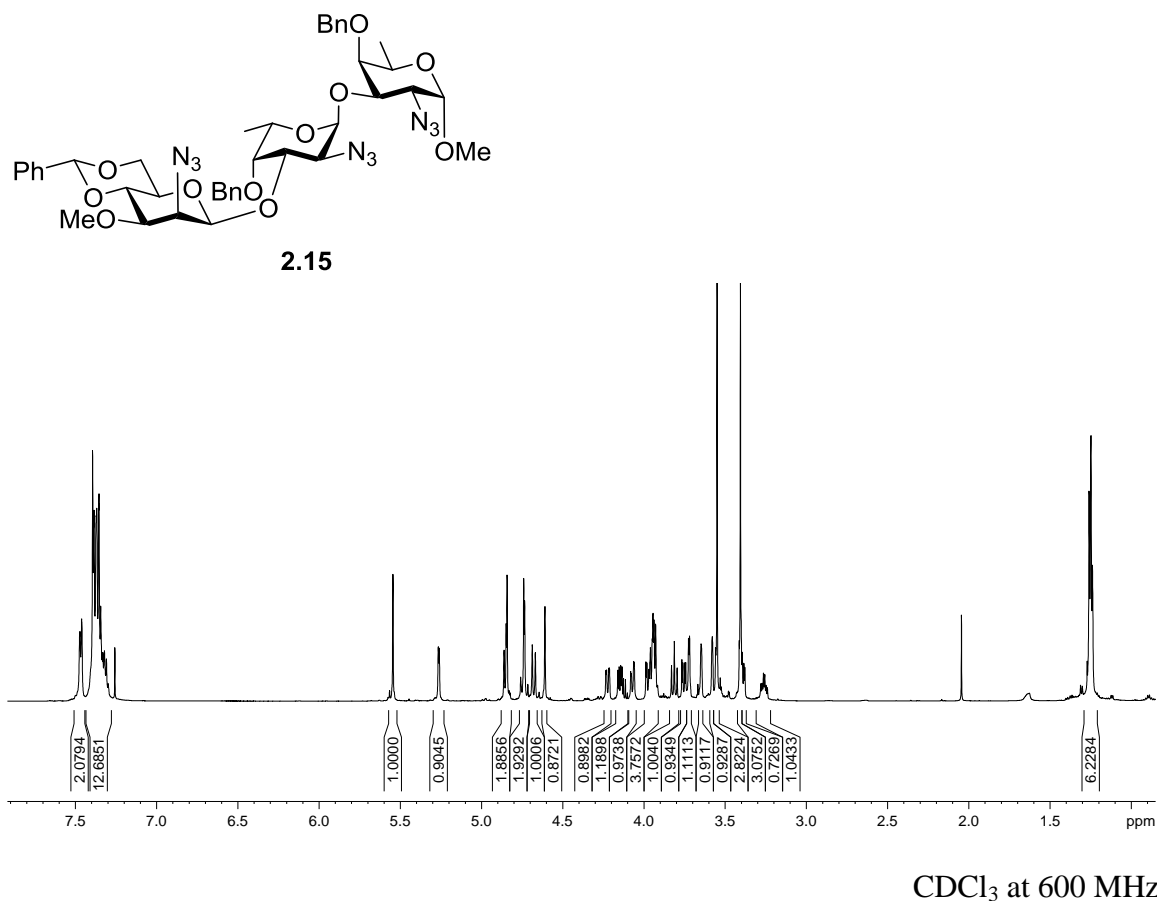


Figure A-16: ^1H NMR spectrum of Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.15**)

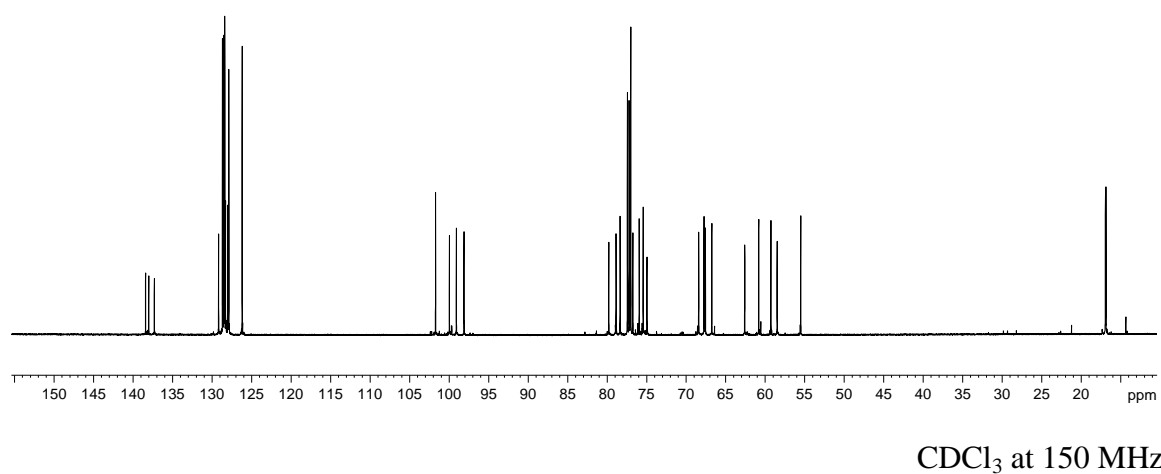
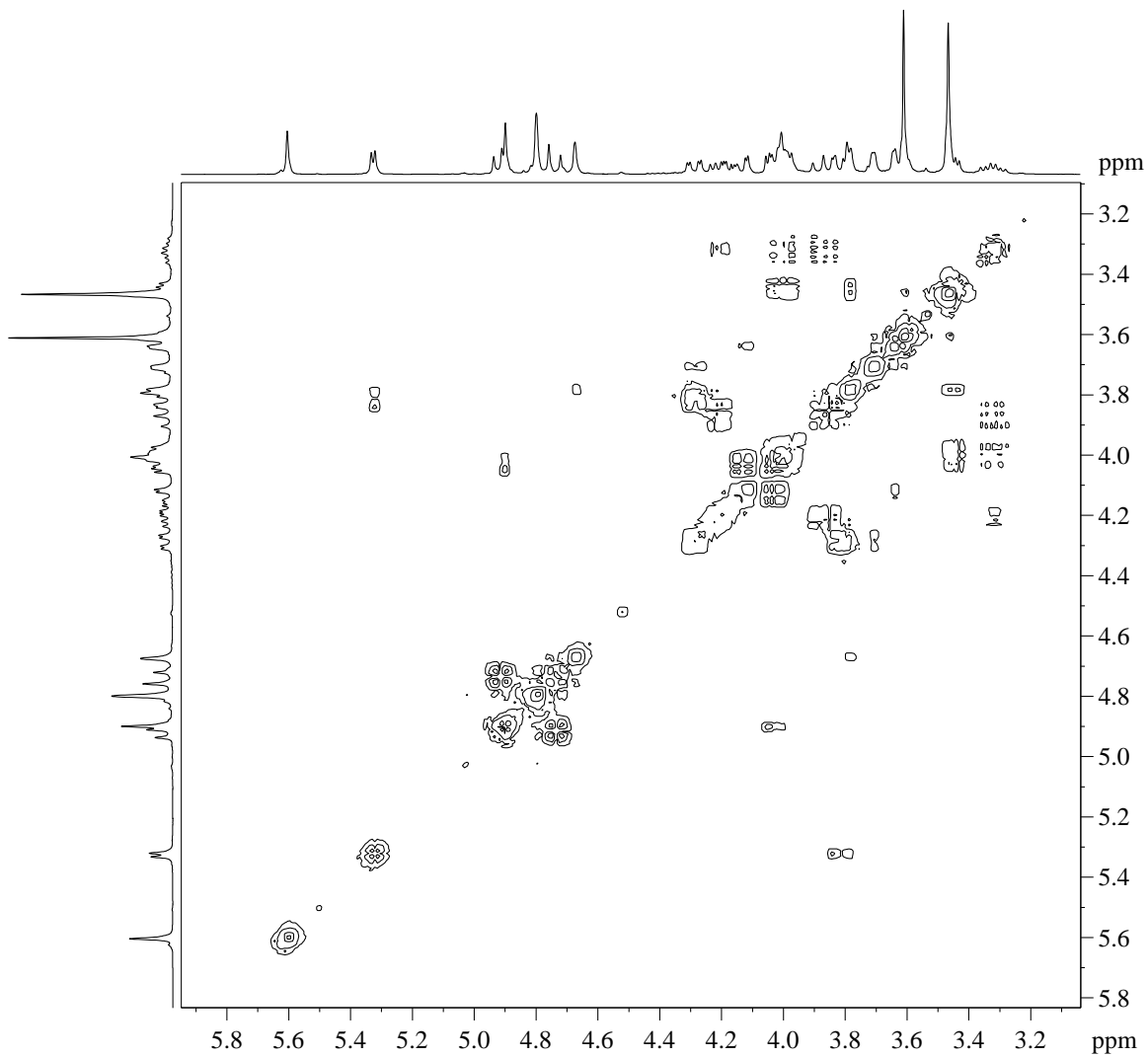
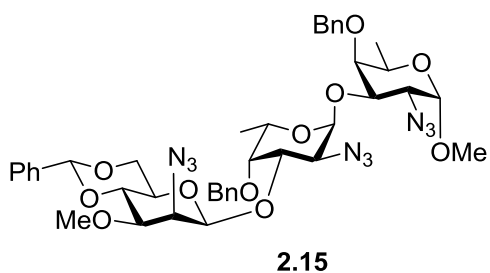


Figure A-17: ^{13}C NMR spectrum of Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.15**)



CDCl₃ at 300 MHz

Figure A-18: 2-D NMR COSY spectrum of Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.15**)

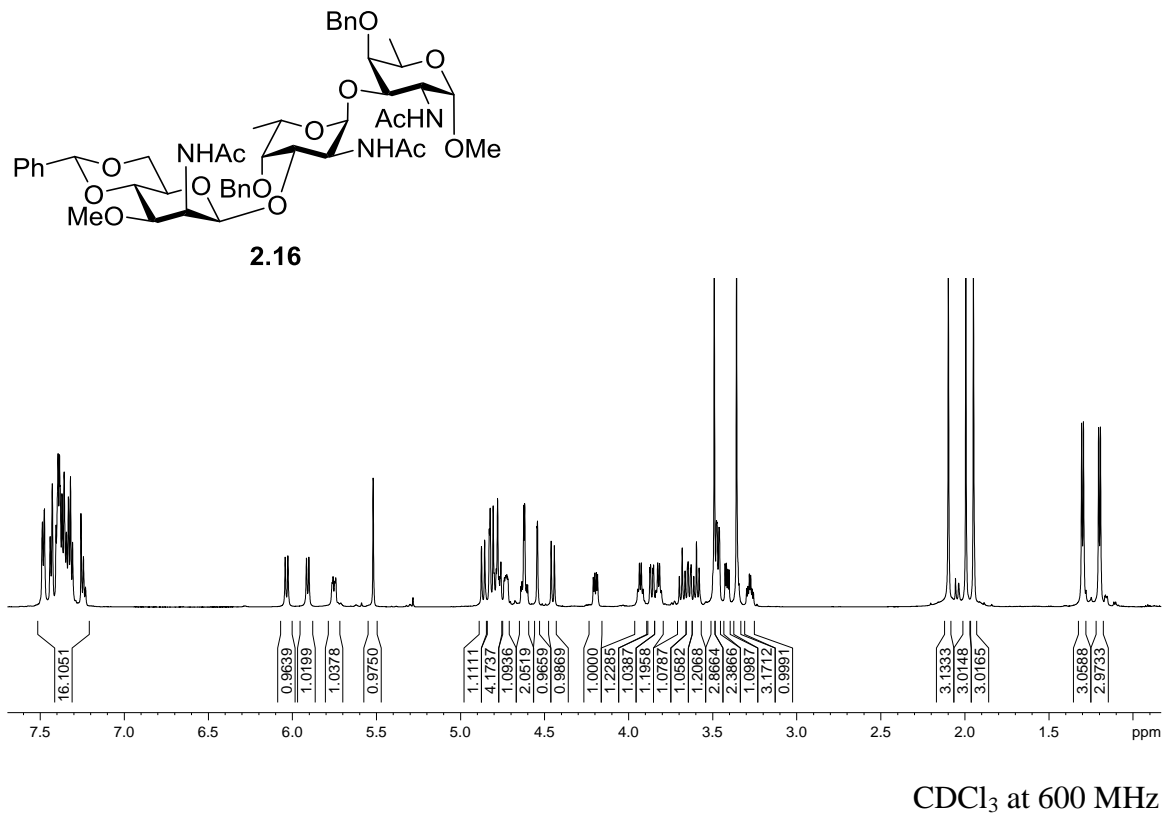


Figure A-19: ^1H NMR spectrum of Methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.16**)

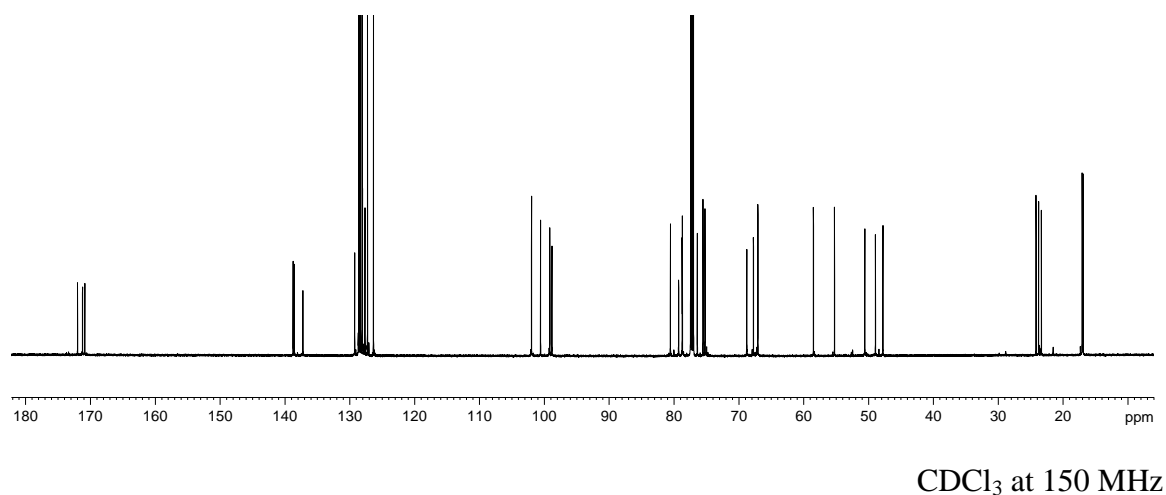


Figure A-20: ^{13}C NMR spectrum of Methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.16**)

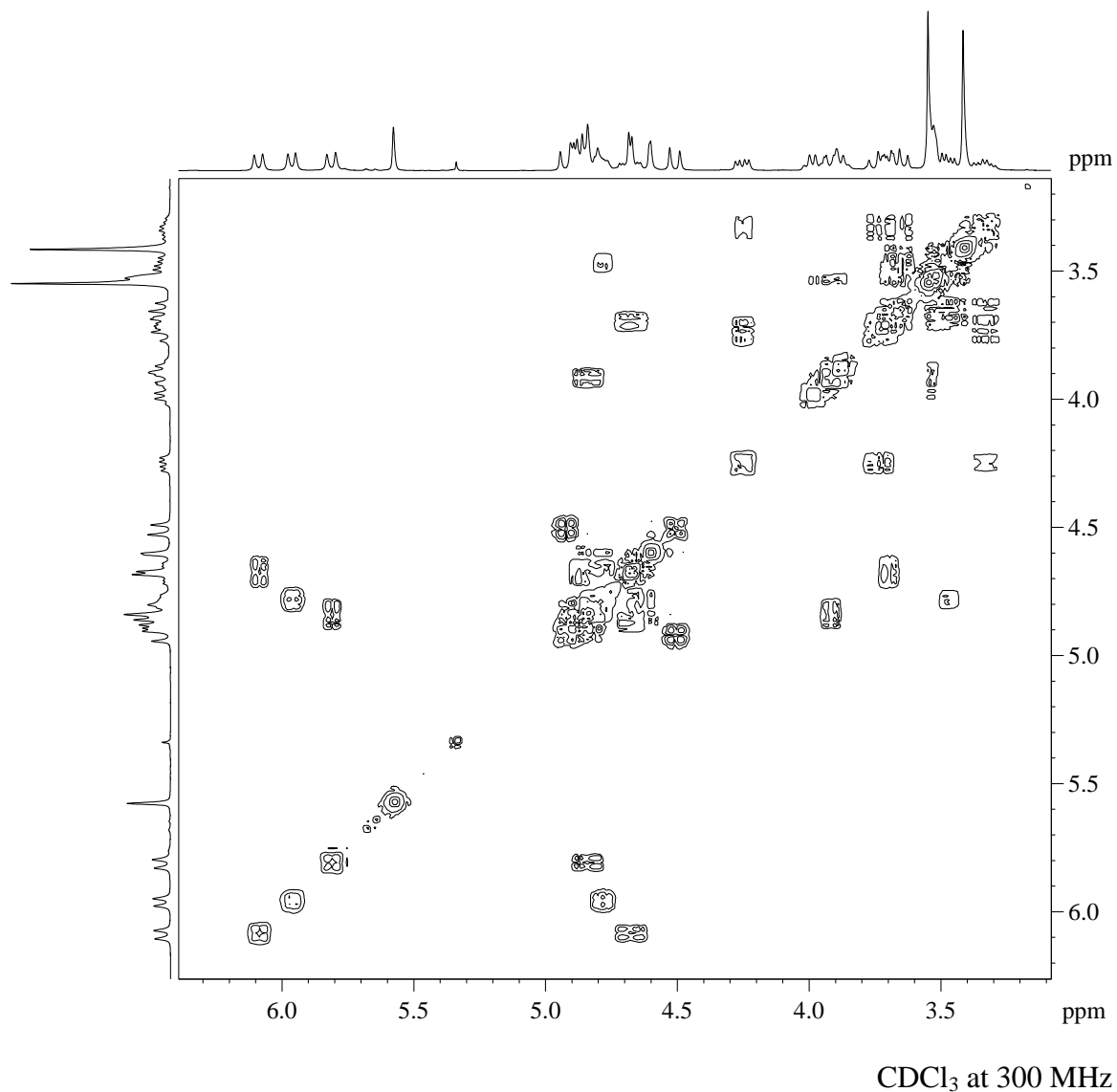
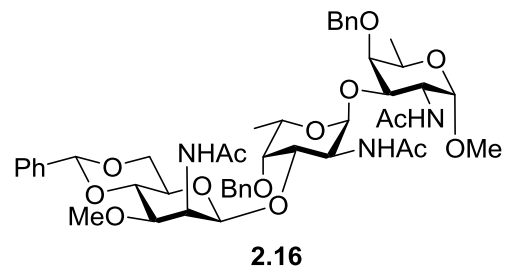


Figure A-21: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**2.16**)

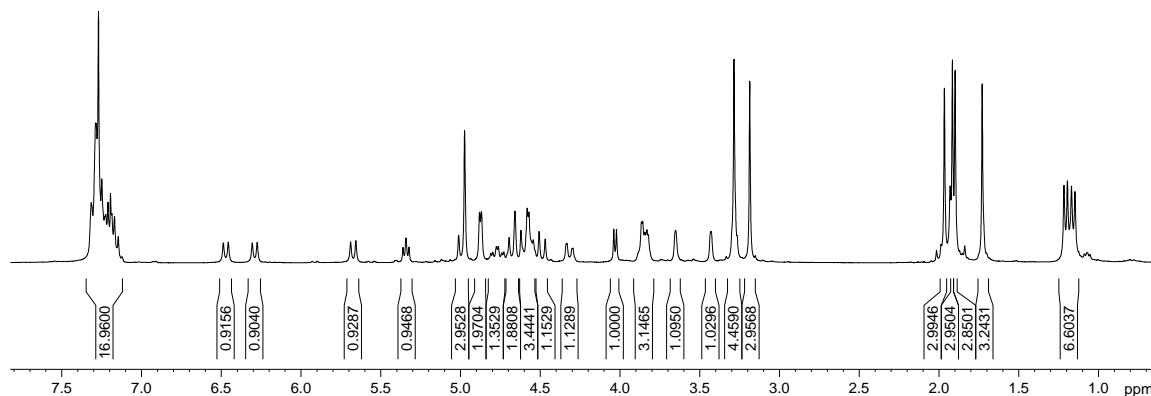
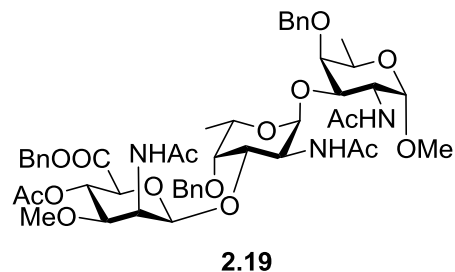
CDCl₃ at 300 MHz

Figure A-22: ¹H NMR spectrum of Methyl *O*-(benzyl 2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl uronate)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**2.19**)

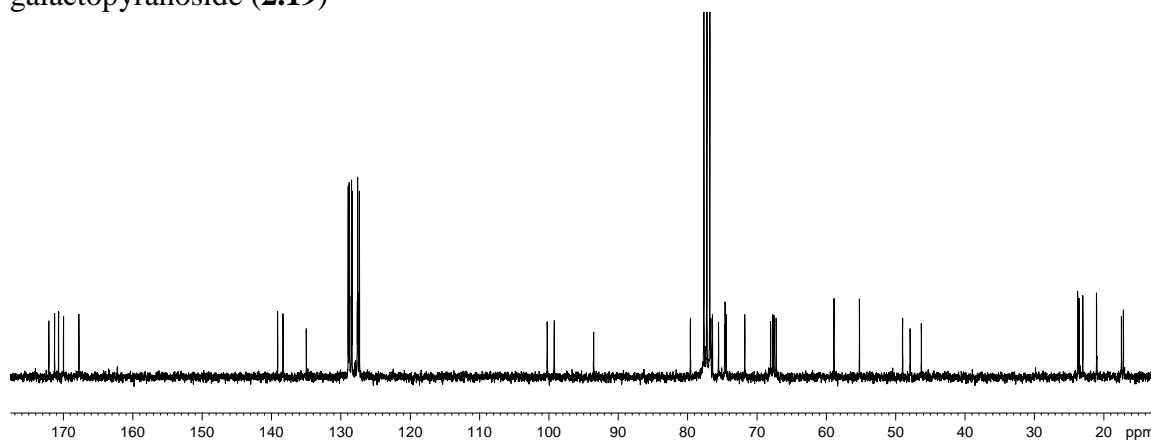
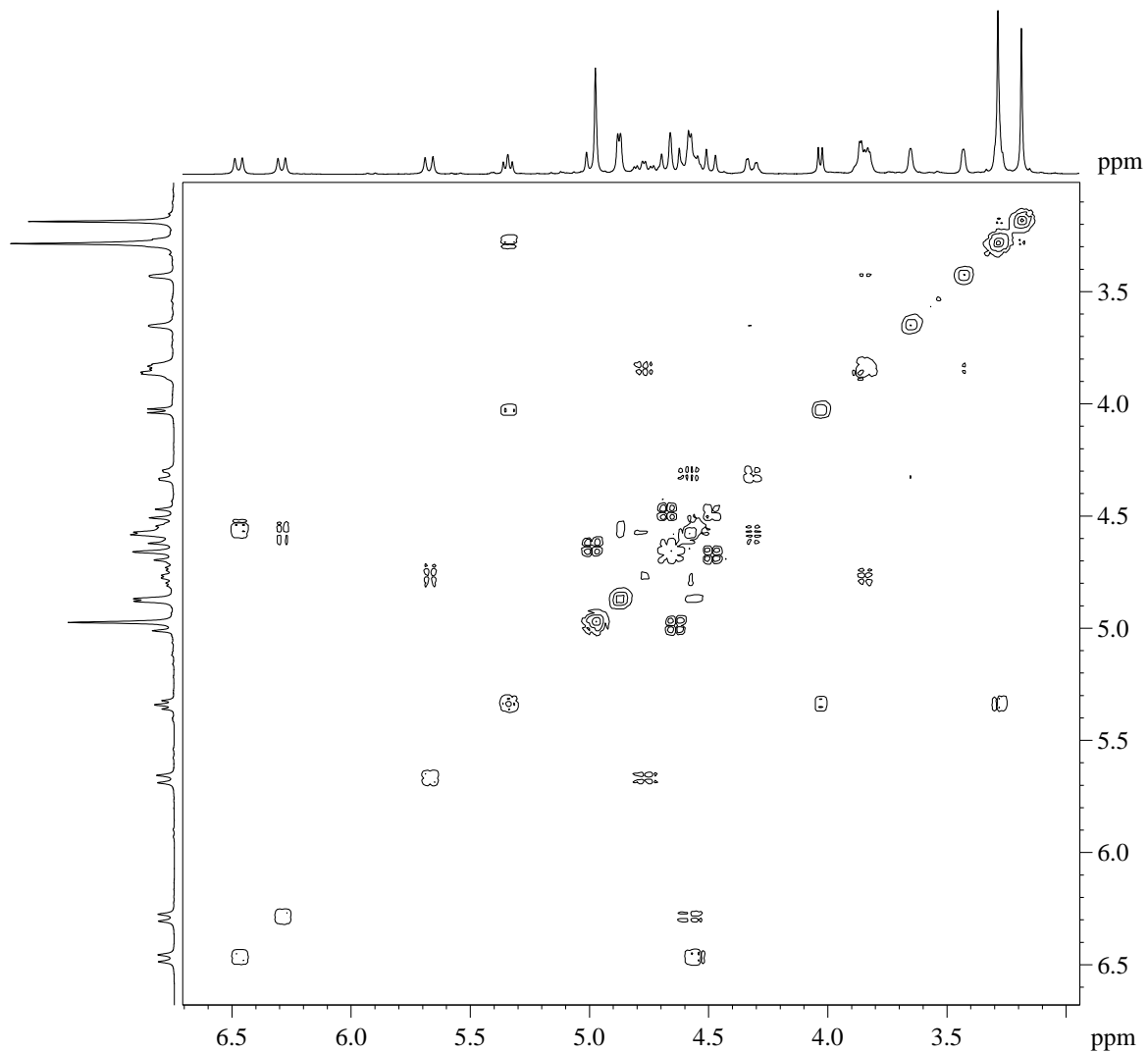
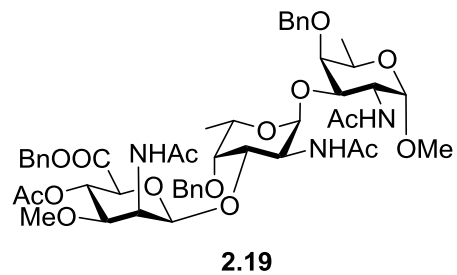
CDCl₃ at 75 MHz

Figure A-23: ¹³C NMR spectrum of Methyl *O*-(benzyl 2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl uronate)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**2.19**)



CDCl_3 at 300 MHz

Figure A-24: 2-D NMR COSY spectrum of Methyl *O*-(benzyl 2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl uronate)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**2.19**)

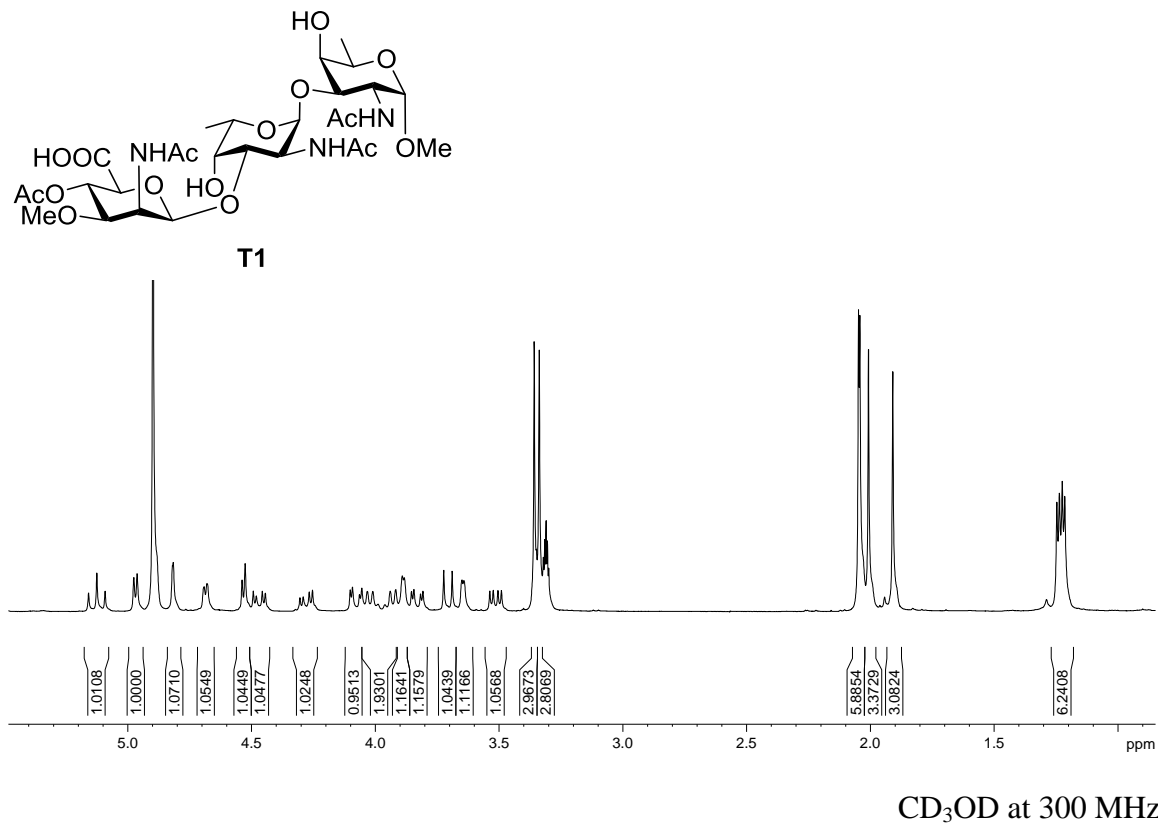


Figure A-25: ¹H NMR spectrum of Methyl *O*-(2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl-β-*D*-mannopyranosyluronic acid)-(1→3)-*O*-(2-acetamido-2,6-dideoxy-α-*L*-fucopyranosyl)-(1→3)-2-acetamido-2,6-dideoxy-α-*D*-galactopyranoside (**T1**)

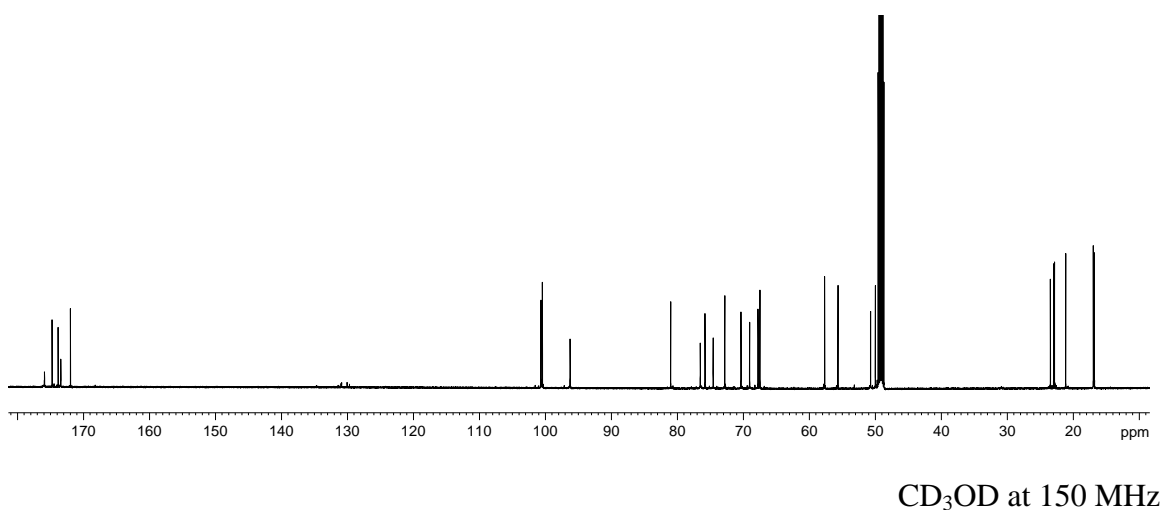


Figure A-26: ¹³C NMR spectrum of Methyl *O*-(2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl-β-*D*-mannopyranosyluronic acid)-(1→3)-*O*-(2-acetamido-2,6-dideoxy-α-*L*-fucopyranosyl)-(1→3)-2-acetamido-2,6-dideoxy-α-*D*-galactopyranoside (**T1**)

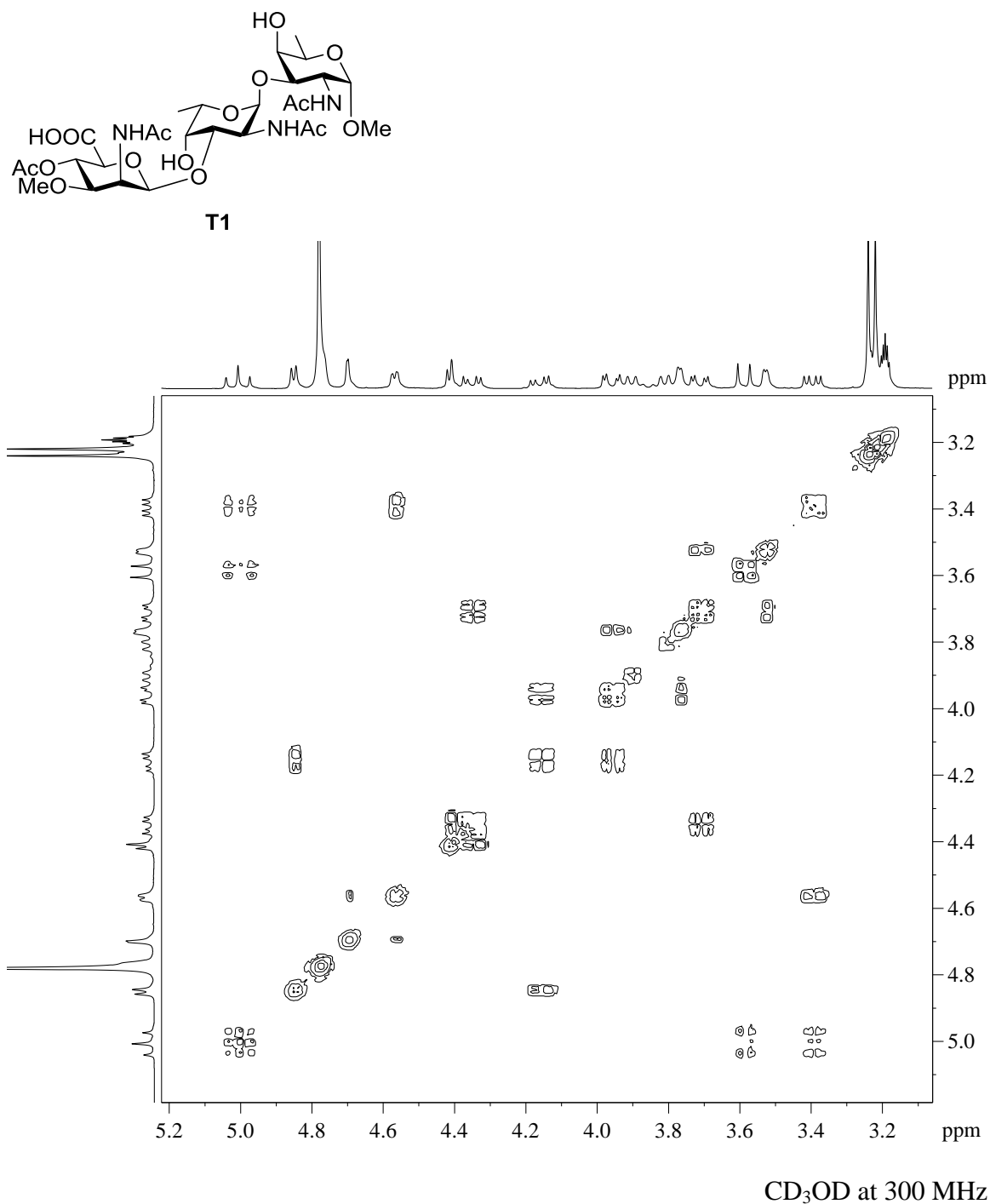


Figure A-27: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl- β -D-mannopyranosyluronic acid)-(1 \rightarrow 3)-*O*-(2-acetamido-2,6-dideoxy- α -L-fucopyranosyl)-(1 \rightarrow 3)-2-acetamido-2,6-dideoxy- α -D-galactopyranoside (**T1**)

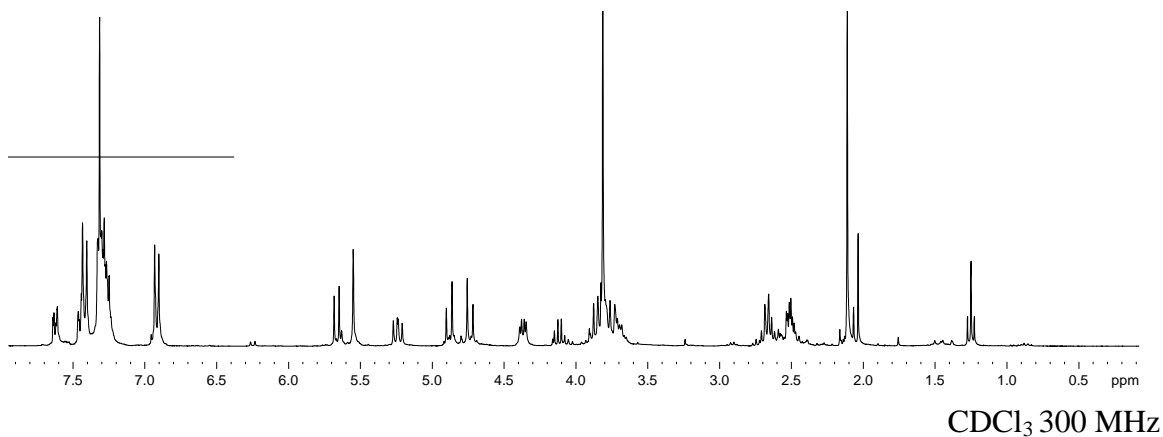
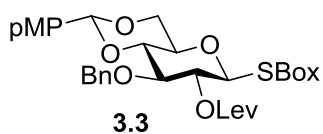


Figure A-28: ¹H NMR spectrum of Benzoxazolyl 3-*O*-benzyl-2-*O*-levulinoyl-4,6-*O*-(*p*-methoxybenzylidene)-1-thio-β-*D*-glucopyranoside (**3.3**)

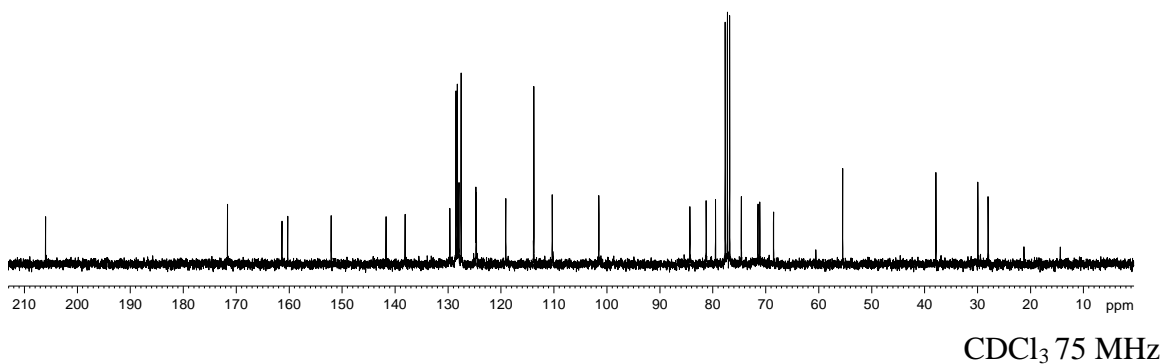


Figure A-29: ¹³C NMR spectrum of Benzoxazolyl 3-*O*-benzyl-2-*O*-levulinoyl-4,6-*O*-(*p*-methoxybenzylidene)-1-thio-β-*D*-glucopyranoside (**3.3**)

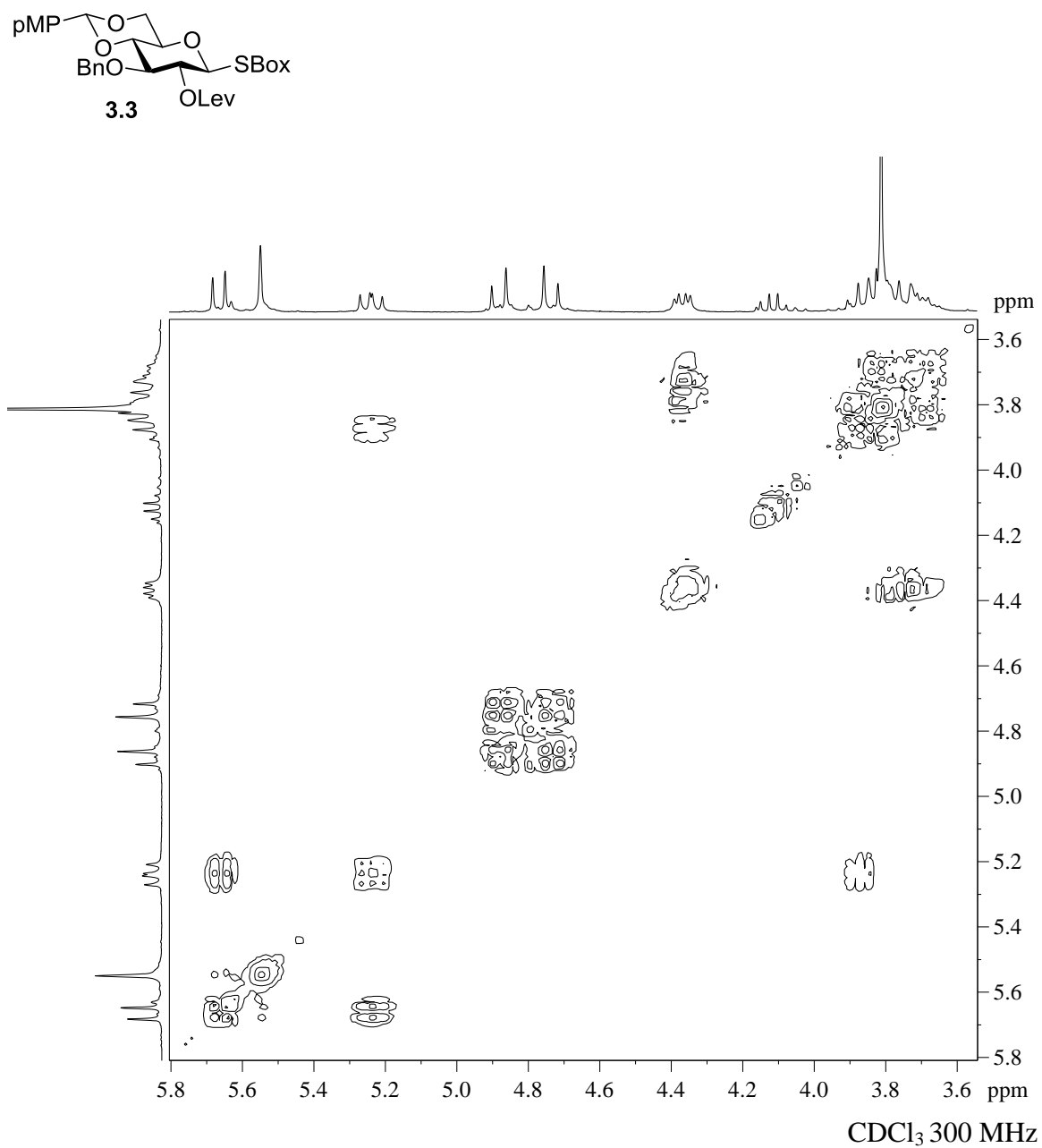


Figure A-30: 2-D NMR COSY spectrum of Benzoxazolyl 3-*O*-benzyl-2-*O*-levulinoyl-4,6-*O*-(*p*-methoxybenzylidene)-1-thio- β -D-glucopyranoside (**3.3**)

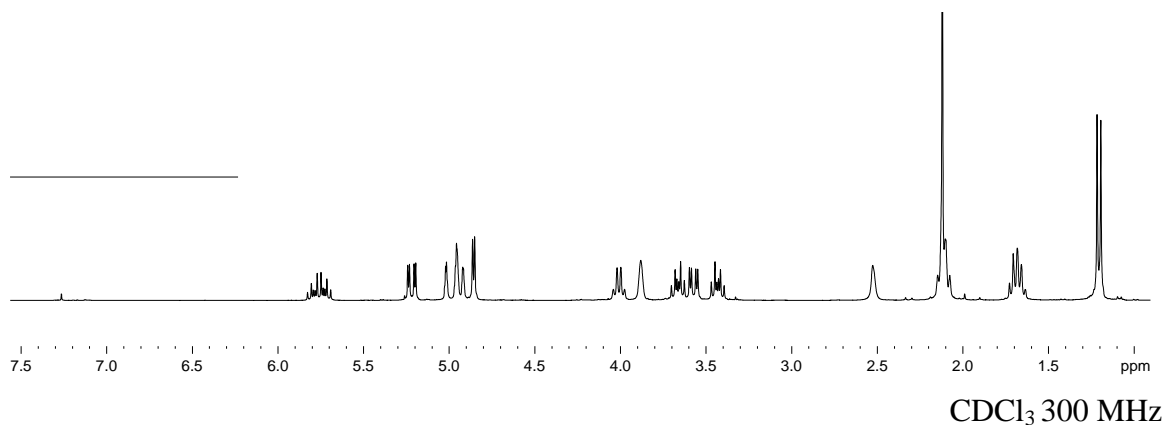
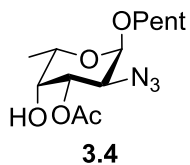


Figure A-31: ¹H NMR spectrum of 4-Pentenyl 3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (**3.4**)

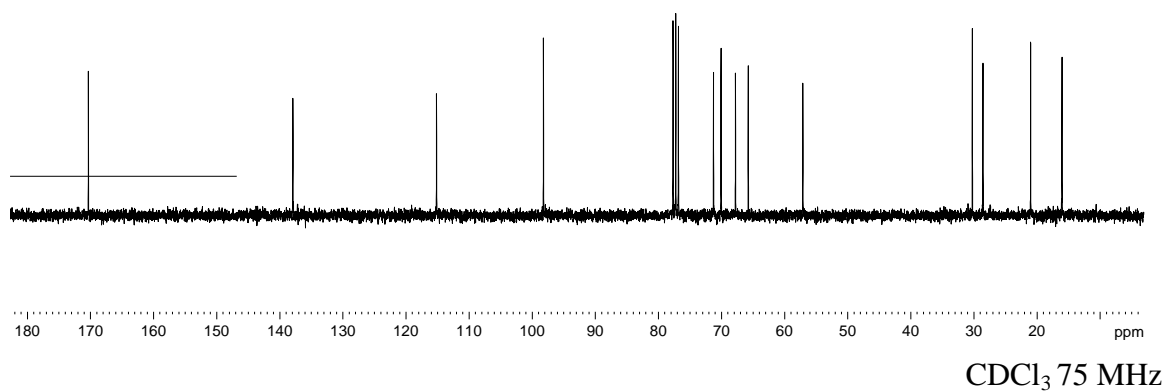


Figure A-32: ¹³C NMR spectrum of 4-Pentenyl 3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (**3.4**)

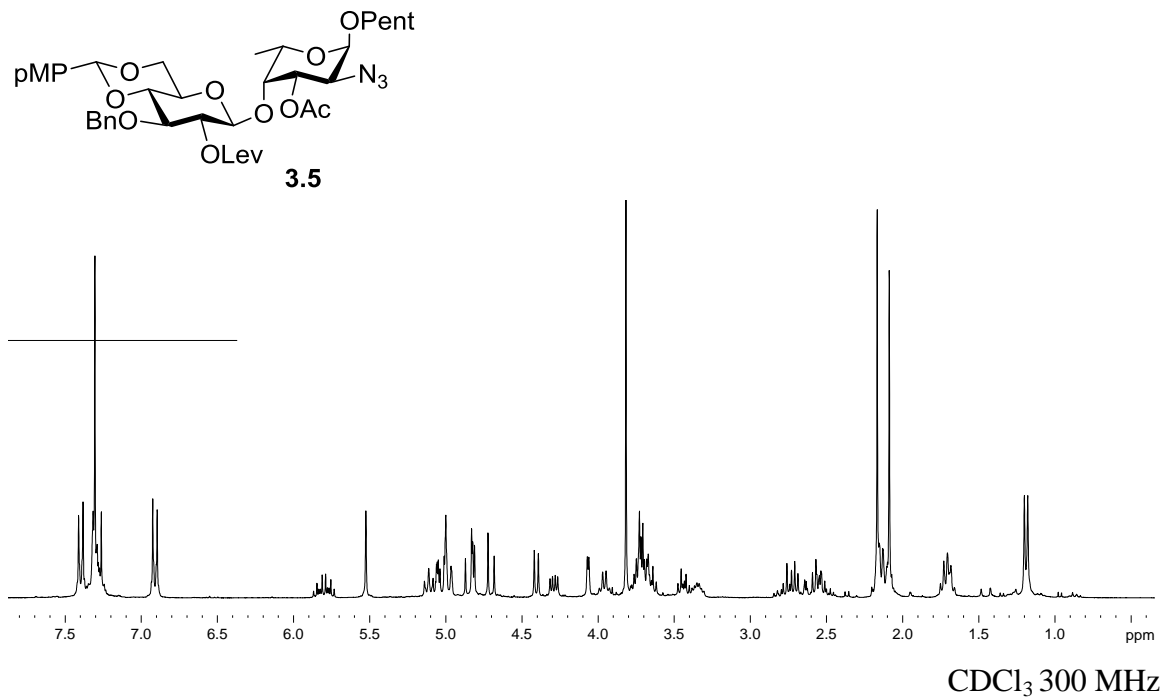


Figure A-34: ¹H NMR spectrum of 4-Pentenyl *O*-(3-*O*-benzyl-2-*O*-levulinoyl-4,6-*O*-*p*-methoxybenzylidene- β -D-glucopyranosyl)-(1 \rightarrow 4)-3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (**3.5**)

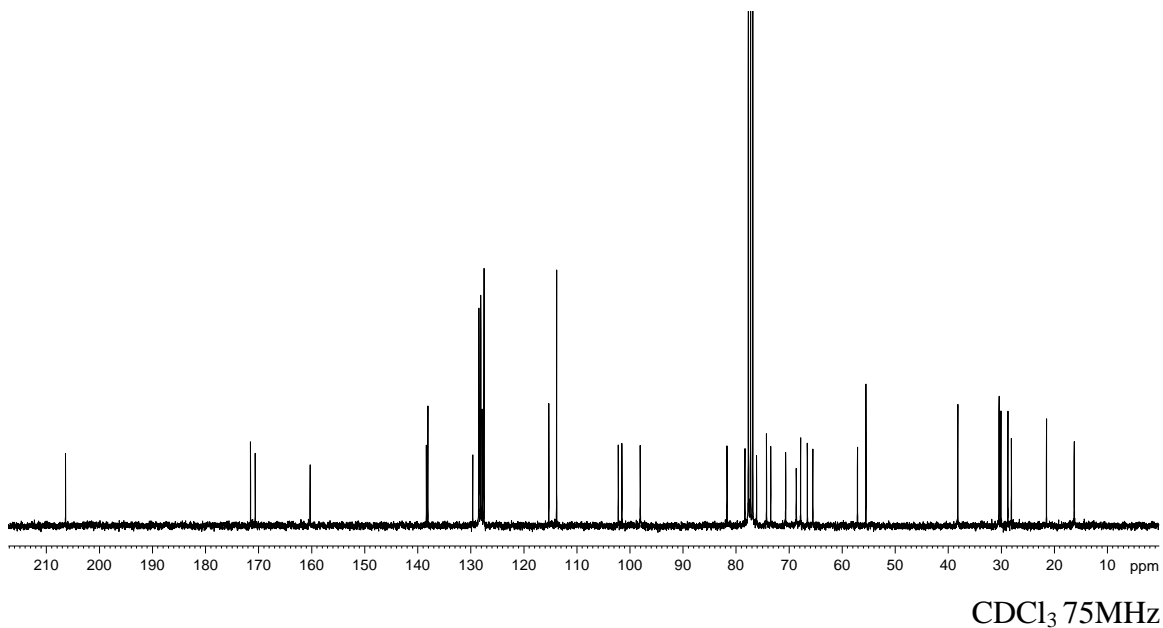


Figure A-35: ¹³C NMR spectrum of 4-Pentenyl *O*-(3-*O*-benzyl-2-*O*-levulinoyl-4,6-*O*-*p*-methoxybenzylidene- β -D-glucopyranosyl)-(1 \rightarrow 4)-3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (**3.5**)

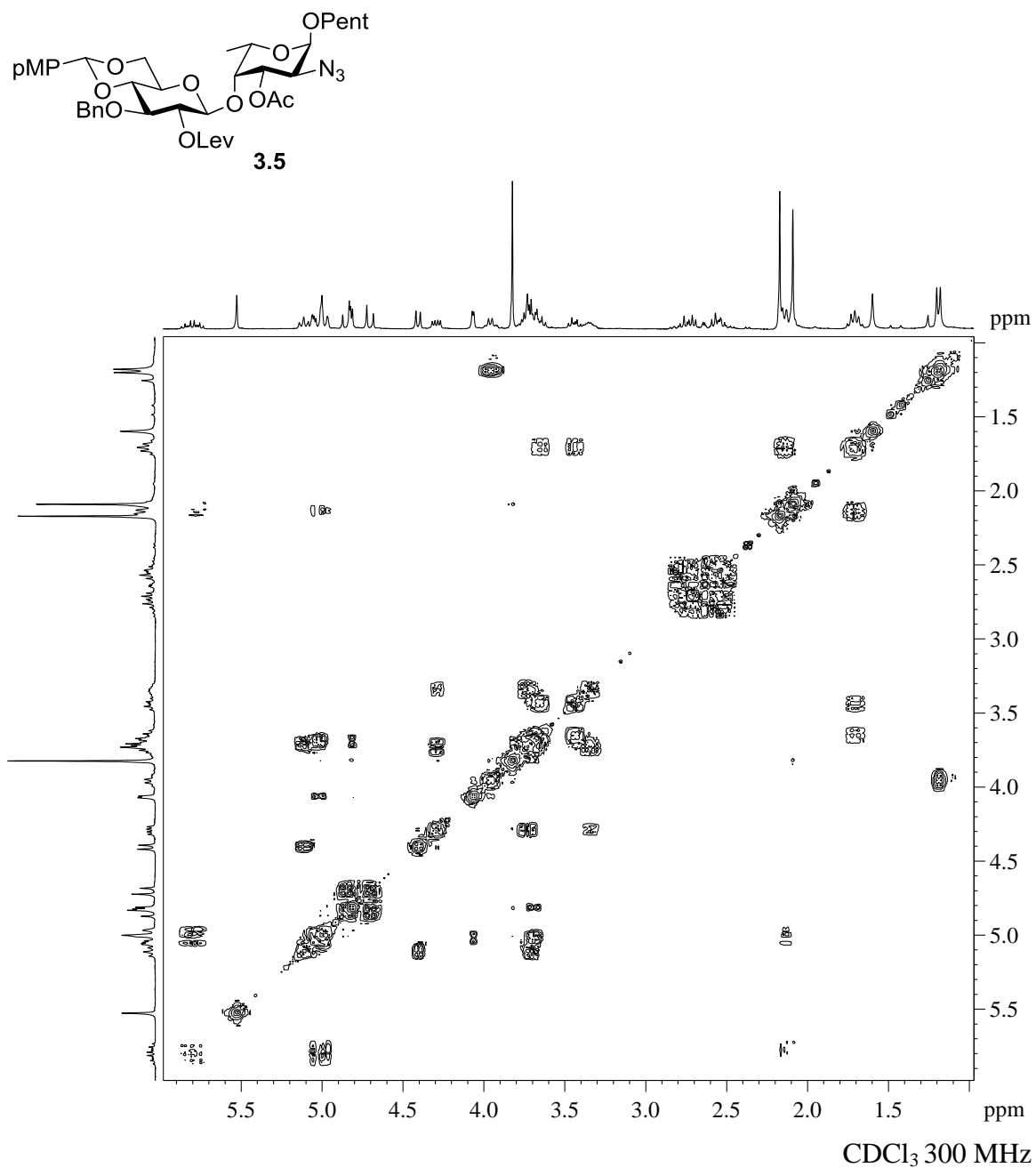


Figure A-36: 2-D NMR COSY spectrum of 4-Pentenyl *O*-(3-*O*-benzyl-2-*O*-levulinoyl-4,6-*O*-*p*-methoxybenzylidene- β -D-glucopyranosyl)-(1 \rightarrow 4)-3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (**3.5**)

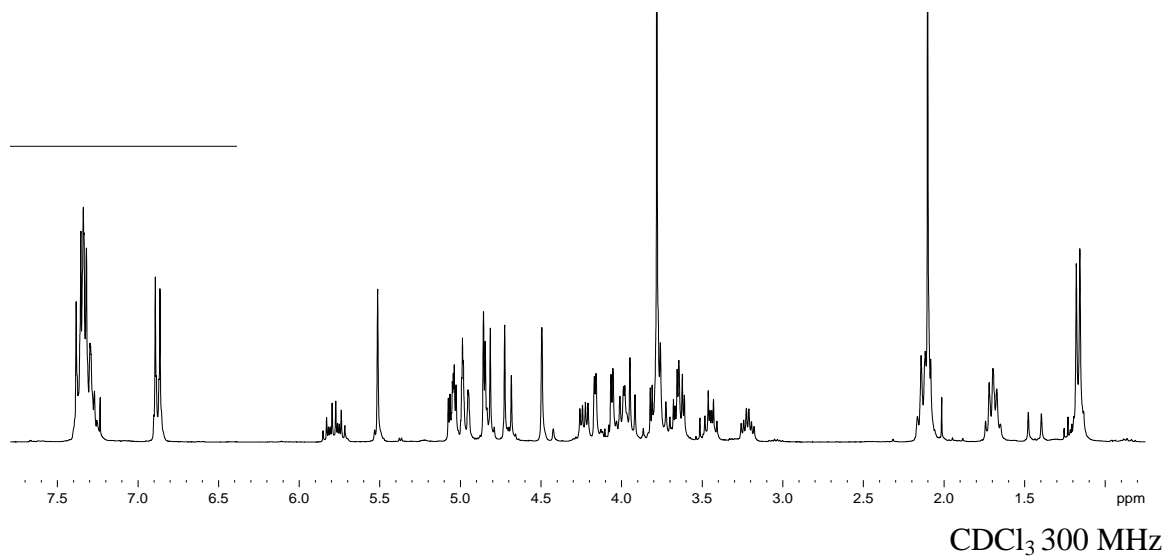
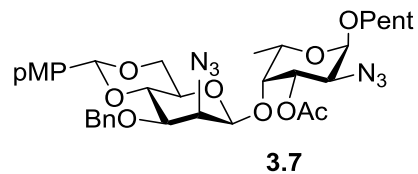


Figure A-37: ¹H NMR spectrum of 4-Pentenyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-β-D-mannopyranosyl)-(1→4)-3-*O*-acetyl-2-azido-2,6-dideoxy-α-L-galactopyranoside (**3.7**)

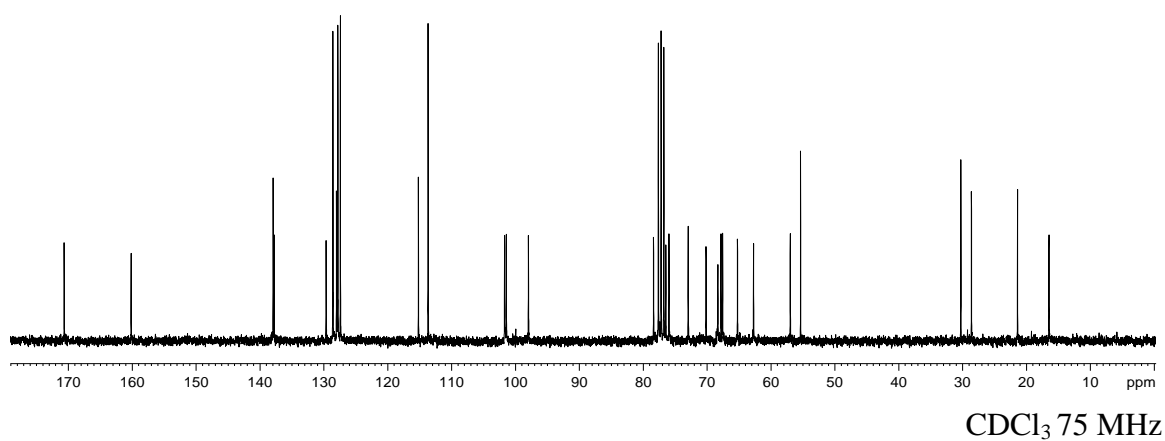


Figure A-38: ¹³C NMR spectrum of 4-Pentenyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-β-D-mannopyranosyl)-(1→4)-3-*O*-acetyl-2-azido-2,6-dideoxy-α-L-galactopyranoside (**3.7**)

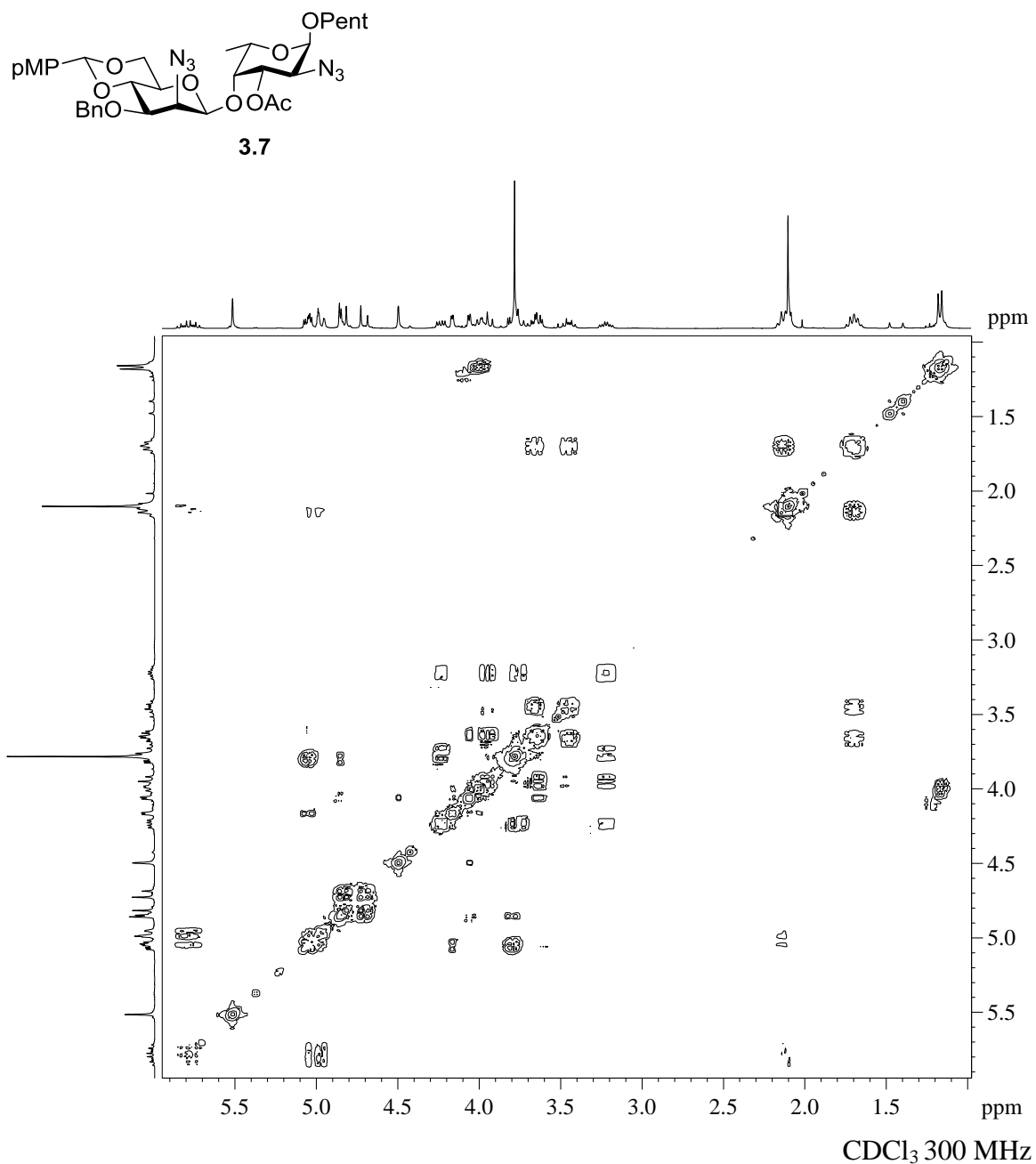


Figure A-39: 2-D NMR COSY spectrum of 4-Pentenyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-*p*-methoxybenzylidene- β -D-mannopyranosyl)-(1 \rightarrow 4)-3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranoside (**3.7**)

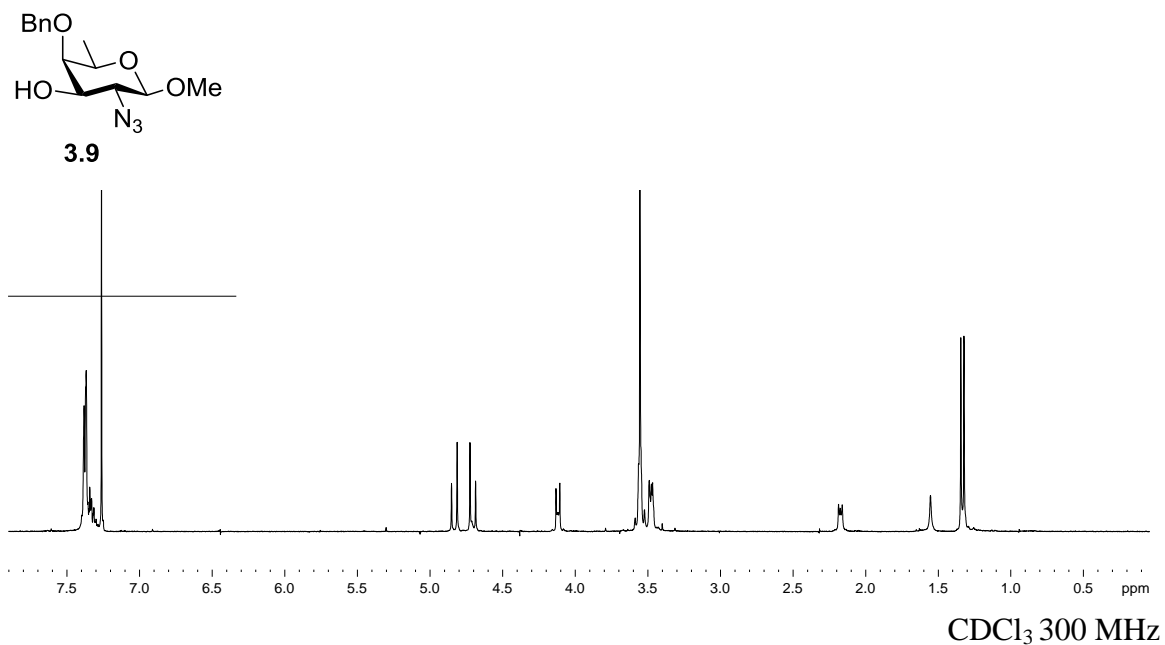


Figure A-40: ¹H NMR spectrum of Methyl 2-azido-4-*O*-benzyl-2,6-dideoxy-β-D-galactopyranoside (**3.9**)

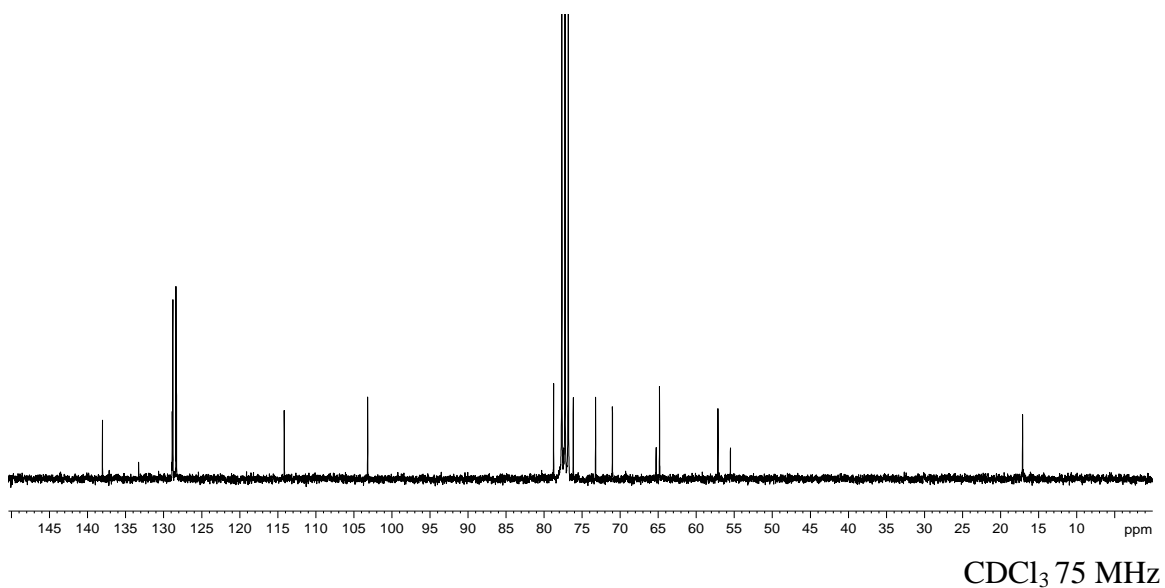


Figure A-41: ¹³C NMR spectrum of Methyl 2-azido-4-*O*-benzyl-2,6-dideoxy-β-D-galactopyranoside (**3.9**)

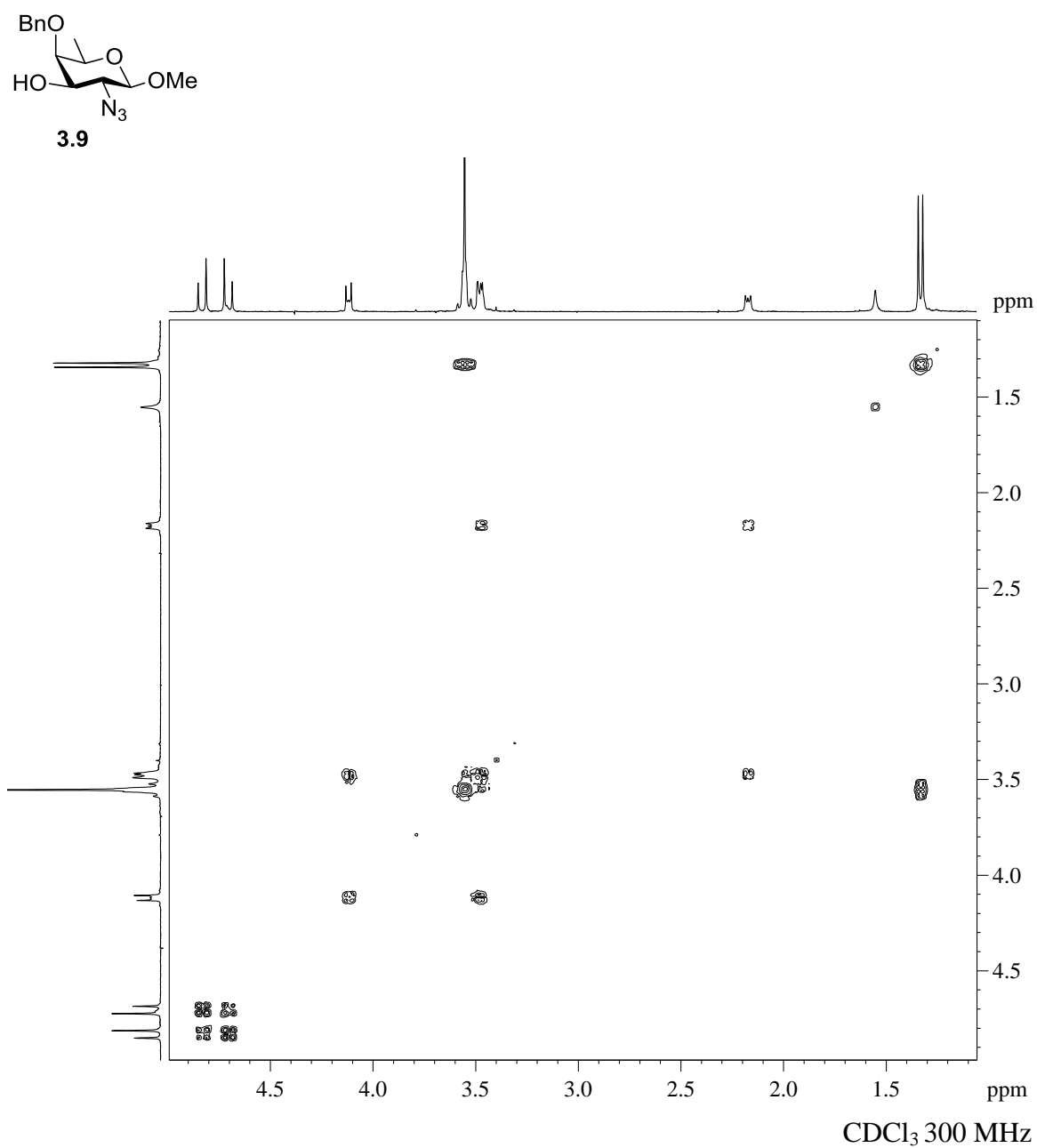


Figure A-42: 2-D NMR COSY spectrum of Methyl 2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.9**)

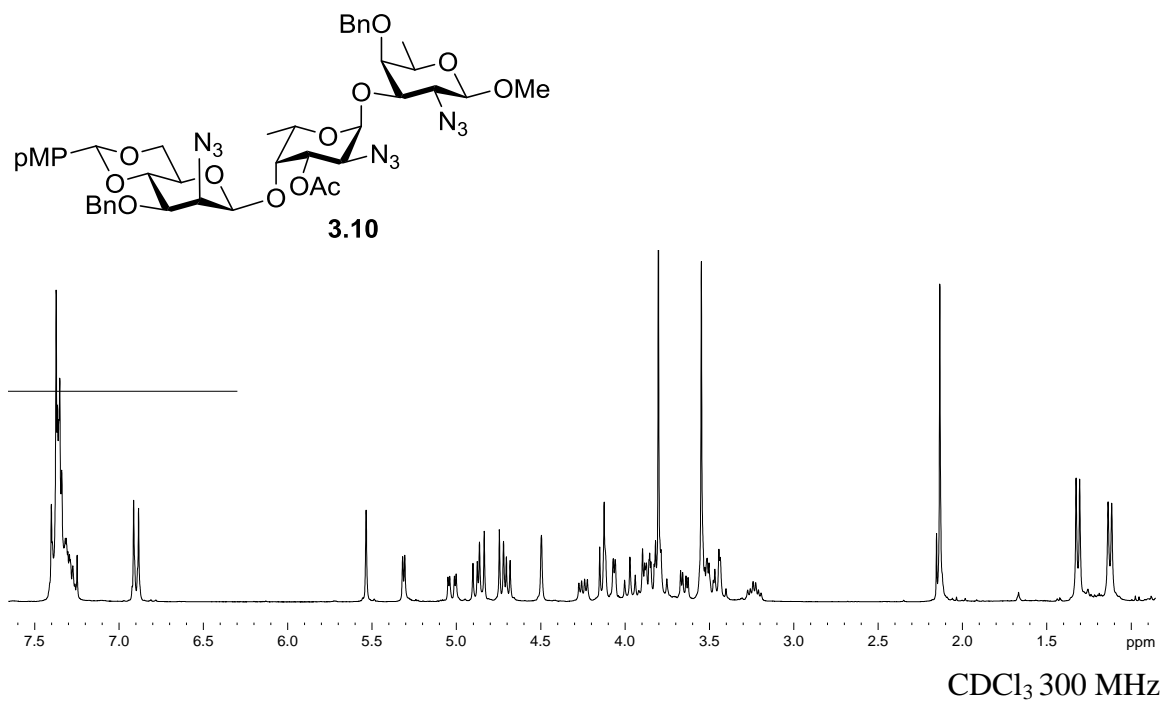


Figure A-43: ¹H NMR spectrum of Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-*p*-methoxybenzylidene- β -D-mannopyranosyl)-(1→4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.10**)

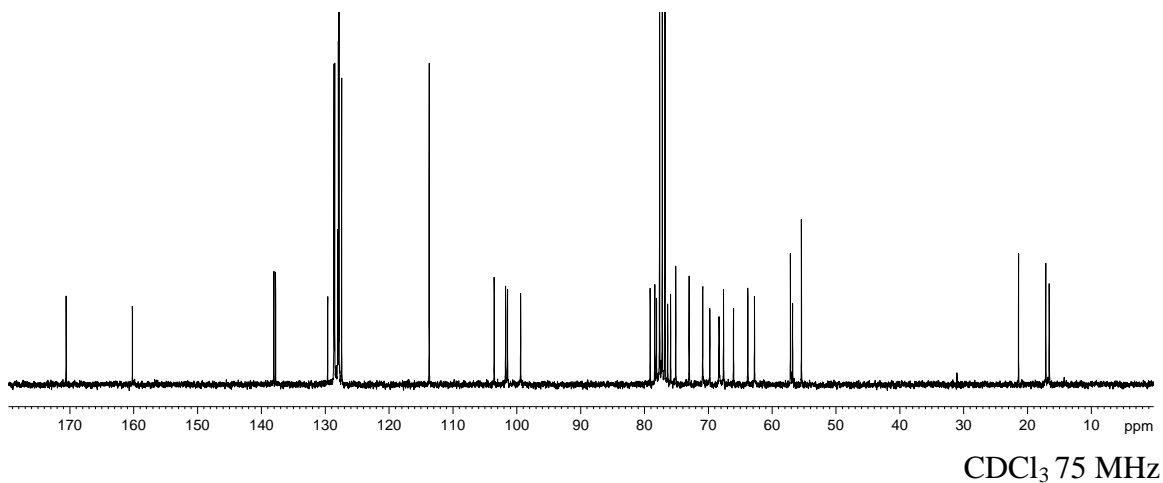


Figure A-44: ¹³C NMR spectrum of Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-*p*-methoxybenzylidene- β -D-mannopyranosyl)-(1→4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.10**)

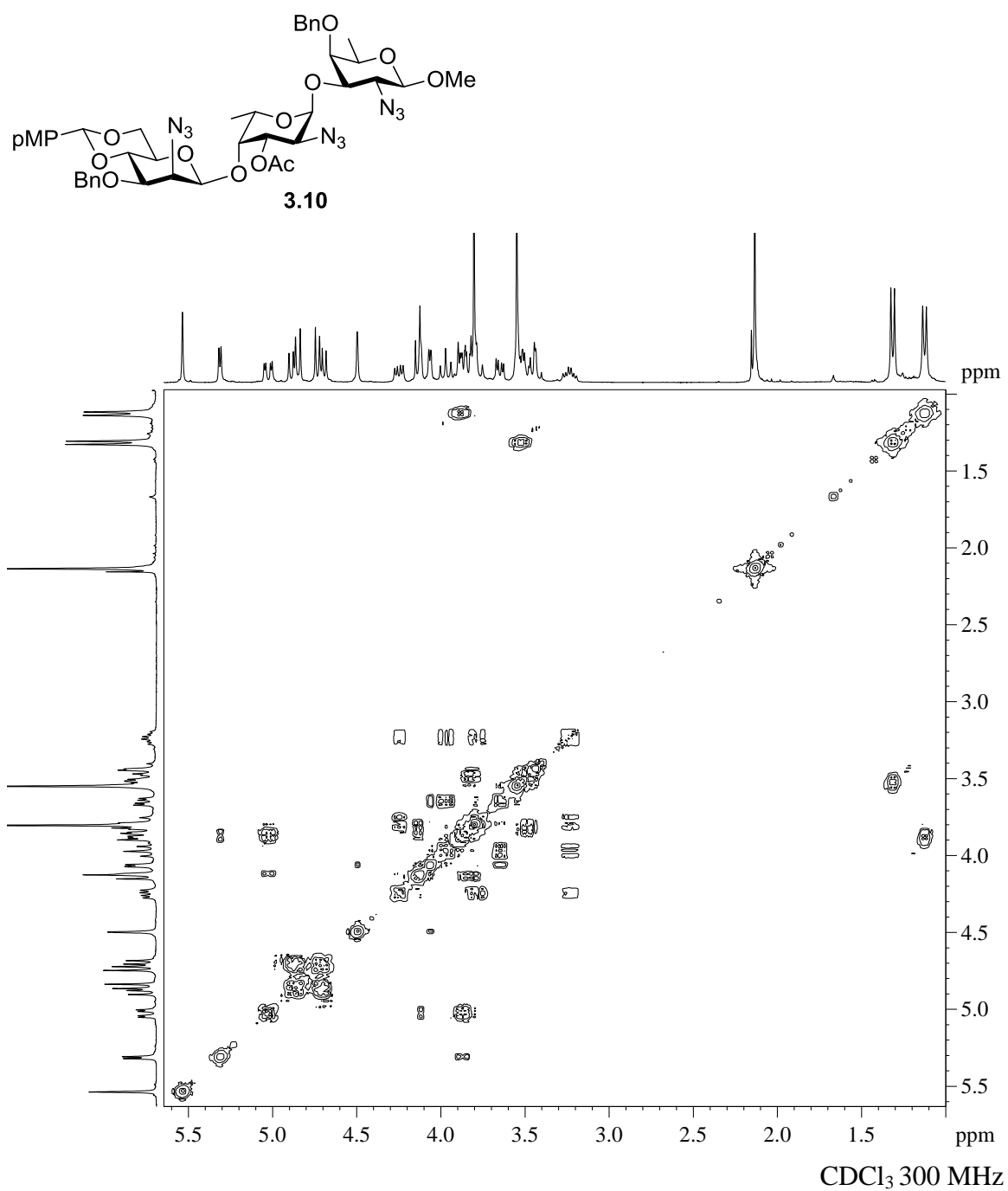


Figure A-45: 2-D NMR COSY spectrum of Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-*p*-methoxybenzylidene- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.10**)

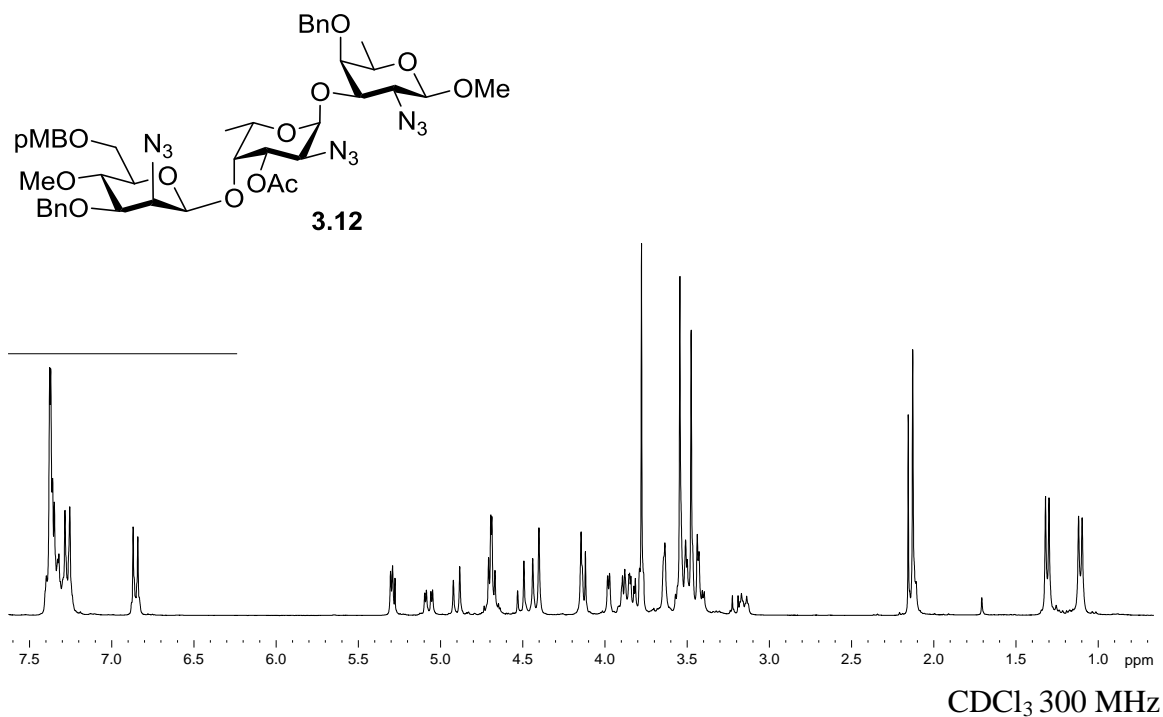


Figure A-46: ¹H NMR spectrum of Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-4-*O*-methyl-β-D-mannopyranosyl)-(1→4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-β-D-galactopyranoside (**3.12**)

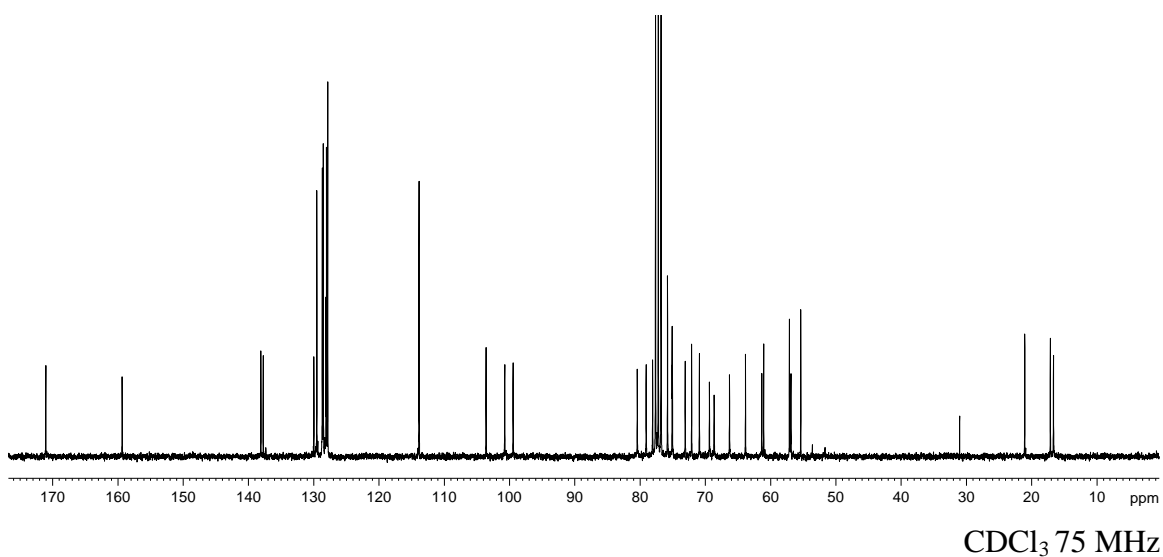


Figure A-47: ¹³C NMR spectrum of Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-4-*O*-methyl-β-D-mannopyranosyl)-(1→4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-β-D-galactopyranoside (**3.12**)

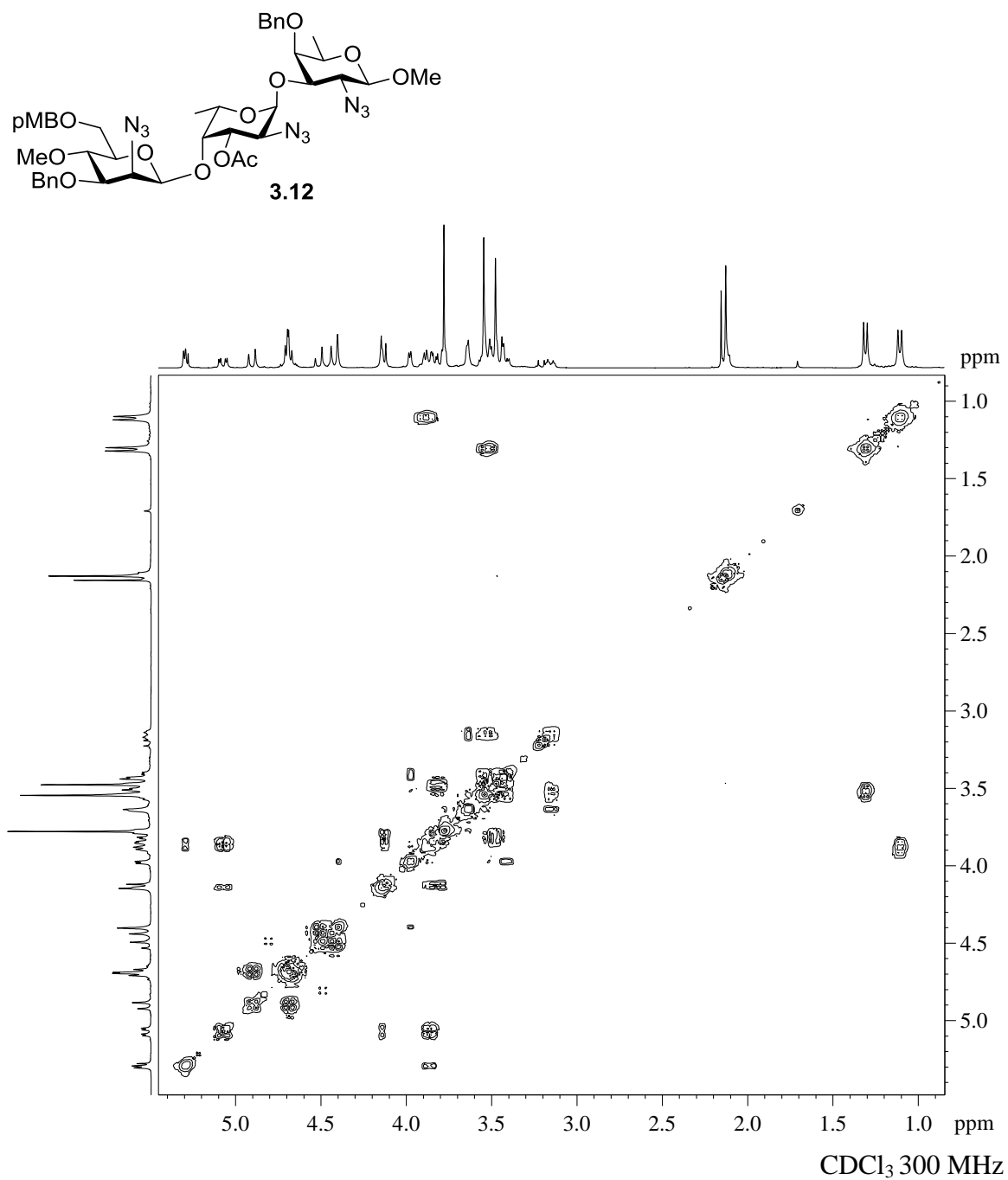


Figure A-48: 2-D NMR COSY spectrum of Methyl *O*-(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-4-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(3-*O*-acetyl-2-azido-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.12**)

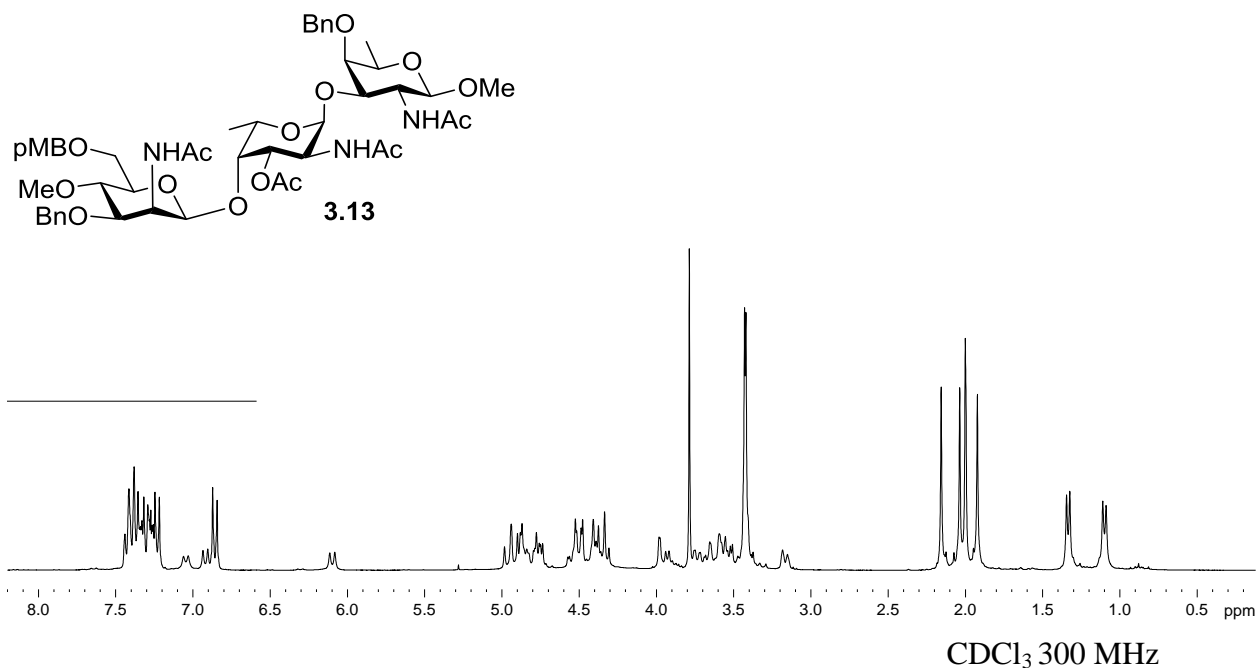


Figure A-49: ^1H NMR spectrum of Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-4-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.13**)

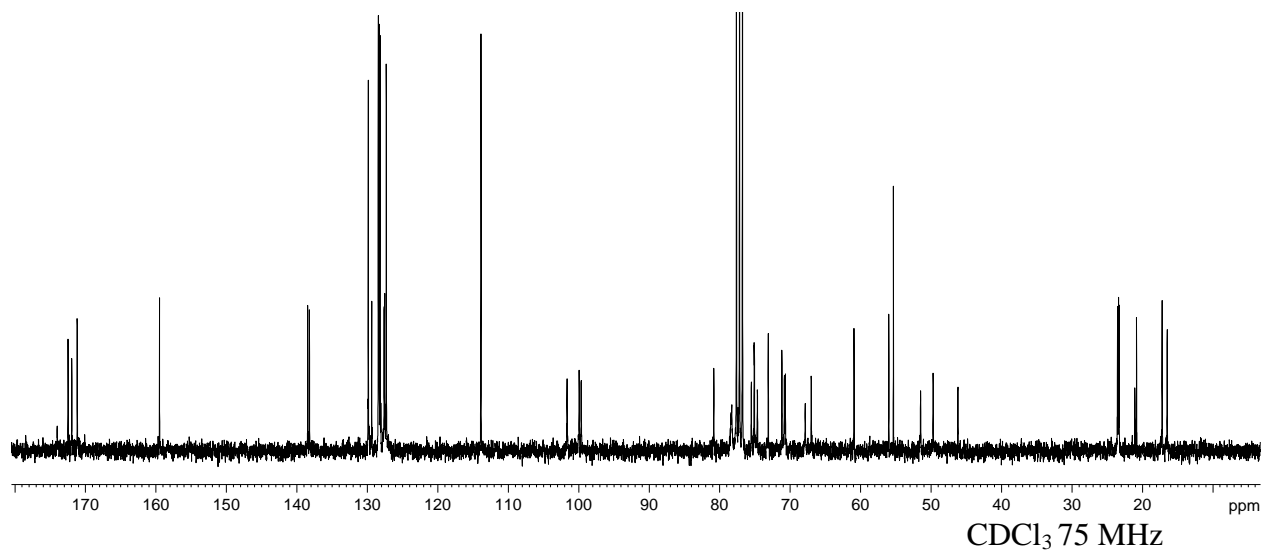


Figure A-50: ^{13}C NMR spectrum of Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-4-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.13**)

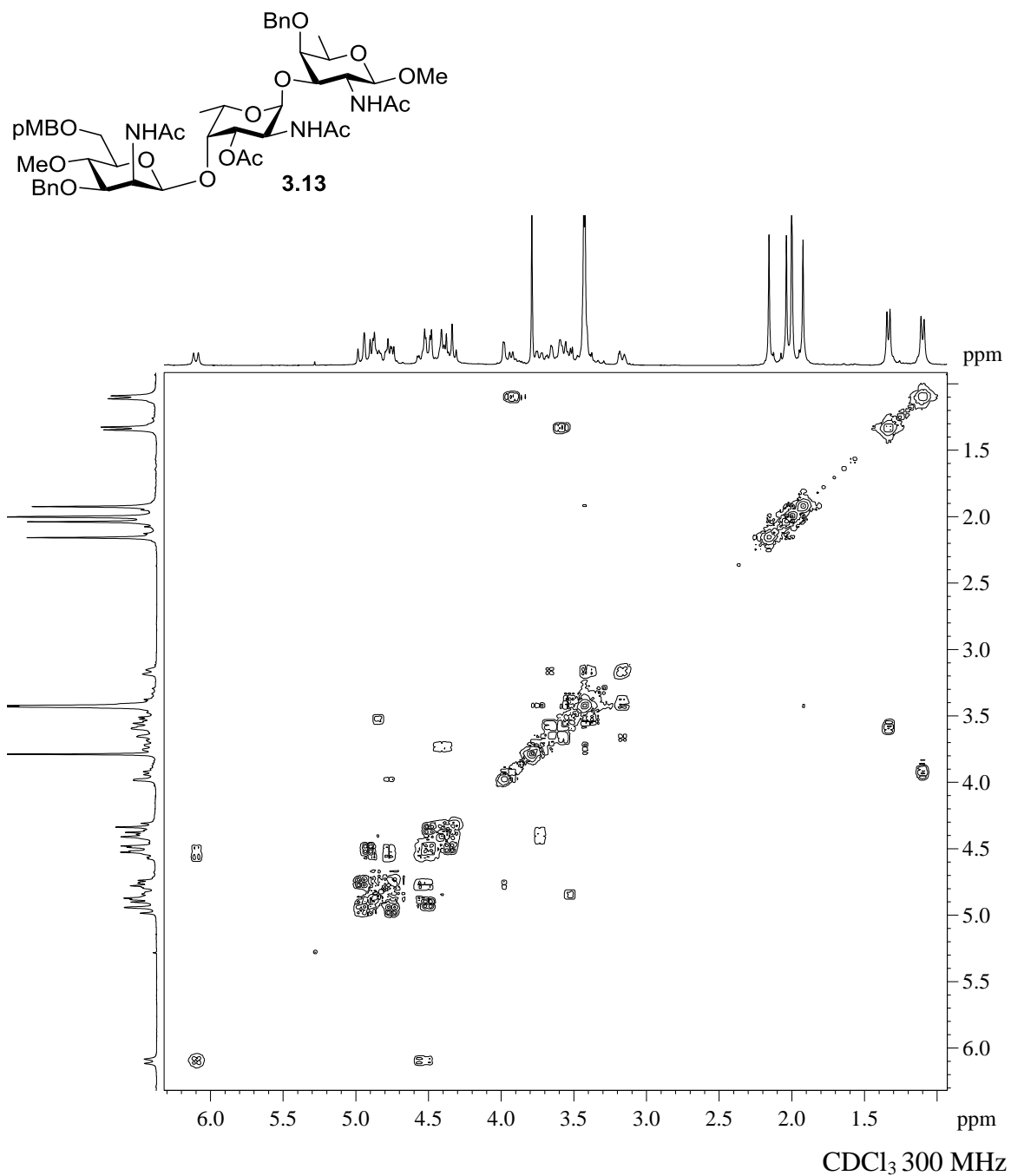


Figure A-51: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-4-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**3.13**)

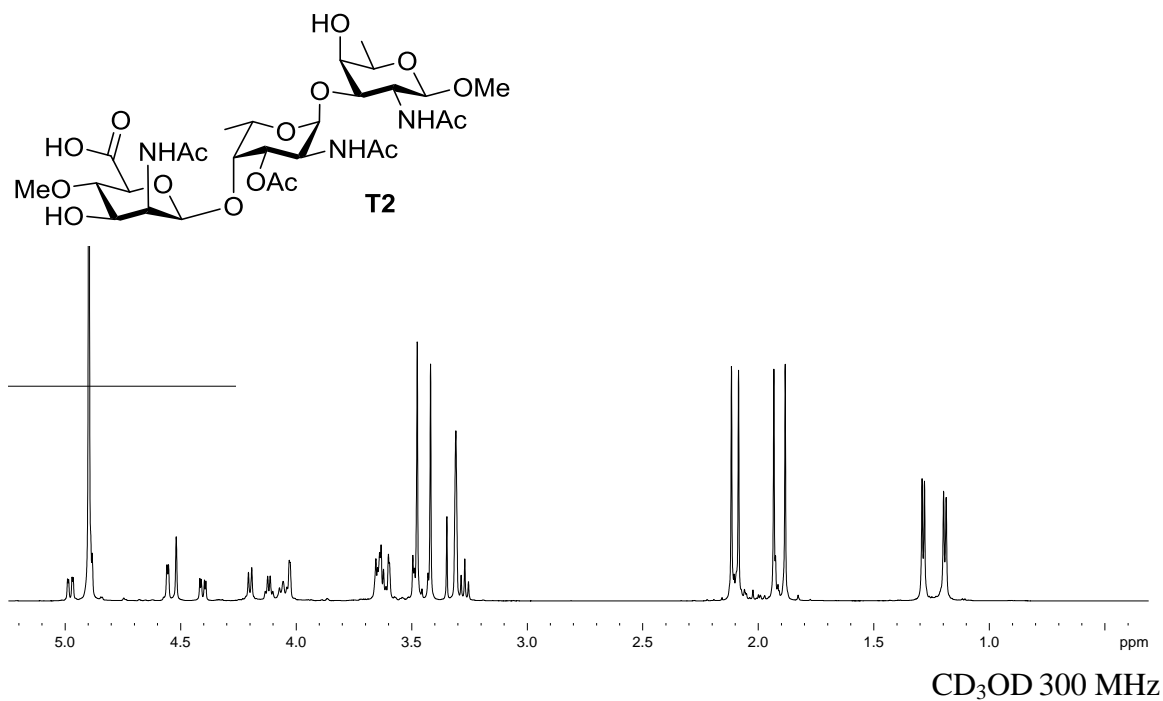


Figure A-52: ¹H NMR spectrum of Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-mannopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-β-D-galactopyranoside (**T2**)

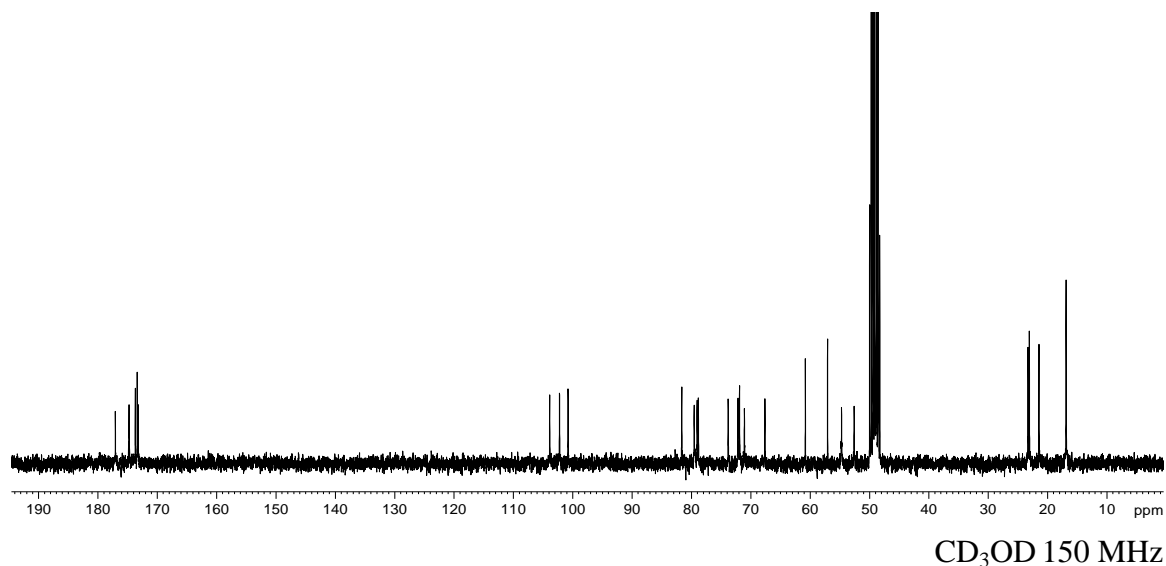


Figure A-53: ¹³C NMR spectrum of Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-mannopyranosyluronic acid)-(1→4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-β-D-galactopyranoside (**T2**)

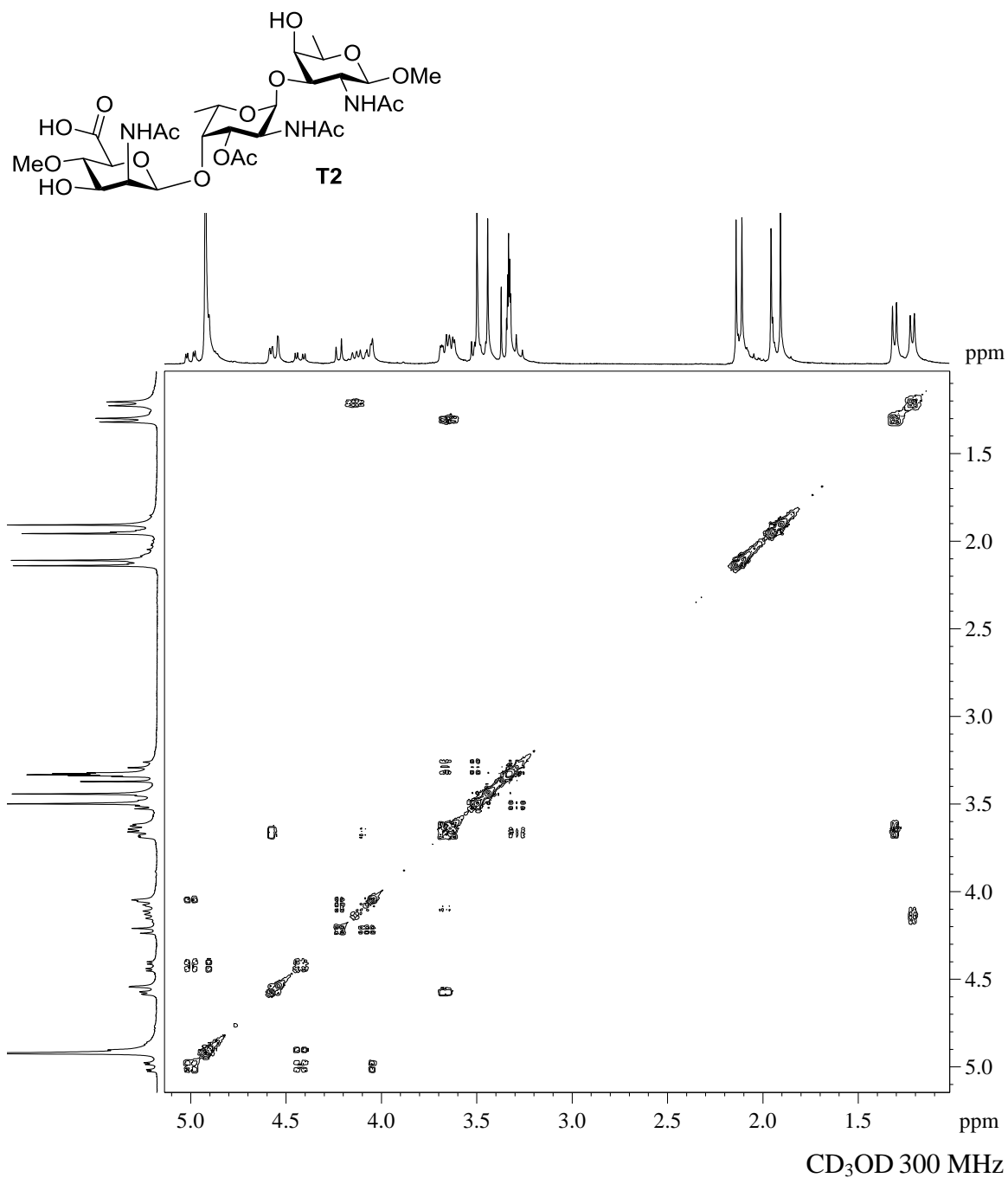


Figure A-54: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-methyl- β -D-mannopyranosyluronic acid)-(1 \rightarrow 4)-*O*-(2-acetamido-3-*O*-acetyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**T2**)

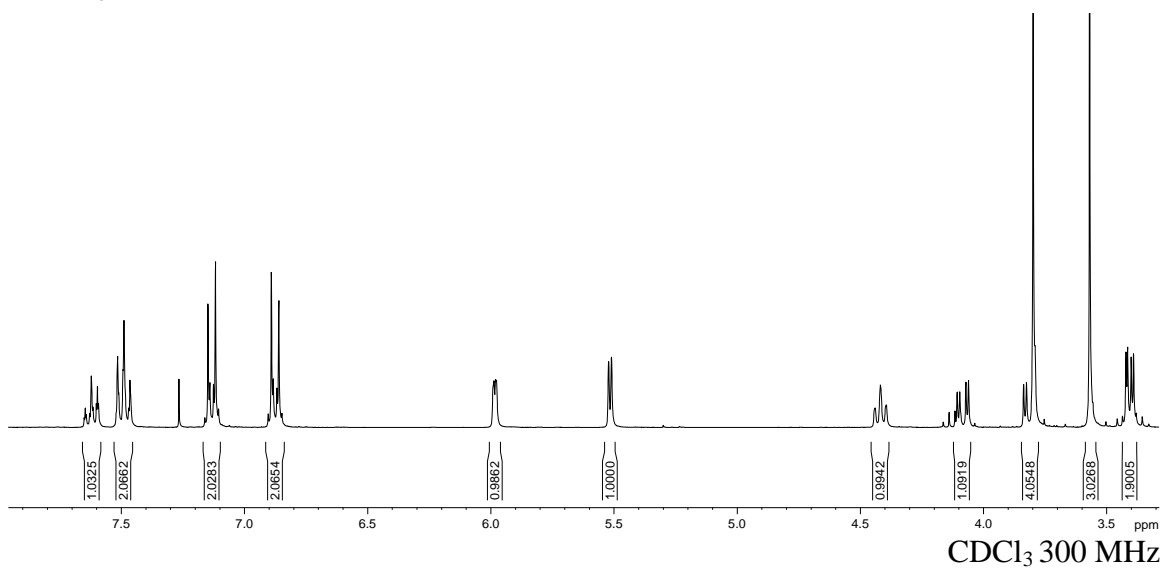
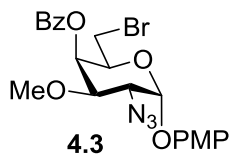


Figure A-55: ¹H NMR spectrum of 4-Methoxyphenyl 2-azido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranoside (**4.3**)

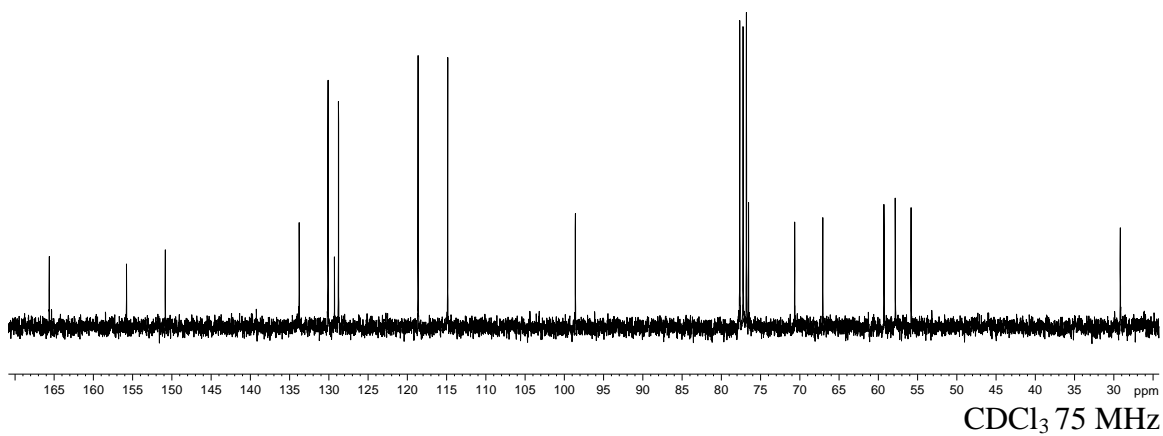


Figure A-56: ¹³C NMR spectrum of 4-Methoxyphenyl 2-azido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranoside (**4.3**)

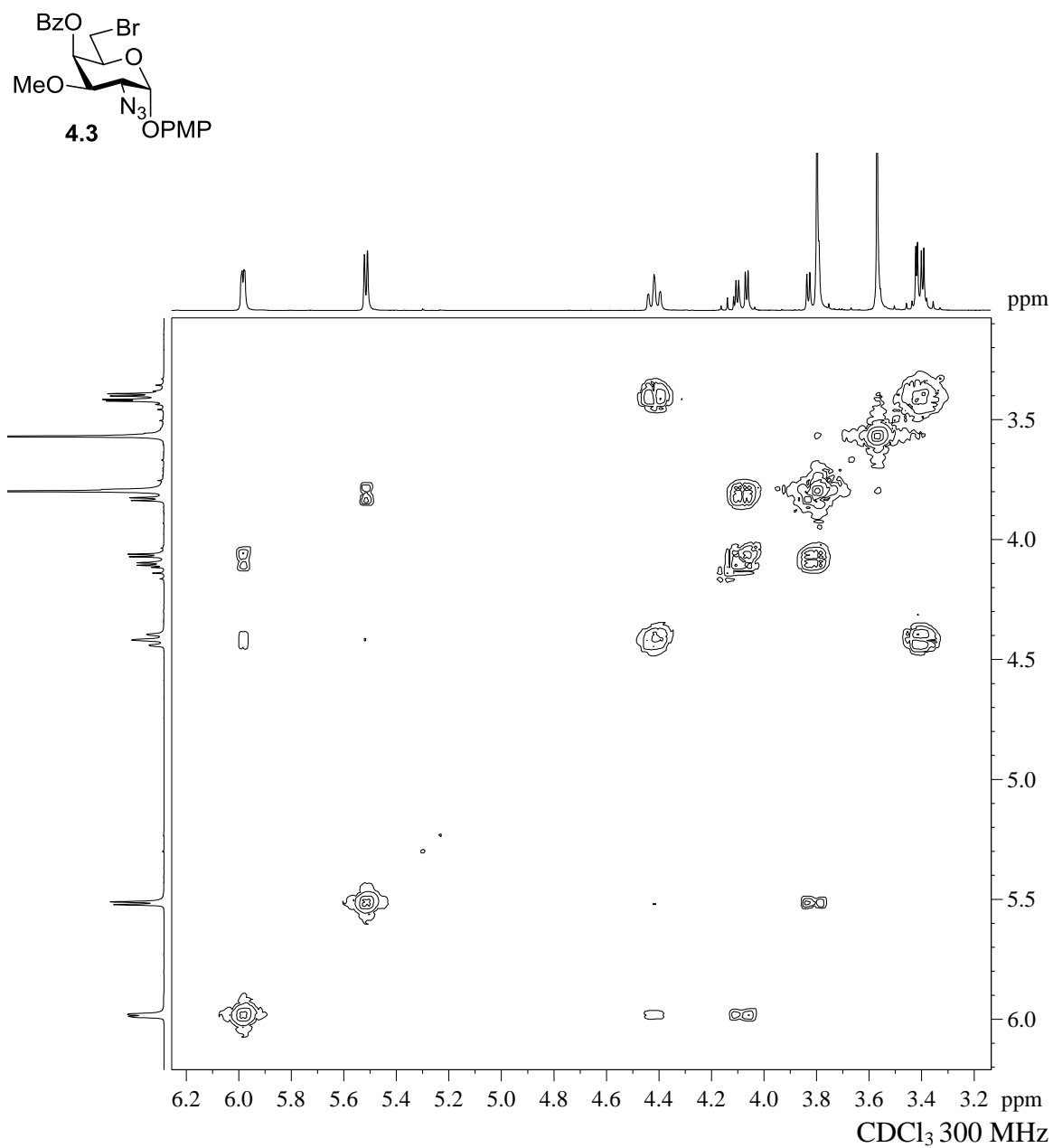


Figure A-57: 2-D NMR COSY spectrum of 4-Methoxyphenyl 2-azido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranoside (**4.3**)

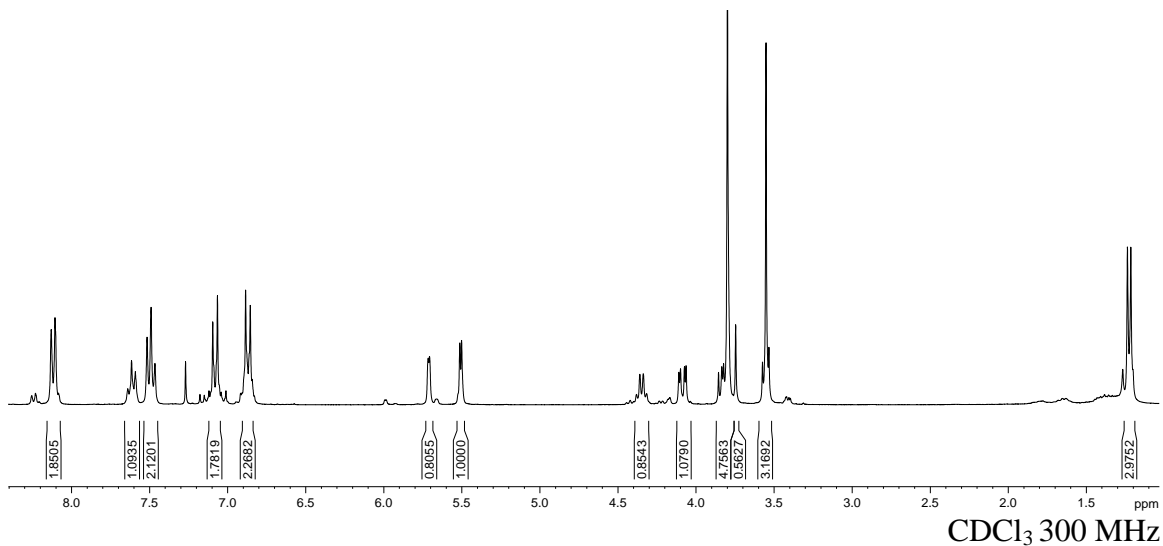
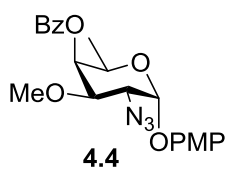


Figure A-58: ¹H NMR spectrum of 4-Methoxyphenyl 2-azido-4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranoside (**4.4**)

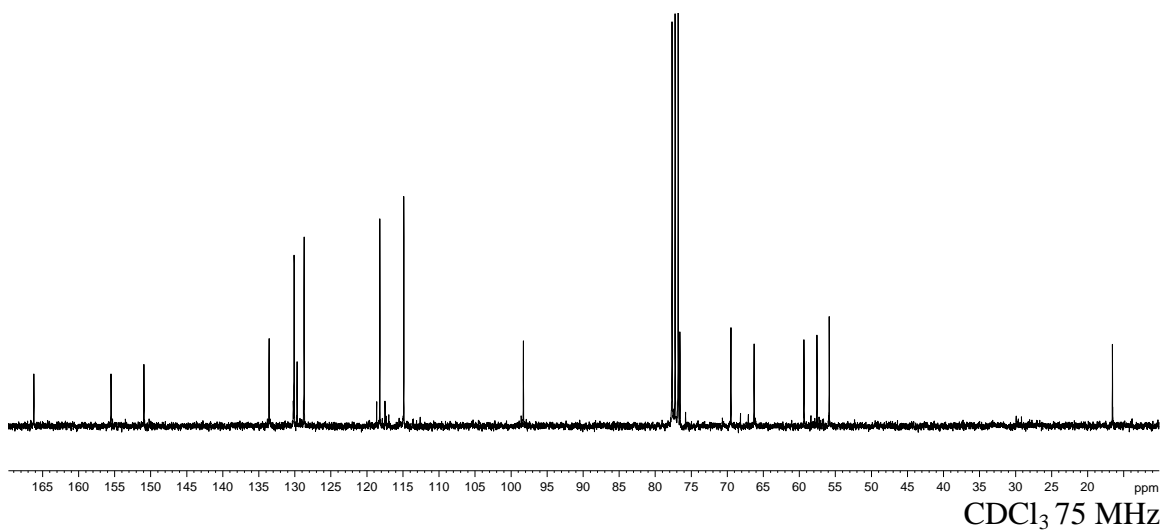


Figure A-59: ¹³C NMR spectrum of 4-Methoxyphenyl 2-azido-4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranoside (**4.4**)

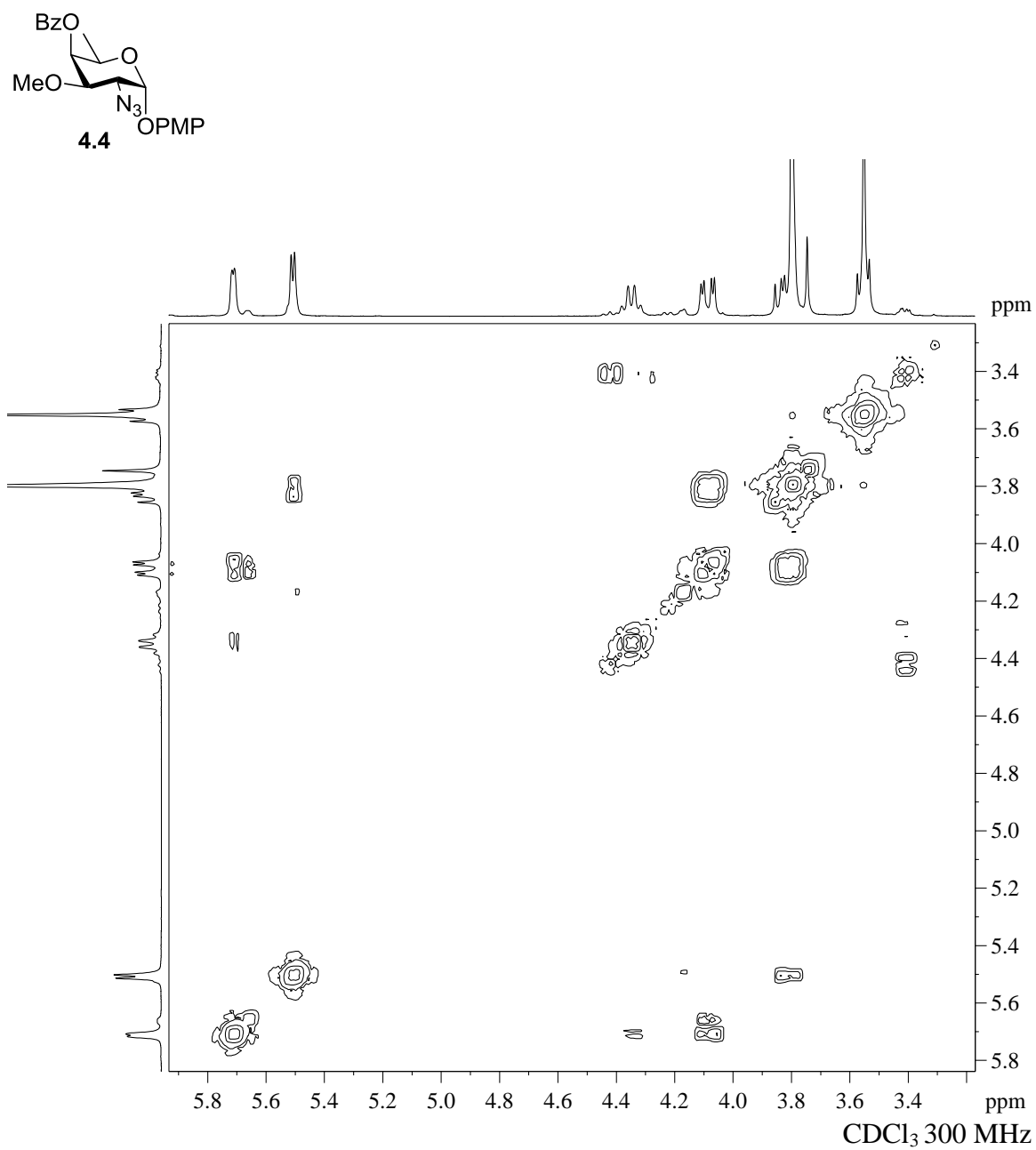


Figure A-60: 2-D NMR COSY spectrum of 4-Methoxyphenyl 2-azido-4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranoside (**4.4**)

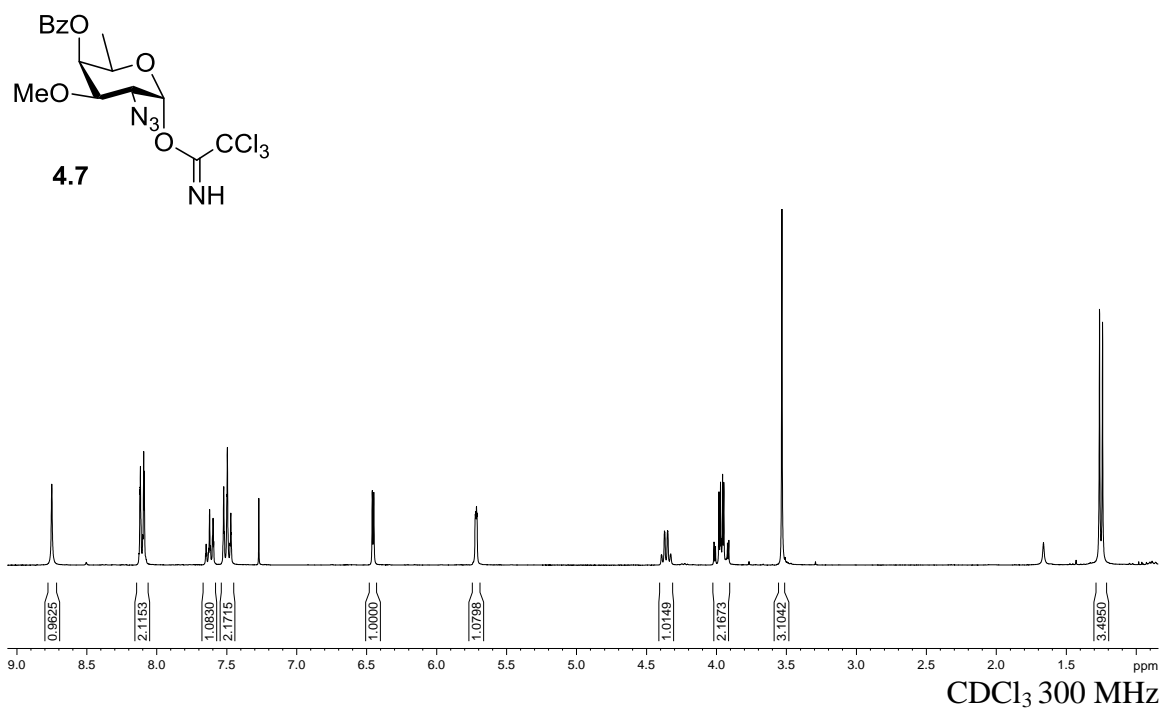


Figure A-61: ^1H NMR spectrum of 2-Azido-4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl trichloroacetimidate (4.7)

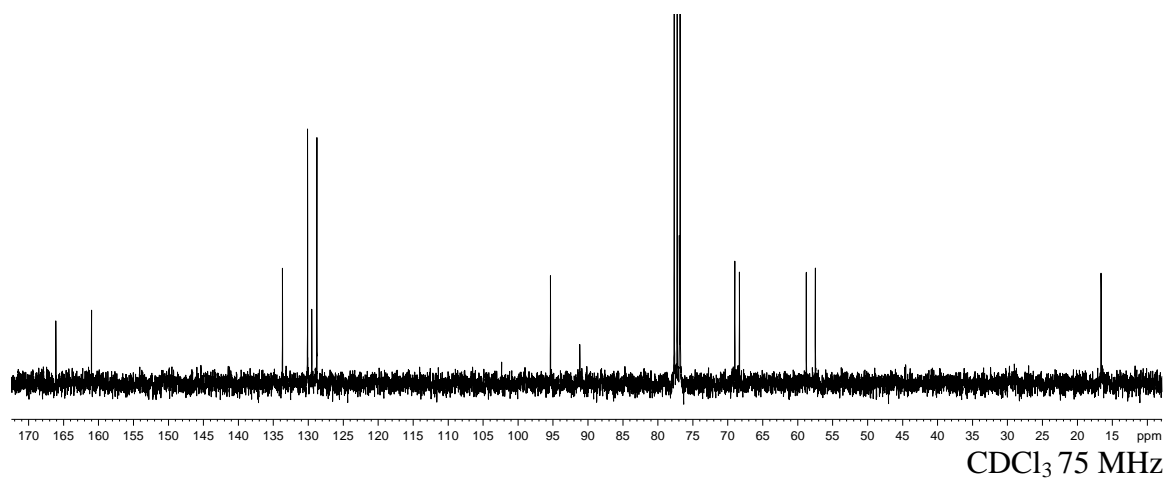


Figure A-62: ^{13}C NMR spectrum of 2-Azido-4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl trichloroacetimidate (4.7)

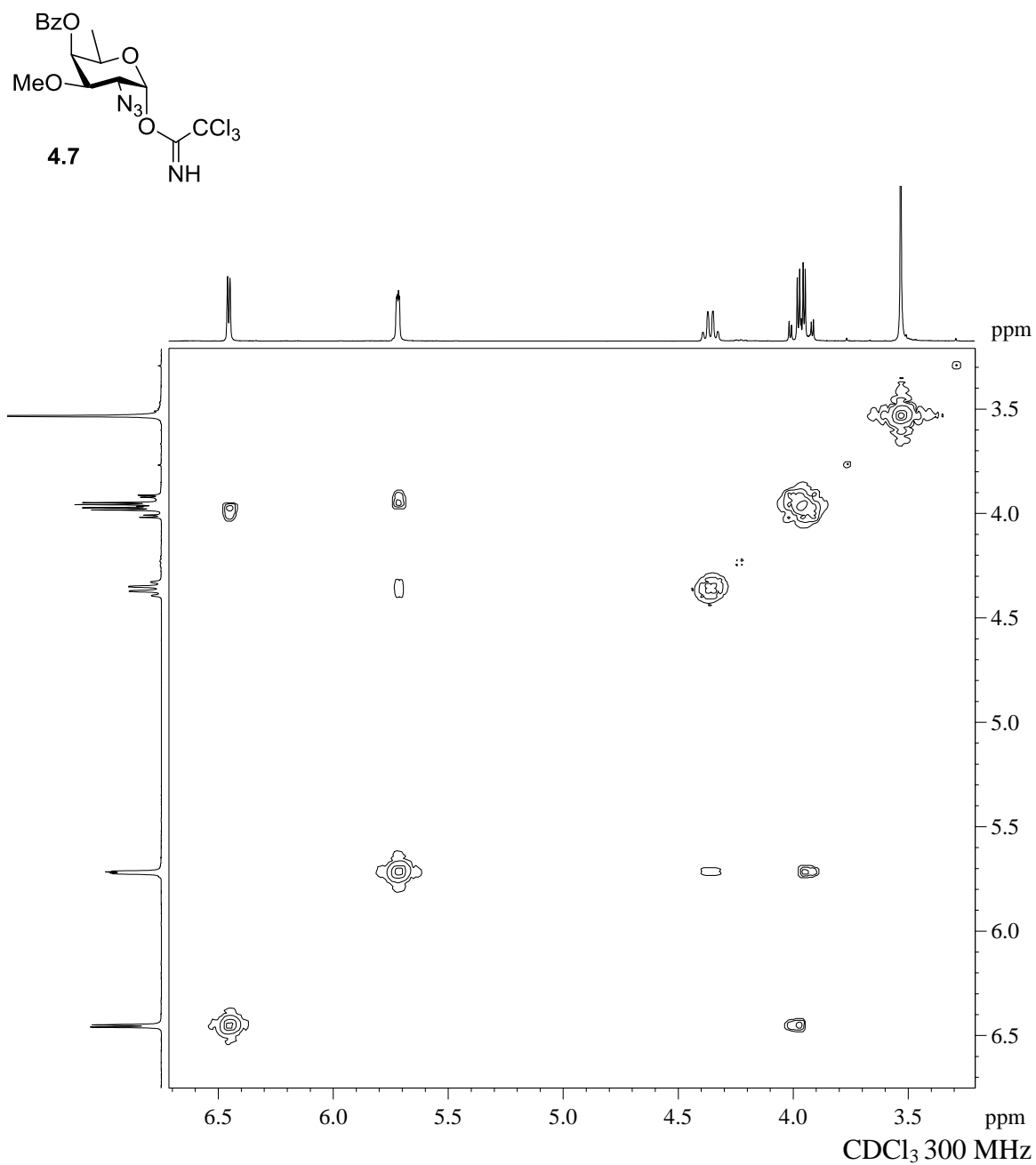


Figure A-63: 2-D NMR COSY spectrum of 2-Azido-4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl trichloroacetimidate (**4.7**)

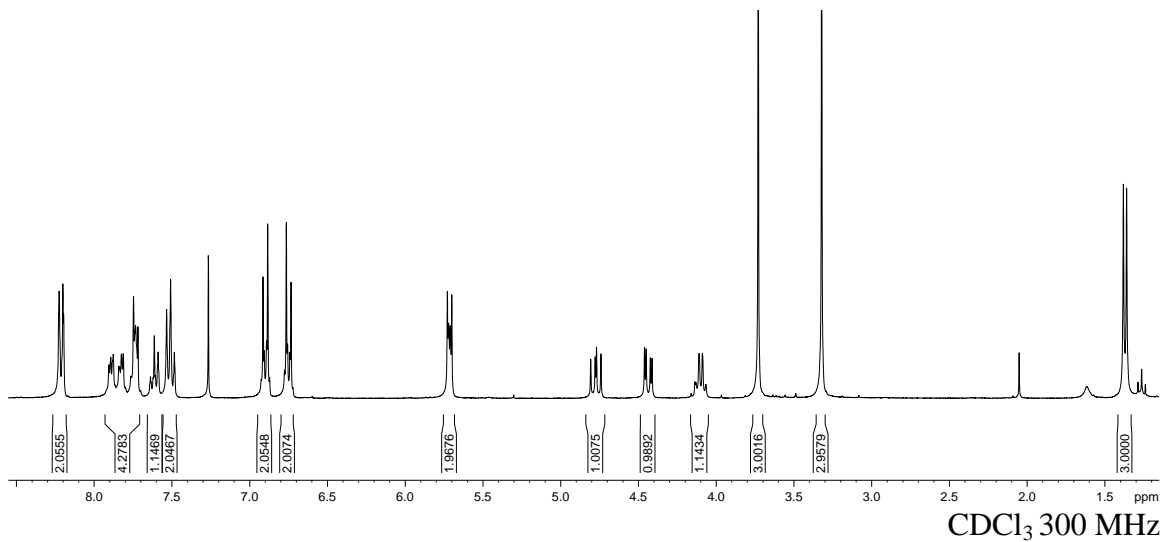
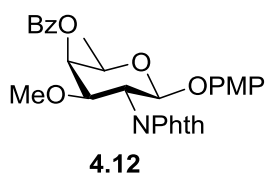


Figure A-64: ¹H NMR spectrum of 4-Methoxyphenyl 4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl-2-phthalimido-β-D-galactopyranoside (**4.12**)

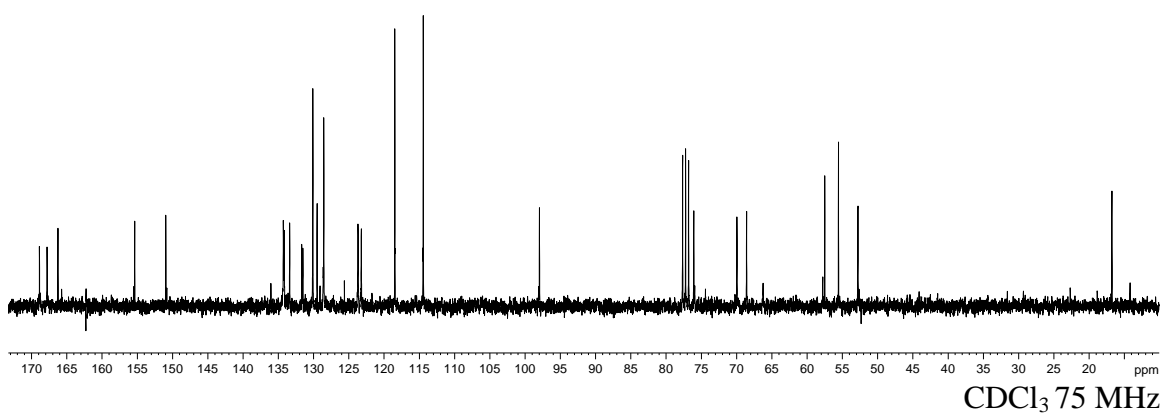


Figure A-65: ¹³C NMR spectrum of 4-Methoxyphenyl 4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl-2-phthalimido-β-D-galactopyranoside (**4.12**)

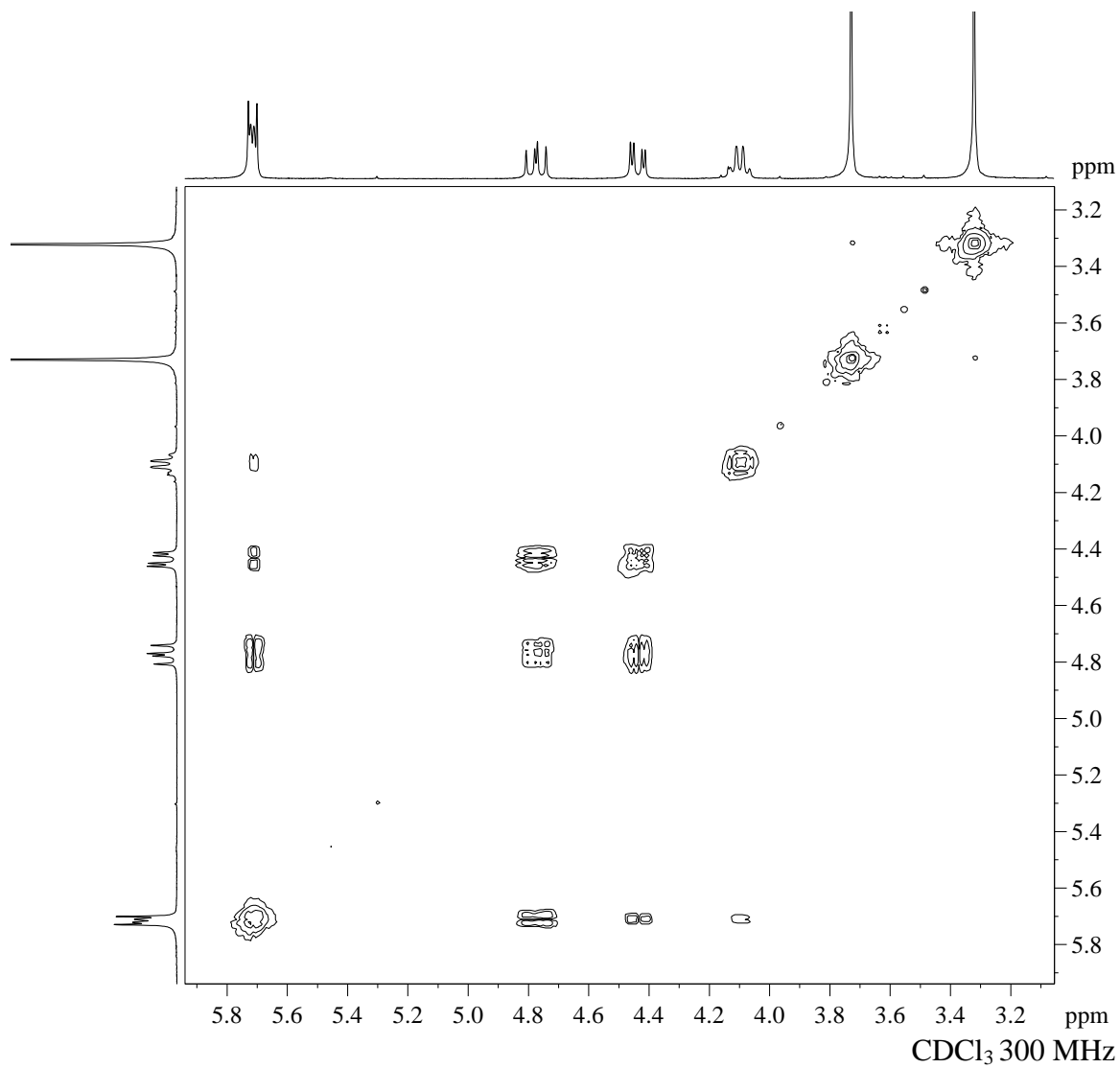
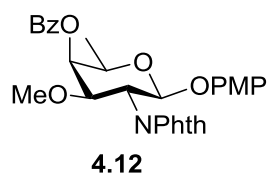


Figure A-66: 2-D NMR COSY spectrum of 4-Methoxyphenyl 4-*O*-benzoyl-2,6-dideoxy-3-*O*-methyl-2-phthalimido- β -D-galactopyranoside (**4.12**)

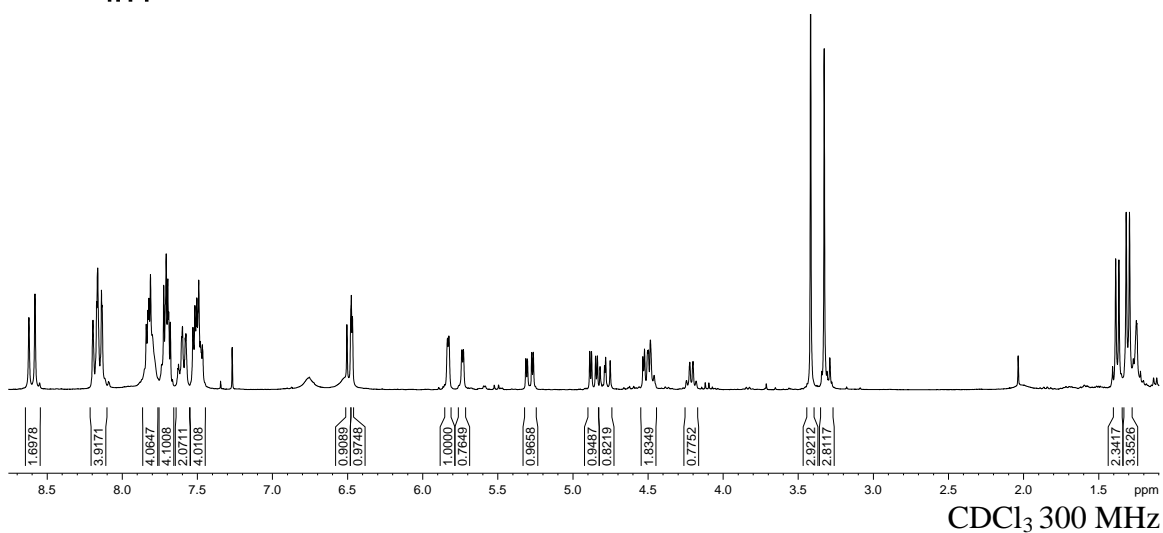
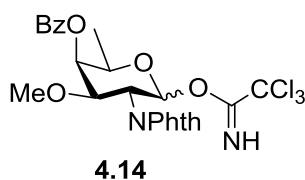


Figure A-67: ¹H NMR spectrum of 4-*O*-Benzoyl-2,6-dideoxy-3-*O*-methyl-2-phthalimido-*D*-galactopyranosyl trichloroacetimidate (**4.14**)

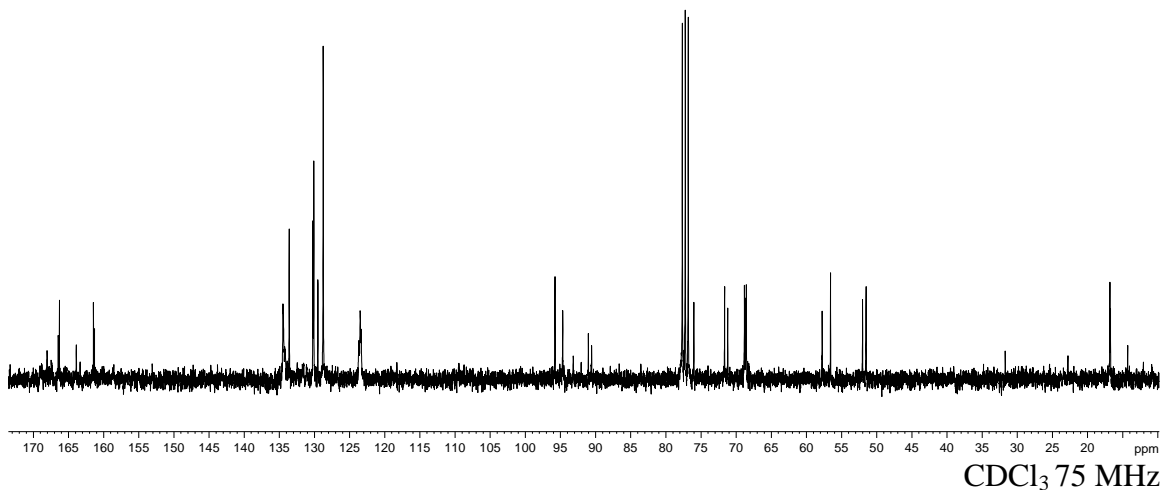


Figure A-68: ¹³C NMR spectrum of 4-*O*-Benzoyl-2,6-dideoxy-3-*O*-methyl-2-phthalimido-*D*-galactopyranosyl trichloroacetimidate (**4.14**)

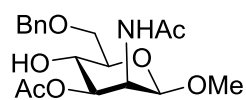
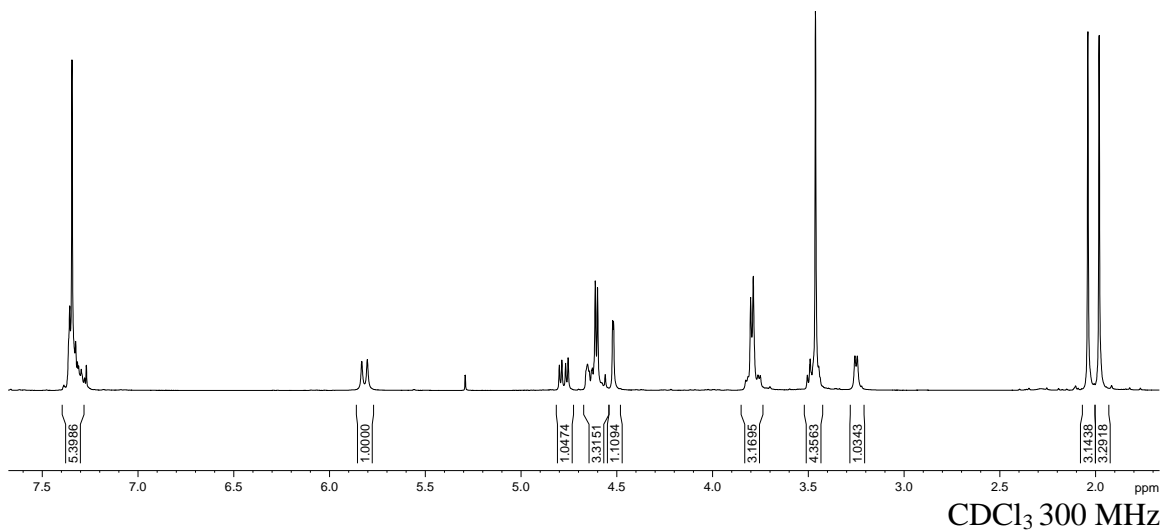
**4.18**

Figure A-70: ¹H NMR spectrum of Methyl 2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-mannopyranoside (**4.18**)

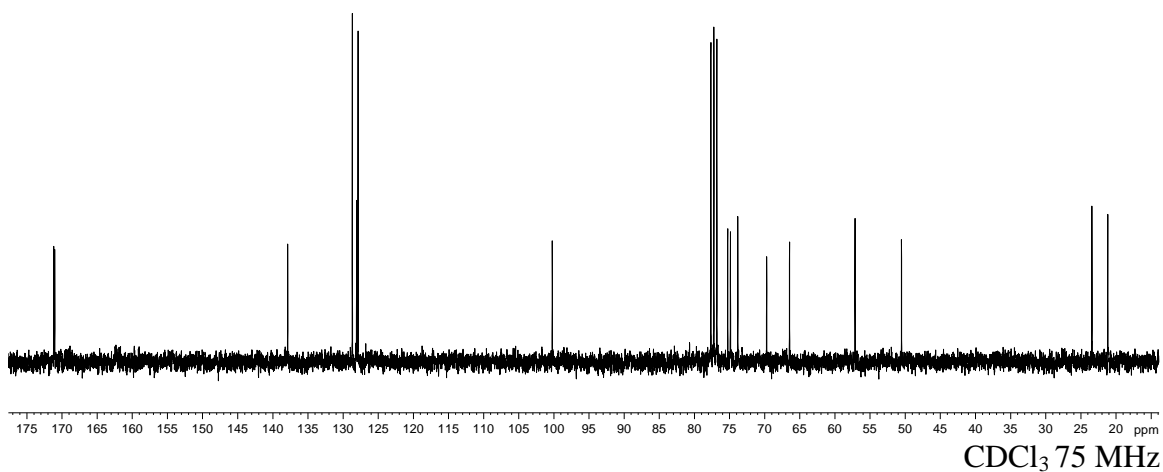
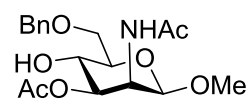
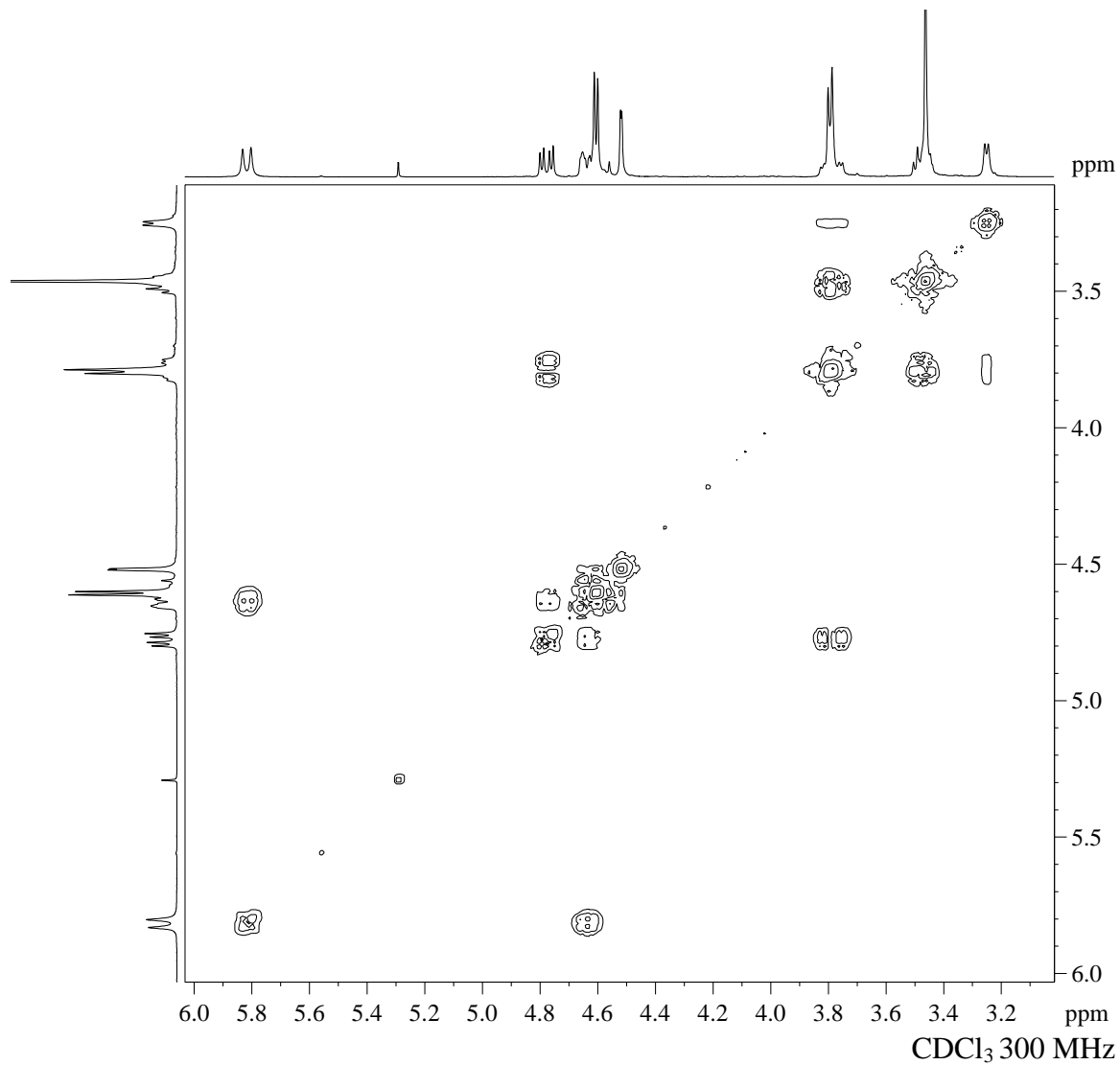


Figure A-71: ¹³C NMR spectrum of Methyl 2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-D-mannopyranoside (**4.18**)

**4.18****Figure A-72:** 2-D NMR COSY spectrum of Methyl 2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy- β -D-mannopyranoside (**4.18**)

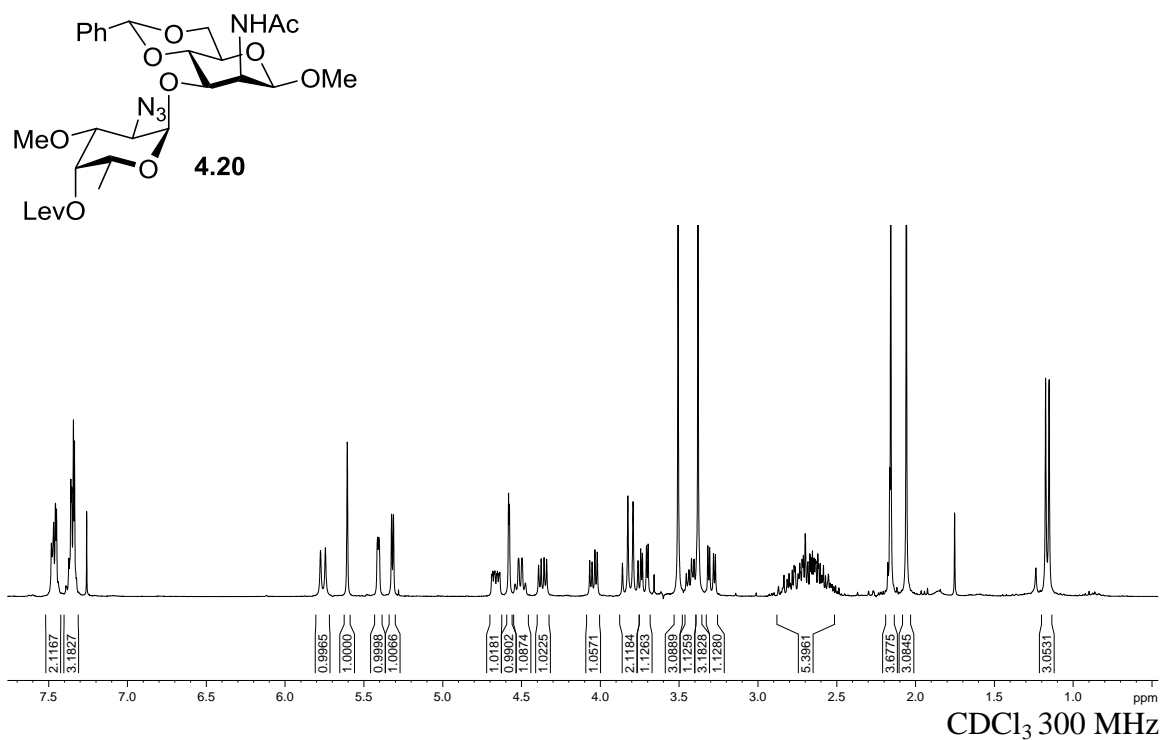


Figure A-73: ^1H NMR spectrum of Methyl *O*-(2-azido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-2-deoxy-4,6-*O*-benzylidene- β -D-mannopyranoside (**4.20**)

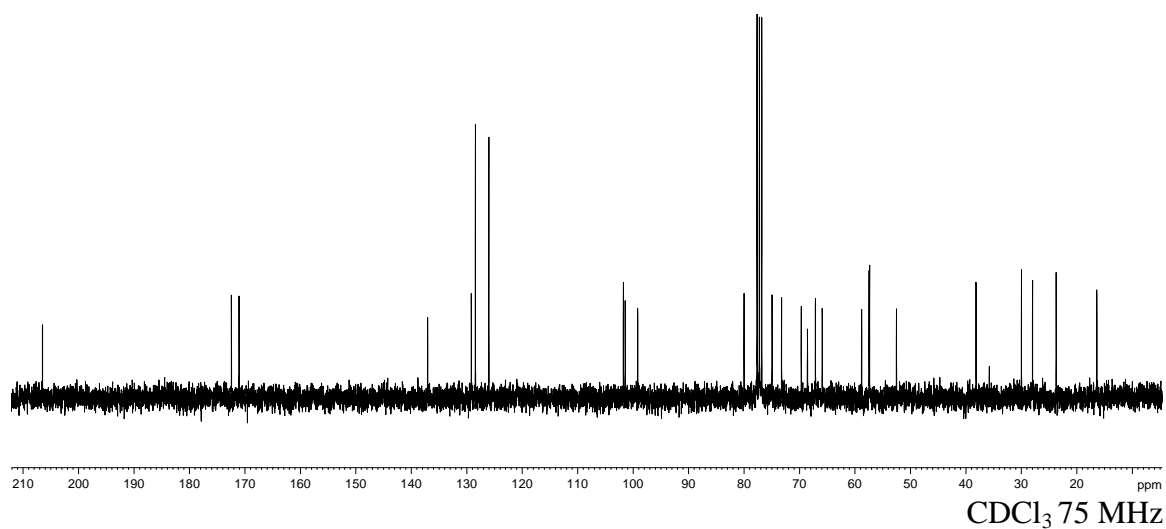


Figure A-74: ^{13}C NMR spectrum of Methyl *O*-(2-azido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-2-deoxy-4,6-*O*-benzylidene- β -D-mannopyranoside (**4.20**)

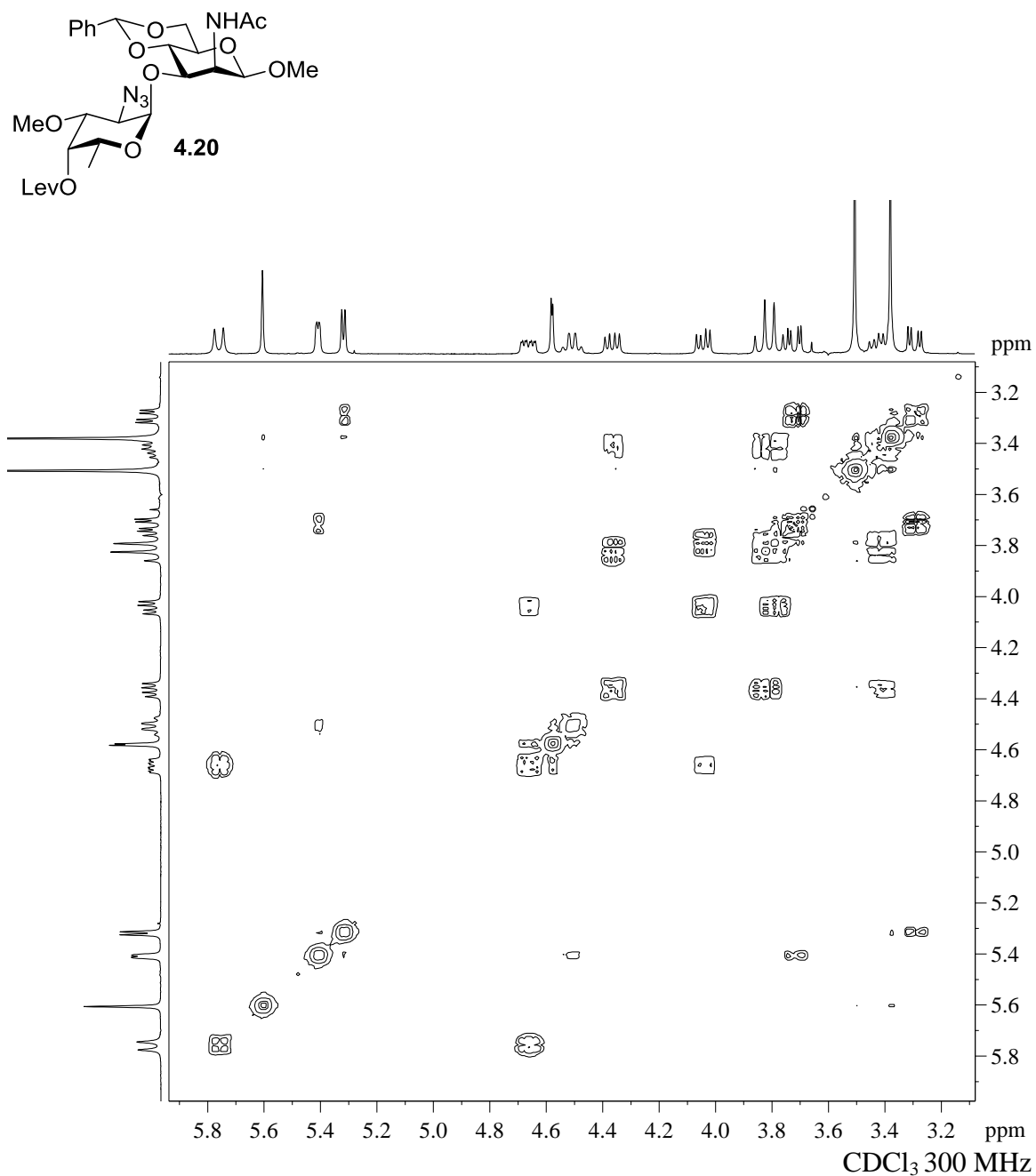


Figure A-75: 2-D NMR COSY spectrum of Methyl *O*-(2-azido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-2-deoxy-4,6-*O*-benzylidene- β -D-mannopyranoside (**4.20**)

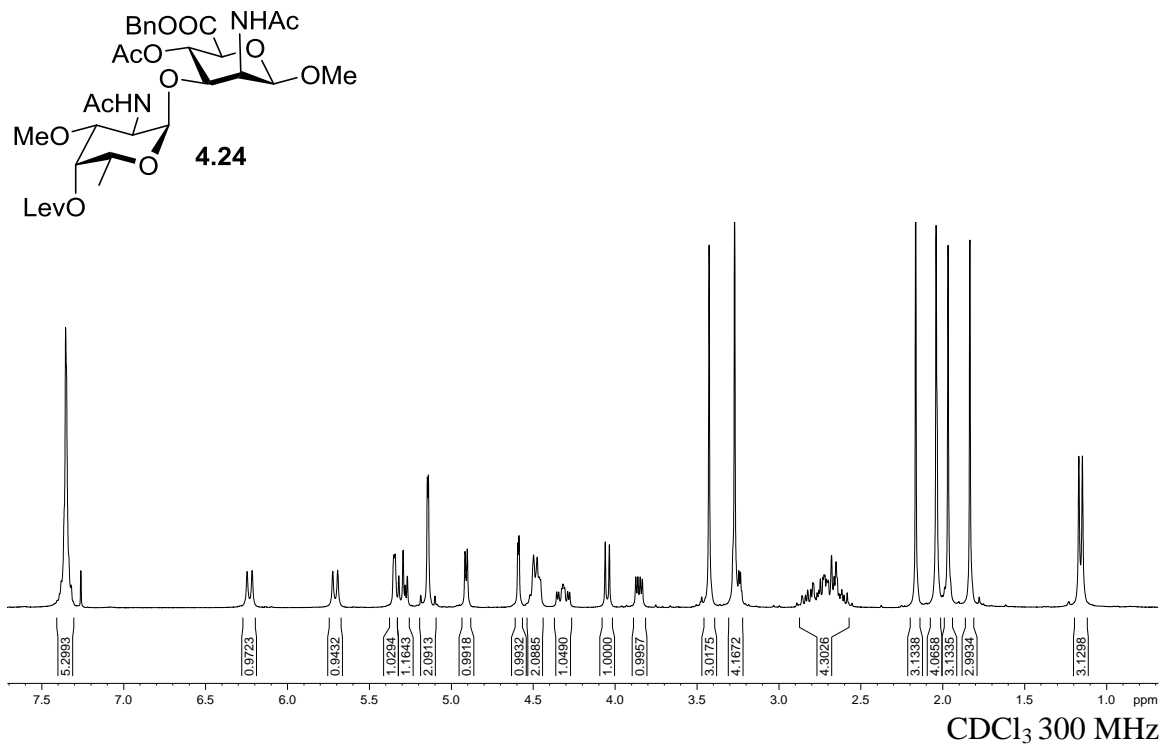


Figure A-76: ^1H NMR spectrum of Methyl *O*-(2-acetamido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-(benzyl 2-acetamido-4-*O*-acetyl-2-deoxy- β -D-mannopyranosid)uronate (**4.24**)

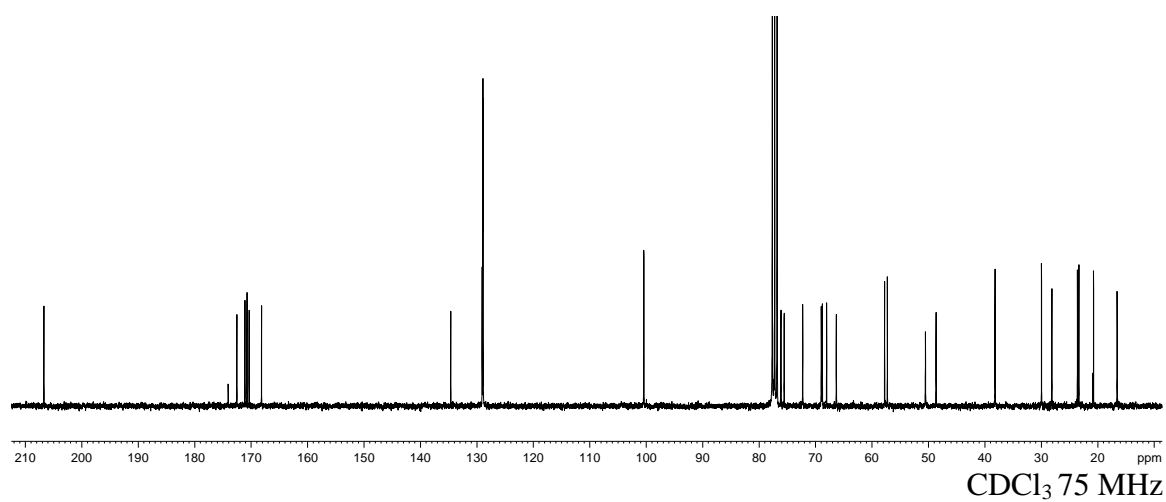


Figure A-77: ^{13}C NMR spectrum of Methyl *O*-(2-acetamido-2,6-dideoxy-4-*O*-levulinoyl-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-(benzyl 2-acetamido-4-*O*-acetyl-2-deoxy- β -D-mannopyranosid)uronate (**4.24**)

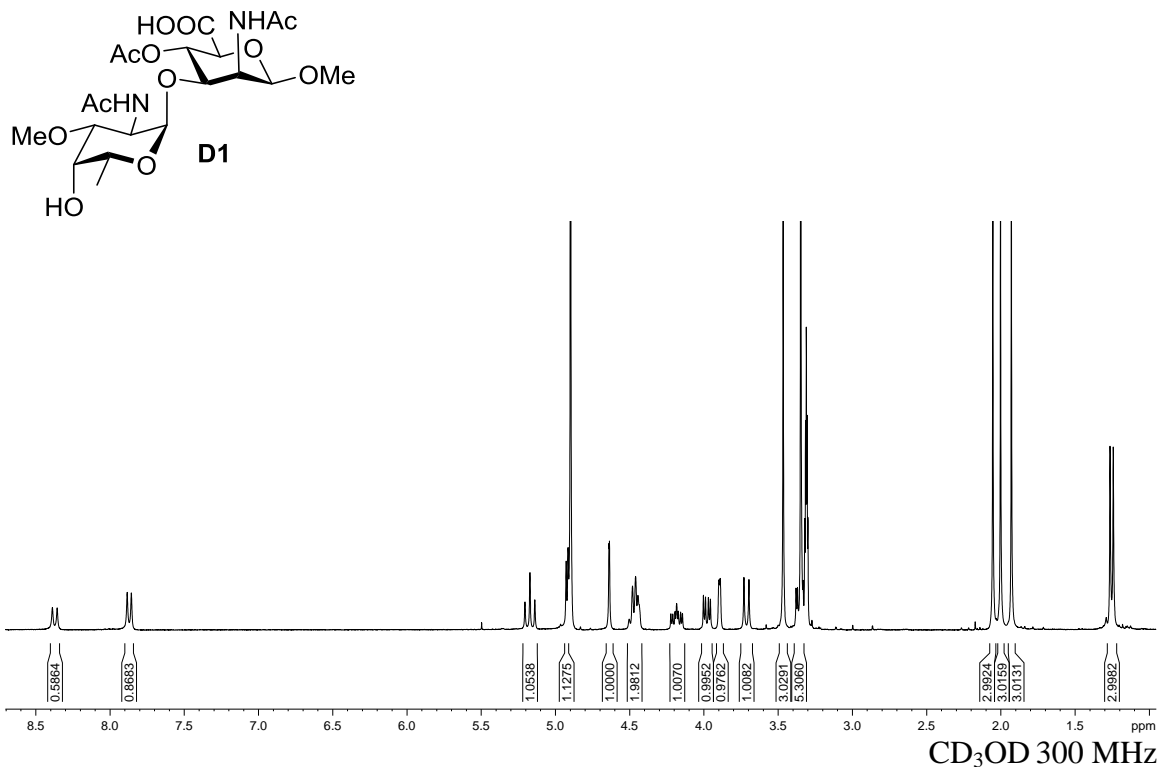


Figure A-79: ^1H NMR spectrum of Methyl *O*-(2-acetamido-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-(2-acetamido-4-*O*-acetyl-2-deoxy- β -D-mannopyranosid)uronic acid (**D1**)

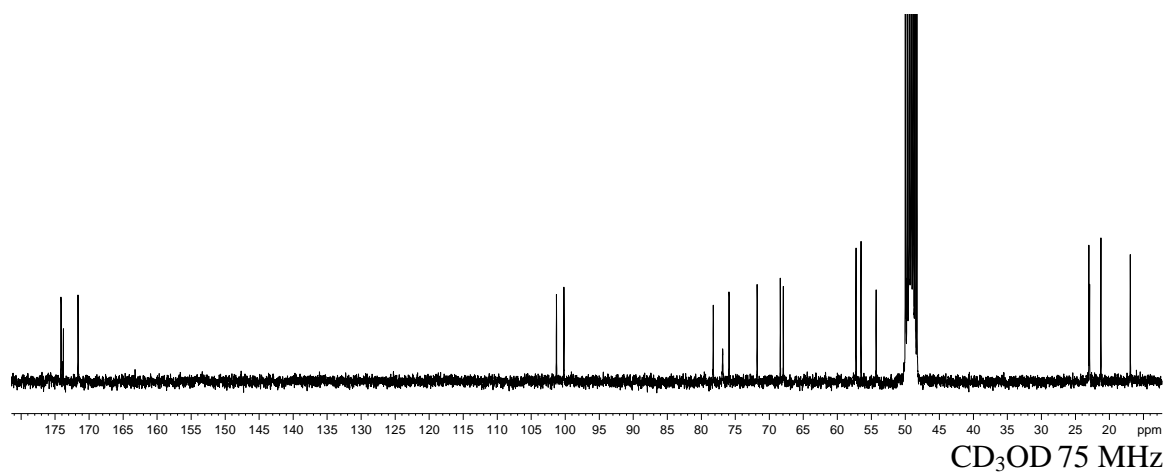


Figure A-80: ^{13}C NMR spectrum of Methyl *O*-(2-acetamido-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-(2-acetamido-4-*O*-acetyl-2-deoxy- β -D-mannopyranosid)uronic acid (**D1**)

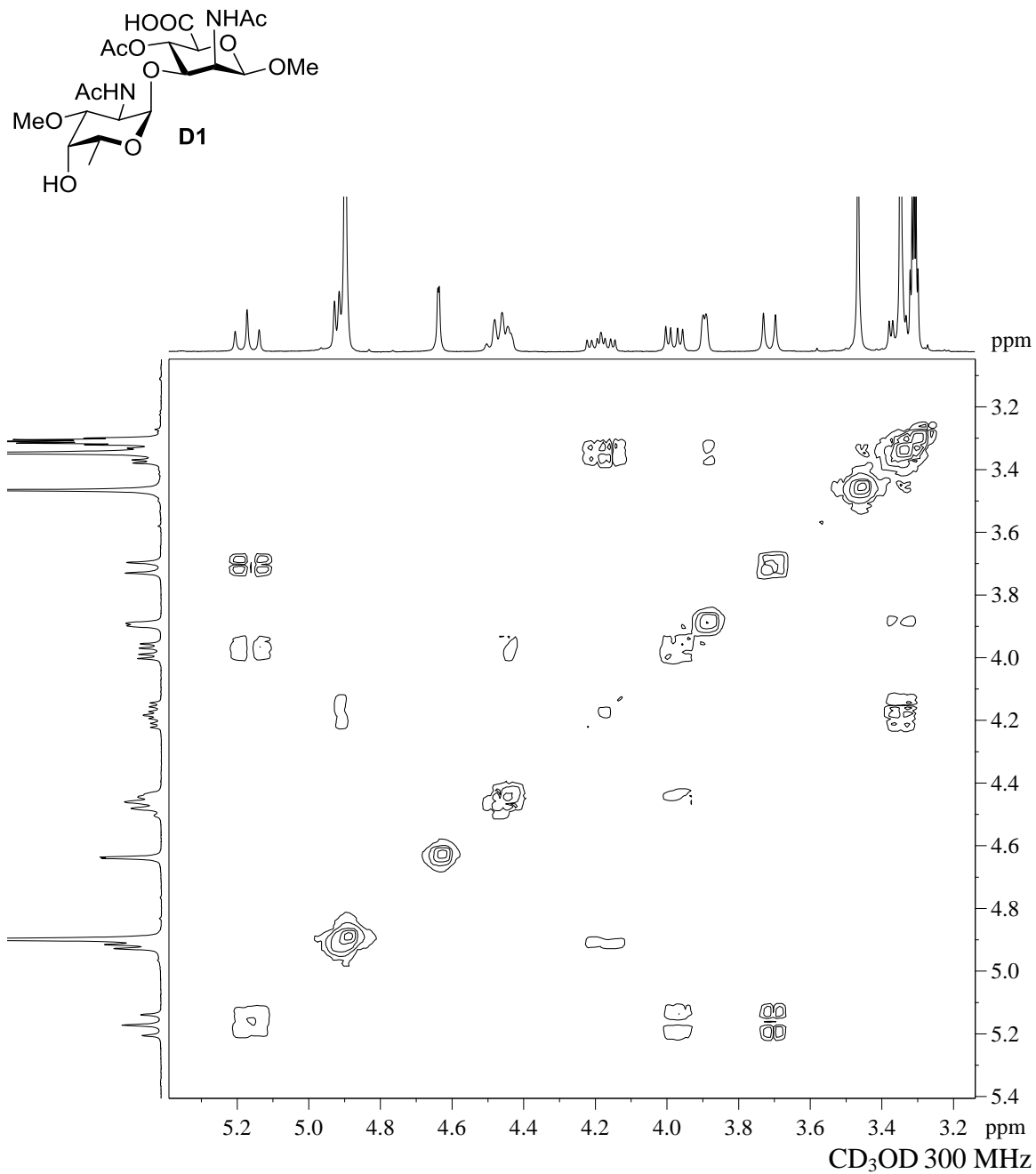


Figure A-81: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-(2-acetamido-4-*O*-acetyl-2-deoxy- β -D-mannopyranosid)uronic acid (**D1**)

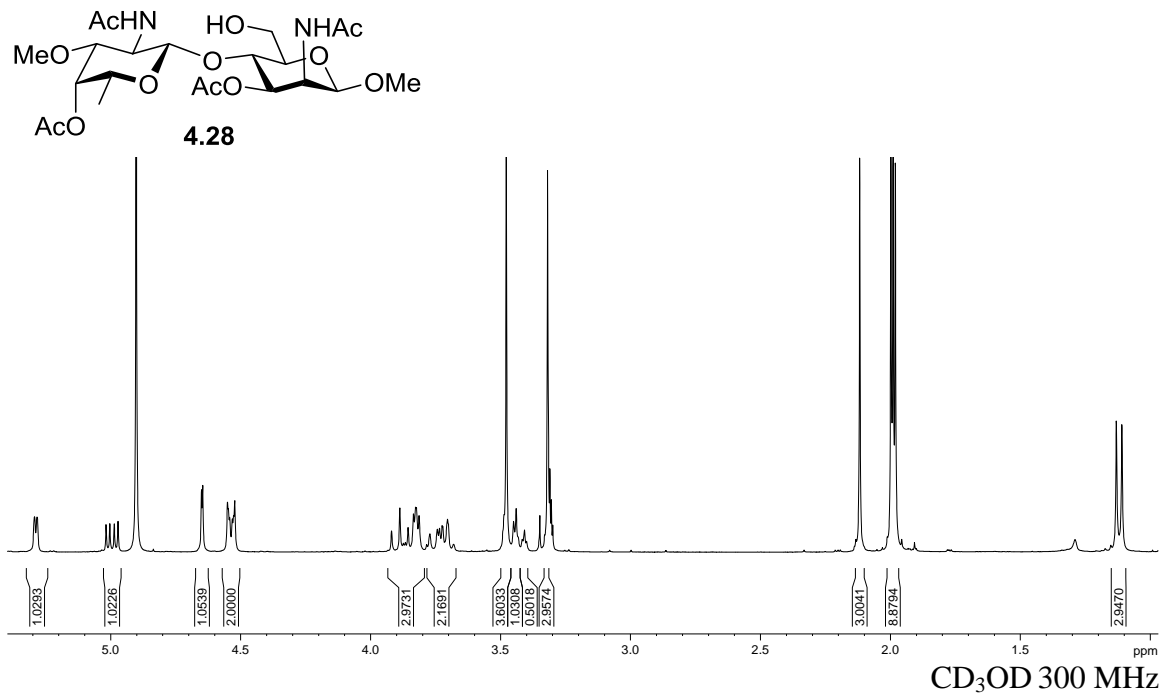


Figure A-82: ^1H NMR spectrum of Methyl *O*-(2-acetamido-4-*O*-acetyl-2,6-dideoxy-3-*O*-methyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2-acetamido-3-*O*-acetyl-2-deoxy- β -D-mannopyranoside (**4.28**)

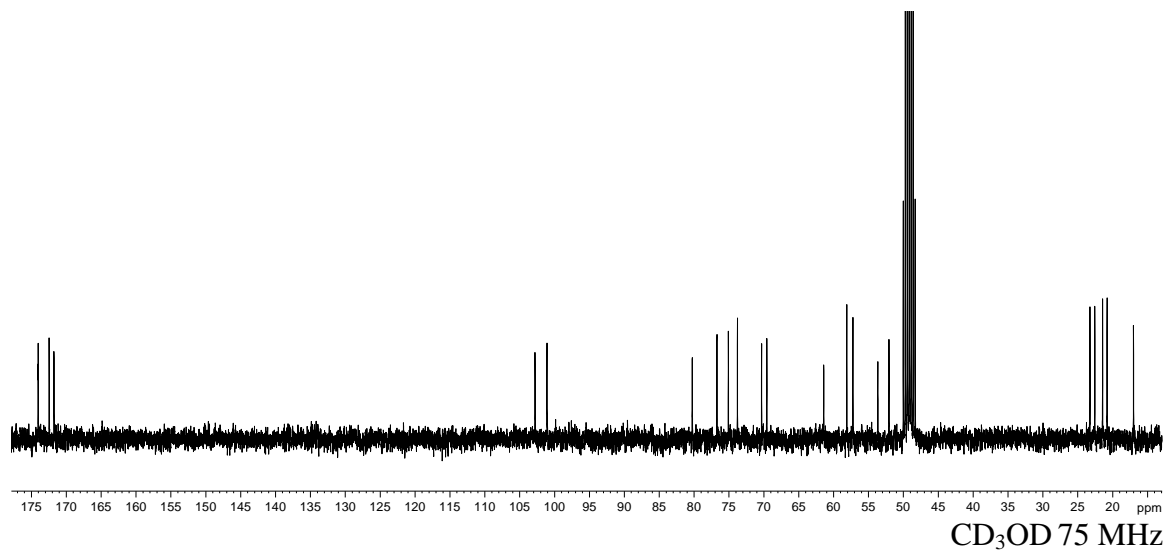


Figure A-83: ^{13}C NMR spectrum of Methyl *O*-(2-acetamido-4-*O*-acetyl-2,6-dideoxy-3-*O*-methyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2-acetamido-3-*O*-acetyl-2-deoxy- β -D-mannopyranoside (**4.28**)

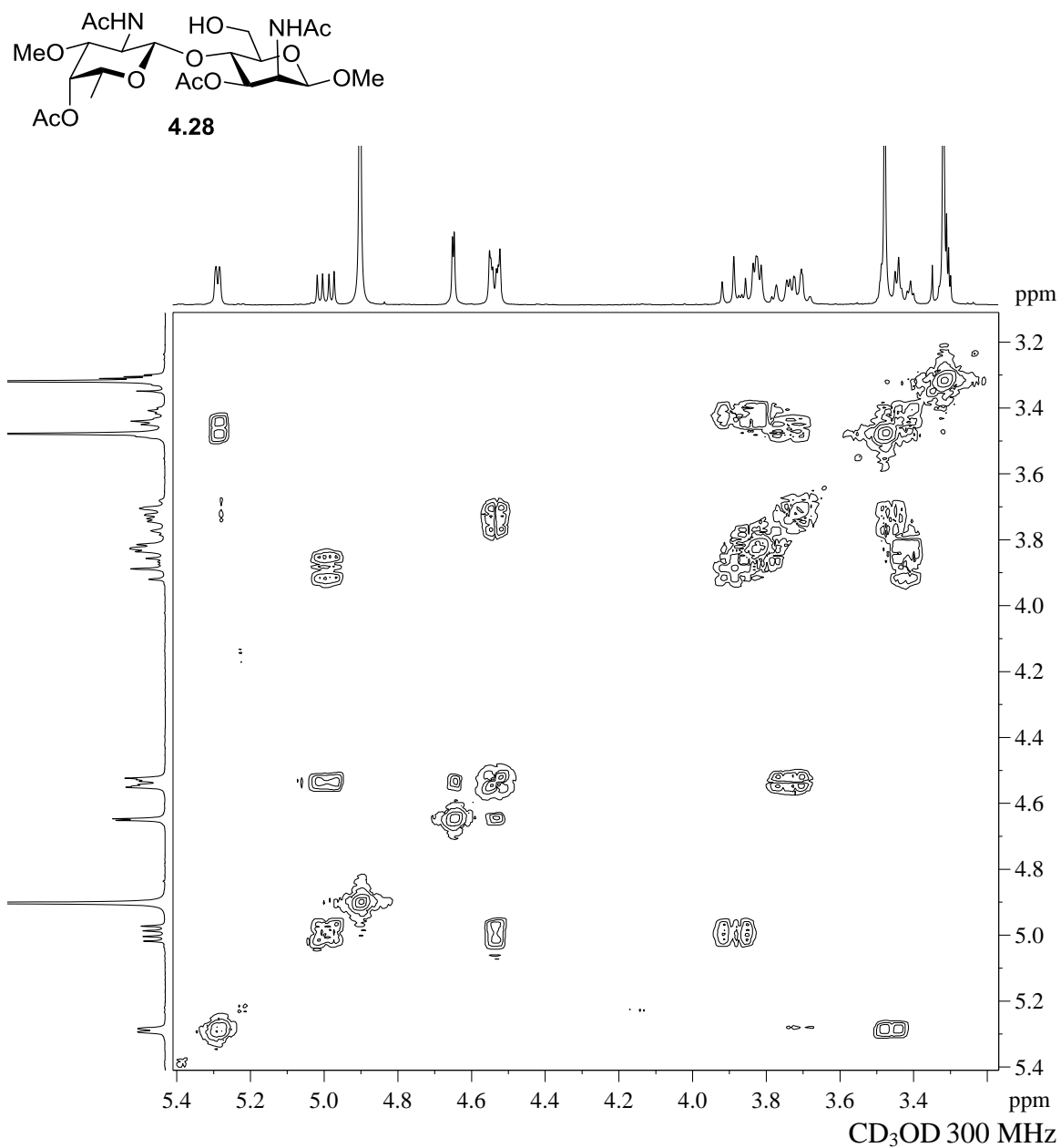


Figure A-84: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-4-*O*-acetyl-2,6-dideoxy-3-*O*-methyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2-acetamido-3-*O*-acetyl-2-deoxy- β -D-mannopyranoside (**4.28**)

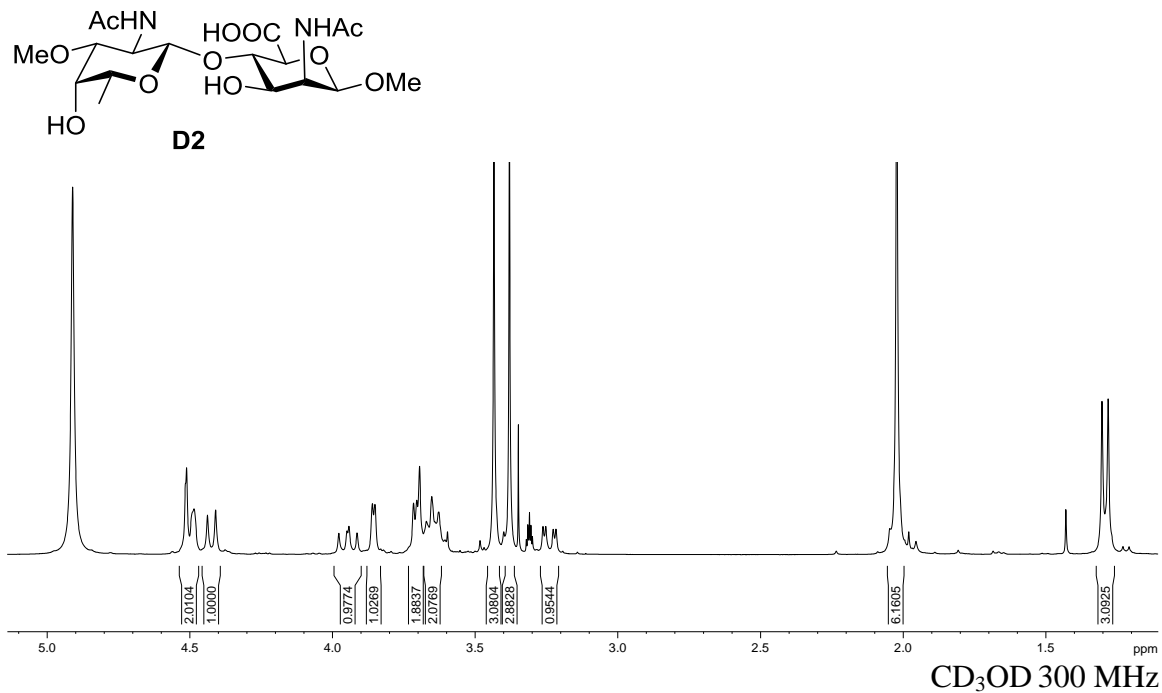


Figure A-85: ¹H NMR spectrum of Methyl *O*-(2-acetamido-2,6-dideoxy-3-*O*-methyl-β-D-fucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-mannopyranosid)uronic acid (**D2**)

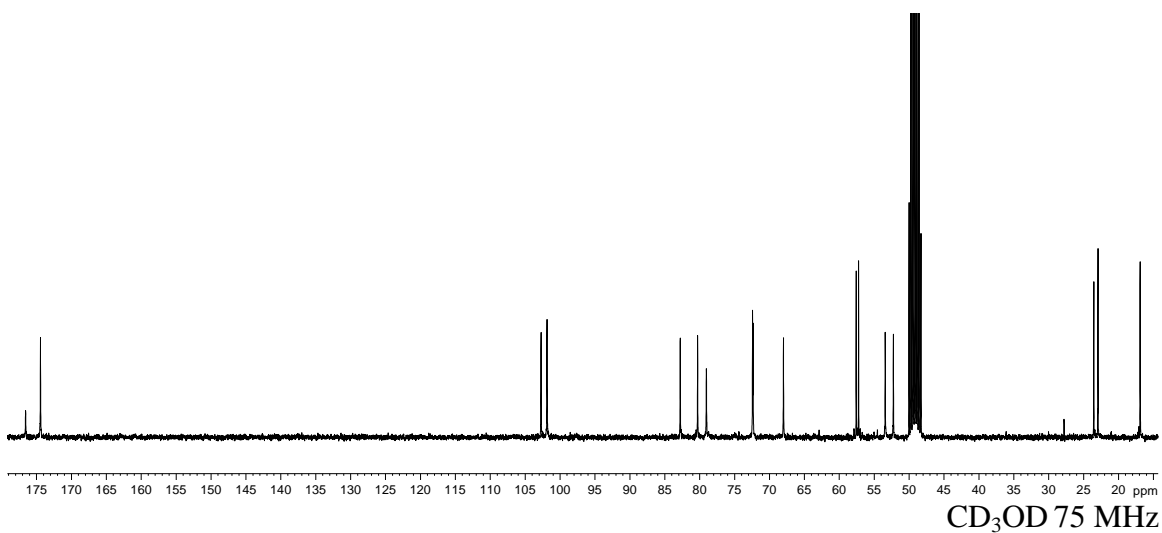


Figure A-86: ¹³C NMR spectrum of Methyl *O*-(2-acetamido-2,6-dideoxy-3-*O*-methyl-β-D-fucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-mannopyranosid)uronic acid (**D2**)

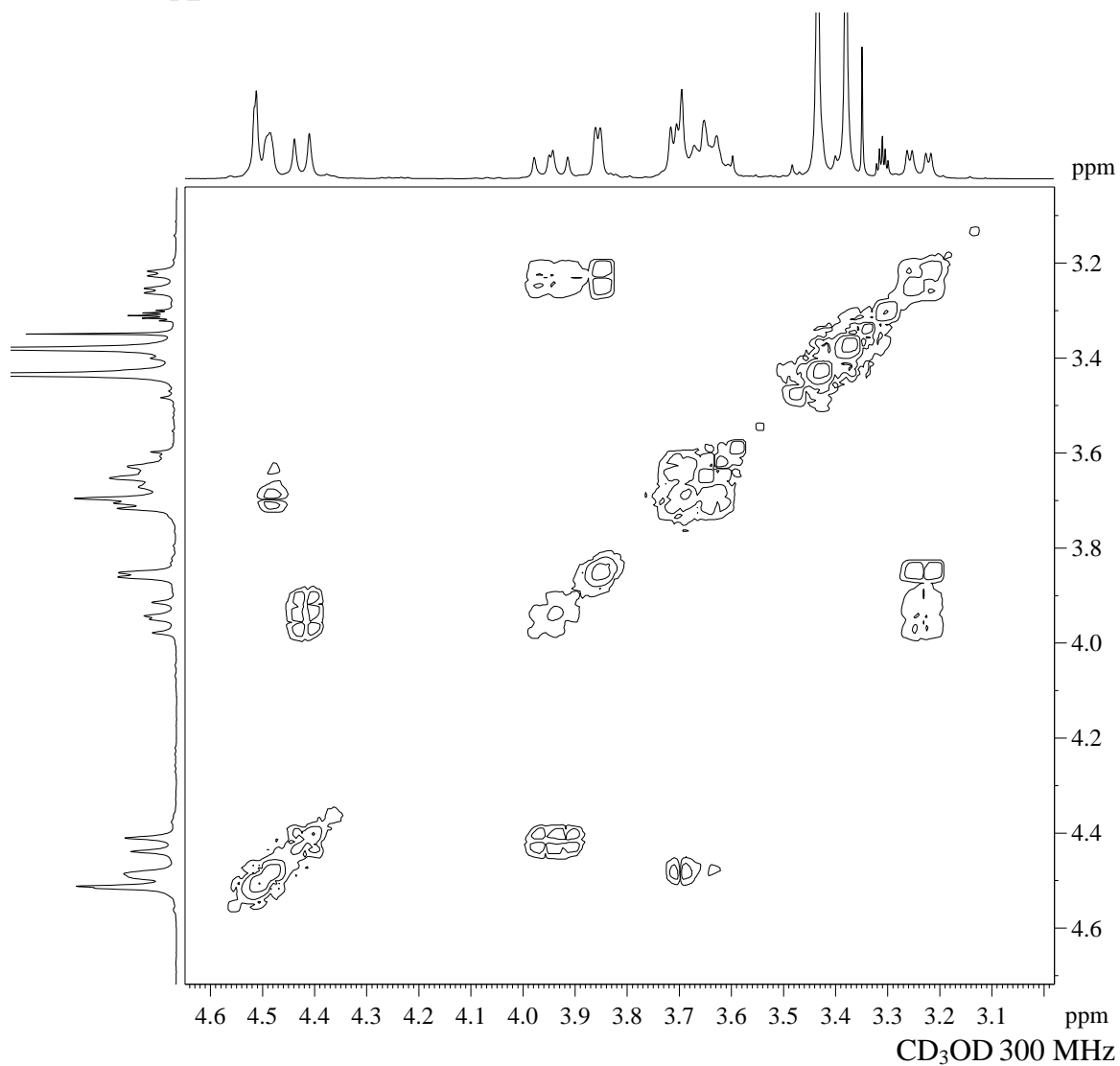
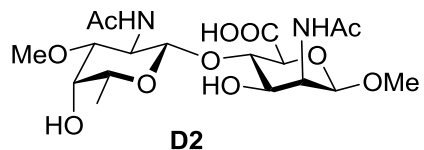


Figure A-87: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-2,6-dideoxy-3-*O*-methyl- β -D-fucopyranosyl)-(1 \rightarrow 4)-(2-acetamido-2-deoxy- β -D-mannopyranosid)uronic acid (**D2**)

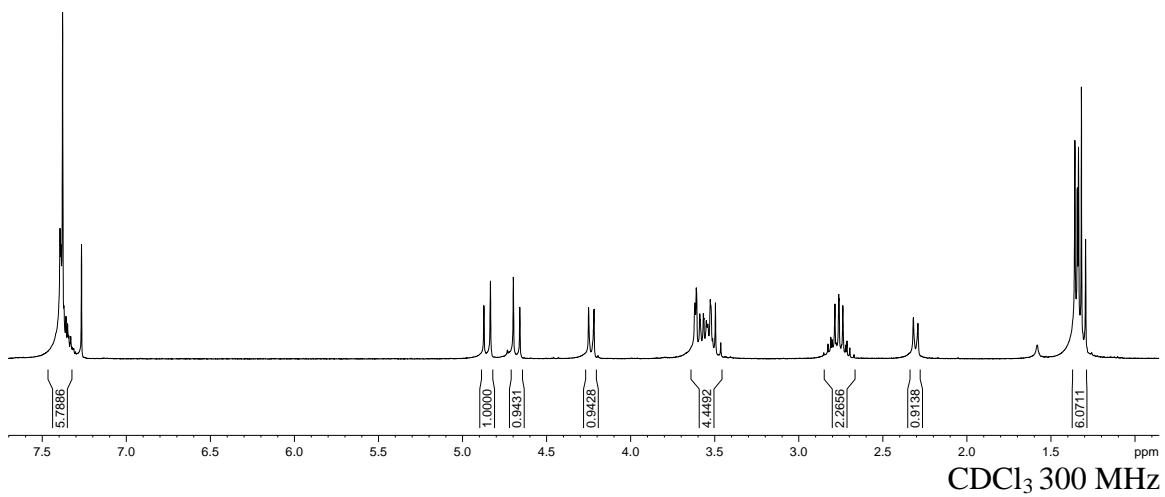
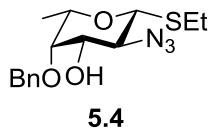


Figure A-88: ¹H NMR spectrum of Ethyl 2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio-β-L-galactopyranoside (**5.4**)

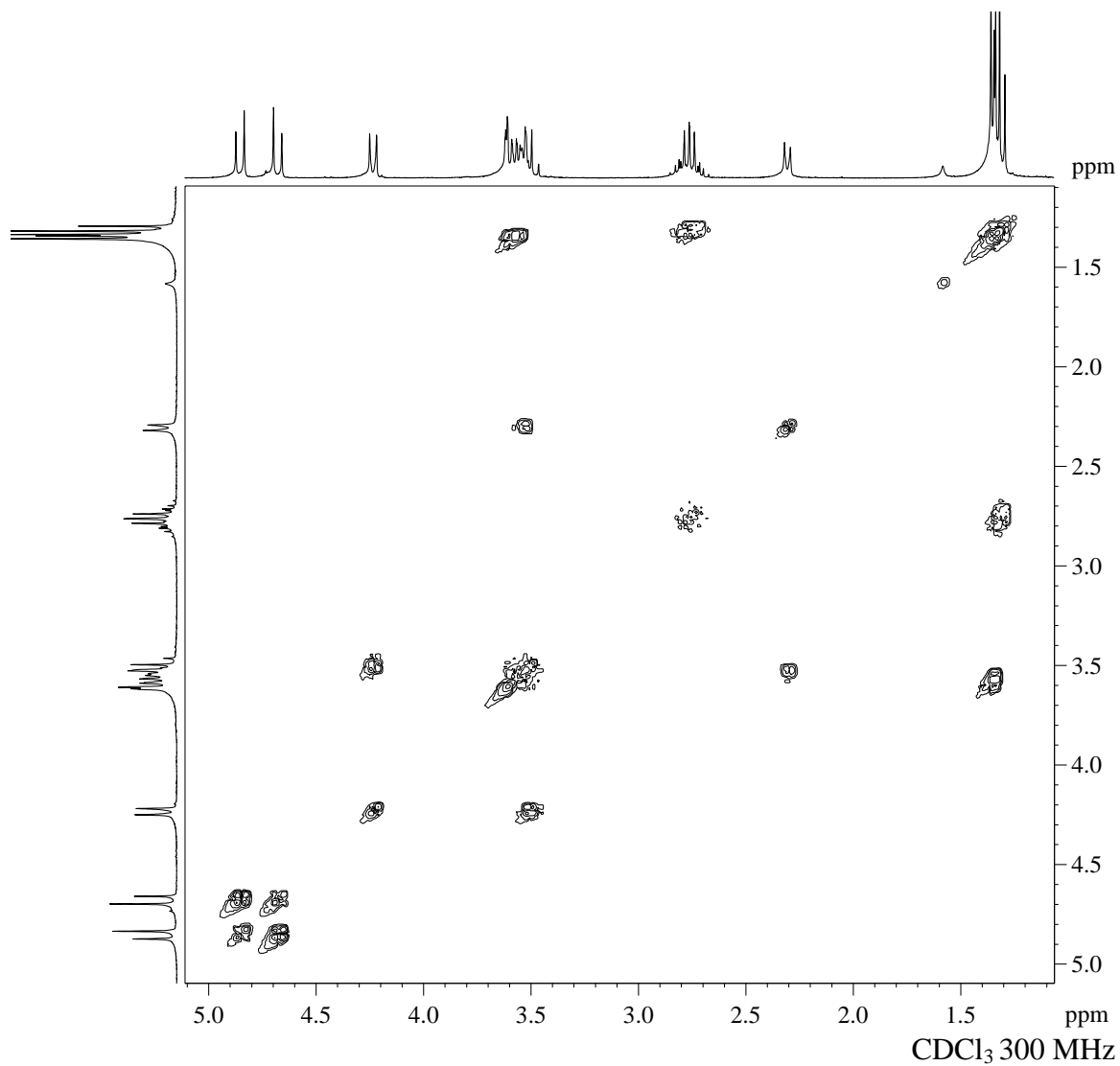
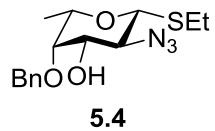


Figure A-89: 2-D NMR COSY spectrum of Ethyl 2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio- β -L-galactopyranoside (**5.4**)

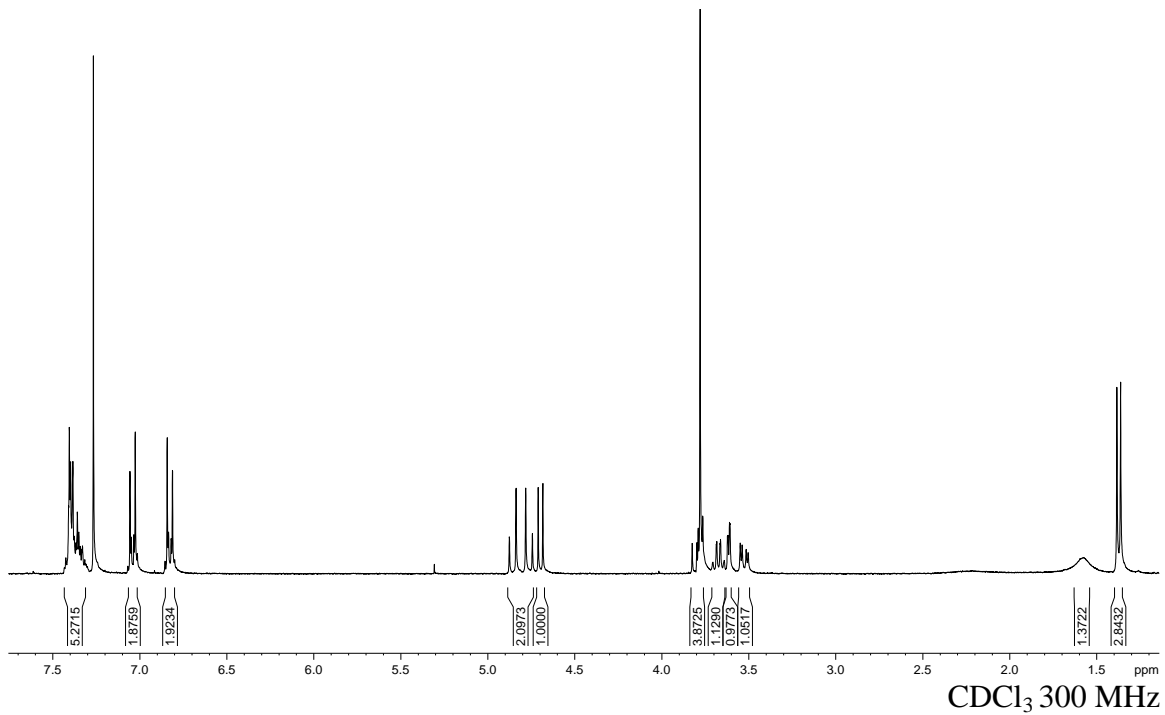
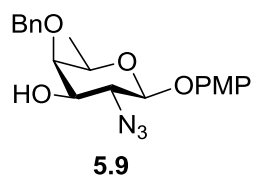


Figure A-90: ¹H NMR spectrum of 4-Methoxyphenyl 2-azido-4-*O*-benzyl-2,6-dideoxy-β-D-galactopyranoside (**5.8**)

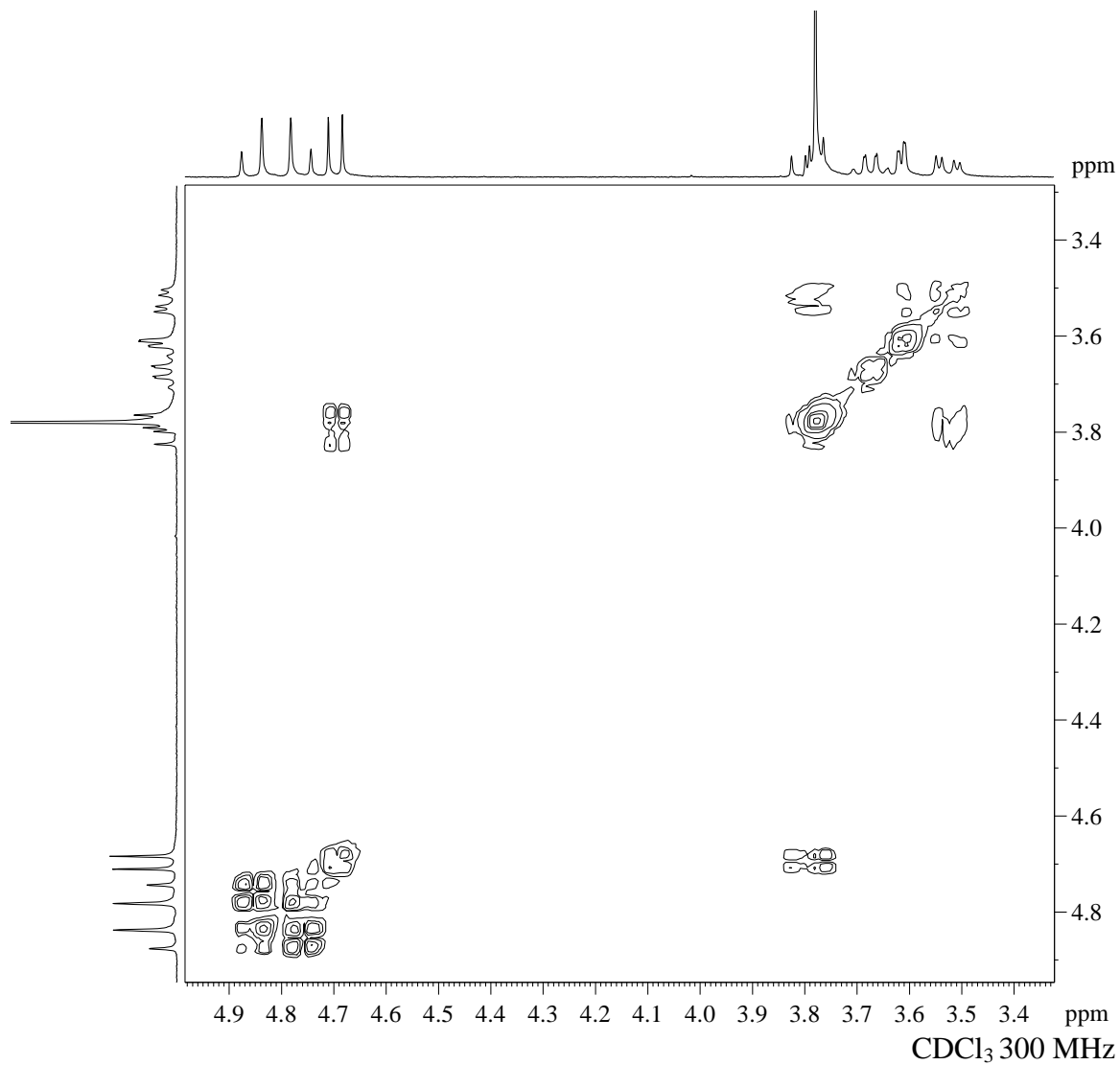
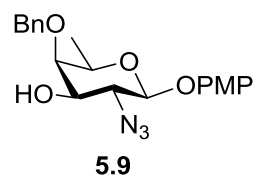


Figure A-91: 2-D NMR COSY spectrum of 4-Methoxyphenyl 2-azido-4-*O*-benzyl-2,6-dideoxy- β -D-galactopyranoside (**5.9**)

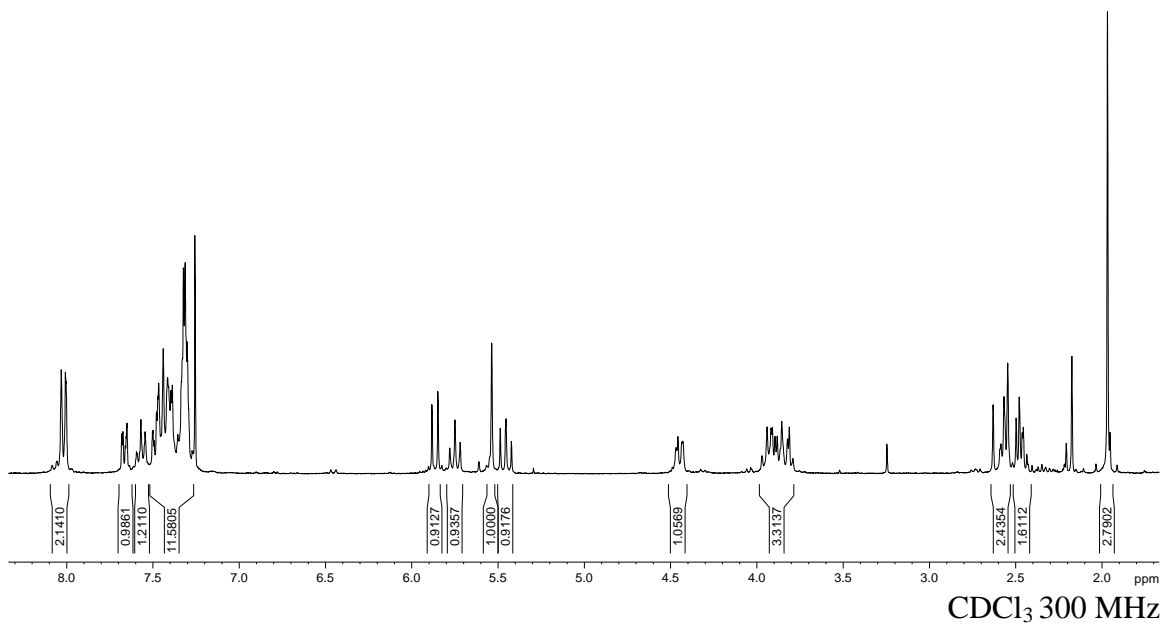
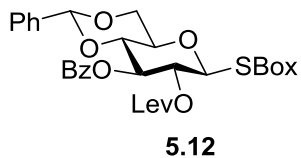


Figure A-92: ¹H NMR spectrum of Benzoxazolyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-levulinoyl-1-thio-β-D-glucopyranoside (**5.12**)

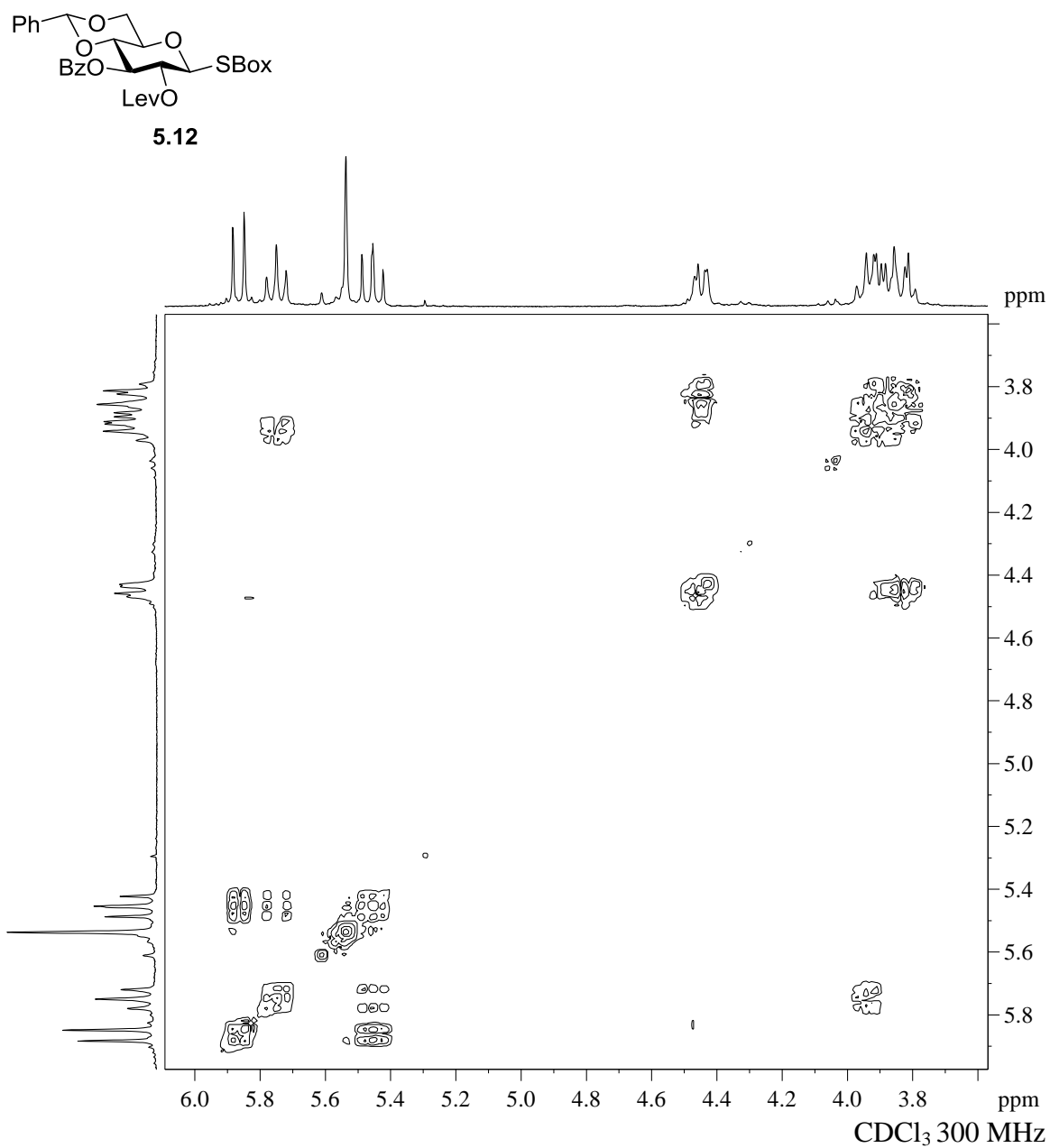
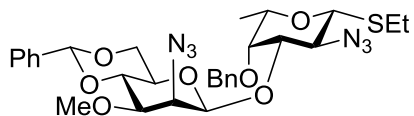


Figure A-93: 2-D NMR COSY spectrum of Benzoxazolyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-levulinoyl-1-thio- β -D-glucopyranoside (**5.12**)



5.16

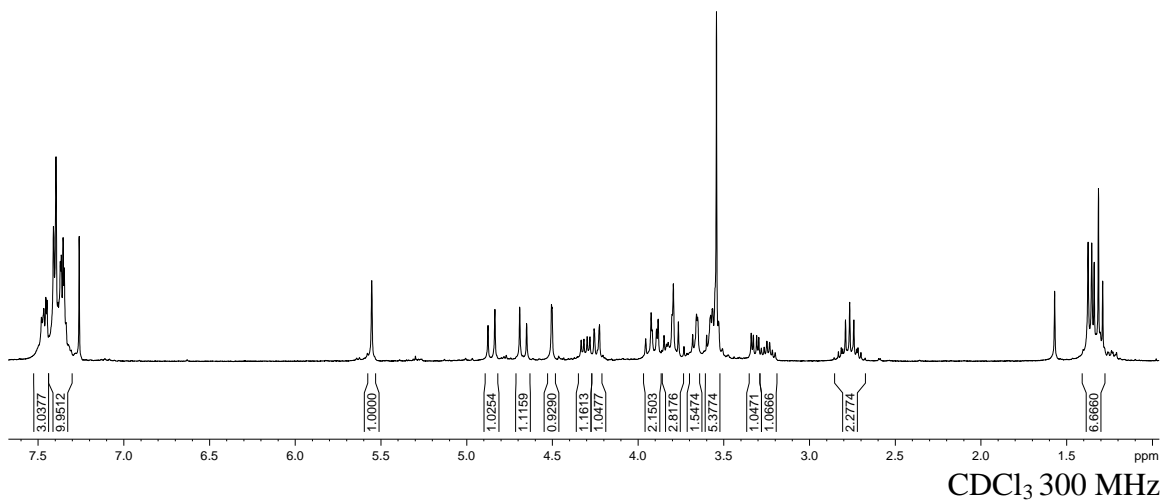


Figure A-94: ¹H NMR spectrum of Ethyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio-β-L-galactopyranoside (**5.16**)

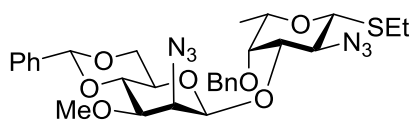
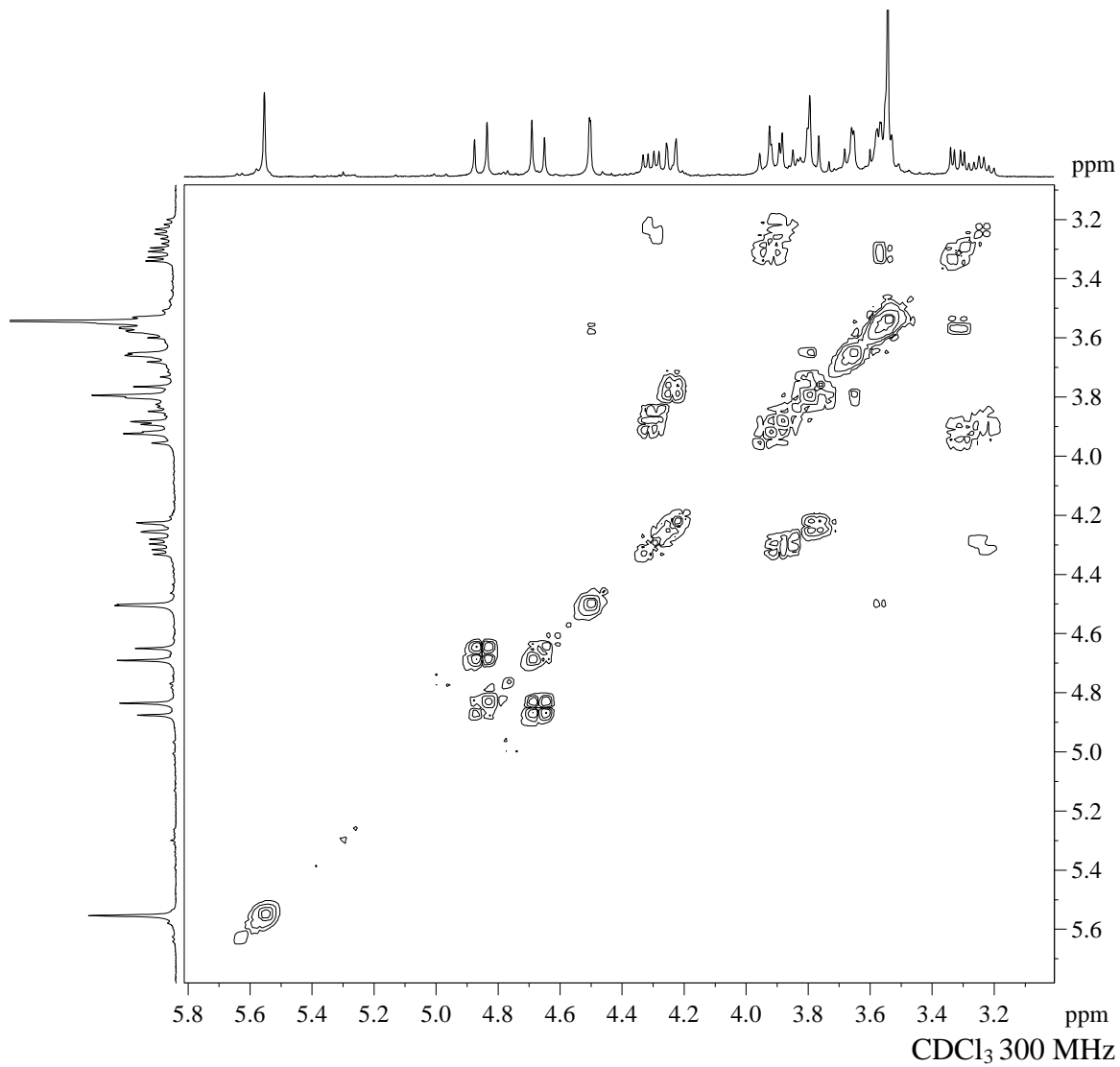
**5.16**

Figure A-95: 2-D NMR COSY spectrum of Ethyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio- β -L-galactopyranoside (**5.16**)

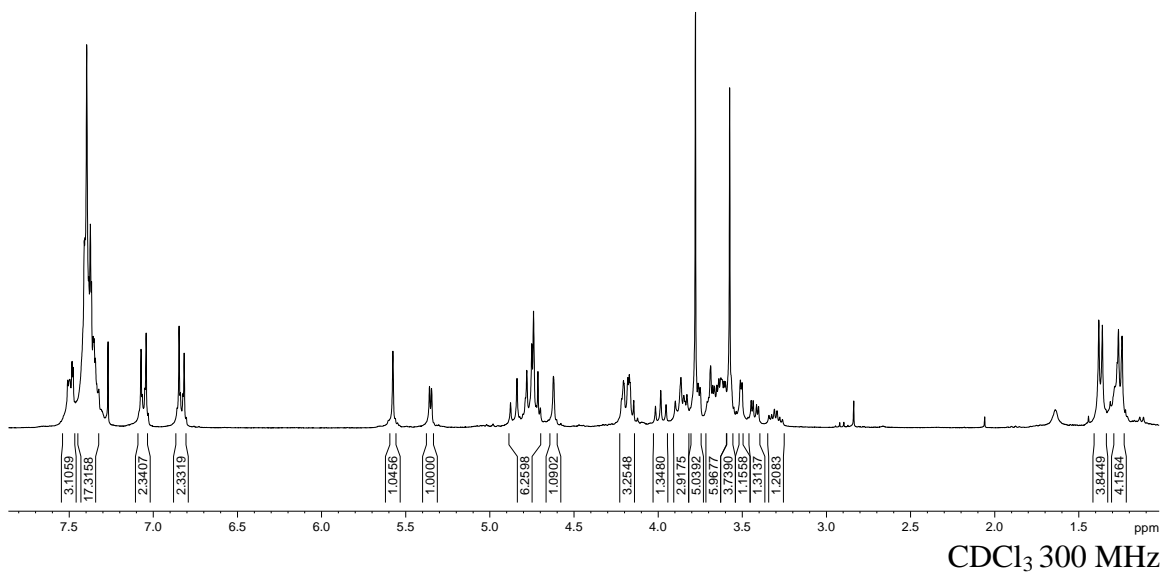
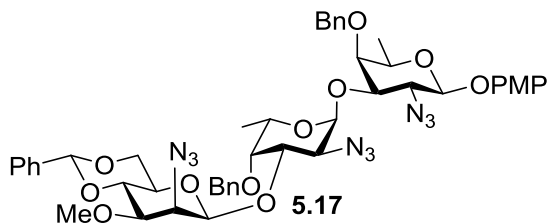


Figure A-96: ¹H NMR spectrum of 4-Methoxyphenyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (5.17)

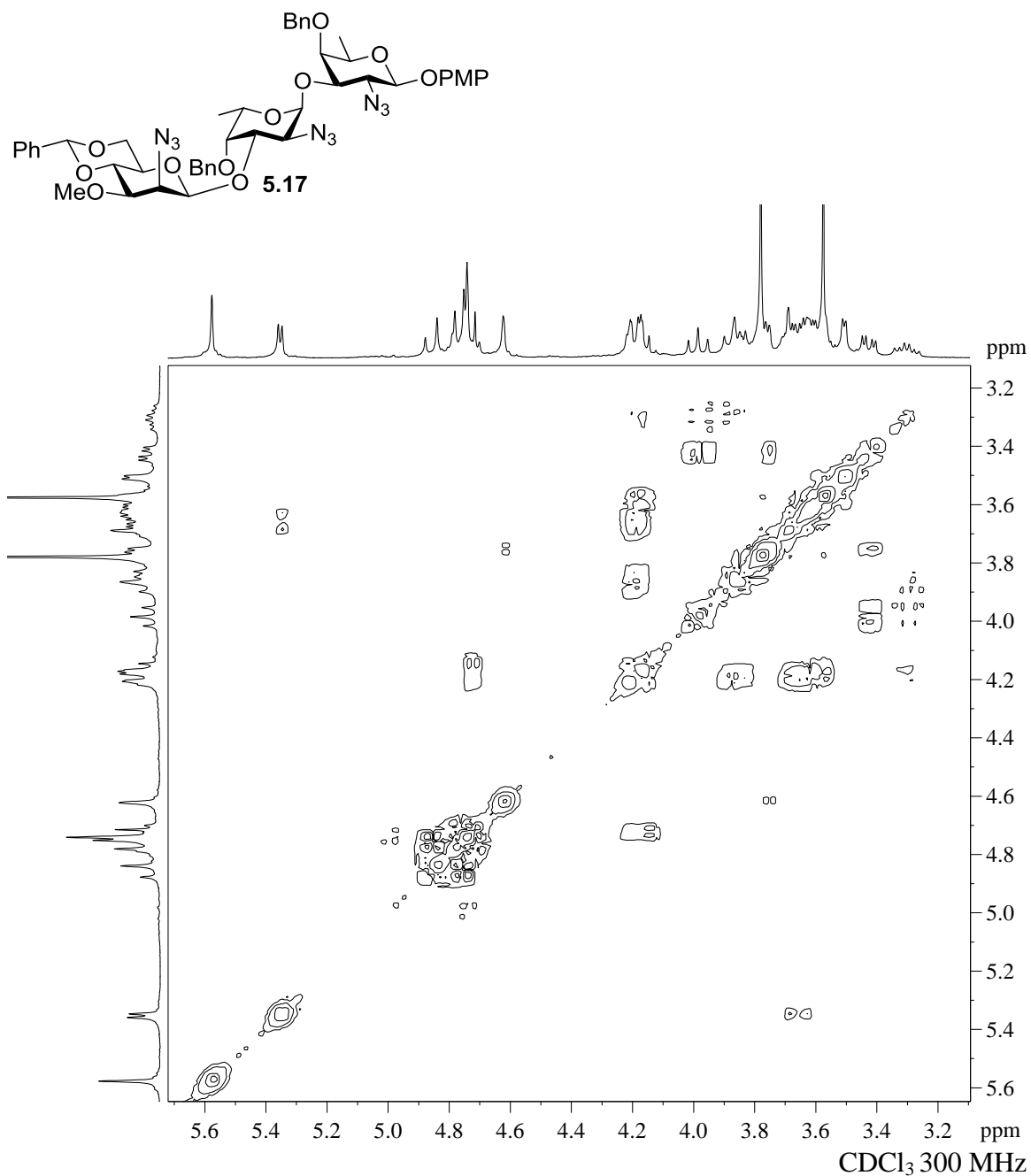


Figure A-97: 2-D NMR COSY spectrum of 4-Methoxyphenyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.17**)

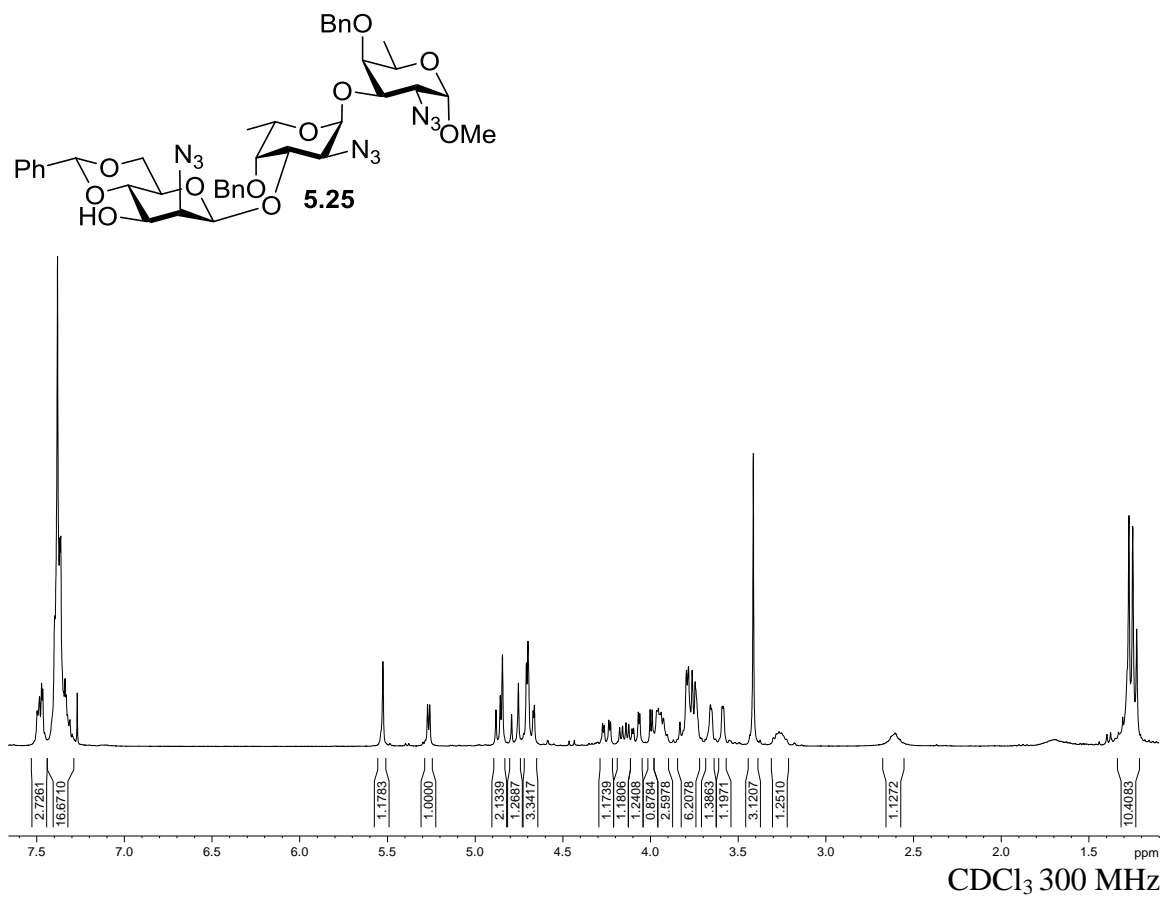


Figure A-98: ^1H NMR spectrum of Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.25**)

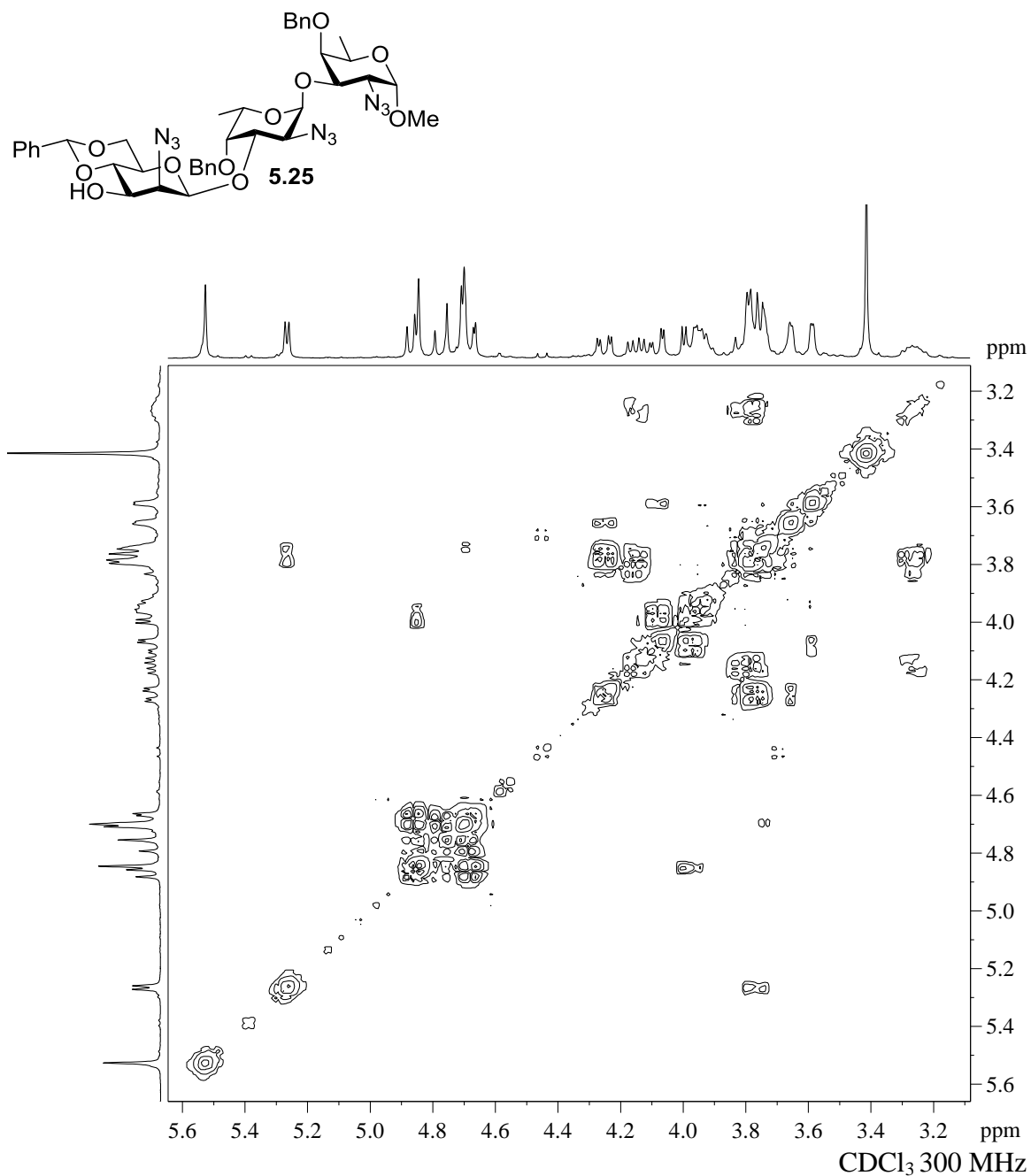


Figure A-99: 2-D NMR COSY spectrum of Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.25**)

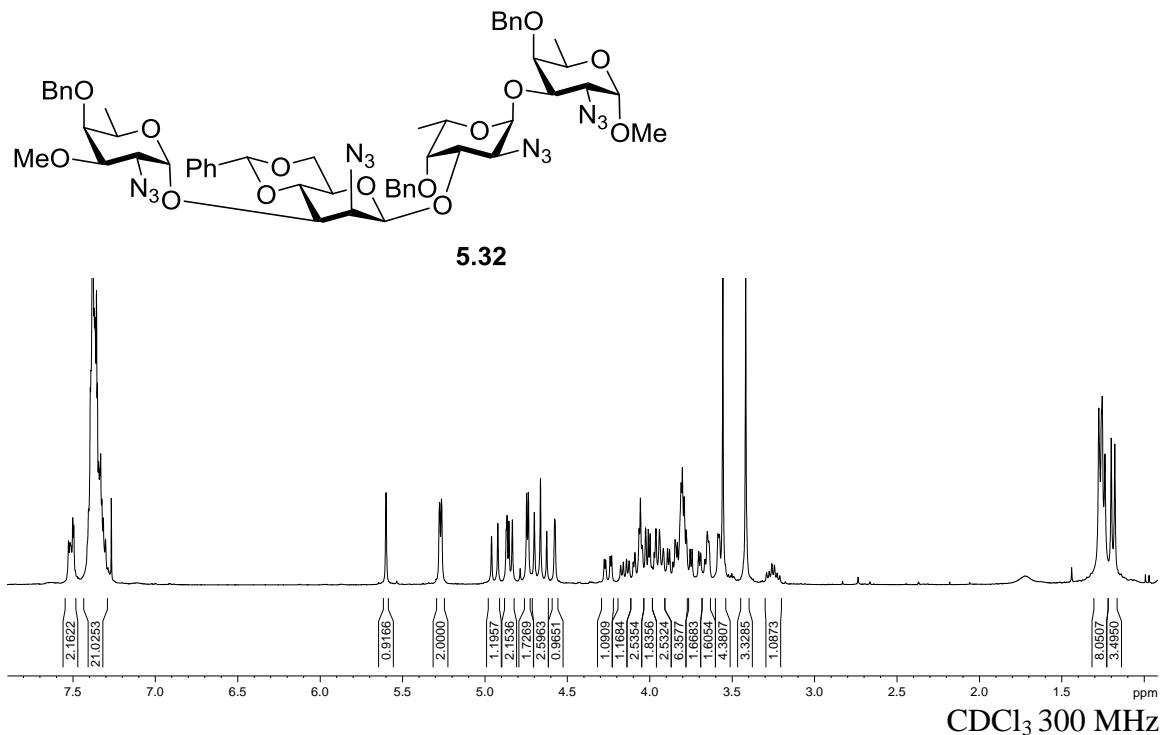


Figure A-100: ¹H NMR spectrum of Methyl *O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.32**)

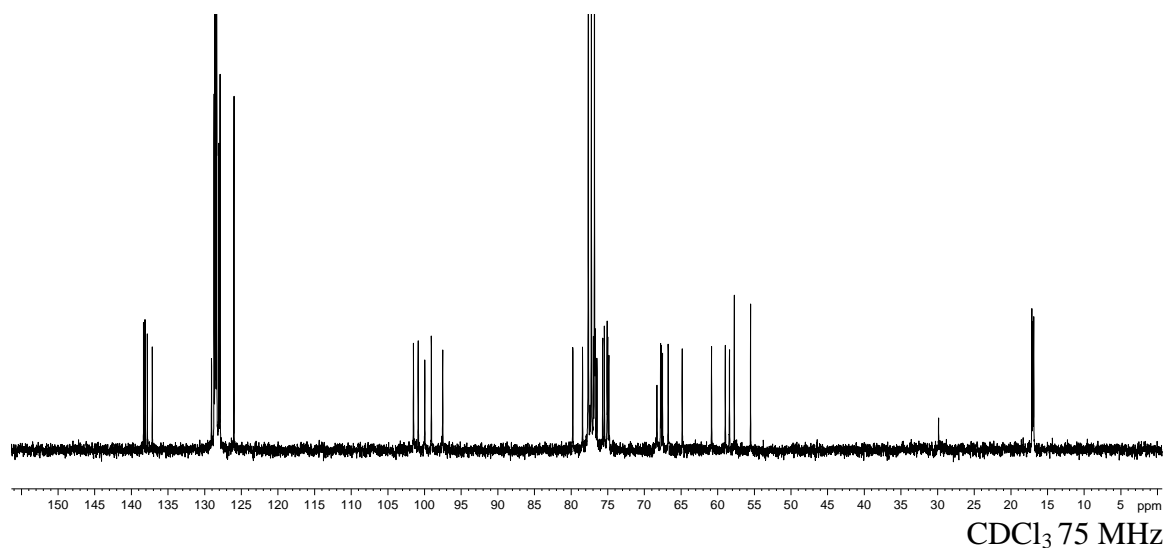


Figure A-101: ¹³C NMR spectrum of Methyl *O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.32**)

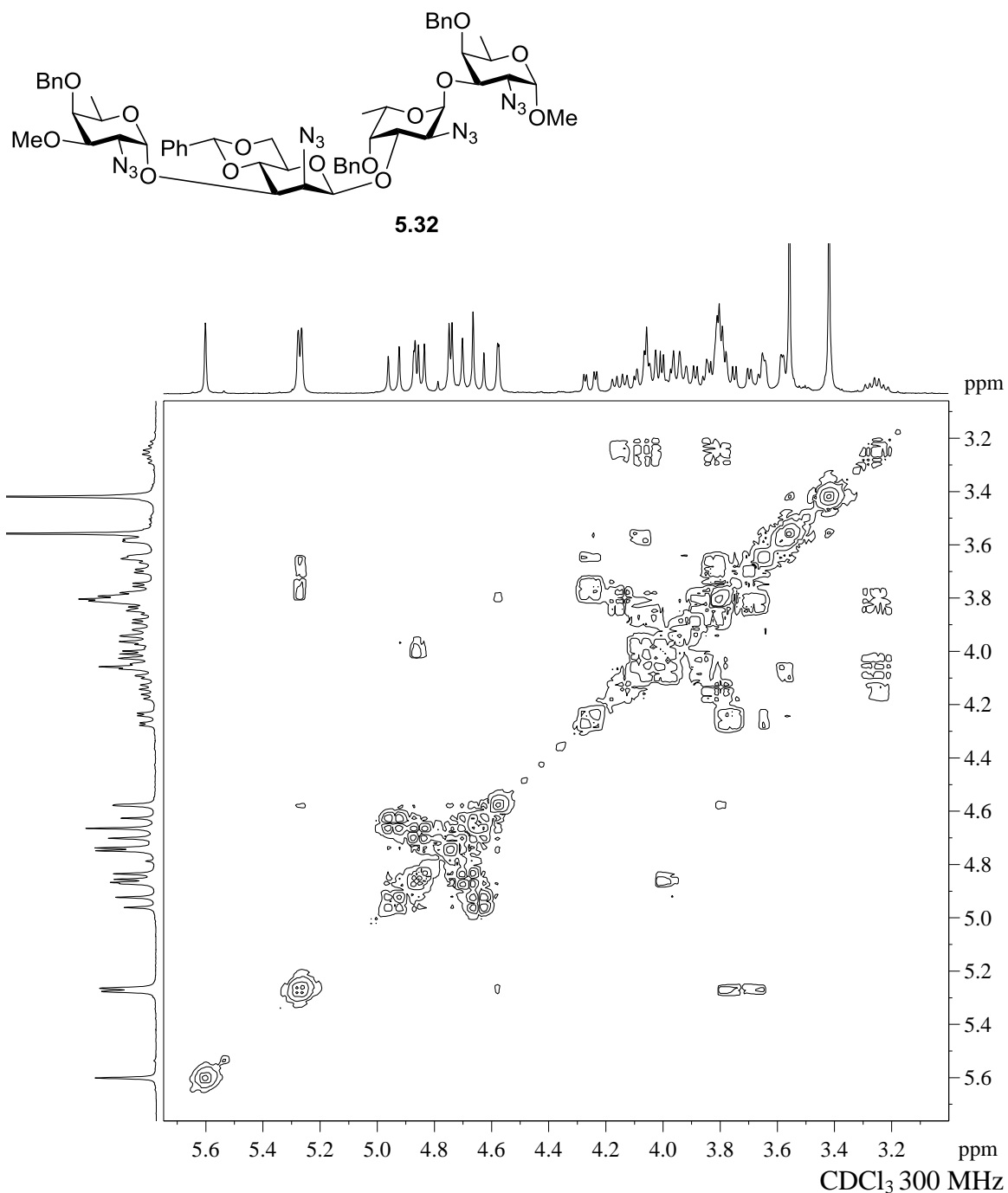


Figure A-102: 2-D NMR COSY spectrum of Methyl *O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-3-*O*-methyl- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (5.32)

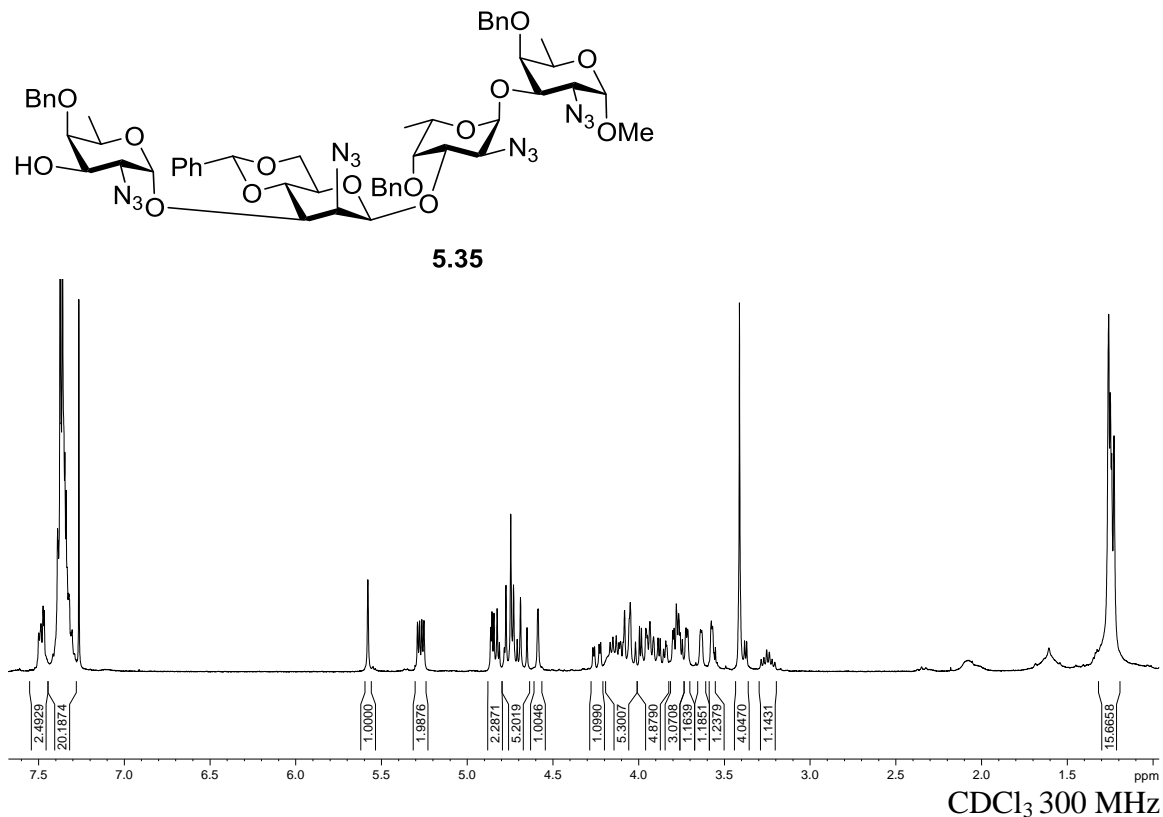


Figure A-103: ¹H NMR spectrum of Methyl *O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.35**)

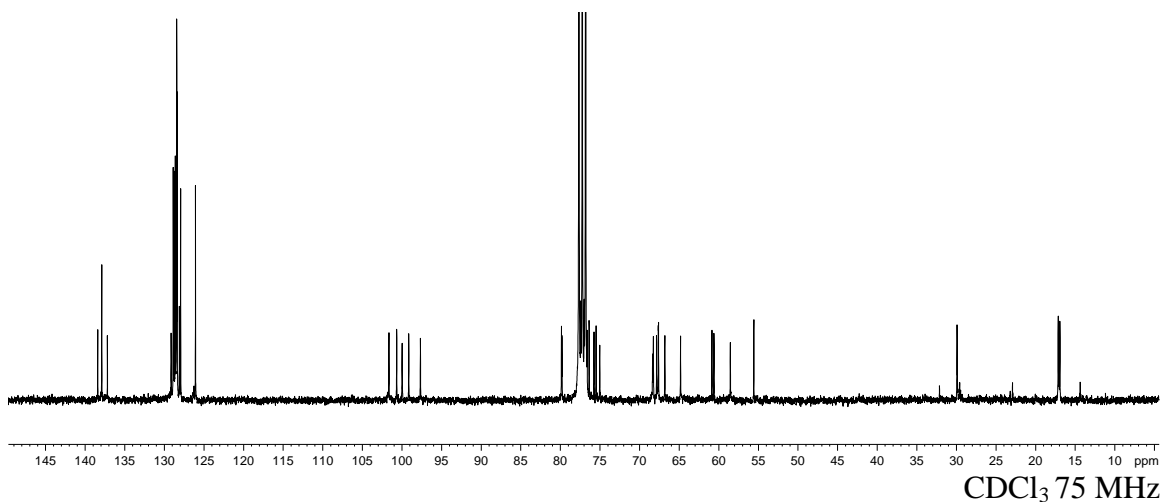


Figure A-104: ¹³C NMR spectrum of Methyl *O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.35**)

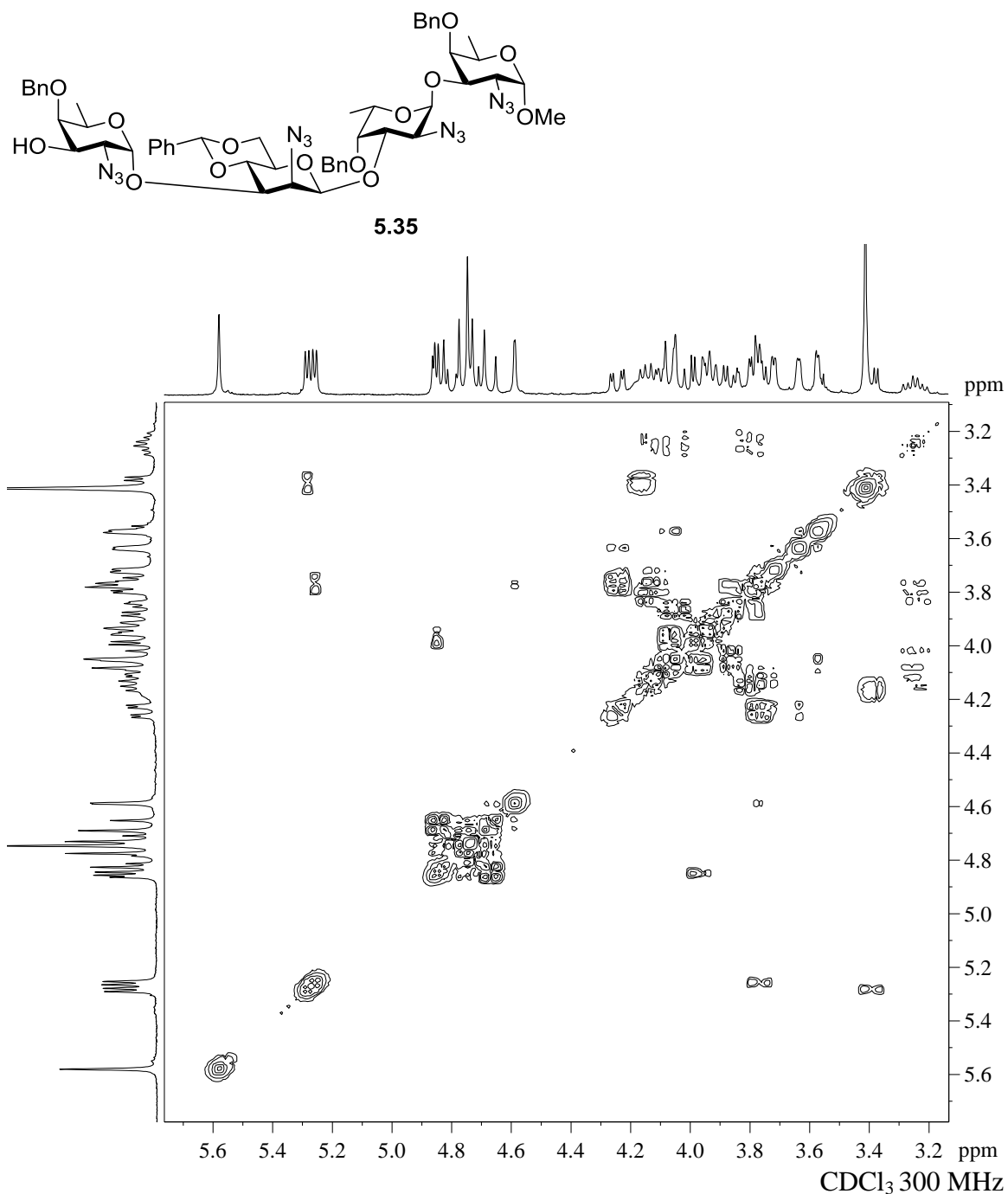


Figure A-105: 2-D NMR COSY spectrum of Methyl *O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy- β -D-mannopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.35**)

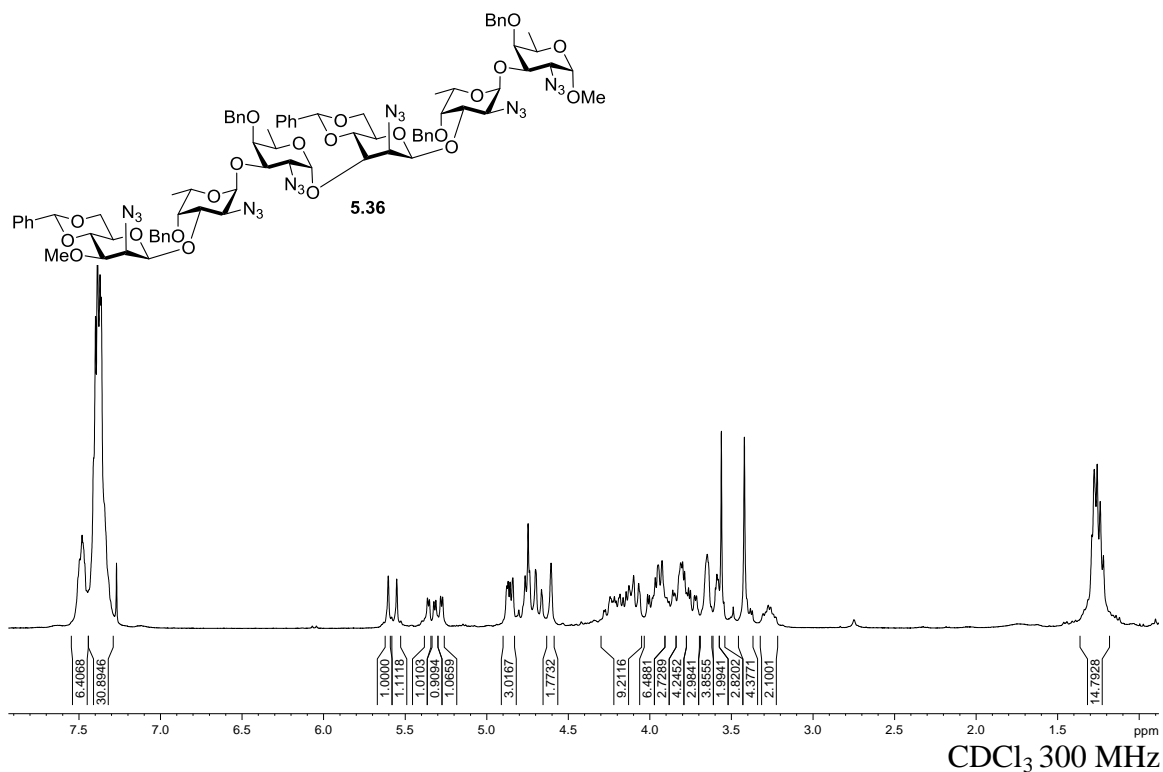


Figure A-106: ¹H NMR spectrum of Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.36**)

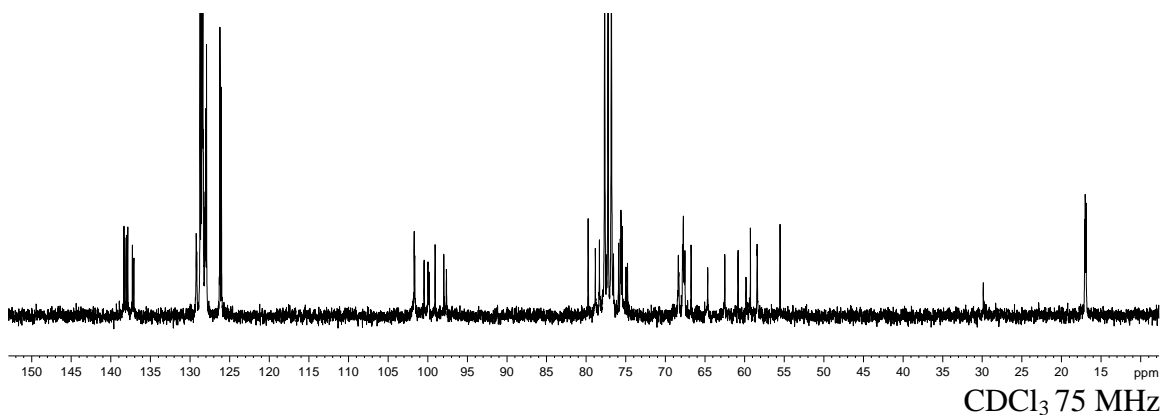


Figure A-107: ¹³C NMR spectrum of Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.36**)

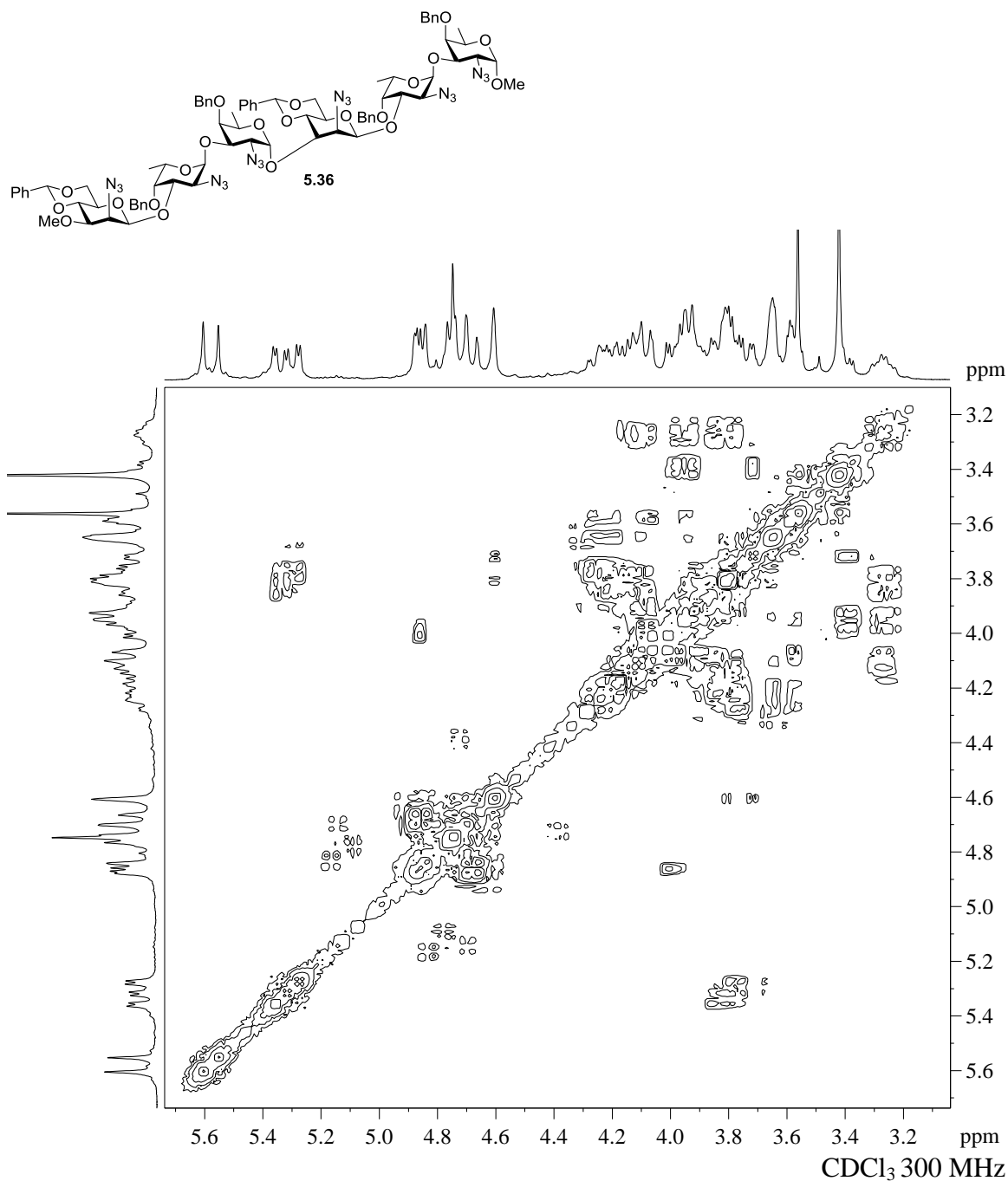


Figure A-108: 2-D NMR COSY spectrum of Methyl *O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.36**)

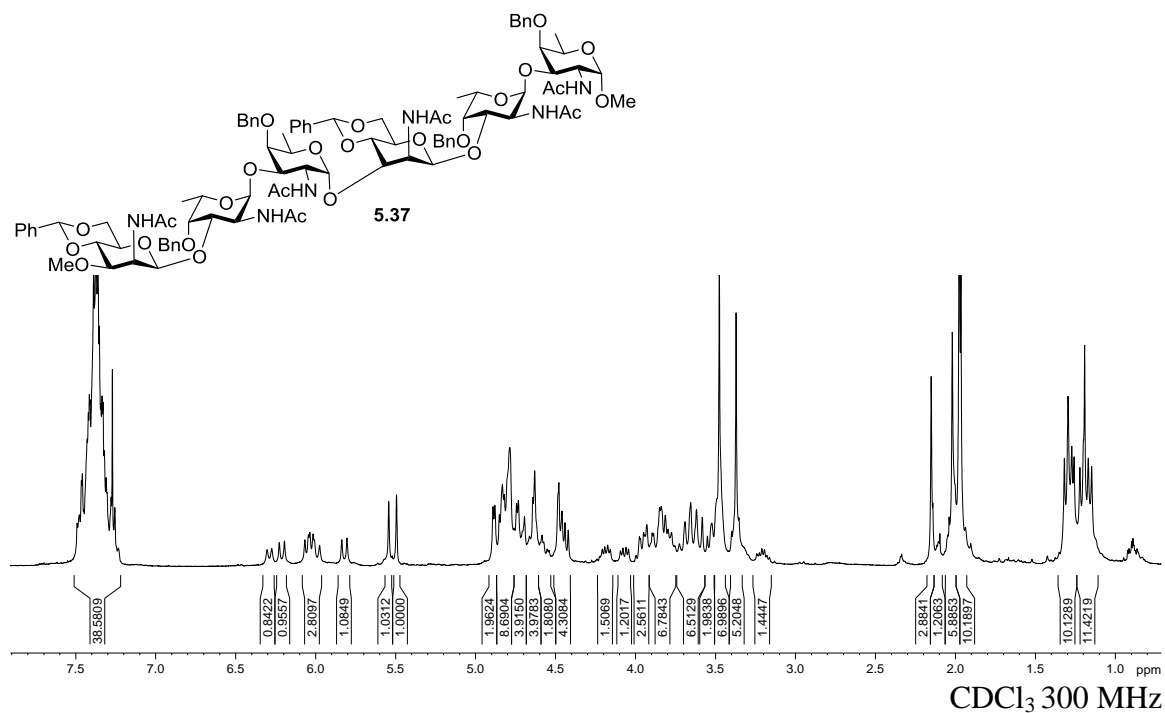


Figure A-109: ¹H NMR spectrum of Methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (5.37)

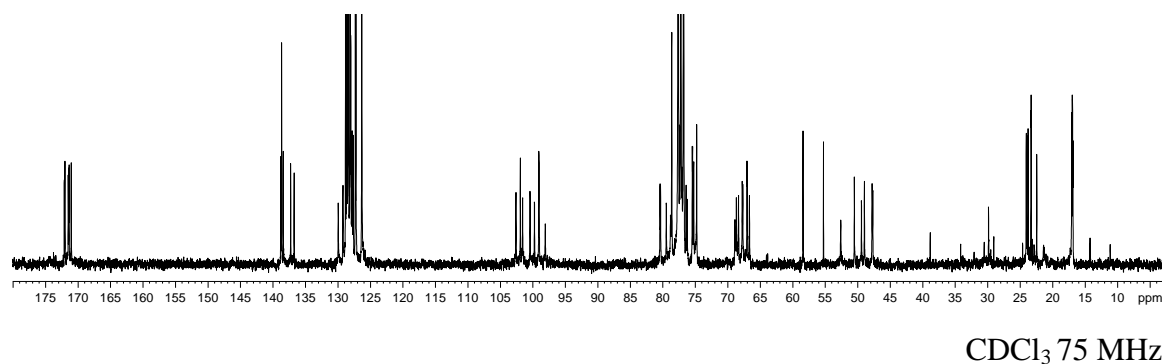


Figure A-110: ¹³C NMR spectrum of Methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (5.37)

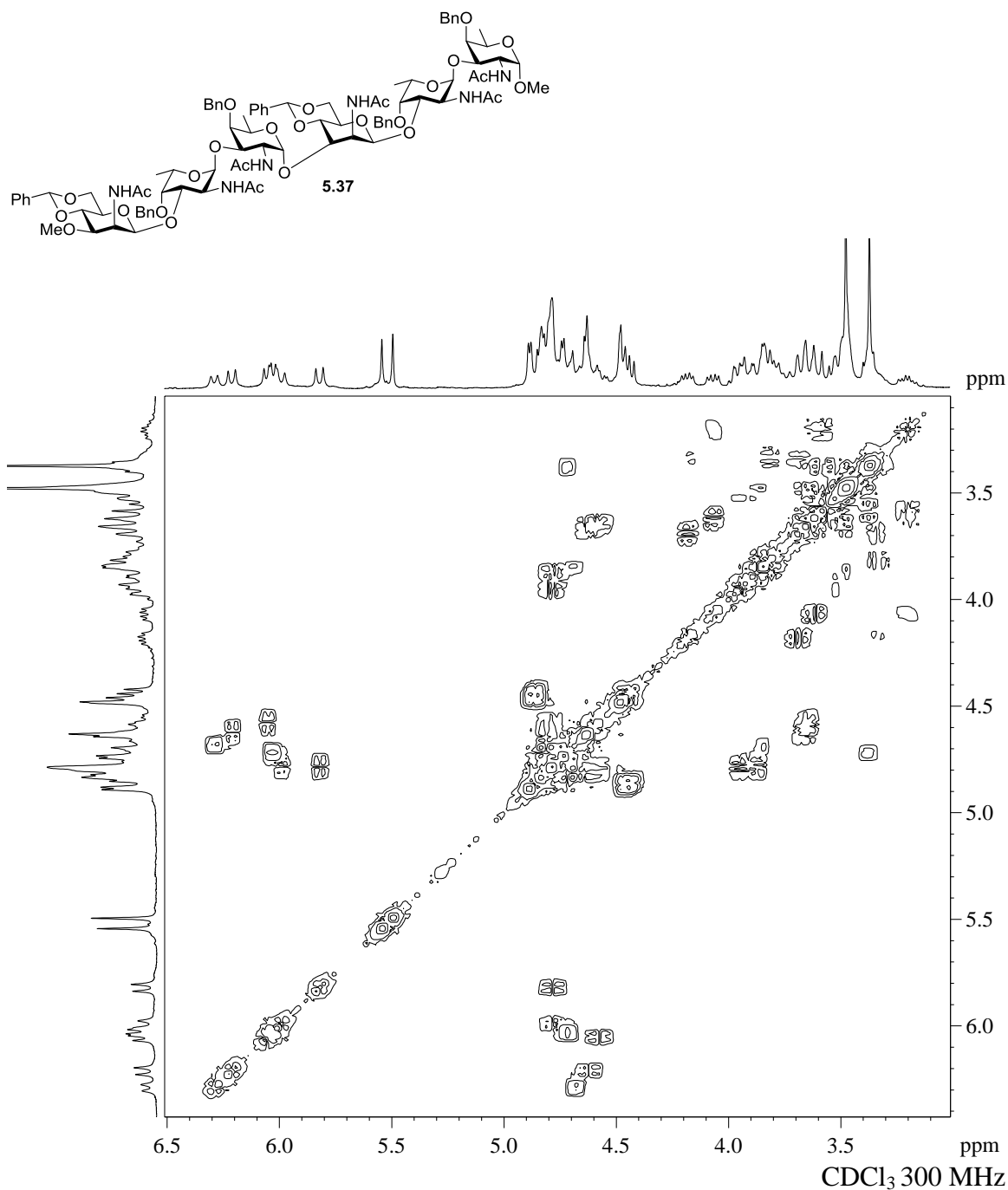


Figure A-111: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-β-*D*-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-*L*-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-*D*-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-*L*-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-*D*-galactopyranoside (**5.37**)

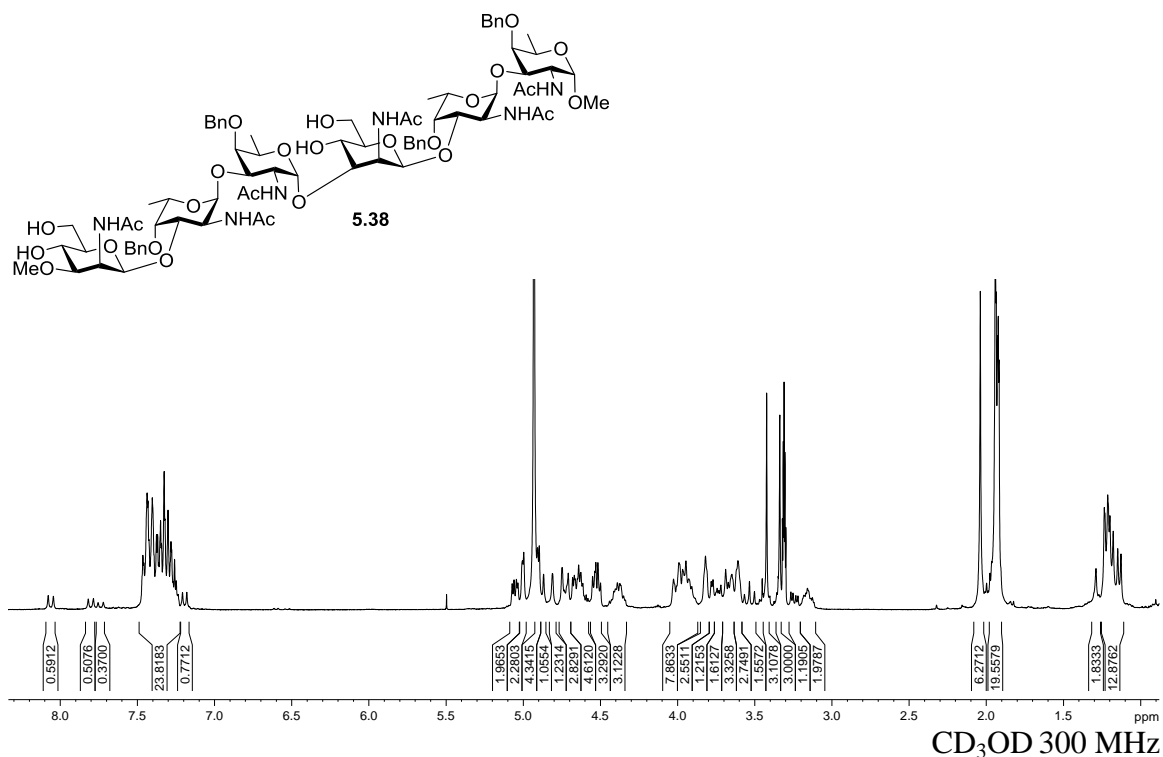


Figure A-112: ¹H NMR spectrum of Methyl *O*-(2-acetamido-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.38**)

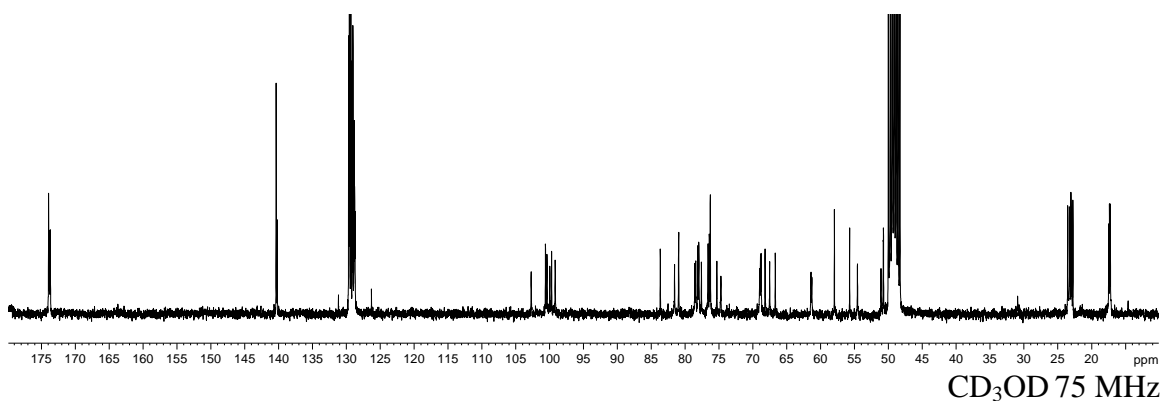


Figure A-113: ¹³C NMR spectrum of Methyl *O*-(2-acetamido-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.38**)

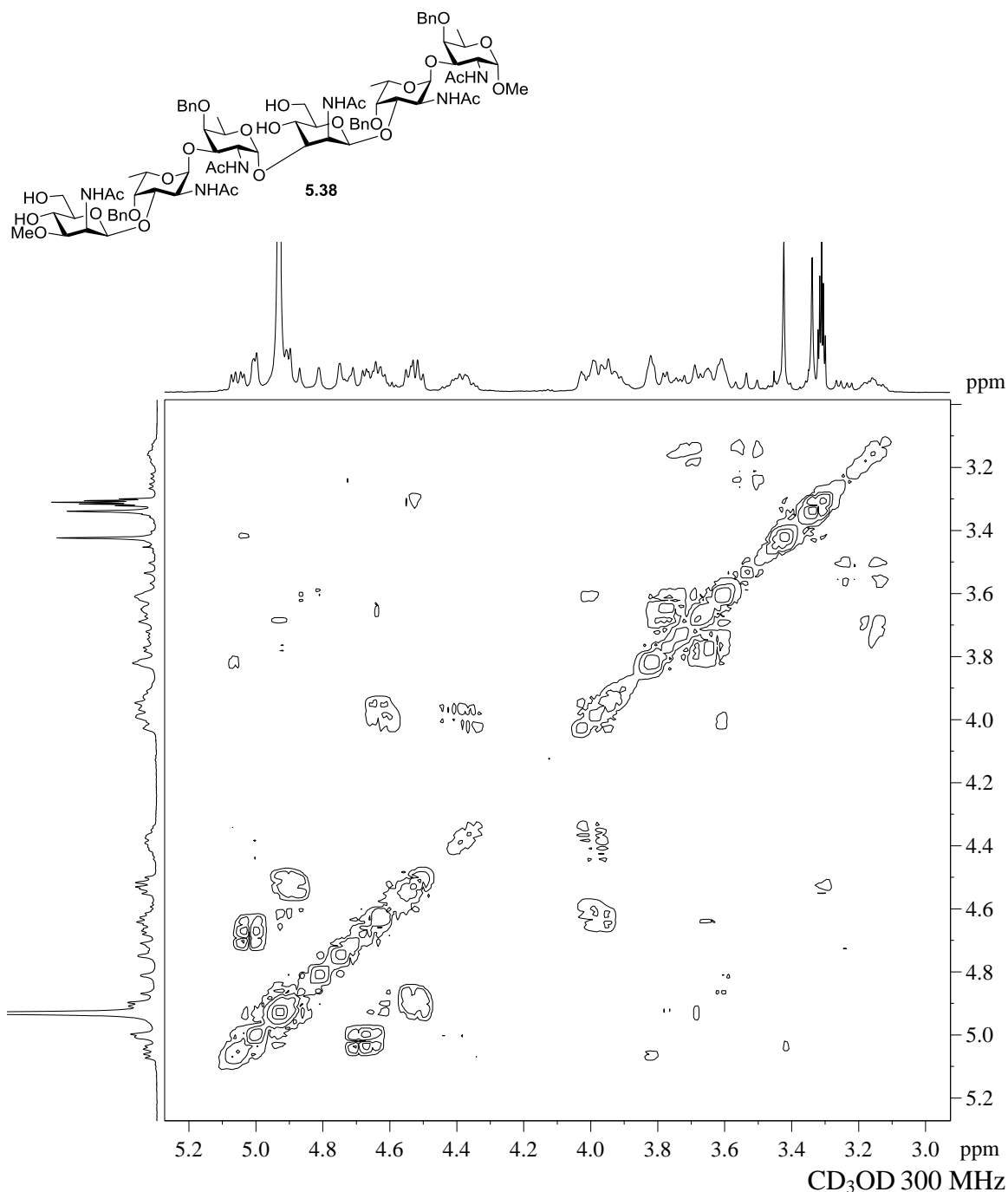


Figure A-114: 2-D NMR COSY spectrum of Methyl *O*-(2-acetamido-2-deoxy-3-*O*-methyl-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-3-*O*-benzoyl-2-deoxy-β-D-mannopyranosyl)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.38**)

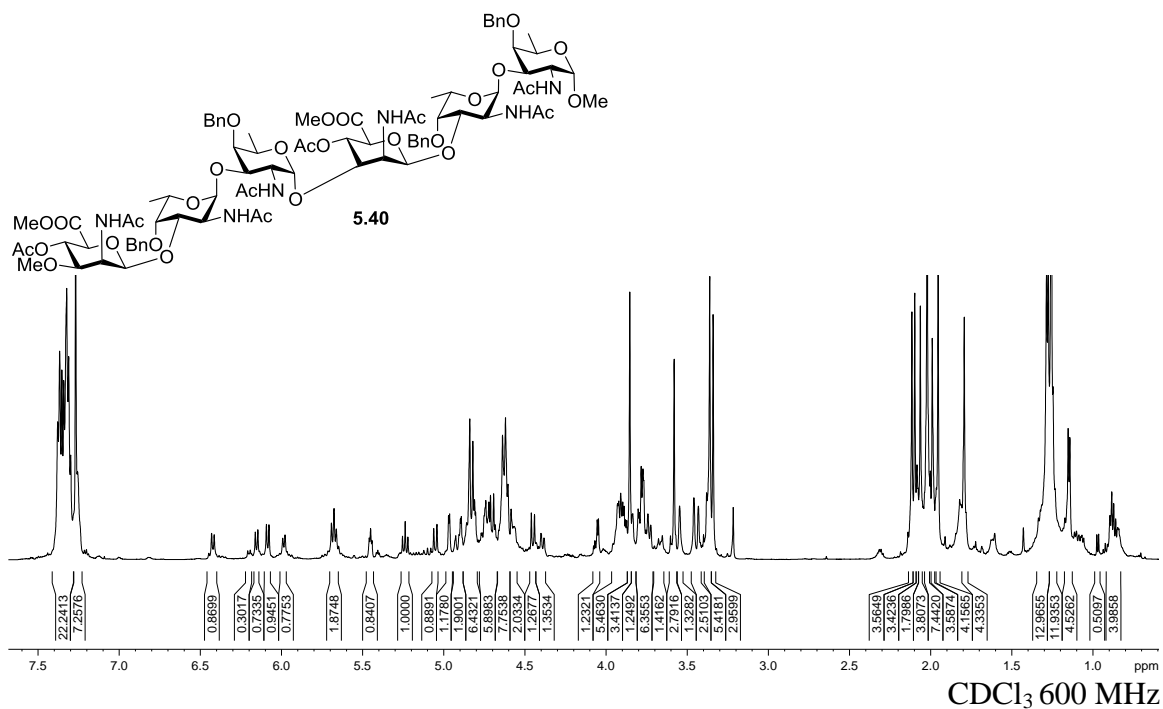


Figure A-115: ¹H NMR spectrum of Methyl *O*-(methyl-2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl-β-D-mannopyranosyluronate)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(methyl-2-acetamido-4-*O*-acetyl-3-*O*-benzoyl-2-deoxy-β-D-mannopyranosyluronate)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.40**)

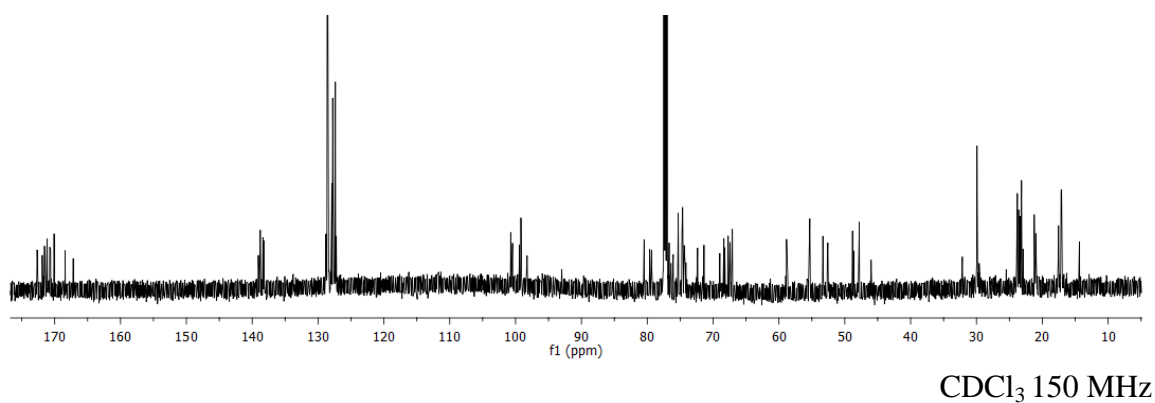


Figure A-116: ¹H NMR spectrum of Methyl *O*-(methyl-2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl-β-D-mannopyranosyluronate)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranosyl)-(1→3)-*O*-(methyl-2-acetamido-4-*O*-acetyl-3-*O*-benzoyl-2-deoxy-β-D-mannopyranosyluronate)-(1→3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-L-galactopyranosyl)-(1→3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy-α-D-galactopyranoside (**5.40**)

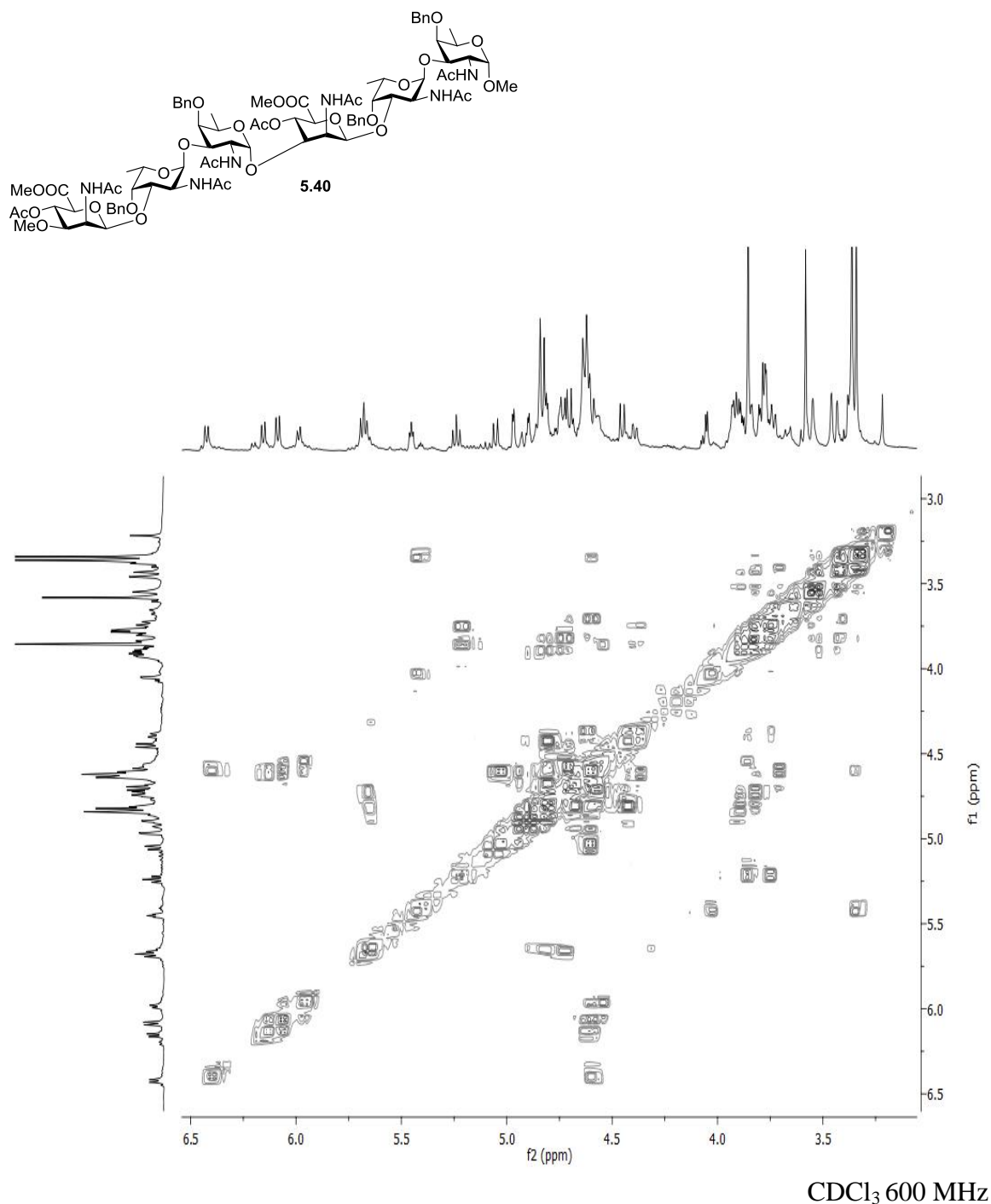


Figure A-117: 2-D NMR COSY spectrum of Methyl *O*-(methyl-2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-methyl- β -D-mannopyranosyl uronate)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-*O*-(2-azido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranosyl)-(1 \rightarrow 3)-*O*-(methyl-2-acetamido-4-*O*-acetyl-3-*O*-benzoyl-2-deoxy- β -D-mannopyranosyluronate)-(1 \rightarrow 3)-*O*-(2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -L-galactopyranosyl)-(1 \rightarrow 3)-2-acetamido-4-*O*-benzyl-2,6-dideoxy- α -D-galactopyranoside (**5.40**)