Molecular Recognition and Membrane Transport with Mixed-Ligand Borates†

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Introduction

Recently, there has been a surge of interest in the use of boronic acid compounds as artificial receptors for a variety of guest structures.[1−6] In particular, boronic acids show a remarkable ability to form reversible, covalent complexes with diol-containing compounds in aqueous solution. This has resulted in the production of a range of molecular devices such as carriers for membrane transport,[1] solid supports for chromatography,[2] enzyme inhibitors,[3] and chemosensors.[4,5] While progress in this research area has been rapid, much of the future work is likely to be slowed due to the difficulty in synthesizing the next generation of structurally more-complicated receptors, particularly those with the capability of enantioselective recognition.[5] These thoughts have motivated us to consider a new strategy for molecular recognition using boron acids. The strategy is predicated on the ability of boric acid to form reversible, covalent 1:2 mixed-ligand complexes.[7] As shown in Figure 1, one of the borate ligands is considered to be the host, and the other ligand to be the guest. The mixed-ligand borate, **1**, is thus the host/guest complex.

Compared to the boronic acid approach, this mixed-ligand borate strategy introduces certain advantages, as well as disadvantages. A significant advantage is the host is no longer a boronic acid, but a bidentate chelating compound which in this study is a diol (Figure 1). This is an attractive feature, from the point of view of synthesis, as the methodology for diol preparation is highly advanced (particularly enantioselective synthesis).[8] In addition, diols are generally easier to purify and are more stable than boronic acids. Another advantage is that borate complexation is generally well understood. A number of research groups have conducted careful, long-term studies on borate complexation reactions with a range of chelating compounds.[7,9] The most apparent disadvantage is the likelihood of forming non-functional 1:2 borate complexes with both ligands being host or guest compounds. However, as proved by this report, there are a number of applications where this complication should not be disabling. Here, we demonstrate the utility of this new recognition strategy by inducing selective glycoside transport through a liquid organic membrane.[10]

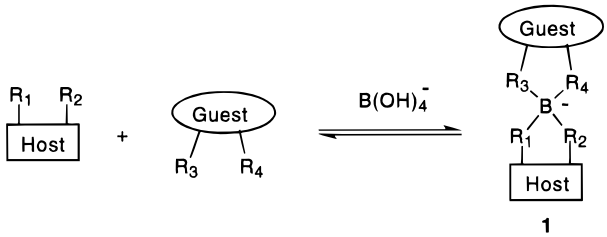

Figure 1. In this study, R_1, R_2, R_3, R_4 = OH.

Results and Discussion

Previously, we have reported on the ability of boronic acids to transport hydrophilic *p*-nitrophenyl *β*-D-glycosides through bulk, liquid membranes (BLMs).[11,12] Depending on the experimental conditions, two transport pathways were found to operate. One pathway involved the formation of transient trigonal boronate esters, whereas the other pathway involved a lipophilic ion-pair comprised of tetrahedral boronate anion and quaternary ammonium cation as the transported species. In this current study, a BLM transport system was devised around the equilibrium shown in Figure 1. Transport rates were determined by standard U-tube experiments where an aqueous departure phase, containing boric acid buffered at pH 9.0 (pK_a of boric acid = 8.98),[7] was separated from an identical aqueous receiving phase by a chloroform layer containing a lipophilic diol and a phase-transfer quaternary ammonium salt.[12] The transport experiment was initiated by adding an aliquot of concentrated *p*-nitrophenyl *β*-D-glycoside solution to the departure phase, after which the rate of appearance of glycoside in the receiving phase was determined from the absorption at 302 nm. In a separate set of experiments, the membrane extraction ability of each carrier system was determined. A two-phase aqueous−organic mixture, with concentrations and volumes equal to the those used in the transport experiments, was vigorously shaken until equilibration was reached. The amount of glycoside extracted from aqueous into organic was measured by UV absorption. Table 1 summarizes the transport rates and percent extraction observed for *p*-nitrophenyl *β*-D-glucopyranoside (glucoside), *p*-nitrophenyl *β*-D-galactopyranoside (galactoside), and *p*-nitrophenyl *β*-D-mannopyranoside (mannoside), mediated by a chloroform layer containing an admixture of tetrapentylammonium chloride (TPAC), and one of the carrier diols, **2**−**5**.

† Molecular recognition with boron acids, part 13. For part 12, see reference 1.

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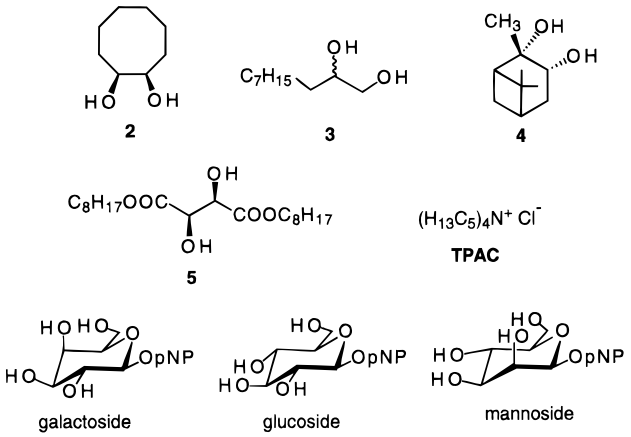
Table 1. Transport Rates and Percent Extractions for Glycosides Mediated by Different Carrier Admixtures

entry	aqueous phase buffer[a]	membrane carrier[b]	transport rate[c] (% extracted)[d] galactoside	mannoside	glucoside
1	$KHCO_3$	TPAC	1.6 (0.4)	1.8 (0.4)	0.8 (0.4)
2	$B(OH)_3$	TPAC	2.2 (0.4)	1.7 (0.4)	0.9 (0.3)
3	$B(OH)_3$	TPAC, **2**	20 (1.7)	16 (1.6)	12 (0.8)
4	$B(OH)_3$	TPAC, **3**	4.2 (0.8)	4.3 (0.9)	4.4 (0.5)
5	$B(OH)_3$	TPAC, **4**	3.6 (1.0)	7.6 (1.6)	2.5 (0.7)
6	$B(OH)_3$	TPAC, **5**	2.1 (0.7)	2.3 (0.8)	1.4 (0.6)

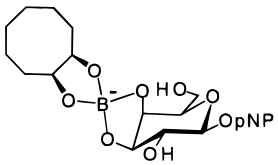
[a] Both aqueous phases contained 100 mM buffer at pH = 9.0. Departure phase started with 1.36 mM glycoside. [b] All membrane additives were 1 mM. [c] Rate (10^{-8} M min^{-1} ± 15%) that glycoside appeared in receiving phase. [d] $[glycoside]_{extracted\ into\ organic}/[glycoside]_{initially\ in\ aqueous} \times 100$. Reproducibility ± 10%.



As seen in Table 1, adding carrier diols, **2**−**5**, to a chloroform membrane containing TPAC had a variable effect on extraction and transport. In terms of extraction ability, there was a clear order of **2** > **4** > **3** ∼ **5**. This matches the expected order of diol complexation stabilities for borate, particularly the prediction that cyclic *cis*-α,*β*-diols complex better than acyclic α,*β*-diols.[9] The general order of glycoside extraction was galactoside ∼ mannoside > glucoside, which reflects the expectation that extraction is highest for glycosides containing a *cis*-α,*β*-diol (present in galactoside and mannoside, but absent in glucoside).[9,11]

The glycoside transport rates did not exactly mirror the extraction values. The best transport enhancement was observed with a carrier admixture of TPAC and *cis*-1,2-cyclooctanediol, **2**, which was able to increase glycoside transport rates 10−15 times the background rate determined in the absence of **2** (compare entry 3 with entry 2 in Table 1). Transport rates with the linear carrier diols **3** and **5** were low, reflecting their poor extracting abilities (entries 4 and 6). Transport rates were also quite low with the carrier pinanediol, **4**, even though extraction values were similar to those found with **2**. The reasons for this are unclear, but it may be that in the case of the sterically hindered **4**, the rates of complexation are sufficiently slowed as to become rate-determining, *i.e.*, the transport process is no longer diffusion-controlled, but kinetically-controlled.[11] The lipophilicity of the quaternary ammonium cation was also surveyed (data not shown). As expected, extraction values increased substantially when phase-transfer ammonium salts were used that were more lipophilic than TPAC. However, glycoside transport rates did not increase with these more lipophilic ammonium carriers and in many cases actually decreased. This result is readily attributed to the known bell-shaped relationship between BLM transport rates and extraction constants. Transport increases with extraction until an optimum extraction value, $K_{ex(max)}$, is reached, after which transport begins to decrease. This is because the rate-determining step in the diffusion-controlled transport process changes from glycoside extraction at the departure/membrane interface, to stripping of the glycoside at the membrane/receiving interface. Increasing extraction beyond $K_{ex(max)}$ (which apparently occurs when very lipophilic ammonium salts are used) further slows this stripping step and produces a build up of complexed carrier in the membrane phase.[11]

The structures of the transported species were investigated using spectroscopic methods. For example, the organic layer from the galactoside extraction experiment using TPAC and **2** (entry 3) was examined by mass spectroscopy. The negative ion FAB spectrum showed a peak at m/z = 452, corresponding to a mixed-ligand borate structure such as **6**. In addition, there was a peak at m/z = 295 corresponding to the homogeneous 1:2 borate, where both borate ligands were carrier **2**. There was no peak for the 1:2 borate where both ligands were galactoside. The ^{1}H NMR spectrum of the organic layer was difficult to interpret due to broadened and overlapping signals; however, ^{11}B NMR showed a single peak at −9.9 ppm (relative to external $B(OMe)_3$) in agreement with the assignment as structure **6**.[9]



In conclusion, the ability of boric acid to form reversible 1:2 mixed-ligand borates has been used to induce glycoside transport through a bulk liquid membrane. Besides membrane transport,[10] this new approach to molecular recognition with boron acids should be useful in other applications such as chromatography[2] and chemosensing.[4,5] Indeed, an enantioselective separation process has already been reported.[13] A particularly appealing feature of this strategy is the ready availability of chiral bidentate chelators, such as diols, hydroxy acids, and amino acids, to act as host compounds.[8]

Experimental Section

Dioctyl L-tartrate, 5. 1-Octyl mesylate (1.16 g, 3.3 mmol) was added to a mixture of L-tartaric acid (0.25 g, 1.6 mmol) and $KHCO_3$ (0.42 g, 4.2 mmol) in DMF (20 mL). After stirring at 90 °C for 24 h, the solvent was removed and the remaining solid dissolved in chloroform (30 mL). The organic layer was washed with water (3 × 30 mL), dried ($MgSO_4$), filtered, and then removed under reduced pressure to give a white waxy solid in 32% yield: mp 42−44 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, t), 1.23 (6H, s), 1.65 (4H, br), 3.62 (2H, t), 4.16, (4H, t), 4.22 (2H, t); ^{13}C NMR ($CDCl_3$) δ 14.1, 18.1, 22.7, 25.8, 28.5, 29.1, 29.3, 29.4, 29.6, 31.9, 32.8, 64.1, 161.1.

Transport Experiments.[12] A solution of chloroform (7 mL, with or without 1 mM tetraalkylammonium chloride and 1 mM diol) was shaken in a separatory funnel with an equal volume of boric acid solution (100 mM, pH 9.0). After the layers were separated, the organic phase was placed in the bottom of a U

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tube apparatus (1.2 cm internal diameter, 10 cm high, 2.5 cm between each arm) equipped with a "spectral" stir bar (Fischer) and a magnetic stirrer. Half of the aqueous phase was carefully added to the departure arm of the U tube and the other half to the receiving arm. Great care was taken to ensure the organic layer was stirred at the same rate (475 rpm) for every experiment and that the U tube was clamped at the same position relative to the magnetic stirrer. To begin the experiment, 150 μL of the appropriate *p*-nitrophenyl β-D-glycopyranoside (33 mM) in water was added to the departure arm. Every 30 min an aliquot (1.0 mL) was removed from the receiving arm, its absorbance at 302 nm quickly determined, and the aliquot returned to the receiving arm. All transport runs were repeated at least once and often many times. The reproducibility of observed rate constants was usually less than $\pm$10% and always less than $\pm$15%.

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