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SYNTHESIS OF BRANCHED POLY-N-ACETYL-LACTOSAMINE TYPE PENTAANTENNARY PENTACOSASACCHARIDE: GLYCAN PART OF A GLYCOSYL CERAMIDE FROM RABBIT ERYTHROCYTE MEMBRANE[1]

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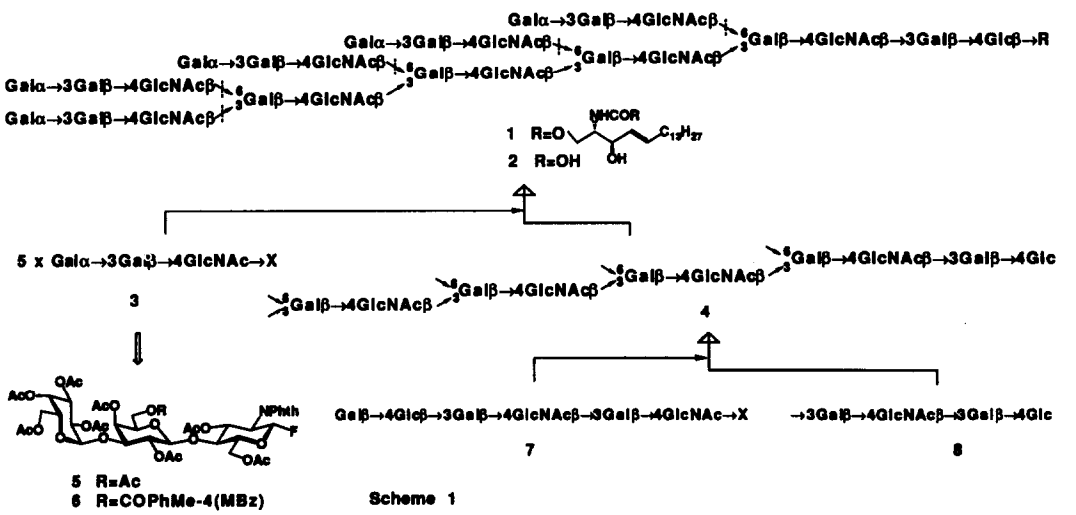
Abstract: A stereocontrolled approach to the synthesis of poly-N-acetyl-lactosamine type pentacosasaccharide was achieved for the first time.

In 1988, pentacosa-saccharide ceramide[2] of blood group I-activity was isolated from Rabbit erythrocyte membranes and its structure was elucidated as **1**. Since the significant structural changes of poly-N-acetyl-lactosamine type glycans as the surface markers were observed during development and differentiation as well as malignant transformation of epithelia cells[3], we have been interested in developing versatile synthetic routes toward poly-N-acetyl lactosamine type glycosyl ceramides. So far we have reported the synthesis of such glycosyl ceramides that carry biantennary octa-[4] as well as triantennary pentadeca-saccharides[5]. In this communication, we describe a regio- and stereo-controlled synthesis of pentaantennary pentacosasaccharide **2**, a glycan part of a most complex glycosyl ceramide **1**.

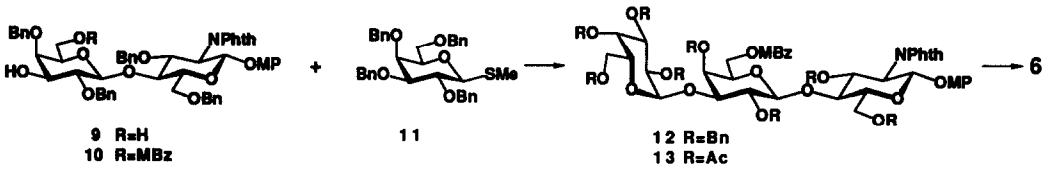
Based on a retrosynthetic analysis of **2**, a key intermediate glycotriosyl donor **3** was designed as **6** which carries one toluoyl group as a signal marker for ^{1}H-nmr spectra. This modification of the available donor **5**[5] is expected to make it easier to follow the progress of glycosylation by ^{1}H-nmr. A synthetic route toward an another key intermediate glycodecaosyl acceptor **4** may be planned according to a strategic



Scheme 1

bond disconnection of **4** into a glycohexaosyl donor **7** and glycotetraosyl acceptor **8**.

A synthesis of the glycotriosyl donor **6** was straightforward. Regioselective acylation of diol **9**[6] with p-MeBzCl in pyridine gave 60% of **10**[7] which was glycosylated with **11** in the presence of $CuBr_2$ and n-Bu_4NBr[8] in $(ClCH_2)_2$ to give 72% of **12**[7]. Conversion of **12** into **6**[7] was performed via **13**[7] in 4 steps (*1* 10% Pd-C, H_2 in 10:5:1 MeOH-EtOAc-H_2O; *2* Ac_2O, DMAP in Py; *3* CAN[9] in 50:38:25 CH_3CN-MePh-H_2O; *4* DAST[10] in $(ClCH_2)_2$; 54% overall).



9 R=H
10 R=MBz

11

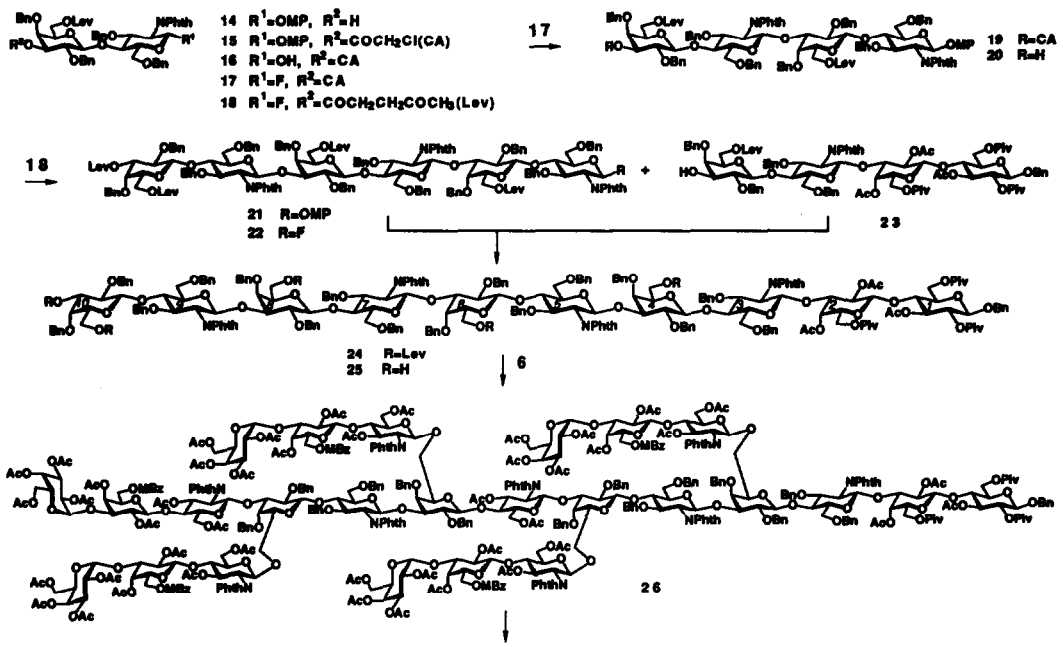
12 R=Bn
13 R=Ac

Scheme 2 (MP = PhOMe-4)

Since we have already reported compound **23**[4] that should be a synthetic equivalent to a glycotetraosyl acceptor **8**, a key intermediate glycodecaosyl acceptor **4** and a glycohexaosyl donor **7** were now designed as **25** and **22** respectively. Compounds **22** and **25** were synthesized as follows. Compound **14** was readily available from diol **9** by treatment with 2-chloro-1-methylpyridinium iodide (CMPI)[11], levulinic acid and DABCO in $(ClCH_2)_2$ in 74%. Conversion of **14** into fluoride **17** was smoothly carried out via **15** and **16** in 3 steps (*1* $(ClCH_2CO)_2O$, DMAP in Py; *2* CAN in 6:5:3 CH_3CN-PhMe-H_2O; *3* DAST in $(ClCH_2)_2$; 72% overall). Glycosylation of **14** with **17** in the presence of $Cp_2Hf(OTf)_2$[12] in $(ClCH_2)_2$ at -23° gave 98% of the desired tetrasaccharide **19**[7]. Selective removal of chloroacetyl group was smoothly achieved by treatment with thiourea[13] in DMF to afford 98% of **20**[7]. Further $Cp_2Hf(OTf)_2$ promoted glycosylation of **20** with fluoride **18** which is readily available[4] from **14** afforded 93% of **21**. Conversion of **21** into fluoride **22** was achieved in 2 steps by the same sequence of reactions as described for **17** in 80% overall yield. Now coupling between glycotetraosyl acceptor **23** with **22** was again executed in the presence of $Cp_2Hf(OTf)_2$ to give 77% of the glycodecaoside **24** that was further transformed into the designed acceptor **25** in 95% yield through cleavage of levuloyl groups by $NH_2NH_2 \cdot AcOH$[14] in 5:1 EtOH-PhMe. Crucial $Cp_2Hf(OTf)_2$ promoted glycosylation of **25** with 2.8 equivalents of glycosyl donor **6** in $(ClCH_2)_2$ at -23° and subsequent purification of the product (*1* biobeads SX-1 in PhMe; *2* preparative tlc) afforded 37% of the desired completely protected pentacosasaccharide **26**[7]. The structure of **26** was deduced from ^{1}H-nmr which contained signals for 5 PhMe as two singlets at δ 2.385 and 2.394 in a ratio of 3:2.

Finally complete deprotection of **26** into **2** was successfully performed in 4 steps. (*1* $NaBH_4$[15] in 4:1 iPrOH-H_2O for 21 h at 25°, then AcOH for 6 h at 120°; *2* Ac_2O; DMAP in Py; *3* MeONa in MeOH for 2 h at 70°; *4* $Pd(OH)_2$-C, H_2 in 1:1 MeOH-H_2O; 37% overall). ^{1}H-nmr data for **2** was in agreement with those[16] for the related natural products, thus providing a synthetic evidence for poly-N-acetyl-lactosamine

structures such as **1**.



Scheme 3

In summary, pentacosasaccharide **2**, one of the most complex I-type poly-N-acetyl-lactosamine structures isolated from Nature was synthesized by employing fluorides **6** and **22** as key glycosyl donors, and a linear glycodecaosyl acceptor **25** as a key glycosyl acceptor. It is to be noted that $Cp_2Hf(OTf)_2$ promoted glycosylation of polyfunctional glycosyl acceptors such as **25** by glycosyl fluorides carrying C-2 N-phthalimido group was found to be remarkably efficient in these experiments.

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References and Notes

1 Part 93 in the series "Synthetic Studies on Cell-Surface Glycans". For part 92, see T. Nakano, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, submitted.

2 P. Hanfland, M. Kordowiez, J. Peter-Katalinic, H. Egge, J. Dabrowski, and U. Dabrowski, *Carbohydr. Res.*, **178** 1 (1988).

3 T. Feizi, *Frends Biochem. Sci.*, **6** 333 (1981); A. Kapadia, T. Feizi, and M. J. Evans, *Exp. Cell Res.*, **131** 185 (1981).

4 Y. Matsuzaki, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, in press.

5 Y. Matsuzaki, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, in press.

6 T. Nakano, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, **31** 1597 (1990).

7 Physical data for new compounds are given below, values of $[\alpha]_D$ and $\delta_{H,C}$ were measured at 25°±3° for solutions in $CHCl_3$ and $CDCl_3$, respectively, unless noted otherwise. Signal assignment such as 1^3 stands for a proton at C-1 of sugar residue *3*. **2**: $[\alpha]_D$ +9.0° (c 0.05, H_2O); ESI MS $(M+O)^+$ 4440; δ_H (D_2O, 70°) 2.045 (s, 4Ac), 2.028 (s, 5Ac), 4.443 (d, 7.7Hz, Galβ), 4.458 (d, 7.7Hz, 4 x Galβ), 4.524 (d, 7.7Hz, 4 x Galβ), 4.538 (d, 6.2Hz, Galβ), 4.615 (d, 7.4Hz, 4 x GlcNAcβ→6), 4.645 (d, 0.7H, 7.7Hz, Glcβ), 4.728 (d, 8.1Hz, 5 x GlcNAcβ→3), 5.135 (d, 3.7Hz, 5 x Galα), 5.209 (d, 0.3H, 4.0Hz, Glcα). **6**: R_F 0.47 in 1:1 toluene-EtOAc; δ_H 1.945-2.172 (7s, 8Ac), 2.422 (s, PhMe), 5.413 and 5.447 (2d, 2.0Hz, 4^2 and 4^3), 6.076 (dd, 8.0 and 52.4Hz, 1^1). **10**: R_F 0.61 in 3:1 toluene-EtOAc; $[\alpha]_D$ +62.7° (c 0.9); δ_H 2.452 (s, PhMe), 3.699 (s, OMe), 5.624 (d, 8.1Hz, 1^1). **12**: R_F 0.28 in 10:1 toluene-EtOAc; $[\alpha]_D$ +60.8° (c 1.1); δ_H 2.446 (s, PhMe), 3.685 (s, OMe), 5.206 (d, 3.3Hz, 1^3), 5.577 (d, 8.4Hz, 1^1). **13**: R_F 0.49 in 1:1 toluene-EtOAc; $[\alpha]_D$ +66.3° (c 1.0); δ_H 1.944-2.174 (8s, 8Ac), 2.413 (s, PhMe), 3.725 (s, OMe), 5.418 and 5.448 (2d, 2.9Hz, 4^2 and 4^3), 5.838 (d, 8.4Hz, 1^1). **14**: R_F 0.35 in 1:1 hexane-EtOAc; $[\alpha]_D$ +44.7° (c 1.0); δ_H 2.195 (s, LevMe), 3.696 (s, OMe), 4.452 (d, 7.3Hz, 1^2), 5.624 (d, 8.1 Hz, 1^1). **15**: R_F 0.53 in 1:1 hexane-EtOAc, $[\alpha]_D$ +49.3° (c 1.4); δ_H 2.212 (s, LevMe), 3.700 (s, OMe), 4.490 (d, 7.7 Hz, 1^2), 4.859 (dd, 3.3 and 10.3Hz, 3^2), 5.615 (d, 8.4Hz, 1^1). **16**: R_F 0.22 in 1:1 hexane-EtOAc; δ_H 2.164 and 2.184 (2s, LevMe). **17**: R_F 0.50 in 1:1 hexane-EtOAc; δ_H 5.635 (dd, 0.17H, 2.6 and 53.8Hz, $1^1\alpha$), 5.844 (dd, 0.83H, 7.5 and 53.5Hz, $1^1\beta$). **19**: R_F 0.46 in 2:1 toluene-EtOAc; $[\alpha]_D$ +29.3° (c 0.9); δ_H 2.135 and 2.155 (2s, 2LevMe), 3.666 (s, OMe), 3.713 and 3.761 (2d, 15.0Hz, $COCH_2Cl$), 5.394 and 5.441 (2d, 8.4Hz, 1^1 and 1^3). **20**: R_F 0.30 in 2:1 toluene-EtOAc; $[\alpha]_D$ +20.7° (c 1.2); δ_H 2.122 and 2.156 (2s, 2LevMe), 3665 (s, OMe), 5.399 and 5.442 (2d, 8.4Hz, 1^1 and 1^3). **21**: R_F 0.21 in 3:2 EtOAc-toluene; $[\alpha]_D$ +14.7° (c 0.9); δ_H 2.055, 2.126, 2.151 and 2.156 (4s, 4LevMe), 3.622 (s, OMe), 5.201, 5.405, and 5.422 (3d, 8.4Hz, 1^1, 1^3, and 1^5). **22**: R_F 0.56 in 1:1 toluene-EtOAc; δ_H 2.057, 2.126, 2.135, and 2.155 (4s, 4LevMe), 5.199 and 5.407 (2d, 8.4Hz, 1^3 and 1^5), 5.508 (dd, 0.11H, 3.3 and 52.8Hz, $1^1\alpha$), 5.655 (dd, 0.89H, 8.1 and 53.8Hz, $1^1\beta$). **24**: R_F 0.34 in 10:1 $CHCl_3$-THF; $[\alpha]_D$ -3.6° (c 2.0); δ_H 1.065, 1.162, and 1.186 (3s, 3Piv), 1.610, 1.867, and 2.019 (3s, 3Ac), 2.044, 2.055, 2.124, and 2.152 (4s, 5LevMe), 5.187, 5.222, and 5.340 (3d, ~8.1Hz, 1^5, 1^{7x2}, and 1^9), 5.209 (d, 4.4Hz, 4^2). **25**: R_F 0.41 in 1:1 toluene-EtOAc; $[\alpha]_D$ -3.0° (c 1.1); ESI MS $(M+O)^+$ 4066; δ_H 1.066, 1.163, and 1.186 (3s, 3Piv), 1.605, 1.868, and 2.039 (3s, 3Ac), 5.189, 5.230, and 5.410 (3d, ~8.4Hz, 1^5, 1^1, and 1^9), 5.216 (d, 4.8Hz, 4^2). **26**: R_F 0.36 in 2:3 toluene-EtOAc; $[\alpha]_D$ +16.8° (c 0.9); ESI MS $(M+O)^+$ 9416; δ_H 1.064, 1.164, and 1.180 (3s, 3Piv), 2.385 (s, 3PhMe), 2.394 (s, 2PhMe).

8 S. Sato, M. Mori, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, **155** C6 (1986).

9 T. Fukuyama, A. A. Laird, and L. M. Hotchkiss, *Tetrahedron Lett.*, **26** 6291 (1985).

10 Wm. Rosenbrook, Jr., D. A. Riley, and P. A. Lartey, *Tetrahedron Lett.*, **26** 3 (1985); G. H. Posner and S. R. Haines, *ibid.*, **26** 935 (1985).

11 K. Saigo, M. Usui, K. Kikuchi, E. Shimada, and T. Mukaiyama, *Bull. Chem. Soc., Japan*, **50** 1863 (1977); H.-J. Koeners, J. Verhoeven, and J. H. van Boom, *Rec. Trav. Chim. Pays-Bas*, **100** 65 (1981).

12 T. Matsumoto, H. Maeta, K. Suzuki, and G. Tsuchihashi, *Tetrahedron Lett.*, **29** 3567 (1988); K. Suzuki, H. Maeta, T. Matsumoto, and G. Tsuchihashi, *ibid.*, **29** 3571 (1988); K. Suzuki, H. Maeta, and T. Matsumoto, *Tetrahedron Lett.*, **30** 4853 (1989).

13 F. A. Cook and P. T. Maichuk, *J. Org. Chem.*, **35** 1940 (1970).

14 J. H. van Boom, and P. M. J. Burgers, *Tetrahedron Lett.*, 4875 (1976), *idem.*, *Recel. Trav. Chim. Pays-Bas*, **97** 73 (1978).

15 J. O. Osby, M. G. Martin, and B. Ganem, *Tetrahedron Lett.*, **25** 2093 (1984).

16 H. Egge, J. Dabrowski, and P. Hanfland, *Pure Appl. Chem.*, **56** 807 (1984).

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