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Preliminary communication

Synthesis of 1-deoxynojirimycin-containing glycans related to the Lewis X and sialyl-Lewis X epitopes recognized by LEC-CAMs *

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LEC-CAMs (selectins)[2,3], a family of cell adhesion receptors, mediate leukocyte trafficking and recruitment to sites of inflamation on vascular endothelium. These receptor proteins contain an *N*-terminal carbohydrate recognition domain homologous to various calcium-dependent mammalian lectins. Very recently, the sialyl-Lewis X epitope, found on neutrophils, monocytes, and tumor cells, has been identified[4–8] as a minimal carbohydrate ligand recognized not only by endothelial–leukocyte adhesion molecule 1 (ELAM-1, E-selectin) but also by other LEC-CAMs, such as platelet activation-dependent granule external membrane protein[9] (PADGEM, P-selectin) and LECAM-1[10] (MEL-14, L-selectin). In the course of synthetic studies on sialoglycoconjugates, we have recently succeeded[11] in the first total synthesis of sialyl-Lewis X ganglioside and its analogs. Some of the cell adhesion studies[7–10] of LEC-CAMs just described have been accomplished by employing those chemically synthesized gangliosides.

1-Deoxynojirimycin (DNJ) and related compounds have been shown[12–17] not only to be potent inhibitors of α-glucosidases and glycoprotein-processing enzymes, but also to be of potential clinical value as antidiabetic, antineoplastic, and anti-HIV agents. We have synthesized a variety of DNJ derivatives[18,19], DNJ-containing disaccharides[20], and sialyl oligosaccharides[21] designed as novel biofunctional glycans. The present paper describes the synthesis of DNJ-containing Lewis X and sialyl-Lewis X epitopes.

4,6-*O*-Benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol[20] (**1**) was treated with chloroacetyl chloride (1.2 mol equiv) at $-20°$ in CH_2Cl_2 containing 2,6-lutidine (2 mol equiv), to give a 70% yield of the 3-*O*-chloroacetyl derivative

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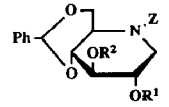
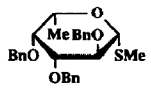


	R^1	R^2
1	H	H
2	H	ClAc
3	Ac	H

Z = benzyloxycarbonyl
ClAc = chloroacetyl



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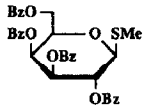
Bn = benzyl



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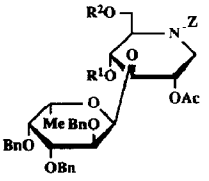
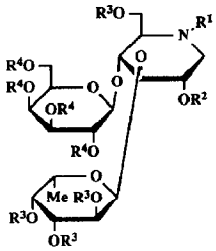
Bz =benzoyl

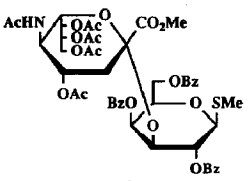


	R^1	R^2
5	benzylidene	
6	H	Bn



	R^1	R^2	R^3	R^4
8	Z	Ac	Bn	Bz
9	H	H	H	H

(Lewis X epitope)



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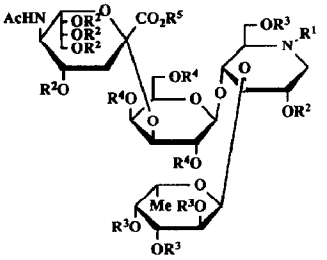

	R^1	R^2	R^3	R^4	R^5
11	Z	Ac	Bn	Bz	Me
12	H	Ac	H	Bz	Me
13	H	H	H	H	H

(sialyl-Lewis X epitope)

2 having $[\alpha]_D$ −5.2° (CH_2Cl_2) and showing in its ^{1}H-NMR spectrum a triplet at δ 5.04 ($J_{2,3} = J_{3,4} = 8.4$ Hz, H-3). Following 2-*O*-acetylation of **2**, dechloroacetylation with aq pyridine gave the glycosyl acceptor **3**, $[\alpha]_D$ −12.5° (CH_2Cl_2), quantitatively. In the ^{1}H-NMR spectrum of **3** H-2 appeared at δ ~4.83 as a one-proton multiplet partially overlapping H-6*eq*. Other significant signals were at δ 2.04 (s, CH_3CO), 3.17 (dd, J_{gem} 13.9, $J_{1ax,2}$ 8.2 Hz, H-1*ax*), 4.11 (dd, $J_{1eq,2}$ 4.4 Hz, H-1*eq*), 3.79 (dd, $J_{2,3}$ 6.6, $J_{3,4}$ 9 Hz, H-3), 3.70 (t, $J_{4,5}$ 9 Hz, H-4), and 3.37 (ddd, $J_{5,6ax}$ 10.8, $J_{5,6eq}$ 4.2 Hz, H-5). Glycosylation of **3** with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**4**) in the presence of dimethyl(methylthio)sulfonium triflate[22,23] (DMTST) and molecular sieves 4A in benzene for 2.5 h, with warming from 7° to room temperature, gave the desired disaccharide **5**, $[\alpha]_D$ −94° (CH_2Cl_2), in 92%

yield. The ^{1}H-NMR spectrum of **5** showed a three-proton doublet at δ 0.83 ($J_{5,6}$ 6.6 Hz, CH_3CH) and a one-proton doublet at δ 5.21 ($J_{1,2}$ 4 Hz, H-1), characteristic of the α-L-fucopyranosyl unit. Reductive ring-opening[24] of the benzylidene group in **5** with sodium cyanoborohydride–hydrogen chloride in dry ether afforded the disaccharide glycosyl acceptor **6**, $[\alpha]_D$ −51° (CH_2Cl_2), in 81% yield. Significant signals in the ^{1}H-NMR spectrum of **6** were at δ 3.38 (dd, J_{gem} 15.4, $J_{1ax,2}$ 2.9 Hz, H-1*ax* of DNJ), 4.12 (~ d, J_{gem} 15.4, $J_{1eq,2} < 2$ Hz, H-1*eq* of DNJ), 4.91 (d, $J_{1,2}$ 3.7 Hz, H-1 of Fuc), and 4.35–5.21 (10 d, CH_2Ph).

The glycosylation of **6** with methyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**7**) was performed in the presence of *N*-iodosuccinimide[25–27] (NIS, 4 mol equiv) and trifluoromethanesulfonic acid (TfOH, 0.4 mol equiv) in CH_2Cl_2, to give the desired trisaccharide **8**, $[\alpha]_D$ −10.4° (CH_2Cl_2), in 70% yield. Hydrogenolytic removal of the benzyloxycarbonyl and benzyl groups over palladium black in MeOH–formic acid, and subsequent *O*-deacylation with NaOMe in MeOH, gave the 1-deoxynojirimycin-containing Lewis X epitope **9**, $[\alpha]_D$ −24° (2:1 H_2O–EtOH), in quantitative yield. Significant signals in the ^{1}H-NMR spectrum of **9** (in D_2O–CD_3OD) were at δ 1.14 (d, $J_{5,6}$ 6.8 Hz, CH_3CH of Fuc), 2.97 (dd, J_{gem} 12, $J_{1ax,2}$ 4.4 Hz, H-1*ax* of DNJ), 4.51 (d, $J_{1,2}$ 7.7 Hz, H-1 of Gal), and 5.37 (d, $J_{1,2}$ 3.9 Hz, H-1 of Fuc), indicating the desired trisaccharide structure.

Coupling of **6** 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] (**10**) was carried out by using NIS (3 mol equiv) and a catalytic amount of TfOH (0.3 mol equiv) as described for **8**, to afford, **11** $[\alpha]_D$ −11° (CH_2Cl_2), in 61% yield. In the ^{1}H-NMR spectrum of **11**, a one-proton doublet at δ 4.64 ($J_{1,2}$ 8 Hz, H-1 of Gal) showed the newly formed glycosidic linkage to be β. Other significant signals were at δ 3.81 (s, CO_2CH_3) and 2.45 (dd, J_{gem} 12.8, $J_{3eq,4}$ 4.6 Hz, H-3*eq* of sialic acid).

The benzyloxycarbonyl and benzyl groups in **11** were simultaneously removed by catalytic hydrogenolysis over palladium black in 1:1 MeOH–acetic acid to give a quantitative yield of **12** $[\alpha]_D$ −20° (MeOH), which was converted, by treatment with NaOMe in MeOH and subsequent saponification of its methyl ester group, into the 1-deoxynojirimycin-containing sialyl-Lewis X epitope **13** $[\alpha]_D$ −14° (3:1 H_2O–EtOH), obtained in quantitative yield after chromatography on a column of Sephadex LH-20. In the ^{1}H-NMR spectrum of **13** the two anomeric protons of residues bound to the DNJ moiety appeared at δ 4.62 ($J_{1,2}$ 7.9 Hz, H-1 of Gal) and 5.40 ($J_{1,2}$ 4 Hz, H-1 of Fuc), respectively, showing the desired β- and α-glycosidic linkages. Very recently, a stepwise synthesis of the sialyl Lewis X epitope itself was reported[28].

In conclusion, by employing methyl thioglycosides **4**, **7**, and **10** as glycosyl donors, and suitably protected mono- and di-saccharide derivatives of 1-deoxynojirimycin (**3** and **6**) as glycosyl acceptors, we achieved syntheses of novel 1-deoxynojirimycin-containing glycans structurally related to the Lewis X and sialyl-Lewis X epitopes recognized by LEC-CAMs.

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