of a complex between the lithium reagent and ketone, through which further reactions should take place, was the only fate of the present reactions. The X-ray and NMR studies evidently provide the first structural characterization of the intermediate adduct of the lithium reagent and ketones.[16]

Acknowledgment. We are grateful for the financial support of the Ministry of Education, Science, and Culture of Japan (Specially Promoted Research No. 02102004) and for a Kurata Research Grant.

Supplementary Material Available: Tables of X-ray experimental data, atomic parameters, anisotropic temperature factors, bond distances, and bond angles (10 pages); a table of observed and calculated structure factors for **4a** (21 pages). Ordering information is given on any current masthead page.

(15) Sitzmann, H.; Boese, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 971 and literature cited therein.

(16) Very recently, stable complexes between esters and lithium hexamethyldisilazide were characterized by X-ray diffraction; see: Williard, P. G.; Liu, Q.-Y.; Lochmann, L. *J. Am. Chem. Soc.* **1992**, *114*, 348. For $(Ph_2P)_2CHLi·Ph_2CO$, see: Issleib, V. K.; Abicht, H. P. *J. Prakt. Chem.* **1970**, *B312*, 456.

Polymerized Liposomes Containing *C*-Glycosides of Sialic Acid: Potent Inhibitors of Influenza Virus in Vitro Infectivity

Wayne Spevak,[†,‡] Jon O. Nagy,[‡] Deborah H. Charych,[‡] Mary E. Schaefer,[§] James H. Gilbert,[§] and Mark D. Bednarski*[,†,‡]

Department of Chemistry
University of California at Berkeley
Berkeley, California 94720
The Center for Advanced Materials
Lawrence Berkeley Laboratory
Berkeley, California 94720
Department of Microbiology and Immunology
Glycomed, Inc., Alameda, California 94501

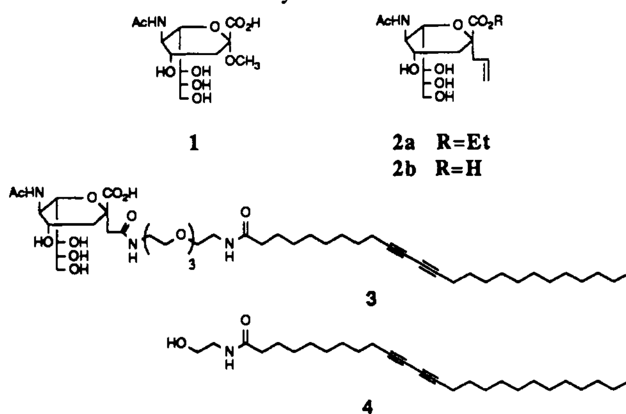
Received September 4, 1992

The surface lectin of the influenza virus, hemagglutinin, binds to terminal α-glycosides of *N*-acetylneuraminic acid (NeuAc) on cell-surface glycoproteins and glycolipids.[1] Viral binding to cells expressing terminal NeuAc residues can be inhibited by α-*O*-glycosides of NeuAc (*O*-sialosides).[2–5] Recently, dramatic enhancements in the inhibition of viral adhesion to erythrocytes have been achieved using synthetic polyvalent sialosides.[6–9] The inhibitory potencies of these polyvalent materials approach those of the most potent naturally occurring hemagglutination inhibitors, equine and guinea pig α_2-macroglobulins.[5,6] Despite intensive efforts in designing polyvalent sialosides to inhibit hemagglutination, no evidence exists that these synthetic *O*-sialoside materials can be used to arrest viral infectivity.[9] In this communication, we report that polymerized liposomes containing α-*C*-glycosides of sialic acid are potent inhibitors of influenza virus in vitro infectivity. Our results also indicate that the capacity to inhibit hemagglutination does not necessarily reflect the capacity to inhibit in vitro infectivity.

Table I. Hemagglutination Inhibition (HAI) and Plaque Reduction Assays of Liposome Preparations I–VI

			plaque reduction	
entry	inhibitor	HAI[a] [**3**], M	[**3**], mM	reduction,[b] %
1	liposome I (0%, **3**)	0 (–)	0.000	0
2	liposome II (1%, **3**)	4.0×10^{-6} (–)	0.003	96
3	liposome III (5%, **3**)	5.7×10^{-7} (+)	0.016	97
4	liposome IV (10%, **3**)	3.3×10^{-7} (+)	0.030	46
5	liposome V (30%, **3**)	8.0×10^{-5} (–)	3.750	0
6	liposome VI (60%, **3**)	1.5×10^{-4} (–)	7.500	0

[a] A (+) indicates complete inhibition while a (–) indicates that no inhibition was observed at the given concentrations of **3**. [b] The values represent the percent reduction in the number of plaques per well due to viral lysis of infected cells.



Sialoside lipid **3** was synthesized from **2a**,[10] and mixed liposomes[11] composed of compounds **3** and **4** were prepared using a modified probe sonication method.[12] The liposome preparations

† University of California at Berkeley.
‡ Lawrence Berkeley Laboratory.
§ Glycomed, Inc.

(1) Paulson, J. C. In *The Receptors*; Conn, M., Ed.; Academic Press: New York, 1985; Vol. 2, pp 131–219. Wiley, D. C.; Skehel, J. J. *Annu. Rev. Biochem.* **1987**, *56*, 365–394. Weis, W.; Brown, J. H.; Cusack, S.; Paulson, J. C.; Skehel, J. J.; Wiley, D. C. *Nature* **1988**, *333*, 426–431. Sauter, N. K.; Bednarski, M. D.; Wurzburg, B. A.; Hanson, J. E.; Whitesides, G. M.; Skehel, J. J.; Wiley, D. C. *Biochemistry* **1989**, *28*, 8388–8396. Wharton, S. A.; Skehel, J. J.; Wiley, D. C. *Virology* **1986**, *149*, 27–35. Stegmann, T.; Hoekstra, D.; Scherphof, G.; Wilschut, J. *J. Biol. Chem.* **1986**, *261*, 10966–10969. White, J.; Kielian, M.; Helenius, A. *Q. Rev. Biophys.* **1983**, *16*, 151–195.

(2) Toogood, P. L.; Galliker, P. K.; Glick, G. D.; Knowles, J. R. *J. Med. Chem.* **1991**, *34*, 3138–3140.

(3) Pritchett, T. J.; Brossmer, R.; Rose, U.; Paulson, J. C. *Virology* **1987**, *160*, 502–506.

(4) Rogers, G. N.; Pritchett, T. J.; Lane, J. L.; Paulson, J. C. *Virology* **1983**, *131*, 394–408.

(5) Pritchett, T. J.; Paulson, J. C. *J. Biol. Chem.* **1989**, *264*, 9850–9858. Hanaoka, K.; Pritchett, T. J.; Takasaki, S.; Kochibe, N.; Sabesan, S.; Paulson, J. C.; Kobata, A. *J. Biol. Chem.* **1989**, *264*, 9842–9849.

(6) Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686–687.

(7) Sabesan, S.; Duus, J. Ø.; Neira, S.; Domaille, P.; Kelm, S.; Paulson, J. C.; Bock, K. *J. Am. Chem. Soc.* **1992**, *114*, 8363–8375. Glick, G. D.; Toogood, P. L.; Wiley, D. C.; Skehel, J. J.; Knowles, J. R. *J. Biol. Chem.* **1991**, *266*, 23660–23669. Glick, G. D.; Knowles, J. R. *J. Am. Chem. Soc.* **1991**, *113*, 4701–4703. Sabesan, S.; Duus, J. Ø.; Domaille, P.; Kelm, S.; Paulson, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 5865–5866. Byramova, N. E.; Mochalova, L. V.; Belyanchikov, I. M.; Matrosovich, M. N.; Bovin, N. V. *J. Carbohydr. Chem.* **1991**, *10*, 691–700. Matrosovich, M. N.; Mochalova, L. V.; Marinina, V. P.; Byramova, N. E.; Bovin, N. V. *FEBS Lett.* **1990**, *272*, 209–212. Roy, R.; Laferrière, C. A. *Carbohydr. Res.* **1988**, *177*, C1–C4.

(8) Nagy, J. O.; Wang, P.; Gilbert, J. H.; Schaefer, M. E.; Hill, T. G.; Callstrom, M. R.; Bednarski, M. D. *J. Med. Chem.* **1992**, *35*, 4501–4502.

(9) Gamian, A.; Chomik, M.; LaFerrière, C. A.; Roy, R. *Can. J. Microbiol.* **1991**, *37*, 233–237. Suzuki, Y.; Nagao, Y.; Kato, H.; Matsumoto, M.; Nerome, K.; Nakajima, K.; Nobusawa, E. *J. Biol. Chem.* **1986**, *113*, 17057–17061. Klenk, H. D.; Rott, R. *Adv. Virus Res.* **1988**, *34*, 247–281.

(10) Nagy, J. O.; Bednarski, M. D. *Tetrahedron Lett.* **1991**, *32*, 3953–3956.

(11) Although most lipids that form liposomes consist of two alkyl chains, synthetic liposome-forming lipids with only one alkyl chain also exist. Hupfer, B.; Ringsdorf, H.; Schupp, H. *Chem. Phys. Lipids* **1983**, *33*, 355–374. Bader, H.; Ringsdorf, H.; Skura, J. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 91–92. Hub, H.; Hupfer, B.; Koch, H.; Ringsdorf, H. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 938–940.

(12) New, R. R. C. In *Liposomes: a practical approach*; New, R. R. C., Ed.: Oxford University Press: Oxford, 1990; pp 33–104.

(13) Kingery-Wood, J. E.; Williams, K. W.; Sigal, G. B.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 7303–7305.

0002-7863/93/1515-1146$04.00/0 © 1993 American Chemical Society

were polymerized by irradiation at 254 nm. Transmission electron microscopy of the liposomes revealed ellipsoid structures averaging on the order of 40 nm in length and 15 nm in width. The indicated percentage of sialoside presented at the liposome surface (0%, 1%, 5%, 10%, 30%, or 60%) represents the mole percentage of lipid monomer **3** used in the liposome preparation.

Liposome preparations I–VI were tested for binding to influenza virus using a standard hemagglutination inhibition (HAI) assay (Table I).[4] To achieve 50% inhibition of viral binding, the α-*O*-methyl glycoside of sialic acid (compound **1**) required a concentration of 2 mM[3] and compound **2b** required a concentration of 10 mM.[8] In sharp contrast, liposome preparations III and IV (5% and 10% of sialoside **3**) required as little as 5.7×10^{-7} and 3.3×10^{-7} M concentrations of the sialoside to achieve complete inhibition of agglutination. This represents an increase in potency of approximately 30 000 times over the corresponding monovalent sialic acid derivatives, making it one of the most potent synthetic inhibitors of hemagglutination reported to date. Interestingly, as the percentage of sialoside **3** is increased from 10% to 30% and 60%, inhibition of hemagglutination is no longer observed at these higher concentrations (compare entry 4 to entries 5 and 6). Liposome preparation II (entry 2), which contains only 1% of sialoside **3**, also showed no inhibition of hemagglutination. A similar trend in which high and low percentages of sialoside diminish the capacity of polyvalent materials to inhibit hemagglutination has been observed in sialoside polymers.[6,13]

We next tested the capability of liposome preparations I–VI to prevent infectivity in cell culture using Madin–Darby canine kidney (MDCK) cells in a standard plaque reduction assay (Table I).[14] To our surprise, liposome preparation II (1% sialoside **3**, entry 2) strongly inhibited viral infectivity (96% inhibition at a concentration of only 3 μM sialoside lipid) even though this liposome preparation did not inhibit hemagglutination. In contrast, liposome preparation IV (10% sialoside **3**, entry 4), which showed potent inhibition of hemagglutination, demonstrated only a modest capability to inhibit infectivity (46% inhibition at a concentration of 30 μM sialoside **3**). Liposome preparation III (5% sialoside **3**, entry 3), which has plaque reduction activity equivalent to liposome preparation II, was also a potent inhibitor of hemagglutination. Liposome preparations I (0% sialoside **3**), V (30% sialoside **3**), and VI (60% sialoside **3**) showed no inhibition of plaque formation and no inhibition of agglutination. Our data indicate that synthetic sialosides which are poor inhibitors of hemagglutination (e.g., liposome preparation II, entry 2) can stop infectivity, while strong inhibitors of hemagglutination (e.g., liposome preparation IV, entry 4) may not effectively stop infectivity. The relationship between inhibiting viral binding to the erythrocyte cell surface and the capacity to prevent infectivity is currently under further investigation.

In summary, we have synthesized a polymerizable sialoside lipid **3** and formed mixed liposome preparations that are potent inhibitors of influenza virus in vitro infectivity. We have also shown that the capacity of a sialoside to inhibit hemagglutination does not necessarily reflect its capacity to inhibit infectivity. Polymerized liposome preparations should serve as important models for understanding pathogen–cell interactions and for developing therapeutic agents based on multivalent carbohydrate structures.

Acknowledgment. This research was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Division of Materials Sciences, by the Division of Energy Biosciences of the U.S. Department of Energy (DE-AC03-76SF0098), and by the National Institutes of Health (Award No. R29 GM43037-02). M.B. thanks the American Cancer Society for a Junior Faculty Award and Eli Lilly for a Young Investigator Grant. W.S. thanks the NIH for a predoctoral training grant.

Note Added in Proof. During the preparation of this manuscript, results similar to those reported here were disclosed by Whitesides and co-workers.[13]

(14) Tobita, K.; Sugiura, A.; Enomoto, C.; Furuyama, M. *Med. Microbiol. Immunol.* **1975**, *162*, 9–14.

Supplementary Material Available: Listings of experimental data for the synthesis of compounds **3** and **4**, procedure for liposome formation, procedure for HAI assay, and procedure for plaque reduction assay (6 pages). Ordering information is given on any current masthead page.

Polycavernoside A: A Novel Glycosidic Macrolide from the Red Alga *Polycavernosa tsudai* (*Gracilaria edulis*)

Mari Yotsu-Yamashita,[†] Robert L. Haddock,[‡] and Takeshi Yasumoto*,[†]

Faculty of Agriculture, Tohoku University
1-1 Tsutsumidori-Amamiyamachi
Aoba-ku, Sendai 981, Japan
Health and Social Services, Government of Guam
P.O. Box 2816 Agana, Guam 96910

Received October 20, 1992

Human intoxication resulting from ingestion of the red alga *Polycavernosa tsudai* (formerly *Gracilaria edulis*) occurred in Guam in late April, 1991.[1] Thirteen people became ill, three of whom died. As the alga had been eaten widely with no previous record of potential risk, identification of the toxin was imperative. In this communication, we report the isolation of two toxins, polycavernoside A (**1**) and B (**2**), and the planar structure of **1**, which is a novel macrolide disaccharide.

P. tsudai (2.6 kg) was collected on June 4, 1991, at Tanguisson Beach, Guam, where the causative alga had previously been collected. Toxins were extracted from the alga with acetone, freed of polar contaminants by partition between water and CH_2Cl_2, and purified by column chromatography,[2] guided by mouse bioassays. Both **1** (400 μg, recovery 14%) and **2** (200 μg, recovery 7%) were obtained as colorless solids: LD_{99} in mice (ip) was 200–400 μg/kg for both. ^{1}H–^{1}H COSY spectra of **1** and **2** suggested their structural similarity, but further analysis of **2** was hampered by sample size. [**1**: UV_{max} (MeCN) 259 (ϵ 25 000), 270 (32 000), 280 (26 000) nm; IR (film) 1630, 1730, 1738 cm^{-1}; HR-FABMS $[M + Na]^+$ m/z 847.4483 (calcd for $[C_{43}H_{68}O_{15}Na]^+$ m/z 847.4455).] Partial structures H2–H8, H11–H13, H15–H23,28, H1′–H5′, and H1″–H6″ were deduced from detailed analyses of ^{1}H–^{1}H COSY and 2D HOHAHA spectra.[3] The conjugated triene (H16–H21) was also supported by the UV maxima; the $^3J_{HH}$ value (15 Hz) determined by the 2D *J* spectrum pointed to *E,E,E* geometry.

The ^{13}C NMR spectrum (CD_3CN) confirmed the presence of a ketone (δ 207.4) and an ester (δ 172.1) suggested by the IR bands. A ^{13}C decoupled HMQC spectrum led to assignments of all ^{1}H and ^{13}C signals except those of two quaternary carbons, C10 (δ 103.9) and C14 (δ 40.5). HMBC spectra[3] clarified the connectivities around quaternary and carbonyl carbons by giving cross peaks due to $^{2,3}J_{CH}$ between C1/H2a, C1/H15, C9/H8a, C10/H12a, C10/H12b, C10/Me25, C13/Me26, C13/Me27, C14/H15, C14/Me26, C14/Me27, C15/Me26, C15/Me27, C26/Me27, and C27/Me26. The structural features around C14 were supported by NOEs (NOESY 270 MHz, ROESY 400

[†] Tohoku University.
[‡] Government of Guam.

(1) Haddock, R. L.; Cruz, O. L. T. *Lancet* **1991**, *338*, 195–196.

(2) Chromatography was done successively on silica gel with CH_2Cl_2/MeOH [1:0, 99:1(**2**) and 9:1(**1**)], ODS-Q and Develosil ODS-7 with CH_3CN/H_2O (85:15), and Cosmosil 5C18-AR with CH_3CN/H_2O (4:1).

(3) ^{1}H–^{1}H COSY spectra were recorded on a JEOL GSX-400 (400 MHz) spectrometer in $CDCl_3$, CD_3CN, or C_5D_5N; 2DHOHAHA and HMQC in CD_3CN; and HMBC in CD_3CN or CD_3CN/D_2O (9:1). In the HMBC experiment (J_{CH} 6.0 Hz), the column width for ^{13}C NMR was reduced to about one-fifth of the conventional width to enhance the digital resolution. The column widths and spectral centers were adjusted so as to avoid overlapping of ^{13}C signals when they were folded back.

 © 1993 American Chemical Society