A New Ternary Zinc(II) Complex with [12]aneN$_4$ (=1,4,7,10-Tetraazacyclododecane) and AZT (=3′-Azido-3′-deoxythymidine). Highly Selective Recognition of Thymidine and Its Related Nucleosides by a Zinc(II) Macrocyclic Tetraamine Complex with Novel Complementary Associations[1]

Mitsuhiko Shionoya,[†] Eiichi Kimura,*[,†] and Motoo Shiro[‡]

Contribution from the Department of Medicinal Chemistry, School of Medicine, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan, and Rigaku Corporation, Matsubaracho 3-9-12, Akishima, Tokyo 196, Japan

Received December 28, 1992

Abstract: Novel complementary associations have been found in a newly synthesized ternary Zn^{II} complex of a macrocyclic tetraamine, [12]aneN$_4$ (=1,4,7,10-tetraazacyclododecane, cyclen, L), and N(3)-deprotonated AZT (=3′-azido-3′-deoxythymidine), **8**. The X-ray crystal analysis of **8** revealed a distorted square-pyramidal N$_5$-coordinate structure where a strong interaction occurs between Zn^{II} and the N(3)-deprotonated anion of the pyrimidine ring (Zn^{II}–N(3) = 2.053(8) Å). Although the distances between the two pyrimidine carbonyls and the symmetrical cyclen NH groups (O(2)–N(17) = 3.22(1) Å; O(2)–N(20) = 3.34(1) Å; O(4)–N(11) = 3.34(1) Å; O(4)–N(14) = 3.33(1) Å) appear long in the solid state, a little wagging of the cyclen ring along the Zn^{II}–N(3) axis in solution may allow the formation of the two (direct or indirect) hydrogen bonds between them to contribute to the stability of **8**. Crystals of **8**·ClO$_4$·2H$_2$O ($C_{18}H_{36}N_9O_{10}ClZn$) are monoclinic, space group $P2_1$ with a = 8.950(4) Å, b = 34.966(4) Å, c = 8.843(3) Å, β = 93.13(3)°; V = 2763(2) Å^3, and Z = 4. Full-matrix least-squares refinement converged at R = 0.078 and R_w = 0.105 for 3290 independent reflections. Potentiometric titrations of deoxyribonucleosides, dA (=2′-deoxyadenosine), dG (=2′-deoxyguanosine), dC (=2′-deoxycytidine), and dT (=thymidine), and related compounds in the presence of Zn^{II}–[12]aneN$_4$ complex **4** disclosed extremely selective binding of **4** to dT and its derivatives, AZT, U (=uridine), Ff (=ftorafur, 5-fluoro-1-(tetrahydro-2-furyl)uracil), and riboflavin. The UV absorption spectral measurement of dT in the presence of **4** has confirmed that **4** binds to dT only in the N(3)-deprotonated form. By these two analytical methods, the anion affinity constants, K(ZnL–S) (=[ZnL–S]/[ZnL][S], where S represents "imide" N-deprotonated substrate), were determined at 25 °C and I = 0.10 (NaClO$_4$) for dT (log K = 5.6), AZT (5.6), U (5.2), Ff (4.6), and riboflavin (5.6). Other nucleosides containing an amino group in place of the carbonyl oxygen of dT (i.e., dG) or containing no "imide" hydrogen (i.e., dA and dC) did not bind to **4** at all, presumably due to the nonbonding steric interaction between their amino groups or to the lack of N$^-$ anion formation. Ino (=inosine) that lacks one carbonyl group at C(2) from dT showed a weaker affinity (log K = 4.2). We conclude that the two possible hydrogen bonds between the carbonyl oxygens of dT and the cyclen amino groups appreciably contribute to the stability of the ternary complexes. Thus, the present study provides a novel type of complementary nucleoside recognition by the Zn^{II} macrocyclic tetraamine complex with high selectivity in aqueous solution.

Introduction

The molecular recognition of nucleic acid constituents is responsible for a wide range of biochemical processes such as complementary base pairings in genetic information storage and transfer,[2] enzymatic reactions performed by coenzymatic nucleotides,[3] oligonucleotide recognition by ribozymes[4] and restriction enzymes,[5] etc. Recently, by the mimicking of such a biochemical concept, a number of artificial receptor molecules have been synthesized for nucleobases, nucleosides, and nucleotides.[6-11] The molecular architecture in these receptors was basically instituted as an assembly of naturally occurring

† Hiroshima University.
‡ Rigaku Corp.

(1) This work was presented by E.K. in his Izatt-Christensen Award Lecture at the 17th International Symposium on Macrocyclic Chemistry, Provo, UT, Aug 1992.
(2) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. *The Molecular Biology of the Cell*; Garland Publishing: New York, 1983; p 437.
(3) Reference 2, p 129.
(4) Cech, T. R. *Science* **1987**, *236*, 1532.
(5) McClerin, J. A.; Frederik, C. A.; Wang, B.-C.; Greene, P.; Boyer, H. W.; Grable, J.; Rosenberg, J. M. *Science* **1987**, *234*, 1526.
(6) (a) Kimura, E.; Kodama, M.; Yatsunami, T. *J. Am. Chem. Soc.* **1982**, *104*, 3182. (b) Kimura, E. *Top. Curr. Chem.* **1985**, *128*, 131 and 141. (c) Umezawa, Y.; Kodama, M.; Takami, W.; Kimura, E.; Koike, T.; Nada, H. *Anal. Chem.* **1988**, *60*, 2392.
(7) (a) Hosseini, M. W.; Lehn, J.-M.; Mertes, M. P. *Helv. Chim. Acta* **1983**, *66*, 2454. (b) Hosseini, M. W.; Lehn, J.-M.; Maggiora, L.; Mertes, K. B.; Mertes, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 537. (c) Hosseini, M. W.; Lehn, J.-M. *J. Am. Chem. Soc.* **1987**, *109*, 7047. (d) Blackburn, G. M.; Thacher, G. R. J.; Hosseini, M. W.; Lehn, J.-M. *Tetrahedron Lett.* **1987**, *28*, 2779. (e) Echevarren, A.; Galan, A.; Lehn, J.-M.; de Mendoza, J. *J. Am. Chem. Soc.* **1989**, *111*, 4994. (f) Hosseini, M. W.; Lehn, J.-M.; Jones, K. C.; Plute, K. E.; Mertes, K. B.; Mertes, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 6330. (g) Brienne, M.-J.; Gabard, J.; Lehn, J.-M.; Stibor, I. *J. Chem. Soc., Chem. Commun.* **1989**, 1868. (h) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 3896. (i) Lehn, J.-M.; Mascal, M.; DeCian, A.; Fischer, J. *J. Chem. Soc., Chem. Commun.* **1990**, 479.
(8) (a) Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1987**, *109*, 5035. (b) Hamilton, A. D.; Pant, N.; Muehldorf, A. *Pure Appl. Chem.* **1988**, *60*, 533. (c) Muehldorf, A. V.; Van Engen, D.; Warner, J. C.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 6561. (d) Goswami, S.; Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 3425. (e) Hamilton, A. D.; Little, D. *J. Chem. Soc., Chem. Commun.* **1990**, 297.

0002-7863/93/1515-6730$04.00/0

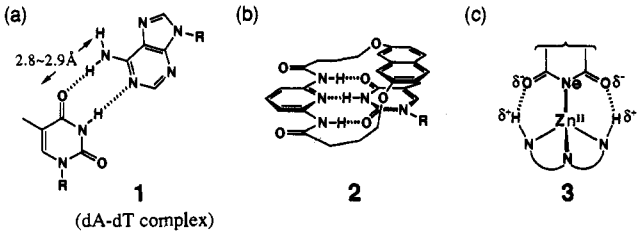
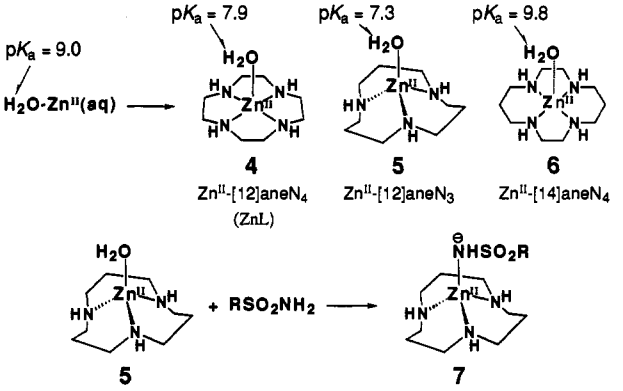


Figure 1. Three types of thymidine recognition: (a) Natural type; (b) artificial type (Hamilton);[8a] (c) our envisioned type.

binding elements, e.g., hydrogen bonding, hydrophobic interactions, or electrostatic interactions, etc., that match complementary features of the host–guest molecules.[12,13] Thymine base, for instance, is recognized in nature through two hydrogen bonds by adenine (see **1**, Figure 1a) and artificially through three hydrogen bonds and a stacking interaction by Hamilton's host molecule **2** (Figure 1b).[8a] However, these interactions are not so strong individually (i.e., −7 kcal·mol^{-1} for a Watson–Crick type hydrogen bond pair between dA and dT, **1**)[14] and hence easily collapse in aqueous solution, unless they are in polymeric assemblies such as DNA duplexes or in nonaqueous solution.

Macrocyclic polyamines and their metal complexes are useful and versatile ("intelligent") in coordination, bioinorganic, biomimetic, and catalysis chemistries.[15] In particular, it is now well established that cyclic polyammonium cations recognize ATP^{4-} and other phosphate anions in aqueous solution at neutral pH,[6,7a-f,h,11b-g] where one of the main electrostatic interactions is due to N–H$^{\delta+}$- - -O$^{\delta-}$ hydrogen bonding.

Scheme I



We have recently demonstrated by comparative studies of a series of Zn^{II} macrocyclic polyamine complexes (**4**–**6**) that the acidity of Zn^{II} ion is reinforced by complexation with [12]aneN$_4$ (=1,4,7,10-tetraazacyclododecane, cyclen, L) and [12]aneN$_3$ (=1,5,9-triazacyclododecane), which resulted in lowered pK_a values of the Zn^{II}-bound H$_2$O from 9.0 (for aquated Zn^{II} ion)[16] to 7.9 and 7.3, respectively, at 25 °C (Scheme I).[17] Another characteristic feature of the Zn^{II} effected by the macrocyclic coordination is the promotion of H$^+$ dissociation (at physiological pH) from sulfonamides –SO$_2$NH– (normally its pK_a values are within 7–11)[17,18] and carboxamides –CONH– (pK_a ~ 14),[19] which is rendered more favorable by the strong interactions between the Zn^{II} and anionic –$^-$NHSO$_2$– and –$^-$NCO–.

We assumed that in those Zn^{II} macrocyclic complexes the hydrogens attached to the nitrogens bound to the central Zn^{II} ions would be acidified. It was figured that, as depicted in **3** (Figure 1c), when the Zn^{II} complexes interact with "imide" functionalities, Zn^{II} would first dissociate the "imide" proton to form a Zn^{II}–N$^-$ bond, whereupon the "imide" carbonyls with developing negative charges might become better acceptors for the acidic NH hydrogens at complementary positions. In testing such a proposal, we suspected that AZT (=3′-azido-3′-deoxythymidine; see Chart I) might be the best probe, because (1) its thymine part seems to offer an ideal structure for such a complementary association with an "imide" hydrogen (pK_a = 9.65) and (2) we have discovered that Zn^{II}–macrocyclic complexes are effective against HIV-1 (human immunodeficiency virus type 1) and hence a complex formed with this AIDS (acquired immune deficiency syndrome)-cure drug might show improved effects.[20]

Indeed, we found the formation of a ternary Zn^{II} complex with [12]aneN$_4$ and N(3)-deprotonated AZT in slightly alkaline aqueous solution. This new type of ternary complex was isolated as crystals, **8**·ClO$_4$·2H$_2$O, to be subjected to X-ray analysis. Subsequently, this novel interaction was examined in aqueous solution in comparison to other nucleoside bases. It has been proven that **4** shows a strong affinity to the "imide" parts of dT

(9) (a) Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Jones, S.; Nemeth, D.; William, K. *J. Am. Chem. Soc.* **1987**, *109*, 5033. (b) Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Costero, A.; Jones, S.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 6866. (c) Rebek, J., Jr.; Williams, K.; Parris, K.; Ballester, P.; Jeong, K.-S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1244. (d) Lindsey, J. S.; Kearney, P. C.; Duff, R. J.; Tjivikna, P. T.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1988**, *110*, 6575. (e) Askew, B.; Ballester, P.; Buhr, C.; Jeong, K.-S.; Jones, S.; Parris, K.; Williams, K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1082. (f) Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245.

(10) (a) Furuta, H.; Magda, D.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 978 and references cited therein. (b) Kral, U.; Sessler, J. L.; Furuta, H. *J. Am. Chem. Soc.* **1992**, *114*, 8704 and references cited therein.

(11) (a) Chen, C.-W.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1978**, *100*, 4921. (b) Tabushi, I.; Kobuke, Y.; Imuta, J. *J. Am. Chem. Soc.* **1980**, *102*, 1744. (c) Yohannes, P. G.; Mertes, M. P.; Mertes, K. B. *J. Am. Chem. Soc.* **1985**, *107*, 8288. (d) Behr, J.-P. *Tetrahedron Lett.* **1986**, *27*, 5861. (e) Marecek, J. F.; Burrows, C. J. *Tetrahedron Lett.* **1986**, *27*, 5943. (f) Wilson, H. R.; Williams, R. J. P. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 1885. (g) Bianchi, A.; Micheloni, M.; Paoletti, P. *Inorg. Chim. Acta* **1988**, *151*, 269. (h) Ebmeyer, F.; Vogtle, F. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 7. (i) Aoyama, Y.; Tanaka, Y.; Sugahara, S. *J. Am. Chem. Soc.* **1989**, *111*, 5397. (j) Zimmerman, S.; Weiming, W. *J. Am. Chem. Soc.* **1989**, *111*, 8054. (k) Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055. (l) Slavin, L. L.; Bose, R. N. *J. Chem. Soc., Chem. Commun.* **1990**, 1256. (m) Schneider, H.-J.; Blatter, T.; Palm, B.; Pflingstag, U.; Rudiger, V.; Theis, I. *J. Am. Chem. Soc.* **1992**, *114*, 7704.

(12) Few earlier studies have taken advantage of metal coordination in recognition of nucleic acid constituents, although metal ions should play critical roles in numerous biological processes. The importance of ligand–ligand interaction in recognition using metal complexes has rarely been reported; however, see, for *cis*-*β*-[Co^{III}(trien)Cl$_2$]$^+$: (a) Marzilli, L. G.; Kistenmacher, T. J.; Darcy, P. E.; Szalda, D. J.; Beer, M. *J. Am. Chem. Soc.* **1974**, *96*, 4688. For reviews: (b) Marzilli, L. G.; Kistenmacher, T. J. *Acc. Chem. Res.* **1977**, *10*, 146. For [Rh^{III}(tren)(H$_2$O)$_2$]$^{3+}$: (c) Torres, L. M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1991**, *113*, 4678 and references cited therein.

(13) For reviews and articles for interactions of metal ions or complexes with nucleic acid constituents, see: (a) Marzilli, L. G. *Prog. Inorg. Chem.* **1977**, *23*, 255. (b) Hodgson, D. J. *Prog. Inorg. Chem.* **1977**, *23*, 211. (c) Wu, F. Y.-H.; Wu, C.-W. *Met. Ions Biol. Syst.* **1983**, *15*, 157. (d) Kalbitzer, H. R. *Met. Ions Biol. Syst.* **1987**, *22*, 81. (e) Sigel, H. *Metal Ions in Biological Systems*; Marcel Decker: New York, 1979; Vol. 8. (f) Reedijk, J. *Pure Appl. Chem.* **1987**, *59*, 181. (g) Lippard, S. J. *Pure Appl. Chem.* **1987**, *59*, 731. (h) Lippert, B. *Prog. Inorg. Chem.* **1989**, *37*, 1. (i) Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1990**, *100*, 293. (j) Pyle, A. H.; Barton, J. K. *Prog. Inorg. Chem.* **1990**, *38*, 413.

(14) Kudritskaya, Z. G.; Danilov, V. I. *J. Theor. Biol.* **1976**, *59*, 303.

(15) For example: (a) Kimura, E. In *Crown Ethers and Analogous Compounds*; Hiraoka, M., Ed.; Elsevier: Amsterdam, 1992; p 381. (b) Kimura, E. *Tetrahedron* **1992**, *48*, 6175. (c) Kimura, E. In *Crown Compounds: Toward Future Applications*; Cooper, S. R., Ed.; VCH Publishers: New York, 1992; p 81.

(16) Mock, W. L.; Tsay, J. *J. Biol. Chem.* **1988**, *263*, 8635.

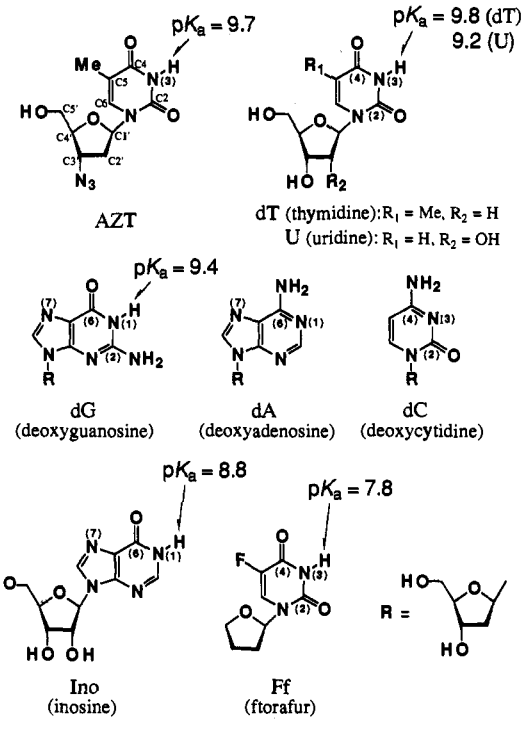
(17) (a) Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805. (b) Kimura, E.; Koike, T. *Comments Inorg. Chem.* **1991**, *11*, 285.

(18) Koike, T.; Kimura, E.; Nakamura, I.; Hashimoto, Y.; Shiro, M. *J. Am. Chem. Soc.* **1992**, *114*, 7338.

(19) Kimura, E.; Koike, T.; Shiota, T.; Iitaka, Y. *Inorg. Chem.* **1990**, *29*, 4621.

(20) Inouye, Y.; Kanamori, T.; Yoshida, T.; Bu, X.; Shionoya, M.; Koike, T.; Kimura, E. Submitted for publication. The affinity constant of the Zn^{II}–[12]aneN$_4$ complex for chloride ion is log K = 1.3 ± 0.1 at 25 °C and I = 0.10.[17b] Accordingly, unless the concentration of the AZT–Zn^{II}–[12]aneN$_4$ complex in the plasma complex goes up to several mM or more, it would rapidly dissociate owing to the presence of competing anions including chloride ion, as pointed out by a reviewer. However, simultaneous use of Zn^{II}–[12]-aneN$_4$ complex and AZT (or use of the AZT–Zn^{II}–[12]aneN$_4$ complex) might heighten their original anti-HIV-1 activities, because their primary actions are quite different (i.e. the former inhibits syncytium formation, while the latter inhibits reverse transcriptase).

Chart I



(=thymidine) and U (=uridine) while it does not bind to other DNA and RNA nucleobases at all.

Experimental Section

General Information. All reagents and solvents used were of analytical grade. AZT (=3′-azido-3′-deoxythymidine), dT (=thymidine), dG (=2′-deoxyguanosine), dC (=2′-deoxycytidine), dA (=2′-deoxyadenosine), U (=uridine), Ff (ftorafur = 5-fluoro-1-(tetrahydro-2-furyl)uracil), Ino (=inosine), and (-)-riboflavin were all purchased from Sigma Chemical Co. Ltd. Zn^{II}–[12]aneN$_4$ complex, 4·2ClO$_4$, was prepared as previously described.[21] IR and UV spectra were recorded on a Shimadzu FTIR-4200 and a Hitachi U-3200 spectrophotometer, respectively. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL GX-400 spectrometer. Tetramethylsilane (TMS, Merck) was used as the internal reference.

Synthesis of (N(3)-Deprotonated-AZT–Zn^{II}–[12]aneN$_4$)·ClO$_4$·2H$_2$O, 8·ClO$_4$·2H$_2$O. AZT (59 mg, 0.22 mmol) was added to 4·2ClO$_4$ (99 mg, 0.22 mmol) and LiOH·H$_2$O (9 mg, 0.22 mmol) in 2 mL of H$_2$O (pH 9.5), and the mixture was heated at 40–50 °C for 10 min. A 0.5 mL volume of 1 M NaClO$_4$ aqueous solution was added to the mixture, and then it was allowed to stand in a refrigerator. Colorless prisms were obtained (81 mg, 58% yield). The above composition (i.e. C$_{18}$H$_{32}$N$_9$O$_8$ClZn·2H$_2$O) was determined by the X-ray analysis of an air-dried prism. IR (KBr pellet): 3488, 3250, 2953, 2083, 1655, 1632, 1570, 1549, 1462, 1449, 1397, 1377, 1366, 1302, 1287, 1269, 1186, 1144, 1121, 1091, 1047, 1015, 974, 951, 864, 812, 785, 636 cm^{-1}. Characteristic IR and ^{1}H and ^{13}C NMR data are listed in Tables I and II, respectively. The prisms were dried under vacuum at 60 °C until a constant weight was reached (~2 h) and then subjected to elemental analysis. The crystals became opaque upon drying due to efflorescence. Anal. Calcd for C$_{18}$H$_{32}$N$_9$O$_8$ClZn·H$_2$O: C, 34.79; H, 5.52; N, 20.29. Found: C, 34.82; H, 5.41; N, 20.25. For solution studies, air-dried prisms (i.e. 8·ClO$_4$·2H$_2$O) were employed.

Potentiometric pH Titrations. The preparation of the test solutions and the calibration of the electrode system (Orion Research Expandable Ion Analyzer EA920 and Orion Research Ross Combination pH Electrode 8102BN) have been described earlier.[22] All samples were kept under an argon (>99.999% purity) atmosphere. The solution temperature was maintained at 25.0 ± 0.1 °C, and the ionic strength was adjusted to 0.10 M with NaClO$_4$. Aqueous solutions (50 mL) of Zn^{II}–[12]aneN$_4$ complex ([4] = 1.0 × 10^{-3} M) with (or without) substrates ([HS] = 1.0 × 10^{-3} M) were titrated with carbonate-free 0.100 M NaOH aqueous solution. The proton dissociation constants (K_S = [S]a_{H^+}/[HS]; HS and S represent the substrates in the neutral form and the N(3)- (or N(1)- for dG and Ino) deprotonated anionic form, respectively) were calculated by the same method as described previously.[19,23] The values of K_w' (=[H$^+$][OH$^-$]), f_{H^+}, and f_{OH^-} used in the computation were 10$^{-13.79}$, 0.83, and 0.75, respectively.

Measurement of the anion affinity constants, K(ZnL–S) (=[ZnL–S]/([ZnL][S], M^{-1}), for a variety of nucleosides and riboflavin was conducted by a potentiometric titration method at 25 °C and I = 0.10 (NaClO$_4$). pH Titration data for 4 (1 mM) in the presence of dT (1 mM) are plotted in Figure 3c. The following equilibria and equations were considered to take place in the buffer region ($0 < a < 2$) (a is the number of equivalents of base added; a_{H^+} is the activity of H$^+$ ion):

$$ZnL + H_2O \rightleftharpoons ZnL(OH^-) + H^+$$

$$K_{OH} = [ZnL(OH^-)]a_{H^+}/[ZnL] \quad (1)$$

$$ZnL + S \rightleftharpoons ZnL\text{–}S \qquad K(ZnL\text{–}S) = [ZnL\text{–}S]/([ZnL][S]) \quad (2)$$

Substrate mass balance:

$$C_{S(total)} = [HS] + [S] + [ZnL\text{–}S] \quad (3)$$

Metal mass balance:

$$C_{ZnL(total)} = [ZnL] + [ZnL(OH^-)] + [ZnL\text{–}S] \quad (4)$$

Proton mass balance:

$$C_a = aC_{ZnL(total)} + [H^+] - [OH^-] = [ZnL\text{–}S] + [ZnL(OH^-)] + [S] \quad (5)$$

From the above equations the following equations are derived:

$$C_{S(total)} = [ZnL\text{–}S] + \beta[S] \quad (6)$$

$$C_{ZnL(total)} = [ZnL\text{–}S] + (1 + \gamma)[ZnL] \quad (7)$$

$$C_a = [ZnL\text{–}S] + \gamma[ZnL] + [S] \quad (8)$$

Here β = ([HS] + [S])/[S] and $\gamma = K_{OH}/a_{H^+}$. From eqs 6–8 one can derive the following equations:

$$[S] = \{C_a - C_{S(total)} + \gamma(C_a - C_{ZnL(total)})\}/(1 - \beta + \gamma) \quad (9)$$

$$[ZnL] = (C_a - [ZnL\text{–}S] - [S])/\gamma \quad (10)$$

$$[ZnL\text{–}S] = C_{S(total)} - \beta[S] \quad (11)$$

Finally, we can obtain anion affinity constants K(ZnL–S) values from pH and a values at each titration point ($0 < a < 2$) by the use of the eqs 9–11.

Spectrophotometric Study. The UV absorption of dT with or without Zn^{II}–[12]aneN$_4$ 4 ([4]/[dT] = 0–10) was measured at 25 °C and pH 8.6 (50 mM TAPS buffer, I = 0.10 (NaClO$_4$), TAPS = N-(tris(hydroxymethyl)methyl)-3-aminopropanesulfonic acid (Dojindo Laboratories)) with a 2-mm cell, where [4] is 0, 0.5, 1.0, 1.5, 2.5, or 5.0 mM and [dT] = 0.5 mM. From the UV spectral changes, the apparent affinity constant K_{app} (=[ZnL–dT]/([dT$_f$][ZnL$_f$]), where [dT$_f$] = [dT] + [N(3)-deprotonated dT] and [ZnL$_f$] = [ZnL] + [ZnL(OH$^-$)]) was calculated by Connors' method.[24] The anion affinity constant K(ZnL–S) for dT was calculated from the K_{app} value, K_{OH}, and K_S.

Crystallographic Study. A colorless prismatic crystal of 8·ClO$_4$·2H$_2$O with dimensions 0.4 × 0.2 × 0.1 mm was used for data collection. The lattice parameters and intensity data were measured on a Rigaku AFC5R diffractometer with graphite-monochromated Cu Kα radiation and a 12-kW rotating anode generator. The structure was solved by direct methods, and the nonhydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 3290 observed reflections to give R = 0.078 and R_w = 0.105. All calculations

(21) Norman, P. R.; Tate, A.; Rich, P. *Inorg. Chim. Acta* **1988**, *145*, 211.
(22) Kimura, E.; Koike, T.; Uenishi, K.; Hediger, M.; Kuramoto, M.; Joko, S.; Arai, Y.; Kodama, M.; Iitaka, Y. *Inorg. Chem.* **1987**, *26*, 2975.
(23) Kimura, E.; Yamaoka, M.; Morioka, M.; Koike, T. *Inorg. Chem.* **1986**, *25*, 3883.
(24) Connors, K. A. In *Binding Constants*; John Wiley & Sons: New York, 1987; Chapter 4, p 141.

Table I. Comparison of Characteristic IR Data (cm^{-1})

compd	condition	$\nu_{N=N=N}$	$\nu_{C=O}$, $\nu_{C=C}$
AZT	KBr pellet	2120 s, 2089 s	1699 vs, 1661 vs
	in DMSO-d_6	2105 s	1701 vs
8·ClO$_4$·2H$_2$O	KBr pellet	2083 s	1655 vs, 1632 sh, 1570 vs, 1549 vs
	in DMSO-d_6	2101 s	1663 vs, 1652 vs, 1588 vs
deuterated 8[a]	KBr pellet	2105 s	1653 vs, 1574 vs, 1553 vs
		cyclen ν_{ND}	2460 br
	in DMSO-d_6	2101 s	1663 vs, 1647 vs, 1588 vs
		cyclen ν_{ND}	2598 br
dT			1699 vs, 1663 vs
H$_{-1}$dT–ZnL[b]			1653 vs, 1636 sh, 1566 vs, 1550 vs

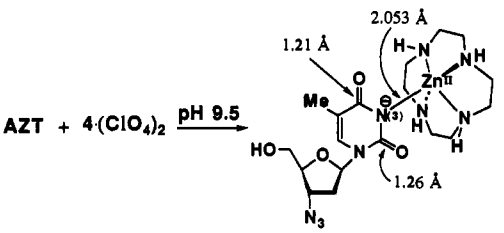
[a] Deuterated 8 was obtained by recrystallization of 8·ClO$_4$·2H$_2$O from D$_2$O as colorless prisms. In this complex, the four hydrogens attached to cyclen nitrogens and one hydrogen of the hydroxyl group at C(5′) are deuterated. [b] This complex was prepared by concentrating an aqueous solution of dT and 4·2ClO$_4$ containing equimolar NaOH to dryness. The crystallization was unsuccessful. H$_{-1}$dT represents the N(3)-deprotonated dT.

were performed using TEXSAN crystallographic software package developed by Molecular Structure Corp. (1985).

Two crystallographically independent 8, two ClO$_4^-$ anions, and four water molecules are in the asymmetric unit. An ORTEP drawing (50% probability ellipsoids) of 8·ClO$_4$·2H$_2$O, with the atom-numbering system, is presented in Figure 2. Crystallographic parameters and selected bond distances (Å), interligand bond distances (Å), and bond angles (deg) of 8·ClO$_4$·2H$_2$O are listed in Tables III and IV, respectively.

Results

Isolation and Characterization of a Ternary Zn^{II}–[12]aneN$_4$ Complex 4 with N(3)-Deprotonated AZT, 8·ClO$_4$·2H$_2$O. From an equimolar mixture of AZT and Zn^{II}–[12]aneN$_4$ complex, as 4·2ClO$_4$, in aqueous solution at pH 9.5, colorless prisms were obtained. Its elemental composition (8·ClO$_4$·2H$_2$O) was determined by the X-ray analysis of an air-dried prism. Its elemental analysis (C, H, N) after drying under vacuum at 60 °C for ~2 h fits to a 1:1 ternary complex 8 with a monoanion ClO$_4^-$ and one water molecule, implying that the other anion is the deprotonated thymidine N(3) (see Experimental Section).



8

A piece of evidence for the structure of imide N(3)-deprotonated complex, 8, comes from the lowered C=O stretching frequencies (KBr pellet, $\nu_{C=O}$) from 1699 cm^{-1} for AZT to 1655 and 1632 cm^{-1} for 8·ClO$_4$·2H$_2$O (Table I), reflecting the longer carbonyl C=O bonds due to deprotonation of the N(3)–H group. No significant change was found in the azide stretching frequency $\nu_{N=N=N}$ upon coordination: 2120, 2089 cm^{-1} for AZT and 2083 cm^{-1} for 8. A similar tendency was also seen in their IR spectra in solution. The C=O stretching frequencies ($\nu_{C=O}$) in DMSO-d_6 vary with the deprotonation of the N(3)–H group from 1701 cm^{-1} for AZT to 1663 and 1652 cm^{-1} for 8. No significant change was found in the azide stretching frequency $\nu_{N=N=N}$ upon coordination: 2105 cm^{-1} for AZT and 2101 cm^{-1} for 8.

Table II. Comparison of ^{1}H and ^{13}C NMR Data for AZT and 8·ClO$_4$·2H$_2$O[a]

	AZT	8·ClO$_4$·2H$_2$O
	^{1}H NMR	
5-CH$_3$	1.77 (d, J = 0.6 Hz)	1.78 (3H, s)
2′-H	2.27 (ddd, J = 13.7, 5.3, 6.5 Hz)	2.17 (d*pseudo*-t, J_d = 13.8, J_t = 6.6/4.4 Hz)
2″-H	2.37 (d*pseudo*-t, J_d = 13.7, J_t = 7.0/6.7 Hz)	2.31 (d*pseudo*-t, J_d = 13.8, J_t = 6.7/6.6 Hz)
5′-H	3.59 (ddd, J = 12.1, 5.3, 4.2 Hz)	3.60 (m, 2H for 5′- and 5″-H)
5″-H	3.64 (ddd, J = 12.1, 5.1, 4.0 Hz)	
4′-H	3.81 (d*pseudo*-t, J_d = 4.9, J_t = 4.2/4.0 Hz)	~3.81 (overlapped with NH peaks)
3′-H	4.40 (d*pseudo*-t, J_d = 7.0, J_t = 5.3/4.9 Hz)	4.38 (d*pseudo*-t, J_d = 6.7, J_t = 4.9/4.4 Hz)
OH	5.20 (*pseudo*-t, J_t = 5.3/5.1 Hz)	5.14 (*pseudo*-t, J_t = 5.1 Hz)
1′-H	6.09 (*pseudo*-t, J_t = 6.7/6.5 Hz)	6.22 (*pseudo*-t, J_t = 6.6 Hz)
6-H	7.67 (m)	7.53 (m)
imide-H	11.31 (s)	none
cyclen		NH 3.79 (br, 2H)
		NH 3.80 (br, 2H)
		–CH$_2$– 2.55–2.70 (m, 8H)
		–CH$_2$– 2.70–2.95 (m, 8H)
	^{13}C NMR	
C2	150.33	156.20
C4	163.33	170.79
C5	109.44	109.67
C6	135.97	134.87
C1′	83.92	83.44
C2′	36.12	36.01
C3′	60.10	60.67
C4′	83.34	83.27
C5′	60.75	61.13
CH$_3$	12.13	13.06
cyclen		43.51

[a] δ values (ppm) based on TMS in DMSO-d_6. ^{1}H NMR spectrum of 4·2ClO$_4$ showed the complete dissociation of the Zn^{II} complex. The complex 8 is thus extremely stabilized by the N(3)$^-$ anion coordination and remains intact even in DMSO which may possibly bind to Zn^{II}. For the numbering, see Chart I.

Table II compares ^{1}H and ^{13}C NMR data for AZT and 8·ClO$_4$·2H$_2$O in DMSO-d_6. The N(3)-deprotonated structure of 8 is supported by the lack of the imide proton N(3)–H of AZT in the ^{1}H NMR spectrum of 8. The N(3) coordination in 8 is also deduced from the upfield shift of the 6-H resonance (from 7.67 to 7.53 ppm) relative to that of free AZT. Moreover, with 8 two kinds of peaks at δ = 3.79 ppm (br, 2 H), and 3.80 ppm (br, 2 H) are observed for NH protons of the [12]aneN$_4$ part at 27 °C, which might indicate two different kinds of NH's such as those near to and distant from the carbonyl oxygens. The ^{13}C NMR spectra also see some structural changes upon complexation of the pyrimidine part. The carbonyl carbons at both C(2) and C(4) are shifted downfield by $\Delta\delta$ 5.87 ppm (from 150.33 to 156.20 ppm) and $\Delta\delta$ 7.46 ppm (from 163.33 to 170.79 ppm), respectively, as a result of the N(3) deprotonation. It is also noted that no significant difference is found for the furanose moiety in both NMR spectra before and after complexation. More conclusive evidence for such interactions was obtained by the X-ray analysis of 8 and the solution properties, as described below.

X-ray Crystal Structure of 8·ClO$_4$·2H$_2$O. The deprotonated "imide" anion-coordinating structure of 8 was established by the X-ray analysis. Figure 2 shows the ORTEP drawing of 8 with 50% probability thermal ellipsoids. Crystal data and data collection parameters are displayed in Table III. Selected bond distances, intermolecular distances, and bond angles are listed in Table IV.

It is evident that AZT firmly binds to the Zn^{II}–[12]aneN$_4$ complex. Thus, Zn^{II} is coordinated in a square pyramidal manner by four nitrogens N(11), N(14), N(17), and N(20) of [12]aneN$_4$ and the imide N(3)$^-$ anion of AZT. All the four NH groups of the [12]aneN$_4$ unit are spatially directed toward the AZT bound to the central Zn^{II} ion *via* the deprotonated-N(3). The Zn^{II}–N(3)$^-$ bond distance of 2.053(8) Å is shorter than the average Zn^{II}–NH ([12]aneN$_4$) bond distance of 2.153 Å. Such a high affinity of

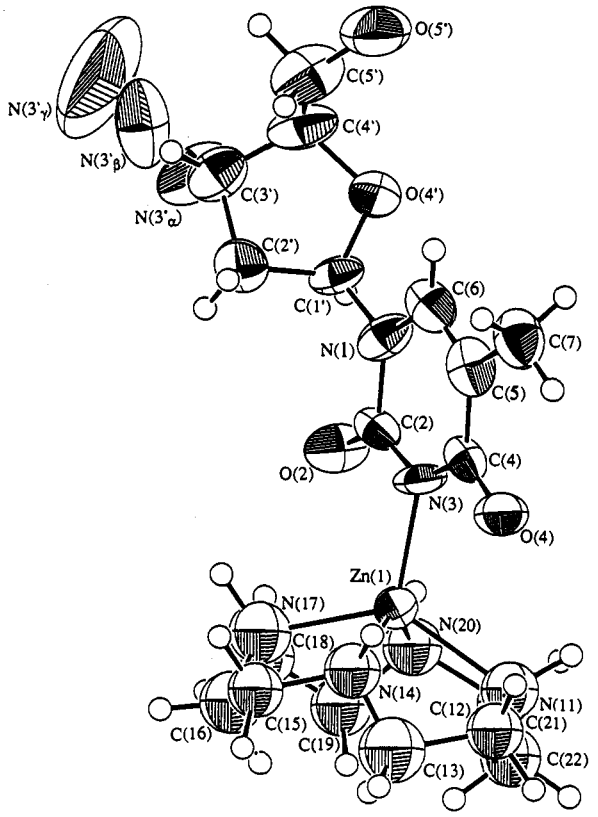
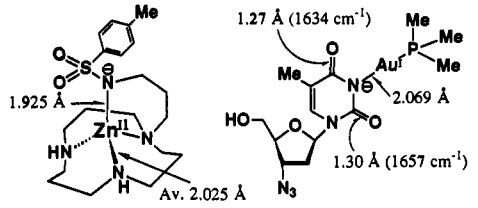


Figure 2. ORTEP drawing (50% probability ellipsoids) of **8**. A perchlorate anion and two H_2O molecules are omitted for clarity.

a Zn^{II} macrocyclic complex to anionic amines has been observed in the Zn^{II}–N^- (sulfonamide) bond distance (1.925 Å) *vs* the average Zn^{II}–NH bond distance of 2.025 Å in (tosylamido)-propyl-[12]aneN$_3$–Zn^{II} complex **9**.[18] The present structure is compatible with our earlier notion that the acidity-strengthened Zn^{II} ions in macrocyclic complexes prefer N^- anion to neutral nitrogen donors.



9[18] **10**[25]

The AZT pyrimidine ring diagonally bisects the symmetrical N4 plane of the Zn^{II}–[12]aneN$_4$ unit. The bond distances between the two pyrimidine carbonyls and the NH groups of [12]aneN$_4$ are O(2)–N(17) = 3.22(1) Å, O(2)–N(20) = 3.34(1) Å, O(4)–N(11) = 3.34(1) Å, and O(4)–N(14) = 3.33(1) Å. It was suggested by an inspection of the difference Fourier map that the tetraamine ring might be disordered at two locations related by a rotation around Zn^{II}–N(3) bond. However, the structure could not be successfully refined even after considering such a disordered model. The comparatively large R value may be due to the insufficient quantity and quality of the intensity data or to the disordered structure of the tetraamine ring. The hydrogen atoms of the NH and CH groups were located by geometrical calculations. Although the distances between the two pyrimidine carbonyl oxygens and the calculated positions of NH hydrogen atoms (2.8–3.1 Å when the NH bonds are assumed to be 1 Å) look long for hydrogen bond formation, a little wagging of the tetraamine ring in solution would permit closer contacts between them for the formation of the two direct (or indirect through water molecules) diagonal hydrogen bonds.

Recently, an Au^I–trimethylphosphine complex with AZT, **10**, was reported[25] with an attempt to develop a potent anti-HIV agent.[26] The X-ray analysis of **10** showed the Au^I–N^- bond distance of 2.069(9) Å, a little longer than Zn^{II}–N(3)$^-$ in **8**. Evidently, there is no possibility of complex stabilization by hydrogen bonds in this Au^I complex. However, the stability of **10** in aqueous solution was not described. Medicinally unfortunately, the anti-HIV activity of **10** was no more than that of AZT itself. We suspect that this is due to dissociation of **10** into AZT and Au^I–PMe$_3$. On the other hand, the Zn^{II}–[12]aneN$_4$ complex, **4**, has already been found to exhibit an anti-HIV-1 activity.[20] Simultaneous use of **4** and AZT might exhibit a synergistic activity. The anti-HIV-1 activity of **8** is now under investigation.

Determination of the Affinity of 4 to Thymidine and Its Homologues and Other Nucleosides. To assess the interactions between the Zn^{II}–[12]aneN$_4$ complex, **4**, and the "imide" functionality of dT in aqueous solution, potentiometric pH

Table III. Crystallographic Parameters of **8**·ClO_4·$2H_2O$

formula	$C_{18}H_{36}N_9O_{10}ClZn$
fw	639.37
cryst color, habit	colorless, prismatic
cryst dimens, mm	0.4 × 0.2 × 0.1
cryst system	monoclinic
space group	$P2_1$
lattice params	a = 8.950(4) Å
	b = 34.966(4) Å
	c = 8.843(3) Å
	β = 93.13(3)°
	V = 2763(2) Å^3
Z	4
ρ_c, g cm^{-3}	1.537
radiation	Cu Kα (λ = 1.541 78 Å)
μ, cm^{-1}	27.29
$2\theta_{max}$, deg	120.7
refinement	full-matrix least-squares method
no. of measd reflcns	4478
no. of indep reflcns ($\|I_o\| > 3\sigma(I_o)$)	3290
R	0.078
R_w	0.105

Table IV. Selected Bond Distances (Å), Intermolecular Distances (Å), and Bond Angles (deg) of **8**·ClO_4·$2H_2O$[a]

Zn(1)–N(3)	2.053(8)	Zn(1)–N(11)	2.17(1)
Zn(1)–N(14)	2.15(1)	Zn(1)–N(17)	2.15(1)
Zn(1)–N(20)	2.15(1)	O(2)–C(2)	1.26(1)
O(4)–C(4)	1.21(1)	N(3)–C(2)	1.27(1)
N(3)–C(4)	1.31(1)		

O(2)- - -N(17)	3.22(1)
O(2)- - -H(17)	2.9[b]
O(2)- - -H(17)–N(17)	100[b]
O(2)- - -N(20)	3.34(1)
O(2)- - -H(20)	2.8[b]
O(2)- - -H(20)–N(20)	108[b]
O(4)- - -N(11)	3.34(1)
O(4)- - -H(11)	3.1[b]
O(4)- - -H(11)–N(11)	91[b]
O(4)- - -N(14)	3.33(1)
O(4)- - -H(14)	2.8[b]
O(4)- - -H(14)–N(14)	113[b]

N(3)–Zn(1)–N(11)	114.0(4)	N(3)–Zn(1)–N(14)	109.2(4)
N(3)–Zn(1)–N(17)	113.3(5)	N(3)–Zn(1)–N(20)	115.9(4)
N(11)–Zn(1)–N(14)	84.3(4)	N(11)–Zn(1)–N(17)	132.7(5)
N(11)–Zn(1)–N(20)	79.9(5)	N(14)–Zn(1)–N(17)	79.3(5)
N(14)–Zn(1)–N(20)	134.8(5)	N(17)–Zn(1)–N(20)	81.1(5)

[a] ESD in parentheses. [b] Values from the calculated H positions (N–H = 1 Å).

(25) Pill, T.; Polborn, K.; Kleinschmidt, A.; Erfle, V.; Breu, W.; Wagner, H.; Beck, W. *Chem. Ber.* **1991**, *124*, 1541 and references cited therein.
(26) Mitsuya, H.; Broder, S. *Nature* **1987**, *325*, 773.

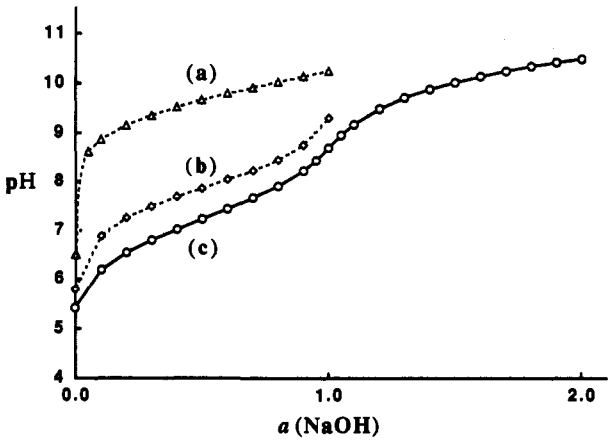
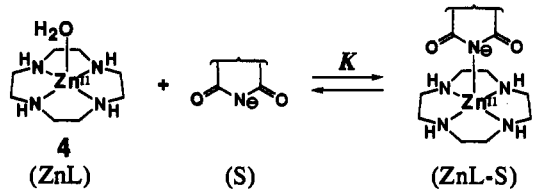


Figure 3. pH titration data (experimental and theoretical) for Zn^{II}–[12]aneN$_4$ (**4**·2ClO$_4$) at I = 0.10 (NaClO$_4$) and 25 °C: (a) 1.0 mM dT; (b) 1.0 mM **4**·2ClO$_4$; (c) 1.0 mM **4**·2ClO$_4$ + 1.0 mM dT. a(NaOH) is the number of equivalents of base added.

Scheme II



titrations of **4** (1 mM) have been conducted in the presence of dT or its derivatives (1 mM) including AZT, U, Ino, Ff, and riboflavin at 25 °C and I = 0.10 (NaClO$_4$). For comparison, other nucleosides, dG, dA, and dC, were also examined. pH Titration data for **4** (1 mM) with 0.100 M NaOH aqueous solution in the absence or in the presence of dT (1 mM) are plotted in Figure 3.

The evaluation of the titration data according to Scheme II yielded the anion affinity constants K(ZnL–S) (=[ZnL–S]/([ZnL][S]), M^{-1}), where S represents "imide" or "amide" N-deprotonated substrate (see Experimental Section). This analytical method was also applied to other nucleosides (AZT, dG, U, Ino, Ff) and riboflavin[27] that would commonly interact with **4** with concomitant deprotonation at the N(3) (or N(1) for dG and Ino) site. All the results are summarized in Table V.

From the UV spectral change of dT as a function of pH, the pK_a value for N(3)–H was determined to be 9.6 ± 0.1 at 25 °C and I = 0.10 (NaClO$_4$). This agrees with pK_a = 9.76 ± 0.01, as determined by a potentiometric pH titration under the same conditions (Table V). With an increase in the concentration of the deprotonated dT, the maximal absorption at 267 nm decreased with a constant isosbestic point at 247 nm. We have tested whether the ease of N(3)-deprotonation in the presence of **4** fits to the log K(ZnL–S) of 5.6 determined by the above potentiometric pH titration method. The UV absorption changed as the ratio [**4**]/[dT] changes (0–10) at 25 °C and pH 8.6 [TAPS buffer, I = 0.10 (NaClO$_4$)], as shown in Figure 4, where [**4**] is 0, 0.5, 1.0, 2.5, or 5.0 mM and [dT] is 0.5 mM. With an increase in the ratio, a similar change in the UV absorption spectra occurred with a decrease in absorption at 267 nm and with a constant isosbestic point at 242 nm. From the UV spectral changes at 267 nm, the apparent affinity constant, log K_{app} of 3.6 ± 0.1 for dT (K_{app} = [ZnL–dT]/([dT$_f$][ZnL$_f$]), where [dT$_f$] = [dT] + [N(3)-deprotonated dT] and [ZnL$_f$] = [ZnL] + [ZnL(OH$^-$)]), was obtained using the same method as described by Connors.[24] The anion affinity constant log K(ZnL–S) of 5.6 was calculated from the K_{app} value of 3.6 and the two pK_a values (9.76 for the N(3)–H of dT and 7.88 for Zn^{II}-bound H_2O in **4**). This log K(ZnL–S) value is in good agreement with that of 5.6 determined for dT by a potentiometric pH titration method. Hence, the UV spectra of dT at various concentrations of **4** indicated the binding of **4** only to the N(3)–deprotonated anionic dT.

Although N(1)–H of dG is even more acidic (pK_a = 9.36) and hence more easily deprotonated than the corresponding N(3)–H of thymidine, the *interaction with 4 was too small to evaluate.* The pH titration of **4** in the presence of dG exhibited two independent deprotonation processes (the same pK_a values

Table V. Comparison of pK_a Values (for Deprotonation of the "Imide" or "Amide" NH's) and Anion Affinity Constants, log K(ZnL–S) [L = [12]aneN$_4$, S = N(3)– (or N(1)– for dG and Ino) Deprotonated Substrates or Anionic Species]

substrate	pK_a[a]	log K(ZnL–S)[b]
dT	9.76 ± 0.01	5.6 ± 0.1 (5.6 ± 0.1)[c]
AZT	9.65 ± 0.02	5.6 ± 0.1
dG	9.36 ± 0.01	*d*
U	9.19 ± 0.01	5.2 ± 0.1
Ino	8.81 ± 0.01	4.2 ± 0.1
Ff	7.82 ± 0.01	4.6 ± 0.1
riboflavin	9.92 ± 0.02	5.6 ± 0.1
OH$^-$[e]	15.7	6.0
$CH_3CO_2^-$[e]	4.5	1.7
SCN$^-$[e]	0.9	2.1

[a] At 25 °C and I = 0.10 (NaClO$_4$). [b] Determined by a potentiometric pH titration at 25 °C and I = 0.10 (NaClO$_4$). K(ZnL–S) is defined as [ZnL–S]/([ZnL][S]), M^{-1}. [c] Calculated from log K_{app} of 3.6, which was determined by a spectrophotometric method (see Experimental Section and text). [d] No change was observed in the presence of 1–2 mM of dG in the potentiometric pH titration. No data using more than 3 mM of dG were available due to its poor solubility. [e] Reference 17b.

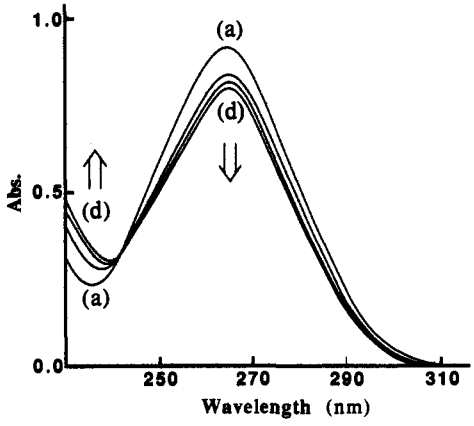
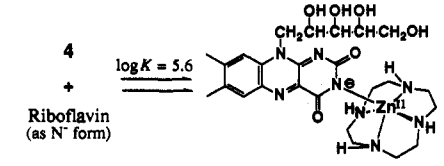


Figure 4. Selected UV absorption spectra of 0.5 mM dT at 25 °C and pH 8.6 (50 mM TAPS buffer, I = 0.10 (NaClO$_4$)) with 2-mm cell: (a) 0.5 mM dT; (b) solution (a) + 0.5 mM **4**·2ClO$_4$; (c) solution (a) + 1.0 mM **4**·2ClO$_4$; (d) solution (a) + 2.5 mM **4**·2ClO$_4$.

(27) The anion affinity constant (log K) for riboflavin, which possesses an "imide" group, was similarly determined to be 5.6

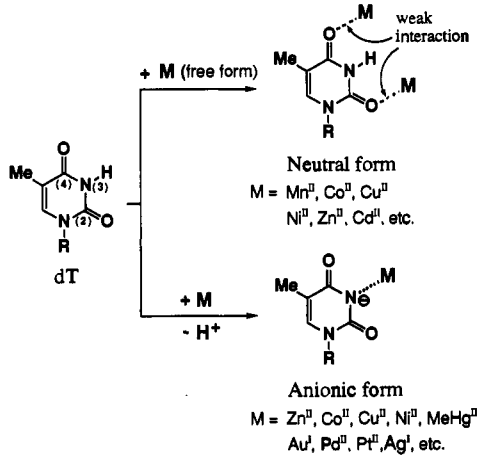


which is comparable to that predicted from its pK_a value of 9.92 ± 0.02 at 25 °C and I = 0.10 (NaClO$_4$) on the basis of the linear relationship in Figure 5. This result clearly indicates that riboflavin binds to **4** through its deprotonated imide N$^-$ anion like dT. Some flavoenzymes are known to contain metal ions as prosthetic groups (Hatei, Y.; Stigall, D. L. In *Enzymes*, Boyer, P. D., Ed.; Academic Press: New York, 1976; Vol. XIII, p 175). However, little has been known for the intrinsic roles of the metal ions. Very recently, it was found that N(3)–H dissociation of 10-methylisoalloxazine, a homologue of naturally occurring flavin derivatives such as FAD and FMN, plays an important role for a rate-accelerating metal ion effect (Zn^{2+} > Co^{2+} > Ni^{2+}) on the oxidation of *N*-benzyldihydronicotinamide, an NADH model compound, in acetonitrile (Tominami, T.; Ikeda, K.; Nabeshima, T.; Yano, Y. *Chem. Lett.* **1992**, 2293). We will report elsewhere interesting properties of the ternary complex formed by N(3)-deprotonated riboflavin and **4**.

of 7.88 for the deprotonation of Zn^{II}–bound H_2O in **4** and 9.36 for N(1)–H of dG). *With dA and dC (with no dissociable NH), the titration curves of 4 did not change at all.* Should the uncharged exocyclic amines or neutral aromatic nitrogens in these nucleosides, dG, dA, and dC, bind to Zn^{II} in **4**, the buffer pH region of **4** (corresponding to the deprotonation of the Zn^{II}-bound H_2O) would have to be raised.

Discussion

2,4-Dioxopyrimidine nucleosides such as dT and U tend to exhibit two alternative binding modes with metal ions.[28] In neutral and basic solutions metal ions bind with N(3)$^-$, whereas in acids or in nonpolar solvents the deprotonation of N(3)–H is impeded; hence, the available binding sites are restricted to the carbonyl oxygens at C(2) and/or C(4).



Several systematic studies have been conducted on the preferred binding sites of alkali, alkaline earth, and transition metal ions.[28] Free Zn^{II} ion is claimed by a potentiometric pH titration method to bind to N(3)$^-$ of U with the 1:1 anion affinity constant, log K, being 3.67 at 25 °C and I = 0.1 (KNO_3).[29] However, there is no firm evidence for this structural assignment. We repeated the same titration at 25 °C and I = 0.10 ($NaClO_4$) but observed only the precipitation due to the formation of Zn^{II} hydroxide.

On the Zn^{II}–[12]aneN$_4$ complex, **4**, one face is available for an incoming substrate for coordinating to Zn^{II} and the four NH groups of [12]aneN$_4$ are spatially directed toward it. It seems reasonable to expect that, if dT coordinates to Zn^{II} at the deprotonated N(3), the two carbonyl oxygens of dT form two hydrogen bonds with the two NH groups of [12]aneN$_4$, since these functionalities are in suitable positions. The highly selective binding of Zn^{II}–[12]aneN$_4$ complex, **4**, to dT and its related nucleosides is thus rationalized.

Among the thymidine derivatives, the affinity order dT (log K = 5.6), AZT (5.6) > U (5.2) > Ff (4.6) is consistent with the order of the basicities of the conjugate base N(3)$^-$; see the linear relationship between log K(ZnL–S) and pK_a values of the conjugate acid in Figure 5. This indicates that **4** binds to these thymidine derivatives in the same manner, as was observed in the AZT complex **8**. Earlier, we found that the Zn^{II} ion in **4** binds with various anions.[17] It is remarkable that the present N(3)$^-$ anions in nucleosides have much stronger affinities to **4** than CH_3COO^- (log K = 1.9) and SCN^- (2.1), which are almost comparable to that of the strongest anion donor OH^- (6.0).[17b]

An attempt was made to find direct evidence for the possible hydrogen bonding between the pyrimidine carbonyl oxygens and the two NH groups of the [12]aneN$_4$ part. Deuterated **8** was

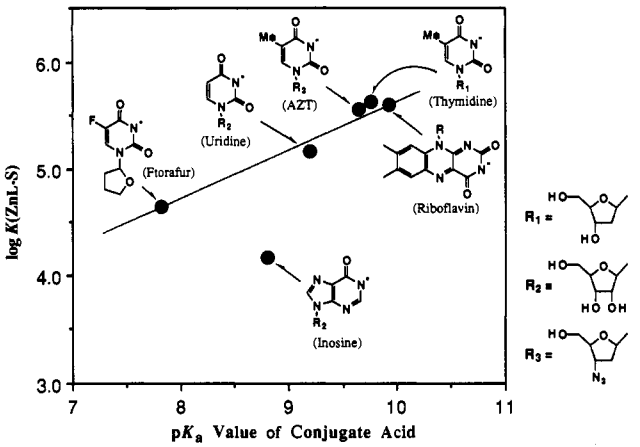
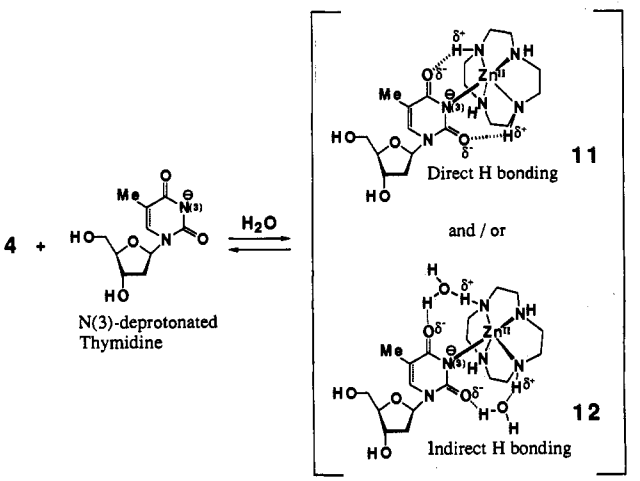


Figure 5. Plot of the anion affinity constants for N(3)– (or N(1)– for dG and Ino) deprotonated substrates, log K(ZnL–S), against pK_a values for the conjugate acids.

Scheme III



prepared by the recrystallization of **8**·ClO_4·2H_2O from D_2O, where the four hydrogen atoms attached to cyclen nitrogens and the hydrogen atom of the ribose O(5′) hydroxyl group were readily exchanged. The IR spectra were compared with those of **8**·ClO_4·2H_2O in both the solid state (KBr pellet) and solution (in DMSO-d_6), as shown in Table I. A new peak appeared in both spectra was assigned to ND frequencies, 2460 cm^{-1} for KBr pellet and 2598 cm^{-1} for DMSO-d_6 solution. However, these peaks are too broad to permit conclusion on the hydrogen bonding.

As for Ino, which lacks one carbonyl group at the C(2) position compared to dT (see Chart I), the affinity with **4** is smaller (log K = 4.2) than predicted from the amide pK_a value of 8.81; see Figure 5. This indirectly shows that the two hydrogen bonds accepted by the two carbonyl groups of thymidine homologues (see **11**) may jointly serve to supplement the stability of the complex **8** in aqueous solution (Scheme III). One may also consider the indirect hydrogen bonds, depending on the state of Zn^{II}–N(3)$^-$ axis rotation (see **12**).[30]

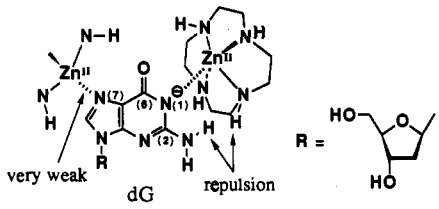
The deprotonated N(1) is the most favored donor in dG for transition metal ions such as Cu^{II}, Ni^{II}, Co^{II}, and Zn^{II} in aqueous solutions.[28b] N(1) of dG might similarly like to bind to Zn^{II} in **4** with concomitant deprotonation. Moreover, one may consider possible direct coordination of the Zn^{II} complex to N(7) of dG

(28) Goodgame, M.; Jakubovic, D. A. *Coord. Chem. Rev.* **1987**, *79*, 97 and references cited therein.

(29) Kahn, B. T.; Rajn, R. M.; Zakeeruddin, S. M. *J. Coord. Chem.* **1987**, *16*, 237.

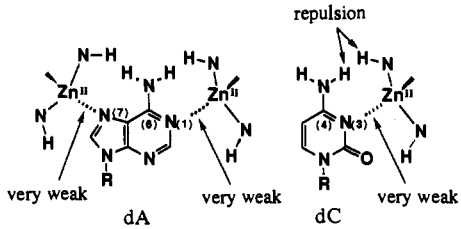
(30) We have recently synthesized a Zn^{II}–*N*-acridinylmethyl–[12]aneN$_4$ complex. It exhibited a stronger affinity for dT: log K = 7.2 ± 0.1 at 25 °C and I = 0.10 ($NaNO_3$). In its NMR spectrum in D_2O the formation of the two hydrogen bonds is evident (due to the extremely slow D-exchange) between the two pyrimidine carbonyl oxygens and the two NH groups of the [12]-aneN$_4$ unit. This is because a rotation that may occur around the Zn^{II}–N(3)$^-$ bond is most likely restricted by the stacking interaction between the thymine base and the acridine aromatic ring. The details will be reported later.

with potential hydrogen bonding available from the macrocyclic amines to the O(6) position. However, no interaction was observed between them in the pH titration. The repulsive interaction between the NH groups in **4** and the amino group at C(2) would block the closer access of **4** to dG (see **13**). Further, N(7) of dG



dG

13



dA dC

14 **15**

does not bind to **4** because the N(7) is sp^2-hybridized and has a very low pK_a. Should N(7) bind to Zn^{II} in **4**, the pH of the buffer region, corresponding to the deprotonation of the Zn^{II}-bound H_2O in **4**, would have to be raised, as is readily derived from the stipulated mass balances. No data using more than 3 mM of dG were available due to its poor solubility. By consideration of the experimental errors and limitations, the log K(ZnL–S) value for dG was estimated to be much less than 3.

N(1) and N(7) of dA tend to bind to transition metal ions in aqueous media.[28b] However, Zn^{II} in **4** is coordinatively almost saturated by four nitrogens,[17b] and hence the binding of **4** to such neutral nitrogen donors, which are sp^2-hybridized within an aromatic ring system and have very low pK_a values, would not be feasible in aqueous media (see **14**).

With dC, aquated Zn^{II} ion can bind to N(3).[28b] A weak (if any) interaction with a neutral sp^2-hybridized N(3) and a repulsive interaction with the amino group at C(4) would hinder the complexation of dC with **4** (see **15**). *Thus, the Zn^{II} complex **4** is very specific only to the thymidine homologues due to novel complementary associations.*

Summary and Conclusion

The Zn^{II}–[12]aneN$_4$ complex, **4**, is a new type of artificial receptor that *in aqueous solution* recognizes and binds specifically to thymidine and its homologues containing an "imide" functionality. The centrosymmetric linear arrangement of the three-point functional groups in **4** comprises the acidic Zn^{II} acting to yield the "imide" anion to form a stable Zn^{II}–N(3)$^-$ bond and the two hydrogens attached to cyclen nitrogens to form two complementary hydrogen bonds with each of the "imide" carbonyls. These electronic and structural fittings permit formation of extremely strong 1:1 complexes of **4** with dT, AZT, U, Ff, and riboflavin. We further found that **4** interacts less strongly with Ino and does not interact with other DNA-related nucleosides, i.e., dG, dA, and dC. The data show that the major bonding is between Zn^{II} and the N$^-$ anionic group of each nucleobase, which is controlled by attractive or repulsive interactions between the cyclen NH groups and the substituents on the nucleobase rings. These complementarities make the binding of the Zn^{II}–macrocyclic tetraamine complex **4** to nucleosides highly selective. The present new molecular recognition will find wide applications in DNA and RNA biochemistry as well as medicinal chemistry.[31]

Acknowledgment. We thank Professor Luigi G. Marzilli (Emory University) for helpful discussion. E.K. is thankful to the Ministry of Education, Science, and Culture in Japan for financial support by a Grant-in-Aid for Scientific Research (A) (No. 04403024) and a Grant-in-Aid for Scientific Research on Priority Areas "Bioinorganic Chemistry" (No. 03241105). M.S. expresses his appreciation to Dr. Scott McNiven (Hiroshima University) for his continuous interest and support.

Supplementary Material Available: For **8**·ClO_4·$2H_2O$, tables of atomic coordinates and equivalent isotropic temperature factors, anisotropic temperature factors, and bond distances and angles and an ORTEP diagram showing atom numbering (11 pages); a listing of observed and calculated structure factors (23 pages). Ordering information is given on any current masthead page.

(31) Aliphatic imide compounds (e.g., barbituric acids) also strongly interact with the Zn^{II}–[12]aneN$_4$ complex, **4** (unpublished results).