Carriers for Nucleotide 5'-Triphosphates. 2. Liquid Membrane and Liposome Transport

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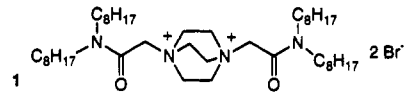
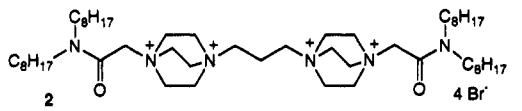
We describe a series of new cationic carriers for the transport of nucleotide 5'-triphosphates across liquid organic membranes. The tetracationic compound **5**, with two diquaternary 1,4-diaza[2.2.2]-bicyclooctane (DABCO) units, forms carrier–nucleotide complexes of 1:1 stoichiometry in dichloromethane and strongly accelerates nucleotide transport across this solvent. As a result of a favorable balance of hydrophobicity and hydrophilicity, complexes of **5** do not leak out of the organic phase into the aqueous source or receiving phases. Liposome transport studies demonstrate that caution is strongly advised when extrapolating from liquid membrane studies to the transport across liposomes or cell membranes. The DABCO-based cationic carriers do not mediate specific nucleotide transport across the liposome walls but rather act as detergents to break the liposomal structure.

Introduction

2',3'-Dideoxynucleotide 5'-triphosphates designed to act as potent chain-terminating inhibitors of HIV reverse transcriptase[1,2] and antisense oligonucleotides[3] designed to hybridize to specific viral mRNA sequences are prime targets in AIDS therapy. However, these highly charged nucleotides cannot penetrate across cell membranes,[4] and therefore, the development of molecular carriers to enhance cellular uptake has become the subject of increasing current research efforts.[5-7] We have recently shown that compound **1** forms complexes of 2:1 stoichiometry with nucleotide triphosphates, e.g. adenosine 5'-triphosphate (ATP), 3'-azido-2'-deoxythymidine (AZTTP), cytidine 5'-triphosphate (CTP), and 2',3'-dideoxythymidine 5'-triphosphate (ddTTP), and transports these anionic substrates efficiently across chloroform as liquid organic membrane.[8] In contrast, compound **2** showed poor carrier properties since it presumably forms highly water-soluble associations which prefer distributing into the aqueous phase or into boundary layers rather than into the liquid membrane.





Although the carrier performance of **1** is acceptable in terms of transport rate, nucleotide selectivity is modest. For the selective transport of a nucleotide triphosphate such as AZTTP, it is desirable to attach to the carrier hydrogen-bonding shapes for specific base recognition. Such modification should be more easily achieved with carriers that form 1:1 complexes rather than with compounds like **1** which form associations of 2:1 stoichiometry.[9] Therefore, we targeted the development of new transport

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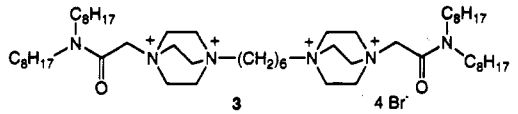
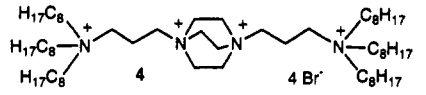
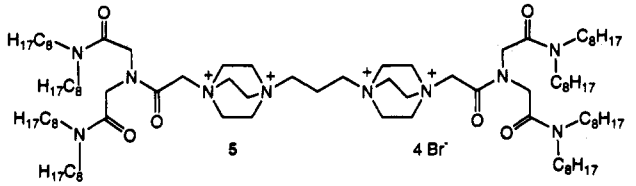
agents that associate with nucleotide triphosphates under formation of complexes with 1:1 stoichiometry. Here, we report on the preparation and carrier performance of the three tetracationic bis(DABCO) derivative 3–5. In addition to liquid membrane transport, the carriers are evaluated in studies of liposome transport, a better model system for cellular uptake.



3 4 Br⁻



4 4 Br⁻

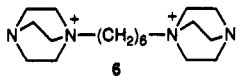


5 4 Br⁻

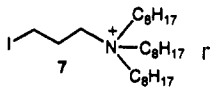
Results and Discussion

Design and Synthesis of Carriers 3–5. CPK molecular model examinations indicated that the size of the C_3-linker between the two DABCO units in 2 was possibly too short to allow for a proper alignment between the four cationic centers of the carrier and the anionic centers of the triphosphates. To test whether a poor ion pairing geometry was at the origin of the failure of 2 to act as a carrier, we prepared compound 3 with a C_6-chain between the DABCO units. The reaction between two DABCO units and 1 equiv of 1,6-diiodohexane afforded the diammonium salt 6 which was diquaternized with 1-iodo-*N*,*N*-dioctylacetamide to give, after ion exchange, 3.

Compound 4 introduces a new geometric array of four quaternary centers and, to increase the carrier lipophilicity, contains six octyl chains compared to four in the derivatives 1–3. The increase in lipophilicity should ensure that the carrier and its nucleotide complexes partition during transport experiments into the liquid organic membrane and do not leak out into the aqueous phases. Compound 4 is prepared in one step, followed by ion exchange, from DABCO and the (iodopropyl)ammonium salt 7.



6 2 I⁻



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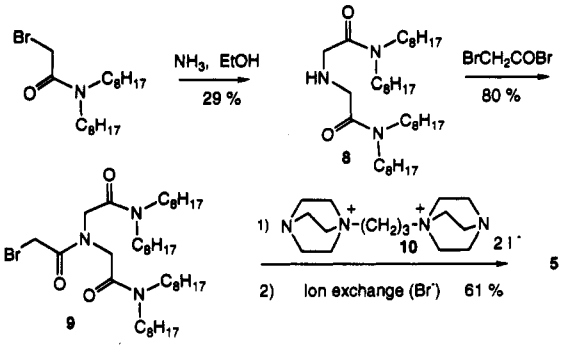
A further enhancement in lipophilicity is achieved in carrier 5 which contains a total of eight octyl chains. Its synthesis is shown in Scheme I. All three new carriers are readily soluble in CH_2Cl_2 and are highly insoluble in water.

Liquid Membrane Transport Studies. In analogy to the determination of the 2:1 stoichiometry for the 1·ATP complex,[8] the stoichiometry of the new carrier complexes was analyzed in concentration-dependent extraction experiments. Figure 1 shows the amounts of ATP extracted into dichloromethane by carrier 5 as a function of the initial nucleotide concentration in the aqueous phase. The titration curve indicates that 5 forms a very stable 1:1 complex with ATP in CH_2Cl_2. In this experiment, the amount of nucleotide extracted into the organic phase was directly determined by electron absorption spectroscopy.

Scheme I



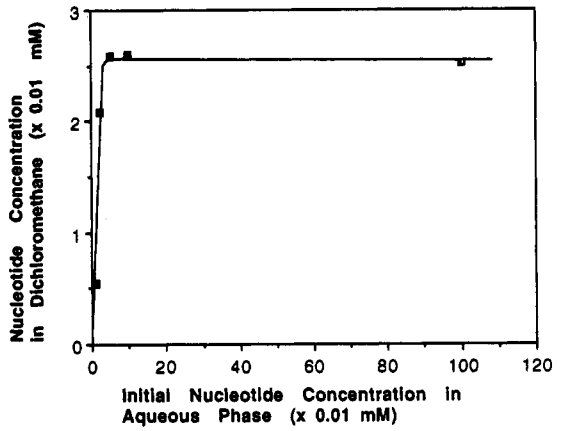


Figure 1. Extraction of ATP from aqueous solutions containing various nucleotide concentrations with a CH_2Cl_2 solution of 5 (2.5×10^{-5} M).

The binding behavior of the new carriers 3 and 4 as well as of previously prepared 2 could not be studied in the same titration experiment since their nucleotide complexes are not very soluble in CH_2Cl_2. Instead, upon extraction or transport, a new stable and well separated boundary layer forms at the water–dichloromethane interface. However, the spectroscopic determination of the amount of nucleotide that disappeared from the aqueous solution in extraction studies provided strong evidence that these three carriers also form 1:1 complexes with ATP. In these experiments, an aqueous solution of ATP (6.0 mL, 1.0×10^{-4} M, pH = 7.0) was shaken with an equal volume of dichloromethane containing the carrier ($c = 5.0 \times 10^{-5}$ M). Under these conditions, compounds 2–4 extract, predominantly into a boundary layer, exactly 1 equiv of ATP (5.0×10^{-5} M) from the aqueous phase. Increasing the initial ATP concentration to 2.0×10^{-4} M does not change the amount of ATP which disappears from the aqueous phase. The stoichiometrical assessment by measuring the disappearance of the nucleotide from the aqueous solution represents a valid method. This is demonstrated by the findings that such experiments give the same stoichiometry for the complexes formed by 1 (2:1 carrier/nucleotide) and 5 (1:1 carrier/nucleotide) than full titrations such as that shown in Figure 1.

(9) A DABCO derivative similar to 1 had been claimed by Tabushi et al. to form 1:1 complexes with ATP (refs 6b–d). Our studies (ref 8) clearly showed that the stoichiometry of the carrier–nucleotide complex is 2:1.

Table I. Rates of Nucleotide Transport across CH_2Cl_2 in a U-Tube-Type Cell[a,b]

carrier	ATP	rate (10^{-9} mol cm^{-2} h^{-1}) CTP	ddTTP	AZTTP
5	0.8	0.25	1.0	0.35
1	8.1	7.2	5.4	1.6
none	<0.005	<0.008	<0.008	<0.008

[a] U-tube cell diemsnions: 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 CH_2Cl_2, 12 mL. Receiving phase: Na_3PO_4 (0.20 M) in water, pH 7.0, 6.0 mL. [b] Reproducibility: ±15%.

Although compounds 2–5 all form 1:1 complexes with ATP, the solubility of these species in the organic phase obviously is very different. In the case of carriers 2–4, only a small amount of ATP (<10% of the material disappeared from the aqueous phase) is found in the CH_2Cl_2 phase, the rest being partitioned into the boundary layer formed between the aqueous and organic phase. In contrast, no new boundary layer is observed in the extractions with 5, and the ATP which disappeared from the aqueous solution is found quantitatively in the CH_2Cl_2 layer. All these observations indicate that the complexes formed by 2–4 are insoluble in CH_2Cl_2 and that only 5, with its eight *n*-octyl groups, reaches the delicate balance of hydrophobicity to hydrophilicity required for the complete partitioning and solubilization of a quadruply ion-paired nucleotide complex into the dichloromethane layer.

The carrier behavior of compounds 1–5 was studied in transport experiments performed in a standard U-tube cell.[8] The rate of delivery of substrates into the aqueous receiving phase was monitored by UV spectroscopy. The tetraquaternary derivatives 2, 3, and 4 all failed to transport nucleotide triphosphates most likely because of the poor complex solubility discussed above. On the other hand, the new carrier 5 transported all the nucleotide triphosphates tested. Experimental conditions were similar to those described earlier[8] except for two modifications (Table I). (i) The liquid organic membrane is dichloromethane instead of chloroform. (ii) In the previous transport experiments, sodium bromide was used in the receiving phase to help dissociate the carrier–nucleotide complex at the membrane interface.[8] Now, we switched to a sodium phosphate solution as the receiving phase since we found that, under this more biorelevant condition, the release of the nucleotide from the complex is more effective. For example, upon switching from 0.2 M NaBr to 0.2 M Na_3PO_4 as receiving phase, the rate of ATP transport by carrier 5 was increased from 0.45×10^{-9} to 0.8×10^{-9} mol cm^{-2} h^{-1}. In extraction experiments, ATP complexed to 5 is rapidly and quantitatively released from the organic phase into 0.2 M Na_3PO_4 (pH 7.0). Table I shows the rates of transport mediated by 5.

Carrier 5 represents the first efficient nucleotide 5′-triphosphate transport agent which forms complexes with 1:1 carrier–nucleotide stoichiometry. The transport rates mediated by 5 are approximately 1 magnitude slower than those in the presence of 1 which undergoes 2:1 carrier–nucleotide complexation.

Liposome Transport. To evaluate the carrier potential of the quaternary DABCO derivatives under conditions closer to those for cellular uptake, nucleotide 5′-triphosphate transport across liposomes was investigated. The liposomes for this study were prepared by sonication of lipid extract from *E. coli*.[10] To monitor transport, radiolabeled ATP was introduced into the interior of the liposomes. Optimal loading was achieved by adding the nucleotide to the thawed liposomes, vigorously mixing, and subsequently freezing, thawing, and sonicating.[11] For example, when 10 μL of [2,8-^{3}H]ATP (1 mCi/1 mL) was incorporated this way into 40 μL of liposome suspension, the scintillation reading of 1 μL of the resulting mixture was 7738 ± 2. In comparison, when 10 μL of [2,8-^{3}H]ATP was added to 40 μL of prethawed and presonicated liposome suspension, the scintillation reading of 1 μL of this suspension was only 278. In each case, nonentrapped solute was carefully removed prior to scintillation counting by passing the mixture through a Sephadex spin column.

Table II. Radioactivity of Liposomes after Incubation with Carriers[a]

carrier	[2,8-^{3}H]ATP	[1-^{14}C]lactose	L-[5-^{3}H]proline
none	10444 (2.0)[b]	25115 (2.0)	2384 (2.9)
1	5072 (2.0)	10207 (2.0)	341 (7.7)
2	3104 (2.5)	6500 (2.0)	390 (7.2)
5	1399 (3.8)	11232 (2.0)	115 (13)
Triton-X-100	9335 (2.0)	23155 (2.0)	2019 (3.2)

[a] Incubation time 30 min; carrier concentration 5.0×10^{-5} M. [b] Percentage error (±x%) of scintillation counting.

After 30 min of incubation by adding the desired carrier (5.0×10^{-5} M) to a suspension of the loaded liposomes in phosphate buffer (25 mM, pH 7.0), the residual radioactivity of the liposome was measured and the decrease in scintillation counts taken as an assay for the carrier-mediated transport. Two kinds of control experiments were carried out. One used liposomes containing [1-^{14}C]-lactose or L-[5-^{3}H]proline to check for nucleotide transport specificity. Another control used Triton-X-100 to check for any detergent effect of our carriers.[12] The results obtained with compounds 1, 2, and 5 are listed in Table II.

Table II shows that [2,8-^{3}H]ATP leakage from the liposomes can be induced upon addition of all three carriers. However, under the same conditions, both [1-^{14}C]-lactose and L-[5-^{3}H]proline also leak out of the liposomes. It was also observed that the rate of leakage of [2,8-^{3}H]-ATP was not time dependent. Increasing the incubation time by the carrier from 30 min to 3 h did not change the amount of [2,8-^{3}H]ATP that leaked out of the liposomes. The leakage is strongly dependent on the carrier concentration. For example, upon lowering the carrier concentration from 5.0×10^{-5} to 3.0×10^{-6} M, no leakage of [2,8-^{3}H]ATP was observed, even after overnight incubation. All these results indicate that, at higher concentration, the quaternary DABCO derivatives 1, 2, and 5 induce [2,8-^{3}H]ATP leakage not by acting as specific nucleotide triphosphate carriers but rather by acting as detergents which break the liposomal structure.[13]

The detergent Triton X-100 is well-known to induce liposome leakage by breaking its structure and is commonly used for this purpose. The data in Table II clearly show that our compounds are much more effective than Triton-

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X-100 in inducing liposomal leakage, a result which might find some interesting application.

As pointed out earlier, the solubility of the carrier and its complexes in the organic membrane is crucial to efficient transport. The interior of the liposomal bilayers consists of hydrocarbon chains, and our carriers and their nucleotide complexes are most likely not soluble in such greasy environment. This could be the reason why our carriers fail to transport [2,8-^{3}H]ATP in the lower concentration ranges where they do not act as detergents.

Conclusions

With the tetraquaternary bis(DABCO) derivative **5**, we have prepared a molecular carrier which forms complexes of 1:1 stoichiometry with a variety of nucleotide 5′-triphosphates in dichloromethane. These complexes do not leak out of a CH_2Cl_2 liquid membrane and, hence, efficient transport of nucleotide 5′-triphosphates is observed in U-type cell experiments. However, we find that our carriers, including **5**, do not mediate the specific transport of nucleotide 5′-triphosphates across liposomes but, above a certain concentration, rather act as detergents breaking the liposomal structure and leading to nonspecific leakage from the liposomal interior. Our experiments strongly advise caution when extrapolating from carrier efficiencies in liquid organic membrane transport experiments to liposome or cellular membrane transport. In the future design of molecular carriers, it will be important to introduce hydrogen-bonding shapes for nucleotide base specificity,[4a] as well as to eliminate their detergent properties. On the other hand, the remarkable liposome breaking properties of **1**, **2**, and **5**, which are superior to those of commonly used Triton-X-100, might well find some useful and interesting application. Investigations into the mechanism of this detergent activity are planned.

Experimental Section

General. Commercial chemicals were used directly unless otherwise noted. For the sources of nucleotide 5′-triphosphates, see ref 8. [2,8-^{3}H]ATP was purchased from Moravak Biochemicals. [1-^{14}C]Lactose and L-[5-^{3}H]proline were purchased from Amersham. The *E. coli* lipid extracts were purchased from Avanti Polar-Lipids, Inc. Bio-Safe II counting cocktail was purchased from Research Products International Corp. and was added to the sample prior to the scintillation counting. The bath sonicator was the 80-W, 80-kHz generator Model G112SP1G, tank Model G112SP1T from Laboratory Supplies Co., Inc. (Hicksville, NY). Mass spectra were determined by fast atom bombardment (FAB) with *m*-nitrobenzyl alcohol as the matrix. Elemental analyses were obtained from Desert Analytics, Tucson, AZ.

4-Aza-1-azoniabicyclo[2.2.2]octane, 1,1′-(1,6-Hexanediyl)bis-, Diiodide (6). A solution of 1,6-diiodohexane (2.0 mL, 0.0121 mol) and 1,4-diazabicyclo[2.2.2]octane (DABCO, 4.1 g, 0.036 mol) in acetone (50 mL) was stirred for 14 h at 20 °C. The collected precipitate was washed with a small amount of acetone to give **6** (6.5 g, 96%): mp 255 °C dec; ^{1}H NMR (360 MHz, Me_2SO-d_6) δ 1.32 (m, 4 H), 1.69 (m, 4 H), 3.01 (t, J = 7.4 Hz, 12 H), 3.21 (m, 4 H), 3.30 (t, J = 7.3 Hz, 12 H); ^{13}C NMR (Me_2SO-d_6) δ 20.85, 25.30, 44.61, 51.46, 62.96; MS 435 [(M − I)$^+$, 100]. Anal. Calcd for $C_{18}H_{36}N_4I_2$ (561.8): C, 38.45; H, 6.45; N, 9.96. Found: C, 38.48; H, 6.48; N, 9.89.

1,4-Diazoniabicyclo[2.2.2]octane, 1,1′-(1,6-Hexanediyl)bis-[4-[2-(dioctylamino)-2-oxoethyl]-, Tetrabromide (3). A mixture of **6** (500 mg, 0.89 mmol) and 1-iodo-*N*,*N*-dioctylacetamide[8] (1.1 g, 2.7 mmol) in DMF (4 mL) was stirred at 70 °C for 12 h. The formed tetrakis(ammonium iodide) was isolated after removal of the solvent and washing with hexanes followed by ethyl acetate. Ion-exchange chromatography (Br$^-$) afforded **3** (530 mg, 50%): mp 220 °C dec; IR (film) ν(C=O) 1656 cm^{-1}; ^{1}H NMR [360 MHz, $CDCl_3$/CD_3OD (9/1)] δ 0.89 (t, J = 6.6 Hz, 12 H), 1.2–1.45 (m, 40 H), 1.45–1.7 (m, 12 H), 1.99 (m, 4 H), 3.2–3.55 (m, 8 H), 3.86 (m, 4 H), 4.35 (m, 12 H), 4.58 (m, 12 H), 4.92 (s, 4 H); ^{13}C NMR ($CDCl_3$/CD_3OD (9/1)) δ 13.67 (2×), 20.78, 22.28 (2×), 23.99, 26.54, 26.64, 27.11, 28.35, 28.89 (4×), 31.45 (2×), 46.46, 47.38, 50.73, 51.50, 61.18, 64.15, 161.21; MS 1114 (M − Br)$^+$. Anal. Calc for $C_{54}H_{108}N_6O_2Br_4$·H_2O (1211.1): C, 53.55; H, 9.15; N, 6.94. Found: C, 53.40; H, 9.16; N, 6.70.

1,4-Diazoniabicyclo[2.2.2]octane, 1,4-Bis[3-(trioctylammonio)propyl]-, Tetrabromide (4). A mixture of *N*-(3-iodopropyl)-*N*,*N*-dioctyl-1-octanaminium iodide[8] (**7**, (370 mg, 0.573 mmol) and DABCO (20 mg, 0.179 mmol) in DMF (1 mL) was stirred at 80 °C for 48 h. The solvent was removed and the residue purified by column chromatography (SiO_2, 5% EtOH then 15% EtOH in CH_2Cl_2). The dichloromethane solution of the product was washed with HI (0.5 N) and then with NaI (0.5 N). Workup yielded the expected tetrakis(ammonium iodide) which, upon ion-exchange chromatography (Br$^-$), gave the desired product **4** (197 mg, 90%): mp 143–145 °C; ^{1}H NMR [360 MHz, $CDCl_3$/CD_3OD (10/1)] δ 0.89 (t, J = 6.6 Hz, 18 H), 1.2–1.5 (m, 60 H), 1.73 (m, 12 H), 2.47 (m, 4 H), 3.33 (t, J = 7.8 Hz, 12 H), 3.52 (t, J = 7.4 Hz, 4 H), 4.04 (t, J = 7.4 Hz, 4 H), 4.38 (s, 12 H); ^{13}C NMR [$CDCl_3$/CD_3OD (10/1)] δ 13.54, 16.77, 21.70, 22.16, 25.94, 28.66 (2×), 31.22, 50.95, 54.79, 59.11, 60.42; MS 1144 (M − Br)$^+$. Anal. Calcd for $C_{60}H_{126}N_4Br_4$·$2H_2O$ (1257.6): C, 57.23; H, 10.41; N, 4.45. Found: C, 57.17; H, 10.43; N, 4.39.

Acetamide, 2,2-Iminobis[*N*,*N*-dioctyl- (8). 1-Bromo-*N*,*N*-dioctylacetamide[8] (2.0 g, 5.5 mmol) was added to 20 mL of EtOH saturated with ammonia. After the mixture was stirred at 20 °C for 2 h, the solvent was removed and the residue redissolved in hexanes. The solution was washed with $NaHCO_3$ and then with water. After removal of the solvent, the residue was dissolved in EtOH (20 mL). More 1-bromo-*N*,*N*-dioctylacetamide (0.60 g, 1.7 mmol) and Et_3N (0.25 mL, 1.7 mmol) were added. The mixture was stirred at 20 °C for 2 h and then worked up as described above. The residue was purified by column chromatography (50% EtOAc in hexanes, EtOAc, then 10% EtOH in EtOAc) to give the desired secondary amine **8** (610 mg, 29%): IR (film) ν(C=O) 1650 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.8–0.95 (m, 12 H), 1.15–1.35 (m, 40 H), 1.45–1.6 (m, 8 H), 3.14 (t, J = 7.7 Hz, 4 H), 3.29 (t, J = 7.7 Hz, 4 H), 3.47 (s, 4 H); ^{13}C NMR ($CDCl_3$) δ 13.75 (2×), 22.32 (2×), 26.57, 26.75, 27.43, 28.64, 28.90, 28.94, 29.00, 29.09, 31.46, 31.50, 45.67, 46.67, 49.69, 169.87; MS 581 (M + H)$^+$; HRMS [(M + H)$^+$, $C_{36}H_{74}N_3O_2$] calcd 580.5777, obsd 580.5793.

Acetamide, 2-Bromo-*N*,*N*-bis[2-(dioctylamino)-2-oxoethyl]- (9). Bromoacetyl bromide (225 μL, 2.58 mmol) was added slowly to a mixture of **8** (1.4 g, 2.46 mmol) and triethylamine (362 μL, 2.60 mmol) in CH_2Cl_2 (30 mL). The resulting solution was stirred at 20 °C for 15 min. Additional CH_2Cl_2 (100 mL) was added and the mixture washed with NH_4Cl (1 N), $NaHCO_3$ (1 N), and water. The residue obtained after solvent evaporation was purified by flash chromatography (EtOAc/hexanes, 1:7 then 1:4) to yield **9** (1.4 g, 80%): IR (film) ν(C=O) 1650, 1659 cm^{-1}; ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.75–0.85 (m, 12 H), 1.1–1.3 (m, 40 H), 1.35–1.55 (m, 8 H), 3.05–3.3 (m, 8 H), 3.73 (s, 2 H), 4.18 (s, 2 H), 4.35 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.89 (4×), 22.39 (4×), 26.00, 26.63 (2×), 26.80, 26.83, 27.48 (2×), 28.84 (2×), 28.96, 28.99, 29.03 (2×), 29.07, (3×), 29.13, 31.52, 31.54 (2×), 31.56, 46.14, 46.35, 47.03, 47.35, 47.49, 49.96, 166.47, 167.16, 167.27; MS 701 [(M + H)$^+$, 100]; HRMS [(M + H)$^+$, $C_{38}H_{75}N_3O_3Br$] calcd 700.4988, obsd 700.5005.

1,4-Diazoniabicyclo[2.2.2]octane, 1,1′-(1,3-Propanediyl)bis[4-[2-[bis[2-(dioctylamino)-2-oxoethyl]amino]-2-oxoethyl]-, Tetrabromide (5). A mixture of **9** (94 mg, 0.13 mmol) and **10**[8] (20 mg, 0.038 mmol) was stirred at 50 °C for 5 h. Then more of **9** (94 mg, 0.13 mmol) was added, and the mixture was stirred overnight at 50 °C. Another portion of **9** (50 mg, 0.072 mmol) was added, and after stirring for an additional 5 h, the solvent was removed. The residue was purified by column chromatography [5% MeOH then 8% MeOH in CH_2Cl_2, followed by 1% NaI in CH_2Cl_2/MeOH (9/1)]. The crude product was dissolved in CH_2Cl_2 and passed through an ion-exchange resin (Br$^-$) to give **5** (42 mg, 61%): IR (film) ν(C=O) 1650, 1660 cm^{-1}; ^{1}H NMR [360 MHz, $CDCl_3$/CD_3OD (10/1)] δ 0.8–0.95 (m, 24 H), 1.2–1.4

(m, 80 H), 1.45–1.65 (m, 16 H), 2.66 (m, 2 H), 3.15–3.4 (m, 16 H), 3.85–4.0 (m, 4 H), 4.16 (s, 4 H), 4.25–4.5 (m, 28 H), 4.73 (s, 4 H). ^{13}C NMR [$CDCl_3/CD_3OD$ (10/1)] δ 13.61 (4×), 17.36, 22.26 (4×), 26.51 (2×), 26.67, 26.76, 27.26, 27.35, 28.53, 28.66, 28.96 (8×), 31.44 (4×), 46.44, 46.98, 47.28 (2×), 48.26, 49.59, 51.13, 51.74, 60.00, 61.15, 163.84, 165.98, 166.46; $C_{91}H_{178}N_{10}O_6Br_4$ (MW 1828.03); MS 1748 $(M - Br)^+$.

Extraction and Liquid Membrane Transport Studies. The detailed procedures described in ref 8 are followed, and the same U-type transport cell has been used. Additional information relevant to this work is included in the captions to Figure 1 and Table I. The only two changes compared to the previous work are (i) dichloromethane (instead of chloroform) acts as liquid membrane and (ii) the receiving phase in the U-type cell is 0.20 M Na_3PO_4 (instead of 0.20 M NaBr).

Liposome Transport Studies. 1. Preparation of Liposome Suspensions. An *E. coli* lipid extract (150 mg) was stirred for approximately 30 min in 3.0 mL of water until a uniform suspension was formed. The mixture was then sonicated in a bath sonicator until it became semiclear (approximately after 10 min). A total of 2.28 mL of this solution and 0.19 mL of a 15% solution of octyl glucoside in water was added to 8.0 mL of buffer solution (1 mM Tris, pH = 7.0). The mixture was incubated for 30 min on ice and then diluted to 365 mL with cold buffer (1 mM Tris, pH 7.0, 8 °C). The solution was centrifuged for 2 h at 40 000 rpm. The precipitate was dissolved in 0.50 mL of buffer solution. The resulting liposome stock solution was divided into 40-μL portions which were kept frozen.

2. Loading of the Liposomes. A 40-μL sample of liposome suspension was thawed at room temperature. Subsequently, 10 μL of [2,8-^{3}H]ATP (1 mCi/1 mL) or of one of the other compounds to be loaded was added. The mixture was mixed via a vortex mixer and then frozen to liquid nitrogen temperature. The frozen liposome suspension was then thawed to room temperature followed by sonication for 10 s.

3. Transport Assay. A total of 4.0 μL of the suspension containing the loaded liposomes was added to 200 μL of 25 mM sodium phosphate buffer, pH 7.0. The solution was passed through two Sephadex G-50-80 spin columns to remove free [2,8-^{3}H]ATP. To 100 μL of the filtrate was added the desired carrier in 5 μL of Me_2SO. The mixture was incubated for a desired time (30 min to 3 h) and then passed through a Sephadex spin column. The radioactivity of the filtrate was counted. The spin column was run in the following manner. A total of 3 mL of preswollen Sephadex G-50-80 was added to a disposable 3-mL syringe. If the Sephadex gel had not been swelled in the desired buffer, it should be washed with that buffer at this time. The syringe was spinned at maximum rate on a clinical centrifuge for 3 min. Subsequently, the sample, diluted in 100 μL of buffer was added to the column. The column was then spinned at the maximum rate of the clinical centrifuge (4000 rpm) for 2 min.

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