Synthesis of complex α2-3 sialooligosaccharides, including sulfated and fucosylated ones, using Neu5Acα2-3Gal as a building block

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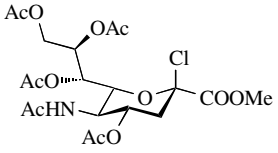
3-Aminopropyl glycosides of Neu5Acα2-3Galβ, Neu5Acα2-3Galβ1-4GlcNAcβ, Neu5Acα2-3Galβ1-4(6-HSO_3)GlcNAcβ, Neu5Acα2-3Galβ1-3GalNAcα, Neu5Acα2-3Galβ1-3(6-HSO_3)GalNAcα, Neu5Acα2-3Galβ1-3GlcNAcβ, Neu5Acα2-3Galβ1-3(6-HSO_3)GlcNAcβ, Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ and Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ were synthesised using protected Neu5Acα2-3Gal bromide as a glycosyl donor at the key stage.

Glycoprotein and glycolipid carbohydrate chains terminated by sialic acids play an especial role in immune response and cell recognition being specific receptors for siglec and selectin families of carbohydrate-recognising proteins.[1] An approach to the systematic understanding of structure–function relationships in sialylated glycoconjugates necessitates efficient regio- and stereoselective glycosylation.

Due to the presence of the electron-withdrawing carboxyl group and the lack of a participating substituent adjacent to the anomeric centre, sialylation often proceeds with low stereoselectivity (especially when a primary hydroxyl group is used as an acceptor[2]) and high 2,3-elimination rate. For searching more effective sialylation methods, the efforts were focused on modifying the catalyst promoter, glycosyl donor and reaction conditions. Thus, silver silicate, silver salicylate and a combination of silver carbonate with silver triflate together with donor **1** and also thioglycoside donors in a combination with various electrophilic promoters were used.[3] An additional group (Cl, Br or SeR; compound **2**) was introduced at the 3-position of neuraminic acid for co-participation in glycosylation resulting in an improved yield and selectivity; however, the group should be removed after the reaction.[4] The additional N-acylation of the 5-MeCONH group or its replacement with N_3 (compound **3**) improved the efficacy of glycosylation.[2,5,6] Besides, the hydroxyl groups at C^4 and C^9 were acylated by acetoxyacetic acid (compound **4**) which significantly increased α-stereoselectivity.[7] Although the above studies promoted an increase in the yield and/or α-stereoselectivity of sialylation, the advances were abolished by a multi-step synthesis of complicated Neu5Ac donors and the need of the elimination of auxiliary substituents.



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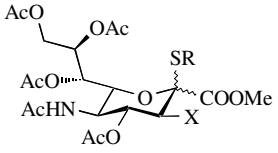
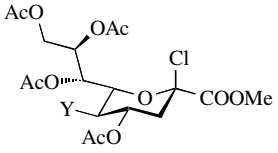


2

X = H, Cl, Br, SeR



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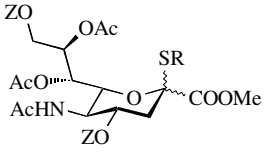
Y = Ac_2N, Boc(Ac)N, N_3



4

Z = $COCH_2OAc$

Recently, we reported a practical method of 2-6 sialooligosaccharide synthesis based on the classical Ag_2CO_3-promoted Koenigs–Knorr glycosylation.[8] Unfortunately, this simple approach failed in the case of 2-3 sialylation, thus confirming once more that 2-3 sialylation remains a difficult task. An alternative route in the 2-3 sialooligosaccharide synthesis is based on the use of a Neu5Acα2-3Gal block as a glycosyl donor. This approach allowed to synthesise a series of 2-3 sialooligosaccharides using peracetylated sialyl-galactosyl trichloroacetimidate.[9–11] We suggest a block synthesis strategy based on the use of acetylated Neu5Acα2-3Gal methyl ester bromide as a glycosyl donor. Once obtained, this building block further permits to synthesise in a divergent manner a wide set of complex oligosaccharides **5**–**13** containing a Neu5Acα2-3Gal motif, including fuco and sulfo derivatives, which are indispensable compounds from the glycobiological viewpoint.

Neu5Acα2-3Galβ-O$(CH_2)_3NH_2$ **5**

Neu5Acα2-3Galβ1-4GlcNAcβ-O$(CH_2)_3NH_2$ (3′SLN) **6**

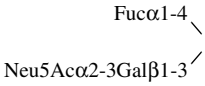
Neu5Acα2-3Galβ1-4(6-HSO_3)GlcNAcβ-O$(CH_2)_3NH_2$ (6-Su-3′SLN) **7**

Neu5Acα2-3Galβ1-3GalNAcα-O$(CH_2)_3NH_2$ (SiaTF) **8**

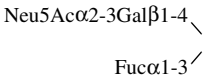
Neu5Acα2-3Galβ1-3(6-HSO_3)GalNAcα-O$(CH_2)_3NH_2$ (6-Su-SiaTF) **9**

Neu5Acα2-3Galβ1-3GlcNAcβ-O$(CH_2)_3NH_2$ (SiaLe^c) **10**

Neu5Acα2-3Galβ1-3(6-HSO_3)GlcNAcβ-O$(CH_2)_3NH_2$ (6-Su-SiaLe^c) **11**



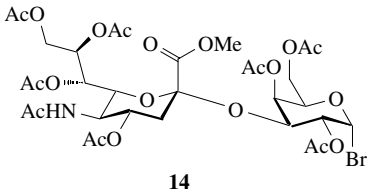
GlcNAcβ-O$(CH_2)_3NH_2$ (SiaLe^a) **12**



GlcNAcβ-O$(CH_2)_3NH_2$ (SiaLe^x) **13**

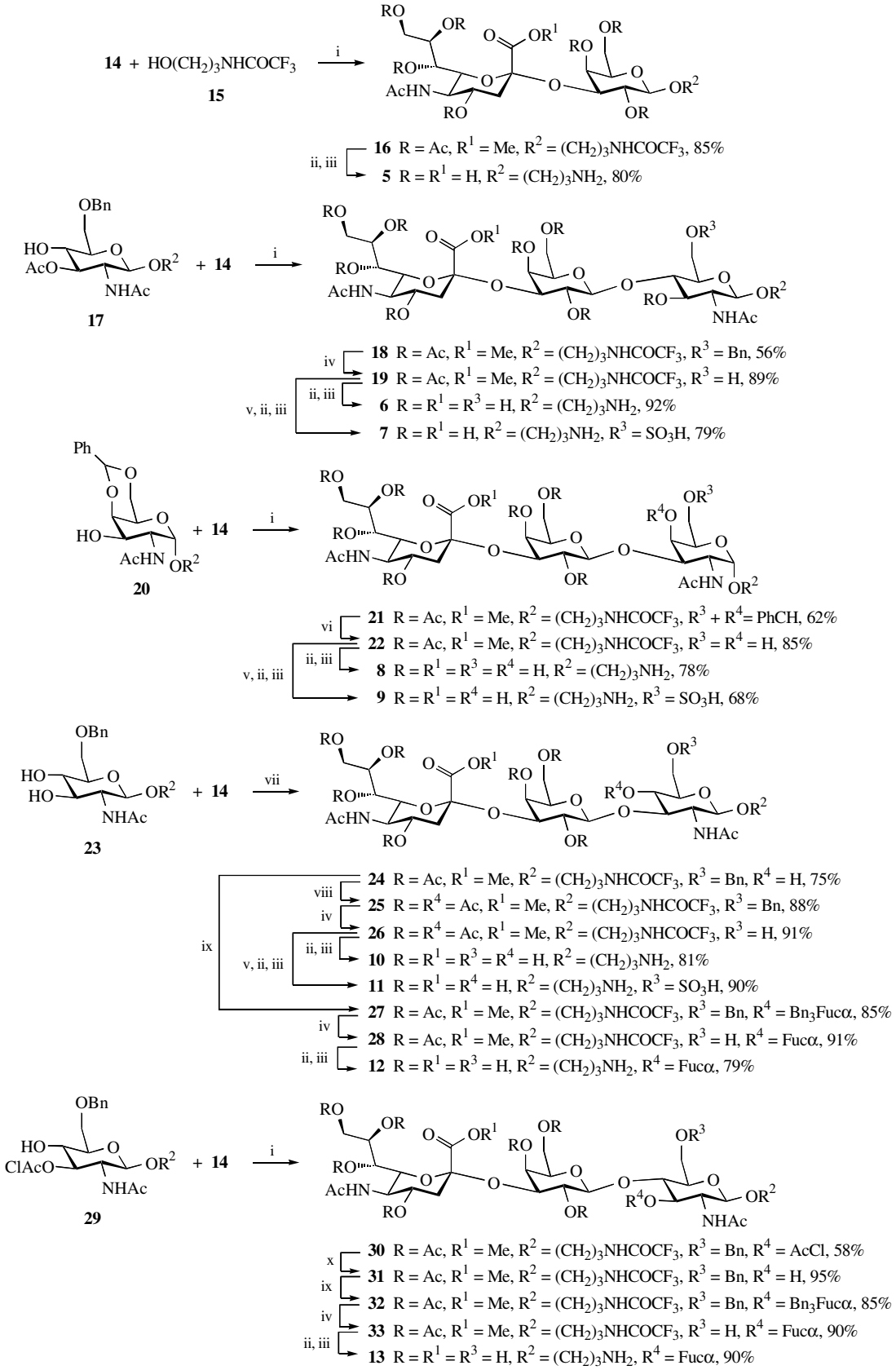
The glycosylation of glycosyl acceptors **17**,[12] **20**,[13] **23**[12] and **29**[14] with acetylated bromide **14**, which was obtained by treating a corresponding peracetate† with HBr in quantitative yield, was the key stage of the synthesis of spacered oligosaccharides **5**–**13**‡ (Scheme 1). Glycosylation positions and anomeric configurations were confirmed for protected compounds using ^1H NMR spectroscopy including spin decoupling and COSY. The positions of sulfation were confirmed by a low-field shift of hexosamine H-6 signals.

Glycosylation was performed in methylene chloride in the presence of silver triflate, tetramethylurea and molecular sieve 4A at room temperature for 3–15 h. Donor **14** was taken in a



14

1.5-fold excess, except for the glycosylation of acceptor **15**, when a two-fold excess of the glycosyl acceptor was used. The yields of Neu5Acα2-3Galβ glycosylation products were 56–85%; by-products (< 10%, presumably corresponding Neu5Acα2-3Galα isomers) were easily removed by chromatography. The AgOTf-promoted glycosylation of diol **23** proceeded non-regiospecifically [56% of the 1-3 isomer, 11% of the 1-4 isomer and 9% of the bis(glycosylation) product], so the preparative synthesis was performed in the presence of $Hg(CN)_2$ as a



Scheme 1 Synthesis of spacered oligosaccharides **5**–**13**. *Reagents and conditions*: i, AgOTf; ii, MeONa, MeOH; iii, NaOH, H_2O; iv, H_2, Pd/C; v, SO_3·Py, Py; vi, AcOH; vii, $Hg(CN)_2/HgBr_2$; viii, Ac_2O; ix, Bn_3FucBr; x, ethylenethiourea.

promoter. Under these conditions, target 1-3 bound trisaccharide **24** was obtained in 75% yield. The presence of the free hydroxyl group at C^4 in trisaccharide **24** allowed us to obtain protected tetrasaccharide **27** by fucosylation. The deblocking of **27** gave rise to free spacered tetrasaccharide SiaLe^a **12** identical to that obtained earlier by another method.[15] To synthesise isomeric tetrasaccharide SiaLe^x **13**,[15] chloroacetyl derivative **29** was converted into trisaccharide **30**, which was subjected to mild dechloroacetylation followed by the fucosylation of resulting acceptor **31**.

The synthetic strategy proposed enabled us to synthesise oligosaccharides containing both sialic acid at the 3-position

of galactose and sulfate at the 6-position of hexosamine, *e.g.*, trisaccharides **7**, **9** and **11**, which are of great biological interest as fragments of mucin-type glycoproteins. These mucins take part, in particular, in the interaction with L-selectin and are receptors for pathogenic bacteria and viruses. The selective deblocking of the hydroxyl group at C-6 of hexosamine in **18**, **21** and **25**, the sulfation of corresponding trisaccharides **19**, **22** and **26** by the SO_3·Py complex, and the final deblocking resulted in the aimed oligosaccharides **7**, **9** and **11**. The chemical synthesis of deblocked trisaccharides **7**, **9** and **11** was not reported earlier.

The oligosaccharide derivatives synthesised were deprotected by conventional methods,[6] namely, sequential hydrogenolysis and treating with sodium methylate and an alkali. An amount of dimeric products, in which the carboxy group of sialic acid of one oligosaccharide molecule acylated the spacer group of the second molecule, was formed in several cases at the final stage of saponification. These dimers were quantitatively converted into the target compounds by treating with 1 N alkali for 24 h at room temperature. De-N-acetylation was not observed under these conditions.

To summarise, Neu5Acα2-3Gal methyl ester bromide is effective in 2-3 sialooligosaccharide synthesis. The advantages of this glycosyl donor are the simplicity of preparation from the corresponding peracetate, compatibility with various protecting groups (including acetate), and convenient conditions of glycosylation – the reactions proceed at room temperature for 3–15 h.

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† The synthesis of the peracetate of Neu5Acα2-3Gal methyl ester will be described elsewhere. In addition, the disaccharide is commercially available from Syntesome GmbH (Munich).

‡ *Spectral characteristics for free oligosaccharides* **5**–**11**. ^{1}H NMR spectra were recorded at 30 °C with a Bruker WM 500 MHz instrument. Mass spectra (MALDI-TOF) were recorded on a VISION 2000 mass spectrometer. Optical rotations were measured with a JASCO DIP-360 polarimeter at 20 °C.

5 (inner salt): ^{1}H NMR (500 MHz, D_2O) δ: 1.806 (dd, 1H, H-3ax Neu5Ac, $J_{3ax,3eq}$ 12.5 Hz, $J_{3ax,4}$ 12.0 Hz), 2.020 (m, 2H, CH_2 sp), 2.051 (s, 3H, NCOMe), 2.782 (dd, 1H, H-3eq Neu5Ac, $J_{3eq,4}$ 4.7 Hz, $J_{3ax,3eq}$ 12.5 Hz), 3.195 (m, 2H, NCH_2 sp), 3.585 (dd, 1H, H-2 Gal, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.8 Hz), 3.616 (dd, 1H, H-7 Neu5Ac, $J_{6,7}$ ~1 Hz, $J_{7,8}$ ~9 Hz), 3.654 (dd, 1H, H-6 Neu5Ac, $J_{5,6}$ 10.5 Hz, $J_{6,7}$ ~1 Hz), 3.650–3.910 (m, 9H), 3.965 (dd, 1H, H-4 Gal, $J_{3,4}$ 2.9 Hz, $J_{4,5}$ ~1 Hz), 4.087 (m, 1H, OCH sp), 4.110 (dd, 1H, H-3 Gal, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 2.9 Hz), 4.513 (d, 1H, H-1 Gal, $J_{1,2}$ 7.8 Hz). MS, *m/z*: 530 (529 + 1) [M$^+$ + H$^+$]. $[\alpha]_D$ –2.4° (*c* 1, H_2O).

6 (inner salt): ^{1}H NMR (500 MHz, D_2O) δ: 1.813 (dd, 1H, H-3ax Neu5Ac, $J_{3ax,3eq}$ 12.5 Hz, $J_{3ax,4}$ 12.0 Hz), 1.969 (m, 2H, CH_2 sp), 2.052 and 2.063 (2s, 2×3H, 2NCOMe), 2.779 (dd, 1H, H-3eq Neu5Ac, $J_{3eq,4}$ 4.7 Hz, $J_{3ax,3eq}$ 12.5 Hz), 3.109 (m, 2H, NCH_2 sp), 3.593 (dd, 1H, H-2 Gal, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.8 Hz), 3.600–3.930 (m, 16H), 3.976 (dd, 1H, H-4 Gal, $J_{3,4}$ 2.9 Hz, $J_{4,5}$ ~1 Hz), 4.029 (dd, 1H, H-6a GlcNAc, $J_{5,6a}$ 1.5 Hz, $J_{6a,6b}$ 11.5 Hz), 4.037 (m, 1H, OCH sp), 4.130 (dd, 1H, H-3 Gal, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 2.9 Hz), 4.533 (d, 1H, H-1 GlcNAc, $J_{1,2}$ 8.1 Hz), 4.568 (d, 1H, H-1 Gal, $J_{1,2}$ 7.8 Hz). MS, *m/z*: 733 (732 + 1) [M$^+$ + H$^+$]. $[\alpha]_D$ –14.0° (*c* 0.5, H_2O).

7 (Na salt): ^{1}H NMR (500 MHz, D_2O) δ: 1.823 (dd, 1H, H-3ax Neu5Ac, $J_{3ax,3eq}$ 12.2 Hz, $J_{3ax,4}$ 12 Hz), 1.980 (m, 2H, CH_2 sp), 2.050 and 2.062 (2s, 2×3H, 2NCOMe), 2.771 (dd, 1H, H-3eq Neu5Ac, $J_{3eq,4}$ 4.5 Hz, $J_{3ax,3eq}$ 12.2 Hz), 3.134 (m, 2H, NCH_2 sp), 3.578 (dd, 1H, H-2 Gal, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 10.0 Hz), 3.621 (dd, 1H, H-7 Neu5Ac, $J_{6,7}$ 1.8 Hz, $J_{7,8}$ 9.1 Hz), 3.657 (dd, 1H, H-6 Neu5Ac, $J_{5,6}$ 10.3 Hz, $J_{6,7}$ 1.8 Hz), 3.640–3.860 (m, 10H), 3.871 (dd, 1H, H-5 Neu5Ac, $J_{4,5} \approx J_{5,6} \approx 10$ Hz), 3.906 (dd, 1H, H-9a Neu5Ac, $J_{8,9a}$ 2.4 Hz, $J_{9a,9b}$ 12.0 Hz), 3.933 (ddd, 1H, H-8 Neu5Ac, $J_{7,8}$ 9.1 Hz, $J_{8,9a}$ 2.4 Hz, $J_{8,9b}$ 6.1 Hz), 3.987 (dd, 1H, H-4 Gal, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ ~1 Hz), 4.013 (m, 1H, OCH sp), 4.142 (dd, 1H, H-3 Gal, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.2 Hz), 4.352 (dd, 1H, H-6b GlcNAc, $J_{5,6b}$ 4.3 Hz, $J_{6a,6b}$ 11.3 Hz), 4.454 (dd, 1H, H-6a GlcNAc, $J_{5,6a}$ 1.5 Hz, $J_{6a,6b}$ 11.3 Hz), 4.569 (d, 1H, H-1 GlcNAc, $J_{1,2}$ 8.3 Hz), 4.622 (d, 1H, H-1 Gal, $J_{1,2}$ 8.1 Hz). MS, *m/z*: 810 [M$^+$]. $[\alpha]_D$ –8.4° (*c* 0.5, H_2O).

8 (inner salt): ^{1}H NMR (500 MHz, D_2O) δ: 1.800 (dd, 1H, H-3ax Neu5Ac, $J_{3ax,3eq}$ 12.5 Hz, $J_{3ax,4}$ 11.7 Hz), 2.016 (m, 2H, CH_2 sp), 2.046 and 2.049 (2s, 2×3H, 2NCOMe), 2.778 (dd, 1H, H-3eq Neu5Ac, $J_{3eq,4}$ 4.6 Hz, $J_{3ax,3eq}$ 12.5 Hz), 3.150 (m, 2H, NCH_2 sp), 3.569 (dd, 1H, H-2 Gal, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.0 Hz), 3.586 (m, 1H, OCH sp), 3.608 (dd, 1H, H-7 Neu5Ac, $J_{6,7}$ ~2 Hz, $J_{7,8}$ 9.1 Hz), 3.640 (dd, 1H, H-6 Neu5Ac, $J_{5,6}$ 10.5 Hz, $J_{6,7}$ ~2 Hz), 3.660 (dd, 1H, H-9b Neu5Ac, $J_{9a,9b}$ 12.0 Hz, $J_{8,9b}$ 6.4 Hz), 3.706 (ddd, 1H, H-4 Neu5Ac, $J_{3ax,4}$ 11.7 Hz, $J_{3eq,4}$ 4.6 Hz, $J_{4,5}$ 10.0 Hz), 3.730–3.825 (m, 5H), 3.830 (m, 1H, OCH sp), 3.836 (dd, 1H, H-5 Neu5Ac, $J_{4,5} \approx J_{5,6} \approx 10.0$ Hz), 3.867 (dd, 1H, H-9a Neu5Ac, $J_{9a,9b}$ 12.0 Hz, $J_{8,9a}$ 2.2 Hz), 3.896 (ddd, 1H, H-8 Neu5Ac, $J_{7,8}$ 9.1 Hz, $J_{8,9a}$ 2.2 Hz, $J_{8,9b}$ 6.4 Hz), 3.948 (dd, 1H, H-4 Gal, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ ~1 Hz), 3.982 (m, 1H, H-5 GalNAc), 4.057 (dd, 1H, H-3 GalNAc, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 2.9 Hz), 4.084 (dd, 1H, H-3 Gal, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.2 Hz), 4.261 (dd, 1H, H-4 GalNAc, $J_{3,4}$ 2.9 Hz, $J_{4,5}$ ~1 Hz), 4.344 (dd, 1H, H-2 GalNAc, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 11.0 Hz), 4.556 (d, 1H, H-1 Gal, $J_{1,2}$ 7.8 Hz), 4.941 (d, 1H, H-1 GalNAc, $J_{1,2}$ 3.7 Hz). MS, *m/z*: 733 (732 + 1) [M$^+$ + H$^+$]. $[\alpha]_D$ +57.0° (*c* 0.8, H_2O).

9 (Na salt): ^{1}H NMR (500 MHz, D_2O) δ: 1.803 (dd, 1H, H-3ax Neu5Ac, $J_{3ax,3eq}$ 12.5 Hz, $J_{3ax,4}$ 11.7 Hz), 2.030 (m, 2H, CH_2 sp), 2.044 and 2.050 (2s, 2×3H, 2NCOMe), 2.778 (dd, 1H, H-3eq Neu5Ac, $J_{3eq,4}$ 4.6 Hz, $J_{3ax,3eq}$ 12.5 Hz), 3.153 (m, 2H, NCH_2 sp), 3.568 (dd, 1H, H-2 Gal, $J_{1,2}$ 8.1 Hz, $J_{2,3}$ 9.8 Hz), 3.611 (dd, 1H, H-7 Neu5Ac, $J_{6,7}$ 1.5 Hz, $J_{7,8}$ 8.8 Hz), 3.610–3.920 (m, 11H), 3.951 (dd, 1H, H-4 Gal, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ ~1 Hz), 4.061 (dd, 1H, H-3 GalNAc, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 2.9 Hz), 4.086 (dd, 1H, H-3 Gal, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.2 Hz), 4.194 (dd, 1H, H-6b GalNAc, $J_{6a,6b}$ 12.2 Hz, $J_{5,6b}$ 9.5 Hz), 4.261 (m, 2H, H-5 and H-6a GalNAc), 4.316 (dd, 1H, H-4 GalNAc, $J_{3,4}$ 2.9 Hz, $J_{4,5}$ ~1 Hz), 4.351 (dd, 1H, H-2 GalNAc, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 11.0 Hz), 4.552 (d, 1H, H-1 Gal, $J_{1,2}$ 8.1 Hz), 4.950 (d, 1H, H-1 GalNAc, $J_{1,2}$ 3.7 Hz). MS, *m/z*: 810 [M$^+$]. $[\alpha]_D$ +50.0° (*c* 0.5, H_2O).

10 (inner salt): ^{1}H NMR (500 MHz, D_2O) δ: 1.772 (dd, 1H, H-3ax Neu5Ac, $J_{3ax,3eq}$ 12.5 Hz, $J_{3ax,4}$ 12.0 Hz), 1.945 (m, 2H, CH_2 sp), 2.022 and 2.032 (2s, 2×3H, 2NCOMe), 2.754 (dd, 1H, H-3eq Neu5Ac, $J_{3eq,4}$ 4.6 Hz, $J_{3ax,3eq}$ 12.5 Hz), 3.082 (m, 2H, NCH_2 sp), 3.487 (m, 1H, H-5 GlcNAc), 3.530 (dd, 1H, H-2 Gal, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.8 Hz), 3.520–3.810 (m, 12H), 3.834 (dd 1H, H-5 Neu5Ac, $J_{4,5} \approx J_{5,6} \approx 10$ Hz), 3.835 (dd, 1H, H-9a Neu5Ac, $J_{9a,9b}$ 12.0 Hz, $J_{8,9a}$ 2.4 Hz), 3.864 (m, 1H, H-8 Neu5Ac), 3.927 (dd, 1H, H-6a GlcNAc, $J_{5,6a}$ 2.2 Hz, $J_{6a,6b}$ 12.5 Hz), 3.928 (dd, 1H, H-4 Gal, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 1 Hz), 4.020 (m, 1H, OCH sp), 4.074 (dd, 1H, H-3 Gal, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.2 Hz), 4.500 (d, 1H, H-1 Gal, $J_{1,2}$ 7.8 Hz), 4.535 (d, 1H, H-1 GlcNAc, $J_{1,2}$ 8.3 Hz). MS, *m/z*: 733 (732 + 1) [M$^+$ + H$^+$]. $[\alpha]_D$ –27.0° (*c* 0.5, H_2O).

11 (Py$^+$ salt): ^{1}H NMR (500 MHz, D_2O) δ: 1.846 (dd, 1H, H-3ax Neu5Ac, $J_{3ax,3eq}$ 12.5 Hz, $J_{3ax,4}$ 12.0 Hz), 1.976 (m, 2H, CH_2 sp), 2.040 and 2.046 (2s, 2×3H, 2NCOMe), 2.761 (dd, 1H, H-3eq Neu5Ac, $J_{3eq,4}$ 4.6 Hz, $J_{3ax,3eq}$ 12.5 Hz), 3.119 (m, 2H, NCH_2 sp), 3.550 (dd, 1H, H-2 Gal, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.8 Hz), 3.580–3.910 (m, 15H), 3.951 (dd, 1H, H-4 Gal, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 1 Hz), 4.006 (m, 1H, OCH sp), 4.113 (dd, 1H, H-3 Gal, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 3.2 Hz), 4.238 (dd, 1H, H-6b GlcNAc, $J_{5,6b}$ 5.6 Hz, $J_{6a,6b}$ 11.3 Hz), 4.385 (dd, 1H, H-6a GlcNAc, $J_{5,6a}$ ~2.0 Hz, $J_{6a,6b}$ 11.3 Hz), 4.521 (d, 1H, H-1 Gal, $J_{1,2}$ 7.8 Hz), 4.585 (d, 1H, H-1 GlcNAc, $J_{1,2}$ 8.6 Hz), 8.109 (m, 2H, Py), 8.657 (m, 1H, Py), 8.811 (m, 2H, Py). $[\alpha]_D$ –15.0° (*c* 1, H_2O).

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