

## Stereo- and regioselective synthesis of spacer armed $\alpha$ 2-6 sialooligosaccharides

Galina V. Pazykina, Svetlana V. Tsygankova, Marina A. Sablina, Alexander S. Paramonov, Alexander B. Tuzikov and Nicolai V. Bovin

### *Spectral characteristics for oligosaccharides.*

$^1\text{H}$  NMR spectra were registered on a Bruker AVANCE spectrometer at 303 K. Chemical shifts  $\delta$  for characteristic protons are given in ppm referenced to HOD ( $\delta$  4.750) or  $\text{CHCl}_3$  ( $\delta$  7.270), coupling constants  $J$ , are given in Hz. The signals in  $^1\text{H}$  NMR spectra were assigned using a technique of spin–spin decoupling and 2D- $^1\text{H}$ ,  $^1\text{H}$ -COSY experiments.  $^{13}\text{C}$  NMR spectra were recorded on a Bruker BioSpin GmbH instrument at 175 MHz. The values of optical rotation were measured on a digital polarimeter Perkin Elmer 341 at 25°C. Mass spectra were registered on a MALDI-TOF Vision-2000 spectrometer using dihydroxybenzoic acid as a matrix.

**Neu5Ac $\alpha$ 2-6Gal $\beta$ -O(CH $_2$ ) $_3$ NH $_2$  (13):**  $^1\text{H}$  NMR (700 MHz, D $_2$ O): 1.693 (dd  $\approx$  t, 1H, H-3 $_{\text{ax}}$ b,  $J$  12.2); 1.969–2.098 (m, 2H, CH $_2$  sp); 2.060 (s, 3H, NCOCH $_3$ ); 2.769 (dd, 1H, H-3 $_{\text{eq}}$ b,  $J_{3_{\text{ax}},3_{\text{eq}}}$  12.5,  $J_{3_{\text{eq}},4}$  4.7); 3.168–3.267 (m, 2H, NCH $_2$  sp); 3.537 (dd, 1H, H-2a,  $J_{1,2}$  8.0,  $J_{2,3}$  9.9); 3.603–3.690 (m, 4H, H-7b, H-9'b, H-3a, H-6'a); 3.715 (ddd, 1H, H-4b,  $J_{3_{\text{ax}},4}$  11.8,  $J_{3_{\text{eq}},4}$  4.7,  $J_{4,5}$  9.9); 3.742 (dd, 1H, H-6b,  $J_{5,6}$  10.5,  $J_{6,7}$  1.6); 3.801–3.859 (m, 2H, H-5a; H-5b,  $J_{4,5}\approx J_{5,6}\approx 10.1$ ); 3.872–3.920 (m, 2H, H-8b, H-9''b); 3.922–3.967 (m, 2H, OCH sp, H-4a); 3.977 (dd, 1H, H-6''a,  $J_{6',6''}$  10.4,  $J_{6'',5}$  8.1); 3.999–4.055 (m, 1H, OCH sp); 4.442 (d, 1H, H-1a,  $J_{1,2}$  8.0).  $^{13}\text{C}$  NMR (176 MHz, D $_2$ O):  $\delta$  175.17 (N-C(O)-CH $_3$ ); 173.31 (C-1b); 103.05 (C-1a); 100.49 (C-2b); 73.54, 72.68, 72.60, 71.83, 70.66, 68.70, 68.23, 68.21, 68.18 (C-2a, C-3a, C-4a, C-5a, C-4b, C-6b, C-7b, C-8b, NCH $_2$ CH $_2$ CH $_2$ O sp); 63.73, 62.70 (C-9b, C-6a); 51.93 (C-5b); 40.32 (C-3b); 37.73 (NCH $_2$ CH $_2$ CH $_2$ O sp); 26.72 (NCH $_2$ CH $_2$ CH $_2$ O sp); 22.07 (N-C(O)-CH $_3$ ).  $R_f$  0.54 (MeOH-MeCN-H $_2$ O, 3:3:2). MS,  $m/z$ : 529 [M+H $^+$ ] (calc. for C $_{20}$ H $_{36}$ N $_2$ O $_4$ ,  $m/z$ : 528,22 [M] $^+$ ). [ $\alpha$ ] $_{546\text{ nm}}$  -15 (c 1; MeCN-H $_2$ O, 1:1).

**Neu5Ac $\beta$ 2-6Gal $\beta$ -O(CH $_2$ ) $_3$ NH $_2$  (13 $\beta$ ):**  $^1\text{H}$  NMR (700 MHz, D $_2$ O): 1.682 (dd  $\approx$  t, 1H, H-3 $_{\text{ax}}$ b,  $J$  12.3); 1.955–2.129 (m, 2H, CH $_2$  sp); 2.072 (s, 3H, NCOCH $_3$ ); 2.394 (dd, 1H, H-3 $_{\text{eq}}$ b,  $J_{3_{\text{ax}},3_{\text{eq}}}$  13.2,  $J_{3_{\text{eq}},4}$  4.9); 3.152–3.265 (m, 2H, NCH $_2$  sp); 3.484 (dd, 1H, H-6'a,  $J_{5,6'}$  7.0,  $J_{6',6''}$  9.9); 3.549

(dd, 1H, H-2a,  $J_{1,2}$  7.9,  $J_{2,3}$  9.9); 3.562 (br. d, 1H, H-7b,  $J$  9.6); 3.669 (dd, 1H, H-3a,  $J_{2,3}$  9.9,  $J_{3,4}$  3.3); 3.692 (dd, 1H, H-9'b,  $J_{8,9'}$  5.8,  $J_{9',9''}$  12.1); 3.777 (dd, 1H, H-6''a,  $J_{6',6''}$  9.9,  $J_{6'',5}$  4.7); 3.817–3.954 (m, 6H); 3.969 (dd  $\approx$  d, 1H, H-4a,  $J$  3.0); 4.016–4.084 (m, 1H, OCH sp); 4.116 (ddd, 1H, H-4b,  $J_{3ax,4}$  11.4,  $J_{3eq,4}$  4.9,  $J_{4,5}$  9.9); 4.465 (d, 1H, H-1a,  $J_{1,2}$  7.9).  $R_f$  0.24 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS,  $m/z$ : 529 [M+H<sup>+</sup>] (calc. for C<sub>20</sub>H<sub>36</sub>N<sub>2</sub>O<sub>14</sub>,  $m/z$ : 528,22 [M]<sup>+</sup>).

**Neu5Ac $\alpha$ 2-6GalNAc $\beta$ -O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (15)**: <sup>1</sup>H NMR (800 MHz, D<sub>2</sub>O): 1.681 (dd  $\approx$  t, 1H, H-3<sub>ax</sub>b,  $J$  12.1); 1.922–1.998 (m, 2H, CH<sub>2</sub> sp); 2.044 and 2.052 (2s, 2 x 3H, NCOCH<sub>3</sub>); 2.752 (dd, 1H, H-3<sub>eq</sub>b,  $J_{3ax,3eq}$  12.4,  $J_{3eq,4}$  4.7); 3.109–3.166 (m, 2H, NCH<sub>2</sub> sp); 3.608 (dd, 1H, H-7b,  $J_{6,7}$  1.2,  $J_{7,8}$  9.0); 3.650 (dd, 1H, H-9' b,  $J_{9',9''}$  11.9,  $J_{8,9'}$  6.1); 3.655 (dd, 1H, H-3 a,  $J_{2,3}$  10.2,  $J_{3,4}$  3.5); 3.698 (ddd, 1H, H-4b,  $J_{3ax,4}$  11.8,  $J_{3eq,4}$  4.8,  $J_{4,5}$  10.1); 3.708–3.742 (m, 2H, H-6b, H-6'a); 3.785 (br.dd, 1H, H-5a,  $J_{5,6'}$  4.0,  $J_{5,6''}$  7.8); 3.797–3.845 (m, 2H, OCH sp, H-5 b); 3.863–3.925 (m, 3H, H-2 a, H-8 b, H-9'' b); 3.953 (dd  $\approx$  d, 1H, H-4a,  $J$  2.9); 3.957–4.010 (m, 2H, H-6''a, OCH sp); 4.443 (d, 1H, H-1a,  $J_{1,2}$  8.5).  $R_f$  0.50 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS,  $m/z$ : 570 [M+H<sup>+</sup>] (calc. for C<sub>22</sub>H<sub>39</sub>N<sub>3</sub>O<sub>14</sub>,  $m/z$ : 569.24 [M]<sup>+</sup>). [ $\alpha$ ]<sub>546 nm</sub> –4 (c 0.3, MeCN:H<sub>2</sub>O 1:1).

**Neu5Ac $\beta$ 2-6GalNAc $\beta$ -O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (15 $\beta$ )**: <sup>1</sup>H NMR (800 MHz, D<sub>2</sub>O): 1.665 (dd, 1H, H-3<sub>ax</sub>b,  $J_{3ax,3eq}$  13.1,  $J_{3ax,4}$  11.6); 1.924–1.994 (m, 2H, CH<sub>2</sub> sp); 2.057 (2s, 2 x 3H, NCOCH<sub>3</sub>); 2.384 (dd, 1H, H-3<sub>eq</sub>b,  $J_{3ax,3eq}$  13.2,  $J_{3eq,4}$  4.9); 3.095–3.158 (m, 2H, NCH<sub>2</sub> sp); 3.484 (dd, 1H, H-6'a,  $J_{6',6''}$  9.9,  $J_{6',5}$  6.7); 3.548 (dd, 1H, H-7b,  $J_{6,7}$  0.7,  $J_{7,8}$  9.1); 3.677 (dd, 1H, H-9'b,  $J_{9',9''}$  11.8,  $J_{8,9'}$  5.6); 3.733 (dd, 1H, H-3a,  $J_{3,4}$  3.3,  $J_{2,3}$  10.9); 3.759–3.927 (m, 8H); 3.982 (dd  $\approx$  d, 1H, H-4a,  $J$  3.0); 3.993–4.030 (m, 1H, OCH sp); 4.103 (ddd, 1H, H-4b,  $J_{3ax,4}$  11.5,  $J_{3eq,4}$  4.9,  $J_{4,5}$  9.7); 4.472 (d, 1H, H-1a,  $J_{1,2}$  8.5).  $R_f$  0.71 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS,  $m/z$ : 570 [M+H<sup>+</sup>] (calc. for C<sub>22</sub>H<sub>39</sub>N<sub>3</sub>O<sub>14</sub>,  $m/z$ : 569.24 [M]<sup>+</sup>).

**Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4Glc $\beta$ -O(CH<sub>2</sub>)<sub>2</sub>NH<sub>2</sub> (17)**: <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O): 1.726 (dd  $\approx$  t, 1H, H-3<sub>ax</sub>c,  $J$  12.2); 2.020 (s, 3H, NCOCH<sub>3</sub>); 2.704 (dd, 1H, H-3<sub>eq</sub>c,  $J_{3ax,3eq}$  12.4,  $J_{3eq,4}$  4.7); 3.266 (m  $\approx$  t, 2H, NCH<sub>2</sub> sp,  $J$  5.1); 3.397 (dd, 1H, H-2a,  $J_{1,2}$  8.1,  $J_{2,3}$  9.1); 3.526 (dd, 1H, H-2b,  $J_{1,2}$  7.9,  $J_{2,3}$  10.0); 3.557 (dd, 1H, H-7c,  $J_{6,7}$  1.8,  $J_{7,8}$  9.1); 3.600 (dd, 1H, H-6'b,  $J_{5,6'}$  3.6,  $J_{6',6''}$  10.4); 3.617–3.724 (m, 7H); 3.788–3.899 (m, 5H); 3.918–3.992 (m, 4H, OCH sp, H-4b, H-6''b, H-6''a); 4.103–4.148 (m, 1H, OCH sp); 4.421 (d, 1H, H-1b,  $J_{1,2}$  7.9); 4.542 (d, 1H, H-1a,  $J_{1,2}$  8.0). <sup>13</sup>C NMR (176 MHz, D<sub>2</sub>O):  $\delta$  174.99 (N-C(O)-CH<sub>3</sub>); 173.49 (C-1c); 103.18, 101.87, 100.34 (C-1b, C-1a, C-2c); 79.47, 74.72, 74.50, 73.79, 72.70, 72.57, 72.44, 71.85, 70.83, 68.57, 68.46, 68.42, 65.78 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-4c, C-6c, C-7c, C-8c, NCH<sub>2</sub>CH<sub>2</sub>O sp); 63.68, 62.71, 60.20 (C-9c, C-6b, C-6a); 51.83 (C-5c); 40.13 (C-3c); 39.46 (NCH<sub>2</sub>CH<sub>2</sub>O sp);

22.10 (N-C(O)-CH<sub>3</sub>). R<sub>f</sub> 0.34 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS, *m/z*: 677 [M+H<sup>+</sup>] (calc. for C<sub>25</sub>H<sub>44</sub>N<sub>2</sub>O<sub>19</sub>, *m/z*: 676.25 [M]<sup>+</sup>). [α]<sub>546 nm</sub> -18 (c 0.4, MeCN:H<sub>2</sub>O 1:1).

**Neu5Acβ2-6Galβ1-4Glcβ-O(CH<sub>2</sub>)<sub>2</sub>NH<sub>2</sub> (17β)**: <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O): 1.633 (dd, 1H, H-3<sub>ax</sub>c, *J*<sub>3ax,3eq</sub> 13.0, *J*<sub>3ax,4</sub> 11.6); 2.041 (s, 3H, NCOCH<sub>3</sub>); 2.403 (dd, 1H, H-3<sub>eq</sub>c, *J*<sub>3ax,3eq</sub> 13.1, *J*<sub>3eq,4</sub> 4.9); 3.266 (m ≈ t, 2H, NCH<sub>2</sub> sp, *J* 5.1); 3.382 (dd, 1H, H-2a, *J*<sub>1,2</sub> 8.0, *J*<sub>2,3</sub> 9.1); 3.488–3.557 (m, 3H, H-2b, H-6'b, H-7c); 3.607–3.718 (m, 5H); 3.736 (dd, 1H, H-6''b, *J*<sub>5,6''</sub> 4.3, *J*<sub>6',6''</sub> 10.2); 3.790–3.901 (m, 6H); 3.921–3.966 (m, 2H, H-4b, OCH sp); 3.984 (dd 1H, H-6''a, *J*<sub>5,6''</sub> 2.0, *J*<sub>6',6''</sub> 12.2 ); 4.083 (ddd, 1H, H-4c, *J*<sub>3ax,4</sub> 11.6, *J*<sub>3eq,4</sub> 4.9, *J*<sub>4,5</sub> 10.3); 4.107–4.151 (m, 1H, OCH sp); 4.439 (d, 1H, H-1b, *J*<sub>1,2</sub> 7.8); 4.548 (d, 1H, H-1a, *J*<sub>1,2</sub> 8.0). R<sub>f</sub> 0.11 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS, *m/z*: 677 [M+H<sup>+</sup>] (calc. for C<sub>25</sub>H<sub>44</sub>N<sub>2</sub>O<sub>19</sub>, *m/z*: 676.25 [M]<sup>+</sup>).

**Neu5Acα2-6Galβ1-3GalNAcα-O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (19)**: <sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O): 1.684 (dd ≈ t, 1H, H-3<sub>ax</sub>c, *J* 12.1); 1.987–2.055 (m, 2H, CH<sub>2</sub> sp); 2.044 and 2.058 (2s, 2 x 3H, NCOCH<sub>3</sub>); 2.751 (dd, 1H, H-3<sub>eq</sub>c, *J*<sub>3ax,3eq</sub> 12.4, *J*<sub>3eq,4</sub> 4.7); 3.128–1.198 (m, 2H, NCH<sub>2</sub> sp); 3.532 (dd, 1H, H-2b, *J*<sub>1,2</sub> 7.8, *J*<sub>2,3</sub> 9.9); 3.578–3.615 (m, 1H, OCH sp); 3.609 (dd, 1H, H-7c, *J*<sub>6,7</sub> 1.5, *J*<sub>7,8</sub> 9.0); 3.631 (dd, 1H, H-3b, *J*<sub>2,3</sub> 9.9, *J*<sub>3,4</sub> 3.5); 3.662 (dd, 1H, H-9'c, *J*<sub>9',9''</sub> 12.5, *J*<sub>8,9'</sub> 6.8); 3.677–3.737 (m, 3H, H-4c, H-6c, H-6'b); 3.747 (br. dd, 1H, H-5b, *J*<sub>5,6'</sub> 4.5, *J*<sub>5,6''</sub> 7.7, *J*<sub>4,5</sub> ≤ 1); 3.820–3.858 (m, 4H, H-6'a, H-6''a, OCH sp; H-5c, *J* 10.0); 3.877–3.913 (m, 2H, H-8c, H-9''c); 3.932 (dd, 1H, H-6''b, *J*<sub>5,6''</sub> 7.7, *J*<sub>6',6''</sub> 10.5); 3.946 (dd ≈ d, 1H, H-4b, *J* 2.9); 4.004 (br. t, 1H, H-5a, *J* 6.1); 4.063 (dd, 1H, H-3a, *J*<sub>2,3</sub> 11.1, *J*<sub>3,4</sub> 3.1); 4.262 (dd ≈ d, 1H, H-4a, *J* 2.8); 4.354 (dd, 1H, H-2a, *J*<sub>1,2</sub> 3.8, *J*<sub>2,3</sub> 11.1); 4.471 (d, 1H, H-1b, *J*<sub>1,2</sub> 7.8); 4.924 (d, 1H, H-1a, *J*<sub>1,2</sub> 3.8). R<sub>f</sub> 0.52 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS, *m/z*: 754 [M+Na<sup>+</sup>] (calc. for C<sub>28</sub>H<sub>49</sub>N<sub>3</sub>O<sub>19</sub>, *m/z*: 731.30 [M]<sup>+</sup>). [α]<sub>546 nm</sub> +61 (c 0.4, MeCN:H<sub>2</sub>O 1:1).

**Neu5Acβ2-6Galβ1-3GalNAcα-O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (19β)**: <sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O): 1.668 (dd, 1H, H-3<sub>ax</sub>c, *J*<sub>3ax,3eq</sub> 13.0, *J*<sub>3ax,4</sub> 11.6); 1.973–2.059 (m, 2H, CH<sub>2</sub> sp); 2.050 and 2.072 (2s, 2 x 3H, NCOCH<sub>3</sub>); 2.396 (dd, 1H, H-3<sub>eq</sub>c, *J*<sub>3ax,3eq</sub> 13.1, *J*<sub>3eq,4</sub> 4.9); 3.124–3.195 (m, 2H, NCH<sub>2</sub> sp); 3.443 (dd, 1H, H-6'b, *J*<sub>5,6'</sub> 5.2, *J*<sub>6',6''</sub> 9.2); 3.521–3.567 (m, 2H, H-2b, H-7c); 3.584–3.620 (m, 1H, OCH sp); 3.640 (dd, 1H, H-3b, *J*<sub>2,3</sub> 9.9, *J*<sub>3,4</sub> 3.4); 3.684 (dd, 1H, H-9'c, *J*<sub>9',9''</sub> 12.2, *J*<sub>8,9'</sub> 6.2); 3.727–3.916 (m, 9H); 3.993 (br. dd, 1H, H-5a, *J*<sub>5,6'</sub> 4.9, *J*<sub>5,6''</sub> 7.9); 4.008 (dd ≈ d, 1H, H-4b, *J* 3.4); 4.060 (dd, 1H, H-3a, *J*<sub>2,3</sub> 11.1, *J*<sub>3,4</sub> 3.1); 4.075–4.132 (m, 1H, H-4c); 4.238 (dd ≈ d, 1H, H-4a, *J* 2.9); 4.357 (dd, 1H, H-2a, *J*<sub>1,2</sub> 3.8, *J*<sub>2,3</sub> 11.1); 4.500 (d, 1H, H-1b, *J*<sub>1,2</sub> 7.8); 4.919 (d, 1H, H-1a, *J*<sub>1,2</sub> 3.8). R<sub>f</sub> 0.21 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS, *m/z*: 754 [M+Na<sup>+</sup>] (calc. for C<sub>28</sub>H<sub>49</sub>N<sub>3</sub>O<sub>19</sub>, *m/z*: 731.30 [M]<sup>+</sup>).

**Neu5Ac $\alpha$ 2-6(Gal $\beta$ 1-3)GalNAc $\alpha$ -O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (20):** <sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O): 1.695 (dd  $\approx$  t, 1H, H-3<sub>axc</sub>, *J* 12,2); 2.010–2.052 (m, 2H, CH<sub>2</sub> sp); 2.043 and 2.057 (2s, 2 x 3H, NCOCH<sub>3</sub>); 2.736 (dd, 1H, H-3<sub>eqc</sub>, *J*<sub>3ax,3eq</sub> 12.4, *J*<sub>3eq,4</sub> 4.7); 3.145 (m  $\approx$  t, 2H, NCH<sub>2</sub> sp, *J* 7.6); 3.545 (dd, 1H, H-2b, *J*<sub>1,2</sub> 7.9, *J*<sub>2,3</sub> 9.9); 3.575–3.624 (m, 2H, H-7c, OCH sp); 3.635 (dd, 1H, H-3b, *J*<sub>2,3</sub> 9.9, *J*<sub>3,4</sub> 3.4); 3.649–3.734 (m, 5H, H-6'a, H-5b, H-4c, H-6c, H-9'c); 3.750 (dd, 1H, H-6'b, *J*<sub>5,6'</sub> 4.1, *J*<sub>6',6''</sub> 11.8); 3.806 (dd, 1H, H-6''b, *J*<sub>5,6''</sub> 8.1, *J*<sub>6',6''</sub> 11.8); 3.814–3.850 (m, 1H, OCH sp); 3.846 (dd  $\approx$  t, 1H, H-5c, *J* 10.1); 3.878–3.948 (m, 4H, H-6''a, H-4b, H-8c, H-9''c); 4.026 (dd, 1H, H-3a, *J*<sub>3,4</sub> 3.1, *J*<sub>2,3</sub> 11.1); 4.071 (br. dd, 1H, H-5a, *J*<sub>5,6'</sub> 5.9, *J*<sub>5,6''</sub> 8.2); 4.286 (dd  $\approx$  d, 1H, H-4a, *J* 2.9); 4.346 (dd, 1H, H-2a, *J*<sub>2,3</sub> 11.1, *J*<sub>1,2</sub> 3.8); 4.471 (d, 1H, H-1b, *J*<sub>1,2</sub> 7.8); 4.912 (d, 1H, H-1a, *J*<sub>1,2</sub> 3.8). <sup>13</sup>C NMR (176 MHz, D<sub>2</sub>O):  $\delta$  175.09, 174.54 (2 x N-C(O)-CH<sub>3</sub>); 173.45 (C-1c); 104.78 (C-1b); 100.42 (C-2c); 97.35 (C-1a); 77.17, 75.05, 2x72.62, 71.83, 70.63, 69.42, 68.70, 68.66, 68.31, 68.28, 65.23 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-4c, C-6c, C-7c, C-8c, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O sp); 63.83, 62.73, 61.05 (C-9c, C-6b, C-6a); 51.90 (C-5c); 48.57 (C-2a); 40.14 (C-3c); 37.22 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O sp); 26.83 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O sp); 22.08, 22.02 (2 x N-C(O)-CH<sub>3</sub>). R<sub>f</sub> 0.53 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS, *m/z*: 732 [M+H<sup>+</sup>] (calc. for C<sub>28</sub>H<sub>49</sub>N<sub>3</sub>O<sub>19</sub>, *m/z*: 731.30 [M]<sup>+</sup>). [ $\alpha$ ]<sub>546 nm</sub> +95 (c 0.3, MeCN:H<sub>2</sub>O 1:1).

**Neu5Ac $\beta$ 2-6(Gal $\beta$ 1-3)GalNAc $\alpha$ -O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (20 $\beta$ ):** <sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O): 1.669 (dd  $\approx$  t, 1H, H-3<sub>axc</sub>, *J* 12.3); 1.973–2.059 (m, 2H, CH<sub>2</sub> sp); 2.046 and 2.073 (2s, 2 x 3H, NCOCH<sub>3</sub>); 2.384 (dd, 1H, H-3<sub>eqc</sub>, *J*<sub>3ax,3eq</sub> 13.1, *J*<sub>3eq,4</sub> 4.9); 3.153 (t  $\approx$  m, 2H, NCH<sub>2</sub> sp, *J* 7.6); 3.408 (dd, 1H, H-6'a, *J*<sub>5,6'</sub> 5.4, *J*<sub>6',6''</sub> 9.6); 3.541–3.584 (m, 2H, H-2b, H-7c); 3.604–3.648 (m, 1H, OCH sp); 3.651 (dd, 1H, H-3b, *J*<sub>2,3</sub> 9.9, *J*<sub>3,4</sub> 3.4); 3.665–3.714 (m, 2H, H-5b, H-9'c); 3.763 (dd, 1H, H-6''a, *J*<sub>5,6''</sub> 8.2, *J*<sub>6',6''</sub> 9.6); 3.780–3.848 (m, 3H, OCH sp, H-6'b, H-6''b); 3.866–3.927 (m, 4H, H-5c, H-6c, H-8c, H-9c); 3.940 (dd  $\approx$  d, 1H, H-4b, *J* 3.3); 4.055 (dd, 1H, H-3a, *J*<sub>2,3</sub> 11.1, *J*<sub>3,4</sub> 3.0); 4.046–4.091 (m, 1H, H-4c); 4.125 (br. t, H-5a, *J* 6.7); 4.345–4.389 (m, 2H, H-2a, H-4a); 4.505 (d, 1H, H-1b, *J*<sub>1,2</sub> 7.8); 4.913 (d, 1H, H-1a, *J*<sub>1,2</sub> 3.7). R<sub>f</sub> 0.25 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS, *m/z*: 732 [M+H<sup>+</sup>] (calc. for C<sub>28</sub>H<sub>49</sub>N<sub>3</sub>O<sub>19</sub>, *m/z*: 731.30 [M]<sup>+</sup>).

**Neu5Ac $\alpha$ 2-6(Gal $\alpha$ 1-3)GalNAc $\alpha$ -O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (21):** <sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O): 1.708 (dd  $\approx$  t, 1H, H-3<sub>axc</sub>, *J* 12.2); 1.995–2.085 (m, 2H, CH<sub>2</sub> sp); 2.057 and 2.074 (2s, 2 x 3H, NCOCH<sub>3</sub>); 2.745 (dd, 1H, H-3<sub>eqc</sub>, *J*<sub>3ax,3eq</sub> 12.5, *J*<sub>3eq,4</sub> 4.7); 3.153 (m  $\approx$  t, 2H, NCH<sub>2</sub> sp, *J* 7.6); 3.602 (dd, 1H, H-7c, *J*<sub>6,7</sub> 1.6, *J*<sub>7,8</sub> 8.8); 3.616–3.651 (m, 1H, OCH sp); 3.665 (dd, 1H, H-9'c, *J*<sub>9',9''</sub> 12.4, *J*<sub>8,9'</sub> 6.9); 3.688–3.725 (m, 1H, H-4c); 3.713 (dd, 1H, H-6c, *J*<sub>5,6</sub> 10.3, *J*<sub>6,7</sub> 1.7); 3.735–3.799 (m, 4H, H-6'a, H-3b, H-6'b, H-6''b); 3.800–3.920 (m, 6H); 3.940 (dd, 1H, H-6''a, *J*<sub>5,6''</sub> 8.2, *J*<sub>6',6''</sub> 10.7); 3.999 (dd  $\approx$  d, 1H, H-4b, *J* 3.1); 4.018 (dd, 1H, H-3a, *J*<sub>2,3</sub> 11.0, *J*<sub>3,4</sub> 3.0); 4.036 (br. dd, 1H, H-5a,

$J_{5,6'}$  4.0,  $J_{5,6''}$  8.2); 4.272 (dd  $\approx$  d, 1H, H-4a,  $J$  2.8); 4.402 (dd, 1H, H-2a,  $J_{1,2}$  3.8,  $J_{2,3}$  11.1); 4.919 (d, 1H, H-1a,  $J_{1,2}$  3.8); 5.147 (d, 1H, H-1b,  $J_{1,2}$  3.9).  $^{13}\text{C}$  NMR (176 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  175.10, 174.29 ( $2 \times \text{N-C(O)-CH}_3$ ); 173.44 (C-1c); 100.46 (C-2c); 97.35, 95.12 (C-1a, C-1b); 72.66, 71.85, 71.38, 69.50, 69.44, 69.23, 68.30, 68.29, 68.07, 65.28, 64.78, 63.84, 62.74, 61.10 (C-3a, C-4a, C-5a, C-6a, C-2b, C-3b, C-4b, C-5b, C-6b, C-4c, C-6c, C-7c, C-8c, C-9c,  $\text{NCH}_2\text{CH}_2\text{CH}_2\text{O}$  sp); 51.89 (C-5c); 47.92 (C-2a); 40.10 (C-3c); 37.21 ( $\text{NCH}_2\text{CH}_2\text{CH}_2\text{O}$  sp); 26.87 ( $\text{NCH}_2\text{CH}_2\text{CH}_2\text{O}$  sp); 22.08, 22.05 ( $2 \times \text{N-C(O)-CH}_3$ ).  $R_f$  0.50 (MeOH-MeCN- $\text{H}_2\text{O}$ , 3:3:2). MS,  $m/z$ : 732 [ $\text{M}+\text{H}^+$ ] (calc. for  $\text{C}_{28}\text{H}_{49}\text{N}_3\text{O}_{19}$ ,  $m/z$ : 731.30 [ $\text{M}^+$ ]).  $[\alpha]_{546\text{ nm}}^{25} +148$  (c 0.85, MeCN: $\text{H}_2\text{O}$  1:1).

**Neu5Ac $\beta$ 2-6(Gal $\alpha$ 1-3)GalNAc $\alpha$ -O(CH $_2$ ) $_3$ NH $_2$  (21 $\beta$ ):**  $^1\text{H}$  NMR (700 MHz,  $\text{D}_2\text{O}$ ): 1.671 (dd, 1H, H-3 $_{\text{ax,c}}$ ,  $J_{3_{\text{ax}},3_{\text{eq}}}$  13.0,  $J_{3_{\text{ax}},4}$  11.6); 1.971–2.074 (m, 2H,  $\text{CH}_2$  sp); 2.072 and 2.076 (2s,  $2 \times 3\text{H}$ ,  $\text{NCOCH}_3$ ); 2.403 (dd, 1H, H-3 $_{\text{eq,c}}$ ,  $J_{3_{\text{ax}},3_{\text{eq}}}$  13.1,  $J_{3_{\text{eq}},4}$  4.9); 3.145 (m  $\approx$  t, 2H,  $\text{NCH}_2$  sp,  $J$  7.6); 3.443 (dd, 1H, H-6'a,  $J_{5,6'}$  5.4,  $J_{6',6''}$  9.9); 3.566 (br.d, 1H, H-7c,  $J$  9.1); 3.633–3.676 (m, 1H,  $\text{OCH}$  sp); 3.687 (dd, 1H, H-9'c,  $J_{8,9'}$  5.7,  $J_{9',9''}$  11.8); 3.743–3.830 (m, 5H); 3.854 (dd, 1H, H-2b,  $J_{1,2}$  3.9,  $J_{2,3}$  10.4); 3.868–3.928 (m, 5H); 4.009 (dd  $\approx$  d, 1H, H-4b,  $J$  3.0); 4.029 (dd, 1H, H-3a,  $J_{2,3}$  11.1,  $J_{3,4}$  2.9); 4.058–4.090 (br. t, 1H, H-5a,  $J$  6.8); 4.107 (ddd, 1H, H-4c,  $J_{3_{\text{ax}},4}$  11.3,  $J_{3_{\text{eq}},4}$  4.9,  $J_{4,5}$  9.0); 4.354 (dd  $\approx$  d, 1H, H-4a,  $J$  2.5); 4.419 (dd, 1H, H-2a,  $J_{1,2}$  3.8,  $J_{2,3}$  11.1); 4.921 (d, 1H, H-1a,  $J_{1,2}$  3.8); 5.201 (d, 1H, H-1b,  $J_{1,2}$  3.9).  $R_f$  0.23 (MeOH-MeCN- $\text{H}_2\text{O}$ , 3:3:2). MS,  $m/z$ : 732 [ $\text{M}+\text{H}^+$ ] (calc. for  $\text{C}_{28}\text{H}_{49}\text{N}_3\text{O}_{19}$ ,  $m/z$ : 731.30 [ $\text{M}^+$ ]).

**Neu5Ac $\alpha$ 2-6(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ -O(CH $_2$ ) $_3$ NH $_2$  (22):**  $^1\text{H}$  NMR (700 MHz,  $\text{D}_2\text{O}$ ): 1.247 (d, 1H, H-6c,  $J_{5,6}$  6.6); 1.718 (dd  $\approx$  t, 1H, H-3 $_{\text{ax,d}}$ ,  $J$  12.2); 1.945–2.005 (m, 2H,  $\text{CH}_2$  sp); 2.048 and 2.089 (2s,  $2 \times 3\text{H}$ ,  $\text{NCOCH}_3$ ); 2.715 (dd, 1H, H-3 $_{\text{eq,d}}$ ,  $J_{3_{\text{ax}},3_{\text{eq}}}$  12.4,  $J_{3_{\text{eq}},4}$  4.6); 3.116 (m  $\approx$  t, 2H,  $\text{NCH}_2$  sp,  $J$  6.9); 3.543 (m  $\approx$  t, 1H, H-5a,  $J$  6.9); 3.574 (dd, 1H, H-7d,  $J_{6,7}$  1.5,  $J_{7,8}$  8.9); 3.610–3.849 (m, 15H); 3.870–3.914 (m, 3H, H-3b, H-8d, H-9'd); 3.930 (dd  $\approx$  d, 1H, H-4b,  $J$  3.3); 3.973 (dd, 1H, H-6'b,  $J_{5,6'}$  8.2,  $J_{6',6''}$  10.2); 3.993 (dd, 1H, H-6''a,  $J_{5,6''}$  1.2,  $J_{6',6''}$  12.0); 4.025–4.068 (m, 1H,  $\text{OCH}$  sp); 4.232 (br. q, 1H, H-5c,  $J_{5,6}$  6.6); 4.538 (d, 1H, H-1a,  $J_{1,2}$  8.2); 4.549 (d, 1H, H-1b,  $J_{1,2}$  7.8); 5.359 (br. s, 1H, H-1c).  $^{13}\text{C}$  NMR (176 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  174.98, 174.79 ( $2 \times \text{N-C(O)-CH}_3$ ); 173.44 (C-1d); 101.06 (C-1b); 100.60 (C-1a); 100.42 (C-2d); 99.26 (C-1c); 77.89, 75.90, 75.20, 73.52, 73.48, 72.65, 71.78, 71.74, 69.63, 69.02, 68.44, 68.37, 68.26, 68.03, 66.94 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-2c, C-3c, C-4c, C-5c, C-4d, C-6d, C-7d, C-8d,  $\text{NCH}_2\text{CH}_2\text{CH}_2\text{O}$  sp); 63.41 (C-9d); 62.74, 60.55 (C-6b, C-6a); 55.39 (C-2a); 51.87 (C-5d); 40.10 (C-3d); 37.75 ( $\text{NCH}_2\text{CH}_2\text{CH}_2\text{O}$  sp); 26.66 ( $\text{NCH}_2\text{CH}_2\text{CH}_2\text{O}$  sp); 22.29, 22.09 ( $2 \times \text{N}$

C(O)-CH<sub>3</sub>); 15.35 (C-6c). R<sub>f</sub> 0.40 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS, *m/z*: 878 [M+H<sup>+</sup>] (calc. for C<sub>34</sub>H<sub>59</sub>N<sub>3</sub>O<sub>23</sub>, *m/z*: 877.35 [M]<sup>+</sup>). [α]<sub>546 nm</sub> -96 (c 0.4, MeCN:H<sub>2</sub>O 1:1).

**Neu5Acβ2-6(Fuca1-2)Galβ1-4GlcNAcβ-O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (22β)**: <sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O): 1.253 (d, 1H, H-6c, *J*<sub>5,6</sub> 6.6); 1.661 (dd ≈ t, 1H, H-3<sub>ax</sub>d, *J* 12.3); 1.942–2.003 (m, 2H, CH<sub>2</sub> sp); 2.067 and 2.074 (2s, 2 x 3H, NCOCH<sub>3</sub>); 2.397 (dd, 1H, H-3<sub>eq</sub>d, *J*<sub>3ax,3eq</sub> 13.1, *J*<sub>3eq,4</sub> 4.9); 3.106 (t ≈ m, 2H, NCH<sub>2</sub> sp, *J* 6.9); 3.496–3.560 (m, 3H, H-5a, H-6<sup>′</sup>b, H-7d); 3.682 (dd, 1H, H-9<sup>′</sup>d, *J*<sub>8,9<sup>′</sup></sub> 5.9, *J*<sub>9<sup>′</sup>,9<sup>′′</sup></sub> 12.2); 3.699–3.853 (m, 12H); 3.855–3.914 (m, 4H); 3.970 (dd ≈ d, 1H, H-4b, *J* 3.4); 4.012 (dd, 1H, H-6<sup>′</sup>a, *J*<sub>5,6<sup>′</sup></sub> 1.2, *J*<sub>6<sup>′</sup>,6<sup>′′</sup></sub> 11.7); 4.027–4.068 (m, 1H, OCH sp); 4.094 (ddd, 1H, H-4d, *J*<sub>3ax,4</sub> 11.2, *J*<sub>3eq,4</sub> 5.0, *J*<sub>4,5</sub> 10.4); 4.241 (br. q, 1H, H-5c, *J*<sub>5,6</sub> 6.6); 4.518 (d, 1H, H-1a, *J*<sub>1,2</sub> 8.3); 4.575 (d, 1H, H-1b, *J*<sub>1,2</sub> 7.7); 5.352 (br. s, 1H, H-1c). R<sub>f</sub> 0.19 (MeOH-MeCN-H<sub>2</sub>O, 3:3:2). MS, *m/z*: 878 [M+H<sup>+</sup>] (calc. for C<sub>34</sub>H<sub>59</sub>N<sub>3</sub>O<sub>23</sub>, *m/z*: 877.35 [M]<sup>+</sup>).

**(23a)**: <sup>1</sup>H NMR (800 MHz, CDCl<sub>3</sub>): 1.774–1.825, (m, 1H, CH sp); 1.857–1.936 (m, 2H, CH sp; H-3<sub>ax</sub>e, *J* 12.2); 1.897, 1.912, 1.960, 1.968, 2.033, 2.045, 2.056, 2.071, 2.082, 2.092, 2.110, 2.123(2), 2.128, 2.147, 2.149 and 2.176 (17s, 17 x 3H, COCH<sub>3</sub>); 2.558 (dd, 1H, H-3<sub>eq</sub>e, *J*<sub>3ax,3eq</sub> 12.8, *J*<sub>3eq,4</sub> 4.6); 3.227–3.278 (m, 1H, NCH sp); 3.359 (dd, 1H, H-6<sup>′′</sup>d, *J*<sub>6<sup>′</sup>,6<sup>′′</sup></sub> 10.6, *J*<sub>5,6<sup>′′</sup></sub> 7.6); 3.538–3.665 (m, 5H, OCH and NCH sp, H-5a, H-5c, H-2c); 3.728–3.775 (m, 3H, H-4a, H-3b, H-6<sup>′</sup>d); 3.801 (ddd ≈ t, 1H, H-5b, *J* 6.7); 3.808 (s, 3H, COOCH<sub>3</sub> e); 3.853 (dd ≈ t, 1H, H-4c, *J* 9.1); 3.877–3.913 (m, 2H, H-5d, OCH sp); 3.995 (ddd ≈ q, 1H, H-5e, *J* 10.4); 4.010 (ddd ≈ q, 1H, H-2a, *J* 9.1); 4.023–4.092 (m, 5H, H-6<sup>′</sup> and H-6<sup>′′</sup> b, H-6<sup>′′</sup>c, H-9<sup>′′</sup>e, H-6e); 4.120 (dd, 1H, H-6<sup>′′</sup>a, *J*<sub>6<sup>′</sup>,6<sup>′′</sup></sub> 11.8, *J*<sub>5,6<sup>′′</sup></sub> 5.2); 4.348 (dd, 1H, H-9<sup>′</sup>e, *J*<sub>9<sup>′</sup>,9<sup>′′</sup></sub> 11.6, *J*<sub>8,9<sup>′</sup></sub> 1.9); 4.401 (2d, 2H, H-1a; H-1b, *J*<sub>1,2</sub> 7.9); 4.502 (dd, 1H, H-6<sup>′</sup>a, *J*<sub>6<sup>′</sup>,6<sup>′′</sup></sub> 11.8, *J*<sub>5,6<sup>′</sup></sub> 2.2); 4.596 (d, 1H, H-1d, *J*<sub>1,2</sub> 7.9); 4.670 (d, 1H, H-1c, *J*<sub>1,2</sub> 7.7); 4.735 (dd, 1H, H-6<sup>′</sup>c, *J*<sub>6<sup>′</sup>,6<sup>′′</sup></sub> 11.8, *J*<sub>5,6<sup>′</sup></sub> 2.1); 4.883 (ddd, 1H, H-4e, *J*<sub>3ax,4</sub> 11.8, *J*<sub>3eq,4</sub> 4.6, *J*<sub>4,5</sub> 10.7); 4.996–5.043 (m, 3H, H-3a, H-2b, H-3d); 5.091 (dd, 1H, H-2d, *J*<sub>1,2</sub> 7.9, *J*<sub>2,3</sub> 10.4); 5.127 (d, 1H, NHAc e, *J*<sub>2,NH</sub> 9.9); 5.175 (dd, 1H, H-3c, *J*<sub>3,4</sub> 9.2, *J*<sub>2,3</sub> 9.8); 5.306–5.364 (m, 2H, H-7e, H-8e); 5.339 (dd ≈ d, 1H, H-4b, *J* 3.3); 5.413 (dd ≈ d, 1H, H-4d, *J* 3.1); 5.495 (d, 1H, NHAc e, *J*<sub>2,NH</sub> 8.8); 5.821 (d, 1H, NHAc a, *J*<sub>2,NH</sub> 8.9); 7.402–7.441 (m, 1H, NHCF<sub>3</sub> sp). R<sub>f</sub> 0.35 (CHCl<sub>3</sub>-MeOH, 7:1).

**Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-O(CH<sub>2</sub>)<sub>3</sub>NH<sub>2</sub> (23)**: <sup>1</sup>H NMR (800 MHz, D<sub>2</sub>O): 1.733 (dd ≈ t, 1H, H-3<sub>ax</sub>e, *J* 12.1); 1.943–1.998 (m, 2H, CH<sub>2</sub> sp); 2.047, 2.063 and 2.073 (3s, 3 x 3H, NCOCH<sub>3</sub>); 2.689 (dd, 1H, H-3<sub>eq</sub>e, *J*<sub>3ax,3eq</sub> 12.5, *J*<sub>3eq,4</sub> 4.6); 3.104 (m ≈ t, 2H, NCH<sub>2</sub> sp, *J* 6.9); 3.540–3.586 (m, 3H, H-5a, H-2b, H-7c); 3.598–3.637 (m, 3H, H-5c, H-2d, H-6<sup>′</sup>d); 3.645–3.924 (m, 21H); 3.943 (dd ≈ d, 1H, H-4d, *J* 3.4); 3.968 (dd, 1H, H-6<sup>′′</sup>a, *J*<sub>6<sup>′</sup>,6<sup>′′</sup></sub> 12.3, *J*<sub>5,6<sup>′′</sup></sub>

1.6); 3.994–4.055 (m, 3H, H-6''d, H-6''c, OCH sp); 4.175 (dd  $\approx$  d, 1H, H-4b,  $J$  3.3); 4.473, 4.481 and 4.532 (3d, 3 x 1H, H-1a, H-1b, H-1d,  $J_{1,2}$  8.0); H-1c in H<sub>2</sub>O. <sup>13</sup>C NMR (176 MHz, D<sub>2</sub>O):  $\delta$  174.98, 174.95, 174.74 (3 x N-C(O)-CH<sub>3</sub>); 173.56 (C-1e); 103.48, 102.98, 102.59, 101.23 (C-1a, C-1b C-1c, C-1d); 100.21 (C-2e); 82.07, 80.54, 78.63, 74.95, 74.78, 74.34, 73.76, 72.59, 72.50, 72.26, 71.76, 70.79, 70.01, 68.48, 68.42, 68.31, 68.26, 67.97 (C-3a, C-4a, C5a, C-2b, C-3b, C-4b, C-5b, C-3c, C-4c, C-5c, C-2d, C-3d, C-4d, C-5d, C-4e, C-6e, C-7e, C-8e, NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O sp); 63.40 (C-9e); 62.73, 61.01, 60.24, 60.06 (C-6a, C-6b, C-6c, C-6d); 55.04 (C-2a, C-2c); 51.94 (C-5e); 40.14 (C-3e); 37.67 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O sp); 26.69 (NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O sp); 22.34, 22.17, 22.08 (3 x N-C(O)-CH<sub>3</sub>). R<sub>f</sub> 0.20 (MeOH-MeCN-H<sub>2</sub>O, 3:3:1.5). MS,  $m/z$ : 1098 [M+H<sup>+</sup>] (calc. for C<sub>42</sub>H<sub>72</sub>N<sub>4</sub>O<sub>29</sub>,  $m/z$ : 1097.03 [M]<sup>+</sup>). [ $\alpha$ ]<sub>546 nm</sub> -21 (c 0.2, MeCN:H<sub>2</sub>O 1:1).