Highly Selective Recognition of Thymidine Mono- and Diphosphate Nucleotides in Aqueous Solution by Ditopic Receptors Zinc(II)−Bis(cyclen) Complexes (Cyclen = 1,4,7,10-Tetraazacyclododecane)

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Abstract: Highly selective and efficient recognition of thymidine and uridine nucleotides such as 3′-dTMP (thymidine 3′-monophosphate), 5′-dTMP (thymidine 5′-monophosphate), 2′-UMP (uridine 2′-monophosphate), 3′-UMP (uridine 3′-monophosphate), 5′-UMP (uridine 5′-monophosphate), 5′-dTDP (thymidine 5′-diphosphate), 5′-dTTP (thymidine 5′-triphosphate), AZTMP (3′-azido-3′-deoxythymidine 5′-monophosphate), and AZTDP (3′-azido-3′-deoxythymidine 5′-diphosphate) with ditopic dimeric zinc(II) complexes of macrocyclic 12-membered tetramines, *meta*- and *para*-xylyl-bis(Zn^{2+}-cyclen)s (Zn_2L^4 and Zn_2L^5) (cyclen = 1,4,7,10-tetraazacyclododecane) has been studied by potentiometric pH titration, isothermal titration calorimetry, UV spectrophotometric titration, and NMR titration. The apparent 1:1 complexation constants for 5′-dTMP, K_{app} ($= [(Zn_2L)-(S^- - OPO_3^{2-})]/[Zn_2L]_{free}[S-OPO_3^{2-}]_{free}$ (M^{-1})), where S^- denotes the imide-deprotonated thymine part), with Zn_2L^4 or Zn_2L^5 determined by potentiometric pH titration showed a more stable complex with Zn_2L^5 ($\log K_{app} = 6.4$) than with Zn_2L^4 ($\log K_{app} = 5.5$) at pH 7.6 with $I = 0.1$ ($NaNO_3$) and 25 °C. These values are much greater than $\log K_{app}$ ($K_{app} = [ZnL^3-dT^-]/[ZnL^3]_{free}[dT]_{free}$ (M^{-1})) of 3.2 for a nucleoside thymidine (dT) complex with a monomeric Zn^{2+}-benzylcyclen (ZnL^3). The 1:1 complexation was confirmed by the FAB mass spectroscopic data for Zn_2L with 3′- and 5′-dTMP. The combined data from the spectrophotometric UV titration and ^1H NMR measurements of 5′-dTMP and 5′-dTDP with Zn_2L^5 in D_2O at pD 7.8 and 35 °C indicated that the terminal phosphate dianion interacted with one of the (Zn^{2+}-cyclen)s and the imide anion of dT bound to the other Zn^{2+}-cyclen.

Introduction

Among various nucleoside anti-HIV drugs, typically prescribed now are 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxycytidine (ddC), and 2′,3′-dideoxyadenosine (ddA).[1−3] These drugs go into cells mainly by simple passive diffusion[4] and then undergo phosphorylation to each ultimate nucleotide triphosphate derivative to be active against HIV reverse transcriptase.[5] The step from their monophosphate to diphosphate esters is rate-determining in the phosphorylation,[5a] and some kinase-deficient cells such as macrophages become reservoirs for HIV.[6] Therefore, development of lipophilic carriers for these nucleotide phosphates would be of great help to increase the efficacy of the drugs.

Until now, a number of artificial carriers for nucleotides have been reported.[7−13] Examples include polyammonium cations[7−9] (e.g., **1**[7]), a guanidino cation receptor,[10] cationic cyclophane

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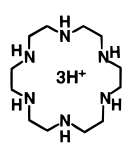
(QCP66),[11] bis(intercaland) receptors,[12] and mono- and dicationic sapphyrins (e.g., monocationic **2**).[13] However, most of these were monotopic receptors for phosphate anions and lacked base selectivities. Exceptionally, the ditopic receptor **2** succeeded in extracting 5′-AMP and 5′-GMP from an aqueous phase of pH 5.0−7.0 to $CHCl_3$ phase due to recognition of A and G by the cytosine moiety and of the phosphate dianion part by the protonated sapphyrin.[13a] On the other hand, selective carriers for thymidine (dT) or uridine (U) nucleotides are unknown, which, if available, would be extremely useful, for instance, in effective administration of AZT.



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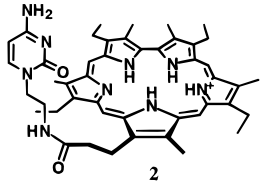
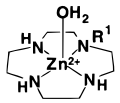
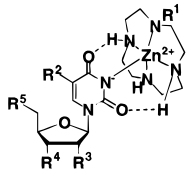


2

We earlier discovered that Zn^{2+}-cyclen complex **3a** (ZnL^1) acted as a monotopic receptor for dT and U among all of the nucleosides at physiological pH in aqueous solution, yielding stable 1:1 complexes **4** by a Zn^{2+}-imide N^- anion bonding and two complementary hydrogen bonds (cyclen = 1,4,7,10-tetraazacyclododecane).[14,15] Recently, a lipophilic hexadecylcyclen **3b** (ZnL^2)[16] was developed as a new type of dT-selective transporter of dT, U, AZT, and the relevant nucleoside compounds.[17] We also found that **3a** acted as a good monotopic receptor for dianionic phosphate monoesters to produce **5** in aqueous solution.[18] For example, the 1:1 complexation constants, log K_{app} (K_{app} = [**5a** (or **5b**)]/[**3c**]$_{free}$[phosphate]$_{free}$ (M^{-1})), of **3c** with dianions of mono(4-nitrophenyl) phosphate and mono(phenyl) phosphate were 3.0 and 3.6, respectively, at pH 7.6 and 25 °C with I = 0.1 ($NaNO_3$).[18,19]



3a : R^1 = H (ZnL^1)
3b : R^1 = $C_{16}H_{33}$ (ZnL^2)
3c : R^1 = CH_2Ph (ZnL^3)



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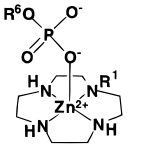

5a : R^6 = 
5b : R^6 =

It is now of interest to investigate whether bis(Zn^{2+}-cyclen) complexes, *m*-dimer Zn_2L^4 **6** and *p*-dimer Zn_2L^5 **7**, that we synthesized earlier as ditopic receptors for dianionic barbital[20] could be good ditopic receptors[15j,21] for dT nucleotides to produce 1:1 complexes such as **8**−**11**. Moreover, **6** and **7** and their free ligands were discovered to possess an extremely potent anti-HIV activity.[22] Herein we present the selective recognition of thymidine 3′-monophosphate (3′-dTMP), thymidine 5′-monophosphate (5′-dTMP), uridine 2′-monophosphate (2′-UMP), uridine 3′-monophosphate (3′-UMP), uridine 5′-monophosphate (5′-UMP), thymidine 5′-diphosphate (5′-dTDP), thymidine 5′-triphosphate (5′-dTTP), AZT 5′-monophosphate (AZTMP), and AZT 5′-diphosphate (AZTDP) by **6** and **7** (Scheme 1).

Results and Discussion

FAB (Fast Atom Bombardment) Mass Study of 1:1 dTMP Complexes with Zn_2L^4 6 and Zn_2L^5 7. To confirm the 1:1 complexation of dTMPs with bis(Zn^{2+}-cyclen)s, we ran FAB mass (positive) experiment for a mixture of 3′- or 5′-dTMP (5 mM) and **6** or **7** (5 mM) in H_2O (pH 7.5 ± 0.1). The experimental mass spectra for 1:1 **6**/(3′-dTMP)$^{3-}$ and 1:1 **7**/(5′-dTMP)$^{3-}$ (both at *m*/*z* 895 with Zn isotopic peaks at *m*/*z* 893, 897, and 899 etc.) fit to the theoretical mass distribution spectra ($C_{34}H_{58}N_{10}O_8PZn_2$) for **8** and **11** (see Supporting Information).

The Potentiometric pH Titration of 3′-dTMP, 5′-dTMP, 3′-UMP, and 5′-UMP with Zn_2L^4 6 and Zn_2L^5 7. The potentiometric pH titration of thymidine mononucleotide (3′- and 5′-dTMP) and uridine mononucleotide (3′- and 5′-UMP) with **6** and **7** at 25 °C with I = 0.10 ($NaNO_3$) was studied to

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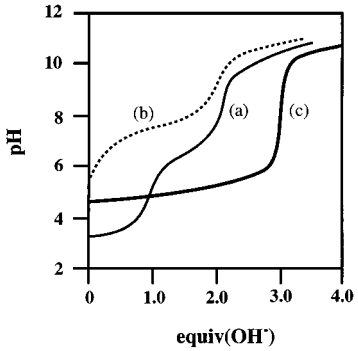
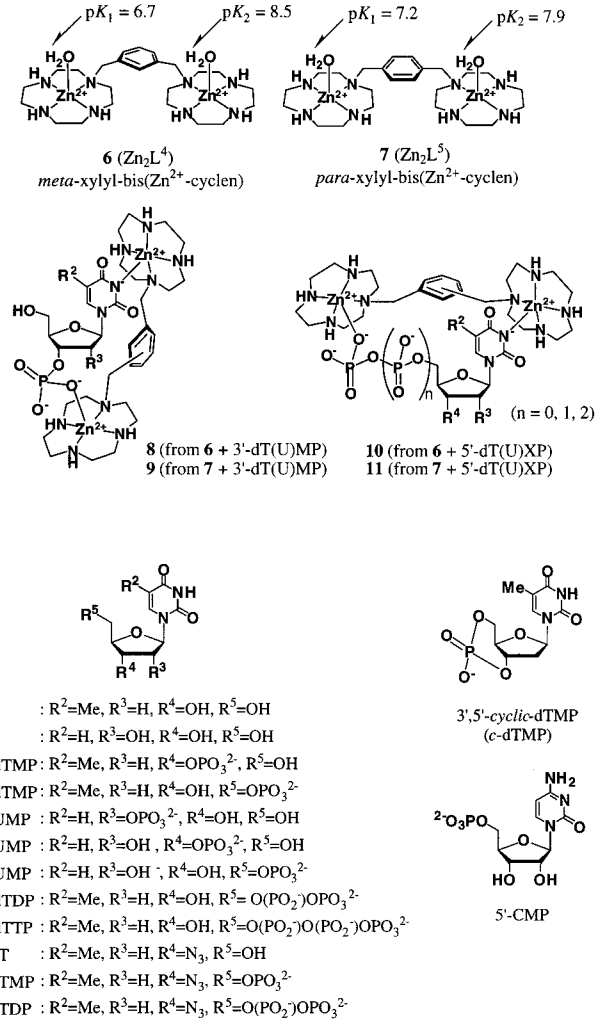


Figure 1. Typical pH titration curves of (a) 1 mM 5′-dTMP, (b) 1 mM Zn_2L^5 (**7**), and (c) 1 mM 5′-dTMP + 1 mM Zn_2L^5 (**7**) at 25 °C with $I = 0.1$ ($NaNO_3$), where equiv(OH^-) is the number of equivalents of base added.

Scheme 1



see details in the 1:1 complexation. Figure 1 shows the typical titration curves for 1 mM 5′-dTMP (curve a), 1 mM **7** (curve b), and 1 mM 5′-dTMP + 1 mM **7** (curve c).

The deprotonation constants of Zn(II)-bound waters in **7**, pK_1 and pK_2 (defined by eqs 1 and 2), were determined from Figure 1b to be 7.2 and 7.9 at 25 °C with $I = 0.10$ ($NaNO_3$), as earlier reported.[20] From Figure 1a, the deprotonation constants of 5′-dTMP, pK_3, pK_4, and pK_5 (defined by eqs 3−5, where S is thymine part and $S-OPO_3H_2$, $S-OPO_3H^-$, $S-OPO_3^{2-}$, and $S^--OPO_3^{2-}$ denote free, mono-deprotonated, di-deprotonated, and tri-deprotonated species of 5′-dTMP, respectively) were <2, 6.5 ± 0.1, and 10.2 ± 0.1, respectively. The intrinsic complexation constant, log K_s defined by eq 6, at 25 °C with $I = 0.10$ ($NaNO_3$) was determined to be 9.6 ± 0.1 by the program "BEST".[23] An apparent complex formation constant, log K_{app}, defined by eqs 7−9, at pH 7.6 was then calculated to be 6.4.

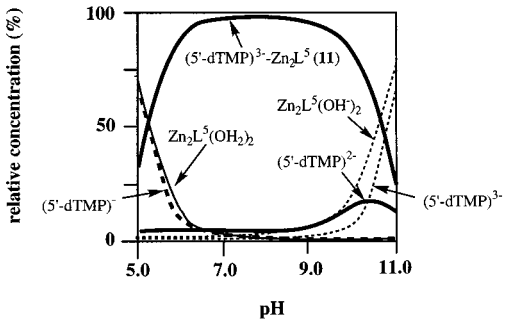


Figure 2. Speciation diagram for the 5′-dTMP (1 mM) and Zn_2L^5 (**7**) (1 mM) as a function of pH at 25 °C with $I = 0.1$ ($NaNO_3$). Other species which exist in less than 5% are omitted for clarity.

$$Zn_2L(OH_2)_2 \rightleftarrows Zn_2L(OH_2)(OH^-) + H^+:$$
$$K_1 = [Zn_2L(OH_2)(OH^-)]a_{H^+}/[Zn_2L(OH_2)_2] \quad (1)$$

$$Zn_2L(OH_2)(OH^-) \rightleftarrows Zn_2L(OH^-)_2 + H^+:$$
$$K_2 = [Zn_2L(OH^-)_2]a_{H^+}/[Zn_2L(OH_2)(OH^-)] \quad (2)$$

$$S-OPO_3H_2 \rightleftarrows S-OPO_3H^- + H^+:$$
$$K_3 = [S-OPO_3H^-]a_{H^+}/[S-OPO_3H_2] \quad (3)$$

$$S-OPO_3H^- \rightleftarrows S-OPO_3^{2-} + H^+:$$
$$K_4 = [S-OPO_3^{2-}]a_{H^+}/[S-OPO_3H^-] \quad (4)$$

$$S-OPO_3^{2-} \rightleftarrows S^--OPO_3^{2-} + H^+:$$
$$K_5 = [S^--OPO_3^{2-}]a_{H^+}/[S-OPO_3^{2-}] \quad (5)$$

$$Zn_2L + S^--OPO_3^{2-} \rightleftarrows Zn_2L-(S^--OPO_3^{2-}):$$
$$K_s = [(Zn_2L)-(S^--OPO_3^{2-})]/[Zn_2L(OH_2)_2][S^--OPO_3^{2-}](M^{-1}) \quad (6)$$

$$K_{app} = [(Zn_2L)-(S^--OPO_3^{2-})]/[Zn_2L]_{free}[S-OPO_3^{2-}]_{free}$$
$$(\text{at designated pH})(M^{-1}) \quad (7)$$

$$[Zn_2L]_{free} = [Zn_2L(OH_2)_2] + [Zn_2L(OH_2)(OH^-)] + [Zn_2L(OH^-)_2] \quad (8)$$

$$[S-OPO_3^{2-}]_{free} = [S-OPO_3H_2] + [S-OPO_3H^-] + [S-OPO_3^{2-}] + [S^--OPO_3^{2-}] \quad (9)$$

Figure 2 depicts a speciation diagram for 5′-dTMP (1 mM) and **7** (1 mM) as a function of pH at 25 °C with $I = 0.1$ ($NaNO_3$), which shows that the population of the 1:1 complex (**11**) is over 95% at 6.6 < pH < 8.8.

The log K_s and log K_{app} values for the 1:1 complexes, **8** (**6**−(3′-dTMP) or **6**−(3′-UMP)), **9** (**7**−(3′-dTMP) or **7**−(3′-UMP)), **10** (**6**−(5′-dTMP) or **6**−(5′-UMP)), and **11** (**7**−(5′-dTMP) or

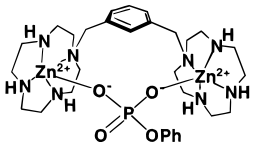
Table 1. Apparent Complexation Constants (log K_{app}) for Imide-Containing Nucleotides with Zinc(II)-cyclen Complexes at pH 7.6 and 25 °C Determined by Potentiometric pH Titration,[a] Isothermal Titration Calorimetry (in 50 mM HEPES Buffer),[b] and UV Titration (in 50 mM HEPES Buffer)[c] with I = 0.1 ($NaNO_3$)

	3c	**6**	**7**
dT	3.2[a] (5.7)[d]	3.2, 3.2[b]	
	3.4[c]	(**6**:dT = 1:2)	
c-dTMP	3.3[c]	3.5, 3.5[b]	
		(**6**:*c*-dTMP = 1:2)	
5′-CMP	3.3[a] (3.7)[d]	3.2[a] (4.3)[d]	
	3.3[b]	3.4[b]	
3′-dTMP		5.2[a] (8.6)[d]	5.9[a] (8.9)[d]
		5.3[b]	5.8[b,e]
		5.4[c,f]	5.8[c,f]
5′-dTMP	3.4, 3.4[b]	5.5[a] (9.3)[d]	6.4[a] (9.6)[d]
	(**3c**:5′-dTMP = 2:1)	5.5[b]	> 6[b,e]
		5.7[c,f]	> 6[c,f]
2′-UMP		5.7[b]	
3′-UMP		4.8[a] (7.8)[d]	5.5[a] (8.5)[d]
		5.2[c,f]	5.7[c,f]
5′-UMP		5.4[a] (8.3)[d]	6.2[a] (8.8)[d]
		5.5[c,f]	> 6[b]
			> 6[c,f]
5′-dTDP		5.6[b]	> 6[b]
		5.5[c,f]	> 6[c,f]
5′-dTTP		5.0[b]	5.6[b]
5′-AZTMP		5.5[b]	> 6[b,e]
		5.7[c,f]	> 6[c,f]
5′-AZTDP		5.3[b]	5.9[b]
		5.5[c]	> 6[c,f]

[a,b,c] For the definition of K_{app} and experiment conditions, see the text. The same titration was carried out at least twice, and the experimental errors were ±3%. [d] The intrinsic complexation constants K_s defined by eq 6 in the text. [e] Titrations were carried out at [5′-dTMP] = 0.2 mM and 0.1 mM, and the average values are listed. [f] Titrations were carried out at [nucleotide] = 0.1 mM and 50 μM, and the average values are listed.

7−(5′-UMP)) determined by the similar potentiometric pH titrations are also included in Table 1.[24,25]

The potentiometric pH titration of 5′-CMP (non-dT mononucleotide)[26] with **6** gave the log K_{app} value of 3.2 ± 0.1 at pH 7.6 (log K_s = 4.3 ± 0.1), almost the same as log K_{app} of 3.5 (log K_s = 4.6) for the 1:1 complex **12** from **6** and mono(phenyl) phosphate at pH 7.6.[18c]



12

Isothermal Titration Calorimetry of dT (U) Nucleotides with Zn_2L^4 6 and Zn_2L^5 7. We measured the complexation constants of all the dT nucleosides and nucleotides with a monotopic ZnL^3 (**3c**) and the ditopic receptors **6** and **7** by isothermal titration calorimetry (ITC)[27] at pH 7.6 (50 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C. In comparison to the potentiometric pH titrations, the ITC gave only the K_{app} values at the given pH in the buffer, but it was simpler to measure. Typical titration curves for 3′-dTMP and 5′-dTMP (both 0.2 mM) with **6** and **7** (both 10.0 mM) are shown in Figure 3, in which cumulative heats (mJ) of the complexation reaction are plotted (the reaction was exothermic). The obtained values (5.3 ± 0.1, 5.8 ± 0.1, 5.5 ± 0.1, and >6 for the 1:1 complexes, **8**, **9**, **10**, and **11**, respectively)[28] for log K_{app} (defined by eq 7) at pH 7.6 in 50 mM HEPES buffer matched well with the corresponding log K_{app} values (5.2, 5.9, 5.5, and 6.4) obtained by the potentiometric pH titration data (see Table 1).

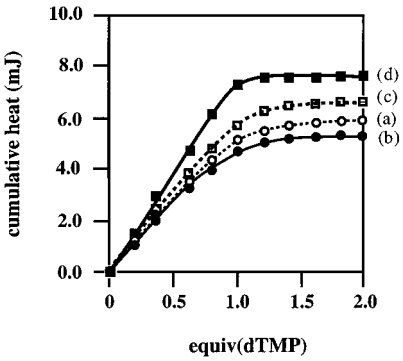


Figure 3. Isothermal titration calorimetry curves for (a) **6** (0.2 mM) + 3′-dTMP, (b) **7** (0.2 mM) + 3′-dTMP, (c) **6** (0.2 mM) + 5′-dTMP, and (d) **7** (0.2 mM) + 5′-dTMP at pH 7.6 (50 mM HEPES with I = 0.1 ($NaNO_3$)) and 25 °C. Equiv(dTMP) is the number of equivalents of [dTMP] added against [Zn_2L].

The present log K_{app} value of 3.4 for the 1:1 complexation of a nucleoside dT with ZnL^3 **3c** was identical to the one determined earlier by the potentiometric pH titration.[15,21] The 1:1 complexation of thymidine 3′,5′-*cyclic*-monophosphate (*c*-dTMP) with **3c** occurred only through a coordination bond between the imide-N^- and zinc(II) cation.[15] As a consequence, it gave log K_{app} of 3.3, almost the same value for the 1:1 **3c**-dT^- complex. Thus, the monoanionic cyclic phosphate part would have little interaction with Zn^{2+}-cyclen.[29] The 1:2 complexation of dT with **6** occurred independently and the log K_{app} for the first and second binding were both 3.2.

The ITC for 3′-dTMP, 5′-dTMP, 5′-dTDP, 5′-dTTP, AZTMP, and AZTDP with the ditopic receptors showed all the 1:1 complexations with log K_{app} values in the range of 5.0−5.6 with **6** and more than 5.6 with **7**. It is most significant that the complexation of those nucleotides with the ditopic receptors is ∼40−1000 times more favorable than that (log K_{app} = 3.4) of dT with the monotopic **3c**, due to the additive binding effect of the dibasic phosphate^{2-} to the second Zn^{2+}-cyclen moieties in **6** and **7**. Furthermore, the *p*-isomer **7** was generally a better

(23) (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.

(24) The pK_3, pK_4, and pK_5 values for 3′-dTMP (<2, 6.1 ± 0.1, and 9.9 ± 0.1, respectively), 3′-UMP (<2, 5.8 ± 0.1, and 9.5 ± 0.1, respectively), and 5′-UMP (<2, 6.2 ± 0.1, and 9.5 ± 0.1, respectively) were determined in this study.

(25) By HPLC using ODS columns, it was confirmed that any chemical conversion of nucleotides did not occur during the potentiometric pH titration, ITC, and UV experiments.

(26) The pK_3 and pK_4 values for 5′-CMP were 4.5 ± 0.1 and 6.6 ± 0.1, respectively.

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

(28) From our standpoint it appears that the apparent complexation constants (K_{app}) for zinc(II) complexes, **3c**, **6**, and **7**, with dT, 3′-dTMP, 5′-dTMP, 3′-UMP, and 5′-UMP were most accurately determined by potentiometric pH titration, which were then checked by the ITC and UV titrations. The ITC experiments with lower concentrations of [Zn_2L] < 0.1 mM) gave too many experimental errors in the measured heat. We carried out the UV titrations of 3′- and 5′-dTMP, 3′- and 5′-UMP, 5′-dTDP, AZTMP, and AZTDP with **6** and **7** at two different concentrations of nucleotide ([nucleotide] = 0.1 mM and 50 μM) and obtained almost the same K_{app} values at these two concentrations (the average values are listed in Table 1). As for the extremely strong complexation of **7**-(5′-dTMP) (see Figure 3d for ITC and Figure 4d for UV titration) and **7**-(5′-dTDP) (not shown), the log K_{app}, values were barely calculable at 6.3 ± 0.1 and 6.7 ± 0.1, respectively, from both ITC and UV titration. However, it would be a safer estimate to put them at >6.

(29) It is of interest to compare with Sessler's protonated sapphyrin which interacted with *c*-dTMP through the monobasic phosphate$^-$ chelation with K_{app} of ∼10^2 (M^{-1}) at pH 6.1 (10 mM bis-Tris buffer) (ref 13d, e).

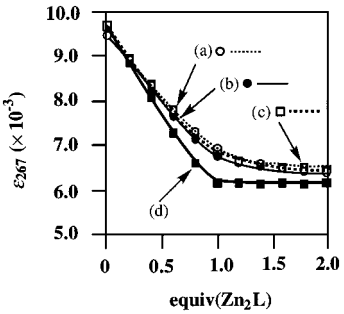


Figure 4. The change of ϵ values of 3′-dTMP and 5′-dTMP at 267 nm on increasing concentration of Zn_2L at pH 7.6 (50 mM HEPES with $I = 0.1$ ($NaNO_3$)) and 25 °C: (a) 3′-dTMP (50 μM) + **6**; (b) 3′-dTMP (50 μM) + **7**; (c) 5′-dTMP (50 μM) + **6**; (d) 5′-dTMP (50 μM) + **7**. Equiv(Zn_2L) is the number of equivalents of [Zn_2L] added against [dTMP].

receptor than the *m*-isomer **6**, probably due to the appropriate distance for the better interaction. Such a strong ditopic complexation was also seen between 2′-UMP and the *m*-dimer **6**, but not with the *p*-isomer **7**, possibly because the distance between the two zinc(II) cations in **7** is too long for the simultaneous binding to 2′-phosphate and the uracil moiety. Among 5′-dTMP, 5′-dTDP, and 5′-dTTP, the diphosphate 5′-dTDP seemed to form the most stable complex with **7**.[28]

The phosphorylation of AZTMP to AZTDP catalyzed by thymidylate kinase (ATP:dTMP phosphotransferase) is rate-determining in the metabolic pathway of AZT.[5] Therefore, the most effective administration form may be AZTDP or AZTTP rather than AZT, the former nucleotide, however, being difficult in cell permeation due to the highly ionic characters. Because **6** and **7** are good hosts for AZTMP and AZTDP, their derivatization into lipophilic forms may make a new effective AZT administration form.

The UV Spectrophotometric Titration of dT (or U) nucleotides with 6 and 7. The UV spectrophotometric titration of 3′-dTMP and 5′-dTMP served to confirm the deprotonated imide functionality in the 1:1 complexes as depicted by **8**−**11**. Earlier, we saw that the imide deprotonation caused a decrease in the ϵ values of dT in the UV absorption at 267 nm.[15a] Figure 4 shows the decreases in the ϵ_{267} values of 3′- and 5′-dTMP (initial concentration = 50 μM) at pH 7.6 (50 mM HEPES with $I = 0.10$ ($NaNO_3$)) and 25 °C.

From curves as shown in Figure 4, the log K_{app} values for **6**−(3′-dTMP), **7**−(3′-dTMP), **6**−(5′-dTMP), and **7**−(5′-dTMP), (these titrations were carried out at two different concentrations with [nucleotide] = 0.1 mM and 50 μM and [**6** or **7**] = 5 mM) were calculated to be 5.4 ± 0.1, 5.8 ± 0.1, 5.7 ± 0.1, and >6, respectively, which agreed with the values obtained by the potentiometric pH titration and ITC measurements. It should be remarked that the ϵ_{267} values of 5′-dTMP decreased by 31 and 36% upon 1:1 complexation with **6** and **7**, respectively, which were significantly greater than the ϵ_{267} decrease of dT upon 1:1 complexation with **3c** (16% decrease).[15a] The difference might come from the tighter binding of the dT moiety (i.e., greater deprotonation) with Zn^{2+}-cyclen in **11** than in **4**.

Similarly, the log K_{app} values for **6**−(3′-UMP), **7**−(3′-UMP), **6**−(5′-UMP), and **7**−(5′-UMP), **6**−(5′-dTDP), **7**−(5′-dTDP), **6**−AZTMP, **7**−AZTMP, **6**−AZTDP, and **7**−AZTDP were determined, which showed the identical behaviors as 3′- and 5′-dTMP (see Table 1).

^{1}H NMR Titration of 5′-dTMP with 7 in Aqueous Solution. The ^{1}H NMR (500 MHz) spectra of the 5′-dTMP complex with **7** in D_2O at pD 7.8 ± 0.1 and 35 °C might inform us more about structure of the complex **11**. Figure 5a and b show the aromatic and anomeric region, and methyl region for **7** (1 mM) and 5′-dTMP (1 mM), respectively. With addition of **7** (0.2 and 0.6 mM) to 1 mM 5′-dTMP (Figure 5c and d), two independent sets of peaks appeared, indicating that (1) the quantitative 1:1 complexation of 5′-dTMP with **7** occurred at the millimolar order of concentration and that (2) the 1:1 complex was kinetically inert on the NMR time scale (10^{-2}−10^{-3} sec).[30,31]

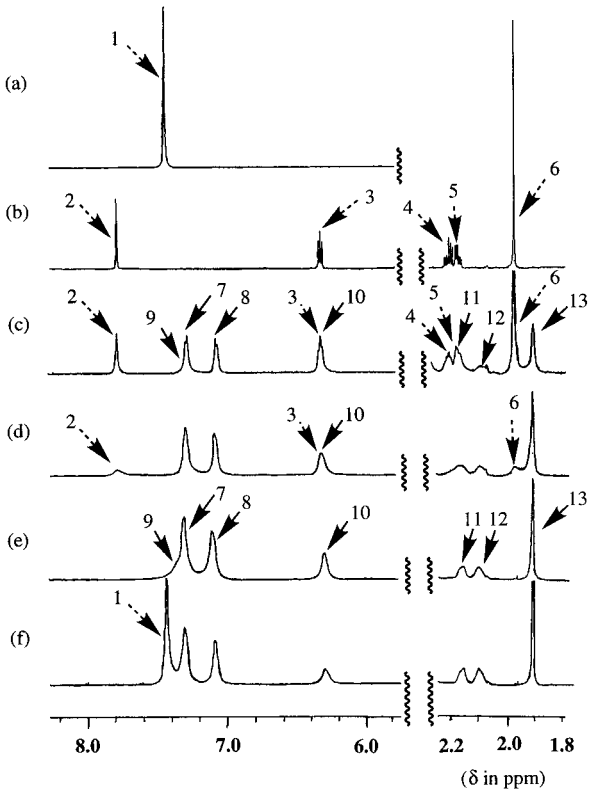


Figure 5. ^{1}H NMR (500 MHz) spectral change of 5′-dTMP (1 mM) in D_2O (pD 7.8 ± 0.1) with increasing concentration of **7** at 35 °C. The ratio of 5′-dTMP:**7** is (a) 0:1, (b) 1:0, (c) 1:0.2, (d) 1:0.6, (e) 1:1, and (f) 1:2, respectively. The dashed arrows indicate peaks of the uncomplexed species, and the plain arrows are peaks of complexed species. Peak 1 is aromatic protons (ArH) of uncomplexed **7**. Peaks 2, 3, 4, 5, and 6 are H(6), H(1′), H(2′β), H(2′α), and Me(5) of uncomplexed 5′-dTMP, respectively (for numbering, see Scheme 2). Peaks 7 and 8 are ArH of **7** complexed with 5′-dTMP. Peaks 9, 10, 11, 12 and 13 correspond to H(6), H(1′), H(2′α), H(2′β), and Me(5) of complexed 5′-dTMP, respectively.

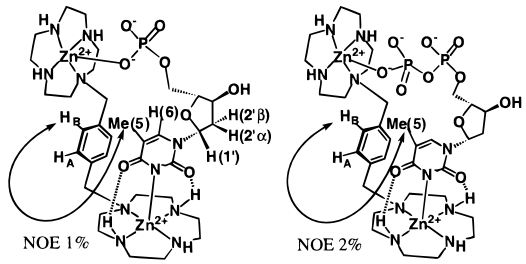
Furthermore, the initial singlet peak of the aromatic protons of the uncomplexed **7** (peak 1 in Figure 5a) changed to two doublet of doublets-like (peaks 7 and 8 in Figure 5c−f presumably corresponding to H_A and H_B, respectively, in Scheme 2), implying that the two Zn^{2+}-cyclen moieties of **7** became non-equivalent in the complex **11**.

The NOE cross-peaks were observed between CH_3(5) of 5′-dTMP (peak 13 in Figure 5) and 5′-dTDP and aromatic protons (peak 8 (= H_B in Scheme 2)) of **7** (1 and 2%, respectively) in **11**, suggesting that the thymine ring and the aromatic ring of **7**

(30) Lian, L.-Y.; Roberts, G. C. K. In *NMR of Macromolecules*; Roberts, G. C. K., Ed.; IRL Press: New York, 1993; pp 153−182.

(31) A singlet peak of phosphorus of 5′-dTMP (3.0 mM) appeared at δ 6.3 and 6.9 (ppm) in the absence and presence of 1 equiv of **7**, respectively, on the ^{31}P NMR in D_2O at pD 7.8 ± 0.1 and 5 °C. In the presence of 0.6 equiv (1.8 mM) of **7**, these two peaks were observed separately, supporting that phosphorus oxygen is bound to Zn^{2+}-cyclen in the kinetically inert 1:1 complex **11** (see Supporting Information).

Scheme 2



7–(5′-dTMP) complex **7**–(5′-dTDP) complex

are closely fixed as depicted in Scheme 2. The $\pi-\pi$ stacking interactions between the benzene ring and thymine ring in **11** may also contribute to the observed hypochromic effect in ϵ_{267} values in UV titration.

The ^{1}H NMR spectrum of 1:1 complex **11** of 5′-dTMP with **7** (0.5 mM) did not collapse by addition of 100 equiv of Na_2HPO_4 in D_2O at pD 7.8 ± 0.1 and 35 °C, implying that 5′-dTMP was far better guests than HPO_4^{2-} for the host **7** and thus large excess of phosphate anions did not hinder the efficient recognition of 5′-dTMP. This fact may be important in case the bis (Zn^{2+}-cyclen)s were used for AZTMP (or AZTDP) transporter in biological conditions.

Conclusions

The *m*- and *p*-bis(Zn^{2+}-cyclen) complexes Zn_2L[4] **6** and Zn_2L[5] **7** have been shown to be the first highly selective ditopic receptors for various derivatives of dT (and U) nucleotides including AZTMP and AZTDP at physiological pH in aqueous solution. The resulting complexes **8**−**11** in general are kinetically inert (on the NMR time scale) and thermodynamically much more stable than each labile component of **3c**−dT$^-$ (**4**) and **3c**−phosphate^{2-} (**5**) at pH 7.6. For instance, the log K_{app} values were 5.5−5.7 and ∼6.4 for 5′-dTMP with **6** and **7**, respectively, at pH 7.6. It is due to the simultaneous bindings of the thymine moieties and dianionic phosphate moieties to the two Zn^{2+}-cyclen units in Zn_2L. Although the log K_{app} values for 1:1 complexation of dT (U) nucleotide and ditopic receptors at pH 7.6, which are nearly the sum of log K_{app} values for **3c**−dT$^-$ and **3c**−phosphate^{2-}, may not be so surprising, the selective recognition of dT (U) nucleotides by the gain in complex stability by a factor of 10^2-10^3 is important, especially in aqueous solution. The fact that the speciation of **11** is over 95% at wide pH range (6.6−8.8) in Figure 2 is noteworthy. The *p*-isomer Zn_2L[5] **7** in general formed more stable complexes than the *m*-dimer Zn_2L[5] **6**. Appropriate attachment of lipophilic functions to **6** or **7** would make novel transport agents for dT-nucleotide drugs. These derivatives also would be useful in separation and detection of various nucleotides.[32] The bis(Zn^{2+}-cyclen) **6** and **7** and their free ligands possess potent anti-HIV activities.[22] It was proposed that bis(macrocyclic tetraamine)s work as specific inhibitors of the interaction between HIV gp120 and a coreceptor of T cell (chemokine receptors such as CXCR4), thereby blocking the invasion of HIV into T cells.[33] It will be interesting to see if the combination of the two mechanistically different kinds of anti-HIV active agents, AZT nucleotides and the bis(Zn^{2+}-cyclen) (and their derivatives), may produce a new cocktail for AIDS treatment.

Experimental Section

General Information. All reagents and solvents used were of the highest commercial quality and used without further purification. The zinc(II) complexes **3c**,[18c] **6**,[18c,d] and **7**[20,21] were synthesized as we previously described. 3′-dTMP, 5′-dTMP, 5′-dTDP, 5′-dTTP, *c*-dTMP, 2′-UMP, 3′-UMP, 5′-UMP, AZTMP, and 5′-CMP were purchased from Sigma, and their purity was checked by HPLC (ODS columns), ^{1}H and/or ^{31}P NMR, or potentiometric pH titration. All aqueous solutions were prepared using deionized and redistilled water. The buffer solution for ITC and UV titration was HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, (pK_a = 7.6 at 20 °C). The ionic strength of the buffer was adjusted to 0.10 with $NaNO_3$. IR spectra for AZTDP were recorded on a Shimadzu FTIR-4200 spectrometer by applying the sample on IR cards (type 61, 3M Co. LTD). ^{1}H NMR spectra were recorded on a JEOL Lambda (500 MHz) or Alpha (400 MHz) spectrometer. 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (tsp) were used as an internal reference for ^{1}H and ^{13}C NMR measurements in D_2O. A D_2O solution of 85% phosphoric acid was used as an external reference for ^{31}P HMR. The external The pD values in D_2O were corrected for a deuterium isotope effect using pD = [pH-meter reading] + 0.40. FAB mass (positive) spectra were recorded on JEOL JMS-SX-101 using glycerin as a matrix.

3′-Azido-3′-deoxythymidine-5′-diphosphate (AZTDP).[5b] Phosphorus oxychloride (55 μL, 0.59 mmol) was added to a mixture of AZT[34] (124 mg, 0.47 mmol) and 1,8-bis(dimethylamino)naphthalene (Proton Sponge) (154 mg, 0.72 mmol) in anhydrous trimethyl phosphate (0.5 mL) at 0 °C, and the whole was stirred at 0−5 °C.[35] After 3 h, a suspension of tetra-*n*-butylammonium phosphate (809 mg, 2.4 mmol) and tri-*n*-butylamine (0.40 mL, 1.7 mmol) in DMF (6 mL) was added quickly at 0 °C and the reaction mixture was turned into a colorless clear solution. After 2 min, 10 mL of 0.2 M triethylammonium carbonate buffer (pH 7.5) was added to the reaction mixture and stirred for 10 min. The whole was evaporated in vacuo, and then 5 mL of liquid NH_3 was added at 0 °C. After stirring overnight at room temperature, the whole was evaporated. The residue was purified by column chromatography on QAE-Sephadex A-25 with triethylammonium bicarbonate buffer (0.2−0.5 M, pH 7.5). The final purification was carried out by reversed-phase HPLC (PREP-ODS, GL Science, Inc.) with the gradient 0−10% MeOH in 10 mM triethylammonium bicarbonate buffer (pH 7.5). Concentration of AZTDP in a stock aqueous solution was determined by ^{31}P NMR in the presence of Na_2HPO_4 (22% yield). IR (IR card) 2988, 2951, 2106 (N_3), 1702, 1692, 1667, 1454, 1400, 1248, 1110, 1060, 959, 837, 515 cm^{-1}. ^{1}H NMR (400 MHz, D_2O/tsp) δ 1.25 (9H, t, J = 7.2 Hz, $HN(CH_2CH_3)_3$), 1.91 (3H, s, CH_3(5)), 2.42−2.48 (2H, m, H(2′)), 3.18 (6H, q, J = 7.2 Hz, $HN(CH_2CH_3)_3$), 4.14−4.24 (3H, m, H(3′) and H(5′)), 4.53−4.56 (1H, m, H(4′)), 6.25 (1H, dd, H(1′), J = 6.6, 6.8 Hz), 7.73 (1H, s, H(6)). ^{31}C NMR (100 MHz, D_2O) δ 9.14, 12.49, 37.17, 47.65, 61.81, 66.30, 83.94 (d C(5′), J_{C-P} = 9.1 Hz), 85.83, 112.73, 138.19, 152.61, 167.43. ^{31}P NMR (162 MHz, D_2O/85% H_3PO_4) δ −8.3 (d, α-P, J_{P-P} = 22.2 Hz), −7.6 (d, β-P, J_{P-P} = 22.2 Hz).

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.[15,16,18] 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 and intrinsic complexation

(32) Gosselin, G.; Imbach, J.-L.; Sommadossi, J.-P. *Bull. Inst. Pasteur* **1994**, *92*, 181−196.

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constants defined in the text were determined by means of the program BEST.[23] All of the sigma fit values defined in the program are smaller than 0.05. 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.[23]

Isothermal Calorimetric Titrations.[27] The heats of 1:1 complexation of nucleosides and nucleotides with zinc(II) complexes were recorded on a Calorimetry Science Corporation Isothermal Titration Calorimeter 4200 at 25.0 ± 0.1 °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 **3c** (2.0 mM), **6**, (0.2 or 0.5 mM) or **7** (0.1, 0.2, or 0.5 mM, depending on the affinity) in 50 mM HEPES was put into a calorimeter cell. After the cell temperature had become constant at 25.0 °C, the solution of a nucleoside (50 mM), a phosphate (50 mM), or a nucleotide (10 or 50 mM) in 50 mM HEPES was portionwise loaded. The titrations were run at least twice. The obtained calorimetric data was analyzed for ΔH values and apparent complexation constants, K_{app}, using the program Data Works and Bind Works (Calorimetry Sciences Corp).

UV Titrations. UV spectra were recorded on a Hitachi U-3500 spectrophotometer at 25.0 ± 0.1 °C. The solution (2.3−2.5 mL) of a nucleotide ([nucleotide] = 0.1 mM or 50 μM) in 50 mM HEPES (pH 7.6 with $I = 0.1$ ($NaNO_3$)) was put into a quartz cell. After the cell temperature had become constant at 25.0 °C, the solution of a zinc(II) complex (concentration was 5.0 mM) in 50 mM HEPES was portionwise added. The titrations were run at least twice. The program Bind Works (Calorimetry Sciences Corp) mentioned above was used for analysis of UV titration to determine apparent complexation constants, K_{app}, for which the decreases in ϵ_{267} values (for dT and AZT derivatives) or those in ϵ_{262} values (for U derivatives) were used instead of the heat of reaction. The molar absorption coefficients (ϵ) ($M^{-1} \cdot cm^{-1}$) of the nucleotides at pH 7.6 and 25 °C used for determination of their concentrations in aqueous buffer solutions were as follows: 3′-dTMP, λ_{max} 267 nm (ϵ 9.5×10^3); 5′-dTMP, λ_{max} 267 nm (ϵ 9.7×10^3), 3′-UMP, λ_{max} 262 nm (ϵ 1.0×10^4); 5′-UMP, λ_{max} 262 nm (ϵ 1.0×10^4); 5′-dTDP, λ_{max} 267 nm (ϵ 9.7×10^3); AZTMP, λ_{max} 267 nm (ϵ 9.4×10^3), and AZTDP, λ_{max} 267 nm (ϵ 9.4×10^3), respectively.

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Supporting Information Available: ^1H, ^{13}C, and ^{31}P NMR spectra of AZTDP, FAB mass spectra of **6**−(3′-dTMP) and **7**−(5′-dTMP) complexes with the theoretical mass distribution spectra, and ^{31}P NMR spectra of 5′-dTMP in the absence and the presence of **7** in D_2O at pD 7.8 ± 0.1 and 5 °C (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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