Table I. Administration of Labeled (2*S*,6*R*)-*S*-(2-Carboxy-*n*-propyl)cysteine to *A. cepa*

expt no.	labeling pattern in precursor **3**	isotopic ratio $^3H/^{35}S$ in precursor **3**	isotopic ratio $^3H/^{35}S$ in product **1**[a]	% ^3H retention
1	(3*S*)-[3-^{3}H,^{35}S]	6.94	4.87	70
2	(3*R*)-[3-^{3}H,^{35}S]	5.17	0.53	10

[a] The ratios are corrected for ^{35}S decay.

treated with sodium 2,4,6-trinitrobenzenesulfonate to remove primary amino compounds from the crude cycloalliin.[15] The recovered cycloalliin was converted to its hydrochloride salt, purified chromatographically, and then recrystallized repeatedly to constant isotopic ratio and constant specific activity. The results of the two incorporation experiments (Table I) demonstrate that CPC is converted into PCS with loss of the 3 *pro-R* hydrogen atom.[16] Since the configuration at C-2 of CPC is *S*, these results indicate that the oxidative decarboxylation reaction involved in the conversion of CPC to PCS proceeds with anti geometry. The formation of PCS from CPC therefore exhibits the same stereochemical preference as the decarboxylations associated with porphyrin and terminal alkene biosynthesis, and it appears that the mechanisms of these three decarboxylation processes may be closely related.[17]

Acknowledgment. We are pleased to acknowledge financial support of this research by the National Science Foundation (CHE8604611) and The Robert A. Welch Foundation (C-729).

(16) The tritium retention figure for (3*R*)-[3-^{3}H]CPC, which is derived from *R*-Alpine-Borane, is close to the theoretical value since the optical purity of *R*-Alpine-Borane is ca. 91%. However, that for (3*S*)-[3-^{3}H]CPC is somewhat lower than expected since the optical purity of *S*-Alpine-Borane is ca. 87%. This is undoubtedly due to the fact that the optical purity of the tritium label in the 3*S* isomer is low. NMR analysis of the chirality of the (1*R*)-(1-^{2}H$_1$)isobutanol obtained from the product of *S*-Alpine-Borane reduction of the deuterated aldehyde indicated that only about 70% of the deuterium label was in the *pro-R* position. This degree of stereoselectivity is lower than is usually encountered in Alpine-Borane reductions. Since the reductions were carried out with an excess of the reagent, the lower optical purity of the alcohol obtained from the *S*-Alpine-Borane reduction may be due to a difference in the rate of reduction of the chiral aldehydes by the two enantiomeric forms of Alpine-Borane.

(17) It is interesting to note that the same hydrogen atom in an absolute stereochemical sense is removed from C-3 of CPC and from C-3 of fatty acids by the corresponding plant enzyme systems.

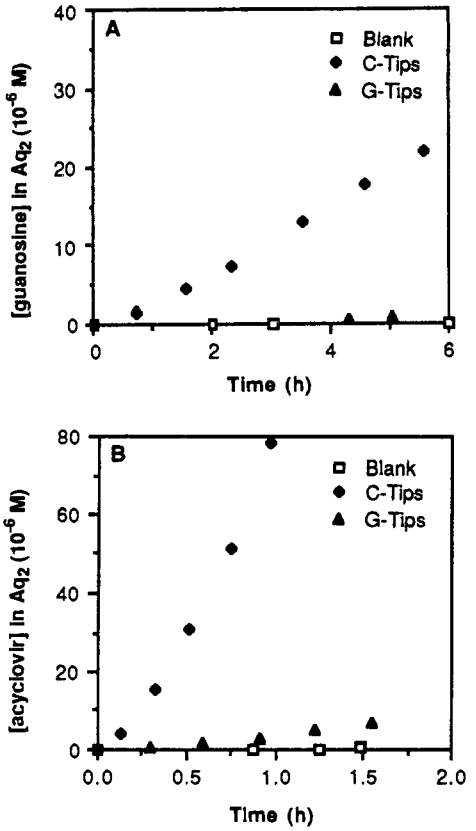

Figure 1. Time course of guanosine (frame A) and acyclovir (frame B) transport through a liquid chloroform membrane effected by using the silylated species C-Tips (**6**) and G-Tips (**7**). Blank refers to control experiments carried out without any added carrier.

Enhanced Transport of Nucleosides and Nucleoside Analogues with Complementary Base-Pairing Agents

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Received January 28, 1991

Recently, nucleoside analogues have been the focus of attention because of their potential utility in antiviral chemotherapy.[1] For example, 9-[(2-hydroxyethoxy)methyl]-9*H*-guanine (acyclovir, **1**)[2] is used to treat herpes infections, and a broad family of nucleosides containing a 2′,3′-dideoxyribose, including 2′,3′-dideoxycytidine (ddC, **2**)[3] and 3′-azido-2′,3′-dideoxythymidine (AZT, **3**)[4] (an approved drug for the treatment of AIDS), have been shown to have anti-HIV activity.[5] Mechanistically, after these substances enter the cells by simple diffusion[6] or with help of membrane-bound transport proteins,[7] phosphorylation in the cytoplasm produces active nucleotide analogues which can inhibit an essential viral enzyme, such as DNA polymerase, and/or terminate the growing virus DNA chain.[8] Therefore, a first requirement for drug activity is the transport of these nucleoside analogues into diseased cells through the lipophilic membrane barrier.[9] If selective carriers were available for various nucleoside-type prodrugs, they could be used to enhance into-cell transport of these substances. We now report a new approach to through-membrane transport based on complementary base-pairing.

Prior studies with various three-phase [Aq_1]-[hydrophobic membrane]-[Aq_2] systems (Aq = aqueous) have shown that substrate binding, substrate–carrier complex diffusion, and sub-

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Table I. Transport Rates of Guanosine, Cytidine, and Analogues[a]

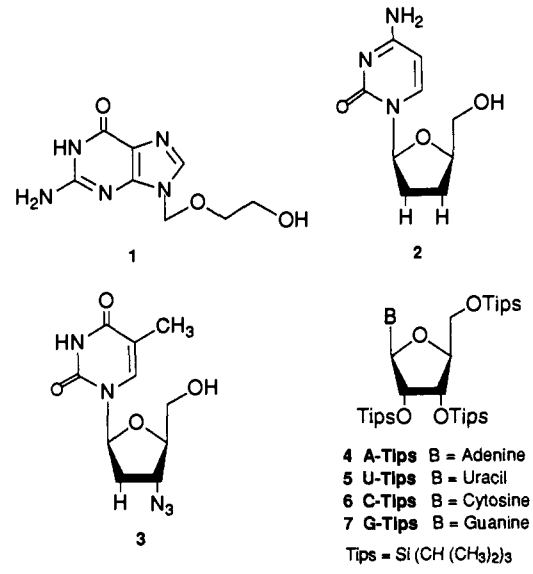
substrate	carrier	k_T,[b] 10^{-9} mol/(cm²·h)	K_b,[c] M^{-1}
guanosine	A-Tips (**4**)	≤0.005 (0.46)[d]	na
	U-Tips (**5**)	≤0.005 (1.35)[d]	na
	C-Tips (**6**)	6.47 (199)[d]	10^4–10^5
	G-Tips (**7**)	0.30 (8.55)[d]	10^3–10^4
	blank	≤0.005 (0.48)[d]	
cytidine	A-Tips (**4**)	0.01 (24.6)[e]	na
	U-Tips (**5**)	0.04 (19.6)[e]	na
	C-Tips (**6**)	0.18 (20.2)[e]	28
	G-Tips (**7**)	4.48 (553)[e]	10^4–10^5
	blank	0.08 (20.9)[e]	

[a] Transport experiments were performed by using a glass U-tube at 28 °C. The conditions were as follows. Aq_1: 10 mM nucleoside, pH 7.0, 1 mL of H_2O. Membrane: 10 mM carrier, 6 mL of $CHCl_3$. Aq_2: 1 mL of H_2O. The release of substrates into the receiving phase was monitored at various times by quantitative reverse-phase HPLC. Experimental errors: <±5%. Blank refers to control experiments carried out in the absence of an added carrier. [b] Rate constants were calculated from the linear region of concentration vs time curves such as those given in Figure 1. [c] From ref 12a; na means not available. [d] The values in parentheses are the k_T constants for acyclovir (**1**). [e] The values in parentheses are the k_T constants for ddC (**2**).

strate release are all important in regulating the efficacy of carrier-enhanced transport for both charged[10] and neutral[11] species. On this basis, we considered that nucleoside transport could be achieved by using hydrophobic recognition agents that interact with nucleoside substrates via complementary base-pairing. Since base-pairing interactions are known to be strong in apolar media,[12] it was thought that recognition would be enhanced within a hydrophobic, membranelike environment but not be so strong as to preclude final substrate release.

To test the above ideas, we have synthesized lipophilic triisopropyl (Tips) substituted nucleoside derivatives (**4**–**7**)[13] and examined their efficacy as transport agents for a variety of nucleoside systems, including adenosine, uridine, cytidine, guanosine, acyclovir (**1**), ddC (**2**), and AZT (**3**), in a simple (H_2O–$CHCl_3$–H_2O) liquid membrane system.[14]

In a first series of experiments, guanosine transport was investigated. In the absence of carrier or in the presence of A- and U-Tips (**4** and **5**) only trace quantities of guanosine were transported (Table I). On the other hand, as shown in Figure 1, frame A, strongly enhanced transport was achieved with C-Tips (**6**) and moderate facilitation effected with G-Tips (**7**). When cytidine transport was studied, G-Tips, in turn, showed the greatest enhancement (Table I). For adenosine and uridine transport, carrier effects were also observed, although in these cases both the degree of carrier selectivity and the overall enhancement were much smaller than in the guanosine and cytidine cases.[15] During these transport experiments, the Tips-substituted nucleosides were stable and remained in the $CHCl_3$ layer.



The remarkable enhancements observed are readily rationalized in terms of the known strong base-pairing interactions (Hoogsteen and Watson–Crick) which take place in $CHCl_3$. In this solvent the binding constant (K_b) for G–C pairing is reported to be 10^4–10^5 M^{-1}.[12] Thus, strong interactions between guanosine and C-Tips (or cytidine and G-Tips) are expected to exist in the membrane of the present three-phase system.[16] The moderate enhancement effected by G-Tips for guanosine transport can also be rationalized in terms of the large self-association constants for G–G base-pairing in $CHCl_3$ (K_b = 10^3–10^4 M^{-1}).[12] In fact, the overall correlation between K_b and transport rate (k_T) (cf. Table I) indicates that base-pairing interactions control transport in the present system.

The transport of nucleoside analogues such as acyclovir (**1**), ddC (**2**), and AZT (**3**) was also investigated. The rate of acyclovir transport (k_T) was enhanced by a factor of 400 by C-Tips and that of ddC transport by a factor of 22 by G-Tips (Figure 1B and Table I). Interestingly, for acyclovir transport with C-Tips and G-Tips, the ratio of k_T is almost the same as that observed for guanosine transport (22–23 times). This, apparently, is just a further reflection of the different K_b values for G–C and G–G pairing under the present transport conditions. Finally, we could also observe a small enhancement for AZT transport using **4**, **5**, and **7**, although in this case the selectivity was much smaller, reflecting, in all likelihood, the high intrinsic lipophilicity of this particular nucleoside analogue.[6]

In conclusion, we have demonstrated effective through-liquid-membrane transport of nucleosides and analogues using an artificial, base-pairing-mediated, lipophilic nucleoside carrier approach. Current efforts involve extensions into the area of nucleotide recognition[17] and transport.

Acknowledgment. This work was supported by the Texas Advanced Research Program (Grant No. 3658-016). J.L.S. also expresses gratitude to the National Science Foundation (PYI 1986), the Camille and Henry Dreyfus Foundation (Teacher-Scholar, 1988–1993), and the Sloan Foundation (fellowship, 1989–1991).

Supplementary Material Available: Experimental details for the synthesis and characterization of the Tips-substituted nucleosides (**4**–**7**) (2 pages). Ordering information is given on any current masthead page.

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