Preparation of a Diglycosylated Hydroxylysine Building Block Used in Solid-Phase Synthesis of a Glycopeptide from Type II Collagen

Johan Broddefalk, Mattias Forsgren, Ingmar Sethson, and Jan Kihlberg*

Organic Chemistry, Umeå University, SE−901 87 Umeå, Sweden

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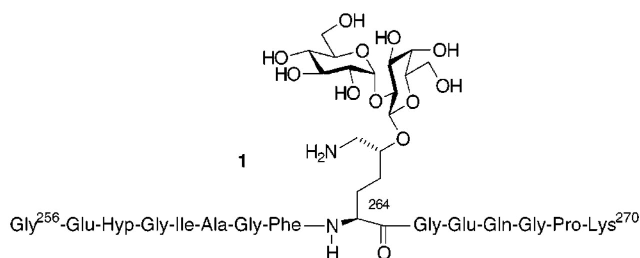
Collagen is the most abundant protein in mammals. In slightly different forms this fibrous protein is found in nearly all organs, where it has structural functions. In collagen, lysine residues located in the sequence Gly-Xaa-Lys can become posttranslationally hydroxylated and then glycosylated, either with a β-D-galactopyranosyl or an α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl moiety.[1] Immunization of mice with type II collagen (CII), the major protein of joint cartilage, leads to collagen-induced arthritis,[2a] i.e., it induces symptoms identical to those of patients suffering from rheumatoid arthritis. Using this animal model it was recently demonstrated that recognition of a peptide epitope found between residues 256 and 270 of CII [CII(256−270)] by autoimmune helper T cells is a key step in eliciting disease.[2b,c] Furthermore, glycosylation of hydroxylysine located at position 264 of CII(256−270) with a galactose moiety was found to be important for the autoimmune response. Access to a hydroxylysine derivative that carries an α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl moiety[3] is required to fully characterize the immune response and in efforts to induce tolerance, i.e., to cure the disease. Such a glycosylated building block should also be useful in studies of the function of collagen in other situations.

Stepwise assembly, using N^{α}-fluoren-9-ylmethoxycarbonyl (Fmoc)-protected glycosylated amino acids as building blocks, is the method of choice for efficient preparation of *O*-linked glycopetides on solid support.[4] Traditionally, the carbohydrate moieties of the glycosylated building blocks have been protected with acetyl or benzoyl groups. These groups enhance the stability of glycosidic linkages toward acid, but base-mediated deprotection may lead to side reactions such as β-elimination or epimerization of peptide stereocenters, especially for the more persistent *O*-benzoates.[5] Benzyl ethers have also been used in solid-phase glycopetide synthesis.[6] However, cysteine, methionine, tyrosine, and to some extent the indole moiety of tryptophan are not compatible with hydrogenolytic removal of benzyl groups.[7] Moreover, during acid-catalyzed cleavage from the solid support, several partially de-*O*-benzylated glycopeptides are formed, thereby complicating isolation of the target glycopeptides.[6a]

We have developed a different protective group strategy based on the use of acid-labile protective groups for the sugar moieties of glycopeptides. This allows an acid-catalyzed, global deprotection step simultaneously with cleavage of the glycopeptide from the solid support. Furthermore, the acid-labile carbohydrate protective groups should be stable both during preparation of the glycosylated building block and during peptide synthesis. Thereby, potentially problematic protective group manipulations can be avoided and the number of synthetic steps required for preparation of complex glycosylated amino acids is reduced. We now report on the use of this strategy to prepare glycopeptide **1**, which corresponds to residues 256−270 of type II collagen.



Results and Discussion

We have recently reported the synthesis of β-D-galactosylated 5-hydroxy-L-norvaline and (5*R*)-5-hydroxy-L-lysine (cf. **2** and **3**, Scheme 1) carrying acid-labile protective groups for both the carbohydrate moiety and the ϵ-amino group of hydroxylysine.[2c,8] Cleavage of the benzyl ester transformed **2** and **3** into glycosylated building blocks that were incorporated in glycopeptides related to type II collagen. Furthermore, the hydroxynorvaline derivative **2** could be glycosylated using donor **4** under promotion by *N*-iodosuccinimide and silver trifluoromethanesulfonate[9] to give the desired α-glucoside **8** in a high yield.[8] However, attempted attachment of an α-D-glucopyranosyl unit to galactosylated hydroxylysine **3** using glucosyl donor **4** was not successful (Scheme 1).

* Corresponding author. Phone: +46-90-786 68 90. Fax: +46-90-13 88 85. E-mail: jan.kihlberg@chem.umu.se.

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(3) Short glycopeptides in which hydroxylysine carried an α-D-Glc-(1→2)-β-D-Gal moiety have previously been prepared in solution (Koeners, H. J.; Schattenkerk, C.; Verhoeven, J. J.; van Boom, J. H. *Tetrahedron* **1981**, *37*, 1763−1771). However, this synthesis was less flexible because it involved glycosylation of hydroxylysine incorporated in a dipeptide. Moreover, the α- and ϵ-amino groups of hydroxylysine carried identical protective groups, thereby preventing extension of the peptide at the *N*-terminus.

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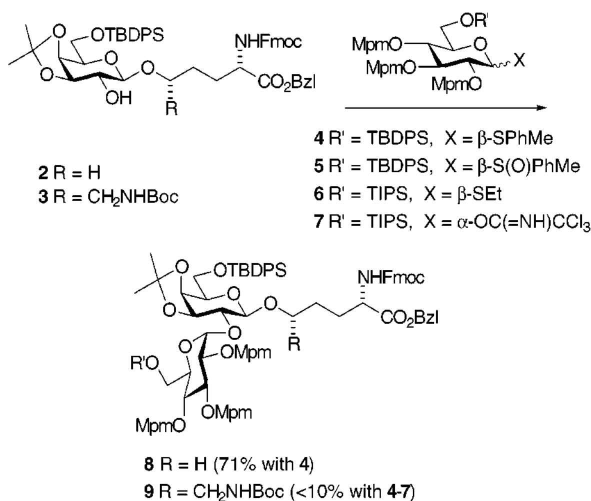
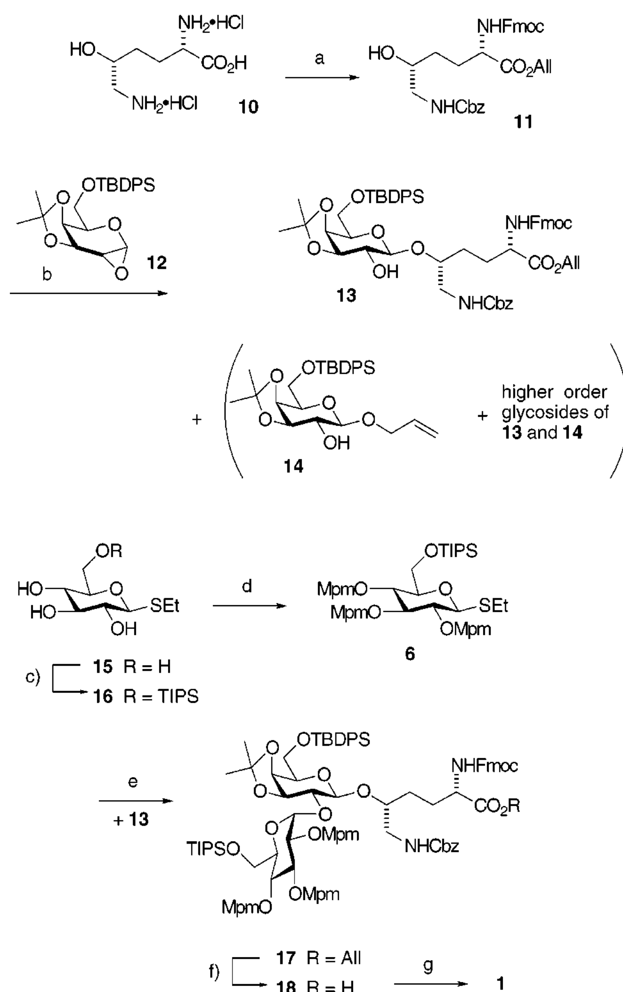
Scheme 1. Attempted Coupling of an α-D-Glucosyl Unit to the Galactosylated Hydroxynorvaline 2 and Hydroxylysine 3



Promotion by various thiofilic reagents[10] resulted in formation of several byproducts,[11] and the yield of diglycosylated hydroxylysine **9** never exceeded ~10%. Use of glucoside donors **5**−**7**, which had similar protective groups as **4** but different leaving groups at the anomeric center, were then investigated. Activation of sulfoxide **5** with trifluoromethanesulfonic anhydride[12] in the presence of base or of the reactive thioethyl glucoside **6** with various soft Lewis acids[13] gave disappointing results, just as did promotion of trichloroacetimidate **7** by a catalytic amount of trimethylsilyl trifluoromethanesulfonate.[14]

Because the problems in glycosylation of **3** originated in the lability of the *N*-*tert*-butoxycarbonyl (Boc) group under the acidic conditions of glycosylations, a more stable protective group had to be used for the ϵ-amino group of hydroxylysine. Preferably, such a protective group should still be removed during acid-catalyzed cleavage of the target glycopeptide from the solid phase to maintain the overall requirement for one global deprotection step. Acid-catalyzed cleavage of the *N*-benzyloxycarbonyl (Cbz) group is usually performed with strong acids such as HBr or sulfonic acids, which cause degradation of glycosidic bonds. However, an early investigation on the synthesis of Met-enkephalin in solution revealed that the Cbz group could be cleaved by trifluoroacetic acid, provided that thioanisole was included as nucleophile.[15] Because these conditions could be compatible with the integrity of *O*-glycosidic bonds, we decided to evaluate the use of the Cbz group in solid-phase synthesis of glycopeptides. The N^{ϵ}-Cbz-protected hydroxylysine derivative **11**, having orthogonally cleavable allyl ester and N^{α}-Fmoc protective groups, was therefore prepared (Scheme 2). This was done essentially as for the analogous Fmoc-Hyl(Boc)-OBzl,[2c] i.e., via formation of a cupric chelate that allowed selective N^{ϵ}-Cbz protection,[16] followed by introduction of the Fmoc group and formation of the allyl ester using a cesium carboxylate.[17] Lactonization was a significant problem in the esterification even when using a large excess of allyl bromide. This reduced the overall yield, as compared to the analogous synthesis of Fmoc-Hyl(Boc)-OBzl.[2c]

Scheme 2[a]



[a] Reagents and conditions: (a) (i) $CuCO_3 \cdot Cu(OH)_2$, CbzCl, H_2O, then Chelex 100 (H^+-form); (ii) FmocCl, Na_2CO_3, H_2O/dioxane (1:1); (iii) Cs_2CO_3, allyl bromide, DMF; 37% overall. (b) $ZnCl_2$, THF, AW-300 MS, −50 °C → rt, 30%. (c) TIPSCl, imidazole, DMF, 96%. (d) MpmCl, NaH, DMF, 74%. (e) NIS, AgOTf, CH_2Cl_2, 4 Å MS, −45 °C, 47%. (f) $(Ph_3P)_4Pd(0)$, PhNHMe, THF, 92%. (g) Solid-phase peptide synthesis, 23%.

β-Galactosylation of protected hydroxylysine **11** was performed in the same manner as reported recently in the synthesis of **3**.[2c] Treatment of the α-1,2-anhydrosugar **12**[8] with **11** in the presence of zinc chloride[18] gave an

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(11) Promotion was attempted by (a) *N*-iodosuccinimide and silver trifluoromethanesulfonate, (b) *N*-iodosuccinimide and trifluoromethanesulfonic acid, (c) *N*-iodosuccinimide and trimethylsilyl trifluoromethanesulfonate, (d) iodonium dicollidine trifluoromethanesulfonate, and (e) methylsulfenyl bromide and silver trifluoromethanesulfonate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine.

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(13) Promotion was attempted by (a) *N*-iodosuccinimide and silver trifluoromethanesulfonate, and (b) *N*-iodosuccinimide and trifluoromethanesulfonic acid.

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anomeric mixture (β/α = 3.8:1 in 51% yield), from which the desired β-glycoside **13** could be isolated in 30% yield. This reaction was also accompanied by lactonization of hydroxylysine **11**, which led to release of allyl alcohol that in turn gave β-allyl galactoside **14** as a byproduct. In addition, small quantities of less polar compounds were obtained and identified as higher order glycosides of **13** and **14** by FABMS. Use of the softer Lewis acid silver trifluoromethanesulfonate[19] in an attempt to reduce lactonization[20] during opening of epoxide **12** gave poor results. The anomeric selectivity shifted in favor of the undesired α-glycoside,[21] while the yield was unchanged (α/β = 1.6:1, 46%).

Coupling of an α-linked glucose unit to galactosylated hydoxylysine **13** was then attempted with the reactive thioethyl glucoside **6** (Scheme 2). This donor was prepared from ethyl 1-thio-β-D-glucopyranoside **15**[22] in two steps. Treatment of **15** with triisopropylsilyl chloride and imidazole gave exclusive silylation of the primary hydroxyl group (→ **16**) and was followed by protection of the secondary hydroxyls using 4-methoxybenzyl chloride and sodium hydride to give **6** in 71% overall yield. Activation of **6** with *N*-iodosuccinimide and silver trifluoromethanesulfonate[9] in dichloromethane allowed coupling to HO-2 of **13**. An anomeric mixture of glycosides (α/β = 3.3:1, 80% yield) was obtained from which pure **17** could be isolated in 47% yield. Attempts to improve the α-selectivity by using a mixture of toluene and diethyl ether as solvent for this glycosylation[23] were not successful (α/β = 2.6:1, 66% yield). Conversion of thioglycoside **6** into a chloro sugar by treatment with iodine monochloride[24] and use of this for glycosylation of **13** promoted by silver trifluoromethanesulfonate in the presence of 2,4,6-trimethylpyridine also affected the preparation of **17** in a negative manner (α/β = 2.1:1, 68% yield). The lower α-selectivity obtained in formation of hydroxylysine derivate **17**, as compared to hydroxynorvaline derivate **8** (α/β = 8:1, 89% yield), indicates a steric influence from the Cbz-protected ϵ-amino group. Finally, deprotection of the allyl ester in **17** using *N*-methylaniline and a catalytic amount of tetrakis(triphenylphosphine) palladium(0)[25] gave diglycosylated hydroxylysine building block **18** (92% yield).

The target type II collagen-derived glycopeptide **1** was prepared from **18** on a polystyrene resin grafted with poly(ethylene glycol) spacers (TentaGel resin). The synthesis was performed according to the Fmoc strategy under standard conditions,[2c,8] employing only a slight excess of diglycosylated building block **18** (1.2 equiv), activated as an azabenzotriazolyl ester.[26] When solid-phase synthesis had been completed, the resin was treated with trifluoroacetic acid containing water, thioanisole, and ethanedithiol as scavengers during 3 h to liberate the glycopeptide from the solid support. Importantly, these conditions were also found to remove the N^{ϵ}-Cbz group of hydroxylysine, as well as the eight different protective groups used for the peptide part and the disaccharide moiety. As revealed by analytical reversed-phase HPLC, this was achieved without affecting the glycosidic bonds, which would have been degraded using the strong acids that are usually employed for removal of the Cbz group. Purification by reversed-phase HPLC then gave glycopeptide **1** in 23% overall yield based on the resin capacity.

Glycopeptide **1** was used to evaluate the specificity of a panel of 29 T cell hydridomas obtained previously by immunization of mice with type II collagen.[2b] It was found that two of the hybridomas recognized **1** when presented by antigen-presenting cells. Previously, 20 of the hydridomas have been shown to recognize the glycopeptide analogue of **1** that lacks the α-D-glucose moiety.[2c] The remaining seven hybridomas responded to peptides that had either lysine or hydroxylysine at position 264. Consequently, the type II collagen fragment CII(256–270) carrying either a galactosyl or a glucosyl-galactosyl residue on hydroxylysine 264 appears to play a crucial role for disease development in the mouse model for rheumatoid arthritis.

In conclusion, use of a Cbz group for protection of the ϵ-amino group of hydroxylysine was essential for the successful synthesis of building block **18** having an α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl moiety linked to hydroxylysine. This building block for the first time allows incorporation of diglycosylated hydroxylysine into glycopeptides, such as the type II collagen fragment **1**, by Fmoc solid-phase synthesis. The protective groups of **18**, including the N^{ϵ}-Cbz group, were all removed during TFA-induced cleavage from the solid phase without degradation of the glycosidic bonds. Access to synthetic glycopeptide fragments from collagen is important in research aimed at understanding how rheumatoid arthritis occurs and in efforts to develop cures for this disease.

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 and THF were distilled from calcium hydride and sodium–potassium/benzophenone, respectively. DMF was distilled and then dried over 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 solvents of HPLC grade or distilled technical grade. Organic solutions were dried over Na_2SO_4 before being concentrated.

^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.27 ppm) or $CDCl_3$ (δ_C 77.0 ppm) as internal standard] or in a 1:1 mixture of CD_3OD and $CDCl_3$ [residual CD_2HOD (δ_H 3.31 ppm) or CD_3OD (δ_C 49.0 ppm) as internal standard] at 300 K. The ^{1}H NMR spectrum of glycopeptide **1** was recorded at 600 MHz in a 9:1 mixture of H_2O and D_2O [H_2O (δ_H 4.95 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,[27a] TOCSY,[27b] and NOESY[27c] experiments. Resonances for aromatic protons and resonances that could not be assigned are not reported. Ions for positive fast atom bombardment mass spectra were produced by a beam of Xenon

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atoms (6 keV) from a matrix of glycerol and thioglycerol. In the amino acid analysis glutamine was determined as glutamic acid.

Analytical normal-phase HPLC was performed on a Kromasil silica column (100 Å, 5 μm, 4.6 mm × 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 mm × 250 mm) with a flowrate of 20 mL/min. Glycopeptide **1** was analyzed on reversed phase using a Kromasil C-8 column (100 Å, 5 μm, 4.6 mm × 250 mm) and 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; *B*, 0.1% trifluoroacetic acid in CH_3CN). Purification of crude **1** was performed on a Kromasil C-8 column (100 Å, 5 μm, 20 mm × 250 mm) using the same eluant and a flow rate of 11 mL/min.

(5*R*)-N^{α}-(Fluoren-9-ylmethoxycarbonyl)-N^{ϵ}-benzyloxycarbonyl-5-hydroxy-L-lysine Allyl Ester (11). Compound **11** was prepared from (5*R*)-5-hydroxy-L-lysine dihydrochloride monohydrate (**10**), benzyl chloroformate (1.6 equiv), 9-fluorenylmethyl chloroformate (1 equiv), and allyl bromide (5 equiv) as described for (5*R*)-N^{α}-(fluoren-9-ylmethoxycarbonyl)-5-hydroxy-L-lysine benzyl ester.[2c] After purification by flash column chromatography (heptane/*tert*-butyl methyl ether/EtOH, 7:3:1) **11** was obtained in 37% overall yield: $[\alpha]^{20}_{D}$ −2° (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.90 (ddt, 1 H, *J* = 5.8, 10.7, 17.0 Hz, OCH_2CHCH_2), 5.59 (d, 1 H, *J* = 7.1 Hz, NHα), 5.33 (bd, 1 H, *J* = 17.2 Hz, OCH_2CHCH_2), 5.19 (bs, 1 H, NHϵ), 5.26 (dd, 1 H, *J* = 1.0, 10.2 Hz, OCH_2CHCH_2), 5.11 (ABd, 1 H, *J* = 12.8 Hz, $PhCH_2O$), 5.08 (ABd, 1 H, *J* = 12.8 Hz, $PhCH_2O$), 4.64 (d, 2 H, *J* = 5.5 Hz, OCH_2CHCH_2), 4.46−4.39 (m, 1 H, Hα), 4.44 (ABdd, 1 H, *J* = 7.1, 10.0 Hz, $FmocCH_2$), 4.38 (ABdd, 1 H, *J* = 6.8, 10.3 Hz, $FmocCH_2$), 4.21 (t, *J* = 6.9 Hz, 1 H, FmocCH), 3.75 (bs, 1 H, Hδ), 3.39−3.31 (m, 1 H, Hϵ), 3.13−3.05 (m, 1 H, Hϵ), 2.84 (bs, 1 H, OH), 2.09−2.00 (m, 1 H, Hβ), 1.87−1.73 (m, 1 H, Hβ), 1.59−1.47 (m, 2 H, Hγ), 1.44 (s, 9 H, *t*Bu); ^{13}C NMR ($CDCl_3$) δ 172.0, 157.2, 156.2, 143.8, 143.7, 141.3, 136.3, 131.4, 128.5, 128.2, 128.1, 127.7, 127.1, 125.0, 120.0, 120.0, 119.2, 70.8, 67.1, 67.0, 66.1, 53.5, 47.1, 46.9, 30.0, 29.2; HRMS (FAB) calcd for $C_{32}H_{35}N_2O_7$ 559.2444 (M + H^+), found 559.2421.

(5*R*)-N^{α}-(Fluoren-9-ylmethoxycarbonyl)-N^{ϵ}-benzyloxycarbonyl-5-*O*-(6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-5-hydroxy-L-lysine Allyl Ester (13). A solution of **11** (305 mg, 0.546 mmol) in THF (3 mL) and crushed molecular sieves (AW-300, 200 mg) was added to freshly prepared 1,2-anhydro-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-α-D-galactopyranoside[8] (**12**, 0.273 mmol), and the mixture was stirred for 20 min at room temperature and then cooled to −50 °C. Zinc chloride (300 μL, 1.0 M in Et_2O, 0.300 mmol) was added, and the reaction mixture was allowed to attain room temperature over 20 h. The mixture was then diluted with EtOAc, filtered (Hyflo-Supercel), and washed with water. The aqueous phase was extracted twice with EtOAc, and the combined organic phases were dried and concentrated. Flash column chromatography (toluene/EtOAc, 7:3 → 1:1) followed by purification by normal-phase HPLC (linear gradient 0% → 20% *tert*-butyl methyl ether in CH_2Cl_2 during 160 min) gave **13** (81 mg, 30%), the corresponding α-anomer (30 mg, 7%), and unreacted acceptor **11** contaminated with the corresponding lactone (182 mg). **Data for 13**: $[\alpha]^{20}_{D}$ +9° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.91 (ddt, 1 H, *J* = 5.7, 10.6, 17.2 Hz, OCH_2CHCH_2), 5.67 (bt, 1 H, *J* = 5.2 Hz, NHϵ), 5.62 (d, *J* = 7.5 Hz, 1 H, NHα), 5.34 (d, 1 H, *J* = 17.2 Hz, OCH_2CHCH_2), 5.27 (d, 1 H, *J* = 10.3 Hz, OCH_2CHCH_2), 5.00 (ABd, 1 H, *J* = 12.3 Hz, $PhCH_2O$), 4.87 (ABd, 1 H, *J* = 12.2 Hz, $PhCH_2O$), 4.65 (bd, 2 H, *J* = 5.6 Hz, OCH_2CHCH_2), 4.44 (ABdd, 1 H, *J* = 7.0, 10.6 Hz, $FmocCH_2$), 4.43−4.37 (m, 1 H, Hα), 4.37 (ABdd, 1 H, *J* = 6.9, 10.4 Hz, $FmocCH_2$), 4.21 (t, 1 H, *J* = 7.1 Hz, FmocCH), 4.20 (bd, 1 H, *J* = 5.5 Hz, H-4), 4.18 (d, 1 H, *J* = 8.3 Hz, H-1), 4.02 (dd, 1 H, *J* = 5.6, 7.0 Hz, H-3), 3.94−3.91 (m, 2 H, H-6), 3.89−3.84 (m, 1 H, H-5), 3.70 (bs, 1 H, Hδ), 3.52 (dt, 1 H, *J* = 3.0, 7.6 Hz, H-2), 3.42−3.36 (m, 1 H, Hϵ), 3.31 (d, 1 H, *J* = 2.9 Hz, OH), 3.22 (dt, 1 H, *J* = 5.6, 14.3 Hz, Hϵ), 2.03−1.84 (m, 2 H, Hβ), 1.71−1.55 (m, 2 H, Hγ), 1.48 and 1.32 (2 s, each 3 H, CH_3), 1.32 (s, 9 H, *t*Bu); ^{13}C NMR ($CDCl_3$) δ 171.8, 156.8, 156.0, 143.8, 143.7, 141.2, 136.6, 135.6, 135.5, 133.1, 133.0, 131.4, 129.8, 128.4, 128.0, 127.9, 127.8, 127.7, 127.7, 127.1, 125.1, 120.0, 120.0, 119.3, 110.1, 102.9, 80.0, 78.9, 73.6, 73.5, 73.0, 67.0, 66.4, 66.2, 62.6, 53.5, 47.1, 44.8, 28.2, 28.1, 28.0, 26.7, 26.2, 19.1; HRMS (FAB) calcd for $C_{57}H_{66}N_2O_{12}SiNa$ 1021.4283 (M + Na^+), found 1021.4305.

Ethyl 6-*O*-Triisopropylsilyl-1-thio-β-D-glucopyranoside (16). Triisopropylsilyl chloride (1.14 mL, 5.35 mmol) was added to a solution of ethyl 1-thio-β-D-glucopyranoside[22] (**15**, 1.00 g, 4.45 mmol) and imidazole (759 mg, 11.2 mmol) in DMF (10 mL) at 0 °C. After 5 h the mixture was partitioned between EtOAc and saturated aqueous NH_4Cl. The aqueous phase was extracted with, and the combined organic phases were dried and concentrated. Flash column chromatography (toluene/EtOH, 10:1) of the residue gave **16** (1.63 g, 96%): $[\alpha]^{20}_{D}$ −49° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.35 (d, 1 H, *J* = 9.7 Hz, H-1), 4.05 (dd, 1 H, *J* = 4.9, 10.0 Hz, H-6), 3.89 (dd, 1 H, *J* = 7.0, 10.0 Hz, H-6), 3.71 (s, 1 H, OH), 3.64 (t, 1 H, *J* = 8.8 Hz, H-4), 3.61 (t, 1 H, *J* = 8.7 Hz, H-3), 3.44 (ddd, 1 H, *J* = 4.9, 7.0, 8.8 Hz, H-5), 3.41 (bt, 1 H, *J* = 8.9 Hz, H-2), 3.08 (s, 1 H, OH), 2.74 (dq, 1 H, *J* = 7.5, 12.6 Hz, SCH_2CH_3), 2.70 (dq, 1 H, *J* = 7.4, 12.7 Hz, SCH_2CH_3), 2.65 (s, 1 H, OH), 1.30 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 1.17−1.04 (m, 21 H, *i*Pr); ^{13}C NMR ($CDCl_3$) δ 85.7, 77.7, 77.5, 73.7, 72.1, 65.8, 24.3, 17.9, 15.4, 11.8; HRMS (FAB) calcd for $C_{17}H_{36}O_5SSiNa$ 403.1950 (M + Na^+), found 403.1965. Anal. Calcd for $C_{17}H_{36}O_5SSi$: C, 53.6; H, 9.5. Found: C, 53.4; H, 9.6.

Ethyl 6-*O*-Triisopropylsilyl-2,3,4-tri-*O*-(4-methoxybenzyl)-1-thio-β-D-glucopyranoside (6). Sodium hydride (473 mg, 60% in mineral oil, 11.8 mmol) was added to a solution of **16** (1.00 g, 2.63 mmol) and 4-methoxybenzyl chloride (2.14 mL, 15.8 mmol) in DMF (10 mL) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at room temperature for a further 32 h. MeOH (5 mL) was then added, and the mixture was partitioned between toluene and saturated aqueous NH_4Cl. The organic phase was washed with saturated aqueous NaCl and water, dried, and concentrated. Flash column chromatography (toluene/EtOAc, 30:1 and heptane/EtOAc, 3:1) of the residue gave **6** (1.45 g, 74%): $[\alpha]^{20}_{D}$ 4° (*c* 4.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.85 (ABd, 1 H, *J* = 10.4 Hz, $MeOPhCH_2O$), 4.83 (d, 1 H, *J* = 9.5 Hz, $MeOPhCH_2O$), 4.80 (ABd, 1 H, *J* = 10.9 Hz, $MeOPhCH_2O$), 4.78 (d, 1 H, *J* = 10.5 Hz, $MeOPhCH_2O$), 4.68 (d, 1 H, *J* = 9.9 Hz, $MeOPhCH_2O$), 4.63 (d, 1 H, *J* = 10.5 Hz, $MeOPhCH_2O$), 4.43 (d, 1 H, *J* = 9.7 Hz, H-1), 3.94 (dd, 1 H, *J* = 1.7, 11.2 Hz, H-6), 3.85 (dd, 1 H, *J* = 4.5, 11.2 Hz, H-6), 3.81, 3.80, and 3.80 (3 s, each 3 H, OCH_3), 3.64 (t, 1 H, *J* = 9.1 Hz, H-3), 3.60 (t, 1 H, *J* = 9.1 Hz, H-4), 3.38 (dd, 1 H, *J* = 8.7, 9.7 Hz, H-2), 3.27 (ddd, 1 H, *J* = 1.7, 4.4, 9.3 Hz, H-5), 2.78 (dq, 1 H, *J* = 7.4, 12.5 Hz, SCH_2CH_3), 2.68 (dq, 1 H, *J* = 7.5, 12.5 Hz, SCH_2CH_3), 1.29 (t, 3 H, *J* = 7.4 Hz, SCH_2CH_3), 1.15−1.04 (m, 21 H, *i*Pr); ^{13}C NMR ($CDCl_3$) δ 159.3, 159.3, 159.2, 130.8, 130.5, 130.4, 129.9, 129.6, 129.5, 113.9, 113.8, 86.5, 84.3, 81.7, 80.3, 77.3, 75.5, 75.1, 74.6, 62.6, 55.3, 55.2, 24.1, 18.0, 18.0, 15.0, 12.0; HRMS (FAB) calcd for $C_{41}H_{60}O_8SSiNa$ 763.3676 (M + Na^+), found 763.3658.

(5*R*)-N^{α}-(Fluoren-9-ylmethoxycarbonyl)-N^{ϵ}-benzyloxycarbonyl-5-*O*-{6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-2-*O*-[6-*O*-triisopropylsilyl-2,3,4-tri-*O*-(4-methoxybenzyl)-α-D-glucopyranosyl]-β-D-galactopyranosyl}-5-hydroxy-L-lysine Allyl Ester (17). A mixture of **6** (52 mg, 70 μmol), **13** (58 mg, 58 μmol), and powdered molecular sieves (4 Å, 150 mg) in CH_2Cl_2 (2 mL) was stirred at room temperature for 15 min. *N*-Iodosuccinimide (18 mg, 70 μmol) was added, and the mixture was cooled to −45 °C and protected from light. Silver trifluoromethanesulfonate (7 mg, 28 μmol) was added, the reaction mixture was stirred for 80 min at −45 °C, and the reaction was then quenched by addition of triethylamine (41 μL, 0.29 mmol). After 5 min, the mixture was diluted with CH_2Cl_2, filtered (Hyflo Supercel), and washed with 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The organic layer was dried, concentrated, and subjected to flash column chromatography (toluene/EtOAc, 8:1) to give **17** and the corresponding β-isomer (78 mg, α/β = 3.3:1, ∼80%). Purification of the mixture by normal-phase HPLC (linear gradient 0% → 8% *tert*-butyl methyl ether in CH_2Cl_2 during 160 min) gave **17** (46 mg, 47%): $[\alpha]^{20}_{D}$ +28° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.85 (ddt, 1 H, *J* = 5.5, 10.7, 17.2 Hz, OCH_2CHCH_2), 5.65 (bs, 1 H, NHϵ), 5.64 (d, 1 H, *J* = 7.2 Hz, NHα), 5.28 (bd, 1 H, *J* = 17.2 Hz, OCH_2CHCH_2), 5.22 (d, 1 H, *J* = 3.5 Hz, H-1′), 5.20 (dd, 1 H, *J* = 1.0, 10.5 Hz, OCH_2CHCH_2), 4.98 (bs, 2 H, $PhCH_2O$), 4.81 (d, 2 H, *J* = 9.9 Hz, $MeOPhCH_2O$), 4.76 (ABd, 1 H, *J* = 10.5 Hz, $MeOPhCH_2O$), 4.68 (d, 1 H, *J* = 10.2 Hz, $MeOPhCH_2O$), 4.67 (bs, 2 H, $MeOPhCH_2O$), 4.57 (bd, 2 H, *J* = 5.3 Hz, OCH_2CHCH_2), 4.46 (d, 1 H, *J* = 7.8 Hz, H-1),

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

residue	NH	H-α	H-β	H-γ	H-δ	others
Gly^{256}		3.76, 3.61				
Glu^{257}	8.70	4.60	1.97, 1.77	2.22[b]		
Hyp^{258}		4.45	2.27, 1.01	4.56	3.85, 3.79	
Gly^{259}	8.67	3.90, 3.84				
Ile^{260}	8.05	4.12	1.81	1.35, 1.11	0.77	0.85 (β-CH_3)
Ala^{261}	8.62	4.21	1.31			
Gly^{262}[c]	8.43	3.83[b]				
Phe^{263}	8.10	4.60	3.01[b]			7.27, 7.17 (arom.)
Hyl^{264}	8.46	4.19	1.87, 1.63	1.58[b]	4.00	3.09, 2.91 (Hϵ), 7.61 (ϵ-NH_2), GlcGal[d]
Gly^{265}[c]	7.87	3.81[b]				
Glu^{266}	8.46	4.22	1.99, 1.85	2.22[b]		
Gln^{267}	8.63	4.27	2.07, 1.91	2.32[b]		7.58, 6.90 ($CONH_2$)
Gly^{268}	8.40	4.09, 3.90				
Pro^{269}		4.34	2.20, 1.90	1.93[b]	3.53[b]	
Lys^{270}	8.14	4.08	1.75, 1.63	1.34[b]	1.59[b]	2.90[b](Hϵ), 7.50 (ϵ-NH_2)

[a] Obtained at 600 MHz, 278 K, and pH = 5.4 with H_2O as internal standard (δ_H 4.95 ppm). [b] Degeneracy has been assumed. [c] The assignment of Gly^{262} and Gly^{265} was exchanged by mistake in refs 2c and 8. [d] Chemical shifts (δ, ppm) for the disaccharide moiety: Gal 4.53 (H-1), 3.85 (H-4), 3.71 (H-6,6), 3.62 (H-5), 3.65 (H-3), 3.58 (H-2); Glc 5.31 (H-1′), 3.99 (H-5′), 3.76 (H-6′), 3.70 (H-6′), 3.68 (H-3′), 3.41 (H-2′), 3.37 (H-4′).

4.45−4.38 (m, 1 H, $FmocCH_2$), 4.21−4.10 (m, 3 H, Hα, FmocCH, $FmocCH_2$), 4.12 (dd, 1 H, *J* = 5.5, 7.1 Hz, H-3), 4.09 (dd, 1 H, *J* = 1.6, 5.8 Hz, H-4), 4.05 (dd, 1 H, *J* = 1.8, 11.1 Hz, H-6), 3.97−3.92 (m, 1 H, H-4′), 3.96 (t, 1 H, *J* = 9.4 Hz, H-3′), 3.90 (dd, 1 H, *J* = 3.0, 10.3 Hz, H-6′), 3.90−3.86 (m, 1 H, H-6), 3.85−3.68 (m, 4 H, H-5, H-5′, H-6′, Hδ), 3.79, 3.79, and 3.74 (3 s, each 3 H, OCH_3), 3.63 (t, 1 H, *J* = 7.1 Hz, H-2), 3.49 (dd, 1 H, *J* = 3.6, 9.7 Hz, H-2′), 3.40−3.30 (m, 2 H, Hϵ), 1.80−1.58 (m, 4 H, Hβ, Hγ), 1.44 and 1.23 (2 s, each 3 H, CH_3), 1.14−1.03 (m, 21 H, *i*Pr), 1.01 (s, 9 H, *t*Bu); ^{13}C NMR ($CDCl_3$) δ 171.8, 159.2, 159.1, 157.2, 156.2, 143.9, 143.8, 141.2, 136.4, 135.6, 135.5, 133.0, 133.0, 131.6, 131.1, 131.0, 130.4, 129.8, 129.5, 128.8, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 127.1, 127.1, 125.2, 125.2, 119.9, 118.8, 113.8, 113.8, 109.7, 101.7, 96.7, 81.8, 79.8, 78.7, 78.0, 77.2, 76.2, 75.3, 74.7, 73.5, 73.1, 72.6, 71.4, 67.0, 66.7, 65.9, 63.0, 61.9, 55.3, 55.2, 53.8, 47.1, 43.0, 28.0, 27.5, 26.7, 26.1, 19.1, 18.1, 18.0, 12.0, 11.9; HRMS (FAB) calcd for $C_{96}H_{120}N_2O_{20}Si_2Na$ 1699.7871 (M + Na^+), found 1699.7887. Anal. Calcd for $C_{96}H_{120}N_2O_{20}Si_2$: C, 68.7; H, 7.2; N, 1.7. Found: C, 68.5; H, 7.3; N, 1.8.

(5*R*)-*N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*N*$^{\epsilon}$-benzyloxycarbonyl-5-*O*-{6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-2-*O*-[6-*O*-triisopropylsilyl-2,3,4-tri-*O*-(4-methoxybenzyl)-α-D-glucopyranosyl]-*β*-D-galactopyranosyl}-5-hydroxy-L-lysine (18). *N*-Methylaniline (7.6 μL, 70 μmol) and tetrakis(triphenylphosphine) palladium(0) (3 mg, 2 μmol) were added to a solution of **17** (39 mg, 23 μmol) in THF (1 mL) at room temperature. The reaction mixture was protected from light and stirred for 40 min. The mixture was then diluted with EtOAc and washed with saturated aqueous NH_4Cl. The organic layer was dried, concentrated, and subjected to flash column chromatography (CH_2Cl_2/MeOH, 50:1 → 10:1) to give **18** (35 mg, 92%): $[\alpha]^{20}_D$ +28° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 5.23 (d, 1 H, *J* = 3.6 Hz, H-1′), 4.93 (ABd, 1 H, *J* = 12.4 Hz, $PhCH_2O$), 4.89 (ABd, 1 H, *J* = 12.3 Hz, $PhCH_2O$), 4.78 (d, 1 H, *J* = 10.5 Hz, $MeOPhCH_2O$), 4.75 (d, 1 H, *J* = 10.4 Hz, $MeOPhCH_2O$), 4.66 (ABd, 1 H, *J* = 10.3 Hz, $MeOPhCH_2O$), 4.64 (d, 1 H, *J* = 10.4 Hz, $MeOPhCH_2O$), 4.58 (ABd, 1 H, *J* = 10.5 Hz, $MeOPhCH_2O$), 4.49 (d, 1 H, *J* = 7.8 Hz, H-1), 4.27 (dd, 1 H, *J* = 6.4, 9.2 Hz, $FmocCH_2$), 4.19−4.06 (m, 5 H, H-3, H-4, Hα, FmocCH, $FmocCH_2$), 3.99 (dd, 1 H, *J* = 1.8, 11.0 Hz, H-6), 3.92 (bt, 1 H, *J* = 10.2 Hz, H-4′), 3.90 (t, 1 H, *J* = 9.3 Hz, H-3′), 3.91−3.82 (m, 4 H, H-5, H-6, H-6′, H-6′), 3.75, 3.74, and 3.67 (3 s, each 3 H, OCH_3), 3.75−3.64 (m, 2 H, H-5′, Hδ), 3.59 (dd, 1 H, *J* = 6.1, 7.6 Hz, H-2), 3.44 (dd, 1 H, *J* = 3.6, 9.7 Hz, H-2′), 3.34−3.31 (m, 1 H, Hϵ), 3.23 (dd, 1 H, *J* = 5.6, 14.5 Hz, Hϵ), 1.87−1.78 (m, 1 H, Hβ), 1.73−1.58 (m, 3 H, Hβ, Hγ, Hγ), 1.44 and 1.23 (2 s, each 3 H, CH_3), 1.14−1.03 (m, 21 H, *i*Pr), 1.01 (s, 9 H, *t*Bu); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 174.8, 159.9, 159.9, 159.8, 158.2, 157.6, 144.5, 141.9, 141.9, 137.1, 136.2, 136.1, 133.7, 133.6, 133.2, 133.1, 132.6, 132.5, 131.6, 131.5, 130.8, 130.4, 130.1, 130.1, 129.9, 129.4, 129.3, 128.9, 128.5, 128.4, 128.3, 127.7, 127.6, 125.7, 120.4, 114.4, 114.3, 110.3, 101.7, 97.2, 82.3, 80.2, 78.7, 77.8, 77.1, 75.9, 75.3, 73.9, 73.2, 72.0, 67.5, 67.2, 63.4, 62.6, 55.6, 55.5, 54.4, 47.7, 44.1, 28.4, 27.1, 26.4, 19.6, 18.4, 18.4, 12.6; HRMS (FAB) calcd for $C_{93}H_{115}N_2O_{20}Si_2Na_2$ 1681.7377 (M − H + 2Na^+), found 1681.7383. Anal. Calcd for $C_{93}H_{116}N_2O_{20}Si_2$: C, 68.2; H, 7.1; N, 1.7. Found: C, 68.2; H, 7.2; N, 1.7.

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-lysyl]-glycyl-L-glutam-1-yl-L-glutaminyl-glycyl-L-prolyl-L-lysine (1). Glycopeptide **1** was synthesized in a mechanically agitated reactor on a resin consisting of a cross-linked polystyrene backbone grafted with poly(ethylene glycol) chains. The resin carried the *C*-terminal lysine on a *p*-hydroxymethylphenoxy linker (TentaGel S PHB, Rapp Polymere, Germany). Reagent solutions and DMF for washing were added manually to the reactor. *N*$^{\alpha}$-Fmoc-amino acids (Bachem, Switzerland) with the following protective groups were used: triphenylmethyl (Trt) for glutamine; *tert*-butyl for glutamic acid and hydroxyproline; and *tert*-butoxycarbonyl (Boc) for lysine.

The *N*$^{\alpha}$-Fmoc-amino acids were activated as 1-benzotriazolyl esters[28] and then added to the resin (15 μmol). Activation was performed by reaction of the appropriate *N*$^{\alpha}$-Fmoc-amino acid (60 μmol), 1-hydroxybenzotriazole (HOBt, 14 mg, 90 μmol), and 1,3-diisopropylcarbodiimide (58.5 μmol, 0.15 mL of a 0.39 mM solution in DMF) in DMF (0.1 mL) for 30 min. The acylation was performed during 1−15 h and was monitored by addition of bromophenol blue (11 nmol, 7.5 μL of a 0.15 mM solution in DMF). *N*$^{\alpha}$-Fmoc deprotection of the peptide resin was effected by treatment with 20% piperidine in DMF during 10−20 min. The glycosylated amino acid **18** (27 mg, 16.5 μmol) was activated as described above using 1,3-diisopropylcarbodiimide (16.5 μmol) and 1-hydroxy-7-azabenzotriazole[26] (HOAt, 6.7 mg, 50 μmol). Activated **1** was then coupled during 24 h, and the coupling was monitored by bromophenol blue as described above.

After completion of the synthesis, the resin carrying protected peptide **1** was washed with CH_2Cl_2 and dried under vacuum. The glycopeptide−resin was cleaved, 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, 14 mL) for 3 h followed by filtration. Acetic acid was added to the filtrate, the solution was concentrated, and acetic acid was added twice more followed by concentration after each addition. The residue was triturated with diethyl ether, which gave a solid, crude glycopeptide that was dissolved in a mixture of acetic acid and water and freeze-dried. Purification by preparative reversed-phase HPLC (linear gradient 0% → 100% *B* in *A* during 60 min) gave **1** (9 mg, 71% peptide content, 23% overall yield): ^{1}H NMR data are given in Table 1; MS (FAB) calcd 1828 (M + H^+), found 1827. Amino acid analysis: Ala 0.98 (1), Glu 3.03 (3), Gly 4.90 (5), Hyl 1.02 (1), Hyp 1.01 (1), Ile 1.02 (1), Lys 1.03 (1), Phe 1.01 (1), Pro 1.01 (1).

(28) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788−798.

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Supporting Information Available: The procedure used for synthesis of compound **11**, as well as ^{1}H and ^{13}C NMR spectra for compounds **6**, **11**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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