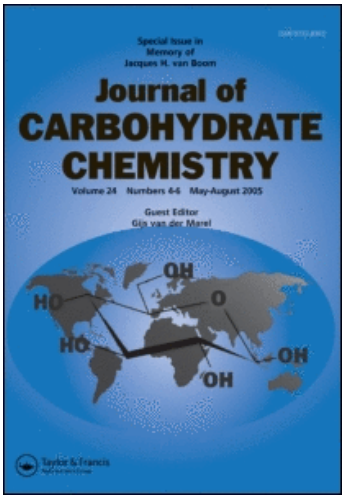

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthetic Studies on Sialoglycoconjugates 45: Synthesis of 1-Deoxynojirimycin-Containing Oligosaccharides Related to the Cancer-Associated Sialyl-Lewis a Antigen Recognized by Lec-Cams (Selectins)

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To cite this Article Kiso, Makoto , Furui, Hiroyasu , Ando, Keiko and Hasegawa, Akira(1993) 'Synthetic Studies on Sialoglycoconjugates 45: Synthesis of 1-Deoxynojirimycin-Containing Oligosaccharides Related to the Cancer-Associated Sialyl-Lewis a Antigen Recognized by Lec-Cams (Selectins)', Journal of Carbohydrate Chemistry, 12: 4, 673 — 677
To link to this Article: DOI: 10.1080/07328309308019416
URL: http://dx.doi.org/10.1080/07328309308019416

J. CARBOHYDRATE CHEMISTRY, 12(4&5), 673-677 (1993)

COMMUNICATION

SYNTHETIC STUDIES ON SIALOGLYCOCONJUGATES 45: SYNTHESIS OF 1-DEOXYNOJIRIMYCIN-CONTAINING OLIGOSACCHARIDES RELATED TO THE CANCER-ASSOCIATED SIALYL-LEWIS A ANTIGEN RECOGNIZED BY LEC-CAMs (SELECTINs)[1]

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Received October 6, 1992 - Final Form January 21, 1993

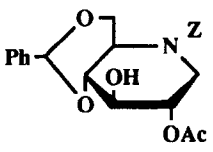
Sialyl-Lewis a (sLe^a), mainly expressed on cancer cells of the digestive organs,[2] has been known as an important cancer-associated carbohydrate antigen.[3] Very recently, it has been demonstrated[4-9] that the sLe^a antigen is one of the possible ligands recognized by selectins, a family of lectin-type cell adhesion molecules (LEC-CAMs). These findings suggest that the sLe^a antigen may be involved in the process of hematogeneous metastasis of cancer cells. 1-Deoxynojirimycin (DNJ) and related compounds have been shown[10] not only to be potent inhibitors of α-glycosidases and glycoprotein-processing enzymes, but also to be of potential clinical value as antidiabetic, antineoplastic and anti-HIV agents. In a preceding paper we reported the synthesis of DNJ-containing sialyl-Lewis x antigen which has been identified as a major carbohydrate ligand for leukocyte adhesion on vascular endothelium mediated by selectins. The present paper describes the first synthesis of 1-deoxynojirimycin-containing cancer-associated sLe^a antigen and related compounds.

2-*O*-Acetyl-4,6-*O*-benzylidene-*N*-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-D-glucitol[11] (**1**, 1 mol equiv), prepared from DNJ *via* the 3-*O*-chloroacetyl derivative, was coupled with methyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (**2**, 2 mol

equiv) in the presence of *N*-iodosuccinimide[12-14] (NIS, 4 mol equiv), trifluoromethanesulfonic acid (TfOH, 0.4 mol equiv) and molecular sieves 4Å at -20 °C to room temperature, to give the desired disaccharide **3**, $[\alpha]_D$ +20° (CH_2Cl_2), in a quantitative yield. The ^1H NMR spectrum of **3** showed a one-proton doublet at δ 5.16 ($J_{1,2}$ = 8.1 Hz, H-1 of Gal), characteristic of the β-glycosidic linkage. Reductive ring opening[15] of the benzylidene group in **3** with sodium cyanoborohydride-hydrogen chloride in dry ether afforded the next glycosyl acceptor **4**, $[\alpha]_D$ +62° (CH_2Cl_2), in 90% yield. Significant signals in the ^1H NMR ($CDCl_3$) spectrum of **4** appeared at δ 1.59 (s, OAc), 3.27 (dd, Jgem = 15.6, $J_{1ax,2}$ = 2.7 Hz, H-1ax), 4.00 (d, Jgem = 15.6, $J_{1eq,2}$ < 2 Hz, H-1eq) and 4.70 (narrow m, H-2) for the DNJ moiety, and δ 4.94 (d, $J_{1,2}$ = 8.1 Hz, H-1), 5.63 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 3.5 Hz, H-3), 5.80 (dd, H-2) and 5.98 (~d, J = 3.3 - 3.5 Hz, H-4) for the Gal moiety. These ^1H NMR data show that the preferred chair conformation[16] of the DNJ moiety shifted to the $^1C_4$ form by the reductive ring opening of the benzylidene group.

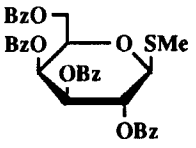
The glycosylation of **4** (1 mol equiv) with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**5**, 1.5 mol equiv) was performed in the presence of dimethyl (methylthio)sulfonium triflate[17,18] (DMTST, 4 mol equiv) and molecualr sieves 4Å in benzene for 3.5 h at 7 °C to room temperature, to give the desired trisaccharide **6**, $[\alpha]_D$ -25° (CH_2Cl_2), quantitatively. When the trisaccharide **6** was hydrogenolyzed over palladium black in MeOH-formic acid for 7~10 days, N-methylation of the DNJ moiety occurred to give **7**, $[\alpha]_D$ -13° (MeOH), almost quantitatively. In the ^1H NMR spectrum (CD_3OD) of **7**, a three-proton doublet at δ 1.41 ($J_{5,6}$ = 6.6 Hz, CH_3 of Fuc) and a one-proton doublet at δ 5.17 ($J_{1,2}$ = 3.7 Hz, H-1 of Fuc) indicated the newly formed glycosidic linkage to be α. The other significant signals appeared at δ 2.14, 2.31 (2s, OAc and N-Me of DNJ), 5.30 (d, J = 7.5 Hz, H-1 of Gal) and 6.00 (~d, J = 3.1 Hz, H-4 of Gal). Treatment of **7** with NaOMe in MeOH, and subsequent purification by Sephadex LH-20 column chromatography gave the DNJ-containing Lewis a (Le^a) epitope **8** (quant), $[\alpha]_D$ +1° (2:1 H_2O-EtOH). The ^1H NMR spectrum of **8** (D_2O-CD_3OD, δ from internal TMS) showed two anomeric protons at δ 4.79 (d, $J_{1,2}$ = 7.2 Hz, H-1 of Gal) and 5.15 (d, $J_{1,2}$ = 3 Hz, H-1 of Fuc). In the ion-spray mass spectrum (positive ion mode), two significant peaks at m/z 486.6 (base peak) $(M+H)^+$ and 507.9 $(M+Na)^+$ were observed.

Coupling of **1** 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[19] (**9**) was carried out by using NIS (3 mol equiv) and a catalytic amount of TfOH (0.3 mol equiv) as described for **3**, to afford **10**, $[\alpha]_D$ +9° ($CHCl_3$), in 90% yield. The structure of **10** was confirmed from the ^1H NMR data of



1

Z = benzyloxycarbonyl



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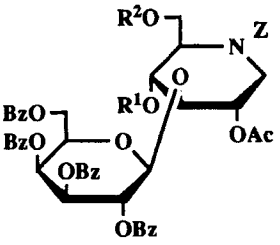
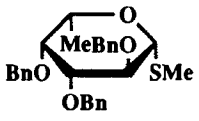
Bz =benzoyl

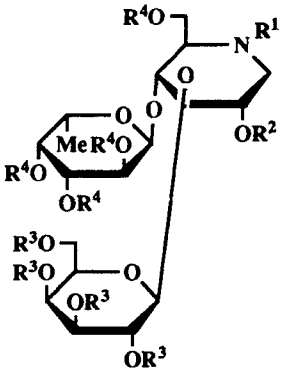


	R^1	R^2
3	benzylidene	
4	H	Bn



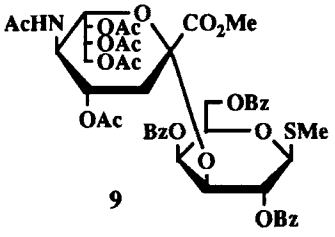
5

Bn = benzyl

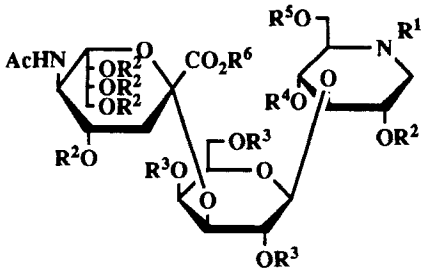


	R^1	R^2	R^3	R^4
6	Z	Ac	Bz	Bn
7	Me	Ac	Bz	H
8	Me	H	H	H

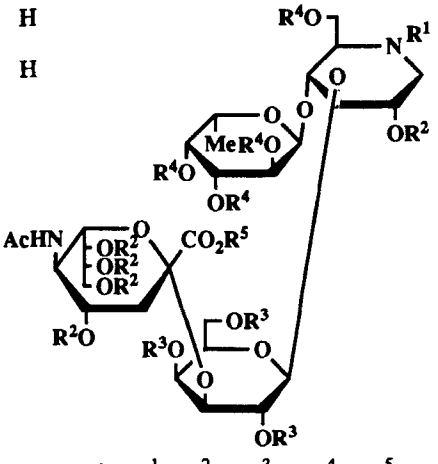
(Le^a epitope)



9



	R^1	R^2	R^3	R^4	R^5	R^6
10	Z	Ac	Bz	benzylidene		Me
11	Z	Ac	Bz	H	Bn	Me
12	Me	Ac	Bz	H	H	Me



	R^1	R^2	R^3	R^4	R^5
13	Z	Ac	Bz	Bn	Me
14	Me	Ac	Bz	H	Me
15	Me	H	H	H	H

(sialyl-Le^a epitope)

12 which was prepared by the hydrogenolysis of **10**. The significant ^{1}H NMR data of **12** were as follows: for Gal moiety, δ 5.09 (d, $J_{1,2}$ = 7.9 Hz, H-1), 5.41 (~d, J = 2.9 Hz, H-4), 5.47 (dd, $J_{2,3}$ = 9.9 Hz, H-2) and 7.4-8.2 (m, Ph-H) and for Neu5Ac moiety, δ 3.85 (s, CO_2Me), 4.77 (m, H-4), 4.94 (dd, $J_{5,6}$ = 10, $J_{6,7}$ = 3.3 Hz, H-6), 5.27 (dd, $J_{7,8}$ = 9.5 Hz, H-7), and 5.60 (m, H-8). Six singlets of acetyl groups appeared at δ 1.61, 1.68, 1.77, 1.90, 2.08, and 2.19, respectively.

Reductive ring opening of the benzylidene group in **10** as described for **4** gave **11** (quant), $[\alpha]_D$ +26° (CH_2Cl_2), which was then coupled with **5** in the presence of DMTST as a glycosyl promoter to afford the desired tetrasaccharide **13** (89%), $[\alpha]_D$ -16° (CH_2Cl_2). Hydrogenolysis of **13** was performed as described for **6** to give **14** (quant), $[\alpha]_D$ -26° (MeOH). In the ^{1}H NMR spectrum of **14**, a significant one-proton doublet was observed at δ 5.10 ($J_{1,2}$ = 3.5 Hz, H-1 of Fuc) to show the newly formed glycosidic linkage to be α.

Compound **14** was then treated with NaOMe in MeOH and the remaining methyl ester group was saponified by adding 2 M KOH, to give the desired DNJ-containing sialyl-Le^a epitope **15**, $[\alpha]_D$ -26° (4:1 H_2O-EtOH) in a quantitative yield after chromatography on a column of Sephadex LH-20. The significant ^{1}H NMR data of **15** (D_2O, δ relative to internal acetone, 2.225 ppm) were as follows: δ 1.20 (d, $J_{5,6}$ = 6.6 Hz, C*H*$_3$ of Fuc), 1.80 (~t, J = 12~13 Hz, H-3ax of Neu5Ac), 2.03 (s, C*H*$_3$CO), 2.76 (dd, J = 12.4, 4.7 Hz, H-3eq of Neu5Ac), 2.96 (s, N-C*H*$_3$ of DNJ), 4.80 (d, J = 7.7 Hz, H-1 of Gal), and 5.10 (d, J = 2.8 Hz, H-1 of Fuc). The mass spectrum of **15** (negative ion mode) showed the significant base peak at m/z 775.3 $(M-H)^-$.

In conclusion, the first syntheses of 1-deoxynojirimycin (DNJ)-containing Le^a and sialyl-Le^a antigens (**8** and **15**) were achieved by employing methyl thioglycosides **2, 5** and **9** as glycosyl donors, and suitably protected mono-, di- and trisaccharide derivatives of 1-deoxynojirimycin (**1, 4** and **11**) as glycosyl acceptors. In these syntheses, we have found that the *N*-benzyloxycarbonyl group in the DNJ part can be hydrogenolyzed directly to give the *N*-methyl-DNJ derivatives.

ACKNOWLEDGMENT

This work was supported in part by a grant-in-aid (No. 03660133) for scientific research from the Japanese Ministry of Education, Science and Culture.

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