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O-Glycosyl α-amino acids as building blocks for glycopeptide synthesis †

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Abstract: A review of *O*-glycosyl α-amino acid structures representing most of the glycosylation sites of known *O*-glycoproteins found in nature, their preparation by chemical methods and application as building blocks for either solution or solid-phase synthesis of *O*-glycopeptides is presented. © 1997 Elsevier Science Ltd

1. Introduction

After realising that most proteins found in nature carry structurally diverse *O*- and *N*-linked oligosaccharide moieties, there is no doubt that a new dimension in many fields of biochemistry[1]

† This review is dedicated to Professor Raymond A. Dwek, Director of The Glycobiology Institute, Department of Biochemistry, University of Oxford, who introduced me to Glycobiology.

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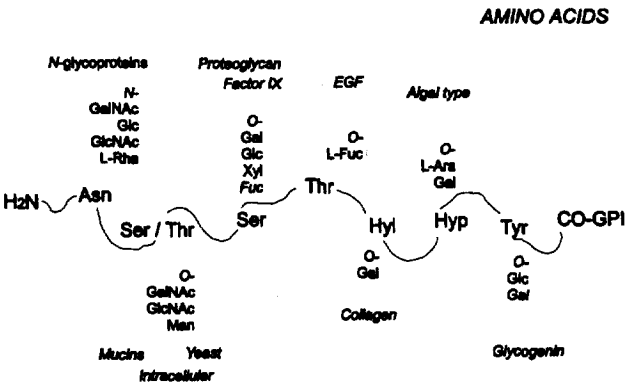
has emerged. As a consequence, during the last decade information regarding the physiochemical and biological functions of the protein bound oligosaccharides has been accumulating in many glycobiology laboratories. However, until now, well defined physiological roles for many of these oligosaccharide moieties have yet to be unveiled.

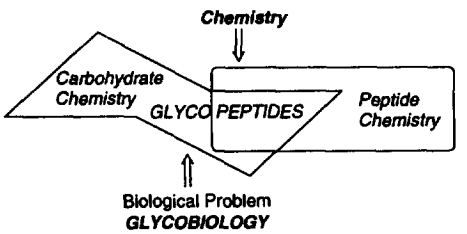
Depending on their functionalities sugars are bound at different amino acid side chains of protein backbones. An important group of them are those that carry an *O-linked protein glycosylation*.[2] This concept is schematically represented in Scheme 1 where the known forms of protein glycosylation are summarised. Such a drawing originally was a static and predictable representation which is starting to become a very dynamic picture due to the continuing discovery of new forms of glycosylation.[3]



Scheme 1.

In common with other studies requiring the isolation of target molecules from biological sources, the study of glycoproteins faces the problem of low concentration in raw materials. The availability of glycoproteins from natural sources is limited because of the microheterogeneity of biological glycoconjugates. This feature is widespread and has been observed for natural as well as for recombinant DNA glycoproteins. In spite of this, the existence of microhetereogeneity gives rise to many intereresting questions regarding the origin of this phenomenon and about its relevance for the biological functions of the **glycoforms**.

Owing to these problems the understanding of the roles conferred by the carbohydrate moieties upon glycoproteins had to be based on the use of synthetic rather than natural models. Such a need for unequivocally synthesised molecular models of glycoproteins has been fuelling the marriage between both carbohydrate[4] and peptide chemistries with the aim to prepare glycopeptides (Scheme 2). After all this effort, glycopeptides can now be made available with variations in both the peptide and carbohydrate part in higher quantities and superior purities than for their parent glycoproteins, therefore, becoming the model compounds of choice for biomedical and structural investigations where glycoproteins are involved.



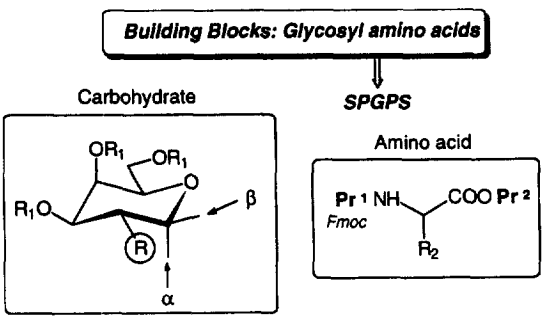
Scheme 2.

The synthesis of GLYCOPEPTIDES[5] requires a combination of synthetic methods from both carbohydrate and peptide chemistry which are different if applied to *N*- or *O*-glycopeptide preparation. By focusing at *O*-glycopeptide synthesis, the present review intends to cover one of the areas where a great deal of novel and recent developments have occurred. Moreover, unlike other glycopeptides, features such as the stereochemistry and lability to acids/bases of the *O*-glycosidic bond makes *O*-glycopeptide chemistry specialized enough to be considered separately.

In theory, two alternatives can be foreseen for the chemical synthesis of *O*-glycopeptides. However, in spite of very recent references,[6] one of the alternatives (based on the direct glycosylation of preformed polypeptides with a given oligosaccharide) has only been described by a few authors[7] and does not constitute a feasible route to *O*-linked glycopeptides.

Instead the most general and effective synthetic methodology employs glycosylated amino acids for the stepwise assembly of glycopeptides, preferably on solid-phase. Thus, synthetic routes to suitably protected glycosylated amino acid building blocks constitute a key to success for the synthesis of *O*-glycopeptides and are *the main subject matter of the present review*. Only chemical methods for *O*-glycoamino acid building block synthesis will be reviewed. Other methods using, *i.e.* enzymatic catalysis and glycoamino acid containing unnatural sugar or amino acid moieties are out of the scope of this work.

Versatility is one of the main advantages of the stepwise 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. On the other hand, since glycosylation of amino acids, with rare exceptions, requires that both the N^{α}-amino acid and the C^{α}-carboxyl functions are blocked, **a protection scheme** is one of the first requirements (Scheme 3).



Scheme 3.

However, protecting groups for glycosylated amino acids to be used in solid-phase peptide synthesis (SPPS) have to be judiciously chosen as the glycosydic bonds are acid-labile (e.g. hydrogen fluoride[8]), and *O*-linked glycopeptides undergo β-elimination on treatment with strong base.[9] This labile character limits the variety of protecting groups that can be applied for glycopeptide synthesis. In spite of these limitations, *O*-glycopeptides allow different acid and basic treatments. Thus, it has been demonstrated[10] that *O*-glycopeptides are stable to bases, e.g. morpholine, piperidine, DBU, that are commonly used to remove the Fmoc protecting group for SPPS. *O*-Glycosidic linkages also appear to be entirely stable to treatment with concentrated TFA in the absence or presence of carbocation scavengers, particularly while the saccharide retains ester type protecting groups.

But in any event, cleavage of *N*- and *C*-groups and removal of saccharidic *O*-protecting groups must always be performed under conditions in which side-reactions such as β-elimination of *O*-linked carbohydrates, epimerization of stereocenters and aspartimide formation can be prevented.

All these considerations have led to a continuous effort for adapting and developing protecting groups that comply with characteristics such as orthogonality and mild deprotection conditions. A concise overview of the achievements for each class of functional groups on an *O*-glycoamino acid structure is provided before entering into a detailed analysis of the different *O*-glycoamino acid building blocks reported in recent literature.

2. Protection scheme

2.1. *Protection of the amino acid*

2.1.1. *α-Amino group protection*

An early aproach to solid-phase synthesis of *O*-linked glycopeptides relied on the *tert*-butyloxycarbonyl (Boc) group for α-amino group protection.[11] However, this approach is no longer required since repeated N^{α}-deprotection with trifluoracetic and final cleavage from the solid phase under strong acid conditions are likely to cleave glycosidic bonds.

In most current studies the N^{α}-fluoren-9-ylmethoxycarbonyl **(Fmoc) group**[12] has been used for α-amino protection of the glycosylated building blocks since it allows protection of amino acid side chains, and linkers to solid-phase, which are cleaved by trifluoroacetic. It is ideally stable for the acidic conditions normally used in *O*-glycosylations and can be removed under mild basic conditions[13] such as morpholine or piperidine without β-elimination.

2.1.2. C-*terminal* C *[α]-carboxyl group protection in the amino acid*

For building block synthesis the *C*-terminal group has been used either protected or unprotected. When protected, a semipermanent protecting group[14] is kept in place during glycosylation and can be removed before conversion into an activated amino acid. Alternatively, in the "active ester" approach an Fmoc amino acid is normally protected as a Pfp ester that can be directly glycosylated to provide building blocks for SPPS. Pfp esters survive exposure to strong Lewis acids (equimolar) in organic solvents, are reasonably stable toward oxygen nucleophiles under weakly acidic and neutral conditions, and can be purified by silica gel chromatography with dry organic solvents. These properties make Pfp a suitable C^{α}-carboxyl protecting group for the glycosylation step, serving a dual role of protection and activation, and saves protecting group manipulation steps.

Another approach is to use Fmoc amino acids where the α-carboxyl group of the amino acid remains free as described in Eloffson's approach.[15] This approach has been successfully used for amino acids such as Ser, Thr, Cys, HomoCys and Tyr. We have also applied this strategy to obtain glycosylated Hyp derivatives.[16]

In some cases, a given N^{α}-amino Fmoc protected amino acid may be orthogonally protected during the glycosylation step with a group such as *t*-Bu, allyl (All) or Bn, which later provide a building block suitable for activation and coupling in the context of Fmoc SPPS.

2.2. *Protection of sugars:* O-, N-*and other carbohydrate protecting groups*

2.2.1. *Carbohydrate* O-*protective groups*

Acetyl protecting groups have been employed on the carbohydrate moiety in order to stabilise the *O*-glycosidic linkages during cleavage with TFA. Even though this may not be an absolute requirement for common monosaccharides such as Glc, Gal, GlcNAc, GalNAc, a slight increase in the acid lability of the *O*-linked saccharide seems to necessitate protection of the hydroxyl groups with electron-withdrawing acyl functions. *For instance*, acyl protection has been found to be important for glycosides of the 6-deoxysugar L-fucose which undergo acid catalysed hydrolysis only 5–6 times faster than glycosides of the corresponding non-deoxygenated monosaccharide galactose.

In addition, **2-*O*-acyl groups** are used for the formation of 1,2-*trans* glycosidic bonds and, in general, acyl protective groups are commonly employed also for more stable saccharides in glycopeptides.

Most frequently employed protective groups are esters, acetates and benzoates because they are

easily removed under mild and homogeneous conditions.[17] Thus it has been shown that base removal of Ac does not epimerize[18] the amino acids.

Building blocks have also been prepared carrying useful ether type protective groups such as **silyl**. Silyl protective groups are removed during acid catalysed cleavage of the glycopeptide[19] from the solid phase and thus a separate-mediated deacetylation step is avoided.

2.2.2. *Carbohydrate* N-*protective groups*

In the 20 years since Lemieux *et al.* developed general methods for the installation and use of phthalimido group on glycosyl donors, access has been provided to a large number and variety of amino glycans. Unfortunately the literature shows that the methodology is challenged by the difficulties facing the deprotection even with procedures that can compromise base sensitive protecting groups elsewhere in the molecules as well as epimerizable centers and delicate functionalities, making isolation of valuable compounds difficult.

In addition to phthaloyl, several other *N*-protecting groups have been also employed in amino sugar synthesis. However, current synthetic efforts in the construction of oligosaccharides, glycoproteins, glycolipids and other glycoconjugates containing a variety of 2-amino-2-deoxy sugars have led to the development of new methods for NITROGEN protection. These new nitrogen-protecting groups have been removed from substrates using mild and often chemoselective conditions. The preparation, present uses, and deprotection of amino sugar derivatives employing these new agents are described in a specialised review.[20]

Of particular importance is the azido moiety which may serve either as a latent function or as a protecting group because it permits both stereoselective α- and β-glycosidations.

Compatibility with hydroxyl protection in the carbohydrate moiety is a necessary requirement but the most important feature of these protection schemes is the orthogonality concept among different *N*-protecting groups in carbohydrate moiety. For instance, a selective reduction of a *N*-Dts in the presence of azido groups in solution and on the solid-phase is described in a recent report.[21] Such a methodology allows the synthesis of multiple glycosylated glycopeptides using different building blocks containing *O*-β-Glc*N*-protection and *O*-α-GalNAc precursors.

2.2.3. *Other carbohydrate protective groups*

The versatility of use of glycosyl amino acids as building blocks envisages new protection schemes in carbohydrate to be added, for instance, *either* those containing sialyl-T_N derivatives in which the carboxylic groups have to be protected during the glycosylation step *or* new building blocks containing other modifications, such as phosphate or sulfate in the carbohydrate moiety. A great advantage will be those deprotection methods that allow the modification of the sugar moiety of the glycosyl amino-acid once it has been incorporated in the solid-phase.

3. *O*-Glycosyl amino acids containing 2-acetamido-2-deoxy-α-D-galactopyranosyl linkage

3.1. *Mucins. T_N antigen synthesis*

The *N*-acetylgalactosamine-Ser/Thr bond was first demonstrated in mucins[22] and is mainly found among serum and cell membrane glycoproteins and high molecular weight mucins which line the gastrointestinal tract and bronchial airways.

These widely so-called mucin-type glycoproteins show a high degree of structural heterogeneity. Therefore a number of different core structures have been identified.

The main characterization of the diversity of possible *O*-linked glycosylation patterns has been carried out on secreted mucin glycoproteins. From the sequence data eight different core regions containing GlcNAc, Gal or GalNAc substitutions of GalNAc can be defined (Table below).

Core 1: Gal(β1→3)GalNAcα-Ser/Thr-R
Core 2: GlcNAc(β1→6)[Gal(β1→3)]GalNAcα-Ser/Thr-R
Core 3: GlcNAc(β1→3)GalNAcα-Ser/Thr-R
Core 4: GlcNAc(β1→6)[GlcNAc(β1→3)]GalNAcα-Ser/Thr-R
Core 5: GalNAc(α1→3)GalNAcα-Ser/Thr-R
Core 6: GlcNAc(β1→6)GalNAcα-Ser/Thr-R
Core 7: GalNAc(α1→6)GalNAcα-Ser/Thr-R
Core 8: Gal(α1→3)GalNAcα-Ser/Thr-R

Core 1, 2 and 3 are the most common core structures in mucins as well as in other secreted and cell surface glycoproteins. Core 1 structure, the so-called T-antigen, is widespread in nature and occurs for instance in blood groups, in the antifreeze glycoproteins of polar fish and in human oncofetal fribronectin. Core 1 is not usually exposed in glycoproteins but often these highly hetereogeneous structures are further galactosylated at the 4-position and/or monosialylated at the 3-[SA (α2→3)Gal(β1→3)GalNAc-] and 6-[SA (α2→6)Gal(β1→3)GalNAcα-] positions or disialylated (SA (α2→6)[SA (α2→3)Gal(β1→3)]GalNAcα-). Much effort has been devoted to the preparation of fragments of the mucin glycoproteins. In particular the T- and so called T_N antigens missing the galactose in the core 1 structure have gained much attention.

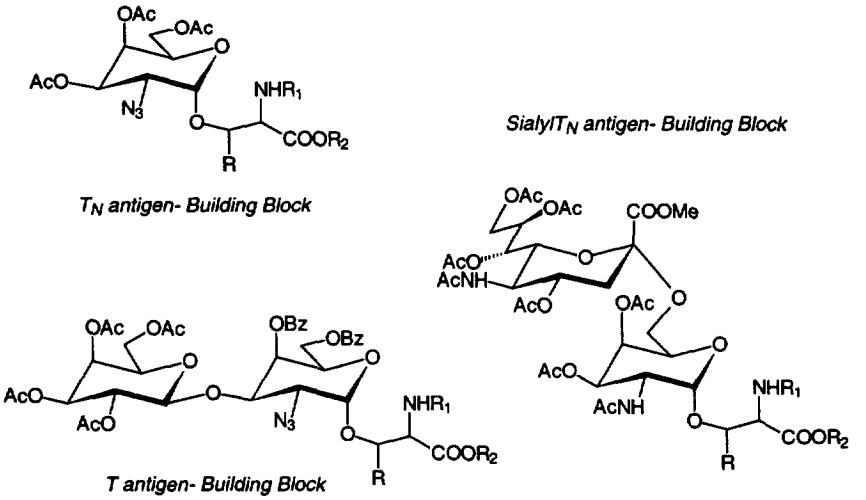
O-Glycans with core 3 and 4 have only been found on mucins. Core 4 was first characterized in sheep gastric mucins[23] and has since been identified in several other animal and human mucins. Core 5 occurs in glycoproteins from several species, and was found in sialylated form of human adenocarcinoma[24] and in meconium.[25] To date, oligosaccharides with core 6 have only been reported on human glycoproteins, including human meconium[26] and ovarian cyst mucins and in bovine κ-casein. It can not be excluded that core 6 structures arise due to degradation by human β-galactosidase acting on Gal(β1→3) and thus converting core 2 to 6. Core 7 has recently been described in bovine submaxillary mucin[27] and core 8 in human respiratory mucin.[28] With the exception of core 7 which to date has only been found as such, all core structures may occur unsubstituted, elongated, or carrying terminal antigens.

Owing that mucins are expressed by cancer cells of the main practical interest behind the study of core molecules is the identification of tumor associated[29] carbohydrate structures[30] such as T_N (GalNAcα-*O*-Ser/Thr), TF or T (Galβ1-3GalNAcα-*O*-Ser/Thr) and ST_N (NeuAcα2-6GalNAcα-*O*-Ser/Thr) in view of their potential for cancer therapy. Thus, a first example is the segments of membrane anchor tumor mucins such as MUC-1, which in the form of glycopeptides, have already been under investigation as possible **vaccines for the immunotherapy** of a variety of cancers of epithelial origin (breast, ovaries, lung, pancreas, colon, etc.)(Scheme 4).

A second example is the disaccharide **βGal(1→3)GalNAc**, *O*-linked to serine or threonine found in mucins and glycoproteins, which is classified as biosynthetic core 1 glycan and popularly known as carcinoma associated **Thomsen–Friedenreich** (TF or T) antigen. Cancer associated mucins from gastric[31] and rectal[32] epithelium were found to express this disaccharide in abnormally increased quantities. This disaccharide is also found on the cell surface of chronic and acute myelogenous leukemic leukocytes.[33] Sialylated versions of TF, particularly α(2→6) sialyl-TF, have been found on the mucins expressed by human breast cancer cell lines.[34] This evidence has led to the proposal that synthetic glycopeptides derived from cancer associated mucins, bearing this structure, may be used as immunogens to stimulate immune responses against cancer cells expressing this disaccharide.[35]

The bottle-neck in fulfilling expectations is the synthesis of glycopeptides. Chemical synthesis of α-*O*-linked *N*-acetylgalactosamine based mucin type glycopeptides depends in turn on the accessibility to large amounts of *O*-glycosylated amino acids bearing the proper carbohydrate sequence. However, problems are associated with the synthesis of these α-*O*-linked GalNAc amino acids.

(1) Assembly of the correct core oligosaccharide
(2) Stereoselective formation of the α-D-GalNAc to Ser/Thr linkage. Use of non-participating precursors of NHAc such as N_3 or steric control (Biomira's approach) using 2-NHAc.
(3) Conversion of the precursor into the acetamido group



Scheme 4. Structures T_N, TF (core 1) and ST_N.

(4) Conversion of the glycosyl amino acid into a suitable building block for peptide synthesis
(5) Incorporation of α-D-NeuNAc residues by either chemical or enzymatic means.

These and other problems are now discussed and exemplified with selected syntheses of core structures already reported in the literature.

3.1.1. *Synthesis of the 2-azido-2-deoxy precursors*

Formation of the α-glycosidic bond between 2-acetamido-2-deoxy-D-galactose and the hydroxyl group of serine or threonine has proved to be a rather difficult task. In theory, the synthesis of such a 1,2-*cis*- glycoside can be established with a reasonable stereoselectivity using a sugar donor with a *non* participating neighbouring substituent at C-2. However, use of *N*-acetyl galactosamine for the synthesis of α-*N*-acetylgalactosaminides has not been possible due to the collapse of the donor during glycosylation reactions to bicyclic oxazoline derivatives.

Paulsen[36] and Lemieux[37] have devised an elegant method to overcome the interference of 2-acetamido group with the anomeric carbon which is based on a 2-azido precursor. The 2-azido function[38] does not participate but can be easily converted into an *N*-acetyl group.

Up to now, it is well established that the azido[39] function plays a pivotal role in the synthesis of 1,2-*cis* linked amino sugars. Thus, the azido derivatives have been prepared by azidonitration of glycals, addition of halogenoazides to glycals, and diazo transfer of 2-amino-2-deoxy-aldoses. However, the most widely used reaction for the preparation of the 2-azido-2-deoxy analogues is the azidonitration,[40] either D-glucal or D-galactal developed by Lemieux *et al.* in 1979. This reaction occurs by the addition of ceric ammonium nitrate and sodium azide to protected glycals regiospecifically affording epimeric mixtures of 2-azido-2-deoxy-1-*O*-nitropyranoses. This reaction has been further optimized recently.[41]

In the past, other non-participating masked amino functions *e.g.* imino compounds have also been proposed. Some examples are the oximino intermediates prepared by addition of nitrosyl chloride to glycals, glycosylations with 2-[(2,4-dinitrophenyl)-amino] donors which was supposed to be a non-participating group, and glycosylations using 2-[-(4-methoxybenzylidene)amino] donors.

3.1.2. *Synthesis of the 2-azido-2-deoxy glycosyl donors*

The precursors discussed above have been transformed in a wide variety of glycosyl donors such as: glycosyl halides, glycosyl imidates, thioglycosides,[42] pent-4-enyl glycosides,[43] vinyl glycosides, and recently, selenoglycosides.[44]

One particular example by Garegg *et al.*[45] derives from recent work by Vasella *et al.*[46] on a diazo transfer reaction. This contribution led to pure anomeric compounds such as 2-azido-2-deoxy thioglycosides and 4-pentenyl 2-azido-2-deoxy-β-D-glucopyranoside with suitable protection in the 3,4,6-positions.

Another example are the halogenides of 2-azido-2-deoxy-D-galactose. These halogenides[47] are readily prepared by replacement of the nitrate group in position 1 using an appropriate soluble halide salt. Bromination of the crude substrate with lithium bromide gives the α-bromide, which after treatment with tetraethyl ammonium chloride[48] provides the β-chloride.

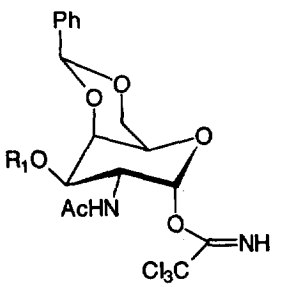
3.1.3. *Reduction of the azide in the α-glycoside*

Considering the need for compatibility with other protecting groups and for efficiency required in solid-phase synthesis new methods for the transformation of the amine precursor N_3 into an acetamido group have been developed. This transformation can take place *before or after* incorporating the building block onto the solid support.

Among others, the following can be cited as examples of the two steps transformation: i) reduction of the azido group by hydrogenolysis over Pd/C and subsequent acetylation (pyridine and acetic anhydride); ii) sodium borohydride[49] reduction in the presence of nickel chloride and acetylation; iii) reduction with H_2S in pyridine, that is compatible with the presence of the Fmoc group. Moreover, procedures that yield the acetamido group in a one-pot single reaction are either a) the Staudinger method[50] (with acetic acid phosphines such as with tri-*n*-octyl phosphine), which readily convert the azido group into the phosphimine which reacts with the dry acetic acid affording the target acetamido group[51] or b) the thioacetic acid[52] procedure. With this latter reagent the transformation of the azido groups into acetamido functions can take place after the completion of the peptide chain on the solid support. However, such a method is cumbersome and inconvenient due to the long reaction times required and the inherent formation of *N*-thioacetates as impurities. To circumvent these difficulties, thiolytic reduction of the azido sugars with dithiols has been also assayed either i) prior to incorporation to MCPS by Zn reduction in THF–Ac_2O–AcOH or ii) after incorporation on the solid phase by employing dithiothreitol (DTT) or *N*-methyl-α-mercaptoacetamide (MCA) as reducing agents in the presence of ethyldiisopropylamine (DIPEA).

3.1.4. *Steric control using 4,6-O-benzyliden-N-acetylgalactosamine derivatives*

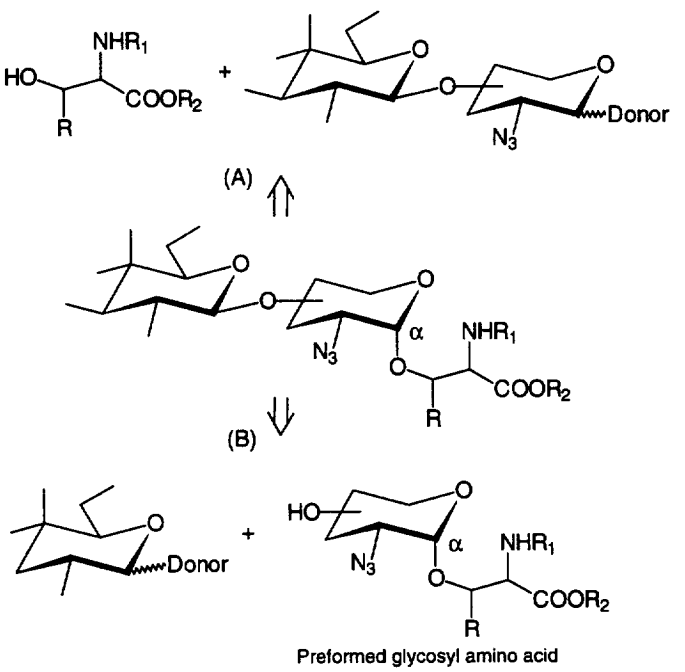
Leaving behind the idea of a non-participating group, a novel strategy based on a steric control concept has been devised by an industrial research laboratory. This method can be adapted for the **commercial production of glycopeptides** that required α-GalNAc based *O*-linked carbohydrate structures whose synthesis hitherto has not been commercially viable. Biomira's chemists have found that 4,6-*O*-benzyliden-*N*-acetylgalactosamine can be converted into a stable donor which reacts with serine or threonine, forming α-glycosides in moderate yields. These building blocks[53] may also serve as intermediates for further extension for the synthesis of other mucin derived glycopeptides (Scheme 5).



Scheme 5. Biomira building block.

3.2. *Approaches to the construction of T antigen (core 1) structures*

Having considered the different solutions given in the literature for the synthesis of all core common partial structures of the 2-azido-2-deoxy glycosyl amino acid, the synthesis of selected core structure is now discussed. The most biologically relevant structure is the **β-D-Gal-(1→3)-α-D-GalNAc** unit. Successful approaches to synthesize a building block with this core structure have been realized using two types of strategies. The first (A) deals with glycosylation of the amino acid by 2-azido disaccharide glycosyl donor while the second (B), uses a preformed 2-azido glycosyl amino acid (or T_N antigen building block) which has been widely adopted for the chemical synthesis of other core structures (Scheme 6).



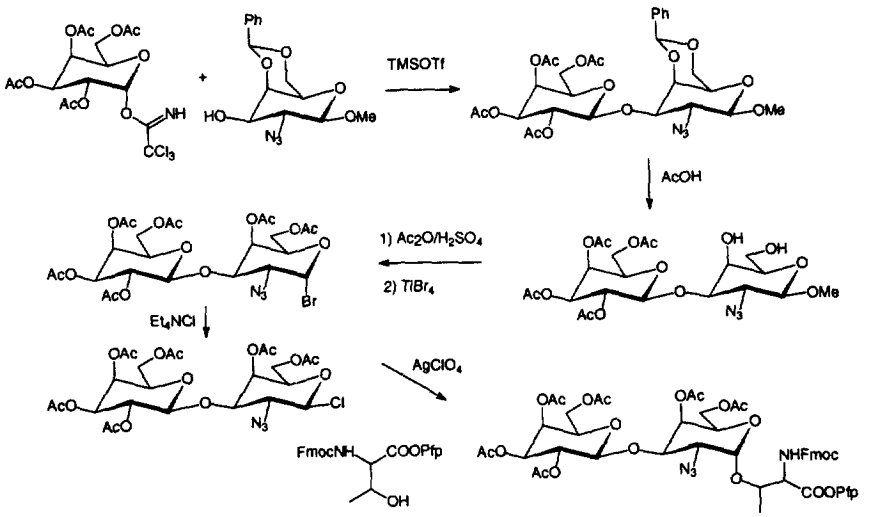
Scheme 6. Conceptual scheme to Core 1 (T antigen).

Among the reported procedures of type (A) common features for the synthesis of the *2-azido-2-deoxy disaccharide* are either formation of the azido group in a preformed disaccharide glycal or glycosylation of the 2-azido monosaccharide to obtain the disaccharide, conversion to the appropriate glycosyl donor and glycosylation of various acceptors. In a recent example given by Magnusson *et al.*,[54] the bromide was prepared from protected 2-(trimethylsilyl)ethyl glycoside carrying a 2-azido functionality. This key intermediate was transformed in high yield into the 2-azido glycosyl bromide by first treating with TFA to remove the TMSEt ether and then reaction with oxalyl bromide in dichloromethane.

Type (B) schemes for T_N and T antigen (core 1) preparation normally proceed throughout glycosylation reactions of amino acids as glycosyl acceptors using either monosaccharide or disaccharide 2-azido-2-deoxy-sugar donors to obtain T_N and T antigen (core 1), respectively. The amino acid preferentially contains an orthogonal protecting group pattern which allows the selective deprotection of either the *N*- or *C*-terminal positions.

3.2.1. *Using glycosyl halides as donors*

Examples of the synthesis of the 2-azido glycosyl amino acids from halides can be accomplished by coupling the 2-azido-α-bromide or the 2-azido-β-chloride or the corresponding 2-azido disaccharide halide to the protected amino acid using several catalysts, *e.g.* silver perchlorate/silver carbonate, TMS triflate and silver triflate. Different *N*- or *C*-protecting groups combinations have been assayed on the amino acid moiety. Among others, the following pairs: Fmoc/Fen[55] (Mono-, Di-); Fmoc/tBu[56] Mono-; Fmoc/Bn (Mono- and Di-),[57] (Di-);[58] Fmoc/All[59] (Mono-, Di-); Z/Bn[60] Di-; Z/tBu[61] Di-; Fmoc/tBu[62] Di-, have helped to conclude that the best option is the Fmoc/Pfp choice (Mono[63]-, Di-[64]) (Mono- and Di-)[65] which yields already activated glyco-amino acid building blocks for stepwise solid-phase peptide synthesis. A typical Fmoc/Pfp scheme is provided in Scheme 7.



Scheme 7.

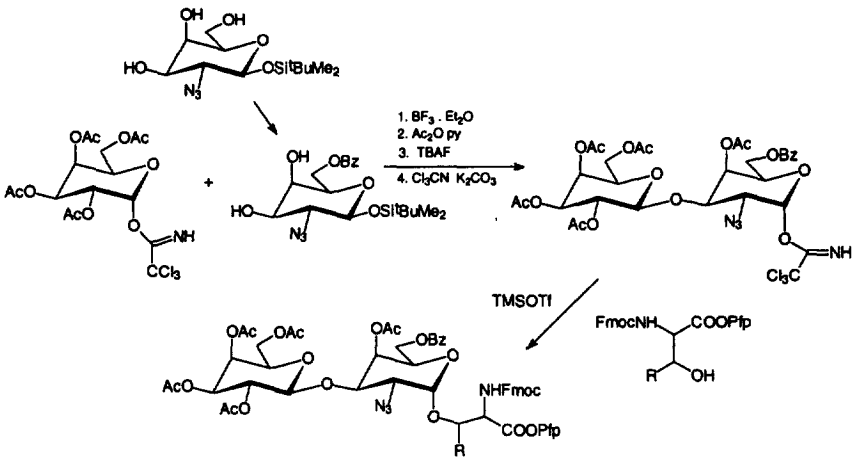
3.2.2. *Using glycosyl imidates as donors*

A number of parallel procedures rely on imidates as glycosyl donors. In this case, the stereochemistry of the glycosylation reactions using **imidates** as leaving groups of 2-azido-2-deoxy-donors was shown to be strongly dependent not only on the promoters, but also on the nature of the acceptor and the orientation of the leaving groups. Also in this case, a series of *N*- or *C*-protecting groups pairs such as: Mono-(Z/Bn,[66] Fmoc/All,[67] Fmoc/Ph[68]); Di-(Z/Bn,[69] Fmoc/Pfp,[70] Fmoc/Fen[71]) have been assayed reaching identical conclusions about suitability of the Fmoc/Pfp pair. The following scheme illustrates a typical example (Scheme 8).

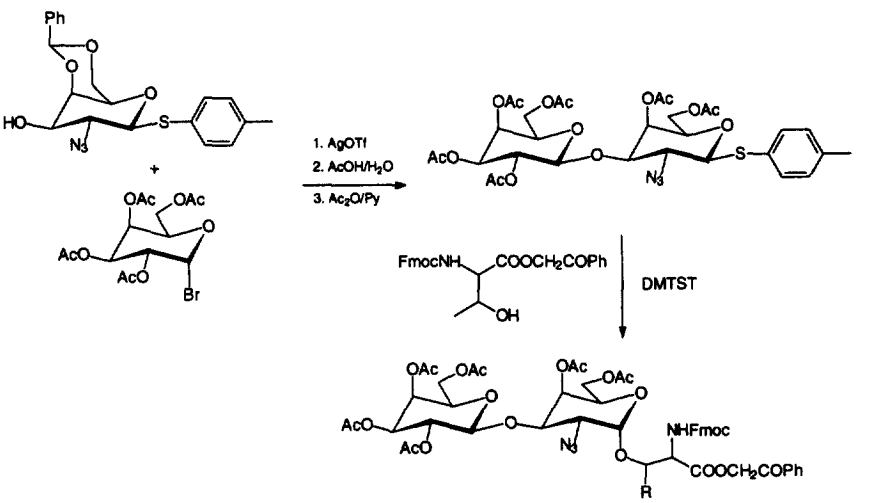
3.2.3. *Using glycosyl thioglycosides as donors*

Among other glycosyl donors the **thioglycosides** most often have been used in conjunction with dimethyl(thiomethyl)sulfonium triflate (DMTST) as catalyst. Other promoters are: methyl triflate, dimethyl(thiomethyl)sulfonium tetrafluoroborate (DMTSB), alkenyl sulfenyl triflates, phenylselenyl triflate, nitrosyl tetrafluoroborate or halonium-forming species along different amino acid protecting group pairs such as: Fmoc/Bn[72] (Mono-); Fmoc/tBu[73] (Mono- and Di-); Z/Bn[74] (Di-), have also been tested. To illustrate these procedures the synthetic route of Scheme 9 is given.

It is remarkable that a free 1-OH reducing 2-azido-2-deoxy-galactose has also been used as the donor with Ser or Thr acceptors in the presence of triflic anhydride in acetonitrile.[75]



Scheme 8.



Scheme 9.

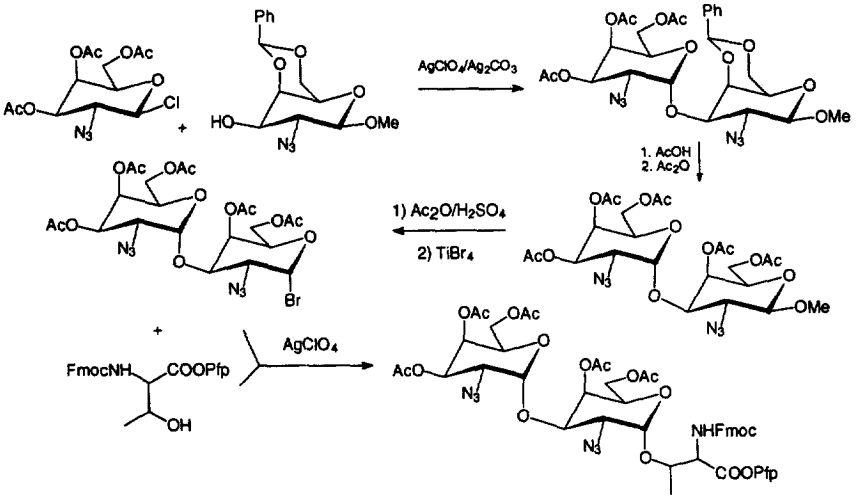
3.3. *Other cores*

Preparation methods for the other core structures have much in common with procedures for the synthesis of core 1.

The natural occurrence of closely related saccharide structures such as the two disaccharides, α-D-GalNAc-(1→3)-α-D-GalNAc and α-D-GalNAc-(1→6)-α-D-GalNAc suggests the existence of two different *N*-acetylgalactosaminyl transferases which facilitates the formation of the α(1→3) and α(1→6) linkages. However until now none of these enzymes responsible for the biosynthesis of the core 5 and core 7 structures have been characterised. Therefore, strategies for the preparation of these core structures can not yet benefit from an enzymatic tool.

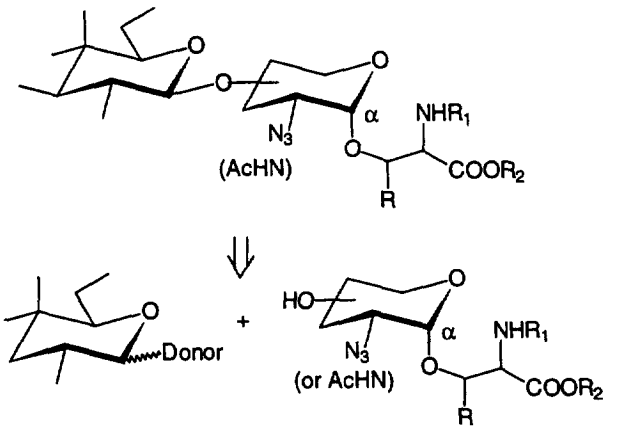
Thus, a suitably activated disaccharide glycosyl donor is coupled to a preformed hydroxyamino acid derivative. In the case of core 5, the only difference with core 1 is that the disaccharide has to be linked through an α(1→3) linkage instead of a β(1→3) linkage and a $GalN_3$ is used in place of a Gal moiety.[76] In the example of Scheme 10 the coupling reaction yielding the α(1→3) disaccharide is performed by $Ag_2CO_3/AgClO_4$ catalysis and the resulting disaccharide is later converted into the

bromide donor by titanium tetrabromide. Finally, the FmocThrOPfp ester is subsequently glycosylated with the obtained α-bromide disaccharide donor in the presence of $Ag_2CO_3/AgClO_4$ to give the desired building block precursor of core 5 in a 73% yield.



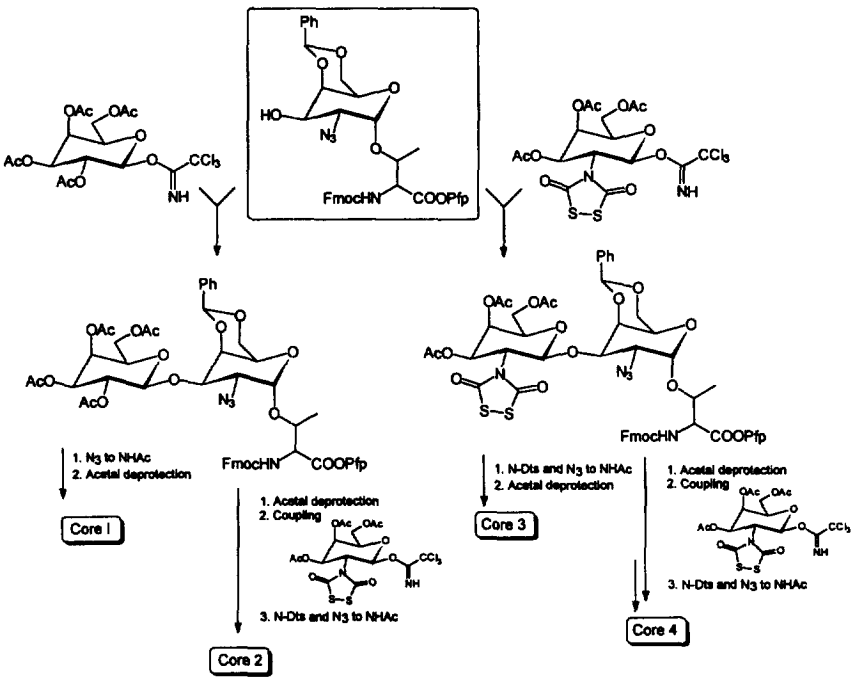
Scheme 10.

However, the most common approach used nowadays to prepare other more structurally complex cores is based on the *glycosylation of a preformed glycosylated amino acid*, such a glycosyl acceptor moiety may be either a α-D-$GalN_3$-Ser/Thr or α-D-GalNAc-Ser/Thr structure depending on wether the desired α-anomer requires preparation throughout the azido or the 2-acetamido precursor, respectively (see Scheme 11). Following these routes, syntheses for the vast majority of synthetic building blocks leading to most cores have appeared in the literature very recently.



Scheme 11.

An illustration of how this strategy may lead to the synthesis of many precursor core structures is summarised in Scheme 12. Thus, stereoselective glycosylation of different FmocThr(α-D-$GalN_3$)OPfp derivatives with a peracetylated galactose imidate or 2-dithiasuccinimido glycosyl donor (see scheme), followed by *in situ* reduction of the *N*-Dts and azido functionalities affords the protected building blocks which can be directly used in solid-phase synthesis of core 1, core 2, core 3 and core 4 mucin *O*-glycopeptides without further modifications of the carbohydrate moiety on the solid phase.



Scheme 12.

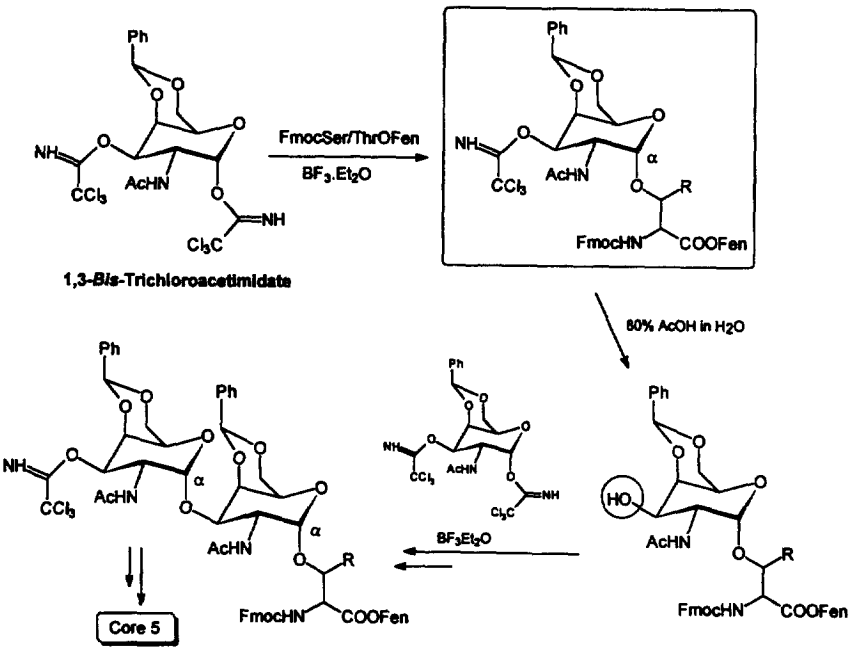
Thus, core 1 and the precursor to the core 2 building block were obtained[77] by stereoselective glycosylation of the 4,6-*O*-benzylidene-*N*-FmocThr(α-D-GalN_3)OPfp derivative with two equivalents of the peracetylated galactose imidate. Core 1 was obtained by reduction of the azido group of the intermediate with simultaneous acetylation using zinc (activated with 2% aq. $CuSO_4$) in THF, acetic anhydride and acetic acid (3:2:1) in 76% yield. The conversion of this intermediate to the core 2 building block began with acid-catalysed hydrolysis of the benzylidene group with warm aqueous acetic acid in 75% yield. The diol obtained was used for further glycosylation with the 2-*N*-Dts 1-imidate using TMSOTf as a catalyst to yield the trisaccharide precursor of core 2 in 67% yield. This trisaccharide-threonine derivative could be successfully converted into the core 2 building block by *simultaneous reduction* of the *N*-Dts and N_3 group in a mixture of THF/Ac_2O/AcOH (3:2:1).

In addition, by similar pathways, i.e. by stereoselective glycosylation of the 4,6-*O*-benzylidene-*N*-FmocThr(α-D-GalN_3)OPfp derivative with mole equivalents of the *N*-Dts peracetylated galactose imidate, the building blocks for core 3 and the precursor to the trisaccharide-threonine core 4 were obtained in moderate yields.

Other core structures such as core 5 have been prepared following a steric control concept. This approach is based on 4,6-benzylidenyl-*N*-acetylgalactosamine donors to prepare these α-GalNAc glycosides of Ser and Thr. An interesting feature of this method is the simultaneous use of trichloroacetimidate groups as both leaving groups for glycosylation and acid sensitive protecting groups.

As shown in Scheme 13 such a versatile donor forms exclusively the α-glycoside with Ser/Thr, and serves as a facile precursor of a free OH at position 3 which can be generated in acid medium without affecting either the 4,6-acetal protecting group or the protecting groups of the Ser/Thr functional groups. In addition, the use of trichloroacetimidate as a protecting group may avoid additional protection/deprotection steps in repetitive glycosylations and may be useful in generating a succession of 1,3-linkages to produce linear oligosaccharides and a variety of *O*-linked glycoamino acid building

blocks. This is possible since the trichloroacetimidate group at the 3-position is unusually stable under glycosylation conditions.



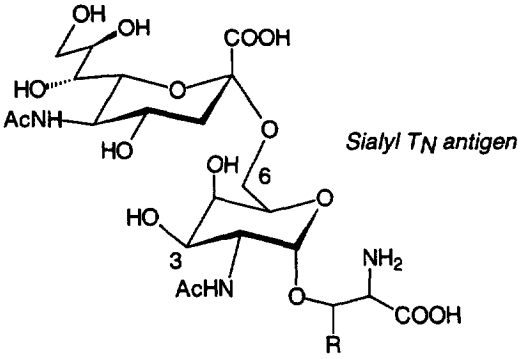
Scheme 13.

Accordingly, the synthesis of cancer associated carbohydrate core 5 and its sialylated analog are accomplished through the use of this donor.[78] Protected cores 2 and 6 and a gastric cancer associated structure[79] related to core 6, namely, F1-α, were synthesized[80] using the 4,6-diol of T_N-Ser/Thr and TF-Ser/Thr.

3.4. *Complex mucin glycopeptides with sialyl-T_N antigenic structures*

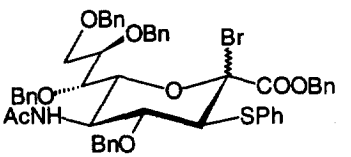
Owing to the potential properties of incomplete glycan side-chains of mucins, such as T_N (GalNAcα-*O*-Ser/Thr) and the T antigen (Galβ1-3GalNAcα-*O*-Ser/Thr) structures as potential cancer immunity raising molecules, several laboratories have reported the synthesis of these tumour-associated cell surface structures. In contrast, there have been less investigations into the synthesis of *O*-glycoprotein glycans containing sialic acid residues[81] and only a few reports describing the synthesis of **sialyl T_N antigen** (NeuAcα2-6GalNAcα-*O*-Ser/Thr)[82] have appeared. The T_N structure and its sialylation product, the sialyl-T_N antigen are even more tumor-selective that the T-antigen (Scheme 14).[83]

Sialylations are generally difficult to perform and only a limited number of donors give high yields and high stereoselectivity. Early sialylations were predominantly carried out using sialyl chlorides and to a lesser extent bromides, but yields and stereoselectivity were in most cases low or at best moderate. A good example is the work reported by Ogawa *et al.*[84] on the syntheses of sialic acid-containing di-, tri- and tetrasaccharides linked to L-serine. The sialic acid residue was introduced with moderate efficiency to *O*-6 of Gal*p*NAc and *O*-3 of Gal*p* residues using sialyl glycosyl chloride and AgOTf as a promoter. Ogawa has also synthesized sialyl-Tn conjugates by coupling of the sialyl containing disaccharide with *N*-benzyloxycarbonyl(Z)-serine benzyl ester using the trichloroacetimidate method and incorporating the amino acid in the later stages. After hydrogenolysis of the benzyl groups, the NeuAc methyl ester of the no longer CH-acidic conjugate could be subjected to alkaline hydrolysis. In the glycosylations of the tri-(*O*-6-sialyl T) and tetra-(disialyl T) saccharides, the reaction was not stereoselective with the undesired β-glycoside being preferentially produced.



Scheme 14.

Novel strategies for the incorporation of sialic acid into clusters of T_N antigens are also due to Ogawa *et al.*[85] The distinctive features of these methods are that an α-glycosidic linkage was established at *an early stage of the synthesis*, by the use of glycosyl fluorides and promoters[86] such as zircocene–perchlorate ($Cp_2ZrCl_2/AgClO_4$) or hafnocene promoters ($Cp_2HfCl_2/AgClO_4$) and the development of a stereoselective synthesis of **sialyl α-glycosides** for the glycosylation with 5-*N*-acetylneuraminic acid derivatives. In particular, zirconocene-promoted glycosylation ($Cp_2ZrCl_2/AgClO_4$) of Fmoc/phenacyl protected Ser/Thr derivatives with a 2-azido-2-deoxy-α-D-galactopyranosyl fluoride donor yield the T_N building block precursor. The α-anomer formed as the main compound is afterwards glycosylated with a 5-*N*-acetylneuraminic acid derivative (NeuAc donor), containing an α-directing thiophenyl group at the 3-position of the per-*O*-benzylated NeuNAc bromide (Scheme 15), which is temporarily required to achieve stereoselective glycosylation. Helferich glycosylation ($Hg(CN)_2$ and $HgBr_2$ in CCl_4) affords a single coupling precursor of the sialyl T_N building block product having a new (2→6) linkage.



Scheme 15.

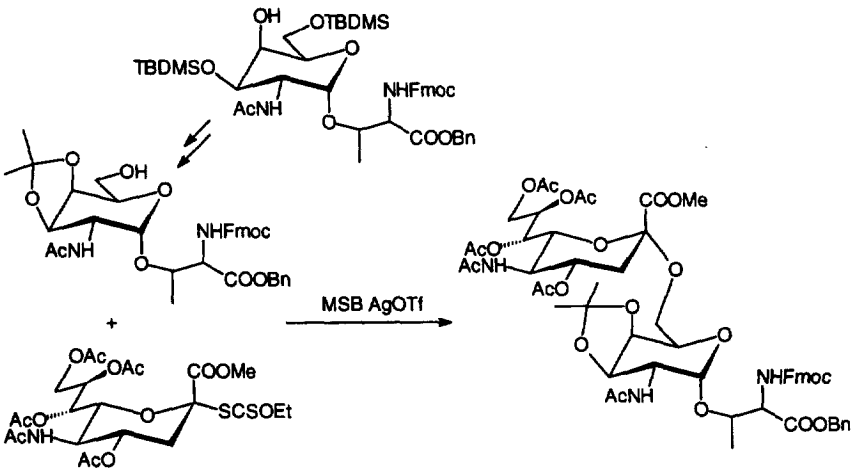
After condensation the thiophenyl group is removed by an improved desulfurization process involving an intermittent addition of AIBN to the refluxing mixture of the thioether and a large excess of triphenyltin hydride in benzene. In summary, the introduction of a participating group, *such as the phenylsulfenyl group*, at C-3 of the sialyl donor increases the yields and selectivity, but several additional synthetic steps are required for the preparation of the donor and the subsequent removal of the participating group.

The important role of sialic acid conjugates in various aspects has spurred the development of new efficient *sialyl donors* such as phosphites, thioglycosides and xanthates which avoid the need of a participating group. Especially the xanthate has been found to give high yields and good stereoselectivity when employed for sialylations promoted by methyl sulfenyl bromide/silver triflate (MSB/AgOTf) at low temperature with mixtures of acetonitrile and dichloromethane.

In spite of these remarkable achievements most already reported sialyl T_N building blocks are not suitable for direct use in solid-phase glycopeptide synthesis.

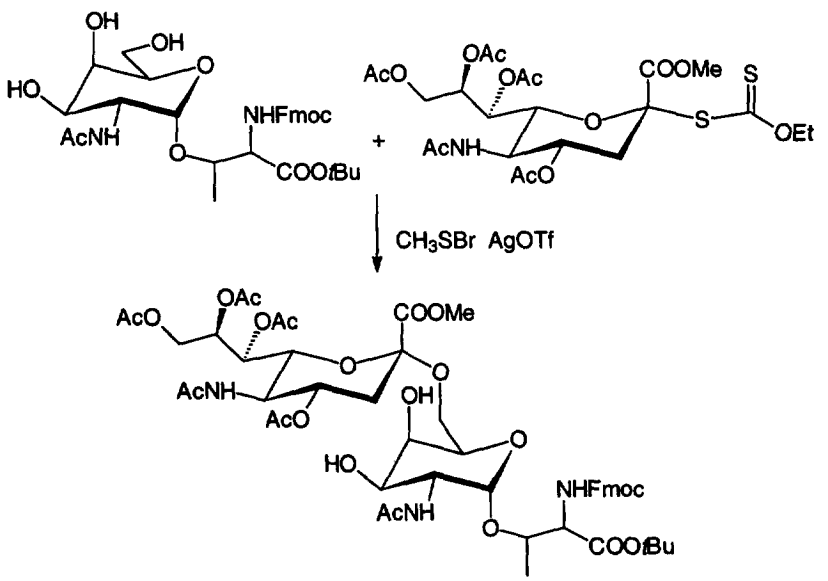
This gap has been filled by Kunz *et al.*[87] This alternative strategy is based on the regioselective coupling of the simple acetyl-protected methyl ester of neuraminic acid to the Z-[*O*-(2-

acetamidogalactosyl)-threonine ester, which is unprotected at the glycan position, to furnish the sialyl-T_N structure. However, a prerequisite for synthesis of sialyl-T_N glycopeptides by this principle is that the NeuNAc methyl ester has to be removed from the glycopeptide without epimerization of amino acid or β-elimination of the glycan side chain. Furthermore, solid-phase synthesis require a Fmoc-protected sialyl-T_N Thr or Ser building block. The selective removal of the *O*-acetyl groups from the base sensitive Fmoc bearing *O*-glycosyl threonine ester is much more difficult than for the Z-protected analogue. To solve this problem, Kihlberg *et al.*[88] recently pursued a multistep methodology via *O*-silyl-protected galactosamine derivatives (Scheme 16).



Scheme 16.

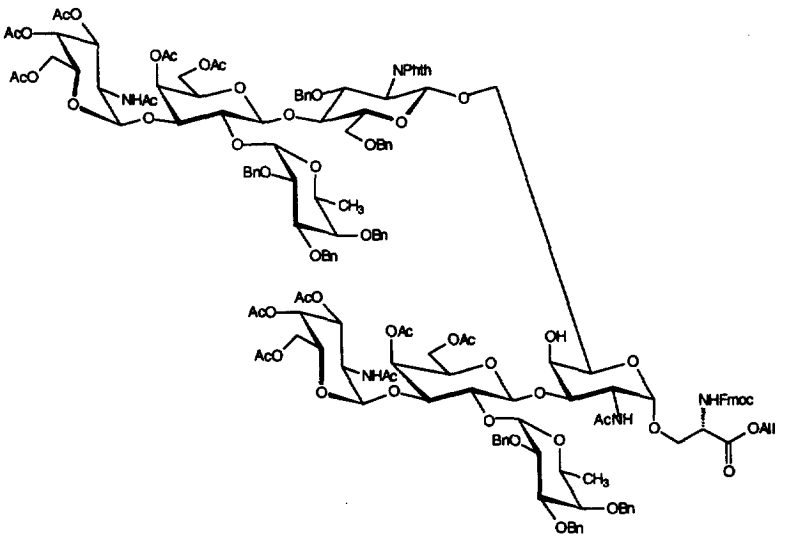
The latest improvements come, however, from Kunz group[89] by preparing the shortest strategy based on a regioselective sialylation of the Fmoc-protected T_N building block (Scheme 17).



Scheme 17.

After acetylation of the 3,4-positions and acidolysis of the *tert*-butyl ester, the Fmoc-sialyl-T_N threonine component is isolated in a very convenient form that can be readily used in solid-phase synthesis of biologically interesting sialyl-T_N antigen glycopeptides. As expected, the hydrolysis of the NeuAc methyl ester was difficult as it demanded stronger basic conditions than for the hydrolysis of *O*-acetyl groups, but β-elimination of the carbohydrate was hardly detected.

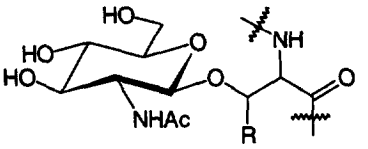
At least for Ogawa's group, the complexity of the glycan part has not, so far, been a limiting factor for the synthesis of related building block structures. Thus, even more complex tetrasaccharide building blocks synthesis has been also reported[90] employing Gal*p*N_3, Gal*p*, Ser/Thr and Neu*p*Ac synthons prepared in stereocontrolled glycosylations. Further examples arise from Ogawa's interest in the development of general synthetic approaches toward *O*-linked glycoproteins with blood group activity. In accordance, he has reported the synthesis of complex glycosyl amino acid such as a glycotetraosyl serine,[91] a partial structure of an ovarian cyst mucin glyoprotein of blood group A activity. This glycosyl amino acid was later used by the authors to build for first time a fully protected glycooctaosyl serine[92] identified as a blood group A mucin-type determinant isolated from blood group A human ovarian mucin. The very value of this work is that it represents an efficient and stereocontrolled synthetic route towards a protected octaosyl serine glycopeptide (Scheme 18), whereas isolation from the natural source only generates the equivalent reduced octasaccharide alditol structure.



Scheme 18.

4. *O*-Glycosyl amino acids containing 2-acetamido-2-deoxy-β-D-galactopyranosyl linkage

It is well known that 2-acetamido-2-deoxy-β-D-glycosides widely exist as part of important biomolecules such as peptidoglycans, glycoproteins, mucopolysaccharides, and blood group determinants. However, recently, a major new form[93] of protein glycosylation termed *O*-GlcNAc has been described. *O*-GlcNAc is a simple, unmodified monosaccharide moiety β-glycosidically linked to the side-chain hydroxyls of serine or threonine that is found in all eukaryotes (Scheme 19). Up to now an increasing number of *O*-GlcNAc modified proteins have been described. These include nuclear, cytoplasmic structural proteins, viral proteins, and transcription related proteins. Although the function of *O*-GlcNAc is still not known, its process of addition to protein appears to be highly dynamic and it has been postulated that this modification plays a regulatory role in many ways analogous to protein phosphorylation.[94]



R: H Ser(O-β-D-GlcNAc)
R: CH_3 Thr(O-β-D-GlcNAc)

Scheme 19.

The natural occurrence of these natural *N*-acetyl glucosamine glycosides and the need for understanding of the role of *O*-GlcNAc modification, has stimulated the development of essential and versatile tools based on glycosylation reactions using D-glucosamine derivatives aimed at the synthesis of *O*-GlcNAc glycopeptides which may help to delineate particular biological functions.

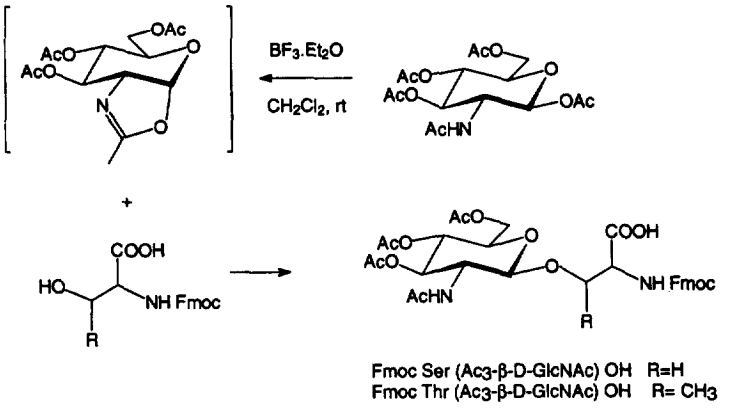
Glycosylation with 2-amino-2-deoxy-sugar derivatives[95] has received particular attention since the 1,2-*trans* glycosylations require glycosyl donors containing participating protective groups in the 2 position. Therefore, it is obvious that the choice of 2-amino protecting group will have a strong influence in the synthesis of the desired glycoamino acid building blocks. A further important factor in choosing a set of orthogonal protecting groups is the lability of the Ser/Thr *O*-glycosidic bond to strong acids and bases conditions.

Accordingly, new protecting groups have been developed to comply with these requirements for attempting the synthesis of 2-acetamido glucopyranosyl amino acids which will be discussed thoroughly in this section.

4.1. *Glycosyl donors with a 2-N-acetyl function*

Few routes have been found for the synthesis of 2-acetamido-2-deoxy-β-D-glycosides of Ser or Thr having a β conformation. The traditional Köenigs–Knorr[96] type reaction is one of the most suited processes for the synthesis of glucosamine glycosides. In these cases, 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl halides (Halide: Cl, F) are always used as glycosyl donors[97] and both amino and carboxyl protected Ser and Thr as glycosyl acceptors.

Alternatively, acid catalyzed reaction of oxazoline derivatives[98] is another major process for the synthesis of these glucosamine glycosides. The oxazoline procedure, an extension of Köenigs–Knorr glycosylation method, and its various improvements, has allowed the successful synthesis of both simple 1,2-*trans*-2-acetamido-2-deoxy glycosides and oligosaccharides. The most commonly used sugar oxazolines[99] are the methyl oxazolines. After glycosylation, activated by an acid[100] or Lewis acid[101] these reactive intermediates allow nucleophilic attack by a glycosyl acceptor, affording anomerically pure β-glycosides already possessing the **natural** *N*-acetyl function which is an extremely useful feature. The fact that the *N*-acetyl group serves as both a protecting and activating group is our contribution[102] to the synthesis of *O*-GlcNAc building blocks by development of a direct glycosylation of the FmocSerOH and FmocThrOH amino acids by a "one pot reaction" system. The oxazoline of the readily available 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose, prepared using an excess of boron trifluoride etherate as Lewis acid promoter, is used as glycosyl donor for the *in situ* glycosylation of the hydroxyl groups of the side chains of FmocSerOH and FmocThrOH (Scheme 20). These building blocks are ready to be used in stepwise solid-phase synthesis since the combination of protecting groups is compatible with peptide synthesis protocols. This oxazoline approach has been recently applied by Waldmann *et al.*[103] for the synthesis of a PhAcOZ-protected serine *O*-β-GlcNAc glycoside.

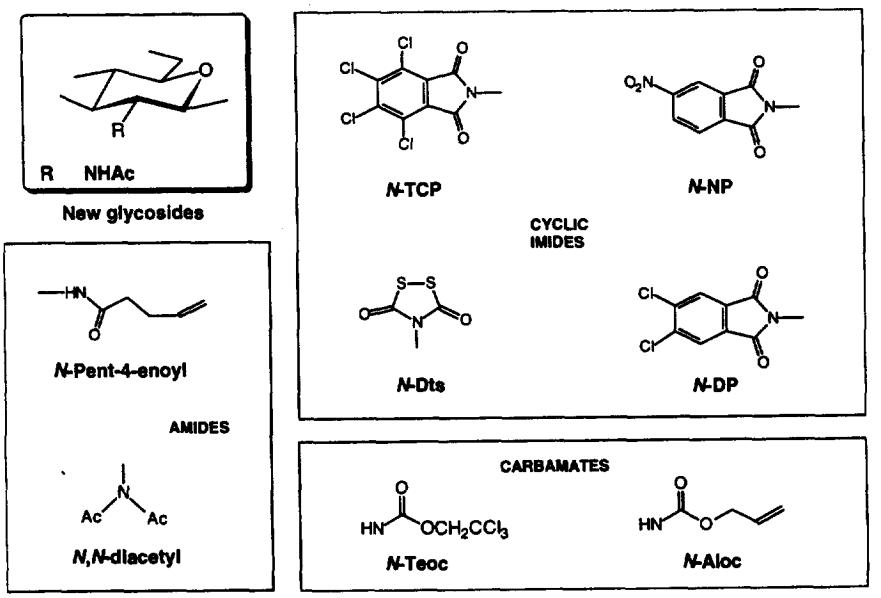


Scheme 20.

4.2. *Glycosyl donors with a 2-N-protected function*

Apart from *O*-GlcNAc glycopeptides aminated carbohydrates are widespread among the growing number of complex biologically important oligosaccharides. In all these structures, amino protecting group manipulation procedures must not only be mild but also preferably chemoselective as to allow for differentiation between several aminated sites.

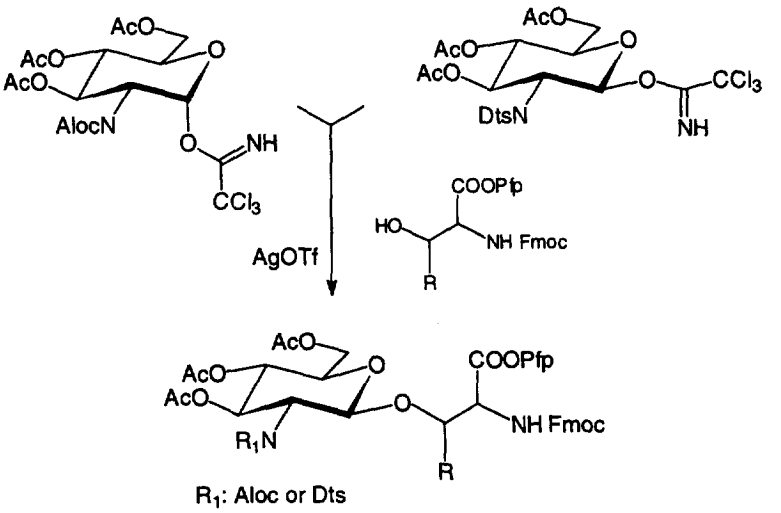
Several new amine protecting groups have been described recently for amino protection in synthetic carbohydrate chemistry. Some of them were inherited from peptide chemistry.[104] They belong to different groups, amongst them cyclic imides as such as phthalimides, carbamates or protected amides which are exemplified below (Scheme 21).



Scheme 21. Development of new building blocks used for *O*-GlcNAc Ser/Thr building blocks.

4.2.1. N-*Dts*, N-*Aloc*

Alternative strategies for *O*-GlcNAc building block preparation have been described by Meldal *et al.*[105] They are based on the direct glycosylation of active esters derivatives of Ser and Thr by employing the *N*-allyloxycarbonyl (*N*-Aloc) and the *N*-dithiasuccinoyl[106] (*N*-Dts) *amino protective groups*. In these building blocks the final *N*-acetyl group can alternatively be generated after glycopeptide assembly on solid phase (*N*-Dts) or prior to its incorporation onto a peptide (*N*-Aloc and *N*-Dts) (Scheme 22).



Scheme 22.

The reduction of the amino precursor *N*-Dts is an important transformation in the solid-phase synthesis of glycopeptides for the introduction of amino functions. The *N*-Dts functionality can be removed efficiently either: (i) by Zn reduction in THF–Ac_2O–AcOH with simultaneous *N*-acetylation in solution or, (ii) by thiolytic reduction[107] with different thiols such as dithiothreitol (DTT), β-mercaptoethanol (BME), *N*-methyl-α-mercaptoacetamide (MCA) or propanedithiol (PDT), in the presence of diisopropylethylamine (DIPEA) as catalyst in the solid-phase, being DTT and BME the most efficient ones. The advantage of using *N*-Dts is that it offers the possibility of glycopeptide labelling by attaching chromophores to the amino group in the solid-phase.

Some advantages of the **Aloc**[108] group are the following: it could be involved in C-2-anchimeric assistance to afford 1,2-*trans* glycoside, it can be easily removed in a chemospecific manner, and it has previously been used as an orthogonal protecting group in SPPS.[109] It is also well known, that the Aloc groups can be removed under practically neutral conditions by using palladium (0) catalyst in the presence of a nucleophile. Pd(0) reacts with the Aloc moiety to form a π-allyl Pd complex which is attacked by morpholine, dimedone, *N,N'*-dimethylbarbituric acid, or Bu_3SnH to give carbon dioxide and the free amine. However, sometimes the liberated amino group competes with those nucleophiles and *N*-allylation may arise as side reaction. This is prevented by using Bu_3SnH which reacts with the π-allyl Pd complex, leading to a tributyl carbamate and propene. The tin carbamate is cleaved *in situ* with a proton donor or an activated electrophilic carbonyl group, including Pfp esters. Pd(0)-catalysed allyl tranfer from the *N*-allyl oxycarbonyl group in the presence of acetic anhydride leads to the final 2-acetamido function.

Meldal has found that the trichloroacetimidate glycosylation method superior to the glycosyl bromide approach. Therefore the imidate was treated with Ser and Thr/Pfp esters in the presence of AgOTf at r.t. and the desired β-glycosides were furnished stereoselectively in 87–90% yield, respectively. They applied these building blocks to the synthesis of *O*-GlcNAc glycopeptide fragments

from the carboxy-terminal domain (CTD) repeating unit of RNA polymerase II and the light polypeptide chain of mammalian neurofilament (NF-L, 61 kDA).

4.2.2. N-*Troc*

Another procedure also used in the synthesis of *O*-GlcNAc Ser/Thr building blocks, relies upon the use of *N*-trichloroethoxycarbonyl (Troc)[110] for the protection of the amino group. It has been used in glycosylations primarily with the corresponding glycosyl halides,[111] but a few examples of Lewis acid promoted glycosylation with 1-*O*-acetyl sugars,[112] and ethyl thioglycosides[113] have also appeared. Thus, for instance, the *N*-Troc glucosamine donors[114] in combination with AgOTf activation affords excellent yields[115] of the desired β-glycosides. Magnusson *et al.*[116] has recently described glycosylations of Fmoc SerOH using *N*-Troc donors in the presence of boron trifluoride etherate as catalyst.

The *N*-Troc has a number of advantages that make it a useful protecting group in the assembly of these building blocks: i) the neighbouring group participation of *N*-Troc leads to the expected β-glycosides; ii) the undesired oxazoline formation is prevented during glycosylation and, iii) the Troc group can be easily removed under mild conditions in a chemospecific manner compatible with the sensitive Pfp ester. The "one-step" *N*-Troc deprotection and *N*-acetylation can be readily accomplished via reduction with zinc dust in THF/Ac_2O/acetic acid (3:2:1). In addition the Troc group is stable under a range of standard conditions used for carbohydrate synthesis. It is sensitive to alcoholysis under basic conditions, which permits convenient transformations into other carbamates. The *N*-Troc group is relatively stable under acidic conditions, which permits removal of *O*-acetyl groups by methanolic HCl. However, attempted selective *O*-deacetylation under basic conditions is unsuccessful, the *N*-Troc group is transformed into the corresponding carbamate. Recently, a mild and selective *O*-deacetylation reagent[117] has been described that leaves the *N*-Troc group intact.

4.2.3. *Phth*

Owing to the fact that a glycosyl donor carrying a *N*-Phth group at the C-2 position will predictably give a 1,2-*trans* β-glycoside in a highly stereoselective manner, Phthaloyl[118] is a valuable amino-protecting group which was one of the first groups to be used for amino protection in synthetic carbohydrate chemistry.

However, while deprotection into an amine can be achieved in a reasonably convenient manner by such nucleophiles as hydrazine, *n*-butylamine, methylamine and $NaBH_4$, this process frequently requires a high temperature and extended reaction time, particularly when applied to a large oligosaccharide carrying multiple NPhth groups. As a result, the course of dephthaloylation is often difficult to monitor, and extensive trial and error efforts are required to find the most favorable conditions for a specific substrate.

To address this problem the use of Phth analogues, such as the 4-nitro-,[119] the di- and the tetrachlorophthaloyl, all substituted on the aromatic ring with electron-withdrawing groups has been proposed.

4.2.4. *TCPhth (TCP)*/N-*pent-4-enoyl*

Fraser-Reid *et al.*[120] have reported recently that the pent-4-enoyl and tetrachlorophthaloyl (TCP) groups serve as protecting devices for amine functionalities that can be removed orthogonally and chemoselectively. They have used[121] this group in the synthesis of *O*-GlcNAc Ser/Thr building blocks. Thus, a 2-*N*-TCP protected 1-pentenyl glycoside was used to glycosylate ZSerOBn in the presence of NIS/TESOTf as catalyst in dichloromethane. TCP belongs to the class of cyclic imidic protecting groups including the parent phthaloyl, maloyl,[122] and dithiasuccinoyl. TCP offers a wealth of advantages in synthetic manipulations where a primary amine must be carrried in a protected form. Of special importance is the survival of carboxylate esters during TCP cleavage.

The TCP group has been found to be stable to a wide range of reagents normally used in standard oligosaccharide transformations. TCP is able to exercise neighbouring group participation. Phth and TCP are comparable from the standpoints of ease of installation and stereodirecting properties. It can be removed with as little as 1.5 equiv of ethylenediamine from sensitive substrates. The cleavage by-product is insoluble in many commonly used solvents and it is therefore conveniently removed by filtration. However TCP is unstable to the strongly basic conditions necessary for standard NaH/BnBr benzylations.

Fraser-Reid's efforts in developing hydroxyl protecting groups that could be installed and/or removed under neutral conditions have provided pentenyl glycoside (NPG) chemistry which has been explored in his group mainly over the last seven years[123] (NPG and glycosyl *N*-pentenoates). One of the features of the NPG chemistry is that primary and secondary amines are readily protected as *N*-pent-4-enoyl derivatives which are crystalline. Deprotection is effected under mild conditions by treatment with 3 equivalents of iodine in aqueous THF solution. Owing to the importance of D-glucosamine in glycoscience, special attention was devoted to some reactions of this substrate. Acylation of D-glucosamine hydrochloride with pent-4-enoic anhydride and subsequent treatment with acetyl chloride yields the glucosyl chloride ready for Köenigs–Knorr type of coupling reactions. This protecting group has *not* yet been used in the synthesis of *O*-GlcNAc Ser/Thr building blocks.

4.2.5. N-*DCPhth*

In connection with synthetic studies for producing polylactosamine-type glycoconjugates, Ogawa *et al.*[124] disclosed some results on developing a 4,5-dichlorophthaloyl (DCPhth) group in oligosaccharide synthesis which has a strong 1,2-*trans*-directing nature as Phth, yet is easily removable under substantially mild conditions. The DCPhth group can be expected to be more stable than its tetrachloro counterpart. It can be removed under mild conditions by using ethylenediamine or hydrazine hydrate/methanol at r.t. (24 h).

4.2.6. N,N-*diAc*

In all the above-discussed methodologies the need for regeneration of free amino groups is disadvantageous, therefore methods for retaining the *N*-acetyl functionality in the activated species are of great interest. A convenient solution of this problem is the formation of *N,N*-diacetyl derivatives of amino sugars[125] which could lead, upon anomeric activation, to highly reactive *N*-acetyl-oxazolinium intermediates. Reaction with the acceptor HOR at the anomeric center will then provide, via stereocontrolled β-attack, the desired β-linked glycoside, which can be transformed into the target molecule under very mild conditions, avoiding the intermediacy of the free amine. This kind of protecting group has not yet been used in the synthesis of *O*-GlcNAc Ser/Thr building blocks.[125a]

In summary, in spite of these already versatile alternatives, new insight is still required before an optimal amine protecting group fulfils most of the requirements to be found in the chemistry of the aminosugar.

5. *O*-Glycosyl amino acids containing other sugar units with hydroxyl groups in the 2 position of the sugar linked to the amino acid

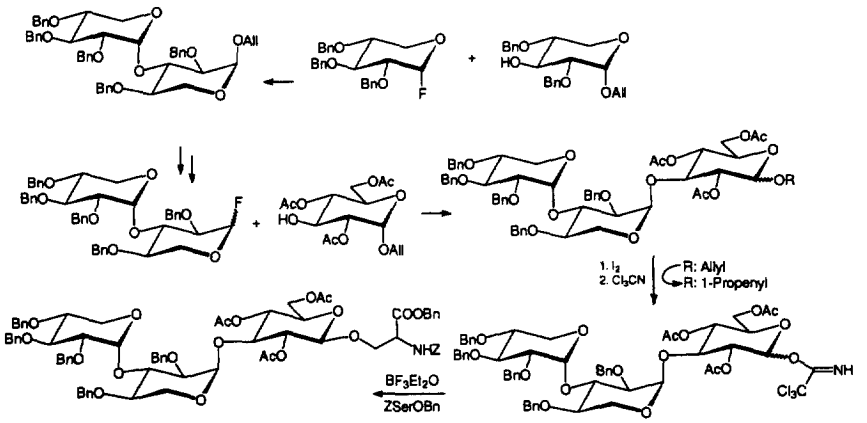
5.1. *With a Glc-Ser/Thr linkage*

Among the most interesting examples of glycoproteins containing Glc-Ser/Thr moieties are the blood clotting factors VII and IX. Recent structural studies have further shown that the first epidermal growth factor (EGF)-like domains of human and bovine blood-clotting factors VII and IX, as well as human and bovine Protein Z and bovine thrombospondin, contain unique *O*-linked sugar chains consisting of glucose and xylose. Bovine proteins have a trisaccharide composed of 1 unit of Glc and 2 units of Xyl. For instance, the trisaccharide of bovine factor IX was identified as (α-Xyl-1→3-α-D-Xyl-1→3-β-D-Glc) and bound to Ser 53 by a β-D-linkage. Bovine factor VII and protein

Z have the same trisaccharide. In the corresponding human proteins, the above trisaccharide[126] and disaccharide[127] whose structure was proposed to be a (α-D-Xyl-1→3-β-D-Glc) are located at the same position indicating microheterogeneity of these *O*-linked sugar chains. The above proteins all contain a conserved sequence of amino acids consisting of a Cys-X-Ser-X-Pro-Cys sequence.

The chemical syntheses of the disaccharide and trisaccharide conjugates to serine have already been carried out. In all cases, D-glucose derivatives were coupled via the α-D-glucosyl bromide with neighbouring participating groups to afford β-glycosides.

Typically, the trisaccharide α-D-Xyl-(1→3)-α-D-Xyl-(1→3)-D-Glc was coupled to a Z/Bn protected serine derivative via the corresponding trichloroacetimidate as glycosyl donor in the presence of boron trifluoride etherate as catalyst, in 66% yield (Scheme 23).[128]



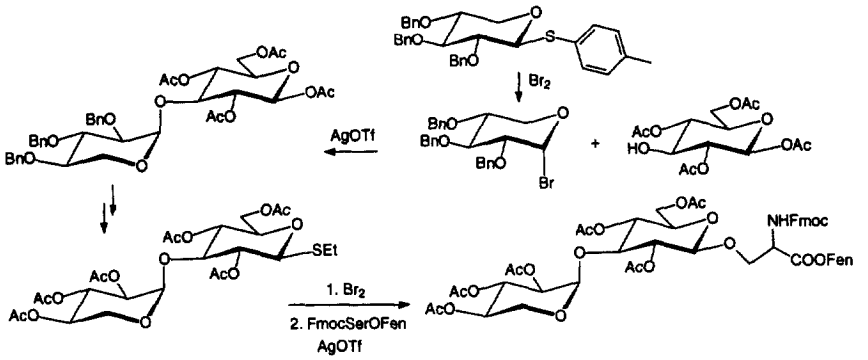
Scheme 23.

Such compound was prepared by reaction of 2,3,4-tri-*O*-benzyl-α-D-xylopyranosyl fluoride with allyl 2,4-di-*O*-benzyl-α-D-xylopyranose in the presence of tin(II) chloride and silver perchlorate, which proceeded to give the α-anomer disaccharide preferentially. By isomerization of the allyl group using an iridium complex the propenyl glycoside is obtained. Removal of this group with iodine and subsequent reaction with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate and triethylamine gives the corresponding glycosyl fluoride. Coupling between the fluoride and an allyl glycoside yields the α-linked trisaccharide. The related trichloroacetimidate is prepared first by isomerization of the allyl group, then further removal and reaction of the free hydroxyl with trichloroacetonitrile. The final building block was also prepared[129] by treatment of the unprotected glycosylated serine compound with *N*-(fluoren-9-ylmethoxycarbonyl)succinimide in 1,4-dioxane followed by treatment with pentafluorophenol and dicyclohexylcarbodiimide in tetrahydrofuran for its use in SPPS.

Similarly, peracetylated α-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl Fmoc glycosyl serine derivative has been prepared[130] by glycosylation of Fmoc Ser phenacyl ester with a derivative of the disaccharide α-D-Xyl*p*-(1→3)-β-D-Glcp) bromide glycosyl donor under silver triflate catalysis (Scheme 24).

The disaccharide was isolated in a 54% yield from the corresponding ethyl thioglycoside by reaction with bromide. Glycosylation of 2,3,4-tri-*O*-benzyl xylopyranosyl bromide with tetraacetylated glucose under silver triflate catalysis yielded the disaccharide. Hydrogenolysis of the Bn ethers, subsequent acetylation, and reaction with boron trifluoride etherate and ethanethiol afforded the disaccharide thioglycoside donor.

The synthesis of other complex oligosaccharides with the Glc-Ser linkage has also been reported. The reaction of the trisaccharide β-D-Glc-(1→4)-β-D-Glc-(1→4)-D-Glc, activated as thioglycoside under promotion of methyltriflate and a non-participating substituent at C-2, with a serine acceptor yielded a mixture of anomeric glycosides.[131] A complex pentasaccharide, available by partial enzymatic



Scheme 24.

degradation of starch, was acetylated and transformed into the glycosyl bromide. This compound could be coupled[132] in the presence of silver triflate with the Fmoc/Pfp protected serine derivative. The desired β-glycoside was obtained in a yield of 42%.

5.2. *With a Fuc-Ser/Thr linkage*

A novel type of *O*-glycosylation presenting a Fuc-Ser/Thr moiety has been recently reported by several authors investigating the *N*-terminal Epidermal Growth Factor (EGF) domains of various coagulation and fibrinolytic proteins,[133] including factors VII and XII, tissue plasminogen activator and urokinase.[134] The same fucose peptide linkage was also found in a smaller peptide, named PMP-C 1, isolated from the insect *Locusta migratoria*[135] and reported to be a serine protease inhibitor.[136]

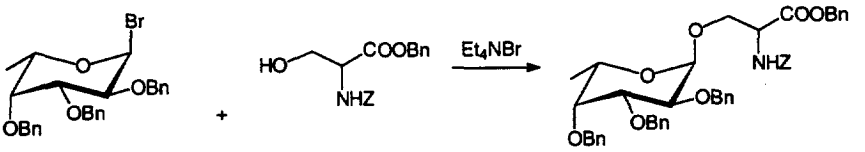
In most cases, L-fucose has been found α-glycosidically linked to serine or threonine residues within the peptide motif Cys-X-X-Gly-Gly-Thr/Ser-Cys, suggesting that new *O*-fucosylated proteins may eventually be identified using protein data bases. More recently Harris[137] identified the tetrasaccharide NeuAc-α (2→6)Gal-β-(1→4)GlcNAc-β-(1→3)-Fuc-α-1→*O*-linked to serine 61 of Human Factor IX.

The biological function of this unusual glycosylation is unknown and therefore a chemical synthesis of fucosylated peptides would be interesting for elucidating its biochemical roles. Furthermore, L-fucose is known to be an important residue of branched oligosaccharides in glycoconjugates that bind to selectins, and synthetic fucosylated glycopeptides might be potential mimetics of selectin-binding oligosaccharide.

Fucosylated Thr and Ser derivatives have been prepared by methods leading to α-glycoside linkages by the use of non-participating protecting groups such as benzyl ethers in the fucosyl donor. This otherwise good choice of orienting groups has important drawbacks which are related to the acid lability of either the free hydroxyl or benzyl protected α-L-fucosyl intermediates. Moreover, benzyl protection is not fully compatible with peptide chemistry protocols, especially when the presence of sulfur-containing amino acids leads to impaired hydrogenations. In spite of these difficulties, the reported procedures proceed throughout benzylated glycosyl donors activated either as bromides or phosphites. In addition, on the amino acid moiety which is to be glycosylated, the orienting groups, in turn, dictate the choice of *N*-protecting groups of the serine acceptor. Thus because the Fmoc group is not completely stable under the hydrogenolytic conditions required for the removal of benzyl ethers, the benzyloxycarbonyl (Z) group is a compulsory alternative during the glycosidation steps. The resulting intermediates only become compatible with solid-phase procedures after replacement of benzyl for *O*-acetyl groups. A later exchange with Fmoc yields the best suited intermediates for solid-phase protocols.

In practice, it is well established that α-fucosides can be synthesized in high stereoselectivity with benzyl-protected fucose donors by application of the *in situ* anomerisation procedure[138] as discussed. The synthesis of the building block is performed[139] by reaction of a serine derivative protected at the

amino and carboxylic group (ZSerOBn) and a 2,3,4-tri-*O*-benzyl L-fucosyl derivative (bromide)[140] which is activated at the anomeric position (Scheme 25).

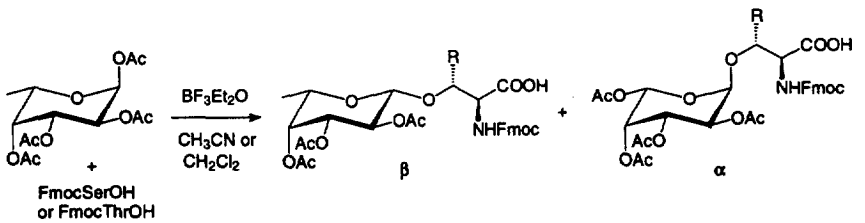


Scheme 25.

Another way to perform glycosylation of serine derivatives uses a fucosyl phosphite derivative donor. It has been reported that tribenzylfucosyl phosphite[141] which has been successfully coupled to BocThrOEt with TfOH or TMSOTf as catalyst to give the α-glycoside in 80% yield.[142]

Alternatives to benzyl protection are strategies using Mpm groups. Their application to the synthesis of fucosyl amino acids proceeds by glycosylation of the FmocThrOtBu with methoxybenzylether thioethylfucoside activated with dimethylthiomethylsulfonium triflate (DMTST) giving the glycoside in 57% yield.[143] For application of this conjugate in glycopeptide synthesis, a sufficient stability of the fucoside bond and protecting group compatibility had to be ensured. This was accomplished in three steps: first, removal of the Mpm with CAN, subsequent acetylation of the hydroxyl groups and lastly, cleavage of the *t*-Bu ester with formic acid to yield a fucosyl serine derivative that is ready to be incorporated in solid-phase schemes.

As seen, Bn or Mpm protection add several steps to the synthetic sequences reducing the overall yields. Recently, a method was described[144] that uses acetates as protective groups of the hydroxyls in the carbohydrate moiety of the glycosyl donor. Glycosylated amino acids having a 1,2-*cis*-α-*O*-glycosidic linkage can also be prepared using carbohydrate peracetates as glycosyl donors, provided that sufficient amounts of Lewis acid are added as promoter, and that the reaction is allowed to proceed towards the thermodynamically more stable α-glycoside. Although the present procedure reduces the synthetic steps from four or six to one and the overall yield is increased, the need for HPLC purification still prevents their preparation in bulk quantities (Scheme 26).



Scheme 26.

5.3. *With a Xyl-Ser linkage*

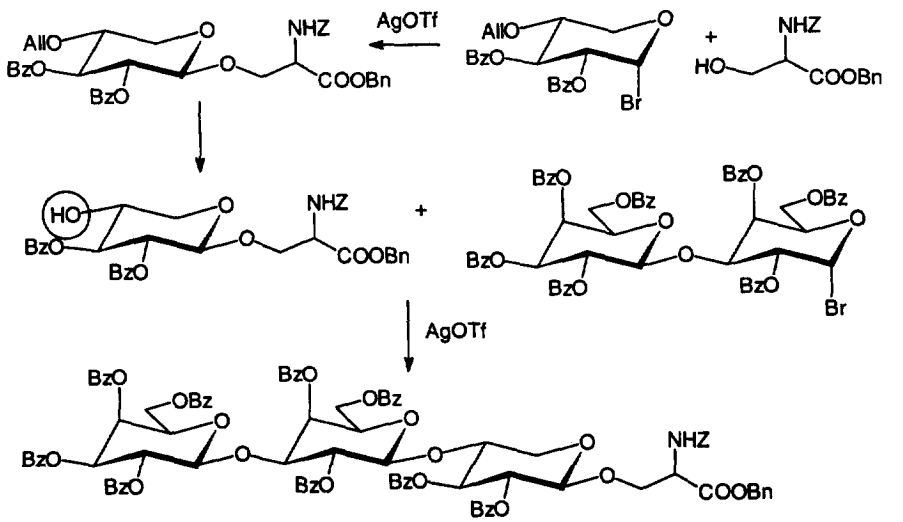
The second most abundant type of *O*-glycoprotein contains a β-glycosidic linkage between D-xylose and the hydroxy group of serine. These structures are found mainly in the proteoglycans. Proteoglycans are complex macromolecules that consist of a core protein to which a variable number of glycosaminoglycan chains are covalently attached.[145] In most mammalian proteoglycans, namely those carrying chondroitin sulfate, dermatan sulfate, heparin and heparan sulfate, the glycosaminoglycan is assumed to be linked to an L-serine residue of the core via D-xylose through a specific tetrasaccharide sequence.[146] However, a most common structure is the trisaccharide β-D-Gal(1→3)-β-D-Gal-(1→4)-β-D-Xyl linked to serine where the two galactose residues are followed by a glucuronic *N*-acetylhexosamine pair, constituting the first repeating disaccharide unit of the polysaccharide chain.

The biosynthesis of the polysaccharide chains of the xylose-containing proteoglycans is initiated by transfer of Xyl from UDP-D-Xyl to specific serine residues in the core protein. The other residues are later added stepwise in reactions catalysed by specific glycosyltranferases. Owing to the difficulties inherent in the preparation of native substrates for the various glycosyltransferases participating in proteoglycan biosynthesis, these enzymes are presently being studied with oligosaccharides of appropriate structure or with serine-linked saccharides.

Obviously, further knowledge on this field is dependent on the newly synthesised linkage region and on new methods for preparing them.

Glycosyl amino acids representing partial structures of this core unit, (β-D-Xyl-Ser, β-D-Gal-(1→4)-β-D-Xyl-Ser and β-D-Gal(1→3)-β-D-Gal-(1→4)-β-D-Xyl-Ser) as well as β-xylopyranosyl peptides with four amino acids have been synthesised. The formation of these β-linked glycosides is easier compared to the mucin type structures. The formation of such 1,2-*trans* glycosides can be performed stereoselectively with a neighbouring participating substituent at the C-2-atom of the sugar. The syntheses of β-D-Xyl-Ser structures have mostly been performed by glycosylating *C*- and *N*-terminal protected serine derivatives with acetylated or benzoylated xylopyranosyl halides with Ser with the following C/N protecting pairs: Fmoc/Bn[147] and Z/Bn.[148] The glycosyl amino acid[149] β-D-Gal-(164)-β-D-Xyl-Ser was also obtained in 72% yield by glycosylation of ZSerOBn with the corresponding disaccharide bromide under silver triflate promotion. Preparation of larger xylopyranosyl peptides[150] of this core type can also be synthesised.[151] In these samples the trichloroacetimidate method has proved to be useful for the connection of D-xylose containing oligosaccharides with serine derivatives.[152]

The synthesis[153] of β-D-Gal(1→3)-β-D-Gal-(1→4)-β-D-Xyl-Ser has been done by glycosylation of the Xyl-Ser a glycon with the galactobiosyl bromide under a silver triflate-collidine complex as a promoter to give the Gal-Gal-Xyl-Ser derivative in a 72% yield. Condensation of the 4-*O*-allyl-2,3-tri-*O*-benzoyl-β-D-xylopyranosyl bromide with ZSerOBn, under a silver triflate–collidine complex as a promoter yielded the Xyl-Ser glycosyl amino acid. The *allyl ether was isomerized to the corresponding propen-1-yl-ether*, using Wilkinson's catalyst. This ether was cleaved by treatment with mercury (II) acetate in 90% aqueous acetone to give the partially protected Xyl-Ser aglycon (Scheme 27).



Scheme 27.

The presence of a *phosphate group* at *O*-2 of β-Xyl residue in both chondroitin sulfate from the Swarm Rat Sarcoma and heparan sulfate from bovine lung was demonstrated by Oegema, Jr. *et al.*[154] in 1984 and by L.A. Fransson *et al.*[155] in 1985. In 1988, Sugahara *et al.*[156] isolated and chemically characterised neutral and *sulfated* glycohexaosylserines from the linkage regions of chondrotin 4-

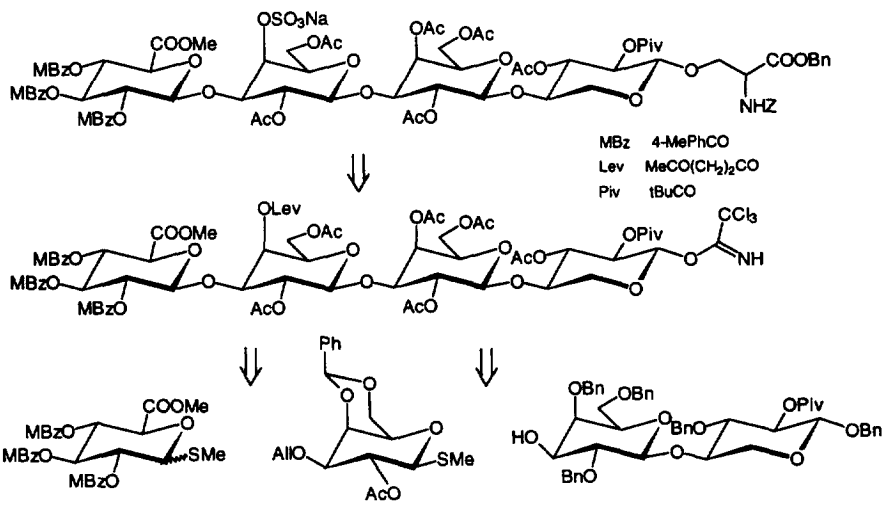
sulfate proteoglycan of Swarm Rat chondrosarcoma after exhaustive enzymatic digestions. The discovery of the presence of a sulfate group at the linkage region particularly at *O*-4 of Gal of chondroitin 4-sulfate is believed to be of significant interest.

One possibility is that such anionic groups could serve as recognition signals for the transportation of the biosynthetic precursor molecules to a specific subcellular multienzymic compartment in the Golgi apparatus where specified repeating disaccharides are to be assembled.

To probe any such assumption, model structures and substrates for enzymatic studies are required. Regrettably, isolation of glycopeptides from this linkage region by chemical means is complicated by the sensitivity of the xylose–peptide bond to acidic or basic conditions. Thus, chemical synthesis is the alternative of choice.

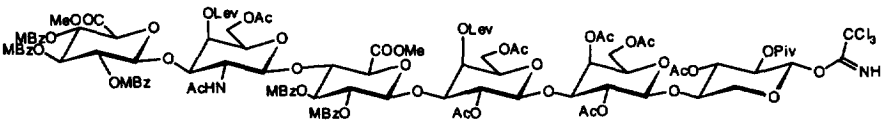
Working in the elucidation of the biosynthetic pathways of proteoglycans the stereocontrolled synthesis of sulfated glycotetraosyl serine[157] and glycohexaosyl serines[158] was reported for the first time by Ogawa *et al.* Other groups[159] devoted to conformational studies of glycopeptides have also conducted the synthesis of both sulfated glycotetraosyl-L-serine and phosphorylated tetrasaccharide dipeptides from the linkage region.

Retrosynthetic analysis is provided inScheme 28 to prepare such a complex structure. The crucial point is the preparation of the glycotetraosyl imidate (or the glycohexaosyl imidate) that is to be coupled to ZSerOBn in the presence of boron trifluoride etherate as catalyst. The glycotetraosyl serine derivative contains levunoyl (Lev) groups that are chemoselectively removed by treatment with hydrazine acetate in toluene–ethanol (1:5) at low temperature and the free hydroxyls are then sulfated with $Me_3N.SO_3$ in DMF at 50°C to give the desired sulfate groups.



Scheme 28.

The synthesis of the complex glycohexaosyl serine was also reported by glycosylation of the same amino acid derivative using the trichloroacetimidate in the presence of boron trifluoride etherate catalyst to afford the glycohexaosyl derivative in a 64% yield (Scheme 29).



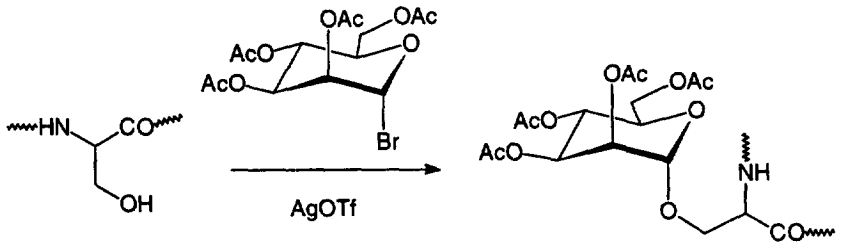
Scheme 29.

5.4. *With a Man-Ser/Thr linkage*

The increasing interest in using yeast in the production of recombinant proteins of therapeutic interest has raised the need for studying the glycosylation patterns induced by these systems.

It is known that α-D-mannopyranosyl residues bound by *O*-linkages to Ser and Thr are present in glycoproteins from yeast and molds. Thus this class of compounds has also been found while attempting glucoamylase G1 in *Aspergillus niger*[160] and human insulin-like growth factor (IGF-I, Somatomedin C) expressed in *Saccharomyces cerevisiae*.[161] In the latter case, the glycosylation site was determined to be threonine-29 and it was mainly substituted with the α-D-Man*p*(1→2)-α-D-Man*p*-(1→3).

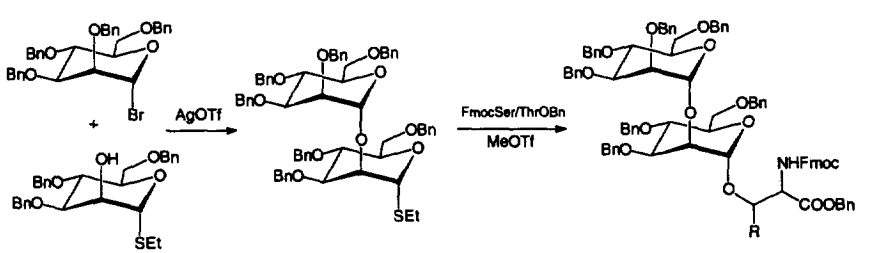
Establishing an α-glycosyl connection of D-mannose is fairly straightforward. α-Mannosyl chloride and α-mannosyl bromide[162] were used for Köenigs–Knorr glycosylations of the amino acids FmosSerOBn and FmocSerOPfp, respectively (89% and 60%) under silver triflate catalysis. This approach was used to achieve mannosylated amino acid linkages involving glycosylation of resin-bound peptides. Although this has been achieved to the extent of 20–23% considerable improvement is needed before it can offer general utility (Scheme 30).



Scheme 30.

This linkage between a single Man residue and Ser or Thr has also been accomplished[163] by glycosylation of FmocSerOAll with peracetylated α-mannosyl trichloroacetimidate under TMSOTf catalysis yielding the mannosyl serine derivative. The trichloroacetimidate[164] has been further used to glycosylate FmocSerOH having unprotected carboxylic acid under boron trifluoride catalysis.

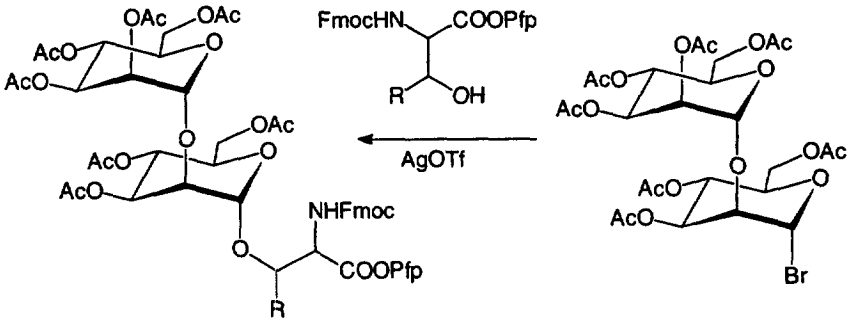
The synthesis of α-D-mannopyranosyl-α-(1→2)-mannopyranosyl derivatives of L-serine and threonine have been described by two methods based on different disaccharide glycosyl donors. In the first approach (Scheme 31), the disaccharide donor is the 1-ethyl thioglycoside that was reacted under methyl triflate catalysis with the FmocSerOBn and FmocThrOBn yielding the glycosides in a 75 and 80% yield, respectively. The 1-ethylthio disaccharide was obtained by reaction of the unprotected 1-ethylthiomannoside in position 2 with perbenzylated mannopyranosyl bromide under silver triflate catalysis.[165]



Scheme 31.

In the second approach (Scheme 32), the disaccharide donor was a glycosyl bromide. The per-*O*-acetyl-α-D-mannopyranosyl-(1→2)-α-mannopyranosyl-Fmoc-L-threonine Pfp derivative was used as a building block in one of the first examples of solid-phase synthesis of a glycopeptide. The silver triflate promoted reaction of the mannose disaccharide donor with the FmocThrOPfp yields the α-

glycoside in 80%.[166] The advantage of this last method is that the resulting disaccharide glycosyl amino acid is ready for its incorporation in solid-phase synthesis schemes.



Scheme 32.

The synthesis of di-mannosylated amino acids has also been described using enzymatic procedures. Particularly remarkable is the use of whole cells of the recombinant *E. coli* as catalysts. To demonstrate the feasibility of the concept, several acceptors for the enzymatic mannosylation reaction with GDP-Man as a mannosyl donor were described and the hydroxyl free Z-α[Manα1,2Man]ThrOMe was prepared in a moderate yield.

The synthesis of more complex structures having mannosyl bonds have been also reported. A first example relies on the fact that mannose-6-phosphate (Man-6-P) has been shown to be an inhibitor of inflammation in the central nervous system.[167] Therefore, it was thought to be of interest to synthesize glycopeptides,[168] containing 6′-*O*-phosphorylated α(1→2) and or α(1→6) linked disaccharides. In these structures the peptide acts as a template which mimics the scaffolding core structure of the natural oligosaccharide ligand. For the synthesis of these building blocks, the crucial step is to obtain the corresponding protected phosphorylated disaccharides. In these syntheses 2,2,2-trichloroethyl (TCE) was probed to be an effective protection of the phosphate group which was quantitatively removed by treatment with Zn and silver carbonate in pyridine containing 10% acetic acid.

Complex Man structures have also been described in a partial structure of an active phytoalexin-elicitor glycoprotein in which a reducing terminal mannosyl residue of the trisaccharide, β-D-Glc-(1→6)-α-D-Man-(1→6)-α-D-Man is *O*-glycosidically attached to serine in the protein portion. A stereocontrolled synthesis of this triglycosyl-serine and its derivatives of a model glycopeptide[169] was described for a detailed investigation of the structural requirements of the bioactive glycoprotein.

Glycosylation of the disaccharide, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate, with *N*-Z-2,3,4-tri-*O*-acetyl-α-D-manno-pyranosyl)-(1→3)-L-serine methyl ester in the presence of AgOTf afforded the desired trisaccharide-serine derivative. The mannosyl serine linkage was effected by coupling of perbenzoylated mannosyl bromide with ZSerOMe, under catalysis with $Hg(CN)_2$ and $HgBr_2$ in a 39% yield in dichloromethane.

5.5. *With a Glc-Tyr linkage*

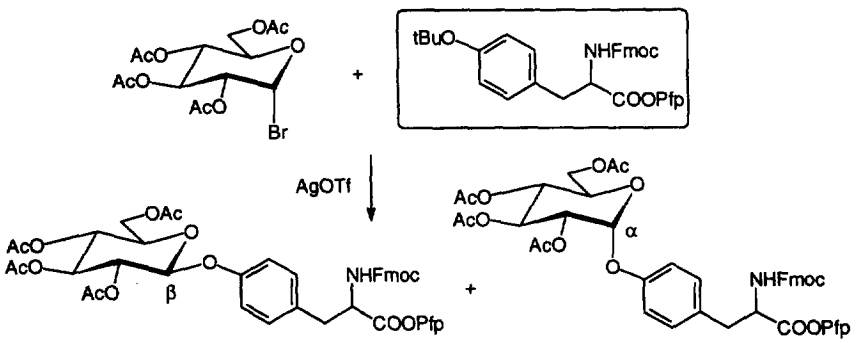
Glycosyl Tyr and glycosylated Tyr containing glycopeptide moieties are not widely spread in nature. *O*-Glycosylated Tyr has been identified in glycogenin.[170] Glycogenin, initiates glycogen synthesis in muscle and other tissues and then remains covalently bound to the glycan part of the molecule.

The anomeric configuration of the glycosidic linkage of Tyr-194 to glucose has not been determined unequivocally. Therefore, both the 1,2-*trans* and 1,2-*cis* aryl glycosides with ester type protecting group are targets in the synthesis of glycogenin-related glycopeptides. Similarly, the presence of a novel *O*-glycosidic linkage via Tyr has been recently detected on the surface layer (*S*-layer) of the prokaryotic eubacterium *Clostridium thermohydrosulfuricum* strains.[171] In two of the strains it was

established that the phenolic hydroxy group was glycosylated with a linear hexasaccharide through a β-galactosidic bond.

The free glycosyl amino acid, β-D-glucosyl-*O*-tyrosine has been shown to be a transient metabolite for Tyr storage for the production of tanning diphenol substrates in Lepidoptera.[172] Related compounds such as a number of polycyclic glycopeptide antibiotics of the vancomycin and ristocetin group carry aromatic glycosylation with β-D-glucose and β-mannose linked to phenolic rings.

A particular feature associated with aromatic glycosylations (in particular, tyrosine glycosides) is the lower nucleophilicity of the phenolic hydroxy groups in comparison with aliphatic hydroxy groups. This means that glycosylation of aromatic hydroxyl groups is inherently more difficult than aliphatic glycosylations because of the low nucleophilicity of the phenols resulting from the donation of electrons to the neighbouring π-electron system. Therefore, quite harsh conditions were often used for these glycosylations. Improved procedures rely on increasing the reactivity of either the donor or the acceptor or by raising the reaction temperature. Thus, for instance, it has been reported that an enhancement of the nucleophilicity of the acceptor hydroxy group may be achieved, *e.g.* using *t*-butyl ether protection of the hydroxyl phenolic group of Tyr such as in the FmocTyr(*t*Bu)OPfp[173] intermediate (Scheme 33).



Scheme 33.

Apart from the classical Köenigs–Knorr synthesis only a few reports on the successful glycosylation of Tyr have been published. Earlier attempts to glycosylate Tyr involved the synthesis[174] of ZTyr(Ac_4-β-D-Glc)OMe and the glycoside was obtained in 47% yield by reaction of peracetylated sugars in the presence of Lewis acids (Helferich conditions) at elevated temperatures. Horvat *et al.*[175] coupled 2,3,4,6-tetra-*O*-benzyl-glycopyranose with ZTyrO*t*Bu and ZTyrOBn at 80°C by *in situ* activation with carbodiimide (DCCI) and $CuCl_2$, and obtained a mixture of anomers in 40–68% yield. Smiatacz *et al.*[176] treated ZTyrOMe and BocTyrOMe with dimeric 3,4,6,tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride in DMF at r.t. for 24 h.

A systematic study of the direct glycosylation of FmocTyrOAll and FmocTyrOPfp with several glucopyranosyl and maltosyl donors has also been published.[177] The Fmoc/Pfp-protected tyrosine derivative was glycosylated with various saccharides. As an example the perbenzoylated maltose bromide was coupled with the tyrosine derivative in acetonitrile under promotion by silver triflate to afford the desired α-glycoside in 33% yield and the corresponding β-anomer in 30% yield. It was demonstrated that the most convenient glycosylations were obtained when peracylated glycosyl bromides were used with silver trifluoromethanesulfonate in dichloromethane at −10°C or in acetonitrile at r.t.

Again, the lower nucleophilicity of a phenolic, as compared to an aliphatic hydroxyl group, which makes glycosylations of tyrosine more difficult than that of serine and threonine has also been demonstrated by glycosylation experiments of FmocTyrOH having unprotected carboxylic acid with peracetylated glycosyl derivatives under boron trifluoride etherate catalysis.[178] As expected, glyco-

sylation of FmocTyrOH with β-D-galactose pentaacetate required longer times to reach completion and gave lower yields compared to FmocSer or Thr. Unlike the glycosylations of aliphatic hydroxyl groups in amino acids, the anomeric ratio of the product was significantly affected by the solvent. In dichloromethane the β-galactoside was obtained as a major product. In contrast, when acetonitrile was used the β- and α-glycosides were obtained. A similar loss of diastereoselectivity has previously been observed upon replacing dichloromethane with acetonitrile as solvent in the silver triflate promoted reactions of allyl and pentafluorophenyl esters of FmocTyr with perbenzoylated or peracetylated glucosyl bromides.

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