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A TOTAL SYNTHESIS OF DIMERIC Le^x ANTIGEN, $III^3V^3Fuc_2nLc_6Cer$: PIVALOYL AUXILIARY FOR STEREOCONTROLLED GLYCOSYLATION[1)]

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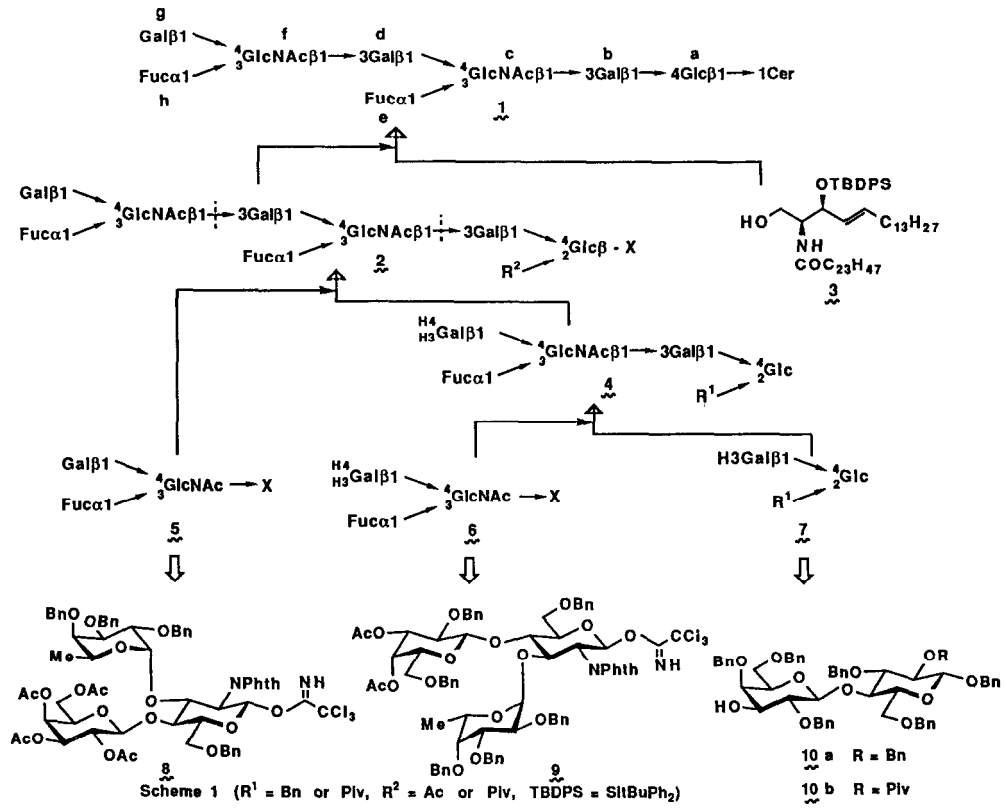
Abstract: A first total synthesis of dimeric Le^x glycooctaosyl ceramide was achieved in a stereocontrolled manner. Synthetic experiments were designed so as to evidence the advantage for the use of O-2a pivaloyl over O-2a acetyl group as a stereocontrolling auxiliary.

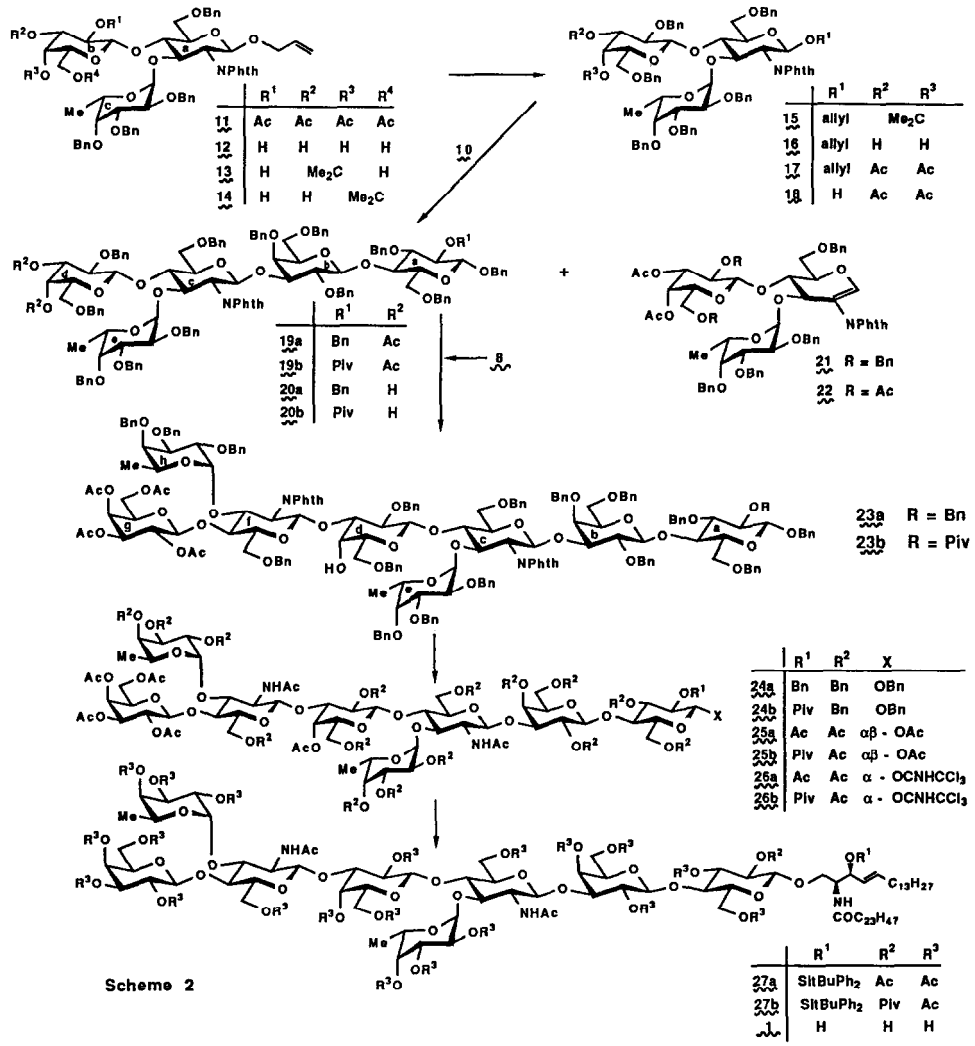
Trisaccharide determinant termed Le^x (or X) (Galβ1→4[Fucα1→3]GlcNAcβ1→R) and its multimeric forms are chemically well-characterized[2)] and recognized as developmentally regulated[3)] stage specific embryonic antigen-1 (SSEA-1) as well as tumor associated antigens[4)] defined by their specific monoclonal antibodies. Accumulation of difucosyl neolactonorhexaosylceramide **1** and trifucosyl analogue is a characteristic membrane phenotype of various human cancers[5)].

As part of our project on the synthesis of a series of SSEA-1 glycosphingolipids, we now describe a first total synthesis of so called dimeric Le^x antigen **1**. Synthetic strategy for **1** (scheme 1) is designed to employ glycooctaosyl donors **2**, which carry either acetyl or pivaloyl group at O-2a as a stereocontrolling auxiliary[6)] in order to compare their efficiency for the



coupling with a ceramide derivative **3**. The donor **2** was retrosynthesized into a glycotriaosyl synthon **5** which was designed as the imidate **8**[7], and a glycopentaosyl synthon **4** which in turn was disconnected into a glycotriaosyl synthon **6** and a glycobiosyl synton **7**. The synthetic equivalent of **7** was already available either as a glycobiosyl acceptor **10a**[8] or **10b**[6], being designed so as to function as a precursor for the synthesis of **2** (R^2=Ac or Piv). Preparation of the imidate **9**, a synthetic equivalent of **6**, was carried out starting from trisaccharide **11**[7]. A diol **13**[9] was obtained along with an isomer **14** (16%) from **11** in 2 steps (1. NaOMe in MeOH-THF, 2. $Me_2C(OMe)_2$-TsOH in DMF, 74% overall). Conversion of **13** into **9** was performed in 5 steps in a usual way via **15-18** (1. BnBr-Ag_2O-KI in DMF, 2. TFA-H_2O-THF, 3. Ac_2O-DMAP in Py, 4. $PdCl_2$-AcONa in aq.AcOH[10], 5. CCl_3CN-DBU in $(ClCH_2)_2$, 30% overall). Being all the designed synthons **8**, **9** and **10ab** available, glycan chain elongation was examined starting from the reducing end synthon **10** of the glycan chain **2**.

Trifluoroborane etherate promoted glycosylation[11] of **10ab** with **9** in $(ClCH_2)_2$ afforded **19** (a: 52%, b: 53%) accompanied by the formation of the glycal **21** (about 10%). Treatment of **19ab**



Scheme 2

with 0.04M NaOMe in 5:2 MeOH-THF afforded **20** (**a**: 88%, **b**: 98%).

Further reaction of the glycosyl acceptor **20ab** which is equivalent to the synthon **4**, with a glycotriaosyl donor **8** was successfully achieved in a regiocontrolled manner[12] to give **23** in agreement with our previous observation[7] (BF_3•OEt_2-MS-AW300 in $(ClCH_2)_2$, **a**: 78%, **b**: 62%) along with **22** (~12%). Conversion of **23** into glycooctaosyl donor **26** was smoothly carried out in 6 steps via **24-25** (1. 2% NH_2NH_2•H_2O in EtOH, 2. and 4. Ac_2O-DMAP in Py, 3. 10% Pd-C, H_2 in 1:1 MeOH-AcOH, 5. NH_2NH_2•AcOH in DMF[13], 6. Cl_3CCN-DBU in $(ClCH_2)_2$, **a**: 50%, **b**: 53% overall).

Crucial glycosylation of **3** with **26** gave **27** (BF_3•OEt_2-MS-AW300 in $(ClCH_2)_2$, **a**: 4.8%, **b**: 31.7%), which clearly confirmed the advantage for the use of pivaloyl over acetyl as a stereocontrolling auxiliary at O-2a not only for the previously reported glycotriaosyl donors[6] but also for such an extended glycooligosyl donor as **2**. The glycooctaosyl ceramide **27ab** was completely deblocked to give **1** in 2 steps (1. Bu_4NF in THF, 2. MeONa in MeOH-THF), ^{1}H-nmr data of which was in good agreement with those reported for the natural product[14].

In summary, total synthesis of dimeric Le^x antigen **1** was accomplished by using either acetyl or pivaloyl as an O-2a auxiliary of the glycosyl donor **2** for stereocontrolled glycosylation of the protected ceramide **3**.

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9) Physical data for synthetic compounds are given below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were measured for the solution in $CHCl_3$ and $CDCl_3$, respectively, at 25° unless noted otherwise. **12**: $[\alpha]_D$ -19.9° (c 0.9); δ_H 5.219 (d, 8.8 Hz, 1a), 4.873 (d, 2.7 Hz, 1c), 4.489 (d, 7.0 Hz, 1b); δ_C 99.9 (1b), 98.7 (1a), 97.0 (1c), 55.9 (2a). **13**: $[\alpha]_D$ -7.0° (c 0.8); δ_H 5.195 (d, 8.5 Hz, 1a), 4.784 (d, 3.4 Hz, 1c), 4.396 (d, 8.2 Hz, 1b), 1.469, 1.313 (2 s, CMe_2), 1.052 (d, 6.4 Hz, 6c); δ_C 110.2 (CMe_2), 99.5 (1b), 98.7 (1a), 97.0 (1c), 55.9 (2a). **14**: $[\alpha]_D$ +2.5° (c 0.6); δ_H 5.113 (d, 8.5 Hz, 1a), 4.833 (d, 3.7 Hz, 1c), 4.551 (d, 7.6 Hz, 1b), 1.392, 1.301 (2 s, CMe_2),

(c 0.6); δ_H 5.113 (d, 8.5 Hz, 1a), 4.833 (d, 3.7 Hz, 1c), 4.551 (d, 7.6 Hz, 1b), 1.392, 1.301 (2 s, CMe_2), 1.098 (d, 6.4 Hz, 6c); δ_C 101.4 (1b), 98.7 (CMe_2), 97.7 (1a), 97.5 (1c), 56.4 (2a). **15**: $[\alpha]_D$ -2.2° (c 0.9); δ_H 5.134 (d, 8.5 Hz, 1a), 4.805 (d, 3.2 Hz, 1c); δ_C 109.5 (CMe_2), 101.5 (1b), 97.9 (1a), 97.5 (1c), 56.5 (2a). **16**: $[\alpha]_D$ -11.4° (c 0.8); δ_H 5.162 (d, 8.5 Hz, 1a), 1.086 (d, 6.4 Hz, 6c); δ_C 101.7 (1b), 97.7 (1a), 97.5 (1c), 56.3 (2a). **17**: $[\alpha]_D$ -7.9° (c 0.8); δ_H 5.328 (d, 2.7 Hz, 4b), 5.109 (d, 8.5 Hz, 1a), 1.911, 1.749 (2 s, 2 Ac), 1.128 (d, 6.4 Hz, 6c); δ_C 102.0 (1b), 97.8 (1a), 97.5 (1c), 56.4 (2a). **18**: $[\alpha]_D$ +1.6° (c 0.8); δ_H 5.324 (d, 2.7 Hz, 4b), 1.917, 1.745 (2 s, 2 Ac), 1.135 (d, 6.4 Hz, 6c). **9**: $[\alpha]_D$ +6.5° (c 1.0); δ_H 8.528 (s, NH), 6.394 (d, 8.8 Hz, 1a), 5.328 (d, 2.9 Hz, 4b), 4.627 (d, 8.0 Hz, 1b), 1.915, 1.759 (2 s, 2 Ac), 1.300 (d, 6.6 Hz, 6c). **19a**: $[\alpha]_D$ -23.5° (c 0.5); δ_H 5.351 (d, 3.6 Hz, 4d), 5.345 (d, 8.2 Hz, 1c), 1.926, 1.740 (2 s, 2 Ac), 1.131 (d, 6.7 Hz, 6e); δ_C 102.5 (1ab), 102.0 (1d), 100.0 (1c), 97.6 (1e), 57.1 (2c). **19b**: $[\alpha]_D$ -25.5° (c 0.9); δ_H 5.350 (d, 2.4 Hz, 4d), 5.337 (d, 8.2 Hz, 1c), 5.014 (dd, 7.9, 9.5 Hz, 2a), 1.925, 1.735 (2 s, 2 Ac), 1.130 (d, 6.4 Hz, 6e), 1.090 (s, tBu); δ_C 102.3 (1b), 102.1 (1d), 99.5 (1ac), 97.4 (1e). **20a**: $[\alpha]_D$ -21.6° (c 0.7); δ_H 5.400 (d, 8.5 Hz, 1c), 1.087 (d, 6.4 Hz, 6e); δ_C 102.5 (1ab), 101.8 (1d), 100.0 (1c), 97.7 (1e). **20b**: $[\alpha]_D$ -24.2° (c 0.5); δ_H 5.390 (d, 8.5 Hz, 1c), 5.013 (dd, 7.9, 9.4 Hz, 2a); δ_C 102.6 (1b), 101.8 (1d), 99.7 (1ac), 97.5 (1e). **21**: $[\alpha]_D$ -41.4° (c 0.7); δ_H 6.714 (s, 1a), 5.439 (d, 2.7 Hz, 4b), 4.955 (dd, 3.6, 10.4 Hz, 3b), 4.797 (d, 3.4 Hz, 1c); δ_C 107.7 (1a), 102.8 (1b), 98.7 (1c). **22**: $[\alpha]_D$ -36.9° (c 0.5); δ_H 6.686 (s, 1a), 5.372 (d, 2.4 Hz, 4b), 5.259 (dd, 8.2, 10.7 Hz, 2b), 4.984 (dd, 3.3, 10.7 Hz, 3b), 4.824 (d, 3.0 Hz, 1c). **23a**: $[\alpha]_D$ -27.4° (c 0.9); δ_H 5.316 (d, 8.3 Hz, 1f), 5.236 (d, 2.7 Hz, 4g), 5.148 (d, 8.3 Hz, 1c), 1.999, 1.982, 1.947, 1.824 (4 s, 4 Ac), 1.194, 0.999 (2 d, 6.4 Hz, 6eh). **23b**: $[\alpha]_D$ -28.0° (c 0.8); δ_H 5.316 (d, 8.5 Hz, 1f), 5.233 (d, 3.3 Hz, 4g), 5.140 (d, 8.2 Hz, 1c), 1.999, 1.981, 1.948, 1.819 (4 s, 4 Ac), 1.192, 0.994 (2 d, 6.4 Hz, 6eh), 1.089 (s, tBu). **24a**: $[\alpha]_D$ -30.9° (c 1.1); δ_H 5.447 (d, 3.3 Hz, 4d), 5.278 (d, 3.0 Hz, 4g), 2.019, 1.947, 1.934, 1.862, 1.832, 1.469, 1.266 (7 s, 7 Ac), 1.170 , 1.114 (2d, 6.4 Hz, 6eh). **24b**: $[\alpha]_D$ -41.0° (c 0.5); δ_H 5.446, 5.275 (2d, 3.3 Hz, 4dg), 2.022, 1.947, 1.933, 1.851, 1.841, 1.621, 1.458 (7s, 7Ac), 1.166, 1.111 (2d, 6.4 Hz, 6eh), 1.119 (s, tBu). **25a**: δ_H 6.254 (d, 0.5 H, 3.7 Hz, 1aα), 5.660 (d, 0.5 H, 8.2 Hz, 1aβ), 1.203, 1.156 (2d, 6.4 Hz, 6eh). **25b**: δ_H 6.291 (d, 0.5 H, 3.9 Hz, 1aα), 5.694 (d, 0.5 Hz, 8.1 Hz, 1aβ). **26a**: $[\alpha]_D$ -25.0° (c 1.0); δ_H 8.651 (s, C=NH), 6.482 (d, 3.7 Hz, 1a), 1.200, 1.153 (2d, 6.6 Hz, 6eh). **26b**: $[\alpha]_D$ -24.6° (c 0.4); δ_H 8.654 (s, C=NH), 6.504 (d, 3.6 Hz, 1a). **27a**: $[\alpha]_D$ -29.3° (c 0.06); δ_H 2.188~1.900 (23s, 23Ac), 1.202, 1.155 (2d, 6.4 Hz, 6eh), 1.004 (s, tBu), 0.881 (2t, 7.0 Hz, $2CH_2CH_3$). **27b**: $[\alpha]_D$ -31.8° (c 0.6); δ_H 2.193~1.903 (22s, 22Ac), 1.156 (d, 6.4 Hz, 6e or h), 1.122 (s, piv), 0.993 (s, tBu), 0.879 (2t, 7.0 Hz, $2CH_2CH_3$). **1**: $[\alpha]_D$ -33.0° (c 0.1, MeOH); δ_H(50:1 DMSOd6-D_2O, 40°), 5.537 (dt, 15.2, 6.5 Hz, Cer5), 5.342 (dd, 7.4, 15.5 Hz, Cer4), 4.873 (d, 3.6 Hz, 1eh), 4.738, 4.723 (2d, 7.3 Hz, 6.9 Hz, 1cf), 4.637, 4.623 (2q, 5.8 Hz, 5eh), 4.345 (d, 6.4 Hz, 1d), 4.301 (d, 7.0 Hz, 1g), 4.276 (d, 7.0 Hz, 1b), 4.172 (d, 7.6 Hz, 1a).

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