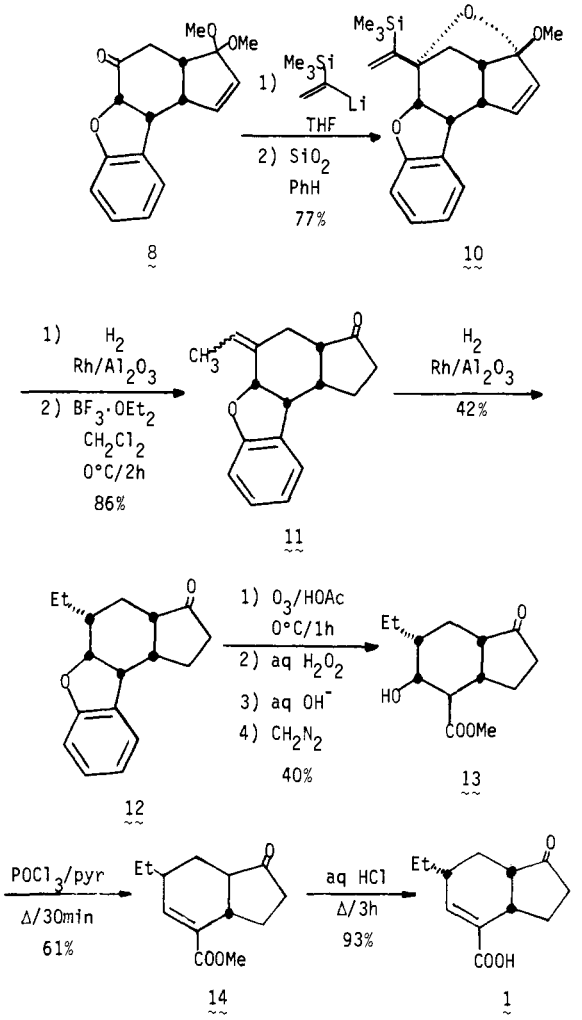
Scheme II



sponding alcohols under these conditions. It is presumably the great steric bulk of the Grignard reagent which causes elimination of hydride from the β position[11] to be preferred over simple addition.[12]

Since a vinyllithium should not undergo facile β-elimination-reduction, the use of the α-trimethylsilylvinyllithium offered another alternative to the Wittig process (Scheme II). Reaction of this organometallic reagent[13,14] with the ketone **8** followed by stirring in benzene over silica gel afforded the internal ketal **10**[7] in good yield. Catalytic hydrogenation produced the tetrahydro compound which now underwent clean desilylative olefin formation upon treatment with boron trifluoride to give the mixture of keto olefins **11**[7] in excellent yield.[15] This three-step alternative for ethylidene formation should be generally useful. Catalytic hydrogenation of **11** produced a mixture of products from which the major product **12**[7] could be purified without chromatography by direct crystallization from the reaction mixture (mp 140–141 °C). The desired product **12** corresponds to reduction of the olefin from the less hindered convex face of the molecule. The stereochemistry of all centers in **12** was determined by a single-crystal X-ray diffraction.[16]

Having served as the protected form of the carboxylate function, the aromatic ring in **12** was now converted into the ester **13** by ozonolysis in acid, oxidation, hydrolysis, and esterification in 40% yield. The final conversion to coronafacic acid (**1**) was effected in 57% overall yield by elimination of the β-hydroxy function with phosphorus oxychloride–pyridine to give the ester **14**[17] which was hydrolyzed in acid to afford **1**.[18] The synthetic product was thus produced by a nine-step process in an overall yield of 5%. It was identical in all respects with an authentic sample.[19] Since coronafacic acid (**1**) has been coupled with coronamic acid (**2**),[4] this report also constitutes a formal total synthesis of coronatine (**3**).

Acknowledgment. We thank the National Cancer Institute, the National Science Foundation, and Eli Lilly Co., for their generous support of our synthetic program.

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(7) All new compounds had spectral properties (NMR, IR, mass spectra) in complete accord with the assigned structures. Also satisfactory elemental analyses were obtained for all crystalline compounds.
(8) We also attempted to use the simple analogous dihydrofuryl system corresponding to **8** which we had prepared earlier[3] for the preparation of **1**. However when selective hydrogenation of the cyclopentene double bond could not be effected, this approach was abandoned.
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(15) These isomers could be separated by chromatography to give a crystalline (mp 145–146 °C) isomer and an oil. However, since hydrogenation of the purified isomers offered no advantages the mixture was used in the subsequent step.
(16) This study was carried out by Professor Charles Strouse and Mr. Larry Goldsmith in our department and will be described in detail in the full paper.
(17) The ester **14** is a mixture of two major compounds in the approximate ratio of 4:1 (NMR integration). This mixture was not separated but rather used directly in the next step.
(18) We assume that coronafacic acid has the structure indicated by **1** as proposed by Ichihara. His evidence for this structure is very strong, although two alternative structures with the ethyl group having the β-stereochemistry cannot be absolutely eliminated since there is the possibility of epimerization of the hydrogen γ to the α,β-unsaturated carboxylate in the final acidic ester hydrolysis.
(19) We thank Professor Ichihara for kindly providing us with an authentic sample of natural coronafacic acid and the spectral data for both the natural and their synthetic material.
(20) Camille and Henry Dreyfus Teacher-Scholar, 1978–1983; Alfred P. Sloan Foundation Fellow, 1979–1981.
(21) Recipient of the Winstein Dissertation Award.

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Secondary Deuterium Isotope Effects on Epoxide Methanolysis Reactions

Sir:

Secondary kinetic deuterium isotope effects have recently come into widespread use as probes of reaction mechanism and transition state structure.[1-7] They are particularly useful for detailed comparison of transition states for enzymatic and chemical model reactions because isotopic substitution, in contrast to introduction of chemical substituent groups, does not change the potential energy surface of the reaction pathway. These effects are presumed to arise by one of two major

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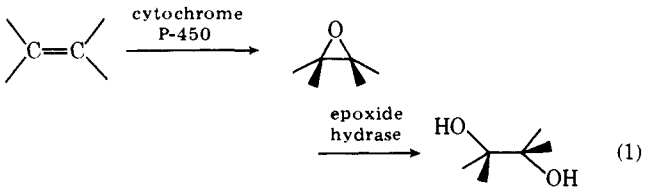
Table I. Rate Constants, Regiospecificities, and Secondary Deuterium Isotope Effects for Acid and Base Methanolysis of *p*-Nitrostyrene Oxide

reaction	$k_{obsd} \times 10^4$, s^{-1}	regiospecificity[a]	k_7^H/k_7^D	k_8^H/k_8^D
		f_7		
acid				
d_0	88.63 ± 0.68	0.943 ± 0.003		
7-d_1	86.90 ± 0.58	0.942 ± 0.001	1.021 ± 0.032[b]	1.002 ± 0.031[c]
8,8-d_2	100.15 ± 0.58	0.944 ± 0.001	0.884 ± 0.027[c]	0.901 ± 0.020[b]
		f_8		
base				
d_0	0.6470 ± 0.0141	0.846 ± 0.003		
7-d_1	0.7056 ± 0.0061	0.847 ± 0.006	0.922 ± 0.020[b]	0.916 ± 0.022[c]
8,8-d_2	0.6793 ± 0.0102	0.849 ± 0.002	0.971 ± 0.026[c]	0.949 ± 0.024[b]

[a] Regiospecificity indexes f_7 and f_8 are defined by $f_7 = k_7/(k_7 + k_8)$ and $f_7 + f_8 = 1$, where $k_7 = k_{obsd} f_7$ (see Scheme I). [b] For nucleophilic attack at the isotope-bearing center. [c] For nucleophilic attack vicinal to the isotope-bearing center.

fundamental processes. Isotopic substitution at the reacting carbon gives rise to the so-called "α effect" due to differences in the energetics of out-of-plane bending of C-H and C-D bonds; these effects are usually interpreted in terms of changes in *hybridization* at the reacting carbon as the reaction processes.[8,9] Alternatively isotopic substitution at carbons adjacent to the reacting carbon leads to a "β effect" presumably arising from differences in the ability of C-H and C-D bonds to interact with orbitals on the reacting carbon by means of *hyperconjugation*.[10,11]

In connection with mechanistic studies of the epoxide-diol pathway (eq 1) we have used secondary deuterium isotope



(1)

effects to compare epoxidation of olefins by cytochrome P-450[12] with olefin epoxidation by *m*-chloroperbenzoic acid,[13] a commonly adopted chemical model for P-450 systems. We now report on observations of secondary deuterium isotope effects on the acid- and base-catalyzed methanolysis of *p*-nitrostyrene oxide (Scheme I), a chemical model system for the epoxide hydrase reaction.

Pseudo-first-order rate constants (k_{obsd}) for reactions of **1a–c** in 0.25 M H_2SO_4-CH_3OH and 1.0 M $NaOCH_3$-CH_3OH were determined at 30.0 °C by monitoring the reactions spectrophotometrically at 295 nm for 3–5 half-lives (r = 0.9999). Product analysis by LC showed that **2** and **3** were the sole products, as indicated in Scheme I. Their ratio, and thus the ratio k_7/k_8, was determined by electronic integration of their peak areas. The relevant rate constant, regiospecificity, and isotope effect data are given in Table I.

Most previous studies of epoxide hydration and solvolysis reactions have centered on the questions of degree of carbonium-ion formation vs. requirement of nucleophilic participation in ring opening under acidic conditions. These considerations are also extremely pertinent to current interest in the relationship between the chemical properties and biological effects of epoxides.

Scheme I

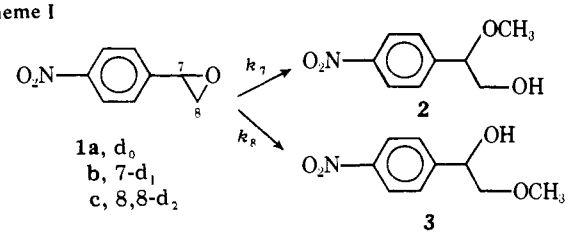
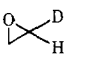
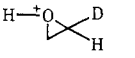


Table II. Fractionation Factors Used to Calculate Equilibrium Isotope Effects on Model Transition State Structures for Reactions of Epoxides[a]

	1.029[b]		1.095[d]
$(CH_3)_2CHD$	1.103[b]		1.12[e]
			1.14[f]
CH_2=CHD	0.924[b]		
		$CH_3CHD(OHR)^+$	1.20[e]
DCH_2NH_2	1.036[b]		1.25[f]
$DCH_2NH_3^+$	1.058[b]	CH_3CHDO^-	1.15[g]
			1.13[h]
CH_3CHDOR	1.174[c]		

[a] Fractionation factors are given relative to ethane at 25 °C. [b] Taken from Hartshorn and Shiner.[35] [c] Taken from Gray et al.[2] [d] Calculated from the fractionation factor ratio $(1.029)(1.174)\cdot(1.103)^{-1}$. [e] Calculated by multiplying the fractionation factor for the neutral molecule by 1.021, a "charge correction term" obtained from the ratio of the *calculated* fractionation factors $(DCH_2NH_2)\cdot(DCH_2NH_3^+)^{-1} = (1.058)(1.036)^{-1}$. [f] Calculated by multiplying the fractionation factor for the neutral molecule by 1.042, a "charge correction term" derived from the experimentally determined pK_a values of $CH_3NH_3^+$.[36] [g] Calculated as in footnote *e* above, except that the reciprocal of the charge correction term was used since the charge is negative in this case. [h] Calculated as in footnote *f* above, except that the reciprocal of the charge correction term was used since the charge is negative in this case.

In acid, the essential features of oxirane ring opening are as follows. Preequilibrium protonation occurs, giving rise to a solvent deuterium isotope effect $k(H_2O)/k(D_2O) = 0.5$.[14] Ring opening occurs largely at the carbon best able to accommodate the development of positive charge,[15,16] although, except for *arene* oxides,[17] there is little evidence to suggest the intermediacy of carbonium ions.[18,19] Rather, the reaction is best characterized as involving nucleophilic attack by solvent at the most positively charged oxirane carbon. Thus styrene oxide reacts in acidic methanol mainly (89%) by *inversion* of configuration at the benzylic carbon,[20] and the reaction is characterized by a sizable negative entropy of activation (ca. −12 to −13 eu) and a Hammett ρ value of −4.1.[21] From a number of studies it appears that the reactivity of epoxides toward ring opening by *nucleophilic* attack is increasingly enhanced by intramolecular hydrogen bonding < metal ion chelation < protonation.[22-24]

The data for reactions of **1a–c** in acidic methanol (Table I) show that there is a high degree of regiospecificity for reaction at the benzylic carbon, C-7. For this reaction, deuteration of the nonreacting carbon, C-8, produces a large inverse "β effect". Since the C-8–H(D) bonds are essentially orthogonal to the C-7–O bond, this isotope effect cannot arise from hy-

Table III. Estimated Kinetic Isotope Effects for Acid-Catalyzed Methanolysis of *p*-Nitrostyrene Oxide

transition state model	k_H/k_D, reaction at C-7		k_H/k_D, reaction at C-8	
	7-d_1	8,8-d_2	7-d_1	8,8-d_2
protonated epoxide	1.00 (0.96)[a]	1.00 (0.92)	1.00 (0.96)	1.00 (0.92)
carbonium ion	1.19	1.00-0.86[b]	1.00-0.93[b]	1.41
tight S_N2 attack on protonated epoxide	0.93	1.00-0.86[b]	1.00-0.93[b]	0.86
protonated product	0.93 (0.90)	0.86	0.93	0.86 (0.79)
product	0.93	0.86	0.93	0.86

[a] Values in parentheses include a charge correction factor as explained in footnote *f* of Table II. [b] This represents the range from no ring opening to a fully opened epoxide ring at the transition state.

perconjugative interactions[25-27] and must therefore arise from changes in C-H(D) bending modes as the oxirane ring opens and C-8 is rehybridized from the $sp^{2.22}$ ground state[28] to a transition state which is more sp^3-like.

The fact that deuteration of C-7 produces a negligible isotope effect on this reaction indicates that *net* bonding at this carbon has changed relatively little in the transition state, with bond making more or less compensating for bond breaking. For the (minor) reaction at C-8 in acid, deuteration at C-7 does not cause a large isotope effect, suggesting relatively little C-8-O bond breaking (i.e., ring opening) in the transition state for this reaction. In contrast, deuteration at C-8 produces a large inverse isotope effect, implying that this reaction requires much tighter approach of the nucleophile and that bond making probably leads bond breaking.

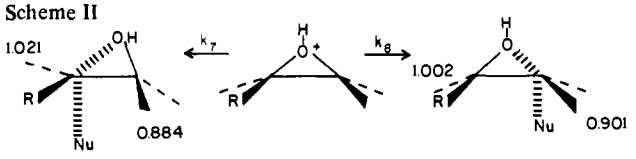
Support of a quantitative nature for the intuitive interpretation given above may be obtained by using the fractionation factors listed in Table II to calculate equilibrium isotope effects for D exchange between stable structures chosen to model various potential transition states for the reaction in question. These values are then estimates for kinetic isotope effects associated with the transition state being modeled; several examples are given in Table III. Thus, for reaction at C-7 via a transition state resembling the protonated epoxide, the isotope effect will lie between unity (if no charge correction is applied) and the value of the fractionation factor ratio $(1.095)(1.14)^{-1} = 0.96$ for one D or $(0.96)^2 = 0.92$ for two D; the latter values include a charge correction as indicated in Table II. The isotope effect for reaction at C-7 via a loose carbonium-ion-like transition state can be approximated from the fractionation factors for epoxide and ethylene as $(1.095)(0.924)^{-1} = 1.19$ for one D at C-7. With two D at C-8 the isotope effect for reaction at C-7 will lie somewhere between 1.00 and 0.86, depending on how much ring opening has occurred in the transition state.[29] For an extremely tight S_N2-like transition state the isotope effect can be estimated[2] using

$$\phi_T = (0.99)(\phi_R\phi_P)^{0.70} \quad (2)$$

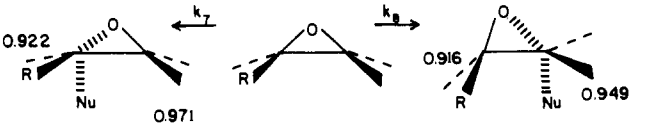
$$k_H/k_D = \phi_R/\phi_T \quad (3)$$

where ϕ_R, ϕ_T, and ϕ_P are the appropriate fractionation factors for the reactant, transition state, and product, respectively. In this way a transition state resembling tight nucleophilic attack by methanol at C-7 of the protonated epoxide would be expected to generate an isotope effect of $(1.095)\cdot(0.99)^{-1}(1.095)^{-0.70}(1.174)^{-0.70} = 0.93$ for one D at C-7. Again the isotope effect for two D at C-8 would be 1.00-0.87 depending on the degree of ring opening in the transition state. A similar analysis for the (minor) reaction at C-8 in acid again supports the qualitative conclusions reached above. Thus the transition states for acid methanolysis of **1** may be depicted as shown in Scheme II.

Scheme II



Scheme III



Similar considerations apply to analysis of the nucleophilic ring opening of **1** by methoxide. The major reaction occurs at C-8, the least hindered carbon. The sizable inverse isotope effect for deuteration of C-7 implies some degree of oxirane ring opening in the transition state. The relatively smaller (per deuterium) inverse isotope effect at the reacting carbon reflects close approach of the nucleophile, such that out-of-plane C-H(D) bending at C-8 is more restricted in the transition state than the ground state. For the minor reaction with methoxide at C-7 the converse obtains, i.e., a large inverse isotope effect from deuteration of C-7 but little effect from deuteration at C-8. This suggests a transition state involving close approach of the nucleophile but relatively little oxirane ring opening. As illustrated in Table III for the reactions of **1** in acid, these qualitative descriptions can be fully supported by quantitative estimates of isotope effects expected for various model transition state structures. Accordingly, the transition states for reaction of **1** with methoxide may thus be depicted as shown in Scheme III.

Several interesting results emerge form this study. Apparently this is the first observation of "an α effect at the β carbon" [30] or, in other words, a secondary effect at a neighboring carbon *not* attributable to hyperconjugation phenomena. Such effects might be expected but are not observed in the solvolysis of β-arylethyl tosylates[31-33] and in the thermal cis-trans isomerization of cyclopropane derivatives.[34] Another interesting result of this study is the parallelism that the major reactions in acid and base both involve "late" or product-like transition states, while both minor reactions involve "early" or reactant-like transition states.[37] Lastly, these studies have shown that secondary deuterium isotope effects provide a sensitive and predictable probe for mechanistic studies of epoxide reactions. We are currently extending this approach to other epoxide reaction systems, both chemical and enzymatic.

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(29) This isotope effect, an "α effect at the β carbon", is presumed to be a measure of ring opening in the transition state. A value of 1.00 would correspond to a reactant-like transition state. For a ring-opened product-like transition state the calculated value of 0.86 for two D is based on the fractionation factors for propane (1.103) and cyclopropane, i.e., $(1.029)\cdot(1.103)^{-1} = 0.93$ and $0.93^2 = 0.86$. *Between these limits the isotope effect may or may not vary linearly with the degree of ring opening in the transition state.* In this regard it was brought to our attention by a referee that the fractionation factor given by Hartshorn and Shiner[35] for cyclobutane is in fact identical with that given for cyclopropane. This somewhat surprising fact may have several interpretations. One is that the "α effect at the β carbon" is *not* linearly related to the degree of ring opening, but instead varies little from unity as the O–C–C angle increases from 60 to 90° and then begins to rise steeply as the O–C–C angle passes beyond 90° toward the tetrahedral limit. This would imply an *extremely* late or product-like transition state for the reaction of 1 at C-7 in acid. A second interpretation would be that the equality of the fractionation factors for cyclopropane and cyclobutane is fortuitous. The plausibility of this interpretation is supported by the fact that, for none of the terms which enter the calculation of the fractionation factor, are the values for cyclopropane and cyclobutane equal; only their products are equal (Table VIII of ref 35). Nevertheless, the issue of the manner in which the "α effect at the β carbon" varies with geometry is one which requires careful consideration in attempts to deduce transition state structures from kinetic secondary deuterium isotope effects.
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(37) This contradicts expectations based on the Hammond postulate. Westaway and Ali have observed a similar situation for the reaction of thiophenoxide ion with benzyldimethylanilinium ion, and have given an extensive discussion of the possible sources for such discrepancy between theory and experiment. See K. C. Westaway and S. F. Ali, *Can. J. Chem.*, **57**, 1354 (1979).
(38) Supported by an NIH Predoctoral Training Grant, GM-07775.

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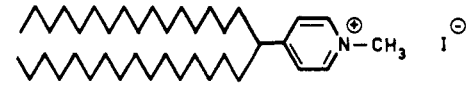
Vesicle Formation by Two Novel Synthetic Amphiphiles Carrying Micropolarity Reporter Head Groups

Sir:

Phospholipid vesicles or liposomes are of great interest as models for biological membranes.[1,2] Usually the vesicles were built up from naturally occurring or synthetically prepared phospholipid molecules. Recently it was shown that vesicles can also be obtained from simple synthetic organic amphiphiles,[3] which are in most cases composed of long-chain dialkyldimethylammonium halides.[4-7] There are, however, also reports on stable bilayer structures formed from amphiphiles with anionic,[8,9] zwitterionic,[10] or nonionic[10] head groups.

Herein we report a study of the aggregation behavior of two novel synthetic doubly chained surfactants, 17-(4-*N*-methylpyridinium)tritriacontane iodide (**1**)[11] and 3,5-dicarbo-*n*-



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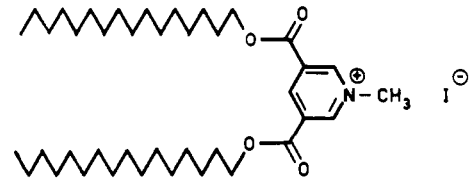

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hexadecyloxy-1-methylpyridinium iodide (**2**).[11] These amphiphilic molecules were chosen for investigation since the head groups exhibit charge-transfer (CT) transitions which are very sensitive to changes in the immediate microenvironment.[12] Thus, sonication of **1** and **2** in Tris·NaCl buffer solutions[16] gave transparant mixtures of uni- and multilamellar vesicles as revealed by electron microscopy.[17] Typical electron micrographs are shown in Figure 1. The diameter of these stable vesicles ranges from 500 to 5000 Å (**1**) and from 500 to 2500 Å (**2**), respectively. The micrographs resemble closely the patterns found for vesicles formed from phospholipids[18] and from other synthetic organic amphiphiles.[3a,4,5,8] When the sonication was performed in the presence of 1% (by weight) uranyl acetate, the micrographs showed lamellae of parallel and curved hydrocarbon layers which evoke a "fingerprint" impression (Figure 1).[19] The thickness of the bilayer (35 ± 5 Å) is estimated from the micrograph and corresponds with the expected value of ~37 Å for twice a fully extended *n*-hexadecyl hydrocarbon chain. The spacing between the bilayers is ~20 Å. It was also possible to prepare mixed vesicles (diameter 200–4000 Å) from both **1** and **2** with egg yolk phosphatidylcholine (PC).

The presence of *closed* vesicles was established by using the technique of Weinstein et al.[20] which takes advantage of the efficient self-quenching of the fluorescence of carboxyfluorescein (CF) upon entrapment of the dye in vesicles in relatively high concentration (100 mM). Escape of CF from the vesicle compartments results in vast dilution of the dye and the rapid buildup of fluorescence. Thus, after sonic dispersal of **1** or **2** in 1.5 mL of 0.1 M aqueous CF solution (pH 7.4) and centrifugation, nonentrapped CF was removed from the supernatant by column chromatography on Sephadex G-100 using a Tris·NaCl buffer solution[16] as the eluant. The vesicles were recovered in the void volume of the column. After dilution with a Hepes·NaCl buffer solution[21] (pH 7.4), the appearance of fluorescence, which monitores leakage of CF through the vesicle membranes, was recorded as a function of time at room temperature.[22,23] It is found that CF molecules are rapidly released from vesicles prepared from **1** by sonication at 0 °C (80% release after 20 min). No vesicles could be obtained upon sonication of **1** at 50 °C. By contrast, vesicles were readily formed upon sonic dispersal of **2** at 50 °C and these vesicles released CF only very slowly (9% release after 20 min). This temperature-dependent effect is reminescent of that observed previously for phospholipid vesicles[24] and probably originates from the formation of unannealed vesicles when sonication is

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