Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3085–3112

BIOORGANIC & MEDICINAL CHEMISTRY

Review

Synthesis of Tumor-Associated Glycopeptide Antigens

Constanze Brocke and Horst Kunz*

Institut für Organische Chemie, Johannes Gutenberg-Universität Mainz, Duesbergweg 10-14, 55128 Mainz, Germany

Received 18 January 2002; accepted 19 April 2002

Dedicated to Professor Christian Birr (Orpegen Pharma, Heidelberg) on the occasion of his 65th birthday.

Abstract—Carbohydrates and peptides linked together in glycoproteins constitute important components of the molecular communication between cells in multicellular organisms. Cell morphogenesis and tumorigenesis are accompanied by changes in the glycoprotein profiles of the outer cell membranes. Glycopeptide fragments of glycoproteins that have altered structures in tumor cells are of interest as tumor-associated antigens for the distinction between normal cells and tumor cells. In contrast to glycoproteins isolated from biological sources, synthetic glycopeptides are obtained in pure form and exactly specified structures. The methods developed for the synthesis of glycopeptides with tumor-associated antigen structure are outlined in this article by means of a series of typical examples. Beginning with *O*-glycopeptides of the relatively simple α-*O*-galactosamine-serine/threonine (T_N-antigen) type, glycopeptide antigens of increasing complexity are described. The review includes syntheses of the saccharide components, the glycosylation reactions to furnish the *O*-glycosyl amino acid building blocks, their selective *C*- and *N*-terminal deprotection and the use of these building blocks for glycopeptide syntheses both in solution and on the solid support. Particular attention is given to glycopeptides containing sialic acid residues, whose syntheses are demanding since reversible protection of the sialic carboxylic group is required. Synthetic methods for the construction of *N*-glycopeptides carrying the important cell adhesion ligands sialyl Lewis x and sialyl Lewis a antigen are also described. Strategies for the construction of glycopeptides of this type require methods compatible with the presence of the sialic acid residue, as well as with the acid-sensitivity of the fucoside bonds.

Contents

*Corresponding author. Tel.: +49-6131-39-22272; fax: +49-6131-39-24786; e-mail: hokunz@mail.uni-mainz.de

0968-0896/02/$ - see front matter
PII: S0968-0896(02)00135-9

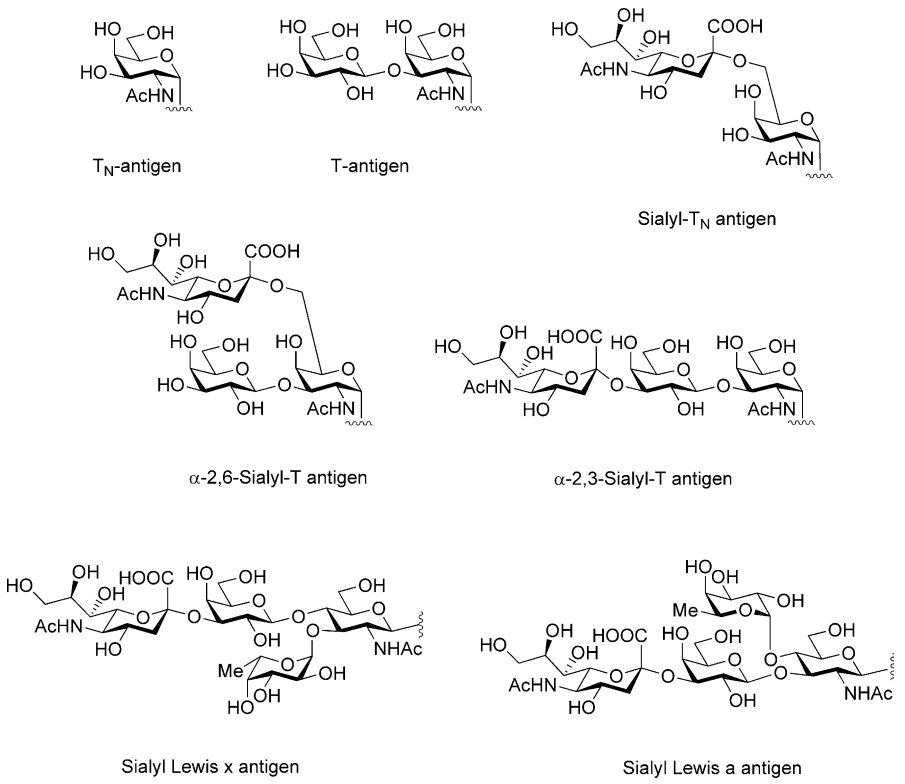


Figure 1. Tumor-associated carbohydrate antigens discussed in this article.

Introduction

Many glycoproteins of the outer cell membrane are decisive ligands and receptors involved in biological recognition processes including, for instance, immunodifferentiation, cell adhesion, cell differentiation and regulation of cell growth.[1–3] In a number of cases, the carbohydrate portion of the membrane glycoproteins plays an important role in biological selectivity.[4] In addition, glycosylation stabilizes the conformations of glycoproteins, protects them from proteolysis and modifies their physicochemical properties. The conformation of a glycoprotein can have a significant influence on its immunogenicity. Intercellular recognition processes can be affected by modulations of protein–protein interactions resulting from altered glycosylation patterns of the components. Therefore, aberrant glycosylation can be associated with autoimmune and infectuous diseases as well as with cancer.[5]

Glycoconjugates expressed predominantly on cancer cells can expose so-called tumor-associated antigens.[6] Examples of such tumor-associated carbohydrate antigens are the T$_N$- (αGalNAc-), T- (*Thomsen-Friedenreich*, βGal-

Abbreviations: AA, amino acid; Ac, acetyl; All, allyl; AMPS, (aminomethyl)polystyrene; Bn, benzyl; Boc, *tert*-butyloxycarbonyl; BSA, bovine serum albumin; *t*Bu, *tert*-butyl; Bz, benzoyl; CAN, ceric ammonium nitrate; CMP, cytidine monophosphate; *p*Cr, *para*-cresyl; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC, dicyclohexylcarbodiimide; Dhbt, 3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl; DIC, diisopropylcarbodiimide; DIPEA, diisopropylethylamine; DMAP, (dimethylamino)pyridine; DMDO, dimethyldioxirane; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DTT, 1,4-dithiothreitol; EEDQ, 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline; Et, ethyl; FACS, fluorescence-activated cell sorter; Fmoc, 9-fluorenylmethoxycarbonyl; Fuc, fucose; Gal, galactose; GalNAc, 2-acetamido-2-deoxygalactose; GlcNAc, 2-acetamido-2-deoxyglucose; HATU, 2-(1*H*-9-azobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU, 2-(1*H*-9-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HMBA, 4-hydroxymethylbenzoic acid; HMPA, hydroxymethyl-phenoxyacetic acid; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; HYCRON, (*E*)-17-hydroxy-4,7,10,13-tetraoxa-15-heptadecanoyl; Ig, immunoglobulin; IIDQ, 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline; KLH, keyhole limpet hemocyanine; Le^a, Lewis a; Le^x, Lewis x; MAG, multiple antigenic glycopeptide; Me, methyl; Mes, β-morpholinylethanesulfonic acid; MHC, major histocompatibility complex; MP, methoxyphenyl; MS, molecular sieves; MSNT, 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole; Mtr, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; NBS, *N*-bromosuccinimide; NEM, *N*-ethylmorpholine; NeuNAc, *N*-acetyl neuraminic acid; NIS, *N*-iodosuccinimide; NMM, *N*-methylmorpholine; NMP, *N*-methylpyrrolidone; PEGA, polyethyleneglycol-dimethylacrylamide copolymer; Pfp, pentafluorophenyl; Ph, phenyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; POEPOP, polyoxyethylene-polyoxypropylene copolymer; Pr, propyl; *i*Pr, *iso*-propyl; QOTf, tetrabutylammonium triflate; RP-HPLC, reversed phase high-performance liquid chromatography; SAMA-OPfp, *S*-acetylthioglycolic pentafluorophenyl ester; SASRIN, super acid-sensitive resin; sLe^a, sialyl Lewis a; sLe^x, sialyl Lewis x; SPGS, solid-phase glycopeptide synthesis; SPPS, solid-phase peptide synthesis; Su, succinimidyl; TBAF, tetrabutylammonium fluoride; TBDMS, *tert*-butyldimethylsilyl; TBDPS, *tert*-butyldiphenylsilyl; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; Tf, trifluoromethanesulfonyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIPS, tri-*iso*-propylsilyl; TMS, trimethylsilyl; TMSE, 2-(trimethylsilyl)ethyl; Trt, trityl; Ts, *para*-toluenesulfonyl. List of amino acids: Ala/A, alanine; Arg/R, arginine; Asn/N, asparagine; Asp/D, apartic acid; Cys/C, cysteine; Gln/Q, glutamine; Glu/E, glutamic acid; Gly/G, glycine; His/H, histidine; Ile/I, isoleucine; Leu/L, leucine; Lys/K, lysine; Met/M, methionine; Nle, norleucine; Orn, ornithine; Phe/F, phenylalanine; Pro/P, proline; Ser/S, serine; *h*Ser, homoserine; Thr/T, threonine; Trp/W, tryptophan; Tyr/Y, tyrosine; Val/V, valine.

Core-1: βGal-1,3-αGalNAc-1,*O*-Ser/Thr

Core-2: βGal-1,3-[βGlcNAc-1,6-]αGalNAc-1,*O*-Ser/Thr

Core-3: βGlcNAc-1,3-αGalNAc-1,*O*-Ser/Thr

Core-4: βGlcNAc-1,3-[βGlcNAc-1,6-]αGalNAc-1,*O*-Ser/Thr

Core-5: αGalNAc-1,3-αGalNAc-1,*O*-Ser/Thr

Core-6: βGlcNAc-1,6-αGalNAc-1,*O*-Ser/Thr

Core-7: αGalNAc-1,6-αGalNAc-1,*O*-Ser/Thr

Core-8: αGal-1,3-αGalNAc-1,*O*-Ser/Thr

Figure 2. Mucin core structures.

1,3-αGalNAc-), sialyl-T_N (αNeuNAc-2,6-αGalNAc-) and sialyl-T (αNeuNAc-2,3-βGal-1,3-αGalNAc- and βGal-1,3-[αNeuNAc-2,6-]αGalNAc-) antigens, all of which are linked to serine or threonine in the peptide backbone, and the sialyl Lewis x (αNeuNAc-2,3-βGal-1,4-[αFuc-1,3-]βGlcNAc-) and sialyl Lewis a (αNeuNAc-2,3-βGal-1,3-[αFuc-1,4-]βGlcNAc-) antigens, which terminate the oligosaccharide at the non-reducing end (Fig. 1). The monosaccharidic T_N-antigen is expressed on the membrane of human epithelial tumors.[7] Its sialylated analogue, the sialyl-T_N antigen, is mainly observed in breast, prostate and ovarian carcinomas.[8] The T-antigen is even reported to be tumor-specific in breast tissue.[9] Both regioisomeric sialyl-T antigens are found on leukocytes of patients suffering from acute myeloid leukemia.[10] In addition, the α-2,3-sialyl-T antigen occurs on colon[11] and breast[12] cancer cells. Lewis x is associated with carcinoma in the gastrointestinal tract,[13] and the expression of sialyl Lewis a is markedly enhanced in transformed colonrectal adenocarcinoma.[14]

Tumor-associated carbohydrate antigens were first discovered in mucins. Mucins are excessively *O*-glycosylated proteins found on the surface of various types of epithelial cells.[15] The apoproteins of mucins contain domains of tandem repeat sequences rich in serine, threonine and proline. In normal tissue, the protein backbone carries complex oligosaccharides derived from glycan core structures (Fig. 2) characterized by a GalNAc-unit α-*O*-linked to serine or threonine. In tumor cells, the expression of mucins is usually increased, and the carbohydrate side-chains are altered due to incomplete glycosylation and premature sialylation.[16] As a result, the conformation of the peptide chain can be influenced by the incompletely formed glycans and thus form new tumor-specific peptide epitopes.[17] Both the peptide and the carbohydrate portion of the glycoprotein can be of immunological importance. For instance, the PDTR-motif present in the tandem repeat of polymorphic epithelial mucin MUC 1 has been identified as the preferred target for the majority of peptide-specific anti-MUC 1 antibodies. At the same time, glycosylation of this tandem repeat region was found to increase the antibody binding.[18]

An enhanced level of tumor-associated antigens expressed on the surface of carcinoma cells often correlates with a poor prognosis for the patient.[5] Vaccinations based on synthetic tumor-associated glycopeptides[19] hold potential for targeting the immune system to the cancer cells in order to generate a tumor-specific immune response. Immunization studies have confirmed that glycopeptide antigens are immunogenic and able to induce a carbohydrate-specific T-cell response.[20] To evoke a humoral as well as a cellular immune response, the glycopeptide antigens are commonly conjugated with immunogenic carrier proteins such as KLH or BSA. The antibodies directed against these synthetic glycopeptide haptens recognize tumor cells expressing the corresponding epitopes.[21] High titers of antibodies have been shown to mediate the eradication of tumor cells by the immune system and to prevent the metastasis of tumor cells, therefore increasing the survival of tumor-bearing mice.[22]

For the application of tumor-associated glycopeptide antigens as epitopes in cancer research, model glycopeptides of exactly defined structure are required. Since glycoproteins are products of post-translational enzymatic glycosylation, they are not readily accessible by genetechnological methods. Furthermore, the isolation of well-defined glycopeptides or glycoproteins from natural sources is difficult due to the microheterogeneity of naturally occurring glycoproteins. Hence, chemical synthesis is a powerful tool for the preparation of large quantities of well-characterized glycopeptides. This review focuses on the preparation of immunologically relevant glycopeptides by chemical and chemoenzymatic methods. Recent advances in the synthesis of glycopeptides containing the tumor-associated T_N-, T-, sialyl-T_N, sialyl-T, sialyl Lewis x and sialyl Lewis a antigens[23] will be discussed on the basis of selected examples. Other reviews are recommended for further information about glycopeptide syntheses.[24]

Methods of Glycopeptide Synthesis

During the last decade, significant progress has been made in the synthesis of complex glycopeptides. While the elaboration of efficient solution-phase methods affords access to large quantities of the desired target compounds, the development of novel strategies for solid-phase glycopeptide synthesis has opened up the possibility of automated glycopeptide preparation.

A number of different strategies have been established for glycopeptide synthesis.[24] Most commonly, the so-called building block approach is employed. Glycosyl amino acid building blocks are prepared and then incorporated into the peptide chain formation by either fragment condensation reactions or by sequential solution or solid-phase peptide synthesis. Other methods in glycopeptide chemistry include the glycosylation of preformed peptides and glycopeptides in solution or on a solid support as well as convergent solid-phase glycopeptide synthesis using polymer-bound carbohydrates.

The solid-phase glycopeptide synthesis according to the building block approach offers an effective and general

route to synthetic glycopeptides, providing high yields by employing excess of amino acids and reagents. For the stepwise solid-phase peptide synthesis, which is usually based on the Fmoc-strategy, the preparation of suitably protected glycosyl amino acid building blocks is a crucial prerequisite.[25] A set of orthogonal protecting groups has to be employed for both the carbohydrate and the amino acid functionalities, ensuring mild deprotection of the assembled glycopeptide. The Fmoc-group used for temporary amine protection of the peptide chain can be removed by careful treatment with morpholine or piperidine. Glycopeptides containing *O*-glycosyl serine or threonine units are prone to base-catalyzed β-elimination of the glycan moiety. In addition, application of strong bases may cause racemization of amino acid residues. On the other hand, *O*-saccharide linkages are labile under acid conditions. In order to stabilize the acetal linkages, *O*- and *N*-acetyl groups should be used for the protection of the glycan portion. Different protecting group strategies are discussed in detail later (see sections *O*-Glycopeptides containing carbohydrate antigens and *N*-Glycopeptides containing carbohydrate antigens).

The syntheses of the building blocks for *O*- and *N*-glycopeptides are fundamentally different.[26] The building blocks for *O*-glycopeptides containing a GalNAc core unit α-*O*-glycosidically linked to a threonine or serine residue are commonly prepared by glycosylation of the corresponding amino acid. More complex glycan structures can be assembled by further glycosylation reactions in order to extend the glycan core. Alternatively, glycosylation of the amino acid can be carried out using the preformed oligosaccharide as a donor. In *N*-glycoproteins, the carbohydrate side-chains are *N*-glycosidically linked via a β-*N*-acetyl-glycosamine glycosidic bond. Therefore, a key step in the synthesis of building blocks for *N*-glycopeptides consists in the condensation of a glycosyl amine and aspartic acid.

The *O*-glycosylation reactions can be performed by using a variety of known glycosyl donors. Commonly employed donors such as glycosyl bromides and chlorides,[27] trichloroacetimidates[28] and thioalkyl or thioaryl glycosides[29] can be activated by silver salts, catalytic amounts of Lewis acid or by soft electrophiles. In addition, 1,2-oxiranes derived from glycal precursors have been successfully applied to glycosylation reactions using activation with $ZnCl_2$.[30] The stereoselectivity of the glycosylation is influenced by the substituent at the C-2 position of the glycosyl donor. Taking advantage of the neighboring group participation induced by acyl functions present in acetyl or benzoyl groups during activation of the anomeric position, stereoselective 1,2-*trans*-, in most cases β-glycosylation is achieved. In contrast, non-participating groups such as benzyl ethers or, in case of GalNAc-derived donors, an azido function give rise to the corresponding α-glycoside, which is favored by the anomeric effect.

Major difficulties appear in glycosylation reactions with sialic acid donors. Although anomeric xanthates,[31] thioalkyl and thioaryl donors,[32] as well as sialosyl phosphites[33] have been shown to be versatile donors in α-2,3- and α-2,6-sialylation reactions, the yields and the stereoselectivity of sialylation reactions are often unsatisfactory. The problems in these sialylation reactions can be attributed to the destabilization of the intermediate glycosidic cation in the neighborhood of the carboxy function and to the lack of a neighboring group at C-3. Furthermore, selective protection of the sialic acid carboxy function is required for the differentiation of this group from the amino acid carboxy functions during glycopeptide synthesis. The formation of an intramolecular lactone found in α-2,3-linked sialic acid derivatives[34] as described later (see sections Glycopeptides containing the α-2,3-sialyl-T antigen and Glycopeptides containing sialyl Lewis x) provides a solution to this problem. Investigations of the stereoselective performance of sialylation reactions have recently been reviewed.[35]

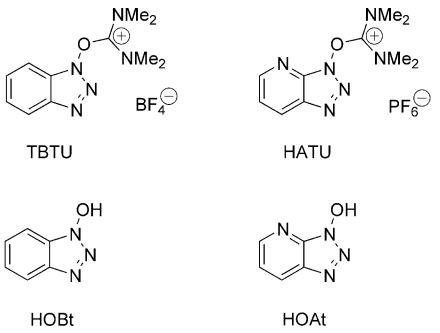


Figure 3. Examples of coupling reagents employed in glycopeptide synthesis.

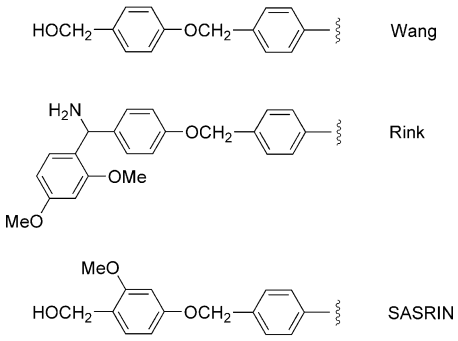


Figure 4. Examples of linkers employed in solid-phase glycopeptide synthesis.

In the solid-phase[36] glycopeptide synthesis according to the Fmoc-strategy, the coupling cycles start with the removal of the Fmoc-protection from the *N*-terminus of the peptide chain. Subsequent coupling reactions are carried out in excess of both amino acid and coupling reagents. In most cases, the condensing reagents form intermediate active esters of the amino acid. Commonly used coupling reagents are TBTU,[37] HATU,[38] DIC/HOBt[39] and HOAt[38] (Fig. 3). In addition, preactivated amino acid esters such as Fmoc-AA-OPfp are commercially available. To avoid side reactions, the amino acid side-chain functionalities are usually blocked by semi-permanent protecting groups. After completion of the

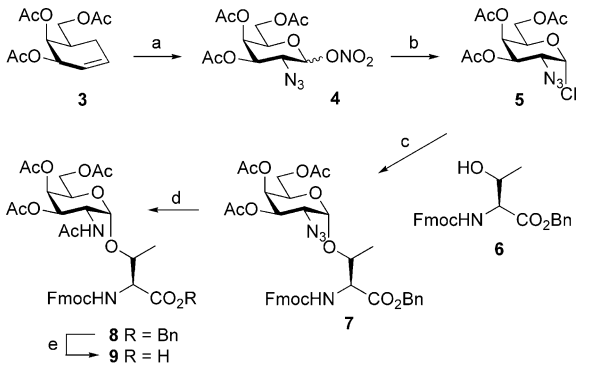
coupling, unreacted amino acids are capped by acetylation in order to prevent the formation of deletion sequences often difficult to separate from the target glycopeptide.

Careful selection of the polymeric support and a linker compatible with the protecting group strategy chosen is necessary for a successful solid-phase glycopeptide synthesis. A variety of resins and linkers are commercially available. Most resins consist of polystyrene-based copolymers with high swelling capacity: either hydrophobic resins such as (aminomethyl)polystyrene, or hydrophilic resins, for example polyethyleneglycol-grafted polymers, such as TentaGel[40] and PEGA.[41] Linkers can be distinguished by their cleavage properties. Acid-labile anchors such as the Wang[42] and the Rink[43] linkers (Fig. 4) are cleaved with 95% TFA. While the glycosidic bonds in the glycopeptide are not effected under these conditions, all acid-labile amino acid side-chain protecting groups can be concomitantly removed. In order to obtain glycopeptide fragments suitable for further manipulations, a super acid-sensitive resin such as SASRIN,[44] which is cleaved by 1% TFA, can be employed. Allyl type linkers are cleavable under almost neutral conditions. They are orthogonally stable to the commonly used acid- and base-labile protecting groups. Details about the different linkers will be given in the context of the corresponding solid-phase syntheses (see sections *O*-Glycopeptides containing carbohydrate antigens and *N*-Glycopeptides containing carbohydrate antigens).

Apart from the chemical methods for glycopeptide synthesis, chemoenzymatic strategies are valuable tools for the preparation of complex glycopeptides. Enzymatic transformations usually proceed regio- and stereoselectively, thus saving complicated protecting group manipulations. However, the application of enzymatic reactions is limited by the substrate selectivity and the availability of the corresponding glycosyltransferases. The *Glycopeptides containing the sialyl-T antigen* section provides an insight into the successful chemoenzymatic synthesis of glycopeptides.

O-Glycopeptides Containing Carbohydrate Antigens Glycopeptides Containing the T_N-Antigen

The T_N-antigen α-*O*-glycosidically linked to serine or threonine is an abundant carbohydrate motif found in natural *O*-glycoproteins. This monosaccharide accumulates on the surface of tumor cells due to incomplete glycan formation in malignant cells. Since the T_N-antigen building blocks are readily accessible by chemical synthesis, a variety of glycopeptides containing the αGalNAc motif have been prepared. In an early study, the synthesis of a tripeptide corresponding to the *N*-terminus of glycophorin A_M bearing two adjacent T_N-antigenic glycosylation sites and its direct conjugation to BSA for the induction of antibodies were described (**1**, Fig. 5).[45] Recently, glycopeptides with clustered arrays of T_N-glycosylated threonine and serine were synthesized and coupled to the carrier proteins KLH and BSA via a mercaptoacetamide spacer (**2**, Fig. 5).[46] Immunological evaluation proved these conjugates to be immunogenic, showing the highest antibody response in mice to clustered T_N-antigens linked to KLH. Vaccines containing this component are currently being tested in patients with prostate cancer.



Scheme 1. (a) CAN, NaN_3, CH_3CN, −15 °C, 75%; (b) Et_4NCl, CH_3CN, 74%; (c) $AgClO_4/Ag_2CO_3$, CH_2Cl_2/toluene, 60%α and 4%β; (d) 1. $NaBH_4/NiCl_2$; 2. Ac_2O, pyridine, 85%; (e) H_2/Pd.

Strategies for the solid-phase glycopeptide synthesis of αGalNAc containing glycopeptides including the corresponding T_N-threonine and T_N-serine building blocks are now well-established. Standard syntheses of these building blocks employ the non-participating azido group for masking the 2-acetamido function of the *N*-acetylgalactosamine.[47] The saccharide is converted into a glycosyl bromide or glycosyl chloride and subjected to a Koenigs-Knorr type α-glycosylation of suitably protected serine or threonine. As an example, the preparation of orthogonally protected T_N-threonine

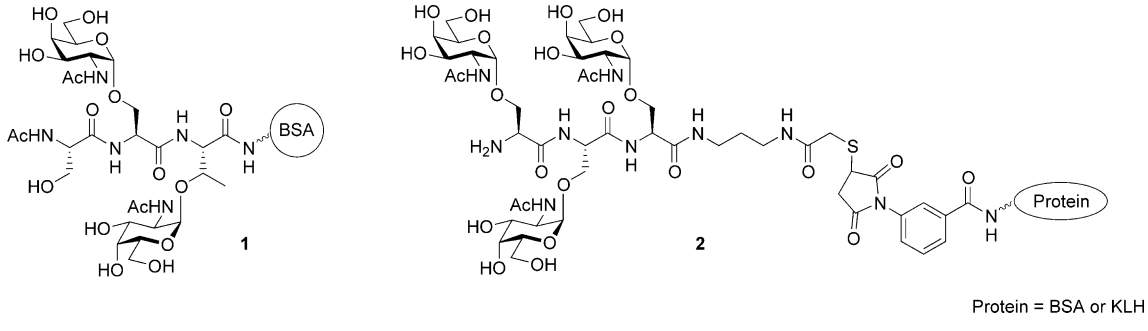
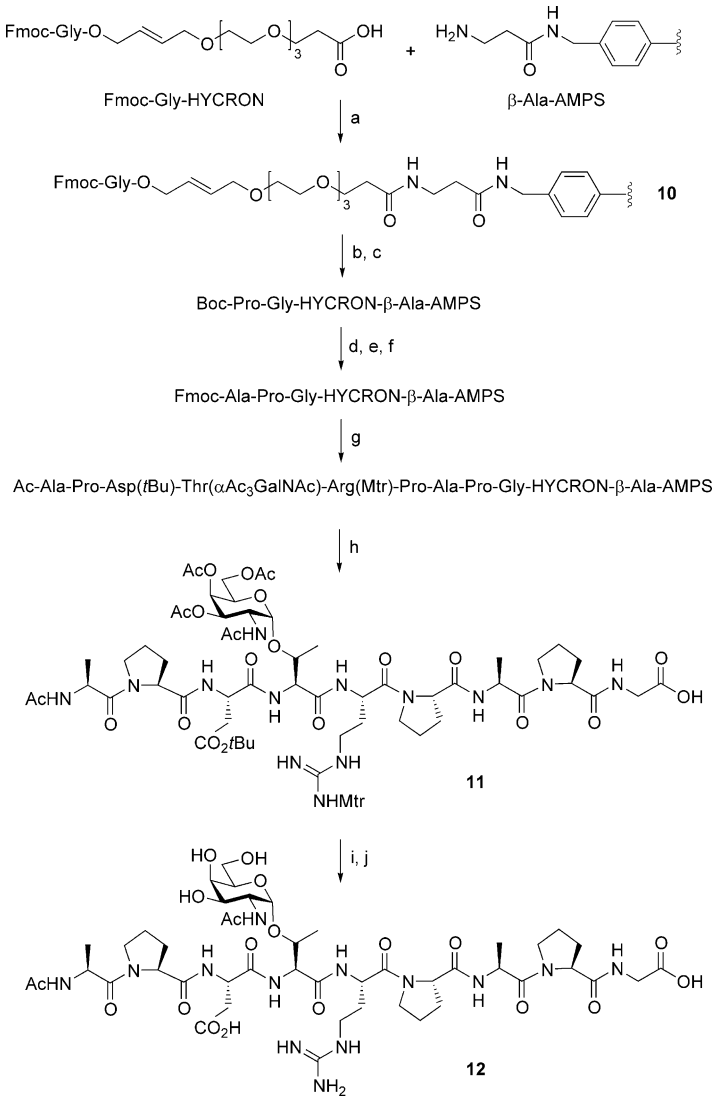


Figure 5. Immunogenic glycopeptides containing clustered T_N-antigens.



Scheme 2. (a) DIC/HOBt, CH_2Cl_2; (b) morpholine/DMF (1/1); (c) Boc-Pro-OH, DIC/HOBt; (d) TFA/CH_2Cl_2 (1/1); (e) iPr$_2$NEt/CH_2Cl_2 (1/10); (f) Fmoc-Ala-OH, DIC/HOBt; (g) SPGS: see text; (h) [Pd(PPh$_3$)$_4$], morpholine, DMF/DMSO (1/1); (i) TFA/MeSEt/PhOMe (40/1/1); (j) NaOMe/MeOH (pH 8.5), 74% overall yield.

building block **9** and its incorporation in the solid-phase synthesis of a glycononapeptide representing the immunodominant region of MUC 1 is described.[48]

In this particular case, *O*-acetyl groups were used for the protection of the carbohydrate, allowing mild deprotection by Zemplén transesterification, which does not cause β-elimination of the carbohydrate portion later in the synthesis. Starting with tri-*O*-acetyl galactal **3**, introduction of the 2-azido group and simultaneous functionalization of the anomeric center were achieved by azidonitration reaction following the procedure of Lemieux and Ratcliffe (Scheme 1).[49] The resulting glycosyl nitrate **4** was converted into the anomeric chloride **5**. Glycosylation of Fmoc-Thr-OBn **6** with donor **5** under in situ anomerization conditions[50] in the presence of $AgClO_4$ and Ag_2CO_3 gave the corresponding glycosyl amino acid derivative **7** in high α-selectivity (60% yield).[45] Reduction of the azido function and subsequent acetylation afforded the protected T_N-threonine building block **8**, which was used in the solid-phase glycopeptide synthesis after hydrogenolytic removal of the benzyl ester (**9**).[51]

The solid-phase synthesis was performed on β-alanyl AMPS resin modified with an allylic HYCRON linker loaded with Fmoc-glycine (**10**, Scheme 2).[48] Allylic anchors can be removed under almost neutral conditions, but they are stable to acids and bases commonly used in peptide chemistry. Hence, it is possible to apply both the Boc- and the Fmoc-group for temporary *N*-terminal protection in the solid-phase peptide synthesis. The HYCRON anchor consists of a hydrophilic triethylene glycol spacer attached to the start amino acid via an allyl type ester, which can be cleaved by irreversible Pd(0)-catalyzed allyl transfer to a scavenger nucleophile such as morpholine, *N*-methylaniline or dimedone.[52]

Extension of the peptide chain was carried out according to the Fmoc-strategy. In every coupling cycle, the *N*-terminal Fmoc-group of the resin-bound peptide was

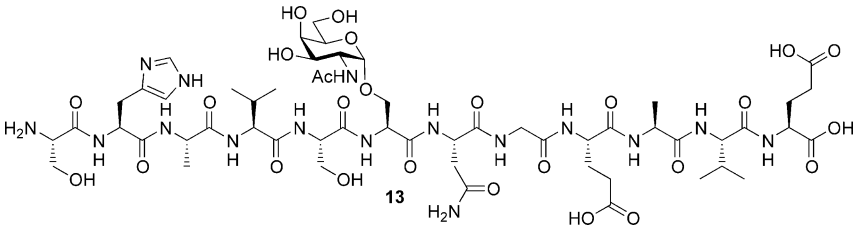


Figure 6. The cadherin-derived glycopeptide **13**.

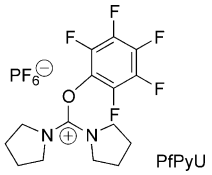


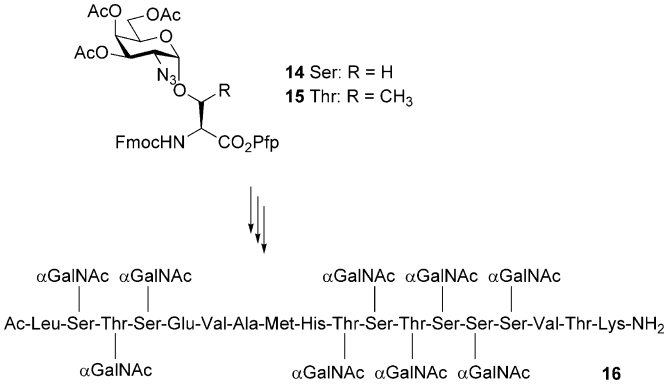
Figure 7. *N*,*N*,*N*′,*N*′-bis(Tetramethylene)-*O*-pentafluorophenyluronium hexafluorophosphate.

removed by treatment with morpholine. The subsequent coupling reaction was performed using excess of Fmoc-amino acid and activation with DIC/HOBt. To monitor the assembly of the peptide backbone analytically, the UV-absorption of the fulvene-morpholine adduct resulting from the Fmoc-deprotection step was recorded. Unreacted amino groups were capped by acetylation after every coupling step. To avoid the formation of diketopiperazines often observed, in particular in a prolyl-glycine *C*-terminal sequence, the second amino acid was introduced as the Boc-proline (Scheme 2). The acidolysis of the Boc-group gave an ammonium salt, which does not undergo undesired intramolecular aminolysis. After this step, the Fmoc-strategy was applied. For the sterically demanding couplings of the *O*-glycosyl threonine and the following aspartic acid building block the more active coupling reagents TBTU/HOBt/NMM were used. The *N*-terminal amino acid alanine was acetylated after Fmoc-removal, and the glycopeptide was detached from the resin (**11**). Acidolytic deprotection of the amino acid side-chains with TFA and removal of the *O*-acetyl groups of the glycan portion by Zemplén transesterification afforded glyconanopeptide **12** in an overall yield of 74%.

The same synthetic strategy was applied to the solid-phase synthesis of an αGalNAc containing glycodecapeptide derived from the loop sequence of the homophilic recognition domain of epithelial cadherin.[53] Cadherins are involved in the regulation of cell adhesion and morphogenesis. They are considered as tumor suppressing molecules, since their down regulation observed in tumor cells leads to the acquisition of invasiveness.[54] The solid-phase synthesis of target molecule **13** (Fig. 6) was accomplished on β-alanyl Tentagel S resin according to the HYCRON methodology described above. Highest yields were obtained using the novel coupling reagent PfPyU[55] (Fig. 7), an *O*-pentafluorophenyluronium salt, which is useful for a mild and efficient carboxylic activation. After solid-phase synthesis and detachment from the resin, the glycododecapeptide was obtained in an overall yield of 55%.

Glycophorin A_N is an important transmembrane sialoglycophorin found in erythrocytes, and is characterized by a large number of glycosylated hydroxy amino acids carrying tetrasaccharides such as αNeuNAc-2,3-βGal-1,3-[αNeuNAc-2,6-]αGalNAc.[56] The αGalNAc motif, representing the first sugar unit of the core structure directly linked to the amino acid chain, decisively influences the conformation of the peptide backbone. Highly glycosylated glycopeptides carrying clustered αGalNAc residues are interesting targets for biological studies.

The solid-phase synthesis of a glycooctadecapeptide corresponding to the *N*-terminal sequence of human glycophorin A_N, carrying three and six adjacent T_N-antigenic glycosylation sites, was carried out in a continuous-flow peptide synthesizer equipped with PEGA resin derivatized with the Rink linker.[57] For the couplings, three equivalents of commercially available Fmoc-amino acid Pfp-esters were used. Dhbt-OH was



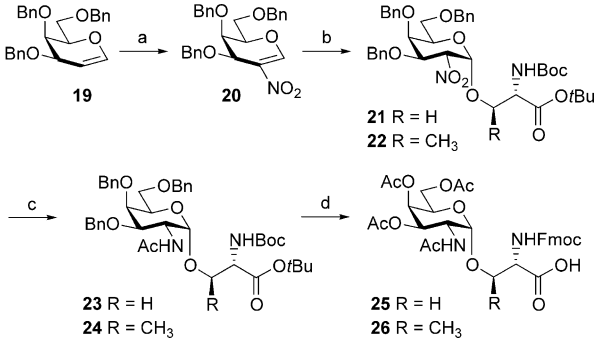
Scheme 3. Solid-phase synthesis of the glycophorin A_N-derived glycopeptide **16**.

Figure 8. Examples of multiple antigenic glycopeptides.

added in order to monitor the reaction by UV-absorption.[58] To introduce the glycosylated amino acid derivatives, 1.5 equivalents of the Fmoc-Ser/Thr-OPfp building blocks **14** and **15** were employed (Scheme 3). After completion, the terminal leucine was *N*-acetylated. The transformation of the azido groups of the glycan moieties into acetamido groups was achieved on the polymeric support using thioacetic acid. Subsequent *O*-deacetylation of the carbohydrate portions as a solid-phase reaction with hydrazine in methanol was followed by acidolytic cleavage of the glycopeptide from the resin and simultaneous deprotection of the amino acid side-chain functions. The target compound **16** was isolated in an overall yield of 51%.

Recently, the immunogenicity of a synthetic multiple antigenic glycopeptide (MAG) displaying four clustered trimeric T_N-epitopes was examined.[59] In contrast to the often used conjugation of the antigen containing glycopeptide to carrier proteins such as BSA or KLH, the antigenic motif was presented via an oligomeric branched lysine core. Active immunotherapy requires both the stimulation of antibodies and a cellular anti-tumor response. Short synthetic glycopeptides bearing multiple T_N-antigens as B-cell epitopes and a poliovirus-derived tridecapeptide $CD4^+$ T-cell epitope were shown to induce T_N-specific antibody responses.[60] The dendrimeric target immunoconjugate **18** (Fig. 8) contains clusters of three consecutive T_N-epitopes linked to the poliovirus T-cell epitope KLFAVWKITYKDT.

Conventional solid-phase synthesis of the peptidic core structure was performed according to the Fmoc-methodology using Wang resin. Assembly of the lysine core was achieved by successive coupling of two levels of Fmoc-Lys(Fmoc)-OH, providing four amino groups readily elongated with the peptide sequence of the poliovirus epitope, respectively.[61] The T_N-threonine building blocks bearing unprotected carbohydrate functions were then coupled as their Pfp-esters to the four branches. Repeated coupling of T_N-threonine followed by attachment of an analogous T_N-serine building block resulted in the multiple antigenic glycopeptide, which was cleaved from the resin after deprotection.



Scheme 4. (a) 1. HNO_3, Ac_2O; 2. NEt_3, CH_2Cl_2; (b) Boc-Ser/Thr-O*t*Bu, KO*t*Bu, toluene, **21**: 80%α and 13%β, **22**: 98%α (based on consumed **20**); (c) Raney-Ni T4-Pt, H_2, EtOH, **23**: 89%, **24**: 84%; (d) 1. Pd/C, H_2, MeOH, AcOH; 2. Ac_2O, pyridine; 3. TFA, CH_2Cl_2; 4. Fmoc-*ON*-Su, $NaHCO_3$, CH_3CN, H_2O.

Conjugate **18** was tested in mice both as a prophylactic and as a therapeutic cancer vaccination, showing high immunogenicity and good protection against the development of T_N-expressing tumor cells. These results correspond well with investigations of therapeutic immunization performed with the analogous MAG **17** (Fig. 8) containing four monomeric T_N-antigens, which increased the survival of tumor-bearing mice.[62]

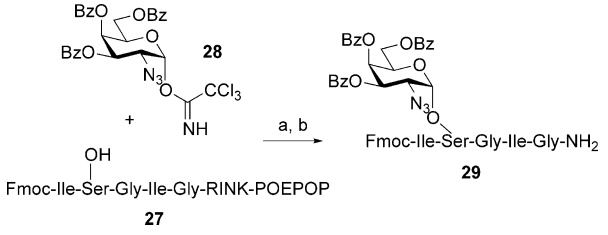
Other strategies for the preparation of αGalNAc serine or threonine building blocks for glycopeptide synthesis have also been reported, employing enzymatic cleavage of protecting groups[63] or different glycosyl donors to react with the amino acid acceptors.[64] A novel methodology was recently developed by Winterfeld et al.[65] The 2-nitrogalactal **20**, available by addition of acetyl nitrate to tri-*O*-benzyl-galactal **19** followed by elimination of acetic acid, was subjected to a Michael addition of Boc-protected serine and threonine derivatives in the presence of catalytic amounts of KO*t*Bu to give the α-glycosides **21** and **22** in high yield (Scheme 4). Stereoselective α-glycosylation was achieved using galactose derivatives with sterically demanding *tert*-butyldiphenylsilyl protection at 6-OH.[66] Reduction of the nitro group with platinized Raney-Ni/H_2 and subsequent acetylation

yielded the corresponding 2-acetamido derivatives **23** and **24**. Further protecting group manipulations provided the glycosylated Fmoc-serine and Fmoc-threonine building blocks **25** and **26**.[67]

A different synthetic strategy for the solid-phase glycopeptide synthesis consists in the solid-phase glycosylation of peptides with appropriate carbohydrate donors. A successful α-glycosylation of the serine side-chain of pentapeptide **27** attached to a POEPOP[68] copolymer using excess of tri-*O*-benzoylated 2-azidogalactosyl trichloroacetimidate **28** has been reported (Scheme 5).[69] After cleavage from the resin, the protected glycopentapeptide **29** was obtained in 78% yield. This methodology is considered promising for the creation of glycopeptide libraries containing a variety of saccharide antigens.

Glycopeptides Containing the T-Antigen

The T-antigen, the βGal-1,3-T_N-antigen, is one of the mucin core structures, and is exposed on the surface of cancer cells. In 1982, Paulsen and Hölck reported the synthesis of T-antigen linked to serine or threonine by galactosylation of a T_N-antigen precursor.[50b]

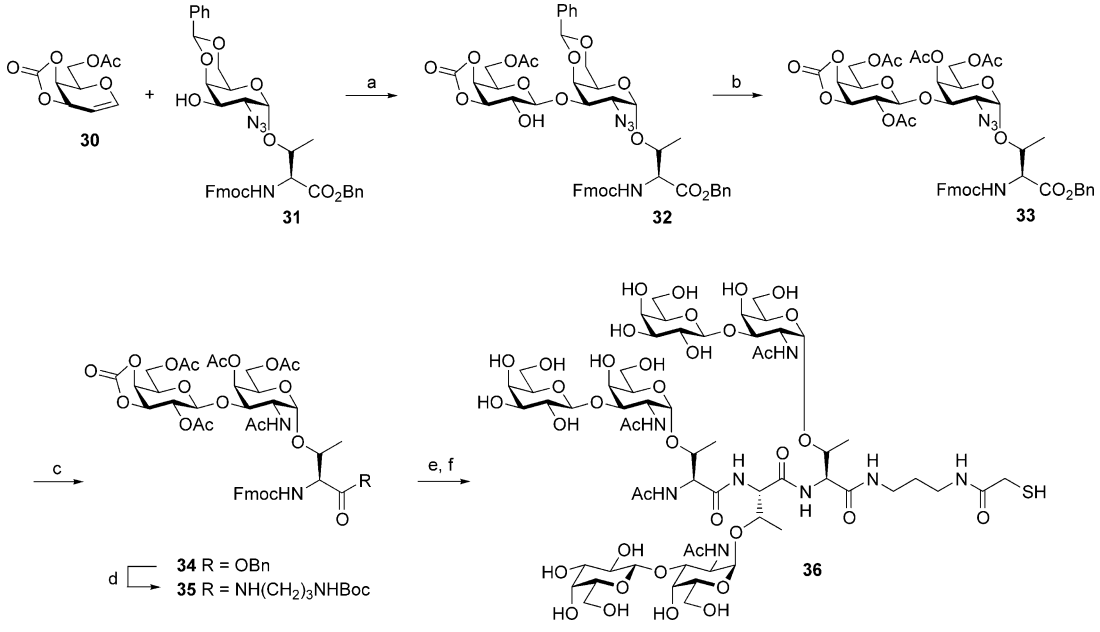


Scheme 5. (a) TMSOTf, CH_2Cl_2; (b) TFA/H_2O, HPLC, 78% (two steps).

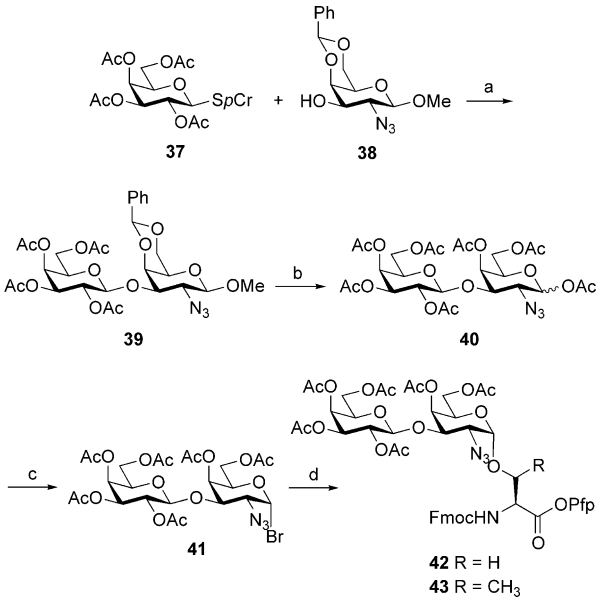
For the generation of synthetic glycopeptide-based vaccines, a trimeric cluster of the T-antigen was prepared.[46] The key step was the glycosylation of the suitably protected T_N-threonine derivative **31** with the epoxide derived from galactal **30**, activated with $ZnCl_2$, yielding 97% of the β-linked disaccharide **32** (Scheme 6). The benzylidene protection in **32** was exchanged for acetyl groups (**33**), and T-threonine building block **34** was obtained after reductive acetylation of the azido function with thioacetic acid. For a conjugation with carriers, a Boc-protected diamine linker was attached to the *C*-terminus after hydrogenolytic cleavage of the benzyl ester (**35**).

The glycopeptide was prepared by three subsequent coupling cycles, including removal of the *N*-terminal Fmoc-protection with KF in the presence of 18-crown-6 and HOAt/HATU promoted acylation. After assembly of the glycotripeptide, the Boc-group was removed by acidolysis, and the linker was converted into a thioglycolic amide. Zemplén transesterification of the *O*-acetyl groups of the glycan portions gave target compound **36** (Scheme 6), ready for covalent attachment to carrier proteins for immunological evaluation.

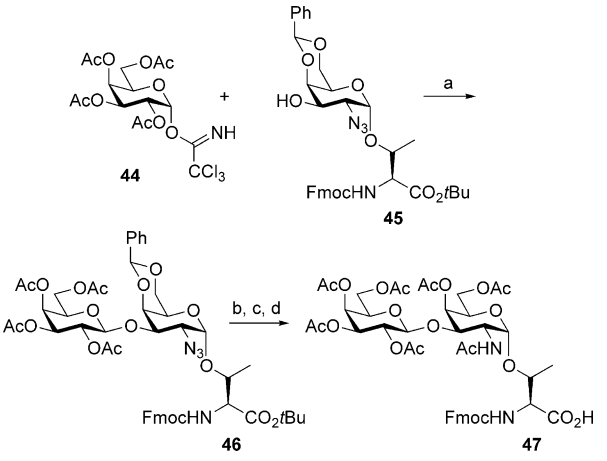
The solid-phase syntheses of a set of human salivary mucin (MUC 7) derived glycopeptides displaying up to three subsequent T-antigens have also been reported.[70] MUC 7 was found to be important in the maintenance of oral health by exhibiting multiple host-defense functions.[71] In order to study the influence of the *O*-glycosylation pattern on the biological activity of human salivary mucin, two different undecapeptide sequences from the tandem repeat of the secreted MUC 7 bearing subsequent glycosylation sites were selected.



Scheme 6. (a) 1. DMDO, CH_2Cl_2, 0 °C; 2. $ZnCl_2$, THF, −78 °C to rt, 97%; (b) 1. 80% AcOH, 70 °C; 2. Ac_2O, DMAP, TEA, CH_2Cl_2, 93%; (c) AcSH, 87%; (d) 1. Pd/C, H_2; 2. $NH_2(CH_2)_3NHBoc$, HOAt, HATU, collidine, DMF, 84%; (e) glycopeptide synthesis: see text; (f) 1. TFA, CH_2Cl_2; 2. SAMA-OPfp, *i*Pr_2NEt, CH_2Cl_2; 3. NaOMe, MeOH, 60%.



Scheme 7. (a) NIS, TfOH, CH_2Cl_2, CH_3CN, −45 °C, 98%; (b) 1. 80% AcOH, 94%; 2. Ac_2O, H_2SO_4, −20 °C; (c) $TiBr_4$, CH_2Cl_2, EtOAc, 78%; (d) Fmoc-Ser/Thr-OPfp, $AgClO_4$, CH_2Cl_2, toluene, −40 °C, **42**: 51%, **43**: 68%.



Scheme 8. (a) TMSOTf, 4Å MS, $ClCH_2CH_2Cl$, 0 °C, 68%; (b) 80% AcOH, 80 °C, 87%; (c) 1. Zn, Ac_2O, AcOH, THF; 2. Ac_2O, pyridine, 82%; (d) 95% TFA, 97%.

The solid-phase glycopeptide synthesis was performed using the building block approach. Glycosylated T-serine and T-threonine building blocks were synthesized by glycosylation of the suitably protected amino acids with a disaccharide donor under Koenigs-Knorr conditions.[72] The disaccharide was assembled in high yield by reacting glycosyl acceptor **38** with thioglycoside **37** using NIS and TfOH as promoters (Scheme 7). Acidolytic cleavage of the benzylidene protecting group in **39** was followed by simultaneous acetylation of the hydroxy functions and exchange of the anomeric methoxy group for an acetoxy function (**40**). Subsequent treatment of **40** with $TiBr_4$ provided disaccharide donor **41**. Glycosylation of Fmoc-Ser-OPfp was carried out at −40 °C in the presence of $AgClO_4$ to give an anomeric mixture (α/β 5/1) of compound **42**. The desired α-glycosylated building block **42** was isolated in 51% yield. Similar reaction of disaccharide bromide **41** with Fmoc-Thr-OPfp afforded predominantly α-glycosylated threonine building block **43** in 68% yield.

MUC 7:

Pro-Ala-Pro-Pro-Ser-Ser-Ser-Ala-Pro-Pro-Glu

Pro-Ala-Pro-Pro-Ser-Ser-Ser*-Ala-Pro-Pro-Glu

Pro-Ala-Pro-Pro-Ser-Ser*-Ser*-Ala-Pro-Pro-Glu

Pro-Ala-Pro-Pro-Ser*-Ser*-Ser*-Ala-Pro-Pro-Glu

Ala-Pro-Pro-Glu-Thr-Thr-Ala-Ala-Pro-Pro-Thr

Ala-Pro-Pro-Glu-Thr-Thr*-Ala-Ala-Pro-Pro-Thr

Ala-Pro-Pro-Glu-Thr*-Thr*-Ala-Ala-Pro-Pro-Thr

Figure 9. * Denotes βGal-1,3-αGalNAc-1,*O*-Ser/Thr.

The solid-phase synthesis of the two peptide sequences PAPPSSSAPPE and APPETTAAPPT containing different numbers of T-antigen (Fig. 9) was performed according to the standard Fmoc-protocol on a Wang resin. Coupling of the glycosylated building blocks was carried out in the presence of HOBt/DIPEA. After completion of the peptide chain, reductive *N*-acetylation of the azido group of the glycan portion with thioacetic acid was carried out on the polymer-bound glycopeptide. Removal of the Fmoc-group and acidolytic cleavage of the protected glycopeptide from the resin was followed by *O*-deacetylation of the glycan functions. The target glycopeptides were analyzed by circular dichroism spectroscopy, revealing that the carbohydrate moieties attached to the surface of the peptide polyproline helical backbone are readily accessible for various biological functions.

In order to study the biosynthesis of the oligosaccharide side-chains of human intestinal mucins, a library of MUC 2 and MUC 3 derived glycodecapeptides carrying saccharide core structures at different positions of the peptide chain has been prepared by multiple column solid-phase synthesis.[73] T_N-threonine and T-threonine building blocks were incorporated in the peptide chain.

The key step in the preparation of the T-threonine building block was the glycosylation of suitably protected glycosyl amino acid **45** with trichloroacetimidate **44** catalyzed by TMSOTf (**46**, Scheme 8). Acidolytic cleavage of the benzylidene acetal was followed by conversion of the azido group into an acetamido function and concomitant *O*-acetylation, giving the desired *core-1* building block **47** after acidolytic removal of the *tert*-butyl group.

The solid-phase synthesis was performed in a parallel fashion using a 20-column multiple synthesizer and a Wang resin. Following the standard Fmoc-procedure, the glycosylated building blocks were activated with TBTU, and the non-glycosylated amino acids were introduced as their Pfp-esters in the presence of Dhbt-OH

MUC 2:

Thr-Thr-Thr-Val-Thr-Pro-Thr-Pro-Thr*-Gly

Thr-Thr-Thr-Val-Thr-Pro-Thr*-Pro-Thr-Gly

Thr-Thr-Thr-Val-Thr*-Pro-Thr-Pro-Thr-Gly

Thr-Thr-Thr*-Val-Thr-Pro-Thr-Pro-Thr-Gly

Thr-Thr*-Thr-Val-Thr-Pro-Thr-Pro-Thr-Gly

Thr-Thr-Thr*-Val-Thr-Pro-Thr*-Pro-Thr-Gly

MUC 3:

Thr-Glu-Thr-Thr-Ser-His-Ser-Thr*-Pro-Gly

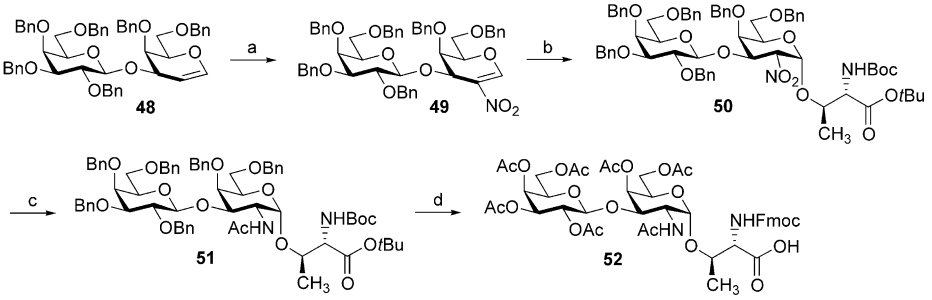
Thr-Glu-Thr-Thr*-Ser-His-Ser-Thr-Pro-Gly

Thr-Glu-Thr*-Thr-Ser-His-Ser-Thr-Pro-Gly

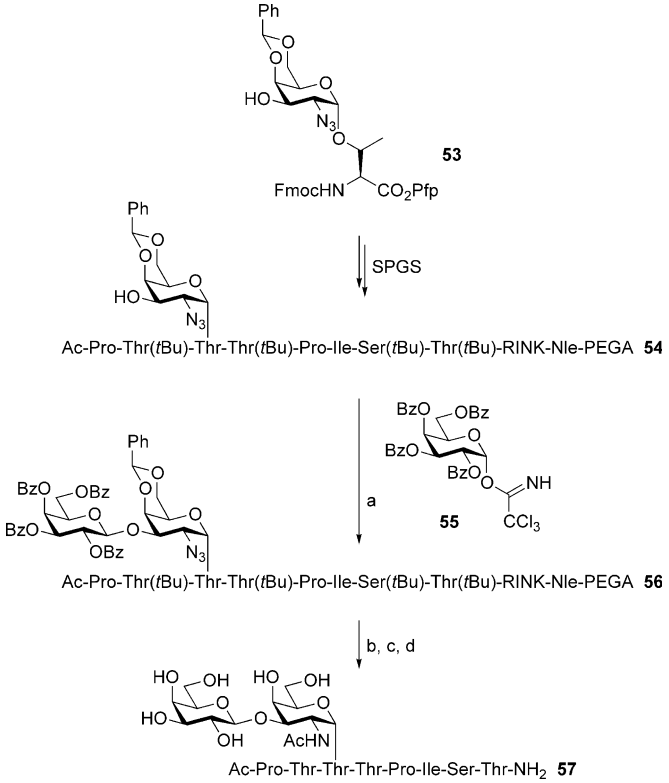
Figure 10. * Denotes βGal-1,3-αGalNAc-1,*O*-Ser/Thr.

using *tert*-butyl or Boc-protection for the amino acid side-chains. After completion of the peptide chains, the glycopeptides were cleaved from the resin by acidolysis. The *O*-acetyl groups were removed under Zemplén conditions. Figure 10 shows an excerpt from the glycodecapeptide library obtained in this way.

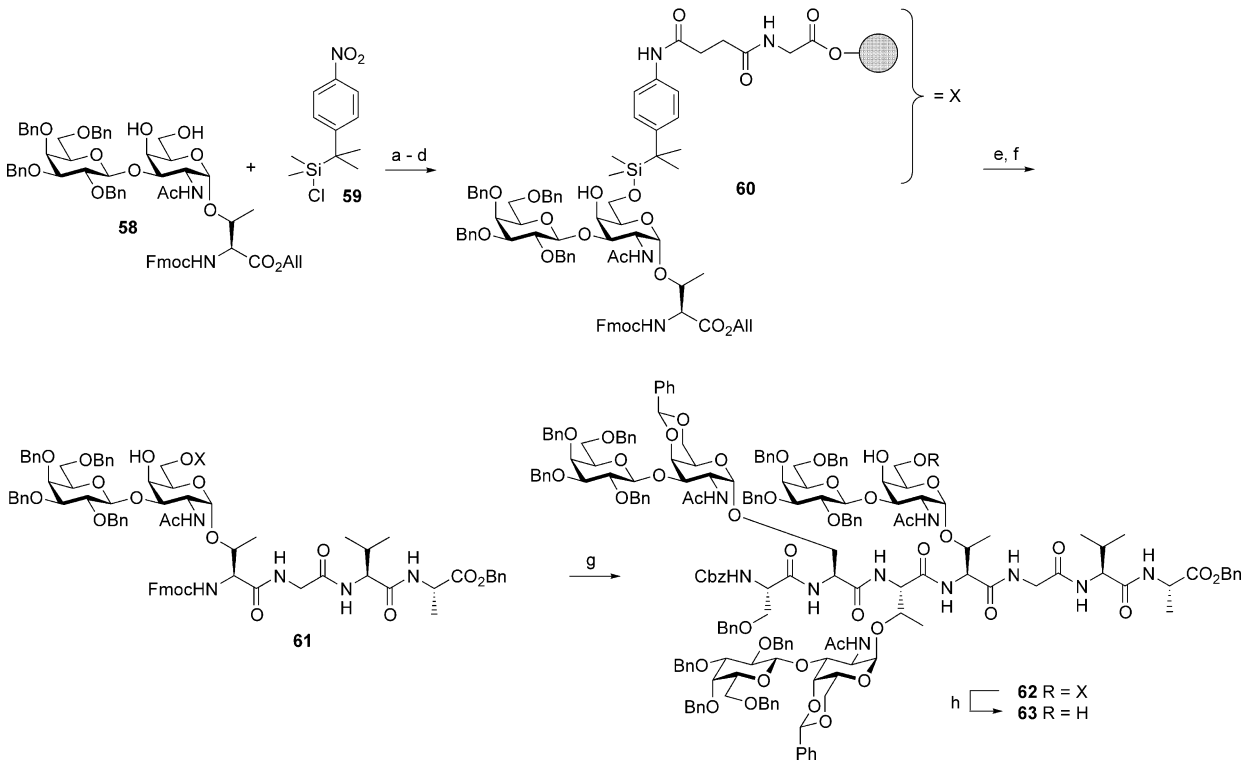
As in case of the T_N-antigen synthesis, the galactosyl-galactal **48** can be converted into a suitable Michael acceptor by nitration with acetyl nitrate to give **49** (Scheme 9).[66] Addition of Boc-Thr-O*t*Bu to the Michael acceptor **49** catalyzed by KO*t*Bu stereoselectively afforded the α-*O*-linked glycoside **50** in 93% yield. Conversion of the nitro into an acetamido group was carried out by hydrogenolytic reduction with Raney nickel followed by acetylation. Exchange of the protecting groups of **51** finally yielded the T-antigen building block **52**, which had been successfully employed for solid-phase glycopeptide synthesis in earlier work.[64,74]



Scheme 9. (a) 1. HNO_3, Ac_2O; 2. Et_3N, CH_2Cl_2, 88%; (b) Boc-Thr-O*t*Bu, KO*t*Bu, toluene, 93%; (c) 1. Raney-Ni T4-Pt, H_2, EtOH; 2. Ac_2O, pyridine, 81%; (d) 1. Pd/C, H_2, MeOH, AcOH; 2. Ac_2O, pyridine; 3. TFA, CH_2Cl_2; 4. Fmoc-*ON*-Su, $NaHCO_3$, CH_3CN, H_2O, 85%.



Scheme 10. (a) TMSOTf, 4Å MS, CH_2Cl_2, −30 °C; (b) 95% TFA, 67% (two steps); (c) Zn, Ac_2O, AcOH, THF, 75%; (d) NaOMe, MeOH, 82%.



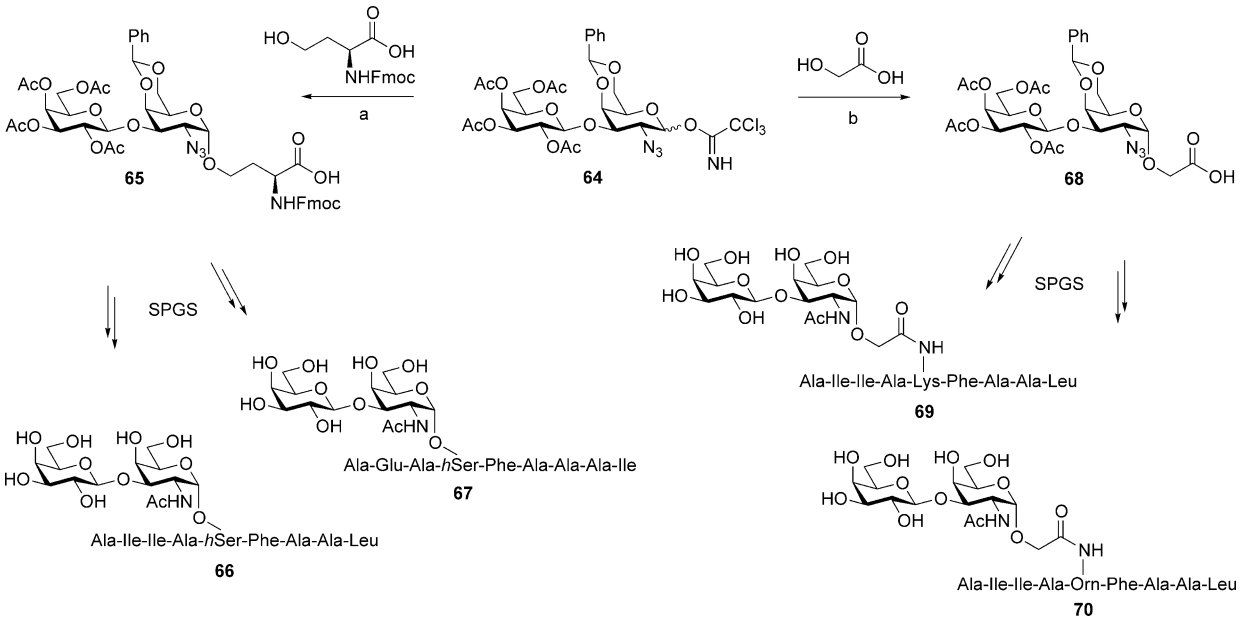
Scheme 11. (a) imidazole, THF, 0 °C, 86%; (b) Zn, AcOH, THF, 81%; (c) succinic anhydride, CH_2Cl_2, 90%; (d) H-Gly-HMP-resin, HBTU, HOBt, iPr$_2$NEt, NMP; (e) [Pd(PPh)$_4$], dimedone, THF; (f) H-Gly-Val-Ala-OBn, HBTU, HOBt, iPr$_2$NEt, NMP; (g) SPGS: see text; (h) TBAF, AcOH, THF, 55% overall yield.

Glycosylation of glycopeptides attached to the solid support, in order to extend the saccharide chain, proved to be useful for the preparation of a partial sequence of MUC 2 carrying the T-antigen.[75] The type of resin used in solid-phase glycosylation reactions has an important influence on the reactivity and the stereoselectivity of the products. In this case, PEGA resin equipped with a Rink linker served as the solid support. For the construction of the solid-phase bound glycopeptide acceptor **54** (Scheme 10), Fmoc-methodology was used. The coupling reactions were performed with Pfp- or Dhbt-esters. Glycosylated threonine building block **53** was incorporated in the peptide chain as a suitably protected glycosyl acceptor bearing a free 3-hydroxy group. Best results in the solid-phase glycosylation reaction were obtained using trichloroacetimidate **55** as a glycosyl donor. The glycosylation was performed in CH_2Cl_2 at −30 °C with eight equivalents of the glycosyl donor and TMSOTf as a catalyst. The reaction proceeded stereoselectively providing the desired β-glycosylated product **56** in a yield of 67%. Acidolytic detachment from the resin resulted in concomitant removal of the *tert*-butyl side-chain protecting groups and cleavage of the benzylidene group of the saccharide residue. Transformation of the azido group into an acetamido function and transesterification of the benzoyl groups gave target compound **57**.

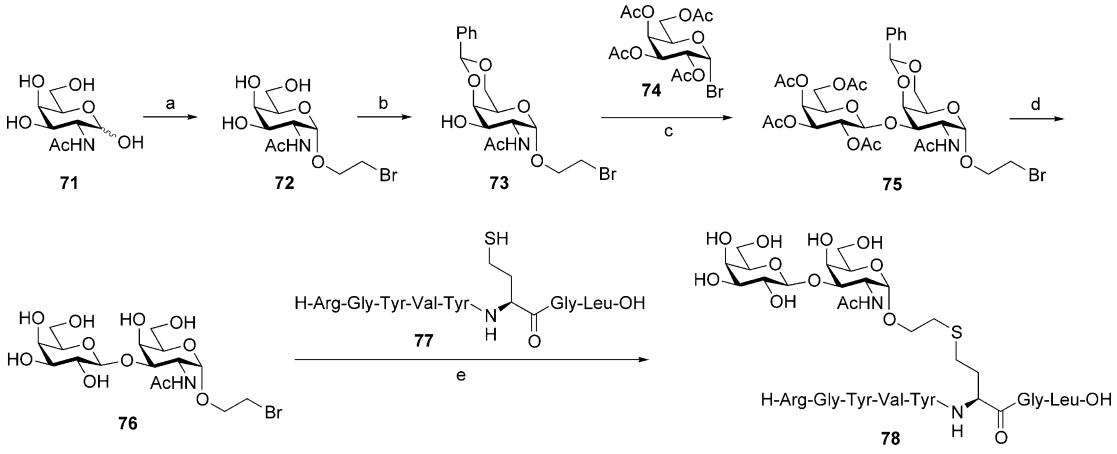
Nakahara et al.[76] developed a novel silyl-ether type linker for solid-phase glycopeptide synthesis, which allows the attachment of an alcoholic function of the carbohydrates to the solid support. Employing this method, peptide coupling reactions at both the *N*- and *C*-termini of the resin-bound glycopeptide fragments can be performed. The silyl linker is cleaved by fluoridolysis, thus releasing the synthesized glycopeptide from the resin in a protected form. The silyl ether concept is compatible with the Fmoc-strategy commonly used for the assembly of glycopeptides on the solid support. The *N*-terminal asialo glycoheptapeptide fragment of glycophorin A_M carrying three subsequent *core-1* structures was synthesized using this methodology.[77]

In the course of this synthesis, silylation of suitably protected glycosylated threonine building block **58** was carried out at the primary hydroxy function of the carbohydrate portion with silicon derivative **59** in the presence of imidazole in THF (Scheme 11). Reduction of the nitro function gave the aniline derivative, which was reacted with succinic anhydride. The product of this procedure was activated with HBTU/HOBt in NMP and coupled to glycine preloaded Wang resin to give **60**. Extension of the *C*-terminal peptide chain was achieved by Pd(0)-catalyzed cleavage of the allyl ester and subsequent fragment condensation with a tripeptide to yield **61**. Stepwise extension of the peptide chain at the *N*-terminus was performed according to the Fmoc-strategy. Subsequent coupling of a T-antigen threonine building block, a T-antigen serine building block and a serine derivative provided target glycoheptapeptide **62**. After detachment from the resin with TBAF/AcOH in THF, the protected glycopeptide **63** was isolated in 55% overall yield.

Both the nature of the peptide and the glycan portion in immunogenic glycopeptides are important for an



Scheme 12. (a) TMSOTf, CH_2Cl_2/THF (3/1), −50 °C, 66%; (b) TMSOTf, CH_2Cl_2/THF (2/1), −50 °C, 77%.



Scheme 13. (a) $BrCH_2CH_2OH$, HCl(g), 55–60 °C, 52%; (b) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, 98%; (c) AgOTf, CH_2Cl_2/toluene (1/1), −25 °C, 50%; (d) 1. 80% aq TFA, 0 °C, 70%; 2. NaOMe, MeOH, 84%; (e) Cs_2CO_3, DMF, 58%.

immune response. In order to study the carbohydrate-specific T-cell response against the tumor-associated T-antigen, neoglycopeptides have been synthesized bearing the T-antigenic carbohydrate motif not directly attached to peptide sequences, which are known to bind to class I MHC molecules. These neoglycopeptides have been constructed by three different strategies.

In the first approach, the glycopeptides were assembled using a T-homoserine building block instead of the regularly employed serine analogue.[78] The building block was prepared by glycosylation of Fmoc-protected homoserine with trichloroacetimidate **64**, affording an anomeric mixture (α/β 20/1) of T-homoserine derivative **65**, from which the α-product was isolated in 66% yield (Scheme 12). The building block **65** was employed for the solid-phase synthesis of two different polyalanine peptide sequences with high binding affinity to the murine class I MHC, resulting in neoglycopeptides **66** and **67** carrying the T-antigen attached at a distance increased by one carbon atom compared to the serine analogue.

The second strategy involved glycosylation of glycolic acid using the same disaccharide donor **64** providing the desired α-product **68** stereoselectively in 77% yield (Scheme 12).[78] To synthesize the target neoglycopeptides, direct acylation was carried out on the corresponding resin-bound Fmoc-protected peptides bearing an unprotected lysine or ornithine residue. The two neoglycopeptides obtained by this procedure contained glycans attached to the peptide chain via an amide bond. The solid-phase glycopeptide synthesis was performed on $PEGA_{800}$ resin modified with a base-labile HMBA-linker following an Fmoc-protocol using Boc-protection of the lysine and ornithine side-chains. The attachment of the T-antigen precursor **68** to the peptide chain on the solid support was mediated by TBTU/NEM and resulted in the formation of a glycan portion linked to the peptide chain via eight or nine spacer

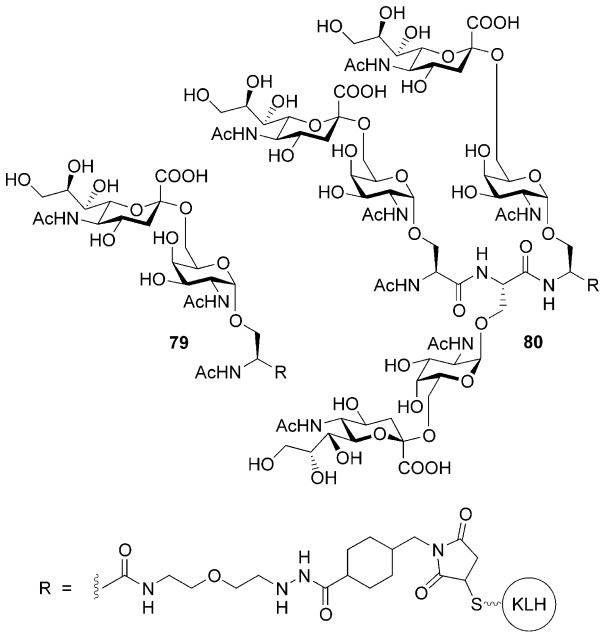


Figure 11. Sialyl-T_N antigen containing glycopeptides used for immunization studies.

atoms in the lysine- and ornithine-derived neoglycopeptides, **69** and **70**, respectively.

In a different approach, a 2-bromoethyl T-antigen glycoside was used to alkylate a homocysteine residue incorporated in a peptide from vesicular stomatitis virus nucleocapsid protein exhibiting class I MHC restricted binding affinity.[79] The synthesis of the T-antigen glycoside started with the Fischer glycosylation of *N*-acetylgalactosamine **71** with 2-bromoethanol at 60 °C. In spite of the participating acetamido group, the desired α-glycoside **72** was formed in 52% yield (Scheme 13). After protection of the 4- and 6-hydroxy groups as benzylidene acetal **73**, stereoselective glycosylation of the 3-hydroxy function with peracetylated galactosyl bromide **74** under Koenigs-Knorr conditions provided β-linked disaccharide **75** in 50% yield. Acidolysis of the benzylidene group and removal of the *O*-acetyl protection by transesterification afforded completely unprotected T-antigen analogue **76**. The octapeptide **77** bearing a homocysteine residue was prepared by standard Fmoc solid-phase synthesis. Alkylation of the peptide sulfhydryl group with neoglycoside **76** was achieved with Cs_2CO_3 as the base in 58% yield, directly furnishing the desired unprotected neoglyopeptide **78** in which the T-antigen is attached to the peptide chain via a spacer.

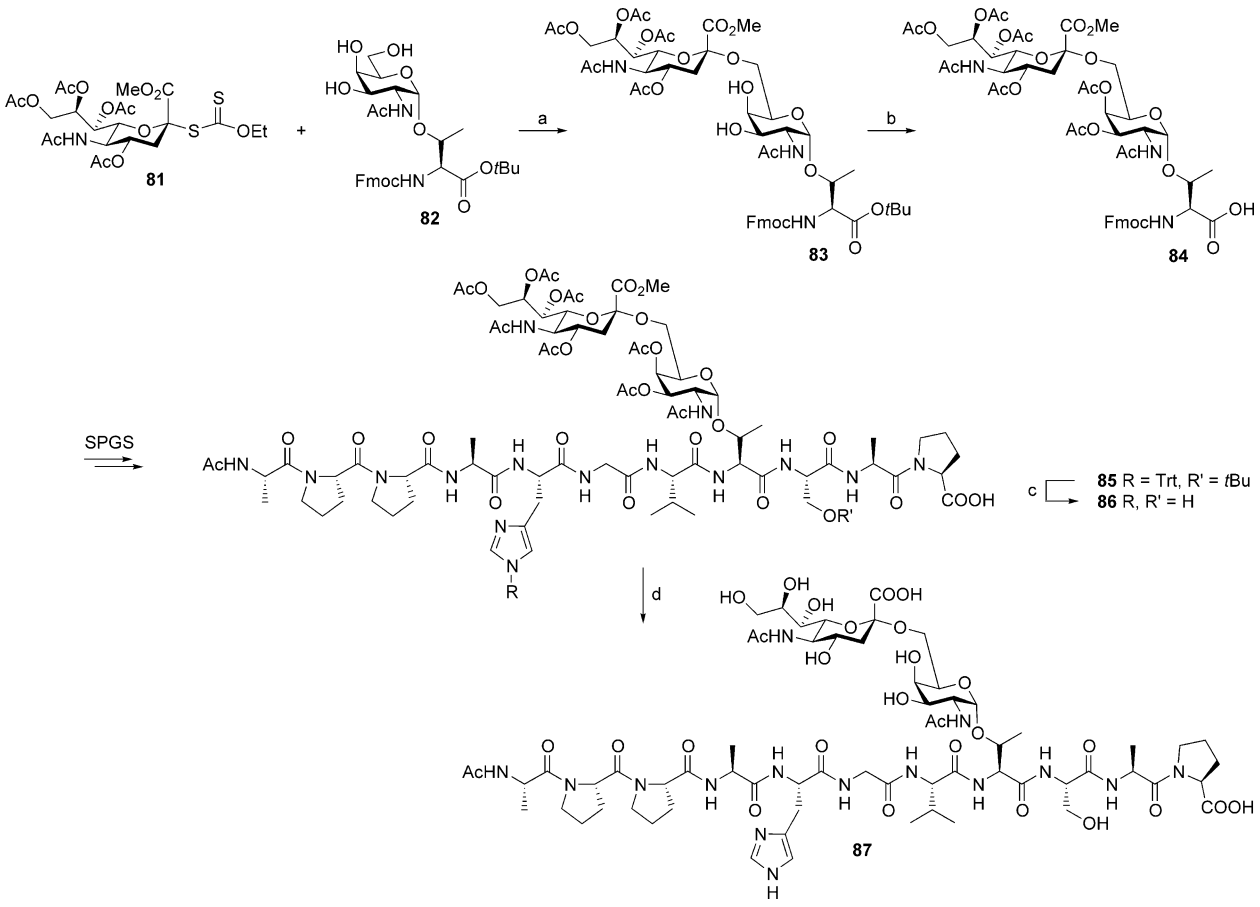
Glycopeptides Containing the Sialyl-T_N Antigen

The sialyl-T_N epitope is a mucin-associated disaccharide antigen, *O*-linked to serine or threonine of mucins expressed on a variety of epithelial cancer cells.[80] It occurs in clustered and unclustered forms.[81] Both active and passive immunotherapy studies have identified the sialyl-T_N antigen as a uniquely effective target for antibody-mediated cancer immunotherapy.[82] Monomeric and trimeric sialyl-T_N serine glycopeptides were synthesized and conjugated with thiolated KLH via a spacer (Fig. 11).[83] This study showed that mice immunized with these conjugates produced IgM and IgG antibodies reacting strongly with ovine submaxillary mucin (OSM) and with LS-C colon cancer cells. The clustered sialyl-T_N antigen KLH conjugate **80** induced a higher titer of antibodies against the natural sialyl-T_N epitope targets OSM and LS-C than its monomeric analogue **79**.[84]

The first solid-phase synthesis of a tumor-associated sialyl-T_N antigen glycopeptide was reported in 1997.[85] The target glycoundecapeptide was derived from the repeating unit of polymorphic epithelial mucin MUC 1[86] carrying a sialyl-T_N threonine epitope. The solid-phase glycopeptide synthesis was performed according to the Fmoc-protocol by applying the building block strategy.

An Fmoc-protected sialyl-T_N threonine building block was required, in which the carboxy protection of the sialic acid is orthogonally stable to the protecting groups used for the peptide functionalities. The methyl ester was chosen as the protecting group, as it is removable from the glycopeptide without epimerization of amino acids or β-elimination of the glycan, provided that optimized reaction conditions are applied. The key step of the building block synthesis was the regio- and stereoselective sialylation of Fmoc-Thr(αGalNAc)-O*t*Bu **82**, which was achieved with sialyl xanthogenate **81**[31] and MeSOTf[87] as a promoter (Scheme 14). Low temperature and intermediate acetonitrile coordination favored the formation of the α-sialoside. Besides the desired α-2,6-*O*-linked sialyl-T_N derivative **83** and its β-anomer (α/β 4/1, 32% yield of pure α-anomer after preparative RP-HPLC), the glycal of sialic acid was formed as the only byproduct. After acetylation of the 3-OH and 4-OH positions and acidolysis of the *tert*-butyl ester, the sialyl-T_N threonine building block **84** was isolated. Its *O*-acetyl protection of the hydroxy functions in the carbohydrate portion ensured sufficient acid-stability in the subsequent solid-phase synthesis.

For the solid-phase synthesis of the glycoundecapeptide, AMPS resin and the allylic HYCRON anchor loaded with Fmoc-proline were employed.[48] Following a typical Fmoc-protocol, the coupling reactions were carried out with *N*-protected amino acids promoted by TBTU, HOBt and NMM. To prevent diketopiperazine formation, the first coupling resulting in the polymer-linked dipeptide was performed with Boc-alanine. Further chain extensions were accomplished using Fmoc-amino acids. The reaction time extended from 4.5 to 15 h for the coupling of the sialyl-T_N unit **84**, whereas 19 h were required for the final two couplings. After the last coupling reaction, the terminal Fmoc-group was exchanged for an acetyl group. The sialyl-T_N undecapeptide **85** was detached from the resin by Pd(0)-catalyzed cleavage of the allylic anchor in 98% yield. Removal of the acid-labile side-chain protecting groups gave glycopeptide **86** in 42% overall yield (after preparative RP-HPLC). Deprotection of the glycan portion was carried out by hydrolysis with NaOH first in methanol then in water. The *O*-acetyl groups were cleaved off at pH 10–11 followed by hydrolysis of the methyl ester in the *N*-acetyl-



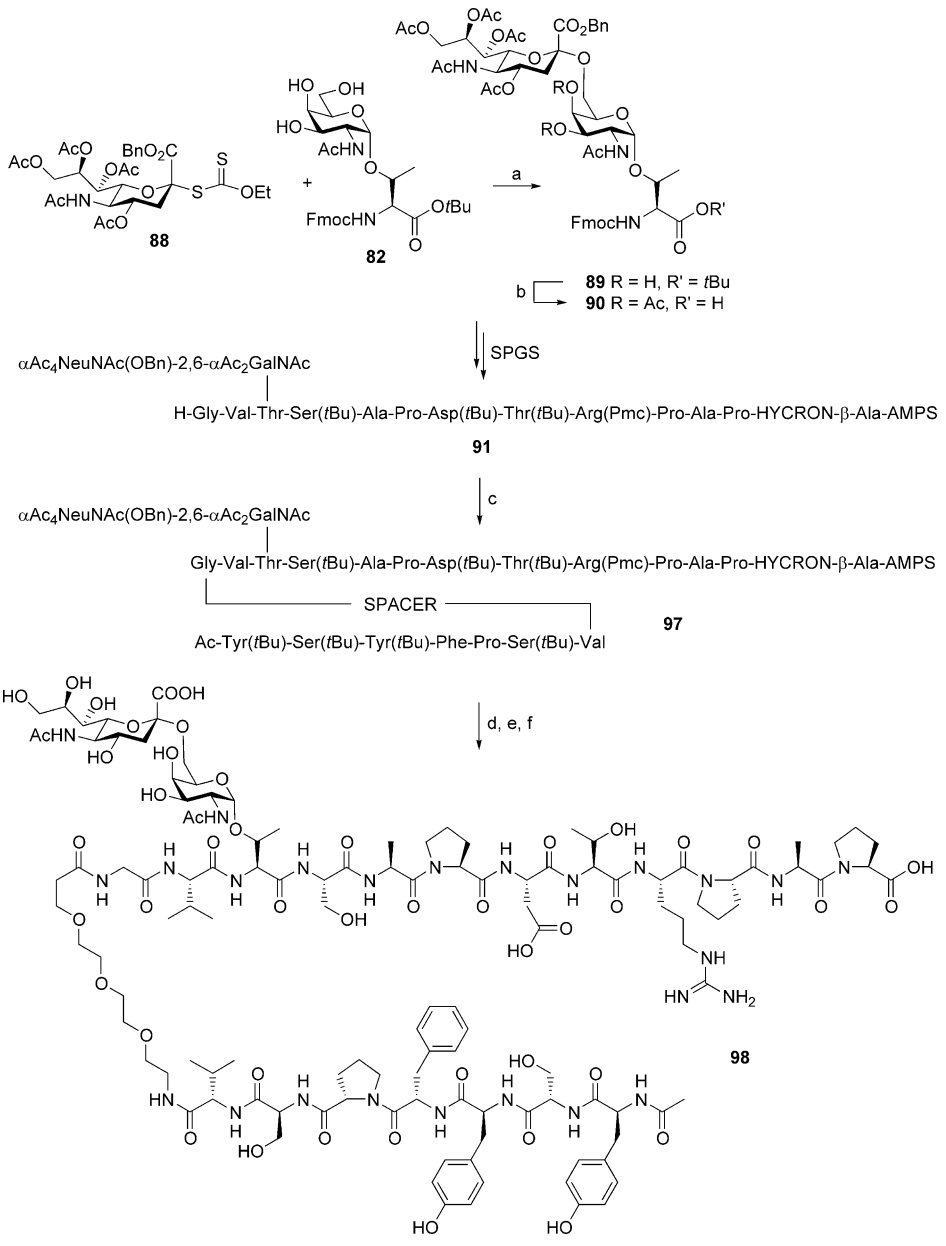
Scheme 14. (a) MeSBr, AgOTf, CH_3CN, CH_2Cl_2, −62 °C, RP-HPLC, 32%α and 8%β; (b) 1. Ac_2O, pyridine, 88%; 2. TFA/anisole (13/1), quant.; (c) TFA, anisole, EtSMe, 42% (yield based on Fmoc-Pro-HYCRON-β-Ala-AMPS); (d) 1. NaOH, MeOH; 2. aq NaOH, 76%.

neuraminic acid portion at pH 11.5. Under these conditions almost no β-elimination of the carbohydrate was detected, and the completely deblocked sialyl-T_N antigen sequence **87** of the tandem repeat of MUC 1 was obtained in 76% yield over the two steps.

Recently, the preparation of a synthetic vaccination conjugate consisting of a tumor-associated MUC 1 glycopeptide bearing the sialyl-T_N antigen, combined with a tetanus toxin epitope via a flexible spacer, has been reported.[88] This conjugate represents a new development in the construction of antitumor vaccines, as it contains both a T-cell epitope and a B-cell epitope in which the peptide motif PDTRPAP of the tandem repeat of MUC 1 is incorporated as the immunodominant domain.[89] The key step in the synthesis of the immunoconjugate was the solid-phase fragment condensation of the T-cell spacer portion and the glycosylated MUC 1 B-cell epitope.

The solid-phase synthesis of the fully protected sialyl-T_N containing MUC 1 glycododecapeptide was carried out by applying the Fmoc-strategy as described above.[85] The sialyl-T_N precursor **89** was synthesized in 60% yield from T_N-threonine derivative **82** and neuraminic acid donor **88**, in which the carboxy function was protected as a benzyl ester (Scheme 15).[90] After *O*-acetylation of **89** and removal of the *tert*-butyl ester, the sialyl-T_N building block **90** was incorporated in the solid-phase synthesis of the B-cell epitope. Starting with Fmoc-proline linked to β-alanyl AMPS resin via the HYCRON anchor,[48] a change to the Boc-strategy was made for the second amino acid alanine in order to avoid the formation of diketopiperazine after *N*-deprotection. The remainder of the synthesis of the resin-bound sialyl-T_N glycododecapeptide **91** was performed according to the Fmoc-strategy.

Parallel to this assembly, the synthesis of a tetanus toxin epitope conjugated with a spacer was carried out. To this end, the precursor **92**[91] was converted into the corresponding amino spacer derivative **93** (Scheme 16). This product was condensed with Fmoc-valine (**94**) and, after cleavage of the *tert*-butyl ester, coupled to the HYCRON linker and attached to the polymer (**95**). The tetanus toxin peptide spacer conjugate was then prepared according to the Fmoc-strategy. To avoid the formation of diketopiperazine, a dipeptide was coupled in the first chain extension step. Finally, the terminal Fmoc-group was exchanged for an acetyl group, and the protected T-cell epitope spacer conjugate **96** was obtained in 44% overall yield by Pd(0)-catalyzed cleavage of the allylic anchor. After activation of its carboxy group with HATU/HOAt, the fragment **96** was coupled to the solid-phase bound B-cell epitope **91** to give conjugate **97** in 42% yield (Scheme 15). Cleavage of

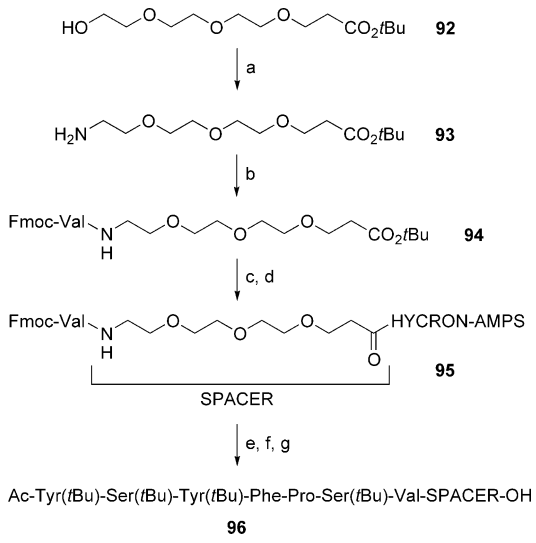


Scheme 15. (a) MeSBr, AgOTf, CH_3CN/CH_2Cl_2 (2/1), −62 °C, 60%; (b) 1. Ac_2O, pyridine, 0 °C, 84%; 2. TFA/CH_2Cl_2 (1/1), anisole, quant.; (c) **96**, HOAt/HATU/NMM, DMF, 42%; (d) Ac_2O/pyridine (1/3); (e) $[Pd(PPh_3)_4]$, morpholine, DMF/DMSO (1/1), 20% overall yield (based on Fmoc-Pro-HYCRON-β-Ala-AMPS); (f) 1. Pd/C, H_2, MeOH; 2. CH_2Cl_2/TFA/thioanisole/ethanedithiol (10/10/1/1); 3. NaOMe, MeOH, pH 8.5, 47% (three steps).

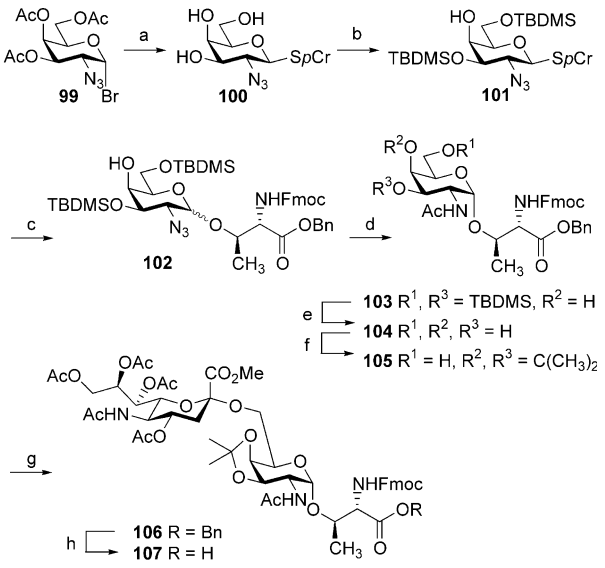
the HYCRON anchor gave the corresponding protected glycopeptide derivative in 20% yield (based on the loading with the start amino acid proline). The pure immunoconjugate **98** was isolated after hydrogenolysis of the sialic acid benzyl ester, acidolysis of the side-chain protecting groups and mild methanolysis of the *O*-acetyl groups in 47% yield.

For immunological evaluation, the proliferation of cytotoxic T-cells was examined after stimulation with the synthetic conjugate **98**. For comparison, the sialyl-T_N carrying MUC 1 glycododecapeptide, the corresponding MUC 1 dodecapeptide, the sialyl-T_N threonine building block and the tetanus toxin spacer conjugate were administered to human lymphocytes. Characterization of the proliferating lymphocytes by antigen-specific monoclonal antibodies in a FACS analysis showed that the conjugate of tumor-associated MUC 1 glycopeptide and T-cell epitope **98** induced proliferation of cytotoxic $CD8^+$ T-cells, while its partial structures only stimulated proliferation of $CD3^+$ T-cells. These results are promising for the development of antitumor vaccines based on synthetic glycopeptide antigens combined with T-cell epitopes.

A different strategy for the production of a sialyl-T_N building block for solid-phase synthesis was reported by Elofsson et al.[92] A T_N-building block carrying sterically demanding TBDMS-groups in the GalNAc-moiety was prepared and further converted into a sialyl-T_N building



Scheme 16. (a) 1. MesCl, NEt_3, CH_2Cl_2; 2. NaN_3, DMF, 60 °C; 3. Raney-Ni, H_2, *i*PrOH, 73%; (b) Fmoc-Val-OH, EEDQ, CH_2Cl_2, 70%; (c) 1. TFA; 2. Br-HYCRON-O*t*Bu, Bu_4NBr, $NaHCO_3$, CH_2Cl_2, 75%; (d) 1. TFA, 95%; 2. H-AMPS, TBTU, HOBt, NMM, DMF, 74%; (e) SPPS: see text; f) 1. morpholine/DMF; 2. Ac_2O/pyridine; (g) $[Pd(PPh_3)_4]$, morpholine, DMF/DMSO (1/1), 44% based on **95**.



Scheme 17. (a) *p*-Thiocresol, NaOH, $CHCl_3$, EtOH, 68%; (b) TBDMSCl, imidazole, DMF, 99%; (c) Fmoc-Thr-OBn, NBS, QOTf, CH_2Cl_2, −28 °C, 71%; (d) AcSH, pyridine, 67%α and 13%β; (e) AcOH, MeOH, THF, 55–66 °C; (f) dimethoxypropane, *p*-TsOH, Et_3N, MeOH, H_2O, reflux, 85%; (g) **81**, MeSBr, AgOTf, 3Å MS, CH_2Cl_2, CH_3CN, −78 °C, 49%; (h) H_2, Pd/C, EtOAc, 88%.

block, which was employed in the solid-phase synthesis of fragments from HIV gp 120 following the Fmoc-protocol.

T_N- and sialyl-T_N epitopes were discovered on the envelope glycoprotein gp 120 of the human immunodeficiency virus (HIV),[93] which is one of the most heavily glycosylated proteins known to date. While most of the viral glycans are *N*-linked and generally not immunogenic, cross-reactivity experiments support the hypothesis that a small number of *O*-linked T_N- and sialyl-T_N epitopes on gp 120 may be potential targets for immune intervention.[94]

The construction of the sialyl-T_N building block for solid-phase glycopeptide synthesis was based on a 2-azidogalactosyl thioglycoside donor as a key compound, in order to enable incorporation of silyl protecting groups for the hydroxy functions of the T_N-antigen. Azidogalactosyl bromide **99** was, therefore, converted into the corresponding *p*-cresylthio glycoside **100**[95] by treatment with *p*-thiocresol and NaOH (Scheme 17). Protection of the triol **100** using TBDMSCl afforded the desired 3,6-disilylated glycosyl donor **101**. Due to steric hindrance, protection of the axial 4-OH in **101** was not necessary. Subsequent glycosylation of Fmoc-Thr-OBn was achieved by activation with NBS and QOTf[96] and provided T_N-threonine derivative **102** as an inseparable mixture of α- and β-anomers (α/β 5.2:1, 71% yield). Reductive acetylation of the azido group in **102** with thioacetic acid furnished α-glycoside **103** in 67% yield (after HPLC). After removal of the TBDMS-groups, triol **104** could not be directly subjected to regioselective sialylation at 6-OH due to its low solubility in organic solvents. Instead, it was converted into the 3,4-isopropylidene protected acceptor **105** and then sialylated with xanthogenate **81**, promoted by MeSBr/AgOTf, to give the α-glycoside **106** in 49% yield. Subsequent hydrogenolysis of the benzyl ester furnished sialyl-T_N building block **107**.

This building block was incorporated into the solid-phase synthesis of glycopeptide **108** (Fig. 12), consisting of amino acids 312–327 from HIV gp 120 with a cysteine residue added at the *C*-terminus in order to allow conjugation to carrier proteins. The glycopeptide was assembled in a peptide synthesizer using a PEG-grafted polystyrene resin derivatized with an acid labile linker. The coupling reactions were performed with HOBt/DIC as reagents. One equivalent of the sialyl-T_N derivative **107** was coupled manually to the peptide

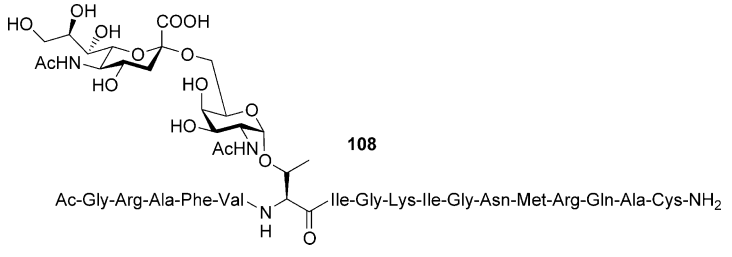
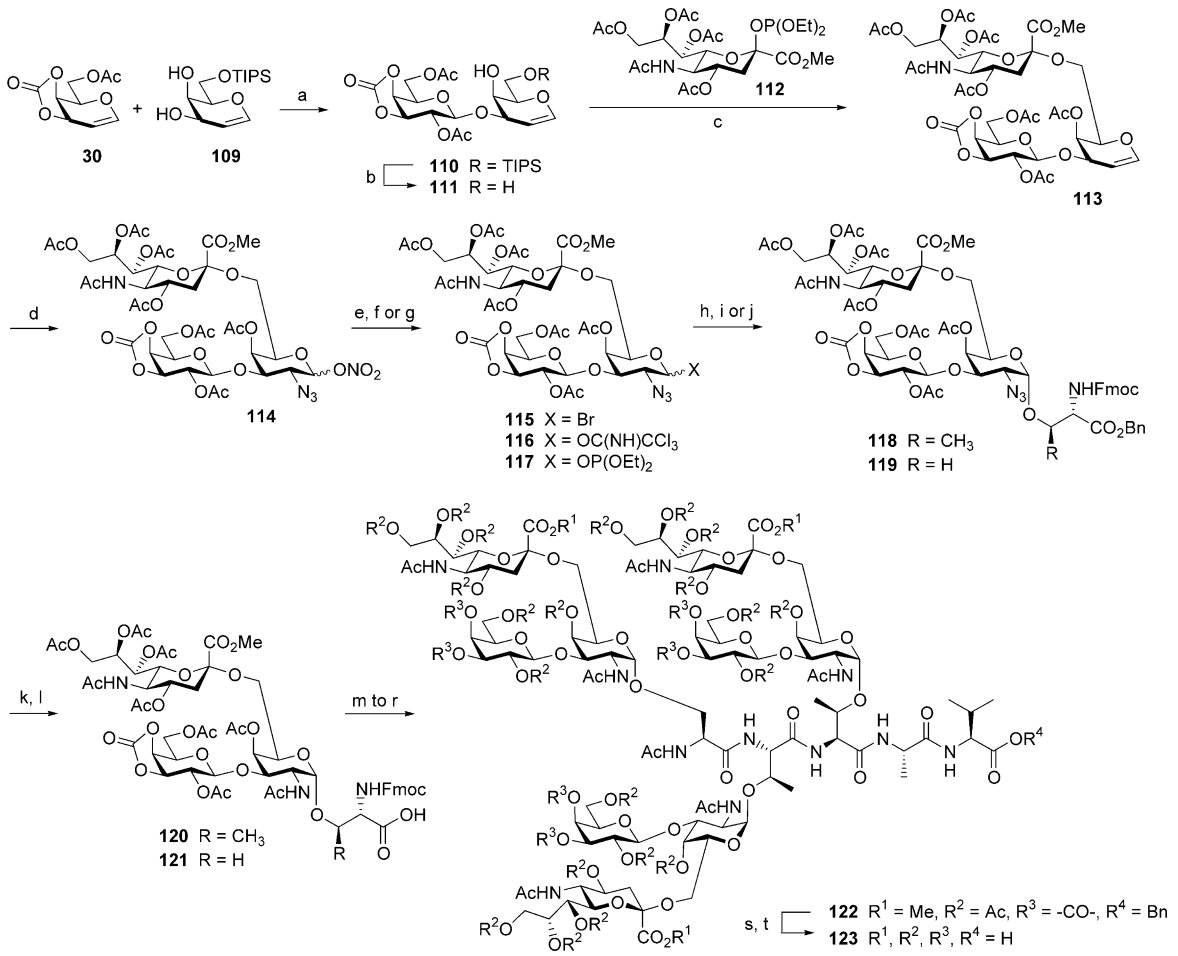


Figure 12. The HIV-derived glycopeptide **108**.



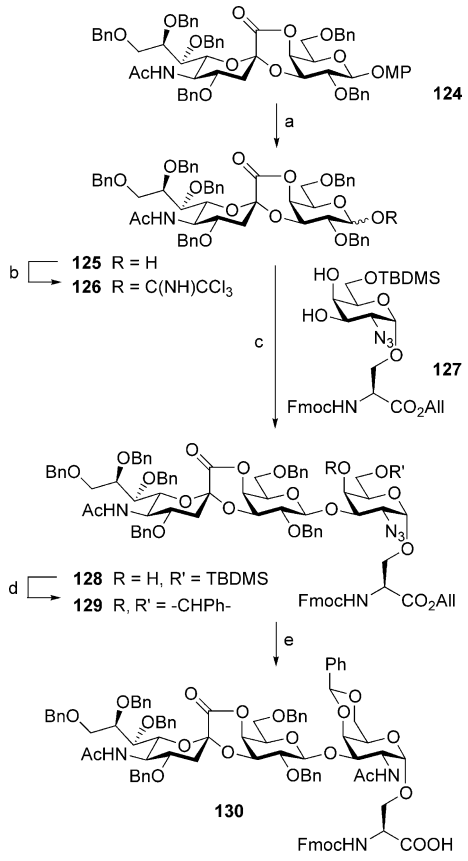
Scheme 18. (a) 1. DMDO, CH_2Cl_2; 2. $ZnCl_2$, −78 to 0 °C; 3. Ac_2O, Et_3N, DMAP, 75%; (b) TBAF, AcOH, THF, 80%; (c) TMSOTf, THF/toluene (1/1), −60 to −45 °C, 84%, α/β 4/1; (d) NaN_3, CAN, CH_3CN, −15 °C, 60%; (e) LiBr, CH_3CN, 75%, α only; (f) 1. PhSH, *i*Pr_2NEt, CH_3CN, 82%; 2. CCl_3CN, K_2CO_3, CH_2Cl_2, 80%; (g) 1. PhSH, *i*Pr_2NEt; 2. $ClP(OEt)_2$, *i*Pr_2NEt, THF, 72%; (h) **115**, Fmoc-Thr/Ser-OBn, $AgClO_4$, CH_2Cl_2, **118**: 74%, α only, **119**: 70%, α/β 2.6/1; (i) **116**, Fmoc-Thr/Ser-OBn, BF_3-OEt_2, THF, −30 °C, **118**: 60%, α only, **119**: 66%, α/β 4/1; or **116β**, Fmoc-Thr/Ser-OBn, BF_3-OEt_2, THF, −30 °C, **118**: 63%, α only, **119**: 65%, α/β 12/1; (j) **117**, Fmoc-Thr/Ser-OBn, BF_3-OEt_2, THF, −30 °C, **119**: 30%, α/β 30/1; (k) AcSH, 78%; (l) H_2, Pd/C, MeOH, H_2O, quant.; (m) **120**, H_2N-Ala-Val-OBn, IIDQ, CH_2Cl_2, 85%; (n) KF, DMF, 18-crown-6, 95%; (o) **120**, IIDQ, 87%; (p) KF, DMF, 18-crown-6, 93%; (q) **121**, IIDQ, 90%; (r) 1. KF, DMF, 18-crown-6; 2. Ac_2O, CH_2Cl_2; (s) H_2, Pd/C, MeOH/H_2O (15/1); (t) aq NaOH, MeOH, pH 10–10.5, 80%.

resin via its azabenzotriazolyl ester. After completion of the solid-phase synthesis, the resin was treated with TFA to cleave the glycopeptide from the solid support, to remove the protecting groups from the amino acid side-chains and to hydrolyze the isopropylidene group of the GalNAc-unit. Finally, the *O*-acetyl groups and the methyl ester were cleaved by careful treatment with NaOMe/MeOH and aqueous NaOH, respectively. The desired glycopeptide **108** was obtained in 12% overall yield. Due to the *C*-terminal cysteine residue, about 1% of the corresponding disulfide derivative was also formed.

Glycopeptides Containing the Sialyl-T Antigen

A significant part of the humoral antitumor response of cancer patients is directed towards the epithelial cell membrane carbohydrate recognition motifs, such as those expressed on mucins. In mammalian tissues, these glycoproteins are glycosylated with the *core-1* structure (Fig. 2). The T-antigen attached to a threonine or a serine of the peptide sequence is further elongated with lactosamine units in normal tissue. In malignant epithelial tumors, the down-regulation of *N*-acetylglucosamine transferase has been observed, and instead of extension, the T-antigen is directly terminated by sialylation at the 6-position of the galactosamine residue to give the α-2,6-sialyl-T antigen, or at the 3-position of the galactose residue, resulting in the α-2,3-sialyl-T antigen (Fig. 1).[97] These structures, which are not observed in normal tissues, represent potential targets for glycopeptide vaccines, which are expected to be effective tools in cancer treatment.

Glycopeptides containing the α-2,6-sialyl-T antigen. The α-2,6-sialyl-T antigen was found to be selectively expressed on myelogenous leukemia cells.[10] An amino terminal pentapeptide fragment of mucin CD 43[98] containing these clustered carbohydrate epitopes was therefore prepared for the purpose of developing anticancer vaccines.[99] The synthetic approach in this case involved the construction of the complete glycodomain in the first stage, followed by convergent glycosylation of the amino acids threonine and serine, and subsequent incorporation of the glycosyl amino acid units into a peptide chain.



Scheme 19. (a) CAN in toluene/CH_3CN/H_2O, 77%; (b) CCl_3CN, DBU, 97%, α/β 3/1; (c) BF_3-OEt_2 in toluene/CH_2Cl_2, −15 to −5 °C, 53%β and 16%α; (d) 1. aq TFA in CH_2Cl_2, 83%; 2. $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, 95%; (e) 1. AcSH, pyridine; 2. $[Pd(PPh_3)_4]$-MeNHPh in THF, 70%.

The α-2,6-sialyl-T building block was synthesized by the glycal assembly strategy. Oxidation of readily available glycal **30** with DMDO and subsequent reaction of the resultant epoxide with 6-*O*-TIPS-galactal **109** promoted by $ZnCl_2$ gave the disaccharide **110** after acetylation in 75% yield (Scheme 18). After removal of the TIPS-group (**111**), the sialylation was carried out using diethyl phosphite **112** and TMSOTf as the activating agent. Trisaccharide **113** was obtained in 84% yield as a separable mixture of anomers (α/β 4/1). Azidonitration of **113** resulted in formation of the latent galactosamine subunit **114**, which could be converted into a variety of trisaccharide donors such as bromide **115**, trichloroacetimidate **116** and diethyl phosphite **117**. The glycosylation of Fmoc-Thr-OBn with bromide **115**, activated by AgOTf, and with trichloroacetimidate **116**, promoted by BF_3-OEt_2, exclusively afforded the desired α-*O*-linked product **118** in 74% and 60–63% yield, respectively. When Fmoc-Ser-OBn was used as the acceptor, the glycosylation did not proceed with complete stereocontrol. The highest stereoselectivity (α/β 30/1) was observed with diethyl phosphite **117** in the presence of BF_3-OEt_2, but the desired product **119** was isolated in only 30% yield. Bromide **115** and trichloroacetimidate **116** afforded higher yields (up to 70%), although the stereoselectivity was poor (**115**: α/β 2.6/1, **116**: α/β 4/1). Using the pure β-anomer of trichloroacetimidate **116** instead of an anomeric mixture, the formation of the α-*O*-linked product **119** was increased to a ratio of α/β 12/1. Reduction of the azido group in **118** and **119** with thioacetic acid, followed by hydrogenolytic removal of the benzyl ester, furnished the α-2,6-sialyl-T threonine and serine building blocks **120** and **121**, respectively.

The synthesis of the glycopeptide backbone was initiated by coupling the trisaccharide building block **120** to Ala-Val-OBn using activation with IIDQ. Iterative peptide coupling steps between the released *N*-terminus of the peptide and the protected glycosyl amino acids **120** and **121** gave the desired glycopentapeptide **122** in an average yield of 85% for each coupling step. Final Fmoc-deprotection was achieved by treatment with KF in the presence of 18-crown-6 followed by acetylation of the *N*-terminus of the glycopeptide. The completely deblocked target compound **123** was obtained by hydrogenolysis of the benzyl ester and saponification of the three methyl esters, the cyclic carbonates and the *O*-acetyl groups of the glycan portion with aqueous NaOH in 80% yield.

Glycopeptides containing the α-2,3-sialyl-T antigen. The first solid-phase synthesis of a glycopeptide containing the α-2,3-sialyl-T antigen was reported in 1997.[100] An α-2,3-sialyl-T serine building block was employed for the synthesis of the B-chain of human α2HS-glycoprotein, a cysteine-containing heptacosaglycopeptide. α2HS-Glycoprotein is a human plasma globulin involved in a number of significant biological functions, such as bone mineralization, endocytosis and opsonization.[101] It is composed of two polypeptide subunits; the minor subunit, the B-chain, has been characterized as a heptacosapeptide carrying the α-2,3-sialyl-T antigen.[102]

The key step in the synthesis of the perbenzylated α-2,3-sialyl-T serine building block was the glycosylation of a 2-azidogalactosyl serine derivative with a sialylated galactose unit (Scheme 19). Starting with disaccharide **124**,[103] the anomeric 4-methoxyphenyl group was removed by oxidative cleavage with CAN to afford the hemiacetal **125**, which was converted into the corresponding trichloroacetimidate donor **126** (α/β 3/1). Glycosylation of acceptor **127** with trichloroacetimidate **126** was promoted by BF_3-OEt_2 to furnish the desired β-glycoside **128** in 53% yield. In addition, 16% of the corresponding α-isomer was formed. Acidolytic desilylation of **128** and introduction of a benzylidene acetal yielded **129**, which was converted into the α-2,3-sialyl-T serine building block **130** by reductive *N*-acetylation of the azido function with thioacetic acid and Pd(0)-catalyzed cleavage of the allyl ester.

The solid-phase glycopeptide synthesis was performed according to the Fmoc-strategy using HMP-resin. The coupling reactions were conducted in a peptide synthesizer by activation with DCC/HOBt in NMP. After assembly of the henicosapeptide containing the amino acid residues 7–27, the activated α-2,3-sialyl-T serine building block **130** was manually attached to the solid-phase bound peptide. The linear peptide chain was completed by automated coupling of the five terminal

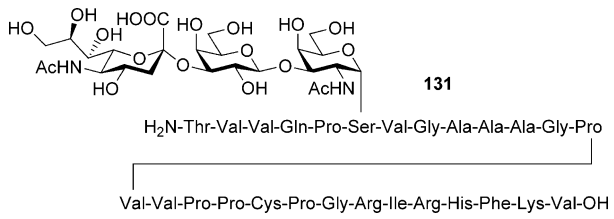


Figure 13. The α2HS-glycoprotein derived glycopeptide **131**.

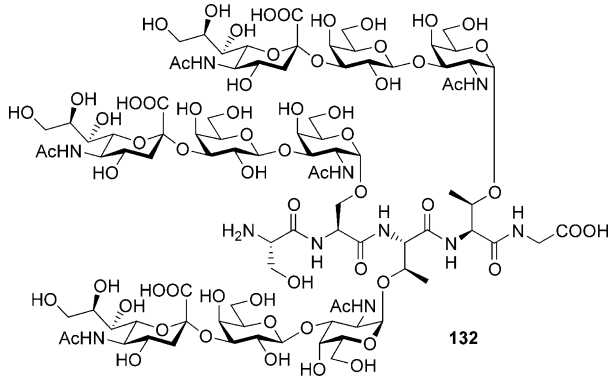


Figure 14. The glycophorin A_M-derived glycopeptide **132**.

amino acids. The Fmoc-group was removed from the glycopeptide, which was then cleaved from the resin with TFA. The hexa-*O*-benzylated glycopeptide was isolated in 52% yield, together with its penta- and tetrabenzylated analogues, in 31% and 4% yield, respectively. Complete deprotection of the combined collected samples was achieved using TMSOTf/TFA in the presence of thioanisole; under these conditions, the *O*-benzyl groups have obviously been removed. Gel-permeation chromatography resulted in the separation of two product fractions, corresponding to the dimeric (58%) and the desired monomeric (42%) glycopeptide, respectively. Due to incomplete hydrolysis of the lactone unit in the dimeric product, additional treatment with $NaHCO_3$ in D_2O was necessary in this case. Final cleavage with 1,4-dithiothreitol gave the monomeric glycopeptide **131** (Fig. 13). However, it must be noted that in the absence of antioxidizing agents, target compound **131** is readily oxidized to reform the dimer.

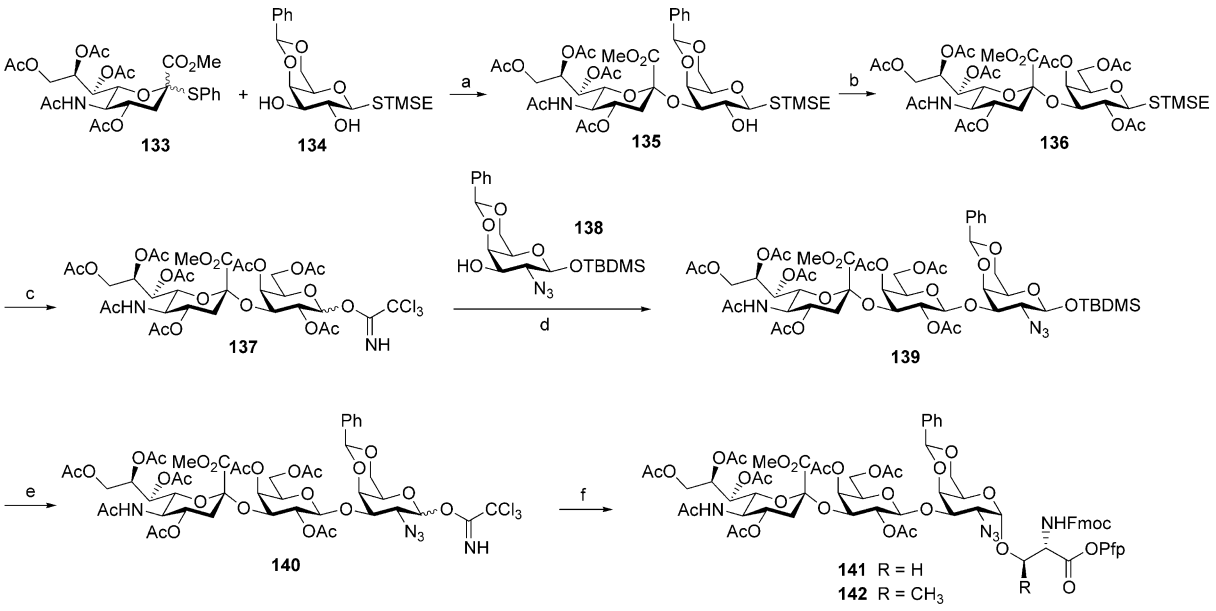
The α-2,3-sialyl-T serine building block **130** and its threonine analogue were also used in the solid-phase synthesis of the *N*-terminal glycopentapeptide sequence of human glycophorin A_M, bearing the consecutive sialyl-T antigen.[104] The glycopeptide was synthesized on a HMPB-BHA resin loaded with the *C*-terminal glycine using HATU/HOAt/DIPEA in NMP as coupling reagents. After coupling of two sialyl-T threonine units, one sialyl-T serine unit and the *N*-terminal Boc-Ser(Bn)-OH to the resin, the glycopeptide was detached from the solid-support with TFA in the presence of benzaldehyde. The fully protected glycopentapeptide was obtained in 81% overall yield. Complete deprotection was accomplished by treatment with TMSOTf and thioanisole in TFA. Subsequent lactone hydrolysis with $NaHCO_3$ in D_2O afforded target glycopeptide **132** (Fig. 14), containing three α-2,3-sialyl-T antigens, which represents the *N*-terminal human blood group type M sequence.

Studies of glycopeptide recognition by T-cells have revealed that substitution of Asn-72 in the self peptide sequence VITAFNEGLK, derived from mouse hemoglobin Hb(67–76), by threonine carrying the tumor-associated T_N-antigen, resulted in highly carbohydrate-specific T-cell immunogenicity.[105] In order to study the effect of sialylation on glycopeptide immunogenicity, the same peptide, containing the α-2,3-sialyl-T threonine and serine antigen at position 72, was prepared by solid-phase glycopeptide synthesis.[106]

The synthesis of the α-2,3-sialyl-T threonine and serine building blocks for application in the solid-phase synthesis was performed by a block condensation approach.[107] The key step in the assembly of the trisaccharide portion was the glycosylation of a 2-azidogalactosyl acceptor with a suitably protected α-2,3-sialyl-galactosyl trichloroacetimidate. After conversion of the sialic acid containing trisaccharide into a trichloroacetimidate donor, coupling to the side-chain of the protected amino acids threonine and serine was carried out. This strategy afforded access to both threonine and serine building blocks carrying the α-2,3-sialyl-T antigen.

The critical sialylation reaction of the suitably protected galactosyl acceptor **134** using sialic acid phenylthioglycoside **133** and TfOH/NIS as promoters was performed initially to afford the α-2,3-sialyl-galactosyl derivative **135** in 65% yield (Scheme 20).[108] After acetylation of the unreacted 2-hydroxy function, the benzylidene acetal was removed by reductive cleavage. Subsequent acetylation gave disaccharide derivative **136**, which was converted into the corresponding trichloroacetimidate **137** after acidolysis of the anomeric TMSE-group. Stereoselective β-glycosylation of 2-azidogalactosyl derivative **138** with disaccharide donor **137** was carried out in the presence of TMSOTf at low temperature. Under these conditions, no orthoester formation was observed. The corresponding trisaccharide **139** was isolated in 80% yield. Further conversion of **139** into the trichloroacetimidate donor **140** was achieved by acidolytic deprotection of the anomeric position and subsequent reaction with trichloroacetonitrile and DBU. The final glycosylation of Fmoc-Ser/Thr-OPfp was promoted by AgOTf and gave the desired α-glycosides **141** and **142** in 33% and 41% yield, respectively. The corresponding β-glycosides were isolated in 24% and 10% yield. The α-2,3-sialyl-T threonine and serine building blocks were directly used in solid-phase glycopeptide synthesis.

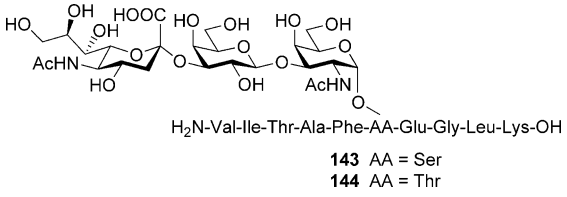
For the assembly of the glycopeptides, PL-$PEGA_{1900}$ resin modified with the HMPA-linker was employed. Attachment of the start amino acid lysine to the anchor was activated by 1-methylimidazole and MSNT.[109] Further coupling reactions were performed according to the Fmoc-strategy using three equivalents of amino acids and Dhbt-OH as a catalyst. Only one equivalent of the glycosylated amino acids **141** or **142** was coupled, respectively. For this step, the reaction time extended



Scheme 20. (a) NIS, TfOH, MS 3Å, CH_2Cl_2, −30 °C, 65%; (b) 1. Ac_2O, pyridine, quant.; 2. Pd/C, AcOH, 63%; 3. Ac_2O, pyridine, 98%; (c) 1. TFA, CH_2Cl_2, 94%; 2. CCl_3CN, DBU, CH_2Cl_2, 0 °C, 80%; (d) TMSOTf, CH_2Cl_2, MS 4Å, 0 °C to rt, 80%; (e) 1. TBAF, THF, pyridine, 0 °C, 81%; 2. CCl_3CN, DBU, CH_2Cl_2, 0 °C; 96%; (f) Fmoc-Ser/Thr-OPfp, AgOTf, CH_2Cl_2, MS 4Å, 0 °C, **141**: 33%, **142**: 41%.

from 24 h to 3 days. The 10th amino acid Boc-Val-OH was attached to the peptide chain by activation with TBTU. After assembly of the glycodecapeptide, the azido group of the carbohydrate moiety was converted into an *N*-acetyl function on the solid support using DTT and DBU in DMF followed by treatment with acetic anhydride. After acidolytic cleavage from the resin, the methyl ester of the sialic acid residue and the *O*-acetyl groups were removed with 30% NaOMe in aqueous MeOH at −30 °C. Despite the basic conditions (pH 14), β-elimination of the sialic acid unit was avoided due to the low reaction temperature. Since the *O*-acetyl group located at the 2-position of the galactose unit turned out to be resistant to hydrolysis under these conditions, complete deprotection was achieved after additional treatment with hydrazine hydrate to yield the glycodecapeptides **143** and **144** (Fig. 15) carrying the α-2,3-sialyl-T antigen.[106]

Chemoenzymatic synthesis. Enzymatic reactions are valuable tools in the synthesis of oligosaccharides and glycopeptides. Using glycosyltransferases[110] and glycosidases,[111] numerous protecting group manipulations can be avoided, and regio- and stereoselective formation of glycosidic linkages can be achieved. Successful enzymatic chain extensions of saccharides have been described in the literature; these procedures are of particular interest for the formation of oligosaccharides and glycopeptides containing sialic acid.[112] However, the different functional groups of the enzymatically prepared sialyl glycoconjugates have to be blocked by selectively removable protecting groups, before introduction of these compounds as building blocks into chemical glycopeptide syntheses can be performed.

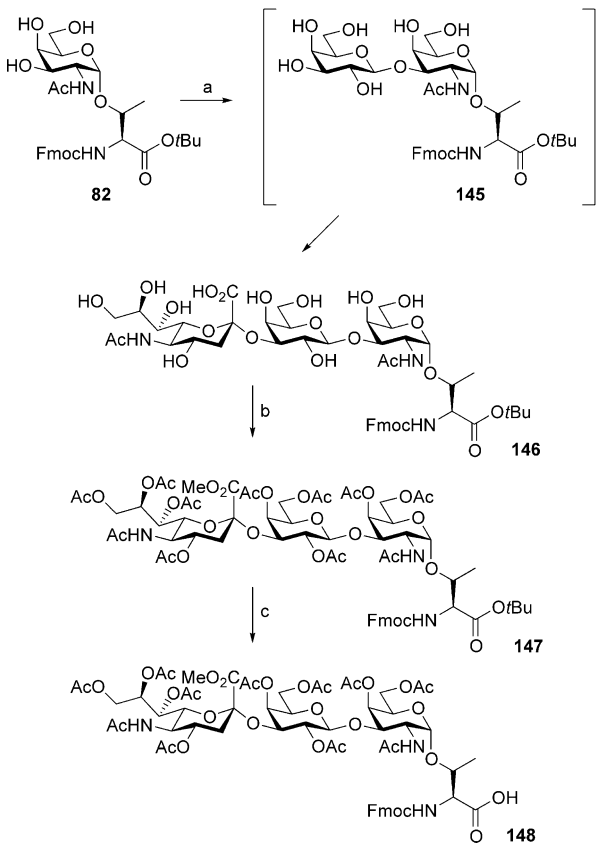


143 AA = Ser
144 AA = Thr

Figure 15. The hemoglobin-derived glycopeptides **143** and **144**.

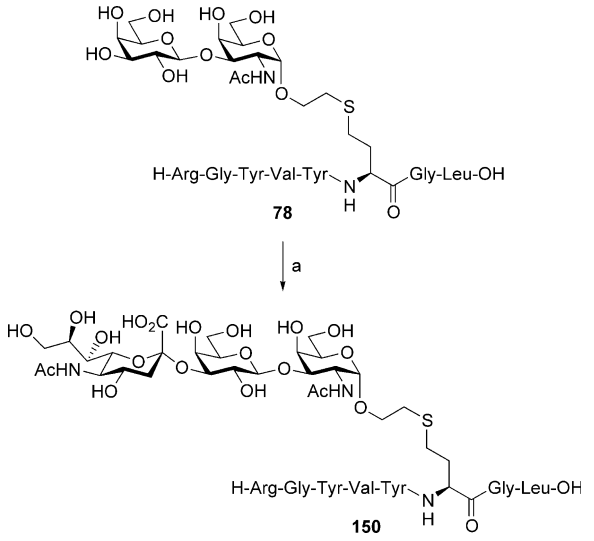
Recently, the chemoenzymatic synthesis of an α-2,3-sialyl-T threonine building block and its application to the solid-phase synthesis of the *N*-terminal sequence of leukemia-associated leukosialin (CD 43) has been reported.[113] Leukosialin[114] bearing the tumor-associated α-2,3-sialyl-T antigen is found on leukocytes of patients suffering from acute myeloid leukemia,[10] as well as on colon carcinoma cells.[11]

The synthesis of the sialyl-T threonine building block was achieved by a regio- and stereoselective enzymatic glycosylation cascade employing a β-galactosidase and an α-2,3-sialyltransferase in a one-pot reaction (Scheme 21). Starting with the protected *O*-glycosyl amino acid **82**, prepared by chemical synthesis as described above,[85] the glycan chain was extended using a commercially available galactosidase from bovine testes, in combination with the *core-1* specific human α-2,3-sialyltransferase (ST3-GalI).[115] Transglycosylation from lactose as a galactose donor to Fmoc-Thr(αGalNAc)-O*t*Bu catalyzed by the galactosidase resulted in the formation of the intermediate T-antigen derivative **145**, which was sialylated in situ to give the α-2,3-sialyl-T derivative **146** in 50% yield (on preparative scale).[116] To improve the low solubility of the protected amino acid glycan **82** in water, β-cyclodextrin dimethyl ether was added to the reaction mixture as a complexing agent. The one-pot procedure was carried out at pH 6.5 using alkaline phosphatase as a third enzyme in order to prevent product inhibition of the sialyltransferase by cytidine phosphate.



Scheme 21. (a) Lactose, β-galactosidase (bovine testes), CMP-Neu5Ac, sialyltransferase and calf intestine alkaline phosphatase in the presence of BSA, 2,6-di-*O*-methyl-β-cyclodextrin in water, pH 6.5, 50%; (b) 1. Ac_2O, pyridine; 2. MeOH, iPr_2NEt; 3. Ac_2O, pyridine, 61%; (c) TFA, 84%.

Before applying the α-2,3-sialyl-T threonine derivative **146** to the solid-phase glycopeptide synthesis, all functional groups on the carbohydrate portion, particularly the sialic acid carboxy group, had to be blocked by protecting groups of orthogonal stability to those within the peptide portion. Treatment of **146** with acetic anhydride in pyridine resulted in complete *O*-acetylation of the hydroxy functions and formation of a mixed anhydride, which was solvolyzed by the addition of DIPEA in methanol to furnish the methyl ester of the *N*-acetylneuraminic acid. Repeated treatment with acetic anhydride in pyridine to reverse the accompanying deacetylation gave the fully protected sialyl-T threonine building block **147** in 61% yield. After acidolysis of the *tert*-butyl ester, the Fmoc-protected α-2,3-sialyl-T threonine building block **148** was incorporated into the solid-phase synthesis of the *N*-terminal glycoheptadecapeptide of leukosialin.



Scheme 22. (a) CMP-Neu5Ac, sialyltransferase (ST3-GalI), calf intestinal phosphatase, Tris–HCl buffer (25 mM, pH 6.5), 37 °C, 68%.

The assembly of the glycopeptide was established in a peptide synthesizer using TentaGel S resin and the acid-labile Wang linker loaded with Fmoc-Thr(*t*Bu). The coupling reactions were performed according to the Fmoc-strategy. After removal of the Fmoc-group with piperidine in NMP, the resin was treated with a fivefold excess of Fmoc-amino acid, HBTU, HOBt and DIPEA, and unreacted amino groups were capped by acetylation. After the first seven coupling cycles, the Fmoc-group of the resin-linked octapeptide was cleaved off manually using morpholine in DMF. The following coupling reaction was carried out over 4 days using only 1.1 equivalents of the valuable sialyl-T threonine building block **148**, HATU, HOAt and NMM. After acetylation, the last eight amino acids were coupled in 40-fold excess by activation with HBTU/HOBt/DIPEA in the peptide synthesizer. Finally, the *N*-terminal Fmoc-group of the assembled glycoheptadecapeptide was exchanged for an acetyl group. Detachment of the completely protected sialyl-T glycopeptide from the polymeric support with TFA induced simultaneous cleavage of all *tert*-butyl side-chain protecting groups to give the corresponding sialyl-T glycoheptadecapeptide in 43% overall yield (after purification by preparative HPLC, yield based on loading with start amino acid). The *O*-acetyl groups were removed from the carbohydrate portion with a catalytic amount of NaOMe in MeOH (pH 8.2–8.5), and hydrolysis of the methyl ester of the *N*-acetylneuraminic acid portion using aqueous NaOH (pH 11–11.5) furnished the pure glycoheptadecapeptide **149** (Fig. 16) in 40% yield (after preparative HPLC).[117]

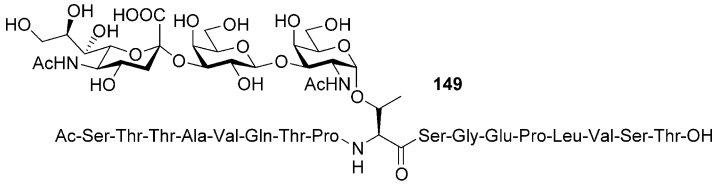


Figure 16. The leukosialin-derived glycopeptide **149**.

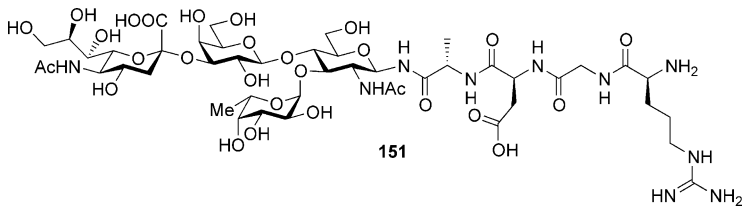


Figure 17. The disintegrine-derived glycopeptide **151**.

Interestingly, it was shown that the enzymatic sialylation of glycopeptides can proceed even where an unnatural linkage between the carbohydrate portion and the peptide backbone in the substrate occurs.[79] For the synthesis of a T-cell stimulating peptide carrying the α-2,3-sialyl-T antigen, precursor **78**, in which the T-antigen is attached to the peptide chain via a spacer, was subjected to enzymatic glycosylation using sialyltransferase ST3-GalI as a catalyst and CMP-Neu5Ac as a sialic acid donor. The target glycopeptide **150** was obtained in 68% yield (Scheme 22).

N-Glycopeptides Containing Carbohydrate Antigens

Sialyl Lewis x (sLe^x) and sialyl Lewis a (sLe^a) are regioisomeric tetrasaccharides, which have been identified as tumor-associated antigens, tissue-specifically expressed on carcinoma cells and involved in metastasis.[118] In normal tissue, the sLe^x and sLe^a saccharides are derived from sialylation of the blood group determinants Lewis x and Lewis a, respectively. They constitute important ligands for selectins, which are carbohydrate recognizing receptors on the surface of endothelial cells responsible for cellular adhesion.[119] In tumors, the degree of accumulation of these antigens in the cell membrane is well correlated with the extent of loss of cell–cell recognition normally controlling cell growth.[120] Therefore, glycoproteins carrying the tumor-associated sLe^x and sLe^a epitopes are supposed to play an important role in the hematogenous metastasis of cancer cells.

In this section, the chemical synthesis of glycopeptides containing the sLe^x and sLe^a saccharide antigens *N*-linked to the peptide backbone, which are interesting target structures for the investigation of cell adhesion phenomena, will be dicussed. However, it should be noted in this context that sLe^x glycopeptides with unnaturally *O*-linked saccharide epitopes have been prepared by chemoenzymatic means.[121,122]

Glycopeptides Containing Sialyl Lewis x

Sialyl Lewis x is mainly found on transformed epithelial cells of the liver, lung and stomach. Complex glycoproteins bearing the sLe^x epitope have been identified as efficient ligands for selectins.[123] Synthetic glycoconjugates such as glycopeptide **151** (Fig. 17) containing the saccharidic sLe^x antigen, as well as the RGD disintegrine peptide motif, have been shown to exhibit strong binding to P-selectin.[124,125] According to the cluster model, multivalent interactions between ligand and protein increase the efficacy of the selectin-glycoprotein binding. Hence, cyclic *N*-glycopeptides bearing three sLe^x antigens were prepared using a synthetic strategy based on a combination of solid-phase peptide synthesis and fragment condensation in solution.[34] The key step was the threefold condensation of the cyclic heptapeptides with partially blocked sLe^x tetrasaccharides.

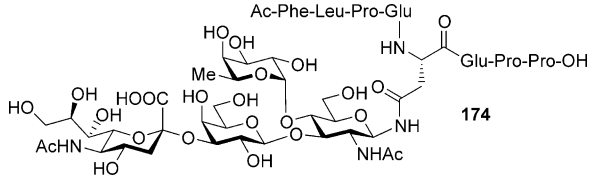
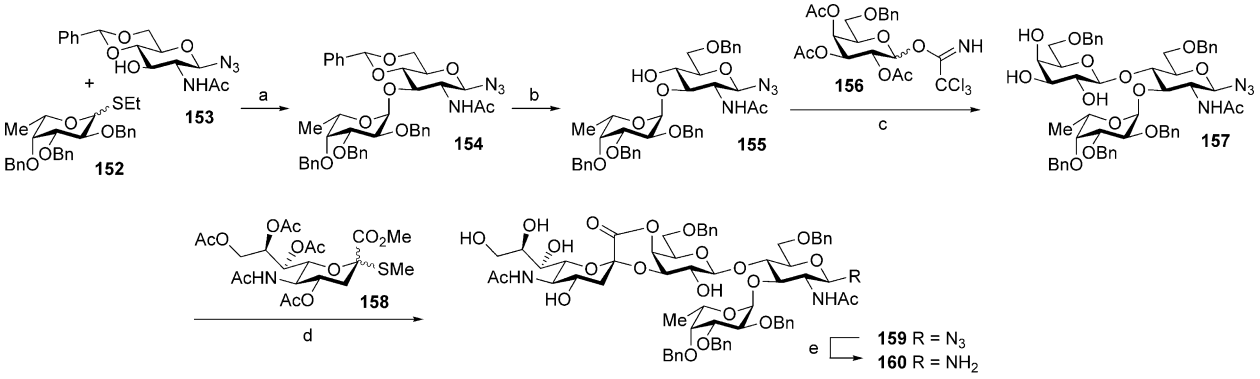
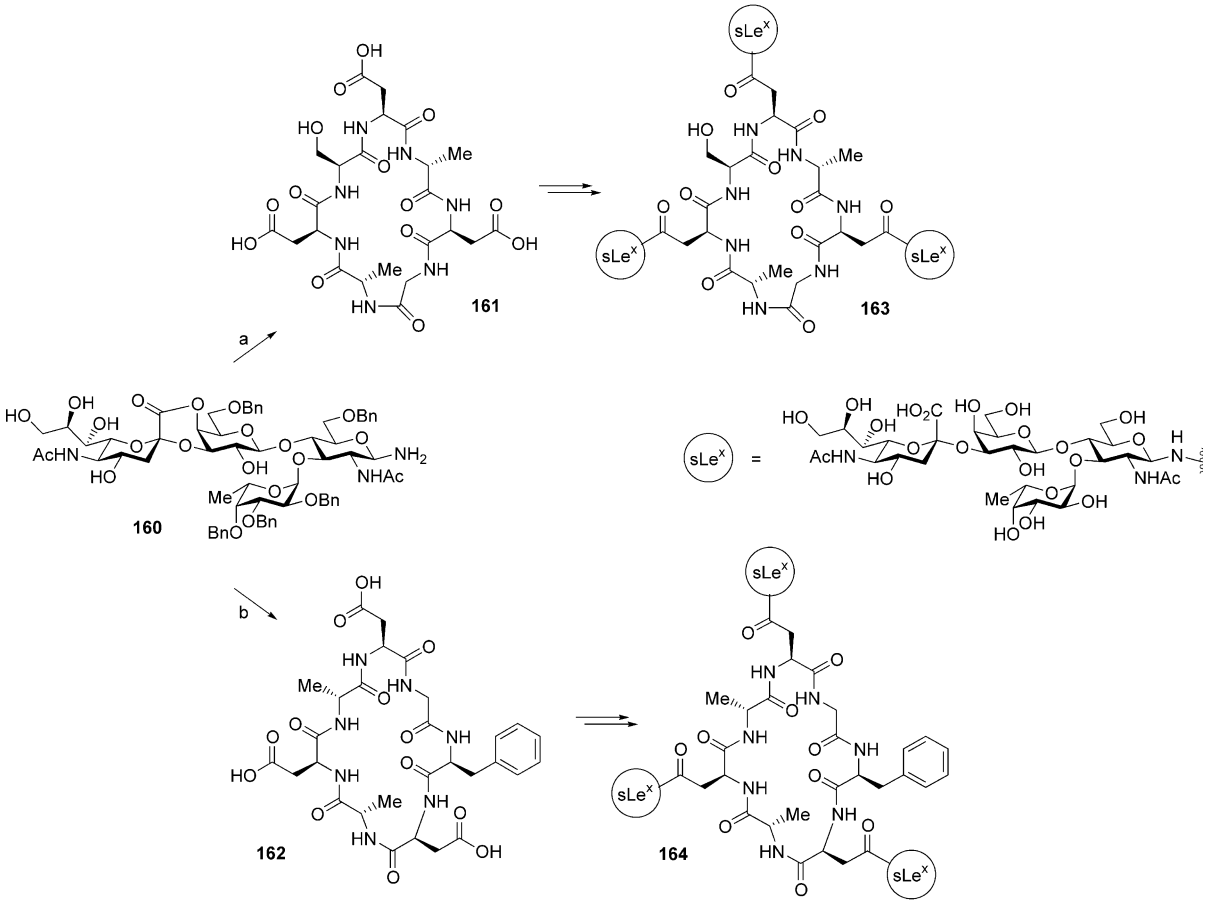


Figure 18. The PSGL-1 derived glycopeptide **174**.

First, a sLe^x tetrasaccharide unit suitable for condensation to the aspartic acid side-chain of the peptide backbone had to be synthesized. This was achieved by a



Scheme 23. (a) $CuBr_2$, Bu_4NBr, CH_2Cl_2/DMF (1/1), MS 3Å, 92%; (b) $NaCNBH_3$, HCl/Et_2O, MS 4Å, 81%; (c) 1. TMSOTf, CH_2Cl_2; 2. NaOMe, MeOH, Amberlite IR 120, 44%; (d) 1. MeSBr/AgOTf, CH_2Cl_2/CH_3CN (5/1), MS 3Å, −40 °C; 2. NaOMe, MeOH, Amberlite IR 120, 42%; (e) Raney-Ni, H_2, *i*PrOH/H_2O (9/1), 95%.



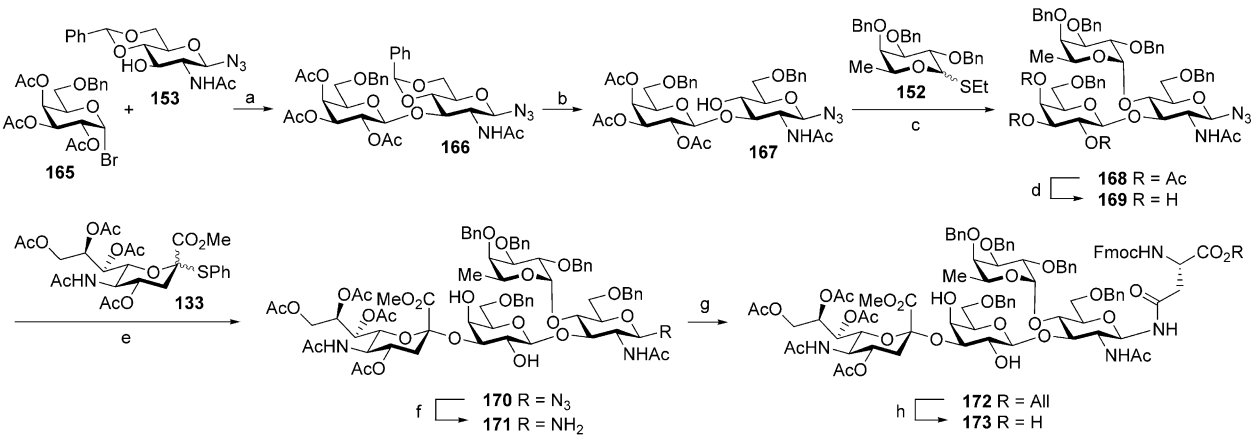
Scheme 24. Synthesis of the cyclic glycopeptides **163** and **164**.

series of glycosylation reactions, starting with a glucosamine derivative carrying an azido group at the anomeric position. The azido function served as an anomeric protecting group, which could be selectively converted into the amine functionality required for the coupling to the peptide side-chain later. This strategy had previously been applied to the synthesis of a bivalent *N*-glycopeptide bearing Lewis x antigenic side-chains.[126]

Glucosaminyl azide **153**[127] was α-selectively glycosylated with thioethyl fucoside **152**[128] carrying non-participating benzyl protecting groups (Scheme 23). The fucosylation was activated by Bu_4NBr and $CuBr_2$,[129] and gave the disaccharide **154** in 92% yield. Disaccharide **154** was converted into glycosyl acceptor **155** by regioselective opening of the benzylidene acetal with $NaCNBH_3$/HCl. β-Galactosylation of the sterically hindered disaccharide acceptor **155** involved neighboring group participation of the 2-acetyl function of trichloroacetimidate donor **156**[130] and was achieved using TMSOTf as a promoter. Subsequent *O*-deacetylation of the galactose moiety provided Le^x trisaccharide **157** in 44% yield (two steps). Regio- and stereoselective α-sialylation of **157** with thiomethyl donor **158**[131] was carried out in CH_2Cl_2/CH_3CN promoted by MeSBr/AgOTf. After removal of the *O*-acetyl groups and concurrent formation of the 1–4′-lactone of the neuraminic acid, sLe^x derivative **159** was obtained in 42% yield (two steps). The 1–4′-lactone, cleavable under mild conditions, was employed for internal protection during the synthesis, masking the carboxy function of the partially blocked sLe^x unit and consequently removing the need for additional protecting group manipulations. Hydrogenolysis of the anomeric azido function of **159** with Raney nickel finally yielded the desired sLe^x building block **160**.

Two cyclic heptapeptides **161** and **162** (Scheme 24) were synthesized, both containing D-alanine in order to facilitate efficient cyclization. First, the linear peptide chains were assembled by solid-phase peptide synthesis according to the Fmoc-strategy. The linear precursor for cycloheptapeptide **161** was prepared on acid-labile SASRIN resin, from which it was detached with retention of the orthogonally stable *tert*-butyl aspartic acid side-chain protecting groups. The cyclization of the peptide chain deblocked at the *N*- and *C*-termini was achieved in a dilute solution by activation with HATU/HOAt/DIPEA in 50% yield. Acidolytic removal of the side-chain protection furnished the cyclic heptapeptide template **161**. The cycloheptapeptide **162** was synthesized analogously by employing acid-labile TCP resin;[132] in this case, cyclization proceeded in 83% yield.

The simultaneous fragment condensation of cyclopeptide **161** and three sLe^x building blocks **160** was accomplished using HATU/HOAt/DIPEA and gave the partially protected sLe^x glycopeptide in 48% yield. Hydrogenolytic removal of the benzyl ether protection of the carbohydrate moiety and subsequent opening of



Scheme 25. (a) $Hg(CN)_2$, CH_2Cl_2/CH_3NO_2 (3/1), MS 3Å, 63%; (b) $NaCNBH_3$, HCl/Et_2O, THF, MS 4Å, 78%; (c) $CuBr_2$, Bu_4NBr, CH_2Cl_2/DMF (1/1), MS 3Å, 93%; (d) NaOMe, MeOH, 90%; (e) NIS, TfOH, CH_3CN, MS 3Å, 44%; (f) Raney-Ni, H_2, *i*PrOH/H_2O (9/1); (g) Fmoc-Asp-OAll, HATU/HOAt, *i*Pr_2NEt, DMF, 74% (two steps); (h) *N*-methylaniline, $[Pd(PPh_3)_4]$, THF, 90%.

the lactone by mild alkaline hydrolysis yielded the target sLe^x glycopeptide **163** (Scheme 24, route a). The analogous fragment condensation of cyclopeptide **162** and sLe^x unit **160** was achieved in 92% yield. The trivalent sLe^x cyclopeptide **164** was obtained after complete deprotection of the carbohydrate portion in 80% overall yield (Scheme 24, route b).

Cell adhesion tests proved sLe^x glycopeptide **164** to be an active synthetic ligand for human E-selectin. In a competitive assay, the trivalent sLe^x glycopeptide **164** inhibited the adhesion of tumor cells carrying sLe^x ligands (cell line HL_{60}) with an IC_{50} of 0.35–0.6 mM.

Glycopeptides Containing Sialyl Lewis a

The sialyl Lewis a epitope is expressed on intestinal, pancreatic and small-cell lung carcinomas.[133] Complex glycoproteins carrying sLe^a have been identified as selectin ligands,[134] which are interesting target molecules for studies of pathological cell adhesion processes involved in tumor metastasis. Well-characterized selectin ligands such as PSGL-1 (P-selectin glycoprotein ligand 1)[135] may be used to block the corresponding selectin receptor in order to prevent cell adhesion. Recently, the solid-phase synthesis of a glycooctapeptide sequence derived from the binding domain of PSGL-1, in which the natural *O*-glycosyl threonine is substituted by a sLe^a asparagine moiety, has been reported.[136]

The target glycopeptide **174** (Fig. 18) contains a sLe^a saccharide attached to the peptide backbone via an amide bond to the asparagine side-chain. The corresponding sLe^a asparagine building block was prepared by first assembling the saccharide moiety, employing an azido group for the anomeric protection of the glucosamine unit.[137] As shown for the synthesis of an analogous Lewis a asparagine conjugate, the azido function remains stable throughout the synthesis of the saccharide unit and can easily be converted into an amine group required for conjugation with the aspartic acid residue.[138]

The first step in the synthesis of the sLe^a tetrasaccharide was the β-glycosylation of appropriately protected glucosaminyl azide **153** with galactosyl bromide **165**, activated by $Hg(CN)_2$,[139] affording lactosaminyl azide **166** stereoselectively in 63% yield (Scheme 25). The benzylidene acetal was opened regioselectively with $NaCNBH_3$/HCl to furnish 4-acceptor **167**. This compound was stereoselectively α-glycosylated with ethylthio fucoside **152** promoted by Bu_4NBr and $CuBr_2$ in 93% yield. After *O*-deacetylation of the galactosyl portion of Le^a trisaccharide **168**, regio- and stereoselective sialylation of **169** was achieved in 44% yield using phenylthio glycoside **133**, activated by NIS and TfOH, as the donor. Hydrogenolytic reduction of the anomeric azido function of **170** with Raney nickel provided tetrasaccharide amine **171**, which was coupled to the side-chain carboxy function of Fmoc-Asp-OAll using HATU/HOAt/DIPEA as the condensing reagents. The allyl ester protecting group of sLe^a asparagine conjugate **172** was finally removed by Pd(0)-catalyzed allyl transfer to *N*-methylaniline to give the desired sLe^a asparagine building block **173**.

The solid-phase glycopeptide synthesis was performed on TentaGel resin with the allylic HYCRON anchor[48] carrying Fmoc-proline as the *C*-terminal amino acid. In the first chain extension step to give the dipeptide, Boc-proline was coupled in order to minimize the formation of diketopiperazine. After removal of the Boc-group, the assembly of the peptide chain was continued following the standard Fmoc-protocol using four equivalents of the corresponding Fmoc-amino acids and TBTU/HOBt/DIPEA and *sym*-collidine as activating reagents. Three equivalents of the sLe^a asparagine building block **173** were employed in the glycopeptide synthesis. The glycooctapeptide was detached from the resin by Pd(0)-catalyzed cleavage of the allylic anchor using *N*-methylaniline as a scavenger, affording the completely protected target molecule in 46% overall yield. After removal of the *N*-terminal Fmoc-group and hydrogenolytic *O*-debenzylation with catalytic Pd/C, the sLe^a glycopeptide was subjected to *O*-acetylation in order to stabilize the fucosidic bond towards treatment

with acids. Subsequent treatment with trifluoroacetic acid in the presence of cation scavengers resulted in removal of the *tert*-butyl protecting groups, accompanied by partial cleavage of the *N*-acetyl neuraminic methyl ester via intermediate lactone formation. *O*-Deacetylation with methanolic NaOH was followed by hydrolysis of the remaining sialic methyl ester in aqueous NaOH and final neutralization, yielding sLe^a glycooctapeptide **174** (Fig. 18) as a mixture of sodium salts.

Conclusions

In this article, recent developments in glycopeptide synthesis have been presented by means of selected examples. Focusing on glycopeptides carrying tumor-associated carbohydrate motifs, the efficiency of chemical and chemoenzymatic methodologies for the preparation of glycoconjugates, including derivatives containing saccharides particularly sensitive to chemical influence such as sialic acid and fucose, has been illustrated. A variety of glycosyl amino acid building blocks is available for the assembly of complex glycopeptides in solution and on the solid support.

The biological significance of the target compounds has briefly been discussed. Tumor-associated glycopeptide antigens represent important structures for the design of immunogens. Antibodies directed against the carbohydrate antigens of these glycopeptides are of interest for tumor diagnostics. Future research effort will certainly include the direction of an immune response against cancer cells by immunization with tumor-associated glycopeptide antigens in order to eradicate circulating metastasis and for the purpose of prophylactic anti-tumor vaccinations.

References and Notes

1. Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1.
2. Varki, A. *Glycobiology* **1993**, *3*, 97.
3. Montreuil, J.; Schachter, H.; Vliegenthart, J. F. G. *Glycoproteins*; Elsevier Science B. V.: Amsterdam, 1995.
4. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.
5. Kim, Y. J.; Varki, A. *Glycoconjugate J.* **1997**, *14*, 569.
6. Springer, G. F. *Science* **1984**, *224*, 1198.
7. Springer, G. F.; Desai, P. R.; Murthy, M. S.; Scanlon, E. F. *ACS Symp. Ser.* **1978**, *80*, 311.
8. (a) Itzkowitz, S. H.; Yuan, M.; Montgomery, C. K.; Kjeldsen, T.; Takahashi, H. K.; Bigbee, W. L.; Kim, Y. S. *Cancer Res.* **1989**, *49*, 197. (b) Itzkowitz, S. H.; Bloom, E. J.; Kolcal, W. A.; Modin, G.; Hakomori, S.-J.; Kim, Y. S. *Cancer* **1990**, *66*, 1960.
9. Karsten, U.; Papsdorf, G.; Pauly, A.; Vojtesek, B.; Moll, R.; Lane, E. B.; Clausen, H.; Stosiek, P.; Kasper, M. *Differentiation* **1993**, *54*, 55.
10. (a) Fukuda, M.; Carlsson, S. R.; Klock, J. C.; Dell, A. *J. Biol. Chem.* **1986**, *261*, 12769. (b) Saitoh, O.; Gallagher, R. E.; Fukuda, M. *Cancer Res.* **1991**, *51*, 2854.
11. Baeckström, D. *J. Biol. Chem.* **1997**, *272*, 11503.
12. Lloyd, K. O.; Burchell, J.; Kudryashov, V.; Yin, B. W. T.; Taylor-Papadimitrou, J. *J. Biol. Chem.* **1996**, *271*, 33325.
13. Hakomori, S.-I.; Kannagi, R. *J. Natl. Cancer Inst.* **1983**, *71*, 231.
14. Koprowski, H.; Herlyn, M.; Steplewski, Z.; Sears, H. F. *Science* **1981**, *212*, 53.
15. Hilkens, J.; Ligtenberg, M. J. L.; Vos, H. L.; Litvinov, S. L. *Trends Biochem. Sci.* **1992**, *17*, 359.
16. (a) Orntoft, T. F.; Harving, N.; Langklide, N. C. *Int. J. Cancer* **1990**, *45*, 666. (b) David, L.; Nesland, J. M.; Clausen, H.; Carneiro, F.; Sobrinho-Siñoes, M. *APMIS* **1992**, *100*, 162 (Suppl. 27). (c) Kushima, R.; Jancic, S.; Hattori, T. *Int. J. Cancer* **1993**, *55*, 904.
17. Brockhausen, I.; Yang, J.-M.; Burchell, J.; Whitehouse, C.; Taylor-Papadimitriou, J. *Eur. J. Biochem.* **1995**, *233*, 607.
18. Spencer, D. I. R.; Missailidis, S.; Denton, G.; Murray, A.; Brady, K.; Matteis, C. I. D.; Searle, M. S.; Tendler, S. J. B.; Price, M. R. *Biospectroscopy* **1999**, *5*, 79.
19. Seitz, O.; Liebe, B.; Braun, P.; Bruch, K. v. d.; Kunz, H. In *Control Mechanisms of Carcinogenesis*; Hengstler, J., Oesch, F., Eds.; Institut für Toxikologie: Mainz, Germany, 1996; pp 338–354.
20. Kihlberg, J.; Elofsson, M. *Curr. Med. Chem.* **1997**, *4*, 85.
21. Koganty, R. R.; Reddish, M. A.; Longenecker, B. M. *Drug Discovery Today* **1996**, *1*, 190.
22. Livingston, P. O. In *Molecular Diagnosis, Prevention and Treatment of Melanoma*; Kirkwood, J. K., Ed.; New York, 1997.
23. The syntheses and biological evaluation of the tumor-associated carbohydrate antigens Globo-H, KH-1, Lewis y, N3 and Fucosyl-GM_1 have recently been reviewed: Danishefsky, S. J.; Allen, J. R. *Angew. Chem.* **2000**, *112*, 882; *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 836.
24. (a) Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495. (b) Seitz, O. *ChemBioChem* **2000**, *1*, 214. (c) St. Hilaire, P. M.; Meldal, M. *Angew. Chem.* **2000**, *112*, 1210; *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 1162. (d) Koeller, K. M.; Wong, C.-H. *J. Chin. Chem. Soc.* **1999**, *46*, 659. (e) Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1999**, *10*, 3045.
25. Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1997**, *8*, 2839.
26. Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317.
27. Koenigs, W.; Knorr, E. *Chem. Ber.* **1901**, *34*, 957.
28. Schmidt, R. R. *Angew. Chem.* **1986**, *98*, 213; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212.
29. Fügedi, P.; Garegg, P. J.; Lönn, H.; Norberg, T. *Glycoconjugate J.* **1987**, *4*, 97.
30. Seeberger, P. H.; Bilodeau, M. T.; Danishefsky, S. J. *Aldrichimica Acta* **1997**, *30*, 75.
31. Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *187*, 35.
32. Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1991**, *212*, 277.
33. (a) Martin, T. J.; Brescello, R.; Toepfer, A.; Schmidt, R. R. *Glycoconjugate J.* **1993**, *10*, 16. (b) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260.
34. Sprengard, U.; Schudok, M.; Schmidt, W.; Kretzschmar, G.; Kunz, H. *Angew. Chem.* **1996**, *108*, 359; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 321.
35. Boons, G.-J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539.
36. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
37. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
38. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
39. König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
40. Bayer, E.; Rapp, W. *Chem. Pept. Protein* **1986**, *3*, 3.
41. Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077.
42. Wang, R. B. *J. Am. Chem. Soc.* **1972**, *94*, 1328.
43. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.

44. Mergler, M.; Tanner, R.; Gosteli, J.; Grogg, P. *Tetrahedron Lett*. **1988**, *29*, 4005.
45. (a) Kunz, H.; Birnbach, S. *Angew. Chem*. **1986**, *98*, 354; *Angew. Chem. Int. Ed. Engl*. **1986**, *25*, 360. (b) Kunz, H.; Birnbach, S.; Wernig, P. *Carbohydr. Res*. **1990**, *202*, 207.
46. Kuduk, S. D.; Schwarz, J. B.; Chen, X.-T.; Glunz, P. W.; Sames, D.; Ragupathi, G.; Livingston, P. O.; Danishefsky, S. J. *J. Am. Chem. Soc*. **1998**, *120*, 12474.
47. Ferrari, B.; Pavia, A. A. *Carbohydr. Res*. **1980**, *79*, C1.
48. (a) Seitz, O.; Kunz, H. *Angew. Chem*. **1995**, *107*, 901; *Angew. Chem. Int. Ed. Engl*. **1995**, *34*, 803. (b) Seitz, O.; Kunz, H. *J. Org. Chem*. **1997**, *62*, 813.
49. Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem*. **1979**, *57*, 1244.
50. (a) Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc*. **1975**, *97*, 4056. (b) Paulsen, H.; Hölck, J.-P. *Carbohydr. Res*. **1982**, *109*, 89.
51. Schultheiβ-Reimann, P.; Kunz, H. *Angew. Chem*. **1983**, *95*, 64; *Angew. Chem. Int. Ed. Engl*. **1983**, *22*, 62.
52. Kunz, H.; Waldmann, H. *Angew. Chem*. **1984**, *96*, 49; *Angew. Chem. Int. Ed. Engl*. **1984**, *23*, 71.
53. Habermann, J.; Kunz, H. *Tetrahedron Lett*. **1998**, *39*, 265.
54. (a) Takeichi, M. *Curr. Opin. Cell Biol*. **1993**, *5*, 806. (b) Vleminckx, K.; Varkaet, L., Jr; Mareel, M.; Fiers, W.; van Roy, F. *Cell* **1991**, *66*, 107. (c) Birchmeier, W. *Bioessays* **1995**, *17*, 97.
55. Habermann, J.; Kunz, H. *J. Prakt. Chem*. **1998**, *340*, 233.
56. Jamieson, G. A., Greenwalt, T. J., Eds. *Glycophorines of Blood Cells and Plasma*. J. P. Lipincott Company: Philadelphia, Toronto, 1971.
57. Klich, G.; Paulsen, H.; Meyer, B.; Meldal, M.; Bock, K. *Carbohydr. Res*. **1997**, *299*, 33.
58. Atherton, E.; Holder, J. L.; Meldal, M.; Sheppard, R. C.; Valerio, R. M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2887.
59. Lo-Man, R.; Vichier-Guerre, S.; Bay, S.; Dériaud, E.; Cantacuzène, D.; Leclerc, C. *J. Immunol*. **2001**, *166*, 2849.
60. Vichier-Guerre, S.; Lo-Man, R.; Bay, S.; Dériaud, E.; Nakada, H.; Leclerc, C.; Cantacuzène, D. *J. Peptide Res*. **2000**, *55*, 173.
61. Bay, S.; Lo-Man, R.; Osinaga, E.; Nakada, H.; Leclerc, C.; Cantacuzène, D. *J. Peptide Res*. **1997**, *49*, 620.
62. Lo-Man, R.; Bay, S.; Vichier-Guerre, S.; Dériaud, E.; Cantacuzène, D.; Leclerc, C. *Cancer Res*. **1999**, *59*, 1520.
63. Braun, P.; Davies, G. M.; Price, M. R.; Williams, P. M.; Tendler, S. J. B.; Kunz, H. *Bioorg. Med. Chem*. **1998**, *6*, 1531.
64. Leuck, M.; Kunz, H. *J. Prakt. Chem*. **1997**, *339*, 322.
65. Winterfeld, G. A.; Ito, Y.; Ogawa, T.; Schmidt, R. R. *Eur. J. Org. Chem*. **1999**, 1167.
66. Winterfeld, G. A.; Schmidt, R. R. *Angew. Chem*. **2001**, *113*, 2718; *Angew. Chem. Int. Ed. Engl*. **2001**, *40*, 2654.
67. Paulsen, H.; Adermann, K. *Liebigs Ann. Chem*. **1989**, 751.
68. Renil, M.; Meldal, M. *Tetrahedron Lett*. **1996**, *37*, 6185.
69. Schleyer, A.; Meldal, M.; Renil, M.; Paulsen, H.; Bock, K. *Angew. Chem*. **1997**, *109*, 2064; *Angew. Chem. Int. Ed. Engl*. **1997**, *36*, 1976.
70. Satyanarayana, J.; Gururaja, T. L.; Naganagowda, G. A.; Ramasubbu, N.; Levine, M. J. *J. Peptide Res*. **1998**, *52*, 165.
71. Schenkels, L. C. P. M.; Gururaja, T. L.; Levine, M. J. In *Oral Mucosal Drug Delivery*; Rathbone, M. J., Ed.; Marcel Dekker: New York, 1996; pp 191–220.
72. For glycosylation of amino acids with T-antigen derived disaccharide donors see Paulsen, H.; Peters, S.; Bielfeldt, T.; Meldal, M.; Bock, K. *Carbohydr. Res*. **1995**, *268*, 17.
73. Mathieux, N.; Paulsen, H.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2359.
74. Lüning, B.; Norberg, T.; Tejbrant, I. *Glycoconjugate J*. **1989**, *6*, 5.
75. Paulsen, H.; Schleyer, A.; Mathieux, N.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 281.
76. Nakamura, K.; Hanai, N.; Kanno, M.; Kobayashi, A.; Ohnishi, Y.; Ito, Y.; Nakahara, Y. *Tetrahedron Lett*. **1999**, *40*, 515.
77. Nakamura, K.; Ishii, A.; Ito, Y.; Nakahara, Y. *Tetrahedron* **1999**, *55*, 11253.
78. St. Hilaire, P. M.; Cipolla, L.; Franco, A.; Tedebark, U.; Tilly, D. A.; Meldal, M. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3559.
79. George, S. K.; Holm, B.; Reis, C. A.; Schwientek, T.; Clausen, H.; Kihlberg, J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 880.
80. Kurosaka, A.; Kitagawa, H.; Fukui, S.; Numata, Y.; Nakada, H.; Funakoshi, I.; Kawasaki, T.; Ogawa, T.; Iijima, H.; Yamashina, I. *J. Biol. Chem*. **1988**, *263*, 8724.
81. Zhang, S.; Walberg, L. A.; Ogata, S.; Itzkowitz, S. H.; Koganty, R. R.; Reddish, M.; Gandhi, S. S.; Longenecker, B. M.; Lloyd, K. O.; Livingston, P. O. *Cancer Res*. **1995**, *55*, 3364.
82. Fung, P. Y. S.; Madej, M.; Koganty, R. R.; Longenecker, B. M. *Cancer Res*. **1990**, *50*, 4308.
83. Ragupathi, G.; Koganty, R. R.; Qiu, D.; Lloyd, K. O.; Livingston, P. O. *Glycoconjugate J*. **1998**, *15*, 217.
84. Ragupathi, G.; Howard, L.; Cappello, S.; Koganty, R. R.; Qiu, D.; Longenecker, B. M.; Reddish, M. A.; Lloyd, K. O.; Livingston, P. O. *Cancer Immunol. Immunother*. **1999**, *48*, 1.
85. (a) Liebe, B.; Kunz, H. *Angew. Chem*. **1997**, *109*, 629; *Angew. Chem. Int. Ed. Engl*. **1997**, *36*, 2830. (b) Liebe, B.; Kunz, H. *Helv. Chim. Acta* **1997**, *80*, 1473.
86. Gendler, S. J.; Lancaster, C. A.; Taylor-Papadimitriou, J.; Duhig, T.; Peat, N.; Burchell, J.; Pemberton, L.; Lalani, E.-N.; Wilson, D. *J. Biol. Chem*. **1990**, *265*, 15286.
87. (a) Dasgupta, F.; Garegg, P. J. *Carbohydr. Res*. **1988**, *177*, C13. (b) Birberg, W.; Lönn, H. *Tetrahedron Lett*. **1991**, *32*, 7457.
88. Keil, S.; Claus, C.; Dippold, W.; Kunz, H. *Angew. Chem*. **2001**, *113*, 379; *Angew. Chem. Int. Ed. Engl*. **2001**, *40*, 366.
89. (a) Burchell, J.; Taylor-Papadimitriou, J.; Boshell, M.; Gendler, S. J.; Duhig, T. *Int. J. Cancer* **1989**, *44*, 691. (b) Price, M. R.; Hudecz, F.; O'Sullivan, C.; Baldwin, R. W.; Edwards, P. M.; Gendler, S. J. *Mol. Immunol*. **1990**, *62*, 795.
90. Furuhata, K.; Kimio, K.; Ogura, H.; Hata, T. *Chem. Pharm. Bull*. **1991**, *39*, 255.
91. Gewehr, M.; Kunz, H. *Synthesis* **1997**, 1499.
92. Elofsson, M.; Salvador, L. A.; Kihlberg, J. *Tetrahedron* **1997**, *53*, 369.
93. (a) Hansen, J.-E. S.; Clausen, H.; Nielsen, C.; Teglbjaerg, L. S.; Hansen, L. L.; Nielsen, C. M.; Dabelsteen, E.; Mathiesen, L.; Hakomori, S.-I.; Nielsen, J. O. *J. Virol*. **1990**, *64*, 2833. (b) Hansen, J.-E. S.; Nielsen, C.; Arendrup, M.; Olufsson, S.; Mathiesen, L.; Nielsen, J. O.; Clausen, H. *J. Virol*. **1991**, *65*, 6461. (c) Hansen, J.-E. S.; Clausen, H.; Hu, S. L.; Nielsen, J. O.; Olufsson, S. *Arch. Virol*. **1992**, *126*, 11.
94. Hansen, J.-E. S.; Hofman, B.; Sørensen, T.; Clausen, H. In *Complex Carbohydrates in Drug Research*; Bock, K., Clausen, H. Eds.; Alfred Benzon symposium 36: Munksgaard: Copenhagen, 1994, 1989; pp 414–427.
95. Lüning, B.; Norberg, T.; Tejbrant, J. *Glycoconjugate J*. **1989**, *6*, 5.
96. Fukase, K.; Hasuoka, A.; Kinoshita, I.; Aoki, Y.; Kusumoto, S. *Tetrahedron* **1995**, *51*, 4923.
97. Burchell, J.; Poulsom, R.; Hanby, A.; Whitehouse, C.; Cooper, L.; Clausen, H.; Miles, D.; Taylor-Papadimitriou, J. *Glycobiology* **1999**, *9*, 1307.
98. Carlstedt, I.; Davies, J. R. *Biochem. Soc. Trans*. **1997**, *25*, 214.
99. (a) Sames, D.; Chen, X.-T.; Danishefsky, S. J. *Nature* **1997**, *389*, 587. (b) Schwarz, J. B.; Kuduk, S. D.; Chen, X.-T.; Sames, D.; Glunz, P. W.; Danishefsky, S. J. *J. Am. Chem. Soc*. **1999**, *121*, 2662.

100. (a) Nakahara, Y.; Nakahara, Y.; Ito, Y.; Ogawa, T. *Tetrahedron Lett*. **1997**, *38*, 7211. (b) Nakahara, Y.; Nakahara, Y.; Ito, Y.; Ogawa, T. *Carbohydr. Res*. **1998**, *309*, 287. (c) For a similar synthesis of the sialyl-T threonine conjugate see ref. 99 (b).
101. Gejyo, F.; Schmid, K. *Biochim. Biophys. Acta* **1981**, *671*, 78.
102. Gejyo, F.; Chang, J.-L.; Bürgi, W.; Schmid, K.; Offner, G. D.; Troxler, R. F.; Halbeek, H. v.; Dorland, L.; Gerwig, G. J.; Vliegenthart, J. F. G. *J. Biol. Chem*. **1983**, *258*, 4966.
103. Nakahara, Y.; Iijima, H.; Ogawa, T. *Tetrahedron Lett*. **1994**, *35*, 3321.
104. Ando, S.; Nakahara, Y.; Ito, Y.; Ogawa, T.; Nakahara, Y. *Carbohydr. Res*. **2000**, *329*, 773.
105. Galli-Stampino, L.; Meinjohanns, E.; Frische, K.; Meldal, M.; Jensen, T.; Werdelin, O.; Mouritsen, S. *Cancer Res*. **1997**, *57*, 3214.
106. Komba, S.; Werdelin, O.; Jensen, T.; Meldal, M. *J. Peptide Sci*. **2000**, *6*, 585.
107. Komba, S.; Meldal, M.; Werdelin, O.; Jensen, T.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1999**, 415.
108. Komba, S.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. Chem*. **1996**, *4*, 1833.
109. Blankemeyer-Menge, B.; Nimtz, M.; Frank, R. *Tetrahedron Lett*. **1990**, *31*, 1701.
110. (a) Ichikawa, Y.; Wang, R.; Wong, C.-H. *Methods Enzymol*. **1994**, *247*, 107. (b) Gambert, U.; Thiem, J. *Top. Curr. Chem*. **1997**, *186*, 21.
111. Kren, V.; Thiem, J. *Chem. Soc. Rev*. **1997**, *24*, 463.
112. (a) Unverzagt, C.; Kunz, H.; Paulson, J. C. *J. Am. Chem. Soc*. **1990**, *112*, 930. (b) Unverzagt, C.; Kelm, S.; Paulson, J. C. *Carbohydr. Res*. **1994**, *251*, 285.
113. Bézay, N.; Dudziak, G.; Liese, A.; Kunz, H. *Angew. Chem*. **2001**, *113*, 2350; *Angew. Chem. Int. Ed. Engl*. **2001**, *40*, 2292.
114. Fukuda, M. *Glycobiology* **1991**, *1*, 347.
115. (a) Sadler, J. E.; Rearick, J. I.; Paulson, J. C.; Hill, R. L. *J. Biol. Chem*. **1979**, *254*, 4434. (b) Chang, M. L.; Eddy, R. L.; Shows, T. B.; Lau, J. T. Y. *Glycobiology* **1995**, *5*, 319.
116. Dudziak, G.; Bézay, N.; Schwientek, T.; Clausen, H.; Kunz, H.; Liese, A. *Tetrahedron* **2000**, *56*, 5865.
117. Throughout the editing of this article, the chemoenzymatic synthesis of a MUC 1-derived glycopeptide containing the α-2,3-sialyl-T antigen has been reported: George, S. K.; Schwientek, T.; Holm, B.; Reis, C. A.; Clausen, H.; Kihlberg, J. *J. Am. Chem. Soc*. **2001**, *123*, 11117.
118. Renkonen, R.; Mattila, P.; Majuri, M.-L.; Räbina, J.; Toppila, S.; Renkonen, J.; Hirvas, L.; Niitimäki, J.; Turmen, J. P.; Renkonen, O.; Paavonen, T. *Glycoconjugate J*. **1997**, *14*, 593.
119. Kansas, G. S. *Blood* **1996**, *88*, 3259.
120. Kannagi, R. *Glycoconjugate J*. **1997**, *14*, 577.
121. Seitz, O.; Wong, C.-H. *J. Am. Chem. Soc*. **1997**, *119*, 8766.
122. Matsuda, M.; Nishimura, S.-I. *J. Med. Chem*. **2001**, *44*, 715.
123. (a) Patel, T. P.; Goelz, S. E.; Lobb, R. R.; Parekh, R. B. *Biochemistry* **1994**, *33*, 14815. (b) Stroud, M. R.; Handa, K.; Ito, K.; Salyan, M. E. K.; Fang, H.; Levery, S. B.; Hakomori, S.; Reinhold, B. B.; Reinhold, V. N. *Biochem. Biophys. Res. Commun*. **1995**, *209*, 777.
124. Sprengard, U.; Kretzschmar, G.; Bartnik, E.; Hüls, C.; Kunz, H. *Angew. Chem*. **1995**, *107*, 1104; *Angew. Chem. Int. Ed. Engl*. **1995**, *34*, 990.
125. Sprengard, U.; Kunz, H.; Hüls, C.; Schmidt, W.; Seiffge, D.; Kretzschmar, G. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 509.
126. Bruch, K. v. d.; Kunz, H. *Angew. Chem*. **1994**, *106*, 87; *Angew. Chem. Int. Ed. Engl*. **1994**, *33*, 101.
127. (a) Kunz, H.; Unverzagt, C. *Angew. Chem*. **1988**, *100*, 1763; *Angew. Chem. Int. Ed. Engl*. **1988**, *27*, 1697. (b) Unverzagt, C.; Kunz, H. *J. Prakt. Chem*. **1992**, *334*, 570.
128. Lönn, H. *Carbohydr. Res*. **1985**, *139*, 105.
129. Sato, S.; Mori, M.; Ito, Y.; Ogawa, T. *Carbohydr. Res*. **1986**, *155*, C6.
130. Schmidt, R. R.; Michel, J. *Angew. Chem*. **1980**, *92*, 763; *Angew. Chem. Int. Ed. Engl*. **1980**, *19*, 731.
131. Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res*. **1991**, *212*, 277.
132. Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiou, G.; Wenqing, Y.; Schäfer, W. *Tetrahedron Lett*. **1989**, *30*, 394.
133. Zhang, S.; Zhan, H. S.; Cordon-Cardo, D.; Reuter, V. E.; Singhal, A. K.; Lloyd, K. O.; Livingston, P. O. *Int. J. Cancer* **1997**, *73*, 50.
134. Varki, A. *Proc. Natl. Acad. Sci. U.S.A*. **1994**, *91*, 7390.
135. (a) Wilkins, P. P.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem*. **1996**, *272*, 18732. (b) Leppänen, A.; Mehta, P.; Ouyang, Y.-B.; Ju, T.; Helin, J.; Moore, K. L.; van Die, I.; Canfield, W. M.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem*. **1999**, *274*, 24838.
136. Peilstöcker, K.; Kunz, H. *Synlett* **2000**, *6*, 823.
137. Peilstöcker, K.; Kunz, H. *Synlett* **2000**, *6*, 820.
138. März, J.; Kunz, H. *Synlett* **1992**, *7*, 589.
139. Helferich, B.; Wedemeyer, F. *Liebigs Ann. Chem*. **1949**, *563*, 139.

Constanze Brocke was born in Hannover, Germany, in 1974. She graduated in chemistry from the University of Hannover in 1999; her degree course included spells at Stanford University (USA) and at the Massachusetts Institute of Technology (USA). Currently she is working on her doctoral thesis under the supervision of Professor H. Kunz at the Johannes Gutenberg University in Mainz. Her research focuses on the synthesis of mucin-derived glycopeptides.

Horst Kunz was born in Frankenhausen, Germany, in 1940. He studied chemistry at the Humboldt University in Berlin and at the University Of Mainz and completed his PhD under the supervision of Professor L. Horner. His habilitation project dealt with ester analogues of acetylcholine and their application in protecting group chemistry. He was appointed Associate Professor for Organic Chemistry in 1979, and Professor for Bioorganic Chemistry at the University of Mainz in 1988. His research centers on stereoselective synthesis, the chemistry of peptides and carbohydrates, solid-phase synthesis, and combinatorial chemistry. He received the Max Bergmann medal in 1992, the Emil Fischer medal in 2000, and the Adolf Windaus medal from the University of Göttingen in 2001. In 1998 he was elected member of the Saxony Academy of Sciences in Leipzig.