# Facilitated Catecholamine Transport through Bulk and Polymer-Supported Liquid Membranes

**Marie-France Paugam,† Jeffrey T. Bien,† Bradley D. Smith,*,† Lysander A. J. Chrisstoffels,‡ Feike de Jong,*,‡ and David N. Reinhoudt*,‡**

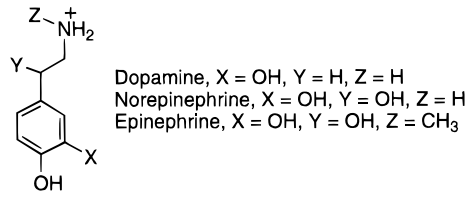
*Contribution from the Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, and Department of Chemical Technology, University of Twente, 7500 AE Enschede, The Netherlands*

*Received May 6, 1996*[⊗]

**Abstract:** A series of crown boronic acids, **1**−**4**, were synthesized and studied as carriers for catecholamine transport through bulk liquid membranes (BLMs) and supported liquid membranes (SLMs). Carrier **1** greatly facilitated the transport of primary catecholamines through BLMs; whereas, the more lipophilic analogues **3** and **4** were less effective. A combination of kinetic, mass spectral, and NMR evidence suggests that the transported species in BLMs is the cyclic, zwitterionic, 1:1 complex **7**. The SLM transport studies used a liquid membrane of 2-nitrophenyl octyl ether supported by a thin, flat sheet of porous polypropylene. In the absence of carrier there was neglible dopamine transport ($<5 \times 10^{-9}$ mol/m$^2$•s) at pH 7.2. When the membrane contained carrier **3** (33 mM or about 2% wt), facilitated catecholamine transport was observed in the order of dopamine ($5 \times 10^{-7}$ mol/m$^2$•s) > epinephrine ($1.5 \times 10^{-7}$ mol/m$^2$•s) > norepinephrine ($0.8 \times 10^{-7}$ mol/m$^2$•s). SLMs containing carrier **3** were stable, implying that carrier **3** is a very good candidate for transport mechanism studies. Crown boronic acid **4** was an even better transport carrier of primary catecholamines with a transport order of norepinephrine ($4.7 \times 10^{-6}$ mol/m$^2$•s) > dopamine ($3.5 \times 10^{-6}$ mol/m$^2$•s) ≫ epinephrine ($3 \times 10^{-8}$ mol/m$^2$•s). It is 10 times more effective than an equimolar mixture of boronic acid **5** and crown **6**, which is one of best examples of ditopic cooperativity yet observed in SLM transport. SLMs containing **4**, however, did not exibit long-term stability. Overall, it is possible that a device based on SLMs containing crown boronic acid carriers can be developed to selectively extract catecholamines from clinical samples.

## Introduction

Catecholamines play an important role in health and disease. Changes in catecholamine levels have been correlated with stress, heart disease, changes in blood pressure and thyroid hormone levels, catecholamine-secreting tumors, neuromuscular disorders, and various types of mental illness.[1,2] Numerous methods have been proposed for the determination of catecholamines and their metabolites in biological fluids such as urine and plasma. The most widely used are spectrometric, fluorometric, and radioenzymatic methods and HPLC with electrochemical detection.[1,3] The metabolites are often the primary assay target because of their higher concentrations and greater stability. However, in certain cases the concentration levels of the parent catecholamines are more diagnostic. This means highly sensitive, specific, and reliable methods are required for measuring the low amounts of dopamine, norepinephrine, and epinephrine. In most cases, a preliminary extraction and purification of the biological sample is necessary. The most common pretreatments involve alumina adsorption, ion-exchange, and/or boric acid chromatography.[1,3] These methods are labor-intensive and require a fair amount of technical expertize to ensure reliable results. A more straightforward and automated method of extracting catecholamines from clinical samples would be desirable.




We are attempting to develop a membrane-based catecholamine purification system. Our approach is to design carrier compounds that can selectively and actively transport catecholamines through liquid organic membranes.[4] Two types of liquid membranes were initially considered: (i) bulk liquid membranes (BLMs), where the aqueous source and receiving phases are separated by an immiscible organic liquid, and (ii) supported liquid membranes (SLMs), which have essentially the same configuration as BLMs but the organic liquid is contained within the pores of a thin porous polymer.[5]

Our preliminary studies used BLMs, which were chosen for a number of reasons.[4] BLM transport experiments are comparatively cheap and easy to conduct. Also, there are few restrictions on carrier structure, *e.g.*, BLM carriers are not required to be highly lipophilic. BLMs, however, are not practical in an industrial setting and are primarily used as a screening assay for carrier candidates. Since the final device

* Author to whom correspondence should be addressed.
† University of Notre Dame.
‡ University of Twente.
⊗ Abstract published in *Advance ACS Abstracts,* October 1, 1996.

(1) Whitley, R. J.; Meikle, A. W.; Watts, N. B. In *Tietz Textbook of Clinical Chemistry*, 2nd ed.; Burtis, C. A., Ashwood, E. R., Eds.; Saunders: Philadelphia, 1994; pp 1739−1764.

(2) Neumeyer, J. L.; Booth, R. G. In *Principles of Medicinal Chemistry*, 4th ed.; Foye, W. O., Lemke, T. L., Williams, D. A., Eds.; Lea and Febiger: Philadelphia, 1995; Chapter 13.

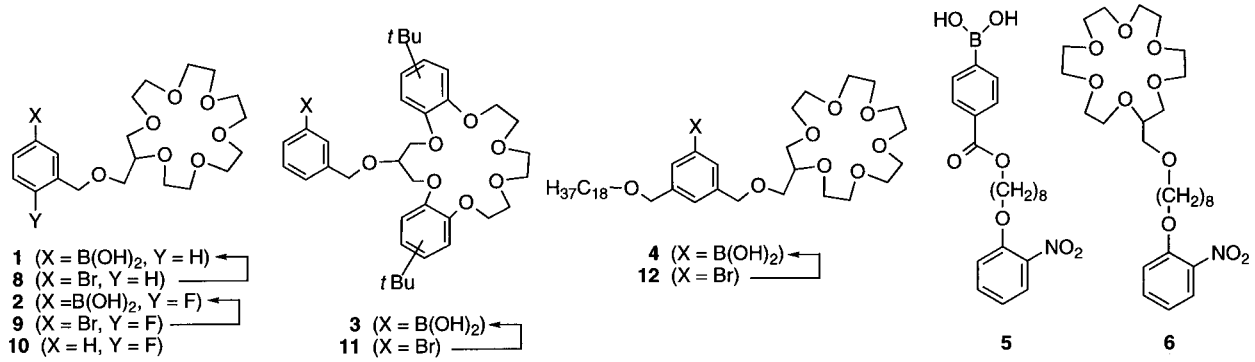
(3) (a) Rosano, T. G.; Swift, T. A.; Hayes, L. W. *Clin. Chem.* **1991**, *37*, 1854−1867. (b) *Quantitative Analysis of Catecholamines and Related Compounds*; Krstulovic, A. M., Ed.; Ellis Horwood: Chichester, 1986.

(4) Paugam, M.-F.; Valencia, L. S.; Boggess, B.; Smith, B. D. *J. Am. Chem. Soc.* **1994**, *116*, 11203−11204.

(5) (a) Visser, H. C.; Reinhoudt, D. N.; de Jong, F. *Chem. Rev.* **1994**, *23*, 75−82. (b) *Liquid membranes: Chemical Applications*; Araki, T., Tsukube, H., Eds.; CRC Press: Boca Raton, FL, 1990. (c) Fyles, T. M. In *Inclusion Aspects of Membrane Chemistry*; Osa, T., Atwood, J. L., Eds.; Kluwer: Boston, 1991.

S0002-7863(96)01507-7 CCC: $12.00 

**Scheme 1**

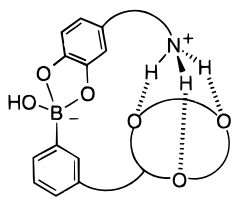



was envisioned to be small, robust, and automated, it was decided that an SLM-based system would be more appropriate. The large surface areas, well-defined diffusional pathway, and sturdy membranes associated with SLMs ensure fast and reproducible transport rates. Furthermore, rates can be controlled by modifying the shape of the polymer support, *e.g.*, changing from flat sheets to hollow fibers increases the effective surface area and consequently raises the total transport rate.[5]

An initial communication of this work focused on the remarkable selectivity of carrier **1** (Scheme 1) to transport dopamine through a BLM.[4] In this paper, we present additional BLM transport studies, as well as a structural elucidation of the transported species in BLMs using probe compound **2**. We also describe various catecholamine carriers that operate efficiently in SLMs. Specifically, we have synthesized and studied the transport abilities of carriers **3**−**6**. This work is the first example of facilitated catecholamine transport through SLMs.

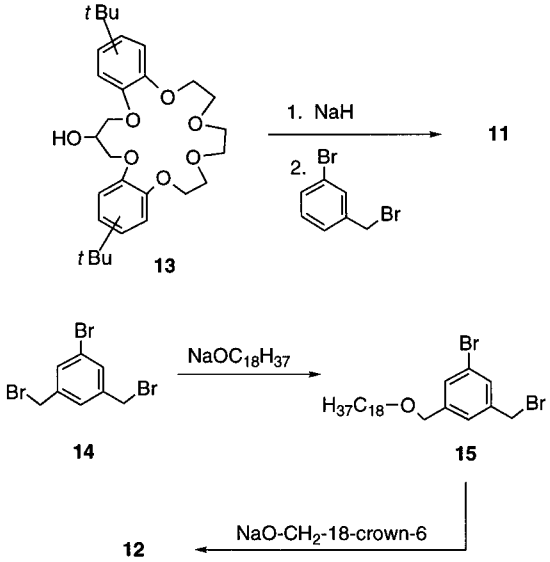
## Carrier Design and Synthesis

The p$K_a$ values of the boronic acid residues in carriers **1**−**4** (Scheme 1) are all approximately 9.0, so that at neutral pH the carriers are uncharged.[6] Transport of a non-diol-containing ammonium cation at neutral pH would require cotransport of an accompanying anion. This is an energetically demanding process which is very dependent on the lipophilicity of the anion.[7] Condensation of a boronic acid with a catechol unit, however, produces a boronate ester of greater acidity than the parent boronic acid, such that at neutral pH the tetrahedral boronate anion is formed.[8,9] The generic structure of a cyclic, 1:1 complex between any of the carriers **1**−**4** and a primary catecholamine is the covalent adduct **7**, a lipophilic zwitterionic species that is able to diffuse through a nonpolar membrane.[10]



**7**

**Scheme 2**




Thus, carriers **1**−**4** were predicted to selectively facilitate catecholamine transport through a liquid organic membrane.

Carriers **3** and **4** are more lipophilic versions of **1** and were designed to operate in SLMs. An examination of CPK models indicated they could assume conformations capable of ditopic binding with a catecholamine molecule. Compounds **5** and **6** were designed to be used together in an SLM as a carrier admixture. Both of their structures incorporate (2-nitrophenoxy)octyl groups to ensure good solubility in the supported, 2-nitrophenyl octyl ether membrane.[11]

Carriers **1**−**4** were synthesized in a straightforward fashion by metalation of their respective bromo precursors **8**, **9**, **11**, and **12** with butyllithium and subsequent treatment with trimethyl borate (Scheme 1). Intermediate **11** was prepared by alkylation of crown **13** (Scheme 2), which in turn was made using the method of Bartsch.[12] Carriers **5** and **6** were synthesized by alkylating the commercially available precursors with 1-bromo-8-(2-nitrophenoxy)octane.[11]

(6) Torsell, K. In *Progress in Boron Chemistry*; Steinberg, H., McCloskey, A. L., Eds.; Pergamon: New York, 1964; p 385.

(7) Lamb, J. D.; Christensen, J. J.; Izatt, S. R.; Bedke, K.; Astin, M. S.; Izatt, R. M. *J. Am. Chem. Soc.* **1980**, *102*, 3399−3403.

(8) (a) Smith, B. D. *Supramol. Chem.* **1996**, *7*, 55−60. (b) James, T. D.; Samankumara Sandanayake, K. R. A.; Shinkai, S. *Supramol. Chem.* **1995**, *6*, 141−157.

(9) London, R. E.; Gabel, S. A. *J. Am. Chem. Soc.* **1994**, *116*, 2562−2569.

(10) Other examples of synthetic ditopic receptors for catecholamines include: (a) Imada, T.; Kijima, H.; Takeuchi, M.; Shinkai, S. *Tetrahedron* **1996**, *52*, 2817−2826. (b) Kimura, E.; Fujioka, H.; Kodama, M. *J. Chem. Soc., Chem. Commun.* **1986**, 1158−1159. (c) Saigo, K.; Kihara, N.; Hashimoto, Y.; Lin, R.; Fujimura, H.; Suzuki, Y.; Hasegawa, M. *J. Am. Chem. Soc.* **1990**, *112*, 1144−1150. (d) Dumont, B.; Schmitt, M.-F.; Joly, J.-P. *Tetrahedron Lett.* **1994**, *35*, 4773−4776. (e) Sutherland, I. O. In *Advances in Supramolecular Chemistry*; Gokel, G. W., Ed.; JAI Press: Greenwhich, CT, 1990; Vol. 1.

(11) Visser, H. C.; Vink, R.; Snellink-Ruel, B. H. M.; Kokhuis, S. B. M.; Harkema, S.; de Jong, F.; Reinhoudt, D. N. *Recl. Trav. Chim. Pays-Bas* **1995**, *114*, 285−294.

(12) Charewicz, W. A.; Heo, G. S.; Bartsch, R. A. *Anal. Chem.* **1982**, *54*, 2094−2097. Heo, G. S.; Bartsch, R. A.; Schlobohm, L. L.; Lee, J. G. *J. Org. Chem.* **1981**, *46*, 3575−3576.

**Table 1.** Initial Fluxes for Dopamine Transport through BLMs Containing Different Carriers

| carrier[a] | flux[b] ($10^{-7}$ mol/m$^2$•s) |
|---|---|
| **1** | 18 |
| none | 0.1 |
| phenylboronic acid | 0.3 |
| dicyclohexyl-18-crown-6 | 0.2 |
| dicyclohexyl-18-crown-6 + phenylboronic acid | 6 |
| 18-crown-6 + phenylboronic acid | 4 |
| **3** | 1.6 |
| **3** + dicyclohexyl-18-crown-6 | 1.7 |
| **13** + phenylboronic acid | 0.1 |
| dibenzo-18-crown-6 + phenylboronic acid | 0.1 |
| **4** | 5 |
| **6** + phenylboronic acid | 1.4 |

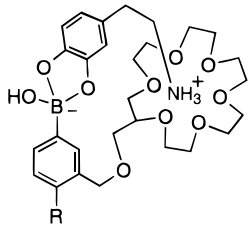
[a] Source phase: sodium phosphate (100 mM, pH 7.4), sodium dithionite (10 mM), dopamine (41 mM). Organic phase: carrier(s) (1 mM) in chloroform. Receiving phase: sodium phosphate (100 mM, pH 7.4), sodium dithionite (10 mM). Temperature: 293 K. [b] Initial dopamine flux extrapolated to $t = 0$, ±15%.

### Transport Studies Using BLMs

The dopamine fluxes through a BLM (chloroform) containing various carrier mixtures are summarized in Table 1. Qualitative aqueous/chloroform partition measurements with carrier **1** indicate that it is only moderately lipophilic. Nonetheless, the more lipophilic carriers **3** and **4** are less efficient at promoting dopamine transport. It appears that in the BLM experiment there is an advantage to having the carrier partially dissolved in the source phase where additional complexation can occur followed by movement of the complexed carrier into the BLM.

### Identification of Transported Species in BLMs

Our initial attempts to elucidate the structure of the transported species in BLMs relied heavily on kinetic and mass spectral evidence. A combination of FAB and electrospray mass spectrometry produced strong evidence for structure **16** as the transported species when carrier **1** was used to transport dopamine through a layer of chloroform (*e.g.*, negative ion FAB spectrum of aqueous and organic phases showed $m/z = 564$ [**16** − H]$^-$).[4] Attempts to corroborate this structural assignment using NMR spectroscopy were initially unclear. A $^1$H NMR spectrum of the organic layer produced ambiguous results due to broadened and overlapping signals, and the solutions were too dilute for $^{13}$C and $^{11}$B analyses. Thus, we resorted to a $^{19}$F NMR method using the fluoro-substituted carrier **2**. The method was based on observations made by London and Gabel that the $^{19}$F signal for 4-fluorobezeneboronic acid moves about 7 ppm upfield when the boronic acid forms a tetrahedral boronate complex with a catechol derivative.[9] This upfield shift reflects the change in boron hybridization from sp$^2$ to sp$^3$, which is transmitted to the para-substituted fluorine by resonance delocalization. The $^{19}$F NMR signal for carrier **2** in aqueous solution (4 mM), buffered at pH 7.4, occurred at −39.1 ppm relative to external $CF_3CH_2OH$.[13] Addition of 40 mM dopamine moved the signal upfield to −45.6 ppm. Extraction of this solution with chloroform and subsequent $^{19}$F NMR analysis of the organic phase also produced a signal at −45.6 ppm (all $^{19}$F NMR spectra are reproduced as supporting information). This upfield change in $^{19}$F chemical shift correlates with the results of London and Gabel and indicates that after treatment with dopamine the carrier boron atom has become sp$^3$ hybridized in both aqueous and organic phases. This is consistent with structure **17**, the fluoro-substituted analogue of **16**.



**16** R = H
**17** R = F

### Transport Studies Using SLMs

The apparatus used in the SLM transport studies has been described before.[14] The liquid membrane was 2-nitrophenyl octyl ether supported by a thin, flat sheet (15 cm$^2$) of porous polypropylene (Accurel). Although a flat sheet is not the most efficient membrane geometry (hollow fibers provide a greater surface area), its surface area can be measured accurately, which greatly facilitates the mechanistic interpretation. Typically, the source and receiving phases (50 mL) contained sodium phosphate (100 mM) buffered at pH 7.2 and sodium dithionite (1 mM) as an antioxidant. At the beginning of the transport experiment the source phase also contained 50 mM catecholamine. The initial catecholamine flux in the receiving phase was determined from the change in UV absorption extrapolated to $t = 0$.

The results of the SLM transport studies are shown in Table 2. Control experiments showed no detectable leakage of the liquid 2-nitrophenyl octyl ether or any of the carriers **3**−**6** into the receiving phase. In the absence of carrier there was neglible dopamine transport ($< 5 \times 10^{-9}$ mol/m$^2$•s) through the SLM. When the liquid membrane contained crown **6** (33 mM or about 2% wt), a small dopamine flux ($4 \times 10^{-8}$ mol/m$^2$•s) was observed which increased 10-fold ($4 \times 10^{-7}$ mol/m$^2$•s) when an equimolar mixture of crown **6** and boronic acid **5** was used. A similar dopamine flux ($5 \times 10^{-7}$ mol/m$^2$•s) was observed with carrier **3**, which combines the boronic acid and crown binding groups into a single molecule. While this was an encouraging result, producing a transport enhancement of >100 times the rate of background diffusion, an even higher flux was expected as the strategy of covalently attaching two monotopic carriers and forming a single ditopic carrier should in principle give rise to improved binding and transport.[15] It appeared that the problem was due to the 19-crown-6 group in **3**, which was likely a poor ammonium binder because of the weakened

(13) The sample of carrier **2** used in the $^{19}$F NMR studies was contaminated with 8% of the protiodeboronated analogue **10**. This impurity was not expected to prejudice the outcome of the experiment and in fact was a benefit because it acted as an internal reference signal (see Supporting Information).

(14) Stolwijk, T. B.; Sudhölter, E. J. R.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1987**, *109*, 7042−7047. van Straaten-Nijenhuis, W.; de Jong, F.; Reinhoudt, D. N. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 317−324.

(15) While there have been many studies of ditopic membrane carriers, surprisingly few have unambiguously demonstrated an increase in ditopic cooperativity due to covalent conjugation, *i.e.*, the ditopic receptor is a more efficient transporter than an equimolar mixture of two appropriate monotopic half-receptors. See, for example: Rudkevich, D. M.; Mercer-Chalmers, J. D.; Verboom, W.; Ungaro, R.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1995**, *117*, 6124−6125. Reetz, M. T.; Huff, J.; Rudolph, J.; Töllner, K.; Deege, A.; Goddard, R. *J. Am. Chem. Soc.* **1994**, *116*, 11588−11589. Mohler, L. K.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 2998−2999. Andreu, C.; Galán, A.; Kobiro, K.; de Mendoza, J.; Park, T. K.; Rebek, J.; Salmerón, A.; Usman, N. **1994**, *116*, 5501−5502. Král, V.; Andrievsky, A.; Sesslor, J. L. *J. Chem. Soc., Chem. Commun.* **1995**, 2349−2351. Sesslor, J. L.; Furuta, H.; Král, V. *Supramol. Chem.* **1993**, *1*, 209−220. Schwabacher, A. W.; Lee, J.; Lei, H. *J. Am. Chem. Soc.* **1992**, *114*, 7597−7598. Seel, A.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 442−444. Aoyama, Y.; Asakawa, M.; Yamagishi, A.; Toi, H.; Ogoshi, H. *J. Am. Chem. Soc.* **1990**, *112*, 3145−3151.

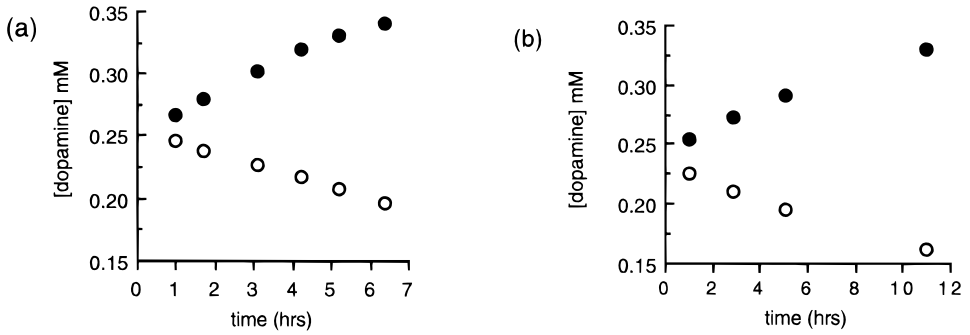



**Figure 1.** Uphill transport of dopamine from (○) source phase at pH 7.4 or (●) receiving phase at pH 5.5. Initially all aqueous layers contained 0.25 mM dopamine, 0.1 M sodium phosphate buffer, and 1 mM sodium dithionite. (a) BLM experiment with chloroform membrane containing **1** (1 mM). (b) SLM experiment with 2-nitrophenyl octyl ether membrane containing **4** (33 mM). Note the aqueous phase volumes in the SLM experiment are approximately 15 times greater than those in the BLM experiment.

**Table 2.** Initial Fluxes for Catecholamine Transport through SLMs Containing Different Carriers[a]

| solute | carrier[a] | flux[b] ($10^{-7}$ mol/m$^2$·s) |
|---|---|---|
| dopamine | none | <0.05 |
| norepinephrine | none | <0.05 |
| epinephrine | none | 0.2 |
| dopamine | **3** | 5 |
| norepinephrine | **3** | 0.8 |
| epinephrine | **3** | 1.5 |
| dopamine | **4** | 35 |
| norepinephrine | **4** | 47 |
| epinephrine | **4** | 0.3 |
| uridine | **4** | <0.1 |
| dopamine | **5** | <0.1 |
| dopamine | **6** | 0.4 |
| dopamine | **5** + **6** | 4 |
| norepinephrine | **5** + **6** | 3 |
| epinephrine | **5** + **6** | 0.8 |

[a] Transport through a liquid membrane supported by a thin, flat sheet of Accurel. Source phase: sodium phosphate (100 mM, pH 7.2), sodium dithionite (1 mM), dopamine (50 mM). Liquid membrane: carrier(s) (33 mM or about 2% wt) dissolved in 2-nitrophenyl octyl ether. Receiving phase: sodium phosphate (100 mM, pH 7.2), sodium dithionite (1 mM). Temperature: 298 K. [b] Initial catecholamine flux extrapolated to $t = 0$, ±10%.

basicity of its four phenoxy oxygens.[16] Consequently, we were very pleased to find that ditopic carrier **4**, which contains an 18-crown-6 group, induced an impressive dopamine flux of 3.5 × $10^{-6}$ mol/m$^2$·s. This is 1 order of magnitude more effective than the **5**/**6** mixture and is one of best examples of ditopic cooperativity yet observed in SLM transport.[15]

The abilities of **3**, **4**, and **5**/**6** to transport epinephrine and norepinephrine were also determined (Table 2). The transport order with carrier **4** and with carrier mixture **5**/**6** was norepinephrine ∼ dopamine ≫ epinephrine. This is in reasonable agreement with the order of BLM transport rates found with carrier **1** and was expected because 18-crown-6 groups are selective for primary ammonium cations over secondary ammonium cations.[4] The transport order with the less efficient carrier **3** was dopamine > epinephrine > norepinephrine. The change in selectivity is attributed to the weaker ammonium binding ability of the 19-crown-6 moiety in **3**, which means the lipophilicity of the catecholamine plays a more important role in controlling transport permeability (as reflected by the background fluxes in Table 1, epinephrine is more lipophilic than norepinephrine).

The stabilities of the various SLMs were investigated. Membranes containing carrier **3** appeared to be quite stable as repeated runs with the same membrane produced little change in flux. Thus carrier **3** is a very good candidate for further mechanistic studies. Unfortunately, the more efficient carrier **4** did not display good long-term stability, as repeated runs with the same membrane showed a decline in observed fluxes (approximately 15% decline with each repetition). As the transport experiment progressed, the physical appearance of the SLM containing **4** was observed to change from translucent to white. HPLC studies showed no evidence of leaching of the carrier into the aqueous phases. Instead, it seemed that the carrier−catecholamine complex was slowly precipitating within the 2-nitrophenyl octyl ether.[17]

An eventual application of this technology will most likely require that the catecholamine transport be not only rapid and selective but also active, *i.e.*, it must be capable of concentrating the catecholamine in the receiving phase. Moreover, due to the susceptibility of catecholamines to decompose under basic conditions, the most practical active transport system would be one driven by a neutral to acid pH gradient. The binding to produce complex **7** is an acid-producing equilibrium. Thus, a neutral to acid pH gradient is predicted to drive catecholamine transport uphill. Uphill transport studies were conducted using a BLM containing carrier **1** and an SLM containing carrier **4**. In both cases, uphill transport from a departure phase at pH 7.4 into a receiving phase at pH 5.5 was readily achieved (Figure 1).

## Conclusions

1. Crown boronic acid **1** greatly enhances the transport of primary catecholamines through BLMs;[4] whereas, the more lipophilic carriers **3** and **4** are less effective. It appears that in the BLM transport experiment there is an advantage to having the carrier partially dissolved in the source phase where additional complexation can occur followed by movement of the complexed carrier into the BLM.

2. A combination of kinetic, mass spectral, and NMR evidence strongly suggests that the transported species in a chloroform membrane is the cyclic, 1:1 complex **7**.[17]

3. Crown boronic acid **3** greatly facilitates the transport of catecholamines through SLMs, with a transport order of dopamine > epinephrine > norepinephrine. SLMs containing carrier **3** are stable, implying that carrier **3** is a very good candidate for transport mechanism studies.

4. Crown boronic acid **4** is an outstanding carrier of primary catecholamines with a transport order of norepinephrine >

(16) Aldag, R.; Scröder, G. *Liebigs Ann. Chem.* **1984**, 1036−1048.

(17) The ditopic recognition sites in **1** are essentially identical to those in **4**. Thus, it is reasonable to expect that **1** and **4** would have the same general transported structure, *i.e.*, the 1:1 cyclic zwitterion **7**. One important difference, however, is that the membrane concentration of **4** in the SLM study was 33 times higher than the concentration of **1** in the BLM study. Although there is strong evidence that **1** forms structure **7**, it is possible that **4** may be form a larger oligomeric analogue(s), which may explain its propensity to precipitate from the membrane.

dopamine ≫ epinephrine. It is initially 10 times more effective than an equimolar mixture of boronic acid **5** and crown **6**, which is one of best examples of ditopic cooperativity yet observed in SLM transport.[15] SLMs containing **4**, however, do not exibit long-term stability. Future work needs to focus on developing analogues of **4** that maintain its impressive transport performance and have high SLM stability.

5. It is possible that a device based on SLMs containing crown boronic acid carriers can be developed to selectively extract catecholamines from clinical samples.

## Experimental Section

The materials and general experimental methods have been described before.[14,18]

**Crown Boronic Acid 1.** Compound **8** (0.86 mmol) was treated with butyllithium (0.90 mmol) in THF (100 mL) at −100 °C. The reaction was stirred for 1 h at −100 °C, treated with $B(OMe)_3$ (5.3 mmol), and allowed to warm up to room temperature overnight. The solution was poured onto a mixture of crushed ice (50 g)/concentrated hydrochloric acid (30 mL). The aqueous solution was extracted with diethyl ether (3 × 20 mL), which removed all impurities. Subsequent extraction with chloroform (4 × 20 mL) gave compound **1** in 32% yield: $^1$H NMR (300 MHz, acetone-$d_6$) $\delta$ 7.83 (s, 1H), 7.60 (d, 1H, $J$ = 7.5 Hz), 7.38 (d, 1H, $J$ = 7.5 Hz), 7.30 (t, 1H, $J$ = 7.5 Hz), 7.10 (s, exchanged with $D_2O$), 4.51 (s, 2H), 3.72 (m, 3H), 3.57 (m, 20 H), 3.29 (m, 2H) ppm; $^{13}$C NMR (10% $D_2O$/acetone-$d_6$) $\delta$ 138.7, 134.9, 134.8, 131.2, 129.7, 128.9, 79.2, 74.4, 72.3, 71.6, 71.4, 71.3, 70.8, 70.3 ppm; HRMS (FAB) *m/z* 485.2558, calcd for $C_{23}O_{10}H_{37}B$ [M + H]$^+$ 485.2558.

**Crown Bromofluorobenzene 9.** A solution of 2-(hydroxymethyl)-18-crown-6 (0.53 mmol) in THF (1 mL) was added dropwise to a solution of NaH (0.80 mmol) in THF (50 mL) at room temperature. The mixture was stirred at room temperature for 3 h and then treated with 5-bromo-2-fluorobenzyl bromide (0.53 mmol, prepared by NBS bromination of 5-bromo-2-fluorotoluene) in THF (5 mL). The mixture was heated at reflux for 4 h, cooled, filtered, and evaporated. The residue was purified by flash chromatography (silica gel, 30:1 dichloromethane/methanol) to afford the desired material as a tan oil in 90% yield: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.56 (1H, dd, $J$ = 6.0, 2.0 Hz), 7.35 (1H, m), 6.91 (1H, t, $J$ = 9.0 Hz), 4.57 (2H, s), 3.82 (3H, m), 3.67 (20H, s), 3.58 (2H, m) ppm; $^{13}$C NMR ($CDCl_3$, 75 MHz) $\delta$ 160.4 (d, $J$ = 255.0 Hz), 132.9 (d, $J$ = 52.0 Hz), 128.7 (d, $J$ = 22.0 Hz), 117.5 (d, $J$ = 22.0 Hz), 78.8, 78.7, 71.4, 71.3, 71.2, 71.1, 70.3, 66.5 ppm; HRMS (FAB) *m/z* 503.1027, calcd for $C_{20}H_{30}O_7BrFNa$ [M + Na]$^+$ 503.1057.

**Crown Fluorobenzeneboronic Acid 2.** Precursor **9** was converted to the boronic acid using the same procedure described for **1**. The crude product was 90% **2** and 10% **10** as proved by comparison with authentic material. Purification by flash chromatography followed by reverse phase HPLC reduced this contaminant to 8%. The final yield of **2** was 10%: $^1$H NMR (500 MHz, acetone-$d_6$/10% $D_2O$) $\delta$ 7.97 (1H, dd, $J$ = 8.0, 2.0 Hz), 7.82 (1H, ddd, $J$ = 14.0, 8.0, 2.0 Hz), 7.06 (1H, dd, $J$ = 11.0, 8.0 Hz), 4.58 (2H, s), 3.73 (3H, m), 3.57 (22H, s) ppm; HRMS (FAB) *m/z* 469.1990, calcd for $C_{20}H_{32}O_9BFNa$ [M + Na]$^+$ 469.2025.

**Crown Bromobenzene 11.** 3-Bromobenzyl bromide (1.5 mmol) was alkylated with crown **13**[12] (1.5 mmol) according to the procedure described for **9**. The crude material was purified by flash chromatography (silica gel, 1:3 ethyl acetate/hexanes) to give **11** in 98% yield. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.56 (s, 1H), 7.38 (d, 1H, $J$ = 8.0 Hz), 7.29 (m, 1H), 7.19 (t, 1H, $J$ = 8.0 Hz), 6.85 (m, 12H), 4.81 (m, 2H), 4.21 (m, 4H), 3.86 (m, 4H), 3.78 (m, 4H), 1.26, 1.28 (s, 18H) ppm; MS (FAB) *m/z* 673/671 [M]$^+$.

**Crown Boronic Acid 3.** Compound **11** (0.54 mmol) was treated with butyllithium (1.1 mmol) in THF (10 mL) at −100 °C. The reaction mixture was stirred at −100 °C for 1 h and then brought to −40 °C over 2 h. The solution was then recooled to −100 °C and treated with $B(OMe)_3$ (2.7 mmol). After being warmed to room temperature overnight, the mixture was quenched with 10% HCl (5 mL). The solution was extracted with methylene chloride (3 × 10 mL). The organic layers were dried ($MgSO_4$), filtered, and evaporated. Subsequent purification by flash chromatography (silica gel, ethyl acetate as eluent until the first fractions were collected and then 4:1 ethyl acetate/methanol) gave **3** as a mixture of three isomers in 48% yield: $^1$H NMR (300 MHz, acetone-$d_6$/10% $D_2O$) $\delta$ 7.90 (s, 1H), 7.77 (d, 1H, $J$ = 7.0 Hz), 7.47 (d, 1H, $J$ = 7.0 Hz), 7.30 (t, 1H, $J$ = 7.0 Hz), 6.88 (m, 8H), 4.81 (br s, 2H), 4.15 (m, 9H), 3.80 (m, 4H), 3.68 (br s, 4H), 1.26, 1.23 (s, 18 H) ppm; $^{13}$C NMR (75 MHz, acetone-$d_6$/10% $D_2O$) $\delta$ 149.9, 149.8, 149.2, 149.1, 148.3, 148.2, 147.4, 146.1, 145.0, 138.5, 138.4, 134.4, 134.3, 134.2, 134.0, 130.4, 130.5, 128.0, 119.7, 119.6, 118.5, 117.6, 117.3, 116.1, 115.9, 114.4, 112.7, 77.8, 77.7, 77.6, 72.6, 71.8, 71.5, 71.1, 70.1, 69.7, 69.5, 34.7, 34.5, 31.6 ppm; HRMS (FAB, glycerol matrix) *m/z* 692.3707, calcd for $C_{39}O_{10}H_{53}B$ [M + glycerol]$^+$ 692.3739.

**1-Bromo-3,5-bis(bromomethyl)benzene (14).** A mixture of 5-bromo-*m*-xylene (28.6 mmol), *N*-bromosuccinimide (58.0 mmol), and α,α′-azobisisobutyronitrile (150 mg) was heated at reflux in dry $CCl_4$ (200 mL) for 18 h. The solution was allowed to cool, then filtered, and evaporated. The desired compound was crystallized from hexanes as white needles: yield 33%; $^1$H NMR (300 MHz, $CDCl_3$ ) $\delta$ 7.48 (s, 2H), 7.37 (s, 1H), 4.42 (s, 4H) ppm; MS (EI) *m/z* 344/342 [M]$^+$.

**Crown Bromobenzene 12.** Octadecanol (4.5 mmol) was alkylated with **14** (4.5 mmol) according to the procedure described for **9**. The crude product was partially purified by chromatography (silica gel, petroleum ether until the first fractions were collected and then 8:1 petroleum ether/ethyl acetate) to give a mixture of **14**, **15**, and 1-bromo-3,5-[bis(1-octadecanoxy)methyl]benzene, **18**. The residue was recrystallized from 4:1 ethanol/methylene chloride at 0 °C to give a mixture of **15** and **18** (2:1). The mixture (1.38 mmol of **15**) was alkylated with 2-(hydroxymethyl)-18-crown-6 (1.38 mmol) according to the procedure described for **9**. The crude product was purified by flash chromatography (alumina, 1:3 petroleum ether/ethyl acetate, $R_f$ = 0.43) to give **12** as an oil in 77% yield: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.40 (s, 2H), 7.18 (s, 1H), 4.50 (s, 2H), 4.45 (s, 2H), 3.80 (m, 3H), 3.67 (br s, 20H), 3.55 (m, 2H), 3.46 (t, 2H, $J$ = 6.5 Hz), 1.61 (p, 2H, $J$ = 7.0 Hz), 1.25 (s, 30H), 0.88 (t, 3H, $J$ = 7.0 Hz); IR (KBr) 2920, 2850, 1670, 1450, 1350, 1300, 1100 cm$^{-1}$; MS (FAB) *m/z* 769/767 [M + H]$^+$.

**Crown Benzeneboronic Acid 4.** Compound **12** (0.17 mmol) was treated with butyllithium (0.35 mmol) in THF (7 mL) at −75 °C. The reaction mixture was gradually brought to −55 °C over 1 h and stirred an additional 6 h. The solution was recooled to −75 °C and treated with $B(OMe)_3$ (1 mmol). After quenching and workup similar to the procedure described for **3**, the crude material was purified by flash chromatography (alumina, ethyl acetate/methanol gradient) to give compound **4** as an oil in 55% yield. $^1$H NMR (300 MHz, acetone-$d_6$/10% $D_2O$) $\delta$ 7.41 (s, 1H), 7.37 (s, 1H), 7.15 (s, 1H), 4.51 (s, 2H), 4.55 (s, 2H), 3.72 (m, 3H), 3.57 (br s, 20H), 3.53 (m, 2H), 3.44 (t, 2H, $J$ = 6.5 Hz), 1.56 (p, 2H, $J$ = 7.0 Hz), 1.25 (s, 30H), 0.84 (t, $J$ = 7.0 Hz) ppm; $^{13}$C NMR (75 MHz, acetone-$d_6$/10% $D_2O$) $\delta$ 136.6, 136.1, 131.7, 131.6, 127.5, 76.4, 71.8, 71.3, 69.6, 69.0, 68.8, 68.7, 68.6, 68.5, 67.9, 67.3, 30.5, 28.2, 24.8, 21.2 ppm; HRMS (FAB) *m/z* 789.5297, calcd for $C_{42}H_{75}O_{11}BNa$ [M + Na]$^+$ 789.5308.

**4-[[[8-(2-Nitrophenoxy)octyl]oxy]carbonyl]benzeneboronic Acid (5).** A suspension of 4-carboxybenzeneboronic acid (3.0 mmol) and potassium hydrogen carbonate (6.0 mmol) in *N*,*N*-dimethylformamide (25 mL) was treated with 1-bromo-8-(2-nitrophenoxy)octane[11] (3.1 mmol) and stirred at 65 °C for 48 h. The solution was allowed to cool, acidified (0.33 M sulfuric acid, pH = 2−3), and extracted with methylene chloride (3 × 20 mL), which was evaporated to afford an oil. Subsequent purification by MPLC (ethyl acetate/methanol gradient) gave **5** in 65% yield: $^1$H NMR (300 MHz, acetone-$d_6$/10% $D_2O$) $\delta$ 7.94 (s, 4H), 7.78 (d, 1H, $J$ = 8.0 Hz), 7.59 (t, 1H, $J$ = 8.0 Hz), 7.28 (d, 1H, $J$ = 8.0 Hz), 7.07 (t, 1H, $J$ = 8.0 Hz), 4.29 (t, 1H, $J$ = 6.5 Hz), 4.15 (t, 1H, $J$ = 6.5 Hz), 1.70 (m, 4H), 1.30 (m, 8H) ppm; $^{13}$C NMR (75 MHz, acetone-$d_6$/10% $D_2O$) $\delta$ 211.2, 167.0, 152.5, 141.0, 134.9, 134.7, 128.9, 125.5, 120.9, 115.5, 70.0, 65.5, 30.0, 29.7, 29.6, 29.5, 29.4 ppm; HRMS (FAB, glycerol matrix) *m/z* 472.2122, calcd for $C_{24}H_{31}O_8NB$ [M + glycerol]$^+$ 472.2143.

**2-[[[8-(2-Nitrophenoxy)octyl]oxy]methyl]-18-crown-6 (6).** 1-Bromo-8-(2-nitrophenoxy)octane[11] (1.01 mmol) was alkylated with 2-(hy-

(18) Morin, G. T.; Paugam, M.-F.; Hughes, M. P.; Smith, B. D. *J. Org. Chem.* **1994**, *59*, 2724−2728.

droxymethyl)-18-crown-6 (1.0 mmol) according to the procedure described for **9**. The residue was purified by flash chromatography (alumina, 1:1 ethyl acetate/hexanes) to give **6** as a yellow oil in 33% yield: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.80 (d, 1H, $J$ = 8.0 Hz), 7.48 (d, 1H, $J$ = 8.0 Hz), 7.04 (d, 1H, $J$ = 8.0 Hz), 6.98 (t, 1H, $J$ = 8.0 Hz), 4.07 (t, 2H, $J$ = 6.5 Hz), 3.79 (t, 2H, $J$ = 5.0 Hz), 3.64 (m, 21H), 3.46 (m, 4H), 1.78 (p, 2H, $J$ = 8.0 Hz), 1.45 (m, 8H) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 152.3, 139.9, 133.8, 125.4, 119.9, 114.3, 78.3, 71.7, 71.5, 70.7, 70.6, 70.5, 64.5, 62.8, 32.6, 29.5, 29.2, 29.1, 28.5, 25.9, 25.7, 25.5 ppm; HRMS (FAB) *m/z* 566.2952, calcd for $C_{27}H_{45}O_{10}NNa$ $[M + Na]^+$ 566.2947.

**Transport of Catecholamines through BLMs.** The apparatus and methodology used in the BLM transport experiments have been described in detail.[18] Briefly, a solution of sodium phosphate buffer (0.1 M, pH 7.4, 7 mL) containing sodium dithionite ($Na_2S_2O_4$, 0.01 M) as an antioxidant and a solution of carrier in chloroform (1 mM, 7 mL) were shaken in a separatory funnel. The organic layer was then placed in the bottom of a U-tube apparatus (internal diameter 1.20 cm, height 10 cm, and 2.5 cm between the arms) and the aqueous phase added in halves to the two arms of the appartaus. Only the organic layer was stirred (475 rpm). The transport experiment was initiated by adding a small aliquot of concentrated catecholamine solution to the source phase. The appearance of catecholamine in the receiving phase was monitored spectroscopically at 279 nm. All transport runs were repeated at least once, and the observed fluxes were always within ±15% of each other.

**Transport of Catecholamines through SLMs.** The design of the SLM transport cells has been described before.[14] A series of near-identical, water-jacketed cells were used, each with source and receiving phase volumes of approximately 50 mL and membrane surface areas close to 15 cm$^2$. The exact dimensions were taken into consideration when computing fluxes. The source and receiving phases contained sodium phosphate (100 mM) buffered at pH 7.2 and sodium dithionite (1 mM) as an antioxidant. The source phase also contained 50 mM catecholamine. The membrane was a thin, flat sheet of Accurel that had been soaked in 2-nitrophenyl octyl ether containing 33 mM (about 2% wt) of carrier. The temperature was maintained at 298 K. The appearance of catecholamine in the receiving phase was determined from the change in UV absorption at 279 nm. Continuous measurement of the receiving phase was achieved by cycling through a flow-through UV cell. Initial fluxes were determined by curve-fitting analysis after 50 min of transport. All transport runs were repeated at least once, and the observed fluxes were always within ±10% of each other.

**$^{19}$F NMR Studies.** An aqueous solution of **2** (4 mL, 4 mM) and dopamine (40 mM), buffered at pH = 7.4, was shaken and equilibrated with an equal volume of chloroform. Both layers were analyzed by $^{19}$F NMR relative to external $CF_3CH_2OH$.

**Acknowledgment.** This work was supported by a NATO collaborative reseach grant and the U.S. National Science Foundation. B.D.S. acknowledges a Cottrell Scholar award of Research Corporation.

**Supporting Information Available:** $^1$H and $^{13}$C NMR spectra of carriers **1**, **3**, **4**, and **5** and $^{19}$F NMR spectra of carrier **2** with and without dopmaine (9 pages). See any current masthead page for ordering and Internet access instructions.

JA9615076