Pergamon

Tetrahedron 54 (1998) 12047–12070

TETRAHEDRON

Use of Acid-labile Protective Groups for Carbohydrate Moieties in Synthesis of Glycopeptides Related to Type II Collagen

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Received 15 April 1998; accepted 23 July 1998

Abstract

β-D-Galactopyranosyl and α-D-glucopyranosyl-β-D-galactopyranosyl moieties carrying silyl, isopropylidene and 4-methoxybenzyl protective groups have been attached to the amino acid 5-hydroxy-L-norvaline. The resulting glycosylated building blocks were used in Fmoc solid-phase synthesis of glycopeptides related to a fragment from type II collagen which is immunodominant in a mouse model for rheumatoid arthritis. The protective groups used for the carbohydrate moieties were completely removed during acid catalyzed cleavage of the glycopeptides from the solid phase under conditions which left the *O*-glycosidic bonds intact.

Keywords: Glycopeptides; Protecting groups; Solid-phase synthesis

1 Introduction

In a cellular immune response T-cells recognize processed protein antigens as short peptides bound to major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells [1-4]. Even though most eucaryotic proteins are glycosylated it has not been established if glycoproteins can be processed to glycopeptides that are recognized by T cells when complexed by MHC molecules [5,6]. In collagen-induced arthritis, a mouse

PII: S0040-4020(98)00714-5

model for rheumatoid arthritis [7,8], autoimmune helper T cells are elicited by antigens obtained by processing of type II collagen (CII), the major glycoprotein of joint cartilage. T cell recognition of CII was recently revealed to involve a determinant encompassed within the fragment CII(256-270) and it was suggested that the recognition was dependent on the carbohydrates of CII [9,10]. The peptide fragment CII(256-270) has two lysine residues susceptible to posttranslational modifications [11], *i.e.* hydroxylation and subsequent glycosylation with either β-D-galactose or α-D-glucosyl-(1→2)-β-D-galactose moieties (cf. **1**). Glycopeptides with different levels of glycosylation are required in order to investigate the binding of CII(256-270) to the class II MHC molecule and the subsequent recognition of the complexes by helper T cells. As a first step in such efforts we now report the synthesis of glycopeptide analogues of CII(256-270) in which amino acids 264 and 270 have been replaced by 5-hydroxy-L-norvaline (Hnv) carrying Galβ- and Glcα(1→2)Galβ- residues [12].

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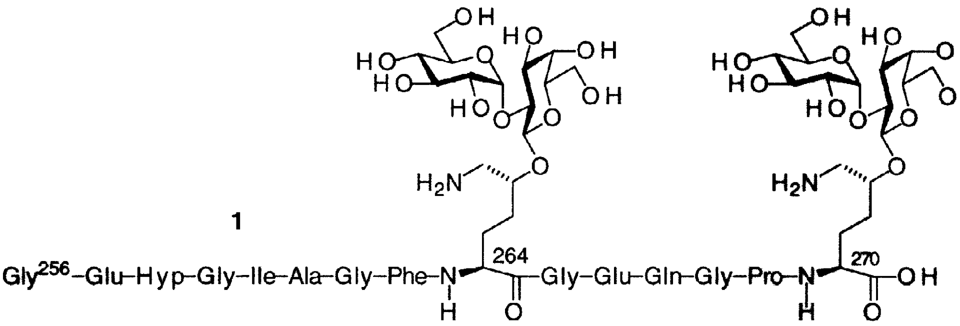


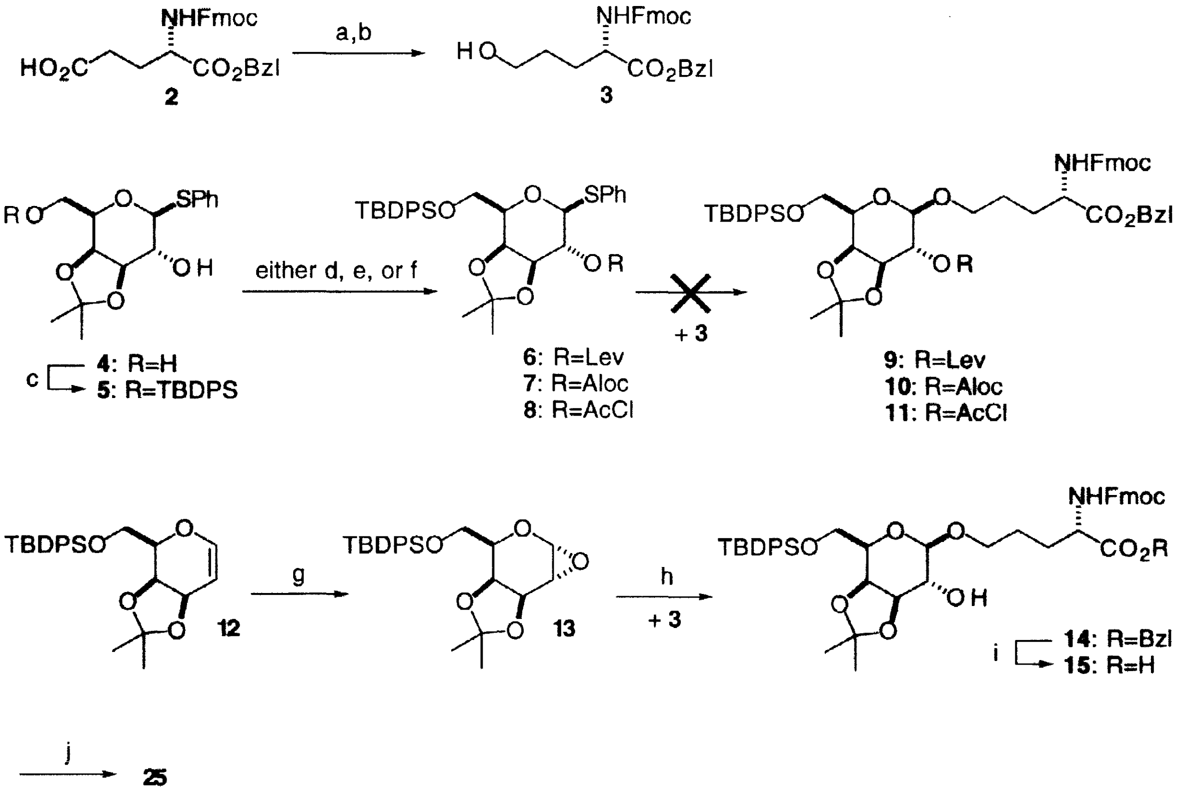
The use of glycosylated amino acids as building blocks in a stepwise assembly of glycopeptides on solid phase is an efficient method for preparation of *O*-linked glycopeptides [13,14,5,15]. The most frequently used protective group pattern for the glycosylated building blocks involves the fluoren-9-ylmethoxycarbonyl (Fmoc) group [16] for the α-amino group and acyl protection of the carbohydrate moiety, usually with acetyl or benzoyl groups. The acyl groups may be regarded as a precaution, which is required for deoxysugars such as fucosides [17,18], in order to stabilize the glycosidic bonds towards the acidic conditions used for cleavage of the glycopeptide from the solid phase and deprotection of the amino acid side chains [5]. Unfortunately the use of acetyl and benzoyl protective groups has some drawbacks. During coupling of the glycosylated amino acid to the peptide-resin *O* → *N* acyl migration may occur and terminate the growing peptide chain [19,20]. Base-mediated deprotection of *O*-acyl groups, especially debenzoylation of *O*-glycosylated serine and threonine residues may be accompanied by β-elimination, as well as epimerization of peptide stereocenters [21-24]. Other side-reactions have also been reported during deacylation of glycopeptides, *e.g.* cystein induced degradation [25] and hydrazide formation of aspartic acid residues [18]. Moreover, in the synthesis of a glycopeptide from protein S the low solubility of a *N*-linked peracetylated chitobiosyl building block made handling of the compound difficult [26]. Benzyl ethers, which are commonly used in synthetic carbohydrate chemistry, have recently found use also for building blocks employed in solid-phase glycopeptide synthesis [27-29]. However, deprotection of the benzyl groups by hydrogenolysis is incompatible with sulphur-containing residues, *i.e.* cystein and methionine, and to some extent with the indole moiety of tryptophan [30]. In addition, treatment with acid to liberate *O*-benzylated glycopeptides from the solid support leads to several partially de-*O*-benzylated products. Alternative strategies have utilized glycosylated amino acids in which the sugar hydroxyl groups were either left unprotected [31,32,23,33] or carried TMS protective groups [34]. Such strategies, however, severely restrict the possibilities for further synthetic manipulations of the glycosylated building blocks. Assembly of the glycosylated amino acid from carbohydrate moieties carrying protective groups which are removed during acidic cleavage of the target glycopeptide from the resin, but that are still sufficiently stable to withstand conditions encountered in carbohydrate and peptide synthesis, constitutes a more versatile approach. We therefore used this approach to prepare glycosylated derivatives of 5-hydroxy-L-norvaline which have isopropylidene, *tert*-butyldiphenylsilyl, *tert*-butyldimethylsilyl and 4-metoxybenzyl ether protective groups for the carbohydrate moiety. The resulting glycosylated amino acids were then used in solid-phase synthesis of some *O*-linked glycopeptide analogues of CII(256-270).

2 Results and Discussion

The 5-hydroxy-L-norvaline derivate **3**, required for the synthesis of glycopeptides related to type II collagen, was prepared from N^{α}-Fmoc-glutamic acid benzyl ester (**2**) [35] (Scheme 1). Treatment with isobutyl chloroformate converted **2** into a mixed carbonic anhydride, and subsequent reduction [36,37] with sodium borohydride in methanol gave the alcohol **3**.

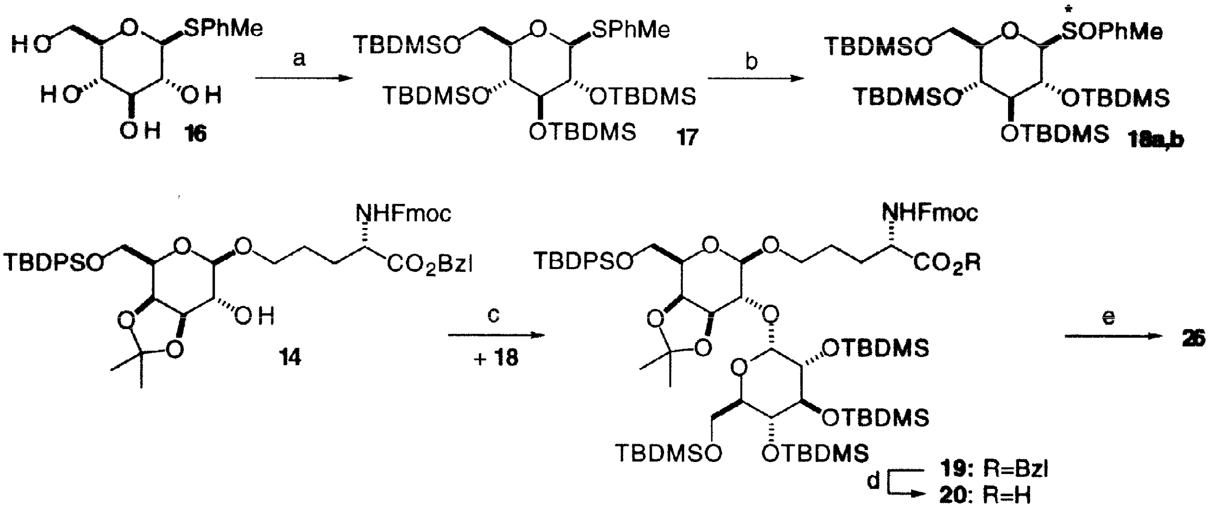
Galactosyl donors that have participating and orthogonally cleavable protective groups at *O*-2 were first investigated in attempts to link a β-D-galactose moiety to acceptor **3**. These donors, **6-8**, which have levulinyl (Lev) [38], allyloxycarbonyl (Aloc) [39], and chloroacetyl (AcCl) [40] protective groups at *O*-2, were prepared in two steps from phenyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (**4**) [41]. Monosilylation of the diol with *tert*-butyldiphenylsilyl chloride (TBDPSCl) [42] gave **5** and subsequent protection with levulinic anhydride [43], allyl chloroformate and chloroacetyl chloride, respectively, gave the galactosyl donors **6-8** (84-94% overall yields). However, treatment of donors **6-8** with different thiophilic promotors [44] and acceptor **3** led to poor results. The desired β-glycosides **9-11** were formed in less than 10% yields due to extensive decomposition or formation of the corresponding orthoester/orthocarbonate.

Alternatively, α-1,2-anhydrosugars, formed by epoxidation of glycals, can be used as glycosyl donors in the synthesis of β-glycosides [45,46]. Epoxidation of 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-D-galactal **12** [47] with dimethyldioxirane gave the corresponding α-1,2-anhydrosugar **13** which was immediately used for glycosylation of **3** (1 equivalent) using zinc chloride as promoter and THF as solvent in the presence of molecular sieves. The desired β-glycoside **14** was obtained in 50% yield together with the corresponding α-glycoside (6%), both of which had unprotected hydroxyl groups at *C*-2 of the galactose moiety. When dichloromethane was used as solvent instead of THF both yield and selectivity dropped (33%, α/β 2:1). The Fmoc group is known to be slowly cleaved during hydrogenolysis but use of ethyl acetate as solvent and adjustment of the amount of Pd/C allowed selective removal [48] of the more reactive benzyl ester in **14** to give the galactosylated building block **15** (93%).



Scheme 1. (a) *i*BuOCOCl, NMM, THF, -10 °C; (b) $NaBH_4$, MeOH, 0 °C, 84% (two steps); (c) TBDPSCl, DMAP, Et_3N, CH_2Cl_2, 98%; (d) Lev_2O, DMAP, pyridine, 89%; (e) AlocCl, DMAP, pyridine, CH_2Cl_2, 0 °C → RT, 86%; (f) ClAcCl, 2,4,6-collidine, DMAP, CH_2Cl_2, -5 °C, 96% (g) dimethyldioxirane, acetone, CH_2Cl_2, 0 °C; (h) $ZnCl_2$, THF, AW-300, -50 °C → RT, 50% (two steps); (i) H_2, 10% Pd/C, EtOAc, 93%; (j) solid phase peptide synthesis, 45%.

A first approach for attaching an α-glucosyl residue to *OH*-2 of **14** employed glycosyl donors protected with the *tert*-butyldimethylsilyl (TBDMS) group (Scheme 2). Donors **18a,b** were prepared from 4-methylphenyl 1-thio-β-D-glucopyranoside (**16**) [49] in two steps. Treatment of **16** with *tert*-butyldimethylsilyl trifluoromethanesulfonate in the presence of a catalytic amount of 4-dimethylaminopyridine in pyridine gave **17** which was then oxidized with 3-chloroperoxybenzoic acid to give a diastereomeric mixture (7.8:1) of sulfoxides **18a,b**. The severe steric interactions induced by the bulky silyl groups distorted the pyranose ring in **17** and **18a,b** [50,51]. The vicinal proton-proton coupling constants (Table 1) and long-range proton coupling constants ($^4J_{2,4}$ and $^4J_{3,5}$ ≈ 1 Hz) for the thioglucoside **17** and the minor sulfoxide **18b** indicated a $^3S_1$ skewed boat conformation of the pyranose ring. In the case of the major sulfoxid **18a**, which has $^4J_{1,3}$ and $^4J_{2,4}$ ≈ 1 Hz, the vicinal proton-proton coupling pattern agreed well with that calculated for an $^OS_2$ skewed boat [52].

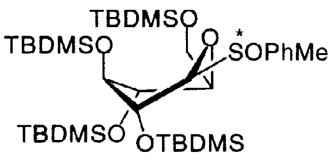


Scheme 2. (a) TBDMSOTf, DMAP, pyridine, 87%; (b) MCPBA, CH_2Cl_2, -78 → -40 °C, 93%, *(diastereomeric ratio 7.8:1); (c) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine, PhMe, -78 °C, 28%; (d) H_2, 10% Pd/C, EtOAc, 83%; (e) solid phase peptide synthesis, 26%.

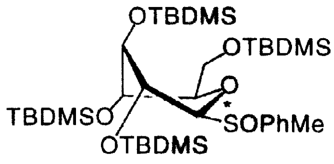
Table 1.

Vicinal Proton - Proton Coupling Constants (Hz)

Compd	$^3J_{1,2}$	$^3J_{2,3}$	$^3J_{3,4}$	$^3J_{4,5}$
17	7.8	1.0	3.1	
18a	1.0	3.1	~0.8	7.3
18b	7.7	1.1	2.8	



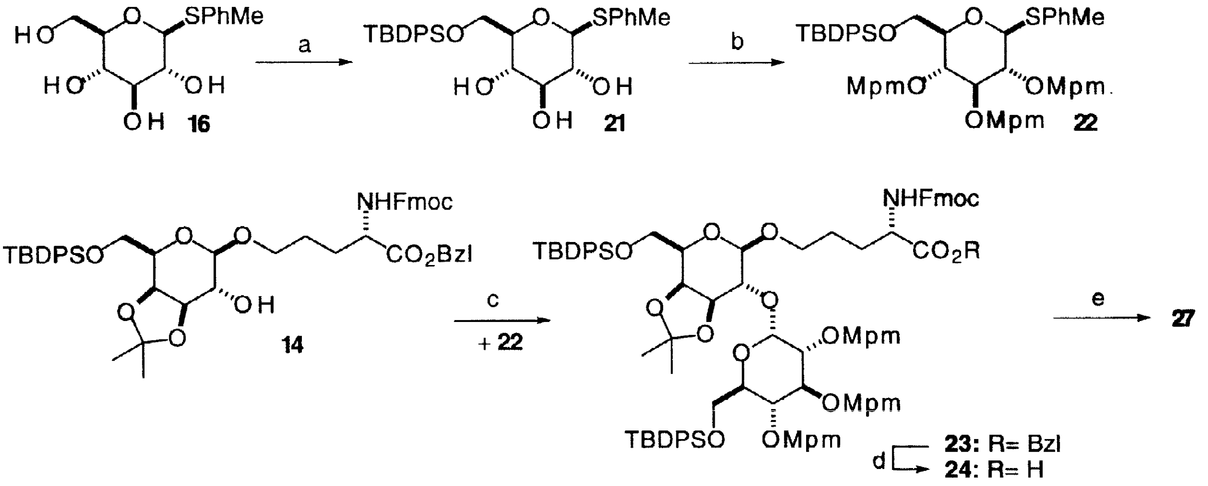
18a $^OS_2$



18b $^3S_1$

Treatment [53] of sulfoxide **18a** or **18b** with trifluoromethanesulfonic anhydride and 2,6-di-*tert*-butyl-4-methylpyridine, followed by acceptor **14** in toluene, gave the desired α-glycoside **19** (28%), the corresponding β-glycoside (28%) and acceptor having a TBDMS group at *O*-2 of the galactose moiety (~10%). An attempt to improve the stereoselectivity of the glycosylation by using a mixture of dichloromethane and diethyl ether (1:4) as solvent did not affect the α:β ratio, but reduced the total yield to 28% due to increased formation of the silylated acceptor. In contrast, α-selective glycoside synthesis using the skewed boat galactosyl donor, phenyl 6-*O*-acetyl-2-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside sulfoxide, has recently been achieved by others [54]. Attempted glycosylation of **14** with the thioglucoside **17**, or the corresponding chloro- or bromo sugars, under a variety of conditions [55] gave very low yields of the desired **19**. Removal of the benzyl ester in **19** by catalytic hydrogenolysis in ethyl acetate then gave the diglycosylated 5-hydroxy-L-norvaline **20** in 83% yield.

In order to improve the α-selectivity in attaching a glucose moiety to **14** alternative protecting group patterns for the glucosyl donor were investigated (Scheme 3). These relied on the sensitivity of 4-methoxybenzyl (Mpm) ethers [56] towards acid, which was recently utilized in oligosaccharide synthesis [57]. The tetra-*O*-Mpm protected glucoside donor, prepared from **16**, was however prone to form the corresponding 1,6-anhydrosugar upon treatment with various promoters. Therefore a donor with a 6-*O*-silyl group and Mpm groups at *O*-2, 3, and 4 was prepared. Silylation of **16** with TBDPSCl in pyridine gave **21** and subsequent metoxybenzylation with 4-methoxybenzyl chloride and sodium hydride gave donor **22** (62% yield over two steps) together with the tetra-*O*-Mpm protected donor. Coupling of donor **22** with acceptor **14** promoted by *N*-iodosuccinimide and silver trifluoromethanesulfonate [58] in dichloromethane improved the α-selectivity and gave the α-linked **23** (71%) and a small amount of the corresponding β-glycoside (9%). To avoid removal of the Mpm-ethers during catalytic hydrogenolysis of the benzyl ester of **23**, ammonium acetate was used to poison the palladium catalyst [59], allowing building block **24** to be obtained in 87% yield.



Scheme 3. (a) TBDPSCl, pyridine, 98%; (b) MpmCl, NaH, DMF, 63%; (c) NIS, AgOTf, CH_2Cl_2, 4Å MS, -45 → -15 °C, 71%; (d) H_2, 10% Pd/C, NH_4OAc, EtOAc, 87%; (e) solid phase peptide synthesis, 38%.

The target type II collagen glycopeptide analogues **25**, **26**, and **27** were synthesized in an automatic peptide synthesizer on a polystyrene resin grafted with polyethylene glycol spacers (TentaGel^{TM} resin). Glycopeptides **25** and **26**, which are glycosylated at position 264, correspond to CII(256-270) and were prepared using a 4-alkoxybenzyl alcohol linker. Esterification of this linker requires use of a large excess of activated amino acid in order to reach completion and avoid racemization. Synthesis of a CII(256-270) analogue, glycosylated also at position 270, would thus require use of substantial amounts of glycosylated 5-hydroxy-L-norvaline, available only in limited quantities. In order to circumvent this problem we instead chose to prepare the extended CII fragment 256-271, which allowed incorporation of the building block **24** via the more efficient formation of an amide bond. Since the CII(256-271) glycopeptide **27** has a *C*-terminal glycine it was prepared using a trityl linker to avoid potential diketopiperazine formation [60] after incorporation of **24** and subsequent Fmoc-cleavage. In the peptide synthesizer N^{α}-Fmoc amino acids (4 equivalents, as compared to the capacity of the resin) having standard side-chain protective groups (*i.e. t*-Boc for Lys, *t*-Bu for Glu and Hyp, and Trt for Gln) were activated as benzotriazolyl esters [61] with 1,3-diisopropyl carbodiimide in DMF. The monoglycosylated building block **15** (1.2 equivalents) and the diglycosylated building blocks **20** (1.0 equivalents) and **24** (1.2 equivalents) were activated as azabenzotriazolyl esters due to the higher coupling efficiency displayed by such esters as compared to benzotriazole esters in peptide synthesis [62]. Coupling of activated **15**, **20**, and **24** was performed manually after removal of the peptide-resin from the synthesizer in order to allow use of a minimal volume of DMF as solvent. After incorporation of the glycosylated building blocks, and capping of unreacted amino groups with acetic anhydride in the syntheses of **20** and **24**, the resins were reinserted in the automatic synthesizer and the syntheses were completed. All couplings were monitored spectrophotometrically with bromophenol blue as an indicator [63] of unacylated amino groups. N^{α}-Fmoc deprotections were effected with piperidine in DMF and were monitored using the absorbance of the dibenzofulvene-piperidine adduct [64]. Cleavage from the resins after completion of the syntheses was performed with trifluoroacetic acid containing water, thioanisole, and ethanedithiol as scavengers. These conditions also removed the protective groups used both for the peptide and saccharide moieties without effecting the glycosidic bonds. Inspection of the analytical reversed-phase HPLC chromatograms for crude glycopeptides **25-27** (Figure 1) indicated that more biproducts were formed in the synthesis of **25** from **15** than in preparation of **26** and **27**. This is probably due to that glycine^{265} was not capped after coupling of **15** and to attachment of amino acids to the unprotected hydroxyl group at *C*-2 of the galactose moiety during elongation of the peptide chain. Purification by reversed-phase HPLC furnished glycopeptides **25**, **26**, and **27** in 45, 26, and 38% overall yields, respectively, based on the resin capacity. All glycopeptides were characterized by ^{1}H NMR spectroscopy, FAB MS, and amino acid analysis.

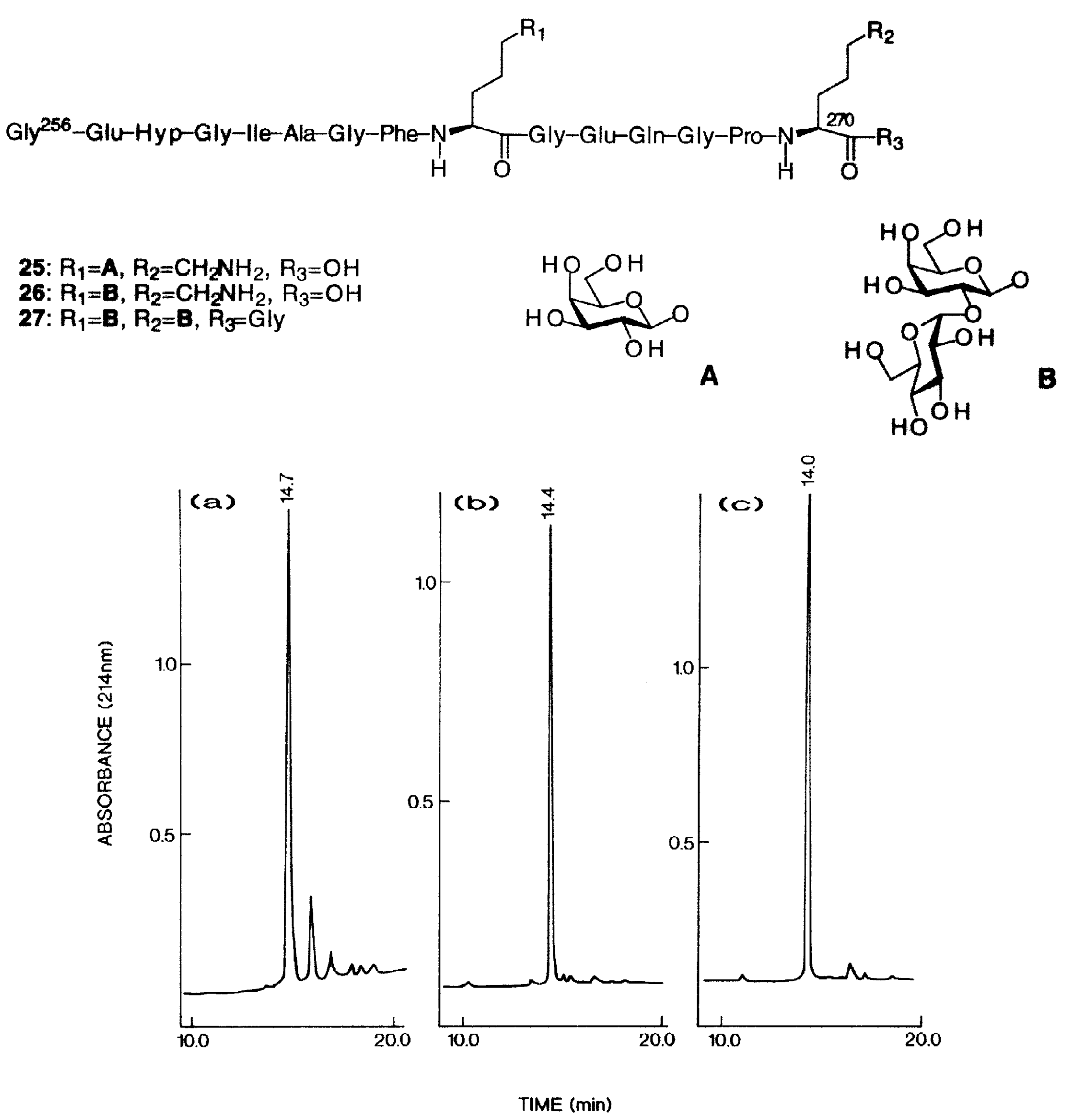


Figure 1. Analytical reversed-phase HPLC chromatograms of crude glycopeptides **25** (**a**), **26** (**b**), and **27** (**c**). HPLC conditions are described in the general procedure for solid-phase glycopeptide synthesis in the experimental section.

3 Conclusions

Three *O*-linked glycosylated amino acid building blocks, **15**, **20**, and **24**, carrying acid labile protective groups have been assembled and used in solid-phase glycopeptide synthesis of three analogues of an immunodominant fragment from type II collagen. The protective group pattern for the carbohydrate moieties involved isopropylidene, *tert*-butyldiphenylsilyl, *tert*-butyldimethylsilyl and 4-metoxybenzyl ether groups. As expected these protective groups were compatible with synthetic transformations such as α- and β-selective glycoside synthesis. Moreover, they were completely removed during acidic cleavage of the glycopeptide from the solid support under conditions which left the *O*-glycosidic bonds intact.

4 Experimental Section

General Methods and Materials.—All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH_2Cl_2 was distilled from calcium hydride. THF and toluene were distilled from sodium-benzophenone. Pyridine was dried over 4 Å molecular sieves, DMF was distilled and then sequentially dried over two portions of 3 Å molecular sieves. TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light and charring with aqueous sulfuric acid or phosphomolybdic acid/ceric sulfate/aqueous sulfuric acid. Flash column chromatography was performed on silica gel (Matrex, 60 Å, 35-70 μm, Grace Amicon) with distilled solvents. Organic solutions were dried over Na_2SO_4 before being concentrated.

The ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX-360, Bruker DRX-400 or a Bruker ARX-500 spectrometer, for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.27 ppm) or $CDCl_3$ (δ_C 77.0 ppm) as internal standard], CD_3OD [residual CD_2HOD (δ_H 3.35 ppm) or CD_3OD (δ_C 49.0 ppm) as internal standard] or in a 1:1 mixture of CD_3OD and $CDCl_3$ [residual CD_2HOD (δ_H 3.35 ppm) or CD_3OD (δ_C 49.0 ppm) as internal standard] at 300 K. The ^1H NMR spectra of glycopeptides **25**, **26**, and **27** were recorded with a Bruker ARX-500 spectrometer for solutions in a 9:1 mixture of H_2O and D_2O [H_2O (δ_H 4.98 ppm) as internal standard] at 278 K. First-order chemical shifts and coupling constants were obtained from one-dimensional spectra and proton resonances were assigned from COSY [65], NOESY [66], TOCSY [67] and ROESY [68] experiments. Resonances for aromatic protons and resonances that could not be assigned are not reported. Optical rotations were measured with a Perkin-Elmer 141 or a Perkin-Elmer 343 polarimeter. Ions for positive fast atom bombardment mass spectra (FAB MS) were produced by a beam of Xenon atoms (6 keV) from a matrix of glycerol and thioglycerol. In the amino acid analyses, 5-hydroxy-L-norvaline was not determined and glutamine was determined as glutamic acid.

Analytical normal phase HPLC was performed on a Kromasil silica column (100 Å, 5 μm, 4.6 x 250 mm) with a flowrate of 2 mL/min and detection at 254 nm. Preparative purifications were performed on a Kromasil silica column (100 Å, 5 μm, 20 x 250 mm) with a flowrate of 20 mL/min.

The preparation of N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-glutamic acid benzyl ester (**2**) [35], phenyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (**4**) [41], 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-D-galactal (**12**) [47], and 4-methylphenyl 1-thio-β-D-glucopyranoside (**16**) [49] has been described previously.

General Procedure for Solid-Phase Glycopeptide Synthesis.—Glycopeptides **25**, **26**, and **27** were synthesized in a custom made, fully automatic continuous flow peptide synthesizer constructed essentially as described [69]. A resin consisting of a cross-linked polystyrene backbone grafted with polyethyleneglycol chains was used for the syntheses. The Resin carried the *C*-terminal lysine on a *p*-hydroxymethylphenoxy linker (TentaGel S PHB^{TM}, Rapp Polymere, Germany) in the synthesis of **25** and **26**, and *C*-terminal glycine on a trityl linker (TentaGel S Trt^{TM}, Rapp Polymere, Germany) in the synthesis of **27**. N^{α}-Fmoc-amino acids (Bachem, Switzerland) with the following side-chain protective groups were used: triphenylmethyl (Trt) for glutamine; *tert*-butyl for glutamic acid and hydroxyproline; and *tert*-butoxycarbonyl (Boc) for lysine. DMF was distilled before being used.

In the synthesis of glycopeptide **26** 68 µmol of resin was used in the peptide synthesizer. The N^{α}-Fmoc-amino acids were activated as 1-benzotriazolyl esters [61]. These were prepared in situ by reaction of the appropriate N^{α}-Fmoc-amino acid (0.272 mmol), 1-hydroxybenzotriazole (HOBt) (0.408 mmol) and 1,3-diisopropylcarbodiimide (0.265 mmol) in DMF (1.3 mL). After 45 min bromophenol blue (51 nmol, 0.4 mL of a 0.15 mM solution in DMF) was added to the 1-benzotriazolyl ester solution which was then recirculated through the column containing the resin. The acylation was monitored [63] using the absorbance of bromophenol blue at 600 nm, and the peptide-resin was automatically washed with DMF after 1 h or when monitoring revealed the coupling to be complete. N^{α}-Fmoc deprotection of the peptide resin was performed by a flow of 20% piperidine in DMF (2 mL/min) through the column for 12.5-27.5 min, and was monitored [64] using the absorbance of the dibenzofulvene-piperidine adduct at 350 nm. After completion of the N^{α}-Fmoc deprotection the peptide-resin was again washed automatically with DMF. The glycosylated amino acid **20** (68 µmol) was activated separately in DMF (1.2 mL) at room temperature during 35 min by addition of 1,3-diisopropylcarbodiimide (68 µmol) and 1-hydroxy-7-azabenzotriazole [62] (HOAt, 0.17 mmol). Compound **20** was then coupled manually to the peptide resin which had been removed from the synthesizer. The coupling of **20** was performed in a mechanically agitated reactor during 23 h, and it was monitored by bromophenol blue as described above. After coupling of **20** unreacted amino groups were capped by addition of acetic anhydride and the glycopeptide resin was reinserted in the synthesizer and coupling of the remaining amino acids was performed as outlined above.

Glycopeptides **25** and **27** were synthesized essentially as described for **26** using 50 and 60 µmol of resin, respectively. In the synthesis of **25** and **27**, the glycosylated building blocks **15** (60 µmol) and **24** (72 µmol) were activated as 1-(7-azabenzotriazolyl) esters [62], which were then coupled manually to the peptide resins during 23 h, as described above for the synthesis of **26**. After coupling of **24** unreacted amino groups were capped by addition of acetic anhydride.

After completion of the synthesis, the resins carrying the protected glycopeptides **25**, **26** and **27** were washed with CH_2Cl_2 and dried under vacuum. For each glycopeptide-resin the glycopeptide was then cleaved from a portion of the peptide-resin (*c.f.* details given for each glycopeptide), the amino acid side chains were deprotected, and acid-labile carbohydrate protective groups were removed, by treatment with trifluoroacetic acid/water/thioanisole/ethanedithiol (87.5:5:5:2.5, ~20 mL/200 mg of glycopeptide resin) for 2 h followed by filtration. Acetic acid (5 mL) was added to the filtrate, the solution was concentrated, and acetic acid (2 x 5 mL) was added again followed by concentration after each addition. The residue was triturated with diethyl ether (15 mL) which gave a solid, crude glycopeptide which was dissolved in a mixture of acetic acid and water (10 mL) and freeze dried. Purification by preparative HPLC gave pure **25**, **26**, and **27**.

The glycopeptides were analyzed on a Kromasil C-8 column (100 Å, 5 µm, 4.6 x 250 mm) using a linear gradient of 0→100% of *B* in *A* over 60 min with a flow rate of 1.5 mL/min and detection at 214 nm (solvent systems *A*: 0.1% aqueous trifluoroacetic acid and *B*: 0.1% trifluoroacetic acid in CH_3CN). Purification of the crude glycopeptides was performed on a Kromasil C-8 column (100 Å, 5 µm, 20 x 250 mm) using the same eluant and a flow rate of 11 mL/min.

The peptide content of the purified glycopeptide, as determined by amino acid analysis, has been taken into account in calculating the final yields for the glycopeptides. For example, 23.4 mg of **25** with a peptide content of 79% was obtained and the yield was therefore based on 18.5 mg (23.4 mg x 0.79).

N^{α}*-(Fluoren-9-ylmethoxycarbonyl)-5-hydroxy-*L*-norvaline benzyl ester* (**3**).—4-Metylmorpholine (303 µl, 2.75 mmol) and isobutyl chloroformate (360 µl, 2.75 mmol) were added to a solution of **2** (1.26 g, 2.75 mmol) in THF (15 mL) at -10 °C and the mixture was stirred at -10 °C for 30 min. The precipitate was filtered off (Celite) and washed with cold THF (0 °C, 8 mL). Sodium borohydride (142 mg, 3.75 mmol) was added to the filtrate in one portion. Then, MeOH (30 mL) was added to the mixture over a period of 15 min at 0 °C and the solution was stirred for an additional 30 min at 0 °C. The mixture was diluted with EtOAc (70 mL) and washed with 1 M aqueous HCl (50 mL). The aqueous phase was extracted with EtOAc (50 mL) and the combined organic phases were washed with saturated aqueous $NaHCO_3$ (70 mL), dried, and concentrated. Flash column chromatography (toluene—MeCN, 5:1) of the residue gave **3** (1.03 mg, 84%): $[\alpha]_D^{25}$ -4° (*c* 3.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.54 (d, 1 H, *J* = 7.9 Hz, NH), 5.23 (ABd, 1 H, *J* = 12.2 Hz, PhC*H*₂O), 5.18 (ABd, 1 H, *J* = 12.1 Hz, PhC*H*₂O), 4.49 (m, 1 H, *J* = 5.4, 7.8 Hz, Hα), 4.42 (d, 2 H, *J* = 7.0 Hz, OCOC*H*₂CH), 4.23 (bt, 1 H, *J* = 7.0 Hz, OCOCH₂C*H*), 3.67-3.63 (m, 2 H, Hδ), 2.03-1.94 (m, 1 H, Hβ), 1.85-1.76 (m, 1 H, Hβ), 1.61-1.55 (m, 2 H, Hγ); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.3, 156.0, 143.9, 143.7, 141.3, 135.2, 128.6, 128.5, 128.3, 127.7, 127.0, 125.1, 120.0, 120.0, 67.2, 67.0, 62.0, 53.6, 47.1, 29.3, 28.0; HRMS (FAB): calcd for $C_{27}H_{28}NO_5$ 446.1967 (M+H$^+$), found 446.1964. Anal. Calcd for $C_{27}H_{27}NO_5$: C, 72.8; H, 6.1; N, 3.1. Found: C, 72.6; H, 6.1; N, 3.2.

*Phenyl 6-O-*tert*-butyldiphenylsilyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside* (**5**).—*tert*-Butyldiphenylsilyl chloride (916 μL, 3.52 mmol) was added to a solution of **4** (1.00 g, 3.20 mmol), 4-dimethylaminopyridine (16 mg, 0.13 mmol), and triethylamine (535 μL, 3.84 mmol) in CH_2Cl_2 (10 mL) at room temperature. After 17 h the mixture was diluted with CH_2Cl_2 (60 mL) and washed with saturated aqueous NH_4Cl (60 mL) and water (60 mL). The organic layer was dried, concentrated, and subjected to flash column chromatography (heptane—EtOAc, 3:1) to give **5** (1.72 g, 98%): ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.48 (d, 1 H, *J* = 10.2 Hz, H-1), 4.32 (dd, 1 H, *J* = 1.8, 5.4 Hz, H-4), 4.12 (dd, 1 H, *J* = 5.5, 6.8 Hz, H-3), 3.60 (ddd, 1 H, *J* = 2.2, 7.0, 10.0 Hz, H-2), 2.47 (d, 1 H, *J* = 2.3 Hz, OH), 1.45 and 1.37 (2 s, each 3 H, CH_3), 1.10 (s, 9 H, *t*Bu); ^{13}C NMR (91 MHz, $CDCl_3$) δ 135.6, 133.3, 132.4, 132.3, 129.7, 129.9, 127.9, 127.7, 127.6, 110.1, 88.3, 78.9, 77.2, 73.3, 71.6, 62.9, 28.1, 26.7, 26.3, 19.2.

*Phenyl 6-O-*tert*-butyldiphenylsilyl-3,4-O-isopropylidene-2-O-levulinyl-1-thio-β-D-galactopyranoside* (**6**).—A solution of levulinic anhydride [43] (311mg, 1.45 mmol) in pyridine (2 mL) was added to a solution of **5** (400 mg, 0.726 mmol) and 4-dimethylaminopyridine (9 mg, 0.07 mmol) in pyridine (8 mL) at room temperature. After 20 h MeOH (5 mL) was added and the mixture was concentrated. The residue was coevaporated with toluene, dissolved in CH_2Cl_2 (40 mL), and washed with saturated aqueous $NaHCO_3$ (40 mL) and water (40 mL). The organic layer was dried, concentrated, and subjected to flash column chromatography (heptane—EtOAc, 3:1) to give **6** (420 mg, 89%): $[\alpha]_D^{20}$ +23° (*c* 1.0, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.05 (dd, 1 H, *J* = 7.1, 10.1 Hz, H-2), 4.64 (d, 1 H, *J* = 10.1 Hz, H-1), 4.32 (dd, 1 H, *J* = 1.3, 5.4 Hz, H-4), 4.21 (dd, 1 H, *J* = 5.4, 7.0 Hz, H-3), 2.93-2.63 (m, 4 H, $COCH_2CH_2CO$), 2.23 (s, 3 H, $COCH_3$), 1.52 and 1.36 (2 s, each 3 H, CH_3), 1.09 (s, 9 H, *t*Bu); ^{13}C NMR (91 MHz, $CDCl_3$) δ 206.2, 171.5, 135.6, 135.6, 133.7, 133.2, 131.8, 131.6, 129.7, 128.9, 127.7, 127.7, 127.6, 110.4, 86.1, 77.1, 77.0, 73.4, 71.8, 62.9, 38.0, 29.9, 28.1, 27.6, 26.8, 26.3, 19.2.

*Phenyl 2-O-allyloxycarbonyl-6-O-*tert*-butyldiphenylsilyl-3,4-O-isopropylidene-1-thio-β-D-galactopyranoside* (**7**).—Allyl chloroformate (926 μL, 8.71 mmol) was added to a solution of **5** (400 mg, 0.726 mmol) and 4-dimethylaminopyridine (18 mg, 0.15 mmol) in CH_2Cl_2/pyridine (5 mL, 3:2) at 0 °C. The reaction mixture was stirred at 0 °C for 40 min and then at room temperature for a further 11 h. The mixture was then diluted with CH_2Cl_2 (40 mL) and washed with water (40 mL) and saturated aqueous $NaHCO_3$ (40 mL). The organic phase was dried, concentrated, and subjected to flash column chromatography (heptane—EtOAc, 3:1) to give **7** (397 mg, 86%): $[\alpha]_D^{20}$ +27° (*c* 1.0, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.00 (ddt, 1 H, *J* = 5.7, 10.5, 17.2 Hz, $CH_2C\mathit{H}CH_2O$), 5.43 (dq, 1 H, *J* = 1.5, 17.2 Hz, $C\mathit{H}_2CHCH_2O$), 5.33 (dq, 1 H, *J* = 1.2, 10.5 Hz, $C\mathit{H}_2CHCH_2O$), 4.86 (dd, 1 H, *J* = 7.1, 10.2 Hz, H-2), 4.76 (ABddt, 1 H, *J* = 1.4, 5.7, 13.1 Hz, $CH_2CHC\mathit{H}_2O$), 4.68 (ABddt, 1 H, *J* = 1.4, 5.7, 13.1 Hz, $CH_2CHC\mathit{H}_2O$), 4.63 (d, 1 H, *J* = 10.2 Hz, H-1), 4.34 (dd, 1 H, *J* =

1.8, 5.3 Hz, H-4), 4.27 (dd, 1 H, *J* = 5.3, 7.0 Hz, H-3), 1.55 and 1.37 (2 s, each 3 H, CH_3), 1.09 (s, 9 H, *t*Bu); ^{13}C NMR (91 MHz, $CDCl_3$) δ 154.2, 135.6, 135.6, 133.3, 133.2, 132.1, 131.4, 129.7, 128.9, 127.7, 127.7, 118.9, 110.5, 86.0, 77.0, 76.9, 75.6, 73.5, 68.9, 62.8, 27.7, 26.8, 26.3, 19.2.

*Phenyl 6-*O-tert*-butyldiphenylsilyl-2-*O*-chloroacetyl-3,4-*O*-isopropylidene-1-thio-*β-D-*galactopyranoside* (**8**).—A solution of chloroacetyl chloride (87 μL, 1.09 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a solution of **5** (400 mg, 0.726 mmol), 2,4,6-collidine (347 μL, 2.61 mmol), and 4-dimethylaminopyridine (9 mg, 0.07 mmol) in CH_2Cl_2 (5 mL) at -5 °C. The reaction mixture was stirred for 2 h at -5 °C and was then diluted with CH_2Cl_2 (40 mL) and washed with water (40 mL) and saturated aqueous $NaHCO_3$ (40 mL). The organic layer was dried, concentrated, and subjected to flash column chromatography (heptane—EtOAc, 3:1) to give **8** (439 mg, 96%): $[\alpha]_D^{20}$ +28° (*c* 1.0, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$) δ 5.10 (dd, 1 H, *J* = 7.3, 10.3 Hz, H-2), 4.65 (d, 1 H, *J* = 10.4 Hz, H-1), 4.34 (dd, 1H, *J* = 1.4, 5.2 Hz, H-4), 4.23 (dd, 1 H, *J* = 5.3, 7.2 Hz, H-3), 4.17 (s, 2 H, $COCH_2Cl$), 1.53 and 1.36 (2 s, each 3 H, CH_3), 1.10 (s, 9 H, *t*Bu); ^{13}C NMR (91 MHz, $CDCl_3$) δ 166.1, 135.6, 135.6, 133.2, 131.9, 129.8, 129.0, 127.8, 127.7, 127.7, 110.7, 85.7, 77.1, 76.9, 73.5, 73.3, 62.8, 40.9, 27.7, 26.8, 26.3, 19.2.

N$^{\alpha}$-*(Fluoren-9-ylmethoxycarbonyl)-5-*O*-(6-*O-tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-*β-D-*galactopyranosyl)-5-hydroxy-*L-*norvaline benzyl ester* (**14**).—A solution of dimethyldioxirane [70] in acetone (20.5 mL, ca 0.07M, 1.44 mmol) was added to the glucal **12** (339 mg, 0.798 mmol) in CH_2Cl_2 (8 mL) at 0 °C and the mixture was protected from light and stirred at 0 °C for 60 min. Concentration of the reaction mixture under reduced pressure yielded the α-1,2-anhydrosugar **13**: ^1H NMR (400 MHz, $CDCl_3$) δ 4.94 (t, 1 H, *J* = 1.8 Hz, H-1), 4.58 (dd, 1 H, *J* = 0.8, 6.1 Hz, H-3), 4.29 (dt, 1 H, *J* = 1.2, 6.1 Hz, H-4), 4.05-4.02 (m, 1 H, H-5), 3.89 (dd, 1 H, *J* = 8.2, 9.6 Hz, H-6), 3.83 (dd, 1 H, *J* = 6.0, 9.6 Hz, H-6), 2.89-2.88 (m, 1 H, H-2), 1.47 and 1.41 (2 s, each 3 H, CH_3), 1.06 (s, 9 H, *t*Bu). A solution of **3** (317 mg, 0.712 mmol) in THF (10 mL) and crushed molecular sieves (AW-300, 600 mg) were added to **13** and the mixture was stirred for 1h at room temperature and then cooled to -50 °C. Zinc chloride (854 μL, 1.0 M in Et_2O, 0.854 mmol) was added and the reaction mixture was allowed to attain room temperature over 18 h. The mixture was then diluted with EtOAc (100 mL), filtered (Celite), and washed with water (100 mL). The aqueous phase was extracted with EtOAc (2 x 50 mL) and the combined organic phases were dried and concentrated. Flash column chromatography (heptane—EtOAc, 3:2 and toluene—MeCN, 6:1) and purification by normal phase HPLC (linear gradient 0→15% EtOH in hexane fraction during 160 min) of the residue gave **14** (313 mg, 50%) and the corresponding α anomer (36 mg, 6%). Compound **14** had: $[\alpha]_D^{25}$ +4° (*c* 0.64, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.43 (d, 1 H, *J* = 8.2 Hz, NH), 5.18 (ABd, 1 H, *J* = 12.2 Hz, $PhCH_2O$), 5.14 (ABd, 1 H, *J* = 12.3 Hz, $PhCH_2O$), 4.49 (dt, 1 H, *J* = 6.1, 7.5 Hz, Hα), 4.41 (ABdd, 1 H, *J* = 7.1, 10.7 Hz, OCOC*H*$_2$CH), 4.35 (ABdd, 1 H, *J* = 7.2, 10.6 Hz, OCOC*H*$_2$CH), 4.26 (dd, 1 H, *J* = 2.1, 5.4 Hz, H-4), 4.19 (bt, 1 H, *J* = 6.9 Hz, OCOCH$_2$C*H*), 4.10 (d, 1 H, *J* = 8.3 Hz, H-1), 4.05 (dd, 1 H, *J* = 5.6, 7.2 Hz, H-3), 3.97 (dd, 1 H, *J* = 7.4, 9.8 Hz, H-6), 3.94-3.90 (m, 1 H, Hδ), 3.90 (dd, 1 H, *J* = 6.2, 9.8 Hz, H-6), 3.85-3.82 (m, 1 H, H-5), 3.50 (bt, 1 H, *J* = 7.8 Hz, H-2), 3.46-3.42 (m, 1 H, Hδ), 3.13 (bs, 1 H, OH), 1.93-1.88 (m, 1 H, Hβ), 1.88-1.80 (m, 1 H, Hβ), 1.71-1.67 (m, 1 H, Hγ), 1.61-1.57 (m, 1 H, Hγ), 1.48 and 1.34 (2 s, each 3 H, CH$_3$), 1.05 (s, 9 H, *t*Bu); ^{13}C NMR (101 MHz, CDCl$_3$) δ 172.2, 156.0, 143.8, 143.7, 141.3, 135.6, 135.5, 135.2, 133.4, 133.3, 129.7, 128.6, 128.5, 128.3, 127.7, 127.6, 127.1, 125.1, 120.0, 119.9, 109.9, 102.3, 78.7, 73.7, 73.5, 73.1, 68.5, 67.2, 67.0, 62.6, 53.4, 47.1, 29.6, 28.2, 26.7, 26.3, 24.9, 19.2; HRMS (FAB): calcd for $C_{52}H_{60}NO_{10}Si$ 886.3987 (M+H$^+$), found 886.3946. Anal. Calcd for $C_{52}H_{59}NO_{10}Si$: C, 70.5; H, 6.7; N, 1.58. Found: C, 70.4; H, 6.6; N, 1.62.

N$^{\alpha}$-*(Fluoren-9-ylmethoxycarbonyl)-5-*O*-(6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-*β-D*-galactopyranosyl)-5-hydroxy-*L*-norvaline* (**15**).—A solution of **14** (150 mg, 0.169 mmol) in EtOAc (10 mL) was subjected to hydrogenolysis (1 atm) over 10% Pd-C (150 mg) at room temperature for 3 h. The catalyst was then filtered off (Celite) and washed with EtOAc and EtOH. The combined filtrates were concentrated and flash column chromatography (toluene—EtOH, 15:1→3:1) of the residue gave **15** (125 mg, 93%): $[\alpha]_D^{25}$ +5° (*c* 1.0, CHCl$_3$); ^{1}H NMR (500 MHz, CD$_3$OD) δ 4.33 (d, 2 H, *J* = 7.0 Hz, OCOC*H*$_2$CH), 4.28 (dd, 1 H, *J* = 2.0, 5.4 Hz, H-4), 4.21 (bt, 1 H, *J* = 7.0 Hz, OCOCH$_2$C*H*), 4.20 (d, 1 H, *J* = 8.2 Hz, H-1), 4.19-4.15 (m, 1 H, Hα), 4.02 (dd, 1 H, *J* = 5.5, 7.2 Hz, H-3), 3.98 (dt, 1 H, *J* = 1.7, 6.6 Hz, H-5), 3.90 (dd, 1 H, *J* = 6.2, 10.0 Hz, H-6), 3.89-3.85 (m, 1 H, Hδ), 3.86 (dd, 1 H, *J* = 6.8, 10.0 Hz, H-6), 3.56-3.52 (m, 1 H, Hδ), 3.40 (dt, 1 H, *J* = 7.8 Hz, H-2), 1.99-1.92 (m, 1 H, Hβ), 1.80-1.74 (m, 1 H, Hβ), 1.74-1.67 (m, 2 H, Hγ), 1.45 and 1.32 (2 s, each 3 H, CH$_3$), 1.03 (s, 9 H, *t*Bu); ^{13}C NMR (101 MHz, CD$_3$OD) δ 176.4, 158.7, 145.4, 145.2, 142.6, 136.7, 136.7, 134.5, 134.5, 130.9, 128.8, 128.8, 128.2, 126.3, 120.9, 110.9, 103.7, 80.8, 74.9, 74.7, 74.5, 70.0, 67.9, 64.1, 55.4, 48.4, 29.7, 28.5, 27.2, 27.1, 26.6, 20.0; HRMS (FAB): calcd for $C_{45}H_{53}NO_{10}SiNa$ 818.3336 (M+Na$^+$), found 818.3353.

*4-Methylphenyl 2,3,4,6-tetra-*O*-*tert*-butyldimethylsilyl-1-thio-*β-D*-glucopyranoside* (**17**).—A solution of **16** (700 mg, 2.44 mmol) and 4-dimethylaminopyridine (30 mg, 0.24 mmol) in pyridine (16 mL) was treated at 0 °C with *tert*-butyldimethylsilyl trifluoromethanesulfonate (4.49 mL, 19.6 mmol). The mixture was stirred at 0 °C for 20 min and then at room temperature for a further 48 h. MeOH (5 mL) was then added and the reaction mixture was concentrated. The residue was partitioned between CH$_2$Cl$_2$ (100 mL) and saturated aqueous NaHCO$_3$ (100 mL). The organic phase was washed with water (100 mL), dried, and concentrated. Flash column chromatography (heptane—toluene, 4:1) of the residue gave **17** (1.59 g, 87%): $[\alpha]_D^{25}$ -25° (*c* 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.97 (d, 1 H, *J* = 7.8 Hz, H-1), 3.97 (dt, 1 H, *J* = 1.4, 3.1 Hz, H-4), 3.87-3.86 (m, 1 H, H-3), 3.84-3.81 (m, 1 H, H-5), 3.77 (dt, 1 H, J = 1.0, 7.7 Hz, H-2), 2.33 (s, 3 H, CH_3Ph), 0.91, 0.91, 0.90, and 0.89 (4 s, each 9 H, *t*Bu), 0.12, 0.12, 0.11, 0.10, 0.09, 0.09, 0.07, and 0.06 (8 s, each 3 H, $SiCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.3, 132.1, 130.5, 129.4, 86.5, 83.2, 78.0, 75.9, 70.0, 64.2, 26.0, 26.0, 25.8, 25.8, 21.1, 18.4, 18.1, 17.9, 17.9, -4.0, -4.1, -4.3, -4.4, -4.7, -4.7, -5.2; HRMS (FAB): calcd for $C_{37}H_{74}O_5SSi_4Na$ 765.4232 (M+Na+), found 765.4208. Anal. Calcd for $C_{37}H_{74}O_5SSi_4$: C, 59.8; H, 10.0. Found: C, 59.8; H, 9.9.

*4-Methylphenyl 2,3,4,6-tetra-*O-tert-*butyldimethylsilyl-1-thio-*β-D-*glucopyranoside Sulfoxides* (**18a,b**).—A solution of 3-chloroperoxybenzoic acid (150 mg, 85%, 0.740 mmol) in CH_2Cl_2 (44 mL) was added dropwise during 40 min to a solution of **17** (500 mg, 0.673 mmol) in CH_2Cl_2 (12 mL) at -78 °C. The reaction mixture was allowed to attain -40 °C over 7.5 h. The mixture was then diluted with CH_2Cl_2 (40 mL) and washed with saturated aqueous $NaHCO_3$ (80 mL) and water (80 mL). The organic phase was dried, concentrated, and subjected to flash column chromatography (heptane—EtOAc, 20:1→10:1) to give **18a** (421 mg, 82%) and **18b** (53 mg, 11%). Compound **18a** had: $[\alpha]_D^{25}$ +53° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.48 (dt, 1 H, J = 1.1, 3.1 Hz, H-2), 4.31 (t, 1 H, J = 1.0 Hz, H-1), 4.03-3.99 (m, 1 H, H-4), 3.93-3.91 (m, 1 H, H-3), 2.42 (s, 3 H, CH_3Ph), 0.95, 0.90, 0.90, and 0.88 (4 s, each 9 H, *t*Bu), 0.24, 0.17, 0.17, 0.16, 0.13, 0.09, 0.03, and -0.01 (8 s, each 3 H, $SiCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.2, 140.7, 129.3, 125.4, 97.5, 78.3, 76.2, 70.8, 68.6, 62.2, 25.9, 25.8, 25.8, 25.8, 21.4, 18.2, 18.0, 18.0, 17.9, -3.8, -3.9, -4.6, -4.7, -4.9, -5.4, -5.5; HRMS (FAB): calcd for $C_{37}H_{74}O_6SSi_4Na$ 781.4181 (M+Na+), found 781.4188. Compound **18b** had: $[\alpha]_D^{25}$ -70° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.40 (d, 1 H, J = 7.7 Hz, H-1), 4.11 (dt, 1 H, J = 1.1, 7.7 Hz, H-2), 3.98 (m, 1 H, H-4), 3.91 (dt, 1 H, J = 1.0, 2.8 Hz, H-3), 3.76 (dd, 1 H, J = 7.9, 10.3, H-6), 3.68 (dd, J = 5.2, 10.3 Hz, H-6), 3.56-3.51 (m, 1 H, H-5), 2.42 (s, 3 H, CH_3Ph), 0.93, 0.90, 0.87, and 0.86 (4 s, each 9 H, *t*Bu), 0.16, 0.13, 0.12, 0.11, 0.09, 0.04, 0.03, and 0.02 (8 s, each 3 H, $SiCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.0, 138.1, 129.5, 125.3, 93.8, 83.2, 78.6, 71.4, 69.9, 63.3, 25.9, 25.8, 21.4, 18.3, 18.0, 17.8, -3.7, -3.8, -4.4, -4.6, -4.8, -4.9, -5.3, -5.4; HRMS (FAB): calcd for $C_{37}H_{74}O_6SSi_4Na$ 781.4181 (M+Na+), found 781.4177.

N^{α}-*(Fluoren-9-ylmethoxycarbonyl)-5-*O*-[6-*O-tert-*butyldiphenylsilyl-2-*O*-(2,3,4,6-tetra-*O-tert-*butyldimethylsilyl-*α-D-*glucopyranosyl)-3,4-*O*-isopropylidene-*β-D-*galactopyranosyl]-5-hydroxy-*L-*norvaline benzyl ester* (**19**).—To a solution of **18a** (514 mg, 0.677 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (278 mg, 1.35 mmol) in toluene (15 mL) at -78 °C was added trifluoromethanesulfonic anhydride (114 μL, 0.677 mmol). After 8 min at -78 °C, **14** (300 mg, 0.339 mmol) in toluene (5 mL) was added dropwise over a 7 min period. The reaction mixture was stirred for 60 min at -78 °C and was then quenched by addition of triethylamine (471 μL, 3.39 mmol). After 5 min, the mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated aqueous $NaHCO_3$ (100 mL) and saturated aqueous NaCl (100 mL). The organic layer was dried, concentrated, and subjected to flash column chromatography (heptane—EtOAc, 10:1) to give a 1:1 αβ-mixture (291 mg, 57%). Flash column chromatography (toluene—MeCN, 70:1) of the mixture gave **19** (144 mg, 28%): $[\alpha]_D^{25}$ +18° (*c* 0.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.24 (d, 1 H, *J* = 8.6 Hz, NH), 5.14 (ABd, 1 H, *J* = 12.2 Hz, PhC*H*$_2$O), 5.09 (ABd, 1 H, *J* = 12.3 Hz, PhC*H*$_2$O), 5.03 (d, 1 H, *J* = 3.5 Hz, H-1'), 4.42-4.36 (m, 2 H, Hα, OCOC*H*$_2$CH), 4.26 (dd, 1 H, *J* = 2.0, 5.8 Hz, H-4), 4.24 (d, 1 H, *J* = 7.3 Hz, H-1), 4.22 (t, 1 H, *J* = 6.1 Hz, H-3), 4.18 (bt, 1 H, *J* = 7.2 Hz, OCOCH$_2$C*H*), 3.95 (dd, 1 H, *J* = 7.3, 9.8 Hz, H-6), 3.89 (dd, 1 H, *J* = 6.2, 9.8 Hz, H-6), 3.71 (dd, 1 H, *J* = 6.2, 7.2 Hz, H-2), 3.48-3-43 (m, 1 H, Hδ), 1.90-1.84 (m, 1 H, Hβ), 1.74-1.59 (m, 3 H, Hβ, γ), 1.50 and 1.33 (2 s, each 3 H, CH_3), 1.04, 0.90, 0.86, 0.86, and 0.84 (5 s, each 9 H, *t*Bu), 0.09, 0.08, 0.07, 0.07, 0.05, 0.05, 0.03, and 0.02 (8 s, each 3 H, $SiCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 155.9, 143.9, 143.8, 141.2, 135.6, 135.6, 135.2, 133.5, 133.3, 129.6, 128.6, 128.4, 128.2, 127.7, 127.6, 127.1, 125.2, 119.9, 109.5, 103.2, 96.1, 78.1, 76.5, 76.2, 74.2, 73.2, 73.0, 71.3, 71.1, 68.4, 67.2, 67.1, 62.6, 62.4, 53.8, 47.1, 29.1, 28.1, 26.7, 26.2, 26.0, 25.9, 25.8, 25.7, 19.2, 18.3, 18.3, 18.0, 17.8, -3.8, -4.1, -4.4, -4.5, -4.7, -4.8, -4.8, -5.3; HRMS (FAB): calcd for $C_{82}H_{125}NO_{15}Si_5Na$ 1526.7793 ($M+Na^+$), found 1526.7815. Anal. Calcd for $C_{82}H_{125}NO_{15}Si_5$: C, 65.4; H, 8.4; N, 0.93. Found: C, 65.4; H, 8.3; N, 0.99.

N^{α}-*(Fluoren-9-ylmethoxycarbonyl)-5-*O*-[6-*O-tert*-butyldiphenylsilyl-2-*O*-(2,3,4,6-tetra-*O-tert*-butyldimethylsilyl-*α-D*-glucopyranosyl)-3,4-*O*-isopropylidene-*β-D*-galactopyranosyl]-5-hydroxy-*L*-norvaline* (**20**).—A solution of **19** (135 mg, 0.090 mmol) in EtOAc (5 mL) was treated with 10% Pd-C (135 mg) under hydrogen (1 atm) at room temperature for 4.5 h. Additional 10% Pd-C (135 mg) was then added and the reaction was continued for another 4.5 h. The mixture was filtered (Celite), concentrated, and subjected to flash column chromatography (toluene—EtOH, 40:1) to give **20** (105 mg, 83%): $[\alpha]_D^{25}$ +29° (*c* 0.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.32 (d, 1 H, *J* = 7.3 Hz, NH), 5.09 (d, 1 H, *J* = 3.0 Hz, H-1'), 4.41-4.35 (m, 2 H, Hα, OCOC*H*$_2$CH), 4.30 (d, 1 H, *J* = 7.0 Hz, H-1), 4.25 (dd, 1 H, *J* = 1.9, 5.9 Hz, H-4), 4.23 (t, 1 H, *J* = 5.8 Hz, H-3), 4.19 (bt, 1 H, *J* = 7.2 Hz, OCOCH$_2$C*H*), 3.77 (dd, 1 H, *J* = 5.8, 6.8 Hz, H-2), 3.53-3.48 (m, 1 H, Hδ), 2.01-1.93 (m, 1 H, Hβ), 1.86-1.78 (m, 1 H, Hβ), 1.71-1.62 (m, 2 H, Hγ), 1.50 and 1.32 (2 s, each 3 H, CH_3), 1.04, 0.90, 0.87, 0.87, and 0.86 (5 s, each 9 H, *t*Bu), 0.10, 0.09, 0.08, 0.08, 0.07, 0.06, 0.04, and 0.03 (8 s, each 3 H, $SiCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.0, 156.0, 143.8, 143.8, 141.3, 135.6, 135.6, 133.4, 133.3, 129.7, 127.7, 127.6, 127.1, 125.1, 120.0, 109.5, 103.0, 95.5, 77.7, 76.4, 76.2, 74.1, 73.1, 72.9, 71.4, 71.3, 68.0, 67.1, 62.7, 62.4, 53.4, 47.1, 28.6, 28.0, 26.7, 26.1, 26.0, 25.9, 25.9, 25.2, 19.2, 18.3, 18.0, 17.9, -3.6, -4.0, -4.3, -4.5, -4.7, -4.7, -4.9, -5.3; HRMS (FAB): calcd for $C_{75}H_{119}NO_{15}Si_5Na$ 1436.7284 ($M+Na^+$), found 1436.7284.

*4-Methylphenyl 6-*O-tert*-butyldiphenylsilyl-1-thio-*β-D*-glucopyranoside* (**21**).—*tert*-Butyldiphenylsilyl chloride (999 μL, 3.84 mmol) was added to a solution of **16** (1.00 g, 3.49

mmol) in pyridine (10 mL) at room temperature. After 17.5 h the mixture was concentrated and the residue was partitioned between Et_2O (100 mL) and saturated aqueous $CuSO_4$ (100 mL). The organic phase was washed with water (100 mL), dried, and concentrated. Flash column chromatography (toluene—EtOH, 12:1) of the residue gave **21** (1.79 g, 98%): $[\alpha]_D^{25}$ -23° (*c* 0.70, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.67 (d, 1 H, *J* = 9.6 Hz, H-1), 3.97 (ABdd, 1 H, *J* = 4.5, 11.2 Hz, H-6), 3.94 (ABdd, 1 H, *J* = 4.6, 11.3 Hz, H-6), 3.64 (bt, 1 H, *J* = 9.0 Hz, H-4), 3.60 (bt, 1 H, *J* = 8.9 Hz, H-3), 3.45 (dt, 1 H, *J* = 4.5, 9.1 Hz, H-5), 3.32 (bt, 1 H, *J* = 8.8 Hz, H-2), 3.21, 3.09, 2.73 (3 s, each 1 H, OH), 2.33 (s, 3 H, CH_3Ph), 1.08 (s, 9 H, *t*Bu); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.4, 135.6, 135.6, 133.3, 132.9, 132.8, 129.9, 129.9, 129.8, 127.8, 127.8, 127.6, 87.9, 78.8 77.7, 71.4, 71.2, 64.3, 26.8, 21.1, 19.2; HRMS (FAB): calcd for $C_{29}H_{36}O_5SSiNa$ 547.1950 ($M+Na^+$), found 547.1942. Anal. Calcd for $C_{29}H_{36}O_5SSi$: C, 66.4; H, 6.9. Found: C, 66.4; H, 6.6.

*4-Methylphenyl 6-*O-tert-*butyldiphenylsilyl-2,3,4-tri-*O*-(4-methoxybenzyl)-1-thio-*β-D-*glucopyranoside* (**22**).—Sodium hydride (286 mg, 60% in mineral oil, 7.15 mmol) was added to a solution of **21** (1.00 g, 1.91 mmol) and 4-methoxybenzyl chloride (1.55 mL, 11.4 mmol) in DMF (10 mL) at 0 °C. The mixture was stirred at 0 °C for 10 min and then at room temperature for a further 19 h. MeOH (5 mL) was then added and the mixture was partitioned between toluene (200 mL) and saturated aqueous NH_4Cl (200 mL). The organic phase was washed with saturated aqueous NaCl (200 mL) and water (2 x 200 mL), dried, and concentrated. Flash column chromatography (toluene—EtOAc, 40:1 and heptane—EtOAc, 3:1) of the residue gave **22** (1.06 g, 63%): $[\alpha]_D^{25}$ -16° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 4.85 (ABd, 1 H, *J* = 10.5 Hz, MeOPhC*H*$_2$O), 4.82 (d, 1 H, *J* = 9.8 Hz, MeOPhC*H*$_2$O), 4.81 (ABd, 1 H, *J* = 10.6 Hz, MeOPhC*H*$_2$O), 4.81 (d, 1 H, *J* = 10.4 Hz, MeOPhC*H*$_2$O), 4.68 (d, 1 H, *J* = 9.9 Hz, MeOPhC*H*$_2$O), 4.61 (d, 2 H, *J* = 9.8 Hz, H-1, MeOPhC*H*$_2$O), 3.99 (dd, 1 H, *J* = 1.4, 11.0 Hz, H-6), 3.92 (dd, 1 H, *J* = 3.8, 11.3 Hz, H-6), 3.83, 3.82, and 3.80 (3 s, each 3 H, OCH_3), 3.75 (t, 1 H, *J* = 9.3 Hz, H-4), 3.68 (dd, 1 H, *J* = 8.6, 9.2 Hz, H-3), 3.50 (dd, 1 H, *J* = 8.7, 9.7 Hz, H-2), 3.35 (ddd, 1 H, *J* = 1.6, 3.6, 9.5 Hz, H-5), 2.31 (s, 3 H, CH_3Ph), 1.11 (s, 9 H, *t*Bu); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.3, 159.2, 159.2, 137.4, 135.9, 135.7, 133.5, 133.0, 132.4, 130.7, 130.5, 130.3, 130.2, 129.8, 129.6, 129.6, 129.6, 129.6, 129.5, 127.7, 127.6, 113.9, 113.8, 113.8, 87.8, 86.6, 80.5, 79.9, 77.1, 75.6, 74.9, 74.7, 62.6, 55.3, 26.9, 21.1, 19.3; HRMS (FAB): calcd for $C_{53}H_{60}O_8SSiNa$ 907.3676 ($M+Na^+$), found 907.3693.

N$^{\alpha}$*-(Fluoren-9-ylmethoxycarbonyl)-5-*O*-[6-*O-tert-*butyldiphenylsilyl-2-*O*-(6-*O-tert-*butyldiphenylsilyl-2,3,4-tri-*O*-{4-methoxybenzyl}-*α-D-*glucopyranosyl)-3,4-*O*-isopropylidene-*β-D-*galactopyranosyl]-5-hydroxy-*L-*norvaline benzyl ester* (**23**).—A mixture of **14** (73 mg, 82 µmol), **22** (88 mg, 99 µmol), and powdered molecular sieves (4Å, 100 mg) in CH_2Cl_2 (2.5 mL) was stirred at room temperature for 10 min. *N*-Iodosuccinimide (22 mg, 99 µmol) was added and the mixture was cooled to -45 °C and protected from light. Silver trifluoromethanesulfonate (5 mg, 20 µmol) was added and the reaction mixture was allowed to attain -15 °C over 3 h. Triethylamine (57 µL, 0.41 mmol) was then added and the mixture was diluted with CH_2Cl_2 (25 mL), filtered (Celite), and washed with 10% aqueous $Na_2S_2O_3$ (25 mL) and saturated aqueous $NaHCO_3$ (25 mL). The organic layer was dried, concentrated, and subjected to flash column chromatography (toluene—EtOAc, 40:1→14:1) and purification by normal phase HPLC (linear gradient 0→5% *tert*-butyl methyl ether in CH_2Cl_2 during 140 min) to give **23** (96 mg, 71%) and the corresponding β anomer (12 mg, 9%). Compound **23** had: $[\alpha]_D^{25}$ +21° (*c* 0.68, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.38 (d, 1 H, *J* = 3.7 Hz, H-1'), 5.28 (d, 1 H, *J* = 8.2 Hz, NH), 5.14 (ABd, 1 H, *J* = 12.3 Hz, $PhCH_2O$), 5.10 (ABd, 1 H, *J* = 12.2 Hz, $PhCH_2O$), 4.93 (d, 1 H, *J* = 10.2 Hz, $MeOPhCH_2O$), 4.89 (d, 1 H, *J* = 10.2 Hz, $MeOPhCH_2O$), 4.78 (d, 1 H, *J* = 10.2 Hz, $MeOPhCH_2O$), 4.74 (d, 1 H, *J* = 10.2 Hz, $MeOPhCH_2O$), 4.72 (d, 1 H, *J* = 11.4 Hz, $MeOPhCH_2O$), 4.57 (d, 1 H, *J* = 11.4 Hz, $MeOPhCH_2O$), 4.42-4.37 (m, 1 H, Hα), 4.37 (d, 1 H, *J* = 8.4 Hz, H-1), 4.36 (dd, 1 H, *J* = 7.1, 10.5 Hz, $OCOCH_2CH$), 4.25 (dd, 1 H, *J* = 7.1, 10.5 Hz, $OCOCH_2CH$), 4.17 (dd, 1 H, *J* = 1.9, 5.3 Hz, H-4), 4.15 (bt, 1 H, *J* = 7.1 Hz, $OCOCH_2CH$), 4.08 (dd, 1 H, J = 5.4, 7.1 Hz, H-3), 3.82, 3.82, and 3.74 (3 s, each 3 H, OCH_3), 3.59 (dd, 1 H, *J* = 3.7, 9.1 Hz, H-2'), 3.57 (t, 1 H, *J* = 7.7 Hz, H-2), 3.50-3.44 (m, 1 H, Hδ), 1.89-1.80 (m, 1 H, Hβ), 1.74-1.60 (m, 3 H, Hβ, γ, γ), 1.15 (s, 3 H, CH_3), 1.08 (s, 9 H, *t*Bu), 1.07 (s, 3 H, CH_3), 1.05 (s, 9 H, *t*Bu); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 159.1, 155.8, 143.8, 143.7, 141.2, 135.9, 135.6, 135.6, 135.5, 135.2, 133.9, 133.4, 133.2, 133.1, 131.2, 131.0, 130.4, 129.7, 129.5, 129.5, 129.0, 128.6, 128.5, 128.2, 127.7, 127.6, 127.5, 127.0, 125.1, 125.1, 119.9, 113.8, 113.8, 113.8, 109.5, 102.7, 96.4, 81.8, 80.1, 78.0, 77.1, 75.5, 74.9, 73.1, 73.0, 71.9, 70.8, 68.5, 67.2, 67.0, 62.5, 62.0, 55.3, 55.2, 53.7, 47.0, 29.2, 28.0, 26.9, 26.7, 26.2, 25.7, 19.4, 19.2; HRMS (FAB): calcd for $C_{98}H_{111}NO_{18}Si_2Na$ 1668.7237 ($M+Na^+$), found 1668.7251. Anal. Calcd for $C_{98}H_{111}NO_{18}Si_2$: C, 71.5; H, 6.8; N, 0.8. Found: C, 71.5; H, 6.6; N, 0.8.

*N^α-(Fluoren-9-ylmethoxycarbonyl)-5-*O*-[6-*O*-*tert*-butyldiphenylsilyl-2-*O*-(6-*O*-*tert*-butyldiphenylsilyl-2,3,4-tri-*O*-{4-methoxybenzyl}-α-D-glucopyranosyl)-3,4-*O*-isopropylidene-β-D-galactopyranosyl]-5-hydroxy-L-norvaline* (**24**).—Methanolic ammonium acetate (494 µL, 0.1 M, 49 µmol) was added to a solution of **23** (407 mg, 0.247 mmol) in EtOAc (25 mL). The mixture was subjected to hydrogenolysis (1 atm) over 10% Pd-C (407 mg) at room temperature for 5 h. The catalyst was then filtered off (Celite) and washed with EtOAc and EtOH. The combined filtrates were concentrated and flash column chromatography (toluene—EtOH, 40:1 and toluene—EtOH, 25:1) of the residue gave **24** (334 mg, 87%): $[\alpha]_D^{25}$ +27° (*c* 0.56, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$—CD_3OD, 1:1) δ 5.40 (d, 1 H, *J* = 3.7 Hz, H-1'), 4.85 (d, 1 H, *J* = 10.4 Hz, $MeOPhCH_2O$), 4.82 (d, 1 H, *J* = 10.4 Hz, $MeOPhCH_2O$), 4.72 (d, 1 H, *J* = 10.3 Hz, $MeOPhCH_2O$), 4.71 (d, 1 H, *J* = 11.2 Hz, $MeOPhCH_2O$), 4.69 (d, 1 H, *J* = 10.3 Hz, $MeOPhCH_2O$), 4.52 (d, 1 H, *J* = 11.2 Hz, $MeOPhCH_2O$), 4.37 (d, 1 H, *J* = 8.4 Hz, H-1), 4.27 (dd, 1 H, *J* = 7.1, 10.4 Hz, OCOCH_2CH), 4.19 (dd, 1 H, J = 7.1, 10.3 Hz, OCOCH_2CH), 4.16 (dd, 1 H, J = 1.6, 5.4 Hz, H-4), 4.10 (bt, 1 H, J = 7.2 Hz, OCOCH$_2$CH), 4.07 (dd, 1 H, J = 5.5, 6.9 Hz, H-3), 3.78, 3.78, and 3.71 (3 s, each 3 H, OCH$_3$), 3.55 (dd, 1 H, J = 3.5, 9.0 Hz, H-2'), 3.54 (t, 1 H, J = 7.7 Hz, H-2), 3.51-3.45 (m, 1 H, Hδ), 1.89-1.80 (m, 1 H, Hβ), 1.77-1.60 (m, 3 H, Hβ, γ, γ), 1.12 (s, 3 H, CH$_3$), 1.04 (s, 9 H, *t*Bu), 1.04 (s, 3 H, CH$_3$), 1.01 (s, 9 H, *t*Bu); ^{13}C NMR (101 MHz, CDCl$_3$—CD$_3$OD, 1:1) δ 174.9, 159.9, 159.8, 157.4, 144.5, 144.4, 141.9, 136.5, 136.2, 136.1, 136.1, 134.4, 133.8, 133.7, 133.6, 131.6, 131.4, 130.8, 130.3, 130.3, 130.3, 130.2, 130.1, 130.0, 129.8, 128.3, 128.3, 128.2, 128.1, 127.6, 125.7, 125.6, 120.4, 114.3, 110.2, 103.2, 96.9, 82.3, 80.6, 78.6, 77.2, 76.0, 75.4, 73.9, 73.8, 72.5, 71.5, 69.4, 67.4, 63.2, 62.6, 55.6, 55.5, 54.3, 47.6, 29.2, 28.4, 27.3, 27.0, 26.6, 26.5, 19.8, 19.6; HRMS (FAB): calcd for $C_{91}H_{105}NO_{18}Si_2Na$ 1578.6768 (M+Na$^+$), found 1578.6738. Anal. Calcd for $C_{91}H_{105}NO_{18}Si_2$: C, 70.2; H, 6.8; N, 0.9. Found: C, 70.2; H, 6.5; N, 0.9.

Table 2.
^{1}H NMR Data (δ, ppm) for Glycopeptide **25** in Water Containing 10% D_2O[a]

Residue	NH	H-α	H-β	H-γ	H-δ	Others
Gly^{256}		3.72, 3.57				
Glu^{257}	8.69	4.57	1.94, 1.73	2.20[b]		
Hyp^{258}		4.43	2.25, 1.98	4.54	3.76[b]	
Gly^{259}	8.66	3.84[b]				
Ile^{260}	8.03	4.09	1.78	1.32, 1.08	0.72	0.81 (β-CH$_3$)
Ala^{261}	8.61	4.18	1.29			
Gly^{262}	7.79	3.83, 3.74				
Phe^{263}	8.11	4.50	3.00, 2.98			7.24, 7.14 (arom.)
Hnv^{264}	8.42	4.21	1.80, 1.61	1.55, 1.48	3.80, 3.53	Galβ[c]
Gly^{265}	8.40	3.79[b]				
Glu^{266}	8.38	4.18	1.96, 1.82	2.20[b]		
Gln^{267}	8.60	4.26	2.05, 1.88	2.28[b]		7.55, 6.87 (CONH$_2$)
Gly^{268}	8.36	4.06, 3.86				
Pro^{269}		4.31	2.17, 1.87	1.91[b]	3.50[b]	
Lys^{270}	8.14	4.05	1.72, 1.60	1.32[b]	1.56[b]	2.88[b] (Hε), 7.49 (ε-NH$_2$)

[a] Obtained at 500 MHz, 278 K and pH = 5.4 with H_2O as internal standard (δ_H 4.98 ppm).

[b] Degeneracy has been assumed.

[c] Chemical shifts (δ, ppm) for the galactose moiety: 4.26 (H-1), 3.81 (H-4), 3.66[b] (H-6), 3.56 (H-5), 3.53 (H-3), 3.38 (H-2).

*Glycyl-*L*-glutam-1-yl-*trans*-4-hydroxy-L-prolyl-glycyl-*L*-isoleucyl-*L*-alanyl-glycyl-*L*-phenylalanyl-5-*O*-(*β*-*D*-galactopyranosyl)-5-hydroxy-L-norvalyl-glycyl-L-glutam-1-yl-L-glutaminyl-glycyl-L-prolyl-*L*-lysine* (**25**).—Synthesis, cleavage of the resin-bound glycopeptide (169 mg, 25 μmol) with simultaneous deprotection, and then purification by reversed-phase HPLC (linear gradient 0→100% *B* in *A* during 60 min), according to the general procedure, gave **25** (23.4 mg, 79% peptide content, 45% overall yield): ^{1}H NMR data, see Table 2; MS (FAB): calcd 1638 (M+H$^+$), found 1639; amino acid analysis: Ala 1.00 (1), Glu 2.91 (3), Gly 4.97 (5), Hyp 0.96 (1), Ile 1.15 (1), Lys 1.00 (1), Phe 1.00 (1), Pro 1.01 (1).

*Glycyl-L-glutam-1-yl-*trans*-4-hydroxy-L-prolyl-glycyl-L-isoleucyl-L-alanyl-glycyl-L-phenylalanyl-5-O-(2-O-α-D-glucopyranosyl-β-D-galactopyranosyl)-5-hydroxy-L-norvalyl-glycyl-L-glutam-1-yl-L-glutaminyl-glycyl-L-prolyl-L-lysine* (**26**).—Synthesis, cleavage of the resin-bound glycopeptide (87 mg, 14 μmol) with simultaneous deprotection, and then purification by reversed-phase HPLC (linear gradient 0→100% *B* in *A* during 60 min), according to the general procedure, gave **26** (9.5 mg, 70% peptide content, 26% overall yield): ^{1}H NMR data, see Table 3; MS (FAB): calcd 1799 (M+H$^+$), found 1799; amino acid analysis: Ala 1.00 (1), Glu 3.00 (3), Gly 4.95 (5), Hyp 1.02 (1), Ile 1.00 (1), Lys 1.01 (1), Phe 1.01 (1), Pro 1.01 (1).

Table 3.

^{1}H NMR Data (δ, ppm) for Glycopeptide **26** in Water Containing 10% D_2O^a

Residue	NH	H-α	H-β	H-γ	H-δ	Others
Gly^{256}		3.76, 3.62				
Glu^{257}	8.73	4.63	2.00, 1.78	2.28^b		
Hyp^{258}		4.47	2.30, 2.02	4.58	3.81^b	
Gly^{259}	8.70	3.89^b				
Ile^{260}	8.07	4.14	1.82	1.37, 1.12	0.77	0.86 (β-CH_3)
Ala^{261}	8.65	4.22	1.32			
Gly^{262}	7.91	3.87, 3.79				
Phe^{263}	8.12	4.54	3.04, 3.01			7.26, 7.17 (arom.)
Hnv^{264}	8.48	4.22	1.84, 1.64	1.60, 1.53	3.86, 3.58	Glcα1,2Galβ^c
Gly^{265}	8.43	3.83^b				
Glu^{266}	8.36	4.24	2.02, 1.85	2.29^b		
Gln^{267}	8.63	4.31	2.09, 1.92	2.32^b		7.59, 6.91 ($CONH_2$)
Gly^{268}	8.40	4.10, 3.91				
Pro^{269}		4.36	2.21, 1.91	1.95^b	3.54^b	
Lys^{270}	8.19	4.11	1.77, 1.65	1.36^b	1.60^b	2.92^b (Hε), 7.53 (ε-NH_2)

[a] Obtained at 500 MHz, 278 K and pH = 5.4 with H_2O as internal standard (δ_H 4.98 ppm).

[b] Degeneracy has been assumed.

[c] Chemical shifts (δ, ppm) for the disaccharide moiety; Gal: 4.44 (H-1), 3.85 (H-4), 3.71^b (H-6), 3.65 (H-3), 3.60 (H-5), 3.55 (H-2); Glc: 5.26 (H-1), 3.97 (H-5), 3.72 (H-6), 3.67 (H-3), 3.66 (H-6), 3.43 (H-2), 3.37 (H-4).

Glycyl-L-glutam-1-yl-trans-4-hydroxy-L-prolyl-glycyl-L-isoleucyl-L-alanyl-glycyl-L-phenylalanyl-5-O-(2-O-α-D-glucopyranosyl-β-D-galactopyranosyl)-5-hydroxy-L-norvalyl-glycyl-L-glutam-1-yl-L-glutaminyl-glycyl-L-prolyl-5-O-(2-O-α-D-glucopyranosyl-β-D-galactopyranosyl)-5-hydroxy-L-norvalyl-glycine (**27**).—Synthesis, cleavage of the resin-bound glycopeptide (164 mg, 20 μmol) with simultaneous deprotection, and then purification by reversed-phase HPLC (linear gradient 0→100% *B* in *A* during 60 min), according to the general procedure, gave **27** (19.4 mg, 85% peptide content, 38% overall yield): ^{1}H NMR data, see Table 4; MS (FAB): calcd 2166 (M+H$^+$), found 2167; amino acid analysis: Ala 1.02 (1), Glu 2.93 (3), Gly 5.93 (6), Hyp 1.02 (1), Ile 1.08 (1), Phe 1.01 (1), Pro 1.02 (1).

Table 4.
^{1}H NMR Data (δ, ppm) for Glycopeptide **27** in Water Containing 10% D_2O[a]

Residue	NH	H-α	H-β	H-γ	H-δ	Others
Gly^{256}		3.74, 3.59				
Glu^{257}	8.70	4.59	1.96, 1.75	2.22[b]		
Hyp^{258}		4.45	2.27, 2.00	4.56	3.78[b]	
Gly^{259}	8.68	3.86[b]				
Ile^{260}	8.04	4.12	1.80	1.34, 1.10	0.75	0.84 (β-CH_3)
Ala^{261}	8.63	4.20	1.30			
Gly^{262}	7.85	3.86, 3.77				
Phe^{263}	8.11	4.52	3.02, 2.99			7.26, 7.15 (arom.)
Hnv^{264}	8.45	4.21	1.82, 1.62	1.57, 1.51	3.83, 3.56	Glcα1,2Galβ[c]
Gly^{265}	8.42	3.80[b]				
Glu^{266}	8.37	4.20	1.98, 1.82	2.22[b]		
Gln^{267}	8.62	4.27	2.06, 1.91	2.31[b]		7.58, 6.89 ($CONH_2$)
Gly^{268}	8.40	4.10, 3.88				
Pro^{269}		4.35	2.21, 1.87	1.93[b]	3.53[b]	
Hnv^{270}	8.58	4.28	1.90, 1.71	1.68, 1.61	3.87, 3.60	Glcα1,2Galβ[d]
Gly^{271}	8.05	3.67[b]				

[a] Obtained at 500 MHz, 278 K and pH = 5.4 with H_2O as internal standard (δ_H 4.98 ppm).

[b] Degeneracy has been assumed.

[c] Chemical shifts (δ, ppm) for the disaccharide moiety; Gal: 4.42 (H-1), 3.83 (H-4), 3.67[b] (H-6), 3.63 (H-3), 3.58 (H-5), 3.51 (H-2); Glc: 5.24 (H-1), 3.95 (H-5), 3.75 (H-6), 3.68 (H-6), 3.64 (H-3), 3.41 (H-2), 3.35 (H-4).

[d] Chemical shifts (δ, ppm) for the disaccharide moiety; Gal: 4.44 (H-1), 3.83 (H-4), 3.67[b] (H-6), 3.63 (H-3), 3.58 (H-5), 3.52 (H-2); Glc: 5.26 (H-1), 3.95 (H-5), 3.75 (H-6), 3.68 (H-6), 3.65 (H-3), 3.43 (H-2), 3.35 (H-4).

6 Acknowledgments

This work was funded by grants from the Swedish Natural Science Research Council, the Swedish National Board for Industrial and Technical Development and the Swedish Medical Research Council.

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