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Preliminary communication

Total synthesis of sulfated Le^a pentaosyl ceramide

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In 1992 Feizi and co-workers reported that an equimolar mixture of sulfated Le^x and Le^a tetrasaccharides derived from an ovarian cystoadenoma glycoprotein were strongly bound to E- and L-selectins [1]. In a recent communication, they concluded that sulfated Le^a tetrasaccharide [2] and pentasaccharide [3] emerge as the most potent E-selectin ligands so far studied [4]. Those observations prompted us to synthesize sulfated Le^x and Le^a pentaosyl ceramide.

In our preceding paper [5], we described the total synthesis of sulfated Le^x pentaosyl ceramide. In connection with our project on the synthesis of glycosphingolipids, we herein deal with a stereocontrolled, facile, first total synthesis of sulfated Le^a pentaosyl ceramide **1** for further chemical and biological scrutiny. The overall strategy is depicted in Scheme 1. Retrosynthetic analysis of a suitable route to **1** (Scheme 1) led us to design a putative glycosyl donor **2** that could be coupled with ceramide derivative **3** [6]. Donor **2** was expected to be constructed from synthons derived from D-galactose, 2-amino-2-deoxy-D-glucose, L-fucose, and lactose (compounds **4**–**6** [7] and **7** [8], respectively, all of which are prepared from readily available compounds).

Glycosylation of **4** (1.5 equiv) with **5** in dichloromethane in the presence of MeOTf at room temperature afforded an 89% yield of the desired β-(1 → 3)-linked compound **8** {$[\alpha]_D$ −20.9° (*c* 1.0); R_f 0.38 (3 : 1 toluene–AcOEt)}. [1] The β configuration of **8** was assigned from the ^{1}H NMR data that showed a signal for H-1d at δ_H 4.547 (d, J = 8.5

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[1] Optical rotations were determined for solutions in $CHCl_3$ at 25°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 24°C, unless noted otherwise. Mass spectra were determined using electrospray-ionization (ESIMS) and fast-atom bombardment mass spectrometry (FABMS) techniques.

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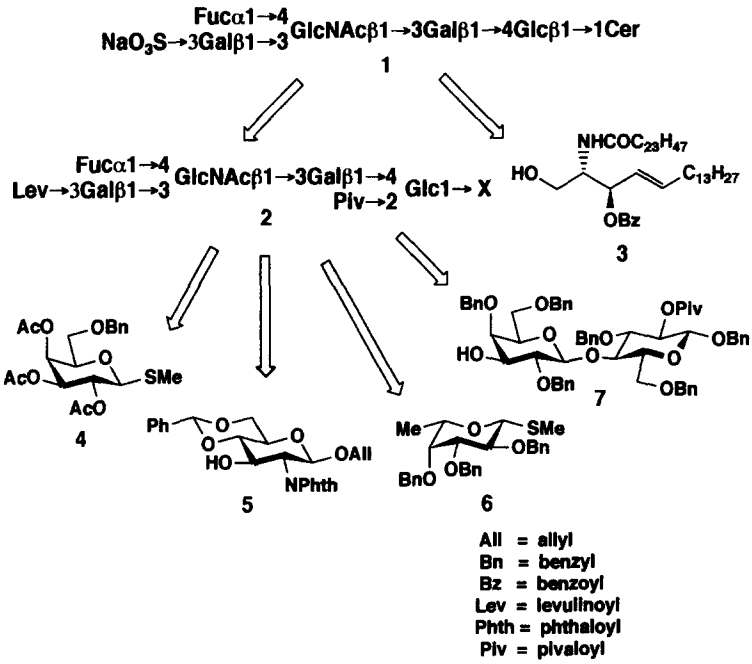
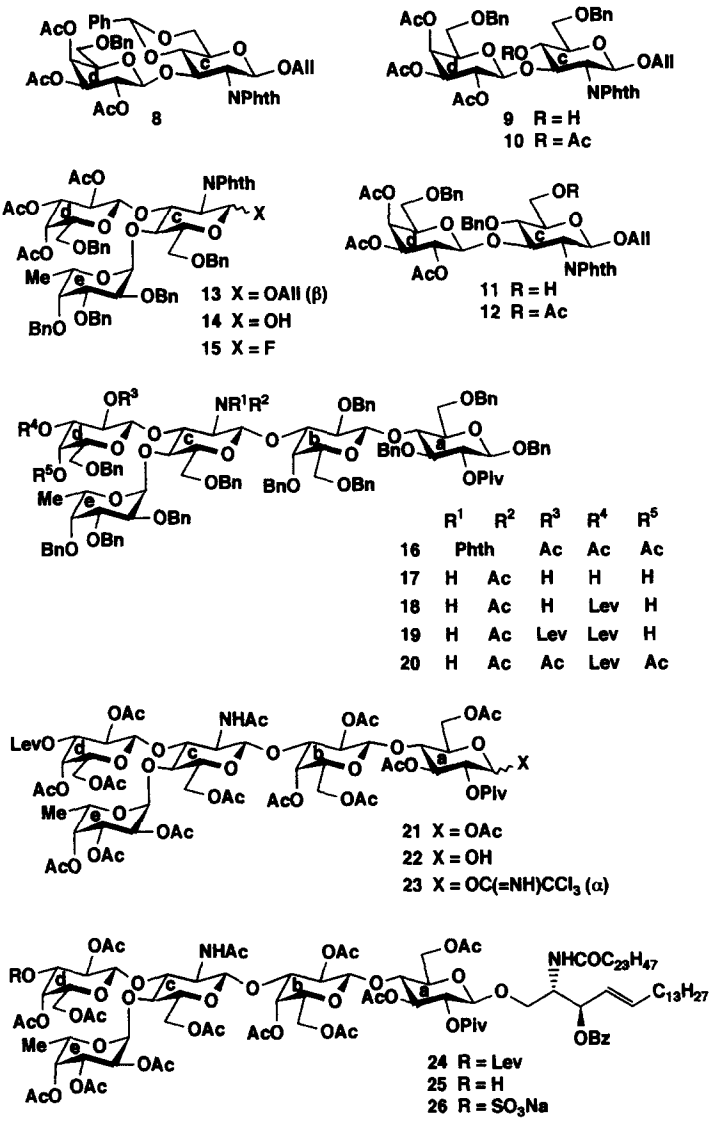


Scheme 1.

Hz). We carried out the reductive ring opening of the benzylidene acetal in compound **8** using some different conditions in THF as solvent {(a) $BH_3 \cdot NMe_3/AlCl_3$ [9a], (b) $BH_3 \cdot NMe_3/BF_3 \cdot OEt_2$, (c) $BH_3 \cdot NMe_3$/TMSOTf, (d) $NaBH_3CN$/etheral HCl [9b]}. Among them, condition (c) was best performed, due to its high conversion yield and high regioselectivity; **9** {89%, $[\alpha]_D$ −16.9° (*c* 1.0); R_f 0.24 (3:1 toluene–AcOEt)}, **11** {4%, $[\alpha]_D$ −37.5° (*c* 0.60); R_f 0.19 (3:1 toluene–AcOEt)}. The regiochemistry outcome of **9** and **11** were deduced by converting both compounds into their respective acetate **10** {$[\alpha]_D$ −11.3° (*c* 1.2); R_f 0.27 (3:1 toluene–AcOEt)} and **12** which showed in the ^{1}H NMR spectrum a deshielded signal for H-4c of **10** at δ_H 4.987 (t, J = 9.9 Hz), which was consistent with our previous data [5].

The crucial α-glycosylation of **9** with methyl thioglycoside **6** (2.0 equiv) in MeCN under the agency of MeOTf afforded a 76% yield of the desired α-(1 → 4)-linked trisaccharide **13** {$[\alpha]_D$ −29.1° (*c* 1.0); R_f 0.45 (3:1 toluene–AcOEt)}. The successful introduction of the L-fucosyl residue was confirmed by the ^{1}H NMR data for **13** that showed a signal for H-1e at δ_H 5.159 (d, J = 4.0 Hz). Deallylation of **13** with (1) $[Ir(COD)(PMePh_2)_2]PF_6$ [10] (0.1 equiv) in THF and (2) I_2 in aq THF afforded hemiacetal **14** in 97% yield. Compound **14** was converted to an α,β mixture ($\alpha:\beta$ 1:3) of the glycosyl fluoride **15** [R_f 0.55 (1:1 toluene–AcOEt); δ_H 5.678 (dd, 0.75 H, J = 7.5, 54.0 Hz, H-1cβ), 5.483 (dd, 0.25 H, J = 3.0, 54.0 Hz, H-1cα)] in 99% yield with diethylaminosulfur trifluoride (DAST) [11] at −15°C.



Scheme 2.

Having prepared the trisaccharide donor **15**, and having the glycosyl acceptor **7** in hand, the crucial glycosylation reaction was examined in the following manner. Glycosylation between **15** and **7** (1.3 equiv) under Mukaiyama conditions [12] [$SnCl_2$–AgOTf in 1 : 1 MeCN–EtCN at −15°C] afforded the desired pentasaccharide **16** in 82% yield {$[\alpha]_D$ −35.4° (*c* 1.0); R_f 0.70 (1 : 1 hexane –AcOEt)}. The configuration of the newly introduced anomeric carbon C-1c was expected to be β, due to the presence of the *N*-2 phthaloyl group in the glycosyl donor, which favors the formation of the 1,2-*trans*

stereochemistry. Indeed, the ^{1}H NMR spectral data showed the anomeric proton of H-1c as a doublet at δ_H 5.193 ($J = 8.0$ Hz), thus confirming the β configuration.

Simultaneous cleavage of the phthaloyl and acetyl groups of **16** was achieved by treatment with hydrazine hydrate in refluxing EtOH [13], and the amino alcohol thus obtained was *N*-acetylated by Ac_2O in MeOH to afford **17** in 87% yield {$[\alpha]_D$ −46.8° (*c* 1.0); R_f 0.42 (1:6 toluene–AcOEt)}. Treatment of **17** with levulinic anhydride afforded *O*-3d-levulinoylated **18** in 54% yield {$[\alpha]_D$ −26.3° (*c* 1.40); R_f 0.39 (1:3 hexane–AcOEt)} along with O-2d, O-3d-dilevulinoylated **19** in 40% yield {$[\alpha]_D$ −35.8° (*c* 1.27); R_f 0.71 (1:3 hexane–AcOEt)}. The assignment of **18** was deduced by the transformation into its acetate **20**, which showed in the ^{1}H NMR spectrum a newly deshielded signal for H-4d at δ_H 5.415 (d, $J = 4.0$ Hz) and H-2d at δ_H 5.022 (dd, $J = 8.5$, 10.5 Hz). Conversion of **20** into the completely acylated glycopentaose **21** was carried out in two steps in 78% 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 **21** was obtained as a 1:1 mixture of $\alpha:\beta$ anomers at C-1a [R_f 0.47 (20:1 $CHCl_3$–MeOH); δ_H 6.295 (d, $J = 4.0$ Hz, H-1aα) and δ_H 5.704 (d, $J = 8.5$ Hz, H-1aβ)].

Chemoselective cleavage of the anomeric acetate of **21** with piperidinium acetate [14] in THF at 50°C afforded an 85% yield of hemiacetal **22**, along with recovered starting material **21** (10%). Compound **22** was converted to α-trichloroacetimidate **23** in 90% yield {$[\alpha]_D$ −3.4° (*c* 1.32); R_f 0.51 (20:1 $CHCl_3$–MeOH); δ_H 6.510 (d, $J = 4.0$ Hz, H-1a)} by CCl_3CN and DBU in $(ClCH_2)_2$ [15]. The crucial coupling between **23** and **3** was performed in freshly distilled $CHCl_3$ in the presence of $BF_3 \cdot OEt_2$ to afford a 35% yield of β-glycoside **24** {$[\alpha]_D$ −20.0° (*c* 1.04); R_f 0.75 (20:1 $CHCl_3$–MeOH)}. The newly formed glycosidic linkage was shown to be β as revealed in the HOHAHA NMR spectrum of **24** [δ_H 4.415 (d, $J = 7.5$ Hz, H-1a)].

Further conversion of **24** to the target glycolipid was executed as follows. Removal of the levulinoyl group of **24** by hydrazinium acetate in EtOH [16] at room temperature afforded **25** in 98% yield {$[\alpha]_D$ −12.8° (*c* 0.51); R_f 0.67 (20:1 $CHCl_3$–MeOH)}. Compound **25** was converted to O-3d-sulfated compound **26** {$[\alpha]_D$ −14.9° (*c* 0.43)} in 97% yield by agency of the $SO_3 \cdot NEt_3$ complex in Me_2NCHO at 90°C. The structure of **26** was confirmed by the COSY and HOHAHA NMR experiments in CD_3OD, which showed that sulfated group had indeed been introduced at O-3d as revealed by the downfield shift of the H-3d [δ_H 4.432 (dd, $J = 3.5$, 10.5 Hz)].

Deprotection of **26** with N NaOH in 1:1 MeOH–THF at 40°C for 4.5 h afforded **1** in 34% yield (1.5 mg), after gel filtration through Sephadex LH-20 using 12:6:1 $CHCl_3$–MeOH–H_2O.

Physicochemical data for **1**: ^{1}H NMR (49:1 Me_2SO-d_6–D_2O, room temperature); δ_H 5.532 (dt, 1 H, $J = 15.5$, 8.5 Hz, H-5Cer), 5.345 (dd, 1 H, $J = 7.0$, 15.5 Hz, H-4Cer), 4.774 (d, 1 H, $J = 3.5$ Hz, H-1e), 4.740 (d, 1 H, $J = 8.0$ Hz, H-1c), 4.602 (q, 1 H, $J = 7.5$ Hz, H-5e), 4.418 (d, 1 H, $J = 7.0$ Hz, H-1d), 4.283 (d, 1 H, $J = 7.0$ Hz, H-1b), 4.164 (d, 1 H, $J = 8.0$ Hz, H-1a), 3.992 (brs, 1 H, H-4d), 1.810 (s, 3 H, NAc), 1.185 (d, 3 H, $J = 6.5$ Hz, H-6e), 0.852 (t, 6 H, $J = 7.0$ Hz, 2 CH_2Me); ESIMS: m/z $(M + Na)^+$ 1610, $(M - Na)^-$ 1564; FABMS (S-Gho matrix): m/z $(M + Na)^+$ 1610, (TEA matrix): m/z $(M - Na)^-$ 1564.

In summary, a stereocontrolled synthesis of the sulfated Le^a pentaosyl ceramide **1** was achieved for the first time using the glycopentaosyl trichloroacetimidate **23** as the key glycosyl donor. The biological properties of **1** are currently being studied.

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References

[1] P.J. Green, T. Tamatani, T. Watanabe, M. Miyasaka, A. Hasegawa, M. Kiso, C.-T. Yuen, M.S. Stoll, and T. Feizi, *Biochem. Biophys. Res. Commun.*, 188 (1992) 244–251.
[2] K.C. Nicolaou, N.J. Bockovich, and D.R. Carcanague, *J. Am. Chem. Soc.*, 115 (1993) 8843–8844.
[3] A. Lubineau, J.L. Gallic, and R. Lemoine, *J. Chem. Soc., Chem. Commun.*, (1993) 1419–1420.
[4] C.-T. Yuen, K. Bezouska, J. O'Brien, M. Stoll, R. Lemoine, A. Lubineau, M. Kiso, A. Hasegawa, N.J. Bockovich, K.C. Nicolaou, and T. Feizi, *J. Biol. Chem.*, 269 (1994) 1595–1598.
[5] S. Nunomura, M. Iida, M. Numata, M. Sugimoto, and T. Ogawa, *Carbohydr. Res.*, 263 (1994) C1–C6.
[6] K. Koike, Y. Nakahara, and T. Ogawa, *Glycocoj J.*, 1 (1984) 107–109; K. Koike, M. Numata, M. Sugimoto, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, 158 (1986) 113–123.
[7] H. Lönn, *Carbohydr. Res.*, 139 (1985) 105–113;115–121.
[8] S. Sato, S. Nunomura, T. Nakano, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, 29 (1988) 4097–4100.
[9] (a) M. Ek, P.J. Garegg, H. Hultberg, and S. Oscarson, *J. Carbohydr. Chem.*, 2 (1983) 305–311; (b) D.A. Horne and A. Jordan, *Tetrahedron Lett.*, 16 (1978) 1357–1358.
[10] L.M. Haines and E. Singleton, *J. Chem. Soc., Dalton Trans.*, (1972) 1891–1896; J.J. Oltvoort, C.A.A. van Boeckel, J.H. De Koning, and J.H. van Boom, *Synthesis*, (1981) 305–308.
[11] Wm. Rosenbrook, Jr., D.A. Riley, and P.A. Lartey, *Tetrahedron Lett.*, 26 (1985) 3–4; G.H. Posner and S.R. Haines, *Tetrahedron Lett.*, 26 (1985) 935–938.
[12] T. Mukaiyama, Y. Murai, and S. Shoda, *Chem. Lett.*, (1981) 431–432.
[13] R.U. Lemieux, T. Takeda, and B.Y. Chung., *ACS Symp. Ser.*, 39 (1976) 90–115.
[14] T. Nakano, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, 31 (1990) 1597–1600.
[15] R.R. Schmidt and J. Michel, *Angew. Chem., Int. Ed. Engl.*, 19 (1980) 731–732.
[16] H.J. Koeners, J. Verhoeven, and J.H. van Boom, *Rec. Trav. Chim. Pays-Bas*, 100 (1981) 65–72.