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THE FIRST, EFFICIENT SYNTHESIS OF NOVEL SLe^x NEOGLYCOLIPIDS CONTAINING N-DEACETYLATED AND LACTAMIZED SIALIC ACID: KEY LIGAND STRUCTURES FOR SELECTIN BINDING[1]

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COMMUNICATION

**THE FIRST, EFFICIENT SYNTHESIS OF NOVEL
SLe^x NEOGLYCOLIPIDS CONTAINING
N-DEACETYLATED AND LACTAMIZED SIALIC
ACID: KEY LIGAND STRUCTURES FOR
SELECTIN BINDING¹**

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Sialyl Lewis x (sLe^x) has been recognized² as a common carbohydrate ligand for E-, P- and L-selectin, a family of C-type lectins implicated in lymphocyte homing, leukocyte recruitment to sites of inflammation, thrombosis, cancer metastasis, and so on. Recently, it has been suggested^{3,4} that the novel sLe^x variants containing *N*-deacetylated and lactamized sialic acid may be involved in the ligand processing pathway for human L-selectin, raising a new regulation mechanism of ligand activity based on the heterogeneity of sialic acid in the sLe^x determinant (Figure 1). This paper reports the first, efficient synthesis of novel sLe^x neoglycolipids which contain *N*-deacetylated and lactamized sialic acid as the key ligand structures for selectin binding.

Phenyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(4-methoxybenzyl)-2-phthalimido-1-thio-β-D-glucopyranoside⁵ (**1**, 1.71 mmol) was coupled with **2** (1.23 mmol) which was readily prepared from 2-(trimethylsilyl)ethyl 3-*O*-benzyl-β-D-galactopyranoside,⁶ in the presence of *N*-iodosuccinimide (NIS, 3.37 mmol), trifluoromethanesulfonic acid (TfOH, 0.17 mmol) and molecular sieves 4 Å (MS 4A, 2.0 g) in CH₂Cl₂ at -20°C, to give **3**, [α]_D +21° (CHCl₃), in 92% yield (Scheme 1). Treatment of **3** with hydrazine monohydrate in EtOH for 24 h under reflux, followed by successive *N*-acetylation and *O*-benzoylation, gave **4**, [α]_D +31° (CHCl₃), in 91% yield. The benzylidene group in **4** was cleaved by acid hydrolysis, and the resulting **5** was treated with *p*-methoxyphenol (MPOH), PPh₃ and diethylazodicarboxylate (DEAD) in THF to afford **6**, [α]_D +28° (CHCl₃), in 96% yield.

Glycosylation of **6** (0.37 mmol) with the suitably protected *N*-trifluoroacetylneuraminyl-α-(2→3)-galactose donor **7** (0.46 mmol), which was prepared

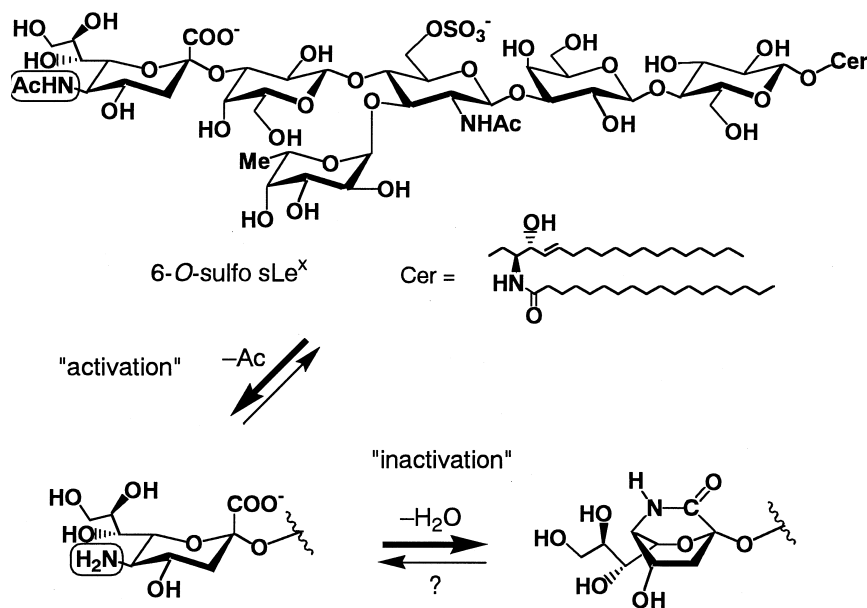
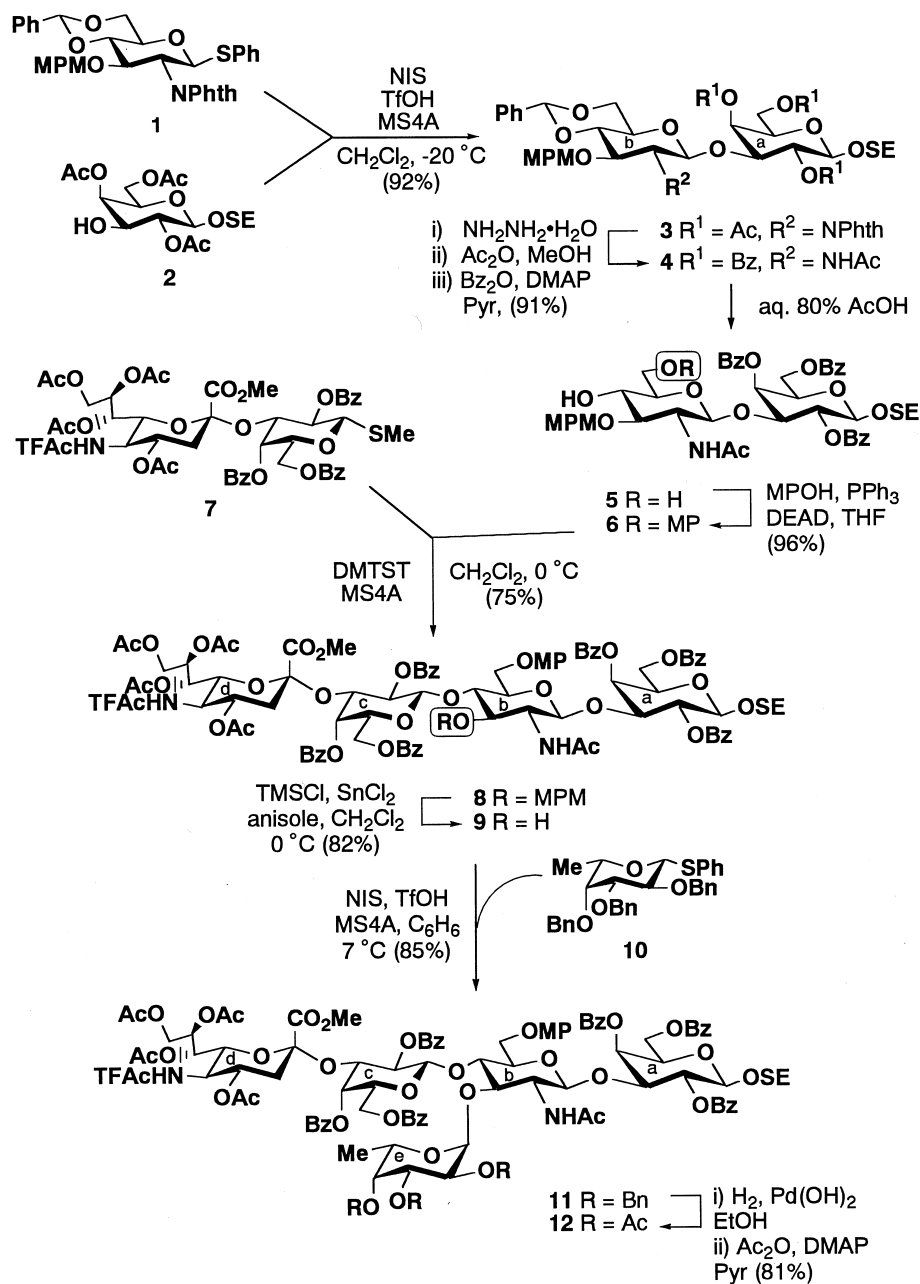


Figure 1. Hypothetical ligand-processing pathway for human L-selectin.^{3,4}

from the corresponding trichloroacetimidate derivative by the similar manner reported previously,³ promoted by dimethyl(methylthio)sulfonium triflate⁷ (DMTST, 1.85 mmol) and MS 4A (1.0 g) in CH₂Cl₂ at 0°C, gave **8**, [α]_D +38° (CHCl₃), in 75% yield. In the ¹H NMR spectrum of **8**, a significant one-proton doublet ($J_{1,2}$ = 8.0 Hz, H-1c) appeared at δ 5.06, showing the newly formed glycosidic linkage to be β . The *p*-methoxybenzyl (MPM) group at C-3 of GlcNAc in **8** was selectively removed (82%) by treatment with TMSCl, SnCl₂ and anisole in CH₂Cl₂ at 0°C, and the resulting **9** (0.23 mmol) was fucosylated by **10** (0.71 mmol) in the presence of NIS (2.1 mmol) and TfOH (0.56 mmol) in benzene at 7°C to afford the desired pentasaccharide **11**, [α]_D +3.0° (CHCl₃), in 85% yield. Hydrogenolytic removal of the benzyl groups in the fucose moiety and the following *O*-acetylation gave **12** (Scheme 1) in 81% yield. In the ¹H NMR spectrum of **12**, a three-proton doublet at δ 0.76 ($J_{5,6}$ = 6.4 Hz, H-6e), a one-proton doublet of doublets at δ 4.90 ($J_{1,2}$ = 3.6, $J_{2,3}$ = 9.9 Hz, H-2e), and a one-proton doublet at δ 5.16 ($J_{1,2}$ = 3.6 Hz, H-1e) were clearly detected, indicating the newly formed glycoside to be an α -L-fucopyranoside. The pentasaccharide **12** was then converted to the imidate derivative **13** (α : β = 5:1) by the removal of SE group (quant.) and activation as the trichloroacetimidate in 89% yield.

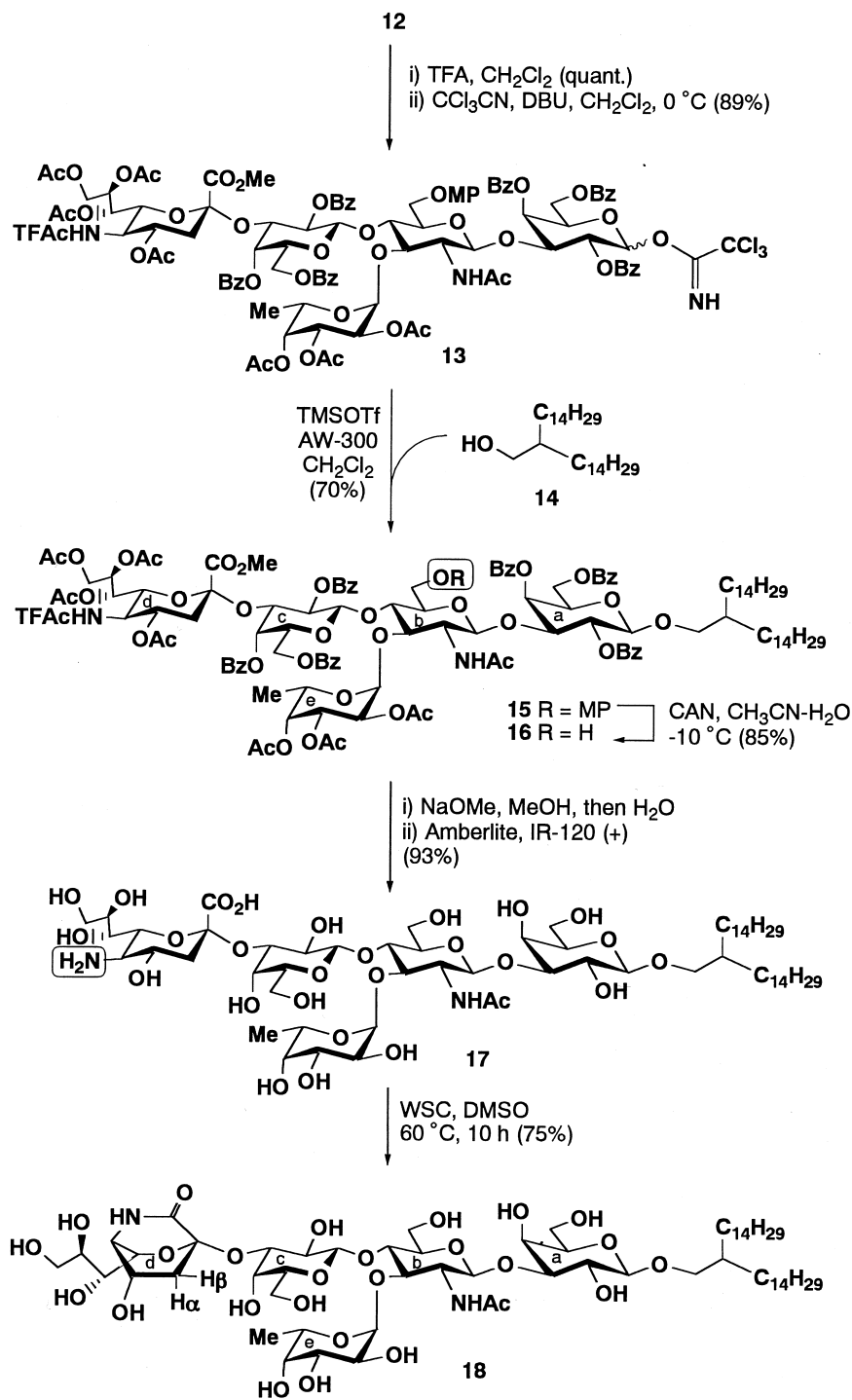
Coupling of **13** (0.09 mmol) and 2-(tetradecyl)hexadecanol⁸ **14** (0.3 mmol) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 8.02 μ mmol) and molecular sieves AW-300 in CH₂Cl₂ gave the desired neoglycolipid **15**, [α]_D +8.4° (CHCl₃), in 70% yield (Scheme 2). Significant signals in the ¹H NMR spectrum of **15** were a six-proton triplet at δ 0.88 (J_{vic} = 6.0 Hz, 2Me), fifty-three alkyl protons at δ 0.93–1.52 (26CH₂ and CH) and a one-proton doublet at δ 4.33





Scheme 1. MPM=*p*-methoxybenzyl, SE=2-(trimethylsilyl)ethyl, MP=*p*-methoxyphenyl, TFAc=trifluoroacetyl.

($J_{1,2} = 8.0$ Hz, H-1a), characteristic of the desired β -linked 2-(tetradecyl)hexadecyl glycoside. The MP group was selectively cleaved by treatment with diammonium cerium(IV) nitrate (CAN) at -10°C in $\text{CH}_3\text{CN-H}_2\text{O}$ to give **16**, [α]_D -20° (CHCl_3) in 85%. Removal of the *O*-acyl and *N*-trifluoroacetyl groups with NaOMe



Scheme 2.



in MeOH, and subsequent saponification of the methyl ester group by addition of water afforded the desired *N*-deacetylated sLe^x neoglycolipid **17**, $[\alpha]_D -19^\circ$ (3:1 MeOH-CHCl₃), in 93% yield.

Treatment of **17** (10.3 μmol) with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 0.1 mmol) in dimethyl sulfoxide (DMSO, 2 mL) for 10 h at 60°C gave the desired lactamized sLe^x **18**, $[\alpha]_D -16.3^\circ$ (3:2 CHCl₃-MeOH), in 75% yield. In the ¹H NMR spectra (500 MHz) of **17** and **18** in CD₃OD, H-3 of the *N*-deacetylated sialic acid moiety appeared at δ 1.73 as a one-proton triplet ($J_{gem} = J_{3,4} = 12.6$ Hz, H-3*dax*), and at δ 2.86 as a one-proton doublet of doublets ($J_{3eq,4} = 4.2$ Hz, H-3*deq*), respectively, showing the usual ²C₅ chair conformation. In contrast, H-3 of the lactamized sialic acid moiety in **18** appeared at δ 2.03 ($J_{gem} = 13.9$, $J_{3\alpha,4} = 4.8$ Hz, H-3*dα*) and δ 2.29 ($J_{gem} = 13.9$, $J_{3\beta,4} = 10.6$ Hz, H-3*dβ*), respectively, as a one-proton doublet of doublets, obviously indicating a typical B^{5,2} boat conformation. These ¹H NMR data are consistent with those reported⁹ for the ganglioside GM4 analogs containing *N*-deacetylated and lactamized sialic acid.

In conclusion, an efficient synthesis of the novel sLe^x neoglycolipids containing *N*-deacetylated and lactamized sialic acid was achieved for the first time.

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REFERENCES AND NOTES

1. Synthetic Studies on Sialoglycoconjugates, Part 120. For Part 119, see Sawada, N.; Ito, M.; Ishida, H.; Kiso, M. The First Synthesis of Glycan Parts of Lactoganglio- and Neolactoganglio-Series Gangliosides. *Tetrahedron Lett.* **2001**, in press.
2. Simanek, E. E.; McGarver, G. J.; Jablonowski, J. A.; Wong, C.-H. Selectin-carbohydrate Interactions: from Natural Ligands to Designed Mimics. *Chem. Rev.* **1998**, *98*, 833–862.
3. Komba, S.; Galustian, C.; Ishida, H.; Feizi, T.; Kannagi, R.; Kiso, M. The First Total Synthesis of 6-Sulfo-de-*N*-acetylsialyl Lewis x Ganglioside: A Superior Ligand for Human L-Selectin. *Angew. Chem. Int. Ed.* **1999**, *38*(8), 1131–1133.
4. Mitsuoka, C.; Ohmori, K.; Kimura, N.; Kanamori, A.; Komba, S.; Ishida, H.; Kiso, M.; Kannagi, R. Regulation of Selectin Binding Activity by Cyclization of Sialic Acid Moiety of Carbohydrate Ligands on Human Leukocytes. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 1597–1602.
5. Kameyama, A.; Ehara, T.; Yamada, Y.; Ishida, H.; Kiso, M.; Hasegawa, A. A Total Synthesis of Sialyl Dimeric Le^x Ganglioside. *J. Carbohydr. Chem.* **1995**, *14*, 507–523.
6. Hasegawa, A.; Hotta, K.; Kameyama, A.; Ishida, H.; Kiso, M. Total Synthesis of Sialyl-α(2-6)-lactotetraosylceramide and Sialyl-α(2-6)-neolactotetraosylceramide. *J. Carbohydr. Chem.* **1991**, *10*, 439–459.



7. Kiso, M.; Hasegawa, A. Synthesis of Ganglioside GM3 and Analogs Containing Modified Sialic Acids and Ceramides. In *Methods in Enzymology*; Abelson, J. N.; Simon, M. I. Eds.; Academic Press.; San Diego, **1994**, 242, 173–182.
8. Hasegawa, A.; Suzuki, N.; Ishida, H.; Kiso, M. Synthesis of Ganglioside GM3 and GM4 Analogs Containing 2- or 3-Branched Fatty-Alkyl Residues in Place of Ceramide. *J. Carbohydr. Chem.* **1996**, *15*(5), 623–637.
9. Otsubo, N.; Ishida, H.; Kiso, M. Synthesis of Novel Ganglioside GM4 Analogues Containing *N*-Deacetylated and Lactamized Sialic Acid: Probes for Searching New Ligand Structures for Human L-Selectin. *Carbohydr. Res.* **2001**, *330*(1), 1–5. The ¹H NMR data (500 MHz, CD₃OD) for *N*-deacetylated and lactamized sialic acid in the GM4 analogues are as follows: δ 1.71 (t, J = 12.1 Hz, H-3 α) and δ 2.87 (dd, J_{gem} = 12.5, J_{3,4} = 4.8 Hz, H-3 eq) for the *N*-deacetylated sialic acid; δ 2.02 (dd, J_{gem} = 13.9, J_{3 α ,4} = 4.8 Hz, H-3 α) and δ 2.29 (dd, J_{gem} = 13.9, J_{3 β ,4} = 10.3 Hz, H-3 β) for the lactamized sialic acid. The FT-IR (KBr) spectrum showed a significant absorption band at 1690 cm⁻¹ (lactam).

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