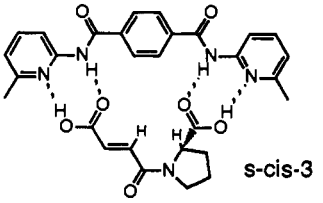
in a gauche conformation, would allow *s-trans*-**1b** to form two, albeit less favorable, bidentate hydrogen bonds to **2**.

To test this we have prepared the more rigid fumaramide **3**, which has an unaffected s-cis:s-trans ratio of 1:4.[15a] However, in a 1:1 mixture with **2** in $CDCl_3$, **3** shows a strong (4:1) preference for the s-cis rotamer, as expected for the formation of a complex of type **4**.[16] The shift in equilibrium is clearly seen in the proline αH region of the ^1H NMR spectra of **3** and **2**-**3** (Figure 4, parts a and b). The rotamer assignments were made by chemical shift comparisons[2c,14] and by the observation (from the s-cis form) of an intramolecular NOE between the resonance at 4.62 ppm (Figure 4b) and that of the fumaramide αCH. In the *s-cis*-**3** complex the fumaramide αCH also shows an intermolecular NOE with the terephthaloyl protons and is shifted 0.33 ppm further downfield than in the *s-trans*-**3** complex, reflecting its position close to the periphery of the terephthaloyl spacer (as in **4**). *s-cis*-**3** is able to form two bidentate contacts with **2** while *s-trans*-**3** presumably forms a single contact between the more acidic amino acid carboxylate and one aminopyridine in **2**.

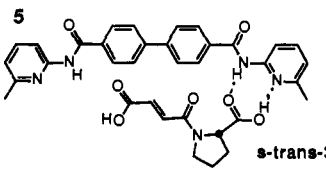
4



s-cis-3

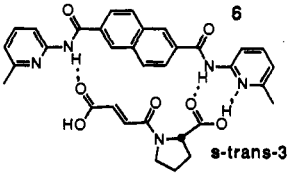
The conformational selectivity can be varied by changing the position of the aminopyridine binding groups. Addition of biphenyl receptor **5** (NH-NH, 11.7 Å) to **3** leads to a much smaller downfield shift in the amide NH resonances (1.7 ppm), no shift in the fumaramide αCH, and a reversion of the s-cis:s-trans ratio back to 1:4. These results suggest that **5** is too long to form any

5



s-trans-3

6



s-trans-3

more than a single bidentate contact with either *s-cis*- or *s-trans*-**3** and so has little effect on either the ratio or the binding-induced NMR shifts. In contrast, naphthyl receptor 6 (NH-NH, 9.39 Å) has a positioning of binding groups intermediate between **2** and **5**. Molecular modeling[11] has suggested that, in addition to the single bidentate contact of **5**, **6** can form a third hydrogen bond to the s-trans fumaramide carboxylate. A 1:1 mixture of **6** and **3** shows a substantial shift in the equilibrium toward the trans rotamer (s-cis:s-trans, 1:8), as seen in the ^1H NMR proline αH region (Figure 4c). Further support for this complex comes from the 0.24 ppm downfield shift of the fumaramide βCH and the observation of an intermolecular NOE between it and the naphthyl protons.

The 32-fold difference in rotamer equilibrium between **2**-**3** and **6**-**3** is less than might be expected for the change in hydrogen-bonding environment, suggesting that these simple hosts are not yet optimized for their substrates. Design improvements as well as modifications to recognize the twisted amide transition state are currently underway.

Acknowledgment. We thank the National Institutes of Health (GM 35208) and the Office of Naval Research for financial support of this work. We are also grateful to the Spanish Ministerio de Educacion y Ciencia for postdoctoral fellowships to C.V. and F.G.-T., the Fulbright Commission for a fellowship to F.G.-T., and Prof. Russell Petter for many valuable discussions.

(15) (a) Measured in $CDCl_3$-CD_3OD (8:2) due to the insolubility of the diacid in $CDCl_3$. (b) Measured in $CDCl_3$.

(16) The K_a for **2**-**3** was too high to be measured accurately by ^1H NMR and was estimated at 10^4-10^5 M^{-1}.

Specific Enzyme-Induced Decapsulation

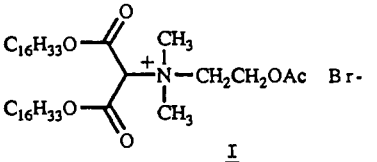
F. M. Menger* and D. E. Johnston, Jr.

Department of Chemistry, Emory University
Atlanta, Georgia 30322

Received April 8, 1991

Little difficulty exists in finding chemotherapeutic agents that kill pathological cells; the problem lies in identifying agents that do so without damaging normal cells as well.[1] One possible route to selectivity plays on the fact that certain pathological cells produce excessive amounts of a particular enzyme (e.g., bone cancer/alkaline phosphatase;[2] neuroblastoma/acetylcholinesterase[3]). If such an enzyme is capable of breaking open specially designed vesicles that encapsulate a cytotoxic drug, then selective therapeutic activity toward the enzyme-exuding cell can be achieved.[4] Since this mechanism of selectivity depends on the encapsulating vesicle, and not on the drug within, the choice of drug becomes less restrictive.

We describe herein compound I which, in concert with acetylcholinesterase (AcE),[5] models the above process.[6,7] Since I



I

has an ionic headgroup plus two long hydrocarbon tails, it possesses the two features necessary for vesicle formation.[8] And since I also incorporates an acetylcholine-like moiety, it reacts with AcE to form a primary alcohol (eq 1, Scheme I). The hydroxyl can then engage in an intramolecular attack upon the carbonyl (eq 2, Scheme I) to eject one of the two tails. But single-chain amphiphiles do not form bilayers and, in fact, they are often used to destroy vesicles.[9,10] Consequently, the vesicular system will experience an enzyme-induced "lesion" and ultimate destruction. Vesicular contents would, naturally, escape.

Compound I was synthesized by monobromination of dihexadecyl malonate (Br_2, refluxing CCl_4, 87%)[11] followed by reaction with 2-(dimethylamino)ethyl acetate[12] (5-fold excess of amine, no solvent, 45 °C, 48%). The product was purified by three or four crystallizations and characterized by ^1H and ^{13}C NMR, IR, elemental analysis, and FAB-MS.

Vesicles of I were formed by bath sonication of a 5-mg film of I with 2.0 mL of buffer at 25 °C for 5 min and then at a temperature 5-10 deg above the phase-transition temperature of 46 °C (a value determined by differential scanning calorimetry). Quasi-elastic light scattering[13] on the clear solutions revealed that

(1) Sneader, W. *Drug Discovery: The Evolution of Modern Medicines*; Wiley: New York, 1985; Chapter 16.

(2) Bodansky, O. *Biochemistry of Human Cancer*; Academic Press: New York, 1975; p 80.

(3) Kimhi, Y.; Mahler, A.; Saya, D. *J. Neurochem.* **1980**, *34*, 554. Stieger, S.; Buetikofer, P.; Wiesmann, U. N.; Brodbeck, U. *J. Neurochem.* **1989**, *52*, 1188.

(4) Phospholipid vesicles are degraded in nature by phospholipases. The goal here is, in effect, to mimic this process with synthetic lipids and with enzymes normally intended for other purposes.

(5) Quinn, D. M. *Chem. Rev.* **1987**, *87*, 955.

(6) This work is abstracted from the Ph.D. Thesis of D. E. Johnston, Jr. (Emory University, 1990), with a title identical with that of this article. Full experimental details are contained therein.

(7) The CA index name for I is 2-propanaminium, *N*-[2-(acetyloxy)-ethyl]-1,3-bis(hexadecyloxy)-*N*,*N*-dimethyl-1,3-dioxo-, bromide.

(8) Kunitake, T.; Okahata, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3860.

(9) Zaslavskii, B. Y.; Borovskaya, A. A.; Lavrinenko, A. K.; Lisichkin, A. Y.; Davidovich, Y. A.; Rogozhin, S. V. *Chem. Phys. Lipids* **1980**, *26*, 49.

(10) Sunamoto, J.; Kondo, H.; Yoshimatsu, A. *Biochem. Biophys. Acta* **1978**, *510*, 52.

(11) Palmer, C. S.; McWherter, P. W. *Organic Syntheses*; Gilman, H., Ed.; Wiley: New York, 1941; Collect. Vol. I, p 245.

(12) Purchased from Kodak.

(13) Menger, F. M.; Lee, J.-J.; Aikens, P.; Davis, S. *J. Colloid Interface Sci.* **1989**, *129*, 185.

0002-7863/91/1513-5467$02.50/0 © 1991 American Chemical Society

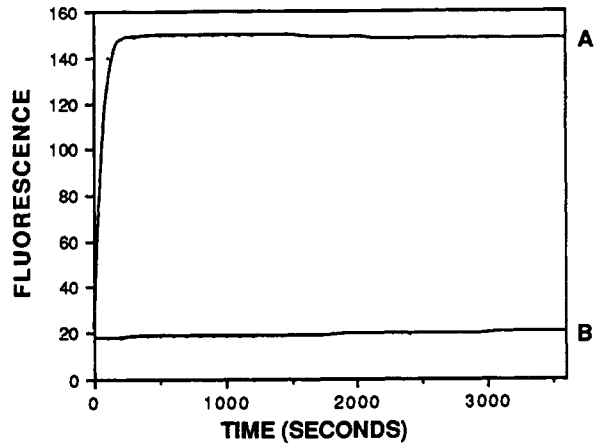
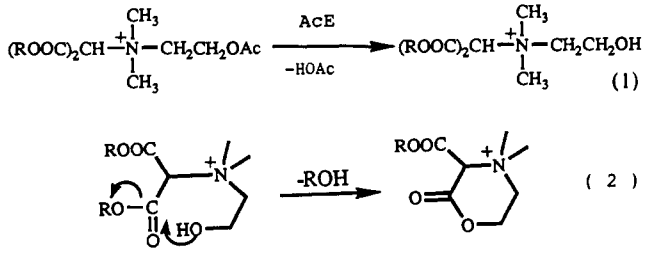


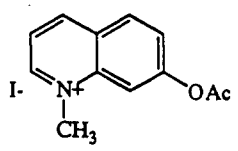
Figure 1. (A) Fluorescence vs time for addition of acetylcholinesterase (1.1×10^{-8} M) to vesicles of I (6.5×10^{-5} M) encapsulating II (2.2×10^{-7} M) at pH = 6.8 and 30.0 °C. Excitation and emission wavelengths are 400 and 500 nm, respectively. The enzyme frees II and hydrolyzes it to a fluorescent product. (B) Base line obtained when no enzyme, or denatured enzyme, was added under equivalent conditions.

Scheme I



92-100% of the vesicles had diameters of 54-63 nm. The size distribution remained constant over at least 6 days, and the preparations kept their clarity for weeks.

How was the enzyme-induced disruption of the vesicles measured? Compound II (which is rapidly hydrolyzed by AcE to a fluorescent 7-hydroxyquinolinium salt)[14] was cosonicated with I to give encapsulated II mixed with free II. Gel filtration



II

(Sephadex G-75-120) removed the latter. Final conditions were 6.5×10^{-5} M I, 2.2×10^{-7} M II, pH = 6.8 phosphate buffer, 0.05 M NaCl, and 30.0 °C. When AcE (1.1×10^{-8} M) was added to the system,[15] an immediate burst of fluorescence ensued (Figure 1). The burst represented quantitative hydrolysis of II and was independent of how long the system aged prior to addition of AcE. Thus, II cannot escape vesicles of I in the absence of AcE (a fact no doubt related in part to the cationic nature of I and II). AcE itself must serve two functions: (a) It attacks the vesicles, allowing II to leak out. (b) It hydrolyzes II (following its release from captivity) to produce the observed fluorescence.

The diethyl ester analogue of I exists as a monomer in water, where it serves as an excellent AcE substrate. Its k_{cat} is only 16-fold less than that of acetylcholine (whose reported rate with AcE is $>10^8$ M^{-1} s^{-1} for an acceleration of 2×10^{17}).[16] NMR studies (8.3 mM analogue, 2.0×10^{-7} M AcE, pD = 6.67) showed a clear preference for cleavage of the acetyl group over the ethyl esters. Therefore, AcE-catalyzed hydrolysis of vesicular I should also be rapid barring any steric problems at the bilayer surface. Our observation that AcE is indeed able to reach and react with vesicular acetyl groups may stem from one or more of the following: (a) AcE is a nonspecific "sloppy" enzyme.[5,17] (b) The acetyl groups are situated at the periphery of the bilayer walls. (c) Individual molecules of I might make themselves accessible to AcE by protruding (transiently or otherwise) from the bilayer surface. Loss of a tail from such a molecule should destabilize the immediate environs[9,10] and, hence, facilitate further reaction at that site.

Control studies with $(EtOOC)_2CHN(CH_3)_3^+$ showed that the compound does not ionize below pH = 8, so that enolization is not a complication at pH = 6.7, where we operated. Neither is spontaneous ester hydrolysis since the control hydrolyzed at pD = 6.7 with a half-life of ca. 2 weeks. Equation 2 of Scheme I was verified by TLC identification of hexadecanol when AcE was added to 2.4×10^{-4} M vesicular I. Hexadecanol could, in fact, be observed visually as the solutions became increasingly cloudy. Finally, no burst of fluorescence was evident when the vesicle system was exposed to heat-denatured AcE or to a different enzyme, acid phosphatase. Thus, as hoped, the vesicles of I are endowed with an enzyme-specific destructability (although it is presently unknown exactly how much damage AcE must do to a vesicle before the contents are no longer retained).[18]

If the science of chemotherapy is to be improved, it must become tuned to a specific knowledge of tumor biochemistry. The present communication describes a small step in this direction.[19]

Acknowledgment. The work described herein was supported by the National Institutes of Health.

Supplementary Material Available: Experimental details on the synthesis of I, the preparation of vesicles, and kinetic procedures (20 pages). Ordering information is given on any current masthead page.

(14) Rosenberry, T. L.; Bernhard, S. A. *Biochemistry* **1972**, *11*, 4308.
(15) AcE was titrated with *N*-methyl-7-(dimethylcarbamoxy)quinolinium iodide according to the following: Rosenberry, T. L.; Bernhard, S. A. *Biochemistry* **1971**, *10*, 4114.
(16) Kovach, I. M.; Huber, J. H.-A.; Schowen, R. L. *J. Am. Chem. Soc.* **1988**, *110*, 590.
(17) Hasan, F. B.; Cohen, S. G.; Cohen, J. B. *J. Biol. Chem.* **1980**, *255*, 3898.
(18) Distearoylphosphatidylcholine vesicles containing a small percent of I gave no reaction with AcE.
(19) Another approach, examples of which are already known, is to covalently link a cleavable substrate to a drug. See: Bundgaard, H. *Adv. Drug Delivery Rev.* **1989**, *3*, 39.

Chiroptical Molecular Switch

Ben L. Feringa,* Wolter F. Jager, and Ben de Lange

Department of Organic Chemistry
University of Groningen
Nijenborgh 16. 9747 AG Groningen, The Netherlands

Egbert W. Meijer†

Philips Natuurkundig Laboratorium
Post Office Box 80.000
5600 JA Eindhoven, The Netherlands

Received January 30, 1991
Revised Manuscript Received May 8, 1991

Photochemically switchable bistable molecules have recently attracted much attention due to possible applications in reversible optical data storage and optical computing.[1,2] To be suitable for optical memory devices, such molecules should meet the following requirements: (a) thermal stability of both isomers, (b) a repeatable switching cycle without loss of activity, and (c) ready

† Present address: DSM Research, P.O. Box 18, 6160 MD Geleen, The Netherlands.

(1) Emmelius, M.; Pawlowski, G.; Vollmann, H. W. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1445.
(2) Engler, E. M. *Adv. Mater.* **1990**, *2*, 166.

0002-7863/91/1513-5468$02.50/0 © 1991 American Chemical Society