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Synthesis and Biological Activity of High-Molecular *N*-Glycosides

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Abstract—A new synthetic approach to high molecular *N*-glycosides has been developed on the basis of radical polymerization of vinyl-containing *N*-glycosides. Homo- and copolymers derived from *N*-(D-glucopyranosyl)-, *N*-(D-mannopyranosyl)-, and *N*-(L-rhamnopyranosyl)-4-vinylaniline have been synthesized and characterized. It has been found for the first time that polymeric *N*-glycosides exhibit antibacterial and immunomodulatory activity.

Keywords: *p*-aminostyrene, *N*-glycosides, carbon chain polymers, biological activity

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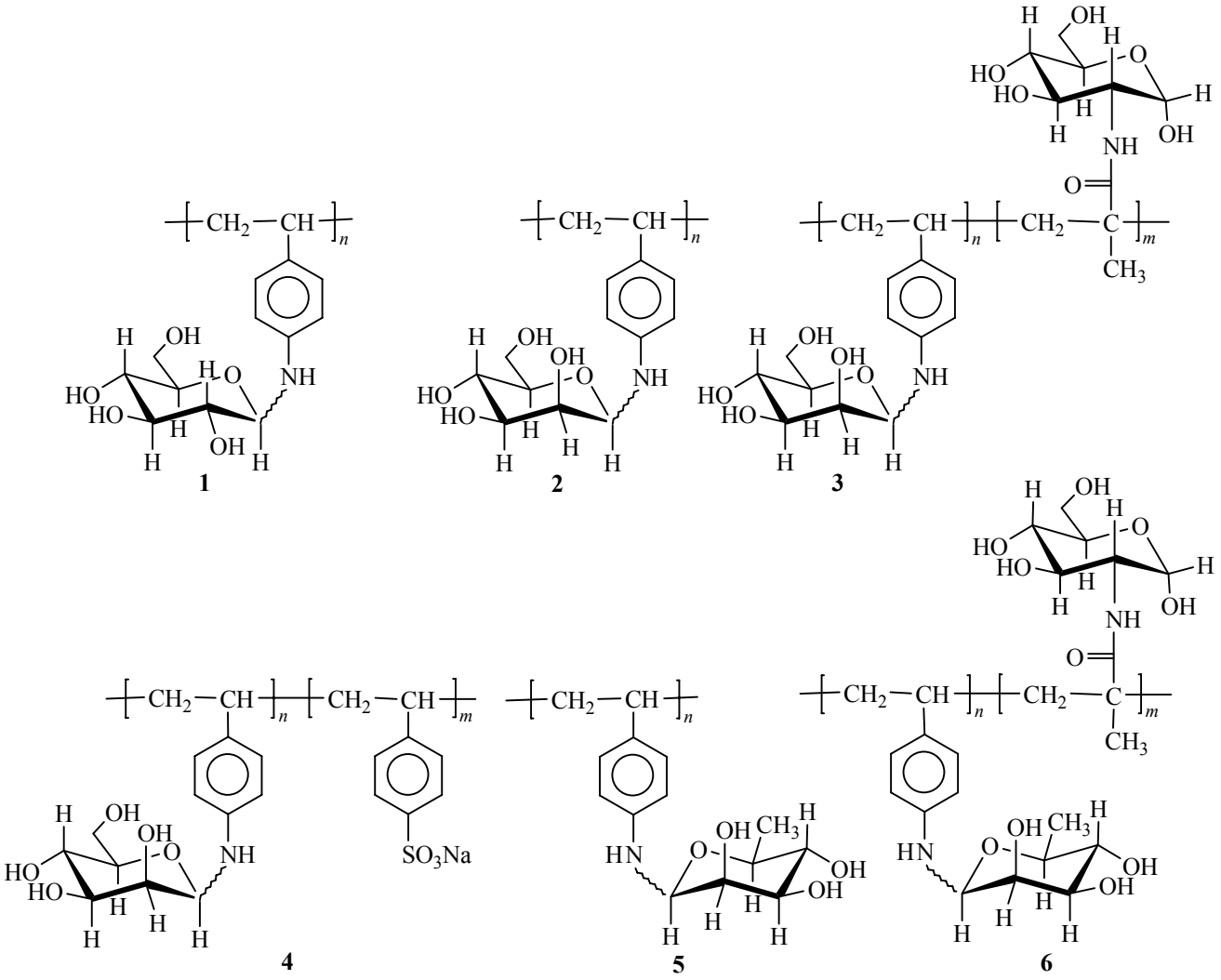
It is known that synthetic high-molecular *N*-glycosides in which, as in most natural proteins, the glycoside fragments reside in side chains exhibit biological activity. Up to now, only a few representatives of high-molecular *N*-glycosides have been synthesized and studied. They are obtained by polymeranalogous transformations of such polymers as proteins [1], synthetic polypeptides [2], and polyvinylamine [3–5]. Introduction of D-glucose and D-glucosamine fragments, as well as of their acetyl derivatives, into polymer molecules have been reported. For example, poly-*N*-glycoside obtained from polyvinylamine, in which *N*-acetyl-β-D-glucosamine residues are linked to the main chain through HN=C(NH–)$_2$ spacers, showed pronounced antitumor effect on parenteral administration [5]. Thus, development of new methods of synthesis of poly-*N*-glycosides is a topical problem.

We previously reported a new procedure for the synthesis of high-molecular *N*-glycosides by radical polymerization of *N*-glucopyranosyl-4-vinylaniline [6]; in addition, the synthesis of other vinyl-containing *N*-glycosides was described [7–10]. The present work was aimed at synthesizing homo- and copolymers from *N*-glycosyl-4-vinylaniline by radical polymerization in solution in the presence of an azo initiator and studying their antibacterial and immunomodulatory activity. The initial monomeric *N*-glycosides were *N*-(D-glucopyranosyl)-, *N*-(D-mannopyranosyl)-, and *N*-(L-rhamnopyranosyl)-4-vinylanilines, and *N*-methacryloyl-α,β-D-glucosamine and sodium 4-vinylbenzenesulfonate were used as comonomers.

As a result, poly{1-[4-(D-glucopyranosylamino)-phenyl]ethylene} (**1**), poly{1-[4-(D-mannopyranosylamino)phenyl]ethylene} (**2**), copolymers **3** and **4** of *N*-(D-mannopyranosyl)-4-vinylaniline with *N*-methacryloyl-α,β-D-glucosamine and sodium 4-vinylbenzenesulfonate, poly{1-[4-(L-rhamnopyranosylamino)phenyl]ethylene} (**5**), and copolymer **6** of *N*-(L-rhamnopyranosyl)-4-vinylaniline with *N*-methacryloyl-α,β-D-glucosamine were isolated. The polymerization was carried out in an anhydrous solvent under in an inert atmosphere using 2,2′-azobis(isobutyronitrile) (AIBN) as initiator. These conditions made it possible to avoid hydrolytic or oxidative decomposition of saccharides. In the synthesis of **1**, the solvent was a mixture of methanol with ethanol which simultaneously acted as precipitant. Two polymer fractions with different stabilities in aqueous solution were thus isolated. On storage, a solid separated from aqueous solutions of both fractions of polymer **1** with equal concentrations due to partial elimination of D-glucose fragments. One fraction, presumably with a longer polymer chain, was stable for 15 h at 5°C, while the other fraction can be stored for a month at 25–30°C without appreciable decomposition (Scheme 1).

Scheme 1.



Poly-*N*-glycosides **2**–**6** were synthesized in DMF which favors gel formation using more concentrated (20%) monomer solutions, so that the yields were considerably improved. Homopolymers **2** and **5** are insoluble, whereas copolymers **3**, **4**, and **6** are soluble in water and other solvents. Thus, introduction into a polymer chain of D-glucosamine or benzenesulfonate groups makes the polymer hydrophilic and endows it with additional functionality. The structure of the synthesized polymers in solution and in the solid phase was determined by NMR spectroscopy.

Polymeric *N*-glycosides **2**–**6** were tested for antibacterial and immunomodulatory activity. Polymer **2** showed bacteriostatic effect against *E. coli* (c_{ing} = 500 μg/mL) and (c_{ing} = 100 μg/mL), while copolymer **3** exhibited bacteriostatic activity against *S. aureus* (c_{ing} = 500 μg/mL) and *E. coli* ($c_{ing} \geq$ 10 μg/mL) and bacteriostatic (c_{ing} = 100 μg/mL) and bactericidal activity (c_{ing} = 500 μg/mL) against *C. albicans*.

All polymers **2**–**6** turned out to act as strong immunosuppressants (see table) in mice on oral and parenteral administration. This is typical of both water-soluble and insoluble compounds. It was found that the immunosuppressive effect at a dose of 10 mg/kg is stronger than at a dose of 25 mg/kg. An exception was polymer **5** whose efficiency was higher at a dose of 25 mg/kg; however, it is necessary to take into consideration a relatively high *p* value in the determination of the immune response factor (IRF) at a dose of 10 mg/kg. The effects of copolymer **3** on different modes of administration were almost similar (IRF 0.60, 10 mg/kg) and approached that of homopolymer **2** (IRF 0.55, 10 mg/kg). It may be concluded that only the presence of *N*-glycoside fragments is responsible

Effect of polymers **2**–**6** on the antibody response in mice[a]

Dose, mg/kg (number of animals)	Spleen cellularity, $\times 10^{-7}$ (m, mg)	Number of antibody-forming cells per spleen, 10^{-2}	Number of antibody-forming cells per 10^6 nuclear spleen cells	Immune response factor
		2 (oral)		
25 (8)	12.5±2.1, $p < 0.1$	242.0±61.6, p 0.1	204.6±30.0, p 0.1	0.75
10 (8)	13.2±1.6, $p < 0.1$	204.6±29.1, $p < 0.01$	158.0±10.8, $p < 0.001$	0.55
Control (8)	13.6±1.3	408.6±63.15	272.4±25.3	
		3 (oral)		
25 (8)	19.0±1.5, $p < 0.1$	268.0±30.0, $p < 0.1$	154.8±15.6, $p < 0.5$	0.70
10 (8)	16.2±1.7, $p < 0.1$	215.0±47.7, $p < 0.1$	140.1±18.7, $p < 0.02$	0.60
Control (8)	14.5±1.2	329.0±87.8	218.0±26.6	
		3 (parenteral)		
25 (8)	11.0±1.6	177.5±14.9, $p < 0.1$	169.5±8.0, $p < 0.1$	1.02
10 (8)	11.2±0.6	125.3±19.8, $p < 0.1$	113.0±13.1, $p < 0.02$	0.60
Control (8)	10.7±0.9	182.0±36.8	165.5±18.5	
		4 (oral)		
25 (9)	18.8±3.8, $p > 0.1$ (242.8±24.1)	286.7±70.4, $p > 0.1$	159.0±25.6, $p > 0.1$	0.80
10 (9)	19.5±0.1, $p < 0.001$(213.0±11.2)	201.9±17.4, $p < 0.02$	105.0±12.5, $p < 0.02$	0.56
Control (8)	24.1±0.8 (242.6±16.1)	423.0±42.5	185.0±27.9	
		4 (parenteral)		
25 (9)	13.6±1.1, $p > 0.1$ (182.7±9.5)	177.0±20.1, $p > 0.1$	120.0±12.6, $p > 0.1$	0.89
10 (9)	14.8±1.0, $p > 0.1$(187.0±14.4)	92.4±13.5, $p < 0.0001$	57.0±4.9, $p < 0.001$	0.42
Control (8)	15.7±1.0 (193.6±5.8)	206.0±19.0	133.7±9.7	
		5 (oral)		
25 (8)	24.0±1.8, $p < 0.05$ (293.7±16.7)	283.0±38.7, $p > 0.1$	160.0±17.4, $p < 0.02$	0.70
10 (9)	18.4±1.2, $p > 0.1$ (251.6±14.0)	371.0±47.0, $p > 0.1$	218.0±12.3, $p > 0.1$	1.0
Control (9)	18.5±2.0 (220.8±15.9)	402.0±71.7	215.5±16.4	
		6 (oral)		
25 (9)	16.5±2.15, $p > 0.5$ (228.0±14.5)	367.3±64.3, $p > 0.1$	246.4±12.8, $p < 0.02$	1.17
10 (8)	18.6±1.9, $p > 0.5$ (231.0±11.7)	390.8±59.9, $p > 0.1$	214.9±17.9, $p > 0.5$	1.02
Control (8)	21.4±1.9 (225.0±11.9)	413.1±34.8	210.0±7.3	
		6 (parenteral)		
25 (9)	18.6±1.7 (217.0±12.1)	401.4±59.4, $p > 0.1$	211.0±16.4, $p < 0.01$	0.64
10 (8)	14.8±1.9, $p = 0.05$ (186.6±11.1)	233.0±49.2, $p < 0.002$	159.5±16.7, $p < 0.001$	0.49
Control (8)	19.4±1.1 (256.6±10.7)	609.0±93.4	325.5±16.7, $p < 0.001$	

[a] p stands for statistical significance; immune response factor is defined as the number of antibody-forming cells per 10^6 spleen cells divided by the same for control group of animals. The immune response factor for the control group was assumed to be unity.

for the biological activity of **2** and **3** and that the second monomer units only make the polymer soluble.

Copolymer **4** also showed a high activity at a dose of 10 mg/kg on oral (IRF 0.56, $p < 0.02$) and parenteral administration (IRF 0.42, $p < 0.001$). Presumably, its biological activity is contributed by both monomer units. Like other polymers containing *para*-substituted benzenesulfonate fragments [11], copolymer **4** may be expected to exhibit antiviral activity. The effect of copolymer **6** was higher on parenteral administration, especially at a dose of 10 mg/kg (IRF 0.49).

In summary, a new procedure has been developed for the synthesis poly-*N*-glycosides. Carbon-chain homo- and copolymers obtained from *N*-glycosyl-4-vinylanilines exhibit antibacterial and immunosuppressive activity. No effect of the configuration of glycosyl fragments on the suppressive activity was observed.

EXPERIMENTAL

Anhydrous alcohols, diethyl ether, and DMF were used. Methanol (99.5%) and ethanol (94.5%) were preliminarily dried by heating under reflux over excess calcium hydride and were then distilled over metallic sodium. Dimethylformamide was treated with phosphoric anhydride, distilled, and finally distilled over a small amount of calcium hydride. Initial *N*-glycosides were synthesized as described in [9, 10].

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance II 400 (400.1 and 100.6 MHz, respectively; solutions in DMFA-d_7, D_2O, D_2O–DMSO-d_6) and Bruker Avance III 500 spectrometers (125.76 MHz for ^{13}C; solid samples). The UV spectra were measured on an SF-256 UVI spectrophotometer (LOMO). The viscosity of copolymer solutions were determined at 25°C using Ubbelode (V = 10 cm^3) and Cannon-Manning 9721-X59 (C 639) (V = 1 cm^3) capillary viscometers.

Polymers **1**–**6** were synthesized according to the procedure reported in [12]. The products were dried first in air until constant weight and then under reduced pressure over phosphoric anhydride.

Poly{1-[4-(D-glucopyranosylamino)phenyl]ethylene} (1). A solution of 0.0045 g (1.0 wt %) of AIBN in 0.5 mL of ethanol was added to a solution of 0.45 g of *N*-(D-glucopyranosyl)-4-vinylaniline in a mixture of 1 mL of anhydrous methanol and 1 mL of anhydrous ethanol, an the mixture was heated for 5 h at 70°C under argon. The solution was separated from the precipitate by decanting and poured into 10 mL of anhydrous ethanol, and the precipitate was filtered off, washed with ethanol, and dried to obtain 0.03 g of hygroscopic polymer **1** which turned colored on exposure to air. The precipitate was ground in anhydrous ethanol, filtered off, washed with ethanol, and dried to isolate 0.04 g of **1**; overall yield 0.07 g (15.6%). UV spectrum (H_2O): λ_{max} 244.9 nm (D = 1.41, c = 0.052 mg/mL). ^{1}H NMR spectrum (D_2O), δ, ppm: 0.8–2.8 m (CH_2, CH, main chain), 3.0–4.4 m (CHOH, CH_2OH), 4.8 s (β-1-H), 4.6–5.0 m (NH), 6.3–7.4 m (H_{arom}).

Polymers **2**–**6** were synthesized in a similar way by heating a 20% solution of the monomer in anhydrous DMF containing 1.1 wt % of AIBN for 7 (**5**, **6**), 9 (**4**), or 11 h (**2**, **3**) at 70±1°C. The product was precipitated with anhydrous methanol, filtered off, and washed with methanol and anhydrous diethyl ether.

Poly{1-[4-(D-mannopyranosylamino)phenyl]-ethylene} (2) was synthesized from 1.2659 g (4.5 mmol) of *N*-(β-D-mannopyranosyl)-4-vinylaniline using 5.06 g of DMF and 0.0139 g of AIBN. Yield 0.96 g (75.8%). ^{13}C NMR spectrum (solid), δ_C, ppm: 39.87 (CH_2), 46.78 (CH), 61.95 (C^6), 66.66 (C^4), 72.49–76.04 (C^2, C^3, C^5), 82.36 (C^1), 116.62 (C^o), 126.95–131.38 (C^m), 137.77 (C^p), 143.56 (C^i).

***N*-(β-D-Mannopyranosyl)-4-vinylaniline–*N*-methacryloyl-α,β-D-glucosamine copolymer (3)** was synthesized from 0.9846 g (3.5 mmol) of *N*-(β-D-mannopyranosyl)-4-vinylaniline and 0.3709 g (1.5 mmol) of *N*-methacryloyl-α,β-D-glucosamine in 5.42 g of DMF containing 0.0149 g of AIBN. Yield 1.0 g (73.8%), white powder, $[\eta]^{25}$ = 0.06 (H_2O), 0.321 dL/g (DMF); D-mannopyranose fraction 76 mol % (NMR). UV spectrum (H_2O), λ_{max}, nm: 242.9, 290.9. ^{13}C NMR spectrum (DMF-d_7), δ_C, ppm: 21.16 (CH_3); 39.94, 44.85, 47.03, 47.88, 50.79, 56.14 (CH_2, CH); 60–82 (C^2–C^6), 83.58 (α-C^1, Man); 85.89, 86.03 (β-C^1, Man); 92.21, 92.32 (α-C^1, Glu), 114.73 (C^o), 129.39 (C^m); 136.45, 136.85 (C^p); 145.04, 145.99 (C^i).

***N*-(β-D-Mannopyranosyl)-4-vinylaniline–sodium 4-vinylbenzenesulfonate copolymer (4)** was synthesized from a mixture of 0.60 g (2.1 mmol) of *N*-(β-D-mannopyranosyl)-4-vinylaniline and 0.60 g (2.9 mmol) of sodium 4-vinylbenzenesulfonate in 5.1 mL of DMF in the presence of 0.0132 g of AIBN. Yield 0.99 g (82.5%), $[\eta]^{25}$ = 0.30 dL/g (0.1 N NaCl). UV spectrum (H_2O): λ_{max} 221.0 nm.

Poly{1-[4-(L-rhamnopyranosylamino)phenyl]-ethylene} (5) was synthesized from 0.90 g (3.4 mmol) of *N*-(β-L-rhamnopyranosyl)-4-vinylaniline in 3.8 mL of DMF containing 0.0099 g of AIBN. Yield 0.45 g (50.0%). ^{13}C NMR spectrum (solid), δ_C, ppm: 18.54 (C^6), 39.88 (CH_2), 46.01 (CH); 66.51, 73.71 (C^2, C^3), 82.48 (C^1), 116.70 br.s (C^o), 126.76–131.71 (C^m), 137.52 (C^p), 143.60 (C^i).

***N*-(β-L-Rhamnopyranosyl)-4-vinylaniline–*N*-methacryloyl-α,β-D-glucosamine copolymer (6)** was synthesized from 0.60 g (2.26 mmol) of *N*-(β-L-rhamnopyranosyl)-4-vinylaniline and 0.20 g (0.87 mmol) of *N*-methacryloyl-α,β-D-glucosamine in 4.2 mL of DMF containing 0.0088 g of AIBN. Yield 0.36 g (45%), white solid, $[\eta]^{25}$ = 0.083±0.008 dL/g (DMF). UV spectrum (H_2O), λ_{max}, nm: 242.9, 288.9. ^{1}H NMR spectrum, δ, ppm: 0.39–2.40 m (CH_3, CH_2, CH), 3.1–4.3 m (2-H, 3-H, 4-H, 5-H, OH, Rham), (2-H, 3-H, 4-H, 5-H, 6-H, OH, Glu), 4.89 s (1-H, Rham), 5.53 s (1-H), 6.0–7.1 m (H_{arom}), 7.1–7.6 m (CONH). ^{13}C NMR spectrum (DMF-d_7), δ_C, ppm: 18.73 (C^6, Rham); 72.83, 73.88, 76.02 (C^2–C^5), 82.84 (C^1, Rham), 114.54 (C^o), 129.14 (C^m), 131–139 (C^p), 145.14 (C^i).

Poly[sodium 1-(4-sulfonatophenyl)ethylene] was synthesized from 1.236 g (6.0 mmol) of sodium 4-vintlbenzenesulfonate in 4.7 mL of DMF in the presence of 0.0136 g of AIBN. Yield 1.14 g (92.3%), $[\eta]^{25}$ = 0.56 dL/g (0.1 N NaCl), M_η 215450. UV spectrum (H_2O): λ_{max} 225.0 nm.

Antibacterial testing. The minimum inhibitory concentrations (c_{ing}) of polymers **2** and **3** were determined at a bacterial concentration of 10^6 CFU/mL by the serial dilution method in a liquid nutrient medium (beef extract broth, medium no. 1, and *Saburo*). Suspension of polymer **2** and solutions of copolymer **3** with concentrations of 10, 100, amd 500 μg/mL were prepared. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 90028), *Aspergillus niger* (clinical strain), and *Mycobacterium tuberculosis* (H37Rv) were used as test cultures. *E. coli* and *S. aureus* were preliminarily grown in the broth for 18–20 h at 37±2°C; *C. albicans* and *Asp. niger* were incubated on *Saburo* medium for 48 h; and *M. tuberculosis* was incubated for 48–72 h on medium no. 1.

Nutrient medium, 1 mL, was added to 1 mL of a polymer suspension or solution, and 0.2 mL of test culture was then added, and the mixtures were incubated under the above conditions. The growth of bacteria was estimated by the turbidity. The c_{ing} values were determined by inoculation onto a solid medium.

Testing for immunomodulatory activity. Polymeric *N*-glycosides **2**–**6** were tested for immunomodulatory activity in outbred male mice (BALB/C strain) sensitized with *Ovis aries* erythrocytes. Preliminarily prepared aqueous solutions of the co-polymers and aqueous suspensions of the homopolymers were administered orally, and copolymer solutions were also administered intraperitoneally. The immunosuppressive effect was evaluated by the immune response factor (IRF).

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