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Efficient Dopamine Transport by a Crown Phosphonate

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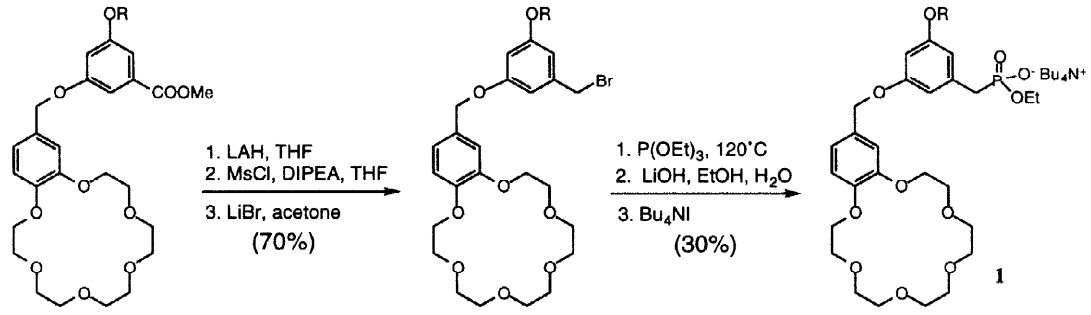
Abstract: Crown phosphonate **1** as a ditopic receptor-based carrier compound showed efficient dopamine transport through organic liquid membranes. A combination of extraction, transport, ^{1}H NMR, and mass spectral data strongly suggests that the transport complex in a chloroform phase is about 2:1 mixture of the cyclic, 1:1 complex **6** and 2:1 complex **8**.

Selective extraction and purification of catecholamines from clinical samples are important for the reason that catecholamine levels are often low and used as clinical indicators of various types of illnesses.[1] The most widely used methods for the determination of catecholamines in clinical samples are spectrometric, fluorometric, radioenzymatic, and chromatographic methods.[1a,2] Recently, Smith and coworkers have developed a membrane-based dopamine purification system in which a series of crown boronic acids were used for catecholamine transport through bulk liquid membranes and supported liquid membranes.[3] In their dopamine carriers, the crown ether functions as an ammonium binding site and the boronic acid moiety as a reversible covalent binding motif for the catechol. There are a few ditopic receptors known which are capable of recognizing both the ammonium and the remote catechol.[3,4] In attempts to develop another dopamine separation and purification system, we designed crown phosphonate **1** as a ditopic receptor-based carrier compound that can display effective and selective transport of dopamine through organic liquid membranes. In our carrier molecule **1**, the phosphonate moiety is expected to be used as a catechol binder through hydrogen bonding interaction.[5] In



Scheme 1

addtion, the supramolecular complex between the crown phosphonate and dopamine is charge balanced and does not need an accompanying anion for transport, which is an energetically demanding process.[6]

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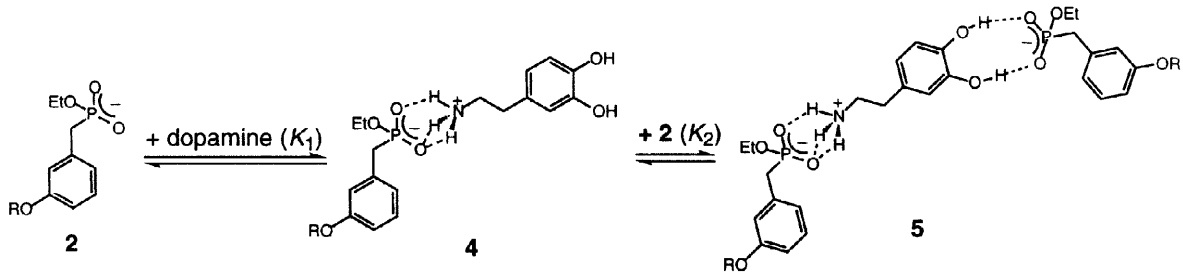
Table 1. Extraction and Transport of Dopamine by Synthetic Carriers

entry	carrier[a]	extraction (%)[b]	flux (10^{-9} mol/m^2·s)[c]
1	none	3.5	0.76 (1)
2	**1**	41.4	218 (287)
3	**2**	8.1	4.12 (5.4)
4	**3**	9.2	2.54 (3.3)
5	**2** + **3**	10.5	11 (14.5)

[a] Departure phase: sodium phosphate (100 mM, pH 7.4), sodium hydrosulfite (10 mM), and dopamine hydrochloride (41 mM). Organic phase: 1 mM of each carrier in chloroform. Receiving phase: sodium phosphate (100 mM, pH 7.4) and sodium hydrosulfite (10 mM). [b] Aqueous phase: sodium phosphate (100 mM, pH 7.4), sodium hydrosulfite (10 mM), and dopamine hydrochloride (0.3 mM). Organic phase: 3 mM of each carrier in chloroform. [c] Initial transport rate (relative rate in parentheses).

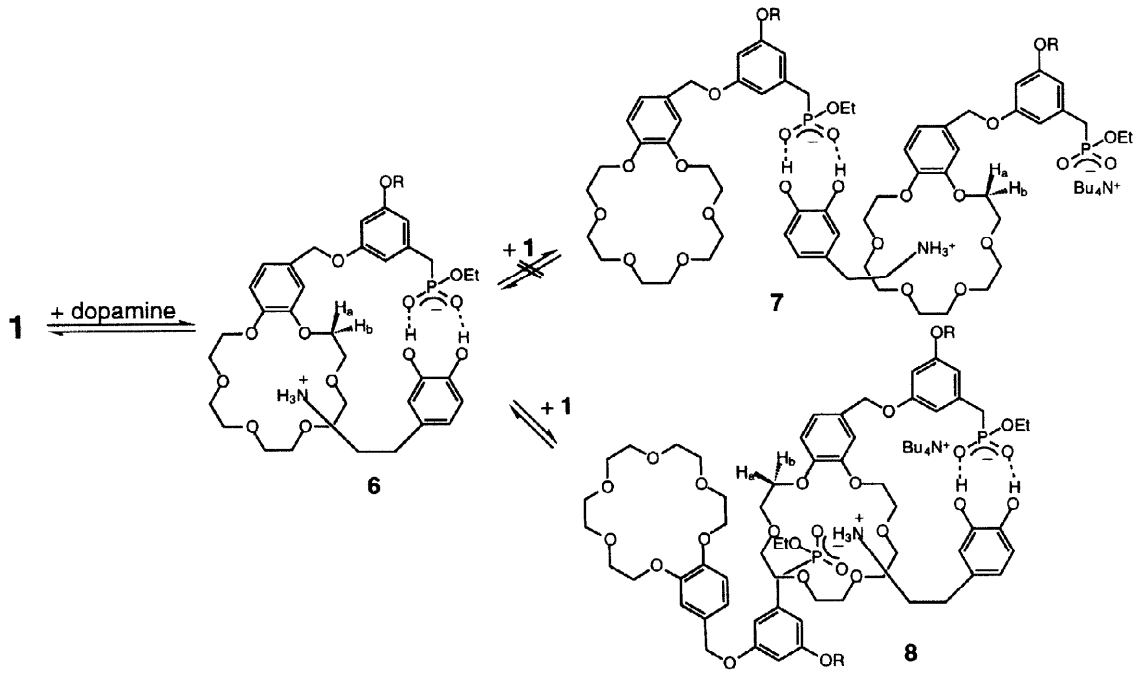
Compound **1** was obtained by the route described in Scheme 1.[7] Reference carriers as monotopic receptors (**2** and **3**) were prepared to test the efficiency of the covalently linked conjugate molecule **1**.

Transport experiments were performed using the standard U tube methodology.[8] The transport rate was monitored at 280 nm by the initial dopamine appearance in the receiving phase. Dopamine extraction and transport data are summarized in Table 1. In the absence of carrier, negligible dopamine transport was observed. Carrier **1** showed a transport rate enhancement of 287 times the background diffusion rate (entries 1 and 2). Both crown carrier **3** and phosphonate **2** showed a small dopamine flux. Transport experiments with co-carrier mixtures of **2** and **3** also exhibited rate acceleration, but not to the same extent as that achieved with the covalently linked system **1** (see entries 2 and 5), which reflects an entropic disadvantage in the case of the joint co-carrier system. Control experiments showed that both the phosphonate and the crown ether moieties were necessary for efficient dopamine transport (entries 3 and 4). In order to determine the binding mode for the complexation with phosphonate **2**, we performed ^{1}H NMR titrations of carrier **2** with dopamine hydrotriflate in CD_3CN, with benzyl ammonium chloride and catechol, respectively in DMSO-d_6. The binding constant for **2**/benzyl ammonium chloride (1600 M^{-1}) is larger than that for **2**/catechol (60 M^{-1}), which clearly indicates that phosphonate **2** binds ammonium moiety more strongly as shown in structure **4** rather than the catechol group of dopamine by both electrostatic and hydrogen bonding interaction. As expected, ^{1}H NMR titration of **2** with dopamine hydrotriflate in CD_3CN showed that phosphonate **2** can recognize both ammonium and catechol moiety, as shown below (K_1 = 188 M^{-1}, K_2 = 10 M^{-1}), which was also confirmed by Job analysis.[5,9] In contrast,



crown ether **3** binds very strongly to dopamine hydrotriflate in CD_3CN with $K_a \approx$ 36,000 M^{-1},which might explain decreased transport rate by **3** due to slower decomplexation of dopamine into the receiving phase compared to phosphonate **2**. Analysis of titration data of crown phosphonate **1** with dopamine hydrotriflate showed that the binding isotherms didn't fit well to a 1:1 or 2:1 binding scheme with association so high even in more polar solvent system (e. g., 10% CD_3OD/CD_3CN). A Job titration[9] (Figure 1) between crown

phosphonate **1** and dopamine hydrotriflate conducted in 10% CD_3OD/CD_3CN showed that maximum signal change was observed at 0.30 ~ 0.35 mol fraction of **1**, indicative of 2:1 binding as shown in **7** or **8**. Whereas a



methylene proton signal adjacent to phenyloxyl group of **3** slightly moved to downfield on addition of dopamine hydrotriflate without splitting the signal into two separate peaks, broad singlet of methylene proton signals (H_a and H_b: 4.14 ppm) on the benzo-18-crown-6 moiety of **1** in the absence of guest splitted into two signals (4.04, 4.18 ppm) on addition of 0.5 equiv. of dopamine hydrotriflate to **1**, which presumably excludes the similar binding mode (benzocrown ether/ammonium interaction between **3** and dopamine) as in **7** but rather indicates 2:1 binding mode as shown in **8**. This is also supported by the fact that 2:1 binding mode **7** is entropically less favorable than the ditopic binding mode **6** and therefore the equilibrium between **6** and **7** is an energetically unfavorable process. In the termolecular complex **8**, the stabilizing interaction is the same as in **6** with the secondary stabilizing interaction between ammonium and phosphonate moiety of another crown phosphonate.

The possible structure for the supramolecular transport complex between carrier **1** and dopamine can be drawn from experimental evidence. Crown phosphonate **1** showed much better dopamine extractability than phosphonate **2** and crown ether **3**. This means that dopamine interacts with both the crown and phosphonate as might be depicted in structure **6**, **7**, or **8**. A concentration-dependent extaction study (Figure 2) supports

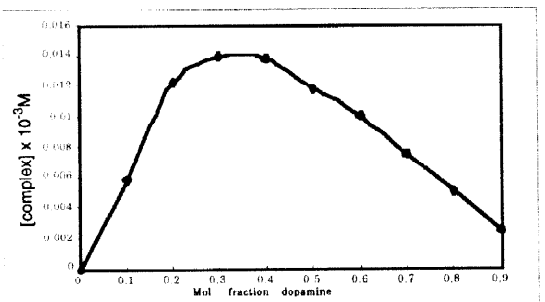


Figure 1. Job plot of **1**-dopamine at a total concentration of 1 mM in 10% CD_3OD/CD_3CN.

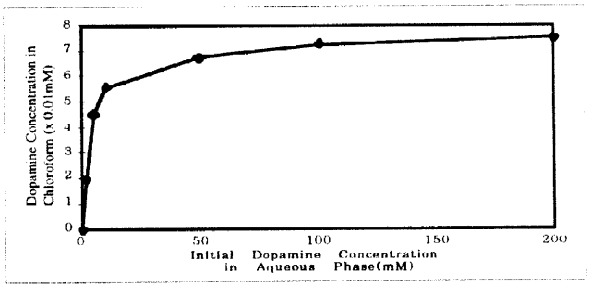


Figure 2. Extraction of dopamine from aqueous solutions containing various dopamine concentrations with a $CHCl_3$ solution of **1**.

possible structures for the neutral supramolecular transport complexes between the crown phosphonate conjugate carrier **1** and dopamine hydrochloride as depicted in **6** and **8**. When aqueous solutions containing varying amounts of dopamine hydrochloride were shaken with a chloroform solution of **1** (1.0×10^{-4} M), the maximum extractability of dopamine hydrochloride was 75% at pH 7.4. This result strongly suggests that the transport complex in a chloroform phase is about 2:1 mixture of the cyclic, 1:1 complex **6** and 2:1 complex **8**, consistent with an NMR titration data. A calculated structure for the complex **6**[10] showed that the ammonium moiety and catechol unit of dopamine are in good position to interact with crown ether and phosphonate of **1**, respectively. Mass spectrometric evidence for structure **6** as one of plausible binding modes was found in the chloroform phase: positive ion FAB, m/z 962 $[\mathbf{6} + H]^+$. Schrader recently showed that primary and secondary ammoniums had a little difference in their binding affinity to phosphonates.[11] However, carrier **1** turned out to be a more efficient transporter for dopamine (primary ammonium) than for epinephrine (secondary ammonium), which reflects not only the absence of primary interaction of phosphonate with ammonium (e.g., **4**) but also the weaker complexation of a secondary ammonium ion to the crown ether moiety.[12] This also demonstrates that carrier **1** binds to dopamine ditopically as shown in structure **6** and **8**.

In conclusion, we have developed a new dopamine carrier which consists of the phosphonate and the crown ether group. Crown phosphonate **1** as a ditopic receptor-based carrier compound showed efficient dopamine transport through organic liquid membranes. A combination of extraction, transport, ^{1}H NMR, and mass data strongly suggests that the transport complex in a chloroform phase is about 2:1 mixture of the cyclic, 1:1 complex **6** and 2:1 complex **8**.

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7. (a) Spectral data for **1**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3 H, $ArO(CH_2)_{17}CH_3$), 1.00 (t, J = 7.3 Hz, 12 H, $N((CH_2)_3CH_3)_4$), 1.18 (t, J = 7.0 Hz, 3 H, OCH_2CH_3), 1.26 (br s, 32 H, $ArOCH_2(CH_2)_{16}CH_3$), 1.42 (m, 8 H, $N((CH_2)_2CH_2CH_3)_4$), 1.65 (m, 8 H, $N(CH_2CH_2CH_2CH_3)_4$), 2.95 (d, J = 21.3 Hz, 2 H, $ArCH_2P(=O)$-), 3.33 (t, J = 8.3 Hz, 8 H, $N(CH_2(CH_2)_2CH_3)_4$), 3.69 - 4.13 (m, 24 H, crown ether OCH_2, $ArOCH_2(CH_2)_{16}CH_3$, $ArCH_2P(=O)(OCH_2CH_3)$-), 4.85 (br s, 2 H, $ArOCH_2Ar'$), 6.28 (s, 1 H), 6.56 (s, 1 H), 6.63 (s, 1 H), 6.81 (d, J = 8.6 Hz, 1 H), 6.88 (s, 2 H); IR (neat) 2922, 2853, 1596, 1515, 1458, 1257, 1121, 955 cm^{-1}.; MS (negative ion FAB) m/z 807 $[\mathbf{1} - Bu_4N]^-$.
(b) The starting material for the synthesis of **1** was prepared by alkylation of methyl 3-hydroxy-5-octadecyloxybenzoate with 4'-hydroxymethylbenzo-18-crown-6 under the traditional Mitsunobu condition (DEAD and PPh_3) in 50% yield.
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10. The minimum energy structure for the complex **6** was obtained from the MC/SD conformational searching with MacroModel V5.5 (Still, C., Columbia University) utilizing the MM2* force field.
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12. Under the same transport condition, the transport rate for epinephrine was 1.19×10^{-9} mol/m^2·s.