Enhanced fructose, glucose and lactose transport promoted by a lipophilic 2-(aminomethyl)-phenylboronic acid

Peter J. Duggan [a,b,*], Todd A. Houston [c], Milton J. Kiefel [c], Stephan M. Levonis [c], Bradley D. Smith [d], Melanie L. Szydzik [b]

[a] CSIRO Molecular and Health Technologies, Bag 10, Clayton South, Victoria 3169, Australia
[b] School of Chemistry, Monash University, Clayton, Victoria 3800, Australia
[c] Institute for Glycomics, Gold Coast Campus, Griffith University, Queensland 4222, Australia
[d] Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

ABSTRACT

A lipophilic 2-(aminomethyl)-phenylboronic acid (**1a**) was prepared and used to transport saccharides across a thin supported liquid membrane. The result was the highest recorded fructose flux promoted by a monoboronic acid under near-neutral conditions, and one of the highest fructose/glucose transport selectivities (12.9). The application of a pH gradient across the membrane further enhanced carrier-mediated fructose flux but fructose/glucose selectivity was decreased. The first effective transport of a disaccharide, lactose, was achieved using supported liquid membranes containing a high concentration of **1a** (250 mM). A strong carrier concentration effect on lactose transport and a battery of mass spectrometry evidence point to the involvement of 2:1 boronate/lactose esters in this transport process.

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1. Introduction

There is great potential for membrane processes to be used in environmentally benign industrial sugar production. We have been particularly interested in the use of thin supported liquid membranes containing boronic acid carriers for use in this application, and results obtained in this area have been extensively reviewed.[1] The transport process under investigation involves the transient formation of tetrahedral sugar/boronate esters (Fig. 1). The advantages of employing the tetrahedral form of the organoboron species are several-fold. The negatively charged tetrahedral boronate esters are generally more stable than the corresponding neutral trigonal boronates[2] and the formation of such tetrahedral esters also allows the use of lipophilic quaternary ammonium salts to improve initial extraction into the membrane. The rate of ester formation at the departure phase–membrane interface is also likely to be faster if the boronic acid is in the negatively charged tetrahedral form.[3c] The mechanism by which the sugars are thought to pass through the membrane as tetrahedral boronates is shown in Figure 1.

Although significant progress has been made towards an industrially feasible process, further improvements are required. One issue that has received much of our attention is flux enhancement.
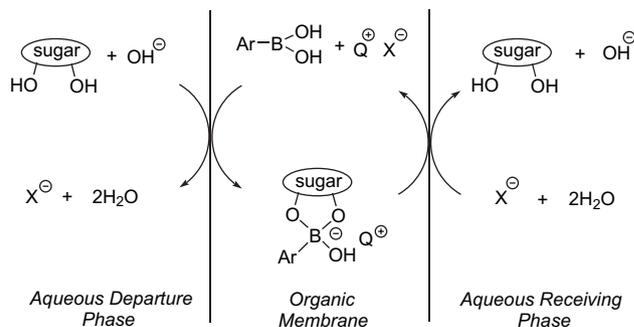

Figure 1. Accepted 'tetrahedral' mechanism by which arylboronic acids, in conjunction with lipophilic ammonium salts (Q^+X^-), assist the passage of sugars through a lipophilic organic membrane.

Successful approaches have included the attachment of multiple boronic acid functionalities to a semi-rigid scaffold[4] and the incorporation of a quaternary ammonium group within the carrier.[5]

There has been much interest for over a decade in 2-(aminomethyl)-arylboronic acids **1** (Fig. 2) due to their application in sensor research.[3] These compounds were first investigated in detail by Wulff for chromatographic applications,[6] but it was later found that when the amine is attached to a fluorophore, they provide a means of sensing the presence of sugars. This is because the fluorescence that is initially quenched by intramolecular photo-electron transfer from the amine is enhanced when the boronic

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* Corresponding author. Tel.: +61 3 9545 2560; fax: +61 3 9545 2446.
  E-mail address: peter.duggan@csiro.au (P.J. Duggan).
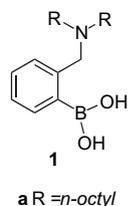
Figure 2. General structure of 2-(aminomethyl)-phenylboronic acids.

acid binds a sugar.[3] The amine also improves the stability of boronate esters[7] and lowers the pK_a of the boronic acid ($\sim$6.5 cf. 8.8 for phenylboronic acid), so that tetrahedral forms are present at neutral pH. Until recently the nature of the interaction between the nitrogen and boron in these boronate esters was thought to involve exclusively a dative B–N bond (**3**, Scheme 1). This was called into question by Wang and Franzen[8] and thorough investigations recently published by Anslyn and co-workers[9] have shown that in the presence of protic solvents at neutral pH, the boronate ester exists in equilibrium between **3** and a 'solvent-inserted' form (**4** when the solvent is water), as illustrated in Scheme 1. In aqueous solvent the zwitterionic **4** tends to dominate.

In the past, our best membrane transport results have been obtained using a basic departure phase to ensure that the tetrahedral transport mechanism was in operation. We were encouraged to investigate the sugar transport properties of 2-(aminomethyl)-arylboronic acids because their lower pK_a may allow good transport to occur at near-neutral pH and might also negate the need for a quaternary ammonium salt in the membrane.

Our quest for a membrane-based technology for the production of high fructose syrup has focused most of our energies on improving fructose flux and selectivity over glucose. There are, however, other potential applications of boronic acid-containing membranes. Lactose, for example, is used in the food, feed, pharmaceutical and cosmetics industries. A major source of lactose is cheese whey, but existing lactose manufacturing processes are time consuming and complex, resulting in low lactose yield and/or poor product purity and consistency. There is a need to develop new technologies for the isolation and purification of lactose from feed streams, particularly from dairy protein whey. Membrane technologies may have an impact here and we were interested to learn if boronic acid-mediated transport through thin organic membranes could be feasible with lactose. Sucrose is the only other disaccharide to be tested extensively in boronic acid assisted membrane transport,[1c] and results with this sugar have been

universally disappointing, presumably due to the more hydrophilic nature of the disaccharide cf. monosaccharides, and its low association constant with boronic acids.[2] The binding constant of lactose to phenylboronic acid is only slightly stronger than that of sucrose,[2] but the greater transport efficiency expected for 2-(aminomethyl)-arylboronic acids gave us hope that lactose transport with these compounds might be viable. Herein we report the preparation and superior fructose, glucose and lactose transport properties of the lipophilic 2-(aminomethyl)-phenylboronic acid **1a**.

2. Results

2.1. Synthesis of 1a

The 2-(aminomethyl)-phenylboronic acid **1a** used in this study was prepared from o-formylphenylboronic acid **6** and dioctylamine as shown in Scheme 2. This involved the initial formation of the imine **7**.[10] This reaction could be significantly accelerated by heating at reflux for 1 h in the presence of molecular sieves and catalytic acetic acid. Careful reduction of **7** at 0 °C with sodium borohydride provided **1a** in 64% isolated yield.

2.2. Transport studies

The competitive transport of fructose and glucose promoted by **1a** was first examined. The membrane consisted of 2-nitrophenyl octyl ether (NPOE) supported on a thin sheet of porous polypropylene Accurel, as previously described in detail.[11] The results are shown in Table 1 and compared to that previously obtained with a reference monoboronic acid **8** (Fig. 3),[11] which has a similar molecular weight to **1a**.

Transport experiments were also performed with lactose present in the departure phase (0.3 M). Initially, conditions that gave the highest fluxes with the monosaccharides (condition (c) in Table 1) were used. With a carrier concentration of 50 mM in the membrane, negligible lactose transport was detected even after two days. This is similar to what has previously been observed with sucrose.[5] Increasing the carrier concentration in the membrane to 250 mM, however, produced strong lactose transport with **1a** (89.4×10^{-8} mol m^{-2} s^{-1}) and was found to be >20 times the flux measured with the reference monoboronic acid **8**[11] (4.1×10^{-8} mol m^{-2} s^{-1}).

The nature of the boronate esters formed between **1a** and lactose was studied using electrospray ionisation mass spectrometry.
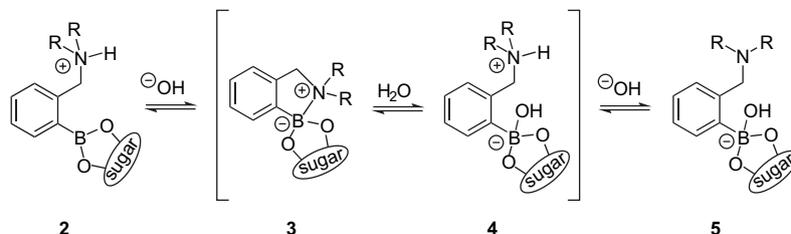
Scheme 1. The various protonation states of 2-(aminomethyl)-phenylboronate sugar esters.
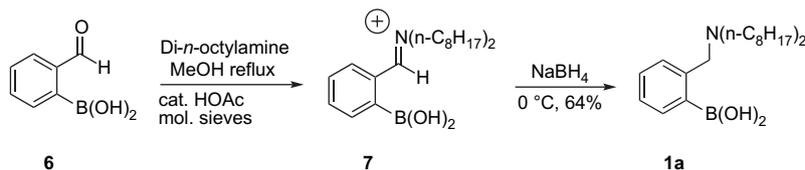
Scheme 2. Synthesis of lipophilic 2-(aminomethyl)-phenylboronic acid sugar transporter (**1a**).

Table 1
Fructose and glucose fluxes through supported liquid membrane

Boronic acid	Conditions[a]	Flux (10^{-8} mol m^{-2} s^{-1})[b]		Ratio of fluxes
		Fructose	Glucose	
1a	a	3.2	0.54	7.0
1a	b	14.2	1.10	12.9
1a	c	84.1	18.1	4.7
8[c]	c	42.3	10.6	4.0

[a] [Boronic acid] in membrane=50–52 mM, [sugars] in departure phase=0.30 M each, T=298 K. Other conditions: (a) pH departure and receiving phases=7.4 (0.1 M sodium phosphate buffer); (b) same as (a) with addition of 1 equiv Aliquat 336 to membrane; (c) same as (b) but with pH departure phase=11.3 (0.1 M sodium carbonate) and pH receiving phase=6.0 (0.1 M sodium phosphate).
[b] Fluxes are the averages of 2–3 independent runs and uncertainties are estimated to be ±10%.
[c] Data from Ref. 11.
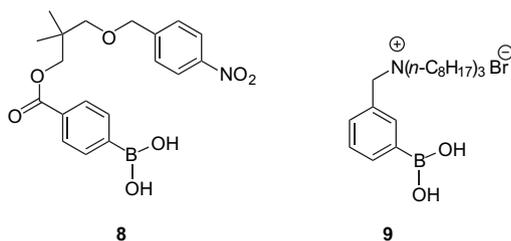
Figure 3. Structure of reference boronic acid (**8**) used in this study and the boronic acid–quaternary ammonium conjugate (**9**) used in a previous study.[5]

The positive ion high resolution spectrum of a methanol solution of **1a** and lactose, made slightly basic by the addition of a trace of aqueous ammonia, showed two strong peaks (m/z=682.4329, 1021.7522), which together with their isotopic distribution are characteristic of protonated 1:1 and 2:1 boronate esters formed between **1a** and lactose (see Supplementary data). A similar experiment with phenylboronic acid in the place of **1a** showed only a weak signal for the lactose/PBA monoester and no evidence for diester formation. When the mass spectrometry experiment was repeated with **1a** and fructose, a strong signal for a 1:1 boronate ester was observed and only a very weak signal for a 2:1 boronate ester. Interestingly, evidence for strong association between the lactose boronate esters (e.g., **10**) and chloride could be observed in the low resolution *negative* ion spectrum (Fig. 4). Association of the fructose monoester with chloride was also observed in its negative ion spectrum. No strong evidence for the formation of 'solvent-inserted' boronate esters similar to **4** was observed in any mass spectra recorded with **1a**.

3. Discussion

The monosaccharide fluxes promoted by **1a** alone when the departure and receiving phases were set to pH 7.4 are unremarkable and similar to those previously observed for simple mono-boronic acids under similar conditions.[12] The addition of Aliquat 336 (mainly trioctylmethylammonium chloride), however, increased the fructose flux by more than four-fold, giving the highest reported fructose flux promoted by a boronic acid under near-neutral conditions. This is a significant result because when simple boronic acid carriers are used, the addition of Aliquat at near-neutral conditions has very little effect on saccharide fluxes.[12] The addition of Aliquat to the membrane had a weaker effect on glucose flux, leading to one of the best fructose/glucose transport selectivities (12.9) observed thus far.

The ability of fructose and lactose boronate esters of **1a** to readily associate with chloride is evident from the negative ion mass spectrometry experiments. This suggests that the source of the enhanced fluxes observed when the departure phase has a pH of 7.4 and Aliquat is added to the membrane results from an enhanced extraction of the sugar into the membrane due to an association of sugar boronate esters similar to **3** with the chloride of Aliquat.

The application of a pH gradient (departure phase pH 11.3; receiving phase 6.0) caused the fructose/glucose selectivity to drop to the level normally seen for simple monoboronic acids under these conditions, but the overall saccharide flux was still significantly
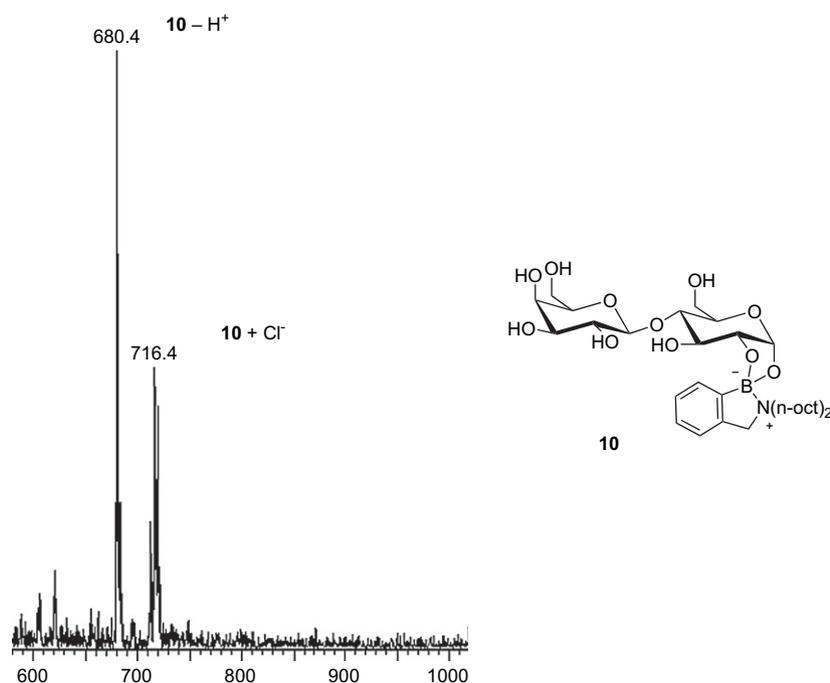
Figure. 4. Low resolution negative ion electrospray mass spectrum of a methanol solution of **1a**+lactose with suggested chemical structure of the detected ions.

enhanced. The fructose flux observed in this system is second only to that recorded with the boronic acid-quaternary ammonium conjugate **9** (5.2×10^{-6} mol m^{-2} s^{-1}).[5] In the present case, where the departure phase is quite basic, the extracted and transported species is most likely similar to **5**, associated with the lipophilic ammonium ion of Aliquat. Here the superior fluxes most likely result from the greater stability of the tetrahedral boronate esters as well as a stronger association with the cation of Aliquat.

The effect of the *ortho*-aminomethyl group on boronic acid promoted transport was even more dramatic with lactose. With disaccharides, fluxes are typically very low, but increasing the membrane concentration of the carrier and Aliquat to 250 mM provided strong lactose fluxes. The positive ion mass spectrometry results show facile formation of 2:1 boronate esters between **1a** and lactose, which explains the strong enhancement of lactose flux with rising membrane carrier concentration. As carrier concentration is increased in the membrane, the equilibrium is shifted in favour of the highly lipophilic boronate diesters, and hence extraction into membrane is promoted. It is most likely that the individual lactose boronate esters are formed at 1,2-position of the glucosyl moiety and either 3,4- or 4,6-position of the galactosyl moiety;[3b] a preference for trigonal boronate ester formation at 4,6-position of galactosides having been previously demonstrated in organic solvents.[13]

4. Conclusions

A lipophilic 2-(aminomethyl)-phenylboronic acid (**1a**) was prepared and used to transport saccharides across a thin supported liquid membrane consisting of 2-nitrophenyl octyl ether. Under near-neutral conditions, and in the absence of any other additives, observed transport fluxes were similar to those obtained with other monoboronic acids. However, when Aliquat 336 was added to the membrane, the highest reported fructose flux promoted by a monoboronic acid under near-neutral conditions and one of the highest fructose/glucose transport selectivities (12.9), were observed. The results of mass spectrometry experiments suggest that the flux enhancement produced by Aliquat reflects an association between the zwitterionic saccharide ester and chloride. The application of a pH gradient across the membrane resulted in one of the highest fructose fluxes ever recorded. The superior transport properties of **1a** were further demonstrated by transport experiments conducted with the disaccharide, lactose. A strong concentration effect on lactose transport and further mass spectrometry evidence point to the involvement of 2:1 boronate/lactose esters in this transport process. This suggests that bis(aminoboronate) compounds of proper spacing may be highly effective lactose transporters.

5. Experimental

5.1. General

Commercial starting materials (Aldrich) and reagents were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer. Methanol was distilled and stored over 3 Å molecular sieves prior to use. Chemical shifts are expressed as parts per million (ppm, δ) relative to MeOD (3.3 ppm in ^{1}H and 49.0 ppm in ^{13}C). Low resolution positive and negative ion electrospray mass spectra were acquired with a VG Platform mass spectrometer using a cone voltage of 50 V. The source was maintained at 80 °C and a solvent flow rate of 0.04 ml/min was used. High resolution positive ion mass spectra was acquired on a Bruker Daltonics Apex III 4.7e Fourier Transform Mass Spectrometer fitted with an Apollo API source.

5.2. Synthesis of 2-((*N,N*-di-*n*-octyl)aminomethyl)-phenylboronic acid (1a)

2-Formylphenylboronic acid (250 mg, 1.67 mmol) was combined with dioctylamine (500 μL, 400 mg, 1.66 mmol) in anhydrous methanol (5 mL) containing one drop acetic acid catalyst. This was stirred at reflux along with 3 Å molecular sieves under argon for 1 h. The reaction mixture was then brought to 0 °C before the addition of NaBH$_4$ (120 mg, 3.2 mmol) portionwise over half an hour. The reaction mixture was further stirred while being allowed to warm to room temperature over half an hour. After concentration of the reaction mixture under vacuum, the residue was dissolved in dichloromethane and filtered to produce a dark oil (670 mg) that was shown by ^{1}H NMR to contain both the desired product and some unreacted dioctylamine. This oil was dissolved in hexanes (50 mL) and shaken vigorously with 0.04 M HCl (50 mL). At the phase boundary a white solid formed. The hexane layer was collected and to this was added potassium carbonate (200 mg) and the mixture was agitated before filtering. The filtrate was finally concentrated under vacuum to produce **1a** (395 mg, 64%) as a colourless oil.

^{1}H NMR (300 MHz, MeOD) δ 0.89 (t, 6H, J=6.9 Hz, 2×CH$_3$), 1.30 (m, 20H, 10×CH$_2$), 1.65 (m, 4H, 2×CH_2CH$_2$N), 2.92 (t, 4H, J=8.4 Hz, 2×CH$_2$CH_2N), 4.19 (s, 2H, CH$_2$–Ar), 7.13 (d, 1H, J=4.2 Hz, ArH), 7.24 (m, 2H, 2×ArH), 7.67 (d, 1H, J=6.9 Hz, ArH) ppm. ^{13}C NMR (75 MHz, MeOD) δ 14.5, 23.7, 24.4, 27.8, 30.2, 30.4, 32.9, 51.7, 61.0, 127.3, 127.4, 128.8, 131.6, 136.1 ppm. m/z (+ve ESI): (33% aq MeCN/NH$_4$OH) 376.2 (M+H)$^+$, 715.7 (dimeric anhydride+H)$^+$, 1073.3 (trimeric anhydride+H)$^+$. HRMS (+ve FTMS): calcd for C$_{23}$H$_{43}$BNO$_2$, 375.3418; found, 375.3413.

5.3. Transport experiments

Apparatus and methods used in the transport of fructose and glucose have previously been described in detail.[11] Lactose concentrations in the receiving phase were measured using a similar enzymatic method to that used for measuring glucose concentration, with the addition of β-galactosidase to catalyse the hydrolysis of lactose to glucose and galactose.

Supplementary data

Exact positive ion mass spectra of lactose boronates formed with **1a** are provided. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.05.052.

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