Reprinted from Journal of Medicinal Chemistry, 1993, 36.

379

Neuraminidase-Resistant Hemagglutination Inhibitors: Acrylamide Copolymers Containing a C-Glycoside of *N*-Acetylneuraminic Acid[1]

Michelle A. Sparks,[2] Kevin W. Williams,[3] and George M. Whitesides*

Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

Received September 14, 1992

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 α-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 $K_i^{(HAI)} \sim 10^{-7}$ 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.[4] 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).[5] Monomeric α-2-methyl-*N*-acetylneuraminic acid (**1**, Neu5Acα2Me) binds weakly to HA.[6] A variety of structurally modified, monomeric Neu5Ac derivatives have been tested as inhibitors of hemagglutination;[7-9] only recently have monomeric derivatives been prepared that are significantly more effective than Neu5Acα2Me, **1**.[8,9] 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.[10] A number of potent, naturally-occurring, polyvalent compounds that inhibit the adhesion of influenza virus to cells in vitro are known.[11] In addition, we[12,13] and others[14-16] 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.[12-16] All Neu5Ac-based inhibitors, whether natural or synthetic in origin, are susceptible to glycosidic cleavage by neuraminidase (NA, EC 3.2.1.18),[17-19] 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[17-19] and aid in the release of newly formed virion from the infected cell.[20]

In order to circumvent the neuraminidase-catalyzed cleavage of terminal Neu5Ac residues from synthetic, polyvalent copolymers presenting Neu5Ac groups as side chains,[12,14,15] 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[21] (**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)[22] in vitro by employing a standard hemagglutination inhibition (HAI) assay.[23] 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.[17]

The synthesis of **5** began with glycosyl chloride **2**.[24] This compound was converted to an anomeric mixture of Neu5Ac C-glycosides **3** (α-2-*C*-allylNeu5Ac) and **4** (β-2-*C*-allylNeu5Ac) under the allylation conditions developed by Paulsen[25] and Bednarski.[26] Separation of **3** from **4**,[25,26] followed by photolytic addition of 2-aminoethanethiol hydrochloride to the double bond terminus of **3**,[14,27] 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_2O (pH 10) produced the desired Neu5Ac-acrylamide **5** in 85% purified yield.

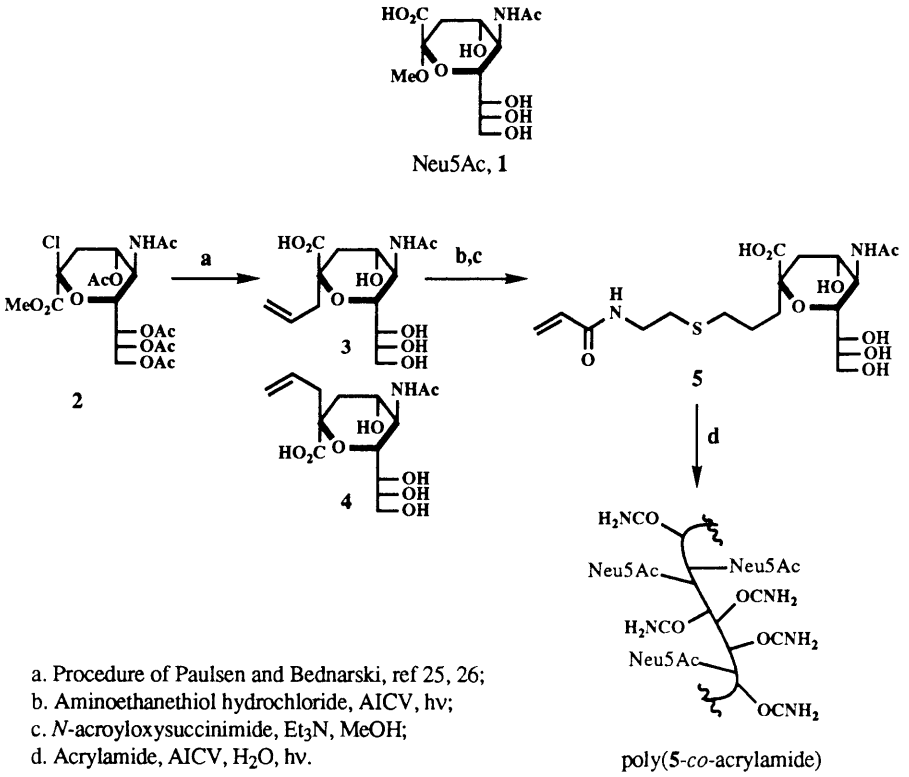
The copolymers were synthesized by photochemically initiated copolymerization of acrylamide and **5**. In all preparations, the initial concentration of acrylamide was fixed at 1.0 M. The initial concentration of **5** was varied from 4.0 mM to 4.0 M; this range corresponded to a mole fraction of **5**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

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

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

0022-2623/93/1836-0778$04.00/0

Scheme I. Synthesis of Copolymer 5 and Acrylamide



a. Procedure of Paulsen and Bednarski, ref 25, 26;
b. Aminoethanethiol hydrochloride, AICV, hν;
c. *N*-acroyloxysuccinimide, Et_3N, MeOH;
d. Acrylamide, AICV, H_2O, hν.

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.[12]

Unlike their O-glycosidic counterparts, the C-glycoside copolymers of Neu5Ac are resistant to neuraminidase. Incubation of a sample of C-glycoside copolymer (χ_{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.[19]

Aliquots of the monomers 3 and 5, and of each poly(5-*co*-acrylamide) (χ_{Neu5Ac} = 0.005–0.8), 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[22,23] 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, $K_i^{(HAI)}$, 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 (±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

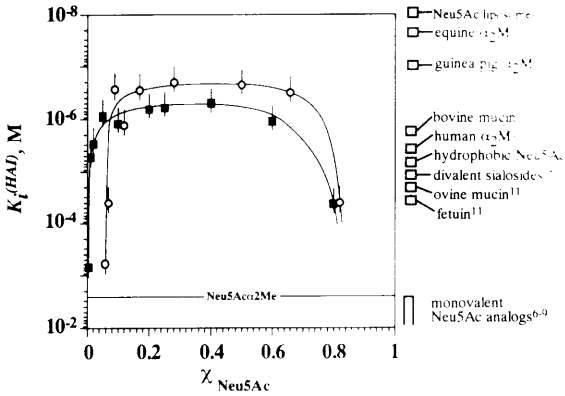


Figure 1. Inhibitory potency, $K_i^{(HAI)}$, of the C- and O-glycosidic copolymers of Neu5Ac as a function of mole fraction, χ_{Neu5Ac}, of Neu5Ac covalently incorporated into an acrylamide copolymer. The data points presented for the C- and O-glycosidic copolymers are averages of four to six sets of data obtained from independently prepared samples of copolymer. The data for the O-glycosidic copolymers have been previously published from this laboratory.[12] $K_i^{(HAI)}$ 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 Neu5Acα2Me 1 ($K_i^{(HAI)}$ = 2.8 × 10^{-3} M)[6] assayed under identical conditions. The error bars represent ±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.[12] The data are expressed in terms of $K_i^{(HAI)}$ versus χ_{Neu5Ac}. For both sets of copolymers, the antihemagglutination potency (measured by $K_i^{(HAI)}$ of the C- and O-glycosidic copolymers of Neu5Ac) reaches a maximum value at $\chi_{Neu5Ac}^{max} \approx 0.3$–0.4. We have attributed the bell shape of these curves to a combination of entropy and optimal glycoside spacing effects.[12] The horizontal line in Figure 1 represents $K_i^{(HAI)}$ for Neu5Acα2Me 1 (2.8 ×

Table I. Antihemagglutination Inhibitory Potency χ_{Neu5Ac}^{max} and χ_{Neu5Ac} for the C- and O-Glycoside Acrylamide Copolymers of Neu5Ac

copolymer	$K_i^{(HAI)}$, μM	χ_{Neu5Ac}^{max} [a]	$\chi_{Neu5Ac}^{0.5}$ [b]
C-glycoside Neu5Ac	0.4	0.3–0.4	0.01
O-glycoside Neu5Ac	0.2	0.3–0.4	0.09

[a] χ_{Neu5Ac}^{max} is the mole fraction of copolymer-linked Neu5Ac that yields the maximum antiagglutination potency. [b] $\chi_{Neu5Ac}^{0.5}$ 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 Neu5Acα2Me in solution ($K_i^{(HAI)} = 2.8 \times 10^{-3}$ M).

10^{-3} M)[6] and is shown as a reference for the $K_i^{(HAI)}$ 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 $\chi_{Neu5Ac} = 0.4$ showed inhibition of hemagglutination with $K_i^{(HAI)} = 2 \times 10^{-7}$ to 6×10^{-7} M in Neu5Ac groups. A parallel set of experiments conducted with copolymers containing O-glycosidic Neu5Ac gave $K_i^{(HAI)}$ of 2×10^{-7} to 3×10^{-7} M in Neu5Ac groups.[12] The slightly weaker inhibition of hemagglutination for the C-glycosidic copolymer (at $\chi_{Neu5Ac} > 0.1$) relative to the O-glycosidic copolymer suggested by Figure 1 is within the margin of experimental error in the hemagglutination assay (±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. $K_i^{(HAI)} = 2.5 \times 10^{-3}$ M for Neu5Acα2Me, **1**),[6] $\chi_{Neu5Ac}^{0.5}$, is shifted from 0.09 for the O-glycosidic copolymer to 0.01 for the C-glycosidic copolymer. Together with χ_{Neu5Ac}^{max}, the mole fraction of copolymer-linked Neu5Ac that yields the maximum antiagglutination potency, the $\chi_{Neu5Ac}^{0.5}$ index provides a convenient means to compare the effectiveness of different types of copolymers containing neuraminic acid (Table I).

The monomeric α-C-glycosides **3** and **5** were also tested in an HAI assay. For these monomers, $K_i^{(HAI)}$ fell between 2.5 and 5 mM; these values are similar to the values of $K_i^{(HAI)}$ obtained for most monomeric Neu5Ac analogs.[6-8] The β-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 β-analog of **5** (0.05–0.2 mole fraction of β-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".[28] 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 χ_{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 χ_{Neu5Ac} is not reflected in an increase in the maximum value observed for $K_i^{(HAI)}$ 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[29] and viruses.[30] 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.[31] A number of studies have examined the effects of polyvalency[32,33] and have reported the syntheses of compounds that are polyvalent in known bacterial and/or virus carbohydrate-based ligands.[12-16] 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[12] 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 60_{F254} (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^+ 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: $CHCl_3$ in $CDCl_3$ at 7.24 ppm, HOD in D_2O at 4.80 ppm, and CDH_2OH in CD_3OD at 3.30 ppm for the proton spectra. For carbon spectra the references are 77.0 ppm for $CDCl_3$, 49.9 ppm for CD_3OD, and 1.30 ppm for CH_3CN in D_2O.

N-Acetylneuaminic acid was obtained from extraction of edible Chinese swiftlet's nest.[34] 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_2HPO_4, and 2 g of KH_2PO_4 in 1 L of distilled H_2O. This stock solution was diluted 1 part in 10 in distilled H_2O and then adjusted to pH 7.2 with 1 N NaOH.

Hemagglutination Inhibition Assay.[22,23] 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^{-4} to 4×10^{-2} 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 [$K_i^{(HAI)}$] 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).[35] The inhibition of hemagglutination by monomeric and copolymeric sialosides was measured relative to Neu5Acα2Me **1** [$K_i^{(HAI)}$ = 2.8 mM].[6]

5-Acetamido-2,6-anhydro-3,5-dideoxy-2-*C*-(2-propenyl)-D-*erythro*-L-*manno*-non-2-ulosonic acid (3) was prepared as described previously:[25,26] mp 104 °C dec; ^1H NMR (400 MHz, CD_3OD) 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); ^{13}C NMR (100 MHz, CD_3OD) 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)$^+$, calcd for $C_{14}H_{24}O_8N$, 334.1502].

5-Acetamido-2,6-anhydro-3,5-dideoxy-2-*C*-(2-propenyl)-D-*erythro*-L-*gluco*-non-2-ulosonic acid (4) was prepared as described previously:[25,26] mp 110–114 °C dec; ^1H NMR (400 MHz, D_2O) 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); ^{13}C NMR (100 MHz, D_2O with CH_3CN 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)$^+$, calcd for $C_{14}H_{24}O_8N$, 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[27] and Roy:[14] C-glycoside **3** (85.9 mg, 0.26 mmol) was added to a 5-mL round-bottom Pyrex flask and dissolved in distilled H_2O (1 mL) at room temperature. Aminoethanethiol hydrochloride (2.0 equiv, 0.52 mmol, 58.3 mg) and *N,N'*-azobis(isocyanovaleric 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 ^1H 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; ^1H NMR (400 MHz, D_2O) 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); ^{13}C NMR (100 MHz, CD_3OD) 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 $C_{16}H_{30}N_2O_8SNa$, 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_2O (4.5 mL) at room temperature. Triethylamine (300 μL) and *N*-(acroyloxy)succinimide (prepared from acroyl 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_3N. 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; ^1H NMR (400 MHz, CD_3OD) 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); ^{13}C NMR (100 MHz, D_2O with CH_3CN 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)$^+$, calcd for $C_{19}H_{33}O_9N_2S$, 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; ^1H NMR (400 MHz, D_2O with CH_3CN 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); ^{13}C NMR (100 MHz, D_2O with CH_3CN 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, 23.4, 23.4, 22.6; high-resolution mass spectrum (FAB) *m/z* 433.1628 [(M + Na)$^+$, calcd for $C_{16}H_{30}N_2O_8SNa$, 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: ^1H NMR (400 MHz, D_2O) 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); ^{13}C NMR (100 MHz, D_2O with CH_3CN 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)$^+$, calcd for $C_{19}H_{33}O_9N_2S$, 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_{Neu5Ac} = 0.2.

An aqueous solution of acrylamide (710.1 mg, 10 mmol) and *N,N'*-azobis(isocyanovaleric acid) (AICV, 74.7 mg, 0.02 mmol) in 10 mL of distilled H_2O 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_2O was added in order to dissolved 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.

References

(1) This work is part of a collaboration with Professors J. J. Skehel, J. R. Knowles, M. Karplus, and D. C. Wiley. Support was provided by NIH Grant GM 39589.

(2) NIH postdoctoral fellow 1991–1993 (CA 09127-01).

(3) NSF postdoctoral fellow 1990–1992 (CHE-9002653).

(4) Matrosovich, M. N. Towards the Development of Antimicrobial Drugs Acting by Inhibition of Pathogen Attachment to Host Cells: A Need for Polyvalency. *FEBS Lett.* **1989**, *252*, 1–4.

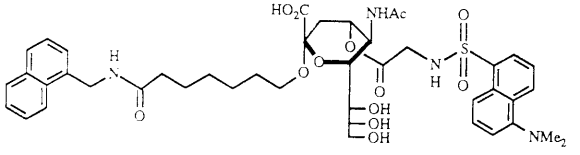
(5) Weis, W.; Brown, J. H.; Cusack, S.; Paulson, J. C.; Skehel, J. J.; Wiley, D. C. Structure of the Influenza Virus Hemagglutinin Complexed with Its Receptor Sialic Acid. *Nature* **1988**, *333*, 426–431. Wiley, D. C.; Skehel, J. J. The Structure and Function of the Hemagglutinin Membrane Glycoprotein of Influenza Virus. *Annu. Rev. Biochem.* **1987**, *56*, 365–394. Ruigrok, R. W. H.; Andre, P. J.; Hoof Van Huysduynen, R. A. M.; Mellema, J. E. Characterization of Three Highly Purified Influenza Virus Strains by Electron Microscopy. *J. Gen. Virol.* **1985**, *65*, 799–802.

(6) Sauter, N. K.; Bednarski, M. D.; Wurzburg, B. A.; Hanson, J. E.; Whitesides, G. M.; Skehel, J. J.; Wiley, D. C. Hemagglutinins from Two Influenza Variants Bind to Sialic Acid Derivatives with Millimolar Dissociation Constants: A 500-MHz Proton Nuclear Magnetic Resonance Study. *Biochemistry* **1989**, *28*, 8388–8396. Pritchett, T. J.; Brossmer, R.; Rose, U.; Paulson, J. C. Recognition of Monovalent Sialosides by Influenza Virus H3 Hemagglutinin. *Virology* **1987**, *160*, 502–506.

(7) DeNinno, M. P. The Synthesis and Glycosidation of *N*-Acetylneuraminic Acid. *Synthesis* **1991**, 483–493. Appropriately placed aromatic substituents have been reported to enhance monomeric binding: Toogood, P. L.; Galliker, P. K.; Glick, G. D.; Knowles, J. R. Monovalent Sialosides That Bind Tightly to Influenza A Virus. *J. Med. Chem.* **1991**, *34*, 3140–3143. See also ref 5 and Paulson, J. C. Interactions of Animal Viruses with Cell Surface Receptors. In *The Receptors*; Conn, P. M., Ed.; Academic: Orlando, 1985; Vol. 2, pp 131–219.

(8) Hydrophobic *N*-acyl analogs of neuraminic acid have been prepared and examined by HAI: Sparks, M. A.; Williams, K. W.; Lucaks, C.; Schrell, A.; Priebe, G.; Spaltenstein, A.; Whitesides, G. M. Synthesis of Potential Inhibitors of Hemagglutination by Influenza Virus: Chemoenzymic Preparation of N-5 Analogs of *N*-Acetylneuraminic Acid. *Tetrahedron* **1993**, *49*, 1–12. Schmid, W.; Avila, L. Z.; Williams, K. W.; Whitesides, G. M. Synthesis of Methyl α-Sialosides N-Substituted with Large Alkanoyl Groups, and Investigation of Their Inhibition of Agglutination of Erythrocytes by Influenza A Virus, *BioMed. Chem. Lett.*, submitted.

(9) A new Neu5Ac derivative, 6′-(((napthylmethyl)amino)carbonyl)-hexyl 5-acetamido-3,5-dideoxy-4-*O*-(((5-(dimethylamino)napth-1-yl)sulfonyl)glycyl)-D-*glycero*-α-D-*galacto*-nonulopyranosidonic acid (**i**), has been prepared that inhibits hemagglutination at 3.7×10^{-6} M: Knowles, J. R.; Weinhold, E. Design and Evaluation of a Tightly Binding Fluorescent Ligand for Influenza A Hemagglutinin. *J. Am. Chem. Soc.* **1992**, *114*, 9270–9275.



i

(10) Polymers of other carbohydrates have been prepared in attempts to mimic ligands for a variety of lectins: Roy, R.; Tropper, F. D.; Romanowska, A. New Strategy in Glycopolymer Synthesis. Preparation of Antigenic Water-Soluble Poly(acrylamide-*co*-*p*-acrylamidophenyl β-lactoside). *Bioconj. Chem.* **1992**, *3*, 256–261. Chernyak, A. Y.; Sharma, G. V. M.; Kononov, L. O.; Krishna, P. R.; Rao, A. V. R.; Kochetkov, N. K. Synthesis of Glycuronamides of Amino Acids, Constituents of Microbial Polysaccharides and Their Conversion into Neoglycoconjugates of Copolymer Type. *Glycoconjugate J.* **1991**, *8*, 82–89. Kosma, P.; Waldstatten, P.; Daoud, L.; Schulz, G.; Unger, F. M. Synthesis of Poly(acrylamide)-copolymers Containing 3,5-Dideoxy-D-arabino-2-octulopyranosylonic (5-deoxy-KDO) Residues. *Carbohydr. Res.* **1989**, *194*, 145–154. Kallin, E.; Lonn, H.; Norberg, T.; Elofsson, M. Derivatization Procedures for Reducing Oligosaccharides, Part 3: Preparation of Oligosaccharide Glycosylamines, and Their Conversion to Oligosaccharide-Acrylamide Copolymers. *Glycoconjugate J.* **1989**, *8*, 597–611. Roy, R.; Tropper, F. D. Synthesis of Copolymer Antigens Containing 2-Acetamido-2-deoxy-α- or β-D-glucopyranosides. *Glycoconjugate J.* **1988**, *5*, 203–206. Roy, R.; Tropper, F. D. Synthesis of Antigenic Carbohydrate Polymers Recognized by Lectins and Antibodies. *J. Chem. Soc., Chem. Commun.* **1988**, 1058–1060. Horeksi, V.; Smolek, P.; Kocourek, J. Water-soluble O-glycosyl Polyacrylamide Derivatives for Specific Precipitation of Lectins. *Biochim. Biophys. Acta* **1978**, *538*, 293–298.

(11) Equine α_2-macroglobulin (equine α_2M) is the most potent inhibitor of influenza viral adhesion known, see: Pritchett, T. J.; Paulson, J. C. Basis for the Potent Inhibition of Influenza Virus Infection by Equine and Guinea Pig α_2-Macroglobulin. *J. Biol. Chem.* **1989**, *264*, 9850–9858. Other naturally occurring polyvalent inhibitors include orosomucoid (Morawiecki, A.; Lisowska, E. Polymerized Orsomucoid-An Inhibitor of Influenza Virus Hemagglutination. *Biochem. Biophys. Res. Commun.* **1965**, *18*, 606–610), fetuin (Spiro, R. G. Studies on Fetuin, a Glycoprotein of Fetal Serum. *J. Biol. Chem.* **1960**, *235*, 2860–2869), and mucin (Gottschalk, A.; McKenzie, H. A. Studies on Mucoproteins VIII. On the Molecular Size and Shape of Ovine Submaxillary Gland Mucoprotein. *Biochim. Biophys. Acta* **1961**, *54*, 226–235). See also ref 15.

(12) Spaltenstein, A.; Whitesides, G. M. Polyacrylamides Bearing Pendant α-Sialoside Groups Strongly Inhibit Agglutination of Erythrocytes by Influenza A Virus. *J. Am. Chem. Soc.* **1991**, *113*, 686–687.

(13) Liposomes that incorporate a sialyl ganglioside inhibit hemagglutination as well as or better than the best-known natural inhibitors (see ref 11): Kingery-Wood, J. E.; Williams, K. W.; Sigal, G. B.; Whitesides, G. M. The Agglutination of Erythrocytes by Influenza Virus is Strongly Inhibited by Liposomes Incorporating an Analog of Sialyl Gangliosides. *J. Am. Chem. Soc.* **1992**, *114*, 7303–7305.

(14) Roy, R.; Laferriere, C. A. Synthesis of Antigenic Copolymers of *N*-Acetylneuraminic Acid Binding to Wheat Germ Agglutinin and Antibodies. *Carbohydr. Res.* **1988**, *177*, C1–C4.

(15) Matrosovich, M. N.; Mochalova, L. V.; Marinina, V. P.; Byramova, N. E.; Bovin, N. V. Synthetic Polymeric Sialoside Inhibitors of Influenza Virus Receptor-Binding Activity. *FEBS Lett.* **1990**, *272*, 209–212. Roy, R.; Laferriere, C. A. Synthesis of Protein Conjugates and Analogues of *N*-Acetylneuraminic Acid. *Can. J. Chem.* **1990**, *68*, 2045–2054. Roy, R.; Laferriere, C. A. Michael Addition as the Key Step in the Synthesis of Sialyloligosaccharide-Protein Conjugates from N-Acroylated Glycopyranosylamines. *J. Chem. Soc., Chem. Commun.* **1990**, 1709–1711. Gamian, A.; Jennings, H. J.; Laferriere, C. A.; Roy, R. Inhibition of Influenza A Virus Hemagglutinin and Induction of Interferon by Synthetic Sialylated Glycoconjugates. *J. Carbohydr. Chem.* **1987**, *6*, 161–165.

(16) Several compounds that are divalent in Neu5Ac have been prepared and shown to inhibit hemagglutination approximately 10^2 better than Neu5Acα2Me (1): Glick, G. D.; Toogood, P. L.; Wiley, D. C.; Skehel, J. J.; Knowles, J. R. Ligand Recognition by Influenza Virus: The Binding of Bivalent Sialosides. *J. Biol. Chem.* **1991**, *266*, 23660–23669. Glick, G. D.; Knowles, J. R. Molecular Recognition of Bivalent Sialosides by Influence Virus. *J. Am. Chem. Soc.* **1991**, *113*, 4701–4703. For the synthesis and HAI data concerning cluster inhibitors (divalent in Neu5Ac) see: Sabesan, S.; Duus, J. O.; Domaille, P.; Kelm, S.; Paulson, J. C. Synthesis of Cluster Sialoside Inhibitors for Influenza Virus. *J. Am. Chem. Soc.* **1991**, *113*, 5865–5866.

(17) Burmeister, W. P.; Ruigrok, R. W.; Cusack, S. The 2.2 Å Resolution Crystal Structure of Influenza B Neuraminidase and Its Complex with Sialic Acid. *EMBO J.* **1992**, *11*, 49–56. Air, G. M.; Laver, W. G. The Neuraminidase of Influenza Virus. *Proteins* **1989**, *6*, 341–356.

(18) NA activity presumably prevents these molecules from acting as inhibitors in vivo, as several Neu5Ac-containing glycoproteins have been shown to prevent binding of virus to cell in vitro: see ref 11 and Hanaoka, K.; Pritchett, T. J.; Takasaki, S.; Kochibe, N.; Sabeson, S.; Paulson, J. C.; Kobata, A. 4-O-Acetyl-*N*-acetylneuraminic Acid in the N-Linked Carbohydrate Structures of Equine and Guinea Pig α_2-Macroglobulins, Potent Inhibitors of Influenza Virus Infection. *J. Biol. Chem.* **1989**, *264*, 9842–9849.

(19) Polymers containing side chains terminating in O-glycosidic Neu5Ac groups are rendered ineffective in inhibiting viral hemagglutination of chicken red blood cells by pretreatment with NA: Spaltenstein, A.; Whitesides, G. M. Unpublished results.

(20) Palese, P.; Tobita, K.; Ueda, M.; Compans, R. W. Characterization of Temperature Sensitive Influenza Virus Mutants Defective in Neuraminidase. *Virology* **1974**, *61*, 397–410.

(21) The complete IUPAC name for this compound is 5-acetamido-2,6-anhydro-3,5-dideoxy-2-*C*-[3-[[2-(*N*-acryloylamino)ethyl]-thio]propyl]-D-*erythro*-L-*manno*-nonulosonic acid.

(22) Kilbourne, E. D. *Bull. W.H.O.* **1969**, *41*, 643.

(23) Rogers, G. N.; Pritchett, T. J.; Laue, J. L.; Paulson, J. C. Differential Sensitivity of Human, Avian, and Equine Influenza A Viruses to a Glycoprotein Inhibitor of Infection: Selection of Receptor Specific Variants. *Virology* **1983**, *131*, 394–408. *W.H.O. Tech. Rep. Ser.* **1953**, *64*, 1–32.

(24) Ogura, H.; Furuhata, K.; Itoh, M.; Shitori, Y. Synthesis of 2-O-Glycosyl Derivatives of *N*-Acetyl-D-Neuraminic Acid. *Carbohydr. Res.* **1986**, *158*, 37–51.
(25) Paulsen, H.; Matschulat, P. Synthesis of a C-glycoside of *N*-Acetylneuraminic Acid and Derivatives. *Liebigs Ann. Chem.* **1991**, 487–495.
(26) Nagy, J. O.; Bednarski, M. D. The Chemical-Enzymatic Synthesis of a Carbon Glycoside of *N*-Acetylneuraminic Acid. *Tetrahedron Lett.* **1991**, *32*, 3953–3956.
(27) Lee, R. T.; Lee, Y. C. Synthesis of 3-(2-Aminoethylthio)propyl Glycosides. *Carbohydr. Res.* **1974**, *37*, 193–201.
(28) Everett, D. H. *Basic Principles of Colloid Science*; Royal Society of London: London, 1988; pp 45–51.
(29) Lindahl, M.; Brossmer, R.; Wadstrom, T. Carbohydrate Receptor Specificity of K99 Fimbriae of Enterotoxigenic *Escherichia coli*. *Glycoconjugate J.* **1987**, *4*, 51–58. Ishikawa, H.; Isayama, Y. Evidence for Sialyl Glycoconjugates as Receptors for *Bordetella bronchiseptica* on Swine Nasal Mucosa. *Infect. Immun.* **1987**, *55*, 1607–1609. Lindberg, A. A.; Brown, J. E.; Stromberg, N.; Westling-Ryd, M.; Schultz, J. E.; Karlsson, K.-A. Identification of the Carbohydrate Receptor for Shiga Toxin Produced by *Shigella dysenteriae* Type 1. *J. Biol. Chem.* **1987**, *262*, 1779–1785. Murray, P. A.; Levine, M. J.; Reddy, M. S.; Tabak, L. A.; Bergey, E. J. Preparation of a Sialic Acid Binding Protein from *Streptococcus mitis* KS32AR. *Infect. Immun.* **1986**, *53*, 359–365. Bose, S. K.; Smith, G. B.; Paul, R. G. Influence of Lectins, Hexoses and Neuraminidase on the Association of Purified Elementary Bodies of *Chlamydia Trachomatis* UW-31 with HeLa Cells. *Infect. Immun.* **1983**, *40*, 1060–1067. Levy, N. J. Wheat Germ Agglutinin Blockage of Chlamydial Attachment Site: Antagonism by *N*-Acetyl-D-Glucosamine. *Infect. Immun.* **1979**, *25*, 946–953. Ofek, I.; Mirelman, D.; Sharon, N. Adherence of *Escherichia coli* to Human Mucosal Cells Mediated by Mannose Receptors. *Nature* **1977**, *265*, 623–625. Kuo, C.-C.; Wang, S.-P.; Grayston, J. T. Effect of Polycations, Polyanions and Neuraminidase on the Infectivity of Trachoma-Inclusion Conjunctivitis and Lymphogranuloma Venereum Organisms in HeLa Cells: Sialic Acid Residues as Possible Receptors for Trachoma Inclusion Conjunctivitis. *Infect. Immun.* **1973**, *8*, 74–79. Carbohydrates in bacterial adhesion, reviews: Isberg, R. R. Discrimination Between Intracellular Uptake and Surface Adhesion of Bacterial Pathogens. *Science* **1991**, *252*, 934–938 and references cited therein. Sharon, N. Bacterial Lectins, Cell-Cell Recognition and Infectious Disease. *FEBS Lett.* **1987**, *217*, 145–157. Reid, G.; Sobel, J. D. Bacterial Adherence in the Pathogenesis of Urinary Tract Infection: A Review. *Rev. Infect. Dis.* **1987**, *9*, 470–487. Beachey, E. H. Bacterial Adherence: Adhesin-Receptor Interactions Mediating the Attachment of Bacteria to Mucosal Surfaces. *J. Infect. Dis.* **1981**, *143*, 325–345.
(30) Tavakkol, A.; Burness, A. T. H. Evidence for a Direct Role for Sialic Acid in the Attachment of Encephalomyocarditis Virus to Human Erythrocytes. *Biochemistry* **1990**, *29*, 10684–10690. Eaton, B. T.; Crameri, G. S. The Site of Bluetongue Virus Attachment to Glycophorins from a Number of Animal Erythrocytes. *J. Gen. Virol.* **1989**, *70*, 3347–3353. Paul, R. W.; Lee, P. W. K. Glycophorin is the Reovirus Receptor on Human Erythrocytes. *Virology* **1987**, *159*, 94–101. Allaway, G. P.; Burness, A. T. H. Site of Attachment of Encephalomyocarditis Virus on Human Erythrocytes. *J. Virol.* **1986**, *59*, 768–770. Fried, H.; Cahan, L. D.; Paulson, J. C. Polyoma Virus Recognizes Specific Sialyloligosaccharide Receptors on Host Cells. *Virology* **1981**, *109*, 188–192.
(31) See ref 15, Matrosovich et al. Rao, B. N. N.; Moreland, M.; Brandley, B. K. The Potential of Carbohydrates as Cell Adhesion Inhibitors. *Med. Chem. Res.* **1991**, *1*, 1–8.
(32) Ponpipom, M. M.; Bugianesi, R. L.; Robbins, J. C.; Doebber, T. W.; Shen, T. Y. Cell-Specific Ligands for Selective Drug Delivery to Tissues and Organs. *J. Med. Chem.* **1981**, *24*, 1388–1395. Lee, Y. C. Synthesis of Some Cluster Glycosides Suitable for Attachment to Proteins and Solid Matrices. *Carbohydr. Res.* **1978**, *67*, 509–514. Kawasaki, T.; Etoh, R.; Yamashina, I. Isolation and Characterization of Mannan-Binding Proteins from Rat Liver. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 1018–1024. Stahl, P. D.; Rodman, J. S.; Miller, M. J.; Schlesinger, P. H. Evidence for Receptor-Mediated Binding of Glycoproteins, Glycoconjugates and Lysosomal Glycosidases by Alveolar Macrophages. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 1399–1403. Kornfield, R.; Kornfield, S. Comparative Aspects of Glycoprotein Structure. *Annu. Rev. Biochem.* **1976**, *45*, 217–237.
(33) Singer, S. J. Intercellular Communication and Cell-Cell Adhesion. *Science* **1992**, *255*, 1671–1677. For discussions concerning polyvalency in cellular communication of the immune system see Springer, T. A. Adhesion Receptors of the Immune System. *Nature* **1990**, *346*, 425–434.
(34) Czarniecki, M. F.; Thornton, E. R. Carbon-13 Nuclear Magnetic Resonance Spin-Lattice Relaxation in the N-Acylneuraminic Acids. Probes for Internal Dynamics and Conformational Analysis. *J. Am. Chem. Soc.* **1977**, *99*, 8273–8279.
(35) Since the inhibition values ($K_i^{(HAI)}$) are calculated based on the concentration of Neu5Ac functional groups in solution they can be compared directly for monomeric and polymeric derivatives of Neu5Ac.