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Effects of Bis(aromatic) Pendants on Recognition of Nucleobase Thymine by Zn^{2+}-1,4,7,10-tetraazacyclododecane (Zn^{2+}-cyclen)

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We have previously shown that the nucleobase thymine binding to Zn^{2+}-cyclen (cyclen = 1,4,7,10-tetraazacyclododecane) complex became stronger by appending acridine, naphthalene, or quinoline rings to the cyclen. Amongst these, the pendant bis((1-naphthyl)methyl) or bis((4-quinolyl)methyl) groups yielded the most effective thymine-recognizing Zn^{2+}-cyclen complexes [*J. Am. Chem. Soc.*, 121 (1999) 5426]. The present study was undertaken to find causes of the bis(aromatic) ring effect by X-ray crystal structure analysis and NMR studies. The crystal structure of the Zn^{2+}-bis((1-naphthyl)methyl)-cyclen complex with a deprotonated 1-methylthymine (1-MeT) failed to show the anticipated evidence for the double π−π stacking interactions between the two naphthalenes and the Zn^{2+}-bound 1-MeT^- (1-MeT^- = N(3′)-deprotonated 1-MeT). Crystal data: formula $C_{36}H_{47}N_7O_7Zn$, M_r = 755.19, monoclinic, space group $P2_1/c$ (No. 14), a = 15.438(2) Å, b = 14.093(3) Å, c = 16.726(2) Å, β = 90.53(1)° V = 3638.7(8) Å^3 Z = 4, R = 0.035, R_w = 0.049. However, the ^{1}H NMR studies of Zn^{2+}-bis((4-quinolyl)-methyl)-cyclen with 1-MeT in varying H_2O/CH_3CN solution showed increasing upfield shifts of Me(5′) and H(6′) of the Zn^{2+}-bound 1-MeT in more aqueous media, indicating that the double intercalation with the two quinolines became more significant in more protic environments. We conclude that the double π−π stacking effect accounts for the enhanced recognition of thymine base by the appended bis((1-naphthyl)methyl) or bis((4-quinolinyl)methyl) groups.

Keywords: X-ray crystal structure; Bisintercalators; Cyclen complex; NMR; zinc; thymine

INTRODUCTION

Several years ago, we discovered that the Zn^{2+}-cyclen complex **1** selectively formed 1:1 complexes **7** with thymidine (dT) derivatives such as AZT through coordination between the Zn^{2+} and the deprotonated N3 in neutral pH aqueous solution with an apparent dissociation constant, K_d, of ca. 1 mM [1–13]. Later, in an effort to search for more efficient dT-recognizing Zn^{2+}-cyclen complexes, we synthesized a Zn^{2+} complex of (9-acridinyl)methyl-pendant cyclen, **2**, which formed a complex **8** with dT with ca. 50 times higher affinity (Scheme 1) [14]. On the basis of the X-ray crystal structure and hypochromic effects in the UV spectroscopic study, we concluded that a π−π stacking interaction between the Zn^{2+}-bound dT and the pendant acridine accounts for the enhanced affinity. The remarkable effect of the pendant acridine was manifested as the efficient dT-selective interaction of the Zn^{2+}-cyclen derivatives with native DNA [15–17]. Although a similar poly(dA−dT) sequence recognition pattern is well known for some conventional DNA-interacting drugs such as distamycin, [18–20] this mechanism is entirely different [21–23].

Bisintercalators [24–32] occur naturally (e.g. triostin A [25], echinomycin [26,27] and calzinophilin A [28]) and have been synthesized artificially [29–32] (e.g. bis(adenine) [29] and ditercalinium [30]). We designed a cyclen derivative with two appended acridines in order to obtain a complex with a much higher affinity for thymine by the formation of a double π−π stacked arrangement of acridine surrounding the Zn^{2+}-bound thymine ring. It was disappointing, however, to find that the Zn^{2+}-bis((9-acridinyl)methyl)-cyclen complex was too insoluble

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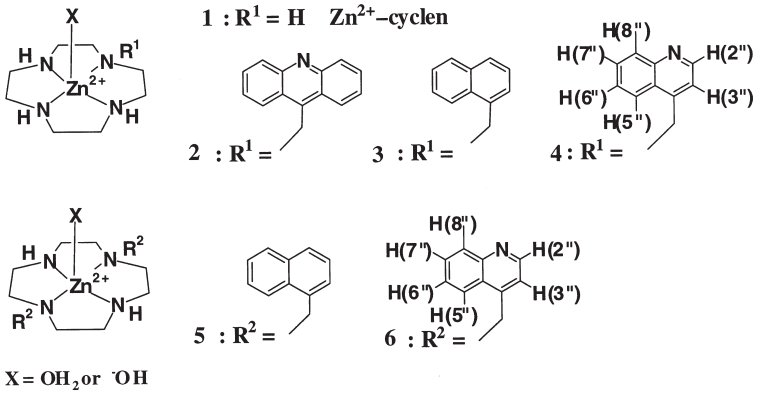


in water to be extensively studied. We then synthesized Zn^{2+}-bis((1-naphthyl)methyl)-cyclen **5** and Zn^{2+}-bis((4-quinolyl)methyl)-cyclen **6** [21–23]. For reference, we also prepared Zn^{2+}-((1-napthyl)-methyl)-cyclen **3** and Zn^{2+}-((4-quinolyl)methyl)-cyclen **4**. In an earlier study involving potentiometric pH titrations, we determined that 1:1 affinity constants for **3**, **4** and **6** with dT [21]. Indeed, we saw the highest affinity for dT with **6** (Table I). The Zn^{2+}-bis((1-naphthyl)methyl)-cyclen complex **5** was not soluble enough to permit the potentiometric pH titration. With DNase I and micrococcal nuclease foot print assays, we found that the bispendant complexes **5** and **6** recognized poly(dT) regions of native DNA (150 bp from *E. coli*) more efficiently than the monopendant complex **2**, **3**, or **4** [21]. In the present study, we have investigated more extensively interaction of dT and 1-methylthymine (1-MeT) with **3**–**6** to see the effects of the double pendants by X-ray crystal structure analysis, ^{1}H NMR titrations, and isothermal calorimetric titrations.

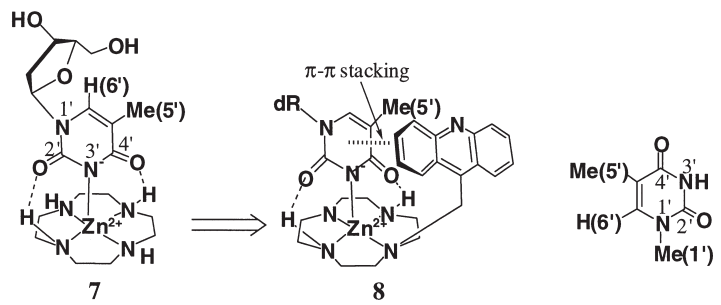
EXPERIMENTAL SECTION

General Information

All reagents and solvents used were of the highest commercial quality and used without further purification. The zinc(II) complexes **3**, **4**, **5**, and **6** were synthesized as we previously described in Ref. [21]. All aqueous solutions were prepared using deionized and redistilled water. A buffer solution for UV titration was EPPS (3-(4-(2-hydroxyethyl)-1-piperazinyl)propanesulfonic acid; $pK_a = 8.0$ at 20°C). The ionic strength of the buffer was adjusted to 0.10 or 0.05 with $NaNO_3$. ^{1}H NMR spectra were recorded on a JEOL Lambda (500 MHz) spectrometer: 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (tsp) was used as an internal reference for ^{1}H NMR measurements in D_2O. The pD values in D_2O were corrected for a deuterium isotope effect using pD = [pH−meterreading] + 0.40.

Isolation Of 5–(1-MeT$^-$) Complex (9·NO_3·2H_2O)

A solution of **5**·2NO_3 (257 mg, 0.4 mmol) [21] in 3:2 CH_3CN/H_2O (20 ml) was added to a solution of 1-methylthymine (56 mg, 0.4 mmol) in 0.1 M NaOH (4 ml) and the reaction mixture was concentrated slowly *in vacuo*. Colorless prisms of **5**–(1-MeT$^-$) complex·NO_3·2H_2O (**9**·NO_3·2H_2O) (255 mg, 75% yield) were obtained (mp > 250°C). IR (KBr): $\nu = 3456$, 3003, 1655, 1635, 1576, 1443, 1385, 1096, 947 cm^{-1}. ^{1}H NMR (500 MHz, CD_3CN, 35°C, TSP): $\delta = 1.82$ (3H, d, $J = 1.2$ Hz, CH_3 (5′) of 1-MeT),



SCHEME 1

TABLE I Comparison of affinity constants ($\log K_{aff}$) of Zn^{2+}–cyclens with dT at 25°C

	$\log K(ZnL{-}S^-)$*	$\log K_{app}(ZnL{-}S^-)$† at pH 8.0	
Zn^{2+}–cyclens	pH-metric	pH-metric	calorimetric‡
1	5.6	3.4	3.2
2	7.2	4.7	
3	6.3	4.2	4.1
4	6.8	4.3	4.4
5	N.D.¶	N.D.¶	4.9
6	7.7	5.0	5.1

* $K(ZnL{-}S^-) = [ZnL{-}S^-]/[ZnL][S^-]$ (M^{-1}) where S = N(3′)-deprotonated thymidine. † $K_{app}(ZnL{-}S^-) = [ZnL{-}S^-]/[ZnL]_{free}[S^-]_{free}$ (at designated pH) (M^{-1}) where $[ZnL]_{free} = [ZnL(OH_2)] + [Zn_2L(OH^-)]$ and $[S]_{free} = [S] + [S^-]$. ‡Carried out at $[ZnL]_{initial} = 0.5$ mM in 10:90 CH_3CN/10 mM EPPS (pH 8.0). ¶ N.D. = not determined.

2.52–2.58 (4H, m, CH_2, of rings), 2.76–2.82 (4H, m CH_2 of cyclen rings), 2.96–3.01 (4H, m, CH_2 of cyclen rings), 3.19–3.27 (4H, m, CH_2 of cyclen rings), 3.23 (3H, s, CH_3 (1′) of 1-MeT), 4.33 (4H, brs, ArCH_2), 7.10 (1H, d, $J = 1.4$ Hz, H (6′) of 1-MeT), 7.43–7.60 (8H, m, ArH), 7.88–794 (4H, m, ArH), 8.17 (2H, d, $J =$ 8.30 Hz, ArH). ^{13}C NMR (125 MHz, DMSO-d_6, 35°C); $\delta = 12.6$, 43.2, 50.4, 95.2, 108.3, 123.3, 123.5, 124.9, 125.5, 126.3, 128.5. 128.6, 129.3, 129.8, 132.6, 133.4, 141.8, 157.4, 172.0. Anal. Calcd. for $C_{36}H_{47}N_7O_7Zn$: C 57.26, H 6.27, N 12.98. Found: C 57.41, H 6.15, N 12.74.

Isolation Of 6–(1-MeT$^-$) Complex (10·ClO_4·2H_2O)

A solution of **6**·2NO_3·H_2O (99 mg, 0.15 mmol) [21] in 3:2 CH_3CN/H_2O (5 ml) was added to a solution of 1-methylthymine (21 mg, 0.15 mmol) in 0.1 M aq. NaOH (0.75 ml). After addition of $NaClO_4$ (122 mg, 1.0 mmol) in H_2O (3 ml), the whole mixture was filtered and concentrated slowly *in vacuo* and colorless prisms of **6**–(1-MeT) complex·ClO_4·2H_2O (**10**·ClO_4·2H_2O) (67 mg, 56% yield) were obtained (mp > 250°C) (although we have not experienced the explosion of ClO_4 salts of zinc complexes, the standard warning of their hazards should be noted). IR (KBr): $\nu = 3440$, 1655, 1639, 1574, 1363, 1333, 1096, 957, 774, 625 cm^{-1}. ^{1}H NMR (500 MHz, D_2O, 35°C TSP): $\delta = 1.40$ (3H, brs, CH_3(5′) of 1-MeT), 2.86–2.91 (4H, m, CH_2 of cyclen rings), 3.00–3.04 (4H, m, CH_2 of cyclen rings), 3.11–3.14 (4H, m, CH_2 of cyclen rings), 3.37–3.41 (4H, m, CH_2 of cyclen rings), 4.14 (4H, brs, ArCH_2), 6.67 (s, 1H, H (6′) of 1-MeT), 7.41 (2H, br, ArH); 7.54 (2H, br, ArH); 7.72 (2H, dd, $J =$ 7.0, 7.0 Hz, ArH); 7.88 (2H, d, $J = 7.0$ Hz, ArH); 7.92 (2H, d, $J = 8.5$ Hz, ArH); 8.64 (2H, br, ArH). ^{13}C NMR (125 MHz, DMSO-d_6, 35°C): $\delta = 12.5$, 35.3, 43.3, 49.5, 50.3, 95.3, 108.3, 123.2, 123.5, 126.7, 127.5, 129.1, 129.8, 139.1, 141.7, 149.4, 157.3, 171.9. Anal. Calcd. for $C_{34}H_{45}N_8O_8ClZn$: C 51.39, H 5.71, N 14.10. Found: C 51.27, H 5.43, N 14.01.

Crystallographic Study Of 5–(1-MeT$^-$) Complex·NO_3·2H_2O (9·NO_3·2H_2O)

A colorless prismatic crystal of ($C_{36}H_{47}N_8O_8Zn$, $M_r = 755.19$) having approximate dimensions of $0.30 \times 0.30 \times 0.20$ mm^3 was mounted in a loop. Intensity data were collected on a Rigaku RAXIS-RAPID imaging plate diffractometer with Mo Kα radiation at of 298.2°C. Cell constants and an orientation matrix for data collection corresponded to primitive monoclinic cell with dimensions: $a =$ 15.438(2) Å, $b = 14.093(3)$ Å, $c = 16.726(2)$ Å, $\beta =$ 90.53(1)° $V = 3686.7(8)$ Å^3. For $Z = 4$ and $M_r =$ 755.19, the calculated density (D_{calcd}) is 1.378 g cm^{-3}. The systematic absence of: hk0l: $h + 1 = 2n + 1$ and 0k0: $k = 2n + 1$ uniquely determine the space group was determined to be: $P2_1/c$ (No. 14). Total of 7536 unique reflections were collected. The structure was solved by direct methods (SIR97) and refined by full-matrix least-squares base on 5473 observed reflections ($I > 3\sigma(I)$) and 154 variable parameters. The values of R_w, $R1$, and S (goodness of fit) were 0.0489, 0.0349, and 1.20, respectively: $R_w = ((\sum w(F_0^2 - F_c^2)^2 / \sum w(F_0^2)^2)^{0.5}) = 0.0489$. $R1 = (\sum ||F_0| - |F_c|| / \sum |F_0|) = 0.0349$ for $I > 2.0\sigma(I)$ data. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.33 and $-0.34\,e^-$Å^{-3}, respectively. All calculations were performed with the teXsan crystallographic software package (Molecular Structure Corporation and Rigaku, 1999).

Crystallographic data (excluding structure factors) for the **5**–(1-MeT$^-$) complex·NO_3·2H_2O have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-173499. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Potentiometric PH Titrations

The preparation of the test solutions and the calibration method of the electrode system (Potentiometric Automatic Titrator AT-400 and Auto Piston Buret

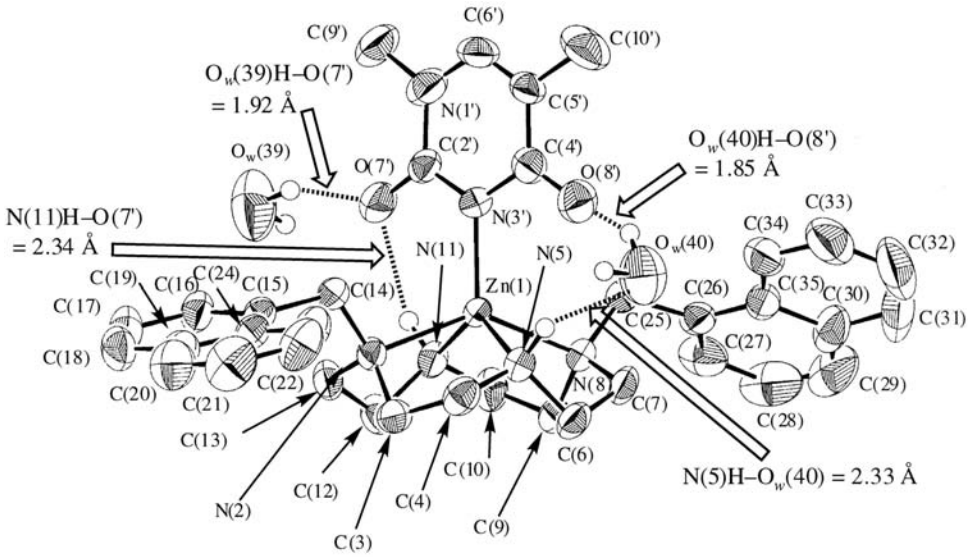


FIGURE 1 ORTEP drawing (Thermal ellipsoids drawn to the 50% probability level) of the **5**–(1-MeT$^-$) complex (corresponding to the structure **9a** in Scheme 2, where dR = Me. Two hydrogen bondings are seen for carbonyl O(7′) with O_w(39) (O_w donates an oxygen atom of water) and N(11)-H of **5** and a hydrogen bonding network is formed between carbonyl O(8′) and N(5)-H via carbonyl O_w(40). Full details of this structure are available from the Cambridge Crystallographic Database.

APB-410 (Kyoto Electronics Manufacturing, Co. Ltd) with Orion Research Ross Combination pH Electrode 8102BN) were described earlier [1,9,11,12,14]. All the test solutions (50 ml) were kept under an argon (>99.999% purity) atmosphere. The potentiometric pH titrations were carried out by addition of 0.1 N aq. NaOH 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 constants defined in the text were determined by means of the program BEST [33]. All the sigma fit values defined in the program are smaller than 0.05. The K_W(= a_{H^+}, a_{OH^-}), K'_W(= $[H^+] \times [OH^-]$) 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 [10–12]

The heats of 1:1 complexation of 1-MeT with zinc(II) complexes were recorded on a Calorimetry Science Corporation Isothermal Titration Calorimeter 4200 at 25.0 ± 0.1°C. 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 **3**–**6**, (0.5 mM) is 10/90 CH_3CN/10 mM EPPS (pH 8.0 with $I = 0.05$ or 0.1 ($NaNO_3$) was put into a calorimeter cell. After the cell temperature had become constant at 25.0°C, the solution of dT (25 mM) in 10 mM EPPS 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).

RESULTS AND DISCUSSIONS

X-ray Crystal Structure of Zn^{2+}-Bis ((1-naphthyl)methyl)-cyclen-1-methylthymine Complex (9)

Mixing Zn^{2+}-bis(1-naphthyl)methyl)-cyclen **5** or Zn^{2+}-bis((4-quinolyl)methyl)-cyclen **6** with an equivalent amount of 1-MeT in basic CH_3CN/H_2O

TABLE II Selected X-ray crystal data of the **5**–(1-MeT$^-$)·$NO_3$2·H_2O complex (**9**·NO_3·2H_2O)

Formula	$C_{36}H_{47}N_7O_7Zn$
M_r	755.19
Cryst syst	monoclinic
Space group	$P2_1/c$ (No. 14)
a(Å)	15.438(2)
b(Å)	14.093(3)
c(Å)	16.726(2)
β (deg)	90.53(1)
V(Å^3)	3638.7(8)
Z	4
D_{calc}(g cm^{-3})	1.378
Radiation	μ(MoK_α)(= 0.733 mm^{-1})
$2\theta_{max}$	55.2
R	0.035
R_w	0.049
No. of reflections used for least squares ($I > 3\sigma(I)$)	5473
Number of variables	460

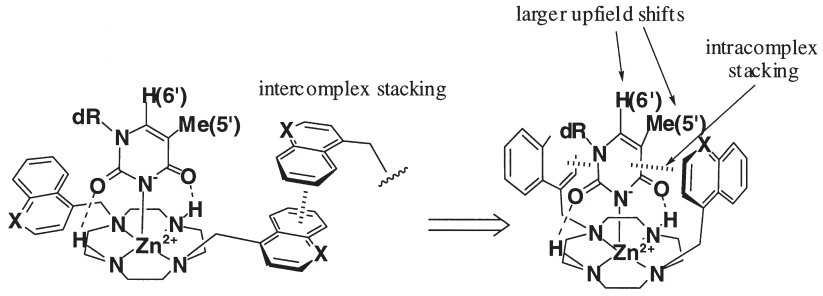
TABLE III Selected bond distances (Å), bond angles (°), and dihedral angles (°) of the **5**–(1-MeT$^-$)·NO_3·$2H_2O$ complex (**9**·NO_3·$2H_2O$)

Bond distances	Distance (Å)
Zn(1)–N(3′)	1.979(2)
Zn(1)–N(5)	2.100(2)
Zn(1)–N(11)	2.106(2)
O(8′)–C(4′)	1.242(3)
N(1′)–C(6′)	1.363(4)
Zn(1)–N(2)	2.240(2)
Zn(1)–N(8)	2.224(2)
O(7′)–C(2′)	1.239(3)
N(1′)–C(2′)	1.412(3)
N(1′)–C(9′)	1.487(4)
Bond angles	**angle(°)**
N(2)–Zn(1)–N(3′)	109.33(8)
N(2)–Zn(1)–N(8)	139.99(7)
N(3′)–Zn(1)–N(5)	118.60(8)
N(3′)–Zn(1)–N(11)	111.94(8)
Zn(1)–N(8)–C(25)	112.1(1)
N(8)–C(25)–C(26)	115.0(2)
N(2)–Zn(1)–N(5)	81.11(8)
N(2)–Zn(1)–N(11)	82.04(7)
N(3′)–Zn(1)–N(8)	110.63(8)
Zn(1)–N(2)–C(14)	110.6(1)
N(2)–C(14)–C(15)	116.2(2)
Dihedral angles	**angle(°)**
N(2)–Zn(1)–N(3′)–C(2′)	−62.6(2)
N(2)–C(14)–C(15)–C(24)	99.3(3)
N(8)–Zn(1)–N(3′)–C(4′)	−62.1(2)
N(8)–C(25)–C(26)–C(35)	−96.8(3)
N(11)–Zn(1)–N(3′)–C(4′)	−151.0(2)
N(2)–C(14)–C(15)–C(16)	−83.8(3)
N(5)–Zn(1)–N(3′)–C(4′)	29.7(2)
N(8)–C(25)–C26–C(27)	83.6(3)
N(11)–Zn(1)–N(3′)–C(2′)	26.4(2)

solution yielded 1:1 complexes **9** (crystallized as a NO_3 salt) and **10** (as a ClO_4 salt). We succeeded in obtaining an X-ray crystal structure analysis of **9**, as shown in Fig. 1. The crystal data are shown in Table II and the selected bond distances, bond angles, and dihedral angles are summarized in Table III. The crystal structure of **9** revealed the N(3′)-deprotonated 1-MeT (1-MeT) bound to Zn^{2+} with the two naphthalene pendants splayed apart rather than stacking with 1-MeT$^-$ (**9a** in Scheme 2). This pendant conformation in the solid state may be favored by the lattice packing forces wherein naphthalene rings are stacked face-to-face with the other naphthalene rings of adjacent complexes (the distance between two naphthalene rings is 3.395(3) Å as shown in Fig. 2). It was interesting to see if these intercomplex aromatic–aromatic stacking in the solid would be replaced by intracomplex aromatic–thymine–aromatic stacking in solution. Such stacking may be detectable via upfield shifts of ^{1}H NMR resonances of the thymine rings in **9** or **10** in CD_3CN/D_2O solution, as found for **8** [14].

^{1}H NMR Studies Of 10 In $CD_3CN\backslash/D_2O$ Solution

The ^{1}H NMR chemical shifts (δ in ppm) of Zn^{2+}-cyclen **1** (5 mM), Zn^{2+}-mono((4-quinolyl)methyl)-cyclen **4** (5 mM) and Zn^{2+}-bis((4-quinolyl)methyl)-cyclen **6** (3 mM) in the absence and presence of an equimolar amount of dT in D_2O at pD 8.5 (where dT is complexed almost quantitatively with **1**, **4**, and **6**) are summarized in Table IV(**4** and **6** were chosen because these two complexes were more soluble than **3** and **5**). Comparison of the **4**–(dT$^-$) complex, the **6**–(dT$^-$) complex **10**, and the **1**–(dT$^-$) complex **7** revealed remarkable upfield shifts of the thymine ring protons (Me(5′) and H(6′)) presumably caused by the monoquinoline pendant arm of **4**: e.g. Me(5′) (for numbering, see Scheme 1), $\Delta\delta = -0.24$ ppm and for H(6′), $\Delta\delta = -0.33$ ppm, and even more remarkable upfield shift caused by the bisquinoline pendant arms of **6**: for Me(5′), $\Delta\delta = -0.40$ ppm, and for H(6′), $\Delta\delta = -0.43$ ppm (note that Me(5′) corresponds to C(10′) in Fig. 1). The ^{1}H signals of quinoline rings also showed similar upfield shifts. For reference, in **8**, the monoarcridine pendant arm effects caused shifts of $\Delta\delta = -0.39$ ppm for Me(5′) and $\Delta\delta = -0.44$ ppm for H(6′) [14]. Thus, $\Delta\delta$ values indicate considerable



9a : X = CH
10a : X = N

in solids and in aprotic solvents

9b : X = CH
10b : X = N

in protic solvents

SCHEME 2

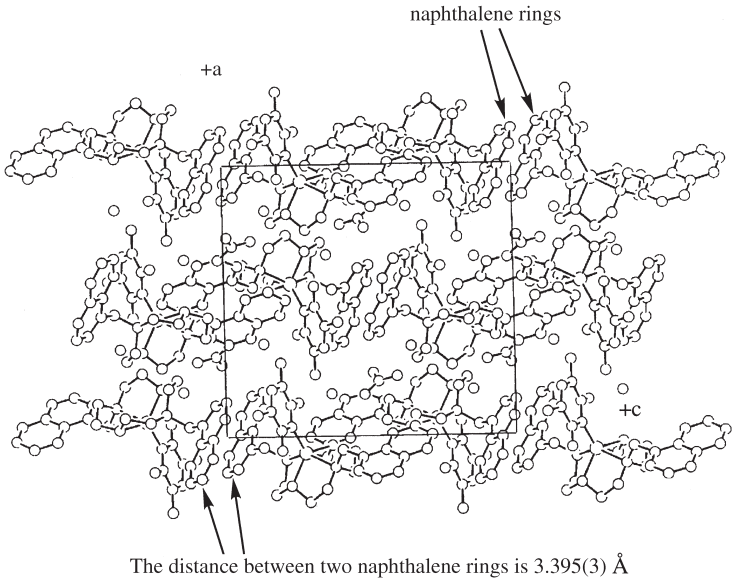


FIGURE 2 Crystal packing of the **5**–(1-MeT$^-$) complex (**9a**) drawn down the *b* crystallographic axis.

stacking for **4**–(1-MeT$^-$) and an even greater degree of stacking for **6**–(1-MeT$^-$) in aqueous solution, results consistent with higher stability ($\log K$ (ZnL–S$^-$) = 7.7) of the thymine complex of **6** with respect to the dT complex with **4** ($\log K$(ZnL–S$^-$) = 6.8) (Table I).

The strength of the $\pi-\pi$ stacking interactions may be solvent dependent. We thus measured ^{1}H NMR spectra of the **6**–(1-MeT$^-$) complex **10** (1 mM) in various ratio of mixed solvents (CD_3CN and D_2O (at pD 8.0)) at 25°C. The results are shown in Fig. 3. In going from the most possible aprotic (80% CD_3CN) to deuteriotic solution (100% D_2O), the conformational change of the quinoline pendants may be postulated as depicted in Scheme 2. As the D_2O concentration increases, the intracomplex stacking tendencies increse (**10a** → **10b**). The most significant stacking becomes evident in 100% D_2O by the maximal $\Delta\delta$ values: −0.35 for Me(5′) and −0.4 for H(6′).

Solvent effects were similarly measured by ^{1}H NMR experiments for the 1-MeT complexes of **3**, **4**, and **5** (Fig. 4). Evidently the bis(aromatic) complexes **5** and **6** display more significant upfield shifts than the mono(aromatic) complexes **3** and **4**, a fact supporting our postulate that the Zn^{2+}-bound 1-MeT is sandwiched between the two aromatic (quinoline or naphthalene) rings in polar environments as illustrated in **9b** and **10b**.

Isothermal Calorimetric and UV Spectrophotometric Titrations

We determined the complexation, constants, $\log K_{app}$(ZnL–S$^-$) for **3**–**6** with dT by isothermal titration calorimetry (ITC) [10–12] in 10/90 CH_3CN/10 mM EPPS (pH 8.0) at 25°C (Fig. 5), from which it is immediately evident that 1-MeT complexes with **5** and **6** are more stable that those with **3** and **4**. In our earlier potentiometric pH titration study, the complexation constants for **5** could not be determined, due to the insufficient solubility of **5** [21]. All the calculated results are summarized in Table I. The bis(aromatic) cyclen complexes, **5** and **6** show larger $\log K_{app}$(ZnL–S$^-$) values than the corresponding mono(aromatic)

TABLE IV ^{1}H NMR chemical shifts (δ in ppm) of **1** (5 mM), **4** (5 mM) and **6** (3 mM) in the absence and presence of an equimolar amount of dT in D_2O at pD 8.5 and 25°C

	d*t**		Quinoline moiety†					
	H(6′)	Me(5′)	H(2″)	H(5″)	H(8″)	H(7″)	H(6″)	H(3″)
dT‡	7.50	1.87						
4			8.87	8.24	8.14	7.91	7.78	7.59
6			8.87	8.20	8.14	7.92	7.80	7.59
1 + dT	7.57	1.87						
4 + dT	7.24 (−0.33)¶	1.63 (−0.24)¶	8.65	8.02	7.97	7.77	7.64	7.43
6 + dT	7.12 (−0.43)¶	1.47 (−0.40)¶	8.58	7.94	7.77	7.77	7.64	7.34

*For assignment, see Scheme 1. †For assignment, see the structures **4** and **6**. ‡Measured at pD > 12. ¶ $\Delta\delta$ values against **1** − dT complex.

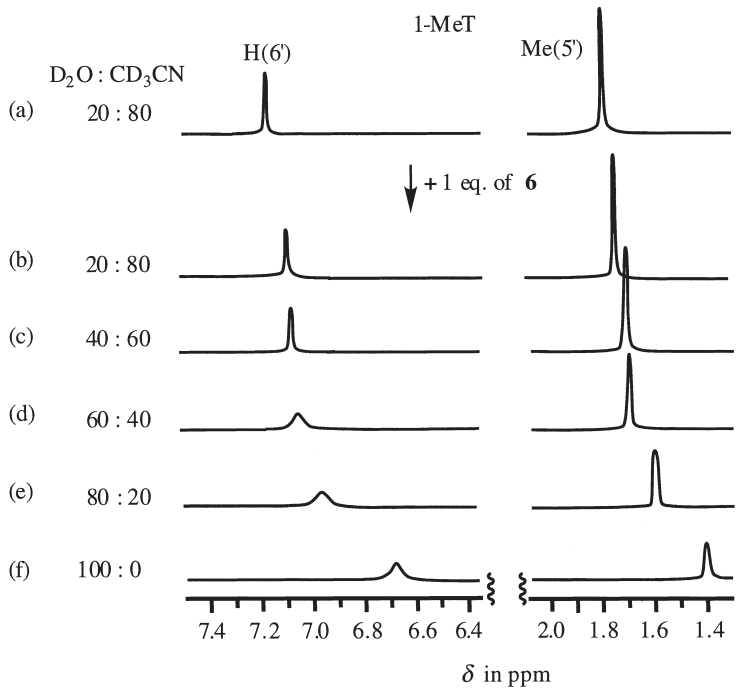


FIGURE 3 ^{1}H NMR (500 MHz) spectral change of a mixture of 1 mM 1-MeT and 1 mM **6** in a various ratio of D_2O and CD_3CN at 35°C. Figure (a) is 1-MeT at pD > 12. Figures (b–f) show H(6′) and Me(5′) of 1-MeT in a 20:80 (b), 40:60 (c), 60:40 (d), and 80:20 (e) mixture of D_2O/CD_3CN, respectively.

complexes, **3** and **4**. The hypochromicities were observed for the pendant quinoline in **4** (17%) and **6** (20%) with an equimolar dT^-, which also supports the stacking between one and two quinoline groups and the thymine ring.

CONCLUSIONS

In this study, we have examined the causes of the greater effect of bis(aromatic) (bis(1-naphthyl)methyl and bis(4-quinolyl)methyl groups) functionalization on the stability of the ternary dT or 1-MeT complexes than the corresponding mono(aromatic) functionalization of Zn^{2+}–cyclens. On the basis of an X-ray crystal structure analysis and ^{1}H NMR spectroscopic experiments, it has been concluded that intracomplex quinoline-thymine-quinoline double stacking is responsible for stronger binding of the thymine ring by **6** in more protic solutions, while intercomplex quinoline–quinoline stacking is preferred in solid states.

We have previously reported that Zn^{2+}-cyclen complexes appended with bis(aromatic) groups, **5** and **6**, bind to double-stranded DNA more strongly than the mono-functionalized counterparts, **3** and **4**. For example, the IC_{50} (concentration required for 50% inhibition) values for **5** and **6** in the complexation of TBP(TATA box binding protein) with DNA (AT region called TATA box, transcription promoter region of SV40 early gene) were 4 and 2.5 μM, respectively, while they were >100 and 70 μM for **3** and **4** [23]. The results described here are consistent with our proposal that the dT complexes of **5** (or **6**) are stabilized by naphthalene–thymine–naphthalene (or quinoline–thymine–quinoline) stacking in aqueous solution. The present findings add further to

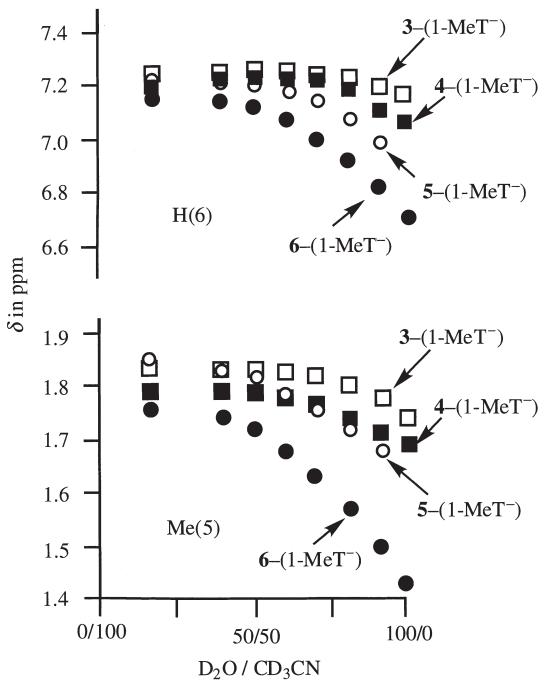


FIGURE 4 ^{1}H NMR (500 MHz) chemical shift change of 1-MeT complexed with **3**, **4**, **5**, **6** in a various ratio of D_2O/CD_3CN at 35°C.

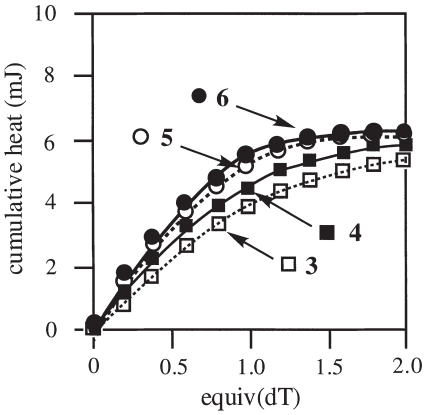


FIGURE 5 Isothermal titration calorimetry curves for **3** (0.5 mM) + dT(blanksquare), **4** (0.5 mM) + dT(blacksquare), **5** (0.5 mM) + dT (blankcircle), and **6** (0.5 mM) + dT(blackcircle), in 10/90 CH_3CN/10 mM EPPS (pH 8.0) at 25°C. Equiv(dT) is the number of equivalents of [dT] added against [ZnL].

our understanding of the recognition processes occurring in these systems and will be useful in the future design of genetic manipulation agents based upon Zn^{2+}-cyclen complexes.

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