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Stereoselective total synthesis of glycopeptides bearing the dimeric and trimeric sialosyl-Tn epitope*,†

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ABSTRACT

The dimeric and trimeric sialosyl-Tn epitopes, [α-D-Neu*p*5Ac-(2→6)-α-D-Gal*p*NAc-(1→3)-L-Ser]$_n$-L-Val (n = 2 and 3), which represent part of a clustered carbohydrate region of glycophorin A, a human erythrocyte glycoprotein, have been synthesised stereoselectively. 2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-galactopyranosyl fluoride (Gal*p*NAc unit), Fmoc-L-serine phenacyl ester (Ser unit), and benzyl 5-acetamido-4,7,8,9-tetra-*O*-benzyl-5-deoxy-3-*S*-phenyl-3-thio-D-*erythro*-L-*gluco*-2-nonulopyranosylonate bromide (Neu*p*5Ac unit) were the key intermediates for stereoselective glycosylation. 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline-promoted elongation of the peptide chain and then hydrogenolysis afforded the title compounds.

INTRODUCTION

During investigations on the total synthesis of cell-surface glycans[1], we have been especially interested in mucin-type glycopeptides that contain sialic acid residues.

The monoclonal antibodies B72.3 (ref. 2) and MLS102 (ref. 3), raised against human metastatic breast cancer and colonic cancer cell lines, respectively, which recognise the sialosyl-Tn epitope[4,5], α-D-Neu*p*5Ac-(2→6)-D-Gal*p*NAc, and synthetic molecules[6] of the monomeric epitope, were used to identify the structure of the epitope. In the immunoassay study of MLS102 with various mucins, a strongly enhanced immunoreaction was observed with ovine submaxillary mucin (OSM) that carried clusters of the epitopic disaccharide[4]. Our attention was then directed towards the synthesis of the clustered sialosyl-Tn epitope system by a novel sequence. Asialoglycopeptides with T and Tn epitope clusters have been synthesised by other groups[7].

As a first attempt to synthesise sialoglycopeptides[8], we now report syntheses of **1** and **2** with the di- and tri-meric sialosyl-Tn epitopes that correspond to the partial structures of glycophorin A, a major glycoprotein of human erythrocyte membrane[9]. Based on a strategy of stepwise elongation of the peptide chain, a suitably protected amino acid fragment with the sialosyl-Tn epitope **22** was designed which, in turn, would be obtainable by stereocontrolled condensation of D-Gal*p*NAc, L-Ser, and D-Neu*p*5Ac synthons (**9–11** and **17**).

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* Dedicated to Professor Grant Buchanan on the occasion of his 65th birthday.
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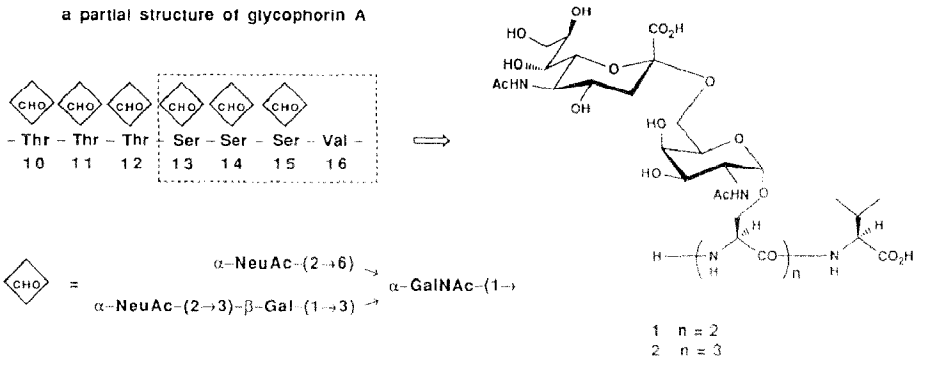
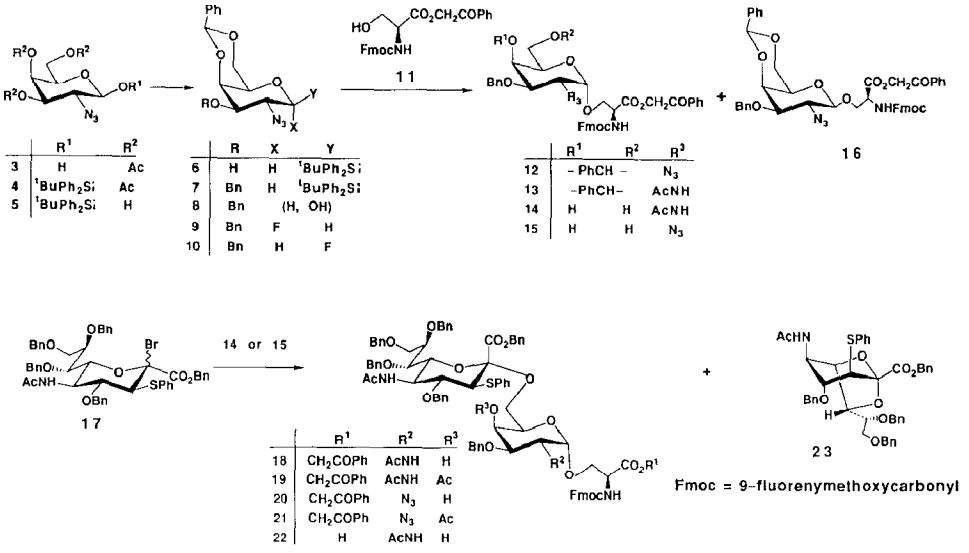


RESULTS AND DISCUSSION

Synthons **11**[10] and **17**[11] are known, and synthons **9** and **10** were obtained as follows. The hemiacetal **3**, derived from D-galactose in 4 steps[12], was protected with a bulky silyl group (^{t}BuPh$_2$Si–) to give exclusively the β anomer **4** (83%), which was converted into the 3-*O*-benzyl-4,6-*O*-benzylidene derivative **7** by deacetylation (→ **5**, quantitatively), benzylidenation (→ **6**, 91%), and benzylation (85%). Desilylation of **7** with tetrabutylammonium fluoride–acetic acid[13] in tetrahydrofuran gave **8** (97%), which, on treatment with diethylaminosulfur trifluoride[14] in tetrahydrofuran, produced the easily separable isomers **9** (78%) and **10** (13%).

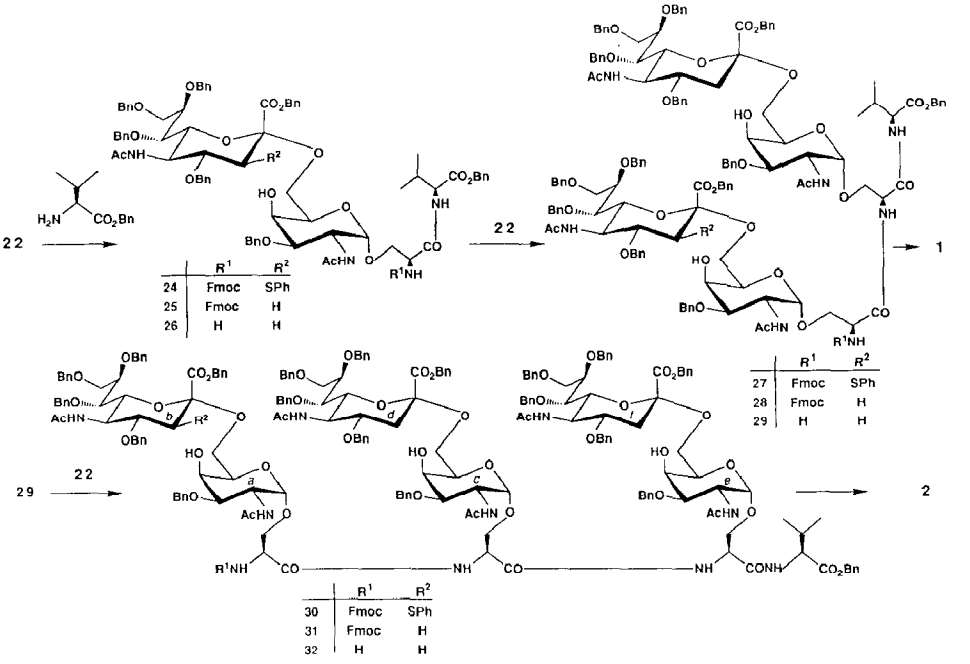
The α-fluoride **9** was coupled with the L-serine derivative **11**, using Cp_2ZrCl_2–silver perchlorate[15] as a promotor in dichloromethane to give the α-glycoside **12** (68%, δ_H 5.10, $J_{1,2}$ 2.4 Hz; δ_C 100.2, $J_{C-1,H-1}$ 172 Hz) and the β isomer **16** (18%, δ_C 102.1, $J_{C-1,H-1}$ 161 Hz). Use of the β anomer **10** in this glycosylation reaction gave **12** and **16** (76% combined yield) with the same stereoselectivity (α, 79%; β, 21%). Cp_2HfCl_2–silver perchlorate[15] also promoted this glycosylation. Treatment of **12** with thioacetic acid[16] gave **13** (81%), which was debenzylidenated with aq. 80% acetic acid at 60° to yield the diol **14** (89%). Attempts to glycosylate **14** with the sialic acid donor **17** in the presence of mercury(II) cyanide/bromide or silver trifluoromethanesulfonate in dichloromethane, dichloromethane–carbon tetrachloride, chloroform, or acetonitrile resulted in poor conversions of **14** accompanied by formation of a bicyclic compound **23**[17], and < 30% of the desired **18** was produced. The unsatisfactory results were due probably to the low solubility of **14** in the solvents.

On the other hand, **15** (83%), derived from **12** by hydrolysis, was more soluble and reacted smoothly with **17**, using mercury(II) cyanide/bromide in dichloromethane–carbon tetrachloride, to afford **20** as a sole product (85%). Neither stereo- nor regioisomeric products were obtained. The newly formed (2→6) linkages in **18** and **20** were assigned by the synthesis sequence and confirmed by ^{1}H-n.m.r. data of the corresponding acetates **19** (δ 5.44, bs, H-4a) and **21** (δ 5.48, bs, H-4a), respectively, whereas the anomeric configuration was presumed to be α because of the neighboring participation of the β-phenylthio group at C-3 in **17**[18]. Compound **20** was converted readily into **18** (91%) on exposure to thioacetic acid. Since an attempt to utilise radical conditions[17] for



the selective cleavage of the phenylthio group from **18** was unsuccessful without affecting the labile phenacyl group, **18** was converted into **22** (97%) with zinc–acetic acid, which was condensed with L-valine benzyl ester[19], using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in dichloromethane, to give dipeptide **24** (82%).

When **24** was heated with triphenyltin hydride and α,α′-azobis(isobutyronitrile) (AIBN) in toluene, the desulfurised product **25** (54%) was obtained. Treatment of **25**



with morpholine exposed the amino group (→**26**, 96%) necessary for the next condensation with **22**. EEDQ-promoted coupling of **22** and **26** afforded the tripeptide derivative **27** (74%), which was desulfurised as above, but in benzene, to give **28**. Retreatment of unreacted **27**, isolated by chromatography, and repetition of the procedure eventually gave 79% of **28**. Treatment of **28** with morpholine removed the Fmoc group and gave the amine **29** (95%), which was condensed with **22** (→**30**, 86%), desulfurised (→**31**, 68%), and deblocked with morpholine to afford the benzylated trimeric sialosyl-Tn epitope **32**.

Hydrogenolysis of **29** and **32** in aqueous methanol and purification by gel filtration followed by ion-exchange chromatography completed the syntheses of **1** and **2**, respectively. The ^{1}H-n.m.r. spectra of **1** and **2** confirmed the structures when compared with those reported for the related natural glycoproteins[20,21]. Notably, the resonances of H-3a and H-3b of the Neu*p*5Ac residues in **1** and **2** were characteristic for α-glycosides.

EXPERIMENTAL

General. — Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter, for solutions in $CHCl_3$, unless noted otherwise. Column chromatography was performed on silica gel (Merck 70–230 mesh). Flash chromatography was performed on Wako Gel C-300 (200–300 mesh). T.l.c. and h.p.t.l.c. were performed on Silica Gel 60 F_{254} (Merck). N.m.r. spectra were recorded with a JEOL GX 500 [^{1}H (500 MHz)] or FX90Q [^{13}C (22.50 MHz)] spectrometer. Chemical shifts are expressed in p.p.m. downfield from the signal for internal Me_4Si, for solutions in $CDCl_3$, unless noted otherwise, and for solutions in D_2O, in p.p.m. downfield from the signal for Me_4Si, by reference to internal Me_2COH (1.230 p.p.m.). F.a.b.-mass spectra were obtained with a JEOL HX110 (HF) mass spectrometer, using glycerol as the matrix.

tert-*Butyldiphenylsilyl 3,4,6-tri-O-acetyl-2-azido-2-deoxy-β-D-galactopyranoside* (**4**). — The hemiacetal **3** (14.8 g, 44.7 mmol) was heated at 60° with ^{t}BuPh$_2$SiCl (16.0 g, 58.2 mmol) and imidazole (8.5 g, 124.9 mmol) in dry *N*,*N*-dimethylformamide (90 mL). After 2.5 h, the mixture was poured into ice–water (300 mL) and extracted with ether. The extract was washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo* to give crude crystals, which were washed with hexane–ether (3:1) to afford **4** (18.2 g). The washings were concentrated and column chromatography of the residue on silica gel (50 g) with hexane–EtOAc (3:1) gave more **4** (2.9 g, 82.8% in total), m.p. 102–104° (from ether), $[\alpha]_D^{20}$ −8.0° (*c* 2.4); R_f 0.48 (3:2 hexane–EtOAc). N.m.r. data: ^{1}H, δ 1.13 (s, 9 H, ^{t}Bu), 1.91 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 2.17 (s, 3 H, Ac), 3.53 (dt, 1 H, *J* 1.2 and 6.6 Hz, H-5), 3.73 (dd, 1 H, *J* 7.8 and 11 Hz, H-2), 3.89 (dd, 1 H, *J* 6.6 and 11.2 Hz, H-6a), 3.96 (dd, 1 H, *J* 6.6 and 11.2 Hz, H-6b), 4.46 (d, 1 H, *J* 7.8 Hz, H-1), 4.68 (dd, 1 H, *J* 3.4 and 10.7 Hz, H-3), 5.23 (dd, 1 H, *J* 1.0 and 3.4 Hz, H-4), 7.36–7.45 (m, 6 H, Ar), 7.69–7.74 (m, 4 H, Ar); ^{13}C, δ 19.1 (CMe_3), 20.4 (CH_3CO), 26.8 [$C(CH_3)_3$], 61.2, 63.6, 66.5, 70.7, 71.2, 96.9 (C-1), 127.4–135.8 (Ar), 169.5 (C=O), 169.9 (C=O).

Anal. Calc. for $C_{28}H_{35}N_3O_8Si$: C, 59.03; H, 6.19; N, 7.38. Found: C, 58.93; H, 6.19; N, 7.37.

tert-*Butyldiphenylsilyl 2-azido-2-deoxy-β-*D-*galactopyranoside* (**5**). — To a solution of **4** (13.9 g, 24.4 mmol) in MeOH (120 mL)–toluene (30 mL) was added 0.1M NaOMe–MeOH (5 mL). The mixture was stirred at room temperature for 3 h, then treated with an excess of Amberlyst 15 (H^+) resin, filtered, and concentrated to give syrupy **5**, $[\alpha]_D^{20}$ +45° (*c* 1.6); R_F 0.49 (9:1 $CHCl_3$–MeOH). N.m.r. data: ^1H ($CDCl_3$–D_2O), δ 1.12 (s, 9 H, ^{t}Bu), 3.06 (bt, 1 H, H-5), 3.30 (dd, 1 H, *J* 3.3 and 10.3 Hz, H-3), 3.48 (dd, 1 H, *J* 4.9 and 11.7 Hz, H-6a), 3.57 (dd, 1 H, *J* 5.9 and 11.7 Hz, H-6b), 3.59 (dd, 1 H, *J* 7.8 and 10.3 Hz, H-2), 3.79 (d, 1 H, *J* 3.3 Hz, H-4), 4.79 (d, 1 H, *J* 7.8 Hz, H-1), 7.36–7.47 (m, 6 H, Ar), 7.70–7.76 (m, 4 H, Ar); ^{13}C, δ 19.1 (*C*Me_3), 26.8 [C(*C*H_3)$_3$], 61.9, 66.7, 68.6, 72.1, 74.1, 97.3 (C-1), 127.4–135.8 (Ar).

Anal. Calc. for $C_{22}H_{29}N_3O_5Si$: C, 59.57; H, 6.59; N, 9.47. Found: C, 59.36; H, 6.49; N, 9.46.

tert-*Butyldiphenylsilyl 2-azido-4,6-*O-*benzylidene-2-deoxy-β-*D-*galactopyranoside* (**6**). — A mixture of **5** (22 g, 49.6 mmol), α,α-dimethoxytoluene (22.4 mL, 148.6 mmol), and *p*-TsOH·H_2O (270 mg) in dry MeCN (400 mL) was stirred at room temperature for 1.5 h, then neutralised with aq. $NaHCO_3$, and concentrated *in vacuo*. The residue was extracted with ether, and the extract was washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography of the residue on silica gel (800 g) with hexane–EtOAc (3:2) afforded **6** (24 g, 91%), $[\alpha]_D^{19}$ −3.4° (*c* 2.2); R_F 0.47 (1:1 hexane–EtOAc). N.m.r. data: ^1H, δ 1.14 (s, 9 H, ^{t}Bu), 2.68 (b, 1 H, OH), 2.90 (bs, 1 H, H-5), 3.36 (b, 1 H; H-3), 3.64 (dd, 1 H, *J* 7.6 and 10.1 Hz, H-2), 3.79 (bd, 1 H, *J* 12.2 Hz, H-6a), 3.90 (d, 1 H, *J* 12.5 Hz, H-6b), 3.96 (d, 1 H, *J* 3.7 Hz, H-4), 4.39 (d, 1 H, *J* 7.6 Hz, H-1), 5.44 (s, 1 H, PhC*H*), 7.33–7.42 (m, 9 H, Ar), 7.50 (m, 2 H, Ar), 7.72–7.81 (m, 4 H, Ar); ^{13}C, δ 19.2 (*C*Me_3), 26.9 [C(*C*H_3)$_3$], 66.3, 66.8, 68.7, 71.4, 74.4, 96.8 (C-1), 101.3 (Ph*C*H), 126.5–137.6 (Ar).

Anal. Calc. for $C_{29}H_{33}N_3O_5Si$: C, 65.51; H, 6.26; N, 7.90. Found: C, 65.71; H, 6.31; N, 8.00.

tert-*Butyldiphenylsilyl 2-azido-3-*O-*benzyl-4,6-*O-*benzylidene-2-deoxy-β-*D-*galactopyranoside* (**7**). — A mixture of **6** (1.5 g, 2.8 mmol) and 60% NaH in oil (22.5 mg, 5.6 mmol) in dry tetrahydrofuran (20 mL) was stirred at 60° for 1 h, benzyl bromide (860 mg, 5.0 mmol) was added, and stirring was continued at 60° overnight. The mixture was cooled, small pieces of ice were added, and the mixture was concentrated *in vacuo*. The residue was extracted with ether–EtOAc (1:1), and the extract was washed with water and brine, dried (Na_2SO_4), and concentrated. Crystallisation of the residue from hexane–EtOAc afforded needles of **7** (1.36 g). From the mother liquor, more **7** (0.14 g) was obtained after column chromatography on silica gel (85.3% total yield); m.p. 147.5°, $[\alpha]_D^{22}$ +24.5° (*c* 1.3); R_F 0.57 (7:3 hexane–EtOAc). N.m.r. data: ^1H, δ 1.13 (s, 9 H, ^{t}Bu), 2.85 (d, 1 H, *J* 0.7 Hz, H-5), 3.23 (dd, 1 H, *J* 3.7 and 10.3 Hz, H-3), 3.77 (dd, 1 H, *J* 1.8 and 12.5 Hz, H-6a), 3.89–3.93 (m, 3 H, H-2,4,6b), 4.38 (d, 1 H, *J* 8.1 Hz, H-1), 4.68 (s, 2 H, PhCH_2), 5.38 [s, 1 H, PhC*H*(O)$_2$], 7.27–7.41 (m, 14 H, Ar), 7.53–7.54 (m, 2 H, Ar), 7.71–7.80 (m, 4 H, Ar); ^{13}C, δ 19.3 (*C*Me_3), 26.9 [C(*C*H_3)$_3$], 64.8, 66.3, 68.9, 71.5, 72.4, 77.9, 96.9 (C-1), 101.1 [Ph*C*H(O)$_2$], 126.5–138.0 (Ar).

Anal. Calc. for $C_{36}H_{39}N_3O_5Si$:, C, 69.54; H, 6.32; N, 6.76. Found: C, 69.50; H, 6.35; N, 6.73.

*2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-*D*-galactopyranose* (**8**). — To a stirred mixture of **7** (1.39 g, 2.2 mmol) and AcOH (1.28 mL, 22.4 mmol) in tetrahydrofuran (30 mL) was added 0.23M *n*-Bu_4NF–tetrahydrofuran (39 mL, 9 mmol) at 0°. Stirring was continued at 0° → room temperature overnight. The mixture was then concentrated *in vacuo*, the residue was extracted with ether–EtOAc (1:1), and the extract was washed with water, aq. $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography of the residue on silica gel (100 g) with toluene–EtOAc (3:2) gave **8** (0.83 g, 96.8%), $[\alpha]_D^{23}$ +138° (*c* 1.0); R_F 0.25 (7:3 hexane–EtOAc).

Anal. Calc. for $C_{20}H_{21}N_3O_5$: C, 62.65; H, 5.52; N, 10.96. Found: C, 62.58; H, 5.55; N, 10.68.

2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α- (**9**) *and -β-*D*-galactopyranosyl fluoride* (**10**). — To a stirred solution of **8** (750 mg, 2.0 mmol) in dry tetrahydrofuran (15 mL) at 0° was added diethylaminosulfur trifluoride (420 μL, 3.2 mmol). The mixture was stirred at room temperature for 30 min, MeOH (0.5 mL) was added, and the mixture was concentrated *in vacuo*. The residue was extracted with ether–EtOAc (1:1), and the extract was washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography of the crude product on silica gel (100 g) with toluene–EtOAc (7:3) gave **9** (591 mg, 78.4%) and **10** (99 mg, 13.1%).

Compound **9** had m.p. 108–109° (needles from hexane–EtOAc), $[\alpha]_D^{20}$ +173.5° (*c* 1.1); R_F 0.60 (7:3 toluene–EtOAc). N.m.r. data: ^1H, δ 3.83 (bs, 1 H, H-5), 3.96–4.06 (m, 3 H, H-2,3,6a), 4.25 (d, 1 H, *J* 2.2 Hz, H-4), 4.29 (dd, 1 H, *J* 1.5 and 12.8 Hz, H-6b), 4.74 (d, 1 H, *J* 12.1 Hz, PhCH_2), 4.78 (d, 1 H, *J* 12.1 Hz, PhCH_2), 5.48 [s, 1 H, PhC*H*$(O)_2$], 5.74 (dd, 1 H, *J* 2.6 and 53.5 Hz, H-1), 7.30–7.52 (m, 10 H, Ar); ^{13}C, δ 58.6 (d, *J* 23.1 Hz, C-2), 64.9, 68.8, 71.3, 72.2, 74.2, 100.8 [Ph*C*H$(O)_2$], 106.9 (d, *J* 225.8 Hz, C-1), 126.1–137.3 (Ar).

Anal. Calc. for $C_{20}H_{20}FN_3O_4$: C, 62.33; H, 5.23; N, 10.90; F, 4.93. Found: C, 62.32; H, 5.24; N, 10.86; F, 5.08.

Compound **10** had m.p. 107–109° (needles from hexane–EtOAc), $[\alpha]_D^{20}$ +55.7° (*c* 1.1); R_F 0.38 (7:3 toluene–EtOAc). N.m.r. data: ^1H, δ 3.41 (bs, 1 H, H-5), 3.44 (ddd, 1 H, *J* 1.1, 3.3, and 10.3 Hz, H-3), 3.98 (ddd, 1 H, *J* 7.7, 10.3, and 12.5 Hz, H-2), 4.02 (bd, *J* 12.8 Hz, H-6a), 4.09 (bs, 1 H, H-4), 4.33 (dd, 1 H, *J* 1.5 and 12.5 Hz, H-6b), 4.75 (s, 2 H, PhCH_2), 5.00 (dd, 1 H, *J* 7.7 and 52.8 Hz, H-1), 5.48 [s, 1 H, PhC*H*$(O)_2$], 7.32–7.54 (m, 10 H, Ar); ^{13}C, δ 62.0 (d, *J* 20.8 Hz, C-2), 66.7 (d, *J* 6.1 Hz, C-3), 68.6, 71.6, 77.4 (d, *J* 9.8 Hz, C-5), 101.1 [Ph*C*H$(O)_2$], 102.2 (d, *J* 213.6 Hz, C-1), 112.9–137.4 (Ar).

Anal. Found: C, 62.38; H, 5.23; N, 11.01; F, 5.18.

*N-(9-Fluorenylmethoxycarbonyl)-O-(2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α- and -β-*D*-galactopyranosyl-(1→3)-*L*-serine phenacyl ester* (**12** and **16**). — *(a) By coupling of* **9** *and* **11**. A mixture of Cp_2ZrCl_2 (121 mg, 414 μmol), $AgClO_4$ (172 mg, 830 μmol), and well-dried powdered molecular sieves 4A (1 g) in dry CH_2Cl_2 (4 mL) was stirred at −20° for 30 min under Ar. A solution of **9** (80 mg, 207 μmol) and **11** (108 mg, 242 μmol) in dry CH_2Cl_2 (4 mL) was added, and the mixture was stirred at −20° to −10°

for 1.5 h, then quenched with pyridine (2 mL), diluted with $CHCl_3$, filtered through Celite, washed with water, aq. $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography of the residue on silica gel (30 g) with toluene–EtOAc (4:1) afforded **12** (115 mg, 68.3%), **16** (31 mg, 18.4%), and **11** (17 mg).

(b) By coupling of **10** *and* **11**. Reaction of the β-fluoride **10** (48 mg, 125 μmol) and **11** (58 mg, 130 μmol) was carried out with Cp_2ZrCl_2 (55 mg, 188 μmol), $AgClO_4$ (78 mg, 376 μmol), and molecular sieves 4A (0.6 g) in CH_2Cl_2 (4 mL), as described above, to afford **12** (61 mg, 60.4%), **16** (16 mg, 15.8%), and **11** (12 mg).

Compound **12** had m.p. 115° (wet)–121° (from hexane–EtOAc), $[\alpha]_D^{25}$ +103° (*c* 1.1); R_F 0.59 (7:3 toluene–EtOAc). N.m.r. data: ^1H, δ 3.75 (s, 1 H, H-5), 4.37 (bt, 1 H, C*H*Ar_2), 4.74–4.77 (m, 3 H, Ser α-H and PhCH_2), 5.10 (d, 1 H, *J* 2.4 Hz, H-1), 5.41 (d, 1 H, *J* 6.1 Hz, CH_2COPh), 5.45 [s, 1 H, PhC*H*$(O)_2$], 5.46 (d, 1 H, *J* 6.1 Hz, CH_2COPh), 5.96 (d, 1 H, *J* 7.9 Hz, NH), 7.26–7.90 (m, 23 H, Ar); ^{13}C, δ 47.1, 54.6, 58.8, 63.5, 66.9, 67.3, 69.2, 71.3, 73.0, 74.2, 100.2 ($^1J_{C,H}$ 172 Hz, C-1), 100.8 [Ph*C*H$(O)_2$], 150.5 (NHCO), 169.5 (*C*OOCH_2), 191.0 (CH_2*C*OPh).

Anal. Calc. for $C_{46}H_{42}N_4O_{10}$: C, 68.14; H, 5.22; N, 6.91. Found: C, 67.99; H, 5.15; N, 6.59.

Compound **16** had $[\alpha]_D^{25}$ +9.6° (*c* 1.4), R_F 0.39 (7:3 toluene–EtOAc). N.m.r. data: ^1H, δ 3.30 (s, 1 H, H-5), 3.40 (dd, 1 H, *J* 3.4 and 10.4 Hz, H-3), 3.89 (dd, 1 H, *J* 8.2 and 10.1 Hz, H-2), 3.97 (dd, 1 H, *J* 1.5 and 12.5 Hz, H-6a), 4.07 (d, 1 H, *J* 3.4 Hz, H-4), 4.22–4.27 (m, 2 H, H-6b and C*H*Ar_2), 4.32–4.40 (m, 3 H, H-1, CH_2OOCNH and Ser β-H), 4.46 (dd, 1 H, *J* 3.4 and 10.7 Hz, Ser β-H), 4.74 (s, 2 H, PhCH_2), 4.78 (m, 1 H, Ser α-H), 5.38 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.45 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.46 [s, 1 H, PhC*H*$(O)_2$], 6.05 (d, 1 H, *J* 8.2 Hz, NH), 7.26–7.81 (m, 23 H, Ar); ^{13}C, δ 47.1, 54.5, 62.1, 66.5, 67.1, 67.5, 69.0, 69.2, 71.6, 72.3, 77.8, 101.1 [Ph*C*H$(O)_2$], 102.1 ($^1J_{C,H}$ 161 Hz, C-1), 153.3 (CONH), 169.4 (*C*OOCH_2), 191.6 (CH_2*C*OPh).

Anal. Found: C, 67.72; H, 5.23; N, 6.83.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(2-acetamido-3*-O-*benzyl-4,6*-O-*benzylidene-2-deoxy*-α-D-*galactopyranosyl-(1→3)*-L-*serine phenacyl ester* (**13**). — To a solution of **12** (490 mg, 0.6 mmol) in CH_2Cl_2 (1.5 mL) was added thioacetic acid (3 mL). The mixture was stirred at room temperature for 2 days, then concentrated *in vacuo*. The resulting solid was washed with ether–EtOAc (1:1) and filtered to give crystalline **13** (367 mg). The filtrate was concentrated and the residue was again treated with thioacetic acid (0.5 mL) to give more (37 mg) **13** (total yield, 80.8%), m.p. 203–205° (from $CHCl_3$), $[\alpha]_D^{25}$ +86° (*c* 0.3, 4:1 chloroform–methanol); R_F 0.41 (12:7:1 EtOAc–toluene–acetone). N.m.r. data ($CDCl_3$–CD_3OD): ^1H, δ 1.87 (s, 3 H, Ac), 3.67 (s, 1 H, H-5), 5.06 (d, 1 H, *J* 3.1 Hz, H-1), 5.38 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.49 [s, 1 H, PhC*H*$(O)_2$], 5.56 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 7.23–7.91 (m, 23 H, Ar); ^{13}C, δ 22.6 (*C*H_3CO), 99.3 (C-1), 100.9 [Ph*C*H$(O)_2$], 172.3 (C–O).

Anal. Calc. for $C_{48}H_{46}N_2O_{11}\cdot 2H_2O$: C, 65.44; H, 5.95; N, 3.18. Found: C, 65.20; H, 5.22; N, 3.11.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(2-acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl-(1→3)*-L-*serine phenacyl ester* (**14**). — A mixture of **13** (245 mg, 0.30

mmol), $CHCl_3$ (0.8 mL), and aq. 80% AcOH (10 mL) was heated at 60° for 3.5 h, then concentrated. Column chromatography of the residue on silica gel (40 g) with $CHCl_3$–MeOH (19:1) gave **14** (194 mg, 88.6%), $[\alpha]_D^{25}$ +54.5° (*c* 0.9, 4:1 chloroform–methanol); R_f 0.40 (9:1 $CHCl_3$–MeOH). N.m.r. data: ^1H, δ 1.90 (s, 3 H, Ac), 4.93 (d, 1 H, *J* 3.1 Hz, H-1), 5.30 (d, 1 H, *J* 16.2 Hz, CH_2COPh), 5.60 (d, 1 H, *J* 16.2 Hz, CH_2COPh), 5.81 (d, 1 H, *J* 7.9 Hz, NH), 6.49 (d, 1 H, *J* 9.8 Hz, NH), 7.23–7.87 (m, 18 H, Ar); ^{13}C ($CDCl_3$–CD_3OD), δ 22.9 (CH_3CO), 47.1, 48.1, 54.2, 62.3, 66.6, 67.1, 68.2, 70.9, 71.4, 76.4, 98.7 (C-1), 119.9–143.7 (Ar), 150.5 (NHCOO), 169.9 (C=O), 171.2 (C=O), 192.1 (CH_2COPh).

Anal. Calc. for $C_{41}H_{42}N_4O_{11}$: C, 66.66; H, 5.73; N, 3.79. Found: C, 66.39; H, 5.72; N, 3.81.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(2-azido-3-O-benzyl-2-deoxy-α-*D*-galactopyranosyl)-(1→3)-*L*-serine phenacyl ester* (**15**). — A mixture of **12** (170 mg, 0.21 mmol) and aq. 80% AcOH (7 mL) was kept at 60° for 2 h, then concentrated *in vacuo*. Column chromatography of the residue on silica gel (30 g) with $CHCl_3$–MeOH (19:1) gave **15** (125 mg, 82.5%), $[\alpha]_D^{23}$ +57° (*c* 1.2); R_f 0.18 (1:1 toluene–EtOAc). N.m.r. data: ^1H, δ 3.71 (dd, 1 H, *J* 3.7 and 10.4 Hz, H-2), 3.77 (m, 1 H, H-6a), 3.86–3.92 (m, 2 H, H-5,6b), 3.99 (dd, 1 H, *J* 2.7 and 10.4 Hz, H-3), 4.04 (dd, 1 H, *J* 3.1 and 11.0 Hz, Ser β-H), 4.12 (d, 1 H, *J* 2.4 Hz, H-4), 4.25 (t, 1 H, *J* 6.9 Hz, Ar_2CH), 4.31 (dd, 1 H, *J* 3.7 and 11.0 Hz, Ser β-H), 4.44 (m, 2 H, CH_2OOCNH), 4.69 (d, 1 H, *J* 11.3 Hz, $PhCH_2$), 4.74 (m, 2 H, $PhCH_2$ and Ser α-H), 5.01 (d, 1 H, *J* 3.4 Hz, H-1), 5.41 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.46 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 6.18 (d, 1 H, *J* 8.6 Hz, NH), 7.30–7.89 (m, 18 H, Ar); ^{13}C, δ 47.1, 59.1, 62.7, 66.9, 67.2, 70.5, 71.8, 75.7, 99.5 (C-1), 120.0–143.8 (Ar), 156.0 (NHCO), 169.6 ($COOCH_2$), 191.3 (CH_2COPh).

Anal. Calc. for $C_{59}H_{58}N_4O_{10}\cdot 0.5H_2O$: C, 64.01; H, 5.37; N, 7.66. Found: C, 64.26; H, 5.27; N, 7.63.

N-*(9-Fluorenylmethoxycarbonyl*-O-{O-*[benzyl (5-acetamido-4,7,8,9-tetra-O-benzyl-5-deoxy-3-S-phenyl-3-thio-*D*-erythro-α-*L*-gluco-2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3-O-benzyl-2-deoxy-α-*D*-galactopyranosyl}-(1→3)-*L*-serine phenacyl ester* (**18**). — *(a) By coupling of* **14** *and* **17**. A mixture of **14** (39 mg, 53 μmol), $Hg(CN)_2$ (35 mg, 139 μmol), $HgBr_2$ (15 mg, 42 μmol), and dried powdered molecular sieves 4A (300 mg) in dry CH_2Cl_2 (3 mL) was stirred under reflux under Ar. A solution of **17** (79 mg, 85 μmol) in dry CCl_4 (4 mL) was added dropwise during 20 min, and the mixture was stirred under reflux for 30 min, then cooled, diluted with EtOAc, filtered through Celite, washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography of the residue on silica gel (15 g) with $CHCl_3$–MeOH (19:1) gave a mixture (94 mg) of less polar materials and **14** (25 mg). Preparative t.l.c. (1:1 toluene–EtOAc) of the mixture afforded benzyl 5-acetamido-2,7-anhydro-4,8,9-tri-*O*-benzyl-5-deoxy-3-*S*-phenyl-3-thio-D-*erythro*-α-D-*gluco*-2-nonulopyranosonate (**23**, 41 mg), R_f 0.51 (1:1 toluene–EtOAc), and **18** (24 mg, 28.6%), $[\alpha]_D^{25}$ +36° (*c* 1.1); R_f 0.20 (1:1 toluene–EtOAc). N.m.r. data: ^1H, δ 1.64 (s, 3 H, Ac), 1.80 (s, 3 H, Ac), 4.84 (d, 1 H, *J* 3.1 Hz, H-1a), 5.07 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.10 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.27 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.48 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.65 (d, 1 H, *J* 8.2 Hz, NH), 6.11 (d, 1 H,

J 9.5 Hz, NH); ^{13}C, δ 23.2 (CH_3CO), 23.6 (CH_3CO), 47.1 (Ar_2CH), 98.7 (C-1a), 101.0 (C-2b), 119.9–143.7 (Ar), 167.1 (C=O), 169.5 (C=O), 170.0 (C=O), 191.6 (C=O).

Anal. Calc. for $C_{93}H_{93}N_3O_{19}S$: C, 70.30; H, 5.90; N, 2.64; S, 2.02. Found: C, 69.87; H, 5.88; N, 2.64; S, 2.12.

N.m.r. data for **23**: ^{1}H, δ 2.02 (s, 3 H, Ac), 3.49–3.54 (m, 2 H, H-8,9a), 3.59 (bs, 1 H, H-3), 3.68 (m, 1 H, H-9b), 3.83 (d, 1 H, *J* 0.9 Hz, H-4), 4.30 (dd, 1 H, *J* 1.5 and 9.2 Hz, H-5), 4.66–4.70 (m, 2 H, H-6,7), 5.17 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.25 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 6.38 (d, 1 H, *J* 9.2 Hz, NH), 6.98–7.36 (m, 20 H, Ar); ^{13}C, δ 23.3 (CH_3CO), 48.3, 51.7, 67.6, 69.0, 71.3, 72.5, 73.4, 76.7, 78.0, 78.7, 79.4, 104.7 (C-2), 127.5–138.0 (Ar), 165.2 (C=O), 169.0 (C=O).

(b) By reductive N-*acetylation of* **20**. A mixture of **20** (78 mg, 49.6 μmol), CH_2Cl_2 (150 μL), and thioacetic acid (800 μL) was stirred at room temperature for 2 days, then concentrated *in vacuo*. Column chromatography of the residue on silica gel (15 g) with toluene–EtOAc (1:1) gave **18** (72 mg, 91.4%).

^{1}H-N.m.r. data of the acetate (**19**) of **18**: δ 1.67 (s, 3 H, Ac), 1.80 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 3.49 (d, 1 H, *J* 7.3 Hz, H-3b), 4.89 (d, 1 H, *J* 2.8 Hz, H-1a), 5.05 (b, 2 H, CO_2CH_2Ph), 5.25 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.43 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.44 (bs, 1 H, H-4a), 5.69 (d, 1 H, *J* 8.5 Hz, NH), 6.03 (d, 1 H, *J* 9.2 Hz, NH).

N-*(9-Fluorenylmethoxycarbonyl)*-O-{O-*[benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-5-deoxy-3-S-phenyl-3-thio-*D-erythro-α-L-gluco-*2-nonulopyranosyl)onate]-(2→6)-2-azido-3-*O-*benzyl-2-deoxy-*α-D-*galactopyranosyl}-(1→3)-*L-*serine phenacyl ester* (**20**). — A mixture of **15** (80 mg, 111 μmol), $Hg(CN)_2$ (97 mg, 384 μmol), $HgBr_2$ (43 mg, 119 μmol), and dried powdered molecular sieves 4A (1 g) in dry CCl_4–CH_2Cl_2 (8 mL and 4 mL) was stirred at room temperature under Ar for 30 min, then cooled to −20°, and treated with a solution of **17** (220 mg, purity 72%, ~170 μmol) in dry CCl_4 (7 mL). The mixture was stirred at −20° → room temperature overnight, diluted with EtOAc, filtered through Celite, washed with water and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography of the residue on silica gel (50 g) with toluene–EtOAc (4:1) gave slightly crude **20** (220 mg), which was further purified by gel-permeation chromatography on Bio-beads S x 3 (50 mL) with toluene to afford **20** (148 mg, 85%), $[\alpha]_D^{23}$ +45° (*c* 1.2); R_F 0.41 (7:3 toluene–EtOAc). N.m.r. data: ^{1}H, δ 1.66 (s, 3 H, Ac), 3.43 (d, 1 H, *J* 7.6 Hz, H-3b), 4.94 (d, 1 H, *J* 3.4 Hz, H-1a), 5.08 (d, 1 H, *J* 11.9 Hz, CO_2CH_2Ph), 5.14 (d, 1 H, *J* 11.9 Hz, CO_2CH_2Ph), 5.33 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.42 (d, 1 H, *J* 16.5 Hz, CH_2COPh), 5.94 (d, 1 H, *J* 8.2 Hz, NH); ^{13}C, δ 23.7 (CH_3CO), 47.2 (Ar_2CH), 98.9 (C-1a), 101.1 (C-2b), 119.9–143.9 (Ar), 167.1 (C=O), 169.5 (C=O), 191.1 (C=O).

Anal. Calc. for $C_{91}H_{85}N_5O_{18}S$: C, 69.49; H, 5.70; N, 4.45. Found: C, 69.08; H, 5.70; N, 4.41.

^{1}H-N.m.r. data of the acetate (**21**) of **20**: δ 1.69 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 3.48 (d, 1 H, *J* 7.6 Hz, H-3b), 4.99 (d, 1 H, *J* 3.1 Hz, H-1a), 5.08 (bs, 2 H, CO_2CH_2Ph), 5.26 (d, 1 H, *J* 16.2 Hz, CH_2COPh), 5.41 (d, 1 H, *J* 16.2 Hz, CH_2COPh), 5.48 (bs, 1 H, H-4a), 6.03 (d, 1 H, *J* 8.2 Hz, NH).

N-(9-Fluorenylmethoxycarbonyl)-O-{O-[benzyl (5-acetamido-4,7,8,9-tetra-O-benzyl-5-deoxy-3-S-phenyl-3-thio-D-erythro-α-L-gluco-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3-O-benzyl-2-deoxy-α-D-galactopyranosyl}-(1→3)-L-serine* (**22**). — A mixture of **20** (84 mg, 52.9 μmol) and Zn dust (1.0 g) in aq. 80% AcOH (2.5 mL) was stirred at room temperature for 2.5 h, then diluted with EtOAc, filtered through Celite, and concentrated *in vacuo*. Column chromatography of the residue on silica gel (25 g) with $CHCl_3$–MeOH–AcOH (92:3:5) gave **22** (75 mg, 96.5%), $[\alpha]_D^{20}$ +75° (*c* 1.2). ^{1}H-N.m.r. data: δ 1.63 (s, 3 H, Ac), 1.66 (s, 3 H, Ac).

Anal. Calc. for $C_{88}H_{97}N_3O_{18}S\cdot 2H_2O$: C, 67.76; H, 6.09; N, 2.79; S, 2.13. Found: C, 67.28; H, 5.70; N, 3.35; S, 1.98.

N-(9-Fluorenylmethoxycarbonyl)-O-{O-[benzyl (5-acetamido-4,7,8,9-tetra-O-benzyl-5-deoxy-3-S-phenyl-3-thio-D-erythro-α-L-gluco-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3-O-benzyl-2-deoxy-α-D-galactopyranosyl}-(1→3)-L-seryl-L-valine benzyl ester* (**24**). — A solution of L-valine benzyl ester (29 mg, 142 μmol) in dry CH_2Cl_2 (1.8 mL), prepared from L-valine benzyl ester *p*-toluenesulfonate and Et_3N in CH_2Cl_2, was added under Ar to a mixture of **22** (70 mg, 47.6 μmol) and EEDQ (35 mg, 141.5 μmol) in dry CH_2Cl_2 (1 mL). The mixture was stirred at room temperature for 3 days, diluted with $CHCl_3$, washed successively with M HCl, water, aq. $NaHCO_3$, water, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. Preparative t.l.c. of the residue with EtOAc–toluene (3:2) afforded a fraction that contained **24**, which was treated with Amberlyst 15 (H^+) resin to remove quinoline and gave **24** (65 mg, 82.3%), $[\alpha]_D^{23}$ +46° (*c* 1.0); R_f 0.43 (3:7 toluene–EtOAc). N.m.r. data: ^{1}H, δ 0.83 (d, 3 H, *J* 6.7 Hz, Val Me), 0.88 (d, 3 H, *J* 6.7 Hz, Val Me), 1.64 (s, 3 H, Ac), 1.93 (s, 3 H, Ac), 2.16 (m, 1 H, Val β-H), 3.39 (bd, 1 H, *J* 10.1 Hz, H-3b), 3.89 (d, 1 H, *J* 2.7 Hz, H-4a), 3.94 (bt, 1 H, Ar_2CH), 4.96 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.07 (d, 1 H, *J* 11.9 Hz, CO_2CH_2Ph), 5.12 (d, 1 H, *J* 11.9 Hz, CO_2CH_2Ph), 5.18 (d, 1 H, *J* 11.9 Hz, CO_2CH_2Ph), 5.85 (bd, 1 H, *J* 6.1 Hz, NH), 6.03 (bd, 1 H, *J* 7.3 Hz, NH), 6.89 (b, 1 H, NH); ^{13}C, δ 17.8 (CH_3), 23.3 (CH_3CO), 23.6 (CH_3CO), 31.3 (Me_2CH), 47.2 (Ar_2C), 98.7 (C-1a), 101.0 (C-2b), 155.8 ($NHCO_2$), 167.3 (C=O), 169.6 (C=O), 169.7 (C=O), 170.0 (C=O), 171.8 (C=O).

Anal. Calc. for $C_{97}H_{102}N_4O_{19}S$: C, 70.19; H, 6.19; N, 3.38. Found: C, 69.97; H, 6.21; N, 3.44.

N-(9-Fluorenylmethoxycarbonyl-O-{O-[benzyl (5-acetamido-4,7,8,9-tetra-O-benzyl-3,5-dideoxy-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3-O-benzyl-2-deoxy-α-D-galactopyranosyl}-(1→3)-L-seryl-L-valine benzyl ester* (**25**). — A stirred mixture of **24** (61 mg, 36.7 μmol), M Ph_3SnH in benzene (310 μL), and α,α′-azobis(isobutyronitrile) (1 mg) in dry toluene (1.8 mL) was heated at 100° with stirring under Ar overnight, then concentrated *in vacuo*. Flash chromatography of the residue on silica gel (20 g) with toluene–EtOAc (1:1) gave a mixture of **24** and **25**, which was again treated as described above. After flash chromatography of the product, the fraction that contained **24** and **25** was subjected to preparative t.l.c. with toluene–EtOAc (7:3) to afford **24** (5.5 mg) and **25** (30.5 mg, 53.5%), $[\alpha]_D^{19}$ +38° (*c* 0.9); R_f 0.31 (7:3 EtOAc–toluene). N.m.r. data: ^{1}H, δ 0.82 (d, 3 H, *J* 7.0 Hz, Val Me), 0.88 (d, 3 H, *J* 6.8 Hz, Val Me), 1.73 (s, 3 H, Ac), 1.92 (s, 3 H, Ac), 2.17 (m, 1 H, Val β-H), 2.75 (dd, 1 H, *J* 4.5 and 12.8 Hz, H-3bβ), 4.02 (bs, 1 H,

H-4a), 4.99 (d, 1 H, *J* 12.3 Hz, CO_2CH_2Ph), 5.08 (d, 1 H, *J* 12.3 Hz, CO_2CH_2Ph), 5.15 (d, 1 H, *J* 12.0 Hz, CO_2CH_2Ph), 5.17 (d, 1 H, *J* 12.0 Hz, CO_2CH_2Ph), 5.67 (bd, 1 H, *J* 6.3 Hz, NH), 6.12 (bd, 1 H, *J* 7.0 Hz, NH), 6.57 (b, 1 H, NH); ^{13}C, δ 17.7 (CH_3), 19.0 (CH_3), 23.3 (CH_3CO), 23.7 (CH_3CO), 31.4 (Me_2CH), 37.2 (C-3b), 47.2 (Ar_2C), 99.2 (C-1a,2b), 155.8 ($NHCO_2$), 168.0 (C=O), 169.6 (C=O), 170.0 (C=O), 170.3 (C=O), 172.0 (C=O).

Anal. Calc. for $C_{91}H_{98}N_4O_{19}$: C, 70.43; H, 6.37; N, 3.61. Found: C, 70.29; H, 6.47; N, 3.56.

O-{O-[*Benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*nonulopyranosyl)onate*]-(2→6)-2-*acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl*}-(1→3)-L-*seryl*-L-*valine benzyl ester* (**26**). — A solution of **25** (29 mg, 18.7 μmol) in morpholine (0.7 mL) was stirred at room temperature for 1 h, then diluted with toluene, and concentrated *in vacuo*. Flash chromatography of the residue on silica gel (10 g) with toluene–EtOH (7:1) afforded **26** (23.8 mg, 95.8%), $[\alpha]_D^{20}$ +33° (*c* 0.8); R_F 0.52 (5:1 toluene–EtOH). N.m.r. data: ^1H, δ 0.80 (d, 3 H, *J* 7.0 Hz, Val Me), 0.83 (d, 3 H, *J* 7.0 Hz, Val Me), 1.77 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 2.15 (m 1 H, Val β-H), 2.74 (dd, 1 H, *J* 4.3 and 12.8 Hz, H-3bβ), 3.47 (ddd, 1 H, *J* 4.3, 10.1, and 11.4 Hz, H-4b), 3.97 (d, 1 H, *J* 2.5 Hz, H-4a), 4.73 (d, 1 H, *J* 4.0 Hz, H-1a), 5.10 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.12 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.16 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.18 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.97 (d, 1 H, *J* 9.2 Hz, NH).

Anal. Calc. for $C_{76}H_{88}N_4O_{17}$: C, 68.66; H, 6.67; N, 4.21. Found: C, 68.58; H, 6.68; N, 4.22.

N-(*9-Fluorenylmethoxycarbonyl*)-O-{O-[*benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-5-deoxy-3-S-phenyl-3-thio*-D-erythro-α-L-gluco-*2-nonulopyranosyl)onate*]-(2→6)-2-*acetamido*-}3-O-[*benzyl-2-deoxy*-α-D-*galactopyranosyl*}-[(1→3)]-L-*seryl*-O-{-[*benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-(2→6)-2-*acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl*}-(1→3)-L-*seryl*-L-*valine benzyl ester* (**27**). — A mixture of **22** (20 mg, 13.6 μmol), **26** (16.2 mg, 12.2 μmol), and EEDQ (10 mg, 40.4 μmol) in dry CH_2Cl_2 (0.8 mL) was stirred at room temperature for 3 days, then concentrated *in vacuo*. Gel-permeation chromatography of the residue on Bio-beads S x 3 (120 mL) with toluene–EtOAc (4:1) gave a fraction that contained **27**, which was subjected to preparative t.l.c. with toluene–EtOAc–EtOH (8:1:1) to afford **27** (25.1 mg, 74.0%), $[\alpha]_D^{20}$ +54° (*c* 1.0); R_F 0.48 (5:1 toluene–EtOH). ^1H-N.m.r. data: δ 0.78 (d, 3 H, *J* 7.0 Hz, Val Me), 0.79 (d, 3 H, *J* 6.4 Hz, Val Me), 1.63 (s, 3 H, Ac), 1.71 (s, 3 H, Ac), 1.90 (s, 3 H, Ac), 1.93 (s, 3 H, Ac), 2.07 (m, 1 H, Val βH), 2.74 (dd, 1 H, *J* 4.0 and 12.8 Hz, H-3dβ), 4.86 (d, 1 H, *J* 3.7 Hz, H-1a or H-1c), 4.95 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.04–5.19 (m, 5 H, CO_2CH_2Ph), 5.86 (bd, 1 H, *J* 6.4 Hz, NH), 6.14 (bd, 1 H, *J* 7.6 Hz, NH), 6.48 (bd, 1 H, *J* 8.6 Hz, NH), 6.89 (b, 1 H, NH).

Anal. Calc. for $C_{161}H_{173}N_2O_{34}S \cdot 5H_2O$: C, 67.32; H, 6.42; N, 3.41. Found: C, 67.39; H, 6.07; N, 3.43.

N-(*9-Fluorenylmethoxycarbonyl*)-O-{O-[*benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-(2→6)-2-*acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl*}-(1→3)-L-*seryl*-O-{O-[*benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)*-

onate]-(2→6)-2-acetamido-3-O-benzyl-2-deoxy-α-D-galactopyranosyl}-(1→3)-L-seryl-L-valine benzyl ester (**28**). — A mixture of **27** (26.8 mg, 9.6 μmol), M Ph_3SnH/benzene (400 μL), and α,α′-azobis(isobutyronitrile) (1 mg) in dry benzene (100 μL) was heated at 90° for 2 h, then concentrated *in vacuo*. Flash chromatography of the residue on silica gel (13 g) with toluene–EtOAc–EtOH (10:10:1) gave a mixture of **27** and **28** (~1:1), which was again treated as above. After three repetitions of the procedure, the mixture of **28** and **27** (95:5 by h.p.l.c.) was purified by h.p.l.c. on a column of Cosmosil ODS (20 × 250 mm) in aq. 97% MeOH to afford **28** (20.4 mg, 79.2%), $[\alpha]_D^{21}$ +46° (*c* 1.0); R_F 0.29 (7:1 toluene–EtOH). ^{1}H-N.m.r. data: δ 0.78 (d, 3 H, *J* 7.0 Hz, Val Me), 0.80 (d, 3 H, *J* 8.2 Hz, Val Me), 1.72 (s, 3 H, Ac), 1.92 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 2.09 (m, 1 H, Val β-H), 2.73 (m, 2 H, H-3bβ,3dβ), 4.01 (bs, 2 H, H-4a,4c), 4.96 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.06 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.08 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.14 (d, 2 H, *J* 11.9 Hz, CO_2CH_2Ph), 5.15 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.72 (b, 1 H, NH), 6.34 (bd, 2 H, NH), 6.93 (b, 1 H, NH), 6.98 (bd, 1 H, NH).

Anal. Calc. for $C_{138}H_{160}N_2O_{34}$·6H_2O: C, 66.92; H, 6.56; N, 3.52. Found: C, 66.89; H, 6.13; N, 3.45.

O-{O-[*Benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl}-(1→3)*-L-*seryl*-O-{O-[*benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl}-(1→3)*-L-*seryl*-L-*valine benzyl ester* (**29**). — Compound **28** (17.3 mg, 6.5 μmol) was treated with morpholine as described for the preparation of **26**. Flash chromatography of the product on silica gel (7 g) with toluene–EtOH (8:1) afforded **29** (15.0 mg, 94.6%), $[\alpha]_D^{21}$ +43.5° (*c* 1.4); R_F 0.45 (5:1 toluene–EtOH). ^{1}H-N.m.r. data: δ 0.80 (d, 3 H, *J* 7.0 Hz, Val Me), 0.83 (d, 3 H, *J* 6.7 Hz, Val Me), 1.73 (s, 3 H, Ac), 1.76 (s, 3 H, Ac), 1.94 (s, 3 H, Ac), 1.96 (s, 3 H, Ac), 2.11 (m, 1 H, Val β-H), 2.74 (m, 2 H, H-3bβ,3dβ), 3.95 (bs, 1 H) and 4.01 (bs, 1 H) (H-4a and H-4c), 4.72 (d, 1 H, *J* 3.7 Hz) and 4.80 (d, 1 H, *J* 3.4 Hz) (H-1a,1c), 4.98 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.06 (d, 1 H, *J* 11.9 Hz, CO_2CH_2Ph), 5.08 (d, 1 H, *J* 11.9 Hz, CO_2CH_2Ph), 5.13 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.14 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.15 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 6.30 (d, 1 H, *J* 9.2 Hz, NH), 6.48 (d, 1 H, *J* 8.2 Hz, NH), 6.68 (d, 1 H, *J* 8.0 Hz, NH).

Anal. Calc. for $C_{140}H_{159}N_3O_{35}$·3H_2O: C, 67.10; H, 6.64; N, 3.91. Found: C, 67.09; H, 6.30; N, 3.92.

N-(*9-Fluorenylmethoxycarbonyl*-O-{O-[*benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-5-deoxy-3-S-phenyl-3-thio*-D-erythro-α-L-gluco-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl}-(1→3)*-L-*seryl*-O-{O-[*benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl}-(1→3)*-L-*seryl*-O-{O-[*benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl}-(1→3)*-L-*seryl*-L-*valine benzyl ester* (**30**). — Reaction of **22** (13.2 mg, 9.0 μmol) and **29** (15.8 mg, 6.4 μmol) was performed with EEDQ (7 mg, 28

μmol) in dry CH_2Cl_2 (0.6 mL) at room temperature for 4 days. As described for **27**, the product was purified by gel-permeation chromatography and preparative t.l.c., to afford **30** (21.6 mg, 85.5%), $[\alpha]_D^{21}$ +51° (*c* 1.1); R_F 0.56 (5:1 toluene–EtOH). ¹H-N.m.r. data [$(CD_3)_2SO$, 80°]: δ 0.73 (d, 3 H, *J* 6.7 Hz, Val Me), 0.76 (d, 3 H, *J* 6.7 Hz, Val Me), 1.56 (bt, 2 H, H-3dα,3fα), 1.77 (s, 3 H, Ac), 1.82 (s, 6 H, 2 Ac), 1.83 (s, 9 H, 3 Ac), 1.95 (m, 1 H, Val β-H), 2.67 (dd, 2 H, *J* 3.7 and 12.2 Hz, H-3dβ,3fβ), 3.24 (d, 1 H, *J* 9.8 Hz, H-3bα), 4.87 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.04–5.10 (m, 4 H, CO_2CH_2Ph), 5.15 (d, 1 H, *J* 12.5 Hz, CO_2CH_2Ph), 5.24 (d, 2 H, *J* 12.2 Hz, CO_2CH_2Ph), 6.77 (d, 1 H, *J* 9.8 Hz, NH), 6.84 (d, 1 H, *J* 9.8 Hz, NH), 6.99 (d, 1 H, *J* 9.5 Hz, NH), 7.63 (d, 1 H, *J* 7.9 Hz, NH), 7.96 (b, 1 H, NH), 8.03 (d, 1 H, *J* 8.2 Hz, NH).

Anal. Calc. for $C_{225}H_{244}N_{10}O_{49}S \cdot 3H_2O$: C, 68.27; H, 6.37; N, 3.54. Found: C, 68.18; H, 6.21; N, 3.50.

N-*(9-Fluorenylmethoxycarbonyl)*-O-{O-*[benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy-α*-D-*galactopyranosyl*}*-(1→3)*-L-*seryl*-O-{O-*[benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy-α*-D-*galactopyranosyl*}*-(1→3)*-L-*seryl*-O-{O-*[benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy-α*-D-*galactopyranosyl*}*-(1→3)*-L-*seryl*-L-*valine benzyl ester* (**31**). — Compound **30** (20 mg, 5.1 μmol) was treated with Ph_3SnH and α,α′-azobis(isobutyronitrile) as described for the preparation of **28**. The procedure was repeated three times and the product (a 93:7 mixture of **31** and **30**) was subjected to h.p.l.c. to give **31** (13.2 mg, 67.9%), $[\alpha]_D^{21}$ +47° (*c* 0.6); R_F 0.54 (5:1 toluene–EtOH). ¹H-N.m.r. data [$(CD_3)_2SO$]: δ 0.68 (d, 3 H, *J* 6.4 Hz, Val Me), 0.73 (d, 3 H, *J* 6.7 Hz, Val Me), 1.50 (m, 3 H, H-3bα,3dα,3fα), 1.77 (s, 3 H, Ac), 1.81 (s, 3 H, Ac), 1.82 (s, 3 H, Ac), 1.86 (bs, 9 H, 3 Ac), 2.69 (bd, 3 H, H-3bβ, H-3dβ, and H-3fβ), 5.04–5.28 (m, 8 H, CO_2CH_2Ph), 7.03 (bd, 1 H, NH).

Anal. Calc. for $C_{219}H_{240}N_{10}O_{49} \cdot 4H_2O$: C, 68.00; H, 6.46; 3.62. Found: C, 67.80; H, 6.35; N, 3.56.

O-{O-*[Benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy-α*-D-*galactopyranosyl*}*-(1→3)*-L-*seryl*-O-{O-*[benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy-α*-D-*galactopyranosyl*}*-(1→3)*-L-*seryl*-O-{O-*[benzyl (5-acetamido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate]-(2→6)-2-acetamido-3*-O-*benzyl-2-deoxy-α*-D-*galactopyranosyl*}*-(1→3)*-L-*seryl*-L-*valine benzyl ester* (**32**). Compound **31** (11 mg, 2.9 μmol) was stirred with morpholine (0.5 mL) at room temperature for 1.5 h, and the product was purified by flash chromatography and gel-permeation chromatography to give **32** (9.8 mg, 94.6%), $[\alpha]_D^{22}$ +50° (*c* 0.5); R_F 0.46 (5:1 toluene–EtOH). ¹H-N.m.r. data [$(CD_3)_2SO$, 80°]: δ 0.73 (d, 3 H, *J* 7.0 Hz, Val Me), 0.77 (d, 3 H, *J* 7.0 Hz, Val Me), 1.58 (m, 3 H, H-3bα,3dα,3fα), 1.77 (s, 3 H, Ac), 1.82 (s, 6 H, 2 Ac), 1.83 (s, 9 H, 3 Ac), 1.95 (m, 1 H, Val β-H), 2.66 (m, 3 H, H-3bβ,3dβ,3fβ), 4.90 (d, 1 H, *J* 12.5 Hz, CO_2CH_2Ph), 5.06 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph),

5.08 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.09 (d, 1 H, *J* 12.5 Hz, CO_2CH_2Ph), 5.10 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.22 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.23 (d, 1 H, *J* 12.2 Hz, CO_2CH_2Ph), 5.24 (d, 1 H, *J* 12.5 Hz, CO_2CH_2Ph), 6.82 (bd, 1 H, NH), 7.02 (bd, 1 H, NH), 7.63 (b, 2 H, NH), 7.97 (bd, 1 H, NH), 8.03 (bd, 1 H, NH).

Anal. Calc. for $C_{204}H_{330}N_{16}O_{47}\cdot 3H_2O$: C, 67.53; H, 6.56; N, 3.86. Found: C, 67.73; H, 6.32; N, 3.26.

O-[O-(*5-Acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*)-(*2→6*)-*2-acetamido-2-deoxy*-α-D-*galactopyranosyl*]-(*1→3*)-L-*seryl*-*O*-[*O*-(*5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*)-(*2→6*)-*2-acetamido-2-deoxy*-α-D-*galactopyranosyl*]-(*1→3*)-L-*seryl*-L-*valine* (**1**). — A mixture of **29** (10.5 mg, 4.3 μmol) and 20% $Pd(OH)_2/C$ (10 mg) in aq. 80% MeOH was stirred under hydrogen at room temperature for 5 days, then filtered through Celite, and concentrated *in vacuo*. The residue was washed through a short column of Sephadex LH-20 with water, and the resulting crude product was subjected to anion-exchange chromatography on Mono-Q (HR5/5) with a gradient of NH_4HCO_3 buffer [0.05M (5 min)–0.15M (20 min)–0.30M (30 min)]. The fractions that contained **1** were combined and concentrated *in vacuo*, and NH_4HCO_3 was removed by evaporation of water several times from the residue which was then lyophilized to leave **1** (3.1 mg), $[\alpha]_D^{25}$ +88° (*c* 0.2, water). ^{1}H-N.m.r. data (D_2O, 20°): δ 0.92 (d, 3 H, *J* 6.7 Hz, Val Me), 0.93 (d, 3 H, *J* 7.0 Hz, Val Me), 1.68 (bt, 2 H, *J* 12.5 Hz, NeuAc H-3α), 2.02 (s, 9 H, 3 Ac), 2.03 (s, 3 H, Ac), 2.15 (m, 1 H, Val β-H), 2.71 (dd, 1 H, *J* 4.5 and 12.5 Hz, NeuAc H-3β), 2.74 (dd, 1 H, *J* 4.6 and 12.2 Hz, NeuAc H-3β), 3.57 (dd, 2 H, *J* 1.5 and 8.9 Hz, NeuAc H-7), 3.63 (dd, 2 H, *J* 6.7 and 12.5 Hz, NeuAc H-9), 3.82 (t, 2 H, *J* 10.1 Hz, NeuAc H-5), 3.96 (bs, 2 H, GalNAc H-4), 4.40 (bt, 1 H, Ser α-H), 4.80 (bt, 1 H, Ser α-H), 4.86 (d, 1 H, *J* 4.0 Hz, GalNAc H-1), 4.88 (d, 1 H, *J* 3.4 Hz, GalNAc H-1). F.a.b.-mass spectrum (positive ion): *m/z* 1280 (M^+ + 1), 1302 (M^+ + Na).

O-[O-(*5-Acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*)-(*2→6*)-*2-acetamido-2-deoxy*-α-D-*galactopyranosyl*]-(*1→3*)-L-*seryl*-O-[O-(*5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*)-(*2→6*)-*2-acetamido-2-deoxy*-α-D-*galactopyranosyl*]-(*1→3*)-L-*seryl*-O-[O-(*5-acetamido-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid*)-(*2→6*)-*2-acetamido-2-deoxy*-α-D-*galactopyranosyl*]-(*1→3*)-L-*seryl*-L-*valine* (**2**). — Compound **32** (9.0 mg, 2.5 μmol) was hydrogenolysed and the crude product was purified, as described for the preparation of **1**, to afford **2** (3.5 mg), $[\alpha]_D^{25}$ +70° (*c* 0.1, water). ^{1}H-N.m.r. data (D_2O, 20°): δ 0.89 (d, 3 H, *J* 7.0 Hz, Val Me), 0.90 (d, 3 H, *J* 6.7 Hz, Val Me), 1.69 (m, 3 H, NeuAc H-3α), 2.02 (s, 9 H, 3 Ac), 2.04 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.74 (m, 3 H, NeuAc H-3β), 4.41 (b, 1 H, Ser α-H); (D_2O, 60°), δ 4.68 (bt, 1 H, Ser α-H), 4.74 (b, 1 H, Ser α-H), 4.88 (d, 1 H, *J* 3.1 Hz, GalNAc H-1), 4.89 (d, 1 H, *J* 3.4 Hz, GalNAc H-1), 4.92 (d, 1 H, *J* 3.4 Hz, GalNAc H-1). F.a.b.-mass spectrum (positive ion): *m/z* 1861 (M^+ + 1), 1883 (M^+ + Na).

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