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Synthesis of mono-, di-, and tri-O-sulfated *N*-acetylglucosamines in a form suitable for glycochip printing

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Spectral characteristics for oligosaccharides. ^1H NMR spectra were recorded at 30°C with Bruker WM 500 MHz instrument. Mass spectra (MALDI-TOF) were recorded on VISION 2000 mass spectrometer. Optical rotations were measured with JASCO DIP-360 polarimeter at 20°C.

2: ^1H NMR (500 MHz, $\text{CDCl}_3:\text{CD}_3\text{OD}$ 3:1), δ : 1.861, 1.980, 2.000, 2.014, 2.043 (5s, 15H, COMe), 3.205 (m, 1H, NCH_2 sp), 3.398 (m, 1H, NCH_2 sp), 3.452 (m, 1H, H-5''), 3.54 – 3.62 (m, 2H, H-5', OCH_2 sp), 3.677 (dd, 1H, H-6a'', $J_{6a,6b}$ 11.7 Hz, $J_{6a,5}$ 5.4 Hz), 3.732 (dd, 1H, H-6b'', $J_{6a,6b}$ 11.7 Hz, $J_{6b,5}$ 5.6 Hz), 3.741 (dd, 1H, H-4', $J_{3,4}$ 8.6 Hz, $J_{4,5}$ 9.1 Hz), 3.892 (dd, 1H, H-2', $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.8 Hz), 3.905 (m, 1H, OCH_2 sp), 4.005 (dd, 1H, H-4'', $J_{3,4}$ 3.4 Hz, $J_{4,5}$ ~ 1 Hz), 4.048 (dd, 1H, H-6b', $J_{6a,6b}$ 11.9 Hz, $J_{5,6b}$ 5.4 Hz), 4.405 (d, 1H, H-1'', $J_{1,2}$ 7.8 Hz), 4.435 (dd, 1H, H-6a', $J_{6a,6b}$ 11.9 Hz, $J_{5,6a}$ 2.7 Hz), 4.493 (d, 1H, H-1', $J_{1,2}$ 7.8 Hz), 4.770 (dd, 1H, H-3'', $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 3.4 Hz), 5.002 (dd, 1H, H-3', $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 8.6 Hz), 5.116 (dd, 1H, H-2'', $J_{2,3}$ 10.3 Hz, $J_{1,2}$ 7.8 Hz).

4: ^1H NMR (500 MHz, $\text{CDCl}_3:\text{CD}_3\text{OD}$ 3:1), δ : 1.943 (m, 2H, CH_2 sp), 2.061, 2.163, 2.170, 2.238 (4s, 12H, COMe), 3.370 (m, 1H, NCH_2 sp), 3.520 (dd, 1H, H-3'', $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.4 Hz), 3.560 – 3.665 (m, 4H, NCH_2 , OCH_2 sp, H-5', H-5''), 3.834 – 3.925 (m, 3H, H-4'', H-

6a', H-6b'), 3.982 (dd, 1H, H-4', $J_{3,4}$ 8.8 Hz, $J_{4,5}$ 9.8 Hz), 4.023 (m, 1H, OCH₂ sp), 4.086 (dd, 1H, H-2', $J_{1,2}$ 8.3 Hz, $J_{2,3}$ 10.3 Hz), 4.332 (dd, 1H, H-6b'', $J_{6a,6b}$ 11.4 Hz, $J_{6b,5}$ 6.8 Hz), 4.396 (dd, 1H, H-6a'', $J_{6a,6b}$ 11.4 Hz, $J_{6a,5}$ 5.6 Hz), 4.444 (d, 1H, H-1'', $J_{1,2}$ 8.1 Hz), 4.519 (d, 1H, H-1', $J_{1,2}$ 8.3 Hz), 4.628 (d, 1H, CH₂Ph, J_{rem} 12.1 Hz), 4.838 (d, 1H, CH₂Ph, J_{rem} 12.1 Hz), 4.961 (dd, 1H, H-2'', $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 10.0 Hz), 5.112 (dd, 1H, H-3', $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 8.8 Hz), 7.417 – 7.530 (m, 6H, Ph, NHCOCF₃).

8: ¹HNMR (500 MHz, CDCl₃), δ : 1.846 (m, 2H, CH₂ sp), 1.901 (s, 3H, COMe), 1.919 (dd, 1H, H-3ax''', $J_{3ax,3eq} \approx J_{3ax,4} \approx 12.8$ Hz), 1.966, 1.977, 2.033, 2.059, 2.064, 2.100, 2.147, 2.194 (8s, 27H, 9COMe), 2.382 (m, 1H, 6-OH), 2.557 (dd, 1H, H-3eq''', $J_{3eq,3ax}$ 12.8 Hz, $J_{3eq,4}$ 4.5 Hz), 3.265 (m, 1H, NCH₂ sp), 3.419 (dd, 1H, H-6b'', $J_{6a,6b}$ 11.0 Hz, $J_{5,6b}$ 8.1 Hz), 3.449 (m, 1H, H-5'), 3.580 (m, 1H, H-6b'), 3.648 (m, 1H, NCH₂ sp), 3.720 (dd, 1H, H-6a'', $J_{6a,6b}$ 11.0 Hz, $J_{6a,5}$ 5.8 Hz), 3.801 (m, 1H, OCH₂ sp), 3.826 (s, 3H, OCH₃'''), 3.92 – 4.07 (m, 5H, H-5'', H-2', H-4', H-6', H-5'''), 4.075 – 4.135 (m, 2H, H-6''', H-8b'''), 4.343 (dd, 1H, H-9a''', $J_{9a,9b}$ 12.0 Hz, $J_{9a,8}$ 2.2 Hz), 4.419 (d, 1H, H-1', $J_{1,2}$ 8.1 Hz), 4.673 (d, 1H, H-1'', $J_{1,2}$ 7.6 Hz), 4.888 (ddd, 1H, H-4''', $J_{3eq,4}$ 4.5 Hz, $J_{3ax,4}$ 12.5 Hz, $J_{4,5}$ 10.5 Hz), 5.037 (dd, 1H, H-3'', $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 3.2 Hz), 5.061 (dd, 1H, H-3', $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 8.8 Hz), 5.085 (dd, 1H, H-2'', $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 10.3 Hz), 5.119 (d, 1H, NH''', $J_{\text{NH},2}$ 9.8 Hz), 5.309 (m, 2H, H-7''', H-8'''), 5.442 (dd, 1H, H-4'', $J_{3,4}$ 3.2 Hz, $J_{4,5} \sim 1$ Hz), 5.857 (d, 1H, NH', $J_{\text{NH},5}$ 8.8 Hz), 7.485 (m, 1H, NHCOCF₃ sp).

6-O-Su-Gal β 1-4(6-O-Su)GlcNAc β O(CH₂)₂NH₂ (10): (500 MHz, D₂O) δ : 2.068 (s, 3H, NCOMe), 3.208 (m, 1H, NCH sp), 3.277 (m, 1H, NCH sp), 3.568 (dd, 1H, H-2'', $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.9 Hz), 3.722 (dd, 1H, H-3'', $J_{2,3}$ 9.9 Hz, $J_{3,4}$ 3.3 Hz), 3.76 – 3.86 (m, 3H', H-2', H-3' and H-4'), 3.888 (m, 1H, H-5'), 3.95 – 4.07 (m, 4H, H-4'', H-5'', OCH₂ sp), 4.221 (m, 2H, H-6a'', H-6b''), 4.343 (dd, 1H, H-6b', $J_{6a,6b}$ 11.1 Hz, $J_{5,6a}$ 5.2 Hz), 4.472 (dd, 1H, H-6a', $J_{6a,6b}$ 11.1 Hz, $J_{6,6a}$ 1.7 Hz), 4.565 (d, 1H, H-1', $J_{1,2}$ 7.9 Hz), 4.628 (d, 1H, H-1'', $J_{1,2}$ 7.9 Hz). MS, m/z : 653 [M + Na⁺]. $[\alpha]_{\text{D}} -12.0^\circ$ (*c* 1, H₂O).

3,6-di-O-Su-Gal β 1-4(6-O-Su)GlcNAc β O(CH₂)₂NH₂ (11): (500 MHz, D₂O) δ : 2.069 (s, 3H, NCOMe), 3.203 (m, 1H, NCH sp), 3.274 (m, 1H, NCH sp), 3.709 (dd, 1H, H-2'', $J_{1,2}$ 7.9 Hz,

$J_{2,3}$ 9.6 Hz), 3.78 – 3.87 (m, 3H, H-2', H-3' and H-4'), 3.908 (m, 1H, H-5'), 3.985 (m, 1H, OCH sp), 4.044 (m, 1H, OCH sp), 4.059 (m, 1H, H-5''), 4.240 (m, 2H, H-6a'' and H-6b''), 4.339 (dd, 1H, H-6b', $J_{6a,6b}$ 11.3 Hz, $J_{5,6b}$ 5.5 Hz), 4.366 (dd, 1H, H-4'', $J_{3,4}$ 3.2 Hz, $J_{4,5}$ ~1Hz), 4.391 (dd, 1H, H-3'', $J_{2,3}$ 9.7 Hz, $J_{3,4}$ 3.2 Hz), 4.494 (dd, 1H, H-6a', $J_{6a,6b}$ 11.3 Hz, $J_{5,6a}$ 1.8 Hz), 4.625 (d, 1H, H-1', $J_{1,2}$ 7.6 Hz), 4.676 (d, 1H, H-1'', $J_{1,2}$ 7.9 Hz). MS, m/z : 732 [M + Na⁺]. $[\alpha]_D -0.6^\circ$ (*c* 1, H₂O)

6-O-Su-Gal β 1-4GlcNAc β O(CH₂)₂NH₂ (**12**): (500 MHz, D₂O) δ : 2.040 (s, 3H, NCOMe), 3.210 (m, 2H, NCH₂ sp), 3.542 (dd, 1H, H-2'', $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.0 Hz), 3.644 (m, 1H, H-5'), 3.680 (dd, 1H, H-3'', $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.4 Hz), 3.690 – 3.803 (m, 3H, H-2', H-3' and H-4'), 3.840 (dd, 1H, H-6b', $J_{6a,6b}$ 12.2 Hz, $J_{5,6b}$ 5.2 Hz), 3.888 (m, 1H, OCH sp), 3.94 – 4.01 (m, 3H, H-4'' and H-5'', H-6a', $J_{6a,6b}$ 12.2 Hz, $J_{5,6a}$ 2.1 Hz), 4.046 (m, 1H, OCH sp), 4.190 (m, 2H, H-6a'' and H-6b''), 4.507 (d, 1H, H-1'', $J_{1,2}$ 7.8 Hz), 4.572 (d, 1H, H-1', $J_{1,2}$ 7.9 Hz). MS, m/z : 551 [M + Na⁺]. $[\alpha]_D -23.4^\circ$ (*c* 1, H₂O)

4,6-di-O-Su-Gal β 1-4GlcNAc β O(CH₂)₂NH₂ (**13**): (500 MHz, D₂O) δ : 2.072 (s, 3H, NCOMe), 3.239 (m, 2H, NCH₂ sp), 3.597 (dd, 1H, H-2'', $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.0 Hz), 3.688 (m, 1H, H-5'), 3.73 – 3.83 (m, 3H, H-2', H-3' and H-4'), 3.848 (dd, 1H, H-3'', $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz), 3.878 (dd, 1H, H-6b', $J_{6a,6b}$ 12.3 Hz, $J_{5,6a}$ 5.2 Hz), 3.923 (m, 1H, OCH sp), 4.026 (dd, 1H, H-6a', $J_{6a,6b}$ 12.3 Hz, $J_{5,6a}$ 1.9 Hz), 4.074 (m, 1H, OCH sp), 4.139 (ddd, 1H, H-5'', $J_{4,5}$ ~1 Hz, $J_{5,6b}$ 9.0 Hz, $J_{5,6a}$ 2.9 Hz), 4.227 (dd, 1H, H-6b'', $J_{5,6b}$ 9.0 Hz, $J_{6a,6b}$ 11.3 Hz), 4.385 (dd, 1H, H-6a'', $J_{5,6a}$ 2.9 Hz, $J_{6a,6b}$ 11.4 Hz), 4.602 (m, 2H, H-1'', H-1'), 4.726 (dd, 1H, H-4'', $J_{3,4}$ 3.3 Hz, $J_{4,5}$ ~1 Hz). MS, m/z : 653 [M + Na⁺]. $[\alpha]_D -15.5^\circ$ (*c* 1, H₂O)

4-O-Su-Gal β 1-4GlcNAc β O(CH₂)₂NH₂ (**14**): (500 MHz, D₂O) δ : 2.069 (s, 3H, NCOMe), 2.997 (m, 2H, NCH₂ sp), 3.583 (dd, 1H, H-2'', $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.1 Hz), 3.638 (m, 1H, H-5'), 3.72 – 3.90 (m, 8H), 3.922 (m, 1H, OCH sp), 4.026 (dd, 1H, H-6b', $J_{5,6b}$ 2.2 Hz, $J_{6a,6b}$ 12.3 Hz), 4.078 (m, 1H, OCH sp), 4.548 (d, 1H, H-1'', $J_{1,2}$ 7.8 Hz), 4.602 (d, 1H, H-1', $J_{1,2}$ 8.1 Hz), 4.693 (dd, 1H, H-4'', $J_{3,4}$ 3.2 Hz, $J_{4,5}$ ~1 Hz). MS, m/z : 551 [M + Na⁺]. $[\alpha]_D -17.8^\circ$ (*c* 1, H₂O)

3,4-di-O-Su-Gal β 1-4GlcNAc β O(CH $_2$) $_3$ NH $_2$ (**15**): (500 MHz, D $_2$ O) δ : 1.968 (m, 2H, CH $_2$ sp), 2.070 (s, 3H, NCOMe), 3.104 (m, 2H, NCH $_2$ sp), 3.691 (m, 1H, H-5'), 3.72 – 3.79 (m, 5H, OCH sp, H-2'', H-2', H-3' and H-4'), 3.821 (dd, 1H, H-6b'', J $_{6a,6b}$ 12.0 Hz, J $_{5,6b}$ 4.0 Hz), 3.845 (dd, 1H, H-6a'', J $_{6a,6b}$ 12.0 Hz, J $_{5,6a}$ 4.0 Hz), 3.887 (dd, 1H, H-6b', J $_{6a,6b}$ 12.3 Hz, J $_{5,6b}$ 4.9 Hz), 3.910 (m, 1H, H-5''), 3.99 – 4.06 (m, 2H, H-6a', OCH sp), 4.440 (dd, 1H, H-3'', J $_{3,4}$ 3.1 Hz, J $_{2,3}$ 10.1 Hz), 4.592 (m, 1H, H-1'), 4.654 (d, 1H, H-1'', J $_{1,2}$ 7.9 Hz), 4.976 (dd, 1H, H-4'', J $_{3,4}$ 3.1 Hz, J $_{4,5}$ ~1 Hz). MS, m/z : 667 [M + Na $^+$]. [α] $_D$ -3.6° (c 1, H $_2$ O)

3-O-Su-Gal β 1-4(6-O-Su)GlcNAc β O(CH $_2$) $_3$ NH $_2$ (**16**): (500 MHz, D $_2$ O) δ : 1.981 (m, 2H, CH $_2$ sp), 2.065 (s, 3H, NCOMe), 3.136 (m, 2H, NCH $_2$ sp), 3.695 (dd, 1H, H-2'', J $_{1,2}$ 7.8 Hz, J $_{2,3}$ 9.9 Hz), 3.746 (dd, 1H, H-2', J $_{1,2}$ 8.2 Hz, J $_{2,3}$ 10.4 Hz), 3.74 – 3.88 (m, 7H, OCH sp, H-5'', H-6a'' and H-6b'', H-3, H-4 and H-5'), 4.015 (m, 1H, OCH sp), 4.316 (dd, 1H, H-4'', J $_{3,4}$ 3.2 Hz, J $_{4,5}$ ~1 Hz), 4.343 (dd, 1H, H-6b', J $_{6a,6b}$ 11.3 Hz, J $_{5,6}$ 4.3 Hz), 4.367 (dd, 1H, H-3'', J $_{3,4}$ 3.2 Hz, J $_{2,3}$ 9.9 Hz), 4.466 (dd, 1H, H-6a', J $_{6a,6b}$ 11.3 Hz, J $_{5,6a}$ 1.3 Hz), 4.575 (d, 1H, H-1', J $_{1,2}$ 8.2 Hz), 4.652 (d, 1H, H-1'', J $_{1,2}$ 7.8 Hz). MS, m/z : 667 [M + Na $^+$]. [α] $_D$ -0° (c 1, H $_2$ O)

3-O-Su-Gal β 1-4GlcNAc β O(CH $_2$) $_2$ NH $_2$ (**17**): (500 MHz, D $_2$ O) δ : 2.071 (s, 3H, NCOMe), 3.245 (m, 2H, NCH $_2$ sp), 3.645 (m, 1H H-5'), 3.710 (dd, 1H, H-2'', J $_{2,3}$ 9.9 Hz, J $_{1,2}$ 7.9 Hz), 3.73 – 3.85 (m, 6H, H-2', H-3' and H-4', H-5, H-6a'' and H-6b''), 3.887 (dd, 1H, H-6b', J $_{6a,6b}$ 12.4 Hz, J $_{5,6b}$ 5.0 Hz), 3.928 (m, 1H, OCH sp), 4.026 (dd, 1H, H-6a', J $_{6a,6b}$ 12.4 Hz, J $_{5,6a}$ 2.1 Hz), 4.079 (m, 1H, OCH sp), 4.312 (dd, 1H, H-4'', J $_{3,4}$ 3.4 Hz, J $_{4,5}$ ~1 Hz), 4.357 (dd, 1H, H-3'', J $_{2,3}$ 9.9 Hz, J $_{3,4}$ 3.4 Hz), 4.607 (d, 1H, H-1', J $_{1,2}$ 8.2 Hz), 4.610 (d, 1H, H-1'', J $_{1,2}$ 7.8 Hz). MS, m/z : 551 [M + Na $^+$]. [α] $_D$ -9.6° (c 1, H $_2$ O)

3,6-di-O-Su-Gal β 1-4GlcNAc β O(CH $_2$) $_2$ NH $_2$ (**18**): (500 MHz, D $_2$ O) δ : 2.073 (s, 3H, NCOMe), 3.238 (m, 2H, NCH $_2$ sp), 3.66 – 3.71 (m, 1H, H-5'), 3.717 (dd, 1H, H-2'', J $_{1,2}$ 7.9 Hz, J $_{2,3}$ 9.5 Hz), 3.75 – 3.85 (m, 3H, H-2', H-3' and H-4'), 3.885 (dd, 1H, H-6b', J $_{5,6b}$ 5.2 Hz, J $_{6a,6b}$ 12.4 Hz), 3.925 (m, 1H, OCH sp), 4.027 (dd, 1H, H-6a', J $_{5,6a}$ 2.1 Hz, J $_{6a,6b}$ 12.4 Hz), 4.037 (m, 1H, H-5''), 4.073 (m, 1H, OCH sp), 4.238 (m, 2H, H-6a'', H-6b''), 4.362 (dd, 1H, H-4'', J $_{3,4}$ 3.4

Hz, $J_{4,5} \sim 1$ Hz), 4.381 (dd, 1H, H-3'', $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 3.4 Hz), 4.604 (m, 1H, H-1'), 4.657 (d, 1H, H-1'', $J_{1,2}$ 7.8 Hz). MS, m/z : 653 [M + Na⁺]. $[\alpha]_D -5.8^\circ$ (c 1, H₂O)

Neu5Ac α 2-6Gal β 1-4(6-O-Su)GlcNAc β O(CH₂)₃NH₂ (**20**): (500 MHz, D₂O) δ : 1.728 (dd, 1H, H-3ax''', $J_{3ax,3eq}$ 12.4 Hz, $J_{3ax,4}$ 12.0 Hz), 1.985 (m, 2H, CH₂ sp), 2.047 (s, 3H, NCOMe), 2.089 (s, 3H, NCOMe), 2.689 (dd, 1H, H-3eq''', $J_{3eq,3ax}$ 12.4 Hz, $J_{3eq,4}$ 4.7 Hz), 3.140 (m, 2H, NCH₂ sp), 3.52 – 3.59 (m, 3H, H-7''', H-2'' and H-6''), 3.663 (dd, 1H, H-9b''', $J_{9a,9b}$ 12.1 Hz, $J_{8,9b}$ 6.0 Hz), 3.66 – 3.72 (m, 3H), 3.723 (dd, 1H, H-6''', $J_{6,7}$ 1.5 Hz, $J_{5,6}$ 10.5 Hz), 3.76 – 3.93 (m, 9H), 3.948 (dd, 1H, H-4'', $J_{3,4}$ 3.4 Hz, $J_{4,5} \sim 1$ Hz), 3.99 – 4.05 (m, 2H, OCH sp, H-6a''), 4.314 (dd, 1H, H-6b', $J_{6a,6b}$ 11.3, $J_{5,6b}$ 5.8 Hz), 4.471 (dd, 1H, H-6a', $J_{6a,6b}$ 11.3 Hz, $J_{5,6a}$ 1.5 Hz), 4.496 (d, 1H, H-1'', $J_{1,2}$ 7.8 Hz), 4.598 (d, 1H, H-1', $J_{1,2}$ 8.2 Hz). MS, m/z : 879 [M + Na⁺]. $[\alpha]_D -19.0^\circ$ (c 1, H₂O)