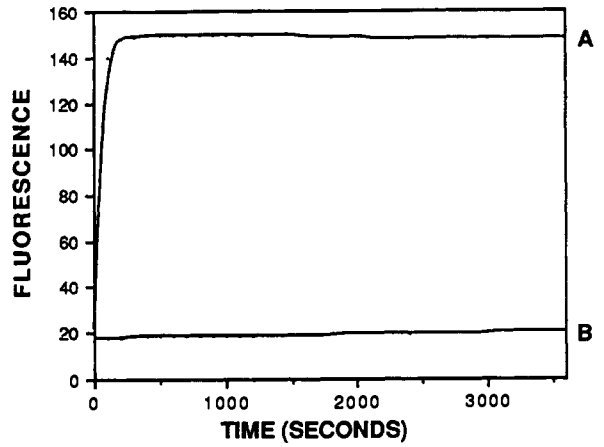
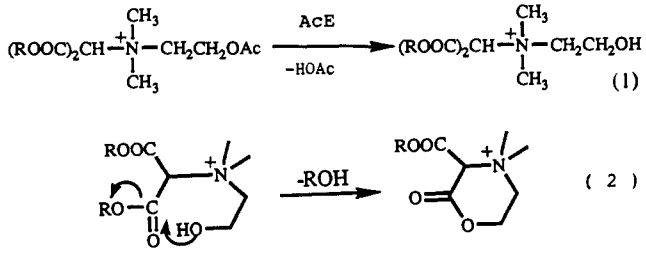


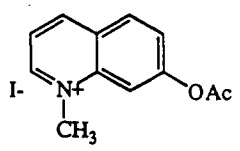
Figure 1. (A) Fluorescence vs time for addition of acetylcholinesterase (1.1×10^{-8} M) to vesicles of I (6.5×10^{-5} M) encapsulating II (2.2×10^{-7} M) at pH = 6.8 and 30.0 °C. Excitation and emission wavelengths are 400 and 500 nm, respectively. The enzyme frees II and hydrolyzes it to a fluorescent 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.

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Chiroptical Molecular Switch

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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

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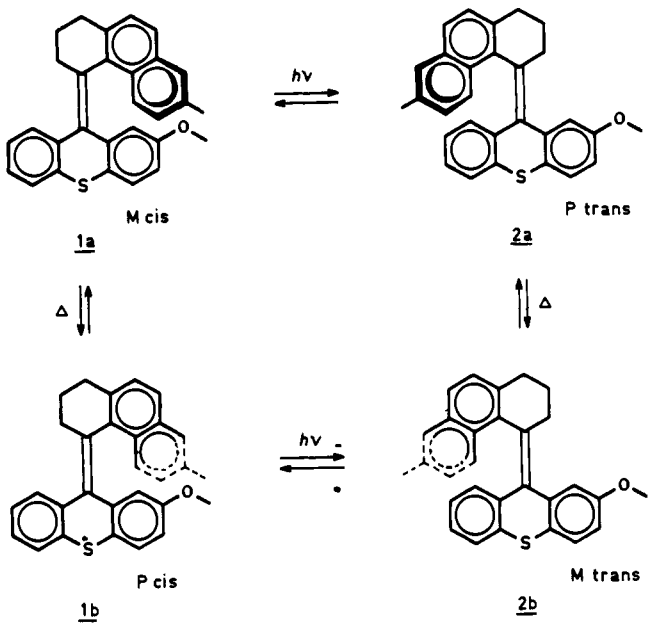
Scheme I



detectability of both forms. Published experimental work has so far focused on cis–trans isomerization of (aza)stilbenes[3-5] and reversible photocyclization reactions,[6-8] and detection of both bistable states has been limited mainly to observation of differences in UV/vis spectra.

We report a unique photoswitchable molecular system based on "pseudoenantiomeric" forms,[9] P and M′, of a chiral helical compound (eq 1). Detection of the photochemically induced

$$\mathrm{P}\ (+) \underset{\lambda_2}{\overset{h\nu,\ \lambda_1}{\rightleftharpoons}} \mathrm{M}'\ (-) \qquad (1)$$

interconversion of P and M′ helices is based on circular dichroism (CD). The enantiomers of either *cis*- or *trans*-4-[9′-(2′-methoxythioxanthylidene)]-7-methyl-1,2,3,4-tetrahydrophenanthrene (**1** and **2**) form the bistable system (Scheme I). Both chiral compounds are sterically overcrowded olefins.[10] This class of compounds was first reported by Feringa and Wynberg.[11]

The synthesis of **1** and **2** is based on the use of the Barton–Kellogg method[12,13] for the formation of the sterically hindered central alkene, starting from 2-methoxy-9*H*-thioxanthene-9-thione[14] and 2,3-dihydro-7-methyl-4(1*H*)-phenanthrenone hydrazone.[15,16] Pure cis-**1** (35% yield, mp 179.0–179.4 °C) was obtained by two crystallizations from ethanol. The *cis*-**1** and *trans*-**2** isomers of these inherently dissymmetric olefins are readily distinguished by their ^{1}H NMR spectra.[16] The MeO singlet at 3.9 ppm in **2** is shifted upfield to 3.0 ppm in cis-**1** due to the shielding effect of the naphthalene moiety. The mixture of **1** and **2** was separated into the four stereoisomers M-cis (**1a**), P-cis (**1b**), P-trans (**2a**), and M-trans (**2b**) by HPLC (Scheme I).[17,18] The CD spectra of **1a** and **2a** are roughly mirror images of each other in accord with the P and M helicities.[18]

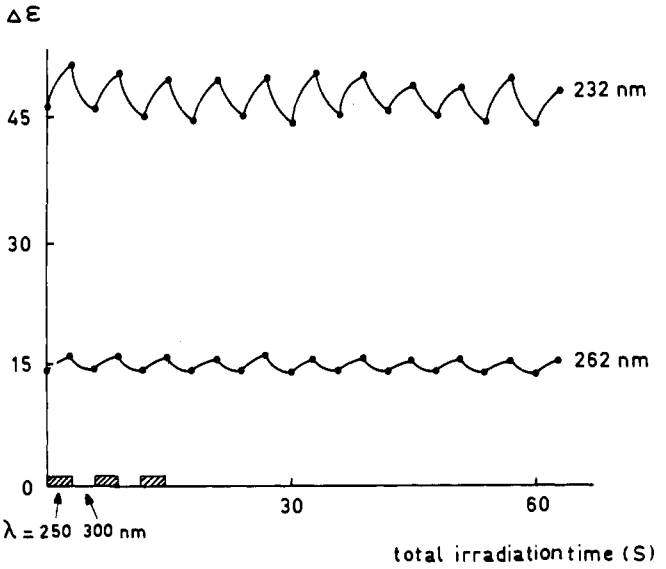


Figure 1. Plot of $\Delta\epsilon$ vs irradiation time at λ = 232 nm (upper curve) and λ = 262 nm (lower curve) for **1a**/**2a** (3.0×10^{-5} mol·L^{-1} in *n*-hexane/2-propanol, 9:1) irradiated alternately at λ = 250 nm and λ = 300 nm; switching time 3 s.

Unlike other overcrowded ethylenes,[10] the enantiomers of **1** and **2** are stable at room temperature. The thermal racemization of M-cis (**1a**) into P-cis (**1b**), as determined by polarimetry in the temperature range 10–90 °C in *p*-xylene, showed first-order kinetics with a racemization barrier of 26.4 kcal·mol^{-1}. This value is much higher than the barriers of 12, 18, and 22 kcal·mol^{-1} found for bisfluorenylidenes,[19] dixanthylidenes,[20] and biacridanes,[21] respectively. Furthermore, no thermal cis–trans isomerization (**1a** ⇄ **2a**) occurred under ambient conditions as determined by ^{1}H NMR and HPLC analyses.

This exceptional behavior might be due to the presence of the tetrahydrophenanthrene unit, which is bulky enough to prevent fast racemization but which has sufficient conformational flexibility to prevent excessive distortion of the central olefinic bond. On irradiation, however, using 300- or 250-nm UV light,[16] cis–trans isomerization of **1** and **2** does occur readily (Scheme I). Irradiation of pure M-cis **1a** (or P-trans **2a**) at 300 nm yielded a mixture of 64% M-cis **1a** and 36% P-trans **2a** as determined by HPLC. No P-cis **1b** or M-trans **2b** could be detected in these experiments. The CD spectra also confirmed the exclusive formation of P-trans out of the M-cis enantiomer and vice versa. Irradiation at 250 nm gave a photostationary state containing 68% M-cis and 32% P-trans.

Using the fact that irradiation at 250 and 300 nm yields different M-cis/P-trans photostationary states, we demonstrated the feasibility of an optical molecular switch based on the intrinsic chiral properties of the system, i.e., a *chiroptical molecular switch*. When either enantiomerically pure M-cis **1a** or P-trans **2a**[16] and

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300-nm light are employed, the same photostationary state is reached. This results in a decrease of the CD absorptions at 232 and 262 nm to 33% of the original values for M-cis **1a**. Alternating irradiation at two wavelengths, λ_1 = 250 nm and λ_2 = 300 nm, results in a modulated CD signal at 232 and 262 nm. A typical example using a switching time of 3 s is shown in Figure 1. Similar cycles were found using switching times between 0.5 and 60 s. The cycle between the two photostationary states could be repeated at least 10 times without racemization or changes in UV and CD spectra.

Several features should be emphasized that were essential for the successful demonstration of this new principle of a chiroptical molecular switch: the remarkable thermal stability, the photoisomerization between two "pseudoenantiomeric"[9] helical systems without racemization, the stability toward photodegradation, and the large chiroptical effects that arise from the inherently dissymmetric structure.

Approaches to optimize the structure–property relations are currently under investigation.

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Supplementary Material Available: Synthetic scheme and spectroscopic and analytical data for **1**, **2**, and precursors and details of HPLC separations (5 pages). Ordering information is given on any current masthead page.

Lithium Cyanocuprates, RCu(CN)Li: First Observation of Two-Bond ^{13}C–^{13}C NMR Couplings in Organocuprates[1]

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NMR coupling constants have proven to be a rich source of structural information on organic and organometallic compounds.[2-5] For example, ^6Li–^{13}C J-coupling has allowed the structures of many organolithium compounds to be elucidated.[4,5] Unfortunately, ^6Li–^{13}C and ^7Li–^{13}C couplings are generally not observed for organocuprates,[6] making the determination of their solution structures a difficult problem. Consequently, investigations were undertaken to find other coupling constants that would provide structural information on organocuprates. It can now be reported that under appropriate conditions two-bond ^{13}C–^{13}C couplings ^2J can be observed for some types of organocuprates.

When 1 equiv of $CH_3CH_2{}^6Li$ is added to $Cu^{13}CN$ in tetrahydrofuran-d_8 (THF-d_8), the ^1H-decoupled ^{13}C NMR spectrum obtained at −78 °C shows singlets at δ 149.1 and 15.54 ppm for the ^{13}CN and ethyl C2 resonances and a doublet (J = 22 Hz) for the ethyl C1 resonance at 1.64 ppm (Table I). In contrast, the use of natural abundance CuCN results in a singlet for C1 at 1.68 ppm. These results establish that both the Et and the CN are bonded to the same Cu, which is consistent with the designation of this reagent as $[EtCuCN]^-Li^+$ or, more commonly, EtCu(CN)Li.

For $EtCu(^{13}CN)Li$ in ether-d_{10} at −78 °C, C1 is a singlet at δ 1.85 ppm (Table I). Upon cooling to −100 °C, C1 is a doublet (J = 21 Hz). Alternatively, if hexamethylphosphoramide (HMPA, ~10 % by volume) is added to the ether solution at −78 °C, C1 is a doublet at 2.04 ppm (J = 22 Hz). Apparently, some exchange mechanism removes the coupling at −78 °C in ether. The effect of HMPA suggests that Li^+ is involved in the exchange process, since its complexation by HMPA slows the exchange sufficiently to allow the coupling to be observed.

Upon the addition of a second equivalent of Et^6Li to the THF-d_8 solution of $EtCu(^{13}CN)^6Li$, and ^{13}C NMR spectrum at −100 °C contains three singlets at 158.7, 17.35, and 4.76 ppm for the CN, C2 and C1, respectively. Even in the presence of HMPA (~10 vol %, −78 °C) or HMPA and 12-crown-4 (12-C-4, 2 equiv, −78 °C and −100 °C), the CN–C1 coupling is absent in the 2:1 reagent, which we represent as $Et_2CuLi{\cdot}LiCN$.

The addition of $^{13}CH_3{}^6Li$ to $Cu^{13}CN$ in THF-d_8 yields $^{13}CH_3Cu(^{13}CN)Li$, which must be cooled to −110 °C in order for a ^1H-decoupled ^{13}C NMR spectrum comprising two doublets (J = 21 Hz) at 149.0 ppm (CN) and −12.46 ppm (Me) to be observed. At −78 °C and −100 °C the spectrum is a pair of singlets; however, upon the addition of HMPA (~10 vol %) to the solution, coupling (J = 22 Hz) is observed at −78 °C as well as at −100 °C.

As in the ethyl case, exchange in $CH_3Cu(^{13}CN)Li$ is more facile in ether-d_{10} than in THF-d_8: the methyl singlet at −78 °C broadens at −100 °C and splits into a partially resolved doublet (J = 12 Hz) at −110 °C and finally into a doublet (J = 22 Hz) at −120 °C. The addition of HMPA (~10 vol %) allows the doublet (J = 22 Hz) to be observed at −78 °C.[7] Apparently, exchange is more facile in MeCu(CN)Li than in EtCu(CN)Li in both THF-d_8 and ether-d_{10}.

At −100 °C in THF-d_8, $(^{13}CH_3)_2CuLi{\cdot}Li^{13}CN$ has a spectrum that consists of two singlets (158.8 ppm, CN; −9.20 ppm, CH_3) that are not split into doublets by the addition of HMPA (~10 vol %, −78 °C and −100 °C) or HMPA and 12-crown-4 (2 equiv, −78 °C), as is also true for $Et_2CuLi{\cdot}Li^{13}CN$ (above).

In the case of $PhCu(^{13}CN)Li$ in THF-d_8, C1 is a singlet (δ 166.0 ppm) at −78 °C, a broad doublet (166.0 ppm, J = 13 Hz) at −100 °C, and a sharp doublet (δ 165.9 ppm, J = 23 Hz) at −110 °C. In contrast to the alkyl cases, exchange in $PhCu(^{13}CN)Li$ is slower in ether-d_{10} than in THF-d_8 and the full coupling is observed at −100 °C. The addition of HMPA at −78 °C results in a doublet (J = 24 Hz) in both THF-d_8 and ether-d_{10}.

In analogy with both $(^{13}CH_3)_2CuLi{\cdot}Li^{13}CN$ and $Et_2CuLi{\cdot}Li^{13}CN$, $Ph_2CuLi{\cdot}Li^{13}CN$ has a singlet for C1 at −100 °C in THF-d_8 or ether-d_{10}. Addition of HMPA (~10 vol %, −78 °C) or HMPA and 12-crown-4 (2 equiv, −78 °C) did not cause coupling to be observed.

As may be seen in Table I, the addition of 12-crown-4 to the solutions of $RCu(^{13}CN)Li$ in THF-d_8 or ether-d_{10} containing HMPA does not significantly change the coupling constants.[8] The addition of HMPA to RCu(CN)Li causes an upfield shift of the CN resonance due to increased back-bonding into CN π* orbitals, which decreases the multiple-bond character.

This study establishes that the reagents prepared from 1 equiv of RLi and CuCN are indeed cyanocuprates RCu(CN)Li in which R and CN are both bonded to Cu. This is important because in the case of the so-called "higher order" cyanocuprates, which have been represented as $R_2Cu(CN)Li_2$, evidence has been presented that the CN is not bonded to Cu;[9] consequently, we represent them

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(8) The 12-crown-4 salts are not soluble without the HMPA present as a cosolvent.

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