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Synthesis of novel ganglioside GM4 analogues containing *N*-deacetylated and lactamized sialic acid: probes for searching new ligand structures for human L-selectin☆

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Abstract

Novel ganglioside GM4 analogues, which contain *N*-deacetylated or lactamized sialic acid instead of usual *N*-acetylneuraminic acid, were synthesized in a highly efficient manner. (Methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-D-galacto-pyranosyl trichloroacetimidate was coupled with 2-(tetradecyl)hexadecanol to give the desired β-glycoside in high yield. Successive O- and N-deacylation, and saponification of the methyl ester group afforded the *N*-deacetylated sialyl derivative that was converted by treatment with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in Me_2SO into the lactamized sialic acid-containing ganglioside GM4 analogue.

Keywords: Novel ganglioside GM4 analogues; Lactamized sialic acid; *N*-deacetylated sialic acid

1. Introduction

Selectins (E-, P-, and L-)[2] are a family of carbohydrate-binding proteins implicated in lymphocyte homing, leukocyte recruitment to sites of inflammation, thrombosis, cancer metastasis, and so on. Sialyl Lewis X (sLe^x) has been assumed to be a common carbohydrate ligand for the three selectins,[3,4] but a considerable molecular heterogeneity is noted in sLe^x-like determinants. We have demonstrated with chemically synthesized gangliosides[5] that sLe^x sulfated at C-6 of the *N*-acetylglucosamine residue (6-*O*-sulfo sLe^x) serves as the major ligand for L-selectin on high endothelial venules (HEV) in human lymph nodes.[6,7] Very recently, it has been shown that a novel *N*-deacetylated sialyl derivative of 6-*O*-sulfo sLe^x is a superior ligand for human L-selectin,[8,9] raising a new regulation mechanism of ligand activity based on the heterogeneity of sialic acid in the sLe^x structures[9] (Fig. 1). The lactamized form of the *N*-deacetylated sialyl derivative may func-

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tion as a dormant pool after activation of selectin ligands. We report herein a highly efficient synthesis of *N*-deacetylated (**4**) and lactamized (**5**) ganglioside GM4 analogues as probes for searching novel selectin ligands as well as a new regulation mechanism of ligand activity for selectins.

2. Results and discussion

For the synthesis of *N*-deacetylated GM4 analogue **4**, we employed (methyl 4,7,8,9-tetra-*O* - acetyl - 3,5 - dideoxy - 5 - trifluoroacetamido-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-D-galactopyranosyl trichloroacetimidate (**1**) as a glycosyl donor.[1,8] In this glycosyl donor **1**, the amino group at C-5 of neuraminic acid is suitably protected by the trifluoroacetyl (TFAc) group, which is stable under acidic conditions but can be readily removed by alkaline treatment.

Glycosylation[10] of 2-(tetradecyl)hexadecanol (**2**) with **1** in dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and powdered 4 Å molecular sieves (AW-300) gave the desired β-glycoside **3** in 72% yield. A significant signal in the ^{1}H NMR spectrum of **3** was a one-proton doublet at δ 4.68 ($J_{1,2}$ 8.1 Hz, H-1a), showing the newly formed glycosidic linkage to be β. Removal of the *O*-acetyl and *N*-trifluoroacetyl groups with sodium methoxide in methanol at 50 °C, and subsequent saponification of the methyl ester group by addition of water afforded the desired *N*-deacetylated GM4 analogue **4** in quantitative yield.

Treatment of **4** with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dimethyl sulfoxide (Me_2SO) for 12 h at 60 °C gave the desired lactamized GM4 analogue **5** in 71% yield. The compound was resistant to alkaline treatment with NaOMe in MeOH, which showed that the product **5** is not a lactone (Scheme 1).

In the ^{1}H NMR spectra (500 MHz) of **4** and **5** in CD_3OD, H-3 of the *N*-deacetylated sialic acid moiety appeared at δ 1.71 (t, $J_{gem} = J_{3,4}$ 12.1 Hz, H-3b*ax*) and 2.87 (dd, $J_{gem} = 12.5$, $J_{3,4}$ 4.8 Hz, H-3b*eq*), respectively, and H-5 was detected at δ 3.07 as a one-proton triplet ($J_{4,5} = J_{5,6}$ 10.1 Hz), showing an ordinary $^2C_5$ chair conformation (Fig. 2). In contrast, H-3 of the lactamized sialic acid moiety in **5** appeared at δ 2.02 (dd, J_{gem} 13.9, $J_{3\alpha,4}$ 4.8 Hz, H-3bα) and δ 2.29 (dd, J_{gem} 13.9, $J_{3\beta,4}$ 10.3 Hz, H-3bβ) as a one-proton doublet of doublets, respectively, indicating a typical $B^{5,2}$ boat conformation. This was further supported by a one-proton doublet of doublets of H-5b ($J_{4,5}$ 3.2, $J_{5,6}$ 2.1 Hz) at δ 3.47. These ^{1}H NMR data are consistent with those reported for the fully protected sialic acid lactam.[11,12] In the FTIR (KBr) spectrum, a significant absorp-

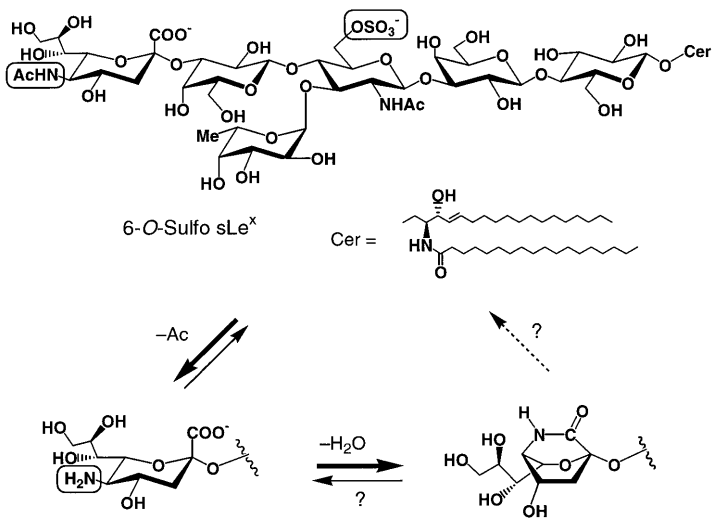
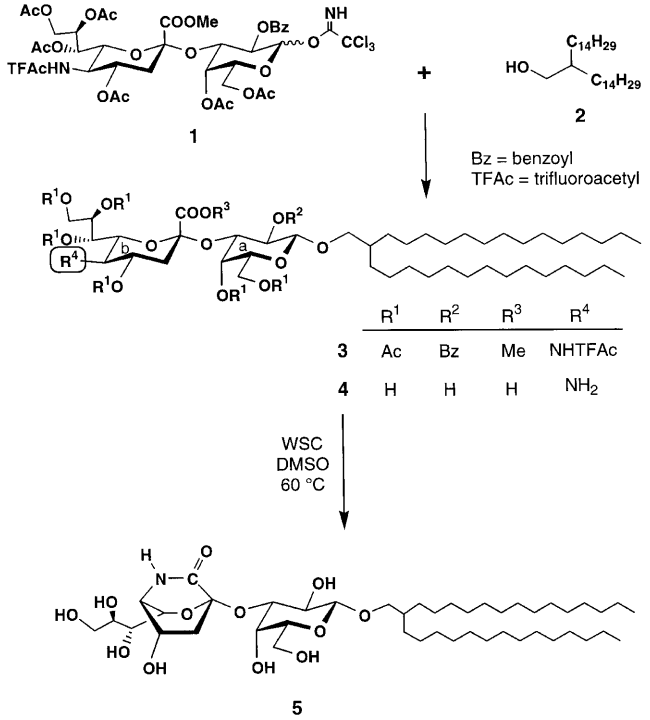


Fig. 1. Hypothetical ligand-processing pathways for the selectins.[8,9]



Scheme 1.

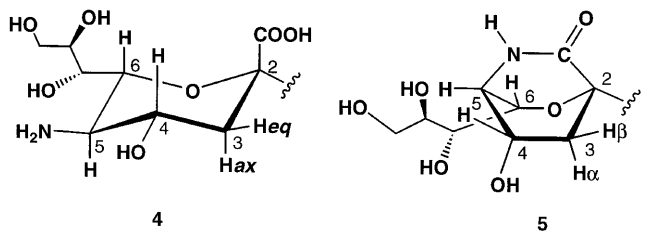


Fig. 2. $^2C_5$ (**4**) and $B^{5,2}$ (**5**) conformations of the neuraminic acid moiety based on ^{1}H NMR assignments.

tion band at 1690 cm^{-1} was clearly detected indicating the desired lactam structure. In the FABMS spectra of **4** and **5**, the molecular ions of $[M-H]^-$ were clearly detected at m/z 848.72 and m/z 830.69, respectively, which gave the common fragment ion at m/z 599.6 resulting from elimination of *N*-deacetylated (-250 Da) or lactamized (-232 Da) sialic acid, providing unambiguous evidence for the structure assigned.

The occurrence of *N*-deacetylated sialic acid has been reported among gangliosides (GM3 and GD3) in certain cell lines and tumor tissues,[13–15] but the lactam derivatives of neuraminic acid have only been created by chemical synthesis.[11,12] Further investigation on the biological functions of *N*-deacetylated and lactamized sialic acid in oligosaccharides is in progress.

3. Experimental

General procedures.—Specific rotations were determined with a Horiba SEPA-300 high sensitivity polarimeter at 25 °C, and ^{1}H NMR spectra were recorded on Varian Unity Inova (400 and 500 MHz) spectrometers with TMS as the internal standard. ^{19}F NMR spectra were recorded on a Varian Unity Inova 400 (476.5 MHz) spectrometer, and the chemical shifts were measured in ppm relative to fluorobenzene. FAB mass spectra were recorded on a JEOL JMS-SX 120A mass spectrometer/JMA-DA 7000 data system. Preparative thin-layer chromatography (TLC) was performed on Silica Gel 60 (E. Merck), and column chromatography on silica gel (Fuji Silysia Co., 300 mesh) was accomplished with the solvent systems (v/v) specified. Concentrations and evaporations were conducted in vacuo.

*2-(Tetradecyl)hexadecyl (methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-(2→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-*D*-galactopyranoside* (**3**).—To a soln of **1** (275 mg, 0.26 mmol) and 2-(tetradecyl)hexadecanol (**2**), (289 mg, 0.66 mmol) in dry CH_2Cl_2 (10 mL) were added powdered 4 Å molecular sieves (AW-300; 1.0 g), and the mixture was stirred for 4 h at rt, then cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 3.57 μL, 18.4 μmol) was added to the mixture, and it was stirred for 12 h at rt. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings was washed with 1 M $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography (70:1 $CHCl_3$–MeOH) of the residue on silica gel gave **3** (245 mg, 72%) as a white solid: $[\alpha]_D$ +20.6° (*c* 1.8, $CHCl_3$); IR (film) 3350, 2950, 1750, 1680, 1520, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 6 H, *J* 6.9 Hz, 2 CH_3), 1.06–1.36 (m, 53 H, 26 CH_2 and CH), 1.41, 1.95, 2.06, 2.07, 2.13, 2.20 (6 s, 18 H, 6 AcO), 1.74 (t, 1 H, $J_{3ax,4} = J_{gem}$ 12.5 Hz, H-3b*ax*), 2.58 (dd, 1 H, $J_{3eq,4}$ 4.7 Hz, H-3b*eq*),

3.28 (dd, 1 H, J_{gem} 9.1, J_{vic} 7.3 Hz, OCH_2C), 3.81 (dd, 1 H, J_{vic} 5.1 Hz, OCH_2C), 3.85 (s, 3 H, COOMe), 3.99 (dd, 1 H, $J_{8,9}$ 5.4, J_{gem} 12.4 Hz, H-9b), 4.34 (dd, 1 H, $J_{8,9'}$ 5.4 Hz, H-9′b), 4.67 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 2.9 Hz, H-3a), 4.68 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1a), 4.99 (m, 1 H, H-4b), 5.01 (d, 1 H, H-4a), 5.18 (dd, 1 H, $J_{6,7}$ 2.5, $J_{7,8}$ 9.2 Hz, H-7b), 5.27 (dd, 1 H, H-2a), 5.55 (m, 1 H, H-8b), 7.43 (t, 2 H, J 7.3 Hz, *m*-H of Ph), 7.57 (t, 1 H, *p*-H of Ph), 8.13 (d, 2 H, *o*-H of Ph); ^{19}F NMR ($CDCl_3$, C_6H_5F): δ 36.91 (s, 3 F, CF_3CO). Anal. Calcd for $C_{67}H_{104}F_3NO_{21}$ (1316.55): C, 61.12; H, 7.96; N, 1.06. Found: C, 61.01; H, 7.76; N, 0.91.

2-(Tetradecyl)hexadecyl (5-amino-3,5-dideoxy-D-glycero-α-D-galacto-*2-nonulopyranosylonic acid)-(2→3)-β*-D-*galactopyranoside* (**4**).—To a solution of **3** (210 mg, 0.16 mmol) in MeOH (10 m) was added a catalytic amount of NaOMe, and the mixture was stirred for 96 h at 50 °C, then water (0.5 mL) was added. After completion of the reaction (12 h), the mixture was neutralized with Amberlite IR-120 (H^+) resin and filtered. The resin was washed with MeOH, and the combined filtrate and washings was concentrated. Column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave **4** (135 mg, quant) as an amorphous mass: $[\alpha]_D$ −15.2° (*c* 1.1, 1:1 $CHCl_3$–MeOH); IR (KBr) 3550, 3350, 2950 cm^{-1}; ^1H NMR (CD_3OD): δ 0.89 (t, 6 H, J 7.3 Hz, 2 CH_3), 1.22–1.36 (m, 53 H, 26 CH_2 and CH), 1.71 (t, 1 H, $J_{3ax,4} = J_{gem}$ 12.1 Hz, H-3b*ax*), 2.87 (dd, 1 H, $J_{3eq,4}$ 4.8 Hz, H-3b*eq*), 3.07 (t, 1 H, $J_{4,5} = J_{5,6}$ 10.1 Hz, H-5b), 3.87 (dd, 1 H, $J_{6,7}$ 2.7 Hz, H-6b), 4.02 (dd, 1 H, $J_{2,3}$ 8.8, $J_{3,4}$ 2.9 Hz, H-3a), 4.23 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1a); FABMS (negative-ion mode, 2:2:1 diethanolamine–tetramethylurea–*m*-nitrobenzylalcohol matrix): *m*/*z* 848.72 $[M-H]^-$ ($C_{45}H_{86}NO_{13}$ MW, exact 848.6099, ave. 849.1767), 599.6 [M − Neu5NH$_2$ − H]$^-$.

2-(Tetradecyl)hexadecyl (5-amino-3,5-dideoxy-D-glycero-α-D-galacto-*2-nonulopyranosyl-1,5-lactam)-(2→3)-β*-D-*galactopyranoside* (**5**).—To a solution of **4** (25.0 mg, 29.4 μmol) in Me_2SO (3 mL) was added 1-(3-dimethylaminopropyl) - 3 - ethylcarbodiimide hydrochloride (WSC; 56 mg, 0.29 mmol), and the mixture was stirred for 12 h at 60 °C. After completion of the reaction, the mixture was concentrated. Column chromatography (1:1 $CHCl_3$–MeOH) of the residue on Sephadex LH-20 gave a crude product, which was further purified by silica gel column chromatography (6:1 $CHCl_3$–MeOH) to give **5** (17.4 mg, 71%) as an amorphous mass: $[\alpha]_D$ −9.3° (*c* 0.8, MeOH); IR (KBr) 3550, 3350, 2950, 1690 cm^{-1}; ^1H NMR (CD_3OD): δ 0.80 (t, 6 H, J 7.0 Hz, 2 CH_3), 1.14–1.54 (m, 53 H, 26 CH_2 and CH), 2.02 (dd, 1 H, $J_{3\alpha,4}$ 4.8, J_{gem} 13.9 Hz, H-3bα), 2.29 (dd, 1 H, $J_{3\beta,4}$ 10.3 Hz, H-3bβ), 3.47 (dd, 1 H, $J_{4,5}$ 3.2, $J_{5,6}$ 2.1 Hz, H-5b), 3.53 (m, 1 H, H-8b), 3.54 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.8 Hz, H-2a), 3.91 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3a), 4.01 (m, 1 H, H-4b), 4.07 (d, 1 H, H-4a), 4.14 (d, 1 H, H-1a), 4.33 (dd, 1 H, $J_{6,7}$ 1.6 Hz, H-6b); FABMS (negative-ion mode, triethanolamine matrix): *m*/*z* 830.69 $[M-H]^-$ ($C_{45}H_{84}NO_{12}$ MW, exact 830.5994, ave. 831.1615), 599.6 [M − lactamized neuraminic acid − H]$^-$.

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