dom chromosomal changes involving chromosome 1 and especially chromosome 6 have been reported (*12, 21–23*), and for breast cancer, loss of heterozygosity on chromosome 11 (*10*) in addition to chromosome 13 also gives support to this concept. Inheritance of a mutation in one of these loci will probably enhance the transformation process but be insufficient for tumorigenesis.

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24. Funds for this research were provided by a Research Grant Award CA44754 from the National Cancer Institute to Y.K.T.F. and the Clayton Foundation for Research and Margaret Bundy Scott Trust to A. L. M. We wish to thank Chin Du, Jin Qian, J. Swallow, and Xiang-He Shi for technical assistance; Ke-Cheng Chen for photographic art work; and C. Stutters and D. Boyd for help in preparing the manuscript. J.M.V. acknowledges ICI for financial support.

26 May 1988; accepted 1 August 1988

Recognition and Transport of Adenine Derivatives with Synthetic Receptors

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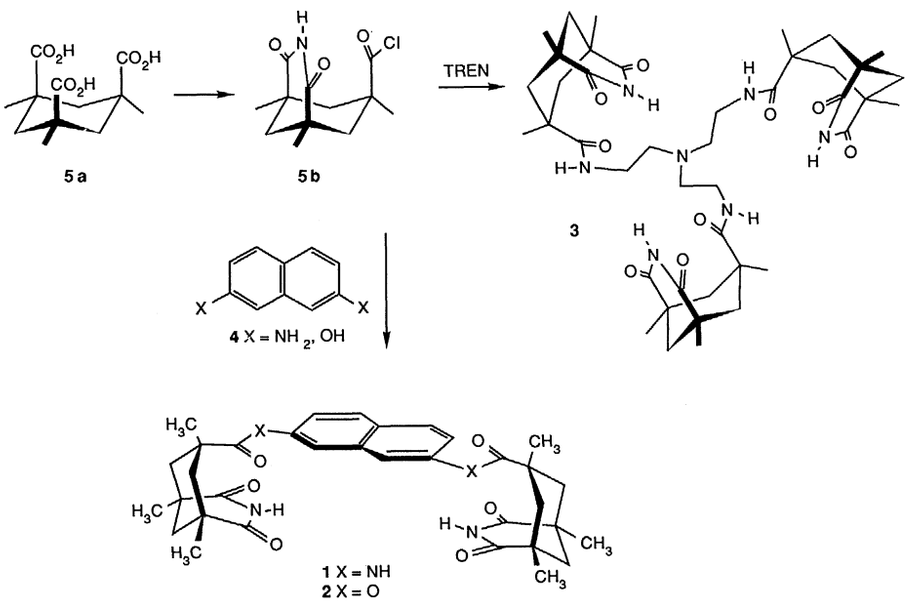
Several new synthetic agents show high affinity for binding adenine derivatives. The structures feature complementary hydrogen bonds that cause the molecular chelation of the purine nucleus. The high lipophilicity of the new agents permits the transport of adenosine and deoxyadenosine across organic liquid membranes. The use of synthetic receptors for small biological targets may have application in drug delivery.

MODEL RECEPTORS HAVE BEEN useful for exploring the intermolecular forces involved in the molecular recognition of adenine derivatives (*1*). Watson-Crick and Hoogsteen base pairing, bifurcated hydrogen bonds, and aryl stacking interactions have all been identified in such systems and their relative contributions to binding selectivity have been assessed. The binding studies have been performed in organic solvents that compete poorly for hydrogen bonds, such as $CDCl_3$. The extraordinary affinity of structures such as the diimide **1** for adenine derivatives raised the possibility of their use in more competitive hydrogen-bonding media. Accordingly, these substances (**1**, **2**, and **3**) were tested for their ability to extract adenine from its aqueous solutions (*2*). We report these results, including their application as vehicles for transport of adenine derivatives across liquid membranes.

The new molecular chelates were prepared by acylating the appropriate spacer groups **4** or TREN (2,2′,2″-triaminotriethylamine) with the imide acid chloride **5b** (scheme 1); the latter is readily available from Kemp's triacid **5a** (*3*).

Both the diamide **1** and the diester **2** extracted one equivalent of adenine **6a** from H_2O into $CDCl_3$. The nuclear magnetic resonance (NMR) spectra of the extracts were consistent with simultaneous Watson-Crick, Hoogsteen, and aryl stacking interactions in the complexes (**7a**) (*4*). The more flexible TREN derivative **3** extracted ~100% of capacity (based on a 1:1 stoichiometry). Parallel experiments with adenosine **6b** (0.1*M*) showed that even this compound was extracted by the molecular chelates. The relative efficiency of extractions was the diamide **1** (45% of capacity) > the TREN derivative **3** (15% of capacity) >> the diester **2** (undetected). With deoxyadenosine **6c**, the order was diamide **1a** (~90% of capacity) > diester **2** (30%) > TREN **3** (10%).

Transport of these adenine derivatives



Scheme 1.

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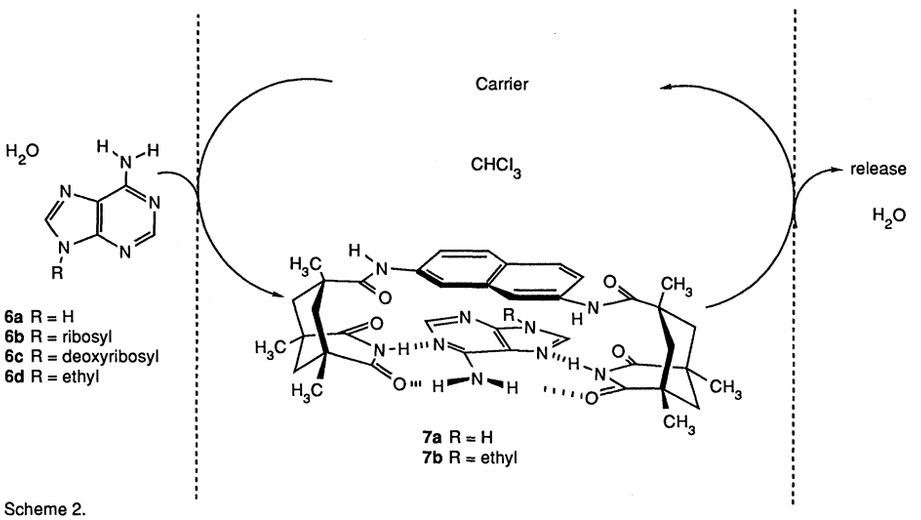
across a $CHCl_3$ liquid membrane was studied with an apparatus previously described for amino acid transport (*5*). Release of adenine derivatives into the receiving phase (scheme 2) was monitored by ultraviolet (UV) absorption at 259.5 nm (Fig. 1 and Table 1). The facilitation factors permit comparisons of transport rates in systems featuring diffusion control, that is, systems at equilibrium with respect to the formation of the complex (*6*). Experiments in which either cytidine or guanosine were used under these conditions showed no transport with any of these carriers.

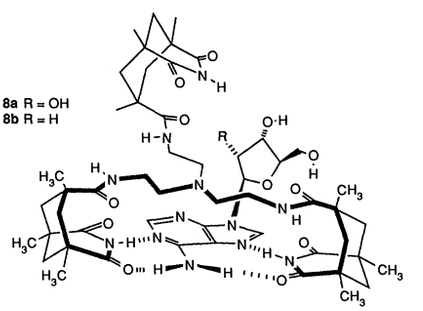
The facilitated transport of adenine derivatives observed with these carriers can be related to specific interactions involved in molecular recognition. Molecular mechanics calculations (*7*) and molecular models suggested the chelate structure **7a** for the complex derived from the diamide. Aromatic stacking interactions and at least three hydrogen bonds are easily achieved; these structural features are supported by nuclear Overhauser effect (NOE) experiments on the complexes with 9-ethyl-adenine **7b**. Association constants for binding of this substrate with **1**, **2**, and **3** are given in Table 2. The value for **3** is corrected for intramolecular hydrogen bonding between two of the imide functions that occur to the extent of 80% in $CDCl_3$. The hydrogen bonding of the C_5' hydroxyl group to one of the imide sites is also likely with either adenosine or deoxyadenosine substrates. With **3**, we can envision similar structures involving the chelation of adenine derivatives between two of the three imide functions. Additional hydrogen bonds involving the third imide function and the C_2' hydroxyl of **6b** may also exist, as suggested in **8a**. Such contacts would be consistent with the high relative affinity of **3** for adenosine observed in the extraction experiments.



Scheme 2.

Table 2. Association constants for binding of 9-ethyl-adenine, **6d**, to synthetic receptors at 25°C in $CDCl_3$.

Receptor	K_a (M^{-1})
1	11,000
2	2,500
3	3,000



The present study reveals that both high affinity and selectivity can be engineered into synthetic carriers through complementary hydrogen bonding and stacking interactions. Recently, other small, biorelevant molecules, such as amino acids (*8*), cyclic dipeptides (*9*), heterocyclic bases (*10*), uric acid (*11*), urea (*12*), and carboxylic acids (*13*) have been bound within synthetic receptors. These results augur well for the development of synthetic pharmaceutical agents of intermediate size that are directed toward small, endogenous targets. Many of the new systems feature a molecular cleft lined with hydrogen-bonding donors and acceptors as the structural entity responsible for selective complexation. It should be possible to incorporate additional complementary functions for the transport of charged derivatives, such as nucleotides.

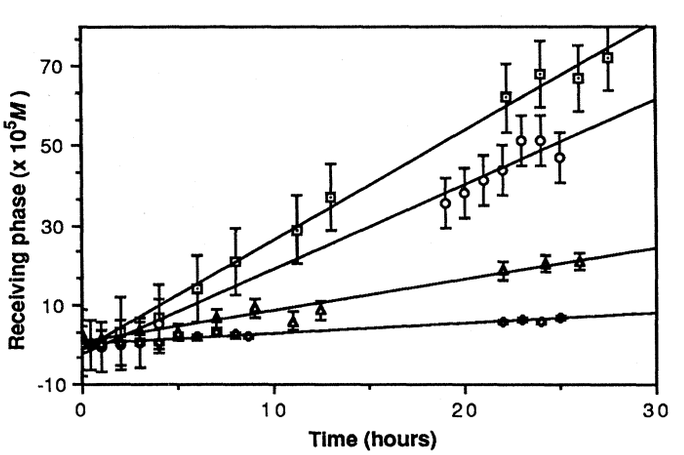


Fig. 1. Transport of $10^{-2}M$ adenosine between water layers separated by a chloroform membrane. Data for one run each with carriers **1**, **2**, and **3**, as well as the blank transport, are shown. **1** (○), $2 \times 10^{-4}M$; **2** (△), $1 \times 10^{-3}M$; **3** (⊡), $1 \times 10^{-3}M$; and blank (◇). Error bars shown represent the standard error of the data.

Table 1. Carrier-mediated transport of adenosine and deoxyadenosine across a $CHCl_3$ liquid membrane. Flux values represent the average of two runs and are reported with a 95% confidence level.

Carrier	Carrier concentration ($M \times 10^{-3}$)	Flux ($\times 10^5$ mmol hour^{-1} cm^{-2})	Facilitation factor	Relative efficiencies
		Adenosine		
1	0.2	4.1 ± 0.25	8.5	14.7
2	1.0	1.4 ± 0.09	2.9	1.0
3	1.0	4.8 ± 0.25	10.0	3.4
Blank		0.48 ± 0.05	1.0	
		Deoxyadenosine		
1	0.26	11 ± 0.47	1.7	4.7
2	1.0	12 ± 0.75	1.9	1.4
3	1.0	9 ± 0.45	1.4	1.0
Blank		6.4 ± 0.15	1.0	

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17 June 1988; accepted 23 August 1988

Sarafotoxin, a Novel Vasoconstrictor Peptide: Phosphoinositide Hydrolysis in Rat Heart and Brain

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Sarafotoxins, a group of 21-residue cardiotoxic peptides from snake venom that induce coronary vasoconstriction, show high-affinity binding to rat atrial and brain membranes and activate the hydrolysis of phosphoinositides. Neither their binding nor their activity is affected by blockers or activators of known receptors and ion channels, suggesting that sarafotoxins act either directly on the phosphoinositide phosphodiesterase system or on a novel receptor. Their amino acid sequence shows a high degree of homology with that of endothelin, a recently described 21-residue vasoconstrictor peptide found in porcine aortic endothelium. This is remarkable, since endothelin is a natural compound of the mammalian vascular system while sarafotoxins are highly toxic components of snake venom.

SARAFOTOXINS S6 (SRTs A, B, AND C) are a group of 21-residue isocardiotoxic peptides, isolated from the venom of the snake *Atractaspis engaddensis*, which are rich in cysteine (four residues per molecule) and show a high sequence homology (*1*). Two of these, a and b, are highly lethal and cause cardiac arrest and death in mice within minutes of intravenous administration. [The median lethal dose (LD_{50}) is ~15 μg per kilogram of body weight (*2*).]

Although the mechanism of action of the sarafotoxins is not fully understood, it has been shown that they have three independent effects on the mouse and rat hearts: a rapid and marked vasoconstriction of the coronary vessels, a severe atrioventricular block, and a slower but very strong positive inotropic effect that is not blocked by either α- or β-adrenergic blockers (*2*). The venom of *Atractaspis* also causes a transient hypertension, which is followed by fluctuation of arterial blood pressure (*2*) and reversible contraction of the ileum in rats. These powerful effects of sarafotoxins could be explained by an increase in intracellular Ca^{2+} (smooth muscle contraction and disturbances in the atrioventricular conducting system), suggesting that the mechanism of their action may involve binding to and activation of a specific protein associated with Ca^{2+} mobilization. In this report we show that sarafotoxins bind specifically and with high affinity to rat atrial and brain membranes and induce the hydrolysis of phosphoinositides in these tissues.

Sarafotoxin S6b (SRT-b), which contains a tyrosyl residue (*1*), was labeled with ^{125}I and used for the binding studies. The toxicity of the ^{125}I-labeled SRT-b was similar to that of the noniodinated toxin. The toxin binds specifically and rapidly to rat atrial membranes (for example, 1600 cpm was recorded with 2.5 n*M* ^{125}I-SRT-b, whereas only 200 cpm was recorded in the presence of 1 μ*M* SRT-b, and the association half-time was 2.5 min with 4 n*M* toxin). Binding activity was not detected in the cytosolic fraction of atrial homogenates. Binding of ^{125}I-SRT-b to the atrial membranes was reversible (dissociation half-time, 10 to 12.5 min) and saturable (Fig. 1). Scatchard analysis (Fig. 1, inset) indicated that the toxin binds to a homogeneous population of sites [maximal binding capacity, 110 fmol per milligram of protein; dissociation constant (K_d), 3 to 5 n*M*]. Ligands of various receptors and ion channels as well as a variety of biologically active peptides (see legend to Fig. 2) failed to inhibit the binding of ^{125}I-SRT-b to the atrial membranes. However, the native toxins SRT-a (Fig. 2), SRT-b, and SRT-c were potent binding inhibitors, with median inhibitory values (I_{50}) of 30, 25, and 100 n*M*, respectively. Evidently, at least one disulfide bond in the sarafotoxins is important for their function and binding, since reduction and carboxymethylation of SRT-b resulted in a loss of toxicity and a marked decrease in affinity (I_{50} > 5000

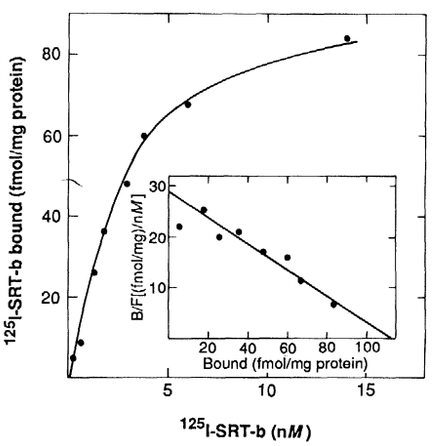

Fig. 1. Binding of ^{125}I-SRT-b to rat atrial membranes, as a function of its concentration. Rat atrial membranes were prepared in 25 m*M* tris-HCl buffer (*p*H 7.4) containing antiproteases [aprotinin (5 U/ml), pepstatin A (5 μg/ml), 0.1 m*M* phenylmethylsulfonylfluoride, 3 m*M* EDTA, and 1 m*M* EGTA) as detailed elsewhere (*9*). Aliquots (50 μl) of membrane preparations (200 μg of protein) were incubated at 25°C for 60 min with various concentrations of ^{125}I-SRT-b (specific activity, 4.7×10^{16} cpm/mol) in a total volume of 200 μl of tris-HCl buffer. Reactions were terminated by the addition of 3 ml of ice-cold tris-HCl buffer and filtration through GF/C filters over vacuum. The filters were washed twice with 3 ml of the buffer, and their radioactivity was estimated by liquid spectrometry. Separate kinetic experiments showed that the bound toxin did not dissociate during ~10-s washings at 4°C (dissociation half-time at 25°C was ~10 min). Assays were performed in triplicate (total binding) together with triplicate samples containing 1 μ*M* unlabeled SRT-b (nonspecific binding). Data are expressed as specific binding per milligram of protein (SEMs are less than 7% of the plotted values). Inset: Scatchard plot.

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