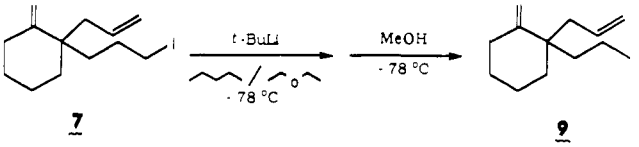

7 9

TMEDA was added to a −78 °C solution of **8** in n-C_5H_{12}–Et_2O, the cooling bath was removed, and the reaction mixture was allowed to warm for 17 min (to ca. +12 °C) before the addition of an excess of electrophile (Table I). As demonstrated by the results summarized in Table I, the product organolithium (**11**, Scheme II) can be trapped with any of a variety of electrophiles to give high (60–80%) isolated yields of functionalized product.[11] It is to be noted that the only other material detected in greater than trace amount from these tandem cyclization reactions was 10–15% of the easily removed *unfunctionalized* parent diene **9**. The presence of diene in the product mixture was not unexpected since, as noted elsewhere,[2] the formation of hydrocarbon formally derived from reduction of the halide is a general occurrence in lithium–halogen exchange reactions involving *t*-BuLi.

The results presented above demonstrate the potential of anion-initiated tandem cyclization as a route to functionalized polycarbocyclic products. Three features of the methodology are worthy of note: (1) The generation of the initial C–Li bond is easily and cleanly accomplished by low-temperature lithium–iodine interchange. (2) Tandem 5-exo-trig cyclization of a diolefinic alkyllithium in the presence of TMEDA is more rapid than competing reactions that consume the anion. (3) The tandem cyclization product is easily functionalized by reaction with any of a variety of electrophiles. We are in the process of extending this approach to the construction of more stereochemically complex systems through higher order sequential (tandem, triplet, etc.) cyclization of suitably constituted substrates.

Acknowledgment. We are grateful to the Humphrey Chemical Company of North Haven, CT for generous unrestricted support of our research.

(11) Tandem cyclization of **8**, followed by addition of an electrophile, afforded mixtures of the exo and endo isomers of 8-CH_2E derivatives of [4.3.3]propellane (Table I) which, in our hands, could not be separated chromatographically.

A Novel, Functional Variant of Cytochrome *c*: Replacement of the Histidine Ligand with Arginine via Site-Directed Mutagenesis

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An u derlying tenet of bioinorganic chemistry is the simple notion tnat a metalloprotein is a large coordination complex in which the protein plays the role of a chelating ligand. Thus, it has been possible to mimic the coordination chemistry of metal ions in proteins with low-molecular-weight synthetic analogues.[2] As an alternate strategy to probing metalloprotein structure and function by studying synthetic models, techniques of genetic manipulation may be useful for preparing mutant proteins that differ from the natural system in the ligation of the metal ions. At least three applications of ligand mutagenesis can be envisaged: (1) to generate coordination environments that have no naturally occurring analogues; (2) to define ranges for a protein's natural function (e.g., redox potential for an electron transport protein); and (3) to change the protein's natural function. One example of this last application has been reported recently for cytochrome b_5 in which the normal electron-transfer function has been replaced by peroxidase activity.[3] In this paper we report our preliminary work on yeast cytochrome *c* that illustrates the other two applications.

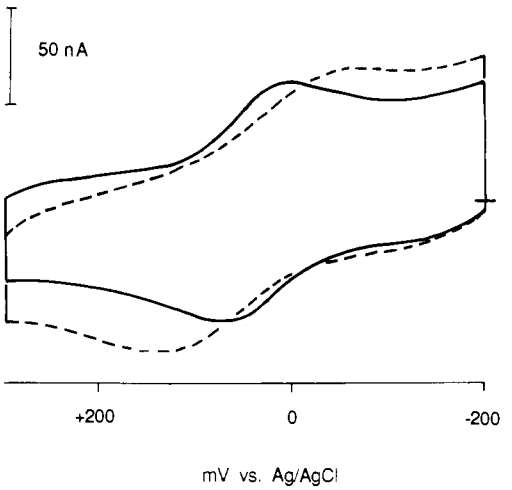

Figure 1. Cyclic voltammetry at a tin-doped indium oxide electrode of wild-type iso-2-cytochrome *c* (—) and C2-18R (– – –). Scan rate = 2 mV/s; protein concentration = 10^{-5} M in 0.8 M NaCl, 0.1 M phosphate buffer, pH 7.8. The potential of the Ag/AgCl reference electrode is +0.23 V vs NHE. The working electrode (0.3 cm^2) pretreatment and horizontal mounting (for slow scan rates) were done as described in ref 22.

Site-directed mutagenesis was carried out on the yeast iso-2-cytochrome c[4] gene (CYC7-H2)[5] at the position corresponding to histidine-18 (vertebrate numbering system).[6] Different plasmids, each containing a separate mutation of CYC7-H2, were used to transform a yeast strain lacking cytochrome c,[12] and the

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(6) Methodologies for mutagenesis at the His-18 codon are described in ref 7, and other materials and methods are given in ref 8. The wild-type gene CYC7-H2 was cloned into pUC118.[9] The 25-mer synthetic oligonucleotide used for mutagenesis contained a mixed population of all four nucleotides at the three positions corresponding to the histidine-18 codon. The oligonucleotide was mixed with uridine-incorporated template in a 2:1 molar ratio, heated to 65 °C, and allowed to cool slowly to room temperature. The mixture was treated with T4 DNA polymerase and T4 DNA ligase and incubated at 37 °C for 1 h. The resultant mixture was transformed in *E. coli* JM109 cells[10] and plated for single colonies which were selected for their resistance to ampicillin. Single strand template DNA prepared[9] from each colony was sequenced by the dideoxy method.[11] After identification of mutants, plasmid DNA was prepared, and the plasmids were used to transform yeast strain E924-4D.[12]
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resulting single colonies were plated on a glycerol medium.[15] Growth resulted in only four cases: one transformant contained a plasmid with a different codon for histidine (CAC); the other three had plasmids coding for arginine (CGA, CGG, or CGT) at position 18.[16,17] Yeast containing cytochrome *c* with the Arg-18 substitution, designated C2-18R, grew more slowly on glycerol or lactate media than yeast having the wild-type protein or the protein derived from the gene with the CAT → CAC mutation.[18]

Small amounts (<1 mg/10 L culture) of C2-18R have been prepared and purified by the literature procedure.[19] The absorption spectra of both reduced and oxidized forms of the protein are nearly identical with those for wild-type iso-2-cytochrome *c* (Supplementary Material). The oxidized form displays a peak at 695 nm, confirming coordination of the methionine-80 ligand,[20] and reduced C2-18R does not react with either CO or O_2, indicating that the heme is still six-coordinate in the variant.

The electrochemical kinetic behavior of C2-18R is significantly different from that of native cytochrome *c*, as shown in Figure 1. At tin-doped indium oxide electrodes,[21,22] wild-type iso-2-cytochrome *c* exhibits an electrochemically reversible response with a peak separation of 60 mV at a scan rate of 2 mV/s. In contrast, the peak separation for C2-18R is 200 mV under the same conditions. Thus, although the formal potential is unaffected by the replacement of histidine by arginine ($E^{\circ\prime}$ = 0.04 V vs Ag/AgCl in both cases), which allows C2-18R to function in the electron transport system, the rate of electron transfer is slower for the variant. The heterogeneous electron-transfer rate constant ($k^{\circ\prime} \approx 10^{-4}$ cm/s for C2-18R) is between 2 and 10 times smaller than $k^{\circ\prime}$ for the wild-type protein, as determined by analysis[23] of cyclic voltammetry data acquired over appropriate ranges of scan rate. Additional electrochemical studies are in progress.

The generation of this new protein is significant because the evolutionariy *invariant* histidine-18 of a cytochrome *c* has been altered without destroying the electron transport function of the protein. Sherman has stated[24] that any replacement at this position would be expected to abolish function, presumably by altering the redox potential of the heme or by changing the protein folding pattern. However, he also notes and presents several examples for proof, that evolutionary invariance does not necessarily imply functional invariance.[24] Whether the guanidyl group of arginine is actually ligating the heme is uncertain, and we are currently preparing well-defined synthetic models of guanidyl-heme coordination to better define the expected properties of this type of complex.[25,26]

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Registry No. Cytochrome *c*, 9007-43-6; L-histidine, 71-00-1; L-arginine, 74-79-3.

Supplementary Material Available: The CYC7-2H (iso-2-cytochrome *c*) gene sequence and autoradiogram of the mutated region of the gene coding for C2-18R and the absorption spectra for reduced and oxidized C2-18R (2 pages). Ordering information is given on any current masthead page.

(12) The yeast strain used (E924-4D) has the following genotype: MATα *cyc*1-1 *cyc*7-67 *ura*3-52 *can*1 *leu*2-3,112 *trp*1-Δ1. The *cyc*1-1 allele is a complete deletion of the iso-1-cytochrome *c* structural gene,[13] and the *cyc*7-67 is a deletion of the iso-2-cytochrome *c* structural gene.[14]

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(16) Several other mutants at the CAT site (His-18) were identified: TAT (Tyr), ACA (Thr), TGT (Cys), CAG (Gln), TCG (Ser), and CTA (Leu). Plasmids containing the CYC7-H2 gene with these codons did not grow on a glycerol medium; yeast cells containing these plasmids grown on 1% S had whole cell spectra that lacked the bands for cytochrome *c*. These mutants provide a valuable control showing that not just any substitution at this position gives a viable protein. They provide indirect evidence that the arginine must be coordinated, otherwise other mutations would be expected to give functional cytochromes.

(17) Plasmids reisolated from yeast grown on glycerol were sequenced to ensure that a reversion had not taken place. The sequence data are included in the Supplementary Material.

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Biomimetic Ion Transport: A Functional Model of a Unimolecular Ion Channel[1]

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The study of biomimetic model systems of ion transport serves to elucidate key features of natural transport processes[2] while at the same time explores the potential for chemical applications in sensors and separations.[3] Although the majority of such studies have focused on mobile carriers,[4] natural transport systems exploit transmembrane channels formed from single or aggregated transport proteins.[5] Only a few studies report the synthesis of functional biomimetic ion channels based on cyclodextrin[6] or polyisocyanide[7] frameworks.[8,9]

We report here the synthesis of a new type of biomimetic ion channel structure and its activity in bilayer vesicles. Our design proposal, sketched in Figure 1, locates a crown ether framework near the bilayer midplane (C). Channel walls (W) would lie above and below the crown ether, extending to the bilayer surfaces. The structure would be maintained by the cooperative action of the polar head groups (H) in contact with the aqueous phases, hydrophobic contacts with the lipids of the bilayer, and the marked

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