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Glycopeptides and Glycoproteins: Focus on the Glycosidic Linkage

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Dedicated to Professor Ralph Hirschmann and Professor Harold W. Heine on the occasions of their 75th birthdays.

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Contents

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Abbreviations

Amino Acids: Ala (alanine), Arg (arginine), Asn (asparagine), Asp (aspartic acid), Cys (cysteine), Gln (glutamine), Glu (glutamic acid), His (histidine), Hyl (hydroxylysine), Hyp (hydroxyproline), Ile (isoleucine), Leu (leucine), Lys (lysine), Met (methionine), Phe (phenylalanine), Pro (proline), Ser (serine), Thr (threonine), Trp (tryptophan), Tyr (tyrosine), Val (valine).

Monosaccharides: Ara (arabinose), Fuc (fucose), Gal (galactose), GalNAc (2-*N*-acetyl-2-deoxygalactose), Glc (glucose), GlcNAc (2-*N*-acetyl-2-deoxyglucose), Man (mannose), Rha (rhamnose), Xyl (xylose).

Other Abbreviations: Ac (acetyl), AcHmb (*N*-2-acetoxy-4-methoxybenzyl), AFGP (antifreeze glycoprotein), AIBN (2,2'-azobisisobutyronitrile), Alloc (allyloxycarbonyl), Ar (aryl group), Bn (benzyl - CH_2Ph), Boc (*tert*-butoxycarbonyl), BOP [benzotriazolyl-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate], ^{t}Bu [*tert*-butyl, $C(CH_3)_3$], Bz [benzoyl, C(=O)Ph], *C* (carbon), Cbz (carbobenzyloxy), DCC (dicyclohexylcarbodiimide), DHQD (dihydroquinidine), DIC (diisopropylcarbodiimide), DMAP (4-dimethylaminopyridine), DME (dimethoxyethane), DMF (dimethylformamide), DMSO (dimethylsulfoxide), DMTST [dimethyl(methylthio)sulfonium triflate], DPPA (diphenylphosphoryl azide), EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide], EEDQ (2-ethoxy-1-ethoxylcarbonyl-1,2-dihydroquinoline, ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate), eq. (equivalent/s), Et (ethyl, CH_3CH_2), FAB (fast atom bombardment), Fmoc (fluorenylmethoxycarbonyl), HATU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate], HBTU (*O*-benzotrioazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate)), Hep [heptyl, $CH_3(CH_2)_6$], HMPA (hexamethylphosphoramide), HOAt (1-hydroxy-7-azabenzotriazole), HOBt (hydroxybenztriazole), ^{i}Pr [isopropyl, $CH(CH_3)_2$], LH-RH (lutenizing hormone - releasing hormone), Me (methyl, CH_3), NBS (*N*-bromosuccinimide), NIS (*N*-iodosuccinimide), NMM (*N*-methylmorpholine), NMR (nuclear magnetic resonance), OT (oligosaccharyl transferase), pfp (pentafluorophenyl), Ph (phenyl, C_6H_5), PhAcOx (*p*-phenylacetoxybenzyloxycarbonyl), Pht (phthalimido), Piv [pivaloyl, $(CH_3)_3CCO$], R (alkyl or carbohydrate group), RNA (ribonucleic acid), RNAse (ribonuclease), RT (room temperature), SLe^x (sialyl Lewis x), TBDPS (*tert*-butyldiphenylsilyl), TFA (trifluoroacetic acid), Tf_2O (triflic anhydride), THF (tetrahydrofuran), TMSOTf (trimethylsilyltriflate), TsOH (*p*-toluenesulfonic acid), X (a good leaving group)

1. INTRODUCTION

Glycopeptides are a rapidly growing family of molecules which contain a carbohydrate domain and a peptide domain. Glycoproteins are larger variants which contain more than about 50 amino acids per peptide (or protein) component. The carbohydrate can be a single monosaccharide or a complex, possibly branched, oligosaccharide containing up to about 20 monosaccharide units.

The biological functions of glycopeptides are many and varied, and scientists have probably only uncovered a fraction of these novel biomolecules to-date. The carbohydrate often alters the structure and function of the peptide/protein. Carbohydrates exposed on the surface of a protein can serve as recognition elements - either on the molecular level, between molecules and cells, or on an intercellular level. The nature, and indeed existence of the carbohydrate can vary during the life cycle of a cell, as a sophistocated battery of glycosyl transfer enzymes operate on it.

The level of interest in this field has grown exponentially in the past 10-15 years. As a consequence several excellent reviews have appeared; some have have focussed on structure,[1-4] others on function,[5,6] biosynthesis,[7] and chemical synthesis.[8-11] The intention of this review is to concentrate solely on the glycosidic linkage (*i.e.*, the crucial covalent bond/s which join the carbohydrate and the peptide/protein).

In order to investigate fully the role of glycopeptides in biology, and begin to manipulate their behaviour for medical purposes, we need to identify first the detailed structure of the glycoconjugates in pertinent biological systems. Discoveries and knowledge in this area are discussed in §2, with emphasis on the carbohydrate-protein linkage. An effort has been made to include unusual glycopeptides, as well as the more common, and arguably more important, types of glycosidic linkages.

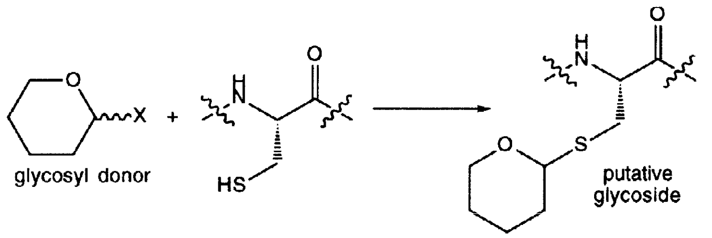
The structure elucidation of these large, complex molecules is often made difficult by the small amounts of material available from nature. Moreover, nature tends to produce heterogeneous glycoproteins which vary in many aspects, including the extent of glycosylation. Separating individual compounds from such families of closely related compounds is not trivial.

Studies of the behaviour of glycopeptides in biochemical systems are greatly facilitated by milligram quantities of prototypical compounds in homogeneous form. To this end, chemical synthesis plays an important part. In §3, the methods used by chemists to form glycosidic linkages to peptides will be surveyed. It will become evident, particularly in the field of *C*-glycosides, that many compounds have been prepared by chemical synthesis which have no counterparts in nature. These "glycopeptidomimetics" are potentially very useful because they emulate the natural compounds, but have enhanced stability.

2. GLYCOSIDIC LINKAGES IN NATURE

Of the 20 coded amino acids, seven contain either no side chain (Gly), or purely hydrocarbon side chains (Ala, Val, Leu, Ile, Phe, Pro). No evidence has so far been found for *C*-glycosylation of the side chains of these residues.

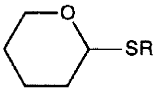
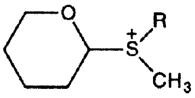
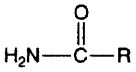
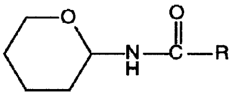
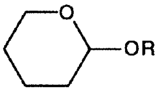
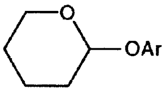
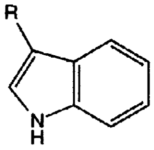
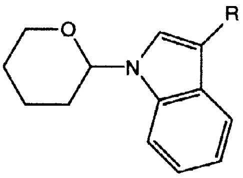
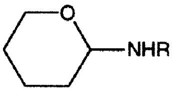
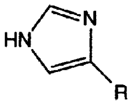
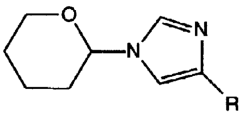
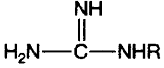
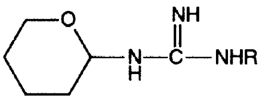
The remaining 13 coded amino acids contain various functional groups in their side chains, which suggest possible sites for glycosylation. These possibilities are summarized in Table 1. For example, the first entry is for Cys; this amino acid has a thiol in its side chain. If the thiol were pyranosylated, this would give rise to the putative glycoside shown in Scheme 1. This kind of glycosidic linkage is known in nature, as indicated in the final column of Table 1.



Scheme 1. Glycosylation of a Cys Residue.

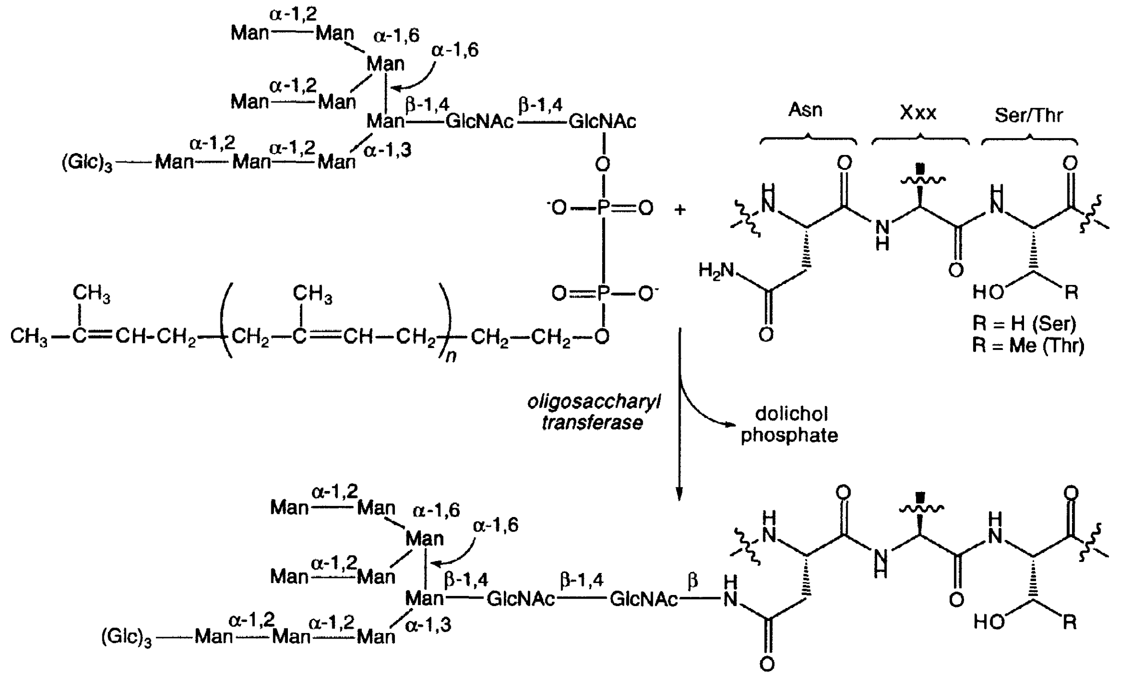
Some non-coded amino acids have also been identified at the linkage site in glycoconjugates: 4-hydroxyproline, 5-hydroxylysine and τ-histidinoalanine (*vide supra*).

Table 1. Possible Glycosides of Coded Amino Acid Side Chains

Functional Group	Amino Acids	Putative Pyranoside	Occurence
thiol R-SH	Cys		see §2.4
thioether $R\text{-}SCH_3$	Met		unknown
amide  $H_2N-\overset{\overset{\displaystyle O}{\|}}{C}-R$	Asn Gln		Asn: see §2.1.1 Gln: unknown
alcohol R-OH	Ser Thr		see §2.2.1
phenol Ar-OH	Tyr		see §2.2.4
indole 	Trp		see §2.1.2
amine $R\text{-}NH_2$	Lys		unknown
imidazole 	His		see §2.1.2
guanidine  $H_2N-\overset{\overset{\displaystyle NH}{\|}}{C}-NHR$	Arg		unknown

2.1 N-*Glycosides*

2.1.1 N-*Glycosides: Linked via the Side Chain of Asparagine Residues.*[12] By far the most commonly observed site of peptide/protein glycosylation is the amide nitrogen of Asn side chains. An enzyme known as oligosaccharyl transferase (OT) transfers the oligosaccharide depicted in Scheme 2 from dolichol phosphate to the amide nitrogen of Asn residues in the rough endoplasmic reticulum. This is a co-translational modification. Glycosylation is only observed when Asn is embedded in the consensus sequence Asn-Xxx-Ser/Thr. In this tripeptide sequence, Xxx can be any amino acid, although Pro is not favoured. A Ser or Thr residue, in the next-but-one position is an absolute requirement for glycosylation. Various glycosyl hydrolases and transferases then act upon the oligosaccharide, in what is referred to as the trimming process. Monosaccharide residues are added and hydrolyzed, but the core pentasaccharide remains in all derived *N*-glycosides.



Scheme 2. Nature's approach to *N*-glycosylation

In recent years, other monosaccharides and oligosaccharides have been found attached to Asn side chains including β-D-glucose[13] and β-*N*-acetylgalactosamine.[14] Messner and Sleytr described a trisaccharide of L-rhamnose attached to an Asn residue in GP I of *Bacillus stearothermophilus*.[15] In these cases, too, the Asn-Xxx-Ser/Thr motif is a prerequisite to glycosylation.

There are two known exceptions to this. Firstly, a glycopeptide called nephritogenoside (**1**) with glucose α-linked to the amide side chain in the sequence Asn-Pro-Leu (Fig. 1).[16]

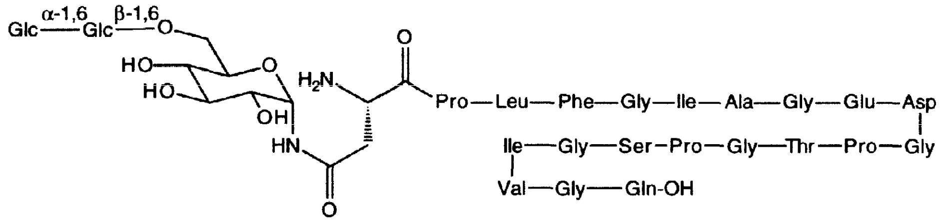


Figure 1. Nephritogenoside (**1**)

The second example is the heavy chain of bovine protein C.[17] This protein contains three carbohydrate groups. The first two are attached in typical environments: **Asn**^{93}Tyr^{94}Thr^{95} and **Asn**^{154}Arg^{155}Thr^{156}. The third site of glycosylation, however, is **Asn**^{170}Ala^{171}Cys^{172}. A Cys residue occupies the next-but-one position, and this Cys residue is part of a disulfide bridge.

These glycosylated Asn residues, in unusual sequences appear to be of quite different biosynthetic origins to those outlined in Scheme 2.

2.1.2 N-Glycosides: Unusual N-Linkages. The theonellamides are a family of cyclic peptides, typified by theonellamide A (**2**) (Fig. 2). These peptides contain a novel τ-histidinoalanine residue, which bridges the cyclic structure. Some congeners are glycosylated on the π-nitrogen of the imidazole ring: theonegramide (β-D-arabinose),[18] theonellamide A (β-D-galactose),[19] theonellamide D (β-L-arabinose)[19] and theonellamide E (β-D-galactose).[19]

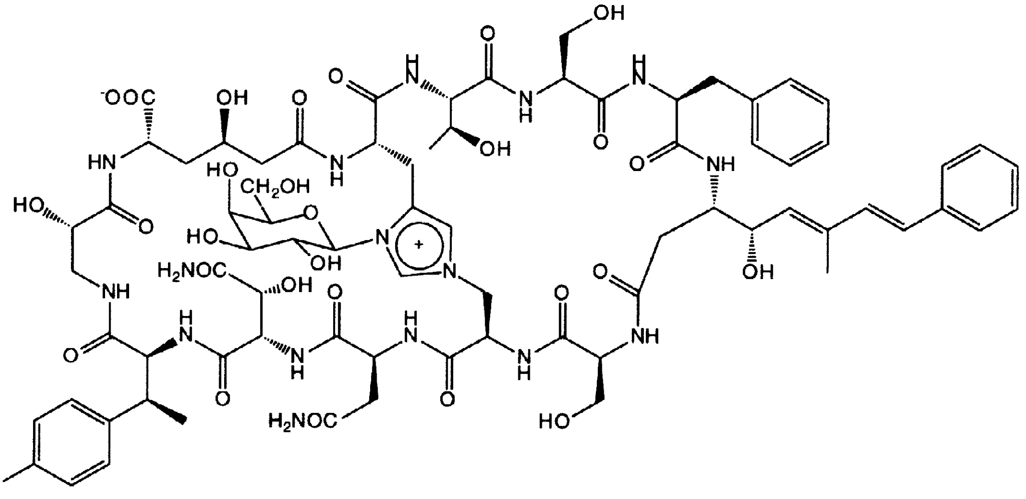
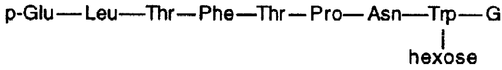


Figure 2. Theonellamide A (**2**)

Gäde *et al.* isolated a glycopeptide called Cam-HrTH-II (**3**) (Fig. 3) from the corpus cardiacum of the Indian stick insect, *Carausius morsus*.[20] This decapeptide was shown, by FAB mass spectrometry, to have a hexose sugar attached to the indole of the Trp side chain. They stated that "the prime candidate would be attachment to the nitrogen of the indole ring." No further reports have appeared. It seems more likely, based on recent NMR data for a related peptide (see §2.5), that attachment might be via C2 of the indole (*i.e.*, a *C*-glycoside rather than a *N*-glycoside).



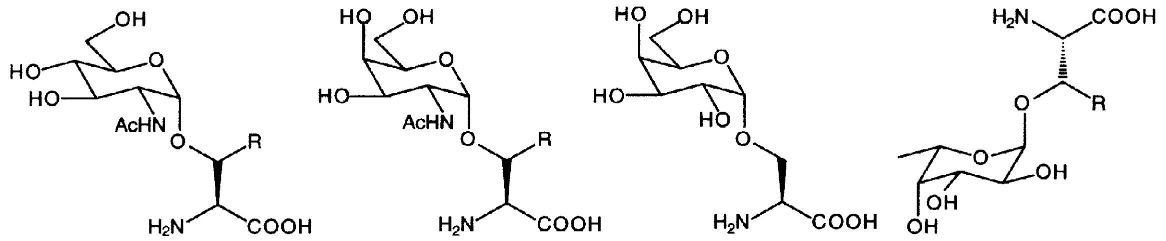
$$\text{p-Glu—Leu—Thr—Phe—Thr—Pro—Asn—}\underset{\text{hexose}}{\underset{|}{\text{Trp}}}\text{—Gly—Thr-NH}_2$$

Figure 3. Cam-HrTH-II (**3**)

2.2 *O-Glycosides*

2.2.1 O-Glycosides: Linked via the Side Chain of Serine or Threonine Residues. The most common *O*-glycosidic linkage is an α-linkage between GlcNAc and the hydroxyl group of Ser and Thr (Fig. 4).[5,21,22] Glycoproteins containing such linkages are found within cells (*i.e.*, in the nucleus or the cytoplasm). The carbohydrate is a monosaccharide unit. An α-D-GalNAc unit attached to a Ser or Thr is a common linkage in tumour-associated glycoproteins. The α-D-Gal-Ser unit has been described in plant glycoproteins (*e.g.*, extensin and potato lectin) and also in "cellulosomes" - extracellular complexes of cellulases produced by cellulolytic bacteria.[23]

The first example of a fucose residue attached to a Thr side chain was reported in 1975.[24] From each litre of normal human urine, 0.2-0.3 mg of 3-*O*-β-D-Glc-α-L-Fuc-L-Thr could be isolated. There have subsequently been several reports of L-Fuc α-linked to Ser and Thr side chains.[25-28]


α-GlcNac-Thr (R=H)
α-GlcNac-Thr (R = Me)

α-GalNac-Ser (R=H)
α-GalNac-Thr (R = Me)

α-Gal-Ser

α-L-Fuc-Ser (R=H)
α-L-Fuc-Thr (R=Me)

Figure 4. Common monosaccharides attached to Ser/Thr residues

A β-D-Glc-Ser linkage occurs in compound **4**, a residue in bovine blood clotting factor IX (Fig. 5).[29] Blood clotting factor IX is a plasma glycoprotein involved in the middle phase of the intrinsic pathway of blood coagulation. It has a molecular weight of 55,400 and consists of a single chain of 416 amino acids. There are oligosaccharides attached to four Asn residues,[30] as well as the unusual β-D-Glc-Ser linkage. The same group reported β-D-Glc-Ser linkages in the first epidermal growth factor-like domains of a number of other glycoproteins.[31]

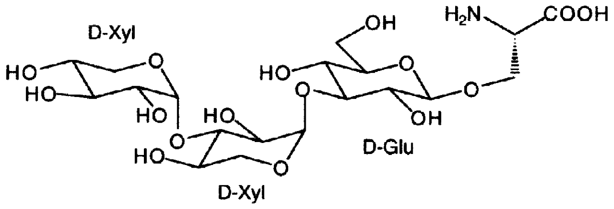


Figure 5. Trisaccharide bound to Ser^{53} (**4**) in Bovine Blood Clotting Factor IX

When human insulin-like growth factor-I (IGF-I) was expressed in yeast, a new form of the IGF-I was formed in addition to IGF-I. It was found that Thr^{29} of the 155 amino acid protein was glycosylated by a disaccharide containing two mannose residues (**5**), as depicted in Fig. 6. This is interesting because it highlights the different specificities of mannosyltransferase enzymes in man and yeast, and cautions that expression of foreign proteins in species such as yeast may not always produce what is expected.[32]

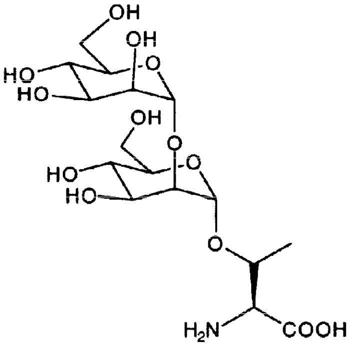
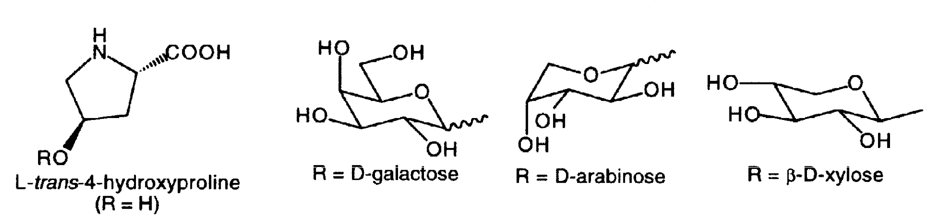


Figure 6. α-(1,2)-D-Man-α-D-Man-Thr (**5**)

2.2.2 *O-Glycosides: Linked via the Side Chains of Hydroxyproline Residues.* In 1969, Lamport proposed that the role of hydroxyproline in extensin was to provide a site for a covalent linkage between the protein and the cell wall.[33] Treatment of the cell walls of tomatoes with a crude cellulase preparation liberated glycopeptides, which yielded galactose and arabinose on acid hydrolysis, suggesting that these residues were directly attached to the protein (Fig. 7). The site of glycosylation was shown to be the 4-hydroxy group of *trans*-4-hydroxyproline. The stereochemistry of the glycosidic linkage was not determined.

Nielsen *et al.* isolated a protein, which they called SP2, a class IV chitinase from leaves of sugar beet (*Beta vulgaris*), which had been infected with *Cercospora beticola*.[34] The mature protein contains 261 amino acids, including five hydroxyproline residues. Mass spectrometry indicated the presence of up to seven pentose sugars. SP2 reacted with Xyl-p, an antibody which is specific for β-1,4-linked xylose.


L-*trans*-4-hydroxyproline (R = H)

R = D-galactose

R = D-arabinose

R = β-D-xylose

Figure 7. Glycosidic Linkages to Hydroxyproline

2.2.3 *O-Glycosides: Linked via the Side Chain of Hydroxylysine Residues.* Butler and Cunningham reported the identification of a disaccharide attached as an *O*-glycoside to the side chain of hydroxylysine in tropocollagen isolated from guinea pig skin.[35] This occurs in a special region of the collagen molecule in the sequence depicted in Fig. 8. The disaccharide was determined to be 2-α-D-glucopyranosyl-β-D-galactopyranose.[36] Levine and Spiro isolated the same Glc-Gal-Hyl unit (**6**) from a bovine glomerular basement membrane glycoprotein.[37]

—Gly—Met—Hyl—Gly—His—Arg—

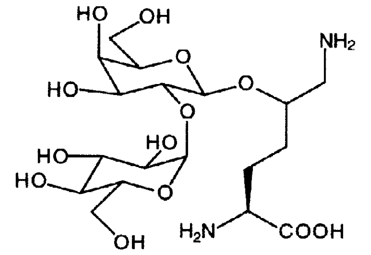

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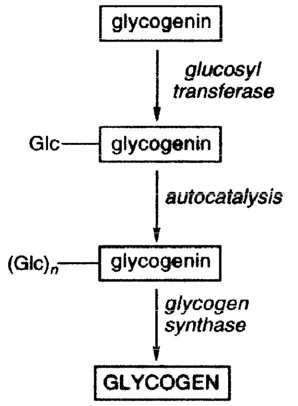
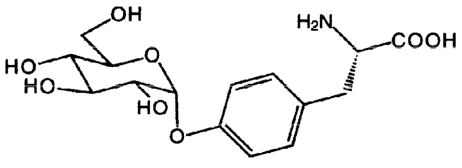
Figure 8. Glycosylation of Hydroxylysine

2.2.4 *O-Glycosides: Linked via the Phenol of Tyrosine Residues.* Glycogen is an α-1,4-linked polymer of glucose, which has long been recognised for its role in energy storage. The synthesis of glycogen begins with a priming protein called glycogenin (Fig. 9). Glycogenin is a glycoprotein, which bears an oligosaccharide of α-1,4-linked glucose units. The reducing end of the oligosaccharide is α-linked[38] to the phenolic group of a Tyr residue (**7**) of the protein. Rodriguez and Whelan identified the glycoprotein in rabbit liver muscle.[39] At about the same time, Aon and Curtino identified glycogenin linked via Tyr in tissue from the retina.[40] Campbell and Cohen determined the 332 amino acid sequence of glycogenin from rabbit skeletal muscle and identified a single glycosylation site (Tyr^{194}).[41] Glycogenin catalyzes the addition of about eight more glucose units to itself, then an enzyme called glycogen synthase takes over. Glycogen synthetase must be bound to glycogenin to be catalytically active.





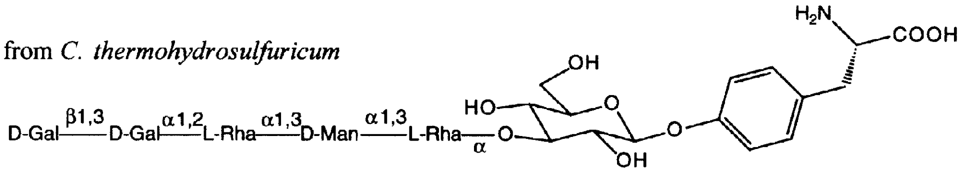
α-Glc-Tyr (7)

Figure 9. Glycosidic Linkage in Glycogenin

As early as 1978, Chen *et al.* identified β-Glc-Tyr in the mature larvae of *D. busckii*. This glucoside accumulates in the late third-star larvae and then suddenly disappears at pupariation. It has been suggested that this is a storage mechanism for Tyr, which is used in the sclerotization of the pupal case.[42]

In 1992, a glycosylated tyrosine residue (**8**) was identified in the hydrosylates of the crystalline surface layer glycoprotein of *Clostridium thermohydrosulfurium* S102-70.[43] A L-rhamnose-rich oligosaccharide was linked, via a β-D-glucose moiety to the side chain phenol of a tyrosine residue (Fig. 10). A similar oligosaccharide was found as a β-*O*-glycoside (**9**), linked via a galactose residue to the side chain of Tyr in the crystalline surface layer glycoprotein of *Thermoanaerobacter thermohydrosulfuricus* L111-69.[44]

from *C. thermohydrosulfuricum*



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from *T. thermohydrosulfuricum*

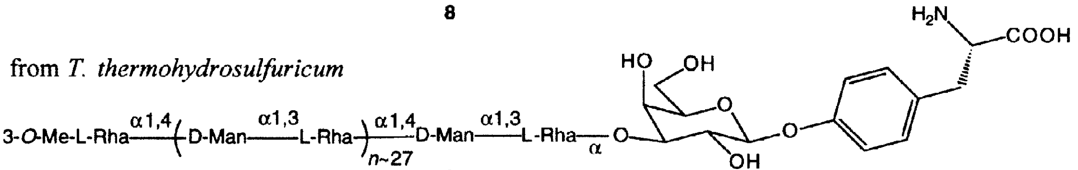

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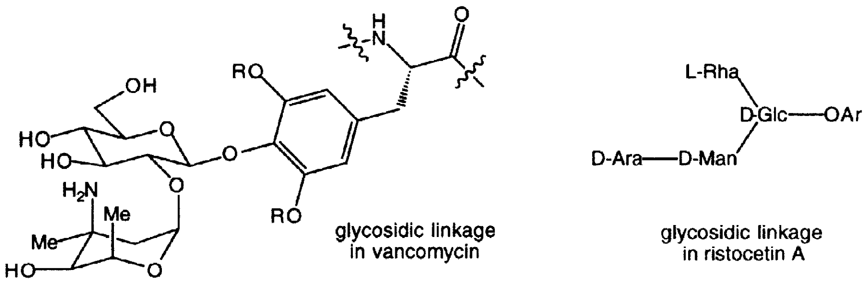
Figure 10. Tyrosine Glycosides

Vancomycin is a heptapeptide antibiotic with a disaccharide attached via the *p*-OH of a 3,4,5-trihydroxyphenylalanine residue (Fig. 11). The unusual sugar is called vancosamine. The linkage is from a β-D-glucose residue. Ristocetin A (*a.k.a.* ristomycin) contains a branched tetrasaccharide, also linked via a β-D-glucose residue to a phenol. These polycyclic glycopeptide antibiotics were reviewed by Williams *et al.*[45]



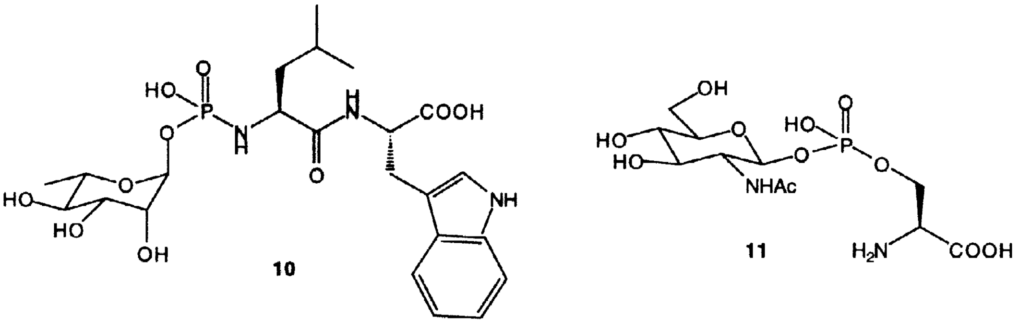
glycosidic linkage in vancomycin

glycosidic linkage in ristocetin A

Figure 11. Partial Structures of Vancomycin and Ristocetin A

2.3 *Phosphorus-Linked Glycopeptides*

Phosphoramidon **(10)** is a glycosylated dipeptide, (Fig. 12), which has L-Rha attached via a phosphonamide to the *N*-terminus of Leu-Trp.[46] Phosphoramidon is a potent inhibitor of metallopeptidases and its discovery from nature stimulated the development of phosphorus-based enzyme inhibitors. Proteinase 1, a lysosomal cysteine protease, of *Dictyostelium discoideum* contains a GlcNAc residue, attached via a phosphodiester linkage to the side chain hydroxyl of Ser (compound **11**).[47] Sugars linked to proteins via a phosphodiester hinge are known to function as immunological determinants. Under regular physiological conditions, it has been suggested that the role of the phosphonate group is to direct the protease towards basic substrates.



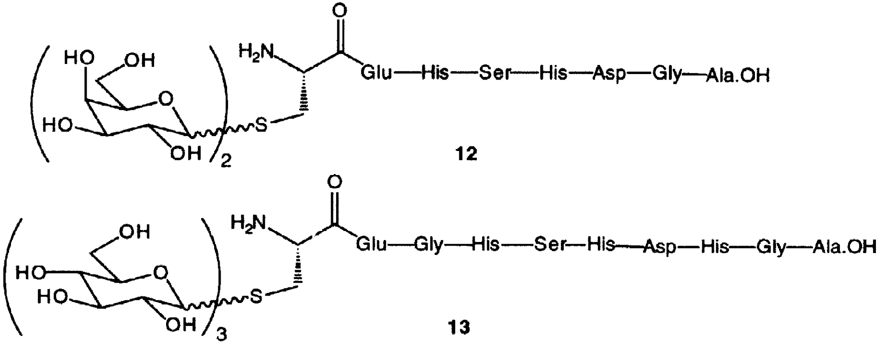
10

11

Figure 12. *P*-Linked Glycopeptides

2.4 *S-Glycosides.*

While *S*-glycosidase enzymes have been known for some time,[48] it was not until 1971 that a *S*-glycosidic linkage was identified in a peptide.[49-51] Lote and Weiss isolated the octapeptide **12** from normal human urine. The digalactose unit was attached to the side chain of the *N*-terminal cysteine residue via a *S*-glycosidic linkage (Fig. 13).[49,50] The same workers isolated a similar decapeptide **(13)** from erythrocyte membranes. Again, the cysteine was the *N*-terminal residue, with an adjacent Glu residue.[51] The oligosaccharide in this case was a trimer of glucose, although the details were not reported. The peptide sequence was very similar.



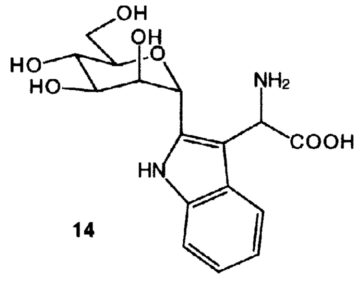
12

13

Figure 13. Component of naturally occurring *S*-glycosides

2.5 *C-Glycosides.*

Hofsteenge *et al.* recently identified a post-translational modification to Trp[7] in RNase Us, from human urine.[52] They provided conclusive evidence that there is a hexose sugar attached to the C2 position of the indole nucleus **(14)** (Fig. 14). The stability of the *C*-glycosidic linkage made it difficult to identify the nature of the monosaccharide. However, using NMR, it was estabilished that the sugar is mannose (the absolute configuration is uncertain) and that it is attached via a α-*C*-glycosidic bond.[53] Subsequent work has proven that this unusual *C*-glycoside does not arise artificially during the isolation procedures. Löffler *et al.* have identified the (C2-Man)Trp residue (**14**) in the intact protein by NMR.[54] They have also demonstrated that this type of *C*-glycoside is not unique to RNase 2 from urine; it has also been identified in RNase 2 from human erythrocytes.

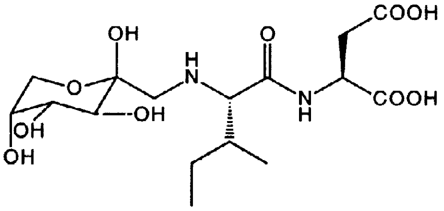


14

Figure 14. Glycosidic linkage in RNase Us

While screening for enkephalinase inhibitors, Vértesy and coworkers isolated a family of compounds (**15-20**) called the enkastines from *Streptomyces albus*, ATCC 21 838.[55] As shown in Table 2, some of these compounds are potent inhibitors of enkephalinase. They were demonstrated to be reasonably selective amongst proteolytic enzymes. These *C*-glycosides are effectively products of an Amadori reaction between D-glucose and the corresponding dipeptide (see §3.4).

Table 2. Enkastines



N-(β-1-deoxyfructos-1-yl)-isoleucylaspartate
(**15**)

Enkastine		IC_{50} *(mol* L^{-1}*)*
N-(β-1-deoxyfructos-1-yl)-isoleucylaspartate	(**15**)	1.8×10^{-9}
N-(β-1-deoxyfructos-1-yl)-valylaspartate	(**16**)	6.3×10^{-9}
N-(β-1-deoxyfructos-1-yl)-valylglutamate	(**17**)	3.0×10^{-8}
N-(β-1-deoxyfructos-1-yl)-isoleucylglutamate	(**18**)	5.6×10^{-8}
N-(β-1-deoxyfructos-1-yl)-alanylaspartate	(**19**)	1.7×10^{-7}
N-(β-1-deoxyfructos-1-yl)-alanylglutamate	(**20**)	1.2×10^{-5}

3. CHEMICAL SYNTHESIS OF GLYCOSIDIC LINKAGES[9-11]

3.1 N-*Glycosides*

3.1.1 N-*Glycosides: Via Glycosylamines.* Direct glycosylation of the amide side chain of Asn has not been reported (Fig. 15a). The routinely adopted approach for the formation of such *N*-glycopeptides is via reaction of a glycosylamine and a suitably protected Asp derivative (Fig. 15b). While this leads to an *N*-glycosidic linkage, the chemistry is that of amide bond formation rather than glycosylation.

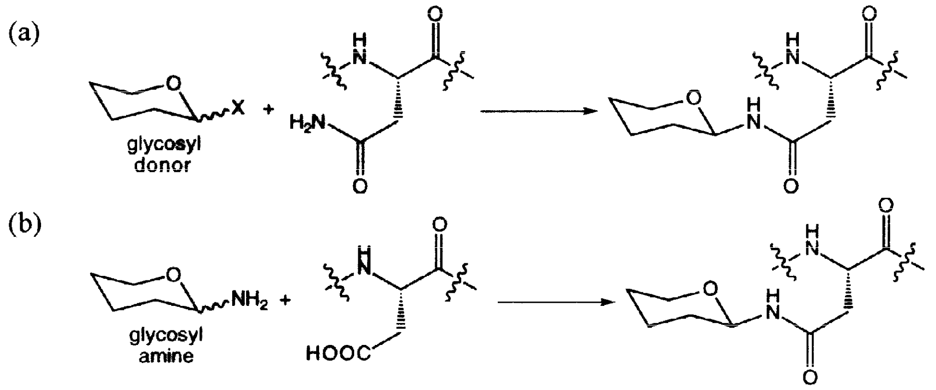
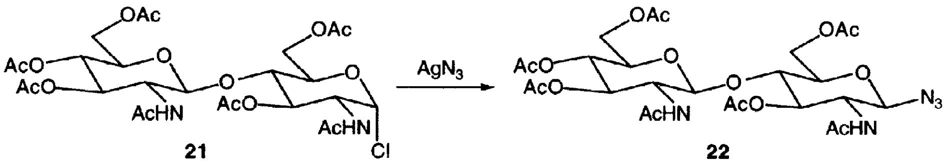


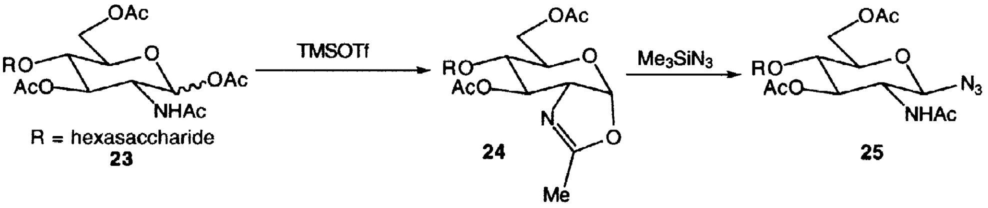
Figure 15. (a) Direct Glycosylation of an Asn Residue; (b) Amide Bond Formation

A prerequisite to this approach is obviously access to glycosylamines. These have been prepared by displacement of glycosyl bromides with ammonia.[56] However, the glycosyl halide is more often converted to the glycosyl azide (Scheme 3) and then reduced. Spinola and Jeanloz reported the treatment of chitobiose derivate **21** with silver azide to give **22**.[57] The same type of conversion has been accomplished under milder conditions, using sodium azide and phase transfer catalysis.[58-60]



Scheme 3

Alternatively, treatment of **23** with trimethylsilyltriflate leads to formation of the oxazole **24**. Opening of the oxazole with trimethylsilylazide gave the β-azide **25** (Scheme 4).[61]



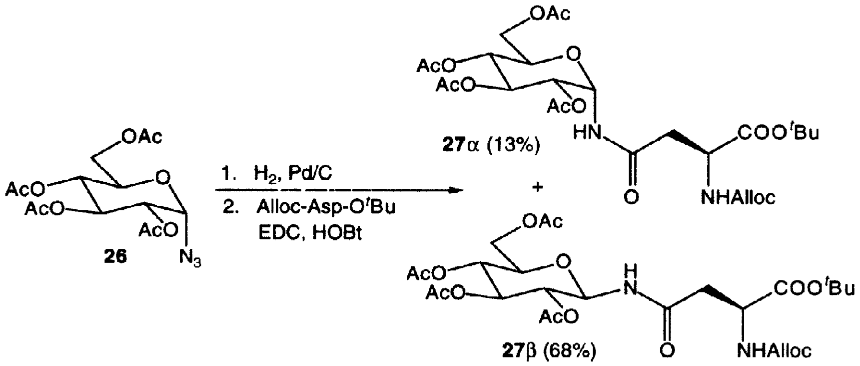
Scheme 4

Reduction of anomeric azides to the corresponding glycosylamines has been accomplished in a number of ways, as summarized in Table 3.

Table 3. Reduction of Glycosyl Azides

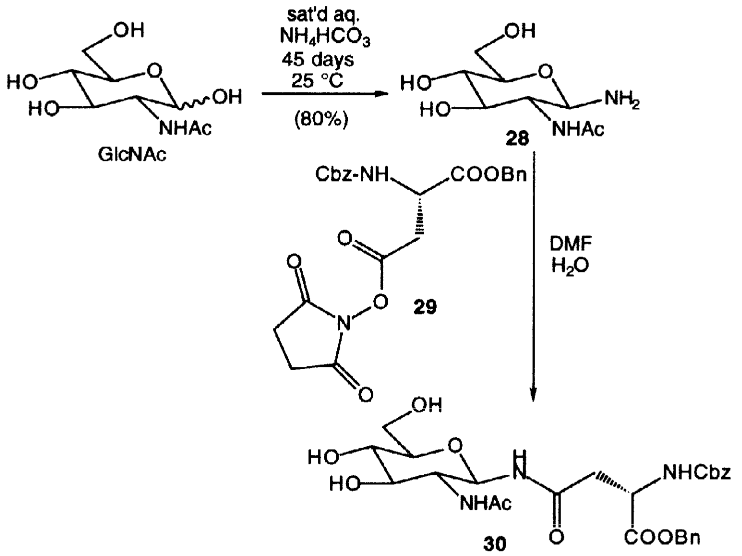
ReactionConditions	*Reference/s*
H_2, Pd/C	60,62,63
H_2, neutral Raney Ni(W-2)	64,65
H_2, Lindlar's catalyst	61
$HSCH_2CH_2CH_2SH$, Et_3N, MeOH	66

Glycosylamines readily anomerize. Under acidic reaction conditions, the amine is protonated and the species equilibrates to favour the β-anomer as a result of the reverse anomeric effect. Noble metal catalysts have also been shown to accelerate anomerization. For example, Teshima *et al.* reduced the α-azide **26** under standard hydrogenolytic conditions (Scheme 5).[63] The resulting amine was coupled directly with Alloc-AspO^{t}Bu . Considerable anomerization had occurred, giving rise to predominantly the β-product (**27β**). Thus, conditions for azide reduction are preferably basic and without metal. A potentially general solution, seems to be the use of propane-1,3-dithiol[67] in the presence of triethylamine.



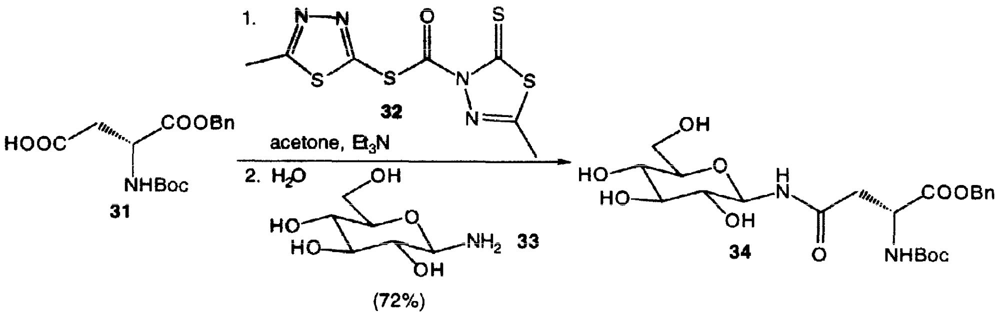
Scheme 5

Glycosylamines can also be prepared directly from unprotected sugars, using ammonium hydrogen carbonate, or ammonia (Scheme 6).[68] In the example shown below, Likhosherstov *et al.* prepared **28** from GlcNAc and coupled it with Asp derivative **29** to give **30**.[69]



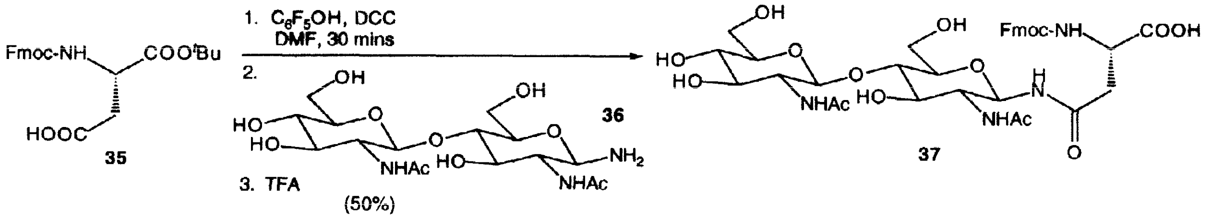
Scheme 6

Activation of the side chain carboxylic acid of **31** with 5-methyl-2-thioxo-1,3,4-thiadiazole-3(2*H*)-carbothioic acid *S*-(5-methyl-1,3,4-thiadizol-2-yl) ester (**32**)[70] followed by coupling with an unprotected aminosugar **33**, yielded the glycoconjugate **34** in good yield (Scheme 7).[71]



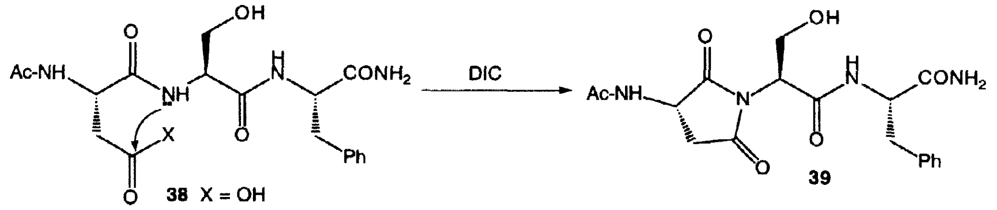
Scheme 7

Otvos and coworkers reported the synthesis and utilization of a chitobiose building block in which the carbohydrate was unprotected (Scheme 8).[72,73] Formation of the β-pentafluorophenyl ester of Asp derivative **35** and reaction with β-1-amino chitobiose (**36**), followed by deprotection of the α-carboxylic acid gave building block **37** in 50% overall yield. This derivative was successfully employed in the solid-phase synthesis of a T cell epitopic dodecapeptide containing a glycosylated Asn in a mid-chain position.

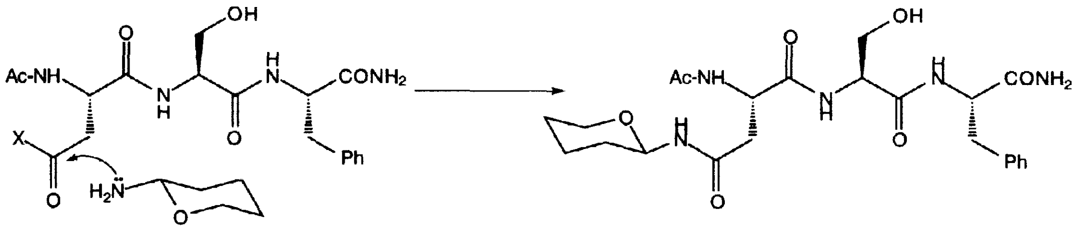


Scheme 8

The formation of a *N*-glycosidic linkage to a preformed peptide (*c.f.*, an α-ester of an amino acid) is more difficult. This transformation is complicated by a side reaction involving intramolecular succinimide formation (Scheme 9). This issue has been thoroughly investigated by Anisfeld and Lansbury.[74] As shown in Scheme 9, activation of Ac-Asp-Ser-Phe-NH_2 (**38**) with diisopropylcarbodiimide (DIC) led to the formation of considerable amounts of **39**. However, under carefully optomized reaction conditions, reasonable yields of glycosides were obtained using BOP and HBTU as coupling reagents (Scheme 10). The amount of base present has a significant effect on the amount of succinimide formation observed. Best results were obtained using 2 eq. of the glycosylamine (no added base) or else 1 eq. of the glycosylamine accompanied by 1.5 eq. of diisopropylethylamine.

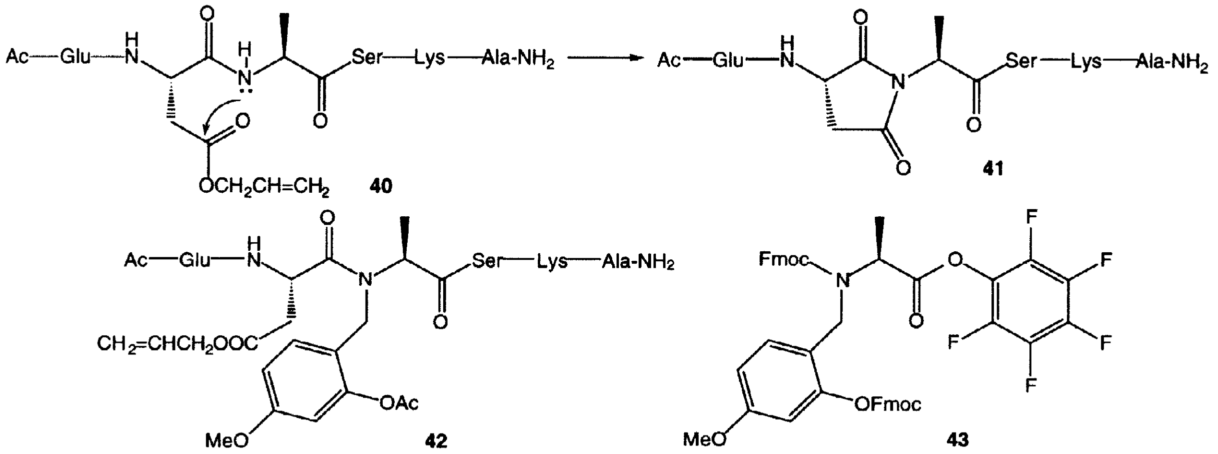


Scheme 9. Aspartimide Formation



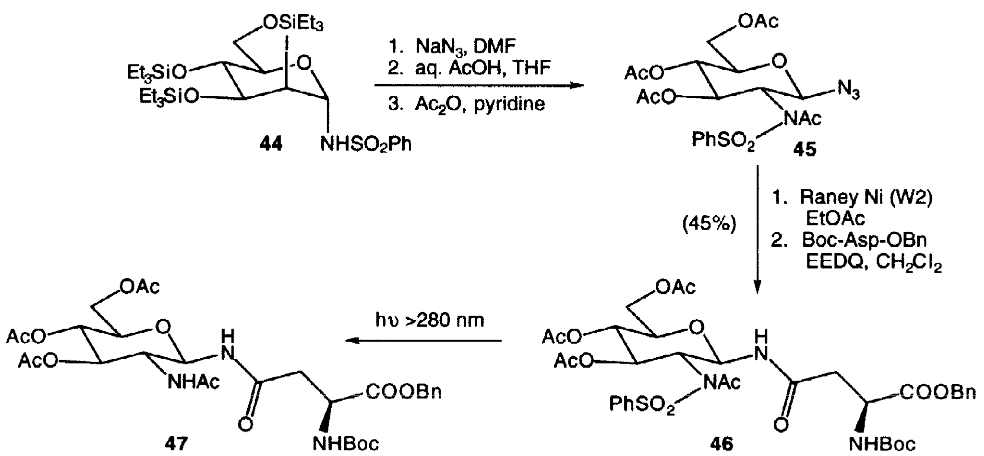
Scheme 10. Glycosylation

Johnson and coworkers have developed a solution to the problem of aspartimide formation. They protect the amide nitrogen with the *N*-(2-acetoxy-4-methoxybenzyl) (AcHmb) group.[75] In the heptapeptide sequence depicted in Scheme 11, the side chain of the Asp residue (to be glycosylated) was orthogonally protected as its allyl ester. Compound **40** was found to be prone to aspartimide formation (**41**), even before removal of the allyl group. The amide nitrogen was protected by incorporating the alanine residue as protected secondary amine **43**. The phenolic hydroxyl at the 2-position of the benzene ring was reprotected as the acetate. No evidence of aspartimide formation was found with compound **42**, either during the assembly of the peptide or during deprotection and activation of the β-carboxylate.[76] Removal of the amide protecting group was initiated by hydrazinolysis of the acetate, which rendered the protecting group acid sensitive.



Scheme 11

McDonald and Danishefsky reported a route to *N*-Asn linked glycopeptides, extending their azaglycosylation methodology (Scheme 12).[64] The 2-iodo-1-*N*-sulfonamide **44** reacted with azide in such a way that it installed a β-azide, with stereoselective migration of the sulfonamide to the 2-position (**45**). Reduction of the β-azide using neutral Raney Ni and coupling with an Asp derivative gave **46**. The phenylsulfonyl group was removed photolytically to give **47**.



Scheme 12

More than one oligosaccharide can be attached to a peptide simultaneously. Scheme 13 details the synthesis of a complex glycoprotein bearing the sialyl Lewis^x epitope. The cyclic heptapeptide, **49**, bearing three appropriately spaced Asp residues, was synthesized (Fig. 16). Coupling with 3 eq. of partially protected glycosylamine **48** led to the formation of three *N*-glycosidic linkages.[77] Compound **50** inhibited the adhesion of HL60 cells 2-3 times more effectively than sialyl Lewis^x. This may be attributable to its multivalency.

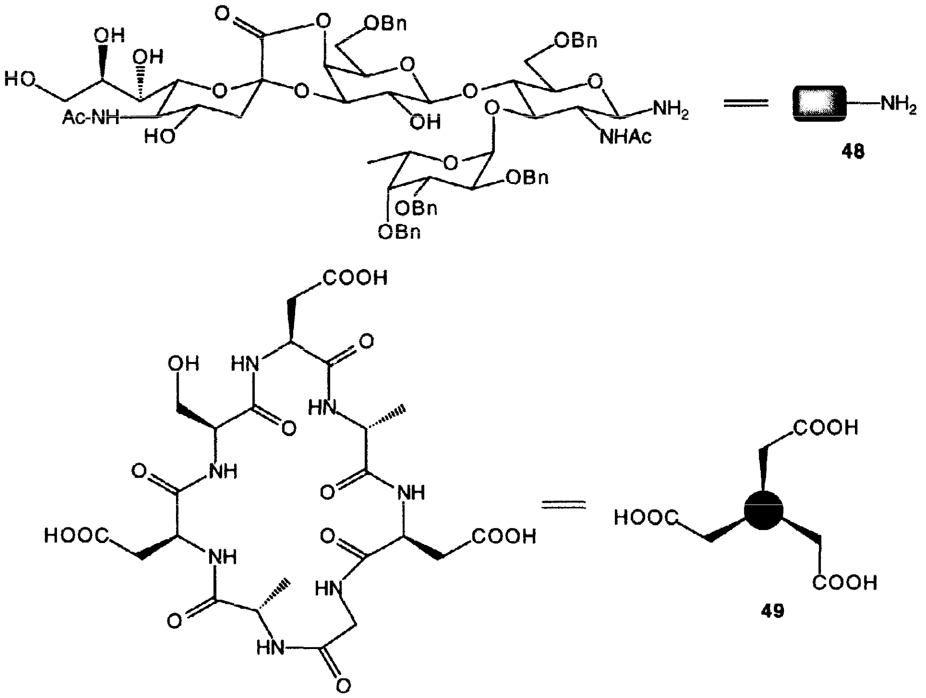
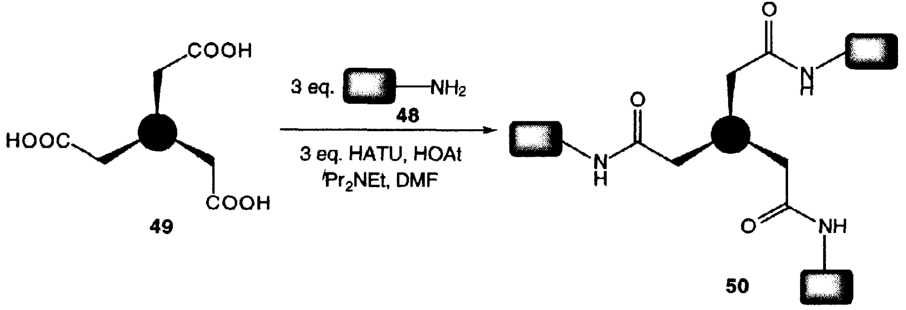
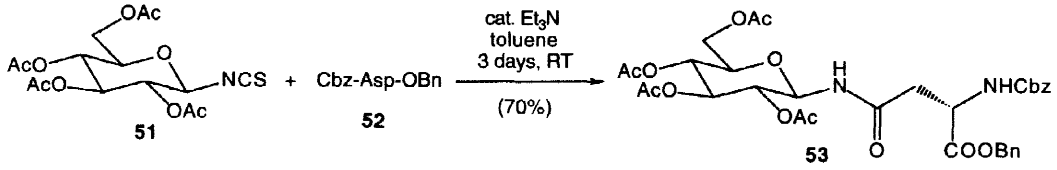


Figure 16. Compounds **48** and **49**.

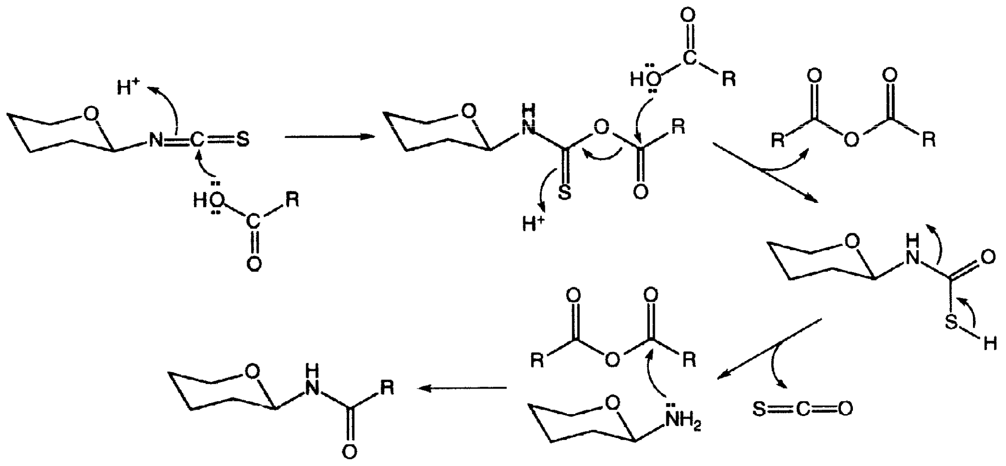


Scheme 13

3.1.2 N-*Glycosides: Via Isothiocyanates.* Zurabyan and coworkers introduced glycosylisothiocyanates as precursors to glycosyl amides. The following example (Scheme 14) involves reaction of glycosylisothiocyanate **51** with Asp derivative **52** to give **53**.[78,79] The mechanism is not known, but Scheme 15 details a possible explanation.[78] Nucleophilic attack of the isothiocyanate by the carboxylic acid activates it toward attack by another equivalent of the carboxylic acid. This leads to the formation of a symmetrical anhydride and a glycosyl thiocarbamic acid. Loss of S=C=O gives the glycosylamine, which reacts with an anhydride to form the amide.

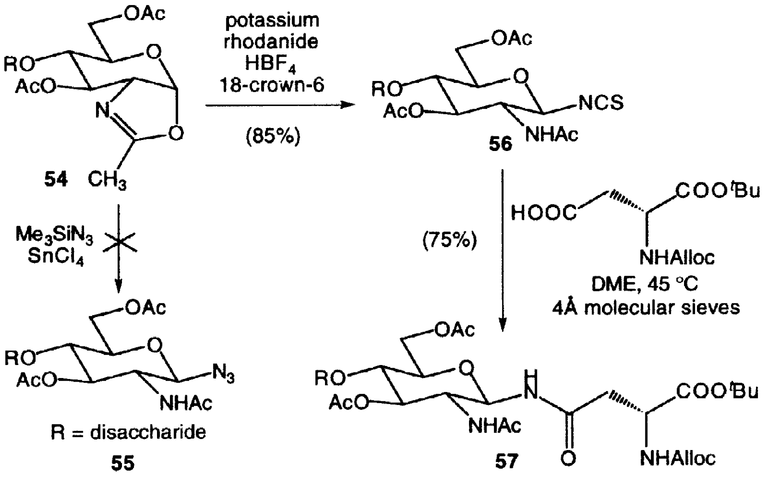


Scheme 14



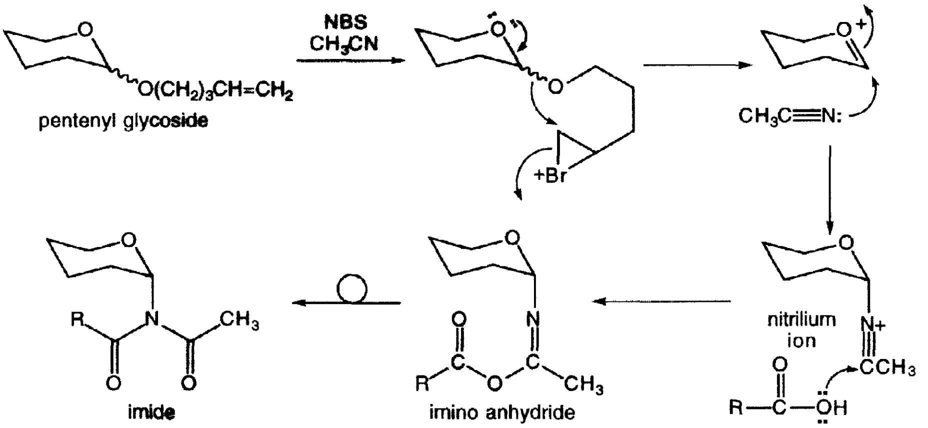
Scheme 15

Günther and Kunz reported that they were unable to form azide **55** from the oxazole **54** (Scheme 16). This was surprising, given the similarilty of the disaccharide to the reducing end of the hexasaccharide described in Scheme 4. They were, however, able to form the isothiocyanate **56**, which coupled with Alloc-Asp-O^{t}Bu to give a good yield of the *N*-linked glycopeptide **57**.[80] They reported that the coupling reaction required strict exclusion of water and that the formation of *N,N'*-bisglycosylthioureas was impossible to circumvent completely.



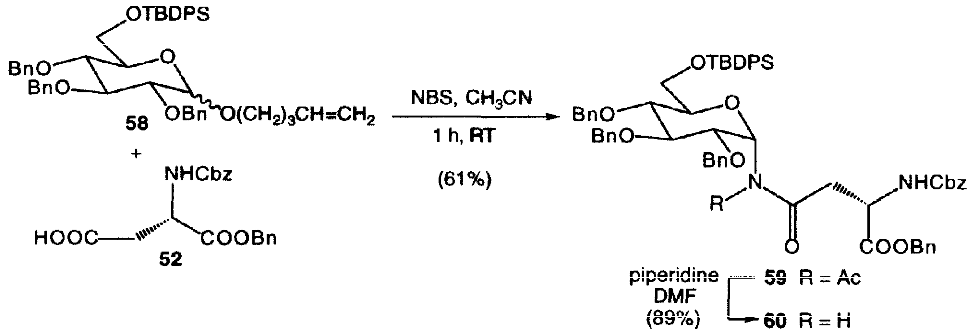
Scheme 16

3.1.3 N*-Glycosides: Via Pentenyl Glycosides.* Fraser-Reid and coworkers have developed a novel approach to the synthesis of *N*-glycosides (Scheme 17). Treatment of either anomer of a pentenyl glycoside with *N*-bromosuccinimide (NBS) in acetonitrile is proposed to lead to the formation of an oxonium ion. This is trapped by acetonitrile to give an α-nitrilium ion, contrary to what would be predicted by the reverse anomeric effect. A carboxylic acid can then add to the nitrilium ion, giving rise to an imino anhydride, which rearranges to give an imide.



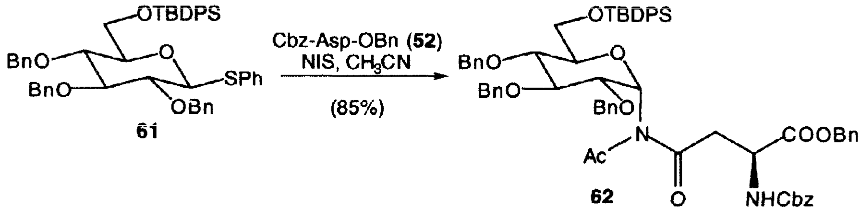
Scheme 17

To demonstrate the utility of this methodology, pentenyl glycoside **58** and Asp derivative **52** were coupled to give **59** in 61% yield (Scheme 18). The acetyl group was removed efficiently by treatment with piperidine in DMF to give **60**.[81,82] This was a key step in their synthesis of nephritogenoside (Fig. 1).



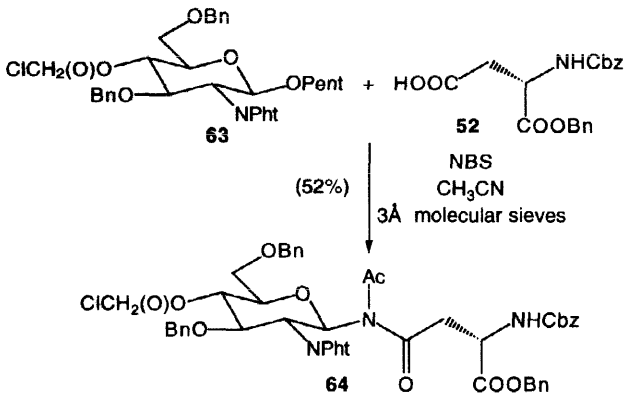
Scheme 18

In a conceptually related synthesis of nephritogenoside, Sasaki and Tachibana treated thioglycoside **61** with Cbz-Asp-OBn (**52**) and *N*-iodosuccinimide in acetonitrile to obtain the *Nα*-glycoside **62** in good yield (Scheme 19).[83] They observed no imide formation when *N*-bromosuccinimide was employed; instead a mixture of the corresponding esters was obtained.



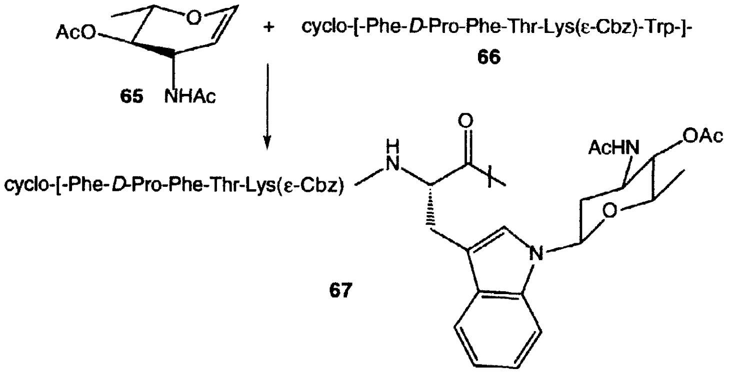
Scheme 19

In recognition of the fact that the more widespread linkage to Asn is β and involves a GlcNAc residue, Handlon and Fraser-Reid have extended this methodology to form such a glycosidic bond (Scheme 20).[84] They invoked neighbouring group participation, by the *N*-phthalimido group at C2 of **63** (NHAc was ineffective) to direct nitrilium ion formation to the β-face, resulting in glycoside **64**.



Scheme 20

3.1.4 N-Glycosides: An Unusual N-Glycosidic Linkage. An unexpected example of *N*-glycosylation was reported by Kessler and co-workers.[85] The intention of the following reaction was to glycosylate the side chain of the Thr residue in the cyclic hexapeptide **66** with glycal **65**. However, the Thr residue appears to occupy the *i*-position of a β-turn and so the hydroxy group is involved in a hydrogen bond and is unreactive towards glycosylation. Instead, *N*-glycosylation of the indole ring was observed to give **67**, with β-stereochemistry (Scheme 21).

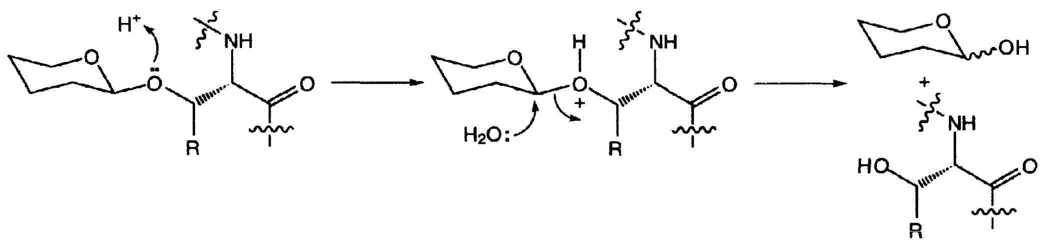


Scheme 21

3.2 *O-Glycosides*[86]

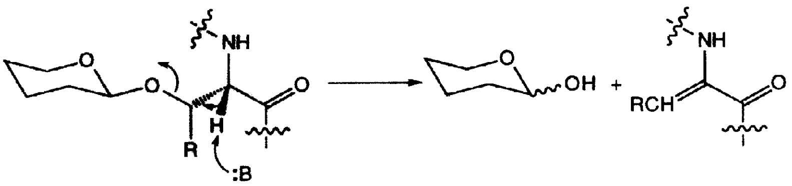
3.2.1 O-Glycosides: Glycosylation of Primary and Secondary Alcohols. The synthesis of *O*-linked glycopeptides is complicated by:

(a) The acid sensitivity of the glycosidic linkage;



Scheme 22. Acid Sensitivity of *O*-Glycosides

(b) The sensitivity of the Ser and Thr glycosides towards a base-promoted retro-Michael reaction. This reaction has been utilized to release *O*-linked carbohydrates from glycoproteins.[87]



Scheme 23. Base Sensitivity of *O*-Glycosides

For these reasons, protecting group manipulations and glycosylation reactions need to be carried out under essentially neutral conditions. Meldal states that the fear of β-elimination (Scheme 23) is exaggerated. Meldal and coworkers have demonstrated that *O*-glycosidic linkages are stable to the reagents normally used in Fmoc-based solid phase peptide synthesis.[88] While many protecting group strategies have been employed over the years, one strategy which has been widely adopted involves Fmoc protection of the α-amino group and protection/activation of the carboxylic acid as its pentafluorophenyl (pfp) ester (Fig. 17).[89,90] The pfp esters appear to be stable to many glycosylation reaction conditions. Moreover, the pfp ester subsequently serves as an activated form of the building block in a coupling reaction with an amine.

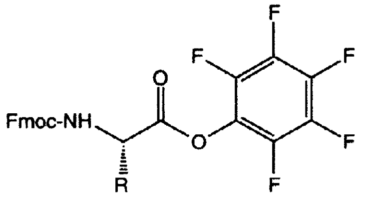


Figure 17

The glycosylation of a primary or secondary alcohol in the side chain of an amino acid is not so conceptually different to forming a glycosidic linkage between two monosaccharide units. Indeed, most standard glycosylation methods have been applied.

Polt *et al.* claim that early work in this area was plagued by low yields and poor α/β selectivities, attributable to low nucleophilicity of the glycosyl acceptor.[91] The hydroxyl group of a Ser or Thr residue is involved in a hydrogen bond with the amide proton (Fig. 18). This decreases electron density on the oxygen, rendering it less nucleophilic. To test this hypothesis, and circumvent the problem, they prepared Schiff bases between the α-amino group and benzophenone. This reverses the direction of the hydrogen bond, and should therefore increase the nucleophilicity of the alcohol. This approach has met with some success, for example the reaction of **68** and **69** (Scheme 24). The resulting Schiff bases (*e.g.*, **70**) can be converted to the free amine or protected amine under either mildly acidic reaction conditions or by catalytic hydrogenolysis.

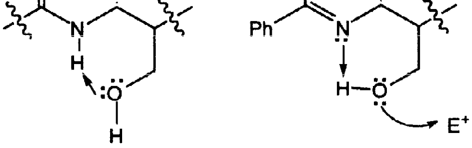
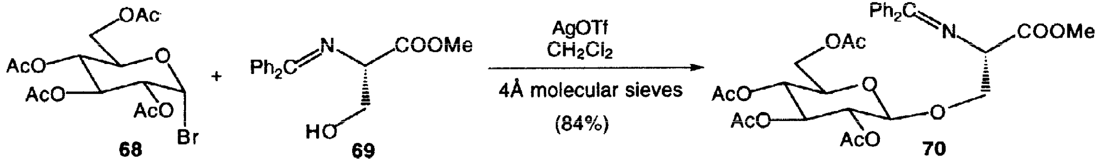
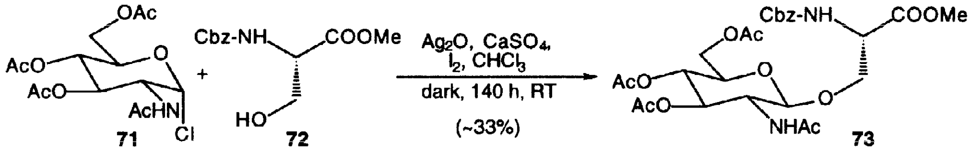


Figure 18



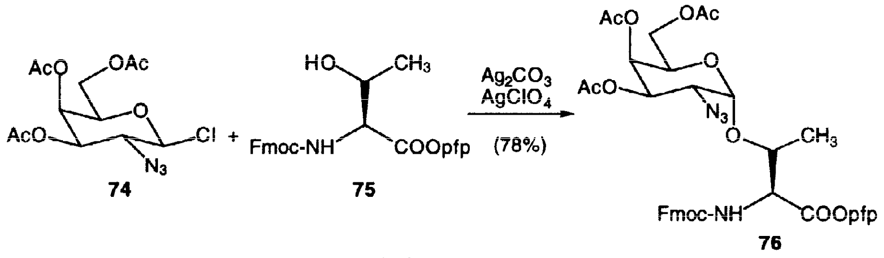
Scheme24

Koenigs—Knorr Coupling[92] In 1961, Jones and coworkers were not at all sure how peptides and oligosaccharides were linked in glycopeptides. Nevertheless, they synthesized a variety of possibilities including the formation of a β-(GlcNAc)-Ser residue (**73**), using a Koenigs—Knorr coupling betwwen α-glucosyl chloride **71** and Ser derivative **72** (Scheme 25).[93] We now know that the corresponding α-glycosidic linkage is widespread in nature.



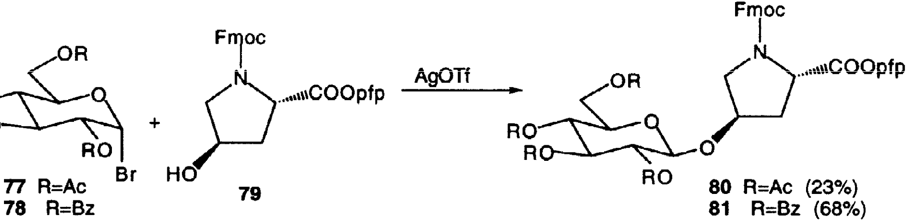
Scheme 25

The following example involves the synthesis of a useful GalNAc-Thr building block (**76**) (Scheme 26). The Koenigs—Knorr coupling reaction between **74** and **75** yields a 14:1 mixture of anomers (α:β).[94] This building block has been used in the synthesis of partial structures of the octapeptides of human glycophorin A^N.[95]



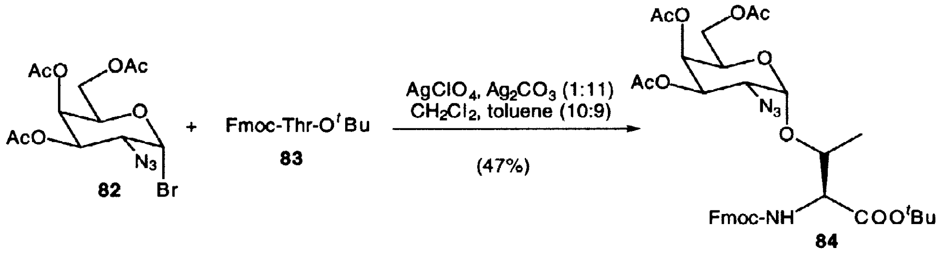
Scheme 26

Utilizing the same protecting group strategy, Arsequell *et al.* prepared glucosylated derivatives of hydroxyproline. As shown in Scheme 27, reaction of glycosyl bromide **77** with Hyp derivative **79** gave glycoside **80**.[96] The yield of the coupling reaction was markedly improved when **78** was used as the glycosyl donor, leading to glycoside **81**.



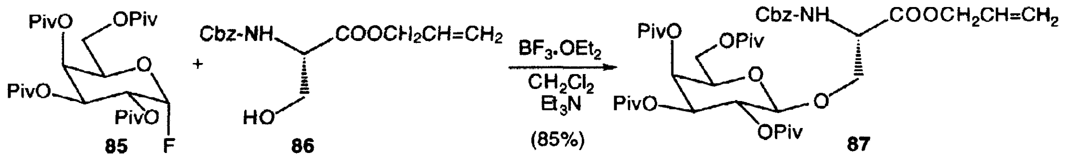
Scheme 27

In their recent synthesis of a tumour-associated sialyl-T_N antigen, Liebe and Kunz prepared their Thr building block (**84**) using a Koenigs—Knorr glycosylation (Scheme 28).[97] Reaction of glycosyl bromide **82** and Thr derivative **83** led to formation exclusively of α-glycoside **84**. The azido group was then converted to an acetamido group with thiolacetic acid, to which the glycosidic linkage was reported to be stable.



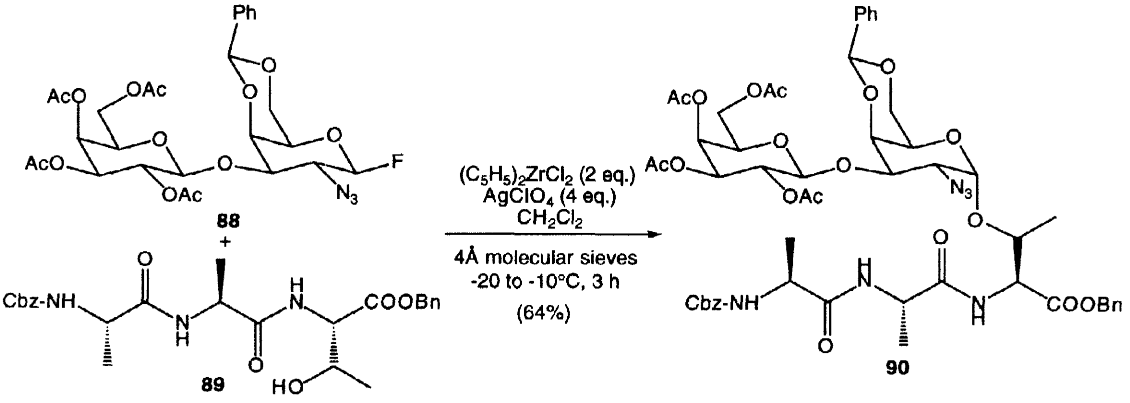
Scheme 28

Glycosyl Fluorides. Conceptually related to the traditional Koenigs—Knorr coupling, is the Lewis acid catalyzed reaction of glycosyl fluorides with nucleophiles. The major advantage of this system is that the reaction mixture is homogeneous. This glycosylation method was introduced to the field of glycopeptides by Kunz and Sager.[98] Reaction of perpivaloylated glycosyl fluoride **85** with Ser derivative **86** gave a good yield of β-glycoside **87** (Scheme 29).



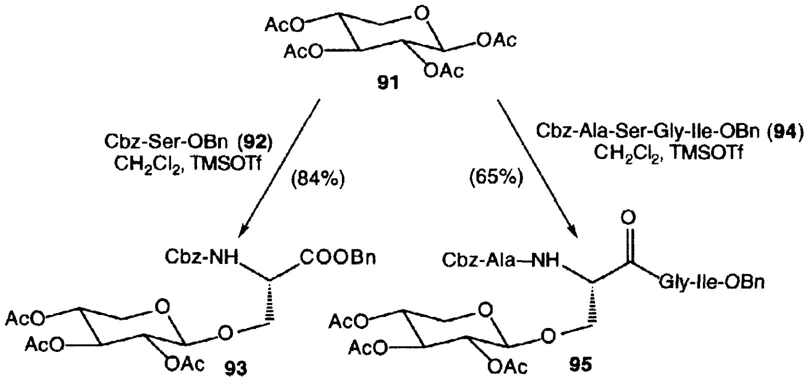
Scheme 29

A recent, topical example was reported by Nishimura and Tsuda in their construction of a synthetic antifreeze glycoprotein (AFGP). Reaction of glycosyl fluoride **88** with three equivalents of tripeptide **89** gave the corresponding glycopeptide **90** (Scheme 30). After deprotection, this compound was polymerized using diphenylphosphoryl azide (DPPA).[99]



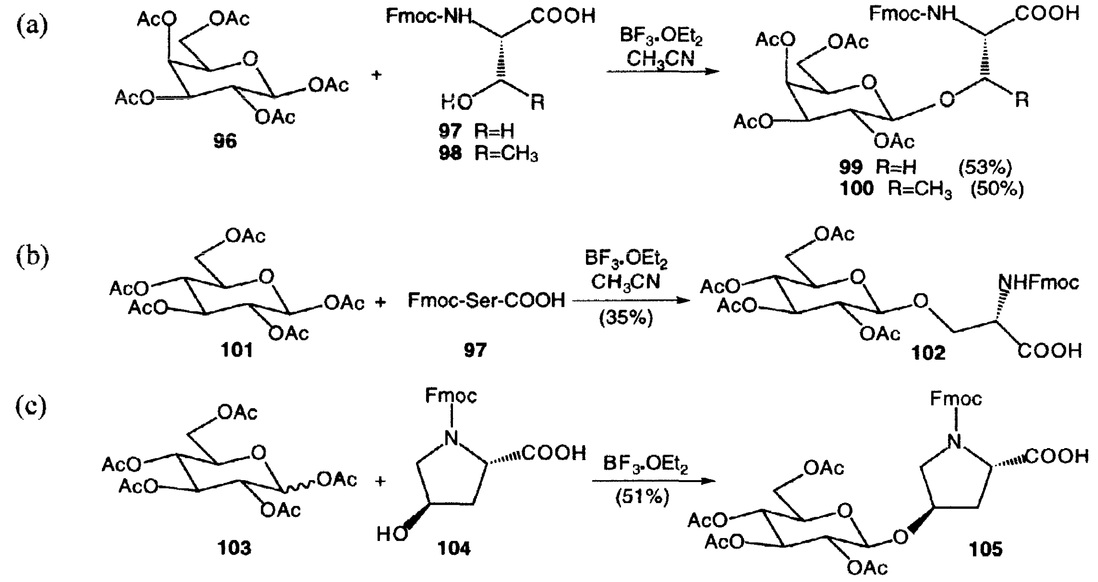
Scheme 30

Activation of Anomeric Acetates. Paulsen and Brenken reported the glycosylation of Ser derivative **92** and a tetrapeptide (**94**), using the tetraacetate of xylose (**91**) (Scheme 31). The reaction was catalysed by trimethylsilyltriflate and gave good yields of the *O*-glycosides **93** and **95** respectively.[100]



Scheme 31

Elofsson *et al.* discovered that anomeric acetates can be used to glycosylate amino acids in which the carboxylic acid is unprotected.[101] This was a major breakthrough because the approach circumvents the deprotection of that carboxylic acid prior to incorporation into a peptide. Coupling of peracetylated galactose (**96**) with *N*-Fmoc protected Ser (**97**) and Thr (**98**) gave reasonable yields of the corresponding glycosylated amino acids **99** and **100** respectively (Scheme 32a). The solvent of choice was found to be acetonitrile, and $BF_3 \cdot OEt_2$ was a better catalyst for the reaction than $SnCl_4$. Steffan *et al.*[102] reported the formation of the Glc-Ser building block **102**, on gram scale, from **101** and **97** (Scheme 32b). Arsequell *et al.* formed the β-glucoside of Hyp (**105**) in the same manner, from **103** and **104** (Scheme 32c).[96] Recall that a related Koenigs—Knorr reaction (Scheme 27) gave a yield of only 23 %.



Scheme 32

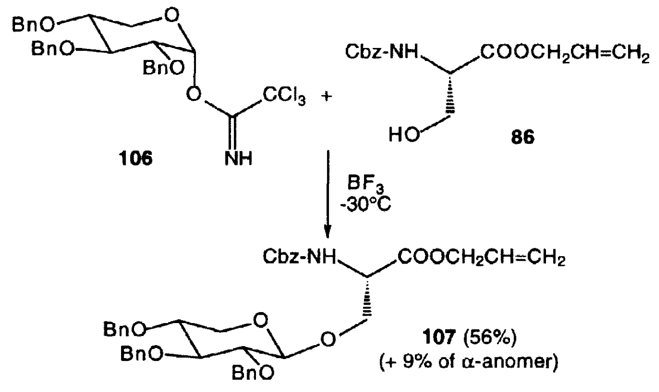
While the yields of these glycosylation reactions are not spectacular, there are some undeniable advantages:

(a) Peracetylated sugars are typically available in one step;

(b) The Fmoc-protected amino acids are commercially available;

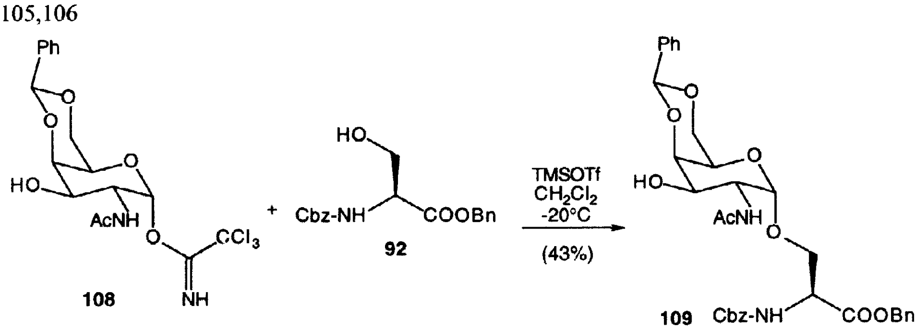
(c) The carboxylic acid is unprotected, giving considerable flexibility in the coupling methods used to introduce the building block into a peptide.

Trichloroacetimidate Method. Kunz and Waldmann used trichloroacetimidate **106** to glycosylate Ser derivative **86** (Scheme 33).[103] This work showcased the allyl group for *C*-protection. The allyl group was removed from **107** [$Pd(PPh_3)_4$, morpholine, THF] in 97% yield.



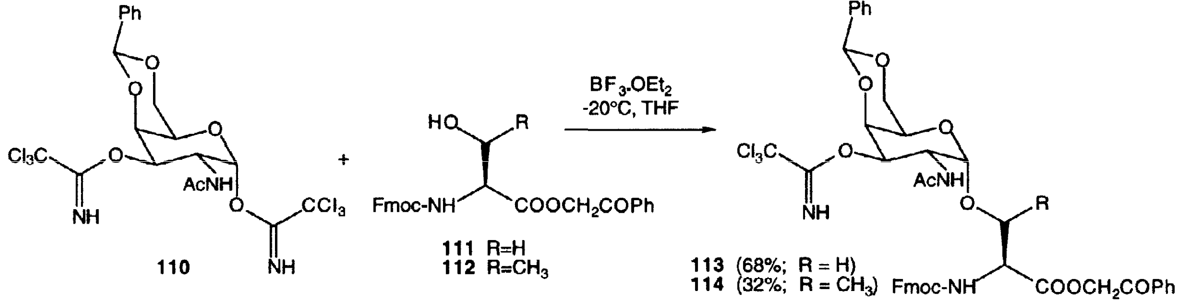
Scheme 33

Another early example of the use of the trichloroacetimidate method was reported by Grundler and Schmidt. Reaction of **108** with **92** in the presence of trimethylsilyltriflate gave the α-glycoside **109** (Scheme 34).[104] Kinzy and Schmidt continued with this approach to the synthesis of mucin-type glycopeptides.[105,106]



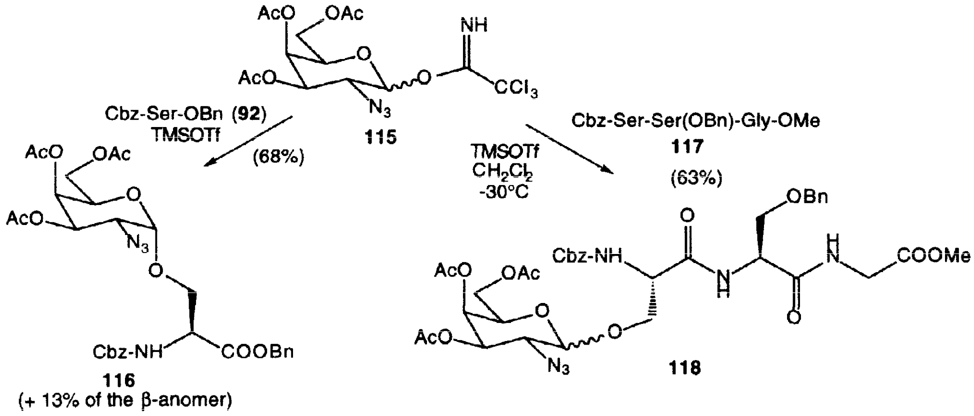
Scheme 34

Qiu and Koganty have developed a novel trichloroacetimidate donor **110** which has proven useful in the synthesis of Core 5 type mucin glycopeptides (Scheme 35).[107] Compound **110** was used to glycosylate both Ser (**111**) and Thr (**112**) building blocks, with formation exclusively of the α-glycosides **113** and **114** respectively. The trichloroacetimidate at the 3-position was removed by stirring in 80% aqueous acetic acid overnight.



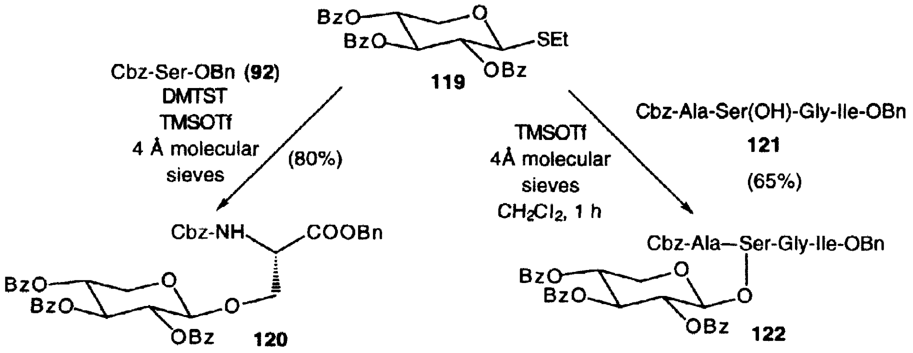
Scheme 35

A mixture of the trichloroacetimidates **115** (α and β) has been used to glycosylate the side chain of Ser residues (Scheme 36). Toyokuni *et at.* prepared the building block **116** as a mixture of anomers, which were separable by flash chromatography.[108] Kanemitsu *et al.* used the same trichloroacetimidate to glycosylate the tripeptide **117** in their synthesis of suppresein A.[109] They also obtained a mixture of anomers (**118**), but did not report the ratio. Suppresein A suppresses the production of pisatin, a pea phytoalexin.



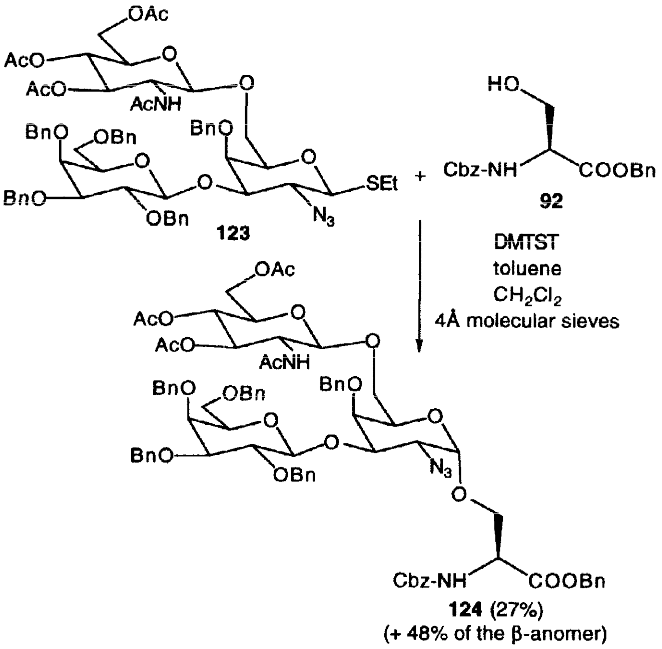
Scheme36

Thioglycosides. The utility of **119**, a thioglycoside derived from xylose, is illustrated in Scheme 37. It has been used to glycosylate Cbz-Ser-OBn (**92**), giving **120** in good yield.[110] The other example is the synthesis of **122** through glycosylation of a tetrapeptide **121**, which corresponds to the 3-6 segment of the protein core of a proteodermatan sulfate. This example shows that this glycosylation method is suitable, not just for the formation of glycosylated amino acids, but also to the glycosylation of oligopeptides.[110]



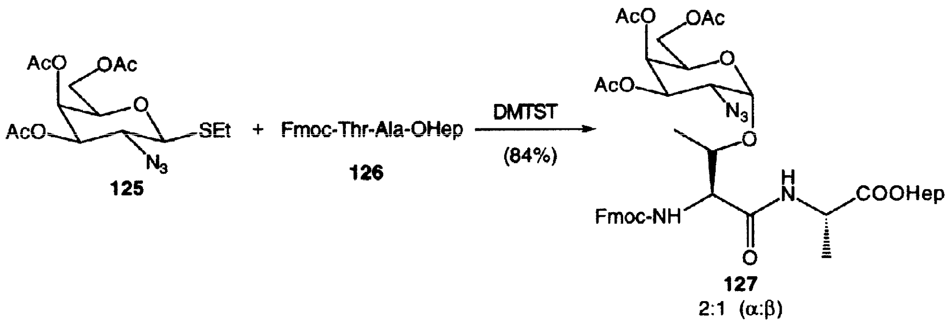
Scheme37

Activation of thioglycosides is also a suitable method for the attachment of oligosaccharides (*c.f.*, monosaccharides) to an amino acid building block. The example in Scheme 38 demonstrates attachment of the core mucin trisaccharide **123** to a serine residue (**92**) to give **124**.[111] Unfortunately the glycosylation is not very stereoselective.



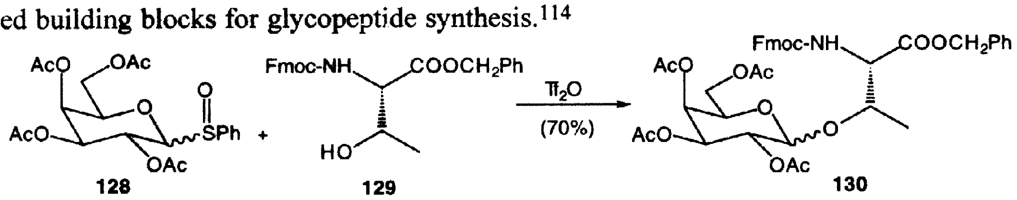
Scheme38

Braun and coworkers reported the preparation of GalNAc-Thr-Ala derivative **127** from **125** and **126** (Scheme 39). The glycosylation proceeded in good yield. The heptyl ester at the *C*-terminus was hydrolysed using lipase M at pH 7 and 37 °C in 95% yield.[112]



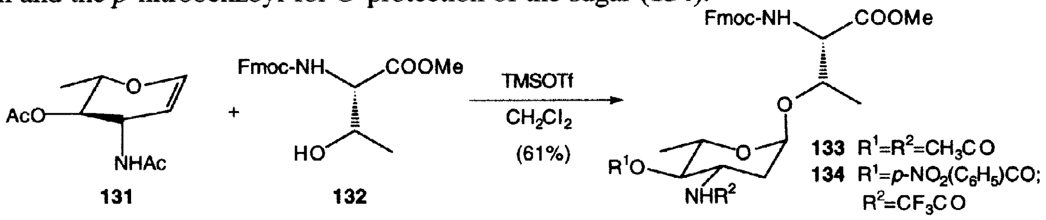
Scheme 39

Sulfoxide Method. Andreotti and Kahne used the sulfoxide method for the glycosylation of **129** with **128** to give a 1:1 mixture of α- and β-anomers of **130** (Scheme 40).[113] These were separated chromatographically and the α-anomer incorporated into a glycopeptide. They also used a disaccharide sulfoxide to prepare glycosylated building blocks for glycopeptide synthesis.[114]



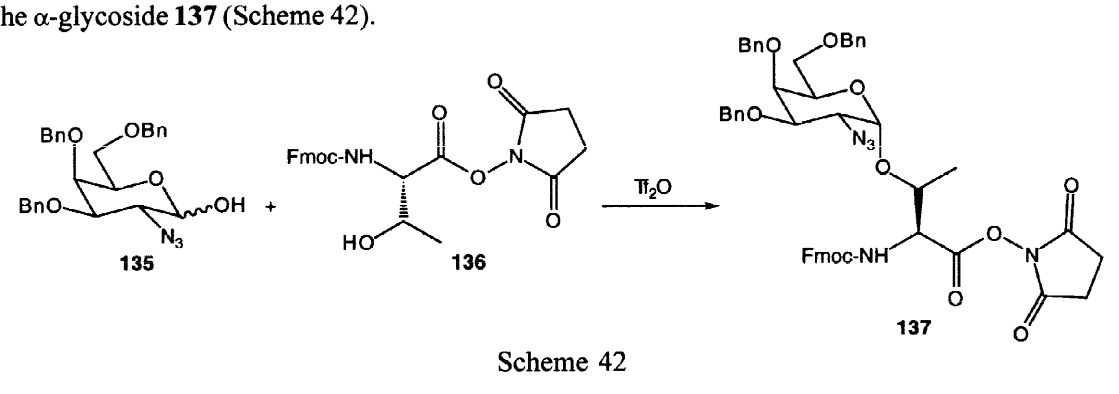
Scheme 40

Employing Glycals. Kessler and coworkers coupled glycal **131** with the protected Thr derivative **132** to give **133** (Scheme 41). Ordinarily, the Ferrier rearrangement is observed under such conditions, but apparently not when there is an *N*-acetyl group at the 3-position. Unfortunately, attempts to hydrolyse the acetate ester in **133** destroyed the glycosidic linkage. A solution was found using the trifluoroacetyl group for *N*-protection and the *p*-nitrobenzoyl for *O*-protection of the sugar (**134**).[115]



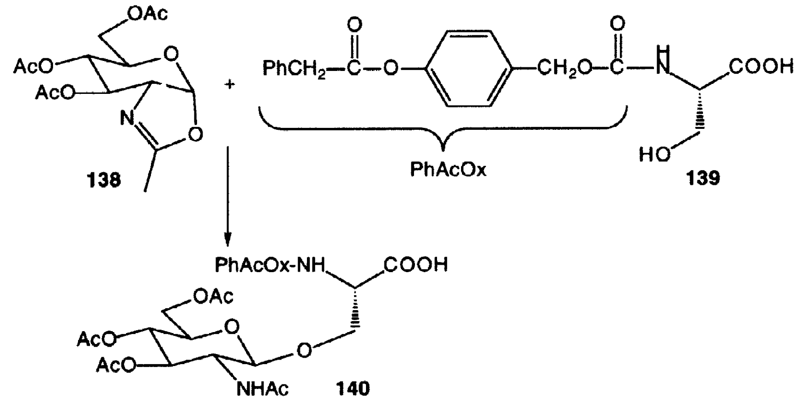
Scheme 41

Triflic Anhydride Method. In their synthesis of the *N*-terminal portion of human glycophorin A^M, the main erythrocyte membrane glycoprotein, Ferrari and Pavia used triflic anhydride as the coupling reagent.[116] Combination of the lactol **135** and the *N*-hydroxysuccinimide ester **136**, in the presence of triflic anhydride gave the α-glycoside **137** (Scheme 42).



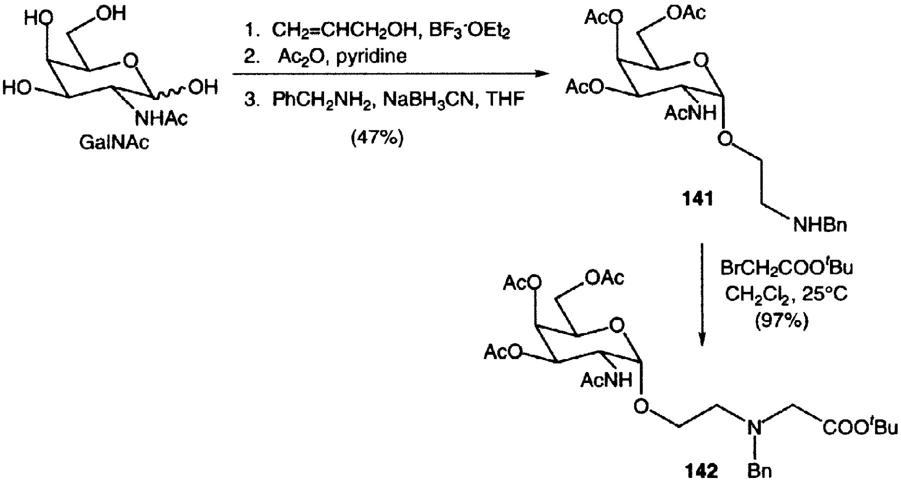
Scheme 42

Via an Oxazole. The use of oxazoles as precursors to glycosyl azides was discussed in §3.1.1. The oxazole ring blocks the lower face of the pyranose ring, directing incoming nucleophiles to the β-face. For monosaccharides with an NHAc group at C2, oxazoles are also possible intermediates for the preparation of *O*-glycosides. Pohl and Waldmann reacted oxazole **138** with the *p*-phenylacetoxybenzyloxycarbonyl protected Ser (**139**) to give the β-glycoside **140** (Scheme 43).[117] This *N*-protecting group can be removed by pencillin G synthase. This work was part of the synthesis of a partial structure of the large subunit of mammalian RNA polymerase II.



Scheme 43

Via an *O*-Allyl Glycoside. An unusual *O*-glycosidic linkage was designed and synthesized by Kim and Roy in their preparation of "glycopeptoids" related to the cancer T_N-antigen.[118] Reaction of GalNAc with allyl alcohol under Lewis acid catalysis led to an *O*-glycoside. Acetylation of the three alcohols, ozonolysis of the double bond to give an aldehyde, and addition of benzylamine under reducing conditions led to secondary amine **141** (Scheme 44). Reaction with *tert*-butylbromoacetate gave the peptoid building block **142**.

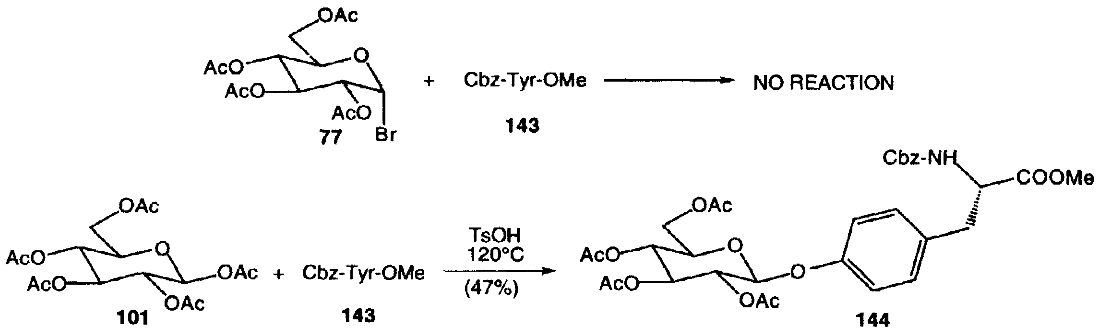


Scheme 44

3.2.2 Glycosylation of Phenols

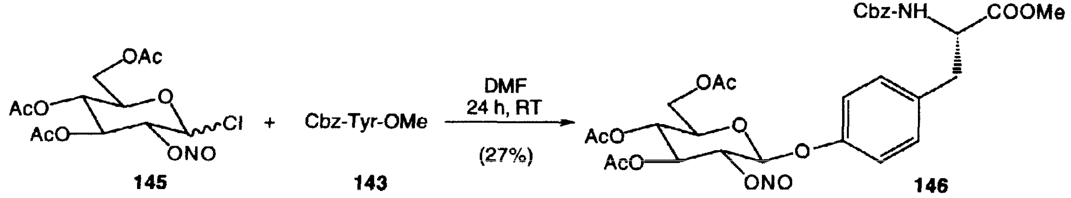
This is more difficult than the glycosylation of an aliphatic alcohol, owing to the reduced nucleophilicity of the phenolic oxygen. Several methods have, however, been successfully applied to the glycosylation of tyrosine side chains.

The first attempts to form such a glycosidic linkage were reported by Lu *et al.*[119] No product was isolated from the Koenigs—Knorr coupling in Scheme 45, using the following catalysts: mercuric cyanide, silver oxide and a mixture of mercuric bromide and mercuric oxide. The authors suggested that the phenol reacts with the catalysts to form insoluble salts. They were, however, able to form the glycoside **144** using an acid catalyzed condensation between peracetylated glucose (**101**) and a suitably protected Tyr residue (**143**).



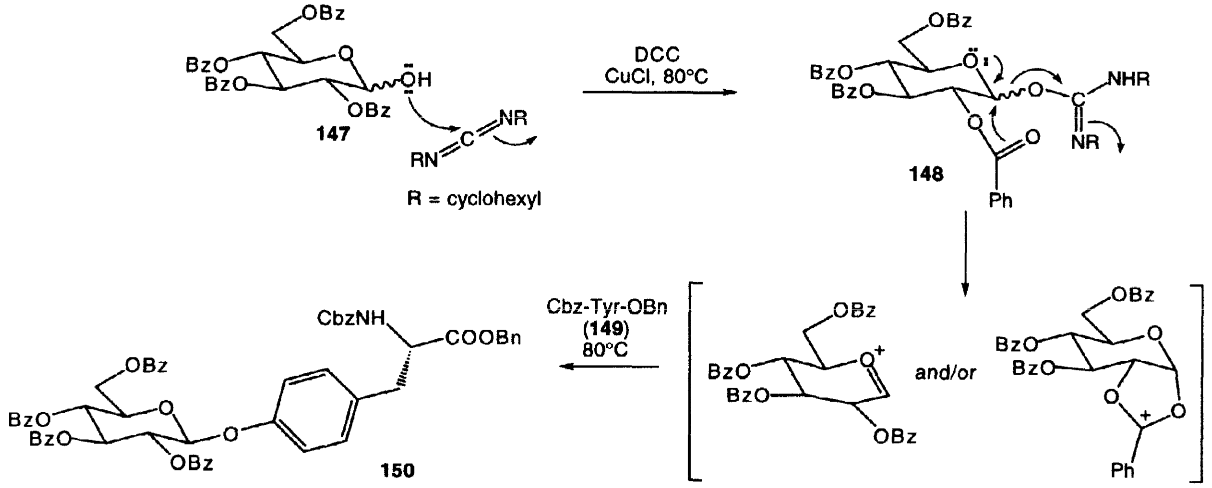
Scheme 45

Smiatacz and coworkers reported isolation of the glycoside **146** in poor yield from the reaction of **145** and **143** (Scheme 46).[120,121]



Scheme 46

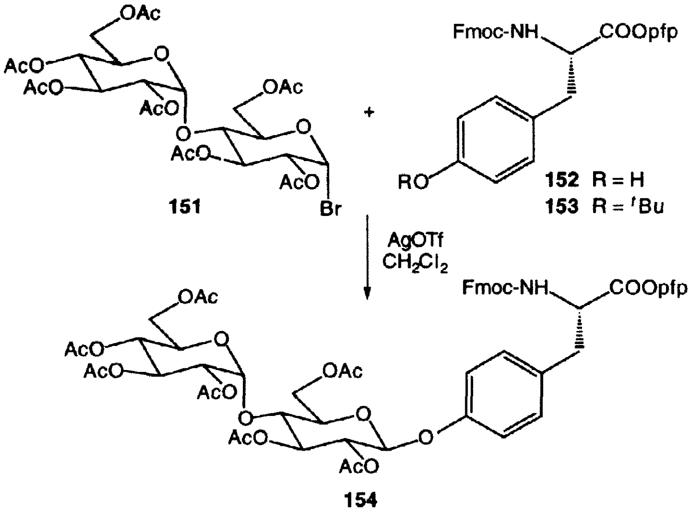
The phenolic oxygen of tyrosine side chains has been glycosylated using *O*-glycosylpseudoureas as intermediates.[122,123] Treatment of a mixture of lactol anomers **147** with DCC in the presence of catalytic copper(I) chloride gave the activated glycoside **148**. Reaction with protected tyrosine derivative **149** gave the corresponding *O*-glycoside **150** in 34-79% yield, with high β-selectivity (Scheme 47).



Scheme 47

The direct glycosylation of Fmoc-protected Tyr esters (*e.g.*, pentafluorophenyl) was reported, using silver triflate as the promotor (Scheme 48).[124] Interestingly, in glycosylations with **151**, the *tert*-butyl ether

(**153**) gave better yields of glycoside **154** than the free phenol (**152**). In a subsequent paper, Vargas-Berenguel *et al.* proposed that the bulky *tert*-butyl group forces the oxygen lone pairs out of conjugation with the pi electrons of the aromatic ring, thereby increasing reactivity.[125]



Scheme 48

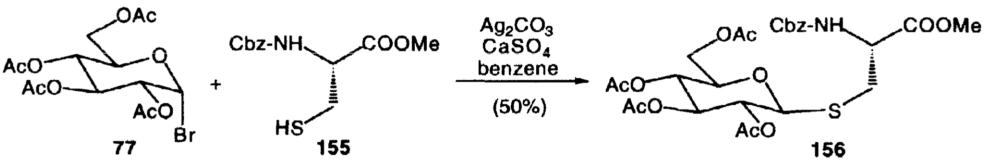
Table 4

R	Reaction Time	Yield
H	120 mins	42%
^{t}Bu	<30 mins	81%

3.3 *S-Glycosides*

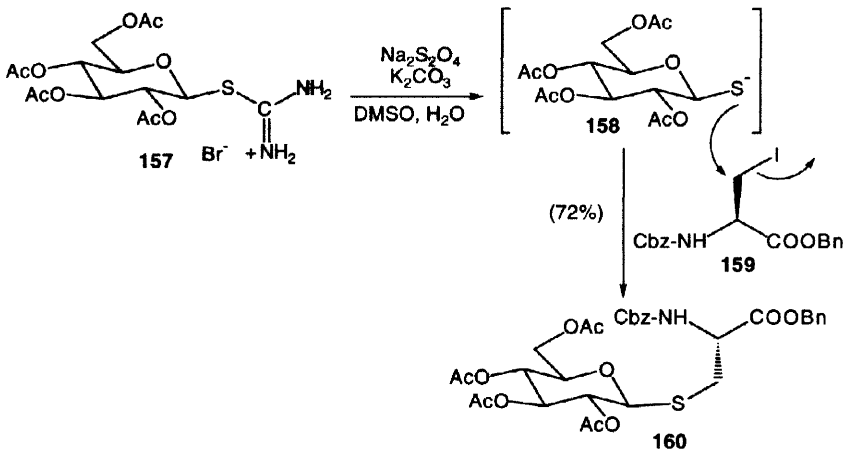
As described in §2.4, the only naturally occurring *S*-glycosides involve either Gal or Glc linked to a *N*-terminal Cys residue. Several attempts have been made to emulate these linkages by chemical synthesis. A variety of glycosylation methods have been applied.

Baran and Drabarek performed a Koenigs—Knorr coupling between **77** and **155** to give a 50% yield of thioglycoside **156** (Scheme 49).[126] Although they do not discuss the stereochemistry of the glycosidic linkage, it is reasonable to assume a preponderance of the β-anomer.



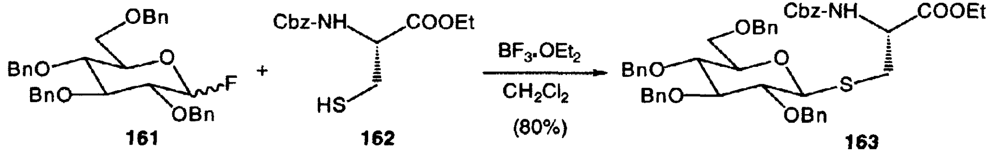
Scheme 49

Monsigny *et al.* coupled a pyranosylisothiouronim salt (**157**) derived from glucose with a β-iodoserine derivative (**159**) (Scheme 50).[127] Under basic conditions, isothiouronium salts are cleaved to generate a nucleophilic thiolate anion (**158**). The yield of the thioglycoside (**160**) compares favourably with that for the Koenigs—Knorr reaction above.



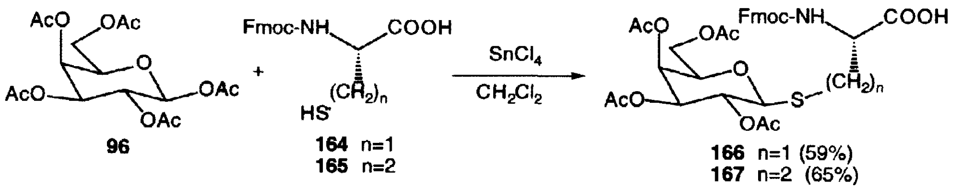
Scheme 50

In a demonstration of the utility of glycosyl fluorides, Nicolaou *et al.* formed the same type of β-thioglycosidic linkage (Scheme 51).[128] Reaction of glycosyl fluoride **161** with cysteine derivative **162** gave **163** in good yield.



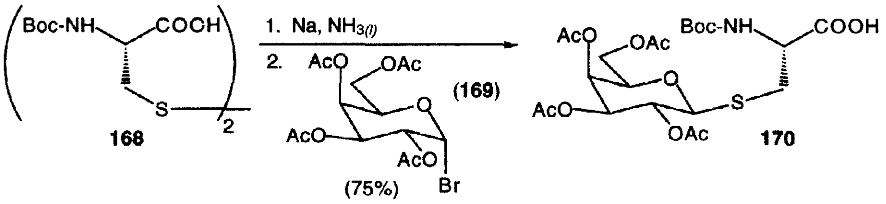
Scheme 51

Lewis acid catalyzed glycosylation of Fmoc-protected Cys (**164**) and homocysteine (**165**), with peracetylated galactose (**96**) was possible without protection of the carboxylic acid and gave thioglycosides **166** and **167** respectively (Scheme 52).[101,129]



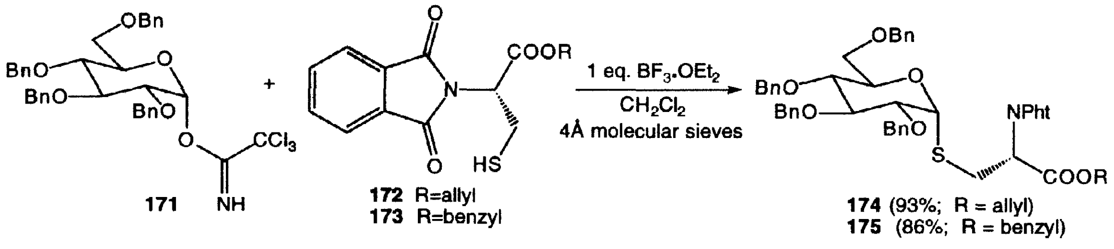
Scheme 52

Kessler and coworkers used a Koenigs—Knorr reaction in which the nucleophile was a thiolate anion rather than a neutral thiol. Reduction of Boc-protected cystine (**168**) and formation of the dianion was accomplished with sodium in liquid ammonia (Scheme 53). Reaction of the thiolate anion with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosylbromide (**169**) led to a good yield of the β-glycoside **170**.[130]



Scheme 53

All examples above involve formation of a β-linkage, which is readily obtained using neighbouring group participation of the protecting group at C2. The synthesis of an *S*-α-D-glycosyl cysteine has recently been reported by Käsbeck and Kessler (Scheme 54).[131] To form the α-linkage in compounds **174** and **175**, they used thiols **172** and **173** respectively in combination with trichloroacetimidate **171**. In terms of the cysteine component in the glycosylation, carbamate protecting groups were found to be unsuitable due to unfavourable hydrogen bonding (see §3.2.1). An *N*-protecting group without a hydrogen bond donor, *viz.* phthalimido, proved superior.



Scheme 54

While *S*-glycosides have been isolated from nature, the driving force behind the synthesis of *S*-glycosides, most noteably by Kessler and coworkers, has been the production of analogues of *O*-glycosides with enhanced stability.

Buserelin (**176**) (Fig. 19) is a potent agonist of LH-RH, with many possible applications in medicine. For paraenteral application, the water solubility of the peptide needed improvement. It was known that glycosylation of the D-Ser residue greatly increased the solubility in aqueous solution, without any loss of activity. Kessler and coworkers reasoned that incorporation of an unnatural *S*-glycosidic linkage, would enhance resistance of the peptide towards chemical and enzymatic degradation. Indeed, incorporation of the L-rhamnose-D-Cys residue (**177**) at the aforementionned position gave a compound with the same activity as buserelin itself. The α-trichloroacetimidate of L-rhamnose (**178**) was used to glycosylate **179** to give the *S*-glycoside **180** in moderate yield (Scheme 55).[132]

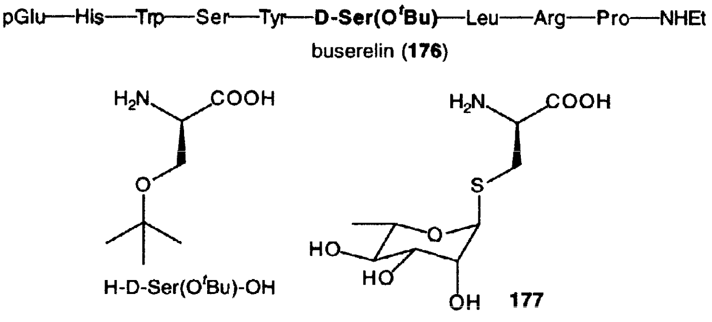
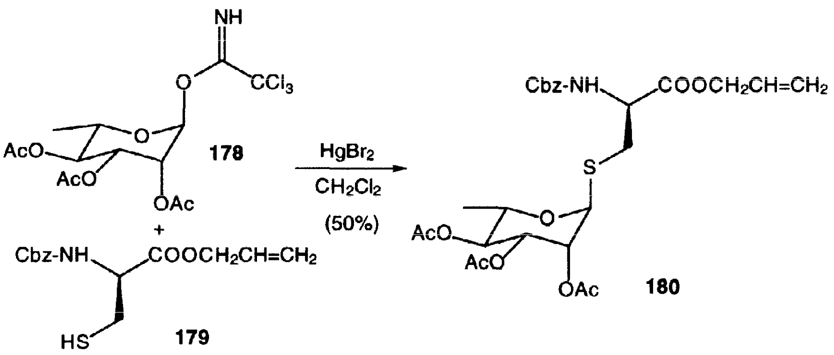


Figure 19



Scheme 55

3.4 C-Glycosides

3.4.1 Glycosyl α-Amino Acids. This group of *C*-glycosides, so far unknown in nature, involve a linkage between the anomeric carbon of a sugar to the α-carbon of an amino acid, most often glycine (Fig. 20). The sugar moiety is effectively the side chain of the amino acid.

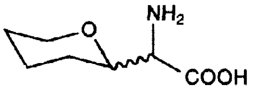
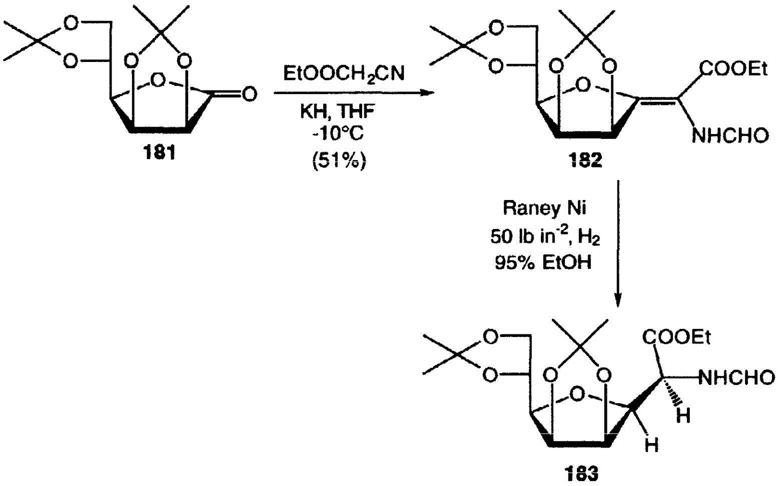


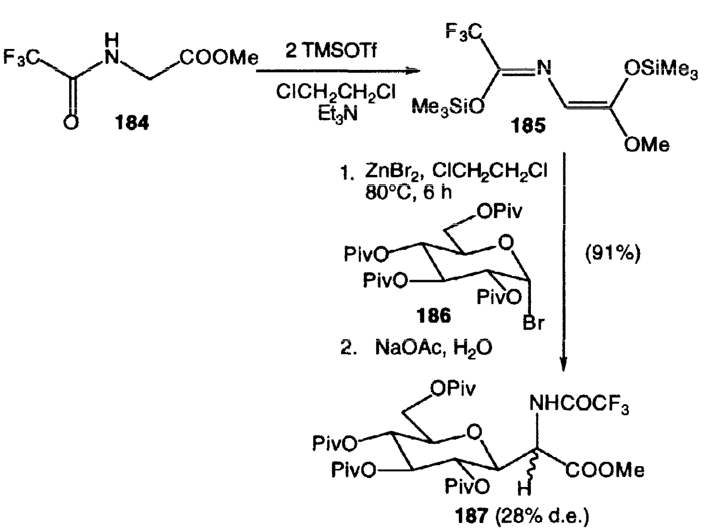
Figure 20. Glycosyl α-Amino Acids

The first example of this kind was reported as early as 1975 by Bischofberger, Hall and Jordaan.[133,134] Addition of the anion of ethyl isocyanate to 2,3:5,6-di-*O*-isopropylidene-D-mannono-1,4-lactone (**181**) gave **182**, with a predominance of the *E*-isomer (86 % d.e.) (Scheme 56). Catalytic hydrogenation gave **183**.



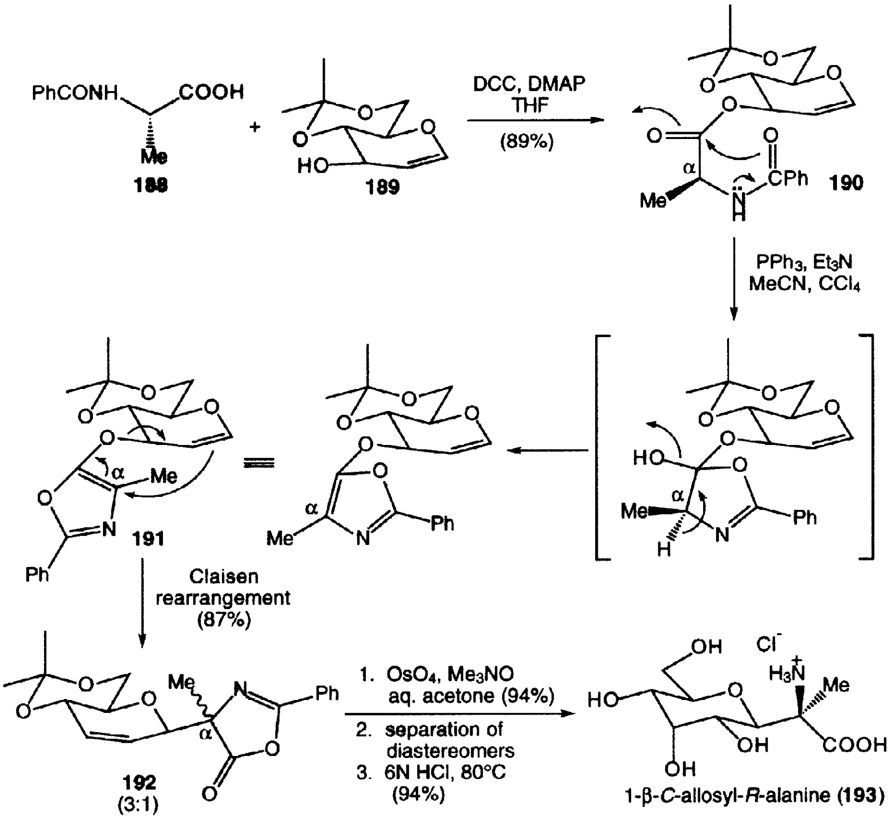
Scheme 56

Fifteen years later, Simchen and Pürkner reported the Lewis acid catalyzed reaction of ketene acetals (*e.g.*, **185**), derived from *N*α-trifluoroacetylamino acid esters (*e.g.*, **184**), with α-glucosyl bromides (*e.g.*, **186**) (Scheme 57).[135] While the chemical yield of **187** was excellent, the diastereoselectivity of the addition was poor.



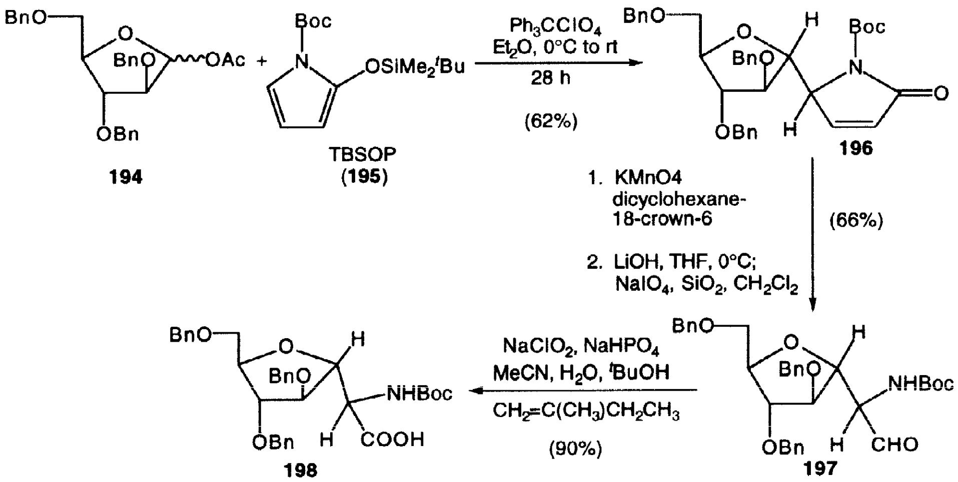
Scheme 57

Columbo, Casiraghi and Pittalis applied the Claisen rearrangement to the formation of a *C*-glycosidic bond (Scheme 58).[136] Formation of an ester linkage between glycal **189** and alanine derivative **188** gave **190**. Under dehydrating conditions an oxazole (**191**) is generated, with concomitant loss of chirality at Cα. During the Claisen rearrangement to give **192**, this stereogenic centre is regenerated, but with only modest stereoselectivity. Oxidative degradation of the oxazolone ring, followed by *cis*-hydroxylation of the glycal double bond and acid-catalyzed cleavage of the ketal gave 1-β-*C*-allosyl-*R*-alanine (**193**).



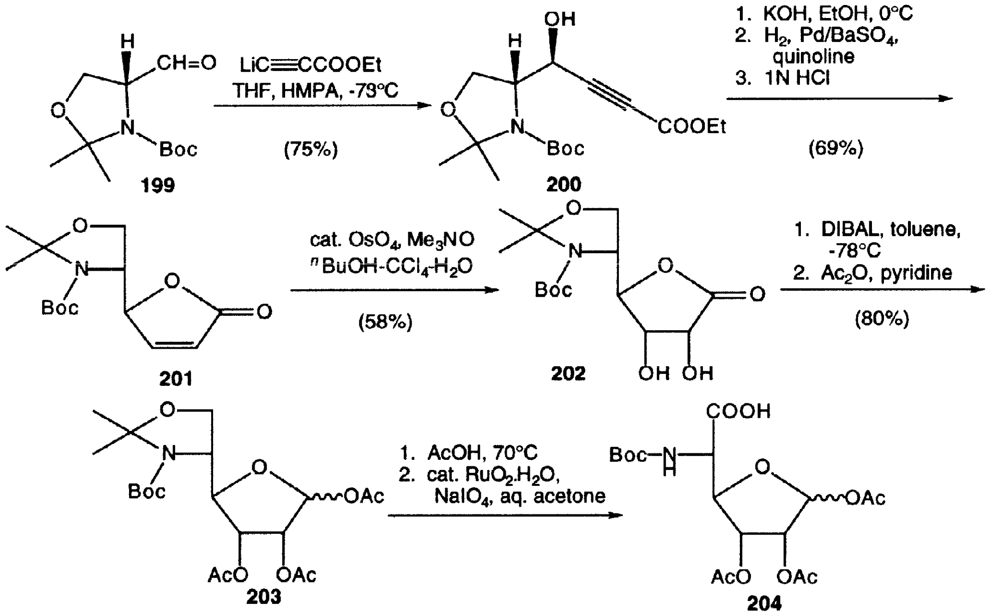
Scheme 58

Rassu *et al.* recently described the addition of TBSOP (**195**, a glycine anion equivalent) to tri-2,3,5-*O*-benzyl-1-*O*-acetyl-arabinofuranose (**194**) to give **196** (Scheme 59).[137] Further manipulations led to the α-*C*-arabinofuranosyl glycine **198**, via aldehyde **197**.



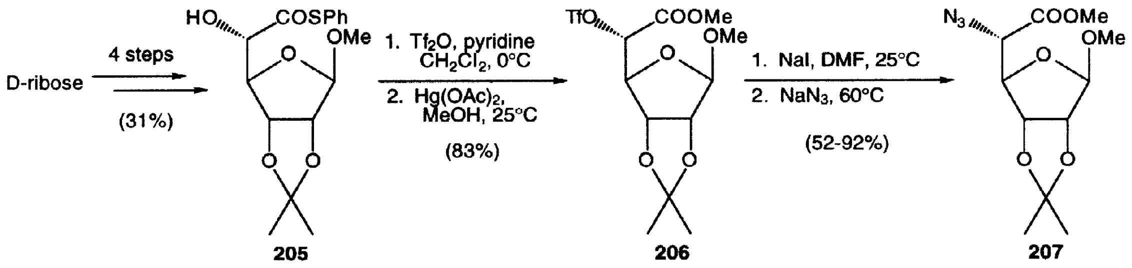
Scheme 59

As an integral part of their synthesis of the peptidyl nucleoside antibiotics such as the polyoxins, Garner and Park developed a stereocontrolled synthesis of a furanosyl α-amino acid (Scheme 60).[138] Beginning with their trademark aldehyde **199**, they accomplished a stereoselective addition of a lithium acetylide to give secondary alcohol **200**. The ethyl ester of **200** was converted to its potassium salt and then the alkyne reduced to the corresponding *cis* olefin. Upon acidification, the hydroxyacid cyclized to give α,β-unsaturated lactone **201**. Dihydroxylation of the olefin gave exclusively **202**. Reduction of the lactone to the lactol and acetylation gave **203**. Acid-catalyzed hydrolysis of the acetonide and oxidation of the resulting primary alcohol gave α-furanosyl amino acid **204**.



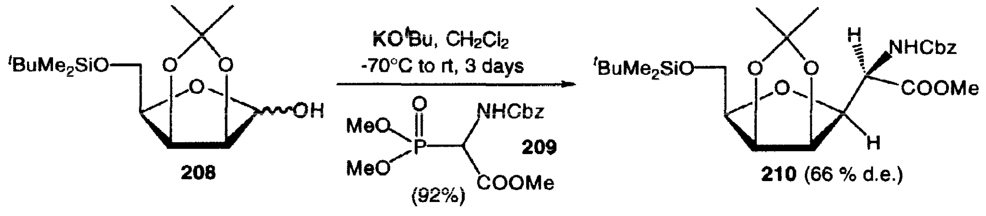
Scheme 60

In a total synthesis of polyoxin C, Barrett and Lebold formed an α-ribosyl-α-azido acid (Scheme 61).[139] The secondary alcohol in compound **205** was converted to the triflate, and the thioester converted to a methyl ester (**206**). The triflate was displaced with iodide, then azide to give **207**. Later in the synthetic scheme, the azide was reduced to the primary amine using triphenylphosphine.



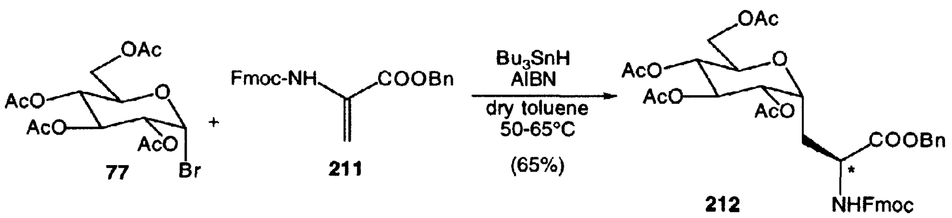
Scheme61

Addition of the phosphonate-stabilized anion derived from **209** to lactol **208** gave the corresponding glycoside **210** in excellent chemical yield with reasonable diastereoselectivity (Scheme 62).[140]



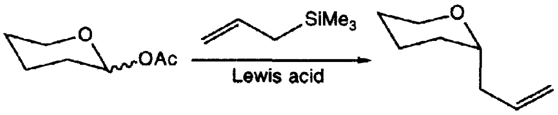
Scheme 62

3.4.2 Glycosyl β-Amino Acids. Kessler *et al.* used a radical reaction between glycosyl bromides and dehydroalanine derivatives. Reaction of **77** and **211** gave **212** (Scheme 63).[141] The reaction is >97% selective for formation of the α-glycoside.[142] However, a mixture of diastereomers is obtained at the α-carbon(*) of the amino acid; in this case the ratio was 2.5:1.0 in favour of the *S*-configuration.



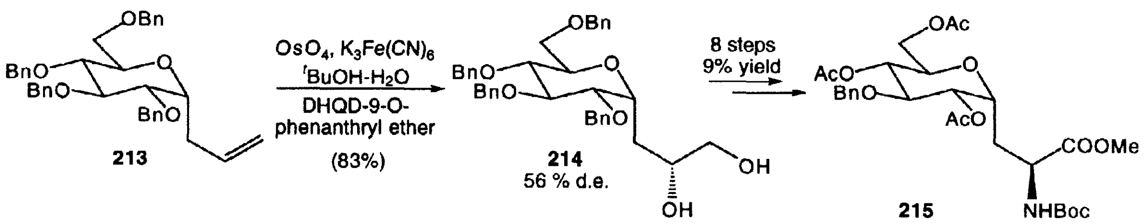
Scheme 63

A useful reaction for introducing a carbon chain at the anomeric centre is the reaction of an anomeric acetate with an allyl silane under Lewis acid catalysis (Scheme 64).



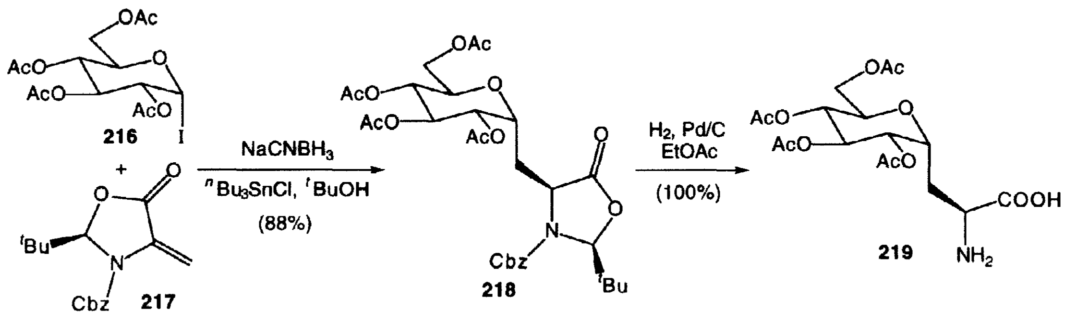
Scheme 64

Such an intermediate (**213**) was utilized by Gurjar *et al.* in the synthesis of β-glucosylalanine derivative **215** (Scheme 65).[143] To introduce the stereochemistry of the α-amino acid, they invoked the Sharpless asymmetric dihydroxylation reaction to give **214**; the diastereoselectivity was only modest. Several standard steps led to the β-glycosylamino acid.



Scheme 65

During an investigation of radical additions to methyleneoxazolidinones, Axon and Beckwith reported the highly diastereoselective addition of a glucosyl radical (derived from **216**) to enone **217**, which resulted in compound **218** (Scheme 66).[144] Glucosyl radicals have a propensity to form new bonds in the α-orientation.[142] Hydrogenolysis of **218** gave the amino acid **219**.



Scheme 66

3.4.3 Glycosyl γ-Amino Acids. Glycosylation at the γ-carbon leads to potentially more useful compounds than those described in §3.4.1 and §3.4.2. The point of attachment of the sugar moiety is analogous to that in naturally occurring *O*-glycosides (Fig. 21). They are mimics of the corresponding glycopeptides, in the sense that the oxygen atom in the glycosidic linkage is replaced by an isosteric -CH_2- group.

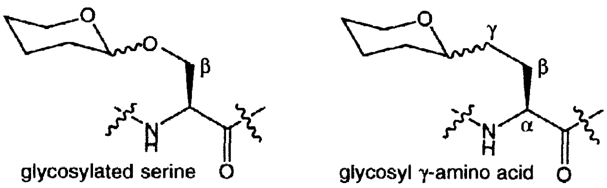
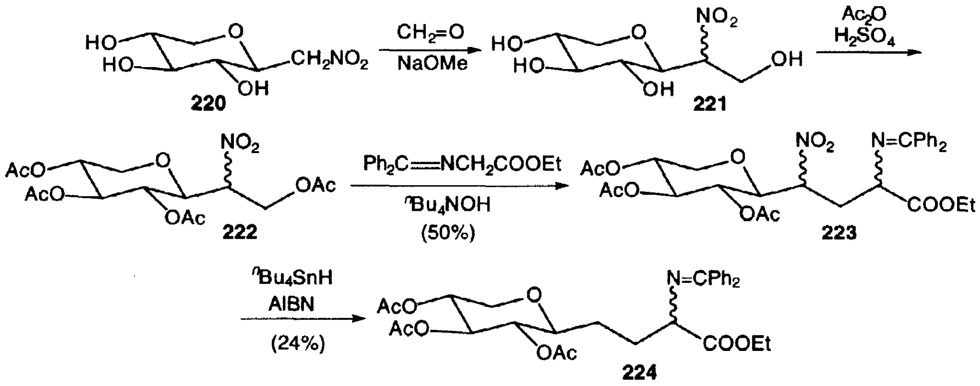

glycosylated serine
glycosyl γ-amino acid

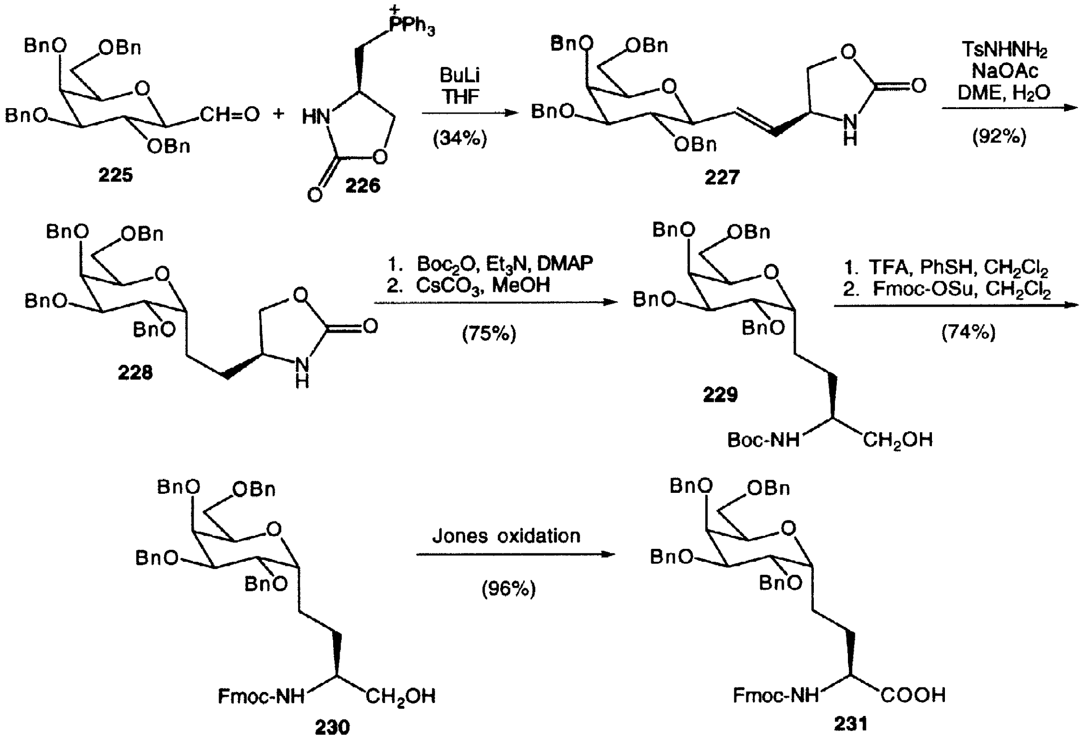
Figure 21. Glycosyl γ-Amino Acids Mimic Glycosylated Serine

Petrus and BeMiller prepared the *C*-glycosyl analogue of *O*-(β-D-xylopyranosyl)-L-serine (**224**) according to Scheme 67.[145] A nitroaldol reaction between D-xylose derivative **220** and formaldehyde gave **221**. Peracetylation gave **222**. Addition of a glycine anion equivalent gave a mixture of all four possible stereoisomers of **223**. Radical reduction to remove the nitro group gave **224**.



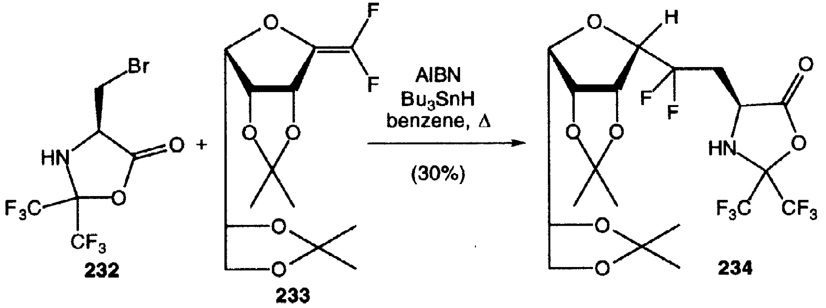
Scheme 67

Bertozzi *et al.* prepared a *C*-glycosyl analogue of the β-galactoside of Ser (Scheme 68).[146] Wittig reaction of aldehyde **225** and phosphonium salt **226** gave a mixture of alkenes **227**. Diimide reduction of the mixture led to **228**. Boc-protection of the nitrogen and opening of the oxazolinone gave **229**. At this stage the Boc group was swapped for Fmoc, giving **230**. Oxidation of the primary alcohol to the corresponding carboxylic acid afforded **231**.



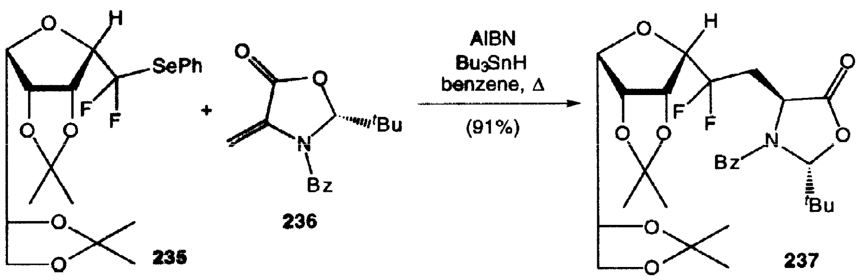
Scheme 68

Herpin *et al.* recently introduced the difluoromethylene isostere to produce another kind of γ-glycosyl amino acid.[147] The tetrahedral, electronegative -CF_2- unit has been shown to be a good isosteric and isoelectronic replacement for an oxygen atom. Addition of the radical derived from bromoalanine derivative **232** to *gem*-difluoro enol ether **233** under tributylstannane mediated conditions gave the *C*-glycoside **234** (Scheme 69).



Scheme 69

They were able to reverse the roles of the carbohydrate and amino acid components, generating a radical from the phenylselenide **235** and having this add to an oxazolidinone **236** (Scheme 70). The chemical yield of **237** was excellent and a d.e. of 60% was observed, favouring formation of the *S*-amino acid.[147]



Scheme 70

3.4.4 Miscellaneous Compounds. Retroinversopeptides have enhanced stability to proteolytic enzymes (Fig. 22a). The same concept of inverting the direction of the amide bond has been applied to the *N*-glycosidic linkage between glucose and the side chain of Asn residues (Fig. 22b).[148] The stability and utility of these compounds has yet to be reported.

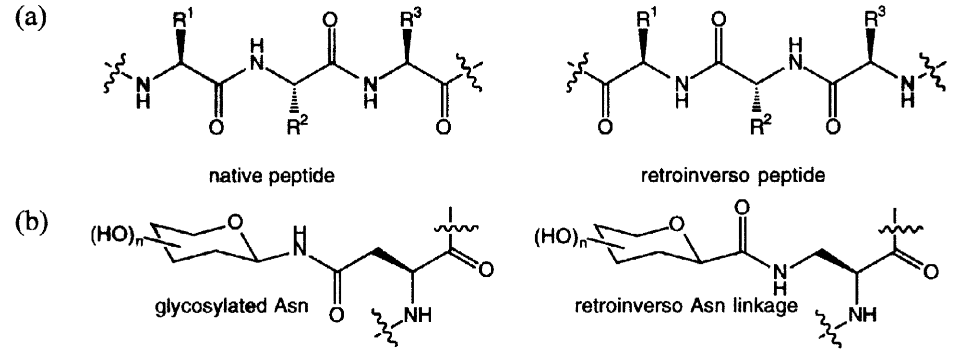
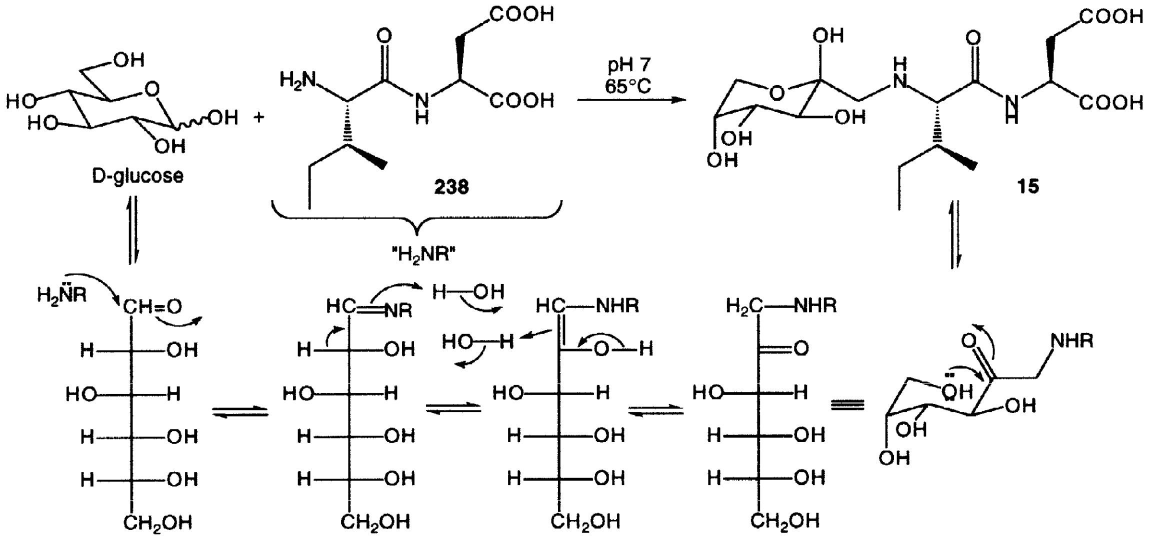


Figure 22. (a) Concept of a Retroinverso Peptide; (b) Retroinverso Asn-Linked Glycopeptide

The Amadori rearrangement has been used by Vértesy *et al.* in a biomimetic synthesis of the enkastines (see §2.5).[55] Reaction of D-glucose and unprotected dipeptides (*e.g.*, **238**), in borate buffer, led to the isolation of the rearranged products (*e.g.*, **15**) in reasonable yield (Scheme 71). A possible mechanistic pathway for the coupling and rearrangement is proposed.



Scheme 71

Wong and coworkers have synthesized some *C*-glycosides as mimics of sialyl Lewis^x (Fig. 23). In mimic **239**[149] the β-hydroxyamino acid is believed to substitute for the essential 4-OH and 6-OH groups of the Gal residue of SLe^x (Fig. 24). Compound **239** was shown to have an IC_{50} of 0.3 mM (*c.f.*, 0.5 mM for SLe^x itself). In another paper, compound **240** was synthesized.[150] The D-mannose is intended to mimic the L-fucose and the Glu side chain the carboxylic acid of sialic acid.

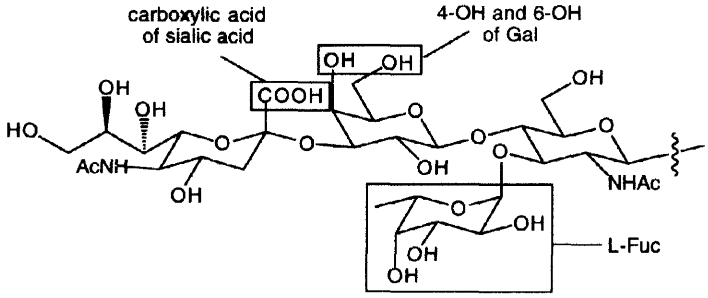
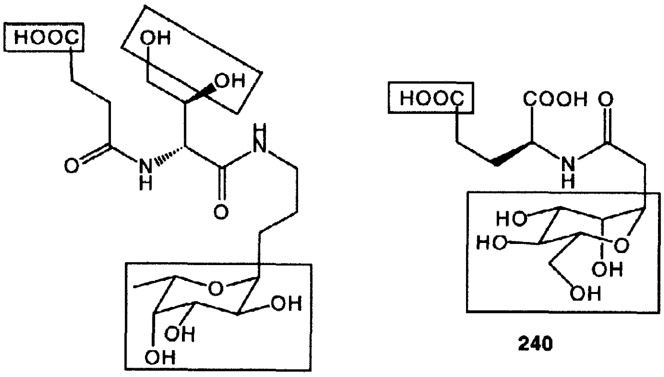


Figure 23. Sialyl Lewis^x

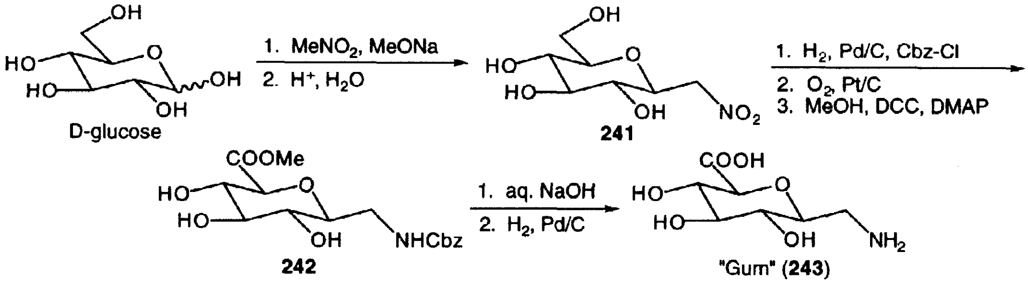


239

240

Figure 24. *C*-Glycoside Mimics of Sialyl Lewis^x

Von Roedern and Kessler have developed a monosaccharide derived peptidomimetic, which they call "Gum" (**243**);[151] the synthesis of **243** is outlined in Scheme 72. A nitroaldol reaction of D-glucose and nitromethane gave **241**. Reduction of the nitro group, protection of the resulting amine as its benzyl carbamate, selective oxidation of the primary alcohol and esterification led to **242**. Hydrolysis of the methyl ester and cleavage of the Cbz group afforded **243**. The pyranose ring of **243** adopts a $^4C_1$ conformation and the unit can be used to replace two adjacent amino acids linked by a *trans* amide bond.



Scheme 72

4. SUMMARY

This review has aimed to showcase research which has enlightened us about the structure of naturally occurring glycopeptides, and those contributions which have led to the chemical synthesis of glycopeptides. By focussing discussion around the key linkage between the carbohydrate and the peptide/protein, it has been possible to draw information together in a new way. The more global strategic issues regarding assembly of the carbohydrate and peptide portions of the molecules, have not been contemplated. This has been done well elsewhere,[1,8-11,86] and has made it possible to consider the unique structural features of this class of molecules which set them apart from either carbohydrates or peptides.

It might have been expected that enzymes would have been featured in §3, but alas, enzymes have been covered in this review only to the extent that they have been used to remove protecting groups under mild, selective conditions.[112] The expression "enzymatic glycoprotein synthesis" is used widely,[152,153] but the use of enzymes is invariably restricted to the formation of glycosidic linkages between sugar units. Little is known about the enzymes which catalyze the formation of the key glycosidic linkages between the carbohydrate and the peptide. An exception to this is oligosaccharyl transferase, which has been isolated and studied.[154,155] Perhaps the isolation, characterization and application of these glycosyl transferases is a future direction in the field of glycopeptides.

It might well be asked why nature employs different kinds of glycosidic linkages in different glycopeptides. Is it related to the environment in which the protein has to perform? Is it related to the tasks that the protein has to perform? Is it related to the kinds of modifications that the glycoprotein undergoes during its lifetime? These, and many more questions, remain to be answered. A key tool in addressing them will undoubtedly be the study of synthetic compounds in biological systems. While it is now possible to assemble glycopeptides of some complexity, it must be recognized that this work is being done by a relatively small group of highly specialized organic chemists. The chemistry is plagued by the nuances of both carbohydrate and peptide chemistry. Much work has yet to be done in the development of general methods for the construction of glycosidic linkages between carbohydrates and peptides.

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Biographical sketch

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Carol Taylor received her B.Sc. in 1987, and her M.Sc. in 1988 from the University of Auckland, New Zealand. Her research at the University of Auckland, with Professor Con Cambie, involved the synthesis of ambergris odorants employing New Zealand natural products as starting materials. In 1987 and 1988 Carol spent two summers investigating the chemistry of *ortho*-quinone monoimides with Professor Harold W. Heine of Bucknell University (PA, USA). She received her Ph.D. in 1993 from the University of Pennsylvania (USA) under the direction of Professor Ralph Hirschmann and Professor Amos B. Smith, III. Her Ph.D. thesis involved the development of catalytic antibodies for peptide bond formation. From 1993 to 1994, Carol was a Research Associate at Princeton University (NJ, USA) with Professor Daniel Kahne, working in the area of solid phase oligosaccharide synthesis. In 1995 Carol returned to New Zealand to the position of Lecturer at the University of Auckland. Current research focusses on the design, synthesis and evaluation of novel peptides, including phosphonopeptides and glycopeptides.