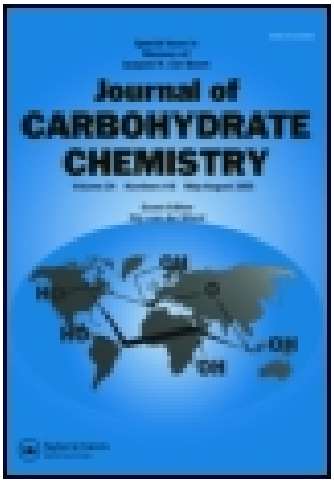

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Studies on Selectin Binding Inhibitors: Synthesis of Sialyl-Lewis X and Sialyl-Lewis A Epitope Analogs Containing 2-Acetamido Derivative of N-Methyl-1-Deoxynojirimycin[1]

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STUDIES ON SELECTIN BINDING INHIBITORS: SYNTHESIS OF SIALYL-LEWIS X AND SIALYL-LEWIS A EPITOPE ANALOGS CONTAINING 2-ACETAMIDO DERIVATIVE OF *N*-METHYL-1-DEOXYNOJIRIMYCIN[1]

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ABSTRACT

Synthesis of sialyl-Lewis x (**15**) and sialyl-Lewis a (**17**) epitope analogs containing the 2-acetamido derivative of *N*-methyl-1-deoxynojirimycin has been achieved. A suitably protected 2-acetamido-1-deoxynojirimycin derivative **5**, prepared from 1-deoxynojirimycin *via* the epoxide intermediate **3**, was successively coupled with methyl-1-thioglycosides of L-fucose (**6**) and α-sialyl-(2→3)-D-galactose (**9**). The resulting tetrasaccharides (**10** and **13**) were each converted, by reductive *N*-methylation and deprotection, into the desired epitope analogs.

INTRODUCTION

The sialyl-Lewis x (sLe^x) and sialyl-Lewis a (sLe^a) carbohydrate epitopes have been identified not only as tumor-associated antigens[2] but also as the minimal carbohydrate ligands for selectins,[3] a family of lectin-type cell adhesion molecules

involved in leukocyte traffic and recruitment to the site of inflammation. It has also been suggested that both sLe^x and sLe^a antigens may be involved in the processes of hematogeneous metastasis of cancer cells.[4]

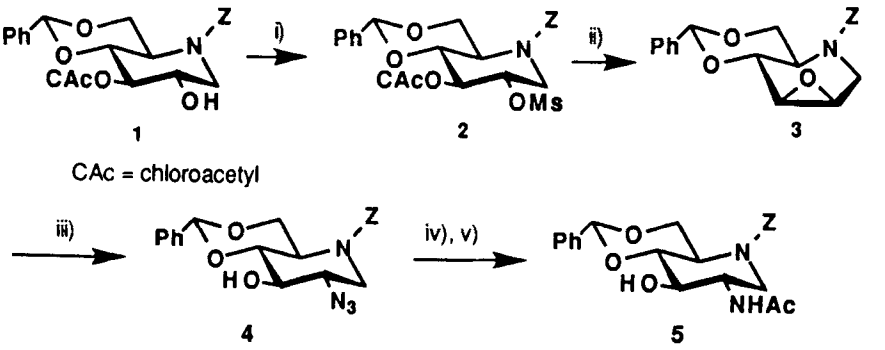
In a series of investigations[5] on structure-function relationship in selectin carbohydrate ligands, we have systematically synthesized[6] various sLe^x relevant gangliosides and their analogs. Among those, the *N*-methyl-1-deoxynojirimycin (*N*-Me-DNJ)-containing sLe^x and sLe^a type tetrasaccharides[7] exhibited potential inhibitory activity against selectin binding *in vitro*. This paper describes the synthesis of novel sLe^x and sLe^a epitope analogs in which the *N*-Me-DNJ part is replaced by its 2-acetamido derivative.

RESULTS AND DISCUSSION

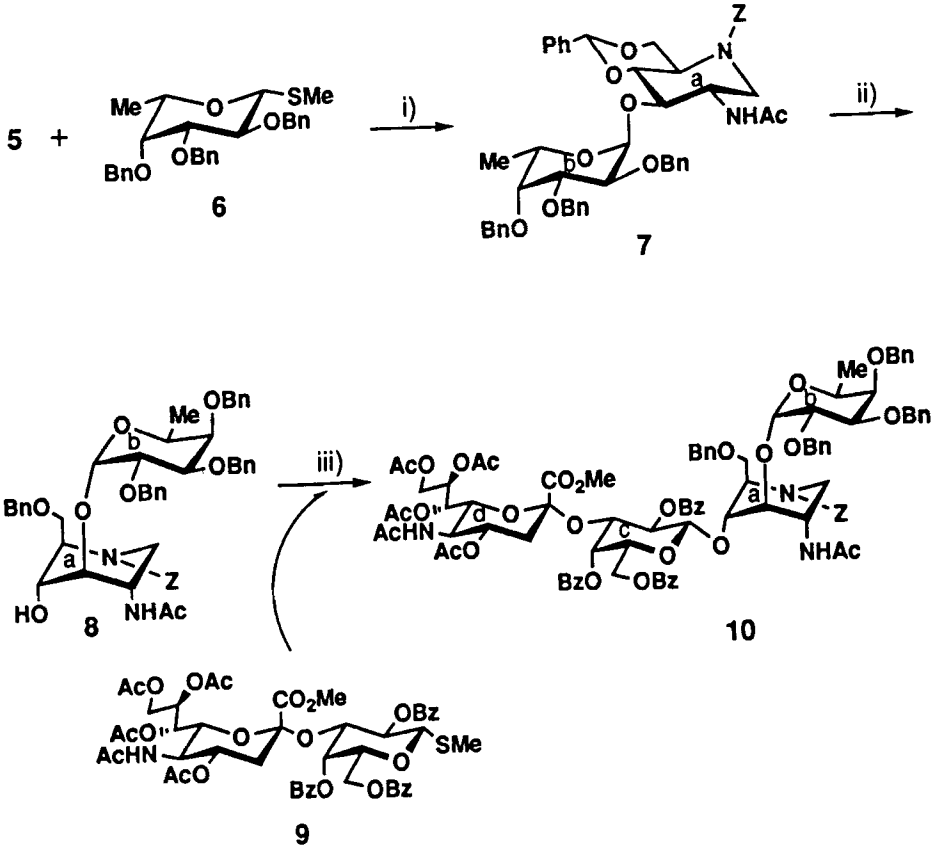
Treatment of 4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-3-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-D-glucitol[7] (**1**) with methanesulfonyl chloride gave **2** which was converted to the epoxide **3** in excellent yields. The epoxide ring was cleaved with sodium azide in *N*,*N*-dimethylformamide to give **4** (40%) and the 3-azido derivative (~30%) as reported[8] for the *N*-*t*-butoxycarbonyl derivative of **3**. The selective reduction of azide with triphenylphosphine and water in dichloromethane, and *N*-acetylation afforded **5** in 93% yield (Scheme 1).

Glycosylation of **5** by methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**6**) in the presence of dimethyl(methylthio)sulfonium triflate[9] (DMTST) and molecular sieves 4Å in benzene gave the desired disaccharide **7** almost quantitatively. Reductive ring opening of the benzylidene group in **7** and iodonium promoted coupling[10] of **8** with methyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside[11] (**9**) afforded the protected sLe^x type tetrasaccharide **10** (Scheme 2).

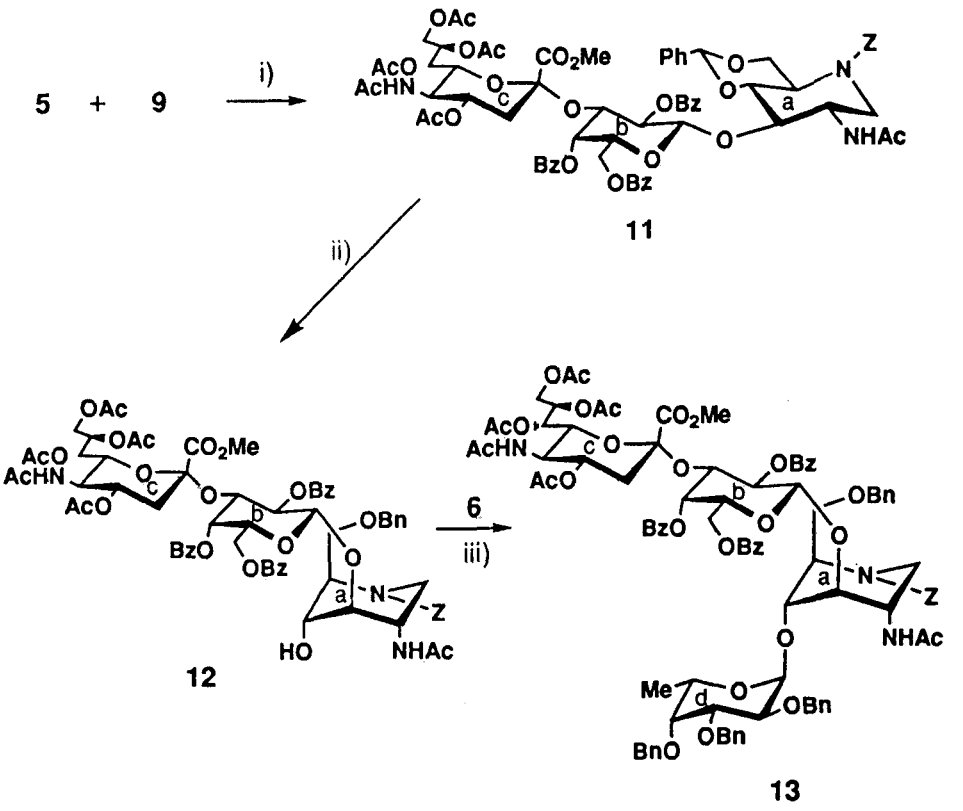
Coupling of **5** with **9** under the similar reaction condition employed for **10** provided the desired trisaccharide **11** which was converted, by reductive ring opening of the benzylidene group, to the next glycosyl acceptor **12**. Iodonium promoted



Scheme 1 i) $MeSO_2Cl$, pyr, ii) NaOMe, MeOH, 1,4-dioxane, iii) NaN_3, DMF, iv) Ph_3P, H_2O, CH_2ClCH_2Cl, v) Ac_2O, MeOH



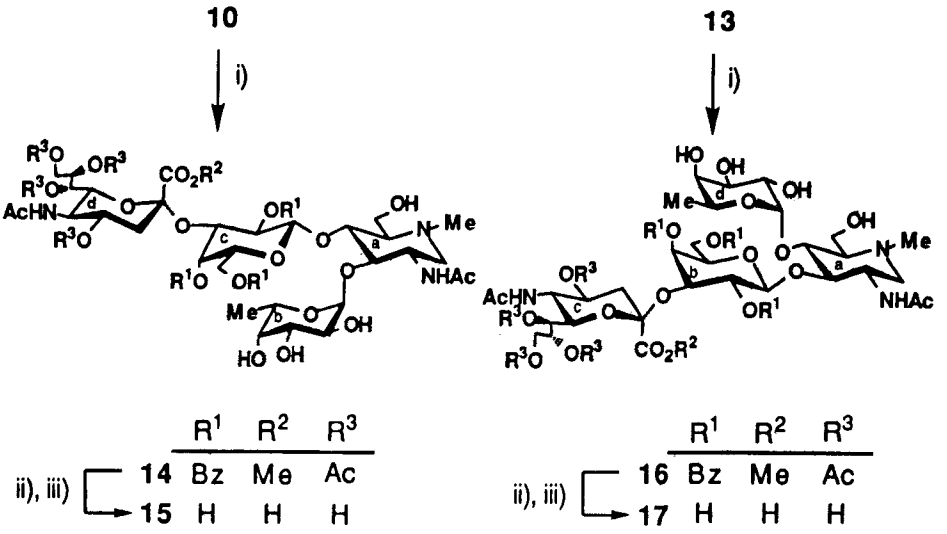
Scheme 2 i) DMTST, benzene, ii) $NaBH_3CN$, HCl/Et_2O, THF iii) NIS, TfOH, CH_2Cl_2



Scheme 3 i) NIS, TfOH, CH_2Cl_2 ii) $NaBH_3CN$, HCl/Et_2O, THF, iii) NIS, TfOH, benzene

glycosylation of **12** with **6** was performed in benzene to give the protected sLe^a type tetrasaccharide **13** (Scheme 3). Hydrogenolysis of **10** and **13** over Pd-C in $MeOH/HCO_2H$, and following *O*-deacylation with methanolic NaOMe and saponification of the methyl ester yielded the corresponding sLe^x (**15**) and sLe^a (**17**) epitope analogs, quantitatively (Scheme 4).

The structures of **15** and **17** thus obtained were analyzed by ion-spray MS, MS/MS, ^1H and ^{13}C NMR spectrometry. The molecular ion peaks were clearly detected both in positive (*m/z* 818.3) and in negative (*m/z* 816.7 or 816.2) modes, respectively, showing the molecular weight calculated for $C_{32}H_{55}N_3O_{21}$. In the



Scheme 4 i) Pd-C, MeOH, HCO_2H, ii) NaOMe, MeOH, iii) 0.2M KOH

positive MS/MS spectra (P = 818), eight significant daughter ions were detected at *m/z* 672(M - Fuc + H)$^+$, 526 (M - NeuAc + H)$^+$, 381 (M - Fuc - NeuAc + H)$^+$, 365 (M - NeuAc - Gal + H)$^+$, 292 (NeuAc fragment)$^+$, 274 (292 - H_2O)$^+$, 219 (protonated 2-acetamido-*N*-Me-DNJ moiety)$^+$ and 201 (219 - H_2O)$^+$, providing the unambiguous evidence for the structures assigned. In the ^{1}H NMR spectra, the two anomeric protons of Gal and Fuc residues bound to the 2-acetamido-*N*-Me-DNJ moiety appeared at δ 4.65 ($J_{1,2}$ = 8 Hz, H-1 of Gal) and 5.39 ($J_{1,2}$ = 3.7 Hz, H-1 of Fuc) for **15**, and δ 4.60 ($J_{1,2}$ = 8 Hz, H-1 of Gal) and 5.05 ($J_{1,2}$ = 3.6 Hz, H-1 of Fuc) for **17**, respectively, indicating the desired β- and α-glycosidic linkages.

A number of studies on selectin binding have been achieved[5] by using various synthetic oligosaccharide derivatives[12,13] related to the sLe^x and sLe^a determinants. Both sLe^x (**15**) and sLe^a (**17**) type tetrasaccharides described here exhibited potential inhibitory activity against selectin binding *in vitro* as previously described[7] for the *N*-Me-DNJ-containing sLe^x and sLe^a epitope analogs.

EXPERIMENTAL

General methods. Optical rotations were determined using a Union PM-201 polarimeter at 25 °C, and IR spectra were recorded on a Jasco IRA-100 spectrophotometer. ^{1}H NMR spectra were recorded on JEOL JNM-GX 270 (270 MHz) or JNM-GX 400 (400 MHz) spectrometers using deuterated solvents ($CDCl_3$, CD_3OD, D_2O) with TMS (d = 0.00 ppm) or acetone (d = 2.225 ppm) as the internal standards. ^{13}C NMR spectra were recorded on a JEOL JNM-GX 400 (100 MHz) spectrometer. Ion-spray mass spectra were recorded on an API-III triple quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments) fitted with an atmospheric pressure ionization source.

All reactions were monitored by TLC (Merck silica gel aluminum plates 60 F-254) and preparative column chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. Concentrations were conducted *in vacuo*.

4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-3-*O*-chloroacetyl-1,5-dideoxy-1,5-imino-2-*O*-mesyl-D-glucitol (2). Methanesulfonyl chloride (0.38 mL) was added to a solution of **1** (1.27 g) in 10:1 CH_2Cl_2-pyridine (55 mL) at -20 °C, and the mixture was stirred for 8 h at 0 °C. The product was extracted with CH_2Cl_2, and the extract was washed with ice-cold 2M HCl and H_2O, dried (Na_2SO_4), and the solvent was evaporated to leave **2** (1.48 g, 99.7%): $[\alpha]_D$ -16.3° (*c* 1.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.14 (s, 3H, Mesyl), 3.34 (dd, 1H, J_{gem} = 14 Hz, $J_{1a,2}$ = 9.7 Hz, H-1a), 3.50 (dt, 1H, $J_{4,5} = J_{5,6a}$ = 9.7 Hz, $J_{5,6e}$ = 4.6 Hz, H-5), 4.20, 4.27 (2d, 2H, J_{gem} = 15 Hz, C*H*$_2$Cl), 4.49 (~t, 1H, H-6a), 4.54 (dd, 1H, J_{gem} = 14 Hz, $J_{1e,2}$ = 4.8 Hz, H-1e), 4.82 (m, 1H, $J_{2,3}$ = 8 Hz, H-2), 4.91 (dd, 1H, J_{gem} = 11.5 Hz, $J_{5,6e}$ = 4.6 Hz, H-6e), 5.25 (s, 2H, OC*H*$_2$Ph), 5.40 (dd, 1H, $J_{2,3}$ = 8 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 5.63 (s, 1H, C*H*Ph), 7.4-7.6 (m, 10H, Ph-*H*).

Anal. Calcd for $C_{24}H_{26}NO_9SCl$ (539.99): C, 53.38; H, 4.85; N, 2.59. Found: C, 53.40; H, 4.62; N, 2.87.

2,3-Anhydro-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-mannitol (3). Compound **2** (1.4 g) in dry 1,4-dioxane (5 mL) and MeOH (15 mL) was treated with methanolic sodium methoxide (28%, 0.9 mL) for 5 min at 0 °C. Solvents were evaporated at 20 °C and the residue was taken up in CH_2Cl_2, washed with H_2O, dried, and the solvent was evaporated. Column chromatography (400:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **3** (0.93 g, 98%): $[\alpha]_D$ +46.7° (*c* 0.9, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 3.05 (dt, 1H, $J_{4,5} = J_{5,6a}$ = 10 Hz, $J_{5,6e}$ = 4.2 Hz, H-5), 3.21, 3.35 (2d, 2H, $J_{2,3}$ = 3.7 Hz, H-2,3), 3.39 (d, 1H, J_{gem} = 14 Hz, H-1a), 4.03 (d, 1H, $J_{4,5}$ = 10 Hz, H-4), 4.46 (dd, 1H, J_{gem} = 11.4 Hz, $J_{5,6e}$ = 4.2 Hz, H-6e), 4.55 (d, 1H, J_{gem} = 14 Hz, H-1e), 4.72 (broad dd, 1H, H-6a), 5.11 (s, 2H, OC*H*$_2$Ph), 5.62 (s, 1H, C*H*Ph), 7.3-7.5 (m, 10H, Ph-*H*).

Anal. Calcd for $C_{21}H_{21}NO_5$ (367.40): C, 68.65; H, 5.76; N, 3.81. Found: C, 68.64; H, 5.90; N, 3.61.

2-Azido-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-glucitol (4). A mixture of **3** (3.71 g) and sodium azide (6.56 g) in *N*,*N*-dimethylformamide (15 mL) was heated for 6 h at 110 °C, and the solvent was removed by evaporation. The residual syrup was taken up in CH_2Cl_2, washed with water, dried (Na_2SO_4), and the solvent was evaporated. Column chromatography (3:1 hexane–AcOEt) of the residue on silica gel gave **4** (1.66 g, 40%) and the 3-azido isomer (~30%). Compound **4** had $[\alpha]_D$ -16° (*c* 1.2, CH_2Cl_2); IR (KBr) 3500 (OH), 2100 (N_3) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.67 (dd, 1H, J_{gem} = 14 Hz, $J_{1a,2}$ = 11 Hz, H-1a), 3.19 (dt, 1H, $J_{4,5} = J_{5,6a}$ = 10 Hz, $J_{5,6e}$ = 4.76 Hz, H-5), 3.49 (m, 1H, $J_{1a,2}$ = 11 Hz, $J_{1e,2}$ = 4.95 Hz, $J_{2,3}$ = 8.61 Hz, H-2), 3.55-3.69 (2t, 2H, H-3 and H-4), 4.30 (dd, 1H, J_{gem} = 14 Hz, $J_{1,2e}$ = 4.95 Hz, H-1e), 4.41 (~t, 1H, J_{gem} = 11.54 Hz, $J_{5,6a}$ = 10.62 Hz, H-6a), 4.78 (dd, 1H, $J_{5,6e}$ = 4.76 Hz, H-6e), 5.11 (2d, 2H, OC*H*$_2$Ph), 5.53 (s, 1H, C*H*Ph), 7.3-7.5 (m, 10H, Ph-*H*).

Anal. Calcd for $C_{21}H_{22}N_4O_5$ (410.43): C, 61.46; H, 5.40; N, 13.65. Found: C, 61.56; H, 5.29; N, 13.70.

2-Acetamido-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-glucitol (5). A mixture of **4** (280 mg) and triphenylphosphine

(358 mg) in dichloromethane was stirred for 30 min at 45 °C. To this mixture, water (0.15 mL) was added and the stirring was continued overnight at 45 °C. The mixture was concentrated to a residue which was treated with acetic anhydride (71 μL) in MeOH (10 mL) for 4 h at room temperature. Pyridine (2 mL) was added at 0 °C and the mixture was concentrated. The residue was taken-up in CH_2Cl_2, washed with 2M HCl and water, dried, and concentrated. Column chromatography (80:1 and 50:1 CH_2Cl_2–MeOH) on silica gel of the residue gave **5** (270 mg, 93%): $[\alpha]_D$ +3.2° (*c* 0.74, CH_2Cl_2); IR (KBr) 3500-3400 (OH, NH), 1650, 1540 (amide); ^1H NMR ($CDCl_3$): δ 1.97 (s, 3H, AcN), 2.74 (dd, 1H, J_{gem} = 13 Hz, $J_{1a,2}$ = 10 Hz, H-1a), 3.25 (dt, 1H, H-5), 3.84 (m, 1H, H-2), 4.37 (~t, 1H, $J_{gem} = J_{5,6a}$ = 10-12 Hz, H-6a), 4.40 (dd, 1H, J_{gem} = 13 Hz, $J_{1e,2}$ = 4.2 Hz, H-1e), 4.79 (dd, 1H, J_{gem} = 11.54 Hz, $J_{5,6e}$ = 4.58 Hz, H-6e), 5.05-5.2 (2d, 2H, OC*H*$_2$Ph), 5.54 (s, 1H, C*H*Ph), 5.87 (d, 1H, N*H*), 7.3-7.5 (m, 10H, Ph-*H*).

Anal. Calcd for $C_{23}H_{26}N_2O_6$ (426.47): C, 64.78; H, 6.15; N, 6.57. Found: C, 64.51; H, 6.36; N, 6.39.

***O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-glucitol (7).** A mixture of **5** (250 mg), methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**6**, 327 mg, 1.2 equiv), and powdered molecular sieves 4Å (MS-4Å, 700 mg) in benzene (20 mL) was stirred overnight at room temperature then cooled to 7 °C. Dimethyl(methylthio)sulfonium triflate (DMTST, 608 mg, 4 equiv) was added and the mixture was stirred for 3 h at 7 °C. Methanol (10 mL) was added at 0 °C and the solution was neutralized with triethylamine. The solids were filtered off and the combined filtrate and washings were concentrated. The residual syrup was taken-up in CH_2Cl_2, washed with water, dried, and concentrated. Column chromatography (2:1 hexane–AcOEt) of the residue on silica gel afforded **7** (494 mg) in almost quantitative yield: $[\alpha]_D$ -83° (*c* 0.97, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.08 (d, 3H, $J_{5,6}$ = 6.41 Hz, H-6b), 2.04 (s, 3H, AcN), 2.52 (dd, 1H, J_{gem} = 13.4 Hz, $J_{1ax,2}$ = 9 Hz, H-1a,ax), 3.26 (dt, 1H, $J_{4,5} = J_{5,6ax}$ = 10 Hz, $J_{5,6eq}$ = 4.4 Hz, H-5a), 3.93 (dd, 1H, $J_{1,2}$ = 3.48 Hz, $J_{2,3}$ = 10 Hz, H-2b), 4.45 (~t, 1H, $J_{gem} = J_{5,6ax}$ = 10-12 Hz, H-6a,ax), 4.86 (dd,

1H, J_{gem} = 11.54 Hz, $J_{5,6eq}$ = 4.4 Hz, H-6a,eq), 5.06-5.16 (2d, 2H, CO_2CH_2Ph), 5.26 (d, 1H, $J_{1,2}$ = 3.48 Hz, H-1b), 5.60 (s, 1H, C*H*Ph), 7.2-7.6 (m, 25H, Ph-*H*).

Anal. Calcd for $C_{50}H_{54}N_2O_{10}$ (842.99): C, 71.24; H, 6.46; N, 3.32. Found: C, 71.31; H, 6.36; N, 3.61.

***O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-glucitol (8).** To a stirred mixture of **7** (494 mg) and molecular sieves 3Å (MS-3Å, 1 g) in dry THF (30 mL), was gradually added sodium cyanoborohydride ($NaBH_3CN$, 600 mg). After the reagent had dissolved, saturated HCl in ether was added dropwise at room temperature until the evolution of gas ceased. The reaction mixture was stirred for 4 h at room temperature and neutralized with Et_3N. The solids were removed by filtration and washed with MeOH and CH_2Cl_2, and the combined filtrate and washings were concentrated. The residue was taken up in CH_2Cl_2, washed with water, dried, and concentrated. Column chromatography (1:1 AcOEt-hexane) of the residue on silica gel gave **8** (495 mg) in almost quantitative yield: $[\alpha]_D$ -15.4° (*c* 0.93, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.07 (d, 3H, $J_{5,6}$ = 6.41 Hz, H-6b), 1.78 (s, 3H, AcN), 3.36 (bdd, 1H, J_{gem} = 14 Hz, $J_{1ax,2}$ = 3 Hz, H-1a,ax), 4.93 (d, 1H, $J_{1,2}$ = 3.67 Hz, H-1b), 5.07-5.19 (2d, 2H, CO_2CH_2Ph), 7.1-7.4 (m, 25H, Ph-*H*).

Anal. Calcd for $C_{50}H_{56}N_2O_{10}$ (845.00): C, 71.07; H, 6.68; N, 3.32. Found: C, 71.28; H, 6.81; N, 3.28.

***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*-benzoyl-β-D-galactopyranosyl)-(1→4)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-2-acetamido-6-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-glucitol (10).** To a solution of **8** (130 mg) and methyl *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*-benzoyl-1-thio-β-D-galactopyranoside (**9**, 230 mg, 1.5 equiv) in CH_2Cl_2 (20 mL) was added MS-4Å (400 mg), and the mixture was treated with *N*-iodosuccinimide (NIS, 110 mg, 3 equiv) and trifluoromethanesulfonic acid (TfOH, 5 μL, 0.3 equiv) overnight at -20°C. The solids

were filtered off and washed with CH_2Cl_2. The combined filtrate and washings were washed with sat. $NaHCO_3$, M $Na_2S_2O_3$ and water, dried, and concentrated. Column chromatography (2:1 Ac_2OEt-hexane) of the residue on silica gel afforded **10** (111 mg, 40% based on acceptor) as a syrup: $[\alpha]_D$ -7° (*c* 1.2, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 0.86 (d, 3H, $J_{5,6}$ = 6.23 Hz, H-6b), 1.79, 1.80 (2s, 6H, 2AcN), 1.89, 2.04, 2.126, 2.134 (4s, 12H, 4AcO), 2.41 (dd, 1H, J_{gem} = 13 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3d,eq), 3.20 (bdd, 1H, J_{gem} = 14 Hz, $J_{1ax,2}$ = 3 Hz, H-1a,ax), 3.79 (s, 3H, CO_2CH_3), 7.1-7.6, 7.95-8.2 (m, 40H, Ph-*H*).

Anal. Calcd for $C_{97}H_{105}N_3O_{30}$ (1792.90): C, 64.98; H, 5.90; N, 2.34. Found: C, 65.11; H, 6.18; N, 2.35.

***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*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-glucitol (11).** To a solution of **5** (150 mg) and **9** (525 mg, 1.5 equiv) in CH_2Cl_2 (15 mL) was added MS-4Å (800 mg), and the mixture was stirred 5 h at room temperature, then cooled to -20 °C. NIS (237 mg, 3 equiv) and TfOH (10 μL, 0.3 equiv) were added and stirring continued overnight at -20°C. Work-up and column chromatography (3:1 and 4:1 AcOEt-hexane) on silica gel as described for **10** gave **11** (220 mg, 46% based on acceptor) as an amorphous mass: $[\alpha]_D$ -0.24° (*c* 0.816, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.36, 1.42, 1.77, 1.92, 2.01, 2.19 (6s, 18H, 2AcN, 4AcO), 1.64 (t, 1H, J = 12.45 Hz, H-3c,ax), 2.47 (dd, 1H, J_{gem} = 12.45 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3c,eq), 2.78 (dd, 1H, J_{gem} = 13 Hz, $J_{1ax,2}$ = 9.7 Hz, H-1a,ax), 3.83 (s, 3H, CO_2CH_3), 4.99 (d, 1H, $J_{1,2}$ = 10 Hz, H-1b), 5.06, 5.12 (2d, 2H, CO_2CH_2Ph), 5.58 (s, 1H, C*H*Ph), 5.71 (m, 1H, H-8c), 7.1-7.7, 7.8-8.4 (m, 25H, Ph-*H*).

Anal. Calcd for $C_{70}H_{75}N_3O_{26}$ (1374.36): C, 61.18; H, 5.50; N, 3.06. Found: C, 61.11; H, 5.69; N, 3.21.

***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*-benzoyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-6-*O*-benzyl-*N*-**

benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-glucitol (12). To a stirred mixture of **11** (190 mg) and MS-3Å (400 mg) in THF (30 mL) was gradually added $NaBH_3CN$ (170 mg), and the mixture was processed as described for **8**. Work-up and column chromatography (4:1 AcOEt-hexane) on silica gel gave **12** (190 mg) in quantitative yield: $[\alpha]_D$ +16° (*c* 0.723, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.58, 1.71, 1.79, 1.90, 2.08, 2.17 (6s, 18H, 2AcN, 4AcO), 2.47 (dd, 1H, J_{gem} = 12.5 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3c,eq), 2.78 (bdd, 1H, J_{gem} = 14 Hz, $J_{1ax,2}$ = 2~3 Hz, H-1a,ax), 3.83 (s, 3H, CO_2CH_3), 4.85 (m, 1H, H-4c), 4.98 (d, 1H, $J_{1,2}$ = 8 Hz, H-1b), 5.02, 5.08 (2d, 2H, CO_2CH_2Ph), 5.24 (dd, 1H, H-7c), 5.43 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-4b), 5.44 (dd, 1H, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 10 Hz, H-2b), 5.63 (m, 1H, H-8c), 7.2-7.6, 8.0-8.2 (m, 25H, Ph-*H*).

Anal. Calcd for $C_{70}H_{77}N_3O_{26}$ (1376.38): C, 61.09; H, 5.64; N, 3.05. Found: C, 60.82; H, 5.55; N, 3.11.

***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*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-2-acetamido-6-*O*-benzyl-*N*-benzyloxycarbonyl-1,5-imino-1,2,5-trideoxy-D-glucitol (13).** To a stirred mixture of **12** (70 mg), **6** (35 mg) and MS-4Å (200 mg) in benzene (15 mL) was added NIS (35 mg, 3 equiv) and TfOH (1.5 μL, 0.3 equiv) at 7°C. The mixture was stirred for 5 h at 7 °C and work-up as described for **11**. Column chromatography (3:1 AcOEt-hexane) on silica gel gave **13** (50 mg, 55% based on acceptor): $[\alpha]_D$ -19° (*c* 1.46, CH_2Cl_2); ^1H NMR ($CDCl_3$): δ 1.06 (d, 3H, $J_{5,6}$ = 6 Hz, H-6d), 1.63 (t, 1H, J = 13 Hz, H-3c,ax), 2.46 (dd, 1H, J_{gem} = 13 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3c,eq), 2.92, 3.36 (2bd, 2H, J_{gem} = 14 Hz, $J_{1,2}$ = 2~3 Hz, H-1a), 3.81 (s, 3H, CO_2CH_3), 5.27 (dd, 1H, J = 9 and 3 Hz, H-7c), 5.41 (d, 1H, $J_{3,4}$ = 3.7 Hz, H-4b), 5.62 (m, 1H, H-8c), 7.1-7.6, 8.0-8.2 (m, 40H, Ph-*H*).

Anal. Calcd for $C_{97}H_{105}N_3O_{30}$ (1792.90): C, 64.98; H, 5.90; N, 2.34. Found: C, 65.13; H, 6.01; N, 2.62.

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-[(α-L-fu-**

copyranosyl)-(1→3)]-2-acetamido-1,5-imino-*N*-methyl-1,2,5-trideoxy-D-glucitol (15). Compound **10** (66 mg) in MeOH (10 mL) and formic acid (10 mL) was hydrogenolyzed in the presence of palladium-black catalyst (66 mg) for 10 days at room temperature. The catalyst was filtered off and washed with MeOH. The combined filtrate and washings were concentrated to dryness. Column chromatography (15:1 CH_2Cl_2–MeOH) of the residue on silica gel gave **14** (48 mg), which was dissolved in dry MeOH (10 mL) and treated with a catalytic amount of NaOMe overnight at room temperature, then with 0.2M KOH (5 mL) for 24 h. The solution was neutralized with Amberlite IR-120 (H^+) ion-exchange resin and filtered. The resin was washed with MeOH/H_2O, and the combined filtrate and washings were concentrated. Column chromatography (3:1 H_2O-MeOH) of the residue on Sephadex LH-20 gave **14** (29 mg) as an amorphous mass: $[\alpha]_D$ -4° (*c* 0.97, 4:1 H_2O-EtOH); ^1H NMR (D_2O): δ 1.19 (d, 1H, $J_{5,6}$ = 6.6 Hz, H-6b), 1.79 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12 Hz, H-3d,ax), 2.02, 2.03 (2s, 6H, 2AcN), 2.78 (dd, 1H, J_{gem} = 12 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3d,eq), 2.86 (s, 3H, N-C*H*$_3$), 3.00 (bt, 1H, $J_{gem} = J_{1ax,2}$ = 12-13 Hz, H-1a,ax), 3.45 (bdd, J_{gem} = 13 Hz, $J_{1eq,2}$ = 3-4 Hz, H-1a,eq), 4.65 (d, 1H, J = 8 Hz, H-1c), 5.39 (d, 1H, J = 3.7 Hz, H-1b); ion-spray MS (positive ion mode) *m/z* 818.3 $[M + H]^+$, (negative ion mode) *m/z* 816.7 $[M - H]^-$, MS/MS (daughter ions derived from *m/z* 818) *m/z* (relative intensity) 818.2 (67), 672.1 $[M - Fuc + H]^+$ (16), 526.3 $[M - NeuAc + H]^+$ (5.4), 381.2 $[M - Fuc - NeuAc + H]^+$ (31), 365.4 $[M - NeuAc - Gal + H]^+$ (5.5), 292.1 [NeuAc fragment]$^+$ (10), 274.1 [NeuAc fragment - H_2O]$^+$ (30), 219.0 $[M - Fuc - NeuAc - Gal + H]^+$ (100), 200.6 [219 - H_2O]$^+$ (12).

Anal. Calcd for $C_{32}H_{55}N_3O_{21}$ (817.79): C, 47.00; H, 6.78; N, 5.14. Found: C, 46.97; H, 6.48; N, 5.06.

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→3)-*O*-[(α-L-fucopyranosyl)-(1→4)]-2-acetamido-1,5-imino-*N*-methyl-1,2,5-trideoxy-D-glucitol (17).** Compound **13** (61 mg) in MeOH (10 mL) and formic acid (10 mL) was hydrogenolyzed in the presence of palladium-black catalyst (60 mg) for 7 days at room temperature. Work-up and column chromatography as described for **14** to afford

16 (44 mg), a part of which (30 mg) was successively treated with NaOMe in MeOH and then with 0.2M KOH, and processed as described for **15** to give **17** (19 mg) as an amorphous mass: $[\alpha]_D$ -23.5° (*c* 0.23, 4:1 H_2O-EtOH); ^1H NMR (D_2O): δ 1.19 (d, 1H, $J_{5,6}$ = 6 Hz, H-6d), 1.77 (t, 1H, $J_{gem} = J_{3ax,4}$ = 12 Hz, H-3c,ax), 2.03 (s, 6H, 2AcN), 2.77 (dd, 1H, J_{gem} = 12 Hz, $J_{3eq,4}$ = 4.4 Hz, H-3c,eq), 2.83 (s, 3H, N-C*H*$_3$), 4.60 (d, 1H, $J_{1,2}$ = 8 Hz, H-1b), 5.05 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1d); ^{13}C NMR (D_2O): δ 16.69 (C-6d), 23.37, 23.67 (Me of 2AcN), 99.76, 100.78 104.21 (anomeric carbon), 175.16 (CO of AcN), 176.30 (C-1c); ion-spray MS (positive ion mode) *m/z* 818.3 $[M + H]^+$, (negative ion mode) *m/z* 816.2 $[M - H]^-$; MS/MS (daughter ions derived from *m/z* 818) *m/z* (relative intensity) 817.6 (64), 672.3 $[M - Fuc + H]^+$ (5.4), 527.1 $[M - NeuAc + H]^+$ (16), 381.2 $[M - Fuc - NeuAc + H]^+$ (14), 365.2 $[M - NeuAc - Gal + H]^+$ (39), 292.1 [NeuAc fragment]$^+$ (14), 274.1 [NeuAc fragment - $H_2O]^+$ (34), 219.0 $[M - Fuc - NeuAc - Gal + H]^+$ (100), 201.0 $[219 - H_2O]^+$ (10).

Anal. Calcd for $C_{32}H_{55}N_3O_{21}$ (817.79): C, 47.00; H, 6.78; N, 5.14. Found: C, 46.80; H, 6.50; N, 5.16.

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