Transport of Glycosides through Liquid Organic Membranes Mediated by Reversible Boronate Formation Is a Diffusion-Controlled Process†

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Abstract: The ability of phenylboronic acid, [3-(1-adamantylcarboxamido)phenyl]boronic acid, and diphenylborinic acid to extract and transport *p*-nitrophenyl β-D-glucopyranoside (glucoside), *p*-nitrophenyl β-D-galactopyranoside (galactoside), and *p*-nitrophenyl β-D-mannopyranoside (mannoside) through a liquid organic membrane, in the presence of trioctylmethylammonium or tetrabutylammonium chloride, was determined. Under the conditions examined, glycoside transport was facilitated by the reversible formation of covalent tetrahedral, anionic glycoside–boronate complexes, which partitioned into the organic membrane as lipophilic ion pairs. The results of various experiments indicated the rate-limiting step in the transport process was diffusion of the solutes through the narrow unstirred boundary layers adjacent the organic/aqueous interfaces. A plot of glycoside transport rate versus glycoside extraction constant, K_{ex}, formed an approximate bell-shaped relationship. Maximal transport occurred when the carrier admixture had an extraction constant of log $K_{ex(max)} \sim 2.2$. Under low extraction conditions ($K_{ex} < K_{ex(max)}$), movement of the glycoside from the receiving phase into the organic membrane was the rate-determining step, and under high extraction conditions ($K_{ex} > K_{ex(max)}$), exit from the membrane into the receiving phase was rate-determining. Because transport was dependent on K_{ex}, an analysis of the structural and environmental factors that controlled transport could be reduced to an analysis of the factors that changed K_{ex} relative to $K_{ex(max)}$. The factors examined included the following; pH, boron acid acidity, diol structure, polarity of the organic layer, boron acid lipophilicity, glycoside lipophilicity, quaternary ammonium lipophilicity, and the presence of competing lipophilic anions. The importance of $K_{ex(max)}$ as the parameter determining transport stereoselectivity is discussed.

Introduction

Synthetic ionophores have been studied extensively as carriers of metal cations through liquid organic membranes.[1] For the majority of these carriers, the observed transport rates and cation selectivities can be successfully rationalized by a transport model that is controlled by diffusion processes within the three-phase system.[1-3] Over the past few years there has been increased emphasis on the facilitated transport of anionic and neutral compounds.[1a,4] However, very few of these studies have attempted to explore the kinetic factors that control the transport cycle.[5] Recently, we and others have reported on the ability of organoboron acids to facilitate the transport of saccharide derivatives through liquid organic membranes.[6] Studies with glycoside derivatives have established that, depending on the experimental conditions, boron acids can mediate glycoside transport via two distinct mechanisms, a neutral trigonal boronate pathway and an ion-paired tetrahedral boronate pathway.[6b] The ion-pair mechanism is the focus of this report. Complexation of the boron acid with the diol function of the glycoside produces a tetrahedral glycoside–boronate anion, which in the presence of a quaternary ammonium cation, Q^+, partitions into the organic layer as a lipophilic ion-pair. Although the overall transport cycle results in translocation of a neutral glycoside, the process is formally an anion transport mechanism. As shown in Scheme 1, the stoichiometry of the ion-pair pathway depends on the ionization state of the boron acid. Mechanism A occurs when the pH of the transport system is below the pK_a of the boron acid (*i.e.*, the glycoside is transported symport with respect to OH^- and antiport with respect to the counterion, A^-). When the pH is above the boron acid pK_a, the pathway reduces to mechanism B.

† Molecular recognition with boron acids, part 5. See ref 6a for parts 1 and 2, Westmark, P. R.; Valencia, L. S.; Smith, B. D. *J. Chromatogr.* **1994**, *664*, 123–128 for part 3, and ref 6b for part 4.

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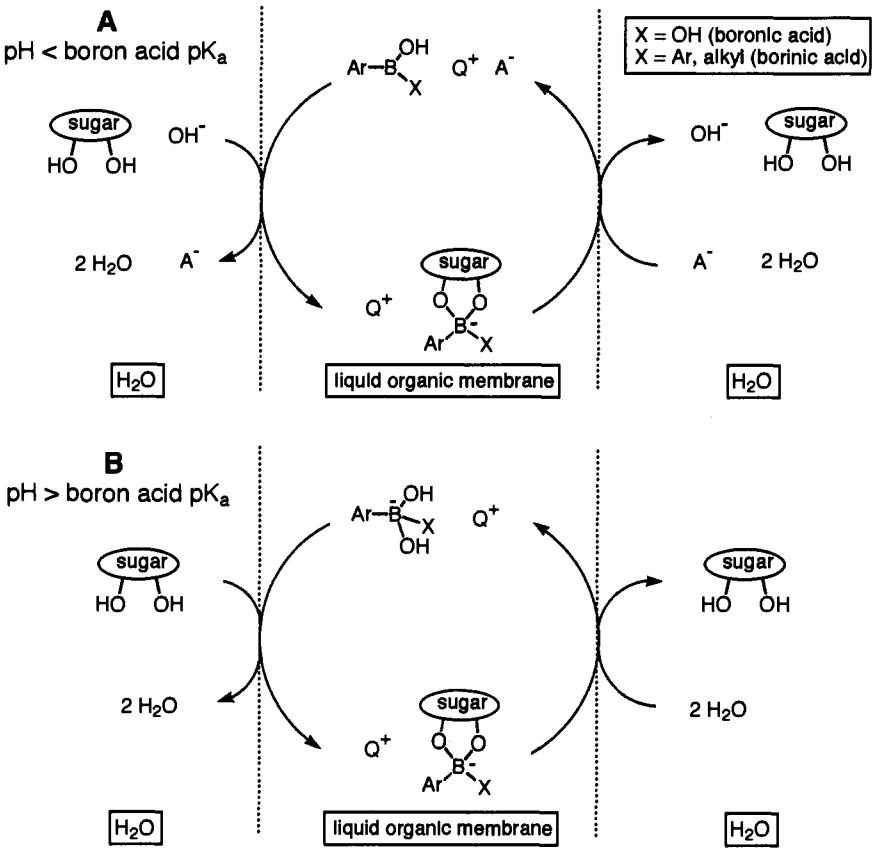
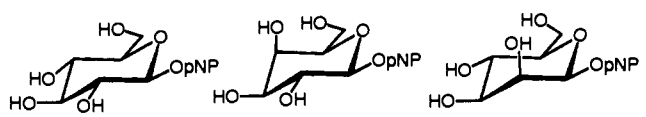
Scheme 1

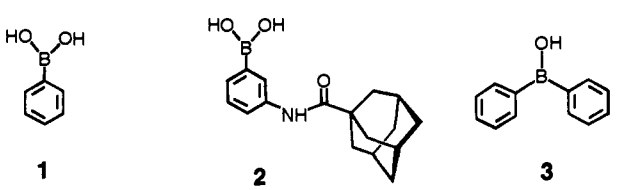


In this report we describe our detailed studies of the ion-pair transport pathway. We summarize the factors that control transport rate, and in particular, we report evidence that transport is a diffusion-controlled process. Our observations allow us to describe a detailed kinetic transport model that predicts how the various transport parameters can be optimized to achieve different transport results such as maximum rate, or high glycoside stereoselectivity. On a more general level, our studies clearly demonstrate that the rules for cation transport,[3] as well as the concepts behind "ion-pair extraction",[7] can be directly applied to the facilitated transport of anionic (and neutral) hydrophilic compounds.

Results

Transport rates for *p*-nitrophenyl β-D-galactopyranoside (galactoside), *p*-nitrophenyl β-D-glucopyranoside (glucoside), and *p*-nitrophenyl β-D-mannopyranoside (mannoside) through liquid organic membranes were determined in the presence of three types of organoboron acids, phenylboronic acid (**1**), [3-(1-adamantylcarboxamido)phenyl]boronic acid (**2**), and diphenylborinic acid (**3**), in combination with trioctylmethylammonium





(TOMA), or tetrabutylammonium (TBA) chloride. Downhill transport was determined using the same U-tube apparatus described previously.[6b] A dichloroethane layer at the bottom of a U-shaped tube separated an aqueous phase in the departure arm of the tube from an aqueous phase in the receiving arm. Only the organic layer was stirred, and the carrier admixture was pre-equilibrated between the three layers. The downhill transport experiments were started by addition of the glycoside to the departure phase, after which the rate of appearance in the receiving phase was monitored by the absorption at 302 nm. The percent extraction was also determined via UV absorption, using the same volumes and concentrations as the transport experiments.

Table 1 summarizes the transport rates and percent extraction determined for the different boron acid–quaternary ammonium admixtures at various aqueous phase pH levels. The data showed a number of general trends. For each glycoside examined, the transport rates mediated by either **1**–TOMA or **2**–TOMA admixtures displayed an approximate bell-shaped relationship with increasing pH. Extractions, however, increased proportionally with pH up to pH 11.4, after which they generally tapered off. With the more acidic and more lipophilic **3**–TOMA carrier system,[8] extraction was observed to be very efficient even at neutral pH, but the transport rate was very low. Changing from **3**–TOMA to the less lipophilic **3**–TBA admixture resulted in much lower extraction but increased transport (compare entries 2, 8, and 14).

To gain insight into which step in the transport process was rate-determining, a number of the transport experiments were repeated. In one series, the glycoside levels in both of the aqueous phases were monitored simultaneously.[9] Simple mass balance provided the amount in the organic layer. Figure 1 shows the

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(8) The pK_a of diphenylborinic acid is 6.2. Rao, G.; Philipp. M. J. *J. Org. Chem.* **1991**, *56*, 1505–1512. There was no sign of borinic acid decomposition under the conditions of the transport experiments.

(9) For an excellent example of the insight gained by monitoring all phases in a transport experiment see: Menger, F. M.; Lee, J.-J. *J. Org. Chem.* **1993**, *58*, 1909–1916.

Table 1. Transport Rates, Percent Extraction, and log K_{ex} for GLycosides Mediated by Different Carrier Admixtures

			transport rate[a] (% extracted,[b] log K_{ex})[c]						
entry	glycoside	pH[d]	no carrier	1	1–TOMA	2–TOMA	3	3–TOMA	3–TBA
1	glucoside	6.4	–	–	14 (1.0, 0.9)	24 (1.4, 1.2)	–	–	–
2		7.4	2.5 (0.05)	4.0 (0.1)	25 (2.0, 1.4)	42 (2.6, 1.5)	3.0 (0.05)	19 (34, 3.0)	35 (0.3)
3		8.4	–	–	37 (3.8, 1.6)	35 (3.9, 1.6)	–	–	–
4		11.4	–	–	45 (26, 2.7)	23 (34, 3.0)	–	–	–
5		12.4	–	3.0 (0.1)	27 (21, 2.5)	13 (43, 3.3)	–	–	–
6		12.4/7.4[e]	–	–	86	–		–	–
7	galactoside	6.4	–	–	47 (3.7, 1.6)	–	–	–	–
8		7.4	3.5 (0.05)	45 (2.5)	66 (6.6, 1.9)	58 (8.6, 2.0)	6.0 (0.3)	3.0 (48, 3.4)	32 (3.4)
9		8.4	–	–	48 (11, 2.2)	–	–	–	–
10		11.4	–	–	38 (32, 2.9)	–	–	–	–
11		12.4	–	4.0 (0.1)	10 (27, 2.8)	–	–	–	–
12		12.4/7.4[e]	–	–	95	–	–	–	–
13	mannoside	6.4	–	–	18 (1.3, 1.2)	20 (2.4, 1.4)	–	–	–
14		7.4	3.5 (0.05)	6.0 (0.2)	36 (3.4, 1.6)	40 (5.2, 1.8)	2.5 (0.05)	8.0 (32, 2.9)	26 (0.4)
15		8.4	–	–	30 (6.8, 1.9)	36 (23, 2.6)	–	–	–
16		11.4	–	–	20 (28, 2.8)	2.0 (33, 3.0)	–	–	–
17		12.4	–	3.5 (0.2)	4.0 (24, 2.7)	1.0 (40, 3.2)	–	–	–
18		12.4/7.4[e]	–	–	63	–	–	–	–

[a] Rate (10^{-8} M min^{-1} ± 15%) that glucoside appeared in receiving phase. Starting conditions were 1.36 mM glycoside in departure, and 1 mM carrier in organic. [b] $[\text{glucoside}]_{\text{extracted into organic}}/[\text{glucoside}]_{\text{initially in aqueous}}$. Reproducibility ± 10%. [c] A single number in parentheses represents the value for % extraction. [d] pH in both aqueous layers buffered with sodium phosphate, 10 mM. [e] Departure phase pH/receiving phase pH.

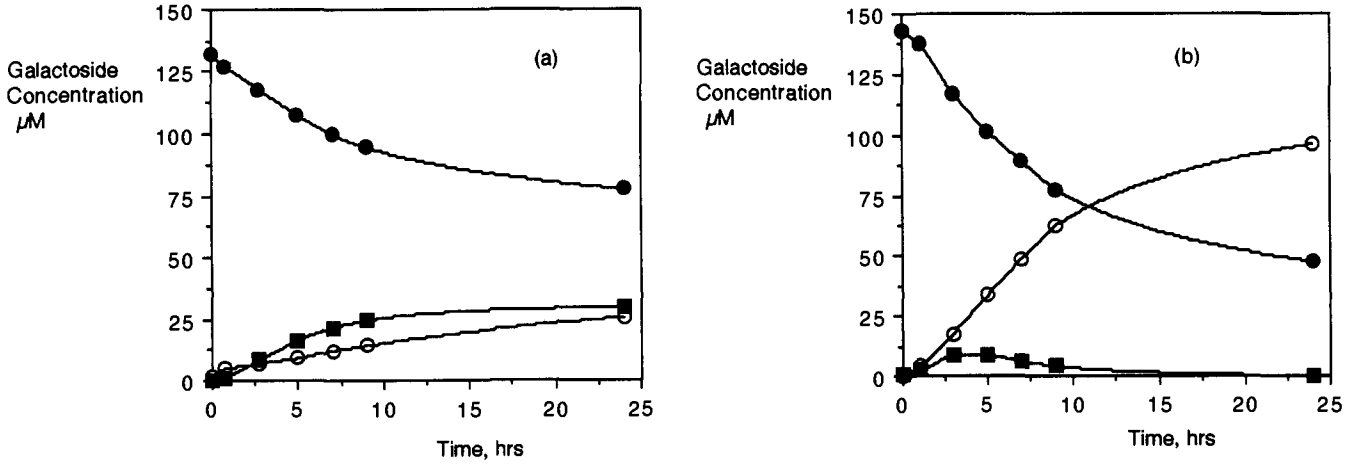


Figure 1. Galactoside concentrations in (●) departure, (○) receiving, (■) organic, for transport mediated by 1–TOMA. (a) Both departure and receiving phases at pH 12.4. (b) Departure at pH 12.4, and receiving at pH 7.4. Other starting conditions: departure, 0.13 mM galactoside, 10 mM sodium phosphate; organic, 1 mM 1–TOMA; receiving, 10 mM sodium phosphate.

results obtained for galactoside transported by 1–TOMA under two different conditions: (a) both aqueous phases at pH 12.4 and (b) departure at pH 12.4 and receiving at pH 7.4. Similar results were also obtained with mannoside and glucoside, but the plots are not shown. Figure 2 shows galactoside transported by 3–TOMA with both aqueous phases at pH 7.4. (Again, similar results were obtained with the other glycosides, but they are not shown.) In another series of transport experiments the effect of stirring rate was examined. Figure 3 shows a linear relationship between organic phase stirring rate and glucoside transport mediated by 1–TOMA with both aqueous phases at pH 12.4. This series was repeated with the aqueous phases at pH 7.4, and a very similar linear relationship was observed (data not shown).

The importance of salts added to the aqueous phases and the polarity of the organic membrane were other factors examined. As described in Table 2, addition of sodium perchlorate to the aqueous phase greatly diminished the glucoside extraction ability of 1–TOMA and 3–TOMA (entry 20 vs entry 19). This was reflected in the transport experiments, where addition of sodium perchlorate to both aqueous layers resulted in very low transport rates. An identical addition of sodium chloride had a much weaker effect on both extraction and transport (entry 21). In the case of the highly extracting 3–TOMA carrier system, where movement of the complexed glycoside from the organic layer into the receiving aqueous phase was rate-limiting, it was found that glucoside

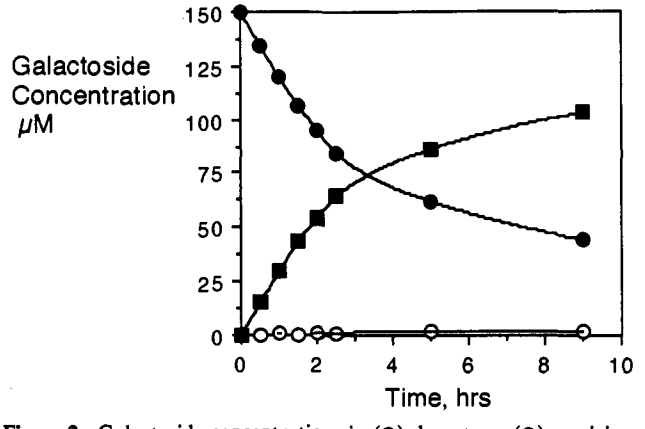


Figure 2. Galactoside concentrations in (●) departure, (○) receiving, (■) organic, for transport mediated by 3–TOMA. Starting conditions: departure, 0.15 mM galactoside, 10 mM sodium phosphate, pH 7.4; organic, 1 mM 3–TOMA; receiving, 10 mM sodium phosphate, pH 7.4.

transport could be greatly improved by adding salts to the receiving phase alone. Addition of sodium chloride produced about a 2-fold increase in transport (entry 23 vs entry 19), whereas addition of sodium perchlorate produced a 10-fold increase (*i.e.*, almost 100-fold increase in glucoside transport compared to a control with

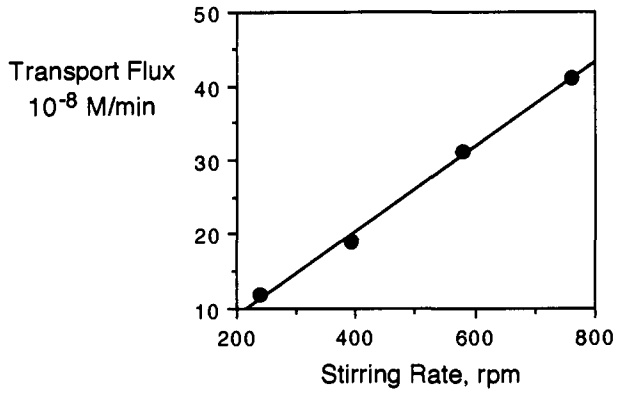


Figure 3. Plot of glucoside transport rate versus stirring rate. Starting conditions: departure, 1.36 mM glucoside, 10 mM sodium phosphate, pH 12.4; organic, 1 mM 1–TOMA; receiving, 10 mM sodium phosphate, pH 12.4.

Table 2. Transport Rates and Percent Extraction for Glucoside as a Function of Organic Layer, and Salts Added to the Aqueous Phases

	salt added[c]			transport rate[a] (% extracted[b])	
entry	departure	receiving	organic layer	1–TOMA	3–TOMA
19	none	none	$ClCH_2CH_2Cl$	25 (2.0)	19 (34)
20	$NaClO_4$	$NaClO_4$	$ClCH_2CH_2Cl$	0 (0.2)	2.6 (0.1)
21	NaCl	NaCl	$ClCH_2CH_2Cl$	14 (1.0)	37 (44)
22	none	$NaClO_4$	$ClCH_2CH_2Cl$	17	200
23	none	NaCl	$ClCH_2CH_2Cl$	29	49
24	none	none	CCl_4	18 (3.3)	7.6 (68)

[a] Rate (10^{-8} M min^{-1} ± 15%) that glucoside appeared in receiving phase. All aqueous phases contained sodium phosphate, 10 mM, pH 7.4. Starting conditions were 1.36 mM glycoside in departure and 1 mM in organic. [b] $[glucoside]_{extracted\ into\ organic}/[glucoside]_{initially\ in\ aqueous}$. Reproducibility ± 10%. [c] 10 mM.

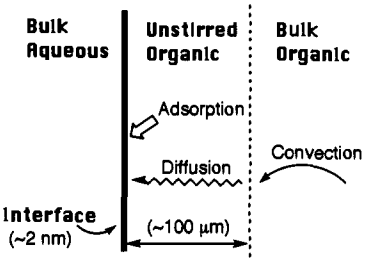
no carrier present, entry 2 vs entry 22). In the case of 1–TOMA, where the rate-limiting step was extraction of the boronate from the departure phase into the organic layer, salt addition to the receiving phase had very little effect. Changing the polarity of the organic layer produced an interesting result. Glucoside extraction was observed to increase when the organic layer was changed from dichloroethane to the less polar carbon tetrachloride (entry 24). Despite the increase in extraction, transport mediated by 1–TOMA and 3–TOMA was found to be lower (entry 19 vs entry 24).

Discussion

Participating Transport Pathways. The extent to which each of the different boronate transport mechanisms (trigonal, tetrahedral A and/or B) participates in a specific transport experiment depends heavily on the experimental conditions. The optimal conditions for trigonal transport were recently summarized.[6b] In short, trigonal transport by arylboronic acids is most likely to occur when the glycoside contains a *cis*-α,γ-diol, and the aqueous phase is near neutral pH. For all transport systems reported here, the trigonal boronate pathway can be ignored, except when galactoside is transported from an aqueous phase near neutral pH, by boronic acids **1** or **2** (entries 7–9).

As stated in the introduction, the relationship of the aqueous phase pH to the boron acid pK_a determines the stoichiometry of the tetrahedral ion-pair mechanism. Most of the transport experiments utilized boronic acids **1** and **2**, whose pK_a's are both approximately 8.8.[6a] Therefore, mechanism A in Scheme 1 is the major pathway for the experiments conducted at pH 8.4 and below, and mechanism B predominates when the pH is 11.4 and above. All experiments with borinic acid **3** (pK_a = 6.2[8]) were conducted at pH 7.4. Under these conditions, mechanism B is expected to be the major pathway, and mechanism A a minor contributor. The differences between the two tetrahedral mechanisms are subtle. In mechanism A, the rate and extent of

Scheme 2



glycoside transport will be influenced by the concentration of the symport ion, OH^-, and antiport ion, A^-. Mechanism B, on the other hand, is predicted to be insensitive to these factors. As discussed below, the rates of boronate formation are always rapid when compared to the rates of organic boundary layer diffusion. Therefore, differences in boronate formation kinetics have no effect on transport rates and can be ignored.

Diffusion-Controlled Transport Model. Since the following discussion is based on the currently accepted model for diffusion-controlled transport, a brief summary is pertinent. Mathematical descriptions of carrier transport have been known for some time;[10] however, it was Behr, Kirch, and Lehn,[11] who successfully used the membrane-diffusion mechanism to explain carrier-mediated transport through liquid organic membranes.[1-3] The applicability of this model to a variety of cation transport systems has been confirmed by various workers,[1] particularly Fyles,[2,12] who has also uncovered some exceptions. The general model assumes the kinetics of carrier complexation are rapid, and the rate-determining step in the transport process is diffusion of the solutes through the unstirred layers (Nernst layers) of the three-phase system. Transport rate through the unstirred layers is in turn determined by the carrier extraction equilibrium constant, K_{ex}. The experimentally observed correlation between rate and K_{ex} is a bell-shaped relationship, where rate is a maximum at an optimum extraction value, $K_{ex(max)}$, and decreases for systems with lower or higher values of K_{ex}. An intuitive physical picture of this correlation can be described in the following way.[2] Transport is a multistep process involving extraction of the solute from the departure phase, movement of the carrier/solute complex through the organic layer, and subsequent stripping of the complex into the receiving phase. Under conditions of weak extraction, transport is slow due to the low amounts of solute moving from the departure phase into the organic layer. Under conditions of high extraction, it is the low solute concentrations moving from the organic layer into the receiving phase that is the rate-determining process.

The applicability of the diffusion-controlled model to the transport of anionic or neutral molecules has not been demonstrated in detail before.[5,11] For the U-tube apparatus used in this work, the organic layer was the only phase stirred. Therefore, it is assumed that solute concentration levels are maintained at a constant level throughout the bulk of the organic layer, except for the narrow unstirred organic boundaries (~100 μm) adjacent to the aqueous/organic interfaces.[13] This allows the transport process to be described as a series of microscopic steps. As an illustration, the likely sequence for the tetrahedral boronate transport mechanism A is described (refer to Scheme 2).[2]

1. Diffusion of the carrier system (boron acid and the quaternary ammonium chloride) from the bulk organic phase through the unstirred boundary layer toward the departure/organic interface.

(10) (a) Rosenberg, T.; Wilbrandt, W. *Pharmacol. Rev.* **1961**, *13*, 109–183. (b) Reusch, C. F.; Cussler, E. L. *AIChE J.* **1973**, *19*, 736–741.
(11) Behr, J.-P.; Kirch, M.; Lehn, J.-M. *J. Am. Chem. Soc.* **1985**, *107*, 241–246.
(12) Fyles, T. M. *J. Membr. Sci.* **1985**, *24*, 229–243.
(13) Although the aqueous phases are unstirred, diffusion through these layers is ignored as a rate-limiting factor. The transport experiments measured initial rates, and under such conditions any concentration gradient of glycoside through the departure phase boundary is assumed to be negligible.

2. Formation of the anionic glycoside–boronate. Depending on the lipophilicity of the boron acid this will occur at the departure/organic interface, or in the bulk aqueous phase followed by diffusion and adsorption of sodium glycoside–boronate to the interface.[13]

3. Adsorption of the quaternary ammonium chloride to the departure/organic interface.

4. Ion exchange at the interface to produce the quaternary ammonium glycoside–boronate ion pair.

5. Desorption of the lipophilic quaternary ammonium glycoside–boronate ion pair from the interface into the organic membrane.

6. Diffusion of the quaternary ammonium glycoside–boronate ion pair through the unstirred organic boundary, toward the bulk organic phase.

The ion-pair (like the other solutes) moves rapidly through the bulk organic phase due to the convection created by the stirring. At the receiving/organic interface, the reverse sequence, 6 through 1, occurs. When considering the dynamics of this transport model, two limiting-case regimes can be envisaged. One is a kinetically-controlled process, where the rate(s) of the chemical steps 2, 3, 4, and/or 5 are rate-limiting, and the other is a diffusion-controlled model where the chemical steps occur rapidly, and steps 1 or 6 are rate-determining.

Relationship between Extraction and Transport Rate. As described in Table 1, the percent glycoside extracted by **1** and **2** generally increased with aqueous phase pH until pH 11.4, after which it decreased slightly. This was expected because the fraction of the tetrahedral diol–boronate increases with pH until the hydroxide ion concentration is high enough to compete with the diol.[14] In terms of glycoside structure, extraction was found to be selective for galactoside > mannoside > glucoside (compare for example, entries 2, 8, and 14). This is in agreement with established tetrahedral boronate stabilities for cyclic diols of *cis*-α,β-diol > *cis*-α,γ-diol > *trans*-α,γ-diol $\gg$ *trans*-α,β-diol. Glycoside transport, on the other hand, displayed a bell-shaped relationship with aqueous phase pH, and by extension a bell-shaped relationship with extraction. Factors other than pH also affected extraction, and to account for this, an extraction equilibrium was defined. As a way of avoiding the differences in stoichiometry associated with the three different extraction mechanisms, an extraction constant, K_{ex}, was calculated using the following expression:[15]

$$\underset{(aq)}{G} + \underset{(org)}{B} \overset{K_{ex}}{\rightleftharpoons} \underset{(org)}{GB}$$

where G = uncomplexed glycoside, B = uncomplexed boron acid (trigonal and tetrahedral), GB = glycoside–boronate (trigonal and tetrahedral).

For each of the percent extraction entries shown in Table 1, a value of log K_{ex} was computed and plotted against transport rate (Figure 4). This plot shows an approximate bell-shaped correlation, with maximal transport rate occurring for a carrier admixture of log $K_{ex(max)} \sim 2.2$. One of the models suggested by this correlation is a transport process that is at least partially diffusion-controlled. In other words, under low extraction conditions the rate-determining step is diffusion of the glycoside–boronate through the unstirred organic boundary at the departure/organic interface (step 6 above), and under conditions of high extraction, the rate-determining step is return of the unoccupied carrier through the unstirred organic boundary at the receiving/organic interface (reverse of step 1 above). A corollary of the diffusion mechanism is if the extracting ability of the membrane is made asymmetric (that is, the departure interface is made highly extracting and the receiving interface weakly extracting), then downhill transport should increase.[2,16] This prediction was tested in two ways.

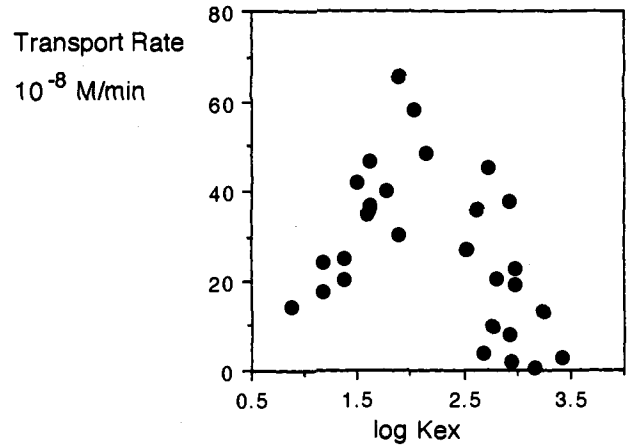


Figure 4. Plot of transport rate versus log K_{ex}. Points taken from Table 1.

The first approach was to affect glycoside extraction using pH. When transport mediated by **1**–TOMA was conducted with both aqueous phases at pH 12.4, the rates were very low even though extraction was very high (entries 5, 11, and 17). Making the membrane asymmetric, by keeping the departure phase at pH 12.4 (highly extracting), and changing the receiving phase pH to neutral (weakly extracting) accelerated transport by 3–15-fold (compare entries 6, 12, 18 with 5, 11, 17). To confirm the origin of this effect, the experiments were repeated, but this time the amounts of glycoside in both aqueous phases were monitored simultaneously.[9] As shown in Figure 1a, when both aqueous phases were at pH 12.4, appearance of glycoside in the receiving layer was slower than loss into the departure phase, implying significant build-up of the glycoside–boronate in the organic layer. Changing the receiving phase to neutral pH facilitated stripping of the boronate; thus there was a much smaller amount in the organic layer at steady state (Figure 1b). Further examples of build-up in the organic layer were obtained with the **3**–TOMA carrier system (Figure 2). Because of the increased acidity and lipophilicity of **3**, there was considerable extraction at neutral pH.[8] But as shown in Figure 2, release into the receiving phase was rate-limiting, and hence a large build-up in the membrane was observed.

The second approach to making the membrane asymmetric was to control extraction by adding competing anions to the aqueous phases.[7] When lipophilic perchlorate anions were added to the aqueous phase, extraction of the glycoside–boronate anion into the organic layer was greatly decreased due to anion competition (entry 19 vs entry 20). As noted in the paragraph above, glucoside extraction by **3**–TOMA at pH 7.4 was very high, and transport was subsequently low due to slow exit of the glucoside from the organic layer. Addition of sodium perchlorate to the receiving phase greatly decreased glucoside extraction ability at that membrane interface, resulting in improved glucoside transfer and dramatically accelerated transport (entry 22).[17] This

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(15) If transport is diffusion-controlled then extraction can be treated as a "black box" phenomenon, where the critical parameter determining transport rate is the amount of extracted glycoside–boronate, GB; the precise extraction mechanism being irrelevant. Since Q^+ is never in limiting amounts, it is assumed that GB partitions completely into the organic layer. Thus, the expression actually describes $K_{complexation}$ which is assumed to be equal to, or at least proportional to, K_{ex}.

(16) Another corollary is that active transport is possible. For a membrane with unequal extraction constants at each interface, the final equilibrium glycoside concentrations are related by $[G]_{rec}/[G]_{dep} = K_{ex(dep)}/K_{ex(rec)}$.

(17) The fact that lipophilic counteranions, such as perchlorate, have a strong effect on transport by **3**–TOMA can be considered an experimental artifact. Transport mediated by **3**–TOMA is expected to be predominantly via ion-pair mechanism B, which is predicted to be insensitive to counteranions, A^-. However, perchlorate anions added to the receiving phase can displace some of the anionic **3**–glucoside complex from the organic membrane. This produces a "burst" in apparent glycoside concentration in the receiving phase. If the **3**–TOMA admixture was in catalytic amounts this displacement would eventually result in catastrophic loss of the carrier from the membrane and

accelerating effect was lost, however, when sodium perchlorate was added to both aqueous phases (entries 20). In this case, extraction decreased so much that movement of the glucoside from the departure phase into the membrane became extremely slow and rate-limiting. Repeating these experiments with more hydrophilic sodium chloride produced similar, but attenuated effects on both extraction and transport (entries 21 and 23).

Strong evidence against a mechanism controlled by the kinetics of boronate formation was the observation that transport was sensitive to the identity of the quaternary ammonium cation. Changing from 3–TOMA to the less lipophilic 3–TBA resulted in decreased extraction but increased transport (*e.g.*, entry 8). This result is not consistent with a complexation-controlled transport mechanism, which predicts the rate to be essentially independent of ammonium cation structure, whereas it is readily rationalized by a diffusion-controlled model, *i.e.*, changing from a carrier admixture of $K_{ex} > K_{ex(max)}$ to a less lipophilic carrier decreases K_{ex} to a value that is closer to $K_{ex(max)}$ thus increasing transport.

Unambiguous proof that transport was diffusion-controlled was gained by showing that transport rate was proportional to the organic stirring rate.[2,18] The unstirred layers associated with the organic membrane are predicted to become narrower as the organic stirring rate is increased, the precise relationship being dependent on the hydrodynamics of the apparatus. When considering the transport process in terms of the microscopic steps described above, chemical steps 2–5 are predicted to be unaffected by changes in the stirring rate. However, diffusion steps 1 and 6 are predicted to be dependent on the thickness of the unstirred layer and hence sensitive to the stirring rate. The transport of glucoside mediated by 1–TOMA was monitored at both pH 7.4 (ion-pair mechanism A predicted to predominate) and pH 12.4 (mechanism B predominating). In both cases the rate was found to be linearly proportional to the stirring rate. The determination at pH 12.4 (Figure 3) is notable because boronate formation is known to be an acid-catalyzed equilibrium; thus reaction rates are expected to decrease with increasing pH.[14,19] Even under conditions likely to produce the slowest boronate formation kinetics, transport was still clearly diffusion-controlled.

Although there is convincing evidence that a diffusion-controlled mechanism is the cause of the bell-shaped plot in Figure 4, the scatter in the plot deserves further comment, since it is too large to be attributed to uncertainty in the measurements. Probable sources for the scatter include the following: (i) The organic boundary layer diffusion co-efficient, D (from Fick's first law), is unlikely to be the same for all solutes.[2] Presumably, D is dependent on solute structure. Therefore, $K_{ex(max)}$ should be different for the various glycoside and carrier mixtures. In addition, $K_{ex(max)}$ should vary with transport mechanism. (ii) The extraction equilibrium defined above assumes the extracted glycoside–boronate to be monomeric within the organic membrane. This may not be true as ion-pair aggregation may be significant.[7] (iii) Diffusion through the aqueous unstirred layers is ignored as a rate-limiting factor because it is assumed that the glycoside concentration gradient in the departure phase is negligible.[13] This assumption rapidly breaks down under very high extraction conditions.

Factors Controlling Transport Rate. The fact that transport is a diffusion-controlled process means that K_{ex} is the critical variable determining transport rate. Therefore, an analysis of the factors that control transport can be reduced to an analysis of the factors that control K_{ex}.[2] Although the kinetics of glycoside–boronate formation can be ignored, the stoichiometry is still important as it suggests which parameters can be altered to influence extraction. A consideration of these parameters, as well as the concepts behind "ion-pair extraction",[7] provides the following list of important structural and environmental factors that control glycoside transport.

Glycoside extraction via the ion-pair mechanism A is an anion antiport process, where a hydrophilic counteranion (chloride) is exchanged for a hydroxide ion that is "transiently disguised" as a lipophilic glycoside–boronate anion. Glycoside extraction increases with the concentration of tetrahedral glycoside–boronate. Formation of the boronate is favored by increasing the pH and increasing boron acid acidity. As well, the arrangement of diols within the glycoside is very important; the usual order of stability is *cis*-α,β-diol > *cis*-α,γ-diol > *trans*-α,γ-diol ≫ *trans*-α,β-diol.[6b] Furthermore, extraction is improved by making the organic layer less polar (compare entries 19 and 24) and by increasing the respective lipophilicities of the boron acid, the glycoside, and the quaternary ammonium cation. Extraction is decreased by including competing lipophilic anions in the aqueous layer. This effect is often utilized in anion transport systems as a method of facilitating anion release from the organic layer into the receiving phase.[4a,h,j,l–n] In principle, such a strategy can be used in any counter-transport process.[2] For mechanism A, with catalytic amounts of carrier, the effect will be maximized by using a counteranion of lipophilicity equal to the transported glycoside–boronate anion.

Inspection of ion-pair mechanism B predicts transport rates to be insensitive to changes in pH and counteranion, A^-. In certain circumstances this may not be true. At very high pH levels (where mechanism B is usually operating), the hydroxide ion concentration is high enough to compete with the diol, such that the fraction of tetrahedral diol–boronate begins to decrease.[14] As well, very lipophilic anions added to the aqueous phases can displace the entire glycoside-boronate anion from the organic membrane without it dissociating into free boronate and glycoside, thus affecting the extraction stoichiometry.[17]

The factors listed above can be used as a set of guidelines for optimizing experimental conditions to obtain a desired stereoselective transport result. For example, if maximum rate is the desired result, then the carrier system should be adjusted either structurally or environmentally to achieve $K_{ex(max)}$. If a more subtle result is desired, such as maximizing the difference in rate between two solutes, then a more sophisticated analysis is required. As an illustration, consider the hypothetical stereoselective separation of glucoside from mannoside. Because mannoside incorporates a *cis*-2,3-diol, it is usually extracted better than glucoside. Under low extraction conditions (*e.g.*, 1–TOMA as the carrier at pH 7.4, Table 1), where both K_{ex}(Glu) and K_{ex}(Man) < $K_{ex(max)}$ the mannoside is transported more rapidly, but if the system is changed to high extraction conditions (*e.g.*, 1–TOMA as the carrier at pH 11.4), where both K_{ex}(Glu) and K_{ex}(Man) > $K_{ex(max)}$, the mannoside is transported more slowly. At the midpoint where K_{ex}(Glu) < $K_{ex(max)}$ < K_{ex}(Man), it is possible that both rates of transport will be identical (*e.g.*, 2–TOMA as the carrier at pH 8.4). These results highlight the important point that unless $K_{ex(max)}$ is known, competitive transport selectivities cannot be predicted from inspection of the relative rates obtained under noncompetitive conditions.[2,11] Moreover, if one or more of the solutes in a competitive transport experiment has a $K_{ex} > K_{ex(max)}$, then transport selectivities will change rapidly with the extent of transport.

Conclusions

1. The transport of glycosides through liquid organic membranes mediated by reversible tetrahedral glycoside-boronate formation occurs via two related ion-pair mechanisms of different stoichiometry (Scheme 1). Mechanism A occurs when the pH of the transport system is below the pK_a of the boron acid, and mechanism B predominates when the pH is above the boron acid pK_a.

a drop in transport rate. Because the carrier is in relatively high amounts (typically the carrier was 1 mM, and glycoside 1.36 mM) only the "burst" is observed, and the point where the carrier becomes exhausted is never reached.

(18) For an example of another interfacial process that is sensitive to stirring rate, see: Menger, F. *J. Am. Chem. Soc.* **1970**, *92*, 5965–5971.

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

2. In all cases, the kinetics of glycoside–boronate formation are sufficiently fast that the rate-limiting step in the transport cycle is diffusion of the solutes through the narrow unstirred boundary layers adjacent to the organic/aqueous interfaces. Any difference in formation kinetics between the transport mechanisms has no effect on transport rates and can be ignored. The critical variable controlling glycoside transport rate is the glycoside extraction constant, K_{ex}. A plot of transport rate versus K_{ex} forms a bell-shaped relationship (Figure 4). Maximal transport rate occurs when the carrier admixture has an extraction constant of log $K_{ex(max)} \sim 2.2$. Under low extraction conditions ($K_{ex} < K_{ex(max)}$), movement of the glycoside from the receiving phase into the organic membrane is the rate-determining step, and under high extraction conditions ($K_{ex} > K_{ex(max)}$), exit from the membrane into the receiving phase is rate-determining.

3. Because transport is dependent on K_{ex}, an analysis of the structural and environmental factors that control transport can be reduced to an analysis of the factors that change K_{ex} relative to $K_{ex(max)}$. This is a general corollary of the diffusion-controlled transport mechanism and is applicable to all transport systems, regardless of whether the transported solutes are cationic, anionic, or neutral compounds.

Experimental Section

Instrumental and Materials. UV spectra were determined using a Perkin-Elmer Lambda 2 spectrophotometer. NMR spectra were collected on either GE GN 300 MHz or Varian Unity 500 MHz instruments. The *p*-nitrophenyl β-D-glycopyranosides were purchased from Sigma and used without further purification. Trioctylmethylammonium chloride, tetrabutylammonium chloride, and phenylboronic acid, **1**, were purchased from Aldrich. [3-(1-Adamantylcarboxamido)phenyl]boronic acid, **2**, was synthesized during a previous study.[6b] Diphenylborinic acid, **3**,was obtained by deprotecting diphenylborinic acid ethanolamine ester (Aldrich) according to the method of Coates and Livingstone.[20]

Transport and Extractions. The transport and extraction experiments reported in Tables 1 and 2 used the identical apparatus and procedures described previously.[6b] Typical starting concentrations were: departure phase, 1.36 mM glycoside in 10 mM sodium phosphate buffer; organic layer, 1 mM of each carrier component; receiving phase, 10 mM sodium phosphate buffer. For the experiments described in Figures 2 and 3, the beginning departure phase glycoside concentration was reduced to approximately 0.14 mM. All experiments were reproduced at least in duplicate. The reproducibility of observed rate constants was always less than 20% and usually less than 10%. The reproducibility of the extractions was ±10%. Unless stated otherwise all stirring rates (determined with a stroboscope) were 470 ± 15 rpm.

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(20) Coates, G. E.; Livingstone, J. G. *J. Chem. Soc.* **1961**, 4909–4911.