In conclusion, both chemical shift range and favorable relaxation parameters lead us to believe that ^{57}Fe NMR can become a powerful tool for study of molecular structure. Its low sensitivity can be partially overcome by use of selectively enriched materials and by study at high magnetic field, where relaxation times are more favorable. We have extended the known chemical shift scale to include compounds where iron is coordinated to nitrogen atoms and have shown the sensitivity of ^{57}Fe chemical shifts to substituent effects. Further study of ^{57}Fe NMR in hemes and other biologically important molecules is under way in our laboratories.

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Registry No. Fe(PP-IX)(CO)(py), 89210-16-2; $Fe(bpy)_3Cl_2$, 14751-83-8; $Fe(bpy)_3Cl_2$(^{15}N and ^{57}Fe enriched), 89196-90-7; *tert*-butylferrocene, 1316-98-9; 1,1'-bis(chlorocarbonyl)ferrocene, 12288-74-3; (chlorocarbonyl)ferrocene, 1293-79-4; 1,1'-dimethylferrocene, 1291-47-0.

Polymer-Encased Vesicles[1]

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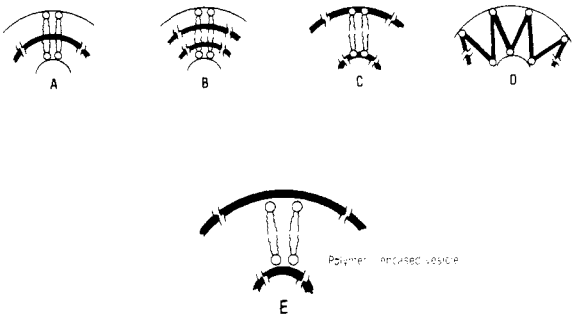
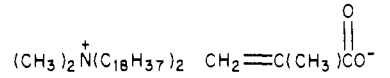
In this communication we introduce a new form of polymerized vesicles in which *a lipid bilayer is encased within two concentric polymerized monolayers*. Such vesicles exhibit improved stability while maintaining the monomeric state of the amphiphile within the bilayer.

Polymerized vesicles are receiving intense interest as models for biomembranes, carriers of drugs, and devices for solar energy conversion.[3-11] They possess many of the structural and physical characteristics found in conventional vesicles but are substantially more stable. All polymerized vesicles that have been reported thus far fall into four classes: those having a polymeric backbone running (A) through the center of the lipid bilayer, (B) through the lipid chains of inner and outer monolayers, (C) through the polar head groups of each monolayer, or (D) through a monolayer lipid membrane. In this report we describe the synthesis and preliminary characterization of polymerized vesicles derived from dioctadecyldimethylammonium methacrylate (DODAM). The





DODAM

uniqueness of these vesicles stems from the fact that the lipid bilayer is not covalently linked together but is, instead, ionically encased within two concentric poly(methacrylate) monolayers (structure E).

Dioctadecyldimethylammonium bromide was converted into DODAM by passage through an anion-exchange resin, AG1-X2, bearing methacrylate ion.[12] Vesicles were prepared by sonic dispersal of 3.0 mg of the surfactant in 2.4 mL of distilled water at 50 °C by using procedures similar to those previously described.[3] Thin-layer chromatography indicated that no lipid decomposition occurred during sonication (R_f = 0.8, silica gel, 3:1 $CHCl_3$/CH_3OH). Vesicle polymerization was carried out by direct UV irradiation at 254 nm (120 min).[13,14] Thin-layer chromatography, using the above conditions, indicated a single spot at the origin and the complete disappearance of the monomer. Electron micrographs recorded on a Philips 400 TEM microscope, using 2% uranyl acetate as a staining agent, confirmed the presence of closed vesicles having diameters ranging between 300 and 600 Å. Significantly, temperature-dependent turbidity measurements (400 nm) confirmed the presence of bilayers within DODAM vesicles, before and after polymerization; both exhibited a well-defined phase transition in the expected range, 44–48 °C.[15,16] Further evidence for closed vesicles comes from the entrapment of (^{14}C) sucrose. By use of procedures similar to those previously described,[3,14] nonpolymerized DODAM vesicles entrapped 1.6% of the radioactive marker and retained 75% of the trapped label after 24 h of dialysis against distilled water; polymerized vesicles showed similar entrapment and retained 88%. Dialysis of polymerized and nonpolymerized vesicles against 23% ethanol (v/v) for 1 h at room temperature resulted in 89% and 20% retention of the sucrose, respectively. In contrast to their nonpolymerized counterparts, which begin to the precipitate on standing after 5 days at room temperature, photopolymerized dispersions of DODAM showed no detectable change after 30 days.

Treatment of polymerized DODAM (derived from a 126-mg-scale vesicle preparation) with 1.0 M HCl (48 h, 23 °C) followed by freeze-drying and repeated solubilization in CH_3OH and precipitation with anhydrous ether afforded a 49% yield of poly(methacrylic acid) having an IR spectrum that was identical with that of an authentic sample; the viscosity-average molecular weight, determined in 0.002 M HCl, was 85 000.[17,18] Analysis

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(12) The chloride form of AG1-X2 was first converted into a hydroxide form and then treated with excess methacrylic acid. DODAM: ^1H NMR ($CDCl_3$) δ 0.88 (t, 6 H, CH_3CH_2), 1.25 (s, 64 H, CH_3CH_2), 1.92 (s, 3 H, $CH_3C{=}$), 3.18–3.45 (m, 10 H, $(CH_3)_2N(CH_2)_2$), 5.26 (m, 1 H, vinyl), 5.88 (m, 1 H, vinyl).

(13) The temperature of the sample during UV irradiation was ca. 40 °C. Specific photopolymerization procedures used were similar to those previously described.[14]

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of a DMF-d_7 solution of this polymer, by ^{1}H NMR (250 MHz) at 100 °C, further revealed that it was 72% isotactic, 15% syndiotactic, and 13% atactic in nature.[19]

Polymer-encased vesicles, such as those derived from DODAM, combine the best features found in synthetic membrane models with those of polymerized analogues; i.e., they retain a monomeric lipid assembly and are stabilized by polymeric counterions. For model studies that focus on the orientational properties and chemical effects of biomembranes, such vesicles may prove extremely valuable.[20] Moreover, the antiviral activity, tumor growth inhibition, and interferon induction associated with poly(acrylic acid) suggest that DODAM and related vesicles may serve as unique polymeric drugs as well as drug carriers.[21] Finally, the ready availability of polymerizable surfactants of the type described herein should greatly facilitate the entry of other workers into the polymerized vesicle area.

Efforts now under way are aimed at expanding this new and unique class of vesicles and applying it to drug delivery, membrane modeling, and solar energy conversion.

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(22) **Note Added in Proof**: Since submission of this work we have learned that 4-vinylpyridine has been shown to spontaneously polymerize on acidic liposomal surfaces, yielding a stable polymeric coating: Aliev, K. V.; Ringsdorf, H.; Schlarb, B.; Leister, K.-H.; *Makromol. Chem. Rapid Commun.*, in press. We are grateful to Professor Ringsdorf for bringing this to our attention.

On the Origin of the Isocyano Function in Marine Sponges[1]

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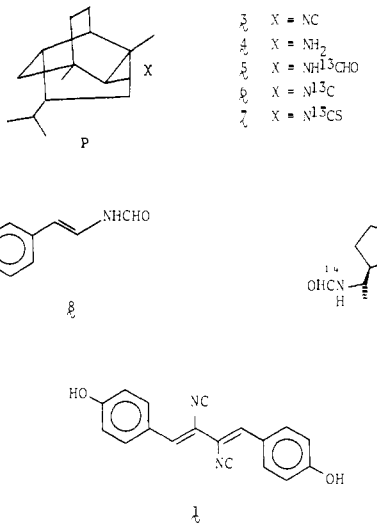
The biogenetic origin of the isocyano function has posed an intriguing, yet unsolved question ever since the first naturally occurring isocyano compound, the *Penicillium* metabolite xanthocillin (**1**), was characterized.[2] Labeling experiments by Achenbach and Grisebach[3] proved that tyrosine furnished the backbone of **1**, but neither formate nor methionine were incorporated into the isocyano group. Later, when we encountered in a sponge an isocyanosesquiterpene and a -diterpene, each accompanied by a formamide and an isothiocyanate, we[4] and others[5] conjectured that the formamides might well be the proximate precursors of the isocyanides. Sodano and co-workers[6] disproved this hypothesis. They injected an ethanolic solution of ^{14}C-labeled



axamide (**2**) into the sponge *Axinella cannabina*, which had been placed in a 10-L aquarium, yet failed to detect labeled isocyanide after 5 days. Up to 15% of radioactivity was recovered in the formamide.

In our experiments on the origin of the isocyano function in sponges, we left the animal, *Hymeniacidon* sp., in its natural habitat until completion of the experiment.[7] We used scuba to embed ^{13}C-labeled precursor encased in double gelatin capsules in the live animal. At the conclusion of the experiment, we removed the sponge and analyzed the extract by GC-MS. In this fashion we confirmed Sodano's[6] earlier finding that the formamide is not a precursor of the isocyanide. We further showed that the isothiocyanate also is not transformed into the isocyanide. We sucessfully demonstrated that, instead, both formamide and isothiocyanate are biosynthesized from isocyanide. In analogy with the xanthocillin experiments,[3] we also found that formate is not used by the sponge as a source of the isocyano carbon.

The labeled precursors were synthesized from 2-isocyanopupukeanane (**3**), mp 78–83 °C, isolated as previously described[8] from *Hymeniacidon* sp.,[9] as shown in Scheme I; the sponges were collected by scuba at Shark's Cove, Oahu, at −10 to −15 m.

Incorporation experiments were conducted at Shark's Cove, Oahu. For each experiment a relatively small sponge (estimated wet wt about 200 g) was selected, and a nearby animal was used as control. Labeled precursors were placed inside two gelatin capsules and carried to the dive site in a plastic jar. An incision was made in the sponge with a sharpened spatula. The capsule was quickly inserted and the incision was capped with a piece of sponge from a nearby animal.[10] At the end of the incubation (1 or 2 weeks) the sponge and a control animal were harvested and frozen until workup. Each sponge was extracted with acetone, the extract partitioned with hexane, and the organic layer injected into the Grobb injection port interfaced with a 30-m DB-5 fused silica capillary column (J&W Scientific), which was coupled to a Finnigan MAT OWA Model 30B mass spectrometer. Since labeled and unlabeled compounds coeluted, the criterion for incorporation was the enhancement of the $M^+ + 1$ and $M^+ + 1 -$ Me peaks, since few other fragments below m/z 217/216 retain the functional group. In the experiments with ^{13}C-labeled **5** and

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(10) We thank Dr. P. Bergquist for suggesting this technique.
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