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SYNTHESIS OF *C*-GLYCOSYL AMINO ACIDS AS STABLE BUILDING BLOCKS FOR MODIFIED GLYCOPEPTIDE SYNTHESIS

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DEDICATION

TO

WHOM I LOVE

MY

MOTHER, FATHER

BROTHERS, SISTERS

Nephews ANS Nieces

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ABBREVIATIONS

Amino Acids:

Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Cys	cysteine
Gly	glycine
His	histidine
Hyl	hydroxylysine
Hyp	hydroxyproline
Ile	isoleucine
Leu	leucine
Lys	lysine
Phe	phenylalanine
Pro	proline
Ser	serine
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Val	valine
Xxx	general amino acid

Monosaccharides:

Gal	galactose
GalNAc	2-*N*-acetyl-2-deoxy-galactopyranose
Glc	glucose
GlcNAc	2-*N*-acetyl-2-deoxy-glucopyranose
Man	mannose
Xyl	xylose

Other abbreviations:

Ac	acetyl
B	base
Bn	benzyl
Boc	*tert*-butoxycarbonyl
t-Bu	*tert*-butyl

C	carbon
DBAD	di-*tert*-butyl azodicarboxylate
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
Bu	butyl
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DIBALH	diisobutylaluminium hydride
DMAP	4-(dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMP	dimethoxypropane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
Dol	dolichol
DPPA	diphenylphosphoryl azide
Et	ethyl
Fmoc	fluorenylmethoxycarbonyl
HIV	human immunodeficiency virus
HMPA	hexamethylphosphoramide
LDA	lithium diisopropylamide
Me	methyl
MeOTf	methyl triflate
MPM	*p*-methoxybenzyl
Nu	nucleophile
OT	oligosaccharyl transferase
PCC	pyridinium chlorochromate
pfp	pentafluorophenyl
Ph	phenyl
Py	pyridine
r.t.	room temperature
SPPS	solid-phase peptide synthesis
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimetylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TsCN	p-toluenesulfonyl cyanide
MW	Microwave
MCPA	*meta*-Chloroperbenzoic acid
MeCN	acetonitrile

SYNTHESIS OF C-GLYCOSYL AMINO ACIDS AS STABLE BUILDING BLOCKS FOR MODIFIED GLYCOPEPTIDE SYNTHESIS

By

MOHAMMAD MOUSA ALDHOUN

ABSTRACT

In this thesis, we have studied and synthesized new class of *C*-glycosly amino acids whose structure features a hetrocycle ring holding the carbohydrate and the amino acid fragments. Pyridine and tetrazole rings were used as hetrocycle linkers in this project. This class of *C*-glycosyl amino acids is of interest as new chealtors and as building building blocks for cotranslational glycopeptides synthesis. In the first part, C-Glycosylmethyl pyridylalanines were synthesized via thermally induced Hantzsch-type cyclocondensation using an aldehyde-ketoester-enamino ester system. To one of these reagents was attached a C-glycosyl residue, while to another was bound an amino acid fragment. In a one-pot optimized methodology, the dihydropyridine was not isolated while its purification was carried out by removal of unreacted material and side products using polymer-supported scavengers. Then the dihydropyridine (mixture of diastereoisomers) was oxidized by a polymer-bound oxidant to give the target pyridine bearing the two bioactive residues. In this way, a range of eight compounds (58-68% yield) was prepared in which the elements of diversity were (i) the gluco and galacto configurations of the pyranose ring, (ii) the α- and β-configurations at the anomeric center, and (iii) the positions of the carbohydrate and amino acid sectors in the pyridine ring. The orthogonal functional group protection in these amino acids allowed their easy incorporation into oligopeptides via sequential amino and carboxylic group coupling.

In the second part, tetrazole moiety was constructed via Huisgen 1,3-dipolar cycloaddition between nitriles and organic azides. Two sets of compounds have been prepared, one being constituted of *C*-galactosyl and *C*-ribosyl *O*-tetrazolyl serines, while the other contains *S*-tetrazolyl cysteine derivatives. In both cases, the synthetic scheme involved a two-step route: the first one being the thermal cycloaddition of a sugar azide with *p*-toluensulfonyl cyanide (TsCN) to give a 1-substituted 5-sulfonyl tetrazole and the second the replacement of the tosyl group with a serine or cysteine residue. For the high efficiency and operational simplicity, the azide-TsCN cycloaddition appears to be a true click process. Finally, one of the amino acids prepared was incorporated into a tripeptide

Key words: C-glycosyl amino acid, C-Glycosylmethyl pyridylalanines, dihydropyridine, glycopeptide synthesis, polymer-supported scavengers, Hantzsch multiconmponent reaction, orthogonal functional group, Click chemistry, Huisgen 1,3-dipolar cycloaddition, 1-substituted 5-sulfonyl tetrazole, *C*-ribosyl *O*-tetrazolyl serines, *C*-galactosyl *O*-tetrazolyl serines,

Sintesi di *C*-glicosil amminoacidi quali intermedi reattivi per la sintesi di *C*-glicopeptidi modificati

Riassunto:

In questo lavoro abbiamo studiato e sintetizzato una nuova classe di *C*-glicosil amminoacidi la cui struttura presenta un anello eterociclico funzionalizzato con un residuo di amminoacido e di carboidrato. In questo progetto sono stati utilizzati come spaziatori delle due unità biologicamente attive (amminoacido e carboidrato) strutture piridiniche e tetrazoliche. L'interesse verso questa nuova classe di *C*-glicosil amminoacidi risiede nelle loro potenzialità come chelanti e nel loro utilizzo come intermedi reattivi per la sintesi co-traslazionale di glicopeptidi modificati.

Nella prima parte del progetto, sono state sintetizzate *C*-glicosilmetil piridil alanine mediante ciclocondensazione termica di Hantzsch a tre componenti di aldeidi, enamino-esteri e β-cheto-esteri. Uno di questi reagenti è stato opportunamente derivatizzato con un residuo *C*-glicosidico, mentre un altro componente è stato funzionalizzato con un'unità amminoacidica. Nella metodologia one-pot ottimizzata, il derivato diidropiridinico intermedio è stato purificato dai componenti non reagiti utilizzando reattivi supportati su polimero.

Successivamente l'intermedio diidropiridinico presente come miscela di diastereoisomeri è stato ossidato mediante l'uso di un ossidante supportato su polimero fornendo la corrispondente piridina funzionalizzata con le due unità biologicamente attive. Mediante questa strategia sono stati sintetizzati otto composti (resa: 56-68%) che presentavano i seguenti elementi di diversità: i) la configurazione *gluco* o *galatto* dell'anello piranosidico, ii) la configurazione α o β in posizione anomerica, e iii) la posizione relativa delle unità di zucchero ed amminoacido sull'anello piridinico. La presenza di gruppi protettivi ortogonali sulle molecole target ha poi permesso il loro facile inserimento in sequenze oligopeptidiche attraverso accoppiamento sequenziale del gruppo amminico e carbossilico.

Nella seconda parte del progetto, è stata costruita la struttura tetrazolica mediante cicloaddizione 1,3-dipolare di Huisgen di nitrili ed azidi organiche. In questo modo è stato possibile sintetizzare due categorie di molecole target, ovvero *C*-galattosil o *C*-ribosil-*O*-tetrazolil serine e i corrispondenti *S*-tetrazolil derivati cisteinici.

In entrambe i casi lo schema sintetico si è completato attraverso due stadi di reazione,:il primo ha previsto la cicloaddizione termica del para-toluensulfonil cianuro (TsCN) con un'azide funzionalizzata con un residuo di carboidrato per dare 5-sulfonil tetrazoli-1-sostituiti; il secondo stadio ha comportato la sostituzione del gruppo tosile con un residuo di serina o cisteina. Data l'alta efficienza e la semplicità della procedura, si ritiene che la cicloaddizione di azidi e TsCN possa essere considerata una reazione click. Infine, uno degli amminoacidi preparati è stato incorporato in un tripeptide.

Parole chiave: *C*-glicosil amminoacidi, *C*-glicosilmetil piridil alanine, diidropiridine, sintesi di glicopeptidi, sequestranti supportati su polimero, reazione multicomponente di Hantzsch, gruppo funzionale ortogonale, click chemistry, cicloaddizione 1,3-dipolare di Huisgen, 5-sulfonil tetrazoli-1-sostituiti, *C*-ribosil-*O*-tetrazolil serine, *C*-galattosil-*O*-tertazolil serine.

1. *C*-GLYCOSYL AMINO ACIDS AS STABLE BUILDING BLOCKS FOR MODIFIED GLYCOPEPTIDE SYNTHESIS

Glycosyl amino acids are molecules that combine the structural features of simple amino acids (amino and carboxylic acid functions) with those of simple carbohydrate (cyclic polyols). The resulting hybrids are highly substituted polyfunctionalized building blocks which can be used for the synthesis of compound libraries by means of combinatorial synthesis with high potential of diversity, to generate potential glycomimetics and peptidomimetics, and due to a high degree of functionalization can be used as multivalent scaffolds or platforms for projecting pharmacophore groups. The importance of glycopeptides as anticancer vaccines, [1] antiviral vaccines against HIV and influenza, [1] antibiotics, [2] and their use as mimetics of natural carbohydrates[3] reflect the importance of glycosyl amino acids as building blocks for them.

Glycosyl amino acids of type **A** and **B** are components of naturally occurring *O*- and *N*-linked glycoproteins. They are building blocks for co-translation of glycopeptide synthesis. Many natural glycopeptide and glycoproteins are considered as therapeutic agents, and indeed some are currently in clinical use.[4] However, the lability of the glycosidic bond towards chemical and enzymatic degradation in vivo results in low bioavailability of carbohydrate derivatives and prevents their oral application.[5] Chemists have observed that substitution of the glycosidic *C*-*O* or *C*-*N* bonds found in *O*-linked and *N*-linked glycoproteins, respectively, with a *C*-*C* bond confers resistance to enzymatic hydrolysis.[6] In addition, *C*-glycosides demonstrate solution conformations and biological activities similar to their native counterparts.[7] Accordingly, much effort has been devoted to the synthesis of *C*-glycosyl amino acids of general structure **C**.[8]

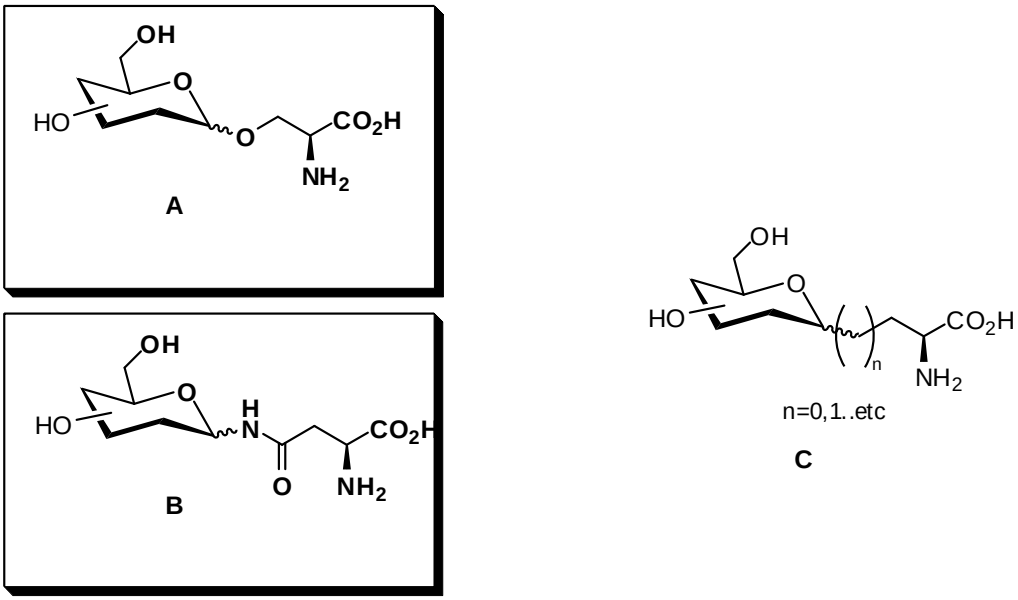


Figure1: Natural (**A** and **B)** and unnatural **C** glycosyl amino acids.

Hence, glycopeptide mimics are promising leads for novel pharmaceuticals. Different designs and manipulations on the glycosyl amino acid structure have been applied, and as a result broad spectrums of *C*-glycosyl amino acids have been synthesized.[8] New families of general formula (**D**) have appeared and have attracted considerable attention for several reasons.

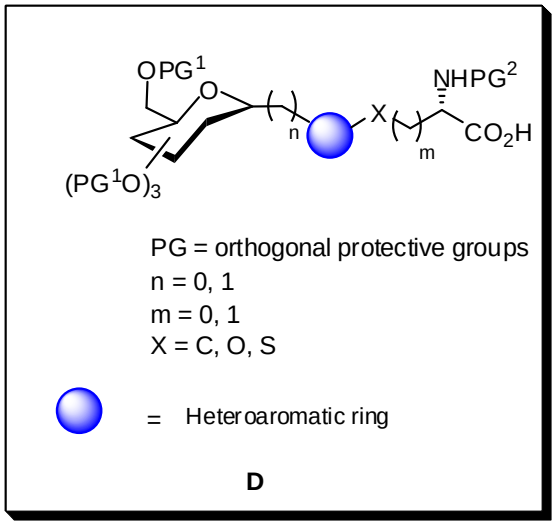


Figure 2: General formula of new families of glycosyl amino acids **D**

The structures of this family are not very different than natural structures of *C*-mannosyl tryptophan **E,** which has been identified in several biologically important proteins, [9] and *O*-glycosyl tyrosine **H,** frequently encountered in glycopeptide antibiotics[2] and as humoral factors in the pheromone gland of the Silkmoth.[10]

Simple structure comparison of synthetic molecules[11] **F** and **G** (derived from formula **D** when n = 0, X=C, m = 0, ring= isoxazol, triazole) with the chemical structure of naturally occurring *C*-mannosyl tryptophan may show the differences (i.e. different heteroaromatic ring and regio modification)

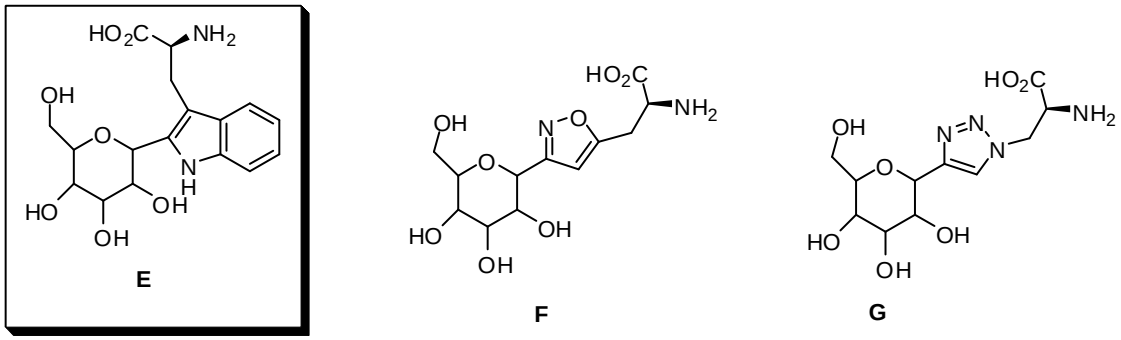


Figure 3: structures comparison of natural *C*-mannosyl tryptophan **E** and synthetic glycosyl amino acids **F** and **G.**

Another example, the modified structure **L,** can be derived from the general formula **D**. This structure has two modifications that differ from natural *O*-glycosyl tyrosine **H (**i.e**.** *C-C* bond and heteroaromatic ring instead of *C-O* bond and phenyl ring, respectively)

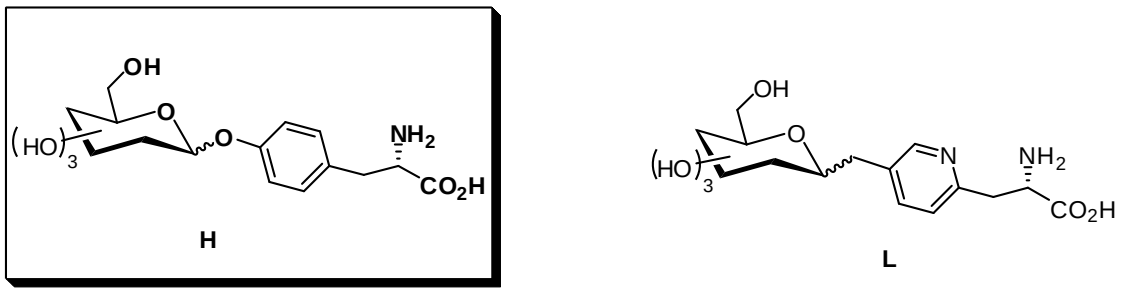


Figure 4: structures comparison of natural O-glycosyl tyrosine **H** and glycosyl amino acid **L**

Such modifications can afford important traits such as chemical stability, enzymatic stability, and chelatability (tridentate ligand) which could be used as ligands for radioactive compounds.[12] Moreover the heterocyclic ring of glycopeptide could be involved in molecular recognition processes. Finally, these molecules are building blocks for the cotranslational modification of glycopeptides and glycoprotein and can be utilized as effective molecular tools to probe and understand mechanisms of key cellular processes of natural proteins.

1.1. Definition, distribution, and functions of natural glycoproteins

For a long time, carbohydrates and proteins were considered as separate classes of natural products; however, it is now well established that nearly 50% of proteins are glycosylated, [13] in which the proteins are covalently linked to carbohydrate (glycan).
Carbohydrates have the ability to convey large amounts of information due to high permutation potential (i.e. possible linkage between two pyranoses are 1-2, 1-3, 1-4, 1-5,1-6, substituted, sulphated, phosphated, α, β…ect). Proteins are biopolymers of amino acids or polypeptides. Unlike carbohydrates that are linear and branched in nature, proteins commonly are linear in nature. The alliance between carbohydrates and proteins has produced a family of compounds that are found in almost all living organisms from unicellular yeast to humans. They occur in cells, in both soluble and membrane-bound forms, as well as in the intercellular matrix and extracellular fluids, where they include numerous biologically active macromolecules.[14]The ubiquity of this family in nature reflects their broad functions as markers in cell-cell communication events that determine microbial virulence, [15] inflammation, [16] and host immune responses.[17] Moreover, correct glycosylation of proteins is critical for expression and folding,[18] increases thermal and proteolytic stability,[19] stabilizes proteins, affects physical properties such as solubility and viscosity, helps for correct orientation in the membrane, and improves recognition to other biochemical molecules or cells (figure 5). Many proteins released by cells to the blood and other fluids are glycoproteins. One set of glycoproteins also carry the blood group determinants.[14] Glycoproteins are involved in many physiological and pathological processes. Many hormones and an increasing number of enzymes have been shown to

contain covalently bound sugars.[20] The collagens, the structural proteins essentials in the formation of connective tissues, and interferon, the antiviral agent produced by mammals, are glycoproteins. Moreover, glycoproteins serve as antigenic determinants, virus receptors, and markers of cellular identity.[21, 17a]

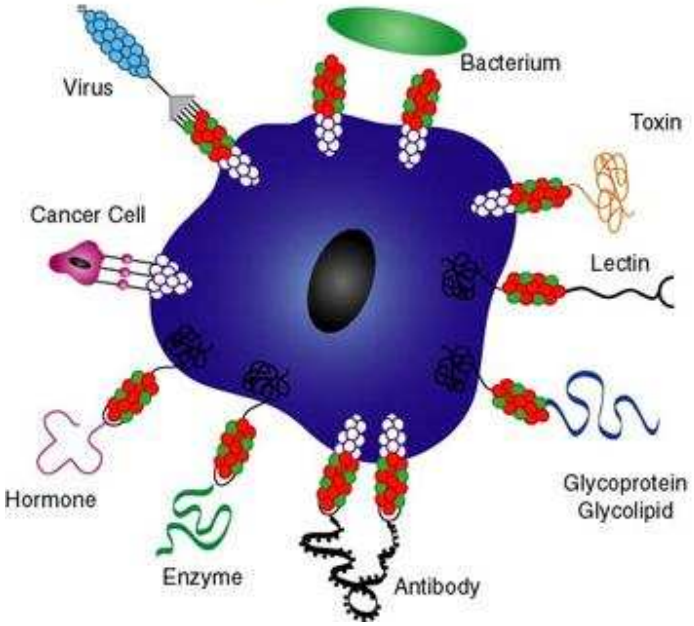


Figure 5: Cell-surface Carbohydrates involved in Molecular Recognition

Glycoproteins are macromolecules containing protein cores to which oligosaccharide chains are linked by glycosidic bonds. Glycopeptides are partial structures of the connecting regions of glycoproteins and, therefore, always contain glycosidic bonds between the carbohydrate and peptide components. Another group of compounds in which carbohydrates are covalently bound to proteins, but differ from the typical glycoproteins, are proteoglycans of connective tissue. They may be considered a special class because they contain long unbranched polysaccharide chains, with molecular weights of 20,000 or more, and are composed largely of repeating disaccharide units. To the contrary, the carbohydrate moieties of typical glycoproteins vary in size and the chains can be a single monosaccharide or a complex, possibly branched, oligosaccharide containing up to about 30 monosaccharide units per peptide (or protein) component. The nature, and indeed existence, of the carbohydrate moieties can vary during the life cycle of a cell, as a sophisticated battery of glycosyl transfer enzymes operate on it. As such,

the carbohydrate component may account for as little as 1% or up to 80-85% of the total weight of the glycoproteins but, in general, its dimensions are significant when compared with the size of the proteins to which it is bound (see Figure 6)

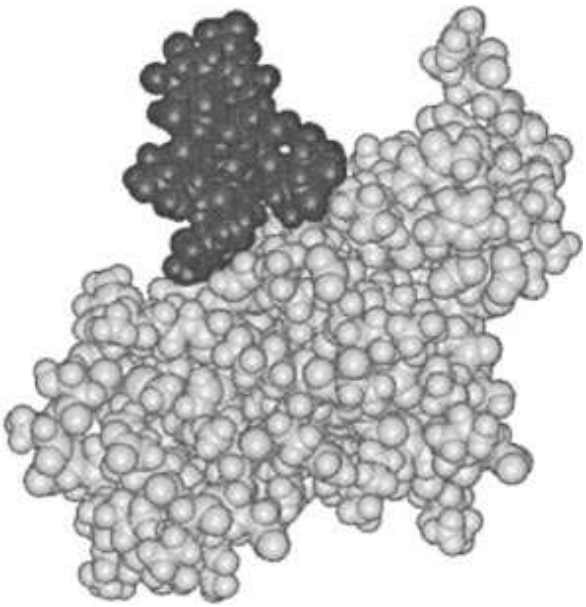


Figure 6: Space-filling model of a portion of the glycoprotein CD2[22] illustrating the relative dimensions of the carbohydrate (black) and protein (gray) moieties.

Although numbers of monosaccharides are known in nature, only ten have been detected in glycoproteins, consisting of 2-acetamido monosaccharides (2-acetamido-2-deoxy-D-galacto- and -D-glucopyranose), neutral monosaccharides (D-galactose, D-glucose, D-mannose, L-fucose, L-arabinose, L-rhamnose or D-xylose), and the sialic acid with its N-acetylated, N-glycolated, and in some cases *O*-acetylated derivatives (Figure 7).

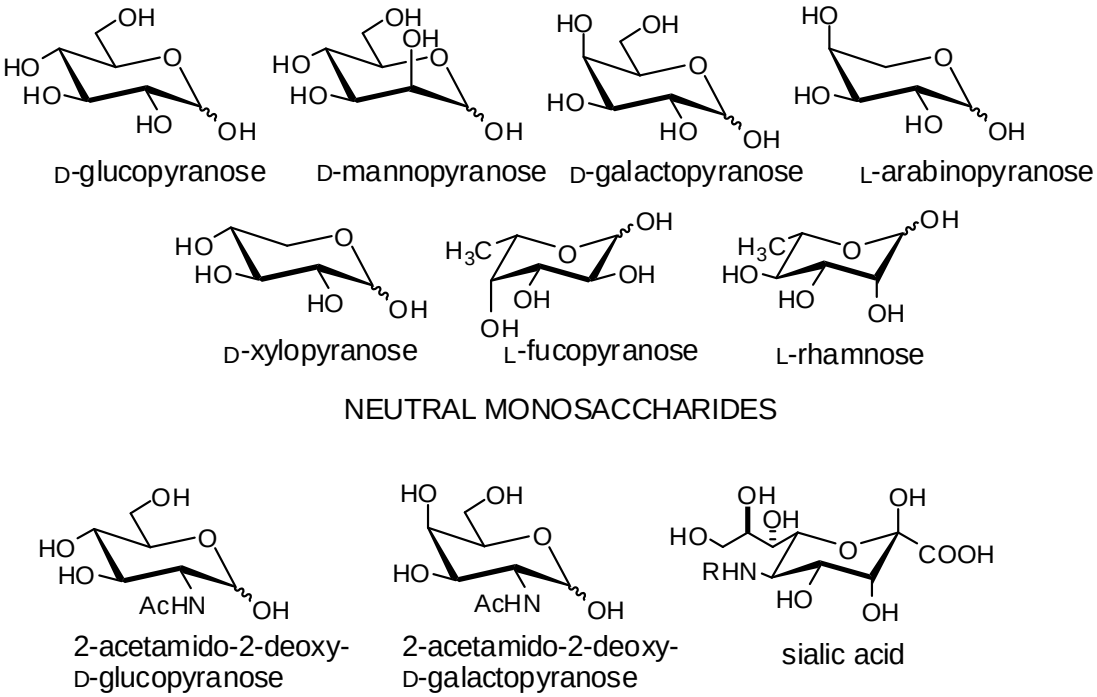


Figure7: Monosaccharides constituting the oligosaccharide units occurring in glycoproteins

Glycosylation in the cells is a post translation process, it is not under direct genetic control but is determined by enzymes, and many different enzyme reactions are involved in the processing pathways. Each individual enzyme reaction may not go to completion, giving rise to heterogeneous range of glycoforms[17a] that possess the same peptide backbone but differ in the nature and site of glycosylation. This occur in all natural glycoproteins, For example, chicken ovalbumin contains a single glycosylated amino-acid residue (Asn-293), but more than a dozen different oligosaccharides have been identified at that site, even in a preparation isolated from a single egg of a purebred hen.[23] The external and internal environment of the individual cell in which the protein is glycosylated may be influenced by physiological changes such as pregnancy and also by some diseases which may affect one or more of the enzymes in the cell give rise different glycosylations.[17b] In the conclusion glycoprotein can be defined as a set of glycoforms, these mixtures of glycoforms could provide a spectrum of activities that can be biased in one direction or another as means of fine-tuning.[24]

1.2. Biological roles of sugar chains in natural glycoproteins

Glycoproteins occur naturally in a number of glycoforms, [17a] i. e. glycosylated fragments that differ for the type of oligosaccharide and the site of attachment to the protein backbone. Each component of the mixture has different properties[24, 25] that could present regulatory difficulties[26] and problems in determining exact function through structure activity relationships, but also could provide a broad spectrum of activities that would work as means of fine-tuning[24]. As previously described, the carbohydrate moiety of glycoproteins can exert a wide range of biological functions, such as in fertilization, embryogenesis, neuronal development, hormonal activities, proliferation of cells and their organization into specific tissues, immune surveillance, and inflammatory reactions[27] they can also modulate protein folding, intra- and intercellular trafficking, and receptor binding and signaling.[17] Remarkable changes in cell surface carbohydrates occur with tumor progression, which appears to be intimately associated with the dreaded state of metastasis.[28] Furthermore, carbohydrates of host cells are often employed by pathogens for cell entry or immunological evasion.[29]

Though clear dramatic effects display on the physical, chemical, and biological properties of the naturally occurring glycoproteins and their mimics, as a result of the presence of carbohydrate units no single common theory has emerged yet for the role of carbohydrate units in glycoproteins, even where the function of the complete molecule is well understood.

A feasible approach to understand the biological roles of sugar chains consists in the removal or modification of carbohydrate moieties with different techniques[30]. The consequences of altering these types of glycosylation range from being essentially undetectable to the complete loss of particular functions.

The biological roles of the sugar chains of major classes of glycoproteins in eukaryotic cells have been reviewed in a recent work by Varky[17a]. This includes:

i) a structural role. Some oligosaccharides offer an aid in the maintenance of protein solubility and conformation: in fact many proteins that are incorrectly glycosylated fail to fold properly and can not exit the endoplasmic reticulum and they are consequently degraded.

ii) an interaction role. For example carbohydrates of membrane glycoproteins constitute recognition signals in the regulation of the cell growth and cell differentiation.[31] In according with these results, it has been found that the glycoprotein profiles in the cell membranes of normally growing cells and of tumor cells are very different[28].

iii) a protection role. The oligosaccharide portion of the glycoproteins often offers protection against the attack of proteolytic enzymes.[32]

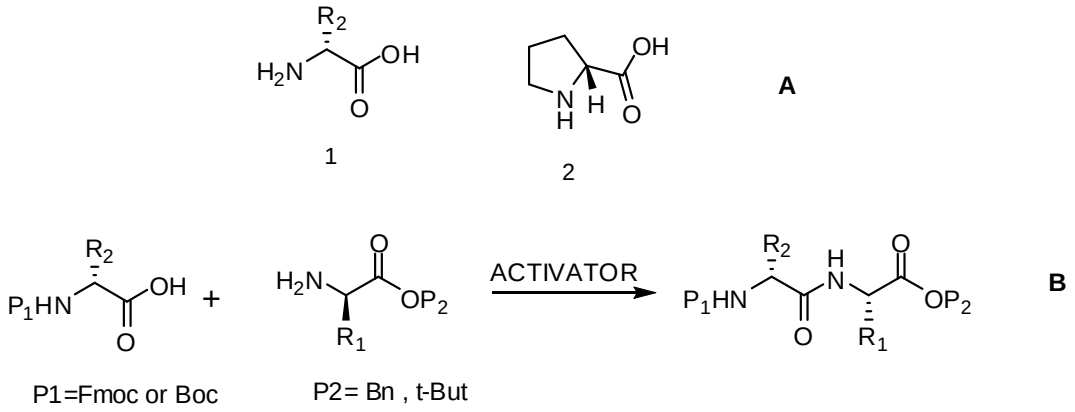
iv) a modulation role. There are several cases in which glycosylation can modulate the interactions of glycoproteins with others molecules. For example the binding process of some cell surface receptors for growth factors is affected only when the attachment of the carbohydrate side chains to the protein is blocked. Thus, glycosylation and deglycosylation are able to mediate a “switching" effect.

1.3. Synthesis of natural glycopeptides: use of glycosyl amino acids

Owing to the decisive functions exhibited by carbohydrates of glycoproteins in the regulation of cell metabolism, host-pathogen interactions, tumor cell metastasis, and cell development (see § 1.2.), and in order to fully define the roles carbohydrates play in these processes, the availability of these glycoconjugate structures is receiving increased interest. The isolation of these complex molecules is often made difficult by low concentrations in raw materials. Moreover, nature tends to produce heterogeneous glycoproteins (glycoforms) which vary in many aspects, including the extent of glycosylation. Separating individual compounds from such families of closely related molecules is not trivial. Moreover, the gene-technological production of these glycoconjugates is still limited because, in contrast to other proteins, glycosylated proteins are posttranslational products resulting from the activity of glycosyl hydrolases and transferases.[33] Therefore, organic synthesis provides a valuable alternative for the preparation of model glycopeptides as partial sequences of glycoproteins bearing one or many saccharide side chains. Synthetic glycopeptides do not have the disadvantages of microheterogeneity and are available, with variations in both the peptide and carbohydrate part, in higher quantities and superior purities than for the natural glycoconjugates. For these reasons, synthetic glycopeptides are becoming the model

compounds of choice for biomedical and structural investigations where glycoproteins are involved.

Nature synthesizes proteins by stepwise assembly of amino acid building blocks, a menu comprising 19 L-α-amino acids of general structure **1** and L-Proline **2** can be involved in such assembly (scheme 1a). For chemical synthesis of particular dipeptide, protection the amino group of one amino acid and the carboxyl group of the other amino acid selectively is required (scheme 1b). The most commonly used amino protecting groups are t-butoxycarbonyl (BOC) and fluorenylmethoxycarbonyl (Fmoc), while t-butyl ester, benzyl ester, methyl ester and allyl ester can be used to protect the carboxyl group.[34]

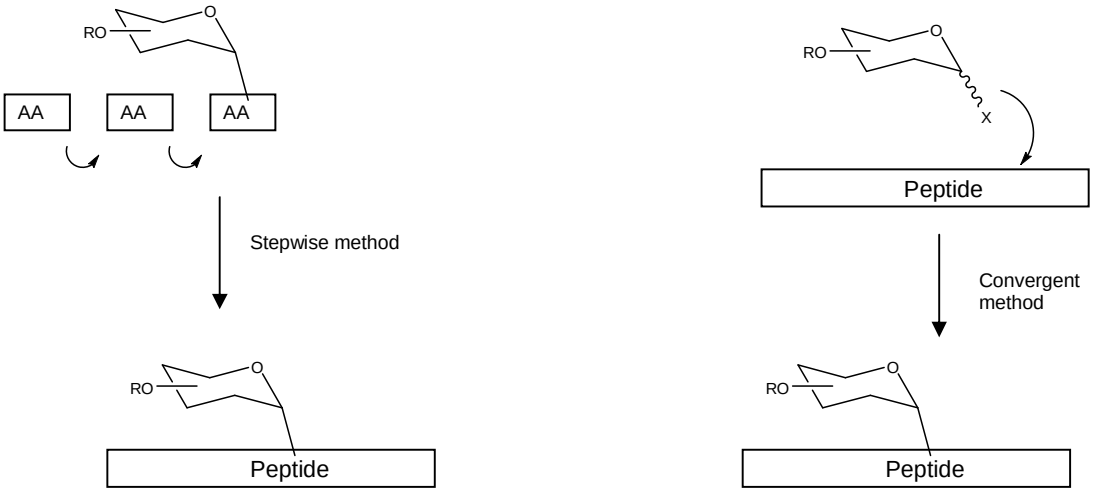


Scheme 1: a) general structures of L-α-amino acids, b) Chemical synthesis of particular dipeptide

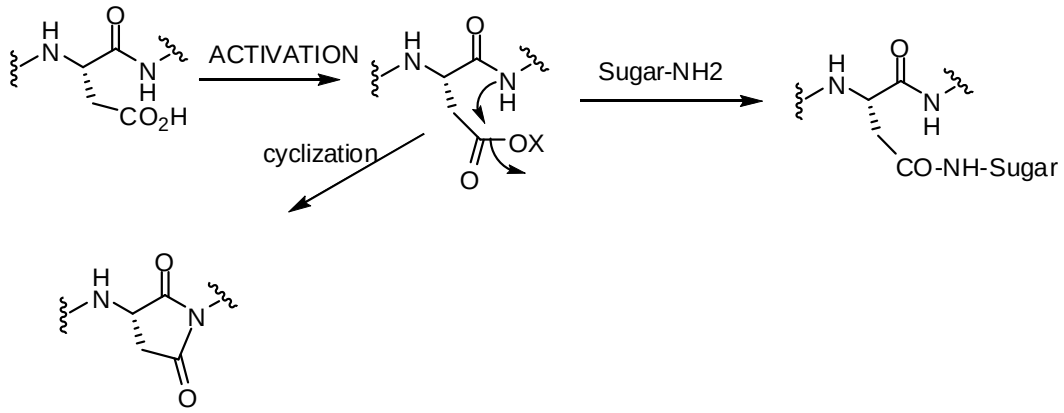
The peptide backbone can be built up either in solution or solid phase. In solution phase, the troublesome isolation of the products makes the approach cumbersome. Unlike this approach, the solid phase is used widely for synthesis of more complex peptides. Small solid beads, insoluble yet porous, are treated with functional units (linkers) in which peptide chains can be built. The peptide will remain covalently attached to the bead until cleaved from it by reagents such as TFA or HF. The peptide is thus immobilized in the solid-phase and can be retained during a filtration process, whereas liquid-phase reagents and by-products of the synthesis are flushed away. Solid-phase peptide synthesis is

usually performed from *C*-terminus to *N*-terminus; the *C*-terminus of *N*-protected amino acid is introduced to the linker in the presence of coupling reagents, and this would be the nucleus for peptide chain. The *N*-protecting group of the peptide nucleus is cleaved, and the deprotected amino functionality is coupled to the *C*-terminus of another *N*-protected amino acid. The cycle of coupling–deprotection is repeated to build up the peptide chain. Finally, the protecting groups are removed and the peptide chain is released from the solid resin. A crucial step in the chemical synthesis of glycopeptides is the incorporation of the saccharide into the peptide. To accomplish this, two approaches may be used: a convergent method or a stepwise method (scheme 2).

i) In the convergent method **(post-translational**), direct glycosylation of properly protected oligopeptides is employed either chemically or enzymatically. The success of *O*-glycosylation by this method is limited because of the low solubility of peptides under the conditions commonly employed for glycosylation and the low reactivity of the side chains of hydroxyls of Serine and Threonine**.**[35] The advantage of the direct glycosylation method is that the route is more convergent, permitting fast access to glycopeptides with different glycan structures but this method suffers from low yields due to steric factors, difficulty of glycosylation, and there is the potential for an intramolecular cyclization reaction[36] (scheme 3).



Scheme 2: glycopeptide synthesis by stepwise and convergent methods



Scheme 3: intramolecular cyclization side reaction in glycopeptide synthesis

The chemical mediated coupling has only been described by a few authors[37-40] reporting moderate success in the preparation of glycopeptides. However, this methodology does not constitute a feasible route to complex glycopeptides containing longer (more than 3-5 amino acids) peptides with multiple attachment sites. Regarding enzymatic glycopeptides synthesis, it is important to stress that the use of enzymes is restricted to the formation of glycosidic linkages between sugar units and to the segmented coupling of peptides or small glycopeptide fragments.[41] little is known about the enzymes which catalyze the formation of the key glycosidic linkages between the carbohydrates and the peptide. An exception to this is oligosaccharyl transferase (OT) which has been isolated and studiated.[22] But the low availability of the enzymes at present, restricts the widespread use this type of glycosylation

ii) stepwise approach(**co- translational)**

This methodology employs glycosylated amino acids, molecules composed of the carbohydrate moiety bound to a first amino acid through a glycosidic linkage, as building blocks for the stepwise assembly of glycopeptides, preferably on solid-phase (Figure 8). Versatility is one of the main advantages of this procedure which allows the synthesis of

glycopeptides with variations at the peptide part and structures containing more than one carbohydrate residue as well. It is also an advantage that the difficulties of generating the crucial bond (stereoselective connection) between the oligosaccharide and the amino acid are solved before the glycosyl amino acid is incorporated into the peptide.

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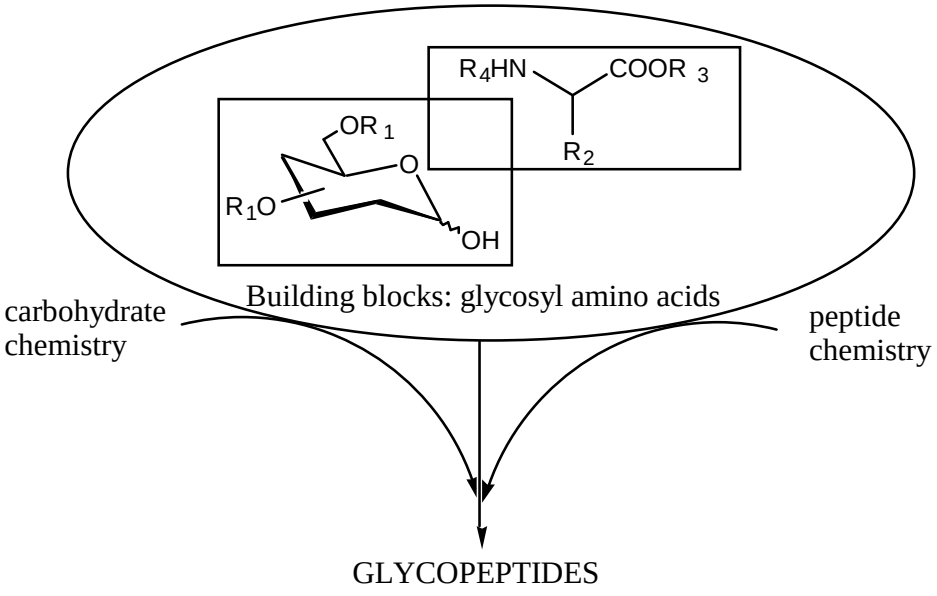


Figure 8: Use of glycosyl amino acids as building blocks for stepwise glycopeptide synthesis

1.4. Glycosidic linkages in nature: *N*- and *O*-glycosyl amino acids

Owing to the efficiency demonstrated by suitably protected glycosylated amino acid building blocks in the preparation of synthetic glycopeptides (see § 1.3.), investigations on the forms of protein glycosylation are receiving increasing interest. Figure 9 shows all the known types of glycosidic linkage between the carbohydrate and the peptide occurring in natural glycoproteins; such a draw is a dynamic picture due to the continuing discovery of new forms of glycosylation.[42]

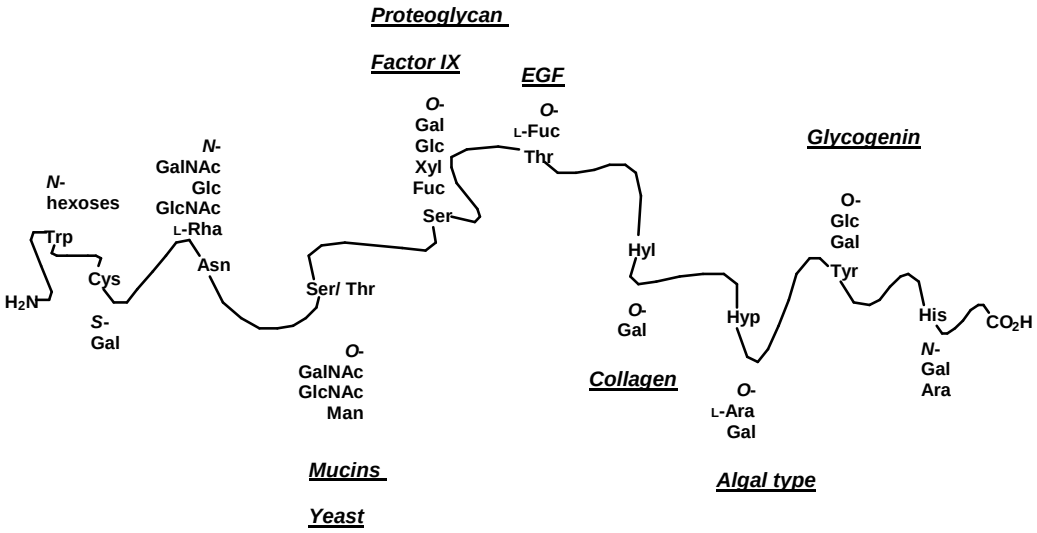


Figure 9: Forms of protein glycosylation

Bonding of sugars to protein involves only ten monosaccharide (see § 1.1.). Of the 20 coded amino acids, seven contain either no side chain (Gly), or purely hydrocarbon side chains (Ala, Val, Leu, Ile, Phe, Pro) and no evidence has so far been found for *C*-glycosylation of the side chains of these residues. The remaining 13 coded amino acids contain various functional groups in their side chains but only asparagine (Asn), serine (Ser), and threonine (Thr) are known to form linkages with carbohydrates. Only in few cases, tryptophan (Trp), histidine (His), cysteine (Cys) and two non coded amino acids, 4-hydroxyproline (Hyp) and 5-hydroxylysine (Hyl) have also been identified at the linkage site in glycoproteins. Therefore there are only three important types of connection between the carbohydrate and peptide portions in natural glycoproteins. Most common are *N*-glycoproteins **3**, in which the side chain amide function of an asparagine unit is joined to the monosaccharide residue (GlcNac, GalNAc, Glc) through a β-*N*-glycosydic linkage (Figure 10).

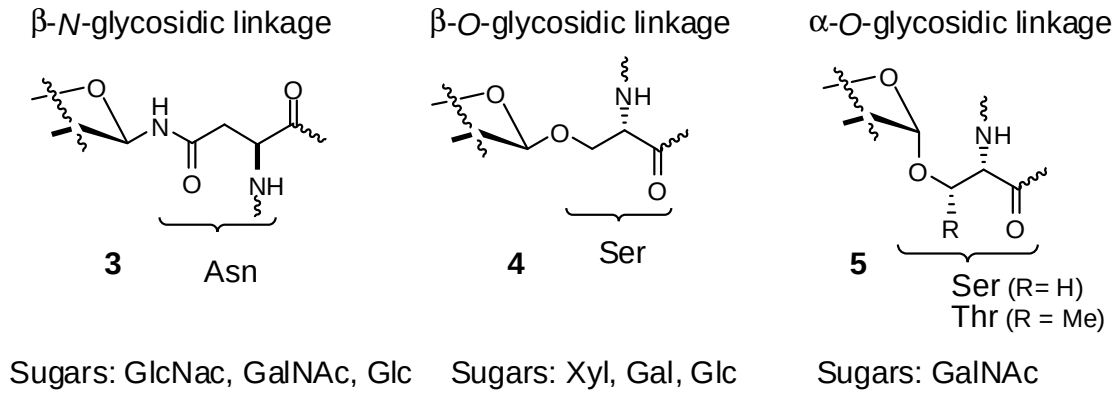


Figure 10: Main types of glycodidic linkages in natural glycopeptides

N-linked glycoproteins[14] are synthesized and modified within two membrane-bound organelles in the cell: the rough endoplasmic reticulum and the Golgi apparatus. The protein component of the glycoprotein is assembled on the surface of the rough endoplasmic reticulum by the sequential addition of amino acids, creating a linear polymer of amino acids called a polypeptide. Twenty different amino acids can be used for the synthesis of polypeptides. The specific order of amino acids in polypeptide is critical to function and is referred to as the amino acid sequence. The carbohydrate of the N-linked glycoprotein is always attached to the amide nitrogen of an aspargin side chain, and is always found in amino acid sequences where the asparagine is followed by some other amino acid and then a serine or threonine residue (-Asn-Xaa-Ser/Thr). Carbohydrate is not attached to the polypeptide one sugar at a time. Rather, a large preformed carbohydrate containing fourteen or more sugar residues(dolichol-linked Glc3Man9GlcNAc2 oligosaccharide) is transferred en bloc to an Asn-X-Ser/Thr sequon on newly synthesized polypeptides through the action of a multi-subunit oligosaccharide transferase complex(figure 11)

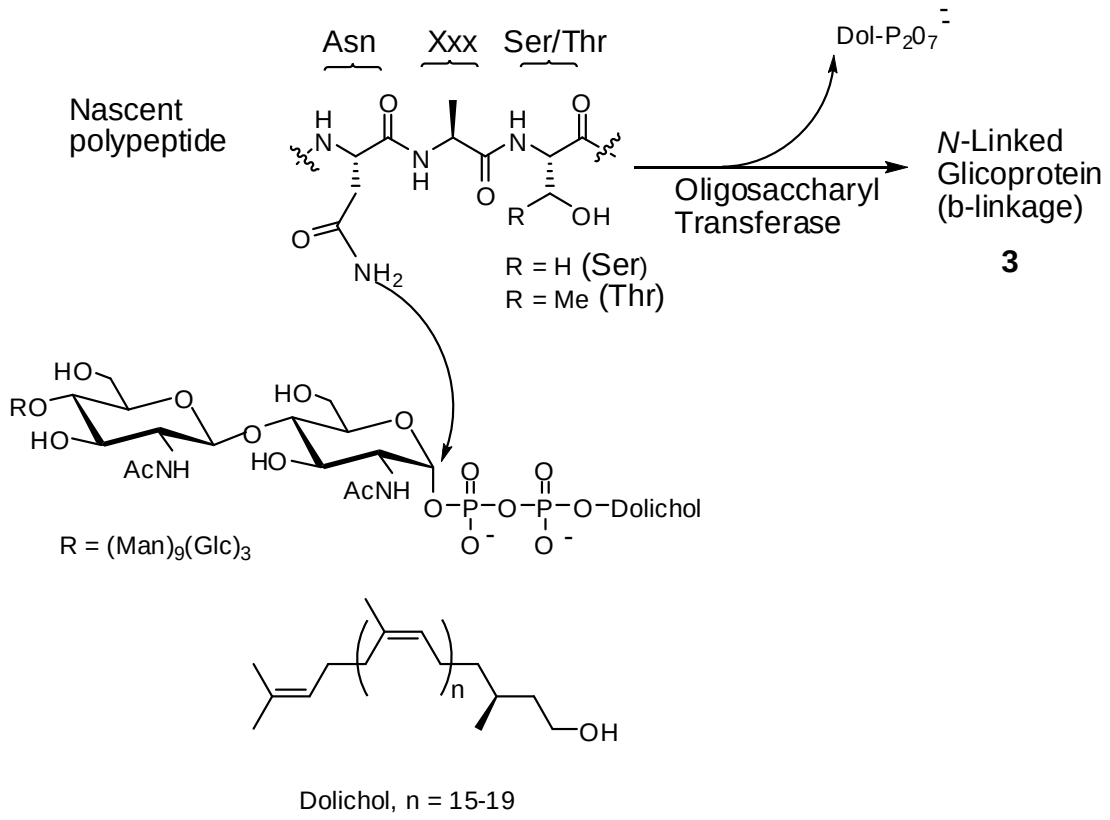
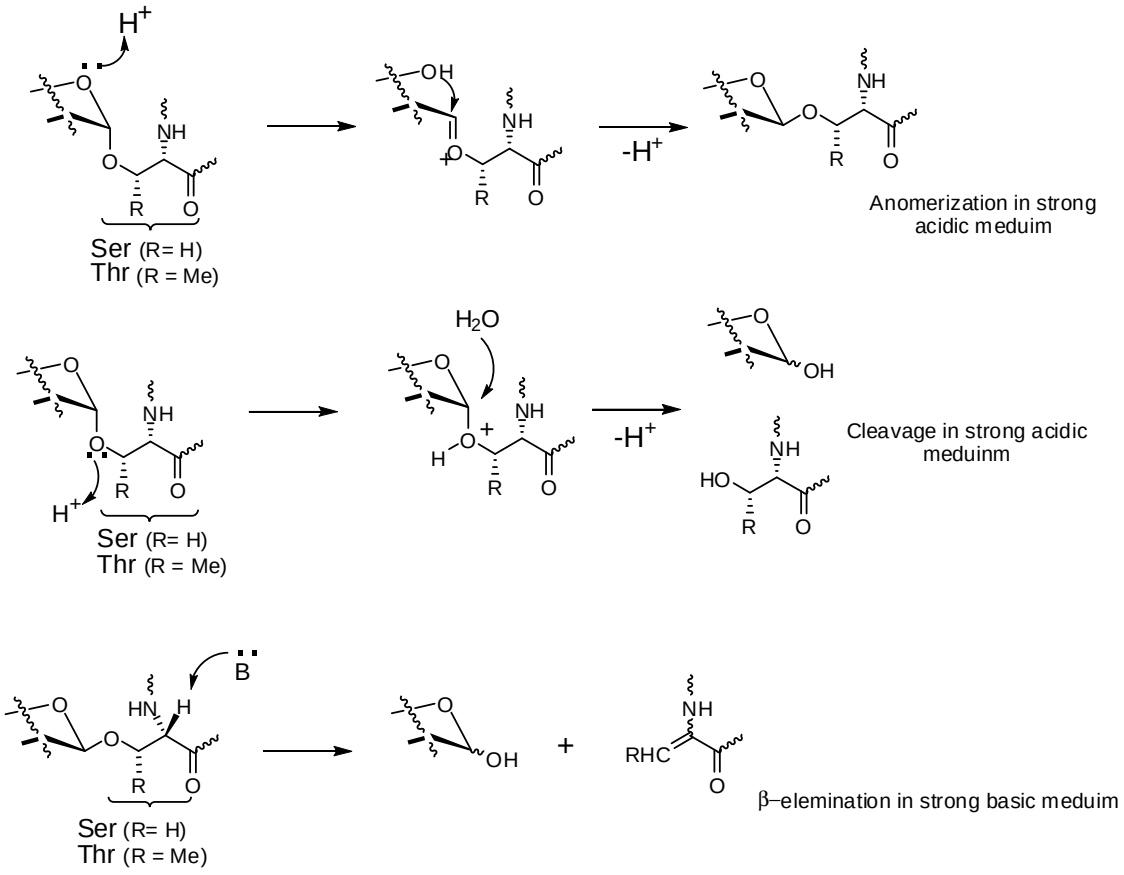


Figure 11: Nature's approach to *N*-glycosylation

Subsequent trimming and processing of the transferred oligosaccharide results in a Man3GlcNAc2 core structure, which is transported to the middle complex of the Golgi apparatus where maturation of the oligosaccharide gives rise to an extreme structural diversity.[22] Another type of glycosidic linkage that occurs in natural glycoproteins involves the side chain hydroxyl function of serine and threonine through an α- and β-O-glycosidic linkage (Figure 10)· O-linked glycoproteins are usually synthesized in the Golgi apparatus, where the GalNAc moiety of UDP-GalNAc is transferred to the hydroxyl side chain of serine or threonine and catalyzed by polypeptide GalNAc transferase[43] Unlike N-linked glycoproteins, O-linked glycoproteins are synthesized by the addition of a single sugar residue at a time.

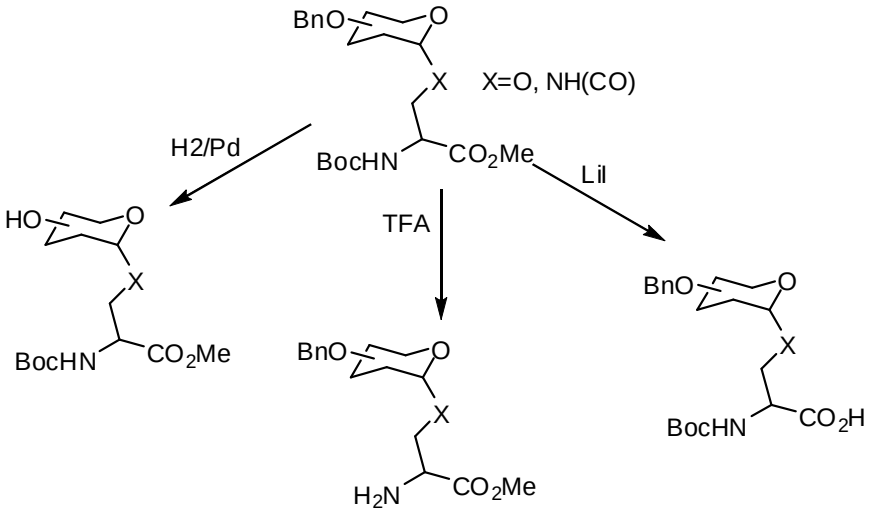
1.5. Natural and unnatural glycopeptide synthesis

The most reliable and efficient methodology for natural glycopeptide synthesis is the use of glycosylated amino acid building blocks in step-wise assembly of glycopeptides , either in solution or preferably on solid phase (see § 1.3). The natural glycosyl amino acids are molecules composed of a carbohydrate moiety bound to an amino acid through different glycosidic linkages: all these types of connection have been reviewed in § 1.4. Synthetic routes to suitably protected amino acid building blocks constitute a key to success for the synthesis of natural glycopeptides. The construction of glycosyl amino acids of exactly specified structure requires the combined application of synthetic methods and protecting group strategies of both carbohydrate and peptide chemistry. Dealing with natural glycopeptides and their building blocks are more complicated due to the high potential for anomerization, racemization and β-elimination when treated either with strong acidic[44] or basic medium[45] (scheme 4).



Scheme 4: sensitivity of the glycosidic linkage in acidic and basic conditions

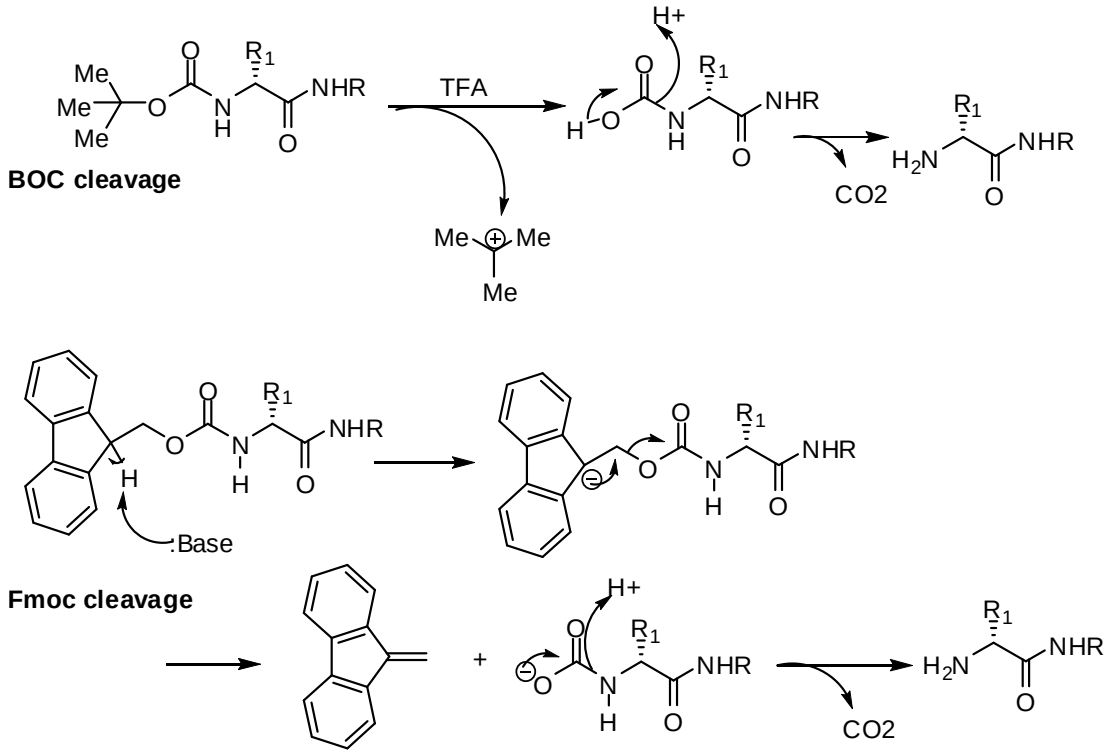
In order to avoid hard reaction condition that could lead to racemization and β-elimination, protecting groups for glycosyl amino acids used in glycopeptide synthesis have to be judiciously chosen.[46] Two main common orthogonal strategies can be considered for this goal. The first is the classical strategy, which uses BOC to protect the amino group[34]. In this strategy, the benzyl ether protects the hydroxyls of the sugar and the carboxyl can be free acid or methyl ester[47]. The three protecting groups are orthogonal with each other, which mean that any of the protecting groups could be removed without affecting the others (scheme 5). The reaction conditions required to cleave them causes neither racemization nor β-elimination.



Scheme 5: The orthogonality of BOC strategy

The BOC group is stable under basic medium, catalytic hydrogenation and Nucleophilic reagents but can be removed by treating with acid such as TFA (scheme 6). The hydroxyls of the sugar are protected as benzyl ether, which is stable under both acidic and basic medium that are required to cleave the BOC and methyl ester, respectively. Benzyl ether can be cleaved by catalytic hydrogenation over Pd but benzyl ether is rarely used as protecting group when the peptide contains cysteine residues. The problem arises from the poising of the Pd catalyst by the sulfur atom of the cysteine. Most chemists use benzyl protecting groups for assembly of oligosaccharide moieties and replace it with another group that is suitable for glycopeptide synthesis. While the BOC group is

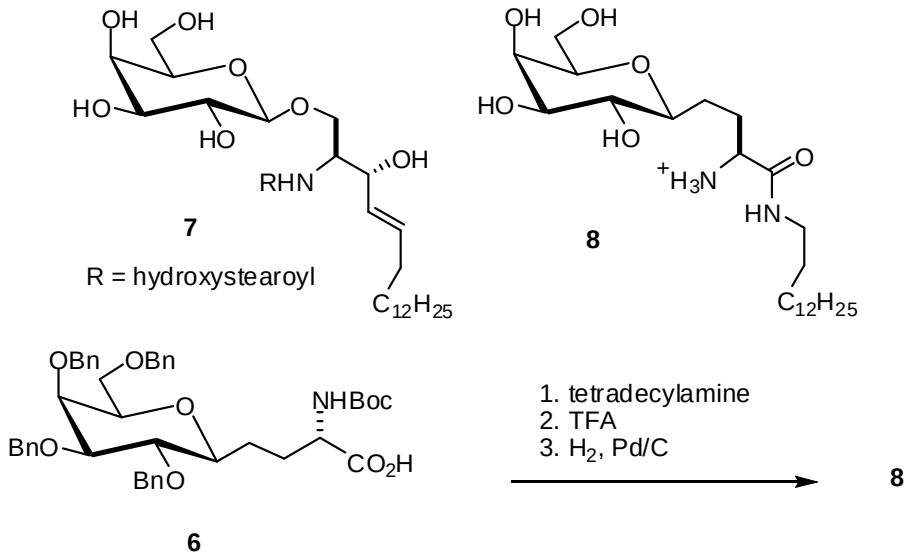
removed by moderately strong acid such as TFA, the cleavage from the solid-phase requires strong acids such as HF. As a result, the glycosidic bonds don't survive this treatment. In conclusion, the BOC is convenient for solution not for solid phase natural glycopeptide synthesis.



Scheme 6: a) BOC cleavage in acidic medium b) Fmoc cleavage in basic medium

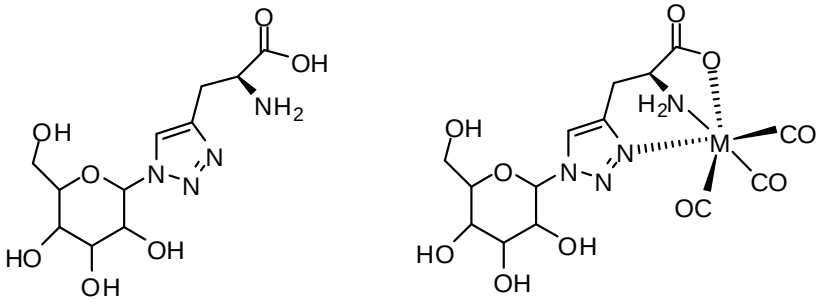
The second strategy is sufficiently mild for solid phase synthesis of glycopeptides using glycosylated amino acids as building blocks, [48] it employs Fmoc to protect the amino group. The Fmoc group is stable in acidic medium, but hydrolyzed in basic conditions such as 20% piperidine, morphaline. (Scheme6**).** The hydroxyls of the sugar are usually protected with acetyl groups that stabilize the glycosidic bonds toward the acidic treatment used for side chain deprotection and cleavage from the solid phase[49, 44c]. The carbohydrate acetyl protective groups can be removed before or after cleavage from the solid phase by treating with dilute sodium methoxide in methanol, while the carboxyl group can be free acid, activated ester such as pentafluorophenyl ester, t-butyl ester, or any other orthogonal ester with Fmoc.

The development of new solid-phase supports, protecting groups and coupling reagents has solved the problem of natural glycopeptide synthesis [35, 48c]. However, the lability of the glycosidic bond towards chemical and enzymatic degradation in vivo is an important limitation in using N- and O-glycopeptides as potential drugs. The substitution of the glycosidic C-O or C-N bonds found in O-linked and N-linked glycoproteins, respectively, with a C-C bond confers resistance to enzymatic hydrolysis[6]. In addition, C-glycosides demonstrate solution conformations and biological activities similar to their native counterparts[7]. For example, the water soluble galactosphingolipid **8** was shown to bind specifically to HIV-1 gp 120 and therefore represented potential inhibitoin of the first step in the infection process causing the AIDS. This compound is the carbon-linked analogue of the natural product **7,** compound **8**, synthesized by using C-glycosyl amino acid **6**[50]



Scheme 7

Various C-glycosyl amino acids have appeared[8] as a result, of mimicry of the natural products, such as C-glycosyl serine, C-glycosyl asparagine, C-glycosyl tyrosine, C-glycosyl tryptophan and broad spectrum of more modified structure such as compound **9** which can be used as chealtors for technetium-99m (Tc). The product **10** can be used in radio diagnosis and radiotherapy.[12]



9 10

Figure 12

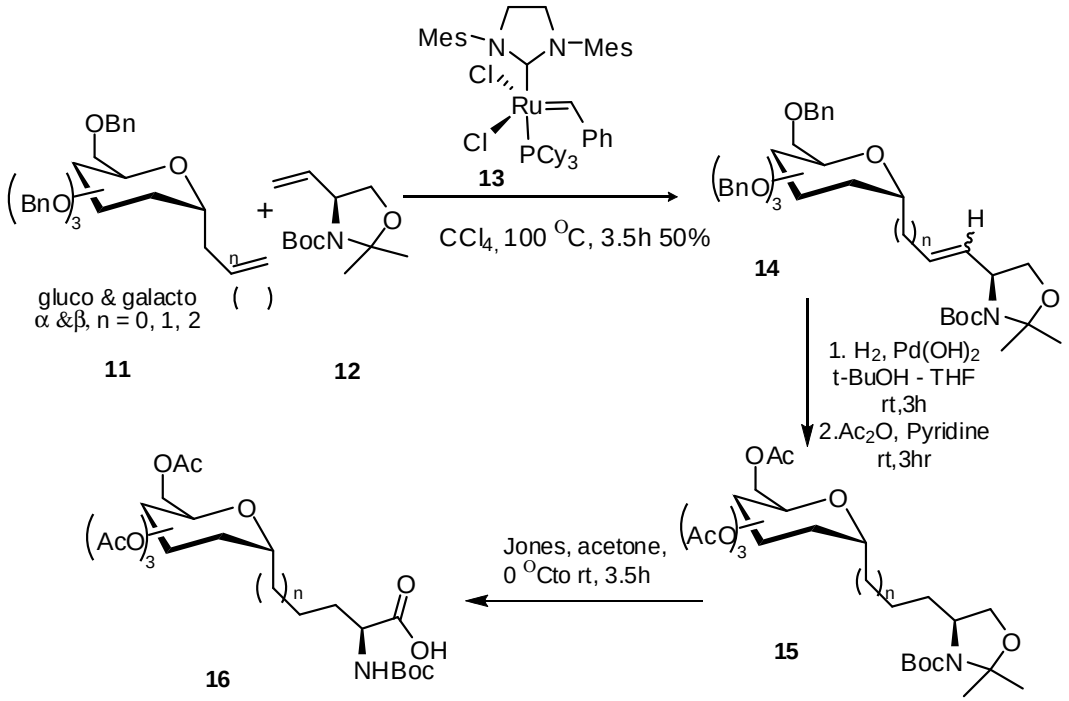
Due to many applications of carbon-glycopeptides used in cancer, HIV, immunotherapy, and infectious and pathogenic researches, interest in the stereoselective synthesis of different *C*-glycosyl amino acids as precursors for them is becoming an important issue in organic chemistry.

2. SYNTHESES OF C-GLYCOSYL AMINO ACIDS: THE STATE OF THE ART

Methods for the synthesis of *C*-glycosyl amino acids until 2000 have been reviewed.[8] Methods that have been reported between 2000 and 2008 are reviewed and described below.

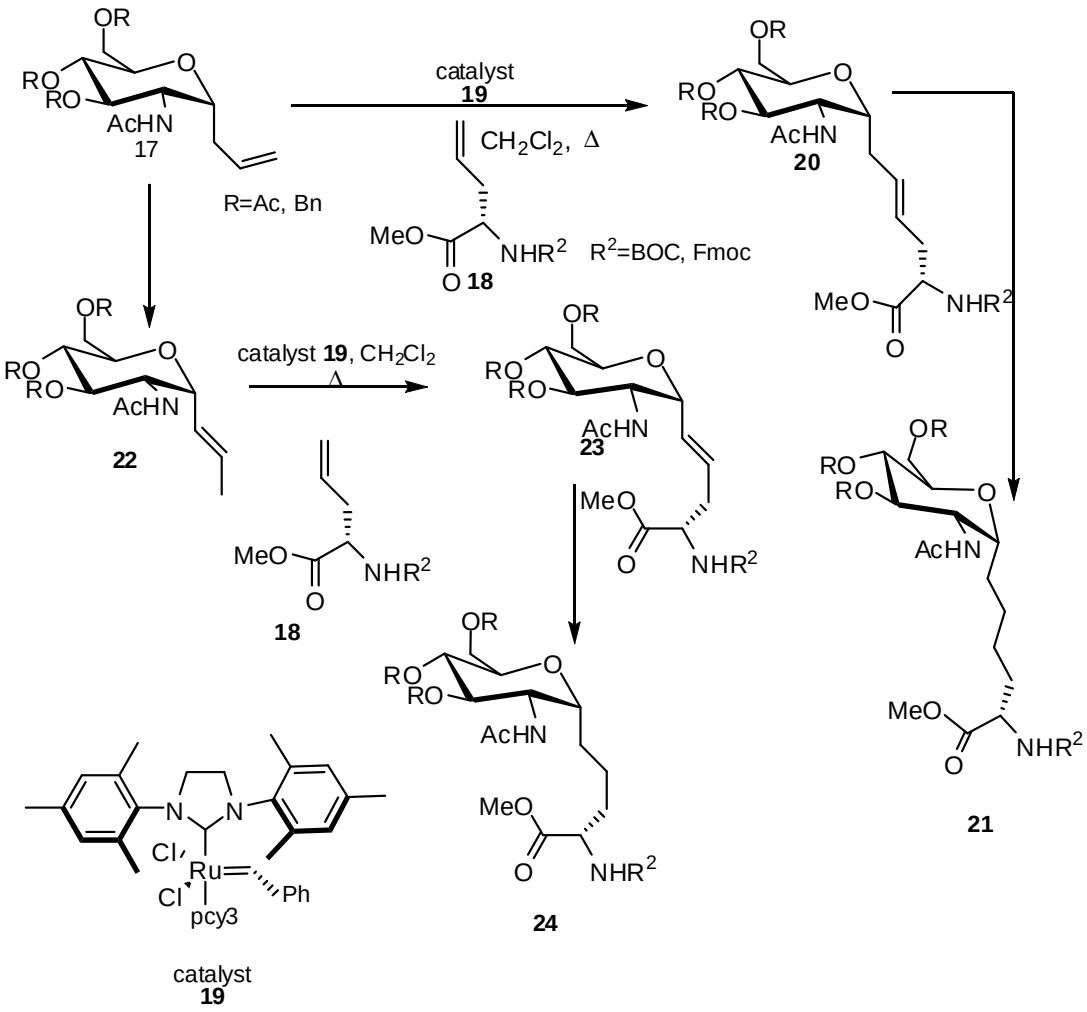
1. Cross metathesis

Olefin cross metathesis between different olefinic α-amino acids and unsaturated C-glycosides has been extensively used for the synthesis of C-glycosyl amino acids. In 2001, Dondoni et al[51] carried out cross metathesis catalyzed by second generation Grubbs catalyst **13** of perbenzylated C-alkenyl glycosides **11** with a vinyl oxazolidine **12**, derived from Garner**'**s aldehyde to afford **14** in ~50% yield. The alkene **14** was transformed into the corresponding peracetylated oxazolidine derivative **15** by hydrogenation over Pd $(OH)_2$ and acetylation. Finally, oxidative cleavage of oxazolidine ring by Jones reagent (CrO_3 aq. H_2SO_4) affords glycosyl amino acid **16** with 2 to 4 carbons linking the sugar anomeric position to the glycinyl moiety (Scheme 8). This methodology has also been extended to prepare a potential glycopeptide nephritogenoside mimetic.



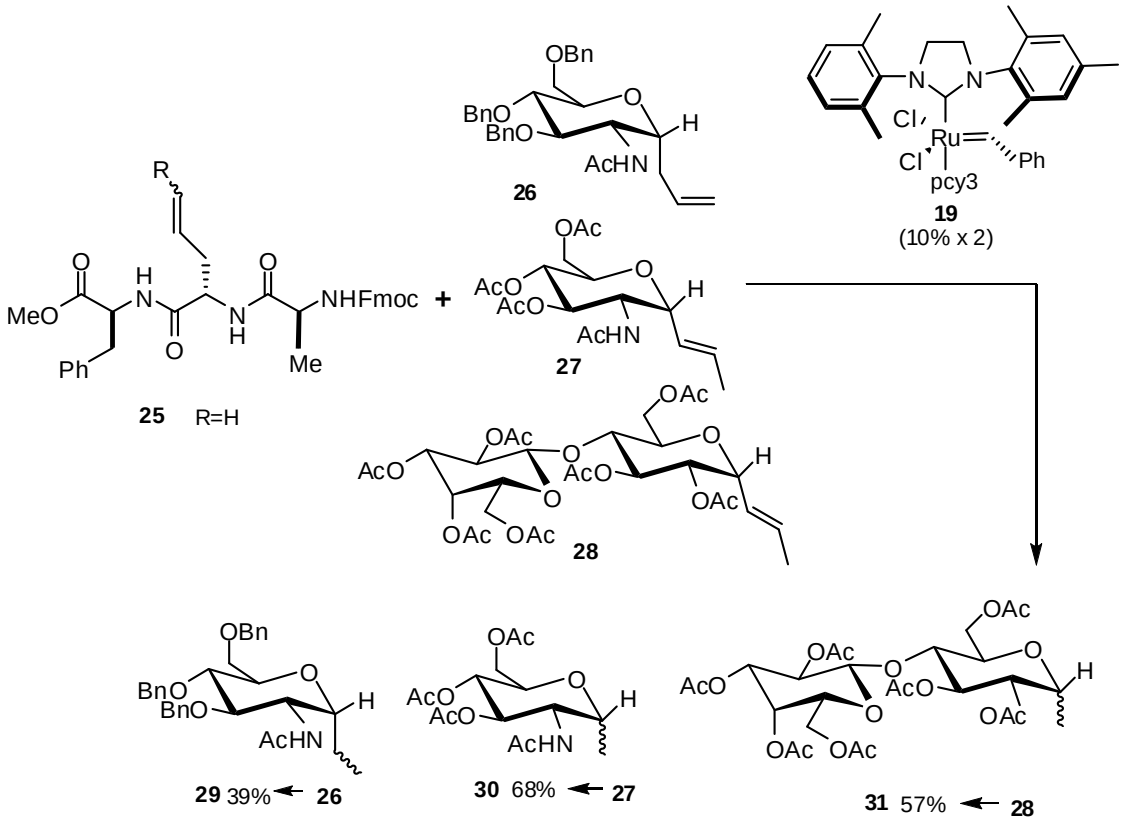
Scheme 8

- In 2002, McGarvey et al.[52] described a successful cross metathesis-based approach between *C*-alkenyl glycosylamines (**17**and **22)** and L-allyl glycine **18** using Grubbs' Catalyst **19** (10-20 mole %) to produce alkene **20** and **23** in very good yields. Hydrogenation of **20** and **23** with catalytic Pd $(OH)_2$ afforded glycosyl amino acid **21** and **24** featuring 3 or 4 carbon tethers (Scheme 9).



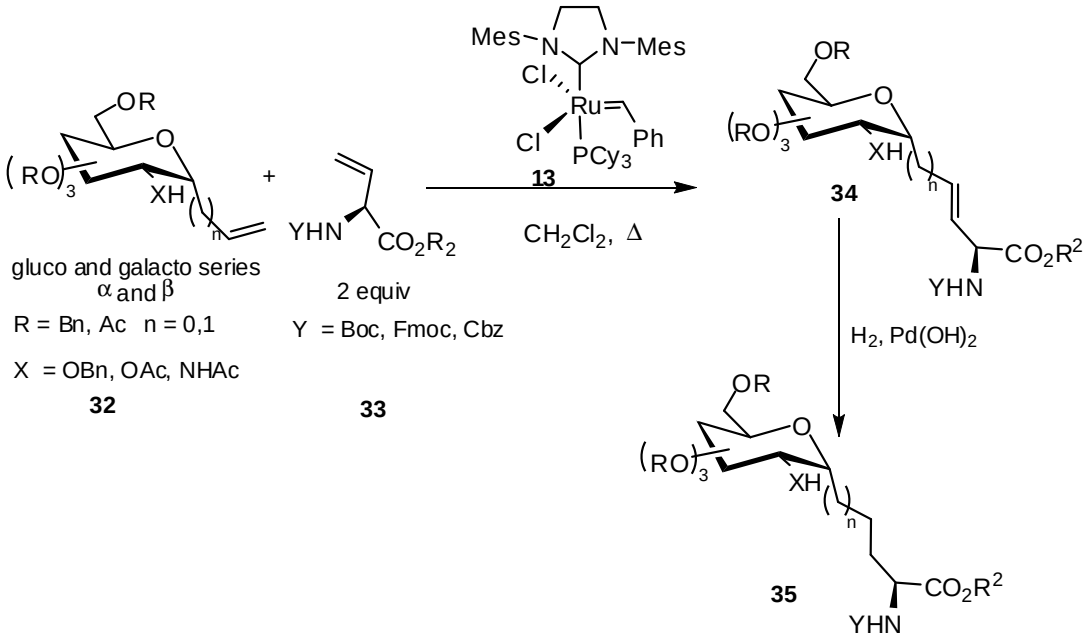
Scheme 9

McGarwey has succeeded also in post-translational metathesis by subjecting peptides carrying olefinic functionality **25** with *C*-glycosides **26**, **27**, and **28** in the presence of 20 mol % of catalyst **19** (Scheme 10).



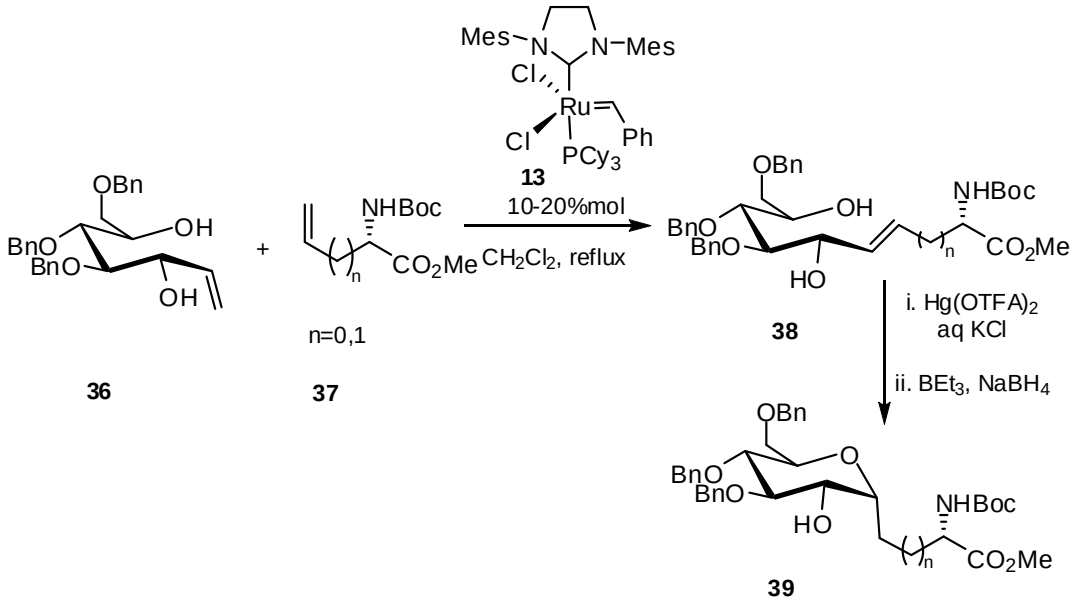
Scheme10: Post-translational metathesis

- In 2003 Nolen et al[53] chronicled olefin cross metathesis between *C*-allyl glycoside **32** and L-vinyl glycine **33** for *C*-glycosyl amino acid synthesis **35** in very good yields (60-86%) (Scheme: 11). This reaction was much less efficient with vinyl glycosides as the yields of the cross coupling product were only 11%.



Scheme 11

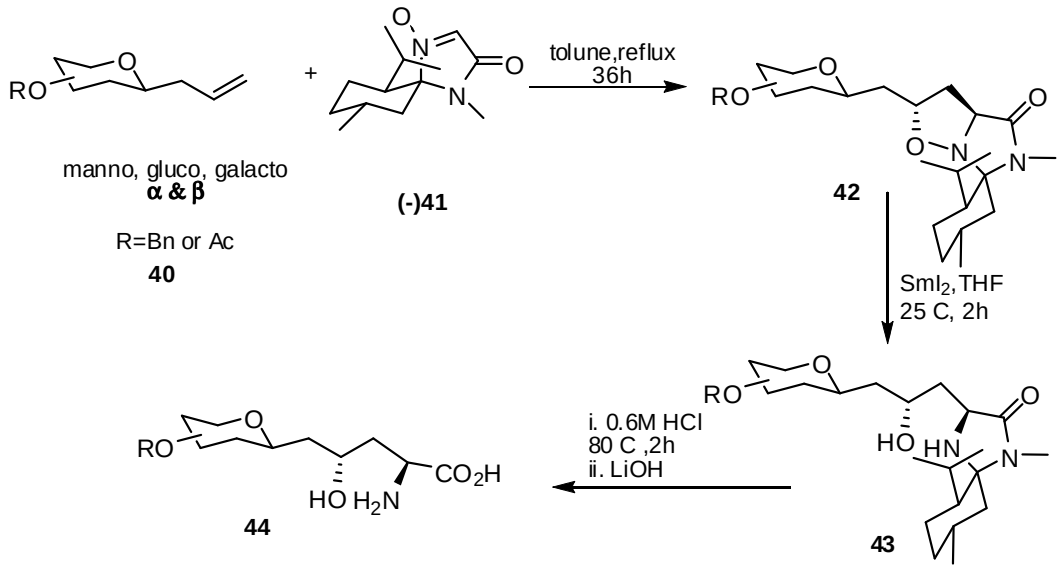
- Neolen et al.[54] in 2005 utilized a cross metathesis / cyclization strategy to produce *C*-glucosyl serine and alanine **39** with high stereoselectivity. Alkenyl sugars in their ring opening form were used to vary the reactivity for cross-metathesis olefination. For example, the cross metathesis on glucoheptenitol **36** with ally or vinyl glycine **37** produced acyclic gluco-amino acid alkene **38,** which upon electrophilic cyclization afforded the desired *C*-glycosyl serine (one carbon) or *C*-glycosyl alanine (two carbons) (Scheme 12). However, this methodology didn't succeed in the case of *C*-mannose derivatives.



Scheme 12

2. Chiral auxiliary based approach toward the synthesis of C-glycosyl amino acids (1,3-dipolar cycloaddition reaction)

In 2001 Westermann and coworkers[55] used a chiral auxiliary based approach with cycloaddition reaction to synthesize C-glycosyl amino acid 45. They used the 1,3-dipolar cycloaddition of ally glycoside 40 and nitrone (-) 41 (glycine equivalent) to give isoxazolidine 42. The reductive cleavage of the N-O bond in 42 by Sm(II) produced the corresponding 1,3- amino alcohol 43 in high yield . Then, the N, N-acetal group of **43** was quantitatively hydrolyzed by 0.6M aqueous HCl and small amounts of acetic acid, and the amidic bond was cleaved with an equimolar amount of lithium hydroxide to give **44**. Both hydrolytic steps were nearly quantitative (Scheme 13).

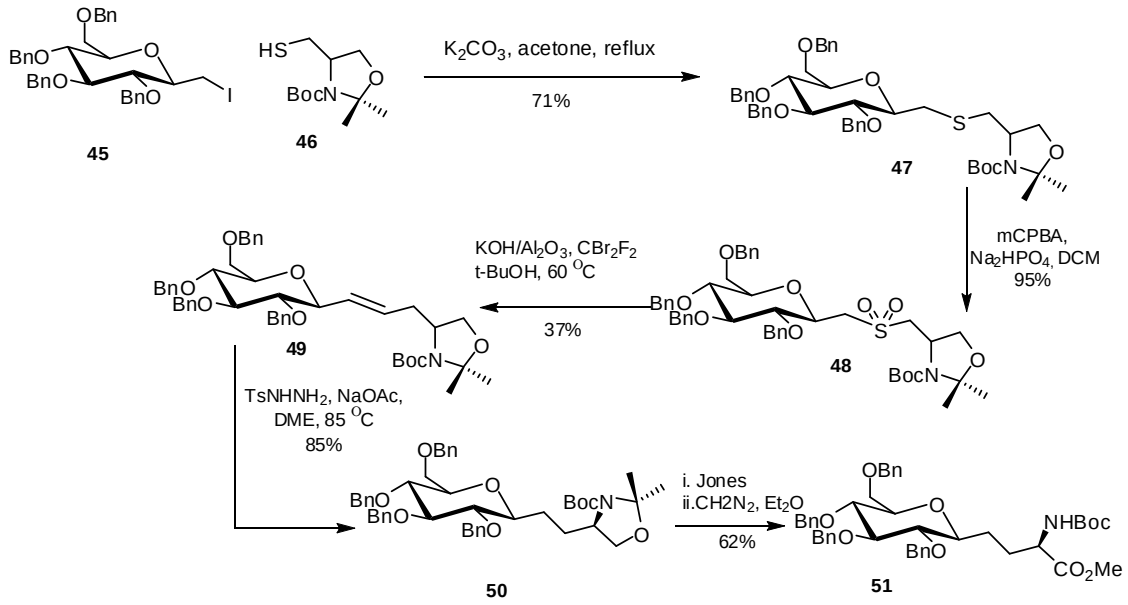


Scheme 13

The broad applicability of this methodology to different glycosides (gluco, galacto, manno, GlcNAc derivatives, vinyl derivative pseudo-glucal α & β), the ability to synthesize both diastereomers in high regioselectivity and stereoselectivity with high percentage yields by using the appropriate nitrone (- or +), the orthogonal protecting group strategy, and the ease of the preparation of building blocks (glycosides and nitrone), considered to be advantages of this methodology.

3. Ramberg-Bäcklund rearrangement

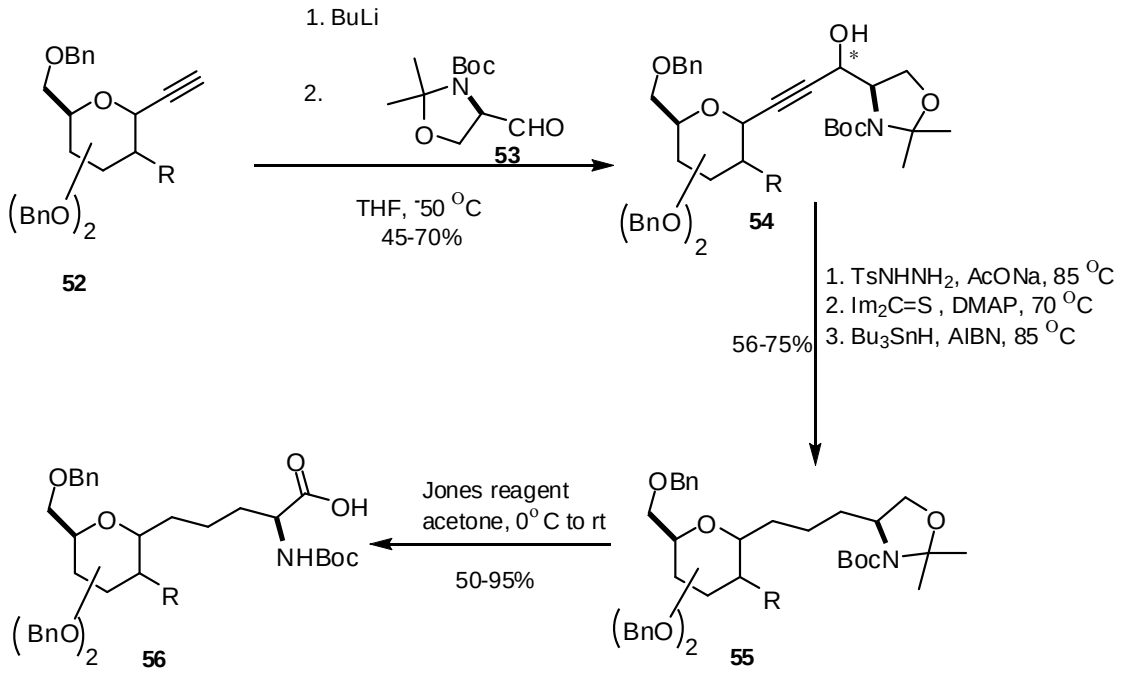
In 2002 Taylor's et al.[56] established a Ramberg-B**ä**cklund approach to synthesize *C*-glycosyl amino acid **51**. The method involves alkylation of thiol **46,** a cysteine equivalent, with glycosylmethyl iodide **45** to give sulfide **47.** The resulting sulfide was oxidized to sulfone **48,** which was then subjected to Chan's halogenative Ramberg-Bäcklund conditions to give alkene **49** in ~37% yield. The resulting alkene **49** was reduced with diimide generated in situ from tosyl hydrazine and the amino acid moiety was unmasked from the N-Boc oxazolidine ring using the Jones reagent to afford the glycosyl amino acid as a single diastereomer. This was isolated and characterized as methyl ester **51** (Scheme14).



Scheme 14

4. Acetylide coupling

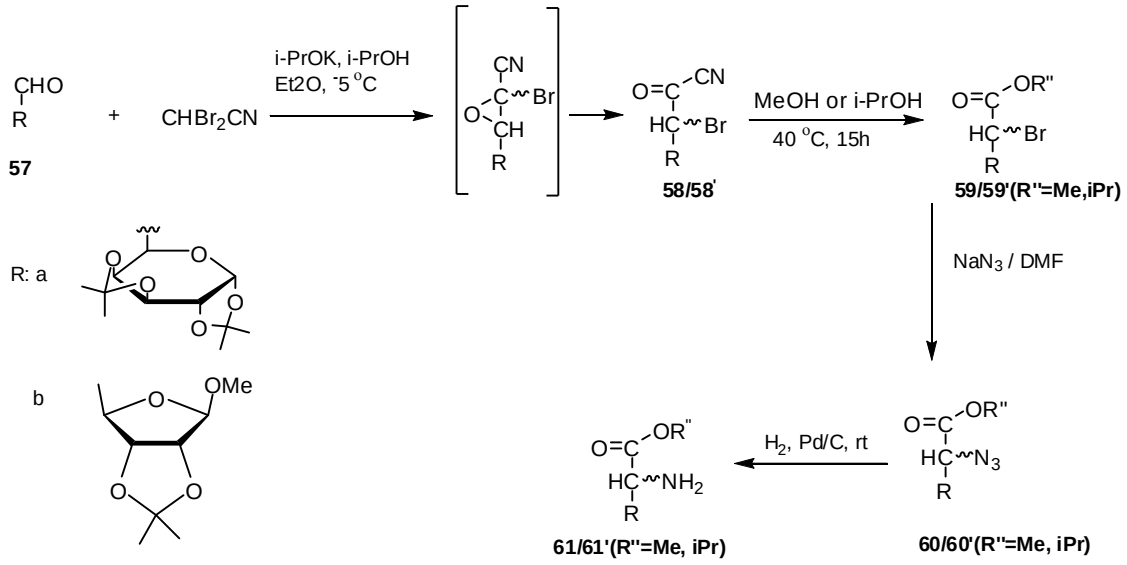
In the same year, Dondoni et al[57] used coupling of the lithium derivative of ethynyl galactoside **52** with Garner aldehyde **53** to give the alcohol **54** in good yield. This was transformed into the intermediate **55** via reduction of the triple bond with tosylhydrazine and deoxygenation by using the Barton-McCombie method. Finally, the oxidative cleavage of the oxazolidine ring of **55** afforded the target amino acid **56** (Scheme: 15).



Scheme 15

5. Homologation of dialdoses

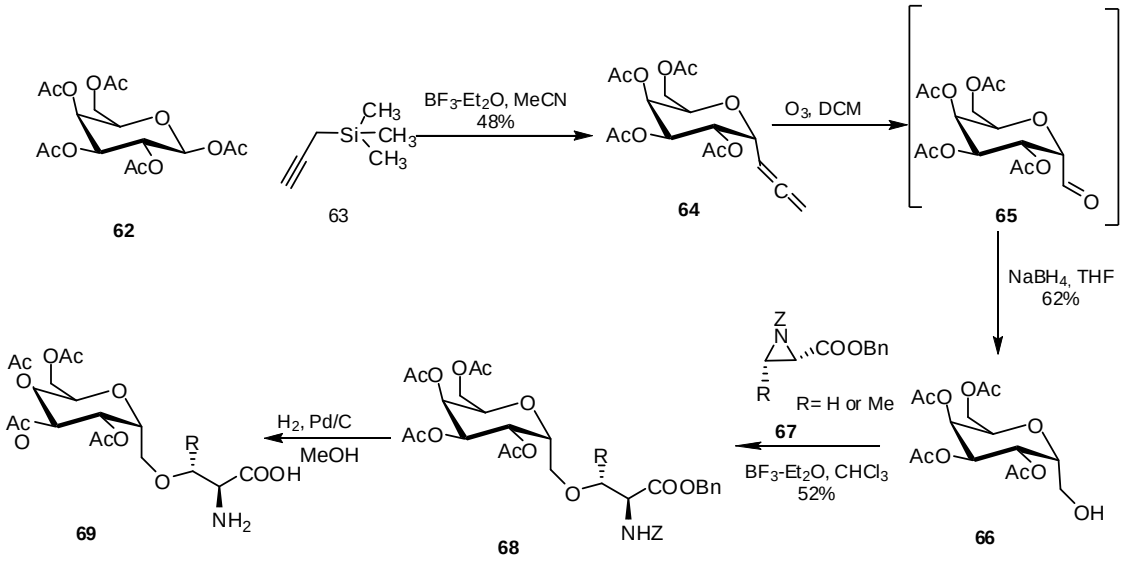
In 2003, Grison and coworkers[58] prepared glycosyl amino acids **61** and **61'** in pure form in three steps starting from dialdose **57**. The reaction of these sugar aldehydes with potassium dibromoacetonitrile carbanion provided the corresponding β-bromo-α-ketonitriles **59/59'** as a mixture of diastereomers that were easily transformed into α-bromo esters **59/59'** by treatment with methanol or isopropanol. Substitution of bromine by the azide group gave the azides **60/60'**. The authors utilized the difference in rate of hydrogenation of epimers **60/60'** to obtain epimer **61' (**D configuration**)** as a pure isomer, after 4 hr of hydrogenation, while the unreacted epimer **60** was totally recovered. This methodology can be considered the most rapid access to the key α-glycosyl amino acid moiety of polyoxins in high yield.



Scheme 16

6. Ring opening of chiral activated Aziridine

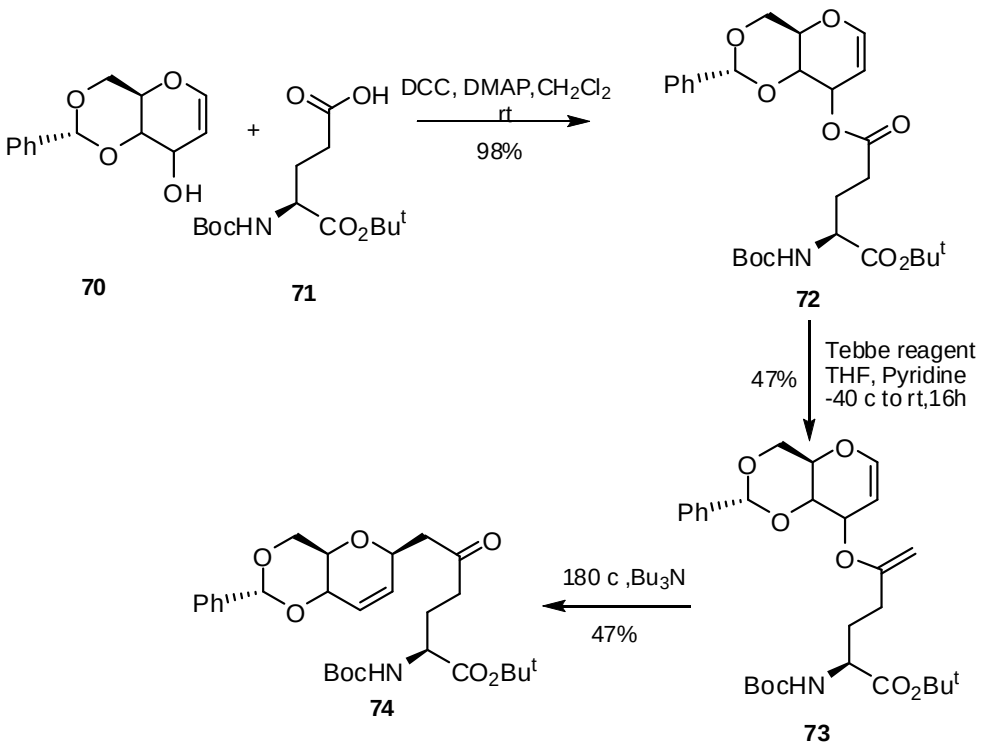
In the same year, Thiem's group[59] synthesized glycosyl amino acid **69** by using the Lewis acid catalyzed ring opening of activated aziridine **67** by alcohol **66** as a key step (Scheme:17). Alcohol **66** was synthesized by reacting peracetylated galactopyranose **62** with propargyl trimethylsilane **63** in the presence of Lewis acid. The α-allene **64** formed in this way was cleaved by treatment with ozone to give the labile aldehyde **65,** which was reduced without isolation to alcohol **66**.



Scheme 17

7. The tandem Tebbe/Claisen rearrangement

In 2004, Fairbanks and coworkers[60] applied the tandem Tebbe/Claisen approach to synthesize *C*-glycosyl amino acid **74**. This approach initially involves esterification of the hydroxy-free glycal **70** with N-Boc glutamic acid monoester **71** to give ester **72**. Tebbe methylenation of this ester gave **73** which underwent [3,3] sigmatropic Claisen rearrangement yielding the *C*-glycosyl amino acid **74** with entirely stereoselective fashion (Scheme:18).

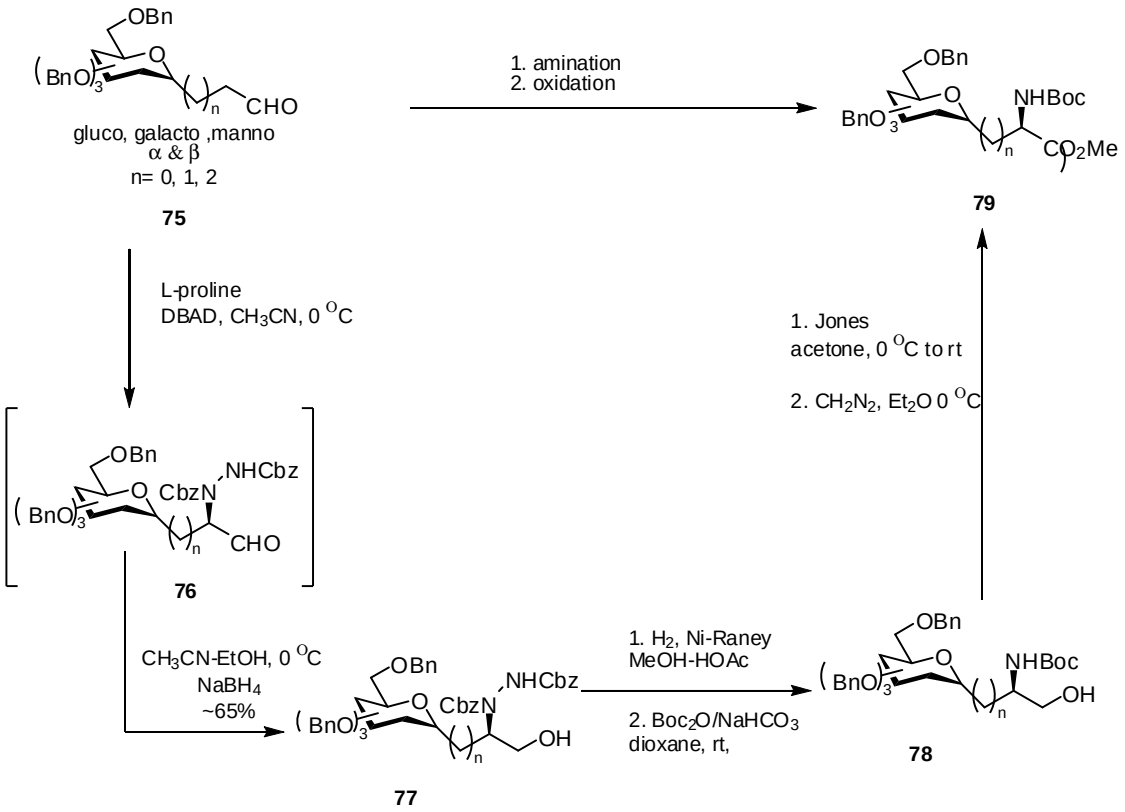


Scheme 18

It is worth mentioning that this approach was limited to the use of protected glutamic acid and did not succeed with other α-amino acids such as aspartic acid. Also the use f 4, 6-O- benzylidene protected glucal was a prerequisite for a successful reaction.

8. Proline–catalyzed direct electrophilic α-amination

In 2008, Dondoni et al.[61] synthesized *C*-glycosyl amino acid **79** via a proline-catalyzed electrophilic α-amination approach. Glycosylmethyl aldehyde **75** was reacted with the electrophilic nitrogen source dibenzyl azodicarboxylate (DBAD) in the presence of 30% of L-proline to afford α-hydrazino aldehyde **76** as single diastereomer. Crude **76** was reduced by sodium borohydride and ethanol to give alcohol **77** in very good yield and high diastereomeric purity. Subsequently, hydrogenation of alcohol **77** over Raney Nickel selectively removed the Cbz protective group and cleaved the N-N bond to give free amine which was treated with Boc_2O to afford the N-Boc- protected amino alcohol **78.** The alcohol **78** was oxidized and esterified to give the target product in high yield (Scheme: 19).

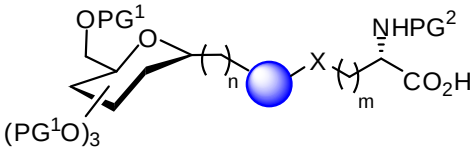


Scheme 19

This methodology has the ability to afford the two diasteroisomers in pure form by using the appropriate proline (L or D).

3- RESEARCH PROJECT: AIM AND METHODS

Broad spectrums of *C*-glycosyl amino acids as potential building blocks for *C*-glycopeptide synthesis have been synthesized.[8] Recently, considerable attention has been given to a class of artificial *C*-glycosyl amino acids whose structure features a hetrocycle ring holding carbohydrate and amino acid fragments (figure 2), isoxazole, and triazole moieties, were synthesized via Huisgen cycloaddition reaction,[11] whereas tetrazole, and pyridine moieties were planned to be synthesized in this project.



PG = orthogonal protective groups
n = 0, 1
m = 0, 1
X = C, O, S

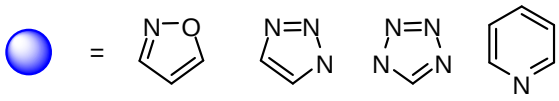
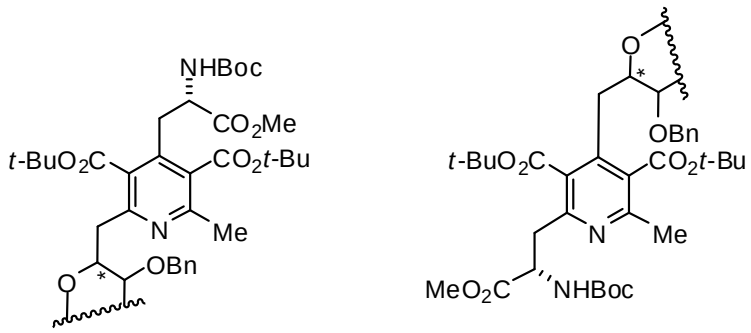


Figure 2

These compounds are of interest as new chelating agents for the binding of radioactive metal ions (Tc, Re). Their complexes can be used in radiodiagnosis, radiotherapy, [12] and radiolabeling[62]. Radiolabeled glycopeptides can be used for angiogenesis imaging. For example, ^{99m}Tc-glycopetide was used to assess efficiency of anti-angiogenic cancer therapy.[62] Moreover, because the carbohydrate unit is bound to the polyamide backbone through an artificial tether resistant to chemical and enzymatic degradation, synthetic glycopeptides can be used as probes in biological studies for a deeper understanding of the effect of glycosylation on protein structure and function in molecular detail.[13] These products can be used in drug-discovery for carbohydrate-based metabolic disorders. We envisaged the heterocycle ring in the constructed glycopeptides to serve as a rigid and robust linker as well as an active site for molecular recognition processes through H-bonding and dipolar interactions. In addition, the efficient construction of the heterocycle-tethered glycosyl amino acid architecture would allow the preparation of a collection of strictly related compounds in which structural and stereochemical diversity can be explored by varying

the nature of the sugar residue as well as its position in the heterocyclic rings. Indeed, the search for structural changes and new biological properties of peptides induced by introduction of non-natural heterocycle amino acid fragments has been actively pursued since several years.[63]

Thus, the first part of the project was the construction of carbohydrate-pyridine -amino acid hybrids. Specifically: the two regioisomers; 2-(*C*-Glycosylmethyl)-4-alaninylpyridines **80** and 4-(*C*-Glycosylmethyl)-2-alaninylpyridines **81** (figure 13).

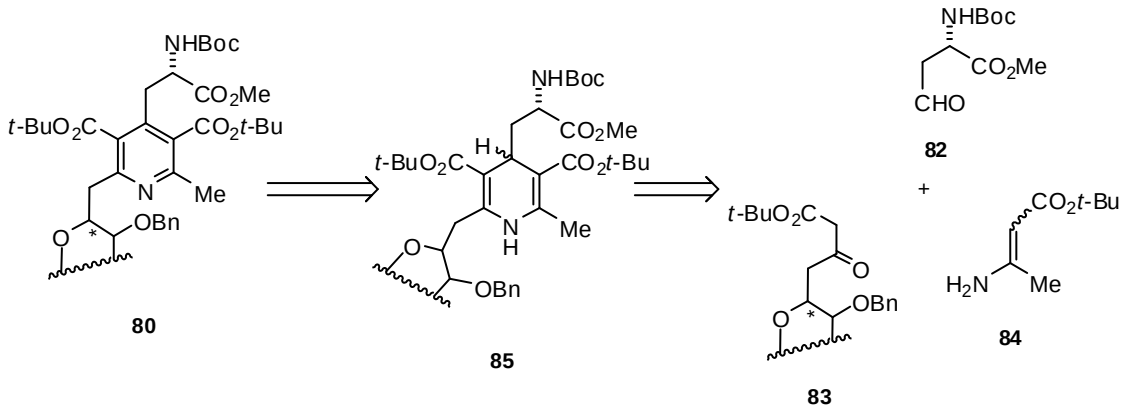


80 **81**

Figure 13

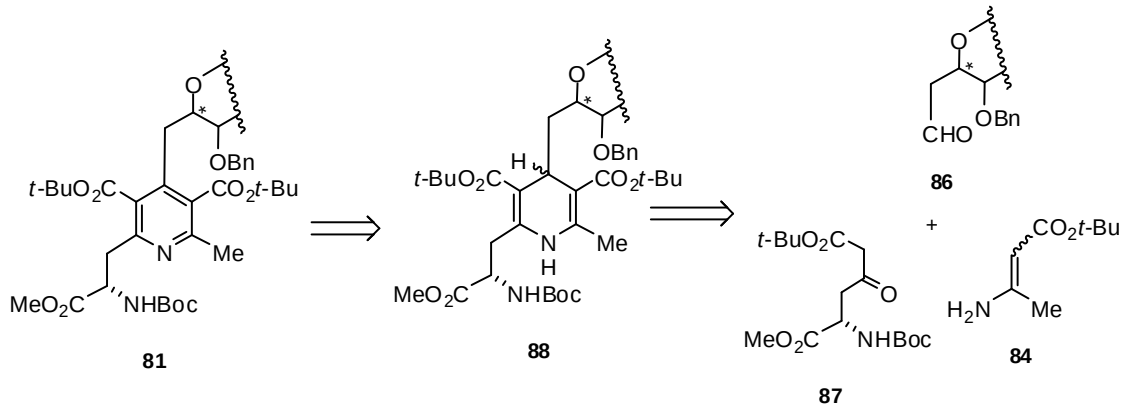
The pyridine ring plays a key role in several biological processes. Most notably is its role in oxidation/reduction processes[64]. The pyridine ring can be constructed via a Hantzsch multicomponent reaction,[65] which involves coupling of an aldehyde-ketoester-enamino ester system in a one-step reaction to generate dihydropyridine, which can be easily oxidized to the pyridine ring. We planned to use the same system in which one of these reagents was planned to carry C-glycosyl residue, while to another was bound an amino acid fragment. With carbohydrate linked to the ketoester and the amino acid as a part of aldehyde, we set the two components of the first regioisomer **80.** Alteration the positions of the carbohydrate and the amino acid was used to set the two components of the second regioisomer **81**, in which the carbohydrate was a part of aldehyde and the amino acid was linked to the ketoester. Since our purpose was to use these building blocks for glycopeptide synthesis, the first issue was to design reagents with functional protective groups that are stable under the reaction conditions and orthogonal in the product. To achieve this purpose, N-Boc and methyl ester were chosen to protect the glycine moiety, benzyl ethers were used to protect the hydroxyls of the carbohydrate, and t-butyl ester

was chosen as electron withdrawing group in both ketoester and enamino ester. Thus, the three reagents for the preparation of the first regioisomer **80** were planned to be aldehyde **82**, ketoester **83**, and enamino ester **84** (scheme21).



Scheme 21: Retrosynthetic pathways

The three reagents for preparation of the second regioisomer **81** were planed to be aldehyde **86**, ketoester **87**, and enamino ester **84** (scheme 22).

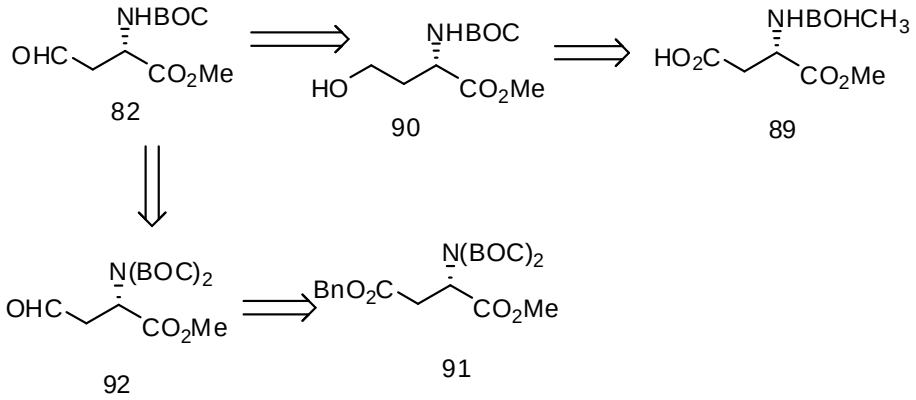


Scheme 22: Retrosynthetic pathways

Cyclocondensation of the three reagents (either (**82, 83,** and **84**) or (**86**,**87,** and **84**)) was done in *t*-BuOH at 70 °C to give, after 24 h, the dihydropyridine (either **85** or **88**) as a mixture of diastereoisomers. This mixture was transformed into the target **(80** or **81**) by oxidation with pyridinium chlorochromate (PCC). Substantial improvement to method efficiency was achieved by executing the cyclocondensation under MW irradiation and then performing product purification and oxidation using polymer supported sequestrants.

MW irradiation at 120 °C reduced the cyclocondensation time from 24 to 1.5 h. Then, the reaction mixture was treated with a mixed-resin bed constituted of three polymer-bound reagents which were chosen as specific scavengers of unreacted material and side products. Specifically: (i) the supported sulfonic acid A-15 removed the residual enamine, (ii) the strong base Ambersep sequestered the ketoester, and (iii) the aminomethylated polystyrene (AM-resin) subtracted the aldehyde. This supported amine also removed the Knoevenagel adduct that was formed as initial condensation product between the aldehyde and the ketoester. After filtration of the resins, the diastereomeric dihydropyridines were oxidized to pyridine with PCC supported on silica gel.

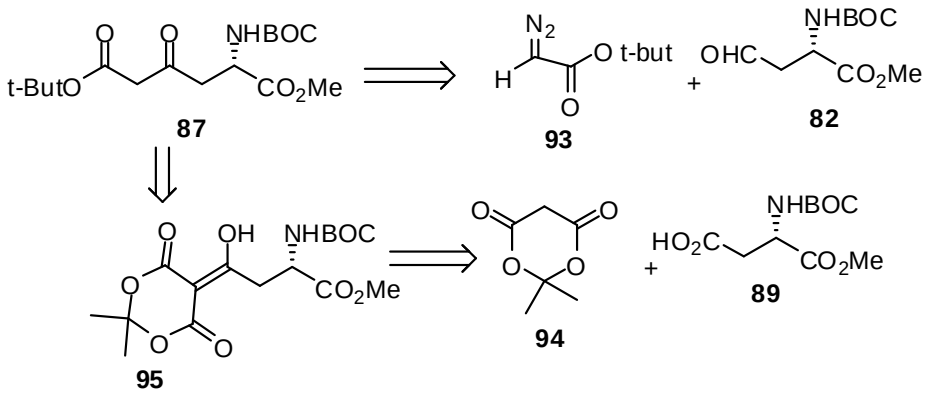
The glycine moiety in the first regioisomer **80** was in the form of aldehyde **82,** which was prepared from the readily available aspartate mono ester **89** in two steps by reduction – oxidation sequence.[66] However, this method afforded the aldehyde in low yield because of the lactonization of the alcohol intermediate **89.**[67] Hence, the preparation was more conveniently carried out in gram quantities, starting from the aspartate diester **91** according to the procedure of Martin and co-workers.[68] (scheme23)



Scheme 23: Retrosynthetic pathways

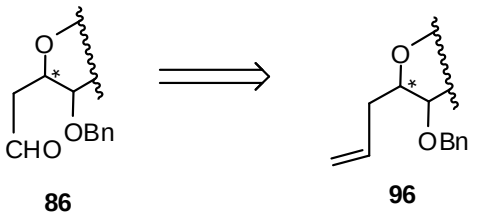
The glycine moiety in the second regioisomer **81** was the ketoester **87** which was first synthesized by reaction of aldehyde **82** with t-butyl diazoacetate **93** in the presence of boron triflorid[69] (scheme 24). The yield was low. Hence, an alternative synthesis of **87** was performed starting from the N-Boc aspartic acid monoester **89** by a procedure similar to that described by Frank and co-workers.[66] This method involves the transformation of

89 into the Meldrum's acid derivative **95** and then treatment of this crude material with refluxing t-BuOH. This method affords **87** in good yield with identical optical value of product obtained by the other route from **82** (scheme 24).



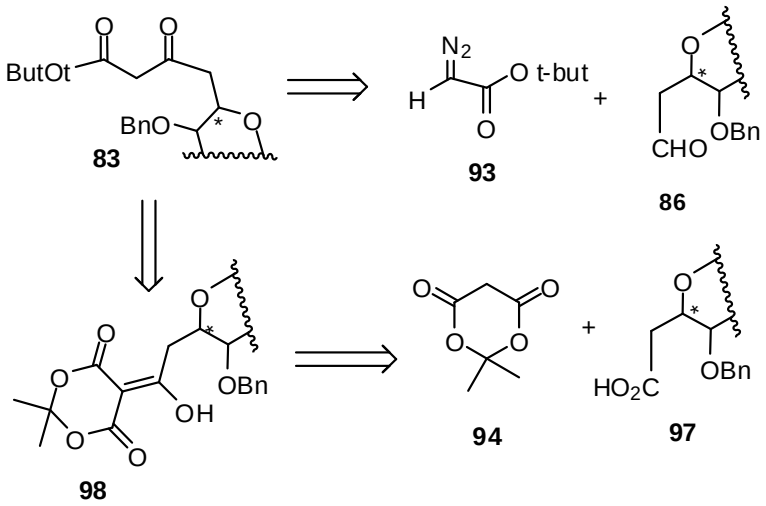
Scheme 24

The C-glycosyl group in the second regioisomer **85** was in the form of aldehyde **86,** which was prepared from double bond oxidative cleavage of the corresponding C-ally glycoside **96** [70] (scheme 25).



Scheme 25

The C-glycosyl moiety in the first regioisomer **80** was in the form of ketoester **83,** which was prepared from reaction of aldehyde **86** with t-butyl diazoacetate **93** in the presence of boron triflorid.[71] However, this method afforded **83** in low yield, and therefore another procedure was followed. This involved the nearly quantitative oxidation of **86** to the carboxylic acid **97** and treatment of the latter with Meldrum's acid **94** in the presence of isopropenyl chloroformate. Then, the crude product **98** was cleaved by treatment with t-BuOH and MW irradiation at 120° C.



Scheme 26

In the second part of the project, we planned to construct carbohydrate-tetrazole-amino acid hybrids. Specifically: C-glycosyl O-tetrazolyl serines **99** and C-glycosyl S-tetrazolyl cysteines **100**. These glycosyl amino acids were planned to be suitable building blocks for glycopeptide synthesis. Hence, N-Boc, methyl ester, and benzyl ether were used as suitable orthogonal protecting groups in case of C-glycosyl O-tetrazolyl serines **99**, while N-Fmoc and O-acetyl were used as suitable orthogonal protecting groups in case of C-glycosyl S-tetrazolyl cysteines **100** (figure 14).

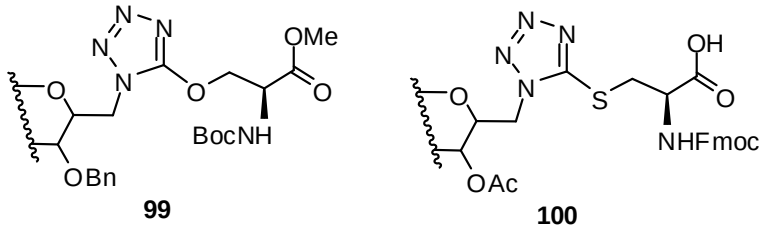
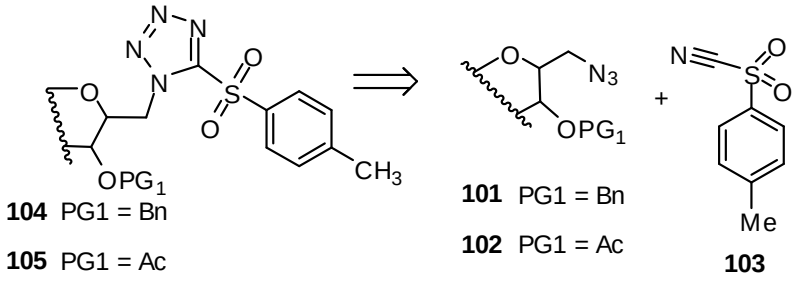


Figure 14

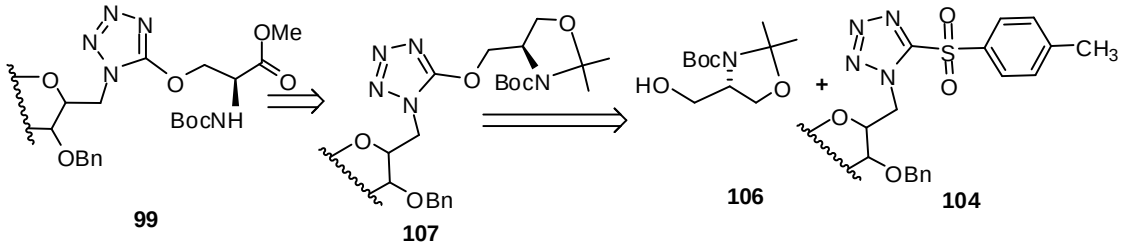
Tetrazole moiety is quite stable under a variety of reaction conditions and can serve as a metabolically stable surrogate for a carboxylic or amide group.[72] The simplest tetrazole synthesis is provided by the Huisgen-type azide-nitrile 1,3-dipolar cycloaddition.[73] The scope and efficiency of this reaction, however, are limited due to the need of using nitriles activated by strong electron-withdrawing groups and harsh reaction conditions. While the former point still remains a major drawback for this transformation, safety concerns have been recently addressed by Vilarrasa and Bosch, who reported on the first tentative metal-catalyzed “click” protocol for the organoazide-nitrile coupling.[74] These

authors found that simple 1,5-disubstitued tetrazole derivatives can be prepared in excellent yields in dichloromethane at ambient temperature in the presence of 1-10 mol% of soluble $Cu_2(OTf)_2 \cdot C_6H_6$ catalyst, while 1,4-disubstituted tetrazoles are obtained as major regioisomers under heterogeneous conditions with 50-100 mol% of the same catalyst. Foregoing work in Sharpless[75] and our own laboratories[76] solved the problem of azide-nitrile ligation of more complex substrates. A two-step reaction sequence has been developed involving the cycloaddition of azides with an activated nitrile such p-toluenesulfonyl cyanide and then substitution of the tosyl group in the tetrazole thus formed by a suitable nucleophile. On applying this strategy to the synthesis of *C*-glycosyl tetrazole amino acids, we reacted the benzylated and acetylated glycosylmethyl azide **101,102** with p-toluenesulfonyl cyanide **103** in the absence of solvent to give the 1,5-disubstituted tetrazole **104,105,** which were used as starting material to produce target glycosyl amino (Scheme 27).



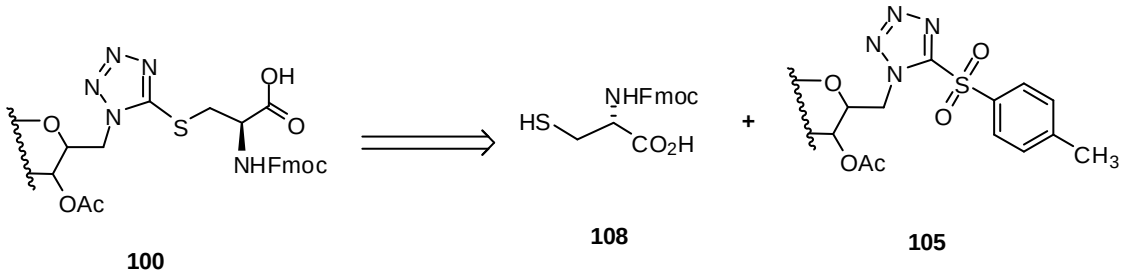
Scheme 27

1,5-disubstituted tetrazole **104** was subjected to the alcohol **106**, a masked serine form, under basic conditions to give the product **107** from which the amino ester **99** was unmasked by *N*-Boc oxazolidine ring cleavage and esterification (scheme 28).



Scheme 28

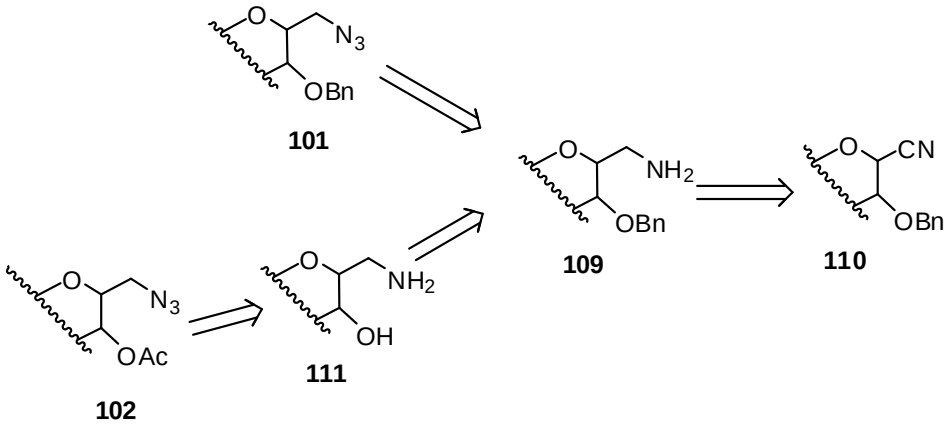
C-glycosyl tetrazolyl cysteines **100** were obtained from the direct replacement of the tosyl group by the sulfur nucleophile of protected cysteine **108** in mild basic condition (scheme 29).



100 **108** **105**

Scheme 29

To the best of our knowledge, no one has used amino acid moieties as nucleophiles to replace the tosyl group of the 1-substituted, 5-sulfonyl tetrazole. Most reactions were done using simple nucleophiles such as methoxide ion, pyrrolidine, and thiophenol.[75] Moreover, the noticeable development was the new method that we followed to synthesize glycosylmethyl azides **101** and **102**. In this method, we used C-glycosylmethyl amine **109** to prepare C-glycosylmethyl azides **101** and **102**. Azide **101** was obtained directly from amine **109** in one step by using imidazole-1-sulfonyl azide hydrochloride as diazotransfer reagent, [77] while azide **102** was obtained using two steps starting from amine **109**, involving debenzylation then azidation and acetylation. Glycosylmethyl amines **109** of both forms α and β were synthesized by reducing the corresponding glycosyl cyanide **110** and these cyanides easily to synthesize in the two anomers α and β by cyanation of glycosyl acetate with trimethylsilyl cyanide (scheme 30). [78]



101 **109** **110** **102** **111**

Scheme 30

4- RESULTS AND DISCUSSION

In this work, we have studied and synthesized new class of *C*-glycosly amino acids whose structure features a hetrocycle ring holding the carbohydrate and the amino acid fragments. As described in chapter three, the two regioisomers; 2-(*C*-Glycosylmethyl)-4-(alaninyl)-pyridines **80** and 4-(*C*-Glycosylmethyl)-2-(alaninyl)-pyridines **81** were synthesized using Hantzsch multicomponent reaction. Cyclocondensation of the three reagents (**(82**, **83**, and **84**) or (**86**, **87**, and **84**)) under MW irradiation, produced dihydropyridines (**85** or **88**).The dihydropyridine was not isolated while its purification was carried out by removal of unreacted material and side products using polymer-supported scavengers. Then the dihydropyridine (mixture of diastereoisomers) was oxidized by a polymer-bound oxidant to give the target pyridine bearing the two bioactive residues. In this way, a range of eight compounds (58-68% yield) was prepared in which the elements of diversity were (i) the gluco and galacto configurations of the pyranose ring, (ii) the α- and β-configurations at the anomeric center, and (iii) the positions of the carbohydrate and amino acid sectors in the pyridine ring. The orthogonal functional group protection in these amino acids allowed their easy incorporation into oligopeptides via sequential amino and carboxylic group coupling.

In the second part, tetrazole moiety was constructed via Huisgen 1,3-dipolar cycloaddition between nitriles and organic azides. Two sets of compounds have been prepared, one being constituted of *C*-galactosyl and *C*-ribosyl *O*-tetrazolyl serines while the other contains *S*-tetrazolyl cysteine derivatives. In both cases, the synthetic scheme involved a two-step route, the first one being the thermal cycloaddition of a sugar azide with *p*-toluensulfonyl cyanide (TsCN) to give a 1-substituted 5-sulfonyl tetrazole and the second the replacement of the tosyl group with a serine or cysteine residue. For the high efficiency and operational simplicity, the azide-TsCN cycloaddition appears to be a true click process. Finally, one of the amino acids prepared was incorporated into a tripeptide.

More details on results and discussion could be found in the attached papers.

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JOC Article

Hantzsch-Type Three-Component Approach to a New Family of Carbon-Linked Glycosyl Amino Acids. Synthesis of *C*-Glycosylmethyl Pyridylalanines†,‡

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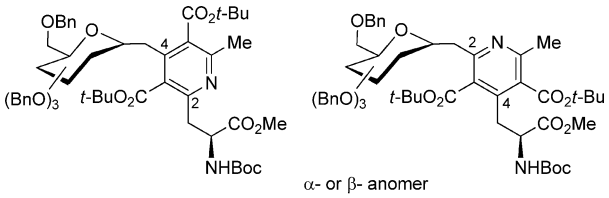

α- or β- anomer

gluco-galacto series

C-Glycosylmethyl pyridylalanines reported in this paper constitute a novel family of glycosyl amino acids that contain a pyridine ring linking the carbohydrate and amino acid residues. These amino acids may serve to prepare nonnatural glycopeptides displaying firmly bound carbohydrate fragments through a rigid and highly stable tether. A viable route to these new hybrid molecules has been opened via thermally induced Hantzsch-type cyclocondensation using an aldehyde−ketoester−enamino ester system. To one of these reagents was attached a *C*-glycosyl residue, while to another was bound an amino acid fragment. In a one-pot optimized methodology, the dihydropyridine was not isolated while its purification was carried out by removal of unreacted material and side products using polymer-supported scavengers. Then the dihydropyridine (mixture of diastereoisomers) was oxidized by a polymer-bound oxidant to give the target pyridine bearing the two bioactive residues. In this way a range of eight compounds (58−68% yield) was prepared in which the elements of diversity were (i) the gluco and galacto configurations of the pyranose ring, (ii) the α- and *β*-configurations at the anomeric center, and (iii) the positions of the carbohydrate and amino acid sectors in the pyridine ring. The orthogonal functional group protection in these amino acids allowed their easy incorporation into oligopeptides via sequential amino and carboxylic group coupling.

Introduction

Because of their potential use as probes in glycobiology and leads in carbohydrate-based drug discovery, synthetic glycopeptides in which the native *O*- or *N*-glycosidic linkages have been replaced by *C*- or *S*-linkages have garnered in recent years an increasing attention by researchers in industry and academia.[1] This modification is considered to provide substantial resistance to chemical and enzymatic deglycosylation while retaining the original biological properties of the peptide. Hence, several methods for the synthesis of *C*-glycosyl[2,3] and *S*-glycosyl[1i] amino acids have been reported in view of their use in co-translational modification of natural glycopeptides. Moreover, in order to introduce in peptides a more substantial modification which may

† This work is part of the lecture presented by A.D. in the *Symposium on Asymmetric Synthesis of Amino Acids* at the 233rd ACS National Meeting, Chicago, March 25−29, 2007.

‡ The paper is dedicated to the memory of the late Professor Yoshihiko Ito, Kyoto University, a good friend to A.D. and a respected colleague.

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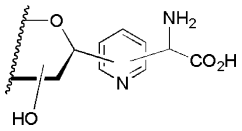


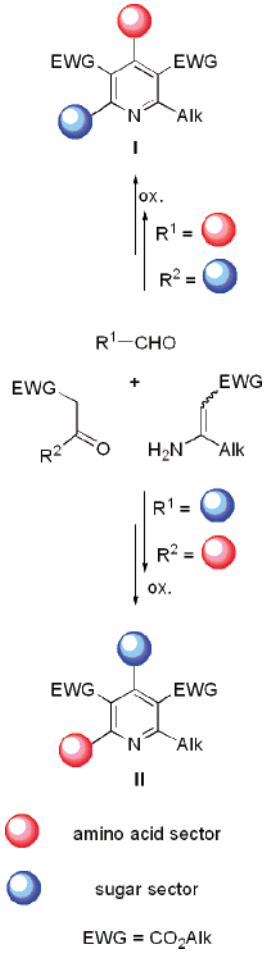
FIGURE 1. Basic structure of designed pyridine-tethered *C*-glycosyl amino acids.

induce changes in physicochemical and biological properties, *C*-glycosyl amino acids with rigid linkers such the acetylene group[4] and phenyl ring[5] have been prepared. In this context, we have recently reported[6] on the first synthesis of *C*-glycosyl amino acids with isoxazole and triazole bridges via alkyne−nitrile oxide and azide−alkyne cycloaddition (Huisgen-type reactions),[7] respectively. The latter reaction was carried out both thermally and under Cu(I) catalysis according to the recent discovery of Sharpless and Meldal and their co-workers.[8] A similar work was reported almost at the same time by another group.[9] We report here the synthesis of a novel family of heterocycle-linked *C*-glycosyl amino acids in which the flat and rigid pyridine ring holds the two chiral bioactive entities (Figure 1). The presence of nitrogen heterocycles bearing carbohydrate residues in *C*-glycopeptides may be quite beneficial since these heteroaromatic bridges can participate in hydrogen-bonding and dipole interactions, which can favor the binding to biomolecular targets and improve solubility as well as membrane transport properties.[10] Only one natural *C*-glycosyl amino acid, identified as an α-*C*-mannopyranosyltryptophan,[11] has been found so far in biologically important glycoproteins. Therefore, quite interestingly, this compound features the rigid heteroaromatic indole ring holding the sugar and amino acid moieties. Very recently, the *β*-isomer and analogues of this natural product have been synthesized by the Nishikawa and Isobe group.[12]

Results and Discussion

Multicomponent one-pot reactions[13] have emerged in recent years as a useful tool to maximize synthetic efficiency,[14] an aspect of increasing relevance in modern chemistry toward the ideal synthesis.[15] Hence, we decided to approach the construction of carbohydrate−heterocycle−amino acid hybrids depicted in Figure 1 by the three-component version of the pyridine

SCHEME 1. Synthesis Plan via Hantzsch Reaction for Pyridine-Tethered *C*-Glycosyl Amino Acid Regioisomers



Hantzsch synthesis[16] comprising the aldehyde−ketoester−enamino ester system (Scheme 1). To one of these reagents is bound the *C*-glycosyl residue and to another the amino acid. With the carbohydrate linked to the ketoester and the amino acid as a part of the aldehyde, the dihydropyridine formed from the cyclocondensation and the pyridine **I** obtained afterward will bear the amino acid at C-4 and the carbohydrate at C-2 of the ring. The regioisomer **II** will be instead obtained using a *C*-glycosyl aldehyde and a ketoester with incorporated the glycinyl group. We planned to use in both routes the same enamino ester with a methyl group adjacent to the amino group in order to keep as low as possible the steric encumbrance in this reagent. Evidently, this methodology will lead to a densely substituted pyridine ring. However, we considered the presence of the two ester groups as an advantage because these func-

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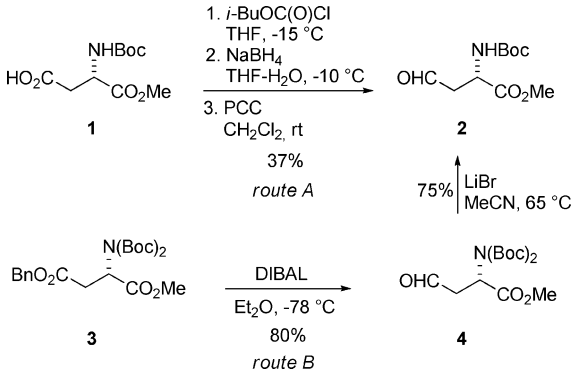
tionalities can be varied in library preparations or serve as reactive points for the introduction of the glycosyl amino acid in molecular frameworks other than peptides. We were spurred to adopt the Hantzsch multicomponent reaction in this project as a logical evolution of our recent research on the synthesis of dihydropyridine *C*-glycoconjugates and piridylalanines.[14b,17] The information collected in the earlier work constituted a precious guide in the choice of suitable reagents and conditions to perform the construction of the pyridine ring bearing both sugar and amino acid residues.

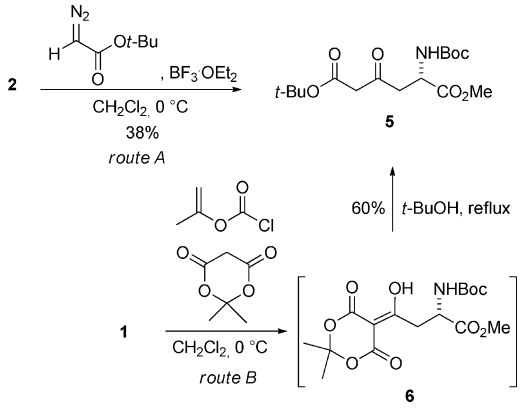
A. Design and Synthesis of Reagents for the Three-Component Hantzsch Reaction (3CHR). The first issue to be addressed in this project was to design reagents with functional protective groups which are stable under the reaction conditions and orthogonal in the product. The latter condition was crucial for the elaboration of the product via selective reactions at each functional group at will. In particular, the protection of the NH_2 of the glycinyl group should preserve the configurational integrity of the α-stereocenter and permit the introduction of the product into peptides via sequential carboxylic and amino group coupling reactions. Toward this goal, the *N*-Boc and methyl ester protection of the glycinyl moiety was previously demonstrated to be a quite convenient combination.[17c,e] Moreover, the *tert*-butyl group in the ketoester and enamino ester appeared to be a convenient choice as well because it differentiated the carboxylic groups of the ring from that of the amino acid and avoided the lactam formation via intramolecular reaction with the free amino acid group.[17c] Finally, the *O*-benzyl protective group in the carbohydrate moiety was demonstrated in several instances to be orthogonal with the *N*-Boc methylglycinate[1g,2,6] and proved to be readily removed in the presence of the pyridine ring by Pd-catalyzed hydrogenolysis.[17e]

Following the above reasoning, we first examined the synthesis of aldehyde and ketoester starting reagents bearing the *N*-Boc methyl glycinate group. Because of the easy elimination of this group from the dihydropyridine ring in the oxidation step to pyridine,[17c] we decided to prepare reagents holding the glycinate residue through a methylene spacer. Hence, the *N*-Boc-*O*-methyl aspartate semialdehyde **2** was prepared in two steps by a reduction−oxidation sequence from the readily available aspartate monoester **1**[18] (Scheme 2). However, this method afforded the aldehyde in low yield because of the lactonization of the alcohol intermediate.[19] Hence, the preparation of **2** was more conveniently carried out in gram quantities, starting from the aspartate diester **3**[20] according to the procedure of Martin and co-workers.[21] However, it is worth noting that the optical rotation value of **2** ($[\alpha]_D = 34.5$) obtained by both routes was more than double that previously registered by these authors (lit.[21b] $[\alpha]_D = 16.4$).[22]

SCHEME 2. Synthesis of *N*-Boc-*O*-methyl Aspartate Semialdehyde 2



SCHEME 3. Synthesis β-Keto Ester 5



With the aldehyde **2** available in gram scale, the synthesis of the β-ketoester **5** was first carried by reaction of **2** with *tert*-butyl diazoacetate and $BF_3{\cdot}Et_2O$ (Scheme 3) according to the procedure already employed in our laboratory.[17c,e] In contrast to previous results, the yield of isolated **5** was only 38%. Hence, an alternative synthesis of **5** was performed starting from the *N*-Boc aspartic acid monoester **1**[18] by a procedure similar to that described by Frank and co-workers.[23] This method involves the transformation of **1** into the Meldrum's acid derivative **6** and then treatment of this crude material with refluxing *t*-BuOH. The overall yield of **5** was 60%, and its optical rotation value ($[\alpha]_D = 25.4$) was identical to that of the product obtained by the other route from **2**.

For reasons given below in section B, aldehydes and ketoesters with a directly linked *C*-glycosyl group appeared to be unsuitable reagents in the planned 3CHR. On the other hand, *C*-glycosylmethyl derivatives turned out to be the reagents of choice. Fortunately enough, the *C*-glucosyl (Glc) acetaldehydes α-**7a**[24] and β-**7a**[24,25] as well as the *C*-galactosyl (Gal) derivatives

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(22) In a control experiment, the aldehyde **2** was reduced to alcohol ($NaBH_4$, MeOH, 0 °C) which spontaneously cyclized (CH_2Cl_2, rt) to give in quantitative overall yield the known (*S*)-*tert*-butyl 2-oxotetrahydrofuran-3-ylcarbamate whose optical rotation value $[\alpha]_D = -29.2$ was identical to that reported in the literature ($[\alpha]_D = -29.5$).[19]

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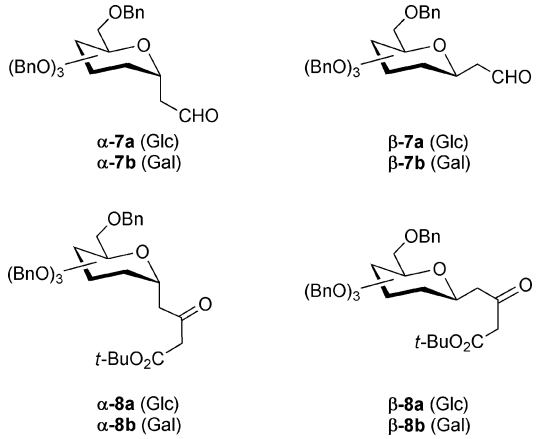
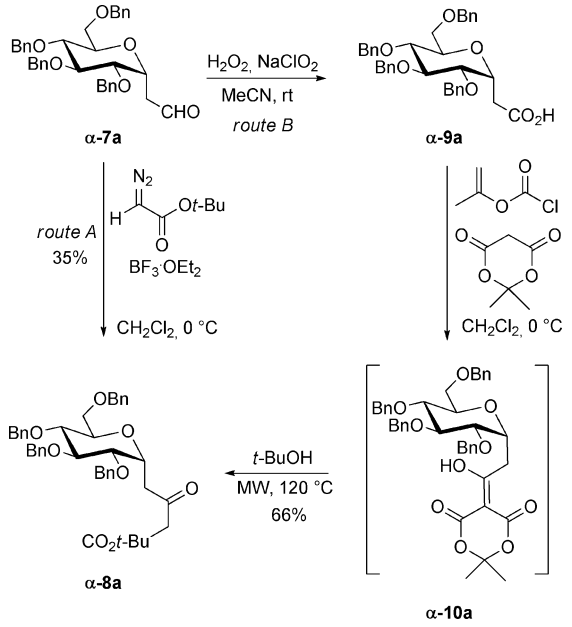


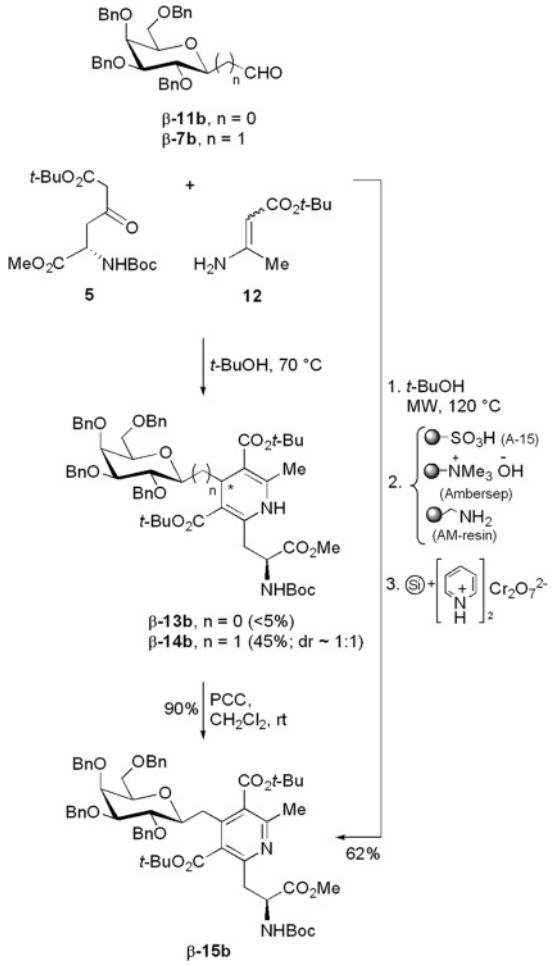
FIGURE 2. *C*-Glucosyl (Glc) and *C*-galactosyl (Gal) acetaldehydes **7** and ketoesters **8**.

SCHEME 4. Synthesis of Sugar *β*-Keto Esters α-8a



α-**7b**[26] and *β*-**7b**[26] (Figure 2) were readily available by double-bond oxidative cleavage of the corresponding *C*-allyl glycosides.[24b,26,27] Then, the four sugar aldehydes **7** were used for the preparation of the ketoesters **8** (Figure 2). As an example, the synthesis of one of these compounds is illustrated in Scheme 4. In one approach, the aldehyde α-**7a** was transformed into the ketoester α-**8a** by reaction with *tert*-butyl diazoacetate as described for other sugar ketoesters.[28] However, this method afforded α-**8a** in low yield (35%), and therefore, another procedure was followed. This involved the nearly quantitative oxidation of α-**7a** to the carboxylic acid α-**9a** and treatment of the latter with Meldrum's acid in the presence of isopropenyl chloroformate. Then the crude product α-**10a** was cleaved by treatment with *t*-BuOH and MW irradiation at 120 °C. The crude ester α-**8a** obtained in this way was purified and isolated in fairly good overall yield (66%). All of the sugar ketoesters shown in Figure 2 were prepared in good yields by this two-step route.

SCHEME 5. Optimization of Reaction Conditions for the Synthesis of 4-(*C*-Galactosylmethyl)-2-(alaninyl)pyridine *β*-15b



B. Synthesis of 4-(*C*-Glycosylmethyl)-2-alaninylpyridines 15. In recent disclosures from this laboratory, we reported that *C*-glycosyl formaldehydes readily available via thiazole-mediated chemistry[29] take part in 3CHR to give the corresponding *C*-glycosyl dihydropyridines.[17a,b] Hence we considered a model reaction constituted of the *C*-galactosyl formaldehyde *β*-**11b**,[29] the *β*-ketoester **5**, and *tert*-butyl aminocrotonate **12** (Scheme 5). Under the usual standard conditions used in our previous work of Hantzsch reactions (*t*-BuOH, 70 °C, 24 h) and even under MW irradiation at 120 °C for 4 h, only traces of the desired dihydropyridines *β*-**13b** were detected by MALDI-TOF analysis of the crude reaction mixture. Instead, various side products were isolated including the glycal arising from debenzylation of the aldehyde *β*-**11b** and carboxylic acids derived from the hydrolysis of the ester groups of **5** and **12**. Therefore, we considered the use of the *C*-galactosyl acetaldehyde *β*-**7b** with the hope that the presence of a methylene spacer between the formyl group and the carbohydrate residue would reduce the steric congestion of the system and the cyclocondensation could take place. The change turned out to be quite

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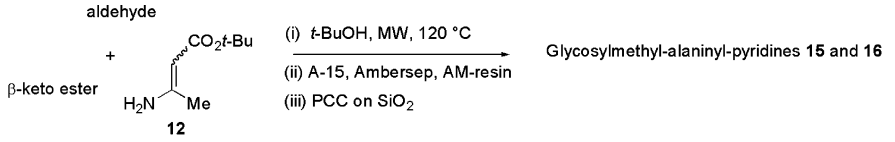
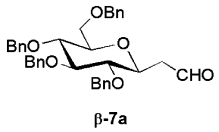
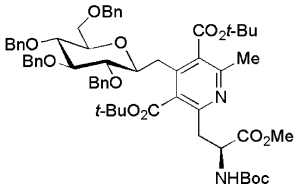
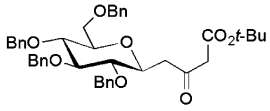
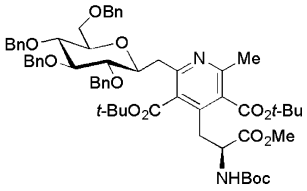
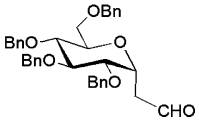
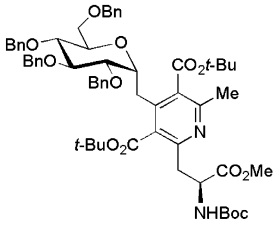
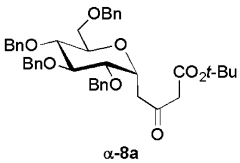
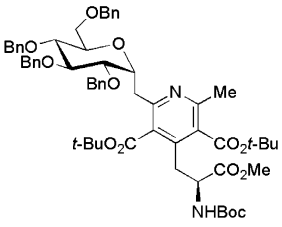
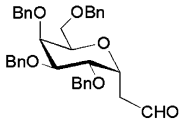
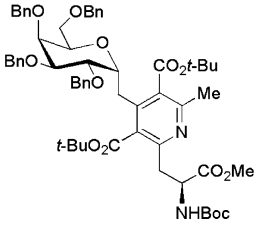
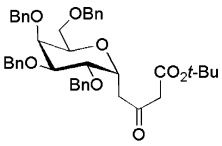
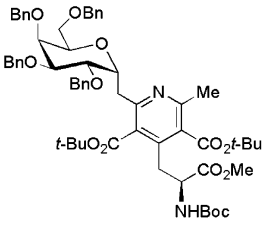
(29) (a) Dondoni, A.; Marra, A. *Chem. Rev.* **2004**, *104*, 2557−2599. (b) Dondoni, A. *Pure Appl. Chem.* **2000**, *72*, 1577−1588. (c) Dondoni, A.; Scherrmann, M.-C. *J. Org. Chem.* **1994**, *59*, 6404−6412.

TABLE 1. Glycosylmethylalaninylpyridines Prepared[a]

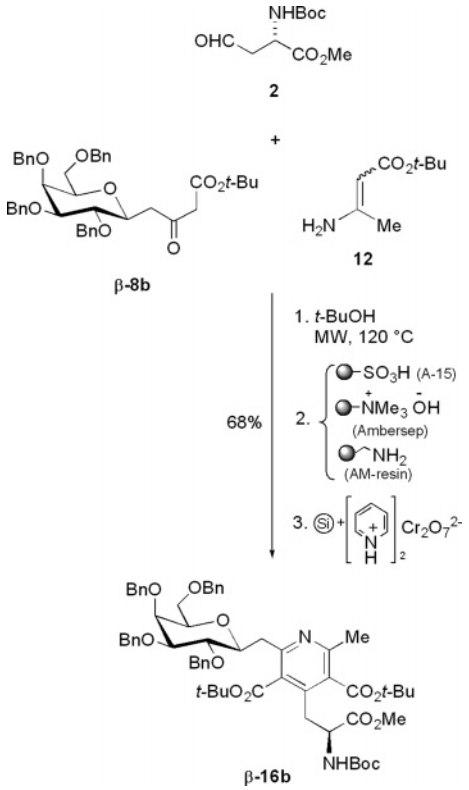


aldehyde and ketoester	product[b,c]	ketoester and aldehyde	product[c,d]
 β-**7a** and **5**	 β-**15a** (61%)	 β-**8a** and **2**	 β-**16a** (62%)
β-**7b** and **5**	β-**15b** (62%) (see Scheme 5)	β-**8b** and **2**	β-**16b** (68%) (see Scheme 6)
 α-**7a** and **5**	 α-**15a** (55%)	 α-**8a** and **2**	 α-**16a** (58%)
 α-**7b** and **5**	 α-**15b** (58%)	 α-**8b** and **2**	 α-**16b** (62%)

[a] All Hantzsch cyclocondensations were run with 0.50 mmol of each component (see the Experimental Section). [b] Time of microwave irradiation was 1.5 h. [c] Isolated yield by chromatography. [d] Time of microwave irradiation was 2.5 h.

beneficial because this new substrate combination upon heating at 70 °C in *t*-BuOH for 24 h afforded the Hantzsch dihydropyridine *β*-**14b** in fair yield (45%) as a mixture of diastereoisomers in 1:1 ratio. The lack of asymmetric induction by the chiral sugar fragment is very likely due to its distance from the newly formed stereocenter and the harsh reaction conditions employed. The mixture of diastereoisomers was oxidized with PCC to give the pyridine *β*-**15b** as a single product (40% overall yield), thus confirming, as already observed in our earlier work,[17e] the conservation of the configurational integrity of the amino acid stereocenter during the Hantzsch cyclocondensation. In order to achieving higher efficiency and establish the conditions for an automatable process, the above reaction sequence was performed in one-pot using polymer-bound reagents. Hence, the cyclocondensation of the aldehyde *β*-**7b**, ketoester **5**, and aminocrotonate **12** in a 1:1:1 ratio was carried out by MW irradiation at 120 °C, which reduced the reaction time from 24 to 1.5 h. Then, the reaction mixture was treated with a mixed-resin bed constituted of three polymer-bound reagents, each one acting as specific scavenger of unreacted material and side products. Specifically, the supported sulfonic acid A-15 removed the residual enamine **12**, the strong hydroxylic base Ambersep sequestered the ketoester **5**, and the aminomethylated polystyrene (AM-resin) subtracted the aldehyde *β*-**7b**. This supported amine removed also the Knoevenagel adduct formed as initial condensation product between the aldehyde and the ketoester.[17e] After filtration of the resins, the diastereomeric dihydropyridines were oxidized to pyridine with PCC supported on silica gel. Also this operation was quite convenient because the noxious chromium salts anchored to the resin were easily removed by filtration. A final column chromatography of the crude product afforded pure *C*-galactosylmethyl-pyridylalanine methyl ester *β*-**15b** in 62% yield. This compound was identical in all respect (NMR, $[\alpha]_D$) to that obtained via the two-step procedure illustrated above and involving the isolation of the dihydropyridine *β*-**14b**. Accord-

SCHEME 6. Synthesis of 2-(*C*-Galactosylmethyl)-4-(alaninyl)pyridine *β*-16b



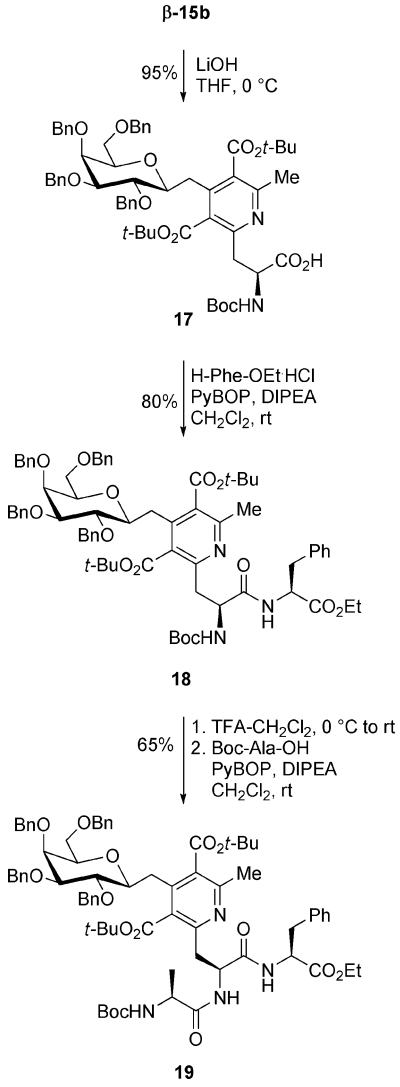
ingly, treatment of crude *β*-**14b** with the set of resins employed in the direct route afforded again authentic *β*-**15b**. This observation opens the route to an automatable and operatively simple synthesis with total preservation of stereocenter integrity and functional group protection.

Three more sugar amino acids with the same substitution pattern in the pyridine ring as in *β*-**15b**, i.e. the sugar sector linked to C-4 and the amino acid fragment linked to C-2, were prepared using the *C*-glycosyl acetaldehydes *β*-**7a**, α-**7a**, and α-**7b** with carbohydrate configuration corresponding to *β*-Glc, α-Glc, and α-Gal respectively (Table 1). The reactions were carried out by MW irradiation and processed by the use of polymer-bound reagents as described above. The three further amino esters *β*-**15a**, α-**15a**, α-**15b** thus prepared were isolated in comparable yields (55−61%) and stereochemically pure form as judged by NMR analysis.

C. Synthesis of 2-(*C*-Glycosylmethyl)-4-alaninylpyridines 16. The approach to this class of compounds started by examining a three-component model reaction (Scheme 6) constituted of the methyl *C*-galactosylacetoacetate *β*-**8b**, the *N*-Boc-*O*-methyl aspartate semialdehyde **2**, and the already exploited valuable aminocrotonate **12**.[30] In this case, the two-step procedure involving the isolation of the dihydropyridine was not explored. Instead, the same reaction conditions and workup operations of the one-pot process illustrated in Scheme 5 were used. In this way, the sugar−pyridine−amino acid *β*-**16b** was isolated in more than 90% purity. This product was mainly

(30) A parallel experiment involving the microwave-assisted 3CHR of **2**, **12**, and *tert*-butyl 3-oxo-3-(2′,3′,4′,6′-tetra-*O*-benzyl-*β*-D-galactopyranosyl)propanoate, i.e. the lower homologous of *β*-**8b**, resulted in no isolation of the corresponding dihydropyridines due to the steric congestion of the system, as previously observed in the preparation of regioisomers *β*-**13b** (Scheme 5).

SCHEME 7. Synthesis of Tripetide 19



contaminated by the residual sugar ketoester *β*-**8b** because the basic resin Ambersep turned out to be a scarcely efficient scavenger toward this bulky compound. However, filtration of the crude mixture through a short column of silica (cyclohexane−AcOEt) afforded the analytically pure amino ester *β*-**16b** in 68% isolated yield. This product resulted to be stereochemically pure as judged by NMR analysis. By changing the configuration of the carbohydrate moiety in the ketoester, the stereoisomers *β*-**16a**, α-**16a**, and α-**16b** (Table 1) were prepared in comparable fair yields (52−68%).

D. Synthesis of Pyridine-Tethered Glycopeptides. In order to demonstrate the potential of the *C*-glycosylmethyl amino esters **15** and **16** as orthogonally protected building blocks for the co-translational modification of glycopeptides, the product *β*-**15b** was selected as a prototype in this crucial validating test (Scheme 7). Toward this goal, we first carried out the selective hydrolysis of the ester functionality of the glycinyl group. The use of very mild conditions (LiOH in THF at 0 °C) afforded the *N*-Boc alanine **17** in excellent yield (95%). Then, the condensation of this product with H-Phe-OEt under activation of the condensation agent (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and the presence of the Hünig base diisopropylethylamine (DIPEA), afforded the dipeptide **18** in fair yield. The *N*-Boc group in this compound

was removed under slightly acid conditions (diluted TFA), thus liberating the NH_2 group that was used in the condensation with Boc-Ala-OH under the same coupling conditions illustrated above. The tripeptide **19** featuring a pendant galactosylmethyl residue linked through a rigid pyridine ring to the peptide backbone was isolated in 65% yield.

Conclusions

After about one and half century after its discovery,[31] the Hantzsch pyridine synthesis continues to manifest its prolific nature and potential as a tool in synthetic organic methodology. In the present work, we have further validated these exceptional prerogatives by assembly in one-pot procedure, without added catalysts and under the simple cooperation of a clean physical energy, such as MW irradiation, three densely functionalized substrates, such as a carbohydrate, a pyridine, and an amino acid. This simple yet efficient chemistry was performed with the assistance of an orchestrated sequence of polymer supported reagents. In this way, only *one* chromatographic purification of the final product was required. The configurational integrity of the chiral reagents was preserved throughout the whole synthetic procedure. In this way a collection of eight news optically pure *C*-glycosylmethyl pyridine amino acids (*C*-glycosyl pyridylalanines) was prepared. It is noteworthy that all products have the sugar residue linked to the pyridine ring through an all carbon tether, thus providing high stability toward glycosidases. Moreover, the orthogonal protection of the various functional groups allows the use of these amino acids in glycopeptide co-translational modification. The way has been now paved for the preparation of a larger library of these amino acids by the change of the carbohydrate and amino acid residues in the reagents employed in the Hantzsch cyclocondensation. This work has been carried out with a view to biological and pharmaceutical applications. In this context, focus on the heterocycle-based ligation strategy of carbohydrate fragments to a peptide backbone via Cu(I)-catalyzed azide−alkyne cycloaddition has been recently brought by Danishefsky and co-workers in their continuous efforts to develop carbohydrate-based anticancer vaccines.[32]

Experimental Section

Aspartates **1**[18] and **3**[20] and aldehydes **2**,[21b] **4**,[21a] α-**7a**,[24] β-**7a**,[24,25] α-**8a**,[26] and β-**8a**[26] are known compounds. Spectroscopic data of intermediate acids β-**9a** and β-**9b** were identical to those reported.[33,25] PCC immobilized on silica gel was prepared according to the procedure described by Eynde and co-workers.[34]

(2*S*)-Methyl 2-(*tert*-Butoxycarbonylamino)-4-oxobutanoate (2). Route A. To a cooled (−15 °C), stirred solution of Boc-L-Asp-OMe **1**[18] (1.00 g, 4.05 mmol) in anhydrous THF (2 mL) were added 4-methylmorpholine (0.45 mL, 4.05 mmol) and isobutyl chloroformate (0.53 mL, 4.05 mmol). The suspension was stirred at −15 °C for an additional 10 min, and then salts were filtered off and washed thoroughly with cold THF (2 × 15 mL). The combined filtrates were cooled to −10 °C, and then a solution of $NaBH_4$ (230 mg, 6.08 mmol) in H_2O (1.5 mL) was added in one portion. The solution was stirred for an additional 10 min and then diluted with AcOEt (75 mL) and H_2O (50 mL). The separated organic layer was washed with 10% citric acid (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL) and then dried ($NaSO_4$) and concentrated. A mixture of the resulting crude alcohol, activated 4-Å powdered molecular sieves (1.00 g), and anhydrous CH_2Cl_2 (40 mL) was stirred at room temperature for 15 min, and then pyridinium chlorocromate (1.64 g, 7.40 mmol) was added in one portion. The suspension was stirred for 45 min, and then cyclohexane (40 mL) and Et_2O (80 mL) were added. The mixture was stirred for an additional 30 min, filtered through a pad of silica gel, and concentrated. The residue was eluted from a column of silica gel with 1.5:1 cyclohexane−Et_2O to afford **2**[21b] (347 mg, 37%) as a yellow oil: $[\alpha]_D = 34.0$ (*c* 2.1, $CHCl_3$) [lit.[21b] $[\alpha]_D = 16.4$ (*c* 5, $CHCl_3$)].

Route B. To a cooled (−78 °C), stirred solution benzyl ester **3**[20] (1.23 g, 2.81 mmol) in anhydrous Et_2O (30 mL) was added dropwise DIBAL (3.2 mL, 3.20 mmol of a 1.0 M solution in hexane). The mixture was stirred at −78 °C for 15 min and then quenched with H_2O (0.5 mL). The suspension was warmed to room temperature, stirred for an additional 30 min, dried (Na_2SO_4), filtered through a pad of Celite, and then concentrated. The residue was eluted from a column of silica gel with 1.5:1 cyclohexane−AcOEt to afford **4**[21a] (745 mg, 80%) as an oil: $[\alpha]_D = -55.4$ (*c* 2.3, $CHCl_3$) [lit.[21a] $[\alpha]_D = -54.9$ (*c* 2, $CHCl_3$)]. A mixture of the aldehyde **4**[21a] (700 mg, 2.11 mmol), lithium bromide (550 mg, 6.34 mmol), and CH_3CN (20 mL) was warmed to 65 °C, stirred at that temperature for 5 h, and then cooled to room temperature and concentrated. The residue was suspended in AcOEt (50 mL) and washed with H_2O (3 × 5 mL). The organic phase was dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with 1.5:1 cyclohexane−Et_2O to afford **2**[21b] (366 mg, 75%) as a yellow oil: $[\alpha]_D = 34.5$ (*c* 3.0, $CHCl_3$).

(2*S*)-6-*tert*-Butyl 1-methyl 2-(*tert*-butoxycarbonylamino)-4-oxohexanedioate (5). Route A. A mixture of aldehyde **2** (462 mg, 2.00 mmol), *tert*-butyl diazoacetate (0.33 mL, 2.40 mmol), activated 4-Å powdered molecular sieves (300 mg), and anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature for 15 min and then cooled to 0 °C. To the mixture a solution of $BF_3{\cdot}Et_2O$ (127 μL, 1.00 mmol) in anhydrous CH_2Cl_2 (1 mL) was added drop by drop, controlling the N_2 evolution at a low steady rate. The mixture was stirred at 0 °C for an additional 30 min, diluted with 10% $NaHCO_3$ (10 mL), warmed to room temperature, filtered through a pad of Celite, and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane−Et_2O to give **5** (262 mg, 38%) as a ∼10:1 mixture of ketone and enol isomers: $[\alpha]_D = 25.3$ (*c* 0.9, $CHCl_3$); ^1H NMR (DMSO-d_6, ketone isomer) $\delta = 7.22$ (d, 1 H, $J_{2,NH} = 8.0$ Hz, NH), 4.35 (ddd, 1 H, $J_{2,3a} = 5.5$ Hz, $J_{2,3b} = 8.5$ Hz, H-2), 3.62 (s, 3 H, OCH_3), 3.53 and 3.46 (2d, 2 H, $J_{5a,5b} = 14.0$ Hz, 2 H-5), 3.00 (dd, 1 H, $J_{3a,3b} = 16.5$ Hz, H-3a), 2.85 (dd, 1 H, H-3b), 1.38 (s, 9 H, *t*-Bu); MALDI-TOF MS 368.4 ($M^+ +$ Na), 384.2 ($M^+ +$ K). Anal. Calcd for $C_{16}H_{27}NO_7$ (345.39): C, 55.64; H, 7.88; N, 4.06. Found: C, 55.68; H, 7.80; N, 4.10.

Route B. A mixture of Boc-L-Asp-OMe **1**[18] (1.00 g, 4.05 mmol), Meldrum's acid (648 mg, 4.50 mmol), DMAP (1.10 g, 8.98 mmol), and anhydrous CH_2Cl_2 (10 mL) was cooled to −5 °C, and then a solution of isopropenyl chloroformate (0.51 mL, 4.66 mmol) in anhydrous CH_2Cl_2 (5 mL) was added dropwise under vigorous magnetic stirring. The mixture was stirred at −5 °C until complete disappearance of the starting material was detected (TLC analysis, ∼1 h). Then a 10% aqueous solution of $KHSO_4$ (8 mL) was added to the solution, the cooling bath was removed, and an additional portion of 10% aqueous solution of $KHSO_4$ (8 mL) was added. The mixture was diluted with CH_2Cl_2 (80 mL), and then the two phases were separated. The organic phase was washed with brine (2 × 5 mL), dried (Na_2SO_4), and concentrated to give the adduct **6**, which was used for the following reaction without any purification. A mixture of the above crude adduct **6**, anhydrous toluene

(31) Hantzsch, A. *Justus Liebigs Ann. Chem.* **1882**, *215*, 1−82.
(32) Wan, Q.; Chen, J.; Chen, G.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8244−8249.
(33) (a) Sutherlin, D. P.; Stark, T. M.; Hughes, R.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 8350−8354. (b) Orsini, F.; Di Teodoro, E. *Tetrahedron: Asymmetry* **2003**, *14*, 2521−2528.
(34) Eynde, J. V.; Mayence, A.; Maquestiau, A. *Tetrahedron Lett.* **1992**, *48*, 463−468.

(24 mL), and anhydrous *t*-BuOH (12 mL) was heated under reflux for 5 h and then concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane−Et_2O to give **5** (838 mg, 60%) as a ∼10:1 mixture of ketone and enol isomers: $[\alpha]_D = 25.4$ (*c* 0.9, $CHCl_3$).

General Procedure for the Synthesis of Sugar Acids 9a,b. To a stirred solution of sugar aldehyde (1.00 mmol) in CH_3CN (10 mL) were added 35% aqueous H_2O_2 (0.2 mL), 1.2 M aqueous KH_2PO_4 (1.0 mL), and 0.17 M aqueous $NaClO_2$ (7.0 mL). The mixture was stirred at room temperature for 2 h and then acidified with 1 N aqueous HCl to pH = 2, diluted with AcOEt (150 mL), and washed with H_2O (2 × 10 mL). The aqueous phase was extracted with AcOEt (2 × 50 mL), and then the combined organic phase was dried (Na_2SO_4) and concentrated to give the corresponding crude acid **9** in almost quantitative yield and at least 95% pure as judged by ^{1}H NMR analysis.

Crude acid α-**9a**: ^{1}H NMR δ = 7.40−7.00 (m, 20 H, Ph), 4.92 and 4.80 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.82 and 4.50 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.72 and 4.66 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.67 (ddd, 1 H, $J_{2a,3}$ = 5.5 Hz, $J_{2b,3}$ = 9.0 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.64 and 4.48 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 3.84−3.62 (m, 6 H, H-4, H-5, H-6, H-7, 2 H-8), 2.82 (dd, 1 H, $J_{2a,2b}$ = 15.0 Hz, H-2a), 2.74 (dd, 1 H, H-2b). Anal. Calcd for $C_{36}H_{38}O_7$ (582.68): C, 74.21; H, 6.57. Found: C, 74.35; H, 6.44.

Crude acid α-**9b**: ^{1}H NMR δ = 7.40−7.10 (m, 20 H, Ph), 4.72 and 4.60 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.69 and 4.57 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.60 and 4.50 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.57 and 4.49 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.46 (ddd, 1 H, $J_{2a,3}$ = 9.0 Hz, $J_{2b,3}$= 4.5 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.16 (ddd, 1 H, $J_{6,7'}$ = 3.0 Hz, $J_{7,8a}$ = 8.0 Hz, $J_{7,8b}$ = 4.0 Hz, H-7), 4.03 (dd, 1 H, $J_{5,6}$ = 4.5 Hz, H-6), 3.95 (dd, 1 H, $J_{8a,8b}$ = 11.0 Hz, H-8a), 3.78−3.70 (m, 2 H, H-4, H-5), 3.68 (dd, 1 H, H-8b), 2.73 (dd, 1 H, $J_{2a,2b}$ = 16.0 Hz, H-2a), 2.57 (dd, 1 H, H-2b). Anal. Calcd for $C_{36}H_{38}O_7$ (582.68): C, 74.21; H, 6.57. Found: C, 74.31; H, 6.48.

General Procedure for the Synthesis of Sugar β-Ketoesters 8a,b. Route B. A mixture of crude sugar acid **9** (∼1.00 mmol), Meldrum's acid (159 mg, 1.10 mmol), DMAP (269 mg, 2.20 mmol), and anhydrous CH_2Cl_2 (8 mL) was cooled to −5 °C, and then a solution of isopropenyl chloroformate (131 μL, 1.20 mmol) in anhydrous CH_2Cl_2 (4 mL) was added dropwise under vigorous magnetic stirring. The mixture was stirred at −5 °C until the complete disappearance of the starting material was detected (TLC analysis, ∼1 h). A 10% aqueous solution of $KHSO_4$ (2 mL) was then added to the solution, the cooling bath was removed, and an additional portion of 10% aqueous solution of $KHSO_4$ (2 mL) was added. The mixture was diluted with CH_2Cl_2 (80 mL), and then the two phases were separated. The organic phase was washed with brine (2 × 5 mL), dried (Na_2SO_4), and concentrated to give the corresponding adduct **10**, which was used for the following reaction without any purification.

A 2.0−5.0 mL process vial was filled with the above crude Meldrum adduct **10** and anhydrous *t*-BuOH (2.5 mL). The vial was sealed with the Teflon septum and aluminum crimp by using an appropriate crimping tool. The vial was then placed in its correct position in the Biotage Initiator cavity where irradiation for 15 min at 120 °C was performed. After the full irradiation sequence was completed, the vial was cooled to room temperature and then opened. The mixture was diluted with AcOEt (10 mL) and then concentrated. The residue was eluted from a column of silica gel with the suitable elution system to give the corresponding sugar β-keto ester **8**.

***tert*-Butyl 3-Oxo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-galactopyranosyl)butanoate (β-8b).** Column chromatography with 7:1 cyclohexane−AcOEt afforded β-**8b** (476 mg, 70%) as a ∼10:1 mixture of ketone and enol isomers: $[\alpha]_D = -8.2$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (ketone isomer) δ = 7.40−7.20 (m, 20 H, Ph), 4.94 and 4.61 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.92 and 4.61 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.74 and 4.65 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.44 and 4.39 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.01 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ ∼ 0.5 Hz, H-4′), 3.74 (ddd, 1 H, $J_{1',2'}$ = 9.0 Hz, $J_{1',4a}$ = 3.5 Hz, $J_{1',4b}$ = 8.5 Hz, H-1′), 3.68 (dd, 1 H, $J_{2',3'}$ = 9.2 Hz, H-2′), 3.62 (dd, 1 H, H-3′), 3.59−3.48 (m, 3 H, H-5′, 2 H-6′), 3.35 and 3.31 (2 d, 2 H, $J_{2a,2b}$ = 15.0 Hz, 2 H-2), 2.85 (dd, 1 H, $J_{4a,4b}$ = 15.5 Hz, H-4a), 2.70 (dd, 1 H, H-4b), 1.40 (s, 9 H, *t*-Bu); MALDI-TOF MS 703.9 (M^+ + Na). Anal. Calcd for $C_{42}H_{48}O_8$ (680.83): C, 74.09; H, 7.11. Found: C, 74.15; H, 7.12.

***tert*-Butyl 3-Oxo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-glucopyranosyl)butanoate (β-8a).** Column chromatography with 6:1 cyclohexane−AcOEt afforded β-**8a** (422 mg, 62%) as a ∼8:1 mixture of ketone and enol isomers: $[\alpha]_D = 2.6$ (*c* 1.2, $CHCl_3$); ^{1}H NMR (ketone isomer) δ = 7.40−7.20 (m, 20 H, Ph), 4.92 and 4.87 (2 d, 2 H, J = 10.5 Hz, PhCH_2), 4.90 and 4.56 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.81 and 4.62 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.58 and 4.49 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 3.76 (ddd, 1 H, $J_{1',2'}$ = 8.5 Hz, $J_{1'}$,4a = 3.5 Hz, $J_{1'}$,4b = 8.5 Hz, H-1′), 3.68 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, $J_{3',4'}$ = 8.5 Hz, H-3′), 3.71−3.62 (m, 3 H, H-4′, 2 H-6′), 3.43 (ddd, 1 H, $J_{4',5'}$ = 9.0 Hz, $J_{5',6'a}$ = 3.0 Hz, $J_{5',6'b}$ = 3.5 Hz, H-5′), 3.38 and 3.33 (2 d, 2 H, $J_{2a,2b}$ = 14.5 Hz, 2 H-2), 3.32 (dd, 1 H, H-2′), 2.84 (dd, 1 H, $J_{4a,4b}$ = 15.5 Hz, H-4a), 2.66 (dd, 1 H, H-4b), 1.42 (s, 9 H, *t*-Bu); MALDI-TOF MS 703.1 (M^+ + Na), 720.0 (M^+ + K). Anal. Calcd for $C_{42}H_{48}O_8$ (680.83): C, 74.09; H, 7.11. Found: C, 74.18; H, 7.15.

***tert*-Butyl 3-Oxo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-galactopyranosyl)butanoate (α-8b).** Column chromatography with 6:1 cyclohexane−AcOEt afforded α-**8b** (462 mg, 68%) as a ∼7:1 mixture of ketone and enol isomers: $[\alpha]_D = 23.8$ (*c* 2.2, $CHCl_3$); ^{1}H NMR (ketone isomer) δ = 7.40−7.20 (m, 20 H, Ph), 4.72 and 4.57 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.68 and 4.54 (2 d, 2 H, J = 10.5 Hz, PhCH_2), 4.58 (ddd, 1 H, $J_{1',2'}$ = 3.0 Hz, $J_{1',4a}$ = 7.5 Hz, $J_{1',4b}$ = 6.0 Hz, H-1′), 4.55 and 4.47 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.10−3.98 (m, 2 H, H-4′, H-5′), 3.90−3.82 (m, 2 H, H-2′, H-6′a), 3.71 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, $J_{3',4'}$ = 3.5 Hz, H-3′), 3.68 (dd, 1 H, $J_{5',6'b}$ = 5.5 Hz, $J_{6'a,6'b}$ = 11.0 Hz, H-6′b), 3.42 and 3.32 (2 d, 2 H, $J_{2a,2b}$ = 15.0 Hz, 2 H-2), 2.86 (dd, 1 H, $J_{4a,4b}$ = 16.0 Hz, H-4a), 2.78 (dd, 1 H, H-4b), 1.42 (s, 9 H, *t*-Bu); MALDI-TOF MS: 703.5 (M^+ + Na), 719.8 (M^+ + K). Anal. Calcd for $C_{42}H_{48}O_8$ (680.83): C, 74.09; H, 7.11. Found: C, 74.00; H, 7.01.

***tert*-Butyl 3-Oxo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-glucopyranosyl)butanoate (α-8a).** Column chromatography with 7:1 cyclohexane−AcOEt afforded α-**8a** (449 mg, 66%) as a ∼6:1 mixture of ketone and enol isomers: $[\alpha]_D = 27.7$ (*c* 2.9, $CHCl_3$); ^{1}H NMR (ketone isomer) δ = 7.40−7.05 (m, 20 H, Ph), 4.92 and 4.80 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.82 and 4.50 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.74 (ddd, 1 H, $J_{1',2'}$ = 4.5 Hz, $J_{1'}$,4a = 5.5 Hz, $J_{1'}$,4b = 8.0 Hz, H-1′), 4.64 and 4.60 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.62 and 4.48 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 3.78 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, H-2′), 3.78−3.60 (m, 5 H, H-3′, H-4′, H-5′, 2 H-6′), 3.42 and 3.32 (2 d, 2 H, $J_{2a,2b}$ = 15.0 Hz, 2 H-2), 3.03 (dd, 1 H, $J_{4a,4b}$ = 15.8 Hz, H-4a), 2.85 (dd, 1 H, H-4b), 1.42 (s, 9 H, *t*-Bu); MALDI-TOF MS 703.5 (M^+ + Na), 719.8 (M^+ + K). Anal. Calcd for $C_{42}H_{48}O_8$ (680.83): C, 74.09; H, 7.11. Found: C, 74.05; H, 7.06.

Route A. A mixture of aldehyde α-**7a** (1.13 g, 2.00 mmol), *tert*-butyl diazoacetate (0.33 mL, 2.40 mmol), activated 4-Å powdered molecular sieves (300 mg), and anhydrous CH_2Cl_2 (20 mL) was stirred at room temperature for 15 min and then cooled to 0 °C. To the mixture was added a solution of BF_3•Et_2O (127 μL, 1.00 mmol) in anhydrous CH_2Cl_2 (1 mL) drop by drop, controlling the N_2 evolution at a low steady rate. The mixture was stirred at 0 °C for an additional 30 min, diluted with 10% $NaHCO_3$ (10 mL), warmed to room temperature, filtered through a pad of Celite, and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was eluted from a column of silica gel with 7:1 cyclohexane−AcOEt to afford α-**8a** (476 mg, 35%) as a ∼6:1 mixture of ketone and enol isomers: $[\alpha]_D = 27.9$ (*c* 2.0, $CHCl_3$).

(4*R*,2‴*S*)- and (4*S*,2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-galactopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-meth-

oxycarbonylethyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (*β*-14b). A screw-capped vial, containing a magnetic bar, was charged with aldehyde *β*-**7b** (283 mg, 0.50 mmol), *β*-ketoester **5** (173 mg, 0.50 mmol), aminocrotonate **12** (79 mg, 0.50 mmol), powdered 4 Å molecular sieves (100 mg), and *t*-BuOH (3 mL). The mixture was then vigorously stirred, degassed under vacuum, and saturated with argon (by an Ar-filled balloon) three times. The mixture was stirred at 70 °C for 24 h and then cooled to room temperature, diluted with AcOEt (10 mL), filtered through a pad of Celite, and concentrated. The residue was eluted from a column of silica gel with 4:1 cyclohexane−AcOEt to give *β*-**14b** (232 mg, 45%) as a∼1:1 mixture of C-4 epimers: ^{1}H NMR (selected data) δ = 7.40−7.20 (m, 20 H, Ph), 6.04 (bs, 0.5 H, NH), 5.89 (d, 0.5 H, J = 8.0 Hz, NH), 4.04−4.00 (m, 1 H, H-4′), 4.40−4.20 (m, 1 H, H-2‴), 3.74 and 3.72 (2 s, 3 H, OCH_3), 2.20 and 2.14 (2 s, 3 H, CH_3), 1.48−1.38 (6 s, 27 H, 3 *t*-Bu); MALDI-TOF MS 1056.5 (M^+ + Na), 1072.6 (M^+ + K). Anal. Calcd for $C_{60}H_{76}N_2O_{13}$ (1033.25): C, 69.75; H, 7.41; N, 2.71. Found: C, 69.88; H, 7.62; N, 2.65.

General Procedure for the Synthesis of 4-(*C*-Glycosylmethyl)-2-alaninylpyridines 15. A 2.0−5.0 mL process vial was filled with sugar aldehyde **7** (283 mg, 0.50 mmol), *β*-ketoester **5** (173 mg, 0.50 mmol), aminocrotonate **12** (79 mg, 0.50 mmol), powdered 4 Å molecular sieves (100 mg), and *t*-BuOH (2.5 mL). The vial was sealed with the Teflon septum and aluminum crimp by using an appropriate crimping tool. The mixture was then vigorously stirred, degassed under vacuum, and saturated with argon (by an Ar-filled balloon) three times The vial was then placed in its correct position in the Biotage Initiator cavity where irradiation for 1.5 h at 120 °C was performed. After the full irradiation sequence was completed, the vial was cooled to room temperature and then opened. The mixture was diluted with AcOEt (10 mL), filtered through a pad of Celite, and concentrated. The residue was dissolved in CH_2Cl_2 (8 mL), and then Amberlyst 15 (400 mg), Ambersep 900 OH (400 mg), and aminomethylated polystyrene (185 mg, 0.50 mmol of a 2.7 mmol g^{-1} resin) were added. The suspension was shaken for 2 h, and then the polymers were filtered off and washed thoroughly with CH_2Cl_2. The combined filtrates were concentrated to give the corresponding crude dihydropyridine derivative. A mixture of the above residue, pyridinium chlorochromate immobilized on silica gel[34] (1.87 g, ∼1.50 mmol of a ∼0.8 mmol g^{-1} resin) and anhydrous CH_2Cl_2 (8 mL) was stirred at room temperature for 12 h. Then the immobilized reagent was filtered off and washed thoroughly with CH_2Cl_2. The combined filtrates were concentrated, and the resulting residue was eluted from a column of silica gel with the suitable elution system to give the corresponding pyridine **15**.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-*β*-D-galactopyranosyl-methyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (*β*-15b). Column chromatography with 4:1 cyclohexane−AcOEt afforded *β*-**15b** (320 mg, 62%) as a white foam: $[\alpha]_D$ = 26.7 (*c* 0.9, $CHCl_3$); ^{1}H NMR δ = 7.40−7.10 (m, 20 H, Ph), 5.88 (bd, 1 H, J = 8.0 Hz, NH), 4.97 and 4.70 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.93 and 4.50 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.79 and 4.69 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.72−4.64 (m, 1 H, H-2‴), 4.41 and 4.33 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.02 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ ∼ 0.5 Hz, H-4′), 3.69 (s, 3 H, OCH_3), 3.68−3.60 (m, 2 H, H-2′, H-6′a), 3.56 (dd, 1 H, $J_{2',3'}$ = 9.0 Hz, H-3′), 3.48−3.34 (m, 3 H, H-1′, H-5′, H-6′b), 3.28 (d, 2 H, $J_{1''',2'''}$ = 7.5 Hz, 2 H-1‴), 3.20 (dd, 1 H, $J_{1',1''a}$ = 3.0 Hz, $J_{1''a,1''b}$ = 14.0 Hz, H-1″a), 2.95 (dd, 1 H, $J_{1',1''b}$ = 10.5 Hz, H-1″b), 2.42 (s, 3 H, CH_3), 1.45 and 1.42 (2 s, 27 H, 3 *t*-Bu); ^{13}C NMR δ = 173.0, 167.5, 167.1, 155.8, 154.3, 152.9, 142.9 (2 C), 139.2−130.2 (5 C), 128.9−127.6 (20 C), 85.0, 83.5, 83.1, 79.8, 79.2, 79.0, 77.4, 75.5, 75.1, 74.2, 73.6, 72.1, 68.6, 52.3, 36.8, 33.1, 28.6, (3 C), 28.2 (6 C), 23.1; MALDI-TOF MS 1032.5 (M^+ + H), 1054.2 (M^+ + Na). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.88; H, 7.28; N, 2.66.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-*β*-D-glucopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (*β*-15a). Column chromatography with 3:1 cyclohexane−AcOEt afforded *β*-**15a** (314 mg, 61%) as a white foam: $[\alpha]_D$ = 36.7 (*c* 0.9, $CHCl_3$); ^{1}H NMR δ = 7.40−7.10 (m, 20 H, Ph), 5.88 (bd, 1 H, J = 8.5 Hz, NH), 4.92 and 4.88 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.91 and 4.68 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.82 and 4.64 (2 d, 2 H, J = 10.5 Hz, PhCH_2), 4.68−4.64 (m, 1 H, H-2‴), 4.52 and 4.36 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 3.72−3.62 (m, 3 H, H-3′, H-4′, H-6′a), 3.68 (s, 3 H, OCH_3), 3.62 (dd, 1 H, $J_{5',6'b}$ = 3.5 Hz, $J_{6'a,6'b}$ = 10.5 Hz, H-6′b), 3.44 (ddd, 1 H, $J_{1',2'}$ = 9.0 Hz, $J_{1',1''}$a = 2.5 Hz, $J_{1',1''b}$ = 11.0 Hz, H-1′), 3.30−3.18 (m, 4 H, H-2′, H-5′, 2 H-1‴), 3.17 (dd, 1 H, $J_{1''a,1''b}$ = 14.5 Hz, H-1″a), 2.88 (dd, 1 H, H-1″b), 2.42 (s, 3 H, CH_3), 1.52 and 1.44 (2 s, 27 H, 3 *t*-Bu); ^{13}C NMR δ = 172.7, 167.1, 166.6, 155.6, 154.2, 152.9, 142.4 (2 C), 138.6−137.9 (5 C), 129.7−127.2 (20 C), 87.4, 83.2, 82.8, 82.5, 79.5, 79.4, 78.8, 78.4, 75.7, 75.1, 74.9, 73.0, 69.3, 52.1, 36.6, 32.8, 29.7 (3C), 28.3 (6 C), 22.3; MALDI-TOF MS 1032.8 (M^+ + H), 1054.6 (M^+ + Na). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.75; H, 7.20; N, 2.75.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (α-15b). Column chromatography with 5:1 cyclohexane−AcOEt afforded α-**15b** (299 mg, 58%) as a white foam: $[\alpha]_D$ = 6.8 (*c* 0.8, $CHCl_3$); ^{1}H NMR δ = 7.40−6.90 (m, 20 H, Ph), 5.75 (bd, 1 H, J = 8.5 Hz, NH), 4.70 and 4.52 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.63 (ddd, 1 H, $J_{1'''a,2'''}$ = 6.0 Hz, $J_{1'''b,2'''}$ = 4.5 Hz, H-2‴), 4.56 and 4.52 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.54 and 4.41 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.51 and 4.40 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.23−4.14 (m, 2 H, H-1′, H-6′a), 3.96−3.80 (m, 4 H, H-2′, H-3′, H-4′, H-6′b), 3.59 (s, 3 H, OCH_3), 3.50 (dd, 1 H, $J_{1',1''a}$ = 10.5 Hz, $J_{1''a,1''b}$ = 14.0 Hz, H-1″a), 3.45−3.41 (m, 1 H, H-5′), 3.29 (dd, 1 H, $J_{1'''a,1'''b}$ = 16.0 Hz, H-1‴a), 3.16 (dd, 1 H, H-1‴b), 2.57 (dd, 1 H, $J_{1',1''b}$ = 1.0 Hz, H-1″b), 2.36 (s, 3 H, CH_3), 1.44 and 1.38 (2 s, 27 H, 3 *t*-Bu); ^{13}C NMR δ = 172.7, 167.3, 166.9, 155.6, 154.1, 152.6, 143.5 (2 C), 139.0−137.9 (5 C), 129.8−127.0 (20 C), 83.1, 82.7, 79.6, 77.9, 75.7, 74.2, 73.7, 73.4, 72.8, 72.4, 71.8, 68.4, 65.7, 52.1, 36.6, 30.9, 28.3 (3 C), 27.9 (6 C), 22.9; MALDI-TOF MS 1032.8 (M^+ + H). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.77; H, 7.29; N, 2.69.

(2‴*S*)-4-(2′,3′,4′,6′-tetra-*O*-Benzyl-α-D-glucopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (α-15a). Column chromatography with 5:1 cyclohexane−AcOEt afforded α-**15a** (284 mg, 55%) as a white foam: $[\alpha]_D$ = 30.5 (*c* 0.7, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 120 °C) δ = 7.40−7.05 (m, 20 H, Ph), 6.42 (bs, 1 H, NH), 4.70 and 4.65 (2 d, 2 H, J = 10.5 Hz, PhCH_2), 4.68 and 4.55 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.66 and 4.53 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.56 (dd, 1 H, $J_{1'''a,2'''}$ = 6.0 Hz, $J_{1'''b,2'''}$ = 7.0 Hz, H-2‴), 4.32−4.26 (m, 1 H, H-1′), 4.26 (s, 2 H, PhCH_2), 3.92 (dd, 1 H, J = 4.5 Hz, J = 5.5 Hz, H-3′), 3.81 (ddd, 1 H, J = 3.5 Hz, J = 4.0 Hz, J = 8.0 Hz, H-5′), 3.67−3.52 (m, 4 H, H-2′, H-4′, 2 H-6′), 3.58 (s, 3 H, OCH_3), 3.36 (dd, 1 H, $J_{1',1''a}$ = 9.5 Hz, $J_{1''a,1''b}$ = 14.0 Hz, H-1″a), 3.22 (dd, 1 H, $J_{1'''a,1''b}$ = 15.5 Hz, H-1‴a), 3.07 (dd, 1 H, H-1‴b), 2.86 (dd, 1 H, $J_{1',1''b}$ = 4.0 Hz, H-1″b), 2.20 (s, 3 H, CH_3), 1.53, 1.51, and 1.37 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR δ = 172.7, 167.5, 167.1, 155.6, 154.5, 153.0, 143.6 (2 C), 138.6−137.9 (5 C), 129.4−127.4 (20 C), 83.6, 83.1, 81.4, 79.6, 79.5, 74.2, 74.0, 73.3, 72.9, 72.8, 72.7, 68.8, 52.2, 36.7, 30.0, 28.3 (3 C), 28.0 (6 C), 23.1; MALDI-TOF MS 1054.9 (M^+ + Na). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.90; H, 7.30; N, 2.78.

General Procedure for the Synthesis of 2-(*C*-Glycosylmethyl)-4-alaninylpyridines 16. A 2.0−5.0 mL process vial was filled with aldehyde **2** (116 mg, 0.50 mmol), sugar *β*-ketoester **8** (340 mg, 0.50 mmol), aminocrotonate **12** (79 mg, 0.50 mmol), powdered 4

Å molecular sieves (100 mg), and *t*-BuOH (2.5 mL). The vial was sealed with the Teflon septum and aluminum crimp by using an appropriate crimping tool. The mixture was then vigorously stirred, degassed under vacuum, and saturated with argon (by an Ar-filled balloon) three times The vial was then placed in its correct position in the Biotage Initiator cavity where irradiation for 2.5 h at 120 °C was performed. After the full irradiation sequence was completed, and the vial was cooled to room temperature and then opened. The mixture was diluted with AcOEt (10 mL), filtered through a pad of Celite, and concentrated. The residue was dissolved in CH_2Cl_2 (8 mL), and then Amberlyst 15 (400 mg), Ambersep 900 OH (400 mg), and aminomethylated polystyrene (185 mg, 0.50 mmol of a 2.7 mmol g^{-1} resin) were added. The suspension was shaken for 2 h, and then the polymers were filtered off and washed thoroughly with CH_2Cl_2. The combined filtrates were concentrated to give the corresponding crude dihydropyridine derivative. A mixture of the above residue, pyridinium chlorochromate immobilized on silica gel[34] (1.87 g, ~1.50 mmol of a ~0.8 mmol g^{-1} resin) and anhydrous CH_2Cl_2 (8 mL), was stirred at room temperature for 12 h. Then the immobilized reagent was filtered off and washed thoroughly with CH_2Cl_2. The combined filtrates were concentrated, and the resulting residue was eluted from a column of silica gel with the suitable elution system to give the corresponding pyridine **16**.

(2‴*S*)-2-(2′,3′,4′,6′-tetra-*O*-Benzyl-β-D-galactopyranosyl-methyl)-4-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (β-16b). Column chromatography with 4:1 cyclohexane−AcOEt afforded β-**16b** (351 mg, 68%) as a white foam: $[\alpha]_D = -17.8$ (*c* 0.7, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 140 °C) $\delta = 7.40-7.10$ (m, 20 H, Ph), 6.08 (bs, 1 H, NH), 4.88 and 4.55 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.86 and 4.69 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.81 and 4.69 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.42−4.36 (m, 1 H, H-2‴), 4.38 and 4.32 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.05 (dd, 1 H, $J_{3',4'} = 2.5$ Hz, $J_{4',5'} \sim 0.5$ Hz, H-4′), 3.92−3.84 (m, 1 H, H-1′), 3.78−3.72 (m, 2 H), 3.62−3.52 (m, 2 H), 3.60 (s 3 H, OCH_3), 3.46−3.40 (m, 1 H), 3.24 (dd, 1 H, $J_{1',1''a} = 3.5$ Hz, $J_{1''a,1''b} = 14.0$ Hz, H-1″a), 3.17 (dd, 1 H, $J_{1'''a,2'''} = 6.5$ Hz, $J_{1'''a,1'''b} = 14.5$ Hz, H-1‴a), 2.95 (dd, 1 H, $J_{1',1''b} = 9.0$ Hz, H-1″b), 2.89 (dd, 1 H, $J_{1'''b,2'''} = 9.5$ Hz, H-1‴b), 2.42 (s, 3 H, CH_3), 1.61, 1.52, and 1.30 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR $\delta = 172.8$, 167.7, 167.3, 157.6−154.7 (5 C), 139.1−135.0 (5 C), 129.4−127.3 (20 C), 84.8, 83.9, 79.5, 79.3, 78.9, 78.4, 75.1, 74.5, 74.0, 73.3, 72.0, 68.6, 53.7, 52.2, 38.9, 31.9, 28.0 (3 C), 27.9 (6 C), 23.1; MALDI-TOF MS 1070.9 ($M^+ + K$). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.82; H, 7.23; N, 2.71.

(2‴*S*)-2-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-glucopyranosylmethyl)-4-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (β-16a). Column chromatography with 4:1 cyclohexane−AcOEt afforded β-**16a** (320 mg, 62%) as a white foam: $[\alpha]_D = -23$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 120 °C): $\delta = 7.40-7.10$ (m, 20 H, Ph), 6.25 (bs, 1 H, NH), 4.84 and 4.72 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.83 (s, 2 H, PhCH_2), 4.73 and 4.60 (2 d, 2 H, $J = 11.2$ Hz, PhCH_2), 4.41−4.30 (m, 1 H, H-2‴), 4.40 and 4.33 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 3.97 (ddd, 1 H, $J_{1',1''a} = 3.5$ Hz, $J_{1',1''b} = 8.5$ Hz, $J_{1',2'} = 9.0$ Hz, H-1′), 3.74 (dd, 1 H, $J = 8.8$ Hz, $J = 9.0$ Hz), 3.64−3.51 (m, 2 H, 2 H-6′), 3.59 (s, 3 H, OCH_3), 3.51 (dd, 1 H, $J = 8.0$ Hz, $J = 8.5$ Hz), 3.44 (dd, 1 H, $J = 8.8$ Hz, $J = 9.0$ Hz), 3.39 (m, 1 H, H-5′), 3.27 (dd, 1 H, $J_{1''a,1''b} = 14.5$ Hz, H-1″a), 3.19 (dd, 1 H, $J_{1'''a,2'''} = 8.0$ Hz, $J_{1'''a,1'''b} = 14.0$ Hz, H-1‴a), 3.26 (dd, 1 H, H-1″b), 2.88 (dd, 1 H, H-1‴b), 2.40 (s, 3 H, CH_3), 1.59, 1.56, and 1.28 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR (selected data) $\delta = 172.7$, 87.4, 84.0, 82.1, 79.4, 78.7, 75.6, 74.9, 73.2, 68.9, 52.7, 52.4, 38.5, 31.7, 28.2, 28.0, 23.0; MALDI-TOF MS 1032.4 ($M^+ + H$), 1054.2 ($M^+ + Na$). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.82; H, 7.28; N, 2.71.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-galactopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (α-16b). Column chromatography with 6:1 cyclohexane−AcOEt afforded α-**16b** (320 mg, 62%) as a white foam: $[\alpha]_D = -3.6$ (*c* 1.2, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 140 °C): $\delta = 7.40-7.10$ (m, 20 H, Ph), 6.18 (bs, 1 H, NH), 4.70 and 4.65 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.69−4.62 (m, 1 H, H-2‴), 4.68 and 4.55 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.62 and 4.56 (2 d, 2 H, $J = 11.0$ Hz, PhCH_2), 4.42−4.34 (m, 1 H, H-1′), 4.38 and 4.31 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.11 (ddd, 1 H, $J_{4',5'} = 1.5$ Hz, $J_{5',6'a} = 4.5$ Hz, $J_{5',6'b} = 9.0$ Hz, H-5′), 4.05 (dd, 1 H, $J_{3',4'} = 2.0$ Hz, H-4′), 3.94 (dd, 1 H, $J_{2',3'} = 6.0$ Hz, H-3′), 3.88 (dd, 1 H, $J_{1',2'} = 3.5$ Hz, H-2′), 3.76−3.68 (m, 2 H, 2 H-6′), 3.60 (s, 3 H, OCH_3), 3.24 (dd, 1 H, $J_{1',1''a} = 6.0$ Hz, $J_{1''a,1''b} = 14.0$ Hz, H-1″a), 3.12 (dd, 1 H, $J_{1'''a,2'''} = 8.0$ Hz, $J_{1'''a,1'''b} = 15.0$ Hz, H-1‴a), 3.06 (dd, 1 H, $J_{1'''b,2'''} = 6.0$ Hz, H-1‴b), 2.42 (s, 3 H, CH_3), 1.61, 1.55, and 1.30 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR (selected data): $\delta = 172.8$, 167.5, 167.2, 155.5, 155.3, 154.6, 139.0, 138.2, 138.1, 129.0−127.4 (20 C), 84.2, 84.0, 79.6, 75.6, 74.0, 73.1, 72.9, 72.5, 66.8, 53.6, 52.3, 34.0, 31.6, 28.2 (3 C), 28.0 (6 C), 23.0; MALDI-TOF MS 1032.7 ($M^+ + H$). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.93; H, 7.15; N, 2.70.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-α-D-glucopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-methoxycarbonylethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (α-16a). Column chromatography with 4:1 cyclohexane−AcOEt afforded α-**16a** (299 mg, 58%) as a white foam: $[\alpha]_D = 16.8$ (*c* 0.8, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 120 °C) $\delta = 7.40-7.10$ (m, 20 H, Ph), 6.24 (bs, 1 H, NH), 4.81 and 4.73 (2 d, 2 H, $J = 11.0$ Hz, PhCH_2), 4.80−4.70 (m, 1 H, H-1′), 4.72 and 4.57 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.68 and 4.64 (2 d, 2 H, $J = 10.8$ Hz, PhCH_2), 4.42 and 4.35 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.40−4.30 (m, 1 H, H-2‴), 3.92−3.82 (m, 2 H), 3.76 (dd, 1 H, $J = 4.5$ Hz, $J = 7.5$ Hz), 3.64−3.50 (m, 3 H), 3.60 (s, 3 H, OCH_3), 3.23 (dd, 1 H, $J_{1'''a,2'''} = 6.5$ Hz, $J_{1'''a,1'''b} = 14.0$ Hz, H-1‴a), 3.18 (dd, 1 H, $J_{1',1''a} = 6.5$ Hz, $J_{1''a,1''b} = 14.5$ Hz, H-1″a), 3.12 (dd, 1 H, $J_{1',1''b} = 5.0$ Hz, H-1″b), 2.87 (dd, 1 H, $J_{1'''b,2'''} = 8.0$ Hz, H-1‴b), 2.44 (s, 3 H, CH_3), 1.62, 1.56, and 1.31 (3 s, 27 H, 3 *t*-Bu); ^{13}C NMR $\delta = 172.7$, 167.5, 167.2, 155.5−154.9 (5 C), 139.0−138.2 (5 C), 128.8−127.5 (20 C), 84.5, 84.1, 82.4, 79.7, 78.0, 75.2, 74.7, 73.7, 73.3, 72.8, 71.8, 68.5, 53.7, 52.3, 32.0, 31.7, 28.2 (3 C), 28.0 (6 C), 23.1; MALDI-TOF MS 1054.3 ($M^+ + Na$). Anal. Calcd for $C_{60}H_{74}N_2O_{13}$ (1031.24): C, 69.88; H, 7.23; N, 2.72. Found: C, 69.81; H, 7.18; N, 2.86.

(2‴*S*)-4-(2′,3′,4′,6′-Tetra-*O*-benzyl-β-D-galactopyranosylmethyl)-2-(2‴-*tert*-butoxycarbonylamino-2‴-carboxyethyl)-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Esters (17). To a cooled (0 °C) stirred solution of methyl ester β-**15b** (103 mg, 0.10 mmol) in THF (2 mL) was added dropwise a pre-cooled 0.2 M aqueous solution of LiOH (0.60 mL, 0.12 mmol). The mixture was stirred at 0 °C until the complete disappearance of the starting material was detected (TLC analysis, ~1 h). The mixture was then acidified with 5% aqueous HCl to pH = 2, warmed to room temperature, diluted with CH_2Cl_2 (80 mL), and washed with H_2O (2 × 10 mL). The organic phase was dried (Na_2SO_4) and concentrated to give the crude acid **17** in almost quantitative yield: ^{1}H NMR $\delta = 7.40-7.10$ (m, 20 H, Ph), 5.93 (bd, 1 H, $J = 6.0$ Hz, NH), 4.98 and 4.52 (2 d, 2 H, $J = 10.5$ Hz, PhCH_2), 4.93 and 4.71 (2 d, 2 H, $J = 11.0$ Hz, PhCH_2), 4.80 and 4.68 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.47 and 4.38 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.38 (ddd, 1 H, $J_{1'''a,2'''} = 8.5$ Hz, $J_{1'''b,2'''} = 8.5$ Hz, H-2‴), 4.03 (dd, 1 H, $J_{3',4'} = 3.5$ Hz, $J_{4',5'} \sim 0.5$ Hz, H-4′), 3.72−3.60 (m, 2 H, H-2′, H-6′a), 3.57 (dd, 1 H, $J_{2',3'} = 9.0$ Hz, H-3′), 3.50−3.30 (m, 4 H, H-1″a, H-1′, H-5′, H-6′b), 3.22 (dd, 1 H, $J_{1',1'b} = 9.5$ Hz, $J_{1''a,1''b} = 16.5$ Hz, H-1′′b), 3.10 (d, 2 H, 2 H-1‴), 2.52 (s, 3 H, CH_3), 1.44, 1.42, and 1.28 (3 s, 27 H, 3 *t*-Bu). Anal. Calcd for $C_{59}H_{72}N_2O_{13}$ (1017.21): C, 69.66; H, 7.13; N, 2.75. Found: C, 69.75; H, 7.10; N, 2.70.

(2′*S*,2″*S*,1‴*S*)-4-(2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosylmethyl)-2-[2′-(2″-*tert*-butoxycarbonylamino-3″-methylpropionylamino)-2′-(1‴-ethoxycarbonyl-2‴-phenylethylcarbamoyl)-ethyl]-6-methylpyridine-3,5-dicarboxylic Acid Di-*tert*-butyl Ester

(Boc-Ala-Pal-Phe-OEt) (19). To a cooled (0 °C), stirred solution of crude acid **17** (101 mg, ~0.10 mmol), L-phenylalanine ethyl ester hydrochloride (34 mg, 0.15 mmol), and (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (62 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (52 μL, 0.30 mmol). The solution was warmed to room temperature, stirred for an additional 2 h, and then concentrated. The residue was suspended with AcOEt (80 mL) and washed with H_2O (2 × 10 mL). The organic phase was dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with 1:1 cyclohexane−AcOEt to give dipeptide **18** (94 mg, ~80%) slightly contaminated by uncharacterized byproducts: ^{1}H NMR (selected data) δ = 8.08 (bd, 1 H, J = 7.5 Hz, NH), 7.40−7.10 (m, 25 H, Ph), 6.12 (bd, 1 H, J = 8.05 Hz, NH), 4.96 and 4.68 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.93 and 4.50 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.78 and 4.69 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.43 and 4.38 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.08 (bq, 2 H, J = 7.0 Hz, OCH_2CH$_3$), 4.02 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ ~ 0.5 Hz, H-4s), 3.57 (dd, 1 H, $J_{2,3}$ = 9.0 Hz, H-3s), 2.33 (bs, 3 H, CH_3), 1.44, 1.42, and 1.28 (3 s, 27 H, 3 *t*-Bu), 1.22 (t, 3 H, OCH$_2$CH_3).

To a cooled (0 °C), stirred solution of dipeptide **18** (94 mg, ~0.08 mmol) in CH_2Cl_2 (2.0 mL) was slowly added a solution of TFA−CH_2Cl_2 (0.50 mL−1.50 mL). Stirring was continued at 0 °C for an additional 30 min and then warmed to room temperature. After 30 min at room temperature, the solution was neutralized at 0 °C with saturated aqueous Na_2CO_3 and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to give the corresponding crude free amine (85 mg), which was used for the following reaction without any purification.

To a cooled (0 °C), stirred solution of the above crude amine (85 mg, ~0.08 mmol), *tert*-butoxycarbonyl-L-alanine (22 mg, 0.12 mmol), and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (73 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (60 μL, 0.35 mmol). The solution was warmed to room temperature, stirred for an additional 2 h, and then concentrated. The residue was suspended with AcOEt (80 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with 1.5:1 cyclohexane−AcOEt to give tripeptide **19** (65 mg, 65% from **35**): $[\alpha]_D$ = 23.5 (*c* 0.8, $CHCl_3$); ^{1}H NMR δ = 7.78 (bd, 2 H, J ~ 7.5 Hz, 2 NH), 7.40−7.00 (m, 25 H, Ph), 5.08 (bd, 1 H, J = 8.0 Hz, NH), 4.98 and 4.68 (2 d, 2 H, J = 11.0 Hz, PhCH_2), 4.92 and 4.48 (2 d, 2 H, J = 11.2 Hz, PhCH_2), 4.86−4.78 (m, 2 H, H-2′, H-1‴), 4.78 and 4.70 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.38 and 4.32 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.20−4.12 (m, 1 H, H-2″), 4.08 (q, 2 H, J = 7.0 Hz, OCH_2CH$_3$), 4.01 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ ~ 0.5 Hz, H-4s), 3.64 (dd, 1 H, $J_{1,2}$ = 8.8 Hz, $J_{2,3}$ = 9.0 Hz, H-2s), 3.68−3.60 (m, 1 H, H-5s), 3.57 (dd, 1 H, H-3s), 3.44 (ddd, 1 H, $J_{1,CH2a}$ = 3.0 Hz, $J_{1,CH2b}$ = 10.5 Hz, H-1s), 3.42−3.32 (m, 2 H, 2 H-6s), 3.20−3.06 (m, 5 H, CH_2a, 2 H-1′, 2 H-2‴), 2.95 (dd, 1 H, $J_{CH2a,CH2b}$ = 14.0 Hz, CH_2b), 2.36 (s, 3 H, CH_3), 1.62, 1.42, and 1.40 (3 s, 27 H, 3 *t*-Bu), 1.28 (d, 3 H, J = 7.0 Hz, CH_3), 1.15 (t, 3 H, OCH$_2$CH_3); MALDI-TOF MS 1286.7 (M^+ + Na). Anal. Calcd for $C_{73}H_{90}N_4O_{15}$ (1263.5): C, 69.39; H, 7.18; N, 4.43. Found: C, 69.45; H, 7.20; N, 4.39.

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Supporting Information Available: General experimental methods and NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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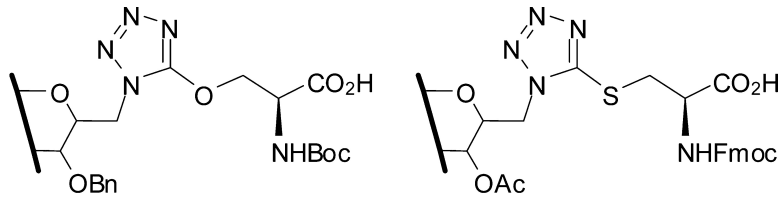

α- or β-anomer
galacto-ribo series

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Click Azide−Nitrile Cycloaddition as a New Ligation Tool for the Synthesis of Tetrazole-Tethered *C*-Glycosyl α-Amino Acids†

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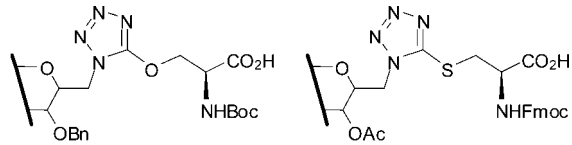

α- or β-anomer
galacto-ribo series

Glycoproteins play a key role in a multitude of biological events in living organisms. Hence, neoglycopeptides obtained from unnatural *C*-glycosyl α-amino acids can be used as synthetic probes in studies aiming at clarifying the role of the carbohydrate domain in glycoprotein biological activity. A new class of *C*-glycosyl α-amino acids featuring a nitrogenated heterocycle ring holding the carbohydrate and glycinyl moiety was designed in our laboratory. Having previously prepared isoxazole-, 1,2,3-triazole-, and pyridine-tethered compounds, the family has now been enlarged by a group of newcomers represented by tetrazole derivatives. Two sets of compounds have been prepared, one being constituted of *C*-galactosyl and *C*-ribosyl *O*-tetrazolyl serines while the other contains *S*-tetrazolyl cysteine derivatives. In both cases, the synthetic scheme involved a two-step route, the first one being the thermal cycloaddition of a sugar azide with *p*-toluensulfonyl cyanide (TsCN) to give a 1-substituted 5-sulfonyl tetrazole and the second the replacement of the tosyl group with a serine or cysteine residue. For the high efficiency and operational simplicity, the azide−TsCN cycloaddition appears to be a true click process. Finally, one of the amino acids prepared was incorporated into a tripeptide.

Introduction

A great deal, about 50%, of proteins in humans are glycosylated.[1] A variety of oligosaccharides that are mostly branched are introduced in the protein backbone mainly through *O*- and *N*-glycosyl amino acids, being serine, threonine, hydroxyproline, and asparagine, the most common derivatives. The carbohydrate domain in glycoproteins is key to vital biological processes including cell−cell recognition and interaction and it affects protein folding, conformation, stability, and biological activity.[2] In this context, defects in the attachment of carbohydrate to protein in humans may be the main cause of undesired biochemical events that result in serious diseases.[3] A striking example is represented by the disease known as congenital disorder of glycosylation (CDG) syndrome. Specifically, following the original observations of a pediatrician in Belgium in the beginning of the 1980s, further studies have revealed that congenital underglycosylation of proteins causes severe health problems in children and typically results in multisystemic presentation involving interference with normal development of the brain and functions of the liver, stomach, and nervous and intestinal systems.[4] On the other hand, the beneficial effects of glycosylation in neuropeptides (e.g., enkephalins) in terms of improved blood−brain barrier penetration and analgesic potency have been recently discussed.[5] The role of antifreeze glycoproteins[6] in Teleost fish is crucial to prevent in vivo ice growth in organisms inhabiting subzero environments. Conse-

† Dedicated to the fond memory of Albert I. Meyers.

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quently, efficient protection of these organisms against cryoinjury and death is achieved. In general, however, numerous mechanisms of carbohydrate action in glycoproteins are at present poorly understood.[1] Natural and unnatural glycopeptides with a well-defined structure and composition can serve as probes in biochemical studies, while the latter are also potential leads for the developments of new drugs against carbohydrate-based metabolic disorders. Hence, a great deal of efforts are made toward the search of efficient synthetic methods of natural *O*- and *N*-linked glycosyl amino and glycopeptides[2b,7] as well as of unnatural *C*-linked analogues[8] to be incorporated into peptidic chains. Attention is also drawn to the synthesis of *S*-linked glycosyl amino acids and thioglycopeptides.[9] While in early studies we have carried out the synthesis of methylene isosteres of glycosyl serines[10] and ethylene isosteres of glycosyl asparagines,[10c,11] more recently we have started a program focused on the synthesis of a newly designed family of *C*-glycosyl amino acids that feature a nitrogenated heterocycle as a tether of the carbohydrate and glycinyl moiety (Figure 1). In our view, the heterocycle should serve not only as a passive yet robust linker but can also act as an additional site of interaction with target substrates through dipolar interactions and hydrogen bonding. Therefore, glycopeptides in which such amino acids are embedded may give rise to highly selective molecular recognition processes. Synthetic approaches to these

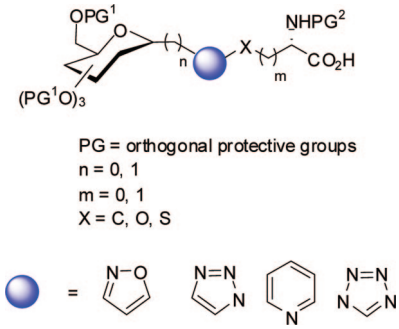

FIGURE 1. Heterocycle-tethered *C*-glycosyl amino acids.

glycosyl amino acids having isoxazole,[12] 1,2,3-triazole,[12] and pyridine rings[13] as linkers have been reported and reviewed.[14] In this paper, we report on the first synthesis of tetrazole-tethered *C*-glycosyl amino acids that relies on an very efficient azide−nitrile cycloaddition as a key step for the heterocyclic ring formation. This ligation strategy is quite attractive because tetrazole is a lipophilic and highly stable unit under a variety of reaction conditions and being densely nitrogenated may induce strong intermolecular interactions via an extensive set of hydrogen bondings. The biological relevance of the tetrazole ring is well-recognized as a metabolically stable surrogate for a carboxylic or amide group.[15]

Results and Discussion

Formation of tetrazole rings by the Huisgen 1,3-dipolar cycloaddition between nitriles and organic azides[16] is in principle the most direct method. However, this reaction is limited in scope because only nitriles activated by strong electron-withdrawing groups can be effectively engaged as dipolarophilic partners by organic azides. A few years ago, Demko and Sharpless have shown for the first time the existence of a reaction window by coupling under solvent-free conditions *p*-toluenesulfonyl cyanide ($TolSO_2$−CN, TsCN)[17] and acyl cyanides[18] with various aromatic and aliphatic azides to give exclusively the corresponding 1,5-disubstituted tetrazoles. As the reactions were carried out using 1 equiv of each reagent and gave nearly quantitative conversion into almost pure products, the process was considered as a case of click chemistry transformation as defined by Sharpless and co-workers.[19] Moreover, earlier work of Gol'tsberg and Koldobskii had previously demonstrated that the 5-sulfonyl substituent in tetrazoles can be readily replaced by a wide range of nucleo-

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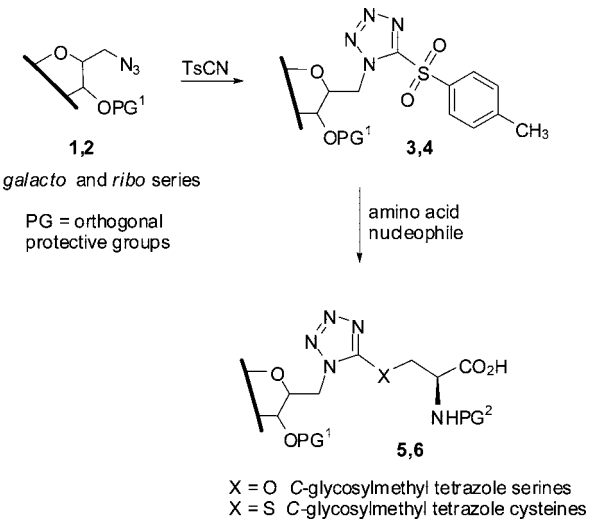
SCHEME 1. Azide−Nitrile Cycloaddition-Based Approach toward the Synthesis of *C*-Glycosylmethyl Tetrazole Serines and Cysteines

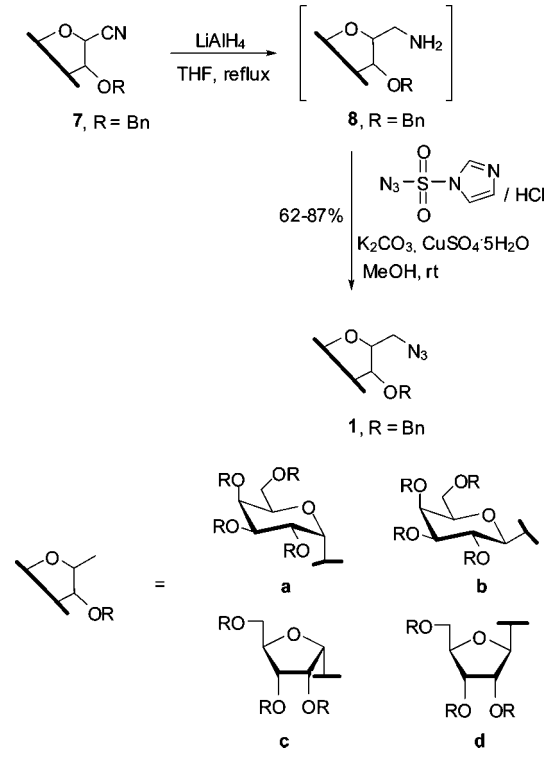


philes.[20] The click azide−TsSCN cycloaddition together with the tosyl substitution in the formed 1-substituted 5-sulfonyl tetrazole established a two-step route toward the synthesis of a variety of 1,5-disubstituted tetrazoles that were hardly accessible by the direct Huisgen azide−nitrile cycloaddition. Recently, we have validated the effectiveness of this click ligation route for the synthesis of complex molecules such as glycoclusters installed on a calix[4]arene platform through tetrazole tethers.[21]

With this in mind, we planned the synthesis of target *C*-glycosylmethyl tetrazole serines **5** and cysteines **6** as shown in Scheme 1. This consisted of the click cycloaddition of benzylated and acetylated glycosylmethyl azides (azidomethyl glycosides) **1** and **2** with TsCN to give 1-glycosylmethyl-5-tosyl tetrazoles **3** and **4** followed by the replacement of the tosyl group by serine and cysteine derivatives acting as *O*- and *S*-nucleophile, respectively. Azides **1** and **2** as starting materials were selected in order to introduce a methylene bridge between the carbohydrate and tetrazole ring, thus creating a robust *C*-glycosidic linkage that would preserve the final product from chemical and enzymatic degradation. Another point of great importance was the careful choice of orthogonal protective groups PG^1 and PG^2 in the carbohydrate and glycinyl moiety in order to make the formed amino acids suitable building blocks in peptide synthesis.

A. Synthesis of Glycosylmethyl Azides 1 and 2. Although the preparation of β-azidomethyl glycosides was reported in previous papers from our laboratory starting from formyl β-*C*-glycosides,[22] we have developed a new and more efficient, general method starting from glycosyl cyanides. These *C*-glycosides are available as either α- or β-anomers by cyanation of glycosyl acetates with trimethylsilyl cyanide (TMSCN).[23] Benzylated galactosyl and ribosyl cyanides **7** were reduced in nearly quantitative yields by lithium aluminum hydride to amines **8**, and these were transformed in very good yields (62−87%) into azides **1** by diazotransfer reaction using imidazole-1-sulfonyl azide (ISA) hydrochloride[24] (Scheme 2). Key in our method was this new, efficient, and shelf-stable diazotransfer reagent. The ISA reagent provided a safer, more effective azide transfer than the commonly used diazo transfer reagent trifluoromethanesulfonyl azide, whose preparation and manipulation require special caution.[25] Following Scheme 2, we have prepared a set of four benzylated glycosylmethyl azides **1a−d** with α- and β-galacto and α- and β-ribo configurations.

SCHEME 2. Synthesis of Perbenzylated Glycosylmethyl Azides



In order to modulate the orthogonal protecting group setting in the final amino acid, acetylated glycosylmethyl azides **2a−d** were conveniently prepared employing aminomethyl glycosides **8** (Scheme 3). Debenzylation of these compounds by hydrogenation over $Pd(OH)_2$ afforded the amines **9** that, as crude material, were subjected to the diazotransfer reaction with ISA hydrochloride to give the azides **10**. These compounds were acetylated to give good overall yields (62−74%) of azides **2a−d**.

B. Synthesis of *C*-Glycosylmethyl *O*-Tetrazolyl Serines 5. The method and conditions for the synthesis of these compounds are illustrated by the synthesis of the β-D-galactosylmethyl derivative **5b** (Scheme 4). Neat tetrabenzyl β-D-galactosylmethyl azide **1b** and TsCN (2.0 equiv) were heated at 100 °C (oil bath). At this temperature, a homogeneous liquid resulted so that the mixture was efficiently stirred by a magnetic bar. As the reaction proceeded, the mixture solidified. The excess of TsCN was simply removed by sublimation under vacuum so that nearly pure 1-galactosylmethyl-5-tosyl tetrazole **3b** was

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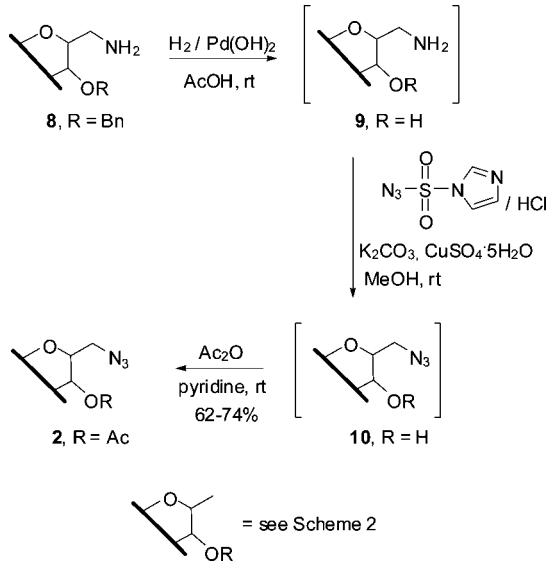
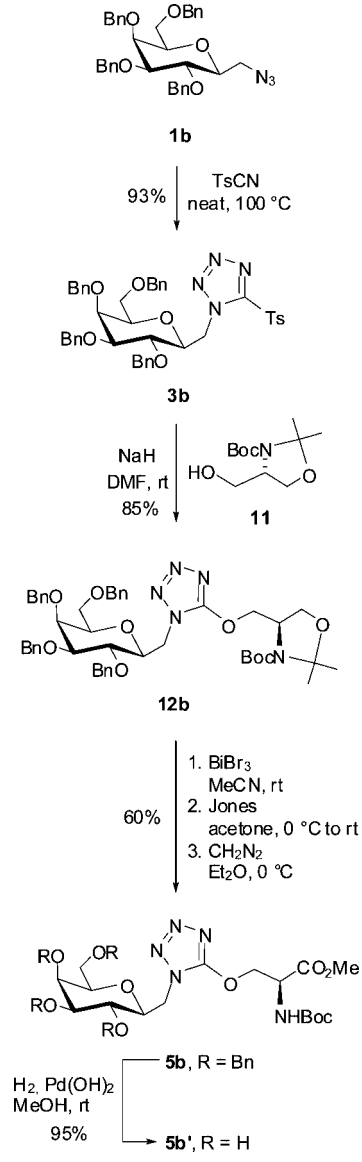
(25) (a) Nyffeler, P. T.; Liang, C.-H.; Koeller, K. M.; Wong, C.-H. *J. Am. Chem. Soc.* **2002**, *124*, 10773–10778. (b) Lundquist, J. T., IV.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781–783.

SCHEME 3. Synthesis of Peracetylated Glycosylmethyl Azides



isolated (93% yield) as the sole regioisomer. The 1,5-substitution pattern in the tetrazole ring was assumed on the basis of previous observations.[17,18,26] A highly performing click azide−nitrile cycloaddition appeared to take place as well with a complex alkyl azide such as **1b**.[27] As the substitution of the tosyl group by an *O*-nucleophile required strong basic conditions, this prevented the use of a protected serine derivative such as *N*-Boc-L-serine methyl ester. The tosyl group of **3b** was replaced by reaction with *N*-Boc-*N*,*O*-isopropylidene-D-serinol **11**[28] and NaH. This reaction provided the masked serine derivative **12b**. It has been demonstrated that compound **11** functions as a configurationally stable equivalent of serine by virtue of the easy transformation of the *N*-Boc oxazolidine ring into the glycinyl group.[10,11] Unexpectedly, treatment of **12b** with the Jones reagent (H_2SO_4−H_2O, CrO_3) at room temperature to achieve one-pot cleavage of the oxazolidine ring and oxidation of the amino alcohol afforded the target amino acid in very poor yield (15%). In addition, the use of aqueous 60−80% AcOH[10] to remove the acetonide protecting group gave poor results (25% yield of the corresponding alcohol). Fortunately, a recent method for cleaving protected cyclic *N,O*-aminals by using catalytic $BiBr_3$ was available.[29] To our satisfaction, treatment of **12b** with $BiBr_3$ in acetonitrile at room temperature, then oxidation of the alcohol with the Jones reagent, and finally esterification with diazomethane afforded the orthogonally protected *N*-Boc methyl serinate **5b** in 60% isolated overall yield. This final product displayed all prerequisites for being a suitable building block in *N*-Boc-based peptide synthesis. Debenzylation of the carbohydrate moiety of **5b** by palladium-catalyzed hydrogenolysis proved to be compatible with the presence of basic nitrogen atoms of the tetrazole ring, thus leading to the amino ester **5b′**.

SCHEME 4. Synthesis of Model Tetrabenzylated *β*-*C*-Glycosylmethyl Tetrazole Serine 5b



The cycloaddition−substitution sequence was successfully applied to the other three benzylated glycosylmethyl azides prepared, i.e., the α-D-galactosyl derivative **1a** and the α-D- and *β*-D-ribosyl derivatives **1c** and **1d**. As quoted in Table 1, uniformly high yields of intermediates **3** and **12** and amino esters **5** were observed in all cases, thus confirming the efficiency of the method.

C. Synthesis of *C*-Glycosylmethyl *S*-Tetrazolyl Cysteines 6. Having in mind the final role of the amino acids under preparation, i.e., their use in cotranslational modification of glycopeptides, we decided to use peracetylated glycosylmethyl azides **2** as starting material. We envisaged the acetyl group removal by saponification of either the final amino acid or the peptide in which it was introduced.[30] In this way, we avoided the risk that the removal of the carbohydrate benzyl groups by Pd-catalyzed hydrogenation could fail due to the presence of the cysteine sulfur atom. Hence, a typical synthesis of a model

(26) Butler, R. N. In *Comprehensive Heterocyclic Compounds*; Katritzky, A. R., Rees, C. W., Scriven, E. F. V., Eds.; Pergamon: Oxford, U.K., 1966; Vol. 4.

(27) The proficuous choice of thermal conditions to perform the cycloaddition of **1b** with TsCN became apparent when the reaction was carried out under Cu(I) catalysis as described by Bosch and Vilarrasa: Bosch, L.; Vilarrasa, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 3926–3930. In our hands, treatment of **1b** with TsCN (2.0 equiv) in CH_2Cl_2 (room temperature) in the presence of $Cu_2(OTf)_2 \cdot C_6H_6$ (0.1 equiv) as the catalyst produced a complex reaction mixture from which the cycloadduct **12b** was isolated in very low yield (18%).

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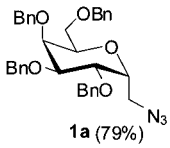
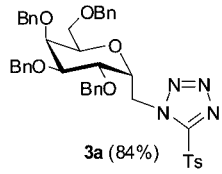
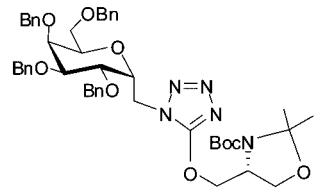
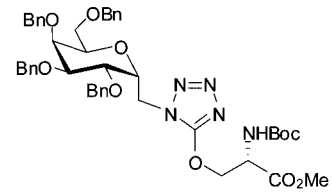
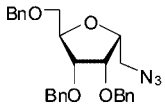
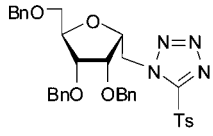
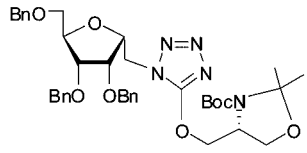
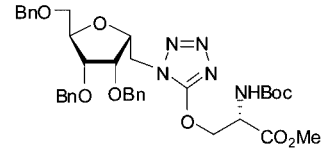
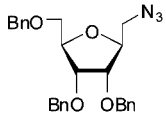
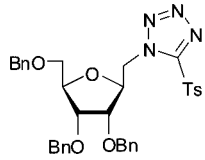
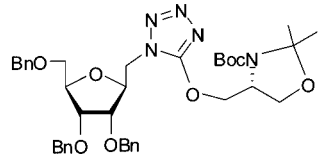
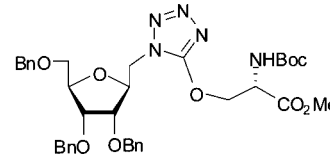
(29) Cong, X.; Hu, F.; Liu, K.-G.; Liao, Q.-J.; Yao, Z.-J. *J. Org. Chem.* **2005**, *70*, 4514–4516. It is worth noting that errors in some references cited in this paper as author given names instead of family names have been reported.

TABLE 1. Glycosylmethyl Tetrazole Serines Prepared[a]

glycosylmethyl azide[b]	glycosylmethyl tetrazole[b]	masked glycosyl amino acid[b]	protected glycosyl amino acid[b]
 **1a** (79%)	 **3a** (84%)	 **12a** (80%)	 **5a** (62%)
1b (87%)	**3b** (93%) see Scheme 4	**12b** (85%) see Scheme 4	**5b** (60%) see Scheme 4
 **1c** (62%)	 **3c** (80%)	 **12c** (83%)	 **5c** (67%)
 **1d** (77%)	 **3d** (96%)	 **12d** (78%)	 **5d** (61%)

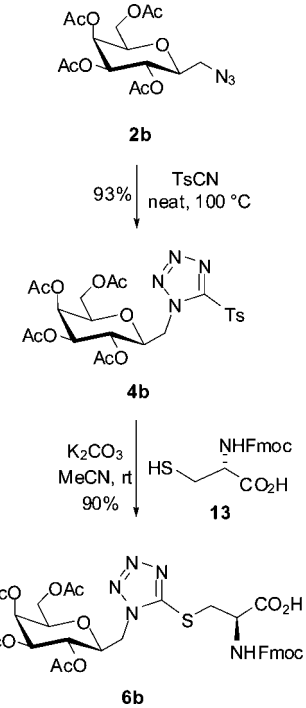
[a] See the Experimental Section for details. [b] Isolated yield by chromatography.

compound is succinctly illustrated here (Scheme 5). The reaction of the tetraacetyl azidomethyl β-D-galactoside **2b** with excess TsCN (2.0 equiv) was run neat at 100 °C, and the isolated 5-tosyl tetrazole **4b** (93% yield) was treated with *N*-fluorenylmethoxycarbonyl (*N*-Fmoc) cysteine **13**[31] (1.1 equiv) under mild basic conditions (K_2CO_3) at room temperature. The cysteine **13** was added in two portions to **4b** in order to reduce its transformation into *N*-Fmoc cystine. The final *C*-galactosylmethyl *S*-tetrazolyl *N*-Fmoc cysteine **6b** was isolated in high yield (90%) without any apparent loss of configurational integrity of the glycinyl group. This approach appeared to be more straightforward than that followed in the synthesis of the serine derivatives **5** (see previous section) in which a masked amino acid nucleophile had to be employed because of the strong basic conditions required for the substitution of the tosyl group.

The same reaction sequence was applied to the other peracetylated glycosylmethyl azides shown in Scheme 3, i.e., compounds **2a**, **2c**, and **2d**. Both the cycloadducts **4a**, **4c**, and **4d** and the tetrazole-tethered *C*-glycosyl cysteines **6a**, **6c**, and **6d** were isolated in good to excellent yields (Table 2). It is worth noting that the set of orthogonal protective groups in amino acids **6a**−**d** makes these compounds all suitable for the *N*-Fmoc-based automated peptide synthesis.[30]

D. Synthesis of Tetrazole-Tethered Glycopeptides. In order to demonstrate the potential of the prepared *C*-glycosyl amino acids as orthogonally protected building blocks for the cotranslational modification of glycopeptides, the *O*-serine derivative

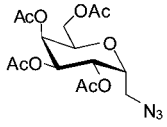
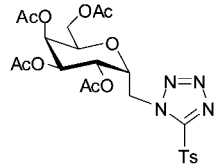
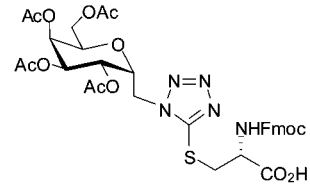
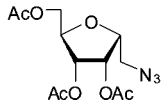
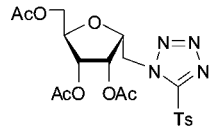
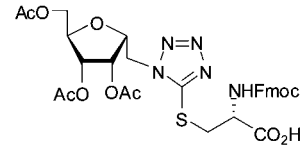
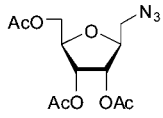
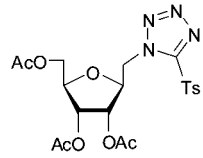
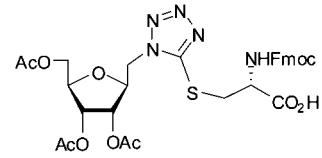
SCHEME 5. Synthesis of Model Tetracetylated *β*-*C*-Glycosylmethyl Tetrazole Cysteine 6b



(30) As a selected example of this protection strategy in glycopeptide synthesis, see: Liu, S.; Ben, R. N. *Org. Lett.* **2005**, *7*, 2385–2388.

(31) Lumbierres, M.; Palomo, J.; Kragol, G. S. R.; Müller, O.; Waldmann, H. *Chem.—Eur. J.* **2005**, *11*, 7405–7415.

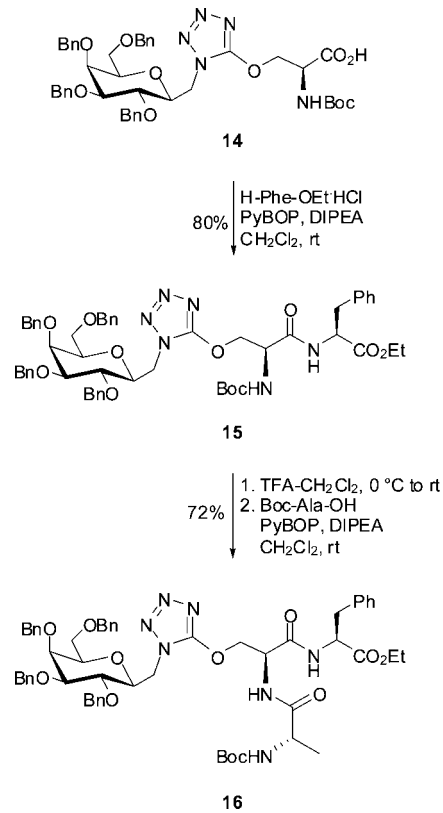
TABLE 2. Glycosylmethyl Tetrazole Cysteines Prepared[a]

glycosylmethyl azide[b]	glycosylmethyl tetrazole[b]	protected glycosyl amino acid[b]
		
2a (64%)	**4a** (94%)	**6a** (92%)
2b (74%)	**4b** (93%) see Scheme 5	**6b** (90%) see Scheme 5
		
2c (66%)	**4c** (98%)	**6c** (91%)
		
2d (68%)	**4d** (90%)	**6d** (90%)

[a] See the Experimental Section for details. [b] Isolated yield by chromatography.

14 was selected as a prototype in this crucial validating test (Scheme 6). A crude sample of this amino acid as obtained from the cleavage of the oxazolidine precursor **12b** was employed in the coupling with phenylalanine ethyl ester hydrochloride (H-Phe-OEt·HCl). The reaction was carried out at ambient temperature under activation of the condensation agent (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) in the presence of the Hünig's base (diisopropylethyl amine, DIPEA). From this reaction, the dipeptide **15** was isolated by chromatography in very good yield (80%). The removal of the *N*-Boc protective group from **15** was readily carried out upon treatment with diluted trifluoroacetic acid (TFA). The amine that formed was used in the condensation with *N*-Boc alanine (Boc-Ala-OH) under the same coupling conditions described above. The tetrazole-tethered glycopeptide **16** was isolated after chromatography in 72% yield. This tripeptide can be inserted in a more complex peptide via *N*-Boc-based peptide synthesis.

SCHEME 6. Synthesis of Tripetide 16



Conclusions

In summary, we have demonstrated the efficiency of the Sharpless−Demko azide−sulfonyl cyanide cycloaddition/sulfonyl substitution route for the synthesis of tetrazole-tethered *C*-glycosyl α-amino acids. A new ligation tool has been established for unnatural *C*-glycosyl α-amino acid synthesis, the building blocks required for the cotranslational modification of glycopeptides. The key step in this strategy was the thermal azide−cyanide cycloaddition, a reaction that is highlighted as a click process when the organic cyanide is activated by a strong

electron-withdrawing substituent such as the sulfonyl group. This and previous work reported from our laboratory[21] demonstrated the fidelity of this thermally induced metal-free process even when applied to complex systems. This reaction combined with the sulfonyl group substitution step appears to have its own value as a new ligation tool. The tetrazole ring that is formed plays the role of a robust molecular keystone holding the biologically active residues. So far the glycosyl amino acids prepared were serine and cysteine derivatives. It can be envisaged that substitution of the sulfonyl group with *N*-, *Se*-, and *C*-nucleophile amino acids or their synthetic equivalents will expand the scope of this new synthetic method.

Experimental Section

Cyanides **7a**−**d**,[23] amines **8b**,[22a] **8d**,[22a] and **9a**,[32a] **9b**,[32] azides **1b**,[22a] **1d**,[22a] and **2b**,[22b] tetrazoles **3b**[21]and **3d**,[21] serinol **11**,[28] and cysteine **13**[31] are known compounds, and their spectroscopic data were identical to those reported.

General Procedure for the Synthesis of Benzylated Amines 8. To a stirred suspension of $LiAlH_4$ (304 mg, 8.00 mmol) in anhydrous THF (10 mL) was slowly added a solution of cyanide **7** (1.10 g, 2.00 mmol) in anhydrous THF (4 mL). The resulting mixture was stirred under reflux until disappearance of starting cyanide (TLC analysis, typically 1 h), cooled to room temperature, and then diluted with 28% aqueous NH_4OH (2 mL). The resulting mixture was stirred at room temperature until the formation of a white precipitate was observed (typically 30 min). The mixture was then filtered over a pad of Celite and washed thoroughly with AcOEt. The combined filtrates were concentrated to give the corresponding crude amine **8** in almost quantitative yield. Each amine **8** was used in the next step without any purification.

(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)methylamine (8a). ^{1}H NMR: δ = 7.50−7.20 (m, 20 H, Ph), 4.75 and 4.61 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.73 and 4.51 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.66 and 4.64 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.56 and 4.52 (2 d, 2 H, J = 12.2 Hz, PhCH_2), 4.10−4.04 (m, 1 H, H-5), 4.02 (dd, 1 H, $J_{4,5}$ = 2.8 Hz, $J_{3,4}$ = 3.8 Hz, H-4), 3.95−3.81 (m, 2 H, H-2, H-3), 3.81−3.71 (m, 2 H, H-1, H-6a), 3.66 (dd, 1 H, $J_{5,6b}$ = 4.2 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6b), 2.97 (dd, 1 H, $J_{1,1'a}$ = 8.9 Hz, $J_{1'a,1'b}$ = 13.5 Hz, H-1′a), 2.78 (dd, 1 H, $J_{1,1'b}$ = 4.2 Hz, H-1′b).

(2,3,5-Tri-*O*-benzyl-α-D-ribofuranosyl)methylamine (8c). ^{1}H NMR: δ = 7.40−7.25 (m, 15 H, Ph), 4.78 and 4.59 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.62 and 4.52 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.57 and 4.54 (2 d, 2 H, J = 12.2 Hz, PhCH_2), 4.20 (ddd, 1 H, $J_{3,4}$ = 7.0 Hz, $J_{4,5a}$ = 3.3 Hz, $J_{4,5b}$ = 4.10 Hz, H-4), 4.08 (dd, 1 H, $J_{1,2}$ = 4.8 Hz, $J_{2,3}$ = 4.8 Hz, H-2), 4.06−3.98 (m, 2 H, H-1, H-3), 3.60 (dd, 1 H, $J_{5a,5b}$ = 10.6 Hz, H-5a), 3.49 (dd, 1 H, H-5b), 3.01 (dd, 1 H, $J_{1,1'a}$ = 6.7 Hz, $J_{1'a,1'b}$ = 13.2 Hz, H-1′a), 2.92 (dd, 1 H, $J_{1,1'b}$ = 5.1 Hz, H-1′b).

General Procedure for the Synthesis of Benzylated Azides 1. A mixture of crude amine **8** (553 mg, ∼1.00 mmol), imidazole-1-sulfonyl azide hydrochloride (251 mg, 1.20 mmol), K_2CO_3 (235 mg, 1.70 mmol), $CuSO_4 \cdot 5H_2O$ (2.5 mg, 0.01 mmol), and MeOH (5 mL) was stirred at room temperature for 4 h. The mixture was then concentrated, diluted with H_2O (15 mL), acidified with concd HCl, and extracted with AcOEt (3 × 25 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with the suitable elution system to give the corresponding azide **1**.

(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl)methyl azide (1a). Column chromatography with 9:1 cyclohexane−AcOEt afforded **1a** (452 mg, 79%) as white foam. $[\alpha]_D$ = 16.2 (*c* 1.3, $CHCl_3$). ^{1}H NMR: δ = 7.42−7.20 (m, 20 H, Ph), 4.72 and 4.59 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.70 and 4.51 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.62 and 4.60 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.60 and 4.53 (2 d, 2 H, J = 12.2 Hz, PhCH_2), 4.22−4.08 (m, 2 H, H-1, H-5), 4.05 (dd, 1 H, $J_{4,5}$ = 2.7 Hz, $J_{3,4}$ = 4.2 Hz, H-4), 3.92(dd, 1 H, $J_{5,6a}$ = 7.3 Hz, $J_{6a,6b}$= 10.7 Hz, H-6a), 3.82−3.70 (m, 3 H, H-2, H-3, H-6b), 3.60 (dd, 1 H, $J_{1,1'a}$ = 8.5 Hz, $J_{1'a,1'b}$ = 12.9 Hz, H-1′a), 3.27 (dd, 1 H, $J_{1,1'b}$ = 4.8 Hz, H-1′b). ^{13}C NMR: δ = 138.3 (2C), 137.8 (2C), 128.5−127.4 (20C), 75.9, 75.5, 73.7, 73.3, 73.1, 73.2, 73.0, 72.9, 70.2, 66.6, 49.2. MALDI-TOF MS: 602.9 (M^+ + Na). Anal. Calcd for $C_{35}H_{37}N_3O_5$ (579.27): C, 75.52; N, 7.25; H, 6.43. Found: C, 75.50; N, 7.27; H, 6.41.

(2,3,5-Tri-*O*-benzyl-α-D-ribofuranosyl)methyl Azide (1c). Column chromatography with 9:1 cyclohexane−AcOEt afforded **1c** (285 mg, 62%) as a white foam. $[\alpha]_D$ = 40.3 (*c* 2.8, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 7.40−7.20 (m, 15 H, Ph), 4.77 and 4.55 (2 d, 2 H, J = 11.5 Hz, PhCH_2), 4.62 and 4.54 (2 d, 2 H, J = 11.8 Hz, PhCH_2), 4.57 and 4.49 (2 d, 2 H, J = 12.0 Hz, PhCH_2), 4.25 (ddd, 1 H, $J_{3,4}$ = 5.5 Hz, $J_{4,5a}$ = 3.6 Hz, $J_{4,5b}$ = 3.4 Hz, H-4), 4.21 (ddd, 1 H, $J_{1,2}$ = 4.8 Hz, $J_{1,1'a}$ = 8.0 Hz, $J_{1,1'b}$ = 5.0 Hz, H-1), 4.12 (dd, 1H, $J_{2,3}$ = 4.8 Hz, H-2), 4.07 (dd, 1 H, H-3), 3.65 (dd, 1 H, $J_{1'a,1'b}$ = 12.8 Hz, H-1′a), 3.59 (dd, 1 H, $J_{5a,5b}$ = 10.7 Hz, H-5a), 3.51 (dd, 1 H, H-5b), 3.46 (dd, 1 H, H-1′b). ^{13}C NMR: δ = 138.0, 137.8, 137.7, 128.4−127.6 (15C), 80.4, 79.0, 78.7, 77.5, 73.4, 73.1, 72.6, 69.9, 50.9. MALDI-TOF MS: 460.4 (M^+ + H). Anal. Calcd for $C_{27}H_{29}N_3O_4$ (459.22): C, 70.57; N, 9.14; H, 6.36. Found: C, 70.53; N, 9.11; H, 6.38.

General Procedure for the Synthesis of Unprotected Amines 9. A vigorously stirred mixture of amine **8** (553 mg, ∼1.00 mmol), 20% palladium hydroxide on carbon (227 mg), and AcOH (8 mL) was degassed under vacuum and saturated with hydrogen (by a H_2-filled balloon) three times. The suspension was stirred at room temperature for 12 h under a positive pressure of hydrogen (8 bar), filtered through a plug of cotton, and concentrated to give crude amine **9** in almost quantitative yield. Each amine **9** was used in the next step without any purification.

(α-D-Ribofuranosyl)methylamine (9c). ^{1}H NMR (D_2O): δ = 4.19 (dd, 1 H, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 4.5 Hz, H-2), 4.15 (ddd, 1 H, $J_{1,1'a}$ = 6.0 Hz, $J_{1,1'b}$ = 4.5 Hz, H-1), 4.03 (dd, 1 H, $J_{3,4}$ = 7.8 Hz, H-3), 3.81 (ddd, 1 H, $J_{4,5a}$ = 2.8 Hz, $J_{4,5b}$ = 5.4 Hz, H-4), 3.66 (dd, 1 H, $J_{5a,5b}$ = 12.5 Hz, H-5a), 3.49 (dd, 1 H, H-5b), 3.15 (dd, 1 H, $J_{1'a,1'b}$ = 13.0 Hz, H-1′a), 3.11 (dd, 1 H, H-1′b). ^{13}C NMR (D_2O): δ = 81.9, 75.9, 72.5, 71.8, 61.5, 39.6.

(β-D-Ribofuranosyl)methylamine (9d). ^{1}H NMR: δ = 3.93 (dd, 1 H, $J_{1,2}$ = 5.1 Hz, $J_{2,3}$ = 5.0 Hz, H-2), 3.90 (ddd, 1 H, $J_{1,1'a}$ = 3.2 Hz, $J_{1,1'b}$ = 13.3 Hz, H-1), 3.84 (dd, 1 H, $J_{3,4}$ = 5.2 Hz, H-3), 3.82 (ddd, 1 H, $J_{4.5a}$ = 3.2 Hz, $J_{4,5b}$ = 4.9 Hz, H-4), 3.17 (dd, 1 H, $J_{1'a,1'b}$ = 13.3 Hz, H-1′a), 2.91 (dd, 1 H, H-1′b). ^{13}C NMR (D_2O): δ = 84.4, 78.5, 72.7, 71.2, 61.5, 41.7.

General Procedure for the Synthesis of Acetylated Azides 2. A mixture of crude amine **9** (193 mg, ∼1.00 mmol), imidazole-1-sulfonyl azide hydrochloride (251 mg, 1.20 mmol), K_2CO_3 (235 mg, 1.70 mmol), $CuSO_4 \cdot 5H_2O$ (2.5 mg, 0.01 mmol), and MeOH (5 mL) was stirred at room temperature for 4 h. The mixture was then concentrated and coevaporated with toluene (2 × 10 mL). Acetic anhydride (3 mL) and pyridine (3 mL) were added to the residue, and the resulting mixture was stirred for an additional 5 h, diluted with H_2O (10 mL), and extracted with AcOEt (3 × 25 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with the suitable elution system to give the corresponding azide **2**.

(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl)methyl azide (2a). Column chromatography with 2:1 cyclohexane−AcOEt afforded **2a** (248 mg, 64%) as a yellow syrup. $[\alpha]_D$ = 53.0 (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 5.44 (dd, 1 H, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 3.5 Hz, H-4), 5.27 (dd, 1 H, $J_{1,2}$ = 4.6 Hz, $J_{2,3}$ = 8.5 Hz, H-2), 5.21 (dd, 1 H, H-3), 4.39 (dd, 1 H, $J_{5.6a}$ = 8.0 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6a), 4.37 (ddd, 1 H, $J_{1,1'a}$ = 8.8 Hz, $J_{1,1'b}$ = 3.7 Hz, H-1), 4.24 (ddd, 1 H, $J_{5,6b}$ = 4.7 Hz, H-5), 4.10 (dd, 1 H, H-6b), 3.61 (dd, 1 H, $J_{1'a,1'b}$ = 13.5 Hz, H-1′a), 3.28 (dd, 1 H, H-1′b), 2.12, 2.10, 2.07, and 2.05 (4s, 12 H, CH_3). ^{13}C NMR: δ = 170.7, 169.8, 169.6, 169.5, 70.8, 69.9, 67.7, 67.6, 66.9, 60.7, 48.3, 20.7 (3C), 20.6. MALDI-TOF MS: 426.4 (M^+ + K). Anal. Calcd for $C_{15}H_{21}N_3O_9$ (387.13): C, 46.51; N, 10.85; H, 5.46. Found: C, 46.50; N, 10.88; H, 5.45.

(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosyl)methyl Azide (2c). Column chromatography with 2.5:1 cyclohexane−AcOEt afforded **2c** (208 mg, 66%) as a white foam. $[\alpha]_D = 55.8$ (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz): $\delta = 5.54$ (dd, 1 H, $J_{1,2} = 4.7$ Hz, $J_{2,3} = 5.0$ Hz, H-2), 5.32−5.26 (m, 1 H, H-3), 4.38 (ddd, 1 H, $J_{1,1'a} = 7.2$ Hz, $J_{1,1'b} = 5.5$ Hz, H-1), 4.33−4.24 (m, 2 H, H-4, H-5a), 4.16−4.08 (m, 1 H, H-5b), 3.51 (dd, 1 H, $J_{1'a,1'b} = 13.0$ Hz, H-1′a), 3.40 (dd, 1 H, H-1′b), 2.16, 2.12, and 2.08 (3s, 9 H, CH_3). ^{13}C NMR: $\delta = 170.6$, 169.6, 169.5, 77.8, 77.6, 71.8, 71.5, 63.4, 50.2, 20.8, 20.6, 20.5. MALDI-TOF MS: 338.1 ($M^+ + Na$). Anal. Calcd for $C_{12}H_{17}N_3O_7$ (315.11): C, 45.71; N, 13.33; H, 5.43. Found: C, 45.74; N, 13.35; H, 5.40.

(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)methyl azide (2d). Column chromatography with 2.5:1 cyclohexane−AcOEt afforded **2d** (214 mg, 68%) as a white foam. $[\alpha]_D = -76.3$ (*c* 1.7, $CHCl_3$). ^{1}H NMR (400 MHz): $\delta = 5.18-5.13$ (m, 2 H, H-2, H-3), 4.34 (dd, 1 H, $J_{4,5a} = 2.9$ Hz, $J_{5b,5a} = 11.9$ Hz, H-5a), 4.21 (ddd, 1 H, $J_{3,4} = 5.5$ Hz, $J_{4,5b} = 5.0$ Hz, H-4), 4.15 (dd, 1 H, H-5b), 4.13 (ddd, 1 H, $J_{1,2} = 5.5$ Hz, $J_{1,1'a} = 3.1$ Hz, $J_{1,1'b} = 4.2$ Hz, H-1), 3.60 (dd, 1 H, $J_{1'a,1'b} = 13.3$ Hz, H-1′a), 3.36 (dd, 1 H, H-1′b), 2.11, 2.09, and 2.07 (3s, 9 H, CH_3). ^{13}C NMR: $\delta = 170.6$, 169.7, 169.6, 80.6, 79.4, 71.6, 71.4, 63.2, 51.6, 20.7, 20.5 (2C). MALDI-TOF MS: 338.4 ($M^+ + Na$). Anal. Calcd for $C_{12}H_{17}N_3O_7$ (315.11): C, 45.71; N, 5.43; H, 35.5. Found: C, 45.73; N, 13.34; H, 5.44.

General Procedure for the Synthesis of 1-Glycosylmethyl-5-tosyl tetrazoles 3 and 4. A mixture of either azide **1** or **2** (0.50 mmol) and commercially available *p*-toluensulfonyl cyanide (181 mg, 1.00 mmol) was stirred at 100 °C in the absence of solvent under a nitrogen atmosphere and then cooled to room temperature. The excess of *p*-toluensulfonyl cyanide was then removed by sublimation under vacuum to give nearly pure tetrazole **3** or **4**. Analytical quality samples of **3** and **4** were obtained by eluting the corresponding crude derivative from a column of silica gel with the suitable elution system.

1-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosylmethyl)-5-(*p*-toluensulfonyl)-1*H*-tetrazole (3a). Column chromatography with 4.5:1 cyclohexane−AcOEt afforded **3a** (319 mg, 84%) as a white amorphous solid. $[\alpha]_D = 47.1$ (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz): $\delta = 8.00-7.90$ (m, 2 H, Ar), 7.50−7.20 (m, 20 H, Ar), 7.18−7.10 (m, 2 H, Ar), 5.10 (dd, 1 H, $J_{1,1'a} = 10.3$ Hz, $J_{1'a,1'b} = 14.2$ Hz, H-1′a), 4.89 (dd, 1 H, $J_{1,1'b} = 3.6$ Hz, H-1′b), 4.75 and 4.57 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.74 and 4.67 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.71 and 4.66 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.65 (ddd, 1 H, $J_{1,2} = 4.5$ Hz, H-1), 4.30 and 4.23 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.18−4.08 (m, 2 H, H-4, H-5), 4.03 (dd, 1 H, $J_{2,3} = 7.5$ Hz, H-2), 3.79 (dd, 1 H, $J_{3,4} = 2.6$ Hz, H-3), 3.63 (dd, 1 H, $J_{5,6a} = 6.0$ Hz, $J_{6a,6b} = 10.4$ Hz, H-6a), 3.60 (dd, 1 H, $J_{5,6b} = 7.0$ Hz, H-6b), 2.43 (s, 3 H, CH_3). ^{13}C NMR: $\delta = 155.1$, 147.2, 138.3, 138.1, 137.6, 137.5, 134.4, 130.3 (2C), 129.2 (2C), 127.9−127.0 (20C), 75.2, 75.1, 73.7, 73.6, 73.4, 73.2, 72.9, 72.8, 70.9, 66.9, 47.7, 21.9. MALDI-TOF MS: 783.4 ($M^+ + Na$). Anal. Calcd for $C_{43}H_{44}N_4O_7S$ (760.29): C, 67.88; N, 7.36; H, 5.83; S, 4.21. Found: C, 67.89; N, 7.32; H, 5.81; S, 4.23.

1-(2,3,5-Tri-*O*-benzyl-α-D-ribofuranosylmethyl)-5-(*p*-toluensulfonyl)-1*H*-tetrazole (3c). Column chromatography with 4:1 cyclohexane−AcOEt afforded **3c** (256 mg, 80%) as a white amorphous solid. $[\alpha]_D = 43.6$ (*c* 2.0, $CHCl_3$). ^{1}H NMR (400 MHz): $\delta = 8.00-7.90$ (m, 2 H, Ar), 7.50−7.30 (m, 15 H, Ar), 7.30−7.10 (m, 2 H, Ar), 5.37 (dd, 1 H, $J_{1,1'a} = 9.9$ Hz, $J_{1'a,1'b} = 14.2$ Hz, H-1′a), 4.82 (dd, 1 H, $J_{1,1'b} = 2.8$ Hz, H-1′b), 4.74 and 4.59 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.70 and 4.63 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.64 (ddd, 1 H, $J_{1,2} = 6.5$ Hz, H-1), 4.53 (ddd, 1 H, $J_{3,4} = 2.8$ Hz, $J_{4,5a} = 3.6$ Hz, $J_{4,5b} = 4.0$ Hz, H-4), 4.51 and 4.43 (2 d, 2 H, $J = 12.2$ Hz, PhCH_2), 4.29 (dd, 1 H, $J_{2,3} = 5.5$ Hz, H-2), 4.06 (dd, 1 H, H-3), 3.47 (dd, 1 H, $J_{5a,5b} = 10.5$ Hz, H-5a), 3.44 (dd, 1 H, H-5b), 2.45 (s, 3 H, CH_3). ^{13}C NMR: $\delta = 155.4$, 147.1, 137.9, 137.7, 137.4, 134.2, 130.2 (2C), 129.1 (2C), 128.5−127.6 (15C), 81.6, 78.2, 77.9, 77.8, 73.5, 72.9, 72.4, 70.2, 50.7, 21.8. MALDI-TOF MS: 641.3 ($M^+ + H$). Anal. Calcd for $C_{35}H_{36}N_4O_6S$ (640.24): C, 65.61; N, 8.74; H, 5.66; S, 5.00. Found: C, 65.63; N, 8.71; H, 5.67; S, 5.02.

1-(2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosylmethyl)-5-(*p*-toluensulfonyl)-1*H*-tetrazole (4a). Column chromatography with 2:1 cyclohexane−AcOEt afforded **4a** (267 mg, 94%) as a white amorphous solid. $[\alpha]_D = 59.7$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz): $\delta = 8.01-7.97$ (m, 2 H, Ar), 7.49−7.42 (m, 2 H, Ar), 5.49 (dd, 1 H, $J_{3,4} = 3.3$ Hz, $J_{4,5} = 3.2$ Hz, H-4), 5.43 (dd, 1 H, $J_{1,2} = 4.8$ Hz, $J_{2,3} = 8.5$ Hz, H-2), 5.28 (dd, 1 H, H-3), 5.06 (dd, 1 H, $J_{1,1'a} = 10.8$ Hz, $J_{1'a,1'b} = 14.5$ Hz, H-1′a), 4.95 (dd, 1 H, $J_{1,1'b} = 3.0$ Hz, H-1′b), 4.75 (ddd, 1 H, H-1), 4.35 (ddd, 1 H, $J_{5,6a} = 8.5$ Hz, $J_{5,6b} = 4.5$ Hz, H-5), 4.21 (dd, 1 H, $J_{6a,6b} = 11.8$ Hz, H-6a), 4.04 (dd, 1 H, H-6b), 2.49 (s, 3 H, CH_3), 2.21, 2.11, 2.08 and 1.93 (4s, 12 H, CH_3). ^{13}C NMR: $\delta =$170.6, 169.8, 169.7, 169.6, 154.9, 147.6, 134.1, 130.4 (2C), 129.3 (2C), 70.4, 69.7, 67.3, 67.0, 66.8, 60.4, 46.4, 21.9, 20.7 (2C), 20.6 (2C). MALDI-TOF MS: 607.5 ($M^+ +$ K). Anal. Calcd for $C_{23}H_{28}N_4O_{11}S$ (568.15): C, 48.59; N, 9.85; H, 4.96; S, 5.64. Found: C, 48.57; N, 9.83; H, 4.92; S, 5.66.

1-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosylmethyl)-5-(*p*-toluensulfonyl)-1*H*-tetrazole (4b). Column chromatography with 2:1 cyclohexane−AcOEt afforded **4b** (264 mg, 93%) as a white amorphous solid. $[\alpha]_D = -7.8$ (*c* 1.4, $CHCl_3$). ^{1}H NMR: $\delta = 8.01-7.95$ (m, 2 H, Ar), 7.47−7.42 (m, 2 H, Ar), 5.42 (dd, 1 H, $J_{3,4} = 3.3$ Hz, $J_{4,5} = 0.5$ Hz, H-4), 5.28 (dd, 1 H, $J_{1,2} = 9.8$ Hz, $J_{2,3} = 9.9$ Hz, H-2), 5.13 (dd, 1 H, H-3), 5.08 (dd, 1 H, $J_{1,1'a} = 9.2$ Hz, $J_{1'a,1'b} = 14.5$ Hz, H-1′a), 4.87 (dd, 1 H, $J_{1,1'b} = 2.5$ Hz, H-1′b), 4.05−3.90 (m, 3 H, H-1, 2H-6), 3.79 (ddd, 1 H, $J_{5,6a} = 6.7$ Hz, $J_{5,6b} = 6.8$ Hz, H-5), 2.49 (s, 3 H, CH_3), 2.18, 2.15, 2.01, and 1.94 (4s, 12 H, CH_3). ^{13}C NMR: $\delta = 170.6$, 170.5, 170.4, 170.2, 155.4, 147.6, 134.3, 130.4 (2C), 129.3 (2C), 76.4, 74.2, 71.5, 67.4, 67.3, 61.1, 50.6, 21.9, 20.7, 20.6, 20.5, 20.4. MALDI-TOF MS: 591.4 ($M^+ + Na$). Anal. Calcd for $C_{23}H_{28}N_4O_{11}S$ (568.15): C, 48.59; N, 9.85; H, 4.96; S, 5.64. Found: C, 48.57; N, 9.83; H, 4.92; S, 5.66.

1-(2,3,5-Tri-*O*-acetyl-α-D-ribofuranosylmethyl)-5-(*p*-toluensulfonyl)-1*H*-tetrazole (4c). Column chromatography with 1.5:1 cyclohexane−AcOEt afforded **4c** (243 mg, 98%) as a white amorphous solid. $[\alpha]_D = 48.8$ (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz): $\delta = 8.01-7.97$ (m, 2 H, Ar), 7.47−7.42 (m, 2 H, Ar), 5.56 (dd, 1 H, $J_{1,2} = 5.5$ Hz, $J_{2,3} = 5.2$ Hz, H-2), 5.34 (dd, 1 H, $J_{3,4} = 5.0$ Hz, H-3), 5.23 (dd, 1 H, $J_{1,1'a} = 9.8$ Hz, $J_{1'a,1'b} = 14.5$ Hz, H-1′a), 4.88−4.80 (m, 2 H, H-1, H-1′b), 4.46 (ddd, 1 H, $J_{4,5a} = 3.2$ Hz, $J_{4,5b} = 4.8$ Hz, H-4), 4.22 (dd, 1 H, $J_{5a,5b} = 12.2$ Hz, H-5a), 4.14 (dd, 1 H, H-5b), 2.50 (s, 3 H, CH_3), 2.19, 2.14, and 2.09 (3s, 9 H, CH_3). ^{13}C NMR: $\delta = 170.4$, 169.7, 169.3, 155.5, 147.5, 134.3, 130.3 (2C), 129.2 (2C), 79.1, 77.4, 71.9, 71.0, 63.2, 49.3, 21.9, 20.7, 20.6, 20.4. MALDI-TOF MS: 497.4 ($M^+ + H$). Anal. Calcd for $C_{20}H_{24}N_4O_9S$ (496.13): C, 48.38; N, 11.28; H, 4.87; S, 6.46. Found: C, 48.35; N, 11.26; H, 4.85; S, 6.47.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosylmethyl)-5-(*p*-toluensulfonyl)-1*H*-tetrazole (4d). Column chromatography with 1.5:1 cyclohexane−AcOEt afforded **4d** (223 mg, 90%) as a white amorphous solid. $[\alpha]_D = -23.6$ (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz): $\delta = 8.02-7.97$ (m, 2 H, Ar), 7.45−7.40 (m, 2 H, Ar), 5.19−5.12 (m, 3 H, H-2, H-3, H-1′a), 5.03 (dd, 1 H, $J_{1,1'b} = 5.8$ Hz, $J_{1'a,1'b} = 14.1$ Hz, H-1′b), 4.54−4.48 (m, 1 H, H-1), 4.20 (ddd, 1 H, $J_{3,4} = 4.0$ Hz, $J_{4,5a} = 3.2$ Hz, $J_{4,5b} = 3.0$ Hz, H-4), 4.11 (dd, 1 H, $J_{5a,5b} = 12.3$ Hz, H-5a), 4.06 (dd, 1 H, H-5b), 2.48 (s, 3 H, CH_3), 2.10, 2.08, and 2.03 (3s, 9 H, CH_3). ^{13}C NMR: $\delta = 170.5$, 169.7 (2C), 155.7, 147.5, 134.5, 130.3 (2C), 129.3 (2C), 80.7, 78.1, 71.9, 71.7, 63.0, 50.2, 21.8, 20.7, 20.5, 20.3. MALDI-TOF MS: 519.7 ($M^+ +$ Na). Anal. Calcd for $C_{20}H_{24}N_4O_9S$ (496.13): C, 48.38; N, 11.28; H, 4.87; S, 6.46. Found: C, 48.35; N, 11.26; H, 4.85; S, 6.47.

General Procedure for the Synthesis of Masked Glycosyl Amino Acids 12 (Table 1). To a stirred solution of D-serinol **11** (92 mg, 0.40 mmol) in anhydrous DMF (4 mL) was added NaH (32 mg, 0.80 mmol of a 60% dispersion in oil). The mixture was stirred at room temperature for 30 min and then diluted with a solution of sugar tetrazole **3** (304 mg, 0.40 mmol) in anhydrous DMF (2 mL). The resulting solution was stirred at room temperature

for 16 h, diluted with saturated aqueous NH_4Cl (10 mL), and extracted with AcOEt (3 × 50 mL). The combined organic phases were dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with the suitable elution system to give the corresponding adduct **12**.

(4R)-2,2-Dimethyl-4-[1-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-galactopyranosylmethyl)-1*H*-tetrazol-5-yloxymethyl)]oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (12a). Column chromatography with 3:1 cyclohexane-AcOEt afforded **12a** (268 mg, 80%) as a white foam. $[\alpha]_D = 37.4$ (*c* 1.3, $CHCl_3$). ^1H NMR (DMSO-d_6, 120 °C) selected data: $\delta = 7.40-7.20$ (m, 20 H, Ph), 4.72 and 4.62 (2 d, 2 H, $J =$ 11.5 Hz, PhCH_2), 4.70 and 4.54 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.25−4.18 (m, 1 H), 4.16−4.08 (m, 1 H), 3.64 (d, 2 H, $J = 6.0$ Hz), 1.45 and 1.43 (2s, 6 H), 1.42 (s, 9 H). MALDI-TOF MS: 858.8 ($M^+ + Na$). Anal. Calcd for $C_{47}H_{57}N_5O_9$ (835.42): C, 67.53; N, 8.38; H, 6.87. Found: C, 67.55; N, 8.34; H, 6.81.

(4R)-2,2-Dimethyl-4-[1-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-galactopyranosylmethyl)-1*H*-tetrazol-5-yloxymethyl)]oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (12b). Column chromatography with 3:1 cyclohexane−AcOEt afforded **12b** (284 mg, 85%) as a yellow syrup. $[\alpha]_D = 1.5$ (*c* 2.3, $CHCl_3$). ^1H NMR (DMSO-d_6, 120 °C): $\delta = 7.40-7.10$ (m, 20 H, Ph), 4.92 and 4.56 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.83 and 4.67 (2 d, 2 H, $J = 11.2$ Hz, PhCH_2), 4.81 and 4.72 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.54 and 4.42 (2 d, 2 H, $J =$ 10.5 Hz, PhCH_2), 4.46−4.34 (m, 3 H), 4.28−4.14 (m, 2 H), 4.06 (dd, 1 H, $J = 1.0$ Hz, $J = 2.5$ Hz), 3.92 (d, 2 H, $J = 4.5$ Hz), 3.82−3.72 (m, 3 H), 3.67 (ddd, 1 H, $J = 1.0$ Hz, $J = 6.0$ Hz, $J =$ 7.5 Hz), 3.56 (dd, 1 H, $J = 6.0$ Hz, $J = 10.5$ Hz), 3.46 (dd, 1 H, $J = 6.5$ Hz, $J = 10.5$ Hz), 1.49 and 1.43 (2s, 6 H), 1.42 (s, 9 H). MALDI-TOF MS: 836.7 ($M^+ + H$). Anal. Calcd for $C_{47}H_{57}N_5O_9$ (835.42): C, 67.53; N, 8.38; H, 6.87. Found: C, 67.54; N, 8.33; H, 6.82.

(4*R*)-2,2-Dimethyl-4-[1-(2′,3′,5′-tri-*O*-benzyl-α-D-ribofuranosylmethyl)-1*H*-tetrazol-5-yloxymethyl)]oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (12c). Column chromatography with 3:1 cyclohexane−AcOEt afforded **12c** (237 mg, 83%) as a white foam. $[\alpha]_D = 49.2$ (*c* 1.4, $CHCl_3$). ^1H NMR (DMSO-d_6, 120 °C): $\delta = 7.40-7.20$ (m, 15 H, Ph), 4.75 and 4.62 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.68 and 4.62 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.57 (dd, 1 H, $J = 4.5$ Hz, $J = 10.5$ Hz), 4.52−4.30 (m, 5 H), 4.48 (s, 2 H, PhCH_2), 4.22−4.15 (m, 2 H), 4.12 (t, 1 H, $J = 4.5$ Hz), 4.00−3.88 (m, 2 H), 3.51 (d, 2 H, $J = 5.5$ Hz), 1.45 and 1.43 (2s, 6 H), 1.42 (s, 9 H). MALDI-TOF MS: 836.7 ($M^+ + K$). Anal. Calcd for $C_{39}H_{49}N_5O_8$ (715.36): C, 65.44; N, 9.78; H, 6.90. Found: C, 65.44; N, 9.72; H, 6.93.

(4*R*)-2,2-Dimethyl-4-[1-(2′,3′,5′-tri-*O*-benzyl-β-D-ribofuranosylmethyl)-1*H*-tetrazol-5-yloxymethyl)]oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (12d). Column chromatography with 3:1 cyclohexane−AcOEt afforded **12d** (223 mg, 78%) as a white foam. $[\alpha]_D = -3.4$ (*c* 1.3, $CHCl_3$). ^1H NMR (DMSO-d_6, 120 °C): $\delta = 7.40-7.20$ (m, 15 H, Ph), 4.62 and 4.53 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.58 (dd, 1 H, $J = 3.2$ Hz, $J = 10.0$ Hz), 4.56 (s, 2 H, PhCH_2), 4.48 (s, 2 H, PhCH_2), 4.44 (dd, 1 H, $J = 7.0$ Hz, $J = 10.0$ Hz), 4.34−4.18 (m, 4 H), 4.14−4.08 (m, 1 H), 4.02−3.94 (m, 4 H), 3.47 (dd, 1 H, $J = 4.5$ Hz, $J = 10.5$ Hz), 3.43 (dd, 1 H, $J =$ 5.0 Hz, $J = 10.5$ Hz), 1.48 and 1.44 (2s, 6 H), 1.42 (s, 9 H). MALDI-TOF MS: 738.4 ($M^+ + Na$). Anal. Calcd for $C_{39}H_{49}N_5O_8$ (715.36): C, 65.44; N, 9.78; H, 6.90. Found: C, 65.41; N, 9.73; H, 6.92.

General Procedure for the Synthesis of Glycosylmethyl Tetrazole Serines 5 (Table 1). To a stirred solution of adduct **12** (167 mg, 0.20 mmol) in MeCN (2 mL) was added $BiBr_3$ (9 mg, 0.02 mmol) in one portion. The resulting mixture was stirred at room temperature for 6 h, diluted with saturated aqueous $NaHCO_3$ solution (5 mL), and extracted with AcOEt (3 × 25 mL). The combined organic phases were dried (Na_2SO_4), and concentrated to give the corresponding crude *N*-Boc amino alcohol.

To a cooled (0 °C), stirred solution of the above crude *N*-Boc amino alcohol (159 mg, ∼0.20 mmol) in acetone (6 mL) freshly prepared 1 M Jones reagent (0.60 mL, 0.60 mmol) was added in one portion. The mixture was allowed to warm to room temperature in 30 min, stirred at room temperature for an additional 1.5 h, and then diluted with *i*-PrOH (0.5 mL). The suspension was neutralized with saturated aqueous $NaHCO_3$, diluted with AcOEt (80 mL), and then washed with brine (2 × 5 mL). The organic phase was dried (Na_2SO_4) and concentrated to afford the corresponding crude carboxylic acid derivative.

To a stirred, cooled (0 °C) solution of the above crude carboxylic acid in CH_2Cl_2 (2.0 mL) was added an ethereal solution of CH_2N_2 until a pale yellow color persisted. The solution was stirred for an additional 15 min at 0 °C and then dried under vacuum. The residue was eluted from a column of silica gel with the suitable elution system to give the corresponding glycosylmethyl tetrazole serine **5**.

(2*S*)-2-*tert*-Butoxycarbonylamino-3-[1-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-galactopyranosylmethyl)-1*H*-tetrazol-5-yloxy]propionic Acid Methyl Ester (5a). Column chromatography with 2.5:1 cyclohexane−AcOEt afforded **5a** (102 mg, 62%) as a white foam. $[\alpha]_D = 16.1$ (*c* 1.1, $CHCl_3$). ^1H NMR (400 MHz): $\delta = 7.44-7.14$ (m, 20 H, Ph), 6.07 (d, 1 H, $J_{2,NH} = 7.5$ Hz, NH), 4.80−4.62 (m, 5 H, 2H-3, 3 C*H*Ph), 4.62−4.46 (m, 4 H, H-2, 3 C*H*Ph), 4.45−4.36 (m, 1 H, H-1′), 4.35 (s, 2 H, CH_2Ph), 4.25 (d, 2 H, $J = 8.0$ Hz, 2H-1″), 4.16−4.09 (m, 1 H, H-5′), 4.02−3.94 (m, 2 H, H-2′, H-4′), 3.76 (dd, 1 H, $J_{2',3'} = 7.6$ Hz, $J_{3',4'} = 2.7$ Hz, H-3′), 3.70−3.60 (m, 1 H, H-6′a), 3.68 (s, 3 H, CH_3), 3.54 (dd, 1 H, $J_{5',6'b} = 4.5$ Hz, $J_{6'a,6'b} = 11.5$ Hz, H-6′b), 1.43 (s, 9 H, *t*-Bu). ^{13}C NMR: $\delta = 169.5$, 161.1, 155.3, 138.0 (2C), 137.7, 137.6, 128.6−127.4 (20C), 80.2, 75.3, 73.8, 73.5, 73.4, 73.3, 73.0, 72.9, 72.3, 70.5, 68.1, 53.1, 52.7, 43.6, 29.7, 28.3 (3C). MALDI-TOF MS: 846.7 ($M^+ + Na$). Anal. Calcd for $C_{45}H_{53}N_5O_{10}$ (823.38): C, 65.60; N, 8.50; H, 6.48. Found: C, 65.62; N, 8.53; H, 6.41.

(2*S*)-2-*tert*-Butoxycarbonylamino-3-[1-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-galactopyranosylmethyl)-1*H*-tetrazol-5-yloxy]propionic Acid Methyl Ester (5b). Column chromatography with 3:1 cyclohexane−AcOEt afforded **5b** (99 mg, 60%) as a white foam. $[\alpha]_D = 4.5$ (*c* 1.0, $CHCl_3$). ^1H NMR (400 MHz): $\delta = 7.45-7.10$ (m, 20 H, Ph), 5.85 (d, 1 H, $J_{2,NH} = 8.5$ Hz, NH), 5.02 and 4.74 (2 d, 2 H, $J = 11.2$ Hz, PhCH_2), 4.90 and 4.52 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.80−4.69 (m, 2 H, 2H-3), 4.76 and 4.66 (2 d, 2 H, $J =$ 11.8 Hz, PhCH_2), 4.66−4.59 (m, 1 H, H-2), 4.42 and 4.36 (2 d, 2 H, $J = 11.8$ Hz, PhCH_2), 4.32 (dd, 1 H, $J_{1',1''a} = 2.8$ Hz, $J_{1''a,1''b} =$ 14.1 Hz, H-1″a), 4.09 (dd, 1 H, $J_{1',1''b} = 8.0$ Hz, H-1″b), 3.96 (dd, 1 H, $J_{3',4'} = 2.6$ Hz, $J_{4',5'} = 0.5$ Hz, H-4′), 3.80 (dd, 1 H, $J_{1',2'} = 9.0$ Hz, $J_{2',3'} = 9.5$ Hz, H-2′), 3.67 (dd, 1 H, H-3′), 3.65 (ddd, 1 H, H-1′), 3.60 (s, 3 H, CH_3), 3.52−3.45 (m, 3 H, H-5′, 2H-6′), 1.45 (s, 9 H, *t*-Bu). ^{13}C NMR: $\delta = 169.4$, 161.5, 155.1, 138.5, 138.0, 137.9, 137.8, 129.0−127.0 (20C), 84.6, 80.5, 77.4, 76.6, 75.7, 75.1, 74.6, 73.4, 72.1, 68.5, 53.2, 52.8, 47.0, 26.7, 28.3 (3C). MALDI-TOF MS: 824.2 ($M^+ + H$). Anal. Calcd for $C_{45}H_{53}N_5O_{10}$ (823.38): C, 65.60; N, 8.50; H, 6.48. Found: C, 65.62; N, 8.53; H, 6.41.

(2*S*)-2-*tert*-Butoxycarbonylamino-3-[1-(2′,3′,5′-tri-*O*-benzyl-α-D-ribofuranosylmethyl)-1*H*-tetrazol-5-yloxy]propionic Acid Methyl Ester (5c). Column chromatography with 2:1 cyclohexane−AcOEt afforded **5c** (94 mg, 67%) as a white foam. $[\alpha]_D = 44.9$ (*c* 1.2, $CHCl_3$). ^1H NMR (400 MHz): $\delta = 7.40-7.20$ (m, 15 H, Ph), 5.63 (d, 1 H, $J_{2,NH} = 8.5$ Hz, NH), 4.83 (d, 2 H, $J = 3.0$ Hz, 2H-3), 4.71 and 4.52 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.74−4.67 (m, 1 H, H-2), 4.67 and 4.61 (2 d, 2 H, $J = 12.0$ Hz, PhCH_2), 4.50−4.34 (m, 6 H, 2H-1", H-1′, H-4′, PhCH_2), 4.22 (dd, 1 H, $J_{1',2'} = 5.5$ Hz, $J_{2',3'} = 5.3$ Hz, H-2′), 4.02 (dd, 1 H, $J_{3',4'} = 3.5$ Hz, H-3′), 3.66 (s, 3 H, CH_3), 3.52 (dd, 1 H, $J_{4',5'a} = 4.5$ Hz, $J_{5'a,5'b} = 10.5$ Hz, H-5′a), 3.47 (dd, 1 H, $J_{4',5'b} = 4.0$ Hz, H-5′b), 1.41 (s, 9 H, *t*-Bu). ^{13}C NMR: $\delta = 169.5$, 161.5, 155.1, 137.8, 137.6, 137.5, 128.5−127.5 (15C), 81.4, 80.5, 77.9, 77.6, 73.5, 72.9, 72.8, 72.3, 70.3, 53.2, 52.8, 46.4, 28.2 (4C). MALDI-TOF MS: 742.8 ($M^+ + K$). Anal. Calcd for $C_{37}H_{45}N_5O_9$ (703.32): C, 63.14; N, 9.95; H, 6.44. Found: C, 63.17; N, 9.91; H, 6.45.

(2*S*)-2-*tert*-Butoxycarbonylamino-3-[1-(2′,3′,5′-tri-*O*-benzyl-β-D-ribofuranosylmethyl)-1*H*-tetrazol-5-yloxy]propionic Acid Methyl Ester (5d). Column chromatography with 2:1 cyclohexane−AcOEt afforded **5d** (86 mg, 61%) as a white foam. $[\alpha]_D = -38.5$ (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 7.50−7.10 (m, 15 H, Ph), 5.83 (d, 1 H, $J_{2,NH}$ = 8.5 Hz, NH), 4.89 (dd, 1 H, $J_{2,3a}$ = 2.8 Hz, $J_{3a,3b}$ = 10.3 Hz, H-3a), 4.62 (ddd, 1 H, $J_{2,3b}$ = 3.4 Hz, H-2), 4.95 and 4.55 (2 d, 2 H, *J* = 11.5 Hz, PhCH_2), 4.54 (dd, 1 H, H-3b), 4.45 (s, 2 H, PhCH_2), 4.38 and 4.20 (2 d, 2 H, *J* = 12.0 Hz, PhCH_2), 4.33−4.27 (m, 3 H, H-1′, 2H-1"), 4.16 (ddd, 1 H, $J_{3',4'}$ = 2.2 Hz, $J_{4',5'a}$ = 3.8 Hz, $J_{4',5'b}$ = 3.3 Hz, H-4′), 3.84 (dd, 1 H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 5.0 Hz, H-2′), 3.75 (s, 3 H, CH_3), 3.63 (dd, 1 H, H-3′), 3.17 (dd, 1 H, $J_{5'a,5'b}$ = 10.2 Hz, H-5′a), 3.13 (dd, 1 H, H-5′b), 1.48 (s, 9 H, *t*-Bu). ^{13}C NMR: δ = 169.6, 161.6, 155.3, 137.5 (3C), 128.5−127.6 (15C), 82.3, 80.4, 78.1, 78.0, 77.2, 73.3, 72.8, 71.8, 69.3, 53.0, 52.9, 45.6, 28.3 (4C). MALDI-TOF MS: 726.4 (M^+ + Na). Anal. Calcd for $C_{37}H_{45}N_5O_9$ (703.32): C, 63.14; N, 9.95; H, 6.44. Found: C, 63.13; N, 9.94; H, 6.43.

(2*S*)-2-*tert*-Butoxycarbonylamino-3-[1-(β-D-galactopyranosylmethyl)-1*H*-tetrazol-5-yloxy]propionic Acid Methyl Ester (5b′). A vigorously stirred mixture of **5b** (165 mg, 0.20 mmol), 20% palladium hydroxide on carbon (80 mg), and MeOH (4 mL) was degassed under vacuum and saturated with hydrogen (by an H_2-filled balloon) three times. The suspension was stirred at room temperature for 4 h under a slightly positive pressure of hydrogen (balloon) and then filtered off through a plug of cotton and washed thoroughly with MeOH (5 mL) and water (2 mL). The combined filtrates were concentrated to afford crude **5b′** (88 mg, 95%) at least 90% pure as established by ^{1}H NMR analysis. ^{1}H NMR (CD_3OD): δ = 4.83−4.75 (m, 2 H, 2H-3), 4.72−4.65 (m, 1 H, H-2), 4.51 (dd, 1 H, $J_{1',1''a}$ = 3.5 Hz, $J_{1''a,1''b}$ = 14.0 Hz, H-1″a), 4.31 (dd, 1 H, $J_{1',1''b}$ = 7.5 Hz, H-1"b), 3.85 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, $J_{4',5'}$ = 0.5 Hz, H-4′), 3.70−3.40 (m, 6 H, H-1′, H-2′, H-3′, H-5′, 2H-6′), 3.77 (s, 3 H, CH_3), 1.45 (s, 9 H, *t*-Bu). ^{13}C NMR (CD_3OD): δ = 169.6, 161.1, 155.8, 78.8, 77.5, 74.7, 71.9, 69.2, 68.9, 61.1, 53.1, 51.9, 47.1, 27.4 (4C). MALDI-TOF MS: 464.8 (M^+ + H), 486.1 (M^+ + Na).

General Procedure for the Synthesis of Glycosylmethyl Tetrazole Cysteines 6 (Table 2). To a stirred mixture of glycosylmethyl tetrazole **4** (142 mg, 0.25 mmol), K_2CO_3 (103 mg, 0.75 mmol), and anhydrous MeCN (2.5 mL) was added cysteine **13** (45 mg, 0.13 mmol) in one portion. The resulting mixture was stirred at room temperature for 2 h, and then a second portion of cysteine **13** (45 mg, 0.13 mmol) was added. The mixture was stirred for an additional 3 h and then concentrated. The resulting residue was eluted from a column of silica gel with the suitable elution system to give the corresponding glycosylmethyl tetrazole cysteine **6**.

(2*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-[1-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-galactopyranosylmethyl)-1*H*-tetrazol-5-ylsulfanyl)propionic Acid (6a). Column chromatography with 32.3:1 AcOEt−AcOH afforded **6a** (174 mg, 92%) as a white amorphous solid. $[\alpha]_D = 47.7$ (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 7.79−7.73 (m, 2 H, Ar), 7.64−7.55 (m, 2 H, Ar), 7.44−7.36 (m, 2 H, Ar), 7.35−7.28 (m, 2 H, Ar), 6.44 (d, 1 H, $J_{2,NH}$ = 6.4 Hz, NH), 5.45 (dd, 1 H, *J* = 3.0 Hz, *J* = 3.5 Hz, H-4′), 5.34−5.21 (m, 2 H, H-2′, H-3′), 4.82−4.68 (m, 1 H, H-2), 4.66−4.57 (m, 1 H, H-1′), 4.57−4.43 (m, 2 H, 2H-1″), 4.43−4.27 (m, 4 H, FmocCH_2, 2H-6′), 4.27−4.19 (m, 1 H, FmocC*H*), 3.97−3.77 (m, 3 H, 2H-3, H-5′), 2.15, 2.09, and 1.97 (3s, 12 H, CH_3). ^{13}C NMR: δ = 171.3, 169.7 (2C), 169.6 (2C), 156.3, 154.1, 143.6 (2C), 141.3 (2C), 127.8 (2C), 127.1 (2C), 125.2 (2C), 120.0 (2C), 70.5, 68.6, 67.8, 67.5, 67.3, 66.3, 59.9, 47.0, 45.8, 36.0, 20.7 (2C), 20.6 (2C). MALDI-TOF MS: 778.4 (M^+ + Na). Anal. Calcd for $C_{34}H_{37}N_5O_{13}S$ (755.21): C, 54.03; N, 9.27; H, 4.93; S, 4.24. Found: C, 54.01; N, 9.26; H, 4.94; S, 4.23.

(2*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-[1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosylmethyl)-1*H*-tetrazol-5-ylsulfanyl)propionic Acid (6b). Column chromatography with 24:1 AcOEt−AcOH afforded **6b** (170 mg, 90%) as a white amorphous solid. $[\alpha]_D = 15.8$ (*c* 1.2, $CHCl_3$). ^{1}H NMR: δ = 7.80−7.73 (m, 2 H, Ar), 7.63−7.55 (m, 2 H, Ar), 7.45−7.35 (m, 2 H, Ar), 7.35−7.27 (m, 2 H, ar), 6.39 (d, 1 H, $J_{2,NH}$ = 6.5 Hz, NH), 5.40 (dd, 1 H, $J_{3',4'}$ = 3.0 Hz, $J_{4',5'}$ = 0.5 Hz, H-4′), 5.15 (dd, 1 H, $J_{1',2'}$ = 9.5 Hz, $J_{2',3'}$ = 9.8 Hz, H-2′), 5.05 (dd, 1 H, H-3′), 4.81−4.70 (m, 1 H, H-2), 4.50−4.26 (m, 4 H, 2H-1″, FmocCH_2), 4.22 (t, 1 H, *J* = 7.0 Hz, FmocC*H*), 4.04 (dd, 1 H, $J_{5',6'a}$ = 6.5 Hz, $J_{6'a,6'b}$ = 11.0 Hz, H-6′a), 3.98−3.80 (m, 3 H, H-3a, H-1′, H-6′b), 3.79−3.61 (m, 2 H, H-3b, H-5′), 2.10, 2.00, and 1.90 (3s, 12 H, CH_3). ^{13}C NMR: δ = 171.4, 171.0, 170.1, 170.0, 169.9, 156.2, 155.4, 143.6 (2c), 141.3 (2C), 127.8 (2C), 127.1 (2C), 125.1 (2C), 120.0 (2C), 76.2, 74.2, 71.4, 67.4, 67.2, 67.1, 61.3, 53.8, 48.7, 47.0, 35.7, 20.7, 20.6, 20.5 (2C). MALDI-TOF MS: 801.6 (M^+ + K). Anal. Calcd for $C_{34}H_{37}N_5O_{13}S$ (755.21): C, 54.03; N, 9.27; H, 4.93; S, 4.24. Found: C, 54.03; N, 9.25; H, 4.91; S, 4.24.

(2*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-[1-(2′,3′,5′-tri-*O*-acetyl-α-D-ribofuranosylmethyl)-1*H*-tetrazol-5-ylsulfanyl)propionic Acid (6c). Column chromatography with 19:1 AcOEt−AcOH afforded **6c** (155 mg, 91%) as a white amorphous solid. $[\alpha]_D = 36.8$ (*c* 1.1, $CHCl_3$). ^{1}H NMR: δ = 7.74−7.60 (m, 2 H, Ar), 7.56−7.40 (m, 2 H, Ar), 7.37−7.20 (m, 2 H, Ar), 7.29−7.10 (m, 2 H, Ar), 6.42 (d, 1 H, $J_{2,NH}$ = 7.5 Hz, NH), 5.49 (dd, 1 H, $J_{1',2'}$ = 5.0 Hz, $J_{2',3'}$ = 5.5 Hz, H-2′), 5.27 (dd, 1 H, $J_{3',4'}$ = 5.5 Hz, H-3′), 4.74 (ddd, 1 H, $J_{2,3a}$ = 3.5 Hz, $J_{2,3b}$ = 6.5 Hz, H-2), 4.59 (ddd, 1 H, $J_{1',1''a}$ = 6.0 Hz, $J_{1',1''b}$ = 6.0 Hz, H-1′), 4.46 (d, 2 H, 2H-1″), 4.42−4.28 (3 H, H-4′, FmocCH_2), 4.22−4.12 (m, 2 H, H-5′a, FmocC*H*), 4.07 (dd, 1 H, $J_{4',5'a}$ = 4.5 Hz, $J_{5'a,5'b}$ = 8.5 Hz, H-5′b), 3.93 (dd, 1 H, $J_{3a,3b}$ = 14.5 Hz, H-3a), 3.76 (dd, 1 H, H-3b), 2.11, 2.05, and 2.01 (3s, 9 H, CH_3). ^{13}C NMR: δ = 171.8, 170.8, 169.6, 169.5, 156.2, 154.6, 143.6, 143.5, 141.2, 141.1, 127.7 (2C), 127.1 (2C), 125.1 (2C), 120.0 (2C), 78.5, 77.2, 71.9, 71.1, 67.3, 63.2, 54.0, 47.3, 46.9, 35.8, 20.7, 20.5 (2C). MALDI-TOF MS: 684.6 (M^+ + H). Anal. Calcd for $C_{31}H_{33}N_5O_{11}S$ (683.19): C, 54.46; N, 10.24; H, 4.87; S, 4.69. Found: C, 54.48; N, 10.23; H, 4.81; S, 4.64.

(2*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-[1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosylmethyl)-1*H*-tetrazol-5-ylsulfanyl)propionic Acid (6d). Column chromatography with 24:1 AcOEt−AcOH afforded **6d** (154 mg, 90%) as a white foam. $[\alpha]_D = -17.4$ (*c* 1.2, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 7.77−7.68 (m, 2 H, Ar), 7.65−7.55 (m, 2 H, Ar), 7.41−7.35 (m, 2 H, Ar), 7.30−7.25 (m, 2 H, Ar), 6.55 (d, 1 H, $J_{2,NH}$ = 7.5 Hz, NH), 5.05 (dd, 1 H, $J_{2',3'}$ = 5.5 Hz, $J_{3',4'}$ = 4.0 Hz, H-3′), 4.96 (dd, 1 H, $J_{1',2'}$ = 7.5 Hz, H-2′), 4.73 (ddd, 1 H, $J_{2,3a}$ = 3.5 Hz, $J_{2,3b}$ = 7.0 Hz, H-2), 4.55 (dd, 1 H, $J_{1',1''a}$ = 4.0 Hz, $J_{1''a,1''b}$ = 14.5 Hz, H-1"a), 4.50 (dd, 1 H, $J_{1',1''b}$ = 4.5 Hz, H-1″b), 4.40−4.30 (m, 3 H, H-1′, FmocCH_2), 4.25−4.10 (m, 3 H, H-4′, H-5′a, FmocC*H*), 4.05 (dd, 1 H, $J_{4',5'b}$ = 3.5 Hz, $J_{5'a,5'b}$ = 12.0 Hz, H-5′b), 3.83 (dd, 1 H, $J_{3a,3b}$ = 14.5 Hz, H-3), 3.73 (dd, 1 H, H-3b), 1.99, 2.06, and 2.07 (3s, 9 H, CH_3). ^{13}C NMR: δ = 171.9, 170.9, 169.8 (2C), 156.3, 155.4, 143.6 (2C), 141.2 (2C), 127.7 (2C), 127.1 (2C), 125.2 (2C), 119.9 (2C), 80.6, 78.2, 71.4, 67.5, 63.2, 53.9, 48.1, 47.0, 35.6, 20.7, 20.5, 20.4. MALDI-TOF MS: 706.4 (M^+ + Na). Anal. Calcd for $C_{31}H_{33}N_5O_{11}S$ (683.19): C, 54.46; N, 10.24; H, 4.87; S, 4.69. Found: C, 54.44; N, 10.21; H, 4.87; S, 4.63.

Dipetide (15). To a cooled (0 °C), stirred solution of crude acid **14** (81 mg, ∼0.10 mmol), L-phenylalanine ethyl ester hydrochloride (34 mg, 0.15 mmol), and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (62 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (52 μL, 0.30 mmol). The solution was warmed to room temperature, stirred for an additional 2 h, and then concentrated. The residue was suspended with AcOEt (80 mL) and washed with H_2O (2 × 10 mL). The organic phase was dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with 1:1 cyclohexane−AcOEt to give dipeptide **15** (79 mg, 80%) as a white foam. $[\alpha]_D = 5.5$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz) selected data: δ = 7.40−7.00 (m, 25 H, Ar), 6.90 (bs, 1 H, NH), 5.60 (bs, 1 H, NH), 5.01 and 4.70 (2 d, 2 H, *J* = 11.5 Hz, PhCH_2), 4.88 and 4.72 (2 d, 2 H, *J* = 12.0 Hz, PhCH_2), 4.42 and 4.34 (2 d, 2 H, *J* = 11.8 Hz, PhCH_2),

4.08 (q, 2 H, $J = 7.0$ Hz, CH_2CH_3), 3.86 (dd, 1 H, $J_{3,4} = 3.5$ Hz, $J_{4,5} = 0.5$ Hz, H-4_{sug}), 3.81 (dd, 1 H, $J_{1,2} = 9.0$ Hz, $J_{2,3} = 9.5$ Hz, H-2_{sug}), 3.68 (ddd, 1 H, $J_{1,1'a} = 1.5$ Hz, $J_{1,1'b} = 2.0$ Hz, H-1_{sug}), 3.62 (dd, 1 H, H-3_{sug}), 3.52−3.46 (m, 3 H, H-5_{sug}, 2H-6_{sug}), 3.06 (dd, 1 H, $J = 6.0$ Hz, $J = 14.5$ Hz), 2.98 (dd, 1 H, $J = 7.0$ Hz, $J = 14.5$ Hz), 1.40 (s, 9 H, *t*-Bu), 1.18 (t, 3 H, $J = 7.0$ Hz, CH_2CH_3). MALDI-TOF MS: 985.8 ($M^+ + H$). Anal. Calcd for $C_{55}H_{64}N_6O_{11}$ (984.46): C, 67.06; N, 8.53; H, 6.55. Found: C, 67.03; N, 8.52; H, 6.58.

Tripeptide (16). To a cooled (0 °C), stirred solution of dipeptide **15** (79 mg, 0.08 mmol) in CH_2Cl_2 (2.0 mL) was slowly added a solution of TFA−CH_2Cl_2 (0.50 mL−1.50 mL). Stirring was continued at 0 °C for an additional 30 min, and then the solution was warmed to room temperature. After 30 min at room temperature, the solution was neutralized at 0 °C with saturated aqueous Na_2CO_3 and extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were dried (Na_2SO_4) and concentrated to give the corresponding crude free amine (70 mg), which was used for the following reaction without any purification.

To a cooled (0 °C), stirred solution of the above crude amine (70 mg, ∼0.08 mmol), *tert*-butoxycarbonyl-L-alanine (22 mg, 0.12 mmol), and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (73 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (2.0 mL) was added *N*,*N*-diisopropylethylamine (60 μL, 0.35 mmol). The solution was warmed to room temperature, stirred for an additional 2 h, and then concentrated. The residue was suspended with AcOEt (80 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4), concentrated, and eluted from a column of silica gel with 1.5:1 cyclohexane−AcOEt to give tripeptide **16** (60 mg, 72% from **15**). $[\alpha]_D = -14.8$ (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz) selected data: $\delta = 7.40-7.00$ (m, 27 H, Ar, 2 NH), 5.08 (bs, 1 H, NH), 5.00 and 4.70 (2 d, 2 H, $J = 11.5$ Hz, PhCH_2), 4.80 and 4.56 (2 d, 2 H, $J = 11.0$ Hz, PhCH_2), 4.09 (q, 2 H, $J = 7.0$ Hz, CH_2CH$_3$), 3.98 (dd, 1 H, $J_{3,4} = 3.5$ Hz, $J_{4,5} = 0.5$ Hz, H-4_{sug}), 3.80 (dd, 1 H, $J_{1,2} = 9.0$ Hz, $J_{2,3} = 9.5$ Hz, H-2_{sug}), 3.73−3.64 (m, 2 H, H-1_{sug}, H-3_{sug}), 3.52−3.40 (m, 3 H, H-5s, 2H-6s), 3.10 (dd, 1 H, $J = 6.0$ Hz, $J = 14.5$ Hz), 3.00 (dd, 1 H, $J = 7.0$ Hz, $J = 14.5$ Hz), 1.40 (s, 9 H, *t*-Bu), 1.28 (d, 3 H, $J = 7.0$ Hz, CH_3), 1.17 (t, 3 H, $J = 7.0$ Hz, CH_2CH_3). MALDI-TOF MS: 1056.9 ($M^+ + H$). Anal. Calcd for $C_{58}H_{69}N_7O_{12}$ (1055.50): C, 65.95; N, 9.28; H, 6.58. Found: C, 65.93; N, 9.23; H, 6.56.

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Supporting Information Available: General experimental methods and NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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