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Synthesis of trisaccharide 6′SiaLe^c and its 6-O-Su derivative

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The synthetic approach to 6′SiaLe^c and its 6-O-Su derivative comprised α-sialylation of a protected Le^c derivative, 2,3-di-OAcGalβ1-3(3-OAc-6-OBn)GlcNAcβ1-Osp (68% yield) as the key stage. Deprotection of the obtained trisaccharide (three stages, 79% overall yield) led to Neu5Acα2-6Galβ1-3GlcNAcβ1-Osp; partial deprotection followed by selective sulfation and total deprotection – to Neu5Acα2-6Galβ1-3(6-O-Su)GlcNAcβ1-Osp (four stages, 72% overall yield).

Mammals have two enzymes capable of synthesizing Neu5Acα2-6Gal motif in glycoproteins and glycolipids, ST6Gal-I and ST6Gal-II. Both sialyltransferases specifically elongate Galβ1-4GlcNAc terminated substrates, whereas isomeric substrate, Galβ1-3GlcNAc (Le^c) motif was found to be ~100 times less potent in *in vitro* assays.[1] Therefore, question about possibility of *in vivo* synthesis of Neu5Acα2-6Galβ1-3GlcNAc terminated glycans remains unclear. Classical works on structures and glycomics also give no certain information about abundance of this and related Neu5Acα2-6Galβ1-3(6-O-Su)GlcNAc motifs (Su = SO_3H), mass spectrometry data cannot differentiate Neu5Acα2-6Galβ1-3GlcNAc *vs*. common Neu5Acα2-6Galβ1-4GlcNAc without standards suitable for MS/MS experimentation. Here, we describe the synthesis of trisaccharides Neu5Acα2-6Galβ1-3GlcNAc and Neu5Acα2-6Galβ1-3(6-O-Su)GlcNAc capable of solving this problem. Additionally, these trisaccharides in a spacer-armed form are convenient substrates for the study of α1-4 fucosyltransferases – enzymes synthesizing glycans with terminations Neu5Acα2-6Galβ1-3(Fucα1-4)GlcNAc and Neu5Acα2-6Galβ1-3(Fucα1-4)(6-O-Su)GlcNAc – analogues of SiaLe^a, well known as cancer-associated antigen CA-19-9. Particular fucosyltransferase(s) responsible in mammalian cells for biosynthesis of these glycans have not been identified yet.

Hg^{II}-catalysed glycosylation of 3,4-diol **1** with acetobromogalactose proceeded preferably with formation of Le^c-derivative **2** (68%). Subsequent deacetylation, benzylidenation, acetylation, and debenzylidenation led to 4′,6′-diol **3** in 67% total yield. Glycosylation of the latter compound with neuraminic acid chloride **4** under conditions described earlier[2,3] (catalysis with Ag_2CO_3) afforded protected 6′-sialylated Le^c derivative (68%). Hydrogenolysis of benzyl ether moiety in this compound led to trisaccharide **5** (88%). The presence of one OH group in GlcNAc fragment allowed us to perform efficient sulfation with Py·SO_3.[4,5] Further deprotection (treatment with MeONa/MeOH, NaOH/H_2O, neutralization with AcOH), purification on Sephadex LH-20 (MeCN–0.01 M aq. Py·AcOH, 1:1, pH 6), and ion-exchange to Na^+ on Dowex gave target 6-O-Su derivative **7** (82%).

Similar deprotection of **5** gave the second target compound **6** (90%) isolated using cation-exchange chromatography on Dowex H^+ resin (elution with 1 M aqueous pyridine).

The structure of all synthesized compounds was confirmed by high resolution ^{1}H NMR spectroscopy.†

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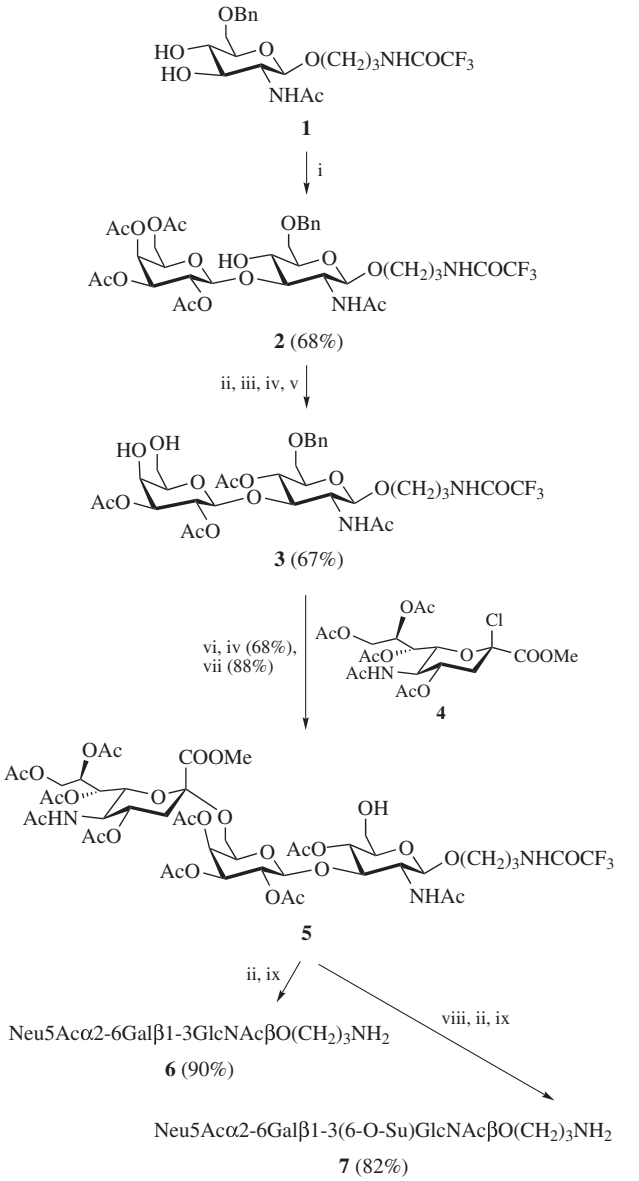

Scheme 1 *Reagents and conditions*: i, Ac_4GalBr, $Hg(CN)_2$/$HgBr_2$, $MeNO_2$/MePh, 15 h; ii, 0.1 M NaOMe/MeOH, 0.5 h; iii, $PhCH(OMe)_2$, TsOH, MeCN, 2 h; iv, Ac_2O, Py, 12 h; v, 80% aq. AcOH, 70 °C, 2 h; vi, Ag_2CO_3, CH_2Cl_2, 70 h; vii, Pd/C, H_2, MeOH, 2 h; viii, Py·SO_3/Py, room temperature, 1 h; ix, 0.1 M NaOH/H_2O, 15 h.

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† ^{1}H NMR spectra were recorded on a Bruker AVANCE 600, 700 and 800 MHz spectrometer at 303 K. Chemical shifts δ for characteristic protons are given in ppm with the use of HOD (δ 4.750), $CHCl_3$ (δ 7.270), or CHD_2OD (δ 3.500) as reference. The signals in ^{1}H NMR spectra were assigned using a technique of spin–spin decoupling (double resonance) and 2D ^{1}H,^{1}H COSY experiments. The values of optical rotation were measured on a Jasco DIP-360 digital polarimeter at 25 °C. Mass spectra were recorded on a MALDI-TOF Vision-2000 spectrometer using dihydroxybenzoic acid as a matrix.

For **2**: ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.757–1.922 (m, 2H, $C^{sp}H_2$), 1.992, 2.014, 2.025, 2.091, 2.166 (5 s, 5×3H, COMe), 3.165 (ddd, 1H, H-2′, $J_{1,2}$ 8.3 Hz, $J_{2,3}$ 10.2 Hz, $J_{NH,2}$ 7.2 Hz), 3.40–3.51 (m, 3H, $NC^{sp}H_2$, H-4′), 3.529 (ddd, 1H, H-5′, $J_{4,5}$ 9.6 Hz, $J_{5,6a}$ 1.9 Hz, $J_{5,6b}$ 6.3 Hz), 3.636 (dd, 1H, H-6′b, $J_{5,6b}$ 6.3 Hz, $J_{6a,6b}$ 10.8 Hz), 3.656–3.692 (m, 1H, $OC^{sp}H$), 3.815 (br. s, 1H, 4-OH), 3.844 (dd, 1H, H-6′a, $J_{5,6a}$ 1.9 Hz, $J_{6a,6b}$ 10.8 Hz), 3.888–3.923 (m, 1H, $OC^{sp}H$), 4.010 (ddd ≈ t, 1H, H-5″, J 6.6 Hz), 4.130 (m ≈ d, 2H, H-6″a, H-6″b, J 6.6 Hz), 4.208 (dd, 1H, H-3′, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 8.3 Hz), 4.558–4.601 (m, 3H, H-1″, CH_2Ph), 4.889 (d, 1H, H-1′, $J_{1,2}$ 8.3 Hz), 5.013 (dd, 1H, H-3″, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.4 Hz), 5.232 (dd, 1H, H-2″, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.5 Hz), 5.387 (dd ≈ d, 1H, H-4″, J 2.9 Hz), 5.795 (d, 1H, NH′, $J_{NH,2}$ 7.2 Hz), 7.27–7.36 (m, 6H, Ph, $NHCOCF_3$).

For **3**: ^{1}H NMR ($CDCl_3$–CD_3OD 3:1, 700 MHz) δ: 1.886–2.015 (m, 2H, $C^{sp}H_2$), 2.149, 2.154, 2.201, 2.212 (4 s, 4×3H, COMe), 3.360–3.398 and 3.590–3.628 (2 m, 2×1H, $NC^{sp}H_2$), 3.64–3.68 (m, 2H, H-5′, H-5″), 3.684 (dd, 1H, H-6′a, $J_{5,6a}$ 5.8 Hz, $J_{6a,6b}$ 10.8 Hz), 3.718 (dd, 1H, H-6′b, $J_{5,6b}$ 2.8 Hz, $J_{6a,6b}$ 10.8 Hz), 3.778–3.804 (m, 1H, $OC^{sp}H$), 3.879 (dd, 1H, H-6″a, $J_{5,6a}$ 5.2 Hz, $J_{6a,6b}$ 11.4 Hz), 3.917 (dd, 1H, H-6″b, $J_{5,6b}$ 6.6 Hz, $J_{6a,6b}$ 11.4 Hz), 3.973 (dd, 1H, H-3′, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 8.6 Hz), 4.011-4.041 (m, 1H, $OC^{sp}H$), 4.149–4.177 (m, 2H, H-2′, H-4″), 4.536 (d, 1H, H-1′, $J_{1,2}$ 8.3 Hz), 4.654 and 4.685 (2 d, 2×1H, CH_2Ph, J_{hem} 11.8 Hz), 4.699 (d, 1H, H-1″, $J_{1,2}$ 7.9 Hz), 4.943 (dd, 1H, H-3″, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.4 Hz), 4.990 (dd ≈ t, 1H, H-4′, J 9.4 Hz), 5.285 (dd, 1H, H-2″, $J_{2,3}$ 10.4 Hz, $J_{1,2}$ 7.9 Hz), 7.41–7.50 (m, 5H, Ph).

For **5**: ^{1}H NMR ($CDCl_3$, 800 MHz) δ: 1.825–1.922 (m, 2H, $C^{sp}H_2$), 1.938 (dd ≈ t, 1H, H-3$^{'''}_{ax}$, J 12.6 Hz), 1.906, 1.971, 2.007, 2.036, 2.059, 2.067, 2,080, 2.144, 2.169, 2.210 (10 s, 10×3H, COMe), 2.569 (dd, 1H, H-3$^{'''}_{eq}$, $J_{3ax,3eq}$ 12.9 Hz, $J_{3eq,4}$ 4.6 Hz), 3.272–3.311 (m, 1H, $NC^{sp}H$), 3.373 (dd, 1H, H-6″a, $J_{5,6a}$ 7.8 Hz, $J_{6a,6b}$ 10.8 Hz), 3.512 (ddd, 1H, H-5′, $J_{4,5}$ 10.0 Hz, $J_{5,6a}$ 4.5 Hz, $J_{5,6b}$ 2.3 Hz), 3.522–3.584 (m, 2H, $NC^{sp}H$, $OC^{sp}H$), 3.609 (dd, 1H, H-6′a, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 12.6 Hz), 3.753 (dd, 1H, H-6′b, $J_{5,6b}$ 2.3 Hz, $J_{6a,6b}$ 12.6 Hz), 3.754 (dd, 1H, H-6″b, $J_{5,6b}$ 6.0 Hz, $J_{6a,6b}$ 10.8 Hz), 3.821 (s, 3H, OMe‴), 3.851 (br. q, 1H, H-2′, J 9.0 Hz), 3.905 (br. t, 1H, H-5″, J 7.0 Hz), 3.976–4.014 (m, 2H, $OC^{sp}H$, H-5‴, $J_{4,5}$ 10.5 Hz, $J_{5,NH}$ 9.9 Hz, $J_{5,6}$ 10.8 Hz), 4.056 (dd, 1H, H-9‴b, $J_{8,9b}$ 5.4 Hz, $J_{9a,9b}$ 12.1 Hz), 4.109 (dd, 1H, H-6‴, $J_{5,6}$ 10.8 Hz, $J_{6,7}$ 1.4 Hz), 4.181 (dd ≈ t, 1H, H-3′, $J_{2,3}$ 9.1 Hz, $J_{3,4}$ 9.3 Hz), 4.373 (dd, 1H, H-9‴a, $J_{8,9a}$ 2.8 Hz, $J_{9a,9b}$ 12.1 Hz), 4.667 (d, 1H, H-1′, $J_{1,2}$ 7.9 Hz), 4.689 (d, 1H, H-1″, $J_{1,2}$ 7.6 Hz), 4.903 (ddd, 1H, H-4‴, $J_{3eq,4}$ 4.6 Hz, $J_{3ax,4}$ 12.2 Hz, $J_{4,5}$ 10.5 Hz), 4.918 (dd, 1H, H-4′, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 10.0 Hz), 4.992 (dd, 1H, H-3″, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.4 Hz), 5.024 (dd, 1H, H-2″, $J_{2,3}$ 10.4 Hz, $J_{1,2}$ 7.6 Hz), 5.115 (d, 1H, NH‴, $J_{NH,5}$ 9.9 Hz), 5.305 (dd, 1H, H-7‴, $J_{6,7}$ 1.7 Hz, $J_{7,8}$ 9.4 Hz), 5.323 (ddd, 1H, H-8‴, $J_{7,8}$ 9.4 Hz, $J_{8,9a}$ 2.8 Hz, $J_{8,9b}$ 5.4 Hz), 5.409 (dd ≈ d, 1H, H-4″, J 3.0 Hz), 6.755 (br. s, 1H, NH′), 7.649 (m ≈ s, 1H, $NHCOC^{sp}F_3$).

For **6**: ^{1}H NMR (D_2O, 700 MHz) δ: 1.721 (dd ≈ t, 1H, H-3$^{'''}_{ax}$, J 12.1 Hz); 1.966–2.005 (m, 2H, $C^{sp}H_2$), 2.060 (s, 2×3H, NCOMe), 2.729 (dd, 1H, H-3$^{'''}_{eq}$, $J_{3ax,3eq}$ 12.4 Hz, $J_{3ex,4}$ 4.7 Hz), 3.123 (m ≈ t, 2H, $NC^{sp}H_2$, J 7.0 Hz); 3.525–3.925 (m, 17H), 3.942 (dd ≈ d, 1H, H-4″, J 3.3 Hz), 3.985–4.035 (m, 2H), 4.040–4.072 (m, 1H, $OC^{sp}H$), 4.416 (d, 1H, H-1″, $J_{1,2}$ 7.8 Hz), 4.581 (d, 1H, H-1′, $J_{1,2}$ 8.5 Hz). $[\alpha]_D$ –36.0 (*c* 0.45; MeCN–H_2O, 1:1). MS, *m*/*z*: 732 $[M]^+$ (calc. for $C_{28}H_{49}N_3O_{19}$, *m*/*z*: 731.71 $[M]^+$).

For **7**: ^{1}H NMR (D_2O, 700 MHz) δ: 1.723 (dd ≈ t, 1H, H-3$^{'''}_{ax}$, J 12.1 Hz); 1.952–2.032 (m, 2H, $C^{sp}H_2$), 2.058 (s, 2×3H, NCOMe), 2.750 (dd, 1H, H-3$^{'''}_{eq}$, $J_{3ax,3eq}$ 12.4 Hz, $J_{3eq,4}$ 4.7 Hz), 3.144 (m ≈ t, 2H, $NC^{sp}H_2$, J 6.8 Hz), 3.546 (dd, 1H, H-2″, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.8 Hz), 3.58–3.99 (m, 17H), 4.006–4.037 (m, 1H, $OC^{sp}H$), 4.266 (dd, 1H, H-6′a, $J_{6a,6b}$ 11.3 Hz, $J_{5,6a}$ 6.1 Hz), 4.438 (d, 1H, H-1″, $J_{1,2}$ 7.9 Hz), 4.442 (dd, 1H, H-6′b, $J_{6a,6b}$ 11.3 Hz, $J_{5,6b}$ 1.9 Hz), 4.597 (d, 1H, H-1′, $J_{1,2}$ 8.5 Hz). $[\alpha]_D$ –22.5 (*c* 0.4; MeCN–H_2O, 1:1). MS, *m*/*z*: 834 $[M]^+$ (calc. for $C_{28}H_{49}N_3NaO_{22}S$, *m*/*z*: 833.76 $[M]^+$).