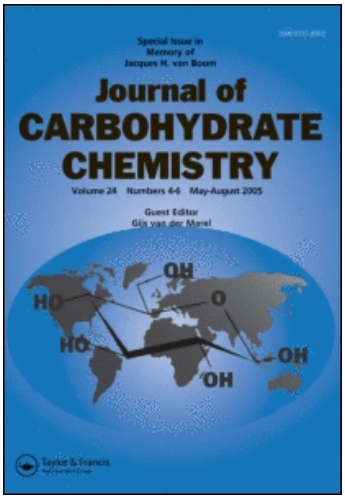

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SYNTHESIS OF SIALIC ACID PSEUDOPOLYSACCHARIDES BY COUPLING OF SPACER-CONNECTED Neu5Ac WITH ACTIVATED POLYMER

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ABSTRACT

A new approach to the synthesis of polyvalent sialosides (pseudopolysaccharides) of Neu5Ac is described. Two monovalent sialosides, namely 4-acetamido- and 4-glycylamidobenzyl α-glycosides of Neu5Ac (and their β-anomers) have been synthesized. The latter, each having a free amino group, have been coupled with poly(4-nitrophenylacrylate) followed by treatment with sodium hydroxide or ethanolamine to give water soluble polyvalent sialosides differing in the nature of polymeric backbone. The coupling proceeded quantitatively providing polymers with a desired number of spacer-connected Neu5Ac residues attached. The polymers are shown to have considerable activity as inhibitors of influenza virus adhesion.

INTRODUCTION

The specific attachment of viral particles to cells during influenza virus (IV) infection proceeds by recognition of the terminal Neu5Ac residues of the host-cell glycoproteins and glycolipids by viral envelope glycoprotein haemagglutinin. Considering the multivalent cooperative nature of virus-cell interaction and data[1] on the competitive inhibition of haemagglutination with different natural sialoglycoproteins we offer here a new route to the synthesis of polyvalent sialosides as potent inhibitors of IV infection.

Roy and co-workers[2-4] have synthesized water-soluble pseudopolysaccharides from the allyl glycoside of Neu5Ac by a copolymerization process.[5,6] We report here another approach to synthesis of polyvalent sialosides by coupling of Neu5Ac derivatives with a spacer arm having a free amino group with the activated polymer.

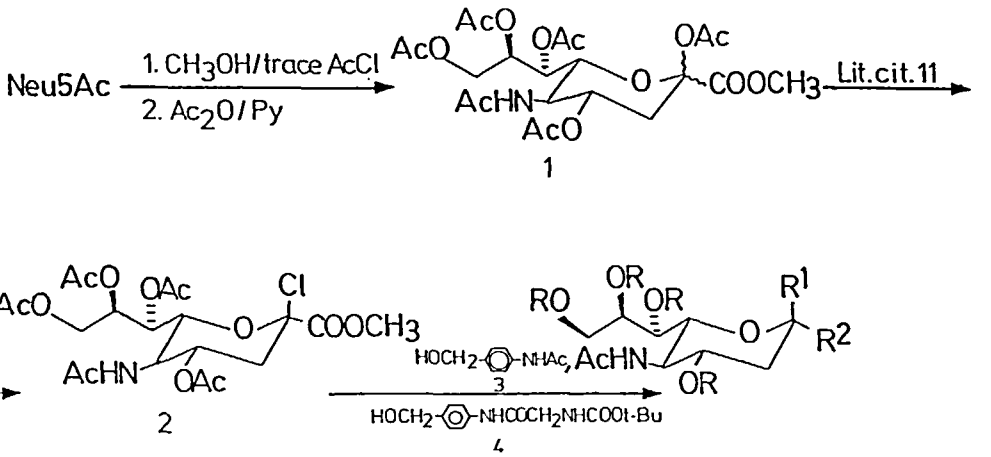
The choice of substituted benzyl sialosides **9** and **17** was made based on the report of Pritchett et al.[7] that among a number of synthetic monovalent sialosides examined, the Neu5Ac α-benzyl glycoside was the most potent inhibitor of A/Memphis/102/72 (H3N2) influenza virus against attachment to erythrocytes.

RESULTS AND DISCUSSION

Koenigs-Knorr reaction of methyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate (**2**) with 4-acetamidobenzyl alcohol (**3**) in acetonitrile-dichloromethane in the presence of mercury bromide - mercury cyanide gave a mixture of α- and β-glycosides (**5**) and (**6**) in 62% yield (3:2 ratio) as revealed from the relative intensities of COOMe signals in the ^{1}H NMR spectrum of the mixture. In the ^{1}H NMR spectrum of the **5**-**6** mixture two sets of signals and coupling constants characteristic of Neu5Ac α- and β-linkage [8] were observed: the H_{3e} dd signal of **6** appeared at δ 2.55, $J_{3a,3e}$ = 13 Hz, $J_{3e,4}$= 5 Hz, and that of **5**, δ 2.65, $J_{3a,3e}$= 13 Hz, $J_{3e,4}$= 4.5 Hz. *O*-Deacetylation of **5** and **6** with sodium methoxide in methanol gave a mixture of **7** and **8** whose ^{1}H NMR spectrum showed a dd for H_{3a} at δ 2.01 and 1.93 for **7** and **8**, respectively. The H_{3e} dd signal appeared at 2.65 (for the α-anomer) and at 2.55 (for the β-anomer) with $J_{3e,4}$= 13 Hz for each anomer and $J_{3e,4}$ = 4.7 Hz for the α- and $J_{3e,4}$= 5 Hz for the β-anomer. Hydrolysis of **7** and **8** with 0.15 M sodium hydroxide afforded the mixture **9** and **10** in 75% yield. Anomers were separated and obtained in pure form using HPLC techniques. In the spectrum of the hydrolyzed mixture the H_{3e} dd appeared at δ 2.77, $J_{3e,3a}$ = 13 Hz, $J_{3e,4}$= 4.5 Hz for the α-anomer and at 2.38, $J_{3e,3a}$= 13 Hz, $J_{3e,4}$= 5 Hz for the β-anomer. Poor yields of coupled products were obtained when silver carbonate-silver triflate as catalyst in dichloromethane-DMF was used.

Coupling of the chloride **2** with the alcohol **4** using mercury bromide-mercury cyanide as catalyst in acetonitrile-dichloromethane gave the mixture of **11** and **12** in 64% yield in the ratio 1:1 as shown by ^{1}H NMR. Two doublet of doublets H_{3e} at δ 2.55 with $J_{3e,3a}$= 13 Hz and $J_{3e,4}$= 5 Hz and another at δ 2.63 with $J_{3e,3a}$= 13 Hz, $J_{3e,4}$ = 4.6 Hz correspond to β-(**12**) and α-(**11**) anomers, respectively. Zemplen deacetylation and hydrolysis of **11** and **12** gave methyl esters **13** and **14** and acids **15** and **16**, respectively. Here again, in the ^{1}H NMR spectrum of each mixture in the *inter alia* signals, H_{3e} dd, with coupling constants and chemical shifts characteristic for α- and β-glycosidic linkages of the Neu5Ac moiety were observed (see EXPERIMENTAL). The overall yield of **15** + **16** was 80% (based on **11** + **12**). After separation using HPLC technique pure nomers α-(**15**) and β-(**16**) were obtained.

Selected properties additionally confirming the anomeric configuration of monovalent sialosides are listed in Table 1.



Compound	R	R^1	R^2
5	AC	COOMe	$OCH_2C_6H_4NHAc$
6	AC	$OCH_2C_6H_4NHAc$	COOMe
7	H	COOMe	$OCH_2C_6H_4NHAc$
8	H	$OCH_2C_6H_4NHAc$	COOMe
9	H	COOH	$OCH_2C_6H_4NHAc$
10	H	$OCH_2C_6H_4NHAc$	COOH
11	AC	COOMe	$OCH_2C_6H_4NHCOCH_2NHBoc$
12	AC	$OCH_2C_6H_4NHCOCH_2NHBoc$	COOMe
13	H	COOMe	$OCH_2C_6H_4NHCOCH_2NHBoc$
14	H	$OCH_2C_6H_4NHCOCH_2NHBoc$	COOMe
15	H	COOH	$OCH_2C_6H_4NHCOCH_2NHBoc$
16	H	$OCH_2C_6H_4NHCOCH_2NHBoc$	COOH
17	H	COOH	$OCH_2C_6H_4NHCOCH_2NH_2$
18	H	$OCH_2C_6H_4NHCOCH_2NH_2$	COOH

Table 1. Selected properties for monovalent sialosides.

Compound, configuration	Chemical shift $^{H}3e$ (dd, δ, ppm)	$^{H}3a$	Coupling const. $^{J}3a,3e$ (Hz)	$^{J}3e,4$	$[\alpha]_D^{20}$ (H_2O)	Inhibitory activity[a]
9, α	2.77	1.67	13	4.5	-21°	active
10, β	2.38	1.68	13.2	5.0	+15.3°	inactive
15, α	2.77	1.68	13	4.5	-24.5°	active
16, β	2.40	1.69	13	5.0	+12.5°	inactive

a, For detailed data see.[9]

Treatment of 4-aminobenzyl alcohol with the *N*-hydroxysuccinimide ester of *N*-Boc-glycine in dry chloroform in the presence of *N*-methylmorpholine resulted in simultaneous *N*- and *O*-acylation. De-*O*-acylation of *N,O*-diacyl derivative with sodium methoxide in methanol gave **4** in 40% yield. Acetylation of 4-aminobenzyl alcohol with acetic anhydride in methanol afforded **3** in 80% yield.

Removal of the Boc-group from **15** and **16** with aqueous 95% trifluoroacetic acid gave glycosides **17** and **18**, which without further purification, were used for coupling with poly(4-nitrophenylacrylate). The coupling was carried out in DMF solution at room temperature in the presence of triethylamine. When coupling was complete the remaining nitrophenyl groups were solvolyzed either with 0.1 N aqueous sodium hydroxide or 10% ethanolamine in DMF. Purification of the resulting material by gel chromatography on Sephadex LH-20 with acetonitrile-water gave the polymers of the basic structure **20** (Table 2).

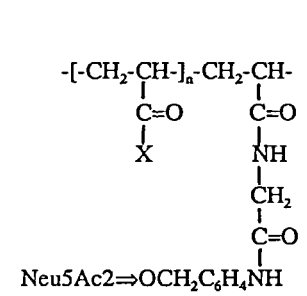
The mole fraction of the attached Neu5Ac residues was assessed spectrophotometrically at 248 nm using glycoside **10** as a standard.

Monomeric sialosides **9, 10, 15, 16** and pseudopolysaccharides **20** obtained were used to investigate their ability to inhibit influenza virus receptor-binding activity.[9] In conclusion, an alternative approach to the synthesis of sialic acid containing pseudopolysaccharides which enabled quantitative coupling of sialic acid derivatives to a synthetic polymeric backbone and variation in the polymer matrix has been developed.

EXPERIMENTAL

General Procedures. Melting point was determined with a Kofler apparatus and is uncorrected. Acetonitrile was refluxed over $KMnO_4$-K_2CO_3, distilled twice from P_2O_5 and from

TABLE 2. Designation of pseudopolysaccharides obtained.

Common formula (20)	X	Anomeric configuration	Mole fraction of Neu5Ac, %	Designation
	ONa	α	5	$P_{\alpha.5.1}$
	ONa	α	10	$P_{\alpha.10.1}$
	ONa	β	10	$P_{\beta.10.1}$
	$HNCH_2CH_2OH$	α	10	$P_{\alpha.10.2}$
	ONa	α	20	$P_{\alpha.20.1}$
	ONa	α	30	$P_{\alpha.30.1}$

CaH_2. Dichloromethane was washed with concd H_2SO_4 and water, dried ($CaCl_2$) and distilled from CaH_2. DMF, dried by azeotropic co-distillation with dry benzene and subsequent distillation of the residue at reduced pressure, was stored over molecular sieves 4A. Before use in coupling reactions with polymer, it was distilled from ninhydrin at reduced pressure. Spots on TLC (Merck) were detected by ultraviolet irradiation (UV) and by charring with 5% sulfuric acid. TLC was performed in the following solvents: propanol - ethyl acetate - water = 4:3:2 (A), toluene - ethyl acetate = 1:5 (B), 1:9 (C), chloroform - methanol = 9:1 (D), 4:1 (E). Column chromatography (CC) was performed on Silica gel L 40-100 μm (Czechoslovakia). HPLC was performed using a Whatman, Partisil 10 ODS-3 column with acetonitrile-water as a mobile phase, the column effluent was monitored with a UV-detector at 240 nm. Optical rotations were determined with a DIP-360 polarimeter. ^1H NMR spectra were recorded with a Bruker WM-250 spectrometer, chemical shifts are expressed in ppm (δ), relative to internal TMS for solutions in $CDCl_3$ or $CDCl_3$ and CD_3OD and an external reference (0.2% solution in $CDCl_3$) for solutions in D_2O. ^1H NMR data were confirmed by use of decoupling techniques. Poly(4-nitrophenylacrylate) (PNA) with degree of polymerization 1500-3000 was a gift of Dr. A. E. Ivanov (Shemyakin Institute of Bioorganic Chemistry). Satisfactory elemental analyses were not obtained for amorphous products, but they were shown to be pure by chromatography and NMR spectroscopy.

Methyl (5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-nonulopyranosyl chloride)onate (2). Methanol (200 mL) was treated at O °C with acetyl chloride (1.4 mL) and added to Neu5Ac (1.36 g, 4.4 mmol), the mixture was kept overnight at room

temperature, then neutralized at O °C with an excess of pyridine (3 mL) and concentrated. Pyridine was repeatedly added and evaporated from the residue (3 x 3 mL). The resulting methyl ester of Neu5Ac was finally dissolved in 20 mL of pyridine, cooled to O °C and treated with acetic anhydride (10 mL). After standing at room temperature overnight the mixture was treated at O °C with methanol (10 mL), the solvents were removed by evaporation, the residue was partitioned between chloroform (100 mL) and water (100 mL), the organic layer was washed with water (50 mL), 1 N hydrochloric acid (50 mL), water, saturated sodium bicarbonate solution, water, filtered through a cotton plug and concentrated. Gradient CC using toluene with increasing amounts of ethyl acetate (⇒70%) gave an amorphous anomeric mixture [10] of α-(R_f 0.33, B, minor) and β-(R_f 0.22, B, major) tetraacetates (2), yield 1.8 g (78%). The mixture was converted [11] to homogeneous chloride (2), which was used immediately without further purification (R_f 0.46, B).

4-Acetamidobenzyl Alcohol (3). To the methanolic solution (10 mL) of 4-aminobenzyl alcohol (200 mg, 1.6 mmol) at 0 °C acetic anhydride (0.5 mL, 5 mmol) was added and allowed to stand at this temperature for 1.5 h. Excess of acetic anhydride was destroyed by adding pyridine (0.5 mL) and the mixture was concentrated. CC of the residue using chloroform with an increasing amount of methanol (⇒15%) gave ninhydrin negative 3 (R_f 0.35, system D, the starting ninhydrin positive amine had R_f 0.45), yield 215 mg (80%), amorphous powder, which crystallized on standing; mp 119-122 °C, lit.[12] mp 120-121 °C (nitromethane). ^{1}H NMR ($CDCl_3$) 2.05 (s, 3H, NAc), 3.4 (near s, 1H, NH), 3.55 (s, 2H, CH_2O-), 4.50 (s, OH), 7.20 - 7.40 (m, 4H, aromatic).

(4-*tert*-Butoxycarbonylglycylamido)benzyl Alcohol (4). To the solution of 4-aminobenzyl alcohol (240 mg, 2 mmol) in chloroform (5 mL) *N*-hydroxysuccinimide ester of Boc-glycine (1 g, 3.7 mmol) and *N*-methylmorpholine (330 μL, 3 mmol) were added and the mixture allowed to stand 24 h at room temperature. The reaction mixture was concentrated and the *N,O*-diacylated product was separated from the residue using CC by elution with a gradient of toluene with an increasing amount of ethyl acetate (⇒25%). The product was *O*-deacylated with 0.02 M sodium methoxide (20 mL) in methanol for 24 h at room temperature. After neutralization with 0.02 M acetic acid in toluene and concentration the residue was subjected to CC in toluene ⇒ ethyl acetate gradient to give the title compound as an amorphous powder; yield 224 mg, (40%), R_f 0.6 (D), *N,O*-diacylated product and starting amine had R_f 0.9 and 0.5, respectively. ^{1}H NMR ($CDCl_3$) 1.41 (s, 9H, CMe_3), 3.84 (m near d, 3H, $COCH_2N$, OH), 4.56 (s, 2H, CH_2OH), 5.35 (broad s, 1H, CH_2NHCOO), 7.21-7.40 (m, aromatic), 8.30 (broad s, 1H, PhNH).

Methyl (4-Acetamidobenzyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α,β-D-*glycero*-D-*galacto*-nonulopranoside)onate (5 + 6). The mixture of 3 (135 mg, 0.8 mmol), mercury cyanide (35mg, 0.09 mmol) and molecular sieves 4A (0.3) in 5 mL of acetonitrile was

stirred 16 h under N_2 at room temperature. The mixture was cooled to 0 °C and the solution of **2**, obtained from 200 mg (0.370 mmol) of **1**, in 5 mL of dichloromethane together with additional portion molecular sieves 4A (0.1 g) were added. The reaction mixture was stirred at 4 °C (24 h) then at room temperature (48 h) until disappearence of **2** (TLC). The mixture was diluted with dichloromethane, filtered, the combined filtrates and washings were concentrated. Solution of the residue in choloroform, washing with 1 M solution of potassium iodide (30 mL), then 2 M sodium chloride (30 mL), filtration through a cotton plug and concentration gave a crude product, 200 mg, which was chromatographed using a gradient of ethyl acetate with increasing amounts of acetone (⇒10 %) to give an inseparable anomeric mixture (3:2) of **5** and **6** (R_f 0.50, E), yield 150 mg (62%). ^{1}H NMR ($CDCL_3$) 1.87, 1.90, 1.99, 2.02, 2.03, 2.04, 2.06, 2.12, 2.14, 2.20, 2.33 (Ac), 2.65 (dd, $J_{3e,4}$ = 4.5 Hz, $J_{3e,3a}$ = 13 Hz, H_{3e} of the α-anomer), 2.55 (dd, $J_{3e,4}$ = 5 Hz, $J_{3e,3a}$ = 13 Hz, H_{3e} of the β-anomer), 3.69 (s, COOMe of the α-anomer), 3.76 (s, COOMe of the β-anomer), 3.98 (dd, $J_{6,7}$ = 2 Hz, $J_{6,5}$ = 10.7 Hz, H-6 of the β-anomer), 4.38 and 4.77 (two d, J_{gem} = 12.5 Hz, CH_2-Ph of the α-anomer), 4.45 and 4.52 (two d, J_{gem} = 12.5 Hz, CH_2-Ph of the β-anomer), 4.87 (dd, $J_{9A,9B}$ = 12.5 Hz, $J_{9A,8}$ = 2,5 Hz, H-9A of the α-anomer), 5.35 (dd, $J_{7,6}$ = 2 Hz, $J_{7,8}$ = 8.5 Hz, H-7 of the α-anomer), 5.45 (dd, $J_{7,6}$ = 2 Hz, $J_{7,8}$ = 4 Hz, H-7 of the β-anomer), 7.15-7.53 (aromatic).

Methyl (4-Acetamidobenzyl 5-Acetamido-3,5-dideoxy-α,β-D-*glycero*-D-*galacto*-non-ulopyranoside)onate (7 + 8). To the mixture of 5 + 6 (150 mg) 0.025 N sodium methoxide in methanol (20 mL) was added and allowed to stand at room temperature for 20 h giving rise to **7** + **8** (R_f = 0.10 and 0.20, respectively, system E). For analytical purposes the 1 mL aliquot of the solution was treated with dry methanol washed Amberlite IR-120 (H^+) to adjust the pH to 7.0, filtered, and concentrated to dryness. ^{1}H NMR-data ($CDCl_3$ + CD_3OD) 1.93 (dd, H_{3a} of β), 2.01 (dd, H_{3a} of a), 2.05 (s, 6H, NAc of two anomers), 2.20 and 2.37 (two s, Ph-NAc of two anomers), 2.55 (dd, $J_{3e,4}$ = 5 Hz, $J_{3e,3a}$ = 13 Hz, H_{3e} of β), 2.65, (dd, $J_{3e,4}$ = 4.7 Hz, $J_{3e,3a}$ = 13 Hz, H_{3e} of α), 3.68 (s, COOMe of β), 3.76 (s, COOMe of α), 3.98, (dd, $J_{6,7}$ = 2.5 Hz, $J_{6,5}$ = 10.5 Hz, H-6 of β), 4.87 (dd, $J_{9A,9B}$ = 12.5 Hz, $J_{9A,8}$ = 2.5 Hz, H-9A of β), 5.35 (dd, $J_{7,6}$ = 2 Hz, $J_{7,8}$ = 8.5 Hz, H-7 of β), 5.43 (dd, $J_{7,6}$ = 2.5 Hz, $J_{7,8}$ = 4 Hz, H-7 of the α-anomer), 7.15-7.45 (m, aromatic).

4-Acetamidobenzyl 5-Acetamido-3,5-dideoxy-α (9) and β-D-*glycero*-D-*galacto*-nonulopyranoside)onic acid (10). To the Zemplen deacetylation solution obtained above water (1 mL) was added. The mixture was concentrated *in vacuo* to the volume of 3 mL and allowed to stand at 40 °C and then 48 h at room temperature. The mixture was deionized with dry Amberlite IR-12O (H^+), filtered, methanol was evaporated from the filtrate. The resulting aqueous solution was freeze dried to give the mixture **9** + **10**; yield 80 mg (75%), (R_f = 0.2 for β, and 0.3 for α, A). Separation of the anomeric mixture was carried out in several runs on C-18 reverse phase column (1 x 25 cm). The compounds were eluted from the column by a gradient of water with increasing amounts of acetonitrile (2 ⇒ 20%), the eluate was monitored at 240 nm,

appropriate fractions were pooled, following acetonitrile evaporation the residue was freeze dried to give **9** (35 mg, R_f 0.3, A) and **10** (35 mg, R_f 0.2, A). Eluted first was the β-anomer (**10**), $[\alpha]_D^{20}$ +15.3° (c 0.55, water), ^{1}H NMR (D_2O) 1.68 (dd, 1H, $J_{3a,3e}$ = 13.2 Hz, $J_{3a,4}$ = 11.25 Hz, H_{3a}), 2.04 (s, 3H, NAc), 2.16 (s 3H, PhNAc), 2.38 (dd, 1H, $J_{3e,4}$ = 5 Hz, H_{3e}), 3.58 (dd, near t, $J_{7,8}$ = 1.5 Hz, $J_{7,8}$ = 9.5 Hz, H-7), 3.68 (dd, $J_{9B,8}$ = 5 Hz, $J_{9B,9A}$ = 12.5 Hz, H-9B), 3.85 (dd, $J_{9A,8}$ = 2.5 Hz, H-9A), 3.95 (m, H-5), 3.99 (m, H-6), 4.04 (m, H-4), 4.26 and 4.59 (two d, J_{gem} = 10.5 Hz, CH_2Ph), 7.45 (m, aromatic). Eluted second was the α-anomer (**9**), $[\alpha]_D^{20}$ = -21° (c 0.47, water) ^{1}H NMR (D_2O) 1.67 (dd near t, $J_{3a,3e}$= 13 Hz, $J_{3a,4} \cong$ 12.5 Hz, H_{3a}), 2.03 (s 3H, NAc), 2.16 (s, 3H, PhNAc), 2.77 (dd, 1H, $J_{3e,4}$ = 4.5 Hz, $J_{3e,3a}$ = 13 Hz, H_{3e}), 3.67 (m, H-4), 3.77 (m, H-9A), 3.83 (near t, $J_{5,6} = J_{5,4}$ = 9.5 Hz, H-5), 3.84 (m, H-9B), 3.60-4.00 (m, H-6, H-8), 4.37 and 4.74 (two d, J_{gem} = 12 Hz, CH_2Ph), 7.40 (near s, aromatic).

Methyl (4-*tert*-Butoxycarbonylglycylamidobenzyl 5-Acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α,β-D-*glycero*-D-*galacto*-nonulopyranoside)onate (11 + 12). The mixture of alcohol **4** (200 mg, 0.7 mmol), mercury cyanide (97 mg, 0.5 mmol), mercury bromide (35 mg, 0.1 mmol), molecular sieves 4A (0.5 g) and acetonitrile (5 mL) was stirred at room temperature for 2 h. The solution of the chloride **2**, prepared from 200 mg (0.37 mmol) of acetate **1** in dichloromethane (4 mL) together with an additional portion of molecular sieves (0.4 g) was added at 0 °C, and the mixture was stirred for 24 h at 4 °C, then 48 h at room temperature. The reaction mixture was diluted with chloroform and worked up as described for the preparation of **5** + **6**, to give 350 mg of the crude product, which was purified using CC by elution first with a gradient of toluene with increasing amounts of ethyl acetate (⇒100%), then a gradient of ethyl acetate with increasing amounts of acetone (⇒ 10%) to give **11** + **12**; R_f 0.20 in the system C, yield 180 mg (64%). ^{1}H NMR ($CDCl_3$) 1.45 and 1.47 (two s, CMe_3), 1.82 - 2.12 (m, Ac), 2.55 (dd, $J_{3e,4}$ = 5 Hz, $J_{3e,3a}$= 13 Hz, H_{3e} of β), 2.63 (dd, $J_{3e,4}$ = 4.6 Hz, $J_{3e,3a}$ = 13 Hz, H_{3e} of α), 3.66 and 3.75 (two s, COOMe of α and β in the ratio 1:1), 3.98 (dd, $J_{6,7}$ = 2 Hz, $J_{6,5}$ = 10.5 Hz, H-6 of the β-anomer), 4.37 and 4.47 (two d, J_{gem} = 12 Hz, CH_2Ph of the α-anomer), 4.43 and 4.52 (two d, J_{gem} = 11.5 Hz, CH_2Ph of the β-anomer), 4.86 (dd, $J_{9A,9B}$ = 12 Hz, $J_{9A,8}$ = 2.5 Hz, H-9A of the β-anomer), 7.15-7.45 (m, aromatic).

Methyl (4-*tert*-Butoxycarbonylglycylamidobenzyl 5-Acetamido-3,5-dideoxy-α, β-D-*glycero*-D-*galacto*-nonulopyranoside)onate (13 + 14). The mixture **11** + **12** (180 mg, 0.24 mmol) was treated with 0.025 M sodium methoxide in methanol (20 mL) for 24 h at room temperature, the cooled (0 °C) solution was made neutral (pH 7) by addition of acetic acid (30 μL) dissolved in 15 mL of methanol. After evaporation of the methanol *in vacuo* the residual solution was applied to the Sephadex LH-20 column (0.8 x 20 cm) equilibrated with methanol. Elution with methanol gave **13** + **14**, yield 112 mg (80%). ^{1}H NMR ($CDCl_3$ + CD_3OD) 1.44 (dd, $J_{3a,4}$ = 10 Hz, $J_{3a,3e}$ = 12.5 Hz, H_{3a} of the β-anomer), 1.60 (dd, $J_{3a,4}$ = 12 Hz, $J_{3a,3e}$ = 13 Hz, H_{3a} of the α-anomer), 1.73 and 1.75 (two s, NAc of the β- and α-anomer), 2.21 (dd, $J_{3e,4}$ = 5 Hz,

$J_{3e,3a}$ = 12.5 Hz, H_{3e} of the β-anomer), 2.51 (dd, $J_{3e,4}$ = 4.5 Hz, $J_{3e,3a}$ = 12.5 Hz, H_{3e} of the α-anomer), 3.49 and 3.52 (two s, COOMe of the α-and β-anomer), 4.16 and 4.50 (two d, J_{gem} = 11 Hz, CH_2Ph of the α-anomer), 7.00-7.24 (m, aromatic).

(4-*tert*-Butoxycarbonylglycylamidobenzyl 5-Acetamido-3,5-dideoxy-α- and β-D-*glycero*-D-*galacto*-nonulopyranoside)onic acid (15 + 16). To the solution of **13** + **14** (100 mg) in methanol (1 mL) 1.5 mL of water and then 260 μL of 2 M sodium methoxide in methanol were added. The solution was heated at 40 °C for 3 h and then kept at room temperature for 48 h. The cooled mixture (0 °C) was neutralized to pH 7 by adding dropwise a solution of hydrochloric acid (50 μL in 10 mL of methanol-water 1:1, v/v). Evaporation and subsequent gel-filtration of the residue on the Biogel P-2 column (1 x 15 cm) in water gave the crude mixture of **15** + **16**. Separation of the anomeric mixture on the reversed phase C-18 column was carried out as described for **9** + **10** separation. Eluted first was the β-anomer (**16**), yield 30 mg, R_f 0.50 (A), $[\alpha]_D^{20}$ +12.6° (c 0.47, water). ^1H NMR (D_2O) 1.44 and 1.50 (two s, 9H, CMe_3), 1.69 (dd near t, $J_{3a,4}$ = 12.5 Hz, H_{3a}), 2.04 (s, 3H, NAc), 2.40 (dd, $J_{3e,4}$ = 5 Hz, $J_{3e,3a}$ = 13 Hz, H_{3e}), 3.59 (dd near d, $J_{7,6}$ < 2 Hz, $J_{7,8}$ = 9.75 Hz, H-7), 3.69 (dd, $J_{9A,9B}$ = 12.5 Hz, $J_{9A,8}$ = 5 Hz, H-9A), 3.86 (dd, $J_{9B,8}$ = 2.8 Hz, H-9B), 3.96 (m, H-5), 3.99 (m, H-6), 4.04 (m, H-4), 4.28 and 4.61 (two d, J_{gem} = 10 Hz, CH_2Ph), 7.45 and 7.50 (two d, aromatic). Eluted second was the α-anomer (**15**), yield 45 mg, R_f 0.60 (A), $[\alpha]_D^{20}$-24.5° (c 0.37, water). ^1H NMR (D_2O) 1.42 and 1.46 (two s, 9H, CMe_3), 1.68 (dd near t, $J_{3a,4}$ = 12.5 Hz, H_{3a}), 2.03 (s, 3H, NAc), 2.77 (s, dd, $J_{3e,4}$ = 4.5 Hz, $J_{3e,3a}$ = 13 Hz, H_{3e}), 3.67 (m, H-4), 3.77 (m, H-9A), 3.83 (near t, $J_{5,6}$ = $J_{5,4}$ = 9.5 Hz, H-5), 3.84 (m, H-9B), 7.40 (near s, aromatic).

Preparation of Pseudopolysaccharides. *Deblocking of* **15** *and* **16.** The solution of **15** or **16** (2 mg, 3.5 μmol) in water (100 μL) was treated with 95% aqueous trifluoroacetic acid (1 mL) at 0 °C for 0.5 h to give ninhydrin-positive **17** and **18** (R_f 0.2 and 0.05, respectively, system A). Repeating co-evaporation of the mixture with dry toluene (3 x 5 mL) gave homogeneous (4-glycylamidobenzyl 5-acetamido-3,5-dideoxy-α- or β-D-*glycero*-D-*galacto*-nonulopyranoside)onic acid (**17** or **18**), which were used immediately without further purification. *Coupling of* **17** *and* **18** *with polymer.* To the solution of the foregoing aminoligand (**17** or **18**) in DMF (200 μL) 10% solution (70 μL) of the NPA (containing 35 μmol of 4-nitrophenyl ester groups) in DMF was added and then treated with triethylamine (20 μL). The reaction mixture was allowed to stand at room temperature for 48-60 h until disappearence of the ninhydrin-positive starting material (TLC). Further modification of the polymer was made by either treating it with 2 mL of 0.1 M sodium hydroxide (procedure 1) or with 2 mL of 10% solution of ethanolamine in DMF (procedure 2), giving rise to the polymers designed as $P_{\alpha(\beta)\text{-}10.1}$ and $P_{\alpha\text{-}10.2'}$, respectively. The reaction mixture was allowed to stand at room temperature for another 48-72 h and 4-nitrophenol was separated by gel-filtration on a Sephadex LH-20 column (1 x 25 cm) in acetonitrile-water (1:1), spectrophotometric monitoring at 240 nm. The

appropriate fractions were pooled, acetonitrile was evaporated and the residue was freeze-dried to give pseudopolysaccharides as white amorphous powders. The yields were 80-90%. Polymers $P_{\alpha 5.1}$, $P_{\alpha 20.1}$ and $P_{\alpha 30.1}$ were obtained by treating a solution of 15 (3.5 μmol) in DMF (200 μL) with 10% solution of PNA (140, 35 and 23 μL, that is containing 70, 17.5 and 11.5 μmol of 4-nitrophenyl ester groups, respectively), the unreacted ester groups being removed according to procedure 1.

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