An Ion Conductor Derived from Spermine and Cholic Acid

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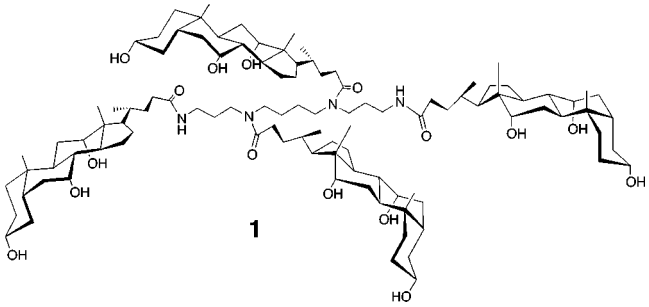
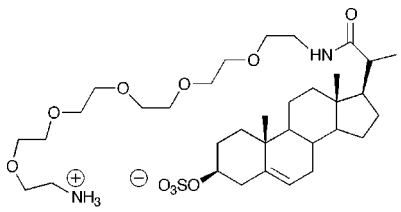
In this paper we show that condensation of two naturally occurring products, spermine and cholic acid, yields an ion conductor (**1**) having high selectivity toward phosphatidylcholine (PC) bilayers of varying thickness. The potential for using conjugates of this type, to exploit subtle differences in bilayer thicknesses from an antimicrobial standpoint, is briefly discussed.



1

Interest in the design of membrane-spanning synthetic ionophores (i.e., ion conductors) has intensified in recent years.[1] Part of this interest stems from the notion that ion conductors may lead to new classes of antibiotics that are less susceptible toward resistance.[2,3] One example of a *sterol*-based ion conductor, which promotes the flow of Na^+ across PC bilayers, is a conjugate derived from 23,24-bisnor-5-cholenic acid and 1,17-diamino-3,6,9,12,15-pentaoxaheptadecane, i.e., **2**.[4]



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Here, we describe a *one-pot* synthesis of a unique ion conductor (**1**), which combines two molecules having entirely different biological roles in nature, i.e., spermine and cholic acid. Although methyl ether derivatives of cholic acid have previously been used

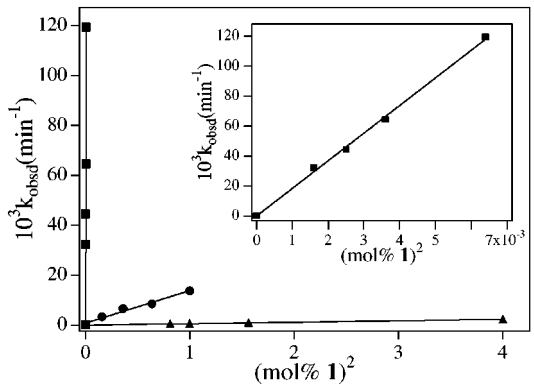
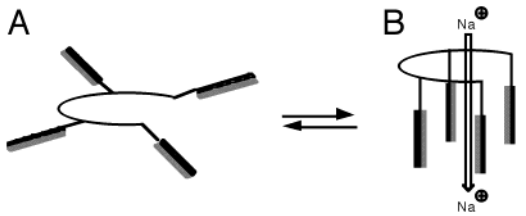


Figure 1. Plot of k_{obsd} versus (mol % **1**)^2 for vesicles made from (■) **C***14*, (●) **C***16*, and (▲) **C***18* at 35 °C. The concentration ranges used in **C***14*, **C***16*, and **C***18* were 0.04−0.08, 0.4−1, and 0.9−2 mol %, respectively. The inset shows expanded *X* and *Y* axes.

Chart 1



to create synthetic ionophores, the direct coupling of two *underivatized* biogenic molecules to give an active ion conductor is without precedent.[1g] We also show that the selectivity of **1** toward bilayer thickness is similar to that of **2**, *when acting on membranes that are four methylene units thicker*. The design principle upon which **1** was based is illustrated in Chart 1. In brief, we envisioned that insertion of **1** into a lipid membrane would favor a conformation in which the hydrophobic face of each sterol (darkened region) would lie in contact with the alkyl chains of neighboring phospholipids, and the hydrophilic face (lightly shaded region) would point toward that of a nearest-neighbor; i.e., conformation **B** would be favored over **A** within the membrane. In principle, dimerization of **B** across the bilayer could then afford a contiguous pathway for Na^+ transport.

The desired target, **1**, was synthesized by direct condensation of cholic acid and spermine. Using experimental procedures similar to those previously described, **1** was incorporated into both leaflets (double-sided addition) of 200 nm unilamellar vesicles of 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine (**C***14*).[4] The rate of entry of Na^+ into these vesicles was then monitored using $^{23}Na^+$ NMR spectroscopy at 35 °C.[4a] Values of pseudo-first-order rate constants, k_{obsd}, showed a second-order dependency on the mol % of **1** (Figure 1). Similar experiments that were carried out with vesicles made from 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine (**C***16*) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (**C***18*) also showed second-order dependencies on the mol % of **1**. These unsaturated phospholipids were specifically chosen to maintain the fluid state at 35 °C.[5]

As discussed previously, a second-order dependency of k_{obsd} on the mol % of **2** in bilayers made from **C***14*, **C***16*, and **C***18*

(1) For a general review, see: Gokel, G. W.; Murillo, O. *Acc. Chem. Res.* **1996**, *29*, 425. For selected examples, see: (a) Fyles, T. M.; Loock, D.; Zhou, X. *J. Am. Chem. Soc.* **1998**, *120*, 2997. (b) Clark, T. A.; Buehler, L. K.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 651. (c) Jin, T.; Kinjo, M.; Kobayashi, Y.; Hirata, H. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 3135. (d) Fritz, M. G.; Walde, P.; Seebach, D. *Macromolecules* **1999**, *32*, 574. (e) Abel, E.; Maguire, G. E. M.; Murillo, O.; Suzuki, I.; DeWall, S. L.; Gokel, G. W. *J. Am. Chem. Soc.* **1999**, *121*, 9043. (f) Hall, C. D.; Kirkovits, G. J.; Hall, A. C. *Chem. Commun.* **1999**, 1897. (g) Kobuke, Y.; Nagatani, T. *Chem. Lett.* **2000**, 298. (h) Renkes, T.; Schafer, H. J.; Siemens, P. M.; Neumann, E. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 2512. (i) Baumeister, B.; Sakai, N.; Matile, S. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 1955.

(2) (a) Stadler, E.; Dedek, P.; Yamashita, K.; Regen, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 6677. (b) Sadownik, A.; Deng, G.; Janout, V.; Bernard, E. M.; Kikuchi, K.; Armstrong, D.; Regen, S. L. *J. Am. Chem. Soc.* **1995**, *117*, 6138.

(3) (a) Sakai, N.; Matile, S. *Chem. Eur. J.* **2000**, *6*, 1731. (b) Winum, J.-Y.; Matile, S. *J. Am. Chem. Soc.* **1999**, *121*, 7961. (c) Robert, F.; Winum, J.-Y.; Sakai, N.; Gerard, D.; Matile, S. *Org. Lett.* **2000**, *2*, 37.

(4) (a) Otto, S.; Osifchin, M.; Regen, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 7276. (b) Otto, S.; Osifchin, M.; Regen, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 10440.

(5) Lewis, B. A.; Engelman, D. M. *J. Mol. Biol.* **1983**, *166*, 211.

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Table 1. Ion Conducting Activity and Membrane Selectivity of **1** and **2**[a]

ion conductor	phospholipid	$10^4 k_2/K$ (min^{-1} mol $\%^{-1}$)	*S*
1	**C***14*	190 000	1500 (**C***14*/**C***16*)
	C*16*	130	22 (**C***16*/**C***18*)
	C*18*	5.8	23 (**C***18*/**C***20*)
	C*20*	0.25	520 (**C***16*/**C***20*)
2[b]	**C***14*	160	38 (**C***14*/**C***16*)
	C*16*	4.2	17 (**C***16*/**C***18*)
	C*18*	0.25	640 (**C***14*/**C***18*)

[a] All kinetic experiments were carried out at 35 °C; the error in k_{obsd} is estimated to be ±10%. [b] Taken from ref 4a.

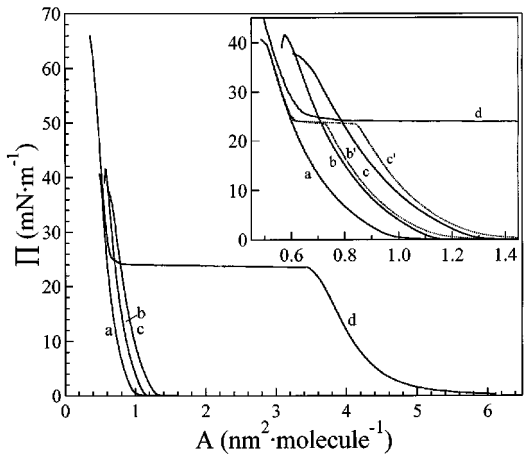

Figure 2. Surface pressure−area isotherms for mixtures of **C***18*/**1** having the following mole percentage of **C***18*: (a) 100, (b) 95.6, (c) 91.7, and (d) 0 at 25 °C. The inset shows expanded scale plus theoretical curves (b′ and c′ correspond to b and c, respectively), assuming ideal mixing.

indicates the existence of transport-active dimers, if it is assumed that only a small fraction of the conjugate is aggregated.[4a] Specifically, it can be shown that $k_{obsd} = k_2[\text{monomer}]^2/K$, where K is the equilibrium constant for dissociation of the dimer, k_2 is the rate constant for Na^+/Li^+ transport, and [monomer] is the analytical concentration of **2** that is present in the dispersion. Similarly, our results with **1** support the existence of transport-active dimers.

Although k_2 and K cannot be separated by this kinetic analysis, one can make operational comparisons between **1** and **2** in each host membrane. Specific values of k_2/K that have been calculated from the slopes in Figure 1 are listed in Table 1. For each membrane type, **1** shows substantially greater activity than **2**. In the case of **C***14* membranes, it is ca. 1200 times more active; with **C***16* and **C***18*, the differences in activities are less pronounced. If we define a membrane-selectivity factor, *S*, as the ratio of $(k_2/K)_m/(k_2/K)_n$, where *m* and *n* refer to two different bilayers that are being compared, then the selectivity of **1** in promoting the transport of Na^+ across **C***14* bilayers, relative to **C***16*, is ca. 1500; a much smaller selectivity (factor of 22) is apparent for **C***16* relative to **C***18*. If one compares membranes that differ by four methylenes per alkyl chain (i.e., **C***14* versus **C***18*), the selectivity jumps to a factor of 33 000! Further inspection of these data reveals that the selectivity of **1** toward bilayer thickness is similar to that of **2**, but *is shifted by two carbons*. In other words, the activity that is observed for **1** in **C***16*, **C***18*, and **C***20* is very similar to that which is found for **2** in **C***14*, **C***16*, and **C***18*, respectively.

The very strong dependence of ion transport activity on membrane thickness that is observed for **1**, in and of itself, provides compelling evidence for a *membrane-spanning* agent.[4a] The two-carbon shift in membrane selectivity can be readily accounted for by a transport-active form of **1** that has an effective length, which is two methylene units longer than that of **2**.

To gain insight into the likely conformation of **1** in lipid bilayers, we examined its monolayer behavior at the air/water interface. As shown in Figure 2, **1** exhibits significant compressibility from 0 to 24 mN·m^{-1}. Extrapolation of the condensed portion of this part of the isotherm to zero surface pressure yields a limiting area of 4.5 nm^2·molecule^{-1}, which is consistent with a structure having the hydrophilic face of each sterol in intimate contact with water; i.e., the entire molecule lies flat on the subphase (conformation **A** in Chart 1). At 24 mN·m^{-1}, a very broad, first-order phase transition occurs. Such a transition implies that a radical change in conformation has taken place. Given the low surface area that **1** occupies at the completion of this transition, it is likely that two of the sterol units have "flipped" up into air and/or down into water. In sharp contrast, mixed monolayers of **1** and **C***18* were much less compressible and *did not exhibit a phase transition*. Extrapolation of the isotherms made from pure **C***18*, **C***18*/**1** (95.6/4.4, mol/mol), and **C***18*/**1** (91.7/8.3, mol/mol) to zero surface pressure yielded limiting areas of 0.70, 0.87, and 1.02 nm^2/molecule, respectively. If one assumes ideal mixing, then the calculated limiting area of **1** in these mixed monolayers is 4.57 and 4.55 nm^2·molecule^{-1}, respectively; at biologically relevant surface pressures (ca. 30 mN·m^{-1}), the calculated areas for **1** are 2.89 and 2.51 nm^2·molecule^{-1}, respectively.[6] The significant reduction in the occupied area of **1**, at this higher surface pressure, is consistent with a model in which each sterol has lifted upward from the water surface such that only the C-3 hydroxyls remain in contact with the aqueous subphase. Thus, these results indicate that **1** *is readily taken up into a compressed phospholipid monolayer in a conformation that approaches* **B**.

In preliminary studies, we have found that a conjugate, formed by acylating all three amine groups of *spermidine* with cholic acid, shows substantially lower activity as compared with **1**. Specifically, k_2/K was approximately 400 times lower in **C***14* membranes; on the basis of its cholic acid content, this conjugate is a factor of 300 times less active. Thus, it appears that the ion conducting property of such conjugates is strongly dependent upon their size.

The high sensitivity of **1** to bilayer thickness reported herein raises the intriguing possibility that one may be able to use such compounds to exploit subtle differences in thickness between mammalian membranes and those of microorganisms, from a therapeutic standpoint. One can imagine, for example, that fungi and/or bacteria may have regions within their plasma membrane that are slightly thinner than mammalian membranes due to the strong condensing (and thickening) effect of cholesterol in the latter.[7−9] The fact that ion conductors such as **1** are derived from naturally occurring precursors adds further to the merit of such compounds, in terms of their potential for being biodegradable and nontoxic.

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Supporting Information Available: Procedure for the synthesis of **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Georgallas, A.; Hunter, D. L.; Lookman, T.; Zuckerman, M. J.; Pink, D. A. *Eur. Biophys. J.* **1984**, *11*, 79.

(7) (a) Vist, M. R.; Davis, J. K. *Biochemistry* **1990**, *29*, 451. (b) Ipsen, J. H.; Mouritsen, O. G.; Bloom, M. *Biophys. J.* **1990**, *57*, 405. (c) Nezil, F. A.; Bloom, M. *Biophys. J.* **1992**, *61*, 1176. (d) Urbina, J. A.; Pekerar, S.; Le, H.; Patterson, J.; Montez, B.; Oldfield, E. *Biochim. Biophys. Acta* **1995**, *1238*, 163.

(8) Although the thickness of bilayers made from a single phospholipid can be determined with high precision, the thicknesses of biological membranes are less certain.[5] Specifically, the likely existence of microdomains, having distinct compositions, is expected to result in a variation in thickness within a single membrane: Welti, R.; Glaser, M. *Chem. Phys. Lipids* **1994**, *73*, 121.

(9) In preliminary experiments, **1** was found to exhibit signficant antifungal activity; i.e., 80% inhibition was observed with three different strains of *Candida albicans* and two different strains of *Cryptococcus neoformans* using concentrations of 1−4 μg/mL after 24 h.