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Preliminary communication

Total synthesis of sulfated Le^x pentaosyl ceramide

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The selectins are a family of adhesion molecules that mediate the binding of leucocytes to endothelial cells and platelets, as well as to lymphocyte-homing receptors. The ligands recognized by L-selectin have been identified as the tetrasaccharides SLe^x and SLe^a [1]. Owing to the biological importance of these ligands in cell-adhesion processes, their efficient chemical synthesis is in demand [2].

Recently Feizi and co-workers reported the isolation of equimolar mixtures of sulfated Le^x and Le^a tetrasaccharides derived from an ovarian cystoadenoma glycoprotein that were more strongly bound to L-selectin than SLe^x or SLe^a [3]. More recently, chemical syntheses of both sulfated Le^x and Le^a were reported from two independent laboratories [4].

As part of our project on the synthesis of glycosphingolipids, we describe herein a stereocontrolled, facile, first total synthesis of sulfated Le^x pentaosyl ceramides **1** and **2** for further chemical and biological scrutiny. The overall strategy is depicted in Scheme 1.

Retrosynthetic analysis of a suitable process to **1** and **2** (Scheme 1) led us to design the putative glycopentaosyl donor **3** that could be coupled with ceramide derivative **4** [5]. Donor **3** was expected to be constructed from synthons derived from D-galactose, 2-amino-2-deoxy-D-glucose, L-fucose, and lactose (compounds **5**, **6** [6], **7** [7], and **8** [8], respectively, all of which are prepared from readily available compounds).

Glycosylation of **5** (1.2 equiv) with **6** in toluene in the presence of MeOTf at $-15°C$ afforded a 61% yield of the desired β-(1→4)-linked compound **9** {$[\alpha]_D$ $-4.8°$ (*c* 0.5);

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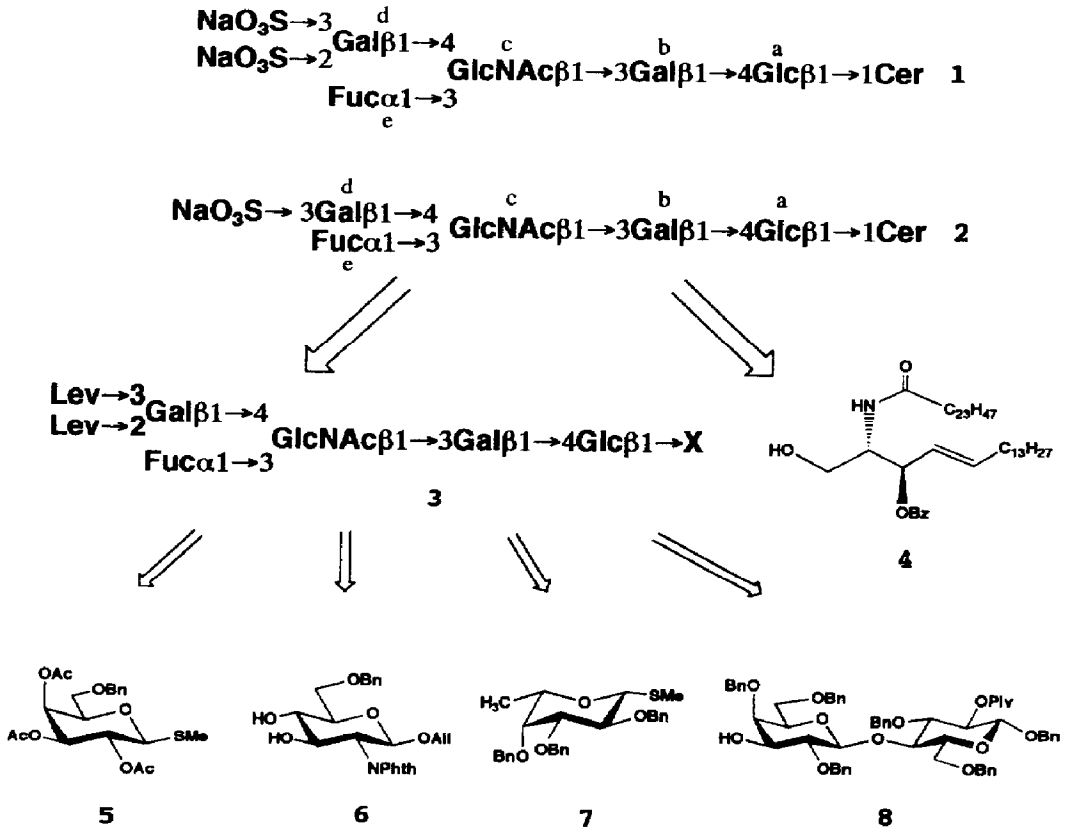


Scheme 1.

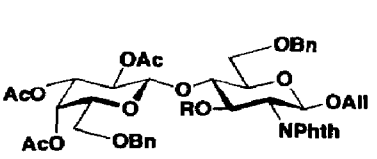
R_f 0.56 (2:1 toluene-EtOAc)}, as well as a 17% yield of its regioisomer **10** {$[\alpha]_D$ −16.9° (*c* 1.0); R_f 0.42 (2:1 toluene–EtOAc)}[1].

The β configurations of **9** and **10** were assignable from the ^{1}H NMR data that showed a signal for H-1d at δ 4.496 (d, *J* 8.1 Hz) and 4.419 (d, *J* 8.1 Hz), respectively. The regiochemistry of the newly introduced glycosidic linkages of **9** and **10** were deduced by converting both compounds into their respective acetates **11** {$[\alpha]_D$ −1.0° (*c* 1.2); R_f 0.29 (3:1 toluene–EtOAc)} and **12** {$[\alpha]_D$ −11.3° (*c* 1.2); R_f 0.27 (3:1 toluene–EtOAc)}, which showed in their ^{1}H NMR spectra deshielded signals for H-3c at δ_H 5.691 (dd, *J* 8.4, 10.6 Hz) and H-4c at δ_H 4.987 (t, *J* 9.9 Hz), respectively.

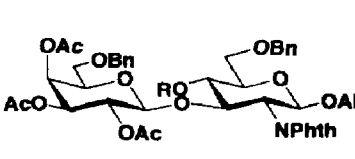
The crucial α-stereoselective glycosylation of **9** with methyl thioglycoside **7** (2.0 equiv) [7] in 5:1 Et_2O–$(ClCH_2)_2$ afforded a 77% yield of trisaccharide **13** {$[\alpha]_D$ −11.1° (*c* 0.4); R_f 0.35 (3:1 toluene–EtOAc)}.

The successful introduction of the L-fucosyl unit was confirmed by the ^{1}H NMR data for **13** that showed a signal for H-1e at δ_H 4.831 (d, *J* 3.3 Hz). Deallylation of **13** with (1) $[Ir(COD)(PMePh_2)_2]PF_6$ [9] in THF and (2) I_2 in aq THF gave hemiacetal **14** in 81%

[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) techniques.



9 R = H
11 R = Ac



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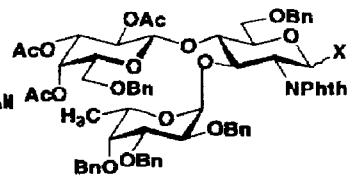
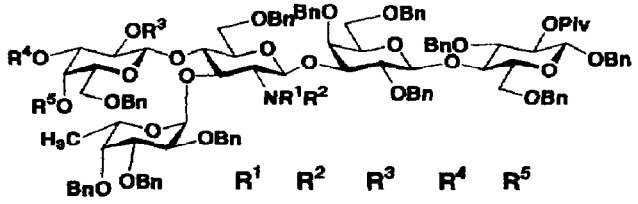
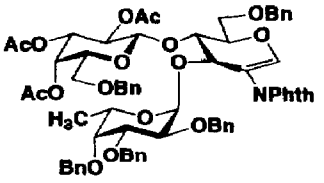


13 X = OAll (β)
14 X = OAc
15 X = F
16 X = OC(=NH)CCl$_3$ (β)



	R^1	R^2	R^3	R^4	R^5
17	Phth		Ac	Ac	Ac
19	Ac	H	Ac	Ac	Ac
20	Ac	H	H	H	H
21	Ac	H	Lev	Lev	H
22	Ac	H	Lev	Lev	Ac



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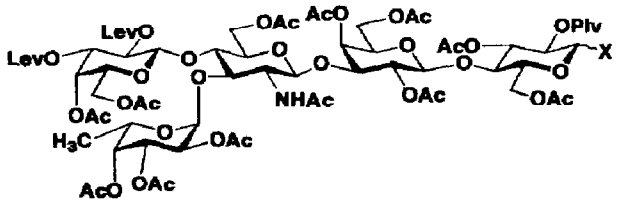
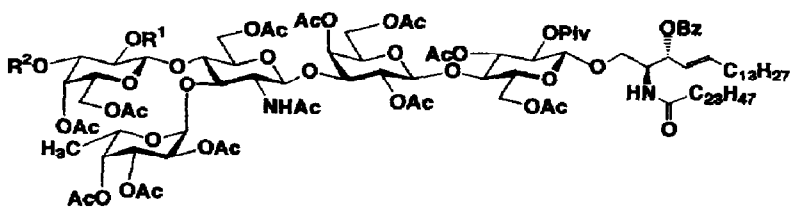


23 X = OAc
24 X = OH
25 X = OC(=NH)CCl$_3$ (α)



	R^1	R^2
26	Lev	Lev
27	H	H
28	Lev	OH
29	H	Lev
30	SO_3Na	SO_3Na
31	Lev	SO_3Na

yield. Compound **14** was transformed into β-trichloroacetimidate **16** {$[\alpha]_D$ +6.2° (*c* 1.0); R_f 0.60 (1:1 toluene–EtOAc); δ_H 6.365 (d, *J* 8.8 Hz, H-1c)} in 78% yield in the presence of CCl_3CN and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [10]. On the other hand, **14** was converted to an α,β-mixture (α:β 1:3) of the glycosyl fluoride **15** [R_f 0.60 (1:1 toluene–EtOAc); δ_H 5.822 (dd, 0.75 H, *J* 7.7, 54.6 Hz, H-1cβ), 5.544 (dd, 0.25 H, *J* 2.7, 54.6 Hz, H-1cα)] in 98% yield with diethylaminosulfur trifluoride (DAST) [11] at −15°C.

Having prepared the trisaccharide donors **15** and **16** so designed, and having the glycosyl acceptor **8** in hand, the crucial glycosylation reaction was examined in the following manner.

Trimethylsilyl triflate-promoted glycosylation [12] between **16** and **8** (2 equiv) in CH_3CN at −38°C afforded the desired pentasaccharide **17** in 42% yield {$[\alpha]_D$ −26° (*c* 0.9); R_f 0.33 (4:1 toluene–EtOAc)}, along with glycal **18** [R_f 0.28 (2:1 hexane–EtOAc); δ_H 6.672 (s, H-1c)] in 54% yield. The glycosylation reaction between **15** (1.3 equiv) and **8**, when carried out under Suzuki conditions [13] [Cp_2HfCl_2–AgOTf in $(ClCH_2)_2$ at −40°C], improved the coupling yield to 58%. 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 1,2-trans stereochemistry. Indeed, the ^1H NMR spectral data showed the anomeric proton of H-1c as a broad doublet at δ_H 5.335 (*J* 8.4 Hz), thus confirming the β configuration.

Simultaneous cleavage of the phthaloyl and acetate groups of **17** was achieved by treatment with hydrazine hydrate in refluxing EtOH [14], and the amino alcohol thus obtained was acetylated to afford **19** in 83% yield [R_f 0.52 (1:1 toluene–EtOAc); δ_H 1.958, 1.957, 1.815, 1.415 (4 s, 4 OAc)]. *O*-Deacetylation of **19** in MeONa–MeOH gave triol **20** in almost quantitative yield {$[\alpha]_D$ −38° (*c* 1.6); R_f 0.29 (1:3 toluene–EtOAc)}. Treatment of **20** with levulinic anhydride afforded the O-2d,O-3d-dilevulinoylated **21** in 77% yield {$[\alpha]_D$ −56° (*c* 0.87); R_f 0.43 (3:5 toluene–EtOAc)}. The assignment of **21** was deduced by converting **21** into acetate **22**, which showed in the homonuclear Hartmann–Hahn (HOHAHA) NMR spectrum a newly deshielded signal for H-4d at δ_H 5.350 (d, *J* 3.7 Hz), as well as signals for the already levulinoylated H-2d [δ_H 4.956 (dd, *J* 8.0, 10.6 Hz)] and H-3d [δ_H 4.822 (dd, *J* 3.7, 10.6 Hz)]. Conversion of **22** into the completely acylated glycopentaose **23** was carried out in two steps in 95% overall yield as follows: (1) H_2 with 20% $Pd(OH)_2$ in 4:1 MeOH–H_2O and (2) Ac_2O and 4-(dimethylamino)pyridine (DMAP) in pyridine. Compound **23** was obtained as a 2:1 mixture of β:α anomers at C-1a [R_f 0.33 (30:1 $CHCl_3$–MeOH); δ_H 6.290 (d, *J* 3.7 Hz, H-1aα) and δ_H 5.699 (d, *J* 8.1 Hz, H-1aβ)]. Chemoselective cleavage of the anomeric acetate of **23** with piperidinium acetate [15] in THF at 40°C afforded a 74% yield of hemiacetal **24**, along with recovered starting material **23** (18%). Compound **24** was treated with CCl_3CN and DBU in $(ClCH_2)_2$ to give α-trichloroacetimidate **25** in 93% yield {$[\alpha]_D$ −0.8° (*c* 0.37); R_f 0.40 (25:1 $CHCl_3$–MeOH); δ_H 6.506 (d, *J* 3.7 Hz, H-1a)}. The crucial coupling between **25** and **4** was performed in freshly distilled $CHCl_3$ in the presence of $BF_3 \cdot OEt_2$ to afford a 61% yield of β-glycoside **26** {$[\alpha]_D$ −19.5° (*c* 1.1); R_f 0.48 (26:1 $CHCl_3$–MeOH)}. The newly formed glycosidic linkage was shown to be β as revealed in the HOHAHA NMR spectrum of **26** [δ_H 4.411 (d, *J* 7.7 Hz, H-1a)].

Further conversion of **26** to the target glycolipid was executed as follows. Removal of the levulinoyl groups of **26** by hydrazinium acetate in EtOH [16] at room temperature gave diol **27** in 75% yield {$[\alpha]_D$ −22° (*c* 1.0); R_f 0.37 (25:1 $CHCl_3$–MeOH)}. It is noteworthy that delevulinoylation of **26** at −12 to −3°C gave a mixture: **27** in 20% yield, **28** in 21% yield {$[\alpha]_D$ −8.2° (*c* 0.2); R_f 0.45 (7:5 toluene–acetone)}, and **29** in 10% yield {$[\alpha]_D$ −15° (*c* 0.46); R_f 0.42 (7:5 toluene–acetone)}. Diol **27** was converted to the disulfated compound **30** {$[\alpha]_D$ −2° (*c* 0.25); R_f 0.30 (4:1 $CHCl_3$–MeOH) in 73% yield by agency of the $SO_3 \cdot NMe_3$ complex in Me_2NCHO at 90°C. The structure of **30** was confirmed by a COSY and a HOHAHA NMR experiment in CD_3OD, which showed that a sulfate group had indeed been introduced as revealed by the downfield shifts of the H-3d [δ_H 4.460 (dd, *J* 3.3, 10.6 Hz)] and H-2d [δ_H 4.880 (dd, *J* 8.1, 10.6 Hz)] signals.

Compound **28** was converted to the monosulfated **31** in the same manner in 77% yield {$[\alpha]_D$ −22° (*c* 0.1); R_f 0.46 (16:3 $CHCl_3$–MeOH)}. The structure of **31** was supported by a HOHAHA NMR experiment in CD_3OD that showed a downfield shift of the H-3d [δ_H 4.565 (dd, *J* 3.3, 10.6 Hz)], indicating sulfation at that position.

Deprotection of **30** and **31** with 1 N NaOH in 1:1 MeOH–THF afforded **1** and **2** in 82 and 97% yields, respectively, after gel filtration through Sephadex LH-20 using 12:6:1 $CHCl_3$–MeOH–H_2O.

Physicochemical data for **1**: R_f 0.48 (2:1:1 BuOH–EtOH–H_2O); ^{1}H NMR (49:1 Me_2SO-d_6–D_2O, 60°C): δ_H 5.560 (dt, *J* 15.4, 7.0 Hz, H-5Cer), 5.373 (dd, *J* 15.4, 7.0 Hz, H-4Cer), 4.972 (d, *J* 7.6 Hz, H-1c), 4.922 (d, *J* 2.9 Hz, H-1e), 4.432 (d, *J* 7.7 Hz, H-1d), 4.316 (d, *J* 3.0 Hz, H-4d), 4.285 (d *J* 8.1 Hz, H-1b), 4.277 (t, *J* 9.5 Hz, H-2d), 4.232 (q, *J* 6.6 Hz, H-5e), 4.174 (d, *J* 7.7 Hz, H-1a), 4.032 (dd, *J* 3.0, 9.5 Hz, H-3d), 1.837 (s, NAc), 1.092 (d, *J* 6.6 Hz, H-6e), 0.860 (t, *J* 7.0 Hz, 2 CH_2Me); ESIMS: *m/z* $(M+Na)^+$ 1713.

Physicochemical data for **2**: R_f 0.55 (2:1:1 BuOH–EtOH–H_2O); ^{1}H NMR (49:1 Me_2SO-d_6–D_2O, 60°C): δ_H 5.556 (dt, *J* 15.4, 7.0 Hz, H-5Cer), 5.376 (dd, *J* 15.4, 7.0 Hz, H-4Cer), 4.881 (d, *J* 7.6 Hz, H-1e), 4.725 (d, *J* 7.7 Hz, H-1c), 4.396 (d, *J* 7.7 Hz, H-1d), 4.280 (d, *J* 7.7 Hz, H-1b), 4.173 (d *J* 7.7 Hz, H-1a), 3.968 (dd, *J* 3.0, 9.5 Hz, H-3d), 3.952 (d, *J* 3.0 Hz, H-4d), 1.833 (s, NAc), 1.017 (d, *J* 6.6 Hz, H-6e), 0.858 (t, *J* 7.0 Hz, 2 CH_2Me); ESIMS: *m/z* $(M+Na)^+$ 1610.

The biological properties of **1** and **2** are currently being studied. In summary, a stereocontrolled synthesis of the sulfated Le^x pentaosylceramides **1** and **2** was achieved for the first time using the glycopentaosyl trichloroacetimidate **25** as the key glycosyl donor.

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