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A TOTAL SYNTHESIS OF FORSSMAN GLYCOLIPID, GLOBOPENTAOSYL CERAMIDE IV^{3}GalNAcαGb$_4$Cer[1)]

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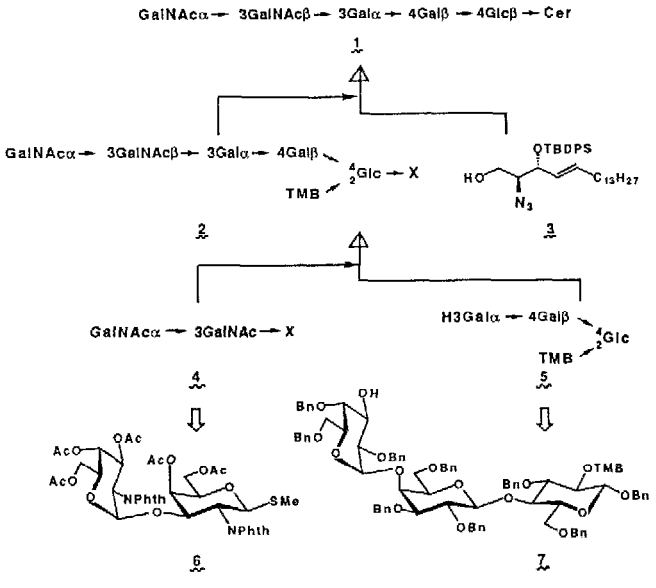
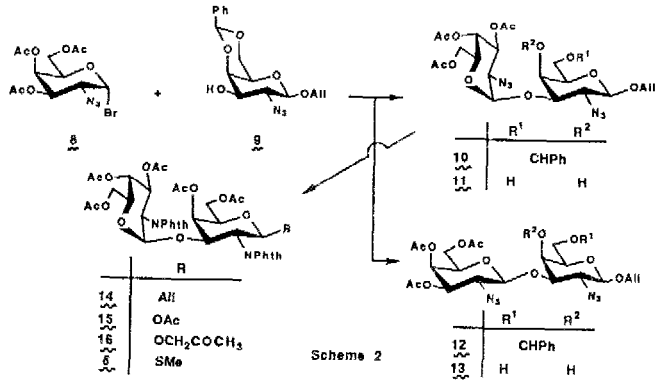
Abstract: A first total synthesis of Forssman antigen, GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer, was achieved in a stereoselective manner by using a glycopentaosyl fluoride as a key glycosyl donor for the crucial coupling with a ceramide equivalent.



Scheme 1 (TMB = 2, 4, 6-trimethylbenzoyl, TBDPS = Bu^{t}Ph$_2$Si)

Forssman antigen that occurs[2] in many species of animals was first described[3] in 1911 and was recently reported[4] as an antigenic marker for a major subpopulation of murine macrophages resided preferentially in spleen and peripheral lymph nodes. The structure of this antigen was proposed[5] in 1971 as globopentaosyl ceramide **1** from enzymic degradation study. In 1982 first synthesis of glycan part of **1** was achieved[6] by Paulsen and Bunsch. We now describe a first total synthesis of **1** in a stereocontrolled manner, the ^{1}H-n.m.r. data of which was found identical with those[7] of natural sample.

A retrosynthetic analysis of **1** led us to design a glycopentaosyl donor **2** that carries a stereocontrolling auxiliary[8], 2,4,6-trimethylbenzoyl group at O-2a, and a reactive glycosyl acceptor, azido alcohol **3**[9], as a ceramide equivalent. Compound **2** was further disconnected into a glycobiosyl donor **4** and a glycotriosyl acceptor **5** which were respectively designed as a thioglycoside **6** and a known[10] glycotrioside **7**.

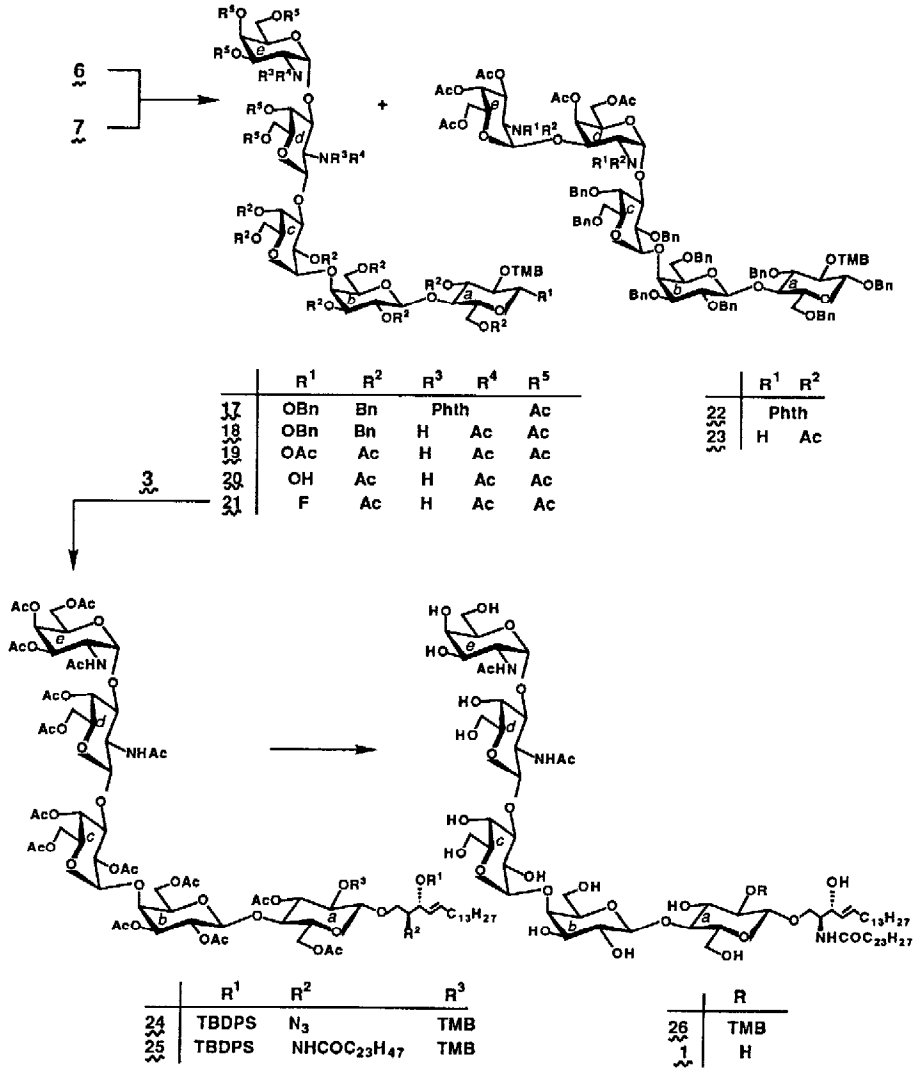


Scheme 2

Preparation of **6** was executed in 7 steps from **9**[11] in 23% overall yield as follows. AgOTf–Powdered molecular sieves 4A (MS4A) promoted glycosylation of **9** with **8**[12] in $(ClCH_2)_2$ gave a 94% yield of an

inseparable 5:1 mixture of **10**[13] and **12**[13] which was directly solvolyzed in 4:1 AcOH–H_2O at 80° and separated by a column of silica gel in 1:1 toluene–EtOAc to give **11**[13] (66%) and **13**[13] (13%). Conversion of **11** into **14**[13] was achieved in 3 steps (1 NaOMe in MeOH for 1 h at 20°, 2 $HS(CH_2)_3SH$[14]–Et_3N in MeOH for 12 h at 20°, 3 phthalic anhydride–Et_3N in Py for 2 h at 75°, then Ac_2O was added and 1 h at 75°, 56% overall). Deallylation of **14** with $PdCl_2$–AcONa in aq. AcOH[15] was found inefficient and gave after acetylation of the products a 1:1 mixture of **15**[13] and **16**[13] in 80% yield. The situation was improved by use of a 1:3 mixture of $(Ph_3P)_3RhCl$–$PdCl_2$ to give a 70% yield of **15** along with a 29% yield of undesired **16**. Treatment of **15** with Bu_3SnSMe and $SnCl_4$ in $(ClCH_2)_2$ for 4 h at 0° afforded thioglycoside **6**[13] in 95% yield.

Having the designed glycobiosyl donor **6** prepared, a key glycosylation of the glycotriosyl acceptor **7** with **6** was performed in CH_3NO_2 in the presence of Bu_4NBr–$CuBr_2$–AgOTf–MS4A[16] for



	R^1	R^2	R^3	R^4	R^5
17	OBn	Bn	Phth		Ac
18	OBn	Bn	H	Ac	Ac
19	OAc	Ac	H	Ac	Ac
20	OH	Ac	H	Ac	Ac
21	F	Ac	H	Ac	Ac

	R^1	R^2
22	Phth	
23	H	Ac

	R^1	R^2	R^3
24	TBDPS	N_3	TMB
25	TBDPS	$NHCOC_{23}H_{47}$	TMB

	R
26	TMB
1	H

Scheme 3

13 h at 20° to give a 78% yield of a 10:1 mixture of desired β-D linked glycopentaoside **17**[13] and the α isomer **22** which were difficult to separate. The mixture was converted into a mixture of acetamido derivatives **18**[13] and **23**[13] in 2 steps (1 $NH_2NH_2 \cdot H_2O$ in EtOH for 16 h at 80°, 2 Ac_2O–DMAP in Py, 93% overall) and then separated by a column of silica gel in 2:1 toluene-acetone. Conversion of **18** into a key glycosyl donor **21** which is equivalent to **2** in scheme 1 was smoothly achieved in 4 steps via **19**[13] and **20**[13] (1 10% Pd–C and H_2 in 7:3 MeOH–H_2O, 2 Ac_2O–DMAP in Py, 3 $NH_2NH_2 \cdot AcOH$ in DMF[17] for 1 h at 20°, 4 DAST[18] in $(ClCH_2)_2$, 63% overall).

Crucial coupling between the key fluoride **21** and azido alcohol **3** was achieved according to Mukaiyama[19] in the presence of $SnCl_2$ and AgOTf in $(ClCH_2)_2$ to give a 23% yield of **24**[13], which was further converted into the target **1**[13] in 5 steps via **25**[13] and **26**[13] (1 Ph_3P in aq. PhH[20] for 19 h at 45°, 2 $C_{13}H_{27}COOH$–2-chloro-1-methylpyridinium iodide–Bu_3N[21] in $(ClCH_2)_2$ for 1 h at 20°, 3 Bu_4NF in THF, 4 1:4 0.1*M* NaOMe–THF for 2 h at 20°, 5 2:1 0.25*M* NaOMe–THF for 3 h at 60°, 67% overall).

In summary, a stereocontrolled total synthesis of globopentaosyl ceramide **1** was achieved for the first time by employing a thioglycoside **6** and a fluoride **20** as two key glycosyl donors, and the natural sample was unambiguously identified with the synthetic **1** through comparison of their 500 MHz ^{1}H-n.m.r. data.

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References and Notes

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13 Physical data for new compounds are compatible with the assigned structures and are given below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were messured for the solution in $CHCl_3$ and $CDCl_3$, respectively,

at 23±3° unless noted otherwise. **1**: $[\alpha]_D$ +46.1° (c 0.02, Py); δ_H (49:1 $(CD_3)_2SO$–D_2O, 60°) 5.543 (td, 6.7 and 15.3 Hz, 5cer), 5.365 (dd, 6.7 and 15.3 Hz, 4cer), 4.824 (d, 3.7 Hz, 1c), 4.751 (d, 3.6 Hz, 1e), 4.564 (d, 7.8 Hz, 1d), 4.278 (d, 7.8 Hz, 1b), 4.179 (d, 7.8 Hz, 1a), 1.844 and 1.834 (2s, 2NAc), 0.854 (t, 6.7 Hz, $2CH_3$). **6**: $[\alpha]_D$ +137° (c 1.0); δ_H 5.961 (dd, 2.8 and 12.2 Hz, 3b), 5.412 (d, 3.7 Hz, 1b), 5.329 (d, 10.3 Hz, 1a); δ_C 94.0 ($^1J_{CH}$ 176 Hz, 1b), 81.1 ($^1J_{CH}$ 156 Hz, 1a). **10** + **12** (5:1 mixture): δ_H 5.593 and 5.581 (2s, in a ratio of 5:1, CHPh), 2.145, 2.050, and 2.044 (3s, 3Ac, **12**), 2.140, 2.063, and 2.038 (3s, 3Ac, **10**). **11**: $[\alpha]_D$ +86.8° (c 1.7); δ_H 5.032 (d, 3.9 Hz, 1b), 4.370 (d, 8.1 Hz, 1a); δ_C 101.5 ($^1J_{CH}$ 160 Hz, 1a), 94.8 ($^1J_{CH}$ 171 Hz, 1b). **13**: $[\alpha]_D$ +29.0° (c 1.4); δ_H 4.574 (d, 7.9 Hz, 1b), 4.357 (d, 7.9 Hz, 1a); δ_C 103.0 ($^1J_{CH}$ 158 Hz, 1b), 101.4 ($^1J_{CH}$ 161 Hz, 1a). **14**: $[\alpha]_D$ +106° (c 0.9); δ_H 5.963 (dd, 3.1 and 12.2 Hz, 3b), 5.394 (d, 3.3 Hz, 1b), 5.324 (d, 8.5 Hz, 1a). **15**: $[\alpha]_D$ +132° (c 1.3); δ_H 6.447 (d, 8.8 Hz, 1a), 5.939 (dd, 2.8 and 12.2 Hz, 3b), 5.412 (d, 3.4 Hz, 1b); δ_C 94.0 ($^1J_{CH}$ 176 Hz, 1b), 90.2 ($^1J_{CH}$ 172 Hz, 1a). **16**: $[\alpha]_D$ +116° (c 1.0); δ_H 6.000 (dd, 3.1 and 12.2 Hz, 3b), 5.388 (d, 3.7 Hz, 1b), 5.271 (d, 8.5 Hz, 1a), 4.183 and 4.116 (2d, 17.4 Hz, OCH_2CO), 2.063, 2.024, 2.007, 2.004, 1.728 and 1.343 (6s, 5Ac and CH_2COMe). **17**: δ_H 6.810 (s, 1.82 H, $PhMe_3H_2$), 5.900 (dd, 3.0 and 12.1 Hz, 3e), 5.462 (d, 8.0 Hz, 1d), 5.390 (dd, 8.1 and 9.5 Hz, 2a), 5.357 (d, 3.6 Hz, 1e), 4.795 (d, 3.6 Hz, 1c), 4.448 (d, 7.7 Hz, 1b); δ_C 103.0 ($^1J_{CH}$ 163 Hz, 1b), 100.1 ($^1J_{CH}$ 170 Hz, 1c), 99.9 ($^1J_{CH}$ 163 Hz, 1a), 99.7 ($^1J_{CH}$ 163 Hz, 1d), 93.9 ($^1J_{CH}$ 175 Hz, 1e). **22**: δ_H 6.748 (s, 0.18 H, $PhMe_3H_2$), 5.965 (dd, 3.0 and 12.0 Hz, 3e). **18**: $[\alpha]_D$ +31.8° (c 1.1); δ_H 6.794 (s, $PhMe_3H_2$), 5.386 (dd, 7.9 and 9.5 Hz, 2a), 5.307 (d, 2.1 Hz, 4e), 5.173 (d, 2.0 Hz, 4d); δ_C 103.0 ($^1J_{CH}$ 163 Hz, 1b), 102.2 ($^1J_{CH}$ 162 Hz, 1d), 100.0 ($^1J_{CH}$ 172 Hz, 1c), 99.9 ($^1J_{CH}$ 162 Hz, 1a), 99.2 ($^1J_{CH}$ 170 Hz, 1e). **23**: $[\alpha]_D$ +28.9° (c 1.0); δ_H 6.820 (s, $PhMe_3H_2$), 5.366 (dd, 7.8 and 9.5 Hz, H-2a), 5.104 (d, 2.4 Hz, H-4e or d); δ_C 102.92 ($^1J_{CH}$ 163 Hz, 1b), 102.89 ($^1J_{CH}$ 172 Hz, 1d), 101.0 ($^1J_{CH}$ 170 Hz, 1c), 100.0 ($^1J_{CH}$ 163 Hz, 1a), 96.9 ($^1J_{CH}$ 170 Hz, 1e). **19**: δ_H 6.843 (s, $PhMe_3H_2$), 6.470 (d, 3.7 Hz, 0.5 H, 1aα), 5.711 (d, 8.3 Hz, 0.5 H, 1aβ). **20**: δ_H 6.854 and 6.843 (2s, in a ratio of 1:3, $PhMe_3H_2$). **21**: δ_H 6.860 (s, $PhMe_3H_2$), 5.880 (dd, 0.3 H, 2.7 and 52.8 Hz, 1aα), 5.472 (dd, 0.7 H, 5.8 and 52.8 Hz, 1aβ), 5.611 (d, 0.3 H, 2.8 Hz, 4cα), 5.592 (d, 0.7 H, 3.6 Hz, 4cβ). **24**: $[\alpha]_D$ +46.5° (c 0.2); δ_H 6.822 (s, $PhMe_3H_2$), 6.571 (d, 10.1 Hz, NH), 6.411 (d, 7.0 Hz, NH), 5.606 (d, 3.0 Hz, 4c), 1.050 (s, Bu^t), 0.881 (t, 6.7 Hz, CH_3). **25**: $[\alpha]_D$ +17.0° (c 0.1); δ_H 6.811 (s, $PhMe_3H_2$), 6.578 (d, 9.7 Hz, NH), 6.550 (d, 8.4 Hz, NH), 5.608 (d, 2.2 Hz, 4c), 5.426 (d, 10.0 Hz, NH), 5.114 (dd, 8.3 and 10.8 Hz, 2a), 1.005 (s, Bu^t), 0.879 (t, 6.6 Hz, $2CH_3$). **26**: $[\alpha]_D$ +32° (c 0.05, Py); δ_H (49:1 $(CD_3)_2SO$-D_2O, 60°), 6.854 (s, $PhMe_3H_2$), 5.518 (td, 7.1 and 15.4 Hz, 5cer), 5.352 (dd, 6.7 and 15.4 Hz, 4cer), 4.849 (t, 9.0 Hz, 2a), 4.793 (d, 3.7 Hz, 1c), 4.731 (d, 4.0 Hz, 1e), 4.552 (d, 8.5 Hz, 1d), 4.544 (d, 8.2 Hz, 1a), 2.232 (s, 3PhMe), 1.826 and 1.831 (2s, 2NAc), 0.843 (t, 7.0 Hz, $2CH_3$).

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