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DESIGN AND SYNTHESIS OF A NOVEL BIS-CROWN ETHER CARRIER MOLECULE

Mimic of (Na^+,K^+)-ATPase

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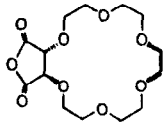
Abstract: A new bis-crown ether carrier molecule **3b** with an ammonium tail was synthesized to mimic some of the properties of (Na^+,K^+)-ATPase activity. At low pH, the ammonium salt shows stabilization by intramolecular hydrogen bonding with the 18-crown-6 portion of the molecule and the compound has some selectivity for the transport of Na^+ over K^+ ions. As the pH is increased, the Na^+ and K^+ ion-binding ability changes and in the free amino form, the host molecule carries both Na^+ and K^+ ions at comparable rates.

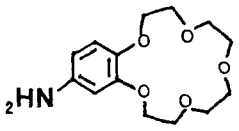
Macrocyclic compounds having functionalized side chains of well-defined stereochemistry have been shown to possess diverse applications in the design and synthesis of a variety of molecular receptors, catalysts and carriers[1]. With this in mind, we became interested in developing a macrocyclic crown ether system that would be a simple model of the (Na^+,K^+)-ATPase pump for selective transport of ions across biological membranes[2]. Suffice to recall that (Na^+,K^+)-ATPase is a transport enzyme which pumps sodium antiport to potassium coupled to ATP hydrolysis. Thusly we report here our first effort in this direction.

The approach is based on the design of a bis-crown ether with two macrocyclic rings of different sizes; one with an affinity mainly for Na^+ ions and the other one for K^+ ions but with the possibility of competing for a side chain primary ammonium group. This molecular architecture has some similarity with other works published on intramolecular sidearm-macrocyclic ring interaction such as the so-called ammonium "tail-biting" group of Shinkai[3] or the "ostrich molecule" complex of Gokel[4]. There are also few examples in the literature of Na^+/K^+ and Ca^{2+}/K^+ cation selective transport across a liquid membrane by macrocyclic carrier molecules[5,6].

The carrier **3b** was synthesized in an overall 75% yield by first condensing the optically active anhydride 1[7] with the 4'-aminobenzo-15-crown-5 molecule **2**[8] using a known procedure[9,10]. After a flash chromatography ($CHC\ell_3$/MeOH/Et_3N, 87:9:4) and a Dowex 50W-8 (H^+ form) ion exchange, the foamy bis-crown ether acid **3a** [MS m/e 618 ($M+H^+$)] was first activated with 1 equiv. of carbonyl-diimidazole in $CH_2C\ell_2$ under strictly anhydrous condition. The reaction was monitored by t.l.c. ($CHC\ell_3$/MeOH/Et_3N-70:8:3) and after 1 hr., no more acid was present. Then 12 equiv. of ethylene diamine was added in one portion and the mixture evaporated to dryness after 1 h. Solvent and remaining imidazole were removed completely by sublimation in a heated bath at 55° under high vacuum for 48 hrs. The desired compound **3b** (free amine form) was isolated as a brownish oily material [^{1}H-NMR ($CDC\ell_3$, 400 MHz) δ 7.69 (s, NH), 7.10 (s, NH), 6.73 (d, J = 8.4 Hz, 1H), 6.28 (d, J = 2.6 Hz, 1H), 6.21 (dd, J_1 = 2.6 Hz, J_2 = 8.4 Hz, 1H), 4.35 (m, 2H), 4.08 (m, 4H), 3.88 (m, 4H), 3.75 (s, 8H), 3.65 (br, 20H), 3.45 (br, 8H), 2.93 (m, 2H), 2.15 (vbr, NH_2); MS m/e 239, 284, 285, 377].



1



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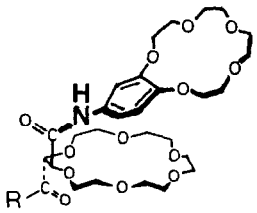
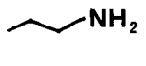


3

a) R = OH

b) R = 

In designing such carrier, one should be aware that the much recognized "hole-size selectivity" principle is not always applicable to a series of simple macrocycles.[11] Nevertheless, in a more complex system such as **3b**, the concerted effect of a benzo-15-crown-5 coupled to a more rigid and doubly substituted 18-crown-6 and the presence of an ammonium side chain should be taken into consideration[8].

Here, the binding of the swinging arm to the crown ether would be controlled by the pH whereby at low pH values the molecule would be in the ammonium form and this group would predominently bind the bottom face of the 18-crown-6 cavity, restricting the complexation of K^+ ions. The molecule would therefore be expected to transport mainly Na^+ ions bound in the other 15-crown-5 cavity. By increasing the pH however, the ammonium side chain would eventually be deprotonated and swing out of the cavity to allow the binding and transport of K^+ ions, as well as Na^+ ions still trapped in the first and smaller crown ether ring. To evaluate this hypothesis, the selective transport of Na^+ and K^+ ions by crown ethers **3a** and **3b**, was determined in a U tube cell (diam. 1.7 cm) in the following way using a three phases system. The crown ether carrier molecule (1 mM) was dissolved in the chloroformic membrane phase (10 mL). The experiments were performed at 20°C and the three layers were mechanically stirred at about 80 rpm. The transport rates were determined by measuring the concentration of Na^+ and K^+ in both aqueous phases by taking small aliquots every 30 min. for at least 8 hrs.

In two separate experiments, the rates of transport of Na^+ and K^+ (as picrates, initial conc. 0.01M) were determined under conditions where the two sides of the cell were filled in one case with 15 mL of a basic (0.05M diethanolamine.HCℓ, pH 9.7) and in the other case with an acidic (0.05M MES.Me_4NOH, pH 6.0) buffer. As expected, in basic medium, the bis-crown ether amine **3b** showed no selectivity for Na^+ over K^+ (5.83 µmol/h in Na^+, 6.24 µmol/h in K^+). However, in acidic medium where the amine group was protonated and presumably bound to the 18-crown-6 cavity by hydrogen bonds, a difference in ion transport was indeed noted. The values obtained were now 6.63 µmol/h for Na^+ and 6.09 µmol/h for K^+. As expected the carrier **3a** behaved differently. The values observed were 10.7 µmol/h of Na^+ and 5.84 µmol/h of K^+ for the acidic medium and 1.94 µmol/h of Na^+ and 2.24 µmol/h of K^+ for the basic condition. The rates were reproducible to ±5%.

Furthermore, the presence of a broad band (3350-2900 cm^{-1}) in the IR spectrum of the protonated form of **3b** gives some evidence of ammonium ion binding by the macrocyclic ring <u>via</u> N-H...O hydrogen bonds[4].

In conclusion, not only the novel carrier **3b** can transport Na^+ and K^+ ions equally well under basic conditions, but shows a slight selectivity for Na^+ ions under acidic conditions. Furthermore and most important, the transport of Na^+ over K^+ can be regulated by the pH. By comparison, the larger values obtained for the acid **3a** are somewhat surprising but could be related to the degree of ionisation of its carboxyl group and the possibility of a salt bridge with the ions.

The study thus showed that such a simple mechanism has some similarity with natural but more complex systems of selective ion transport. With such a modest but selective carrier molecule in hand, we project in the future to explore its potential when incorporated into lipidic artificial membrane.

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References and Notes:

1. J.M. Lehn, Science (Washington, D.C.); **227**, 849-856 (1985).

2. K.J. Sweadner and S.M. Goldin, N. Engl. J. Med., **302**, 777-783 (1980).

3. S. Shinkai, M. Ishihara, K. Ueda and O. Manabe; J. Chem. Soc. Chem. Commun., 727-729 (1984); S. Shinkai,M. Ishihera, K. Ueda, and O. Manabe, J. Chem. Soc. Perkin Trans II, 511 (1985).

4. J.R. Beadle, D.M. Dishong, R.K. Khanna, and G.W. Gokel, Tetrahedron, **40**, 3935-3944 (1984).

5. Y. Nakatasuji, H. Kobayashi, and M. Okahara, J. Chem. Soc. Chem. Commun, 800 (1983).

6. A. Hriciga and J.M. Lehn, Proc. Nat. Acad. Sc. USA, **80**, 6426 (1983).

7. L.A. Frederick, T.M. Fyles, N.P. Gurprassad, and D.M. Whitefield, Can. J. Chem., **59**, 1724-1733 (1981).

8. R. Ungaro, B. El Haj, and J. Smid, J. Amer. Chem. Soc., **98**, 5198-5202 (1976). Although they showed that 4'-amide substituted benzo-15-crown-5 are in fact K^+ selective by a factor of 2 over Na^+, the presence of an ammonium side chain in our case can make the system more Na^+ selective.

9. J.P. Behr, J.M. Lehn, D. Moras, and J. Thierry, J. Amer. Chem. Soc., **103**, 701-703 (1981).

10. New compounds were characterized by high resolution NMR (400 MHz) spectroscopy, high resolution mass spectrometry, and microanalytical data.

11. G.W. Gokel, D.M. Goli, C. Minganti, and L. Echegoyen, J. Amer. Chem. Soc. **105**, 6786 (1983).

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