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Preliminary communication

Synthesis of antigenic copolymers of *N*-acetylneuraminic acid binding to wheat germ agglutinin and antibodies

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Sialic acids are a group of some 30 derivatives of neuraminic acid of importance as constituents of many glycoproteins and glycolipids[1]. Sialic acid residues often occupy positions at the nonreducing ends of oligosaccharide chains, where they may serve as recognition sites for agglutinins, antibodies, toxins, and viruses[2]. This recognition phenomenon is responsible for tissue-specific adherence and colonization by microorganisms. It has also been demonstrated that sialic acids mask recognition sites[3], and this behaviour accounts for the inertness of the immune-defense system towards many sialic acid-bearing antigens. Another important role of the sialic acids is their involvement as differentiation and oncodevelopmental antigens[4,5]. As such, they constitute key functional entities in the design of specific oncogenic markers.

In order to develop a strategy for the production and screening of monoclonal antibodies against *N*-acetylneuraminic (NeuAc, **1**), the most ubiquitous of the sialic acids, and its structural variants, we described the synthesis of NeuAc neoglycoproteins and pseudopolysaccharides[6]. The NeuAc–bovine serum albumin (BSA) and the NeuAc–tetanus toxoid conjugates were found to be highly immunogenic in rabbits. To establish the exact nature of the antigenic determinants recognized by these antibodies, we needed an efficient, water-soluble, multivalent NeuAc pseudopolysaccharide which did not share the antigenicity of the protein carriers. The simple copolymer (**5**) described previously[6], although satisfactory in some binding studies, was only poorly antigenic with wheat germ agglutinin and the rabbit antibodies. This was attributed to the close proximity of the NeuAc residues to the polymeric backbone.

To overcome this problem we undertook the synthesis of a copolymer of acrylamide and an α-glycoside of NeuAc carrying an 8-atom (~11 Å) spacer arm. This new pseudopolysaccharide (**6**), by comparison with **5** has its NeuAc residues

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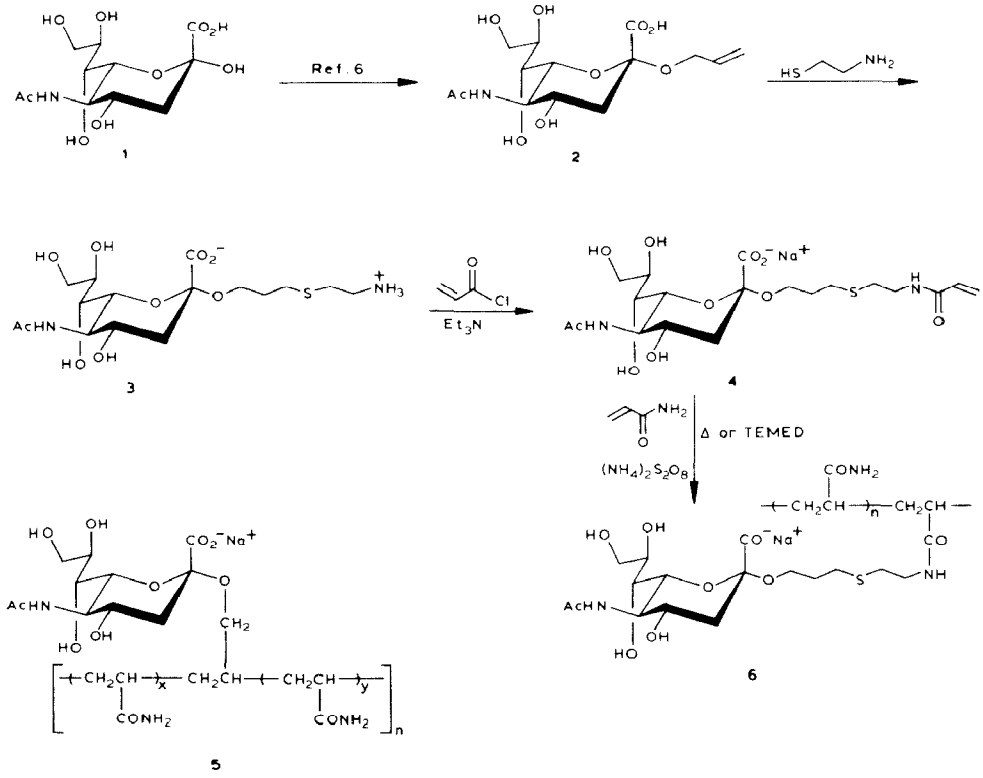

more readily accessible, as demonstrated by double immunodiffusion in agarose gel with both agglutinin and antibodies. The strategy used in preparing **6** has the advantage of permitting the incorporation of various monomers for particular applications, and it also permits the introduction of monomers having a better reactivity ratio than those previously reported[7,8].

The light-induced free-radical addition of 2-aminoethanethiol hydrochloride (cysteamine) to the allyl α-glycoside of NeuAc (**2**; ref. 6) gave the 3-(2-aminoethylthio)propyl glycoside **3** in 60% isolated yield after purification on a Sephadex G-10 column (elution with water). The addition[9] of the thiol group to the double bond occurred in anti-Markovnikov fashion, as shown by the lack of a detectable terminal methyl group signal in the ^{1}H-n.m.r. spectrum of the crude reaction mixture. Compound **3** had $[\alpha]_D$ −8.3° (*c* 1.0, 0.1M pyridine–acetate buffer, pH 5.0); R_F 0.15 in 3:2:1 EtOAc–HOAc–H_2O; f.a.b.-m.s.: *m/z* 427 [(M + 1)$^+$ calc. for $C_{16}H_{30}N_2O_9S$]; δ_H (D_2O): 3.542–3.890 (9 H, NeuAc + OCH_2 of spacer), 3.224 (t, 2 H, *J* 6.5 Hz, CH_2NH_2), 2.857 (t, 2 H, *J* 6.5 Hz, SCH_2), 2.748 (dd, 1 H, $J_{3a,3e}$ 12, $J_{3e,4}$ 4.9 Hz, H-3*e*), 2.658 (t, 2 H, *J* 7.0 Hz, CH_2S), 2.030 (s, 3 H, $COCH_3$), 1.853 (q, 2 H, *J* 5.8 Hz, $OCH_2CH_2CH_2S$), 1.651 (dd, 1 H, $J_{3a,3e}$ 12, $J_{3a,4}$ 12 Hz, H-3*a*); δ_C (D_2O): 176.2 ($COCH_3$), 174.6 (C-1), 101.7 (C-2), 73.7 (C-6), 72.9 (C-8), 69.3 (C-4,7), 63.7 (C-9), 53.0 (C-5), 41.4 (C-3), 23.1 ($COCH_3$), 64.1, 39.4, 29.9, 29.3, and 28.3 (spacer CH_2).

The selective acryloylation of the amino group in **3** was performed in methanol, using triethylamine as the base ("pH" held constant at ~8–9) and an excess of acryloyl chloride dissolved in dioxane. The reaction mixture was purified by gel-permeation chromatography on a Sephadex G-10 column to afford an 88% yield of **4** as a lyophilized white powder; $[\alpha]_D$ −11° (*c* 1.0, 0.1M pyridine acetate buffer, pH 5.0); R_F 0.36 in 3:2:1 EtOAc–HOAc–H_2O; f.a.b.-m.s.: *m/z* 481 [(M + 1)$^+$ calc. for $C_{19}H_{32}N_2O_{10}S$]; δ_H (D_2O): 6.282 (dd, 1 H, J_{trans} 17.0, J_{cis} 9.6 Hz, C*H*=CH_2), 6.183 (dd, 1 H, J_{trans} 17.0, J_{gem} 2.0 Hz, CH=C*H*$_2$), 5.766 (dd, 1 H, J_{cis} 9.6, J_{gem} 2.0 Hz, CH=C*H*$_2$), 3.90–3.54 (9 H), 3.483 (t, 2 H, C*H*$_2$NH, *J* 6.8 Hz), 2.748 (dd, 1 H, $J_{3a,3e}$ 12, $J_{3e,4}$ 4.9 Hz, H-3*e*), 2.744 (t, 2 H, *J* 6.6 Hz, SC*H*$_2$), 2.642 (t, 2 H, *J* 6.0 Hz, C*H*$_2$S), 2.030 (s, 3 H, NCOC*H*$_3$), 1.836 (q, 2 H, *J* 6.8 Hz, $OCH_2CH_2CH_2S$), and 1.645 (dd, 1 H, $J_{3a,3e}$ 12, $J_{3a,4}$ 12 Hz, H-3*a*).

The aqueous copolymerization of the acryloylated derivative **4** with acrylamide was then effected by an electron-transfer polymerization initiated by ammonium persulfate[6–8]. The polymerization could equally well be activated by brief heating (5 min) at 100°, or by *N,N,N′,N′*-tetramethylethylenediamine (TEMED) overnight at room temperature. The copolymers were isolated by exhaustive dialysis against distilled water. The solutions were then freeze-dried to give **6** as a white powder in yields of 30 and 65%, respectively.

In typical runs, the incorporations of NeuAc in the copolymers were in the range of 15% by weight as determined by the resorcinol method[10]. The molar ratio of acrylamide to NeuAc was therefore calculated to be ~22:1, a result also consistent with the ^{1}H-n.m.r. data. Crude estimates, based on comparisons of the relative mobilities in agarose gel with those of similar copolymers[7], gave molecular weight distributions in the range 40–70,000.

As expected, the NeuAc-acrylamide copolymer **6** showed improved immunochemical properties over the previously synthesized copolymer **5**. Radial immunodiffusion in agarose gel gave sharp precipitin bands with wheat germ agglutinin and rabbit polyclonal antibodies raised against NeuAc–BSA and NeuAc–tetanus toxoid conjugates[6]. These preliminary results showed that the NeuAc residues are now more accessible to the binding sites of these biomolecules and that the association constants are higher than those obtained with **5**. Since the NeuAc neoglycoproteins have only their carbohydrate residues in common with **6**, this indicates that the lectin and the antibodies have specificity for NeuAc. The copolymer **6** will be tested for binding to other lectins, such as those of *Limax flavus*, *Limulus polyphemus*, and *Carcinoscorpius rotunda cauda*[11].

In conclusion, a new strategy was described for the synthesis of serologically useful sialic acid antigens. This methodology is now being applied to more complex sialyl oligosaccharides.

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