Research Article

Koenigs-Knorr Glycosylation with Neuraminic Acid Derivatives

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Earlier we reported a convenient and efficient method of preparing α 2-6 sialooligosaccharides in conditions of Koenigs-Knorr reaction. The use of Ag_2CO_3 allowed carrying out α 2-6 sialylation of galacto-4,6-diol of mono- and disaccharides with chloride of acetylated *N*-acetylneuraminic acid methyl ester as glycosyl donor. In this study we applied this approach to other derivatives of neuraminic acid, namely, Neu5Gc, 9-deoxy-9-*N*Ac-Neu5Ac, Neu5Aca2-8Neu5Ac, and Neu5Aca2-8Neu5Aca2-8Neu5Ac as glycosyl donors; eight compounds were synthesized: Neu5Gca-O $(\text{CH}_2)_3\text{NH}_2$ (**8**), Neu5Gca2-6Gal β 1-4GlcNAc β -O $(\text{CH}_2)_3\text{NH}_2$ (**10**), 9-deoxy-9-*N*Ac-Neu5Ac-O $(\text{CH}_2)_3\text{NH}_2$ (**15**), 9-deoxy-9-*N*Ac-Neu5Aca2-6Gal β 1-4GlcNAc β -O $(\text{CH}_2)_3\text{NH}_2$ (**17**), Neu5Aca2-8Neu5Aca-O $(\text{CH}_2)_3\text{NH}_2$ (**23**), Neu5Aca2-8Neu5Aca-OCH₃ (**24**), Neu5Aca2-8Neu5Aca-OCH₂(*p*-C₆H₄)NHCOCH₂NH₂ (**25**), and Neu5Aca2-8Neu5Aca2-8Neu5Aca-O $(\text{CH}_2)_3\text{NH}_2$ (**32**). These sialosides were used for characterization of siglecs and other carbohydrate-binding proteins.

1. Introduction

Sialic acid family comprises over 40 neuraminic acid versions and monosaccharide derivatives; the number of biologically relevant sialic acid oligosaccharides is also very high. Sialic acids are a part of the most important molecules of life, since they occupy the terminal position on cell membrane glycoproteins and glycolipids. Given their location and ubiquitous distribution, sialic acids can mediate or modulate a wide range of physiological and pathological processes and play a role not only in the protection and adaptation of life, but also in being utilized by life-threatening infectious microorganisms [1]. Several families of sialic acid-binding proteins have been discovered over the last few decades, including mammalian selectins and siglecs [2]. Only a limited number of sialomolecules are available as the tools for the study of mammalian and microbial lectins and sialo-modifying enzymes. Due to this, the search of simple and efficient synthetic ways for sialosaccharides is very relevant. The methods of sialylation described in the

literature [3–5] rarely give high yields and α -stereoselectivity but suggest complex modifications of donors; introduction and removal of substituents at C-3, C-4, C-5, and C-9 in order to improve sialylation stereoselectivity presume complex multistage synthesis of such compounds. In case of the use of 2-xantates, thiophosphates and various imidates, sialylation usually proceeds at -50 – 70°C and requires strong Lewis acids as promoters, this posing the corresponding limitations to the repertoire of protective groups in glycosyl acceptor. Recently we have described a simple and efficient method of the synthesis of α 2-6 sialooligosaccharides in conditions of Koenigs-Knorr reaction [6] when the simplest possible sialyl donor is used at room temperature and gives high α -stereoselectivity. It was interesting to study the applicability of this approach to other neuraminic acid derivatives (Neu5Gc, 9-deoxy-9-*N*Ac-Neu5Ac, Neu5Aca2-8Neu5Ac, and Neu5Aca2-8Neu5Aca2-8Neu5Ac). Here, we describe practical chemical syntheses of several spacer-armed sialic acid glycosides, with especial interest to *N*-glycolyl-neuraminic acid version playing role in cancer

immunology and transplantation, and also α 2-8 oligosaccharides known as key motif of polysialic acids, numerous gangliosides, and serving as affinity ligand for siglec-7 [7].

Peracetylated methyl esters of Neu5Gc (**6**) [8], 9-deoxy-9-NAc-Neu5Ac (**13**), Neu5Ac α 2-8Neu5Ac (**19** and **20**) [9], and Neu5Ac α 2-8Neu5Ac α 2-8Neu5Ac (**27–30**) [10] were synthesized starting from free saccharides by esterification in acidic conditions (MeOH/HCl) with subsequent acetylation (Ac₂O/pyridine).

2-Chloroderivatives **7**, **14**, **21** + **22**, and **31**, Sug-Cl, were synthesized from the corresponding peracetylated methyl esters by treatment with HCl in CHCl₃ for 1.5–2 days and were used without further purification.

Glycosylation was performed in dry CH₂Cl₂ in the presence of Ag₂CO₃ or Ag₂CO₃/AgOTf (silver trifluoromethanesulfonate) and molecular sieves 4Å (MS 4Å) at room temperature for 1–7 days; the reaction conditions and yields are given in the Table 1. In this study we have used equivalent amounts of glycosyl donor (Sug-Cl) and acceptor or excess of acceptor. The corresponding glycals were found to be the main side products in all glycosylation reactions. Deprotection resulted in the mixture of the target product with glycal and unreacted acceptor, easily separated using cation-exchange chromatography on Dowex H⁺ resin [11]: elution with water gave glycal (acid), whereas the product (amino acid) had been retained on the column and was completely eluted with 1 M aqueous pyridine; unreacted glycosyl acceptor (amine) was eluted with 1 M NH₄OH. In the case of disialic and trisialic glycosides partial lactonization occurred during purification on Dowex H⁺, so the lactone was treated with aqueous NaOH to give corresponding acid.

All Sug-Cl (see Table 1) have demonstrated considerably less activity in glycosylation compared to chloride of acetylated *N*-acetylneuraminic acid methyl ester (**3**) [6, 8]; in several cases Ag₂CO₃ promoted glycosylation (without AgOTf) either proceeded very slowly or did not proceed at all. The optimal of AgOTf added to Ag₂CO₃ ratio (in respect to the reaction duration and acceptable α/β anomer ratio) was found to be 10% mol using chloride of Neu5Ac (**3**) as glycosyl donor and HO(CH₂)₃NHCOCF₃ as acceptor; larger amount gave more β -isomer and glycal; preferable acceptor/donor ratio was not less than 2. Glycosylation at –10°C did not improve the α/β ratio compared to room temperature.

Glycosylation with chloride of Neu5Gc (**7**) catalyzed by Ag₂CO₃ in absence of AgOTf proceeded slowly; 3-trifluoroacetamidopropanol (Scheme 1, no. 3 in Table 1) gave the mixture of anomers in 68% yield (α/β 92:8). Deprotected product was isolated on Dowex H⁺ resin (elution with 1 M Py) with additional HPLC purification; the corresponding α -glycoside (**8**) was isolated in 57% yield, that was better result compared to NIS/TFS-OH promoted glycosylation with Neu5Gc β Set [8].

Glycosylation of 4',6'-diol of *N*-acetylglucosamine (**9**) with chloride of Neu5Gc **7** in presence of Ag₂CO₃/AgOTf (Scheme 1, no. 4 in Table 1) gave 30% of α/β mixture (43:57); the pure α -glycoside **10** was isolated (HPLC) in

12% yield. Unreacted acceptor **9** (58%) was recovered in deprotected form **11**.

Glycosylation with chloride of 9-deoxy-9-NAc-Neu5Ac **14** in presence of only Ag₂CO₃ proceeded similarly to the reaction with Neu5Ac (Scheme 2, no. 5 and no. 6 in Table 1). 3-Trifluoroacetamidopropanol and 4',6'-diol **16** gave comparable yields of corresponding α -glycosides (29% of **15** and 25% of **17**); β anomers were observed in trace amounts. Unreacted acceptor **16** was recovered (66%) in deprotected form **11**.

Methylation followed by acetylation of Neu5Ac α 2-8Neu5Ac (**18**) or Neu5Ac α 2-8Neu5Ac α 2-8Neu5Ac (**26**) gave the mixtures of acetates of methyl esters and corresponding lactones (preparation of the lactones described in [9, 10]); the mixtures were used without separation. Acetates of disialic acid were the mixture of **19** and **20** (yield 82%); 2-Chloroderivatives used as glycosyl donor were the mixture of **21** and **22** (Scheme 3). Acetates of trisialic acid were the mixture of four substances (**27–30**) (yield 72%); the mixture of corresponding four 2-Chloroderivatives used as glycosyl donor is designated as **31** (Scheme 4).

Glycosylation with Neu5Ac α 2-8Neu5Ac glycosyl chlorides **21** + **22** in absence of AgOTf proceeded very slowly. Spacer alcohol HO(CH₂)₃NHCOCF₃ gave ~20% yield of glycosylation product **23**, α/β ratio 94:6. Glycosylation in the presence of AgOTf proceeded seven times faster resulting in α/β ratio 90:10 (32%) (Scheme 3, no. 7 and no. 8 in Table 1). Synthesis of disialic acid methyl glycoside was carried out at 100-fold excess of methanol (Scheme 3, no. 9 in Table 1). After deprotection and chromatography on silica gel, purification on Sephadex LH-20 and ion exchange to Na⁺ on Dowex the yield of target α -glycoside **24** was 40% (~33% on starting disialic acid) that is comparable to the literature data [12]; only the traces of β glycoside were observed.

In case of *p*-amidoglycyl-benzyl alcohol glycosylation with Neu5Ac α 2-8Neu5Ac the use of 0.1 mol of AgOTf per glycosyl chloride gave only 10% of **25**; 0.6 ratio led to increase of conversion (yield of α + β increased from 20% to 32%), but it was accompanied with the increase of β -anomer formation (Scheme 3, no. 10 and no. 11 in Table 1).

Trisialic chlorides **31** reacted with HO(CH₂)₃NHCOCF₃ only in the presence of AgOTf; increase of mol/mol ratios from 0.1 to 0.5 on chloride gave **32** (~25% yield) accompanied by the increase of conversion degree and reduction of α/β ratio (Scheme 4, no. 12 and no. 13 in Table 1).

The structure of all the studied compounds was confirmed by high resolution ¹H NMR data. Spectra of derivatives **4**, **8**, **10**, and **24** coincided with those described before [8, 12, 13]. Chemical shift of H-3_{eq} (Neu5Ac, Neu5Gc and 9-deoxy-9-NAc-Neu5Ac residues) was 2.6–2.7 ppm for α -anomer and 2.2–2.4 ppm for β -anomer [3]. Upfield shift has been observed in spectra of 9-deoxy-9-NAc-Neu5Ac derivatives for H-9 (3.6 ppm) and H9' (3.3 ppm) as compared to Neu5Ac (3.9 and 3.6 ppm, correspondingly) [14]. Glycosidic α (2-8) bond in disialic and trisialic acid derivatives was characterized by downfield position of H-8 and H-9 (>4.0 ppm) as compared to Neu5Ac [12, 15]. The synthesized sialosides were used for characterization of

SCHEME 1: (a) MeOH/HCl; (b) Ac₂O/Py; (c) AcCl, MeOH/CHCl₃; (d) Ag₂CO₃ (Ag₂CO₃/AgOTf), CH₂Cl₂, MS 4Å; (e) MeONa/MeOH; (f) NaOH/H₂O; (g) H₂, Pd/C.

siglecs [16] and other carbohydrate-binding proteins [17, 18].

2. Experimental

Neu5Ac was from Juelich Enzyme Products GmbH (Wiesbaden, Germany); 9-deoxy-9-NAc-Neu5Ac was obtained as described in [19]; Neu5Ac α 2-8Neu5Ac and Neu5Ac α 2-8Neu5Ac α 2-8Neu5Ac and Neu5Gc were from Nacalai Tesque Inc. (Kyoto, Japan).

2.1. Preparation of Peracetylated Methyl Esters of Neuraminic Acid Derivatives General procedure for 6, 13, 19 + 20, and

27–30. Neuraminic acid derivative (0.5 mmol of 5, 12, 18, or 26 as Na salt) was suspended in 25 ml of dry methanol cooled to 0°C followed by dropwise addition of AcCl (178 μ l, 2.5 mmol). The mixture was stirred at room temperature for 2 h (clear solution is formed). The solution was evaporated at reduced pressure (water jet pump) and coevaporated three times with toluene. The residue was acetylated with 9 ml of Ac₂O/Py (1:2) for 15–20 h at 40°C, coevaporated with toluene, and subjected to chromatography on silica gel (yields 70%–90%). Peracetylated derivatives of di- and trisialic acids (19 + 20 and 27–30) were mixtures of acetates of methyl ester and lactones, which were used without separation.

SCHEME 2: (a) MeOH/HCl; (b) Ac₂O/Py; (c) AcCl, MeOH/CHCl₃; (d) Ag₂CO₃, CH₂Cl₂, MS 4Å; (e) MeONa/MeOH; (f) NaOH/H₂O.

TABLE 1: Sialylation with neuraminic acid derivatives.

#	Sug-Cl	ROH	ROH/ Sug-Cl	Sug-Cl + ROH protected glycosylchloride Ag ₂ CO ₃ /AgOTf or Ag ₂ CO ₃	deprotection	separation	Sug-OR unprotected glycoside	Yield ^a α + β, %	Yield ^a α, %	α / β ^b
1	Neu5Ac (3)	HO(CH ₂) ₃ NHCOCF ₃	5	2	—	2.6 (62 h)	4	71	71	> 98 : 2 [8]
2 ^c		HO(CH ₂) ₃ NHCOCF ₃	2	3	0.1	0.2 (5 h)		47	36	95 : 5
3	Neu5Gc (7)	HO(CH ₂) ₃ NHCOCF ₃	3	3	—	3	8	68	57	92 : 8
4		4',6'-diol-LacNAcβ- O(CH ₂) ₃ NHCOCF ₃ (9) ^d	1	3	0.1	7	10	30	12	43 : 57
5	9-deoxy-9-NAc- Neu5Ac (14)	HO(CH ₂) ₃ NHCOCF ₃	1	3	—	3	15	n.d.	29	Traces of β
6		4',6'-diol-LacNAcβ- O(CH ₂) ₃ NHCOCF ₃ (16) ^e	1	6	—	5	17	n.d.	25	Traces of β
7	Neu5Ac2-8	HO(CH ₂) ₃ NHCOCF ₃	2	3	—	7	23	20	not done	94 : 6
8	Neu5Ac 2-8	HO(CH ₂) ₃ NHCOCF ₃	2	3	0.1	1		36	32	90 : 10
9	(21+22)	MeOH	100	6	0.15	3	24	n.d.	40	Traces of β
10		HOCH ₂ (p- C ₆ H ₄)NHCOCF ₃ NHBoc	2	5	0.1 ^f	3	25	20	10	50 : 50
11		HOCH ₂ (p- C ₆ H ₄)NHCOCF ₃ NHBoc	4	6	0.6	3		32	12	39 : 61
12	Neu5Ac2-8	HO(CH ₂) ₃ NHCOCF ₃	3	3	0.1 ^f	7	32	33	27	81 : 19
13	Neu5Ac2-8 (31)	HO(CH ₂) ₃ NHCOCF ₃	3	3	0.5	7		37	not done	67 : 33

^aPreparative yield of unprotected glycoside Sug-OR calculated on protected precursor of Sug-Cl (n.d.: not determined);

^bα/β ratio according to NMR data;

^cA series of experiments demonstrated that these conditions are optimal for the reaction in the presence of AgOTf;

^dRecovery of the acceptor in the form LacNAcβ-O(CH₂)₃NH₂ (11) 58%;

^eRecovery of the acceptor in the form LacNAcβ-O(CH₂)₃NH₂ (11) 66%;

^fReaction did not proceed without AgOTf.

SCHEME 3: (a) MeOH/HCl; (b) Ac₂O/Py; (c) AcCl, MeOH/CHCl₃; (d) Ag₂CO₃/AgOTf, CH₂Cl₂, MS 4Å; (e) MeONa/MeOH; (f) NaOH/H₂O; (g) CF₃COOH.

2.2. Preparation of Neuraminic Acid Glycosyl Chlorides Derivatives (Sug-Cl) General procedure for 7, 14, 21 + 22, and 31. Peracetylated methyl ester of neuraminic acid derivative (1 mmol of 6, 13, 19 + 20 or 27–30) was dissolved in 10 ml freshly distilled chloroform followed by addition of dry methanol (0.81 ml, 20 mmol). The solution was cooled (ice + salt) and AcCl (2.84 ml, 40 mmol) was added dropwise at cooling and stirring. After 30 min the reaction mixture was thoroughly sealed then warmed slowly and kept at room temperature for 1.5–2 days. The reaction mixture was evaporated at reduced pressure (water jet pump) and coevaporated with dry toluene several times to neutral pH value of the solution. Chlorides obtained in this way

were used without further purification assuming quantitative yield of the reaction (the substances were stored at -18°C).

2.3. Glycosylation General procedure for 4, 8, 10, 15, 17, 23, 24, 25, and 32 (see Table 1 for details). A solution of Sug-Cl (0.1 mmol) in dry CH₂Cl₂ (3 ml) and AgOTf (0.01–0.06 mmol) were added to a mixture of acceptor (0.1–0.4 mmol), Ag₂CO₃ (0.3–0.6 mmol), and MS 4Å (1 g) in dry methylene chloride (5 ml). The reaction mixture was stirred at room temperature (20–25°C) for 0.2–7 days (reaction completion was determined by disappearing of Sug-Cl according to TLC). The reaction mixture was filtered, the residue was washed with CHCl₃/MeOH (1 : 1); the combined

SCHEME 4: (a) MeOH/HCl; (b) Ac₂O/Py; (c) AcCl, MeOH/CHCl₃; (d) Ag₂CO₃/AgOTf, CH₂Cl₂, MS 4Å; (e) MeONa/MeOH; (f) NaOH/H₂O.

filtrate was evaporated, coevaporated with toluene, and dried *in vacuo*.

2.4. Deprotection and Separation. (see Table 1 for yields).

(a) Procedure for **4**, **8**, **15**, and **17**: the dry residue of treated reaction mixture (see above) was dissolved in 6 ml of dry MeOH and 0.3 ml 2 M MeONa/MeOH were added. The mixture was kept for 30 min at r.t., then evaporated followed by addition of 6 ml H₂O. After 10–15 h (r.t.) the solution was evaporated, the residue was dissolved in ~ 1 ml water and the solution was applied on Dowex 50x4-400 (H⁺) ion-exchange resin column (1.5 × 6 cm). The resin was washed sequentially with water (50 ml), 1 M aq. pyridine (50 ml) and 1 M aq. NH₃ (50 ml). Target glycosides were completely eluted with 1 M pyridine. In the case of **10** and **17**, nonreacted acceptor **16** was eluted with 1 M aq. NH₃ in deprotected form (**11**). Pure α -glycoside **8** and corresponding β anomer were obtained by HPLC separation (ODS C₁₈, water).

(b) Procedure for **10** was the same as (a), but included hydrogenolysis before MeONa/MeOH treatment: MeOH, 5% Pd/C, H₂, 2 h. Pure α -glycoside **10** and corresponding β anomer were obtained by HPLC separation (ODS C₁₈, water).

(c) Synthesis of **23** and **32** included procedure (a); products were eluted 1 M aq. pyridine. The obtained material was treated with 1 ml of 0.1 M NaOH for 2 h at r.t. to open lactone rings (partial lactonisation occurs during purification on Dowex H⁺) followed by neutralization with AcOH. Pure products as Na-salts were obtained after chromatography on silica gel (MeOH/MeCN/water, 3:3:1), purification on Sephadex LH-20 (MeCN/water, 1:1), cation exchange on Dowex 50x4-400 (Na⁺), and freeze drying.

(d) Procedure for **25** was the same as (c), with additional treatment with CF₃COOH to remove Boc protection before the first Dowex chromatography. The mixture was dissolved in 0.3 ml CF₃COOH, kept for 1 h at r.t., then coevaporated with toluene and dried *in vacuo*; the followed procedures were performed according to procedure (c).

(e) Synthesis of **24** included deprotection (treatment with MeONa/MeOH, NaOH/H₂O, neutralization with AcOH), column chromatography on silica gel (2-propanol/MeCN/water 4:3:2), purification on Sephadex LH-20 (MeCN/water, 1:1), cation exchange on Dowex 50x4-400 (Na⁺) and freeze drying.

2.4.1. Characteristics of Synthesized Spaced Saccharides. ¹H NMR spectra were recorded at 30°C with a Varian 600 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. Thin-layer chromatography was performed using silica gel 60 F₂₅₄ aluminum sheets (Merck, 1.05554) with detection by charring after 7% H₃PO₄ soaking, or ninhydrine.

9-Deoxy-9-NAc-Neu5Acα-O(CH₂)₃NH₂ (15). ¹H NMR spectrum (D₂O, 303 K, 600 MHz) δ ppm: 1.695 (1H, dd, $J_{3ax,3eq} \approx J_{3ax,4} \approx 12$ Hz, H3ax), 1.953 (2H, m, 2CH), 2.035 and 2.053 (2 × 3H, s, 2COCH₃), 2.722 (1H, dd, $J_{3eq,3ax} 12.5$, $J_{3eq,4} 4.7$ Hz, H3eq), 3.138 (2H, m, 2CHN), 3.266 (1H, dd, $J_{9',9} 14.2$, $J_{9,8} 7.7$ Hz, H9), 3.504 (1H, dd, $J_{6,7} 1.4$, $J_{7,8} 8.8$ Hz, H7), 3.584 (1H, m, OCH), 3.629 (1H, dd, $J_{9',9} 14.2$, $J_{9',8} 2.9$ Hz, H9'), 3.704 (1H, ddd, $J_{4,3eq} 4.7$, $J_{4,3ax} 11.8$, $J_{4,5} 9.3$ Hz, H4), 3.788 (1H, dd, $J_{6,5} 10.4$, $J_{6,7} 1.4$ Hz, H6), 3.839 (1H, dd, $J_{5,6} 10.4$, $J_{5,4} 9.3$ Hz, H5), 3.833 (1H, m, OCH), 3.903 (1H, ddd, $J_{8,9} 2.9$, $J_{8,9'} 7.7$, $J_{8,7} 8.8$ Hz, H8). MS, m/z : 408 [M⁻]. $[\alpha]_D +1.8$ (c 1, H₂O). R_f 0.58 (MeOH/MeCN/water 3:3:2).

9-Deoxy-9-NAc-Neu5Acα2-6Galβ1-4GlcNAcβ-O(CH₂)₃NH₂ (inner salt) (17). ¹H NMR spectrum (D₂O, 303 K, 600 MHz) δ ppm: 1.716 (1H, dd, $J_{3ax,3eq} \approx J_{3ax,4} \approx 12.2$ Hz, H3ax 9-deoxy-9-NAc-Neu5Ac), 1.975 (2H, m, 2CH), 2.037, 2.045, 2.092 (3 × 3H, s, 3COCH₃), 2.677 (1H, dd, $J_{3eq,4} 4.5$, $J_{3eq,3ax} 12.2$ Hz, H3eq 9-deoxy-9-NAc-Neu5Ac), 3.114 (2H, m, 2NCH), 3.303 (1H, dd, $J_{9,8} 7.7$, $J_{9,9'} 14.0$ Hz, H9 9-deoxy-9-NAc-Neu5Ac), 3.462 (1H, dd, $J_{7,6} 1.4$, $J_{7,8} 9.0$ Hz, H7 9-deoxy-9-NAc-Neu5Ac), 3.560 (2H, m, H2, H6 Galβ), 3.611 (1H, dd, $J_{9',8} 2.9$, $J_{9',9} 14.0$ Hz, H9' 9-deoxy-9-NAc-Neu5Ac), 3.61–3.71 (4H, m, H4, H5 GlcNAcβ, $J_{3,4} 3.5$, $J_{3,2} 9.8$ Hz, H3 Galβ, H4 9-deoxy-9-NAc-Neu5Ac), 3.71–3.88 (7H, m, H2, H3 and H6 GlcNAcβ, H5 Galβ, H5, H6 9-deoxy-9-NAc-Neu5Ac, OCH), 3.945 (2H, m, H4 Galβ, H8 9-deoxy-9-NAc-Neu5Ac), 3.97–4.08, (3H, m, OCH, H6' of GlcNAcβ, H6' of Galβ), 4.464 (1H, d, $J_{1,2} 7.9$ Hz, H1 Galβ), 4.560 (1H, m, H1 GlcNAcβ). MS, m/z : 771 [M⁻]. $[\alpha]_D -20$ (c 1, H₂O). R_f 0.41 (MeOH/MeCN/water 3:3:2).

Neu5Acα2-8Neu5Acα-O(CH₂)₃NH₂ (Na⁺ salt) (23). ¹H NMR spectrum (D₂O, 303 K, 600 MHz) δ ppm: 1.646 (1H, dd, $J_{3ax,3eq} \approx J_{3ax,4} \approx 12$ Hz, H3ax Neu5Acα), 1.751 (1H, dd, $J_{3ax,3eq} \approx J_{3ax,4} \approx 12$ Hz, H3ax Neu5Acα2-8), 1.963 (2H, m, 2CH), 2.050 and 2.087 (2 × 3H, s, 2COCH₃), 2.623 (1H, dd, $J_{3eq,3ax} 12.4$, $J_{3eq,4} 4.4$ Hz, H3eq Neu5Acα), 2.790 (1H, dd, $J_{3eq,3ax} 12.4$, $J_{3eq,4} 4.5$ Hz, H3eq Neu5Acα2-8), 3.148 (2H, m, 2CHN), 3.55–3.76 (7H, m, OCH, H4, H9 Neu5Acα, H4, H6, H7, H9 Neu5Acα2-8), 3.79–3.94 (7H, m, OCH, H5, H6, H7 Neu5Acα, H5, H8, H9' Neu5Acα2-8), 4.113 (1H,

dd, $J_{9',8} 3.9$, $J_{9',9} 12.1$ Hz, H9' Neu5Acα), 4.198 (1H, m, H8 Neu5Acα). MS, m/z : 723 [M⁻]. $[\alpha]_D +3.2$ (c 0.5, H₂O). R_f 0.44 (MeOH/MeCN/water 3:3:1).

Neu5Acα2-8Neu5Acα-OCH₂(p-C₆H₄)NHCOCH₂NH₂ (Na⁺ salt) (25). ¹H NMR spectrum (D₂O, 303 K, 600 MHz) δ ppm: 1.651 (1H, dd, $J_{3ax,3eq} \approx J_{3ax,4} \approx 12$ Hz, H3ax Neu5Acα), 1.760 (1H, dd, $J_{3ax,3eq} \approx J_{3ax,4} \approx 12$ Hz, H3ax Neu5Acα2-8), 2.051 and 2.094 (2 × 3H, s, 2COCH₃), 2.705 (1H, dd, $J_{3eq,3ax} 12.2$, $J_{3eq,4} 4.3$ Hz, H3eq Neu5Acα), 2.793 (1H, dd, $J_{3eq,3ax} 12.4$, $J_{3eq,4} 4.6$ Hz, H3eq Neu5Acα2-8), 3.58–3.75 (6H, m, H4, H9 Neu5Acα, H4, H6, H7, H9 Neu5Acα2-8), 3.81–3.95 (6H, m, H5, H6, H7 Neu5Acα, H5, H8, H9' Neu5Acα2-8), 3.954 (2H, s, 2CHN), 4.188 (1H, dd, $J_{9',9} 12.1$, $J_{9',8} 3.8$, H9' Neu5Acα), 4.247 (1H, m, H8 Neu5Acα), 4.504 and 4.504 (2 × 1H, d, $J_{hem} 10.8$ Hz, 2OCH-Ar), 7.478 (4H, m, C₆H₄). MS, m/z : 763 [M⁻]. $[\alpha]_D -17.0$ (c 0.2, H₂O). R_f 0.60 (MeOH/MeCN/water 3:3:2).

Neu5Acα2-8Neu5Acβ-OCH₂(p-C₆H₄)NHCOCH₂NH₂ (Na⁺ salt) (25β). ¹H NMR spectrum (D₂O, 303 K, 600 MHz) δ ppm: 1.620 (1H, dd, $J_{3ax,3eq} \approx J_{3ax,4} \approx 12$ Hz, H3ax Neu5Acα2-8), 1.690 (1H, dd, $J_{3ax,3eq} \approx J_{3ax,4}$, H3ax Neu5Acβ), 2.035 and 2.105 (2 × 3H, s, 2COCH₃), 2.391 (1H, dd, $J_{3eq,3ax} 13.2$, $J_{3eq,4} 4.8$ Hz, H3eq Neu5Acβ), 2.630 (1H, dd, $J_{3eq,3ax} 12.4$, $J_{3eq,4} 4.7$ Hz, H3eq Neu5Acα2-8), 3.52–3.64 (4H, m, H4, H6, H7, H9 Neu5Acα2-8), 3.685 (1H, dd, $J_{9',9} 12.1$, $J_{9',8}$, H9' Neu5Acα2-8), 3.71–3.81 (4H, m, H5 Neu5Acα2-8, H6, H7, H9 Neu5Acβ), 3.87–3.94 (2H, m, H5 Neu5Acβ, H8 Neu5Acα2-8), 3.955 (2H, s, 2CHN), 4.083 (1H, ddd, H4 Neu5Acβ), 4.158 (2H, m, H8, H9' Neu5Acβ), 4.355 and 4.542 (2 × 1H, 2d, $J_{hem} 10.8$ Hz, 2OCH-Ar), 7.527 (4H, m, C₆H₄). MS, m/z : 763 [M⁻]. $[\alpha]_D +10.0$ (c 0.2, H₂O). R_f 0.40 (MeOH/MeCN/water 3:3:2).

Neu5Acα2-8Neu5Acα2-8Neu5Acα-O(CH₂)₃NH₂ (Na⁺ salt) (32). ¹H NMR spectrum (D₂O, 303 K, 600 MHz) δ ppm: 1.652, 1.724 and 1.767 (3 × 1H, dd, $J_{3ax,3eq} \approx J_{3ax,4}$, 3 × H3ax), 1.965 (2H, m, 2CH), 2.051, 2.089 and 2.097 (3 × 3H, s, 3COCH₃), 2.621 (1H, dd, $J_{3eq,3ax} 4.7$, $J_{3eq,4} 12.2$ Hz, H3eq Neu5Acα), 2.701 (1H, dd, $J_{3eq,3ax} 12.0$, $J_{3eq,4} 4.3$ Hz, H3eq 2-8Neu5Acα2-8), 2.784 (1H, dd, $J_{3eq,3ax} 4.7$, $J_{3eq,4} 12.2$ Hz, H3eq Neu5Acα2-8), 3.150 (2H, m, 2CHN), 3.55–3.74 (10H, m, OCH, 3 × H4, 3 × H9; H6, H7 Neu5Acα2-8, H6 2-8Neu5Acα2-8), 3.79–3.97 (9H, m, 3 × H5; H6, H7 Neu5Acα, H7 2-8Neu5Acα2-8, H8, H9' Neu5Acα2-8, OCH), 4.112 (3H, m, H8 Neu5Acα, H8, H9' 2-8Neu5Acα2-8). MS, m/z : 949 [M⁻]. $[\alpha]_D +9.2$ (c 1, H₂O). R_f 0.33 (MeOH/MeCN/water 3:3:1).

Neu5Acα2-8Neu5Acα2-8Neu5Acβ-O(CH₂)₃NH₂ (Na⁺ salt) (32β). ¹H NMR spectrum (D₂O, 303 K, 600 MHz) δ ppm: 1.653, 1.674 and 1.739 (3 × 1H, dd, H3_{ax}), 2.015 (2H, m, 2CH), 2.050, 2.086 and 2.112 (3 × 3H, s, 3COCH₃), 2.328 (1H, dd, $J_{3eq,3ax} 12.9$, $J_{3eq,4} 4.9$ Hz, H3eq Neu5Acβ), 2.730 (1H, dd, $J_{3eq,3ax} 12.4$, $J_{3eq,4} 4.2$ Hz, H3eq 2-8Neu5Acα2-8), 2.775 (1H, dd, $J_{3eq,3ax} 12.3$, $J_{3eq,4} 4.5$ Hz, H3eq Neu5Acα2-8), 3.130 (1H, m, NCH), 3.236 (1H, m, NCH), 3.386

(1H, m, OCH), 3.78–3.93 (6H, m, 3× H5; H7 Neu5Ac β , H7 2-8Neu5Ac α 2-8, H9' Neu5Ac α 2-8), 3.955 (1H, m, H8 Neu5Ac α 2-8), 4.009 (1H, ddd, $J_{4',3eq}$ 4.8, $J_{4,3ax} \approx J_{4,5} \approx 11.0$ Hz, H4 Neu5Ac β), 4.064 (1H, dd, $J_{9',9}$ 12.3, $J_{9',8}$ 2.6 Hz, H9' Neu5Ac β), 4.096 (1H, m, H8 Neu5Ac β), 4.159 (2H, m, H8, H9' 2-8Neu5Ac α 2-8). MS, m/z : 949 [M^-]. $[\alpha]_D^{+9.0}$ (c 0.3, H₂O). R_f 0.22 (MeOH/MeCN/water 3 : 3 : 1).

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