Ribonucleoside Membrane Transport by a New Class of Synthetic Carrier

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Although uncharged, sugars are lipophobic species that do not diffuse through cell membranes rapidly. Included are ribonucleosides such as adenosine, which serve regulatory functions (including coronary vasodilation and inhibition of platelet aggregation)[1] and for which biochemical transport systems have consequently evolved.[2] Biomimetic means of achieving nucleoside transport have been reported in which binding occurs at the nucleic acid base.[3] A complementary mode of reversible association involves boronic acid diol complexation, first employed functionally by Letsinger.[4] We have reported previously that phenylboronic acid (PBA) affords transport selective for ribo- vs deoxyribonucleosides, but only when accompanied by a tetraalkylammonium ("TOMA") phase-transfer reagent;[5] ion pairing of the resulting boronate complex[6] is presumably required for transport. We now report enhanced ribonucleoside transport properties by a new class of synthetic carrier incorporating reversible boronate complexation, charge neutralization, and lipophilicity into a single molecule.

3-Pyridineboronic acid (**1**) exists at pH 7 predominantly as the zwitterionic pyridinium boronate, displaying pK_as of 4.0 and 8.2.[7] N-Alkylation of **1** with **2a–c** at 73 °C in nitromethane for, respectively, 3, 7, and 12 days affords 3-pyridiniumboronic acids **3a–c**.[8] *N*-Methylpyridiniumboronic acid (**3a**) displays a single pK_a of 4.4 (UV, monitored at 262 nm), which corresponds closely to the boronic acid ionization of **1** and characterizes **3a** as a stronger acid than PBA (pK_a 8.8). Both **1** and **3a** are strongly hydrophilic compounds (≥99% partitioning into water vs an equal volume of dichloroethane) and predictably do not effect the transport of nucleosides through liquid membranes.

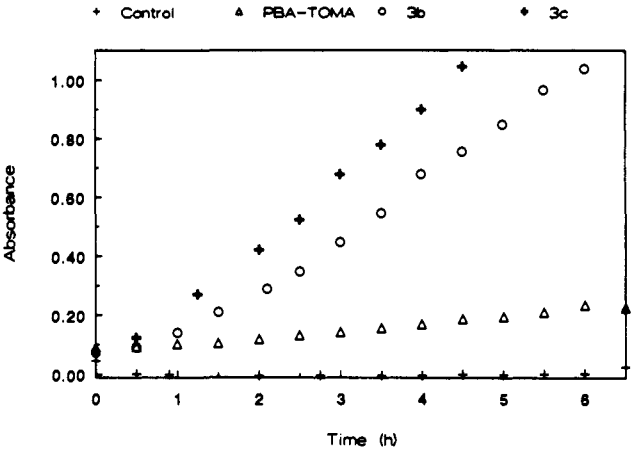
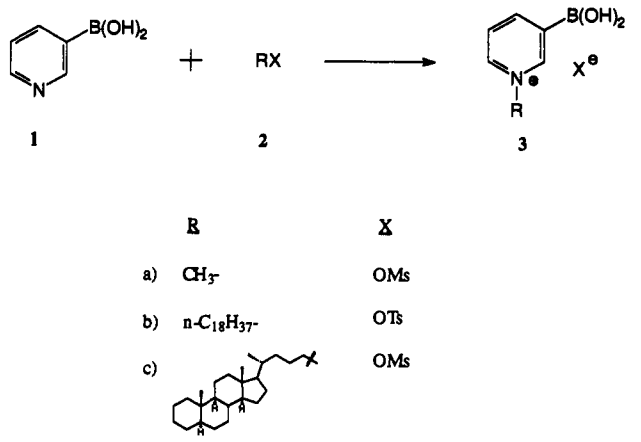


Figure 1. Uridine transport.



	R	X
a)	CH_3-	OMs
b)	n-$C_{18}H_{37}$-	OTs
c)		OMs

By comparison, lipophiles **3b** and **3c** partition between water and dichloroethane mainly into the organic phase (K_{eq} = 12 for **3c**). We observe that each compound, and especially the cholanyl derivative, displays as anticipated enhanced nucleoside transport as compared to the PBA–TOMA system. To measure the rates of facilitated nucleoside transport through a dichloroethane liquid membrane, U-tubes (11.5-mm i.d.) were charged by adding first 0.5 mM solution of the carrier in dichloroethane (7 mL) and then pH 6.5 phosphate buffer (0.1 M; 3.5 mL) to each arm (designated α and β). The dichloroethane layer was stirred using a "flea" stirbar and an electromagnetic stirrer capable of delivering a constant 350 rpm to all experiments; this stir rate was insufficient to generate mechanical transfer of aqueous solution from one arm to the other. At time = 0, nucleoside was added to the α-arm sufficient to provide an initial concentration of 20 mM. The change in absorbance of the β-arm was then monitored by UV as a function of time. Controls without added nucleoside confirmed that the UV increase was not due to decomposition of the carriers. Uridine transport through a $ClCH_2CH_2Cl$ liquid membrane yields the data shown in Figure 1. In the postinduction period rate, PBA–TOMA demonstrates a 17-fold increase in transport rate.

Synthetic carriers **3b** and **3c** are clearly better still, displaying increases of 110- and 140-fold, respectively, as compared to passive

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(8) While the alkylation of **1** by methyl iodide has previously been reported to be unsuccessful (ref 7), we find that the reaction is enabled by use of nonnucleophilic anions such as mesylate and tosylate. Full synthetic details for **3a–c** are included in the supplementary material.

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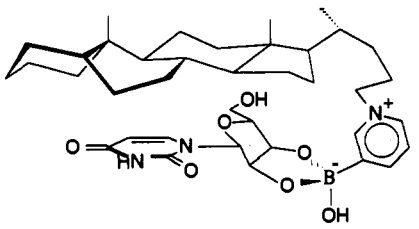

Figure 2.

diffusion. It is notable that the concentration of carriers is 20 times lower in the present studies than in earlier ones; in addition, the nucleoside selectivities are different and complementary.[3] A condensed view of the putative uridine·**3c** complex is shown in Figure 2; a CPK model predicts the potential for cofacial hydrophobic interaction. Relative transport rates using four ribonucleosides, each value an average of three trials, are provided in Table I.

In summary, we report a novel class of compounds that demonstrate efficient ribonucleoside transport. Carriers **3b** and **3c** function as covalent versions of PBA–TOMA, in which the functions of boronate pK_a modulation, internal charge neutralization, and liquid membrane localization are structurally engineered. This approach to polyol recognition is currently being applied in other synthetic receptors.

Table I. Relative Rates of Nucleoside Transport

	no carrier	PBA–TOMA	**3b**	**3c**
adenosine	1.0	6.6	15	13
inosine	1.0	12	13	36
cytidine	1.0	0.5	3.7	10
uridine	1.0	17	110	140

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Supplementary Material Available: Full synthetic details for **3a–c** (2 pages). Ordering information is given on any current masthead page.