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# A Total Synthesis of a Stage Specific Pentasaccharide Embryogenesis Marker

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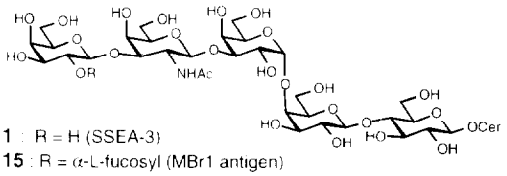
**Abstract:** A thioglycoside coupling mediated by methyl triflate was used to generate Stage Specific Embryonic Antigen-3.

Among the markers for the progression of embyrogenesis are alterations in cell surface carbohydrate expression patterns.[1,2] An interesting example of this is the degree of expression of galactosyl globoside **1**. This compound is found in kidney tissue and, also, in human teratocarcinoma cells.[3,4] Moreover, its degree of expression seems to be correlated with the development of the embryogenesis process.[5] In recognition of this property, **1** is also referred to the Stage Specific Embryonic Antigen-3 (SSEA-3). It has also been reported that changes in the expression of SSEA-3 occur during the course of differentiation of human teratocarcinoma cells. Antibodies which recognize galactosyl globoside *per se* or as substructures of a larger oligosaccharide domain have been found. The heavy and light chain sequences of such antibodies are now known.[6,7]

**Scheme 1**: SSEA-3 and MBr1 antigen

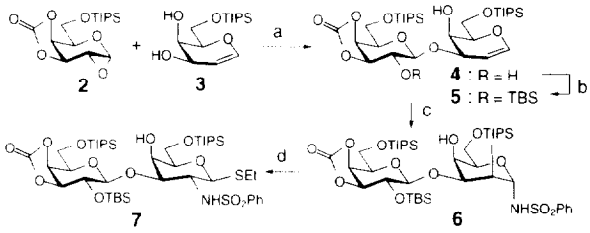



**1** : R = H (SSEA-3)
**15** : R = α-L-fucosyl (MBr1 antigen)

We looked upon the synthesis of SSEA-3 as an opportunity to further explore and develop the strategies of glycal assembly and azaglycosylation[8] in generating N-acetylamino containing oligosaccharides. The total synthesis of an SSEA-3 itself, containing a C 24 fatty acid side chain was reported by Ogawa in 1988 in a rather lengthy process.[9] The problem has been taken up very recently by Magnussen[10] who reported a synthesis of the deprotected carbohydrate portion of the compound, but without an attached ceramide. We looked upon SSEA-3 as an attractive target to probe antibody-carbohydrate-antigen specificity, as well as a testing ground for the power of the glycal assembly methodology. Our total synthesis of SSEA-3 is described in this Letter.

The disaccharide **4** was assembled from building blocks **2** and **3** following now well established principles in glycal assembly. The glycal linkage in **5** was subjected to iodosulfonamidation to produce adduct **6**. It had been hoped that compound **6** itself would function as a donor system with acceptor **10** (*vide infra*). However as will be seen, this possibility could not be realized in our hands. Accordingly, compound **6** was converted to **7** through the action of lithium ethanethiolate.

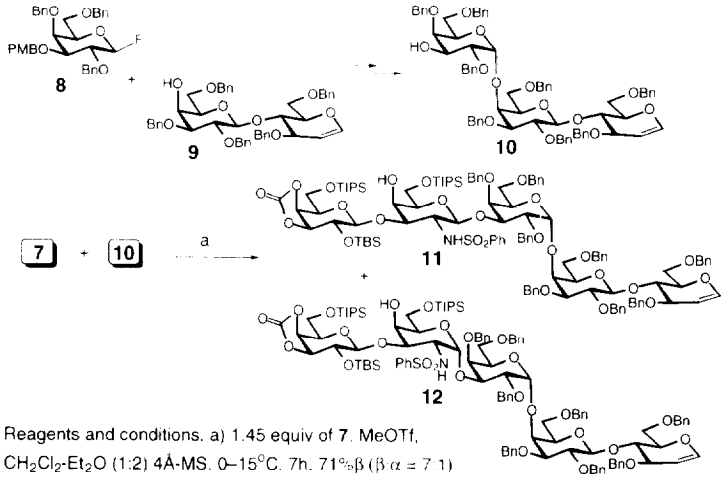
**Scheme 2**



Reagents and conditions, a) $ZnCl_2$, THF, 87%, b) TBSOTf, $Et_3N$, DMAP, $CH_2Cl_2$, -40–0°C, 76%; c) $I(coll)_2ClO_4$, $PhSO_2NH_2$,4Å- MS, THF, 0°C, 62% (α:β = 4:1); d) EtSH, LHMDS, DMF, -40°–rt, 56%

The glycosyl acceptor **10** was assembled from building blocks **8** and **9**, as shown. Unfortunately, reaction of **6** and tributyltin derivative of **10** under standard azaglycosylation conditions was not successful. Apparently, this failure is a consequence of a mismatch in the glycosyl donor and glycosyl acceptor in the putative coupling since related couplings do occur.[8a-d] The failure to achieve this coupling gave us another opportunity to explore the usefulness of the 1β thioethyl 2α phenylsulfonamido donor arrangement in **7**. In the event, reaction of **7** and **10**, under the conditions shown, gave a 61% yield of **11**,[12] accompanied by approximately 10% of the α anomer **12**.

**Scheme 3**

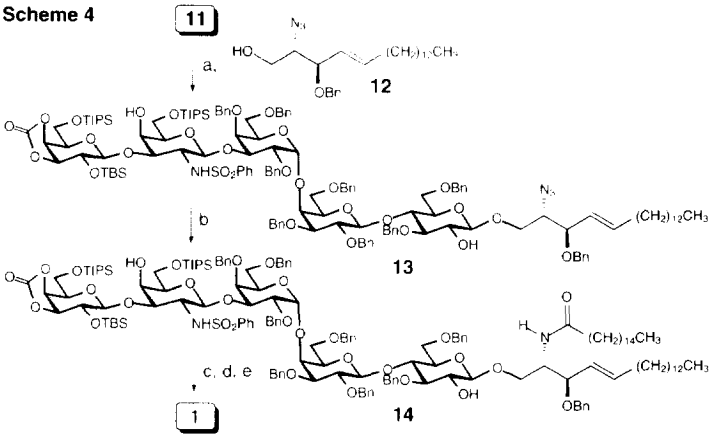


Reagents and conditions, a) 1.45 equiv of **7**, MeOTf, $CH_2Cl_2$-$Et_2O$ (1:2) 4Å-MS, 0–15°C, 7h, 71%β (β:α = 7:1)

With the key pentasaccharide glycal in hand attachment of the ceramide was accomplished as shown. Reaction of **11** with dimethyl dioxirane was followed by coupling of the derived epoxide with sphingosine precursor **12** in the presence of zinc chloride. A 41% yield of **13** was obtained. The elaboration of fatty acid sidechain was completed by reduction of the azide followed by *in situ* trapping of the resultant amine with palmitic anhydride. This sequence afforded a 90% yield of **14**. Deprotection of the pendant oxygens of the pentasaccharide was accomplished in a straightforward way. All silyl groups were cleaved with TBAF and all benzyl groups were cleaved with sodium and ammonia. For purposes of initial isolation the resultant product

was peracetylated to afford the peracetate (structure not shown). Methanolysis of the peracetate with sodium methoxide gave rise to the target antigen **1**[12] in 86% yield from **14**. The structure is clearly that represented. Thus, analysis of the NMR spectra along the sequence was supportive of the proposed structure. Moreover, proton-NMR spectra of the anomeric region of the final product measured in DMSO-$D_6$ in the presence of 2% $D_2O$ were identical to the recorded data.[3b]

**Scheme 4**




Reagents and conditions, a) DMDO, $CH_2Cl_2$, 0°, then **12** (9 equiv), $ZnCl_2$, 41%; b) Lindlar cat., $H_2$, palmitic anh., EtOAc, 90%; c) TBAF, THF; d) Na/$NH_3$, THF, -78°, then $Ac_2O$, DMAP, $Et_3N$-THF-DMF; e) NaOMe, MeOH, 86% (3 steps)

Recently, we described the synthesis of a breast tumor antigen characterized by binding to MBr1 antibody.[11] The breast (and ovarian) tumor antigens differ from SSEA-3 shown herein by the presence of a single α-L-fucosyl residue. The presence of the fucosyl residue is apparently critical to MBr1 antigen recognition since the MBr1 antibody does not recognize compound **1**, but does recognize **15**.[13] The synthesis speaks well for the synthetic economies which are possible by the methodology of glycal assembly in the construction of biologically important oligosaccharide determinants.

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12) Data for **11**, $[\alpha]^{23}_{D}$ = 8.3° (*c* 2.9, $CHCl_3$); IR ($CHCl_3$ film) 2941, 2865, 1792, 1650, 1454, 1162, 1102, 734 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$) δ 7.81-7.79 (2H, m), 7.33-7.02 (43H, m), 6.44 (1H, d, J = 6.2 Hz), 5.14 (1H, d, J = 3.4 Hz), 5.08-5.05 (2H, m), 4.84-4.80 (2H, m), 4.74-4.70 (2H, m), 4.63 (1H, d, J = 8.8 Hz), 4.58-4.39 (9H, m), 4.42-4.19 (9H, m), 4.12-3.78 (15H, m), 3.70-3.58 (3H, m), 3.46-3.24 (7H, m), 2.92 (OH), 1.12-1.04 (42H, m), 0.91 (9H, s), 0.20 (3H, s), 0.16 (3H, s); $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 155.66, 144.50, 140.12, 138.94, 138.68, 138.51, 138.40, 138.01, 137.90, 137.83, 137.77, 132.43, 128.73, 128.51, 128.33, 128.23, 128.18, 128.15, 128.11, 127.98, 127.93, 127.88, 127.81, 127.72, 127.66, 127.62, 127.47, 127.44, 127.40, 127.34, 127.14, 103.33, 102.93, 101.17, 99.64, 97.72, 80.94, 80.51, 78.97, 78.90, 78.43, 76.54, 75.90, 75.20, 75.05, 74.49, 74.14, 73.59, 73.49, 73.14, 73.10, 73.06, 72.62, 72.28, 72.24, 71.29, 70.44, 69.28, 68.72, 68.43, 68.11, 67.94, 67.82, 65.94, 61.78, 61.49, 56.02, 25.57, 17.97, 17.94, 17.87, 17.85, 17.66, 11.83, -4.63, -5.34; HRMS (FAB) calcd for $C_{117}H_{155}O_{26}NSSi_3Na$ $[M+Na]^+$ 2128.9760, found 2128.9850; Data for **1**, mp 200° (dec); $[\alpha]^{23}_{D}$ = +31.3° (*c* 0.4, MeOH); IR (KBr) 3402 (br), 2920, 2851, 1642, 1560, 1468, 1377, 1076 $cm^{-1}$; $^1$H-NMR (400 MHz, $CD_3OD$) δ 5.68 (1H, dt, J = 15.2, 6.6 Hz), 5.43 (1H, dd, J = 15.3, 7.7 Hz), 4.93 (1H, d, J = 3.9 Hz), 4.70 (1H, d, J = 8.4 Hz), 4.40 (1H, m), 4.34 (1H, d, J = 7.6 Hz), 4.29 (1H, d, J = 7.8 Hz), 4.26 (1H, t, J = 6.4 Hz), 4.18-4.14 (2H, m), 4.10-4.03 (3H, m), 3.98-3.64 (17H, m), 3.59-3.39 (10H, m), 3.26 (1H, m), 2.16 (2H, t, J = 7.6 Hz), 2.04-1.99 (2H, m), 1.98 (3H, s), 1.60-1.25 (48H, m), 0.89 (6H, t, J = 7.0 Hz) ; $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 171.81, 170.86, 131.41, 104.68, 104.01, 103.43, 102.73, 100.03, 81.20, 80.17, 79.77, 76.16, 75.31, 75.18, 75.08, 74.70, 74.39, 73.16, 72.88, 72.67, 70.67, 70.56, 70.31, 69.25, 68.05, 67.37, 67.15, 60.43, 60.34, 60.18, 59.13, 52.90, 51.46, 35.58, 31.75, 31.28, 29.09 (several peaks), 28.72, 25.39, 23.21, 22.08, 13.90; HRMS (FAB) calcd for $C_{66}H_{120}O_{28}N_2Na$ $[M+Na]^+$ 1411.7920, found 1411.7940

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