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Highly selective lipophilic diboronic acid that transports fructose as the trisdentate 2,3,6-β-D-fructofuranose ester

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Abstract—The transport of fructose and glucose through supported liquid membranes promoted by a tetrahedral shaped lipophilic monoboronic acid and diboronic acid, both based on a pentaerythritol core, has been studied. The diboronic acid gave a high fructose/glucose selectivity (7.7:1.0). The results of molecular modelling studies and ^{13}C NMR experiments with uniformly labelled ^{13}C-D-fructose suggest that the enhanced selectivity results from the formation of a trisdentate 2,3,6-β-D-fructofuranose diester within the membrane. Experimental evidence for a previously proposed macrocyclic D-fructopyranose diester formed with an *o*-phenylene linked diboronic acid has also been found.

1. Introduction

It is almost fifty years since the esterification of polyols with aryl boronic acids was first examined,[1] and applications of this reversible process in organic synthesis have been investigated for almost as long.[2] Shinbo and co-workers were the first to demonstrate that an aryl boronic acid, in combination with a lipophilic ammonium ion, could facilitate the passage of monosaccharides through a lipophilic liquid membrane.[3] Boronic acids that are able to selectively transport carbohydrates across lipophilic membranes have potential applications in drug delivery[4,5] and in environmentally benign industrial sugar production.[5b,6] With the latter application in mind, we have been investigating the inherent preference of aryl boronic acids to transport fructose over other sugars, with the ultimate aim of developing a new method for the production of high fructose syrup.

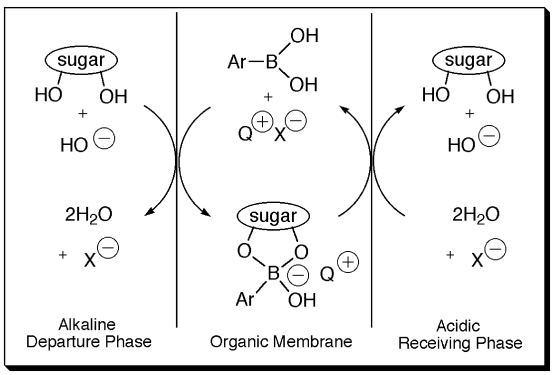

Figure 1. Accepted 'tetrahedral' transport mechanism for the passage of sugars through a lipophilic membrane promoted by an aryl boronic acid and a quaternary ammonium salt (Q^+X^-).

Figure 1 shows how the transport of a sugar out of an alkaline aqueous departure phase, through a lipophilic membrane and into a slightly acidic aqueous receiving phase can be promoted by a boronic acid. This transport process is thought to be diffusion controlled,[5] with the formation of the boronate esters at the interface being rapid and reversible. Since, in this mechanism, every sugar molecule is co-transported with a hydroxide ion, sugar transport can be driven uphill via the application of a pH gradient across the membrane.[6]

Membrane stability and ease of preparation of carriers are of prime importance if this process is to become industrially feasible. In our early experiments with bulk liquid membranes (BLM's),[6] we tried to address these issues through the use of a boronic acid derived from cholesterol (**1**, Fig. 2).[7] This highly lipophilic boronic acid, which is expected to be resistant to leaching, unfortunately possesses very poor transport properties in a BLM, with virtually no sugar transport being observed under standard conditions. We suggested that steroidal boronic acids of this type probably self assemble at the membrane interface, inhibiting transport.[6] This seems reasonable, given that **1** possesses liquid crystalline properties.[7]

Keywords: boronic acids; membrane transport; monosaccharides.
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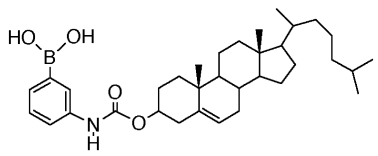

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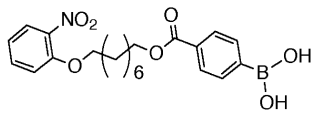

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Figure 2. Previously described monoboronic acids used in this study.

More recently, we have been studying sugar transport through supported liquid membranes (SLM's) because these membranes are more likely to be industrially useful than BLM's, and experiments involving SLM's require much lower quantities of carrier.[8] Here we report the transport properties exhibited by **1** in an SLM, together with those of a new class of carriers based on a pentaerythritol core.[9] The study of the sugar transport properties of these new carriers, as well their interaction with ^{13}C-labelled fructose has allowed us to better understand the sugar transport process promoted by boronic acid carriers, and make predictions on how saccharide selectivity can be improved.

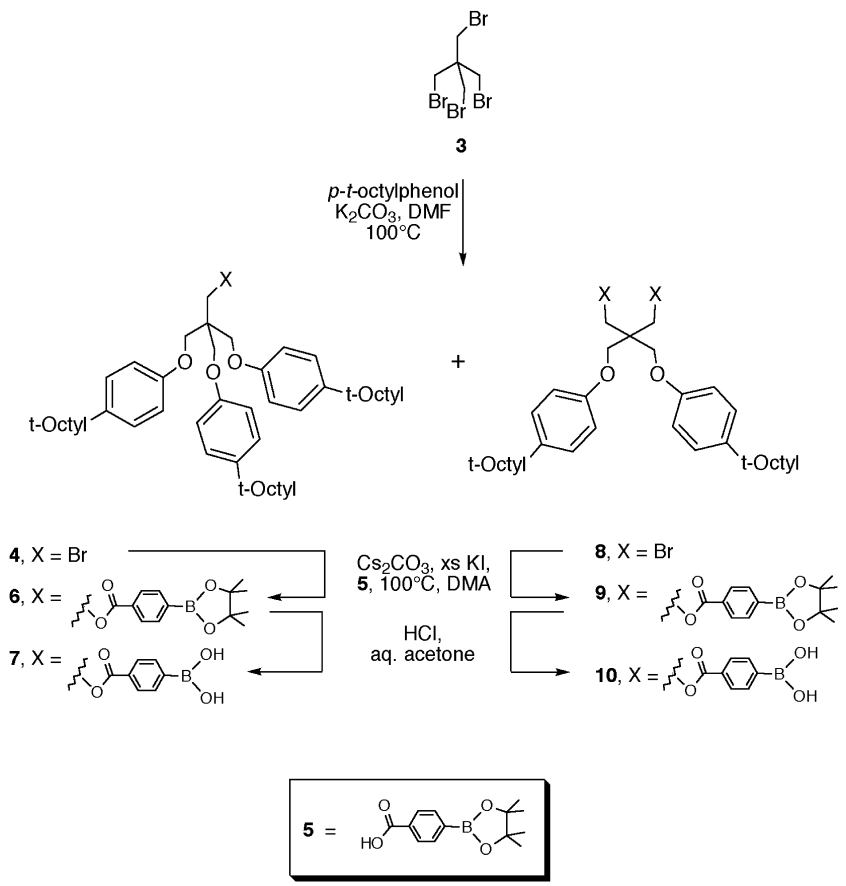
2. Results and discussion

2.1. Carrier design and synthesis

In order to circumvent any possible unfavourable stacking interactions at the aqueous-membrane interface, an overall tetrahedral shape, derived from a pentaerythritol core, was chosen for the new boronic acid carriers. High lipophilicity was incorporated through the attachment of *p-tert*-octylphenyl ethers, while the boronic acid functionality was attached through an ester link to *p*-carboxyphenyl boronic acid. Following this strategy, a mono- and diboronic acid (**7** and **10**) were synthesised, as outlined in Scheme 1. The use of a pinacol protecting group for the boronic acid appears to minimise side reactions during the alkylation of the carboxylic acid (**5**) and makes the boron-containing products (**6** and **9**) easier to isolate and purify. The deprotection of these boronates with aq. HCl/acetone, although slow, was found to be very a clean process.

2.2. Sugar transport

In the production of high fructose syrup, one of the critical steps is the enrichment of the fructose concentration in mixtures that contain similar quantities of glucose and fructose. Hence, in this study, we looked for the enhancement of fructose flux over that of glucose. Competitive SLM transport fluxes were determined using apparatus described previously.[8a] The liquid membrane consisted of the carriers



Scheme 1. Synthesis of tetrahedral shaped lipophilic boronic acids.

Table 1. Sugar fluxes through a supported liquid membrane[a]

Entry	Boronic acid	Flux (10^{-8} mol m^{-2} s^{-1})		ratio of fluxes
		Fructose	Glucose	
1	**2**	28.4	6.7	4.2
2	**1**	7.6	4.1	1.9
3	**7**	17.9	3.5	5.1
4	**10**	26.1	3.4	7.7
5[b]	**11**	21	3.4	6.2

[a] [Boronic acid] in membrane=50 mM, fluxes shown are averages of 2–3 runs, T=298 K, flux uncertainty±10%.
[b] Data obtained in a previous study under similar conditions.[8a]

dissolved in 2-nitrophenyl octyl ether supported by a thin, flat sheet of microporous polypropylene (Accurel®). The membrane separated two aqueous phases, with the source phase containing 0.3 M fructose and glucose buffered at pH 11.3 with Na_2CO_3 and the receiving phase buffered at pH 6.0 with sodium phosphate. The appearance of fructose and glucose in the receiving phase was monitored by enzymatic methods.

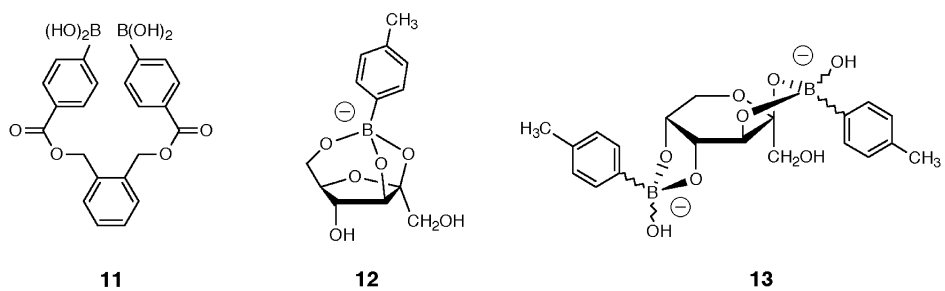
The mono-saccharide transport promoted by a reference monoboronic acid (**2**)[10] is shown in Table 1 (entry 1), compared with that induced by **1** (entry 2), the pentaerythrityl boronic acids (entries 3 and 4) and a previously reported *o*-phenylene linked diboronic acid (**11**, entry 5). In analogy to the results obtained in BLM transport experiments,[6] **1** showed poor SLM transport characteristics, inducing lower sugar fluxes than the reference monoboronic (**2**). The very low fructose/glucose selectivity induced by **1** is a curious result and suggests that the properties of this compound retard fructose transport more than glucose transport.

In contrast to the transport properties of **1**, the highly lipophilic, tetrahedral shaped carrier (**7**) proved to be an excellent sugar transporter. No leaching of this carrier was observed during the course of the transport experiments, and the fructose flux produced by **7** was found to be significantly higher than that of **1**, despite having a substantially higher molecular weight (849 g mol^{-1} cf. 550 g mol^{-1}). The fructose selectivity of **7** was also superior to that of **1**, being more like to that of **2**. The choice of a tetrahedral shape for lipophilic carriers thus appears to be validated. However, the higher molecular weight of **7**, which would be expected to lower the diffusion constant of its sugar boronates within the membrane, does in fact appear to have a detrimental impact on sugars fluxes. The flux promoted by **7** is approximately 30% lower than that of the lighter monoboronic acid (**2**), which has a molecular weight of less than half of that of **7** (415 g mol^{-1} cf. 849 g mol^{-1}).

More remarkable are the transport properties of the diboronic acid (**10**). This compound, while also showing no evidence of leaching during the course of transport experiments, exhibits unexpectedly high selectivity for fructose over glucose (Table 1, entry 4). This selectivity stems from an enhanced fructose flux, compared with that of **7**, a flux that is very similar to that promoted by the much lighter monoboronic acid (**2**; 415 g mol^{-1} cf. 809 g mol^{-1} for **10**). In the following two sections, evidence is presented that supports the notion that the enhanced fructose flux promoted by **10** stems from its ability to simultaneously carry 2 equiv. of fructose through the membrane in the form of a stabilised fructofuranose diester.

2.3. Molecular modelling

It has previously been reported that the *o*-phenylene linked diboronic acid (**11**, Fig. 3) displays enhanced fructose selectivity relative to **2** (Table 1, entry 5),[8a] and based on the results of molecular modelling experiments, it was suggested that this enhanced selectivity is due to the ability of **11** to form a 1:1 macrocyclic diester with fructopyranose, shown in Figure 4(A). The formation of such an ester should minimize the hydrophilic surface area of fructose, thus enhancing extraction into, and promoting smooth passage through, the hydrophobic liquid membrane. Similar modelling experiments[11] with the fructopyranose ester of **10**, however, showed that the spatial arrangement of the boronic acid functionalities of **10** are inappropriate to allow it to form a similarly stable cyclic 1:1 diester. Other possibilities were sought, and after recognizing that in aqueous alkaline solution, the major boronate ester formed between *p*-tolylboronic acid and fructose is the trisdentate 2,3,6-β-D-fructofuranose ester (**12**, Fig. 3),[12] a 2:1 diester of **10** was identified (Fig. 4(B)). These molecular modelling experiments further suggested that this diester could form two intramolecular hydrogen bonds with interatomic distances of less than 1.2 Å.[13] This structure is an attractive possibility as the additional hydrogen bonding interactions should further stabilize the fructofuranose esters, and association between the fructose portions of the esters would reduce the amount of hydrophilic surface exposed to the hydrophobic medium, thus improving fructose extraction and diffusion through the membrane. The ability of each molecule of **10** to readily carry two fructose molecules across the membrane at once, in the form of a frutofuranose diester, similar to that



11 **12** **13**

Figure 3. Previously studied *o*-phenylene linked diboronic acid (**11**)[8a] and previously characterised *p*-tolylboronates of fructose (**12** and **13**).[12]

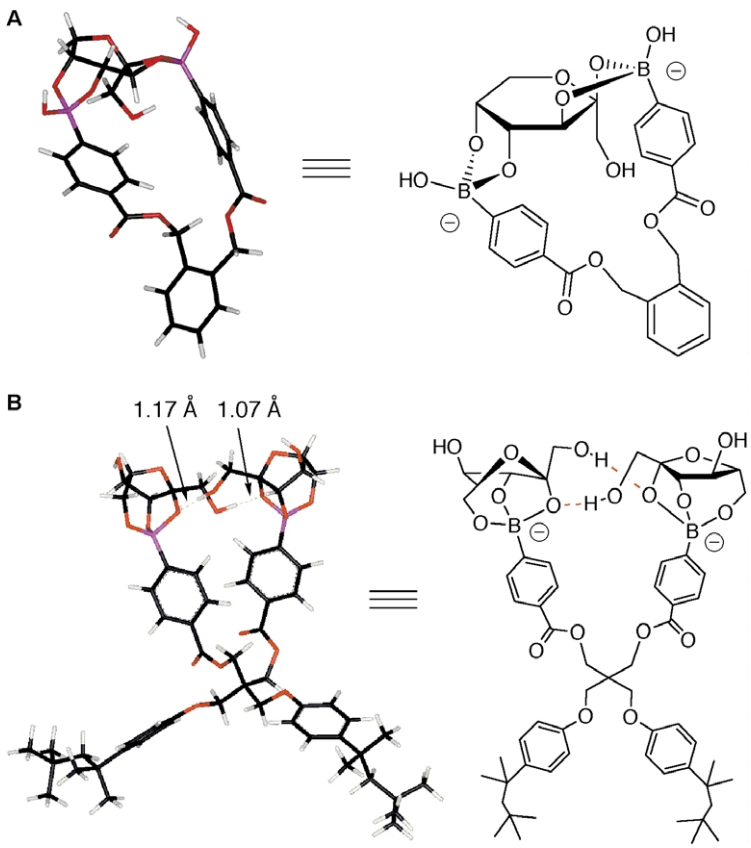


Figure 4. Fructose esters of diboronic acids identified through molecular modelling experiments. (A) Macrocyclic D-fructopyranose diester of **11**.[8a] (B) Trisdentate 2,3,6-β-D-fructofuranose diester of **10**.

shown in Figure 4, may also help to account for the improved fructose flux promoted by this diboronic acid.

2.4. Solution structures of fructose boronates

To test the above predictions, experimental evidence for the structures shown in Figure 4 was sought. Tetrahedral boronate esters are notoriously difficult to characterise, and attempts to identify the fructose esters of **10** and **11**, formed by extraction of fructose from aqueous alkaline solution into organic solutions containing the boronic acid and aliquat 336, with a range of mass spectrometry techniques, including electron impact ionisation (EI), atmospheric pressure chemical ionisation (APCI), electrospray (ES) and, in the case of **11**, fast atom bombardment (FAB), proved fruitless. Attempts to form crystals of these esters suitable for X-ray crystal structure determination also proved unsuccessful.‡ However, the aqueous solution structures of several aryl boronates formed with ^{13}C enriched fructose have previously been identified by ^{13}C NMR spectroscopy through the use of chemical shift correlations and $^{1}J_{CC}$ coupling constants,[12] and modification of that method allowed the identification of the likely structures of the major boronate esters extracted into the membrane during fructose transport promoted by **2** and the diboronic acids (**10** and **11**). This method is especially suitable here as the use of ^{13}C enriched fructose virtually eliminates interference by signals from the carriers and ammonium ions, and a considerable amount of reference data on the various boronate esters that can be formed with fructose is now available.[12,14]

The initial experiment involved the extraction of fructose, uniformally labelled with ^{13}C with an enrichment of 99%, out of D_2O adjusted to ~pD11.0 with Na_2CO_3, into $CDCl_3$ containing Aliquat 336® and **2**. These conditions closely model the initial stages of the transport process facilitated by a typical monoboronic acid, in which fructose is sequestered from the source phase and drawn into the membrane. Thus, the boronate esters formed in the membrane, and in $CDCl_3$ in the extraction experiment, are likely to be very similar. A portion of the resulting ^{13}C NMR spectrum of the $CDCl_3$ solution thus obtained is shown in Figure 5(A), with the major peaks and $^{1}J_{CC}$ coupling constants listed at entry 3 in Tables 2 and 3, respectively. For comparison, data previously obtained with the trisdentate 2,3,6-β-D-fructofuranose *p*-tolylorthoboronate ester (**12**) and the four possible diastereomeric forms of β-D-fructopyranose *p*-tolylhydroxyboronate esters (**13**) in D_2O[12] are shown at entries 1 and 2 in these tables.

On first inspection of Figure 5(A) it is immediately clear that only one major fructose ester is formed with **2** in $CDCl_3$, and by comparison of the NMR chemical shifts and $^{1}J_{CC}$ coupling constants of this ester (Tables 2 and 3) with the data previously obtained for **12** and **13**, it is apparent that this ester is the trisdentate 2,3,6-β-D-fructofuranose ester. In particular, signals at $>\delta$ 110 ppm for C-2 and signals for

‡ Even if such experiments had been successful, the relevance to transport experiments of structures identified in the gas phase or solid state is somewhat questionable.

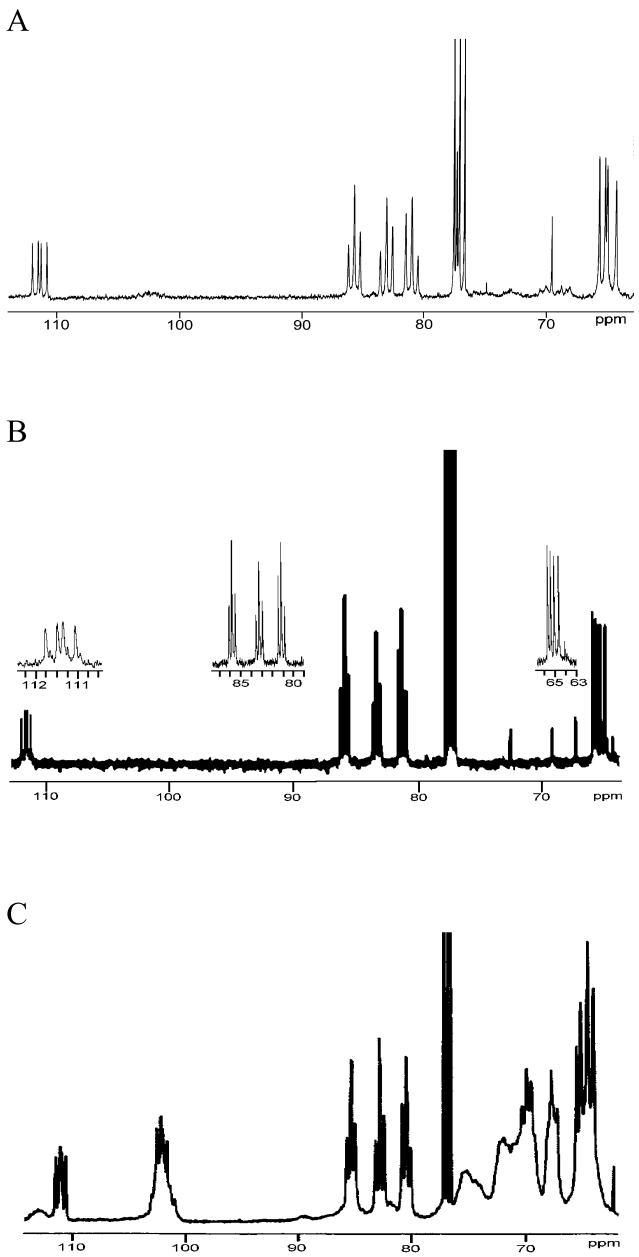


Figure 5. ^{13}C NMR spectra of UL-^{13}C-D-fructose extracted from D_2O (pD~11) into $CDCl_3$ containing **2**, **10** and **11** and aliquat 336. (A) Extraction with **2** (75 MHz). (B) Extraction with **10** (125 MHz). (C) Extraction with **11** (100 MHz).

C-3 to C-5 at >80 ppm are characteristic of such structures. In addition, the $^1J_{CC}$ coupling constants for the fructose ester of **2** are more in line with those of **12** than **13**. The weak, broad signal at δ 101–103 ppm in Figure 5(A) is in a similar position to that observed for C-2 in **13**, suggesting that, although the trisdentate 2,3,6-β-D-fructofuranose ester of **2** is dominant, some pyranose esters are still present in the $CDCl_3$ solution.

Table 3. $^1J_{CC}$ Coupling constants (in Hz) for fructose portion of tetrahedral boronate esters

Entry	Boronate	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
1	**12**[a]	54.1	37.4	40.3	36.9	36.3
2	**13**[a,b]	51.3	38.3	44.3	34.1	38.9
3	**2**·boronate	53.8	36.6	38.4	36.2	36.4
4	**10**·diboronate	53.5	37.1	38.4	36.8	37.0

[a] Data obtained in a previous study,[12] pD=11–12 in D_2O.
[b] These coupling constants are approximate and are within ±0.5 Hz of those found for the four diastereoisomers of **13** (where measured).

Analogous experiments were then performed with the diboronic acids (**10** and **11**). In the case of the pentaerythrityl diboronic acid (**10**), a very similar spectrum to that obtained with **2** was produced, the major fructose derivative observed again being the trisdentate 2,3,6-β-D-fructofuranose ester (see correlations in Tables 2 and 3). In fact, notwithstanding that the signal-to-noise in Figure 5(B) is inferior to that in Figure 5(A), there is no evidence for fructopyranose esters in this spectrum.

Remarkably, the spectrum obtained with the *o*-phenylene linked diboronic acid (**11**) is distinctly different from the previous two (Fig. 5(C)). Although there is evidence for the presence of the trisdentate 2,3,6-β-D-fructofuranose ester, especially in the region just above δ 110 ppm, very strong signals in the region δ 100–103 ppm and in the range δ 63–76 ppm suggest the presence of significant quantities of β-D-fructopyranose esters, thus supporting the previous molecular modelling results.[8a] In addition, significant amounts of polymeric material, both furanose and pyranose in nature, appear to be present, as shown by the broad signals at ~δ 112 ppm, in the region δ 70–77 ppm, as well as those overlapping with many of the sharper peaks throughout the spectrum. Similar results have recently been reported for glucose adducts of ferrocene containing diboronic acids.[15]

3. Conclusions

A new class of tetrahedral shaped lipophilic boronic acids based on a pentaerythritol core (**7** and **10**) have been prepared and tested for their ability to transport fructose and glucose through a lipophilic SLM. These compounds have shown far superior transport properties to those of a previously described cholesterol derivative (**1**), despite

Table 2. ^{13}C Chemical shifts (ppm) for fructose portion of tetrahedral boronate esters

Entry	Boronate	C-1	C-2	C-3	C-4	C-5	C-6
1	**12**[a]	66.1	113.4	84.7	82.1	87.6	67.9
2	**13**[a,b]	69.4±0.2	103.8±0.8	76.4±0.5	73.3±1.0	71.7±0.3	66.7±0.4
3	**2**·boronate	64.8	111.4	83.1	81.0	85.7	65.5
4	**10**·diboronate	64.9	111.4	83.2	81.1	85.8	65.4

[a] Data obtained in a previous study,[12] pD=11–12 in D_2O.
[b] Uncertainties indicate the range over which signals occur for the four diastereoisomers of **13**.

having significantly higher molecular weight. The overall tetrahedral shape of **7** and **10** appears to prevent unfavourable interfacial stacking interactions that are likely to occur with more planar boronic acids such as **1**.

The diboronic acid (**10**) shows unusually high fructose/glucose transport selectivity, which appears to result from the formation within the membrane of a trisdentate 2,3,6-β-D-fructofuranose diester (Fig. 4(B)), which can be stabilized by intramolecular hydrogen bonds. This contrasts markedly with results obtained with a previously described *o*-phenylene linked diboronic acid (**11**), which appears to gain advantage from the formation of a macrocyclic D-fructopyranose diester.

In general, the minimisation of the hydrophilic surface area of the transported sugar should improve flux by enhancing both extraction into the membrane and diffusion through the lipophilic medium. While the formation of macrocyclic Dfructopyranose diesters with diboronic acids appears to be beneficial in this regard, greater advantage can apparently be obtained through the formation of adjacent trisdentate 2,3,6-β-D-fructofuranose diesters. Results described here suggest that these esters gain stability by intramolecular association within the membrane, which also leads to reduced exposure of the hydrophilic surface of the fructose portion of the esters. The implication is that the projection of multiple boronic acids in a parallel fashion from a rigid, hydrophobic scaffold should lead to improved fructose flux and selectivity. Studies that test this design hypothesis have been initiated and the preliminary results are very encouraging.[8b]

4. Experimental

4.1. General

Melting points of solid compounds were measured on a Reichert Hot stage melting point apparatus. All infrared spectra were recorded on a Perkin–Elmer 1600 Series Fourier Transform spectrophotometer, either as Nujol (paraffin) mulls mounted on sodium chloride plates, or as potassium bromide disks. All NMR spectra were recorded of solutions of compounds dissolved in $CDCl_3$ (unless stated otherwise), referenced to tetramethylsilane (δ 0.00 ppm) at spectrometer frequencies of 300 MHz (^{1}H NMR) or 75 MHz (^{13}C NMR), with the exception of the ^{13}C NMR spectrum of the fructose ester of **10**, which was recorded at 125 MHz, and of **11**, which was recorded at 100 MHz. Mass spectra were recorded on a Bruker–Bioapex 47e Fourier Transform mass spectrometer. Unless otherwise stated, compounds were dissolved in methanol and ionised using an electrospray ionisation source and recorded in positive ion mode. Microanalyses were performed by Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. All solvents were AR grade and used as supplied, except dimethyl formamide, which was dried over 4 Å molecular sieves. Column chromatography was performed according to the method reported by Still et al.[16] using the eluents stated, expressed as volume ratios. Compound **5** is commercially available from Boron Molecular Ltd, Noble Pk, Victoria, Australia (http://www.boronmolecular.com).

4.2. Synthesis

4.2.1. 1,1′-[[2-(Bromomethyl)-2-[[4-(1,1,3,3-tetramethylbutyl)phenoxy]methyl]-1,3-propanediyl]bis(oxy)]bis-[4-(1,1,3,3-tetramethylbutyl)-benzene (4). Pentaerythrityl tetrabromide (2.32 g, 6 mmol), *p*-*t*-octyl phenol (3.70 g, 18 mmol) potassium carbonate (4.14 g, 30 mmol) and DMF (30 mL) were combined and stirred at 100°C for 24 h under argon. The reaction mixture was allowed to cool, then filtered. The solvent was removed in vacuo and the residue taken up in dichloromethane and washed with 5% HCl (20 mL), water (2×20 mL), brine (30 mL) and dried over $MgSO_4$. The solvent was then evaporated to give a yellow oil (2.40 g). This oil was submitted to flash chromatography (hexane 100%) to give pure monobromide (**4**) (1.32 g, 28%) and dibromide (**8**) (1.02 g, 22%). Characterisation data for **8** are shown below. **4**, mp 98.5°C. IR (KBr) ν: 1610 w, 1511 m, 1463 s, 1377 m, 1241 m, 1184 m, cm^{-1}. ^{1}H NMR: δ 7.24 (d, J=7.8 Hz, 6H), 6.82 (d, J=7.8 Hz, 6H), 4.23 (s, 6H), 3.90 (s, 2H), 1.69 (s, 6H), 1.33 (s, 18H), 0.72 (s, 27H) ppm. ^{13}C NMR: δ 156.3, 142.8, 127.1, 114.0, 67.3, 57.1, 44.5, 38.2, 34.8, 32.5, 32.1, 31.9 ppm. HRMS calcd for $C_{47}H_{71}BrO_3Na$ (MNa^+): 785.4484. Found: 785.4477. Anal. calcd for $C_{47}H_{71}BrO_3$: C, 73.89; H, 9.37; Br, 10.21. Found: C, 74.35; H, 9.37; Br, 10.46.

4.2.2. Benzoic acid, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-, 3-[4-(1,1,3,3-tetramethylbutyl)phenoxy]-2,2-bis[[4-(1,1,3,3-tetramethylbutyl)phenoxy]methyl]-propyl ester (6). The monobromide (**4**), (0.20 g, 0.26 mmol, **5** (0.06 g, 0.03 mmol), cesium carbonate (0.23 g, 0.78 mmol), potassium iodide (0.55 g, 3.30 mmol) and dimethyl acetamide (30 mL) were combined and heated at 100°C for 24 h under argon. Upon cooling the solvent was removed in vacuo and the residue adsorbed onto a small plug of silica. The plug was washed with hexane then the product was desorbed by washing with dichloromethane. Removal of the dichloromethane under vacuum gave an oil which was submitted to flash chromatography (dichloromethane/hexane 8:2) to give white crystalline product (0.21 g, 75%), mp 114–116°C. IR (Nujol) ν: 1716 m, 1609 w, 1510 m, 1242 m, 1122 m, 1099 m, 1025 m, cm^{-1}. ^{1}H NMR: δ 7.95 (d, J=7.8 Hz, 2H), 7.82 (d, J=7.8 Hz, 2H), 7.24 (d, J=7.8 Hz, 6H), 6.82 (d, J=7.8 Hz, 6H), 4.75 (s, 2H), 4.31 (s, 6H), 1.69 (s, 6H), 1.34 (s, 12H), 1.32 (s, 18H) 0.71 (s, 27H) ppm. ^{13}C NMR: δ 166.3, 156.4, 142.7, 134.7, 132.3, 128.6, 127.1, 113.9, 84.3, 67.1, 64.5, 57.1, 44.5, 38.2, 32.5, 32.0, 31.9, 25.1 ppm. (Carbon attached to boron not observed due to broadening) MS (APCI) m/z 931.4 ($M+1^+$, 100%).

4.2.3. Benzoic acid, 4-borono-, 1-[3-[4-(1,1,3,3-tetramethylbutyl)phenoxy]-2,2-bis[[4-(1,1,3,3-tetramethylbutyl)phenoxy]methyl]propyl] ester (7). The mono pinacol ester (**6**) (0.10 g), hydrochloric acid (3 mL, 1 M) and acetone (30 mL) were combined and stirred for 24 h. The solvent was removed in vacuo and the residue taken up in dichloromethane (30 mL). The organics were washed with water (3×50 mL) and dried with $MgSO_4$. Filtration and removal of the solvent in vacuo gave glass like crystals (0.06 g, 66%), mp 126–128°C. IR (Nujol) ν: 3385 b, 3268 s, 1721 m, 1655 s, 1540 m, 1243 m, 1028 m, cm^{-1}. ^{1}H NMR

(d_6-acetone/D_2O, 9:1): δ 8.02 (d, J=7.8 Hz, 2H), 7.96 (d, J=7.8 Hz, 2H), 7.28 (d, J=7.8 Hz, 6H), 6.92 (d, J=7.8 Hz, 6H), 4.75 (s, 2H), 4.41 (s, 6H), 1.71 (s, 6H), 1.31 (s, 18H) 0.69 (s, 27H) ppm. ^{13}C NMR: δ 166.3, 156.4, 142.7, 133.7, 132.2, 128.9, 127.1, 113.9, 67.0, 64.5, 57.1, 44.5, 38.2, 32.5, 32.0, 31.9 ppm. (Carbon attached to boron not observed due to broadening).

4.2.4. 1,1′-[[2,2-Bis(bromomethyl)-1,3-propanediyl]bis-(oxy)]bis[4-(1,1,3,3-tetramethylbutyl)-benzene (8). Pentaerythrityl tetrabromide (2.00 g, 5.15 mmol) and *p*-*t*-octyl phenol (2.13 g, 10.30 mmol) were treated with potassium carbonate (5.38 g, 16.50 mmol) in DMF (30 mL) in the same manner as that described for the preparation of **4**. Flash chromatography was used to obtain pure dibromide (**8**) (1.58 g, 48%) and monobromide (**4**) (0.42 g 12%). **8**, mp 109–110°C. IR (KBr) ν: 1511 m, 1460 m, 1376 m cm^{-1}. ^{1}H NMR: δ 7.26 (d, J=7.8 Hz, 4H), 6.82 (d, J=7.8 Hz, 4H), 4.13 (s, 4H), 3.79 (s, 4H), 1.69 (s, 4H), 1.33 (s, 12H), 0.72 (s, 18H) ppm. ^{13}C NMR: δ 156.2, 143.3, 127.3, 114.2, 67.6, 57.2, 44.3, 38.2, 35.2, 32.7, 32.2, 32.0 ppm. MS (DCM/MeOH 1:2) m/z: 559.3 (M−Br, 3%), 637.4 (M$^+$(^{79}Br$_2$)+1, 3%). Anal. calcd for $C_{33}H_{50}Br_2O_2$: C, 62.07; H, 7.89; Br, 25.03. Found: C, 61.98; H, 8.01; Br, 24.99.

4.2.5. Benzoic acid, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-, 2,2-bis[[4-(1,1,3,3-tetramethylbutyl)phenoxy]methyl]-1,3-propanediyl ester (9). The dibromide (**8**) (1.00 g, 1.5 mmol) was treated with **5** (0.86 g, 3.47 mmol), cesium carbonate (2.26 g, 6.9 mmol) and potassium iodide (2.62 g, 15.8 mmol) in dimethyl acetamide (30 mL) in the same way as that described for the preparation of **6**. Flash chromatography (eluent 5% ethyl acetate/hexane⟶100% ethyl acetate) was used to obtain **9** as a white crystalline solid (0.40 g, 28%), mp 206–208°C. IR (Nujol) ν: 1718 m, 1243 m, 1114 m, 1018 m, 710 m, ppm. ^{1}H NMR: δ 7.96 (d, J=7.8 Hz, 4H), 7.82 (d, J=7.8 Hz, 4H), 7.23 (d, J=7.7 Hz, 4H), 6.82 (d, J=7.8 Hz, 4H), 4.73 (s, 4H), 4.28 (s, 4H), 1.68 (s, 4H), 1.35 (s, 24H), 1.33 (s, 12H), 0.72 (s, 18H) ppm. ^{13}C NMR: δ 166.1, 156.1, 142.7, 134.6, 131.9, 128.5, 127.0, 113.7, 84.1, 67.0, 64.1, 57.0, 43.9, 38.0, 32.4, 31.9, 31.7, 25.0 ppm. (Carbon attached to boron not observed due to broadening) MS (APCI) m/z 973.3 (M+1$^+$, 33%), 995.2 (M+Na$^+$, 9%).

4.2.6. Benzoic acid, 4-borono-, 1,1′-[2,2-bis[[4-(1,1,3,3-tetramethylbutyl)phenoxy]methyl]-1,3-propanediyl] ester (10). The dipinacol ester (**9**) (0.20 g), 1 M hydrochloric acid (10 mL) and an acetone/water mix (4:1, 150 mL) were combined and stirred at room temperature for 48 h. The solvent was removed in vacuo and a white crystalline precipitate was collected. This was dissolved in chloroform and washed with water (20 mL×3) and the solvent removed to leave white crystals (0.12 g, 73%), mp 110–111°C. IR (Nujol) ν: 3400 b, 3330 s, 1779 m, 1698 s, 1543 m, 1264 m, 1181 m, 1014 m, cm^{-1}. ^{1}H NMR (d_6-acetone/D_2O, 9:1): δ 7.97 (d, J=7.8 Hz, 4H), 7.90 (d, J=7.8 Hz, 4H), 7.26 (d, J=7.7 Hz, 4H), 6.90 (d, J=7.8 Hz, 4H), 4.75 (s, 4H), 4.41 (s, 4H), 1.67 (s, 4H), 1.27 (s, 12H), 0.65 (s, 18H) ppm. ^{13}C NMR: δ 166.3, 156.2, 142.9, 134.8, 132.0, 128.6, 127.1, 113.9, 67.1, 64.2, 57.1, 44.0, 38.2, 32.5, 32.0, 31.9 ppm. (Carbon attached to boron not observed due to broadening).

4.3. Transport experiments

The transport cell consisted of two identical water jacketed cylindrical halves (T=298 K), with a half cell volume of 34 mL and a cell membrane surface area of 12.6 cm^2. Each half cell was stirred by a magnetic stirrer at a rate of 250 rpm. The SLM consisted of a polypropylene support (Accurel® type 1E flat sheet (thickness 0.1 mm pore size 0.1 μm)) obtained from Membrana GmbH, Wuppertal, Germany, coated in a solution of carriers dissolved in 2-nitrophenyl octyl ether. Liquid membranes were prepared in duplicate by dissolving 25 μmol carrier and 25 μmol Aliquat 336® in a suitable solvent, (usually chloroform) dividing the solution into two equal parts, then adding 0.25 g of nitrophenyl octyl ether to each. The solvent was then removed in vacuo to leave oils. Two polymer supports were then coated with these oils and kept under vacuum (~1 Torr) for 24 h. The departure phase consisted of a solution of 0.3 mol dm^{-3} fructose, 0.3 mol dm^{-3} glucose dissolved in a buffer solution of 0.5 mol dm^{-3} Na_2CO_3 at pH 11.3. The sugar solutions were freshly prepared before each transport experiment. The receiving phase consisted of a 0.1 mol dm^{-3} solution of sodium phosphate at pH 6.0.

Aliquots were removed from the receiving phase at hourly intervals and analysed in triplicate for glucose with a coupled hexokinase/glucose-6-phosphate dehydrogenase assay, and fructose with the addition of phosphoglucose isomerase, by modification of a previously described method.[17] The volume of aliquots removed from the receiving phase was adjusted so that the final absorbance change was in the range 0.05–0.50 absorbance units. These aliquots were added to 1 mL plastic disposable cuvettes, and enough NaH_2PO_4 (0.1 mol dm^{-3}, pH 7.5) was added to give a combined volume of 890 μL. ATP (5 μL of a 0.2 mol dm^{-3} soln) and NADP (5 μL of a 0.1 mol dm^{-3} soln) both dissolved in NaH_2PO_4 (0.07 mol dm^{-3}, pH 7.5) were added to each cuvette, and 2.5 units of hexokinase/glucose-6-phosphate dehydrogenase dissolved in 25 μL of NaH_2PO_4 (0.07 mol dm^{-3}, pH 7.5 containing 0.004 mol dm^{-3} $MgCl_2$) was added. The absorbance change at 340 nm could be used to determine [glucose]. Once glucose determination was complete, 14 units of phosphoglucose isomerase dissolved in 25 μL of NaH_2PO_4 (0.07 mol dm^{-3}, pH 7.5 containing 0.004 mol dm^{-3} $MgCl_2$) was added, and the change in absorbance at 340 nm could be used to determine [fructose]. Fluxes were determined from slopes of plots of [Total NADPH Abs (340 nm)] vs time over periods of at least 5 h, according to Eq. (1), and the results averaged over two runs. All transport experiments were performed at least in duplicate and all assays were done in triplicate. Flux uncertainty ±10%.

$$\text{Flux(mol m}^{-2}\text{s}^{-1}) = \frac{\text{slope(min}^{-1}) \times 0.034\text{(L)}}{6220\text{(L mol}^{-1}\text{ cm}^{-1}) \times 1\text{(cm)} \times 0.00126\text{(m}^2) \times 60\text{(s min}^{-1})} \quad (1)$$

4.4. NMR Studies of ^{13}C-fructose boronate esters

Each boronic acid (10 mg) was briefly shaken in an eppendorf tube with Aliquat 336® (1 equiv. for each boronic acid), uniformly labelled ^{13}C-fructose (99% enrichment, 6 equiv.) and 0.5 mol dm^{-3} Na_2CO_3 in D_2O (1.5 mL pD=11.0). $CDCl_3$ (0.7 mL) was then added and the mixture shaken for 1 h. The organic layer was removed, dried ($MgSO_4$) and placed in an NMR tube.

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References

1. (a) Kuivila, H. G.; Keough, A. H.; Soboczenski, E. J. *J. Org. Chem.* **1954**, *19*, 780–783. (b) Wolfrom, M. L.; Solms, J. *J. Org. Chem.* **1956**, *21*, 815–816. (c) Sugihara, J. M.; Bowman, C. M. *J. Am. Chem. Soc.* **1958**, *80*, 2443–2446. (d) Lorand, J. P.; Edwards, J. O. *J. Org. Chem.* **1959**, *24*, 769–774.
2. (a) Ferrier, R. J. *Adv. Carbohydr. Chem. Biochem.* **1978**, *35*, 31–80. (b) Duggan, P. J.; Tyndall, E. M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1325–1339, and references sited therein.
3. Shinbo, T.; Nishimura, K.; Yamaguchi, T.; Sugiura, M. *J. Chem. Soc., Chem. Commun.* **1986**, 349–351.
4. (a) Riggs, J. A.; Hossler, K. A.; Smith, B. D.; Karpa, M. J.; Griffin, G.; Duggan, P. J. *Tetrahedron Lett.* **1996**, *37*, 6303–6306. (b) Westmark, P. R.; Smith, B. D. *J. Pharm. Sci.* **1996**, *85*, 266–269.
5. (a) Smith, B. D. *Supramol. Chem.* **1996**, *7*, 55–60. (b) Smith, B. D.; Gardiner, S. *J. Adv. Supramol. Chem.* **1999**, *5*, 157–202, and references cited therein.
6. Karpa, M. J.; Duggan, P. J.; Griffin, G. J.; Freudigmann, S. J. *Tetrahedron* **1997**, *53*, 3669–3678.
7. James, T. D.; Harada, T.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1993**, 857–858.
8. (a) Gardiner, S. J.; Smith, B. D.; Duggan, P. J.; Karpa, M. J.; Griffin, G. J. *Tetrahedron* **1999**, *55*, 2857–2864. (b) Altamore, T. M.; Barrett, E. S.; Duggan, P. J.; Sherburn, M. S.; Szydzik, M. L. *Org. Lett.* **2002**, *4*, 3489–3491. (c) Duggan, P. J.; Szydzik, M. L. *Aust. J. Chem.* **2003**, *56*, 17–21.
9. A preliminary report of part of the work described here has already appeared; Draffin, S. P.; Duggan, P. J.; Duggan, S. A. M. *Org. Lett.* **2001**, *3*, 917–920.
10. (a) Paugam, M.-F.; Riggs, J. A.; Smith, B. D. *J. Chem. Soc., Chem. Commun.* **1996**, 2539–2540. (b) Paugam, M.-F.; Bien, J. T.; Smith, B. D.; Chrisstoffels, L. A. J.; de Jong, F.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1996**, *118*, 9820–9825.
11. Molecular Mechanics simulations were performed with MSI's Discover Minimisation Module within the Insight II suite of programs. Simulations were performed in the gas phase using the ESFF force field and the 'Structural' analysis option. Further details of the computational procedures used can be found at http://www.accelrys.com/insight/discover.html.
12. Norrild, J. C.; Eggert, H. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2583–2588.
13. Shinkai and co-workers have suggested that a similar type of H-bonding might occur in difructose boronate diesters appended to poly(L-lysine); Kobayashi, H.; Nakashima, K.; Ohshima, E.; Hisaeda, Y.; Hamachi, I.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **2000**, 997–1002.
14. (a) Shull, B. K.; Spielvogel, D. E.; Head, G.; Gopalaswamy, R.; Sankar, S.; Devito, K. *J. Pharm. Sci.* **2000**, *89*, 215–222. (b) He, M.; Johnson, R. J.; Escobedo, J. O.; Beck, P. A.; Kim, K. K.; St Luce, N. N.; Davis, C. J.; Lewis, P. T.; Fronczek, F. R.; Melancon, B. J.; Mrse, A. A.; Treleaven, W. D.; Strongin, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 5000–5009.
15. Norrild, J. C.; Søtofte, I. *J. Chem. Soc., Perkin Trans. 2* **2002**, 303–311.
16. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
17. Kinksy, S. C. *Methods Enzymol.* **1974**, *32*, 501–504.