

## Neuraminidase-Resistant Hemagglutination Inhibitors: Acrylamide Copolymers Containing a C-Glycoside of *N*-Acetylneuraminic Acid<sup>1</sup>

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Copolymers consisting of a polyacrylamide backbone with side chains terminated in C-glycosidic analogs of *N*-acetylneuraminic acid were synthesized by free radical copolymerization of  $\alpha$ -2-C-[3-[[2-(*N*-acryloylamino)ethyl]thio]propyl]-*N*-acetylneuraminic acid (**5**) with acrylamide. Unlike natural and synthetic polyvalent materials that contain *N*-acetylneuraminic acid in O-glycosidic form, these C-glycosidic copolymers resist neuraminidase-catalyzed cleavage of the neuraminic acid residue from the copolymer backbone. Examination of these C-glycosidic copolymers in a hemagglutination inhibition assay indicated that they are as effective in vitro as polyvalent O-glycosidic copolymers in inhibiting agglutination of erythrocytes by influenza virus. The minimum value of the inhibition constant, calculated on the basis of the concentration of Neu5Ac groups in solution, is  $K_i^{(\text{HAI})} \sim 10^{-7}$  M for both copolymers. The inhibitory potency of the C-glycoside-based copolymers becomes more significant at lower concentrations of Neu5Ac moieties in solution than does the inhibitory potency of the O-glycoside-based copolymer.

Polyvalency—the ability of a ligand to bind to a target via multiple chemical interactions—is considered an important factor in many pathogen–host cell interactions that result in infection.<sup>4</sup> Influenza A, an orthomyxovirus responsible for the most severe outbreaks of influenza, adheres to the terminal *N*-acetylneuraminic acid (Neu5Ac) of glycolipids and glycoproteins on the surface of mammalian epithelial cells via the viral surface lectin, hemagglutinin (HA).<sup>5</sup> Monomeric  $\alpha$ -2-methyl-*N*-acetylneuraminic acid (**1**, Neu5Ac $\alpha$ 2Me) binds weakly to HA.<sup>6</sup> A variety of structurally modified, monomeric Neu5Ac derivatives have been tested as inhibitors of hemagglutination;<sup>7–9</sup> only recently have monomeric derivatives been prepared that are significantly more effective than Neu5Ac $\alpha$ 2Me, **1**.<sup>8,9</sup> The detailed mechanism of inhibition by these compounds remains unclear.

An alternative approach to the prevention of infection is the development of *polyvalent* drugs that could, in principle, bind to and block access to receptor sites of the pathogen.<sup>10</sup> A number of potent, naturally-occurring, polyvalent compounds that inhibit the adhesion of influenza virus to cells in vitro are known.<sup>11</sup> In addition, we<sup>12,13</sup> and others<sup>14–16</sup> have developed inhibitors of influenza virus-induced hemagglutination that are polyvalent in Neu5Ac. In vitro, these polyvalent inhibitors prevent hemagglutination based on virus–cell adhesion at concentrations of Neu5Ac groups between  $10^{-6}$  and  $10^{-8}$  M.<sup>12–16</sup> All Neu5Ac-based inhibitors, whether natural or synthetic in origin, are susceptible to glycosidic cleavage by neuraminidase (NA, EC 3.2.1.18),<sup>17–19</sup> a glycosidase that is present on the surface of the influenza virus. Neuraminidase-catalyzed cleavage of the Neu5Ac groups present in the oligosaccharide moieties of glycoproteins is thought to allow the virus particle to escape from naturally occurring polyvalent inhibitors<sup>17–19</sup> and aid in the release of newly formed virion from the infected cell.<sup>20</sup>

In order to circumvent the neuraminidase-catalyzed cleavage of terminal Neu5Ac residues from synthetic, polyvalent copolymers presenting Neu5Ac groups as side chains,<sup>12,14,15</sup> we have prepared random copolymers incorporating C-glycosidic Neu5Ac moieties derived from

the monomeric 2-C-[3-[[2-(*N*-acryloylamino)ethyl]thio]propyl]-*N*-acetylneuraminic acid<sup>21</sup> (**5**, Scheme I).

The copolymers of **5** and acrylamide—poly(5-*co*-acrylamide)—were prepared with a range of mole fractions of **5** attached to an acrylamide backbone. We have measured the ability of these copolymers to inhibit the agglutination of chicken erythrocytes induced by influenza virus (X-31)<sup>22</sup> in vitro by employing a standard hemagglutination inhibition (HAI) assay.<sup>23</sup> These copolymers have the potential to compete with polyvalent cell surfaces for HA (and perhaps NA) binding sites on the viral surface and to inhibit virus–host cell binding, without being susceptible to cleavage and release of the Neu5Ac groups by NA.<sup>17</sup>

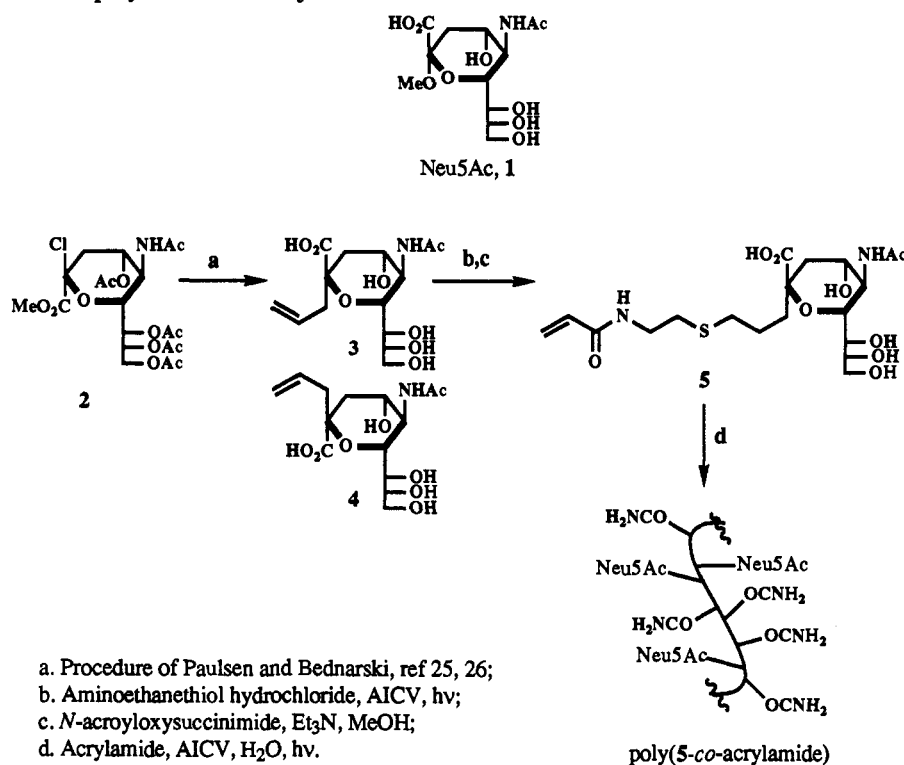
The synthesis of **5** began with glycosyl chloride **2**.<sup>24</sup> This compound was converted to an anomeric mixture of Neu5Ac C-glycosides **3** ( $\alpha$ -2-C-allylNeu5Ac) and **4** ( $\beta$ -2-C-allylNeu5Ac) under the allylation conditions developed by Paulsen<sup>25</sup> and Bednarski.<sup>26</sup> Separation of **3** from **4**,<sup>25,26</sup> followed by photolytic addition of 2-aminoethanethiol hydrochloride to the double bond terminus of **3**,<sup>14,27</sup> was a convenient method for the installation of a linkage group with a terminal primary amine. This amine allowed for attachment of the acrylamide moiety needed for copolymerization. Addition of the 2-aminoethanethiol adduct to a solution of *N*-(acryloyloxy)succinimide in basic MeOH/H<sub>2</sub>O (pH 10) produced the desired Neu5Ac-acrylamide **5** in 85% purified yield.

The copolymers were synthesized by photochemically initiated copolymerization of acrylamide and **5**. In all preparations, the initial concentration of acrylamide was fixed at 1.0 M. The initial concentration of **5** was varied from 4.0 mM to 4.0 M; this range corresponded to a mole fraction of **5** ( $X_{\text{Neu5Ac}}$ ) in total acrylamide moieties of 0.005–0.80 (eq 1). We assume this mole fraction is preserved in the final copolymer.

$$X_{\text{Neu5Ac}} = \frac{[\mathbf{5}]}{[\mathbf{5}] + [\text{acrylamide}]} \quad (1)$$

These copolymers have not been fully characterized. Previous experience in the preparation of acrylamide

## Scheme I. Synthesis of Copolymer 5 and Acrylamide

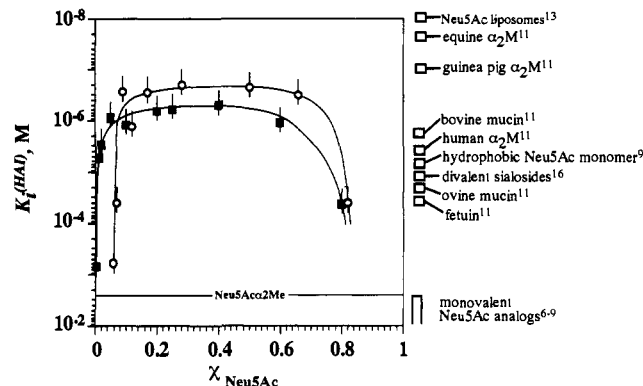


copolymers of the parent O-glycosides of Neu5Ac has, however, shown that copolymers produced by an analogous procedure are retained by a 100 000 MW cutoff dialysis membrane.<sup>12</sup>

Unlike their O-glycosidic counterparts, the C-glycoside copolymers of Neu5Ac are resistant to neuraminidase. Incubation of a sample of C-glycoside copolymer ( $X_{\text{Neu5Ac}} = 0.20$ ) with neuraminidase prior to an HAI assay did not result in a decrease in inhibitory potency when compared to the HAI results of the untreated copolymer sample. Under analogous conditions, the O-glycosidic copolymer lost its inhibitory activity.<sup>19</sup>

Aliquots of the monomers 3 and 5, and of each poly(5-co-acrylamide) ( $X_{\text{Neu5Ac}} = 0.005\text{--}0.8$ ), were prepared for testing in an HAI assay by diluting the potential inhibitor in sterile phosphate buffered saline solution (PBS) and adjusting to pH 7.2. The solutions were employed in a standard HAI assay<sup>22,23</sup> to evaluate the potential of these compounds to inhibit agglutination of chicken erythrocytes induced by influenza virus. This assay involves adding virus to a solution of serially diluted inhibitor in a 96-well titer plate, incubating the mixture for 30 min, adding a suspension of erythrocytes, and determining the lowest concentration of Neu5Ac groups in solution for which hemagglutination is no longer observed under the specified assay conditions. The endpoint of the HAI assay,  $K_i^{(\text{HAI})}$ , is defined as this lowest concentration. This assay depends on a number of variables, not all of which are under complete control (especially the quality of the erythrocytes). The assay is reasonably reproducible ( $\pm 1$  well) provided appropriate controls are followed. The relevance of this *in vitro* assay to *in vivo* infectivity remains to be established.

Figure 1 and Table I summarize the results of the HAI assay obtained with the poly(5-co-acrylamide) copolymers containing C-glycoside Neu5Ac groups and compares these new results with those obtained with the copolymers



**Figure 1.** Inhibitory potency,  $K_i^{(\text{HAI})}$ , of the C- and O-glycosidic copolymers of Neu5Ac as a function of mole fraction,  $X_{\text{Neu5Ac}}$ , of Neu5Ac covalently incorporated into an acrylamide copolymer. The data points presented for the C- and O-glycosidic copolymers are averages of four to six sets of data obtained from independently prepared samples of copolymer. The data for the O-glycosidic copolymers have been previously published from this laboratory.<sup>12</sup>  $K_i^{(\text{HAI})}$  is the minimum concentration of copolymer-linked Neu5Ac residues in solution that inhibits agglutination of chicken erythrocytes *in vitro* at 4 °C. Assay conditions are given in the Experimental Section. The horizontal line denotes the threshold antiagglutination inhibitory potency of monomeric Neu5Ac $\alpha$ 2Me 1 ( $K_i^{(\text{HAI})} = 2.8 \times 10^{-3}$  M)<sup>6</sup> assayed under identical conditions. The error bars represent  $\pm 1$  well in the HAI assay for (■) C-glycosidic copolymer of Neu5Ac, and (○) O-glycosidic copolymers of Neu5Ac.

containing O-glycoside Neu5Ac groups, reported previously.<sup>12</sup> The data are expressed in terms of  $K_i^{(\text{HAI})}$  versus  $X_{\text{Neu5Ac}}$ . For both sets of copolymers, the antihemagglutination potency (measured by  $K_i^{(\text{HAI})}$ ) of the C- and O-glycosidic copolymers of Neu5Ac reaches a maximum value at  $X_{\text{Neu5Ac}}^{\text{max}} \approx 0.3\text{--}0.4$ . We have attributed the bell shape of these curves to a combination of entropy and optimal glycoside spacing effects.<sup>12</sup> The horizontal line in Figure 1 represents  $K_i^{(\text{HAI})}$  for Neu5Ac $\alpha$ 2Me 1 ( $2.8 \times$

**Table I.** Antihemagglutination Inhibitory Potency  $\chi_{\text{Neu5Ac}}^{\text{max}}$  and  $\chi_{\text{Neu5Ac}}^{0.5}$  for the C- and O-Glycoside Acrylamide Copolymers of Neu5Ac

copolymer	$K_i^{(\text{HAI})}$ , $\mu\text{M}$	$\chi_{\text{Neu5Ac}}^{\text{max } a}$	$\chi_{\text{Neu5Ac}}^{0.5 b}$
C-glycoside Neu5Ac	0.4	0.3–0.4	0.01
O-glycoside Neu5Ac	0.2	0.3–0.4	0.09

<sup>a</sup>  $\chi_{\text{Neu5Ac}}^{\text{max}}$  is the mole fraction of copolymer-linked Neu5Ac that yields the maximum antiagglutination potency. <sup>b</sup>  $\chi_{\text{Neu5Ac}}^{0.5}$  is the minimum mole fraction of copolymer-linked Neu5Ac required to exert half of the maximum inhibitory potency above the threshold inhibitory level given by monomeric Neu5Ac $\alpha$ 2Me in solution ( $K_i^{(\text{HAI})} = 2.8 \times 10^{-3}$  M).

$10^{-3}$  M)<sup>6</sup> and is shown as a reference for the  $K_i^{(\text{HAI})}$  of monomeric analogs of Neu5Ac.

Figure 1 permits two important comparisons between the C- and O-glycosidic copolymers. First, both copolymers have similar maximum inhibitory potency in vitro. Four identical, independently prepared poly(5-*co*-acrylamide) polymers having  $\chi_{\text{Neu5Ac}} = 0.4$  showed inhibition of hemagglutination with  $K_i^{(\text{HAI})} = 2 \times 10^{-7}$  to  $6 \times 10^{-7}$  M in Neu5Ac groups. A parallel set of experiments conducted with copolymers containing O-glycosidic Neu5Ac gave  $K_i^{(\text{HAI})}$  of  $2 \times 10^{-7}$  to  $3 \times 10^{-7}$  M in Neu5Ac groups.<sup>12</sup> The slightly weaker inhibition of hemagglutination for the C-glycosidic copolymer (at  $\chi_{\text{Neu5Ac}} > 0.1$ ) relative to the O-glycosidic copolymer suggested by Figure 1 is within the margin of experimental error in the hemagglutination assay ( $\pm 1$  well). Second, the minimum mole fraction that is required to exert half of the maximum inhibitory potency above the threshold inhibitory level (i.e.  $K_i^{(\text{HAI})} = 2.5 \times 10^{-3}$  M for Neu5Ac $\alpha$ 2Me, 1),<sup>6</sup>  $\chi_{\text{Neu5Ac}}^{0.5}$ , is shifted from 0.09 for the O-glycosidic copolymer to 0.01 for the C-glycosidic copolymer. Together with  $\chi_{\text{Neu5Ac}}^{\text{max}}$ , the mole fraction of copolymer-linked Neu5Ac that yields the maximum antiagglutination potency, the  $\chi_{\text{Neu5Ac}}^{0.5}$  index provides a convenient means to compare the effectiveness of different types of copolymers containing neuraminic acid (Table I).

The monomeric  $\alpha$ -C-glycosides 3 and 5 were also tested in an HAI assay. For these monomers,  $K_i^{(\text{HAI})}$  fell between 2.5 and 5 mM; these values are similar to the values of  $K_i^{(\text{HAI})}$  obtained for most monomeric Neu5Ac analogs.<sup>6–8</sup> The  $\beta$ -analogs 4, and the analogous Neu5Ac-acrylamide 5 that was prepared from 4, showed no inhibition at concentrations  $< 20$  mM, the highest concentration employed in the HAI assays. Copolymers prepared from the  $\beta$ -analog of 5 (0.05–0.2 mole fraction of  $\beta$ -Neu5Ac 5 in acrylamide copolymer) also showed no inhibition by HAI.

## Discussion

The mechanism by which these copolymers influence hemagglutination is still being defined. Two broad classes of mechanisms are now being evaluated. In the one, the presence of multiple Neu5Ac moieties on the copolymer would result in entropic enhancement of binding and in a higher occupancy of HA binding sites on the surface of the virus than in the presence of the same concentration of Neu5Ac groups present as monomers. In the other, the presence of a hydrophilic copolymer that binds to virus would result in the formation of a layer of copolymer at the viral surface and would inhibit binding of virus to erythrocytes by a combination of interactions lumped together under the term "steric stabilization".<sup>28</sup> Steric stabilization includes both enthalpic and entropic changes that would accompany compression of the water-swollen

copolymer associated with the surface of the virus as it approached the surface of the erythrocyte. Importantly, this mechanism of stabilization does *not*, in principle, require enhanced binding of Neu5Ac groups to HA binding sites (although it does not prohibit such enhancement); it is based more on the physical characteristics of the copolymer when adsorbed on the surface of the virus than on the biospecificity of that adsorption. Presently available, but incomplete, information suggests that both mechanisms contribute to the inhibition of hemagglutination being observed here.

Two observations from the present work are relevant to the mechanism of action of these copolymers. First, the concentration of Neu5Ac groups present in the C-glycoside copolymer (at low values of  $\chi_{\text{Neu5Ac}}$ ) required to achieve a given extent of inhibition of hemagglutination is *lower* than for the corresponding O-glycosidic copolymer; second, the maximum inhibition capacity of the C- and O-glycosidic copolymers is very similar. We suggest as a hypothesis to be tested in future work, that this difference reflects the ability of the C-glycoside copolymer to interact with the neuraminidase binding site, without hydrolysis, in addition to interacting with the HA binding site. This ability would provide an additional set of interactions that would attach the C-glycosidic copolymer to the virus at low concentrations of Neu5Ac groups. The observation that this enhanced binding at low values of  $\chi_{\text{Neu5Ac}}$  is not reflected in an increase in the maximum value observed for  $K_i^{(\text{HAI})}$  is qualitatively comparable with a mechanism for inhibition of hemagglutination based on steric stabilization; understanding its relevance to entropically enhanced binding is more complicated.

## Conclusion

Carbohydrates present on the surface of mammalian cells are ligands for surface lectins for a number of bacteria<sup>29</sup> and viruses.<sup>30</sup> In most cases that have been examined, the monosaccharides themselves do not bind tightly and do not significantly inhibit pathogen–cell adhesion. This general observation supports the hypothesis that it is the polyvalent character of the pathogen–host interaction that results in tight binding and that promotes infection.<sup>31</sup> A number of studies have examined the effects of polyvalency<sup>32,33</sup> and have reported the syntheses of compounds that are polyvalent in known bacterial and/or virus carbohydrate-based ligands.<sup>12–16</sup> It is clear that, in order to be effective in vivo, these carbohydrate-based compounds must also be resistant to glycosidic cleavage by pathogen and host–cell hydrolases. The C-glycosidic copolymers of Neu5Ac described here are as effective as the analogous O-glycosidic copolymers of Neu5Ac<sup>12</sup> in inhibiting influenza virus-induced agglutination of erythrocytes in vitro and have the added advantage of being resistant to cleavage by viral neuraminidase. At present we do not know whether these compounds and copolymers are biologically active in vivo, and they are currently being evaluated in infectivity assays.

## Experimental Section

**Materials and Methods.** All solvents and reagents were from Aldrich and used without further purification unless otherwise noted. Reaction mixtures were stirred magnetically and monitored by thin-layer chromatography on silica gel precoated glass plates (E. Merck, Darmstadt). Flash column chromatography was performed on silica gel 60F<sub>254</sub> (230–400 mesh, E. Merck) using the solvents indicated. Size-exclusion chromatography was

performed on Biogel P2 resin or Sephadex G10 using distilled water as the eluant. Ion-exchange chromatography was performed on Dowex 50W-X8 (H<sup>+</sup> form) cation exchange resin. Vortexing of copolymer samples was done with a Fischer vortex Genie II.

All melting points were obtained using a Mel-temp apparatus and are uncorrected. Proton and carbon NMR spectra were measured on a Bruker AM-400 MHz NMR spectrophotometer. Chemical shifts are reported in ppm relative to the solvent: CHCl<sub>3</sub> in CDCl<sub>3</sub> at 7.24 ppm, HOD in D<sub>2</sub>O at 4.80 ppm, and CD<sub>2</sub>H<sub>2</sub>OH in CD<sub>3</sub>OD at 3.30 ppm for the proton spectra. For carbon spectra the references are 77.0 ppm for CDCl<sub>3</sub>, 49.9 ppm for CD<sub>3</sub>OD, and 1.30 ppm for CH<sub>3</sub>CN in D<sub>2</sub>O.

*N*-Acetylneuraminic acid was obtained from extraction of edible Chinese swiftlet's nest.<sup>34</sup> Erythrocytes from 2 week old chickens were from Spafas Inc. Influenza virus (X-31) was obtained from the laboratory of Professor John Skehel. Phosphate-buffered saline used in the HAI assays was prepared from 80 g of NaCl, 2 g of KCl, 11 g of Na<sub>2</sub>HPO<sub>4</sub>, and 2 g of KH<sub>2</sub>PO<sub>4</sub> in 1 L of distilled H<sub>2</sub>O. This stock solution was diluted 1 part in 10 in distilled H<sub>2</sub>O and then adjusted to pH 7.2 with 1 N NaOH.

**Hemagglutination Inhibition Assay.**<sup>22,23</sup> The HA titer of a PBS stock solution of X-31 influenza virus was determined by serial dilution of 50 μL of the virus solution through 12 microtiter plate wells (of a 96-well microtiter plate) each containing 50 μL of PBS. A suspension of chicken erythrocytes in PBS (100 μL) were added to each well, mixed, and incubated at 4 °C for 1 h. The HA endpoint is defined as the last dilution well before erythrocyte pellets begin to form and is expressed as a reciprocal of the endpoint dilution.

The monomeric inhibitors 3 and 5 were diluted to 80 mM in PBS (pH 7.2). The copolymers were also diluted in PBS with concentrations of C-glycosidic Neu5Ac 5 ranging from 5 × 10<sup>-4</sup> to 4 × 10<sup>-2</sup> M. The stock solution of the inhibitor (50 μL) was serially diluted through 12 microtiter plate wells containing 50 μL of PBS. To each well was added 50 μL of a suspension of X-31 virus that had been diluted to the concentration of the endpoint in the titer, determined as described above. After a 30-min incubation period (4 °C), 100 μL of a suspension of chicken erythrocytes was added to each well, and the plate was gently agitated and then incubated at 4 °C for an additional 2 h. The HAI endpoint is the last well where a red pellet is observed. This endpoint [*K*<sub>1</sub><sup>(HAI)</sup>] is defined as the lowest concentration of sialic acid residues in solution that gave a 50% inhibition of hemagglutination (incomplete pellet formation) by influenza virus (X-31).<sup>35</sup> The inhibition of hemagglutination by monomeric and copolymeric sialosides was measured relative to Neu5Acα2Me 1 [*K*<sub>1</sub><sup>(HAI)</sup> = 2.8 mM].<sup>6</sup>

**5-Acetamido-2,6-anhydro-3,5-dideoxy-2-C-(2-propenyl)-D-erythro-L-manno-non-2-ulonic acid (3)** was prepared as described previously:<sup>25,26</sup> mp 104 °C dec; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 5.91 (m, 1 H), 4.99 (m, 2 H), 3.85–3.78 (m, 2 H), 3.73–3.66 (m, 2 H), 3.61–3.45 (m, 3 H), 2.64 (dd, 1 H, *J* = 4.8, 12.6 Hz), 2.39 (m, 2 H), 1.92 (s, 3 H), 1.40 (dd, 1 H, *J* = 12.4, 12.6 Hz); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 179.4, 175.5, 135.1, 117.4, 82.5, 75.3, 72.9, 70.4, 69.9, 64.5, 54.6, 46.0, 42.3, 22.6; high-resolution mass spectrum (FAB) *m/z* 334.1502 [(M + H)<sup>+</sup>, calcd for C<sub>14</sub>H<sub>24</sub>O<sub>8</sub>N, 334.1502].

**5-Acetamido-2,6-anhydro-3,5-dideoxy-2-C-(2-propenyl)-D-erythro-L-gluco-non-2-ulonic acid (4)** was prepared as described previously:<sup>25,26</sup> mp 110–114 °C dec; <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 5.72 (m, 1 H), 5.14 (m, 2 H), 4.04 (m, 1 H), 3.89–3.78 (m, 4 H), 3.61 (dd, 1 H, *J* = 6.3, 12.6 Hz), 3.51 (d, 1 H, *J* = 8.8 Hz), 2.92 (dd, 1 H, *J* = 6.0, 15.0 Hz), 2.53 (dd, 1 H, *J* = 8.0, 15.0 Hz), 2.32 (dd, 1 H, *J* = 4.70, 13.0 Hz), 2.01 (s, 3 H), 1.74 (dd, 1 H, *J* = 12.2, 13.0 Hz); <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O with CH<sub>3</sub>CN as an internal standard, 1.3 ppm) δ 176.7, 175.3, 132.0, 119.6, 80.1, 71.0, 70.5, 68.9, 67.2, 63.6, 53.0, 39.3, 35.9, 22.6; high-resolution mass spectrum (FAB) *m/z* 334.1512 [(M + H)<sup>+</sup>, calcd for C<sub>14</sub>H<sub>24</sub>O<sub>8</sub>N, 334.1502].

**α-2-C-[3-[(2-(*N*-Acryloylamino)ethyl)thio]propyl]-*N*-acetylneuraminic Acid (5).** The intermediate α-[3-[(2-aminoethyl)thio]propyl]-C-glycoside was prepared via the method of Lee<sup>27</sup> and Roy:<sup>14</sup> C-glycoside 3 (85.9 mg, 0.26 mmol) was added to a 5-mL round-bottom Pyrex flask and dissolved in distilled H<sub>2</sub>O (1 mL) at room temperature. Aminoethanethiol hydro-

chloride (2.0 equiv, 0.52 mmol, 58.3 mg) and *N,N'*-azobis(isocyanoveraleric acid) (AICV, 1 mg) were added to the reaction mixture, and the solution was purged with argon for 10 min. The mixture was irradiated with a medium-pressure Hg arc lamp for 8 h at which time TLC and <sup>1</sup>H NMR examination of the crude reaction mixture indicated that the starting material had been consumed completely. Purification of the reaction mixture on a Biogel P2 size-exclusion column and lyophilization of the pooled fractions afforded the desired primary amine (90 mg, 0.22 mmol, 85% yield) as a white foam: mp 240 °C dec; <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 3.85–3.52 (m, 9 H), 3.18 (t, 2 H, *J* = 6.3 Hz), 2.81 (t, 2 H, *J* = 6.3 Hz), 2.59 (m, 2 H), 2.03 (s, 3 H), 1.83–1.43 (m, 4 H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 179.8, 175.9, 82.4, 74.3, 72.9, 69.6, 69.1, 63.5, 53.2, 41.9, 39.4, 39.3, 31.5, 28.7, 24.3, 22.8; high-resolution mass spectrum (FAB) *m/z* 433.1621 [(M + Na), calcd for C<sub>16</sub>H<sub>30</sub>N<sub>2</sub>O<sub>8</sub>SNa, 433.1604].

The α-2-C-[3-[(2-aminoethyl)thio]propyl]-*N*-acetylneuraminic acid (183 mg, 0.43 mmol) was dissolved in MeOH (1.5 mL) and H<sub>2</sub>O (4.5 mL) at room temperature. Triethylamine (300 μL) and *N*-(acryloyloxy)succinimide (prepared from acryloyl chloride and *N*-hydroxysuccinimide, 1.3 equiv, 0.56 mmol, 94 mg) were added, and the reaction was stirred for 4 h at room temperature. The reaction mixture was maintained at pH ~10 with Et<sub>3</sub>N. Purification on a biogel P2 size-exclusion column and lyophilization of the pooled fractions afforded the acrylamide 5 (156.2 mg, 0.32 mmol, 76% yield) as a white foam: mp 176 °C dec; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 6.25 (dd, 1 H, *J* = 9.9, 17.0 Hz), 6.12 (d, 1 H, *J* = 17.0 Hz), 5.58 (d, 1 H, *J* = 9.9 Hz), 3.85–3.29 (m, 9 H), 2.72 (t, 2 H, *J* = 6.5 Hz), 2.59 (t, 2 H, *J* = 6.5 Hz), 2.03 (s, 3 H), 1.82–1.66 (m, 2 H), 1.51 (t, 2 H, *J* = 11.7 Hz), 1.27 (m, 2 H); <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O with CH<sub>3</sub>CN as an internal reference, 1.3 ppm) δ 178.8, 175.8, 169.3, 130.7, 128.2, 81.8, 74.3, 72.7, 69.4, 69.1, 63.5, 53.1, 41.4, 39.6, 39.4, 31.8, 31.0, 24.1, 22.8; high-resolution mass spectrum (FAB) *m/z* 464.1891 [(M + H)<sup>+</sup>, calcd for C<sub>19</sub>H<sub>33</sub>O<sub>9</sub>N<sub>2</sub>S, 464.1907].

**β-2-C-[3-[(2-(*N*-Acryloylamino)ethyl)thio]propyl]-*N*-acetylneuraminic acid (5)** was prepared from 4: the β-[3-[(2-aminoethyl)thio]propyl]-C-glycoside was prepared by photolytic addition of aminoethanethiol hydrochloride to 4. Purification on a biogel P2 size-exclusion column and lyophilization of the pooled fractions afforded the β-[3-[(2-aminoethyl)thio]propyl]-*N*-acetylneuraminic acid as a white foam: mp 200–205 °C dec; <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O with CH<sub>3</sub>CN internal standard) δ 3.99 (m, 1 H), 3.86 (m, 1 H), 3.74 (m, 4 H), 3.62 (dd, 1 H, *J* = 5.9, 11.8 Hz), 3.53 (d, 1 H, *J* = 8.5 Hz), 3.17 (t, 2 H, *J* = 6.7 Hz), 2.80 (t, 2 H, *J* = 6.7 Hz), 2.57 (m, 2 H), 2.22 (m, 1 H), 2.08 (m, 1 H), 2.01 (s, 3 H), 1.86 (m, 2 H), 1.65 (m, 2 H); <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O with CH<sub>3</sub>CN internal standard, 1.30 ppm) δ 180.3, 175.9, 81.4, 71.2, 70.6, 68.9, 67.5, 63.7, 53.2, 40.7, 38.8, 31.1, 30.6, 28.4, 23.4, 22.6; high-resolution mass spectrum (FAB) *m/z* 433.1628 [(M + Na)<sup>+</sup>, calcd for C<sub>16</sub>H<sub>30</sub>N<sub>2</sub>O<sub>8</sub>SNa, 433.1621].

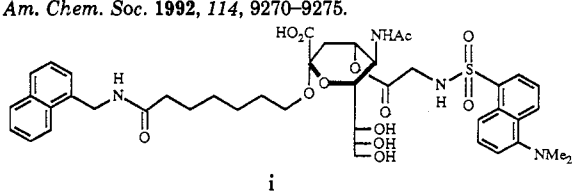
The β-analog of 5 was prepared, as described above for 5, in 90% overall yield from β-2-C-[3-[(2-aminoethyl)thio]propyl]-*N*-acetylneuraminic acid: <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O) δ 6.25 (dd, 1 H, *J* = 9.86, 17.0 Hz), 6.19 (d, 1 H, *J* = 17.0 Hz), 5.75 (d, 1 H, *J* = 9.75 Hz), 3.99 (m, 1 H), 3.86–3.61 (m, 5 H), 3.46 (m, 3 H), 3.36 (t, 2 H, *J* = 5.71 Hz), 2.69 (t, 2 H, *J* = 5.71 Hz), 2.60 (m, 2 H), 2.22 (dd, 1 H, *J* = 4.60, 12.82 Hz), 2.09 (m, 1 H), 2.00 (m, 1 H), 1.75 (m, 1 H), 1.36 (m, 1 H); <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O with CH<sub>3</sub>CN internal standard, 1.30 ppm) δ 180.7, 175.2, 170.0, 130.9, 128.5, 81.4, 71.2, 70.7, 68.9, 67.9, 63.7, 53.3, 40.7, 38.8, 31.1, 31.4, 30.7, 23.4, 22.6; high-resolution mass spectrum (FAB) *m/z* 464.1910 [(M + H)<sup>+</sup>, calcd for C<sub>19</sub>H<sub>33</sub>O<sub>9</sub>N<sub>2</sub>S, 464.1907].

**Preparation of Poly(5-co-acrylamide) Polymers.** All copolymerization reactions were carried out following obvious variations in the procedure described here in detail for the C-glycosidic poly(5-co-acrylamide) with *χ*<sub>Neu5Ac</sub> = 0.2.

An aqueous solution of acrylamide (710.1 mg, 10 mmol) and *N,N'*-azobis(isocyanoveraleric acid) (AICV, 74.7 mg, 0.02 mmol) in 10 mL of distilled H<sub>2</sub>O was prepared. This solution was 1.0 M in acrylamide. Into a 1-mL Pyrex test tube was weighed the Neu5Ac-acrylamide 5 (10 mg, 0.02 mmol). The prepared acrylamide solution (86.2 μL, 0.09 mmol) was syringed into the Pyrex test tube. An additional 100 μL of distilled H<sub>2</sub>O was added in order to dissolve the Neu5Ac-acrylamide 5 completely. The test tube was sealed with a rubber septum and the reaction

solution purged with argon for 20 min. The test tube containing the reaction solution was moved to a photoreaction chamber where the solution was irradiated with a UV lamp (365 nm) for 6 h; as the copolymerization proceeded the solution became viscous. The solution of copolymer was transferred by pipet into an Eppendorf tube and diluted to 0.5 mL with PBS (pH 7.0). The copolymer solutions prepared in this fashion were usually acidic (pH 3.0–4.5) and had to be titrated to pH 7.0 with 1.0 N NaOH. Each solution containing dissolved copolymer was vortexed briefly (10 s) prior to use in an HAI assay.

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