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A HIGHLY STEREOSELECTIVE SYNTHESIS OF DI- AND TRIMERIC SIALOSYL-Tn EPITOPE: A PARTIAL STRUCTURE OF GLYCOPHORIN A[1]

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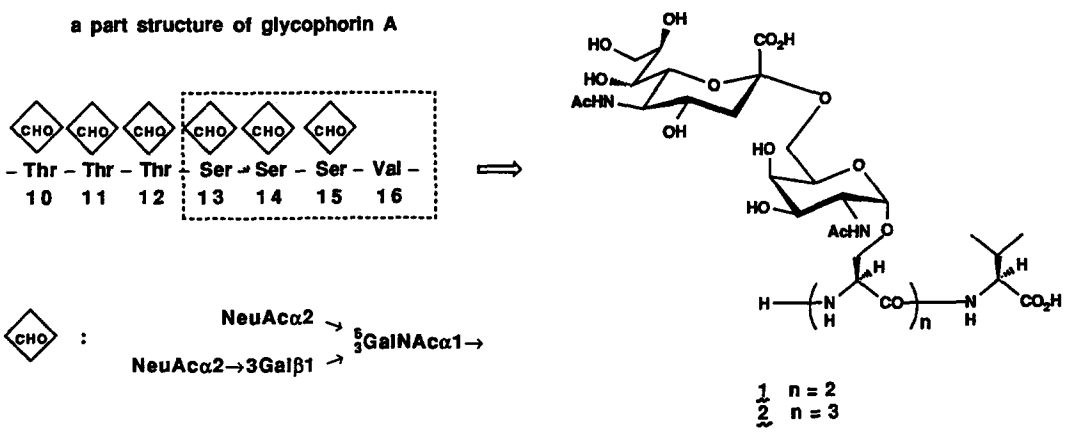
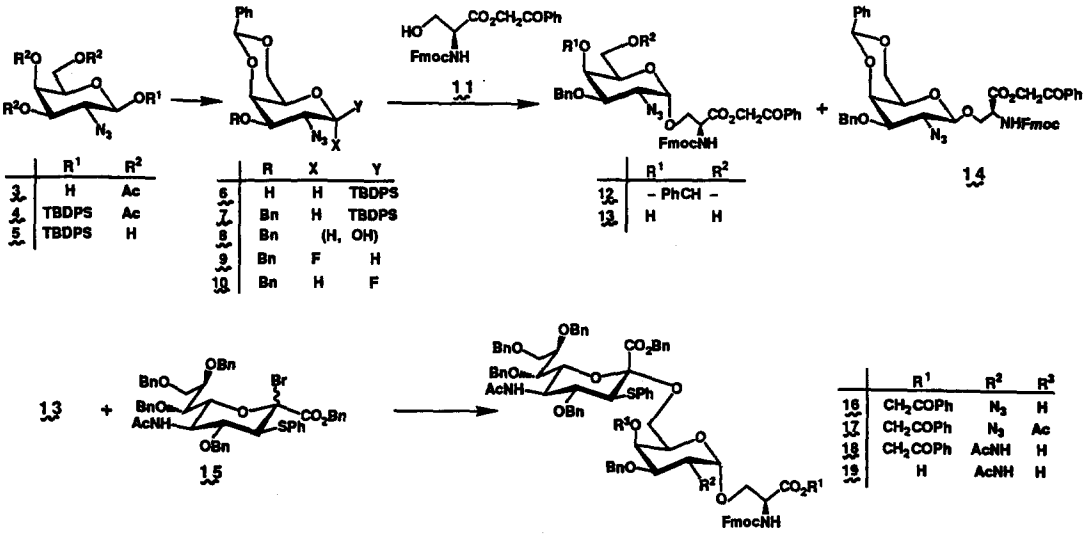
Abstract: Di- and trimeric sialosyl-Tn epitope, prototype molecules of O-linked (mucin type) sialoglycoproteins, were synthesized in a stereocontrolled manner.

Monoclonal antibodies B72.3[2] and MLS 102[3] that had been established using human metastatic breast cancer and colonic cancer cell line, respectively, were recently shown[4,5] to be directed to sialosyl-Tn antigen by use of synthetic monomeric epitopes[6]. Since strongly enhanced immunoreactivity of ovine submaxiallary mucin (OBM) with MLS 102 was ascribed[4] to the presence of a cluster structure of disaccharide α-D-Neu*p*5Ac-(2→6)-α-D-Gal*p*NAc on OBM, we have become interested in the synthesis of sialoglycopeptides bearing such oligosaccharide clusters. It is to be noted that in close connection with this project asialoglycopeptides bearing T and Tn epitope clusters have already been synthesized[7].

We now describe first synthesis of sialoglycopeptides **1** and **2** with di- and trimeric sialosyl Tn epitopes that correspond to the partial structures of glycophorin A, a major glycoprotein of



human erythrocyte membrane[8]. A hemiacetal **3**, available in 4 steps from D-galactose[9], was silylated (t-BuPh$_2$SiCl, imidazole, DMF, 60°, 2.5 h, 83%) to give **4**, which was deacetylated (NaOMe, MeOH) and benzylidenated (1,1-dimethoxytoluene, p-TsOH, CH_3CN) to **6** (91%). Benzylation of **6** (BnBr, NaH, THF, reflux, overnight, 85%) followed by desilylation[10] (n-Bu$_4$NF, AcOH, THF, overnight, 97%) gave **8**, which on treatment with DAST[11] in THF afforded a mixture of fluorides **9**

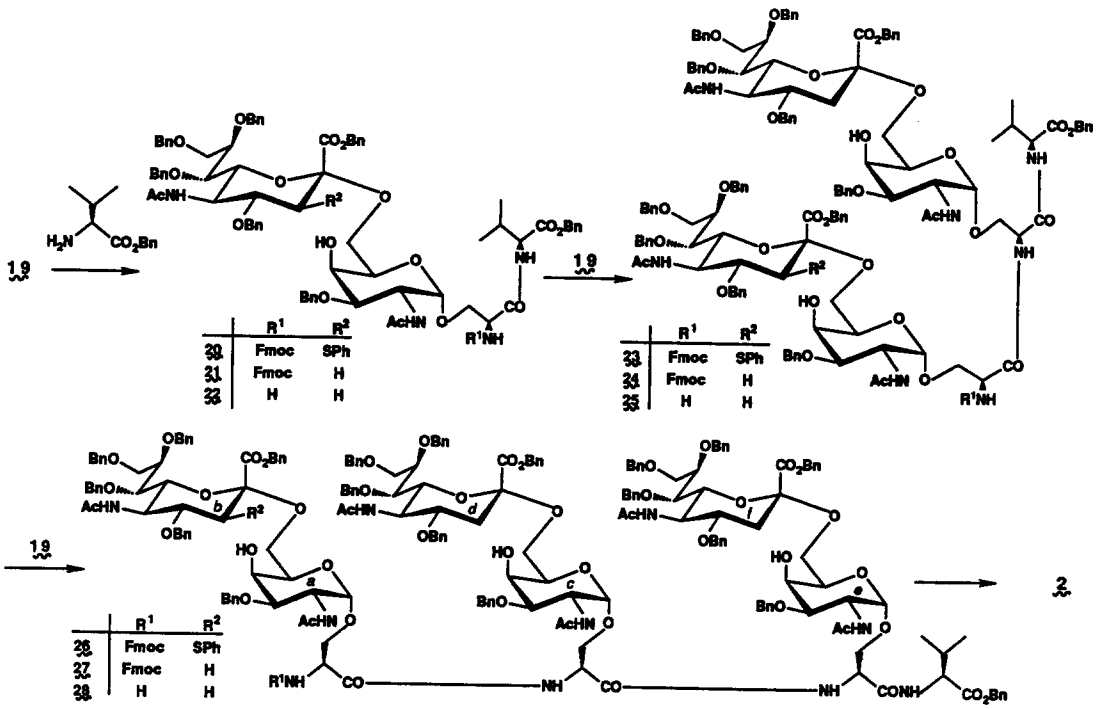


and **10** in 78 and 13% yield, respectively. Glycosylation of an L-serine derivative **11**[12] with **9** (or **10**) in the presence of Cp_2HfCl_2 and $AgClO_4$[13] in CH_2Cl_2 proceeded smoothly to give α- (**12**; 67%) and β-glycosides (**14**; 12%)[14]. After debenzylidenation of **12** (80% AcOH, 60°, 2 h, 83%) to diol **13**, a key glycosylation of **13** with a NeuAc donor **15**[15] was carried out[16] [$Hg(CN)_2$, $HgBr_2$, MS4A, CCl_4-CH_2Cl_2, -25°~room temp. overnight]. The coupling product **16**[14] (85%) was chromatographically and spectroscopically homogeneous, and neither stereo- nor regio-isomeric product could be isolated. The newly formed 2→6 linkage was evidenced by ^{1}H-n.m.r. data of the corresponding acetate **17** (δ_H 5.48, brs, H-4a), while the anomeric configuration was presumed[16] to be α because of the neighboring participation of β-phenylthio group at C-3 in **15**. On exposure to thioacetic acid[17], **16** was converted to acetamide **18**[14] (91%), which was then treated with Zn-AcOH to liberate the carboxyl group of serine moiety in 97% yield. EEDQ promoted coupling (CH_2Cl_2, room temp., 3 days) of **19** and L-valine benzylester[18] afforded an 82% yield of **20**[14], which was reductively desulfurized[16] (Ph_3SnH, AIBN, toluene, 100°) to **21**[14] (79% yield at 55% conversion). Cleavage of Fmoc group (morpholine, room temp., 1h) produced an amine **22** (96%) suitable for the next condensation (EEDQ, CH_2Cl_2 room temp., 3 days) with **19**. The product **23**[14] (74%) was desulfurized (Ph_3SnH, AIBN, benzene, reflux 2h, SiO_2-chromatography, 3 times repetition of the procedure, 79%) to give **24** which was, in turn, treated with morpholine to give **25** (95%).

For the synthesis of trimeric sialosyl Tn epitope **2**, **25** was further condensed with **19** (EEDQ) to give **26** (86%), which was desulfurized (**27**; 68%) and deblocked with morpholine to **28** (95%).

To complete the synthesis of **1** and **2**, **25** and **28** were fully deprotected by hydrogenolysis using 20% $Pd(OH)_2$-C as the catalyst in 80% aq. MeOH for 5 days. Purification of each debenzylated product was achieved by gel filtration and ion exchange chromatography (MonoQ column in FPLC system) to afford the target compounds. The structure of **1** (57%) and **2** (75%) including anomeric configuration of NeuAc residues were assignable by comparison of the ^{1}H-n.m.r. spectra[14] with those reported for the related natural glycoproteins[19].

In conclusion, di- and trimeric sialosyl Tn epitope molecules (1 and 2) of immunological importance were synthesized for the first time in a stereocontrolled manner.



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A similar result was obtained by the use of Cp_2ZrCl_2-$AgClO_4$ as an alternative promotor in this glycosylation.
14) Physical data for key compounds are given below. Values of δ_H and δ_C were measured for the solution in $CDCl_3$ unless noted otherwise. **12**: δ_H 5.10 (d, J 2.4 Hz, H-1); δ_C 100.2 ($^1J_{CH}$ 172 Hz, C-1). **14**: δ_C 102.1 ($^1J_{CH}$ 161 Hz, C-1). **16**: δ_H 3.43 (d, J 7.6 Hz, H-3b), 4.94 (d, J 3.4 Hz, H-1a); δ_C 98.9 (C-1a), 101.1 (C-2b). **18**: δ_H 1.64 (s, Ac), 1.80 (s, Ac). **20**: δ_H 0.83 (d, J 6.7 Hz, Me), 0.88 (d, J 6.7 Hz, Me), 1.64 (s, Ac), 1.93 (s, Ac), δ_C 98.7 (C-1a), 101.0 (C-2b). **21**: δ_H 0.82 (d, J 7.0 Hz, Me), 0.88 (d, J 6.8 Hz, Me), 1.73 (s, Ac), 1.92 (s, Ac), 2.75 (dd, J 4.5, 12.8 Hz, H-3bβ). **23**: δ_H 0.78 (d, J 7.0 Hz, Me), 0.79 (d, J 6.4 Hz, Me), 1.63 (s, Ac), 1.71 (s, Ac), 1.90 (s, Ac), 1.93 (s, Ac), 2.74 (dd, J 4.0, 12.8 Hz, H-3dβ). **24**: δ_H 0.78 (d, J 7.0 Hz, Me), 0.80 (d, J 8.2 Hz, Me), 1.72 (s, Ac), 1.92 (s, Ac), 1.94 (s, Ac), 2.73 (m, H-3bβ, H-3dβ). **26**: δ_H (DMSO-d_6, 80°) 0.73 (d, J 6.7 Hz, Me), 0.76 (d, J 6.7 Hz, Me), 1.56 (brt, H-3dα, H-3fα), 1.77 (s, Ac), 1.82 (s, 2Ac), 1.83 (s, 3Ac), 2.67 (dd, J 3.7, 12.2 Hz, H-3dβ, H-3fβ), 3.24 (d, J 9.8 Hz, H-3bα). **27**: δ_H (DMSO-d_6) 0.68 (d, J 6.4 Hz, Me), 0.73 (d, J 6.7 Hz, Me), 1.50 (m, NeuAc H-3α), 1.77 (s, Ac), 1.81 (s, Ac), 1.82 (s, Ac), 1.86 (s, 3Ac), 2.69 (brd, NeuAc H-3β). **1**: $[\alpha]_D$ +88° (H_2O); δ_H (D_2O) 0.92 (d, J 6.7 Hz, Me), 0.93 (d, J 7.0 Hz, Me), 1.68 (brt, J 12.5 Hz, NeuAc H-3α), 2.02 (s, 3Ac), 2.03 (s, Ac), 2.15 (m, Valβ-H), 2.71 (dd, J 4.5, 12.5 Hz, NeuAc H-3β), 2.74 (dd, J 4.6, 12.2 Hz, NeuAc H-3β), 3.57 (dd, J 1.5, 8.9 Hz, NeuAc H-7), 3.63 (dd, J 6.7, 12.5 Hz, NeuAc H-9), 3.82 (t, J 10.1 Hz, NeuAc H-5), 3.96 (brs, GalNAc H-4), 4.40 (brt, Serα-H), 4.80 (brt, Serα-H), 4.86 (d, J 4.0 Hz, GalNAc H-1), 4.88 (d, J 3.4 Hz, GalNAc H-1). **2**: $[\alpha]_D$ +70° (H_2O); δ_H (D_2O,) 0.89 (d, J 7.0 Hz, Me), 0.90 (d, J 6.7 Hz, Me), 1.69 (m, NeuAc H-3α), 2.02 (s, 3Ac), 2.04 (s, Ac), 2.05 (s, Ac), 2.06 (s, Ac), 2.74 (m, NeuAc H-3β), 4.41 (br, Serα-H), 4.68 (brt, Serα-H), 4.74 (br, Serα-H), 4.88 (d, J 3.1 Hz, GalNAc H-1), 4.89 (d, J 3.4 Hz, GalNAc H-1), 4.92 (d, J 3.4 Hz, GalNAc H-1).
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