Selective Dopamine Transport Using a Crown Boronic Acid†

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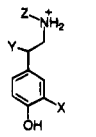
In vivo catecholamine concentrations are used as clinical indicators of a wide range of illnesses, including tumors of the neural crest, cardiovascular disease, and neuromuscular disorders.[1a] The most important compounds are dopamine and its biosynthetic progeny, norepinephrine and epinephrine. Various body

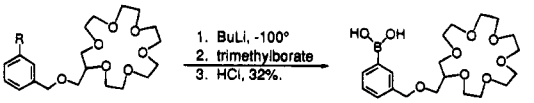


Dopamine, X = OH, Y = H, Z = H
Norepinephrine, X = OH, Y = OH, Z = H
Epinephrine, X = OH, Y = OH, Z = CH_3
Tyramine, X = H, Y = H, Z = H

fluids and tissues are assayed, including urine, plasma, and brain and cerebrospinal fluid. Often the low catecholamine levels, as well as the complicated sample matrices, require the samples to be concentrated and purified prior to HPLC, GC, or radioenzymatic analysis. A number of purification and concentration methods have been described, including alumina adsorption, liquid−liquid extraction, ion-exchange, and boric acid chromatography.[1] In general, these methods are labor intensive. Consequently, simpler catecholamine purification systems that maintain high selectivity and recoverability are of interest.[1b] An approach we are following is the development of carrier compounds with an ability to selectively transport catecholamines through a lipophilic membrane. In this report we describe a carrier with high dopamine transport selectivity. Besides analytical applications,[2] there are potential clinical uses for a dopamine-selective carrier. The most notable is a chemical delivery system to improve dopamine replenishment strategies used to treat diseases such as parkinsonism.[3]

Most of the studies on catecholamine transport have involved derivatives of 18-crown-6, which has a general affinity for primary ammonium ions.[4,5] With these systems, the order of observed transport rates has been primarily determined by the lipophilicity of the catecholamine.[5] Thus, transport selectivity for the highly hydrophilic dopamine has been especially poor. While many hosts have been developed to discriminate between substituents that are close to the ammonium functionality,[4] there are very few ditopic dopamine receptors with an ability to recognize both the ammonium and the remote catechol.[6] The

Scheme 1



2 R = Br
3 R = H

1

crown boronic acid, **1**, was designed as a ditopic dopamine carrier. Besides the obvious design feature that the crown is a binding motif for the ammonium group and the boronic acid a reversible covalent binder of the catechol, there are some more subtle points worth emphasizing. The pK_a of the boronic acid moiety in **1** is approximately 9, so that at physiological pH the carrier is neutral.[7] To transport non-diol-containing ammonium compounds (or metal cations), an accompanying anion has to be cotransported to maintain charge neutrality. This is an energetically demanding process which is very dependent on the lipophilicity of the anion.[8] Condensation of a boronic acid with a catechol, however, produces a boronate ester of greater acidity than the parent boronic acid, such that at neutral pH the tetrahedral boronate anion is formed.[9] Therefore, the structure of a 1:1 complex between the carrier **1** and dopamine is predicted to be the covalent adduct **4**, a lipophilic zwitterionic species able to move directly into a lipophilic membrane. *Unlike all previous designs, ditopic carrier 1 is not only dopamine shape selective, but the resulting host−guest complex, 4, is charge balanced and does not need an accompanying anion for transport. This provides carrier 1 with a novel selectivity mechanism for dopamine transport.*

Compound **1** was obtained as an oil by the route described in Scheme 1.[10] Liquid membrane transport experiments were conducted using the standard U tube methodology.[11] An aqueous departure phase buffered at pH 7.4 with 100 mM sodium phosphate and also containing 10 mM sodium dithionite as an antioxidant was separated from an identical receiving phase by a chloroform layer containing 1 mM carrier. The transport experiment was initiated by adding dopamine (41 mM) to the departure phase, after which its initial rate of appearance in the receiving phase was monitored at 279 nm. As shown in Table 1, carrier **1** increased dopamine transport 160 times greater than the background diffusion observed in the absence of carrier (entries 1 and 2). At higher dopamine concentrations, transport rates became saturated, which is indicative of carrier-mediated transport. Treatment of the data as a classic Michealis−Menten system (see supplementary material)[12] provided a dopamine extraction constant of $K_{ex} = 125\ M^{-1}$.[13] Control experiments

† Molecular recognition with boron acids, part 7. For part 6, see: Westmark, P. R.; Smith, B. D. *J. Am. Chem. Soc.* **1994**, *116*, 9343−9344.

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(10) Attempts to purify **1** by chromatography resulted in moderate amounts of deboronated product **3**. Fortunately, all side products obtained during the final synthetic step could be selectively extracted from aqueous solution with ether. Compound **1** was then obtained in pure form by extraction with chloroform. ^{1}H NMR (300 MHz, acetone-d_6): δ 7.75 (s, 1H), 7.69 (d, 1H, J = 7.5 Hz), 7.35 (d, 1H, J = 7.5 Hz), 7.28 (t, 1H, J = 7.5 Hz), 7.10 (s, disappeared upon addition of D_2O), 4.48 (s, 2H), 3.68 (m, 3H), 3.57 (m, 20 H), 3.52 (m, 2H). ^{13}C NMR (acetone-d_6 with 10% D_2O): δ 137.8, 134.1, 134.0, 130.5, 128.2, 78.3, 73.5, 71.4, 70.7, 70.5, 69.8, 69.4.

(11) The apparatus and methodology used in the transport experiments have been described in detail: Morin, G. T.; Paugam, M.-F.; Hughes, M. P.; Smith, B. D. *J. Org. Chem.* **1994**, *59*, 2724−2728. Briefly, the dimensions of the U tube were internal diameter, 1.20 cm; height, 10 cm; 2.5 cm between the arms. Both aqueous phases were 3.5 mL, and the chloroform layer was 7.0 mL. Only the organic layer was stirred (475 rpm).

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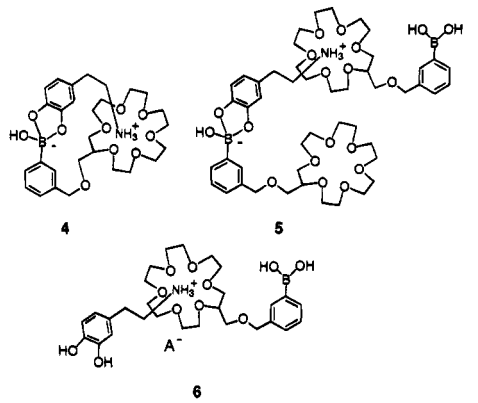
Table 1. Rates of Dopamine Transport

entry	carrier[a]	rate[b] (±15%)
1	**1**	356 (160)
2	none	2.2 (1)
3	phenylboronic acid	6 (3)
4	dicyclohexyl-18-crown-6	4 (2)
5	phenylboronic acid + dicyclohexyl-18-crown-6	123 (55)
6	phenylboronic acid + 18-crown-6	84 (38)
7	**1** + phenylboronic acid	364 (164)
8	**1** + 18-crown-6	348 (156)
9	**1** + $NaClO_4$[c]	467 (210)
10	dicyclohexyl-18-crown-6 + $NaClO_4$[c]	72 (32)

[a] Departure phase: sodium phosphate (100 mM, pH 7.4), sodium dithionite (10 mM), and dopamine (41 mM). Organic phase: 1 mM of each carrier in chloroform. Receiving phase: sodium phosphate (100 mM, pH 7.4) and sodium dithionite (10 mM). [b] Rate (10^{-8} M min^{-1}) at which dopamine initially appeared in the receiving phase (relative rate in parentheses). [c] $NaClO_4$ (41 mM) added to the departure phase.

showed that both the crown and the boronic acid were necessary for efficient transport (entries 3 and 4). Experiments with carrier admixtures of phenylboronic acid and 18-crown-6 compounds produced accelerated transport, but not to the same extent as that achieved with the covalently connected **1** (compare entries 5 and 6 with entry 1).

When considering the structure of the transported dopamine−**1** complex, three types of structures come to mind: the 1:1 zwitterionic covalent complex **4** (or higher cyclic oligomers), a 1:2 complex **5**, and an ion-pair such as **6**. Attempts to



4 **5**

6

determine the transported structure by ^1H NMR analysis of extracted solutions produced ambiguous results, due to broadened and overlapping signals.[14] Mass spectrometry was far more informative. Conclusive evidence was found for structure **4** in both the aqueous phase (electrospray MS, *m/z* 564 [**4** + H]$^+$; negative ion FAB, *m/z* 562 [**4** − H]$^-$) and the chloroform layer (negative ion FAB, *m/z* 562 [**4** − H]$^-$). In addition, further transport experiments indicated **4** to be the kinetically competent species. For example, transport runs with mixtures of carrier **1** plus boronic acid or crown produced no increase in transport rate over **1** alone (compare entries 7 and 8 with entry 1). Transport via a 1:2 complex such as **5** would be expected to increase under these conditions. Evidence against an ion-pair structure like **6** was the observation that addition of lipophilic perchlorate anions to the departure phase had little effect on transport by **1** (compare entries 9 and 1), whereas transport by crown ether alone (which forms an ion-pair analogous to **6**) displayed a large enhancement (compare entries 10 and 4).[8]

Table 2. Transport Rates for Catecholamines, Glycosides, and Uridine in the Presence and Absence of Carrier **1**

entry	transported compound[a]	no carrier (±15%)[f]	carrier **1** (±15%)[f]	rate enhancement[g]
11	dopamine[b]	2.2	356	160
12	norepinephrine[b]	2	120	60
13	epinephrine[b]	3.4	6.8	2
14	tyramine[b]	70	70	1
15	*p*-nitrophenyl β-glucoside[c,d]	1.3	3.9	3
16	*p*-nitrophenyl β-mannoside[c,d]	1.6	3.3	2
17	uridine[c,e]	0.2	0.2	1

[a] Departure phase: sodium phosphate buffer (100 mM, pH 7.4) and sodium dithionite (10 mM). Organic phase: carrier **1** (1 mM) in chloroform. Receiving phase: sodium phosphate buffer (100 mM, pH 7.4) and sodium dithionite (10 mM). [b] Departure side initially contained 41 mM catecholamine. [c] The starting concentration of transported species was adjusted to give a similar rate of background diffusion. [d] Departure side initially contained 1.36 mM glycoside. [e] Departure side initially contained 120 mM uridine. [f] Rate (10^{-8} M min^{-1}) at which transported solute initially appeared in receiving phase. [g] Transport rate in the presence of carrier **1** divided by the rate in the absence of carrier.

The selectivity of carrier **1** was determined for other catecholamines and diol-containing compounds. Inspection of Table 2 shows that carrier **1** is quite a selective dopamine transporter. Of the amine examples, the low epinephrine enhancement (entry 13) reflects the poor binding of a secondary ammonium ion by the 18-crown-6 moiety, and the negligible tyramine transport enhancement (entry 14) is attributable to the absence of a diol. The ability to transport certain saccharide derivatives, namely two glycosides and a nucleoside, was examined, and very weak accelerations were observed (entries 15−17).[15]

To be useful in the separations application described in the opening paragraph, the transport system must not only display good dopamine selectivity but also have active transport ability. Moreover, due to the susceptibility of dopamine to decomposition under basic conditions, the most practical active transport system would be one driven by a neutral to acidic pH gradient. The binding of dopamine with carrier **1** to produce covalent complex **4** is an acid-producing equilibrium.[9] Thus, in the presence of a pH gradient, it is predicted to be an active transport system.[4a] This was indeed the case. Active transport from a departure phase at pH 7.4 into a receiving phase at pH 5.5 was readily achieved.

We have described the novel crown boronic acid, **1**, as a carrier for catecholamine transport. Compound **1** is one of the most selective dopamine transporters yet reported;[5] it has all the fundamental properties needed to be a successful separation and concentration system for quantitative dopamine analysis. Moreover, the transport system can be readily improved, in terms of carrier design[16] and type of lipophilic membrane.[17]

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of carrier **1**, mass spectra of extracted solutions, plots of raw transport data, and derivation of K_{ex} (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(12) This treatment assumes that $K_{ex} < K_{ex(max)}$. Fyles, T. M. In *Inclusion Aspects of Membrane Chemistry*; Osa, T., Atwood, J. L., Eds.; Kluwer: Boston, 1991; p 72.

(13) Czarnik has reported an association constant, K_{assn} of 3030 M^{-1} for 2-anthrylboronic acid and dopamine at neutral pH.[2a] The K_{ex} reported here is significantly lower. Since $K_{ex} = K_{assn}K_{partn}$, K_{partn} for **4** must be quite small, in agreement with the moderate hydrophilicity of carrier **1**.

(14) The extracted solutions were too dilute for ^{13}C or ^{11}B NMR analysis.

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