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]

Acknowledgment is made to the North Carolina Biotechnology Center, NCSU for Biomedical Research Support Grant RR7071, the University Research Council of UNC-CH, and the A. P. Sloan Foundation for support of this research. We especially thank Professor Beverly Errede for providing the CYC7-H2 gene and the cytochrome *c* deficient yeast strain E924-4D and for her helpful advice and expertise with the DNA work.

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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(14) Errede, B.; Company, M.; Hutchinson, C. A., III *Mol. Cell. Biol.* **1987**, *7*, 258-265.

(15) Yeast strains lacking cytochrome *c* are able to grow on a sucrose medium (1% S). In order to grow on the nonfermentable carbon sources such as glycerol or lactate, the electron transport chain must be functional.

(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.

(18) This mutation of the gene still codes for His, thus the *protein* is not a variant.

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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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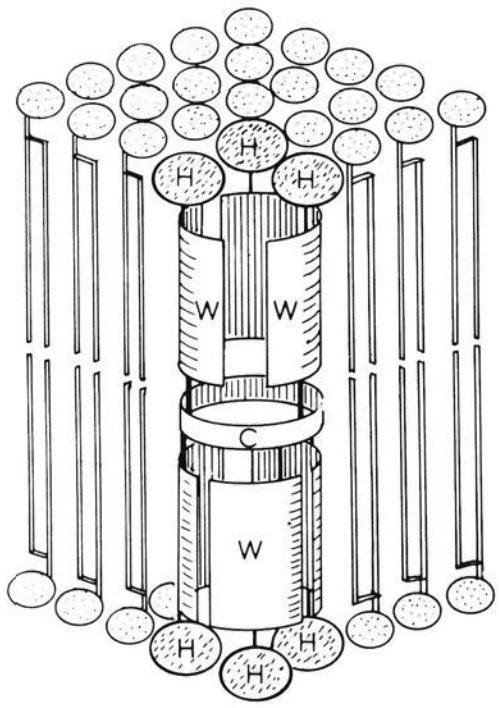
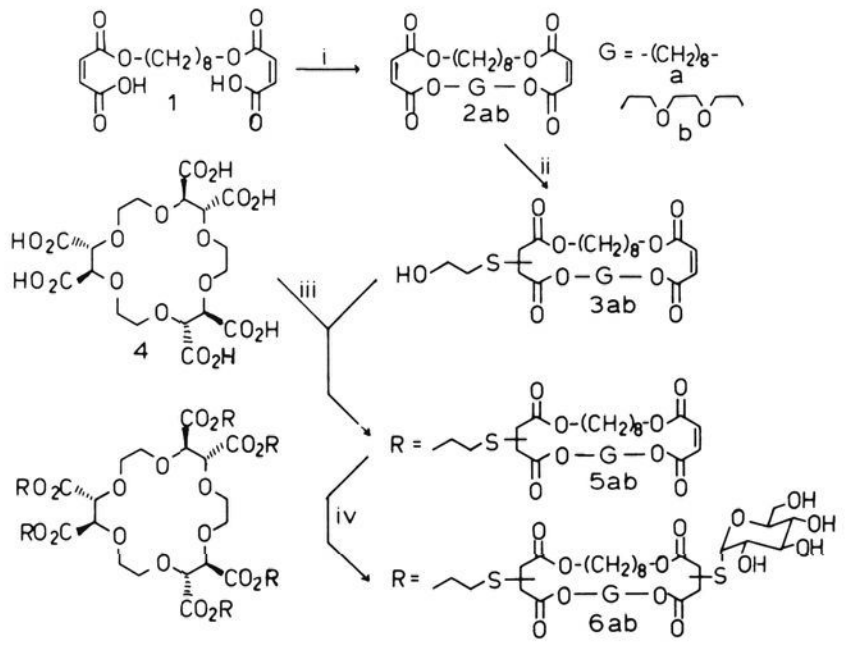


Figure 1. A design proposal for a unimolecular ion channel imbedded in a bilayer membrane: C = crown ether, W = channel wall, H = head group.

Scheme I[a]



[a] Reagents, conditions: (i) HO–G–OH, TsOH, benzene, reflux, azeotropic distillation; (ii) 2-mercaptoethanol, piperidine buffer, pH 8, isopropyl alcohol/water; (iii) (1) $PCl_5/POCl_3$, (2) triethylamine, CH_2Cl_2; (iv) β-1-mercapto-D-glucose, piperidine buffer, pH 8, isopropyl/water.

conformational rigidity of the crown ether core.[10] The speculative structure sketched in Figure 1 serves primarily as a guide to a synthetic strategy as opposed to a mechanistic proposal.

The synthesis is outlined in Scheme I. Diols react with maleic anhydride in a two-step process via the diacid **1** to produce macrocyclic tetraesters[11] **2ab**; the required macrocycles were isolated by chromatography on silica gel followed by gel permeation (Sephadex LH20, yield 8–12%).[12] Treatment of **2ab** with 1 equiv of thioethanol gave the mono adducts **3ab** in 20% yield after chromatography. The crown ether hexaacid **4**[13] was converted to the acid chloride and treated with the alcohols **3ab** to give the hexaenes **5ab** (mixed regio- **5b** and stereoisomers). A

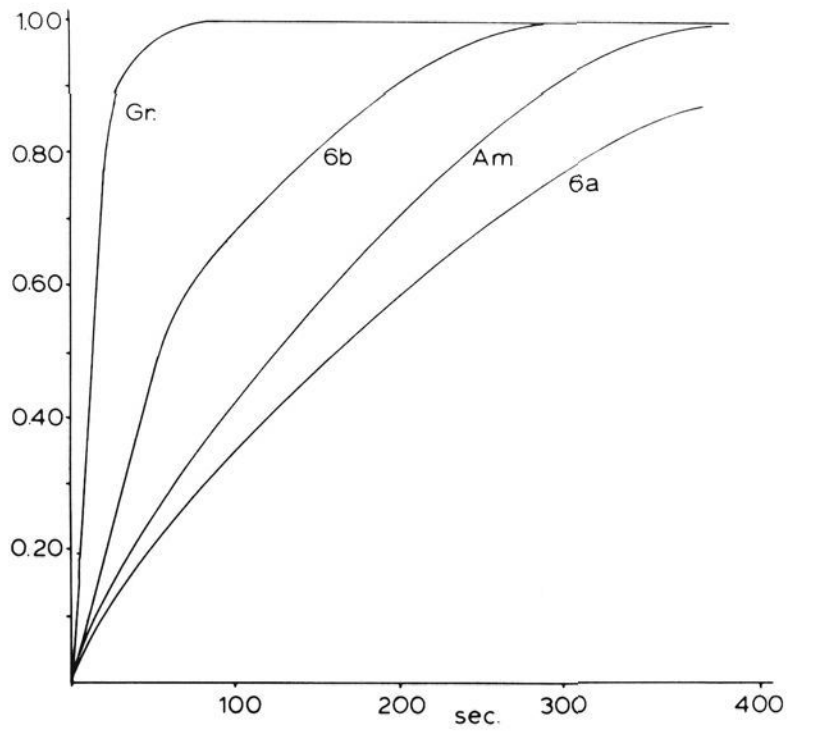


Figure 2. Fractional change in fluorescent intensity $((I_0 - I_t)/(I_0 - I_\infty))$ of vesicle entrapped fluorescein as a function of time for 0.5 nmol of added channel forming compounds:[15] Gr. = gramicidin D; Am. = amphotericin B.

second Michael addition of β-1-mercapto-D-glucose produced the functional mimics **6ab**. Although very complex, the ^1H and ^{13}C NMR spectra of **5ab** and **6ab** were completely consistent with the assigned structures. A molecular ion could not be obtained by FAB-MS, but fragments corresponding to loss of one to three sidearms were observed for **5a** and **6a**. Molecular weight determination by vapor pressure osmometry[14] gave $(4.7 \pm 0.2) \times 10^3$ g/mol for **6b** (calcd for $C_{198}H_{322}O_{108}S_{12}$, 4814.4).

Large unilamellar vesicles containing entrapped carboxyfluorescein were prepared by a reverse evaporation method.[15] In the absence of additional channels or carriers an imposed pH gradient (pH 6.5 inside, pH 5.5 outside) collapses slowly over a period of days. As shown in Figure 2, addition of **6ab** or the natural channel-forming compounds gramacidin D or amphotericin B[16] results in rapid decrease of the fluorescence intensity of the entrapped fluorescein due to collapse of the pH gradient. Under the conditions of Figure 2 there are of the order of 10^2 channels/vesicle, and the gradient collapse is accelerated by a factor of $>10^5$ over the base rate.

The following features of the transport process mediated by **6ab** have been established: (i) The apparent transport rate increases with increasing concentration of **6ab**. At all concentrations, the glycol substituted derivative **6b** is a factor of three to five times more active than the parent hydrocarbon **6a**. (ii) Vesicle lysis by a detergent action has not occurred as there is no change in the vesicle solution turbidity on the addition of either natural or artificial (**6ab**) channel compounds. Neither is there any change of vesicle morphology evident by electron microscopy. In contrast, detergents such as Triton X100 provoke rapid clearing at comparable concentrations. (iii) The addition of the proton carrier FCCP[17] accelerates the collapse of the pH gradient in the presence of channels (**6ab**, gramicidin D) although it has only a minor effect

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(due to vesicle lysis[16]) on the intact vesicles. In the presence of FCCP, pH gradient collapse is known to be coupled to an electroneutral countertransport of metal ions (Na^+ in these experiments).[16] However, the rate of gradient collapse may not only directly reflect the efficiency of the channel as a cation transporter but may also reflect the rate at which very active channels are created in the bilayer.[16]

The precise mode of action of the artificial ion channels **6ab** has yet to be established, and the design proposal of Figure 1 remains highly speculative. It is clear, however, that highly active transporters can be created by simple synthetic approaches. We look forward to reporting our further explorations of this strategy for biomimetic ion transport.

Acknowledgment. The support of this work by the Natural Sciences and Engineering Research Council of Canada through operating grants (T.M.F.) and postgraduate scholarships (P.J.D.) is gratefully acknowledged.

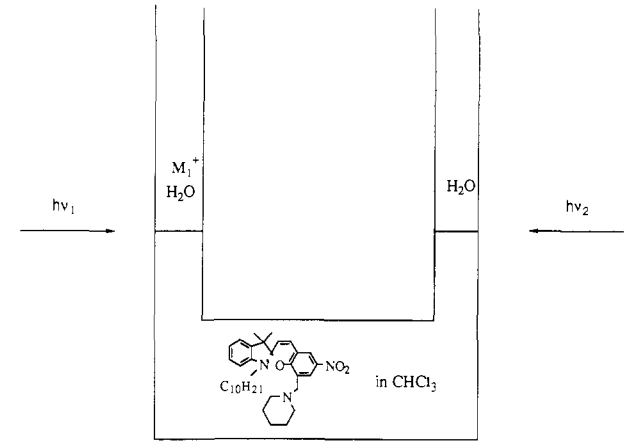

Figure 1.

Photodynamic Transport of Metal Ions

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The spiropyranindolines are an important class of photo- and thermochromic compounds whose unusual properties can be attributed to the equilibrium shown in Scheme I.[3] Previous work from our laboratory has demonstrated that the ca. 3 kcal/mol strain energy induced by the macrocycle shown in Scheme I leads to a dramatic shift in the spiropyranindoline = merocyanine equilibrium.[4] Replacing the macrocyclic strap with a ligating group (L) generates a new photodynamic system, **3** = **4** (Scheme II), which in the presence of an appropriate metal cation could exist as **4**, with the liberated phenolate acting as a second ligating group. Visible irradiation of **4** should then regenerate **3** with expulsion of the metal. We report herein that, using suitably substituted spiropyranindolines, *reversible* metal binding can be observed and that light-driven transport of metal ions across an organic membrane has now been achieved using the **3** = **4** system.[5,6]

While the metal ion-mediated conversion of **3a** to **4a** has been reported by Taylor,[7] the reversibility of the metal binding, a critical property for the development of a metal transport system, had not been described.[8] Reaction of **3a** with zinc (2+) salts[9] to generate **4a**, followed by irradiation with visible light, led to complete conversion of **4a** to **3a**, as determined by both NMR[10] and ultraviolet spectroscopy.[11]

PHOTODYNAMIC TRANSPORT OF Zn (+2)

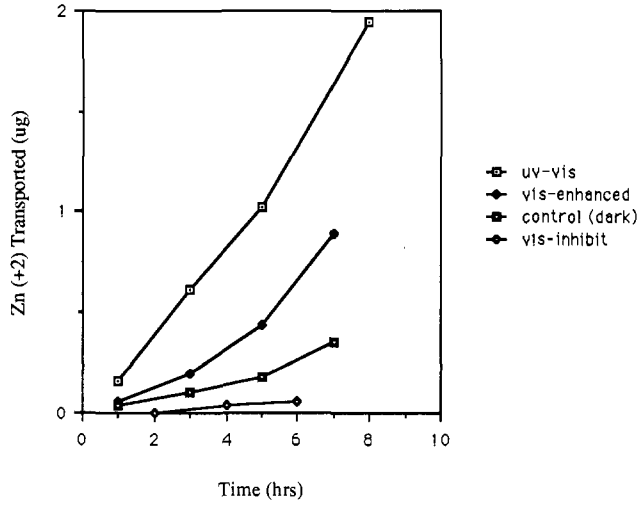
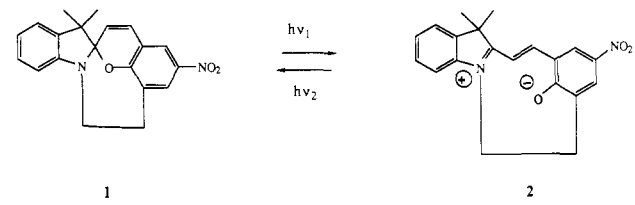
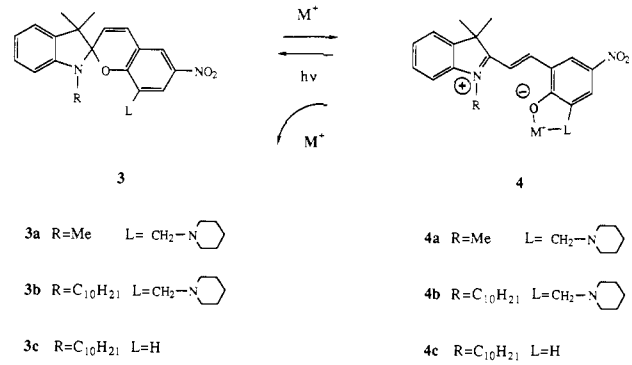


Figure 2.

Scheme I



Scheme II



The ability of **3b**[12] to mediate metal-ion transport across an organic membrane was examined using the apparatus which is

(1) Fellow of the Alfred P. Sloan Foundation (1987-1989). Recipient of a National Institutes of Health Career Development Award (1988-1993) and a Merck Grant for Faculty Development (1985-1986).
(2) Current address: Department of Chemistry, University of California, Los Angeles, Ca 90024.
(3) For a general review of the chemistry of spiropyranindolines, see: Bertelson, R. In *Photochromism*; Brown, G., Ed.; Wiley: New York, 1971; Chapter 3.
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(6) For a recently reported example of a light-powered proton pump, see: Haberfield, P. *J. Am. Chem. Soc.* **1987**, *109*, 6177.
(7) Taylor, L. D.; Nicholson, J.; Davis, R. B. *Tetrahedron Lett.* **1967**, 1585.
(8) For a related study, see: Phillips, J. P.; Mueller, A.; Przystal, F. *J. Am. Chem. Soc.* **1965**, *87*, 4020.
(9) For an example of photoregulated binding of zinc ion, see: Blank, M.; Soo, L.; Wasserman, N.; Erlanger, B. *Science* **1981**, *214*, 71.
(10) NMR experiments were performed using 3 mM solution of the spiropyranindoline with 1 equiv of metal perchlorate in deuteriochloroform. Spectra were recorded at 500 MHz.

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