ABSTRACT

Title of Dissertation: METHODS FOR STEREOSELECTIVE SYNTHESIS OF GLYCOPYRANOSYLAMIDE LINKAGE

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Glycoproteins play an important role in biological processes including intercellular communication, cell-cell recognition, and cell growth regulation. The study of cellular processes is often limited by the availability of glycopeptides or glycoproteins from natural sources. The advances in carbohydrate and peptide chemistry in the last decade have created generally applicable methodologies for the synthesis of glycopeptides. The crucial step of any synthesis of a glycopeptide is the introduction of the carbohydrate residue to the amino acid or peptide in a stereoselective manner under conditions which are compatible with glycosidic linkages and common protecting groups for peptide synthesis.

A novel synthesis of glycopyranosyl isoxazolines has been developed employing readily available 2-acetoxy-glycosyl azides and triphenylphosphine. The synthesis of the isoxazolines proceeded via epimerization of a β-phosphorimine to the

α-phosphorimine followed by cyclization to provide the isoxazoline. This methodology has been employed to synthesize glucopyranosyl, galactopyranosyl, and mannopyranosyl isoxazoline derivatives. The methodology has also been extended with di- and trisaccharide azides.

The stereoselective synthesis of the glycosylamide linkage has been developed utilizing *in situ* generated glycosyl isoxazolines. Results of the coupling studies demonstrated that the optimum conditions for the synthesis of glycosylamides involved the coupling of the isoxazoline with a 2-pyridyl thioester in the presence of copper(II) chloride. The stereoselective syntheses of α-glucosylamide and β-mannosyl amide have been accomplished in high yields. Isoxazoline couplings were applied to the synthesis of α-glucosyl amino acids derivatives also; particularly noteworthy was the coupling with differentially protected aspartic acid analogs. It was shown that the coupling conditions are compatible with different protection strategies used in peptide couplings.

Treatment of 2-NAc- or 2-NPhth-2-deoxyglucopyranosyl azides gave phosphorimine intermediates that failed to cyclize to imidazoline in analogy with the isoxazoline. The phosphorimines, however, coupled efficiently with thiopyridyl esters to provide the glycosyl-β-amide linkage. The yields in these couplings were superior to the yields from the traditional Staudinger reactions utilizing carboxylic acids. Coupling of these phosphorimines with the thiopyridyl ester of asparagine derivatives has proven to be a superior method for the synthesis of N-linked glycopeptide derivatives.

METHODS FOR STEREOSELECTIVE SYNTHESIS OF GLYCOPYRANOSYLAMIDE LINKAGE

by

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TABLE OF CONTENTS

List of Tables v

List of Figures vi

List of Schemes vii

List of Abbreviations x

Introduction 1

- Structural Features of N-Linked Glycoproteins 2
- Importance of N-Glycosylation of Proteins 4
- Biosynthesis of N-Linked Glycoproteins 6
- Synthesis of N-Glycosylamide Linkage 8
  - Oligosaccharidetransferase (OST):Chemoenzymatic Approach 11
  - Glycosyl Thioglycosides: Biomimetic Approach 12
  - Glycosyl Isothiocyanate 12
  - Pentenyl Glycosides 14
  - Glycosylamines 16
  - Glycosyl Phosphorimine: Staudinger Reaction 21
  - Thio Acids and Glycosylazides 26
  - Solid-Phase Synthesis of N-Glycopeptides 27
  - Special Case I: Synthesis of α-N-Glucosylamide Linkages 28
  - Special Case II: Synthesis of β-N-Mannosylamide Linkages 32
- The Goal of the Research 34

Results and Discussion 35

- Synthesis of Glycosyl Isoxazolines 35
- Coupling of Glycosyl Isoxazolines with Acid Derivatives 46
- Attempts Towards the Synthesis of Glycosyl Imidazoline 60
- Couplings Utilizing Thiolesters for the Synthesis of β-Glucosylamide 67

Application of the Methods Developed for the Synthesis of Molecules of Interest 70

Conclusions 73

Experimental 76

References 122

LIST OF TABLE

Table 1. Staudinger-type couplings performed by Inazu 24

Table 2. Staudinger-type couplings performed by Boullanger 25

Table 3. Couplings with anhydrides and acid chlorides 47

Table 4. Couplings with activated esters 48

Table 5. Couplings with 2-pyridyl thiolphenylacetate 50

Table 6. Couplings with 2-pyridyl thiolphenylacetate utilizing transition metals 53

Table 7. Control experiments utilizing glucosyl-α-amide **95** 55

Table 8. Control experiments utilizing isoxazoline **75** 56

Table 9. Couplings of 2-NAc-glucosyl azide to provide glucosyl-β-amide **116** 68

Table 10. Couplings of 2-NPhth-glucosyl azide to provide glucosyl-β-amide **117** 70

Table 11. Couplings with thiolester of aspartic acid derivatives 71

LIST OF FIGURES

Figure 1. Structures of naturally occurring N-glycopeptide linkages 1

Figure 2. The structures of monosaccharides found in N-glycoproteins 2

Figure 3. The structural types of β-GlcNAc linked N-glycoproteins 3

Figure 4. The biosynthesis of complex type N-glycoproteins 7

Figure 5. Three conceptual approaches for the preparation of glycopeptides 10

Figure 6. The pentasaccharide which yields α/β mixtures when reduced 19

Figure 7. Structure of the *N*-glycopeptide Nephritogenoside 29

Figure 8. Compounds containing α-N-glucosylamide linkage. 31

Figure 9. The structure of β-mannobiose derivatives as liposome components 34

Figure 10. Proposed structures for the glucosyl isoxazoline and oxazoline. 37

Figure 11. The *in situ* ^{1}H NMR spectra of glucosyl isoxazoline **75** in $CDCl_3$. 38

Figure 12. The *in situ* ^{1}H NMR of the reaction of β-mannosyl azide with triphenyl phosphine at 83 °C after: a) 20 min, b) 5 h, c) 24 h. 45

Figure 13. The P^{31} NMR spectra of the crude reaction of β-mannosyl azide with triphenylphosphine after: a) 20 min, b) 17 h, c) 24 h. 46

Figure 14. ^{1}H NMR of the α/β mixture of glucosylamide **95**. 54

Figure 15. P^{31} NMR of the reaction of 2-NAc-glucosyl-β-azide **53** with Ph_3P at 55 °C after: a) 30 min, b) 2 h, c) 24 h. 64

Figure 16. The *in situ* ^{1}H NMR spectra of the reaction of 2-NAc-glucosyl-β-azide **53** with Ph_3P at 55 °C after: a) 30 min, b) 5 h, c) 24 h. 65

LIST OF SCHEMES

Scheme 1	11
Scheme 2	12
Scheme 3	13
Scheme 4	13
Scheme 5	14
Scheme 6	15
Scheme 7	15
Scheme 8	18
Scheme 9	19
Scheme 10	20
Scheme 11	20
Scheme 12	21
Scheme 13	22
Scheme 14	22
Scheme 15	23
Scheme 16	26
Scheme 17	27
Scheme 18	29
Scheme 19	30
Scheme 20	31
Scheme 21	32

Scheme 22 33

Scheme 23 33

Scheme 24 35

Scheme 25 36

Scheme 26 39

Scheme 27 40

Scheme 28 41

Scheme 29 42

Scheme 30 43

Scheme 31 43

Scheme 32 49

Scheme 33 51

Scheme 34 51

Scheme 35 57

Scheme 36 57

Scheme 37 58

Scheme 38 58

Scheme 39 59

Scheme 40 60

Scheme 41 61

Scheme 42 62

Scheme 43 66

Scheme 44 67

Scheme 45 71

Scheme 46 72

Scheme 47 72

LIST OF ABBREVIATIONS

Ac	acetyl
All	allyl
Anthr	anthracene
Ar	aryl
Asp	aspartic acid
Bn	benzyl
Boc	tertiary butoxy carbonyl
Bu	butyl
Btz	1-*N*-benzotriazole
Bz	benzoyl
DIEA	diisopropyl ethyl amine
DMF	*N*,*N*-dimethylformamide
ER	endoplasmic reticulum
Et	ethyl
equiv	equivalent(s)
Fmoc	9-fluorenylmethylcarbonyl
hr	hour(s)
HPLC	high-performance liquid chromatography
Hz	Hertz
IDCP	di-sym-collidine iodoniumperchlorate
IR	infrared

Leu	leucine
M^+	molecular ion
Me	methyl
MHz	megahertz
min	minute(s)
NBS	*N*-bromo succinimide
NIS	*N*-iodo succinimide
NMR	nuclear magnetic resonance
Nu	nucleophile
OAc	acetate
OBz	benzoate
Pfb	pentafluorophenyl
Ph	phenyl
Phth	phthalimido
Pyr	2-pyridyl
Rf	retardation factor
SPyr	2-pyridyl thiol
Su	*N*-succinimidyl
TBAF	tetrabutylammonium fluoride
t-Bu	*tertiary*-butyl
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography

TMS	trimethylsilyl
Troc	trichloroethoxycarbonyl
UV	ultraviolet
Z	benzyloxycarbonyl

Introduction

DNA, proteins, carbohydrates, and lipids are the four major classes of macromolecules in biology.[1] Since carbohydrates have two different linkage types (α and β) and several positions for connectivity, oligosaccharides can have an almost unlimited variation in their structure.[2] Most of the carbohydrates present in cells are attached either to proteins, to form the glycoproteins, or to lipids to form glycolipids. A glycoprotein is a protein containing one or more covalently linked carbohydrate groups. Glycoproteins are classified into two principal groups according to the linkage between the carbohydrate and the amino acid residue. The first group is O-linked glycoproteins in which the carbohydrate moiety is attached to the oxygen of an amino acid residue. In most O-linked glycoproteins, an initial GalNAc residue is attached by an α-O-glycosidic linkage to a serine or threonine residue of the protein.[3] The second group is N-linked glycoproteins in which the carbohydrate is attached to the nitrogen of the amide group of an asparagine (Asn) amino acid on the protein.[3] This introduction will focus on the chemistry of N-linked glycoproteins, especially the synthesis of the glycosylamide linkage between carbohydrate and amino acid moieties.

Figure 1. Structures of naturally occurring N-glycopeptide linkages.

Structural Features of N-Linked Glycoproteins

In all of the known N-linked glycoproteins, the carbohydrate unit is linked to the protein through the amido group of the L-asparagine amino acid, with the asparagine as part of the consensus sequence Asn-Xaa-Ser/Thr, where Xaa may be any amino acid with the exception of proline.[4] The occurrence of this sequence is necessary for N-glycosylation, but not sufficient.[5] The only known exception to this pattern is nephritogenoside in which the glycosylated asparagine is contained in the sequence Asn-Pro-Leu.[6]

In most N-linked glycoproteins, N-acetyl glucosamine (GlcNAc) is the monosaccharide which binds the carbohydrate moiety to the asparagine of the protein via a β-linkage at the anomeric center (Figure 1, **1**). Recently, several asparagine linked monosaccharides, that incorporate β-N-glucopyranosyl (Figure 1, **2**), β-N-acetylgalactopyranosyl (Figure 1, **3**), and α-N-glucopyranosyl (Figure 1, **4**) linkages have been reported.[6-8] Figure 2 shows the structures of monosaccharides commonly found in the glycan part of the N-glycoproteins.

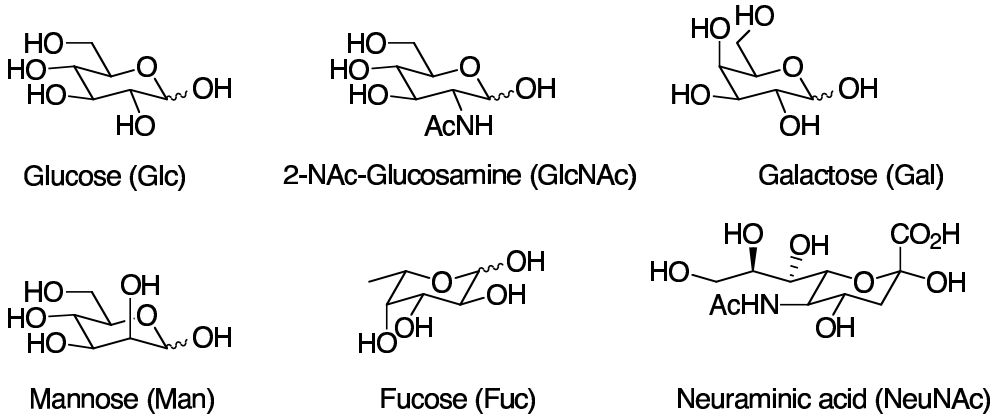


Figure 2. The structures of monosaccharides commonly found in N-glycoproteins.

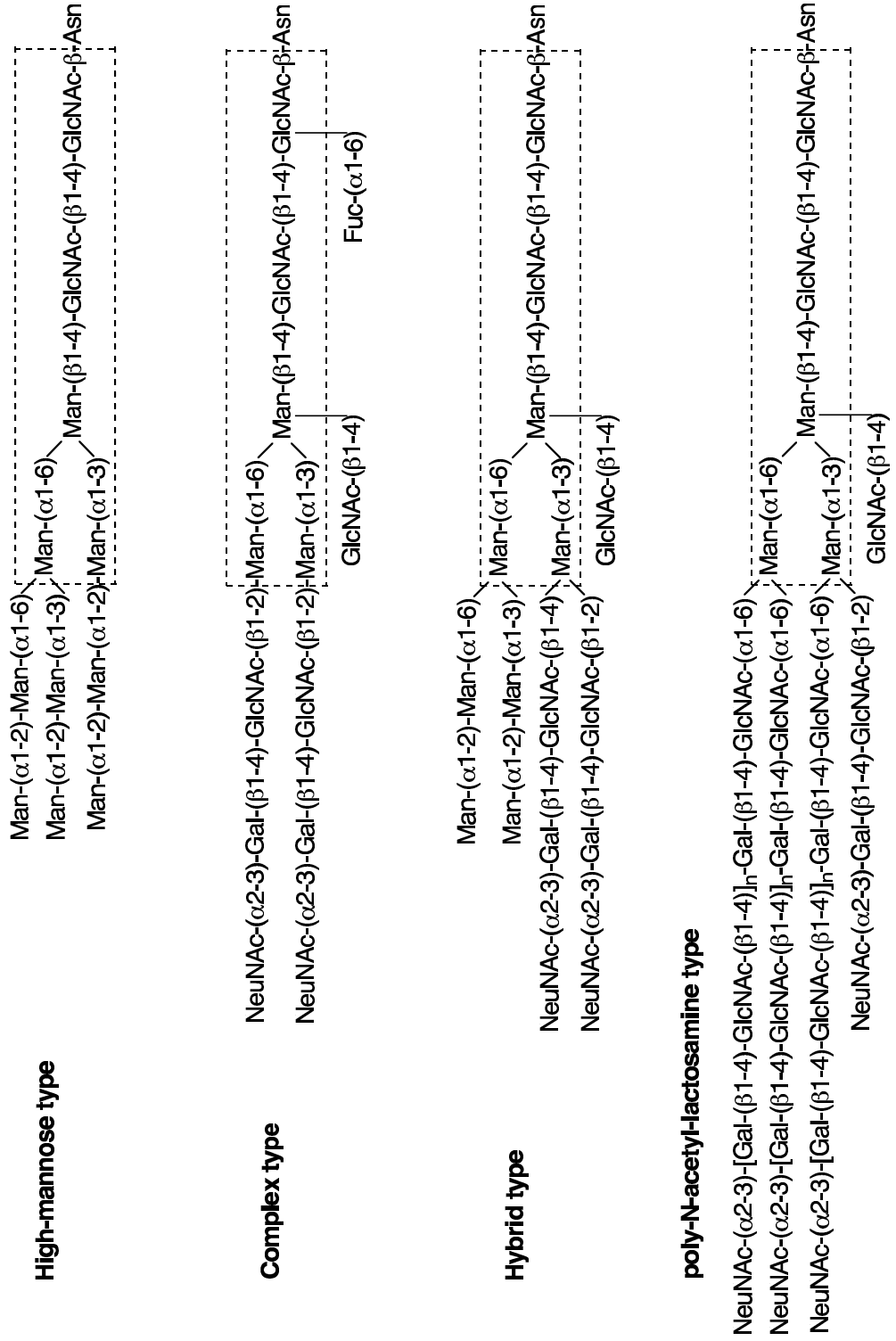


Figure 3. The structural types of β-GlcNAc linked N-glycoproteins.

On the basis of the structure and the location of glycan residues added to the trimannosyl core, N-linked oligosaccharides which are linked to asparagine via β-GlcNAc can be classified into four main groups (Figure 3).[9] These are high-mannose, complex, hybrid, and poly-N-acetyl-lactosamine. All of these structural types have a common pentasaccharide core shown in the dashed area in Figure 3. High-mannose type glycans contain only α-mannosyl residues attached to the common core. In complex type glycans, the core is branched via GlcNAc being the terminal monosaccharide of the branches (antennae). Hybrid glycans have the characteristic features of both complex and high-mannose type glycans. The poly-N-acetyl-lactosamine glycans contain repeating units of [Gal(β1-4)GlcNAc(β1-3)-] attached to the core. All the recently discovered glycoproteins with novel glycosidic linkages mentioned above are exceptions to these classification.

Importance of N-Glycosylation of Proteins

The majority of proteins found in eukaryotic cells and biological fluids are glycosylated and glycoproteins are present in animals, plants, microorganisms and viruses.[10] The diversity of glycosyl systems suggests that there is no single biological role for N-glycosylation, although their major function is to act as recognition markers.[11,12] Examples of the biological importance of N-glycosylation include:[3,13-18]

a) The glycans of proteins correct the folding of proteins during their biosynthesis and favor their secretion from the cell. The immunoglobulins IgA, IgM, IgG were inefficiently secreted when these proteins were synthesized in the presence of tunicamycin, which inhibits the glycosylation of N-linked glycoproteins. Treatment

with tunicamycin prevented secretion of enzymes in yeast and non-glycosylated proteins were entrapped in intracellular membranes.

b) Glycans control the proteolysis to give biologically functional proteins. For example, the non-glycosylated insulin receptor was not processed to the functional insulin receptor, and was not translocated to the plasma membrane.

c) Glycosylation was found to be essential for ligand binding to the epidermal growth factor receptor.

d) Glycosylation also was found to be necessary to stabilize proteins and protect them from proteolytic degradation. Among the receptors protected in this manner are: nerve growth factor receptor, low-density lipoprotein receptor of smooth muscle cells, and the thyroid stimulating hormone.

e) The absence of carbohydrates affected the physicochemical properties of some glycoproteins. The glycosylation of serum proteins has been shown to influence their half-life times *in vivo*. Nonglycosylated erythroprotein has one-tenth the activity of the fully glycosylated molecule, primarily as a result of renal filtration and clearance.

f) It is well known that the glycans of glycoproteins are involved in cell-cell recognition and adhesion, cell differentiation and development, and cell-contact inhibition, thereby intervening in the social life of cells. Cell surface carbohydrates of tumor cells have profoundly modified structures. In addition, some N-glycosylation inhibitors inhibit tumor metastasis when administered to animals. In fact, the modified tumor glycans may act as recognition signals for endothelial cells and play an important role in the origin of secondary tumors. Inhibition of N-glycosylation in

virus-infected cells has dramatic effects on virus multiplication and infectivity of the newly formed virus particles. It was also found that non-glycosylated viral proteins are not inserted into the mammalian cell membrane, and therefore, no adhesion occurred. For example, GP-120 of HIV-1 is highly N-glycosylated and the full pathogenic potential of HIV-1 *in vitro* is manifested only if its viral proteins are N-glycosylated.[18]

g) The number of diseases whose origin lies in the deficiencies of N-linked glycosylation have proven the importance of N-glycosylation for human development. Carbohydrate-deficient glycoprotein syndrome (CDG I or II), a multisystematic disease, is caused by defects in the synthesis of N-glycan. It causes severe development abnormalities, including psychomotor retardation, skeletal deformities, and epilepsy.

Biosynthesis of N-Linked Glycoproteins

N-Linked glycosylation of proteins is a co-translational event.[3] This means that it is initiated during protein synthesis. This is in contrast to O-linked glycosylation, which begins after protein synthesis is complete, and is post-translational. N-Linked oligosaccharide biosynthesis occurs as a stepwise process in the membranes of ER and Golgi complex (Figure 4).[19,20] The first stage of the process involves the synthesis of the precursor oligosaccharide by the stepwise addition of monosaccharides to the polyisoprenoid lipid dolichol. Early reactions occur on the cytoplasmic face of the ER and later additions occur on the luminal face of the ER. The first reaction involves the transfer of a GlcNAc-P from UDP-GlcNAc to Dol-P to

form the lipid intermediate dolichol-PP-GlcNAc. A second GlcNAc residue is incorporated from UDP-GlcNAc to form GlcNAcβ1-4GlcNAc-PP-dolichol. Further reactions occur as a result of the action of mannosyltransferases I-V by addition from GDP-Man yielding $Man_5(GlcNAc)_2$-PP-dolichol structure. After formation of this oligosaccharide in the cytoplasm, the entire molecule is "flipped" into the lumen face of the ER. This partially formed oligosaccharide intermediate is further substituted with four Man and three Glc residues. The donor molecules for these reactions are produced in the cytoplasm (dolichol-P-Man and dolichol-P-Glc) and flipped into lumen of the ER. The attachment of these residues provides the final $Glc_3Man_9(GlcNAc)_2$-PP-dolichol structure (Figure 4).

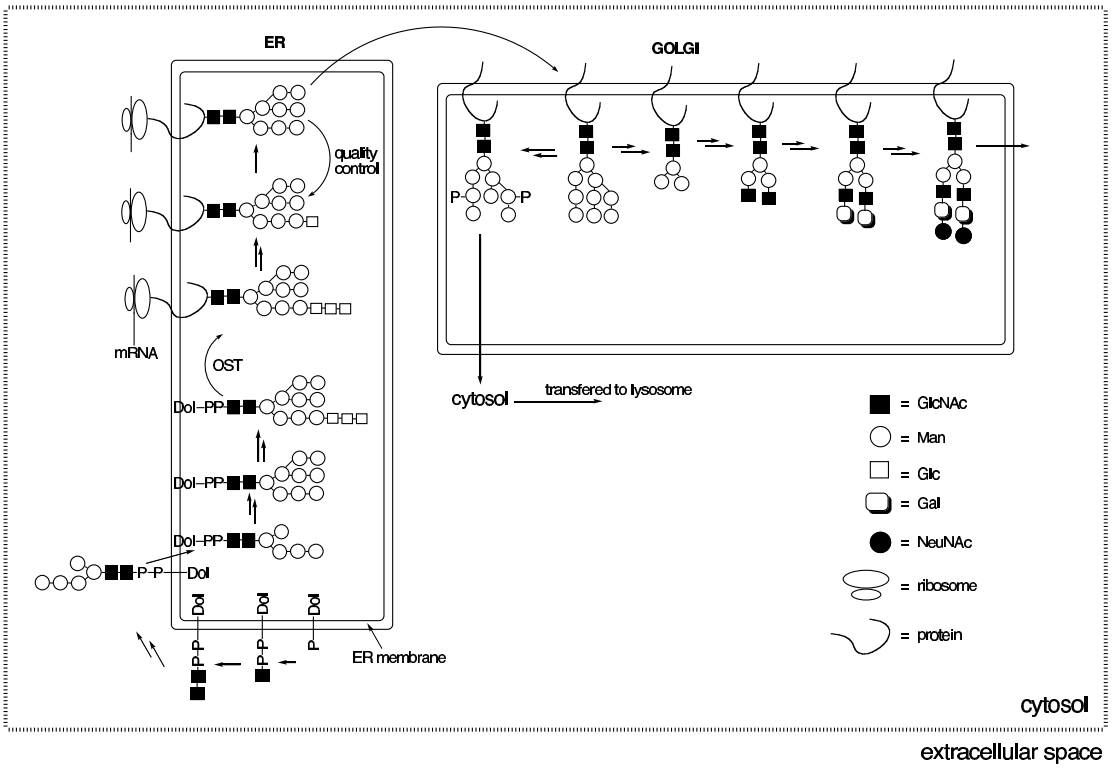


Figure 4. The biosynthesis of complex type N-glycoproteins

The second stage of the biosynthesis of N-linked oligosaccharides involves the transfer of the dolichol-linked oligosaccharide to the growing polypeptide. The oligosaccharide is transferred *en bloc* from dolichol to Asn, which is present in the consensus sequence of Asn-Xaa-Ser/Thr and also in a 'turn' region on the growing peptide while on the luminal side of the ER. The transfer is catalyzed by oligosaccharyltransferase (OST). The third stage of the biosynthesis involves the trimming of three glucose residues and quality control of protein folding. After the removal of the first two Glc residues by glucosidases I and II, the glycosylated peptide is released from the ER membrane. In the lumen of the ER, the polypeptide is allowed to fold with the assistance of chaperone molecules. Following removal of third Glc, the glycopeptide is released from the chaperone molecules and then trimmed further by an α-mannosidase that removes one Man residue. In the case where the process of correct folding fails, the deglucosylated glycopeptide is glucosylated by a specific enzyme for improperly folded glycoproteins. The reglucosylated glycopeptide can be bound again by chaperone to be folded correctly. Uncorrected (misfolded) glycoproteins are transported from the ER-Golgi to the lysosome for degradation; while properly folded glycoproteins are transferred to the Golgi complex. The final stage of processing involves the trimming and branching of the oligosaccharide as the protein is transferred through various Golgi stacks to its ultimate position in the cell.

Synthesis of Glycosylamide Linkage in N-Glycopeptides

It is evident that glycoproteins play an important role in many biological processes. The availability of glycopeptides from natural sources is limited because of

their low concentration. Simplified structures of glycoproteins are needed in large quantity as model compounds for biochemical and structural investigations. The advances in carbohydrate and peptide chemistry in the last decade have provided a variety of methodologies for the synthesis of glycopeptide derivatives.[4,11,12,15,21-25] In this article, only studies towards the synthesis of N-linked glycosylamide linkages between carbohydrates and amino acid or peptides will be discussed.

The crucial step of any synthesis of a glycopeptide is the introduction of the carbohydrate residue to the amino acid or peptide in a stereoselective manner. This should be accomplished under mild conditions that are compatible with existing glycosidic linkages and common protecting groups. There are two general synthetic approaches for the preparation of glycopeptides as outlined in Figure 5. The stepwise approach involves the synthesis of glycosylated asparagine as a building block for the stepwise solid-phase peptide synthesis. Glycosylasparagine building block is synthesized separately either in solution or on solid-phase. This approach is effective for the synthesis of glycopeptides either with a single carbohydrate moiety or with multiple carbohydrates.

The second general approach for glycopeptide synthesis is convergent and there are two types of convergent approaches. The first method involves the condensation of a separately prepared oligosaccharide moiety and a peptide. This method is not suitable for the synthesis of complex glycopeptide synthesis because of the low solubility of peptides in organic solvents and the low yields obtained due to the steric effects imposed by the size of the condensation partners.

The second type of convergent approach consists of the standard peptide synthesis using N-acetyl-glucosylaspartic acid building block. After completion of the peptide chain, the oligosaccharide is extended by either chemical or enzymatic methods. In this approach, the glycosylasparagine building block is synthesized separately. This approach is suitable for solution and solid phase synthesis of either large glycopeptides or glycopeptides with more than one carbohydrate unit. The most effective approach is chosen according to the structural features of the glycopeptide of interest. Following are the different methods for preparation of N-glycosidic linkage and their applications. For any of these general methods to be effective, the stereoselective synthesis of glycopyranosyl asparagine derivatives must be feasible.

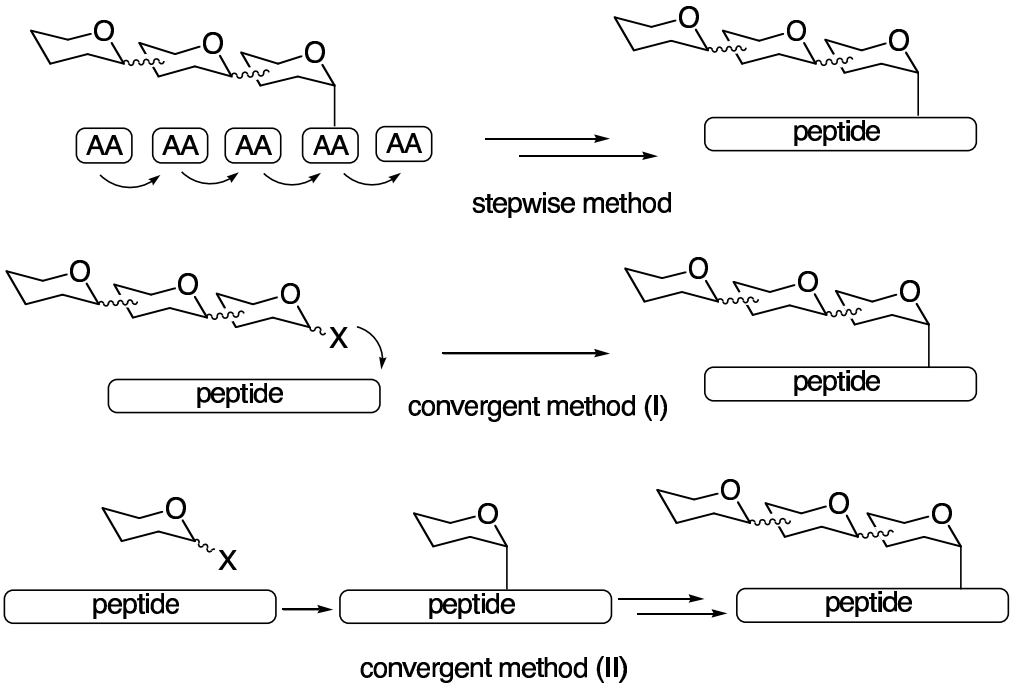


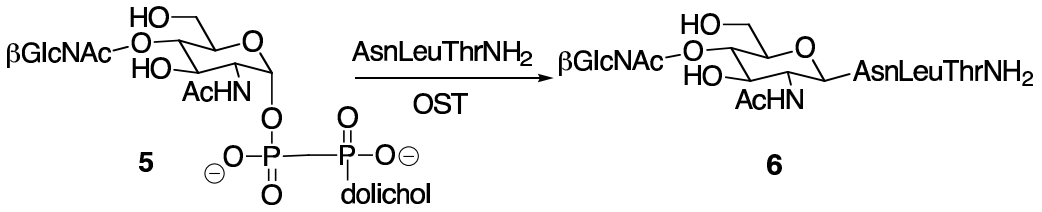
Figure 5. Three conceptual approaches for the preparation of glycopeptides.

Via Oligosaccharidetransferase (OST):Chemoenzymatic Approach

The chemoenzymatic approach requires the coupling of a lipid linked oligosaccharide (i.e. GlcNAc-GlcNAc-PP-dolichol) and peptide using OST enzyme as a catalyst. OST is the enzyme that catalyzes the transfer of the oligosaccharide from oligosaccharide-PP-dolichol to the peptide during the biosynthesis of N-linked glycoproteins.[26-29] It has been shown that smaller oligosaccharide groups, such as chitobiose, linked to dolichol can be transferred to the peptides using OST.[30] However, the peptide undergoing for N-glycosylation must have the Asn-Xaa-Ser/Thr consensus sequence.[31,32]

Coward has prepared glycopeptide **6** by catalyzing the coupling of a tripeptide with chitobiosyl-PP-dolichol (**5**) using enzyme OST (Scheme 1).[33] The advantages of using OST are the excellent stereoselectivity and specificity of the coupling, and the ability to use unprotected sugar and peptide units. Nonetheless, several disadvantages make this method less attractive. The most serious limitation is the inability to isolate significant quantities of this enzyme OST from natural sources. In addition, this enzyme cannot be used to synthesize non-natural glycopeptides lacking the consensus amino acid sequence. Accordingly, the chemoenzymatic approach for the synthesis of glycopeptides is employed for the enzymatic synthesis of the glycan using glycosyltransferases or glycosidases.[24,34]

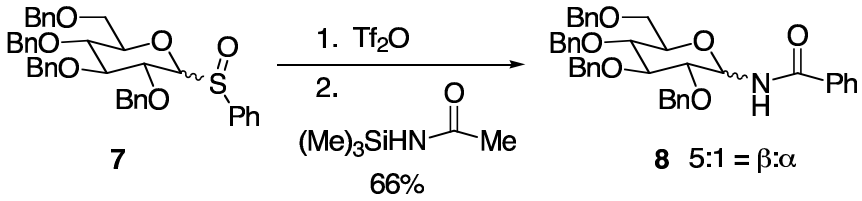
Scheme 1



Via Glycosyl Thioglycosides: Biomimetic Approach

O-Glycosylation based on activation of thioglycosides has been studied extensively; however, there is only one instance where a glycosylthiosulfoxo group has served as a glycosyl donor for the glycosylation of an amide nitrogen (Scheme 2).[35] Kahne has reported that the activation of glycosylthiosulfoxide **7** with triflic anhydride followed by coupling with a silylated amide gave amide **8.** The high β-stereoselectivity of the reaction depended on the polarity of the solvent and C2 neighboring group participation. Further application of this methodology to glycopeptide synthesis has not been demonstrated.

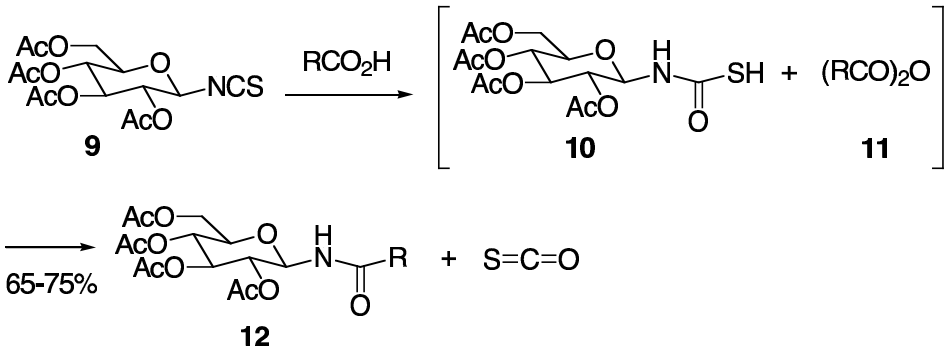
Scheme 2



Via Glycosyl Isothiocyanate

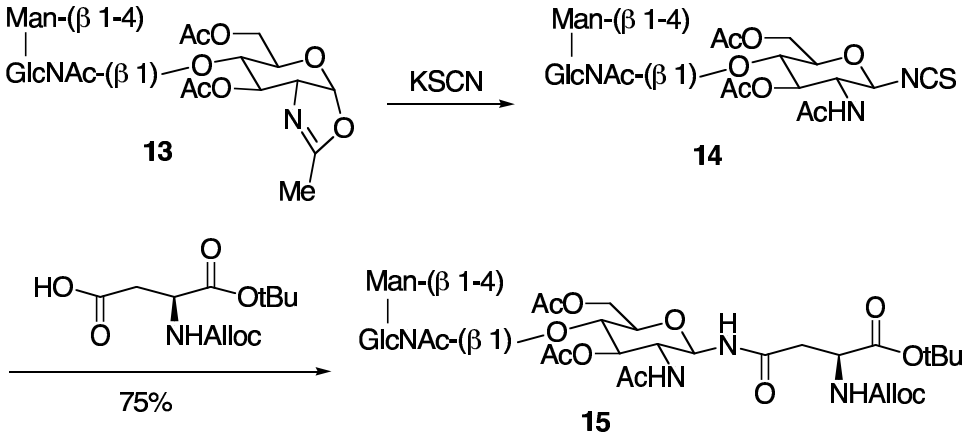
Khorlin showed that the reaction of β-glycosyl isothiocyanate **9** with a slight excess of carboxylic acid in the presence of triethylamine provided the corresponding glycosylamides in moderate to good yields (Scheme 3).[36] The starting β-acetylisothiocyanates (**9**) were obtained in high yields (90%) by treatment of α-glycosyl halides with silver or ammonium thiocyanate.[37] The reaction occurred by nucleophilic attack on the isothiocyanate **9** by the carboxylic acid leading to the formation of the glycosyl thiocarbamic acid **10** and the symmetrical anhydride **11**.

Scheme 3



The loss of SCO from **10** gave the glycosylamine which underwent coupling with the anhydride formed *in situ* to give the glycosylamide **12.** The authors were able to synthesize the aspartoyl derivative of **12** in 70% yield from **9** and a protected aspartic acid derivative.[36]

Scheme 4

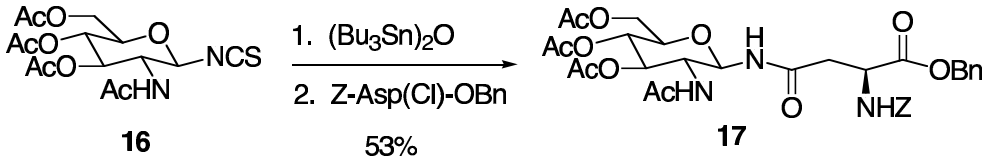


Application of this coupling methodology was reported by the Kunz group for the synthesis of β-mannosyl-chitobiosyl-asparagine conjugate **15** (Scheme 4).[38]

Isothiocyanate **14** was obtained by the reaction of oxazoline **13** with KSCN. It is of the interest to note that attempts to convert oxazoline **13** into the corresponding azide failed. Reaction of isothiocyanate **14** with protected aspartic acid derivative under anhydrous conditions afforded β-amide **15** in 75% yield.

The Santoyo-Gonzalez group developed a modification of this procedure in which a glycosyl isothiocyanate was reduced using bis(tributyltin) oxide to produce a glycosylamine for *in situ* coupling with a carboxylic acid or acid chloride (Scheme 5).[39] For example, isocyanate **16** was coupled with protected aspartic acid chloride and aspartoyl derivative **17** was obtained in 53% yield.

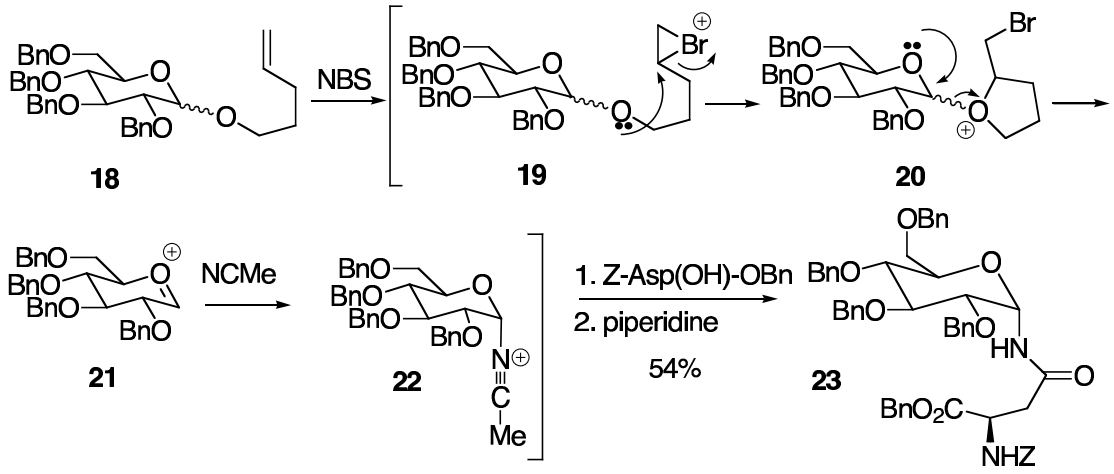
Scheme 5



Via Pentenyl Glycosides

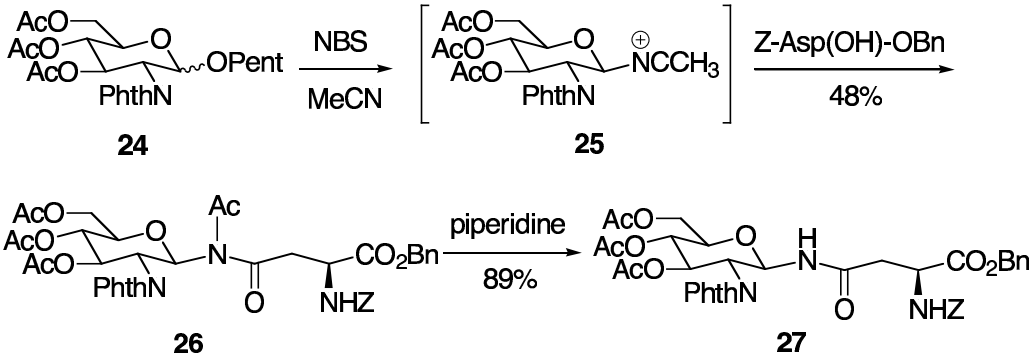
Fraser-Reid has developed an N-glycosylation method which is based on his earlier glycosylation methodology.[40] This new approach allows for the direct coupling of a carboxylic acid with a n-pentenyl glycoside without generating a glycosylamine intermediate.[41-43] The method produces an electrophilic carboxonium ion **20** from the oxidative hydrolysis of an anomeric O-pentenyl glycoside **18** using *N*-bromosuccinimide (NBS) (Scheme 6).

Scheme 6



Trapping of the Koenigs-Knorr intermediate **21** by acetonitrile afforded an α-nitrilium ion **22.** Addition of a carboxylic acid to **22** afforded the α-linked glycosylated amino acid. The acetyl group of the intermediate imide was removed selectively upon treatment with piperidine to give only α-*N*-glycosylamino acid **23.**[44] This methodology is the best to that date for providing α-glycosylamide linkages in a stereoselective manner.

Scheme 7



Synthesis of the naturally occurring β-N-glycosidic linkage was accomplished by generating β-nitrilium ion (**25**) as an intermediate (Scheme 7).[45,46] Neighboring group participation *via* the sterically hindered phthalimide (Phth) group in the C-2 position was responsible for the excellent β-stereoselectivity in the coupling with aspartic acid derivatives.

Via Glycosylamines

The most commonly employed synthetic method for the construction of the glycosylamide linkage is the condensation of glycosylamines with protected acid derivatives in the presence of coupling reagents such as dicyclohexyl carbodiimide (DCC) and N-hydroxybenzotriazole (HOBt).[24,47]

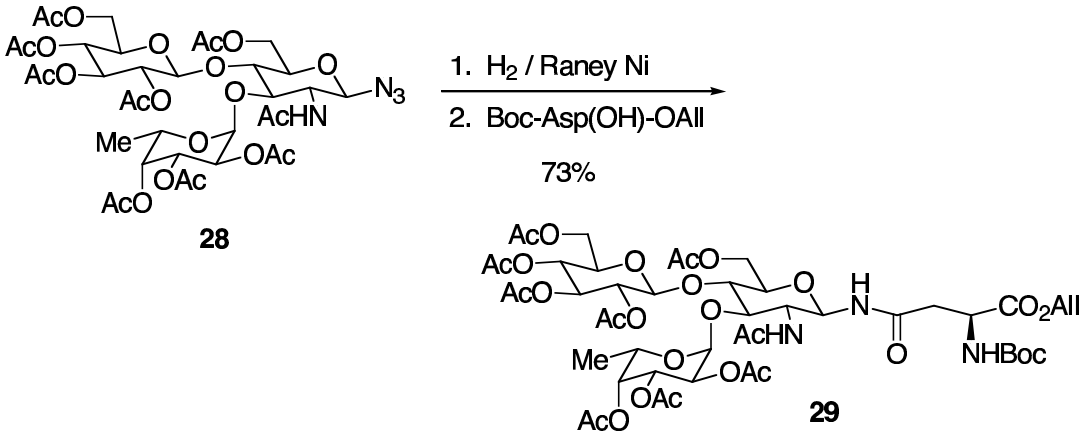
Despite the obvious simplicity, this methodology suffers from several disadvantages stemming from the hydrolytic instability of glycosylamines. Glycosylamines hydrolyze rapidly under acidic or neutral conditions and α/β anomerization is often difficult to control.[48] Under the aminolysis conditions, glycosylamines can dimerize spontaneously to form diglycopranosyl amines (**40**, Scheme 12). A second side reaction is that under reducing conditions there is possibility of O→N acetyl migration.[48,49] Finally, under basic conditions either sugar protection groups or the sensitive glycosidic linkages are prone to cleavage.[49]

I: Protected Glycosylamines via Glycosylazides

This is the most commonly used method for the synthesis of glycopeptides in solution or solid-phase synthesis.[2,22,47] The reduction of glycosyl azides affords glycosylamines. The reduction can be performed by catalytic hydrogenation with the use of variety of metals (Pd/C, Lindlar catalyst, PtO_2, Raney Ni, Al/Hg) or propanedithiol in the presence of triethylamine.[50-55] However, there are limitations to the methodology: under reducing conditions anomerization, acetyl migration, hydrolysis and dimerization commonly.[56,57] The severity of these problems is dependent on the solvent system employed, the protecting groups on the sugar, the size of the carbohydrate moiety, and the catalyst used for hydrogenation. In order to minimize these problems, the labile glycosylamines must be converted immediately into glycosylamides by the treatment with activated aspartic acid derivatives.

The preparation of glycosyl azides has been extensively studied and they can be accessed in excellent yields (80-95%) and with high stereoselectivity.[58-63] Glycosyl azides are prepared from glycosyl halides, acetates, oxazolines, or glycals. Using a glycosyl acetate, oxazoline, or glycal as a precursor provides only β-glycosyl azide, while using β- or α-glycosyl halides can provide either α- or β-glycosyl azides, respectively.[60]

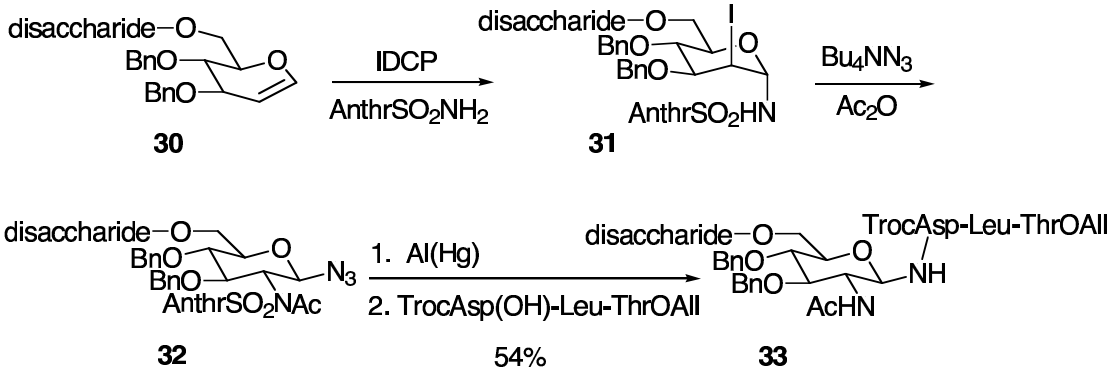
Scheme 8



The Kunz group applied the reductive coupling methodology to the synthesis of glycosylamide **29** (Scheme 8).[2,64] The reduction of the azide **28** by Raney Ni to the corresponding amine followed by immediate condensation with the protected aspartic acid derivative furnished the N-glycosyl asparagine conjugate **29** in 73% yield from the azide.

The Danishefsky group has developed a method for glycosylamide synthesis in which the reaction of a glycal **30** with anthracenesulfonamide in the presence of iodonium di-*sym*-collidine perchlorate (IDCP) afforded *trans*-2-iodo-1-*N*-sulfonamide **31** in 78% yield (Scheme 9).[62,65,66] Treatment of iodosulfonamide **31** with Bu_4NN_3 provided azide **32** quantitatively with stereoselective migration of the sulfonamide group followed by the installation of azide at the anomeric center. Subsequent reduction of azide **32** and removal of sulfonate functionality with aluminum amalgam in the presence of diisopropylamine led to the formation of the corresponding protected glycosylamine that coupled with tripeptide to give glycosylpeptide **33.**

Scheme 9



Although there are problems associated with generating glycosylamine intermediates by reduction, many of these limitations have been overcome in the last decade by the judicious choice of reducing reagent, such as Raney Ni or Pd/C, or protecting groups.[54] However, anomerization during the coupling remains a significant problem. For example, reduction of pentasaccharide azide **34** (Figure 6) under either Raney Ni or Pd/C conditions followed by immediate coupling with a dipeptide gave a 1:1 α/β mixture of the N-glycosylpeptide in 67% yield.[54] This result shows that anomerization remains a limitation in the coupling of complex glycosyl amines obtained by the reduction of the anomeric azide.

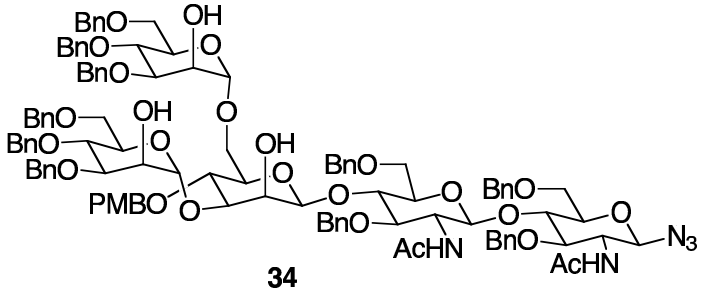
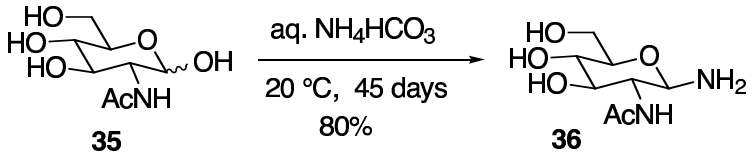


Figure 6. Structure of the pentasaccharide which yields α/β mixtures when reduced.

II: Unprotected Glycosylamines

Coupling methods based on the use of unprotected glycosyl derivatives have been developed to avoid the protection and deprotection steps usually necessary. The reaction of ammonia or primary amines with unprotected sugars yields glycosylamines (Scheme 10). Kochetkov and co-workers reported a one-step synthesis of unprotected β-glycosylamine **36** using aqueous ammonium bicarbonate solution in 50-80% yield.[67] The reaction was highly dependent on the temperature. The yield of **36** was 80% when the reaction was performed at 20 °C for 45 days.

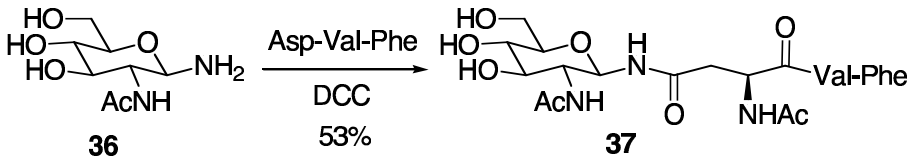
Scheme 10



35 **36**

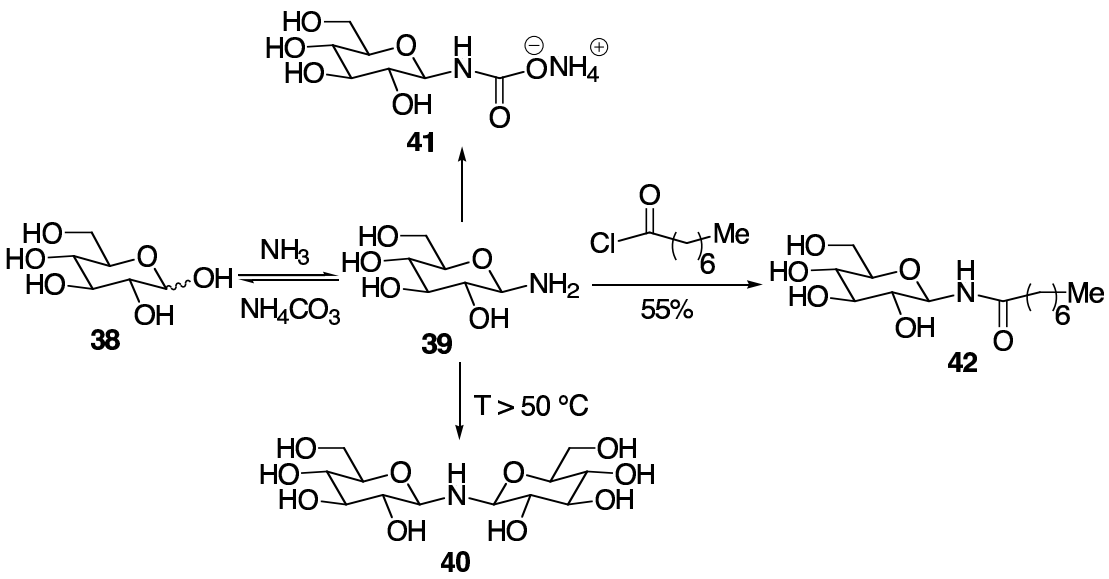
Using Kochetkov's method, the Lansbury group synthesized N-glycopeptide **37** by coupling the tripeptide with glycosylamine **36.** (Scheme 11).[68] However, incomplete removal of the ammonia formed during the synthesis of the glycosylamines caused side reactions. Under optimized conditions unprotected glycosyl peptides were obtained in 50-60% isolated yield. The optimized conditions could be applied also to the convergent solid-phase synthesis of N-glycopeptides.[69]

Scheme 11



36 **37**

Scheme 12

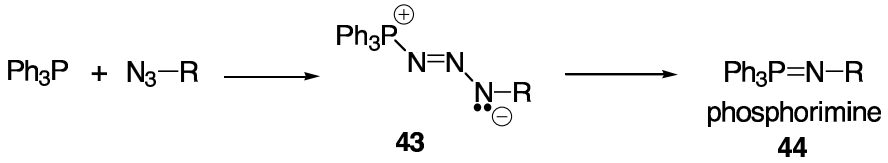


This method was improved by Lubineau et al. by the utilization of an equimolar amount of ammonium bicarbonate in the presence of ammonia (Scheme 12).[48] β-Glucosylamine **39** was synthesized quantitatively by reacting 0.2 M glucose with 16 M ammonia and 0.2 M ammonium bicarbonate at 42 °C for 36 h When the reaction mixture containing **39** was heated above 50 °C, dimerization of the monosaccharide occurred to yield bis-saccharide **40**. When a higher concentration of ammonium bicarbonate was used, glucosyl carbamate **41** became the major product. The reaction of **39** with octyl chloride at 0 °C gave the corresponding N-octanoyl-β-glucosylamine **42** in 55% yield. There are also examples that use this methodology for the coupling of unprotected di, tri- or oligosaccharide amines with amino acid derivatives.[70-73]

Via Glycosyl Phosphorimine: Staudinger Reaction

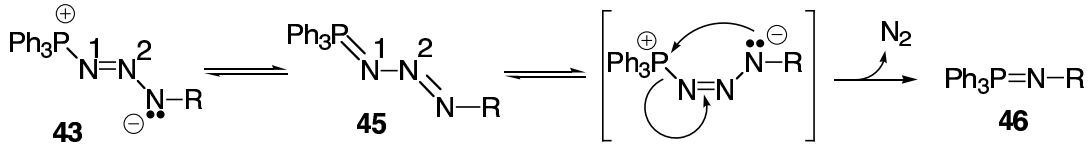
The Staudinger reaction in its classical form is a two-step process involving the initial electrophilic addition of an azide to a phosphine (e.g. trialkyl- or triaryl phosphines) followed by nitrogen elimination from the intermediate phosphazide **43** to give phosphorimine **44** (also called an iminophosphorane or phosphazene) (Scheme 13).[74-76] Usually, the imination proceeds smoothly, almost quantitatively, without the formation of side products.

Scheme 13

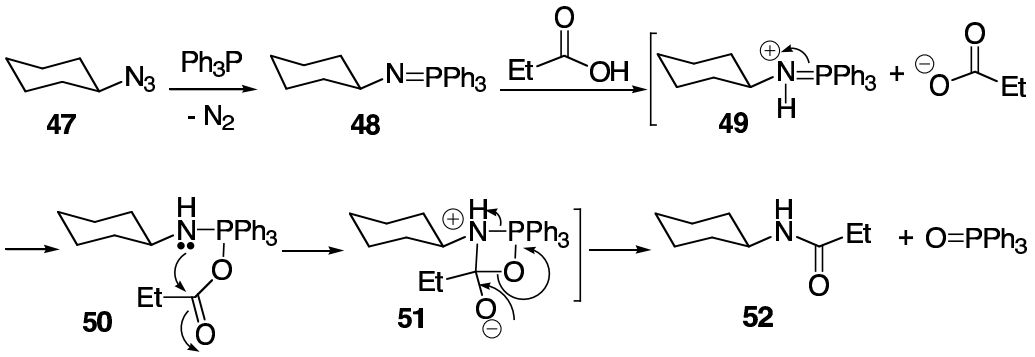


The structure of the phosphazide intermediate was elucidated by X-ray crystallography (Scheme 14).[75] It was found that PN_3 part of a phosphazide is acyclic, which means that the azide is attacked by the P^{+3} site to its terminal nitrogen atom. The chain PNNNC of **45** is nearly planar and has an *E*-configuration with respect to the central N^1-N^2 bond, which partially exhibits significant double bond character.

Scheme 14

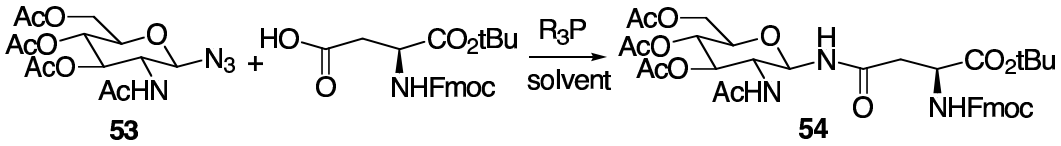


Scheme 15



Since the Staudinger reaction avoids the problematic glycosylamine intermediate and can be performed under mild, neutral conditions, it has been used recently for the synthesis of N-glycosyl amino acid adducts. The first example of the use of the Staudinger reaction for amide formation is reported by the Vilarrasa group (Scheme 15).[77] The scope and limitations of the reaction was determined using alkyl- or aryl azides to couple with aliphatic or aromatic carboxylic acids in the presence of Ph_3P. Phosphorimine **48** was formed from β-azide **47** by the release of nitrogen as described above. The carboxylic acid was deprotonated by phosphorimine **48** and the carboxylate attacked phosphorus. Intermediate **51** was formed by attack of amine, followed by immediate release of triphenylphosphine oxide which gave glycosyl amide **52**.

Table 1. Staudinger-type couplings performed by Inazu

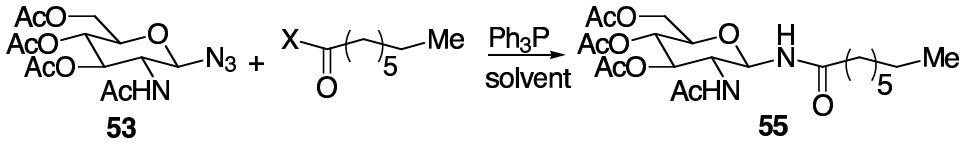


53 **54**

entry	solvent	R_3P	yield (%)
a	CH_2Cl_2	Et_3P	77
b	MeOH	Et_3P	0
c	CH_2Cl_2	Bu_3P	52
d	CH_2Cl_2	Ph_3P	0

The first application of this methodology to the synthesis of *N*-glycopeptides was reported by the Inazu group (Table 1).[78] It was shown that the reaction is highly solvent dependent and worked best in nonpolar solvents such as methylene chloride. In polar, protic solvents no amide was obtained (Table 1, entry b). The couplings utilizing more sterically demanding alkyl phosphines such as tributylphosphine gave lower yields (Table 1, entry c). The coupling reaction using less reactive triphenylphosphine did not give the desired glycosylamide product (Table 1, entry d). The Inazu group improved their yields by performing the coupling reactions at lower temperatures.[79] Later, a derivative of **54** was synthesized in 54% yield using Boc-Asp(OH)-OBn. The resulted glycosylated amino acid was used as a building block for elongation of the peptide moiety followed by enzymatic elongation of the saccharide moiety.[80]

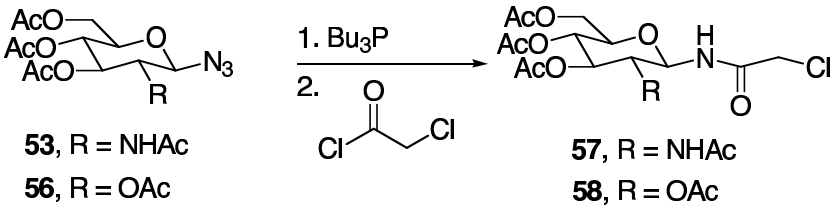
Table 2. Staudinger-type couplings performed by Boullanger.



entry	solvent	X	yield (%)
a	C_6H_6	OH	0
b	C_6H_6	Cl	67
c	CH_2Cl_2	Cl	60
d	toluene	Cl	56

The Boullanger group prepared glycosyl amides using an acid chloride in place of a carboxylic acid, in the presence of less reactive, but easy to handle triphenylphosphine (Table 2).[81,82] Boullanger was able to prepare β-glycosyl amide **55** from β-azide **53** at room temperature. When the same coupling reaction was performed with the corresponding α-glycosyl azide, anomeric mixtures were obtained. The reaction was highly solvent dependent, working best in nonpolar solvents such as benzene, methylene chloride, and toluene (Table 2, entries b-d). The Boullanger group also reported that the coupling of carboxylic acids with glycosyl azides using triphenylphosphine did not provide glycosylamide **55** (Table 2, entry a). When triphenylphosphine was added to a solution of azide, nitrogen release was immediate and the ^{31}P and ^{1}H NMR spectra indicated the formation of β-phosphorimine. In the absence of an acetylating agent, β-phosphorimine epimerized to a 1:1 α/β mixture over 8 h. An equilibrium mixture of 1:4/β:α was reached after 24 h. This result supports the well-known phenomena of anomerization of phosphorimines.[82]

Scheme 16



53, R = NHAc
56, R = OAc

57, R = NHAc
58, R = OAc

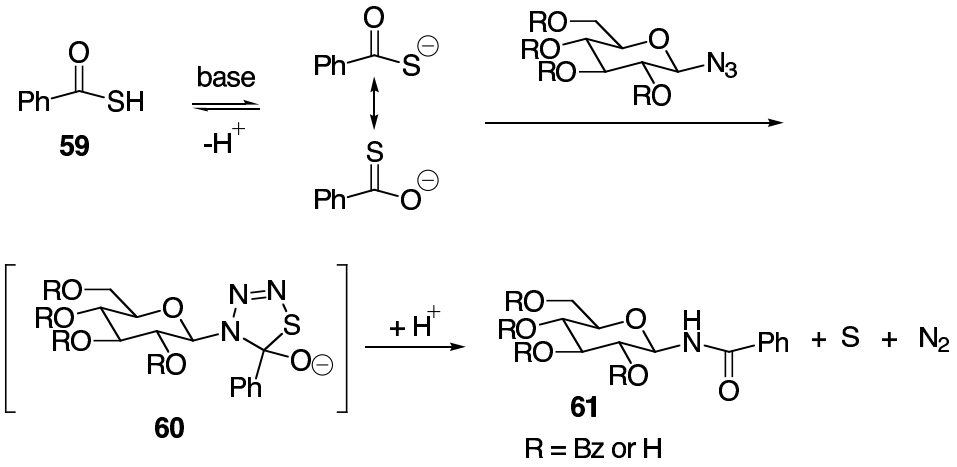
The Santoyo-Gonzalez group also used the Staudinger reaction for the synthesis of chloroacetyl N-glycosides **57** and **58** (Scheme 16).[83,84] When glycosyl azide **56** was coupled with chloroacetic anhydride and Bu_3P under Boullanger's conditions, glycosylamide **58** was obtained in 75% yield. However, in contradiction to the reports of Boulanger and Inazu (shown in Table 1 and Table 2), when 2-N-acetylglucosyl-β-azide **53** was used, glycosylamide **57** was not obtained. This result is attributed to the poor reactivity of the phosphorimine intermediate due to the hydrogen bonding between nitrogen of the phosphorimine and the hydrogen of the C2 amide.

Via Thio Acids and Glycosylazides

Recently a new method was reported by Lawrence for the synthesis of glycosylamides utilizing glycosylazides and thioacids.[85,86] According to the authors this coupling reaction proceeds by a different mechanism than that of the traditional Staudinger reaction. Under basic conditions, the thiocarboxylate reacted with the azide to give intermediate **60** (Scheme 17). Decomposition of **60**, either in a stepwise manner or by retro-[2+3] reaction, gave amide **61**. The coupling procedure was applied to a variety of azides, such as aryl, alkyl, vinyl and glucosyl azides to demonstrate the generality of the methodology. The advantage of this method is the

ability of both protected or unprotected glycosyl azides to undergo the coupling in either chloroform or water. When protected glucosyl azide (R = Bz) was used, amide **61** was obtained in 64% yield; unprotected glucosyl azide (R = H) afforded 83% yield of amide. This method has not been applied yet to the synthesis of the glycopeptides.

Scheme 17



Solid-Phase Synthesis of N-Glycopeptides

The preparation of larger glycopeptides typically requires multistep transformations involving iterative protection-coupling-deprotection reactions and chromatographic purification of intermediates at each stage of the synthesis. Thus, polymer supported peptide and oligosaccharide synthesis has become an attractive to solution phase sequences.[25] In solution phase synthesis, the convergent coupling of carbohydrates and large peptides is difficult due to the problems associated with the separation of the product from the unreacted components.[87] Almost all solid-phase synthesis of glycosylamide linkage have been accomplished by coupling of a

glycosylamine, obtained by the reduction of azide, with an activated ester of the aspartic acid derivative.[88-94]

Recently, the Danishefsky group utilized the unprotected glycosylamine method in solid-phase synthesis of glycopeptide derivatives.[72,95] Similarly, the Toth group showed that the Staudinger reaction is compatible also with both Boc and Fmoc solid-phase glycopeptide strategies on a variety of resins.[96]

The three approaches used in solid-phase glycopeptide synthesis are similar to those for solution-phase synthesis which are summarized in Figure 5.[92] The convergent approach involves coupling of a large oligosaccharide to a polypeptide and suffers from low yields, due to steric factors.

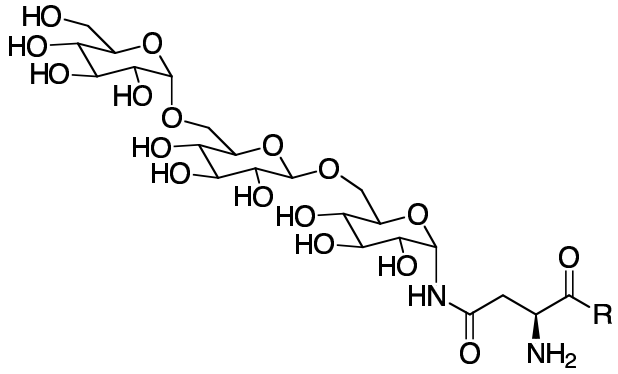
The second approach involves the use of an amino acid derivative with a large pendant oligosaccharide attached. This approach is limited by the rapid drop-off of coupling yields as the oligosaccharide increases in size and branching.[97] For example, Meldal has shown that as the saccharide size increased, yields of the product dropped from 80% to 35%.[98]

The third approach involves the solid-phase synthesis of a singly glycosylated peptide in which a monosaccharide is deprotected selectively and an oligosaccharide is built up by enzymatic or chemical reactions.[93,99]

Special Case I: Synthesis of α-N-Glucopyranosylamide Linkages

The β-2-acetamido-2-deoxy-glucopyranosyl amide linkage is a common link present in most N-linked glycopeptides (Figure 1, **1**) and there are variety of synthetic protocols designed to provide this linkage. The discovery of the naturally occurring

α-N-glucosylamide linkage in the N-glucosyl peptide nephritogenoside led chemists to investigate new stereoselective coupling methodologies.

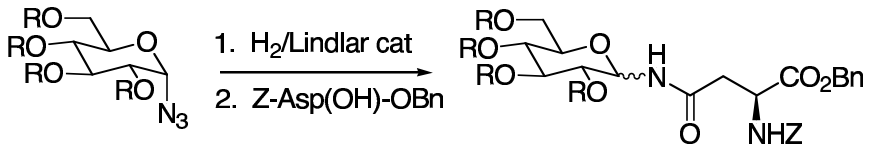


R = NH-Pro-Leu-Phe-Gly-Ile-Ala-Gly-Glu-Asp-Pro-Thr-Gly-Pro-Ser-Gly-Ile-Val-Gly-Gln-OH

Figure 7. Structure of the *N*-glycopeptide Nephritogenoside.

Nephritogenoside (Figure 7) is a strong inducer of chronic progressive glomerulonephritis in animals.[6,100-103] However, isolation and purification of this compound from natural sources are very difficult. Several syntheses of nephritogenoside have been reported.[42,51,104-112] The stereoselective preparation of the α-N-glycopeptide linkage has proven to be the most difficult aspect of these approaches.

Scheme 18



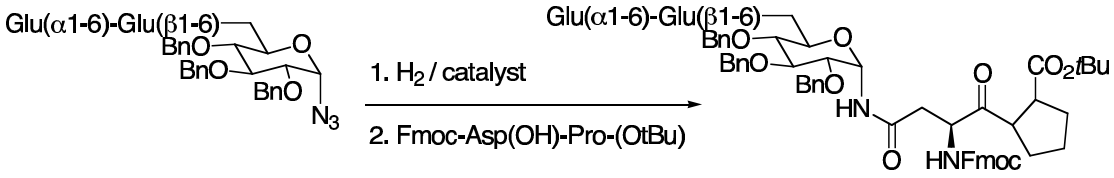
R = Ac, α:β (1:10), 70%

R = Bn, α:β (1:3), 76%

As shown in Scheme 18, the reductive couplings of glucopyranosyl-α-azide with aspartic acid derivatives gave the glucosylamide as mixtures of anomers.[104,113] Anomerization of the intermediate α-glucosylamine occurred under the reducing/coupling conditions, and the ratio of anomers obtained depended upon the catalyst choice and neighboring group participation of substituents at C2 and C6 on the glycosyl moiety.[113]

The key α-N-glucosylamide linkage in these syntheses has been made most frequently by the reduction of triglucosyl-α-azide to its triglucosylamine and subsequent coupling with a dipeptide (Scheme 19).[51,104,105,107] While this approach gave the desired glycodipeptide in good yield (65-80%), the stereoselectivity at the anomeric center was poor: α/β mixtures ratios ranged from 1:5 to 5:1.

Scheme 19

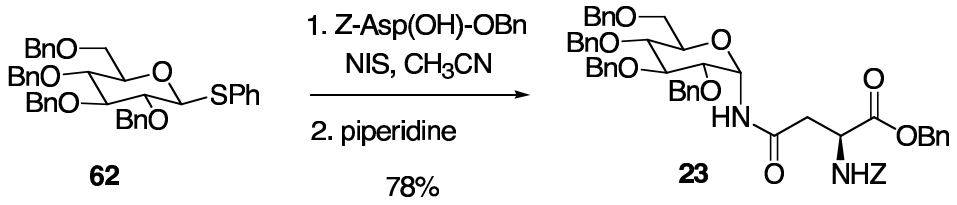


catalyst : Pd/C, α:β (1:5), 81%

catalyst : Lindlar, α:β (5:1), 64-76%

In an effort to limit anomerization, coupling protocols for the synthesis of α-glycopeptide linkages have been developed which avoid intermediate formation of the free amine. Fraser-Reid has shown that condensation of O-pentenyl glycosides with aspartic acid in the presence of NBS and acetonitrile afforded only the α-N-glucosylamide **23** in 54% (Scheme 6).[42]

Scheme 20



The Sasaki group improved the Fraser-Reid method by using phenyl thioglycoside **62** and NIS as activators, which resulted in the formation of α-N-glucosylamide **23** in 78% yield (Scheme 20).[112] The main disadvantage of this method is the need to piperidine making this method incompatible with the Fmoc protection strategy of the peptide portion. In addition, this method has never been applied to the coupling of di- or trisaccharides.

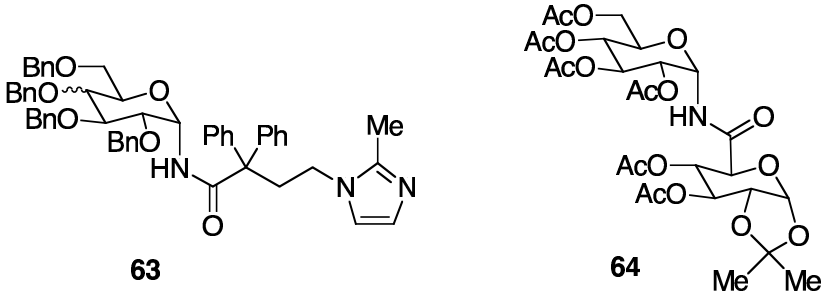


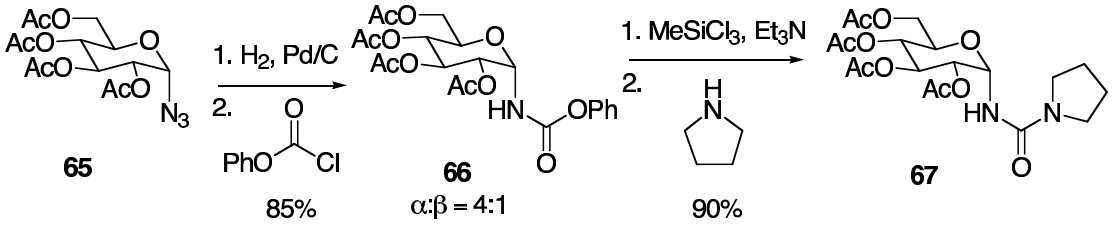
Figure 8. Compounds containing α-N-glucosylamide linkage.

There are also several other compounds possessing the α-N-glucosylamide linkage that have been synthesized either for their biological activity or as part of activity-structure studies. Compound **63** (Figure 8) had higher selectivity for binding muscarinic receptor in smooth muscle than the muscarinic receptor in heart and is a potent antagonist of the muscarinic receptor. Thus, this compound is useful for the

treatment of the pollakiurea (frequent urination) and urinary incontinence.[114] Oligosaccharide mimetic **64** (Figure 8) has been synthesized at DuPont and was shown to be more stable to chemical hydrolysis than its disaccharide analog.[115]

Compounds containing the urea functionality are of biological interest as antimycobacterial agents and inhibitors of HIV protease and series of glycosyl ureas have shown to be α-glucosidase inhibitors.[116-119] Compound **66** has been shown to be a useful synthon for the synthesis of α-N-glucosyl ureas in high yields (Scheme 21).[120] Phenyl carbamate **66** was synthesized by the reductive coupling of α-glucosyl azide **65** with phenyl chloroformate in 85% yield as α/β (4:1) mixture.

Scheme 21



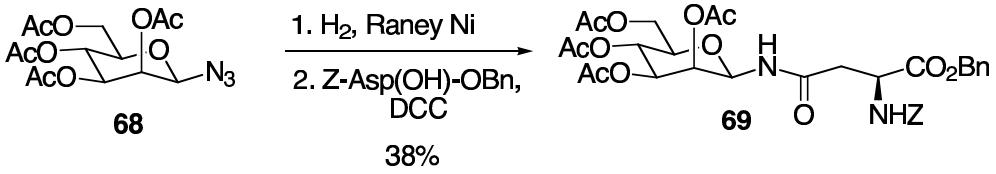
Alternatively, reaction of an α-glycosyl azide with a carboxylic acid in the presence of a tertiary phosphine (the Staudinger reaction) also afforded α-N-linked glycopeptides, although anomerization remains as a significant problem.[121]

Special Case II: Synthesis of β-N-Mannopyranosylamide Linkages

There is one example of a β-O-mannoside linkage found in mammalian oligosaccharides, but this is located at the N-glycan pentasaccharide core of all N-glycoproteins.[24] The formation of this ubiquitous bond has become one the most

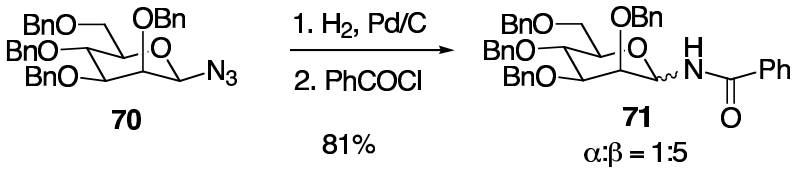
difficult issues in oligosaccharide chemistry. On the other hand, since no naturally occurring β-N-mannosyl linkage has been found, its synthesis has not been significantly explored.

Scheme 22



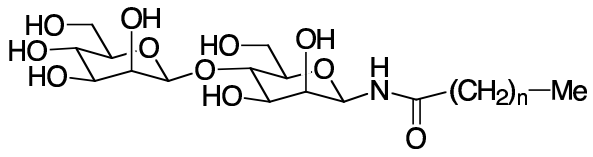
Few attempts have been made to synthesize compounds possessing the β-N-mannosyl linkage. Goals for the synthesis includes the structure-activity studies, determination of biological activities, or use as liposome components. In all cases the β-N-mannosyl linkage was constructed by the reductive coupling of the β-mannosyl azide resulting in α/β mixtures of the glycosylamide. Tanaka and Ponpipom synthesized the β-N-mannosyl aspartic acid derivative **69** in 38% yield from β-azide **68**, in which they separated the β-mannosyl amine from the α-mannosyl amine and then coupled the desired anomer with the aspartic acid derivative (Scheme 22).[122-124]

Scheme 23



The Fraser-Reid group synthesized β-N-mannosyl derivative **71** as α/β (1:5) mixture in 81% yield (Scheme 23).[125] Mannobiose derivatives (**72**) were synthesized

to be useful components of the pharmaceutical preparation of liposome derivatives (Figure 9).[126]



72 n = 10, 12, 16, or 18

Figure 9. The structure of β-mannobiose derivatives used as liposome components.

The Goal of the Research

As discussed above, there are important biologically active natural and unnatural products possessing the N-glycosylamide linkage. Accordingly, a general approach to the stereoselective synthesis of this functionality is required. In spite of significant progress over the few decades, the synthesis of the N-glycosylamide linkage still presents a significant challenge, especially the stereoselective synthesis of α-N-glucopyranosyl amide and β-N-mannopyranosyl amide linkages. One of the goals of this research was to develop a new methodology for the stereoselective synthesis of α-N-glucopyranosyl amide and β-N-mannopyranosyl amide linkages in high yields. This methodology should be compatible with both Boc and Fmoc peptide synthetic strategies and should allow the convergent synthesis of glycosyl amide linkage from an oligosaccharide and a peptide. The second goal of this research was to apply the resulting methodology to the stereoselective synthesis of both α- and β-2-N-acetamido-2-deoxy-glucopyranosyl amide linkages.

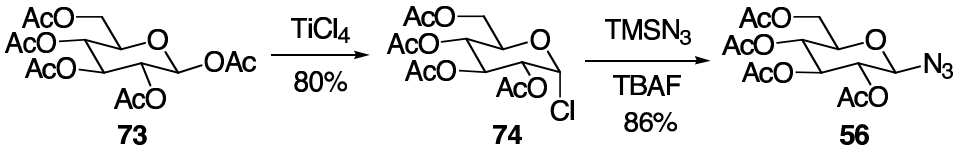
Results and Discussion

Synthesis of Glycosyl Isoxazolines

This research project, designed to develope new methods for glycoprotein synthesis, included three distinct sub-goals: first, stereoselective syntheses of glycopyranosyl azides were to be achieved. Once the azides were prepared, the second phase was to demonstrate that glycosyl isoxazolines could be prepared via phosphorimine-based cyclization. Finally, the use of the isoxazolines for coupling with activated amino acid derivatives were addressed. The results from each phase are discussed below.

For our studies, tetracetate-β-glucopyranosyl azide was selected as the initial coupling partner for investigation in the modified Staudinger methodology. β-Azide **56** was prepared from α-chloride **74** in 85% yield according to the procedure developed in our lab by Soli (Scheme 24).[60]

Scheme 24

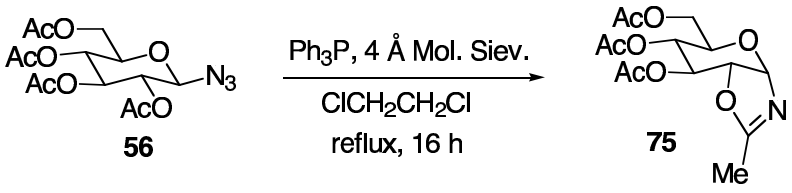


Isoxazoline **75** was isolated from the reaction of azide **56** with triphenylphosphine in the presence of molecular sieves in 55% yield (Scheme 25).[127] The incorporation of molecular sieves into the reaction mixture was crucial to the success of the cyclization although the exact role of the sieves is unclear. We do not believe that sieves are serving solely to remove traces of water from the reaction

mixture since neither the starting material nor the product are highly susceptible to traces of water. It is more likely that molecular sieves function as a weak Lewis acidic catalyst to assist in activation of the ester carbonyl group.

Infrared (IR) spectroscopy, ^{1}H and ^{13}C NMR, 1D-selective nuclear overhauser effect spectroscopy (NOESY), correlated spectroscopy (COSY), and mass spectrometry were employed in the characterization of the structure of isoxazoline 7**5.**

Scheme 25



The peak at 1667 cm^{-1} in the IR spectrum and the lack of an amide proton absorbance at about 3400 cm^{-1} were consistent with the isoxazoline function. According to the mass spectra, the molecular ion peak [$(M+H)^{+}$ = 330], which is 44 less than that of the starting azide **56**, corresponds to a structure which has lost two nitrogen and one oxygen atom relative to the starting material. Since there is a large coupling constant for H1 proton ($J_{1\text{-}2}$ = 7.6), which is usually between 7 and 10 Hz for diaxial proton couplings, and the starting material had also β-configuration, isoxazoline was initially thought to have the trans H1-H2 or β-configuration (Figure 10, see **76**).

However, the coupling constants, $J_{2\text{-}3}$ and $J_{3\text{-}4}$ were 4.0, which are unexpectedly small for diaxial proton couplings. These small coupling constants indicate that the strained five membered ring of isoxazoline causes distortion of the sugar pyranose

ring from its typical chair conformation. It has been shown that isomeric glucopyranosyl oxazoline **77** (prepared from NAc-glucosamine derivative) adopts the distorted chair conformation, resulting in a small coupling constants (2.5 Hz) for $J_{3\text{-}4}$ and large coupling constant (7.5) for $J_{1\text{-}2}$.[128] As determined by X-ray, ^{1}H NMR, and computational analysis, oxazoline **77** adopts the skew conformation of the pyranoid ring as shown in Figure 10. The spectral similarities between oxazoline **75** and **77** suggested that the α-isoxazoline **75** had been formed by reaction of azide **56** with triphenylphosphine. From the selective 1D-NOESY spectrum, there is a relatively stronger H1-H2 NOE (7.5%) than the H2-H3 NOE (3.5%) and no H1-H3 NOE, which is consistent with the H1-H2 syn-relationship.

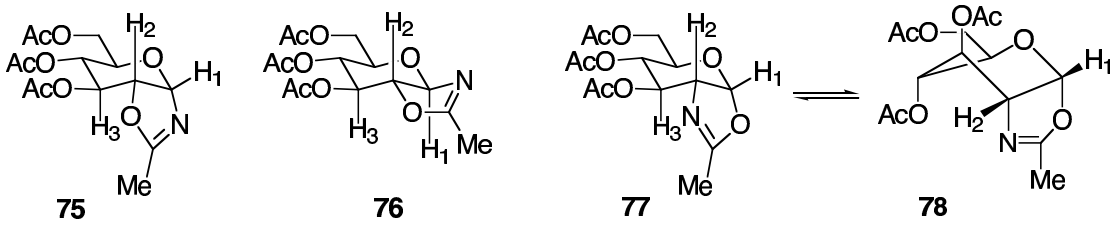


Figure 10. Proposed structures for the glucosyl isoxazoline and oxazoline.

In the ^{13}C NMR spectrum, the methyl carbons of acetyl groups of glycosyl derivatives are normally located between δ 20-21 ppm. The ^{13}C NMR spectrum of isoxazoline **75** has one characteristic peak at δ14.1 ppm that we attributed to the carbon of the methyl group of the isoxazoline ring. The methyl carbon of oxazoline ring in **77** also has the analogous signal at δ13.8 ppm. Subsequent analysis of isoxazoline derivatives (vide infra) demonstrated that the peak at ca. δ14.0 ppm in the ^{13}C NMR spectra was characteristic for all the glucopyranosyl isoxazolines.

The results from these observations and the similarities of ^{1}H spectral data of the reaction product with that of oxazoline **77** led us to conclude that the compound obtained had the α-configuration **75.** Later, the proposed structure was also confirmed by the results of coupling reactions that provided α-glucosylamide products.

The $CDCl_3$ solution of isoxazoline **75** could be kept for two days without decomposition. Refluxing the glucosyl isoxazoline solution at 83 °C for 24 h longer

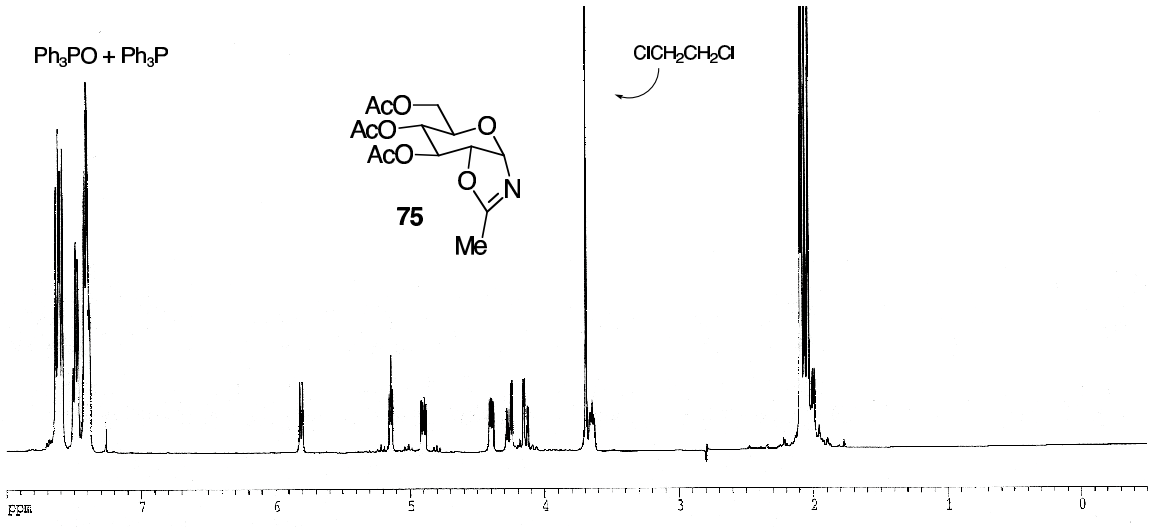
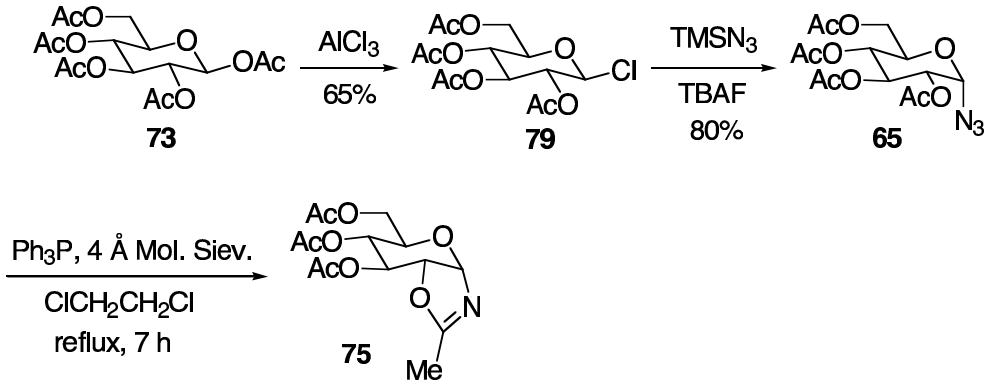


Figure 11. The *in situ* ^{1}H NMR spectra of glucosyl isoxazoline **75** in $CDCl_3$.

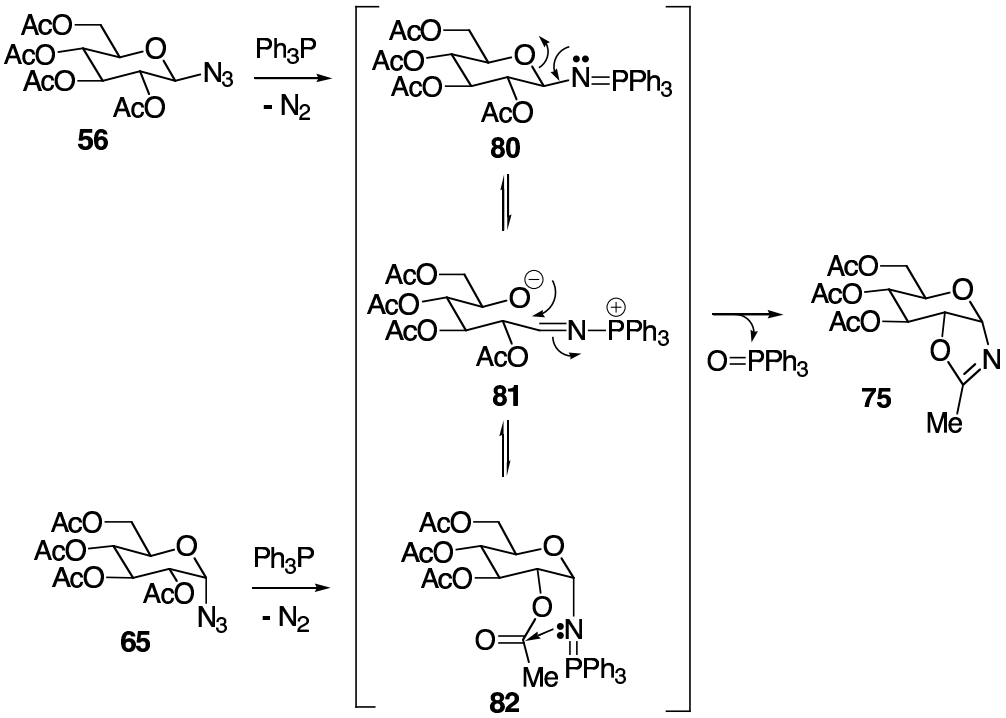
did not cause decomposition either. However, isoxazoline **75** was unstable to chromatography over silica. Since, **75** is the only glucosyl derivative observed in the ^{1}H NMR spectrum of the reaction mixture, in subsequent experiments, **75** was generated *in situ* for coupling reactions. The crude ^{1}H NMR of the generated *in situ* isoxazoline **75** is shown in Figure 11.

Scheme 26



In an attempt to confirm the proposed structure of isoxazoline **75**, α-glucosyl azide **65** was submitted to the same reaction conditions as the β-azide. α-Glucosyl azide **65** was obtained from β-chloride **79** in 80% yield according to the procedure developed by Soli (Scheme 26).[127] The reaction of the α-azide with triphenylphosphine and 4Å molecular sieves in refluxing dichloroethane also yielded isoxazoline **75.** However, the reaction time required to convert the α-azide **65** to form isoxazoline **75** is shorter (7 h) than that of β-azide **56** (16 h).

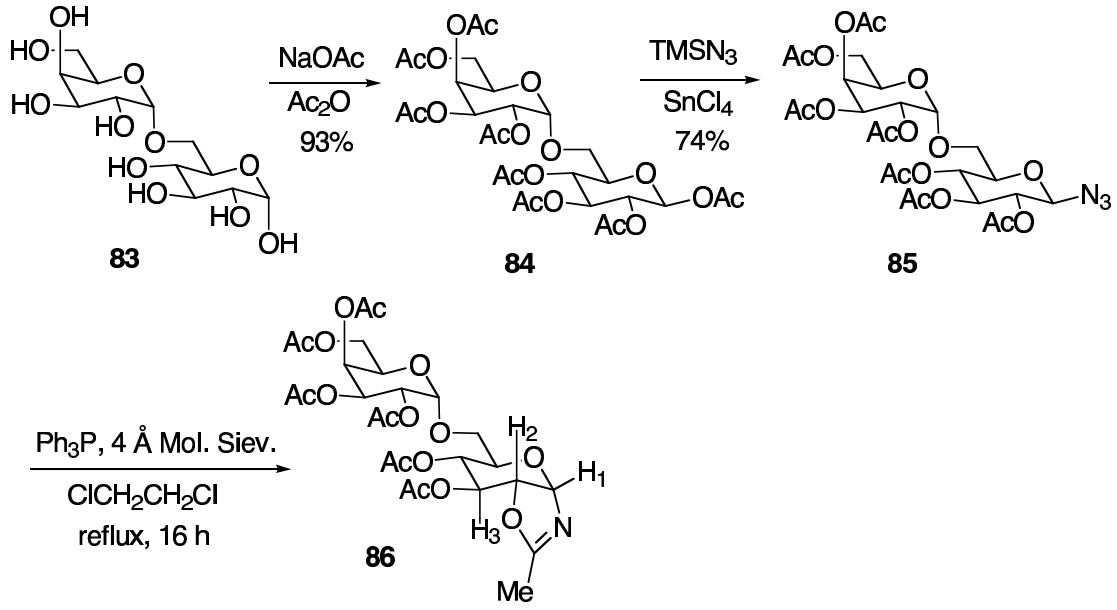
Scheme 27



Formation of isoxazoline **75** from either azide configuration can be explained by the mechanism shown in Scheme 27 involving the α/β anomerization of the intermediate phosphorimines **80** and **82.**[127] In Staudinger-type reactions, it has been proposed that the compounds **80** and **82** are in equilibrium *via* acyclic intermediate **81.** Isoxazoline formation from **80** cannot occur due to the strain present in the resulting product **75**. However, epimerization of **80** to the α-anomer **82** followed by cyclization gave α-isoxazoline **75**.

To demonstrate the generality of the isoxazoline formation, complex glycosyl azides, di- and triglycosyl azides are prepared and subjected to the reaction conditions.

Scheme 28

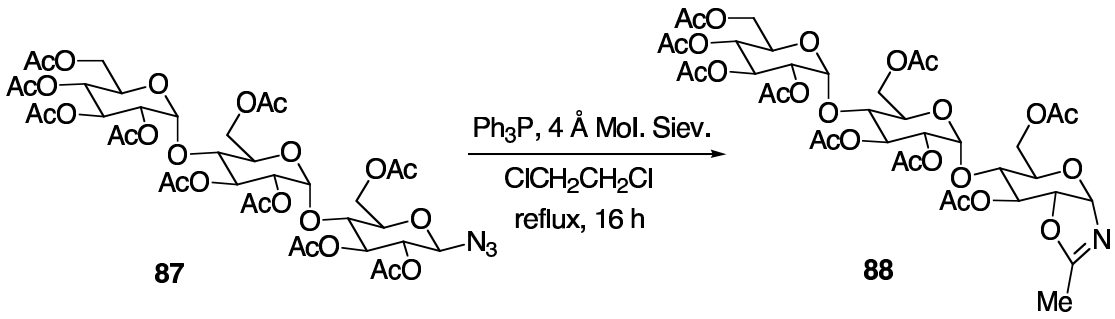


The reaction of melibiose **83** with sodium acetate in refluxing acetic anhydride provided octacetate-β-melibioside **84** in high yield. β-Melibioside **84** was converted into β-azide **85** in one step upon reaction with tin(IV) chloride and $TMSN_3$ (Scheme 28).[129] As expected, reaction of azide **85** under the general isoxazoline formation conditions, provided only isoxazolines **86** as determined by NMR spectroscopy was the respective . The α-configuration of isoxazoline **86** was indicated by a doublet (J = 7.2) at δ 5.82 ppm for H1, a triplet (J = 4.4) at δ 5.20 ppm for H3, and a doublet of doublets (J = 7.2, 4.4) at δ 4.37 for H2 all of which are the characteristic for the α-configuration of the melibiosyl isoxazoline. As mentioned above in the discussion of glucosyl isoxazoline **75**, the absorbance at δ 14.1 ppm in the C^{13} NMR spectrum of isoxazoline **86** is distinctive for the formation of the isoxazoline.

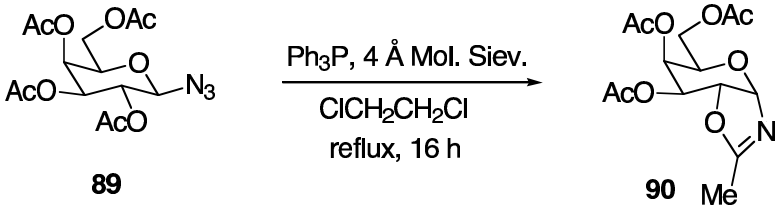
In analogy with the melibiose methodology, peracetylated β-maltotriose azide **87** was prepared from maltotriose.[129] When azide **87** was submitted to the general isoxazoline formation conditions, the only product observed by NMR spectroscopy was isoxazolines **88** (Scheme 29). In the maltotriosyl isoxazoline **88** case, the H2 and H3 absorbences in the ^{1}H NMR spectra are undistinguishable since they appeared with other protons. However, a doublet (J = 7.6) at δ 5.90 ppm for H1 in ^{1}H NMR and carbon peak for the methyl carbon of isoxazoline at δ 13.9 confirmed the formation of maltotriosyl isoxazoline **88** with the α-configuration.

These results demonstrate that di- and triglucosyl azides, including sensitive α-(1-4) and α-(1-6) glycosidic linkages, tolerate the reaction conditions and form the corresponding glycosyl isoxazolines.

Scheme 29



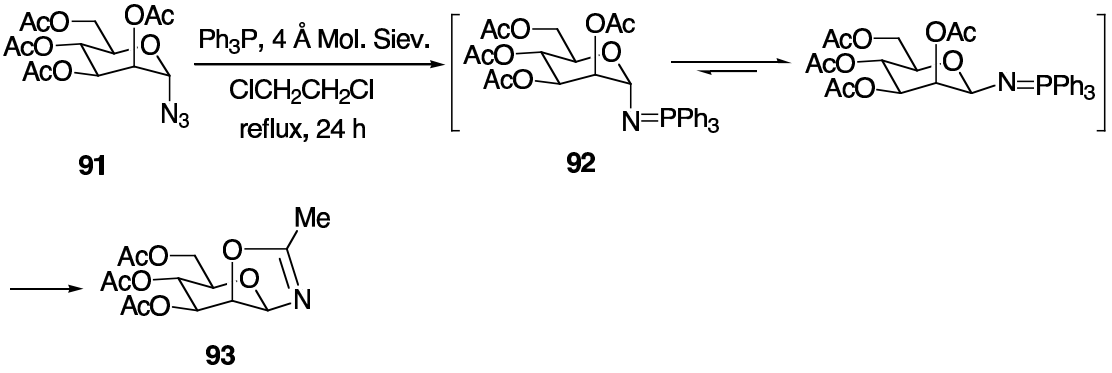
Scheme 30



To demonstrate the generality of isoxazoline formation β-galactopyranosyl azide **89** and α-mannopyranosyl azide **91** were prepared from their commercially available acetates in one step using tin(IV) chloride and $TMSN_3$.[129]

Analogous to glucosyl isoxazoline case, when galactosyl azide was allowed to react with triphenylphosphine in refluxing dichloroethane (Scheme 30), the galactosyl isoxazoline **90** was the only product obtained. ^{13}C NMR spectroscopy of the galactosyl isoxazoline also contained a peak at δ 14.6 ppm, attributed to the methyl carbon on the isoxazoline ring.

Scheme 31



The reaction of α-mannopyranosyl azide with triphenylphosphine was monitored by ^1H NMR and ^{31}P NMR spectroscopy (Scheme 31). The ^1H NMR and ^{31}P

NMR spectra from a time course experiment are shown in Figures 12 and 13. Twenty minutes after the addition of triphenylphosphine at room temperature, the only glycosyl derivative observed was α-mannosyl phosphorimine **92.** After 5 h of refluxing, a 1:2 mixture of β-mannosyl isoxazoline and α-phosphorimine was observed by ^{1}H NMR spectroscopy. Failure to observe the formation of β-phosphorimine by either ^{1}H or ^{31}P NMR spectroscopy suggested that as soon as the α-phosphorimine epimerized to the β-phosphorimine, condensation to form the isoxazoline occurred.

After 17 h at reflux, α-phosphorimine **92** was still present in the mixture as determined by ^{31}P NMR. The reaction went to completion after 24 h at reflux to give β-mannosyl isoxazoline **93**. Isoxazoline **93** was unstable to chromatography; the crude product (>95% pure by ^{1}H NMR spectroscopy) was used without further purification.

In conclusion, stereoselective syntheses of glycopyranosyl isoxazolines, such as α-glucosyl, α-galactosyl, and β-mannosyl isoxazoline, have been demonstrated. The reaction conditions are mild enough to apply to di- and trisaccharide azides bearing sensitive anomeric linkages.

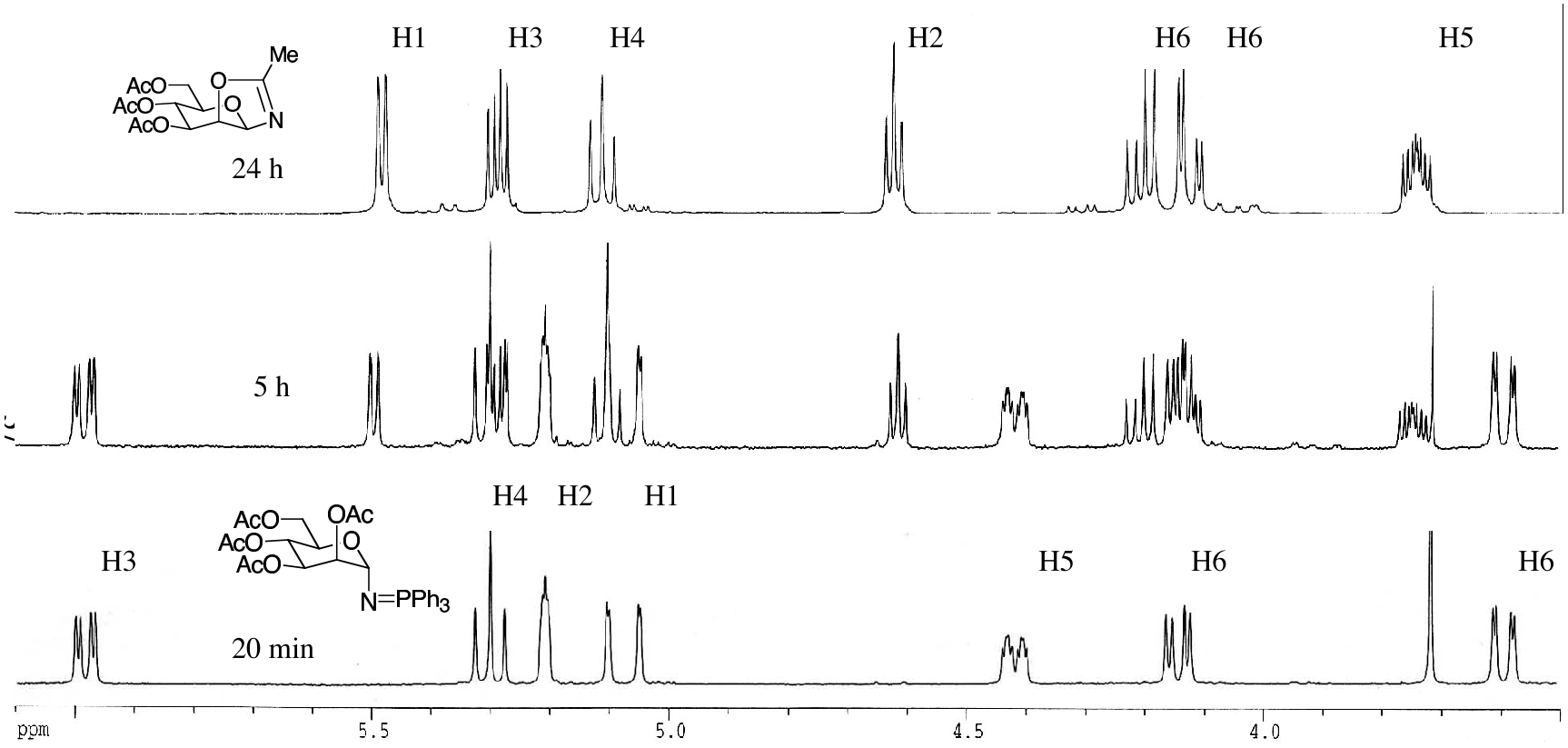


Figure 12. The *in situ* ^{1}H NMR spectra of the reaction of β-mannosyl azide with triphenyl phosphine at 83 °C after: a) 20 min, b) 5 h, c) 24 h.

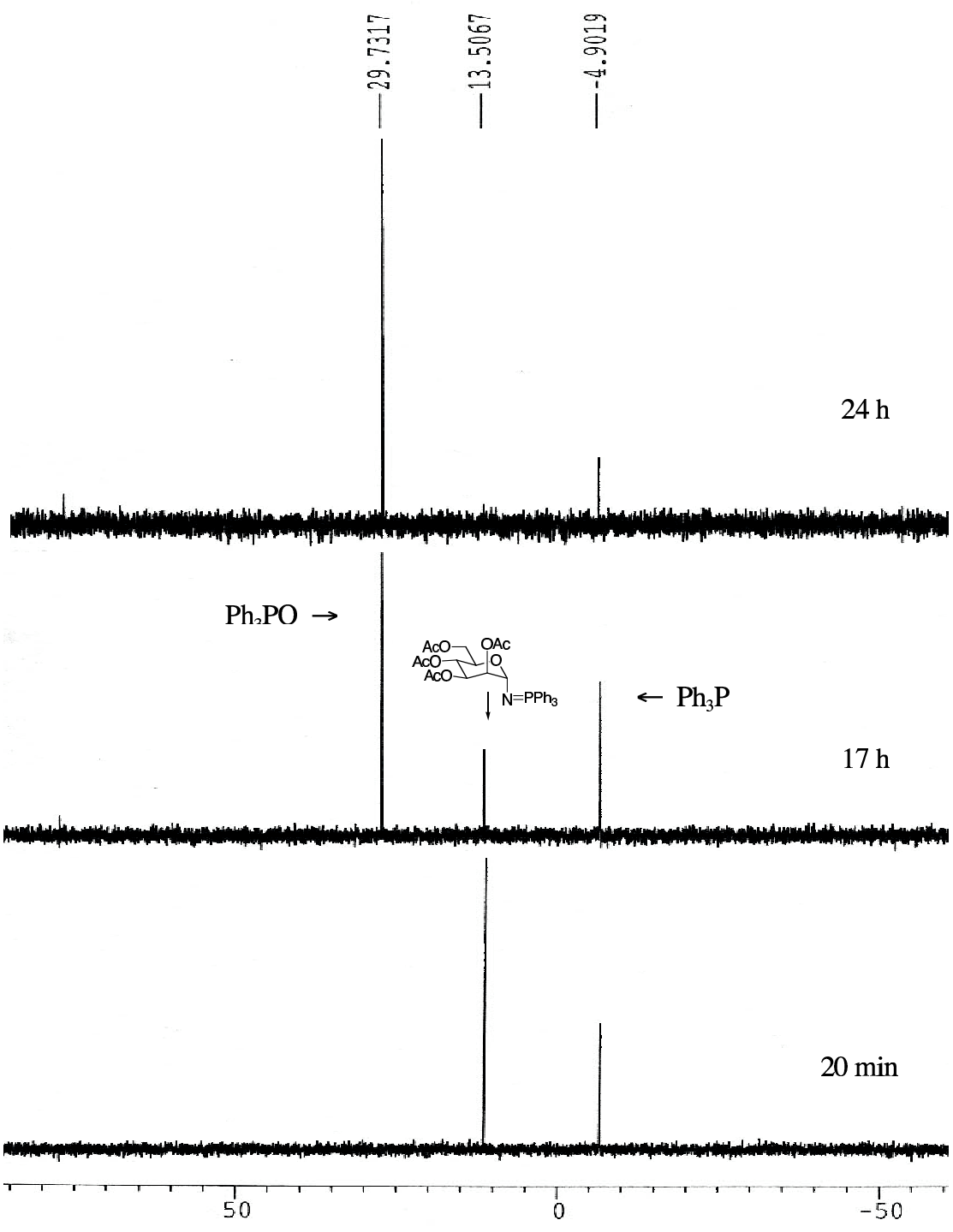
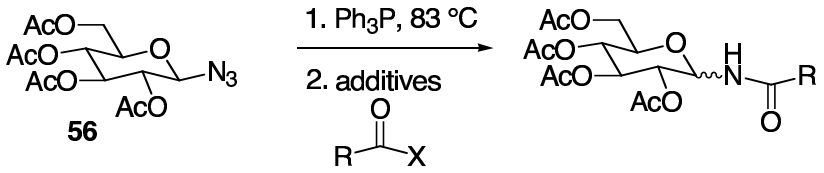


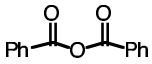
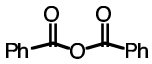
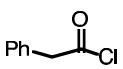
Figure 13. The P^{31} NMR spectra of the crude reaction of β-mannosyl azide with triphenylphosphine after: a) 20 min, b) 17 h, c) 24 h.

Coupling of Glycosyl Isoxazolines with Acid Derivatives

It was our intention to show that glycosyl isoxazolines could be used as a coupling partner to synthesize the glycosylamide linkage in stereoselective manner. Preliminary coupling studies with activated acid derivatives were undertaken to determine the optimum acylating reagents. For this purpose, glucopyranosyl isoxazoline **75**, obtained from the reaction of glucopyranosyl β-azide **56** with triphenylphosphine, was utilized. Since the isoxazoline was relatively sensitive toward chromatography, all the reagents and additives for the acylation were added to the *in situ* generated isoxazoline solution. Control reactions using purified isoxazoline demonstrated that the stereoselectivity of the coupling was not altered whether purified or *in situ* generated isoxazoline was employed in the coupling.

Table 3. Couplings with anhydrides and acid chlorides.

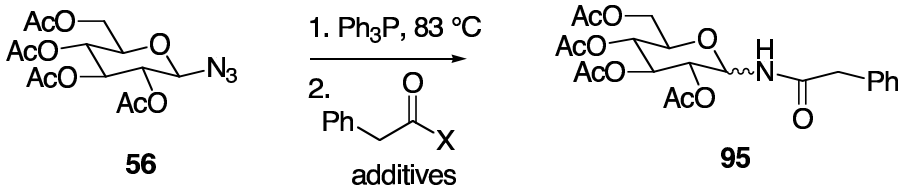


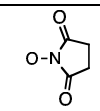
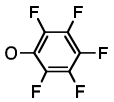
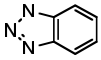
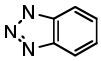
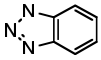
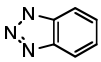
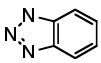
entry	reagent	equiv	additive	temp (°C)	compound (yield,%)[a]	α/β[b]
1		4	-	83	**94** (60)	1/3
2		2	DIEA	83	**94** (25)	6/1
3		1.3	-	83	**94** (65)	17/1
4		1.3	-	25	**95** (65)	>19/1

All the yields reported in tables are the yields of isolated pure product and α/β ratio determined by ^{1}H NMR spectroscopy, as explained on page 55.

The coupling results using anhydrides and acid chlorides are summarized in Table 3. Acylation with benzoic anhydride gave a good yield of the corresponding amido product, although significant anomerization was observed (Table 3, entry 1). The more reactive acid chlorides gave good yields of the adducts with high stereoselectivity (Table 3, entries 2 and 3). The α-stereoselectivity of the coupling reaction with acid chlorides was also considered as a chemical proof of the α-isoxazoline configuration.

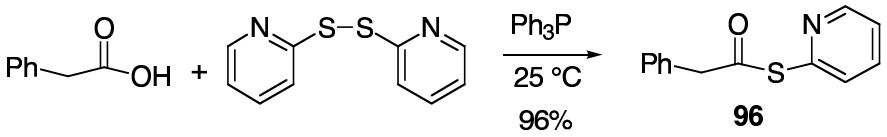
Table 4. Couplings with activated esters of phenylacetic acid.



entry	X	equiv	additive	temp (°C)	yield (%)	α/β
1		4	-	83	25	1/4
2		4	-	83	25	1/3
3		1.3	-	83	0	-
4		1.3	-	25	0	-
5		1.3	$CuCl_2 \cdot 2H_2O$	25	0	-
6		1.3	$ZnCl_2$	25	19	10/1
7		1.3	$FeCl_3 \cdot 6H_2O$	25	11	10/1

Unfortunately, acid chlorides are inappropriate reagents for general glycopeptide synthesis and alternative, more robust derivatives were investigated. For this purpose, the N-hydroxysuccinimidyl, pentafluorophenyl, and N-benzotriazole phenylacetate were prepared. Attempts to utilize *N*-hydroxysuccinimidyl (Table 4, entry 1) or pentafluorophenyl (Table 4, entry 2) esters in the acylation protocol met with mixed success since the yields and α/β selectivity were poor even at elevated temperatures. N-phenylacetylbenzotriazole (Table 4, entries 3 and 4) did not couple at all with the isoxazoline even at reflux. To facilitate the coupling of the isoxazoline with benzotriazole, metal salts were added to increase the nucleophilicity of the carbonyl carbon by coordination of metal ion onto the benzotriazoyl moiety (Table 4, entries 5-7). While the addition of copper(II) salt did not have any effect on the coupling reaction, the addition of zinc and iron (III) salts afforded the coupled product in good stereoselectivity but poor yields.

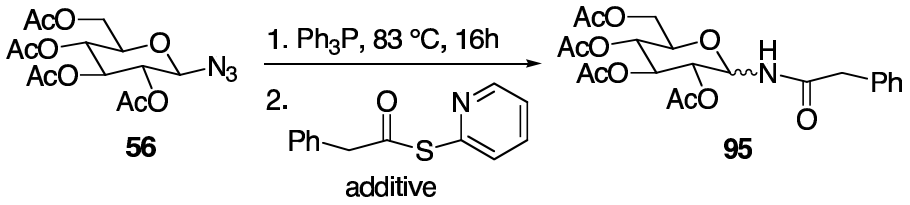
Scheme 32



96

Thioesters have been used in peptide synthesis[130,131]; therefore, thiopyridyl ester **96** was investigated also for coupling reactions. According to the modified procedure of Mukaiyama, thiopyridyl esters can be synthesized by the reaction of carboxylic acid with dipyridyl disulfide and triphenylphosphine at room temperature in high yields (Scheme 32).[132] These reaction conditions are also compatible with different amino acid protection strategies, such as Fmoc, Boc, and benzyloxy carbonyl (Z).

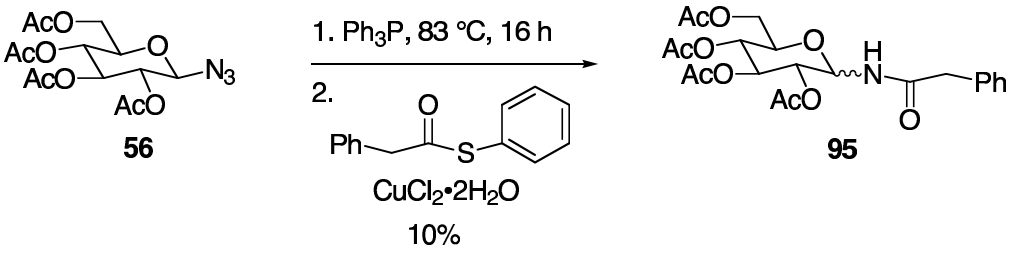
Table 5. Couplings of azide **56** with 2-pyridyl thiolphenylacetate.



entry	additive	temp (°C)	yield (%)	α/β
1	-	83	67	5/1
2	-	RT	50	5/1
3	$CuCl_2 \cdot 2H_2O$	RT	80	>19/1

Under the coupling conditions, the reaction of the *in situ* generated isoxazoline **56** with thiopyridyl ester **96** provided the α-adduct with good selectivity and high yield (Table 5, entry 1). When the reaction was performed at room temperature, the yield decreased slightly while the stereoselectivity was retained (Table 5, entry 2). These results encouraged us to focus on couplings utilizing thiopyridyl ester **96**. The yield and stereoselectivity of the coupling reaction were increased dramatically by the addition of copper (II) chloride (Table 5, entry 3). Presumably, the coordination of copper to the nitrogen of the pyridyl moiety increases the electrophilicity of the ester. This result was the first example in which addition of a copper salt to a thiopyridyl ester coupling to increase the electrophilicity of the ester. Recently, the same approach has been used in peptide forming reactions using thiopyridyl esters.[133,134]

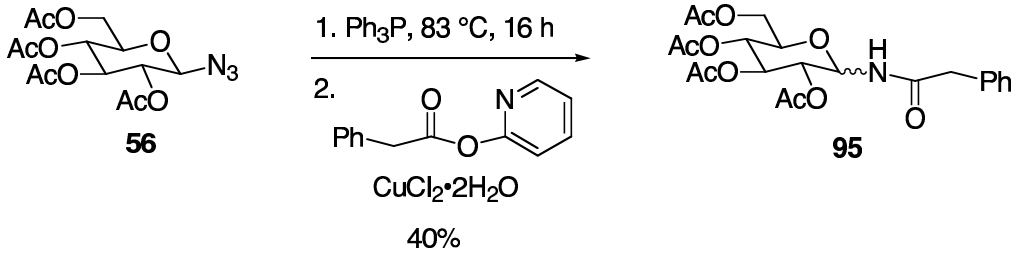
Scheme 33



To determine if the copper was coordinating to the nitrogen of the pyridyl moiety or to the sulfur atom of the thioester, two additional esters were synthesized. When the coupling reaction was attempted with the thiophenyl ester in the presence of copper (II) chloride, only 10% of the coupled product was obtained (Scheme 33).

When the reaction carried out with 2-hydroxy pyridyl ester, 40% of the coupled product obtained (Scheme 34). These results indicate that the nitrogen of the pyridyl moiety of the ester **96** plays more significant role in coordinating with copper than the sulfur atom.

Scheme 34

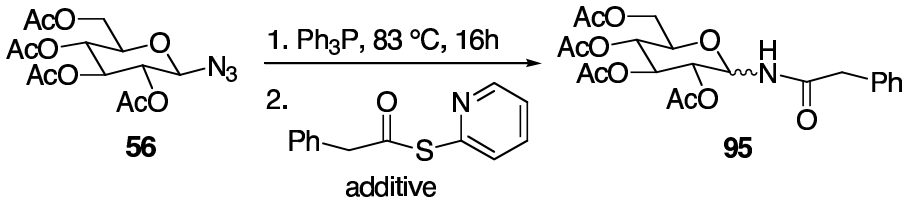


A systematic investigation of transition metal salts was undertaken to maximize the yield of coupling reaction and the results are summarized in Table 6. The low yield and the stereoselectivity obtained by using copper(I) chloride, compared to copper(II) chloride, shows the importance of the oxidation state of the copper (Table 6, entry 3). The results of the entries 4 and 5 demonstrated the importance of the counter ion of the copper in the coupling reaction.

An interesting result was obtained when zinc (II) chloride was employed as metal salt additive (Table 6, entry 6). The coupled product was obtained in good yield (60%) with modest β-stereoselectivity, which is different from the selectivity obtained using copper salts. Preference for the β-configuration was shown also when iron (II) sulfate (Table 6, entry 12) or cadmium (II) acetate (Table 6, entry 9) was employed, although yields were low.

Employing cobalt (II) chloride also resulted in high α-stereoselectivity with low yields (Table 6, entry 13). Couplings with iron (III) chloride also provided the coupled product in good yield (66%) with good α-stereoselectivity.

Table 6. Couplings of 2-pyridyl thiolphenylacetate utilizing metal salts



entry	additive	yield (%)	α/β
1	$CuCl_2 \cdot 2H_2O$	80	>19/1
2	$CuCl_2$	74	>19/1
3	CuCl	26	5/2
4	$CuBr_2$	50	5/1
5	$Cu(NO_3)_2$	0	-
6	$ZnCl_2$	60	1/3
7	$ZnBr_2$	48	3/2
8	$NiCl_2$	30	1/1
9	$Cd(OAc)_2$	25	2/3
10	$FeCl_3$	66	9/1
11	$FeCl_3 \cdot 6H_2O$	61	1/1
12	$FeSO_4 \cdot 7H_2O$	14	1/5
13	$CoCl_2 \cdot 6H_2O$	17	>19/1
14	$TiCl_4$	40	7/1

The stereoselectivity of the coupling reactions (α/β ratio of the coupled product) were readily determined using ^{1}H NMR spectroscopy, which is consistent with the results obtained from HPLC isolation. Because the α- and the β-glucosyl amide product eluted at the same time upon flash column chromatography. The product was collected as a mixture. The ^{1}H NMR spectra of α/β mixture of glucosyl amide **95** is shown in Figure 14.

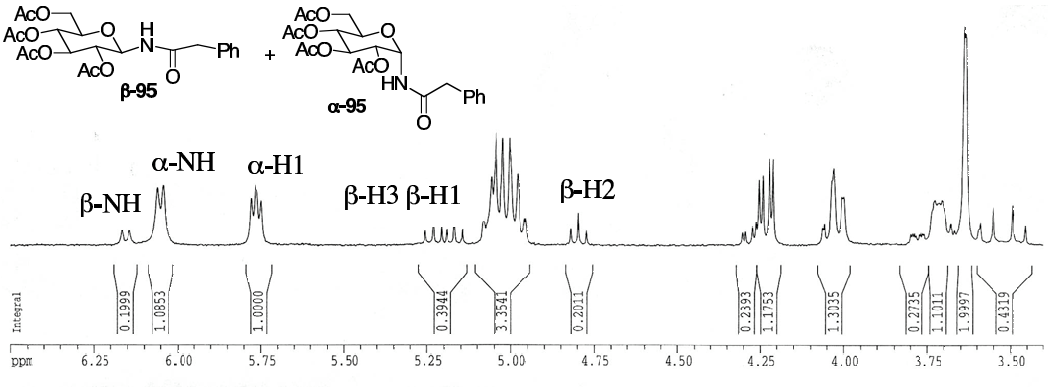
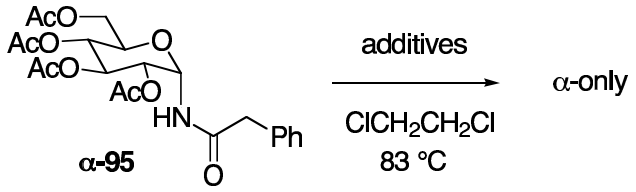


Figure 14. ^{1}H NMR of the α/β mixture of glucosylamide **95**.

Examination of the ^{1}H NMR spectrum indicated that the NH and H1 protons for both the α- and β-adducts appear at different chemical shifts; making it easy to identify the α/β ratio of the mixtures (Figure 14). The coupling pattern of the H1 proton was distinctive also for both α- and β-adducts: the H1 of the α-adduct appears as doublet of doublets with small coupling constants (α-H1: $J_{H1\text{-}NH} = 7.0$, $J_{H1\text{-}H2} = 4.8$) and H1of the β-adduct has triplet with large coupling constant (β-H1 J = 9.6).

Table 7. Control experiments utilizing glucosyl-α-amide **95**.



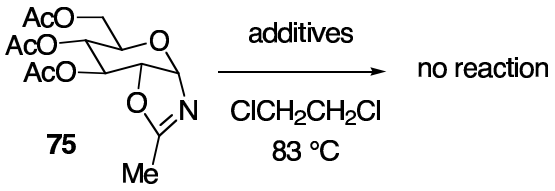
entry	additives
1	-
2	diisopropylethylamine
3	phenyl acetic acid
4	$ZnCl_2$
5	$ZnCl_2$ and 2-pyridine thiol
6	$FeSO_4 \bullet 6H_2O$ and 2-pyridine thiol

Control experiments were conducted to determine at what point the α/β epimerization occurred. Anomerization in glycosyl amide forming reactions has been explained mechanistically by involving acyclic intermediates.[82] Therefore, the first experiment determined whether the epimerization occurred after formation of α-glucosylamide (Table 7). Refluxing α-glucosylamide **95** solutions at 83°C under neutral, acidic, or basic conditions did not result in anomerization (Table 7, entries 1-3). Addition of Lewis acidic metal salts did not lead to epimerization (Table 7, entry 4). Similarly, adding known byproducts in the reaction mixture along with metal salts did not result in any change (Table 7, entries 5 and 6).

The second series of controls involved investigation of the effects of the metal salts on the glucosyl isoxazoline **75** (Table 8). Isoxazoline **75** was recovered

unchanged upon treatment with both $ZnCl_2$ and $CuCl_2$. The results of these control experiments demonstrated that epimerization occurred before the formation of the final α-glucosylamide product and after the first step of the conjugation of the coupling partner with isoxazoline.

Table 8. Control experiments utilizing isoxazoline **75**.

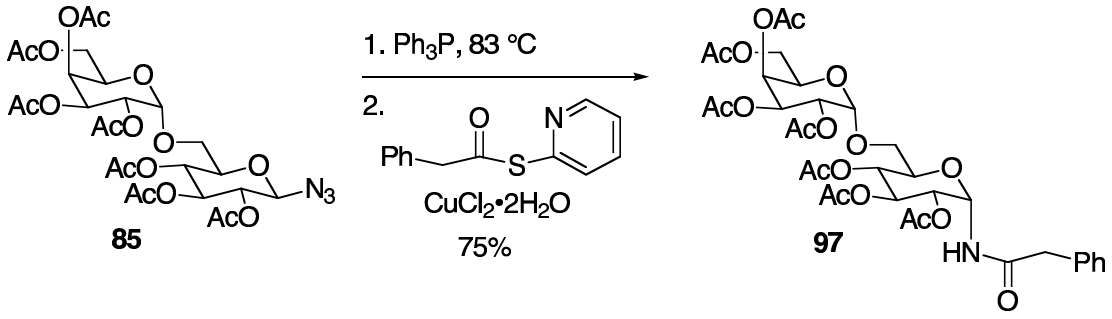


entry	additives
1	$ZnCl_2$
2	diisopropylethylamine and $ZnCl_2$
3	$CuCl_2 \bullet 2H_2O$

Since optimized protocol for the stereoselective synthesis of α-glucosylamide from isoxazolines had been found, coupling reactions involving more complex substrates were investigated.

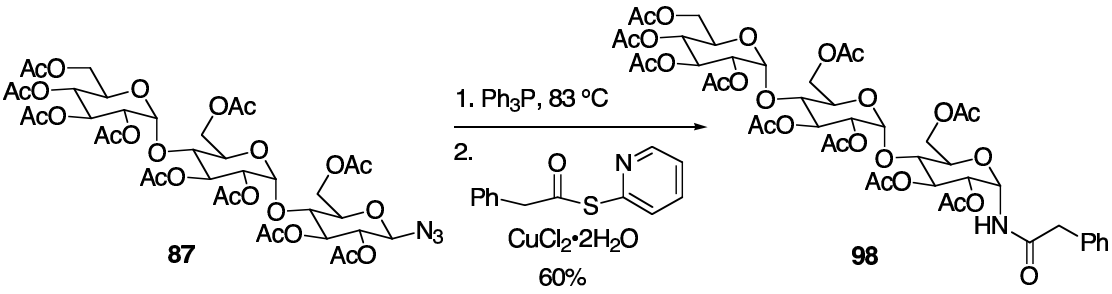
Peracetylated melibiose-β-azide **85** was chosen as an initial point of investigation (Scheme 35). In addition to being disaccharide, the α-(1-6) glycosidic linkage present in this compound is also found in naturally occurring nephritogenoside (Figure 7). Under the optimized coupling conditions, melibiosyl isoxazoline, formed by the reaction of azide **85** and triphenylphosphine, coupled with thioester in the presence of copper (II) chloride to give the melibiosyl-α-amide **97** in 75% yield.

Scheme 35



The second substrate chosen to investigate was β-maltotriosyl azide **87**, as it is a trisaccharide and possesses an α-(1-4) glycosidic linkage. Under the defined coupling conditions, maltotriosyl-α-amide **98** was obtained in 60% yield (Scheme 36).

Scheme 36

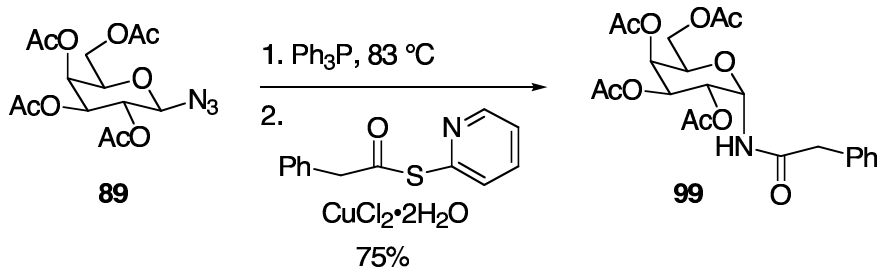


The results of the couplings of the utilizing di- and trisaccharides demonstrate the generality of the coupling methodology using complex carbohydrates for the synthesis of α-glycosylamide linkages. This also shows that the isoxazoline methodology can be used either in a stepwise or convergent fashion to synthesize the glycosyl peptides.

The scope of the coupling methodology was expanded by utilizing glycosyl azides other than glucosyl-based substrates. The reaction of β-galactosyl azide **89**

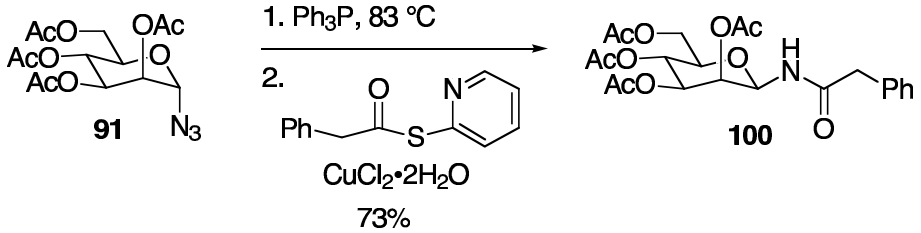
under the coupling conditions provided α-galactosyl amide **99** in 75% yield (Scheme 37).

Scheme 37



As it was discussed earlier under the special case II heading, the synthesis of β-O- and N-mannosides is a difficult task. To date, attempts to synthesize β-N-mannosyl amides usually gave the product in low or moderate stereoselectivity (1:5, α:β). These methods also require starting with the β-mannosyl azide, which is more difficult to obtain than its α-adduct. In our coupling methodology, however, α-mannosyl azide was the starting material for the synthesis of β-mannosyl isoxazoline, which coupled with thiopyridyl ester and provided the β-mannosyl amide **100** in 73% yield with excellent stereoselectivity (Scheme 38).

Scheme 38

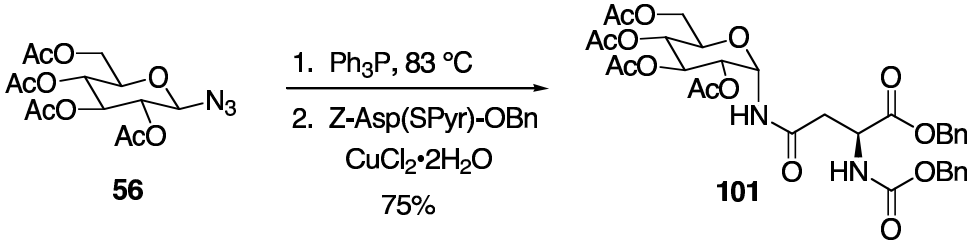


Following the successful development of the isoxazoline based coupling strategy to synthesize α-N-glycosylamide substrates, attention was shifted towards the application of this strategy to the couplings of amino acid derivatives. In order to demonstrate the compatibility of the isoxazoline methodology with different protecting group strategies, thiopyridyl esters of aspartic acid with Fmoc-tBu, Boc-tBu and Z-Bn protecting groups were synthesized.

The synthesis of thiopyridyl esters of aspartic acid derivatives was performed as described previously by reaction of the protected aspartic acid with triphenylphosphine at room temperature (Scheme 32). The purification over the silica generally gave yields between 73% and 98% depending on the protecting groups present in aspartic acid derivative. These results, along with the published results, showed that thiopyridyl esters of the amino acids with different protecting groups were easily accessible in high yields.[130,131]

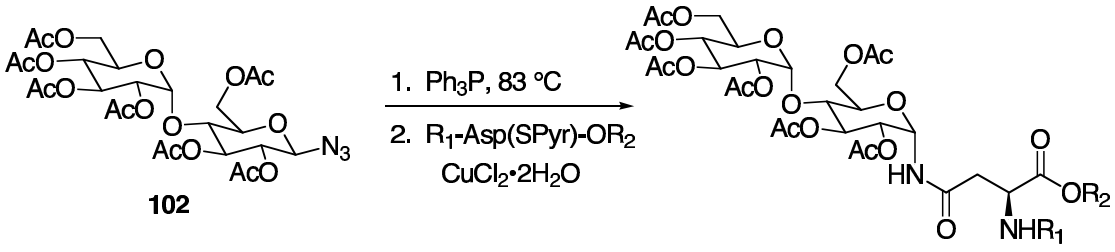
The isoxazoline derived from azide **56** was coupled with the benzyloxycarbonyl and benzyl protected thiopyridyl ester of aspartic acid (Scheme 39). The α-N-glucosyl amino acid **101** was obtained in 75% yield with excellent stereoselectivity.

Scheme 39



Having shown that the isoxazoline methodology can be used to synthesize α-glucosyl amino acids, it was intended to demonstrate the compatibility of this method with different protecting groups. Thus, isoxazoline derived from the disaccharide maltosyl azide **102** was employed to couple with three different aspartic acid derivatives (Scheme 40). In all instances, the coupled products were obtained in good yields.

Scheme 40



103, R_1 = Z, R_2 = Bn: 72%

104, R_1 = Boc, R_2 = tBu: 68%

105, R_1 = Fmoc, R_2 = tBu: 65%

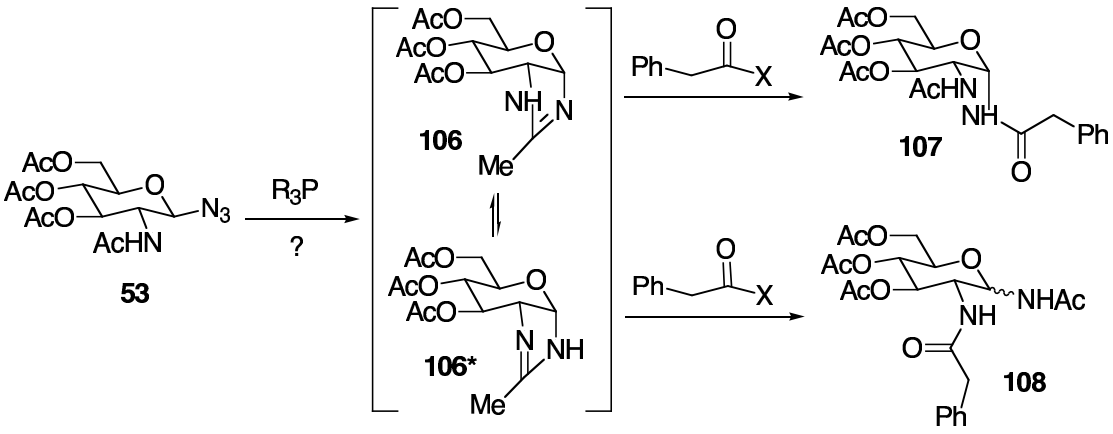
It should be noted that of all the available methodologies for preparing α-glycosylated amino acids such as **101** and **103-105**, the isoxazoline based coupling methodology is the most versatile reported to date, gives good to excellent yields, is compatible with different peptide protection strategies and glycosidic linkages, and is applicable to both stepwise and convergent synthetic methodologies.

Attempts towards the synthesis of glycosyl imidazoline

Once the isoxazoline methodology had been established, focus shifted toward couplings employing 2-NHAc-glucosyl-β-azide **53**. As was discussed earlier, in most

N-linked glycoproteins, 2-N-acetyl-2-deoxy-glucoseamine (Figure 1, **1**) is the carbohydrate that links the oligosaccharide moiety to the asparagine of the protein *via* β-glycosidic linkage. Even though the α-anomer has not been found in Nature, we were chose to develop a methodology to prepare both the α- and β-anomers since the α-anomers can be used in the structure-activity studies in glycoprotein and drug research.

Scheme 41

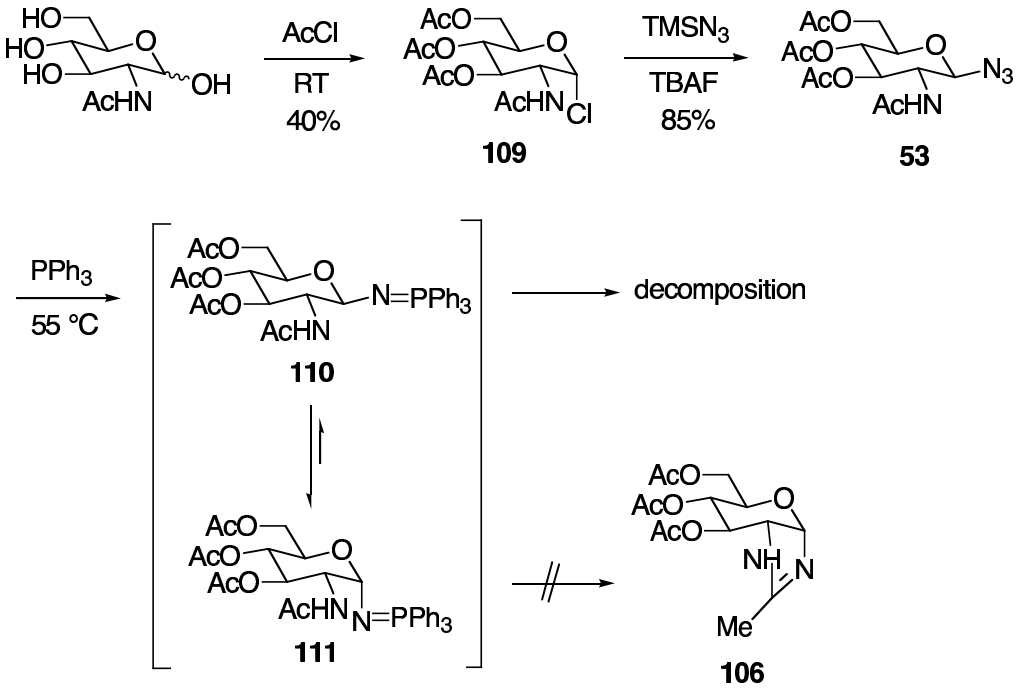


In order to achieve this goal, imidazoline **106** was the key intermediate for the stereoselective synthesis of α-amide **107**. The synthesis of glucosyl imidazoline **106** was expected to be achieved under the isoxazoline conditions (Scheme 41). Once imidazoline **106** had been synthesized, a regioselectivity problem that does not exist in isoxazoline system was anticipated with the couplings of imidazoline, as the results of isoxazoline couplings were confirmed that expectation. There are two nucleophilic nitrogens in the glycosyl imidazoline **106** *via* the tautomerization (**106** ⇌ **106***); either basic nitrogen may participate in the coupling with an electrophile. If acetylation occurs on the C1 nitrogen, α-NHAc-glucosylamide **107** will be the

product. If the acetylation occurs on the C2 nitrogen, glycosylamide derivative **108** should result. Both **107** or **108** would be synthetically useful: amide **107** is the N-linked glycopeptide product; while amide **108** would be a model for the structural units found in glycolipids.

2-Acetamido-glucosyl-β-azide **53** was obtained from α-chloride **109** in 85% yield according to the reported procedures (Scheme 42). Reaction of azide **53** with triphenyl phosphine under the isoxazoline formation conditions resulted in decomposition and the desired imidazoline **106** could not be detected. When the reaction was performed at 55 °C, after 16 hours α-phosphorimine **111** was the major glycosyl derivative observed by ^{1}H and ^{31}P NMR (Scheme 42).

Scheme 42



Reaction was followed by ^{31}P and ^{1}H NMR spectroscopy as shown in Figures 15 and 16, respectively. Thirty minutes after the addition of triphenylphosphine to the azide solution only β-phosphorimine **110** was observed (δ 16.8 ppm in P^{31} NMR). After 2 h of heating at 55 °C, almost 30% of the β-phosphorimine had epimerized to α-phosphorimine **111** observed at δ 15.9 in ^{31}P NMR. After 24 h of heating at this temperature, the α-phosphorimine was the major product and there was only a trace of β-phosphorimine **110** present. Prolonged heating resulted in the formation of triphenylphosphine oxide (δ 29.7 in ^{31}P NMR), and the decomposition of the glucosyl derivative. α-Phosphorimine **111** did not cyclize to form glycosyl imidazoline **106** in analogy to the isoxazoline studies.

The anomeric stereochemistry of phosphorimine **111** was assigned by ^{1}H NMR spectroscopy (Figure 16). The initially formed β-phosphorimine **110** gave a complex ^{1}H NMR spectrum in which H1 and H2 signals were overlapping at δ 5.00-5.10 ppm. However, after heating for 24 h, the ^{1}H and ^{31}P NMR spectrums indicated that a new phosphorimine was present and the α-stereochemistry of **111** was assigned. The H4 and H1 signals overlap at δ 5.10 ppm, but they could be characterized by phosphorus decoupled ^{1}H NMR spectroscopy. The H4 signal was apparent triplet with coupling constant of 9.6 Hz, which is typical for these systems. The ^{1}H-^{31}P decoupling experiments showed that H1 had $J_{H1-H2} = 4.0$ Hz and $J_{H1-P} = 19.6$ Hz. The value of the J_{H1-H2} was consistent with the α-stereochemical assignment.

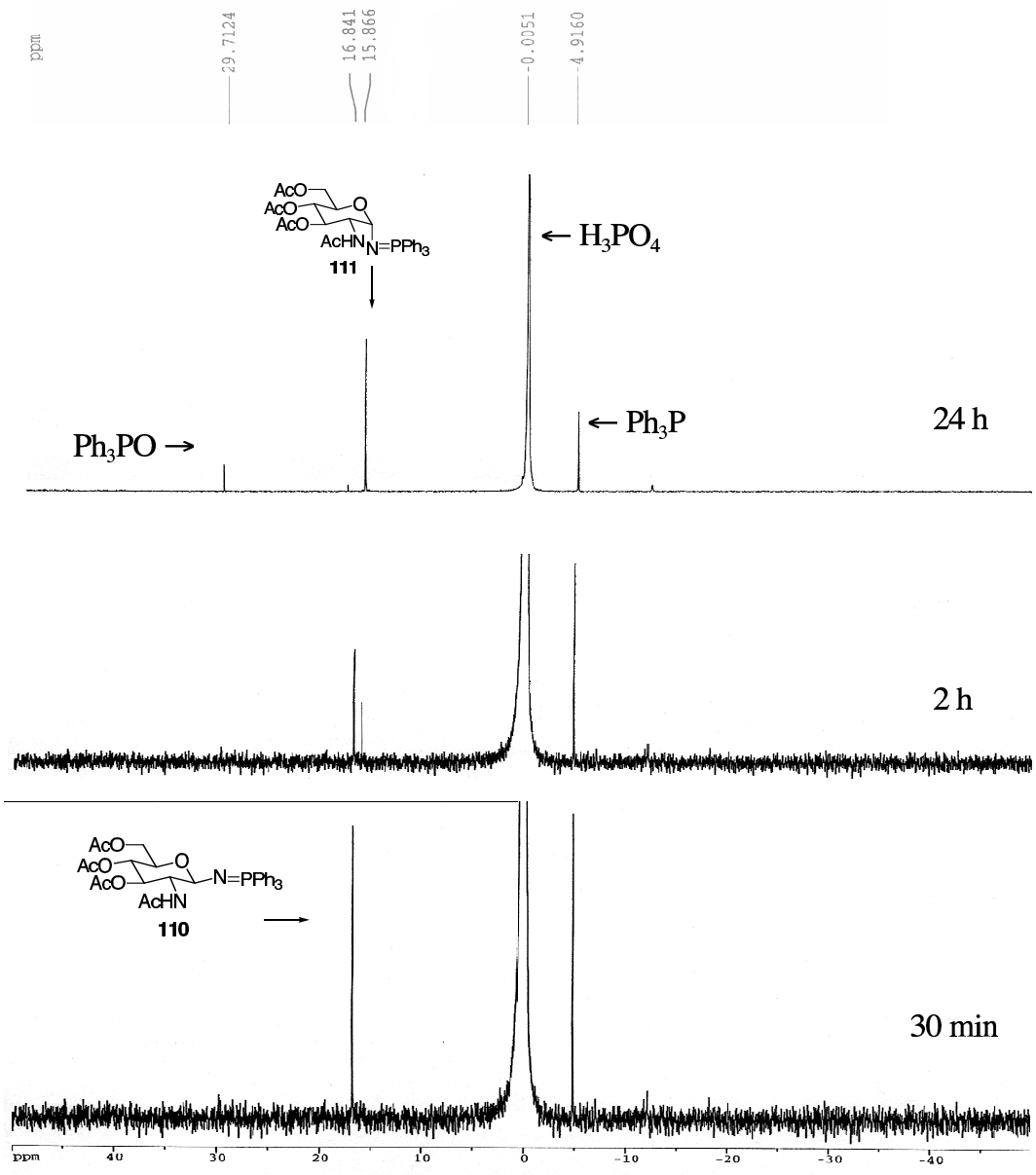


Figure 15. P^{31} NMR of the reaction of 2-NAc-glucosyl-β-azide **53** with Ph_3P at 55 °C after: a) 30 min, b) 2 h, c) 24 h.

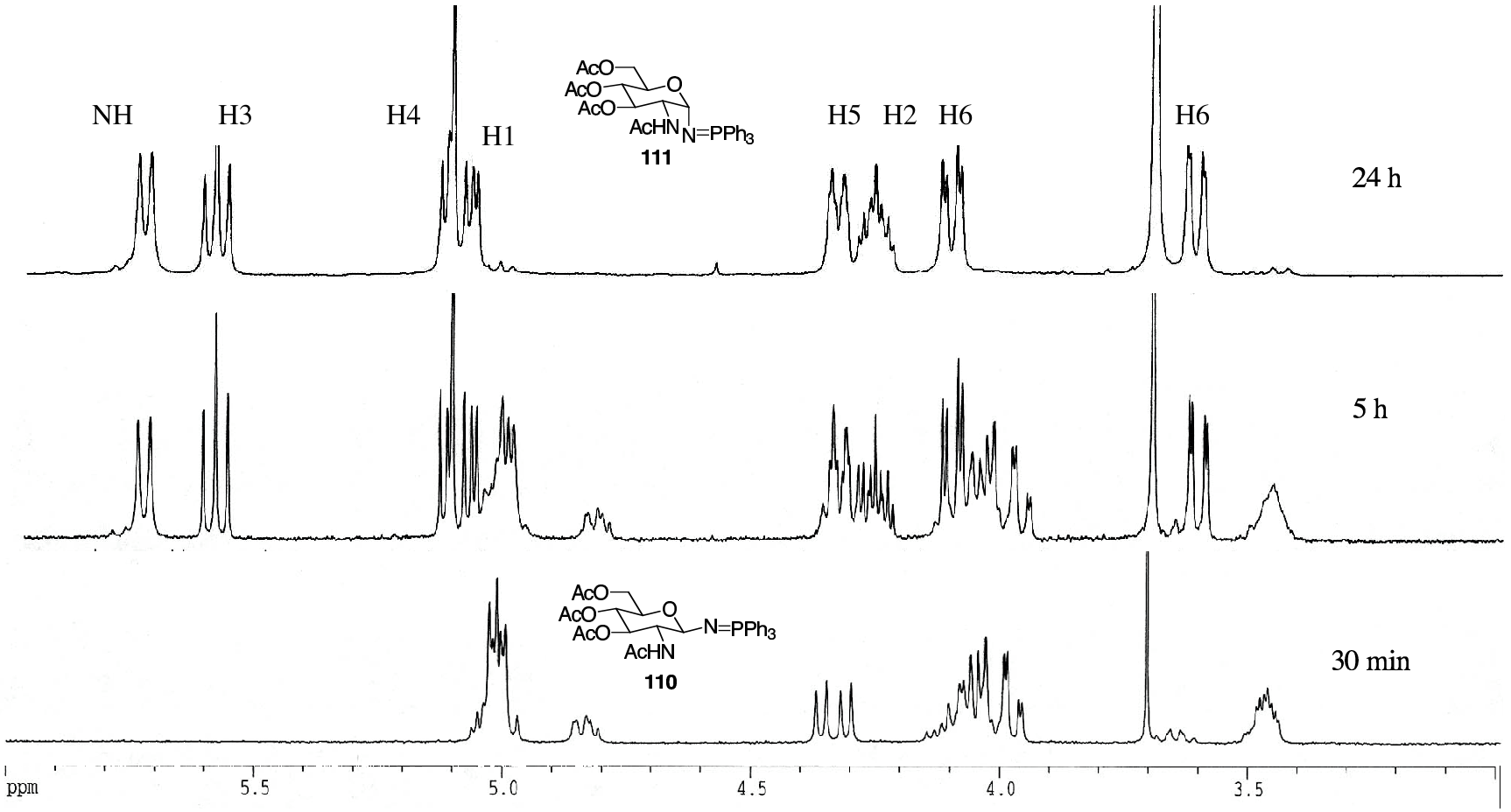


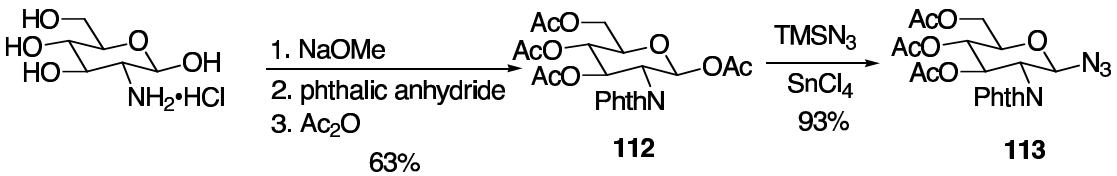
Figure 16. The *in situ* ^{1}H NMR spectra of the reaction of2-NAc-glucosyl-β-azide **53** with Ph_3P at 55 °C after: a) 30 min, b) 5 h, c) 24 h.

Two approaches were considered to overcome the failure of the phosphorimine to undergo cyclization. The first approach involved increasing the nucleophilicity of the phosphorimine nitrogen by replacing triphenylphosphine with a trialkyl phosphine. Thus, α-trimethylphosphorimine was prepared. This phosphorimine has electron donating methyl groups instead of electron withdrawing phenyl groups. However, the use of trimethyl phosphine did not provide the desired imidazoline adduct **106** under any conditions.

The second approach involved increasing the electrophilicity of the carbonyl carbon of the amide by replacing the hydrogen on C2 nitrogen with an electron-withdrawing group. If these approaches does not work alone, the combination of both would be applied.

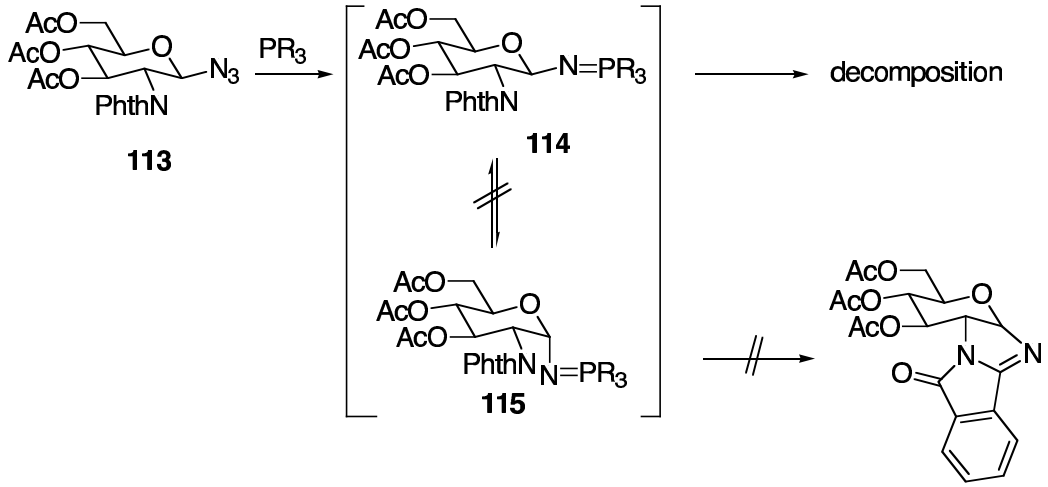
Regarding the second approach, it was decided to replace the acetyl group on C2 nitrogen with a phthalimido group. The presence two carbonyl groups on nitrogen should increase the electrophilicity of the carbonyl carbon. The phthalimido group has been widely used in glycopeptide chemistry as a nitrogen protecting group. Therefore, its conversion to an acetyl group after the coupling reaction has been well developed and can be easily achieved in high yields. 2-N-Phthalimido-glucosyl-β-azide **113** was synthesized from β-acetate **112** in 73% yield (Scheme 43).

Scheme 43



Reaction of azide **113** with triphenylphosphine under the isoxazoline formation conditions resulted in decomposition (Scheme 44). When the reaction was performed at 55 °C, β-phosphorimine **114** was observed, however, it did not epimerize to its α-phosphorimine anomer **115.** Even after 36 h, β-phosphorimine was the only adduct in the reaction mixture as observed by ^{31}P NMR. Similarly, substitution of trimethylphosphine for triphenylphosphine gave identical results; only the β-phosphorimine **114** was observed and the anomer ratio did not change under any conditions.

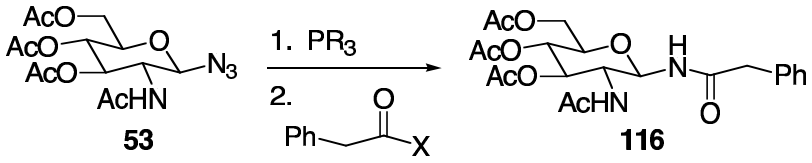
Scheme 44



Couplings Utilizing Thiolesters for the Synthesis of β-Glucosylamide

Following the unsuccessful attempts to synthesize glucosyl imidazoline **106,** it was decided to use α-phosphorimine **111** as the coupling partner to access the α-NHAc-glucosylamide adduct. The capture of the α-phosphorimine with an electrophile should provide the expected α-NHAc-glucosylamide linkage.

Table 9. Couplings of 2-NHAc-glucosyl azide to provide β-glucosylamide **116.**



entry	R	X	additive	temp (°C)	yield (%)
1	Ph	Cl	-	55	20
2	Me	SPyr	-	55	54
3	Me	SPyr	-	25	65
4	Me	SPyr	$CuCl_2 \cdot 2H_2O$	25	0
5	Me	OH	-	25	30

Reaction of the azide **53** with triphenylphosphine at 55 °C followed by the addition of phenylacetyl chloride, gave exclusively β-NHAc-glucosylamide **116** (Table 9, entry 1). The stereoselectivity of this coupling was unexpected since >95% of the α-phosphorimine **111** was present in the reaction mixture (by ^{1}H and ^{31}P NMR) prior to addition of the acid chloride. Assuming that the coupling reaction between the phosphorimine and acid chloride was rapid, the α-amide anomer should have been formed. This unexpected result could be attributed to the minor β-phosphorimine reacting significantly faster than the major α-phosphorimine, which drives the equilibrium towards the formation of the β-adduct. The alternative explanation, in which the α-phosphorimine coupled to give α-glucosylamide product which then

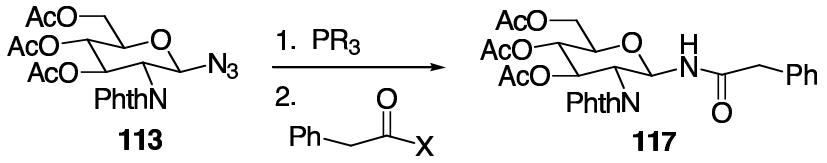
epimerized to the β-glucosylamide product under the reaction conditions, was shown to not be viable in the case of α-glucosylamide **95** by control experiments.

The coupling reaction was optimized using trimethylphosphine and 2-pyridyl thiolphenylacetate as the electrophile under the same conditions. Under these conditions, β-glucosylamide **116** was obtained in 54% yield (Table 9, entry 2). When the reaction utilizing trimethyl phosphine and thioester was performed at room temperature, β-glucosylamide **116** was obtained in 65% yield (Table 9, entry 3). The addition of copper(II) chloride salt, as used in the isoxazoline couplings, resulted in the decomposition of the phosphorimine (Table 9, entry 4). This result might be due to the coordination of the copper(II) to the phosphorimine nitrogen, causing it to become less nucleophilic, and thus less likely to couple.

Under the same conditions employment of phenylacetic acid, a typical electrophile used in Staudinger reactions, resulted in formation of β-glucosylamide in 30% yield (Table 9, entry 5). In typical Staudinger reactions of glycosyl derivatives, the reaction is usually performed at –78 °C in order to obtain high yield. The combination of trimethylphosphine and 2-pyridyl thiolester provided β-glucosylamide **116** in good yield at room temperature.

The coupling of phthalimido-phosphorimine **114**, derived from azide **113,** with phenylacetyl chloride provided β-glucosylamide **117** in 52% yield (Table 10, entry 1). The yield of the condensation was improved by using trimethyl phosphine and pyridyl thiolester. Under these conditions, the coupling reaction gave amide **117** in 73% yield (Table 10, entry 2). The addition of copper(II) chloride also resulted in decomposition of the phosphorimine prior to any coupling (Table 10, entry 3).

Table 10. Couplings of 2-NPhth-glucosyl azide to provide β-glucosylamide **117.**

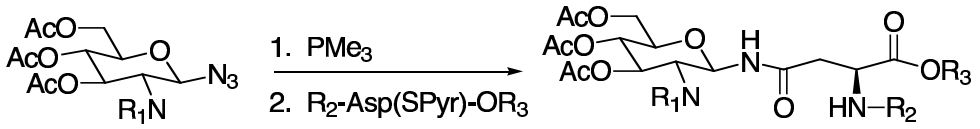


entry	R	X	additive	temp (°C)	yield (%)
1	Ph	Cl	-	55	52
2	Me	SPyr	-	25	73
3	Me	SPyr	$CuCl_2 \cdot 2H_2O$	25	0

Following the successful development of the pyridyl thioester based Staudinger coupling strategy to synthesize β-N-glycosylamide substrates, attention shifted towards the application of this strategy to the coupling with amino acid derivatives. The compatibility of the coupling conditions with different protecting group strategies was demonstrated by performing coupling reactions with the thiolester of aspartic acid derivatives bearing different protecting groups.

Reaction of azides **53** and **113** with trimethyl phosphine at room temperature for 15 min, followed by the addition of the thiolester of the aspartic acid derivative provided the corresponding glucosyl amino acid derivatives in good yields (Table 11, entries 1-4). The yields of adducts are good and the protecting groups were retained under coupling conditions.

Table 11. Couplings with thiopyridyl ester of aspartic acid derivatives.

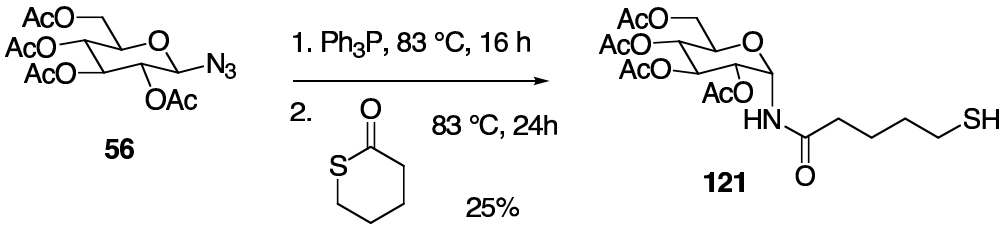


entry	R_1	R_2	R_3	product (yield,%)
1	AcH	Z	Bn	**17** (61)
2	AcH	Boc	tBu	**118** (59)
3	AcH	Fmoc	tBu	**54** (55)
4	Phth	Fmoc	tBu	**119** (59)

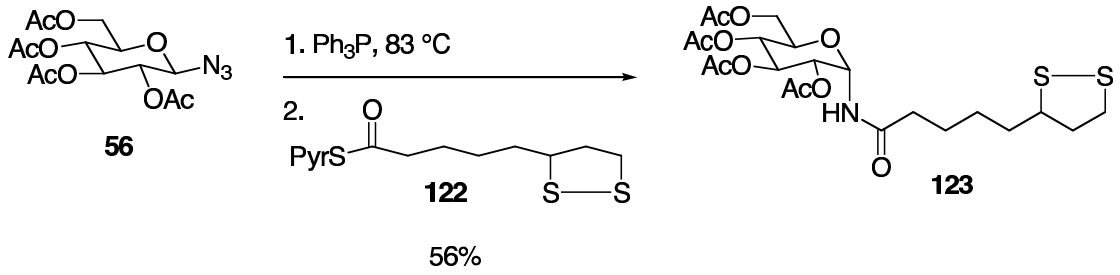
Application of the Methods Developed to the Synthesis of Molecules of Interest

As the part of the carbohydrate modified gold nanoparticle project, carbohydrate molecules possessing a thiol linker were needed. The coupling of the isoxazoline generated from azide **56** with thiolactone **120** provided the α-linked glucosyl thiol **121** in 25% yield (Scheme 45).[135]

Scheme 45



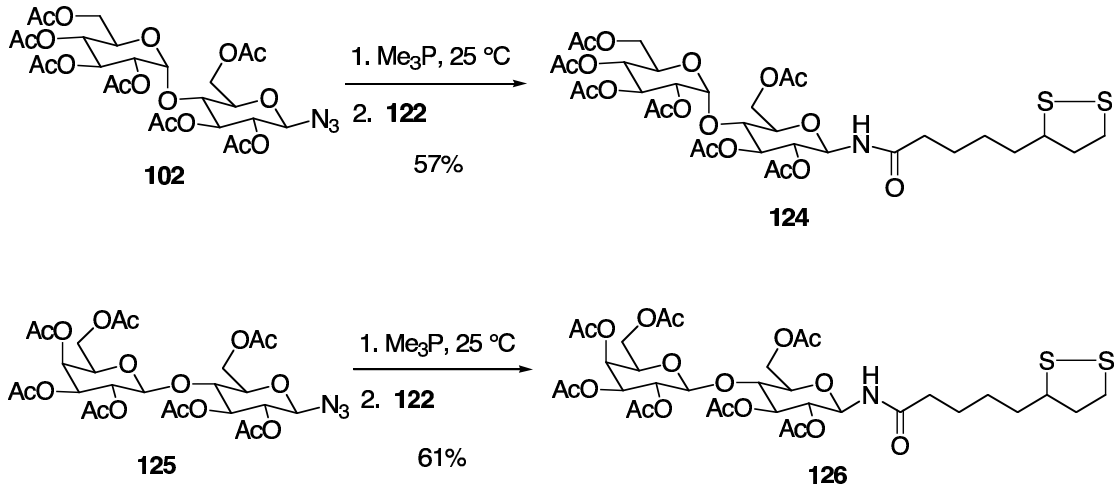
Scheme 46



Similarly, the 2-pyridyl thiolester of thioctic acid underwent coupling with azide **56** to afford glucosyl-α-amide **123** in 56% yield (Scheme 46)

The reaction of maltose azide **102** and lactose azide **125** with trimethylphosphine followed by the addition of thioester **122** provided the corresponding disaccharide β-amides **124** and **126** in 57% and 61% yields, respectively (Scheme 47).

Scheme 47



The studies with compounds **124, 126,** and their deacetylated analogs, attached on gold surface and nanogold particles, are underway.

Conclusions

A stereoselective synthesis of α-glucosyl, α-galactosyl, and β-mannosyl isoxazolines from readily available glycosyl azides has been developed. Synthesis of di- and triglucosyl isoxazolines having α-(1-6) and α-(1-4) glycosidic linkages has also been successfully demonstrated.

Glycosyl isoxazolines were used as coupling partners to synthesize glycosylamide linkages in a highly stereoselective manner. The optimum coupling conditions for the stereoselective synthesis of α-glucosylamide derivatives involved the treatment of the *in situ* generated isoxazoline with a 2-pyridyl thioester in the presence of copper(II) chloride.

It should be noted that of all the available methodologies for preparing α-glucopyranosyl amide and β-mannopyranosyl amides, the isoxazoline-based coupling methodology is the best reported to date with regard to yields, compatibility with protection strategies on both the saccharide and peptide components, different glycosidic linkages, and applicability to both stepwise and convergent synthetic methodologies.

Of note is the general and predictable nature of the isoxazoline couplings, as compared to reductive coupling of azides. While the reductive couplings of azides with acids gave coupled products in good yields, the individual reactions lack generality and often highly depend upon the conditions employed, such as catalyst, solvent, and neighboring group effect.

Application of the isoxazoline coupling strategy to the synthesis of α-glucosyl amino acids derivatives, including disaccharides, demonstrated that this methodology

could be applied to the stereoselective synthesis of the natural glycopeptide nephritogenoside. The compatibility of this method with different protection strategies and its applicability to both stepwise and convergent strategies makes this methodology superior to the other methods available for the synthesis of nephritogenoside.

Attempts using the isoxazoline approach in the synthesis of glycosyl imidazoline derivatives were unsuccessful. Treatment of 2-NHAc-glucopyranosyl-β-azide with phosphines resulted in formation of the β-phosphorimine that epimerized slowly to provide the α-derivative. Unfortunately, the α-phosphorimine derivative failed to cyclize to give the corresponding imidazoline derivative under the reaction conditions. However, 2-NHAc-glucopyranosyl-β-amides could be synthesized from the coupling of thiopyridyl ester with an *in situ* generated 2-NHAc-glycopyranosyl phosphorimine. Both the α- and β-phosphorimines gave exclusively the β-amide, presumably *via* epimerization prior to coupling. This approach to the synthesis of N-linked glycoproteins gave better yields than the traditional Staudinger reaction conditions previously reported. The neutral and mild conditions of this method make it an attractive alternative to the synthesis of sensitive substrates containing 2-NHAc-glucosyl β-amide linkage.

This methodogy should be applicable to the synthesis of glycoprotein analogs via a number of different routes. Having demonstrated the generality of the coupling of complex and simple saccharides to a differentially protected asparagine derivative, it should be possible to use the resulting glycosylated asparagine in automated peptide synthesis to provide hybrid glycoprotein derivatives. Alternatively, the coupling of

complex oligosaccharides with activated peptides containing the aspartic acid residue can be envisioned. Although the synthesis of complex glycoprotein derivatives has not been demonstrated in this thesis, the methodology has been shown to be viable. Exploitation of the methodology to synthesis of more complex glycoprotein analogs is underway.

Experimental

General. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 MHz, Bruker DRX-500 MHz, and Bruker DRX-600 MHz spectrometers. Chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ (7.240). Coupling constants (*J* values) are given in hertz (Hz). Spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), br (broad).

Infrared spectra were recorded on a Nicolet 5DXC FT-IR spectrophotometer. Band positions are given in reciprocal centimeters (cm^{-1}) and relative intensities are listed as br (broad), s (strong), m (medium) or w (weak).

Melting points were taken in Kimax soft glass capillary tubes using a Thomas-Hoover Uni-Melt capillary melting point apparatus equipped with a calibrated thermometer and are corrected.

Low resolution (LRMS) and high resolution (HRMS) mass spectra were obtained on a VG-7070E magnetic sector instrument.

Thin-layer chromatography (TLC) was performed on 0.25 mm Merck silica-coated glass plates treated with a UV-active binder, with compounds being identified in one or more of the following manners: UV (254 nm) or vanillin/sulfuric acid/ethanol charring.

Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium/benzophenone ketyl. Methylene chloride (CH_2Cl_2) and dimethyl formamide (DMF) were distilled from calcium hydride. Methanol (MeOH) was dried and stored

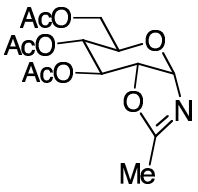
over molecular sieves. 1,2-Dicholoroethane was distilled from calcium hydride and stored over molecular sieves under argon.

Triphenylphosphine (Ph_3P) and benzoic anhydride were recrystallized from hexane. Phenylacetic acid and benzoic acid were recrystallized from petroleum ether.

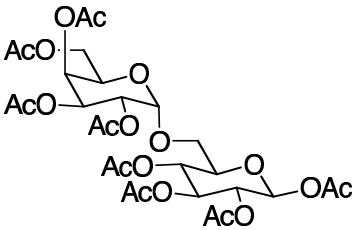
Fmoc-Asp-OtBu, Boc-Asp-OtBu, and Z-Asp-OBzl were purchased from Bachem and used as received. 2,2´-dipyridyl disulfide, benzotriazole, D-maltose monohydrate, α-D-lactose monohydrate, α-D-melibiose hydrate, β-D-glucose pentacetate, β-D-galactose pentacetate, glucosamine hydrochloride, and 2-acetamido-2-deoxy-D-glucose were purchased from Aldrich or Acros and used as received. β-D-Mannose pentacetate was purchased from Sigma and used as received. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (**56**) and 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl azide (**65**) were prepared according to literature.[60]

Glassware used in the reactions dried overnight in an oven at 120 °C. All reactions were performed under an atmosphere of argon unless noted otherwise.

All compounds were determined to be >95% pure by ^{1}H NMR spectroscopy unless otherwise noted.

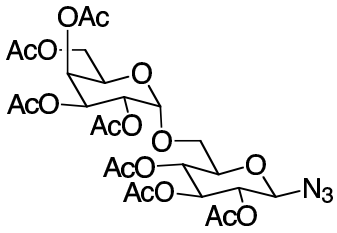


3,4,6-Tri-*O*-acetyl-α-D-glucopyranosyl isoxazoline (75). 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide (**56**) (150 mg, 0.402 mmol) and Ph_3P (116 mg, 0.442 mmol) were dissolved in 1,2-dichloroethane (5 mL) in the presence of 4Å molecular sieves. The resulting solution was heated under reflux for 16 h, then was cooled to ambient temperature and concentrated in vacuo. Purification of the residue by flash chromatography (hexanes:EtOAc:Et_3N, 25:75:1) gave 76 mg (58%) of isoxazoline **75** as colorless oil. R_f = 0.21 (hexanes:EtOAc, 1:3); IR (CCl_4) 2959 (m), 1751 (s), 1667 (m), 1242 (s), 1214 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 3.61 (ddd, *J* = 8.8, 5.2, 2.4, 1H, H5), 4.11 (dd, *J* = 12.4, 2.4, 1H, H6), 4.23 (dd, *J* = 12.4, 5.2, 1H, H6), 4.37 (dd, *J* = 7.6, 4.0, 1H, H2), 4.88 (dd, *J* = 8.8, 4.0, 1H, H4), 5.12 (t, *J* = 4.0, 1H, H3), 5.80 (d, *J* = 7.6, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 20.6, 20.7, 20.7, 63.1, 67.3, 67.7, 70.7, 75.9, 92.8, 168.6, 169.4, 169.5, 170.6; LRMS (FAB) 330 ($(M + H)^+$, 100); HRMS (FAB) calcd for $C_{14}H_{20}O_8N$ 330.1189 $(M + H)^+$, found 330.1194.



2,3,4-Tri-*O*-acetyl-6-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl]-β-D-glucopyranosyl acetate (octa-*O*-acetate-β-melibioside) (84). The acetate was prepared according to the procedure of Wolfrom *et. al.*[136] Melibiose (4.00 g, 11.1 mmol) was added to the refluxing mixture of NaOAc (4.55 g, 55.5 mmol) and Ac_2O (27 mL, 279 mmol). The solution was refluxed for 3 hr and cooled to 100 °C, then immediately transferred into 400 mL of

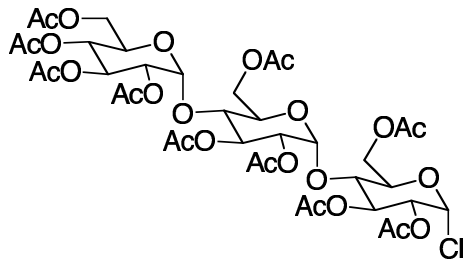
ice-water mixture and stirred vigorously for 4 hr. The water was decanted and the precipitate was diluted with 200 mL $CHCl_3$, washed with 2x200 mL sat. aq. $NaHCO_3$ solution and 2x200 mL H_2O, then dried over Na_2SO_4 and concentrated in vacuo to give colorless gummy oil. The azeotropic removal of remaining $CHCl_3$ by dissolving the oil in 2x100 mL hot EtOH, followed by two successive crystallization from 95% EtOH gave 7.01 g (93%) of ~ 90% pure octa-*O*-acetate-β-melibioside, which was used in the formation of hepta-*O*-acetyl-β-melibiosyl azide **84** without further purification. IR (CCl_4) 2956 (w), 1750 (s), 1218 (s), 1038 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (s, 3H), 1.95 (s, 3H), 1.96 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 3.56 (dd, *J* = 11.6, 2.4 1H, H6), 3.66 (dd, *J* = 11.6, 4.4, 1H, H6), 3.72 (ddd, *J* = 9.6, 4.4, 2.4, 1H, H5), 3.95-4.05 (m, 2H, H6´), 4.10-4.10 (m, 1H, H5´), 4.95-5.10 (m, 4H, H2, H2´, H1´, H4), 5.18 (t, *J* = 9.6, 1H, H3), 5.28 (dd, *J* = 10.0, 3.2, 1H, H3´), 5.35-5.40 (m, 1H, H4´), 5.60 (d, *J* = 8.4, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 20.8, 20.9, 20.9, 20.9, 20.9, 21.0, 21.0, 61.9, 65.8, 66.6, 67.6, 68.2, 68.3, 68.5, 70.4, 73.1, 73.7, 91.8, 96.6, 169.1, 169.5, 169.5, 170.0, 170.3, 170.4, 170.6, 170.8; LRMS (FAB) 685 ($(M + Li)^+$, 100); HRMS (FAB) calcd for $C_{28}H_{38}O_{19}Li$ 685.2167, found 685.2164.



2,3,4-Tri-*O*-acetyl-6-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl]-β-D-glucopyranosyl azide (Hepta-*O*-acetyl-β-melibiosyl azide) (85).

The azide was prepared according to the procedure of Peto *et. al.*[129] Octa-*O*-acetyl-β-melibioside (200 mg, 0.295 mmol) was dissolved in 10 mL

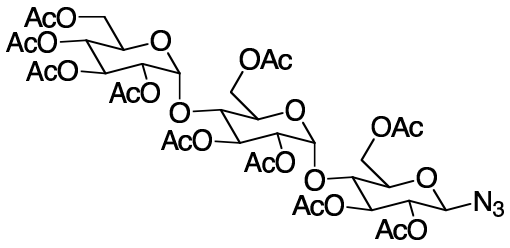
of CH_2Cl_2. Trimethylsilyl azide (55 μL, 0.413 mmol) was added via syringe followed by $SnCl_4$ (18 μL, 0.148 mmol). The solution was stirred for 20 h at 20 °C. The reaction mixture was diluted with 100 mL CH_2Cl_2, washed with 100 mL sat. aq. $NaHCO_3$ solution, and with 2x100 mL H_2O. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give crude product, which was crystallized from absolute ethanol to afford 144 mg (74%) of hepta-O-acetyl-β-melibiosyl azide **85** as a white solid: m.p. 140-142 °C (lit. m.p.[129] 142-143 °C)100; IR (CCl_4) 2964 (w), 2118 (s), 1758 (s), 1225 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.00 (s, 3H), 2.03 (s, 6H), 2.06 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 3.55-3.60 (m, 1H, H5), 3.70-3.80 (m, 2H, H6), 4.07 (d, *J* = 6.8, 2H, H6´), 4.21 (t, *J* = 6.8, 1H, H5´), 4.60 (d, *J* = 9.0, 1H, H1), 4.90 (t, *J* = 9.0, 1H, H2), 5.08 (t, *J* = 9.0, 1H, H4), 5.10 (dd, *J* = 10.8, 3.8, 1H, H2´), 5.15 (d, *J* = 3.8, 1H, H1´), 5.21 (t, *J* = 9.0, 1H, H3), 5.35 (dd, *J* = 10.8, 3.8, 1H, H3´), 5.44 (d, *J* = 3.8, 1H, H4´); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.5, 20.6, 20.6, 20.6, 20.7, 20.8, 61.6, 66.0, 66.4, 67.4, 68.0, 68.0, 68.4, 70.6, 72.6, 74.8, 87.6, 96.2, 169.2, 169.2, 169.9, 170.1, 170.2, 170.4, 170.5; LRMS (FAB) 668 ($(M + Li)^+$, 53); HRMS (FAB) calcd for $C_{26}H_{35}O_{17}N_3Li$ 668.2127, found 668.2135. Since the coupling constant between H4´ and H5´ is smaller than 1 Hz, and it is undistinguishable on the spectrum, it was reported as no coupling. The ^{1}H-NMR and ^{13}C-NMR data were consistent with the data reported by Petö *et al.*[129]



2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-α-D-glucopyranosyl chloride (Deca-*O*-acetyl-α-maltotriosyl chloride).

Deca-*O*-acetyl-β-maltotriose (1.80 g, 1.86 mmol) was dissolved in 20 mL of anhydrous chloroform, followed by addition of 1M titanium tetrachloride solution (2.05 mL, 2.05 mmol) via syringe. The solution was refluxed for 3 h, and cooled to RT. The reaction mixture was diluted with 100 mL CH_2Cl_2, washed twice with 100 mL water, filtered through a plug of silica gel, dried over Na_2SO_4, and concentrated in vacuo to give a yellow oil. The oil was crystallized from mixture of Et_2O and petroleum ether to afford 1.53 g (87%) of ~ 90% pure deca-*O*-acetyl-α-maltotriosyl chloride as a white solid, and was used in the formation of azide **87** without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.08 (s, 6H), 2.11 (s, 3H), 2.16 (s, 3H), 2.19 (s, 3H), 3.90-4.10 (m, 5H), 4.15-4.20 (m, 1H), 4.25-4.35 (m, 3H), 4.50-4.55 (m, 2H), 4.75 (dd, *J* = 10.4, 4.0, 1H, H2´), 4.86 (dd, *J* = 10.4, 4.0, 1H, H2´´), 4.89 (dd, *J* = 10.4, 4.0, 1H, H2), 5.08 (t, *J* = 10.4, 1H, H4´´), 5.30 (d, *J* = 4.0, 1H, H1´), 5.37 (t, *J* = 10.4, 1H, H3´´), 5.40-5.45 (m, 1H, H3´), 5.43 (d, *J* = 4.0, 1H, H1´´), 5.62 (t, *J* = 10.4, 1H, H3), 6.18 (d, *J* = 4.0, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.8, 20.9, 20.9, 21.0,

61.3, 62.1, 62.1, 67.9, 68.4, 69.2, 69.3, 70.1, 70.4, 70.9, 71.2, 71.6, 71.8, 72.2, 72.8, 89.7, 95.6, 95.9, 169.4, 169.5, 169.7, 169.8, 170.0, 170.3, 170.4, 170.5, 170.7, 170.8; LRMS (FAB) 1075 ((M + Cs)$^+$, 45); HRMS (FAB) calcd for $C_{38}H_{51}O_{25}ClCs$ 1075.1462, found 1075.1483.

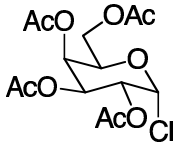


2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranosyl azide.. (deca-*O*-acetyl-β-maltotriosyl azide) (87).

Method A: Deca-*O*-acetyl-β-maltotriose (500 mg, 0.517 mmol) was dissolved in 25 mL of distilled CH_2Cl_2. Trimethylsilyl azide (89 μL, 0.672 mmol) was added via syringe followed by $SnCl_4$ (30 μL, 0.256 mmol) and the solution was stirred for 20 h at 20 °C. The reaction mixture was diluted with 200 mL CH_2Cl_2, washed with 200 mL sat. aq. $NaHCO_3$ solution, and with 2x200 mL H_2O. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give crude product as an oil, which was triturated with absolute ethanol to afford 349 mg (71%) of deca-O-acetyl-β-maltotriosyl azide **87** as a foam.

Method B: The deca-*O*-acetyl-α-maltotriosyl chloride (1.52 g, 1.61 mmol) was dissolved in 15 mL of THF. Trimethylsilyl azide (278 μL, 2.10 mmol) was added via syringe followed by TBAF (2.10 mL, 2.10 mmol). The solution was stirred for 29 h at

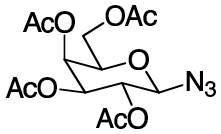
65 °C. The reaction was cooled to ambient temperature, filtered through a plug of silica gel, dried over Na_2SO_4, and concentrated in vacuo to give a yellow oil. The oil was triturated with absolute ethanol to afford 1.22 mg (80%) of deca-*O*-acetyl-β-maltotriosyl azide **87** as foam: IR (CCl_4) 2956 (w), 2117 (m), 1759 (s), 1368 (s), 1227 (s)1053 (s); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.97 (s, 3H), 1.98 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 3.80 (ddd, *J* = 12.4, 4.5, 2.7, 1H, H5), 3.85-4.00 (m, 4H, H5˝, H5′, H4, H4′), 4.03 (dd, *J* = 2.3, 12.4, 1H, H6˝), 4.16 (dd, *J* = 3.6, 12.4, 1H, H6′), 4.23 (dd, *J* = 3.6, 12.4, 1H, H6˝), 4.30 (dd, *J* = 4.5, 12.4, 1H, H6), 4.44 (dd, *J* = 2.3, 12.4, 1H, H6′), 4.48 (dd, *J* = 2.7, 12.4, 1H, H6), 4.69 (d, *J* = 8.9, 1H, H1), 4.72 (dd, *J* = 4.1, 10.2, 1H, H2′), 4.76 (t, *J* = 8.9, 1H, H2), 4.83 (dd, *J* = 4.1, 10.2, 1H, H2˝), 5.05 (t, *J* = 10.2, 1H, H4˝), 5.20-5.25 (m, 2H, H3, H1′), 5.30-5.37 (m, 2H, H3˝, H3′), 5.38 (d, *J* = 4.1, 1H, H1˝); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.7, 20.8, 20.8, 61.3, 62.2, 62.7, 67.8, 68.5, 69.0, 69.3, 70.0, 70.4, 71.5, 71.6, 72.4, 73.4, 74.1, 74.9, 87.4, 95.6, 95.9, 169.4, 169.5, 169.7, 169.8, 170.0, 170.3, 170.4, 170.5, 170.6, 170.6; LRMS (FAB) 956 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{38}H_{51}N_3O_{25}Li$ 956.2972, found 956.2997.



2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl chloride

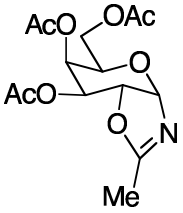
Penta-*O*-acetyl-β-galactose (5.00 g, 12.8 mmol) was dissolved in 25 mL of anhydrous chloroform, followed by addition of 1M titanium tetrachloride solution (13.5 mL, 13.5 mmol) via syringe. The solution was refluxed for 3 h, and cooled to room temperature. The reaction mixture was diluted with 100 mL CH_2Cl_2,

washed twice with 100 mL water, filtered through a plug of silica gel, dried over Na_2SO_4, and concentrated in vacuo to give a yellow oil. The oil was crystallized from mixture of Et_2O and petroleum ether to afford 4.48 g (95%) of tetra-*O*-acetyl-α-galactosyl chloride as a white solid; m.p. 73-75 °C (lit. m.p. 71-73 °C)[137]; IR (CCl_4) 2953 (w), 1751 (s), 1361 (s), 1221 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.04 (s, 3H), 2.10 (s, 3H), 2.13 (s, 3H), 4.08 (dd, *J* = 11.6, 6.4, 1H, H6), 4.14 (dd, *J* = 11.6, 6.4, 1H, H6), 4.50 (t, *J* = 6.4, 1H, H5), 5.23 (dd, *J* = 10.8, 4.0, 1H, H2), 5.39 (dd, *J* = 10.8, 3.2, 1H, H3), 5.50 (d, *J* = 3.2, 1H, H4), 6.35 (d, *J* = 4.0, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 20.8, 20.9, 20.9, 61.2, 67.3, 67.4, 68.1, 69.1, 91.4, 170.0, 170.2, 170.4, 170.6; LRMS (FAB) 367 ($(M + H)^+$, 12); HRMS (FAB) calcd for $C_{14}H_{20}O_9Cl$ 367.0796, found 367.0798.

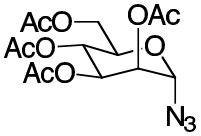


2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide (89). 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl chloride (4.48 g, 12.2 mmol) was dissolved in 20 mL of distilled THF. Trimethylsilyl azide (2.10 mL, 15.8 mmol) was added via syringe followed by 1.0 M TBAF (15.8 mL, 15.8 mmol). The solution was stirred for 30 h at 65 °C. The solution was cooled to RT and filtered through a plug of silica. The filtrate was dried over Na_2SO_4 and concentrated in vacuo to give 3.70 g (81%) of azide **89** as a white solid; m.p. 78-80 °C (lit. m.p. 79 °C)[138]; IR (CCl_4) 2958 (w), 2120 (s), 1757 (s), 1368 (s), 1227 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.18 (s, 3H), 4.02 (dd, *J* = 6.4, 1.0, 1H, H5), 4.10-4.20 (m, 2H, H6), 4.60 (d, *J* = 8.8, 1H, H1), 5.04 (dd, *J* = 10.4, 3.6, 1H, H3), 5.17 (dd, *J* = 10.4, 8.8, 1H, H2), 5.43 (dd, *J* = 3.6, 1.0, 1H, H4); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 20.9, 21.0, 21.1, 21.1, 61.6, 67.2, 68.4, 71.1, 73.3, 88.7, 169.8, 170.4, 170.5, 170.8; LRMS (FAB) 380 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{14}H_{19}O_9N_3Li$ 380.1281, found 380.1297.

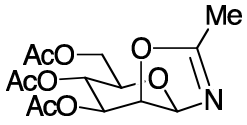


3,4,6-Tri-*O*-acetyl-α-D-galactopyranosyl isoxazoline (90). 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide (**89**)(150 mg, 0.402 mmol) and Ph_3P (116 mg, 0.442 mmol) were dissolved in 1,2-dichloroethane (5 mL) in the presence of 4Å molecular sieves. The resulting solution was heated under reflux for 15h, then was cooled to ambient temperature and concentrated in vacuo. The crude oil was dried under vacuum and characterized without any purification. Therefore, Ph_3P and Ph_3PO, which are marked on NMR spectra but not reported at here, were present in the NMR spectrum of the crude product: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 4.09 (dd, *J* = 11.0, 6.0, 1H, H6), 4.15-4.225 (m, 2H, H6, H5), 4.46 (t, *J* = 7.2, 1H, H2), 4.92 (dd, *J* = 7.2, 3.2, 1H, H3), 5.45 (t, *J* = 3.2, 1H, H4), 5.81 (d, *J* = 7.2, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6, 20.6, 20.7, 20.7, 61.3, 66.3, 69.5, 71.9, 76.7, 93.7, 168.3, 169.9, 170.0, 170.5.



2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl azide (91). The azide was prepared according to the procedure of Györgydeák *et. al.*[139] 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl acetate (2.00 g, 5.13 mmol) was dissolved in 20 mL of distilled CH_2Cl_2. Trimethylsilyl azide (1.02 mL, 7.70 mmol) was added via syringe followed by $SnCl_4$ (300 μL, 2.57 mmol) and the solution was

stirred for 20 h at 25 °C. The reaction mixture was diluted with 200 mL CH_2Cl_2, washed with 200 mL sat. aq. $NaHCO_3$ solution, and with 2x200 mL H_2O. The organic layer was filtered through a plug of silica. The filtrate was dried over Na_2SO_4 and concentrated in vacuo to give 1.82 g (95%) of azide **91** as a clear syrup: IR (CCl_4) 2956 (w), 2118 (s), 1759 (s), 1370 (s), 1228 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.03 (s, 3H), 2.09 (s, 3H), 2.15 (s, 3H), 4.10-4.20 (m, 2H, H5, H6), 4.28 (dd, *J* = 12.4, 5.6, 1H, H6), 5.13 (dd, *J* = 2.8, 2.0, 1H, H2), 5.22 (dd, *J* = 10.0, 2.8, 1H, H3), 5.27 (t, *J* = 10.0, 1H, H4), 5.37 (d, *J* = 2.0, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.7, 20.8, 62.1, 65.6, 68.2, 69.1, 70.6, 87.4, 169.6, 169.7, 169.8, 170.6; LRMS (FAB) 380 ($(M + Li)^+$, 100); HRMS (FAB) calcd for $C_{14}H_{19}O_9N_3Li$ 380.1281, found 380.1291.



3,4,6-Tri-*O*-acetyl-β-D-mannopyranosyl isoxazoline (93). 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl azide (**91**) (150 mg, 0.402 mmol) and Ph_3P (116 mg, 0.442 mmol) were dissolved in 1,2-dichloroethane (5 mL) in the presence of 4Å molecular sieves. The resulting solution was heated under reflux for 24 h, then was cooled to ambient temperature and concentrated in vacuo. Purification of the residue by flash chromatography (hexanes:EtOAc:Et_3N, 25:75:1) gave 64 mg (49%) of isoxazoline **93** as colorless oil: R_f = 0.23 (hexanes:EtOAc, 1:3); IR (CCl_4) 2933 (m), 1754 (s), 1665 (m), 1222 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (s, 3H), 2.04 (s, 3H), 2.10 (s, 6H), 3.74 (ddd, *J* = 9.0, 6.4, 3.2, 1H, H5), 4.11 (dd, *J* = 12.0, 3.2, 1H, H6), 4.23 (dd, *J* = 12.0, 6.4, 1H, H6), 4.61 (t, *J* = 5.2, 1H, H2), 5.10 (t, *J* = 9.0, 1H, H4), 5.28 (dd, *J* = 9.0, 5.2, 1H, H3), 5.49 (d, *J* = 5.2, 1H, H1); ^{13}C NMR

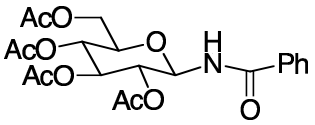
(100 MHz, $CDCl_3$) δ 14.5, 20.7, 20.8, 20.8, 63.4, 66.3, 69.2, 72.9, 76.7, 93.2, 169.4, 170.2, 170.7, 171.3; LRMS (FAB) 330 ($(M + H)^+$, 100); HRMS (FAB) calcd for $C_{14}H_{20}O_8N$ 330.1189 $(M + H)^+$, found 330.1191.

General procedure (A) for the coupling reactions using Ph_3P. Per-*O*-acetyl-β-D-glycopyranosyl azide (1.0 equiv.) and Ph_3P (1.1 equiv.) were dissolved in distilled 1,2-dichloroethane in the presence of 4Å molecular sieves. The resulting solution was heated under reflux for 15h, then was cooled to RT. The acylating reagent (1.3 equiv.) and additive (1.3 equiv.), respectively, were added to the reaction mixture and stirred at given temperature for 24 hr. The reaction mixture was diluted with 100 mL Et_2O and washed with 2x100 mL H_2O. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give crude product, which was purified by flash chromatography.

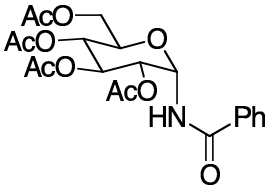
2,3,4,6-Tetra-*O*-acetyl-1-*N*-benzoyl-β-D-glucopyranosylamine (β-94) and 2,3,4,6-Tetra-*O*-acetyl-1-*N*-benzoyl-α-D-glucopyranosylamine (α-94)

For Table 3, entry 1: Compounds **95** were prepared following the general coupling procedure (A) outlined above employing 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (**56**) (125 mg, 0.335 mmol), Ph_3P (96.0 mg, 0.368 mmol), and benzoic anhydride (302 mg, 1.34 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 90.0 mg (60%) of α/β mixture (1:3) of **95** as white solid. TLC R_f = 0.30 (hexanes:EtOAc, 1:1). The anomers were separated by

HPLC using Hypersilica 5μm column (hexane:CH_2Cl_2:MeOH, 90:10:1), and isolated pure anomers were recrystallized from hexanes:EtOAc (1:1) solvent mixture.



(β-94): m.p. 197-199 °C; IR (CCl_4) 3441 (w), 3066 (w), 2960 (w), 1760 (s), 1691 (m), 1223 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (s, 9H), 2.06 (s, 3H), 3.89 (ddd, *J* = 9.6, 4.0, 2.0, 1H, H5), 4.07 (dd, *J* = 12.8, 2.0, 1H, H6), 4.33 (dd, *J* = 12.8, 4.0, 1H, H6), 5.04 (t, *J* = 9.6, 1H, H2), 5.10 (t, *J* = 9.6, 1H, H4), 5.38 (t, *J* = 9.6, 1H, H3), 5.42 (t, *J* = 9.6, 1H, H1), 6.97 (d, *J* = 9.0, 1H, NH), 7.40-7.75 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.6, 20.6, 20.7, 20.7, 61.6, 68.2, 70.8, 72.5, 73.6, 78.9, 127.2, 128.7, 132.4, 132.7, 167.1, 169.6, 169.9, 170.6, 171.5; LRMS (FAB) 452 ((M + H)$^+$); HRMS (FAB) calcd for $C_{21}H_{26}O_{10}N$ 452.1557 (M + H)$^+$, found 452.1561.

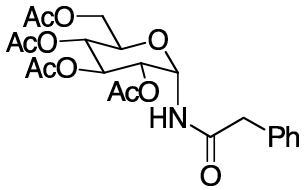


(α-94): m.p. 234-236 °C; IR (CCl_4) 3482 (w), 3062 (w), 2985 (w), 1752 (s), 1667 (m), 1221 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 4.01 (ddd, *J* = 9.6, 4.0, 2.0, 1H, H5), 4.08 (dd, *J* = 12.0, 2.0, 1H, H6), 4.30 (dd, *J* = 12.0, 4.0, 1H, H6), 5.08 (t, *J* = 9.6, 1H, H4), 5.27 (dd, *J* = 9.6, 5.5, 1H, H2), 5.39 (t, *J* = 9.6, 1H, H3), 6.04 (dd, *J* = 5.0, 7.0, 1H, H1), 6.73 (d, *J* = 7.0, 1H, NH), 7.45-7.85 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.7, 20.8, 20.8, 20.9, 61.9, 68.4, 68.6, 68.7, 70.4, 75.0, 127.5, 129.0, 132.6, 134.5, 169.3, 169.6, 170.6, 170.9, 172.0; LRMS (FAB) 452 ((M + H)$^+$); HRMS (FAB) calcd for $C_{21}H_{26}O_{10}N$ 452.1557 (M + H)$^+$, found 452.1557.

2,3,4,6-Tetra-*O*-acetyl-1-*N*-phenyl acetyl-β-D-glucopyranosylamine (β-95) and 2,3,4,6-Tetra-*O*-acetyl-1-*N*-phenyl acetyl-α-D-glucopyranosylamine (α-95).

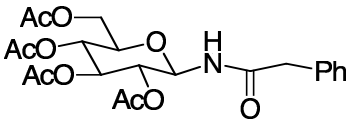
Method A (Table 5, entry 1): Compounds **95** were prepared following the general coupling procedure (A) outlined above employing 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide **56** (200 mg, 0.536 mmol), Ph_3P (155 mg, 0.590 mmol), and 2-pyridyl thiolphenylacetate (160 mg, 0.697 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 166 mg (67%) of α/β mixture (5:1) of **95** as white solid. TLC R_f = 0.33 (hexanes:EtOAc, 1:1). The anomers were separated by HPLC using Hypersilica 5μm column (hexane:CH_2Cl_2:MeOH, 90:10:1), and isolated pure anomers were recrystallized from hexanes:EtOAc (1:1) solvent mixture.

Method B (Table 5, entry 3): Compound **α-95** was prepared following the general coupling procedure (A) outlined above employing 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (**56**) (200 mg, 0.536 mmol), Ph_3P (155 mg, 0.590 mmol), and 2-thiopyridyl phenylacetate (160 mg, 0.697 mmol) as the acylating reagent, $CuCl_2 \cdot 2H_2O$ (119 mg, 0.697 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 199 mg (80%) of **α-95** as a white solid:

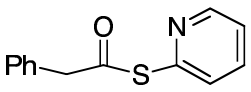


(α-95): m.p. 156-158 °C; IR (CCl_4) 3258 (w), 3062 (w), 2962 (w), 1754 (s), 1655 (m), 1220 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 (s, 3H), 1.98 (s, 6H), 2.02 (s, 3H), 3.61 (s, 2H), 3.74 (ddd, *J* = 9.6, 4.0, 2.4, 1H, H5), 3.99 (dd, *J* = 12.0, 2.4, 1H, H6), 4.22 (dd, *J* = 12.0, 4.0, 1H, H6), 4.95-5.10 (m, 3H, H2, H3, H4), 5.78 (dd, *J* = 4.8, 7.0, 1H, H1), 6.46 (d, *J* = 7.0, 1H, NH), 7.25-7.35 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7,

21.0, 21.0, 21.1, 44.2, 62.0, 68.4, 68.6, 68.7, 70.4, 74.7, 128.1, 129.4, 129.6, 134.7, 169.3, 169.7, 170.7, 170.2, 172.1; LRMS (FAB) 466 ((M + H)$^+$, 16), 43 (100), 91 (57); HRMS (FAB) calcd for $C_{22}H_{28}O_{10}N$ 466.1713 (M + H)$^+$, found 466.1716.



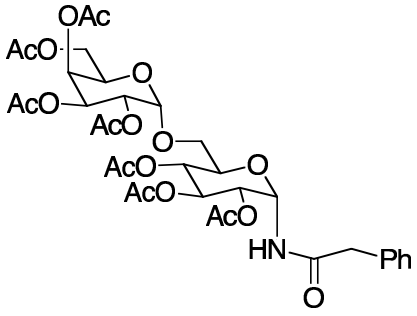
(β-95): m.p. 158-159 °C; IR (KBr) 3457 (w), 3060 (w), 2955 (w), 1750 (s), 1662 (m), 1536 (m) 1229 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.81 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.06 (s, 3H), 3.47 (d, *J* = 15.2, 1H, CH_2), 3.57 (d, *J* = 15.2, 1H, CH_2), 3.78 (ddd, *J* = 9.6, 4.4, 2.0, 1H, H5), 4.05 (dd, *J* = 12.4, 2.0, 1H, H6), 4.28 (dd, *J* = 12.4, 4.4, 1H, H6`), 4.80 (t, *J* = 9.6, 1H, H2), 5.00 (t, *J* = 9.6, 1H, H4), 5.17 (t, *J* = 9.6, 1H, H1), 5.23 (t, *J* = 9.6, 1H, H3), 6.17 (d, *J* = 9.6, 1H, NH), 7.19 (d, *J* = 7.2, 2H), 7.25-7.35 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.3, 20.5, 20.6, 20.7, 43.9, 61.6, 68.1, 70.1, 72.5, 73.6, 78.3, 127.5, 129.1, 129.2, 133.7, 169.5, 169.8, 170.6, 170.6, 171.2; LRMS (FAB) 466 ((M + H)$^+$, 100); HRMS (FAB) calcd for $C_{22}H_{28}O_{10}N$ 466.1713 (M + H)$^+$, found 466.1725.



2-Pyridyl thiolphenylacetate (96)

Phenyl acetic acid (1.70 g, 12.5 mmol), triphenylphosphine (4.21 g, 16.0 mmol), and 2,2'-dithiopyridine (3.53 g, 16.0 mmol) is dissolved in 30 mL distilled THF. The solution stirred at RT for 24 hr. and concentrated in vacuo to give crude product, which was purified by flash chromatography (hexanes:EtOAc, 4:1) to give 2.73 g (96%) of thiolester **96** as orange oil. It was stored under argon in a desiccator: R_f = 0.35 (hexanes:EtOAc, 4:1); IR (CCl_4) 3070 (w), 3033 (m), 2912 (w),

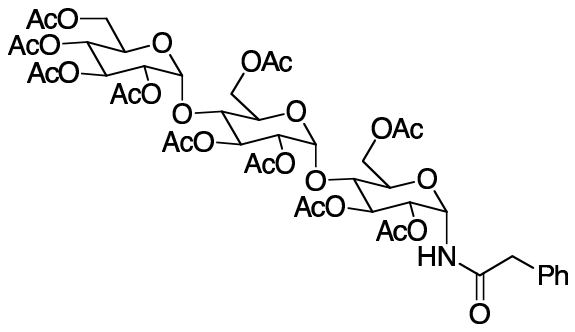
1708 (s), 1420 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 (s, 2H), 7.20-7.25 (m, 1H), 7.25-7.35 (m, 5H), 7.54 (d, *J* = 8.0, 1H), 7.66 (td, *J* = 8.0,1.0, 1H), 8.58 (dd, *J* = 5.0, 1.0, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.0, 124.1, 128.1, 129.2, 130.2, 130.6, 133.2, 137.7, 150.8, 151.8, 194.9; LRMS (EI) 229 ((M^+, 21), 118 (100)), 91 (80); HRMS (EI) calcd for $C_{13}H_{11}ONS$ 229.0561, found 229.0553.



2,3,4-Tri-*O*-acetyl-6-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl]-1-*N*-phenyl acetyl-α-D-glucopyranosylamine (97).

Hepta-*O*-acetyl-1-*N*-phenyl acetyl melibiosyl amine **(97)** was prepared following the general coupling procedure (A) outlined above employing hepta-*O*-acetyl-β-melibiosyl azide (**85**) (125 mg, 0.189 mmol), Ph_3P (55.0 mg, 0.208 mmol), 2-pyridyl thiolphenylacetate (57.0 mg, 0.246 mmol) as the acylating reagent, and $CuCl_2$•$2H_2O$ (42.1 mg, 0.246 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 110 mg (77%) of **97** as a white foam: R_f = 0.19 (hexanes:EtOAc, 1:1); IR (CCl_4) 3398 (w), 3030 (w), 2926 (m), 1754 (s), 1706 (m), 1370 (s), 1227 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 3.60-3.75 (m, 5H, H5, H6, H6, CH2), 4.00 (dd, *J* = 11.2, 6.4, 1H, H6´), 4.11 (dd, *J* = 11.2, 6.4, 1H, H6´), 4.22 (t, *J* = 6.4, 1H, H5´), 4.98 (t, *J* = 9.0, 1H, H4), 4.99 (dd, *J* = 9.0, 5.0, 1H, H2), 5.00-5.10 (m, 3H, H3, H1´, H2´), 5.29 (dd, *J* = 8.8, 3.0, 1H, H3´), 5.44 (d, *J* = 3.0, 1H, H4´), 5.72 (dd, *J* = 7.6, 5.0, 1H, H1), 6.28 (d, *J* = 7.6, 1H, NH), 7.25-7.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 20.6, 20.7,

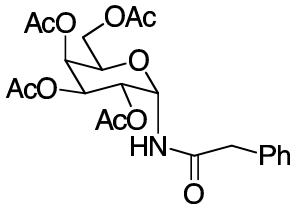
20.7, 20.8, 43.8, 61.5, 66.3, 66.6, 67.4, 68.0, 68.1, 68.4, 69.0, 69.0, 69.9, 73.9, 96.4, 127.6, 129.1, 129.1, 134.3, 168.9, 169.3, 169.9, 170.1, 170.2, 170.5, 170.7, 171.4; LRMS (FAB) 760 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{34}H_{43}NO_{18}Li$ 760.2640, found 760.2639.



2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-glucopyranosyl]-1-*N*-phenyl acetyl-α-D-glucopyranosylamine (98).

Deca-*O*-acetyl-1-α-*N*-phenyl acetyl maltotriosyl amine **(98)** was prepared following the general coupling procedure (A) outlined above employing deca-*O*-acetyl-β-maltotriosyl azide **87** (200 mg, 0.211 mmol), Ph_3P (61.0 mg, 0.232 mmol), 2-pyridyl thiolphenylacetate (63.0 mg, 0.275 mmol) as the acylating reagent, and $CuCl_2•2H_2O$ (47.0 mg, 0.275 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 2:3) gave 138 mg (63%) of **98** as a white foam: R_f = 0.22 (hexanes:EtOAc, 2:3); IR (CCl_4) 3429 (w), 3030 (w), 2958 (w), 1758 (s), 1699 (m), 1368 (s), 1233 (s), 1040 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.11 (s, 3H), 2.11 (s, 3H), 3.65-3.70 (m, 3H, CH2, H4), 3.85-4.00 (m, 4H, H4´, H5,

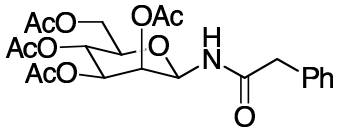
H5´, H5´´), 4.03 (dd, *J* = 2.0, 12.4, 1H, H6´´), 4.11 (dd, *J* = 4.0, 12.4, 1H, H6´), 4.18 (dd, *J* = 5.6, 12.4, 1H, H6), 4.23 (dd, *J* = 3.6, 12.4, 1H, H6´´), 4.29 (dd, *J* = 3.2, 12.4, 1H, H6), 4.43 (dd, *J* = 2.0, 12.4, 1H, H6´), 4.73 (dd, *J* = 4.0, 10.0, 1H, H2´), 4.85 (dd, *J* = 4.0, 10.0, 1H, H2´´), 4.90 (dd, *J* = 4.4, 7.0, 1H, H2), 4.96 (m, 1H, H3), 5.06 (t, *J* = 10.0, 1H, H4´´), 5.15 (d, *J* = 4.0, 1H, H1´), 5.33 (t, *J* = 10.0, 1H, H3´´), 5.34 (t, *J* = 10.0, 1H, H3´), 5.38 (d, *J* = 4.0, 1H, H1´´), 5.76 (dd, *J* = 4.4, 8.4, 1H, H1), 6.25 (d, *J* = 8.4, 1H, NH), 7.29-7.43 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 20.4, 20.6, 20.6, 20.6, 20.7, 20.8, 20.8, 20.8, 20.9, 43.9, 61.3, 62.4, 63.0, 67.8, 68.0, 68.5, 68.8, 69.4, 69.8, 70.1, 70.3, 70.5, 71.9, 72.4, 72.8, 74.3, 95.9, 96.4, 127.7, 129.3, 129.4, 134.2, 169.2, 169.4, 169.7, 169.8, 169.9, 170.3, 170.4, 170.4, 170.5, 170.6, 171.1; LRMS (FAB) 1174 ((M + Cs)$^+$, 12); HRMS (FAB) calcd for $C_{46}H_{59}NO_{26}Cs$ 1174.2380, found 1174.2327.



2,3,4,6-Tetra-*O*-acetyl-1-*N*-phenyl acetyl-α-D-galactopyranosylamine (99)

Compound **99** was prepared following the general coupling procedure (A) outlined above employing 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl azide (**89**) (200 mg, 0.536 mmol), Ph_3P (155 mg, 0.590 mmol), and 2-pyridyl thiolphenylacetate (160 mg, 0.697 mmol) as the acylating reagent, $CuCl_2•2H_2O$ (119 mg, 0.697 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 182 mg (73%) of **α-99** as a white solid: R_f = 0.20 (hexanes:EtOAc, 1:1); m.p. 151-153 °C; IR (CCl_4) 3272 (w), 3068 (w), 1757 (s), 1652 (m), 1223 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.83 (s, 3H), 1.97 (s, 3H), 2.01 (s, 3H),

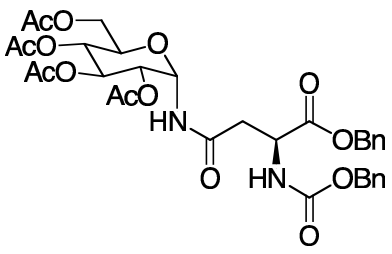
2.10 (s, 3H), 3.63 (s, 2H, CH_2), 3.90-3.95 (m, 1H, H5), 4.00-4.10 (m, 2H, H6, H6), 4.87 (dd, *J* = 10.4, 3.2, 1H, H3), 5.25 (dd, *J* = 10.4, 6.0, 1H, H2), 5.32 (br s, 1H, H4), 5.81 (dd, *J* = 6.0, 1H, H1), 6.00 (d, *J* = 6.0, 1H, NH), 7.20-7.35 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.4, 20.6, 20.7, 20.7, 43.9, 61.2, 66.0, 67.0, 67.3, 67.5, 74.6, 127.8, 129.1, 129.3, 133.2, 169.0, 170.0, 170.1, 170.4, 171.5; LRMS (FAB) 472 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{22}H_{27}O_{10}NLi$ 472.1795 (M + Li)$^+$, found 472.1788.



2,3,4,6-Tetra-*O*-acetyl-1-*N*-phenyl acetyl-β-D-mannopyranosylamine (100)

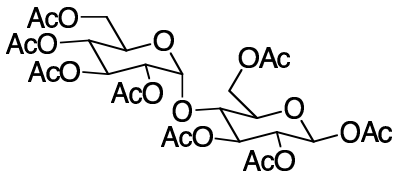
Compound **100** was prepared following the general coupling procedure (A) outlined above employing 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl azide (**91**) (200 mg, 0.536 mmol), Ph_3P (155 mg, 0.590 mmol), and 2-pyridyl thiolphenylacetate (160 mg, 0.697 mmol) as the acylating reagent, $CuCl_2•2H_2O$ (119 mg, 0.697 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 182 mg (73%) of **β-100** as a white solid: R_f = 0.20 (hexanes:EtOAc, 1:1); IR (CCl_4) 3257 (w), 3063 (w), 2960 (w), 1752 (s), 1655 (m), 1217 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.91 (s, 3H), 1.92 (s, 3H), 2.01 (s, 3H), 2.06 (s, 3H), 3.58 (d, *J* = 16.8, 1H, CH_2), 3.62 (d, *J* = 16.8, 1H, CH_2), 3.71 (ddd, *J* = 9.6, 5.6, 2.0, 1H, H5), 4.03 (dd, *J* = 12.4, 2.0, 1H, H6), 4.26 (dd, *J* = 12.4, 5.6, 1H, H6), 5.04 (dd, *J* = 9.6, 3.2, 1H, H3), 5.11 (t, *J* = 9.6, 1H, H4), 5.22 (dd, *J* = 3.2, 1.6, 1H, H2), 5.49 (dd, *J* = 9.6, 1.6, 1H, H1), 5.95 (d, *J* = 9.6, 1H, NH), 7.20-7.35 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.4, 20.5, 20.7, 20.8, 43.5, 62.2, 65.1, 69.7, 71.3, 74.2, 75.8, 127.7, 129.2, 129.7, 133.7,

169.6, 169.8, 170.1, 170.7; LRMS (FAB) 466 ((M + H)$^+$, 100); HRMS (FAB) calcd for $C_{22}H_{28}O_{10}N$ 466.1713 (M + H)$^+$, found 466.1726.



2,3,4,6-Tetra-*O*-acetyl-1-*N*-[1-benzyl-*N*-(benzyloxy)carbonyl-L-aspart-4-oyl]-α-D-glucopyranosylamine (101).

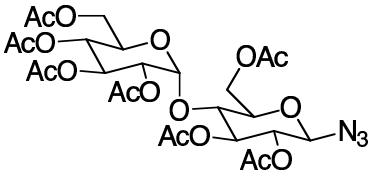
Asparagine derivative **101** was prepared following the general coupling procedure (A) outlined above employing α-azide (**56**) (100 mg, 0.268 mmol), Ph_3P (77.0 mg, 0.295 mmol), Z-Asp(SPyr)-OBn (157 mg, 0.348 mmol) as the acylating reagent, and $CuCl_2$•$2H_2O$ (60.0 mg, 0.348 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 138 mg (75%) of **101** as a white solid: m.p. 159-160 °C; R_f = 0.30 (hexanes:EtOAc, 1:1); IR (CCl_4) 3429 (w), 3345 (w), 3035 (w), 2956 (w), 1754 (s), 1701 (s), 1685 (m), 1220 (s), 1042 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.87 (dd, *J* = 16.0, 3.6, 1H, CH_2), 2.95 (dd, *J* = 16.0, 3.6, 1H, CH_2), 3.84 (m, 1H, H5), 3.97 (dd, *J* = 12.0, 2.0, 1H, H6), 4.24 (dd, *J* = 12.0, 4.0, 1H, H6), 4.67 (m, 1H, CH), 5.03 (t, *J* = 9.2, 1H, H4), 5.05-5.25 (m, 6H, H2, H3, 2xCH_2Ph), 5.80 (t, *J* = 6.0, 1H, H1), 5.92 (br s, 1H, Asp-NH), 6.57 (br s, 1H, NH), 7.30-7.35 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.6, 20.6, 20.7, 38.3, 50.9, 61.5, 67.3, 67.7, 68.0, 68.2, 68.3, 70.0, 74.3, 128.0, 128.1, 128.3, 128.5, 128.5, 128.6, 135.0, 135.9, 156.3, 169.1, 169.4, 169.4, 170.5, 170.6, 170.8; LRMS (EI) 686 ((M$^+$, 12), 91 (100); HRMS (EI) calcd for $C_{33}H_{38}O_{14}N_2$ 686.2323, found 687.2305. ^{1}H NMR and ^{13}C NMR data were consistent with the data reported by Zhang *et al.*[113]



2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,4,6-tetra-*O*-cetyl-α-D-glucopyranosyl]-β-D-glucopyranosyl acetate (octa-*O*-acetyl-β-maltose)

The acetate was prepared according to the procedure of Wolfrom *et. al.*[136] D-Maltose monohydrate (9.00 g, 25.0 mmol) was added in portions over 30 min into the refluxing solution of anhydrous NaOAc (8.20 g, 100 mmol) and acetic anhydride (47.0 mL, 500 mL). The solution was refluxed for 3 hr and cooled to 100 °C, then immediately transferred into 400 mL of ice-water mixture and stirred vigorously for 4 hr. The water was decanted and the precipitate was diluted with 200 mL $CHCl_3$, washed with 2x200 mL sat. aq. $NaHCO_3$ solution and 2x 200 mL H_2O, then dried over Na_2SO_4 and concentrated in vacuo to give colorless gummy oil. The azeotropic removal of remaining $CHCl_3$ by dissolving the oil in 2x100 mL hot EtOH, followed by two successive crystallization from 95% EtOH gave 11.8 g (70%) of octa-*O*-acetyl-β-maltose as white solid: m.p. 156-158 °C (lit. m.p. 159-160 °C)[136]; IR (CCl_4) 2963 (w), 1759 (s), 1336 (s), 1227 (s), 1044 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.08 (s, 6H), 2.12 (s, 3H), 3.81 (ddd, *J* = 9.6, 4.4, 2.4, 1H, H5), 3.91 (ddd, *J* = 10.0, 4.4, 2.4, 1H, H5´), 4.00-4.05 (m, 2H, H6´, H4), 4.20 (dd, *J* = 12.4, 4.4, 1H, H6), 4.21 (dd, *J* = 12.4, 4.4, 1H, H6´), 4.43 (dd, *J* = 12.4, 2.4, 1H, H6), 4.84 (dd, *J* = 10.0, 4.0, 1H, H2´), 4.96 (t, *J* = 8.8, 1H, H2), 5.04 (t, *J* = 10.0, 1H, H4´), 5.27 (t, *J* = 8.8, 1H, H3), 5.33 (t, *J* = 10.0, 1H, H3´), 5.39 (d, *J* = 4.0, 1H, H1´), 5.72 (d, *J* = 8.8, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 206, 20.7, 20.8, 20.8, 20.9, 61.4, 62.5, 67.9, 68.5, 69.3, 69.9, 70.9, 72.3, 72.9,

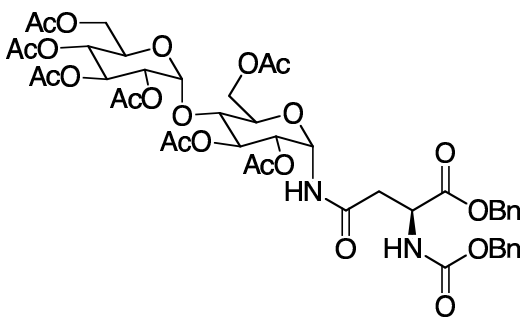
75.3, 91.2, 95.7, 168.8, 169.5, 169.6, 169.9, 170.1, 170.4, 170.5, 170.6; LRMS (FAB) 685 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{28}H_{38}O_{19}Li$ 685.2167, found 685.2151.



2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl]-β-D-glucopyranosyl azide (hepta-*O*-acetyl-β-maltosyl azide)(102).

Octa-*O*-acetyl-β-maltose (8.00 g, 11.8 mmol) was dissolved in 50 mL of distilled CH_2Cl_2. Trimethylsilyl azide (2.19 mL, 16.5 mmol) was added via syringe followed by 1.0 M $SnCl_4$ solution (5.90 mL, 5.90 mmol). The solution was stirred for 20 h at 20 °C. The reaction mixture was diluted with 200 mL CH_2Cl_2, washed with 200 mL sat. aq. $NaHCO_3$ solution, and with 2x200 mL H_2O. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give crude product, which was crystallized from absolute ethanol to afford 6.48 g (83%) of β-azide **102** as a white solid: m.p. 114-116 °C (lit. m.p. 115-116 °C)[129]; IR (CCl_4) 2959 (w), 2119 (s), 1761 (s), 1367 (s), 1229 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 2.14 (s, 3H), 3.76 (ddd, *J* = 9.6, 4.0, 2.4, 1H, H5), 3.92 (ddd, *J* = 10.0, 4.0, 2.4, 1H, H5´), 4.00 (t, *J* = 9.6, 1H, H4), 4.02 (dd, *J* = 12.4, 2.4, 1H, H6´), 4.19 (dd, *J* = 12.4, 4.0, 1H, H6´), 4.24 (dd, *J* = 12.4, 4.0, 1H, H6), 4.49 (dd, *J* = 12.4, 2.4, 1H, H6), 4.68 (d, *J* = 9.0, 1H, H1), 4.77 (t, *J* = 9.0, 1H, H2), 4.83 (dd, *J* = 10.4, 4.4, 1H, H2´), 5.04 (t, *J* = 10.0, 1H, H4´), 5.24 (t, *J* = 9.0, 1H, H3), 5.33 (dd, *J* = 10.0, 1H, H3´), 5.39 (d, *J* = 4.4, 1H, H1´); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.6, 20.6, 20.7, 20.8, 20.9, 61.4, 62.5, 67.9, 68.6, 69.2, 69.9, 71.4, 72.3, 74.2, 75.1, 87.5, 95.7, 169.4, 169.5, 169.9, 170.1, 170.4, 170.5, 170.5; LRMS (FAB) 668 ((M + Li)$^+$,

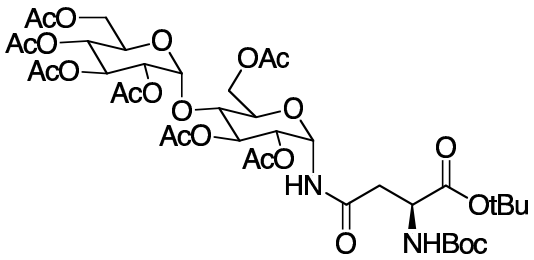
73); HRMS (FAB) calcd for $C_{26}H_{35}O_{17}N_3Li$ 668.2127, found 668.2150. The ^{1}H-NMR and ^{13}C-NMR data were consistent with the data reported by Petö *et. al.*[129]



Hepta-*O*-acetyl-1-*N*-[1-benzyl-*N*-(benzyloxy)carbonyl-L-aspart-4-oyl]-α-maltosylamine (103).

Hepta-*O*-acetyl-1-*N*-aspartoyl-α-maltosylamine derivative (**103**) was prepared following the general coupling procedure (A) outlined above employing hepta-*O*-acetyl-β-maltose azide (**102**) (150 mg, 0.221 mmol), Ph_3P (75.0 mg, 0.287 mmol), Z-Asp(SPyr)-OBzl (129 mg, 0.287 mmol) as the acylating reagent, and $CuCl_2$•$2H_2O$ (49.0 mg, 0.287 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 2:3) gave 155 mg (72%) of **103** as a white foam: R_f = 0.22 (hexanes:EtOAc, 2:3); IR (CCl_4) 3414 (w), 3440 (w), 2964 (m), 1753 (s), 1727 (m), 1698 (w), 1370 (s), 1230 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.06 (s, 6H), 2.87 (dd, *J* = 16.0, 3.6, 1H, CH_2), 3.01(dd, *J* = 16.0, 3.6, 1H, CH_2), 3.84 (dd, *J* = 8.4, 6.8, 1H, H4), 3.90-4.00 (m, 2H, H5, H5´), 4.03(dd, *J* = 12.0, 2.0, 1H, H6´), 4.18 (dd, *J* = 12.0, 4.8, 2H, H6, H6´), 4.28 (dd, *J* = 12.0, 3.0, 1H, H6), 4.60-4.65 (m, 1H, CH), 4.86 (dd, *J* = 10.0, 3.2, 1H, H2´), 4.97 (dd, *J* = 8.0, 5.0, 1H, H2), 5.01 (t, *J* = 10.0, 1H, H4´), 5.09 (d, *J* = 2.0, 2H, CH_2Ph), 5.10-5.20 (m, 3H, H3, CH_2Ph), 5.33 (d, *J* = 3.6, 1H, H1´), 5.35 (t, *J* = 10.0, 1H, H3´), 5.73 (dd, *J* = 8.0, 5.0, 1H, H1), 5.90 (d, *J* = 8.4, 1H, Asp-NH), 6.65 (d, *J* = 8.0, 1H, NH), 7.25-7.30 (m, 10H, 2xPh); ^{13}C NMR (100 MHz, $CDCl_3$) δ

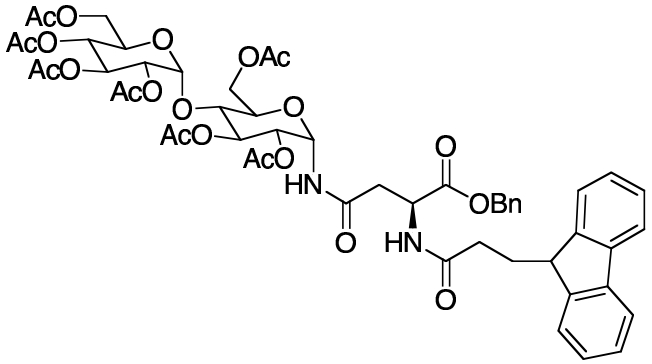
20.5, 20.6, 20.6, 20.6, 20.7, 20.8, 20.9, 38.3, 50.8, 61.5, 62.5, 67.2, 67.7, 68.0, 68.3, 68.4, 69.3, 69.6, 70.1, 71.4, 73.0, 73.1, 96.2, 128.1, 128.1, 128.2, 128.4, 128.5, 128.6, 135.1, 136.0, 156.3, 169.4, 169.4, 170.0, 170.0, 170.4, 170.4, 170.6, 170.6, 170.7; LRMS (FAB) 981 ($(M + Li)^+$, 100); HRMS (FAB) calcd for $C_{45}H_{54}N_2O_{22}Li$ 981.3328, found 981.3304.



Hepta-*O*-acetyl-1-*N*-[1-*tert*-butyl-*N*-(*tert*-butoxy)carbonyl-L-aspart-4-oyl]-α-maltosylamine (104).

Hepta-*O*-acetyl-1-*N*-aspartoyl-α-maltosylamine derivative **(104)** was prepared following the general coupling procedure (A) outlined above employing hepta-*O*-acetyl-β-maltose azide (**102**) (150 mg, 0.221 mmol), Ph_3P (75.0 mg, 0.287 mmol), Boc-Asp(SPyr)-OtBu (110 mg, 0.287 mmol) as the acylating reagent, and $CuCl_2 \bullet 2H_2O$ (49.0 mg, 0.287 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 2:3) gave 136 mg (68%) of **104** as a white foam: R_f = 0.20 (hexanes:EtOAc, 2:3); IR (CCl_4) 3434 (w), 3416 (w), 2961 (m), 1756 (s), 1714 (m), 1700 (w), 1372 (s), 1231 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.46 (s, 9H), 1.99 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.09 (s, 6H), 2.78 (dd, *J* = 15.6, 4.8, 1H, Asp-CH_2), 2.89 (dd, *J* = 15.6, 4.8, 1H, Asp-CH_2), 3.85 (dd, *J* = 8.0, 6.4, 1H, H4), 3.90-4.00 (m, 2H, H5, H5´), 4.03(dd, *J* = 12.4, 2.4, 1H, H6´), 4.15-4.40 (m, 4H, H6, H6, H6´, Asp-CH), 4.86 (dd, *J* = 10.0, 4.0, 1H, H2´), 4.98 (dd, *J* = 8.0, 5.0, 1H, H2), 5.00 (t, *J* = 10.0, 1H, H4´), 5.20 (t, *J* = 8.0, 1H, H3), 5.33 (d, *J* = 4.0, 1H, H1´), 5.33 (t, *J* =

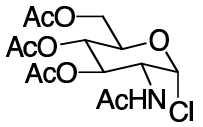
10.0, 1H, H3´), 5.55 (br s, 1H, Asp-NH), 5.75 (dd, *J* = 7.6, 5.0, 1H, H1), 6.72 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 20.7, 20.7, 20.8, 20.9, 27.9, 28.3, 39.0, 50.1, 61.5, 62.7, 68.0, 68.4, 68.4, 69.3, 69.6, 70.0, 71.4, 73.1, 73.2, 80.0, 82.6, 96.3, 155.3, 169.4, 169.4, 169.9, 170.0, 170.0, 170.4, 170.4, 170.5, 170.6; LRMS (FAB) 914 ((M + Li)$^+$, 60); HRMS (FAB) calcd for $C_{39}H_{58}N_2O_{22}Cs$ 1039.2536, found 1039.2550.



Hepta-*O*-acetyl-1-*N*-[1-*tert*-butyl-*N*-(9-fluorenylmethylcarbonyl)-L-aspart-4-oyl]-α-maltosylamine (105).

Hepta-*O*-acetyl-1-*N*-aspartoyl-α-maltosylamine derivative (**105**) was prepared following the general coupling procedure (A) outlined above employing hepta-*O*-acetyl-β-maltose azide (**102**) (150 mg, 0.221 mmol), Ph_3P (75.0 mg, 0.287 mmol), Fmoc-Asp(SPyr)-OtBu (145 mg, 0.287 mmol) as the acylating reagent, and $CuCl_2$•$2H_2O$ (49.0 mg, 0.287 mmol) as the additive. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:2) gave 149 mg (65%) of **105** as a white foam: R_f = 0.20 (hexanes:EtOAc, 2:3); IR (CCl_4) 3440 (w), 3415 (w), 2961 (m), 1753 (s), 1727 (m), 1699 (w), 1367 (m), 1230 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.99 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.04 (s, 6H), 2.07 (s, 3H), 2.09 (s, 3H),

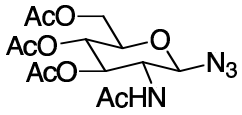
2.84 (dd, J = 16.0, 4.8, 1H, Asp-CH_2), 2.96 (dd, J = 16.0, 4.8, 1H, Asp-CH_2), 3.86 (dd, J = 8.0, 6.4, 1H, H4), 3.90-4.00 (m, 2H, H5, H5´), 4.03(dd, J = 12.4, 2.0, 1H, H6´), 4.15-4.35 (m, 5H, H6, H6, H6´, Fmoc-CH_2), 4.42 (t, J = 9.2, 1H, Fmoc-CH), 4.50 (m, 1H, Asp-CH), 4.86 (dd, J = 10.0, 4.0, 1H, H2´), 4.99 (dd, J = 8.0, 5.2, 1H, H2), 5.04 (t, J = 10.0, 1H, H4´), 5.21 (t, J = 8.0, 1H, H3), 5.33 (m, 2H, H1´, H3´), 5.77 (br t, J = 6.0, 1H, H1), 5.92 (d, J = 7.2, 1H, Asp-NH), 6.72 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 20.6, 20.7, 20.8, 20.9, 27.9, 38.6, 47.0, 51.2, 61.5, 62.6, 67.3, 68.0, 68.3, 68.4, 69.3, 69.7, 70.0, 71.3, 73.1, 73.1, 82.9, 96.2, 120.0, 125.1, 127.1, 127.7, 141.3, 143.7, 156.3, 169.4, 169.4, 169.4, 169.7, 169.7, 170.0, 170.4, 170.5, 170.6; LRMS (FAB) 1035 ((M + Li)$^+$, 50); HRMS (FAB) calcd for $C_{49}H_{60}N_2O_{22}Cs$ 1161.2692, found 1161.2649.



2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (109).

The procedure of Horton D. was followed.[140] The heterogeneous solution of 2-acetamido-2-deoxy-D-glucose (25.0 g, 113 mmol) and acetyl chloride (56 mL, 791 mmol) was stirred in a flask with a condenser under reflux without outside heating. After 20 hr, the homogeneous red solution was diluted with 300 mL of CH_2Cl_2 and poured onto 500 mL of ice water. The mixture was rapidly stirred and transferred onto the mixture 300 g of ice and 300 mL of sat. aq. $NaHCO_3$ solution. The mixture was rapidly shaken until the acid was neutralized. The organic layer was separated and dried over Na_2SO_4. The solution was concentrated at 50 °C to 40mL clear syrup in which 250 mL of distilled ether was rapidly added. After the solution left at RT for 16

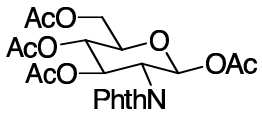
hr for crystallization, it was filtered and dried under vacuum to give 23.1 g of the mixture of α-chloride **109** and α-acetate. Purification of the mixture by flash chromatography (Et_2O:CH_2Cl_2, 4:1) gave 16.5 g (40%) of α-chloride as a white solid; TLC R_f = 0.44 (Et_2O:CH_2Cl_2, 4:1); m.p. 124-126 °C (lit. m.p. 127-128 °C)[140]; IR (CCl_4) 3257 (w), 2960 (w), 1752 (s), 1701(m); ^{1}H NMR (400 MHz, $CDCl_3$) 1.96 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.08 (s, 3H), 4.05-4.15 (m, 1H, H6), 4.20-4.30 (m, 2H, H5, H6), 4.51 (ddd, *J* = 9.6, 7.2, 3.6, 1H, H2), 5.19 (t, *J* = 9.6, 1H, H4), 5.30 (t, *J* = 9.6, 1H, H3), 5.78 (d, *J* = 3.6 1H, NH), 6.16 (d, *J* = 3.6, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) 20.5, 20.5, 20.7, 23.1, 53.5, 61.1, 66.9, 70.1, 70.9, 93.6, 169.1, 170.1, 170.6, 171.5; LRMS (FAB) 366 ($(M + H)^+$, 5); HRMS (FAB) calcd for $C_{14}H_{21}O_8NCl$ 366.0956 $(M + H)^+$, found 366.0950.



2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (53).

2-Acetamido-α-D-glucopyranosyl chloride **(109)** (8.00 g, 21.9 mmol) was dissolved in 150 mL of distilled THF. Trimethylsilyl azide (5.8 mL, 43.8 mmol) was added via syringe followed by 1.0 M TBAF (43.8 mL, 43.8 mmol). The solution was stirred for 3 h at 65 °C. The solution was cooled to RT and filtered through a plug of silica. The filtrate was dried over Na_2SO_4 and concentrated in vacuo, which was crystallized from EtOH-Et_2O mixture to give 6.96 g (85%) of β-azide **53** as a white solid; m.p. 163-165 °C (lit. m.p. 159-161 °C)[141]; IR (CCl_4) 3437 (w), 2964 (w), 2118 (s), 1752 (s), 1704 (m), 1237 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.09 (s, 3H), 3.76 (ddd, *J* = 12.4, 4.8, 2.0, 1H, H5), 3.90 (q, *J* = 9.4, 1H, H2), 4.14 (dd,

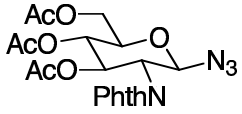
J = 12.4, 2.0, 1H, H6), 4.25 (dd, *J* = 12.4, 4.8, 1H, H6), 4.73 (d, *J* = 9.4, 1H, H1), 5.08 (t, *J* = 9.4, 1H, H4), 5.22 (t, *J* = 9.4, 1H, H3), 5.52 (d, *J* = 9.4, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.7, 23.3, 54.2, 61.8, 67.9, 72.1, 74.0, 88.4, 169.2, 170.3, 170.7, 171.0; LRMS (FAB) 373 ($(M + H)^+$, 8); HRMS (FAB) calcd for $C_{14}H_{21}O_8N_4$ 373.1359, found 373.1375. ^{1}H NMR and ^{13}C NMR data were consistent with the data reported by Gan *et. al.*[141]



1,3,4,6-Tetra-*O*-acetyl-2-phthalimido-2-deoxy-β-D-glucopyranoside (112).

The procedure by Hernández-Torres *et. al.* was followed.[142] A 1M NaOMe solution, which was prepared freshly by dissolving Na metal (2.30 g, 100 mmol) in 130 mL MeOH at –5 °C, was cannulated into a flask containing glucosamine hydrochloride (21.6 g, 100 mmol). The reaction mixture was stirred vigorously for 2 hr at RT, then treated with finely grounded phthalic anhydride (8.50 g, 57.5 mmol) and stirred vigorously for another 1 hr at RT. The second portion of finely grounded phthalic anhydride (8.50 g, 57.5 mmol) was added, followed by the addition of Et_3N (16.0 mL, 115 mmol) and 130 mL MeOH. After the reaction mixture vigorously stirred for 36 hr, light yellow thick paste was obtained. The paste was cooled to –20 °C for hr and filtered, then washed with cold MeOH. The solid was dried under vacuum for 16 hr and added into 300 mL pyridine at 0 °C, then stirred vigorously for 5 min, followed by the treatment with acetic anhydride (163 mL, 115 mmol). The reaction mixture was stirred at RT for 48 hr and quenched with 100 mL cold EtOH. EtOAc was removed by rotary evaporation, followed by azeotropic removal of pyridine by toluene (4x100

mL). The slurry was dissolved in 500 mL $CHCl_3$, washed with 4X200 mL and with 200 mL brine, then dried over Na_2SO_4 and evaporated to dryness. The crude product was dissolved in 40 mL of hot EtOAc, then diluted with 160 mL of hexanes and left to cool at –5 °C. The crystallized product was filtered and washed with cold hexanes, then dried under vacuum to give 30.1 g (63%) of β-acetate **112** as white solid: m.p. 94-95 °C (lit. m.p. 94-95 °C)[143]; IR (CCl_4) 2956 (w), 1763 (s), 1727 (m), 1385 (s), 1215 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.85 (s, 3H), 1.98 (s, 3H), 2.02 (s, 3H), 2.10 (s, 3H), 4.00 (ddd, *J* = 12.4, 4.4, 2.0, 1H, H5), 4.12 (dd, *J* = 12.4, 2.0, 1H, H6), 4.34 (dd, *J* = 12.4, 4.4, 1H, H6), 4.45 (dd, *J* = 9.6, 8.8, 1H, H2), 5.19 (t, *J* = 9.6, 1H, H4), 5.86 (t, *J* = 9.6, 1H, H3), 6.49 (d, *J* = 8.8, 1H, H1), 7.70-7.85 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.6, 20.7, 20.8, 53.5, 61.5, 68.2, 70.5, 72.6, 89.7, 123.8, 123.8, 131.2, 134.5, 167.4, 167.4, 168.6, 169.5, 170.0, 170.7; LRMS (FAB) 484 ($(M + Li)^+$, 35); HRMS (FAB) calcd for $C_{22}H_{23}O_{11}NLi$ 484.1431, found 484.1448. ^{1}H NMR and ^{13}C NMR data were consistent with the data reported by Dasgupta *et. al.*[143]



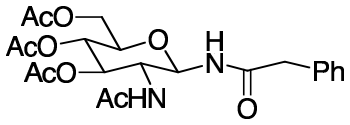
3,4,6-Tetra-*O*-acetyl-2-phthalimido-2-deoxy-β-D-glucopyranosyl azide (113).

1,3,4,6-Tetra-*O*-acetyl-2-phthalimido-2-deoxy-β-D-glucopyranoside (**112**) (5.00 g, 10.5 mmol) was dissolved in 25 mL of distilled CH_2Cl_2. Trimethylsilyl azide (1.95 mL, 14.7 mmol) was added via syringe followed by 1.0 M $SnCl_4$ (10.5 mL, 10.5 mmol). The solution was stirred for 20 h at 25 °C. The reaction mixture was diluted with 200 mL CH_2Cl_2, washed with 200 mL sat. aq. $NaHCO_3$ solution, and with 2x200 mL H_2O. The organic layer was filtered through a plug of silica. The organic layer

was dried over Na_2SO_4 and concentrated in vacuo to give crude product, which was crystallized from the mixture of absolute ethanol and petroleum ether to afford 3.52 g (73%) of β-azide **113** as a white solid: m.p. 139-140 °C (lit. m.p. 142-143 °C)[144]; IR (CCl_4) 2117 (s), 1755 (s), 1722 (s), 1384 (s), 1234 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.81 (s, 3H), 1.99 (s, 3H), 2.08 (s, 3H), 3.95 (m, 1H, H5), 4.15-4.25 (m, 2H, H6, H2), 4.30 (dd, *J* = 12.4, 4.4, 1H, H6), 5.15 (t, *J* = 9.6, 1H, H4), 5.61 (d, *J* = 9.6, 1H, H1), 5.76 (t, *J* = 9.6, 1H, H3), 7.70-7.85 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 20.5, 20.6, 53.9, 61.6, 68.3, 70.1, 73.9, 85.4, 123.7, 123.7, 131.1, 134.5, 167.0, 167.7, 169.3, 169.9, 170.5; LRMS (FAB) 467 ($(M + Li)^+$, 33); HRMS (FAB) calcd for $C_{20}H_{20}O_9N_4Li$ 467.1390, found 467.1400. ^{1}H NMR and ^{13}C NMR data were consistent with the data reported by Unverzagt *et. al.*[145]

General procedure (B) for the coupling reactions using Me_3P.

β-D-glycopyranosyl azide (1.0 equiv.) and 1.0 M solution of Me_3P (1.1 equiv.) were dissolved in distilled 1,2-dichloroethane and the resulting solution was stirred for 15 min at RT. The diisopropylethylamine (DIEA) (1.0 equiv.), acylating reagent (1.3 equiv.) and additive (1.3 equiv.), respectively, were added to the reaction mixture and stirred at given temperature for 20 hr. The reaction mixture was diluted with 100 mL EtOAc and washed with 2x100 mL H_2O. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give crude product, which was purified by flash chromatography.



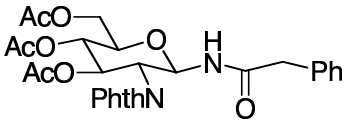
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*N*-phenyl acetyl-β-D-glucopyranosyl amine (116)

Method A (Table 9, entry 3): Compound **116** was prepared following the general coupling procedure outlined above employing 2-acetamido-3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**53**) (150 mg, 0.403 mmol), 1.0 M solution of Me_3P (443 μL, 0.443 mmol), DIEA (70 μL, 0.403 mmol), and 2-pyridyl thiolphenylacetate (120 mg, 0.524 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:4) gave 122 mg (65%) of **β-116** as a white solid.

Method B (Table 9, entry 5): Compound **116** was prepared following the general coupling procedure (B) outlined above (except DIEA) employing 2-acetamido-3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**53**) (150 mg, 0.403 mmol), 1.0 M solution of Me_3P (443 μL, 0.443 mmol), phenylacetic acid (55.0 mg, 0.403 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:4) gave 56 mg (30%) of **β-116** as a white solid.

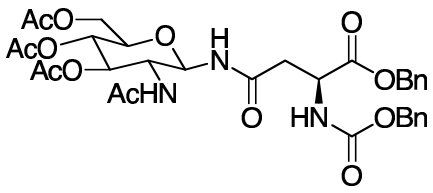
m.p. 240-242 °C; R_f = 0.28 (hexanes:EtOAc, 1:1); IR (CCl_4) 3415 (w), 1753 (s), 1695 (m), 1376 (s) 1227 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.74 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 3.43 (d, *J* = 14.8, 1H, CH_2), 3.53 (d, *J* = 14.8, 1H, CH_2), 3.71 (ddd, *J* = 9.6, 4.0, 2.0, 1H, H5), 4.05 (dd, *J* = 12.4, 2.0, 1H, H6), 4.08 (q, *J* = 9.6, 1H, H2), 4.27 (dd, *J* = 12.4, 4.0, 1H, H6), 4.97 (t, *J* = 9.6, 1H, H3), 5.00 (dd, *J* = 9.6, 8.4, 1H, H1), 5.08 (t, *J* = 9.6, 1H, H4), 5.86 (d, *J* = 8.4, 1H, NHAc), 6.97 (d, *J* = 8.4, 1H, NH), 7.20-7.30 (m, 5H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$) 20.6, 20.7, 20.8, 22.9, 43.8, 53.1, 61.7, 67.5, 72.8, 73.6, 80.5, 127.3, 128.8, 129.2, 134.0, 169.2, 170.7, 171.1, 171.8,

171.9; LRMS (FAB) 465 ((M + H)$^+$, 100); HRMS (FAB) calcd for $C_{22}H_{29}O_9N_2$ (M + H)$^+$ 465.1873, found 465.1888.



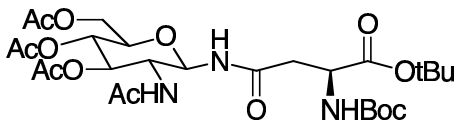
3,4,6-Tetra-*O*-acetyl-2-phthalimido-2-deoxy-1-N-phenyl acetyl-β-D-glucopyranosyl amine (117)

Compound **117** was prepared following the general coupling procedure (B) outlined above employing 3,4,6-tetra-*O*-acetyl-2-phthalimido-2-deoxy-β-D-glucopyranosyl azide (**113**) (150 mg, 0.326 mmol), 1.0 M solution of Me_3P (357 μL, 0.357 mmol), DIEA (57 μL, 0.326 mmol), and 2-pyridyl thiolphenylacetate (97.1 mg, 0.424 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 130 mg (73%) of **β-117** as a white solid: m.p. 152-154 °C; R_f = 0.18 (hexanes:EtOAc, 1:1); IR (CCl_4) 3420(w), 3033 (m), 1756 (s), 1723 (s), 1702 (m), 1381 (s) 1233 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.80 (s, 3H), 2.00 (s, 3H), 2.08 (s, 3H), 3.37 (d, *J* = 15.6, 1H, CH_2), 3.43 (d, *J* = 15.6, 1H, CH_2), 3.97 (ddd, *J* = 9.6, 4.0, 2.0, 1H, H5), 4.08 (dd, *J* = 12.4, 2.0, 1H, H6), 4.11 (t, *J* = 9.6, 1H, H2), 4.33 (dd, *J* = 12.4, 4.0, 1H, H6), 5.08 (t, *J* = 9.6, 1H, H4), 5.83 (d, *J* = 9.6, 1H, NH), 5.94 (t, *J* = 9.6, 1H, H3), 5.97 (t, *J* = 9.6, 1H, H1), 6.90-7.10 (m, 5H, Ph), 7.70-7.75 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.4, 20.6, 20.8, 43.6, 53.8, 61.7, 68.8, 70.4, 73.6, 75.9, 123.6, 124.1, 127.4, 128.8, 128.9, 130.7, 131.3, 133.7, 134.1, 134.7, 167.0, 167.5, 169.6, 169.8, 170.7, 170.8; LRMS (FAB) 552 ((M + H)$^+$, 5); HRMS (FAB) calcd for $C_{28}H_{28}O_{10}N_2$ (M + H)$^+$ 552.1744, found 552.1766.



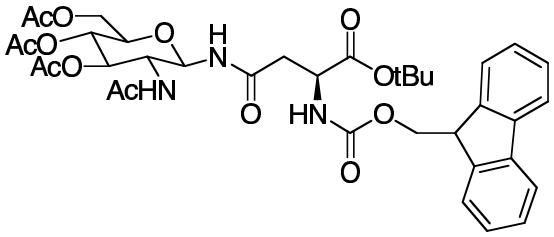
2-Acetamido-3,4,6-Tetra-*O*-acetyl-2-deoxy-1-*N*-[1-benzyl-*N*-(benzyloxy)carbonyl-L-aspart-4-oyl]-β-D-glucopyranosyl amine (17).

Compound **17** was prepared following the general coupling procedure (B) outlined above employing 2-acetamido-3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**53**) (150 mg, 0.402 mmol), 1.0 M solution of Me_3P (442 μL, 0.442 mmol), DIEA (70 μL, 0.402 mmol), Z-Asp(SPyr)-OBn (235 mg, 0.523 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:4) gave 168 mg (61%) of **β-17** as a solid: m.p. 213-215 (decomp) (lit. m.p. 214-215)[36]; R_f = 0.23 (hexanes:EtOAc, 1:4); IR (CCl_4) 3425(w), 3320 (w), 2929 (w), 1752 (s), 1700(m), 1670 (m), 1370 (s) 1231 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.80 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.69 (dd, *J* = 16.4, 4.0, 1H, Asp-CH_2), 2.85 (dd, *J* = 16.4, 4.0, 1H, Asp-CH_2), 3.68 (ddd, *J* = 9.6, 4.0, 1.6, 1H, H5), 4.02 (q, *J* = 9.6, 1H, H2), 4.04 (dd, *J* = 12.4, 1.6, 1H, H6), 4.26 (dd, *J* = 12.4, 4.0, 1H, H6), 4.64 (dt, *J* = 9.0, 4.4, 1H, Asp-CH), 4.89 (dd, *J* = 9.6, 8.4, 1H, H1), 4.94 (t, *J* = 9.6, H3), 5.05-5.10 (m, 4H, H4, 2xCH_2Ph), 5.20 (d, *J* = 12.4, 1H, CH_2Ph), 5.70 (d, *J* = 8.0, 1H, NHAc), 5.98 (d, *J* = 9.0, 1H, Asp-NH), 7.12 (d, *J* = 8.4, 1H, NH), 7.25-7.35 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.6, 20.7, 20.8, 23.0, 37.7, 50.5, 53.4, 61.6, 67.1, 67.2, 67.3, 72.7, 73.6, 80.5, 128.0, 128.1, 128.2, 128.4, 128.5, 128.5, 135.6, 136.2, 156.1, 169.2, 170.7, 170.9, 171.0, 172.0, 172.5; LRMS (FAB) 686 ((M + H)$^+$, 20); HRMS (FAB) calcd for $C_{33}H_{40}O_{13}N_3$ (M + H)$^+$ 686.2561, found 686.2542.



2-Acetamido-3,4,6-Tetra-*O*-acetyl-2-deoxy-1-*N*-[1-*tert*-butyl-*N*-(*tert*-butoxy)carbonyl-L-aspart-4-oyl]-β-D-glucopyranosyl amine (118).

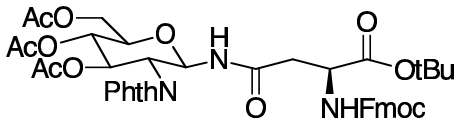
Compound **118** was prepared following the general coupling procedure (B) outlined above employing 2-acetamido-3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**53**) (150 mg, 0.402 mmol), 1.0 M solution of Me_3P (442 μL, 0.442 mmol), DIEA (70 μL, 0.402 mmol), Boc-Asp(SPyr)-OtBu (200 mg, 0.523 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:4) gave 146 mg (59%) of **β-118** as a foam: R_f = 0.22 (hexanes:EtOAc, 1:4); IR (CCl_4) 3423(w), 3350 (w), 2929 (w), 1751 (s), 1701(m), 1685 (m), 1373 (s) 1225 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.41 (s, 18H), 1.96 (s, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.62 (dd, *J* = 16.4, 4.0, 1H, Asp-CH_2), 2.77 (dd, *J* = 16.4, 4.0, 1H, Asp-CH_2), 3.69 (ddd, *J* = 9.6, 4.0, 2.0, 1H, H5), 4.03 (dd, *J* = 12.4, 2.0, 1H, H6), 4.08 (q, *J* = 9.6, 1H, H2), 4.27 (dd, *J* = 12.4, 4.0, 1H, H6), 4.39 (dt, *J* = 8.8, 4.4, 1H, Asp-CH), 4.99 (dd, *J* = 9.6, 8.4, 1H, H1), 5.00 (t, *J* = 9.6, H3), 5.11 (t, *J* = 9.6, H4), 5.57 (d, *J* = 8.8, 1H, Asp-NH), 5.94 (d, *J* = 8.0, 1H, NHAc), 7.07 (d, *J* = 8.4, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) 20.6, 20.7, 20.8, 23.2, 27.9, 28.3, 37.9, 50.5, 53.5, 61.6, 67.4, 72.8, 73.5, 79.7, 80.3, 81.8, 155.6, 169.2, 170.3, 170.7, 171.2, 172.1, 172.2; LRMS (FAB) 624 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{27}H_{43}O_{13}N_3Li$ (M + Li)$^+$ 624.2956, found 624.2983.



2-Acetamido-3,4,6-Tetra-*O*-acetyl-2-deoxy-1-*N*-[1-*tert*-butyl-*N*-(9-fluorenylmethylcarbonyl)-L-aspart-4-oyl]-β-D-glucopyranosyl amine (54).

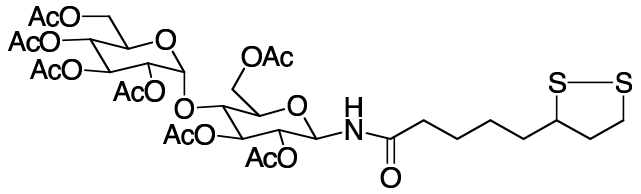
Compound **54** was prepared following the general coupling procedure (B) outlined above employing 2-acetamido-3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (**53**) (150 mg, 0.402 mmol), 1.0 M solution of Me_3P (442 μL, 0.442 mmol), DIEA (70 μL, 0.402 mmol), Fmoc-Asp(SPyr)-OtBu (264 mg, 0.523 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:4) gave 163 mg (55%) of **β-54** as a foam: R_f = 0.23 (hexanes:EtOAc, 1:4); IR (CCl_4) 3423(w), 3323 (w), 2929 (w), 1750 (s), 1697(m), 1660 (m), 1365 (s) 1231 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.43 (s, 9H), 1.95 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.68 (dd, *J* = 16.4, 4.0, 1H, Asp-CH_2), 2.83 (dd, *J* = 16.4, 4.0, 1H, Asp-CH_2), 3.71 (ddd, *J* = 9.2, 4.0, 2.0, 1H, H5), 4.03 (br d, *J* = 12.4, 1H, H6), 4.09 (q, *J* = 9.2, 1H, H2), 4.20 (t, *J* = 7.2, 1H, Fmoc-CH), 4.25-4.30 (m, 2H, H6, Fmoc-CH_2), 4.40 (dd, *J* = 10.4, 7.2, 1H, Fmoc-CH_2), 4.49 (dt, *J* = 8.8, 4.0, 1H, Asp-CH), 5.00 (dd, *J* = 9.2, 7.6, 1H, H1), 5.01 (t, *J* = 9.2, 1H, H3), 5.12 (d, *J* = 9.2, 1H, H4), 5.90 (d, *J* = 8.8, 1H, Asp-NH), 5.93 (d, *J* = 8.0, 1H, NHAc), 7.14 (d, *J* = 7.6, 1H, NH), 7.29 (t, *J* = 7.6, 2H), 7.38 (t, *J* = 7.6, 2H), 7.28 (d, *J* = 7.6, 2H), 7.74 (d, *J* = 7.6, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.6, 20.7, 22.4, 23.1, 27.9, 37.9, 47.1, 50.9, 53.5, 61.5, 67.1, 67.3, 72.8, 73.5, 80.4, 82.2, 120.0, 125.2, 127.1, 127.7, 141.2, 143.8, 143.9, 156.1, 169.2, 169.9, 170.6,

171.1, 172.2, 172.4; LRMS (FAB) 746 ((M + Li)$^+$, 4); HRMS (FAB) calcd for $C_{37}H_{45}O_{13}N_3Li$ (M + Li)$^+$ 746.3112, found 746.3128. There is one more aromatic carbon (7) than expected (6) due to the desymmetrization of the Fmoc group.



3,4,6-Tetra-*O*-acetyl-2-phthalimido-2-deoxy-1-*N*-[1-*tert*-butyl-*N*-(9-fluorenylmethylcarbonyl)-L-aspart-4-oyl]-β-D-glucopyranosyl amine (119). Compound **119** was prepared following the general coupling procedure (B) outlined above employing 3,4,6-tetra-*O*-acetyl-2-phthalimido-2-deoxy-β-D-glucopyranosyl azide (**113**) (150 mg, 0.326 mmol), 1.0 M solution of Me_3P (357 μL, 0.357 mmol), DIEA (57 μL, 0.326 mmol), Fmoc-Asp(SPyr)-OtBu (214 mg, 0.424 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) gave 159 mg (59%) of **β-119** as a foam: R_f = 0.20 (hexanes:EtOAc, 1:1); IR (CCl_4) 3423(w), 3371 (w), 3043 (m), 2933 (m), 1751 (s), 1724 (s), 1700 (m), 1687 (w), 1385 (s) 1240 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.09 (s, 9H), 1.84 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H),2.62 (dd, *J* = 16.4, 4.0, 1H, Asp-CH_2), 2.72 (dd, *J* = 16.4, 4.0, 1H, Asp-CH_2), 3.96 (ddd, *J* = 9.6, 4.0, 2.0, 1H, H5), 4.05 (br d, *J* = 12.0, 1H, H6), 4.15-4.35 (m, 6H, H2, H6, Asp-CH, Fmoc-CH, Fmoc-CH_2), 5.13 (t, *J* = 9.6, 1H, H4), 5.87 (d, *J* = 8.4, 1H, Asp-NH), 5.99 (t, *J* = 9.6, 1H, H3), 6.00-6.05 (m, 1H, H1, NH), 7.25-7.85 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) 20.4, 20.6, 20.7, 27.5, 37.8, 47.0, 50.7, 54.1, 61.6, 67.2, 68.4, 70.4, 73.7, 75.8, 82.0, 120.0, 123.7, 124.0, 125.1, 125.2, 127.1, 127.7, 130.9, 131.4, 134.3, 134.6, 141.2, 143.7, 143.9, 156.1, 167.1, 167.9, 169.4, 169.6, 169.9, 170.5, 170.6; LRMS (FAB) 834 ((M + Li)$^+$,

36); HRMS (FAB) calcd for $C_{43}H_{45}O_{14}N_3Li$ $(M + Li)^+$ 834.3062, found 834.3027. There are two more aromatic carbons (14) than expected (12) due to the desymmetrization of the Fmoc group.

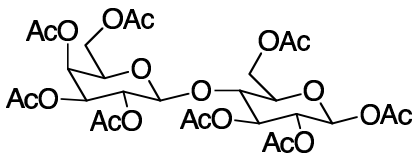


2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl]-1-N-thioctoyl-β-D-glucopyranosyl amine (124)

Method A: Compound **124** was prepared following the general coupling procedure (B) outlined above employing hepta-*O*-acetyl-β-D-maltose azide (**102**) (500 mg, 0.755 mmol), 1.0 M solution of Me_3P (831 μL, 0.831 mmol), DIEA (131 μL, 0.755 mmol), D/L-thioctic acid (156 mg, 0.755 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:3) gave 249 mg (40%) of **β-124** as oil.

Method B: Compound **124** was prepared following the general coupling procedure (B) outlined above employing hepta-*O*-acetyl-β-D-maltose azide (**102**) (500 mg, 0.755 mmol), 1.0 M solution of Me_3P (831 μL, 0.831 mmol), DIEA (131 μL, 0.755 mmol), **122** (271 mg, 0.906 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:3) gave 355 mg (57%) of **β-124** as oil; IR (CCl_4) 3438(w), 2933 (w), 1754 (s), 1708(m), 1368 (s) 1231 (s); ^1H NMR (400 MHz, $CDCl_3$) 1.35-1.45 (m, 2H), 1.55-1.70 (m, 4H), 1.80-1.90 (m, 1H), 1.97 (s, 3H), 1.99 (s,

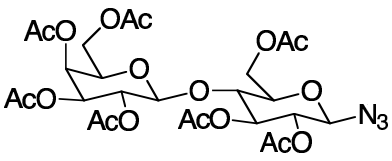
9H), 2.03 (s, 3H), 2.05-2.20 (m, 8H), 2.40-2.45 (m, 1H), 3.00-3.15 (m, 2H), 3.52 (p, *J* = 7.2, 1H), 3.76 (br d, *J* = 9.6, 1H, H5), 3.89 (m, 1H, H5´), 3.96 (t, *J* = 9.6, 1H, H4), 4.00 (dd, *J* = 12.4, 2.4, 1H, H6´), 4.20-4.25 (m, 2H, H6, H6´), 4.39 (br d, *J* = 12.4, 1H, H6), 4.72 (t, *J* = 9.6, 1H, H2), 4.82 (dd, *J* = 9.6, 4.0, 1H, H2´), 5.03 (t, *J* = 9.6, 1H, H4´), 5.24 (t, *J* = 9.6, 1H, H1), 5.32 (t, *J* = 9.6, 1H, H3), 5.35 (t, *J* = 9.6, 1H, H3´), 5.36 (d, *J* = 4.0, 1H, H1´), 6.06 (d, *J* = 9.6, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) 20.5, 2.05, 20.6, 20.6, 20.7, 20.8, 20.8, 24.7, 24.7, 28.6, 28.7, 34.5, 36.2, 36.2, 38.4, 40.2, 56.2, 61.3, 62.7, 67.8, 68.5, 69.2, 69.9, 71.3, 72.5, 73.8, 74.9, 77.6, 95.5, 169.4, 169.6, 169.8, 170.4, 170.5, 170.6, 171.1, 171.2, 172.8, 172.8; LRMS (FAB) 830 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{34}H_{49}O_{18}NS_2Li$ (M + Li)$^+$ 830.2551, found 830.2550. There are five carbons (39) more than expected (34), due to the diastereotopic nature of the molecule.



2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,4,6-tetra-*O*-cetyl-β-D-galactopyranosyl]-β-D-glucopyranosyl acetate (octa-*O*-acetyl-β-lactose)

The acetate was prepared according to the procedure of Wolfrom *et. al.*[136] α-D-Lactose monohydrate (9.00 g, 25.0 mmol) was added in portions over 30 min into the refluxing solution of anhydrous NaOAc (8.20 g, 100 mmol) and acetic anhydride (47.0 mL, 500 mL). The solution was refluxed for 3 hr and cooled to 100 °C, then immediately transferred into 400 mL of ice-water mixture and stirred vigorously for 4 hr. The water was decanted and the precipitate was diluted with 200 mL $CHCl_3$, washed with 2x200 mL sat. aq. $NaHCO_3$ solution and 2x 200 mL H_2O, then dried over

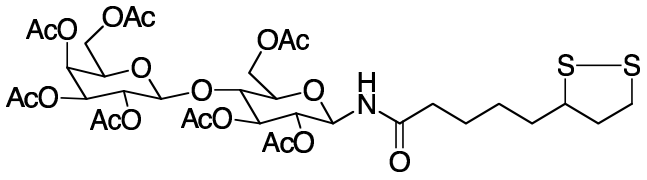
Na_2SO_4 and concentrated in vacuo to give colorless gummy oil. The azeotropic removal of remaining $CHCl_3$ by dissolving the oil in 2X100 mL hot EtOH, followed by two successive crystallization from 95% EtOH gave 12.5 g (74%) of octa-*O*-acetyl-β-lactose as white solid: m.p. 90-92 °C (lit. m.p. 91-93 °C)[146]; IR (CCl_4) 2963 (w), 1756 (s), 1366 (s), 1235 (m), 1051 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (s, 3H), 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.13 (s, 3H), 3.70-3.75 (m, 1H, H5), 3.82 (t, *J* = 9.2, 1H, H4), 3. 83 (m, 1H, H6), 4.00-4.15 (m, 3H, H5´, H6´, H6), 4.43 (dd, *J* = 12.0, 2.0, 1H, H6´), 4.45 (d, *J* = 8.0, 1H, H1´), 4.92 (dd, *J* = 10.4, 3.6, 1H, H3´), 5.02 (dd, *J* = 9.2, 8.4, 1H, H2), 5.09 (dd, *J* = 10.0, 8.0, 1H, H2´), 5.22 (t, *J* = 9.2, 1H, H3), 5.32 (dd, *J* = 3.6, 0.8, 1H, H4´), 5.64 (d, *J* = 8.4, 1H, H1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 207, 20.7, 20.8, 20.8, 20.9, 60.8, 61.7, 66.5, 68.9, 70.5, 70.7, 70.9, 72.6, 73.5, 75.7, 91.5, 101.0, 168.9, 169.0, 169.6, 169.6, 170.1, 170.2, 170.3, 170.4; LRMS (FAB) 685 ((M + Li)$^+$, 58); HRMS (FAB) calcd for $C_{28}H_{38}O_{19}Li$ 685.2167, found 685.2191.



2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-β-D-glucopyranosyl azide (hepta-*O*-acetyl-β-lactosyl azide)(125).

Octa-*O*-acetyl-β-lactose (8.00 g, 11.8 mmol) was dissolved in 50 mL of distilled CH_2Cl_2. Trimethylsilyl azide (2.19 mL, 16.5 mmol) was added via syringe followed by 1.0 M $SnCl_4$ solution (5.90 mL, 5.90 mmol). The solution was stirred for 20 h at 20 °C. The reaction mixture was diluted with 200 mL CH_2Cl_2, washed with 200 mL sat. aq. $NaHCO_3$ solution, and with 2x200 mL H_2O. The organic layer was dried over

Na_2SO_4 and concentrated in vacuo to give crude product, which was crystallized from absolute ethanol to afford 6.41 g (82%) of hepta-*O*-acetyl-β-lactose azide **125** as a white solid: m.p. 74-76 °C (lit. m.p. 74-76 °C)[129]; IR (CCl_4) 2936 (w), 2118 (s), 1759 (s), 1366 (s), 1231 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (s, 3H), 2.02 (s, 6H), 2.04 (s, 3H), 2.05 (s, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 3.65-3.70 (m, 1H, H5), 3.79 (t, *J* = 9.2, 1H, H4), 3.80-3.85 (m, 1H, H6´), 4.00-4.15 (m, 3H, H5´, H6, H6´), 4.45 (d, *J* = 8.4, 1H, H1´), 4.45-4.50 (m, 1H, H6), 4.60 (d, *J* = 9.0, 1H, H1), 4.83 (t, *J* = 9.0, 1H, H2), 4.92 (dd, *J* = 10.4, 3.2, 1H, H3´), 5.08 (dd, *J* = 10.4, 8.4, 1H, H2´), 5.18 (t, *J* = 9.0, 1H, H3), 5.32 (d, *J* = 3.2, 1H, H4´); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 20.6, 20.6, 20.7, 20.8, 60.7, 61.7, 66.5, 69.0, 70.7, 70.9, 70.9, 72.5, 74.7, 75.8, 75.1, 87.7, 101.1, 169.0, 169.5, 169.6, 170.0, 170.1, 170.3, 170.3; LRMS (FAB) 668 ((M + Li)$^+$, 45); HRMS (FAB) calcd for $C_{26}H_{35}O_{17}N_3Li$ 668.2127, found 668.2094. The ^{1}H-NMR and ^{13}C-NMR data were consistent with the data reported by Petö *et. al.*[129]

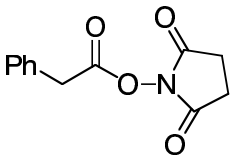


2,3,6-Tri-*O*-acetyl-4-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-1-N-thioctoyl-β-D-glucopyranosyl amine (126)

Method A: Compound **126** was prepared following the general coupling procedure (B) outlined above employing hepta-*O*-acetyl-β-D-lactose azide (**125**) (400 mg, 0.604 mmol), 1.0 M solution of Me_3P (665 μL, 0.665 mmol), DIEA (105 μL, 0.604 mmol), D/L-thioctic acid (125 mg, 0.604 mmol) as the acylating reagent. Purification of the

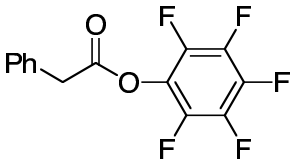
residue by flash chromatography (hexanes:EtOAc, 1:3) gave 140 mg (28%) of **β-126** as oil.

Method B: Compound **126** was prepared following the general coupling procedure (B) outlined above employing hepta-*O*-acetyl-β-D-lactose azide (**125**) (400 mg, 0.604 mmol), 1.0 M solution of Me_3P (665 μL, 0.665 mmol), DIEA (105 μL, 0.604 mmol), **122** (216 mg, 0.723 mmol) as the acylating reagent. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:3) gave 306 mg (61%) of **β-126** as oil; IR (CCl_4) 3432(w), 2934 (w), 1757 (s), 1709(m), 1368 (s) 1231 (s); ^{1}H NMR (400 MHz, $CDCl_3$) 1.35-1.45 (m, 2H), 1.55-1.70 (m, 4H), 1.80-1.90 (m, 1H), 1.93 (s, 3H), 2.01 (s, 6H), 2.02 (s, 3H), 2.04 (s, 3H), 2.05-2.20 (m, 7H), 2.40-2.45 (m, 1H), 3.00-3.15 (m, 2H), 3.52 (p, *J* = 7.2, 1H), 3.70-3.75 (m, 1H, H5), 3.74 (t, *J* = 9.6, 1H, H4), 3.84 (br t, *J* = 7.2, 1H, H5´), 4.00-4.15 (m, 3H, H6´, H6´, H6), 4.39 (m, 1H, H6), 4.41 (d, *J* = 8.0, 1H, H1´), 4.78 (t, *J* = 9.6, 1H, H2), 4.90 (dd, *J* = 10.4, 3.2, 1H, H3´), 5.07 (dd, *J* = 10.4, 8.0, 1H, H2´), 5.17 (t, *J* = 9.6, 1H, H1), 5.26 (t, *J* = 9.6, 1H, H3), 5.32 (br d, *J* = 3.2, 1H, H4´), 6.11 (d, *J* = 9.6, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) 20.5, 2.06, 20.6, 20.6, 20.7, 20.7, 20.9, 24.7, 24.7, 28.6, 28.7, 34.5, 36.2, 36.2, 38.4, 40.2, 40.2, 56.2, 60.8, 61.9, 66.5, 68.9, 68.9, 69.0, 70.6, 70.9, 70.9, 72.2, 74.4, 75.9, 77.9, 100.9, 168.9, 169.3, 170.1, 170.1, 170.3, 170.3, 171.3, 172.8, 172.8; LRMS (FAB) 830 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{34}H_{49}O_{18}NS_2Li$ (M + Li)$^+$ 830.2551, found 830.2566. There are seven carbons (41) more than expected (34), due to the diastereotopic nature of the molecule.



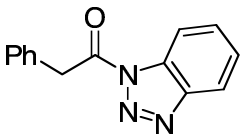
1-Succinimidyl phenylacetate

The ester was prepared according to the procedure of Stefanowicz *et. al.*[147] and the recrystallization (hexanes:benzene) gave the ester as white solid: m.p. 114-116 °C (lit. 118-119 °C); R_f = 0.30 (hexanes:EtOAc, 2:1); IR (CCl_4) 3034 (w), 2936 (m), 2858 (w), 1747 (s), 1704 (m), 1069 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (s, 4H), 3.92 (s, 2H), 7.20-7.35 (m, 5H); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 25.5, 37.6, 127.8, 128.8, 129.2, 131.4, 166.7, 169.0; LRMS (EI) 233 ((M^+, 63), 91 (100); HRMS (EI) calcd for $C_{12}H_{11}O_4N$ 233.0688, found 233.0699.



Pentafluorophenyl phenylacetate

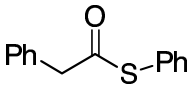
The ester was prepared according to the procedure used for 1-succinimidyl phenylacetate and the purification by flash chromatography (hexanes:EtOAc, 4:1) gave the ester as colorless oil: R_f = 0.62 (hexanes:EtOAc, 4:1); IR (CCl_4) 3068 (w), 3034 (m), 2936 (w), 1790 (s), 1704 (m), 1054 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (s, 2H), 7.30-7.45 (m, 5H); ^{13}C NMR (100 MHZ, $CDCl_3$) δ 40.0, 125.1(t, *J* = 13.8), 127.7, 128.8, 129.2, 132.0, 137.8 (dm, *J* = 250), 139.5 (dm, *J* = 250), 141.1 (dm, *J* = 250), 167.5; LRMS (EI) 302 ((M^+, 8), 91 (100); HRMS (FAB) calcd for $C_{14}H_8O_2F_5$ 303.0444 $(M + H)^+$, found 303.0455.



N-(Phenylacetyl)benzotriazole

A modified procedure of Katritzky *et. al.* was followed.[148,149] Phenylacetyl chloride (3.32 mL, 25.0 mmol) was added into the solution of benzotriazole (2.98 g, 25.0 mmol) and *N*,*N*-diisopropylethylamine (4.35 mL, 25.0

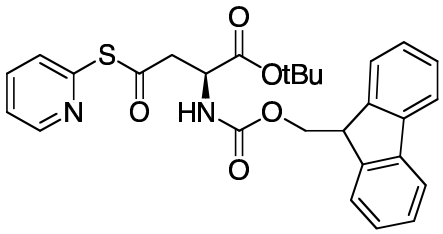
mmol) in 80 mL of distilled CH_2Cl_2 at 0 °C. Th resulting solution was refluxed for 3 hr and cooled to RT. The mixture was diluted with 150 mL CH_2Cl_2 and washed with 2x30 mL 2M NaOH solution, 100 mL sat. aq. $NaHCO_3$ solution, and 100 mL H_2O. After the solution was dried over Na_2SO_4, the solvent was evaporated under vacuum to give crude oil, which was crystallized from the mixture of 2 mL $CHCl_3$ and 30 mL hexanes to give 4.30 g (73%) of acylbenzotriazole as white solid. It was stored under argon in a desiccator: m.p. 65-66 °C (lit. m.p. 66-67 °C)[149]; IR (CCl_4) 3084 (w), 3035 (w), 1734 (s), 1374 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.72 (s, 2H), 7.30-7.40 (m, 3H), 7.44-7.47 (m, 2H), 7.49 (t, *J* = 8.0, 1H), 7.63 (t, *J* = 8.0, 1H), 8.11 (d, *J* = 8.0, 1H), 8.25 (d, *J* = 8.0, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.9, 114.4, 120.1, 126.2, 127.6, 128.8, 129.8, 130.4, 131.1, 132.4, 146.2, 170.2; LRMS (FAB) 238 (((M + H)$^+$, 20), 119 (100)); HRMS (FAB) calcd for $C_{14}H_{12}ON_3$ 238.0980, found 238.0968. ^{1}H NMR and ^{13}C NMR data were consistent with the data reported by Katritzky *et. al.*[149]



Phenyl thiolphenylacetate

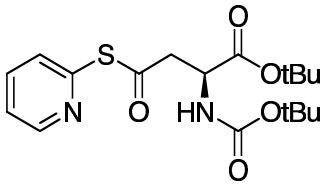
The ester was prepared according to the procedure of Watanabe *et. al.*[150] The solution of 2-fluoro-1-methylpyridinium p-toluenesulfonate (3.06 g, 10.8 mmol) in 10 mL distilled CH_2Cl_2 at –10 °C was cannulated into the solution of phenylacetic acid (1.36 g, 10.0 mmol) and Et_3N (700 μL, 10.0 mmol) in 20 mL distilled CH_2Cl_2 at –10 °C. The mixture was stirred at –10 °C for 1 hr. The solution of benzenethiol (1.10 g, 10.0 mmol) and Et_3N (700 μL, 10.0 mmol) in 20 mL distilled CH_2Cl_2 at –10 °C was cannulated into the mixture and the resulting solution stirred at –10 °C for 2 hr. The solvent was evaporated under vacuum to give crude product,

which was purified by flash chromatography (hexanes) to give 2.05 g (90%) of thiolester **96** as clear oil. It was stored under argon in a desiccator: R_f = 0.25 (hexanes); IR (CCl_4) 3065 (s), 3032 (s), 2916 (w), 1708 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (s, 2H), 7.25-7.35 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.9, 127.5, 127.7, 128.7, 129.1, 129.4, 129.6, 133.2, 134.4, 195.3; LRMS (EI) 228 ((M^+, 26), 91 (100)); HRMS (EI) calcd for $C_{14}H_{12}OS$ 228.0609, found 228.0620.



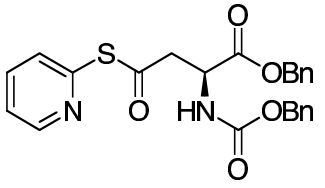
Fmoc-Asp(SPyr)-O*t*Bu

Fmoc-Asp-O*t*Bu (1.00 g, 2.43 mmol), triphenylphosphine (0.828 g, 3.16 mmol), and 2,2'-dithiopyridine (0.695 g, 3.16 mmol) is dissolved in 30 mL distilled THF. The solution stirred at RT for 24 hr. and concentrated in vacuo to give crude product, which was purified by flash chromatography (hexanes:EtOAc, 2:1) to give 0.90 g (73%) of thiolester as light yellow oil. It was stored under argon in a desiccator: R_f = 0.27; IR (CCl_4) 3434 (w), 3071 (w), 2981 (w), 1727 (s), 1701 (s), 1500 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 3.32 (dd, *J* = 16.8, 4.4, 1H, CH_2), 3.42 (dd, *J* = 16.8, 4.4, 1H, CH_2), 4.21 (t, *J* = 7.2, 1H, CH-Fmoc), 4.35 (d, *J* = 7.2, 1H, CH_2-Fmoc) 4.54 (dt, *J* = 8.8, 4.4, 1H, CH), 5.73 (d, *J* = 8.8, 1H, NH), 7.25-7.30 (m, 3H), 7.38 (t, *J* = 7.2, 2H), 7.58 (d, *J* = 7.2, 3H), 7.70-7.75 (m, 3H), 8.61 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9, 45.6, 47.1, 51.1, 67.2, 83.0, 119.9, 123.8, 125.2, 127.1, 127.2, 130.0, 137.2, 141.2, 143.7, 150.5, 150.7, 155.8, 169.0, 194.6; LRMS (FAB) 511 (($M + Li)^+$, 53); HRMS (FAB) calcd for $C_{28}H_{28}O_5N_2SLi$ 511.1879 $(M + Li)^+$, found 511.1891.



Boc-Asp(SPyr)-O*t*Bu

Boc-Asp-O*t*Bu (1.50 g, 5.19 mmol), triphenylphosphine (1.77 g, 6.79 mmol), and 2,2'-dithiopyridine (1.49 g, 6.79 mmol) is dissolved in 30 mL distilled THF. The solution stirred at RT for 24 hr. and concentrated in vacuo to give crude product, which was purified by flash chromatography (hexanes:EtOAc, 2:1) to give 1.95 g (98%) of thiolester as light yellow oil. It was stored under argon in a desiccator: $R_f = 0.27$; IR (CCl_4) 3430 (w), 3047 (w), 2985 (m), 1740 (s), 1714 (s), 1491 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.43 (s, 9H), 3.20 (dd, *J* = 16.8, 4.4, 1H, CH_2), 3.30 (dd, *J* = 16.8, 4.4, 1H, CH_2), 4.44 (dt, *J* = 8.8, 4.4, 1H, CH), 5.40 (d, *J* = 8.8, 1H, NH), 7.27 (ddd, *J* = 7.6, 4.8, 1.0, 1H, Pyr), 7.58 (d, *J* = 7.6, 1H, Pyr), 7.71 (dt, *J* = 7.6, 2.0, 1H, Pyr), 8.59 (ddd, *J* = 4.8, 2.0, 1.0, 1H, Pyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9, 28.3, 45.9, 50.8, 80.0, 82.7, 123.7, 130.0, 137.7, 150.5, 150.9, 155.3, 169.4, 194.5; LRMS (FAB) 389 ((M + Li)$^+$, 100); HRMS (FAB) calcd for $C_{18}H_{26}O_5N_2SLi$ 389.1722 (M + Li)$^+$, found 389.1741.



Z-Asp(SPyr)-OBzl

Z-Asp-OBzl (1.50 g, 4.20 mmol), triphenylphosphine (1.43 g, 5.46 mmol), and 2,2'-dithiopyridine (1.20 g, 5.46 mmol) is dissolved in 30 mL distilled THF. The solution stirred at RT for 24 hr. and concentrated in vacuo to give crude product, which was purified by flash chromatography (hexanes:EtOAc, 2:1) to give 1.70 g (90%) of thiolester as light yellow solid. It was stored under argon in a desiccator: m.p. 79-81 °C; $R_f = 0.26$; m.p.

79-81 °C; IR (CCl_4) 3433 (w), 3069 (w), 2958 (w), 1749 (s), 1734 (s), 1701 (s), 1507 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.29 (dd, *J* = 17.2, 4.4, 1H, CH_2), 3.41 (dd, *J* = 17.2, 4.2, 1H, CH_2), 4.67 (dt, *J* = 8.4, 4.2, 1H, CH), 5.09 (s, 2H, CH_2Ph), 5.16 (s, 2H, CH_2Ph), 5.71 (d, *J* = 8.4, 1H, NH), 7.25-7.35 (m, 11H, 2xPh, Pyr), 7.47 (d, *J* = 7.6,1H, Pyr), 7.69 (dt, *J* = 7.6, 1.4, 1H, Pyr), 8.59 (dd, *J* = 4.8, 1.4, 1H, Pyr); ^{13}C NMR (100 MHz, $CDCl_3$) δ 45.4, 50.7, 67.2, 67.7, 123.8, 128.0, 128.2, 128.4, 128.5, 128.6, 128.6, 130.1, 135.0, 136.0, 137.2, 150.5, 150.5, 155.8, 170.1, 194.6; LRMS (FAB) 451 ((M + H)$^+$, 25); HRMS (FAB) calcd for $C_{24}H_{23}O_5N_2S$ 451.1328 (M + H)$^+$, found 451.1347.

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