The First Selective and Efficient Transport of Imide-Containing Nucleosides and Nucleotides by Lipophilic Cyclen−Zinc(II) Complexes (Cyclen = 1,4,7,10-Tetraazacyclododecane)

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Abstract: Zinc(II) complexes (ZnL) of macrocyclic tetramines, cyclens, bearing alkyl chains of four different lengths (propyl, octyl, dodecyl, and hexadecyl) have been tested as new carriers for highly selective extraction and transport of imide-containing nucleosides and nucleotides (L = cyclen = 1-alkyl-1,4,7,10-tetraazacyclododecane). The most lipophilic Zn^{2+}−hexadecylcyclen was found to most effectively extract thymidine (dT) from an aqueous solution into a $CHCl_3$ layer, while it did not extract other nucleobase derivatives (C, A, and G) at all. More lipophilic thymidine derivatives (HS) such as 3′-azido-3′-deoxythymidine (AZT), 1-methylthymine (1-MeT), and ftorafur (Ff) were almost quantitatively extracted by an equivalent amount of Zn^{2+}−hexadecylcyclen. The ^1H NMR spectra of the $CDCl_3$-extracted AZT by Zn^{2+}−hexadecylcyclen confirmed the formation of the 1:1 complex (S^-−ZnL) bound through imide-N^- anion and zinc(II) cation (S^- denotes the deprotonated dT derivatives). While apparent 1:1 complexation constants, log K_{app} ($K_{app}(S^-$−ZnL) = $[S^-$−ZnL]/$[(HS + S^-)_{free}][ZnL_{free}]$ (M^{-1})), for Zn^{2+}−octylcyclen with dT and AZT at pH 7.6 are the same value of 3.4 ± 0.1 (determined by potentiometric pH titration) in aqueous solution at 25 °C with I = 0.1 ($NaNO_3$), the log K_{app} values for Zn^{2+}−hexadecylcyclen with dT and AZT in the presence of a neutral detergent Triton X-100 (10 mM) at pH 7.6 (50 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C are 3.3 ± 0.1 and 4.4 ± 0.1, respectively (determined by isothermal calorimetric titration). These results support the observation that the extraction of AZT with Zn^{2+}−hexadecylcyclen is more favorable than that of dT. The $CHCl_3$-extracted dT was more easily released into an acidic (pH 6.0) aqueous solution than AZT. As a support of the extraction experiments, the transport of dT and AZT (1 mM) from a pH 9.0 aqueous solution (Aq I) to a pH 5.0 aqueous solution (Aq II) mediated by a liquid $CHCl_3$ membrane containing 1 mM Zn^{2+}−hexadecylcyclen was carried out.

Introduction

Thymidine and uridine nucleoside analogues are useful in antiviral chemotherapy.[1] The most typical example is 3′-azido-3′-deoxythymidine (AZT), an approved drug for the treatment of AIDS.[2] These molecules first go into cells by simple passive diffusion[3] or with help of membrane-bound transport proteins[4] and then undergo phosphorylation[5] in cytoplasm to become triphosphate derivatives to be activated for inhibition of essential viral enzymes such as reverse transcriptase.[1,2] The first requirement for the drug activity, therefore, is efficient transport of these nucleoside analogues into infected cells through a lipophilic membrane barrier.[6] Lipophilic derivatives or selective and efficient carriers may be useful in enhancing membrane transport of these substances, which then helps reducing daily doses of these expensive and toxic drugs.[6,7]

There have been a number of nucleoside (or nucleotide) carriers designed on the basis of Watson−Crick, Hoogsteen, or the relevant associations,[8−11] phosphate anions−cationic

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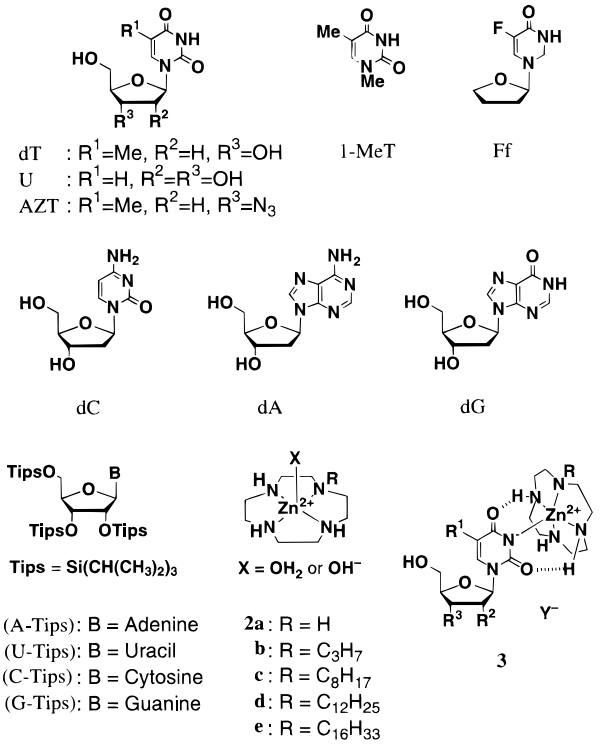
carrier association,[12−17] and so on.[18] For instance, Sessler et al. reported nucleosides protected at sugar hydroxy groups with triisopropylsilyl (Tips) group **1**.[9] Of these, C-Tips (**1c**) and G-Tips (**1d**) selectively transport guanosine (G) and cytidine (C) derivatives, respectively. However, the transport enhancement of thymidine (dT) or AZT by A-Tips (**1a**) was limited. The weaker dT (or U)-A (adenine) base pair association in comparison with G−C association possibly is not sustainable in the $H_2O \rightarrow CHCl_3$ partition. So far, there have practically been no selective and efficient carriers for dT or U derivatives, although selective hosts for dT's in $CHCl_3$ solution were reported.[19] On the other hand, lipophilic cationic carriers (e.g., quarternary ammonium salts) for phosphates[13−16] lack the base selectivity.

In the course of study on intrinsic properties of zinc(II) ion in zinc enzymes,[20] we have discovered that Zn^{2+}−1,4,7,10-tetraazacyclododecane (Zn^{2+}−cyclen, **2a**) interacts only with dT and uridine (U) among all the nucleosides through a Zn^{2+}−imide-N^- anion bonding and the two complementary hydrogen bonds at physiological pH in aqueous solution, resulting in formation of stable 1:1 complexes **3**.[21,22] We recently synthesized a lipophilic hexadecylcyclen **2e** for catalytic hydrolysis of a lipophilic phosphotriester in micellar solution containing a neutral detergent Triton X-100.[23]

In this study, we present **2e** and homologues **2b**−**d** as a new type of the first selective transporters of dT, U, and AZT or their relevant compounds. Furthermore, we have found that

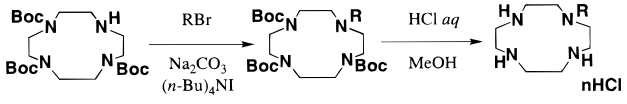


AZT is extracted into organic phase more efficiently than dT by **2e**, despite the fact that the complexation of dT and AZT with a nonlipophilic Zn^{2+}−cyclen **2a** occurs in a similar extent in aqueous solution. We have then studied contrasting complexation properties of **2e** with dT and AZT in micellar solution containing Triton X-100 by isothermal calorimetric titration. The transport of dT and AZT through a liquid $CHCl_3$ membrane containing **2e** is also described.

Results and Discussion

Synthesis of Alkylcyclens and Their Zinc(II) Complexes. Cyclens bearing alkyl chains on a ring nitrogen (**6b**−**e**) were synthesized by *N*-alkylation of 3Boc-cyclen (**4**)[24] with a corresponding alkyl bromide in the presence of Na_2CO_3 and $(n\text{-Bu})_4NI$, as shown in Scheme 1. Successive deprotection of **5b**−**e** with aqueous HCl gave nHCl salts (**6b**−**e**) (n = 3 or 4) of the corresponding alkylcyclen. The two zinc(II) complexes **2d,e** were crystalline. The others, **2b,c**, did not crystallize, and as a result, they were prepared in situ for the following extraction experiments.

Scheme 1



Partition Properties of Zinc(II) Complexes of Alkylcyclen. Before the extraction experiment, the partition mode of the transporters **2b**−**e** between an aqueous phase and an organic phase was studied. A zinc(II) complex (2 μmol) in 2 mL of buffer solution (50 mM CHES at pH 9.0 with I = 0.1 ($NaNO_3$)) plus 2 mL of $CDCl_3$ containing 1 mM naphthalene (for an

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Table 1. Extraction of Nucleosides by $CHCl_3$ Solution Containing Zinc(II)−Cyclen Carriers at 25 °C[a]

run	nucleoside[b]	carrier	pH of aqueous layer[c]	extraction efficiency (%)
1	dT	none	9.0	<1
2	dT	**2e**	6.0	3 ± 1
3	dT	**2e**	7.0	5 ± 1
4	dT	**2e**	7.6	8 ± 1
5	dT	**2e**	8.0	12 ± 1
6	dT	**2e**	9.0	22 ± 2
7	dT	**2e**	10.0	20 ± 1
8	dT	**6e** (free ligand)	9.0	<1
9	dT	**2d**	9.0	18 ± 1
10	dT	**2c**	9.0	12 ± 1
11	dT	**2b**	9.0	<1
12	U	none	9.0	<1
13	U	**2e**	9.0	13 ± 1
14	dC	**2e**	9.0	<1
15	dA	**2e**	9.0	<1
16	dG	**2e**	9.0	<2
17	dT	**2e**[d]	9.0	21 ± 1
18	dT	**2e**[e]	9.0	<1

[a] For the experimental conditions, see text and Experimental Section. [b] The initial concentration of a nucleoside in a buffer solution was 1 mM. [c] Good's buffer (50 mM with $I = 0.1$ ($NaNO_3$)) was used for pH 6.0 (MES), 7.0 (HEPES), 7.6 (HEPES), 8.0 (EPPS), 9.0 (CHES), and 10.0 (CAPS). [d] 1 mM cholesterol 3-sulfonate was added. [e] 1 mM sodium palmitate was added.

internal reference) was vigorously stirred for equilibration for 30 min at 25 °C. The initially turbid $CDCl_3$ layer became clear after the centrifuge (3000 rpm × 10 min at 25 °C). The ratio of the zinc(II) complex partitioned into the $CDCl_3$ layer was determined by ^{1}H NMR; <1% for **2b**, 53% for **2c**, 79% for **2d**, and 98% for **2e**.[25,26]

Extraction of Nucleosides from an Aqueous Phase to a $CHCl_3$ Phase by Zinc(II) Complexes 2b−e. We studied extraction of nucleosides from an aqueous phase to $CHCl_3$ by the Zn^{2+}−cyclen complexes **2b**−**e**. An aqueous solution (2.0 mL) of 1 mM dT in 50 mM Good's buffer (pH 5−10) with $I = 0.1$ ($NaNO_3$) was vigorously stirred for 30 min at 25 °C with a 1 mM solution of **2e** in $CHCl_3$ (2.0 mL), and then two phases were completely separated by centrifuging (3000 rpm × 10 min at 25 °C).[27] The efficiency in the extraction was determined by measuring the remaining nucleoside in the aqueous phase by the UV absorption spectrum and checked by measuring the amount of transferred nucleosides into $CDCl_3$ by ^{1}H NMR. The results are summarized in Table 1. Without the Zn^{2+}−alkylcyclen carriers, dT and U were not extracted (runs 1 and 12). The pH effect of aqueous phase on the extraction of dT with an equivalent amount of **2e** was examined (runs 2−7). The best extraction (22%) was achieved at pH 9.0 (run 5). The free ligand **6e** existing as a diprotonated form (**6e**·$2H^+$) did not extract dT at pH 9.0 (run 8). As expected, the longer is the alkyl chain of Zn^{2+}−alkylcyclen complexes, the more efficient is dT transport to a $CHCl_3$ phase (runs 6 and 9−11). Uridine was extracted at pH 9.0 (run 13), although the efficiency (13%) was not so good as dT, probably due to more hydrophilic nature of U by an additional hydroxyl group on the sugar part and lack of a lipophilic methyl group on the pyrimidine ring.

(25) The partition properties of the corresponding free ligands into a $CDCl_3$ layer from an aqueous phase of pH 9.0 (50 mM CHES with $I = 0.1$ ($NaNO_3$)) were determined by ^{1}H NMR. The partition of **6b** was 13% and **6c**−**e** were extracted almost quantitatively.

(26) It was confirmed by ^{1}H NMR that **2c**−**e** did not dissociate zinc(II) cation when extracted into $CHCl_3$ layer.

(27) The partition of dT is rapid: the extraction of dT from an aqueous phase to a $CHCl_3$ phase finished within 10 min of stirring. The distribution of nucleosides or nucleotides is thus controlled thermodynamically.

Table 2. Extraction of Imide-Containing Nucleosides and Nucleotides by **2e** and **6e** in $CHCl_3$ at 25 °C

run	nucleoside or nucleotide[a]	carrier[a]	pH of aqueous layer[b]	extraction efficiency (%)[c]
1	AZT	none	7.6	17 ± 1
2	AZT	**6e**	7.6	17 ± 1
3	AZT	**2e**	7.6	91 ± 1
4	AZT	none	9.0	14 ± 1
5	AZT	**2e**	9.0	97 ± 1
6	1-MeT	none	7.6	25 ± 1
7	1-MeT	**2e**	7.6	91 ± 1
8	1-MeT	none	9.0	21 ± 1
9	1-MeT	**2e**	9.0	96 ± 1
10	Ff	none	7.0	41 ± 1
11	Ff	**2e**	7.0	>98
12	Ff	none	9.0	5 ± 1
13	Ff	**2e**	9.0	>98
14	5′-dTMP	none	9.0	<1
15	5′-dTMP	**2e**	9.0	18 ± 1
16	5′-dTTP	none	9.0	<1
17	5′-dTTP	**2e**	9.0	23 ± 1
18	5′-AZTMP	none	7.6	<1
19	5′-AZTMP	**6e**	7.6	<1
20	5′-AZTMP	**2e**	7.6	37 ± 1
21	5′-AZTMP	none	9.0	<1
22	5′-AZTMP	**2e**	9.0	36 ± 1
23	d(TpT)[d]	none	9.0	<1
24	d(TpT)[d]	**2e**	9.0	33 ± 1

[a] The initial concentration of a nucleoside (or nucleotide) in an aqueous buffer solution and that of **2e** in $CHCl_3$ was 1 mM unless otherwise described. For abbreviations of nucleosides and nucleotides, see text. [b] Good's buffers, HEPES and CHES (50 mM ($I = 0.1$ ($NaNO_3$)) were used for pH 7.6 and pH 9.0, respectively. [c] The experiments were performed two or three times. [d] 0.5 mM d(TpT) in 50 mM CHES buffer was used.

The dT and U selectivity in the nucleoside extraction is evident in runs 6, 13, and 14−16. *Nucleosides other than dT and U were not transported to an organic phase, indicating that the Zn^{2+}−cyclen complex **2e** is an exclusive dT- and U-selective carrier, as we initially hoped*. This selectivity also implies that the transport of dT and U results from 1:1 dT^- (or U^-)−**2e** complex **3** with counteranions, Y^- (see the following NMR study), but not from simple reverse-micelle formation by the hydrophobic zinc(II) complexes in a $CHCl_3$ phase. Lipophilic anions Y^- might enhance the lipophilicity of the complex for better extraction of dT. However, the addition of 1 mM cholesterol 3-sulfate anions exhibited negligible effect (run 17). The existence of 1 mM sodium palmitate ($C_{16}H_{33}COONa$) inhibited the extraction of dT (run 18), probably because the carboxylate anion may have stronger interaction[28] with **2e** to form a more lipophilic and stable complex in the $CHCl_3$ phase.

Extraction of Other Imide-Containing Nucleosides and Nucleotides from Aqueous Phase to $CHCl_3$ Phase by 2e. The extraction of other imide-containing nucleosides, AZT, 1-methylthymine (1-MeT), or ftorafur (Ff) by **2e** was tested in a similar fashion. The results are summarized in Table 2. These bases alone are fairly well soluble in $CHCl_3$ (runs 1, 4, 6, 8, 10, and 12) because of their lipophilicity.[29] While the diprotonated ligand **6e**·$2H^+$ showed no effect (run 2), its zinc(II) complex **2e** greatly enhanced the extraction efficiency of AZT (run 3). It is of interest that AZT, which has almost the same pK_1 value (deprotonation constant of imide proton) of 9.7 and almost the same apparent complexation constant with **2a**, log K_{app} for

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(29) The partition coefficients of dT and lipophilic AZT for 1-octanol/0.1 M aqueous phosphate buffer (pH 7.0) were reported to be 0.064 and 1.26, respectively (ref 3a).

AZT^-−**2a** = 3.3 at pH 7.6 and 3.7 at pH 9.0, as those of dT (pK_1 = 9.8 and log K_{app} for dT^-−**2a** = 3.2 at pH 7.6 and 3.6 at pH 9.0) in aqueous solution,[21a] was extracted by **2e** much better than dT into a $CHCl_3$ layer from an aqueous phase of pH 7.6 and 9.0 (runs 3 and 5). For definition of the deprotonation constants, the complexation constants, etc., see eqs 1−4,

$$ZnL + (HS + S^-)_{free} \rightleftarrows S^-{-}ZnL:$$

$$K_{app}(S^-{-}ZnL) = [S^-{-}ZnL]/[(HS + S^-)_{free}][ZnL_{free}] \quad (M^{-1}) \quad (1)$$

$$HS \rightleftarrows S^- + H^+: \qquad K_1 = [S^-]a_{H^+}/[HS] \qquad (2)$$

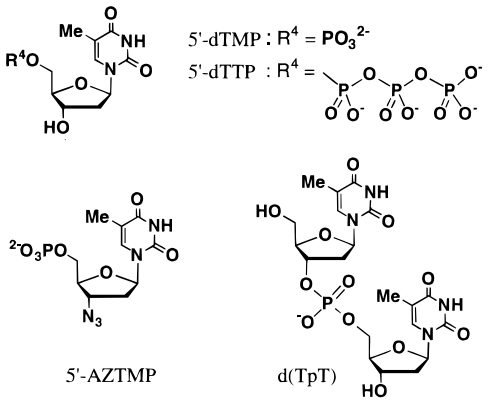
$$ZnL(OH_2) \rightleftarrows ZnL(OH^-) + H^+:$$

$$K_2 = [ZnL(OH^-)]a_{H^+}/[ZnL(OH_2)] \quad (3)$$

$$ZnL + S^- \rightleftarrows S^-{-}ZnL:$$

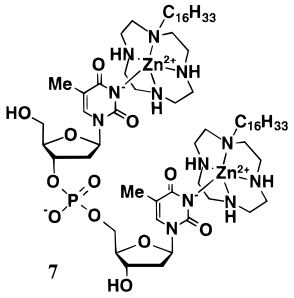
$$K(S^-{-}ZnL) = [S^-{-}ZnL]/[S^-][ZnL](M^{-1}) \quad (4)$$

where ZnL is Zn^{2+}−cyclen, HS and S^- are dT derivatives and their deprotonated species, and a_{H^+} is the activity of H^+, respectively. 1-MeT having a pK_1 value[22d] of 10.0 was extracted as well as AZT (runs 7 and 9). Ftorafur (Ff) (pK_1 = 7.8) was extracted in even better efficiency at lower pH of 7.0 (runs 11 and 13), due to a greater K_{app} value at low pH (log K_{app}(Ff^-−**2a**) = 3.7 at pH 7.6)[21a] and higher lipophilicity of Ff.



Two thymidine nucleotides, thymidine 5-monophosphate (5′-dTMP) and thymidine 5′-triphosphate (5′-dTTP), despite having phosphate anionic charges, were extracted by **2e** as efficiently as dT nucleoside (runs 15 and 17). The extraction of 5′-AZTMP (3′-azido-3′-deoxythymidine 5′-monophosphate) is of interest, since it is a precursor of the biologically active 5′-triphosphate of AZT and phosphorylation of AZT to 5′-AZTMP by cellular kinases is the rate-limiting step, which may be very slow in some cells such as macrophages.[6] It is thus remarkable that **2e** extracted 37% (at pH 7.6) and 36% (at pH 9.0) of the hydrophilic 5′-AZTMP (runs 20 and 22), which, unlike lipophilic AZT, was hardly partitioned into a $CHCl_3$ layer without a carrier (runs 18 and 21). The free ligand **6e** again did not transport 5′-AZTMP (run 19). The thymidilyl(3′-5′)thymidine monophosphate (d(TpT)) (0.5 mM) that can be bound to 2 equiv of **2e** (1 mM) at each T site to yield the 1:2 complex **7**[30] was transferred more efficiently (run 24, 33%) than dT^-−**2e** (22%).

The dT and U selectivity of our lipophilic carrier may be compared with previous lipophilic ammonium cation carriers



developed by Tabushi[13] and Diedrich,[14] which only recognized nucleotides to extract 5′-mono-, di-, and triphosphates of U and dT from an aqueous solution of pH 7−8 into a $CHCl_3$ layer. These quarternary ammonium compounds, moreover, cannot differentiate dT and U from G, A, or C.[31]

^{1}H NMR Spectrum of 1:1 Complex of AZT with 2e in $CDCl_3$. To confirm that the $CHCl_3$-extracted AZT by **2e** was in the form of the 1:1 complex **8** (Scheme 2) but not in uncomplexed AZT, we measured ^{1}H NMR spectra in $CDCl_3$. Parts a and b of Figure 1 show ^{1}H NMR spectra of **2e** and AZT (1 mM) in $CDCl_3$, respectively. Figure 1c is the ^{1}H NMR spectrum of a mere 1:1 mixture of AZT and **2e** in $CDCl_3$ (both 1 mM). Figure 1d shows ^{1}H NMR spectrum of the $CDCl_3$ extract (2.0 mL) from 2.0 mL of 1 mM AZT in 50 mM CHES (pH 9.0 with I = 0.1 ($NaNO_3$)) in the presence of 1 equiv of **2e**. Integration of H(1′) proton of AZT and the terminal Me(5) group of **2e** gives a 1:3 ratio in Figure 1d, proving a 1:1 AZT^-−**2e** ratio in the $CDCl_3$ phase. A mere mixing of AZT with **2e** in pure $CHCl_3$ did not cause the complexation (Figure 1c). Parts c and d of Figure 1 are not identical, although both samples are composed of AZT and **2e** in 1:1 stoichiometry. In comparing Figure 1c,d, we observed upfield shifts of H(6) (δ 7.35 to 7.2) (bold blank arrows), H(1′) (δ 6.04 to 5.90) (plain arrows), H(2′) (δ 2.55 and 2.40 to 2.45 and 2.35, respectively) (dashed arrows), and Me(5) (δ 1.93 to 1.88) (round arrows) protons, as we earlier saw at AZT^-−**2a** complexation in D_2O (for assignment, see **8** in Scheme 2).[21] The imide N−H proton of thymine base indicated by black bold arrows in Figure 1b,c is gone in Figure 1d. The broad peaks pointed by narrow blank arrows in Figure 1a (δ 3.79 and δ 3.87) and in Figure 1d (δ 3.62), which disappear when the sample solution is shaken with D_2O (pD 9.0), were assigned to the N−H protons of the cyclen ring in **2e**. Although we could not observe the behavior of dT derivatives having too low solubility in $CDCl_3$, all of the imide-containing nucleosides and nucleotides should be extracted into the $CHCl_3$ layer in a similar fashion, as depicted by the left half of Scheme 2.

For further check of the complex **8** formation in $CHCl_3$, we prepared AZT^- in $CDCl_3$ using pentaisopropylguanidine (PIG)[32] as a nonligative organic base, whose conjugate acid has a pK_a

(30) Kimura, E.; Kikuchi, M.; Koike, T. a paper submitted for publication.

(31) We carried out the extraction of several phosphates to see whether the phosphate anion is involved in extraction of thymidine nucleotides by **2e** as suggested by a reviewer. However, we could not determine the distribution of 2′-deoxyadenosine 5′-monophosphate (5′-dAMP) and 2′-deoxycytidine 5′-monophosphate (5′-dCMP) because of precipitation. **2e** did not promote the extraction of adenosine 5′-monophosphate (5′-AMP) from an aqueous layer of pH 7.6 into a $CHCl_3$ layer (2% and 3% in the absence and presence of 1 equiv of **2e**, respectively). On the other hand, 1-naphthyl phosphate ($\epsilon_{286} = 5.4 \times 10^3$ at pH 7.6), a nonnaturally occurring phosphate, was extracted in 95% by **2e**, a fact suggesting that the interaction of zinc(II) cation and a phosphate moiety might sometimes contribute to the extraction of some nucleotides.

(32) (a) Barton, D. H. R.; Elliott, J. D.; Géro, S. D. *J. Chem. Soc., Chem. Commun.* **1981**, 1136−1137. (b) Barton, D. H. R.; Elliott, J. D.; Géro, S. D. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2085−2090. (c) Wieland, G.; Simchen, G. *Liebig Ann. Chem.* **1985**, 2178−2193.

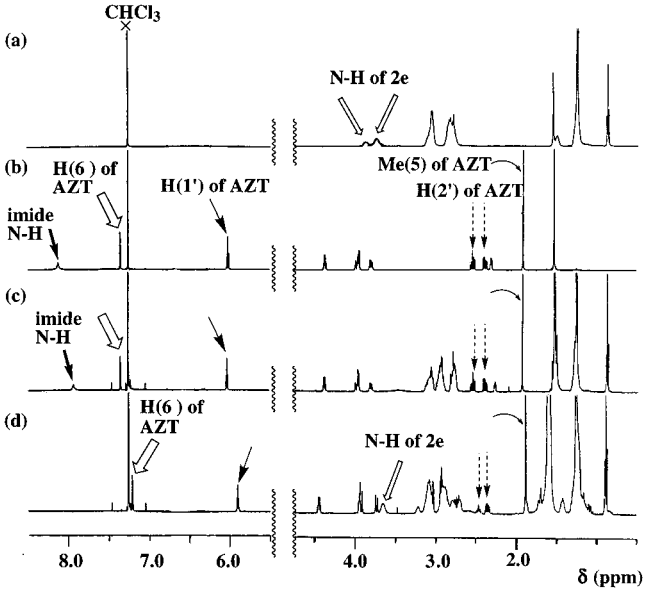
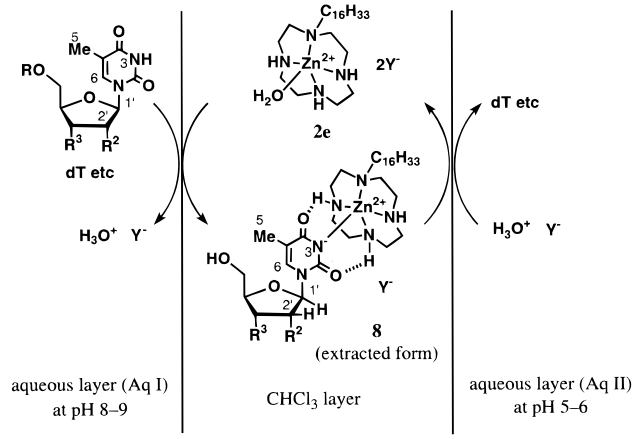
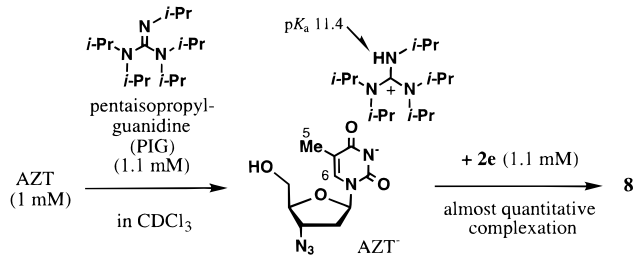


Figure 1. ^{1}H NMR spectra in $CDCl_3$ of **2e** (a), AZT (b), a mixture of **2e** and AZT (c), and the 1:1 complex **8** obtained by extraction from a CHES buffer solution (pH 9.0) (d).

Scheme 2



Scheme 3



value of 11.4.[32c] By addition of 1.1 mM PIG to a solution of 1 mM AZT in $CDCl_3$, the peak of the imide N−H disappeared, confirming that PIG deprotonated the imide proton to yield AZT^- as shown in Scheme 3. Other peaks of AZT did not exhibit any shifts. The addition of 1.1 mM **2e** to this mixture caused upfield shifts of H(1′) (from δ 6.04 to 5.8) and Me(5) (from δ 1.93 to 1.88), implying the formation of **8**, as was observed with Figure 1d. The upfield shifts of these two peaks occurred linearly until 1 equiv and then reached a plateau, indicating that the complexation of AZT^- with **2e** occurred almost quantitatively at $[AZT^-] = [\mathbf{2e}] = 1$ mM in $CHCl_3$ (data not shown).

The Potentiometric pH Titration of dT and AZT with 2c.

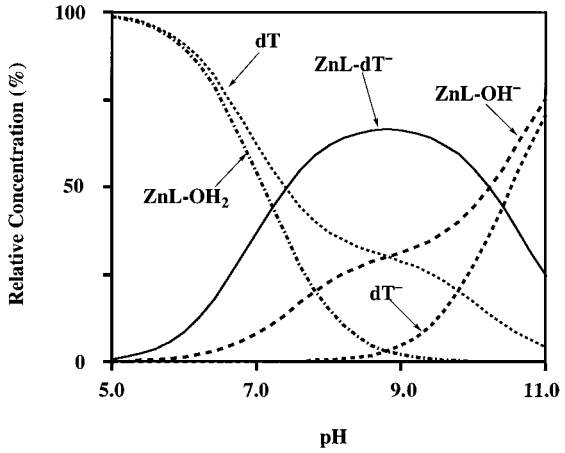


Figure 2. Distribution diagram for the dT and ZnL (**2c**) species for an 1 mM dT/1 mM **2c** mixture as a function of pH at 25 °C with I = 0.1 ($NaNO_3$) (dT^- denotes the deprotonated dT).

We have found far greater extraction of AZT into $CHCl_3$ layer in the form of **8** (91% at pH 7.6, run 3 in Table 2) than a mere 1:1 AZT^-−(Zn^{2+}−cyclen) complex **3** formation constant in aqueous solution predicted; log K_{app} = 3.3 at pH 7.6, i.e., 50% AZT is in the complex at [total AZT] = [total **2e**] = 1 mM. For this inquiry, we have studied the effect of the long alkyl chain on the stability of the dT and AZT complexes by pH-metric titration of 1 mM dT (or AZT) with 1 mM **2c** bearing an octyl group, which though partially lipophilic (see above) is still soluble enough in aqueous solution. The pK_1 values of imide proton in dT and AZT defined by eq 2 are 9.8 ± 0.1 and 9.7 ± 0.1, respectively.[21a] The deprotonation constant of the zinc(II)-bound water, pK_2, of **2c** (eq 3) was 7.8 ± 0.1 in aqueous solution. The complex formation constants, log K(S^-−ZnL) defined by eq 4 for dT and AZT at 25 °C with I = 0.10 ($NaNO_3$), were almost the same (5.7 ± 0.1 and 5.8 ± 0.1), as determined by the program "BEST".[33] From these two log K(S^-−ZnL) values, the apparent 1:1 complexation constants, log K_{app}, of **2c** with dT and AZT at pH 7.6 and 25 °C were calculated to be 3.2 ± 0.1 and 3.4 ± 0.1, i.e., 47% and 53% complexation at pH 7.6 when [total **2c**] = [total dT] = [total AZT] = 1 mM, respectively. It is concluded that the octyl pendant affected little on the dT^-−ZnL and AZT^-−ZnL complex formation.

Figure 2 shows a pH-dependent distribution diagram for five species (HS, S^-, ZnL(OH_2), ZnL(OH^-), and S^-−ZnL complex) at 25 °C when dT and **2c** (both 1 mM) are mixed at 25 °C and I = 0.1 ($NaNO_3$). AZT with **2c** gives a similar diagram. The complexation of dT with **2c** (to give dT^-−ZnL species in Figure 2) increases as the solution pH rises and reaches maximum (65−66% complexation) at pH 8.4−9.4. At higher pH, the displacement of the dT^- by hydroxide anion occurs to ZnL(OH^-), resulting in dissociation of the dT^-−**2c** complex **3**. The dT^-−ZnL formation diagram is in parallel with the pH-dependent extraction efficiency (Figure 3a,c), although the maximum extraction efficiency at pH 9.0 (22%) did not reach the maximum complex formation in the one-phase aqueous solution at the same pH (65−66%). The complex population is less than 10% at low pH 5−7.

Discrepancy between the Complexation Behaviors in Aqueous Solutions and the $CHCl_3$ Partition Behaviors.

(33) (a) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*, 2nd ed.; VCH: New York, 1992. (b) Martell, A. E.; Hancock, R. D. *Metal Complexes in Aqueous Solutions*; Plenum Press: New York, 1996.

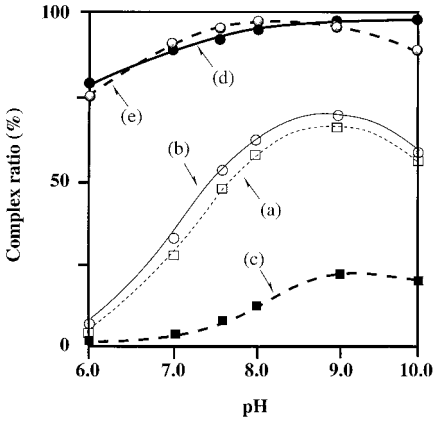


Figure 3. pH-dependent 1:1 complex profile of dT and AZT with **2c** and the extraction profile by **2e**: (a) $dT^{-}-$**2c** and (b) $AZT^{-}-$**2c** complexes in aqueous solution, (c) [dT] and (d) [AZT] extracted into a $CHCl_3$ layer by **2e**. The initial concentration [dT] = [AZT] = 1 mM (at 25 °C with $I = 0.1$ ($NaNO_3$)). An equal volume of buffer solution and $CHCl_3$ were used for the extraction (c and d). For curve e, see text.

Figure 3a,b shows the calculated pH-dependent complexation of the $dT^{-}-$**2c** and $AZT^{-}-$**2c** complexes in aqueous solution. Parts c and d of Figure 3 compare the observed pH-dependent $CHCl_3$-partition percentage of dT and AZT (both 1 mM) in aqua-$CHCl_3$ layers in the presence of equivalent amounts of **2e**. It is now evident that despite the dT and AZT complexation behaviors with **2e** being similar in aqueous solution, the dT and AZT extractabilities by **2e** are quite different. Some extra stability for $AZT^{-}-$**2e** complex in $CHCl_3$ solution may be taken into consideration. The curve d is close to curve e, which is a hypothetical profile for $AZT^{-}-$**2e** complexation at [total **2e**] = [total AZT] = 1 mM, assuming the pK_1 value (9.41) for imide proton of AZT, the pK_2 value (7.56) for the deprotonation of zinc(II)-bound water in **2e**[23] (both are experimental values in an aqueous solution containing 10 mM Triton X-100), and the intrinsic 1:1 complexation constant, log K($AZT^{-}-$**2e**) of 7.8. Accordingly, we propose a hypothetical complexation constant, log K($AZT^{-}-$**2e**) value of 7.8, *in aqua-CHCl₃ system*, which is 100 times greater than the experimental log K($AZT^{-}-$**2c**) value of 5.8 *in aqueous system.* On the other hand, a hypothetical log K($dT^{-}-$**2e**) value in the aqua-$CHCl_3$ system, that fits to the experimental extraction profile (curve c) is estimated to be 4.4−4.5, a value 16−20 times smaller than the experimental log K($dT^{-}-$**2c**) of 5.7 in aqueous solution, using pK_1 value for dT of 9.46 and pK_2 of 7.56 (obtained in 10 mM Triton X-100 solution).

Isothermal Calorimetric Titration for Complexation of 2a,e with dT and AZT in Micellar Solution. Earlier,[23] we showed that Triton X-100 (10 mM) micellar phase dissolves the lipophilic **2e** in aqueous solution. We have assumed that the microenvironments in the micellar condition represent the two-phase interface at the extraction. To obtain some supporting evidence for the proposal that the enhanced extractability of AZT by **2e** being due to enhanced stability of $AZT^{-}-$**2e** complex in $CHCl_3$ solution, we measured the apparent complexation constants, log K_{app}, of hydrophilic **2a** and lipophilic **2e** with dT or AZT in 50 mM HEPES buffer (pH 7.6) in the absence and presence of nonionic detergent Triton X-100 (10 mM) by isothermal calorimetric titration.[34]

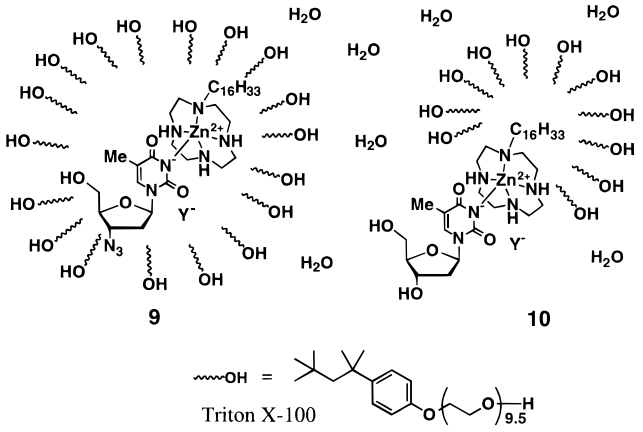
The results are summarized in Table 3. The log K_{app} values for $Zn^{2+}-$cyclen **2a** with dT (3.0 ± 0.1) and AZT (3.1 ± 0.1) in the absence of Triton X-100 were almost identical. The addition of 10 mM Triton X-100 did not significantly affect these values (log K_{app}($dT^{-}-$**2a**) = 3.1 ± 0.1 and log K_{app}-($AZT^{-}-$**2a**) = 3.2 ± 0.1). On the other hand, combination of the lipophilic **2e** with lipophilic AZT gave 13 times more stable complex than with hydrophilic dT; K_{app}($AZT^{-}-$**2e**) = 4.4 ± 0.1 against K_{app}($dT^{-}-$**2e**) = 3.3 ± 0.1 (i.e., 82% against 50% complexation at 1 mM) in a buffer solution containing 10 mM Triton X-100. These facts imply that the lipophilic $AZT^{-}-$**2e** complex gains extra stability by going into the lipophilic co-micellar phase (**9** in Scheme 4), while the less lipophilic $dT^{-}-$**2e** complex (and $AZT^{-}-$**2a**, too) tends to stay in the aqueous phase (**10**).

Table 3. Apparent Affinity Constants (log K_{app}[a]) for 1:1 Complexation of dT and AZT with **2a**,**e** in the Absence or Presence of Triton X-100, Determined by Isothermal Calorimetric Titration at 25 °C

		log K_{app}	
nucleoside	solvent	**2a**	**2e**
dT	50 mM HEPES (pH 7.6)[b]	3.0 ± 0.1	
	50 mM HEPES (pH 7.6)[b] 10 mM Triton X-100	3.1 ± 0.1	3.3 ± 0.1
AZT	50 mM HEPES (pH 7.6)[b]	3.1 ± 0.1	
	50 mM HEPES (pH 7.6)[b] 10 mM Triton X-100	3.2 ± 0.1	4.4 ± 0.1

[a] $K_{app} = [S^{-}-ZnL]/[(HS + S^{-})_{free}][ZnL_{free}]$ (HS is dT or AZT and S^{-} is their deprotonated species). [b] The ionic strength was adjusted to 0.10 with $NaNO_3$.

Scheme 4



Nowick et al. have reported that molecular recognition due to A−T hydrogen bonding between alkylammonium derivatives of thymine and adenine derivatives bearing alkyl chain is much more effective in 30 mM aqueous SDS (sodium dodecyl sulfate) solution.[11] Their suggestion that hydrophobic interactions in a micellar solution contribute by a factor of ca. 10^1-10^2 to K_{app} is consistent with our proposed 100 times and observed 13 times higher stability of $AZT^{-}-$**2e** in $CHCl_3$ and in the co-micellar phase, respectively.

Transport of dT and AZT by 2e through Liquid Membrane (Scheme 2). For membrane transport, the $CHCl_3$ extractants from the first aqueous phase (Aq I) should be released to another aqueous phase (Aq II). This indeed was indicated by the following ^1H NMR experiment. A $CDCl_3$ solution containing 1 mM **2e** which extracted ca. 20% of a 1 mM dT in an aqueous buffer Aq I (pH 9.0) released dT nearly 100% to an aqueous solution Aq II (pH 6.0) after rigorous mixing. Lipophilic nucleosides such as AZT were also released, though to a lesser degree; ca. 21% of AZT went to aqueous

(34) (a) Freire, E.; Mayorga, O. L.; Straume, M. *Anal. Chem.* **1990**, *62*, 950a−959a, 1254a. (b) Wadsö, I. *Chem. Soc. Rev.* **1997**, 79−86.

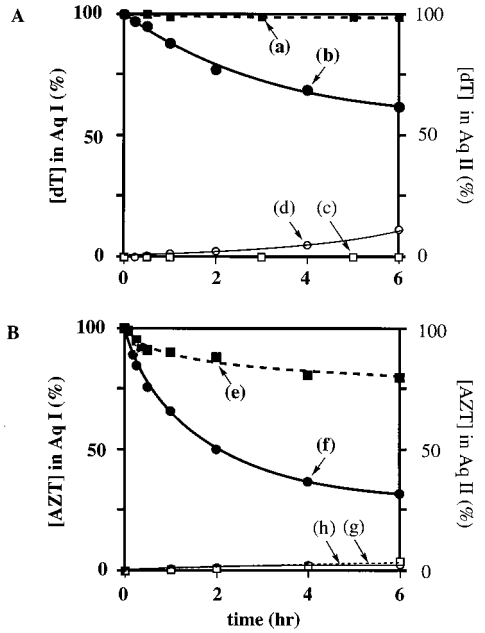


Figure 4. Transport of dT (A) and AZT (B) by **2e** from Aq I (pH 9.0) to from Aq II (pH 5.0) through a $CHCl_3$ phase: Curves a and b show the decreasing [dT] in Aq I in the absence and presence of **2e**, respectively. Curves c and d display the increasing [dT] in Aq II in the absence and presence of **2e**, respectively. Curves e and f show the decreasing [AZT] in Aq I in the absence and presence of **2e**, respectively, and curves g and h are the increasing [AZT] in Aq II in the absence and presence of **2e**, respectively.

solution (Aq II) of pH 6.0.[35] We then assumed that continuous membrane transport of dT and U and the relevant derivatives would be possible by **2e** from an aqueous phase of higher pH (Aq I) to an aqueous phase of lower pH (Aq II), which is coupled with proton antiport (see Scheme 2).

The transport of dT and AZT was compared under the same conditions using a U-type cell (the initial [dT] in Aq I = 1 mM) from Aq I (5.0 mL of 50 mM CHES at pH 9.0 with I = 0.1 ($NaNO_3$)) to Aq II (5.0 mL of 50 mM MES at pH 5.0 with I = 0.1 ($NaNO_3$)) through a $CHCl_3$ layer (10 mL) containing 1.0 mM **2e** with a stirring bar. The results are summarized in Figure 4A,B. Curves a and b display the decreasing [dT] in Aq I in the absence and presence of **2e**, respectively. While the migration of dT from Aq I to the $CHCl_3$ phase was negligible without **2e**, ca. 40% of dT moved from Aq I to the $CHCl_3$ phase by **2e** after 6 h. Further migration of dT to Aq II was slower under the same stirring conditions; 11% after 6 h (see curve d in Figure 4A). Figure 4B shows the behaviors of AZT under the same conditions. AZT migrated from Aq I to $CHCl_3$ phase by 19% (curve e) and 70% (curve f) after 6 h in the absence and presence of **2e**, respectively, while the transfer of AZT into Aq II was extremely slow (1−2% after 6 h) (curves g and h).[36] With a more efficient stirring device, faster and more efficient liquid membrane transport of dT dereivatives should be achieved, as suggested by the extraction experiments.

(35) It has been confirmed by ^{1}H NMR that AZT is extracted as the complex **8** even at pH 6.

(36) Under these conditions, the initial rate of **2e**-mediated dT transport from Aq I to a $CHCl_3$ phase and that from a $CHCl_3$ phase to Aq II were 8.8×10^{-9} (mol·cm^{-2}·min^{-1}) and 7.1×10^{-8} (mol·cm^{-2}·h^{-1}), respectively. Initial rates of AZT transport from Aq I to $CHCl_3$ phase in the absence and presence of **2e** were 1.0×10^{-8} and 4.1×10^{-8} (mol·cm^{-2}·min^{-1}), respectively, indicating that transfer of AZT by **2e** is ca. 4 times faster than the control.

Conclusion

Lipophilic Zn^{2+}−cyclen complexes have been synthesized and found to be the first carriers of imide-containing dT or U nucleosides and nucleotides (HS) for selective and efficient transport from an aqueous phase to a $CHCl_3$ phase. The transport mechanism is entirely new, differing from any previously reported ones, involving the formation of lipophilic 1:1 S^-−ZnL complexes under slightly alkaline conditions. In comparison to the hydrophilic nucleosides and nucleotides, lipophilic nucleosides such as AZT were extracted into an organic layer far better than the complex formation constants, K(S^-−ZnL), in aqueous solution predicted. This is probably due to 1−2 orders of magnitude more favorable complexation of AZT with the lipophilic Zn^{2+}−cyclen **2e** in aqua-organic phase, as supported by the higher complexation constant for AZT^-−**2e** determined by the isothermal calorimetric titration of AZT with **2e** in micellar solutions containing Triton X-100. The $CHCl_3$-extracted nucleosides and nucleotides in the form of S^-−**2e** complexes were dissociated and released as free forms, HS, into acidic (pH 6) aqueous solution (Aq II), although lipophilic AZT was harder in the dissociation. Thus, selective and efficient membrane transport of dT, U, and the relevant derivatives from alkaline pH to acidic pH solutions would be possible by **2e**, which is coupled with proton antiport. In biological applications, these new lipophilic Zn^{2+}−cyclens might make a novel selective and efficient delivery system for dT homologue drugs.[37]

Experimental Section

General Information. The reagents and solvents were purchased at the highest commercial quality and used without further purification. 3′-Azido-3′-deoxythymidine (AZT), ftorafur (Ff), thymidine 5′-monophosphate (5′-dTMP), thymidine 5′-triphosphate (5′-dTTP), 3′-azido-3′-deoxythymidine 5′-monophosphate (5′-AZTMP), and thymidilyl(3′-5′)thymidine (d(TpT)) were purchased from Sigma. $CHCl_3$ was washed with water, dried over $CaCl_2$, and redistilled from CaH_2. All aqueous solutions were prepared using deionized and distilled water. IR spectra were recorded on a Shimadzu FTIR-4200 spectrometer. IR spectra for an amorphous solid or an oily compound were recorded by applying the sample on IR cards (Type 62, 3M Co. LTD). ^{1}H NMR spectra were recorded on a JEOL Lambda (500 MHz) spectrometer. Tetramethylsilane in $CDCl_3$ and CD_3OD and 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt in D_2O were used as internal references for ^{1}H and ^{13}C NMR measurements. The pD values in D_2O were corrected for a deuterium isotope effect using pD = [pH-meter reading] + 0.40. FAB mass spectra were recorded on a JEOL JMS-SX102. Elemental analysis was performed on a Perkin-Elmer CHN Analyzer 2400. Thin-layer chromatography (TLC) and silica gel column chromatography were performed using Merck Art. 5554 (silica gel) TLC plate and Fuji Silysia Chemical FL-100D (silica gel), respectively.

UV spectra were recorded on a Hitachi U-3500 spectrophotometer at 25.0 ± 0.1 °C. Buffer (50 mM) solutions (CAPS, pH 10.0; CHES, pH 9.0; EPPS, pH 8.0; HEPES, pH 7.0 and 7.6; and MES, pH 6.0 and 5.0) were used, and the ionic strengths were all adjusted to 0.10 with $NaNO_3$. The Good's buffers (pK_a at 20 °C) were purchased from Dojindo and were used without further purification: CAPS (3-(cyclohexylamino)propanesulfonic acid, 10.4), CHES (2-(cyclohexylamino)ethanesulfonic acid, 9.5), EPPS (3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid, 8.0), HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, 7.6), and MES (2-morpholinoethanesulfonic acid, 6.2).

Synthesis of 1-Propyl-4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (5b). A solution of 1,4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (**4**)[24] (1.6 g, 3.4 mmol)

(37) The preliminary experiment showed that AZT uptake into human fibroblast cell, TIG-3, was enhanced by **2e** (unpublished results by S. Aoki, K. Anno, T. Ide, and E. Kimura).

and 1-bromopropane (5.4 g, 44 mmol) was stirred with Na_2CO_3 (1.8 g, 17 mmol) and *n*-Bu_4NI (2.5 g, 6.8 mmol) at 80 °C for 3 day. After the reaction mixture was cooled, the insoluble compounds were filtered off and washed with $CHCl_3$. The filtrate was concentrated under reduced pressure, and the remaining residue was purified by silica gel column chromatography (hexane/AcOEt) to yield **5b** (1.5 g, 85%) as a colorless amorphous solid. IR (IR card): 2975, 2876, 1699, 1653, 1472, 1418, 1366, 1250, 1123, 1107, 1040, 970, 772 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 7.3 Hz, CH_3), 1.45−1.50 (29H, m, $CH_2CH_2CH_3$ + $C(CH_3)_3$), 2.47 (2H, t like, NCH_2Et), 2.57−2.69 (4H, m, NCH_2), 3.20−3.58 (12H, m, NCH_2). ^{13}C NMR ($CDCl_3$): δ 12.09, 28.55, 28.73, 47.63, 48.51, 49.97, 53.95, 55.10, 55.25, 79.27, 79.30, 79.49, 155.51, 155.75, 156.19.

1-Octyl-4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (5c) was prepared using the procedure described above for **5b** (CH_3CN was used as a solvent) from **4** and 1-bromooctane; 90% yield (a colorless amorphous solid). IR (IR card): 2975, 2859, 1699, 1472, 1414, 1366, 1267, 1175, 1109, 1042, 976, 951, 861, 773 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 7.0 Hz, CH_3), 1.20−1.33 (10H, m, CCH_2C), 1.38−1.49 (29H, m, CCH_2C + $C(CH_3)_3$), 2.49 (2H, t like, $NCH_2C_7H_{15}$), 2.56−2.68 (4H, m, NCH_2), 3.20−3.58 (12H, m, N CH_2). ^{13}C NMR ($CDCl_3$): δ 14.07, 22.61, 27.91, 28.09, 28.43, 28.55, 28.73, 29.31, 29.62, 31.85, 47.63, 48.03, 49.95, 53.00, 53.90, 55.15, 155.51, 155.75, 155.16.

1-Dodecyl-4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (5d) was prepared using the procedure described above (in $CH_3CN/CHCl_3$ = 1:1) from **4** and 1-bromododecane; 79% yield (a colorless amorphous solid). IR (IR card): 2975, 2926, 2855, 1690, 1455, 1412, 1364, 1171, 976, 774 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 7.4 Hz, CH_3), 1.26−1.32 (18H, m, CCH_2C), 1.43−1.49 (29H, m, CCH_2C + $C(CH_3)_3$), 2.49 (2H, t like, $NCH_2C_{11}H_{23}$), 2.58−2.69 (4H, s, NCH_2), 3.20−3.60 (12H, m, NCH_2). ^{13}C NMR ($CDCl_3$): δ 14.06, 22.65, 27.88, 28.51, 28.70, 29.31, 29.60, 29.61, 29.63, 29.65, 31.89, 47.62, 48.00, 50.26, 52.96, 53.83, 55.13, 79.23, 79.44, 155.48, 155.70, 156.13.

1-Hexadecyl-4,7,10-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane (5e) was prepared using the procedure described above for **5b** (in $CH_3CN/CHCl_3$ = 1:1) from **4** and 1-bromohexadecane; 82% yield (a colorless oil). IR (IR card): 2926, 2855, 1699, 1472, 1412, 1364, 1173, 976, 860, 772, 557 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 7.1 Hz, CH_3), 1.2−1.31 (26H, m, CCH_2C), 1.4−1.49 (29H, brs, CCH_2C + $C(CH_3)_3$), 2.49 (2H, t like, $NCH_2C_{15}H_{31}$), 2.57−2.68 (4H, m, NCH_2), 3.2−3.6 (12H, m, NCH_2). ^{13}C NMR ($CDCl_3$): δ 14.10, 22.66, 22.70, 22.83, 24.20, 25.31, 26.96, 27.92, 28.55, 28.74, 29.37, 29.46, 29.65, 29.68, 29.69, 29.69, 29.71, 29.72, 31.60, 31.94, 47.67, 48.04, 49.96, 52.99, 53.88, 55.14, 79.27, 79.48, 155.51, 155.74, 156.18.

Synthesis of 1-Propyl-1,4,7,10-tetraazacyclododecane Trihydrochloric Acid Salt (6b·3HCl). To a solution of **5b** (1.5 g, 2.9 mmol) in MeOH (35 mL) was added slowly 36% aqueous HCl (5 mL) at room temperature, and the whole was stirred for 2 day at room temperature. After the solvent was evaporated, the resulting colorless solid was recrystallized from MeOH/H_2O to obtain **6b**·3HCl (0.9 g, 94% yield) as colorless needles: mp 262−265 °C. IR (KBr pellet): 2976, 2872, 2736, 1599, 1568, 1472, 1443, 1368, 997, 770 cm^{-1}. Anal. Calcd for $C_{11}H_{29}N_4Cl_3$: C, 40.81; H, 9.03; N, 17.31. Found: C, 41.04; H, 9.22; N, 17.38. FAB-MS *m*/*z* 215 [(M − 3HCl + H)$^+$, $C_{11}H_{27}N_4$]. The $CDCl_3$ solution of the acid-free **6b** for NMR experiment was prepared by extraction with $CDCl_3$ from a solution of **6b** in D_2O. ^1H NMR ($CDCl_3$): δ 0.90 (3H, t, J = 7.3 Hz, CH_3), 1.50 (2H, tq, J = 7.0, 7.3 Hz, $CH_2CH_2CH_3$), 2.38 (2H, dd like, J = 6.1, 7.8 Hz, NCH_2), 2.51−2.53 (4H, m, NCH_2), 2.56−2.58 (4H, m, NCH_2), 2.62−2.64 (4H, m, NCH_2), 2.77−2.79 (4H, m, NCH_2). ^{13}C NMR ($CDCl_3$): δ 11.96, 20.54, 45.32, 46.22, 47.16, 51.74, 56.58.

1-Octyl-1,4,7,10-tetraazacyclododecane Tetrahydrochloric Acid Salt (6c·4HCl) was prepared using the procedure described above from **5c**; 88% yield (colorless prisms) after recrystallized from 3 N HCl: dec 207 °C. IR (KBr): 2993, 2853, 2531, 1576, 1495, 1460, 1420, 1379, 1020, 829, 758 cm^{-1}. Anal. Calcd for $C_{16}H_{40}N_4Cl_4$: C, 44.66; H, 9.37; N, 13.02. Found: C, 44.93; H, 9.54; N, 13.07. FAB-MS *m*/*z* 285 [(M − 4HCl + H)$^+$, $C_{16}H_{37}N_4$]. The $CDCl_3$ solution was prepared as described above. ^1H NMR ($CDCl_3$): δ 0.86 (3H, t, J = 7.0 Hz, CH_3), 1.20−1.27 (10H, m, CCH_2C), 1.44−1.51 (2H, m, CCH_2C), 2.41 (2H, dd like, J = 7.2, 7.7 Hz, NCH_2), 2.51−2.53 (4H, m, NCH_2), 2.55−2.58 (4H, m, NCH_2), 2.62−2.64 (4H, m, NCH_2), 2.77−2.79 (4H, m, NCH_2). ^{13}C NMR ($CDCl_3$): δ 14.11, 22.68, 28.39, 27.55, 29.36, 29.53, 31.88, 45.30, 46.21, 47.14, 51.73, 54.70.

1-Dodecyl-1,4,7,10-tetraazacyclododecane Trihydrochloric Acid Salt Dihydrate (6d·3HCl·2H$_2$O) was prepared using the procedure described above from **5d** in 95% yield (colorless needles) after recrystallized from 3 N HCl: dec 215 °C. IR (KBr): 2923, 2849, 2678, 2454, 1576, 1497, 1460, 1422, 1256, 1044, 995, 829, 762, 725 cm^{-1}. Anal. Calcd for $C_{20}H_{51}N_4O_2Cl_3$: C, 49.43; H, 10.58; N, 11.53. Found: C, 49.63; H, 10.50; N, 11.45. FAB-MS *m*/*z* 341 [(M − 3HCl + H)$^+$, $C_{20}H_{45}N_4$]. The $CDCl_3$ solution of the acid-free **6d** for NMR experiment was prepared as described above. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 7.0 Hz, CH_3), 1.25−1.31 (18H, m, CCH_2C), 1.45−1.48 (2H, m, CCH_2C), 2.41 (2H, dd like, J = 7.3, 7.4 Hz, NCH_2), 2.51−2.53 (4H, m, NCH_2), 2.55−2.57 (4H, m, NCH_2), 2.61−2.63 (4H, m, NCH_2), 2.77−2.79 (4H, m, NCH_2). ^{13}C NMR ($CDCl_3$): δ 14.10, 22.70, 27.36, 27.56, 29.37, 29.60, 29.67, 29.69, 29.71, 29.72, 31.95, 45.24, 46.17, 47.08, 51.69, 54.68.

1-Hexadecyl-1,4,7,10-tetraazacyclododecane Trihydrochloric Acid Salt Dihydrate (6e·3HCl·2H$_2$O) was prepared using the procedure described above from **5e** in 89% yield (colorless needles).[23]

Synthesis of 1-Dodecyl-1,4,7,10-tetraazacyclododecane Zn(ClO$_4$)$_2$ Complex (2d·(ClO$_4$)$_2$). The trihydrochloric acid salt of **6d**, **6d**·3HCl·$2H_2O$ (0.31 g, 0.63 mmol), was dissolved in alkaline aqueous solution (50 mL, pH 12), and then the solution was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layer was dried over anhydrous K_2CO_3 and filtered. The solvent was evaporated in a reduced pressure to obtain acid-free ligand **6d** as a colorless viscous oil, which was successively dissolved in MeOH (20 mL), to which $Zn(ClO_4)_2$·$6H_2O$ (0.26 g, 0.7 mmol) was added and stirred at room temperature for 1 h. The reaction mixture was evaporated, and the resulting solid was recrystallized from AcOEt to give **2d**·$(ClO_4)_2$ (0.24 g, 64% yield) as colorless needles: mp 168−170 °C. IR (KBr): 3140, 2919, 1472, 1146, 1117, 1092, 637, 630 cm^{-1}. ^1H NMR (CD_3OD): δ 0.90 (3H, t, J = 7.2 Hz, CH_3), 1.24−1.40 (18H, m, CCH_2C), 1.58−1.63 (2H, m, CCH_2C), 2.78−3.14 (18H, m, NCH_2), 4.05−4.12 (1H, m, N*H*), 4.15−4.21 (2H, m, N*H*). ^{13}C NMR (CD_3OD): δ 14.43, 23.33, 23.73, 28.37, 30.46, 30.55, 30.71, 30.76, 30.78, 33.08, 43.81, 45.10, 45.21, 46.06, 51.03, 54.48. Anal. Calcd for $C_{20}H_{44}N_4O_8Cl_2$: C, 39.71; H, 7.33; N, 9.26. Found: C, 39.77; H, 7.66; N, 8.99. FAB-MS *m*/*z* 503 [(M − ClO_4)$^+$, $C_{20}H_{44}N_4O_4Cl^{64}Zn$], 505 [(M − ClO_4)$^+$, $C_{20}H_{44}N_4O_4Cl^{66}Zn$], 507 [(M − ClO_4)$^+$, $C_{20}H_{44}N_4O_4Cl^{68}Zn$].

1-Hexadecyl-1,4,7,10-tetraazacyclododecane Zn(ClO$_4$)$_2$ Complex Dihydrate (2e·(ClO$_4$)$_2$·2H$_2$O) was synthesized from **6e**·3HCl·$2H_2O$ according to our previous paper.[23]

Extraction of Nucleosides and Nucleotides from an Aqueous Layer into a CHCl$_3$ Layer. For the extraction experiments, all buffer solutions were vigorously mixed with distilled $CHCl_3$ and separated by centrifuge (3000 rpm × 30 min). A solution of a carrier (**2d**,**e**) (1.0 mM) in $CHCl_3$ (2.0 mL) was vigorously stirred in a sample tube using a stirring bar at 25 ± 0.1 °C for 30 min with a 1.0 mM (or 0.5 mM) solution of a nucleoside or a nucleotide in a 50 mM buffer with I = 0.10 ($NaNO_3$). For the extraction of dT by **2b**,**c**, 50 mM CHES solution (pH 9.0, 2.0 mL) containing 1 mM **6b** (or **6c**), 1 mM $Zn(ClO_4)_2$, and 1 mM dT was prepared and stirred with $CHCl_3$ (2.0 mL). The mixture of two layers were centrifuged (3000 rpm × 10 min at 25 ± 0.1 °C) for a complete separation. The pH values of aqueous layer did not change before and after extraction. The extraction efficiency of a nucleoside or a nucleotide was determined by measuring the remaining nucleoside in the aqueous phase by the UV absorption spectrum (reproducibility ±1%). These values were also checked by measuring the amount of transferred nucleosides or nucleotides into $CDCl_3$ (containing 1 mM naphthalene as an internal reference) by ^1H NMR. The experiments were repeated twice or three times, and the averaged values are listed in Tables 1 and 2. Control experiments without a carrier were conducted simultaneously.

The molar absorption coefficients (ϵ) (M^{-1}·cm^{-1}) of the nucleosides and nucleotides at 25 °C used for determination of their concentrations in aqueous buffer solutions are as follows: dT, λ_{max} 267 nm (ϵ 9.6 ×

10^3 at pH 6.0 and pH 7.0, 9.5×10^3 at pH 7.6 and pH 8.0, 9.1×10^3 at pH 9.0, and 7.8×10^3 at pH 10.0); U, λ_{max} 261 nm (ϵ 8.5×10^3 at pH 9.0), dC, λ_{max} 271 nm (ϵ 8.1×10^3 at pH 9.0); dA, λ_{max} 260 nm (ϵ 13.8×10^3 at pH 9.0), dG, λ_{max} 254 nm (ϵ 11.6×10^3 at pH 9.0); AZT, λ_{max} 267 nm (ϵ 10.2×10^3 at pH 6.0, 10.1×10^3 at pH 7.0, pH 7.6, and pH 8.0, 9.5×10^3 at pH 9.0, and 8.5×10^3 at pH 10.0); 1-MeT, λ_{max} 272 nm (ϵ 8.1×10^3 at pH 7.6 and 8.0×10^3 at pH 9.0); Ff, λ_{max} 270 nm (ϵ 8.3×10^3 at pH 7.0 and 6.8×10^3 at pH 9.0), 5′-dTMP, λ_{max} 267 nm (ϵ 9.5×10^3 at pH 9.0); 5′-dTTP, λ_{max} 267 nm (ϵ 8.9×10^3 at pH 9.0); 5′-AZTMP, λ_{max} 265 nm (ϵ 9.4×10^3 at pH 7.6 and 9.0×10^3 at pH 9.0); d(TpT), λ_{max} 267 nm (ϵ 17.1×10^3 at pH 9.0); and 5′-AMP, λ_{max} 260 nm (ϵ 14.5×10^3 at pH 7.6).

Potentiometric pH Titrations. The preparation of the test solutions and the calibration method of the electrode system (Orion Research Expandable Ion Analyzer EA920 and Orion Research Ross Combination pH Electrode 8102BN) were described earlier.[21−24] All of the test solutions (50 mL) were kept under an argon (>99.999% purity) atmosphere. The potentiometric pH titrations were carried out with $I = 0.10$ ($NaNO_3$) at 25.0 ± 0.1 °C, and at least two independent titrations were performed. Deprotonation constants of Zn^{2+}-bound water K'_2 (=[HO^--bound species][H^+]/[H_2O-bound species]) and complex affinity constants $K(S^- - ZnL)$ (= [S^-−ZnL]/[ZnL][S^-], where ZnL is **2c** and S^- is deprotonated dT or AZT) were determined by means of the program BEST.[33] All the σ fit values defined in the program are smaller than 0.005. The K_W ($=a_{H^+} \cdot a_{OH^-}$), K'_W (=[H^+][OH^-]), and f_{H^+} values used at 25 °C are $10^{-14.00}$, $10^{-13.79}$, and 0.825. The corresponding mixed constants, K_2 (=[HO^--bound species]a_{H^+}/[H_2O-bound species]), are derived using $[H^+] = a_{H^+}/f_{H^+}$. The species distribution values (%) against pH (=−log[H^+] + 0.084) were obtained using the program SPE.[33]

Isothermal Calorimetric Titrations.[34] The heats of 1:1 complexation of **2a**,**e** with dT or AZT were recorded on a Calorimetry Science Corp. Isothermal Titration Calorimeter 4200 at 25.0 °C and pH 7.6 (50 mM HEPES buffer with $I = 0.10$ ($NaNO_3$)). The calorimeter was calibrated by heat (474.7 mJ) of protonation of tris(hydroxymethyl)-aminomethane (250 mM, 1.0 mL) by 10-μL injection of 1.00 mM aqueous HCl at 25.0 °C. The solution (1.0 mL) of 1.7 mM **2a** or 1.50 mM (or 0.73 mM) **2e** in 50 mM HEPES (with or without 10 mM Triton X-100) was put into a calorimeter cell, to which the solution of dT (40 mM) or AZT (33 mM) in 50 mM HEPES was loaded. At least two titrations were carried out. The obtained calorimetric data was analyzed for ΔH values and apparent complexation constants, K_{app}, using the program Data Works and Bind Works provided by the Calorimetry Sciences Corp.

Liquid Membrane Transport of dT and AZT through a $CHCl_3$ phase. The transport experiment was conducted by using a U-type cell of 1.2-cm diameter and 4.5-cm distance from center to center between the two legs. Onto the bottom of the U-cell, 1 mM solution of **2e** in $CHCl_3$ (10 mL) was loaded. Atop the $CHCl_3$ phase in one arm, 5.0 mL of 50 mM CHES at pH 9.0 with $I = 0.1$ ($NaNO_3$) solution containing 1 mM nucleoside was placed as a nucleoside-source phase (Aq I). In the other arm, 5.0 mL of 50 mM MES at pH 5.0 with $I = 0.1$ ($NaNO_3$) was placed as a nucleoside-receiving phase (Aq II). The cell was held in the same position to a magnetic stirring motor in all experiments, and the $CHCl_3$ layer was rotated slowly at a constant speed (ca. 130 rpm) with a magnetic stirrer. The concentration of dT or AZT in Aq I and Aq II was monitored by the UV absorption at specified time intervals. The pHs of both aqueous phase were readjusted carefully by the addition of a tiny amount of 1 N NaOH or 1 N HCl to keep the pH fluctuation within ±0.1 pH unit. Control experiments without **2e** were carried out simultaneously.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **5b**−**e**, **6b**−**d**, and **2d** (17 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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