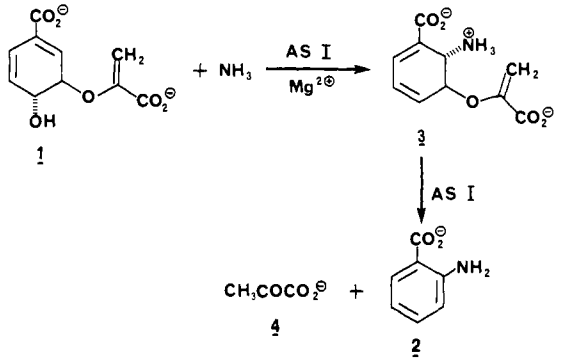
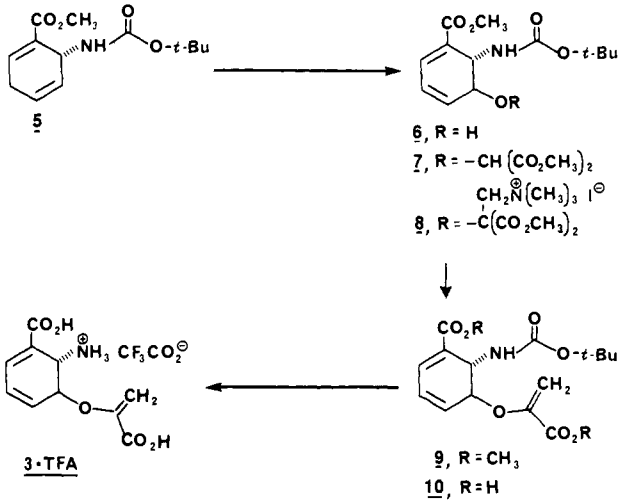
Scheme I



Scheme II



action of **6** with dimethyl diazomalonate and 1 mol % $Rh_2(OAc)_4$ in benzene at 65 °C gave **7** (56%).[13] Reaction of **7** with Eschenmoser's salt [$CH_2{=}N(CH_3)_2{}^+I^-$, $(C_2H_5)_3N$, CH_2Cl_2] and quaternization of the Mannich base (CH_3I, CH_2Cl_2) provided **8** (100%). Base-induced fragmentation (1.5 equiv of NaOH, THF/H_2O, 0 °C, 45 min) gave **9**[14] (46%). Saponification of **9** (2.2 equiv of NaOH, THF/H_2O, 4 °C, 40 h) followed by acidification with Amberlite IR-120 resin afforded **10** (94%). Treatment of **10** with dry, freshly distilled CF_3CO_2H (TFA) at 0 °C for 15 min followed by workup gave salt **3**·TFA (43%).[15,16]

To test compound **3** as a potential intermediate in the enzymic biosynthesis of anthranilate from chorismate and ammonia, samples of the trifluoroacetate salt, **3**·TFA, were incubated with pure *S. marcescens* AS I enzyme.[17] For comparison chorismate,[18] with or without ammonia, was used as control substrate. Compound **3** was an excellent substrate, undergoing enzymic conversion to anthranilate[19] in the absence of NH_4^+ with a K_m of 0.2 mM and V_{max} of 300 (nmol/min)/mg enzyme compared to a K_m of 0.11 mM and V_{max} of 500 (nmol/min)/mg for the natural substrate chorismate[17,20] in the presence of ammonia. In the absence of NH_4^+ ions, chorismate gave no anthranilate. Addition of 50 mM NH_4^+ to enzymic incubations of **3**·TFA did increase V_{max} values ca. 2-fold such that under these conditions **3**·TFA was processed to anthranilate at higher V_{max} than chorismate so **3**·TFA is both a kinetically and chemically competent candidate for a reaction intermediate.

It was anticipated that anthranilate synthase would act stereospecifically on only one of the enantiomers of (±)-**3**·TFA, presumably the 5*S*,6*S* isomer. In incubations containing 0.2–3.2 mM **3**·TFA[15] with varying enzyme levels, we routinely observed 24–27% conversion in the absence of NH_4^+ and 35% in the presence of NH_4^+ by fluorescence assay.[17] In parallel incubations where coproduct pyruvate (**4**) was monitored by coupled in situ reduction by L-lactate dehydrogenase and NADH, 34–35% conversions were detected, with or without added NH_4^+. This is substantial conversion but less than 50% for reasons as yet unclear.[21]

In sum, compound **3** is processed enzymically to anthranilate by the *S. marcescens* synthase at rates that support its role as reaction intermediate and thereby substantiate the mechanism of Scheme I for this enzyme, with trans geometry in the amino enol pyruvyl intermediate.

Acknowledgment. We are grateful to Professor H. Zalkin for a generous gift of AS I from *S. marcescens*, to the National Institutes of Health, Grants GM 20011 and GM 31958, for financial support, and to the National Science Foundation for a predoctoral fellowship to K.G.A.

Supplementary Material Available: Physical data for **6**, **7**, **9**, and **3**·TFA (1 page). Ordering information is given on any current masthead page.

(13) Procedure of Ganem, B.; Ikota, N.; Muralidharan, V. B.; Wade, W. S.; Young, S. D.; Yukimoto, Y. *J. Am. Chem. Soc.* **1982**, *104*, 6787–6788.
(14) Trans stereochemistry for the substituents at C_5 and C_6 of **9** was established from the ^1H NMR spectrum of the products from reaction of **9** with 4-phenyl-1,2,4-triazoline-3,5-dione. Two Diels–Alder adducts were formed. For one adduct J = 2.9 Hz for the two H's derived from H_5 and H_6 of **9**. The corresponding J for the other adduct was 1.8 Hz.
(15) **3**·TFA: mp 93–95 °C; IR (KBr) 3600–3250, 1685, 1630 cm^{-1}; UV (H_2O), 280 nm (ϵ 5900); ^1H NMR (CD_3OD) δ 7.37 (1 H, d, J = 5 Hz), 6.47 (2 H, m), 5.63 (1 H, d, J = 3 Hz), 5.07 (1 H, d, J = 3 Hz), 4.53 (1 H, d, J = 6 Hz), the remaining absorption is obscured by the DOH peak.
(16) Salt **3**·TFA is a white, nonhygroscopic powder. The neutral amino acid (hygroscopic) can be obtained by eluting a cold aqueous solution of **3**·TFA through a column of ion-retardation resin AG11A8 (Bio-Rad Corp). The material is most conveniently handled in salt form.
(17) Assay conditions were adapted from Zalkin and Kling (Zalkin, H.; Kling, D. *Biochemistry* **1968**, *7*, 3566–3573) and were performed at 26 °C with a Perkin-Elmer LS-3 Fluorimeter. Buffers were adjusted to be pH 8.6 with or without NH_4^+.
(18) Chorismate was isolated from culture growth of *K. pneumoniae* 62-1 (formerly *A. aerogenes* 62-1) according to: Gibson, F. *Methods Enzymol.* **1970**, *17A*, 362–364. We thank Professor F. Gibson for a generous gift of *K. pneumoniae* 62-1.
(19) Monitored as in ref 17 and also by TLC on silica plates, developed in 80:18:2 ether:hexane:acetic acid and 93:5:2 $CHCl_3$:CH_3OH:CH_3COOH.
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(21) The extent of conversion did not reflect any inhibition by K^+TFA^-, nor was it increased by additional amounts of enzyme. It is conceivable but not obviously due to enzyme inactivation or to nonenzymic breakdown of **3**·TFA during incubations on the basis of the experimental results reported herein.

^{57}Fe NMR: Relaxation Mechanisms and Chemical Shifts

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Despite the importance of iron in biological, organometallic, and coordination chemistry, only limited studies of ^{57}Fe NMR have been reported.[2-7] ^{57}Fe, the only isotope of iron suitable for

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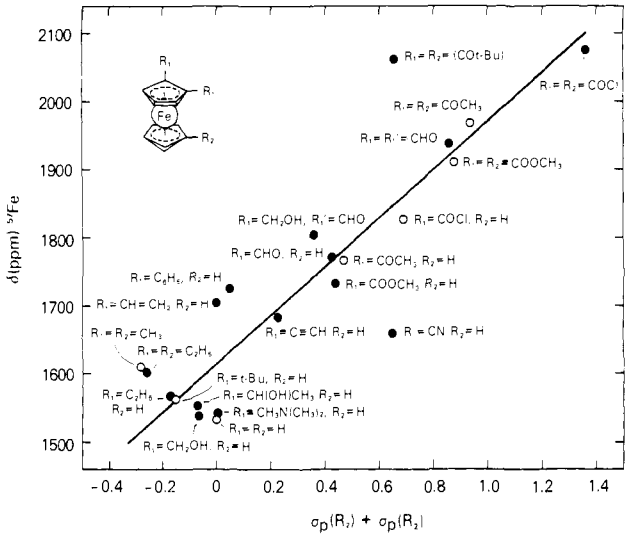

Figure 1. Plot of ^{57}Fe chemical shift vs. the sum of the Hammett constants for the substituent group on the ferrocene moiety. σ_p values from 0. Exner.[8] Excluding shifts measured for di-*tert*-butylacetylferrocene (for steric reasons) and cyanoferrocene (standard T test), a correlation parameter of 0.95 is calculated for 19 compounds. (○) This work; (●) data from ref 7.

NMR study, has spin $^1/_2$ but a sensitivity only 7.4×10^{-7} times that of the proton when natural abundance (2.2%) materials are used. Little has been reported regarding relaxation behavior of ^{57}Fe.

We report here ^{57}Fe NMR results at natural abundance in some compounds and enriched to 90% in others. These data provide information on (1) the chemical shift range expected for iron coordinated to nitrogen, (2) the effect of substituents on ^{57}Fe chemical shifts, and (3) the relaxation mechanisms for ^{57}Fe; thus they define the basic parameters needed for the further development of ^{57}Fe NMR.

We measured the ^{57}Fe chemical shifts in eight ferrocenes and present these data (relative to $Fe(CO)_5$) in Figure 1, along with those reported by Haslinger, et al.[7] These chemical shifts span 500 ppm and correlate rather well with Hammett σ_p parameters. This sizable range of chemical shifts indicates that the paramagnetic term in the chemical shift equation[9] is dominant. More importantly, the sensitivity of the ^{57}Fe chemical shift to substituents removed from the iron center suggests that it may be useful in studying subtle structural changes in other compounds, including those of biochemical interest.

Previous measurements of ^{57}Fe chemical shifts have been restricted largely to compounds in which iron is in oxidation state 0 or 2+ and is coordinated only to carbon ligands. Jenny et al.[6] reported chemical shifts that cover a range of 3000 ppm. We have measured considerably larger chemical shifts in two compounds in which iron (90% enriched in ^{57}Fe) is coordinated to nitrogen—Fe(PP-IX)(CO)(py)(PP-IX = protoporphyrin-IX), 0.05 M in pyridine, 8211 ppm, and tris(2,2′-bipyridine)iron(II) chloride, 0.06 M in D_2O, 11 269 ppm. Recently Nozawa et al.[10] reported the ^{57}Fe chemical shift of two porphyrins in the vicinity of 7300 ppm. Clearly the effect of the ligands is pronounced, but we

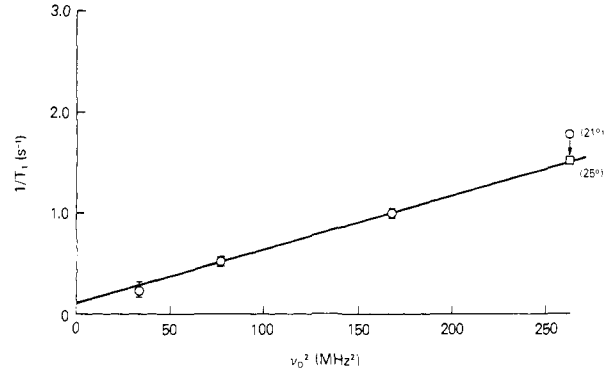

Figure 2. ^{57}Fe spin–lattice relaxation rate in *tert*-butylferrocene vs. square of frequency. Data at 25 °C. Additional data at 270 MHz: 4.1 °C, 0.5 s; 10.5 °C, 1.3 s; 25.0 °C, 1.9 s; 37.5 °C, 3.2 s. At 400 MHz: 10 °C 0.6 s. 80% tert-butylferrocene, 20% acetone-d_6. Samples degassed by three freeze–pump–thaw cycles and sealed under argon. Value at 500 MHz, 25 °C, extrapolated from measured value at 21 °C by assuming same temperature dependence as measured at 270 MHz.

believe that there are too few data available as yet to permit any detailed elucidation of the factors involved. It is interesting to note that the 8772 ppm difference in chemical shifts between $Fe(bpy)_3Cl_2$ and $K_4Fe(CN)_6$ is considerably larger than the 6620 ppm difference for the corresponding ^{59}Co compounds.[11] Jenny et al.[6] have commented on the effect of solvent variation and of temperature change on ^{57}Fe chemical shifts. We too have observed such effects. While small relative to the large range of chemical shifts, they can easily introduce uncertainies of 10–20 ppm.

For efficient NMR study of nuclei, like ^{57}Fe, with weak magnetic moments, it is necessary to be able to estimate relaxation times, which requires a knowledge of relaxation mechanisms. Accordingly, we measured T_1 (spin–lattice) relaxation times of *tert*-butylferrocene at several temperatures and magnetic field strengths.

We found the nuclear Overhauser effect at 4–25 °C, 6.34 Tesla (270 MHz), to be very near zero. Spin–lattice relaxation via dipolar relaxation is unlikely to dominate because of the small ^{57}Fe magnetic moment and relatively long proton-to-iron distances in most compounds. Figure 2 shows that relaxation rate is directly proportional to the square of observation frequency, as expected for relaxation by chemical shift anisotropy,[12] with the small intercept indicating little contribution from other mechanisms, including dipolar relaxation.

These results demonstrate the value in working at the highest possible field strength in order to maximize signal, not only from the more favorable Boltzmann distribution but also from the more rapid pulse repetition permitted by shorter relaxation times. For ^{57}Fe(PP-IX)(CO)(py) at 500 MHz, 23 °C, we have measured T_1 of only 0.17 s. However, even at high field, compounds in which the iron atom is in a more symmetric environment may show much longer relaxation times. For example, the $Fe(bpy)_3Cl_2$ complex at 500 MHz, ca. 23 °C, has an approximate T_1 (null method) of 5 ± 2 s.

With nitrogen ligands, scalar relaxation might affect T_2 and produce undesirably broad lines, even though it is unlikely to influence T_1.[13] For scalar relaxation to occur two conditions must be met: the existence of a significant scalar coupling between ^{57}Fe and ^{14}N and quadrupolar relaxation of ^{14}N within a narrow "intermediate" time range. To assay this effect, we synthesized $Fe(bpy)_3Cl_2$ enriched in ^{15}N (99%) and in ^{57}Fe (90%) and measured from the ^{15}N spectrum $^1J(^{15}N,^{57}Fe) = 8.4$ Hz (confirmed with poorer resolution as a septet in the ^{57}Fe spectrum). From the ^{14}N spectrum of $Fe(bpy)_3Cl_2$ we found a line width of 9200 Hz. By using the standard equations,[13] we obtain a contribution of only 0.01 Hz to the ^{57}Fe line width.

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(13) Reference 12, pp 192–193.

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.

Acknowledgment. We thank P. S. Pregosin for helpful discussions and T. Inubushi for help and advice in sample preparation. The ^{57}Fe data at 13 MHz were obtained at the NSF Regional Facility, University of South Carolina, Grant CHE 82-07445.

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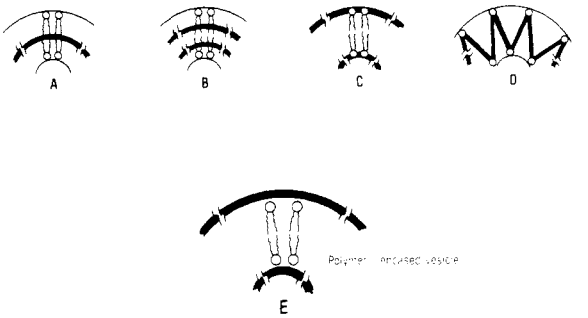
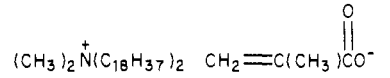
In this communication we introduce a new form of polymerized vesicles in which *a lipid bilayer is encased within two concentric polymerized monolayers*. Such vesicles exhibit improved stability while maintaining the monomeric state of the amphiphile within the bilayer.

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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