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Syntheses of α-dystroglycan derived glycosyl amino acids carrying a novel mannosyl serine/threonine linkage

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α-Dystroglycan (α-DG) is a membrane-associated, extracellular glycoprotein. It is anchored to the cell-membrane by binding to the transmembrane glycoprotein β-dystroglycan (β-DG) to form an α/β-DG-complex. It was discovered that the bovine peripheral nerve α-DG possesses the Ser/Thr linked tetrasaccharide as the major constituent of the O-linked carbohydrates, which was proposed to contribute laminin binding activity of this glycoprotein.

This structure has a striking feature in terms of the mode of linkage between oligosaccharide and the core protein. It has a mannose residue linked to the core protein through Ser/Thr residue. A similar structure was proposed to exist in brain derived HNK-1 immunoreactive O-glycans. Being interested in the structural novelty and potential biological significance of this type of glycan chains, the chemical synthesis of Ser/Thr linked mannose containing tetrasaccharide was investigated. Tetrasaccharide donor was constructed from monosaccharide blocks and coupled with Ser/Thr derivatives. Subsequent deprotection afforded target tetraosyl serine. Furthermore, synthetic routes to lower homologues, namely Gal-β-(1,4)-GlcNAc-β-(1,2)-Man-α-Ser and GlcNAc-β-(1,2)-Man-α-Ser were also provided.

Keywords: **amino acids and derivatives, glycopeptides, glycoproteins, glycosylation**

Introduction

α-Dystroglycan (α-DG) is a membrane-associated, extracellular glycoprotein. It is anchored to the cell-membrane by binding to the transmembrane glycoprotein β-dystroglycan (β-DG) to form an α/β-DG-complex. This complex is widely expressed in various types of tissues including skeletal muscle [1] and Schwannoma cells [2]. It serves as a transmembrane linker between the extracellular matrix and the intracellular skeleton as α-DG binds with high affinity to extracellular matrix components like laminin in the striated muscle whereas the intracellular domain of β-DG binds to cytoskeletal proteins like dystrophin [3]. Disruption of this transmembrane linkage leads to skeletal muscular dystrophies [4].

Endo and coworkers discovered that the bovine peripheral nerve α-DG possesses the Ser/Thr linked tetrasaccharide **1a**/**b** as the major constituent of the O-linked carbohydrates, which was proposed to contribute laminin binding activity of this glycoprotein [1]. This structure has a striking feature in terms of the mode of linkage between oligosaccharide and the core protein. Unlike conventional O-glycans which are linked through α-GalNAc residue, it has a mannose residue linked to the core protein through Ser/Thr residue [5]. A similar structure was proposed to exist in brain derived HNK-1 immunoreactive O-glycans [6]. Being interested in the structural novelty and potential biological significance of this type of glycan chains, the chemical synthesis of Ser/Thr linked tetrasaccharide **1a** was investigated, preliminary account of which was reported previously [7].

Since Fmoc/*t*-Bu (or allyl) protected **20a**/**c** and **20d** were assumed as the immediate precursors of **1a** and **1b**, respectively, it was expected that the synthetic access to glycopeptide sequences of α-DG can be provided based on standard Fmoc-based peptide synthesis protocol. Furthermore, in order to provide molecular probes for detailed biological studies, di-(βGlcNAcl → 2αMan) and trisaccharide (βGall → 4βGlcNAcl → 2αMan) linked to Ser (i.e. **37** and **29**) were also prepared.

The presence of O-glycan structure linked to Ser/Thr via mannose residue has also been discovered in yeast [8]. The anomeric configuration of the O-linked mannose of these molecules was confirmed to be α. Therefore, the mannose

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residue of α-DG derived glycans was tentatively presumed to be α-configured in our synthetic study, although no definitive proof has been provided yet.

Materials and methods

General methods

Starting materials and reagents were purchased from standard vendors and used without purification unless otherwise noted. All reactions sensitive to air and/or moisture were carried out under nitrogen or argon atmosphere with anhydrous solvents. Analytical and preparative thin layer chromatography (0.25 mm and 0.5 mm thickness, respectively) were developed on silica gel 60 F_{256} plates (Merck, Darmstadt, Germany). Silica gel column chromatography was performed on E. Merck silica gel 60 (60–230 mesh or 230–400 mesh, Merck, Darmstadt, Germany). Bio-Gel P-2 used for size exclusion chromatography was obtained from Bio-Rad (Hercules, California, USA). NMR spectra were obtained on a JEOL EX-270, EX-400, α-500, and/or α-600 spectrometer (^{1}H at 270, 400, 500, and/or 600 MHz, and ^{13}C at 67.5, 100, 125, and/or 150 MHz) at ambient temperature unless otherwise noted. *m*-Nitrobenzyl alcohol (NBA) was used as a matrix for FAB-MS aquisition.

Thexyldimetylsilyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranoside (**5**)

A mixture of compounds **3a** (1.50 g, 3.63 mmol) and **2** (3.10 g, 5.94 mmol) and molecular sieves 4 Å (4.0 g) in dry acetonitrile (30 ml) was stirred under argon at −40°C. Then, a solution of *N*-iodosuccinimide (NIS, 2.67 g, 11.3 mmol) in dry acetonitrile (20 ml) was added and stirring continued for additional 30 min, which was followed by the addition of trifluoromethanesulfonic acid (TfOH, 100 µl, 1.12 mmol). After being stirred at the same temperature for 3 h, the reaction was quenched with triethylamine (170 µl, 1.21 mmol), diluted with CH_2Cl_2, filtered through Celite and evaporated *in vacuo*. The residue was dissolved in chloroform, washed with a 10% $KHCO_3/Na_2S_2O_3$ solution (2 × 40 ml), dried over $MgSO_4$, and the volatiles removed *in vacuo* to afford crude **4** as a brown foam (4.24 g); $R_f = 0.30$ ($CHCl_3$/MeOH, 18/1).

The crude **4** was dissolved in pyridine/acetic anhydride (2/1, 90 ml) at 0°C and stirred for 12 h at ambient temperature. Resulting mixture was evaporated and coevaporated with toluene (3 × 50 ml). The residue was dissolved in CH_2Cl_2 and washed successively with 10% HCl solution (3 × 50 ml), 1 M $KHCO_3$ (2 × 50 ml) and H_2O. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to furnish a yellow foam (4.96 g) which was purified by silica gel column chromatography (toluene/acetone, 3/1) to afford 2.04 g (57%) of the title compound; $R_f = 0.50$ ($CHCl_3$/MeOH, 18/1); $[\alpha]_D^{25} = -12.3$ ($CHCl_3$, 1.0); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.31–7.25 (m, 5 H, Ar), 5.53 (ddd, 1 H, $J_{7,8} = 8.8$ Hz, $J_{8,9b} = 5.9$ Hz, $J_{8,9a} = 2.7$ Hz, H-8^{SA}), 5.34 (dd, 1 H, $J_{6,7} = 2.8$ Hz, H-7^{SA}), 5.05 (d, 1 H, $J_{NH,5} = 10.3$ Hz, NH^{SA}), 4.96–4.93 (m, 2 H, H-2^{Gal}, H-4^{Gal}), 4.85 (ddd, $J_{3a,4} = 12.1$ Hz, $J_{3e,4} = 4.6$ Hz, $J_{4,5} = 10.3$ Hz, H-4^{SA}), 4.83 (d, 1 H, $J_{1,2} = 7.7$ Hz, H-1^{Gal}), 4.51 (d, 1 H, $J_{gem} = 11.8$ Hz, benzylic), 4.47 (dd, $J_{2,3} = 10.2$ Hz, $J_{3,4} = 3.4$ Hz, H-3^{Gal}), 4.42 (d, 1 H, $J_{gem} = 11.8$ Hz, benzylic), 4.33 (dd, $J_{gem} = 12.4$ Hz, H-9a^{SA}), 4.04 (ddd, 1 H, $J_{4,5} \sim J_{5,6} \sim J_{5,N} = 10.5$ Hz, H-5^{SA}), 4.00 (dd, 1 H, H-9b^{SA}), 3.82 (s, 3 H, OMe), 3.81–3.78 (m, 1 H, H-5^{Gal}), 3.62 (dd, 1 H, H-6^{SA}), 3.48–3.42 (m, 2 H, H-6ab^{Gal}), 2.56 (dd, 1 H, H-3eq^{SA}), 2.16, 2.13, 2.05, 2.03, 2.01, 1.98, 1.83 (7 s, 21 H, NAc, OAc), 1.71 (dd, $J_{gem} \sim J_{3a,4} = 12.4$ Hz, H-3ax^{SA}), 1.59 (m, 1 H, CH), 0.83–0.87 (m, 12 H, CMe_2), 0.15, 0.16 (2 s, 6 H, $SiMe_2$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.9, 170.5, 170.4, 170.3, 170.2, 169.7, 169.6, 167.9, 138.0, 129.1, 128.3, 127.6, 96.9 (C-1^{Gal}), 95.7 (C-2^{SA}), 73.4 (CH_2), 72.1 (C-5^1), 72.0 (C-6^{SA}, 71.8 (C-2^{Gal}), 71.8 (C-3^{Gal}), 69.4 (C-4^{SA}), 68.5 (C-6^{Gal}), 68.4 (C-4^{Gal}), 67.9 (C-8^{SA}), 67.2 (C-7^{SA}), 62.4 (C-9^{SA}), 53.1 (OMe), 49.1 (C-5^{SA}), 37.5 (C-3^{SA}), 33.9 (*C*HMe_2), 24.8 (NAc), 23.2, 21.4, 21.1, 20.9, 20.8, 20.7 (OAc), 20.0, 19.9, 18.5, 18.5 (*CMe*$_2$), −2.0, −3.3 (SiMe).

FAB-MS (pos. mode, NBA): $m/z = 1008.1$ $[M + Na]^+$; $C_{46}H_{71}NaNO_{20}Si$ requires 1008.4.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3(-2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl trichloroacetimidate (**7**)

A solution of disaccharide **5** (305 mg, 0.309 mmol) in 50 ml dry THF containing 0.30 ml (5.2 mmol) of acetic acid was reacted with tetrabutylammonium fluoride (TBAF, 1 M in THF, 3 ml, 3 mmol). After stirring for 3 d at room temperature, the mixture was concentrated and coevaporated with toluene (×3). The residue was purified by silica gel column chromatography (toluene/acetone, 3/1) to give 236 mg (92%) of **6** as an amorphous; $R_f = 0.39/0.45$ ($CHCl_3$/MeOH, 18/1).

Compound **6** (200 mg, 0.242 mmol) was dissolved in 6.5 ml of dry CH_2Cl_2 and trichloroacetonitrile (0.3 ml, 3 mmol) was added. Under ice-water cooling, the reaction was started by the addition of 4 µl (0.03 mmol) of 1,8-diazabicyclo[5.4.0]uncec-7-ene (DBU) and stirred for 4 h at the same temperature. The mixture was subjected to a column of silica gel (hexane/ethyl acetate, 1/2 → ethyl acetate) to give 222.3 mg (93%) of **7** as a white amorphous; $R_f = 0.53$ ($CHCl_3$/MeOH, 18/1); $[\alpha]_D^{25} = -10.0$ ($CHCl_3$, 0.6); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 8.60 (s, 1 H, C = N*H*), 7.23 (m, 5 H, Ar), 5.87 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1^{Gal}), 5.49 (m, 1 H, H-8^{SA}), 5.28 (m, 1 H, H-7^{SA}), 5.23 (dd, 1 H, $J_{2,3} = 10.2$ Hz, H-2^{Gal}), 5.04 (m, 1 H, H-4^{Gal}), 5.02 (d, 1 H, $J_{NH,5} = 9.9$ Hz, NH^{SA}), 4.83 (m, 1 H, H-4^{SA}), 4.65 (dd, 1 H, $J_{3,4} = 3.1$ Hz, H-3^{Gal}), 4.45 (2 d, 2 H, $J_{gem} = 11.9$ Hz, OCH_2), 4.36 (m, 1 H, H-9a^{SA}), 4.06–3.87 (m, 3 H, H-5^{Gal}, H-5^{SA}, H-9b^{SA}), 3.81 (s, 3 H, OMe), 3.60–3.37 (m, 3 H, H-6^{SA}, H-6ab^{Gal}), 2.52 (dd, $J_{3,4} = 4.3$ Hz, $J_{gem} = 12.5$ Hz, H-3eq^{SA}), 2.11, 2.10, 2.01, 1.97, 1.94 (6s, 18 H, OAc), 1.79 (s, 3 H, NAc) 1.67 (dd, $J_{gem} \sim J_{3a,4} = 12.5$ Hz, H-3$^{SA}_{ax}$); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 170.9, 170.6, 170.5,

170.3, 170.2, 169.7, 169.4, 167.8, 137.8, 129.0, 128.3, 127.8, 96.9 (C-1^{Gal}), 95.7 (C-2^{SA}), 73.4 (CH_2), 72.1 (C-5^{Gal}), 72.0 (C-6^{SA}), 71.8 (C-2^{1}), 71.8 (C-3^{1}), 69.4 (C-4^{SA}), 68.5 (C-6^{Gal}), 68.4 (C-4^{Gal}), 67.9 (C-8^{SA}), 67.2 (C-7^{SA}), 62.4 (C-9^{SA}), 53.1 (OMe), 49.1 (C-5^{SA}), 37.5 (C-3^{SA}), 33.9 (*C*HMe_2), 24.8 (NAc), 23.2, 21.4, 21.1, 20.9, 20.8, 20.7 (OAc).

FAB-MS (pos. mode, NBA): $m/z = 1009.6$ $[M + Na]^+$; $C_{40}H_{53}Cl_3NaN_2O_{20}$ requires 1009.2.

Benzyl O-(4-O-acetyl-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**10**)

A solution of compound **9a** (1.00 g, 1.85 mmol) and thioglycoside **8** (1.38 g, 2.40 mmol) in dry acetonitrile (15 ml) was stirred under argon with freshly activated molecular sieves 3 Å (2.0 g) at −40°C. Then, a solution of *N*-iodosuccinimide (NIS, 1.25 g, 5.28 mmol) in dry acetonitrile (10 ml) was added followed by trifluoromethanesulfonic acid (TfOH, 47 µl, 0.53 mmol). After being stirred at −40°C for 1 h, the reaction was quenched with triethylamine (84 µl, 0.60 mmol) and diluted with CH_2Cl_2, filtered through Celite and evaporated. The residue was diluted with chloroform, washed with 10% $KHCO_3/Na_2S_2O_3$ solution (2 × 40 ml), dried over $MgSO_4$ and evaporated *in vacuo* to afford a brown foam. Purification by silica gel column chromatography (toluene/ethyl acetate, 5/1) gave **10** (1.86 g, 95%) as a white foam; $R_f = 0.63$ (toluene/ethyl acetate, 4/1); $[\alpha]_D^{25} = 9.6$ ($CHCl_3$, 2.4); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.57–6.80 (m, 34 H), 5.38 (d, 1 H, $J_{1,2} = 8.1$ Hz, H-1^{GlcN}), 5.24 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 9.4$ Hz, H-4^{GlcN}), 4.90 (d, $J_{gem} = 10.9$ Hz, OCH_2), 4.87 (d, 1 H, $J_{gem} = 11.5$ Hz, OCH_2), 4.71 (d, 1 H, $J_{1,2} = 1.7$ Hz, H-1^{Man}), 4.61 (d, 1 H, $J_{gem} = 11.5$ Hz, OCH_2), 4.58 (d, 1 H, $J_{gem} = 11.8$ Hz, OCH_2), 4.57 (m, 2 H, H-2^{GlcN}, H-3^{GlcN}), 4.55 (d, 1 H, $J_{gem} = 11.9$ Hz, OCH_2), 4.52 (d, 1 H, $J_{gem} = 11.9$ Hz, OCH_2), 4.25 (m, 1 H, H-2^{Man}), 4.19 (2 d, $J_{gem} = 12.2$ Hz, OCH_2), 3.94 (dd, 1 H, $J_{2,3} = 3.2$ Hz, $J_{3,4} = 8.3$ Hz, H-3^{Man}), 3.88 (m, 1 H, H-5^{GlcN}), 3.80–3.50 (m, 5 H, H-6ab^{GlcN}, H-5^{Man}, H-4^{Man}, H-6a^{Man}), 3.11 (dd, 1 H, $J_{gem} = 11.0$ Hz, $J_{5,6} = 6.2$ Hz, H-6b^{Man}), 2.06 (s, 3 H, OAc); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 169.7 [C=O (OAc)], 138.4, 138.4, 138.2, 137.7, 137.0, 133.6, 131.6, 128.6, 128.3, 128.3, 128.2, 128.1, 128.0, 127.8, 127.8, 127.7, 127.5, 127.4, 127.4, 123.1, 96.8 (C-1^{GlcN}), 96.5 ($J_{C,H} = 171.9$ Hz, C-1^{Man}), 77.5 (C-3^{Man}), 76.7 (C-3^{GlcN}), 74.9 (CH_2), 74.6 (C-4^{Man}), 73.6 (C-2^{Man}), 73.4 (C-5^{GlcN}), 72.8 (CH_2), 72.5 (C-4^{GlcN}), 71.9 (C-5^{Man}), 70.8 (CH_2), 70.2 (C-6^{GlcN}), 69.9 (C-6^{Man}), 69.0 (CH_2), 55.3 (C-2^{GlcN}), 20.9 (OAc).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 1076.0$ $[M + Na]^+$; $C_{64}H_{63}NaNO_{13}$ requires 1076.4.

*Benzyl O-(2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**12**)*

*Preparation of amine **11**.* A solution of disaccharide **10** (1.84 g, 1.75 mmol) in 30 ml of *n*-butanol containing 7.5 ml of ethylenediamine was stirred for 20 h at 100°C. Subsequent evaporation *in vacuo* and coevaporation with toluene (× 3) gave a yellow oil which was passed through a column of silica gel ($CHCl_3$/MeOH, 25/1) to furnish 1.47 g (95.5%) of **11** as a yellow syrup: $R_f = 0.35$ (hexane/acetone, 1.2/1).

Preparation of a TfN_3 solution. NaN_3 (8.00 g, 123 mmol) was dissolved at room temperature in H_2O (20 ml) and 25 ml of CH_2Cl_2 was added. The biphasic mixture was vigorously stirred for 30 min at 0°C and trifluoromethanesulfonic anhydride (Tf_2O, 4.1 ml, 25 mmol) was added over 30 min. The mixture was stirred for 2 h at 0°C, layers separated and the aqueous layer extracted with CH_2Cl_2 (2 × 10 ml). The combined organic layers were washed with sat. aq. $NaHCO_3$ solution (20 ml) and H_2O (20 ml), successively, dried ($MgSO_4$) and filtered. The solution, which was assumed to be 0.53 M, was stored at 4°C over molecular sieves 4 Å.

*Conversion of **11** to **12**.* To a solution of **11** (1.45 g, 1.64 mmol) and 4-(dimethylamino)pyridine (DMAP, 960 mg, 7.85 mmol) in dry acetonitrile (10 ml) was added a solution of TfN_3 in CH_2Cl_2 (15 ml, ca. 8 mmol) over 10 min at 0°C. After being stirred for 24 h at room temperature, the mixture was diluted with CH_2Cl_2 and successively washed with 1 N $KHCO_3$ (25 ml), 1 N HCl (25 ml) and 1 N $KHCO_3$ (25 ml), dried over $MgSO_4$ and evaporated *in vacuo*. The dark yellow syrup was purified by silica gel column chromatography (hexane/acetone, 3/1) to afford 1.41 g (95%) of **12** as a colorless syrup; R_f 0.63 (toluene/ethyl acetate, 4/1); $[\alpha]_D^{25} = -3.8$ ($CHCl_3$, 1.0); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.33–7.05 (m, 30 H, Ar), 5.01 (d, 1 H, $J_{1,2} = 1.3$ Hz, H-1^{Man}), 4.90–4.66 (m, 5 H, OCH_2), 4.52–4.33 (m, 7 H, OCH_2), 4.28 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1^{GlcN}), 4.20 (dd, 1 H, $J_{2,3} = 4.0$ Hz, H-2^{Man}), 3.90 (dd, 1 H, $J_{3,4} = 8.4$ Hz, H-3^{Man}), 3.86–3.45 (m, 8 H, H-5^{Man}, H-4^{Man}, H-6ab^{Man}, H-6ab^{GlcN}, H-4^{GlcN}, H-2^{GlcN}), 3.36 (m, 1 H, H-5^{GlcN}), 3.13 (dd, 1 H, $J_{2,3} = 9.7$ Hz, $J_{3,4} = 8.7$ Hz, H-3^{GlcN}), 2.55 (d, 1 H, $J_{OH,4} = 1.8$ Hz, 4-OH^{GlcN}); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 138.4, 138.3, 138.1, 137.5, 137.2, 128.6, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 127.8, 127.8, 127.7, 127.6, 127.6, 127.4, 100.9 (C-1^{GlcN}), 96.7 ($J_{C,H} = 173.2$ Hz, C-1^{Man}), 82.2 (C-3^{GlcN}), 78.1 (C-3^{Man}), 75.1, 74.9 (CH_2), 74.8 (C-4^{Man}), 74.2 (C-5^{GlcN}), 73.8 (C-2^{Man}), 73.7, 73.1 (CH_2), 72.0 (C-5^{Man}), 71.8 (C-4^{GlcN}), 71.2 (CH_2), 70.6 (CH_2), 70.3 (C-6^{GlcN}), 69.6 (C-6^{Man}), 69.2 (CH_2), 65.5 (C-2^{GlcN}).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 929.8$ $[M + Na]^+$; $C_{54}H_{57}NaN_3O_{20}$ requires 930.4.

Benzyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-2 → 3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**13**)

A solution of compound **12** (500 mg, 0.55 mmol) and trichloroacetimidate **7** (642 mg, 0.66 mmol) in 30 ml dry CH_2Cl_2 was stirred under argon with freshly activated

molecular sieves 4 Å (1.5 g) at −20°C for 1 h. Then, TMSOTf (19 µl, 0.10 mmol) was added and stirring continued for 1 h. The reaction was quenched with triethylamine (8 µl), diluted with CH_2Cl_2 and filtered through a pad of Celite. Filter cake was thoroughly washed with CH_2Cl_2 and the combined filtrate and washings were washed with 1 N $KHCO_3$ and water, successively, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (toluene/acetone, 3/1) and the title compound (798 mg, 84%) was isolated as a white foam; $R_f = 0.36$ ($CHCl_3$/MeOH, 18/1); $[\alpha]_D^{25} = -7.0$ ($CHCl_3$, 0.56); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.34–6.99 (m, 35 H, Ar), 5.52 (m, 1 H, H-8^{SA}), 5.27 (dd, 1 H, $J_{6,7} = 2.7$ Hz, $J_{7,8} = 9.0$ Hz, H-7^{SA}), 5.07 (d, 1 H, $J_{NH,5} = 10.3$ Hz, NH^{SA}), 5.01 (d, 1 H, $J_{1,2} = 1.3$ Hz, H-1^{Man}), 4.95–4.65 (m, 9 H, H-2^{Gal}, H-4^{Gal}, H-1^{Gal}, H-4^{SA}, OCH_2), 4.51–4.21 (m, 12 H, H-3^{Gal}, H-1^{GlcN}, H-2^{Man}, H-9a^{SA}, OCH_2), 4.11–3.79 (m, 6 H, OCH_2, H-5^{SA}, H-3^{Man}, H-4^{GlcN}, H-5^{Man}, H-9b^{SA}), 3.76 (s, 3 H, OMe), 3.74–3.60 (m, 6 H, H-6ab^{Gal}, H-4^{Man}, H-6ab^{Man}, H-5^{Gal}), 3.55 (dd, 1 H, $J_{5,6} = 10.6$ Hz, H-6^{SA}), 3.48–3.24 (m, 5 H, H-2^{GlcN}, H-5^{GlcN}, H-3^{GlcN}, H-6ab^{Gal}), 2.51 (dd, 1 H, $J_{vic} = 4.6$ Hz, $J_{gem} = 13.6$ Hz, H-3eq^{SA}), 2.09, 2.08, 2.03, 1.98, 1.94, 1.93, 1.87, 1.77 (8 s, 24 H, NAc, OAc), 1.64 (dd, 1 H, $J_{vic} = 12.5$ Hz, H-3ax^{SA}); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 170.8, 170.4, 170.4, 170.2, 169.7, 169.6, 167.7, 138.6, 138.4, 138.3, 138.2, 137.9, 137.2, 128.2, 128.0, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3, 127.2, 100.5 ($J_{C,H} = 162.5$ Hz, C-1^{GlcN} or C-1^{Gal}), 100.3 ($J_{C,H} = 161.9$ Hz, C-1^{Gal} or C-1^{GlcN}), 96.9 (C-2^{SA}), 96.6 ($J_{C,H} = 172.6$ Hz, C-1^{Man}), 81.3 (C-3^{GlcN}), 77.9 (C-3^{Man}), 76.3 (C-4^{GlcN}), 75.1 (CH_2), 75.0 (C-5^{GlcN}), 74.8 (CH_2), 74.7 (C-6^{GlcN}), 73.4 (C-2^{Man}), 73.2 (CH_2), 73.1 (CH_2), 72.0 (C-6^{SA}), 71.9 (C-5^{Man}), 71.8 (C-3^{Gal}), 71.6 (C-4^{Man}), 71.1 (C-2^{Gal}), 70.7 (CH_2), 69.5 (C-6^{Man}), 69.3 (C-4^{SA}), 69.1 (C-5^{Gal}), 67.7 (C-4^{Gal}), 67.6 (C-8^{SA}), 67.3 (C-6^{Gal}), 67.1 (C-7^{SA}), 65.8 (C-2^{GlcN}), 62.4 (C-9^{SA}), 53.1 (OMe), 49.0 (C-5^{SA}), 37.5 (C-3^{SA}), 23.3, 23.1 (NAc), 21.3, 21.0, 20.7, 20.7 (OAc).

FAB-MS (pos. mode, NBA): $m/z = 1741.2$ $[M + Na]^+$; average mass for $C_{91}H_{104}NaN_4O_{29}$: 1740.8.

Benzyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4-di-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**14**)

Compound **13** (500 mg, 0.291 mmol) was dissolved in a mixture of pyridine (1.75 ml) and water (0.75 ml) and subsequently reacted with propanedithiol (2.5 ml) and diisopropylethylamine (0.5 ml). After being stirred for 14 h at room temperature, the reaction was judged to be complete; $R_f = 0.68$ ($CHCl_3$/MeOH, 18/1). Acetic anhydride (10 ml) was added and stirring continued for 8 h. The reaction mixture was evaporated under high vacuum and coevaporated with toluene (×3). The residue was purified by silica gel column chromatography (toluene/acetone, 3/1) to afford 495 mg (95%) of **14**; $R_f = 0.71$ ($CHCl_3$/MeOH, 18/1); $[\alpha]_D^{25} = -0.6$ ($CHCl_3$, 0.84); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.37–7.18 (m, 35 H, Ar), 5.81 (d, 1 H, $J_{NH,2} = 7.1$ Hz, NH^{GlcN}), 5.58 (m, 1 H, H-8^{SA}), 5.35 (dd, 1 H, $J_{6,7} = 2.6$ Hz, $J_{7,8} = 9.0$ Hz, H-7^{SA}), 5.20 (d, 1 H, $J_{NH,5} = 10.2$ Hz, NH^{SA}), 5.06 (d, 1 H, $J_{1,2} = 7.3$ Hz, H-1^{GlcN}), 5.02–4.97 (m, 2 H, H-4^{Gal}, H-2^{Gal}), 4.94–4.87 (m, H-4^{SA}, H-1^{Man}, OCH_2), 4.85 (d, 1 H, $J_{1,2} = 7.0$ Hz, H-1^{Gal}), 4.77 (d, 1 H, $J_{gem} = 11.4$ Hz, OCH_2), 4.68 (d, 1 H, $J_{gem} = 11.7$ Hz, OCH_2), 4.59–4.31 (m, 12 H, H-3^{GlcN},H-3^{Gal}, H-9a^{SA}, OCH_2), 4.22 (d, 1 H, $J_{gem} = 11.9$ Hz, OCH_2), 4.19 (m, 1 H, H-2^{Man}), 4.11–3.59 (m, 16 H, H-6ab^{GlcN}, H-6ab^{Man}, H-5^{Gal}, H-5^{GlcN}, H-5^{Man}, H-6^{SA}, H-9b^{SA}, H-4^{GlcN}, H-4^{Man}, H-3^{Man}, H-5^{SA}, OMe), 3.31–3.20 (m, 3 H, H-6ab^{Gal}, H-2^{GlcN}), 2.50 (dd, 1 H, $J_{vic} = 4.6$ Hz, $J_{gem} = 12.6$ Hz, H-3eq^{SA}), 2.17, 2.10, 2.05, 2.01, 2.00, 1.95, 1.85, 1.73 (8 s, 24 H, NAc, OAc), 1.73 (m, 1 H, H-3ax^{SA}); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 171.2, 170.9, 170.4, 170.4, 170.2, 170.1, 169.9, 169.6, 167.8, 139.2, 138.5, 138.4, 138.3, 138.1, 137.9, 137.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.0, 128.0, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 100.0 (C-1^{Gal}), 97.7 (C-1^{GlcN}), 97.4 ($J_{C,H} = 171.3$ Hz, C-1^{Man}), 96.9 (C-2^{SA}), 78.0 (C-3^{Man}), 77.6 (C-3^{GlcN}), 76.6 (C-4^{GlcN}), 75.0 (CH_2), 74.7 (C-6^{Man}), 74.4 (C-6^{GlcN}), 73.7, 73.2, 73.1 (C-2^{Man}), 72.0 (C-5^{Man}), 71.7 (C-4^{Man}, C-3^{Gal}, C-5^{GlcN}, C-5^{Gal}), 71.0 (CH_2, C-2^{Gal}), 69.5 (CH_2), 69.3 (C-4^{SA}), 69.2 (C-6^{SA}), 67.8 (C-4^{Gal}), 67.7 (C-8^{SA}), 67.5 (C-6^{Gal}), 67.2 (C-7^{SA}), 62.4 (C-9^{SA}9, 56.6 (C-2^{GlcN}), 53.1 (OMe), 49.0 (C-5^{SA}), 37.5 (C-3^{SA}), 23.3, 23.1 (NAc), 21.3, 20.9, 20.7, 20.6 (OAc).

FAB-MS (pos. mode, NBA): $m/z = 1757.4$ $[M + Na]^+$; average mass for $C_{93}H_{108}NaN_2O_{30}$: 1756.9.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl acetate (**16**)

A solution of **14** (680 mg, 0.392 mmol) in 50 ml dry methanol and 5 ml acetic acid was hydrogenated over $Pd(OH)_2$/C (100 mg) under atmospheric pressure of H_2 for 16 h. The catalyst was removed by filtration through a Celite pad and the filter cake thoroughly washed with MeOH/HOAc (9:1). The combined filtrate and washings were concentrated *in vacuo*, coevaporated with toluene (3 × 20 ml) and dried under high vacuum. Resultant crude **15** (458 mg); $R_f = 0.62$ (*n*-BuOH/MeOH/H_2O/HOAc, 6/2/2/1) was dissolved in pyridine/acetic anhydride (2/1, 30 ml) at 0°C and stirred for 12 h. Subsequent evaporation and coevaporation with toluene (3 × 50 ml) *in vacuo* led to a brown foam which was dissolved in CH_2Cl_2 and washed successively with 10% HCl (3 × 50 ml), 1 N $KHCO_3$ (2 × 50 ml) and H_2O. The organic layer was dried over $MgSO_4$, and volatiles were removed by evaporation. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 30/1) to afford 528 mg (95%, $\alpha/\beta = 9/1$) of compound **16**; $R_f = 0.33$ ($CHCl_3$/MeOH, 18/1); $[\alpha]_D^{25} = -8.8$

($CHCl_3$, 0.35); ^{1}H-NMR (400 MHz, $CDCl_3$): δ (major isomer) 5.97 (d, 1 H, $J_{1,2} = 1.8$ Hz, H-1^{Man}), 5.89 (d, 1 H, $J_{NH,2} = 9.6$ Hz, NH^{GlcN}), 5.49 (ddd, 1 H, $J_{8,9a} = 2.7$ Hz, $J_{8,9b} = 5.6$ Hz, H-8^{SA}), 5.33 (dd, 1 H, $J_{6,7} = 2.7$ Hz, $J_{7,8} = 9.3$ Hz, H-7^{SA}), 5.28 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 10.1$ Hz, H-4^{Man}), 5.23 (d, 1 H, $J_{NH,5} = 10.2$ Hz, NH^{SA}), 5.19 (dd, 1 H, $J_{2,3} \sim J_{3,4} = 10.5$ Hz, H-3^{Gal}), 4.98 (dd, 1 H, $J_{2,3} = 1.4$ Hz, $J_{3,4} = 10.1$ Hz, H-3^{Man}), 4.86–4.80 (m, 3 H, H-2^{Gal}, H-4^{SA}, H-4^{Gal}), 4.62 (d, 1 H, $J_{1,2} = 7.9$ Hz, H-1^{Gal}), 4.46 (dd, 1 H, $J_{2,3} = 10.2$ Hz, $J_{3,4} = 3.3$ Hz, H-3^{Gal}), 4.41 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1^{GlcN}), 4.37 (dd, 1 H, $J_{gem} = 12.6$ Hz, H-9a^{SA}), 4.31 (m, 1 H, H-6a^{GlcN}), 4.17–3.88 (m, 10 H, H-6a^{Man}, H-6b^{GlcN}, H-2^{Man}, H-2^{GlcN}, H-6b^{Man}, H-6ab^{Gal}, H-5^{SA}, H-9b^{SA}, H-5^{Man}), 3.81–3.75 (m, 5 H, H-4^{GlcN}, H-5^{Gal}, OMe), 3.59 (dd, 1 H, $J_{5,6} = 11.1$ Hz, $J_{6,7} = 2.7$ Hz, H-6^{SA}), 3.53 (m, 1 H, H-5^{GlcN}), 2.52 (dd, 1 H, $J_{gem} = 12.6$ Hz, $J_{vic} = 4.6$ Hz, H-3eq^{SA}), 2.18, 2.10, 2.09, 2.06, 2.05, 2.03, 2.02, 2.01, 2.00, 1.99, 1.98, 1.95, 1.94 (13 s, 39 H, OAc), 1.87, 1.80 (2 s, 6 H, NAc), 1.61 (dd, 1 H, $J_{vic} = 12.5$ Hz, H-3ax^{SA}); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.7, 170.5, 170.4, 170.3, 170.3, 170.2, 170.2, 170.0, 170.0, 169.8, 169.8, 169.3, 169.3, 168.7, 168.6, 167.6, 100.7 ($J_{C,H} = 161.7$ Hz, C-1^{Gal}), 100.5 ($J_{C,H} = 160.9$ Hz, C-1^2), 96.7 (C-2^{SA}), 90.9 ($J_{C,H} = 175.0$ Hz, C-1^{Man}), 75.9 (C-4^{GlcN}), 73.3 (C-2^{Man}), 73.0 (C-3^{GlcN}), 72.7 (C-5^{GlcN}), 72.0 (C-6^{SA}), 71.2 (C-3^{Gal}), 70.9 (C-5^{Man}), 70.5 (C-5^{Gal}), 69.8 (C-2^{Gal}), 69.7 (C-3^{Man}), 69.3 (C-4^{SA}), 67.7 (C-8^{SA}), 67.3 (C-4^{Gal}), 67.0 (C-7^{SA}), 65.1 (C-4^{Man}), 62.5 (C-6^{GlcN}), 62.3 (C-9^{SA}), 61.9 (C-6^{Man}), 61.5 (C-6^{Gal}), 53.3 (C-2^{GlcN}), 53.1 (OMe), 49.0 (C-5^{SA}), 37.4 (C-3^{SA}), 23.2, 23.2 (NAc), 21.6, 21.1, 21.0, 20.9, 20.9, 20.7, 20.7 (OAc).

FAB-MS (pos. mode, NBA): $m/z = 1420.7$ $[M + Na]^+$; average mass for $C_{58}H_{80}NaN_2O_{37}$: 1420.3.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**18**)

A solution of **16** (380 mg, 0.272 mmol) in dry DMF (5 ml) was stirred at 0°C. Hydrazine acetate (44 mg, 0.48 mmol) was added and the stirring continued at 0°C for 6 h. The reaction was quenched with acetic acid (40 µl) and evaporated under high vacuum. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH, 20/1) to afford 350 mg (95%) of **17**; $R_f = 0.38$ ($CHCl_3$/MeOH, 9/1), which was used for the subsequent reaction without extensive characterization.

A solution of **17** (405 mg, 0.30 mmol) in dry CH_2Cl_2 (7 ml) containing 0.35 ml (3.5 mmol) of trichloroacetonitrile was stirred at 0°C. 1,8-Diazabicyclo[5.4.0]uncec-7-ene (DBU, 5 µl 0.04 mmol) was added and the mixture stirred for 3 h at 0°C. The reaction mixture was passed through a column of silica gel (ethyl acetate → acetone) to give 421 mg (94%, α:β = 9:1) of compound **18** as a white amorphous: $R_f = 0.48$ ($CHCl_3$/MeOH, 9/1); $[\alpha]_D^{25} = -10.4$ ($CHCl_3$, 0.73); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.69 (s, 1 H, C = NH), 6.18 (d, 1 H, $J_{NH,2} = 9.6$ Hz, (NH^{GlcN}), 6.14 (d, 1 H, $J_{1,2} < 1.5$ Hz, H-1^{Man}), 5.46 (m, 1 H, H-8^{SA}), 5.33 (d, 1 H, $J_{NH,5} = 10.2$ Hz, NH^{SA}), 5.32 (dd, 1 H, $J_{6,7} = 2.4$ Hz, $J_{7,8} = 8.8$ Hz, H-7^{SA}), 5.32 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 10.3$ Hz, H-4^{Man}), 5.03 (dd, 1 H, $J_{2,3} \sim J_{3,4} = 9.5$ Hz, H-3^{GlcN}), 5.02 (dd, 1 H, $J_{2,3} \sim J_{3,4} = 9.5$ Hz, H-3^{Man}), 4.98 (dd, 1 H, $J_{2,3} = 1.4$ Hz, $J_{3,4} = 10.1$ Hz, H-3^{Man}), 4.86–4.80 (m, 3 H, H-2^{Gal}, H-4^{SA}, H-4^{Gal}), 4.60 (d, 1 H, $J_{1,2} = 7.9$ Hz, H-1^{Gal}), 4.49–4.43 (m, 2 H, H-1^{GlcN}, H-3^{Gal}), 4.38–4.29 (m, 3 H, H-9a^{SA}, H-6a^{GlcN}, H-2^{Man}), 4.13–3.87 (m, 9 H, H-6ab^{Man}, H-6b^{GlcN}, H-2^{GlcN}, H-5^{Man}, H-5^{SA}, H-6ab^{Gal}, H-9b^{SA}), 3.84–3.80 (m, 2 H, H-5^{Gal}, H-4^{GlcN}), 3.78 (s, 3 H, OMe), 3.60 (dd, 1 H, $J_{5,6} = 11.0$ Hz, $J_{6,7} = 2.7$ Hz, H-6^{SA}), 3.57 (m, 1 H, H-5^{GlcN}), 2.52 (dd, 1 H, $J_{gem} = 12.6$ Hz, $J_{vic} = 4.2$ Hz, H-3eq^{SA}), 2.18, 2.10, 2.05, 2.04, 2.03, 2.02, 2.00, 1.95, 1.94, 1.89 (12 s, 36 H, OAc), 1.87, 1.79 (2 s, 6 H, NAc), 1.60 (dd, 1 H, $J_{vic} = 12.4$ Hz, H-3ax^{SA}); ^{13}C-NMR (100 MHz, $CDCl_3$): δ170.6, 170.4, 170.2, 170.1, 170.0, 169.9, 169.5, 169.3, 169.1, 167.6, 160.0 (C=NH), 100.6 ($J_{C,H} = 163.7$ Hz, C-1^{Gal}), 100.5 ($J_{C,H} = 162.8$ Hz, C-1^{GlcN}), 96.6 (C-2^{SA}), 95.0 ($J_{C,H} = 177.5$ Hz, C-1^{Man}), 75.9 (C-4^{GlcN}), 72.8 (C-3^{GlcN}), 72.7 (C-5^{GlcN}, C-2^{Man}), 71.9 (C-6^{SA}), 71.4 (C-5^{Man}), 71.1 (C-3^{Gal}), 70.5 (C-5^{Gal}), 69.9 (C-2^{Gal}), 69.8 (C-3^{Man}), 69.3 (C-4^{SA}), 67.8 (C-8^{SA}), 67.3 (C-4^{Gal}), 66.9 (C-7^{SA}), 64.9 (C-4^{Man}), 62.3 (C-6^{GlcN}), 62.2 (C-9^{SA}), 61.9 (C-6^{Man}), 61.5 (C-6^{Gal}), 53.3 (C-2^{GlcN}), 53.1 (OMe), 49.0 (C-5^{SA}), 37.4 (C-3^{SA}), 23.2, 23.2 (NAc), 21.6, 20.9, 20.9, 20.7 (OAc).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 1521.9$ $[M + Na]^+$; average mass for $C_{58}H_{78}Cl_3NaN_3O_{36}$: 1522.6.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-L-serine tert-butyl ester (**20a**)

A solution of trichloroacetimidate **18** (56.8 mg, 0.0379 mmol) and serine derivative **19a** (40 mg, 0.10 mmol) in 4 ml CH_2Cl_2/toluene (1/1) was stirred under argon with freshly activated molecular sieves 4 Å (400 mg) at −20°C for 1 h. Then, TMSOTf (1.5 µl, 8 µmol) was added and stirring continued for 1 h. The reaction was quenched with triethylamine (1.6 µl, 11 µmol), diluted with CH_2Cl_2 and filtered through a Celite pad. The filter cake was thoroughly washed with CH_2Cl_2 and combined filtrate and washings were washed with 1 N $KHCO_3$ and water, successively, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (toluene/acetone, 1.2/1) and 59 mg (91%) of the title compound was obtained as a white foam; $R_f = 0.65$ ($CHCl_3$/MeOH, 9/1); $[\alpha]_D^{25} = -2.8$ ($CHCl_3$, 0.6); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.78–7.32 (m, 8 H, Ar), 5.83 (d, 1 H, $J_{NH,H\alpha} = 8.5$ Hz, NH^{Ser}), 5.66 (d, 1 H, $J_{NH,2} = 8.7$ Hz, NH^{GlcN}), 5.55 (m, 1 H, H-8^{SA}), 5.39 (dd, 1 H, $J_{6,7} = 2.8$ Hz, $J_{7,8} = 9.4$ Hz, H-7^{SA}), 5.22 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 9.9$ Hz, H-

4^{Man}), 5.19 (m, 1 H, H-3^{GlcN}), 5.15 (d, 1 H, $J_{NH,5} = 10.3$ Hz, NH^{SA}), 5.00 (dd, 1 H, $J_{2,3} = 2.9$ Hz, H-3^{Man}), 4.95–4.86 (m, 3 H, H-2^{Gal}, H-4^{SA}, H-4^{Gal}), 4.74 (m, 1 H, H-1^{Man}), 4.67 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1^{Gal}), 4.57 (d, $J_{1,2} = 8.1$ Hz, H-1^{GlcN}), 4.52 (dd, 1 H, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 3.3$ Hz, H-3^{Gal}), 4.44–4.35 (m, 5 H, α-CH^{Ser}, H-9a^{SA}, αCH$_2^{Fmoc}$, H-6a^{GlcN}), 4.24 (m, 1 H, β-CH^{Fmoc}), 4.20–4.16 (m, 2 H, H-6a^{Man}, H-6b^{GlcN}), 4.09 (d, 1 H, H-2^{Man}), 4.07–3.78 (m, 14 H, H-6b^{Man}, H-5^{SA}, β-CH$_2^{Ser}$, H-9b^{SA}, H-6ab^{Gal}, H-5^{Man}, H-5^{Gal}, H-2^{GlcN}, H-4^{GlcN}, OMe), 3.64 (dd, 1 H, $J^{5,6} = 10.6$ Hz, H-6^{SA}), 3.58 (m, 1 H, H-5^{GlcN}), 2.58 (dd, 1 H, $J_{vic} = 4.6$ Hz, $J_{gem} = 12.5$ Hz, H-3eq^{SA}), 2.24, 2.17, 2.16, 2.09, 2.08, 2.07, 2.06, 2.01, 1.98, 1.90, 1.88, 1.86 (14 s, 42 H, NAc, OAc), 1.68 (dd, 1 H, $J_{vic} = 12.5$ Hz, H-3ax^{SA}), 1.51 (s, 9 H, ^{t}Bu); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.8, 170.8, 170.6, 170.6, 170.5, 170.4, 170.33, 170.30, 170.25, 170.2, 169.7, 169.6, 169.4, 167.9, 155.8, 143.8, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 100.9 ($J_{C,H} = 165.1$ Hz, C-1^{Gal}), 99.7 ($J_{C,H} = 164.1$ Hz, C-1^{GlcN}), 98.3 ($J_{C,H} = 169.9$ Hz, C-1^{Man}), 96.7 (C-2^{SA}), 83.0 (*C*Me$_3$), 76.1 (C-4^{GlcN}), 74.1 (C-2^{Man}), 72.7 (C-5^{GlcN}), 72.4 (C-3^{GlcN}), 72.0 (C-6^{SA}), 71.2 (C-3^{Gal}), 70.5 (C-5^{Gal}), 69.9 (C-3^{Man}), 69.9 (C-4^{Gal}), 69.7 (C-6^{Gal}), 69.3 (C-5^{Man}, C-4^{Gal}), 67.7 (C-8^{SA}), 67.3 (C-4^{SA}), 67.2 (α-C^{Fmoc}), 66.9 (C-7^{SA}), 65.8 (C-4^{Man}), 62.6 (C-6^{Man}), 62.5 (C-6^{GlcN}), 62.2 (C-9^{SA}), 61.6 (β-C^{Ser}), 54.8 (α-C^{Ser}), 54.1 (C-2^{GlcN}), 53.1 (OMe), 49.1 (C-5^{SA}), 47.1 (β-C^{Fmoc}), 37.4 (C-3^{SA}), 28.0 (C*Me*$_3$), 23.1 (NAc), 21.5, 20.9, 20.8, 20.7, 20.7, 20.7, 20.6 (OAc).

FAB-MS (pos. mode, NBA): $m/z = 1743.1$ $[M + Na]^+$; average mass for $C_{78}H_{101}NaN_3O_{40}$: 1743.6.

N^α-(Fluoren-9-ylmethoxycarbonyl)-O-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-L-serine pentafluorophenyl ester (**20b**)

The reaction was performed in a manner as described for **20a** using **18** (40 mg, 0.027 mmol), pentafluorophenyl ester **19b** (24 mg, 0.049 mmol), and TMSOTf (1 µl, 5 µmol). Purification by silica gel column chromatography (toluene/acetone, 1.2/1) afforded 30.8 mg (63%) of **20b**; $R_f = 0.65$ ($CHCl_3$/MeOH, 9/1); $[\alpha]_D^{25} = -9.1$ ($CHCl_3$, 1.33); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.78–7.30 (m, 8 H, Ar), 6.07 (d 1 H, $J_{NH,H\alpha} = 8.5$ Hz, NH^{Ser}), 5.63 (d, 1 H, $J_{NH,2} = 8.3$ Hz, NH^{GlcN}), 5.55 (m, 1 H, H-8^{SA}), 5.40 (dd, 1 H, $J_{6,7} = 2.9$ Hz, $J_{7,8} = 9.3$ Hz, H-7^{SA}), 5.22 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 9.9$ Hz, H-4^{Man}), 5.16 (m, 1 H, H-3^{GlcN}), 5.14 (d, 1 H, $J_{NH,5} = 10.3$ Hz, NH^{SA}), 5.06 (dd, 1 H, $J_{2,3} = 2.9$ Hz, H-3^{Man}), 5.00 (m, 1 H, α-CH^{Ser}), 4.96–4.86 (m, 3 H, H-2^{Gal}, H-4^{SA}, H-4^{Gal}), 4.83 (d, 1 H, $J_{1,2} < 1.0$ Hz, H-1^{Man}), 4.66 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1^{Gal}), 4.62 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{GlcN}), 4.52 (dd, 1 H, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 3.3$ Hz, H-3^{Gal}), 4.48–4.39 (m, 4 H, H-9a^{SA}, α-CH$_2^{Fmoc}$, H-6a^{GlcN}), 4.26–3.96 (m, 11 H, β-CH^{Fmoc}, H-5^{SA}, H-6b^{GlcN}, H-6ab^{Man}, H-6ab^{Gal}, H-9b^{SA}, β-CH$_2^{Ser}$, H-2^{Man}), 3.88–3.79 (m, 7 H, OMe, H-6b^{Man}, H-2^{GlcN}, H-5^{Man}, H-5^{Gal}, H-4^{GlcN}), 3.64 (dd, 1 H, $J_{5,6} = 11.0$ Hz, H-6^{SA}), 3.59 (m, 1 H, H-5^{GlcN}), 2.58 (dd, 1 H, $J_{vic} = 4.4$ Hz, $J_{gem} = 12.3$ Hz, H-3eq^{SA}), 2.24, 2.17, 2.16, 2.09, 2.08, 2.07, 2.06, 2.03, 2.01, 2.00 (12 s, 36 H, OAc), 1.90, 1.86 (2 s, 6 H, NAc), 1.68 (dd, 1 H, $J_{vic} = 12.5$ Hz, H-3ax^{SA}); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.8, 170.7, 170.6, 170.6, 170.5, 170.4, 170.34, 170.28, 170.2, 169.6, 169.4, 167.9, 166.4 (C-1^{SA}), 155.8 (C=O^{Fmoc}), 143.6, 143.5, 141.3, 127.8, 127.1, 125.1, 120.0, 100.9 ($J_{C,H} = 162.1$ Hz, C-1^{Gal}), 99.6 ($J_{C,H} = 162.3$ Hz, C-1^{GlcN}), 98.9 ($J_{C,H} = 168.7$ Hz, C-1^{Man}), 96.8 (C-2^{SA}), 75.9 (C-4^{GlcN}), 73.8 (C-2^{Man}), 72.9 (C-5^{GlcN}), 72.2 (C-3^{GlcN}), 72.0 (C-6^{SA}), 71.2 (C-3^{Gal}), 70.6 (C-5^{Gal}), 69.8 (C-2^{Gal}), 69.7 (β-C^{Ser}), 69.6 (C-5^{Man}), 69.5 (C-3^{Man}), 69.3 (C-4^{Gal}), 67.7 (C-8^{SA}), 67.6 (α-C^{Fmoc}), 67.3 (C-4^{SA}), 66.9 (C-7^{SA}), 65.8 (C-4^{Man}), 62.6 (C-6^{Man}), 62.3 (C-6^{GlcN}), 62.1 (C-9^{SA}), 61.6 (C-6^{Gal}), 54.4 (α-C^{Ser}), 54.1 (C-2^{GlcN}), 53.1 (OMe), 49.1 (C-5^{SA}), 47.0 (β-C^{Fmoc}), 37.4 (C-3^{SA}), 23.1 (NAc). 21.5, 20.8, 20.7, 20.7, 20.6, 20.6, 20.6 (OAc).

FAB-MS (pos. mode, NBA: $m/z = 1853.1$ $[M + Na]^+$; average mass for $C_{80}H_{92}F_5NaN_3O_{40}$: 1853.6.

N^α-(Fluoren-9-ylmethoxycarbonyl)-O-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-L-serine allyl ester (**20c**)

Trichloroacetimidate **18** (80 mg, 0.053 mmol) was reacted with serine derivative **19c** (48 mg, 0.13 mmol) in 4 ml CH_2Cl_2/toluene (1/1) in a similar manner as described for the preparation of **20a** in the presence of TMSOTf (2 µl, 0.01 mmol) and molecular sieves 4 Å (400 mg). Purification by silica gel column chromatography (toluene/acetone, 1.2/1), afforded 76 mg (84%) of the title compound as a white foam; $R_f = 0.58$ ($CHCl_3$/MeOH, 9/1); $R_f = 0.58$ ($CHCl_3$/MeOH, 9/1); $[\alpha]_D^{25} = 0.6$ ($CHCl_3$, 0.6); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.78–7.30 (Ar), 5.99–5.90 (m, 2 H, $CH_2-CH=CH_2$, NH^{Ser}), 5.69 (d, 1 H, $J_{NH,2} = 8.7$ Hz, NH^{GlcN}), 5.54 (m, 1 H, H-8^{SA}), 5.41–5.38 (m, 2 H, H-7^{SA}, $CH_2-CH=C\underline{H}_a\underline{H}_b$), 5.33 (d, 1 H, $J = 15.9$ Hz, $CH_2-CH=CH\underline{H}_a\underline{H}_b$), 5.22 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 9.8$ Hz, H-4^{Man}), 5.18–5.13 (m, 2 H, H-3^{GlcN}, NH^{SA}), 5.00 (dd, 1 H, $J_{2,3} = 2.9$ Hz, H-3^{Man}), 4.95–4.85 (m, 3 H, H-2^{Gal}, H-4^{SA}, H-4^{Gal}), 4.73–4.70 (m, 3 H, *CH*$_2$– CH=CH$_2$, H-1^{Man}), 4.67 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1^{Gal}), 4.59 (m, 1 H, α-CH^{Ser}), 4.56 (d, $J_{1,2} = 7.8$ Hz, H-1^{GlcN}), 4.52 (dd, 1 H, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 3.3$ Hz, H-3^{Gal}), 4.45–4.36 (m, 4 H, H-9a^{SA}, αCH$_2^{Fmoc}$, H-6a^{GlcN}), 4.26–4.15 (m, 3 H, β-CH^{Fmoc}, H-6a^{Man}, H-6b^{GlcN}), 4.11–3.95 (m, 9 H, β-CH$_2^{Ser}$, H-5^{SA}, H-2^{Man}, H-5^{Man}, H-6ab^{Gal}, H-6b^{Man}, H-9b^{SA}), 3.86–3.81 (m, 6 H, OMe, H-2^{GlcN}, H-5^{Gal}, H-4^{GlcN}), 3.63 (dd, 1 H, $J_{5,6} = 10.8$ Hz, $J_{6,7} = 2.4$ Hz, H-6^{SA}), 3.59 (m, 1 H, H-5^{GlcN}), 2.58 (dd, 1 H, $J_{vic} = 4.7$ Hz, $J_{gem} = 12.4$ Hz, H-3eq^{SA}, 2.24, 2.17, 2.16, 2.10, 2.08, 2.07, 2.06, 2.01, 1.99, 1.90, 1.85, 1.86 (14 s, 42 H, NAc, OAc), 1.68 (dd, 1 H, $J_{vic} = 12.5$ Hz, H-3ax^{SA}); ^{13}C-NMR (125 MHz,

$CDCl_3$): δ 170.9, 170.8, 170.7, 170.6, 170.5, 170.4, 170.3, 170.2, 169.7, 169.6, 169.4, 167.9 [C=O (NAc, OAc, COOAll, C-1^{SA})], 155.9 [C=O (Fmoc)], 143.7, 141.3, 141.3 (Ar, Fmoc), 131.4 ($CH_2-CH=CH_2$), 127.8, 127.1, 125.1, 120.0 (Ar, Fmoc), 119.2 ($CH_2-CH=CH_2$), 100.9 ($J_{C,H}=163.8$ Hz, C-1^{Gal}), 99.8 ($J_{C,H}=164.1$ Hz, C-1^{GlcN}), 98.4 ($J_{C,H}=170.4$ Hz, C-1^{Man}), 96.8 (C-2^{SA}), 76.0 (C-4^{GlcN}), 74.2 (C-2^{Man}), 72.8 (C-5^{GlcN}), 72.3 (C-3^{GlcN}), 72.0 (C-6^{SA}), 71.2 (C-3^{Gal}), 70.5 (C-5^{Gal}), 69.9 (C-3^{Man}), 69.9 (C-2^{Gal}), 69.4 (C-4^{Gal}), 69.3 (C-6^{Gal}), 69.2 (C-5^{Man}), 67.8 (C-8^{SA}), 67.3 (C-4^{SA}, α-C^{Fmoc}), 66.9 (C-7^{SA}), 66.5 (CH_2), 65.8 (C-4^{Man}), 62.5 (C-6^{Man}), 62.5 (C-6^{GlcN}), 62.2 (C-9^{SA}), 61.6 (β-C^{Ser}), 54.3 (α-C^{Ser}), 54.0 (C-2^{GlcN}), 53.1 (OMe), 49.1 (C-5^{SA}), 47.1 (β-C^{Fmoc}), 37.4 (C-3^{SA}), 23.1 (NAc), 21.5, 20.9, 20.8, 20.7, 20.7, 20.6, (OAc).

FAB-MS (pos. mode, NBA: $m/z=1726.8$ $[M+Na]^+$; average mass for $C_{77}H_{97}NaN_3O_{40}$: 1726.6.

*N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-L-threonine allyl ester (**20d**)*

Trichloroacetimidate **18** (40 mg, 0.027 mmol) was reacted with threonine derivative **19d** (25 mg, 0.066 mmol) in 2 ml CH_2Cl_2/toluene (1/1) in a similar manner as described for the preparation of **20a** in the presence of TMSOTf (1 µl, 5 mmol) and molecular sieves 4 Å (200 mg). Purification by silica gel column chromatography (toluene/acetone, 1.2/1), afforded 41 mg (89%) of the title compound as a white foam; $R_f=0.68$ ($CHCl_3$/MeOH, 9/1); $[\alpha]_D^{25}=-0.15$ ($CHCl_3$, 0.67); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.75–7.29 (Ar), 5.92 (m, 1 H, $CH_2-CH=CH_2$) 5.69 (d, 1 H, $J_{NH,2}=8.7$ Hz, NH^{GlcN}), 5.57 (d, 1 H, $J_{NH,H\alpha}=9.5$ Hz, NH^{Thr}), 5.52 (m, 1 H, H-8^{SA}), 5.40–5.35 (m, 2 H, H-7^{SA}, $CH_2-CH=CH_aH_b$), 5.28 (d, 1 H, $J=10.9$ Hz, $CH_2-CH=CH_aH_b$), 5.17 (dd, 1 H, $J_{3,4}\sim J_{4,5}=10.0$ Hz, H-4^{Man}), 5.13 (d, 1 H, $J_{NH,5}=8.5$ Hz, NH^{SA}), 5.12 (dd, 1 H, $J_{2,3}\sim J_{3,4}=8.6$ Hz, H-3^{GlcN}), 4.99–4.85 (m, 4 H, H-3^{Man}, H-2^{Gal}, H-4^{SA}, H-4^{Gal}), 4.79 (d, 1 H, $J_{1,2}<1.5$ Hz, H-1^{Man}), 4.67–4.60 (m, 3 H, $CH_2-CH=CH_2$, H-1^{Gal}), 4.50–4.32 (m, 8 H, αCH_2^{Fmoc}, H-1^{GlcN}, H-3^{Gal}, α-H^{Thr}, H-$9a^{SA}$, H-$6a^{GlcN}$, β-H^{Thr}), 4.15 (dd, 1 H, $J_{vic}=7.3$ Hz, β-H^{Fmoc}), 4.12–3.70 (m, 15 H, H-$6b^{GlcN}$, H-$6ab^{Man}$, H-5^{SA}, H-$6ab^{Gal}$, H-2^{Man}, H-5^{Man}, H-$9b^{SA}$, H-2^{GlcN}, OMe, H-4^{GlcN}, H-5^{Gal}), 3.60 (dd, 1 H, $J_{5,6}=10.7$ Hz, $J_{6,7}=2.7$ Hz, H-6^{SA}), 3.54 (m, 1 H, H-5^{GlcN}), 2.57 (dd, 1 H, $J_{vic}=4.4$ Hz, $J_{gem}=12.3$ Hz, H-$3eq^{SA}$), 2.21, 2.14, 2.07, 2.06, 2.04, 2.03, 2.01, 1.97, 1.89, 1.82 (14 s, 42 H, OAc, NAc), 1.62 (dd, 1 H, $J_{vic}=12.3$ Hz, H-$3ax^{SA}$); 1.27 (d, 3 H, $J_{vic}=7.1$ Hz, Me^{Thr}); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.7, 170.5, 170.4, 170.3, 170.2, 170.04, 170.00, 169.5, 169.2, 167.8 [C=O (NAc, OAc, C-1^{SA})], 156.4 [C=O (Fmoc)], 143.7, 143.5, 141.2 (Ar, Fmoc), 131.4 ($CH_2-CH=CH_2$), 127.7, 127.0, 125.1, 119.9 (Ar, Fmoc), 119.0 ($CH_2-CH=CH_2$), 100.9 ($J_{C,H}=160.9$ Hz, C-1^{Gal}), 99.9 ($J_{C,H}=160.9$ Hz, C-1^{GlcN}), 98.8 ($J_{C,H}=171.7$ Hz, C-1^{Man}), 96.7 (C-2^{SA}), 76.8 (β-C^{Thr}), 75.9 (C-4^{GlcN}), 74.5 (C-2^{Man}), 72.7 (C-5^{GlcN}), 72.5 (C-3^{GlcN}), 72.0 (C-6^{SA}), 71.2 (C-3^{Gal}), 70.5 (C-5^{Gal}), 69.9 (C-3^{Man}, C-2^{Gal}), 69.3 (C-4^{SA}, C-5^{Man}), 67.7 (C-8^{SA}), 67.4 (α-C^{Fmoc}), 67.3 (C-4^{Gal}), 66.9 (C-7^{SA}), 66.3 ($CH_2-CH=CH_2$), 66.1 (C-4^{Man}), 62.7 (C-6^{Man}), 62.5 (C-6^{GlcN}), 62.3 (C-9^{SA}), 61.6 (C-6^{Gal}), 58.5 (α-C^{Thr}), 53.8 (C-2^{GlcN}), 53.1 (OMe), 49.1 (C-5^{SA}), 47.1 (β-C^{Fmoc}), 37.4 (C-3^{SA}), 23.2 (NAc), 21.6, 20.9, 20.8, 20.7, 20.7 (OAc), 18.0 (Me^{Thr}).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z=1741.1$ $[M+Na]^+$; $C_{78}H_{99}NaN_3O_{40}$ requires 1740.6.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4,6-tri-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-L-serine (**20e**)

*a) From **20c**: cleavage of allyl ester.* A mixture of **20c** (200 mg, 0.117 mmol), dimedone (197 mg, 1.33 mmol) and $Pd(Ph_3P)_4$ (50 mg, 0.043 mmol) was dissolved in dry THF (6.6 ml) and stirred under Ar atmosphere for 3 h at room temperature. After complete cleavage of the allyl ester was confirmed, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography (0.5% HOAc in $CHCl_3$/MeOH, 20/1) to furnish 178 mg (91%) of **20e** as a white amorphous; $R_f=0.10$ ($CHCl_3$/MeOH, 9/1); $[\alpha]_D^{25}=-2.3$ (0.67, MeOH); ^{1}H-NMR (500 MHz, d_6-DMSO): δ 7.88 (m, 2 H, Ar), 7.78 (d, 1 H, $J_{NH,2}=9.5$ Hz, NH^{GlcN}), 7.73 (d, 1 H, $J_{NH,H\alpha}=7.5$ Hz, NH^{Ser}), 7.71 (m, 2 H, Ar), 7.44–7.19 (2 m, 4 H, Ar), 5.43 (m, 1 H, H-8^{SA}), 5.22 (dd, 1 H, $J_{6,7}=2.5$ Hz, $J_{7,8}=9.2$ Hz, H-7^{SA}), 5.01–4.86 (m, 6 H, H-4^{Man}, H-1^{Man}, H-3^{GlcN}, H-3^{Man}, β-CH_2^{Ser}), 4.72 (ddd, 1 H, $J_{3a,4}=12.1$ Hz, $J_{4,5}=10.1$ Hz, $J_{3e,4}=4.5$ Hz, H-4^{SA}), 4.65 (dd, 1 H, $J_{2,3}=9.7$ Hz, H-2^{Gal}), 4.60 (d, 1 H, $J_{1,2}=7.9$ Hz, H-1^{Gal}), 4.47 (d, 1 H, $J_{1,2}=9.3$ Hz, H-1^{Gal}), 4.46 (m, 1 H, H-3^{Gal}), 4.34–4.20 (m, 6 H, α-CH_2^{Fmoc}, β-CH^{Fmoc}, α-CH^{Ser}, H-$9a^{SA}$, H-$6a^{GlcN}$), 4.05–3.65 (m, 17 H, H-$6b^{GlcN}$, H-2^{Man}, H-$6ab^{Man}$, H-$6ab^{Gal}$, H-$9b^{SA}$, H-5^{SA}, H-5^{Man}, H-5^{Gal}, OMe, H-2^{GlcN}, H-4^{Man}, H-6^{SA}, H-5^{GlcN}), 2.45 (dd, 1 H, $J_{vic}=4.5$ Hz, $J_{gem}=12.3$ Hz, H-$3eq^{SA}$), 2.24, 2.17, 2.16, 2.09, 2.08, 2.07, 2.06, 2.03, 2.01, 2.00 (12 s, 36 H, OAc), 1.90, 1.86, (2 s, 6 H, NAc), 1.34 (dd, 1 H, $J_{vic}=12.1$ Hz, H-$3ax^{SA}$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.0, 169.9, 169.7, 169.4, 169.3, 169.1, 169.04, 168.97, 168.7, 167.3 [C=O (NAc, OAc, CO_2H, C-1^{SA})], 155.8 [C=O (Fmoc)], 143.7, 143.5, 140.6, 127.5, 126.9, 125.2, 120.0, 100.2 ($J_{C,H}=166.7$ Hz, C-1^{Gal}), 99.9 ($J_{C,H}=165.9$ Hz, C-1^{GlcN}), 97.7 ($J_{C,H}=168.7$ Hz, C-1^{Man}), 96.1 (C-2^{SA}), 76.3 (C-4^{GlcN}), 74.4 (C-2^{Man}), 72.9 (C-3^{GlcN}), 71.6 (C-5^{GlcN}), 71.6 (C-6^{SA}), 70.5 (C-3^{Gal}), 70.0 (C-3^{Man}), 69.8 (C-5^{Gal}), 69.5 (C-2^{Gal}, C-4^{Gal}), 69.3 (C-4^{SA}), 68.0 (C-5^{Man}), 67.3 (C-8^{SA}), 67.1 (β-C^{Ser}), 66.5 (C-7^{SA}), 65.7 (α-C^{Fmoc}), 65.0 (C-4^{Man}), 62.2 (C-6^{GlcN}), 61.8 (C-9^{SA}),

61.6 (C-6^{Man}), 60.9 (C-6^{Gal}), 54.4 (α-C^{Ser}), 53.0 (OMe) 52.5 (C-2^{GlcN}), 47.4 (C-5^{SA}), 46.6 (β-C^{Fmoc}), 37.2 (C-3^{SA}), 22.6, 22.5 (NAc), 20.6, 20.5, 20.4, 20.3 (OAc).

*b) From **20a**:* The glycosyl amino acid **20a** (22.0 mg, 12.7 μmol) was dissolved in 500 μl trifluoroacetic acid/H_2O (95/5). After stirring for 1 h, the mixture was concentrated *in vacuo* and coevaporated with toluene (3 × 1 ml) and dried under high vacuum. The residue was purified by silica gel column chromatography (0.5% HOAc in $CHCl_3$/MeOH, 20/1) to give 19.5 mg (92%) of **20e** as a white amorphous.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-L-threonine (**20f**)*

A mixture of **20d** (180 mg, 0.105 mmol), dimedone (180 mg, 1.22 mmol) and $Pd(Ph_3P)_4$ (45 mg, 0.039 mmol) in dry THF (6 ml) was stirred for 3 h under Ar atmosphere at room temperature. The mixture was concentrated *in vacuo* and purified by silica gel column chromatography (0.5% HOAc) in $CHCl_3$/MeOH, 20/1) to furnish 157 mg (89%) of **20f** as a white amorphous; $R_f = 0.09$ ($CHCl_3$/MeOH, 9/1); $[\alpha]_D^{25} = 0.3$ (0.67, MeOH); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.73–7.29 (m, 8 H, Ar), 6.63 (d, 1 H, $J_{NH,2} = 8.1$ Hz, NH^{GlcN}), 5.78 (d, 1 H, $J_{NH,H\alpha} = 9.6$ Hz, NH^{Thr}), 5.48 (m, 1 H, H-8^{SA}), 5.38–5.33 (m, 2 H, H-7^{SA}, NH^{SA}), 5.22 (dd, 1 H, $J_{2,3} \sim J_{3,4} = 8.6$ Hz, H-3^{GlcN}), 5.15 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 10.0$ Hz, H-4^{Man}), 4.98 (m, 1 H, H-3^{Man}), 4.89–4.81 (m, 3 H, H-2^{Gal}, H-4^{Gal}, H-4^{SA}), 4.75 (d, 1 H, $J_{1,2} < 1.5$ Hz, H-1^{Man}), 4.58(d, 1 H, $J_{1,2} = 8.3$ Hz, H-1^{Gal}), 4.49 (d, 1 H, $J_{1,2} = 7.3$ Hz, H-1^{GlcN}), 4.48–4.28 (m, 7 H, H-3^{Gal}, β-H^{Thr}, α-H^{Fmoc}, H-6a^{GlcN}, H-9a^{SA}, α-H^{Thr}), 4.22 (dd, 1 H, $J_{vic} = 7.3$ Hz, β-H^{Fmoc}), 4.17–4.05 (m, 3 H, H-6ab^{Man}, H-6b^{GlcN}), 4.03–3.88 (m, 6 H, H-2^{Man}, H-5^{Man}, H-6ab^{Gal}, H-9b^{SA}, H-5^{SA}), 3.80(s, 3 H, OMe), 3.79–3.70 (m, 3 H, H-5^{Gal}, H-2^{GlcN}, H-4^{GlcN}), 3.64–3.56 (m, 3 H, H-5^{GlcN}, H-6^{SA}), 2.53 (dd, 1 H, $J_{vic} = 4.5$ Hz, $J_{gem} = 12.3$ Hz, H-3eq^{SA}), 2.19, 2.12, 2.07, 2.05, 2.03, 2.00, 1.97, 1.96, 1.91, 1.81 (14 s, 42 H, OAc, NAc), 1.62 (dd, 1 H, $J_{vic} = 12.3$ Hz, H-3ax^{SA}), 1.26 (d, 3 H, $J_{vic} = 5.8$ Hz, Me^{Thr}); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.9, 171.3, 170.6, 170.4, 170.3, 170.2, 169.9, 169.8, 169.3, 169.3, 167.6 [C=O (NAc, OAc, CO_2H, C-1^{SA})], 156.6 [C=O (Fmoc)], 143.7, 143.6, 141.0, 127.5, 126.9, 125.1, 119.8 (Ar), 100.4 ($J_{C,H} = 165.8$ Hz, C-1^{Gal}), 100.3 ($J_{C,H} = 166.7$ Hz, C-1^{GlcN}), 99.6 (C-2^{SA}), 99.1 ($J_{C,H} = 175.0$ Hz, C-1^{Man}), 76.3 (β-C^{Thr}), 75.8 (C-4^{GlcN}), 75.5 (C-2^{Man}), 72.4 (C-5^{GlcN}), 71.9 (C-6^{SA}), 71.8 (C-3^{GlcN}), 71.1 (C-3^{Gal}), 70.6 (C-5^{Gal}), 70.0 (C-3^{Man}), 69.9 (C-2^{Gal}), 69.3 (C-4^{SA}), 69.0 (C-5^{Man}), 67.8 (C-8^{SA}), 67.3 (C-4^{Gal}), 67.2 (α-C^{Fmoc}), 66.8 (C-7^{SA}), 66.1 (C-4^{Man}), 62.6 (C-6^{Man}), 62.5 (C-6^{GlcN}), 62.1 C-9^{SA}), 61.6 (C-6^{Gal}), 58.3 (α-C^{Thr}), 53.8 (C-2^{GlcN}), 53.2 (OMe), 49.1 (C-5^{SA}), 47.2 (β-C^{Fmoc}), 37.5 (C-3^{SA}), 23.2, 23.1, (NAc), 21.6, 21.0, 20.9, 20.8, 20.7, (OAc), 18.9 (Me^{Thr}).

ESI-MS (neg. mode, $CHCl_3$/MeOH, 1/1): $m/z = 1676.4$ $[M-H]^-$; $C_{75}H_{95}N_3O_{39}$ requires 1676.5.

O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-O-(β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-α-D-mannopyranosyl-L-serine (**1a**)*

The glycosylated serine derivative **20e** (14 mg, 8.4 μmol) was dissolved in a mixture of 0.5 ml dry $CHCl_3$ and morpholine (0.5 ml) and stirred for 90 min at room temperature. After complete cleavage of the Fmoc group, the mixture was concentrated *in vacuo*, coevaporated with toluene and thoroughly dried under high vacuum. The residue was dissolved in dry MeOH and reacted with NaOMe (46 μl of a 0.87 M solution) for 1 h (pH ~ 8.5). Second portion of NaOMe (46 μl of a 0.87 M solution) was added and stirring continued for 3 h (pH ~ 9.0–9.5). The reaction mixture was quenched with 10% acetic acid, evaporated and dried. The residue was dissolved in 2 ml MeOH and reacted with 6.12 mM aq. NaOH (4 ml) and stirred for 1 h (pH ~ 9.5–10.0). A subsequent portion of 6.12 mM aq. NaOH (4 ml) was added and stirring continued for 4 h (pH ~ 9.5–10.0). The mixture was quenched with 10% acetic acid, concentrated *in vacuo* and purified by size exclusion chromatography (Bio-Gel® P-2) to afford 7.1 mg (92%) of **1a**; ^{1}H-NMR (500 MHz, D_2O): δ 4.86 (d, 1 H, $J_{1,2} = 1.4$ Hz, H-1^{Man}α), 4.56 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1^{GlcN}β), 4.53 (d, 1 H, $J = 7.9$ Hz, H-1^{Gal}β), 2.74 (dd, 1 H, $J_{3,4} = 4.6$ Hz, $J_{gem} = 12.4$ Hz, H-3eq^{SA}), 2.03, 2.02 (2 s, 6 H, NAc), 1.79 (dd, 1 H, $J_{vic} = 12.2$ Hz, H-3$^{SA}_{ax}$); ^{13}C-NMR (125 MHz, D_2O): δ 175.7, 175.4, 174.6, 172.5 [C=O (NAc, CO_2H)], 103.3 (C-1^{Gal}), 100.5 (C-2^{SA}), 100.2 (C-1^{GN}), 98.3 ($J_{CH} = 172.3$ Hz, C-1^{M}), 40.3 (C-3^{SA}), 23.0, 22.7 (NAc).

ESI-MS (neg. mode, MeOH/H_2O = 1/1): $m/z = 922.2$ $[M-H]^-$; $C_{34}H_{56}N_3O_{26}$ requires 922.3.

Benzyl O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**22**)*

A solution of compound **12** (148.5 mg, 0.164 mmol) and tricholoroacetimidate **21** (133 mg, 0.246 mmol) in 4 ml dry CH_2Cl_2 was stirred under argon with freshly activated molecular sieves 4 Å (300 mg) at −20°C for 1 h. TMSOTf (10 μl, 0.052 mmol) was added and stirring continued for 1 h. The reaction was quenched with triethylamine (5 μl), diluted with CH_2Cl_2, and filtered through a Celite pad. After being thoroughly washed with CH_2Cl_2, combined filtrate and washings were washed with 1 N $KHCO_3$ and water, successively, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 3/1) to afford 183 mg (87%) of the title compound as a white foam: $R_f = 0.70$ (toluene/ethyl acetate, 7/3); $[\alpha]_D^{25} = -30.4$ ($CHCl_3$, 0.5); ^{1}H-NMR (400 MHz,

$CDCl_3$1): δ 7.43–7.15 (m, 35 H, Ar) 5.42 (dd, 1 H, $J_{3,4} = 3.4$ Hz, $J_{4,5} < 1.0$ Hz, H-4^{Gal}), 5.12 (dd, 1 H, $J_{2,3} = 10.0$ Hz, H-2^{Gal}), 5.10 (d, 1 H, $J_{1,2} = 1.6$ Hz, H-1^{Man}), 4.94 (d, 1 H, $J_{gem} = 10.6$ Hz, OCH_2), 4.87 (dd, 1 H, H-3^{Gal}), 4.84 (d, 1 H, $J_{gem} = 10.9$ Hz, OCH_2), 4.81 (d, 1 H, $J_{gem} = 11.7$ Hz, OCH_2), 4.74 (d, 1 H, $J_{gem} = 11.6$ Hz, OCH_2), 4.65 (d, 1 H, $J_{gem} = 11.9$ Hz, OCH_2), 4.61 (2d, 2 H, OCH_2), 4.59 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1^{Gal}), 4.54–4.43 (m, 4 H, OCH_2), 4.35 (d, 1 H, $J_{gem} = 11.9$ Hz, OCH_2), 4.33 (d, 1 H, $J_{1,2} = 8.1$ Hz, H-1^{GlcN}), 4.29 (m, 1 H, H-2^{Man}), 4.21 (d, 1 H, $J_{gem} = 12.1$ Hz, OCH_2), 4.05–3.98 (m, 2 H, H-4^{GlcN}, H-3^{Man}), 3.95–3.82 (m, 2 H, H-5^{Man}, H-4^{Man}), 3.78–3.68 (m, 4 H, H-6ab^{Man}, H-6ab^{GlcN}), 3.57–3.52 (m, 2 H, H-5^{Gal}, H-2^{GlcN}), 3.34–3.28 (m, 4 H, H-3^{GlcN}, H-5^{GlcN}, H-6ab^{Gal}), 1.94, 1.91, 1.90 (3 s, 9 H, OAc); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.6, 169.5, 168.8 [C=O (NAc, OAc)], 138.1, 138.1, 138.0, 138.0, 137.4, 137.2, 136.9, 128.3, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.3, 127.1, 100.6 ($J_{C,H} = 158.4$ Hz, C-1^{Gal}), 99.9 ($J_{C,H} = 163.3$ Hz, C-1^{GlcN}), 96.6 ($J_{C,H} = 168.3$ Hz, C-1^{Man}, 80.7 (C-3^{GlcN}), 78.1 (C-3^{Man}), 75.8 (C-4^{GlcN}), 74.9 (CH_2), 74.8 (C-5^{GlcN}), 74.6 (C-4^{Man}), 73.7 (C-2^{Man}), 73.5, 73.2, 73.0 (CH_2), 71.9 (C-5^{Man}), 71.8 (C-5^{Gal}), 71.0 (C-3^{Gal}), 70.9 (CH_2), 69.7 (C-2^{Gal}), 69.4 (C-6^{Man}), 69.1 (CH_2), 67.9 (C-6^{GlcN}), 67.2 (C-4^{Gal}), 66.5 (C-6^{Gal}), 65.6 (C-2^{GlcN}), 20.7, 20.6, 20.6 (OAc).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 1308.5$ $[M + Na]^+$; $C_{73}H_{79}NaN_3O_{18}$ requires 1308.5.

Benzyl O-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**23**)

Compound **22** (173.1 mg, 0.135 mmol) was dissolved in pyridine/H_2O (7/3, 1.2 ml) and treated with propanedithiol (1.15 ml) and diisopropylethylamine (0.23 ml). After 14 h at room temperature, complete conversion to the amine was observed; $R_f = 0.39$ (hexane/ethyl acetate, 2/1). Acetic anhydride (4.6 ml) was added and stirring was continued for 8 h. Evaporation under high vacuum and coevaporation with toluene (3 × 20 ml) furnished a yellow oil which was purified by silica gel column chromatography (toluene/acetone, 3/1) to give 156 mg (89%) of **23** as a white foam: $R_f = 0.45$ (hexane/ethyl acetate, 2/1); $[\alpha]_D^{25} = -13.9$ ($CHCl_3$, 0.5); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.34–7.15 (m, 35 H, Ar), 5.67 (d, 1 H, $J_{NH,2} = 7.1$ Hz, NH^{GlcN}), 5.36 (d, 1 H, $J_{3,4} = 3.4$ Hz, H-4^{Gal}), 5.07 (dd, 1 H, $J_{2,3} = 7.9$ Hz, H-2^{Gal}), 5.06 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1^{GlcN}), 4.88 (d, 1 H, $J_{1,2} = 1.7$ Hz, H-1^{Man}), 4.85–4.80 (m, 3 H, H-3^{Gal}, OCH_2), 4.74 (d, 1 H, $J_{gem} = 11.1$ Hz, OCH_2), 4.65 (d, 1 H, $J_{gem} = 11.7$ Hz, OCH_2), 4.60–4.38 (m, 8 H, OCH_2), 4.31 (d, 1 H, $J_{gem} = 11.9$ Hz, OCH_2), 4.29 (dd, 1 H, $J_{2,3} \sim J_{3,4} = 8.7$ Hz, H-3^{GlcN}), 4.20 (d, 1 H, $J_{gem} = 12.0$ Hz, OCH_2), 4.14 (bs, 1 H, H-2^{Man}), 3.93–3.87 (m, 3 H, H-3^{Man}, H-4^{Man}, H-4^{GlcN}), 3.70–3.67 (m, 4 H, H-6a^{GlcN}, H6ab^{Man}, H-5^{Man}), 3.60–3.53 (m, 2 H, H-6ab^{Gal}), 3.13 (m, 1 H, H-2^{GlcN}, 1.99, 1.96, 1.95, 3 s, 9 H, OAc), 1.71 (s, 3 H, NAc); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.8, 169.7, 169.6, 169.0 [C=O (NAc, OAc)], 138.8, 138.2, 138.1, 137.9, 137.6, 137.4, 137.1, 128.3, 128.2, 128.1, 128.1, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.0, 99.9 ($J_{C,H} = 162.5$ Hz, C-1^{Gal}), 97.6 ($J_{C,H} = 164.2$ Hz, C-1^{GlcN}), 97.3 ($J_{C,H} = 169.6$ Hz, C-1^{Man}), 78.2 (C-4^{Man}), 77.0 (C-3^{GlcN}), 76.6 (C-4^{GlcN}), 75.0 (CH_2), 74.6 (C-5^{GlcN}), 74.4 (C-3^{Man}), 73.9, 73.5, 73.3 (CH_2), 73.3 (C-2^{Man}), 73.2 (CH_2, 71.8 (C-5^{Gal}), 71.8 (C-5^{Man}), 71.2 (CH_2), 71.0 (C-3^{Gal}), 69.7 (C-2^{Gal}), 69.4 (CH_2), 69.1 (C-6^{GlcN}, 68.6 (C-6^{Man}), 67.3 (C-4^{Gal}), 66.8 (C-6^{Gal}), 56.9 (C-2^{GlcN}), 23.4 (NAc), 20.8, 20.7, 20.7 (OAc).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 1324.8$ $[M + Na]^+$; $C_{75}H_{83}NaNO_{19}$ requires 1324.5.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl acetate (**25**)

Compound **23** (150 mg, 0.115 mmol) was hydrogenated over 50 mg of Pd/C (10%) in 7:3 MeOH-acetic acid (7:3, 10 ml) under atmospheric pressure. After 24 h, insoluble materials were removed by filtration though Celite and the filter cake washed with MeOH/HOAc (9/1). Combined filtrate and washings were concentrated *in vacuo*, coevaporated with toluene (3 × 20 ml) and dried under high vacuum to afford **24** (85 mg); $R_f = 0.67$ (*n*-BuOH/MeOH/H_2O/HOAc, 6/2/2/1), which was used for the subsequent reaction.

Crude **24** was dissolved in pyridine/acetic anhyride (2/1, 15 ml) at 0°C and stirred for 12 h. Resulting mixture was evaporated, coevaporated with toluene (3 × 50 ml), dissolved in CH_2Cl_2 and washed successively with 10% HCl (3 × 50 ml), 1 N $KHCO_3$ (2 × 50 ml) and H_2O. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to furnish a yellow foam which was purified by silica gel column chromatography (hexane/acetone, 1.5/1 → 1/1) to afford **25** (108 mg, 97%, α:β = 6:1); $R_f = 0.33$ ($CHCl_3$/MeO, 18/1); $[\alpha]_D^{25} = -13.9$ ($CHCl_3$, 0.5); ^{1}H-NMR (400 MHz, $CDCl_3$, α-isomer): δ 6.09 (d, 1 H, $J_{NH,2} = 9.4$ Hz, NH^{GlcN}), 5.97 (d, 1 H, $J_{1,2} = 1.7$ Hz, H-1^{Man}), 5.29–5.26 (m, 2 H, H-4^{Man}, H-4^{Gal}), 5.05–4.96 (m, 3 H, H-2^{Gal}, H-3^{GlcN}, H-3^{Gal}), 4.89 (dd, 1 H, $J_{2,3} = 10.4$ Hz, $J_{3,4} = 3.4$ Hz, H-3^{Gal}), 4.48 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1^{GlcN}), 4.42 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{Gal}), 4.33 (m, 1 H, H-6a^{GlcN}), 4.13 (dd, 1 H, $J_{5,6} = 4.7$ Hz, $J_{gem} = 12.4$ Hz, H-6a^{Gal}), 4.09–3.95 (m, 6 H, H-6b^{Gal}, H-2^{Man}, H-6b^{GlcN}, H6ab^{Man}, H-2^{GlcN}), 3.87 (m, 1 H, H-5^{Man}), 3.82 (dd, 1 H, $J_{5,6a} \sim J_{5,6b} = 6.4$ Hz, H-5^{Gal}), 3.70 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 9.1$ Hz, H-4^{GlcN}), 3.53 (m, 1 H, H-5^{GlcN}), 2.11, 2.10, 2.08, 2.07, 2.05, 2.04, 1.99, 1.96, 1.93, 1.89, 1.86 (11 s, 33 H, NAc, OAc); ^{13}C-NMR (100 MHz, $CDCl_3$, α-isomer): δ 170.6, 170.2, 170.1, 170.0, 169.8, 168.8, 168.7, 168.6 [C=O (NAc, OAc)], 100.8 ($J_{C,H} = 160.9$ Hz, C-1^{Gal}, 100.1 ($J_{C,H} = 160.1$ Hz, C-1^{GlcN}), 90.8 ($J_{C,H} = 175.0$ Hz, C-1^{Man}), 76.0 (C-4^{GlcN}), 73.2 (C-2^{Man}), 72.6 (C-5^{GlcN}), 72.2 (C-3^{Man}), 70.8 (C-5^{Man}, C-3^{Gal}), 70.6 (C-5^{Gal}), 69.7 (C-

3^{GlcN}), 69.0 (C-2^{Gal}), 66.6 (C-4^{Gal}), 65.0 (C-4^{Man}), 62.2 (C-6^{GlcN}), 61.9 (C-6^{Gal}), 60.8 (C-6^{Man}), 53.2 (C-2^{GlcN}), 23.0 (NAc), 21.0, 20.9, 20.8, 20.7, 20.6, 20.5 (OAc).

ESI-MS (pos. mode, $CHCl_3$/MeOH = 1/1): $m/z = 988.0$ $[M + Na]^+$; $C_{40}H_{55}NaNO_{26}$ requires 988.3.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-actamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (**27**)

A solution of peracetylated trisaccharide **25** (95 mg, 0.098 mmol) in dry DMF (2 ml) was stirred at 0°C followed by addition of hydrazine acetate (14 mg, 0.15 mmol). After being stirred for 4 h at 0°C, the reaction was quenched with acetic acid (15 µl) and evaporated under high vacuum. The residue was purified by silica gel column chromatography (hexane/acetone, 1/1) to afford 89 mg (97%) of **26**; $R_f = 0.19$ (hexane/acetone, 1.2/1), which was used for the preparation of **27** without further characterization.

A solution of **26** (72 mg, 0.078 mmol) in 2.5 ml dry CH_2Cl_2 containing 0.30 ml (3.0 mmol) of trichloroacetonitrile was stirred at 0°C. 3.0 µl (0.02 mmol) of 1,8-diazabicyclo[5.4.0]uncec-7-ene (DBU) was added and the stirring continued for 2 h at 0°C. Purification by silica gel column chromatography (hexane/acetone, 1.2/1) afforded 75 mg (90%) of the title compound as a white amorphous; $R_f = 0.48$ (hexane/acetone, 1.2/1); $[\alpha]_D^{25} = -3.9$ ($CHCl_3$, 0.33); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.64 (s, 1 H, C = N−H) 6.15 (d, 1 H, $J_{1,2} < 1.5$ Hz, H−1^{Man}), 5.81 (d, 1 H, $J_{NH,2} = 9.0$ Hz, NH^{GlcN}), 5.33 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 9.9$ Hz, H−4^{Man}), 5.32 (m, 1 H, H−4^{Gal}), 5.13–5.03 (m, 3 H, H−3^{Man}, H−3^{GlcN}, H−2^{Gal}), 4.92 (dd, 1 H, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 3.4$ Hz, H−3^{Gal}), 4.57 (d, 1 H, $J_{1,2} = 8.1$ Hz, H−1^{GlcN}), 4.45 (d, 1 H, $J_{1,2} = 8.1$ Hz, H−1^{Gal}), 4.37 (m, 1 H, H−6a^{GlcN}), 4.31 (m, 1 H, H−2^{Man}, 4.17 (m, 1 H, H−6a^{Gal}), 4.10–3.95 (m, 7 H, H−6ab^{Gal}, H−6b^{GlcN}, H−6ab^{Man}, H5^{Man}, H−2^{GlcN}), 3.84 (ddd, 1 H, $J_{4,5} < 1.5$ Hz, $J_{5,6a} \sim J_{5,6b} = 6.7$ Hz, H−5^{Gal}), 3.74 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 8.8$ Hz, H−4^{GlcN}), 3.56 (m, 1 H, H−5^{GlcN}), 2.11, 2.07, 2.06, 2.04, 2.03, 2.01, 2.00, 1.97, 1.92 (9 s, 27 H, OAc), 1.91 (s, 3 H, NAc); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.6, 170.3, 170.1, 170.0, 169.8, 169.7, 168.9 [C=O (NAc, OAc)], 160.0 (C=NH), 100.8 ($J_{C,H} = 161.7$ Hz, C-1^{Gal}), 100.2 ($J_{C,H} = 164.2$ Hz, C-1^{GlcN}), 95.1 ($J_{C,H} = 176.6$ Hz, C-1^{Man}), 75.8 (C-4^{GlcN}), 72.8 (C-5^{GlcN}, C-2^{Man}), 72.0 (C-3^{Man}), 71.5 (C-5^{Man}), 70.8 (C-3^{Gal}), 70.7 (C-5^{Gal}), 69.8 (C-3^{GlcN}), 69.1 (C-2^{Gal}), 66.7(C-4^{Gal}), 65.0 (C-4^{Man}), 62.2 (C-6^{GlcN}), 62.0 (C-6^{Gal}), 60.9 (C-6^{Man}), 53.5 (C-2^{GlcN}), 23.2 (NAc), 20.9, 20.9, 20.8, 20.7, 20.6 (OAc).

ESI-MS (pos. mode): $m/z = 1091.2$; average mass for $C_{40}H_{53}Cl_3N_2NaO_{25}$ 1091.2.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-O-(2-acetamido-3,6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-L-serine allyl ester (**28**)

Trichloroacetimidate **27** (15 mg, 14 µmol) was reacted with serine derivative **19c** (25 mg, 68 µmol) in CH_2Cl_2/toluene (1/1, 0.8 ml) in the presence of molecular sieves 4 Å (150 mg) and TMSOTf (0.6 µl, 3 µmol) as described for the preparation of **20a**. Purification by silica gel column chromatography (hexane/acetone, 1.2/1) afforded the title compound (16 mg, 90%) as a white foam; $R_f = 0.30$ (hexane/acetone, 1.2/1); $[\alpha]_D^{25} = -2.3$ ($CHCl_3$, 1.33); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.76–7.28 (m, 8 H, Ar), 5.92 (m, 1 H, $CH_2-CH=CH_2$), 5.86 (d, 1 H, $J_{NH,H\alpha} = 8.5$ Hz, NH^{Ser}), 5.67 (d, 1 H, $J_{NH,2} = 8.1$ Hz, NH^{GlcN}), 5.37–5.26 (m, 3 H, $CH_2-CH=CH_2$, H-4^{Gal}), 5.21 (dd, 1 H, $J_{2,3} \sim J_{3,4} = 8.4$ Hz, H-3^{GlcN}), 5.18 (dd, 1 H, $J_{3,4} \sim J_{4,5} = 9.9$ Hz, H-4^{Man}), 5.09 (dd, 1 H, $J_{2,3} = 10.6$ Hz, H-2^{Gal}), 5.02 (m, 1 H, H-3^{Man}), 4.94 (dd, 1 H, $J_{3,4} = 3.4$ Hz, H-3^{Gal}), 4.73 (d, 1 H, $J_{1,2} < 1.5$ Hz, H-1^{Man}), 4.70 (m, 2 H, $CH_2-CH=CH_2$), 4.64 (d, 1 H, $J_{1,2} = 7.3$ Hz, H-1^{GlcN}), 4.57 (m, 1 H, α-CH^{Ser}), 4.45 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{Gal}), 4.40–4.28 (m, 3 H, H-6a^{GlcN}, α-CH_2^{Fmoc}), 4.20 (dd, 1 H, $J_{vic} \sim 6.3$ Hz, β-CH^{Fmoc}), 4.17–4.01 (m, 6 H, H-2^{Man}, H-6b^{GlcN}, H-6ab^{Man}, H-6ab^{Gal}), 4.00–3.95 (m, 2 H, β-CH_2^{Ser}), 3.85 (m, 2 H, H-5^{Man}, H-5^{Gal}), 3.75–3.69 (m, 2 H, H-2^{GlcN}, H-4^{GlcN}), 3.58 (m, 1 H, H-5^{GlcN}), 2.13, 2.09, 2.07, 2.05, 2.04, 2.02, 1.99, 1.98, 1.95, 1.89–1.94 (10 s, 30 H, NAc, OAc); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.5, 170.2, 170.1, 170.0, 169.9, 169.8, 169.3, 169.2, 169.0 (C=O), 155.6 [C=O (Fmoc)], 143.6, 143.5, 141.1 (Ar), 131.3 ($CH_2-CH=CH_2$), 127.6, 127.0, 125.0, 119.9 (Ar), 119.1 ($CH_2-CH=CH_2$), 100.9 (C-1^{Gal}), 99.3 (C-1^{GlcN}), 98.3 ($J_{C,H} = 172.2$ Hz, C-1^{Man}), 75.8 (C-4^{GlcN}), 74.3 (C-2^{Man}), 72.7 (C-5^{GlcN}), 71.4 (C-3^{GlcN}), 70.8 (C-5^{Man}, C-3^{Gal}), 69.9 (C-3^{Man}), 69.4 (C-5^{Gal}), 69.3 (β-C^{Ser}), 69.1 (C-2^{Gal}), 67.4 (α-C^{Fmoc}), 66.7 (C-4^{Gal}), 66.5 ($CH_2-CH=CH_2$), 65.9 (C-4^{Man}), 62.6 (C-6^{GlcN}), 62.5 (C-6^{Gal}), 60.9 (C-6^{Man}), 54.4 (α-C^{Ser}), 53.8, 47.2 (β-C^{Fmoc}), 23.3 (NAc), 21.0, 20.8, 20.8, 20.7 (OAc).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 1295.4$ $[M + Na]^+$; $C_{59}H_{72}NaN_2O_{29}$ requires 1295.4.

O-(β-D-Galactopyranosyl)-(1 → 4)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-α-D-mannopyranosyl]-L-serine (**29**)

a) Allyl ester cleavage. A mixture of glycosyl amino acid **28** (20 mg, 16 µmol), dimedone (29 mg, 0.2 mmol) and $Pd(Ph_3P)_4$ (10 mg, 8.5 µmol) in dry THF (1 ml) was stirred for 2 h at room temperature under Ar atmosphere. After complete cleavage of the allyl ester was observed, the mixture was concentrated *in vacuo* and purified by silica gel column chromatography (1% HOAc in hexane/acetone, 1/1) to furnish free acid (19.3 mg, >95%) as a white amorphous; $R_f = 0.30$ (hexane/acetone, 1.2/1).

b) Fmoc cleavage and deacetylation. The free acid (19.0 mg, 15.4 µmol) was dissolved in a mixture of dry $CHCl_3$ (1 ml) and morpholine (1 ml) and stirred for 90 min at room temperature. The mixture was concentrated *in vacuo*, coevaporated with toluene (3 × 1 ml) and dried under high vacuum to furnish an

amorphous glass, which was then dissolved in dry MeOH (5 ml). Methanolic solution of NaOMe (0.87 M, 20 µl) was added and the solution was stirred for 1 h (pH ~ 8.5). Additional amounts; [1) 20 µl (1 h); 2) 20 µl (2 h); 3) 30 µl (3 h); 4) 20 µl (5 h)] of 0.87 M NaOMe solution were added to ensure the complete reaction. After stirring for additional 9 h (pH ~ 9.0–9.5), the reaction mixture was quenched with 10% acetic acid, evaporated and dried under high vacuum. Final purification was achieved by size exclusion chromotography (Bio-Gel® P-2, H_2O) to afford the title compound (8.0 mg, 82%) as a white amorphous; $R_f = 0.52$ (*n*-BuOH/MeOH/H_2O/HOAc, 2/2/2/1); $[\alpha]_D^{25} = 0.5$ (0.53, H_2O); ^{1}H-NMR (500 MHz, D_2O): δ 4.87 (d, 1 H, $J_{1,2} < 1.5$ Hz, H-1^{Man}), 4.57 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1^{GlcN}), 4.46 (d, 1 H, $J_{1,2} = 7.8$ Hz, H-1^{Gal}), 4.09 (m, 1 H, H-2^{Man}), 3.98–3.87 (m, 5 H, H-6a^{GlcN}, H-4^{Gal}, H-3^{Man}, H-6a^{Man}, α-CH^{Ser}), 3.87–3.81 (m, 2 H, H-6b^{GlcN}, β-CH^{Ser}), 3.79–3.70 (m, 7 H, β-CH^{Ser}, H-2^{Gal}, H-3^{GlcN}, H-4^{GlcN}, H-6ab^{Gal}, H-5^{Gal}), 3.68–3.46 (m, 6 H, H-3^{Gal}, H-5^{Man}, H-6b^{Man}, H-5^{GlcN}, H-2^{Gal}, H-4^{Man}), 2.05 (s, 3 H, NAc); ^{13}C-NMR (125 MHz, D_2O): δ 175.4 (NAc), 172.9 (CO_2H), 103.6 ($J_{C,H} = 162.6$ Hz, C-1^{Gal}), 100.2 ($J_{C,H} = 161.9$ Hz, C-1^{GlcN}), 98.3 ($J_{C,H} = 170.2$ Hz, C-1^{Man}), 79.1 (C-4^{GlcN}), 76.9 (C-2^{Man}), 76.0 (C-3^{GlcN}), 75.4 (C-5^{GlcN}), 73.9 (C-5^{Man}), 73.2 (C-3^{Gal}), 72.7 (C-5^{Gal}), 71.6 (C-2^{Gal}), 70.0 (α-C^{Ser}), 69.2 (C-3^{Man}, C-4^{Gal}), 67.9 (C-4^{Man}), 62.3 (C-6^{Man}), 61.7 (C-6^{Gal}, β-C^{Ser}), 60.6 (C-6^{GlcN}), 55.5 (C-2^{GlcN}), 23.0 (NAc).

ESI-MS (neg. mode, MeOH/H_2O, 1/1): $m/z = 631.3$ $[M\text{-}N]^-$; $C_{23}H_{39}N_2O_{18}$ requires 631.2.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-(3,4,6-tri-O-benzyl-2-O-p-methoxybenzyl-α-D-mannopyranosyl)-L-serine benzyl ester (**31**)

a) attachment of the amino acid. A solution of the fluoride **30** (100 mg, 0.174 mmol), serine derivative **19g** (87 mg, 0.21 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (47 mg, 0.23 mmol) in 6 ml dry toluene/CH_2Cl_2 (1/1) was stirred under argon with freshly activated molecular sieves 4 Å (500 mg). Then, a mixture of $SnCl_2$ (43 mg, 0.23 mmol) and $AgClO_4$ (47 mg, 0.23 mmol) was added and stirring continued for 5 d at room temperature. After being diluted with CH_2Cl_2, the mixture was filtered through Celite and the filtrate was washed successively with 1 N $KHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. Purification by silica gel column chromatography (hexane/ethyl acetate, 2.5/1) gave 138 mg (82%) of **31**α/β as a slightly yellow amorphous; $R_f = 0.44$ (hexane/ethyl acetate, 2/1). The separation of the α/β-isomers was performed after subsequent cleavage of the *p*-methoxybenzyl group.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-L-serine benzyl ester (**32**)

The anomeric mixture **31**α/β (2.40 g, 2.47 mmol) was dissolved in 100 ml acetonitrile/H_2O (9:1), and treated with 3.50 g (6.38 mmol) of ammonium cerium (IV) nitrate (CAN) for 6 h at 0°C. The mixture was diluted with CH_2Cl_2 and washed with 1 N $KHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The dark yellow oil was purified by silica gel column chromatography (toluene/ethyl acetate, 4/1) to afford **32** (1.56 g, 74%) together with its stereoisomer **32**β (260 mg, 12%).

32: $R_f = 0.20$ (toluene/ethyl acetate, 4/1); $[\alpha]_D^{25} = 25.8$ ($CHCl_3$, 0.75); ^{1}H-NMR (270 MHz, $CDCl_3$): δ = 7.67 (m, 2 H, Fmoc) 7.54 (m, 2 H, Fmoc), 7.32–7.06 (m, 24 H, Ph, Fmoc), 5.75 (d, 1 H, $J_{NH,H\alpha} = 8.6$ Hz, NH), 5.20 (d, 1 H, $J_{gem} = 12.2$ Hz, OCH_2), 5.04 (d, 1 H, $J_{gem} = 12.2$ Hz, OCH_2), 4.72 (d, 1 H, $J_{gem} = 10.9$ Hz, OCH_2), 4.71 (d, 1 H, $J_{1,2} < 1.5$ Hz, H-1α), 4.56 (bs, 2 H, OCH_2), 4.54 (d, 1 H, $J_{gem} = 12.6$ Hz, OCH_2), 4.53 (m, 1 H, α-CH^{Ser}), 4.43 (m, 2 H, OCH_2), 4.28 (m, 2 H, OCH_2^{Fmoc}), 4.15 (m, 1 H, β-CH^{Fmoc}), 3.91 (m, 2 H, β-CH_2^{Ser}), 3.76–3.58 (m, 6 H, H-5, H-4, H-2, H-3, H-6ab), 2.31 (d, 1 H, $J_{2,OH} = 2.0$ Hz, 2-OH); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 170.5 [C=O (Bn-ester)], 156.7 [C=O (urethane)], 144.5, 141.9, 141.3, 138.9, 138.8, 138.5, 135.9, 129.5, 129.3, 129.0, 129.0, 128.7, 128.5, 128.4, 128.2, 127.8, 127.8, 120.6, 101.1 ($J_{CH} = 174.9$ Hz, C-1), 80.5 (C-3), 75.9 (OCH_2), 74.7 (C-4), 74.1, 72.7 (OCH_2), 72.4 (C-2), 69.8 (β-C^{Ser}), 69.4 (C-6), 68.8 (C-5), 68.1 [OCH_2 (Bn-ester)], 68.0 (α-C^{Fmoc}), 55.2 (α-C^{Ser}), 47.8 (β-C^{Fmoc}).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 872.0$ $[M + Na]^+$; $C_{52}H_{51}NaNO_{10}$ requires 872.3.

32β: $R_f = 0.24$ (toluene/ethyl acetate, 4/1); $[\alpha]_D^{25} = -1.1$ ($CHCl_3$, 2.0); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.66 (m, 2 H, Fmoc) 7.51 (m, 2 H, Fmoc), 7.40–7.11 (m, 24 H, Ph, Fmoc), 5.74 (d, 1 H, $J_{NH,H\alpha} = 8.3$ Hz, NH), 5.12 (bs, 2 H, OCH_2, Bn-ester), 4.81 (d, 1 H, $J_{gem} = 10.9$ Hz, OCH_2), 4.68 (d, 1 H, $J_{gem} = 11.9$ Hz, OCH_2), 4.56 (d, 1 H, $J_{gem} = 11.8$ Hz, OCH_2), 4.54–4.21 (m, 7 H, α-CH^{Ser}, OCH_2, H-1α, OCH_2^{Fmoc}, $CH_aH_b^{Ser}$), 4.13 (m, 1 H, β-CH^{Fmoc}), 3.99 (bs, 1 H, H-2), 3.87–3.56 (m, 4 H, $CH_a\underline{H_b}^{Ser}$, H-4, H-6ab), 3.44 (dd, 1 H, $J_{2,3} = 3.0$ Hz, $J_{3,4} = 8.9$ Hz, H-3), 3.30 (m, 1 H, H-5), 2.18 (bs, 1 H, 2-OH); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 169.7 [C=O (Bn-ester)], 156.0 [C=O (urethane)], 143.8, 143.7, 141.3, 138.2, 138.1, 137.7, 135.2, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.7, 127.6, 127.0, 125.1, 120.0, 99.8 ($J_{CH} = 163.1$ Hz, C-1), 81.2 (C-3), 75.3 (C-5), 75.1 (OCH_2), 74.0 (C-4), 73.4 (OCH_2), 71.4 (OCH_2), 69.0 (C-6), 68.9 (β-C^{Ser}), 67.9 (C-2), 67.4 [OCH_2 (Bn ester)], 67.1 (α-C^{Fmoc}), 54.4 (α-C^{Ser}), 47.1 (β-C^{Fmoc}).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 872.6$ $[M + Na]^+$; $C_{52}H_{51}NaNO_{10}$ requires 872.3.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-(2-azido-3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-β-D-glucopyranosyl-(1 → 2)-O-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-L-serine benzyl ester (**34**)

A solution of mannosyl serine **32** (100 mg, 0.118 mmol) and thioglycoside **33a** (121 mg, 0.240 mmol) in 4 ml dry acetonitrile was stirred under argon with freshly activated molecular

sieves 3Å (500 mg) at −40°C. A solution of *N*-iodosuccinimide (NIS, 103 mg, 0.46 mmol) in 2 ml dry acetonitrile was added and stirring continued for 20 min at the same temperature followed by the addition of trifluoromethanesulfonic acid (TfOH, 4 µl, 0.04 mmol). After being stirred for additional 2 h, the reaction was quenched with triethylamine (84 µl, 0.6 mmol). The mixture was diluted with CH_2Cl_2, filtered through Celite and evaporated. The residue was dissolved in chloroform, washed with 10% $KHCO_3/Na_2S_2O_3$ solution (2 × 40 ml), dried over $MgSO_4$ and evaporated *in vacuo*. Purification by silica gel column chromatography (hexane/acetone, 3/1) gave a mixture of **34** and corresponding α-isomer (123 mg, 81%); $R_f = 0.53$ (α-isomer) and 0.51 (**34**) (hexane/acetone, 2/1), which was subjected to the next reaction without further purification.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-[(2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl]-L-serine benzyl ester (**35**).
Diastereomeric mixture of **34** (95 mg, 0.073 mmol) was dissolved in ethanol (5 ml) and treated with thiourea (21 mg, 0.28 mmol) under reflux for 8 h. The mixture was diluted with ethyl acetate, washed successively with 1 N $KHCO_3$ and brine, successively, dried over $MgSO_4$ and evaporated *in vacuo*. Purification by silica gel column chromatography (hexane/acetone, 3/1) gave **35** (71.2 mg, 81%) together with corresponding stereoisomer (10.1 mg, 10%).

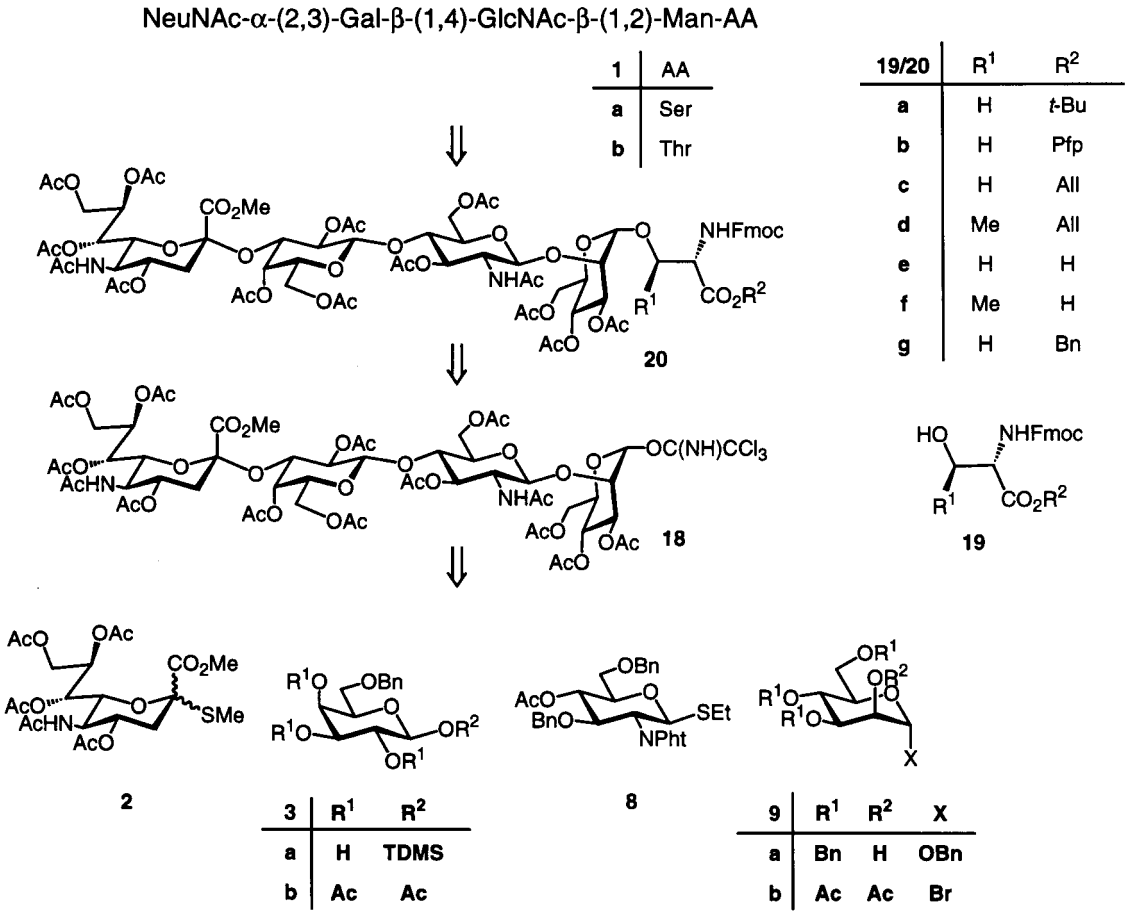
35: $R_f = 0.34$ (toluene/ethyl acetate, 4/1); $[\alpha]_D^{25} = -11.7$ ($CHCl_3$, 0.8); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.67 (m, 2 H, Fmoc) 7.48 (m, 2 H, Fmoc), 7.26–7.04 (m, 24 H, Ar), 5.90 (d, 1 H, $J_{NH,H\alpha} = 8.6$ Hz, NH), 5.15 (d, 1 H, $J_{gem} = 12.6$ Hz, OCH_2), 5.08 (d, 1 H, $J_{gem} = 12.6$ Hz, OCH_2), 4.84 (d, 1 H, $J_{gem} = 11.6$ Hz, OCH_2), 4.82 (d, 1 H, $J_{1,2} < 1.5$ Hz, H-1), 4.71 (d, 2 H, $J_{gem} = 10.9$ Hz, OCH_2), 4.68 (d, 1 H, $J_{gem} = 11.2$ Hz, OCH_2), 4.55 (m, 1 H, α-CH^{Ser}), 4.44–4.36 (m, 5 H, OCH_2), 4.30 (d, 1 H, $J_{gem} = 10.6$ Hz, OCH_2), 4.28–4.23 (m, 2 H, OCH_2), 4.21 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1^{GlcN}), 4.12 (m, 2 H, β-CH^{Fmoc}, CH_a H_b^{Ser}), 4.01 (bs, 1 H, H-2^{Man}), 3.92 (m, 1 H, $CH_aH_b^{ser}$), 3.75–3.50 (m, 8 H, H-3^{Man}, H-5^{Man}, H-4^{Man}, H-6ab^{GlcN}, H-$6_{ab}{}^{Man}$, H-4^{GlcN}), 3.46 (dd, 1 H, $J_{2,3} = 9.6$ Hz, H-3^{GlcN}), 3.35 (m, 1 H, H-5^{GlcN}), 2.57 (bs, 1 H, 4^{GlcN}-OH); ^{13}C-NMR (6.75 MHz, $CDCl_3$): δ 169.9 [C=O (Bn ester)], 156.0 [C=O (urethane)], 143.8, 141.2, 138.2, 138.1, 138.0, 137.4, 135.3, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.4, 127.1, 125.2, 119.9, 101.1 (J_{CH} = 164.8 Hz, C-1^{GlcN}), 98.6 ($J_{CH} = 173.6$ Hz, C-1^{Man}), 8.21 (C-3^{GlcN}), 77.8 (C-3^{Man}), 74.9 (OCH_2), 74.7 (C-4^{Man}), 74.2 (C-2^{GlcN}), 74.0 (C-5^{GlcN}), 73.8, 73.1 (OCH_2), 72.3 (C-5^{Man}), 71.8 (C-4^{GlcN}), 71.3 (OCH_2), 70.6 (C-6^{GlcN}), 69.4 (C-6^{Man}), 69.1 (β-C^{Ser}), 67.3 (OCH_2), 54.5 (α-C^{Ser}), 47.0 (β-C^{Fmoc}).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 1239.9$ $[M + Na]^+$; $C_{72}H_{72}NaN_4O_{14}$ requires 1239.5.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-[(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl]-L-serine benzyl ester (**36**).
The disaccharide **35** (75 mg, 0.062 mmol) was dissolved in dry methanol (2 ml) and acetic anhydride (200 µl). The solution was hydrogenated over Lindlar catalyst (75 mg) under atmospheric pressure at room temperature for 12 h. The catalyst was removed by filtration through a Celite pad and the filter cake was thoroughly washed with methanol. The combined filtrate and washings were concentrated *in vacuo*, coevaporated with toluene (3 × 20 ml) and dried under high vacuum. The residue was purified by silica gel column chromatography (hexane/acetone, 1.5/1) and preparative TLC (hexane/acetone, 1.2:1) to give **36** (52 mg, 68%) as a white foam; $R_f = 0.44$ (hexane/acetone, 1.2/1); $[\alpha]_D^{25} = -2.8$ ($CHCl_3$, 0.7); ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.69 (m, 2 H, Fmoc) 7.51 (m, 2 H, Fmoc), 7.34–7.15 (m, 34 H, Ph, Fmoc), 5.90 (d, 1 H, $J_{NH,H\alpha} = 8.6$ Hz, NH^{Ser}), 5.59 (d, 1 H, $J_{NH,2} = 6.8$ Hz), 5.18 (d, 1 H, $J_{gem} = 12.3$ Hz, OCH_2), 5.12 (d, 1 H, $J_{gem} = 12.3$ Hz, OCH_2), 5.07 (d, 1 H, $J_{1,2} = 8.5$ Hz, H-1^{GlcN}), 4.84 (d, 1 H, $J_{gem} = 11.0$ Hz, OCH_2), 4.66 (d, 1 H, $J_{gem} = 11.4$ Hz, OCH_2), 4.62 (d, 1 H, $J_{1,2} < 1.5$ Hz, H-1^{Man}), 4.61 (bs, 2 H, OCH_2), 4.49 (m, 1 H, H-α^{Ser}), 4.48–4.32 (m, 4 H, OCH_2), 4.30–4.22 (m, 4 H, OCH_2, CH_2^{Fmoc}), 4.14 (dd, 1 H, $J_{vic} = 7.2$ Hz, β-CH^{Fmoc}), 3.98–3.84 (m, 3 H, CH_2^{Ser}, H-2^{Man}), 3.75–3.45 (m, 10 H, H-3^{Man}, H-6ab^{GlcN}, H-6ab^{Man}, H-3^{GlcN}, H-4^{Man}, H-5^{Man}, H-5^{GlcN}, H-4^{GlcN}), 2.91 (ddd, 1 H, $J_{2,3} = 10.0$ Hz, H-2^{GlcN}), 2.62 (bs, 1 H, 4^{GlcN}-OH), 1.65 (s, 3 H, NAc).

ESI-MS (pos. mode, $CHCl_3$/MeOH, 1/1): $m/z = 1255.4$ $[M + Na]^+$; $C_{72}H_{72}NaN_2O_{14}$ requires 1255.5.

O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-α-D-mannopyranosyl-L-serine (**37**)
To a solution of the glycosyl amino acid **36** (42 mg, 0.035 mmol) in ethanol/THF/H_2O (4/2/1, 7 ml) was added $Pd(OH)_2/C$ (33 mg) and stirred under atmospheric pressure of H_2 at room temperature for 12 h. A second portion (30 mg) of $Pd(OH)_2/C$ was added and the stirring continued for another 24 h. The catalyst was once removed by filtration and the filtrate was, after concentration *in vacuo*, again subjected to hydrogenation over 35 mg of $Pd(OH)_2/C$ in 5 ml ethanol/H_2O (4:1). After 24 h, the reaction was found to be complete. The catalyst was filtered off and the filtrate was passed through SepPak® RP18 cartridge (Fa. Waters, MeOH) and concentrated *in vacuo*. Final purification was achieved by size exclusion chromatography (Bio-Gel® P-2, H_2O) to give 16.0 mg (96%) of the title compound; $R_f = 0.20$, (n-BuOH/MeOH/H_2O/HOAc, 6/2/2/1); $[\alpha]_D^{25} = -0.4$ (H_2O, 1.0); ^{1}H-NMR (500 MHz, D_2O): δ 4.72 (d, 1 H, $J_{1,2} = 1.4$ Hz, H-1^{Man}), 4.40 (d, 1 H, $J_{1,2} = 8.6$ Hz, H-1^{GlcN}), 3.97 (m, 2 H, H-β^{Ser}, H-2^{Man}), 3.80–3.77 (m, 3 H, H-β^{Ser}, H-α^{Ser}, H-6a^{GlcN}), 3.76 (m, 1 H, H-6a^{Man}), 3.71 (dd, 1 H, $J_{2,3} = 3.4$ Hz, $J_{3,4} = 9.6$ Hz, H-3^{Man}), 3.61 (dd, 1 H, $J_{gem} = 12.3$ Hz, $J_{5,6b} = 5.4$ Hz, H-6b^{GlcN}), 3.55 (dd, 1 H $J_{2,3} = 10.5$ Hz, H-



Scheme 1. Synthetic design of the tetrasaccharide serine/threonine.

2^{GlcN}), 3.50–3.44 (m, 2 H, H-6b^{Man}, H-5^{Man}), 3.41 (dd, 1 H $J_{3,4} = 8.6$ Hz, H-3^{GlcN}), 3.37 (dd, 1 H $J_{3,4} \sim J_{4,5} = 9.6$ Hz, H-4^{Man}), 3.31–3.30 (m, 2 H, H-4^{GlcN}, H-5^{GlcN}), 1.91 (s, 3 H, NAc); ^{13}C-NMR (125 MHz, D_2O): δ 175.0 [C=O (NAc)], 172.5 [C=O (CO_2H)], 99.9 (C-1^{GlcN}), 97.9 (C-1^{Man}), 76.5 (C-2^{Man}), 76.0 (C-4^{GlcN}), 73.6 (C-3^{GlcN}), 73.5 (C-5^{Man}), 70.1 (C-5^{GlcN}), 69.6 (C-3^{Man}), 67.4 (C-4^{Man}), 66.5 (β-C^{Ser}), 61.8 (C-6^{Man}), 60.8 (C-6^{GlcN}), 55.5 (C-2^{GlcN}), 54.8 (α-C^{Ser}), 22.5 (NAc).

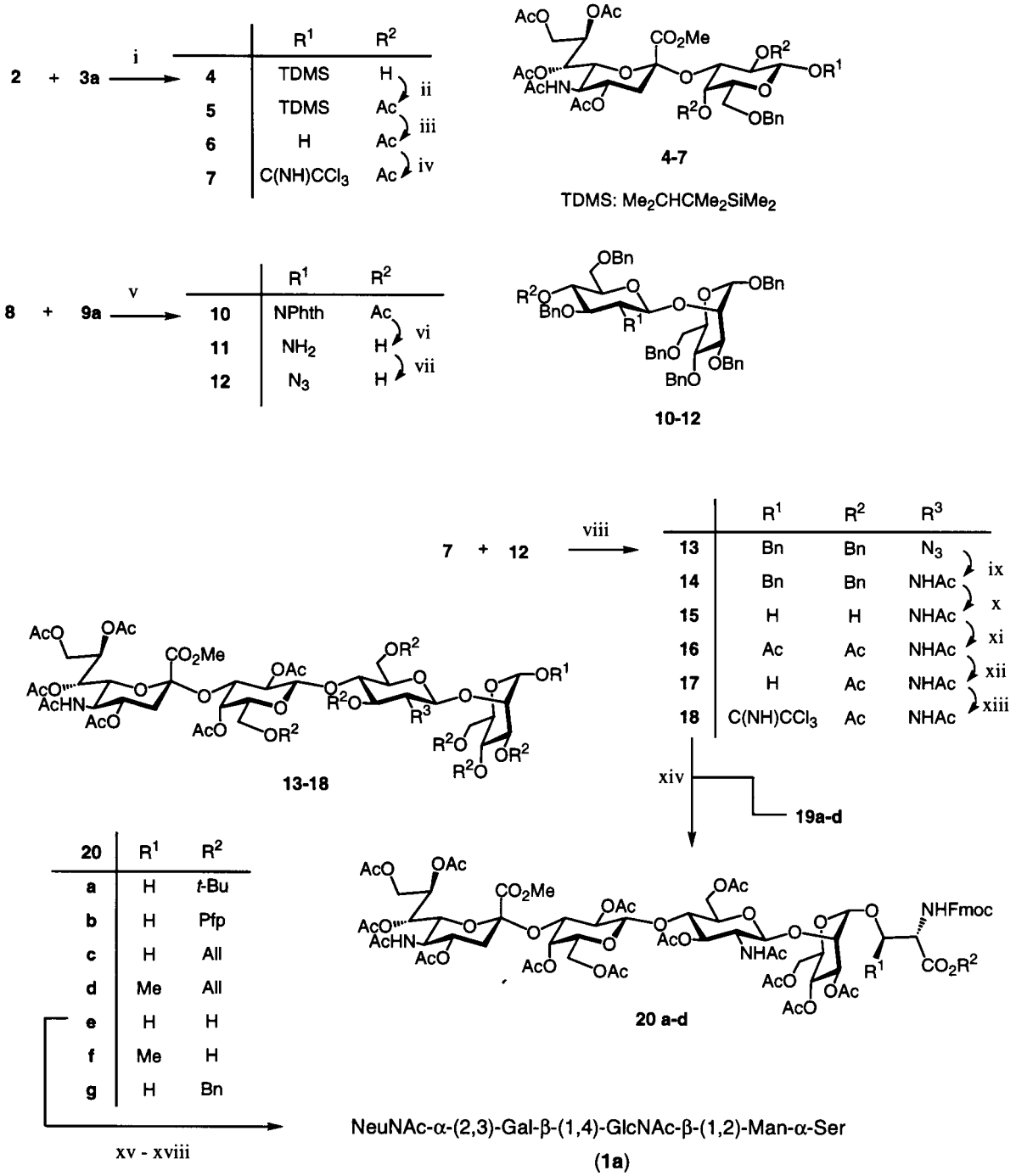
ESI-MS (neg. mode, MeOH/H_2O, 1/1): $m/z = 471.3$ $[M]^-$; $C_{17}H_{30}N_2O_{13}$ requires 470.4.

Results and discussion

As a general design for the synthesis of the Fmoc-protected glycosyl amino acids **20a–d** (Scheme 1), incorporation of the amino acid moiety was envisaged to be performed at the end of the synthesis, by coupling with a tetrasaccharide donor. Because of the intrinsic 1,2-trans directing nature of mannosyl donor, it was expected that α-selective glycosylation with Ser/Thr should be possible under stereoelectronically controlled conditions. By using the tetrasaccharide donor (e.g. **18**) as the common intermediate, maximum flexibility in terms of the amino acid protection pattern can be provided. It was also assumed that technical problem that may be caused by the presence of acid and/or base sensitive and potentially nucleophilic amino acid component can be minimized at the stage of oligosaccharide assembly. Trichloroacetimidate technology [9] was adopted for this purpose, because of its reliability, particularly for complex oligosaccharide condensation [10].

The synthesis of the tetrasaccharide fragment was executed as depicted in Scheme 2. Preparation of the left hand disaccharide fragment **7** was commenced with galactose derivative **3a**, which in turn was prepared from 2,3,4-tri-O-acetyl-6-O-benzyl-D-galactopyranosyl acetate (**3b**) [11] in 3 steps; i) N_2H_4·HOAc, DMF, 0°C (85%); ii) Me_2CHCMe_2Si-Me_2Cl (TDMSCl), imidazole, DMF, 0°C ~ RT [12] (91%); iii) NaOMe/MeOH, −10°C (93%).

Glycosylation with sialic acid donor **2** [13] was effected under standard conditions using *N*-iodosuccinimide (NIS)-trifluoromethanesulfonic acid (TfOH) [13–16] and resultant **4** was isolated after being acetylated to **5** which was obtained in 57%



Scheme 2. Synthesis of tetrasaccharide Ser/Thr: i) NIS, TfOH/MeCN, − 40°C. ii) Ac_2O/pyridine, r.t., 12 h, 57% over 2 steps. iii) Bu_4NF, AcOH/THF, r.t., 3 d, 92%. iv) CCl_3CN, DBU/CH_2Cl_2, 0°C, 93%. v) NIS, TfOH/MeCN, − 40°C, 1 h, 95%. vi) $H_2N(CH_2)_2NH_2$/*n*-BuOH, 100°C, 20 h. vii) TfN_3, 4-DMAP/MeCN-CH_2Cl_2, r.t., 24 h, 95% over 2 steps. viii) TMSOTf/CH_2Cl_2, −20°C, 84%. ix) $SH(CH_2)_3SH$, (*i*-Pr)$_2$NEt/pyridine-H_2O, r.t., 14 h, then Ac_2O, r.t., 8 h, 95%. x) $Pd(OH)_2$/C, H_2/MeOH-AcOH, r.t., 16 h. xi) Ac_2O/pyridine, r.t., 12 h, 95% over 2 steps. xii) NH_2NH_2·AcOH/DMF, 0°C, 6 h, 95%. xiii) CCl_3CN, DBU/CH_2Cl_2, 0°C, 3 h, 94%. xiv) TMSOTf/CH_2Cl_2-toluene (1:1), − 20°C, 1 h, 91%. xv) $Pd(PPh_3)_4$, dimedone/THF, r.t., 3 h, 91%. xvi) morpholine/$CHCl_3$, r.t., 90 min. xvii) NaOMe/MeOH, r.t. xviii) NaOH/aq. MeOH, r.t., 4 h, 92%.

yield. The regioselectivity of the sialylation was unequivocally confirmed at this stage, based on ^{1}H-NMR analysis. Subsequent desilylation with Bu_4NF-AcOH gave **6** that was transformed to the trichloroacetimidate **7** by using trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]uncec-7-ene (DBU).

On the other hand, the right hand fragment **12** was synthesized from GlcNAc derived thioglycoside **8** [17] and Man derivative **9a**. Requisite **9a** was prepared from 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide (**9b**) [18] in 4 steps; i) BnOH, 2,4,6-collidine, MeCN (83%); ii) BnBr, KOH,

toluene; iii) TMSOTf, CH_2Cl_2, MS 4 Å; iv) NaOMe, MeOH, 80% overall. Coupling of these components was effected by NIS-TfOH [14–16] to afford **10** in high yield. Subsequent dephthaloylation [19] led to amine **11** that was masked as azide **12** by treatment with TfN_3 [20].

With the building blocks **7** and **12** in hand, the formation of tetrasaccharide **13** was next examined. Coupling of these fragments was achieved with TMSOTf (0.2 equiv.) as a promoter to give **13** in 83% yield. Subsequent conversion into **18** was performed by a series of straightforward functional group transformations. Thus, reduction of azide with propanedithiol [21] was followed by acetylation to afford **14** that was subjected to debenzylation (to **15**), acetylation (to **16**) and chemoselective deacetylation to give **17**. Subsequent transformation to trichloroacetimidate **18** was performed under standard conditions.

The stereoselective attachment of the Fmoc protected amino acids **19a** [22], **19b** [23], **19c** [24], and **19d** [24] to **18** was effected by the action of TMSOTf (0.2 equiv.) in CH_2Cl_2/toluene. The reaction proceeded cleanly at $-20°C$ to provide the α-linked glycosyl aminoacids **20a, b, c** and **d** as the only identifiable coupled products in 91%, 63%, 84% and 89% yield, respectively.

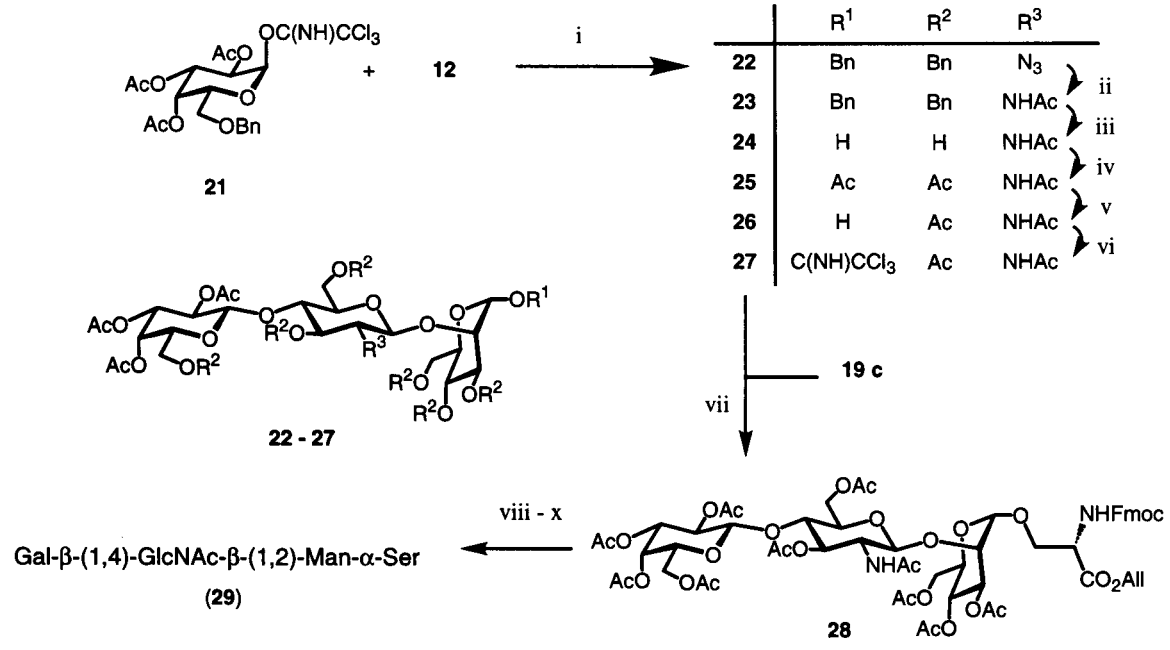
Completely deblocked tetraosyl serine **1a** was obtained from **20c** as follows. First, the allyl ester was cleaved by $Pd(Ph_3P)_4$ in the presence of dimedone to afford free acid **20e**.

Threonine-linked **20d** was deallylated in a similar manner to give **20f**. Subsequent Fmoc removal from **20e** effected by morpholine was followed by complete deprotection of O-acetyl groups (NaOMe/MeOH, pH ≈ 9.0) and saponification of methyl ester (5 mM NaOH/aq. MeOH). Purification by size exclusion chromatography (Bio-Gel® P-2) furnished the deprotected compound **1a** in 81% overall yield from **20c**. The structure of **1a** was securely confirmed by NMR and ESI-mass spectroscopy. During the course of this study, the same compound was synthesized by Matsuo *et al.* by combined use of chemical and enzymatic means [25].

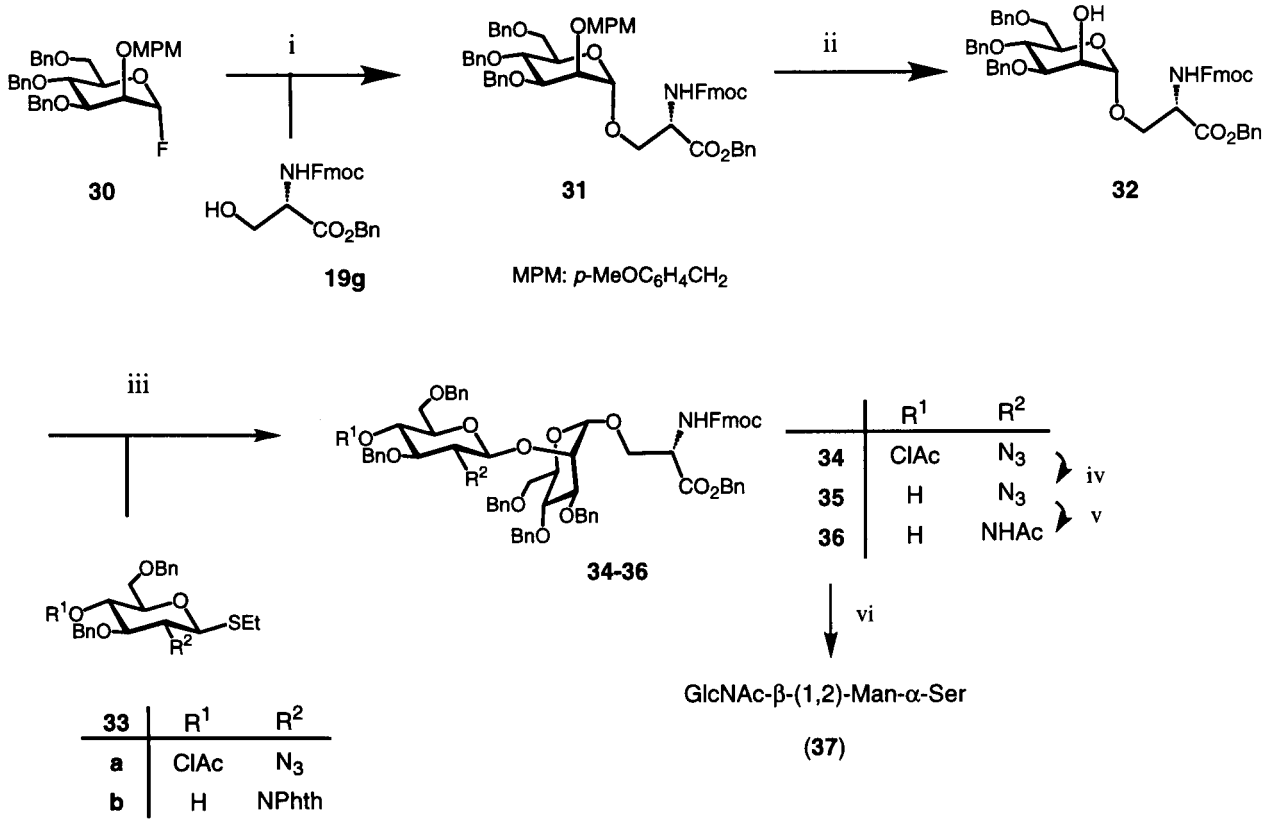
The trisaccharide serine **29** was prepared in a manner analogous to that described for **1a** (Scheme 3). Thus, galactosyl donor **21** derived from **3b** was coupled with **12** ($TMSOTf/CH_2Cl_2$, 87%) to give trisaccharide **22** that was converted to trisaccharide donor **27** via **23, 24, 25** and **26**. Coupling with serine derivative **19c** was again effected by the action of TMSOTf (CH_2Cl_2/toluene, 1/1, $-20°C$), which proceeded stereoselectively to give **28** as a single isomer (90% yield, $^1J_{C,H} = 172$ Hz).

Preparation of completely deprotected glycosyl serine **29** was performed in 3 steps. Purification by size exclusion chromatography (Bio-Gel® P-2) furnished **29** in 78% overall yield from **28**. The structure of **29** was confirmed by NMR and ESI-mass spectroscopy.

For the preparation of disaccahride serine **37**, the possibility was explored to elongate the glycan chain from glycosylated amino acid. (Scheme 4) Mannosylated serine **32** having free 2-OH group called for this purpose was synthesized from fluoride **30** [26] and Fmoc/Bn protected serine **19g** [27,28].



Scheme 3. Synthesis of trisaccharide serine: i) $TMSOTf/CH_2Cl_2$, $-20°C$, 1 h, 87%. ii) $SH(CH_2)_3SH$, $(i\text{-}Pr)_2NEt$/pyridine-H_2O, r.t., 14 h, then Ac_2O, r.t., 8 h, 89%. iii) Pd/C, H_2/MeOH-AcOH, r.t., 24 h. iv) Ac_2O/pyridine, r.t., 12 h, 97% over 2 steps. v) NH_2NH_2·AcOH/DMF, 0°C, 4 h, 97%. vi) CCl_3CN, DBU/CH_2Cl_2, 0°C, 2 h, 90%. vii) $TMSOTf/CH_2Cl_2$-toluene, $-20°C$, 90%. viii) $Pd(PPh_3)_4$, dimedone/THF, r.t., 2 h, 95%. ix) morpholine/$CHCl_3$, r.t., 90 min. x) NaOMe/MeOH, r.t., 82%.



Scheme 4. Synthesis of disaccharide serine: i) $AgClO_4$, $SnCl_2$, 2,6-di-*tert*-butyl-4-methylpyridine/toluene-CH_2Cl_2, r.t., 5 d, 82% (α:β = 6:1). ii) CAN/90% aq. MeCN, r.t., 6 h, 74%. iii) NIS, TfOH/MeCN, $-40°C$, 2 h, 81% (α :β = 1:8). iv) thiourea/EtOH, reflux, 8 h, 81%. v) Lindlar cat., H_2, Ac_2O/MeOH, r.t., 12 h, 68%. vi) $Pd(OH)_2/C$, H_2/EtOH-THF-H_2O, r.t., 96%.

Coupling under Mukaiyama's conditions [29] afforded **31** ($\alpha/\beta = 6/1$; 82%). Although preparative scale separation of diastereomers turned out to be impractical at this stage, subsequent oxidative removal [30] of the *p*-methoxybenzyl (MPM) group allowed for the isolation of stereochemically homogeneous **32** (74%) together with corresponding β-isomer (12%).

As a latent GlcNAc donor, azide carrying thioglycoside **33a** was adopted, because the use of phthaloyl group for 2-NH_2 protection should be avoided due to the presumed instability of the molecule under the conditions required for dephthaloylation. It was prepared from ethyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**33b**) [17]; i) ethylenediamine, *n*-BuOH, 100°C; ii) $CF_3SO_2N_3$ (TfN_3), DMAP, MeCN-CH_2Cl_2; iii) $(ClCH_2CO)_2O$, pyridine, CH_2Cl_2, 0°C, 89% over 3 steps. In order to attain β-selective glycosylation, coupling with **32** was performed in acetonitrile [31] in the presence of NIS-TfOH [13–16]. The reaction afforded a mixture of desired **34** and a minor amount of α-isomer (β:α = 8:1, 81%), which was subjected to the removal of chloroacetyl group. The resulting **35** was separated from its stereoisomer and further converted to acetamide **36**. Hydrogenolytic debenzylation was accompanied by the cleavage of Fmoc group to afford **37**.

Although the use of regioselectively protected GlcNAc donor **33a** for the current purpose is redundant, **34** obtained by this route would provide more direct access to **1a** and its derivatives.

Conclusion

We have established a versatile and efficient stereoselective synthetic route to α-DG derived oligosaccharide. This structure was synthesized in its deprotected (**1a**) as well as in variously protected (**20a–d**) forms. The latter would be valuable for the construction of glycopeptides carrying oligosaccharide characteristic to α-DG. Lower homologues **29, 37** and **32** consist of tri-, di- and monosaccharide, respectively were also synthesized. All of these compounds are structurally well-defined and would be valuable as molecular probe to disclose the biological function and/or biosynthetic pathway of this unique class of glycoprotein glycan chain.

Tentatively assumed α-configuration of Ser/Thr linked mannose residue has not been clarified yet. Comparative studies using analogs having β-mannosidic linkage would be required to remove this uncertainty. Stereocontrolled synthetic access to such β-linked counterparts would be possible by making use of *p*-methoxybenzyl assisted intramolecular

agylcon delivery, which was developed in this laboratory [10,32,33].

Acknowledgements

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