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Selective Fructose Transport Through Supported Liquid Membranes Containing Diboronic Acid or Conjugated Monoboronic Acid-Quaternary Ammonium Carriers.

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Abstract
The design and preparation of two new classes of boronic acid carriers is described along with an evaluation of their abilities to extract and transport the commercially important sugars, fructose, glucose, and sucrose through polymer supported liquid membranes. Transport fluxes with diboronic acid carriers are lower than those observed with monoboronic acids. However, fructose selectivity is improved when the diboronic linker group allows the formation of a 1:1 macrocyclic complex. A conjugated monoboronic acid-quaternary ammonium carrier facilitates fructose transport about twenty times better than an analogous monoboronic acid/quaternary ammonium mixture. The rate-determining step for the transport is diffusion through the membrane.

Keywords: Membranes; Molecular Recognition; Carbohydrates; Boron and Compounds

Introduction

Although membranes have been considered for use in sugar separations, there are very few examples of lipophilic membranes that are selectively permeable to sugars.[1] Even rarer are membranes capable of separating saccharide isomers such as the different hexose diastereomers. Recently, we and others have shown that liquid membranes containing lipophilic boronic acid carriers are selectively permeable to fructose over glucose.[2-5] We have also demonstrated how a fructose-selective Supported Liquid Membrane (SLM) containing the lipophilic boronic acid carrier **1** can be employed in a laboratory scale process to produce high fructose syrup.[2] For use in an industrial setting, however, the following membrane properties need to be improved; (i) membrane stability, (ii) transport flux, (iii) transport selectivity, and (iv) ability to transport uphill. We are attempting to address these concerns by systematically modifying the properties of the various membrane components. The present paper deals with membrane properties (ii) - (iv). Specifically, the design and preparation of two new classes of boronic acid carriers is described along with an evaluation of their abilities to transport the commercially important sugars, fructose, glucose, and sucrose through SLMs.

Boronic acids facilitate the transport of saccharides through liquid membranes by forming labile chelated complexes with the saccharide's vicinal diol groups.[3-5] Depending on the experimental conditions the transported species is a neutral trigonal boronate ester (eq 1), or an anionic tetrahedral boronate that forms an ion-pair with a

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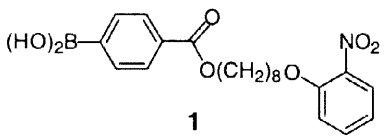
lipophilic phase-transfer cation (eq 2). While the trigonal boronate pathway is apparently more fructose selective than the tetrahedral pathway,[2] the tetrahedral pathway is inherently more attractive for industrial separations because transport can be driven uphill by the application of a basic to acidic pH gradient.[4,5] It is therefore important to develop tetrahedral boronate transport systems with improved fluxes and fructose selectivities.



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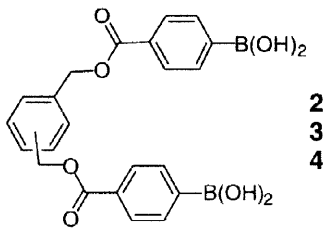
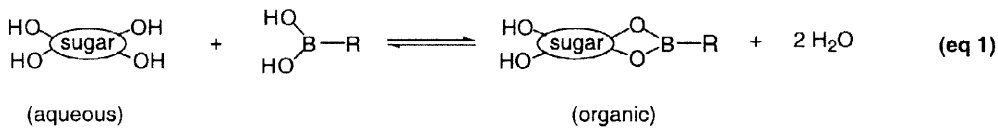
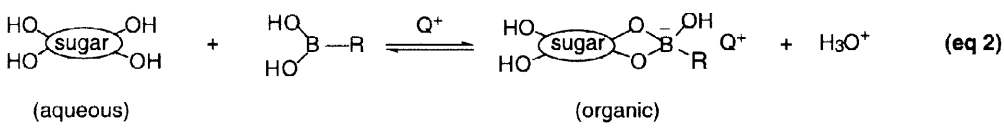


2 ortho
3 meta
4 para

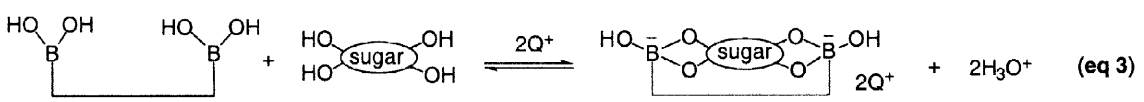


(aqueous) (organic) **(eq 1)**



(aqueous) (organic) **(eq 2)**

There is no previous published study of membrane transport using diboronic acid carriers, however, the sugar binding properties of a number of diboronic acids have recently been investigated.[6] The results of these studies suggest that appropriately designed diboronic acids should be able to transport monosaccharides by forming transient macrocyclic complexes (eq 3), the transport selectivity being controlled by the structure and saccharide recognition ability of the diboronic acid. With eq 3 in mind, we chose to examine the extraction and transport abilities of isomeric diboronic acids **2** - **4**. The advantage of comparing carriers **2** - **4** is that there is little difference in their lipophilicities or membrane diffusion constants. Thus, the observed transport efficiencies can be treated as a measure of the diboronic acid recognition abilities which will depend on the size and shape of the isomeric linker groups connecting the two boronic acids.



(aqueous) (organic) **(eq 3)**

Results and Discussion

Diboronic Acid Carriers

The synthesis of monoboronic acid **1** has been described previously.[2] Diboronic acids **2** - **4** were prepared by reaction of the appropriate isomer of dibromoxylene with two molar equivalents of (4-

carboxybenzene)boronic acid. Initially, competitive extraction studies were carried out (Table 1). The sugars were extracted from buffered aqueous solutions containing a 1:1:1 mixture of fructose, glucose and sucrose, into an organic phase containing an equimolar mixture of one of the boronic acids **1** - **4** and Aliquat 336™ (which is predominantly trioctylmethylammonium chloride). The percentage of sugar extracted was determined by enzymatic analysis of the aqueous phase before and after extraction. As expected, sugar extraction increased significantly once the aqueous pH was higher than the boronic acid pKa (estimated to be around 8 for compounds **1** - **4**).[4] In all cases the extraction selectivity was fructose > glucose >> sucrose. Of the diboronic acids, the *ortho* isomer **2** showed the highest fructose to glucose selectivity at pH 11.

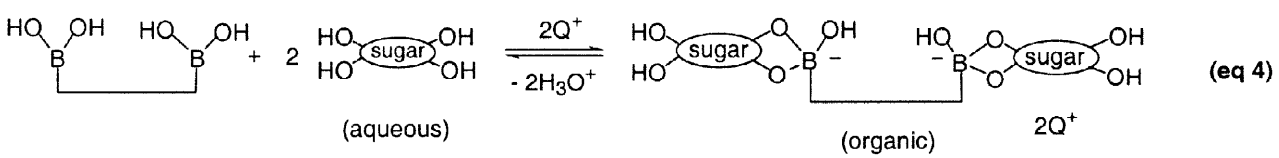
Table 1. Percent of Sugars Extracted at Different pH Values.[a,b]

extractant mixture	fructose				glucose				sucrose			
	pH				pH				pH			
	5	6	7	11	5	6	7	11	5	6	7	11
1/Aliquat	0	12	6	47	8	8	7	22	7	0	4	10
2/Aliquat	9	10	12	41	7	6	7	24	2	6	0	10
3/Aliquat	11	11	11	38	7	8	12	28	4	4	6	2
4/Aliquat	3	10	11	38	8	6	9	27	0	1	0	3

[a]Uncertainty of ±3% for fructose and glucose extraction and ±90 % for sucrose extraction. [b]Organic phase (0.5 mL) of chloroform /methanol (10:1) contained 10 mM of boronic acid and 10 mM of Aliquat; aqueous phase (0.5 mL) contained 10 mM of each sugar, 0.1 M potassium carbonate for pH 11, and 0.1 M potassium phosphate for pH 5-7.

Competitive SLM transport fluxes were determined with the same apparatus used in earlier studies.[2] The liquid membrane was a solution of boronic acid/Aliquat dissolved in 2-nitrophenyl octyl ether supported by a thin, flat sheet of microporous polypropylene (Accurel™). The membrane separated two buffered aqueous phases, with the source phase containing a 1:1:1 mixture of fructose:glucose:sucrose. In each case a pH gradient was used (pH 11 in the source and pH 6 in the receiving) since this is the condition that produces uphill transport.[4] The appearance of sugar in the receiving phase was monitored by enzymatic methods.

As seen in Table 2, the fluxes obtained with the diboronic acids **2** - **4** were slightly lower than those for monoboronic acid **1**. This is attributed to the different stoichiometries of the transported species. The monoboronic acid transports as a 1:1 ion pair (eq 2), whereas the diboronic acids can also transport as a 1:2 ion pair (eq 3 or eq 4). These higher aggregates are harder to form for entropic reasons and less likely to partition into the membrane. Also, they are larger and thus likely to have lower diffusion constants. As shown below, the rate-determining step for the transport is diffusion through the liquid organic membrane.



(eq 4)

All of the carriers **1** - **4** exhibited a transport selectivity order of fructose > glucose >> sucrose, which matches the extraction order. Interestingly, the *ortho* diboronic acid isomer **2** showed the highest fructose to glucose selectivity of 6.1. This suggests that it is more capable of forming a macrocyclic bidentate 1:1 complex with fructose (eq 3). Molecular modeling clearly indicates that the spacer width for **2** accommodates a fructose guest better than the spacers in **3** or **4** which are too wide (Figure 1). The modeling also indicates that the spacer in **2** is too wide to easily form an analogous macrocyclic structure with glucose. This is in agreement with previous studies that have shown that smaller diboronic acid cleft distances are needed for good glucose binding.[6]

Table 2. Initial Fluxes for Competive Sugar Transport.[a]

entry	carrier mixture	conc. (mM)	Flux (10^{-8} mol m^{-2} s^{-1}) fructose	glucose	sucrose	fructose/glucose
1	**1**/Aliquat	50	29	6.4	0.7	4.5
2	**2**/Aliquat	50	21	3.4	0.5	6.1
3	**3**/Aliquat	50	14	2.9	0.5	4.8
4	**4**/Aliquat	50	13	3.4	0.5	3.8

[a]Carrier was dissolved in 2-nitrophenyl octyl ether supported by a sheet of Accurel (11.3 cm^2). Source phase (38 mL) was buffered with 0.10 M sodium carbonate pH 11.0 and contained 0.10 M of each sugar. The receiving phase (38 mL) was buffered with 0.10 M sodium phosphate pH 6.0. T = 25 °C. Flux uncertainty ±10%.

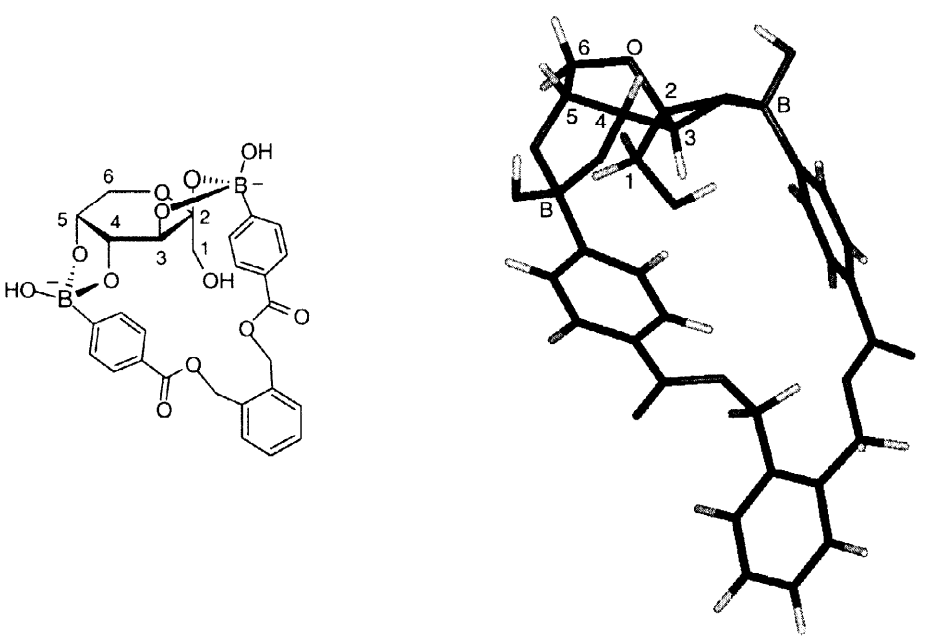
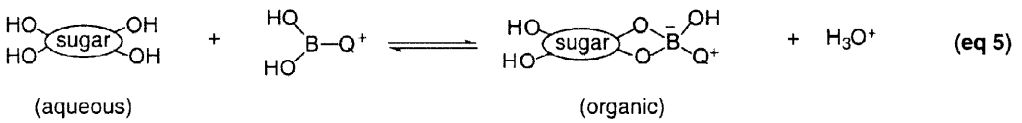
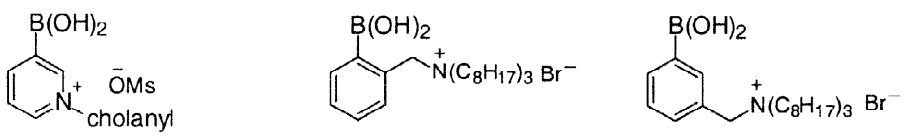


Figure 1. Drawing and molecular model of a likely macrocyclic complex, **5**, formed from *ortho*-diboronic acid **2** and β-fructopyranose. The modeling was based on the structure of β-D-fructopyranose 2,3:4,5-bis(tolylboronate) recently elucidated by Norrild and Eggert.[7] To improve model clarity, the β-fructopyranose carbons and ring oxygen are labeled, as well as the two tetrahedral borons.

Conjugated Boronic Acid-Quaternary Ammonium Carrier

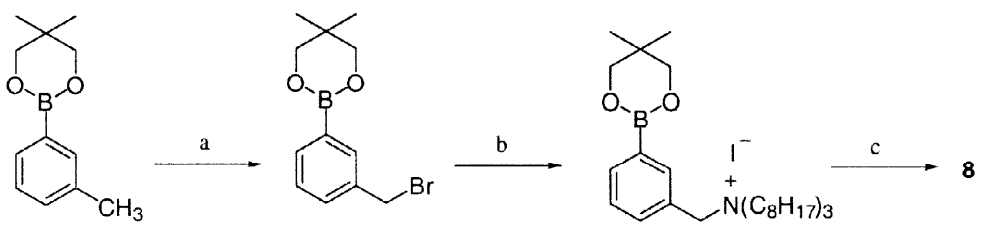
The monoboronic acid transport studies described above used a binary carrier mixture of boronic acid and quaternary ammonium salt, and extracted saccharides according to eq 2. This extraction equilibrium can be made more favourable if the two carriers are covalently linked together, as this reduces the entropic cost of forming the transported complex (compare eq 2 to eq 5). This effect was first demonstrated by Czarnik who showed that pyridinium boronic acid **6** transports nucleosides through bulk liquid membranes at higher rates than a mixture of phenylboronic acid/Aliquat.[8] More recently, Shinkai found enhanced glucoside extraction using the boronic acid-quaternary ammonium conjugate **7**.[9] Here we describe the remarkable SLM transport ability of the isomeric boronic acid-quaternary ammonium conjugate **8**.





6 **7** **8**

Scheme 1



9 **10** **11**

Reagents and Conditions: a) NBS, AIBN, CCl_4, Δ; b) trioctylamine, KI; c) HBr/$CHCl_3$; (6% overall yield).

Compound **8** was prepared by the sequence shown in Scheme 1. Fructose and glucose transport studies were conducted using virtually the same conditions as described above and the results are shown in Table 3. Carrier **8** was found to induce a fructose flux about twenty times higher than a corresponding **1**/Aliquat mixture (entries 6 vs 8). Competition experiments showed a 2.8:1 selectivity for fructose over glucose. Repeated use of the same membrane (entry 8) results in a significant decrease in flux suggesting that **8** slowly partitions into the aqueous phases over time.

Since the fluxes observed with **8** were so high we wondered if the rate-limiting step was still diffusion of the complex across the membrane (the alternative possibility is that diffusion is so fast that the rate limiting step becomes the kinetics of complex dissociation). A series of transport experiments were conducted using a varying number of identical membranes that were stacked together (each membrane contained the same concentration of carrier **8**). As shown in Figure 2, a plot of inverse fructose flux (1/J) versus membrane thickness (d) is essentially

linear with a zero intercept, unambiguous evidence that diffusion through the membrane is still the rate-controlling step.[1]

Table 3. Initial Sugar Fluxes.[a]

entry	carrier	conc. (mM)	Flux (10^{-8} mol m^{-2} s^{-1}) fructose	glucose	fructose / glucose
5[b]	**1**/Aliquat	250	91	17	5.4
6	**1**/Aliquat	40	27	-	-
7[b]	**8**	40	520	187	2.8
8	**8**	40	443 (193)[c]	-	-

[a]Carrier was dissolved in 2-nitrophenyl octyl ether supported by a sheet of Accurel (11.0 cm^2). Source phase (50 mL) was buffered with 0.50 M sodium carbonate pH 10.0 and contained 0.30 M of each sugar. The receiving phase (50 mL) was buffered with 0.50 M sodium acetate pH 6.0. T = 25 °C, Flux uncertainty ±10%. [b]Competitive experiments in which the source phase contained 0.3 M fructose and 0.3 M glucose. [c]Repeat run with the same membrane after it was washed with pH 7.3 buffer for 24 h.

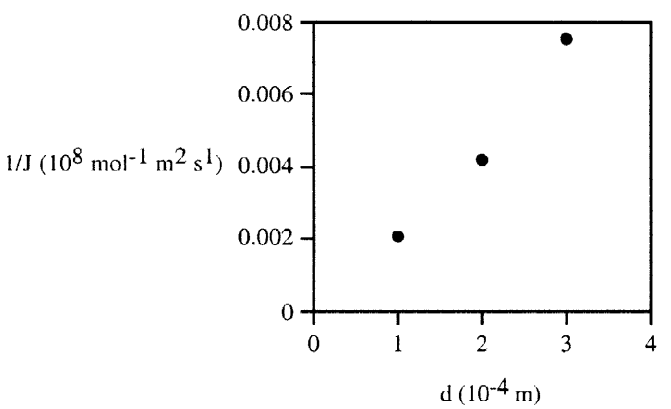


Figure 2. Influence of membrane thickness (d) on inverse fructose flux (1/J).

Summary

1. For industrial sugar separations, the tetrahedral boronate pathway (eq 2) is more attractive than the trigonal pathway (eq 1) because it allows transport to be driven uphill by a pH gradient. The tetrahedral pathway is only moderately fructose selective using monoboronic acid carriers dissolved in supported liquid membranes.

2. Fluxes with the diboronic acid carriers are slightly lower than those observed with monoboronic acids; however, fructose selectivity can be improved slightly by using appropriately designed diboronic acids that are capable of forming macrocyclic complexes (eq 3).

3. The conjugated monoboronic acid-quaternary ammonium carrier **8** facilitates monosaccharide transport about twenty times better than an analogous monoboronic acid/quaternary ammonium mixture. The rate-determining step for transport is diffusion through the membrane. SLMs containing **8** are not stable over time, presumably due to leaching of the carrier into the aqueous phases. Efforts to solve this critical problem are underway and will be reported in due course.

Experimental

Monoboronic Acid 1: The synthesis of **1** has been described previously.[2]

***ortho*-Diboronic Acid 2**: 4-Carboxyphenylboronic acid (0.20 g, 1.2 mmol) and potassium carbonate (0.33 g, 2.4 mmol) were suspended in dimethylacetamide (15 mL) and heated to reflux. α,α'-Dibromo-*o*-xylene (0.158 g, 0.6 mmol) was added and the mixture heated at reflux for 24 hours. After cooling the solution was filtered and the solvent removed under reduced pressure. Methanol (5 mL) was added followed by water (15 mL) and the mixture cooled to precipitate the diboronic acid. The white solid was filtered and dried under vacuum. (0.240 g, 92%), mp 208-210 °C. ^{1}H NMR (300 MHz, $CD_3COCD_3 + D_2O$) δ 5.60 (s, 4H), 7.44 (dd, J=5.7, 3.3 Hz, 2H), 7.55 (dd, J=5.7, 3.3 Hz, 2H), 7.94 (d, J=8.3 Hz, 4H), 8.00 (d, J=8.3 Hz, 4H) ppm. ^{13}C NMR (75 MHz, $CD_3COCD_3 + D_2O$) δ 64.5, 121.4, 128.8, 130.5, 131.9, 134.7, 135.3, 167.3 ppm (C directly attached to B not observed due to broadening). HRMS (FAB$^+$/glycerol matrix) calcd for $C_{28}H_{29}B_2O_{10}$ (glycerol ester) 547.1946, found 547.1963.

***meta*-Diboronic Acid 3**: The procedure was the same as for **2**. ^{1}H NMR (300 MHz, $CD_3COCD_3 + D_2O$) δ 5.42 (s, 4H), 7.35 (s, 1H), 7.50 (m, 2H), 7.67 (m, 1H), 7.97 (d, J=8.4 Hz, 4H), 8.02 (d, J=8.4 Hz, 4H) ppm. ^{13}C NMR (75 MHz, CD_3OD) δ 66.1, 122.4, 126.45, 128.4, 135.6, 137.5, 138.1, 147.1, 148.6, 151.3, 167.4 ppm (C directly attached to B not observed due to broadening). MS (FAB$^+$/pinacol matrix) $C_{34}H_{41}B_2O_8$ (pinacol ester) m/z 599.

***para*-Diboronic Acid 4**: The procedure was the same as for **2**. ^{1}H NMR (300 MHz, $CD_3COCD_3 + D_2O$) δ 5.35 (s, 4H), 7.51 (s, 4H), 7.93 (m, 8H), 7.97 (s, 2H) ppm. ^{13}C NMR (75 MHz, $CD_3COCD_3 + D_2O$) δ 66.6, 122.2, 128.0, 132.3, 134.5, 136.9, 167.2 ppm (C directly attached to B not observed due to broadening). MS (FAB$^+$/pinacol matrix) $C_{34}H_{41}B_2O_8$ (pinacol ester) m/z 599.

Boronic Ester 9: A solution of 3-tolylboronic acid (0.500 g, 3.68 mmol) and 2,2-dimethylpropanediol (0.38 g, 3.68 mmol) in methanol (50 mL) was stirred for 24 hours, then evaporated to leave a white solid (0.496 g, 99 %). ^{1}H NMR (300 MHz, CD_3COCD_3) δ 0.99 (s, 6H); 2.06 (s, 3H); 3.77 (s, 4H); 7.21 (m, 2H), 7.54 (m, 2H) ppm. ^{13}C NMR (75 MHz, CD_3COCD_3) δ 22.5, 32.4, 34.6, 72.7, 125.2, 129.9, 130.2, 134.5, 142.9 ppm (C directly attached to B not observed due to broadening). HRMS (FAB$^+$/glycerol) calcd for $C_{12}H_{17}BO_2$ (glycerol ester) 204.1324, found 204.1320.

Boronic Ester 10: Compound **9** (0.30 g, 1.43 mmol) was dissolved in carbon tetrachloride (50 mL) under a nitrogen atmosphere. N-bromosuccinimide (0.25 g, 1.437 mmol) and azodiisobutyronitrile (0.05 g, catalytic) were added and the mixture was refluxed until the orange color disappeared and a white precipitate formed (2 h). The solution was cooled to 0 °C, and the succinimide removed by filtration. The solvent was evaporated and the residue recrystallized from hexanes (0.213 g, 52 %). ^{1}H NMR (300 MHz, $CD_3COCD_3 + D_2O$) δ 0.99 (s, 6H), 3.79 (s, 4H), 4.65 (s, 2H), 7.33 (t, J=7.5 Hz, 1H), 7.51 (d, J=7.8 Hz, 1H), 7.68 (d, J=7.5 Hz, 1H), 7.81 (s, 1H) ppm. ^{13}C NMR (75 MHz, $CD_3COCD_3 + D_2O$) δ 21.8, 32.4, 34.6, 72.7, 128.7, 132.51, 134.9, 135.5, 138.4 ppm (C directly attached to B not observed due to broadening). HRMS (FAB$^+$/glycerol) calcd for $C_{12}H_{16}BrBO_2$ (glycerol ester) 283.0507, found 283.0523.

Boronic Ester 11: Compound **10** (0.20g, 0.70 mmol) was dissolved in acetonitrile (75 mL), potassium iodide (0.30 g, 3.535 mmol) and trioctylamine (1.24 g, 3.53 mmol) were added and the mixture refluxed under a N_2 atmosphere for 48 hours. The acetonitrile was removed by rotary evaporation leaving a dark oil, which was purified by column chromatography (silica gel/hexane, then 10:1 hexane: methanol). ^{1}H NMR (300 MHz, $CD_3COCD_3 + D_2O$) δ 0.86 (t, J=6.6 Hz, 9H), 1.04 (s, 4H), 1.28 and 1.40 (both bs, 36H), 3.34 (m, 6H), 3.76 (s, 4H), 4.83 (s, 2H), 7.41 (t, J=7.2 Hz, 1H), 7.64 (d, J=6.6 Hz, 1H), 7.96 (d, J=7.2 Hz, 1H), 8.05 (s, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 19.3, 21.8, 22.6, 26.2, 27.3, 29.1, 31.6, 31.9, 58.6, 62.8, 72.3, 125.8, 128.8, 134.5, 136.2, 137.4 ppm (C directly attached to B not observed due to broadening). HRMS (FAB$^+$/glycerol) calcd for $C_{36}H_{67}BO_2N$ (glycerol ester) 556.5271, found 556.5277.

Boronic Acid-Trioctylammonium Conjugate 8: Compound **11** was dissolved in chloroform (5 mL) and concentrated HBr (20 mL) was added, the two phase mixture was stirred overnight and the organic layer was separated, washed with sodium bicarbonate and dried giving a mixture of protected boronate and free boronic

acid. Longer reaction times did not increase the yield of deprotection. Column chromatography (silica gel / 20:1 hexane : MeOH) produced **8** as a yellow oil (0.05 g, 13% for **10** to **8**). ^{1}H NMR (300 MHz, CD_3COCD_3 + D_2O) δ 0.86 (t, J=6.6 Hz, 9H), 1.28 and 1.40 (both bs, 36H), 3.36 (m, 6H), 4.75 (s, 2H), 7.47 (t, J=6.9 Hz, 1H), 7.62 (d, J=7.4 Hz, 1H), 7.98 (s, 1H), 8.04 (d, J=6.9 Hz, 1H) ppm. (FAB$^+$/glycerol) calcd for $C_{34}H_{63}NBO_3$ (glycerol ester) 544.4907, found 544.4940.

Extraction Studies: The procedure has been previously described in detail.[4]

Transport Studies: The transport cells consist of two identical water-jacketed cylindrical halves.[10] The data listed in Table 2 was obtained using a half-cell volume of 38 mL and a membrane surface area of 11.3 cm^2, whereas the data listed in Table 3 was obtained using a half-cell volume of 50 mL and a membrane surface area of 11.0 cm^2. Each half-cell was stirred mechanically by a steel turbine driven by an external magnet spinning at 50 rpm. The membrane was a flat sheet of Accurel^{TM} (thickness 0.10 mm) supporting a solution (~0.15 mL) of carrier in 2-nitrophenyl octyl ether. Glucose concentrations were determined using a coupled hexokinase-glucose-6-phosphate dehydrogenase assay which produces an NADPH adsorption at 340 nm.[11] The concentrations of fructose, or fructose plus glucose were obtained by including phosphoglucose isomerase in the glucose assay.[12] Sucrose assays also included invertase. Note two equivalents of NADPH are produced for each sucrose molecule consumed. Initial fluxes were calculated after extrapolating the slopes for sugar appearance in the receiving phase to t = 0. All runs were repeated at least once and observed fluxes were always within ±10%.

Acknowledgments

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