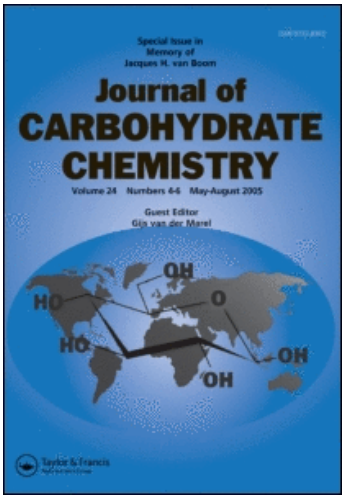

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COMMUNICATION

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

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Sialyl Lewis x (sLe^x) has been recognized[2] 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-glucopyranoside[5] (**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 4Å (MS 4A, 2.0 g) in CH_2Cl_2 at −20°C, to give **3**, $[\alpha]_D$ +21° ($CHCl_3$), 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**, $[\alpha]_D$ +31° ($CHCl_3$), in 91% yield. The benzylidene group in **4** was cleaved by acid hydrolysis, and the resulting **5** was treated with *p*-methoxyphenol (MPOH), PPh_3 and diethylazodicarboxylate (DEAD) in THF to afford **6**, $[\alpha]_D$ +28° ($CHCl_3$), 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

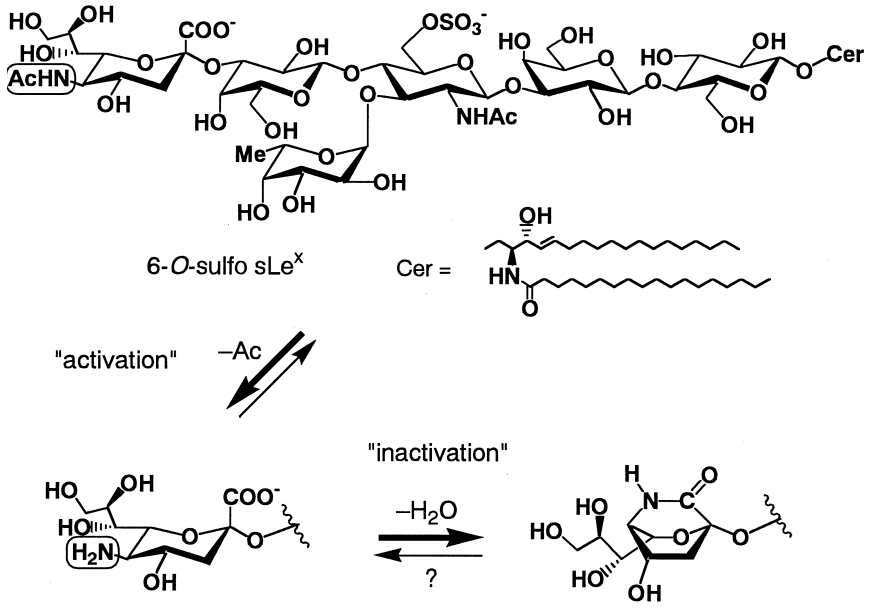
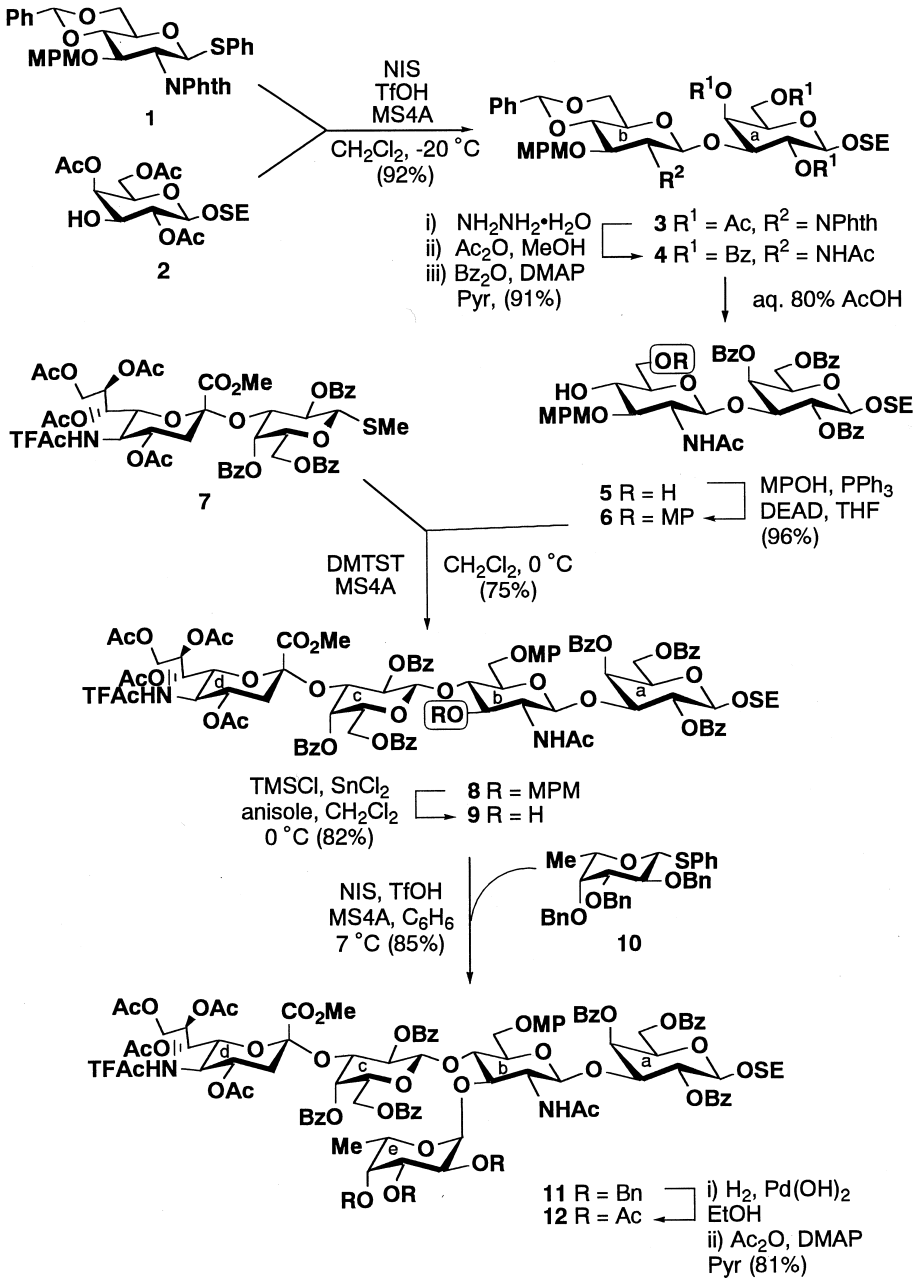


Figure 1. Hypothetical ligand-processing pathway for human L-selectin.[3,4]

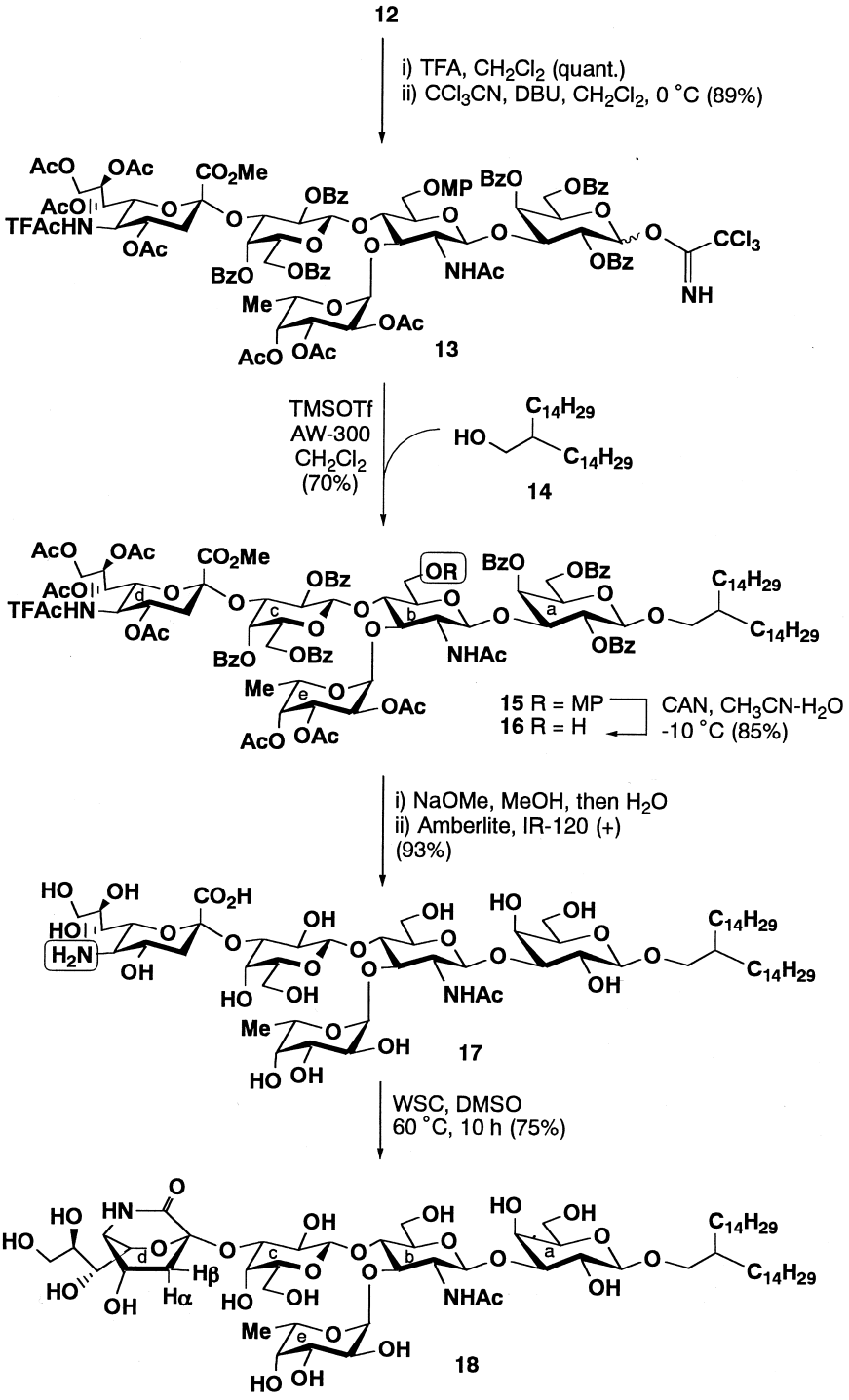
from the corresponding trichloroacetimidate derivative by the similar manner reported previously,[3] promoted by dimethyl(methylthio)sulfonium triflate[7] (DMTST, 1.85 mmol) and MS 4A (1.0 g) in CH_2Cl_2 at 0°C, gave **8**, $[\alpha]_D$ +38° ($CHCl_3$), in 75% yield. In the ^1H NMR spectrum of **8**, 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, $SnCl_2$ and anisole in CH_2Cl_2 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**, $[\alpha]_D$ +3.0° ($CHCl_3$), 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)hexadecanol[8] **14** (0.3 mmol) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 8.02 μmmol) and molecular sieves AW-300 in CH_2Cl_2 gave the desired neoglycolipid **15**, $[\alpha]_D$ +8.4° ($CHCl_3$), 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 ($26CH_2$ 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 CH_3CN-H_2O to give **16**, $[\alpha]_D$ −20° ($CHCl_3$) in 85%. Removal of the *O*-acyl and *N*-trifluoroacetyl groups with NaOMe



Scheme 2.

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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° (3:1 MeOH-$CHCl_3$), 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° (3:2 $CHCl_3$-MeOH), in 75% yield. In the ^{1}H NMR spectra (500 MHz) of **17** and **18** in CD_3OD, 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-3d*ax*), and at δ 2.86 as a one-proton doublet of doublets ($J_{3eq,4} = 4.2$ Hz, H-3d*eq*), respectively, showing the usual $^2C_5$ 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-3dα) and δ 2.29 ($J_{gem} = 13.9$, $J_{3\beta,4} = 10.6$ Hz, H-3dβ), respectively, as a one-proton doublet of doublets, obviously indicating a typical $B^{5,2}$ boat conformation. These ^{1}H NMR data are consistent with those reported[9] 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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