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Preliminary communication

Stereo- and regio-controlled, total synthesis of the Le^b antigen, III^4 FucIV^2 FucLcOse$_4$ Cer*,†

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Le^b glycosphingolipid, which contains[2] lacto-*N*-difucohexaose I as the carbohydrate structure, is known to be present in human tumors[3], human meconium[4], human erythrocytes[5], human plasma[6], and human intestine[7]. Structure **1** was proposed from methylation and enzymic data[3], and supported by ^{1}H-n.m.r. studies on permethylated derivatives[8], peracetylated derivatives[9], and underivatized[10] **1**.

Synthetic studies on Le^b glycolipid **1** have so far been directed to the synthesis of the nonreducing-end tetrasaccharide portion[11], which corresponds to the Le^b active determinant[12]. We now describe a total synthesis of Le^b glycolipid **1** that is unambiguous. Because the ^{1}H-n.m.r. data for our synthetic sample were found to be in good agreement with those of the natural compound[10], synthetic evidence for the proposed structure **1** is now firmly provided.

Retrosynthetic analysis (see Scheme 1) of glycolipid **1** led us to design the unknown glycohexaosyl donor **2** and the already reported glycosyl acceptor **3** (preparable from D-glucose[13]). The glycohexaosyl donor **2** may be "disconnected" into a backbone tetrasaccharide and a fucosyl donor. The former may be designed as the specifically protected derivative **4** and the latter as[14] the methyl 1-thioglycoside **5**.

We discuss, firstly, a synthesis of tetrasaccharide derivative **4**, secondly, the conversion of **4** into the glycohexaosyl donor **2**, and finally, glycosylation of the sphingenine derivative **3** with **2**.

Treatment of benzyl lactoside **6** with α,α-dimethoxytoluene and *p*-TsOH gave benzylidene derivative **7**, and reductive cleavage of the benzylidene ring of **7** by BH_3NMe_3–$AlCl_3$ in THF[15] afforded benzyl hexa-*O*-benzyllactoside **8**, $[\alpha]_D$ −5.7° (*c* 1.5)***, in 80% yield. *O*-Deacetylation of the methyl 1-thioglycoside **9**[16], and benzylidenation of the product with $PhCH(OMe)_2$–*p*-TsOH–DMF afforded benzylidene derivative **10**, $[\alpha]_D$ +2.0° (*c* 0.7), in 72% yield. Reductive cleavage of **10** with BH_3NMe_3–$AlCl_3$ in THF gave diol **11**, $[\alpha]_D$ +4.5° (*c* 0.3); R_F 0.35 in 2:1 EtOAc–

*Part 48 in the series "Synthetic Studies on Cell-Surface Glycans". For Part 47, see ref. 1.

†Structure: α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-(2*R*,3*S*,4*E*)$OCH_2CH(NHCOC_{23}H_{47})CHOHCH{=}CHC_{13}H_{27}$.

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***The values of $[\alpha]_D$ were determined for a solution in $CHCl_3$, unless noted otherwise.

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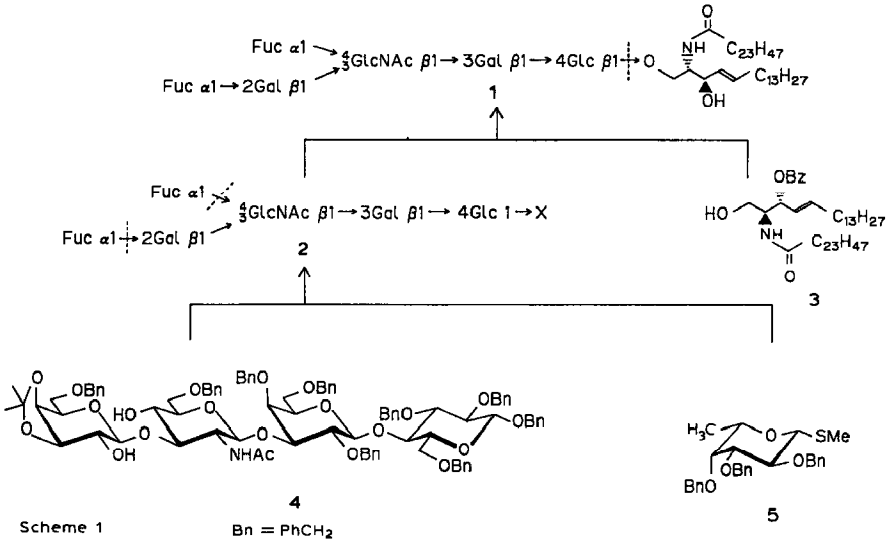


Scheme 1

toluene, in 75% yield; this was acetylated to give diacetate **12**, $[\alpha]_D$ +69° (*c* 0.9), m.p. 166–167°; R_F 0.67 in 3:2 toluene–EtOAc.

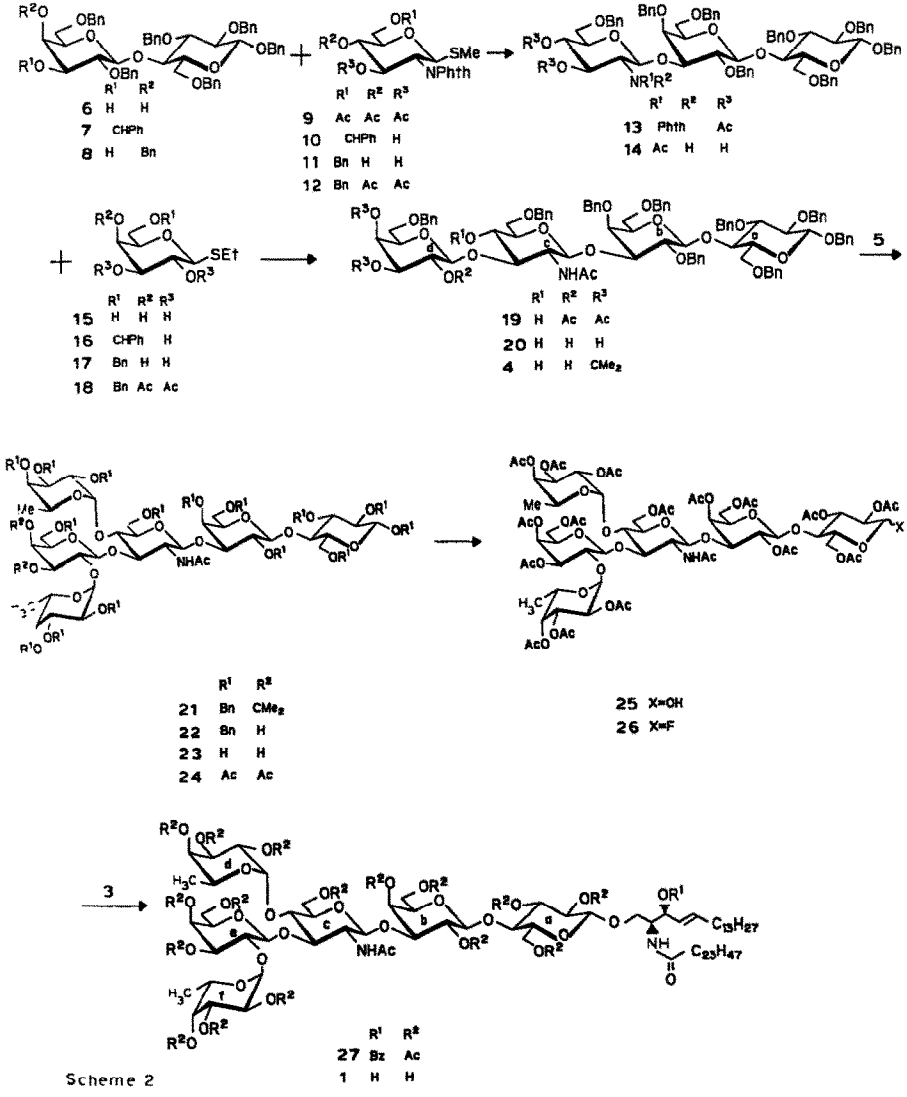
Glycosylation of the lactose derivative **8** with the methyl 1-thioglycoside **12** in the presence of $MeOSO_2CF_3$–molecular sieves 4A[17] in CH_3NO_2 afforded trisaccharide **13**, $[\alpha]_D$ −2.2° (*c* 1.2); R_F 0.57 in 3:1 toluene–EtOAc, which was converted in 3 steps [(*1*) NaOMe–MeOH, (*2*) $H_2NNH_2 \cdot H_2O$–EtOH, (*3*) Ac_2O–MeOH] into diol trisaccharide **14**, $[\alpha]_D$ −14.5° (*c* 0.5) in 58% overall yield from **8**.

The galactosyl donor **18**, $[\alpha]_D$ −32.8° (*c* 0.9), m.p. 53–54°; R_F 0.65 in 2:1 toluene–EtOAc, was prepared in a straightforward manner from 1-thioglycoside **15** (in 36% overall yield) in 3 steps: (*1*) PhCHO–$ZnCl_2$, (*2*) $BH_3 \cdot NMe_3$–$AlCl_3$–THF, (*3*) Ac_2O–pyridine–DMAP. Regioselective glycosylation of diol **14** with the galactosyl donor **18** was performed in the presence of $CuBr_2$–Bu_4NBr–$HgBr_2$–molecular sieves 4A[18], to give 77% yield of the desired product **19**, $[\alpha]_D$ −9.2° (*c* 0.5), R_F 0.64 in 3:2 EtOAc–toluene. The regiochemistry of **19** was assigned from its ^{13}C-n.m.r. data, which showed a shielded signal for C-2c at δ 56.7 relative to a signal at δ 58.6 for C-2c of trisaccharide **14**. *O*-Deacetylation of **19** with NaOMe–MeOH gave **20**, which was converted, by treatment with 2,2-dimethoxypropane and *p*-TsOH, into the designed tetrasaccharide derivative **4** in 71% yield, $[\alpha]_D$ −7.8° (*c* 1.4), R_F 0.56 in 2:1 toluene–acetone.

Stereoselective glycosylation of diol **20** with 1-thioglycoside **5** was achieved in the presence of $CuBr_2$–Bu_4NBr–$HgBr_2$–molecular sieves 4A in $MeNO_2$, to give a 68% yield of the desired hexasaccharide **21**, $[\alpha]_D$ −37° (*c* 0.9), R_F 0.54 in 3:1 toluene–EtOAc. The stereochemistry of glycosylation was determined by means of the ^1H-n.m.r. data for **21** which contained two doublets for H-1d and H-1f, at δ 5.094 and 5.384, with a $^3J_{HH}$ value of 3.7 Hz. *O*-Deisopropylidenation of compound **21** with 2:2:1 CF_3CO_2H–THF–H_2O gave diol **22**, $[\alpha]_D$ −33.6° (*c* 0.7), R_F 0.48 in 2:1 toluene–EtOAc, and hydrogenolysis of **22** in the presence of 10% Pd–C in 1:1 MeOH–AcOH afforded free hexasaccharide **23**, $[\alpha]_D$ −44.3° (*c* 0.6, H_2O), R_F 0.22 in 2:2:1

n-BuOH–EtOH–H_2O. The structure of **23** was established from the reaction sequence of the synthesis route, and supported by the ^{1}H-n.m.r. data (D_2O), which revealed characteristic proton signals at δ 5.204 (d, 0.35 H, *J* 4.0 Hz, H-1aα), 5.137 (d, 1 H, *J* 3.7 Hz, H-1f), 5.012 (d, 1 H, *J* 3.7 Hz, H-1d), 4.857 (q, 1 H, *J* 6.4 Hz, H-5d), 4.646 (d, 2 H, *J* 7.6 Hz, H-1c,e), 4.587 (d, 0.65 H, *J* 8.6 Hz, H-1aβ), 4.403 (d, 1 H, *J* 7.9 Hz, H-1b) and 4.327 (q, 1 H, *J* 6.1 Hz, H-5f). Acetylation of **23** with Ac_2O–pyridine–DMAP gave peracetylated hexasaccharide **24**, R_F 0.49 in 1:1 toluene–acetone, as a mixture of α and β anomers in the ratio of ~3:4, in 91% yield from **22**.

Through chemoselective deacetylation with $H_2NNH_2 \cdot AcOH$ in DMF[19], compound **24** was transformed into hemiacetal **25**, R_F 0.39 in 1:1 toluene–acetone, which,



Scheme 2

upon treatment with DAST[20], afforded a 7:1 mixture of the β and α anomers of fluoride **26**, R_F 0.73 in 1:1 toluene–acetone.

Having prepared fluoride **26**, a synthetic equivalent of the glycohexaosyl donor **2** (see Scheme 1), crucial glycosylation with **26** of the protected ceramide **3**, prepared previously from D-glucose[13], in the presence of $AgOSO_2CF_3$–$SnCl_2$–molecular sieves 4A[21] in 1:1 $CHCl_3$–toluene was found to give a 9% yield of the protected Le^b antigen **27**, $[\alpha]_D$ −46.9° (*c* 0.3), R_F 0.50 in 3:2 toluene–acetone. *O*-Deacylation of **27** with NaOMe–MeOH afforded a quantitative yield of Le^b glycolipid **1**, $[\alpha]_D$ −42.1° (*c* 0.2), R_F 0.52 in 2:1:1 n-BuOH–EtOH–H_2O. The stereochemistry of the final glycosylation was assigned as β-D from the ^{1}H-n.m.r. data (49:1 Me_2SOd_6–D_2O, 60°) of synthetic **1**, which contained signals at δ 5.568 (td, 1 H, *J* 6.7 and 15.3 Hz, H-5cer), 5.378 (dd, 1 H, *J* 7.3 and 15.6 Hz, H-4cer), 4.955 (d, 1 H, *J* 4.0 Hz, H-1f), 4.806 (d, H-1, *J* 3.7 Hz, H-1d), 4.658 (d, 1 H, *J* 8.2 Hz, H-1c), 4.617 (q, 1 H, *J* 7.3 Hz, H-5d), 4.552 (d, 1 H, *J* 7.0 Hz, H-1e), 4.300 (d, 1 H, *J* 6.4 Hz, H-1b), 4.214 (q, 1 H, *J* 6.1 Hz, H-5f), 4.185 (d, 1 H, *J* 7.9 Hz, H-1a), and 3.091 (t, 1 H, *J* 7.6 Hz, H-2a).

In conclusion, a regio- and stereo-controlled, total synthesis of Le^b antigen **1** was accomplished by employing the glycohexaosyl donor **26** and the ceramide derivative **3**.

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