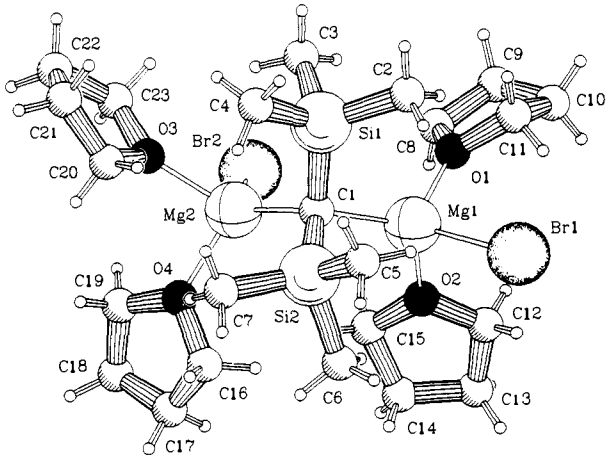

Figure 1. PLUTON drawing of **1** (molecule 1). Selected bond distances (Å) and angles (deg): Mg(1)–C(1), 2.10 (4); Mg(2)–C(1), 2.14 (4); Si(1)–C(1), 1.85 (4); Si(2)–C(1), 1.81 (4); Mg–O, 2.05 (3) (average); Mg(1)–C(1)–Mg(2), 112 (2); Si(1)–C(1)–Si(2), 113 (2); Mg(1)–C(1)–Si(1), 104 (2); Mg(1)–C(1)–Si(2), 107 (2); Mg(2)–C(1)–Si(1), 106 (2); Mg(2)–C(1)–Si(2), 115 (2); C(1)–Mg(1)–Br(1), 121 (1); C(1)–Mg(2)–Br(2), 122 (1); O–Mg–O, 93 (1) (average).

"classical" Grignard reaction from bis(trimethylsilyl)dibromomethane (**2**) with magnesium in diethyl ether and isolated by crystallization (40% yield). Somewhat unexpectedly, **1** turned out to be rather unreactive toward most electrophiles.[3]

In an attempt to better understand this unusual behavior, we undertook an X-ray structure determination of **1**. Crystals of **1** suitable for X-ray crystal structure determination were obtained from a saturated solution in THF/hexane (1:1) on cooling to +5 °C. The asymmetric unit of the orthorhombic unit cell contains two very similar molecules, one of which is shown in Figure 1.[4]

Anticipating **1** to be a crowded molecule and realizing that organomagnesium compounds tend to escape from congested situations by association to form bridged dimers or oligomers,[5] we were surprised to find *monomeric* units of **1** with *two* THF ligands per magnesium in the crystal; association measurements showed that the monomeric state is retained in THF solution. In view of this, the rather "normal" character of **1** noted at first sight was no lesser surprise: the central carbon atom is a slightly distorted tetrahedron with bond angles ranging from 104 (2)° to 116 (2)°, and the two magnesium atoms are tetracoordinated with unexceptional geometries.[5]

Closer examination reveals that all carbon–metal(loid) bonds are slightly shorter than usual. The (average) Mg–C bond distance is 2.12 (4) Å; in ether-coordinated Grignard reagents, Mg–C distances are normally about 2.15 Å.[5] Similarly, the (average) Si–C(1) bonds (1.83 (4) Å) are shorter than normal sp^3-hybridized Si–C bonds (1.88 Å). As **1** is a crowded molecule, we had, on the contrary, expected to find bond lengthening. Apparently, attachment of four electropositive groups to the central carbon leads to accumulation of negative charge at this carbon and thus to electrostatic strengthening and shortening of the highly polar bonds. This counteracts the steric crowding and contributes to the reduced reactivity of **1**.

The crowdedness of the molecule is an interesting feature. Intuitively, we had speculated[3] that steric hindrance by four bulky groups (two $SiMe_3$ groups and two $MgBr(THF)_2$ groups) is a major reason for the decreased reactivity of **1**. As can be seen in Figure 1, the central carbanionic carbon atom is indeed efficiently shielded against approach by large electrophiles.

Finally, we wish to point out that the structure **1** is the first experimentally determined compound carrying two strongly positive metal atoms at the same carbon atom (in addition to the two somewhat less positive silicons!). The only other two relevant structures are those of CD_2Li_2[6] and $Li_2[CH_2\{Al(CH_2SiMe_3)_3\}_2]$;[7] they have strongly different features because they are unsolvated and highly electron deficient, occurring either as a polymer or as an anionic complex with a hexacoordinated central carbon, respectively.

Acknowledgment. These investigations were supported in part (W.J.J.S., A.L.S.) by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for Advancement of Pure Research (NWO).

Supplementary Material Available: Tables of crystal data and details of the structure determination, final coordinates, and equivalent isotropic thermal parameters of the non-hydrogen atoms, hydrogen atom parameters, (an)isotropic thermal parameters, and bond distances and angles and PLUTON and ORTEP plots of the two molecules (17 pages). Ordering information is given on any current masthead page.

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(4) **Crystal data for 1**: $C_{23}H_{50}Br_2Mg_2O_4Si_2$, M_r = 655.23; colorless, block-shaped crystal (0.35 × 0.43 × 0.48 mm), orthorhombic, space group $Pc2_1b$, with a = 11.225 (1) Å, b = 22.620 (2) Å, c = 26.844 (2) Å, V = 6816 (1) Å^3, Z = 8, d_{calcd} = 1.277 g cm^{-3}, $F(000)$ = 2736, μ(Mo Kα) = 24.8 cm^{-1}; 4916 independent reflections ($0.76 < \theta < 24.2°$, $\omega/2\theta$ scan, T = 295 K) were measured on an Enraf-Nonius CAD-4T/rotating anode diffractometer using graphite monochromated Mo Kα radiation (λ = 0.710 73 Å). The crystal reflected poorly and had rather broad reflection profiles. Data were corrected for Lorentz polarization effects, for a small linear increase (5%) of the intensity control reflections, and for absorption (DIFABS; correction range 0.411–1.324). The structure was solved by Patterson (SHELXS86) and difference Fourier techniques. The O(6) THF molecule was found to be disordered over two positions (50:50 ratio); all THF molecules show high thermal motion and were included in the full-matrix least-squares (SHELX76) refinement as rigid groups. Hydrogen atoms were introduced at calculated positions and refined riding on their carrier atoms. Br, Si, Mg, and O were refined with anisotropic thermal parameters, and carbon atoms were refined isotropically in view of the poor quality of the crystals and the limited number of observed reflections. Convergence was reached at R = 0.072, wR = 0.080, $w = 1/[\sigma^2(F) + 0.00071F^2]$ for 1652 reflections with $I > 2.5\sigma(I)$ and 304 parameters. A final difference Fourier map showed no residual density outside −0.38 and 0.49 e/Å^3. Geometrical details of the structure are provided as supplementary material.

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The Agglutination of Erythrocytes by Influenza Virus is Strongly Inhibited by Liposomes Incorporating an Analog of Sialyl Gangliosides[1]

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Infection of a mammalian cell by influenza virus begins with the recognition of sialic acid (SA) groups on the cell surface by a viral surface protein, hemagglutinin (HA). Although virus binds tightly to cells,[3] solubilized HA binds only weakly ($K_d \sim 2.5 \times 10^{-3}$ M) to methyl α-sialoside.[4] This qualitative difference in the strength of binding is the basis for the hypothesis that the binding of virus to cell is controlled by polyvalent interactions.[5]

(1) This work is part of a collaboration with J. J. Skehel, J. R. Knowles, M. Karplus, and D. C. Wiley. Support provided by the NIH Grants GM 39589 and GM 30367.

(2) NSF Postdoctoral Fellow 1990–1992 (CHE-9002635).

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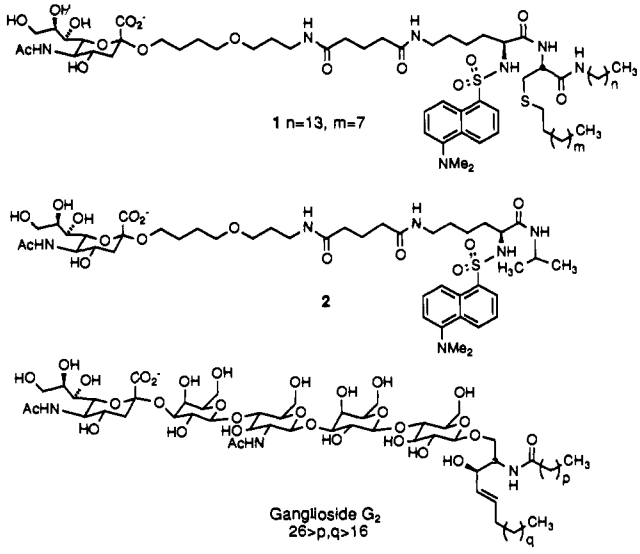
We are exploring the efficiency of polyvalent species presenting multiple SA groups as inhibitors of the binding of virus to cell and have reported that soluble polymers having a polyacrylamide backbone and pendant SA moieties inhibit virus-induced hemagglutination $\sim 10^5$ better than monomeric sialosides, when compared on the basis of SA residues.[6]

Here we compare synthetic and naturally occurring polymeric inhibitors with fluid bilayer liposomes presenting SA groups at their surface. Liposomes have been used as models for the fusion of virus to cell membranes[7] and as drug delivery vehicles.[8] Gangliosides[9] and antibodies[10] have been incorporated into liposome bilayers to probe ligand–receptor interactions. For our purposes, polyvalent, liposome-based inhibitors have three interesting features. First, we expect these systems to interact effectively with HA molecules on the surface of the virus. Second, in a fluid bilayer, the SA moieties can, in principle, move by lateral diffusion to optimize their binding to the virus. Third, the SA groups on the surface of a liposome are more localized in space than are the SA groups on a soluble polymer; they may, therefore, be more susceptible to detailed theoretical analysis.

To anchor the SA residue to the lipid bilayer, we designed and synthesized functionalized lipids that are intended to resemble sphingosine in their properties but are easier to synthesize. Compound **1** is a representative structure. A strategy for attachment of sugar moieties to liposomes using an analog of the naturally occurring glycosphingolipids has several advantages over one derived from natural materials. First, **1** is synthesized from



commonplace, inexpensive starting materials. Second, a convergent synthetic strategy readily accommodates structural variations and minimizes the quantity of saccharide needed. Third, a handle (in the form of an amine group in the dipeptide core) is included to which a fluorescent tag (or a tag with other useful properties) may be attached; this capability to modify and detect the glycolipid analog directly is useful in tracking it in the biological system. For comparison G_2, the major ganglioside on erythrocyte membranes, is shown.[11]

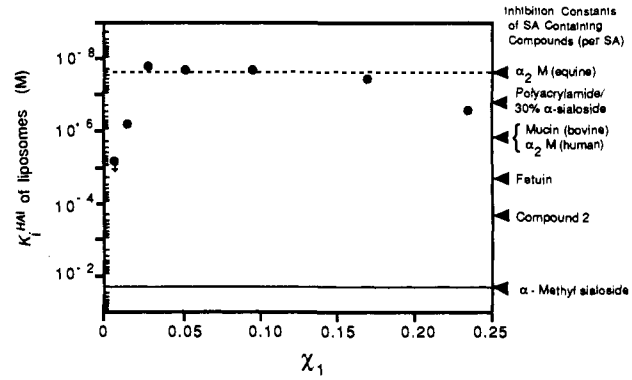


Figure 1. Inhibition of influenza virus-induced hemagglutination of chicken erythrocytes by liposomes incorporating **1**. The inhibition constant K_i^{HAI} is based on the content of SA groups in the system; values for SA groups on liposomes and polymers and the monovalent compounds are therefore directly comparable. The horizontal axis is the mole fraction of **1** used in preparing the liposomes, $\chi_1 = 1/(1 + PC + Chol)$. The data represent the averages of three independent trials in which independent preparations of liposomes were used. Inhibition constants of reference systems are taken from the following: Pritchett, T. J.; Paulson, J. C. *J. Biol. Chem.* **1989**, *264*, 9850 and ref 6.

Synthesis of **1** began with sequential amide formation between Boc-cystine and tetradecylamine, reduction of the disulfide group, and alkylation on the sulfur with bromodecane.[12] We used shorter alkyl chains than those found in gangliosides in the synthesis of **1** to improve solubility and simplify purification. The marker tag, here the fluorescent 5-(dimethylamino)naphthalene-1-sulfonyl (Dans) group, was incorporated by attachment to N_α of the lysine group. Coupling of the cysteine and lysine components by an amide group completed the anchor portion of **1**. The sugar component SA-2-O$(CH_2)_4$O$(CH_2)_3NH_2$[6] was joined to the dipeptide unit with a bridging glutaryl diamide unit. Compound **1** was prepared in six steps and 25% overall yield from Boc-cystine.

Liposomes were prepared using **1** and a lipid mixture comprised of egg phosphatidylcholine (PC) and cholesterol (Chol, 7:2 molar ratio). The appropriate amounts of **1** were added to buffered lipid, and the resulting mixture was sonicated until the turbidity dissipated to form small (25–100 nm) unilamellar liposomes.[13] The biochemical activity of the functionalized liposomes was evaluated using the hemagglutination inhibition (HAI) assay.[14] Briefly, the HAI assay consists of serial dilutions of inhibitors on 96-well microtitre plates followed by the addition of influenza virus X-31 and chicken red blood cells (RBCs). After 1–2 h, the wells are checked for agglutination of the RBCs. We define the hemagglutination inhibition constant K_i^{HAI} as the lowest concentration of SA residues that inhibits agglutination of erythrocytes by influenza virus at 4 °C under these assay conditions.

The HAI assay of the liposomes containing **1** revealed that they are extremely good inhibitors of hemagglutination, calculated on the basis of total SA groups contained in the system: $K_i^{HAI} \sim$ 20 nM (Figure 1). Independent liposome preparations give reproducible results within a factor of 4. The SA concentrations of liposome preparations were determined by taking half of the initial solution concentration of **1** to account for the material that is inaccessible on the inner bilayer surface of the liposome. The optimum mole fraction of SA on the surface of the liposome for inhibition is in the range 2.5–18%. This number is consistent with Haywood's observation that 3% of bovine ganglioside on liposomes is necessary to inhibit Sendai virus using the HAI assay.[10]

When **1** alone was assayed for inhibition, lysis of the RBCs occurred at concentrations above 10 μM. This result was expected for a surfactant such as **1**. Compound **2** was used to provide a

(6) This number reflects the increase in binding obtained when a monomeric SA moiety was incorporated into a polymer. Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686. Similar results have been obtained: Matrosovich, M. N.; Mochalova, L. V.; Marinina, V. P.; Bryamova, N. E.; Bovin, N. V. *FEBS Lett.* **1990**, *272*, 209. Roy, R.; Laferrière, C. A. *Carbohydr. Res.* **1988**, *177*, C1.

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(14) *W. H. O. Tech. Rep. Ser.* **1953**, *64*, 1. Rogers, G. N.; Pritchett, T. J.; Lane, J. L.; Paulson, J. C. *Virology* **1983**, *131*, 394.

soluble molecule against which to compare **1**.[15] A $K_i^{HAI} \sim 200$ μM (average of three trials) was obtained for **2**, a factor of 10^4 higher than for the polyvalent liposome system. Liposomes containing no **1** and liposomes containing the methyl ester of **1** were examined by the HAI assay as controls. Neither system inhibited hemagglutination.

These results establish that arrays of SA groups at the surface of liposomes are moderately more effective in inhibiting agglutination of RBCs by influenza virus than are SA groups linked to soluble polymers. More significantly, these SA functionalized liposomes are as good as or better than the best-known natural inhibitors of hemagglutination, the mucins and macroglobulins. We emphasize that the effective inhibition observed with **1** involves only a monosaccharide rather than a complex polysaccharide (i.e., a glycoprotein or ganglioside): this observation makes it unnecessary to synthesize the complex sialyl polysaccharides found in nature. It remains to be established whether this inhibitory activity is due to enhanced binding of SA to viral HA originating in polyvalency and entropic factors or to steric occlusion of the surface of the virus by bound liposome.[16] We will describe studies of the ability of these liposomes to inhibit infectivity of influenza virus in vivo later.

Supplementary Material Available: Experimental data for compounds **1**, **3**-**7**, and **9**-**12** (9 pages). Ordering information is given on any current masthead page.

(15) Compound **1** (in a liposome) and **2** (in solution) have substantially different steric constraints. In the liposome, the Dans group is predicted to be constrained to an area near the lipid bilayer surface and unavailable to interact with HA; in **2**, the Dans group is sterically unconstrained. We have not yet investigated whether the Dans group in either case plays a role in binding. See: Toogood, P. L.; Galliker, P. K.; Glick, G. D.; Knowles, J. R. *J. Med. Chem.* **1991**, *34*, 3138.

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$[Pt_2(\mu\text{-}CH_3CO_2\text{-}O,O')_4(H_2O)_2](ClO_4)_2$, a Platinum(III) Dimeric Cation with a Very Short, Compressed, Metal-Metal Bond

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Many metal ions form "lantern-shaped" dimeric complexes in which the metal ions are bridged by four carboxylate anions (e.g., rhodium(II) acetate (**1**)[1]), but no such compound has been characterized with platinum.[2] This is remarkable in view of the existence of complexes in which two platinum(III) atoms are bridged by four sulfate[3-5] (**2**) or hydrogenphosphate[5-7] (**3**) anions. Recently,[8] a Pt(III) compound, **4**, has been prepared in which there

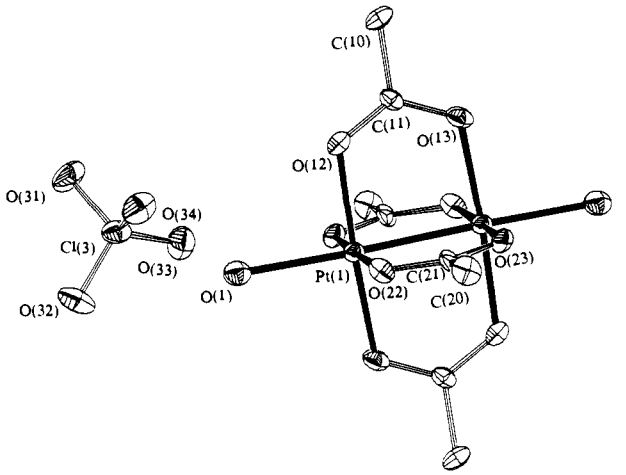
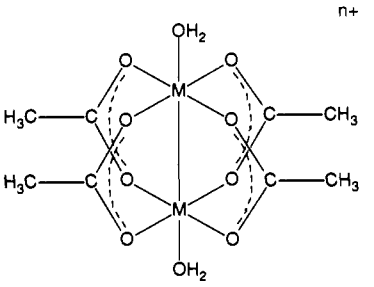


Figure 1. ORTEP diagram of the cation and one of the perchlorate anions of $[Pt_2(\mu\text{-}CH_3CO_2\text{-}O,O')_4(H_2O)_2](ClO_4)_2$, with hydrogen atoms omitted for clarity. Unlabeled atoms are related to those labeled by symmetry.

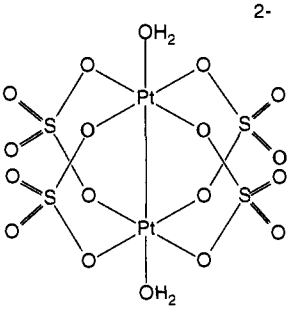
are two $Pt-CH_2C(O)O-Pt$ bridges as well as two "normal" O,O'-bridging acetate ligands. The question could then be asked, whether a Pt(III) complex analogous to **1** could exist.



1 M = Rh, n = 0

5 M = Pt, n = 2

Potassium salts of **2** and **3** have been prepared by reaction of $K_2[Pt(NO_2)_4]$ with sulfuric and phosphoric acid, respectively.[9] A detailed account of the reactions of $K_2[Pt(NO_2)_4]$ with aqueous acetic acid will be published elsewhere.[10] A mixture of platinum(II), platinum(III), and platinum(IV) products was usually obtained, depending on precise reaction conditions. These always contained both acetate and nitrite ligands. However, when a solution of $K_2[Pt(NO_2)_4]$ in a 2:1 mixture (by volume) of glacial acetic acid and 1 M perchloric acid was heated in air, a yellow solid was obtained which was analyzed for $[Pt_2(CH_3CO_2)_4(H_2O)_2](ClO_4)_2$.[11]



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(2) Preparation of $[Pt_2(CH_3CO_2)_6]$ has been claimed, by reduction of $K_2[Pt(OH)_6]$ by formic acid in acetic acid suspension. Characterization was by elemental analysis and IR spectroscopy only (Rudyi, R. I.; Cherkashina, N. V.; Mazo, G. Ya; Salyn', Ya V.; Moiseev, I. I. *Izvest. Akad. Nauk SSSR, Ser. Khim.* **1980**, 754-758). Attempts in this laboratory to reproduce this preparation were unsuccessful.

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(9) We have found that O_2 is also necessary in these reactions.

(10) Appleton, T. G.; Barnham, K. J.; Byriel, K. A.; Hall, J. R.; Kennard, C. H. L.; Mathieson, M. T.; Penman, K. G., to be submitted for publication.

(11) CAUTION: in two separate incidents, very small quantities of the solid have decomposed violently. The compound should be handled in small quantities only and treated with respect. An analogous reaction with CF_3SO_3H gives the trifluoromethanesulfonate salt.

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