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Syntheses of Copolymer Antigens Containing 2-Acetamido-2-Deoxy-α- or β-D-Glucopyranosides

RENÉ ROY* and FRANÇOIS TROPPER

Ottawa-Carleton Chemistry Institute, Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

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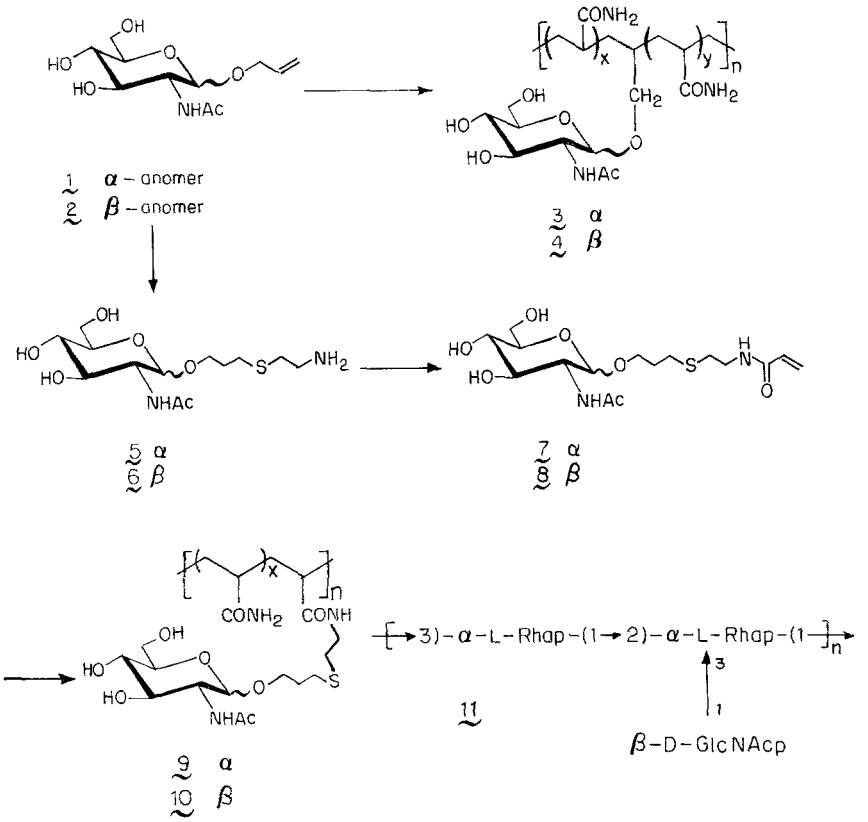
The synthesis of artificial carbohydrate antigens derived from allyl 2-acetamido-2-deoxy-α- and β-D-glucopyranosides and acrylamide is described. The two anomeric glycosyl copolymers were prepared with and without spacer arms and their binding properties to lectins and antibodies are compared.

The carbohydrate sequences of cell surface glycolipids and glycoproteins serve as recognition structures for endogenous ligands and as receptors for infective agents and their toxins [1-4]. Some of these defined carbohydrate residues have been made immunogenic when properly conjugated to protein carriers [5]. In the light of the recent progress made with the production of monoclonal antibodies of narrow specificities, it became of interest to synthesize water soluble carbohydrate-containing copolymers useful for the serological screening of their homologous monoclonal or affinity purified antibodies.

Preliminary studies on antigenic copolymers with immunodominant monosaccharides [6, 7] revealed that antibodies, as opposed to lectins, failed to bind to the copolymers deprived of a spacer arm between the carbohydrate residues and the polymer backbone. In order to examine these seemingly different binding capacities toward artificial carbohydrate antigens, we describe the synthesis of model 2-acetamido-2-deoxy-α- and β-D-glucopyranoside-acrylamide copolymers with spacer arms of ~11 Å. Their binding properties to the lectins from *Triticum vulgaris* (WGA) and *Canavalia ensiformis* (Con A) as well as to the affinity purified rabbit antibodies to the group-specific carbohydrate antigen of the β-hemolytic Group A streptococcal pyogenes [8] were compared with similar copolymers deprived of spacers.

The present approach extends the previous methodology proposed by Horejsi *et al.* [9] which consisted of the direct copolymerization of allyl glycosides with acrylamide. The method was followed by other investigators [10-12] including ourselves in the case of an

* Author for correspondence



N-acetylneuraminic acid copolymer [13]. Transformation of the alkene groups of allyl *N*-acetyl-D-glucopyranosides into *N*-acryloyl spacer arms would provide access to elongated monomers of improved reactivity, giving random copolymers [9-13]. A similar approach has been utilized for the synthesis of cross-linked polyacrylamide gels [14]. The two types of copolymers were synthesized in both anomeric configurations.

Allyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**1**) was prepared with twice the yield (70%) reported in the literature [15] by simply doubling the amount of boron trifluoride etherate catalyst used (0.2 equivalents) in the Fischer glycosidation. The corresponding β-anomer **2** was also prepared according to a published procedure [15]. The copolymers **3** and **4** which did not contain a spacer where obtained in yields of 67% and 47%, respectively. The reactions were performed in water and were initiated by ammonium persulfate at 100°C for a period of 10 min. The copolymer **3** had $[\alpha]_D$ +56.5° (c 1.08, water) and a ratio of propanamide to α-*N*-acetyl-D-glucosaminide of 5:1. Copolymer **4** had $[\alpha]_D$ -11.6° (c 1.12, water) and a 7:1 ratio of incorporated co-monomers. The molecular weights of these copolymers were estimated at 80-100 000 as measured by their relative mobilities in agarose gels.

The copolymers **9** and **10**, possessing a spacer arm of ~11 Å, were also synthesized. Elongation of the alkene group of **1** and **2** by addition of 2-aminoethanethiol was accomplished following the original strategy of Lee and Lee [15]. However, contrary to the

described procedure, the reaction required u.v. irradiation (254 nm). Nevertheless, our physical data agreed with those published for **5** and **6** [15]. Compound **5** had $[\alpha]_D$ +121.1° (c 1.03, water), m.p. 147.8-148.2°C (recrystallized from EtOH/Et_2O); published values [14], $[\alpha]_D$ +126.0°, m.p. 149-151°C: R_F 0.14 in EtOAc/HOAc/MeOH, 3/2/1 by vol. The 300 MHz ^{1}H-NMR spectra were run in DMSO-d_6 at 25°C unless noted otherwise. The ^{1}H-NMR of **5** contained signals at δ (ppm) 4.63 (d, *J* 3.6 Hz, H-1), 3.12 (t, *J* 9.0 Hz,-O-CH_2-C), 2.72 (t, *J* 7 Hz, C-CH_2-C-N), 2.72 (t, *J* 7 Hz, S-C-CH_2-N), 2.58 (t, *J* 7 Hz, O-C-C-CH_2-S), 2.53 (t, *J* 7 Hz, S-CH_2-C-N), 1.83 (s, $NHCOCH_3$), 1.74 (dt, *J* 7 Hz, *J* 9 Hz, O-C-CH_2-C-S). Compound **6** had $[\alpha]_D$ -13.6° (c 0.83, water), m.p. 175.8-177.4°C (absolute EtOH); published values [14], $[\alpha]_D$ -10.8°, m.p. 175-177°C: R_F 0.15 in EtOAc/HOAc/MeOH, 3/2/1 by vol., δH 4.24 (d, *J* 8.1 Hz, H-1), 1.81 (s, $NCOCH_3$), other signals were similar to **5**.

Quantitative conversion of **5** and **6** to the corresponding glycosidic acrylamides **7** and **8** was easily achieved by the dropwise addition of a solution of acryloyl chloride in chloroform to a cooled solution of **5** or **6** in methanol containing anion exchange resin (OH^-). Compound **7** had $[\alpha]_D$ +105.5° (c 1.19, water), m.p. 145.9-147.7°C (CH_3CN); R_F 0.58 in EtOAc/HOAc/MeOH, 3/2/1 by vol., δH 6.22 (dd, J_{cis} 9.8 Hz, J_{trans} 17.1 Hz, CO-CH=C), 6.08 (dd, J_{gem} 2.5 Hz, J_{trans} 17.1 Hz, COC=CH_2), 5.59 (dd, J_{gem} 2.5 Hz, J_{cis} 9.8 Hz, COC-CH_2), 4.62 (d, *J* 3.6 Hz, H-1). Compound **8** had $[\alpha]_D$-9.9° (c 1.22, water), m.p. 135.5-137.1°C (CH_3CN); R_F 0.50 in EtOAc/HOAc/MeOH, 3/2/1 by vol., δH 4.24 (d, *J* 8.3 Hz, H-1).

Copolymerization of **7** and **8** with acrylamide (1:4 molar ratio) under the same conditions specified earlier for the allyl glycosides gave the random copolymers **9**, $[\alpha]_D$ +34.0° (c 1.03, water), and **10**, $[\alpha]_D$ -9.8° (c 0.84, water) in 60 and 53% yields, respectively. The copolymers **9** and **10** also had molecular weights in the range of 80-100 000 and showed sugar contents to acrylamide ratio of 1:7 and 1:4, respectively.

The serological specificities of the copolymers **3**, **4** (without spacer) and **9**, **10** (with spacer) were tested by double radial immunodiffusion according to the method of Ouchterlony [16]. Sharp precipitin bands were obtained with all four copolymers and both lectins WGA and Con A. However, the copolymer **10** was the only one to show a sharp precipitin band with the affinity purified rabbit IgG antibodies raised against the capsular polysaccharide **11** of the β-hemolytic Group A streptococci [17]. The usefulness of these copolymers in other serological experiments (ELISA) and in binding studies has also been established and the results will be published elsewhere.

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