Headline Articles

A Zinc(II)–Cyclen Complex Attached to an Anthraquinone Moiety that Acts as a Redox-Active Nucleobase Receptor in Aqueous Solution

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Recently, we demonstrated that a zinc(II) macrocyclic tetraamine complex, Zn^{II}–cyclen (cyclen=1,4,7, 10-tetraazacyclododecane), could bind to thymidine (dT) and uridine (U) selectively among the DNA/RNA bases in aqueous solution at physiological pH. This paper describes the design and synthesis of the zinc(II) complex of an anthraquinone-pendant cyclen. The interaction of this complex with a variety of nucleosides has been studied by potentiometric pH titration, ^{1}H NMR, and a polarographic technique. The effect of the anthraquinone functionality in this complex is an enhanced 1:1 association with N(3)-deprotonated dT ($\log K=6.6\pm0.1$ against 5.6 for Zn^{II}–cyclen system at 25 °C with $I=0.10$ ($NaNO_3$)) and its congeners, implying an additional anthraquinone-thymine aromatic stacking π–π interaction. This complex, moreover, can electrochemically recognize thymidine and other related organic substrates in aqueous solution, and is therefore a new prototype for electrochemical oligonucleotide recognition.

As part of the growing interest in supramolecular chemistry,[1] redox-active host compounds capable of electrochemically recognizing a guest species are currently attracting a great deal of interest. Although ferrocene,[2] quinone derivatives,[3] and a number of other redox-active moieties[4] have been incorporated into a range of receptor molecules, so far electrochemical binding studies have, with a few exceptions,[5] been directed towards the recognition of cationic[2—4] and anionic[6] guest species. However, the electrochemical response to weaker, but biologically more significant, interactions, such as π–π stacking or hydrogen bonding, has not been investigated.

Recently,[7] Zn^{II}–1, 4, 7, 10- tetraazacyclododecane (Zn^{II}–cyclen) **1** has been shown to bind thymidine (dT) and uridine (U) selectively among the DNA/RNA bases, through the combination of Zn^{II}–N^- (imido) coordination and additional hydrogen bonding between the imidodicarbonyl of the guest and NH groups on the cyclen. We subsequently designed an acridine-pendant Zn^{II}–cyclen **3** that maintains base selectivity and has an increased affinity for the thymine base due to an additional π–π stacking interaction between the acridine moiety of **3** and the thymine base.[8] This paper describes how the unique ability of **1** to bind dT and its derivatives has been put to use with the synthesis of a novel conjugate system **2**, which connects an organic redox system, namely the anthraquinone unit, to the Zn^{II}–cyclen nucleobase binding site. Such a supermolecule might act as a site-selective DNA probe or photonuclease.[9] Here we report on the first example of electrochemical DNA/RNA base sensing by the use of the bifunctional zinc(II) complex **2** in an aqueous solution.

Results and Discussion

Synthesis of Anthraquinone-Pendant Cyclen (5) and Its Zinc(II) Complex (2) (Scheme 1). Anthraquinone-pendant cyclen **5** was synthesized by the reaction of 2-(bromomethyl)-9,10-anthraquinone with ca. four molar amounts of cyclen in anhydrous CH_3CN. The acid-free ligand **5** was obtained as a yellow solid after purification by column chromatography. Characterization of the compound was accomplished using ^{1}H and ^{13}C NMR, as well as elemental analysis (C, H, N).

The protonation constants ($K_n=[H_nL]/[H_{n-1}L]a_{H^+}$, M^{-1}) of anthraquinone-pendant cyclen **5** (L) were determined by potentiometric pH titration with a 0.1 M NaOH aqueous solution (1 M=1 mol dm^{-3}) at 25 °C with $I=0.10$ ($NaNO_3$). The protonation constants of

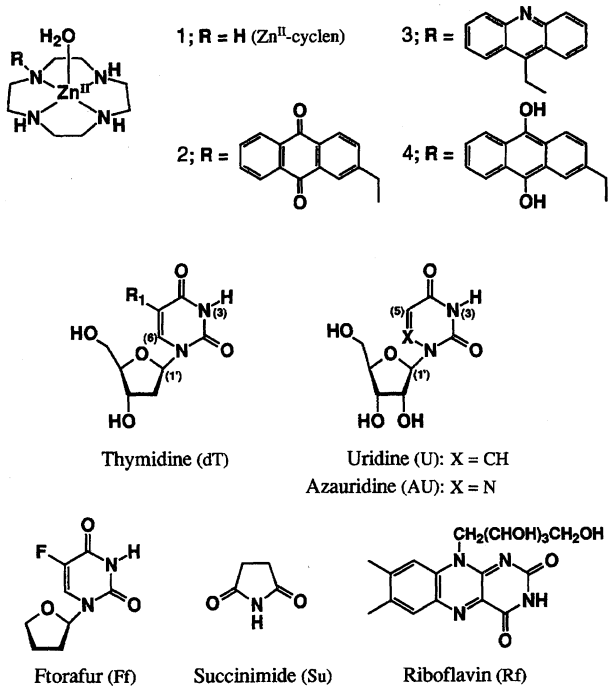
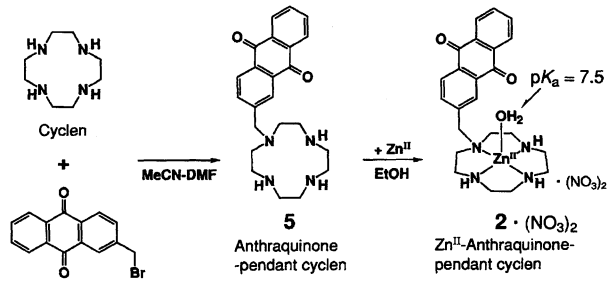


Chart 1.

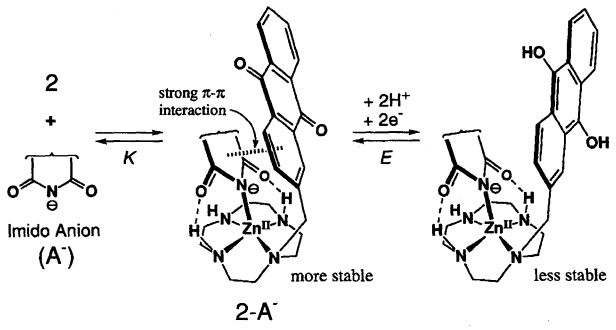


Scheme 1.

the macrocyclic tetraamine moiety, $\log K_1 - \log K_4$, are 10.46±0.03, 8.51±0.02, <2, <2, respectively.

The zinc(II) complex **2** was isolated as its nitrate salt from an EtOH solution of **2** and an equimolar amount of $Zn(NO_3)_2 \cdot 6H_2O$. The pK_a value of the Zn^{II}-bound water in **2** was determined to be 7.48±0.02 by potentiometric pH titration of **2** at 25 °C with I=0.10 ($NaNO_3$). The acidity of Zn^{II} in **1** (pK_a=7.88)[7] or in Zn^{II}–*N*-methyl cyclen (pK_a=7.68)[8] is thus further enhanced in **2**, possibly due to a hydrophobic effect of the anthraquinone. This compound was characterized by ^1H and ^{13}C NMR, as well as elemental analysis (C, H, N).

Determination of Affinities of 2 to dT and Its Homologues. In order to study the interaction of **2** with the "imido" functionalily of nucleobases in aqueous solution, potentiometric pH titrations were performed with equimolar solutions of **2** (1.0 mM) and organic guests dT, U, ftorafur (Ff), azauridine (AU), succinimide (Su), and riboflavin (Rf)[10] (see Chart 1) at 25 °C with I=0.10 ($NaNO_3$). The resulting data are consistent with the 1:1 imido anion complexation of the anthraquinone-pendant Zn^{II}–cyclen (Scheme 2). The



Scheme 2.

same imido anion binding mode was observed for **1**[7] and the zinc(II) complex of acridine-pendant cyclen **3**.[8] For each thymidine homologue that was investigated, the buffer pH region corresponding to the deprotonation of the Zn^{II}-bound H_2O significantly dropped, indicating imido deprotonation with concomitant complexation. The imido anion (A^-) binding constants, $\log K$ (K=[Zn^{II}-complex-A^-]/[A^-][Zn^{II}-complex]), between **2** and these guest anions were evaluated in a similar manner as used before[8] (Table 1).

The open site (i.e., the H_2O-binding site) of **2** is available for an incoming substrate, and the three NH groups of the cyclen ring and the anthraquione plane are spatially directed towards it. It was reasonable to expect that if dT, or its homologues, coordinated to Zn^{II} at the deprotonated N(3), the two contiguous exocyclic carbonyl oxygens would supplement the interaction by forming two hydrogen bonds with two NH groups of the cyclen, and additionally, the anthraquinone ring would reinforce the complex stability by means of a stacking interaciton with the pyrimidine ring.

In fact, the complexation constants with **2** were all found to be greater than those with the unsubstituted Zn^{II} complex **1** (Table 1), supporting our prediction of an additional binding force from a stacking interaciton.

Table 1. A Comparison of Imido Anion Affinity Constants (K) and ΔE Values for the Electrochemical Reduction of **2** to **4** at 25 °C

Anion (A^-)	pK_a[b]	log K[a] **1**[b]	**2**[c]	**3**[c]	**4**[d]	ΔE[e]
Rf^-	9.92	5.6	7.1±0.2	7.3	N.D.	N.D.
dT^-	9.76	5.6	6.6±0.1	7.2	5.9	−20
Su^-	9.52	5.6	5.8±0.1	N.D.	5.5	−8
U^-	9.19	5.2	5.8±0.1	6.9	5.3	−16
Ff^-	7.82	4.6	5.3±0.1	6.6	4.8	−14
AU^-	6.58	4.1	4.3±0.1	6.3	4.0	−10

a) K=[Zn^{II}-complex-A^-]/[A^-][Zn^{II}-complex], b) I= 0.10 ($NaClO_4$) (from Ref. 7 except Su^-), c) I=0.10 ($NaNO_3$)(data for **3** from Ref. 8, N.D. is not determined), d) K(for **2**-A^-)/K(for **4**-A^-) = exp[− 2$F\Delta E/RT$], where F/RT = 38.9 V^{-1}, e) The confidence limit is within ±2 mV, $\Delta E = E$(**2**-A^-/**4**-A^-) − E (**2**/**4** (Zn^{II}–OH^- form)) in mV at I=0.10 ($NaNO_3$), 25 °C and pH 8.5.

Among the "imido"-containing bases, the affinity order dT ($\log K=6.6$) > U (5.8) > Ff (5.3) > AU (4.3) is consistent with the order of the basicities of the conjugate base, $N(3)^-$. This is compatible with our earlier findings that the strongly acidic Zn^{II} in macrocyclic complexes prefers more basic N^- anions.[7,8] Moreover, a linear relationship exists between the $\log K$ and pK_a values of the conjugate acid, as depicted in Fig. 1. This fact indicates that **2** binds to all these nucleosides in the same manner as does the Zn^{II}–N^- coordinate interaciton, acting as the controlling force. In Fig. 1, the slopes of the lines for systems **2** and **3** are different from each other. The magnitude of changes in the basicities of the conjugate base $N(3)^-$ upon stacking interactions is believed to be dependent on the properties of each aromatic pendant.

NMR Studies on the Supercomplexes 1—3. Previously, NMR studies and an X-ray crystal-structure determination attributed the increase in the guest affinity for **3** over **1** (Fig. 1) to an additional π–π stacking interacion between the pendant acridine functionality and the guest.[8] Therefore, for **2** there also appears to be a stacking interaction, although this effect becomes only apparent as the basicity of the anionic guest increases. This is possibly due to a more effective aromatic overlap and/or lower electron density on the imido-N^- as the N^-–Zn^{II} bond becomes stronger. In support of this trend, an ^1H NMR study carried out at pD 8.0 with **3** showed large upfield shifts of 0.55 and 0.59 ppm for the 5-H and 1′-H protons, respectively, of bound AU^-, compared to free deprotonated AU^-, whereas shifts of only 0.06 and 0.16 ppm, respectively, with **2**, and <0.07 ppm with **1**, were observed. Conversely, with both **3** and **2**, upfield shifts of >0.44 ppm and >0.51 ppm were observed for the 6-H and 1′-H protons, respectively, of dT^-, whereas for **1**, again only small shifts (<0.03 ppm) were observed. Further evidence for a π–π stacking interaction is given by the larger affinity ($\log K=7.1$) of **2** with the imido N deprotonated riboflavin (Rf^-) than that predicted from the pK_a value of 9.92, and by the smaller affinity ($\log K=5.8$) between **2** and anionic Su^- than that predicted from the imido pK_a value of 9.52 (Fig. 1), due to the lack of guest aromaticity. The affinity constant of anionic Su^- with **1** ($\log K=5.6$), on the other hand, is approximately the value expected due to the guest's basicity.

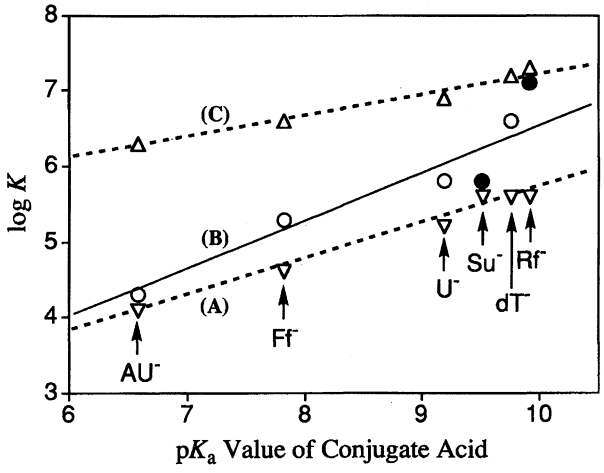


Fig. 1. Plots of the affinity constants: $\log K$ of **1** (line A, ▽, Ref. 6), **2** (line B, ○) [● with Su^- and Rf^-] and **3** (line C, △, Ref. 7) with anions AU^-, Ff^-, U^-, Su^-, dT^-, and Rf^- against pK_a values of the conjugate acid.

Electrochemical Studies. Electrochemical studies using a polarographic technique showed that both the ligand and **2** exhibited a reversible two-electron redox process (i.e., 9,10-anthraquinone+$2H^+$+$2e^-$⇌9,10-anthracenediol)[11] at −0.574 V and −0.562 V, respectively, vs. SCE at pH 8.5 and 25 °C. Upon the addition of excess amounts of each substrate (to ensure >98% complexation) to buffered solutions of **2** (0.4 mM) over the range $7.4 \leq pH \leq 10.2$, a constant negative shift in the E value (ΔE, Table 1) was observed, but no change was found with the acid-free ligand **5** under the same conditions. Furthermore, for dT and its nucleoside derivatives (U, Ff, and AU), the ΔE value was found to be proportional to the pK_a value of the guest conjugate acid, whereas for Su, a significantly smaller shift than that predicted from its pK_a value was observed.

The negative ΔE values mean that the reduced product **4** has a lower affinity for each respective anionic guest.[12] The $\log K$ values for **4** can be evaluated (Table 1), and a linear correlation is found when these are plotted against the guest conjugate acid pK_a values, including that of Su^-; furthermore, this line almost superimposes the correlation line for **1**. Therefore, since the $\log K$ values for both **1** and **4** are now very similar, and the decrease in stability upon the reduction of the **2**–dT-complex ($\Delta\log K=0.7$) is bigger than that for both the **2**–AU^- ($\Delta\log K=0.3$) and **2**–Su^- ($\Delta\log K=0.3$) complexes, it is highly likely that the reduction of **2** is accompanied by a decrease in the π–π stacking interaction between the host and the guest (Scheme 2). Although the origins of π–π stacking is complex,[13] in this case the increased electron density between the two stacking rings leads to a decrease in the interaction. These results indicate that the strength of the π–π interaction between the host **2** and each imido guest is an important factor in determining the degree of electrochemical response to complexation.

As for the other DNA bases, no interaction was found between **2** and 2′-deoxycytosine (dC) or 2′-deoxyadenosine (dA) (likewise with **1** and **3**),[7,8] either by pH titration or by an electrochemical study, due to the lack of any suitable functionality on these guests that can interact with the Zn^{II} center. However, a small negative shift ($\Delta E=-5$ mV) was observed with **2** and excess 2′-deoxyguanosine (dG). It is likely that dG interacts with **2** via the N(7) coordination, as found with studies on **3**.[8] Since this mode of interacion is stabilized by π–π

stacking, no interaction is observed between dG and **1**.[7]

Although the electrochemical response to complexation with the present system is not as large as we had anticipated, **2** is still a possible prototype for the electrochemical sensing of specific oligonucleotide base sequences at physiological pH, especially since anthraquinone derivatives can intercalate with DNA.[9,14]

Experimental

General Information. All of the reagents used were of analytical reagent grade (purity > 99%). Thymidine (dT), 2′-deoxyguanosine (dG), 2′-deoxycytidine (dC), 2′-deoxyadenosine (dA), uridine (U), ftorafur (Ff, 5-fluoro-1-(tetrahydro-2-furyl)uracil), 6-azauridine (AU), succinimide (Su), and riboflavin (Rf) were all purchased from Sigma Chemical Co., Ltd. Cyclen, as tetrahydrochloride salts, was purchased from Tokyo Kasei Co., Ltd. IR spectra were recorded on a Shimadzu FTIR-4200 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM α400 spectrometer. The following Good's buffers (Dojindo) were commercially available and used without further purification: HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pK_a = 7.5), TAPS (3-[tris(hydroxymethyl)methylamino]propanesulfonic acid, pK_a = 8.4), CHES (2-(cyclohexylamino)ethanesulfonic acid, pK_a = 9.3), CAPSO (3-(cyclohexylamino)-2-hydroxypropanesulfonic acid, pK_a = 10.0), and CAPS (3-(cyclohexylamino)propanesulfonic acid, pK_a = 10.4). All aqueous solutions were prepared using deionized and distilled water.

Synthesis of Anthraquinone-Pendant Cyclen, 5. 2-(Bromomethyl)-9,10-anthraquinone[15] (0.42 g, 1.39 mmol) was dissolved in a mixture of MeCN (70 ml) and DMF (10 ml) by gentle heating. This was then added dropwise over a 5 h period to a stirred solution of cyclen (1.0 g, 5.8 mmol) in MeCN (30 ml) at ca. 80 °C. After the addition was completed, the solution was stirred at room temperature for 12 h, and the solvents were removed in vacuo, giving a brown oil. This was treated with 0.2 M HCl (100 ml); the resultant white suspension was filtered through glass wool and the filtrate washed with CH_2Cl_2. The aqueous layer was then made basic (5 M NaOH) and extracted with CH_2Cl_2 (3×100 ml), dried (Na_2SO_4) and the solvent removed in vacuo to give crude **5** as a yellow solid (0.31 g). This was purified by column chromatography on silica gel using CH_2Cl_2/MeOH/28% aq NH_3 (ratio 95 : 4 : 1) as an eluent to give a pure yellow solid **5** (0.19 g, 35%).

5: Mp 145—147 °C (decomp); IR (KBr) 3324, 1676 (C=O), 1593, 1327, 1296, 708 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 2.43 (3H, br, NH), 2.55—2.65 (4H, m, NCH_2), 2.65—2.70 (4H, m, NCH_2), 2.70—2.75 (4H, m, NCH_2), 2.80—2.90 (4H, m, NCH_2), 3.79 (2H, s, $ArCH_2$), 7.77—7.79 (3H, m), 8.26—8.31 (4H, m); ^{13}C NMR ($CDCl_3$) δ = 45.56, 46.52, 47.57, 52.00, 59.34, 127.18, 127.22, 127.48, 127.72, 132.73, 133.62, 133.64, 133.68, 133.98, 134.06, 134.55, 146.69, 182.93, 183.34. Found: C, 67.16; H, 7.18; N, 13.44%. Calcd for $C_{23}H_{28}N_4O_2 \cdot 1H_2O$: C, 67.29; H, 7.37; N, 13.65%.

Synthesis of Anthraquinone-Pendant Zn^{II}–Cyclen Complex, 2·(NO$_3$)$_2$. To a solution of **5**·H_2O (100 mg, 0.24 mmol) in EtOH (6 ml) at 50 °C was added dropwise a solution of $Zn(NO_3)_2 \cdot 6H_2O$ (75 mg, 0.25 mmol) in EtOH (2.3 ml). A precipitate was immediately obtained. The mixture was first allowed to cool to room temperature; then the precipitate was collected by filtration under N_2 as a pale-yellow solid (113 mg, 73%).

2·(NO$_3$)$_2$: IR (KBr) 3436, 1674 (C=O), 1591, 1385, 1327, 1296, 712 cm^{-1}; ^{1}H NMR (D_2O) δ = 2.7—3.0 (8H, m, NCH_2), 3.0—3.1 (4H, m, NCH_2), 3.25—3.35 (4H, m, NCH_2), 3.79 (1H, m, NH), 4.06 (1H, m, NH), 4.10 (2H, s, $ArCH_2$), 7.75 (1H, d, J = 8 Hz, 3-H), 7.82—7.87 (2H, m, 6-H and 7-H), 7.95 (1H, s, 1-H), 8.01—8.07 (3H, m, 4-H, 5-H, and 8-H); ^{13}C NMR (D_2O) δ = 44.99, 45.11, 46.52, 47.40, 47.52, 52.20, 58.26, 129.91, 130.29, 131.92, 135.54, 135.57, 135.60, 135.70, 137.95, 138.00, 140.03, 141.61, 187.05, 187.19. Found: C, 46.36; H, 5.09; N, 13.94%. Calcd for $C_{23}H_{30}N_6O_9Zn_1$: C, 46.05; H, 5.04; N, 14.01%.

Potentiometric pH Titrations. The electrode system (Orion Research Expandable Ion Analyzer EA920 and Orion Research Ross Combination pH Electrode 81028N) was used for potentiometric pH titrations. The calculation method for the protonation constants ($\log K_n$) of the ligand and the deprotonation constants (pK_a) for nucleosides were the same as previously described.[8] Measurements of the complexation constants, $K = [Zn^{II}\text{–cyclen–}A^-]/([Zn^{II}\text{–cyclen}][A^-])$ (M^{-1}), for a variety of nucleosides were conducted at 25 °C and I = 0.10 ($NaNO_3$). The calculation method has been previously described.[7,8]

Electrochemical Studies. Polarographic measurements were carried out with a Yanaco P-1100 polarographic analyzer and a three-electrode cell (a dropping mercury working electrode, an SCE reference electrode, a mercury pool counter electrode).[16] The test solution contained 20 mM Good's buffer (HEPES at pH 7.4 and 7.9, TAPS at 8.4 and 8.8, CHES at 9.2 and 9.6, CAPSO at 9.8 and 10.2, CAPS at 10.4), anthraquinone species (0.40 mM), and ca. 90 mM $NaNO_3$ (I = 0.10). These buffers (0—20 mM) had practically no effect on the half-wave potentials. All criteria for a reversible two-electron process at scan rate 1 mV s^{-1} were fulfilled by the usual log-plot slope of 30 mV/decade.

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References

1) J. M. Lehn, *Angew. Chem., Int. Ed. Engl.*, **29**, 1304 (1990).

2) P. D. Beer, *Adv. Mater.*, **6**, 607 (1994); J. C. Medina, T. T. Goodnow, M. T. Rojas, J. L. Atwood, B. C. Lynn, A. E. Kaifer, and G. W. Gokel, *J. Am. Chem. Soc.*, **114**, 10583 (1992); C. D. Hall, N. W. Sharpe, I. P. Danks, and Y. P. Sang, *J. Chem. Soc., Chem. Commun.*, **1989**, 419.

3) M. Delgado, R. E. Wolf, Jr., J. R. Hartman, G. McCafferty, R. Yagbasan, S. C. Rawle, D. J. Watkin, and S. R. Cooper, *J. Am. Chem. Soc.*, **114**, 8983 (1992); Z. Chen, O. F. Schall, M. Alcala, Y. Li, G. W. Gokel, and L. Echegoyen, *J. Am. Chem. Soc.*, **114**, 444 (1992); L.

Echegoyen, Y. Hafez, R. C. Lawson, J. de Mendoza, and T. Torres, *J. Org. Chem.*, **58**, 2009 (1993), and references cited therein; G. De Santis, L. Fabbrizzi, C. Mangano, A. Poggi, and B. Seghi, *Inorg. Chim. Acta*, **177**, 47 (1990); A. D. Hamilton, "Bioorganic Chemistry Frontiers," ed by H. Dugas, Springer-Verlag, New York (1991), Vol. 2, pp. 115—174.

4) Y. Huang, R. J. Drake, and D. W. Stephan, *Inorg. Chem.*, **32**, 3022 (1993); N. D. Lowe and C. D. Garner, *J. Chem. Soc., Dalton Trans.*, **1993**, 3333; T. Jørgensen, T. K. Hasen, and J. Becher, *Chem. Soc. Rev.*, **23**, 41 (1994).

5) L. Zhang, A. Macias, T. Lu, J. I. Gordon, G. W. Gokel, and A. E. Kaifer, *J. Chem. Soc., Chem. Commun.*, **1993**, 1017; Some transition metal complexes can respond electrochemically to direct coordination by neutral molecules such as CO, see for instance: E. Kimura, T. Koike, M. Kodama, and D. Meyerstein, *Inorg. Chem.*, **28**, 2998 (1989).

6) P. D. Beer, Z. Cheng, A. J. Coulden, A. Graydon, S. E. Stokes, and T. Wear, *J. Chem. Soc., Chem. Commun.*, **1993**, 1834.

7) M. Shionoya, E. Kimura, and M. Shiro, *J. Am. Chem. Soc.*, **115**, 6730 (1993).

8) M. Shionoya, T. Ikeda, E. Kimura, and M. Shiro, *J. Am. Chem. Soc.*, **116**, 3848 (1994).

9) B. Armitage, C. Yu, C. Devadoss, and G. B. Schuster, *J. Am. Chem. Soc.*, **116**, 9847 (1994).

10) The zinc(II) complex **2** was initially designed for electrochemical recognition of riboflavin (Rf) since MM studies suggested that the anthraquinone-pendant arm was able to efficiently overlap all three rings of the Zn^{II}-bound Rf^- to generate a stronger π–π stacking interaction than those found with the thymidine derivatives. Indeed, the affinity of Rf^- with **2** is greater than that predicted from the pK_a value of Rf. However, the Rf^- bound to **2** exhibited a redox process at almost exactly the same potential as that of the anthraquinone moiety, which therefore precluded any measurement of the electrochemical response to Rf^- complexation.

11) G. Dryhurst, K. M. Kadish, F. Scheller, and R. Renneberg, "Biological Electrochemistry," Academic Press, New York (1982), Chap. 1.

12) The pK_a value of the OH group of anthracenediol in **4** (Zn^{II}–OH^- form) was determined to be 9.3±0.2 at 25 °C and I=0.10 ($NaNO_3$) by plotting the E value of **2** against pH. Since attempts at chemically converting **2** to **4** were unsuccessfully, we could not obtain direct evidence for the interaction between the anthracenediol in **4** and the guest nucleosides bound to Zn^{II}.

13) C. A. Hunter, *Angew. Chem., Int. Ed. Engl.*, **32**, 1584 (1993).

14) J. W. Lown, *Chem. Soc. Rev.*, **23**, 165 (1993).

15) R. L. Blankespoor, A. N. K. Lau, and L. L. Miller, *J. Org. Chem.*, **49**, 4441 (1984).

16) E. Kimura, Y. Kuramoto, T. Koike, H. Fujioka, and M. Kodama, *J. Org. Chem.*, **55**, 42 (1990).