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# Development of the Regenerative Glycosylation Approach for Manual and Automated Oligosaccharide Synthesis

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Dissertation Committee

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Abstract

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Prof. Alexei V. Demchenko, Chairperson

It has been recognized that carbohydrates are involved in all phases of life beginning with the embryonic development and cell growth. However, it is the involvement of carbohydrates in the progression of various deadly diseases that gave these natural compounds significant roles in diagnostics and as pharmaceuticals. Although carbohydrates are highly desirable to the biological and medical communities, these molecules are still very challenging targets for chemists. Functionalization, derivatization, controlling anomeric stereoselectivity, purification, and characterization are all existing experimental hurdles towards producing synthetic carbohydrates in large quantities and high purity. Advances in chemistry and biochemistry have certainly facilitated the synthesis and purification of carbohydrates. However, the development of practical and general methods for chemical glycosylation and expeditious oligosaccharide synthesis remain an important and challenging area of research.

This topic has also become the basis for this doctoral dissertation wherein the focus was primarily placed on the development of new leaving groups for chemical glycosylation and designing novel methods and technologies for efficient oligosaccharide synthesis.

One outcome of this study is the development of two new classes of building blocks for chemical glycosylation, O-benzoxazolyl (OBox) and 3,3-difluoro-3*H*-indol-2-yl (OFox) imidates. Based on our observation that the OFox leaving group has the same structure before its introduction and after its departure, 3,3-difluoroxindole (HOFox) aglycone, we developed a new regenerative concept for chemical glycosylation. This method allows for performing glycosylation reactions in a conceptually novel way using catalytic amounts of reagents and activators. Further application of the regenerative concept to the HPLC-assisted synthesis on polymer support, an automated technology being developed in our laboratory, was complementary for refining all chemical aspects of both methods.

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## List of Abbreviations

Å.....Angstrom

Ac.....Acetyl

$Ac_2O$……...…Acetic anhydride

$AgBF_4$ .....Silver tetrafluoroborate

$AgClO_4$ .....Silver perchlorate

$Ag_2CO_3$ .....Silver carbonate

$AgNO_3$.....Silver nitrate

$Ag_2O$.....Silver oxide

AgOBox…….…..…..Silver salt of OBox

AgOTf .....Silver trifluoromethanesulfonate

$AgPF_6$……...…...Silver hexafluorophasphate

HOBox……..……..2-Benzoxazolinone

$BF_3$-$OEt_2$.....Boron trifluoride etherate

$Bi(OTf)_3$……..…...…Bismuth(III) trifluoromethanesulfonate

Bn .....Benzyl

BnBr .....Benzyl bromide

Box .....Benzoxazolyl

BSM……..……....Benzenesulfinyl morpholine

$Bu_4NI$.....Tetrabutylammonium iodide

$Bu_4NBr$.....Tetrabutylammonium bromide

Bz .....Benzoyl

$CaH_2$……..…….……Calcium hydride

CAN…….....Cerric ammonium nitrate

Car……..……..Carbamoyl

$CHCl_3$……..Chloroform

$CH_2I_2$……...……...……...…Diiodomethane

$CH_3NO_2$…….……Nitromethane

ClBox…….....2-Chlorobenzoxazole

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

CPME……..……Cyclopentylmethylether

d.....Doublet

DABCO.....1,4–Diazabicyclo[2.2.2]octane

DAST……..……...Diethylaminosulfur trifluoride

DBU……...…1,8-Diazabicycloundec-7-ene

DCE.....1,2-dichloroethane

dd.....Doublet of doublets

DIPEA…….…….*N*,*N*-Diisopropylethylamine

DDQ……2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

DMAP……..……4-Dimethylaminopyridine

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

DMP…….……...……2,4-Dimethoxyphenyl

DMTST……..….Dimethylsulfonium triflate

DTBMP…….…….2,6-di-tert-butyl-4-methylpyridine

DTBPI…….……...2,6-Lutidinium *p*-toluenesulfonate

DTBS……..……..…5,7-di-*tert*-butylsilylene

E.....Electrophile

EDC……..…..1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

Et ..... Ethyl

EtCN……..……...…….Propionitrile

EtOAc .....Ethyl acetate

$Et_2O$.....Diethyl ether

EtOH .....Ethanol

$Et_3SiH$ .....Triethylsilane

Fmoc……..…….Fluorenylmethoxycarbonyl

Glc.....Glucose

h.....Hour(s)

$HClO_4$…….…...Perchloric acid

$HgBr_2$ .....Mercury(II) bromide

$HgCl_2$ .....Mercury(II) chloride

$Hg(CN)_2$ .....Mercury(II) cyanide

HOBox……..……..……2-Benzoxazolinone

HPLC .....High Performance Liquid Chromatography

HR-EI MS .....High Resolution Electron Ionization mass spectrum

HR-FAB MS .....High Resolution Fast Atom Bombardment mass spectrum

Hz .....Hertz

IAD……..……Intramolecular aglycone delivery

IDCP……..……..…Iodonium dicollidine perchlorate

iPr.....Isopropyl

IR.....Infra red

$K_2CO_3$……..…Potassium carbonate

KIE…….…….Kinetic isotope effect

KOH.....Potassium hydroxide

LCG……..…….Low concentration glycosylation

LG .....Leaving group

$LiClO_4$.....Lithium perchlorate

LiOH……...…….Lithium hydroxide

LPTS…….……..2,6-Lutidinium-*p*-toluenesulfonate

m.....Multiplet

m-CPBA…….……meta-Chloroperoxybenzoic acid

Me.....Methyl

MeCN.....Acetonitrile

MeOH.....Methanol

MeOTf.....Methyl trifluoromethanesulfonate

$MgSO_4$…….…….……Magnesium sulfate

min ..... Minute(s)

MS.....Molecular sieves

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

NaOH.....Sodium hydroxide

NaOMe.....Sodium methoxide

NAP…….Naphthylmethyl ether

NBS .....*N*-Bromosuccinimide

NIS .....*N*-Iodosuccinimide

NMR .....Nuclear magnetic resonance

Nu.....Nucleophile

OFox.....3,3-Difluoroxindole

P.....Protecting group

$PdCl_2$……..……..……Palladium chloride

PFP……..….Pentafluoropropionyl

Ph .....Phenyl

PhSeOTf.……..……Phenylselenyl triflate

Pic……Picolinyl

Pico……...Picoloyl

pMB……..…….……....*p*-Methoxybenzyl

pNB…….……....*p*-Nitrobenzyl

ppm .....Parts per million

p-TolSCl.……...…..*p*-Toluenesulfenyl chloride

p-TolSOTf.…….....*p*-Toluenesulfenyl triflate

PTFAI.....N-phenyl trifluoroacetimidate

p-TsOH…….……para-toluenesulfonic acid

$R_f$ .....Retention factor

rt .....Room temperature

s.....Singlet

SBox.....*S*-Benzoxazolyl

$Sm(OTf)_3$……..……...Samarium(III) triflate

$SnCl_4$……..……..Tin tetrachloride

$SOCl_2$……..……..…Thionyl chloride

SPOG……..…..Solvent participation in a one pot glycosylation strategy

STaz ....……..…..……....S-Thiazolinyl

t.....Triplet

TBDMS.....*tert*-Butyldimethylsilyl

TBSOTf.....…..….*tert*-Butylsilyl trifluoromethanesulfonate

TCAI.....Trichloroacetimidate

TFA.....Trifluoroacetic acid

$Tf_2O$.....Trifluoromethanesulfonic anhydride

TfOH ..... Trifluoromethanesulfonic (triflic) acid

THF..... Tetrahydrofuran

TIPDS……...3'5'-Tetraisopropyldisiloxane

TLC .....Thin layer chromatography

TMO…….……..Trimethylene oxide

TMP…….……..1,3,5-Trimethoxyphenyl

TMS..... Trimethylsilyl

$TMSClO_4$…….…….…..Trimethylsilyl perchlorate

$TMS_2O$…….……..Trimethylsilyl anhydride

TMSOTf.....Trimethylsilyl trifluoromethanesulfonate

Tol.....Tolyl

Tr.....Triphenylmethyl

$TrClO_4$ .....Trityl perchlorate

Ts.....4-Tolylsulfonyl

TTBP.....2,4,6-Tri-*tert*-butylpyrimidine

UV.....Ultra violet

$Yb(OTf)_3$ .....Ytterbium(III) trifluoromethanesulfonate

$ZnCl_2$ .....Zinc(II) chloride

$ZnEt_2$……..……...…..Diethyl zinc

$Zn(OTf)_2$ .....Zinc(II) trifluoromethanesulfonate

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# Chapter 1

# Stereocontrolled 1,2-cis glycosylation as the driving force of progress in synthetic carbohydrate chemistry

## 1.1. Introduction

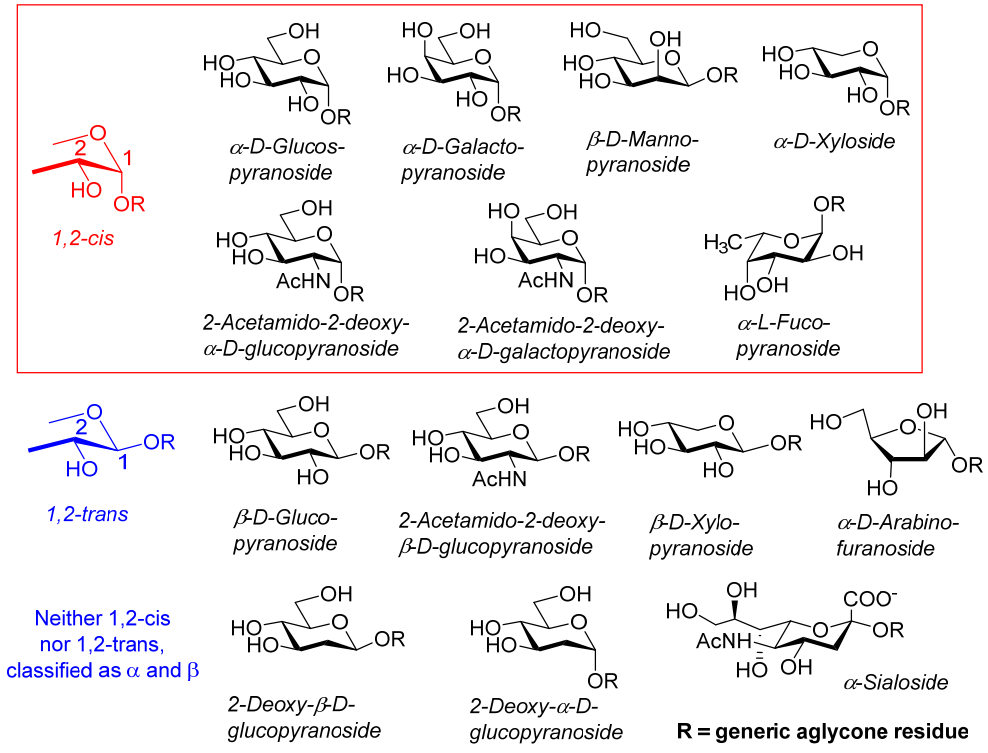
Carbohydrates, as polysaccharides or glycoconjugates, represent the largest class of naturally occurring compounds and are often found as essential components of many bioactive molecules in nature.[1-4] Carbohydrates were initially viewed as energy-storage materials, structural components, and primary metabolites. Now it is known that carbohydrates mediate many fundamental biological processes such as immune defense, fertilization, metastasis, signal transduction, cell growth and cell-cell adhesion. In the past few years, we have been learning that carbohydrates play crucial roles in pathogenesis of diabetes, bacterial and viral infections, inflammation, development and growth of cancers, and many other diseases.[5-9] Clearly, uncovering the contributions of carbohydrates to cell biology would greatly facilitate advances in the field of glycobiology and medicine.[10-13]

For the most part, medicinally important carbohydrates exist as complex oligomers or as conjugates with other biomolecules including natural products,[4,14,15] lipids,[2,16-20] peptides,[21-23] proteins,[3,24-26] etc.[27-31] The carbohydrate part itself exists in various sizes and shapes ranging from monomeric sugars and simple linear chains to highly branched glycoforms. Major obstacles in studying the natural carbohydrates are the difficulties in isolating, characterizing, and synthesizing these molecules due to their low abundance and heterogeneity in nature. While scientists have been able to successfully isolate and characterize certain classes of natural carbohydrates, the availability of pure isolates is still low. As a consequence, the systematic study of these molecules often relies on synthetic chemistry to provide pure compounds in significant quantities.

Among a variety of glycosidic bonds in nature, it is the *O*-glycosidic bonds that are of major interest and challenge to chemists due to their high abundance and difficulty in synthesis. There

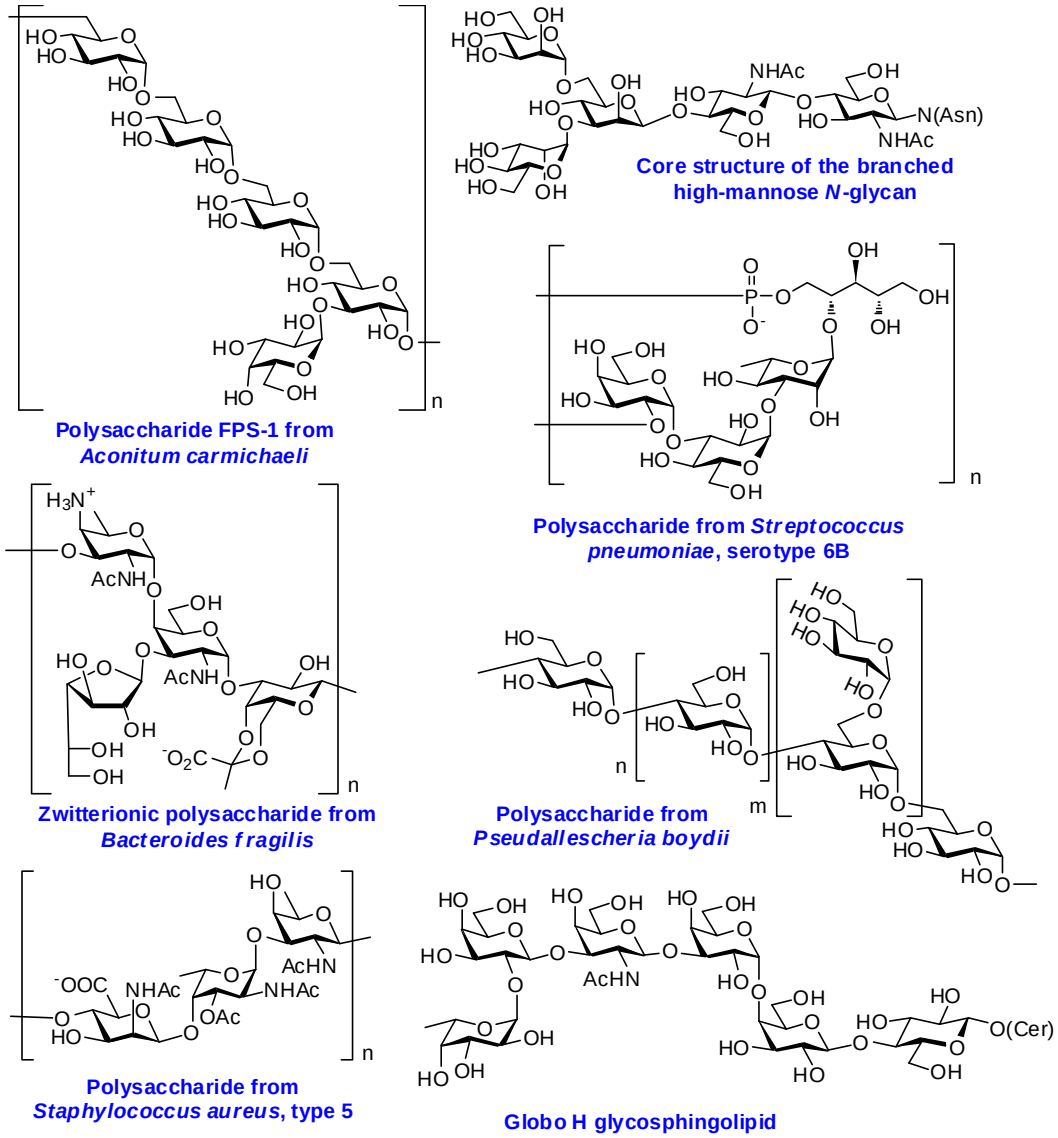
are two major types of *O*-glycosides, which are, depending on nomenclature, most commonly defined as α- and β-, or 1,2-*cis* and 1,2-*trans* glycosides. The 1,2-*cis* glycosyl residues, α-glycosides for D-glucose, D-galactose or β-glycosides for D-mannose, L-rhamnose, as well as their 1,2-*trans* counterparts (β-glycosides for D-glucose, β-glycosides for D-mannose) are both important and abundant classes of linkages and are found as components in a variety of natural compounds. Some other common types of glycosides, for instance 2-deoxyglycosides and sialosides, lack the neighboring substituent. These compounds can neither be defined as 1,2-cis nor 1,2-trans glycosides, hence, these are commonly referred to as α and β glycosides. Representative examples of common glycosides are shown in Figure 1.1.

**Figure 1.1. Common monosaccharide residues found in the mammalian and bacterial glycome.**



Many oligosaccharides containing 1,2-cis *O*-glycosidic linkages are of high importance due to their biological roles and therapeutic potential. Some representative naturally occurring oligosaccharides containing 1,2-cis linkages are shown in Figure 1.2.

**Figure 1.2. Naturally occurring oligosaccharides containing 1,2-*cis* linkages**




For example, immunomodulatory pentasaccharide FPS-1 from *Aconitum carmichaeli* is composed of the α-(1→6) linked glucosyl backbone with some α-(1→3)-glucosyl branching.[32]

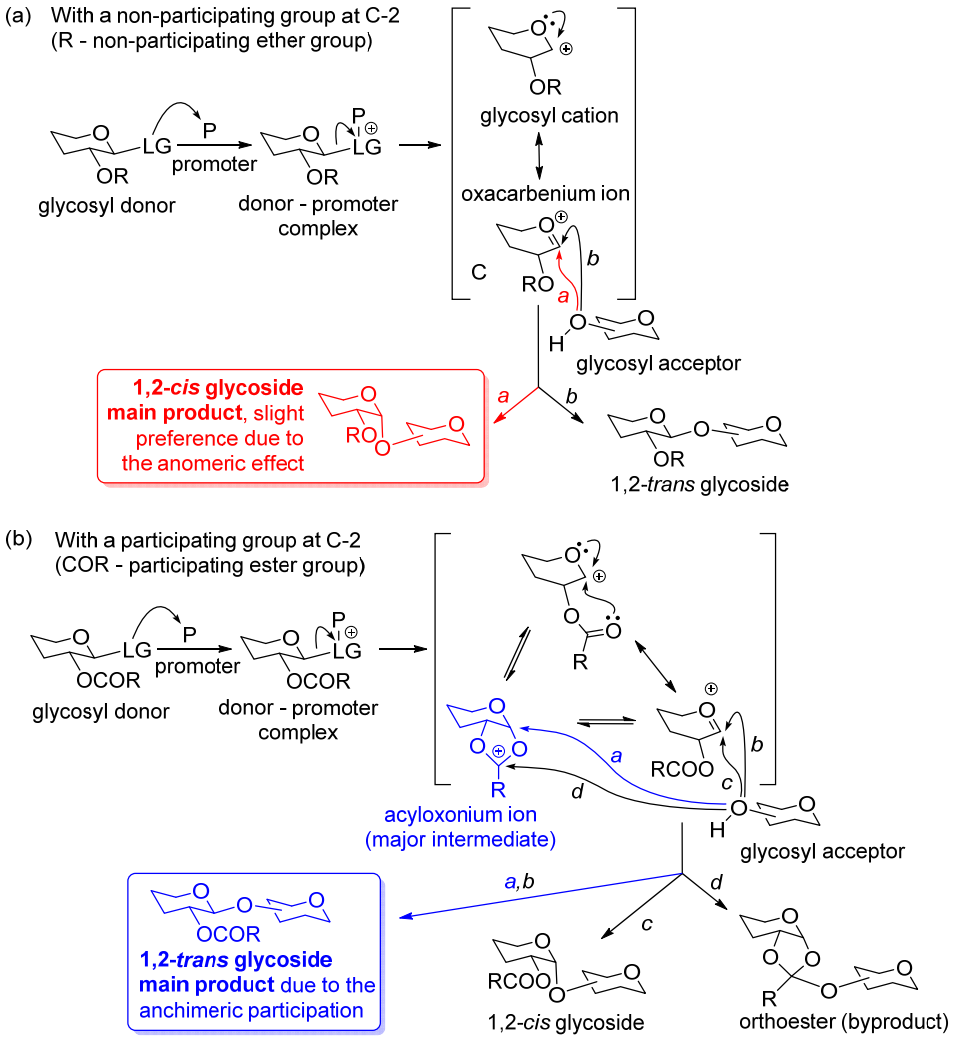
Fungus *Pseudallescheria boydii* consists of the glycogen-like α-(1→4)-linked glucan backbone with occasional α-(1→6)-glucosyl branches.[33,34] Zwitterionic polysaccharide A1 found on the capsule of the bacterium *Bacteroides fragilis* has a 1,2-cis-linked glycosaminoglycan motif.[35] Many pneumococcal polysaccharides possess 1,2-cis glycosidic linkages, for instance polysaccharide from *Streptococcus pneumonia* serotype 6B[36-39] that is included in all current pneumococcal vaccines,[40-44] has α-glucose and α-galactose residues. The trisaccharide repeating unit isolated from *Staphylococcus aureus* type 5[45] possesses uncommon ManNAcA and FucNAc, both 1,2-cis-glycosidically linked. High mannose-type N-linked glycans[46,47] that mediate the pathogenesis of cancers,[27] AIDS,[48] Alzheimer's disease,[49] idiopathic normal pressure hydrocephalus,[49] classical galactosemia,[50] etc. bear an important β-mannose bond. All glycosphingolipids of the globoside family have an α-linked galactose residue. Amongst these, Globo-H, which is a current target for breast and prostate cancer vaccine development,[51,52] has an α-fucose residue as well.

### 1.2. Outline of chemical glycosylation: mechanism, general principles and special cases

Over the last two decades, synthetic carbohydrate chemistry has witnessed a dramatic improvement in the methods available. Glycosylation is arguably the most important albeit challenging reaction in the field of carbohydrate chemistry. Most commonly, it involves the reaction between a glycosyl donor and glycosyl acceptor, in presence of an activator or promoter, to form a glycosidic bond. Upon activation, the promoter-assisted departure of the leaving group results in the formation of a glycosyl cation, which then gets stabilized via an oxacarbenium ion intermediate (Scheme 1.1a). The nucleophile, glycosyl acceptor, can then attack (form the glycosidic bond) either from the top or the bottom face of the flattened ring. This would give rise

to either 1,2-*cis* or 1,2-*trans* glycosides with respect to the neighboring substituent at C-2, and uncontrolled reactions may lead to the mixture of both.

**Scheme 1.1. General outline of glycosylation and the key intermediates involved**




The formation of 1,2-*trans* linkages can be accomplished using the participatory effect of the neighboring 2-acyl substituent. In this case, the oxacarbenium ion can be further stabilized via a bicyclic acyloxonium intermediate, which becomes the key intermediate en route to glycosylation products (Scheme 1.1b). Since the bottom face of the ring can be blocked now, the nucleophilic attack of the glycosyl acceptor would be directed from the opposite, top face,

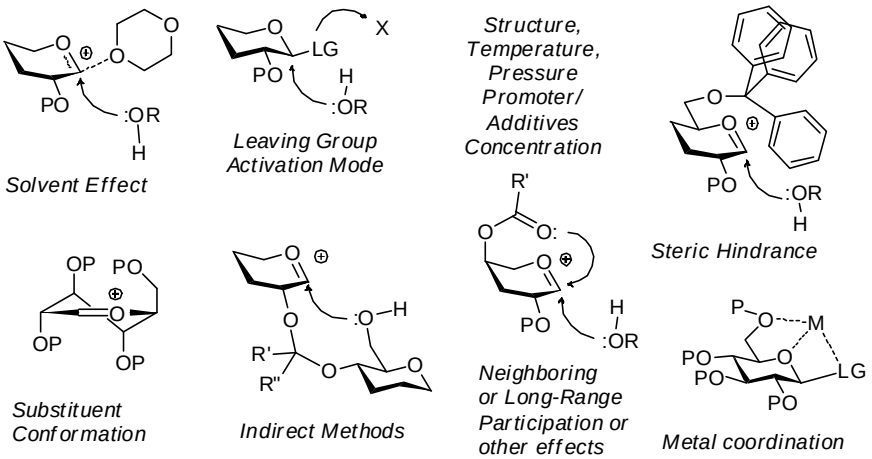
providing access to 1,2-*trans* linkage with a high stereoselectivity. Occasionally, substantial amounts of 1,2-*cis*-linked products are also formed. In these cases, glycosylation assumingly proceeds via oxacarbenium ion, resulting in the formation of 1,2-*trans* and 1,2-cis glycosides, respectively, or most-commonly mixtures thereof. An orthoester formation is another possible process (Scheme 1.1b).

While the stereoselective synthesis of 1,2-*trans* linkages can be reliably achieved with the use of the neighboring group assistance,[53-55] the formation of 1,2-*cis* linkages is typically much more challenging. The presence of a non-participating group is required for the synthesis of 1,2-cis glycosides, but the non-participating group alone cannot ensure the stereoselectivity. Although the α-product is favored by the anomeric effect,[56] the stereoselectivity of glycosylation can be poor and requires other modes of stereocontrol. A variety of reaction conditions and structural elements of the reactants has been developed to obtain excellent 1,2-cis stereoselectivity, no comprehensive method for 1,2-cis glycosylation is yet available. The failure to control the stereoselectivity of glycosylations leads to the formation of a mixture of anomers. Thus, the effective formation of 1,2-*cis* glycosidic linkages with high stereocontrol is a major challenge. [57]

In addition to the apparent complexity of the glycosidation process, there are other competing processes that cannot be disregarded. Side reactions, such as elimination, substitution (formation of unexpected substitution products or hydrolysis at the anomeric center), cyclization (inter- and intramolecular orthoesterification), migration, redox, etc,[58] often complicate stereocontrol and compromise the yields of glycosylations. Several factors are known to affect stereoselectivity and yield of glycosylation and those include temperature, solvent, type of donor used, type of acceptor used, amount and type of promoter used, protecting groups, etc. (Figure 1.3). These

effects and specifically designed methods to control the stereoselectivity of glycosylation will be discussed in the subsequent sections.

**Figure 1.3. Factors affecting the stereoselectivity of glycosylations**




While some sugars follow general trends, there are classes of compounds and glycosidic linkages that require special methods. These special cases of glycosylation require careful selection of techniques, their modification, or design of conceptually new approaches. Indirect or total synthesis-based technologies have been developed and applied specifically to the synthesis of these targets.

Glycosides of 2-amino-2-deoxy sugars, in particular those of the D-gluco and D-galacto series, are widely distributed in living organisms as glycoconjugates (glycolipids, lipopolysaccharides, glycoproteins),[59] or glycosaminoglycans (heparin, heparan sulfate, dermatan sulfate, chondroitin sulfate, hyaluronic acid),[60] etc.[61] Since a vast majority of naturally-occurring 2-amino-2-deoxy sugars are *N*-acetylated, from the synthetic point of view, a 2-acetamido-2-deoxy substituted glycosyl donor would be desirable. For this type of glycosyl donors however, the oxacarbenium ion rearranges rapidly into an unreactive oxazoline intermediate. Therefore, even the synthesis of such 1,2-trans glycosides requires an additional

step and a careful selection of suitable protecting groups. A minimal requirement for the synthesis of 1,2-cis glycosides would be the use of a C-2 non-participating moiety, most commonly azide. 2,3-Oxazolidinone protection introduced by Kerns and *N*-*p*-methoxybenzylidene protection explored by Nguyen also show good promise to become universal approaches to 1,2-cis glycosylation with 2-aminosugars (vide infra).

β-Mannosyl residues are frequently found in glycoproteins. The chemical synthesis of β-mannosides cannot be achieved by relying on the anomeric effect, which would favor axial α-mannosides. In addition, the formation of β-mannosides is further disfavored by the repulsive interactions that would have occurred between the axial C-2 substituent and the nucleophile approaching from the top face of the ring. For many years, the only direct procedure applicable to β-mannosylation - Ag-silicate promoted glycosidation of α-halides - was assumed to follow a bimolecular $S_N2$ mechanism.[62,63] The difficulty of the direct β-mannosylation was addressed by developing a variety of indirect approaches such as C-2 oxidation-reduction, C-2 inversion, anomeric alkylation, and intramolecular aglycone delivery.[64-68] This was the standing in the field before Crich and co-workers discovered that 4,6-*O*-benzylidene protected sulfoxide[69] or thioglycoside[70] glycosyl donors provide excellent β-manno stereoselectivity. Detailed mechanistic and spectroscopic studies by the Crich group[65,71-73] showed that anomeric α-*O*-triflates generated in situ are reactive intermediates that can substituted with high stereocontrol at low temperatures.

In comparison to their six-membered counterparts, furanosides are less abundant. Nevertheless, their presence in a variety of polysaccharides from bacteria, parasites, and fungi makes this type of glycosidic linkage an important synthetic target.[74,75] The synthesis of 1,2-trans furanosides is relatively straightforward and, similarly to that of pyranosides, can be reliably

achieved with the use of glycosyl donors bearing a participating group at C-2. In contrast, construction of 1,2-cis glycofuranosidic linkage is difficult, even more so than with pyranosides due to the lack of the anomeric effect and the conformational flexibility of the five-membered ring. In fact, both electronic and steric effects favor the formation of 1,2-*trans* glycofuranosides. In the past decade, a notable improvement in 1,2-*cis* furanosylation was made possible with glycosyl donors in which the ring has been locked into a single conformation. These examples include 2,3-anhydro,[76-80] 3,5-O-(di-*tert*-butylsilylene),[81,82] and 3,5-*O*-tetraisopropyldisiloxanylidene[83] protected bicyclic glycosyl donors. A recent example wherein stereoselective 1,2-cis glycofuranosylation was accomplished with the assistance of H-bond mediated aglycone delivery will be discussed below.

2-Deoxyglycosides are important constituents of many classes of antibiotics. The development of reliable methods for stereoselective synthesis of both α- and β-2-deoxyglycosides is critical for the synthesis of natural products, drugs and glycomimetics.[84,85] It should be noted that due to the lack of anchimeric assistance from the substituent at C-2, the synthesis of both types of linkages represents a notable challenge. Direct glycosylation of 2-deoxy glycosyl donors often results in the formation of anomeric mixtures, though notable recent progress in the area has to be acknowledged.[86-91]

Sialic acids are nine-carbon monosaccharides involved in a wide range of biological phenomena. Their unique structure is characterized by the presence of a carboxylic group, deoxygenated C-3, glycerol chain at C-6 and differently functionalized C-5. The natural equatorial glycosides and their unnatural axial counterparts are classified as α- and β-glycosides, respectively. In spite of extensive efforts and notable progress, the chemical synthesis of sialosides remains a significant challenge.[92-95] The presence of a destabilizing electron-

withdrawing carboxylic group and the lack of a participating auxiliary often drive sialylation reactions toward competitive elimination reactions resulting in the formation of a 2,3-dehydro derivative and/or in poor stereoselectivity (β-anomer). To overcome these problems, a variety of leaving groups and activation conditions for direct sialylations have been developed. It was also demonstrated that the N-substituent at C-5 plays an influential role in both stereoselectivity of sialylation and the reactivity of sialyl donors.[96] A particular advance in the recent years has been made with 4,5-oxazolidinone derivatives that provide high yields and stereoselectivities in sialylations.[97-100]

### 1.3. Effect of the glycosyl donor

Varying the nature of anomeric leaving groups in the donor has been exploited in many different ways. Glycosylations using trichloroacetimidates[101-104] and thioglycosides[105] as donors have become the most widely studied methods for chemical glycosylation. There are many publications describing the comparison of various glycosylation methods applied for particular targets. Our previous reviews on 1,2-cis glycosylation, thoroughly discusses all pros and cons of using various leaving groups.[106,107] However, only a few principles could be reliably outlined. It has been unambiguously demonstrated that halides activated in the presence of a halide ion (from *e.g.* $Bu_4NBr$) often provide the highest ratios of α/β-glycosides.[108-112] Since glycosylation reactions commonly follow a unimolecular $S_N1$ displacement mechanism, the orientation of the leaving group at the anomeric center is of little importance. However, occasionally glycosylation reactions proceed via an $S_N2$-like mechanism with inversion of the anomeric configuration: β-glycosyl halides formed from their α-counterparts with bromonium ion[113] or from α-thioglycosides in the presence of bromine,[114] glycosyl thiocyanates,[115,116] and anomeric

mannosyl triflates formed in situ from sulfoxides or thioglycosides for the synthesis of □-mannosides.[69,70]

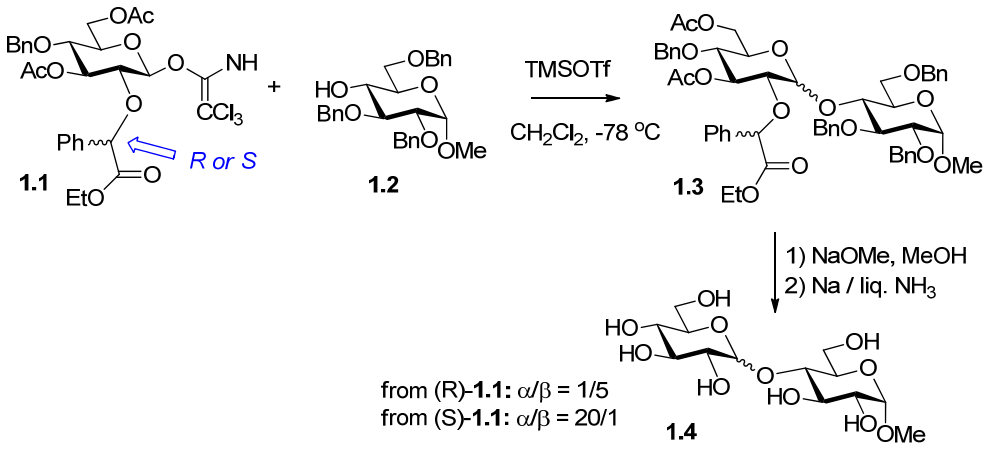
Protecting groups are introduced into a molecule to temporarily mask a functional group, thereby differentiating similar types of functional groups and exposing only the one that needs to be reacted.[117] It is well known that the stereoselectivity of glycosylation can be profoundly influenced by protecting groups.[118] Various protecting groups that may have a significant effect on the stereoselectivity of glycosylation have been discussed below. Neighboring protecting groups at C-2 traditionally known as participating groups for the synthesis of 1,2-trans glycosides can now assist in the formation of either 1,2-cis or 1,2-trans glycosides. Remote protecting groups at positions C-3, 4 and/or 6 may affect the stereoselectivity via remote assistance by means of participation, H-bond mediated aglycone delivery, steric hindrance and/or electron withdrawal. Also discussed in this section are protecting groups that restrict the conformational flexibility of carbohydrates or force carbohydrate molecules to adopt unusual conformation.

### 1.3.1. Neighboring protecting group at C-2

As aforementioned, neighboring acyl-type protecting group offers one of the most powerful tools to direct stereoselectivity toward the formation of a 1,2-*trans*-linked product. Demchenko and co-workers developed glycosyl donors equipped with a 2-picolinyl ether substituent that can also participate and form 1,2-trans glycosides stereoselectively.[119,120] Boons and co-workers took a different approach for developing a participating group capable of the participation from the opposite face of the ring giving rise to 1,2-cis linked glycosides.[121] According to this approach, the C-2 position was protected with a substituted benzyl group that contains a nucleophilic

moiety capable of participation at the anomeric center.[121] On activation of the glycosyl donor, the resulting oxacarbenium ion is attacked by this nucleophilic moiety. This attack, in principle, can lead to the formation of a *cis*- or *trans*-decalin-like system, Boons and co-workers showed that the selectivity is highly dependent on the configuration of the asymmetric center of the chiral auxiliary. To accommodate the bulky phenyl group in the pseudo-equatorial position of the newly formed six-membered ring, auxiliary with (S)-stereochemistry would lead to a *trans*-decalin-like intermediate. This intermediate would help to direct the nucleophilic attack of the glycosyl acceptor from the bottom face and hence lead to a 1,2-*cis*-linked glycoside. Conversely a chiral auxiliary with the opposite (R)-configuration could participate via the *cis*-decalin-like intermediate, thereby producing 1,2-*trans* glycoside. Ethyl mandelate was chosen to test this methodology because both the enantiomers are readily available, conditions required for its installation are compatible with other protecting groups, and it is stable during the glycosylation, but can be readily removed under mild reductive conditions.

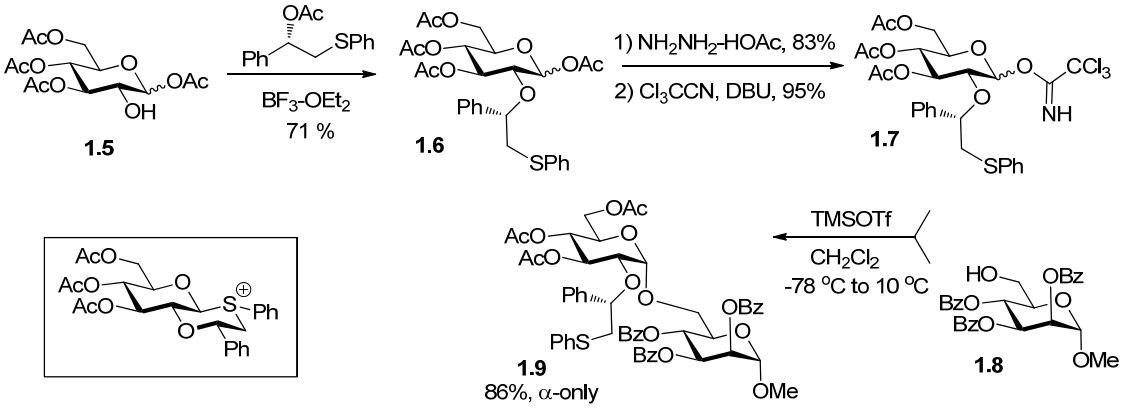
**Scheme 1.2. Stereoselective glycosylations with ethyl (R)- and (S)-mandelate protected glycosyl donor 1.1**



As depicted in Scheme 1.2, when ethyl (S)-mandelate-protected donor (S)-**1.1** was glycosylated with glycosyl acceptor **1.2**, disaccharide **1.3** was obtained with high α-selectivity (α/β = 20/1). Conversely, when (R)-**1.1** was used as the glycosyl donor, reversal of anomeric selectivity was observed (α/β = 1/5). Deprotection of the acyl groups using sodium methoxide in methanol and the benzyl groups, including the chiral auxiliary, under Birch reduction conditions provided disaccharide **1.4**.

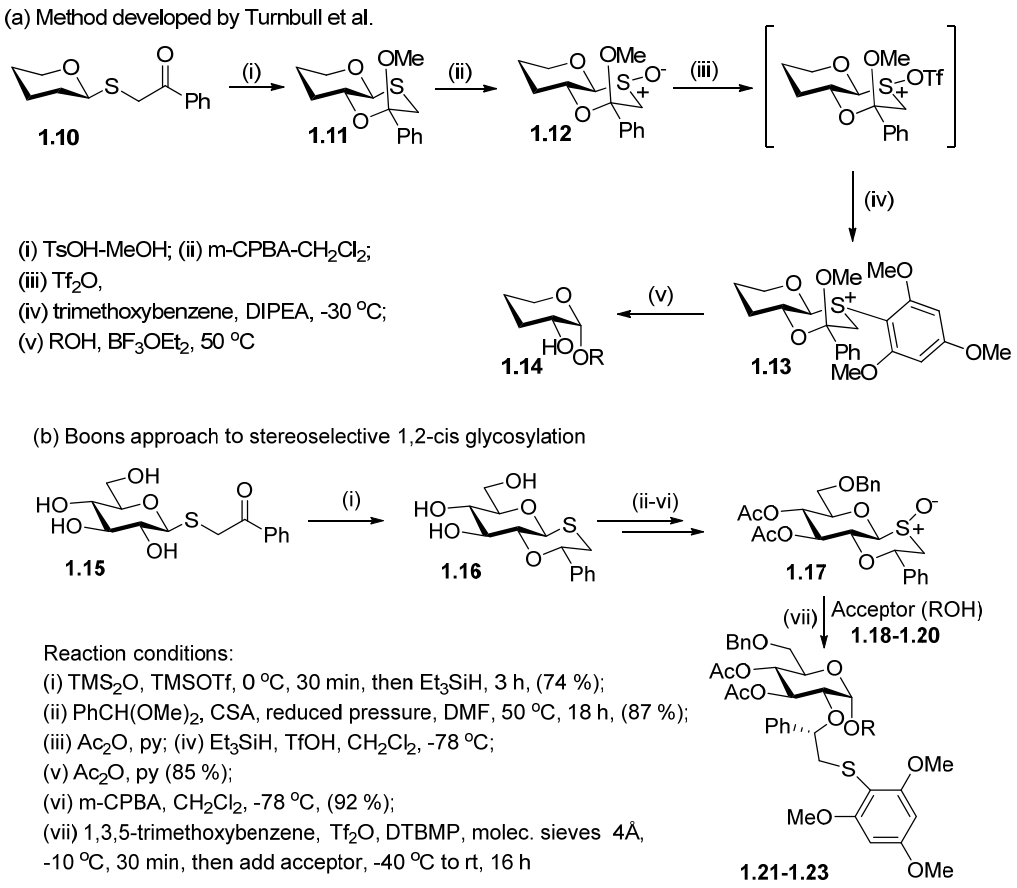
The second generation auxiliary developed to further enhance 1,2-cis stereoselectivity was based on (S)-phenyl-thiomethylbenzyl ether moiety at C-2 of the glycosyl donor.[122,123] It was assumed that this type of moiety would be capable of more efficient and stereoselective participation via the formation of a chair and hence more stable *trans*-decalin-like intermediate. This, in turn, would force the (S)-phenyl group into the equatorial position to avoid unfavorable 1,3-diaxial interactions that would have occurred if the bulky phenyl group was placed into the axial position. As depicted in Scheme 1.3, 1-(S)-phenyl-2-(phenylsulfanyl)ethyl ether protected trichloroacetimidate donor **1.7** was obtained from glucose tetraacetate **1.5** via sequential protection, liberation of the anomeric hydroxyl and the introduction of the imidoyl leaving group. Glycosyl donor **1.7** was then reacted with glycosyl acceptor **1.8** in the presence of TMSOTf to afford α-glycoside **1.9** in 86% yield and with exclusive α-stereoselectivity. The auxiliary can then be removed by acetolysis in the presence of $BF_3$-$OEt_2$ and acetic anhydride. This method has been extended to the polymer-supported synthesis of the repeating unit of the immune-modulatory polysaccharide from *Aconitum carmichaeli* composed of an α-(1→6)-linked glucosyl backbone branched with α(1→3)-linked glucoside moieties.[124]

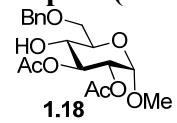
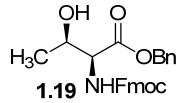
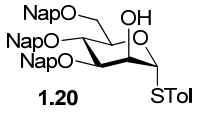
**Scheme 1.3. Synthesis of C-2 (*S*)-phenyl-thiomethylbenzyl ether-protected glycosyl donor 1.6 and its glycosidation**




More recently, to simplify this approach, Boons and co-workers adopted a different direction towards the synthesis of 1,2-cis glycosides.[125] This was certainly inspired by their earlier work on chiral auxiliaries and inherent drawbacks related to the necessity of obtaining pure enantiomeric substrate, and the relatively low stability of the moiety. Additional inspiration came from work by Turnbull et al. who developed a very elegant approach using thioglycoside donors having an integral α-directing group.[126] As depicted in Scheme 1.4a, these reactions proceeded via bicyclic intermediates and the cyclic thioglycoside **1.11** was activated via the oxidation into sulfoxide **1.12** and S-arylation to form reactive sulfonium ions **1.13** en route to O-glycosylation. In the Boons' approach depicted in Scheme 1.4b,[125] sulfoxide donor **1.16** was prepared from thioglycoside **1.15** by the treatment with trimethylsilyl anhydride ($TMS_2O$) in presence of TMSOTf, followed by reduction with $Et_3SiH$. The latter **1.16** was subjected to a series of protecting group manipulations followed by oxidation with m-CPBA to give sulfoxide **1.17**. The glycosidation of donor **1.17** included the treatment with trifluoromethanesulfonic anhydride ($Tf_2O$), arylation with 1,3,5-trimethoxybenzene, followed by addition of the glycosyl acceptor.

**Scheme 1.4. Stereoselective glycosylation via cyclic sulfonium ions**




| Entry | Acceptor (ROH) | Product | Yield, α/β ratio |
|---|---|---|---|
| 1 |  1.18 | **1.21** | 62%, 25/1 |
| 2 |  1.19 | **1.22** | 89%, α-only |
| 3 |  1.20 | **1.23** | 72%, α-only |

As summarized in Scheme 1.4b, glycosyl acceptors **1.18-1.20** provided the corresponding disaccharides **1.21-1.23** in high yields and stereoselectivities.[125] It was observed that while the donors bearing electron-withdrawing groups at C-3, 4, and 6 gave only the α-anomer, their 4,6-diether substituted counterpart suffered from a slight loss of α-anomeric selectivity. It was

concluded that the highly reactive sulfonium ions partially react via oxacarbenium ion intermediate.

Building upon their previous work, Turnbull and co-workers recently designed a new oxathiane donor scaffold where the axial methoxy group was replaced with *O*-substituent constrained in a spirocyclic ring.[127] As in the previous methods, the oxathiane spiroketal donor is then activated via S-arylation. Overall, a novel class of oxathiane glycosyl donors is easily accessible, highly α-selective in glycosylations, and offers high stability towards common protecting group manipulations.

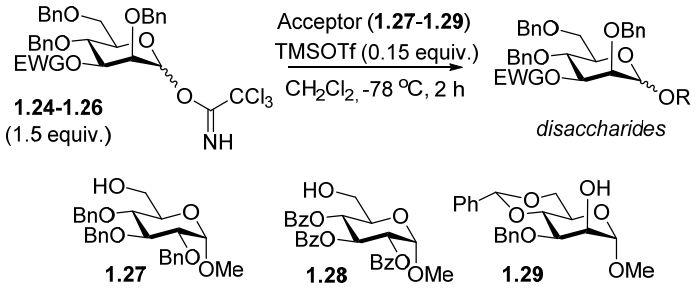
### 1.3.3. Remote protecting groups

The effects of the remote substituents have long been considered of somewhat lesser importance than that of the neighboring substituent at C-2. However, the idea of participating groups at remote positions other than C-2 has been brought to the attention by many researchers. There have been various reports, both in favor and in opposition of the idea of remote participation, starting from long-range 6-O-acyl or carbonate group assisted synthesis of α-glucosides.[128-132] For derivatives of the D-galacto series a remote effect beneficial for the formation of α-galactosides was noted when a participating moiety was present at C-4.[132-135]. Similar effects (including C-3 participation) were also detected for the derivatives of the L-fuco,[136-138] L-rhamno,[139] D-manno,[140] D-gluco[63,141,142] series.

In 2009, Kim presented a dedicated study of the effect of 3- and 6-*O*-acetyl donors on the stereoselectivity of mannopyranosylation.[143] The comparative study showed that there was the remote participation by 3-*O* and 6-*O* acetyl groups, but no participation by 4-*O*-acyl group. It was observed that when mannopyranosyl trichloroacetimidate donors bearing electron

withdrawing ester groups, such as acetyl (**1.24**) or benzoyl (**1.25**) at C-3 position, were coupled with primary acceptors **1.27-1.29** in presence of TMSOTf as a promoter, the corresponding disaccharides were obtained in excellent yields (88-94%) and high β-selectivity (α/β = 1/26-40, entries 1-4, Table 1.1). However, when benzyl sulfoxide was used as an electron withdrawing protecting group at C-3, the selectivity obtained with donor **1.26** was reversed and the corresponding disaccharides were obtained with preferential α-selectivity (α/β = 1/10-16, entries 5 and 6, Table 1.1).

**Table 1.1. Effect of 3-*O*-acyl protection on the stereoselectivity of mannosylation**

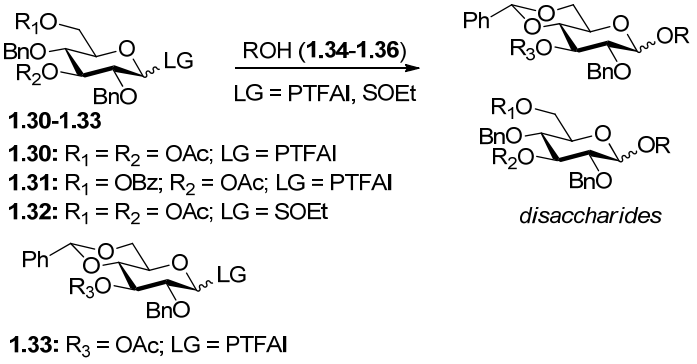



| Entry | Donor (EWG) | Acceptor (1.0 equiv.) | Disaccharide yield | α/β ratio |
|---|---|---|---|---|
| **1** | **1.24** (Ac) | **1.27** | 91% | 1/25.9 |
| **2** | **1.24** (Ac) | **1.28** | 94% | 1/39.0 |
| **3** | **1.24** (Ac) | **1.29** | 92% | 1/40.4 |
| **4** | **1.25** (Bz) | **1.28** | 88% | 1/29.6 |
| **5** | **1.26** ($SO_2Bn$) | **1.27** | 95% | 15.9/1 |
| **6** | **1.26** ($SO_2Bn$) | **1.28** | 93% | 10.2/1 |

Very recently, Nifantiev et al. studied the effect of an acyl substituent at C-3 position on the stereoselectivity obtained with conformationally flexible and conformationally restricted glucosyl donors.[144] Partially acylated *N*-phenyltrifluoroacetimidate and sulfoxide glycosyl

donors showed no significant difference in stereoselectivity between the rigid or flexible 3-*O*-acetylated structures. Irrespectively of the protecting group at C-3, the conformationally rigid 4,6-*O*-benzylidene-protected glucosyl donors gave excellent α-selectivity at higher reaction temperatures (-35 $^{o}$C to rt).

**Table 1.2. Effect of an acyl substituent at C-3 position on the stereoselectivity**



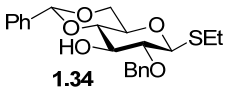
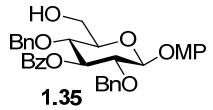
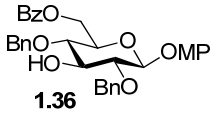
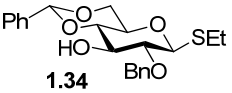
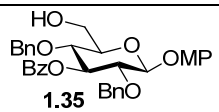

**1.30-1.33**

**1.30:** $R_1 = R_2$ = OAc; LG = PTFAI

**1.31:** $R_1$ = OBz; $R_2$ = OAc; LG = PTFAI

**1.32:** $R_1 = R_2$ = OAc; LG = SOEt

**1.33:** $R_3$ = OAc; LG = PTFAI

| Entry | Donor | Acceptor | Yield, α/β ratio |
|---|---|---|---|
| 1[a] | **1.30** |  **1.34** | 89%, 5.3/1 |
| 2[a] | **1.30** |  **1.35** | 93%, 11.2/1 |
| 3[a] | **1.31** |  **1.36** | 93%, 16.4/1 |
| 4[b] | **1.32** |  **1.34** | 59%, 6.8/1 |
| 5[c] | **1.33** |  **1.35** | 96%, 5.9/1 |

Conditions: [a] - MeOTf, $CH_2Cl_2$, AW-300, -35 → -15 $^{o}$C; [b] - $Tf_2O$, DTBMP, $CH_2Cl_2$, -78 → 0 $^{o}$C; [c] - MeOTf, $CH_2Cl_2$, AW-300, 20 $^{o}$C.

Thus, as depicted in Table 1.2, when glycosyl donor **1.30** bearing acetyl protecting groups at C-3 and C-6 is reacted with glycosyl acceptors **1.34** and **1.35** the corresponding disaccharides are obtained in good yields and with high selectivities (α/β = 5.3-11.2/1, entries 1 and 2). Suprisingly, when glycosyl donor **1.31** wherein the C-6 acetyl was replaced with C-6 benzoyl was used, further increase in selectivity was observed (α/β = 16.4/1, entry 3). In this context, 3,6-di-O-acetyl protected sulfoxide donor **1.32** provided lower yields and stereoselectivities (entry 4). A similar selectivity albeit excellent yield was observed with conformationally restricted 4,6-*O*-benzylidene-protected glucosyl donors **1.33** (96% yield, α/β = 5.9/1, entry 5).

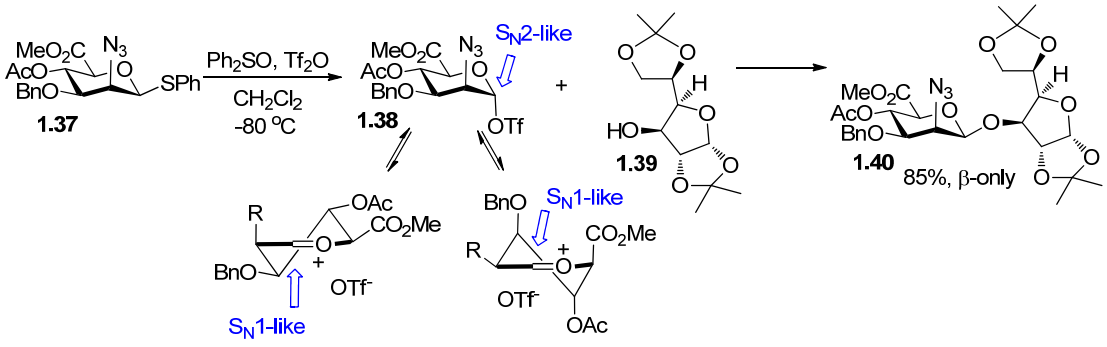
It was also found that steric bulkiness or strong electron-withdrawing properties of remote substituents, particularly those at C-6, are beneficial for 1,2-*cis* glucosylation and galactosylation most likely due to shielding (sterically or electronically) the top face of the ring and therefore favoring the nucleophilic attack from the opposite side.[63,141,145-150] Ito and co-workers also reported that the use of 4-*O*-pentafluoropropionyl group is beneficial for synthesis of α-galactosides.[151] A study with 2-azido sugars revealed an interesting relationship between the stereoselectivity and the effect of remote participating groups in $GalN_3$ and $GlcN_3$ sugars.[152] Over the course of this study it was observed that for $GlcN_3$ sugars, acetyl groups at C-3 and C-6 positions show more α-directing effect whereas 4-*O*-acetyl is more β-directing.[153] Crich showed that bulky 3-O-*tert*-butyldimethylsilyl (TBDMS) can push the axial 2-O-benzyl of mannosyl donors towards the anomeric center, thereby hindering the nucleophilic attack from the top face, leading to poor β-selectivities.[154] Differently, naphthylpropargyl ether protection at C-2 or C-3 favors high β-manno selectivity.[155]

Codee and co-workers investigated the use of 2-azidomannouronate ester donor for glycosidation, and observed high 1,2-*cis* selectivity.[156] On gaining further insight into the

mechanism, it was concluded that the first step involves the formation of a mixture of anomeric triflates in which the triflate with a $^{1}C_4$ chair conformation is the predominant species. This species accommodates the anomeric triflate in the equatorial position. On decomposition of the triflate, oxacarbenium ion intermediate preferentially adopts $^{3}H_4$ half-chair conformation. In this case, the C-5 carboxylate occupies a pseudo-axial position allowing space-stabilization of the positive charge at the anomeric center. Accordingly, when thiophenyl donor **1.37** was activated in the presence of diphenyl sulfoxide and triflic anhydride, anomeric triflate **1.38** was formed. The later was then reacted with acceptor **1.39** to give disaccharide **1.40** in 85% yield and with complete β-selectivity (Scheme 1.5.).

The presence of anomeric triflate, C-5 ester and the C-2 azide together render the anomeric center more electron deficient. As a result, the incoming nucleophile attacks the half chair along a pseudo-axial trajectory on the β-face of the molecule to produce 1,2-*cis* linkage. It was also shown that the stereoselectivity obtained is independent of the type of donor used and does not rely on the preactivation protocol.[157] It was also reported that the stereodirecting effect of the C-5-carboxylate ester is important for obtaining 1,2-*cis* selectivity.

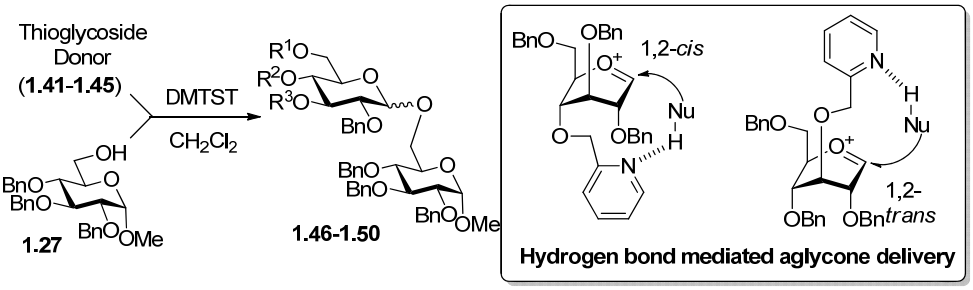
**Scheme 1.5. Rationalization of the high β-selectivity achieved with 2-azidomannouronate thiophenyl donor 1.30**

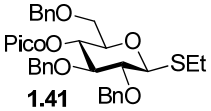
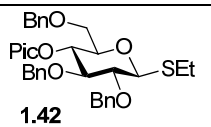
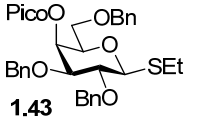
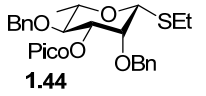
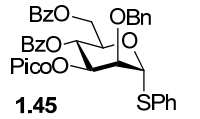


A very different stereodirecting effect was discovered for remote picolinyl (Pic) and picoloyl (Pico) substituents. As aforementioned, picolinyl at C-2 formally participated at the anomeric center and gives 1,2-trans glycosides via six-membered ring intermediate.[120] The action of the remote picolinyl and related picoloyl substituents is totally different. Not being able to participate at the anomeric center directly, picolinyl nitrogen forms the hydrogen bond with the incoming glycosyl acceptor. As a result, very high facial selectivity, always anti in respect to the picolinyl substituent is observed.[158] This, rather unexpected involvement of remote picolinyl substituents was termed as H-bond-mediated aglycone delivery (HAD). Based on the above hypothesis, it was shown that under high dilution conditions (5 mM), 4-*O*-picoloyl or picolinyl glucosyl donors (**1.41**-**1.45**) provide faster coupling times and enhanced selectivity compared to that obtained under standard concentration (50 mM). Thus, glucosyl donors **1.41** and **1.42** provided high levels of α-selectivity, particularly with O-picolinyl protection (α/β = >25/1, entry 1, Table 1.3.). Respectively, galactosyl donor **1.43** and rhamnosyl donor **1.44** gave high β-selectivity (α/β = >1/ 25, entries 3 and 4). An extension to this study, Demchenko and co-workers showed that the presence of 3-*O*-picoloyl group in mannosyl donor **1.45** can effectively provide β-selectivities (α/β = 1/18.5, entry 5).[159]

The applicability of this approach was demonstrated for the synthesis of an oligosaccharide containing both primary and secondary β-mannosidic linkages (Scheme 1.6a). Thus, when 3-*O*-picolylated mannosyl donor **1.45** was reacted with glycosyl acceptor **1.27** in the presence of DMTST β-linked disaccharide **1.50** was obtained with α/β = 1/18.5 selectivity. The picoloyl group at C-3 position of this disaccharide was then selectively deprotected using copper(II) acetate. The resulting acceptor **1.51** was then coupled with mannosyl donor **1.52**, to provide the desired trisaccharide **1.53** in 76% yield and with complete β-selectivity.

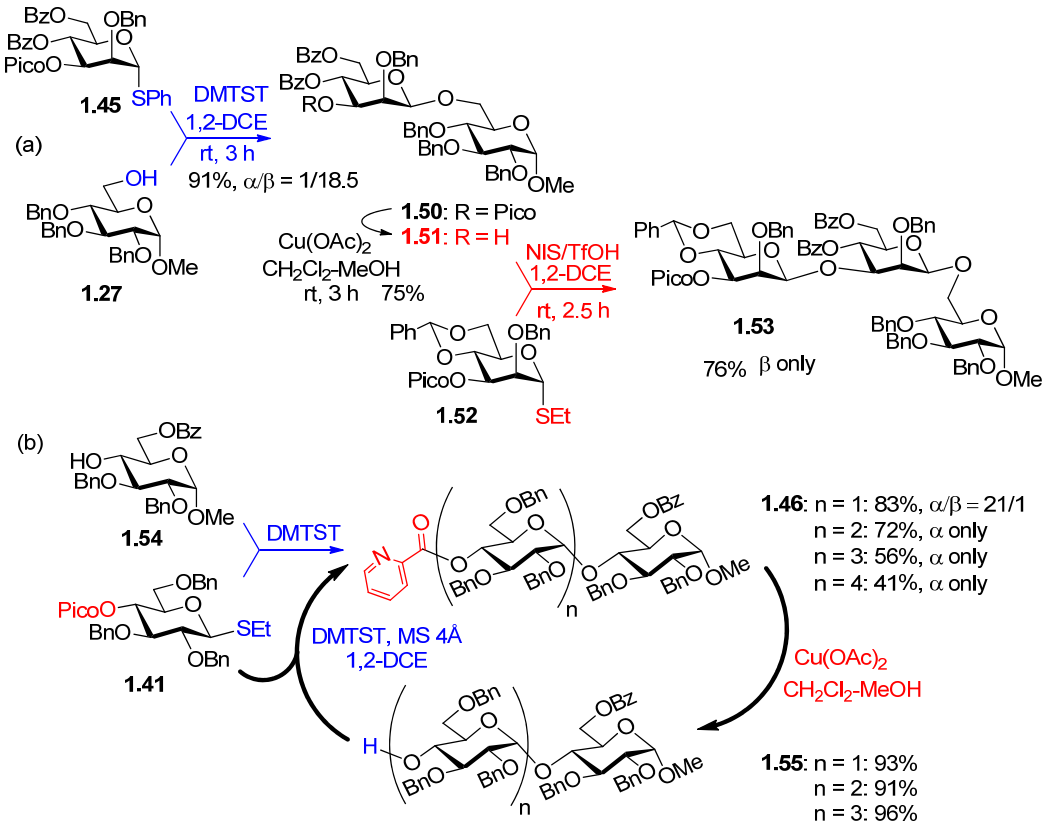
**Table 1.3. Hydrogen-bond-mediated Aglycone Delivery (HAD)**




| Entry | Donor | Conc. | Time | Product (Yield) | α/β Ratio |
|---|---|---|---|---|---|
| 1 |  1.41 | 5mM | 4 h | **1.46** (73%) | >25/1 |
| 2 |  1.42 | 5mM | 5h | **1.47** (86%) | 5.3/1 |
| 3 |  1.43 | 5mM | 1 h | **1.48** (95%) | >1/25 |
| 4 |  1.44 | 50 mM | 15 min | **1.49** (94%) | >1/25 |
| 5 |  1.45 | 5mM | 2.5 h | **1.50** (91%) | 1/18.5 |

Further application of this new stereoselective glycosylation reaction has emerged in application to the synthesis of linear and branched α-glucans.[160] As depicted in Scheme 1.6b, when 4-O-picoloylated glucosyl donor **1.41** was glycosylated with acceptor **1.54** in the presence of DMTST, disaccharide **1.46** was obtained in 83% yield (α/β = 21/1). The picoloyl group was then deprotected with copper(II) acetate to form the second generation glycosyl acceptor **1.55**. The process was reiterated to obtain pentasaccharide **1.46** (n = 4) with 41% yield and complete α-selectivity.

**Scheme 1.6. HAD-assisted synthesis of β-mannan and α-glucan**




Hung and co-workers developed a series of orthogonally protected D-glucoaminyl donors for stereocontrolled α-glycosylation.[161] The most advantageous protecting group pattern was determined to be 2-azido functionality, 2-nphthylmethyl (2-NAP) group at C-4, *p*-bromobenzyl (*p*-BrBn) at C-3, and TBDPS at C-6 positions. Azido protection was preferred since it is a small protecting group and provides easy analysis of the sugar derivatives, 2-NAP group was used because of its flexibility as a temporary or a permanent protecting group, and *p*-BrBn and TBDPS groups were used as sterically bulky groups to prevent the attack of the acceptor from the unwanted top face. This orthogonally protected glucosamine-derived trichloroacetimidate donor was used for synthesis of heparin-related sequences.[161]

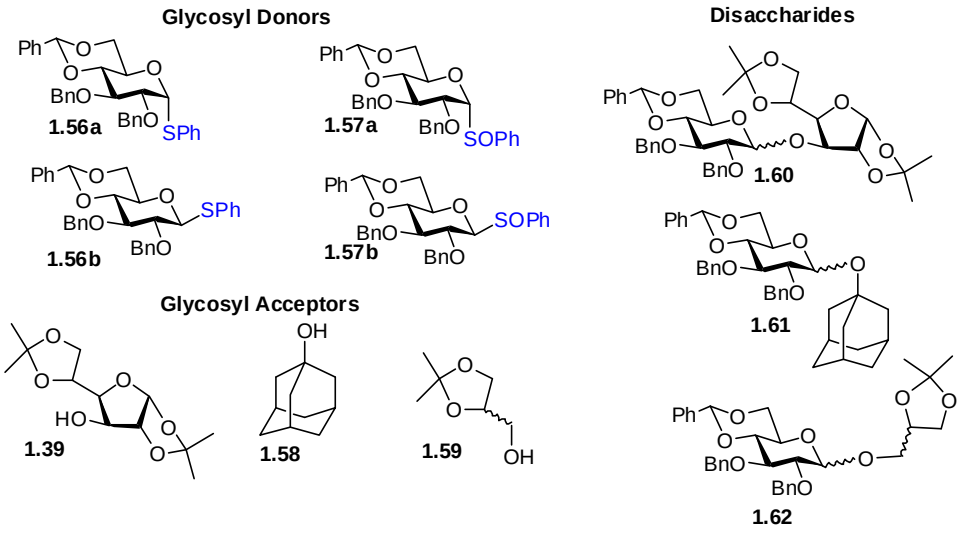
### 1.3.3. Conformation restraining cyclic protecting groups

Torsional effects induced by the cyclic protecting groups may also strongly affect the stereoselectivity at the anomeric center. The best-known example of this effect is the work by Crich and co-workers on the synthesis of β-mannosides. Thus, it has been demonstrated that 4,6-*O*-benzylidene-protected thioglycoside donors give superior β-manno selectivity in comparison to that achieved with donors lacking this type of protection.[65,69,71,162,163] The stereoselectivity observed was rationalized by carrying out experiments in which the benzylidene protected sulfoxide donor[69 164,165] is pre-activated using $Tf_2O$ to form a sulfonium salt, which collapses into the α-triflate that exists in the dynamic equilibrium with the contact ion pair. The presence of glycosyl triflate intermediate in mannosylations was also recognized with thioglycoside,[166] trichloroacetimidate,[167] 2-(hydroxycarbonyl)-benzyl,[168] hemiacetal,[169] pentenoate,[170] and phthalate[171,172] donors, all protected as 4,6-benzylidene acetals. It is believed that the closely associated triflate counterion shields the α-face and β-linked product forms preferentially. An α-deuterium kinetic isotope effect (KIE) study indicated substantial oxacarbenium ion character of this reaction pathway ruling out a possibility of the bimolecular displacement.[163] Similar conclusions were made as a result of KIE experiments with mannosyl iodides.[173] The deactivating effect of benzyl substituent was found to be a combination or torsional strain[174] restricting the conformational flexibility of the ring and enhanced electron-withdrawal.[175] The latter effect is due to locking the hydroxylmethyl group in the conformation wherein the C6-O6 bond is directed away from the O-5. This may cause additional destabilization of the oxacarbenium intermediate that seeks for compensation from tight coordination to the counter anion.

This study revealed that 4,6-*O*-benzylidene protected glycopyranosyl triflates provide high β-selectivities with glycosyl donors of the mannose series, whereas high α-selectivity is obtained

with their glucose configured counterparts.[162] The reaction for synthesis of α-glucosides was found to be independent on the anomeric stereochemistry of the donor employed. They rationalized this finding by the fact that the α-triflate formed undergoes the equilibrium with its more reactive β-counterpart rather than with the oxacarbenium ion intermediate. The rate and equilibrium constant for formation of β-glucosyl triflate are such that it preferentially forms the α-linked product.

**Table 1.4. Stereodirecting effect of 4,6-*O*-benzylidene protected glucosyl donors activated with PhSOTf and $Tf_2O$**

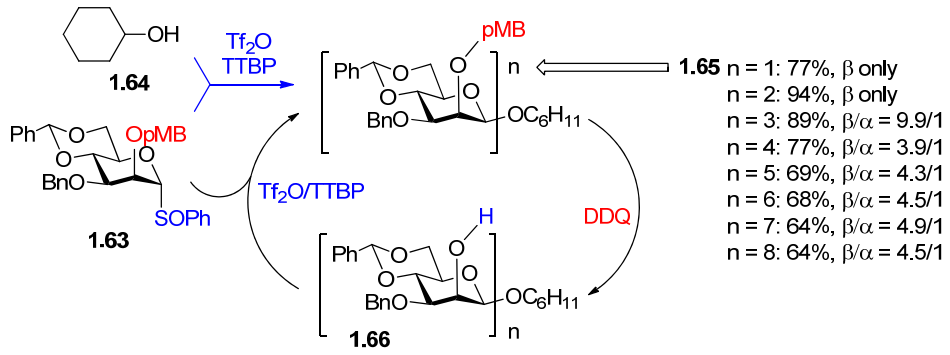



| Entry | Donor, Acceptor | Coupling reagent | Product | Yield, α/β ratio |
|---|---|---|---|---|
| 1 | **1.56a**, **1.39** | PhSOTf | **1.60** | 70%, >95/5 |
| 2 | **1.56b**, **1.39** | PhSOTf | **1.60** | 80%, >95/5 |
| 3 | **1.57a**, **1.59** | $Tf_2O$ | **1.62** | 89%, >95/5 |
| 4 | **1.57b**, **1.58** | $Tf_2O$ | **1.61** | 63%, >95/5 |

Many useful applications have evolved from the Crich methodology. For instance, the direct syntheses of a β-(1→2)-mannooctaose and of a β-(1→4)-mannohexaose represent the power of

this technique.[176] As depicted in Scheme 1.7, the synthesis of the (1→2)-mannan was achieved by means of the sulfoxide coupling protocol. Thus, 2-*O*-paramethoxybenzyl protected sulfoxide donor **1.63** was reacted with cyclohexanol **1.64** in the presence of triflic anhydride and 2,4,6-tri-tert-butylpyrimidine (TTBP) to afford β-mannoside **1.65** (n = 1) in 77% yield. The latter was deprotected with DDQ to give glycosyl acceptor **1.66**. Reiteration of glycosylation deprotection steps led to a series of (1→2)-linked mannan homologs. For instance, octasaccharide **1.65** (n = 8) was obtained in 64% yield (β/α = 4.5/1). In this context, the (1→4)-linked mannan was prepared from the thioglycoside donors activated using sulfinamide methodology.

**Scheme 1.7. β-(1→2)-Linked mannans using sulfoxide coupling protocol**

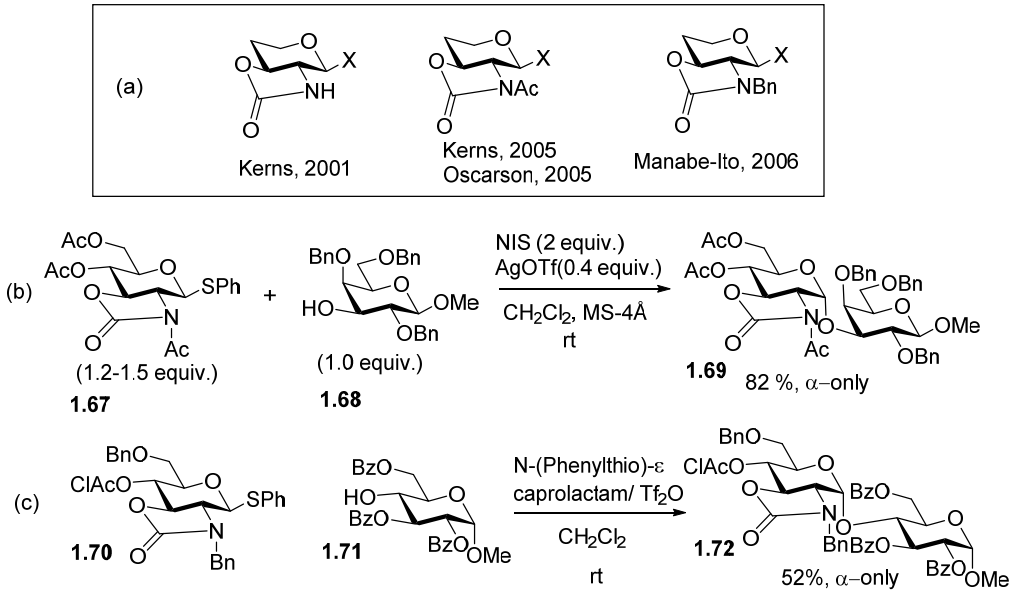



To study the influence of similar conformationally rigid protecting groups, on the selectivity obtained, Werz and co-workers synthesized variety of mannosyl donors with a spiroannulated cyclopropane ring at C-5 bearing one hydroxyl group.[177] Though very high β-selectivity wasn't observed, it was shown that the spiroannulated cyclopropane motif leads to fixation of the chair-like conformation, similar to that shown for 4,6-benzylidene protected sugars.

Kerns discovered that 2,3-*trans*-oxazolidinone-protected glucosaminyl donor provides excellent 1,2-*cis* selectivity in glycosylations (Scheme 1.8a).[178,179] Although high α-selectivity could be obtained, the oxazolidinone protected donor showed propensity to undergo side

reactions, such as *N*-glycosylation or *N*-sulfenylation. As an extension to that, Kerns et al.[180,181] and Oscarson *et al.*[182] reported an N-acetylated version of these oxazolidinones in which the nitrogen is protected.[178,180] These donors showed switchable stereoselectivity of glycosylations that was achieved by tuning the reaction conditions.[183-185] This interesting finding stimulated further experimental and mechanistic studies. Mechanistically it was suggested that the β-linked product is formed initially, which rapidly anomerizes into the corresponding α-anomer. The presence of the oxazolidinone ring is the key for this anomerization to occur, which was found to proceed via the endocyclic C1-O5 bond cleavage.[186,187] For instance, when N-acetyl-2,3-oxazolidinone protected donor **1.67** was reacted with glycosyl acceptor **1.68** in presence of NIS and AgOTf, disaccharide **1.69** was obtained in 82% yield (α-only, Scheme 1.8b.).

**Scheme 1.8. Selective α-glycosylations with N-acetyl- and N-benzyl-2,3-oxazolidinone-protected glycosyl donors 1.67 and 1.70**




Manabe, Ito and their co-workers reported *N*-benzylated 2,3-oxazolidinone donors for 1,2-*cis* glycosylation.[188,189] Thus, when glycosyl donor **1.70** was glycosidated with acceptor **1.71** in

presence of N-(phenylthio)-ε-caprolactam and triflic anhydride at rt, disaccharide **1.72** was obtained in 52% yield with complete α-selectivity (Scheme 1.8b).

Crich et al. showed that the 2,3-*O*-carbonate protecting group is highly α-selective for mannosylations and rhamnosylations.[154,190] In contrast, 3,4-*O*-carbonate protected rhamnosyl donors showed moderate β-selectivities owing to the electron withdrawing but non-participating nature of this group. Crich reported synthesis of β-glucosides using 2,3-*O*-carbonate protected glucosyl donor.[191] It was suggested that the conformation restricting *trans*-fused ring favors the formation of an α-triflate intermediate over the formation of an oxacarbenium ion. The effect of 3,4-*O*-carbonate protection was found to be weaker with slight preference toward β-selectivity.[89] Ye and co-workers studied 2,3-*O*-carbonyl protected glucose and galactose donors for pre-activation based glycosylations.[192] These reactions were generally β-stereoselective, but Lewis acids additives were found to favor α-stereoselectivity (vide infra).

A beneficial effect of a bulky 4,6-O-di-tert-butylsilylene (DTBS) protecting group[193-195] on α-selective galactosylation and galactosamination was recently applied to the synthesis of a series of human ABO histo-blood group type 2 antigens by Kiso and co-workers.[196]

### 1.4. Effect of the glycosyl acceptor

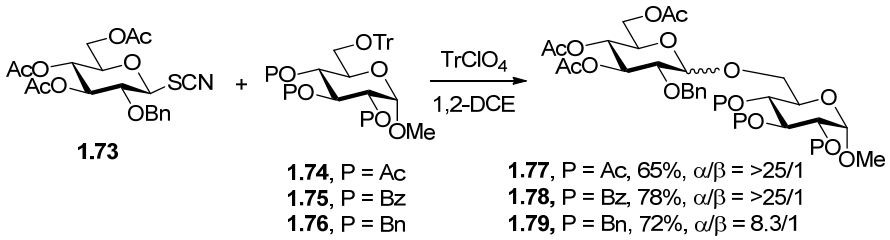
Many examples wherein different glycosyl acceptors have different selectivities can be seen throughout the text of this Chapter. A general principle is that the alcohol reactivity is inversely correlated with the stereoselectivity. Typically the most reactive hydroxyls give the lowest α/β-ratios: the stronger the nucleophile, the faster the reaction, and therefore the more difficult it is to control. Regarding the sugar or aliphatic glycosyl acceptors, the general rule normally states: glycosylation of more reactive primary hydroxyl generally provides poorer stereoselectivity in

comparison to that when the secondary hydroxyls are involved.[197] The same principle is applicable for the synthesis of glycopeptides, thus, glycosylation of the secondary hydroxyl of threonine typically gives higher α-stereoselectivity than when primary hydroxyl group of serine is glycosylated with 2-azido-2-deoxygalactosyl bromide or trichloroacetimidates.[198,199] Occasionally, primary hydroxyls provide somewhat higher stereoselectivity in comparison to that of the secondary hydroxyl groups. This can serve as an evidence of the glycosylation reaction proceeding via the bimolecular mechanism, at least partially. Primary alcohols gave higher stereoselectivity in H-bond-mediated aglycone delivery reactions mediated by the remote picolinyl groups.[158]

It is well established that ester electron-withdrawing substituents reduce electron density of the neighboring hydroxyl group lowering their nucleophilicity.[150,200,201] This may improve stereoselectivity, as the reaction can be carried out in more controlled manner. As an example, glycosylation of axial 4-OH of galactose often gives excellent 1,2-*cis* stereoselectivity, especially in combination with electron-withdrawing substituents (*e.g. O*-benzoyl, OBz).[202] However, less reactive hydroxyls can lose their marginal reactivity completely when surrounded by the deactivating species, resulting in lower glycosylation yields.

Recently, Demchenko and co-workers have shown that electron-withdrawing acyl protecting groups have significant effect on the stereoselectivity obtained with thiocyanates as glycosyl donors.[203] Thus, when thiocyanate **1.73** was reacted with acyl-protected acceptors **1.74** and **1.75**, the corresponding disaccharides **1.77** and **1.78** were obtained with complete α-selectivity (α/β = >25/1, Scheme 1.9). However, when benzyl-protected acceptor **1.76** was used instead, the stereoselectivity dropped (**1.79**, α/β = 8.3/1).

**Scheme 1.9. Acyl protecting groups in acceptor enhance stereoselectivity in glycosylations**




Very recently, Toshima and co-workers reported a novel chemical glycosylation method that makes use of the chiral recognition ability of the aglycone.[204] Thus, chiral catalysts and chiral acceptors may favor the formation of one anomer over another.

## 1.5. Effect of the reaction conditions

### *1.5.1. Temperature*

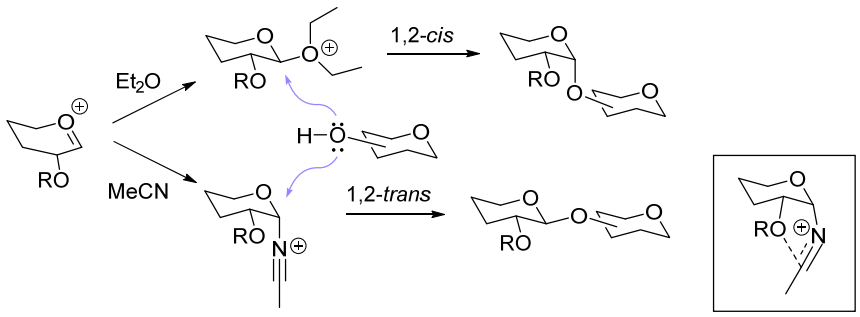
Kinetically controlled glycosylations at lower temperatures generally favor β-glycoside formation,[108,205-209] although converse observations have also been reported.[210,211] Since the α-glycoside is thermodynamically more favored due to the anomeric effect, it is predominantly formed at high temperatures. A number of examples have been discussed throughout other parts of this Chapter.

### *1.5.2. Solvent*

Effect of reaction solvents on the selectivity of glycosylation reaction has been widely studied. In general, polar reaction solvents increase the rate of the β-glycoside formation via charge separation between O-5 and β-O-1. If the synthesis of α-glycosides is desired, $CH_2Cl_2$, $ClCH_2CH_2Cl$ or toluene would be suitable candidates as the reaction solvents. However, there are more powerful forces than simple solvation that have to be taken into consideration. It has

been shown earlier, that ethereal solvents have a tendency to drive the glycosylation in α-selective fashion, while nitrile solvents increase the amount of β-glycoside formation.[129,212] These observations were rationalized as follows: ether type reaction solvents such as diethyl ether,[213] tetrahydrofuran,[213] or dioxane[214] lead to the preferential formation of the equatorial intermediate. On the other hand, if the reactions are performed in acetonitrile, the nitrilium cation formed *in situ* exclusively adopts axial orientation, allowing stereoselective formation of equatorially substituted glycosides (Scheme 3). This approach permits the formation of 1,2-*trans* glucosides with good stereoselectivity even with glycosyl donors bearing a non-participating substituent. Recently, the Mong group proposed a revised mechanism for glycosylations in nitrile solvents.[215] According to their proposed model, the oxacarbenium ion or intermediates interact with the nitrile solvent producing mixtures of α- and β-glycosyl nitrilium intermediates. Though the formation of 1,2-*cis* nitrilium species is favored by the anomeric effect, it is further reinforced through the participation of the oxygen atom of the C-2 ether function. The resultant glycosyl oxazolinium intermediate is then attacked by a nucleophile from the top face leading to the formation of β-product.

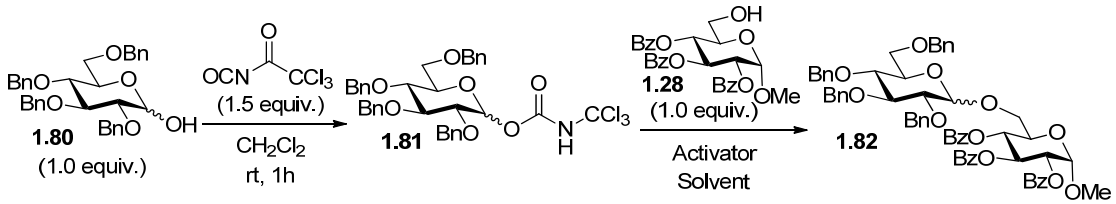
**Scheme 1.10. Effect of the reaction solvents**




Many applications of solvent systems are known. One recent example employing *N*-trichloroacetyl carbamate group introduced by Redlich[216] and Vankar,[217] showed high

selectivities, which can be switched by simply switching the solvent. Thus, Omura et al [218] showed that the α-selective glycosylations could be performed with a catalytic amount of $TMSClO_4$ in $Et_2O$, whereas high β-selectivity could be achieved by activation with TMSOTf in EtCN in the presence of 5 Å molecular sieves. For instance, when N-trichloroacetyl carbamate **1.81** was glycosylated with acceptor **1.28** in the presence of $TMSClO_4$ in diethyl ether as the solvent, disaccharide **1.82** was formed with high α-selectivities (entries 1 and 2, Table 1.5)

**Table 1.5. One-pot synthesis and glycosidation of carbamate 1.81**

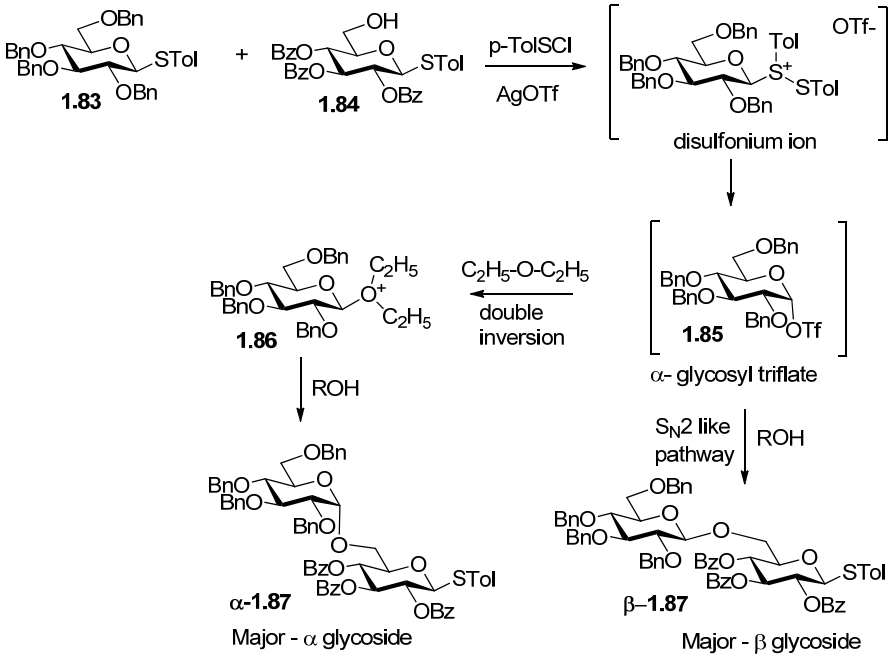



| Entry | Activator | Solvent | Reaction conditions | Yield, α/β ratio |
|---|---|---|---|---|
| 1 | $TMSClO_4$ (1.5 equiv.) | $Et_2O$ | 0 °C, 0.5 h | 99%, 93/7 |
| 2[a] | $TMSClO_4$ (0.2 equiv.) | $Et_2O$ | 0 °C, 0.5 h | 88%, 91/9 |
| 3 | TMSOTf (1.5 equiv.) | EtCN | -40 °C, 0.5 h then -23 °C, 0.5 h | 88%, 8/92 |

[a] – 5 Å molecular sieves were used

Huang *et. al.* have recently studied the effect of solvents and additives on the stereochemical outcome of preactivation-based one-pot glycosylation strategy using thioglycosides as donors.[219] When donor **1.83** dissolved in diethyl ether was pre-activated with 1 equiv. of *p*-TolSOTf, formed in-situ from *p*-TolSCl and AgOTf (3 equiv.), disaccharide **1.87** was obtained in 67% yield (α/β = 1.1/1, Scheme 1.11.). Interestingly, when the amount of AgOTf was decreased to 1.1 equiv., significant change in α-selectivity was observed (α/β = 6/1). In addition, when the reaction was performed by increasing the volume of diethyl ether by 10 fold, further enhancement in α-selectivity was observed (α/β = 10/1).

**Scheme 1.11. Effect of solvent and excess amount of AgOTf used on the selectivity obtained**

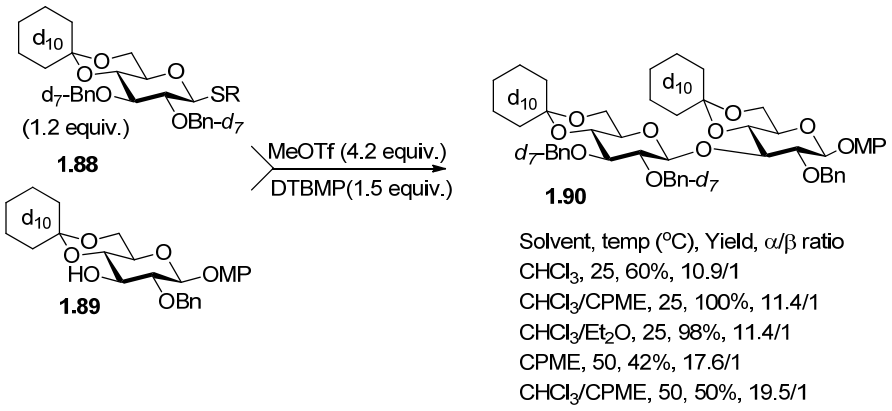



Moreover, when dichloromethane was used as the reaction solvent, the stereoselectivity was switched (α/β = 1/8). With the belief that glycosyl triflates are formed as the reaction intermediates, the observed stereoselectivity was rationalized as follows. The reactions performed in diethyl ether proceed through a double-inversion mechanism. Under dilute conditions and with lower excess of AgOTf, solvent participation becomes more effective, resulting in higher α-selectivity. In case of dichloromethane, due to the non-nucleophilic and non-polar nature of the solvent, the reaction is likely to proceed via $S_N2$-like pathway, directly displacing the α-glycosyl triflate leading to β-glycosides.

Ito and co-workers developed a high-throughput screening system to study the synergistic solvent effect of combined ethereal and halogenated solvents on the course of glycosylation.[212] This study employed the use of glycosyl donors, which were isotopically labeled with per-

deuterated (Bn-$d_7$) benzyl ether protecting groups. The advantage of using Bn-$d_7$ is the "disappearance" of all benzylic methylene signals at around 4-5 ppm, thereby making it easier to estimate the isomeric ratios of the glycosylated products. The labeled donor was glycosidated with various acceptors in the presence of MeOTf as the activator and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) in various solvents. Although ethereal solvents are known to exert α-directing effect, the use of mixed solvent systems containing both halogenated and ethereal solvents was found even more beneficial for enhancing α-selectivity. Thus, a 1/1 (v/v) mixture of $CHCl_3/Et_2O$ or $CHCl_3$/cyclopentyl methyl ether (CPME) provided the best results and the use of such solvent systems was extended to the synthesis of a variety of 1,2-*cis* linkages.[212,220] When per-deuterated benzyl ether protected thioglycoside donor **1.88** was reacted with per-deuterated glycosyl acceptor **1.89** in presence of methyl triflate (MeOTf) as a promoter, disaccharide **1.90** with selectivity up to α/β = 19.5/1 was obtained (Scheme 1.12.).

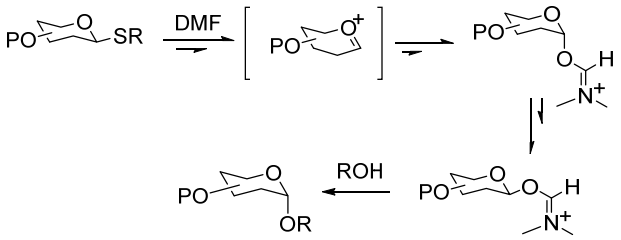
**Scheme 1.12. Solvent and temperature effects on 1,2-cis glycosylation**




Mong and co-workers took a different direction in studying reaction solvent by using dimethylformamide (DMF) as a co-solvent, rather uncommon reaction solvent in glycosylations.[221] This procedure is based on pre-activation, where DMF is employed to trap the glycosyl oxacarbenium ion as an equilibrium mixture of α/β-glycosyl imidates. Being more

reactive, β-imidate reacts preferentially with the glycosyl acceptor producing desired α-glycoside with high selectivity.

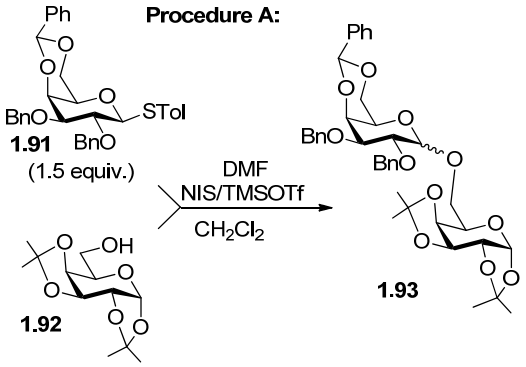
**Scheme 1.13. DMF-mediated glycosylation**



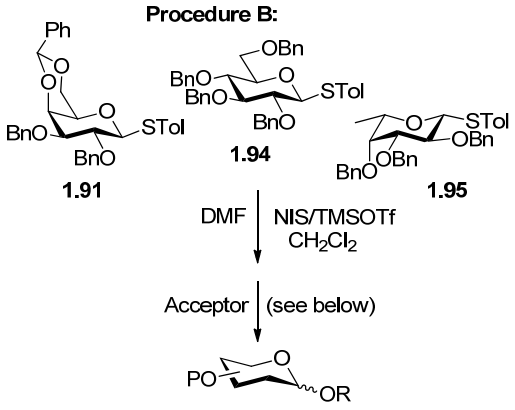

This study employed two conceptually different protocols for glycosylation. First, a conventional method (Procedure A), wherein a mixture of glycosyl donor, acceptor, and DMF was treated with NIS and TMSOTf. Second, a pre-activation method (Procedure B), according to which the glycosyl donor was reacted with NIS and TMSOTf in the presence of DMF followed by the addition of the glycosyl acceptor. Both approaches furnished the desired glycosylation product. As shown in Table 1.6, reaction of benzylated donor **1.91** with glycosyl acceptor **1.92** gave moderate stereoselectivity (82%, α/β = 6/1, entry 1, Table 1.4). It was also demonstrated that the increase in the amount of DMF to 3 and 6 equiv. (entries 4 and 5) translated into a significant increase in α-stereoselectivity (up to α/β = 19/1, entry 5).

Interestingly, the use of ethereal solvents had no benefit for the further improvement of stereoselectivity, irrespective of the type of ethereal solvent used. The results obtained using procedure **A** were then applied to the investigation of the effectiveness of glycosylation procedure **B**. It was observed that due to the modulating effect provided by DMF, all glycosylations between thioglycoside donors **1.91**, **1.94**, or **1.95** and acceptors **1.96-1.100** proceeded with high α-selectivity (α/β = 11-49/1, entries 4-9, Table 1.4).

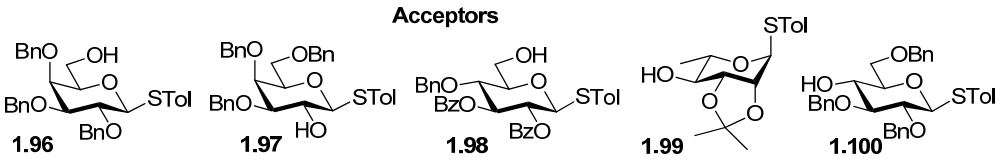
**Table 1.6. Investigation of DMF-mediated glycosylations**




| Entry | DMF (equiv.) | Temp (°C), time (h) | Yield of 1.93, α/β ratio |
|---|---|---|---|
| 1 | 1.5 | -10, 2.0 | 82%, 6/1 |
| 2 | 3.0 | -10, 2.0 | 87%, 15/1 |
| 3 | 6.0 | -10, 2.0 | 87%, 19/1 |

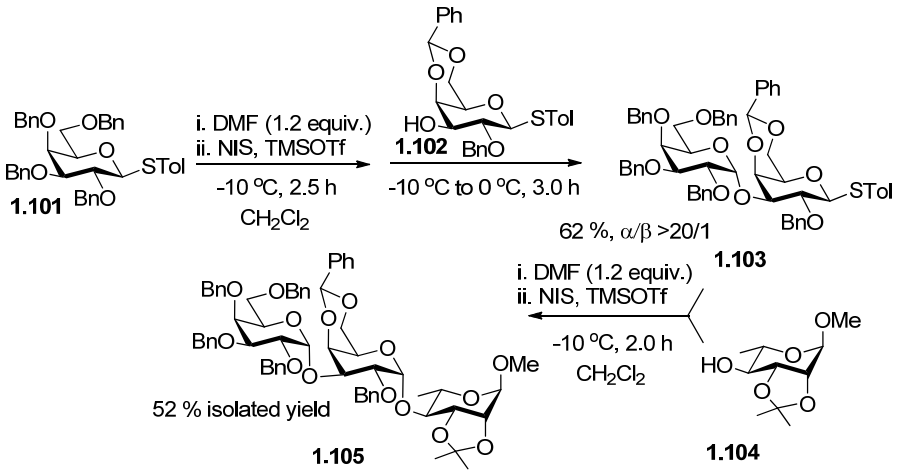



| Entry | Donor | Acceptor | Temp (°C) time (h) | Yield, α/β with DMF |
|---|---|---|---|---|
| 4 | **1.91** | **1.96** | -10, 3.0 | 60%, 36/1 |
| 5 | **1.91** | **1.97** | 0, 3.0 | 55%, 11/1 |
| 6 | **1.91** | **1.98** | -10, 3.0 | 85%, 49/1 |
| 7 | **1.91** | **1.99** | 0, 2.0 | 50%, 13/1 |
| 8 | **1.94** | **1.100** | 0, 5.0 | 50%, 49/1 |
| 9 | **1.95** | **1.97** | -10, 6 | 61%, 49/1 |




Encouraged by the α-stereodirecting effect of DMF, the preactivation protocol was extended to a sequential one-pot oligosaccharide synthesis.[221] Prior to the one-pot synthesis, the efficiency of the procedure was tested by carrying out the synthesis of disaccharide and evaluating the stereoselectivity obtained. An interesting feature of DMF as an additive to a one-pot multi-step synthesis is that it is regenerated after the first coupling and hence can be engaged into the subsequent modulation cycle. As depicted in Scheme 1.14, two contiguous 1,2-*cis* linkages were efficiently assembled and trisaccharide **1.105** was isolated in an overall yield of 52%.

**Scheme 1.14. DMF- mediated synthesis of trisaccharide 1.105**



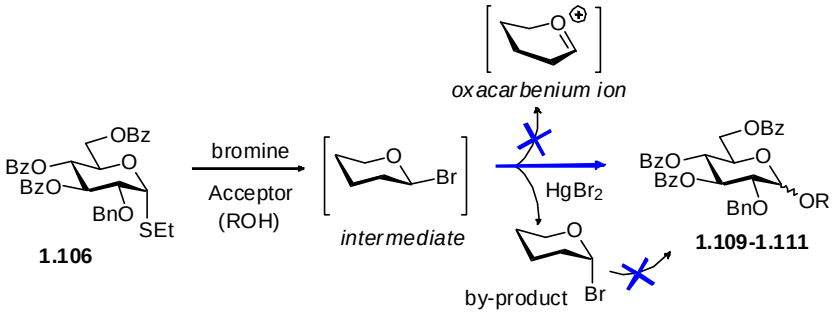

### 1.5.3. Promoter, additives, and chelators

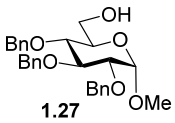
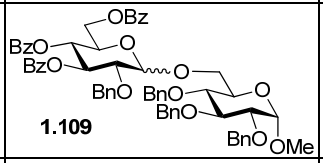
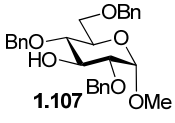
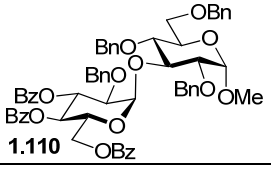
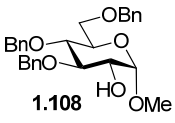
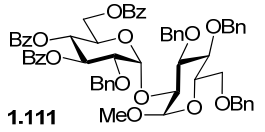
At the early stages of method development, glycosylations of poorly nucleophilic acceptors were sluggish and inefficient. The first attempts to solve this problem gave rise to the development of catalytic systems that were actively involved in facilitating the departure of the leaving group.[222] Zemplen[223] and, subsequently, Helferich[224] assumed that complexation of the anomeric bromides or chlorides with reactive mercury (II) salt catalysts would significantly improve their leaving group ability. This approach has become a valuable expansion of the classic Koenigs-Knorr method.[225] These early attempts to improve the glycosylation process have revealed the necessity to find a delicate balance between the reactivity and stereoselectivity because it was noted that faster reactions often result in decreased stereoselectivity.[226,227] Recently, Demchenko and co-workers investigated the glycosidation of thioglycosides in the presence of bromine.[114] It was demonstrated that bromine-mediated glycosylation of thioglycoside **1.106** leads to exclusive α-selectivity in product **1.109-1.111** (Scheme 1.15.). This reaction was monitored by NMR, and this study demonstrated that β-bromide is the reactive intermediate that leads to the product **1.109-1.111** with complete stereoselectivity. The NMR

experiment showed that β-bromide can undergo a relatively rapid anomerization into the α-linked counterpart, and if this anomerization is not suppressed, the yield of the glycosylation product can be low.

Once formed, α-bromide is totally unreactive under the established reaction conditions, but it can be reactivated in the presence of mercury (II)-based promoters. This, however, can compromise α-selectivity with primary alcohols. This concept complements well-known *in-situ* anomerization procedure introduced by Lemieux and co-workers for reactive bromides[113] and further adapted to iodides by Gervay-Hague.[112,228]

**Scheme 1.15. Stereoselective glycosidation of superdisarmed thioglycoside 1.106 via reactive β-bromide intermediate**

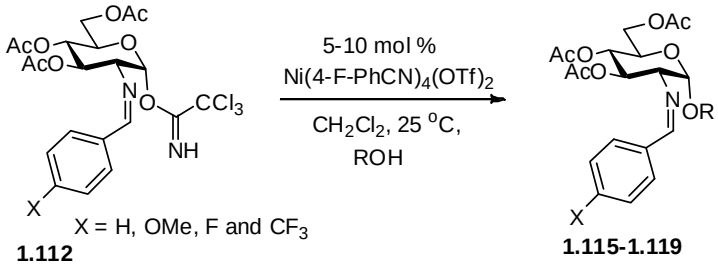


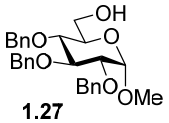
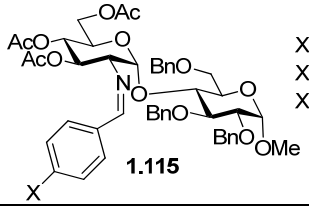
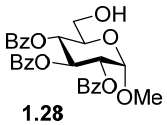
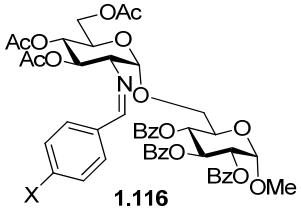
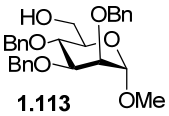
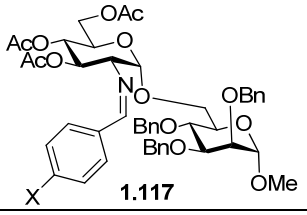
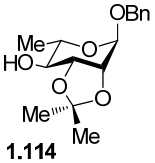
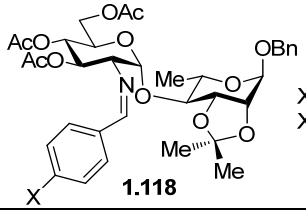
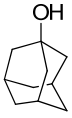
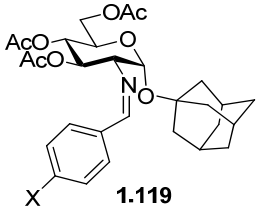

| Entry | Acceptor (ROH) | Product | Yield | α/β ratio |
|---|---|---|---|---|
| **1** |  **1.27** |  **1.109** | 72% | 10.5/1 |
| **2** |  **1.107** |  **1.110** | 89% | >25/1 |
| **3** |  **1.108** |  **1.111** | 87% | >25/1 |

It is a general trend that milder activating conditions are beneficial for 1,2-*cis* glycosylation. Thus, halide ion-catalyzed reactions gave the best results for the glycosylation with glycosyl halides;[113] thioglycosides give higher selectivity when activated with a mild promoter, such as iodonium dicollidine perchlorate (IDCP) [229,230] or bromine.[114]

Many of the current methodologies for glycosylation require the use of stoichiometric amounts of promoters.. The use of transition metal catalysts helps achieve greener and milder way to construct glycosidic linkages and offers new opportunities for stereocontrol.[231] O'Doherty developed a well-rounded methodology for palladium(0)-catalyzed glycosylations, wherein carbohydrate chirality centers are installed post-glycosylationally.[90,232-234] Nguyen and co-workers studied palladium(II)-catalyzed glycosidation of glycosyl trichloroacetimidates using Pd-$(CH_3CN)_4(BF_4)_2$ or similar catalysts.[235,236] This study evolved into the investigation of a series of nickel catalysts providing an efficient means for glycosidation of N-*p*-methoxybenzylidene-protected 2-amino-2-deoxy trichloroacetimidate donor.[237,238] The nature of the ligand on nickel has been found to be the deciding factor in controlling the stereoselectivity of glycosylations. Thus, it was observed that electron-withdrawing substituents help to decrease the reaction time, which is translated into increased α-selectivity. The efficiency of the nickel-catalyzed glycosylations was then extended to glycosylation of a variety of acceptors and applied to the synthesis of number of oligosaccharides. As summarized in Table 1.5, N-benzylidene trichloroacetimidate donor **1.112** bearing different para substituents was reacted with primary (**1.27**-**1.28**) and secondary glycosyl acceptors (**1.58**, **1.113**-**1.114**) under catalysis of Ni(4-F-PhCN)$_4$(OTf)$_2$, to provide disaccharides (**1.115**-**1.119**) with very high α-selectivity.

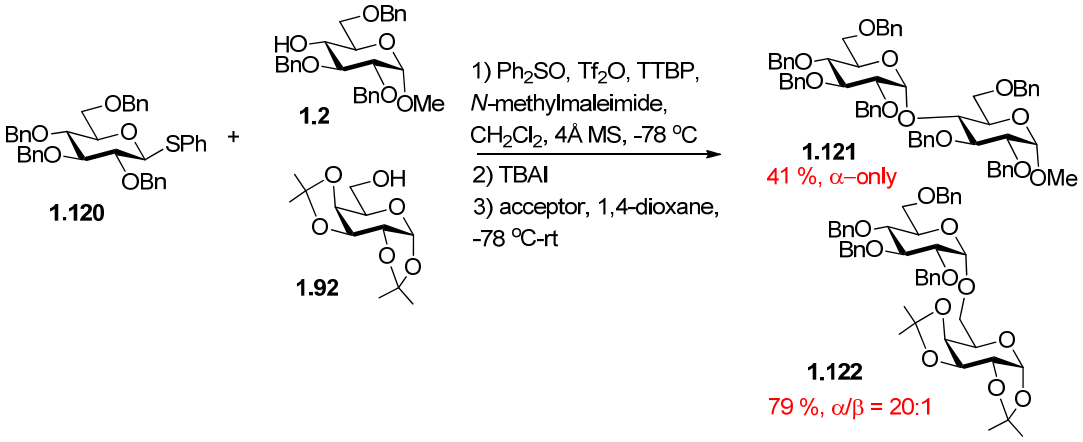
**Table 1.7. Nickel-catalyzed α-selective glycosidation of N-benzylidene trichloroacetimidate donor 1.112**




| Entry | R-OH | Product |
|---|---|---|
| 1 |  1.27 |  1.115 X = OMe, 97%, 6:1<br>X = F, 97%, 10:1<br>X = $CF_3$ , 97%, α-only |
| 2 |  1.28 |  1.116 78%, (15:1) |
| 3 |  1.113 |  1.117 82%, (10:1) |
| 4 |  1.114 |  1.118 X = OMe, 93%, 12:1<br>X = F, 88%, 13:1 |
| 5 |  1.58 |  1.119 96%, (17:1) |

Bennett and co-workers recently applied the activation of thioglycosides with $Ph_2SO$ in the presence of TBAI to the stereoselective synthesis of 1,2-cis glycosides. It was observed that this reaction proceeds via the intermediacy of glycosyl iodides.[239] The underpinning idea of using TBAI is that the conversion of α-glycosyl triflates into β-glycosyl iodides would favor the formation of α-glycosides. Thus, when the armed *S*-phenyl thioglycoside **1.120** was reacted with glycosyl acceptors **1.2** and **1.92** under the $Ph_2SO/Tf_2O$ conditions followed by the addition of TBAI, disaccharides **1.121** (41%, α-only) and **1.122** (79%, α/β = 20/1) were obtained in excellent stereoselectivity (Scheme 1.16).

**Scheme 1.16. Synthesis of 1,2-*cis*-linked glycosides by activation of thioglycosides in the presence of TBAI**



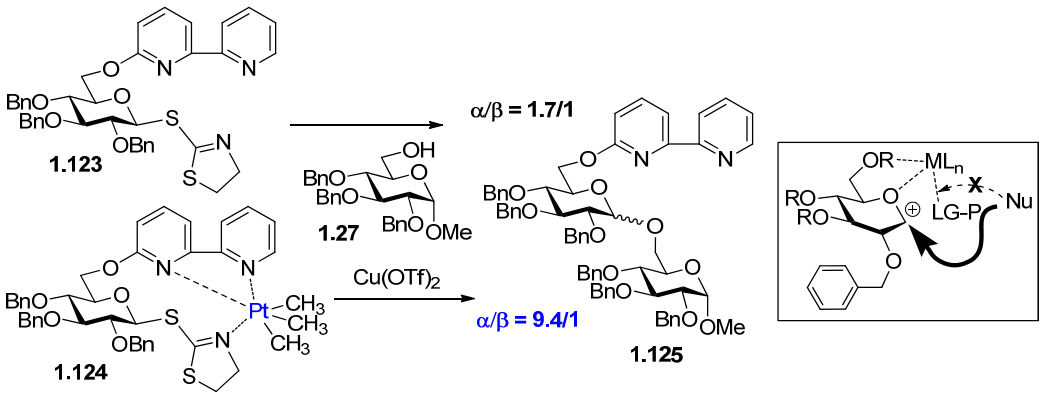

Recently there has been an explosion in the study of gold-catalyzed activation of alkynes to exploit the low oxophilic character of gold and the excellent functional group compatibilities these catalysts exhibit.[240-244] This includes work by Hotha and co-workers where propargyl glycosides were activated using Au(III) chloride to give α/β mixtures of glycosides and disaccharides in good yields. Yu and co-workers conducted a similar study with glycosyl *ortho*-alkynylbenzoates under the catalytic Au(I) activation conditions.[243] [244] Another promising new

field is the use of chiral thioureas as organocatalysts for glycosylations.[245] As of now, this approach is limited to the synthesis of 2-deoxy α-glycosides[246] and β-selective glycosylations with 2-oxygenated sugars.[247]

Various additions to the promoter systems often influence the stereochemical outcome of the glycosylation. Amongst the most remarkable examples is the use of perchlorate ion additive that was found to be very influential in 1,2-cis glycosylations.[248,249] Lately, the effectiveness of use of silver perchlorate as activator in glycosylations of thioimidates and thioglycosides to provide better 1,2-*cis* selectivity than those achieved with more common triflates, has been studied.[250]

Demchenko and co-workers observed that a multi-dentate metal coordination to the leaving group, along with a protecting group at *O*-6 and/or *O*-5, has a strong effect on the stereoselectivity of chemical glycosylation (Scheme 1.16). It was demonstrated that platinum(IV) complexation of 6-*O*-picolinyl or 6-*O*-bipyridyl to the leaving group, such as thiazolinyl, has a pronounced effect on the stereoselectivity of glycosylation.[251] While the glycosidation of thioimidate donor **1.123** with acceptor **1.27** in the presence of $Cu(OTf)_2$ gave the disaccharide **1.125** with poor selectivity (α/β = 1.7/1, Scheme 1.17), the complexed glycosyl donor counterpart **1.124** showed a significant 5-fold increase in 1,2-*cis* stereoselectivity (α/β = 9.4/1).

**Scheme 1.17. Effect of metal complexation on the stereoselectivity of glycosylation**



While studying 2,3-*O*-carbonyl protected glucose and galactose donors, which are generally β-stereoselective, Ye and co-workers observed that Lewis acids additives favor α-stereoselectivity in preactivation-based glycosylations.[192] Thus, catalytic amount of $BF_3$-$OEt_2$ or $AgBF_4$ as well as 1 equiv. of $AgPF_6$ or $SnCl_4$ completely reversed stereoselectivity to give α-linked products. It was assumed that similar to that proven for 2,3-oxazolidinones,[186,187] the initially formed β-linked product anomerizes into the thermodynamically more stable α-anomer, and this anomerization is facilitated by Lewis acid additives.

### 1.6. Other effects and special methods

High pressure applied to the reactions with participating glycosyl donors further enhances 1,2-*trans* selectivity;[252] when the high pressure conditions were applied for glycosylations with a non-participating glycosyl donor, remarkable increase in the reaction yield was noted with only marginal changes in stereoselectivity.[253] Unfavorable steric interactions that occur between glycosyl donor and acceptor in the transition state or other factors or conditions may unexpectedly govern the course and outcome of the glycosylation process. One of the most remarkable effects, so-called *"double stereodifferentiation"* takes place when stereochemical interactions between bulky substituents in glycosyl donor and glycosyl acceptor may outperform even the strong stereodirecting effect of a neighboring participating group. The pair of reagents where these interactions occur is called a *"mismatched pair"*.[254]

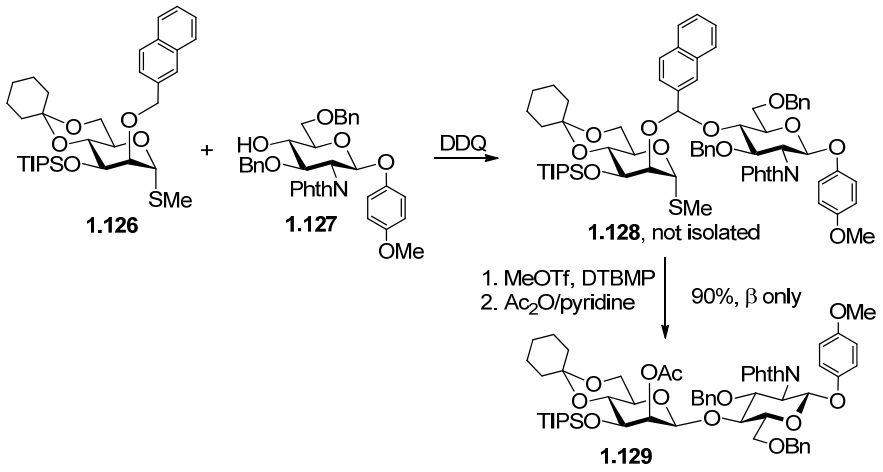
A number of methods have been developed that do not include a formal glycosylation step.[255-257] Typically, these indirect procedures include multistep syntheses and are of lower efficiency than direct glycosylations. Therefore, practical application of these techniques is envisaged for the synthesis of glycosidic linkages that cannot be easily accessed by conventional

technologies. O'Doherty and co-workers developed a new method for the synthesis of di- and oligosaccharides that can be accomplished from non-carbohydrate precursors.[90,232-234] The de novo asymmetric methodology was applied to the synthesis of mono-, di-, and oligosaccharides via palladium-catalyzed reaction. The synthesis of 1,2-cis linkages have not yet been accomplished.

### *1.6.1. Intramolecular aglycone delivery* (IAD)

Barresi and Hindsgaul were the first to apply the idea of intramolecular glycosylation, which was used for the synthesis of β-mannosides.[258] Subsequently, it was demonstrated that silicon bridge-mediated aglycone delivery provides high yields and excellent stereocontrol.[259,260] Further improvement emerged with the introduction of the allyl-mediated strategy that allows high yields and complete stereoselectivity in α-glucosylations and β-mannosylations.[261] More recently Ito and co-workers invented naphthylmethyl ether (NAP)-mediated intramolecular aglycone delivery.[262] The versatility of this approach is that it generally provides significantly higher yields in comparison to that of traditional approaches. Further value of this methodology is that it allows for stereoselective synthesis of various 1,2-*cis* linkages, such as β-Man*p*, β-Ara*f*, and α-Glc*p*. A representative example, the synthesis of disaccharide **1.129**, is depicted in Scheme 1.18. Thus, when 2-*O*-NAP-protected thiomethyl glycosyl donor **1.126** was reacted with acceptor **1.127** in the presence of DDQ, followed by the removal of NAP tether and acetylation, disaccharide **1.129** was obtained in 90% yield with complete β-selectivity.

**Scheme 1.18. β-Mannopyranosylation via NAP-tether mediated intramolecular aglycone delivery (IAD)**

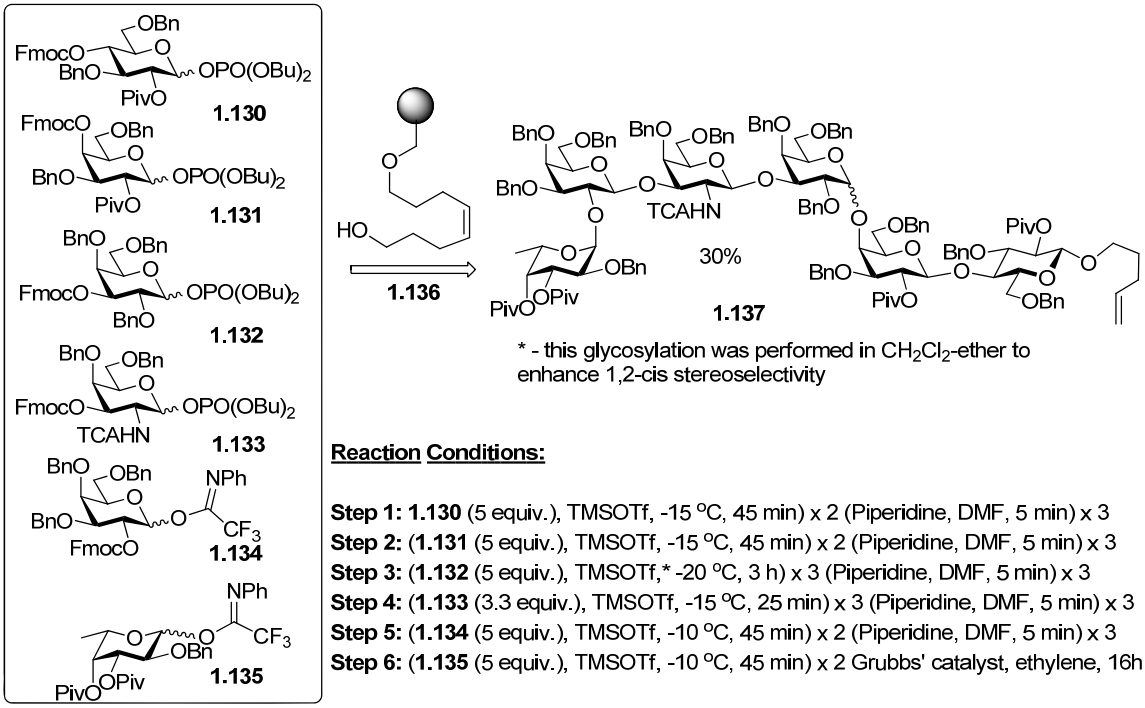



### *1.6.2. Supported and tagged synthesis*

The last decade has witnessed dramatic improvements in the area of solid phase-supported oligosaccharide synthesis.[263-267] Polymer supported synthesis is very attractive because it allows to conduct the synthesis of oligosaccharide sequences without the necessity of purifying (and characterizing) the intermediates. Another important advantage of oligosaccharide synthesis on solid phase support is the ease of excess reagent removal (by filtration). This effort culminated in the automated synthesis by Seeberger, which was the first attempt to conquer the challenge on 1,2-cis glycosidic bond formation using an automated approach.[268] Careful refinement of reaction conditions allowed 1,2-cis galactosylation in dichloromethane-ether and Globo-H sequence was assembled as depicted in Scheme 1.19. First, glycosyl phosphate donor **1.130** was linked to the resin **1.136** via glycosylation using TMSOTf (repeated once) as the promoter, followed by deprotection of the Fmoc substituent with piperidine (repeated twice) to provide polymer-bound acceptor. The general synthetic protocol consists of repetitive cycles of glycosylation using either glycosyl phosphate (**1.130-1.133**) or glycosyl N-phenyl

trifluoroacetimidate donors (**1.134** and **1.135**) followed by the deprotection with piperidine. The final product **1.137** was obtained under an atmosphere of ethylene in the presence of Grubbs' catalyst[269] in an overall yield of 30%.

**Scheme 1.19. Automated synthesis of Globo H hexasaccharide**




**Reaction Conditions:**

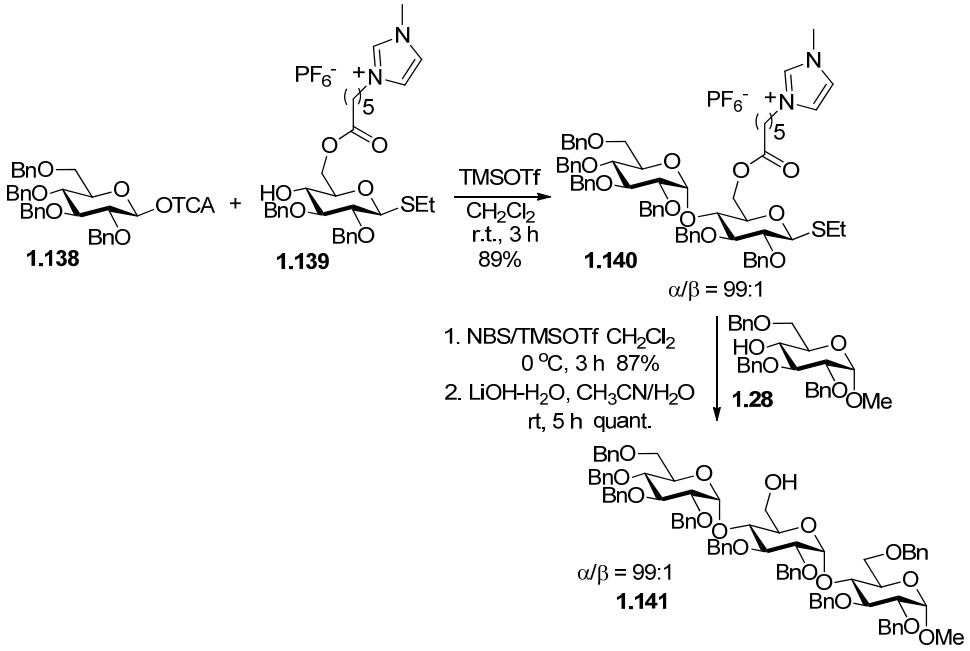
**Step 1: 1.130** (5 equiv.), TMSOTf, -15 °C, 45 min) x 2 (Piperidine, DMF, 5 min) x 3
**Step 2:** (**1.131** (5 equiv.), TMSOTf, -15 °C, 45 min) x 2 (Piperidine, DMF, 5 min) x 3
**Step 3:** (**1.132** (5 equiv.), TMSOTf,* -20 °C, 3 h) x 3 (Piperidine, DMF, 5 min) x 3
**Step 4:** (**1.133** (3.3 equiv.), TMSOTf, -15 °C, 25 min) x 3 (Piperidine, DMF, 5 min) x 3
**Step 5:** (**1.134** (5 equiv.), TMSOTf, -10 °C, 45 min) x 2 (Piperidine, DMF, 5 min) x 3
**Step 6:** (**1.135** (5 equiv.), TMSOTf, -10 °C, 45 min) x 2 Grubbs' catalyst, ethylene, 16h

Very recently the same group has reported the total synthesis of *O*-antigen pentasaccharide repeating unit obtained from pathogenic *E. coli.* O111. With the synthetic challenge of constructing two unnatural and labile coitose units, the total synthesis was achieved in 21 steps with 1.5% overall yield.[270] Boons et al. presented a very elegant synthesis of α-linked oligosaccharide on polymer support using their recent chiral auxiliary –assisted synthesis of 1,2-cis glycosides.[124]

A promising technique for the tagged oligosaccharide synthesis that makes use of an ionic-liquid support has recently emerged.[271,272] As with the polymer-supported and fluorous tag-

supported syntheses,[273-275] ionic liquid-supported assembly expedites oligosaccharide synthesis by eliminating the need for chromatographic purification of the intermediates. [272,276-282] Differently from insoluble polymer beads, ionic liquid supports allow for homogeneous conditions. This approach is illustrated by the synthesis of trisaccharide **1.141** (Scheme 1.20).[283]

**Scheme 1.20. Glycosylation on ionic liquid support and product isolation.**




In this synthetic strategy, the glycosyl acceptor **1.139** was grafted onto an ionic liquid support at C-6 position of the sugar moiety. The resulting tagged glycosyl acceptor **1.139** was reacted with trichloroacetimidate donor **1.138** to afford disaccharide **1.140** in 89% and high α-stereoselectivity. The purification is accomplished by simple washing or liquid-liquid extractions. Disaccharide **1.140** was then reacted with acceptor **1.28** followed by the removal of the ionic liquid tag using LiOH-$H_2O$ to afford trisaccharide **1.141** in 87% yield.

## 1.7. Conclusions and outlook

The progress in the area of chemical glycosylation has significantly improved our ability to synthesize various glycosidic linkages with impressive yields and stereoselectivity. Can we conclude that we have entirely solved the problem of chemical glycosylation? Unfortunately not, and hopefully this Chapter has introduced the reader to the challenge of chemical glycosylation, a variety of factors, conditions, and driving forces influencing all aspects of this complex chemical reaction as well as informing the reader about the specialized material dedicated to particular methods and strategies employed in modern carbohydrate chemistry. Recent progress made in the area of development of new coupling methods and highly efficient strategies for oligosaccharide synthesis will ultimately provide an efficient and trouble-free access to complex saccharides. This goal cannot be achieved without the comprehensive knowledge of the glycosylation mechanism and the driving forces of glycosylation and competing side processes. It is likely that the consecutive scientific development in this field will be focusing on studying the fundamental mechanistic aspects of glycosylation rather than developing additional anomeric leaving groups.

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# Chapter 2

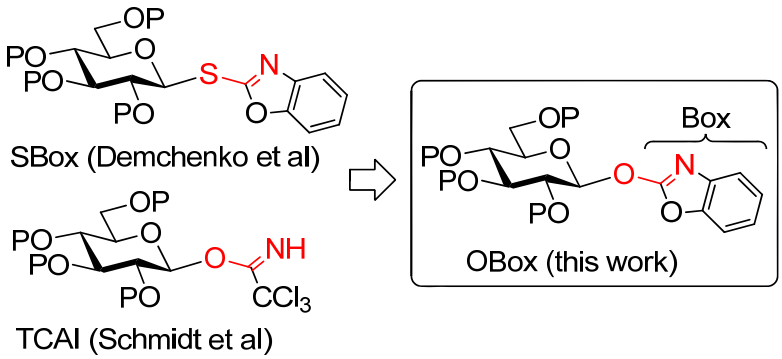
# *O*-Benzoxazolyl imidates as versatile glycosyl donors for chemical glycosylation

Swati S. Nigudkar, Archana R. Parameswar, Papapida Pornsuriyasak, Keith J. Stine, and Alexei V. Demchenko. *O*-Benzoxazolyl imidates as versatile glycosyl donors for chemical glycosylation. *Org. Biomol. Chem.,* **2013**, *11*, 4068-4076.

## 2.1. Introduction

Complex carbohydrates play important roles in many life-sustaining and life-threatening processes including embryonic growth, cell differentiation, immune response, infections, and carcinogenesis.[1] Our understanding of these processes is often dependent on the knowledge of the structure and properties of the carbohydrates involved. Many classes of complex carbohydrates can be isolated from natural sources, but it is chemical or enzymatic synthesis that allows for obtaining sufficient quantities of pure natural compounds or unnatural mimetics thereof.[2] Amongst the synthetic steps required to obtain complex carbohydrates from simple building blocks, chemical glycosylation reactions create the greatest synthetic hurdle for chemists.[3,4] Hence, the development of new leaving groups for chemical glycosylation and the optimization of reaction conditions for their activation represents an important and challenging area of research.[5] The tremendous progress made in the area of glycosciences has resulted in the development of a variety of leaving groups amongst which *O*-trichloroacetimidates (TCAI)[6] and thioglycosides[7] have arguably become the most widespread glycosyl donors to date.

***Figure 2.1.*** **Designing new *O*-benzoxazolyl leaving group**



As a part of the ongoing research effort to develop new methods for chemical glycosylation, our laboratory reported a series of new thioimidoyl leaving groups that have shown superior properties in glycosylation to that of many other methods.[8,9] Amongst these, *S*-benzoxazolyl donors (cyclic *S*-imidate)[10-17] have shown excellent glycosyl donor properties for simple one-step glycosylations and easily fit into a variety of concepts for expeditious oligosaccharide synthesis.[18] Aiming to develop a similar method for chemical glycosylation, we became interested in studying cyclic *O*-imidates. As the first step towards accomplishment of this aim, we report the investigation of a novel leaving group, *O*-benzoxazolyl (OBox), which represents a bridging structure between *O*-imidates and SBox glycosides (Figure 2.1). This study is also expected to complement previous work of Mukaiyama[19-23] and Chirva[24] dedicated to a study of structurally related *O*-imidate derivatives.

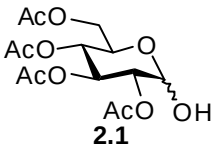
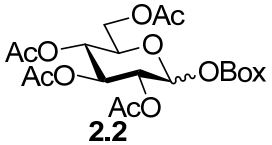
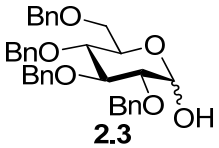
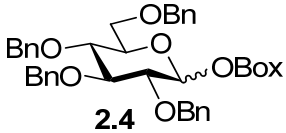
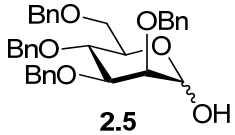
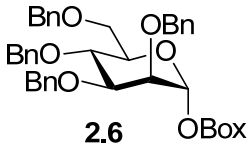
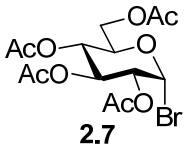
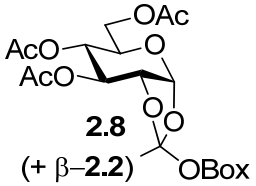
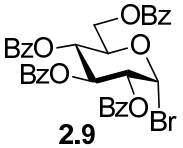
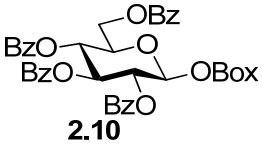
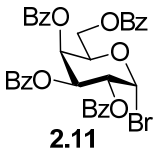
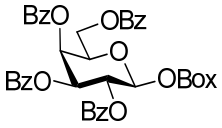
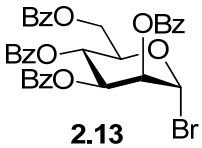
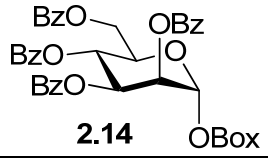
## 2.2. Results and discussion

We determined that the novel OBox leaving group can be introduced by several modes using commercially available and inexpensive precursors. For the sugar part, both hemiacetals and glycosyl bromides were found to be suitable starting materials. In this context, Mukaiyama's imidate was similarly synthesized by direct condensation reaction between anomeric hydroxyl group and 2-chloro-6-nitrobenzthiazole giving a mixture of isomers.[21] As summarized in Table 2.1, a variety of OBox derivatives of the D-gluco (**2.2, 2.4, 2.8, 2.10**), D-manno (**2.6, 2.14**), and D-galacto (**2.12**) series were readily obtained in yields up to 85%. The transformation of hemiacetals **2.1**,[25] **2.3**,[25] and **2.5**[26] into the corresponding OBox imidates **2.2**, **2.4**, and **2.6** involved a base-catalyzed (DBU

or KOH) reaction with 2-chlorobenzoxazole (ClBox, entries 1-3, Table 2.1). With exception of benzylated OBox derivative **2.4** (entry 2) that was obtained as a mixture of anomers ($\alpha/\beta$ = 2.5/1), other substitutions proceeded stereoselectively.

The OBox glycosides could also be obtained from halides using 2-benzoxazolinone (HOBox), but the conversion was more efficient when the corresponding silver salt (AgOBox) was used instead (entries 4-7). To our surprise, conversion of acetylated bromide **2.7**[27] resulted in the formation of a relatively stable OBox orthoester **2.8** along with a small amount of the expected OBox derivative **2.2** (entry 4). Orthoesters have been known for long time and found broad application in regioselective protection of carbohydrates and glycosylation.[28] However, to the best of our knowledge, no occurrence of *O*-imidate-based orthoester derivatives has been reported. On the other hand, reaction of benzobromo derivatives of the gluco **2.9**,[29] galacto **2.11**,[30] and manno series **2.13**[31] with AgOBox in the presence of $Bu_4NI$ and 2,6-lutidine afforded the corresponding OBox imidates **2.10**, **2.12**, and **2.14** in good yields with exclusive 1,2-*trans* anomeric selectivity and no orthoester formation detected. In this context, transformation of the tetrabenzoylated hemiacetal to the corresponding OBox imidate **2.10** was found to be inefficient and incomplete probably due to the reversibility of the reaction. The formation of the *N*-linked glycosides along with *O*- or *S*-linked derivatives upon reaction with bi- or multi-dentate aglycones is not uncommon.[12,32,33] In this particular case, however, no *N*-linked products were isolated.

***Table 2.1.*** **Synthesis of *O*-benzoxazolyl derivatives**

| *Entry* | *Starting material* | *Conditions,*[a] *time* | *Product* | *Yield α/β ratio* |
|---|---|---|---|---|
| 1 |  **2.1** | A, 2 h |  **2.2** | 85% 1.5/1 |
| 2 |  **2.3** | B, 2.5 h |  **2.4** | 80% 2.5/1 |
| 3 |  **2.5** | B, 2 h |  **2.6** | 50% α only |
| 4 |  **2.7** | C, 1.25 h |  **2.8** (+ β–**2.2**) | 79% + 9% (β-**2.2**) |
| 5 |  **2.9** | D, 10 h |  **2.10** | 83% β only |
| 6 |  **2.11** | D, 2.5 h |  **2.12** | 82% β only |
| 7 |  **2.13** | D, 1.5 h |  **2.14** | 81% α only |

[a] – Conditions: A: ClBox, DBU, $CH_2Cl_2$, 0 °C; B: ClBox, KOH, acetone, molec. sieves 3Å, 0 °C; C: AgOBox, $CH_2Cl_2$, molec. sieves 3Å, 40 °C; D: AgOBox, $Bu_4NI$, 2,6-lutidine, $CH_2Cl_2$, rt (entry 6 and 7) or 45 °C (entry 5)

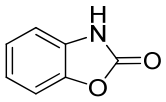
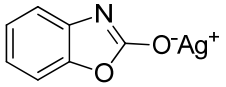
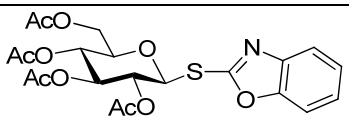
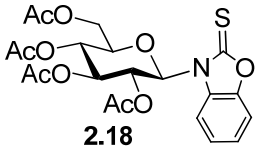
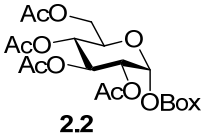
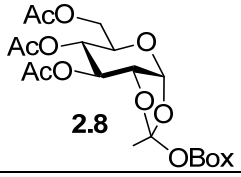
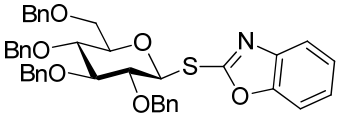
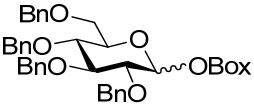
Occasionally, *N*-linked glycosides are also obtained as by-products of the synthesis of *O*- and *S*-imidates. Based on the literature data for per-acetylated SBox glycosides and their N-linked counterparts (**2.17** and **2.18**),[34] we found that UV spectroscopy would

adequately serve our needs for structure determinations.[15] To this end, it should be possible to unambiguously distinguish the isomers by simple and reproducible comparison of the absorption bands. Thus, the thioimide (SH) derivative has two narrow bands ($\lambda_{max}$) at around 280 and 290 nm (C=N), whereas the thioamide (NH) has a single broad band at ~300 nm (C=S, entries 1 and 2, Table 2.2). Resultantly, we anticipated that UV spectroscopy could be used to differentiate *O*-imidates from their *N*-linked counterparts. Indeed, OBox glycoside **2.2** showed two narrow bands at 273 and 279 nm indicative of the imide (C=N) structure (entry 3). Along these lines, HOBox derivative **2.15** exists as an amide (C=O, single band at 275 nm, entry 4), whereas its silver salt **2.16** is an imide (C=N, two bands at 274 and 280 nm, entry 5). In addition to the previously reported data for per-benzylated *S*-benzoxazolyl (SBox) glycosides and their *N*-linked counterparts (**2.19** and **2.20**, entries 7 and 8, respectively),[15] herein we report UV data for per-benzylated OBox derivative **2.4**. The UV spectrum confirms the imide structure (C=N, $\lambda_{max}$ = 270 and 276, entry 8).

Having performed the synthesis and thorough characterization of the novel OBox imidates, we turned our attention to studying their glycosyl donor properties. A typical activation of TCAI is affected under catalysis of a Lewis acid ($BF_3$-$OEt_2$ or TMSOTf) and leads to the corresponding disaccharides in good to excellent yields.[6,35] A typical activation of SBox glycoside requires different reaction conditions and can be affected in the presence of stoichiometric amount of Ag(I), Cu(II), Bi(III), or TMS triflates. Alternatively, SBox glycosides can be activated in the presence of thiophilic promoters, typical for thioglycoside activation,[7] including MeOTf, DMTST, NIS/TMSOTf, etc.[9] With this prior knowledge and a range of OBox donors in hand, we planned on studying

their reactivity in glycosylation reactions with standard glycosyl acceptors **2.21-2.24** (Figure 2.2).[36]

***Table 2.2.*** **Comparative UV data for *O*, *N* and *S*-linked derivatives**

| *Entry* | *Compound* | *Band 1* $\lambda_{max}$ *(nm)* | *Band 2* $\lambda_{max}$ *(nm)* | *Ref.* |
|---|---|---|---|---|
| 1 |  **2.15** | 275 | - | This work |
| 2 |  **2.16** | 274 | 280 | This work |
| 3 |  **2.17** | 280 | 290 | [34] |
| 4 |  **2.18** | 300 | -- | [34] |
| 5 |  **2.2** | 273 | 279 | This work |
| 6 |  **2.8** | 274 | 280 | This work |
| 7 |  **2.19** | 280 | 290 | [15] |
| 9 |  **2.4** | 270 | 276 | This work |

A coupling reaction of per-*O*-benzylated OBox imidate **2.4** with glycosyl acceptor **2.21**[37] in the presence of MeOTf (10 mol %) was completed within 5 min at -78 $^{o}$C and

provided the corresponding disaccharide **2.25**[38] in 97% (α/β = 1/1.7, entry 1, Table 2.3). Activation of OBox imidate **2.4** for reaction with acceptor **2.21** was nearly as efficient in the presence of either TfOH (10 mol %) or TMSOTf (10 mol %) and provided the corresponding disaccharide **2.25** in 85% (α/β = 1/1.2, entry 2) or 95% yield (α/β = 1/4.0, entry 3). Both reactions were completed in less than 5 min at -20 $^{o}$C (entry 2) or -78 $^{o}$C (entry 3). The latter reaction conditions (TMSOTf, -78 $^{o}$C) were applied to glycosylation of secondary glycosyl acceptors **2.22-2.24**[39-41] with OBox donor **2.4**. Again, all couplings were completed within 5 min and provided the corresponding disaccharides **2.26-2.28**[42-44] in 89-95% yields (α/β = 1/1.0-2.4, entries 4-6). Other promoters including AgOTf, MeOTf, $Bi(OTf)_3$, and $Cu(OTf)_2$ also provided very efficient activation of OBox glycosides (vide infra). Although OBox donor **2.4** showed very satisfactory results, relatively low stereoselectivity was observed in practically all glycosidations of **2.4** using 1,2-dichloroethane as the solvent. A similar reactivity profile was recorded for per-benzylated mannosyl donor **2.6** with an exception that a moderate selectivity was obtained with primary acceptor **2.21** and complete 1,2-trans selectivity was observed with secondary acceptors **2.22-2.24** (entries 7-10) to provide the respective disaccharides **2.29-2.32** in excellent yields of 90-94%.

**Figure 2.2. Glycosyl acceptors investigated**

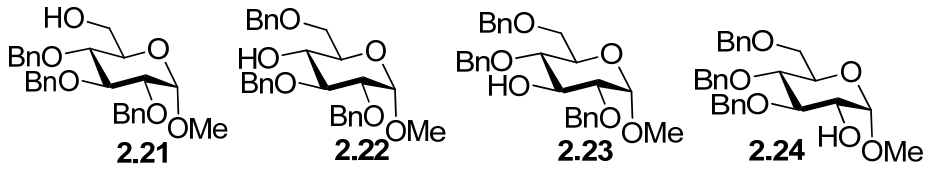
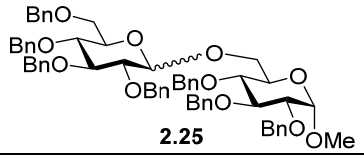
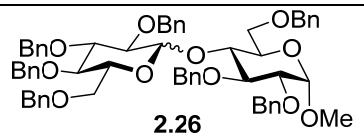
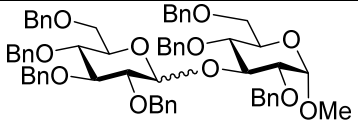
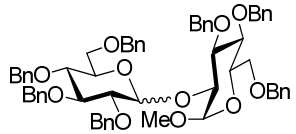
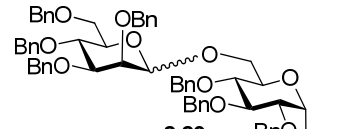
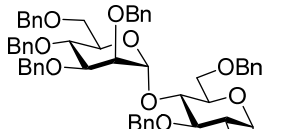
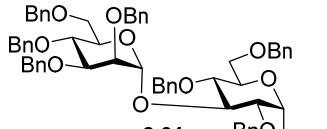
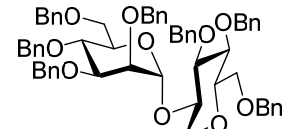


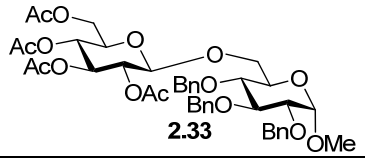
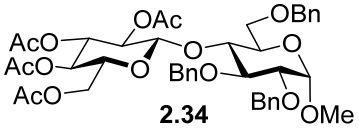
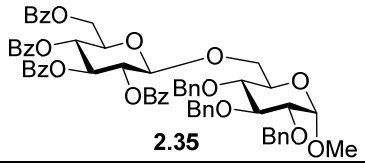
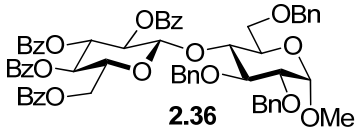
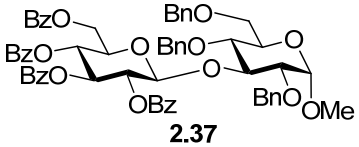
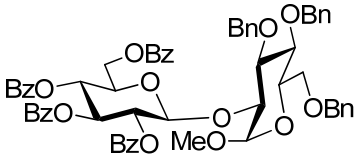
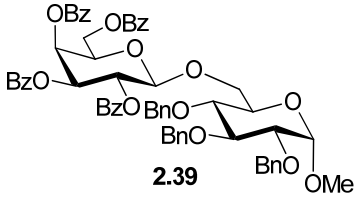
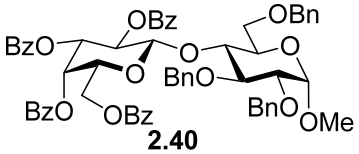
***Table 2.3.*** **Synthesis of 1,2-*cis* glycosides**

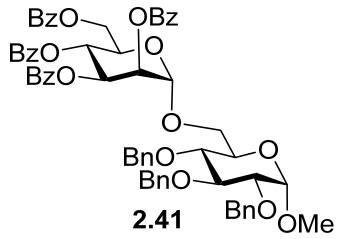
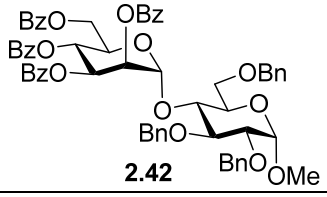
| Entry | Donor + Acceptor, Conditions,[a] time | Product | Yield, α/β ratio |
|---|---|---|---|
| 1 | **2.4** + **2.21** Method A, 15 min |  **2.25** | 97%, 1/1.7 |
| 2 | **2.4** + **2.21** Method B, 5 min | **2.25** | 85%, 1/1.2 |
| 3 | **2.4** + **2.21** Method C, 5 min | **2.25** | 95%, 1/4.0 |
| 4 | **2.4** + **2.22** Method C, 5 min |  **2.26** | 89%, 1/1.5 |
| 5 | **2.4** + **2.23** Method C, 5 min |  **2.27** | 95%, 1/1.0 |
| 6 | **2.4** +**2.24** Method C, 5 min |  **2.28** | 92%, 1/2.4 |
| 7 | **2.6** + **2.21** Method C, 5 min |  **2.29** | 93%, 2.9/1 |
| 8 | **2.6** + **2.22** Method C, 5 min |  **2.30** | 91%, α only |
| 9 | **2.6** + **2.23** Method C, 5 min |  **2.31** | 90%, α only |
| 10 | **2.6** + **2.24** Method C, 5 min |  **2.32** | 94%, α only |

Having investigated the reactivity of per-*O*-benzylated OBox glycosyl donors **2.4** and **2.6**, we turned our attention to studying their per-*O*-acylated counterparts. The disarming (deactivating) effect of electron-withdrawing substituents on the reactivity of building blocks in glycosylation is well known.[45-47] Therefore, we anticipated substantially reduced reactivity of per-acylated OBox derivatives. Surprisingly, the reactivity of per-acylated derivatives was similar to that observed with their per-benzylated counterparts. Although the reactions were fast, per-acetylated OBox glycoside **2.2** provided somewhat low yields (data not shown) when TMSOTf was used as the promoter. We relate this to the competing acetyl migration from the O-2 of the glycosyl donor to the free hydroxyl of the glycosyl acceptor. In this context, $SnCl_4$-promoted glycosidations of glycosyl donor **2.2** with glycosyl acceptors **2.21** and **2.22** were much cleaner, showed minimal acetyl migration, and provided the corresponding 1,2-trans-linked disaccharides **2.33**[44] and **2.34**[48] in good yields of 85% and 77%, respectively (entries 1 and 2, Table 2.4). $SnCl_4$-promoted glycosidation of the acetylated OBox orthoester **2.8** provided a very similar outcome in comparison to the glycosylation of glycosyl imidate **2.2** (entries 3 and 4).

The reactivity of per-benzoylated glucose derivative **2.10** in presence of TMSOTf at -78 $^{o}$C was also quite similar to that observed with its per-benzylated counterpart **2.4**. All glycosidations of donor **2.10** were completed within 5-10 min and provided the corresponding disaccharides **2.35-2.38**[36,49,50] in excellent yields of 87-97% (entries 5-8). Very high reactivity was also observed for per-benzoylated galactosyl and mannosyl donors **2.12** and **2.14,** respectively. All glycosylations in the presence of TMSOTf at -78 $^{o}$C were completed within 5-15 min and provided the corresponding disaccharides **2.39-2.42**[51-53] in excellent yields of 78-97% (entries 9-12).

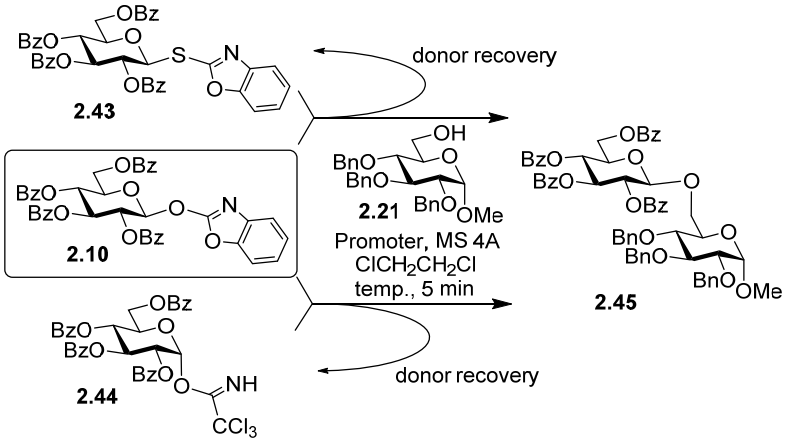
***Table 2.4.*** **Synthesis of 1,2-*trans* glycosides**

| Entry | Donor + Acceptor, Conditions,[a] time | Product | Yield, α/β ratio |
|---|---|---|---|
| 1 | **2.2** + **2.21** Method D, 5 min |  **2.33** | 85%, β only |
| 2 | **2.2** + **2.22** Method D, 5 min |  **2.34** | 77%, β only |
| 3 | **2.8** + **2.21** Method D, 15 min | **2.33** | 82%, β only |
| 4 | **2.8** + **2.22** Method D, 15 min | **2.34** | 75%, β only |
| 5 | **2.10** +**2.21** Method C, 10 min |  **2.35** | 89%, β only |
| 6 | **2.10** + **2.22** Method C, 10 min |  **2.36** | 88%, β only |
| 7 | **2.10** + **2.23** Method C, 10 min |  **2.37** | 88%, β only |
| 8 | **2.10** + **2.24** Method C, 10 min |  **2.38** | 87%, β only |
| 9 | **2.12** + **2.21** Method C, 5 min |  **2.39** | 83%, β only |
| 10 | **2.12** + **2.22** Method C, 5 min |  **2.40** | 78%, β only |

| | | | |
|---|---|---|---|
| 11 | **2.14** + **2.21**<br>Method C, 15 min |  <br>**2.41** | 95%,<br>α only |
| 12 | **2.14** + **2.22**<br>Method C, 15 min |  <br>**2.42** | 97%,<br>α only |

With the high reactivity of OBox glycosides observed even under relatively mild and catalytic activation conditions, we aimed at establishing the comparative reactivity profile of the OBox imidates along with known glycosyl donors. Using the previously established protocol for competitive glycosylations, two per-*O*-benzoylated glycosyl donors, OBox **2.10** and SBox **2.43**,[15] were set to compete for a limited quantity of glycosyl acceptor **2.21**. Competitive glycosylations performed in the presence of 10 mol. % of TMSOTf, AgOTf or $Cu(OTf)_2$ all showed OBox donor **2.10** to be much more reactive. This was judged by nearly complete recovery of the unreacted SBox donor **2.43** (Table 2.5). Similar observations were made in the competitive reaction between OBox donor **2.10** and TCAI donor **2.44**. Again, the OBox donor was more reactive under all of the following activation conditions: TMSOTf (0.05 equiv.), AgOTf (0.1 equiv.), or $Bi(OTf)_3$ (0.03 equiv.), whereas TCAI donor **2.44** was recovered in 82-93% yield (Table 2.5).

***Table 2.5.*** **OBox imidate 2.10 showed consistently higher reactivity in comparison with SBox glycoside 2.43 and trichloroacetimidate 2.44**




| Glycosyl donors | Conditions,[a] temp. | Yield of **2.45** | Donor recovery |
|---|---|---|---|
| **2.10** + **2.43** | Method C, 0 °C | 93% | **2.43**: 93% |
| | Method E, rt | 94% | **2.43**: 92% |
| | Method F, rt | 99% | **2.43**: 95% |
| **2.10** + **2.44** | Method C,[b] -78 °C | 96% | **2.44**: 87% |
| | Method E, rt | 91% | **2.44**: 82% |
| | Method G, rt | 90% | **2.44**: 93% |

[a] – Method C: TMSOTf (0.1 equiv. to donor), molec. sieves 4Å, $ClCH_2CH_2Cl$; Method E: $Cu(OTf)_2$ (0.1 equiv. to donor), molec. sieves 4Å, $ClCH_2CH_2Cl$; Method F: AgOTf (0.1 equiv. to donor), molec. sieves 4Å, $ClCH_2CH_2Cl$; Method G: $Bi(OTf)_3$ (0.03 equiv. to donor), molec. sieves 4Å, $ClCH_2CH_2Cl$, rt; [b] – 0.05 equiv. of TMSOTf was used

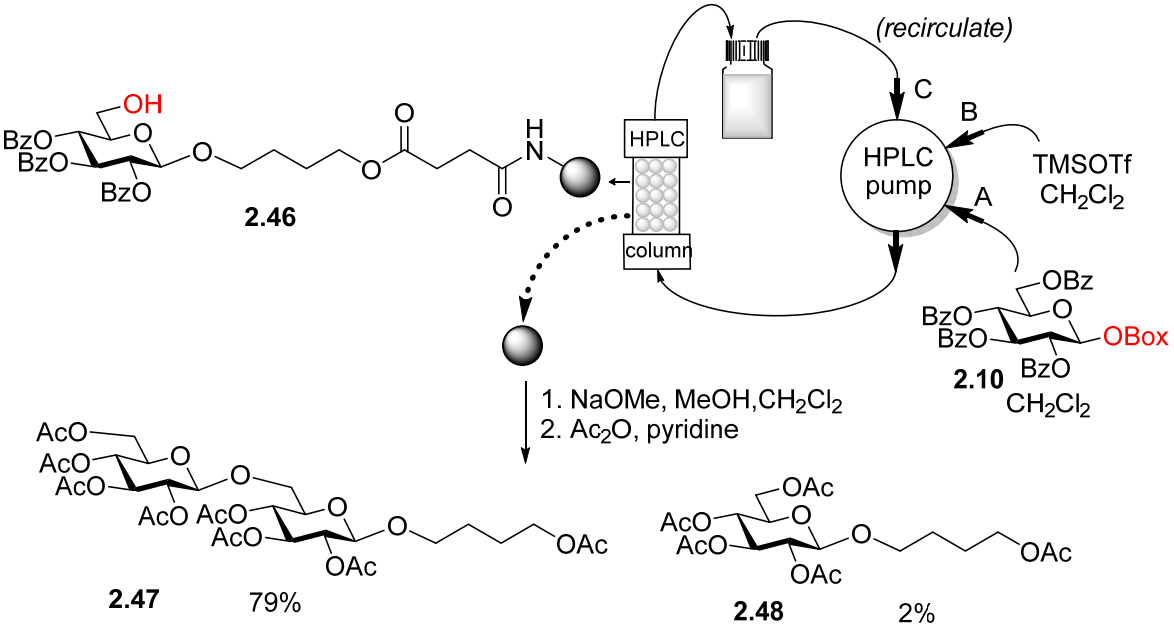
Polymer-supported synthesis is very attractive because it allows for rapid synthesis of oligosaccharide sequences without the necessity of purifying (and characterizing) the intermediates.[54,55] On achieving a series of solution-based experiments, we decided to investigate possible beneficial effects of using highly reactive OBox imidates in glycosylations on polymer support using our recently developed HPLC-assisted automated technology.[56] This experimental setup based on an unmodified HPLC

instrument was executed as follows. The Omnifit SolventPlus chromatography glass column was loaded with the resin-bond glycosyl acceptor. The loaded column was connected to the HPLC and solutions of glycosyl donor and promoter in $CH_2Cl_2$ were then delivered concomitantly using different heads of the multi-headed HPLC pump. Upon completion of glycosylation, as judged by the HPLC detector, the resulting disaccharide can be cleaved off from the polymer support or the oligosaccharide sequencing can be continued as desired.

In this particular application, Tentagel resin-bound glycosyl acceptor **2.46**,[56] attached via the anomeric center, was loaded into the Omnifit column, which was connected to the HPLC equipment (Scheme 2.1). Reagent bottles, one containing a 22 mM solution of glycosyl donor **2.10** in $CH_2Cl_2$ and another one containing a 27 mM solution of TMSOTf in $CH_2Cl_2$ were connected to inlets for pumps A and B, respectively. Pumps A/B were then programmed to deliver the mixed solution of donor/promoter concomitantly in the ratio of 4/1 (v/v) at the total flow rate of 0.3 mL/min. Upon exiting the column, a solution containing unreacted donor and promoter was collected in a separate vial that would be subsequently used as a recirculating chamber connected to Pump C. Upon consumption of the fresh solution of glycosyl donor **2.10** (10 equiv., ~20 min), Pump A was switched to Pump C connected to the recirculating chamber (80%), while Pump B continued to deliver fresh TMSOTf (20%) at a combined flow rate of 0.3 mL/min for 10 min. After that, Pump C was set to 100% flow rate and the information about the consumption of donor **2.10** and accumulation of the corresponding hemiacetal, the product of the competing hydrolysis, was acquired by periodic monitoring of the solution phase by TLC. Upon complete hydrolysis of glycosyl donor **2.10** (about 60 min of recirculation),

pump C was stopped and the column was purged with $CH_2Cl_2$ (pump A, 2.0 mL/min) for 10 min. The formation of disaccharide **2.47**[56] was determined by cleaving off the sugar molecule from the resin using 0.1 M solution of $NaOCH_3$ in $CH_3OH/CH_2Cl_2$ followed by acetylation ($Ac_2O$/pyridine). The treatment with the $NaOCH_3$ solution can be performed using either HPLC set-up directly or by transferring the resin into a separate flask and performing conventional deprotection. Resultantly, product **2.47** was isolated in 79% yield along with 2% of a monosaccharide product **2.48**[56] derived from the unreacted acceptor **2.46**.

***Scheme 2.1.*** **Exploratory study of the coupling efficiency of glycosyl donor 2.10 with the polymer-bound acceptor 2.46**



## 2.3. Conclusions

We have discovered a new class of *O*-imidoyl glycosyl donors that were shown to be more reactive than previously investigated *O*- and *S*-imidates. These donors can be activated using a catalytic amount of a Lewis acid, as little as 3 mol %. High reactivity profile of OBox imidates was confirmed in direct competitive experiments with known glycosyl donors and was applied to HPLC-assisted glycosylation on polymer support. Further application of OBox donors to automated oligosaccharide synthesis and investigation of their structure-reactivity-stereoselectivity profile is underway in our laboratories

## 2.4. Experimental Section

### *2.4.1. General Remarks*

All reactions were conducted under argon with dry, freshly distilled solvents unless otherwise noted. $CH_2Cl_2$, $ClCH_2CH_2Cl$, and acetone were distilled from $CaH_2$ directly prior to application. 2-Chlorobenzoxazole and 2-benzoxazolinone were used as is. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried for 2-3 h directly prior to application. $Cu(OTf)_2$ and $Bi(OTf)_3$ were dried *in vacuo* for 3-4 h directly prior to use. TMSOTf and $SnCl_4$ were used as is. Molecular sieves (3 Å or 4 Å), used for reactions, were crushed and activated overnight at 390 $^oC$ and then for 2-3 h at 390 $^oC$ prior to application. Reactions were monitored by TLC on Kieselgel 60 $F_{254}$ and 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 $^oC$. Column chromatography was performed on silica gel 60 (70-230 mesh). Optical rotations were

measured at 'Jasco P-1020' polarimeter. $^{1}$H-NMR spectra were recorded at 300MHz, $^{13}$C-NMR spectra were recorded at 75MHz (Bruker Avance). The $^{1}$H chemical shifts are referenced to the signal of the residual $CHCl_3$ ($\delta_H$ = 7.27 ppm) for solutions in $CDCl_3$. The $^{13}$C chemical shifts are referenced to the central signal of $CDCl_3$ ($\delta_C$ = 77.23 ppm) for solutions in $CDCl_3$. HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

**Silver benzoxazol-2-olate (AgOBox, 2.16).** A solution of 2-benzoxazolinone (HOBox, 2.0 g, 14.8 mmol) and NaOH (0.6 g, 14.8 mmol) in water (14 mL) was added dropwise to a solution of $AgNO_3$ (2.5 g, 14.8 mmol) in water (14 mL) and the resulting mixture was stirred for 10 min at rt. The solid was filtered off and rinsed successively with water (10 mL), MeOH (3 mL), and water (10 mL). The solid was collected and dried *in vacuo* for 16 h to afford the title compound (3.3 g, 92% yield) as a brown solid. The title compound can be stored for extended period of time but was additionally dried for 4-5 h directly prior to application. Analytical data for **2.16**: $^{1}$H-NMR (300 MHz): δ, 6.77 (t, 1H, aromatic), 6.95 (m, 3H, aromatic); $^{13}$C-NMR (75 MHz): δ, 107.2, 111.5, 118.3, 121.9, 142.5, 146.4, 163.3; UV: $\lambda_{max}$ = 274, 280 nm.

### *2.4.2. Synthesis of OBox glycosyl donors*

**Benzoxazolyl 2,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranoside (2.2).** 2-Chlorobenzoxazole (79.5 μL, 0.7 mmol) and DBU (0.14 mL, 0.93 mmol) were added to a stirring solution of 2,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranose[25] (**2.1**, 0.16 g, 0.46 mmol) in dry $CH_2Cl_2$ (1.6 mL) and the resulting mixture was stirred for 2 h at 0 $^{o}$C. After that,

the solid was filtered off and washed successively with $CH_2Cl_2$. The combined filtrate (~60 mL) was washed with 1% aq. NaOH (2 x 20 mL), water (20 mL), and brine (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 obtain the title compound (187 mg, 85% yield) as a white foam. Analytical data for α-**2.2**: $R_f$ = 0.42 (ethyl acetate/hexanes, 1/1, v/v); $[\alpha]_D^{22}$ +84.5 (*c*= 1.0, $CHCl_3$); $^1$H-NMR (300 MHz): δ, 2.04-2.11 (m, 12H, 4 x $COCH_3$), 4.13 (m, 1H, H-6a), 4.30 (m, 2H, H-5, 6b), 5.25 (m, 2H, $J_{2,3}$ = 9.9 Hz, $J_{4,5}$ = 3.5 Hz, H-2, 4), 5.70 (dd, 1H, $J_{3,4}$ = 9.9 Hz, H-3), 6.57 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 7.20-7.50 (m, 4H, aromatic), 7.44 (d, 1H, aromatic), 7.21-7.50 (m, 4H, aromatic) ppm; $^{13}$C-NMR (75 MHz): δ, 22.7 (x 4), 61.2, 67.6, 69.3, 69.5, 70.1, 96.7, 110.2, 118.6, 123.6, 124.7, 140.4, 148.4, 161.5, 169.4, 169.9, 170.1, 170.6; UV: $\lambda_{max}$ = 273, 279 nm; HR-FAB MS $[M+Na]^+$ calculated for $C_{21}H_{23}NO_{11}Na^+$ 488.1168, found 488.1168.

**Benzoxazolyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranoside (2.4).** 2-Chlorobenzoxazole (0.20 g, 1.9 mmol) and KOH (0.021 g, 0.37 mmol) were added to a stirring solution of 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranose (**2.3**, 0.2 g, 0.37 mmol) in acetone (4.0 mL) and the resulting mixture was stirred for 3 h at 0 $^oC$. After that, the solid was filtered off and washed successively with $CH_2Cl_2$. The combined filtrate (~60 mL) was washed with water (3 x 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate – hexanes gradient elution) to obtain the title compound (195 mg, 80% yield, α/β = 2.5/1) as a colorless syrup. Analytical data for α-**2.4**: $R_f$ = 0.43

(ethyl acetate/hexanes, 1/4, v/v); $^{1}$H-NMR (300 MHz): δ, 3.61 (dd, 1H, $J_{5,6a}$ = 1.6 Hz, $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.77 (m, 3H, H-2, 4, 6b), 4.00 (m, 1H, H-5), 4.11 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 4.48 (dd, 2H, $^{2}J$ = 12.0 Hz, C$H_2$Ph), 4.67 (dd, 2H, $^{2}J$ = 10.7 Hz, C$H_2$Ph), 4.73 (s, 2H, C$H_2$Ph), 4.90 (dd, 2H, $^{2}J$ = 10.9 Hz, C$H_2$Ph), 6.47 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 7.17-7.31 (m, 24H, aromatic) ppm; $^{13}$C-NMR (75 MHz): δ, 68.1, 73.5, 73.7 (x 2), 75.4, 76.0, 76.8, 79.2, 81.6, 98.8, 110.0, 118.6, 123.2, 124.5, 127.9 (x 2), 128.0 (x 3), 128.1 (x 4), 128.2 (x 2), 128.6 (x 9), 137.6, 138.0, 138.3, 138.8, 141.0, 148.4, 162.3; UV: $\lambda_{max}$ = 270, 276 nm; HR-FAB MS $[M+Na]^+$ calculated for $C_{41}H_{39}NO_7Na^+$ 680.2624, found 680.2624.

**Benzoxazolyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (2.6).** 2-Chlorobenzoxazole (0.056 g, 0.37 mmol) and KOH (0.054 g, 0.96 mmol) were added to a stirring solution of 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose[26] (**2.5**, 0.2 g, 0.37 mmol) in acetone (4.0 mL) and the resulting mixture was stirred for 2 h at 0 $^{o}$C. After that, the solid was filtered off and washed successively with $CH_2Cl_2$. The combined filtrate (~60 mL) was washed with water (3 x 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexanes gradient elution) to obtain the title compound (122 mg, 50 % yield) as a colorless syrup. Analytical data for **2.6**: $R_f$ = 0.4 (ethyl acetate/hexanes, 1/4, v/v); $[\alpha]_D^{21}$ +36.2 (*c*= 1.0, $CHCl_3$); $^{1}$H-NMR (300 MHz): δ, 3.65 (dd, 1H, $J_{5,6b}$ =1.8 Hz, $J_{6a,6b}$ = 11.2 Hz, H-6b), 3.74 (dd, 1H, $J_{5,6a}$ = 4.4 Hz, H-6a), 3.90-3.97 (m, 3H, $J_{3,4}$ = 9.4 Hz, H-2, 3, 5), 4.09 (dd, 1H, $J_{4,5}$ = 9.4 Hz, H-4), 4.50 (dd, 2H, $^{2}J$ = *12*.0 Hz, C$H_2$Ph), 4.54 (dd, 2H, $^{2}J$ = 11.7 Hz, C$H_2$Ph), 4.65 (dd, 2H, $^{2}J$ = 10.8 Hz,

C$H_2$Ph), 4.74 (dd, 2H, $^2J$ = 12.3 Hz, C$H_2$Ph), , 6.36 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 7.11-7.51 (m, 24H, aromatic) ppm; $^{13}$C-NMR (75 MHz): δ, 68.90, 72.68, 72.97, 73.51, 73.64, 74.21, 75.01, 75.36, 79.21, 99.56, 110.04, 118.66, 123.36 (x 2), 124.60 (x 2), 127.66 (x 2), 127.97 (x 2), 128.03 (x 2), 128.14 (x 2), 128.22 (x 2), 128.47 (x 2), 128.55 (x 2), 137.89 (x 2), 138.29 (x 2), 138.43 (x 2), 138.47 (x 2), 141.03, 148.54, 161.60; HR-FAB MS $[M+Na]^+$ calculated for $C_{41}H_{39}NO_7Na^+$ 680.2624, found 680.2626.

**3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-benzoxazolyloxyethylidene)-α-D-glucopyranose (2.8).** A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[27] (**2.7**, 0.50 g, 1.2 mmol) and freshly activated molecular sieves (3Å, 1.5 g) in dry $CH_2Cl_2$ (5.0 mL) was stirred under argon for 1 h at rt. After that, AgOBox (**2.16**, 0.59 g, 2.43 mmol) was added and the resulting mixture was stirred for 1.5 h at 40 $^o$C. The solid was filtered off and rinsed successively with $CH_2Cl_2$. The combined filtrate (~100 mL) was washed with 1% aq. NaOH (2 x 40 mL), water (2 x 40 mL), and brine (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-hexanes gradient elution) to afford the title compound (447 mg, 79% yield) as a pale-yellow foam. Analytical data for **2.8**: $R_f$ = 0.5 (ethyl acetate/hexanes, 1/1, v/v); $[\alpha]_D^{26}$ -3.7 (*c* = 1.0, $CHCl_3$); $^1$H-NMR (300 MHz): δ, 2.08, 2.10, 2.13, 2.15 (4 s, 12H, 4 x $COCH_3$), 4.06 (m, 1H, H-5), 4.25-4.28 (m, 3H, $J_{2,3}$ = 2.4 Hz, H-2, 6a, 6b), 4.95 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 5.32 (dd, 1H, $J_{3,4}$ = 2.4 Hz, H-3), 5.82 (d, 1H, $J_{1,2}$ = 5.3 Hz, H-1), 7.10-7.47 (m, 4H, aromatic) ppm; $^{13}$C-NMR (75 MHz): δ, 20.7, 20.8, 20.9, 22.4, 63.0, 67.3, 68.0, 69.3, 73.1, 97.5, 110.2, 111.7, 113.5, 124.0,

124.4, 129.4, 142.5, 151.6, 169.0, 169.7, 170.7 ppm; UV: $\lambda_{max}$ = 274, 280 nm; HR-FAB MS $[M+Na]^+$ calculated for $C_{21}H_{23}NO_{11}Na^+$ 488.1168, found 488.1170.

**Benzoxazolyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (2.10).** A mixture of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[29] (**2.9**, 0.40 g, 0.56 mmol) and freshly activated molecular sieves (3Å, 1.2 g) in dry $CH_2Cl_2$ (4.0 mL) was stirred under argon for 1 h at rt. After that, AgOBox (**2.16**, 0.20 g, 0.84 mmol), 2,6-lutidine (0.09 g, 0.84 mmol), and tetrabutylammonium iodide (TBAI, 0.012 g, 0.033 mmol) were added and the resulting mixture was stirred for 10 h at rt. The solid was filtered off and rinsed successively with $CH_2Cl_2$. The combined filtrate (~60 mL) was washed with 1% aq. NaOH (2 x 20 mL), water (2 x 20 mL), and brine (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-hexanes gradient elution) to afford the title compound (0.36 g, 83% yield) as a pale-yellow amorphous solid. Analytical data for **2.10**: $R_f$ = 0.46 (ethyl acetate/hexanes, 2/3, v/v); $[\alpha]_D^{26}$ +35.9 (*c*= 1.0, $CHCl_3$); $^1$H-NMR (300 MHz): δ, 4.50 (m, 2H, $J_{5,6a}$ = 3.2 Hz, H-5, 6a), 4.70 (dd, 1H, $J_{5,6b}$ = 1.5 Hz, H-6b), 5.76 (m, 1H, $J_{2,3}$ = 5.5 Hz, H-2), 5.87 (dd, 1H, $J_{4,5}$ = 4.6 Hz, H-4), 6.03 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 6.38 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 7.15-7.92 (m, 24H, aromatic), ppm; $^{13}$C-NMR: δ, 62.6, 68.9, 70.9, 72.5, 73.4, 98.7, 110.2, 118.5, 123.6 (x 2), 124.6 (x 2), 128.4 (x 2), 128.4 (x 2), 128.5 (x 4), 128.6, 129.5, 129.8 (x 2), 129.9 (x 4), 130.0 (x 2), 133.1, 133.5, 133.6 (x 2), 140.4, 148.5, 161.6, 164.9, 165.1, 165.7, 166.1 ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{41}H_{31}NO_{11}Na^+$ 736.1795, found 736.1794.

**Benzoxazolyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (2.12).** A mixture of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide[30] (**2.11**, 0.20 g, 0.28 mmol) and freshly activated molecular sieves (3Å, 0.6 g) in dry $CH_2Cl_2$ (2.0 mL) was stirred under argon for 1 h at rt. After that, AgOBox (**2.16**, 0.10 g, 0.42 mmol), 2,6-lutidine (0.045 g, 0.42 mmol), TBAI (0.006 g, 0.016 mmol) were added and the resulting mixture was stirred for 3 h at rt. The solids were filtered off and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL), water (2 x 10 mL), and brine (10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexanes gradient elution) to afford the title compound (177 mg, 82% yield) as a yellow foam. Analytical data for **2.12**: $R_f$ = 0.50 (ethyl acetate/hexanes, 2/3, v/v); $[\alpha]_D^{26}$ +81.5 (*c* = 1.0, $CHCl_3$); $^1$H-NMR (300 MHz): δ, 4.51 (m, 1H, $J_{5,6a}$ = 5.2 Hz, $J_{6a,6b}$ = 9.6 Hz, H-6a), 4.68 (m, 2H, $J_{5,6b}$ = 6.8 Hz, H-5, 6b), 5.78 (dd, 1H, $J_{3,4}$ = 3.4 Hz, H-3), 6.14 (m, 2H, $J_{2,3}$ = 10.2 Hz, H-2, 4), 6.39 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 7.19-8.13 (m, 24H, aromatic), ppm; $^{13}$C-NMR (75 MHz): δ, 61.8, 67.7, 68.8, 71.6, 72.6, 99.1, 110.2, 118.5, 123.6, 124.6, 128.6 (x 6), 128.7, 128.8 (x 4), 129.3, 129.9 (x 4), 129.9 (x 2), 130.1 (x 2), 133.3, 133.5, 133.6, 133.8, 140.4, 148.5, 161.7, 165.1, 165.5 (x 2), 166.0 ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{41}H_{31}NO_{11}Na^+$ 736.1795, found 736.1800.

**Benzoxazolyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (2.14).** A mixture of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[31] (**2.13**, 0.20 g, 0.28 mmol) and freshly activated molecular sieves (3Å, 0.6 g) in dry $CH_2Cl_2$ (2.0 mL) was stirred under argon for 1 h at rt. After that, AgOBox (**2.16,** 0.10 g, 0.42 mmol), 2,6-lutidine (0.045 g,

0.42 mmol), and TBAI (0.006 g, 0.016 mmol) were added and the resulting mixture was stirred for 1.5 h 45 $^{o}$C. The solid was filtered off and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL), water (2 x 10 mL), and brine (10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexanes gradient elution) to afford the title compound (175.2 mg, 81% yield) as a white foam. Analytical data for **2.14**: $R_f$ = 0.44 (ethyl acetate/hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +0.62 (*c* = 1.0, $CHCl_3$); $^1$H-NMR (300 MHz): δ, 4.50 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 12.9 Hz, H-6a), 4.68 (dd, 1H, $J_{5,6b}$ = 2.4 Hz, H-5, 6b), 6.04 (dd, 1H, $J_{2,3}$ = 2.0 Hz, H-2), 6.09 (dd, 1H, $J_{3.4}$ = 10.0 Hz, H-3), 6.25 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 6.64 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 7.25-8.08 (m, 24H, aromatic), ppm; $^{13}$C-NMR (75 MHz): δ, 62.3, 66.0, 68.8, 69.4, 71.3, 97.7, 110.1, 118.9, 123.7, 124.7, 128.4 (x 3), 128.6 (x 3), 128.7 (x 3), 128.8 (x 2), 129.7, 129.7 (x 2), 129.8 (x 2), 129.9 (x 2), 130.0 (x 2), 133.1, 133.5, 133.7, 133.8, 140.5, 148.6, 160.8, 165.1, 165.3, 165.5, 166.0 ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{41}H_{31}NO_{11}Na^+$ 736.1795, found 736.1769.

**2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (2.44).** This compound was obtained from 2,3,4,6-tetra-*O*-benzoyl-α/β-D-glucopyranose[57] by method previously described[58] in 83% yield as a white foam. Analytical data for **2.44**: $R_f$ = 0.44 (ethyl acetate/hexanes, 3/7, v/v); $[\alpha]_D^{27}$ +76.6 (*c*= 1.0, $CHCl_3$); $^1$H-NMR (300 MHz): δ, 4.49 (dd, 1H, $J_{5,6a}$ = 5.4 Hz, $J_{6a,6b}$ = 12.8 Hz, H-6a), 4.64 (m, 2H, $J_{5,6b}$ = 2.6 Hz, H-5, 6b), 5.62 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 5.83 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 6.28 (dd, 1H, $J_{3,4}$ = 9.8 Hz, H-3), 6.84 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 7.26-8.17 (m, 20H, aromatic), 8.64 (s, 1H,

-NH) ppm; $^{13}$C-NMR (75 MHz): δ, 62.7, 68.8, 70.4, 70.9, 90.9, 93.3, 128.6 (x 5), 128.7 (x 5), 128.8 (x 1), 129.0, 129.7, 129.9 (x 2), 130.0 (x 5), 133.4, 133.5, 133.8 (x 2), 160.7, 165.4, 165.6, 165.9, 166.3; HR-FAB MS $[M+Na]^+$ calculated for $C_{36}H_{28}Cl_3NO_{10}Na^+$ 762.0676, found 762.0671.

### *2.4.3. General glycosylation procedures*

***Method A.*** *Typical MeOTf-promoted glycosylation procedure:* A mixture of glycosyl donor (0.13 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3 Å, 90 mg) in 1,2-dichloroethane (0.5 mL) was stirred under argon for 1 h at rt. The mixture was cooled to -20 $^{o}$C, MeOTf (0.013 mmol) was added and the resulting mixture was stirred for 15 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~30 mL) was washed with 1% aq. NaOH (2 x 5 mL) and water (2 x 5 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 - hexanes gradient elution) to obtain the respective disaccharide in a yield listed in Table 2.3.

***Method B.*** *Typical TfOH-promoted glycosylation procedure:* A mixture of glycosyl donor (0.13 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4 Å, 90 mg) in 1,2-dichloroethane (0.5 mL) was stirred under argon for 1 h at rt. The mixture was cooled to -20 $^{o}$C, TfOH (0.013 mmol) was added and the resulting mixture was stirred for 10 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~30 mL) was washed with 1%

aq. NaOH (2 x 5 mL) and water (2 x 5 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 - hexanes gradient elution) to obtain the respective disaccharide in a yield listed in Table 2.3.

***Method C.*** *Typical TMSOTf-promoted glycosylation procedure:* A mixture of glycosyl donor (0.12 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4Å, 90 mg) in 1,2-dichloroethane (0.5 mL) was stirred under argon for 1 h at rt. On most occasions (see Table 2.3) the mixture was cooled to -78 $^oC$, TMSOTf (0.012 mmol) was added and the resulting mixture was stirred for 5-15 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~30 mL) was washed with 1% aq. NaOH (2 x 5 mL) and water (2 x 5 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 - hexanes gradient elution) to obtain the respective disaccharide in a yield listed in Table 2.3.

***Method D.*** *Typical $SnCl_4$-promoted glycosylation procedure:* A mixture of glycosyl donor (0.12 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4 Å, 90 mg) in 1,2-dichloroethane (0.5 mL) was stirred under argon for 1 h at rt. After that, $SnCl_4$ (0.012 mmol) was added and the resulting mixture was stirred for 5-15 min at rt (Table 2.3). The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~30 mL) was washed with 1% aq. NaOH (2 x 5 mL) and water (2 x 5 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 obtain the respective disaccharide in a yield listed in Table 2.4.

### *2.4.4. General procedures for competitive glycosylations*

***Method E.*** *Typical $Cu(OTf)_2$-promoted glycosylation procedure:* A mixture of glycosyl donor A (0.12 mmol), glycosyl donor B (0.12 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4Å, 90 mg) in 1,2-dichloroethane (0.5 mL) was stirred under argon for 1 h at rt. After that, $Cu(OTf)_2$ (0.012 mmol) was added and the resulting mixture was stirred for 5 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~30 mL) was washed with 1% aq. NaOH (2 x 5 mL) and water (2 x 5 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 obtain the respective disaccharide in a yield listed in Table 2.5.

***Method F.*** *Typical AgOTf-promoted glycosylation procedure:* A mixture of glycosyl donor A (0.12 mmol), glycosyl donor B (0.12 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4Å, 90 mg) in 1,2-dichloroethane (0.5 mL) was stirred under argon for 1 h at rt. After that, AgOTf (0.012 mmol) was added and the resulting mixture was stirred for 10 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~30 mL) was washed with 1% aq. NaOH (2 x 5 mL) and water (2 x 5 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 obtain the respective disaccharide in a yield listed in Table 2.5.

***Method G.*** *Typical $Bi(OTf)_3$-promoted glycosylation procedure:* A mixture of glycosyl donor A (0.12 mmol), glycosyl donor B (0.12 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4Å, 90 mg) in 1,2-dichloroethane (0.5 mL) was stirred under argon for 1 h at rt. After that, $Bi(OTf)_3$ (0.004 mmol) was added and the resulting mixture was stirred for 10 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~30 mL) was washed with 1% aq. NaOH (2 x 5 mL) and water (2 x 5 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 obtain the respective disaccharide in a yield listed in Table 2.5.

### *2.4.5. HPLC-mediated synthesis of disaccharide 40*

***General procedure for HPLC-mediated glycosylation of 2.46 with glycosyl donor 2.10.***

A solution of glycosyl donor 10 (78.5 mg, 0.11 mmol, pump A, percentage flow: 80%) in $CH_2Cl_2$ (5.0 mL) and a solution of TMSOTf (11 μL, 0.055 mmol, pump B, percentage flow: 20%) in $CH_2Cl_2$ (2.0 mL) were passed concomitantly through a column containing resin-bound acceptor (50 mg, 0.011 mmol) at the combined flow rate of 0.3 mL min−1 for 20 min. The pump A was then switched to pump C (recirculating chamber, percentage flow: 80%) while pump B remained the same (TMSOTf soln., percentage flow: 20%) and

the resulting solutions were passed concomitantly through a column with the combined flow rate of 0.3 mL min−1 for 10 min. After that, the system was switched to pump C (recirculating chamber, percentage flow 100%) and the solution was recirculated at a flow rate of 0.3 mL min−1 for 1 h. Pump C was stopped and the column was purged with $CH_2Cl_2$ (pump A, 2.0 mL min−1) for 10 min.

***Procedure for cleavage of sugar from resin.*** A 1 M solution of sodium methoxide in methanol (0.3 mL) was added to a suspension or a mixture of resin in methanol (2.0 mL) and the resulting mixture was kept for 24 h at rt. After that, the reaction mixture was neutralized with Dowex H+ resin. The resin was filtered off and rinsed successively with MeOH (10 × 5 mL). The combined filtrate (~50 mL) was separated, dried with $MgSO_4$, concentrated in vacuo, and dried. The crude product obtained from this step was then acetylated according to the procedure described below.

***Acetylation of the crude product.*** $Ac_2O$ (0.3 mL) was added to a solution of the crude deacylated product (~19.7 mg, 0.0079 mmol) in pyridine (0.5 mL) and the resulting mixture was stirred under argon for 16 h at rt. The reaction mixture was quenched by the addition of $CH_3OH$ (~1.0 mL). The volatiles were evaporated under reduced pressure and the residue was co-evaporated with toluene (3 × 5 mL). The resulting residue was purified by column chromatography on silica gel (ethyl acetate–hexanes gradient elution) to afford the corresponding disaccharide **2.47** in 79% yield.

## 2.5. References

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# Chapter 3

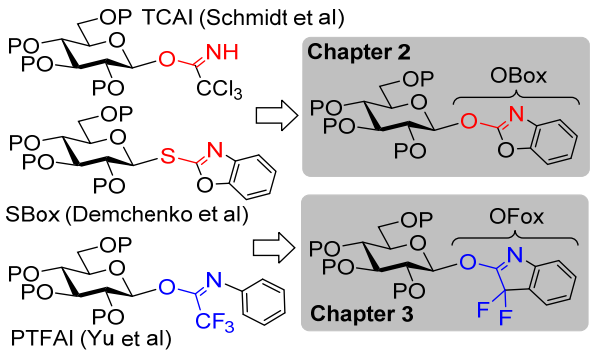
# Regenerative Glycosylation under Nucleophilic Catalysis with HOFox

Swati S. Nigudkar, Keith J. Stine, and Alexei V. Demchenko. Regenerative glycosylation under nucleophilic catalysis, *J. Am. Chem. Soc.,* **2014**, *136*, 921-923.

## 3.1. Introduction

Practically all complex carbohydrates have an oligomeric sequence wherein monosaccharide residues are linked via glycosidic linkages.[1] Both simple methods and sophisticated strategies for glycoside synthesis and oligosaccharide assembly exist.[2,3] However, the complexity of glycosylation reactions[4-7] is responsible for many drawbacks that all current methods experience. In spite of significant progress, chemical glycosylation remains challenging due to the requirement to achieve complete stereocontrol and to suppress side reactions.[8] Amongst a plethora of leaving groups developed, a vast majority of glycosylations make use of thioglycosides[9-12] and *O*-trichloroacetimidates (TCAI).[13-15] Our laboratory has also been developing new leaving groups for chemical glycosylation.[16,17] For instance, we developed *S*-benzoxazolyl (SBox) donors[18] and more recently introduced O-benzoxazolyl (OBox) imidates,[19] which represent a hybrid structure between SBox and TCAI (Scheme 1), but it is more reactive than either.

**Scheme 3.1. *O*-Imidates, established and new.**




Since the early days, stereoselective formation of glycosidic linkages has been the main challenge and the driving force for innovations in the area of carbohydrate chemistry.

Among a plethora of methods developed, glycosyl trichloroacetimidates (TCAI) introduced by Schmidt in 1980,[20] exhibit excellent glycosyl donor properties and often lead to high stereocontrol during glycosidation.[2] Despite their usefulness as glycosyl donors, the high reactivity and poor stability sometimes result in low yields during preparation, isolation, and glycosidation with TCAI.[13-15,21,22] As a modification to this approach, Yu reported glycosyl *N*-phenyl trifluoroacetimidates (PTFAI) as a new class of glycosyl donors.[23,24] Practically at the same time, Iadonisi and co-workers also reported the use of PTFAI as glycosyl donors.[25,26] These O-imidates showed similar properties in terms of their reactivity in glycosylations, but also showed improved stability and higher reaction yields in comparison to those with TCAI.[27]

As described in Chapter 2, we introduced *O*-benzoxazolyl (OBox) imidates that also fall into this generic category of O-imidoyl glycosyl donors. [19] OBox imidates showed higher reactivity profile than similarly structured TCAI counterparts. However it lacked stability, which complicated its handling and reduced its shelf-life. Thus, with an aim of expanding the arsenal of heterocyclic *O*-imidates with enhanced glycosyl donor properties, herein we describe the investigation of novel 3,3-difluoro-3*H*-indol-2-yl (OFox) imidates.

## 3.2. Results and discussion

### 3.2.1. The synthesis of OFox glycosides

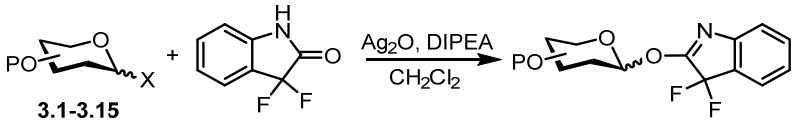
3,3-Difluoroxindole aglycone (HOFox) was readily synthesized by reacting commercially available Isatin with a slight excess of DAST in anhydrous $CH_2Cl_2$ at room temperature.[28] The 3,3-difluoroxindole group was then introduced at the anomeric

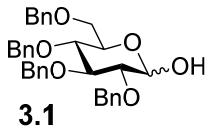
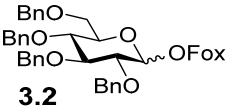
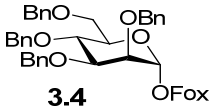
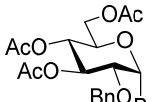
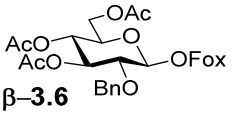
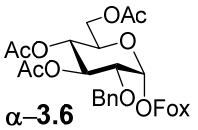
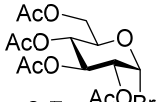
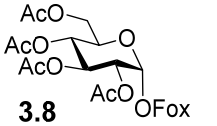
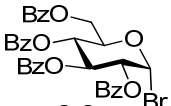
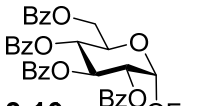
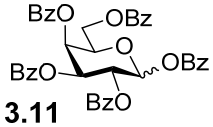
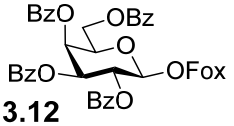
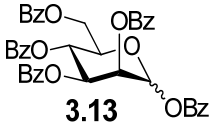
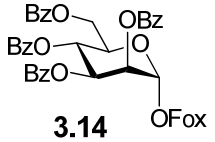
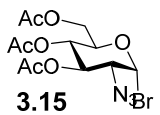
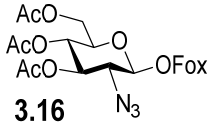
position by reaction of HOFox with different glycosyl halides (X = Br, Cl), used either directly or obtained *in situ* from the corresponding hemiacetals or esters, as suitable starting materials (Table 3.1). It was observed that almost all anomeric substitutions proceeded stereoselectively providing corresponding 1,2-*trans or 1,2-cis*-linked OFox imidates in 60-85% yields. Additionally, the synthesis of OFox glycosides was free of side reactions such as formation of *N*-glycosides or 1,2-dehydro derivatives (glycals).[29] As starting materials, a series of differentially protected glucosyl halides bearing arming (**3.1**), disarming (**3.7, 3.9**), and superdisarming (**3.5**) protecting group patterns have been investigated. The synthesis of common D-galacto (**3.12**), D-manno (**3.4** and **3.14**) and azido (**3.14**) OFox glycosyl donors was also performed. The formation of OFox imidates was confirmed by changes in the $^{19}F$ values from -112.4 ppm for 3,3-difluoro-2-oxindole to -121 to -122 ppm for the coupled product.[30]

### 3.2.2. Comparative investigation of *O*-imidates 3.2, 3.17 and 3.18

After synthesizing a variety of OFox imidates, we turned our focus to studying their properties in glycosylation reactions and stability under various acidic reaction conditions. These studies of OFox glycosyl donors were conducted as a comparison with similarly protected TCAI and PTFAI. It has been previously demonstrated that the activation of TCAI and PTFAI donors can be accomplished in the presence of the catalytic amount of promoters including TMSOTf,[21] $BF_3OEt_2$,[15] *p*-TsOH,[20] $Bi(OTf)_3$,[31] $Yb(OTf)_3$,[32] $Sm(OTf)_3$,[32] and AgOTf[33] Among these, TMSOTf and $BF_3$-$OEt_2$ are arguably the most commonly used promoters. Hence our initial focus has been centered on examining the effect of these activators on OFox glycosides.

*Table 3.1.* **Synthesis of 3,3-difluoro-3*H*-indol-2-yl (OFox) imidates**

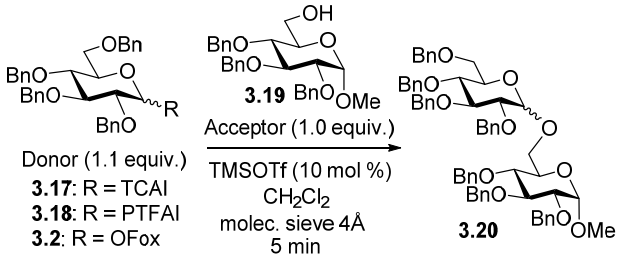



| *Entry* | *Starting material* | *Conditions,[a] temp, time* | *Product* | *Yield, α/β ratio* |
|---|---|---|---|---|
| 1 |  **3.1** | **A**, rt, 8 h |  **3.2** | 62%, 10/1 |
| 2 |  **3.3** | **A**, rt, 12 h |  **3.4** | 61%, α only |
| 3 |  **3.5** | **B**, 0 °C, 40 min |  **β–3.6** | 80%, β only |
| 4 | **3.5** | **B**, rt, 5 h |  **α–3.6** | 81%, α only |
| 5 |  **3.7** | **C**, rt, 6 h |  **3.8** | 80%, α only |
| 6 |  **3.9** | **B**, rt, 10 h |  **3.10** | 75%, α only |
| 7 |  **3.11** | **D**, 0 °C to rt, 6 h |  **3.12** | 75%, β only |
| 8 |  **3.13** | **D**, rt, 2.5 h |  **3.14** | 84%, α only |
| 9 |  **3.15** | **B**, 0 °C to rt, 3 h |  **3.16** | 70%, β only |

[a] – Conditions: **A**: i) $SOCl_2$, DMF, $CH_2Cl_2$, ii) 3,3-difluoroxindole, $Ag_2O$, DIPEA, $CH_2Cl_2$; **B**: 3,3-difluoroxindole, $Ag_2O$, DIPEA, $CH_2Cl_2$; **C**: 3,3-difluoroxindole, $Ag_2O$, DIPEA, toluene; **D**: i) 33% HBr/AcOH, $CH_2Cl_2$, ii) 3,3-difluoroxindole, $Ag_2O$, DIPEA, $CH_2Cl_2$.

A comparative study of different *O*-imidates as glycosyl donors in TMSOTf-promoted glycosylations is summarized in Table 3.2. Thus, coupling of glycosyl donors **3.2**, **3.16**, **3.17** with acceptor **3.18** produced disaccharide **3.19** in 5 min either at -78 $^{o}$C or ambient temperature in comparable yields and stereoselectivities. Similar results were obtained in $BF_3$-$OEt_2$ promoted glycosylations. A further exploratory study of OFox imidates dedicated to investigating various factors that affect stereoselectivity and reactivity of these glycosyl donors was conducted and will be described in the following subchapter.

***Table 3.2.*** **Comparative investigation of glycosylations with *O*-imidates 3.2, 3.17, and 3.18 in the presence of TMSOTf**

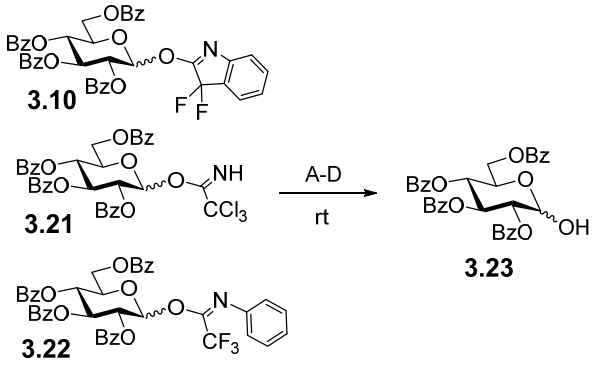



| Entry | Donor | Temp | Yield of **3.20** | Ratio, α/β |
|---|---|---|---|---|
| 1 | **3.17** | -78 $^{o}$C | 92% | 1/15 |
| 2 | **3.17** | rt | 94% | 4.0/1 |
| 3 | **3.18** | -78 $^{o}$C | 70% | 1/4.4 |
| 4 | **3.18** | rt | 91% | 1/2.2 |
| 5 | **3.2** | -78 $^{o}$C | 94% | 1/24 |
| 6 | **3.2** | rt | 93% | 1/1.2 |

For conducting the comparative hydrolytic stability studies, per-benzoylated OFox glycosyl donor **3.10** was compared with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate **3.21** and 2,3,4,6-tetra-*O*-benzoyl-α,β-D-glucopyranosyl *N*-phenyltrifluoroacetimidate **3.22**. These studies summarized in Table 3.3 were performed in the presence of various Lewis acids in wet $ClCH_2CH_2Cl$. A mixture of imidate (**3.10**,

**3.21**, or **3.22**, 0.01 mmol), activator (conditions A-D, 0.013 mmol, Table 3.3) in $ClCH_2CH_2Cl/H_2O$ (0.5 mL, 500/1, v/v) was stirred for 24 h at rt. Quantitative estimates were made at 1, 16 and 24 h time points and are based on the accumulation of 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranose **3.23**, as observed by TLC ($R_f$ = 0.45, ethyl acetate/hexanes, 3/7, v/v). Among these, the first procedure involved hydrolysis in the presence of $BF_3$-$OEt_2$ as the activator (Table 3.3, entries 1-3).

***Table 3.3.*** **Comparative hydrolytic stability study of OFox imidates, TCAI, and PTFAI under various reaction conditions.**




| *Entry* | *Donor* | *Conditions*[a] | *% of hemiacetal **3.23** formed after* *1 h* | *16 h* | *24 h* |
|---|---|---|---|---|---|
| 1 | **3.10** | A | quant. | quant. | quant. |
| 2 | **3.21** | A | 50 | 60 | 60 |
| 3 | **3.22** | A | 40 | 50 | 50 |
| 4 | **3.10** | B | 0 | 0 | 0 |
| 5 | **3.21** | B | 0 | 0 | 0 |
| 6 | **3.22** | B | 0 | 0 | 0 |
| 7 | **3.10** | C | 0 | quant. | quant. |
| 8 | **3.21** | C | 0 | 0 | 20 |
| 9 | **3.22** | C | 0 | 0 | 0 |
| 10 | **3.10** | D | 0 | 0 | 50 |
| 11 | **3.21** | D | 0 | 10 | quant. |
| 12 | **3.22** | D | 0 | 70 | quant. |

[a] Conditions: A: $BF_3$-$OEt_2$ (0.1 equiv.); B: $Bi(OTf)_3$ (0.1equiv.); C: MeOTf (1.0 equiv.); D: $PdCl_2$ (0.1 equiv.)

Under these reaction conditions, OFox imidate **3.10** underwent a nearly quantitative hydrolysis in 5 min, whereas their TCAI (**3.21**) PTFAI (**3.22**) counterparts showed 50% and 40% hydrolysis, respectively, in 5 min or even after 24 h of reaction. Yields listed in Table 3.3 are estimated based on TLC. On the other hand, practically no hydrolysis in cases of all three imidates took place in the presence of $Bi(OTf)_3$ (0.1 equiv., entries 4-6). In a similar series of experiments, we determined that PTFAI **3.22** could be reliably differentiated from the OFox donor **3.10** and TCAI counterpart **3.21** under MeOTf-mediated activation conditions (entries 7-9). Conversely, we found out that $PdCl_2$ mediated reactions OFox donor 3.10 and TCAI donor 3.21 are more stable than their PTFAI counterpart **3.22** (entries 12-14).

### 3.2.4. Glycosidation of OFox donors: optimization of the reaction conditions

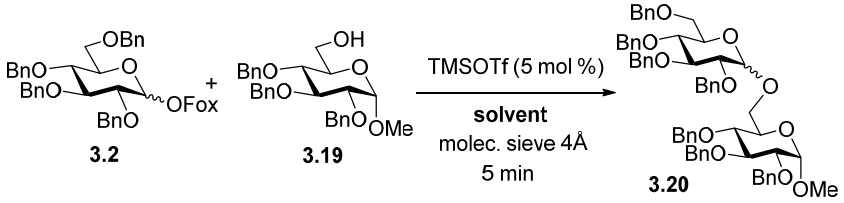
Encouraged by the observed selectivity and overall high reactivity of OFox imidates, we planned on exploring the effect of different experimental conditions and factors including the structure of glycosyl donor, glycosyl acceptor, activator, solvent, and temperature on the reaction outcome.

The effect of solvents on the stereoselectivity of glycosidation has been studied for a long time and resulted in a good level of understanding of the modes by which solvents may affect the reaction. For instance, it has been shown that nitrilic solvents strongly favor the formation of equatorial (β-D) glycosides, whereas ethereal solvents are often beneficial for the synthesis of axial (α-D) glycosides. With the exploration of the solvent effect on the stereoselectivity of glycosidation of OFox donors in mind, glycosylation in $CH_2Cl_2$ was chosen as the benchmark. Along this study, we also investigated the temperature effect of glycosylation with OFox imidates with the anticipation that low

temperatures will favor the equatorial (kinetic) product, whereas high temperature would be beneficial for axial (thermodynamic) product.

Thus, glycosylation of 3,3-difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranoside **3.2** with glycosyl acceptor **3.19** was performed in the presence of TMSOTf (5 mol %) at -40 $^{o}$C. Resultantly, disaccharide **3.20** was obtained in 97% yield and good β-selectivity (α/β = 1/8.0, entry 1, Table 3.4). A notable improvement of already commendable β-selectivity was achieved by changing the reaction solvent from $CH_2Cl_2$ to $CH_3CN$. Under these reaction conditions disaccharide **3.20** was obtained in 87% yield (α/β = 1/14, entry 2). Interestingly, when a solvent mixture of $CH_2Cl_2/CH_3CN$ (1/2, v/v) was used a decrease in stereoselectivity was recorded (α/β = 1/6.0, entry 3).

***Table 3.4.*** **The effect of solvents and temperature on the stereoselectivity of glycosidation of per-benzylated OFox donor 3.2.**



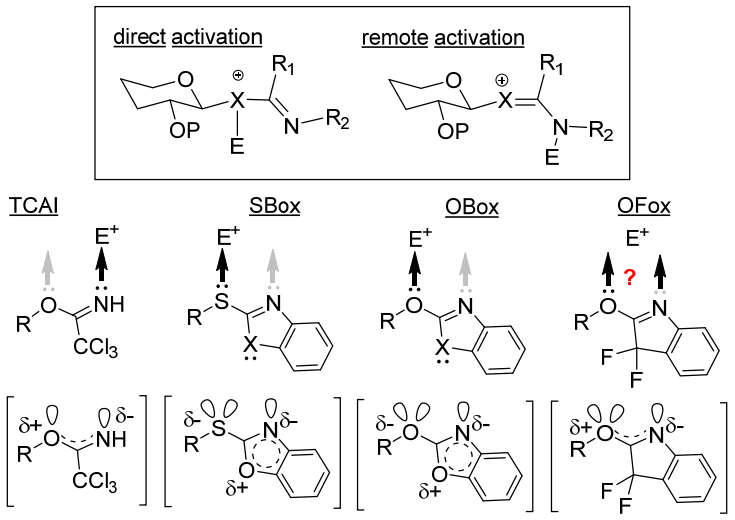

| *Entry* | *Solvent* | *Temp* | *Yield of **3.20*** | *α/β ratio* |
|---|---|---|---|---|
| 1 | $CH_2Cl_2$ | -40 $^{o}$C | 97% | 1/8.0 |
| 2 | $CH_3CN$ | -40 $^{o}$C | 87% | 1/14 |
| 3 | $CH_2Cl_2/CH_3CN$ (1/2, v/v) | -40 $^{o}$C | 96% | 1/6.0 |
| 4 | $CH_2Cl_2$ | -78 $^{o}$C | 94% | 1/24 |
| 5 | $CH_2Cl_2/CH_3CN$/EtCN (1/2/1, v/v/v) | -78 $^{o}$C | 99% | β only |
| 6 | EtCN | -78 $^{o}$C | 99% | β only |
| 7 | $Et_2O$ | -78 $^{o}$C | 84% | 1/5.0 |
| 8 | $Et_2O$ | 50 $^{o}$C | 81% | 1.6/1 |
| 9 | THF | 50 $^{o}$C | 79% | 1.4/1 |

The use of acetonitrile as the reaction solvent has its limitations because the reactions cannot be cooled below -40 $^{o}$C. Therefore, to achieve direct comparison of excellent β-selectivity (α/β = 1/24, entry 4) obtained in $CH_2Cl_2$ at -78 $^{o}$C we incorporated propionitrile (EtCN) in our study. To our delight, when MeCN was used either as an additive or neat, disaccharide **3.20** was obtained in a nearly quantitative yield and complete β-stereoselectivity (entries 5 and 6). Along similar lines, the effect of diethyl ether as the reaction solvent was also investigated. Since diethyl ether favors the formation of α-glucosides, we observed dramatically reduced β-stereoselectivity in reactions at -78 $^{o}$C (α/β = 1/5.0, entry 7). The same reaction performed at higher temperatures of 50 $^{o}$C, gave disaccharide **3.20** with preferential α-stereoselectivity. (α/β = 1.6/1, entry 7). Similar results were obtained using THF as the reaction solvent.

A result of this study confirmed that OFox imidates adequately respond to the solvent and temperature effects. A similar trend to that established for other classes of glycosyl donors was also observed herein. As an expansion of this study we investigated whether different Lewis acid activators would provide further enhancement of the OFox based glycosylation method. A valuable insight into the mode of activation and departure of the OFox leaving group would be another anticipated outcome of this study. A majority of leaving groups in carbohydrate chemistry, even the most reactive ones, would not depart on their own. Instead, the leaving group departure is typically affected via the interaction with electrophilic promoters. Resultantly, the leaving group ability is further enhanced and it departs as neutral species or a complex. For the glycosyl imidate series, the activation can be affected directly, via the anomeric atom (oxygen or sulfur), or remotely,

via the nitrogen atom (Figure 3.1). For instance, it has become common knowledge that TCAI donors are activated via the remote nitrogen.

***Figure 3.1.*** **Direct vs. remote activation of S- and O-imidates.**




In principle, the activation mode can be determined by means of isolation and characterization of the departed aglycone that represents the leaving group-promoter conjugate. As previously shown in our laboratory, S-thiazolinyl donors are activated via the remote nitrogen.[34] In strong contrast, bicyclic leaving groups SBox or OBox are activated via the anomeric sulfur or oxygen atom, respectively (Figure 3.1).[35] This is due to the propensity of the benzoxazolyl group to retain the aromaticity of its heterocyclic ring, which otherwise would have been disrupted if the activation were taking place via the endocyclic nitrogen. In regards to the OFox imidates, the five-membered ring is non-aromatic, therefore, our working hypothesis was that the activation of the OFox leaving group is affected via the remote nitrogen. Further experiments were dedicated to studying various activators, designed with proving the viability of this hypothesis. However, since all the reactions were done in 5-10 min in the presence of only catalytic amount of the

promoter, the isolation of the departed leaving group as an elusive OFox-promoter conjugate was proven impossible. Instead, only "free" HOFox could be isolated from the reaction medium because the departed aglycone would release the activator. The latter would be then available for the next catalytic cycle.

A study dedicated to the activation of OFox imidate **3.2** using common promoters for the activation of imidate donors is summarized in Table 3.5. Glycosylations using TMSOTf as the promoter have been used as the benchmark (entries 1 and 2). Using $BF_3$-$OEt_2$, $Cu(OTf)_2$ or MeOTf as promoters gave very comparable results (entries 3-5) to those achieved with TMSOTf. All of these reactions proceeded with a nearly complete β-selectivity for the formation of disaccharide **3.20**, which is rather unusual for benzylated glycosyl donors.

***Table 3.5.*** **The effect of promoter on the stereoselectivity of glycosidation of per-benzylated OFox donor 3.2 with acceptor 3.19**

| *Entry* | *Promoter (equiv.)* | *Temp, time* | *Yield of **3.20*** | *α/β ratio* |
|---|---|---|---|---|
| 1 | TMSOTf (0.05 equiv.) | -78 $^{o}$C, 5 min | 94% | 1/24 |
| 2 | TMSOTf (0.05 equiv.)[a] | -78 $^{o}$C, 10 min | 84% | 1/5.0 |
| 3 | $BF_3$-$OEt_2$ (0.1 equiv.) | -78 $^{o}$C, 5 min | 93% | 1/20 |
| 4 | $Cu(OTf)_2$ (0.2 equiv.) | -78 $^{o}$C, 5 min | 97% | β only |
| 5 | MeOTf (0.2 equiv.) | -78 $^{o}$C, 10 min | 91% | 1/>25 |
| 6 | AgOTf (0.5 equiv.) | rt, 24 h | 71% | 1/1.6 |
| 7 | $PdCl_2$ (0.3 equiv.) | rt, 36 h | 57% | 1/1.7 |
| 8 | $Bi(OTf)_3$ (0.1 equiv.) | -78 $^{o}$C, 5 min | 85% | 1/7 |
| 9 | $TMSClO_4$ (0.1 equiv.)[a] | rt, 5 min | 75% | 5.0/1 |
| 10 | $TMSClO_4$ (0.1 equiv.)[b] | rt, 5 min | 54% | 6.2/1 |

[a] - $Et_2O$ was used as the reaction solvent

[b] - A mixture of $Et_2O$/1,4-dioxane (1/1, v/v) was used as the reaction solvent

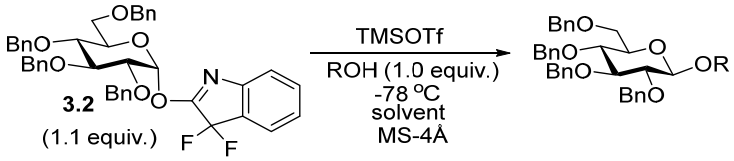
When metal salt-based promoters AgOTf or $PdCl_2$ were used, the reactions became much slower, did not proceed to completion even in 24-36 h, and stereoselectivity also dropped (entries 6 and 7). $Bi(OTf)_3$-promoted reactions were swift (entry 8), but the

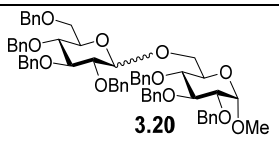
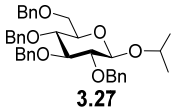
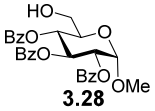
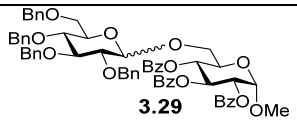
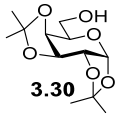
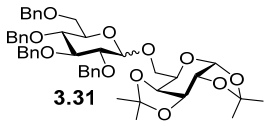
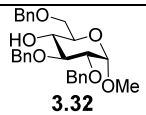
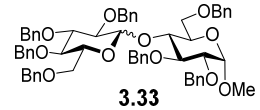
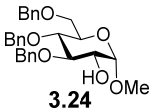
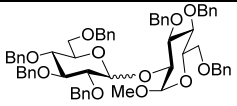
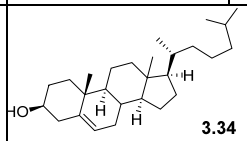
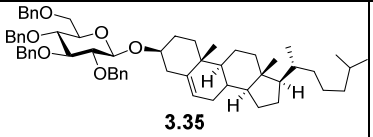
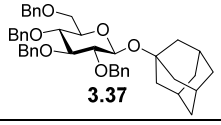
stereoselectivity has dropped in all cases of metal promoter-based activations. As a result of the preliminary screening of reaction conditions; we conclude that reactions in the presence of 5 mol % of TMSOTf in either $CH_2Cl_2$ or EtCN at -78 $^oC$ were found to be the most effective for β-glycosidation of OFox imidate **3.2**.

Having obtained high, and rather unexpected, levels of β-selectivity in glycosylations with OFox imidates, we were curious to investigate whether by changing the activation conditions, OFox glycosides could provide α-selectivity. For this purpose we chose trimethylsilyl perchlorate ($TMSClO_4$) as an activator because our recent study dedicated to the effect of the counter anion on the stereoselectivity showed that perchlorates typically outperform other counter ions and provide superior α-selectivity. To further shift the selectivity towards α-linked products, $TMSClO_4$ was used in cooperation with ethereal solvents that are known to favor axially substituted products. Thus, reaction in diethyl ether gave disaccharide **3.20** in 75% yield in good α-selectivity (α/β = 5.0/1, entry 9). The stereoselectivity was further improved when diethyl ether/1,4-dioxane (1/1, v/v) was used as a solvent mixture (α/β = 6.2/1, entry 10)

With the most favorable reaction conditions for glycosidation of the OFox donors, we were bound to extend the scope of this procedure to glycosylation of various glycosyl acceptors ranging from highly reactive primary alcohols to sterically hindered and less reactive or sterically hindered secondary alcohols. The result of this study is summarized in Table 3.6. As aforementioned, glycosidation of donor **3.2** with primary acceptor **3.19** gave glycoside **3.20** in a nearly quantitative yield and complete β-selectivity using either $CH_2Cl_2$ or EtCN reaction solvent (entries 1 and 2). Glycosidation of donor **3.2** with 2-propanol **3.26** was also quite selective and gave glycoside **3.27** in 77% yield and

***Table 3.6.*** **TMSOTf-promoted glycosidation of OFox donor 3.2 with different acceptors in $CH_2Cl_2$ or EtCN at -78 °C**



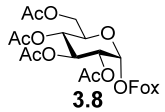
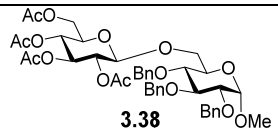
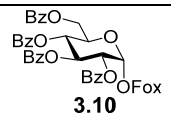
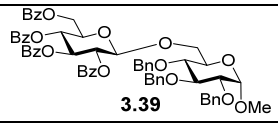
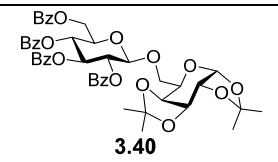
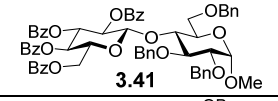
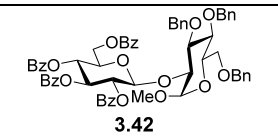
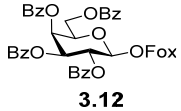
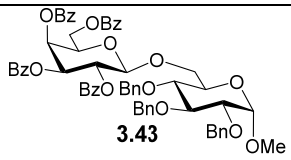
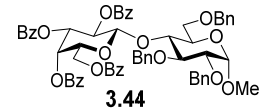
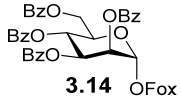
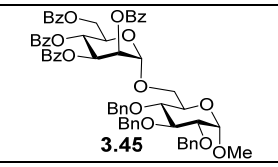
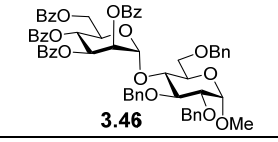

| *Entry* | *Acceptor* | *Solvent* | *Product* | *Yield*[b] | *α/β ratio*[c] |
|---|---|---|---|---|---|
| 1 | **3.19** | $CH_2Cl_2$ |  **3.20** | 94% | 1/24 |
| 2 | **3.19** | EtCN | **3.20** | 99% | β only |
| 3 | 2-propanol **3.26** | EtCN |  **3.27** | 77% | β only |
| 4 |  **3.28** | $CH_2Cl_2$ |  **3.29** | 89% | 1/11 |
| 5 | **3.28** | EtCN | **3.29** | 87% | 1/18 |
| 6 |  **3.30** | $CH_2Cl_2$ |  **3.31** | 85% | 1/12 |
| 7 | **3.30** | EtCN | **3.31** | 89% | β only |
| 8 |  **3.32** | $CH_2Cl_2$ |  **3.33** | 94% | 1/4.0 |
| 9 | **3.32** | EtCN | **3.33** | 92% | 1/12 |
| 10 |  **3.24** | $CH_2Cl_2$ |  **3.25** | 90% | 1/6.0 |
| 11 | **3.24** | EtCN | **3.25** | 88% | 1/15 |
| 12 |  **3.34** | $CH_2Cl_2$ |  **3.35** | 86% | β-only |
| 13 |  **3.36** | $CH_2Cl_2$ |  **3.37** | 88% | 1/23 |

complete β-selectivity (entry 3). Along similar lines, glycosylations with other primary acceptors **3.28** and **3.30** provided similar results, and afforded the corresponding disaccharides **3.29** and **3.31** in good yields and high 1,2-*trans* selectivity (up to α/β = 1/18, entries 4-7). On the other hand, reactions with secondary acceptors **3.32** and **3.34** showed somewhat lower selectivity for the synthesis of disaccharides **3.33** and **3.35** (up to α/β = 1/15, entries 8-11). Glycosylations with sterically hindered alcohols, cholesterol **3.34** and adamantanol **3.36**, proceeded smoothly generating the desired glycosides **3.35** and adamantanol **3.37** in good yields and nearly complete 1,2-*trans* stereoselectivity (entries 12 and 13). These glycosylations indicate that OFox glycosyl donors can be used to obtain β-linked glycosides in high yields and selectivities. It is noteworthy that the high β-selectivity obtained from glycosyl donors without the neighboring participating acyl group is relatively rare.

With success in achieving excellent glycosylation yields and 1,2-trans stereoselectivity using per-O-benzylated (armed) OFox imidate donor **3.2**, we turned our attention to studying their expectedly less reactive acylated counterparts. It should be noted that glycosyl donors of this class would provide an even more straightforward access to 1,2-trans-linked products. As discussed in the previous Chapter, the presence of acyl substituents provides a disarming effect thereby substantially reducing the reactivity of acylated glycosyl donors. However, as in case with OBox glycosides, even acylated OFox imidates showed similar level of reactivity to that observed with their per-benzylated counterparts.

Starting with per-acetylated donor **3.8**, in the reaction with acceptor **3.19** in the presence of TMSOTf we observed very low yields for the formation of **3.38** due to high

***Table 3.7.*** **Glycosylation with per-acylated glucosyl, galactosyl, and mannosyl OFox donors in $CH_2Cl_2$ with TMSOTf as a promoter at rt.**

| *Entry* | *Donor* | *Acceptor* | *Product* | *Yield* |
|---|---|---|---|---|
| 1[a] |  **3.8** | **3.19** |  **3.38** | 80% |
| 2 |  **3.10** | **3.19** |  **3.39** | 94% |
| 3 | **3.10** | **3.30** |  **3.40** | 86% |
| 4 | **3.10** | **3.32** |  **3.41** | 90% |
| 5 | **3.10** | **3.24** |  **3.42** | 93% |
| 6 |  **3.12** | **3.19** |  **3.43** | 98% |
| 7 | **3.12** | **3.32** |  **3.44** | 98% |
| 9 |  **3.14** | **3.19** |  **3.45** | 93% |
| 10 | **3.14** | **3.32** |  **3.46** | 90% |

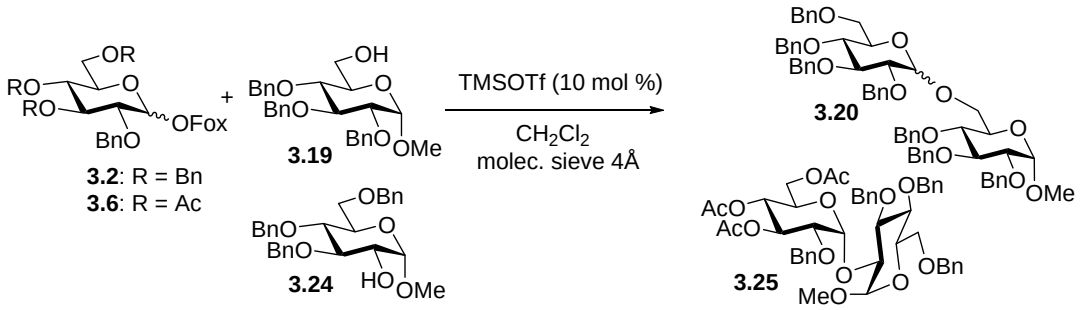
[a] – $SnCl_4$ was used as a promoter to prevent acetyl migration to the C-6 of acceptor that was observed in the presence of TMSOTf.

rates of the competing acetyl migration to the C-6 of the glycosyl acceptor. Interestingly, we were able to overcome this issue by performing essentially the same glycosylation

reaction using $SnCl_4$ as an activator. As a result of this experiment, disaccharide **3.38** was obtained in 80% with very minimal impact from the acetyl migration (Table 3.7, entry 1). Nevertheless, to avoid complications related to possible competing acetyl migration, further study was conducted with per-benzoylated OFox imidates of the D-gluco, D-galacto, and D-manno series (**3.10**, **3.12**, and **3.14,** respectively) using glycosyl acceptors **3.19**, **3.30**, **3.32**, and **3.24**. All these glycosylations proceeded smoothly giving excellent yields and complete 1,2-trans stereoselectivity (entries 2-10).

Since per-benzylated OFox donor **3.2** was generally β-selective, in the expansion of our studies to obtaining 1,2-*cis* glycosides we wanted to investigate whether OFox imidates with the superdisarming 2-O-benzyl-3,4,6-tri-O-acyl protecting group pattern would be beneficial for the synthesis of α-glucosides. For this purpose we obtained OFox imidate **3.6**, which was subjected to comparative glycosylations along with the standard perbenzylated OFox donor **3.2**. Alongside, we wanted to look into the possible effect of the anomeric group orientation on the stereoselectivity of glycosylations. For this purpose, we obtained α-**3.6** and **β-3.6** and glycosylations were performed with primary and secondary acceptors **3.19** and **3.24**. The result of this study is summarized in Table 3.8. Thus, perbenzylated OFox donor **3.2** (α/β = 10/1) gave essentially the same result as that obtained with α-**3.2**. In both cases, disaccharide **3.20** was obtained with excellent selectivity of α/β = 1/24. Even more dramatic result was obtained with the superdisarmed glycosyl donor **3.6**. Thus, both α-3.2 and β-3.2 provided the corresponding disaccharide **3.25** in practically identical yield of 88-89% and complete α-stereoselectivity. As a result of this study, we conclude that irrespective of the orientation of the OFox group of the glycosyl donor, complete 1,2-*cis* stereoselectivity was achieved in all reactions.

***Table 3.8.*** **The effect of anomeric configuration on the stereoselectivity of glycosidation of OFox donors 3.2 and 3.6**




| *Entry* | *Donor* | *Acceptor* | *Temp, time* | *Product, yield, %* | *α/β ratio* |
|---|---|---|---|---|---|
| 1 | **3.2** (α/β = 10/1) | **3.19** | -78 °C, 5min | **3.20**, 94 | 1/24 |
| 2 | **α-3.2** | **3.19** | -78 °C, 5min | **3.20**, 98 | 1/24 |
| 3 | **α-3.6** | **3.24** | rt, 15min | **3.25**, 88 | α only |
| 4 | **β-3.6** | **3.24** | rt, 15min | **3.25**, 89 | α only |

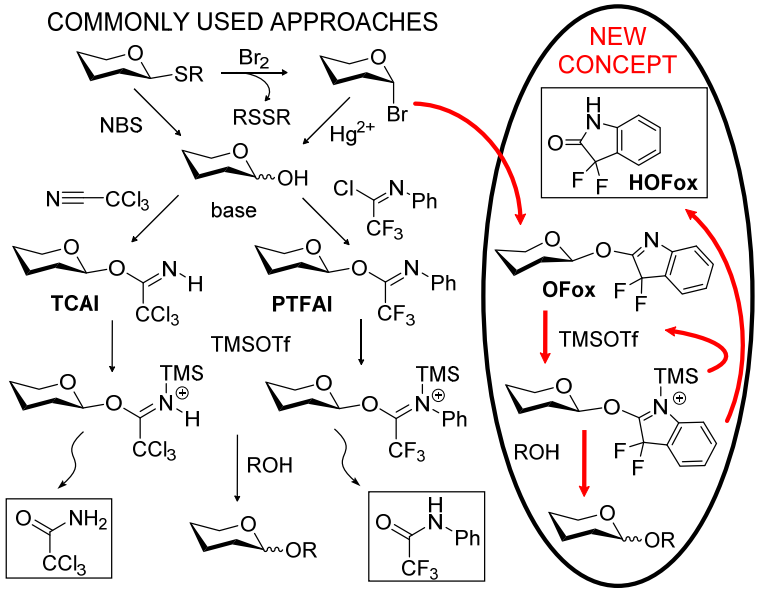
### 3.3. Regenerative glycosylation

While developing the new class of O-imidates we noticed a feature of the OFox leaving group that differentiates it from all others. The aglycone structure needed to introduce OFox and that of the departed leaving group are essentially the same cyclic amide 3,3-difluoroxindole (HOFox).[28,36] The significance of this observation is that, in principle, one should be able to conduct both the introduction and activation of this leaving group in the catalytic "donor-regenerative" fashion, which is routinely done in enzymatic glycosylations,[37,38] but represents a new direction in the field of chemical glycosylation.

Having established basic principles of the synthesis and activation of OFox imidates, we turned our attention to the investigation of the regenerative glycosylation, a concept that sets OFox donors apart from other known methods for glycosylation. Both TCAI and PTFAI are obtained from hemiacetal precursors, using cheap trichloroacetonitrile or

rather costly 2,2,2-trifluoro-N-phenylacetimidoyl chloride, respectively, but their synthesis is often originated from thioglycosides (Scheme 3.2). The use of thioglycosides as general building blocks is very broad. Thioglycosides are also excellent glycosyl donors, but their relatively low reactivity profile and the requirement for stoichiometric promoters limit their application. Conversely, O-imidates are very reactive and require only catalytic amount of the activator. Many O-imidates can be purified, but none can be stored and hence have to be used in glycosidations right away. Leaving groups in the previously studied imidates TCAI or PTFAI depart as unreactive amides, trichloroacetamide or 2,2,2-trifluoro-N-phenylacetamide, respectively, and cannot be reused directly (Scheme 3.2).

**Scheme 3.2. Common approaches and the new concept**

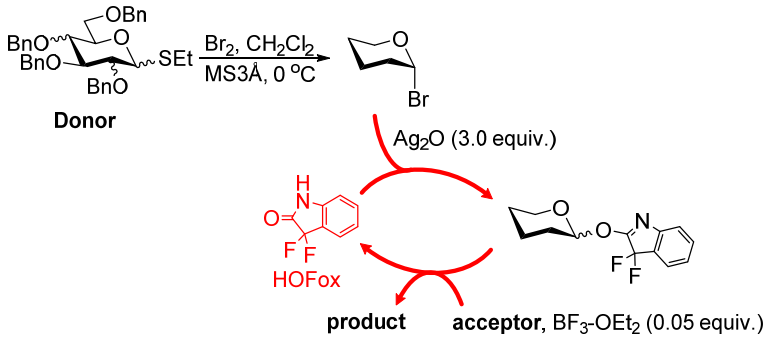


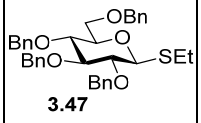
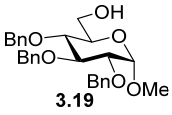
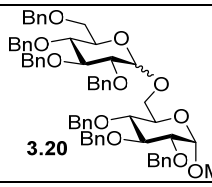
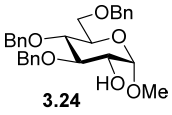
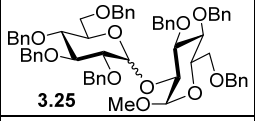
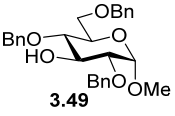
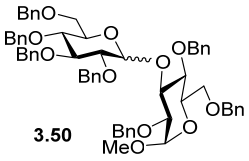
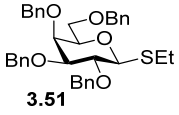
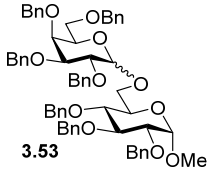
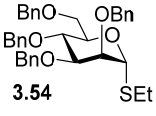
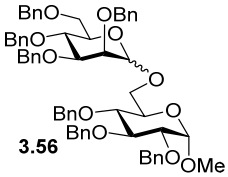

Differently, since both the reagent for the introduction of the OFox leaving group and the departed leaving group are essentially the same (HOFox), both the synthesis and glycosidation of OFox imidates can be conducted using catalytic amount of the HOFox

aglycone. HOFox aglycone will first react with a stable precursor to form highly reactive OFox imidate donor. The latter will then react with the acceptor while regenerating HOFox aglycone, which will be available for the next catalytic cycle to regenerate the OFox donor. Resultantly, only a small amount of the reactive donor is present in the reaction depending on the amount of added HOFox, whereas the OFox donor gets regenerated only upon its consumption (Scheme 3.2).

In our preliminary study of the regenerative concept, we first reacted S-ethyl glycoside **3.47** with stoichiometric bromine to form glycosyl bromide **3.48**. The latter gets readily converted into OFox imidate **3.2** in the presence of HOFox. OFox imidates have reasonable shelf-life but are readily activated with TMSOTf, $Bi(OTf)_3$ or other Lewis acid promoters (10 mol %). The amount of the reactive glycosyl donor present in the reaction medium can be controlled by the amount of HOFox added (range studied 0.1-1.0 equiv.). We managed to achieve reasonable glycosylation rates (5 h) with as little as 10% of HOFox aglycone and with yields between 79-90% (entries 2-6, Table 3.9). For comparison, bromide **3.48** would require at least 16-24 h to react completely under these reaction conditions (entry 1). After confirming the effectiveness of the regenerative glycosylation procedure, we next investigated its scope towards secondary acceptors. For comparison, these reactions were performed using 0.1 and 0.25 equiv. of OFox, and the glycosylations proceeded smoothly providing corresponding disaccharides in 75% and 62% yields respectively (entries 7 and 8). At the same time, the procedure was also performed on galactosyl and mannosyl sugars. However, the reaction yields were on average lower than those observed with glucosyl donor (entries 9 and 10).

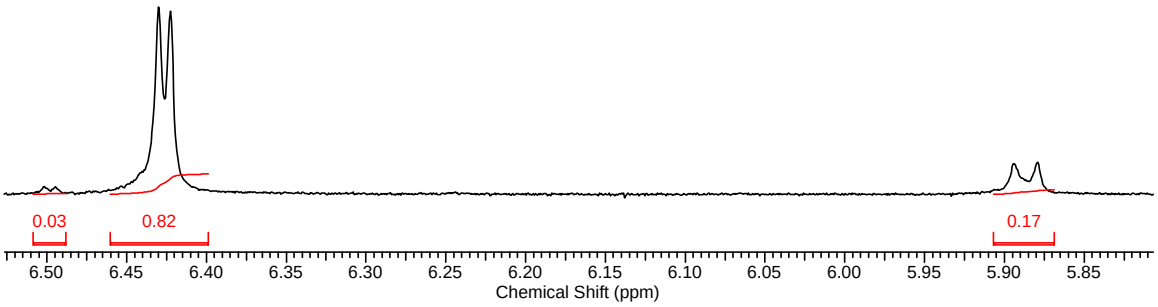
## *Table 3.9.* Regenerative glycosylation




| *Entry* | *Donor* | *Acceptor* | *HOFox (equiv.)* | *Reaction time* | *Product* | *Yield* | *Ratio α/β* |
|---|---|---|---|---|---|---|---|
| 1 |  **3.47** |  **3.19** | 0 | 5 h |  **3.20** | 9% | 1/1.1 |
| 2 | **3.47** | **3.19** | 0.10 | 3 h | **3.20** | 84% | 1/1.9 |
| 3 | **3.47** | **3.19** | 0.25 | 2 h | **3.20** | 79% | 1/1.9 |
| 4 | **3.47** | **3.19** | 0.50 | 40 min | **3.20** | 86% | 1/1.2 |
| 5 | **3.47** | **3.19** | 0.75 | 30 min | **3.20** | 88% | 1/1.2 |
| 6 | **3.47** | **3.19** | 1.0 | 10 min | **3.20** | 90% | 1/1.2 |
| 7 | **3.47** |  **3.24** | 0.1 | 3 h |  **3.25** | 72% | 1/1.7 |
| 8 | **3.47** |  **3.49** | 0.25 | 3 h |  **3.50** | 62% | 1/1.0 |
| 9 |  **3.51** | **3.19** | 0.1 | 2.5 h |  **3.53** | 69% | 1/1.1 |
| 10 |  **3.54** | **3.19** | 0.1 | 3 h |  **3.56** | 75% | 2.5/1 |

To obtain the ultimate proof that the regenerative reaction proceeds via the formal formation of the OFox imidate, we performed an NMR monitoring as follows. A reaction using bromide donor **3.48** and $Ag_2O$ (3 equiv.) in $CD_2Cl_2$ was set up in the standard 5 mm NMR tube at 0 $^oC$ in absence of a glycosyl acceptor. HOFox (0.25 equiv.) was then added and the 1H NMR spectrum recorded after 5 min clearly showed the formation of OFox imidate (Scheme 3.3). Thus, two new peaks, one at around 5.87 ppm, indicative of the formation of β-OFox imidate **3.2**, and one at around 6.50 ppm indicative of the formation of α-OFox imidate were formed. In our opinion, this NMR-monitoring experiment clearly proves that OFox imidates are the essential intermediates en route to the glycosylation prodicts.

**Scheme 3.3. "Regenerative glycosylation" monitored by the NMR**




## 3.4. Conclusions

A new class compounds, OFox imidates, have been synthesized in high yields and employed as versatile glycosyl donors in the synthesis of 1,2-cis and 1,2-trans glycosides. These glycosylations show notable features such as operational simplicity, rapid reaction times, high yields and excellent stereocontrol in presence of Lewis acids used in catalytic amounts. Achieving complete β-stereoselectivity with the use of propionitrile as the

solvent, suggests a possibility of solvent participation during glycosylation. At the same time, good α-selectivities could also be achieved by changing the promoter or using OFox of the superdisarmed series. A new regenerative concept for chemical glycosylation that proceeds via reactive glycosyl OFox intermediates and activation thereof in the catalytic fashion in situ was discovered. The concept has some similarities with the two-step activation by Nicolaou[39-41] and Danishefsky[42-45] and preactivation strategy by Huang and Ye,[46-49] but differs in the catalytic conversion and continuous regeneration of the glycosyl donor. In the previous methods, the entire precursor (thioglycoside or glycal) is first converted into reactive intermediates (Br, F, OTf/$NTf_2$ or epoxide) and then acceptor is added. This regenerative approach is expected to help reduce side reactions commonly found in conventional glycosylations wherein excess of the highly reactive or stoichiometrically preactivated glycosyl donor is present from the beginning.

## 3.5. Experimental Section

### *3.5.1. General Remarks*

All reactions were performed under argon with dry, freshly distilled solvents unless otherwise noted. $CH_2Cl_2$, $ClCH_2CH_2Cl$, toluene, $CH_3CN$, and EtCN were distilled from $CaH_2$ directly prior to application. Anhydrous 1,4-dioxane, tetrahydrofuran, and diethyl ether were used as is. AgOTf was co-evaporated with toluene (3 x 10 mL) and dried in *vacuo* for 2-3 h directly prior to application. TMSOTf, $SnCl_4$, MeOTf, $BF_3$-$OEt_2$, $Cu(OTf)_2$, $PdCl_2$, $TMSClO_4$ and $Bi(OTf)_3$ were used as is. Molecular sieves (3 Å or 4 Å), used for the reactions, were crushed and activated at 390 °C and then for 2-3 h at 390 °C

prior to application. Reactions were monitored by TLC on Kieselgel 60 $F_{254}$ and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at < 40 $^{o}$C. Column chromatography was performed on silica gel 60 (70-230 mesh). Optical rotations were measured at 'Jasco P-1020' polarimeter. $^{1}$H n.m.r. spectra were recorded at 300 MHz, 500 MHz, or 600 MHz. $^{13}$C n.m.r. spectra were recorded at 75 MHz or 150 MHz. $^{19}$F spectra were recorded at 282.2 MHz. The $^{1}$H chemical shifts are referenced to the signal of the residual $CHCl_3$ ($\delta_H$ = 7.27 ppm) for solutions in $CDCl_3$. The $^{13}$C chemical shifts are referenced to the central signal of $CDCl_3$ ($\delta_C$ = 77.23 ppm) for solutions in $CDCl_3$. HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

### *3.5.2. Synthesis of 3,3-difluoroxindole (HOFox, 3,3-difluoroindolin-2-one).*

HOFox was obtained from Isatin and DAST as previously described. Analytical data were in accordance with that previously reported.[28]

### *3.5.3. Preparation of OFox imidates*

*Method A. A typical procedure for the preparation from hemiacetals via glycosyl chlorides.* $SOCl_2$ (81.1 μL, 1.11 mmol) was added dropwise to a solution of hemiacetal (**3.1** or **3.3**, 0.20 g, 0.37 mmol) in dry $CH_2Cl_2$ (2.0 mL) and dry DMF (14.3 μL) and the resulting mixture was stirred under argon for 7 h at rt. Upon completion, the reaction mixture was diluted with $CH_2Cl_2$ (~30 mL) and washed with sat. aq. $NaHCO_3$ (2 x 15 mL) and cold water (2 x 15 mL). The organic phase was separated, dried with $MgSO_4$,

and concentrated *in vacuo*. The residue containing crude glycosyl halide (0.37 mmol) was dried under high vacuum for 4 h. Freshly activated molecular sieves (3 Å, 600 mg) and dry $CH_2Cl_2$ (2.0 mL) were added and the resulting mixture was stirred under argon for 1 h at rt. After that, 3,3-difluorooxindole (69 mg, 0.41 mmol), $Ag_2O$ (258 mg, 1.11 mmol), and diisopropylethylamine (DIPEA, 97 μL, 0.56 mmol) were added and the resulting mixture was stirred for 8-12 h. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 15 mL) and water (2 x 15 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-hexanes gradient elution) to afford the corresponding OFox imidates **3.2** or **3.4** in yields listed in Table 3.1.

*Methods B and C. A typical procedure for the preparation from glycosyl bromides.* A mixture of a glycosyl bromide (**3.5**, **3.7**, **3.9** or **3.15**, 0.15 mmol) and freshly activated molecular sieves (3 Å, 300 mg) in dry $CH_2Cl_2$ (Method B, 1.0 mL) or toluene (Method C, 1.0 mL) was stirred under argon for 1 h at rt. After that, 3,3-difluorooxindole (25.7 mg, 0.15 mmol), $Ag_2O$ (105 mg, 0.45 mmol), and DIPEA (39.7 μL, 0.23 mmol) were added and the resulting mixture was stirred for 40 min-10 h at the temperature indicated in Table 1S. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 15 mL) and water (2 x 15 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-hexanes gradient elution) to afford the corresponding OFox imidates in yields listed in Table 3.1.

*Method D. A typical procedure for the preparation from pentabenzoates via glycosyl bromides.* A 33% solution of HBr in AcOH (0.10 mL, 1.7 mmol) was added to a solution of a 1,2,3,4,6-penta-*O*-benzoyl-D-galacto or mannopyranose (**3.11** or **3.13**, 100 mg, 0.14 mmol) in dry $CH_2Cl_2$ (0.2 mL) and the resulting mixture was stirred under argon for 2-4 h at rt. The reaction mixture was then diluted with $CH_2Cl_2$ (~40 mL) and washed with cold sat. aq. $NaHCO_3$ (2 x 15 mL) and cold water (2 x 15 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue containing crude glycosyl bromide (0.14 mmol) was dried under high vacuum for 4 h. After that, freshly activated molecular sieves (3 Å) and dry $CH_2Cl_2$ (1.4 mL) were added and the resulting mixture was stirred under argon for 1 h at rt. After that, the resulting mixture was cooled to 0 $^oC$ in case of galactose sugar and at rt for mannose sugar, followed by addition of 3,3-difluorooxindole (26.7 mg, 0.16 mmol), $Ag_2O$ (99.2 mg, 0.43 mmol) and DIPEA (37.4 μL, 0.21 mmol) were added and the resulting mixture was stirred for 2-6 h as indicated in Table 3.1. The solids were then filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 15 mL) and water (2 x 15 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-hexanes gradient elution) to afford the corresponding OFox imidates in yields listed in Table 3.1.

**3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranoside (3.2).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **3.1**[50] by Method B in 62% yield (α/β = 10/1) as a white foam. Analytical data for α-**3.2**: $R_f$ = 0.43 (ethyl acetate/hexanes, 1/4, v/v); $^1$H n.m.r. (300 MHz): δ, 3.66 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.79 (dd, 1H, $J_{2,3}$ = 9.3 Hz, H-2, ), 3.79 (dd, 1H, $J_{5,6b}$ = 3.3 Hz, H-6b), 3.83 (dd, 1H, $J_{4,5}$ = 9.3 Hz, H-4), 4.01 (m, 1H, H-5), 4.13 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.52 (dd, 2H, $^2J$ = 12.0 Hz, C$H_2$Ph), 4.69 (dd, 2H, $^2J$ = 10.5 Hz, C$H_2$Ph), 4.73 (s, 2H, C$H_2$Ph), 4.93 (dd, 2H, $^2J$ = 10.9 Hz, C$H_2$Ph), 6.50 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 7.11-7.42 (m, 24H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 68.1, 73.2, 73.5, 73.6 (x 2), 75.2, 75.3, 79.4, 81.5, 99.8, 117.4, 120.4, 123.3, 125.7, 127.8, 127.9, 128.0 (x 4), 128.0 (x 2), 128.1 (x 7), 128.6 (x 8), 133.6, 137.9, 138.0, 138.2, 138.8 ppm; $^{19}$F n.m.r.: δ, -121.3 (s, 2F, $CF_2$) ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{42}H_{39}F_2NO_6Na^+$ 714.2643, found 714.2645.

**3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (3.4).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzyl-α/β-D-mannopyranose **3.3**[51] by Method A in 61% yield as a white foam. Analytical data for **3.4**: $R_f$ = 0.45 (ethyl acetate/hexanes, 2/3, v/v); $[\alpha]_D^{24}$ +13.7 (*c* = 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 3.74 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 3.84 (dd, 1H, $J_{5,6b}$ = 4.3 Hz, H-6b), 3.95-4.03 (m, 3H, H-2, 4, 5), 4.14 (m, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 4.60 (dd, 2H, $^2J$ = 11.6 Hz, C$H_2$Ph), 4.62 (dd, 2H, $^2J$ = 12.1 Hz, C$H_2$Ph), 4.70 (dd, 2H, $^2J$ = 10.7 Hz, C$H_2$Ph), 4.78 (s, 2H, C$H_2$Ph), 6.40 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1), 7.15-7.47 (m, 24H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 69.1, 72.6, 72.8, 73.1, 73.4, 74.0, 74.8, 75.3, 79.0, 97.7, 120.5 (x 2), 123.4 (x 2), 125.9, 126.7, 127.7, 127.7 (x 2), 128.0 (x 3), 128.1 (x 2), 128.2 (x 2), 128.3

(x 3), 128.5 (x 2), 128.6 (x 6), 133.7, 138.0, 138.2, 138.4 (x 2), ppm; $^{19}$F n.m.r.: δ, -121.5 (s, 2F, $CF_2$) ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{42}H_{39}F_2NO_6Na^+$ 714.2643, found 714.2639.

**3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide (3.5).** The title compound was obtained from 1,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl-D-glucopyranose[52] in 90% yield as a white foam as previously described.[53] Analytical data for **3.5**: $R_f$ = 0.43 (ethyl acetate/hexanes, 2/3, v/v); $^1$H n.m.r. (300 MHz): δ, 1.96, 1.98, 2.00 (3s, 9H, 3 x $COCH_3$), 3.57 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 4.12 (m, 1H, $J_{5,6a}$ = 4.1 Hz, H-6a), 4.22 (m, 1H, H-5), 4.27 (dd, 1H, $J_{5,6b}$ = 4.0 Hz, $J_{6a,6b}$ = 12.6 Hz, 6b), 4.63 (dd, 2H, $^2J$ = 12.3 Hz, C$H_2$Ph), 5.06 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 5.48 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 6.34 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1) ppm; $^{13}$C n.m.r. (75 MHz): δ, 20.8, 20.9 (x 2), 61.3, 67.3, 72.2 (x 2), 72.9, 76.5, 89.2, 128.1 (x 2), 128.5, 128.8 (x 2), 137.0, 169.9, 170.1, 170.7 ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{19}H_{23}BrO_8Na^+$ 481.0474, found 481.0483.

**3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-β-D-glucopyranoside (β-3.6).** The title compound was obtained from 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide **3.5** by Method B at rt in 80% yield as a white foam. Analytical data for **β-3.6**: $R_f$ = 0.39 (ethyl acetate/hexanes, 2/3, v/v); $[\alpha]_D^{26}$ +13.7 (*c* = 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 1.95, 2.03, 2.07 (3s, 9H, 3 x $COCH_3$), 3.80 (dd, 1H, $J_{2,3}$ = 7.9 Hz, H-2), 3.95 (m, 1H, H-5), 4.14 (dd, 1H, $J_{5,6a}$ = 2.3 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.34 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, H-6b), 4.74 (dd, 2H, $^2J$ = 11.6 Hz, C$H_2$Ph), 5.11 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 5.27 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 5.94 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 7.18-7.49

(m, 9H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 20.7 (x 2), 20.8, 61.5, 67.9, 72.6, 73.6, 74.7, 77.6, 99.1, 120.6, 123.3, 126.2, 128.1 (x 2), 128.3 (x 3), 128.5 (x 3), 133.7, 137.1, 150.3, 169.7, 170.0, 170.7 ppm; $^{19}$F n.m.r.: δ, -122.0 (d, 2F, $CF_2$) ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{27}H_{27}F_2NO_9Na^+$ 570.1552, found 570.1562.

**3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranoside (α-3.6).** The title compound was obtained from 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide **3.5** by Method B at 0 $^{o}$C in 81% yield as a white foam. Analytical data for **α-3.6**: $R_f$ = 0.38 (ethyl acetate/hexanes, 2/3, v/v); $[\alpha]_D^{25}$ +101.4 (*c* = 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 2.02, 2.03, 2.05 (3s, 9H, 3 x $COCH_3$), 3.81 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-2), 4.06 (dd, 1H, $J_{5,6a}$ = 2.0 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.18 (m, 1H, H-5), 4,29 (dd, 1H, $J_{5,6b}$ = 4.1 Hz, H-6b), 4.67 (s, 2H, C$H_2$Ph), 5.11 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 5.57 (d, 1H, $J_{3,4}$ = 9.7 Hz, H-3), 6.45 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 7.17-7.40 (m, 9H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 20.7 (x 2), 20.8, 61.4, 67.8, 69.8, 71.4, 73.3, 75.7, 95.0, 120.4, 123.3, 125.9, 126.8, 127.9 (x 3), 128.2, 128.6 (x 3), 133.5, 137.2, 150.4, 168.7, 169.8, 170.1 ppm; $^{19}$F n.m.r.: δ, -121.6 (s, 1F, $CF_2^a$), -121.5 (s, 1F, $CF_2^b$); HR-FAB MS $[M+Na]^+$ calculated for $C_{27}H_{27}F_2NO_9Na^+$ 570.1552, found 570.1569.

**3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (3.8).** The title compound was obtained from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **3.7**[54] by Method C in 62% yield as a white foam. Analytical data for **3.8**: $R_f$ = 0.42 (ethyl acetate/hexanes, 1/1, v/v); $[\alpha]_D^{21}$ +5.2 (*c*= 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 1.96, 1.97, 1.98, 2.01 (4s, 12H, 4 x $COCH_3$), 3.95 (m, 1H, H-5), 4.15 (dd, 1H, $J_{5,6a}$ = 2.3 Hz,

$J_{6a,6b}$ = 12.5 Hz, H-6a), 4.30 (dd, 1H, $J_{5,6b}$ = 4.3 Hz, H-6b), 5.17-5.32 (m, 3H, H-2, 3, 4), 5.95 (m, 1H, H-1), 7.14-7.40 (m, 4H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 20.4, 20.6 (x 2), 20.7, 61.4, 67.6, 70.3, 72.4, 72.9, 96.6, 120.5 (x 2), 123.3 (x 2), 126.2, 126.8, 133.6, 150.0, 169.3, 169.4, 170.2, 170.8 ppm; $^{19}$F n.m.r.: δ, -122.4 (s, 1F, $CF_2^a$), -122.3 (s, 1F, $CF_2^b$) ppm; HR-FAB MS $[M+H]^+$ calculated for $C_{22}H_{23}F_2NO_{10}$ 500.1290, found 500.1361.

**3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (3.10).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **3.9**[55] by Method B in 75% yield as a pale yellow foam. Analytical data for **3.10**: $R_f$ = 0.41 (ethyl acetate/hexanes, 3/7, v/v); $[\alpha]_D^{23}$ +45.8 (*c*= 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 4.48 (dd, 1H, $J_{5,6a}$ = 4.7 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.64 (m, 2H, $J_{5,6b}$ = 2.3 Hz, H-5, 6b), 5.69 (dd, 1H, $J_{2,3}$ = 10.2 Hz, H-2), 5.83 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 6.31 (dd, 1H, $J_{3,4}$ = 10.0 Hz, H-3), 6.82 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 7.05-7.99 (m, 24H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 62.4, 68.6, 70.1, 70.3, 70.6, 94.8, 120.6, 123.2, 126.0, 126.5, 128.3, 128.4 (x 2), 128.4 (x 2), 128.5 (x 4), 128.6 (x 2), 128.8, 129.1, 129.5, 129.7 (x 2), 129.8 (x 2), 130.0 (x 4), 133.1, 133.4 , 133.5, 133.6, 133.7, 150.1, 165.2, 165.5, 165.6, 166.0 ppm; $^{19}$F n.m.r.: δ, -121.8 (s, 1F, $CF_2^a$), -121.6 (s, 1F, $CF_2^b$) ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{42}H_{31}F_2NO_{10}Na^+$ 770.1813, found 770.1800.

**3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (3.12).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **3.11**[56] by Method D in 75% yield as a white foam. Analytical data for **3.12**: $R_f$ =

0.39 (ethyl acetate/hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +2.7 (*c*= 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 4.51 (dd, 1H, $J_{5,6a}$ = 5.9 Hz, $J_{6a,6b}$ = 10.6 Hz, H-6a), 4.63 (m, 1H, H-5), 4.72 (dd, 1H, $J_{5,6b}$ = 6.6 Hz, H-6b), 5.75 (dd, 1H, $J_{3,4}$ = 3.4 Hz, H-3), 6.11 (m, 2H, H-2, 4), 6.34 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 7.14-8.14 (m, 24H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 61.8, 67.6, 68.9, 71.4, 72.7, 97.4, 120.4, 123.6, 124.7, 125.4, 128.3, 128.6 (x 4), 128.7, 128.8, 128.9 (x 2), 129.0, 129.2 (x 2), 129.4, 129.9 (x 9), 130.1 (x 2), 133.3, 133.5, 133.6, 133.8, 165.1, 165.5 (x 2), 166.0 ppm; $^{19}$F n.m.r.: δ, -122.2 (s, 2F, $CF_2$); HR-FAB MS $[M+Na]^+$ calculated for $C_{42}H_{31}F_2NO_{10}Na^+$ 770.1813, found 770.1791.

**3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (3.14).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide **3.13**[57] by Method D in 84% yield as a white foam. Analytical data for **3.14**: $R_f$ = 0.43 (ethyl acetate/hexanes, 3/7, v/v); $[\alpha]_D^{21}$ -5.3 (*c* = 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 4.52 (dd, 1H, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.62 (m, 1H, H-5), 4.70 (dd, 1H, $J_{5,6b}$ = 2.2 Hz, H-6b), 6.02 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2), 6.07 (dd, 1H, $J_{3,4}$ = 10.0 Hz, H-3), 6.24 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 6.64 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 7.16-8.09 (m, 24H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 62.5, 66.2, 69.0, 69.6, 71.5, 96.0, 121.0, 123.5, 125.5, 126.4, 126.8, 128.4, 128.5 (x 2), 128.6 (x 2), 128.7 (x 2), 128.8, 128.9 (x 2), 128.9, 129.0, 129.2, 129.8, 129.9 (x 2), 130.0 (x 2), 130.1 (x 4), 133.2, 133.6, 133.8, 134.0, 138.1, 165.2, 165.5, 165.6, 166.1 ppm; $^{19}$F n.m.r.: δ, -121.8 (s, 1F, $CF_2^a$), -121.5 (s, 1F, $CF_2^b$); HR-FAB MS $[M+Na]^+$ calculated for $C_{42}H_{31}F_2NO_{10}Na^+$ 770.1813, found 770.1814.

**3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide (3.15).** The title compound was obtained from 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose[58,59] in 90% yield as a white foam as previously described.{Paulsen, 1988 #2816{Rowan, 2009 #3894} Analytical data for **3.15**: $R_f$ = 0.46 (ethyl acetate/hexanes, 2/3, v/v); $[\alpha]_D^{19}$ +146.9 (*c*= 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 2.06, 2.09, 2.11 (3s, 9H, 3 x $COCH_3$), 3.81 (dd, 1H, $J_{2,3}$ = 10.2 Hz, H-2), 4.12 (m, 1H, $J_{5,6a}$ = 1.9 Hz, $J_{6a,6b}$ = 8.5 Hz, H-6a), 4.33 (m, 2H, $J_{5,6b}$ = 4.1 Hz, H-5, 6b), 5.14 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 5.5 (dd, 1H, $J_{3,4}$ = 10.0 Hz, H-3), 6.42 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1) ppm; $^{13}$C n.m.r. (75 MHz): δ, 20.5, 20.6 (x 2), 61.0, 62.3, 67.3, 71.7, 72.4, 87.4, 169.6, 169.7, 170.4 ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{12}H_{16}BrN_3O_7Na^+$ 416.0069, found 416.0065.

**3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside (3.16).** The title compound was obtained from 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide **3.15** by Method B in 70% yield as a white foam. Analytical data for **3.16**: $R_f$ = 0.43 (ethyl acetate/hexanes, 2/3, v/v); $[\alpha]_D^{24}$ +8.4 (*c*= 1.0, $CHCl_3$); $^1$H n.m.r. (300 MHz): δ, 2.05, 2.08, 2.12 (3s, 9H, 3 x $COCH_3$), 3.88 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 3.93 (m, 1H, H-5), 4.16 (dd, 1H, $J_{5,6a}$ = 2.3 Hz, H-6a), 4.35 (dd, 1H, $J_{5,6b}$ = 4.3 Hz, $J_{6a,6b}$ = 12.6 Hz, H-6b), 5.13 (dd, 1H, $J_{4,5}$ = 8.9 Hz, H-4), 5.17 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 5.82 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 7.19-7.43 (m, 4H, aromatic) ppm; $^{13}$C n.m.r. (75 MHz): δ, 20.7, 20.8, 20.9, 60.6, 63.1, 67.8, 72.7, 73.0, 97.6, 120.7, 123.5, 126.5, 126.7, 127.0, 127.3, 133.8, 150.1, 169.8, 170.0, 170.7 ppm; $^{19}$F n.m.r.: δ, -122.2 (s, 2F, $CF_2$). HR-FAB MS $[M+Na]^+$ calculated for $C_{20}H_{20}F_2N_4O_8Na^+$ 505.1147, found 505.1142.

**2,3,4,6-Tetra-*O*-benzyl-α/β-D-glucopyranosyl trichloroacetimidate (3.17).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **3.1**[50] in 62% yield as a white foam as previously described.[60] Analytical data for is this for α-**3.17**: $R_f$ = 0.43 (ethyl acetate/hexanes, 1/4 v/v); $^1$H n.m.r. (300 MHz): δ, 3.67 (dd, 1H, $J_{5,6a}$ = 1.9 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.74-3.80 (m, 3H, H-2, 4, 6b), 3.99 (m, 1H, H-5), 4.05 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 4.53 (dd, 2H, $^2J$ = *12*.0 Hz, C$H_2$Ph), 4.71 (dd, 2H, $^2J$ = 11.7 Hz, C$H_2$Ph), 4.73 (dd, 2H, $^2J$ = 10.7 Hz, C$H_2$Ph), 4.84 (dd, 2H, $^2J$ = 7.6 Hz, C$H_2$Ph) , 6.50 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 7.12-7.33 (m, 24H, aromatic), 8.57 (s, 1H, NH) ppm; $^{13}$C n.m.r. (75 MHz): δ, 68.1, 73.0, 73.2, 73.6, 75.5, 75.8, 79.5, 81.5, 91.4, 94.5, 127.8 (x 3), 127.9 (x 2), 128.0, 128.1 (x 2), 128.2 (x 4), 128.5 (x 4), 128.6 (x 4), 137.9, 138.1, 138.2, 138.7, 161.4 ppm; HR-ESI MS $[M+Na]^+$ calculated for $C_{36}H_{36}Cl_3NO_6Na^+$ 706.1506, found 706.1500.

**2,3,4-Tri-*O*-benzyl-α,β-D-glucopyranosyl *N*-phenyltrifluoroacetimidate (3.18).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose **3.1**[50] by adapting previously published procedure.[23] To a mixture of 2,3,4,6-tetra-*O*-benzyl-α/β-D-glucopyranose **3.1**[50] (150 mg, 0.28 mmol) and 2,2,2,-Trifluoro-*N*-phenylethanimidoyl chloride (89.4 μL, 0.55 mmol), was added $K_2CO_3$ (55 mg, 0.55 mmol) in acetone (1.5 mL) and the resulting mixture was stirred for 3 h at rt. The solids were filtered off through a pad of Celite and the solvent was concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexanes gradient elution) to afford the corresponding *N*-phenyltrifluoroacetimidate in 72 % yield as a white amorphous solid. Analytical data for β-**3.18**: $R_f$ = 0.5 (ethyl acetate/hexanes, 1/4, v/v); $^1$H

n.m.r. (500 MHz): δ, 3.56-3.95 (m, 6H, H-2, 3, 4, 5, 6a, 6b), 4.58 (dd, 2H, $^{2}J$ = *9*.5 Hz, C$H_2$Ph), 4.69 (dd, 2H, $^{2}J$ = 12.2 Hz, C$H_2$Ph), 4.88 (s, 2H, C$H_2$Ph), 5.00 (dd, 2H, $^{2}J$ = 10.2 Hz, C$H_2$Ph) , 5.74 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 6.78-7.5 (m, 25H, aromatic); $^{13}$C n.m.r. (75 MHz): δ, 73.6, 75.3, 75.4, 75.8 (x 2), 75.9 (x 2), 75.8, 81.1, 84.7, 119.5, 124.5, 127.9, 128.0 (x 2), 128.1 (x 3), 128.2 (x 3), 128.4 (x 2), 128.6 (x 6), 128.7 (x 7), 128.9 (x 2), 137.9, 138.1 (x 2), 138.5, 143.6 ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{42}H_{40}F_3NO_6Na^+$ 734.2705, found 734.2720.

**2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (3.21).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranose in 83% yield as a white foam as previously described.[61] Analytical data for **3.21** was in accordance with that reported previously.[19]

**2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl N-phenyltrifluoroacetimidate (3.22).** The title compound was obtained from 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranose by adapting previously published procedure.[23] To a mixture of 2,3,4,6-tetra-*O*-benzoyl-α/β-D-glucopyranose (2.17 g, 3.64 mmol) and 2,2,2,-trifluoro-*N*-phenylethanimidoyl chloride (0.7 mL, 4.37 mmol), was added $K_2CO_3$ (0.54 g, 5.46 mmol) in acetone (20 mL) and the resulting mixture was stirred for 5 h at rt. The solids were filtered off through a pad of Celite and the solvent was concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexanes gradient elution) to afford the corresponding *N*-phenyltrifluoroacetimidate in 86 % yield as a white foam. Analytical data for α-**3.22**: $R_f$ = 0.5 (ethyl acetate/hexanes, 3/7, v/v); $[\alpha]_D^{27}$ +49.4 (*c*= 1.0, $CHCl_3$);

$^1$H n.m.r. (500 MHz): δ, 4.51 (dd, 1H, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.69 (m, 1H, H-5), 4.77 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, H-6b), 5.67 (dd, 1H, $J_{2,3}$ = 10.3 Hz, H-2), 5.93 (dd, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 6.30 (m, 3H, H-3, 2', 6'), 6.92 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 7.00-8.15 (m, 25H, aromatic) ppm; $^{13}$C n.m.r. (125 MHz): δ, 62.4, 68.6, 70.0, 70.5, 70.8, 92.3, 119.1, 124.4, 128.6 (x 2), 128.7 (x 5), 128.8 (x 5), 128.9, 129.7, 129.9 (x 2), 130.0 (x 8), 133.3, 133.4, 133.7, 133.8, 142.8, 143.0, 165.2, 165.4, 165.7, 166.1; $^{19}$F n.m.r.: δ, -65.51 (s, 3F, $CF_3$) ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{42}H_{31}F_2NO_{10}Na^+$ 790.1876, found 790.1886.

### *3.5.4. General glycosylation procedures and the synthesis of glycosides*

*Method A. A typical TMSOTf-promoted glycosylation procedure.* A mixture of glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4Å, 90 mg) in $CH_2Cl_2$ (0.5 mL) or other solvent as indicated in Tables was stirred under argon for 1 h at rt. The mixture was cooled to -78 $^oC$ or other temperature as indicated in Tables, TMSOTf (0.0055-0.011 mmol) was added, and the resulting mixture was stirred for 10-15 min as indicated in Tables. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford a glycoside derivative in yields listed in Tables.

*Method B. A typical $BF_3$-$OEt_2$-promoted glycosylation procedure.* A mixture of 3,3-difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside **3.2** (32.7 mg, 0.047 mmol), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **3.19** (18.3 mg, 0.043 mmol), and freshly activated molecular sieves (4Å, 95 mg) in $CH_2Cl_2$ (0.6 mL) was stirred under argon for 1 h at rt. The mixture was cooled to -78 $^oC$, $BF_3$-$OEt_2$ (0.3 μL, 0.002 mmol) was added, and the resulting mixture was stirred for 5 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford disaccharide **3.20** in 93% yield (α/β = 1/20, Table 3.5).

*Method C. A typical $Cu(OTf)_2$-promoted glycosylation procedure.* A mixture of donor **3.2** (38.3 mg, 0.055 mmol), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **3.19** (23.4 mg, 0.05 mmol), and freshly activated molecular sieves (3 Å, 110 mg) in $CH_2Cl_2$ (0.64 mL) was stirred under argon for 1 h at rt. *$Cu(OTf)_2$* (2.0 mg, 0.003 mmol) was added and the resulting mixture was stirred for 5 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford disaccharide **3.20** in 97% yield (β only, Table 3.5).

*Method D. A typical MeOTf-promoted glycosylation procedure.* A mixture of donor **3.2** (30.6 mg, 0.044 mmol), acceptor **3.19** (18.7 mg, 0.040 mmol), and freshly activated molecular sieves (4Å, 90 mg) in $CH_2Cl_2$ (0.5 mL) was stirred under argon for 1 h at rt. MeOTf (0.55 μL, 0.0044 mmol) was added and the resulting mixture was stirred for 10 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford disaccharide **3.20** in 91% yield (α/β = 1/>25, Table3.5).

*Method E. A typical AgOTf-promoted glycosylation procedure.* A mixture of donor **3.2** (43.5 mg, 0.062 mmol), acceptor **3.19** (26.7 mg, 0.057 mmol), and freshly activated molecular sieves (4Å, 120 mg) in $CH_2Cl_2$ (0.73 mL) was stirred under argon for 1 h at rt. AgOTf (7.3 mg, 0.028 mmol) was added and the resulting mixture was stirred for 24 h. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford disaccharide **3.20** in 71% yield (α/β = 1/1.6, Table 3.5).

*Method F. A typical $PdCl_2$-promoted glycosylation procedure.* A mixture of donor **3.2** (33.2 mg, 0.048 mmol), acceptor **3.19** (20.3 mg, 0.044 mmol), and freshly activated

molecular sieves (4Å, 90 mg) in $CH_2Cl_2$ (0.55 mL) was stirred under argon for 1 h at rt. $PdCl_2$ (2.55 mg, 0.014 mmol) was added and the resulting mixture was stirred for 36 h. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford disaccharide **3.20** in 57% yield (α/β = 1/1.7, Table 3.5).

*Method G. A typical $TMSClO_4$-promoted glycosylation procedure.* A mixture of donor **3.2** (0.11 mmol), acceptor **3.19** (0.10 mmol), and freshly activated molecular sieves (3 Å, 90 mg) in $Et_2O$ (0.5 mL) or $Et_2O$/1.4-dioxane (0.5 mL, 1/1, v/v) was stirred under argon for 1 h at rt. $TMSClO_4$[62] (0.011 mmol) was added and the resulting mixture was stirred for 5 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford disaccharide **3.20** in 75% (α/β = 5/1, Table 3.5) or 54% yield (α/β = 6.2/1), respectively.

*Method H. A typical $Bi(OTf)_3$-promoted glycosylation procedure.* A mixture of donor **3.2** (41.3 mg, 0.059 mmol), acceptor **3.19** (25.2 mg, 0.054 mmol), and freshly activated molecular sieves (4Å, 120 mg) in $CH_2Cl_2$ (0.7 mL) was stirred under argon for 1 h at rt. The reaction was cooled to -60 $^oC$ followed by the addition of *$Bi(OTf)_3$* (3.9 mg, 0.006

mmol) and the resulting mixture was stirred for 20 min. The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford disaccharide **3.20** in 85% yield, (α/β = 1/7.0, Table 3.5).

*Method I. A typical $SnCl_4$-promoted glycosylation procedure.* A mixture of 3,3-difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside **3.8** (25.8 mg, 0.051 mmol), acceptor **3.19** (21.8 mg, 0.046 mmol), and freshly activated molecular sieves (4Å, 90 mg) in $CH_2Cl_2$ (0.5 mL) was stirred under argon for 1 h at rt. $SnCl_4$ (0.6 μL, 0.005 mmol) was added and the resulting mixture was stirred for 10 min (Table 4). The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~40 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford disaccharide **3.38** in 80% yield (Table 3.5).

**Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (3.20).** The title compound was obtained by Methods A-H from donor **3.2** and acceptor **3.19**[63] in 54-97% yield (α/β ranging from 6.2/1 to β-only, see Tables). Analytical data for **3.20** was in accordance with that reported previously.[64,65]

**2-Propyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (3.27).** The title compound was obtained by Method A from donor **3.2** and isopropanol **3.26** as a white amorphous solid in 77% yield (β only). Analytical data for **3.27** was in accordance with that reported previously.[66]

**Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-α-D-glucopyranoside (3.29).** The title compound was obtained by Method A from donor **3.2** and methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside **3.28**[67] in $CH_2Cl_2$ or EtCN in 89% (α/β = 1/11) or 87% yield (α/β = 1/18), respectively. Analytical data for **3.29** was in accordance with that reported previously.[68]

**6-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.31).** The title compound was obtained by Method A from donor **3.2** and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **3.30** in $CH_2Cl_2$ or EtCN in 85% (α/β = 1/12) or 89% yield (β only), respectively. Analytical data for **3.31** was in accordance with that reported previously.

**Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-α-D-glucopyranoside (3.33).** The title compound was obtained by Method A from donor **3.2** and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **3.32**[63] in $CH_2Cl_2$ or EtCN in 94% (α/β = 1/4) or 92% yield (α/β = 1/12), respectively. Analytical data for **3.33** was in accordance with that reported previously.[68]

**Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.25).** The title compound was obtained by Method A from donor **3.2** and methyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranoside **3.24**[63] in $CH_2Cl_2$ or EtCN in 90% (α/β = 1/6.0) or 88% yield (α/β = 1/15), respectively. Analytical data for **3.25** was in accordance with that reported previously.[29]

**(3β)-Cholest-5-en-3-yl 2,3,4,6-tetra-*O*-benzyl-*β*-D-glucopyranoside (3.35).** The title compound was obtained by Method A from donor **3.2** and (3β)-cholest-5-en-3-ol **3.34** as a white amorphous solid in 86% yield (β-only). Analytical data for **3.35** was in accordance with that reported previously.[69]

**1-Adamantyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside (3.37).** The title compound was obtained by Method A from donor **3.2** and 1-adamantol **3.36** as a white amorphous solid in 88% yield (α/β = 1/23). Analytical data for **3.37** was in accordance with that reported previously.[69]

**Methyl 2-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (40).** The title compound was obtained by Method A from donor α-**12** or donor β-**12** and acceptor **34**[63] as a clear film in 89% or 88% yield (α only), respectively. Analytical data for **40** was in accordance with that reported previously.[29]

**Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3.38).** The title compound was obtained by Method I from donor **3.8**

and acceptor **3.19**[63] as a clear film in 80% yield. Analytical data for **3.38** were essentially the same as reported previously.[70]

**Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3.39).** The title compound was obtained by Method A from donor **3.10** and acceptor **3.19**[63] as a clear film in 94% yield. Analytical data for **3.39** were essentially the same as reported previously.[63]

**6-*O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3.40).** The title compound was obtained by Method A from donor **3.10** and acceptor **3.30** in 86% yield. Analytical data for **3.40** were essentially similar as reported previously.[29]

**Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3.41).** The title compound was obtained by Method A from donor **3.10** and acceptor **3.32**[63] as a clear film in 90% yield. Analytical data for **3.41** were essentially the same as reported previously.[63]

**Methyl 2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (3.42).** The title compound was obtained by Method A from donor **3.10** and acceptor **3.24**[63] as a clear film in 93% yield. Analytical data for **3.42** were essentially the same as reported previously.[63]

**Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3.43).** The title compound was obtained by Method A from donor **3.12** and acceptor **3.19**[63] as a clear film in 98% yield. Analytical data for **3.43** were essentially the same as reported previously.[71]

**Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3.44).** The title compound was obtained by Method A from donor **3.12** and acceptor **3.32**[63] as a clear film in 98% yield. Analytical data for **3.44** were essentially the same as reported previously.[29]

**Methyl 6-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (3.45).** The title compound was obtained by Method A from donor **3.14** and acceptor **3.19**[63] as a clear film in 93% yield. Analytical data for **3.45** were essentially the same as reported previously.[29]

**Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (3.46).** The title compound was obtained by Method A from donor **3.14** and acceptor **3.32**[63] as a clear film in 90% yield. Analytical data for **3.46** were essentially the same as reported previously.[72]

### *3.5.5. A typical procedure for regenerative glycosylation*

A mixture of ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside **3.47**[73] (30 mg, 0.051 mmol) and activated molecular sieves (3 Å, 90 mg) in $CH_2Cl_2$ (0.5 mL) was stirred

under argon for 1 h at rt. The mixture was cooled to 0 $^{o}$C, bromine (0.27 μL, 0.01 mmol) was added, and the resulting mixture was kept for 15 min at 0 $^{o}$C. After that, 3,3-difluorooxindole (0.9 mg - 8.9 mg, 0.0051-0.051 mmol, see Table 2 of the manuscript), and $Ag_2O$ (35.5 mg, 0.16 mmol) were added to the reaction mixture and the resulting mixture was stirred for 1h. Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **3.19** (18.3 mg, 0.039 mmol) and $BF_3$-$OEt_2$ (0.33 μL, 0.0025 mmol) were added and the reaction was stirred for 10 min - 5 h (see Table 2 of the manuscript). The solids were filtered off through a pad of Celite and rinsed successively with $CH_2Cl_2$. The combined filtrate (~20 mL) was washed with 1% aq. NaOH (2 x 10 mL) and water (2 x 10 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexanes gradient elution) to afford disaccharide **3.20** in 9-90% yield (see Table 2 of the manuscript).

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# Chapter 4

# Refinement of the Key Chemical Aspects of the HPLC–Assisted Automated Oligosaccharide Synthesis

## 4.1. Introduction

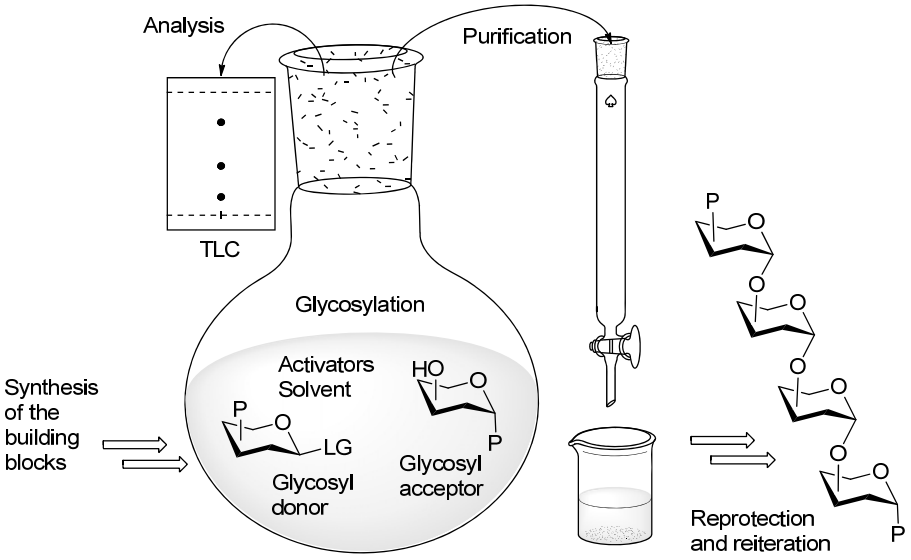
Whether in the form of simple monosaccharides or as a part of complex natural products, conjugates or biomarkers, carbohydrates are involved in a wide variety of fundamental or disease-related processes. As a result, carbohydrates and their conjugates show tremendous diagnostic and therapeutic potential. With the advent of modern technology, the need for elucidating the exact mechanism by which these carbohydrates interact with other biomolecules, and features that are responsible for these interactions, has been particularly stimulating in the field. While scientists have been able to successfully isolate certain classes of natural carbohydrates, the availability of pure natural isolates is still inadequate to address challenges offered by modern glycosciences. As a consequence, chemical synthesis has become a viable means to obtain both natural complex carbohydrates and unnatural analogues thereof. With the immense task of conducting efficient drug design and the search for new pharmaceuticals, the chemical synthesis of complex carbohydrate structures and/or mimetics thereof falls short.

### *4.1.1. Chemical Synthesis of Oligosaccharides*

Practically all complex carbohydrates bear an oligomeric sequence wherein monosaccharide residues are linked via O-glycosidic linkages. This linkage is obtained by a glycosylation reaction that typically involves a nucleophilic displacement of a leaving group (LG, Scheme 4.1.) on the glycosyl donor by a hydroxyl group of the acceptor.[1] The remaining functional groups of both components are temporarily masked with protecting groups (Ps).[2-25] Despite significant progress, chemical glycosylation remains challenging due to the requirements of achieving complete stereocontrol and

suppressing side reactions.[1] Further elongation of the saccharide chain for the synthesis of oligomeric structures requires additional protecting/leaving group modifications between each glycosylation step. Fraser-Reid,[26,27] Nicolaou,[28] Danishefsky,[29] Kahne,[30] Wong,[31] Boons,[32] Crich,[19] Huang,[33] and others[34] developed advanced strategies that allow for streamlining the oligosaccharide synthesis. Nevertheless, chemical synthesis of oligosaccharides of even moderate complexity still remains a considerable challenge. As such, the development of efficient methods for chemical glycosylation[1] and expeditious oligosaccharide[34] and glycoconjugate synthesis remains a demanding area of research.

**Scheme 4.1. Conventional solution-phase synthesis**



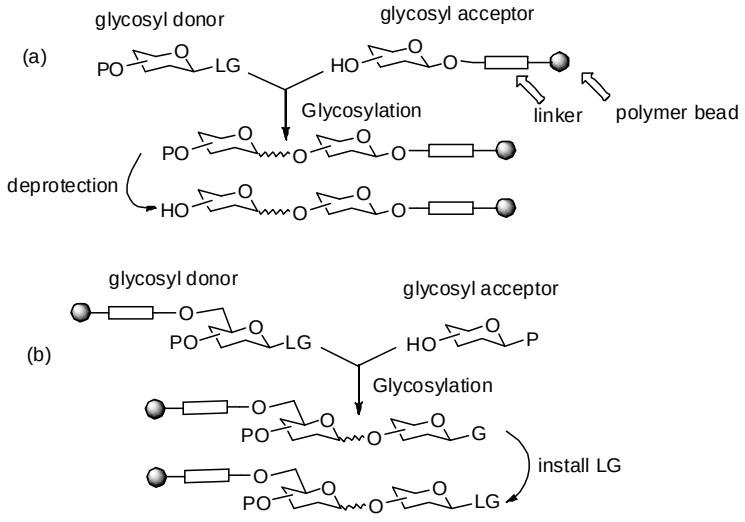

### *4.1.2. Solid phase oligosaccharide synthesis*

Solid-phase synthesis using insoluble polymer supports[35,36] has been widely used in the preparation of many organic molecules[35-38] including peptides[39] and oligonucleotides.[40] The early example of solid phase peptide synthesis by Merrifield[41] was followed by oligosaccharide synthesis by Fréchet and Schuerch that emerged shortly

thereafter.[42] Since early attempts, the polymer-supported oligosaccharide synthesis has become a viable means for the synthesis of oligosaccharide sequences without the necessity of purifying, and characterizing, and even isolating the intermediates.[43-46] Another important advantage of oligosaccharide synthesis on a solid support is the ease of excess reagent removal (usually can be achieved by filtration and rinsing).

There are two main strategies for solid phase saccharide synthesis that differ in the type of the attachment (Scheme 4.2). In strategy A, the glycosyl acceptor unit is bound to the solid support either at the anomeric position or at another suitable hydroxyl. In this case, an excess of the glycosyl donor and promoter are in the solution. The periodic monitoring of the solution phase by TLC can provide information on whether the glycosyl donor is still remaining in the solution. The completeness of the coupling can be determined experimentally by performing the Kaiser test,[47] or by cleaving the product off the polymer support followed by characterization. Routinely, the coupling step is repeated multiple times using fresh reagents to ensure that all glycosyl acceptor is consumed.

**Scheme 4.2. General strategies for glycosylation on polymer support**



In approach B, the glycosyl donor unit, linked to the solid support via a suitable hydroxyl group, is reacted with the excess of the solution phase acceptor. Two-directional techniques, combining approaches A and B are also known.[48,49] To improve the operational simplicity of the oligosaccharide assembly Seeberger developed an automated approach, which is based on strategy A: using a solid phase acceptor and a liquid phase donor (vide infra).[50]

### *4.1.3. Key aspects of the solid phase synthesis*

The solid support, a functionalized polymer used in a majority of applications, is an insoluble material that is usually used in the form of a bead, a gel-like spherical microparticle. The beads have good swelling properties in organic solvents that allows for diffusion and hence facilitates the reagent delivery to the reactive sites.[51] The polymeric bead is then covalently linked to (or loaded with) a sugar moiety, typically via a linker (or a spacer). It is known that the efficiency of the synthesis using solid supports depends on the following factors:

1) The loading efficiency of the resin to the first anchoring unit.
2) The amount of cross-linking: It's believed that lesser the amount of cross-linking, the better is the stability and swelling capacity of the resin; resins wherein 1-2% of monomers are cross-linked are considered ideal.
3) The material from which the support is constructed.
4) Chemical stability. The functional groups present on the resin might also react with (or trap) the reagents, thereby poisoning (contaminating) the resin.

5) Physical stability. Resin beads can disintegrate or collapse if out-of-range (excessive) temperature or pressure is applied.

Among various resins studied, the three dominant resins currently used for oligosaccharide synthesis are chloromethyl-, hydroxymethyl- and aminomethyl-polystyrene resins. Amongst these, use of chloromethylpolystyrene cross-linked with divinyl benzene (Merrifield resin), has been widely used for a variety of applications.[51] The low cost and its compatibility with a variety of reagents and solvents makes the Merrifield resin a popular choice for oligosaccharide synthesis. Nevertheless, the search of other suitable polymer supports is ongoing and other polystyrene based supports, such as JandaGel, Tentagel, ArgoGel, etc. have also shown wide applicability. Though most of the polymer supported oligosaccharide synthesis has been performed on insoluble supports, for oligosaccharides synthesis using soluble polymer supports, fluorous tag or ionic liquid supports have been introduced.

The linker, or a linker/spacer combination, is used to connect (covalently bind) the solid support and the first sugar building block. The chemical composition and the size (length) of the linker are known to have an effect on the outcome of reactions. But it is the type of the bond between the linker and the sugar as well between the linker and the resin that are of the key importance. The linker has to withstand all reaction conditions required for glycosylation and protecting group manipulations, yet it should be easily removable using mild reagents at the end of the synthesis.[52] Many different linkers have been developed and classified based on the reaction conditions required, acidic or basic, reductive or oxidative, for linker cleavage. In the recent years, photo-cleavable or metathesis cleavable linkers have also been developed. Among other linkers, silyl ether,

1,2-di(hydroxymethyl)-benzene,[53] alkene,[54] and acylsulfonamide have been successfully employed in polymer supported oligosaccharide syntheses.[51,55-57]

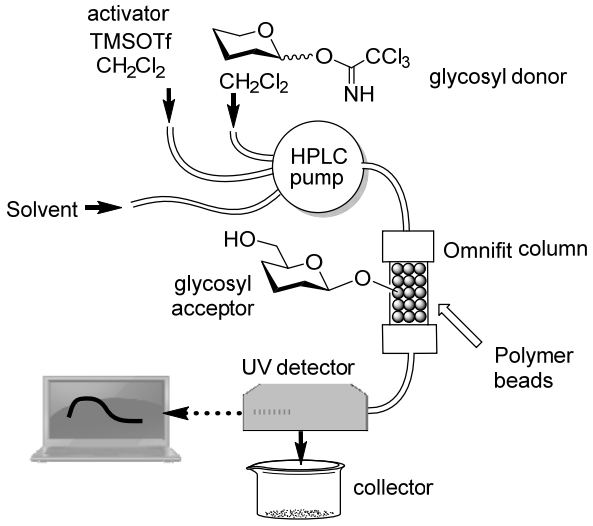
### *4.1.4. Automation of oligosaccharide synthesis*

In spite of remarkable progress, the solid phase technique still suffers from significant limitations: the need for large reagent excess, limited use of molecular sieves, large volume of waste solvent, cumbersome analysis of intermediates, lower anomeric stereoselectivity, loss and poisoning of resin, reagent trapping, etc. To expedite solid-phase oligosaccharide synthesis further, Seeberger and co-workers developed an automated approach in 2001.[50,58,59] The automation was initially accomplished by using a peptide synthesizer that has evolved into "the first fully automated solid-phase oligosaccharide synthesizer" (2012),[60] which was recently commercialized (Glyconeer 2.1, sold by GlycoUniverse). Although the automated platform developed by Seeberger introduces an idea of operational simplicity, the technology remains over-engineered and underdeveloped. It offers very little promise to become universally available, operationally simple or inexpensive.

To overcome these limitations, recently our group has expanded technologies available for the synthesis of oligosaccharides using polymer supports. The new experimental set-up was based on modified HPLC[61] equipment that is broadly available in most of synthetic or analytical laboratories and requires practically no investment for the basic setup. The modular character of HPLC allows for opportunities to implement attachments, vary reagent delivery modes, modify detection systems, accessories, and vary software packages. HPLC-based automation allows for real-time reaction

monitoring, which, in turn, helps reduce the reaction time and the amount of reagents needed. In brief, a chromatography column (Omnifit glass column) was loosely packed with the pre-swelled polymer resin loaded with the glycosyl acceptor. The column was then connected to the HPLC system containing a three-headed pump, a detector, and a computer equipped with the standard HPLC-operating software (Scheme 4.3). The column was then purged with solvent and the glycosyl donor and promoter delivered using a multi-headed HPLC pump. After a relatively short reaction time, typically 60 min, the system was purged with the solvent to rinse off any excess reagents. At this time, the resin was loaded with the disaccharide derivative that can be either cleaved off from the polymer support or the oligosaccharide elongation can be continued via alternating deprotection/glycosylation steps.

**Scheme 4.3. HPLC-based automation of oligosaccharide synthesis**




## 4.2. Results and Discussion

Preliminary work in our laboratory has clearly shown the advantages of the new technology in comparison to that of the state-of-the-art polymer-supported synthesis. This

method has shown a promise in reducing the amount of reagents and solvents via recirculation, achieving faster reaction times, and performing real-time reaction monitoring using a UV detector. With success in developing this new technology to perform automated oligosaccharide synthesis, herein we aimed at optimizing all the aspects of the HPLC-based automated synthesis. For this purpose, we will investigate different types and classes of polymeric supports, linkers, glycosyl donors, and activation conditions to identify the best conditions for reaction set-up, monitoring, and quenching – all performed in the automated manner using standard HPLC-operating software.

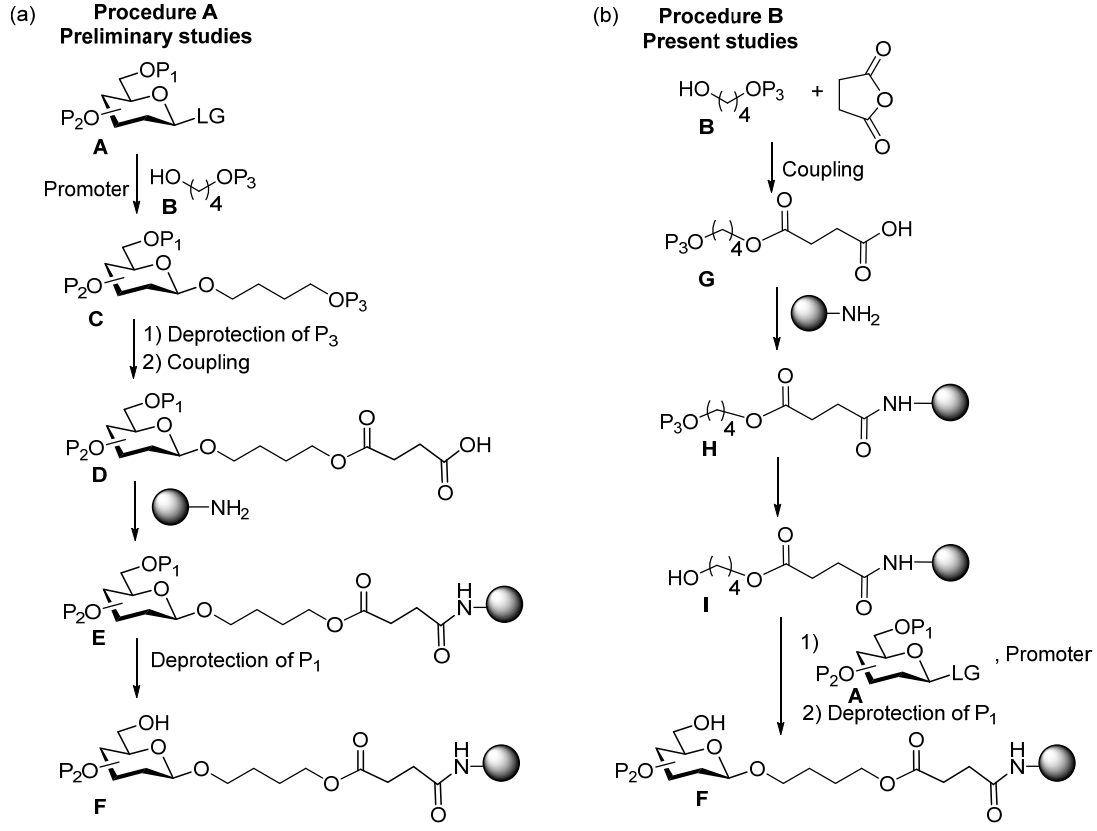
### *4.2.1. Synthesis of the polymer-bound acceptor*

In the preliminary study of the HLPC automation, the monosaccharide glycosyl acceptor was first attached to the linker and spacer and then tethered to the resin at the end (Procedure A, Scheme 4.4a). Precursor **A** bearing a trityl group as the temporary protecting group at the primary C-6 position ($P_1$) was first glycosidated with linker **B**. The resulting conjugate **C** was then attached to the spacer by first carrying out deprotection of the temporary protecting group on the linker ($P_3$) and then coupling with the spacer to yield compound **D**. The latter was then tethered to Tentagel MB-$NH_2$ resin using EDC (3.0 equiv.) and DMAP (1.0 equiv.) mediated coupling to give precursor **E**. The temporary protecting group (trityl) was then cleaved off to afford the polymer bound acceptor **F**.

Inspired by recent work by Seeberger,[60] we have begun loading the spacer/linker first (Procedure B, Scheme 4.4b). Our modified approach begins with the coupling of linker **B** to the spacer unit followed by treatment with EDC (3.0 equiv.) and DMAP (1.0 equiv.)

on Tentagel MB-$NH_2$ resin to form precursor **H**. The temporary protecting group of precursor **H** is then removed to afford acceptor molecule **I**. The latter is then glycosylated with donor **A** and the elongation is performed by simple reiteration of deprotection and glycosylation steps until the oligosaccharide of the desired length is generated.

**Scheme 4.4. Strategic adjustment to the loading sequence**

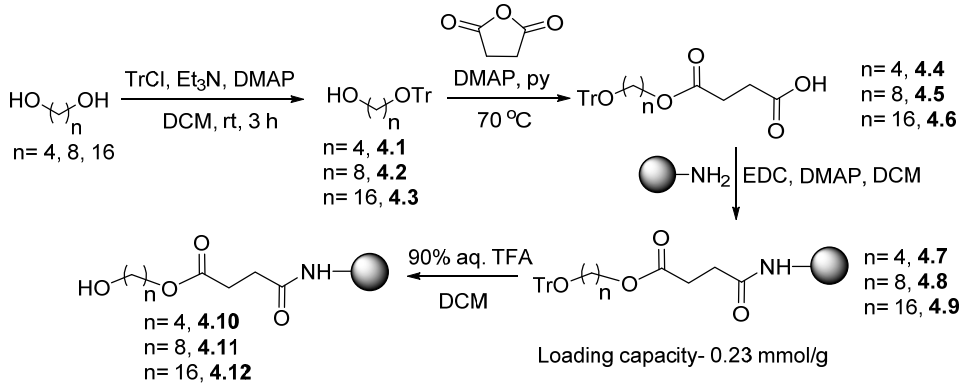



We believe that Procedure B is much more convenient for the HPLC-based automation platform. During the preliminary study the monosaccharide glycosyl acceptor was manually loaded on the resin and only afterwards was it subjected to the automation sequence. In our present study, all the steps starting from loading of compound **G** to the resin are automated. The modified OH resin precursor **I** can also be stored and used for multiple automated oligosaccharide syntheses as needed via the operationally simple

iterative glycosylations and deprotection steps. This strategic adjustment is expected to enhance the reliability of the HPLC-based automation.

Herein, we illustrate the synthesis of the polymer-bound glycosyl acceptor using a C4 (butyl), C8 (octyl) and C16 (hexadecyl) spacer, succinoyl linker and Tentagel MB-$NH_2$ resin as the solid support. The synthesis began with monotritylation of (1,4-butanediol, 1,8-octanediol, and 1,16-hexadecanediol) to give spacer precursors **4.1**, **4.2**, and **4.3** respectively (Scheme 4.5). The latter was then reacted with succinic anhydride in the presence of DMAP to give conjugate **4.4**, **4.5**, and **4.6** respectively. The carboxyl on **4.4**, **4.5**, and **4.6** was then manually coupled with the free amine group of the Tentagel resin using a flask on a shaker in the presence of EDC (3.0 equiv.) and DMAP (1.0 equiv.) to give **4.7**, **4.8**, and **4.9** respectively, with a loading capacity of 0.23/ 0.29/ 0.30 mmol/g, respectively, in 72 h.

**Scheme 4.5. Synthesis of the polymer-bound acceptor 4.4**

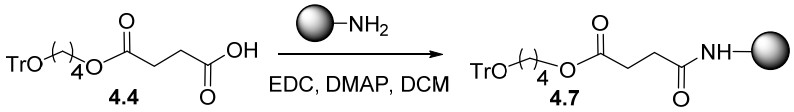



Alternatively, the same coupling was performed using the HPLC-based set up as follows. Tentagel resin was packed into the glass Omnifit column and swelled for 1 h in $CH_2Cl_2$. Linker-spacer conjugate **4.4**, **4.5**, and **4.6** along with the coupling reagents, EDC (1 equiv.) and DMAP, were then passed through the column at a flow rate of 1.0 mL/min

for 3 h. After that, the column was washed with $CH_2Cl_2$ (1 x 10 mL), acetone (1 x 10 mL) and $CH_2Cl_2$ (2 x 10 mL) to ensure complete removal of the remaining reactants. The resin was then dried and the loading capacity (0.23 mmol/g) was calculated based on the increase in weight of the resin. Detritylation was then performed using 90% trifluoroacetic acid in water to yield the polymer bound acceptor **4.10**, **4.11**, and **4.12**, respectively.

During our exploratory study with JandaGel and Tentagel resins, it was observed that the desired loading capacities could be achieved much faster using HPLC-based reagent delivery rather than the manual loading in a flask. Thus, loading capacities of 0.4 and 0.23 mmol/g for JandaGel (entry 1, Table 4.1) and Tentagel (entry 2) respectively, were obtained. Based on the periodic monitoring using Kaiser test, both manual loading procedures required a minimum reaction time of 72 h. However, when the same couplings were performed using the HPLC-assisted experimental set-up, similar loading capacities were obtained within 2-3 h (entries 3 and 4). We also noticed that HPLC-based automation allowed us to reduce the amount of the coupling reagent (EDC) to a very minimal 1.0 equiv. whereas manual experiments required at least 3.0 equiv. of EDC.

**Table 4.1. Comparison of the loading efficiency between manual and HPLC-based automated experiments**




| Entry | Resin | Experimental set-up | EDC (equiv.) | Loading Time | Loading capacity, mmol/g |
|---|---|---|---|---|---|
| 1 | JandaGel | Manual | 3 | 72 h | 0.40 |
| 2 | Tentagel | Manual | 3 | 72 h | 0.23 |
| 3 | JandaGel | HPLC-based | 1 | 2 h | 0.38 |
| 4 | Tentagel | HPLC-based | 1 | 3 h | 0.24 |

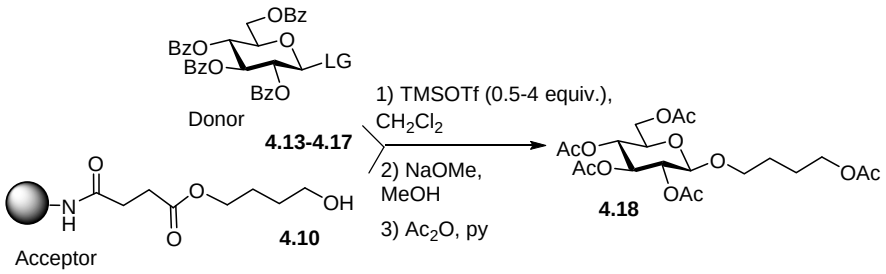
### *4.2.2. Screening of different glycosyl donors*

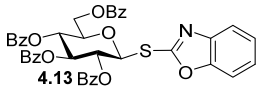
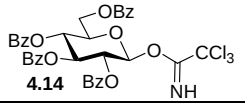
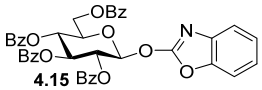
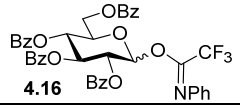
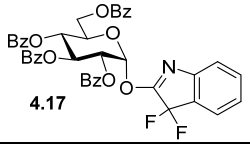
The original development of the HPLC-based synthesis was solely based on utilizing glycosyl trichloroacetimidates (TCAI) as glycosyl donors. In order to expand the utility of this technology, we wanted to investigate whether other classes of glycosyl donors would be suitable. Our laboratory has been developing new methods for chemical glycosylation with the central focus on glycosyl thioimidates and thioglycosides.[62] The use of thioglycosides as donors in glycosylations of solid-supported acceptors has been reported, but the relatively low reactivity profile of thioglycosides and the requirement for stoichiometric promoters limits their application. Also under HPLC-assisted synthesis ethyl, phenyl or tolyl thioglycosides were quite inefficient as glycosyl donors, which resulted in very low yields (~5%) of the coupling products. In this context, we had no success in using glycosyl bromides and fluorides as glycosyl donors.

Based on the previous studies with S-benzoxazolyl (SBox) imidates,[63] we already knew that these glycosyl donors offer superior properties for reactions in solution.[64,65] The SBox donors were also found suitable for the solid phase synthesis using polymer[66] and nanoporous gold supports.[67] In these applications, SBox donors were activated with TMSOTf. When essentially the same activations conditions were applied to glycosylation of glycosyl acceptor **4.10** with SBox donor **4.13** using the HPLC-based experimental set up, compound **4.18** was obtained in a good yield of 67% (entry 1, Table 4.2.). This reaction was performed by passing solutions of the glycosyl donor **4.13** in $CH_2Cl_2$ and TMSOTf in $CH_2Cl_2$ through the column packed with acceptor **4.10** using a multi-headed HPLC pump. The reaction mixture that eluted from the column was then recirculated for 4 h to ensure completion of the reaction. It should be noted that all yields reported here

are isolated yields after HPLC-based coupling and cleavage from the solid support with recirculating solution of MeONa in MeOH, followed by manual acetylation with $Ac_2O$ and pyridine.

**Table 4.2. Comparative study of different S/O-imidoyl donors using HPLC-assisted experimental set-up**




| Entry | Donor, 10 equiv. | Time for recirculation | Yield |
|---|---|---|---|
| 1 |  **4.13** | 4 h | 67% |
| 2 |  **4.14** | 1 h | 83% |
| 3 |  **4.15** | 1 h | 85% |
| 4 |  **4.16** | 2 h | 90% |
| 5 |  **4.17** | 2 h | 87 |

Although acceptable yield were obtained in SBox-mediated couplings, it has also become apparent that highly reactive TCAI donors easily outperform SBox imidates. Thus, a high yield of 83% was obtained in the TMSOTf-promoted synthesis of **4.18** from donor **4.14** and the solid phase-bound acceptor **4.10** (entry 2). As shown in Chapter 2, O-benzoxazolyl (OBox) imidates, which represent a hybrid structure between SBox and

TCAI, are more reactive than either leaving group. Hence, we were curious to investigate whether the higher reactivity of OBox imidates would be beneficial for HPLC-based applications. Indeed, a similar reactivity profile and yields to those obtained with TCAI were achieved with OBox imidates. Thus, on reacting OBox donor **4.15** with acceptor **4.10**, compound **4.18** was obtained in 85% yields (entry 3).

Inspired by promising results obtained with TCAI and OBox leaving groups, we continued the search of suitable glycosyl donors by studying other O-imidates in the HPLC-based applications. Over the course of this study we noticed that although N-phenyl trifluoroacetimidates (PTFAI)[68,69] are more stable than their TCAI or OBox counterparts, they often outperform TCAI in glycosylations. Thus, when glycosylation of glycosyl donor **4.16** with acceptor **4.10** was carried out in the presence of TMSOTf compound **4.18** was obtained in an excellent yield of 90%. However, since PTFAI imidates are less reactive than their TCAI or OBox counterparts, higher amounts of TMSOTf (2.5 equiv.) was used for their activation versus 0.5 equiv. required for the activation of other O-imidates.

This stimulated our interest in investigating structurally similar OFox imidates (see Chapter 3) as glycosyl donors for HPLC-assisted glycosylations. As expected, results similar to those obtained with PTFAI donors were achieved (87% yield, entry 5, Table 4.2.). Under solid-supported synthesis, the OFox glycosides showed similar reactivity to PTFAI glycosides and still required 2 equiv. of TMSOTf. This represents a significantly larger amount of the activator in comparison to that required for the activation of OFox imidates in solution (0.03-0.10 equiv.). As an outcome of this comparison study, we conclude that reactive, but comparatively stable PTFAI and OFox imidates, achieve the

best yields with HPLC-assisted glycosylations. Nevertheless, a relatively high amount of TMSOTf required for their activation represents a drawback. This stimulated our further search of suitable reaction conditions and we decided to apply the regenerative concept for glycosylation to the HPLC-based automated set-up.

### *4.2.3. Application of the regenerative concept to HPLC-assisted automated synthesis*

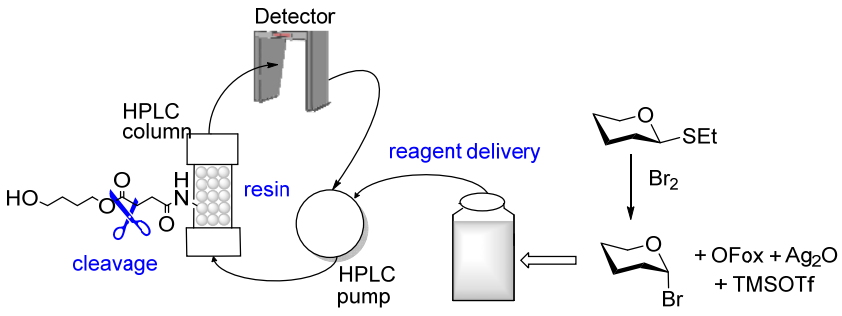
It is widely acknowledged that glycosylations on a solid phase offer different challenges than those in solution. Additional hurdles relate to the mismatch between highly reactive solution-based vs. unreactive solid-phase-immobilized reactants.[45,70] Yet, little has been done to develop dedicated methods, and all solid-phase glycosylations use donors that have been developed for couplings in solution. Low efficiency of solid-phase reactions has been addressed by a "cavalier approach": applying a large excess of the glycosyl donor (5-10 equiv.) and repeating the glycosylation step 2-3 times. In our opinion, this approach is destined to fail, from an efficiency perspective, because 80-90% of the donor goes to waste.[46] Automation offers some operational simplicity, but the entire concept suffers from the inherited drawbacks of conventional methods.

To overcome these limitations, we wanted to investigate whether the conditions established for the OFox leaving group-based regenerative concept (see Chapter 3) would be beneficial to HPLC-assisted synthesis. Since the 3,3-difluoroxindole (HOFox)[71,72] aglycone has the same structure before and after glycosylation, the synthesis and glycosidation of OFox imidates can be conducted using catalytic reagents: HOFox aglycone will first react with a stable precursor to form highly reactive OFox imidate. The latter will then react with the polymer-bound glycosyl acceptor while regenerating

HOFox aglycone, which will be available for the next catalytic cycle to generate additional quantities of the OFox donor, etc. As a result, only a small amount of the reactive OFox donor is present in the reaction medium (which depends on the amount of HOFox added), and the donor gets regenerated only upon its consumption.

In our opinion, the implementation of the OFox-based regenerative concept into the HPLC-based automated set-up would have a conservatively estimated three-fold benefit. First, the reactive donor would be generated in small amounts, which will help to minimize side reactions. Second, the donor would be constantly regenerated ensuring continuous feeding of the system with the "fresh" donor. Third, a stable precursor can be used, and careful monitoring of glycosylation will ensure that only a minimal amount is delivered.

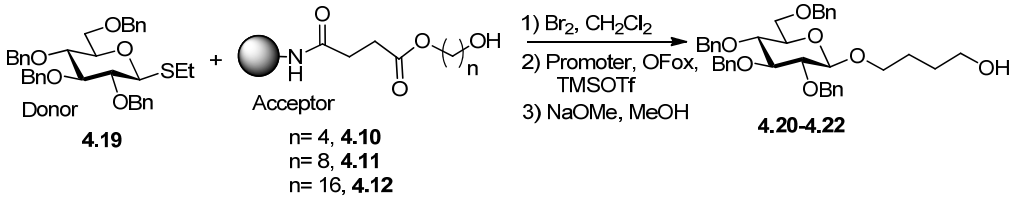
**Scheme 4.6. Outline of the OFox-mediated regenerative glycosylation on solid support**




As illustrated in Scheme 4.6, a stable thioglycoside (SEt) donor is first converted to glycosyl bromide. 3,3-Difluoroxindole and $Ag_2O$ are then added to the same reaction flask. This solution is then passed through a column containing the polymer-bound acceptor. After 1 hour, TMSOTf is added to the same reaction flask to activate the OFox formed. The reaction is continued until all glycosyl bromide has been consumed

(glycosidated or hydrolyzed). Over the course of the reaction, the consumption of HOFox to form the OFox imidate can be monitored using the HPLC-detector. The resin is then washed several times to remove excess reagents. The sugar moiety is then cleaved off from the solid support using NaOMe in MeOH and then purified to get the desired monosaccharide.

**Table 4.3. Manual solid-phase synthesis using regenerative glycosylation**




| Entry | Donor, equiv. | Linker | Amount of OFox added, equiv. | Promoter (equiv.) | Time | Yield of 4.20-4.22 |
|---|---|---|---|---|---|---|
| 1 | 10 | C-4 (**4.10**) | 0.2 | $Ag_2O$ (5) | 48 h | No reaction |
| 2 | 10 | C-8 (**4.11**) | 0.2 | $Ag_2O$ (5) | 48 h | No reaction |
| 3 | 10 | C-16 (**4.13**) | 0.2 | $Ag_2O$ (5) | 48 h | 20% |
| 4 | 10 | C-16 (**4.13**) | 0.2 | AgOTf (4) | 48 h | 21% |
| 5 | 10 | C-16 (**4.13**) | 0.4 | AgOTf (4) | 48 h | 23% |
| 6 | 10 | C-16 (**4.13**) | 0.2 | AgOTf (4)/ TMSOTf (2) | 48 h | 10% |

With these considerations in mind, we began a dedicated study of regenerative glycosylations on a solid phase support using both a manual and HPLC-based experimental set-up. Alongside with this study, we also wanted to investigate the effect of the linker. Starting with the four-carbon alkyl chain linker, we also obtained C8 and C16 linkers using essentially the same method as that described for the synthesis of the C4 linker (see Scheme 4.5). Manual solid-supported synthesis was carried our as follows.

The resin-bound acceptor (**4.10-4.12)** was swelled in dry $CH_2Cl_2$ for 1 h. To the same flask, S-ethyl glycoside **4.19** and bromine were added to form the desired glycosyl bromide. The latter is stable in the presence of TMSOTf, but can be readily converted to the corresponding OFox imidate in the presence of HOFox and $Ag_2O$. The OFox imidate is then reacted with the solid support bound acceptor (**4.10-4.12**) in the presence of TMSOTf. During our initial experimentation, regenerative glycosylations performed using C4 or C8 linker, did not proceed. At this instant, we realized that the longer the linker, the better will be the availability of the free hydroxyl of the glycosyl acceptor since it creates more reaction space by extending the acceptor away from the bulky solid support.The assumption was in accordance to the results obtained from our previous study where we showed that higher reaction yield and improved selectivities were obtained using C8-O-C8 spacer. Inded, when glycosylations were performed using C16 linker, some conversion (~20%, Table 4.3, entry 3) was observed.

While performing solid-phase regenerative glycosylations, we encountered some difficulties with respect to filtering of the resin and further steps associated with the synthesis. The reason behind that was the insolubility of silver oxide in the solvent used for glycosylation. As a result, we decided to use silver triflate instead of silver oxide. Because of its solubility in acetone, AgOTf can be easily washed off during filtration. Also when present in stiochiometric amounts, it activates both bromides and OFox imidates. Thus, regenerative glycosylation using stiochiometric amounts (4.0 equiv.) of silver triflate provided **4.22** in 21% yield. When the same reaction was performed in the presence of AgOTf/TfOH (4.0 equiv./1.0 equiv.) only 10% conversion was observed. When the amount of HOFox was increased from 0.2 equiv. to 0.4 equiv. there was no

significant change in the yields obtained. Further investigation of this regenerative glycosylation approach to solid supported synthesis and HPLC-assisted automated oligosaccharide synthesis are currently underway in our laboratory.

## 4.3. Conclusions

Our laboratory had shown a new automation platform based on HPLC (high-performance liquid chromatography) equipment to be a very promising new technology for the synthesis of oligosaccharides. Refinement of chemical aspects of HPLC-based experimental set-up was performed in terms of studying various linkers, resins, and glycosyl donors. We also implemented the regenerative glycosylation concept to the HPLC-assisted automated synthesis. With the application of the regenerative concept, we have observed faster reaction times and achieved lower reagent excesses in comparison to that of all other known approaches for solid-phase synthesis. With the ultimate goal of developing universal conditions for HPLC-assisted synthesis, we expect that the regenerative concept will significantly enhance our ability to conduct solid-supported glycosylations.

## 4.4. Experimental Section

### *4.4.1. General Remarks*

All reactions were performed under argon with dry, freshly distilled solvents unless otherwise noted. $CH_2Cl_2$, EtCN were distilled from $CaH_2$ directly prior to application. TMSOTf, $BF_3$-$OEt_2$ and $TMSClO_4$ were used as is. Molecular sieves (3Å) used for the reactions were activated at 390 °C and then for 2-3 h at 390 °C under reduced pressure

prior to application. Reactions were monitored by TLC on Kieselgel 60 $F_{254}$ and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at < 40 $^{o}$C. Column chromatography was performed on silica gel 60 (70-230 mesh). Optical rotations were measured at 'Jasco P-1020' polarimeter. $^{1}$H n.m.r. spectra were recorded at 300 MHz, 500 MHz, or 600 MHz. $^{13}$C n.m.r. spectra were recorded at 75 MHz or 150 MHz. $^{19}$F spectra were recorded at 282.2 MHz. The $^{1}$H chemical shifts are referenced to the signal of the residual $CHCl_3$ ($\delta_H$ = 7.27 ppm) for solutions in $CDCl_3$. The $^{13}$C chemical shifts are referenced to the central signal of $CDCl_3$ ($\delta_C$ = 77.23 ppm) for solutions in $CDCl_3$. HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

### *4.4.2. Synthesis of linkers and glycosyl acceptors*

**4-Trityloxybutan-1-ol (4.1).** The title compound was obtained from 1,4-butanediol according to the procedure reported previously[73] in 85% yield as a white amorphous powder. Analytical data for **4.1**: $R_f$ = 0.44 (ethyl acetate/hexanes, 3/7, v/v); $^{1}$H-n.m.r. (300 MHz): δ, 1.67 (m, 4H, -C*H*$_2$), 3.08 (t, 2H, $^{2}J$ = 5.9 Hz, -C*H*$_2$), 4.08 (t, 2H, $^{2}J$ = 5.9 Hz, -C*H*$_2$), 7.20-7.42 (m, 15H, aromatic); $^{13}$C-n.m.r. (75 MHz): δ, 26.8, 30.2, 63.1, 63.7, 86.8, 127.1 (x 3), 128.0 (x 6), 128.8 (x 6), 144.4 (x 3) ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{23}H_{24}O_2Na^+$ 355.1674, found 355.1676.

**4-(4-Trityloxy)butoxy-4-oxobutanoic acid (4.4).** The title compound was obtained from **4.1** and succinic anhydride as described previously[61] in 93 % yield as a colorless

syrup. Analytical data for **4.4**: $R_f$ = 0.42 (methanol/dichloromethane, 1/9, v/v); $^1$H-n.m.r. (300 MHz): δ, 1.71 (m, 4H, $CH_2$), 2.61 (m, 4H, $CH_2$), 3.08 (t, 2H, $^2J$ = 6.2 Hz, $CH_2$), 4.08 (t, 2H, $^2J$ = 6.2 Hz, $CH_2$), 7.19-7.48 (m, 15H, aromatic); $^{13}$C-n.m.r. (75 MHz): δ, 25.8, 26.7, 29.1, 63.2, 65.1, 86.6, 127.1 (x 4), 127.9 (x 7), 128.8 (x 7), 144.5 (x 3), 172.4, 177.3 ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{27}H_{28}O_5Na^+$ 455.1834, found 455.1824.

**8-Trityloxyoctan-1-ol (4.2).** The title compound was obtained from 1,8-octanediol as described previously[74] in 73 % yield as an amorphous powder. Analytical data for **4.2**: $R_f$ = 0.5 (ethyl acetate/hexanes, 2/3 v/v); $^1$H-n.m.r. (300 MHz): δ, 1.32 (m, 6H, 3 x $CH_2$), 1.58 (m, 6H, 3 x $CH_2$), 3.04 (t, 2H, $^2J$ = 6.6 Hz, $CH_2$), 3.63 (t, 2H, $^2J$ = 6.6 Hz, $CH_2$), 7.20-7.49 (m, 15H, aromatic); $^{13}$C-n.m.r. (75 MHz): δ, 26.8, 30.2, 63.1, 63.7, 86.8, 127.0 (x 3), 127.9 (x 6), 128.9 (x 6), 144.7 (x 3) ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{27}H_{32}O_2Na^+$ 411.2300, found 411.2296.

**4-(8-Trityloxy)octyloxy-4-oxobutanoic acid (4.5).** The title compound was obtained from **4.2** and succinic anhydride as described previously[61] in 76% yield as a colorless syrup. Analytical data for **4.5**: $R_f$ = 0.38 (ethyl acetate/hexanes, 2/3 v/v); $^1$H-n.m.r. (300 MHz): δ, 1.20-1.40 (m, 9H, -OH, $CH_2$), 1.61 (m, 4H, $CH_2$), 2.64 (t, 4H, $CH_2$), 3.03 (t, 2H, $^2J$ = 6.6 Hz, $CH_2$), 4.08 (t, 2H, $^2J$ = 6.7 Hz, $CH_2$), 7.19-7.49 (m, 15H, aromatic); $^{13}$C-n.m.r. (75 MHz): δ, 26.0, 26.4, 28.7, 29.1, 29.3, 29.5, 30.2, 63.8, 65.2, 86.5, 127.0, 127.9, 128.1, 144.7, 172.4, 178.0 ppm; HR-FAB MS $[M+Na]^+$ calculated for $C_{31}H_{36}O_5Na^+$ 511.2460, found 511.2459.

**16-Trityloxyhexadecane-1-ol (4.3).** The title compound was obtained from 1,16-hexadecanediol as described previously[74] in 52% yield as a colorless syrup. Analytical data for **4.3**: $R_f$ = 0.33 (ethyl acetate/hexanes, 1/4 v/v); $^1$H- n.m.r. (500 MHz): δ, 1.19-1.34 (m, 28H, 16 x $CH_2$), 1.46-1.58 (m, 4H, 2 x $CH_2$), 2.98 (t, 2H, $^2J$ = 6.6 Hz, $CH_2$), 3.57 (t, 2H, $^2J$ = 6.6 Hz, $CH_2$), 7.16-7.45 (m, 15H, aromatic); $^{13}$C- n.m.r. (75 MHz): δ, 26.0, 26.5, 29.7 (x 2), 29.8 (x 7), 29.9, 30.3, 33.1, 63.3, 64.0, 86.6, 127.0, 127.5, 127.9 (x 4), 128.1 ( x3), 128.2 (x 3), 129.0 (x 4), 144.9, 147.2 ppm.

**4-(16-Trityloxy)hexadecyloxy-4-oxobutanoic acid (4.6).** The title compound was obtained from **4.3** and succinic anhydride as described previously[61] in 72% yield as a colorless syrup. Analytical data for **4.6**: $R_f$ = 0.44 (ethyl acetate/hexanes, 1/1 v/v); $^1$H-n.m.r. (300 MHz): δ, 1.12-1.42 (m, 27H, $CH_2$), 1.52-1.68 (m, 4H, $CH_2$), 2.58-2.70 (m , 4H, $CH_2$), 3.04 (t, 2H, $^2J$ = 6.6 Hz, $CH_2$), 4.09 (t, 2H, $^2J$ = 6.7 Hz, $CH_2$), 7.19-7.48 (m, 15H, aromatic); $^{13}$C- n.m.r. (75 MHz): δ, 28.8, 29.2 (x 2), 29.4, 29.7 (x 2), 29.8 (x 6), 29.9 (x 3), 30.3, 63.9, 65.2, 86.6, 127.0 (x 2), 127.4, 127.8 (x 4), 128.1 (x 2) 128.2 (x 2), 128.9 (x 4), 144.8 (x 2), 147.2, 172.4, 177.3 ppm.

### *4.4.3. Synthesis of glycosyl donors*

***S*-Benzoxazolyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (4.13).** The title compound was obtained in 75% yield as a pale yellow foam as previously described.[75] Analytical data for **4.13** was in accordance with that reported previously.[75]

**2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (4.14).** The title compound was obtained in 83% yield as a white foam as previously described.[76] Analytical data for **4.14** was in accordance with that reported previously.[77]

**Benzoxazolyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (4.15).** The title compound was obtained in 75% yield as a pale yellow foam as previously described.[77] Analytical data for **4.15** was in accordance with that reported previously.[77]

**2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl N-phenyltrifluoroacetimidate (4.16).** The title compound was obtained in 86% yield as a white foam as previously described.[68] Analytical data for **4.16** was in accordance with that reported previously.[78]

**3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (4.17).** The title compound was obtained in 75% yield as a pale yellow foam as previously described.[78] Analytical data for **4.17** was in accordance with that reported previously.[78]

**Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (4.19).** The title compound was obtained as previously described.[79]

### *4.4.4. Manual and HPLC-mediated polymer supported synthesis*

*General glycosylation procedure using HPLC-assisted automated set-up.* A solution of glycosyl donor **4.13-4.17** (39 mM in $CH_2Cl_2$, Pump A, percentage flow: 80%), and a solution of TMSOTf (0.28 mM in $CH_2Cl_2$, Pump B, percentage flow: 20%) were passed

concomitantly through a column containing resin-bound glycosyl acceptor **4.10-4.12** at the combined flow rate of 0.3 mL/min for 20 min. The pump A was then switched to pump C (recirculating chamber, percentage flow: 80%) while pump B remained the same (TMSOTf soln., percentage flow: 20%) and the resulting solutions were passed concomitantly through a column with the combined flow rate of 0.3 mL/min for 10 min. After that, the system was switched to pump C (recirculating chamber, percentage flow 100%) and the solution was recirculated at a flow rate of 0.3 mL/min for 1-4 h. Pump C was stopped and the column was purged with $CH_2Cl_2$ (pump A, 10.0 mL/min), acetone (pump A, 10.0 mL/min), and again with $CH_2Cl_2$ (pump A, 20.0 mL/min).

*Procedure for manual cleavage of the products from the resin.* A 1 M solution of sodium methoxide in methanol (0.3 mL) was added to a suspension or a mixture of resin in methanol (2.0 mL) and the resulting mixture was kept for 24 h at rt. After that, the reaction mixture was neutralized with Dowex H+ resin. The resin was filtered off and rinsed successively with MeOH (10 × 5 mL). The combined filtrate (~50 mL) was separated, dried with $MgSO_4$, concentrated in vacuo, and dried. The crude product obtained from this step was then acetylated according to the procedure described below.

*Acetylation of the crude product.* To a stirred solution of crude monosaccharide (0.0482 mmol) in pyridine (2 mL) $Ac_2O$ (73 mL, 0.771 mmol) was added dropwise in the presence of catalytic DMAP. The reaction mixture was stirred under argon for 6 h at room temperature. The reaction mixture was quenched with $CH_3OH$ (1 mL) and the resulting mixture was concentrated under reduced pressure. The residue was purified by

column chromatography on silica gel (ethyl acetate – toluene gradient elution) to afford corresponding monosaccharide **4.18** in 67-90% yield.

*Procedure for the manual regenerative glycosylation on solid phase.* A mixture of resin-bound acceptor **4.10**-**4.12** (0.028 mmol) and ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside **4.19**[79] (0.28 mmol) in dry dichloromethane (2 mL) was agitated for 2 h at room temperature. After that, bromine (0.01 mmol) was added, and the resulting mixture was kept for 30 min at rt. After that, 3,3-difluorooxindole (0.056-0.14 mmol), and $Ag_2O$/AgOTf (1.4/1.12 mmol) were added to the reaction mixture and the resulting mixture was stirred for 1 h at rt. After 1h, TMSOTf (0.56 mmol) was added and the mixture was agitated for 24-36 h at room temperature under argon. The resin was separated by sintered filter, washed successively with dichloromethane (5 x 5 mL), acetone (5 x 5 mL) and again dichloromethane (5 x 5 mL) followed by drying in vacuo. The residual resin was suspended in dry dichloromethane (1 mL) before adding 1 N sodium methoxide solution in methanol (2 mL). The reaction mixture was agitated at room temperature for 5 h; the pH was monitored and adjusted within pH~8-9 range with 1 N sodium methoxide solution, if needed. The resin was separated by sintered filter, washed successively with methanol (3 x 5 mL) and dichloromethane (3 x 5 mL), and dried in vacuo. The combined filtrate was neutralized with Dowex ($H^+$), the resin was filtered off, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate – hexanes gradient elution) to afford corresponding monosaccharide.

*Procedure for HPLC-mediated regenerative glycosylation.* A mixture of ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside **4.19**[79] (0.028-0.28 mmol) and activated molecular sieves beads (3 Å, 90 mg) in $CH_2Cl_2$ (0.5 mL) was stirred under argon for 1 h at rt. The mixture was cooled to 0 $^oC$, bromine (0.01 mmol) was added, and the resulting mixture was kept for 15 min at 0 $^oC$. After that, 3,3-difluorooxindole (0.0056-0.mmol, and $Ag_2O$ (36 mg, 0.16 mmol) were added to the reaction mixture and the resulting mixture connected to HPLC (Pump A, percentage flow: 100%), and recirculated through the column containing the resin bound acceptor **4.10-4.12** for 1h. Following that, TMSOTf (4.5 μL) was added to the chamber containing the bromide, HOFox and $Ag_2O$ and passed concomitantly through the column at a flow rate of 0.5 mL/min for 5 h. Pump A was stopped and the column was purged with $CH_2Cl_2$ (pump A, 10.0 mL/min), acetone (pump A, 10.0 mL/min), and again with $CH_2Cl_2$ (pump A, 20.0 mL/min).

*Procedure for manual cleavage of the products from the resin.* A 1 M solution of sodium methoxide in methanol (0.3 mL) was added to a suspension or a mixture of resin in methanol (1.0 mL) and the resulting mixture was kept for 24 h at rt. After that, the reaction mixture was neutralized with Dowex H+ resin. The resin was filtered off and rinsed successively with MeOH (10 × 5 mL). The combined filtrate (~50 mL) was separated, dried with $MgSO_4$, concentrated in vacuo, and dried. The crude product obtained from this step was then acetylated according to the procedure described below.

**4-Acetoxybutyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (4.18).** The title compound was obtained in yields shown in Tables as a white solid. Analytical data for **4.18** was in accordance with that reported previously.[80]

## 4.5. References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# Appendix

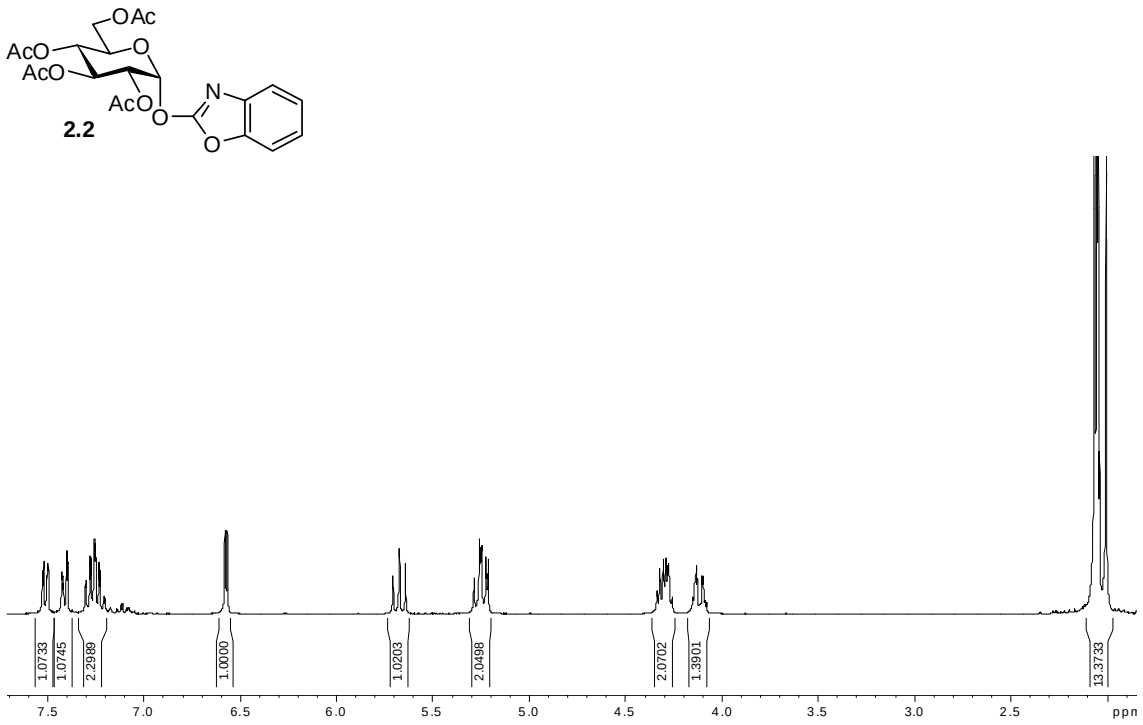



**Figure A-1: $^{1}$H NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (2.2)**

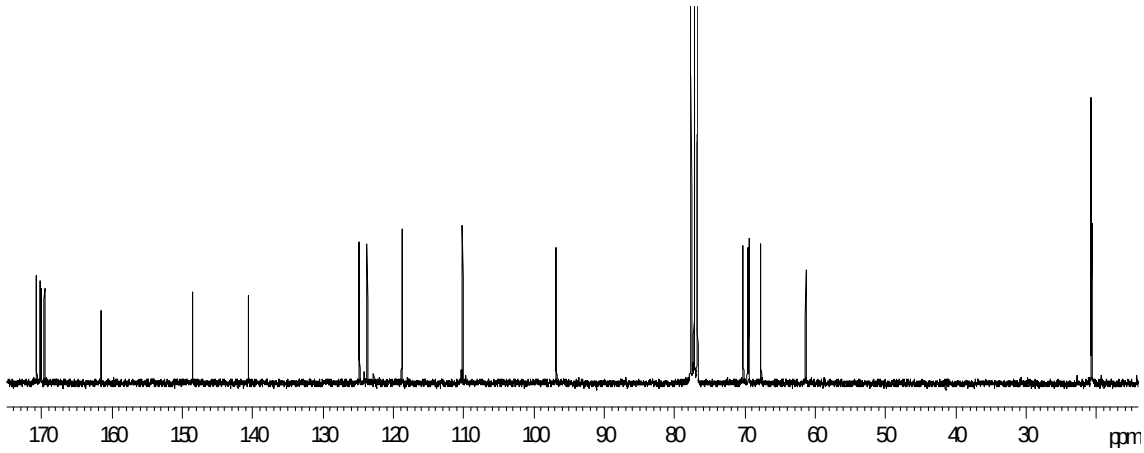



**Figure A-2: $^{13}$C NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (2.2)**

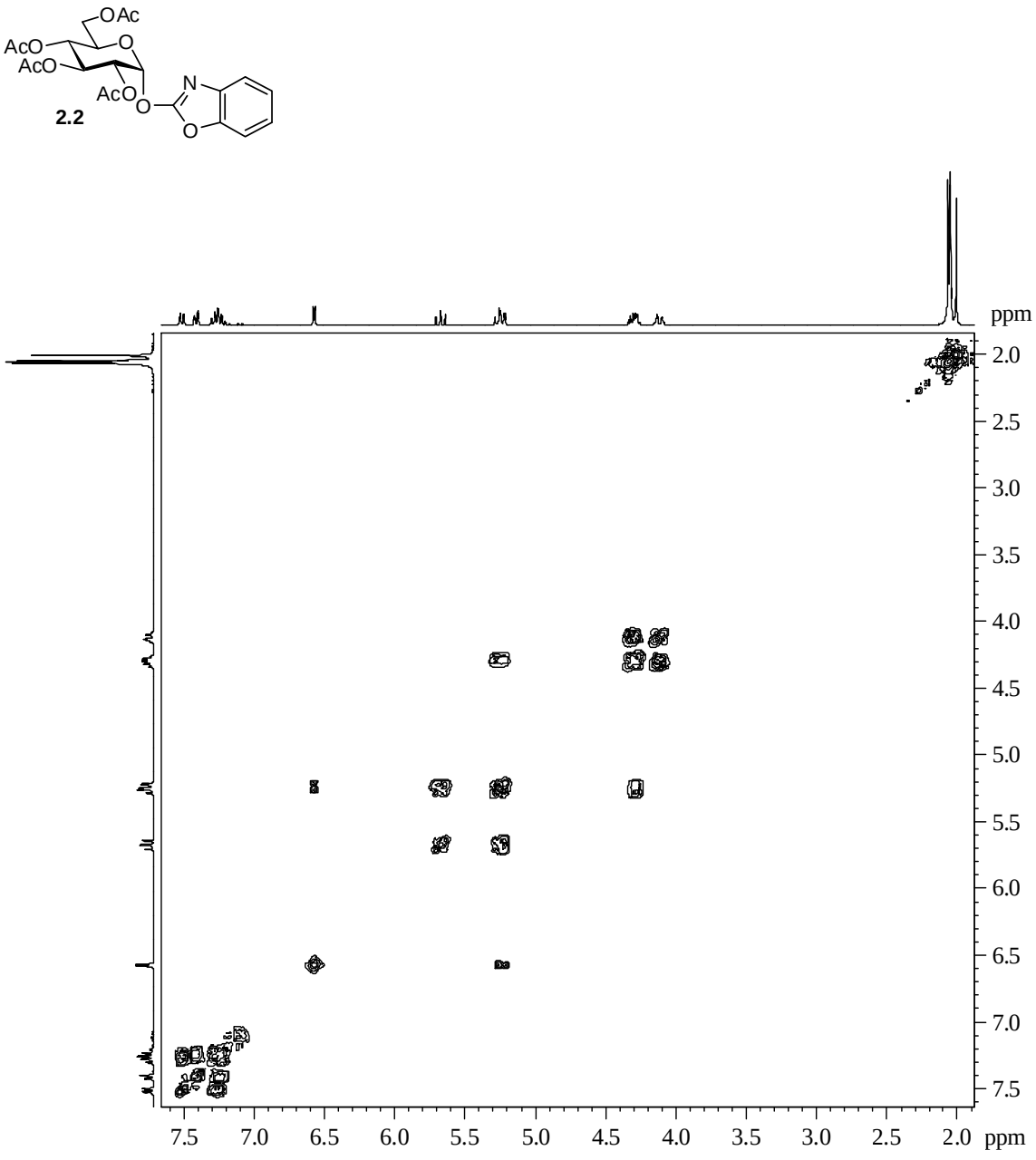



**Figure A-3: 2D NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (2.2)**

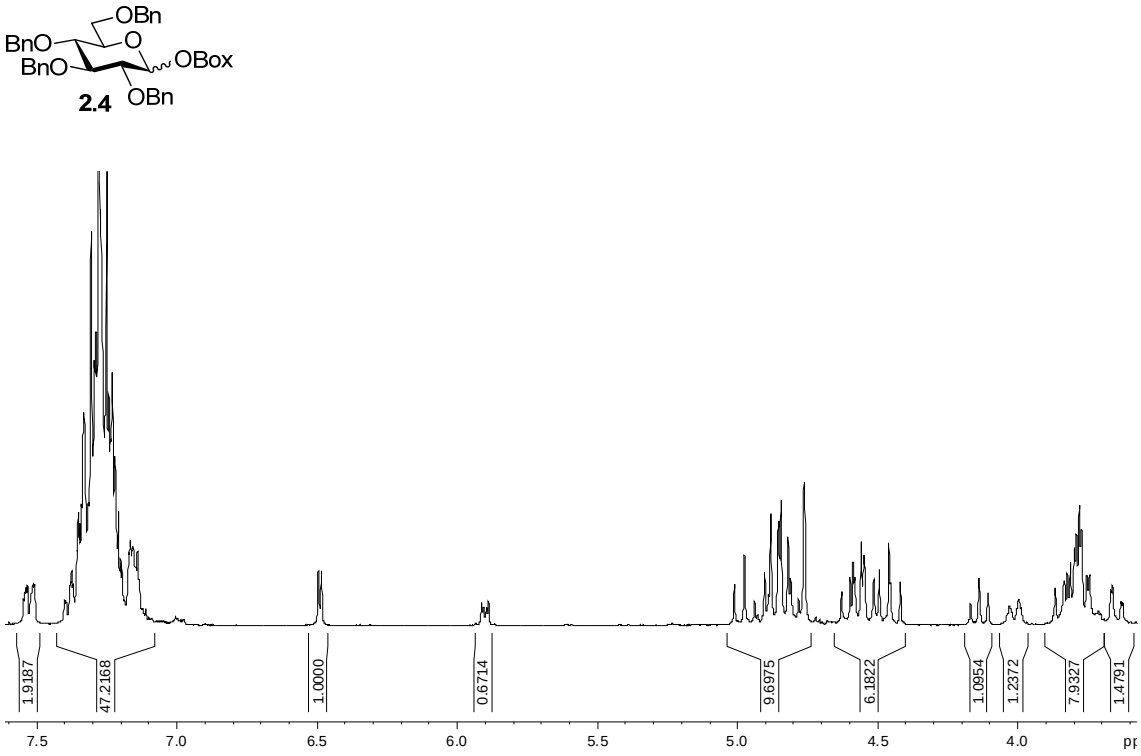



**Figure A-4: $^{1}$H NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranoside (2.4)**

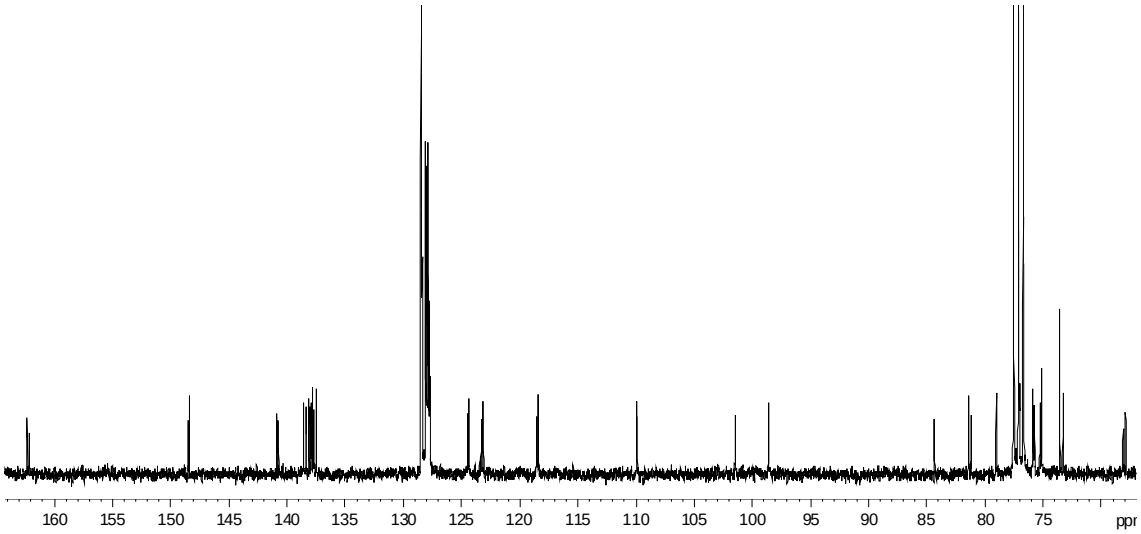



**Figure A-5: $^{13}$C NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranoside (2.4)**

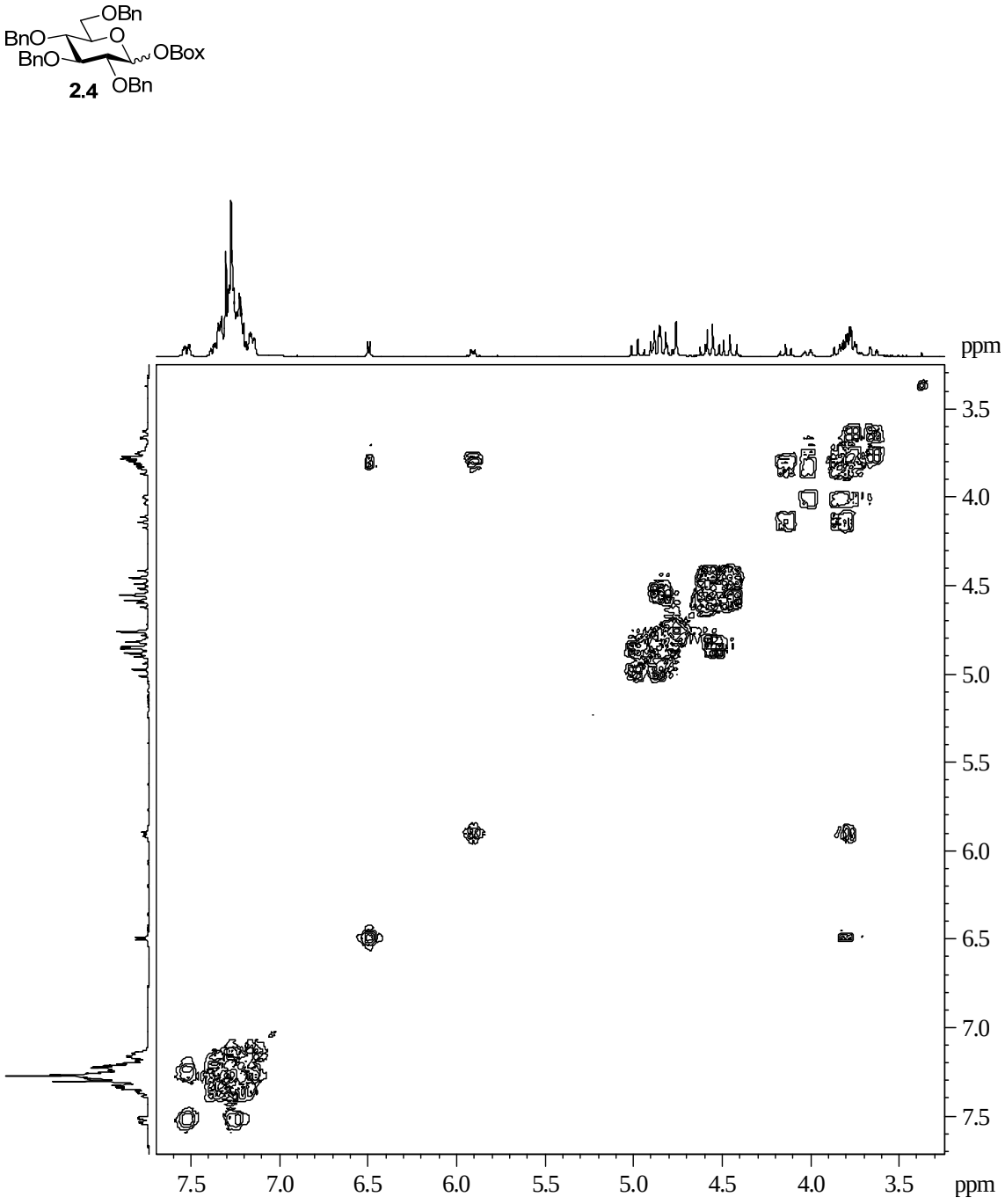



**Figure A-6: 2D NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranoside (2.4)**

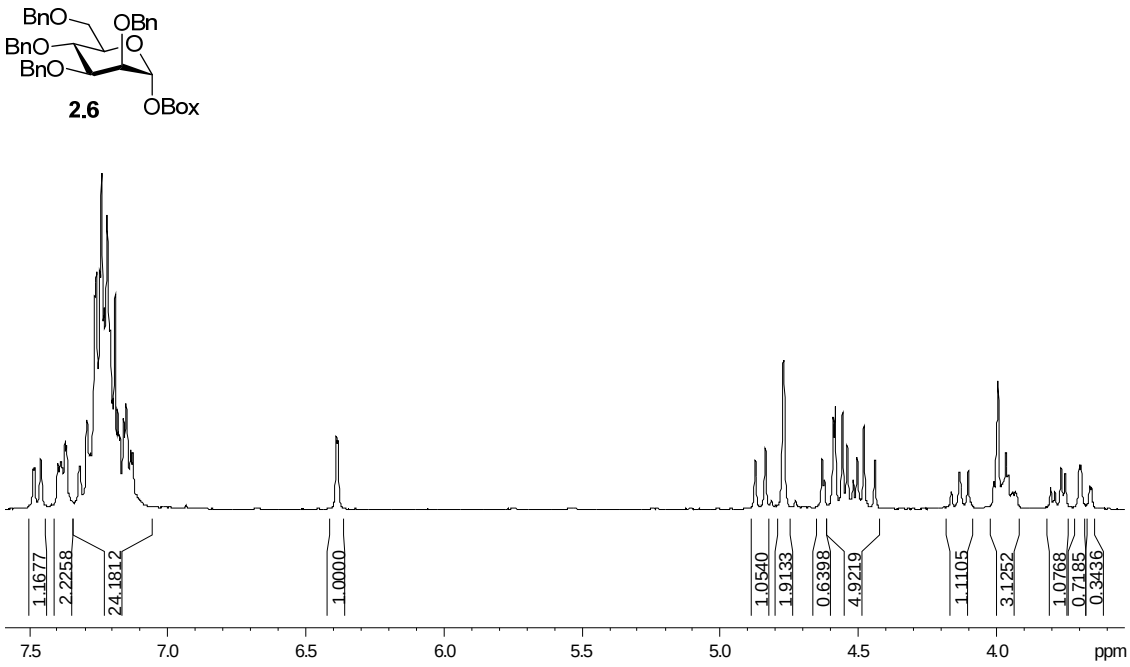



**Figure A-7: $^{1}$H NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranoside (2.6)**

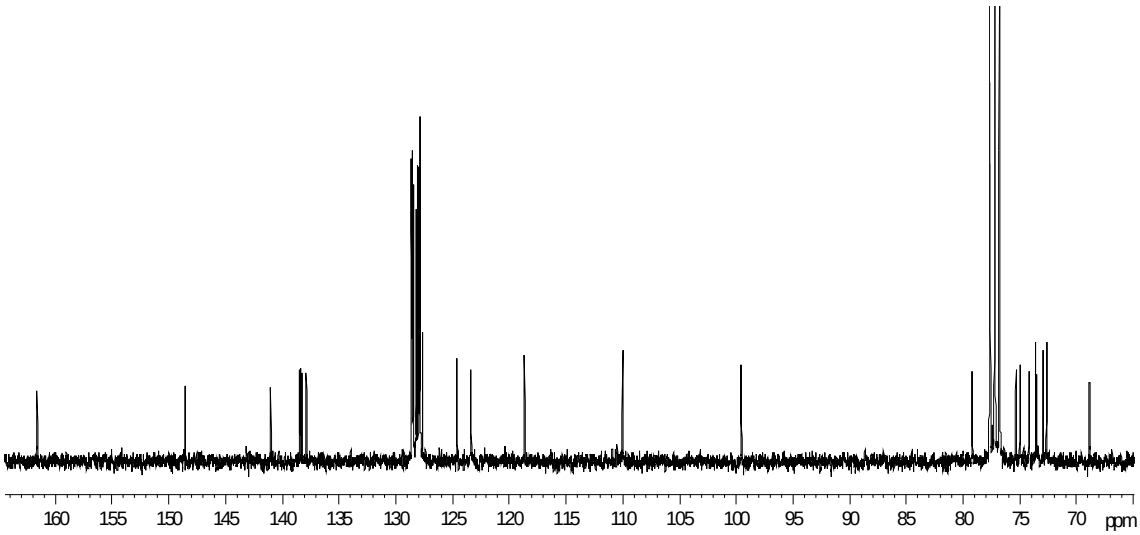



**Figure A-8: $^{13}$C NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranoside (2.6)**

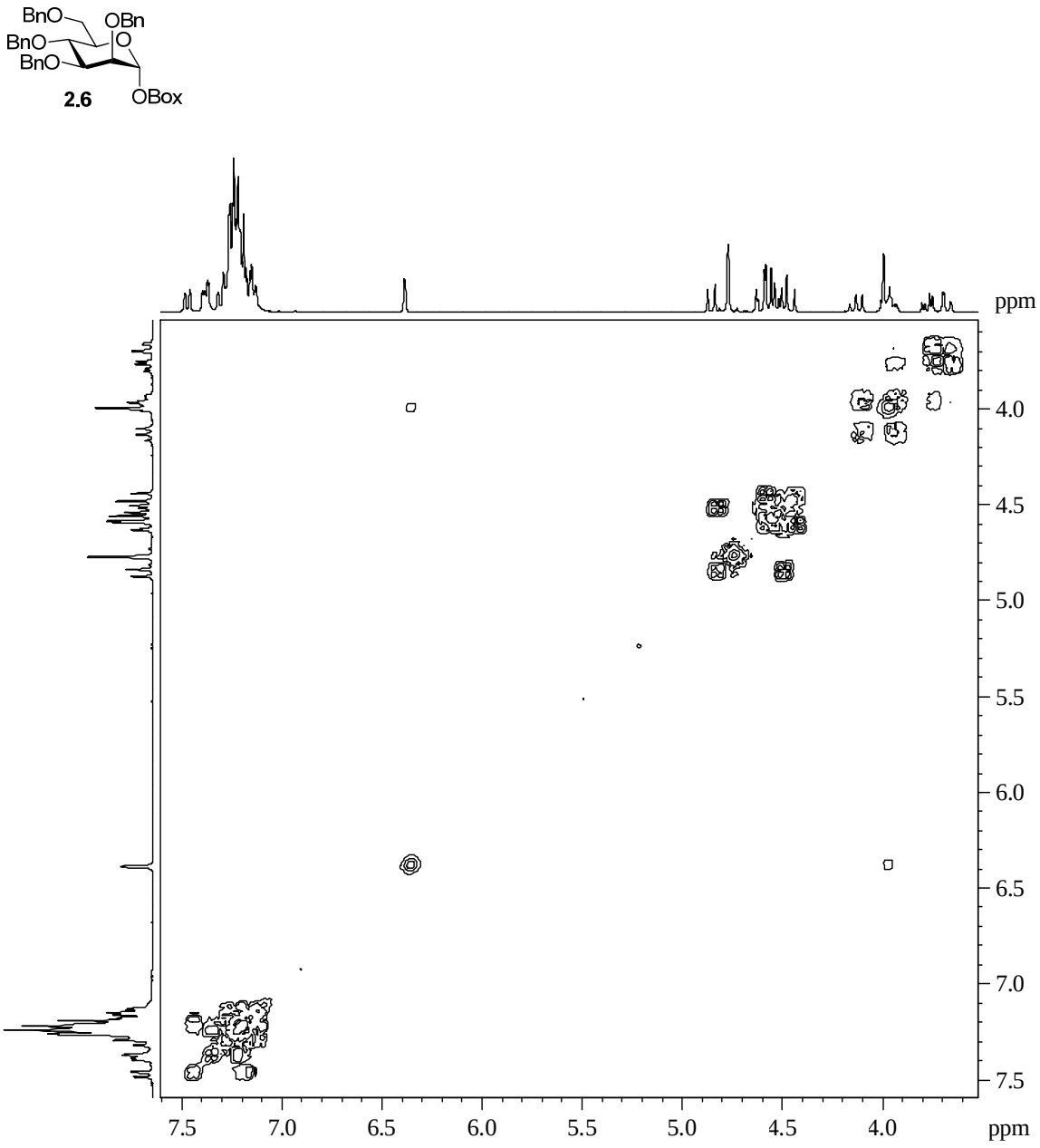



**Figure A-9: 2D NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzyl-α-D-mannopyranoside (2.6)**

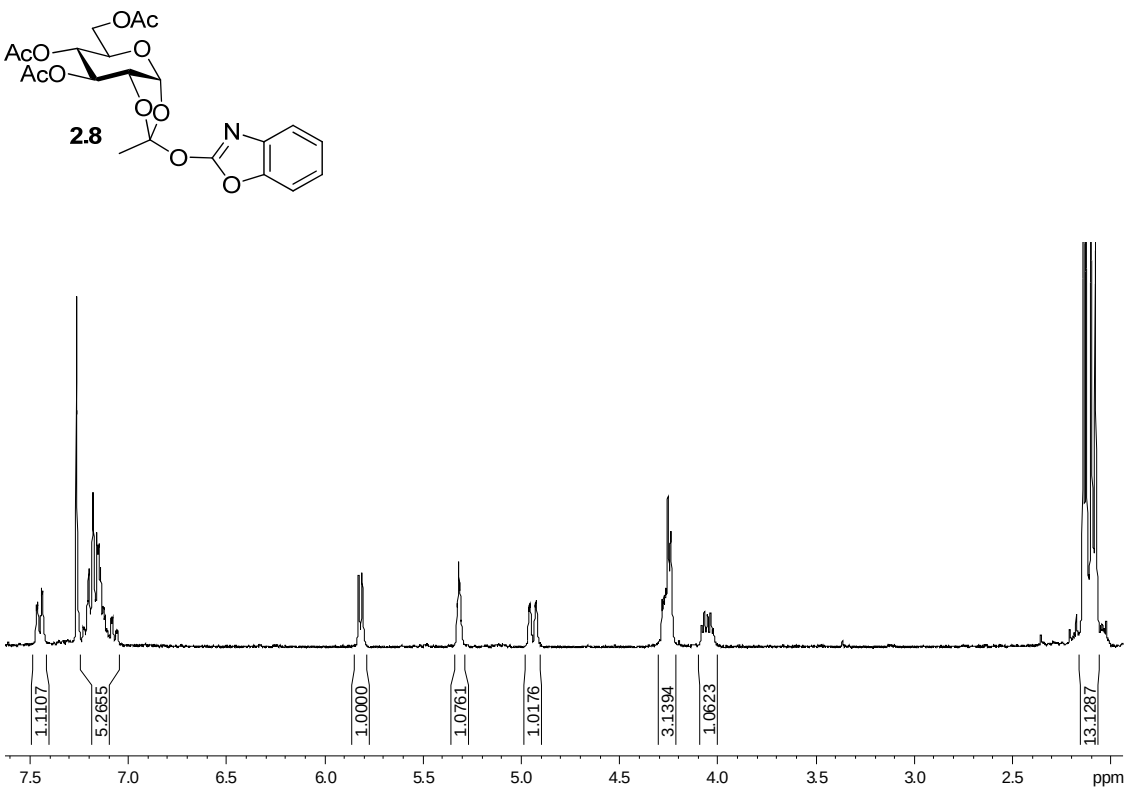



**Figure A-10: $^{1}$H NMR spectrum of Benzoxazolyl 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-benzoxazolyloxyethylidene)-α-D-glucopyranose (2.8)**

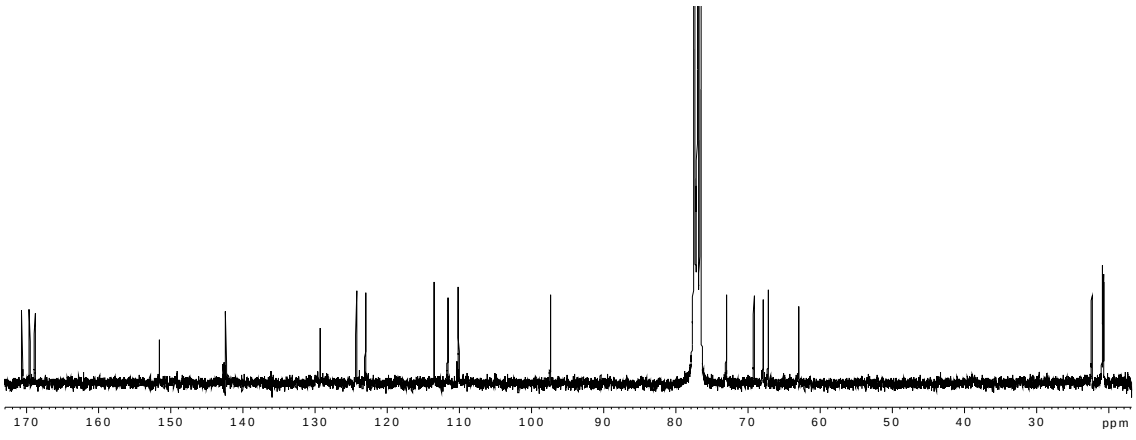



**Figure A-11: $^{13}$C NMR spectrum of Benzoxazolyl 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-benzoxazolyloxyethylidene)-α-D-glucopyranose (2.8)**

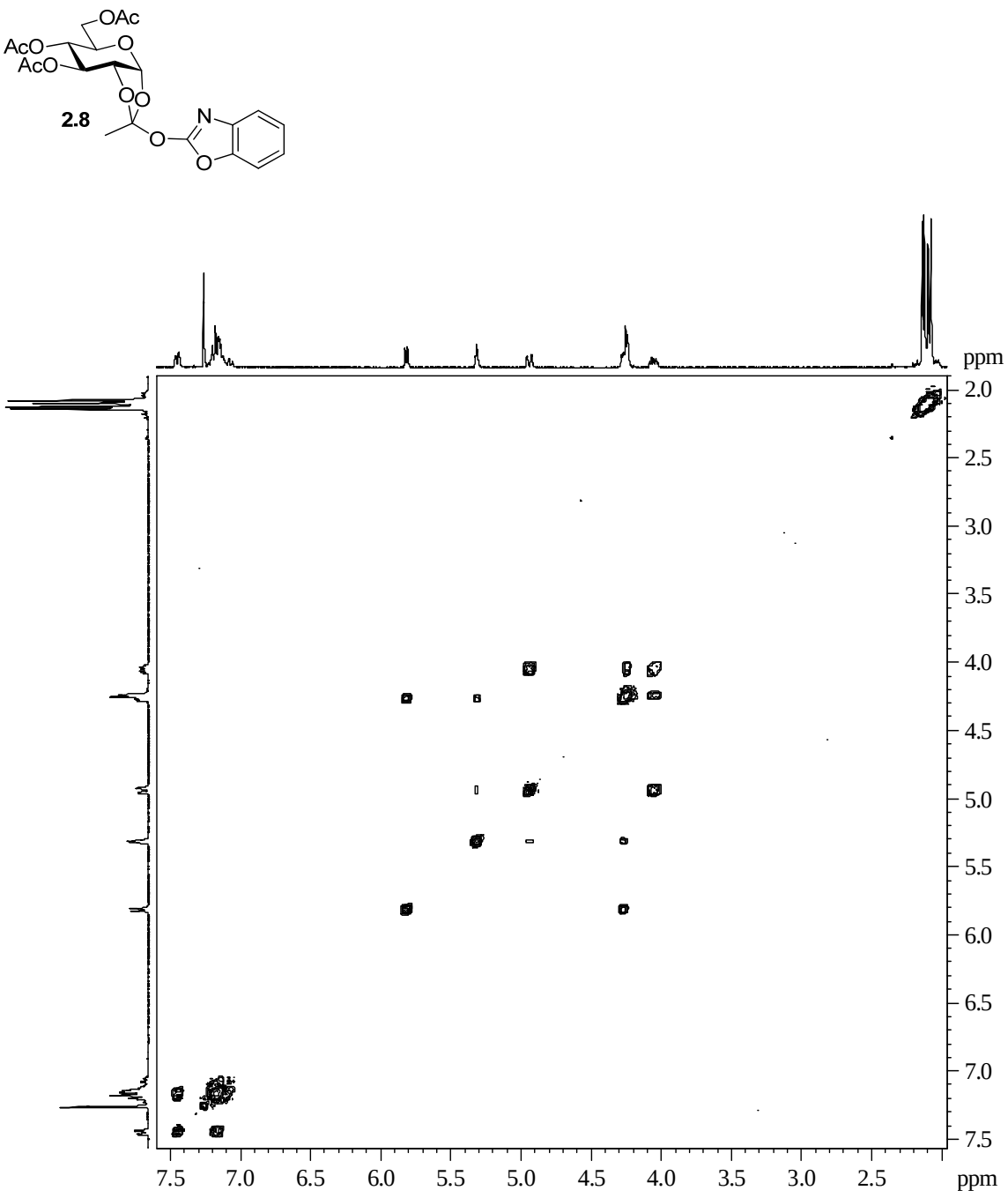



**Figure A-12: 2D NMR spectrum of Benzoxazolyl 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-benzoxazolyloxyethylidene)-α-D-glucopyranose (2.8)**

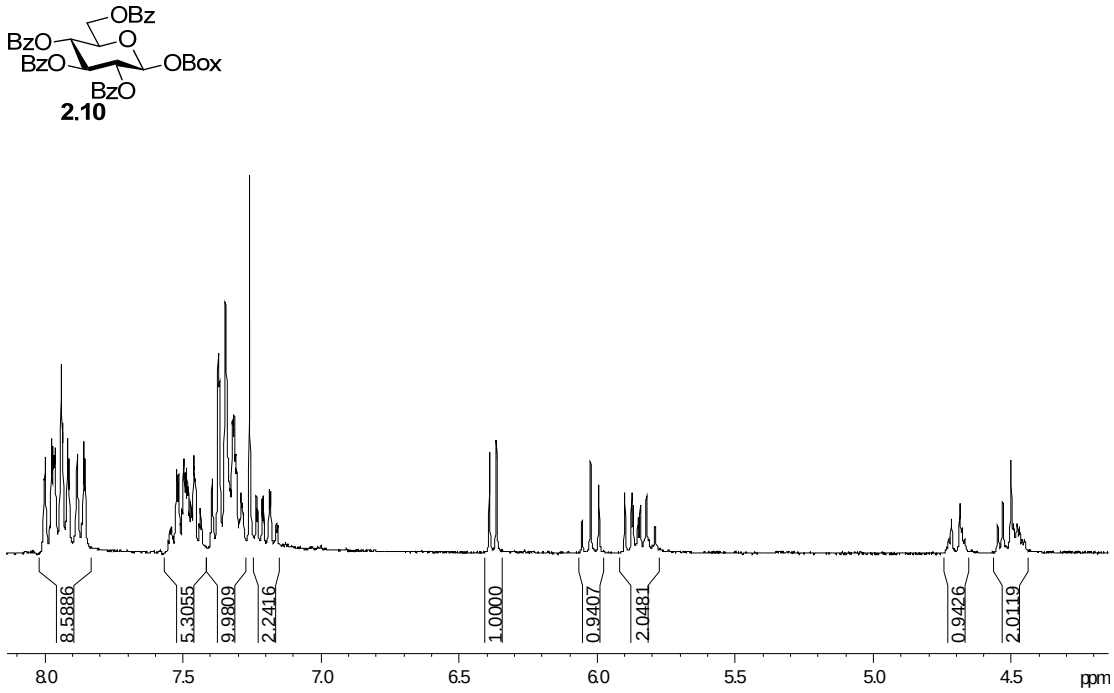



**Figure A-13: $^{1}$H NMR spectrum of Benzoxazolyl Benzoxazolyl 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranoside (2.10)**

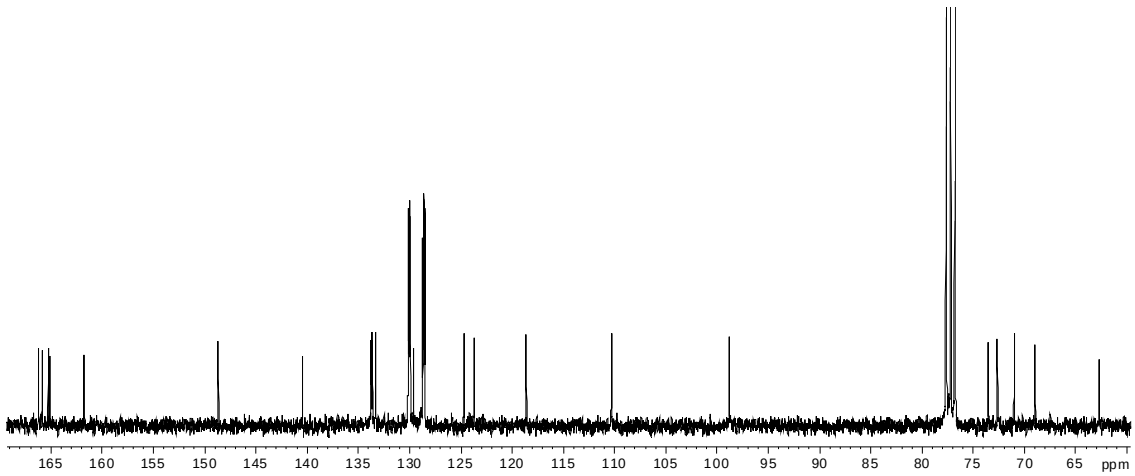



**Figure A-14: $^{13}$C NMR spectrum of Benzoxazolyl Benzoxazolyl 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranoside (2.10)**

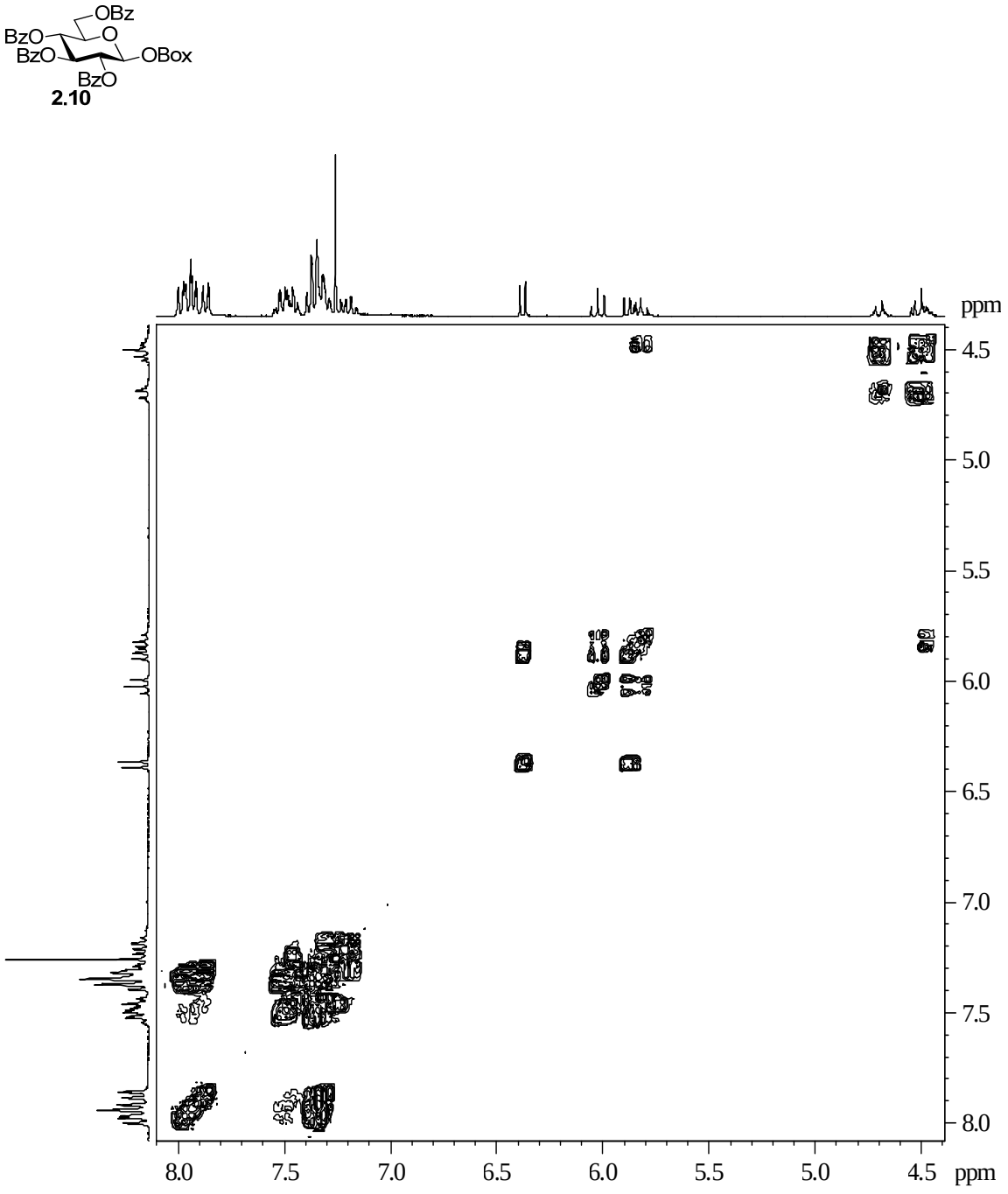



**Figure A-15: 2D NMR spectrum of Benzoxazolyl Benzoxazolyl 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranoside (2.10)**

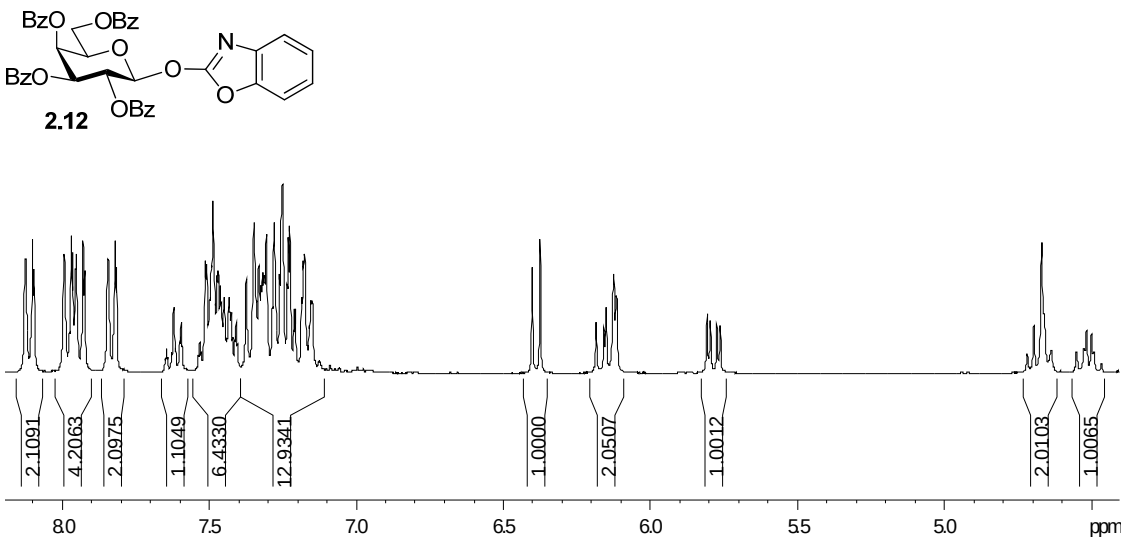



**Figure A-16: $^{1}$H NMR spectrum of Benzoxazolyl-2,3,4,6-tetra-O-benzoyl-α-D-galactopyranoside (2.12)**

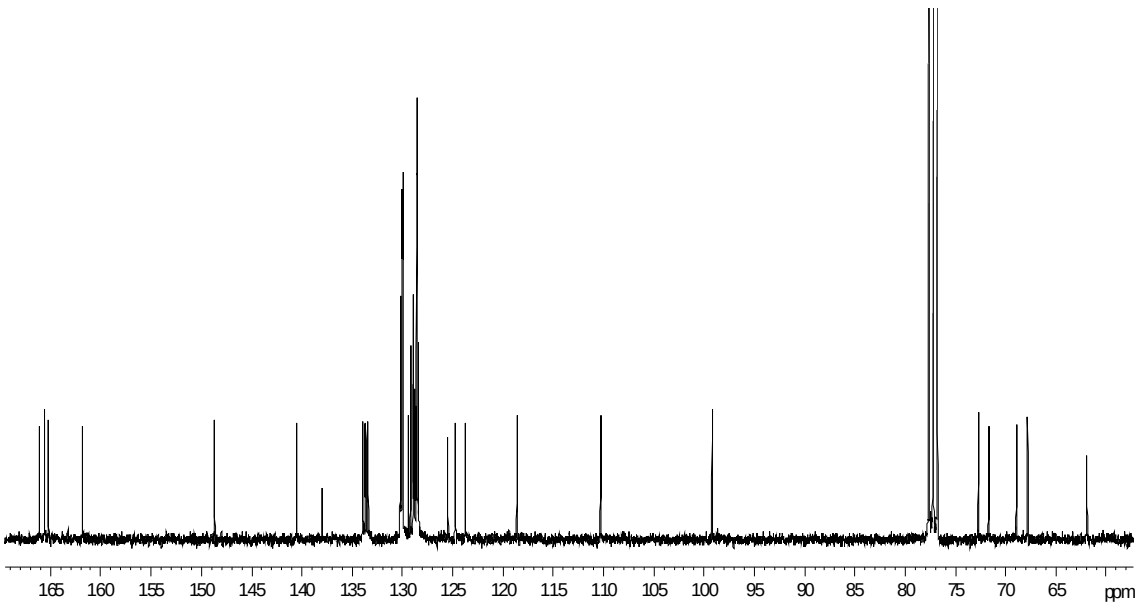



**Figure A-17: $^{13}$C NMR spectrum of Benzoxazolyl-2,3,4,6-tetra-O-benzoyl-α-D-galactopyranoside (2.12)**

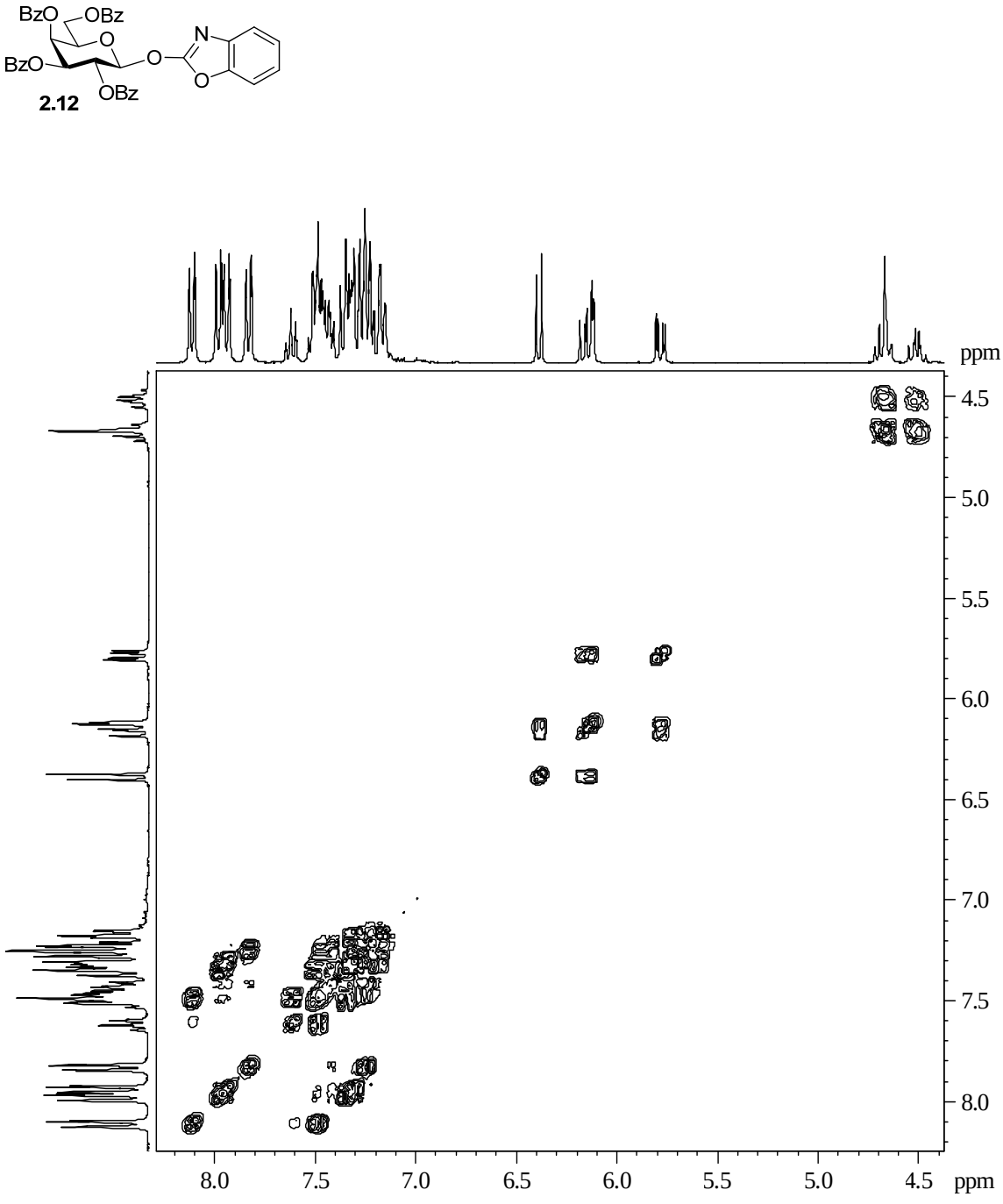



**Figure A-18: 2D NMR spectrum of Benzoxazolyl-2,3,4,6-tetra-O-benzoyl-α-D-galactopyranoside (2.12)**

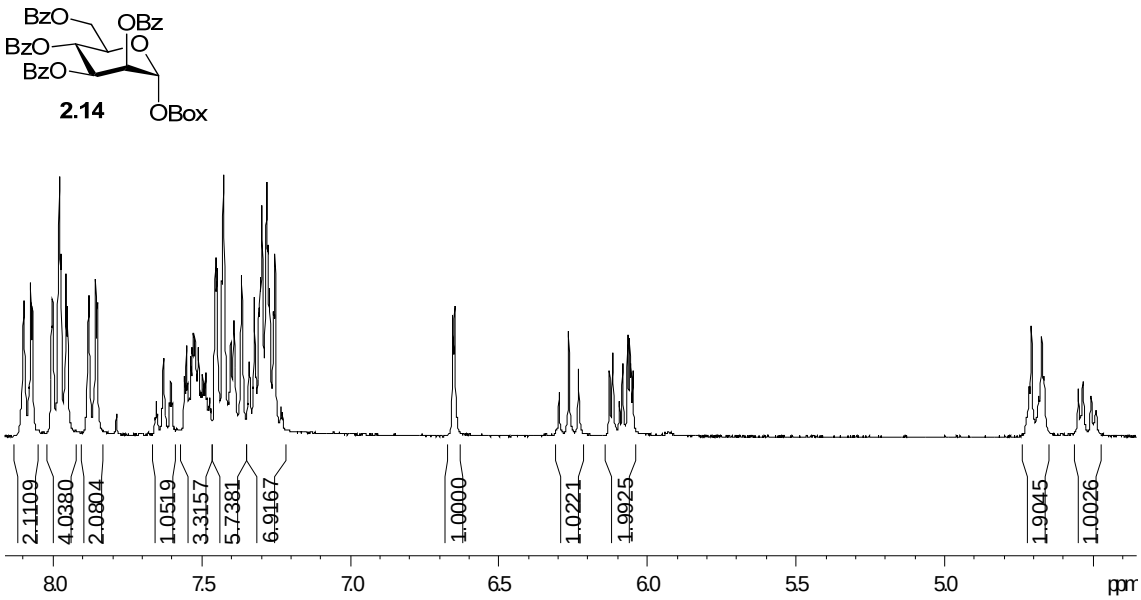



**Figure A-19: $^{1}$H NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranoside (2.14)**

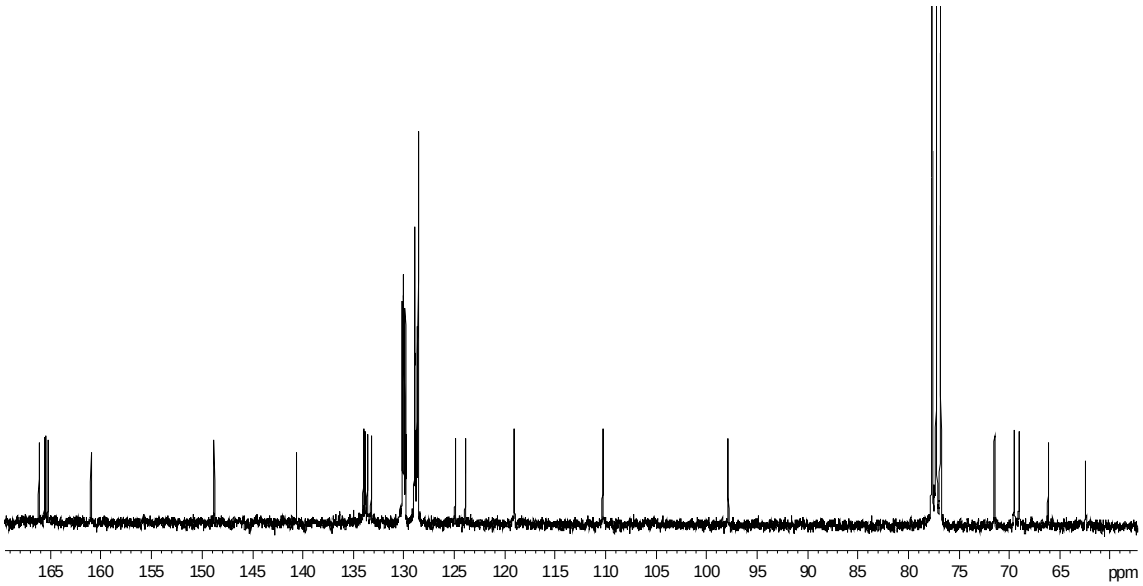



**Figure A-20: $^{13}$C NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranoside (2.14)**

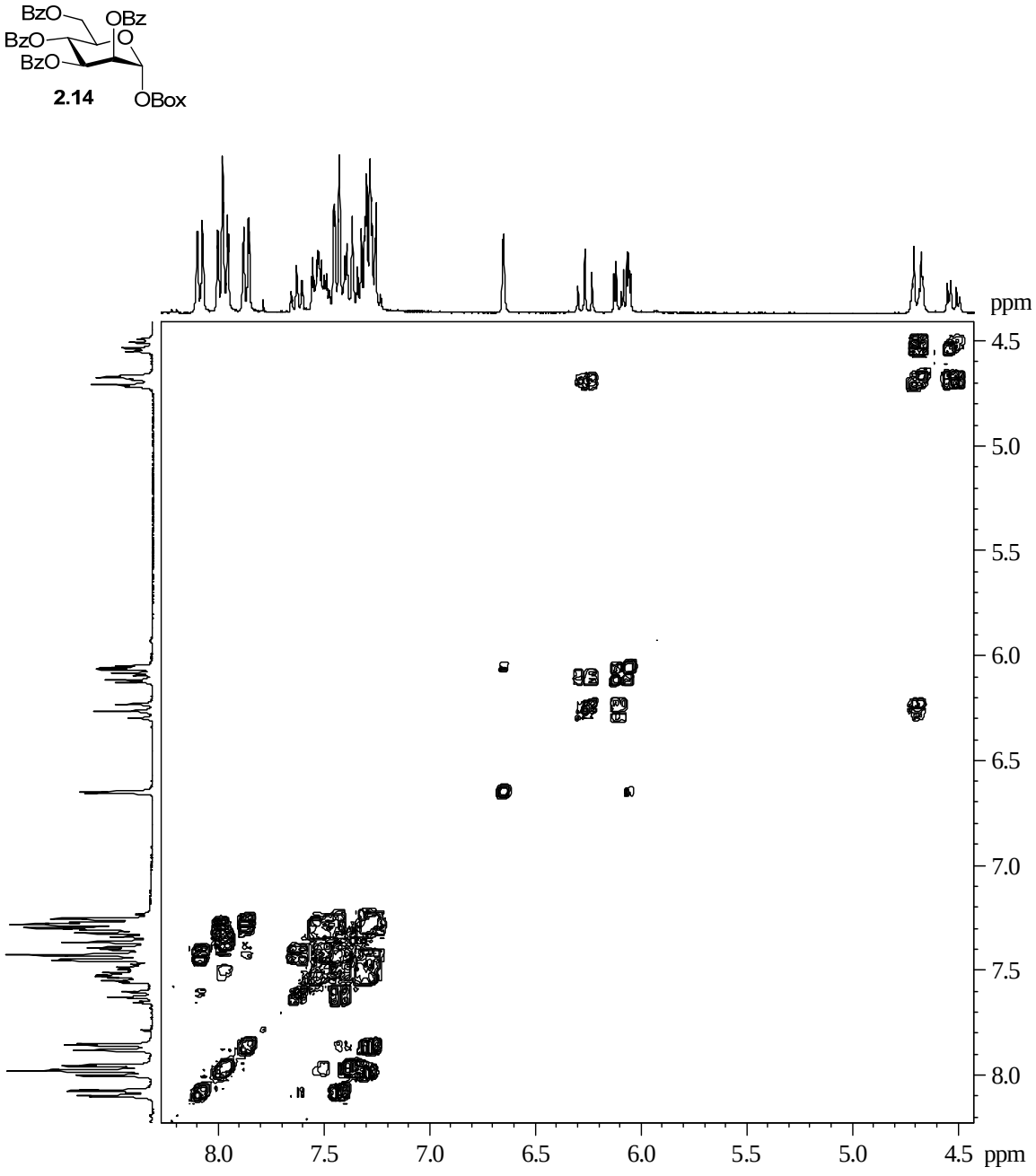



**Figure A-21: 2D NMR spectrum of Benzoxazolyl 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranoside (2.14)**

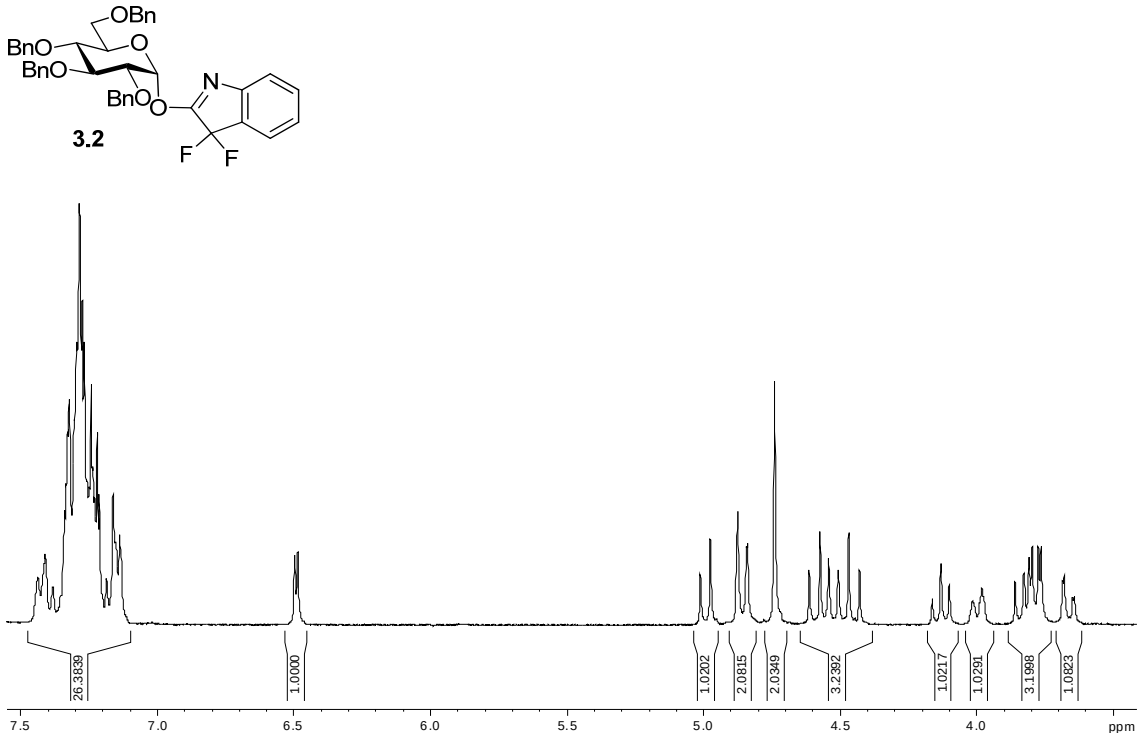



**Figure A-22: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (3.2)**

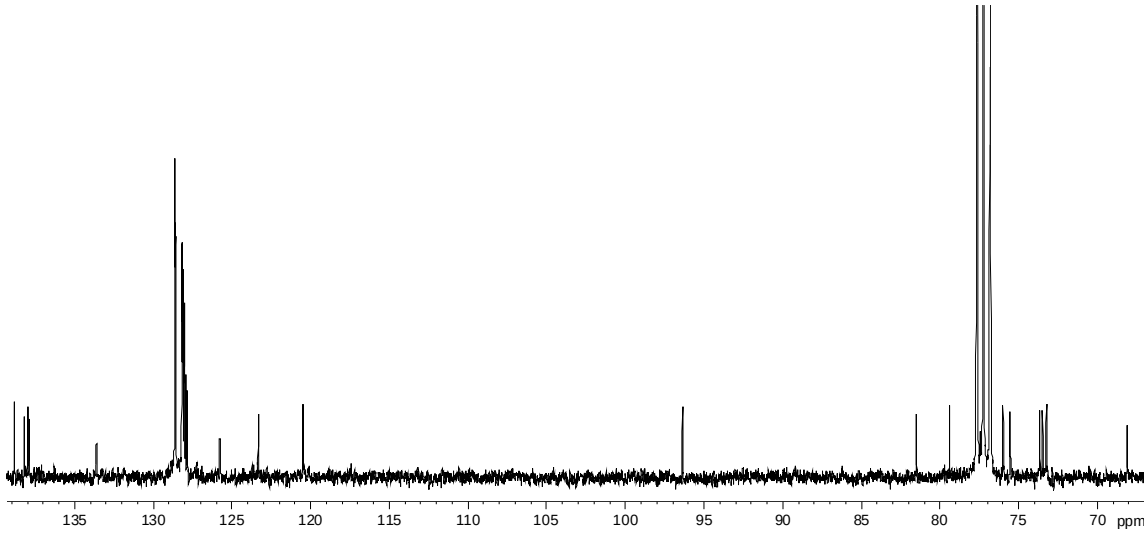



**Figure A-23: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (3.2)**

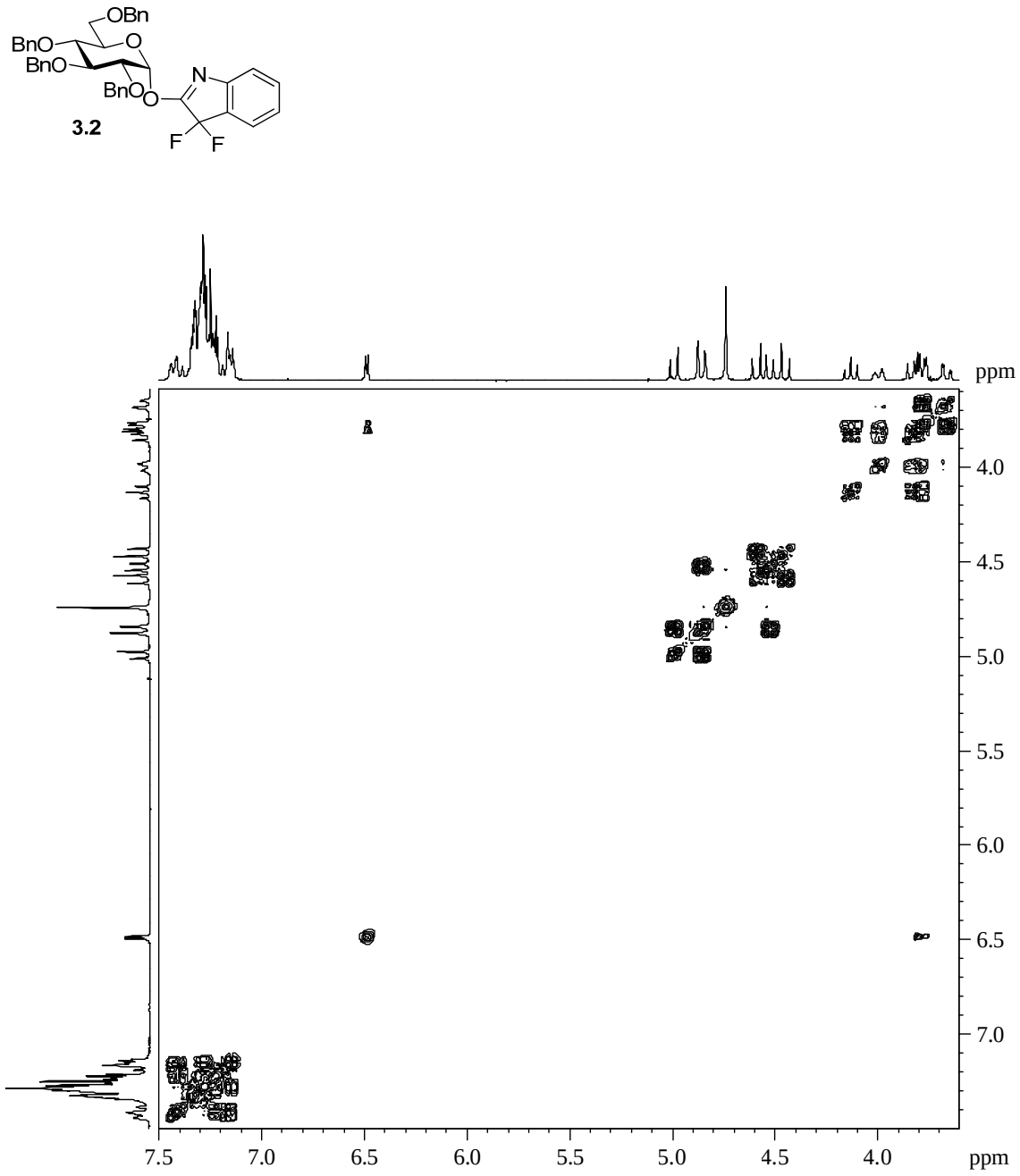



**Figure A-24: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (3.2)**

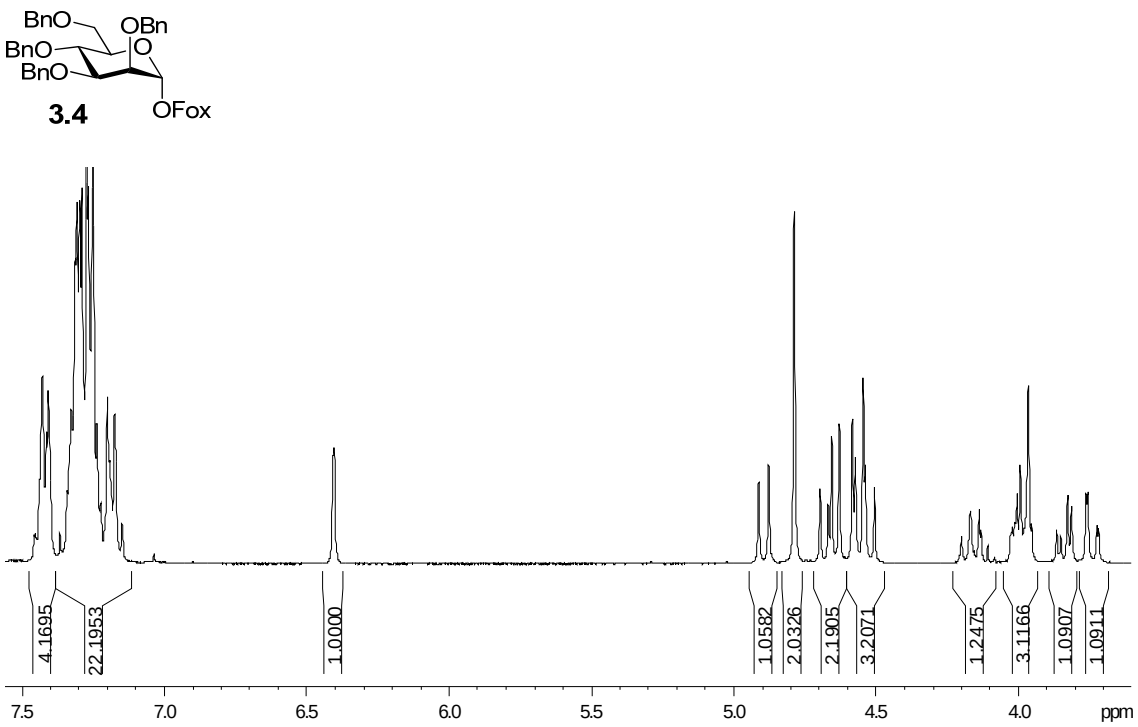



**Figure A-25: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (3.4)**

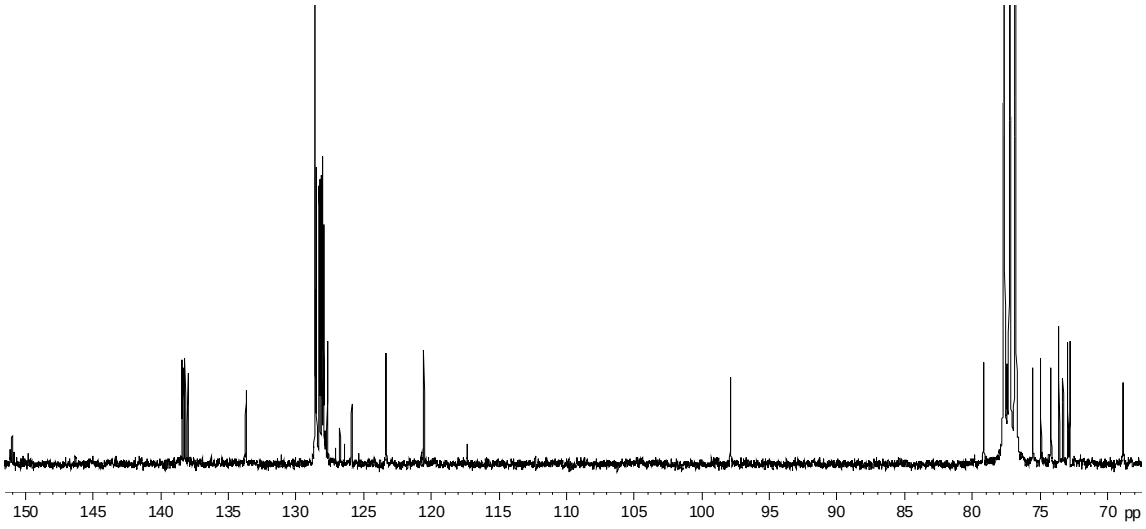



**Figure A-26: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (3.4)**

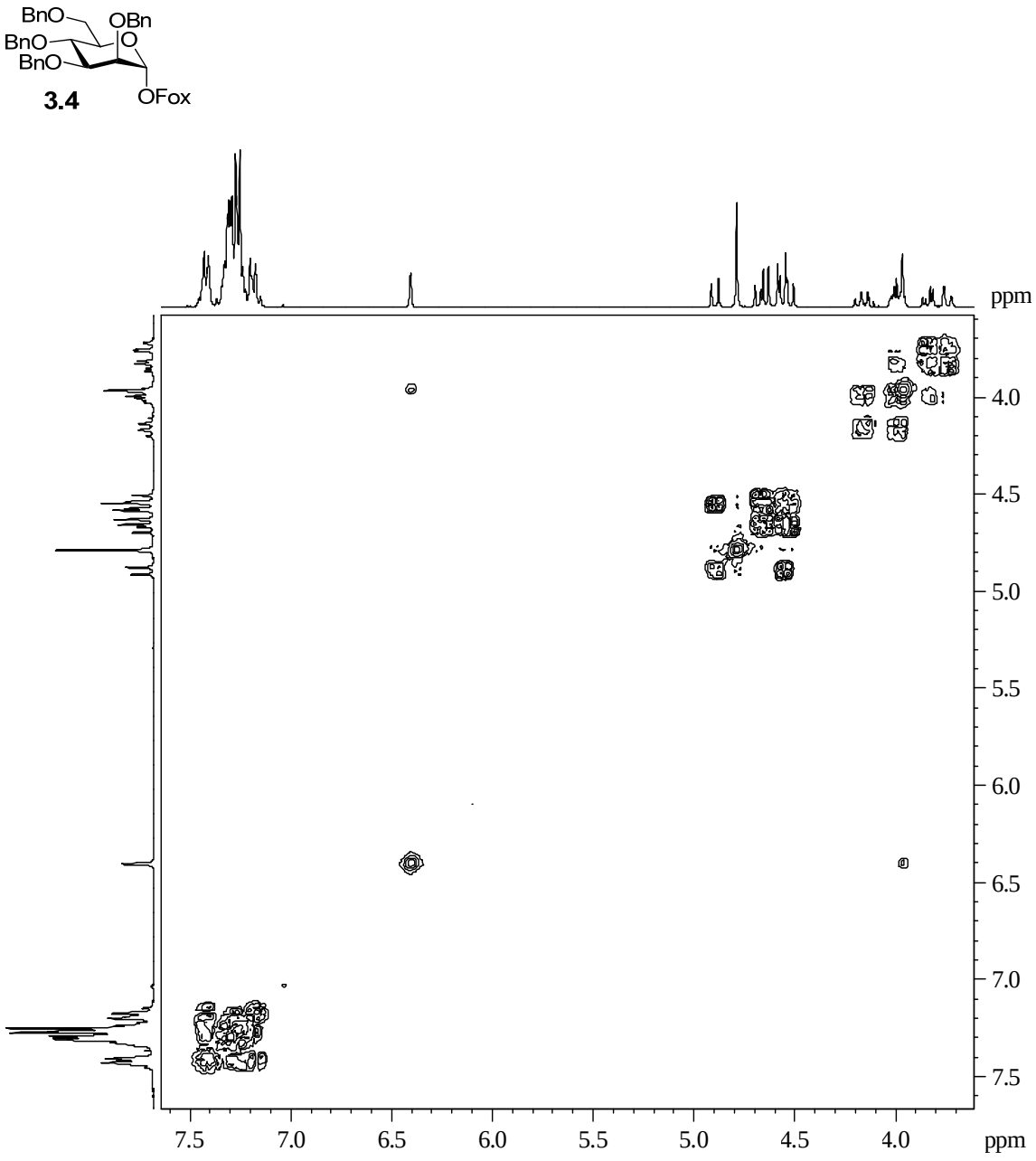
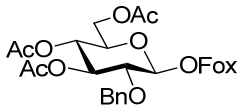



**Figure A-27: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (3.4)**




β–**3.6**

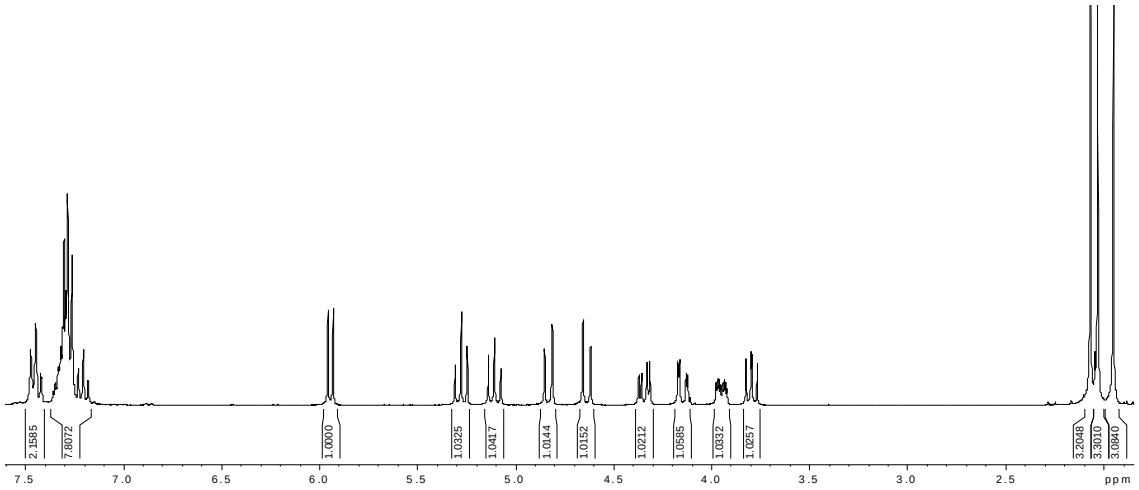



**Figure A-28: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-β-D-glucopyranoside (β-3.6)**

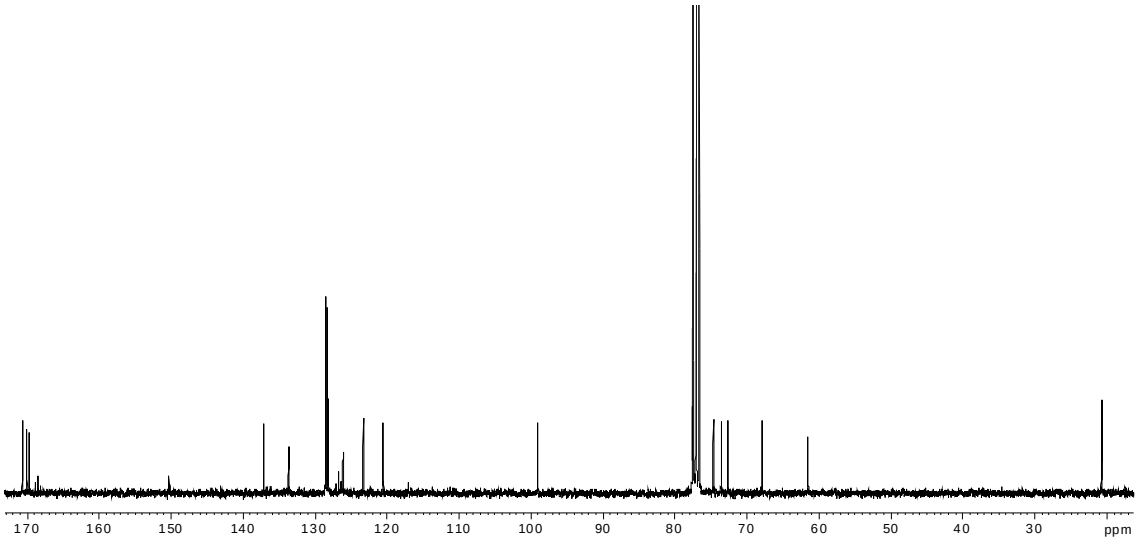



**Figure A-29: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-β-D-glucopyranoside (β-3.6)**

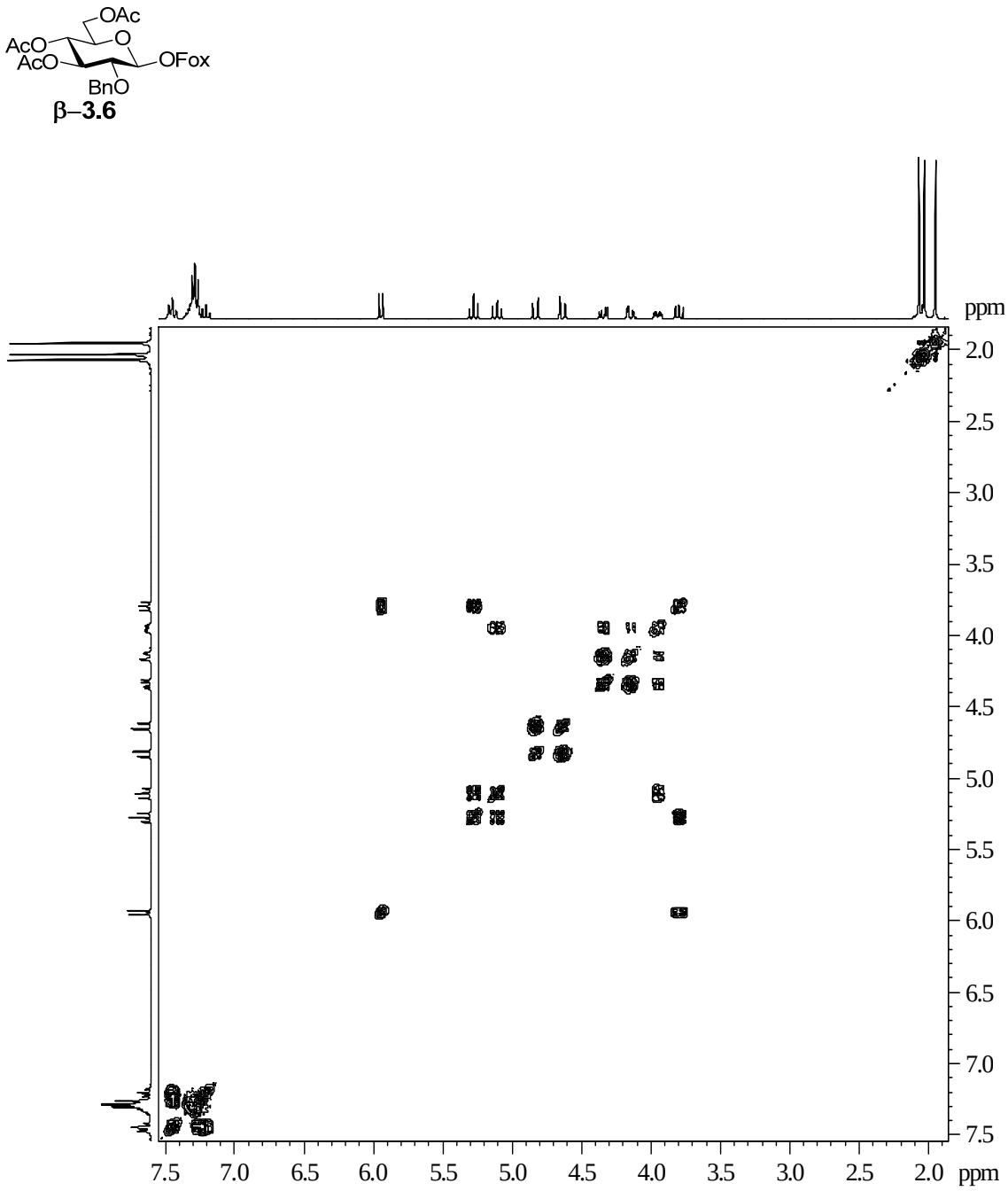



**Figure A-30: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-β-D-glucopyranoside (β-3.6)**

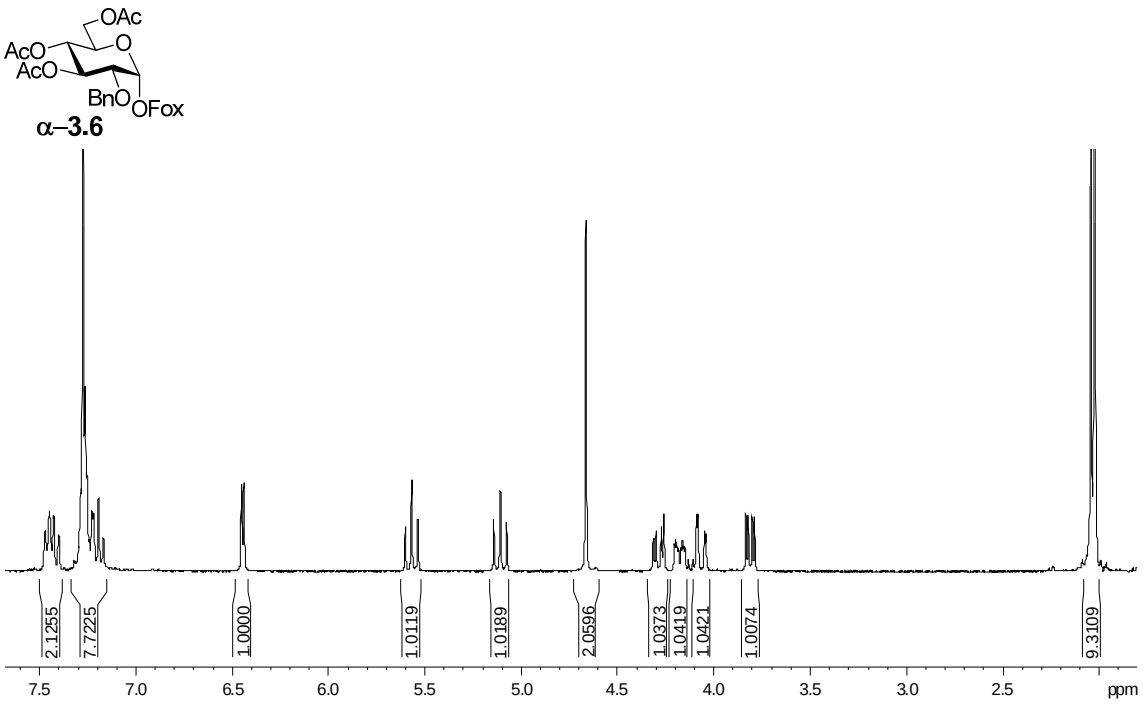



**Figure A-31: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranoside (α-3.6)**

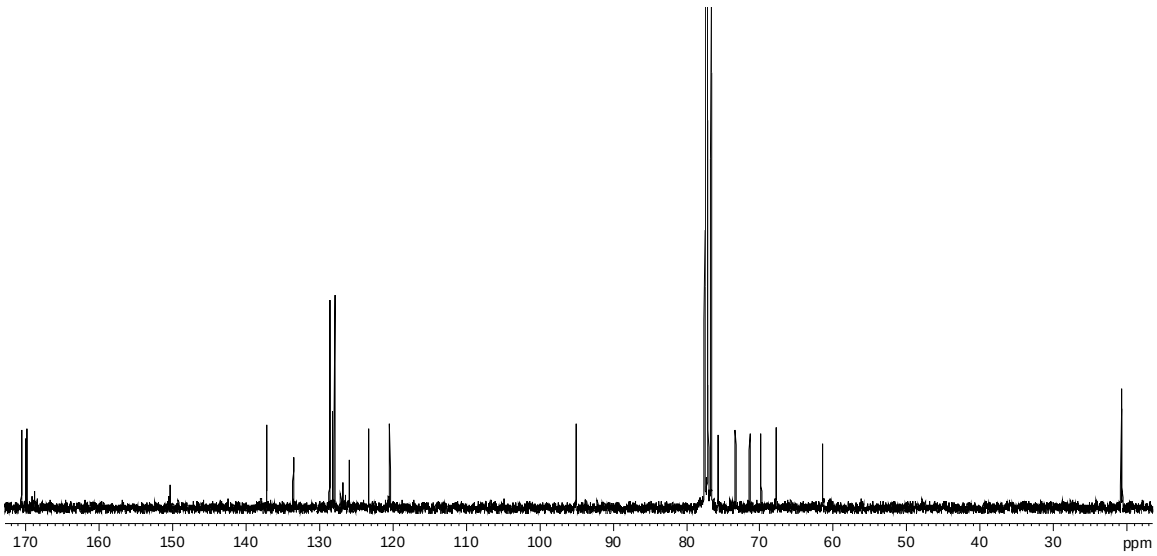



**Figure A-32: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranoside (α-3.6)**

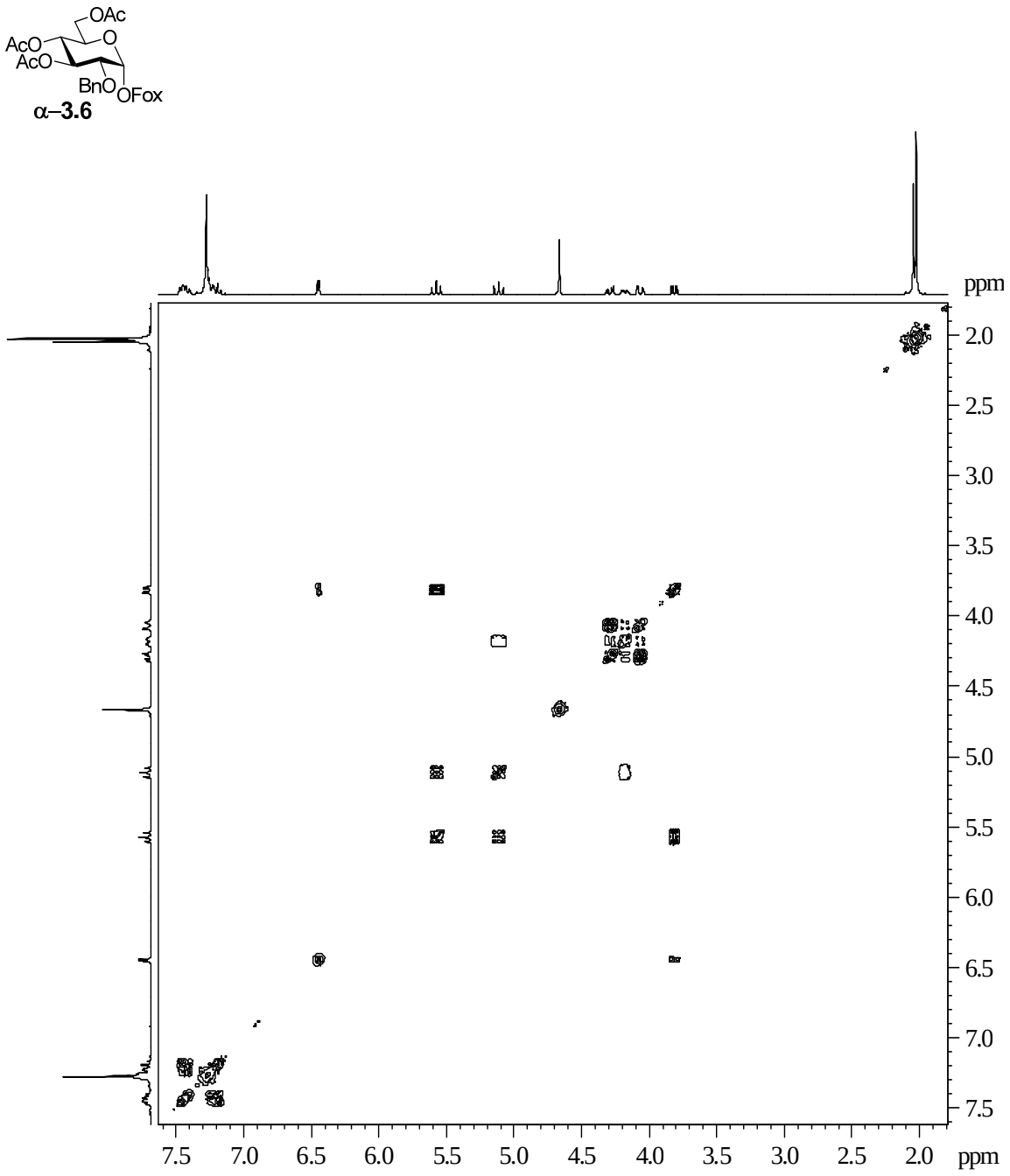



**Figure A-33: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranoside (α-3.6)**

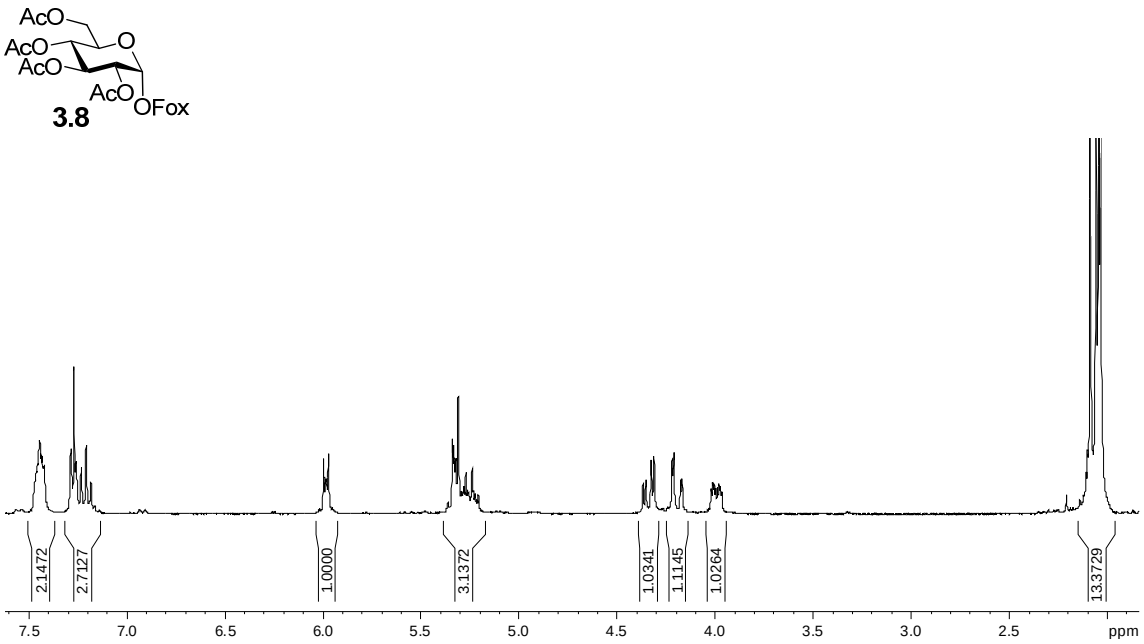



**Figure A-34: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (3.8)**

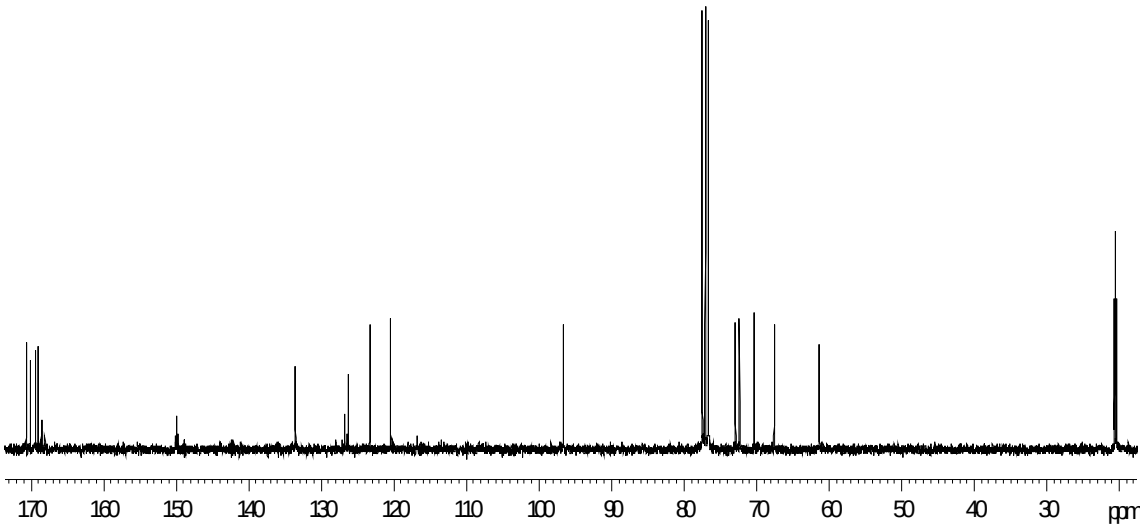



**Figure A-35: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (3.8)**

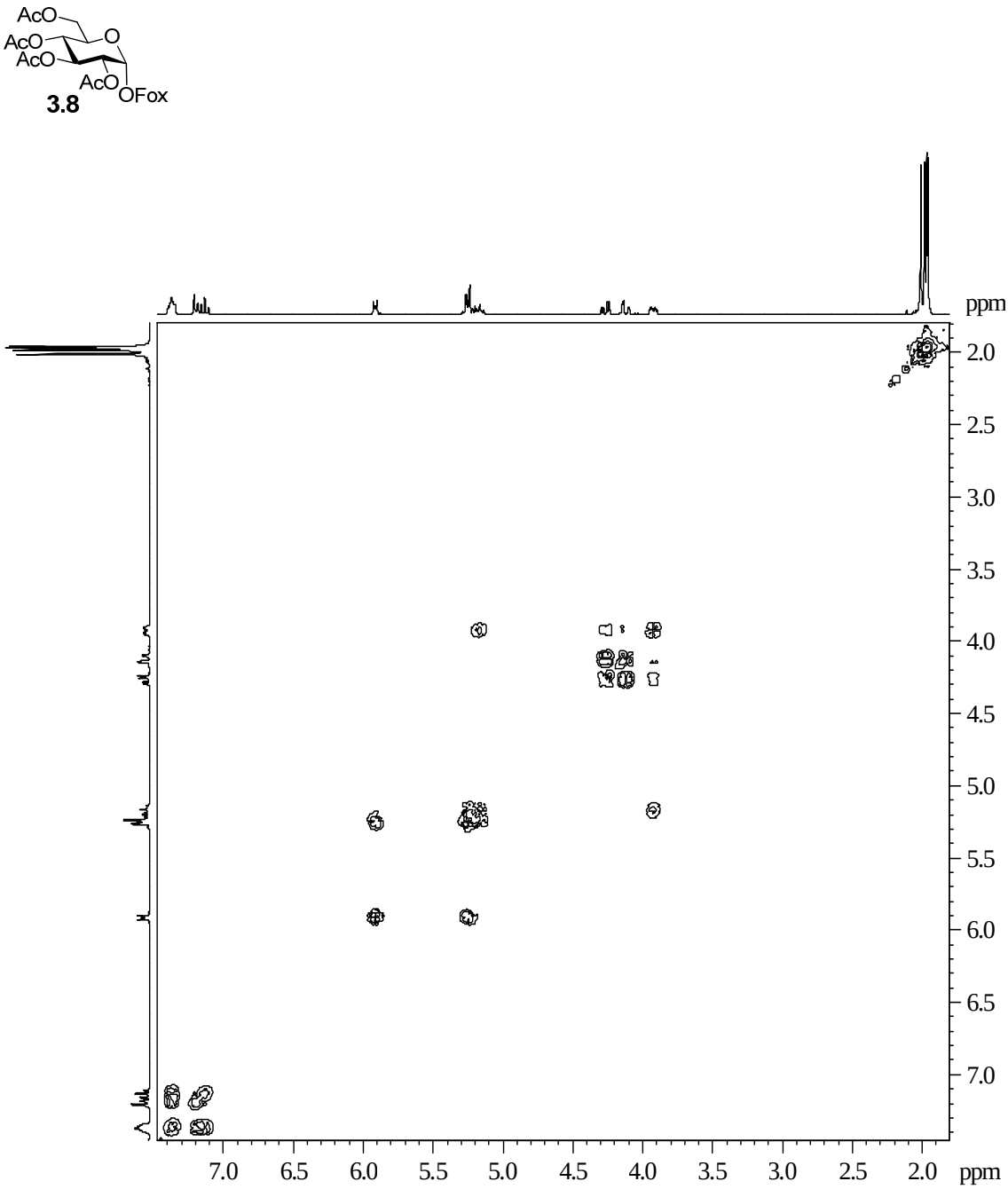



**Figure A-36: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (3.8)**

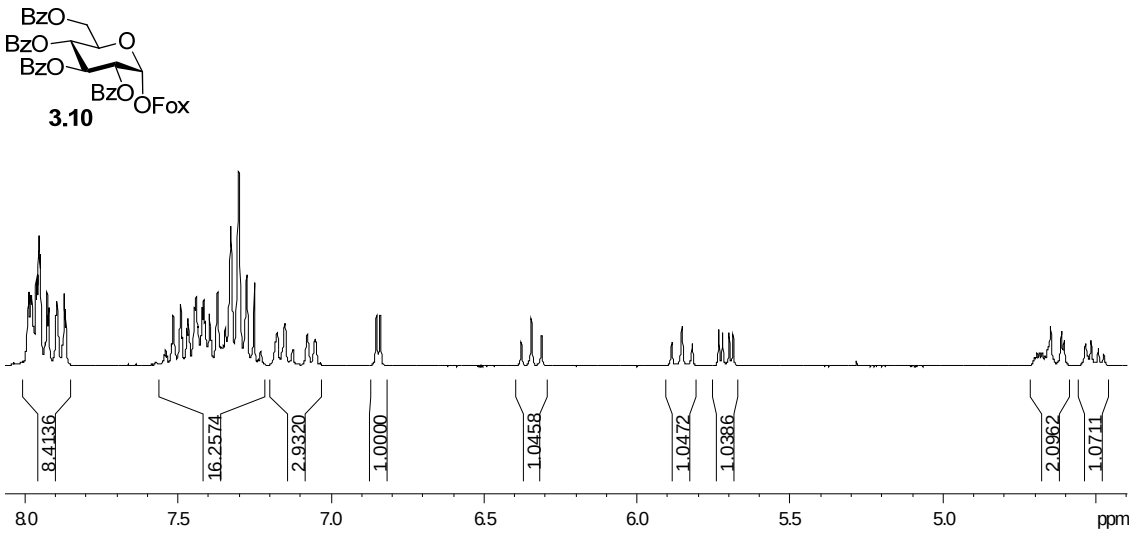



**Figure A-37: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (3.10)**

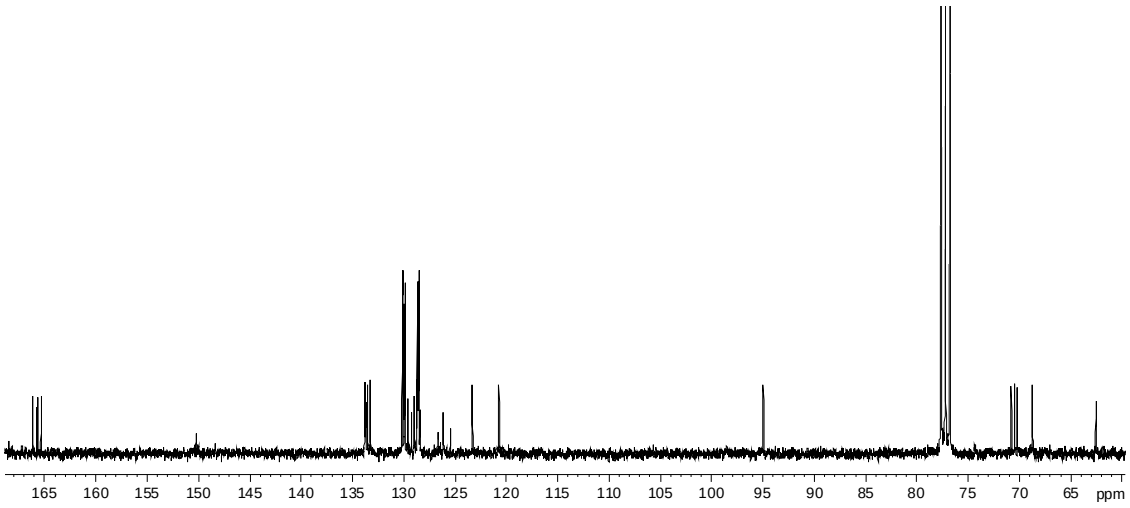



**Figure A-38: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (3.10)**

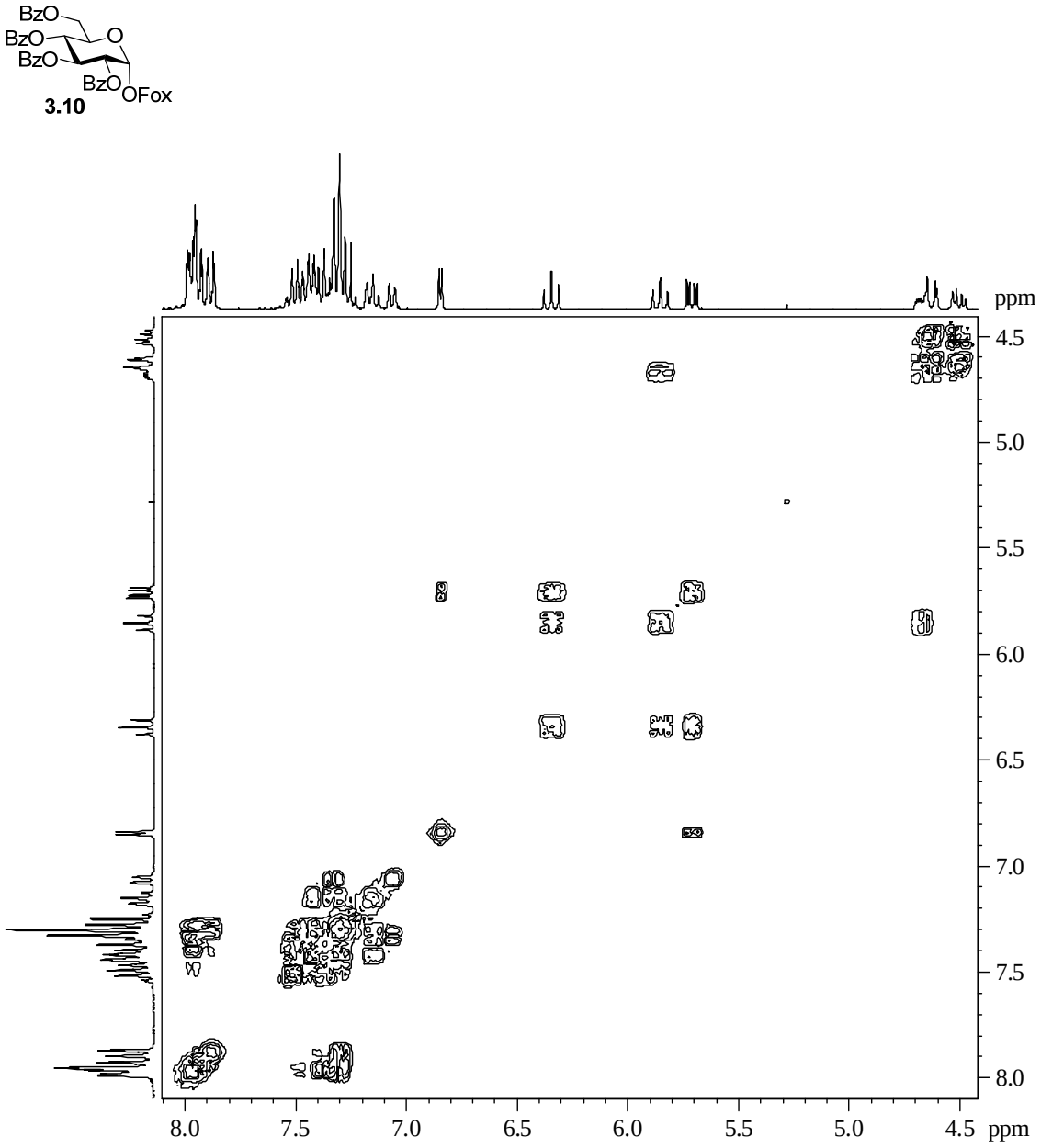
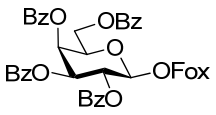



**Figure A-39: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (3.10)**




**3.12**

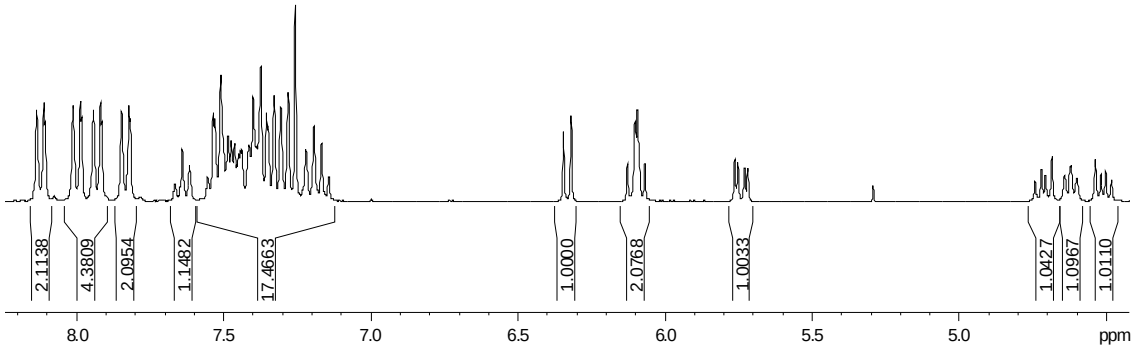



**Figure A-40: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (3.12)**

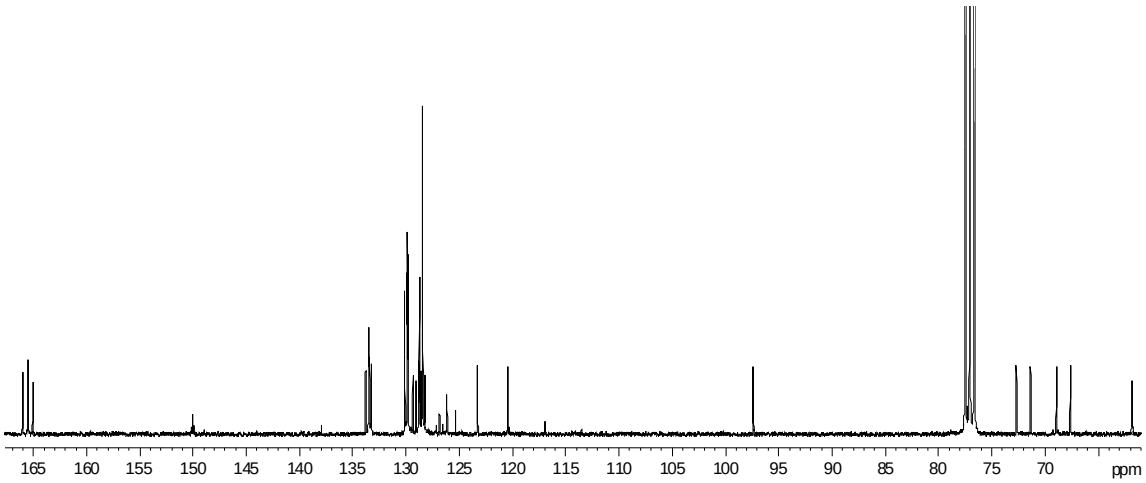
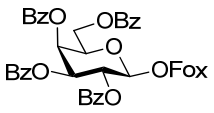



**Figure A-41: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (3.12)**




**3.12**

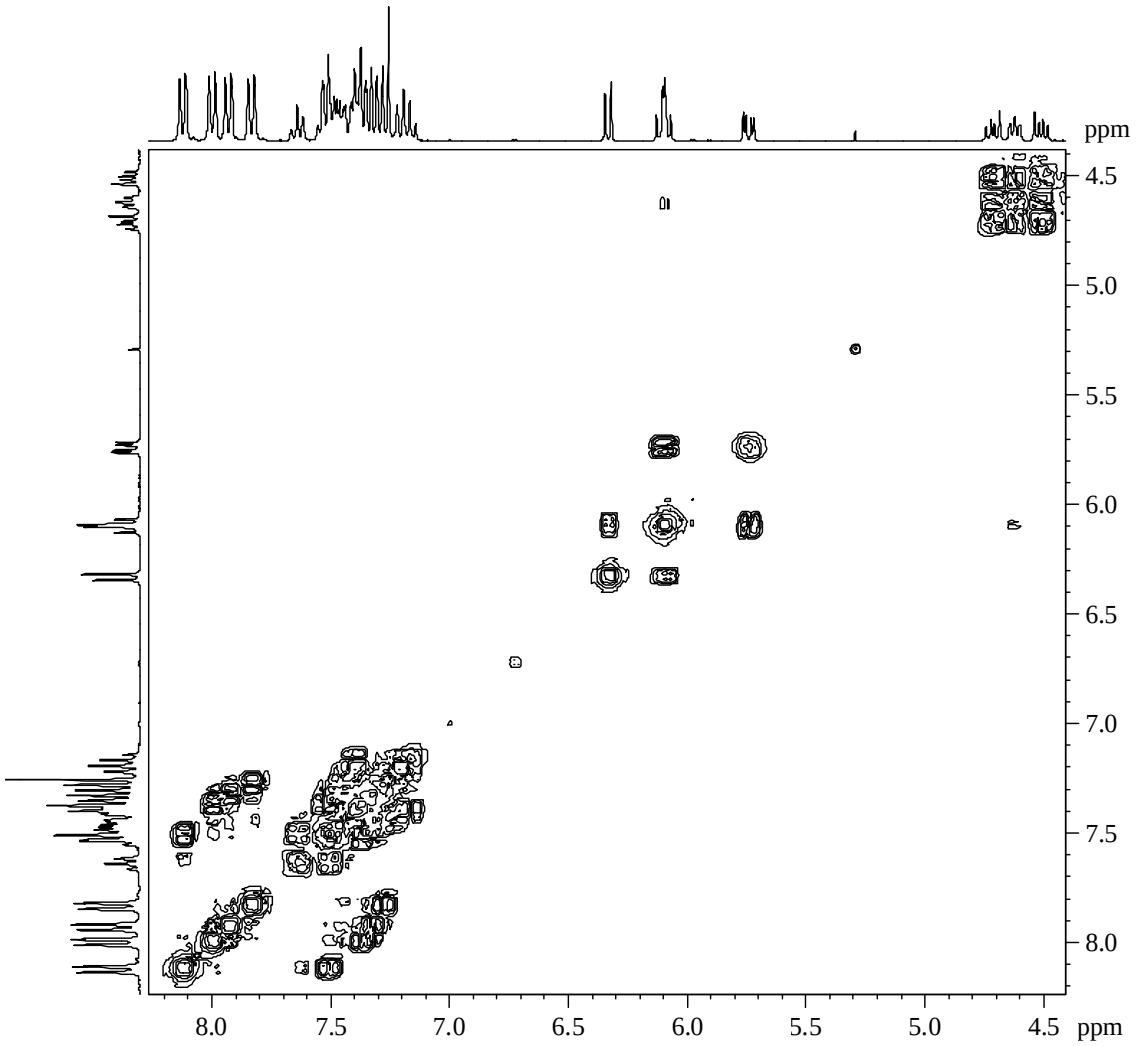



**Figure A-42: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (3.12)**

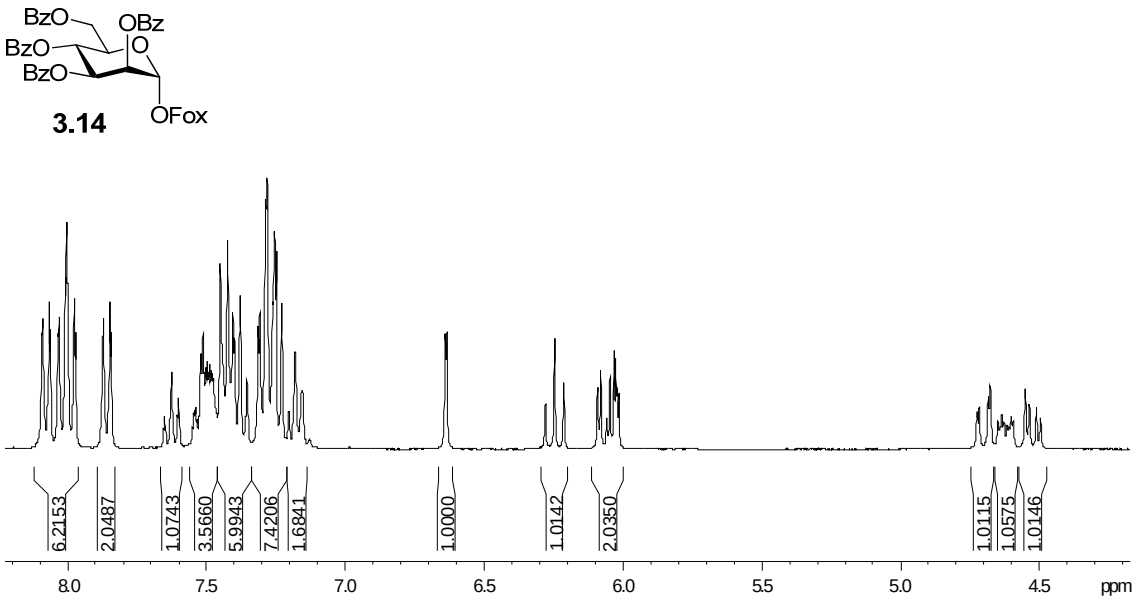



**Figure A-43: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (3.14)**

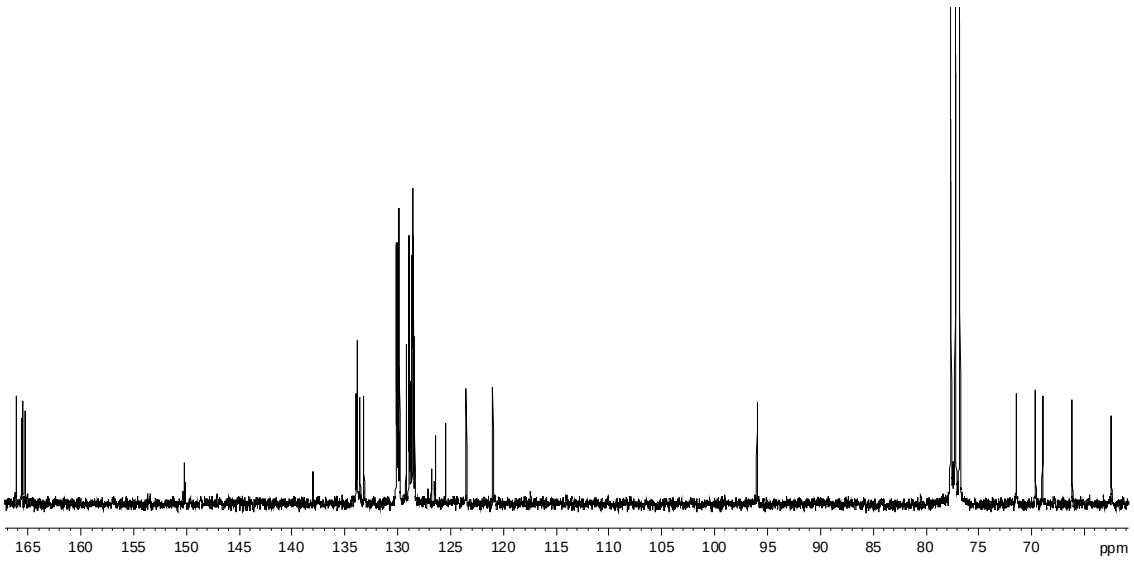
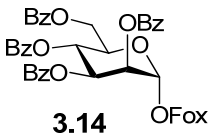



**Figure A-44: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (3.14)**




**3.14**

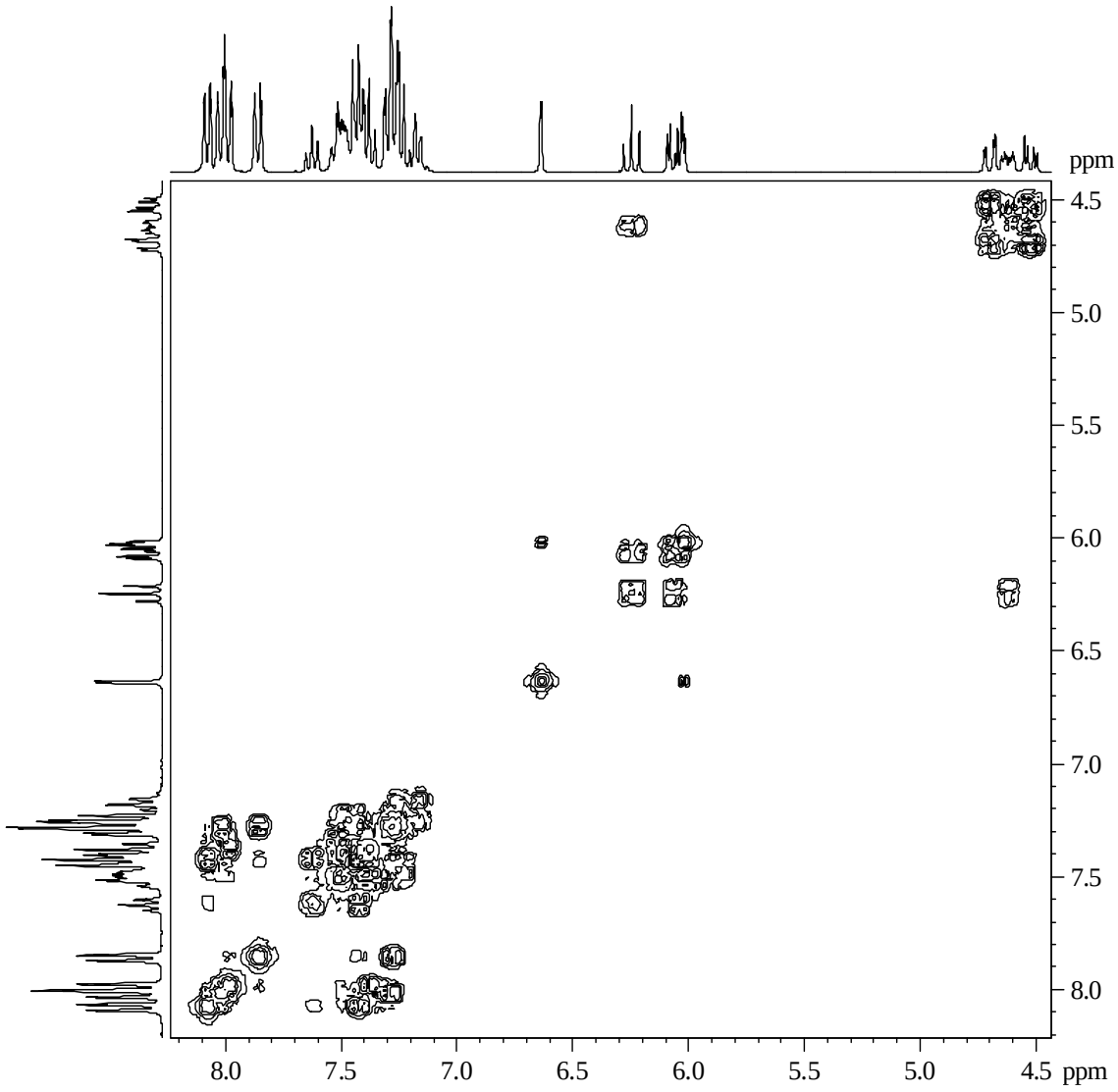
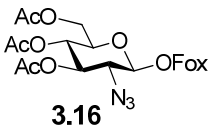



**Figure A-45: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (3.14)**




3.16

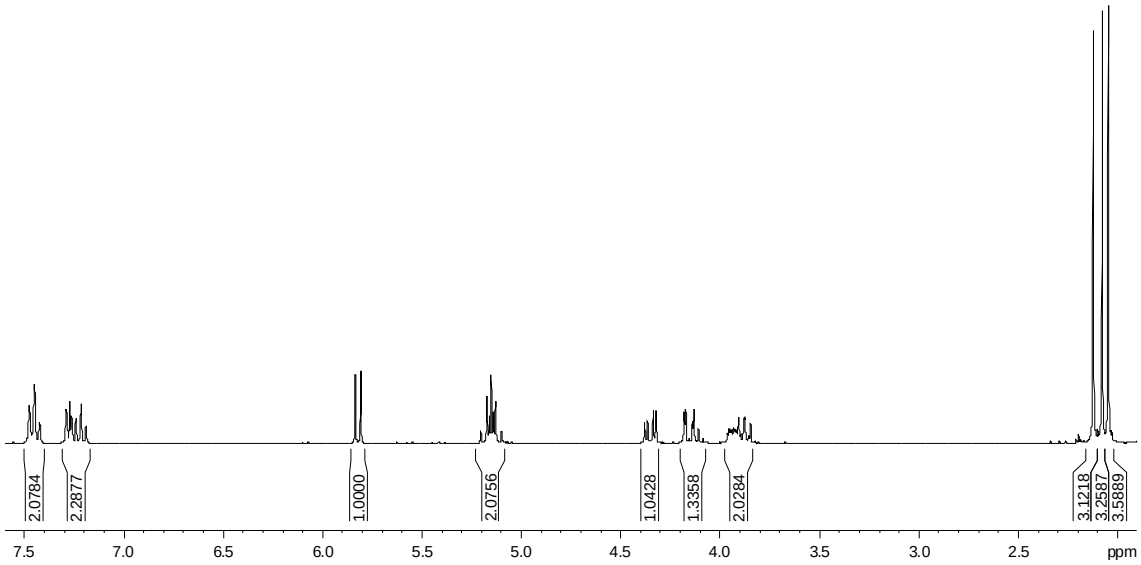



**Figure A-46: $^{1}$H NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside (3.16)**

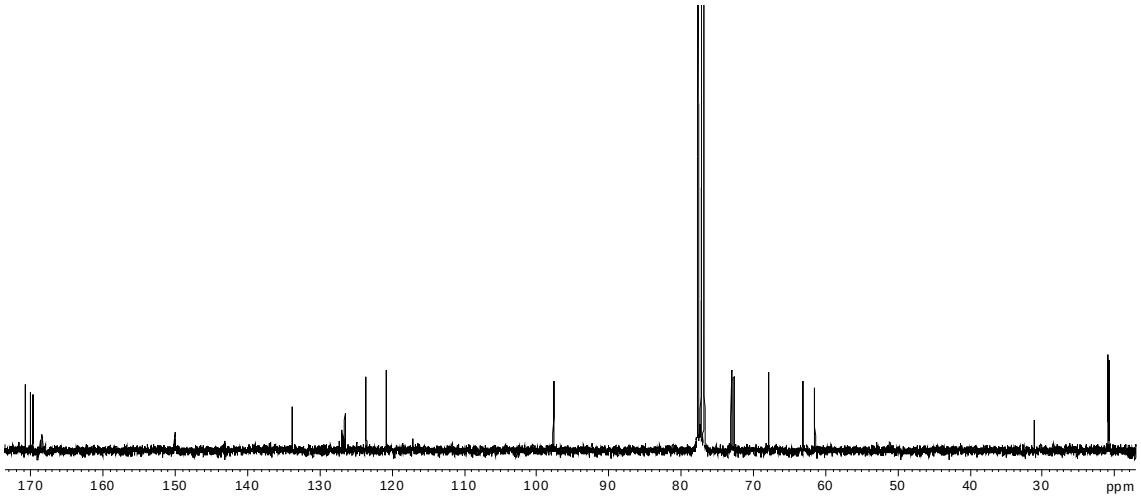



**Figure A-47: $^{13}$C NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside (3.16)**

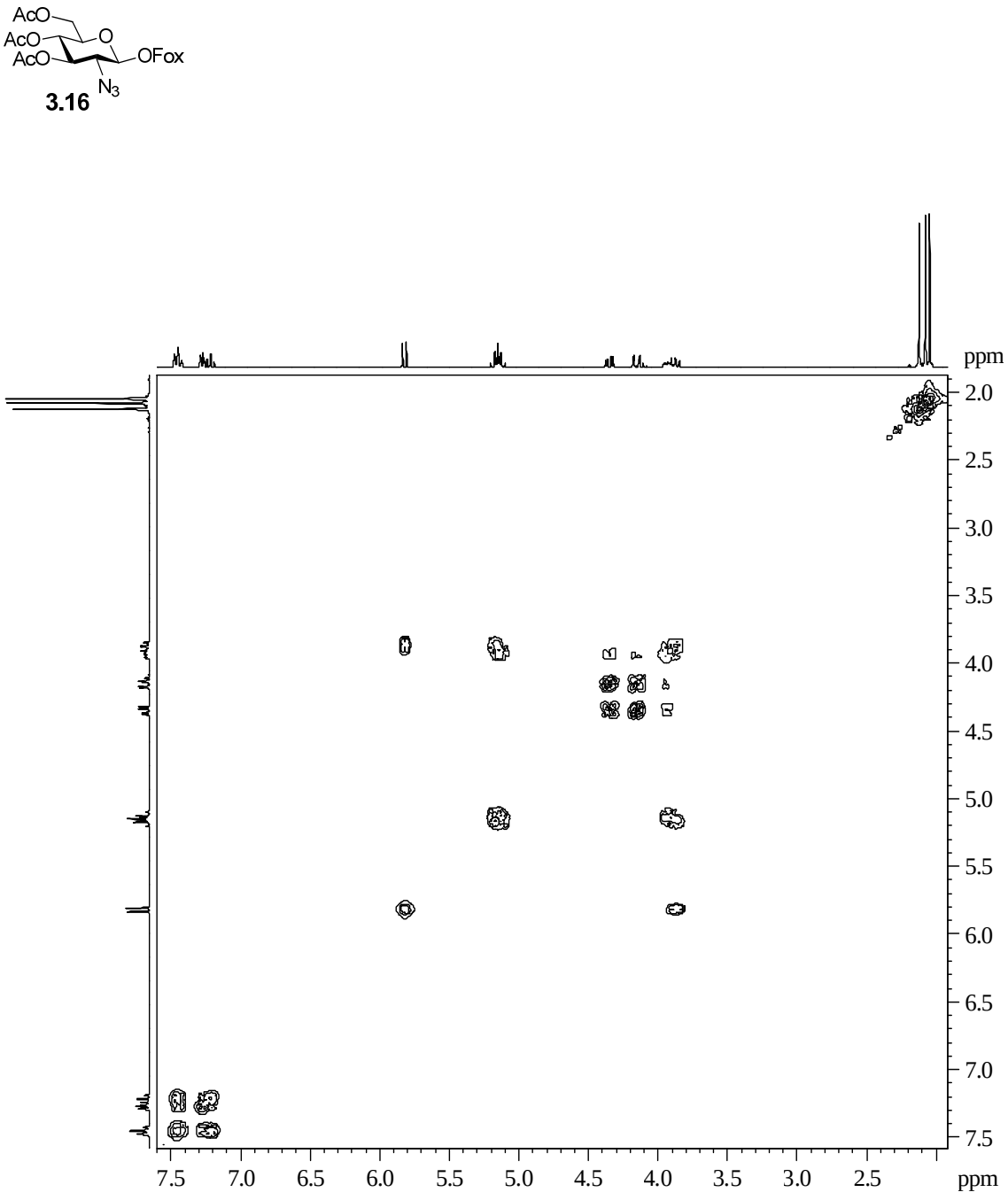



**Figure A-48: 2D NMR spectrum of 3,3-Difluoro-3*H*-indol-2-yl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside (3.16)**

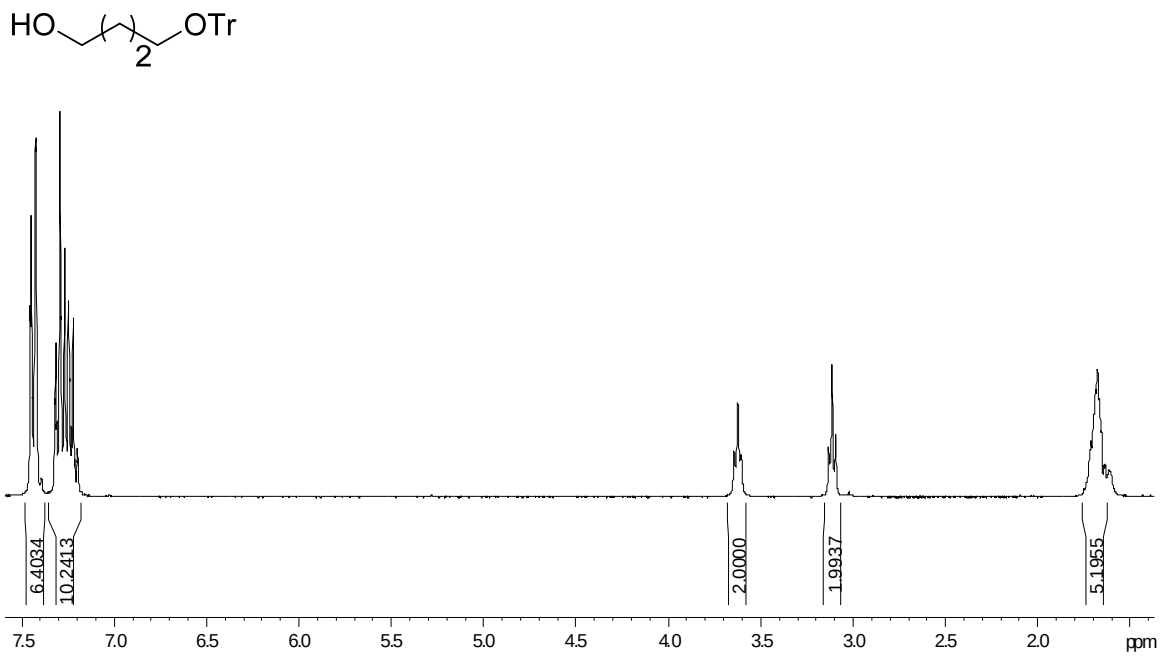



**Figure A-49: $^{1}$H NMR spectrum of 4-Trityloxybutan-1-ol (4.1)**

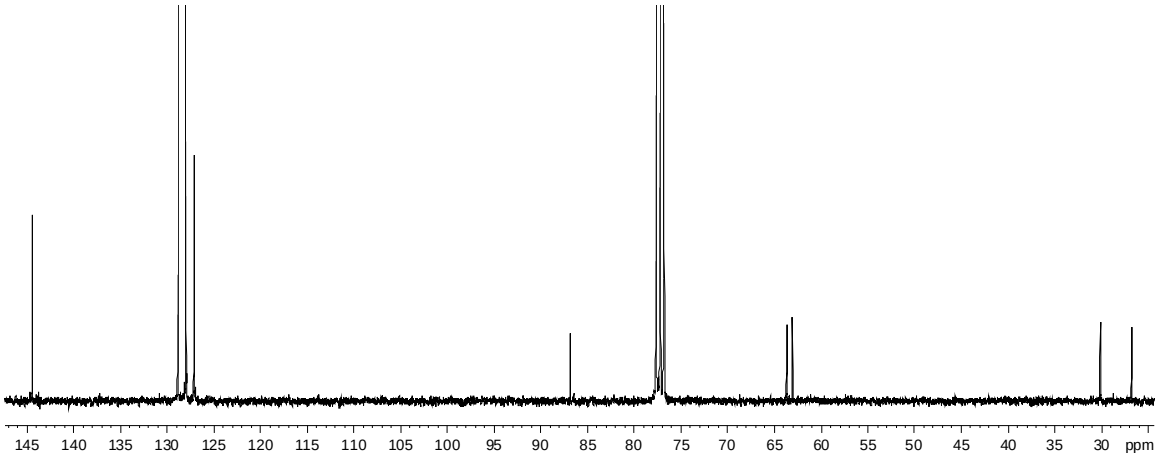



**Figure A-50: $^{13}$C NMR spectrum of 4-Trityloxybutan-1-ol (4.1)**

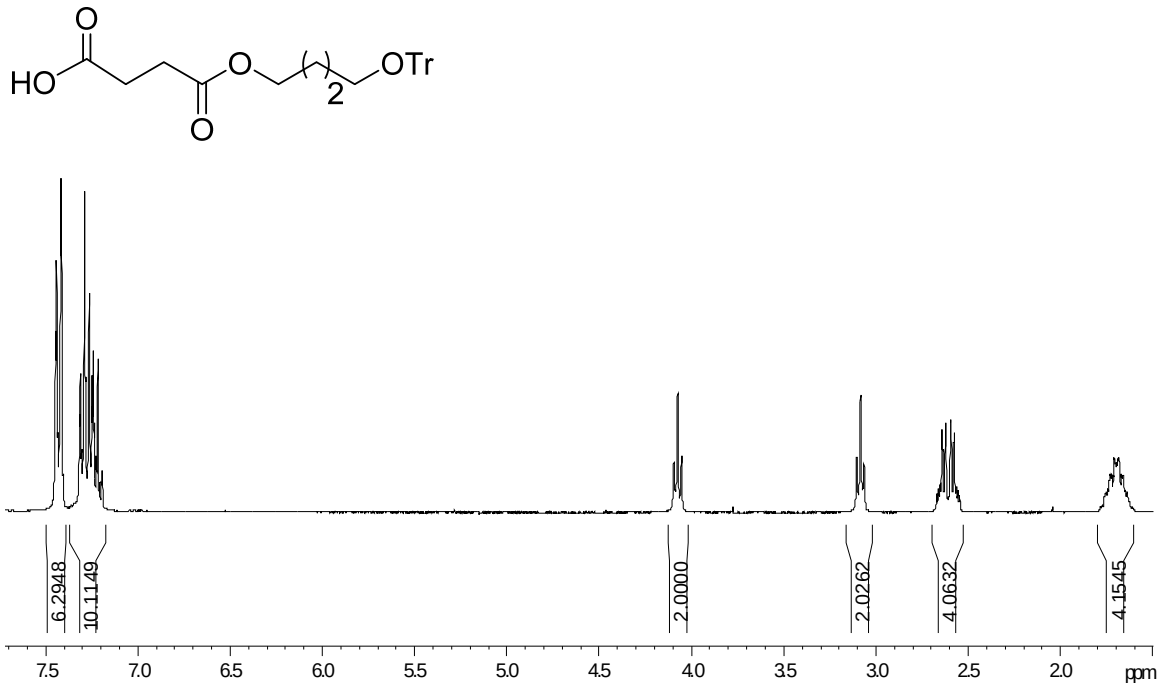



**Figure A-51: $^{1}$H NMR spectrum of 4-(4-Trityloxy)butoxy-4-oxobutanoic acid (4.4)**

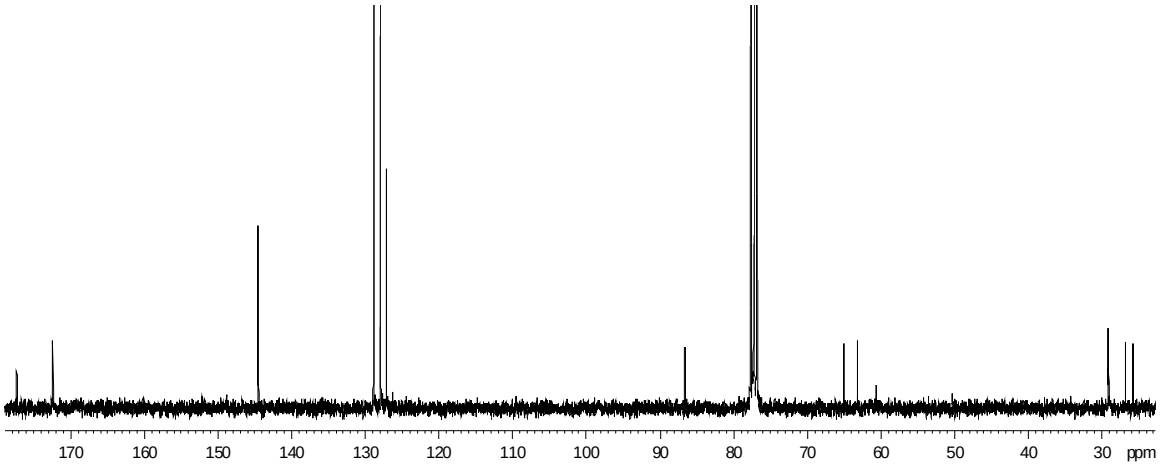



**Figure A-52: $^{13}$C NMR spectrum of 4-(4-Trityloxy)butoxy-4-oxobutanoic acid (4.4)**

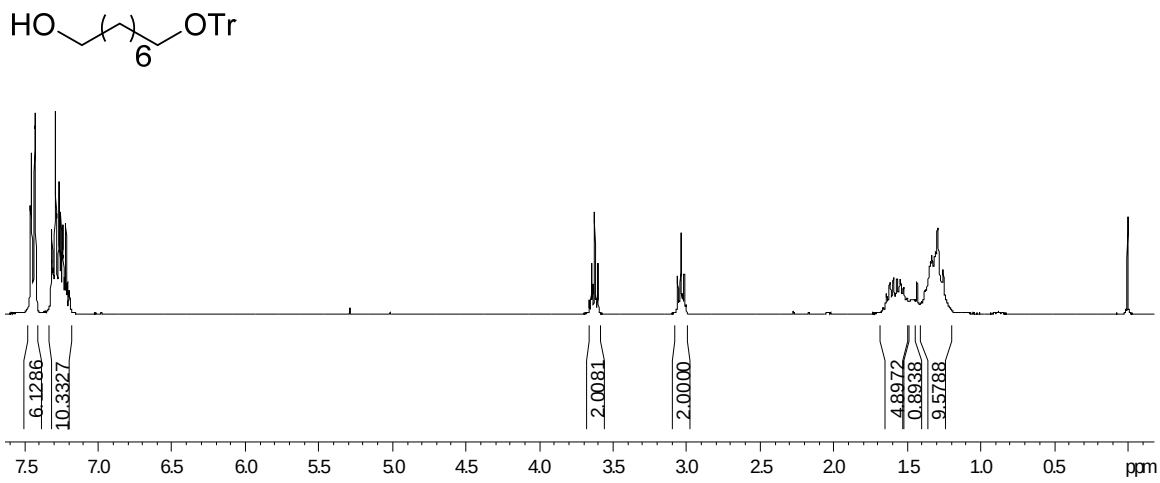



**Figure A-53: $^{1}$H NMR spectrum of 8-Trityloxyoctan-1-ol (4.2)**

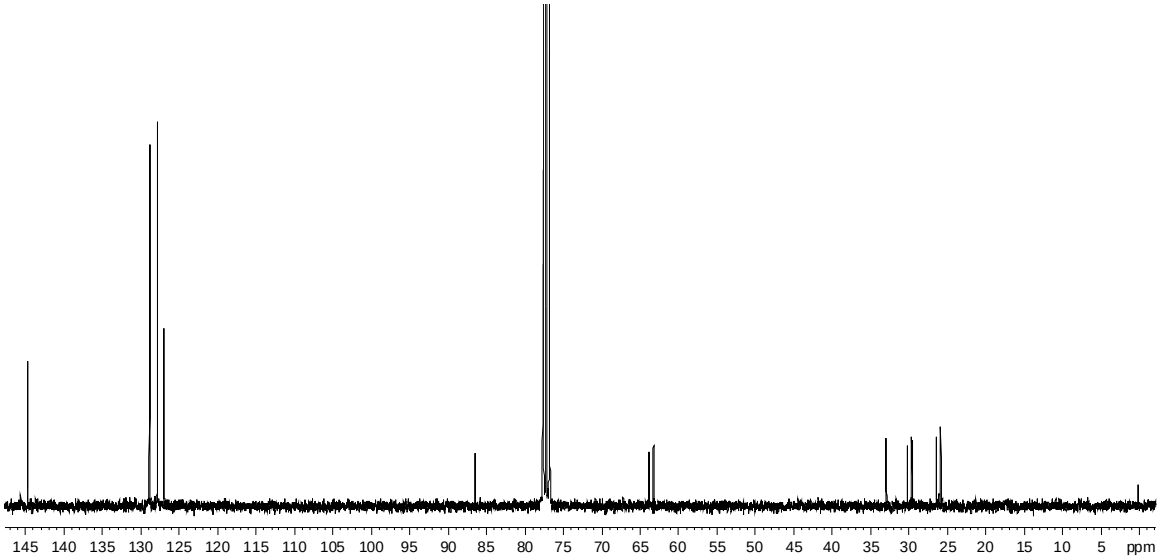



**Figure A-54: $^{13}$C NMR spectrum of 8-Trityloxyoctan-1-ol (4.2)**

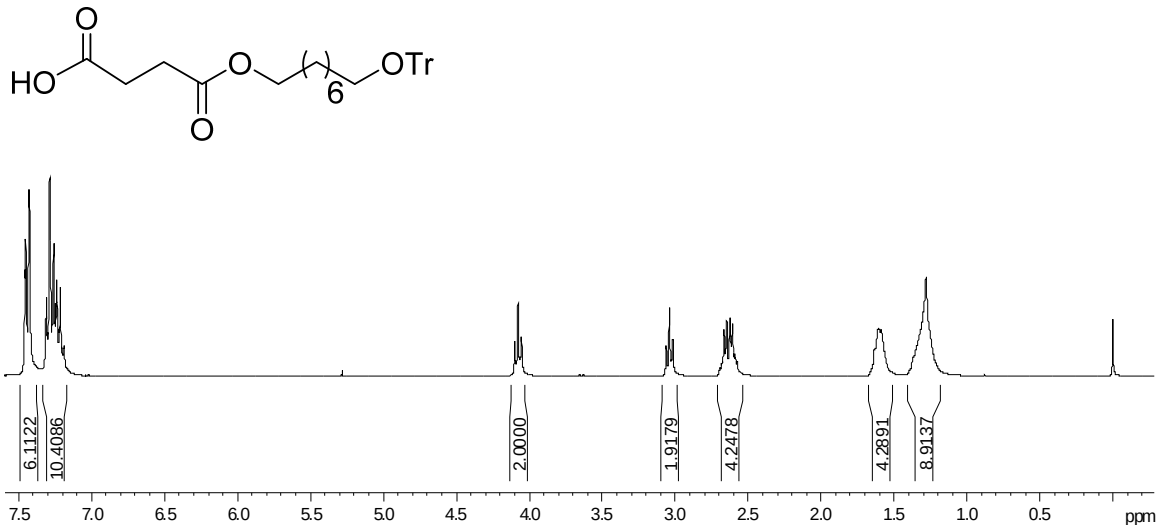



**Figure A-55: $^{1}$H NMR spectrum of 4-(8-Trityloxy)octyloxy-4-oxobutanoic acid (4.5)**

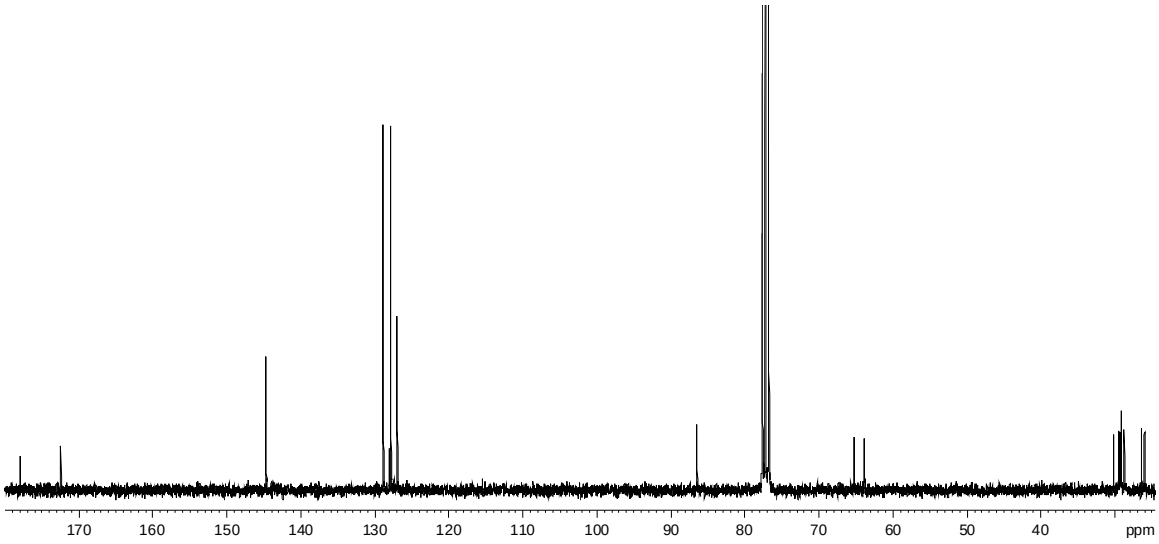



**Figure A-56: $^{13}$C NMR spectrum of 4-(8-Trityloxy)octyloxy-4-oxobutanoic acid (4.5)**

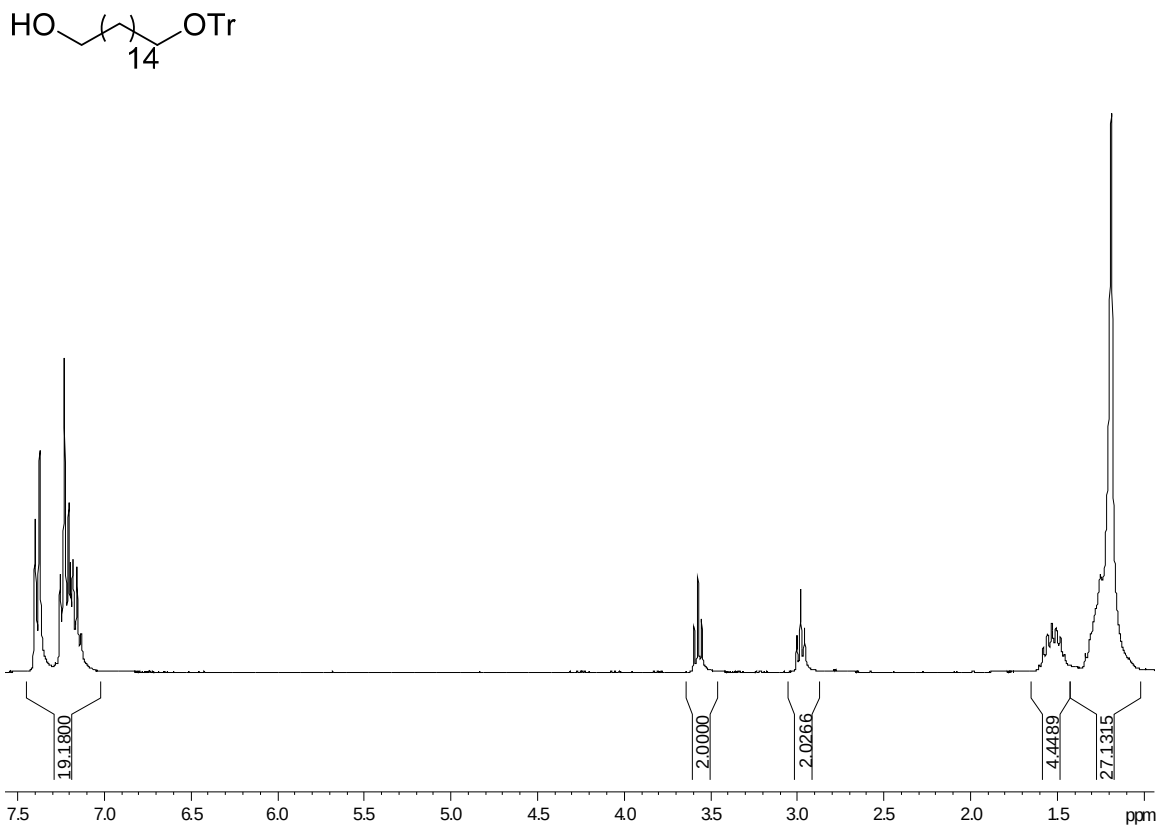



**Figure A-57: $^{1}$H NMR spectrum of 16-Trityloxyhexadecane-1-ol (4.3)**

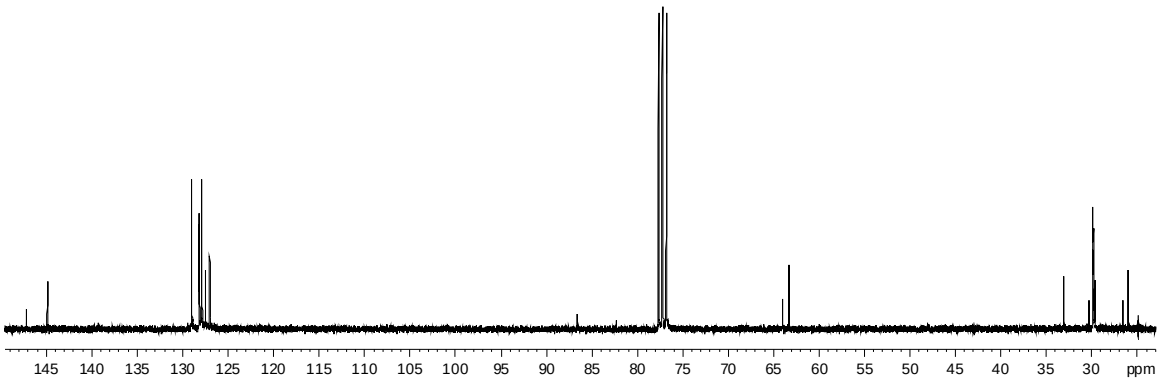



**Figure A-58: $^{13}$C NMR spectrum of 16-Trityloxyhexadecane-1-ol (4.3)**

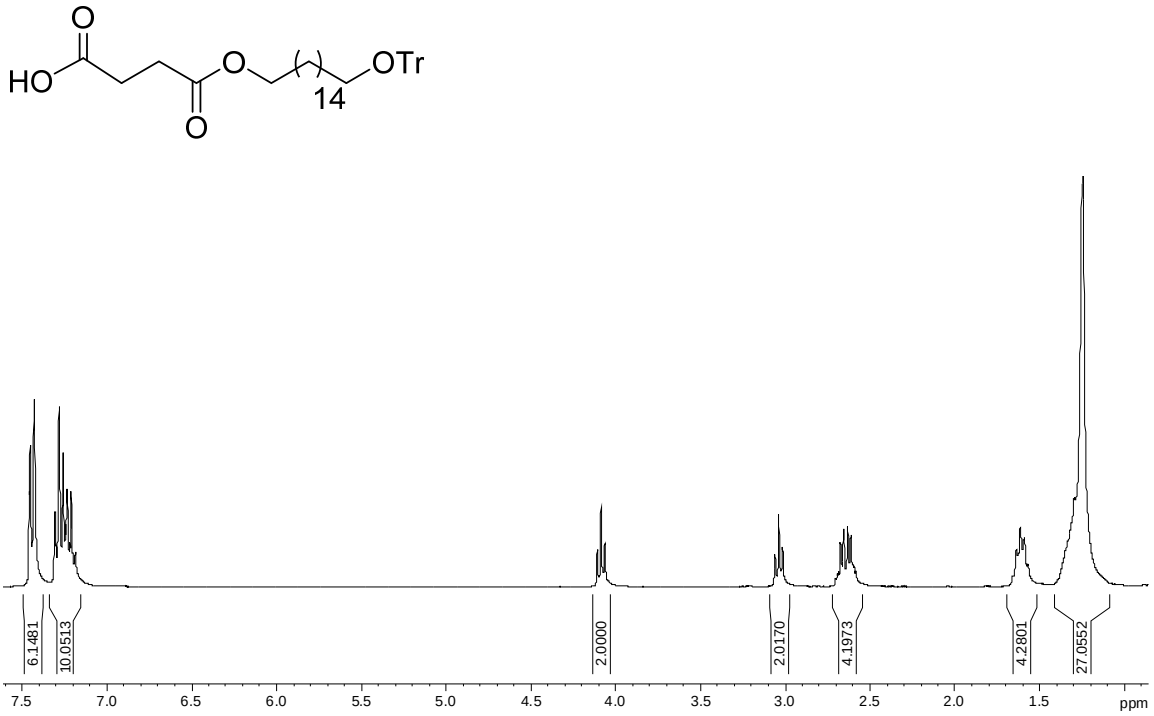



**Figure A-59: $^{1}$H NMR spectrum of 4-(16-Trityloxy)hexadecyloxy-4-oxobutanoic acid (4.6)**

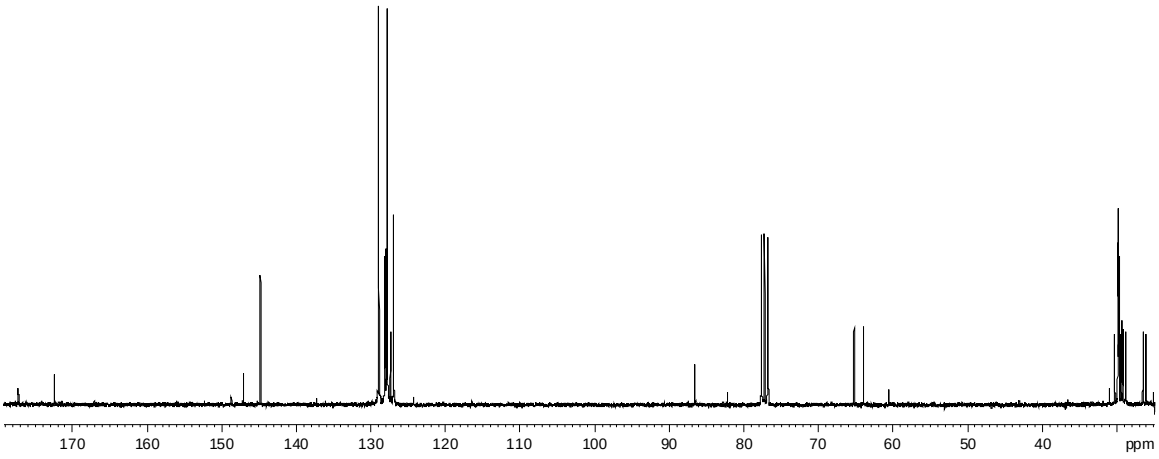



**Figure A-60: $^{13}$C NMR spectrum of 4-(16-Trityloxy)hexadecyloxy-4-oxobutanoic acid (4.6)**