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TOTAL SYNTHESIS OF A STAGE SPECIFIC EMBRYONIC ANTIGEN-1 (SSEA-1) A GLYCOHEPTAOSYL CERAMIDE $V^3FucnLc_6Cer$[1)]

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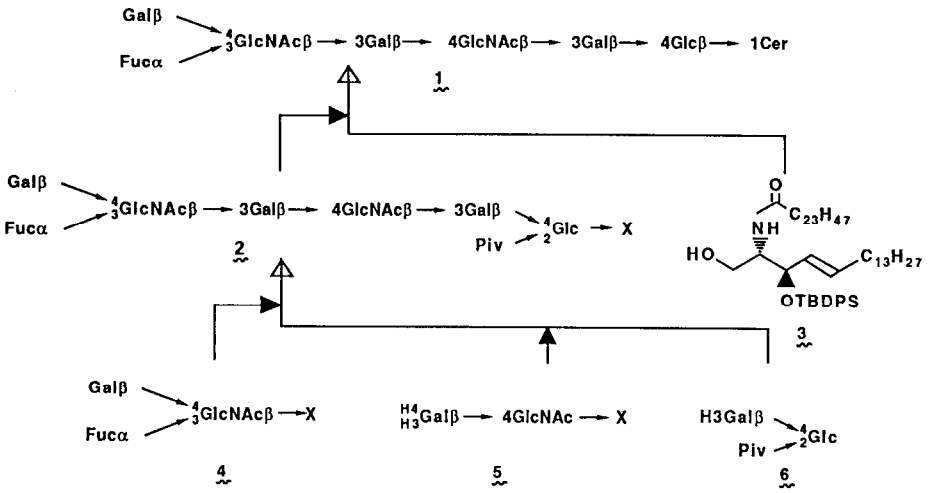
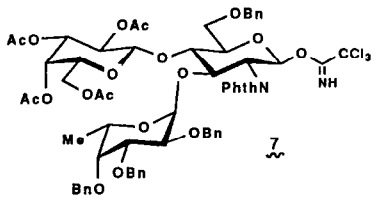
Abstract: Total synthesis of SSEA-1 glycoheptaosyl ceramide was for the first time achieved in a regio- and stereocontrolled manner using pivaloyl group as a stereocontrolling auxiliary.

Carbohydrate structures defined by a monoclonal antibody to murine stage specific embryonic antigen 1 (SSEA-1) appear at the late 8-cell stage of the mouse embryo[2)]. SSEA-1 may also be expressed in early stages of human development. A fucolipid **1**, one of the fucolipids which are reactive with anti-SSEA-1 antibody, has been isolated from human erythrocyte stroma[3)], adenocarcinoma tissue[4)], human granulocytes[5)], and chemically characterized. The increased expression of SSEA-1 is an oncodevelopmental marker[4)] for human adenocarcinoma and may be a preneoplastic change in human colon.



Scheme 1 (Piv. = pivaloyl)

Since only scarce amount of SSEA-1 were available from natural sources, development of a practical total synthesis route should be a prerequisite to supply **1** in a reasonable amount for biological studies. According to the strategy developed in a preceding paper[1)], a glycoheptaosyl donor **2** was designed to have O-2a protected with pivaloyl group (scheme 1). **2** was retrosynthesized into two glycosyl donors **4** and **5**, and a glycosyl acceptor **6**, which were,



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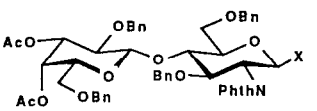
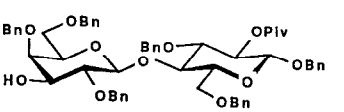


8 X = $OCNHCCl_3$
9 X = SMe



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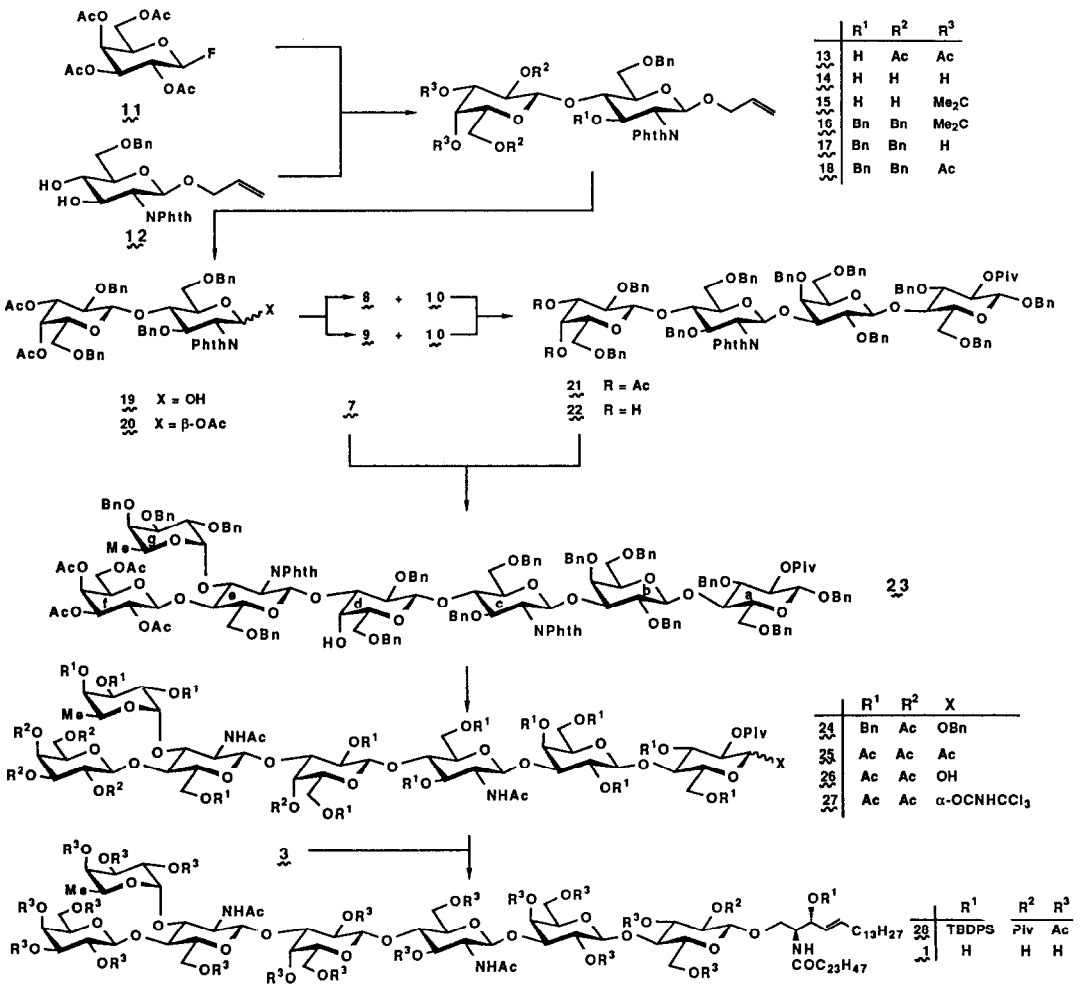
respectively, designed as properly functionalized synthons **7**, **8** or **9**, and **10**. **7** was a most suitable synthon for a regioselective elongation[6] of the glycotriaosyl unit at O-3 in 3,4-diol of galactopyranosyl residues and **10** was designed[1] so that a smooth coupling between glycan chains and protected ceramides should be achieved. Both the non-reducing end glycotriaosyl, (X-determinant) synthon **7**[6] and the reducing end glycobiosyl synthon **10**[1] have already been reported. Hence, synthesis of internal glycobiosyl synthons **8** and **9** was first pursued.

Glycosylation of diol **12**[6] with galactopyranosyl fluoride **11** under Mukaiyama condition[7] ($SnCl_2$-AgOTf-MS4A in 5:1 $(ClCH_2)_2$-toluene) afforded a β(1→4) disaccharide **13**[6] (64%) along with a β(1→3) (23%) and an α(1→3) (1%) isomers. Conversion of **13** into lactol **19**[8] was carried out in 6 steps (1. NaOMe-MeOH, 2. $Me_2C(OMe)_2$-TsOH in DMF; then Amberlist 15 in MeOH, 3. BnBr-Ag_2O-KI in DMF, 4. TFA-H_2O-THF, 5. Ac_2O-DMAP in Py, 6. $PdCl_2$-NaOAc in aq. $AcOH$[9]), 41% overall) via **14**-**18**. Treatment of **19** with DBU and Cl_3CCN in CH_2Cl_2 gave imidate **8**[8] (72%). On the other hand, acetylation of **19** and S-glycosylation of **20**[8] with $MeSSnBu_3$-$SnCl_4$[10] in $(ClCH_2)_2$ gave **9**[8] (62%). Being the key synthons **7**, **8**, **9**, and **10** available, crucial condensations of these synthons were



Scheme 2

next examined. $BF_3 \cdot OEt_2$ promoted glycosylation of **10** with **8** in $(ClCH_2)_2$ according to Schmidt[11] afforded a tetrasaccharide **21**[8] (38%). **21** could be obtained in a somewhat higher efficiency by use of the thioglycoside **9**. Thus, $CuBr_2$-Bu_4NBr-AgOTf-MS4A[12] promoted glycosylation of **10** with **9** in CH_3NO_2 gave a slightly improved yield of **21** (51%) while use of MeOTf-MS4A[13] in $(ClCH_2)_2$ gave a 43% yield of **21**. The obtained yields are moderate but reasonable due to the steric impediment[14] for the glycosylation at O-3 of a 2,4,6-tri-O-benzyl-galactopyranosyl system. Use of more sterically accessible glycosyl acceptors, 3,4-diol of a 2,6-di-O-benzyl-galactopyranosyl system, would lead to the formation of a regioisomeric mixture[15] upon reaction with lactosaminyl donors.

Deacetylation of **21** to the 3,4-diol **22**[8] and $BF_3 \cdot OEt_2$ promoted glycosylation of **22** with the glycotriaosyl imidate **7** afforded a $\beta(1\rightarrow3)$ linked glycoheptaoside **23**[8] as a sole regioisomer (77%, overall), in agreement with our previous observation[6]. Conventional transformation of **23** into a glycosyl donor **27**[8] was achieved in 6 steps (1. 2% $NH_2NH_2 \cdot H_2O$ in EtOH, 2. Ac_2O-DMAP in Py, 3. 10% Pd-C, H_2 in MeOH-AcOH, 4. Ac_2O-DMAP in Py, 5. $NH_2NH_2 \cdot AcOH$ in DMF[16], 6. Cl_3CCN-DBU in $(ClCH_2)_2$, 51% overall) via **24**, **25** and **26**. Crucial glycosylation of a protected ceramide **3** with the α-imidate **27** under a standard condition[11], $BF_3 \cdot OEt_2$-MSAW300 in $(ClCH_2)_2$, afforded a 28% yield of a completely protected glycoheptaosyl ceramide **28**[8] which was deblocked to give **1** in 2 steps (1. Bu_4NF in THF-MeOH, 2. NaOMe in THF-MeOH, 65% overall). The ^1H-nmr data for synthetic **1** was in good agreement with those of the natural **1**[17].

In conclusion, suitably designed key glycosyl synthons **7**, **9**, and **10** were successfully employed for the first total synthesis of SSEA-1 glycoheptaosyl ceramide **1** with a high regio- and stereocontrol.

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Reference and Notes

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8) Physical data for key compounds are described below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were measured for $CHCl_3$ and $CDCl_3$ solutions, respectively, at 25°, unless noted otherwise. **1**: $[\alpha]_D$ -26.6° (c 1.2, MeOH); δ_H(99:1, DMSOd_6-D_2O, 60°) 5.556 (dt, 15.2, 6.4 Hz, H-5cer), 5.371 (dd, 8.5, 15.2 Hz, H-4cer), 4.883 (d, 3.7 Hz, H-1g), 4.748 (d, 7.6 Hz, H-1e), 4.681 (d, 8.5 Hz, H-1c), 4.583 (q, 7.3 Hz, H-5g), 4.3 (3d, overlapped, H-1bdf), 4.174 (d, 7.6 Hz, H-1a), 3.060 (t, 7.9 Hz, H-2a). **8**: $[\alpha]_D$ +31.2° (c 1.0); δ_H 8.542 (s, NH), 6.403 (d, 8.5 Hz, H-1a), 5.360 (d, 2.7 Hz, H-4b). **9**: $[\alpha]_D$ +24.5° (c 1.3); δ_H 5.361 (d, 2.4 Hz, H-4b), 5.132 (d, 10.1 Hz, H-1a), 4.873 (dd, 3.6, 10.1 Hz, H-3b), 4.545 (d, 7.6 Hz, H-1b), 2.127 (s, SMe); δ_C 102.5 (1b), 54.1 (2a), 11.0 (SMe). **14**: $[\alpha]_D$ -9.4° (c 1.3); δ_C 103.5 (1b), 97.4 (1a), 56.3 (2a). **15**: $[\alpha]_D$ +6.5° (c 0.8); δ_H 5.224 (d, 8.2 H, H-1a), 4.239 (d, 8.2 Hz, H-1b), 1.496, 1.326 (2s, CMe_2); δ_C 110.0 (CMe_2), 102.9 (1b), 97.3 (1a), 56.0 (2a). **16**: $[\alpha]_D$ +47.8° (c 0.6); δ_H 5.149 (d, 8.5 Hz, H-1a), 4.413 (d, 7.9 Hz, H-1b), 1.369, 1.331 (2s, CMe_2); δ_C 109.6 (CMe_2), 102.2 (1b), 97.5 (1a), 55.7 (2a). **17**: $[\alpha]_D$ +27.8° (c 1.3); δ_H 3.956 (d, 1.8 Hz, H-4b); δ_C 102.9 (1b), 97.5 (1a), 55.8 (2a). **18**: $[\alpha]_D$ +16.5° (c 0.8); δ_H 5.353 (d, 2.7 Hz, H-4b), 5.153 (d, 8.2 Hz, H-1a), 4.861 (dd, 3.6, 10.4 Hz, H-3b), 4.505 (d, 7.9 Hz, H-1b); δ_C 102.5 (1b), 97.5 (1a), 55.6 (2a). **19**: $[\alpha]_D$ +28.0° (c 0.8); δ_H 5.355 (d, 3.4 Hz, H-4b), 4.839 (dd, 3.4, 10.1 Hz, H-3b); δ_C 102.6 (1b), 93.0 (1a), 57.5 (2a). **20**: $[\alpha]_D$ +22.6° (c 0.8); δ_H 6.282 (d, 8.8 Hz, H-1a), 5.344 (d, 3.4 Hz, H-4b), 4.805 (dd, 3.7, 10.1 Hz, H-3b); δ_C 102.4 (1b), 90.4 (1a), 54.6 (2a). **21**: $[\alpha]_D$ -7.5° (c 0.7); δ_H 5.380 (d, 8.5 Hz, H-1c), 5.366 (d, 2.5 Hz, H-4d), 5.021 (dd, 8.2, 9.2 Hz, H-2a), 4.900 (dd, 3.4, 10.1 Hz, H-3d), 1.958, 1.947 (2 s, 2 Ac), 1.087 (s, CMe_3). δ_C 102.5 (1bd), 99.7 (1ac). **22**: $[\alpha]_D$ -5.3° (c 0.6); δ_H 5.379 (d, 8.5 Hz, H-1c), 5.017 (dd, 7.9, 9.4 Hz, H-2a), 4.119 (dd, 8.5, 10.1 Hz, H-2c), 1.084 (s, CMe_3); δ_C 103.0 (1d), 102.4 (1b), 99.7 (1ac). **23**: $[\alpha]_D$ -6.6° (c 1.1); δ_H 5.327 (d, 8.2 Hz, H-1e), 5.244 (d, 2.7 Hz, H-4g), 5.189 (d, 7.9 Hz, H-1c); δ_C 102.5 (1bd), 99.6 (1acef), 97.5 (1g). **24**: $[\alpha]_D$ -22.5° (c 0.5); δ_H 5.480 (d, 3.3 Hz, H-4d), 5.278 (d, 3.0 Hz, H-4g), 2.027, 1.945, 1.938, 1.932, 1.848, 1.517, 1.434 (7 s, 7 Ac), 1.156 (d, 6.4 Hz, H-6f), 1.119 (s, CMe_3). **25**: α:β=1:1, δ_H 6.291 (d, 0.5 H, 3.6 Hz, H-1aα), 5.695 (d, 0.5 H, 8.5 Hz, H-1aβ). **26**: $[\alpha]_D$ -6.2° (c 0.5). **27**: $[\alpha]_D$ +4.3° (c 0.5); δ_H 8.651 (s, NH), 6.501 (d, 3.6 Hz, H-1a). **28**: $[\alpha]_D$ -20.0° (c 1.2); δ_H 4.657, 4.614, 4.416 (d, J 7.9 Hz, H-1 x 3), 1.252 (s, CMe_3), 1.122 (s, CMe_3).

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