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Note

A Convenient and Efficient Synthesis of Sialyl Lewis X[†]

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A convenient synthesis of the sialyl Lewis X (sLe^x) tetrasaccharide, NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAc (8), as a carbohydrate ligand for selectins is described. The key step is the reaction between NeuAcα2-3GalSMe (5) as a glycosyl donor and the suitably protected Fucα1-3GlcNAc derivative (4) as the glycosyl acceptor by using dimethyl(methylthio)sulfonium triflate (DMTST) as the promoter.

Key words: selectin; glycosylation; sialyl Lewis X

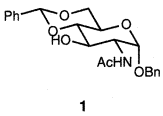
Selectins are a family of cell adhesion molecules (CAMs) that play an important role in the initial interaction of leukocyte homing, platelet binding, and neutrophil extravasation. There is now general agreement that E-selectin (ELAM-1), P-selectin (GMP-140) and L-selectin (LECAM-1) can all recognize sLe^x.[2,3] Therefore, many analogs of sLe^x have been designed and synthesized.[4] However, there have been few attempts[5] at a convenient and efficient synthesis of sLe^x itself, especially by using a disaccharide acceptor and a disaccharide donor in the key step.

It is thus very important to develop a convenient synthetic method for sLe^x. As part of our studies on selectin blockers,[6] we describe here a regio- and stereo-controlled synthesis of the sLe^x tetrasaccharide.

Glycosylation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**1**)[7] with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**2**)[8] in CH_2Cl_2 for 30 min at 0°C in the presence of dimethyl(methylthio)sulfonium triflate (DMTST)[9,10] and 4 Å molecular sieves (MS-4A) exclusively gave α-glycoside **3** in a 94% yield. Reductive ring-opening of the benzylidene group in **3** with sodium cyanoborohydride-hydrogen chloride in ether regioselectively gave **4** in a 77% yield.

Compound **4** was then used as the glycosyl acceptor to react 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 (**5**)[11] as the glycosyl donor for the synthesis of sLe^x.

Glycosylation of **4** with **5** in CH_2Cl_2 for 6 days at room temperature in the presence of DMTST and MS-4A gave desired tetrasaccharide **6** in a 60% yield. Significant signals in the ^{1}H-NMR spectrum of **6** were a three-proton doublet at δ 1.50 ($J_{5,6}$=6.4 Hz, CH_3 of the fucose moiety), six three-proton singlets at δ 1.70, 1.86, 1.95, 1.99, 2.08 and 2.24 (N-$COCH_3$, O-$COCH_3$), a three-proton singlet at δ 3.70 (O-CH_3), a complex resonance at δ 7.12–8.25 ($8C_6H_5$), and a one-proton doublet at δ 5.10 ($J_{1,2}$=8.4 Hz, H-1 of the benzoylated Gal moiety), indicating the newly formed glycosidic linkage to be β. *O*-Deacylalion of **6** with sodium methoxide in methanol for 3 days at 40°C and subsequent saponification of the methyl ester group gave compound **7** in a 95% yield. Finally, catalytic hydrogenolysis (20% Pd-C) of the benzyl groups of **7** in methanol-water for 2 days at room temperature yielded desired sLe^x **8** in a 95% yield, after chromatography in a column of Sephadex LH-20.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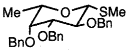
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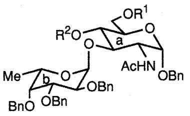
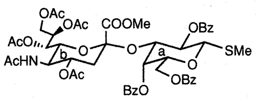

3 R^1, R^2=benzylidene
4 R^1=Bn, R^2=H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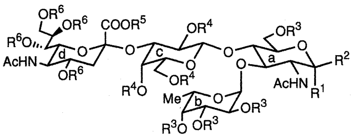

	R^1	R^2	R^3	R^4	R^5	R^6
6	OBn	H	Bn	Bz	Me	Ac
7	OBn	H	Bn	H	H	H
8	H, OH		H	H	H	H

In conclusion, a facile and good yielding synthesis of sLe^x was achieved by glycosylating disaccharide acceptor **4** with disaccharide donor **5**.

Experimental

All melting point (mp) data are uncorrected. Optical rotation values were determined with a Horiba SEPA-300 instrument at 25°C, and ^{1}H- and ^{13}C-NMR spectra were recorded by a Jeol JNM-GSX 270 spectrometer, using tetramethylsilane as the internal standard. FABMS data were recorded by a Jeol JMS-SX 102 mass spectrometer, and preparative chromatography was performed on silica gel (Merck Silica Gel 60) with the specified solvent systems.

[†] Synthetic studies on sialoglycoconjugates, part 105. For part 104, see ref. 1.
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Benzyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (**3**). To a solution of **1** (1.5 g, 3.8 mmol) and **2** (2.5 g, 5.3 mmol) in CH_2Cl_2 (80 ml) was added MS-4A (2 g). The mixture was stirred for 1.5 h at room temperature and cooled to 0°C before DMTST (3.6 g, 14 mmol) was added. After stirring had been continued for 30 min at 0°C, the mixture was filtered and washed with CH_2Cl_2 (50 ml). The combined filtrate and washings were washed with 1 M Na_2CO_3, dried (Na_2SO_4) and concentrated. The residue was crystallized from EtOAc to give **3** (2.9 g, 94%) as colorless crystals; mp 177°C; $[\alpha]^{25}_{D}$-0.09° (c 0.33, 3:1 CH_3OH–$CHCl_3$); NMR ($CDCl_3$) δ_H: 1.07 (d, 3H, $J_{5,6}=6.3$ Hz, H-6b), 1.42 (s, 3H, AcN), 5.19 (d, 1H, $J_{1,2}=3.4$ Hz, H-1b), 5.22 (d, 1H, $J_{1,2}=3.4$ Hz, H-1a), 5.59 (s, 1H, benzylidene-H), 6.50 (d, 1H, NH), and 7.0–7.6 (m, 25H, 5Ph); LRMS *m/z* (FAB-negative) 814; HRMS *m/z* (FAB-negative): calcd. for $C_{49}H_{52}O_{10}N$, 814.3591; found, 814.3618.

Benzyl O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-6-O-benzyl-2-deoxy-α-D-glucopyranoside (**4**). To a solution of **3** (2.0 g, 2.5 mmol) in THF (160 ml) were added MS-4A (4 g) and Congo Red (10 mg), and the mixture was stirred for 1 h at room temperature. After $NaBH_3CN$ (1.5 g, 25 mmol) had been added, the mixture was cooled, and 2 N HCl in Et_2O was added dropwise at 0°C until a blue color was obtained. After being neutralized with Et_3N, the mixture was filtered and washed with CH_2Cl_2 (50 ml). The combined filtrate and washings were washed with water, dried (Na_2SO_4) and concentrated. The residue was chromatographed in a silica gel column (2:1 *n*-hexane-EtOAc) to give **4** (1.6 g, 77%) as colorless crystals; mp 162°C; $[\alpha]^{25}_{D}+0.25°$ (c 1.0, $CHCl_3$); NMR ($CDCl_3$) δ_H: 1.15 (d, 3H, $J_{5,6}=6.3$ Hz, H-6b), 1.40 (s, 3H, AcN), 4.97 (d, 1H, $J_{1,2}=3.4$ Hz, H-1b), 5.22 (d, 1H, $J_{1,2}=3.4$ Hz, H-1a), 5.95 (d, 1H, NH), and 7.15–7.45 (m, 25H, 5Ph); LRMS *m/z* (FAB-negative) 816; HRMS *m/z* (FAB-negative): calcd. for $C_{49}H_{54}O_{10}N$, 816.3747; found, 816.3773.

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-(2,4,6-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-2-deoxy-α-D-glucopyranoside (**6**). To a solution of **4** (1.0 g, 1.2 mmol) and **5** (2.0 g, 2.0 mmol) in CH_2Cl_2 (25 ml) was added MS-4A (8 g). The mixture was stirred for 24 h at room temperature and cooled to 0°C before DMTST (2.6 g, 10 mmol) in CH_2Cl_2 (15 ml) was added. Stirring was continued for 6 days at room temperature, after which the solids were filtered off and washed with CH_2Cl_2 (100 ml). The combined filtrate and washings were successively washed with 1 M Na_2CO_3 and water, dried (Na_2SO_4) and concentrated. Column chromatography (1:4 *n*-hexane-EtOAc) of the residue on silica gel (300 g) gave **6** (1.3 g, 60%) as an amorphous mass; mp 122°C; $[\alpha]^{25}_{D}+0.11°$ (c 0.44, CH_3OH); NMR (CD_3OD) δ_H: 1.50 (d, 3H, $J_{5,6}=6.4$ Hz, H-6b), 1.70–2.24 (6s, 18H, 4AcO, 2AcN), 2.50 (dd, 1H, $J_{gem}=12.5$, $J_{3eq,4}=4.9$ Hz, H-3d*eq*), 3.70 (s, 3H, MeO), 4.90 (dd, 1H, $J_{2,3}=9,9$, $J_{3,4}=3.5$ Hz, H-3c), 5.10 (d, 1H, $J_{1,2}=8.4$ Hz, H-1c), 5.25 (dd, 1H, $J_{6,7}=2.5$, $J_{7,8}=9.9$ Hz, H-7d), 5.30 (d, 1H, $J_{3,4}=3.5$ Hz, H-4c), 5.35 (d, 1H, $J_{1,2}=3.5$ Hz, H-1b), 5.55 (dd, 1H, $J_{1,2}=8.4$, $J_{2,3}=9.9$ Hz, H-2c), and 7.12–8.25 (m, 40H, 8Ph); LRMS *m/z* (FAB-negative) 1763. *Anal.* Found: C, 65.07; H, 6.12; N, 1.62%. Calcd. for $C_{96}H_{104}N_2O_{30}$ (1765.9): C, 65.30; H, 5.94; N, 1.59%.

Benzyl O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-O-β-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 (**7**). To a solution of **6** (1.3 g, 0.74 mmol) in MeOH (100 ml) was added NaOMe (320 mg). The mixture was stirred for 3 days at 40°C, the course of the reaction being monitored by TLC. Water (0.5 ml) was added, and the mixture was stirred for an additional 18 hours at room temperature before being neutralized with Amberlist 15 (H^+) resin. The resin was filtered off and washed with MeOH, and the combined filtrate and washings were concentrated. Column chromatography (2:1:0.1 $CHCl_3$–MeOH–H_2O) of the residue on silica gel (300 g) gave **7** (890 mg, 95%) as an amorphous mass; mp 178°C; $[\alpha]^{25}_{D}+0.02°$ (c 0.53, CH_3OH); NMR (CD_3OD) δ_H: 1.17 (d, 3H, $J_{5,6}=6.4$ Hz, H-6b), 1.88, 2.03 (2s, 6H, 2AcN), 2.90 (dd, 1H, $J_{gem}=12.3$, $J_{3eq,4}=3.5$ Hz, H-3d*eq*), 4.30 (dd, 1H, $J_{1,2}=3.5$, $J_{2,3}=10.4$ Hz, H-2a), 4.45 (d, 1H, $J_{1,2}=7.4$ Hz, H-1c), 5.30 (d, 1H, $J_{1,2}=3.5$ Hz, H-1b), and 7.21–7.44 (m, 25H, 5Ph); LRMS *m/z* (FAB-negative) 1269. *Anal.* Found: C, 62.17; H, 6.71; N, 2.08%. Calcd. for $C_{66}H_{82}N_2O_{23}$ (1271.4): C, 62.35; H, 6.50; N, 2.20%.

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-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-D-glucopyranose (**8**). A solution of **7** (840 mg, 0.66 mmol) in MeOH (40 ml) and H_2O (20 ml) was hydrogenated in the presence of 20% Pd-C (840 mg) for 48 h at 25°C, before being filtered and concentrated. Column chromatography (MeOH) of the residue on Sephadex LH-20 (50 g) gave **8** (514 mg, 94.8%) as an amorphous mass; $[\alpha]^{25}_{D}+3.0°$ (c 0.13, CH_3OH); NMR (CD_3OD) δ_H: 1.17 (d, 3H, $J_{5,6}=6.4$ Hz, H-6b), 1.98, 2.01 (2s, 6H, 2AcN), 2.90 (dd, 1H, $J_{gem}=12.3$, $J_{3eq,4}=3.5$ Hz, H-3d*eq*), 4.15 (dd, 1H, $J_{1,2}=3.4$ Hz, $J_{2,3}=10.7$ Hz, H-2a), 4.50 (d, 1H, $J_{1,2}=7.8$ Hz, H-1c), 5.00 (d, 1H, $J_{1,2}=3.4$ Hz, H-1a), and 5.05 (d, 1H, $J_{1,2}=3.9$ Hz, H-1b); NMR (CD_3OD) δ_c: 175.4, 174.9, 173.8, 104.0, 100.9, 100.1, 93.0, 78.0, 76.7, 75.8, 75.0, 74.3, 73.8, 73.0, 72.8, 71.2, 70.9, 70.2, 70.0, 69.4, 68.9, 67.6, 64.7, 63.1, 61.4, 55.8, 54.0, 42.4, 22.8, 22.6, 16.5; LRMS *m/z* (FAB-negative) 819; HRMS *m/z* (FAB-negative): calcd. for $C_{31}H_{51}O_{23}N_2$, 819.2881; found, 819.2855.

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