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Preliminary communication

Stereocontrolled synthesis of sialyl Lewis X ceramide consisting of a pentasaccharide recognized by the selectin family *

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Glycoconjugates containing sialic acid have received much attention owing to their important functions in biological systems[2]. Very recently, it has been demonstrated[3] that the selectin family, such as E-selectin (endotherial leukocyte adhesion molecule-1, ELAM-1), P-selectin (GMP-140), and L-selectin (LECAM-1), recognizes the sialyl Le^X determinant, α-Ne5Ac-(2 → 3)-β-D-Gal-(1 → 4)-[α-L-Fuc-(1 → 3)]-β-D-GlcNAc, which is found as the terminal carbohydrate structure in both glycolipids and glycoproteins.

Previously, we have reported the synthesis of sialyl Le^X ganglioside[4] (a hexasaccharide), sialyl (2 → 6)αLe^X ganglioside[5], and analogues[1], and have examined recognition activity by E-selectin. The data indicated that both the fucose and sialic acid residues were required for recognition and sialyl (2 → 6)αLe^X ganglioside was not recognized. In view of these facts, it is of interest to elucidate the more detailed structural requirements necessary for selectin recognition. We describe herein the stereocontrolled synthesis of sialyl Le^X ceramide, α-Neu5Ac-(2 → 3)-β-D-Gal-(1 → 4)-[α-L-Fuc-(1 → 3)]-β-D-GlcNAc-(1 → 3)-β-D-Gal-(1 → 1)-ceramide, which is a pentasaccharide.

The glycosylation (Scheme 1) of 2-(trimethylsilyl)ethyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**), prepared from 2-(trimethylsilyl)ethyl β-D-galactopyranoside (**1**, ref. 6) via dibutyltin oxide-mediated, selective 3-*O*-4-methoxybenzylation, *O*-benzylation, and oxidative removal of the 4-methoxybenzyl group with 2,3-dichloro-5,6-dicyanobenzoquinone[7], was effected with 2,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide[8] (**5**). Reaction in the presence of silver carbonate, silver perchlorate, and powdered 4A molecular sieves (MS-4A) in

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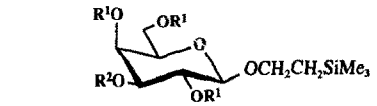
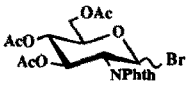


1 R^1=R^2=H

2 R^1=H, R^2=MPM

3 R^1=Bn, R^2=MPM

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



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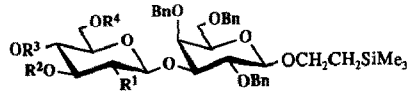
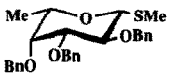


	R^1	R^2	R^3	R^4
6	NPhth	Ac	Ac	Ac
7	NHAc	H	H	H
8	NHAc	H	-benzylidene-	



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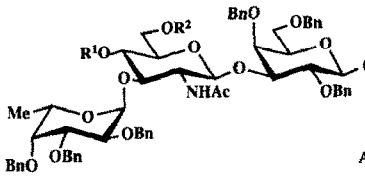
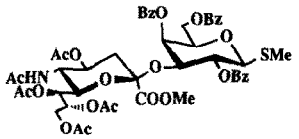


10 R^1=R^2=benzylidene

11 R^1=H, R^2=Bn



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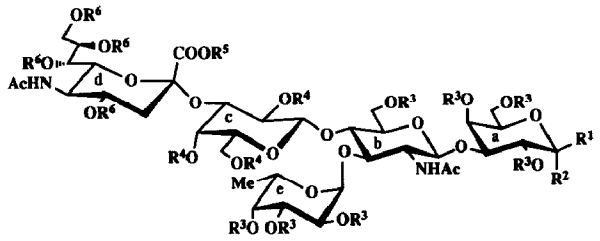
MPM = *p*-methoxybenzyl
Bn = benzyl
Bz = benzoyl

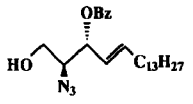
Scheme 1.

dichloromethane gave a 76% yield of the desired β-glycoside **6** {mp 140–142°, $[\alpha]_D$ −4.5° ($CHCl_3$)}, showing in its ^{1}H-NMR spectrum a one-proton doublet at δ 5.81 ($J_{1,2}$ 8.4 Hz, H-1), characteristic of the 2-phthalimido-β-D-glucopyranosyl unit. *O*-Deacetylation of **6**, followed by heating with hydrazine hydrate in 95% aqueous ethanol, and *N*-acetylation of the product afforded 2-(trimethylsilyl)ethyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**7**) {mp 94–96°, $[\alpha]_D$ −22° ($CHCl_3$)} in 82% yield. Treatment of **7** with benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid gave the benzylidene derivative **8** in 88% yield. Compound **8** was used as the glycosyl acceptor. The glycosylation of **8** with methyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[9] (**9**) in benzene for 10 h at 5–10°, in the presence of dimethyl(methylthio)sulfonium triflate (DMTST)[10] and MS-4A, afforded the desired trisaccharide **10** {$[\alpha]_D$ −63° ($CHCl_3$)} in 97% yield, showing in its ^{1}H-NMR spectrum a three-proton doublet at δ 0.87 ($J_{5,6}$ 6.4 Hz, CH_3CH) and a one-proton



	R^1	R^2	R^3	R^4	R^5	R^6
13	OSE	H	Bn	Bz	Me	Ac
14	OSE	H	Ac	Bz	Me	Ac
15	OH,H		Ac	Bz	Me	Ac
16	H	$OC(=NH)CCl_3$	Ac	Bz	Me	Ac

SE = 2-(trimethylsilyl)ethyl



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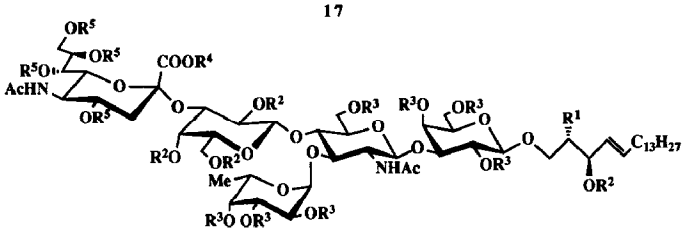

	R^1	R^2	R^3	R^4	R^5
18	N_3	Bz	Ac	Me	Ac
19	$NHCOC_{17}H_{35}$	Bz	Ac	Me	Ac
20	$NHCOC_{17}H_{35}$	H	H	H	H

Scheme 2.

doublet at δ 5.07 ($J_{1,2}$ 3.6 Hz, H-1), characteristic of the α-L-fucopyranosyl unit. Reductive ring-opening of the benzylidene group in **10** with sodium cyanoborohydride–hydrogen chloride in dry tetrahydrofuran, according to the method of Garegg et al.[11] afforded compound **11** in 77% yield. The glycosylation of **11** 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[12] (**12**), in dichloromethane for 16 h at 0° in the presence of DMTST and MS-4A, gave a 57% yield of the pentasaccharide **13** {$[\alpha]_D$ −26° ($CHCl_3$)}, which had the desired stereochemistry (Scheme 2). Significant signals in the ^{1}H-NMR spectrum of **13** were a three-proton doublet at δ 1.15 ($J_{5,6}$ 6.4 Hz, CH_3CH, fucose unit), two three-proton singlets, at δ 1.58 and 1.61 (N-$COCH_3$), four three-proton singlets, at δ 1.80, 1.93, 1.98, and 2.15 ($OCOCH_3$), a three-proton singlet at δ 3.79

(OCH_3), and a complex resonance at δ 7.14–8.22 (7 C_6H_5), a one-proton doublet of doublets at δ 5.46 ($J_{1,2}$ 8.1, $J_{2,3}$ 10.1 Hz, H-2 of the benzoylated Gal unit), indicating the newly formed glycosidic linkage to be β-D. Removal of the benzyl groups from **13** by catalytic hydrogenolysis over 10% Pd/C in 5:1 ethanol–formic acid for 48 h at 45°, and subsequent acetylation gave compound **14** {$[\alpha]_D$ −24° ($CHCl_3$)} in 66% yield. Selective removal of the 2-(trimethylsilyl)ethyl group from **14** by treatment[13] with trifluoroacetic acid in dichloromethane for 30 min at 0° gave compound **15** in quantitative yield. Treatment[14] of **15** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 4 h at 0° gave the corresponding α-trichloroacetimidate **16** {$[\alpha]_D$ +6° ($CHCl_3$)} in 85% yield. Significant signals in the ^{1}H-NMR spectrum were a one-proton doublet at δ 6.49 ($J_{1,2}$ 3.8 Hz, H-1 of the acetylated Gal unit) and a one-proton singlet at δ 8.65 (C=N*H*), indicating α-trichloroacetimidate formation.

The final glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[15] **17** with **16** in dichloromethane for 8 h at 0° in the presence of boron trifluoride etherate[14a,15a] and MS-4A, afforded only the expected β-glycoside **18** {$[\alpha]_D$ −18° ($CHCl_3$)}, in 71% yield after column chromatography. Selective reduction[16] of the azide group in **18** with hydrogen sulfide in aqueous pyridine gave the amine, and this latter compound on condensation with octadecanoic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dichloromethane for 24 h at room temperature afforded the sialyl Le^X ganglioside (pentasaccharide) derivative **19** {$[\alpha]_D$ −9.7° ($CHCl_3$)} in 80% yield. Finally, *O*-deacylation of **19** with sodium methoxide in methanol and saponification of the methyl ester group, furnished the end product **20** {$[\alpha]_D$ −19° (5:4:0.7 $CHCl_3$–MeOH–H_2O} in quantitative yield after chromatography on a column of Sephadex LH-20.

In conclusion, by using compounds **5**, **9**, **12**, and **16** as glycosyl donors, and compounds **4**, **8**, **11**, and **17** as glycosyl acceptors, a stereocontrolled synthesis of sialyl Le^X ganglioside (pentasaccharide) was achieved. This ganglioside was recognized[3i] by E-selectin to almost the same degree as the sialyl Le^X ganglioside (hexasaccharide).

New compounds obtained gave elemental analyses and IR and ^{1}H-NMR (270 MHz) data in agreement with the structures assigned.

ACKNOWLEDGMENT

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