these peaks represent the ether and sulfone oxygens, respectively. The free methanesulfonic acid peak appears at 176.2 ppm. The ethanolysis was conducted as before; once again, the signal of the acid liberated was shifted away from the region of interest by metering in praseodymium nitrate as a shift reagent. The kinetics, followed to two half-lives, behaved in excellent first-order fashion; $k_1 = 6.18 \times 10^{-6}$ s^{-1}, with a correlation coefficient of 0.999. Throughout this period, only an ether ^{17}O signal was seen; even at the conclusion of the experiment, no sulfonyl ^{17}O was observed even though this signal is naturally about 8 times sharper than the ether signal (a factor of 2 due to statistics, and another of 4 because of line width). We estimate that even 0.5% scrambling would have been observed (see Figure 1). This is in startling constrast to the *exo*-brosylate; unsolvolyzed material remaining after two half-lives in that case is completely scrambled.[6] It is clear that the reluctance of norbornyl ions to capture nucleophiles on the endo side continues undiminished even if the species to be bound is the nearest neighbor anion that had just departed. As Brown has often pointed out,[1] the leaving anion must be poorly solvated as it begins its journey into the U-shaped cationic cavity. By one possible extension of this reasoning, ionization could lead to a highly crowded endo pair stage from which return might be efficient; clearly, such an extrapolation is not justified.

The facts observed here must be seen in the light of recently reached agreement that the *endo*-sulfonates solvolyze without significant solvent assistance.[7a,b] They lead to several interesting conclusions. The first of these is that the correct value of the exo/endo solvolysis rate ratio is indeed of the order of 10^3. The only possible doubt rests on the notion that return might occur without oxygen scrambling; this notion is, however, without laboratory precedent as noted above. Second, the *exo*- and *endo*-2-norbornyl substrates clearly have different rate-controlling steps in solvolysis: formation of an ion pair in endo and dissociation of the pair in exo. The data permit the statement that in the endo energy profile, the maximum representing the ionization exceeds any other by at least 3 kcal/mol. The third conclusion is that the argument for charge delocalization in the transition state for exo solvolysis that was based on its unusually large volume[13] is now much stronger than before, since it was subject to the caveat that an "earlier" location along the reaction coordinate might be responsible. Now that the reverse of this possibility has been demonstrated, there is no longer a viable alternative to the interpretation that it is due to charge dispersal.

Registry No. ^{17}O, 13968-48-4; ^{17}O-norbornanone, 88393-12-8; norbornanone ethylene glycol ketal, 172-67-8; ^{17}O-*endo*-norbornanol, 88393-13-9; ^{17}O-*endo*-norbornanol brosylate, 88393-14-0; *endo*-norbornanol mesylate, 28627-78-3; ^{18}O-*endo*-norbornanol mesylate, 88393-15-1.

(8) The NMR spectra were measured with a 300-MHz Nicolet spectrometer operating at maximum sensitivity. For natural-abundance spectra, concentrated solutions (20%) in ethanol were used, with about a half million transients (500-μs delay delay time). For the enriched samples, 1-2% solutions were used with about 10^5 transients; the signal to noise ratio was in all cases about 80. Best simulation results were obtained with line widths of 71, 92, and 237 Hz for the sulfone, acid, and ether oxygens, respectively. ^{17}O-labeled norbornanone[9] was obtained in several 100-mg batches by the hydrolysis at 60 °C for 24 h of 200 μL of ethylene glycol ketal[10] with 25 μL of water-^{17}O (20 atom %) and 0.5 μL of concentrated HCl in sufficient dioxane to yield a homogenous solution; it was purified from traces of unreacted ketal by means of GC (Carbowax, 110 °C). Reduction to the *endo*-alcohol[11] and conversion to the brosylate[2] and mesylate[12] followed known procedures.

(9) Meinwald, J.; Crandall, J.; Hymans, W. E. *Org. Synth.* **1965**, *45*, 77.

(10) Renoll, M.; Newman, M. S. "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III, p 502.

(11) Cannon, W. F.; House, H. O. "Organic Syntheses"; Wiley: New York, 1973; Collect. Vol. V, p 294.

(12) Crossland, R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195.

(13) le Noble, W. J.; Yates, B. L. *J. Am. Chem. Soc.* **1965**, *87*, 3515. le Noble, W. J.; Yates, B. L.; Scaplehorn, A. W. *Ibid.* **1967**, *89*, 3751. le Noble, W. J.; Gabrielsen, B. *Tetrahedron Lett.* **1970**, 45; **1971**, 3417. le Noble, W. J.; Bitterman, S.; Staub, P.; Meyer, F. K.; Merbach, A. E. *J. Org. Chem.* **1979**, *44*, 3263. le Noble, W. J.; Schulman, E. M.; Merbach, A. E. *Ibid.* **1981**, *46*, 3352. Jenner, G.; Srivastava, S.; le Noble, W. J. *Tetrahedron Lett.* **1983**, *24*, 2429. Related contributions from other laboratories are referred to elsewhere: Asano, T.; le Noble, W. J. *Chem. Rev.* **1978**, *78*, 407.

Structure Determination of a Tetrasaccharide: Transient Nuclear Overhauser Effects in the Rotating Frame

Aksel A. Bothner-By,* Richard L. Stephens, and Ju-mee Lee

Department of Chemistry, Carnegie-Mellon University Pittsburgh, Pennsylvania 15213

Christopher D. Warren and R. W. Jeanloz

Laboratory for Carbohydrate Research Departments of Biological Chemistry and Medicine Harvard Medical School, Massachusetts General Hospital Boston, Massachusetts 02114

Received October 3, 1983

Homonuclear Overhauser effects (NOE) have been widely used in structure determination.[1] They have proven especially useful for the study of small molecules in which magnetic dipolar relaxation of the nuclei is in the extreme narrowing limit.[2] In such cases the maximum positive effect[3] that can be observed is 50%.

As spectrometer frequencies have risen and as more attention has centered on larger and more complex molecules, the situation has arisen more frequently where $\omega_0\tau_c$, the product of spectrometer angular frequency and molecular rotational correlation time, is equal to or exceeds unity. When $\omega_0\tau_c \approx 1$ no NOE occurs; when it greatly exceeds 1, as in the case of macromolecules, the NOE approaches −1 and specificity is lost due to spin diffusion.[4] Observation of the NOE at short times,[5] or of transient NOEs,[6] overcomes this difficulty in part, but at the price of observing very small effects.

We have found that the observation of transient NOEs in the rotating frame overcomes these difficulties.

We first consider a one-dimensional difference experiment. A reference spectrum, R, is generated by (1) a 90° x pulse, (2) immediate application of a spin-locking field along the y axis during a relaxation period t_{max}, (3) removal of the spin-locking field and acquisition of the free induction decay, and (4) fourier transformation. A cross-relaxation spectrum, C, is generated by the same sequence, except that immediately prior to the 90° x pulse, a selective 180° pulse is applied to one of the signals, inverting it. Finally, a difference spectrum, D, is obtained by subtracting R from C.

As an example, in Figure 1 are shown the spectra R, C, and D, obtained at 600 MHz with a sample of the tetrasaccharide, methyl *O*-(α-D-glucopyranosyluronic acid)-(1→6)-*O*-α-D-glucopyranosyl-1(1→2)-[*O*-α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranoside[7] (GGRR, 1). The 600-MHz proton spectrum of GGRR had been assigned by using standard 2D techniques and decoupling so that all intraring couplings could be observed and measured, establishing that each ring was predominantly in the expected lowest energy chair conformation. It was not possible, however, to observe intra- or interring NOES of the usual kind in order to confirm the sequence of sugars, presumably because $\omega\tau_c \approx 1$ under our conditions.

Observation of a transient effect in the rotating frame was successful, however. In Figure 1, the inverted peak is assigned to the anomeric proton of the rhamnosyl group (ring A), and the positive effects observable in spectrum D arise from the vicinal H_2 of ring A and H_2 and H_3 of the adjacent rhamnosyl ring B. This confirms the ring A–ring B linkage and suggests a particular conformation for the AB glycosidic linkage.

(1) Bothner-By, A. A. In "Biological Applications of Magnetic Resonance"; Shulman, R. G., Ed.; Academic Press: New York, 1979, pp 177-219.

(2) Abragam, A. "The Principles of Nuclear Magnetism"; Clarendon Press: Oxford, 1961, p 279.

(3) Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect"; Academic Press: New York, 1971; p 25.

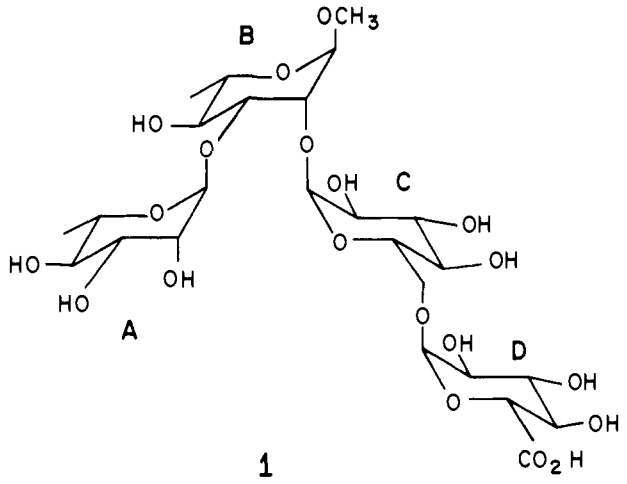
(4) Kalk, A.; Berendson, H. J. C. *J. Magn. Reson.* **1976**, *24*, 343-366.

(5) Wagner, G.; Wuthrich, K. *J. Magn. Reson.* **1979**, *33*, 675.

(6) Gordon, S. L.; Wuthrich, K. *J. Am. Chem. Soc.* **1978**, *100*, 7094.

(7) Schwarzenbach, D.; Jeanloz, R. W. *Carbohydr. Res.* **1981**, *90*, 193-202.

0002-7863/84/1506-0811$01.50/0



1

The theory of the effect in the rotating frame is being fully developed. However a simple analysis based on a two-spin system is instructive. For two spin sets one may write

$$\dot{m}_A = -\rho_2 m_A - \sigma_2 m_B \quad (1)$$

$$\dot{m}_B = -\sigma_2 m_A - \rho_2 m_B \quad (2)$$

where m_A and m_B are the transverse magnetizations, locked along the y axis in the rotating frame, and ρ_2 and σ_2 are the autorelaxation and cross-relaxation rate constants. The initial conditions for the R and C spectra are $m_A = m_B = 1.0$ and $m_A' = -m_B = 1.0$, respectively. These give the solutions

$$m_A = m_B = e^{-(\rho_2+\sigma_2)t} \quad (3)$$

and

$$m_A = -m_B = e^{-(\rho_2-\sigma_2)t} \quad (4)$$

The NOE is therefore

$$\mathrm{NOE} = -e^{-(\rho_2+\rho_2)t} + e^{-(\rho_2-\rho_2)t} \quad (5)$$

which has a maximum value

$$\mathrm{NOE}_{max} = -\left(\frac{\rho_2+\sigma_2}{\rho_2-\sigma_2}\right)^{-(\rho_2+\sigma_2)/2\sigma_2} + \left(\frac{\rho_2+\sigma_2}{\rho_2-\sigma_2}\right)^{-(\rho_2-\sigma_2)/2\sigma_2} \quad (6)$$

at a time, t_{max}, when

$$t_{max} = (1/2\sigma_2)\ \ln\ (\rho_2+\sigma_2)/(\rho_2-\sigma_2) \quad (7)$$

For most molecules $\omega_{eff}\tau_c << 1$. Here $\omega_{eff} = ((\gamma H_1)^2 + \delta\omega^2)^{1/2}$, and $\delta\omega$ is the angular frequency offset between the spin-locking field and the Larmor precessional frequency. This means that the relaxation behavior is very similar to normal transverse relaxation and that ρ_2 and σ_2 are approximated by[8]

$$\rho_2 = \frac{1}{T_2(\mathrm{dis})} = \frac{\gamma^4\hbar^2}{20r^6}\left\{5\tau_c + \frac{9\tau_c}{1+\omega_o^2\tau_c^2} + \frac{6\tau_c}{1+4\,\omega_o^2\tau_c^2}\right\}\ldots \quad (8)$$

$$\sigma_2 = \frac{1}{T_2(\mathrm{ind})} - \frac{1}{T_2(\mathrm{dis})} = \frac{\gamma^4\hbar^2}{20r^6}\left\{4\tau_c + \frac{6\tau_c}{1+\omega_o^2\tau_c^2}\right\}\ldots \quad (9)$$

where $T_2(\mathrm{dis})$ and $T_2(\mathrm{ind})$ are transverse relaxation times for distinguishable and indistinguishable spins. Substitution of these into (7) gives values for NOE_{max} as a function of $\omega_o\tau_c$. A plot of the results is shown in Figure 2. The maximum transient NOE in the rotating frame increases from 38.5% for $\omega_o\tau_c << 1$ to 67.5% for $\omega_o\tau_c >> 1$. This has two important consequences: (1) the NOE is positive and does not vanish for any value of $\omega_o\tau_c$; (2) multispin effects will be minor. The positive sign also ensures that the effect will not be confused with transfer of magnetization by chemical exchange.[9]

(8) Hubbard, P. S. *Rev. Mod. Phys.* **1961**, *33*, 249–264.
(9) Hennig, J.; Limbach, H. H. *J. Magn. Reson.* **1982**, *49*, 322–327.

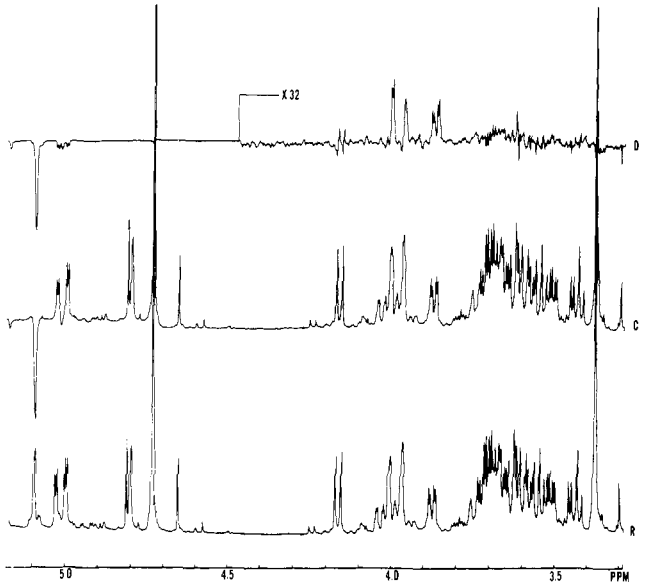


Figure 1. CAMELSPIN spectra of the tetrasaccharide methyl *O*-(α-D-glucopyranosyluronic acid)-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→3)]-α-L-rhamnopyranoside. (R) Spectrum obtained after 250 ms of spinlock; (C) as in R but with preliminary inversion of the anomeric protons of rhamnosyl ring A; (D) difference spectrum.

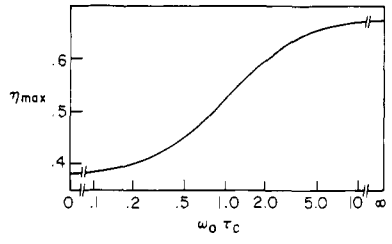


Figure 2. Predicted maximum transverse Overhauser effects in a two spin system relaxed exclusively by magnetic dipole interaction, as a function of rotational correlation time.

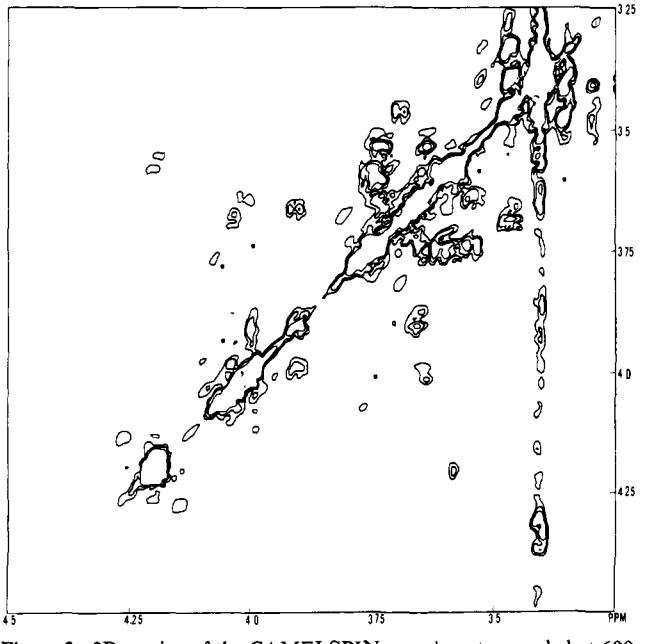


Figure 3. 2D version of the CAMELSPIN experiment, recorded at 600 MHz (data matrix; 512 x 1K) (number of scans, 64); spin-locking time, 250-ms; carbohydrate concentration, approximately 10 mmol.

Generalization of the experiment to a 2-D version is relatively simple. The 2D sequence consists of (1) a 90° x pulse, (2) a variable delay, t_1, during which the spins dephase according to

their Larmor frequencies, (3) application of the spin-locking field (which will rapidly destroy the X components of magnetization) along y for the relaxation period t_{max}, and (4) removal of the spin-locking field and acquisition of the fid, during t_2. Double Fourier transformation yields the 2D spectrum (Figure 3). The spectrum itself is displayed along the diagonal, while cross-peaks represent transient Overhauser effects in the rotating frame.

Interproton effects identified from the 2-D experiment on the tetrasaccharide include (A1,A2), (A2,A3), (A3,A4), (A2,A4-(diaxial)), (A1,B2), (A1,B3), (B2-B3), (B3,B4), (C2,C3), (C4,C5), (C6,C6'), and (D4,D5). An effect tentatively identified as (C2,B5) suggests a particular conformation about the B–C glycosidic linkage.

Relaxation processes in the rotating frame are sensitive to low-frequency motions (of the order of $\omega_1 = \gamma H_1$). Such motions might occur in the form of slow conformational changes, and the influence of these on the effects is of interest. However, most processes will be fast compared to ω_1, and the experiment may be thought of as a way to restore the extreme narrowing condition for higher molecular weight compounds. We think a suitable acronym for this type of experiment is CAMELSPIN (cross-relaxation appropriate for minimolecules emulated by locked spins). Ice-skating enthusiasts will have no difficulty with the origin of the term.

Acknowledgment. This research is supported by NIH grant AM16532 and was performed by using the 600-MHz NMR spectrometer of the NMR Facility for Biomedical Studies, Pittsburgh, PA, supported by NIH Grant RR00292.

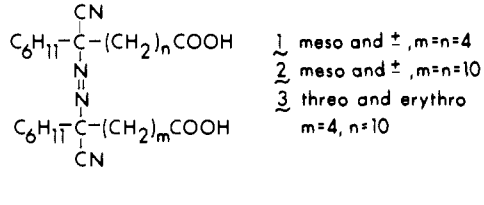
Free Radicals in Lipid Bilayers: New Probes of Lipid Radical Dynamics

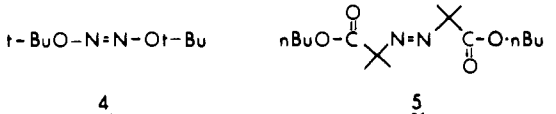
Ned A. Porter,* Russell C. Petter, and William J. Brittain

Department of Chemistry, Duke University
Durham, North Carolina 27706

Received October 11, 1983
Revised Manuscript Received December 10, 1983

Free radical reactions in membranes have been implicated in several instances of destructive membrane oxidation[1] but the details of the initiation and propagation reactions of radicals in membranes are not well understood. In order to investigate the effect of phospholidpid bilayers on the formation and fate of lipidic radicals, we prepared[2] the amphipathic 1,2-diazenes **1**, **2**, and **3**.





4 **5**

Each of these diazenes has two diastereomers which were isolated and studied separately.[3] Other 1,2-diazenes have been studied as free radical initiators in model membranes. For example, di-*tert*-butyl hyponitrite (**4**) and 2,2'-azobis(2-(*n*-butylcarboxy)-propane) (**5**) have been examined as free radical initiators in phospholipid bilayers.[4,5] However, these studies suffer from a lack of information about the microenvironment surrounding the initiators in the bilayers. This uncertainty raises questions about the magnitude of bilayer cage effects in these systems.[4] Initiators **1–3** are designed to be oriented in the bilayer and thus resolve locational ambiguities that plague initiators **4** and **5**.[6]

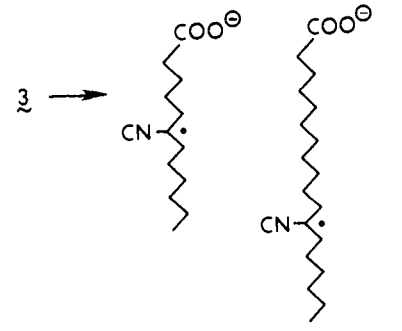


Figure 1.

Table I. Efficiencies for Radical Production from Diazenes 1-3[a] in Phospholipid Bilayers at 60.00 °C

diazene[a]	$k \pm$ SE in C_6H_5Cl[a-c]	$e \pm$ SE in C_6H_5Cl	$k \pm$ SE in DPPC[c,d]	$e \pm$ SE in DPPC[d]
meso-1	21.1 ± 0.4	0.512 ± 0.014	35.5 ± 0.5	0.054 ± 0.001
(±)-1	14.5 ± 0.1	0.596 ± 0.024	5.7 ± 0.2	0.041 ± 0.006
2a	23.7 ± 0.3	0.483 ± 0.021	13.2 ± 0.2	0.077 ± 0.008
2b	12.8 ± 0.4	0.507 ± 0.028	5.2 ± 0.2	0.069 ± 0.011
3a	*e*	*e*	18.7 ± 1.3	0.101 ± 0.017
3b	*e*	*e*	5.3 ± 0.3	0.109 ± 0.011

[a] Stereoisomers of diazenes **2** and **3** have not been identified and are indicated here as "a" and "b".[3] [b] SE = standard error of linear regression. [c] First-order rate constant × 10^6, s^{-1}; weighted mean of at least three independent measurements. [d] DPPC = dipalmitoylphosphatidylcholine in pH 7 buffer with 1 mM EDTA. [e] Not determined.

Free radicals generated by decomposition of diazenes can react with each other or escape the initial encounter and react with atmospheric oxygen at a diffusion-controlled rate.[7] The resulting peroxyl radicals are scavenged by hydrogen atom donors such as α-tocopherol (vitamin E) and the ratio of radicals escaped (and α-tocopherol scavenging) to radicals produced from the diazene is termed the escape efficiency.[8,9]

The efficiencies of our initiators were experimentally determined via reverse-phase HPLC by monitoring the α-tocopherol loss relative to the loss of the diazene in question at 60.00 °C under atomspheric oxygen. Since each α-tocopherol is known to scavenge two peroxyl radicals,[8] the efficiencies were calculated as the coefficient of the linear regression of [α-tocopherol] vs. [diazene]. Efficiencies were independent of diazene concentration in the range studied (3–10% in DPPC), and efficiency plots were linear to at least three diazene half-lives (<0.6% diazene in DPPC). The results thus suggest that the diazene does not, in itself, significantly modify the bilayer medium. Our results are presented in Table I.

In chlorobenzene at 60 °C, 2,2'-azobis(isobutyronitrile) (AIBN) gives escape efficiencies of 0.59.[10] Under the same conditions,

(1) Tappel, A. L. In "Free Radicals in Biology"; Pryor, Ed.; Academic Press: New York, 1980; Vol. IV, p 2.
(2) Petter, R. C.; Mitchell, J. C.; Brittain, W. J.; McIntosh, T. J.; Porter, N. A. *J. Am. Chem. Soc.* **1983**, *105*, 5700.
(3) The diazenes **2** and **3** were prepared in a way analogous to the method used to prepare **1**.[2] Diastereomers were separated by chromatography on a C-18 column with CH_3OH/H_2O/HOAc. A solvent mixture of (v/v) 750/250/2 was used for **1**, 810/190/2 for **3**, and 950/50/2 for **2**. The first eluting isomer is designated "a", the second "b". The configuration of the stereoisomers of **2** and **3** is not known.
(4) Barclay, L. R. C.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6478.
(5) Winterle, J. S.; Mill, T. *J. Am. Chem. Soc.* **1980**, *102*, 6336.
(6) A low-angle X-ray analysis of **1** incorporated into DPPC multilamellar vesicles has been published.[2] This study confirms the fact that **1** forms a homogeneous phase with DPPC. Preliminary results indicate that similar conclusions can be drawn about **2** in DPPC.
(7) Maillard, B.; Ingold, K. U.; Scaiano, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 5095.
(8) Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1983**, *105*, 6472.
(9) Hammond, G. S.; Sen, J. N.; Boozer, C. E. *J. Am. Chem. Soc.* *77*, 3244.

0002-7863/84/1506-0813$01.50/0