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Preliminary communication

A total synthesis of glyconononaosyl ceramide with a sialyl dimeric Le^x sequence ☆

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E-selectin (endotherial-leukocyte adhesion molecule-1), P-selectin (GMP-140) and L-selectin (leukocyte adhesion molecule-1) belong to a family of adhesion molecules that mediate the binding of leucocytes to endothelial cells and platelets, as well as to lymphocyte-homing receptors. The ligand recognized by E-selectin is the SLe^x type determinant [1] which is found as the terminal carbohydrate structure in both glycolipids and glycoproteins. From this background, Hasegawa and co-workers have reported the synthesis of sialyl Le^x gangliosides and analogues to clarify structure–activity relationships in this epitope [2].

Sialyl dimeric Le^x exhibits high potency among the naturally occurring E-selectin binding molecules, but nobody has reported its synthesis as the naturally occurring glycolipid structure due to its molecular complexity, although the carbohydrate portion was synthesized by Nicolaou et al. [3]. It is noteworthy that glyconononaosyl ceramide **1** is identified as tumor-associated ganglioside that accumulates in human colonic adenocarcinoma but is absent in normal colonic mucosa [4]. Owing to the biological importance of **1**, an efficient chemical synthesis is in demand.

☆ For a preliminary report, see M. Iida, S. Nunomura, M. Numata, M. Sugimoto, K. Tomita, and T. Ogawa, *Nippon Nōgeikagaku Kaishi (Japan Society for Bioscience, Biotechnology, and Agrochemistry)*, 67 (1993) 181.

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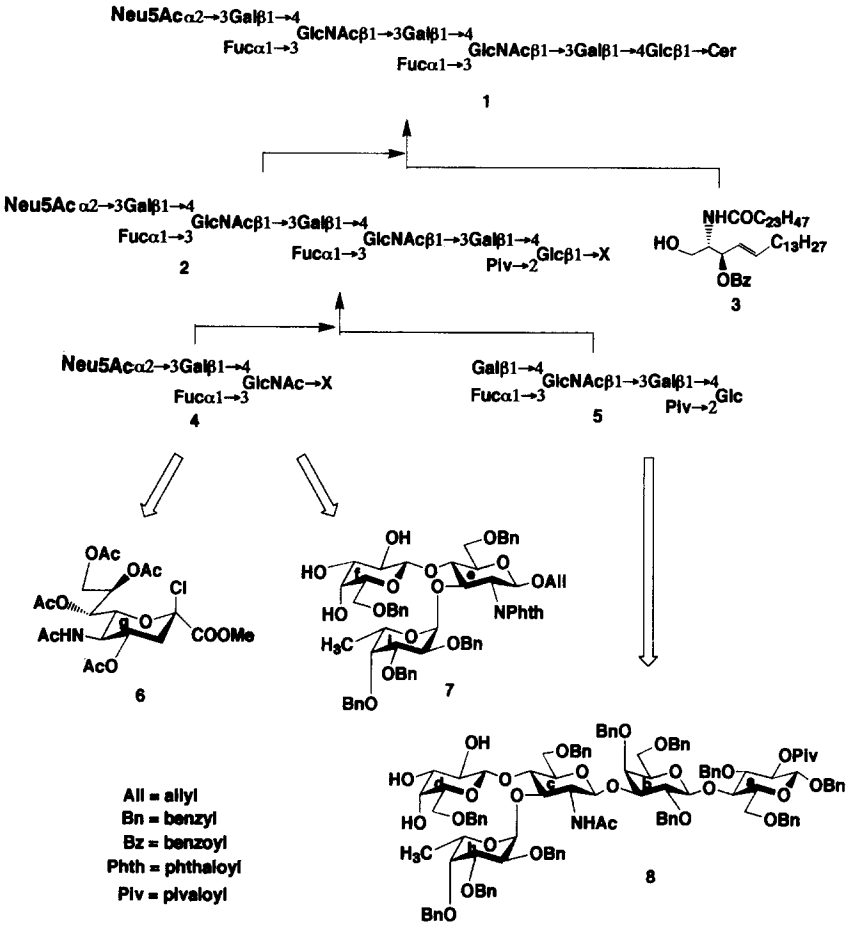
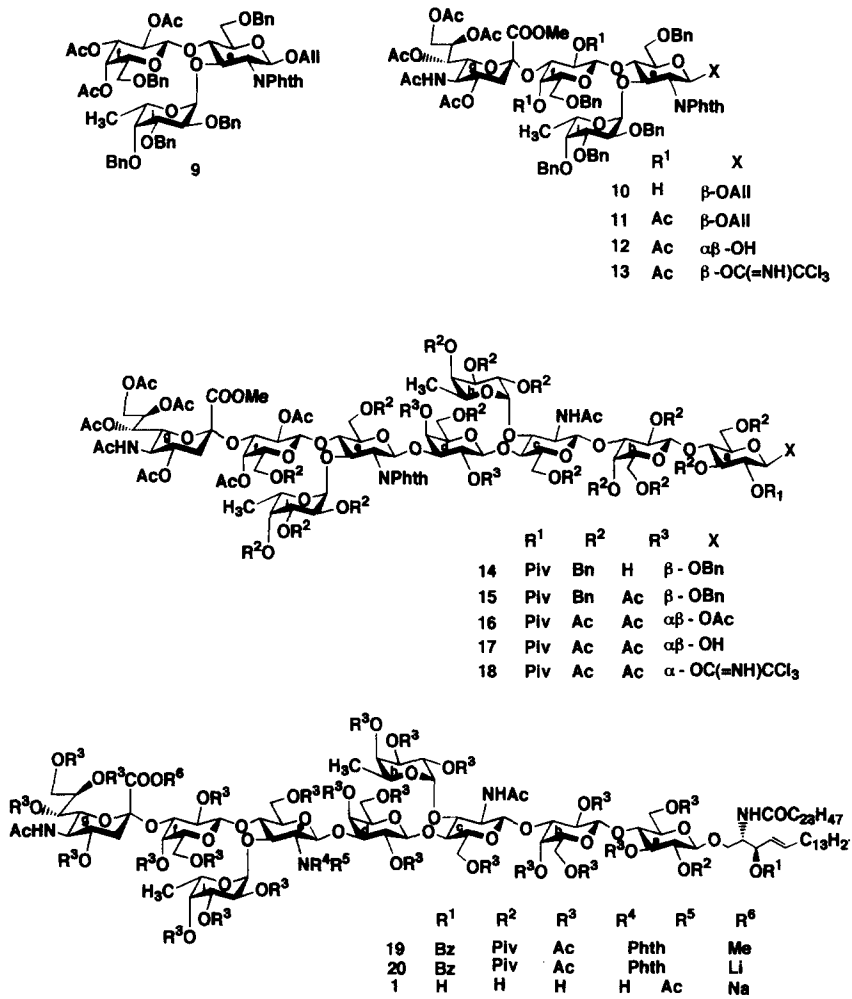


Scheme 1.

As part of our project on the synthesis of glycosphingolipids, we describe herein a stereocontrolled first total synthesis of sialyl dimeric Le^x glycononaosyl ceramide **1**. Retrosynthetic analysis of **1** (Scheme 1) led us to design the putative glycosyl donor **2** that could be coupled with ceramide derivative **3** [5]. The glycosyl donor **2** was then expected to be constructed from putative tetrasaccharide donor **4** and pentasaccharide acceptor **5** [6]. Donor **4** and acceptor **5** were expected to be constructed from compounds **6**, **7** and **8**. The efficiency of the pivaloyl auxiliary group at O-2a of **5** has been established in previous studies [7].

In order to study the reactivity of O-3 at residue f of the building block **7**, we prepared **7** {$[\alpha]_D$ −13.2° (*c* 0.3); R_f 0.61 (12:1 $CHCl_3$–MeOH) from known compound **9** [6] by Zemplén *O*-deacetylation in 97% yield.

Glycosylation of **6** [8] (3 equiv) with **7** in CH_3CN under the influence of $HgBr_2$–$Hg(CN)_2$ at 0°C afforded a 41% yield of the desired α-(2 → 3)-linked tetrasaccharide **10**



Scheme 2.

{$[\alpha]_D$ −4.9° (*c* 0.3); R_f 0.14 (10:1 toluene–MeOH); mp. 193°C}[1] along with 19% yield of its β-isomer. Compound **10** was easily separated from its β-isomer by recrystallization from MeOH. The regiochemistry of the newly introduced glycosidic linkage of **10** was deduced by converting **10** into acetate **11** {$[\alpha]_D$ −9.1° (*c* 0.2); R_f 0.25 (9:1 toluene–MeOH)}, which showed in the homonuclear Hartmann–Hahn

[1] It should be noted that all new compounds described herein gave satisfactory elemental analyses. Optical rotations were determined for solutions in $CHCl_3$ at 22°C. NMR spectra were recorded with a JNM-GX 500 Fourier-transform instrument.

The values of δ_H are expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$ at 25°C, unless noted otherwise. Mass spectra were determined using electrospray-ionization (ESIMS) and fast-atom bombardment mass spectroscopy (FABMS) techniques.

(HOHAHA) NMR spectrum newly deshielded signals for H-2f at δ_H 4.754 (dd, $J = 8.8$, 10.2 Hz), H-4f at δ_H 5.058 (d, $J = 3.3$ Hz). The observed chemical shifts and coupling constants of the Neu5Ac unit for H-3g (δ_H 2.542, $J_{3a,3e} = 12.5$ Hz, $J_{3e,4} = 4.8$ Hz), H-4g (δ_H 4.908), and H-7g (δ_H 5.391, $J_{6,7} = 2.6$ Hz, $J_{7,8} = 9.5$ Hz) are characteristic of the α-glycosidic linkage of Neu5Ac, in good agreement with previous observations [9].

Deallylation of **11** with (1) $[Ir(COD)(PMePh_2)_2]PF_6$ [10] in THF and (2) I_2 in aq THF gave hemiacetal **12** in 84% yield. Compound **12** was transformed into β-trichloroacetimidate **13** {R_f 0.33 (3:1 $CHCl_3$–acetone); δ_H 6.394 (d, $J = 8.8$ Hz, H-1e)} in 80% yield in the presence of CCl_3CN and 1,8-diazabicyclo [5,4,0]undec-7-ene (DBU) [11]. Having prepared the designed tetrasaccharide donor **13** and the glycosyl acceptor **8** [6], crucial glycosylation was examined. Boron trifluoride etherate-promoted glycosylation of **13** (1.5 equiv) with **8** in CH_3CN at -40°C was successfully achieved in a regio- and stereo-controlled manner to give **14** in 52% yield: {$[\alpha]_D$ $-23°$ (c 1.0); R_f 0.48 (3:1 $CHCl_3$–acetone)}. But in the case of trimethylsilyl triflate-promoted glycosylation [12], the coupling yield was decreased to 30%. The configuration of the newly introduced anomeric carbon C-1e was expected to be β, due to the presence of the *N*-2 phthaloyl group in the glycosyl donor that favors the formation of 1,2-*trans* stereochemistry. Indeed the ^1H NMR spectral data showed the anomeric proton of H-1e at δ_H 5.234 (d, $J = 8.4$ Hz), thus confirming the β configuration. The regiochemistry of **14** was deduced by converting **14** into acetate **15** {$[\alpha]_D$ $-19°$ (c 0.8); R_f 0.7 in (3:1 $CHCl_3$–acetone)}$_2$, which showed in the ^1H NMR spectrum a newly deshielded signal for H-4d at δ_H 5.470 (d, $J = 4.0$ Hz) and H-2d at δ_H 4.616 (dd, $J = 8.1$, 9.2 Hz).

Conversion of **15** into the completely acylated glycononaose **16** was carried out in two steps in 99% overall yield as follows: (1) H_2 with 20% $Pd(OH)_2$–C in 4:1 MeOH–H_2O; (2) Ac_2O and 4-(dimethylamino)pyridine (DMAP) in pyridine. Compound **16** was obtained as a 1:1 mixture of $\alpha:\beta$ anomers at C-1a [R_f 0.36 (20:1 $CHCl_3$–MeOH); δ_H 6.284 (d, $J = 3.7$ Hz, H-1aα) and δ_H 5.688 (d, $J = 8.1$ Hz, H-1aβ)]. Chemoselective cleavage of the anomeric acetate of **16** with hydrazinium acetate [13] in DMF at room temperature gave **17** in 98% yield. Compound **17** was treated with CCl_3CN and DBU in $(ClCH_2)_2$ to afford α-trichloroacetimidate **18** in 91% yield: {R_f 0.33 (25:1 $CHCl_3$–MeOH); δ_H 6.489 (d, $J = 3.7$ Hz, H-1a)}. The crucial coupling between **18** and **3** was performed in freshly distilled $CHCl_3$ in the presence of boron trifluoride etherate at -15°C to afford a 39% yield of β-glycoside **19** {$[\alpha]_D$ $-30.6°$ (c 1.0); R_f 0.38 (1:1 toluene–acetone)}. The newly formed glycosidic linkage was shown to be β as revealed in the HOHAHA NMR spectrum of **19** {δ_H 4.403 (d, $J = 7.7$ Hz, H-1a)}. Further conversion of **19** to the target glycolipid [1] was excecuted as follows. Compound **19** was refluxed for 7 h with a large excess of LiI in dry pyridine [14]. Purification of the reaction mixture by gel filtration through Sephadex LH-20 in 1:2 $CHCl_3$–MeOH gave a 93% yield of the lithium salt **20**. Subsequent treatment of **20** with (1) NH_2NHMe in refluxing EtOH, (2) Ac_2O in MeOH and (3) aq NaOH in 1:1 MeOH–THF, afforded the target compound **1** (4.0 mg) in 47% yield, after gel filtration through Sephadex LH–20 using 5:5:1 $CHCl_3$–MeOH–H_2O.

Physicochemical data for **1**: R_f 0.14 (2:1:1 BuOH–EtOH–H_2O); ^1H NMR (50:1 Me_2SO-d_6–D_2O, 60°C): δ_H 5.601 (dt, 1 H, $J = 15.4$, 7.0 Hz, H-5Cer), 5.410 (dd, 1 H,

J = 15.4, 7.0 Hz, H-4Cer), 4.935 (d, 2 H, J = 3.3 Hz, H-1h and H-1i), 4.802 (d, 1 H, J = 7.3 Hz, H-1c or H-1e), 4.788 (d, 1 H, J = 6.6 Hz, H-1e or H-1c), 4.404 (d, 1 H, J = 7.3 Hz, H-1d or H-1f), 4.360 (d, 1 H, J = 7.7 Hz, H-1f or H-1d), 4.334 (d, 1 H, J = 7.0 Hz, H-1b), 4.220 (d, 1 H, J = 8.1 Hz, H-1a), 2.818 (dd, 1 H, J = 5.1, 12.1 Hz, H-3g_{eq}), 1.169 (d, 3 H, J = 6.6 Hz, H-6h or H-6i), 1.086 (d, 3 H, J = 6.6 Hz, H-6i or H-6h), 1.945, 1.881, and 1.875 (3 s, 9 H, 3 NAc), 0.897 (t, 6 H, J = 7.0 Hz, 2CH_2Me); ESIMS: m/z $(M + 2Na)^{2+}$ 1177.9; FABMS (TEA matrix): m/z $(M - Na)^-$ 2287.

The biological properties of **1** are currently being studied. In summary, an unambiguous total synthesis of sialyl dimeric Le^x glycononaosyl ceramide was achieved for the first time in a regio- and stereo-controlled manner using **18** as a key glycosyl donor.

Acknowledgments

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