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A Stereocontrolled Synthetic Approach to Glycopeptides Corresponding to the Carbohydrate–Protein Linkage Region of Cell-Surface Proteoglycans

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Abstract—The glycopeptides **1** (Gly{*O*-β-D-Xyl*p*}-L-Ser-Gly-L-Glu) and **2** (Gly{*O*-{β-D-Glc*p*A-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*}-(1→4)-β-D-Xyl*p*}-L-Ser-Gly-L-Glu), carrying the core structure of serine-linked cell-surface proteoglycans were synthesized in a stereocontrolled manner. The carbohydrate key imidate xylosyl donors **3** and glycotetraosyl donors **4** and **5**, as well as a tetrapeptide glycosyl acceptor **6**, were coupled in the crucial glycosylation step. In these reactions, the application of either trimethylsilyl trifluoromethanesulfonate (TMSOTf) or borontrifluoride etherate (BF_3·Et_2O) as catalysts proved to be highly efficient. The serine linked glycopeptides **34**, **36** and **37** thus obtained yielded target compounds **1** and **2** on complete deprotection.

Introduction

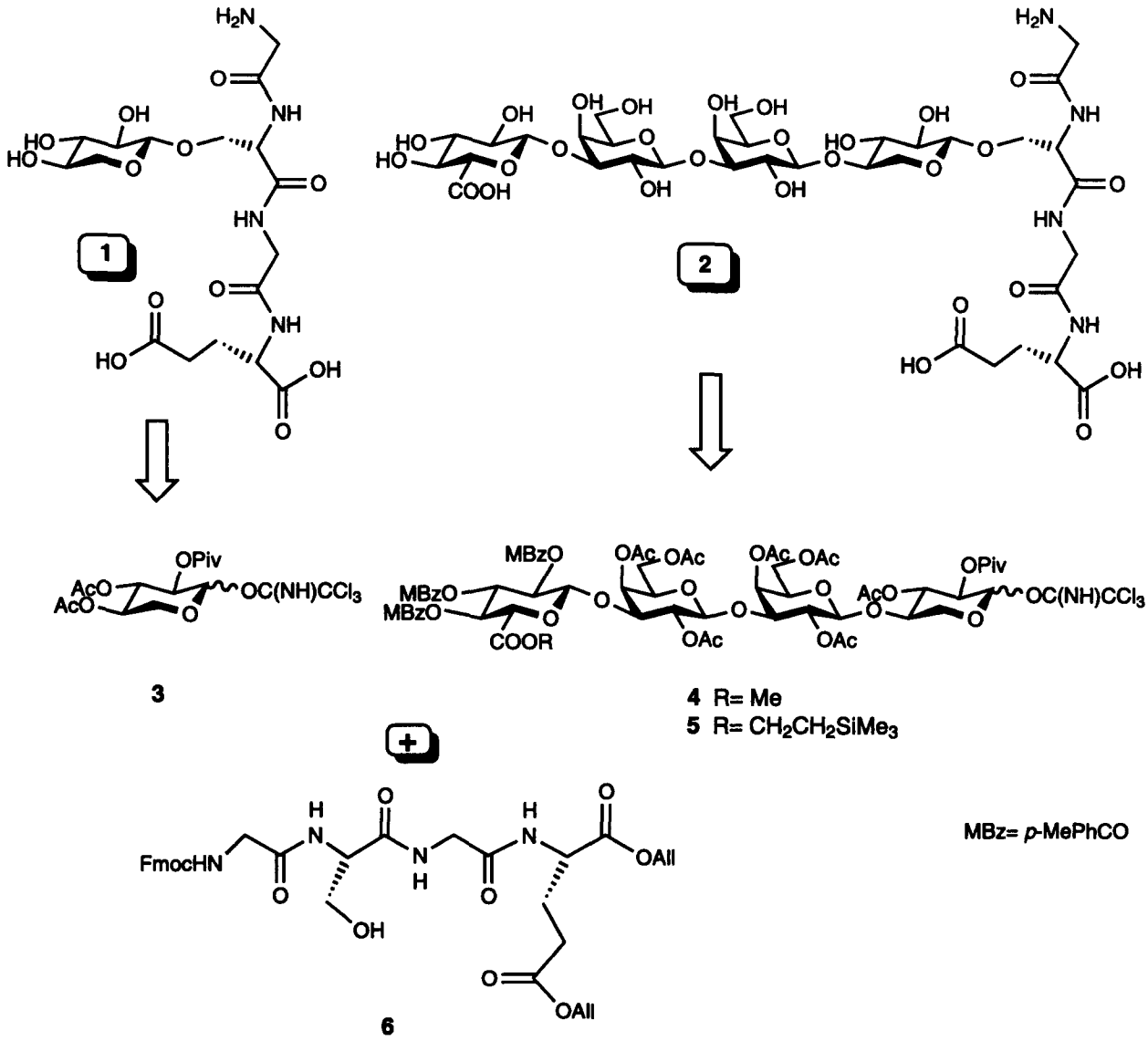
Proteoglycans composed of various glycosaminoglycan chains covalently bound via serine residues to a core protein are believed to participate in a variety of biological processes.[1] Thrombomodulin and Syndecan-1 are cell-surface proteoglycans involved in the regulation of blood coagulation[2] and in binding to extracellular matrix proteins,[3] respectively. Despite their significant biological roles, the biosynthetic sorting mechanism for the inital chain initiation of glycosaminoglycan oligosaccharides during proteoglycan assembly has not yet been clarified.[4] Thrombomodulin[5] and Syndecan-1 [3a,6] from various origins have been shown to consist of an identical tetrapeptide flanking the chondroitin sulfate attachment site. This core protein sequence could have a directing influence on the biological sorting mechanism, hence determining if, where and what type of *O*-linked glycosaminoglycan chain is initated by xylosyltransferase.[7] This prompted us to investigate the synthesis of glycopeptides such as **1** and **2**, which are in themselves highly interesting synthetic targets in addition to being important substrates for enzymatic investigations. We herein report on the first stereoselective synthesis of **1** and **2**.

Results and Discussion

Based on a retrosynthetic analysis (as shown in Scheme 1) of target molecules **1** and **2** , we have designed the key xylosyl (Xyl) donor **3**, as well as glycotetraosyl donors **4** and **5**, which correspond to the glycan part of **1** and **2**, respectively. They were designed as glycosyl imidates such that stereoselective β-coupling with glycosyl acceptor **6** could be achieved taking advantage of the directing participation of the O-2 pivaloyl substituent on the Xyl residue.[8] The acid function of the glucuronic acid (GlcA) residue was protected as either the methyl or 2-(trimethylsilyl)ethyl ester in order to allow final deprotection under mild conditions. It is to be noted that the syntheses of tetrasaccharides β-D-Glc*p*A-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Xyl*p* linked to serine (Ser) and to a dipeptide have been reported.[9]

The key intermediates **4** and **5** may further be disconnected into glycosyl donors **7**–**9** and the glycosyl acceptor **10**. Since compounds **8** and **10** have been reported previously,[9d] we first describe the synthesis of the monosaccharide building blocks **7** and **9**, as well as the Xyl donor **3** (Scheme 2). Conversion of the Xyl derivative **11**[9d] into imidate **3** was efficiently carried out in four steps. Catalytic hydrogenation of **11** followed by acetylation gave the fully protected Xyl intermediate **12**. Subsequent removal of the anomeric acetyl group by the action of hydrazine acetate (H_2NNH_2·AcOH)[10] in *N,N*-dimethylformamide (DMF) furnished hemiacetal **13**, which could be transformed into the Xyl glycosyl imidate donor **3**. Galactose (Gal) and GlcA donors **7** and **9**, designed as building blocks in the synthesis of glycotetraosyl imidates **4** and **5**, were prepared as follows. Synthesis of **7** was performed in two steps starting from **14**.[9d] After cleavage of the benzylidene protective group by employing *p*-toluenesulfonic acid (*p*-TsOH) as a catalyst, the resulting diol **15** was successfully benzylated under carefully monitored conditions (NaH, BnBr in DMF), thus providing an efficient route to the two central Gal units of imidate donors **4** and **5**. The methyl ester of the GlcA donor **8**[9d] was converted into a 2-(trimethylsilyl)ethyl ester by saponification followed by esterification [Me_3SiCl, 2-(trimethylsilyl) ethanol in THF[11]], thus providing facile access to this alternate GlcA donor **9** (72%).

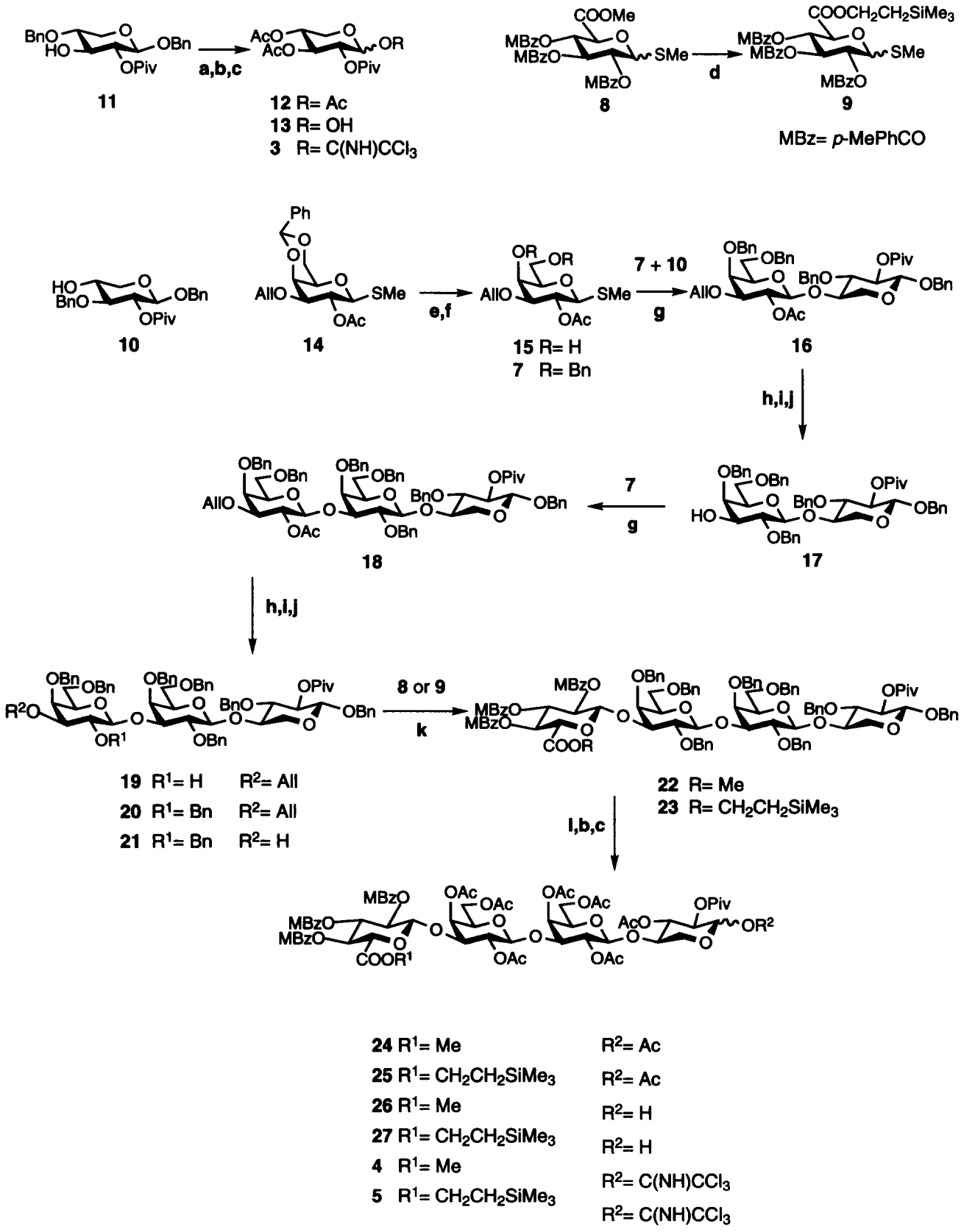
The required glycotetraosyl donors **4** and **5** were then constructed as depicted in Scheme 2. *N*-Iodosuccinimide



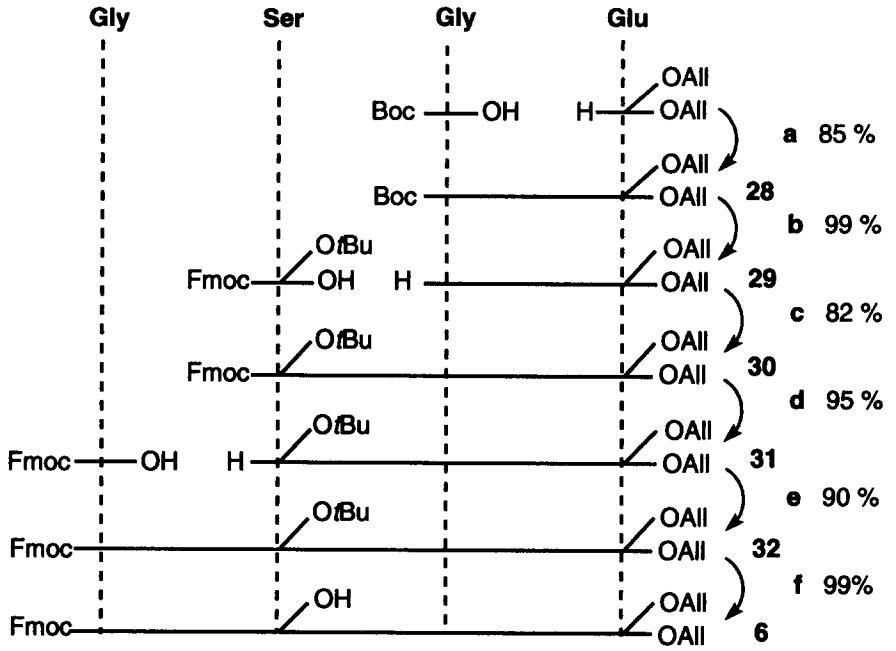
Scheme 1.

(NIS)–trifluoromethanesulfonic acid (TfOH)-MS4A[12a,b] promoted glycosylation of **10** with **7** in 1,2-dichloroethane (DCE):diethyl ether (Et_2O), 4:1, at –25 °C gave 92% of **16** in the desired β-stereoselective manner. Further conversion of **16** was carried out as reported by Goto and Ogawa[9d] to furnish the disaccharide acceptor **17** after three steps. Chain extension of **17**, with again Gal donor **7**, was performed under the same glycosylation conditions (NIS-TfOH-MS4A) as given for the preparation of **16** to yield **18** in 95% yield. Also, this coupling was found to proceed stereoselectively and no undesired α-glycoside could be isolated. The β-linked trisaccharide **18** was then converted into the glycotriosyl acceptor **21.** After the initial removal of the O-2 acetyl group under Corey's conditions,[13] the resulting alcohol **19** was efficiently benzylated (BnBr, KI, Ag_2O in DMF) to give **20** in 90% yield. Deallylation of **20** was achieved by an iridium-catalyzed process,[14] thus furnishing the desired glycotriosyl acceptor **21**. The following $CuBr_2$–AgOTf–Bu_4NBr–MS4A[15] promoted coupling of **21** with thiomethyl GlcA donors **8** and **9** proceeded smoothly at room temperature in CH_2Cl_2 as solvent giving **22** and **23** in 86 and 81% yield, respectively. Necessary protective group manipulations subsequently converted tetrasaccharides **22** and **23** into the key glycotetraosyl donors **4** and **5**. Removal of the benzyl groups in **22** and **23** followed by 4-dimethylaminopyridine (DMAP) catalyzed acetylation gave **24** and **25** which were obtained as mixtures of their α- and β-anomeric acetates in a 1:1 ratio. Treatment of the anomeric acetates with $H_2NNH_2 \cdot AcOH$ provided hemiacetals **26** and **27** which were then transformed into the designed glycotetraosyl donors **4** and **5** in high yields (91 and 93%, respectively).

The tetrapeptide glycosyl acceptor **6** was prepared from commercially available amino acid derivatives[16] and its synthesis was accomplished as outlined in Scheme 3. The peptide linkages were formed in CH_2Cl_2 solutions taking advantage of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) or 2-isobutoxy-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ) as the condensing agent to give **28**, **30** and **32** in 82–90% yield. The *N*-terminal *tert*-butoxycarbonyl (Boc) group in **28** and the side-chain pro-



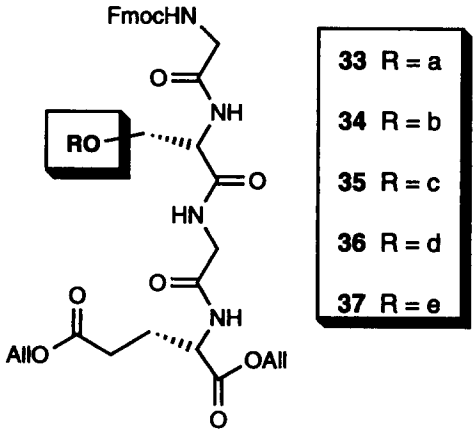
Scheme 2. Reagents and conditions: (a) H_2, 10%Pd-C, MeOH, then Ac_2O, pyridine, DMAP, 0 °C → rt; (b) H_2NNH_2·AcOH, DMF, 50 °C → rt; (c) CCl_3CN, DBU, CH_2Cl_2, rt; (d) 1.25 N LiOH, THF:H_2O (10:3), rt; then 2-(trimethylsilyl)ethanol:THF (2:1), Me_3SiCl, rt; (e) *p*-TsOH, CH_2Cl_2:MeOH (1:1), rt; (f) NaH, BnBr, DMF, 0 °C; (g) NIS, TfOH, DCE:Et_2O (4:1), –25 °C; (h) 30% H_2O_2, 1.25 N LiOH, THF, 0 °C → rt; (i) Ag_2O, BnBr, KI, DMF, 0 °C → rt; (j) [Ir(COD)(PMePh$_2$)$_2$PF$_6$], H_2, THF, rt, then H_2O, I_2, 0 °C → rt; (k) $CuBr_2$, AgOTF, Bu_4NBr, CH_2Cl_2, rt; (l) H_2, 10% Pd-C, MeOH:EtOAc (2:1), then Ac_2O, pyridine, DMAP, 0 °C → rt.

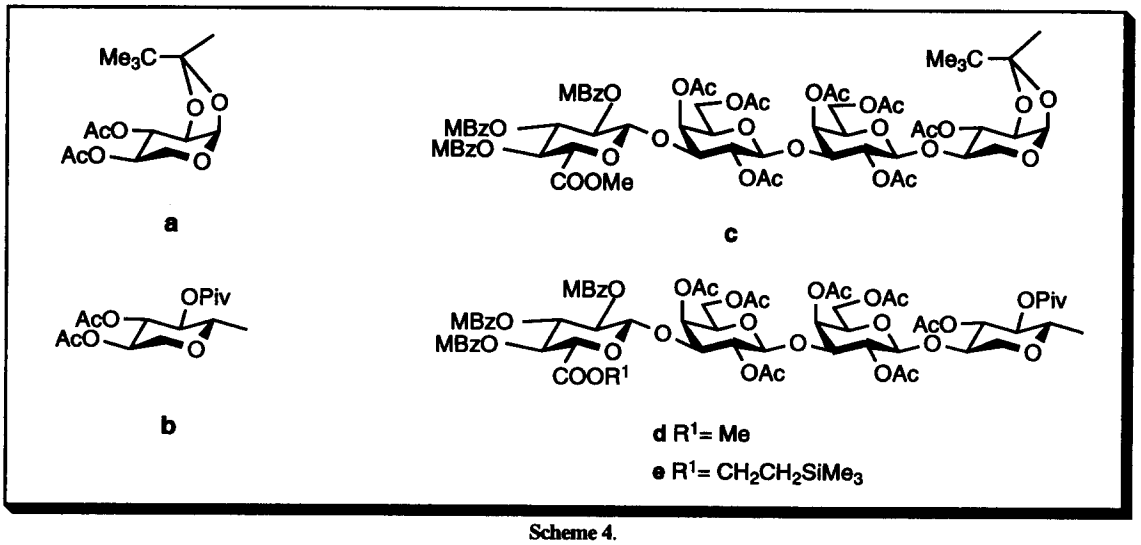


Scheme 3. Reagents and conditions: (a) NEt_3, EEDQ, CH_2Cl_2, rt; (b) CH_2Cl_2:TFA 2:1, rt; (c) IIDQ, CH_2Cl_2, NEt_3, rt; (d) CH_2Cl_2:morpholine (1:1), rt; (e) EEDQ, CH_2Cl_2, rt; (f) TFA, rt.

Donor	Promotor (eq.)	Product	Yield (%)
3	TMSOTf (0.1)	33	57
		34	19
3	TMSOTf (0.1+0.1)	34	88
3	BF_3•Et_2O (0.3)	34	69
4	TMSOTf (0.1)	35	40
		36	52
4	TMSOTf (0.1+0.1)	36	80
4	BF_3•Et_2O (0.3)	36	79
5	TMSOTf (0.1+0.1)	37	81
5	BF_3•Et_2O (0.3)	37	64

Reactions were performed in CH_2Cl_2 solutions at -20°C in the presence of MS4A and acceptor **6** and were finished within 15 min-1h.



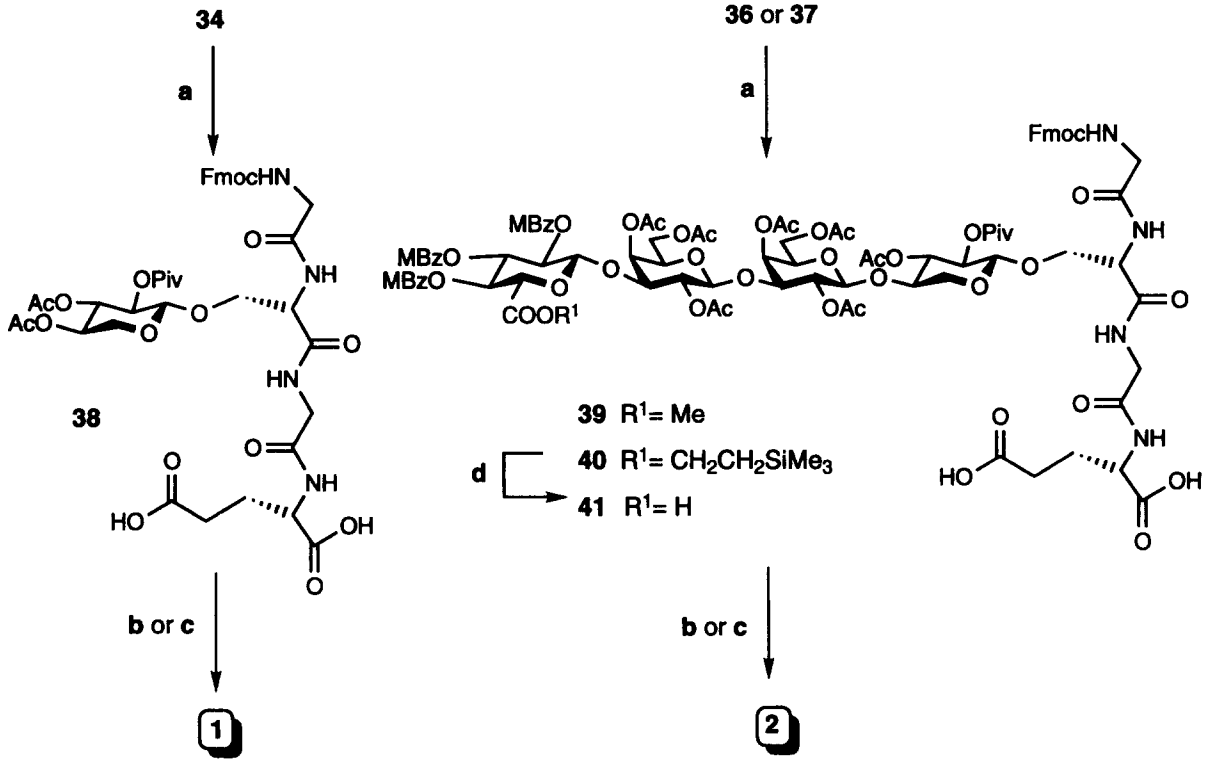


Scheme 4.

tecting *tert*-butyl (*t*Bu) group of **32** were cleaved by action of trifluoroacetic acid (TFA) in 99% yield. The *N*-terminal 9-fluorenylmethoxycarbonyl (Fmoc) group of **30** could be selectively removed with morpholine in CH_2Cl_2[17] to yield **31** (95%).

Having the necessary intermediates in hand, optimized conditions for the crucial coupling between Xyl donor **3** and tetrapeptide **6** were developed (Scheme 4). Initially, the use of TMSOTf (0.1 eq.) as promotor in the presence of MS4A in CH_2Cl_2 at –20 °C gave rise to a mixture of orthoester **33** (57%) and the desired β-linked glycopeptide **34** (19%). Addition of further catalyst to the reaction mixture, however, resulted in the highly efficient (88%) stereoselective formation of **34**.[18] When activation of donor **3** was performed using boron trifluoride etherate ($BF_3{\cdot}Et_2O$), **34** could be obtained in 69% yield. Having these results in hand, the crucial coupling reactions between **6** and glycotetraosyl donors **4** and **5** were carried out. The addition of only 0.1 eq. of TMSOTf to a mixture of **4** and **6** resulted, as in the case of Xyl imidate donor **5**, in the formation of an orthoester **35** (40%) in addition to the desired β-glycoside **36** (52%). As before, the repeated addition of TMSOTf (0.1 + 0.1 eq.) to the mixtures of tetrapeptide **6** and donor **4** or **5** furnished the desired β-linked glycopeptides **36** (80%) and **37** (81%), respectively. The fully protected glycopeptides **36** and **37** could also be obtained (79 and 64%, respectively) when activation of imidates was performed using $BF_3{\cdot}Et_2O$ in CH_2Cl_2.

Finally, smooth deprotection of **34**, **36** and **37** was accomplished as shown in Scheme 5. In all cases, the *C*-terminal allyl ester groups were cleaved under the conditions of Kunz[19] using *N*-methylaniline as a weak base and allyl trapping nucleophile, thus yielding diacids **38–40** (88–98%). Direct conversion of **38** into **1** could be achieved by applying $H_2NNH_2{\cdot}H_2O$[20] or 1 N NaOH in MeOH for the removal of the acyl protective groups (80 and 85% yield). On the other hand, conversion of **39** to glycotetraosyl tetrapeptide **2** required two steps. First, the deprotection of the methyl ester moiety was achieved using 1.25 N LiOH in THF:water (10:3). The resulting acid was, after purification by gel filtration, de-esterified using either $H_2NNH_2{\cdot}H_2O$ or NaOH in MeOH to yield the target compound **2** (79 and 92%, respectively). In the case of diacid **40** the 2-(trimethylsilyl)ethyl ester on the GlcA residue was removed by the action of TFA:CH_2Cl_2 1:1[21] (99%). The target compound **2** was then also obtained by this alternate route after treatment of resulting triacid **41** as described above ($H_2NNH_2{\cdot}H_2O$ or NaOH in MeOH, 85 and 90%, respectively). In our hands, the final deprotection using NaOH in MeOH was easier to monitor by TLC and gave rise to cleaner reaction mixtures as well as higher yields. At this stage care had to be taken in order to ensure the complete removal of the pivaloyl group at O-2 of the xylose residue. Purification of the synthetic target glycopeptides **1** and **2** was performed by size exclusion chromatography (Sephadex LH 20, H_2O) and ion-exchange chromatography (Mono Q, 30–40 mM aq. Tris·HCl). The purity of the synthetic glycopeptides and peptides was established by reverse-phase HPLC (Lichrosphere RP-18, CH_3CN–H_2O–TFA mixtures) and ^1H NMR. In no case was any β-elimination or racemization observed. The assigned structures for synthetic **1** and **2** were deduced from the unambigous synthetic sequence and confirmed by ^1H NMR and FAB-



Scheme 5. Reagents and conditions: (a) $Pd(PPh_3)_4$, PhNHMe, THF, rt; (b) MeOH:$H_2NNH_2{\cdot}H_2O$ (4:1); (c) aq. NaOH, MeOH, pH 8.5–9, rt; (d) CH_2Cl_2:TFA (2:1) 0 °C → rt.

MS data. The ^{1}H NMR spectra of target compounds **1** and **2** (Fig. 1) clearly reveal the presence of one and five anomeric protons, respectively, confirming the correct stereochemistry. For the peptide part characteristic signals were observed for Ser-αH and the glutamic acid residue. Signals derived from the carbohydrate portion are in good agreement with the data reported for sulfated hexaglycosyl serine obtained from natural sources.[22]

In summary, xylosyl tetrapeptide **1** and glycotetraosyl tetrapeptide **2**, key structural elements from the core region of cell-surface proteoglycans, were synthesized for the first time in a stereocontrolled manner.

Experimental

General procedures

Optical rotations were measured at 22 ± 3 °C with a JASCO DIP-310 polarimeter in $CHCl_3$ solutions and ^{1}H NMR spectra were measured with a JEOL EX-270 spectrometer for solutions in $CDCl_3$ with Me_4Si as internal standard, unless otherwise stated. Signal assignment such as 1^3 stands for a proton at C-1 of sugar residue 3 (as counted from the reducing end). Silica gel chromatography, analytical TLC, preparative TLC and high-performance TLC were performed on columns of silica gel 60 (Merck) or glass plates coated with silica gel F_{254} (Merck), respectively. Gels for size exclusion chromatography (Sephadex LH 20) were purchased from Pharmacia. Molecular sieves were purchased from Nakarai Chemical and activated at 180 °C under vacuum prior to use. Melting points were determined with a Yanaco MP apparatus and are uncorrected. Analytical HPLC was executed on a Hitachi LC 655 system with a variable wavelength UV monitor (Hitachi, at 254 nm) and a LiChrospher 100 RP-18 column (Merck–Ciba) using acetonitrile (CH_3CN)–water mixtures at a flow of 1 mL min^{-1}, unless otherwise stated. Fast protein liquid chromatography (FPLC) was performed with a Pharmacia GP-250 system, to P-500 pumps (Pharmacia), a Bromma LKB 2151 variable-wavelength monitor (at 220 nm) and a Mono Q HR 5/5 column (Pharmacia). Buffer systems A (H_2O) and B (aq. Tris·HCl) were used at a flow of 1 mL min^{-1} for a gradient program as follows; 0→5 min: 0% B, 5→25 min: 20%→100% B (linear), 25→30min: 100% B, 30→40 min: 0% B. All reactions in organic solvents were performed under N_2 or Argon atmosphere and organic solvents were distilled according to standard procedures prior to use.

1,3,4-Tri-O-acetyl-2-O-pivaloyl-α/β-D-xylopyranose (12). A mixture of compound **11**[8d] (795 mg) and 10% palladium on carbon (100 mg) in 15 mL of MeOH was vigorously stirred under H_2 atmosphere for 48 h. The reaction mixture was filtered over a short column of silica gel, the volatiles removed *in vacuo* and the residue taken up in CH_2Cl_2 (10 mL). The solution was cooled to 0 °C and Ac_2O (980 mg), NEt_3 (1.56 g) and DMAP (12 mg) were subsequently added. The mixture was allowed to warm up to room temperature for 3 h, diluted with CH_2Cl_2 and successively washed with aq. $NaHCO_3$, 1 N HCl and brine. The organic layer was dried and evaporated *in vacuo*. The remainder was purified by silica gel chromatography (*n*-hexane:EtOAc 2:1) to afford 551 mg of **12** (80%) as a 1:2 mixture of α- and β-anomer; R_f 0.60 (*n*-hexane:EtOAc 2:1); **12α**: ^{1}H NMR δ 6.30 (*d*, $J_{1,2}$ = 3.94 Hz, 1), 5.53 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.91 Hz, 3), 3.95 (*dd*, $J_{4,5eq}$ = 5.59 Hz and $J_{5eq,5ax}$ = 11.20 Hz, 5eq), 3.70 (*dd*, $J_{4,5ax}$ = 9.90 Hz, 5ax), 1.13 (*s*, Piv); **12β**: ^{1}H NMR δ 5.72 (*d*, $J_{1,2}$ = 6.91 Hz, 1), 5.25 (*t*, $J_{2,3}$ = $J_{3,4}$ = 8.58 Hz, 3), 4.15 (*dd*, $J_{4,5eq}$ = 4.94 Hz and $J_{5eq,5ax}$ = 11.85 Hz, 5eq.), 3.95 (*dd*, $J_{4,5ax}$ = 8.56 Hz, 5ax), 1.16 (*s*, Piv).

3,4-Di-O-acetyl-2-O-pivaloyl-α/β-D-xylopyranose (13). A solution of **12** (551 mg) in 10 mL of DMF was heated at 50 °C before H_2NNH_2·AcOH (211 mg) was added. After all of the reagent had dissolved (*ca* 2 min) the mixture was allowed to cool to room temperature for 15 min, diluted with Et_2O (30 mL) and washed with brine. The organic layer was dried, evaporated *in vacuo* and the remainder was subjected to silica gel chromatography (*n*-hexane:EtOAc 1:1) to yield 330 mg (68%) of **13** as a 1:1 mixture of α- and β-anomers; R_f 0.41 (*n*-hexane:EtOAc 1:1); **13α**: ^{1}H NMR δ 5.39 (*dd*, $J_{1,2}$ = 3.94 Hz and $J_{1,OH}$ = 3.29 Hz, 1), 4.76 (*dd*, $J_{2,3}$ = 9.88 Hz, 2), 5.57 (*t*, $J_{3,4}$ = 9.88 Hz, 3), 3.06 (*d*, OH), 2.04 and 2.02 (2*s*, 2 Ac), 1.19 (*s*, Piv); **13β**: ^{1}H NMR δ 5.30 (*t*, $J_{3,4}$ = 10.23 Hz and $J_{2,3}$ = 8.23 Hz), 4.66 (*t*, $J_{1,OH}$ = 8.58 Hz and $J_{1,2}$ = 7.91 Hz, 1), 4.13 (*dd*, $J_{4,5eq}$ = 6.3 Hz and $J_{5eq,5ax}$ = 11.88 Hz, 5eq.), 3.37 (*dd*, $J_{4,5ax}$ = 10.20 Hz, 5ax), 3.76 (*d*, OH).

3,4-Di-O-acetyl-2-O-pivaloyl-α/β-D-xylopyranosyl trichloroacetimidate (3). To a solution of **13** (325 mg) in 3 mL of CH_2Cl_2 were added trichloroacetonitrile (CCl_3CN, 1.44 g) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 30 mg) at room temperature. After 15 min the mixture was directly applied to silica gel chromatography (*n*-hexane:EtOAc 3:1) to give 453 mg (96%) of **3** as a 5:2 mixture of α- and β-anomers; R_f 0.51 (*n*-hexane:EtOAc 2:1); **3α**: ^{1}H NMR δ 8.66 (*s*, NH), 6.50 (*d*, $J_{1,2}$= 3.65 Hz, 1), 5.63 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.88 Hz, 3), 5.05 (*dd*, 2), 2.06 and 2.03 (2*s*, 2 Ac), 1.14 (*s*, Piv); **3β**: δ_H 8.71 (*s*, NH), 6.02 (*d*, $J_{1,2}$ = 4.05 Hz, 1), 2.10 and 2.08 (2*s*, 2 Ac), 1.20 (*s*, Piv).

Methyl 2-O-acetyl-3-O-allyl-1-thio-β-D-galactopyranoside (15). To a solution of **14**[9d] (6.2 g) in 150 mL of CH_2Cl_2:MeOH 1:1 was added *p*-TsOH (0.31 g) and the mixture stirred overnight. After 22 h the reaction was quenched by adding NEt_3, the solution was concentrated *in vacuo* and the remainder purified by silica gel chromatography (*n*-hexane:EtOAc 2:1) to yield 4.05 g (81%) of **15** as a syrup. Further elution gave 0.65 g (10%) of **14**. **15**: R_f 0.22 (*n*-hexane:EtOAc 2:1); $[\alpha]_D$ +11.5° (*c* 0.93); ^{1}H NMR δ 5.91–5.77 (C<u>H</u>=CH$_2$), 5.32–5.19 (CH=C<u>H</u>$_2$), 5.25 (*dd*, $J_{1,2}$ = 9.88 Hz and $J_{2,3}$ = 9.56 Hz, 2), 4.27 (*d*, 1), 3.50 (*dd*, $J_{3,4}$ = 3.32 Hz, 3), 2.17 and 2.11 (*s*, SMe and Ac).

Methyl 2-O-acetyl-3-O-allyl-4,6-di-O-benzyl-1-thio-β-D-galactopyranoside (7). To a cooled solution (0 °C) of **15** (1.36 g) in 30 mL of DMF were added sodium hydride (NaH, 60% dispersion in oil, 446 mg) and then dropwise

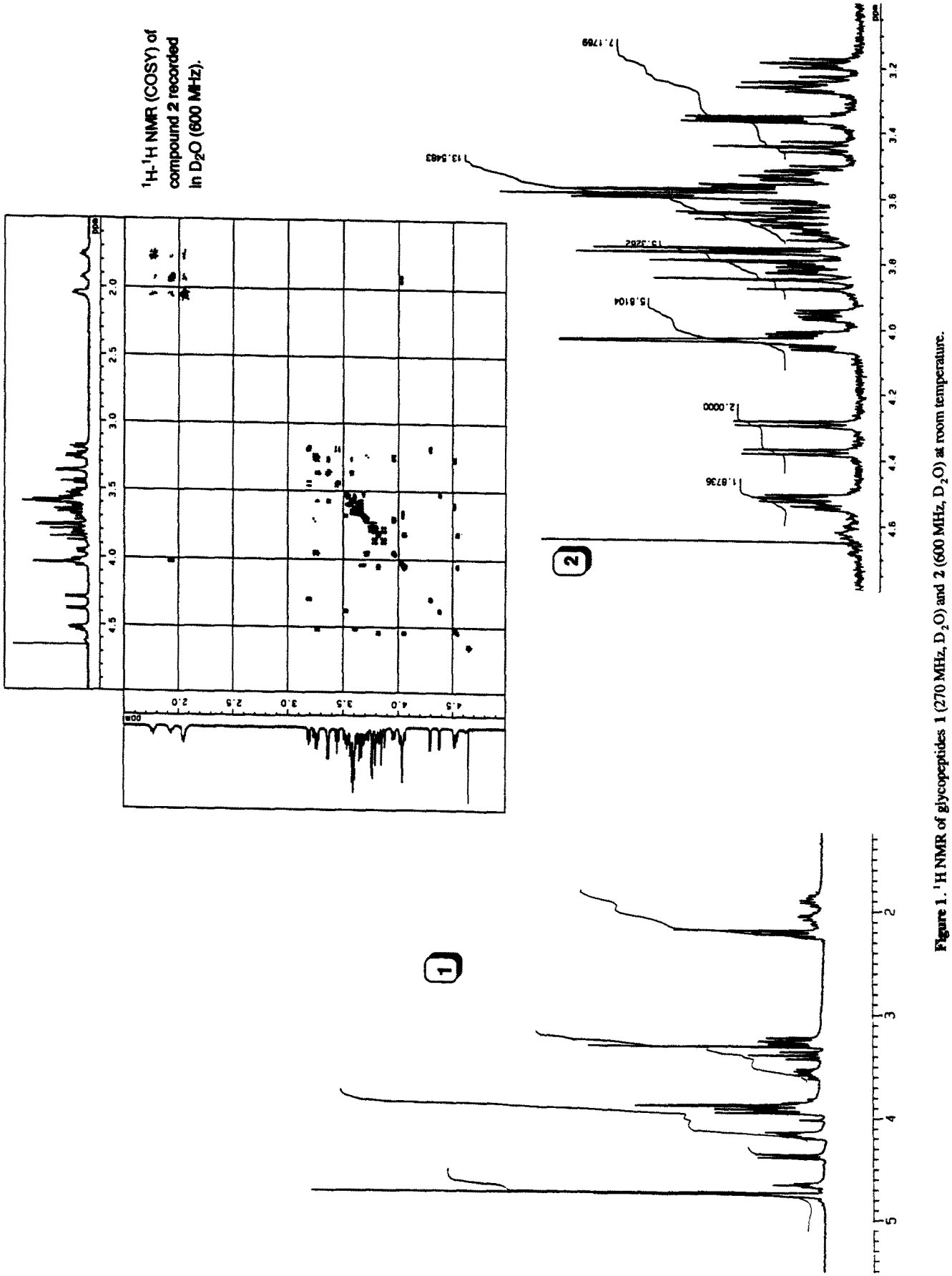


Figure 1. ^{1}H NMR of glycopeptides **1** (270 MHz, D_2O) and **2** (600 MHz, D_2O) at room temperature.

BnBr (3.18 g). After 1 h the mixture was allowed to warm up to room temperature for 1 h, then recooled and 2 mL of MeOH were added. After an additional 30 min the mixture was diluted with Et_2O (80 mL) and washed with brine (2 × 50 mL). The organic layer was dried and the volatiles removed. The resulting syrup was purified by silica gel chromatography (*n*-hexane:EtOAc 5:1) to give 1.76 g (80%) of **7**; R_f 0.24 (*n*-hexane:EtOAc 5:1); $[\alpha]_D$ +0.2° (*c* 1.13); ^{1}H NMR δ 7.35–7.23 (arom. H), 5.91–5.77 (C<u>H</u>=CH$_2$), 5.39 (*dd*, $J_{1,2}$ = 9.90 Hz and $J_{2,3}$ = 9.57 Hz, 2), 5.32–5.19 (CH=C<u>H</u>$_2$), 4.25 (*dd*, 1), 3.96 (*br d*, $J_{3,4}$ = 2.64 Hz, 4), 3.48 (*dd*, $J_{3,4}$ = 2.64 Hz, 3), 2.14 and 2.10 (2*s*, SMe and Ac).

*2-(Trimethylsilyl)ethyl (methyl 2,3,4-tri-*O*-(*p*-methyltoluoyl)-1-thio-β-D-glucopyranoside) uronate (9).* To a cooled solution (–5 °C) of **8** (328 mg) in 13 mL of THF:H_2O (10:3) was added 1.25 N LiOH (0.45 mL) dropwise. After 45 min the mixture was neutralized with 10% AcOH, diluted with $CHCl_3$ (20 mL) and washed with brine (2 × 10 mL). The organic layer was dried, concentrated *in vacuo* and the remainder was dissolved in 7.5 mL of 2-(trimethylsilyl)ethanol:THF (2:1) prior to addition of TMSCl (138 mg). After 21 h the mixture was concentrated *in vacuo* and the residue directly submitted to chromatography on SiO_2 (*n*-hexane:EtOAc 6:1) to yield 271 mg (72%) of **9** as a mixture of α- and β-anomers (5:2) with R_f 0.52 (*n*-hexane:EtOAc 4:1). **9α**: ^{1}H NMR δ 7.97–7.86 and 7.27–7.18 (arom. H), 6.05 (*dd*, $J_{3,4}$ = 8.58 Hz and $J_{2,3}$ = 8.55 Hz, 3), 5.95 (*d*, $J_{1,2}$ = 5.26 Hz, 1), 5.72 (*dd*, $J_{4,5}$ = 8.23 Hz, 4), 5.51 (*dd*, 2), 5.00 (*d*, 5), 4.18 (*m*, C<u>H</u>$_2$SiMe$_3$), 2.44, 2.42 and 2.39 (3*s*, 3 C<u>H</u>$_3$C$_6$H$_4$), 0.80 (*m*, COOC<u>H</u>$_2$), 0.00 (*s*, SiMe$_3$); **9β**: ^{1}H NMR δ 5.97 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.56 Hz, 3), 5.69 (*dd*, $J_{4,5}$ = 9.88 Hz, 4), 5.61 (*dd*, $J_{1,2}$ = 9.91 Hz, 2), 4.75 (*d*, 1), 4.33 (*d*, 5). Anal. calcd for $C_{36}H_{42}O_9SSi$: C, 63.69; H, 6.24; S, 4.72; found: C, 63.57; H, 6.24; S, 4.59.

Benzyl O-(2-O-*acetyl*-3-O-*allyl*-4,6-*di*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→4)-3-O-*benzyl*-2-O-*pivaloyl*-β-D-*xylopyranoside (16).* A mixture of **7** (182 mg), **10**[9d] (127 mg) and molecular sieves (MS), 4A (400 mg), in 7.5 mL of DCE was cooled to –25 °C and stirred for 15 min. To this, a freshly prepared solution of NIS (90 mg) and TfOH (6 mg) in 5 mL of DCE:Et_2O (1:1) was added via a syringe. After 10 min NEt_3 (0.1 mL) was added, the mixture filtered over Celite and successively washed with aq. $NaHCO_3$, aq. $Na_2S_2O_3$ and brine. After drying the organic layer was evaporated *in vacuo* and the remainder purified by flash chromatography on SiO_2 (*n*-hexane: EtOAc 5:2) to yield 238 mg (92%) of **16** as colorless crystals giving the same physical data as previously reported.[9d]

Benzyl O-(2-O-*acetyl*-3-O-*allyl*-4,6-*di*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→3)-(2,4,6-*tri*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→4)-3-O-*benzyl*-2-O-*pivaloyl*-β-D-*xylopyranoside (18).* To a mixture of **7** (100 mg), **17**[9d] (114 mg) and MS4A (300 mg) in 7.5 mL of DCE was added a freshly prepared solution of NIS (48 mg) and TfOH (3.2 mg) in DCE:Et_2O 1:1 (5 mL) at –25 °C. Additional TfOH was injected (3.2 mg each) after 20 and 40 min. After 50 min NEt_3 (20 μL) was added, the mixture filtered over Celite and successively washed with aq. $NaHCO_3$, aq. $Na_2S_2O_3$ and brine. After drying the volatiles were removed *in vacuo* and the remainder purified by flash chromatography on SiO_2 (*n*-hexane:EtOAc 2:1) to give 168 mg (95 %) of **18**; R_f 0.47 (*n*-hexane:EtOAc 2:1); $[\alpha]_D$ –45.3° (*c* 0.68); ^{1}H NMR δ 7.63–7.15 (arom. H), 5.97–5.80 (C<u>H</u>=CH$_2$), 5.42 (*dd*, $J_{2,3}$ = 10.23 Hz and $J_{1,2}$ = 7.91 Hz, 2^3), 5.33–5.16 (CH=C<u>H</u>$_2$), 5.02 (*dd*, $J_{2,3}$ = 8.26 Hz and $J_{1,2}$ = 6.61 Hz, 2^1), 4.84 (*d*, 1^3), 4.49 (*d*, 1^1), 4.36 (*d*, $J_{1,2}$ = 7.23 Hz, 1^2), 3.91 and 3.87 (2*d*, $J_{3,4}$ = 2.64 Hz, 4^2 and 4^3), 3.38 (*dd*, 3^3), 3.27 (*dd*, $J_{5eq,5ax}$ = 11.55 Hz and $J_{4,5ax}$ = 7.59 Hz, $5ax^1$), 1.95 (*s*, Ac), 1.13 (*s*, Piv). Anal. calcd for $C_{76}H_{86}O_{17}\cdot H_2O$: C, 70.83; H, 6.88; found: C, 70.97; H, 6.78.

Benzyl O-(3-O-*allyl*-4,6-*di*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→3)-(2,4,6-*tri*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→4)-3-O-*benzyl*-2-O-*pivaloyl*-β-D-*xylopyranoside (19).* To a solution of **18** (156 mg) in THF (6 mL) were added 1.1 mL of a 30% H_2O_2 solution and 0.45 mL of a 1.25 N LiOH dropwise at 0 °C. After 30 min the reaction was allowed to warm up to room temperature for 11 h, diluted with EtOAc (25 mL) and successively washed with aq. $Na_2S_2O_3$ and brine. The organic layer was dried, filtered and evaporated to yield crude material which was purified by silica gel chromatography (*n*-hexane:EtOAc 3:2): 114 mg (88.5%, based on 22 mg of recovered material) of **19** with R_f 0.25 (*n*-hexane:EtOAc 2:1); $[\alpha]_D$ –44.6° (*c* 0.26); ^{1}H NMR δ 7.35–7.18 (arom. H), 6.02–5.85 (C<u>H</u>=CH$_2$), 5.38–5.19 (CH=C<u>H</u>$_2$), 5.04 (*dd*, $J_{2,3}$ = 7.59 Hz and $J_{1,2}$ = 6.26 Hz, 2^1), 4.53 (*d*, 1^1), 4.39 (*d*, $J_{1,2}$ = 6.91 Hz, 1^2), 3.91 and 3.90 (2*d*, $J_{3,4}$ = 2.97 and 2.64 Hz, 4^2 and 4^3), 2.49 (*br s*, OH), 1.12 (*s*, Piv). Anal. calcd for $C_{74}H_{84}O_{16}\cdot H_2O$: C, 71.29; H, 6.95; found: C, 71.52; H, 6.87.

Benzyl O-(3-O-*allyl*-2,4,6-*tri*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→3)-(2,4,6-*tri*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→4)-3-O-*benzyl*-2-O-*pivaloyl*-β-D-*xylopyranoside (20).* A solution of **19** (42.8 mg) in 1 mL of DMF was cooled (0 °C) and then Ag_2O (48.4 mg) KI (17.3 mg) and BnBr (35.9 mg) were subsequently added before the mixture was allowed to warm up to room temperature. After 3.5 h the mixture was filtered over Celite, diluted with Et_2O and successively washed with aq. $Na_2S_2O_3$ and brine. The organic layer was dried, filtered, evaporated *in vacuo* and the remainder purified by flash chromatography on SiO_2 (*n*-hexane:EtOAc 2:1). Thus, 43.2 mg (94%) of **20** were obtained: R_f 0.43 (*n*-hexane:EtOAc 2:1); $[\alpha]_D$ –39.8° (*c* 0.76); ^{1}H NMR δ 7.34–7.15 (arom. H), 6.01–5.86 (C<u>H</u>=CH$_2$), 5.36–5.15 (CH=C<u>H</u>$_2$), 5.03 (*dd*, $J_{2,3}$ = 7.59 Hz and $J_{1,2}$ = 6.59 Hz, 2^1), 4.82 (*d*, $J_{1,2}$ = 7.59 Hz, 1^3), 4.51 (*d*, 1^1), 4.37 (*d*, $J_{1,2}$ = 7.26 Hz, 1^2), 3.92 and 3.85 (2*d*, $J_{3,4}$ = 2.97 and 2.64 Hz, 4^2 and 4^3), 3.74 (*dd*, $J_{3,4}$ = 9.88 Hz, 2^3), 3.64 (*t*, 3^1), 1.13 (*s*, Piv).

Benzyl O-(2,4,6-*tri*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→3)-(2,4,6-*tri*-O-*benzyl*-β-D-*galactopyranosyl*)-(1→4)-3-O-*benzyl*-2-O-*pivaloyl*-β-D-*xylopyranoside (21).* Through a suspension of [Ir(COD)(PMePh$_2$)$_2$PF$_6$] (0.8 mg)

in 1 mL of THF was bubbled H_2 until a clear solution was obtained. The excess of H_2 was removed and a solution of **20** (57.5 mg) in 2 mL of THF was added via a syringe. After 25 min the mixture was cooled to 0 °C prior to the addition of 0.4 mL of H_2O and I_2 (22.1 mg). After 40 min the mixture was allowed to warm up to room temperature for 40 min, diluted with EtOAc (10 mL) and successively washed with aq. $Na_2S_2O_3$ and brine. The organic layer was dried, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on SiO_2 (*n*-hexane: EtOAc 3:2) to yield 53.3 mg (95.5%) of **21** as syrup: R_f 0.20 (*n*-hexane:EtOAc 2:1); $[\alpha]_D$ −30.3° (*c* 1.04); ^{1}H NMR δ 7.35–7.15 (arom. H), 5.03 (*dd*, $J_{2,3}$ = 7.91 Hz and $J_{1,2}$ = 6.26 Hz, 2^1), 4.82 (*d*, $J_{1,2}$ = 7.91 Hz, 1^3), 4.52 (*d*, 1^1), 4.38 (*d*, $J_{1,2}$ = 7.23 Hz, 1^2), 4.07 (*dd*, $J_{4,5eq}$ = 4.29 Hz and $J_{5eq,5ax}$ = 11.55 Hz, $5eq^1$), 3.94 and 3.86 (2*d*, $J_{3,4}$ = 2.97 Hz, 4^2 and 4^3), 3.80 (*dd*, $J_{2,3}$ = 9.55 Hz, 2^2), 3.65 (*dd*, $J_{3,4}$ = 7.59 Hz, 3^1), 3.32 (*dd*, $J_{4,5ax}$ = 7.59 Hz, $5ax^1$), 2.23 (*br s*, OH), 1.14 (*s*, Piv). Anal. calcd for $C_{81}H_{90}O_{16}$: C, 73.20; H, 6.78; found: C, 73.44; H, 6.81.

Benzyl O-*[methyl 2,3,4-tri*-O-*{*p-*methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*benzyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*benzyl-β-D-galactopyranosyl)-(1→4)-3*-O-*benzyl-2*-O-*pivaloyl-β-D-xylopyranoside (22).* A mixture of $CuBr_2$ (15.8 mg), AgOTf (18.2 mg), Bu_4NBr (4.6 mg) and MS4A (250 mg) in 2 mL of CH_2Cl_2 was stirred at room temperature for 30 min and then cooled to 0 °C. To this a solution of **21** (18.7 mg) and donor **8** (10.9 mg) in 2 mL of CH_2Cl_2 was added. The resulting mixture was allowed to warm up to room temperature for 28 h, filtered over Celite and successively washed with 0.5 N $NaHCO_3$ and brine. The organic layer was dried, filtered and the volatiles removed *in vacuo*. The remainder was purified by preparative TLC (*n*-hexane: EtOAc 3:2) to yield 20.3 mg (86.5% based on 2.1 mg of recovered acceptor **21**) of **22**: R_f 0.55 (*n*-hexane:EtOAc 3:2); $[\alpha]_D$ −32.4° (*c* 0.74); ^{1}H NMR δ 7.85–6.90 (arom. H), 5.82 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.56 Hz, 3^4), 5.64 (*dd*, $J_{4,5}$ = 9.91 Hz, 4^4), 5.61 (*dd*, $J_{1,2}$ = 7.59 Hz, 2^4), 5.35 (*d*, 1^4), 5.01 (*dd*, $J_{2,3}$ = 7.59 Hz and $J_{1,2}$ = 6.26 Hz, 2^1), 4.80 (*d*, $J_{1,2}$ = 7.59 Hz, 1^3), 4.47 (*d*, 1^1), 4.38 (*d*, $J_{1,2}$ = 8.26 Hz, 2^1), 4.22 (*d*, 5^4), 3.68 (*s*, Me), 3.24 (*dd*, $J_{5eq,5ax}$ = 11.53 Hz and $J_{4,5ax}$ = 7.59 Hz, $5ax^1$), 2.37, 2.27 and 2.26 (3*s*, 3 $C\underline{H}_3$-C_6H_4), 1.13 (*s*, Piv). Anal. calcd for $C_{112}H_{118}O_{25}\cdot H_2O$: C, 71.58; H, 6.43; found: C, 71.55; H, 6.38.

Benzyl O-*[2-(trimethylsilyl)ethyl 2,3,4-tri*-O-*{*p-*methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*benzyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*benzyl-β-D-galactopyranosyl)-(1→4)-3*-O-*benzyl-2*-O-*pivaloyl-β-D-xylopyranoside (23).* A mixture of $CuBr_2$ (14.1 mg), AgOTf (16.3 mg), Bu_4NBr (4.1 mg) and MS4A (150 mg) in 2 mL of CH_2Cl_2 was stirred at room temperature for 30 min and then cooled to 0 °C. To this a solution of acceptor **21** (16.7 mg) and **9** (11.2 mg) in 2 mL of CH_2Cl_2 was added. The resulting mixture was allowed to warm up to room temperature for 21 h, filtered over Celite and successively washed with 0.5 N $NaHCO_3$ and brine. The organic layer was dried, filtered and the volatiles removed *in vacuo*. The remainder was purified by preparative TLC (*n*-hexane:EtOAc 2:1) to yield 15.8 mg (81% based on 4.4 mg of recovered acceptor **21**) of **23** as a syrup: R_f 0.31 (*n*-hexane:EtOAc 2:1); $[\alpha]_D$ −32.8° (*c* 0.63); ^{1}H NMR δ 7.85–6.89 (arom. H), 5:82 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.56 Hz, 3^4), 5.62 (*dd*, $J_{1,2}$ = 7.59 Hz, 2^4), 5.61 (*dd*, $J_{4,5}$ = 9.88 Hz, 4^4), 5.34 (*d*, 1^4), 5.01 (*dd*, $J_{2,3}$ = 7.59 Hz and $J_{1,2}$ = 6.26 Hz, 2^1), 4.81 (*d*, $J_{1,2}$ = 7.59 Hz, 1^3), 4.47 (*d*, 1^1), 4.20 (*d*, 5^4), 3.23 (*dd*, $J_{5eq,5ax}$ = 11.53 Hz and $J_{4,5ax}$ = 7.91 Hz, $5ax^1$), 2.37, 2.27 and 2.25 (3*s*, 3 $C\underline{H}_3$-C_6H_4), 1.12 (*s*, Piv), 0.76 (*m*, $COOC\underline{H}_2$), −0.05 (*s*, $SiMe_3$). Anal. calcd for $C_{116}H_{128}O_{25}Si\cdot H_2O$: C, 70.78; H, 6.65; found: C, 70.95; H, 6.57.

O-*[Methyl 2,3,4-tri*-O-*{*p-*methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-1,3-di*-O-*acetyl-2*-O-*pivaloyl-α/β-D-xylopyranose (24).* The compound **22** (9.3 mg) was dissolved in 3 mL of MeOH:EtOAc (2:1), 10% palladium on carbon (10 mg) was added and the mixture vigorously stirred under H_2 atmosphere for 3 days. The mixture was filtered over a short LH 20 Sephadex column, concentrated under reduced pressure and the residue (R_f 0.45 in $CHCl_3$:MeOH 7:1) was taken up in 1 mL pyridine. To this, Ac_2O (0.1 mL) and DMAP (cat.) were added under ice-bath cooling (0 °C). The mixture was allowed to warm up to room temperature for 39 h, diluted with $CHCl_3$ (3 mL) and successively washed with aq. $NaHCO_3$, 1 N HCl and brine. The organic layer was dried, filtered and evaporated *in vacuo*. The residue was purified by preparative TLC (*n*-hexane:EtOAc 1:2) to yield 6.0 mg (83%) of **24** as a 1:1 mixture of α- and β-anomer; R_f 0.25 (*n*-hexane:EtOAc 2:3). **24α**: ^{1}H NMR δ 7.86–7.68 and 7.17–7.06 (arom. H), 6.22 (*d*, $J_{1,2}$ = 3.62 Hz, 1^1), 5.80 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.23 Hz, 3^4), 5.64 (*dd*, $J_{4,5}$ = 9.91 Hz, 4^4), 5.40 (*dd*, $J_{1,2}$ = 6.59 Hz, 2^4), 4.92 (*d*, 1^4), 4.88 (*dd*, $J_{2,3}$ = 10.21 Hz, 2^1), 4.37 and 4.34 (2*d*, $J_{1,2}$ = 7.91 Hz, 1^2 and 1^3), 4.27 (*d*, 5^4), 3.70 (*s*, Me), 2.36, 2.35 and 2.29 (3*s*, 3 $C\underline{H}_3$-C_6H_4), 1.12 (*s*, Piv). **24β**: 5.63 (*d*, $J_{1,2}$ = 6.26 Hz, 1^1), 1.14 (*s*, Piv). Anal. calcd for $C_{69}H_{82}O_{33}$: C, 57.57; H, 5.74; found: C, 57.22; H, 5.80.

O-*[2-(Trimethylsilyl)ethyl 2,3,4-tri*-O-*{*p-*methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-1,3-di*-O-*acetyl-2*-O-*pivaloyl-α/β-D-xylopyranose (25).* A mixture of **23** (9.8 mg) and 10% palladium on carbon (10 mg) in MeOH:EtOAc 2:1 (3 mL) was stirred for 42 h under H_2 atmosphere before filtration over a short LH 20 Sephadex column. The volatiles were removed and the residue (R_f 0.60 in $CHCl_3$:MeOH 7:1) was taken up in 1 mL pyridine. The solution was cooled to 0 °C and Ac_2O (0.1 mL) as well as catalytic amounts of DMAP were added. The mixture was kept at room temperature for 16 h, diluted with $CHCl_3$ and washed with aq. $NaHCO_3$, 1 N HCl and brine. The organic phase was dried, filtered and evaporated under reduced pressure. The residue was purified by preparative TLC (*n*-hexane:EtOAc 3:2) to yield 6.7 mg (88%) of **25** as a 1:1 mixture of α- and β-anomers. **25α**: R_f 0.65 (*n*-hexane:EtOAc 1:2); $[\alpha]_D$ +31.9° (*c* 0.73); ^{1}H NMR δ 7.85–7.69 and 7.17–7.06 (arom. H), 6.22 (*d*, $J_{1,2}$ = 3.97 Hz, 1^1), 5.69 (*dd*, $J_{3,4}$ = 9.56 Hz and $J_{2,3}$ = 9.23 Hz, 3^4), 5.56 (*dd*, $J_{4,5}$ = 9.88 Hz, 4^4), 5.48 (*d*, $J_{3,4}$ = 2.97 Hz, 4^3),

4.92 (*d*, $J_{1,2}$ = 7.23 Hz, 1^4), 4.89 (*dd*, $J_{2,3}$ = 9.88 Hz, 2^1), 4.38 and 4.35 (2*d*, $J_{1,2}$ = 8.56 and 8.26 Hz, 1^2 and 1^3), 4.23 (*d*, 5^4), 2.36, 2.35 and 2.29 (3*s*, 3 CH_3-C_6H_4), 1.12 (*s*, Piv), 0.82 (*m*, $COOCH_2$), –0.06 (*s*, $SiMe_3$). **25β**: R_f 0.58 (*n*-hexane:EtOAc 1:2); ^{1}H NMR δ 5.33 (*d*, $J_{1,2}$ = 7.59 Hz, 1^1), 5.08 (*t*, $J_{2,3}$ = $J_{3,4}$ = 8.91 Hz, 1^3), 3.33 (*dd*, $J_{5ax,5eq}$ = 11.55 Hz and $J_{4,5ax}$ = 9.56 Hz, 5ax^1), 1.14 (*s*, Piv). Anal. calcd for $C_{73}H_{92}O_{33}Si{\cdot}0.5H_2O$: C, 57.13; H, 6.10; found: C, 57.08; H, 5.99.

O-*[Methyl 2,3,4-tri*-O-*{p-methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-3*-O-*acetyl-2*-O-*pivaloyl-α/β-D-xylopyranose (26).* To a solution of **24** (154 mg) in 2 mL of DMF was added $H_2NNH_2{\cdot}$AcOH (14.5 mg) at 50 °C. The mixture was heated until all the reagent had dissolved (2–3 min), was then allowed to cool to room temperature for 25 min and finally diluted with EtOAc (20 mL). The organic phase was washed twice with brine, dried, filtered and then concentrated under reduced pressure. The resulting syrup was purified by silica gel chromatography (*n*-hexane:EtOAc 1:3) to give 143.1 mg (90%) of **26** as colorless foam: R_f 0.28 (*n*-hexane:EtOAc 1:3, a 1:1 mixture of α- and β-anomers). **26α**: ^{1}H NMR δ 7.80–7.69 and 7.17–7.06 (arom. H), 5.80 (*dd*, $J_{3,4}$ = 9.55 Hz and $J_{2,3}$ = 9.23 Hz, 3^4), 5.64 (*dd*, $J_{4,5}$ = 9.88 Hz, 4^4), 5.37 (*dd*, $J_{1,2}$ = 7.23 Hz, 2^4), 4.93 (*d*, 1^4), 4.38 (*m*, 1^2 and 1^3), 4.27 (*d*, 5^4), 3.70 (*s*, Me), 2.36, 2.35 and 2.28 (3*s*, 3 CH_3-C_6H_4), 1.17 (*s*, Piv). **26β**: ^{1}H NMR δ 5.18 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.23 Hz, 3^1), 3.29 (*dd*, $J_{4,5ax}$ = 9.88 Hz and $J_{5eq,5ax}$ = 11.20 Hz, 5ax^1).

O-*[2-(Trimethylsilyl)ethyl 2,3,4-tri*-O-*{p-methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-3*-O-*acetyl-2*-O-*pivaloyl-α/β-D-xylopyranose (27).* A solution of **25** (154.5 mg) in 3 mL of DMF was heated to 50 °C and $H_2NNH_2{\cdot}$AcOH (12.1 mg) was added. After all the reagent had dissolved (3 min), the mixture was stirred at room temperature for 1.5 h, then diluted with EtOAc (15 mL) and washed with brine (twice). The organic phase was dried, filtered and the volatiles removed *in vacuo*. The crude product (R_f 0.49 in *n*-hexane:EtOAc 1:2) was purified by silica gel chromatography (*n*-hexane:EtOAc 1:2) to yield 120.1 mg (86% based on 9.3 mg of recovered starting material) of **27** as a 1:1 mixture of α- and β-anomers. **27α**: ^{1}H NMR δ 7.85–7.73 and 7.19–7.10 (arom. H), 5.83 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.23 Hz, 3^4), 5.70 (*dd*, $J_{4,5}$ = 9.56 Hz, 4^4), 5.53 (*d*, $J_{3,4}$ = 3.29 Hz, 4^3), 5.50 (*m*, 1^1), 5.40 (*dd*, $J_{1,2}$ = 7.26 Hz, 2^4), 4.96 (*d*, 1^4), 4.73 (*dd*, $J_{2,3}$ = 9.88 Hz and $J_{1,2}$ = 3.62 Hz, 2^1), 4.43 (*d*, $J_{1,2}$ = 7.91 Hz, 1^3), 4.41 (*d*, $J_{1,2}$ = 7.91 Hz, 1^2), 4.25 (*d*, 5^4), 2.40, 2.39 and 2.33 (3*s*, 3 CH_3-C_6H_4), 1.21 (*s*, Piv), 0.83 (*m*, $COOCH_2$), 0.00 (*s*, $SiMe_3$). **27β**: ^{1}H NMR δ 5.22 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.23 Hz, 3^1), 4.77 (*dd*, $J_{1,2}$ = 7.91 Hz, 2^1), 4.67 (*br t*, 1^1), 4.38 (*d*, $J_{1,2}$ = 7.91 Hz, 1^2).

O-*[Methyl 2,3,4-tri*-O-*{p-methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-3*-O-*acetyl-2*-O-*pivaloyl-α/β-D-xylopyranosyl trichloroacetimidate (4).* To a solution of **26** (135 mg) in 3 mL of CH_2Cl_2 were added CCl_3CN (139 mg) and DBU (2.9 mg) at room temperature. After 45 min the mixture was directly applied to silica gel chromatography (*n*-hexane:EtOAc 3:1) to yield 135 mg (90.5%) of **4** as a 2:1 mixture of α- and β-anomers: R_f 0.47 (*n*-hexane:EtOAc 3:1). **4α**: ^{1}H NMR δ 8.64 (*s*, NH), 7.84–7.68 and 7.17–7.06 (arom. H), 6.41 (*d*, $J_{1,2}$ = 3.64 Hz, 1^1), 5.80 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.23 Hz, 3^4), 5.65 (*dd*, $J_{4,5}$ = 9.56 Hz, 4^4), 5.36 (*dd*, $J_{1,2}$ = 7.23 Hz, 2^4), 5.05 (*m*, 2^1), 4.97 (*d*, 1^4), 4.39 and 4.38 (2*d*, $J_{1,2}$ = 7.91 Hz, 1^2 and 1^3), 4.23 (*d*, 5^4), 3.72 (*s*, Me), 2.35, 2.34 and 2.28 (3*s*, 3 CH_3-C_6H_4), 1.13 (*s*, Piv). **4β**: ^{1}H NMR δ 8.68 (*s*, NH), 5.93 (*d*, $J_{1,2}$ = 5.59 Hz, 1^1), 5.20 (*t*, $J_{2,3}$ = $J_{3,4}$ = 6.91 Hz, 3^1), 3.56 (*dd*, $J_{4,5ax}$ = 6.59 Hz and $J_{5ax,5eq}$ = 11.88 Hz, 5ax^1), 1.18 (*s*, Piv).

O-*[2-(Trimethylsilyl)ethyl 2,3,4-tri*-O-*{p-methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-3*-O-*acetyl-2*-O-*pivaloyl-α/β-D-xylopyranosyl trichloroacetimidate (5).* To a solution of **27** (119 mg) in 3 mL of CH_2Cl_2 were added CCl_3CN (173 mg) and DBU (2.5 mg) at room temperature. After 45 min the mixture was directly applied to silica gel chromatography (*n*-hexane:EtOAc 1:1) to yield 121 mg (92.5%) of **5** as a 5:2 mixture of α- and β-anomers: R_f 0.30 (*n*-hexane:EtOAc 1:1). **5α**: ^{1}H NMR δ 8.68 (*s*, NH), 7.83–7.73 and 7.19–7.10 (arom. H), 6.48 (*d*, $J_{1,2}$ = 3.62 Hz, 1^1), 5.84 (*dd*, $J_{3,4}$ = 9.56 Hz and $J_{2,3}$ = 9.23 Hz, 3^4), 5.70 (*t*, $J_{4,5}$ = 9.56 Hz, 4^4), 4.98 (*d*, $J_{1,2}$ = 7.23 Hz, 1^4), 4.44 and 4.41 (2*d*, $J_{1,2}$ = 7.91 Hz, 1^2 and 1^3), 4.27 (*d*, 5^4), 2.40, 2.39 and 2.34 (3*s*, 3 CH_3-C_6H_4), 1.18 (*s*, Piv), 0.82 (*m*, $COOCH_2$), 0.00 ($SiMe_3$). **5β**: ^{1}H NMR δ 8.72 (*s*, NH), 5.97 (*d*, $J_{1,2}$ = 5.29 Hz, 1^1), 1.22 (*s*, Piv).

N-*(tert-Butoxycarbonyl)-glycyl-L-glutamic acid di*-O-*allyl ester (28).* To a solution of Boc-Gly-COOH (0.87 g) and L-Glu(COOAll)-COOAll·*p*-TsOH (1.99 g) in 20 mL of CH_2Cl_2 were added NEt_3 (0.51 g) and EEDQ (1.98 g) at 0 °C. The mixture was allowed to warm up to room temperature for 26 h and then washed successively with 0.5 N $NaHCO_3$, 0.5 N HCl and brine. The organic phase was dried, filtered and the volatiles removed *in vacuo*. The remainder was purified by flash chromatography on SiO_2 (*n*-hexane:EtOAc 2:1) to give 1.65 g (85%) of **28** as a chromatographically homogenous syrup with R_f 0.19 (*n*-hexane:EtOAc 2:1); $[\alpha]_D$ +16.7° (*c* 0.51); ^{1}H NMR δ 6.00–5.80 (CH=CH_2), 5.37–5.22 (CH=CH_2), 4.65 (*m*, Glu-αH), 4.62 and 4.57 (2*m*, 2 CH_2CH=), 3.82 (*m*, GlyCH_2), 2.43 (*m*, Glu-γCH_2), 2.26 and 2.05 (2*m*, Glu-βCH_2), 1.45 (*s*, *t*Bu).

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(tert-butyl)-L-seryl-glycyl-L-glutamic acid di*-O-*allyl ester (30).* The protected dipeptide **28** (664 mg) was treated with CH_2Cl_2:TFA 2:1; (7.5 mL) for 25 min. The mixture was evaporated *in vacuo*, then co-evaporated twice with 5 mL of $CHCl_3$: toluene (1:1) and the resulting syrup **29** (674 mg, 99%) was directly used in the following condensation reaction. To a mixture of **29** and Fmoc-Ser(O*t*Bu)-COOH (624 mg) in 15 mL of CH_2Cl_2 were added NEt_3 (165 mg) and HDQ

(790 mg) while stirring at 0 °C. The mixture was allowed to warm up to room temperature for 39 h, then diluted (20 mL CH_2Cl_2) and successively washed with 0.5 N $NaHCO_3$, 0.5 N HCl and brine. The organic layer was dried, filtered and the volatiles removed *in vacuo*. The remainder was crystallized from *n*-hexane:EtOAc (2:1) to yield 867 mg (82 %) of **30**: R_f 0.26 in *n*-hexane:EtOAc 1:1; $[\alpha]_D$ +18.0° (*c* 0.40); mp 125–127 °C; ^{1}H NMR δ 7.79–7.29 (arom. H), 5.96–5.80 (C<u>H</u>=CH$_2$), 5.36–5.19 (CH=C<u>H</u>$_2$), 4.80 (*m*, Glu-αH), 4.62 and 4.55 (2*m*, 2 C<u>H</u>$_2$CH=), 4.24 (*d*, *J* = 6.91 Hz, FmocCH$_2$), 4.14 (*m*, Ser-αH), 4.13 (*t*, Fmoc9H), 4.00 (*m*, GlyCH$_2$), 3.82 (*dd*, ^2J = 8.91 Hz and ^3J = 4.29 Hz, Ser-βCH), 3.46 (*dd*, ^3J = 7.23 Hz, Ser-βCH), 2.22 (*m*, Glu-γCH$_2$), 2.10 and 2.00 (2*m*, Glu-β<u>CH</u>$_2$), 1.20 (*s*, *t*Bu). HPLC retention time: 4.27 min in 80% CH_3CN.

N-*(9-Fluorenylmethoxycarbonyl)-glycyl*-O-*(*tert*-butyl)-L-seryl-glycyl-L-glutamic acid di*-O-*allyl ester (32).* The fully protected tripeptide **30** (260 mg) was treated with 4 mL CH_2Cl_2:morpholine (1:1) for 90 min at room temperature. The mixture was concentrated under reduced pressure and the remainder purified by flash chromatography on SiO_2 to yield amine **31** (162 mg, 95%) which was directly used in the following condensation reaction. To a solution of **31** and Fmoc-Gly-COOH (112 mg) in 5 mL of CH_2Cl_2 was added EEDQ (187 mg) at +5 °C. The mixture was then stirred at room temperature for 16 h and successively washed with 0.5 N $NaHCO_3$, 0.5 N HCl and brine. The organic phase was dried and the obtained solution was concentrated under reduced pressure. The remainder was purified by flash chromatography on SiO_2 (*n*-hexane:EtOAc 1:4→ *n*-hexane:EtOAc:MeOH 2:20:1 gradient) to yield **32** (240 mg, 90%): R_f 0.25 in *n*-hexane:EtOAc (1:4); $[\alpha]_D$ +17.5° (*c* 0.40); mp 100–102 °C (from *n*-hexane:EtOAc 1:2); ^{1}H NMR δ 7.76–7.26 (arom. H), 5.91–5.77 (C<u>H</u>=CH$_2$), 5.31–5.17 (CH=C<u>H</u>$_2$), 4.68 (*m*, Glu-αH), 4.55 (*m*, Ser-αH), 4.55 and 4.50 (2*m*, 2 C<u>H</u>$_2$CH=), 4.35 (*d*, *J* = 6.91 Hz, FmocCH$_2$), 4.20 (*t*, Fmoc9H), 4.15 and 4.10 (2*m*, 2 GlyCH$_2$), 3.78 (*dd*, ^2J = 8.88 Hz and ^3J = 4.61 Hz, Ser-βCH), 3.41 (*dd*, ^3J = 8.26 Hz, Ser-βCH), 2.37 (*m*, Glu-γCH$_2$), 2.20 and 2.00 (2*m*, Glu-β<u>CH</u>$_2$), 1.19 (*s*, *t*Bu). HPLC retention time 8.48 min (0.4 mL min^{-1}) in 80% CH_3CN. Anal. calcd for $C_{37}H_{46}N_4O_{10}$: C, 62.88; H, 6.56; N, 7.93; found: C, 62.88; H, 6.65; N, 8.15.

N-*(9-Fluorenylmethoxycarbonyl)-glycyl-L-seryl-glycyl-L-glutamic acid di*-O-*allyl ester (6).* The *tert*-butyl ether **32** (200 mg) was dissolved in 3 mL of neat TFA for 1 h and the mixture was then concentrated under reduced pressure. The remainder was co-evaporated with toluene (2 × 2 mL) to yield **6** (184 mg, 99%) as chromatographically homogenous syrup (R_f 0.23 in toluene:EtOH 10:1) which could be crystallized from *n*-hexane:EtOAc (1:2); $[\alpha]_D$ +2.9° (*c* 0.48); mp 128–129.5 °C; ^{1}H NMR δ 7.73–7.22 (arom. H), 5.91–5.74 (C<u>H</u>=CH$_2$), 5.28–5.16 (CH=C<u>H</u>$_2$), 4.60–4.50 (2*m*, 2 C<u>H</u>$_2$CH=), 4.52 (*m*, Ser-αH and Glu-αH), 4.34 (*d*, *J* = 6.94 Hz, FmocCH$_2$), 4.20 (*t*, Fmoc9H), 3.98 and 3.92 (2*m*, 2 GlyCH$_2$), 3.95 and 3.73 (2*m*, Ser-β<u>CH</u>$_2$), 2.36 (*m*, Glu-γCH$_2$), 2.18 and 1.98 (2*m*, Glu-βC<u>H</u>$_2$). HPLC retention time 3.98 min in 60% CH_3CN. Anal. calcd for $C_{33}H_{38}N_4O_{10}\cdot H_2O$: C, 59.42; H, 6.05; N, 8.40; found: C, 59.14; H, 5.88; N, 8.90.

N-*(9-Fluorenylmethoxycarbonyl)-glycyl*-O-*(3,4-di*-O-*acetyl-2*-O-*pivaloyl-β-D-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid di*-O-*allyl ester (34).* A mixture of **3** (39.5 mg), **6** (66.6 mg) and MS4A(150 mg) in 3 mL of CH_2Cl_2 was treated with TMSOTf (1.9 mg, 8.5 μL of a 1 M solution in CH_2Cl_2) at –20 °C for 15 min. Additional TMSOTf (1.9 mg) was added and the mixture was quenched by injection of 15 μL of NEt_3 after another 15 min. The mixture was filtered over Celite, evaporated under reduced pressure and purified by silica gel chromatography (EtOAc) to yield glycopeptide **34** (72.3 mg, 88%): R_f 0.52 in EtOAc; $[\alpha]_D$ –7.0° (*c* 0.92); ^{1}H NMR δ 7.77–7.28 (arom. H), 5.94–5.80 (C<u>H</u>=CH$_2$), 5.33–5.19 (CH=C<u>H</u>$_2$), 5.24 (*dd*, $J_{2,3}$ = 9.23 Hz and $J_{3,4}$ = 8.88 Hz, 3), 4.95 (*m*, 4), 4.91 (*dd*, $J_{1,2}$ = 6.91 Hz, 2), 4.6 (*m*, Ser-αH and Glu-αH), 4.58 and 4.54 (2*m*, 2 C<u>H</u>$_2$CH=), 4.53 (*d*, 1), 4.41 (*d*, *J* = 6.91 Hz, FmocCH$_2$), 4.21 (*t*, Fmoc9H), 4.12 (*m*, Ser-βCH), 3.96 and 3.90 (2*m*, 2 GlyCH$_2$), 3.79 (*dd*, ^2J = 10.55 Hz and ^3J = 6.94 Hz, Ser-βCH), 3.37 (*dd*, $J_{5eq,5ax}$ = 10.55 Hz and $J_{4,5ax}$ = 9.23 Hz, 5ax), 2.42 (*m*, Glu-γCH$_2$), 2.27 and 2.08 (2*m*, Glu-β<u>CH</u>$_2$), 2.03 and 2.00 (2*s*, 2 Ac). HPLC retention time: 9.26 min (0.4 mL min^{-1}) in 80% CH_3CN. Anal. calcd for $C_{49}H_{58}N_4O_{17}\cdot 2H_2O$: C, 58.44; H, 6.20; N, 5.56; found: C, 58.30; H, 6.05; N, 5.68.

Glycopeptide **34** was also obtained and then a mixture of **3** (39.5 mg), **6** (61.0 mg) and MS4A (100 mg) in 3 mL of CH_2Cl_2 was treated with TMSOTf (0.9 mg, 4.2 μL of a 1 M solution in CH_2Cl_2) at –20 °C for 15 min. The mixture was quenched by injection of 5 μL of NEt_3, filtered over Celite, evaporated under reduced pressure and purified by silica gel chromatography (EtOAc) to yield glycopeptide **34** (15.5 mg, 19%). Further elution gave orthoester derivative **33** (47.0 mg, 57%): R_f 0.49 in EtOAc, ^{1}H NMR δ 5.52 (*d*, $J_{1,2}$ = 4.29 Hz, 1^1), 4.4 (*m*, 2^1), 2.10 and 2.04 (2*s*, 2 Ac), 1.03 (*s*, Piv), other chemical shifts were close to the ones observed for **34**.

Compound **34** was also obtained as follows. A mixture of **3** (11.6 mg), **6** (24.4 mg) and MS4A (100 mg) in 3 mL of CH_2Cl_2 was treated with $BF_3\cdot Et_2O$ (1.1 mg, 15 μL of a 0.5 M solution in CH_2Cl_2) at –20 °C for 15 min. The mixture was filtered over Celite, diluted with EtOAc (3 mL) and successively washed with 0.5 N $NaHCO_3$ and brine. The organic layer was dried, evaporated *in vacuo* and the residue purified by preparative TLC (EtOAc) to give 16.7 mg (69%) of **34** showing the same physical data as given above.

N-*(9-Fluorenylmethoxycarbonyl)-glycyl*-O-(O-*[methyl 2,3,4-tri*-O-*{*p*-methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-3*-O-*acetyl-2*-O-*pivaloyl-β-D-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid di*-O-*allyl ester (36).* To a mixture of **4** (30.8 mg), **6** (19.5 mg) and MS4A (100mg) in 2 mL of CH_2Cl_2 was added TMSOTf (0.45 mg, 2 μL of a 1 M solution in CH_2Cl_2) at –20 °C. Additional catalyst (0.45

mg) was added after 15 min and the mixture was treated with 5 μL of NEt_3 and filtered over Celite after 30 min. The volatiles were removed *in vacuo* and the remainder subjected to flash chromatography on silica gel to yield 32.5 mg (80%) of **36**: R_f 0.28 in toluene:EtOH 10:1; $[\alpha]_D$ +2.4° (*c* 1.45); ^{1}H NMR δ 7.81–7.06 (arom. H), 5.94–5.80 (C<u>H</u>=CH$_2$), 5.80 (*m*, 3^4), 5.64 (*t*, $J_{3,4} = J_{4,5}$ = 9.56 Hz, 4^4), 5.49 (*d*, $J_{3,4}$ = 3.29 Hz, 4^3), 5.37–5.19 (CH=C<u>H</u>$_2$), 5.30 (*m*, 2^4), 5.12 (*dd*, $J_{2,3}$ = 9.23 Hz and $J_{3,4}$ = 8.91 Hz, 3^1), 4.92 (*d*, $J_{1,2}$ = 6.91 Hz, 1^4), 4.82 (*dd*, $J_{1,2}$ = 7.23 Hz, 2^1), 4.6 (*m*, Ser-αH and Glu-αH), 4.59 and 4.54 (2*m*, 2 C<u>H</u>$_2$CH=), 4.46 (*d*, 1^1), 4.37 (2*d*, $J_{1,2}$ = 7.91 Hz, 1^2 and 1^3), 4.27 (*d*, $J_{4,5}$ = 9.56 Hz, 5^4), 3.96 and 3.90 (2*m*, 2 GlyCH$_2$), 3.70 (*s*, Me), 3.28 (*dd*, $J_{5eq,5ax}$ = 11.23 Hz and $J_{4,5ax}$ = 9.88 Hz, 5ax^1), 2.40 (*m*, Glu-γCH$_2$), 2.36, 2.35 and 2.29 (3*s*, 3 C<u>H</u>$_3$-C$_6$H$_4$), 1.14 (*s*, Piv). HPLC retention time: 10.54 min in 80% CH_3CN. Anal. calcd for $C_{100}H_{116}N_4O_{41}\cdot 2H_2O$: C, 58.25; H, 5.86; N, 2.71; found: C, 58.27; H, 5.67; N, 2.75.

Compound **36** was also obtained when a mixture of **4** (19.3 mg), **6** (12.2 mg) and MS4A (140 mg) in 2 mL of CH_2Cl_2 was treated with $BF_3\cdot Et_2O$ (0.55 mg, 7.5 μL of a 0.5 M solution in CH_2Cl_2) at –20 °C for 45 min. The mixture was filtered over Celite, subsequently washed with 0.5 N $NaHCO_3$ and brine. The organic layer was dried, evaporated *in vacuo* and the remaining crude product was purified by preparative TLC (toluene:EtOH 10:1) to yield 20.2 mg (79.5%) of **36** showing the same physical data as given above.

The glycopeptides **36** (19.4 mg, 52%) and **35** (15.0 mg, 40%) were obtained when a mixture of **4** (28.1 mg), **6** (17.8 mg) and MS4A (100 mg) in 2 mL of CH_2Cl_2 was treated with TMSOTf (0.4 mg, 1.8 μL of a 1 M solution in CH_2Cl_2) at –20 °C. Work-up and purification was performed as given above. **35**: R_f 0.21 in toluene:EtOH 10:1; ^{1}H NMR δ 5.55 (*d*, $J_{1,2}$ = 4.97 Hz, 1^1), 4.4 (*m*, 2^1), 4.45 (*m*, Ser-αH), 1.03 (*s*, Piv), other chemical shifts were similar to those observed for **36**.

*N-(9-Fluorenylmethoxycarbonyl)-glycyl-*O*-(*O*-[2-(trimethylsilyl)ethyl 2,3,4-tri-*O*-{p-methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3-*O*-acetyl-2-*O*-pivaloyl-β-D-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid di-*O*-allyl ester (37).* To a mixture of **5** (12.9 mg), acceptor **6** (7.7 mg) and MS4A (150 mg) in 2 mL of CH_2Cl_2 was added TMSOTf (0.2 mg, 1 μL of a 1 M solution in CH_2Cl_2) at –20 °C. Additional catalyst (0.2 mg) was added after 15 min and the mixture was quenched by adding 5 μL of NEt_3 before filtration over Celite after 30 min. The volatiles were removed *in vacuo* and the remainder subjected to preparative TLC (toluene:EtOAc 10:1) to yield glycopeptide **37** (13.5 mg, 81%): R_f 0.40 in toluene:EtOH (10:1); $[\alpha]_D$ +6.1° (*c* 0.49); ^{1}H NMR δ 7.81–7.06 (arom. H), 5.95–5.80 (C<u>H</u>=CH$_2$), 5.79 (*dd*, $J_{3,4}$ = 9.56 Hz and $J_{2,3}$ = 9.23 Hz, 3^4), 5.66 (*t*, $J_{4,5}$ = 9.56 Hz), 5.49 (*d*, $J_{3,4}$ = 3.29 Hz, 4^3), 5.36–5.19 (CH=C<u>H</u>$_2$), 5.30 (*m*, 2^4), 5.12 (*dd*, $J_{2,3}$ = 9.23 Hz and $J_{3,4}$ = 8.91 Hz, 3^1), 4.91 (*d*, $J_{1,2}$ = 6.94 Hz, 1^4), 4.82 (*dd*, $J_{1,2}$ = 7.26 Hz, 2^1), 4.6 (*m*, Ser-αH and Glu-αH), 4.59 and 4.54 (2*m*, 2 C<u>H</u>$_2$CH=), 4.46 (*d*, 1^1), 4.38 and 4.36 (2*d*, $J_{1,2}$ = 7.91 Hz, 1^2 and 1^3), 4.23 (*d*, 5^4), 4.00 and 3.95 (2*m*, 2 GlyCH$_2$), 3.28 (*dd*, $J_{5eq,5ax}$ = 11.56 Hz and $J_{4,5ax}$ = 9.88 Hz, 5ax^1), 2.40 (*m*, Glu-γCH$_2$), 2.35, 2.34 and 2.29 (3*s*, 3 C<u>H</u>$_3$-C$_6$H$_4$), 2.20 and 2.00 (2*m*, Glu-βCH$_2$), 1.14 (*s*, Piv), 0.78 (*m*, C<u>H</u>$_2$SiMe$_3$), –0.06 (*s*, $SiMe_3$). HPLC retention time: 28.30 min in 80% CH_3CN. Anal. calcd for: $C_{104}H_{126}N_4O_{41}Si\cdot H_2O$: C, 58.58; H, 6.05; N, 2.62; found: C, 58.27; H, 5.91; N, 2.48.

Compound **37** was also obtained when a mixture of **5** (12.3 mg), **6** (7.4 mg) and MS4A (150 mg) in 2 mL of CH_2Cl_2 was treated with $BF_3\cdot Et_2O$ (0.3 mg, 4.5 μL of a 0.5 M solution in CH_2Cl_2) at –20°C. The addition of catalyst was repeated after 25 and 45 min (0.2 mg each). The mixture was filtered over Celite after 1 h and then subsequently washed with 0.5 N $NaHCO_3$ and brine. The organic layer was dried, evaporated *in vacuo* and the crude product was purified by preparative TLC (toluene:EtOH 10:1) to yield 10.2 mg (64%) of **37** showing the same physical data as given above.

*N-(9-Fluorenylmethoxycarbonyl)-glycyl-*O*-(3,4-di-*O*-acetyl-2-*O*-pivaloyl-β-D-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid (38).* A solution of **34** (18.8 mg) in 2 mL of freshly distilled THF was degassed and $Pd(PPh_3)_4$ (4.4 mg) and PhNHMe (41.3 mg) were added. After 20 min the mixture was concentrated, taken up in 6 mL of $CHCl_3$:(*n*-butyl alcohol) (5:1) and washed with 1 N HCl and brine. The organic phase was dried, filtered, and concentrated under reduced pressure until a yellow precipitate was obtained. The mixture was filtered, the filtrate evaporated *in vacuo* and the remainder purified by silica gel chromatography ($CHCl_3$:MeOH:AcOH 90:5:2→ 90:15:4 gradient) to yield 17.2 mg (99%) of **38**: R_f 0.32 in $CHCl_3$:MeOH 1:1; $[\alpha]_D$ –6.8° (*c* 0.35); ^{1}H NMR (CD_3OD) δ 7.90–7.38 (arom. H), 5.28 (*t*, $J_{2,3} = J_{3,4}$ = 8.56 Hz, 3), 4.95 (*m*, 2 and 4), 4.75 (*d*, $J_{1,2}$ = 6.91 Hz, 1), 4.70 (*br t*, *J* = 5.91 Hz, Ser-αH), 4.48 (*d*, *J* = 6.94 Hz, FmocCH$_2$), 4.35 (*m*, Glu-αH), 4.34 (*t*, Fmoc9H), 4.16 (*dd*, $J_{5eq,5ax}$ = 11.52 Hz and $J_{4,5eq}$ = 4.59 Hz, 5eq), 4.10 and 3.83 (2*m*, Ser-βCH$_2$), 4.10 and 3.98 (2*m*, 2 GlyCH$_2$), 3.54 (*dd*, $J_{4,5ax}$ = 8.91 Hz, 5ax), 2.35 (*m*, Glu-γCH$_2$), 2.25 and 2.10 (2*m*, Glu-βCH$_2$), 2.09 and 2.06 (2*s*, 2 Ac), 1.26 (*s*, Piv). HPLC retention time: 2.48 min in 80% CH_3CN (+ 0.1% TFA).

*N-(9-Fluorenylmethoxycarbonyl)-glycyl-*O*-(O-[methyl 2,3,4-tri-*O*-{p-methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3-*O*-acetyl-2-*O*-pivaloyl-β-D-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid (39).* To a solution of **36** (8.8 mg) in 1.5 mL of freshly distilled and degassed THF were added $Pd(PPh_3)_4$ (1.0 mg) and PhNHMe (9.3 mg). After 15 min the mixture was concentrated, taken up in 6 mL of $CHCl_3$:(*n*-butyl alcohol) (5:1) and washed with 1 N HCl and brine. The organic phase was dried, filtered and concentrated under reduced pressure until a yellow precipitate was obtained. The mixture was filtered, the filtrate evaporated *in vacuo* and the remainder purified by preparative TLC ($CHCl_3$:MeOH:AcOH 80:20:2) to yield

7.8 mg (92%) of **39** [R_f 0.43 ($CHCl_3$:MeOH:AcOH 80:20:2); $[\alpha]_D$ +1.8° (*c* 0.55). HPLC retention time: 5.12 min in 80% CH_3CN (+ 0.1% TFA)] which was directly deprotected to yield compound **2** as described below. Anal. calcd for $C_{98}H_{118}N_4O_{41}Si$: C, 57.93; H, 5.84; N, 2.75; found: C, 57.76; H, 5.78; N, 2.53.

N-*(9-Fluorenylmethoxycarbonyl)-glycyl*-O-(O-*[2-(trimethylsilyl)ethyl 2,3,4-tri*-O-*{*p-*methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-3*-O-*acetyl-2*-O-*pivaloyl-β-D-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid (40).* To a solution of **37** (19.8 mg) in 3 mL of freshly distilled and degassed THF were added $Pd(PPh_3)_4$ (2.2 mg) and PhNHMe (20.0 mg). After 15 min the mixture was concentrated, taken up in 6 mL of $CHCl_3$:(*n*-butyl alcohol) (5:1) and washed with 1 N HCl and brine. The organic phase was dried, filtered and concentrated under reduced pressure until a yellow precipitate was obtained. The mixture was filtered, the filtrate evaporated *in vacuo* and the remainder purified by preparative TLC ($CHCl_3$:MeOH:AcOH 90:5:2) to yield 16.6 mg (88%) of **40** [R_f 0.32 ($CHCl_3$:MeOH:AcOH 90:5:2); $[\alpha]_D$ +2.2° (*c* 0.81). HPLC retention time: 10.71 min in 80% CH_3CN (+ 0.1% TFA)], which was directly deprotected to give **41** as described below.

N-*(9-Fluorenylmethoxycarbonyl)-glycyl*-O-(O-*[2,3,4-tri*-O-*{*p-*methyltoluoyl}-β-D-glucopyranosyl uronate]-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri*-O-*acetyl-β-D-galactopyranosyl)-(1→4)-3*-O-*acetyl-2*-O-*pivaloyl-β-D-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid (41).* To a solution of **40** (13.3 mg) in 1 mL of CH_2Cl_2 was added 0.5 mL of TFA dropwise at 0 °C. After 1 h the temperature was allowed to raise to room temperature for 30 min, after which time the mixture was concentrated *in vacuo* and co-evaporated twice with $CHCl_3$:toluene 1:1 (2 mL each). The resulting triacid **41** [13.2 mg (quant.); R_f 0.47 in $CHCl_3$:MeOH:AcOH 90:15:2; $[\alpha]_D$ +1.8° (*c* 0.66). HPLC retention time: 3.86 min in 80% CH_3CN (+ 0.1% TFA)] was directly used for deprotection to yield target **2** as described below.

Glycyl-O-*(β-D-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid (1).* A solution of **38** (7.0 mg) in 0.5 mL of MeOH was treated with 1 N NaOH until pH 8.5 had been adjusted (on wet pH paper, Merck Neutralit). After 1 day the mixture was neutralized by adding 1 M AcOH and directly applied to size exclusion chromatography on LH 20 Sephadex (H_2O) to yield 3.6 mg (85%) of glycopeptide **1** (to ensure complete deprotection of the pivaloyl group at O-2 of the xylose residue the experiment had to be repeated in a MeOH–water mixture under otherwise same conditions for 2d): R_f 0.43 in $CHCl_3$:MeOH:H_2O:AcOH 2:10:2:1; ^1H NMR δ (D_2O) 4.65 (*t*, *J* 4.97 Hz, Ser-αH), 4.38 (*d*, $J_{1,2}$ = 7.59 Hz, 1), 4.15 (*m*, Glu-αH and Ser-βCH), 3.90 (*m*, Ser-βCH, 5eq and 2 $GlyCH_2$), 3.39 (*dd*, $J_{2,3}$ = 9.23 Hz and $J_{3,4}$ = 8.91 Hz, 3), 3.29 (*dd*, $J_{5eq,5ax}$ = 11.20 Hz and $J_{4,5ax}$ = 7.91 Hz, 5ax), 3.26 (*dd*, 2), 2.20 (*m*, Glu-γCH_2), 2.05 and 1.90 (2*m*, Glu-βCH_2). FABMS: 479.0 $(M-H)^-$, $C_{17}H_{28}N_4O_{12}$ requires 480.1. During FPLC compound **1** was eluted after 12.8 min using 30 mM Tris·HCl as buffer system B in a gradient program as given in general procedures. Glycopeptide **1** was also obtained after treatment of **38** (11.1 mg) in 2 mL of MeOH:$H_2NNH_2·H_2O$ (4:1) at 40 °C for 2 h. Purification as described gave 4.8 mg (80%) of **1** showing the same physical data as stated above.

Glycyl-O-(O-*[β-D-glucopyranosyl uronate]-(1→3)-(β-D-galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-*D*-xylopyranosyl)-L-seryl-glycyl-L-glutamic acid (2). From diacid 39*: A mixture of **39** (7.2 mg) in THF:H_2O (5:1; 0.25 mL) was cooled to 0 °C and 17.5 μL of 1.25 N LiOH were added dropwise. After 50 min the mixture was neutralized with 1 M AcOH and submitted to size exclusion chromatography (LH 20 Sephadex, $CHCl_3$:MeOH:H_2O 5:5:1). The glycopeptide containing fractions were collected, concentrated *in vacuo* and taken up in 1 mL of MeOH. The solution was adjusted to pH 9 (on wet pH paper, Merck Neutralit) using 0.25 N NaOH, neutralized after 9 h by adding 1 M AcOH and directly submitted to a LH 20 Sephadex column (H_2O) for size exclusion chromatography to yield 3.4 mg (92%) of target **2** (to ensure complete deprotection of the pivaloyl group at O-2 of the xylose residue the experiment had to be repeated in a MeOH:water mixture under otherwise same conditions for 2d): R_f 0.47 in $CHCl_3$:MeOH:H_2O:AcOH (2:6:2:1); ^1H NMR (600 MHz, D_2O, internal *t*BuOH) δ 4.52 (*t*, *J* = 4.8 Hz, Ser-αH), 4.51 (*d*, $J_{1,2}$ = 7.68 Hz, 1^4), 4.50 (*d*, $J_{1,2}$ = 7.32 Hz, 1^3), 4.38 (*d*, $J_{1,2}$ = 7.68 Hz, 1^2), 4.29 (*d*, $J_{1,2}$ = 7.68 Hz, 1^1), 4.04 (*dd*, Ser-βCH), 4.03 (*br d*, $J_{3,4}$ = 2.97 Hz, 4^2 and 4^3), 4.02 (*dd*, *J* = 8.8 and 4.0 Hz, Glu-αH), 4.96 (*dd*, $J_{5eq,5ax}$ = 11.70 Hz and $J_{4,5eq}$ = 5.16 Hz, $5eq^1$), 3.82 (*dd*, Ser-βCH), 3.83 (*m*, $GlyCH_2$), 3.44 (*t*, $J_{2,3}$ = $J_{3,4}$ = 9.54 Hz, 3^1), 3.28 (*dd*, $J_{2,3}$ = 9.48 Hz, 2^4), 3.24 (*dd*, $J_{4,5ax}$ = 9.48 Hz, $5ax^1$), 3.18 (*dd*, 2^1), 2.05 (*m*, Glu-γCH_2), 1.93 and 1.76 (2*m*, Glu-βCH_2). FABMS: 979.1 $(M-H)^-$, $C_{35}H_{56}N_4O_{28}$ requires 980.2. During FPLC glycopeptide **2** was eluted after 16.6 min using 40 mM Tris-HCl as buffer system B in a gradient program as given in general procedures. Compound **2** was also prepared as follows. A mixture of **39** (9.9 mg) in THF:H_2O (5:1; 0.36 mL) was treated as described above. The remainder was dissolved in 1 mL of MeOH and 0.25 mL of $H_2NNH_2·H_2O$ was added dropwise. The mixture was heated at 40 °C for 4 h and then directly submitted to size exclusion chromatography (LH 20 Sephadex, H_2O) to yield 4.0 mg (79%) of **2**. To obtain a product giving the same NMR data as above, purification by size exclusion chromatography had to be repeated twice.

From triacid 41. To a solution of **41** (6.5 mg) in 0.6 mL MeOH:H_2O (5:1) was added 1 N NaOH (pH adjusted to 8.5–9). The pH was readjusted after 3 and 23 h. After 29 h the mixture was neutralized with 1 M AcOH and directly submitted to size exclusion chromatography (LH 20 Sephadex, H_2O) giving 3.1 mg (90%) of glycopeptide **2**. The same product was obtained and then **41** (5.2 mg) was treated with 0.75 mL MeOH:$H_2NNH_2·H_2O$ (4:1) at +10 °C for 17 h. The mixture was then purified as described to yield 2.3 mg (85%) of **2** giving the same physical data as reported above.

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