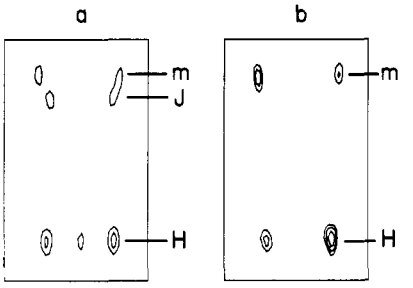

Figure 2. A small region of the intensity contour map of Figure 1 showing the doublets from sites c and d which are coupled to sites m, J, and H at higher fields. The frequencies of the resonances from sites c and d are degenerate, so the connectivity pattern remains ambiguous in spectrum a. However in spectrum b resonances from proton-bearing sites have been suppressed, leaving only the quaternary site c, establishing its connectivity to m and H but not J.

of data acquisition, during which the proton decoupler was switched off, allowing ^{13}C magnetization vectors from all proton-bearing sites to precess into antiparallel alignments, giving zero net signal when the proton decoupler is switched on again.[18,19]

The small region of the contour map corresponding to the chemical shifts of site c and d is enlarged in Figure 2. In the regular spectrum (a) ambiguity remains, but the spectrum (b) from quaternary sites shows that c is coupled to H but not to J. Hence the head-to-tail structure is confirmed.

With the details of the connectivity known, a tentative assignment of the stereochemistry can be proposed.[20] The question of why a single dimer is formed in a photochemically reversible reaction occurring in homogeneous solution remains unanswered.[21]

Registry No. 1, 82902-61-2.

(18) Anet, F. A. L.; Jaffer, N.; Strouse, J. 21st Experimental NMR Conference, Tallahassee, Fl, 1980.
(19) LeCocq, C.; Lallemand, J. *J. Chem. Soc., Chem. Commun.* **1981**, 150–152.
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Formation and Enhanced Stability of Fluoroalkyl Bilayer Membranes

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Contribution No. 665
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Received June 21, 1982

It has been established that the bilayer membrane is formed from totally synthetic amphiphiles.[1] These amphiphiles are composed of cationic, anionic, nonionic, or zwitterionic head groups and of double-chain alkyl groups[2] or single-chain units with rigid segments[3] as hydrophobic moieties. Thus, the bilayer formation can be considered as a general physicochemical phenomenon observed for a large variety of amphiphiles, although these bilayer-forming compounds have been limited to hydrocarbon amphiphiles. Fluorocarbon amphiphiles are known to show surfactant behavior quite different from that of the corresponding hydrocarbon amphiphiles such as low surface tension,[4] limited miscibility with hydrocarbon micelles,[5] and formation of giant micelles.[6,7]

(1) Kunitake, T.; Okahata, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3860–3861 and subsequent papers.
(2) Reviews: Kunitake, T. *J. Macromol. Sci., Chem.* **1979**, *A13*, 587–602. Fendler, J. H. *Acc. Chem. Res.* **1980**, *13*, 7–13.
(3) Kunitake, T.; Okahata, Y.; Shimomura, M.; Yasunami, S.; Takarabe, K. *J. Am. Chem. Soc.* **1981**, *103*, 5401–5413.
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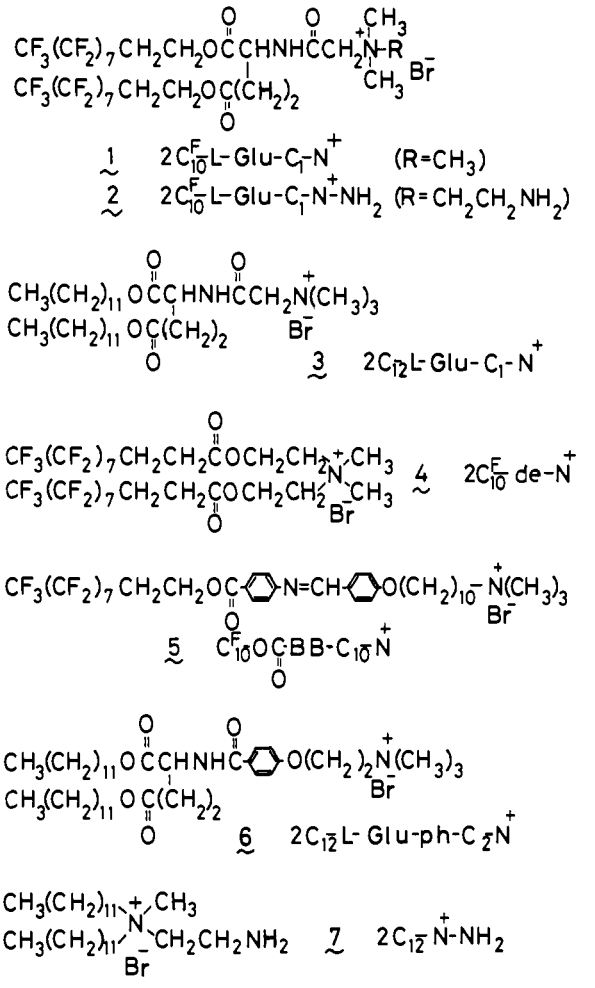
Chart I



Therefore, if bilayer membranes of fluorocarbon amphiphiles are prepared, they would show interesting characteristics that combine organized assemblage of the bilayer membrane and peculiar physicochemical properties of fluorocarbon compounds. We describe in this communication the first examples of fluoroalkyl bilayer membranes.[8]

More than 20 fluoroalkyl amphiphiles have been newly synthesized in these laboratories.[9] Their aggregation characteristics in water are in general similar to those of the hydrocarbon counterparts. For example, a well-sonicated aqueous solution of $2C_{10}^{F}$-L-Glu-C_1-N^+ (**1**, Chart I) gives single- and double-walled vesicles with diameter of 500–2000 Å (Figure 1),[10] and a solution of the hydrocarbon counterpart, $2C_{12}$-L-Glu-C_1-N^+ (**3**) contains small, multiwalled bilayer vesicles. Amphiphile **4** gave multiwalled vesicles. We showed previously that amphiphiles derived from

(6) Hoffmann, H.; Ulbricht, W.; Tagesson, B. *Z. Phys. Chem. (Wiesbaden)* **1978**, *113*, 17–36.
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(8) Similar attempts are being done at the University of Mainz, Germany: Ringsdorf, H., personal communication.
(9) The fluoroalkyl amphiphiles were synthesized from *1H,1H,2H,2H*-perfluorodecanol or *2H,2H,3H,3H*-perfluoroundecanoic acid (Ugine Kuhlmann) by the procedures used for preparation of the corresponding hydrocarbon amphiphiles, and the final products were identified by thin-layer chromatography, NMR spectroscopy, and elemental analysis; Okahata, Y.; Yasunami, S., unpublished results from these laboratories.
(10) The formation of the vesicle structure was confirmed by trapping experiments which had been used for hydrocarbon vesicles.[11] For this purpose, aqueous dispersions of **1** were prepared in the presence of water-soluble amines (D-glucosamine and ethanolamine) and separated from untrapped amines by gel chromatography (Sephadex G-50). Trapped amines were detected by reaction with excess fluorescamine. Trapping of fluorescent riboflavin was also ascertained.
(11) Kunitake, T.; Okahata, Y.; Yasunami, S. *Chem. Lett.* **1981**, 1397–1400.

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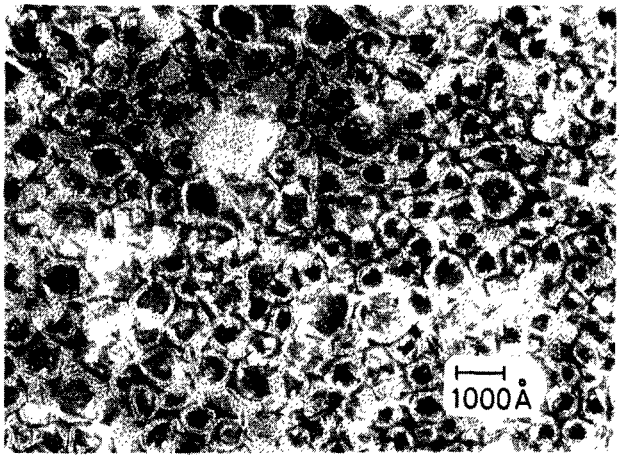
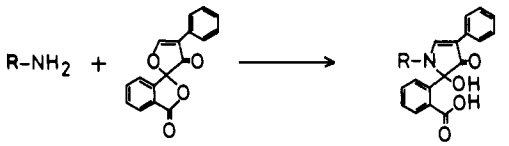


Figure 1. Electron micrograph of fluoroalkyl vesicles (20_{10}^{F}-L-Glu-C_1-N^+ (**1**), 10 nM); stained by uranyl acetate; magnification × 13 333.

dialkyl glutamate give well-developed bilayer structures.[12] This is also the case with fluoroalkyl amphiphiles. The bilayer structure was similarly observed for single-chain ammonium amphiphiles that contain the diphenylazomethine rigid segment and the fluoroalkyl tail (**5**), in consistence with the results obtained for the corresponding amphiphiles with hydrocarbon tails.[13] The aggregation behavior of these new fluorocarbon amphiphiles will be described more fully elsewhere.

One of the most interesting functions expected for fluoroalkyl bilayer vesicles would be their compartmentalization capabilities, since fluorocarbons show much reduced affinities toward organic and inorganic species. Fluorescamine has been used to label amino phospholipids in liposomes by Lee and Forte.[14] In this technique fluorescamine readily reacts with free amino groups to give a highly fluorescent product (eq 1), while neither the hydrolyzed



(1)

fluorescamine fluorescent

fluorescamine nor the reagent itself is fluorescent. Therefore, the increase in fluorescence intensity is a direct measure of the amount of the reacted amine.

Newly synthesized labels **2** and **7** were cosonicated with the fluorocarbon and hydrocarbon vesicles, respectively. These labels are capable of bilayer formation themselves, should not perturb the bilayer matrix extensively, and should be distributed evenly at the outer and inner surfaces of the vesicles. $2C_{12}$-L-Glu-Ph-C_2-N^+, **6**, is selected as the hydrocarbon vesicle, because it forms well-defined, single-walled vesicles.[13]

Figure 2 shows the reaction of fluorescamine with amine-labeled aggregates. In all cases, given amounts of surfactants and labels were sonicated together in water and the solutions allowed to react with fluorescamine at 20 °C.[14,15] In the case of the micelle of nonionic surfactant, Triton-X-100 (curve A), amine label **7** reacts quantitatively with added fluorescamine, in consistence with the globular structure of the micelle (the label is located at the outer surface). In the case of the hydrocarbon vesicle of **6**, approxi-

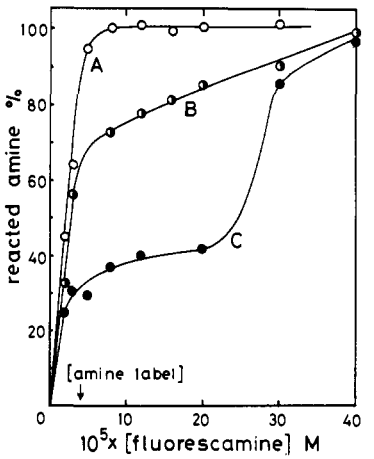
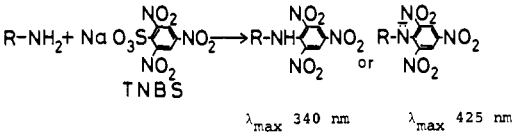


Figure 2. Reaction of fluorescamine with amine-labeled micelles and bilayers (20 °C, 0.02 M borate buffer, pH 8.20, μ 0.01 (KCl)): (A) 5 × 10^{-3} M Triton X-100, 4 × 10^{-5} M $2C_{12}$-N^+-NH_2; (B) 8 × 10^{-4} M $2C_{12}$-L-Glu-Ph-C_2-N^+, 4 × 10^{-5} M $2C_{12}$-N^+-NH_2; (C) 8 × 10^{-4} M $2C_{10}^{F}$-L-Glu-C_1-N^+, 4 × 10^{-5} M $2C_{10}^{F}$-L-Glu-C_1-N^+-NH_2.

mately two-thirds of the label amine reacts readily; however, the rest react more sluggishly in the presence of excess fluorescamine. These results are in agreement with the presence of single-walled vesicles, since two-thirds of the label amine should exist at the outer surface when an even distribution is assumed. The secondary labeling process is probably induced by penetration of fluorescamine into the inner-surface of the vesicle. In the case of the fluorocarbon vesicle, the reaction ceases to proceed at 40%, implying that fluorescamine reacts only with the amine label located at the outer surface of the double-walled vesicle (see Figure 1). The subsequent, drastic increase in the reaction may be produced by formation of the membrane defect due to accumulation of the reacted amine.

Lee and Forte described fluorescence quenching of fluorescamine-labeled liposomes by fusion with liposomes labeled with trinitrobenzenesulfonate (TNBS eq 2).[14]



(2)

This technique can be used conveniently for examination of the ease of mixing of the fluorocarbon vesicle. Four kinds of amine-labeled vesicles (A–D) were prepared by sonication and allowed to react quantitatively with TNBS or fluoroescamine.

A	$2C_{12}$-L-Glu-Ph-C_2-N^+	8 × 10^{-3} M
	$2C_{12}$-N^+-NH_2 (TNBS)	4 × 10^{-4} M
B	$2C_{12}$-L-Glu-Ph-C_2-N^+	8 × 10^{-4} M
	$2C_{12}$-N^+-NH_2 (fluorescamine)	4 × 10^{-5} M
C	$2C_{10}^{F}$-L-Glu-C_1-N^+	8 × 10^{-3} M
	$2C_{10}^{F}$-L-Glu-C_1-N^+-NH_2 (TNBS)	4 × 10^{-4} M
D	$2C_{10}^{F}$-L-Glu-C_1-N^+	8 × 10^{-3} M
	$2C_{10}^{F}$-L-Glu-C_1-N^+-NH_2 (fluorescamine)	4 × 10^{-5} M

The time course of the fluorescence quenching was measured after mixing 100 μL of aqueous solutions of the quenching vesicle (Q vesicle: A and C) with 900 μL of solutions of the fluorescent vesicle (F vesicle: B and D). Figure 3 summarizes the quenching experiment. As shown in curve a, the fluorescence intensity is diminished instantaneously due to dilution when a vesicle solution that does not contain the quencher unit is added. When vesicles A and B are mixed, the instantaneous decrease is accompanied by further rapid decreases (curves b and c). The latter stage is apparently induced by fusion and/or component exchange of F and Q vesicles. In contrast, when the fluorocarbon F vesicle is mixed with the fluorocarbon Q vesicle (curve d) or with the hydrocarbon Q vesicle (curve e), there are observed no decreases

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(15) A labeled vesicle solution (1 mL) was mixed with a given amount of fluorescamine in acetone with vigorous shaking. The mixture (acetone concentration is less than 2%) was maintained for 30–40 min at room temperature, a 100-μL aliquot was added to 1 mL of 5 mM citrate in ethanol, and the fluorescence intensity at 480 nm (excitation wavelength, 390 nm) was determined at 20 °C. The fluorescamine reaction was essentially over in 1–2 min, since the fluorescence intensity did not increase upon further reaction. Excess fluorescamine is rapidly hydrolyzed to a nonfluorescent product.[14]

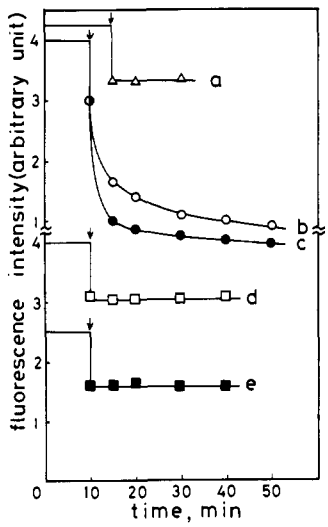

Figure 3. Mixing of fluorescent vesicles (B and D) and quencher vesicles (A and C). The quencher vesicles were added at time indicated by the arrows (see text for vesicle compositions): (a) A (without quencher) + B at 40 °C; (b) A + B at 20 °C; (c) A + B at 40 °C; (d) C + D at 40 °C; (e) D + A at 40 °C.

in the intensity, apart from the instantaneous decrease. This indicates that the fluorocarbon vesicle does not undergo fusion and/or component exchange with the hydrocarbon or fluorocarbon vesicles.

In conclusion, double-chain, fluoroalkyl amphiphiles are shown to form stable bilayer membranes when dispersed in water. The fluoroalkyl bilayer is apparently much less permeable than the hydrocarbon counterpart and does not undergo facile mixing with other bilayers. The peculiar characteristics of the fluorocarbon bilayer such as strong aggregation, O_2 binding, and reduced permeability provide exciting possibilities.

Acknowledgment. We are grateful to Professor M. Takayanagi for the use of an electron microscope (Hitachi H-500).

Registry No. 1, 82838-63-9; **2**, 82838-64-0; **3**, 82838-65-1; **4**, 82838-66-2; **5**, 82838-67-3; **6**, 79508-19-3; **7**, 82838-68-4; TNBS, 5400-70-4; fluorescamine, 38183-12-9.

Hydrogen Bonding in Alcohols: Its Effect on the Carbene Insertion Reaction[1a]

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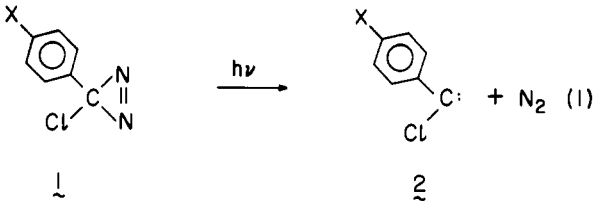
Received April 30, 1982

The insertion reactions of carbenes into the O–H bonds of alcohols have received considerable attention.[2,3] In many instances competitive kinetic studies have been carried out comparing rate constants for insertion of a carbene into different alcohols[2,4] or comparing rate constants for O–H insertion with respect to those for olefin addition.[2,5] The essential assumption has always been that the reaction of the carbene with each of the substrates is first order with respect to that substrate. In this work we report that this simple assumption does not hold for the liquid-phase reactions of carbenes **2a** and **2b** with methanol or *tert*-butyl alcohol.

Laser flash photolysis[6] of diazirines[7] **1a** (0.004 M) and **1b** (0.0067 M) in acetonitrile solvent gave strong absorption spectra due respectively to transients with λ_{max} = 326 and 355 nm. The transient absorption spectrum obtained in the photolysis of **1a** has been characterized as due to phenylchlorocarbene, **2a**, in its singlet state.[10] Using the same experimental criteria[10] on the system containing **1b**, e.g., sensitivity of the transient signal to added oxygen, the failure to observe a triplet EPR signal of the matrix isolated transient at 4 K, etc.,[11] and finding essentially identical results, we assigned that transient absorption spectrum as being due to *p*-anisylchlorocarbene, **2b**, in its singlet state (reaction 1).



1 2

1a, 2a, X = H; 1b, 2b, X = CH_3O

In the absence of added quenchers, **2a** and **2b** decayed predominantly via second-order processes with initial half-lives in the 2–10-μs range, depending on the laser dose.

The transient absorptions due to **2a** and **2b** could be quenched in the normal way by addition of substrates such as tetramethylethylene (TME).[9,12] The reactions were first order with respect to such substrates as evidenced by the excellent linearity of plots obtained by using eq 2. This equation relates the observed

$$k_{obsd} = k_0 + k_2[\text{substrate}] \quad (2)$$

pseudo-first-order rate constant for transient decay, k_{obsd}, to the rate constant for decay in the absence of substrate, k_0, and the rate constant for the reaction of carbene with substrate, k_2.

When methanol was used as a substrate in acetonitrile solvent, plots obtained by using eq 3 showed pronounced curvature (Figure 1). The flash photolysis experiments were repeated in isooctane as solvent, which does not strongly associate with methanol,[14] and the data again showed pronounced curvature. This indicates that the *reactions with the carbenes were not first order with respect to methanol.* In addition, quenching by methanol was about 10 times more efficient in isooctane than in acetonitrile. Spectral properties were similar in both solvents.

Product studies were carried out to verify that the reactions being monitored were indeed the expected insertion processes. Thus, photolysis (λ > 300 nm) of an isooctane solution of **1a** (0.052

(1) (a) Issued as NRCC publication no. 20486. (b) Permanent address: Chemistry Department, University of Prince Edward Island, PEI, Canada.
(2) Kirmse, W. "Carbene Chemistry"; Academic Press: New York, 1964. Jones, M., Jr. "Carbenes"; Wiley: New York, 1973.
(3) For an overview of recent studies in matrices see: Senthilnathan, V. P.; Platz, M. S. *J. Am. Chem. Soc.* **1980**, *102*, 7637–7643.
(4) See, for example: Kerr, J. A.; O'Grady, B. V.; Trotman-Dickenson, A. F. *J. Chem. Soc. A* **1967**, 897–898.
(5) See, for example: Bethell, D.; Stevens, G.; Tickle, P. *Chem. Commun.* **1970**, 792–794. Closs, G. L.; Rabinow, B. E., *J. Am. Chem. Soc.* **1976**, *98*, 8190–8198. Eisenthal, K. B.; Turro, N. J.; Aikawa, M.; Butcher, J. A., Jr.; DuPuy, C.; Hetherington, W.; Korenowski, G. M.; McAuliffe, M. J. *Ibid.* **1980**, *102*, 6563–6565.
(6) The technique and instrument have been described in detail elsewhere; see: Scaiano, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 7747–7753.
(7) The diazirines were prepared following Graham's method[8] and could be obtained analytically pure with appropriate IR and UV characteristics[9] by using chromatographic purification on silica gel. They were always repurified before each experiment since they tend to deteriorate on storage. **Caution**: a cold sample of **1b** decomposed violently on rapid warming to room temperature.
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(11) We thank Drs. K. F. Preston and M. S. Platz for carrying out the EPR measurements.
(12) Griller, D.; Liu, M. T. H.; Scaiano, J. C.; Wong, P. C., to be submitted for publication.
(13) Products were analyzed by using gas chromatography with a mass spectrometer detector. Products could not be analyzed for the reactions of **2b** since the solutions became highly colored shortly after the start of photolysis and screened **1b** from further degradation.
(14) The behavior of neopentyl alcohol is similar to that of methanol and leads to positive curvature.

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