sites lead to similar results. The HOMO region corresponds to lone pairs of the oxygen atoms bonded to the zeolite lattice. The LUMO is located on the Ag^+ ion and corresponds to the empty 5s orbital. On the six-ring, two transitions from the lone pairs of two oxygen atoms to the Ag^+ 5s orbital [$Ag^+(5s) \leftarrow O(n)$] are found (340, 380 nm). The third band at 405 nm is a transition from the oxygen atoms corresponding to the connections to the neighboring α cage. If the Ag^+ ion is coordinated by water only, the $Ag^+(5s) \leftarrow O(n)$ transition is found below 250 nm. The first ionization energy of water is 12.6 eV, which means that the HOMO lies at -12.6 eV. For silicates, however, it is found at -10.7 eV, which explains the long-wavelength situation of the LMCT.[6] The coordination of only one water molecule to a Ag^+ ion which is coordinated to zeolite oxygen atoms leads to a destabilization or a raising of the $Ag^+(5s)$ level, and therefore to a blue shift of the LMCT transitions.

We were able to show that activation at room temperature of silver-containing zeolite A with very low degrees of exchange already leads to a yellow coloring, and that the absorption of light and therefore the impression of yellow coloring are reversible with respect to the desorption/adsorption of water. The dependency of the absorption bands on the degree of exchange can be explained with a simple model of the cation distribution. Quantum-chemical calculations show that electronic transitions from the lattice oxygen atom to the silver ion are allowed, that these transitions are in the visible range, and that they experience a blue shift if coordinated with water. Hence, an observation first described 35 years ago has been quantified and explained in a noncontradictory way.

Experimental Section

Chemically pure zeolite was produced and characterized by a known procedure.[7] For the examinations, 80 mg of zeolite were freshly exchanged each time with 0.1 M $AgNO_3$ (Merck, Titrisol). At degrees of exchange up to six Ag^+ ions, the zeolite was placed in 5 mL of H_2O, and a calculated amount of $AgNO_3$ was added and suspended for 15 min. Quantitative uptake of the Ag^+ ions can be assumed.[8] The zeolite was washed with water twice. The zeolite stored at 92% humidity was assumed to have 27 H_2O molecules per formula unit.[1] To obtain samples fully exchanged with Ag^+ ions, the zeolite was suspended twice in 20 mL of $AgNO_3$ solution for 15 min and washed three times with 15 mL of H_2O. The exchanged zeolite was put into 2 mL of H_2O and transferred to a quartz ampoule (cylinder with a height of 2 cm and a radius of 0.75 cm) which was connected to a HV-Flansch Adapter in a gas-tight way. The ampoule was connected to the pump in a horizontal position so that the zeolite could be deposited evenly at the bottom of the ampoule. The extra water was vaporized at 10 mbar (2–3 h). For activation, the turbo pump (Alcatel) was connected until the final pressure of the apparatus was achieved (48–72 h, $1-2 \times 10^{-7}$ mbar). After activation at room temperature, temperature-treated samples were gradually brought to the desired temperature ($\pm 5\,^{\circ}C$) with an adjustable heat pistol. The heating rate was chosen such that the pressure within the apparatus did not exceed 5×10^{-6} mbar, to avoid irreversible brown color changes. The ampoule with the sample sufficiently adhered to the bottom was separated from the pump with a burner under gas-tight conditions and transferred to the spectrometer. UV/Vis spectra were measured as diffuse reflection spectra at room temperature with a Perkin Elmer Lambda 14 spectrometer with an integration sphere (Labshere RSA-PE-20). Before they were graphically represented, the automatically collected data were converted using the Kubelka–Munk formula.

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A Lysoganglioside/Poly-L-glutamic Acid Conjugate as a Picomolar Inhibitor of Influenza Hemagglutinin**

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Cell surfaces are often covered with oligosaccharides that play important roles in many cellular recognition events, including infection, cancer metastasis, and other intercellular adhesion processes.[1] Hemagglutinins (HAs) of influenza virus are typical examples of such receptor molecules. These trimeric proteins are capable of binding to sialylated oligosaccharides on the epithelial cell surface.[2] The minimum

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structure involved in this recognition is either α-Neu5Ac-(2→3)-β-Gal or α-Neu5Ac-(2→6)-β-Gal as the nonreducing terminal saccharide, depending on the strain of the virus.[3] The HAs are also known to help trigger fusion between the viral membrane and a lysosomal-lipid bilayer through changes in their conformations.[4] Thus, inhibition of either or both of these processes could prevent viral infection. We report here the synthesis of polyvalent inhibitors that contain a hydrophobic moiety designed to disturb the fusion mechanism initiated by the protein–carbohydrate recognition process.

One problem associated with carbohydrate–receptor interactions is their relatively low binding affinity. A possible approach to increase the affinity is to use multivalent ligands with specific monomeric carbohydrate ligands.[5] For this reason, functionalyzed polymers,[5d, 6, 7] peptides,[8] dendrimers,[9] or liposomes that contain sugars[10] have recently attracted interest, and some have been shown to exhibit higher affinities to receptors.[5] Various types of polymers that incorporate sialic acid residues have also been synthesized in an attempt to inhibit the influenza virus.[5d, 7] Because this virus has both HA and neuraminidase (NA) on its surface, polymers with sialic acid residues bound through noncleavable linkages have emerged as stable and potent inhibitors of HA. Alternatively, introduction of a second functionality to provide additional affinity for the target or nearby molecules might increase the affinity. Hydrophobic groups, for example, could be used to enhance binding affinity by their interaction with hydrophobic amino acid side chains and/or the membrane; the latter interaction might in turn interfere with the conformational change of the membrane-fusion domain of HA even after the first inhibition process has had its effect. An infection by influenza virus could thus be inhibited at two different stages: first during initial binding, and later in the course of HA-mediated membrane fusion.

To test the hypothesis that hydrophobic groups in a polymer might contribute to enhanced binding and disruption of the virus, we chose the molecule lysoganglioside GM_3 (lyso-GM_3), α-Neu5Ac-(2→3)-β-Gal-(1→4)-β-Glc-(1→)-sphingosine,[11] which differs from the ganglioside GM_3 by the absence of the stearic acid residue. This was then conjugated with the polymer poly-L-glutamic acid (PGA). The reason for our choice of lyso-GM_3 is that GM_3 is the simplest ganglioside recognized by HA[2]. The amino functionality of the sphingosine is suitable for connection to the polymer. The amphiphilic nature of sphingosine, which is directly attached to the oligosaccharide moiety, could help present the epitope unit to the surface of the synthetic polymer by the formation of either intra- (folded) or intermolecular aggregates (Figure 1). PGA (degree of polymerization = 540) was selected because of its low toxicity, low immunogenicity, and biodegradability.[12] Furthermore, its length is sufficient to meet the criteria for the "chain lock" strategy to be effective, as described above. Another advantage of this strategy is that, because we chose the natural form of the oligosaccharide as ligand, galactosyl residues exposed on the virus surface after cleavage of sialic acids by NA may lead to galactose-mediated endocytosis. We describe here the synthesis of a lyso-GM_3/poly-L-glutamic acid conjugate and its inhibitory effect on HAs that bind the α-sialyl-(2→3)-galactosyl residue.

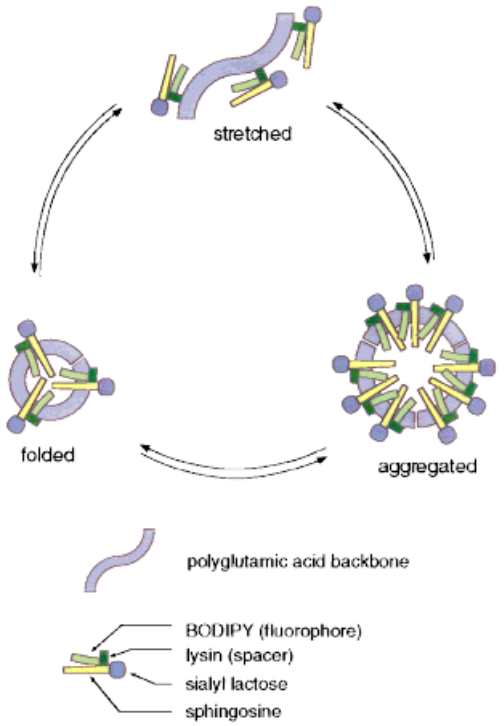

Figure 1. Schematic representation of lyso-GM_3-PGA, which exists in a stretched form in methanol and in either folded or aggregated forms in aqueous media.

The synthesis (Scheme 1) begins with derivatization of L-lysine, which was used as a spacer between lyso-GM_3 and the polymer backbone and as the trifunctional linker for incorporation of a fluorescent dye. The latter was expected to facilitate subsequent quantification and visualization of the disruption process if the polymer–virus complexes were indeed taken up by macrophages. The copper chelate of L-lysine was used to protect the ε-amino functionality with a 9-fluorenylmethyloxylcarbonyl (Fmoc) group under basic conditions (58.2%). The fluorescent tag, a 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-propionyl (BODYPY FL C3) group,[13] was then attached to the α-amino functionality (87%). Compound **3** was next transformed into active ester **4**, which without further purification was coupled with lyso-GM_3 (**5**) to provide condensate **6**[14] (quantitative yield based on thin-layer chromatography with fluorescence detection). The Fmoc group was removed under standard conditions, although it was found that prolongation of the reaction resulted in a poor yield because of decomposition of the fluorescent tag. The coupling reaction between **7** and poly-L-glutamic acid (PGA) activated as its succinimidyl ester (**8**) proceeded smoothly to give, after treatment with sodium bicarbonate and purification by gel-permeation chromatography (GPC) over a Superose-12-pg column (Pharmacia Biotech), a polymer that contained lyso-GM_3 (**9**).[15] The lyso-GM_3 (or sialic acid) content was calculated on the basis of absorption at 508 nm (BODIPY; in MeOH) and was determined to have an average value of 44.6 nmol per mg of polymer (0.72 mol%).

Synthetic polymer **9** was unique in the sense that the green luminescence observed in MeOH disappeared in water,

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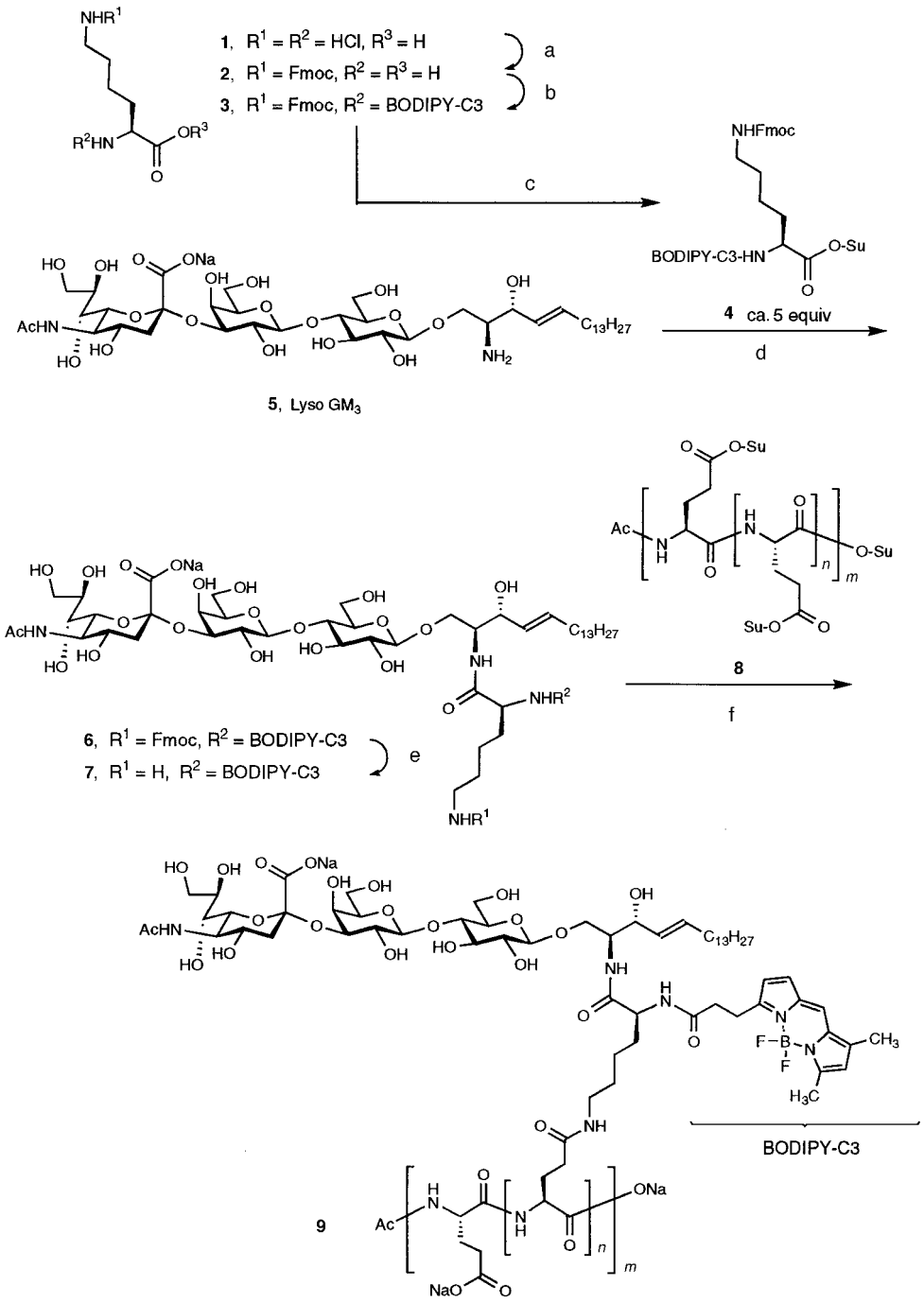

Scheme 1. a) 1. $CuCO_3Cu(OH)_2 \cdot H_2O$, H_2O; 2. Fmoc-O-Su, 10% Na_2CO_3, 1,2-dimethoxyethane, 58.2%; b) BODIPY FL C3-O-Su, 10% Na_2CO_3, MeOH, 87%; c) HO-Su, 1,3-diisopropylcarbodiimide, CH_2Cl_2; d) Et_3N, MeOH/DMF, quantitative; e) piperidine, DMF, 34%; f) DMF/MeOH. Su = succinimidyl; $m = 270$, $n:m \approx 1.5:100$.

whereupon the solution acquired a faint orange color, even though BODYPY FL C3 itself displays a green luminescence in both of these media as well as in NaOAc solution ($c \approx 10\ \text{nmol mL}^{-1}$; Figure 2). Quenching of the fluorescence and the observed shift in the absorption maximum were attributed to folding or aggregation in water caused by the hydrophobicity of sphingosine and the BODIPY fluorophore. The lyso-GM_3 content of the polymer may cause the fluorophore in the synthetic polymer to be highly localized and thus able to interact in the ground state, evidenced by an 83.3% decrease in fluorescence ($\lambda_{em} = 512$, $\lambda_{ex} = 480$ nm) and a slight shift in the absorption maximum (λ_{max} 504.6 and 507.8 nm in MeOH and H_2O, respectively; Figure 3). Neither a shift in emission wavelength nor additional peaks were found to arise from excimer interactions. The BODIPY fluorophore is known to be insensitive to the pH value and largely independent of its environment; however, it can form aggregates, which results in self-quenching in aqueous media. The observed self-quenching is not a result of a high concentration of fluorophore incorporated into the polymer, because quenching did not occur for the free fluorophore under the same conditions. The lyso-GM_3 content was also consistent with the quantities of materials used in the condensation of lyso-GM_3-Lys(BODIPY)-NH_2 (**7**) with activated PGA (**8**). We therefore conclude that the synthetic polymer **9** exists in a stretched form in methanol and as a folded or aggregated form in aqueous media.

We investigated the inhibitory activity of the polymer toward influenza A/PR/8/34 (H1N1) with an enzyme-linked immunosorbent assay (ELISA).[16] The IC_{50} value for **9** was 1.9×10^{-12} M based on the lyso-GM_3 content (or 7.5×10^{-12} M based on the sialic acid content; estimated from the UV absorption of the fluorophore). For comparison, the IC_{50} values for GM_3, lyso-GM_3 (**5**), and sialyllactose (the trisaccharide portion of GM_3) were also determined (1×10^{-9}, 3×10^{-9}, and 1.5×10^{-7} M, respectively). Sialyl-PGA was much less active than **9**. PGA showed no inhibition up to a concentration of 1×10^{-9} M; compound **9** was also assayed at this concentration (Table 1).

We have thus developed a new strategy for the inhibition of influenza virus, and the process itself can now be studied with the aid of an incorporated fluorophore. Experimental evidence was obtained to support the "chain lock" mechanism (Figure 4). Enhancement in the fluorescence emission of

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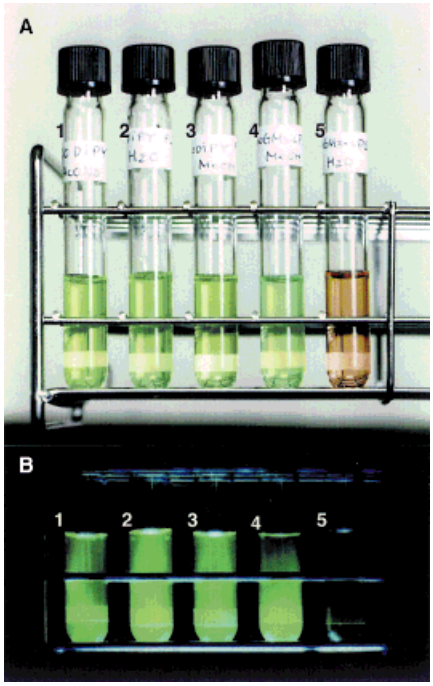

Figure 2. Solutions of lyso-GM_3-PGA (**9**) and BODIPY fluorophore in different media: BODIPY FL C3 in 1) NaOAc, 2) water, and 3) MeOH, as well as solutions of lyso-GM3-PGA in 4) MeOH and 5) water, all at 26 °C. The upper photograph (A) was taken with ambient light; for the lower photograph (B) the solutions were irradiated with UV light (365 nm).

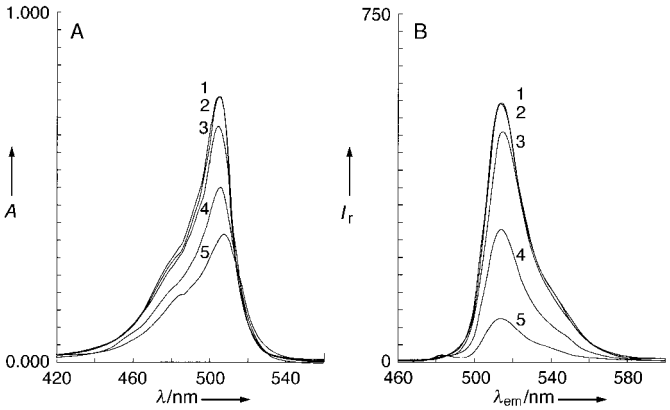

Figure 3. A) UV/Vis (0.1 mg mL^{-1}) and B) fluorescence emission spectra (80 μg mL^{-1}, λ_{ex} = 480 nm) of solutions of lyso-GM_3-PGA (**9**) in 1) MeOH, 2) 75% MeOH, 3) 50% MeOH, 4) 25% MeOH, and 5) water. A = absorbance, I_r = relative fluorescence intensity.

Table 1. Inhibitory activity of synthetic polymer **9** and related compounds with respect to the influenza virus A/PR/8/34 (H1N1).

Compound	IC_{50} [M][a]	Compound	IC_{50} [M][a]
9	1.9×10^{-12}	lyso-GM_3 **5**	3×10^{-9}
9	7.5×10^{-12}[b]	sialyllactose	1.5×10^{-7}
GM_3	1.0×10^{-9}	PGA	–[c]

[a] Relative to the sialyllactose content. [b] Relative to the sialic acid content. [c] No inhibition.

BODIPY (λ_{em} 510, λ_{ex} 480 nm) was observed immediately after the addition of polymer **9** to a virus solution, which suggests an unfolding of the polymer after an initial self-quenching in a folded state. The emission intensity then decreased upon further incubation at 10 °C, perhaps explained by energy transfer to the aromatic amino acid residues of HA. When the tryptophane residue was irradiated (λ_{ex} = 287 nm), emission from BODIPY (λ_{em} 510 nm) was also observed, indicative of energy transfer between BODIPY and the Trp residues.[17] The tight binding may be a consequence of initial multivalent interactions between **9** and the virus, followed by a hydrophobic interaction between the ligand and the receptor. Alternatively, the hydrophobic interaction may occur first. Both GM_3 and lyso-GM_3 might form liposomes and micelles and exhibit a multivalent effect, but not as effectively as **9**.

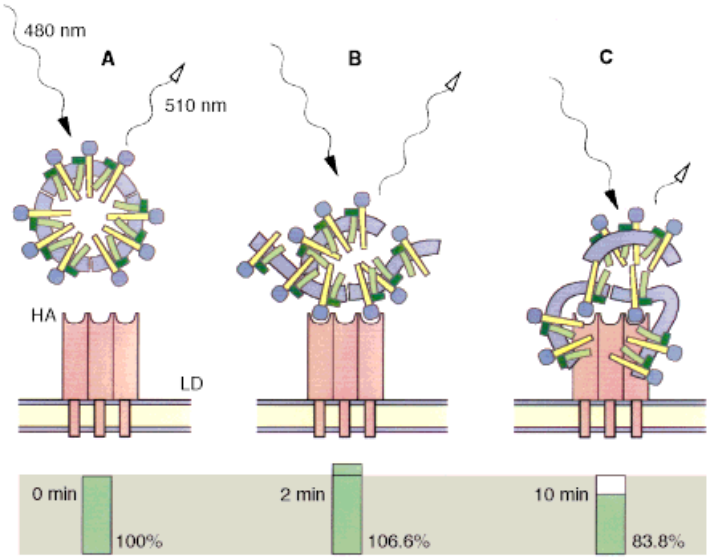

Figure 4. Schematic representation of the "chain lock" inhibition strategy. A) The aggregated polymeric inhibitor with a specific ligand, in this case sialyllactose, approaches the receptor, here hemagglutinin (HA). B) The aggregated polymer interacts with the receptor, whereby the fluorescence intensity increases. Emission from the exposed BODIPY fluorophore is greater than the energy loss caused by energy transfer to a Trp residue at the binding site. C) Hydrophobic interaction occurs to cause formation of a stable complex. The hydrophobic groups in HA, including the aromatic residues, lead to a further decrease in the fluorescence emission of BODIPY as a result of energy transfer. The bar graphs (bottom) indicate the relative fluorescence intensity (λ_{em} = 510, λ_{ex} = 480 nm) of the assay medium. The emission intensity of the mixture at t = 0 min was set to be 100%. See Figure 1 for an explanation of the symbols. LD = lipid double layer of the virus.

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[14] $R_f = 0.27$ ($CHCl_3/CH_3OH$/15 mM $CaCl_2$ (60/35/8, v/v/v), ^1H NMR (600 MHz, CD_3OD): $\delta = 7.34$ (s, 1H, BODIPY), 6.93 (d, 1H, BODIPY), 6.26 (d, 1H, BODIPY), 6.13 (s, 1H, BODIPY), 3.13 (m, 2H, BODIPY-CH_2), 2.62 (t, 2H, BODIPY-CH_2), 2.42 (s, 3H, BODIPY-CH_3), 2.19 (s, 3H, BODIPY-CH_3), 4.2 – 4.3 (1H, C_α-H), 3.03 (m, 2H, C_ε-H), 1.5 – 1.65 (m, 4H, C_β-H, C_γ-H), 1.69 (m, 2H, C_δ-H), 4.21 (d, 1H, $J = 8.1$ Hz, Glc-H1), 4.32 (d, 1H, $J = 8.1$ Hz, Gal-H1), 3.93 – 3.95 (1H, Gal-H3), 3.80 (br. s, 1H, Gal-H4), 2.81 (dd, 1H, Neu5Ac-$H3_{eq.}$), 1.54 (1H, Neu5Ac-$H3_{ax.}$), 1.91 (3H, Neu5Ac-NHCOCH_3), 5.32 – 5.38 (m, 1H, sphingosine, olefinic H4), 5.56 – 5.59 (m, 1H, sphingosine, olefinic H5), 1.19 (16H, sphingosine-CH_2), 0.80 (sphingosine-CH_3); ESI-MS positive ions: calcd for $[M2\mathrm{Na} - \mathrm{H}]^+$: 1362, found: 1362; calcd for $[M+\mathrm{Na}]^+$: 1340, found: 1340; negative ions: calcd for $[M - \mathrm{H}]^-$: 1316, found 1316.

[15] The average molecular weight ($\bar{M}_r$) was estimated to be 87 kDa based on the lyso-GM_3 content (0.72 %; $m = 536.1$, $n = 3.9$) and the degree of polymerization (DP = 540) of PGA. $\bar{M}_r$ of **9** estimated by GPC to be 336 kDa (Waters Ultrahydrogel Liner column (7.8 × 300 mm), elution with 0.1 M $NaNO_3$, flow rate 1 mL min^{-1}, UV detection at 500 nm and 40 °C). Millenium 2010 with GPC software was used to analyze the data. Polyethylene oxides were employed as standards and detected by refractive index. The GPC result is apparently erroneous with respect to the number of introduced lyso-GM_3 groups($\bar{M}_r$(GPC)/$\bar{M}_r$(UV) = 3.86), perhaps as a result of aggregation.

[16] The inhibitory activity of polymer **9** toward influenza virus A/PR/8/34 (H1N1) was determined with an ELISA. Thus, an ethanol solution with 1 nmol of GM_3 was added to each well of 96-microwell plates. The solvent was evaporated at 37 °C, and the remaining binding sites on the wells were blocked for 12 h at 4 °C with 200 μL of phosphate-buffered saline (PBS; 0.15 M NaCl, 8.1 mM Na_2HPO_4, 1.5 mM NaH_2PO_4) that contained 2 % bovine serum albumin (BSA). The wells were then washed five times with PBS. A series of dilutions, each by a factor of two, was prepared from a solution of the polymer (200 pmol) in 0.2 % BSA-PBS. These dilute solutions were preincubated at 4 °C for 2 h with 50 μL of the influenza virus suspension (32 hemagglutinin units (HAU) and then introduced into the wells. The plates were incubated at 4 °C for 12 h, washed five times with PBS, and then incubated for 2 h at 4 °C with a 1000-fold diluted 0.2 % BSA-PBS solution of 50 μL of anti-influenza virus antibodies and treated at 4 °C for 2 h with horse radish peroxidase (HRP)-conjugated protein A diluted 1000-fold with solution A. The virions bound to GM_3 and immobilized in the wells were detected with *o*-phenylenediamine (OPD) solution that contained 4 mg of OPD and 0.01 % H_2O_2 in 100 mM of phosphate buffer (adjusted to pH 5.0 with citric acid). The reactions were terminated by addition of 4 N H_2SO_4, and viral binding activities in the form of color development were determined at 492 nm (reference wavelength 630 nm). Serial dilutions (dilution factor two) of sialyl lactose (2 μmol), PGA (800 pmol), and lyso-GM_3 (20 nmol) were tested as controls in the manner described.

[17] The HA trimer of H1 serotype influenza virus contains a total of 15 Trp residues.

Assembly of DNA/Fullerene Hybrid Materials**

Alan M. Cassell, Walter A. Scrivens, and James M. Tour*

There are biochemical approaches to molecular structures of precisely defined dimensions ranging from 1 nm to 10 cm in length. Conversely, the synthesis of precisely defined unnatural molecular architectures beyond 25 nm in length is often unattainable due to solubility, material through-put, and characterization constraints.[1] Therefore, as nanotechnological needs advance, syntheses could rely upon self-assembling strategies using natural scaffolds as templates for the construction of synthetic nanostructures.[2] DNA is particularly

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