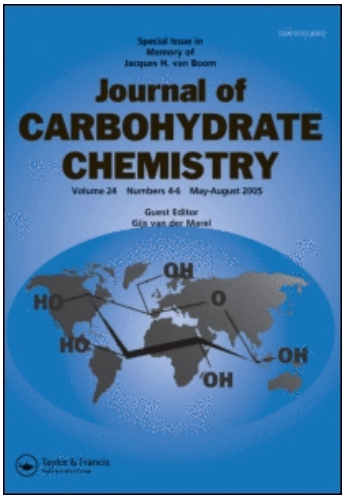

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Gyula Dekany[a]; Karen Wright[a]; Istvan Toth[a]
[a] The School of Pharmacy, University of London, London, UK

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AN ECONOMICAL SYNTHESIS OF LEWIS X, SIALYL LEWIS X AND THEIR α-GALACTOSYL ANALOGUES

Gyula Dekany, Karen Wright and Istvan Toth*

The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK

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ABSTRACT

Economical syntheses of the Lewis X trisaccharide **8** and sialyl Lewis X tetrasaccharide **18** epitopes and the syntheses of the α-galactosyl epimers **9** and **20** of these structures are described. Thioglycosides **2**, **5**, **11** and **15** were used as glycosyl donors to construct the desired compounds in a stepwise manner in dimethyl(methylthio)sulphonium triflate promoted couplings. Benzyl 3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**4**) was a key structure in these syntheses, and was synthesised in multi-gram scale.

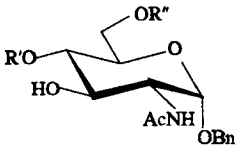
INTRODUCTION

The tetrasaccharide sialyl Lewis X (SLe^X) and trisaccharide Lewis X (Le^X) epitopes have been the subject of intense research interest in recent years because of their involvement in leucocyte migration mediated by selectins in inflammatory processes.[1] Analogues with increased selectin binding activity over the naturally occurring structures have been widely sought. This research requires the use of free SLe^X or Le^X as reference compounds. These oligosaccharides are commercially available, but are very expensive from current sources. We attempted to develop a large scale chemical synthesis of these structures to provide material for use in selectin binding assays.

The Le^X epitope was first synthesised in 1979 by Jacquinet and Sinäy,[2] and the SLe^X tetrasaccharide in 1991 by Nicolaou et al.[3] Hasegawa and co-workers[4] described the total synthesis of SLe^X ganglioside in the same year. Other synthetic routes to the tetrasaccharide have been developed,[5,6] including large scale production based on the use of glycosyltransferases.[7] Recently a large scale chemical synthesis leading to the glycosylamine form of SLe^X has also been reported.[8] We describe a novel, economical large scale synthesis of Le^X and SLe^X and the synthesis of their α-galactosyl epimers.

RESULTS AND DISCUSSION

We adopted a stepwise addition strategy to form the two structures required, and employed stable, easily synthesised thioglycoside donors in glycosylation reactions. The donor sugar units used were synthesised by reported methods in large scale. Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**1**)[9] underwent fucosylation with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**2**)[4] smoothly in the presence of dimethyl(methylthio)sulphonium triflate (DMTST)[10,11] to give the disaccharide **3** in almost quantitative yield.



1 R′ = R″ = benzylidene

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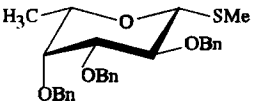

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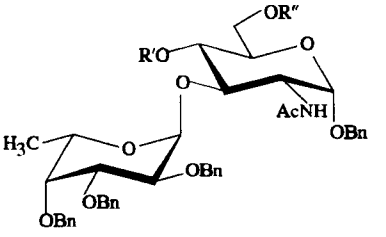
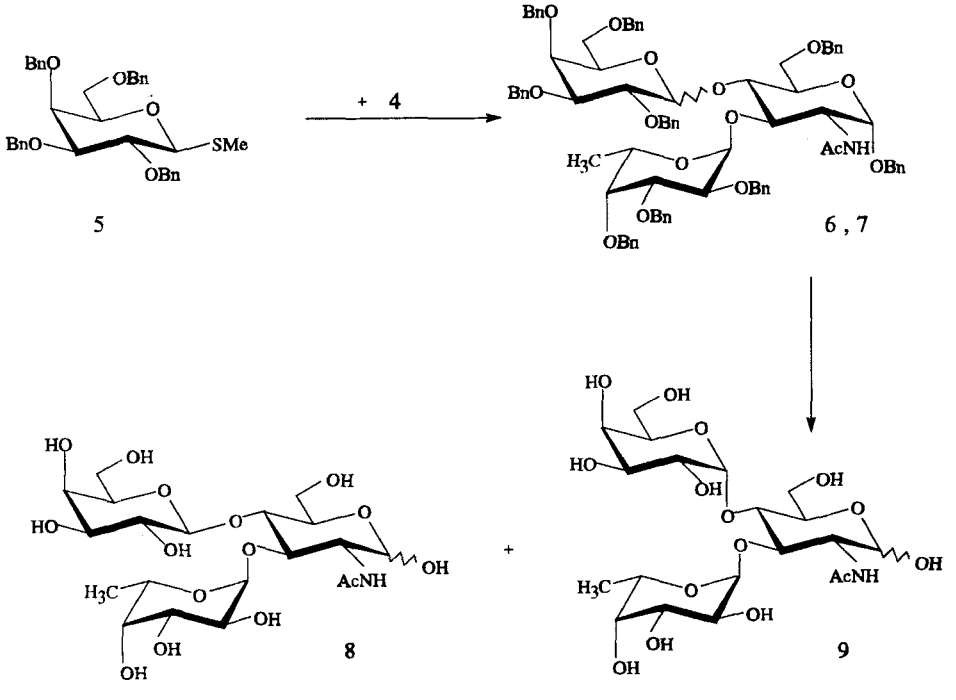


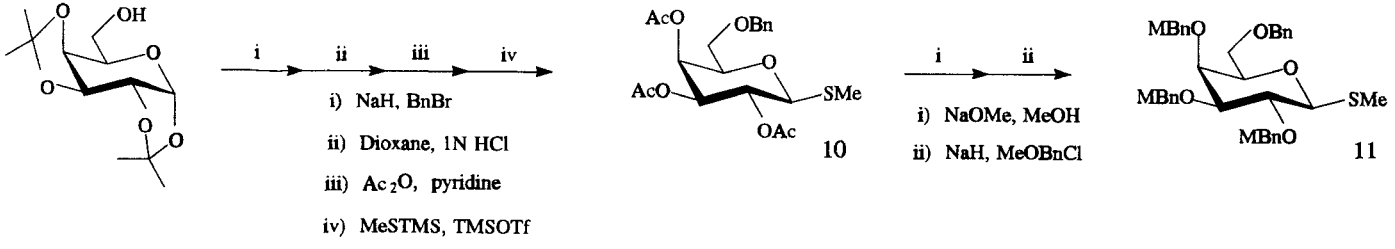
3 R′ = R″ = benzylidene

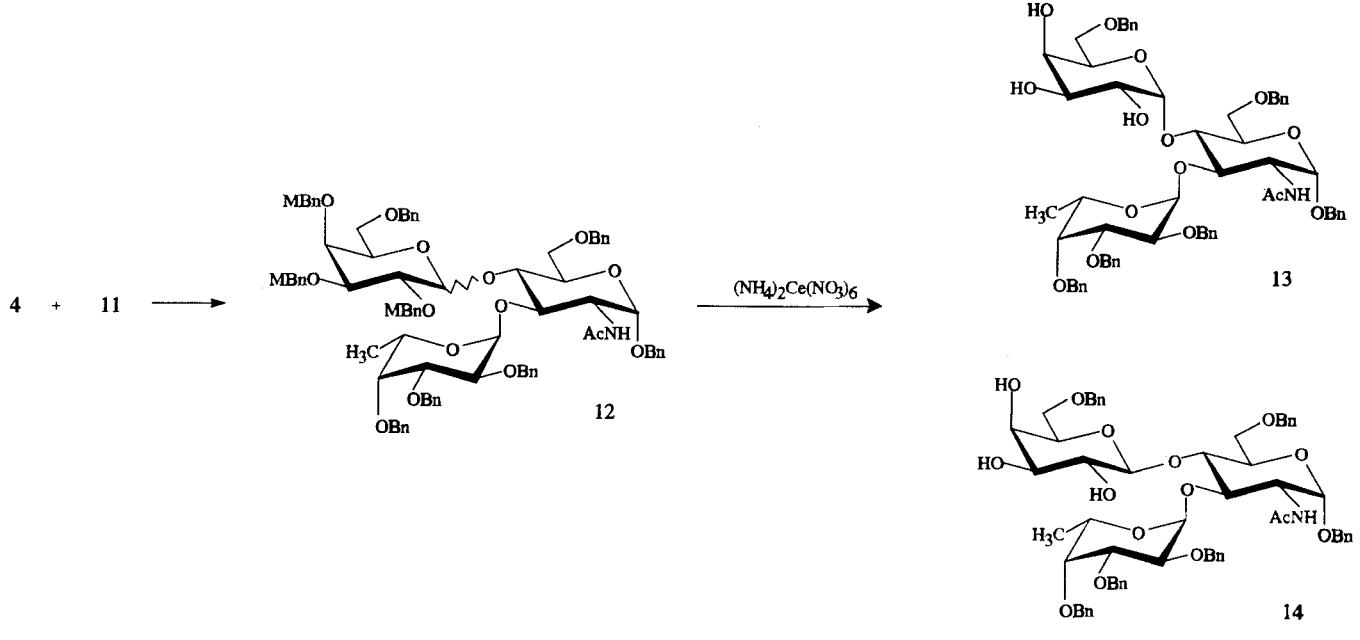
4 R′ = H R″ = Bn

When the more stable, crystalline promoter dimethyl(methylthio)sulphonium tetrafluoroborate (DMTSB)[12] was used, a similar yield of disaccharide **3** was obtained. Reductive ring-opening of the benzylidene acetal of **3** with sodium cyanoborohydride/HCl in THF[13] gave the required acceptor **4** in 72% yield. Some difficulty was experienced in freeing this compound from cyanoborohydride derivatives - purification was only achieved after treatment with a mixed ion exchange resin and recrystallisation from methanol.

Galactosylation of **4** was problematic, due to the poor reactivity of the 4-OH of the glucosamine residue in this compound (as previously remarked by other workers).[8] Acetyl protected thiogalactoside donors failed to produce trisaccharide in more than trace quantities with DMTSB or DMTST promotion. When the protective groups were exchanged to benzyl type groups, galactosylation was successful, but this was at the expense of stereoselectivity. To form the Le^X epitope **6**, **4** was reacted with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**5**)[14] with DMTST as the promoter



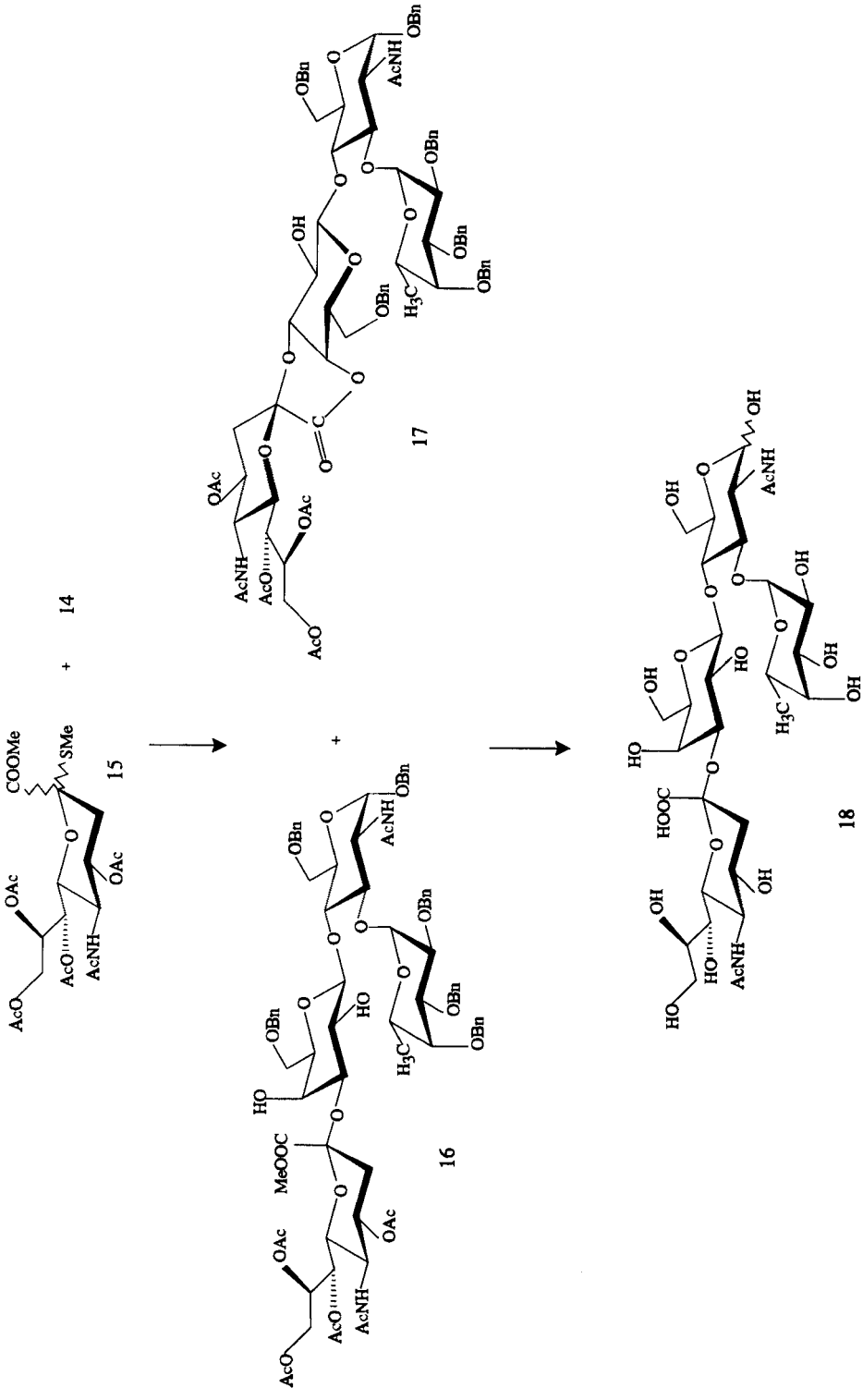




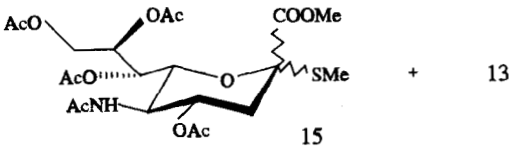
in chloroform at room temperature for 3 hours. This reaction resulted in the desired protected trisaccharide **6** and the α-galactosyl epimer **7** in 61% yield in a ratio of approximately 5:7. The anomers were identified from their ^{1}H NMR spectra by the presence of a doublet at 4.84 ppm ($J_{1,2}$= 7.99Hz) for **6**, and a narrow doublet at 5.12 ppm ($J_{1,2}$= 3.5Hz) for **7**, and by the relative positions of the H-6_{fuc} residue signals at 1.12 ppm for **6** and at 1.27 ppm for **7**. Attempts to influence the stereoselectivity of the glycosylation by using acetonitrile as solvent and low temperatures[15] led to a greatly decreased yield of trisaccharide without significant differences in the α:β ratio. The trisaccharides **6** and **7** were deprotected by catalytic hydrogenation over 10% Pd/C catalyst to give **8** (Le^X) and **9** ("epi-Le^X") in near quantitative yield.

A different protective group pattern was required for the synthesis of SLe^X. It was previously demonstrated that sialylation proceeds regioselectively and in better yield if the galactose acceptor was deprotected at the 2, 3 and 4 positions.[16] For this reason, a galactose donor with temporary protection (methoxybenzyl) at 2, 3 and 4 positions and permanent protection (benzyl) at position 6 was synthesised. Donor **11** can be synthesised in large scale in six steps from commercially available 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose. Benzylation, acetal deprotection with dioxane:1N HCl 1:1 v/v, *O*-acetylation and formation of a methyl thioglycoside with (methylthio)trimethylsilane and trimethylsilyl triflate gave compound **10** as an anomeric mixture. The β-anomer of this mixture was precipitated from methanol, and was *O*-acetyl deprotected with sodium methoxide/methanol and *O*-methoxybenzyl protected with methoxybenzyl chloride to give the desired donor **11**. Glycosylation of compound **4** with donor **11** in the presence of DMTST gave an α,β mixture of trisaccharides **12**, which was not separated at this stage. The mixture was treated with ammonium cerium (IV) nitrate in acetonitrile/water 9:1 v/v[17] for 1 h to give the partially deprotected trisaccharides **13** (α-galactoside, 23% yield from **4**) and **14** (β-galactoside, 25% yield from **4**) which were distinguished from their ^{1}H NMR spectra by the presence of a sharp doublet at 5.18 ppm ($J_{1,2}$ = 3.3Hz) in the spectra of compound **13** and by the relative positions of the H-6_{fuc} signals, at 1.19 ppm for **13** and at 1.04 ppm in the spectra of compound **14.**

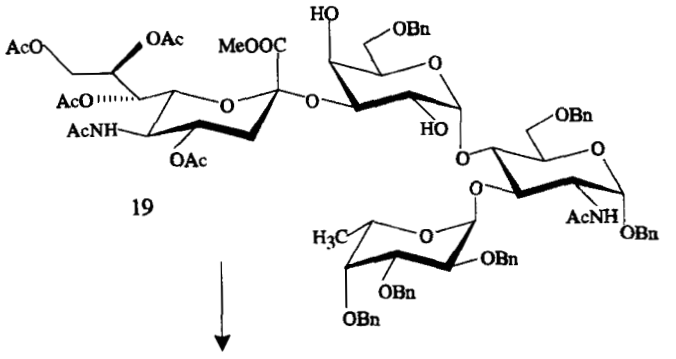
Sialylation of trisaccharide **14** was performed using methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α,β-D-*galacto*-2-



nonulopyranoside)onate (**15**)[18] at -15 °C in acetonitrile with DMTST as promoter for 2 days. The products of sialylation were the methyl ester **16** and the 1→4' lactone **17** which was formed by intramolecular transesterification during glycosylation. The 1→4' conformation of the lactone **17** was determined from the ^{1}H NMR spectrum of **17** by the position of H-2_{gal} at 4.23 ppm, and of H-4_{gal} at 5.31 ppm. Lactone formation was noted in previous syntheses of this tetrasaccharide.[3,8] These products could be only partially



15 + 13



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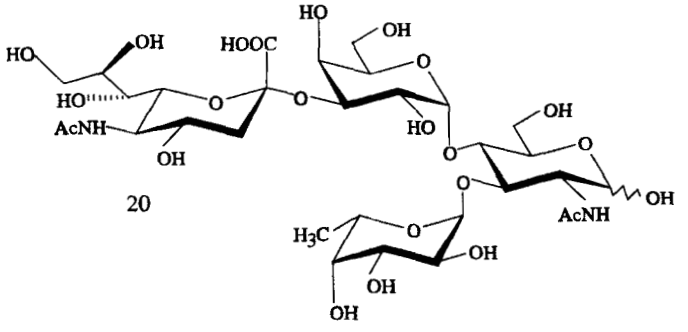

20

separated at this stage. The tetrasaccharide mixture was deprotected by *O*-deacetylation with sodium methoxide in methanol, saponification of the methyl ester/ lactone functions and catalytic hydrogenation over 10% Pd/C catalyst to give the free SLe^X epitope **18** in 78% yield.

To investigate the biological properties of the unnatural, α-galactosyl form of SLe^X **20** was also synthesised. Compound **13** was sialylated in a similar manner as described for the synthesis of **16**. However, in this case no lactone formation occurred and the yield of tetrasaccharide **19** formed was low (18%). Compound **19** was deprotected to give **20** ("epi-SLe^X") in near quantitative yield.

EXPERIMENTAL

General methods. Purification was achieved by chromatography through Sorbsil C60-H40/60, using mobile phases as stated. Reaction progress was monitored by thin layer chromatography on Kieselgel 60 F_{254} using mobile phases as stated. Visualisation was by UV light, iodine, or charring with sulphuric acid. The ion exchange resin used was Amberlite IR-120(H^+). The solvents used in reaction mixtures were water free. Reactions were carried out at room temperature unless otherwise stated. ^1H NMR spectra were obtained with a Bruker AM 500 instrument operating at a field of 500 MHz. Chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were run with a VG Analytical ZAB-SE instrument using fast atom bombardment (FAB) techniques - 20kV Cs^+ ion bombardment, with 2 μL of appropriate matrix, either 3-nitrobenzyl alcohol or thioglycerol with NaI (MeOH) solution added when necessary to produce natriated species when no protonated molecular ions were observed, or on a Fisons matrix assisted laser desorption time of flight spectrometer (MALDI TOF) with a N_2 laser operating at 337nm and 5μL of 2,5 -dihydroxybenzoic acid as matrix.

Benzyl 3-*O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (4). A mixture of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside **1** (6.94 g, 17.39 mmol), methyl 2,3,4-tri-*O*-benzyl-1-thio-ß-L-fucopyranoside **2** (8.07 g, 17.39 mmol) and molecular sieves 4Å (13 g) in tetrahydrofuran (650 mL) was stirred at room temperature. DMTSB (5.0 g, 25.5 mmol) was added and the mixture was stirred for 2 h. The reaction was monitored by TLC using $CHCl_3$:EtOAc

5:1 v/v as the mobile phase. Triethylamine (7 mL) was added, the mixture was filtered and the filtrate was concentrated. The residue was taken up in chloroform (300 mL) and washed with water (50 mL), saturated $NaHCO_3$ solution (50 mL) and water (50 mL). The organic layer was dried with $MgSO_4$, filtered and concentrated. Ethanol/benzene 1:1 v/v (40 mL) was evaporated from the residue, giving 15.6 g of a crude mixture containing benzyl 3-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**3**). The residue was taken up in THF (130 mL) then methyl orange indicator (50 mg) and sodium cyanoborohydride (10 g, 159.13 mmol) were added at 0 °C. THF saturated with HCl was added very slowly until a permanent pink colour was obtained. More THF/HCl (2x10 drops) was added, and the reaction mixture was stirred between 25-40 °C for 4 h. The reaction was monitored by TLC using chloroform:EtOAc 5:1 v/v as the mobile phase. The mixture was neutralised with triethylamine and concentrated. The residue was taken up in chloroform (350 mL) and washed with water (70 mL), saturated $NaHCO_3$ solution (70 mL). The organic layer was treated with Amberlite MB-1 ion exchange resin (60 g), dried over $MgSO_4$, and concentrated. The product **4** was crystallised from methanol (30 mL) (10.2 g 72 %): ^{1}H NMR ($CDCl_3$) δ 7.41-7.16 (m, 25H, ArH), 5.94 (d, 1H, NH), 1.42 (s, 3H, NAc), 1.17 (d, 3H, H-6'); FAB MS $C_{49}H_{55}NO_{10}$ (817.98) *m/z* (%) 840 $[M+Na]^+$ (100), 747 (10), 360 (40), 338 (22).

Benzyl *O*-(2,3,4,6-Tetra-*O*-benzyl-α,ß-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (6, 7). A mixture of **5** (2.44 g, 4.28 mmol) and **4** (1.0 g, 1.22 mmol) was stirred at room temperature with 2 g of molecular sieves 4Å in chloroform (20 mL). DMTST (950 mg, 3.68 mmol) was added and the mixture was stirred for 1 h. A further 600 mg (2.32 mmol) of DMTST was added and the mixture was stirred for 3 h. Chloroform (150 mL) was added and the solution was washed with 2 x 40 mL saturated $NaHCO_3$ solution. The organic layer was separated and dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using hexane:chloroform:ether:acetonitrile 3:10:3:1 v/v/v/v as the mobile phase to give **6** (583 mg, 36%): R_f 0.44 (hexane:chloroform:ether:acetonitrile 3:10:3:1 v/v/v/v); ^{1}H NMR ($CDCl_3$) δ 7.43-7.24 (m, 45H, ArH), 5.77 (d, 1H, NH), 5.29 (d, 1H, H-1_{fuc}), 1.83 (s, 3H,

NHAc), 1.12 (d, 3H, H-6_{fuc}); FAB MS $C_{83}H_{89}NO_{15}$ (1340.54) *m/z* (%) 1363 $[M+Na]^+$ (100), 1273 (12); and **7** (423mg, 26%): R_f 0.48 (hexane:chloroform:ether:acetonitrile 3:10:3:1 v/v/v/v); ^1H NMR ($CDCl_3$) δ 7.01-7.46 (m, 45H, ArH), 5.92 (d, 1H, NH), 5.36 (d, 1H, H-1_{fuc}), 5.12 (d, 1H, H-1_{gal}, $J_{1,2}$=3.5Hz), 1.36 (s, 3H, NHAc), 1.27 (d, 3H, H-6_{fuc}); FAB MS $C_{83}H_{89}NO_{15}$ (1340.54) *m/z* (%) 1363 $[M+Na]^+$ (100), 1273 (12), 471 (10).

***O*-(ß-D-Galactopyranosyl)-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α,ß-D-glucopyranose (8).** 10% Pd/C (1000 mg) was added to **6** (583 mg, 0.43 mmol) in 10 mL acetic acid/methanol 3:1 v/v. The mixture was stirred under H_2 for 2 days at 40 °C, then filtered. The filtrate was lyophilised to give **8** (230 mg, 100%): R_f 0.7 (MeOH:H_2O 4:1 v/v); ^1H NMR (D_2O) δ 4.94 (t, H-$1_{Fuc\ \alpha,ß}$), 4.68 (d, H-1_{GlcNAc}), 4.34 (d, H-1_{Gal}, $J_{1,2}$=7.79Hz), 1.87, 1.85 (2s, NAc_α, $NAc_ß$), 1.02 (d, H-6_{Fuc}); MALDI-TOF MS $C_{20}H_{35}NO_{15}$ (529.48) *m/z* (%) 568 $[M+K]^+$ (31), 552 $[M+Na]^+$ (100), 375 (29), 332 (32), 273 (34).

***O*-(α-D-Galactopyranosyl)-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α,ß-D-glucopyranose (9).** 10% Pd/C (800 mg) was added to **7** (423 mg, 0.315 mmol) in 10 mL acetic acid/methanol 3:1 v/v. The mixture was stirred under H_2 for 2 days at 40 °C, then filtered. The filtrate was lyophilised to give **9** (165 mg, 99%): R_f 0.66 (MeOH:H_2O 4:1 v/v); ^1H NMR (D_2O) δ 8.06 (d, NH), 4.72 (d, H-$1_{GlcNAcß}$, $J_{1,2}$=7.2Hz), 1.89, 1.86 (2s, NAc_α, $NAc_ß$), 1.05 (d, H-6_{Fuc}); MALDI-TOF MS $C_{20}H_{35}NO_{15}$ (529.48) *m/z* (%) 568 $[M+K]^+$ (31), 552 $[M+Na]^+$ (100), 375 (29), 332 (32), 273 (34).

Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-benzyl-1-thio-ß-D-galactopyranoside (10). 1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose (25.0 g, 96.15 mmol) in DMF (50 mL) was added dropwise at 0 °C to a suspension of sodium hydride 60% (5.67 g, 140.35 mmol) in DMF (50 mL). The mixture was stirred at room temperature for 2.5 h, then benzyl bromide (24.0 g, 140.35 mmol) was added dropwise at 0 °C. The mixture was stirred at room temperature overnight. The reaction was monitored by TLC using chloroform/ethyl acetate 1:1 v/v as the mobile phase. The reaction mixture was cooled to 0 °C and methanol (5 mL) was added dropwise, then the mixture was stirred at room temperature for 30 minutes. The mixture was concentrated, and xylene (2 x 15 mL) was distilled from the residue. The residue was taken up in ether (500 mL) and washed with water (2x100 mL). The organic layer was concentrated, the residue was taken up in

methanol (300 mL) and extracted with hexane (2x150 mL). The methanol phase was concentrated to give crude 6-*O*-benzyl-1,2,3,4-di-*O*-isopropylidene-D-galactopyranose (34.2 g). The residue was dissolved in dioxane:1N HCl 1:1 v/v (60 mL) and heated at 100 °C for 3 h. The deprotection was monitored by TLC using $CHCl_3$:methanol 1:1 v/v as the mobile phase. The dioxane was evaporated, and the solution was neutralised with 1M NaOH solution. The solution was concentrated and benzene (3 x 5 mL) was distilled off to give 27.6 g of crude 6-*O*-benzyl-α,β-D-galactopyranose. The residue was dissolved in pyridine (170 mL), and cooled to 0 °C. Acetic anhydride (193 mL) was added slowly, and the mixture was stirred at 0 °C for 30 minutes, then at room temperature for 16 h. The mixture was concentrated, and toluene (2 x 40 mL) was distilled off. The residue was taken up in $CHCl_3$ (300 mL), and washed with water (60 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to give 30.7 g of crude 1,2,3,4-tetra-*O*-acetyl-6-*O*-benzyl-α,β-D-galactopyranose. The residue was dissolved in CH_2Cl_2 (40 mL). (Methylthio)trimethylsilane (13.2 g, 105 mmol) and trimethylsilyl triflate (18.2 g, 77 mmol) were added and the reaction mixture was stirred at room temperature for 16 h. The reaction was monitored by TLC using hexane:EtOAc 8:7 v/v as the mobile phase. The mixture was diluted with CH_2Cl_2 (250 mL), cooled to 0 °C and washed with 1M sodium carbonate solution (100 mL). The organic layer was separated and washed with water (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. Methanol (10 mL) was added to the residue, and the suspension was kept at 0 °C overnight. The resulting white precipitate was filtered off (β-anomer, 12.82 g, 31%): R_f 0.57 (hexane:EtOAc 8:7 v/v); ^1H NMR ($CDCl_3$) δ 7.31 (m, 5H, ArH), 5.51 (d, 1H, H-4), 5.22 (t, 1H, H-2), 5.05 (dd, 1H, H-3), 4.54, 4.42 (2d, 2H, CH_2Ar), 4.37 (d, 1H, H-1, $J_{1,2}$=9.75Hz), 3.57, 3.46 (2m, 2H, H-6, H-6'), 2.17 (s, 3H, SMe), 2.05, 2.04, 1.97 (3s, 9H, 3OAc); FAB MS $C_{20}H_{26}SO_8$ (426.47) *m/z* (%) 449 $[M+Na]^+$ (100), 379 (22). The filtrate was concentrated, and the residue was purified by column chromatography using hexane:ethyl acetate 8:7 v/v as the mobile phase to give a further 16 g (39 %) of an anomeric mixture of thioglycosides.

Methyl 6-*O*-Benzyl-2,3,4-tri-*O*-methoxybenzyl-1-thio-ß-D-galactopyranoside (11). Methyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-1-thio-ß-D-galactopyranoside **10β** (12.80 g, 30.05 mmol) and sodium methoxide (300 mg, 5.63 mmol) were taken up in methanol

(150 mL) and stirred at 40 °C for 2 h. The reaction was monitored by TLC using $CHCl_3$:MeOH 6:1 v/v. The mixture was neutralised with ion exchange resin, filtered and concentrated to give a white solid. The solid was taken up in DMF (30 mL) and added slowly at 0 °C to sodium hydride 60% (9.61 g, 240 mmol) suspended in DMF (50 mL). The mixture was stirred for 45 minutes at room temperature, then cooled to 0 °C. Methoxybenzyl chloride (37.5 g, 240 mmol) was added slowly. The mixture was stirred at room temperature for 16 h. The mixture was cooled to 0 °C and methanol was added to the solution until gas evolution ceased. The mixture was concentrated, and xylene (4x10 mL) and benzene (20 mL) were distilled off. The residue was taken up in $CHCl_3$ (300 mL) and washed with $NaHCO_3$ solution (75 mL) and water (100 mL). The organic layer was concentrated. The residue was taken up in acetonitrile (100 mL) and extracted with hexane (3x200 mL). The acetonitrile phase was separated and concentrated. The residue was taken up in methanol and the resulting precipitate was filtered off. The solid was recrystallised from di-isopropyl ether to give **11** as a white solid (15.61 g, 79 %): R_f 0.45 (hexane:$CHCl_3$:EtOAc 7:7:2 v/v/v); ^{1}H NMR ($CDCl_3$) δ 7.27, 6.84 (2m, 17H, ArH), 4.88 (d, 1H, CHAr), 4.76 (q, 2H, CHAr), 4.66 (s, 2H, CHAr), 4.56 (d, 1H, CHAr), 4.43 (q, 2H, CHAr), 4.31 (d, 1H, H-1, $J_{1,2}$=9.6Hz), 3.92 (d, 1H, H-4), 3.84, 3.81, 3.80 (3s, 9H, CH_3OAr), 3.56 (m, 3H, H-5, H-6, H-6'), 2.20 (s, 3H, SMe); FAB MS $C_{38}H_{44}O_8S$ (660.79) *m/z* (%) 684 $[M+Na]^+$ (100), 479 (22), 441 (37), 326 (37).

Benzyl *O*-(6-*O*-Benzyl-2,3,4-tri-*O*-[4-methoxybenzyl]-α,ß-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (12). **4** (7.67 g, 9.38 mmol) and **11** (15.49 g, 23.47 mmol) with 15 g molecular sieves 4Å in 100 mL chloroform were stirred together at room temperature for 30 minutes. DMTST (11.7 g, 45.35 mmol) was added and the mixture was stirred for 3 h. Triethylamine was added until the pH of the mixture was basic. The mixture was diluted with 200 mL chloroform and filtered. The filtrate was washed with 100 mL water, 100 mL saturated $NaHCO_3$ solution, and another 100 mL water. The organic layer was separated and concentrated to give 26.0 g of crude residue which was deprotected without further purification. An analytical sample was prepared by column chromatography using 1,2-dichloroethane:hexane:ether:acetonitrile 10:3:5:1 v/v/v/v as the mobile phase to give **12** as an anomeric mixture: R_f 0.55 (1,2-

dichloroethane:hexane:ether:acetonitrile 10:3:5:1 v/v/v/v); ^{1}H NMR ($CDCl_3$) δ 7.67-7.16 (m, ArH), 5.87 (d, NHα), 5.66 (d, NHß), 5.38 (d, H-1_{fuc}α), 5.22 (d, H-1_{fuc}ß), 5.14 (d, H-1_{gal}α), 3.81, 3.80, 3.78 (3s, CH_3OAr), 1.78 (s, NHAcß), 1.37 (s, NHAcα), 1.27 (d, H-6_{fuc}α), 1.07 (d, H-6_{fuc}ß); FAB MS $C_{86}H_{95}NO_{18}$ (1430.62) *m/z* (%) 1452 $[M+Na]^+$ (100), 1361 (11), 515 (23), 360 (17).

Benzyl *O*-(6-*O*-Benzyl-α,ß-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (13, 14). **12** (26.0g) was taken up in 400 mL acetonitrile:water 9:1 v/v and cooled to 0 °C. Ammonium cerium (IV) nitrate (100 g, 182.4 mmol) was slowly added and the mixture was stirred at room temperature for 1 h. Dichloromethane (2000 mL) was added, and the mixture was washed with 800 mL saturated $NaHCO_3$ solution and filtered. The organic layer was separated and concentrated. Hexane (500 mL) was added to the residue and heated with stirring until boiling, then the supernatant liquid was decanted. This was repeated with a further 500 mL hexane, and 500 mL petroleum ether. The residue was taken up in 400 mL ether:ethyl acetate 2:1 v/v and washed with 100 mL water. The organic layer was separated, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using dichloromethane:acetonitrile 2:1 v/v as the mobile phase to give **13** (α-anomer, 2.29 g, 23% from **4**): R_f, 0.45 (CH_2Cl_2:MeCN 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.39-7.17 (m, 30H, ArH), 5.18 (d, 1H, H-1_{gal}, $J_{1,2}$= 3.3Hz), 5.15 (d, 1H, H-1_{fuc}, $J_{1,2}$= 3.77), 5.03 (d, 1H, H-1_{gleNAc}, $J_{1,2}$= 3.12Hz), 4.26 (dd, 1H, H-3_{gal}), 1.40 (s, 3H, NHAc), 1.19 (d, 3H, H-6_{fuc}); FAB MS $C_{62}H_{71}NO_{15}$ (1069.17) *m/z* (%) 1092 $[M+Na]^+$ (100), 1000 (10), 441 (47), 326 (33), 199 (49), 173 (47); and **14** (ß-anomer, 2.50g, 25% from **4**): R_f, 0.5 (CH_2Cl_2:MeCN 2:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.28-7.06 (m, 30H, ArH), 5.27 (d, 1H, H-1_{fuc}), 5.07 (d, 1H, H-1_{gleNAc}), 1.36 (s, 3H, NHAc), 1.04 (d, 3H, H-6_{fuc}); FAB MS $C_{62}H_{71}NO_{15}$ (1069.17) *m/z* (%) 1092 $[M+Na]^+$ (100), 1000 (12), 441 (47), 326 (39).

Benzyl *O*-[6-*O*-Benzyl-3-*O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-ß-D-galactopyranosyl]-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (16) and **Benzyl *O*-{3-*O*-[4''-(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylolide)]-6-*O*-benzyl-ß-D-**

galactopyranosyl}-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (17). **14** (2.44 g, 2.28 mmol) and methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α,ß-D-*galacto*-2-nonulopyranoside)onate **15** (2.38 g, 4.56 mmol) were dissolved in acetonitrile (125 mL) and stirred with molecular sieves (4Å) (6.1 g) at 20 °C for 5 h. The reaction mixture was cooled to -30 °C, and DMTST (3.53 g, 13.69 mmol) and molecular sieves (4Å) (2.5 g) were added. The mixture was stirred at -30 °C for 10 minutes and then at -15°C for 24 h. A second portion of **15** (2.38 g, 4.56 mmol) was added. The mixture was cooled to -30 °C and DMTST (3.53 g, 13.69 mmol) with molecular sieves (4Å) (2.5 g) were added. The mixture was stirred at -30 °C for 10 minutes, then at -15 °C for 24 h. Triethylamine (4 mL) was added, and the temperature of the mixture was allowed to rise to 20 °C. The mixture was filtered and concentrated. The residue was taken up in dichloromethane (300 mL) and washed with saturated $NaHCO_3$ solution (50 mL) and water (50 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by multiple column chromatography using ether:ethyl acetate:methanol 10:4:0.25 v/v/v, 1,2-dichloroethane:tetrahydrofuran 2:1 v/v, toluene:acetonitrile 1:1 v/v as mobile phases, to give **16** (410 mg, 12 %): ^{1}H NMR ($CDCl_3$) δ 7.39-7.17 (m, 30H, ArH), 5.47 (m, 1H, H-8_{sia}), 5.42 (d, 1H, H-1_{fuc} $J_{1,2}$=3.2Hz), 5.34 (dd, 1H, H-7_{sia}), 5.15 (m, 2H, NH_{sia}, H-1_{glcNAc}), 4.93 (m, 2H, H-4_{sia}, CH_2Ar_a), 3.54 (s, 3H, OMe), 2.72 (dd, 1H, H-$3_{sia,eq}$), 2.10, 2.09, 2.06, 2.02 (4s, 12H, 4OAc), 1.92 (s, 6H, 2NAc), 1.14 (d, 3H, H-6_{fuc}); MALDI TOF MS $C_{82}H_{98}N_2O_{27}$ (1542.62) *m/z* (%) 1581 $[M+K]^+$ (52), 1565 $[M+Na]^+$ (100), 1475 (20); and **17** (513 mg, 15 %): ^{1}H NMR ($CDCl_3$) δ 7.39-7.06 (m, 30H, 30ArH), 5.38 (m, 3H, H-8_{sia}, H-4_{sia}, NH_{sia}), 5.32 (d, 1H, H-1_{fuc}, $J_{1,2}$=3.4Hz), 5.31 (d, 1H, H-4_{gal}), 5.24 (dd, 1H, H-7_{sia}), 5.14 (d, 1H, H-1_{glcNAc}, $J_{1,2}$=3.5Hz), 4.94 (d, 1H, CH_2Ar_a), 4.79 (d, 1H, H-1_{gal}, $J_{1,2}$=9.3Hz), 4.34 (dd, 1H, H-3_{gal}), 4.23 (t, 1H, H-2_{gal}), 2.49 (t, 1H, H-$3_{sia,\ ax}$), 2.28 (dd, 1H, H-$3_{sia,\ eq}$), 2.16, 2.03, 1.90, 1.89, 1.88, 1.70 (6s, 18H, 4OAc, 2NAc), 1.10 (d, 3H, H-6_{fuc}); MALDI TOF MS $C_{82}H_{96}N_2O_{26}$ (1510.34) m/z (%)= 1549 $[M+K]^+$ (47), 1533 $[M+Na]^+$ (100), 1443 (50), 1353 (27); and a mixture of **16** and **17** (1.13 g, 32 %).

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(ß-D-galactopyranosyl)-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α,ß-D-glucopyranoside (18). A mixture of **16** and **17** (1.23 g, 0.797 mmol) was

dissolved in methanol (32 mL) and sodium methoxide (255 mg, 4.72 mmol) was added. The reaction mixture was stirred at 35 °C for 24 h then water (6.2 mL) was added. The mixture was stirred at room temperature for 6 h and neutralised with Amberlite IR-120 (H^+) ion exchange resin. The resin was filtered off and the filtrate was concentrated to give the deacetylated acid. The residue was dissolved in methanol (130 mL) and hydrogenated over Pd/C (10%) (2.2 g) for 5 days at 35 °C. The mixture was filtered and concentrated. The residue was purified by column chromatography using acetonitrile:water 3:1 v/v as the mobile phase. After lyophilisation, **18** was obtained as a white solid (511 mg, 78%): R_f 0.41 (acetonitrile:water 3:1 v/v); ^{1}H NMR δ (D_2O) 4.95 (m, 1H, H-1 $_{fuc}$), 4.68 (q, 1H, H-1 $_{GlcNAc}$), 4.39, 4.37 (2d, 1H, H-1_{Gal}), 2.62 (dd, 1H, H-$3_{sia,eq}$), 1.88 (s, 6H, 2 NAc), 1.65 (t, 1H, H-$3_{sia,ax}$), 1.03, 1.01 (2d, 3H, H-6_{fuc}); MALDI TOF MS $C_{31}H_{52}N_2O_{23}$ (820.75) *m/z* (%) 897 $[M+2K]^+$ (10), 882 $[M+Na+K]^+$ (25), 866 $[M+2Na]^+$ (47), 859 $[M+K]^+$ (24), 843 $[M+Na]^+$ (100), 575 (55).

Benzyl *O*-[6-*O*-Benzyl-3-*O*-(Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-α-D-galactopyranosyl]-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (19). **13** (307 mg, 0.288 mmol) and methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α,ß-D-*galacto*-2-nonulopyranoside)onate **15** (300 mg, 0.575 mmol) were dissolved in acetonitrile (16 mL) and stirred with molecular sieves (4Å) (1 g) at 20 °C for 3 h. The reaction mixture was cooled to -30 °C, and DMTST (445 mg, 1.72 mmol) and molecular sieves (4Å) (400 mg) were added. The mixture was stirred at -30 °C for 10 minutes and then at -15 °C for 24 h. A second portion of **15** (300 mg, 0.575 mmol) was added. The mixture was cooled to -30 °C and DMTST (445 mg, 1.72 mmol) with molecular sieves (4Å) (400 mg) were added. The mixture was stirred at -30 °C for 10 minutes, then at -15 °C for 24 h. Triethylamine (1 mL) was added, and the temperature of the mixture was allowed to rise to 20 °C. The mixture was filtered and concentrated. The residue was taken up in dichloromethane (30 mL) and washed with saturated $NaHCO_3$ solution (5 mL) and water (5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using ether:ethyl acetate:methanol 10:4:0.4 v/v/v as the mobile phase, to give **19** (80 mg, 18 %): R_f 0.55 (1,2-dichloroethane: tetrahydrofuran 2:1 v/v);

^{1}H NMR δ ($CDCl_3$) 7.14 -7.39 (m, 30H, ArH), 5.23 (d, 2H, H-1_{fuc}, H-1_{gal}), 5.18 (d, 1H, NH_{sia}), 5.01 (d, 1H, H-1_{glcNAc}), 4.93 (m, 1H, H-8_{sia}), 2.67 (dd, 1H, H-$3_{eq\ sia}$), 1.96, 1.98, 2.02, 2.11 (4s, 12H, 4 OAc), 1.56, 1.85 (2s, 6H, 2NAc), 1.14 (d, 3H, H-6_{fuc}); MALDI TOF MS $C_{82}H_{98}N_2O_{27}$ (1542.62) *m/z* (%) 1581 $[M+K]^+$ (23), 1565 $[M+Na]^+$ (100), 1476 (24), 1224 (22), 1150 (16).

(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(α-D-galactopyranosyl)-(1→4)-*O*-[(α-L-fucopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α,ß-D-glucopyranoside (20). 18 (50 mg, 0.032 mmol) was dissolved in methanol (1.25 mL) and sodium methoxide (10 mg, 0.185 mmol) was added. The reaction mixture was stirred at room temperature for 24 h, then water (0.25 mL) was added. The mixture was stirred at room temperature for 2 h and neutralised with Amberlite IR-120 (H^+) ion exchange resin. The resin was filtered off and the filtrate was concentrated to give the deacetylated acid. The residue was dissolved in methanol (10 mL) and hydrogenated over Pd/C (10%) (100 mg) for 7 days at room temperature. The mixture was filtered and concentrated to give **20** (25mg, 96%): R_f 0.37 (acetonitrile: water 3:1 v/v); ^{1}H NMR δ ($CDCl_3$) 2.58 (dd, 1H, H-$3_{eq\ sia}$), 1.86, 1.91 (2s, 6H, 2NAc), 1.66 (t, 1H, H-$3_{ax\ sia}$), 1.03 (m, 3H, H-6_{fuc}); MALDI TOF MS $C_{31}H_{52}N_2O_{23}$ (820.75) *m/z* (%) 882 $[M+K+Na]^+$ (57), 859 $[M+K]^+$ (94), 843 $[M+Na]^+$ (100), 585 (61), 360 (60).

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