Tetrahedron Letters,Vol.29,No.44,pp 5681-5684,1988 0040-4039/88 $3.00 + .C
Printed in Great Britain Pergamon Press plc

A TOTAL SYNTHESIS OF A STAGE SPECIFIC EMBRYONIC ANTIGEN-3 (SSEA-3), GLOBOPENTAOSYL CERAMIDE, IV^{3}GalGb$_4$Cer. USE OF 2,4,6-TRIMETHYLBENZOYL GROUP AS A STEREOCONTROLLING AUXILIARY[1)]

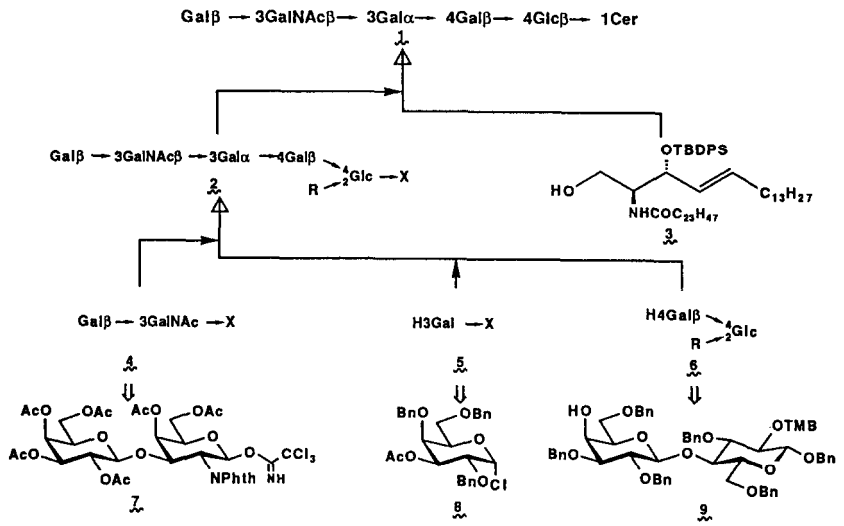
Shigeki Nunomura, and Tomoya Ogawa*
RIKEN (The Institute of Physical and Chemical Research),
Wako-shi, Saitama, 351-01 Japan

Abstract: A first total synthesis of a SSEA-3, Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→ 3Cer, was achieved in an efficient way by using a key glycopentaosyl glycosyl donor that carried a stereocontrolling auxiliary at O-2a.

Globopentaosyl ceramide **1** has been isolated from green monkey kidney[2)], human teratocarcinoma[3)] as well as from human kidney[4)], and was chemically[3)] characterized. **1** showed a strongest reactivity[3)] to a monoclonal antibody directed to 4- to 8-cell stage of murine embryos and has been known[5)] as the stage-specific embryonic antigen 3 (SSEA-3). Cell surface glycolipids such as **1** are claimed to play significant roles as differentiation antigens[6)] during the course of embryogenesis. We describe here a first total synthesis of **1**.

Retrosynthetic analysis of **1** led us to design a glycopentaosyl donor **2** with a stereocontrolling auxiliary[7)] at O-2a and a ceramide derivative **3**[8)]. Compound **2** may be constructed by elongation of glycan chains using two glycosyl donors **4** and **5**, and a glycosyl acceptor **6**. Synthons **4** and **5** may be designed as **7** and **8**, respectively, in order to give with high stereoselectivity either 1,2-trans or 1,2-cis glycosides. Properly protected lactose derivative **9**, a synthetic equivalent of **6**, was designed so that 2,4,6-trimethylbenzoyl group at O-2a of **9** should be functional later at the crucial coupling step between **2** and **3**. It is to be noted that benzoyl[9)] group has already been claimed to have definite advantage over acetyl group as an O-2 stereocontrolling auxiliary for 1,2-trans glycosylation.

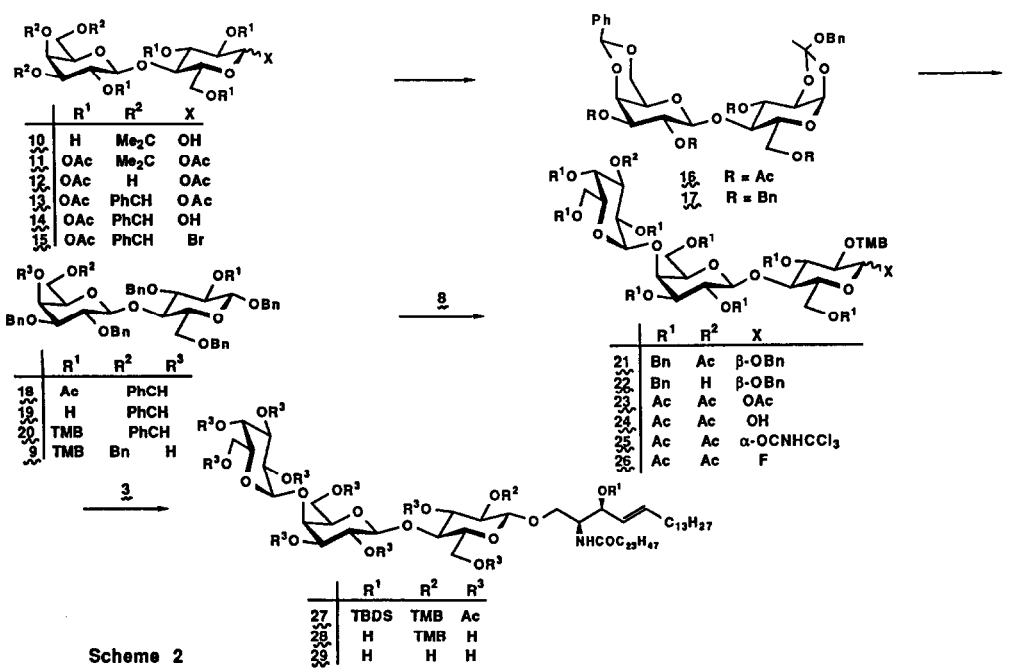
A practical route for the synthesis of a glycosyl acceptor **9** is developed as follows. The



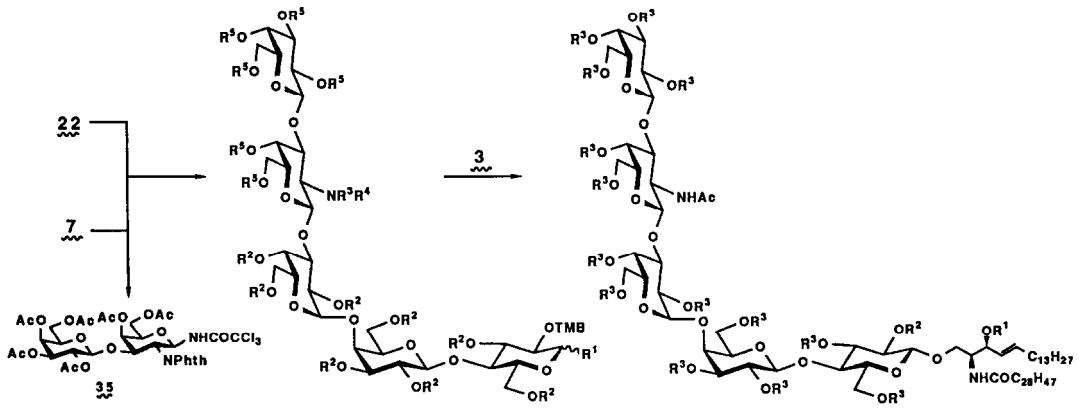
Scheme 1 (TMB = 2, 4, 6-trimethylbenzoyl, TBDPS = $tBuPh_2Si$)

readily available[10] isopropylidene derivative **10** of lactose was converted into bromide **15**[11] in 5 steps via **11-14** (1 Ac_2O in Py, 2 80% AcOH, 3 PhCH(OMe)$_2$-TsOH in CH_3CN, 4 NH_2NH_2•AcOH in DMF[12], 5 CBr_4-$(Me_2N)_3P$[13] in THF, 46% overall). Conversion of **15** into **9** was achieved via orthoester **17** in 7 steps (1 BnOH-n-Bu_4NBr-Et_3N in CH_2Cl_2, 2 NaOMe in MeOH, 3 NaH-BnBr in DMF, 4 TMSOTf-MS4A in $(CH_2Cl)_2$[14], 5 NaOMe in MeOH, 6 TMB chloride-DMAP in Py, 7 BH_3•NMe_3-$AlCl_3$ in THF[15], 47% overall). Having the key glycosyl acceptor **9** prepared, AgOTf-MS4A promoted glycosylation of **9** with a galactosyl donor **8**[7] smoothly afforded the expected product **21** (78%). In order to examine the efficiency of TMB auxiliary at O-2a, conversion of **21** into two glycotriaosyl donors **25** (68% overall) and **26** (81% overall) was performed via **23** and **24** in 4 steps, respectively (1 Pd-C, H_2 in 7:3 MeOH-H_2O, 2 Ac_2O-DMAP in Py, 3 NH_2NH_2•AcOH in DMF, 4 Cl_3CN-DBU in $(CH_2Cl)_2$[16], or DAST in $(CH_2Cl)_2$[17]. Glycosylation of a ceramide derivative **3** with either **25** or **26** was examined in the presence of TMSOTf-MS4A in $(CH_2Cl)_2$ or $SnCl_2$[18]-AgOTf-MS4A in $(CH_2Cl)_2$ to give **27** (27 or 40%, respectively), which was smoothly deblocked via **28** to **29** (1 n-Bu_4NF in THF, 2 0.1M NaOMe-MeOH-THF, 20°, 3 0.5M NaOH in MeOH-THF, 50°, 85% overall). These results clearly proved that as a stereocontrolling auxiliary at O-2a for 1,2-trans glycosylation TMB was as functional as pivaloyl which was previously developed[7]. Therefore, further experiments directed toward a total synthesis of **1** was pursued as follows.

Compound **21** was deacetylated to a glycosyl acceptor **22** which was glycosylated by a readily obtainable **7**[19] to give **30** (BF_3•OEt_2-MSAW300 in $(CH_2Cl)_2$, 22% based on **7**). A major by-product derived from **7** was identified as **35** (44%). Conversion of **30** into a glycopentaosyl imidate **34** was performed in a conventional way via **31-33** in 6 steps (1 NH_2NH_2•H_2O in EtOH, 2 Ac_2O in Py, 3 10% Pd-C and H_2 in 7:3 MeOH-H_2O, 4 Ac_2O-DMAP in Py, 5 NH_2NH_2•AcOH in DMF, 6 Cl_3CCN-DBU in $(CH_2Cl)_2$, 60% overall). A crucial coupling between **3** and **34** was achieved to afford **36**(TMSOTf-MSAW300 in $(CH_2Cl)_2$, 33%), which was deblocked to give the target **1** (1 n-Bu_4NF in THF, 2 NaOMe in MeOH, 3 NaOH in H_2O-MeOH, 55% overall). ^1H Nmr data of synthetic **1** was in complete



Scheme 2



	R^1	R^2	R^3	R^4	R^5
30	β-OBn	Bn	Phth		Ac
31	β-OBn	Bn	NHAc		Ac
32	Ac	Ac	NHAc		Ac
33	OH	Ac	NHAc		Ac
34	α-OCNHCCl$_3$	Ac	NHAc		Ac

	R^1	R^2	R^3
36	TBDS	TMB	Ac
1	H	H	H

Scheme 3

agreement with those reported for natural sample[3].

In conclusion, a first total synthesis of a SSEA-3, globopentaosyl ceramide **1**, was successfully achieved by employing TMB as a stereocontrolling auxiliary at O-2a in the key glycosyl donor **34**.

Acknowledgments. This work was partly supported by Special Coordination Funds of the Science and Technology Agency of the Japanese Government. We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the NMR spectra and Dr. H. Yamazaki and his staff for the elemental analyses. We also thank Ms. A. Takahashi and Ms. K. Moriwaki for their technical assistance.

Rererences and Notes

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11) Physical data for new 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. **1**: $[\alpha]_D$ +9.5° (c 0.1, Py); δ_H(99:1

DMSOd6-D2O, 60°) 5.539 (dt, 15.0, 6.7 Hz, 5cer), 5.362 (dd, 15.5, 7.0 Hz, 4cer), 4.833 (d, 3.9 Hz, 1c), 4.642 (d, 8.1 Hz, 1d), 4.285 (d, 7.3 Hz, 1b), 4.233 (d, 7.6 Hz, 1e), 4.171 (d, 7.8 Hz, 1a), 1.826 (s, NAc). **9**: $[\alpha]_D$ +9.1° (c 0.7); δ_H 5.417 (dd, 7.8, 9.5 Hz, 2a), 4.569 (d, 7.8 Hz, 1b), 4.454 (d, 7.8 Hz, 1a), 2.245 (s, Me), 2.119 (s, 2Me); δ_C 102.5 (160 Hz, 1b), 100.0 (160 Hz, 1a). **11**: α:β=1:13, δ_H 6.253 (d, 0.07 H, 3.9 Hz, 1aα), 5.674 (d, 0.93 H, 8.0 Hz, 1aβ), 1.422, 1.370 (2 s, CMe_2). **12**: α:β=1:4, δ_H 6.248 (d, 0.2 H, 3.5 Hz, 1aα), 5.692 (d, 0.8 H, 8.1 Hz, 1aβ) **13**: α:β=3:2, δ_H 6.263 (d, 0.6 H, 3.6 Hz, 1aα), 5.681 (d, 0.4 H, 8.5 Hz, 1aβ). **14**: $[\alpha]_D$ +82.0° (c 1.3); δ_H 5.474 (s, CHPh). **15**: $[\alpha]_D$ +134° (c 0.5); δ_H 6.544 (d, 4.2 Hz, 1a), 5.566 (t, 9.7 Hz, 3a), 5.481 (s, CHPh), 5.284 (dd, 7.8, 10.3 Hz, 2b), 4.495 (d, 7.8 Hz, 1b), 2.134, 2.092, 2.067, 2.063, 2.045 (5s, 5Ac). **16**: $[\alpha]_D$ +31.0° (c 1.7); δ_H 5.503 (s, CHPh), 5.372 (dd, 8.0, 10.2 Hz, 2b), 4.640 (d, 8.0 Hz, 1b), 2.102, 2.090, 2.072, 2.068 (4s, 4OAc), 1.797 (s, CMe). **17**: $[\alpha]_D$ +13.0° (c 1.1); δ_H 5.697 (d, 5.1 Hz, 1a), 5.482 (s, CHPh), 4.317 (d, 7.8 Hz, 1b), 1.729 (s, CMe). **18**: $[\alpha]_D$ -4.6° (c 1.0); δ_H 5.437 (s, CHPh), 5.055 (dd, 8.0, 9.5 Hz, 2a), 4.447 (d, 7.8 Hz, 1b), 4.400 (d, 8.0 Hz, 1a), 1.942 (s, Ac). **19**: $[\alpha]_D$ +3.9° (c 1.2); δ_H 5.454 (s, CHPh), 4.451 (d, 7.8 Hz, 1b), 4.371 (d, 7.6 Hz, 1a). **20**: $[\alpha]_D$ -7.3° (c 0.6); δ_H 5.427 (dd, 7.8, 8.1 Hz, 2a), 5.417 (s, CHPh), 4.576 (d, 8.1 Hz, 1b), 4.515 (d, 7.8 Hz, 1a), 2.253 (s, Me), 2.120 (s, 2Me). **21**: $[\alpha]_D$ +50.0° (c 1.1); δ_H 5.356 (dd, 8.0, 9.4 Hz, 2a), 5.241 (dd, 2.9, 10.7 Hz, 3c), 5.052 (d, 3.4 Hz, 1c), 2.255 (s, Me), 2.093 (s, 2Me), 1.795 (s, Ac); δ_C 102.9 (161 Hz, 1b), 99.9 (158 Hz, 1a), 99.9 (173 Hz, 1c). **22**: $[\alpha]_D$ +23.0° (c 1.4); δ_H 5.384 (dd, 8.1, 9.5 Hz, 2a), 5.049 (d, 3.5 Hz, 1c), 2.249 (s, Me), 2.072 (s, 2Me); δ_C 102.5 (160 Hz, 1b), 100.0 (159 Hz, 1a), 99.4 (166 Hz, 1c). **23**: α:β=1:1, δ_H 6.462 (d, 0.5 H, 3.7 Hz, 1aα), 5.697 (d, 0.5 H, 8.3 Hz, 1aβ); δ_C 101.2, 100.9 (1b), 99.60, 99.63 (1c), 91.9 (161 Hz, 1aβ), 88.7 (178 Hz, 1aα). **24**: $[\alpha]_D$ +75.0° (c 0.7). **25**: δ_H 8.721 (s, NH), 6.807 (s, 2ArH), 6.693 (d, 3.6 Hz, 1a), 5.638 (dd, 9.1, 10.5 Hz, 3a), 5.581 (dd, 2.2, 4.0 Hz, 4c), 4.998 (d, 3.7 Hz, 1c). **26**: α:β=1:7; $[\alpha]_D$ +70.0° (c 1.0); δ_H 5.853 (dd, 0.13 H, 3.3, 52.8 Hz, 1aα), 5.574 (d, 3.2 Hz, 4c), 5.487 (dd, 0.87 H, 5.2, 52.8 Hz, 1aβ), 4.989 (d, 3.3 Hz, 1c). **27**: $[\alpha]_D$ +17.0° (c 1.0); δ_H 6.803 (s, 2ArH), 5.584 (d, 2.4 Hz, 4c), 4.992 (d, 3.4 Hz, 1c). **28**: $[\alpha]_D$ +15.0° (c 0.5, Py); δ_H(99:1 DMSOd6-D2O) 6.856 (s, 2ArH), 4.873 (t, 8.4 Hz, 2a), 4.794 (d, 3.3 Hz, 1c), 4.572 (d, 7.4 Hz, 1b), 4.308 (d, 7.9 Hz, 1a). **29**: identical with the authentic sample, for the reference please see, K. Koike, M. Sugimoto, S. Sato, Y. Ito, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, **163**, 189 (1987). **30**: $[\alpha]_D$ +15.0° (c 0.7); δ_H 5.409 (dd, 7.8, 9.5 Hz, 2a), 5.333 (d, 2.8 Hz, 4d), 2.284 (s, Me), 2.166 (s, 2Me), 2.108, 2.066, 2.032, 1.909, 1.869, 1.443 (6Ac); δ_C 103.0 (1b), 100.7 (1e), 100.2 (1c), 99.9 (1a,d). **31**: $[\alpha]_D$ +15.0° (c 0.6); δ_H 5.409 (dd, 7.8, 9.5 Hz, 2a), 5.338 (d, 3.4 Hz, 4d); δ_C 103.0 (1b), 102.0 (1d), 100.9 (1e), 99.8 (1a,c). **32**: α:β=1:1; δ_H 6.853 (s, 2ArH), 6.472 (d, 0.5 H, 3.7 Hz, 1a). **33**: $[\alpha]_D$ +72.0° (c 1.5). **34**: $[\alpha]_D$ +57.0° (c 0.6); δ_H 8.750 (s, =NH), 6.835 (s, 2ArH), 6.701 (d, 3.7 Hz, 1a). **35**: $[\alpha]_D$ +37.0° (c 1.0); δ_H 5.701 (t, 9.5 Hz, 1a), 5.578 (d, 3.2 Hz, 4a), 5.275 (dd, 0.7, 3.1 Hz, 4b), 4.519 (d, 7.8 Hz, 1b). **36**: $[\alpha]_D$ +28.0° (c 0.8); δ_H 6.824 (s, 2ArH), 1.005 (s, tBu), 0.881 (t, 6.4 Hz, $2CH_2CH_3$).

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(Received in Japan 29 August 1988)