An Imidazole-Functionalized Phosphatidylcholine Derivative: Nucleophilic Vesicles with Adjustable Reactivity†

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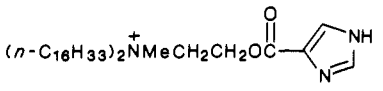
In connection with our quest for locus specific vesicular chemistry,[2] we sought a nucleophile-functionalized, vesicle-forming surfactant[3] with a "natural" acylglycerol backbone. We have already described the (dihexadecylmethylethylammonio)-carboxyimidazole surfactant **1**.[4] We are pleased to report here the synthesis of an imidazole-derivatized phosphatidylcholine (**2**) and the remarkable kinetic behavior of vesicles created from this novel surfactant.[5]



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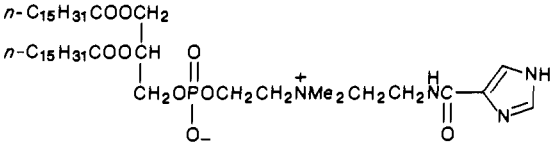

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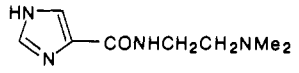
4(5)-Imidazolecarboxylic acid[6] was converted to its acid chloride ($SOCl_2$, reflux, 36 h), and the latter was reacted with *N,N*-dimethylethylenediamine ($CHCl_3$, 25 °C, 4 h), affording 79% of **3** (chromatography, SiO_2, 1:1 MeOH/$CHCl_3$). Reaction with bromoethanol ($CHCl_3$, trace *i*-Pr_2NEt, 70 °C, 48 h, sealed tube) gave hygroscopic salt **4** (83%, chromatography as above). Lyophilized **4** was "condensed" with 1,2-dipalmitoyl-*rac*-glycerol (Sigma) and 2-chlorophenyl *O,O*-bis[1-benzotriazolyl]phosphate,[7] by using the modified phosphotriester method of van Boom,[7] affording **5**, where RO and R′O are residues derived from dipalmitoylglycerol and **4** by deletion of their hydroxyl protons. Without purification, lyophilized **5** was converted to **2** by *p*-chlorophenoxide cleavage with tetramethylguanidinium *p*-nitrobenzaldoximate.[7,8] Pure **2** (25% overall yield) was obtained as the trihydrate (C,H,N analysis) after preparative TLC (SiO_2, 1:1 MeOH/$CHCl_3$) and column chromatography (SiO_2, 5:5:1 MeOH/$CHCl_3$/H_2O).[9]



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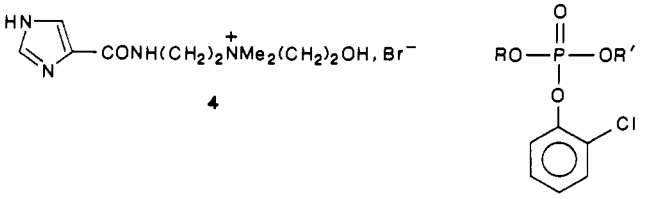

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† Dedicated to the memory of Professor Iwao Tabushi.

(1) Visiting Professor from Università di Padova: Fulbright-Scholar, 1985–86.

(2) Moss, R. A.; Swarup, S. *J. Am. Chem. Soc.* **1986**, *108*, 5341.

(3) Review of functional vesicles: Fuhrhop, J.-H.; Mathieu, J. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 100.

(4) Moss, R. A.; Kim, K. Y. *Isr. J. Chem.* **1985**, *25*, 11.

(5) Earlier studies of imidazole nucleophiles in vesicular media include the following: (a) Kunitake, T.; Sakamoto, T. *J. Am. Chem. Soc.* **1978**, *100*, 4615. (b) Murakami, Y.; Nakano, A.; Okahata, Y. *Ibid.* **1983**, *105*, 6070.

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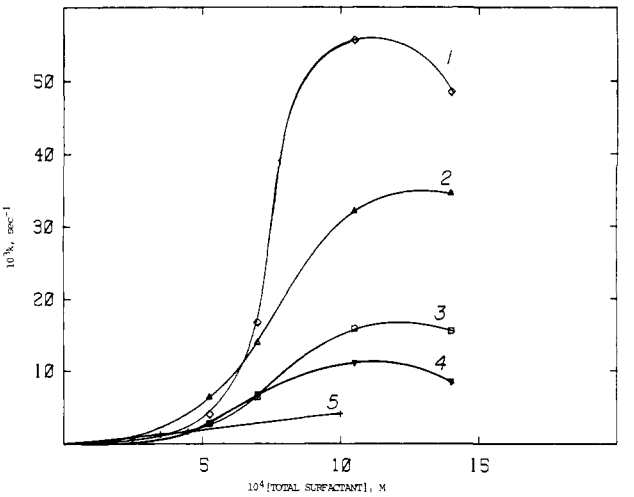

Figure 1. Pseudo-first-order rate constants (k s^{-1}, ordinate) vs. total surfactant concentration (M, abscissa) for cleavages of active ester substrates: 1, ANBS and (**2** + **6**); 2, PNPH and (**2** + **6**); 3, ANBS and (**2** + **7**); 4, PNPH and (**2** + **7**), 5, PNPH and native **2**. (Rate constants for curve 5 have been multiplied by 10 to bring them on scale.) See ref 14a for conditions.

Table I. Kinetic Parameters for Reactions of Vesicular **2** and **1** at 25 °C[a]

case	vesicle[b]	substrate	covesclr additive[c]	k_ψ, s^{-1}	k_2,[d] M^{-1} s^{-1}
1	**2**	PNPH	none	4.2×10^{-4}	0.42
2	**2**	PNPH	**6**	3.2×10^{-2}	91.
3	**2**	PNPH	**7**	1.1×10^{-2}	31.
4	**1**[e]	PNPH	none	11.4	11.4×10^3
5	**2**	ANBS	**6**	5.6×10^{-2}	160.
6	**2**	ANBS	**7**	1.6×10^{-2}	46.
7	**1**[e]	ANBS	none	9.3	9.3×10^3
8	**2**	PNPA	**6**	1.4×10^{-3}	4.0
9	**2**	PNPA	**7**	7.1×10^{-4}	2.0
10	**1**[e]	PNPA	none	1.3	1.3×10^3

[a] See note 14 for kinetic conditions. [b] [**2**] = 3.5×10^{-4} M, except in case 1, where [**2**] = 1.0×10^{-3} M. [c] [additive] = 7.0×10^{-4} M, covesicles created by injection of mixed ethanolic surfactant solutions. [d] $k_2 = k_\psi$/[imidazole-functionalized surfactant]. [e] Data are from ref 4; [**1**] = 1.0×10^{-3} M.

Stable vesicles of **2** were created by fast injection[4,10] of ethanolic solutions into aqueous Tris buffer at 50 °C. These vesicles had a sharp phase transition (T_c) at 36 °C,[11] a unimodal size distribution, and an apparent diameter of 1400 ± 10_3 Å by dynamic light scattering (90°) at pH 8.[12] On the basis of the method of generation and their size, our vesicular **2** should be multilamellar.[13] An electron micrograph of vesicular **2** (stained with 2% uranyl acetate at pH 4 and dried on a carbon-formvar coated copper grid) revealed lozenge-shaped vesicles with diameters of ~800 Å, thicknesses of ~160 Å, and bilayer "widths" of ~200 Å, sug-

(9) Mp 225–227 °C (liquid crystal, 60 °C); the 400-MHz NMR spectrum ($CDCl_3$) is in agreement with structure **2**; the imidazole CH protons are visible at δ 7.63 and 7.59, the CH_2CH_2 fragment of the ethanamido linkage appears at δ 3.7–4.0. The remainder of the spectrum closely resembles that of **7**; see Chapman, D.; Morrison, A. *J. Biol. Chem.* **1966**, *241*, 5044.

(10) Batzri, S.; Korn, E. D. *Biochim. Biophys. Acta* **1973**, *298*, 1015. TLC demonstrates the absence of lysis to palmitic acid when these vesicles are maintained at pH 8 for 24 h. Vesicles prepared by sonication were unstable and deposited precipitates after ~30 min.

(11) T_c was determined from sharp discontinuities in the temperature dependent fluorescence polarization of covesicalized 1,6-diphenylhexatriene: Andrich, M. P.; Vanderkooi, J. M. *Biochem.* **1976**, *15*, 1257.

(12) The light scattering diameter of vesicular **2** is dependent on the scattering angle, suggesting a nonspherical morphology. This is confirmed by electron microscopy; see below.

(13) Hope, M. J.; Bally, M. B.; Mayer, L. D.; Janoff, A. S.; Cullis, P. R. *Chem. Phys. Lipids* **1986**, *40*, 89.

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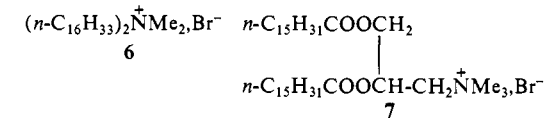
gestive of 3-4 lamellae. The drying process probably accounts for the smaller diameter, as compared to the fully hydrated vesicles studied by light scattering.

Native vesicular **2** is surprisingly unreactive toward active ester substrates such as *p*-nitrophenyl hexanoate (PNPH).[14a] At [**2**] = 1.0×10^{-3} M, k_ψ for this esterolysis is 4.2×10^{-4} s^{-1}, 27 000 times less than $k_\psi = 11.4$ s^{-1} for the analogous reaction of PNPH with vesicular **1**.[4] However, the reactivity of **2** is strongly potentiated in covesicles with nonfunctional cationic surfactants **6**



or **7**; cf., Figure 1 and Table I. Thus, at [**2**] = 3.5×10^{-4} M and [**6** or **7**] = 7.0×10^{-4} M, i.e., [total surfactant] = 1.05×10^{-3} M, k_ψ for PNPH cleavages are increased to 3.2×10^{-2} s^{-1} (by **6**) and 1.1×10^{-2} s^{-1} (by **7**). Correction for [**2**], affords second-order rate constants, k_2, of 91 M^{-1} s^{-1} (**2** + **6**) and 31 M^{-1} s^{-1} (**2** + **7**) for the covesicular cleavages of PNPH, representing kinetic enhancements of 217 and 74, respectively, over native vesicular **2** (Table I, cases 1-3).[14b] Similarly, the covesicles were also reactive (although not as reactive as native vesicular **1**) toward substrates 4-acetoxy-3-nitrobenzene sulfonate (ANBS)[4] and *p*-nitrophenyl acetate (PNPA); see Figure 1 and Table I, cases 5-10.

How is the imidazole moiety of **2** "switched on" in the covesicles? We suggest that the reactivity of vesicular **2** is controlled by the accessibility of the imidazole moieties to substrate. Phosphatidylcholine vesicles feature extensive electrostatic interactions between the N^+ and ^-O-P of adjacent headgroups; consequently, these lie parallel to the bilayer surface.[15] In native phospholipid **2**, this may "bury" the exovesicular imidazole moieties in the vesicular surface, so that they are relatively inaccessible to substrate. Additionally, in multilamellar vesicles of **2** only a small fraction of the imidazoles will be exovesicular; the good packing of the acylglycerol backbones will deny substrate access to the majority of imidazoles on interior lamellae, thus decreasing vesicular reactivity. Covesicles of **2** with **6** or **7**, in contrast, are much more permeable to substrate, their imidazole residues are consequently more accessible, and the reactivity is enhanced.

These suggestions are supported by measurements of the half-times ($\tau_{1/2}$, s) required for the development of fluorescence by added 1,8-anilinonaphthalene sulfonate (ANS) in stopped-flow experiments with vesicular **2**, (**2** + **6**) and (**2** + **7**). $\tau_{1/2}$ is inversely related to the rate constant for permeation of ANS into the vesicles[16,17] and should also reflect the accessibility of the endovesicular imidazoles of **2** toward the substrates. Native vesicles of **2** show *no* ANS permeation below 35 °C ($\tau_{1/2} = 1.63$ s at 40 °C, where T_c is 36 °C by fluorescence[11]), but 1:2 covesicular (**2** + **6**) shows "instantaneous" ($\tau_{1/2} < 5$ ms) ANS permeation at 20-40 °C, and 1:2 covesicular (**2** + **7**) has $\tau_{1/2} \sim 7$ s at 26 °C.[18]

Clearly, cationic covesicular additives **6** and **7** increase the permeability of vesicular **2** and, hence, access to the interior imidazole nucleophiles.[19] The mechanism of additive action may also involve substitution in the N^+-^-O-P headgroup association[15] of vesicular **2**, thus providing greater mobility and accessibility for the exovesicular imidazole moieties.

Although the reactivity of nucleophilic, imidazole-functionalized, vesicular **2** can be "adjusted" by covesicalization with **6** or **7**,[20] the reactivity of the covesicles remains inferior to that of native vesicular **1**. Partly, this may reflect greater accessibility of the imidazole residues in vesicles constructed solely with the dialkylammonium ion backbone. There could also be intrinsic, structure-based reactivity differences between the imidazole groups of **2** and **1**. However, the pK_a for ($ImH^+ \rightleftharpoons Im + H^+$) of covesicular (**2** + **7**) is ~5.3[21] (vs. ~5.5 for **1**[4]), and the solvent isotope effect (k_{H_2O}/k_{D_2O}) is 1.25 for the 1:2 (**2** + **6**) covesicular cleavage of ANBS. These results implicate the neutral imidazole moiety of **2** (perhaps assisted by hydroxide ion at N-H) in the nucleophilic cleavages of the ester substrates, as is also the case for vesicular **1**.[4]

Acknowledgment. We are grateful to the U.S. Army Research Office for support of this work and to Professor L. D. Simon (Waksman Institute) for electron microscopy.

(14) (a) Kinetic conditions: 0.01 M Tris buffer, pH 8.0 ± 0.1, $\mu = 0.01$ (KCl), 4 vol % EtOH, 25 °C, [substrate] = 1.0×10^{-5} M. k_ψ was determined by monitoring *p*-nitrophenoxide ion at 400 nm. Reproducibilities of k_ψ were generally <±2%, although one case featured ±7%. All runs in Table I or Figure 1 conformed to *good* pseudo-first-order kinetics with $r > 0.998$ over >90% of reaction. (b) Vesicles of **6** or **7** are not particularly reactive toward PNPH. Under the standard buffer conditions,[14a] PNPH is cleaved with $k_\psi = 5.43 \times 10^{-5}$ s^{-1}. With 1×10^{-3} M vesicular **6** or **7**, k_ψ increases to 1.34×10^{-4} or 1.18×10^{-4} s^{-1}, respectively, representing enhancements of 2.5 (**6**) or 2.2 (**7**). In contrast, the kinetic enhancements in PNPH cleavage (relative to buffer) are 589 (**2** + **6**) or 203 (**2** + **7**) for the covesicular systems. The differences between (**2** + **6**) and (**2** + **7**) are real and far beyond the reproducibilities of the kinetic data.

(15) Yeagle, P. L. *Acc. Chem. Res.* **1978**, *11*, 321.

(16) Moss, R. A.; Swarup, S.; Wilk, B.; Hendrickson, T. F. *Tetrahedron Lett.* **1985**, *26*, 4827.

(17) Haynes, D. H.; Simkowitz, P. *J. Membr. Biol.* **1977**, *33*, 63.

(18) T_c's for vesicular phase transitions from the "rigid" gel to the more fluid liquid crystalline phases were determined both by fluorescence polarization[11] and from discontinuities in Arrhenius plots (k_ψ vs. $1/T$) for PNPH cleavage. For native vesicles of **2**, $T_c = 36$ °C (fluorescence) or 31 °C (Arrhenius); for 1:2 covesicles of (**2** + **6**) or (**2** + **7**), $T_c = 27$ or 47 °C, respectively, by either method. Plots of fluorescence polarization vs. temperature[11] for (**2** + **6**) or (**2** + **7**) showed significant changes in T_c (in comparison to **2**) but only single, sharp, gel-to-liquid crystal transitions, suggesting both efficient intravesicular mixing of **6** or **7** with **2** and the absence of surfactant "sorting". Neither pure vesicular **2** nor **7** permits ANS permeation below their respective T_c's of 36 or 52 °C. Vesicular **6** is readily permeable at temperatures as low as 15 °C.

(19) The esterolytic reactivities of vesicular (**2** + **6**) and (**2** + **7**) increase by factors of 18 and 6, respectively, at T_c ± 3 °C, further implicating permeability of the substrate and fluidity of the vesicle interiors as reactivity controlling factors. Significantly, the reactivity of holovesicular **2** increases much less (factor of 2.5) at T_c ± 5 °C.

(20) A 2:1 ratio of **6** or **7** to **2** appears optimum for this purpose.

(21) This value comes from a rate constant vs. pH profile for the cleavage of PNPH by 1:2 covesicular (**2** + **7**).

Dimethylsilylene Insertion into Tantalum-Hydride Bonds

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The insertion of silylenes into heteronuclear single bonds is the most well-established type of reaction for these divalent intermediates.[1] As part of our studies of silylene transfer to transition-metal substrates we recently reported the synthesis of dimethylsilyl complexes from molybdenum hydrides by using hexamethylsilacyclopropane (HMS),[2] a source of dimethylsilylene under mild conditions.[3] The apparent insertion of dimethylsilylene into the Mo-H bonds, however, was found to be the net result of a radical chain mechanism, which does not involve dimethylsilylene. We now report the silylation of tantalum hydride complexes with HMS, which apparently proceeds via the insertion

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