Protected-mode Synthesis of *N*-Linked Glycopeptides: Single-step Preparation of Building Blocks as Peracetyl Glycosylated N^{α}Fmoc Asparagine OPfp Esters

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The preparation of N^{α}-(fluoren-9-ylmethoxycarbonyl)asparagine pentafluorophenyl esters (N^{α}-Fmoc-Asn-OPfp) glycosylated with per-*O*-acetylated β-D-glucose, *N*-acetyl-β-D-glucosamine, β-D-mannose, 4-*O*-β-D-glucopyranosyl-β-D-glucose (cellobiose), 4-*O*-β-D-galactopyranosyl-β-D-glucose (lactose) and 4-*O*-α-D-glucopyranosyl-β-D-glucose (maltose) is described. The per-*O*-acetylated glycosylamines were treated selectively with the key compound N^{α}-Fmoc-Asp(Cl)-OPfp **2**, to give, in a single step, the glycosylated building blocks. The acid chloride **2** was prepared in a quantitative one-pot reaction from commercially available N^{α}-Fmoc-Asp(OBu^{t})-OPfp **1**. The acid stability of the *N*-glycosidic linkage was investigated. The building block **7**, containing a maltose moiety, was used in the synthesis of a glycosylated D-Ala^{1} Peptide-T amide analogue **14**. CD spectra were recorded in 85% TFE. All compounds were fully characterized by ^{1}H and ^{13}C NMR spectroscopy.

The importance of carbohydrate recognition in biological events is well established[1] and the configuration and spatial arrangement of the involved carbohydrates is a subject of ongoing research. The mannose-binding protein (MBP) from mammalian serum, which activates the complement cascade[2,3] requires equatorial hydroxy groups on C-3 and C-4 but binds to residues having an axial or equatorial hydroxy group or even an *N*-acetyl group on C-2. A high-resolution (1.7 Å) crystal structure of a mannose-binding protein complexed with an oligosaccharide has recently shown that only non-reducing terminal mannose residues are in direct contact with the carbohydrate-recognition domain (CRD).[4] The hepatic asialoglycoprotein receptor (ASPG-R) binds galactose and exhibits what has been termed the cluster effect,[5] that is, increased binding affinity with up to three terminal galactose residues. The binding affinity is also highly dependent of the spatial arrangement of the galactose residues as determined by oligosaccharide probes.[6] In order to further study ligand–receptor interactions it is clear that a large variety of individually tailored compounds will be necessary.

We now report on the synthesis of fully protected mono- and di-saccharide-containing asparagine building blocks activated as their pentafluorophenyl esters for the solid-phase synthesis of *N*-linked glycopeptides. These compounds may prove useful in the design of glycosylated peptide templates and in the future design of potential diagnostic and therapeutic agents.

Several approaches to the chemical synthesis of *N*-linked glycopeptides have been reported. For example, peptides have been glycosylated with synthetic or naturally isolated oligosaccharides,[7] and glycosylated amino acids have been isolated from naturally occurring glycoproteins and used in a chemical conjugation of polypeptides.[8] However, the use of chemically glycosylated asparagine residues as building blocks in the stepwise assembly of *N*-linked glycopeptides presents probably the most versatile and general method.[9–12] By this methodology the amide bond between the carbohydrate and the amino acid is formed prior to the synthesis of the peptide. The tripeptide sequence Asn-Xxx-Ser/Thr, which is the natural prerequisite for *N*-glycosylation in glycoproteins,[13] can therefore be overcome since there are no requirements made of the peptide amino acid sequence.

Different semipermanent protection groups for the α-carboxylic acid during the glycosylation have been used: benzyl ester,[14–17] ethyl ester,[18] phenacyl[19] and *tert*-butyl ester.[9,20] The hydroxy groups of the carbohydrate moiety have been protected[9,14–19] or left unprotected[20] and after removal of the *C*-terminal protection the glycosyl amino acids have been used in the assembly of glycopeptides activated as esters or with the use of *in situ* coupling reagents. The present strategy for *N*-linked glycopeptide synthesis implies a simple preparation and use of fully protected and activated glycosylated amino acid building blocks.[10] This facilitates automation and multiple glycopeptide synthesis as has already been demonstrated for *O*-linked glycopeptides.[21] Additionally, the protected hydroxy groups confer stability to the *N*-glycosidic bond and, in the case of oligosaccharides, to *O*-glycosidic bonds as well.[22] In the present work the carbohydrate moiety is protected prior to glycosylation with *O*-acetyl groups, which can be removed after the glycopeptide synthesis under mild conditions. The formation of the crucial amide bond between N^{α}-Fmoc asparagine OPfp ester and the carbohydrate is carried out as the last synthetic step and the resulting glycosylated asparagine building block can be purified and used directly in the solid-phase synthesis of *N*-linked glycopeptides.

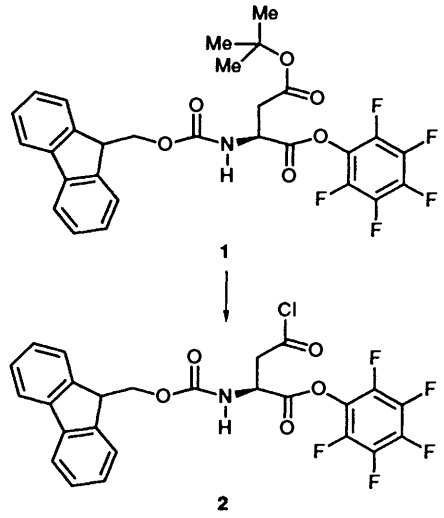
The use of the building blocks described is exemplified by the synthesis of a D-Ala^{1} Peptide-T amide analogue, D-Ala-Ser-Thr-Thr-Thr-Asn(maltose)-Tyr-Thr-NH_2 **14**. The nonglycosylated D-Ala^{1} Peptide-T amide **15** has been shown to be an effective inhibitor of human immunodeficiency virus (HIV) binding to the CD4 receptor of T cells.[23]

Results and Discussion

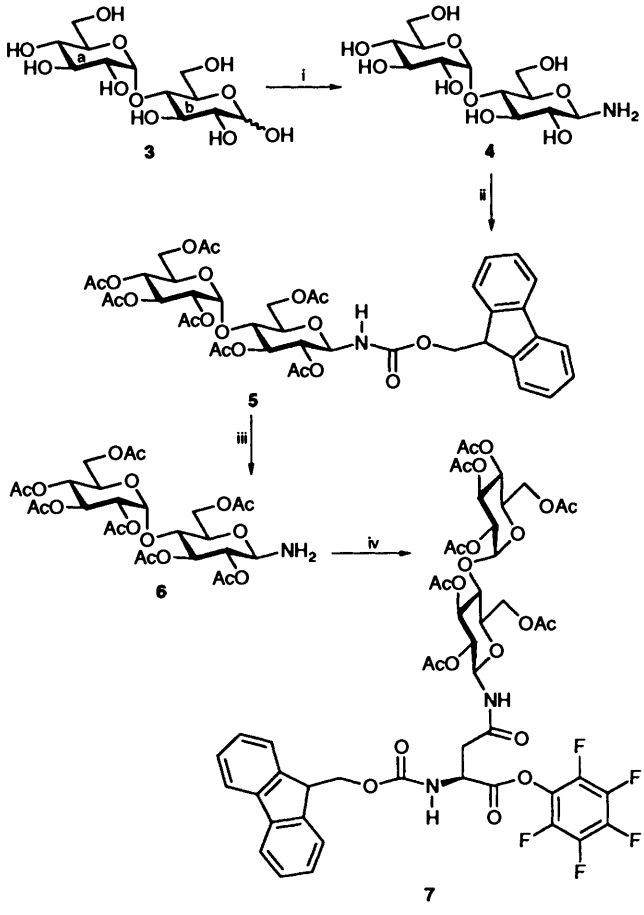
Mono- and di-saccharides were treated with a saturated solution of ammonium hydrogencarbonate as previously reported[20,24,25] to give the β-D-glycosylamines. *N*-Acetyl-D-glusosamine and the three disaccharides D-cellobiose, D-lactose and D-maltose were thus converted into the β-glycosylamines in high yield (> 90%), with no detectable formation of bis-glycosyl amines,[25,26] and were used in the subsequent steps as crude products. However, upon using D-glucose and D-mannose as starting compounds we found 20 and 35%, respectively of the bis-glycosyl amines and attempts to purify the product mixtures by ion-exchange chromatography were unsuccessful. Furthermore, the conversion of D-mannose into glycosylamine was only 70% as determined by NMR spectroscopy and we therefore turned to the old procedure of Isbell and Frush.[26,27] Treatment of D-glucose and D-mannose with methanolic

ammonia afforded the pure β-glycosylamines as crystalline products in high yield on a large scale (40 g). The β-anomer was indicated by a J_{12} coupling constant of ~9 Hz when the reducing end had the *gluco* configuration. In the case of the *manno* configuration the β anomer was indicated by a $^1J_{CH}$ coupling constant of 152 Hz.[28] For temporary amine protection, the glycosylamines were treated with fluoren-9-ylmethoxycarbonyl succinimide ester (Fmoc-OSu) in pyridine and then *O*-acetylation of hydroxy groups was achieved by addition of acetic anhydride, as previously reported.[10] The fully protected glycosylamines were obtained in 32–77% yield. The best yields, 77 and 75%, respectively, were obtained for 2,3,4,6-tetra-*O*-acetyl-*N*-fluoren-9-ylmethoxycarbonyl-β-D-gluco- **16** and -manno-pyranosylamine **17** where the starting materials were the pure, crystalline glycosylamines. Attempts to prepare the protected galactosylamine derivative were unsuccessful and led to formation of several products, which could not be separated. Treatment of the *N*-Fmoc-protected per-*O*-acetylated glycosylamines with piperidine in tetrahydrofuran (THF)[10] or morpholine in dimethylformamide (DMF) gave the Fmoc-deprotected compounds in 66–92% yield. Per-*O*-acetylated glycosylamines of mono- and di-saccharides have been reported previously and were prepared by reduction of the per-*O*-acetylated glycosyl azides[29–31] or by deprotection of the *N*-[2,2-bis(ethoxycarbonyl)vinyl]-protected per-*O*-acetylated glycosylamines.[32,33]

In most previous work on glycosylated asparagine building blocks, the coupling between the amino acid and the carbohydrate moiety has been followed by one or several synthetic steps to convert the building block into a derivative suited for peptide coupling.[9,12,14–20] In all these cases, the use of *in situ* coupling reagents or other methods of activation of the α-carboxy group prior to acylation were necessary resulting in a possibility of unwanted side-reactions. In the present strategy a well-characterized, dually activated asparagine derivative was employed. In this way any loss of the precious glycosylated amino acid and also the need for coupling reagents was avoided, thus minimizing side-reactions. The acid chloride N^{α}-Fmoc-Asp(Cl)-OPfp **2** provided all the necessary requirements for the amino acid: base-labile amine protection, protection of the α-carboxylate function during the glycosylation, and at the same time sufficient activation for solid-phase peptide synthesis, and a highly activated side-chain acid chloride ready for selective aminolysis by the per-*O*-acetylated glycosylamines. The coupling reaction afforded, in a single step, the glycosylated amino acid for use in the solid-phase glycopeptide synthesis. The acid chloride **2** required use of dry organic solvents, therefore the glycosylamine hydroxy groups had to be protected prior to this reaction in order to provide sufficient solubility. Our first approach to the synthesis of compound **2** involved a two-step treatment of commercially available N^{α}-Fmoc-Asp-(OBu^t)-OPfp **1** with trifluoroacetic acid (TFA) followed by thionyl dichloride at ambient temperature.[10] However, this method was found to be unreliable and instead simultaneous treatment with TFA and thionyl dichloride at 40 °C for 24 h gave the acid chloride **2** quantitatively, and the reaction was completely reproducible on a 0.025–5 g scale. NMR and fast-atom–bombardment mass spectroscopy (FAB–MS) revealed the presence of the acid (N^{α}-Fmoc-Asp-OPfp, *m/z* 521.9) and the anhydride [(N^{α}-Fmoc-Asp-OPfp)$_2$O, *m/z* 1022.7] amongst the intermediate products. The preparation of another aspartic acid chloride derivative in the synthesis of amino acids with side-chain diazo functions has recently been reported by Burger *et al.*[34] Condensation of the per-*O*-acetylated glycosylamines and acid chloride **2** proceeded instantaneously at 0 °C in 72–91% yield and no reaction with the Pfp ester was observed. The syntheses of the disaccharide building blocks **7**, **11** and **12** were carried out before the synthetic procedure for obtaining the

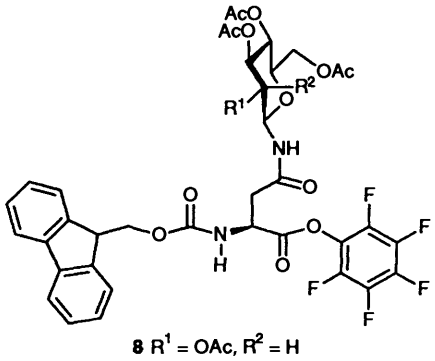


Scheme 1 *Reagents and conditions:* TFA (10 mol equiv.), $SOCl_2$ (100 mol equiv.), 40 °C, 24 h (100%)

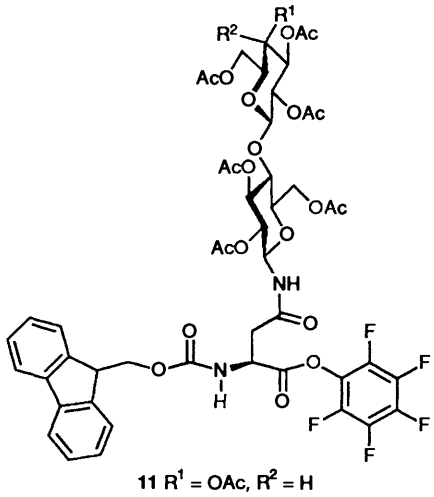


Scheme 2 *Reagents:* i, NH_4HCO_3; ii, Fmoc-OSu, pyridine; then Ac_2O; iii, morpholine/DMF; iv, **2**, *N*-ethylmorpholine, THF.

acid chloride **2** in a pure form had been fully developed and the yields were therefore low, 32–60%. The synthesis of compound **7** was later repeated in 76% yield, demonstrating that the method also gives high yields with disaccharides. The versatility of fully protected sugar derivatives in the solid-phase synthesis of *N*-glycopeptides depends upon the stability of *O*- and *N*-glycosidic bonds under the acidic conditions used in peptide deprotection and cleavage. We therefore synthesized a glycosylated dipeptide H-Asn(per-*O*-acetyl, lactosyl)-Phe-NH-Resin and cleaved it from the resin under different acidic conditions to



8 R^1 = OAc, R^2 = H
9 R^1 = AcNH, R^2 = H
10 R^1 = H, R^2 = OAc



11 R^1 = OAc, R^2 = H
12 R^1 = H, R^2 = OAc

evaluate the stability of the two types of glycosidic linkages. Even after 24 h of treatment with 95% TFA at ambient temperature the intact glycopeptide H-Asn [2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-Phe-NH_2 **18** was recovered in >95% yield as confirmed by HPLC and NMR spectroscopy. It has been shown that the use of unprotected sugar derivatives in solid-phase synthesis, in most cases, does not give decomposition products after 2 h in TFA;[20,35] however, with unprotected lactose 20% decomposition was observed after treatment with TFA for 24 h.[20]

It has been proposed that building blocks with protected carbohydrate moieties are less well incorporated into the peptide than are the unprotected analogues.[12] This result is in sharp contrast to the results of others[11,36,37] and may rather be considered a comparison of Pfp esters *versus* symmetrical anhydrides. The result may even be due to an unsuccessful attempt to prepare the symmetrical anhydride of the glycosylated amino acid.

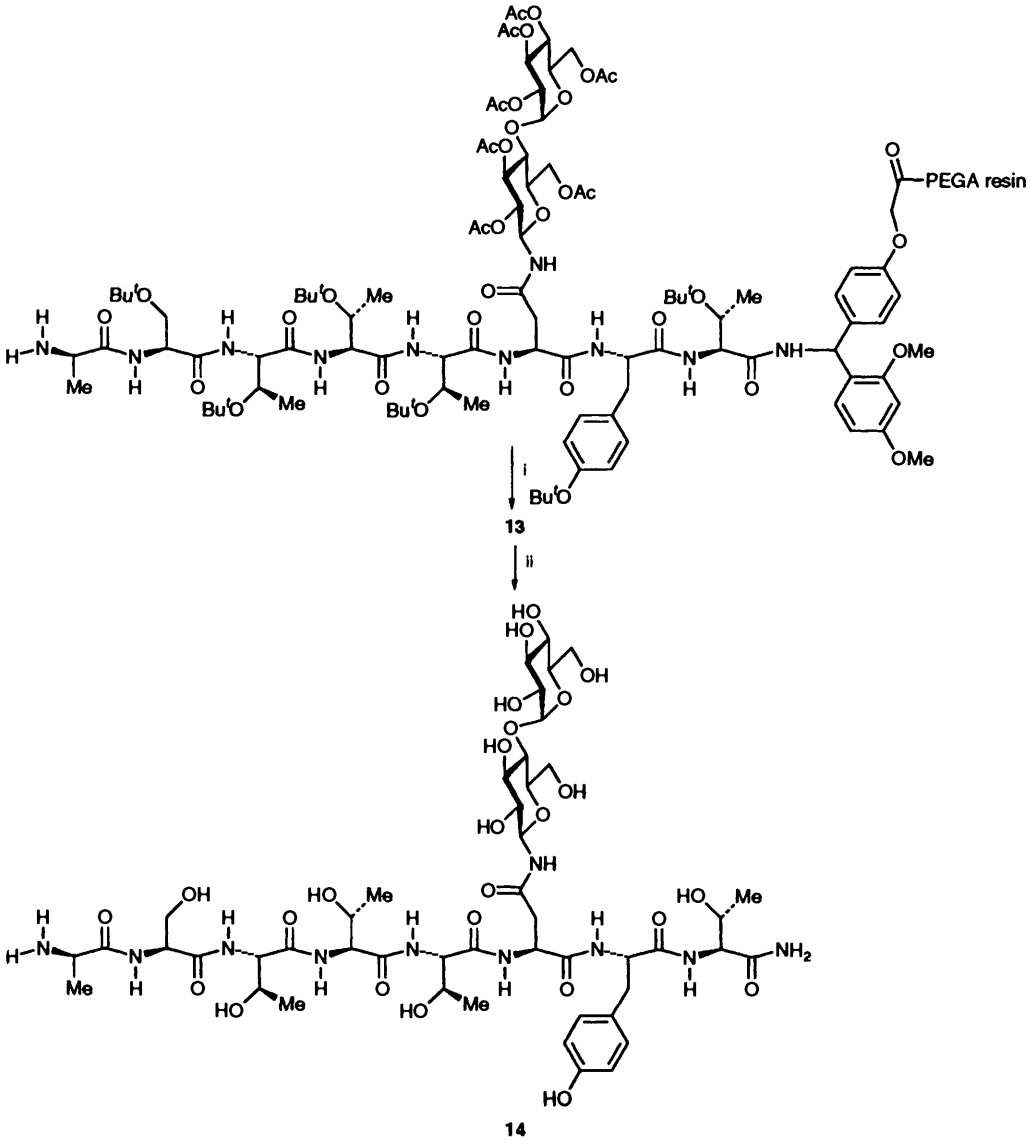
We used the building block **7**, containing a maltose moiety, in the automated continuous-flow solid-phase synthesis of a glycosylated D-Ala[1] Peptide-T amide analogue **14**. Poly(ethylene glycol)/dimethylacrylamide copolymer (PEGA-resin)[38] was used as the solid phase and DMF as the solvent. The synthesis was carried out with 3 mol equiv. of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt) ester-activated Fmoc-protected amino acids with acid-labile *tert*-butyl (OBu[t]) protection of side-chains (Ser, Thr and Tyr). In the coupling of compound **7**, 2 mol equiv. were used and 1 mol equiv. of Dhbt-OH added as an auxiliary nucleophile. N^{α}-Fmoc-D-alanine was coupled as the free acid with *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate (TBTU)[39] as coupling reagent. The acylation steps were monitored at 440 nm by a solid-phase spectrophotometer recording the yellow colouration of the resin due to formation of an ion-pair between Dhbt-OH and unchanged amino groups.[40] Removal of Fmoc groups was performed with 20% piperidine in DMF. The glycopeptide was cleaved from the solid support with TFA, and scavengers and the acid-labile side-chain protection was simultaneously removed. The crude yield of glycopeptide **13** after cleavage with TFA and repeated trituration of the solid product with diethyl ether was 87% with 83% incorporation of the glycosylated amino acid, based on HPLC peak-area integration. The yield after preparative HPLC was 27%. *O*-Deacetylation followed by preparative HPLC gave compound **14** in an overall yield of 17%. The glycopeptide **14** was fully characterized by ^1H and ^{13}C NMR spectroscopy and the NMR data were compared with those of the nonglycosylated D-Ala[1] Peptide-T amide **15**. Only very minor changes in chemical shifts and coupling constants were observed. Circular dichroism (CD) spectra of compound **14**, **15**, and a D-Ala[1] Peptide-T amide conjugate **19**,[41] in which D-glucitol is linked to the asparagine residue, were recorded in 85% 2,2,2-trifluoroethanol (TFE) (Fig. 1) The spectral contributions of the sugar moieties were estimated by subtracting the CD spectra of the 1-*N*-acetates of 4-*O*-α-D-glucopyranosyl-β-D-glucosylamine (D-maltosylamine) and 1-amino-1-deoxy-D-glucitol, respectively, from the CD spectra of the two *N*-linked glycopeptides **14** and **19** (Fig. 2). As can be seen from the two sets of curves the corrected spectrum is substantially different from the uncorrected one. After analysis of the three corrected spectra by the singular value decomposition (SVD) method[42,43] the analogue glycosylated with maltose, **14**, indicated an increase in α-helicity when compared with the nonglycosylated peptide **15**. For small peptides, in this case of eight residues, this may rather be indicative of stabilization of a β-turn structure, as previously reported.[44] Our results indicate that when working with small glycopeptides it is important to note the spectral contribution of the sugar moiety.

The building block **12**, containing a lactose moiety, has been used in the synthesis of a di-*N*-glycosylated cell adhesion inhibitor containing the sequence Arg-Gly-Asp.[45] A full paper on this work will be published elsewhere.

In the present work we have demonstrated the synthesis and use of fully protected glycosylated asparagine building blocks activated as their Pfp esters. With the possibility of varying the glycosyl moiety between mono- and di-saccharides with different configurations, the synthesized building blocks may prove useful in the design of *N*-linked glycopeptide templates for the study of protein–carbohydrate interactions, for studying the influence of *N*-glycosylation, or for the design of new diagnostic and therapeutic agents.

Experimental

Vacuum liquid chromatography (VLC)[46] was performed on Merck Silica Gel 60 H and chromatography under dry conditions was performed on dried Silica Gel (120 °C; >24 h) with distilled solvents kept over molecular sieves. TLC was performed on Merck Silica Gel 60 F_{254} with detection by charring with sulfuric acid, or by UV light when applicable. DMF was freshly distilled by fractional distillation at reduced



Scheme 3 *Reagents:* i, TFA; ii, NaOMe; MeOH

pressure and kept over 3 Å molecular sieves. Thionyl dichloride and pyridine were distilled prior to use. THF was passed through a column of basic alumina, distilled from diphosphorus pentaoxide and kept over 3 Å molecular sieves. Light petroleum was the 60–80 °C fraction. All organic solvents were of p.a. quality or better. Concentrations were performed under reduced pressure at temperatures < 30 °C (bath). *p*-[α-(Fluoren-9-ylmethoxycarbonylamino)-2,4-dimethoxybenzyl]phenoxy-acetic acid (Rink-linker)[36] and suitably protected N^{α}-Fmoc amino acids were purchased from NovaBiochem (Switzerland), TBTU and Dhbt-OH from Fluka (Switzerland), *N*-ethylmorpholine from Merck (Germany) and ethane-1,2-dithiol from Aldrich (USA). The peptides and the glycopeptides were hydrolysed with 6 mol dm^{-3} HCl at 110 °C for 24 h and the amino acid composition was determined on a Pharmacia LKB Alpha Plus amino acid analyser, Asn was determined as Asp. Nomenclature is according to IUPAC recommendations. Positive fast-atom-bombardment mass spectra (FAB–MS) were recorded on a JEOL JMS-HMX/HX110A Tandem Mass Spectrometer. Ions were produced by a beam of xenon atoms, 6 keV, from a matrix of 3-nitrobenzyl alcohol. Optical rotations were measured on a Perkin-Elmer 241 Polarimeter and are given in units of 10^{-1} deg cm^3 g^{-1}. M.p.s were measured with a Büchi apparatus and are uncorrected. Circular dichroism (CD) spectra were recorded at Novo Nordisk, Denmark, on a Jobin Yvon Model IV dichrograph calibrated with (+)-camphor-10-sulfonic acid. All spectra were recorded at 25 °C with a cell pathlength of 0.01 cm. The peptide concentrations were 4.0 mmol dm^{-3}. All spectra were smoothed with a Fourier transform algorithm and the appropriate background spectra were subtracted. The result, $\Delta\varepsilon$, is based on the molar concentration of peptide bonds. Secondary structures were predicted from 180–260 nm CD spectra using SVD combined with variable selection.[42,43,47,48] For this purpose, a set of 32 reference proteins with known CD spectra and X-ray structures was used. NMR spectra were recorded on a Bruker AM-500 or a Bruker AMX-600 MHz spectrometer. The ^1H and ^{13}C resonances were assigned by ^1H, ^{13}C, ^1H–^1H COSY, ^1H–^1H double quantum filtered phase-sensitive COSY, ^{13}C–^1H correlation, NOE in a rotating frame (ROESY) and phase-sensitive 2-dimensional total correlation spectroscopy (TOCSY) experiments. Unit identifications are defined in Scheme 2, structure **3**. 2D NMR spectroscopy was performed as previously described.[21] NMR spectra were recorded in $CDCl_3$, $(CD_3)_2SO$ or water–CD_3CO_2D mixtures and pH (pD) values of NMR samples were measured at room temperature using a PHM63 digital pH meter (Radiometer) equipped with an Ingold electrode with no correction for isotope effects.[49] HPLC was performed on a Waters system with a 600 controller, a 410 differential refractometer or a 991 photodiode array detector,

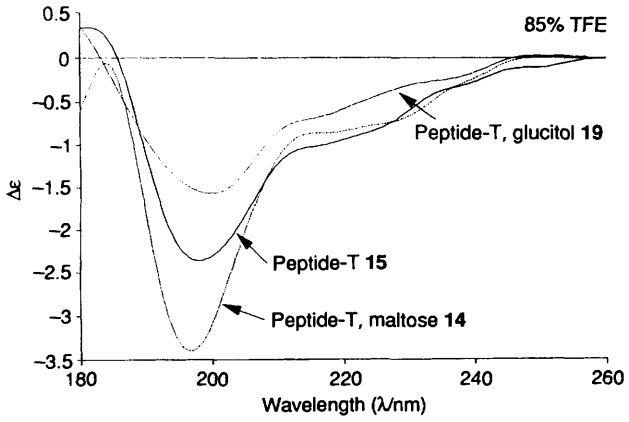


Fig. 1 CD spectra of D-Ala[1] Peptide-T amide **15**, the glycosylated maltose analogue **14** and the glycosylated glucitol analogue **33** without correction for the sugar contribution.

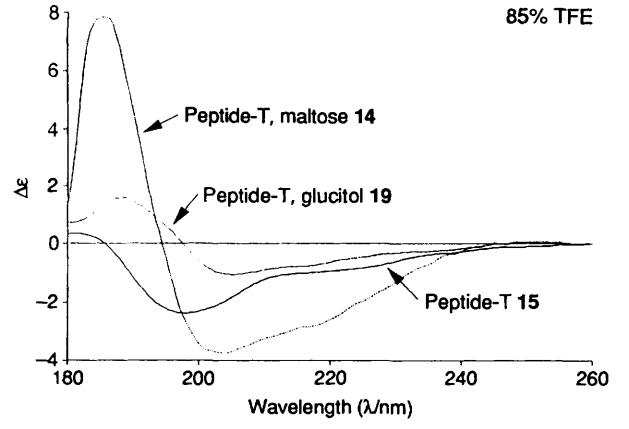


Fig. 2 CD spectra of D-Ala[1] Peptide-T amide **15**, the glycosylated maltose analogue **14** and the glycosylated glucitol analogue **19** after correction for the sugar contribution.

both equipped with preparative flow cells, and a model 600 pump with modified 80 cm^3 min^{-1} pump heads. The system was fitted with switchable analytical RCM (8 × 100 mm) and Deltapak (19 × 300 mm) columns and a preparative radial pack module for columns (50 × 300 mm) packed with reversed-phase C_{18}. Solvent system A: 0.1% TFA and B: 0.1% TFA in 90% acetonitrile–10% water, was employed for both analytical (1 cm^3 min^{-1}) and preparative (10 or 20 cm^3 min^{-1}) separations and detection was at 215 nm. Elemental analyses were carried out at LEO Pharmaceutical Products, Denmark.

Solid-phase Synthesis. General Procedure.—Synthesis of the glycopeptides was performed in DMF with a custom-made, fully automatic, continuous-flow peptide synthesizer or by the plastic syringe technique (described below) using PEGA-resin[38] (0.07 mmol g^{-1}). Amino acids were coupled as their Pfp esters (3 mol equiv.) with Dhbt-OH (1 mol equiv.) added as an auxiliary nucleophile or as their Dhbt esters (3 mol equiv.). The side-chains were protected with Bu[t] for serine, threonine and tyrosine. N^{α}-Fmoc deprotection was effected by treatment with 20% piperidine in DMF for 30 min and the acylation times were determined with a solid-phase spectrophotometer at 440 nm. Glycine was coupled directly to the resin followed by coupling of the Rink-linker by the TBTU procedure.[39] The first amino acid was coupled and unchanged amino groups were capped by addition of acetic anhydride before coupling of the second amino acid. After deprotection of the last amino acid the resin was removed from the column, washed with dichloromethane, and dried overnight. Cleavage of the peptide or the glycopeptide from the solid support was performed by treatment with a mixture of TFA, water and scavengers as described in detail under the individual peptides. After cleavage the resin was poured onto a glass filter and washed three times with TFA followed by 95% aq. acetic acid. The combined filtrates were concentrated and the peptides/glycopeptides were precipitated by several triturations with diethyl ether. Residual solvent was removed under reduced pressure and the peptide was purified by preparative HPLC.

The purified acetylated glycopeptide was dissolved in dry methanol (1 mg cm^{-3}) and sodium methoxide in methanol (1 mol dm^{-3}) was added until a wetted pH-paper indicated pH 11. The mixture was stirred at ambient temperature for 2 h, neutralized with small pieces of solid CO_2 and concentrated. The residue was dissolved in water (1 mg cm^{-3}) and purified by preparative HPLC.

Solid-phase Synthesis: Plastic Syringe Technique.—The peptide H-Asn-Phe-NH_2 **20** and the glycopeptide H-Asn [2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-Phe-NH_2 **18** were synthesized by use of the plastic syringe technique, which is a simple and cheap alternative to automatic peptide synthesizers. The technique is exemplified here with the synthesis of the dipeptide **20**. A 20 cm^3 disposable plastic syringe A (without piston) was fitted with a sintered Teflon filter (pore size 70 μm) and the outlet was connected to the outlet of a 50 cm^3 plastic syringe B *via* a Teflon tube with female Luer adaptors. Syringe B was used as a waste syringe to remove solvents and unused reagents. PEGA-resin (0.5 g, 0.07 mmol g^{-1}) was placed in syringe A and allowed to swell in DMF (10 cm^3) which was carefully added from the top and removed from the bottom by suction with syringe B. N^{α}-Fmoc-L-Gly-OPfp (49 mg, 0.105 mmol) and Dhbt-OH (5.7 mg, 0.035 mmol) were dissolved in DMF (4 cm^3) and the mixture was added to the resin. The acylation time was determined by observation of the yellow colour formed between Dhbt-OH and unchanged amino groups.[40] After coupling the resin was rinsed with DMF (8 × 4 cm^3) before N^{α}-Fmoc deprotection. Piperidine in DMF (20%; 2 × 4 cm^3) was added to the resin in two steps. The first portion was sucked quickly through the resin followed by addition of the second portion, which was removed after 30 min. After a thorough rinse with DMF (8 × 4 cm^3), Rink-linker (57 mg, 0.105 mmol), TBTU (34 mg, 0.105 mmol) and *N*-ethylmorpholine (26 mm^3, 0.21 mmol) were dissolved in DMF (4 cm^3) and added to the resin. After 2 h the resin was rinsed with DMF (8 × 4 cm^3) before *N*-Fmoc deprotection and coupling of N^{α}-Fmoc-L-Phe-OPfp and N^{α}-Fmoc-L-Asn-OPfp as described above. After final deprotection the peptide-resin was rinsed with dichloromethane and dried before cleavage of the peptide from the solid support with TFA–ethane-1,2-dithiol–thioanisole–anisole–water (67:1:1:2.7:2.7) to yield crude dipeptide **20**.

N^{α}-(*Fluoren-9-ylmethoxycarbonyl*)-L-*aspartic* 4-*Acid Chloride* 1-*Pentafluorophenyl Ester* **2**.—N^{α}-Fmoc-L-Asp(OBu[t])-OPfp **1** (2.0 g, 3.47 mmol) was treated with a mixture of TFA (2.57 cm^3, 34.7 mmol) and thionyl dichloride (25.0 cm^3, 347 mmol) at 40 °C for 24 h, then the mixture was concentrated and co-concentrated with THF. The resulting *title compound* **2** was pure according to ^1H and ^{13}C NMR spectroscopy and was used without further purification; δ_H(500 MHz; $CDCl_3$) (*inter alia*) 5.758 (1 H, d, $J_{\alpha N^{\alpha}H}$ 8.0, NH), 5.024 (1 H, m, H^{α}), 3.792 (1 H, dd, $J_{\alpha\beta}$ 4.5, $J_{\beta\beta'}$ 19.0, H^{β}), 3.670 (1 H, dd, $J_{\alpha\beta'}$ 3.5, $H^{\beta'}$), 4.280 (1 H, t, Fmoc CH_2CH) and 4.538 (2 H, br dd, Fmoc CH_2CH); δ_C(125.77 MHz; $CDCl_3$) (*inter alia*) 50.3 (C^{α}), 48.6 (C^{β}), 47.0 (Fmoc CH_2CH) and 67.7 (Fmoc CH_2CH) [Found: FAB–MS *m/z* 539.87 ($M + H^+$). $C_{25}H_{15}ClF_5NO_5$ requires M, 539.06].

Preparation of Glycosylamines.—β-D-Glucopyranosylamine **21** and β-D-mannopyranosylamine monohydrate **22** were

prepared as previously reported.[26,27] The β-D-glycosylamines **23**, **24**, **25** and **4**, of *N*-acetyl-D-glucosamine, 4-*O*-β-D-glucopyranosyl-D-glucose (cellobiose), 4-*O*-β-D-galactopyranosyl-D-glucose (lactose) and 4-*O*-α-D-glucopyranosyl-D-glucose (maltose), respectively, were prepared essentially as previously described,[24,25] except for the fact that ion-exchange chromatography was omitted. Thus, the saccharide was dissolved in water (0.1 mmol cm^3) and treated with an excess of ammonium hydrogencarbonate for 7 days at 30 °C. The solution was concentrated to half its original volume and diluted with water. This procedure was repeated twice. After lyophilization of the solution, the product, which according to NMR spectroscopy was more than 90% pure, was used directly in the next step.

2,3,4,6-*Tetra*-O-*acetyl*-N-(*fluoren*-9-*ylmethoxycarbonyl*)-β-D-*glucopyranosylamine* **16**.—β-D-Glucopyranosylamine[26] **21** (2.0 g, 11.2 mmol) was suspended in pyridine (30 cm^3), Fmoc-OSu (4.0 g, 11.9 mmol) was added, and the mixture was stirred overnight at ambient temperature. Acetic anhydride (19 cm^3, 200 mmol) was added and the reaction mixture was left overnight. After concentration and co-concentration with toluene, the residue was dissolved in dichloromethane (15 cm^3) and filtered through Celite. Purification by VLC [ethyl acetate–light petroleum (1:2)] afforded *title compound* **16** (4.9 g, 77%), $[\alpha]_D^{20}$ −6.4 (*c* 0.88, $CHCl_3$); m.p. 112–114 °C (from THF–hexane) (Found: C, 61.0; H, 5.6; N, 2.8. $C_{29}H_{31}NO_{11}$ requires C, 61.2; H, 5.5; N, 2.5%; *M*, 569.58); ^1H and ^{13}C NMR data are presented in Tables 1 and 4, respectively.

2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-N-(*fluoren*-9-*ylmethoxycarbonyl*)-β-D-*glucopyranosylamine*[10] **26**.—Crude 2-acetamido-2-deoxy-β-D-glucopyranosylamine[24] **23** (10.0 g, 45 mmol) was suspended in pyridine (125 cm^3), Fmoc-OSu (16.9 g, 50 mmol) was added, and the mixture was stirred overnight at ambient temperature. More pyridine (100 cm^3), and acetic anhydride (40.0 cm^3, 424 mmol), were added. After 4 h, water (25 cm^3) was added in small portions followed by additional water (400 cm^3) and cooling to 0 °C. After the mixture had been stirred at 0 °C for 1 h crude compound **26** was isolated by filtration. Recrystallization afforded *title compound* **26** (10.0 g, 39%), $[\alpha]_D^{20}$ −15.2 (*c* 1.50, $CHCl_3$); m.p. 228–230 °C (from THF–hexane) (Found: C, 60.8; H, 5.9; N, 4.8. $C_{29}H_{32}N_2O_{10}$ requires C, 61.3; H, 5.7; N, 4.9%; *M*, 568.59); ^1H and ^{13}C NMR data are presented in Tables 1 and 4, respectively.

2,3,4,6-*Tetra*-O-*acetyl*-N-(*fluoren*-9-*ylmethoxycarbonyl*)-β-D-*mannopyranosylamine* **17**.—*Title compound* **17** was prepared in 75% yield (syrup) from β-D-mannopyranosylamine monohydrate[27] **22** as described for compound **16**; $[\alpha]_D^{20}$ −18.5 (*c* 1.20, $CHCl_3$) (Found: C, 60.1 H, 5.6; N, 2.7. $C_{29}H_{31}NO_{11}$ requires C, 61.2; H, 5.5; N, 2.5%; *M*, 569.58); ^1H and ^{13}C NMR data are presented in Tables 1 and 4, respectively.

2,3,6-*Tri*-O-*acetyl*-N-(*fluoren*-9-*ylmethoxycarbonyl*)-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-β-D-*glucopyranosylamine* **27**.—*Title compound* **27** was prepared in 32% yield from β-D-cellobiosylamine[24] **24** as described for compound **16**; $[\alpha]_D^{20}$ −14.9 (*c* 1.38, $CHCl_3$); m.p. 230–232 °C (from THF–hexane) (Found: C, 57.2; H, 5.6; N, 1.5. $C_{41}H_{47}NO_{19}$ requires C, 57.4; H, 5.5; N, 1.6%; *M*, 857.83); ^1H and ^{13}C NMR data are presented in Tables 2 and 5, respectively.

2,3,6-*Tri*-O-*acetyl*-N-(*fluoren*-9-*ylmethoxycarbonyl*)-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosylamine* **28**.—*Title compound* **28** (syrup) was prepared in 47% yield [VLC with eluent ethyl acetate–light petroleum (7:5)] from β-D-lactosylamine[24] **25** as described for compound **16**. $[\alpha]_D^{20}$ −3.2 (*c* 1.73, $CHCl_3$) (Found: C, 56.5; H, 5.7; N, 1.6. $C_{41}H_{47}NO_{19}$ requires C, 57.4; H, 5.5; N, 1.6%; *M*, 857.83); ^1H and ^{13}C NMR data are presented in Tables 2 and 5, respectively.

2,3,6-*Tri*-O-*acetyl*-N-(*fluoren*-9-*ylmethoxycarbonyl*)-4-O-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*glucopyranosyl*)-β-D-*gluocpyranosylamine* **5**.—*Title compound* **5** was prepared in 37% yield [VLC with eluent ethyl acetate–light petroleum (7:5)] from β-D-maltosylamine[24] **4** as described for compound **16**; $[\alpha]_D^{20}$ +54.5 (*c* 0.48, $CHCl_3$); m.p. 184–186 °C (from THF–hexane) (Found: C, 57.0; H, 5.6; N, 1.7. $C_{41}H_{47}NO_{19}$ requires C, 57.4; H, 5.5; N, 1.6%; *M*, 857.83); ^1H and ^{13}C NMR data are presented in Tables 2 and 5, respectively.

2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosylamine* **29**.—Compound **16** (1.0 g, 1.76 mmol) was suspended in THF (10 cm^3) and piperidine (4.4 cm^3) was added. After 10 min at ambient temperature the mixture was treated with hexane (60 cm^3). Filtration and washing with hexane afforded title compound **29** (0.51 g, 84%), $[\alpha]_D^{20}$ +12.3 (*c* 1.00, $CHCl_3$); m.p. 120–122 °C (decomp., from THF–hexane) {lit.,[32] $[\alpha]_D$ +18 (*c* 0.5, $CHCl_3$); m.p. 122–124 °C; lit.,[31] $[\alpha]_D$ +11 (*c* 0.5, methanol); m.p. 126 °C (Found: C, 47.8; H, 6.1; N, 4.6. Calc. for $C_{14}H_{21}NO_9$: C, 48.4; H, 6.1; N, 4.0%; *M*, 347.32); ^1H and ^{13}C NMR data are presented in Tables 1 and 4, respectively.

2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosylamine*[10] **30**.—Compound **9** (1.5 g, 2.64 mmol) was suspended in THF (40 cm^3) and piperidine (10 cm^3) was added. After 10 min at ambient temperature the solution was clear and after a further 20 min hexane (110 cm^3) was added. Filtration and washing with hexane afforded title compound **30** (0.81 g, 92%), $[\alpha]_D^{20}$ −19.3 (*c* 1.14, $CHCl_3$); the compound turned brown at 140 °C and decomposed at 230 °C {lit.,[29] $[\alpha]_D^{28}$ −5.2 (*c* 1.27, $CHCl_3$); lit.,[30] $[\alpha]_D^{24}$ −22.9 (*c* 1.15, $CHCl_3$)} (Found: C, 48.1; H, 6.6; N, 7.6. Calc. for $C_{14}H_{22}N_2O_8$: C, 48.6; H, 6.4; N, 8.1%; *M*, 346.34); ^1H and ^{13}C NMR data are presented in Tables 1 and 4, respectively.

2,3,4,6-*Tetra*-O-*acetyl*-β-D-*mannopyranosylamine* **31**.—*Title compound* **31** was prepared in 66% yield from compound **17** as described for compound **29**; $[\alpha]_D^{20}$ −10.2 (*c* 1.32, $CHCl_3$); m.p. 148–149 °C (from THF–hexane) (Found: C, 48.7; H, 6.2; N, 4.1. $C_{14}H_{21}NO_9$ requires C, 48.4; H, 6.1; N, 4.0%; *M*, 347.32); ^1H and ^{13}C NMR data are presented in Tables 1 and 4, respectively.

2,3,6-*Tri*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-β-D-*glucopyranosylamine* **32**.—Compound **27** (0.76 g, 1.20 mmol) was dissolved in DMF (2 cm^3) and morpholine (2 cm^3) was added. After 15 min the solution was diluted with toluene (5 cm^3) and concentrated, followed by co-concentration with toluene (10 cm^3) twice. The residue was dissolved in dichloromethane (5 cm^3) and filtered through Celite. Purification by VLC [ethyl acetate–light petroleum (4:1) to (9:1)] afforded *title compound* **32** (0.42 g, 75%), $[\alpha]_D^{20}$ −6.5 (*c* 0.81, $CHCl_3$); m.p. 200–202 °C (from THF–hexane) (Found: C, 49.2; H, 5.9; N, 2.3. $C_{26}H_{37}NO_{17}$ requires C, 49.1; H, 5.9; N, 2.2%; *M*, 635.58); ^1H and ^{13}C NMR data are presented in Tables 2 and 5, respectively.

2,3,6-*Tri*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosylamine* **33**.—*Title compound* **33** was prepared in 70% yield (syrup) from compound **28** as described for compound **32**; $[\alpha]_D^{20}$ +3.3 (*c* 0.46, $CHCl_3$) (Found: C, 48.1; H, 5.8; N, 2.3%); ^1H and ^{13}C NMR data are presented in Tables 2 and 5, respectively.

Table 1 Selected ^{1}H NMR chemical shifts and coupling constants (Hz, in parentheses) of compounds **16**, **26**, **17**, **29**, **30** and **31** measured at 500 MHz on solutions in $CDCl_3$ at 300 K

	16	**26**[a]	**17**	**29**[b]	**30**[a]	**31**
Fmoc CH_2	4.44 (7.0)	4.36 (9.8, 13.5)	4.48 (6.5, 16.0)			
CH_2'	4.39 (7.0)	4.23	4.48 (6.5)			
CH	4.25	4.23	4.25			
N^1H	5.70 (9.5)	8.21 (9.6)	5.62 (10.0)		2.40	2.13 (10.0)
1-H	5.10 (9.5)	5.05 (9.6)	5.38 (1.0)	4.2 (9.5)	4.16 (9.6)	4.49 (1.0)
N^2H		7.97 (9.0)			7.83 (9.6)	
2-H	5.00 (9.5)	3.88 (9.8)	5.46 (1.0)	4.85 (9.5)	3.62 (9.8)	5.44 (3.5)
3-H	5.35 (9.5)	5.15 (9.8)	5.15 (9.0)	5.27 (9.5)	5.04 (9.8)	5.11 (10.0)
4-H	5.12 (9.5)	4.81 (9.8)	5.27 (10.0)	5.06 (9.5)	4.77 (9.8)	5.23 (10.0)
5-H	3.85	3.81	3.81	3.72	3.71	3.70
6-H	4.36 (3.5, 13.0)	4.18 (3.8, 12.5)	4.36 (4.0, 12.0)	4.24 (4.6, 12.1)	4.11 (4.9, 12.1)	4.29 (5.5, 12.0)
6'-H	4.14 (1.5)	3.96 (2.1)	4.13 (1.5)	4.11 (2.6)	3.97 (2.4)	4.15 (2.5)

[a] From ref. 10 NMR spectra were recorded in $(CD_3)_2SO$. [b] From ref. 32.

Table 2 Selected ^{1}H NMR chemical shifts and coupling constants (Hz, in parentheses) of compounds **27**, **28**, **5**, **32**, **33** and **6** measured at 500 MHz on solutions in $CDCl_3$ at 300 K

	27	**28**	**5**	**32**	**33**	**6**
Fmoc CH_2	4.44	4.42	4.43[a]			
CH_2'	4.24	4.22 (7.0, 10.0)	4.28[a]			
CH	4.38	4.35 (7.0)	4.43[a]			
N^1H	5.61 (10.0)	5.70 (9.0)	5.57 (9.0)	1.96[b] (8.0)	1.97[b]	1.96[b] (7.0)
1^b-H	5.03 (9.5)	5.03 (9.0)	5.10 (9.5)	4.19 (8.5)	4.18	4.25 (9.5)
2^b-H	4.90 (10.0)	4.89 (9.0)	4.83 (9.0)	4.77 (9.0)	4.76 (9.5)	4.70 (9.5)
3^b-H	5.32 (9.5)	5.31 (9.0)	5.40 (9.5)	5.25 (9.0)	5.26 (9.5)	5.33 (9.5)
4^b-H	3.78[a] (9.5)	3.81 (9.0)	4.02 (9.0)	3.75 (9.5)	3.76 (9.5)	3.94 (9.5)
5^b-H	3.78[a]	3.76	3.83	3.62	3.64	3.70
6^b-H	4.54 (8.5)	4.46 (12.0)	4.49 (12.8)	4.52 (1.5, 12.0)	4.49 (2.0, 12.0)	4.50 (2.5, 12.0)
6^b-H'	4.17	4.28 (7.5)	4.28	4.11 (5.0)	4.10 (5.5)	4.22 (4.5)
1^a-H	4.53 (8.5)	4.50 (8.0)	5.44 (3.8)	4.54 (8.0)	4.51 (7.8)	5.44 (4.0)
2^a-H	4.97 (9.5)	5.13 (10.0)	4.90 (10.5)	4.96 (8.5)	5.13 (10.5)	4.90 (10.5)
3^a-H	5.18 (9.5)	4.98 (3.5)	5.40 (9.8)	5.18 (9.0)	4.98 (3.5)	5.40 (10.0)
4^a-H	5.11 (10.0)	5.38 (1.0)	5.10 (9.8)	5.10 (9.5)	5.38 (1.0)	5.09 (10.0)
5^a-H	3.70	3.90	3.98	3.69	3.90	4.00
6^a-H	4.41 (4.5, 12.5)	4.15 (6.0, 11.0)	4.28 (12.8)	4.41 (4.5, 12.5)	4.17 (6.5, 11.0)	4.29 (4.0, 12.5)
6^a-H'	4.09 (2.0)	4.10 (7.5)	4.09	4.08 (2.0)	4.11 (7.5)	4.09 (2.5)

[a] Approximate values due to high complexity of the spin systems. [b] Two protons.

Table 3 Selected ^{1}H NMR chemical shifts and coupling constants (Hz, in parentheses) of compounds **8**, **9**, **10**, **11**, **12** and **7** measured at 500 MHz on solutions in $CDCl_3$ at 300 K

	8	**9**[a]	**10**	**11**	**12**	**7**
Asn $N^\alpha H$	6.20 (9.5)	8.12 (8.0)	6.16 (9.0)	6.23 (9.5)	6.21 (9.5)	6.24 (9.5)
H^α	5.06	4.85	5.04	5.04	5.05	5.05
H^β	3.11 (4.5, 12.0)	2.87 (6.5, 16.2)	3.18 (4.5, 17.0)	3.08 (4.0, 17.0)	3.08 (4.0, 17.0)	3.08 (4.0, 17.0)
$H^{\beta'}$	2.91 (4.0)	2.71 (6.9)	2.95 (3.5)	2.89 (3.5)	2.89 (3.5)	2.89 (3.5)
Fmoc CH_2	4.53 (7.0, 10.5)	4.39 (7.0, 10.3)	4.52 (7.0, 10.5)	4.27	4.26	4.27
CH_2'	4.42 (7.5)	4.33 (7.0)	4.40 (7.5)	4.51	4.53 (7.0, 10.5)	4.53 (7.0, 10.5)
CH	4.28	4.23	4.27	4.40[b]	4.41 (7.5)	4.41 (7.0)
$N^\gamma H$	6.44 (9.0)	8.77 (9.3)	6.47 (9.0)	6.42 (9.0)	6.36 (9.0)	6.36 (9.0)
1^b-H				5.24 (9.5)	5.25 (9.0)	5.32 (9.5)
2^b-H				4.86 (9.5)	4.86 (9.5)	4.79 (9.5)
3^b-H				5.34 (9.5)	5.36 (9.5)	5.43 (9.5)
4^b-H				3.80 (9.5)	3.82 (9.5)	4.01 (9.5)
5^b-H				3.75	3.76	3.82
6^b-H				4.51 (2.0, 12.5)	4.48	4.48 (2.0, 12.0)
6^b-H'				4.17	4.18	4.28 (3.5)
1^a-H	5.30 (9.5)	5.18 (9.5)	5.60 (1.0)	4.55 (8.0)	4.50 (8.0)	5.43 (4.0)
2^a-H	4.96 (9.5)	3.88 (9.8, 9.4)	5.40 (3.5)	4.96 (9.5)	5.15 (10.5)	4.90 (10.5)
3^a-H	5.37 (9.5)	5.11 (9.8)	5.16 (10.0)	5.18 (9.5)	4.99 (3.5)	5.40 (10.0)
4^a-H	5.11 (9.5)	4.82 (9.8)	5.28 (10.0)	5.11 (9.5)	5.40 (1.0)	5.10 (10.0)
5^a-H	3.85	3.83	3.82	3.70	3.91	3.96
6^a-H	4.36 (4.5, 12.5)	4.16 (3.8, 12.5)	4.35 (5.0, 12.5)	4.40[b]	4.20 (7.0, 11.5)	4.26
6^a-H'	4.12 (2.0)	3.92 (2.5)	4.11 (2.0)	4.08 (2.0, 12.5)	4.11 (7.0)	4.09

[a] From ref. 10. NMR spectra were recorded in $(CD_3)_2SO$. 2^a-NH δ 7.90. [b] Approximate values due to the high complexity of the spin systems.

2,3,6-*Tri*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*glucopyranosyl*)-β-D-*glucopyranosylamine* **6**.—*Title compound* **6** was prepared in 87% yield from compound **5** as described for compound **31**; $[\alpha]_D^{20}$ +72.9 (*c* 0.63, $CHCl_3$); m.p. 189–190 °C (from THF–hexane) (Found: C, 48.9; H, 5.9; N, 2.3%); ^1H and ^{13}C NMR data are presented in Tables 2 and 5, respectively.

Table 4 Selected ^{13}C NMR chemical shifts of compounds **16**, **26**, **17**, **29**, **30** and **31** measured at 125.77 MHz on solutions in $CDCl_3$ at 300 K

	16	26[a]	17	29	30[a]	31
Fmoc[b] CH	46.8	46.4	46.9			
CH_2	67.6	65.9	67.5			
C=O	155.8	155.7	154.9			
C-1	80.9	80.6	78.4	85.0	85.2	82.2
C-2	70.3	52.1	70.0	72.1	54.3	70.7
C-3	72.7	73.3	71.6	72.7	73.5	72.0
C-4	68.1	68.3	65.0	68.8	69.1	65.9
C-5	73.4	72.0	73.9	73.2	71.3	73.3
C-6	61.6	61.8	62.1	62.3	62.4	62.8

[a] NMR spectra were recorded in $(CD_3)_2SO$. [b] Fmoc aromatic carbons resonate at δ_C 143.5, 141.3, 127.7, 127.1, 125.0 and 120.0.

Table 5 Selected ^{13}C NMR chemical shifts of compounds **27**, **28**, **5**, **32**, **33** and **6** measured at 125.77 MHz on solutions in $CDCl_3$ at 300 K

	27	28	5	32	33	6
Fmoc[a] CH	46.8	46.8	46.9			
CH_2	67.5	67.4	67.6			
C=O	155.2	155.2	155.2			
C-1[b]	80.7	80.6	80.4	84.7	84.6	84.4
C-2[b]	70.5	70.6	71.0	72.4	72.5	72.9
C-3[b]	72.2	72.4	75.1	72.7	73.0	75.7
C-4[b]	76.2	75.9	72.6	76.8	76.6	73.0
C-5[b]	74.3	74.2	73.8	73.7	73.7	73.1
C-6[b]	61.8	61.9	62.7	62.2	62.3	63.1
C-1[a]	100.7	100.8	95.6	100.8	101.0	95.5
C-2[a]	71.6	69.0	70.0	71.6	69.1	70.0
C-3[a]	72.9	70.9	69.3	72.9	71.0	69.3
C-4[a]	67.8	66.6	68.0	67.8	66.6	68.0
C-5[a]	72.0	70.6	68.6	71.9	70.6	68.5
C-6[a]	61.6	60.8	61.4	61.6	60.8	61.5

[a] Fmoc aromatic carbons resonate at δ_C 143.5, 141.3, 127.7, 127.1, 125.0 and 120.0.

N^{α}-(*Fluoren*-9-*ylmethoxycarbonyl*)-N^{γ}-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-L-*asparagine Pentafluorophenyl Ester* **8**.—Compound **29** (0.4 g, 1.15 mmol) was dissolved in THF (5 cm^3) and *N*-ethylmorpholine (152 mm^3, 1.20 mmol) was added. The mixture was added dropwise to a solution of compound **2** (1.19 mmol) in THF (8 cm^3) at 0 °C. *N*-Ethylmorpholine hydrochloride precipitated almost instantaneously. Filtration of the reaction mixture, concentration of the filtrate, and dissolution of the residue in dichloromethane (2 cm^3) and purification (VLC) under dry conditions [ethyl acetate–light petroleum (1:2)] afforded *title compound* **8** (0.7 g, 72%); $[\alpha]_D^{20}$ +18.9 (*c* 0.91, $CHCl_3$); m.p. 193–195 °C (from THF–hexane) (Found: C, 55.0 H, 4.2; N, 3.3. $C_{39}H_{35}F_5N_2O_{14}$ requires C, 55.1; H, 4.1; N, 3.3%; M, 850.71); ^1H and ^{13}C NMR data are presented in Tables 3 and 6, respectively.

N^{γ}-(2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*)-N^{α}-(*fluoren*-9-*ylmethoxycarbonyl*)-L-*asparagine Pentafluorophenyl Ester*[10] **9**.—Compound **30** (0.58 g, 1.70 mmol) was dissolved in THF (17 cm^3) and *N*-ethylmorpholine (0.24 cm^3, 1.90 mmol) was added. The mixture was added dropwise to a solution of compound **2** (1.90 mmol) in THF (5 cm^3) at 0 °C. *N*-Ethylmorpholine hydrochloride precipitated almost instantaneously and filtration of the reaction mixture followed by addition of hexane afforded the crystalline title compound **9** (1.20 g, 84%) which was filtered off, $[\alpha]_D^{20}$ +7.7 (*c* 1.03, $CHCl_3$); the compound turned brown at 120 °C and decomposed at 190 °C (Found: C, 54.5; H, 4.6; N, 4.9. Calc. for $C_{39}H_{36}F_5N_3O_{13}$: C, 55.1; H, 4.3; N, 5.0%; M, 849.73); ^1H and ^{13}C NMR data are presented in Tables 3 and 6, respectively.

N^{α}-(*Fluoren*-9-*ylmethoxycarbonyl*)-N^{γ}-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*mannopyranosyl*)-L-*asparagine Pentafluorophenyl Ester* **10**.—*Title compound* **10** (syrup) was prepared in 91% yield [VLC with eluent ethyl acetate–light petroleum (2:3)] from substrates **31** and **2** as described for compound **8**; $[\alpha]_D^{20}$ +0.3 (*c* 0.76, $CHCl_3$) (Found: C, 54.3; H, 4.6; N, 3.0. $C_{39}H_{35}F_5N_2O_{14}$ requires C, 55.1; H, 4.1; N, 3.3%; M, 850.71); ^1H and ^{13}C NMR data are presented in Tables 3 and 6, respectively.

N^{α}-(*Fluoren*-9-*ylmethoxycarbonyl*)-N^{γ}-[2,3,6-*tri*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-β-D-*glucopyranosyl*]-L-*asparagine Pentafluorophenyl Ester* **11**.—*Title compound* **11** was prepared in 50% yield [VLC with eluent ethyl acetate–light petroleum (1:1)] from substrates **32** and **2** as described for compound **8**; $[\alpha]_D^{20}$ +5.1 (*c* 1.48, $CHCl_3$); m.p. 178–182 °C

Table 6 Selected ^{13}C NMR chemical shifts of compounds **8**, **9**, **10**, **11**, **12** and **7** measured at 125.77 MHz on solutions in $CDCl_3$ at 300 K

	8	9[a]	10	11	12	7
Asn C^{α}	50.1	49.9	50.2	50.2	50.2	50.1
C^{β}	37.7	36.4	37.7	37.6	37.5	37.6
Fmoc[b] CH	47.0	46.5	47.0	47.0	47.0	47.0
CH_2	67.5	65.9	67.6	67.5	67.5	67.5
C=O	155.9	155.8	156.0	155.9	155.9	156.0
Pfp	136.8–142.0	136.8–142.0	136.8–142.0	136.8–142.0	136.8–142.0	136.8–142.0
C-1[b]				78.1	78.0	77.7
C-2[b]				70.8	70.9	71.3
C-3[b]				71.9	72.2	74.8
C-4[b]				76.0	75.8	72.4
C-5[b]				74.7	74.7	74.1
C-6[b]				61.7	61.8	62.6
C-1[a]	78.2	78.1	76.0	100.6	100.8	95.6
C-2[a]	70.6	52.1	69.9	71.5	69.0	70.0
C-3[a]	73.7	73.3	71.5	72.9	70.9	69.3
C-4[a]	67.9	68.3	65.0	67.8	66.6	67.9
C-5[a]	72.4	72.2	74.3	72.0	70.7	68.6
C-6[a]	61.5	61.8	62.1	61.6	60.8	61.4

[a] NMR spectra were recorded in $(CD_3)_2SO$. [b] Fmoc aromatic carbons resonate at δ_C 143.5, 141.3, 127.7, 127.1, 125.0 and 120.0.

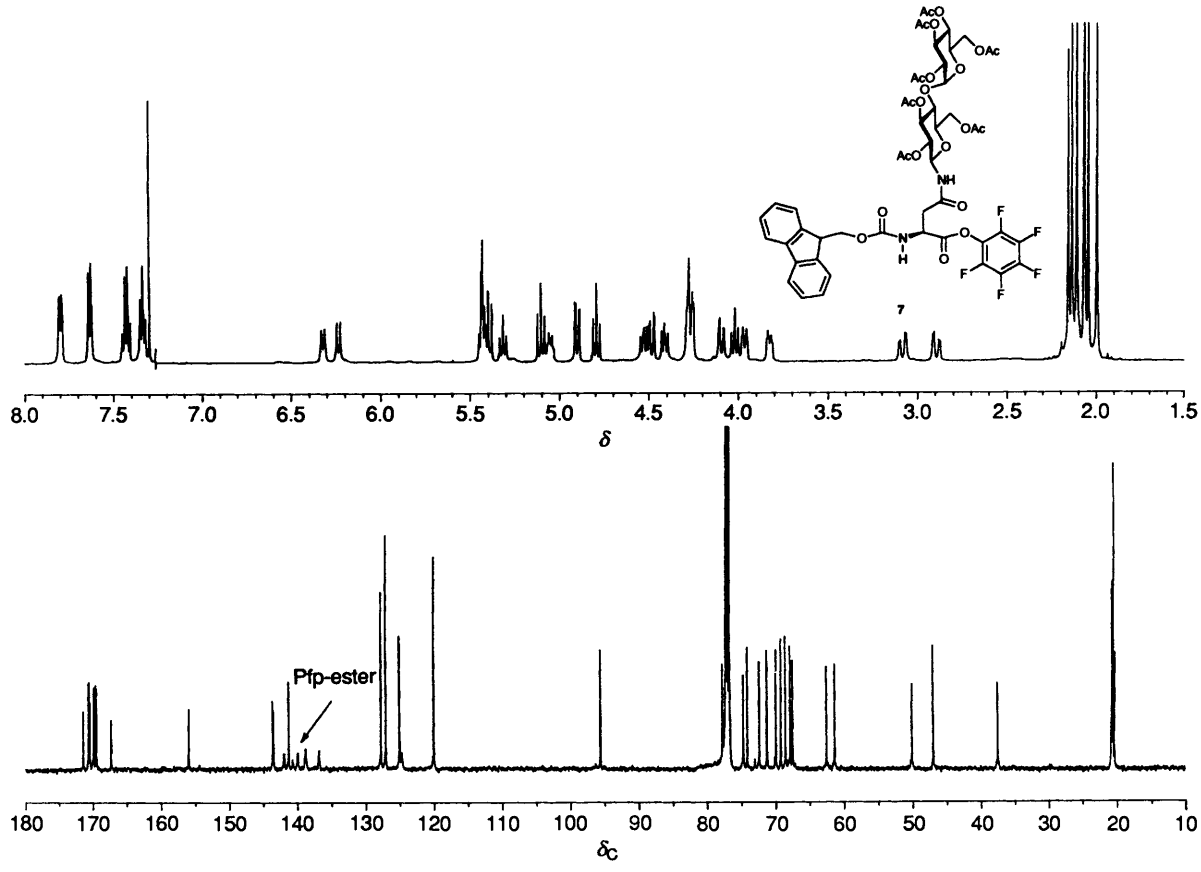


Fig. 3 ^{1}H NMR spectrum (top) and ^{13}C NMR spectrum (bottom) of building block **7**. The pentafluorophenyl carbon atoms are seen at δ_C 136.8–142.0 as small peaks due to carbon–fluorine coupling.

(from THF–hexane) (Found: C, 53.6; H, 4.9; N, 2.5. $C_{51}H_{51}F_5N_2O_{22}$ requires C, 53.8; H, 4.5; N, 2.5%; M, 1138.97); ^{1}H and ^{13}C NMR data are presented in Tables 3 and 6, respectively.

N$^{\alpha}$-(*Fluoren-9-ylmethoxycarbonyl*)-N$^{\gamma}$-[2,3,6-*tri*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-L-*asparagine Pentafluorophenyl Ester* **12**.—*Title compound* **12** was prepared in 36% yield [VLC with eluent ethyl acetate–light petroleum (1:1)] from substrates **33** and **2** as described for compound **8**; $[\alpha]_D^{20}$ +5.9 (*c* 0.96, $CHCl_3$); m.p. 172–174 °C (from THF–hexane) (Found: C, 53.9; H, 4.5; N, 2.4%); ^{1}H and ^{13}C NMR data are presented in Tables 3 and 6, respectively.

N$^{\alpha}$-(*Fluoren-9-ylmethoxycarbonyl*)-N$^{\gamma}$-[2,3,6-*tri*-O-*acetyl*-4-O-(2,3,4,6-*tetra*-O-*acetyl*-α-D-*glucopyranosyl*)-β-D-*glucopyranosyl*]-L-*asparagine Pentafluorophenyl Ester* **7**.—*Title compound* **7** was prepared in 76% yield [VLC with eluent ethyl acetate–light petroleum (5:7)] from substrates **6** and **2** as described for compound **8**; $[\alpha]_D^{20}$ +56.5 (*c* 1.29, $CHCl_3$); m.p. 160–165 °C (from THF–hexane) (Found: C, 53.5; H, 4.6; N, 2.3%); ^{1}H and ^{13}C NMR data are presented in Tables 3 and 6, respectively, and in Fig. 3.

H-Asn-Phe-NH$_2$ **20** *and H-Asn*-[2,3,6-*tri*-O-*acetyl*-4-O-(2,3,-4,6-*tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-β-D-*glucopyranosyl*]-*Phe-NH*$_2$ **18**.—Title compounds **20** and **18** were synthesized by the plastic syringe technique as described above. In the synthesis of compound **18** H-Phe-Resin was coupled with compound **12** (80 mg, 0.07 mmol) to give peptide-resin **A**.

Investigation of the Stability of N-*Glycodipeptide* **18** *towards TFA/Scavenger Mixtures*.—Portions of peptide-resin **A** (50 mg) were treated with four different TFA cleavage-mixtures [2.5 cm^3 of TFA–water (95:5), TFA–dichloromethane (7:3), TFA–ethane-1,2-dithiol–thioanisole–anisole–water (67:1:2.7:2.7) or TFA–dichloromethane-triisopropylsilane (7:2:1)] and samples (0.5 cm^3) were taken out after 30 min, 1 h, 2 h and 24 h. Samples were filtered through a glass filter, concentrated, and triturated with diethyl ether. The residues were dissolved in DMF (0.25 cm^3) and analysed by analytical HPLC using 100% solvent A for 10 min, followed by a linear gradient of 0–100% solvent B during 50 min. The resulting chromatograms showed essentially one peak (> 95%; t_R 39 min) with all the cleavage mixtures and cleavage times tried. Samples were pooled, and purified by preparative HPLC, and *N*-lactosyldipeptide **18** was isolated and its structure confirmed by NMR and amino acid analyses (theoretical value in parenthesis): Asp 0.97 (1), Phe 0.95 (1). As for compound **18**, crude dipeptide **20** was analysed by analytical HPLC (t_R 23 min); δ_H(500 MHz; $CDCl_3$; **18**) (*inter alia*) 4.525 (1 H, d, $J_{1a,2a}$ 8.0, 1^a-H), 5.127 (1 H, dd, $J_{2a,3a}$ 10.5, 2^a-H), 4.997 (1 H, dd, $J_{3a,4a}$ 3.5, 3^a-H), 5.385 (1 H, dd, $J_{4a,5a}$ 1.0, 4^a-H), 3.917 (1 H, m, 5^a-H), 4.177 (1 H, dd, $J_{5a,6a}$ 6.0, $J_{5a,6a'}$ 11.0, 6^a-H), 4.105 (1 H, dd, $J_{5a,6a'}$ 7.5, 6^a-H'), 5.187 (1 H, dd, $J_{1b,2b}$ 9.0, 1^b-H), 4.910 (1 H, dd, $J_{2b,3b}$ 9.5, 2^b-H), 5.275 (1 H, dd, $J_{3b,4b}$ 9.0, 3^b-H), 3.813 (1 H, m, $J_{4b,5b}$ 9.5, 4^b-H), 3.743 (1 H, m, 5^b-H), 4.10 (1 H, m, 6^b-H), 4.53 (1 H, m, 6^b-H'), 4.13 (1 H, m, Asn H$^{\alpha}$), 2.84 (2 H, m, Asn H$^{\beta}$ and H$^{\beta'}$), 8.220 (1 H, d, $J_{N\gamma H1b}$ 9.5, Asn N$^{\gamma}$H), 8.420 (1 H, Phe NH), 4.53 (1 H, m, Phe H$^{\alpha}$), 3.150 (1 H, dd, $J_{\alpha\beta}$ 6.0, $J_{\beta\beta'}$ 13.5, Phe H$^{\beta}$), 3.028 (1 H, dd, $J_{\alpha\beta'}$ 8.5, Phe H$^{\beta'}$), 6.810 (1 H, Phe CONH) and 6.155 (1 H, Phe CONH'); δ_C(125.77 MHz; $CDCl_3$; **18**) (*inter alia*) 100.9 (C-1^a), 69.1 (C-2^a), 70.9 (C-3^a), 66.6 (C-4^a), 70.7 (C-5^a), 60.7 (C-6^a), 77.7 (C-1^b), 70.7 (C-2^b), 72.8 (C-3^b), 75.6 (C-4^b), 74.8 (C-5^b), 61.6 (C-6^b), 50.2 (Asn C$^{\alpha}$), 37.4 (Asn C$^{\beta}$), 55.5 (Phe C$^{\alpha}$) and 37.4 (Phe C$^{\beta}$).

Table 7 ^{1}H NMR chemical shifts and coupling constants (Hz) of compounds **15**[a] and **14**,[b] measured at 500 MHz or 600 MHz at 300 K. Reference: internal acetic acid at δ 2.03

		15	**14**			**15**	**14**
D-Ala^1	α	4.160 (7.0)	4.162 (7.1)	Asn^6	N$^\alpha$H	8.325 (8.0)	8.371 (7.6)
	β	1.536	1.539		α	4.694	4.742
Ser^2	N$^\alpha$H	8.690 (6.8)	8.708 (6.9)		β	2.762 (6.5, 15.5)	2.848 (6.2, 16.1)
	α	4.560	4.568		β′	2.669 (7.5)	2.762 (7.1)
	β	3.911 (5.5, 11.5)	3.911 (5.9, 11.5)		N$^\gamma$H	7.489	8.791 (8.8)
	β′	3.864 (5.0)	3.868 (5.2)		N$^\gamma$H′	6.819	
Thr^3	N$^\alpha$H	8.306 (8.0)	8.325 (7.8)	Tyr^7	N$^\alpha$H	8.183 (7.0)	8.205 (7.0)
	α	4.464 (4.5)	4.471 (4.3)		α	4.600	4.603
	β	4.279 (6.5)	4.284 (6.5)		β	3.045 (7.5, 14.0)	3.052 (7.0, 14.0)
	γ	1.202	1.206		β′	2.961 (8.0)	2.969 (8.0)
Thr^4	N$^\alpha$H	8.183 (7.0)	8.215 (7.5)		δ,δ′	7.110 (8.5)	7.114 (8.4)
	α	4.421 (4.5)	4.430 (4.6)		ε,ε′	6.807	6.812
	β	4.230 (6.5)	4.243 (6.4)	Thr^8	N$^\alpha$H	7.977 (8.0)	8.029 (8.0)
	γ	1.177	1.179		α	4.230 (4.0)	4.240
Thr^5	N$^\alpha$H	8.100 (7.5)	8.119 (7.6)		β	4.204 (6.5)	4.212 (6.3)
	α	4.297 (4.5)	4.298 (4.7)		γ	1.130	1.143
	β	4.108 (6.5)	4.097 (6.3)		CONH	6.968	6.978
	γ	1.095	1.088		CONH′	7.030	7.063

		1-H	2-H	3-H	4-H	5-H	6-H	6′-H
14	α-D-Glucopyranosyl (1 → 4)	5.367 (3.8)	3.561 (9.8)	3.672 (9.5)	3.395 (9.5)	3.697	3.864 (11.4)	3.748
	β-D-Glucopyranosyl	4.90[c]	3.389 (9.2)	3.798 (8.7)	3.625	3.743	3.832 (1.7, 12.2)	3.738 (5.1)

[a] 5.2 mg in 10% CD_3CO_2D in water (600 mm^3, pH 2.3). [b] 4.6 mg in 10% CD_3CO_2D in water (600 mm^3, pH 2.4). [c] Approximate value.

Table 8 Selected ^{13}C NMR chemical shifts of compounds **15**[a] and **14**,[b] measured at 125.77 MHz at 300 K. Reference: internal acetic acid at δ_C 20.0

		15	**14**			**15**	**14**
D-Ala^1	α	49.5	49.6	Asn^6	α	50.5	50.2
	β	16.8	16.9		β	36.2	36.7
Ser^2	α	55.9	55.9	Tyr^7	α	55.7	55.8
	β	61.4	61.4		β	36.3	36.2
Thr^3	α	59.3	59.3		γ	127.9	128.0
	β	67.2	67.3		δ,δ′	130.7	130.7
	γ	19.0	19.0		ε,ε′	115.8	115.9
Thr^4	α	59.3	59.3		ζ	154.8	154.9
	β	67.3	67.3	Thr^8	α	59.0	59.1
	γ	19.0	19.0		β	67.3	67.2
Thr^5	α	59.3	59.3		γ	19.0	19.0
	β	67.3	67.4				
	γ	19.0	19.0				

		C-1	C-2	C-3	C-4	C-5	C-6
14	α-D-Glucopyranosyl (1 → 4)	99.9	72.0	76.8	69.7	73.2	60.8
	β-D-Glucopyranosyl	79.3	72.0	77.2	76.4	72.9	60.8

[a] 15.0 mg in 10% CD_3CO_2D in water (600 mm^3, pH 2.7). [b] 4.6 mg in 10% CD_3CO_2D in water (600 mm^3, pH 2.4).

D-*Ala-Ser-Thr-Thr-Thr-Asn*-(4-O-α-D-*glucopyranosyl*-β-D-*glucopyranosyl*)-*Tyr-Thr-NH*$_2$ **14**.—The solid-phase synthesis was carried out on a custom-made, fully automatic, continuous-flow peptide synthesizer, according to the general procedure as described above. PEGA-resin (0.5 g, 0.07 mmol/g) was used together with 2 mol equiv. of substrate **7** (80 mg, 0.07 mmol) and 1 mol equiv. of Dhbt-OH (5.7 mg, 0.035 mmol). After completion of the coupling (4 h) a mixture of residual substrate **7** and Dhbt-OH was recovered (54 mg, 0.04 mmol). D-Alanine was incorporated as the free acid by the TBTU procedure.[39] After cleavage from the resin with TFA–ethane-1,2-dithiol–thioanisole–anisole–water (67:1:1:2.7:2.7) (22 cm^3; 2 h; ambient temperature) and several triturations with diethyl ether, the precipitated crude glycopeptide, hepta-*O*-acetate **13** (36 mg, 87% based on the amount of **7** consumed in the reaction. The crude material contained one major component.[50]), was purified by preparative HPLC using 10% solvent B for 20 min, followed by a linear gradient of 10–60% solvent B during 100 min (t_R 59.6 min). The yield of compound **13** after HPLC was 11 mg (27%). Deacetylation with sodium methoxide as described above, followed by purification by preparative HPLC using 100% solvent A for 10 min, followed by a linear gradient of 0–30% solvent B during 60 min (t_R 30.6 min) afforded pure title compound **14** (5.5 mg, 17%). ^{1}H and ^{13}C NMR data are presented in Tables 7 and 8, respectively. Amino acid analyses (theoretical value in parenthesis): Ala 1.00 (1), Asn 0.88 (1), Ser 1.06 (1), Thr 4.17 (4), Tyr 0.89 (1).

Analysis of CD Spectra.—The CD spectra of compounds **14**, **15** and **19**[41] were analysed by the SVD method combined with variable selection to give the following compositions for the five structural elements: *H* (α-helix), *A* (antiparallel β-strand), *P* (parallel β-strand), *T* (turn), *O* (other): **14** *H*, 10; *A* 34; *P* 0; *T* 22; *O* 35; total 101%; **15** *H*, 18; *A* 23; *P* 0; *T* 19; *O* 40; total 100%. The analysis of compound **19** did not give a satisfactory total fit when analysed by the SVD method.

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50 Loss of product as a result of irreversible retention of glycopeptides on reversed phase silica is currently under investigation in our laboratory.

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