nitrile/0.1 M aqueous NH_4HCO_3 (5.5:2:1:1.5), respectively. Lyophilization of appropriate fractions provided 177 mg (40%) of diphosphate 8 which was characterized by appropriate ^1H, ^{13}C, and ^{31}P NMR spectra; negative ion-LRFABMS; positive ion FABMS (LR and HR); and combustion analysis for C, H, N, P.[23] Selected data follow: ^1H NMR (300 MHz, D_2O–ND_4OD) δ 1.57 (s, 6 H, $=C(CCH_3)_2$), 1.64 (s, 6 H, $2CH_3$), 1.95–2.11 (m, 8 H, $4CH_2$), 2.43 (br m, 2 H, CH_2), 2.80 (s, 3 H, NCH_3), 3.04 (br, 2 H, NCH_2), 3.30 (br t, 2 H, NCH_2), 4.18 (br, 2 H, CH_2O), 5.13 (m, 3 H, 3CH=); ^{31}P NMR (121.5 MHz, D_2O–ND_4OD, pH 8~9) δ –5.55 (br), –10.03 (br); ^{31}P NMR (121.5 MHz, 1 w/v% EDTA in D_2O–ND_4OD, pH 8~9) δ –5.35 (d, 0.63 P, J = 22 Hz), –9.80 (d, 1 P, J = 22 Hz); calcd for $C_{19}H_{38}NO_7P_2$ 454.2123, found 454.2115. A complete procedure and all characterization data for 8 are provided in ref 15.

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Supplementary Material Available: ^1H NMR spectra of compounds 7, 8, 14a, 23, and 24 (7 pages). Ordering information is given on any current masthead page.

Carriers for Liquid Membrane Transport of Nucleotide 5′-Triphosphates

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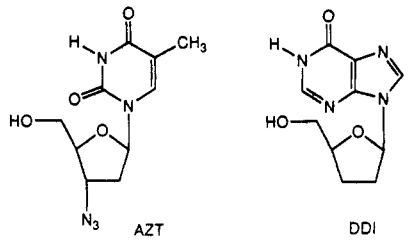
The transport of the nucleotide triphosphates adenosine 5′-triphosphate (ATP), cytidine 5′-triphosphate (CTP), 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP), and 3′-azido-2′-deoxythymidine 5′-triphosphate (AZTTP) across a liquid organic membrane mediated by cationic DABCO-derived carriers was studied. With their branched aliphatic chains, these compounds, which bear two (2), three (3), or four (4) quaternary ammonium centers show excellent solubility in chloroform, the solvent chosen as liquid organic membrane. The bisquaternary mono(DABCO) derivative **2** was found to be the most efficient carrier for all nucleotides. Concentration-dependent extraction studies showed that **2** undergoes formation of a 2:1 carrier–nucleotide complex in the chloroform phase. This stoichiometry differs from the 1:1 stoichiometry previously suggested by Tabushi et al. for the association between nucleotide 5′-triphosphates and a bisquaternary DABCO derivative. The tricationic compound **3** is generally inferior to **2** in its carrier properties but shows an unexpectedly high selectivity for transporting ddTTP. The tetracationic bis(DABCO) derivative **4** shows poor carrier properties since it forms highly water-soluble associations which prefer distribution into the aqueous phase rather than into the liquid membrane.

Introduction

Recently, 2′,3′-dideoxynucleotide 5′-triphosphates have been the focus of attention because of their application in the treatment of AIDS.[1,2] Some of these compounds have been shown to act as potent chain-terminating inhibitors of HIV reverse transcriptase, a prime target in AIDS therapy.[2,3] However, due to their highly charged nature, the triphosphates cannot penetrate across cell membranes. Therefore, the corresponding nucleosides, instead of the nucleotide triphosphates, are administered to patients. In fact, two members of the family of dideoxynucleosides, namely 3′-azido-2′-deoxythymidine (AZT) and 2′,3′-dideoxyinosine (DDI), are now approved for AIDS therapy.[4]



To serve as inhibitors for the HIV reverse transcriptase, the nucleosides must be transformed into nucleotide triphosphates through the action of cellular nucleoside kinases. However, this transformation may not be very efficient, at least in the case of AZT.[5] It is imaginable that the development of an efficient transmembrane carrier could allow the dideoxynucleotide triphosphates to be directly administered to patients, therefore avoiding the need for high concentrations of the modified nucleosides which is problematic in view of their toxicity.[6]

The development of artificial carriers for nucleotide 5′-triphosphate transport has not received much attention in the past.[7,8] Among the early work is the report by

(1) AIDS Report, *Chem. Eng. News*, 1987, *65* (Nov 23), 14–70.
(2) (a) Mitsuya, H.; Broder, S. *Nature* 1987, *325*, 773–778. (b) Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* 1986, *83*, 1911–1915.
(3) Fauci, A. S. *Science (Washington, D.C.)* 1988, *239*, 617–622. See also other articles on AIDS in this issue of *Science*.
(4) DDI approval, *Chem. Eng. News*, 1991, *69* (Oct 14), 17.
(5) Furman, P. A.; Fyfe, J. A.; St. Clair, M. H.; Weinhold, K.; Rideout, J. L.; Freeman, G. A.; Lehrman, S. N.; Bolognesi, D. P.; Broder, S.; Mitsuya, H.; Barry, D. W. *Proc. Natl. Acad. Sci. U.S.A.* 1986, *83*, 8333–8337.
(6) Yarchoan, R.; Mitsuya, H.; Broder, S. *Sci. Amer.* 1988, *259*, 110–119.
(7) Nucleotide recognition studies: (a) Gâlan, A.; de Mendoza, J.; Toiron, C.; Bruix, M.; Deslongchamps, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* 1991, *113*, 9424–9425. (b) Gâlan, A.; Pueyo, E.; Salmerón, A.; de Mendoza, J. *Tetrahedron Lett.* 1991, *32*, 1827–1830. (c) Furuta, H.; Magda, D.; Sessler, J. L. *J. Am. Chem. Soc.* 1991, *113*, 978–985. (d) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* 1990, *112*, 3896–3904. (e) Behr, J.-P. *Tetrahedron Lett.* 1986, *27*, 5821–5864. (f) Schmidtchen, F. P. *Tetrahedron Lett.* 1989, *30*, 4493–4496. (g) Kimura, E. *Top. Curr. Chem.* 1985, *128*, 113–141. (h) Marecek, J. F.; Fischer, P. A.; Burrows, C. J. *Tetrahedron Lett.* 1988, *29*, 6231–6234.
(8) Nucleotide transport studies: (a) Furuta, H.; Cyr, M. J.; Sessler, J. L. *J. Am. Chem. Soc.* 1991, *113*, 6677–6678. (b) Tabushi, I.; Kobuke, Y.; Imuta, J. *J. Am. Chem. Soc.* 1981, *103*, 6152–6157. (c) Tabushi, I.; Kobuke, Y.; Imuta, J. *J. Am. Chem. Soc.* 1980, *102*, 1744–1745. (d) Tabushi, I.; Imuta, J.; Seko, N.; Kobuke, Y. *J. Am. Chem. Soc.* 1978, *100*, 6287–6288. (e) Bergstrom, D. E.; Abrahamson, J. K.; Chan, M. Y.-M. *Biochim. Biophys. Acta* 1991, *1061*, 95–105. (f) Grotjohn, B. F.; Czarnick, A. W. *Tetrahedron Lett.* 1989, *30*, 2325–2328.

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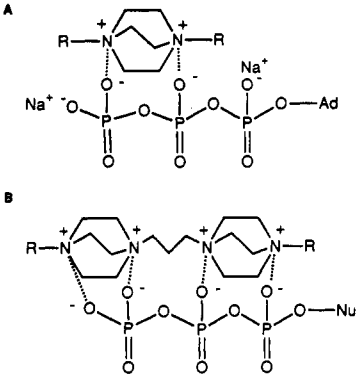
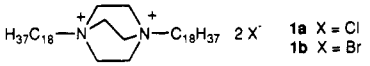


Figure 1. Schematic drawings of possible interaction geometries between nucleotide 5′-triphosphates and diquaternary mono-(DABCO) carriers (A) and tetraquaternary bis(DABCO) carriers (B).

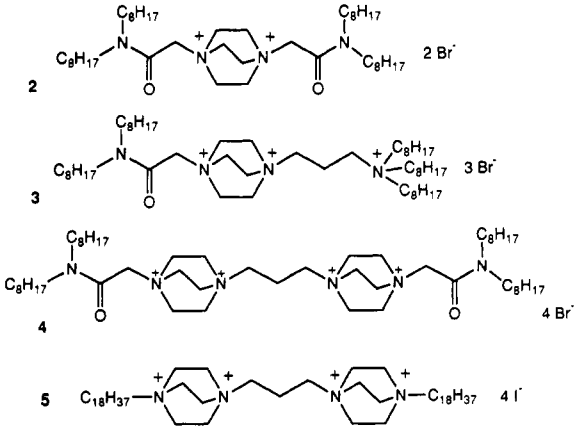
Tabushi et al.[8b] that the lipophilic bis(quaternary amines) **1a/b**, derived from 1,4-diaza[2.2.2]bicyclooctane (DABCO)



1a X = Cl
1b X = Br

and designed to transport adenosine 5′-monophosphate (AMP) and diphosphate (ADP), are also capable of transporting adenosine 5′-triphosphate (ATP) across chloroform. The authors suggested that ATP most likely was forming a 1:1 complex with the diammonium carrier in the organic liquid membrane (Figure 1A).

Since, at physiological pH, the triphosphates are present predominantly in the tri- and tetraanionic forms,[9] it could be expected that the strength of ion pairing (Figure 1B) would increase with carriers that bear three or even four charges. We were interested to study whether such increase in association strength would translate into a faster rate of transport across an organic liquid membrane. Furthermore, we wanted to expand the range of nucleotide 5′-triphosphates in our study and include, among others, AZTTP as a target for transport. Another challenge in this work was the fine-tuning of the balance of hydrophilicity to hydrophobicity in highly charged tri- and tetracationic carriers to achieve their complete distribution into the organic membrane phase and to prevent both the free and ion-paired carriers from leaking out into the aqueous phases. To address these questions, we prepared the new DABCO-derived carriers **2–5**.



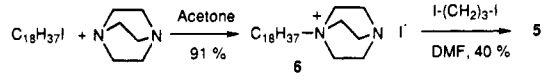
Results and Discussion

Synthesis and Solubility Properties of Carriers 2–5. The preparation of the bis(DABCO) derivative **5** is shown in Scheme I. We found this tetracationic derivative to have poor solubility both in organic solvents like chloroform, dichloromethane, or ethyl acetate as well as in water, which could be explained by the tight packing of the straight aliphatic chains in the solid state. We prepared the Tabushi compounds **1a/b** following the published procedure[8b] and found them also not to be very soluble in chloroform. Even at [**1b**] = 1.0×10^{-4} M, solutions of this derivative are cloudy. Therefore, to increase the general solubility of the carriers, we substituted the close-packing linear alkyl chains by branched chains.

The branched carriers **2–4**, prepared according to Scheme II, indeed showed much improved solubility in chloroform, giving clear solutions in all concentration ranges studied (see below) while, at the same time, exhibiting high insolubility in water. Ion-pairing chromatography[10] on SiO_2 (elution with 1% NaI in CH_3OH/CH_2Cl_2) was particularly effective in the purification of these polyammonium derivatives. The ammonium bromides rather than the iodides were prepared as the target compounds since the optical end-absorption of iodide ions (λ_{max} = 220 nm) interfered with the determination of nucleotide concentrations by UV spectroscopy at λ = 260 nm.

Transport Studies. The transport of nucleotide 5′-triphosphates through a liquid chloroform membrane

Scheme I



Scheme II

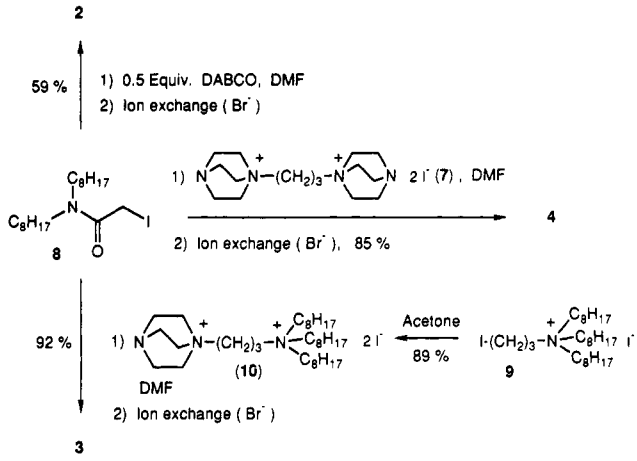


Table I. Rates of Nucleotide Transport across Chloroform in a U-Tube-Type Cell[a]

		rate (10^{-9} mol cm^{-2} h^{-1})[b]		
carrier	ATP	CTP	ddTTP	AZTTP
2	7.1	8.3	10.0	3.7
3	<0.005	<0.04	11.8	0.41
4	<0.005	<0.008	<0.008	<0.008
1b	1.0	<0.008	–[c]	–
none	<0.005	<0.008	<0.008	<0.008

[a] U-tube cell dimensions: 1.34-cm diameter, 4.70-cm center-to-center distance between the two legs. Source phase: nucleotide (1.0×10^{-4} M) in water, pH 7.0, 6.0 mL). Liquid membrane: carrier ($1.0\ 10^{-5}$ M) in $CHCl_3$, 12 mL. Receiving phase: NaBr (0.20 M) in water, pH 7.0, 6.0 mL. [b] Reproducibility: ±15%. [c] Not determined.

(9) Rawn, J. D. *Biochemistry*; Neil Patterson: Burlington, 1989; p 275.

(10) For an example of ion-pairing chromatography, see: Gazdag, M.; Babjak, M.; Kemenes-Bakos, P.; Gorog, S. *J. Chrhomatogr.* **1991**, *550*, 639–644.

Table II. Extraction of Nucleotides from an Aqueous into a Chloroform Phase

		$[nucleotide]_{CHCl_3}/[carrier]_{CHCl_3}$			
carrier	run	ATP	CTP	ddTTP	AZTTP
2	A[a]	0.51	0.52	–[b]	–
	B[a]	0.36	0.40	0.40	0.11
3	A	0.22	0.12	–	–
	B	0.17	0.24	0.40	0.03
4	A	0.00	0.00	–	–
	B	0.04	0.06	0.11	–

[a] Extraction conditions: an equivolume of nucleotide (A: 5.0×10^{-4} M; B: 5.0×10^{-5} M) in water (pH 7.0) with an equivolume of receptor (1.0×10^{-4} M) in $CHCl_3$. [b] Not determined.

mediated by the ammonium bromides **1b** and **2–4** was studied in a standard U-tube cell.[11] The rate of delivery of the substrates in the aqueous receiving phase was monitored by UV spectroscopy. The measured rates of nucleotide transport are given in Table I. Substrates in these studies were ATP, AZTTP, cytidine 5′-triphosphate (CTP), and 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP).

To our initial surprise, we found the tetracationic carrier **4** not to be effective in transporting nucleotide triphosphates; no delivery into the receiving phase could be detected. Careful extraction studies established that while a 1.0×10^{-4} M solution of **4** in chloroform can extract ATP (about 4% in a single extraction) from a very dilute (5.0×10^{-5} M) aqueous solution, the same carrier solution failed to extract the nucleotide from a more concentrated solution (5.0×10^{-4} M). Apparently, a different form of association occurs at higher ATP concentrations leading to a very hydrophilic ion-paired complex that is no longer soluble in chloroform. A double extraction experiment indeed established that most of the receptor had leaked out of the chloroform layer upon attempted extraction of the more concentrated ATP phase. In this experiment, the chloroform solution of **4** (1.0×10^{-4} M) was first extracted with an equal volume of the more concentrated (5.0×10^{-4} M) aqueous ATP solution. Subsequently, the chloroform phase was shaken with an equal volume of the more dilute (5.0×10^{-5} M) ATP solution, conditions under which **4** normally is capable of extracting the nucleotide as discussed above (Table II). However, no ATP was extracted this time, since the carrier had leaked out into the aqueous phase during the first extraction.

The bis(ammonium bromide) **2** was found to be a very effective nucleotide carrier. Under the conditions given in Table I, about 30% of the amount of nucleotide initially present in the source phase can be transported within 20 h into the receiving phase. In all experiments, the carrier-mediated transport was accelerated much more than 1000 times over the rate of passage across pure chloroform. Nucleotide 5′-triphosphate transport through blank chloroform was too slow to be measured, and the rate values given in Table I for this process represent upper limits. The tris(ammonium bromide) **3** shows an interesting selectivity: it fails to mediate effectively the transport of all nucleotides with the exception of ddTTP. At present, we do not have an explanation for this well-reproducible result. The transport of AZTTP by all three carriers **2–4** is shown in Figure 2.

The effects of pH and magnesium ion concentration on the rate of ATP transport by the bis(ammonium bromide) **2** were studied under the conditions shown in Table I. The

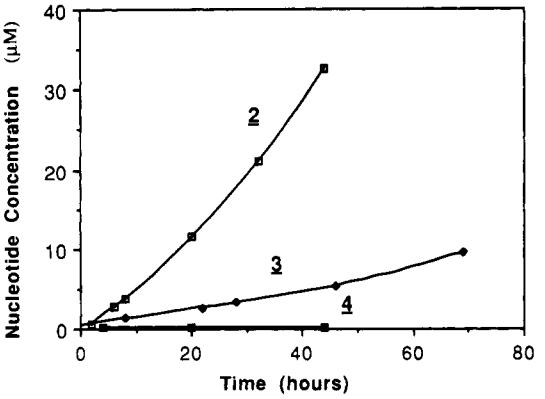


Figure 2. Transport of AZTTP mediated by carriers **2–4**. The nucleotide concentration refers to the concentration in the aqueous receiving phase.

effect of magnesium ions was of particular interest since nucleotide triphosphates form stable complexes with Mg^{2+} and probably exist as such in the cell.[12] The rate increased from 7.1×10^{-9} to 9.2×10^{-9} mol cm^{-2} h^{-1} when the pH of both aqueous phases was lowered from 7.0 to 5.0. The rate increased to 9.0×10^{-9} mol cm^{-2} h^{-1} when magnesium chloride (1.0×10^{-4} M) was added to both aqueous phases. The small increases in transport rates in both cases are probably related to a reduced hydrophilicity of ATP as a result of increased protonation and Mg^{2+} association, respectively.

Similar to Tabushi's compound **1b**,[8b] the bis(ammonium bromide) **2** was also capable of transporting nucleotide diphosphates, and a rate of 6.7×10^{-9} mol^{-1} cm^{-2} h^{-1} was measured for ADP transport. AMP can also be transported by **2**. The transport is slower than with ADP and ATP; a strong nonlinearity in the increase of nucleotide concentration in the receiving phase with time prevented accurate rate assessments.

The new carrier **2** shows significant improvements over the earlier derivative **1b**. As already mentioned above, the linear-chain derivative **1b**, in contrast to the branched-chain derivative **2**, possesses only limited chloroform solubility and transport occurs across cloudy solutions. ATP transport mediated by **1b** is much slower than in the presence of **2**, and CTP transport cannot be effected at all with **1b** (Table I).

To evaluate the efficiency of the extraction from the source phase into the liquid membrane and the release back into the receiving phase in the carrier-mediated transport, we performed a series of extraction experiments. The extractabilities of the nucleotides from aqueous layers into chloroform layers containing **2–4** are shown in Table II. Two different nucleotide concentrations in the aqueous phase were chosen to explore possible concentration dependencies of the extractability. With the bis(ammonium bromide) **2**, all nucleotides are extracted efficiently under both conditions. The extractability increases with higher initial nucleotide concentration in the aqueous phase. As already discussed above, the extractability of nucleotides by the tetrakis(ammonium bromide) **4** strongly decreases with increasing nucleotide concentration.

The back-extraction of the bound nucleotide 5′-triphosphates from chloroform into aqueous sodium bromide solution (0.20 M, pH = 7.0) was shown to be very efficient. Fast and essentially quantitative release of ATP and ddTTP bound to all three carriers **2–4** was observed in single back-extractions.

(11) For experiments in similar U-tube type cells, see: Diederich, F.; Dick, K. *J. Am. Chem. Soc.* **1984**, *106*, 8024–8036.

(12) Zubay, G. *Biochemistry*; Addison-Wesley: London, 1983; p 665.

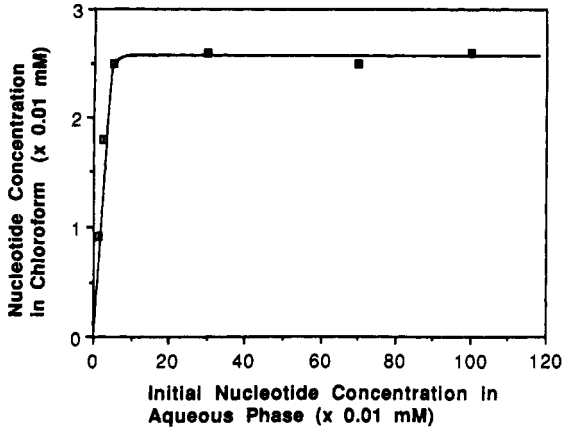


Figure 3. Extraction of ATP from aqueous solutions containing various nucleotide concentrations with a chloroform solution of **2** (5.0×10^{-5} M).

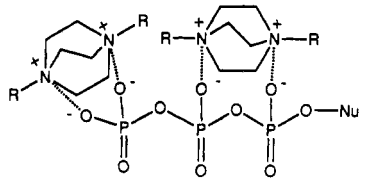


Figure 4. Geometry of the 2:1 carrier (**2**)–nucleotide complex in the chloroform phase supported by the extraction data of Figure 3.

There exist some qualitative correlations between the extraction and the transport data. The bis(ammonium bromide) **2** both transports and extracts nucleotide triphosphates faster than the other carriers. However, a rigid correlation does not exist. The triammonium derivative **3** extracts ATP better than AZTTP but the latter nucleotide is transported much faster (Table I). This difference results from the fact that the extraction is performed under thermodynamic control, whereas transport is kinetically controlled. Although **3** extracts ATP and CTP efficiently, it takes about 30 min to reach equilibrium and clear layer separation. On the other hand, the extraction of AZTTP by **3** reaches equilibrium within less than 5 min. These results probably indicate that the complexes formed by ATP and CTP are not easily soluble in chloroform, while the complex formed in the case of AZTTP is easily soluble in chloroform.

A concentration-dependent extraction study provided valuable information on the mode of interaction between the diammonium carrier **2** and nucleotide 5′-triphosphates. When aqueous solutions containing varying amounts of ATP (1.25 to 100×10^{-5} M) were shaken together with a 5.0×10^{-5} molar chloroform solution of **2**, the maximum amount of ATP that could be extracted in the organic phase was just one half of the receptor concentration (Figure 3). This result strongly supports the formation of a 2:1 carrier–nucleotide complex by **2** (Figure 4) rather than the formation of a 1:1 association which was suggested by Tabushi for the interaction between **1b** and ATP.[8b] Presumably, the latter carrier also prefers the 2:1 ion-pairing stoichiometry shown in Figure 4. However, we were unable to demonstrate this in a similar series of concentration-dependent extractions with **1b**, since the poor solubility of this carrier in chloroform leads to a consistent formation of a boundary layer between aqueous and organic phase.

The observed 2:1 stoichiometry of the interaction between the carrier **2** and ATP suggests that four ammonium centers are needed to transport nucleotide triphosphates efficiently. According to this model, tetracationic bis(DABCO) derivatives like **4** should transport nucleotides efficiently. However, the experiments with **4** did not show any significant nucleotide transport. A reason for this failure may possibly be an incorrect design of the linker between the two DABCO units in **4**, which could prevent a proper alignment between the four cationic centers in the carrier and the anionic centers of the triphosphates. However, the experiments above strongly suggest that the tendency of tetracationic **4** to form highly water-soluble associations, which prefer distributing into the aqueous phases rather than into the liquid membrane, is the major disadvantage with this system.

Conclusion

The transport of nucleotide 5′-triphosphates across a liquid organic membrane mediated by DABCO-derived carriers bearing two (**2**), three (**3**), and four (**4**) quaternary ammonium centers was studied. With their branched aliphatic chains, these compounds show excellent solubility in chloroform, the solvent chosen as liquid membrane. The bis(ammonium bromide) **2** was found to be the most efficient carrier. Concentration-dependent extraction studies showed that **2** undergoes formation of a 2:1 carrier–nucleotide complex in the organic chloroform phase. This stoichiometry differs from the 1:1 stoichiometry previously suggested by Tabushi et al. for the association between ATP and a bisquaternary DABCO derivative (**1b**) which, as a result of a more unfavorable balance of hydrophilicity to hydrophobicity, is far inferior in its transport properties to the new carrier **2**. The tricationic compound **3** is generally inferior to **2** in its carrier properties but shows an unexpectedly high selectivity for transporting ddTTP. The tetracationic bis(DABCO) derivative **4** shows poor carrier properties since it forms highly water-soluble associations which prefer distributing into the aqueous phase rather than into the liquid membrane.

This study demonstrates that charge compensation by highly lipophilic cationic carriers ensures efficient transport of nucleotide 5′-triphosphates across liquid membranes. The new systems described in this paper are now tested for nucleotide transport across liposomes and cell membranes. Hydrogen-bonding shapes for specific base recognition are now being introduced in receptors like **2** in order to achieve the selective transport of AIDS-targeted nucleotide 5′-triphosphates such as AZTTP.

Experimental Section

General. Commercial chemicals were used directly unless otherwise noted. ATP and CTP were purchased from Sigma and ddTTP from Boehringer Mannheim. AZTTP was purchased from Moravek Biochemicals, Inc. Mass spectra were determined by fast atom bombardment (FAB) in *m*-nitrobenzyl alcohol as the matrix. The *m/z* values listed below are followed by relative intensities given in parentheses. Elemental analyses were obtained from Desert Analytics, Tucson, AZ. The general reaction workup included separation of the product-containing organic phase from aqueous layers and evaporation of the solvent in vacuo.

1-Octadecyl-4-aza-1-azoniabicyclo[2.2.2]octane Iodide (6). A solution of 1,4-diazabicyclo[2.2.2]octane (DABCO) (4.20 g, 0.038 mol) and stearyl iodide (11.90 g, 0.031 mol) in acetone (70 mL) was stirred at room temperature overnight. The precipitated product was collected and washed with a small amount of acetone to give **6** (14.0 g, 91%): mp 138–142 °C; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 0.67 (t, J = 6.1 Hz, 3 H), 0.9–1.2 (m, 30 H), 1.60 (m, 2 H), 3.07 (t, J = 6.8 Hz, 6 H), 3.24 (t, J = 8.1 Hz, 2 H), 3.46 (t, J = 6.8 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ 13.49, 21.61, 22.00, 25.76, 28.55, 28.69, 28.81, 28.83, 28.98 (2×), 29.01, 29.04 (5×), 31.24, 44.73, 51.83, 64.05; MS 365 [$(M - I)^+$, 100]; HRMS *m/z* [$(M - I)^+$, $C_{24}H_{49}N_2$] calcd 365.3895, obsd 365.3912.

1,1'-(1,3-Propanediyl)bis[4-octadecyl-1,4-diazoniabicyclo-[2.2.2]octane] Tetraiodide (5). The mono(ammonium iodide) **6** (122 mg, 0.25 mmol) and 1,3-diiodopropane (7.1 μL, 0.06 mmol) in dimethylformamide (DMF, 1 mL) were stirred at 80 °C for 2 days. The solvent was removed and the residue stirred with a small amount of CH_3OH/CH_2Cl_2 (1:5). The insoluble product was collected and washed with the solvent mixture to give **5** (60 mg, 40%): mp 220 °C dec; ^1H NMR (200 MHz, Me_2SO-d_6) δ 0.86 (m, 6 H), 1.24 (m, 60 H), 1.71 (m, 4 H), 2.32 (m, 2 H), 3.55 (m, 8 H), 3.92 (s, 24 H); MS 1153 $(M - I)^+$. Anal. Calcd for $C_{51}H_{104}I_4N_4 \cdot H_2O$ (1299.1): C, 47.15; H, 8.22; N, 4.31. Found: C, 47.21; H, 8.03; N, 4.28.

1,1'-(1,3-Propanediyl)bis[4-aza-1-azoniabicyclo[2.2.2]octane] Diiodide (7). A solution of 1,3-diiodopropane (2.07 mL, 0.018 mol) and DABCO (4.5 g, 0.040 mol) in acetone (70 mL) was stirred for 14 h at 20 °C. The collected precipitate was washed with a small amount of acetone to give **7** (9.1 g, 97%): mp 230 °C dec; ^1H NMR (360 MHz, Me_2SO-d_6) δ 2.25 (m, 2 H), 3.04 (t, J = 7.2 Hz, 12 H), 3.28 (t, J = 8.1 Hz, 4 H), 3.36 (t, J = 7.2 Hz, 12 H); MS 393 $(M - I)^+$. Anal. Calcd for $C_{15}H_{30}I_2N_4$ (520.2): C, 34.63; H, 5.81; N, 10.77. Found: C, 34.48; H, 5.76; N, 10.54.

1-Iodo-*N*,*N*-dioctylacetamide (8). A total of 0.43 mL (5.0 mmol) of α-bromoacetyl bromide was added slowly to a mixture of dioctylamine (1.5 mL, 5.0 mmol) and triethylamine (0.70 mL, 5.0 mmol) in CH_2Cl_2 (20 mL), and the resulting solution was stirred for 15 min at 20 °C. Addition CH_2Cl_2 (100 mL) was added, and the mixture was washed with 1 N HCl, 1 N Na_2CO_3, and water. The residue, obtained after workup, was dissolved in acetone (30 mL), and sodium iodide (3.0 g, 20.0 mmol) was added. After being stirred at 50 °C for 3 h, the mixture was diluted with CH_2Cl_2 and washed with aqueous $NaHSO_3$ and water. Flash chromatography [SiO_2, ethyl acetate/hexanes (1:5)] after workup gave **8** (1.8 g, 88%) as an oil: IR (film) ν(C=O) 1650 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 0.7–0.85 (m, 6 H), 1.1–1.3 (m, 20 H), 1.44 (m, 2 H), 1.54 (m, 2 H), 3.14 (t, J = 7.8 Hz, 2 H), 3.20 (t, J = 7.6 Hz, 2 H), 3.63 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ −2.93, 13.88, 13.90, 22.41, 22.42, 26.59, 26.68, 26.80, 28.89, 28.97, 29.00, 29.06, 29.15, 31.53, 31.58, 45.97, 49.21, 167.21; MS 410 $[(M + H)^+, 100]$; HRMS m/z $[(M + H)^+, C_{18}H_{37}INO]$ calcd 410.1922, obsd 410.1902.

1,1'-(1,3-Propanediyl)bis[4-[2-(dioctylamino)-2-oxoethyl]-1,4-diazoniabicyclo[2.2.2]octane] Tetrabromide (4). A mixture of **8** (590 mg, 1.44 mmol) and **7** (250 mg, 0.48 mmol) in DMF (3 mL) was stirred at 80 °C for 14 h. The solvent was removed in vacuo and the residue purified by chromatography [SiO_2, CH_2Cl_2/CH_3OH (95:5), then 1% NaI in CH_2Cl_2/CH_3OH (6:1)]. The product was dissolved in chloroform, and the solution was washed with water. Workup gave the tetrakis(ammonium iodide) which was converted into the colorless oil **4** (469 mg, 85%) via ion-exchange chromatography (Br^-); IR (film) ν(C=O) 1650 cm^{-1}; ^1H NMR [360 MHz, $CDCl_3/CD_3OD$ (9:1)] δ 0.88 (t, J = 6.5 Hz, 12 H), 1.2–1.4 (m, 40 H), 1.53 (m, 4 H), 1.64 (m, 4 H), 2.78 (m, 2 H), 3.27 (m, 8 H), 4.06 (m, 4 H), 4.43 (m, 12 H), 4.64 (m, 12 H), 4.97 (s, 4 H); ^{13}C NMR [360 MHz, $CDCl_3/CD_3OD$ (9:1)] δ 13.91 (2×), 17.39, 22.48 (2×), 26.69, 26.86, 27.28, 28.52, 29.08 (4×), 31.65 (2×), 46.62, 47.56, 51.60, 51.68, 60.67, 61.49, 161.4; MS 1069 $(M - Br)^+$; HRMS m/z $[(M - Br)^+, C_{51}H_{102}{}^{79}Br_3N_6O_2]$ calcd 1067.5616, obsd 1067.5660.

***N*-(3-Iodopropyl)-*N*,*N*-dioctyl-1-octanaminium Iodide (9).** A mixture of trioctylamine (1.00 mL, 2.29 mmol) and 1,3-diiodopropane (4.0 mL, 34.4 mmol) in ethanol (8 mL) was stirred at 70 °C for 14 h. The solvent was removed and the residue purified by flash chromatography [EtOAc/hexanes (3:7–1:1), then pure EtOAc]. The product was dissolved in CH_2Cl_2 and washed with 0.5 N HI and 0.5 N NaI. Workup yielded **9** (0.83 g, 56%) as a colorless oil which crystallized from EtOAc: mp. 90–92 °C; ^1H NMR (360 MHz, $CDCl_3$) δ 0.88 (t, J = 6.7 Hz, 9 H), 1.2–1.5 (m, 30 H), 1.75 (m, 6 H), 2.34 (m, 2 H), 3.35–3.45 (m, 8 H), 3.62 (t, J = 8.0 Hz, 2 H); MS 522 $[(M - I)^+, 100]$. Anal. Calcd for $C_{27}H_{57}NI_2$ (649.6): C, 49.93; H, 8.84; N, 2.16. Found: C, 49.84; H, 8.80; N, 2.16.

1-[3-(Trioctylammonio)propyl]-4-aza-1-azoniabicyclo-[2.2.2]octane Diiodide (10). A mixture of **9** (200 mg, 0.307 mmol) and DABCO (103 mg, 0.921 mmol) in acetone (2 mL) was stirred at 20 °C for 14 h and then added dropwise under vigorous stirring into 20 mL of anhydrous Et_2O. The precipitate was redissolved in acetone (2 mL) and again added to Et_2O as described. Repeating this process one more time gave **10** (209 mg, 89% after drying at 10^{-3} Torr) as a very hygroscopic solid: ^1H NMR (360 MHz, $CDCl_3$) δ 0.88 (t, J = 6.5 Hz, 9 H), 1.2–1.5 (m, 30 H), 1.77 (m, 6 H), 2.62 (m, 2 H), 3.25 (m, 6 H), 3.43 (m, 6 H), 3.62 (m, 2 H), 3.84 (m, 6 H), 4.10 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.93, 18.15, 22.29, 22.44, 26.22, 28.90 (2×), 31.45, 45.14, 52.59, 55.73, 59.50, 60.18; MS 634 $(M - I)^+$; HRMS m/z $[(M - I)^+, C_{33}H_{69}IN_3]$ calcd 634.4538, obsd 634.4544.

1-[2-(Dioctylamino)-2-oxoethyl]-4-[3-(trioctylammonio)-propyl]-1,4-diazoniabicyclo[2.2.2]octane Tribromide (3). A mixture of **10** (100 mg, 0.13 mmol) and **8** (107 mg, 0.26 mmol) in DMF (1 mL) was stirred at 70 °C for 2 h. The solvent was removed and the residue purified by chromatography [SiO_2; EtOAc/hexanes (1:1), then EtOAc/CH_3CN (1:1), then CH_2Cl_2/CH_3OH (9:1)]. Further workup exactly as described for **9** gave the tris(ammonium iodide) which was transformed into the very hygroscopic solid **3** (125 mg, 92%) via ion-exchange chromatography (Br^-): IR (film) ν(C=O) 1651 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 0.85–0.9 (m, 15 H), 1.2–1.5 (m, 50 H), 1.5 (m, 2 H), 1.63 (m, 2 H), 1.75 (m, 6 H), 2.71 (m, 2 H), 3.27 (t, J = 7.6 Hz, 4 H), 3.36 (t, J = 8.1 Hz, 6 H), 3.66 (m, 2 H), 4.46 (m, 2 H), 4.6–4.75 (m, 12 H), 5.01 (s, 2 H); ^{13}C NMR δ 13.92 (3×), 17.79, 22.07, 22.46 (3×), 26.20, 26.69, 26.88, 27.35, 28.72, 28.93 (3×), 29.05 (2×), 31.49, 31.63, 31.67, 46.71, 47.64, 51.13, 51.87, 55.59, 59.35, 60.25, 61.63, 161.02; MS 949 $(M - Br)^+$; HRMS m/z $[(M - Br)^+, C_{51}H_{105}{}^{79}Br^{81}BrN_4O]$ calcd 949.6629, obsd 949.6653.

1,4-Bis[2-(dioctylamino)-2-oxoethyl]-1,4-diazoniabicyclo-[2.2.2]octane Dibromide (2). A mixture of **8** (1.50 g, 3.66 mmol) and DABCO (0.14 g, 1.2 mmol) in DMF (3 mL) was stirred at 80 °C for 14 h. The solvent was removed and the residue purified by chromatography [SiO_2; EtOAc/hexanes (3:7), then CH_2Cl_2/CH_3OH (9:1)]. Further workup exactly as described for **3** gave the bis(ammonium bromide) **2** (600 mg, 59%): mp 158–159 °C; IR (film) ν(C=O) 1644 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.88 (t, J = 6.6 Hz, 12 H), 1.27 (m, 40 H), 1.51 (m, 4 H), 1.70 (m, 4 H), 3.15–3.4 (m, 8 H), 4.90 (s, 12 H), 5.54 (s, 4 H); MS 756 $(M - Br)^+$. Anal. Calcd for $C_{42}H_{84}Br_2N_4O_2$ (837.0): C, 60.27; H, 10.12; N, 6.69. Found: C, 60.61; H, 10.06; N, 6.81.

Transport Study. All experiments were conducted at T = 293–295 K in a U-tube-type cell of 1.34-cm diameter and 4.70-cm distance from center to center between the two legs containing source and receiving phases. The cell was held in exactly the same position to a magnetic stirring motor in all experiments. A total of 12 mL of receptor in chloroform (1.0×10^{-5} M) was located onto the bottom of the cell as phase II. Atop phase II in one arm was placed a total of 6 mL of nucleotide (1.0×10^{-4} M) in water (pH 7.0) as the source phase I. In the other arm, 6.0 mL of 0.20 M NaBr (pH 7.0) in water was placed as the receiving phase III. Phase II was stirred at a constant rate (740 rpm) in all experiments. At that rate, clean interfaces were obtained between the three clear phases. The delivery of the nucleotide in the receiving phase was monitored by UV absorption spectroscopy. The rates of transport were calculated from the initial linear plots of the nucleotide concentration as a function of time. The following UV absorptions of the nucleotide bases in aqueous solution were evaluated: ATP λ_{max} 260 nm (ϵ 1.4×10^4 L mol^{-1} cm^{-1}); CTP λ_{max} 273 nm (ϵ 8.1×10^3 L mol^{-1} cm^{-1}); ddTTP and AZTTP λ_{max} 270 nm (ϵ 8.6×10^3 L mol^{-1} cm^{-1}). The rates reported in Table I are averages obtained in triplicate runs.

Extraction Studies. (i) Extraction of nucleotides from an aqueous solution into a chloroform layer: A solution of the carrier in chloroform (6.0 mL, 1.0×10^{-4} M) was shaken for 5 min with an equal volume of an aqueous solution (pH 7.0) of the nucleotide (condition A, nucleotide concentration 5.0×10^{-4} M; condition B, nucleotide concentration 5.0×10^{-5} M). The pH of the aqueous phase was readjusted to 7.0 with 0.01 N NaOH or HCl. The flask was shaken again for 5 min. The pH was again adjusted to 7.0. The process was repeated several times until the concentration of the nucleotides in the chloroform layer, monitored by UV spectroscopy, did not change. (ii) Extraction of the bound nucleotide back to aqueous NaBr solution: First, 8 mL of an aqueous solution (pH 7.0) containing the nucleotide (5.0×10^{-5} M) was extracted with an equal volume of chloroform containing the carrier (1.0×10^{-4} M) to generate a solution of nucleotide–receptor complex in chloroform. Subsequently, the concentration of nucleotide bound in the chloroform phase was measured. Then, 6

mL of this chloroform solution was extracted during 5 min with an equal volume of aqueous 0.2 M NaBr (pH 7.0). The pH of the aqueous phase was readjusted to 7.0. The flask was shaken again for 5 more min. At the end of the back-extraction, the nucleotide concentration in chloroform was measured again by UV spectroscopy to calculate the extraction yield. UV absorption of the nucleotide bases in chloroform: ATP λ_{max} 260 nm (ϵ 1.3 $\times 10^4$ L mol^{-1} cm^{-1}); CTP λ_{max} 275 nm (ϵ 6.6 $\times 10^3$ L mol^{-1} cm^{-1}); ddTTP and AZTTP λ_{max} 268 nm (ϵ 8.4 $\times 10^3$ L mol^{-1} cm^{-1}). The extraction yields in Table II are averages of triplicate runs.

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Trichodiene Synthase. Synergistic Inhibition by Inorganic Pyrophosphate and Aza Analogs of the Bisabolyl Cation

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A series of aza analogs of the bisabolyl and α-terpinyl cations were tested as inhibitors of the sesquiterpene cyclase, trichodiene synthase. Both (*R*)- and (*S*)-**16** and (*R*)- and (*S*)-**13** as well as trimethylamine were only weak inhibitors when incubated alone. In the presence of inorganic pyrophosphate, itself a known competitive inhibitor of trichodiene synthase, all five amines showed strong cooperative competitive inhibition with an enhancement factor estimated to be 10–40. The apparent induced inhibition constant αK_J decreased in going from trimethylamine to the monoterpene analogs **13** and was strongest for the sesquiterpene analogs **16**, indicating that both electrostatic and hydrophobic interactions are important in the binding of each intermediate analog. The cyclase showed little discrimination, however, between the individual enantiomers of each inhibitor.

Trichodiene synthase catalyzes the cyclization of farnesyl diphosphate (**1**) (FPP) to trichodiene (**4**),[1] the sesquiterpene hydrocarbon precursor of the trichothecenes, a large family of antibiotic metabolites produced by several genera of phytopathogenic fungi as well as the higher plant genus *Baccharis*.[2] The presence in contaminated grain of trichothecenes, which are potent inhibitors of protein synthesis, has been associated with serious incidents of mycotoxicoses. Trichodiene synthase has been isolated from a variety of fungal sources including *Trichothecium roseum*,[1,3] *Gibberella pulicaris*,[4] and *Fusarium sporotrichioides*.[5] The *F. sporotrichioides* cyclase has been purified to homogeneity and shown to be a homodimer, M_r 90 000. Cyclization of the acyclic substrate *trans,trans*-FPP requires no cofactors other than the divalent metal cation Mg^{2+}, in common with the majority of known terpene cyclases. The calculated k_{cat} of 0.15 s^{-1}, while modest, is typical of such cyclases, and well in excess of the competing Mg^{2+}-catalyzed solvolysis of the allylic substrate. The trichodiene synthase genes of both *F. sporotrichioides* and *G. pulicaris* have been cloned and sequenced, showing 89% homology at the nucleic acid level.[6,7] After deletion of a 60-nt intron, the trichodiene synthase gene has been expressed in *Escherichia coli* and the recombinant cyclase appears to be identical to the native enzyme in all respects.[8]

Extensive mechanistic and stereochemical studies have supported a cyclization mechanism, illustrated in Scheme I, in which *trans,trans*-FPP is initially isomerized to the corresponding tertiary allylic isomer, nerolidyl diphosphate (**2**) (NPP), which by rotation about the newly generated 2,3-single bond can adopt a conformation capable of cyclization to the bisabolyl cation (**3**).[1,9] Further cyclization, followed by a succession of well-documented hydride and methyl migrations, can then yield, after deprotonation, trichodiene. Although the isomerization and cyclization components of this mechanism are clearly distinct reactions, these transformations are believed to take place by

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