Efficient Inhibition of Photo[2 + 2]cycloaddition of Thymidilyl(3′−5′)thymidine and Promotion of Photosplitting of the *cis-syn*-Cyclobutane Thymine Dimer by Dimeric Zinc(II)−Cyclen Complexes Containing *m*- and *p*-Xylyl Spacers

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Abstract: Monomeric and dimeric zinc(II) complexes of cyclen (cyclen = 1,4,7,10-tetraazacyclododecane) inhibited the photo[2 + 2]cycloaddition of thymidilyl(3′−5′)thymidine (d(TpT)) at neutral pH in aqueous solution for a novel mechanism. Comparison of the initial rates and the product yields of the photodimerization of d(TpT) (in Tris buffer at pH 7.6 with $I = 0.10$ ($NaNO_3$)) at 3−5 °C by high-pressure mercury lamp indicates that the dimeric zinc(II) complexes, *p*- and *m*-xylyl-bis(Zn^{2+}−cyclen) (Zn_2L^2 and Zn_2L^3), are effective inhibitors (70−85% inhibition compared with the control reaction at [d(TpT)] = [bis(Zn^{2+}−cyclen)] = 0.2 mM after 20 min irradiation). This inhibition is due to the extremely strong 1:1 complexation of two deprotonated thymidine (dT^-) moieties with two Zn^{2+}−cyclen moieties (apparent complexation constants, log K_{app} ($K_{app} = [Zn_2L^2$ (or Zn_2L^3)−$d(T^-pT^-)]/[Zn_2L^2_{free}$ (or $Zn_2L^3_{free}$)][$d(TpT)_{free}$] (M^{-1})), of 6.4 ± 0.1 at pH 7.6 (50 mM HEPES, I = 0.1 ($NaNO_3$)) and 25 °C, as determined by the isothermal calorimetric titration). A major product, *cis-syn*-cyclobutane thymine dimer (T[*c*,*s*]T), was also found to form complexes with Zn^{2+}−cyclens. The apparent affinity constants for 1:1 complexes of one of the imide sites of T[*c*,*s*]T with a monomeric Zn^{2+}−benzylcyclen (ZnL^1) and with each Zn^{2+}−cyclen unit (ZnL) of *m*-xylyl-bis(Zn^{2+}−cyclen), log K_{app} ($K_{app} = [ZnL^1$ (or ZnL)−$(T[c,s]T)^-]/[ZnL^1_{free}$ (or ZnL_{free})][T[*c*,*s*]T_{free}] (M^{-1})), were 3.7 ± 0.1 and 3.8 ± 0.1, respectively, at pH 7.6 (50 mM HEPES, I = 0.1 ($NaNO_3$)) and 25 °C. The photosplitting of T[*c*,*s*]T, a reverse reaction of the photodimerization, at pH 7.6 (5 mM Tris buffer with $I = 0.1$ ($NaNO_3$)) was kinetically and thermodynamically promoted by *m*-xylyl-bis(Zn^{2+}−cyclen). The ^1H NMR measurement showed that 78% of the cyclobutane of T[*c*,*s*]T (1 mM) was split after 1 h of UV exposure in the presence of an equivalent amount of *m*-xylyl-bis-(Zn^{2+}−cyclen), whereas the control reaction showed 54% splitting. The kinetic and thermodynamic stability of the 1:1 *m*-xylyl-bis(Zn^{2+}−cyclen)−$d(T^-pT^-)$ complex also accounts for the acceleration of photosplitting of T[*c*,*s*]T. The inhibitory effect of *m*-xylyl-bis(Zn^{2+}−cyclen) on the photoreaction of poly(dT) was also revealed.

Introduction

Exposure of cellular nucleic acids to UV radiation leads to a variety of lesions, which, if unrepaired, are potentially carcinogenic, mutagenic, and cytotoxic.[1−4] Among the known photoproducts of DNA, some of the major ones are *cis-syn* (T[*c*,*s*]T) (**3a**), *trans-syn*-I (T[*t*,*s*-I]T) (**3b**) cyclobutane thymine dimers, and a pyrimidine (6−4) pyrimidone photodimer (**4**), which results from the photo[2 + 2]cycloaddition of two adjacent thymidilyl(3′−5′)thymidine (d(TpT)) sites (**1**) (Scheme 1). These base lesions induce base mutations in the p53 tumor suppressor gene[5] and even interfere in the interaction of DNA with proteins such as RNA polymerase II[6] and some transcription factors.[7]

Recent reports on the depletion of the stratospheric ozone layer have led to efforts in the development of novel molecules that protect nucleic acids from UV exposure.[8] However, compounds which efficiently inhibit photodamage of nucleic acids are still scarce, except for the Hg^+ ion,[9] dyes such as

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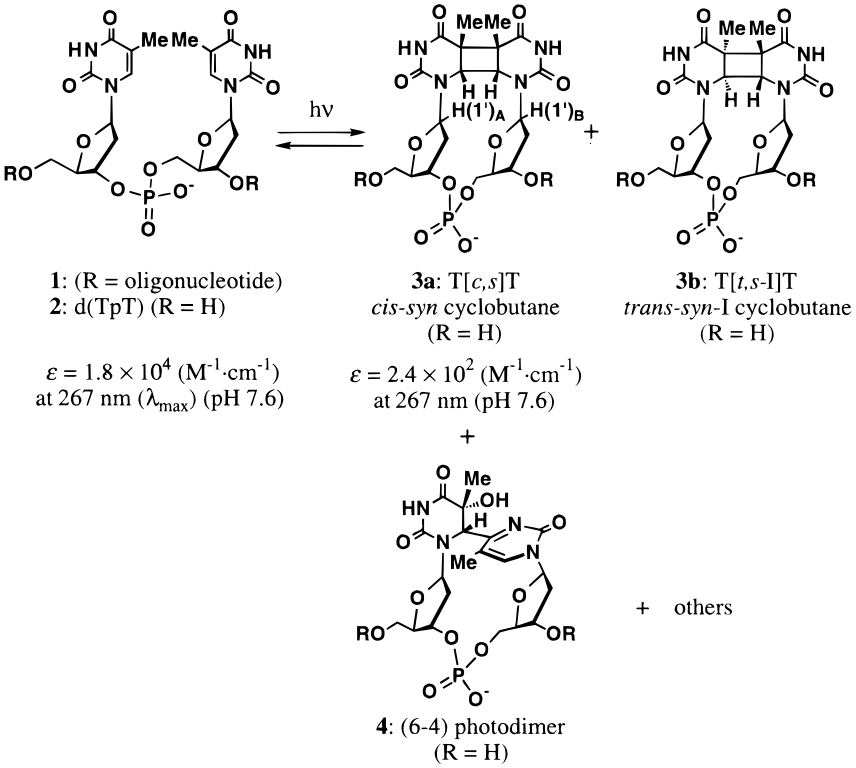
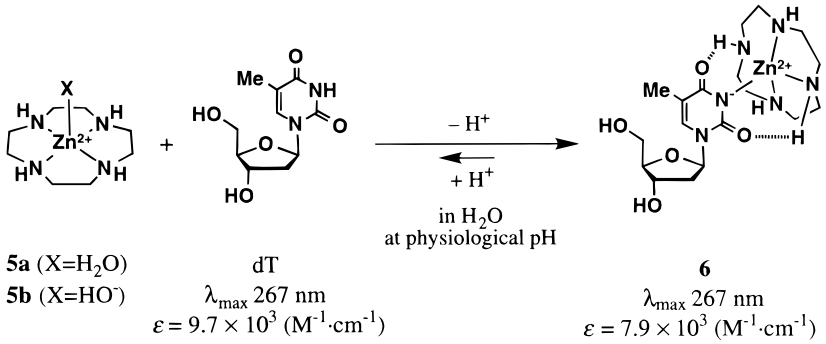
Scheme 1



Scheme 2



proflavin[10] and ethidium bromide,[11] or "sunscreens", which contain UV-absorbing and light-scattering molecules.[12]

We previously reported that zinc(II)−cyclen complex **5** is a highly selective host for dT (thymidine) (λ_{max} 267 nm, $\epsilon = 9.7 \times 10^3$ (M^{-1}·cm^{-1}) at pH 7.6) and U (uridine) in neutral aqueous solution (cyclen = 1,4,7,10-tetraazacyclododecane),[13−15] yielding a 1:1 complex **6** (λ_{max} 267 nm, $\epsilon = 7.9 \times 10^3$ (M^{-1}·cm^{-1}) at pH 7.6), where the imide-deprotonated dT^- (or U^-) binds with a zinc(II) cation and two carbonyl oxygens bind with the two complementary cyclen NH's through two hydrogen bonds (Scheme 2). The intrinsic stability constant of **6**, log K_s (K_s = [**6**]/[**5a**][dT^-] (M^{-1})), is 5.6 ± 0.1 at 25 °C, or the apparent affinity constant, log K_{app} (K_{app} = [**6**]/[$\mathbf{5}_{free}$][dT_{free}] (M^{-1})), is 3.1 ± 0.1 at pH 7.6 and 25 °C with I = 0.1 ($NaNO_3$).[13a]

Moreover, we have recently discovered that the *p*-xylyl-bis-(Zn^{2+}−cyclen) complex (*p*-bis(Zn^{2+}−cyclen) = Zn_2L^2) **8**[16,17]

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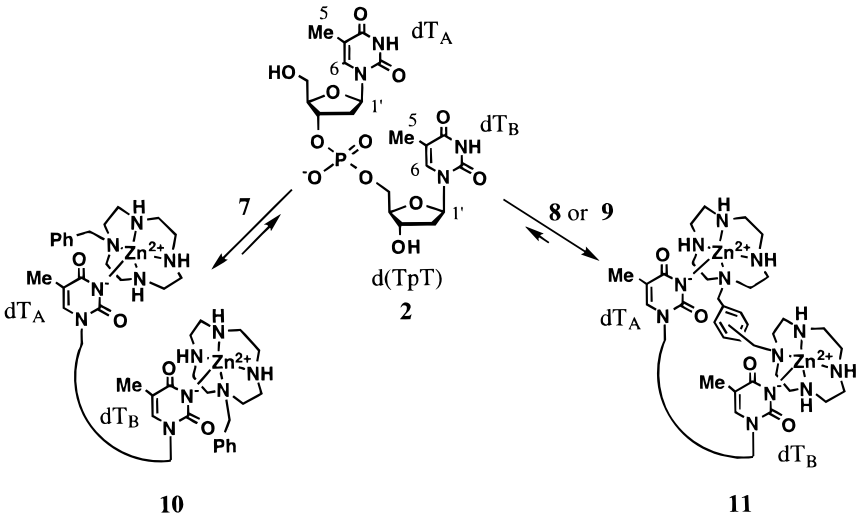
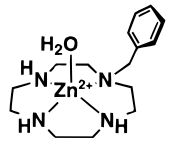
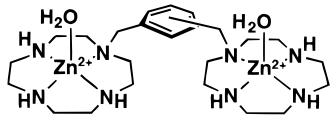
Scheme 3



forms a very stable 1:1 complex **11** with d(TpT) at neutral pH in aqueous solution (Scheme 3), which is ca. 10^3 times more stable than a complex **10** comprising d(TpT) and a monomeric zinc(II) complex **7** in a 1:2 molar ratio,[17] due to a "chelate" effect.[18] An energy-minimization calculation showed complex **11** taking a structure in which the two thymine rings were widely separated by ca. 10 Å. We then supposed that **11** might sterically inhibit the photo[2 + 2]cycloaddition and/or promote reverse photosplitting of the thymine dimers, thereby yielding an unprecedented prototype for protection of the specific sites of nucleic acids against UV light. Herein, we describe the effects of Zn^{2+}−cyclens, especially the dimeric complexes **8** and *m*-xylyl-bis(Zn^{2+}−cyclen) (*m*-bis(Zn^{2+}−cyclen) = Zn_2L^3) **9**,[19] which are inert against ultraviolet light, on the photo[2 + 2]cycloaddition of thymidilyl(3′−5′)thymidine **2** and the photosplitting of the *cis-syn*-cyclobutane thymine dimer (T[*c*,*s*]T) **3a** at neutral pH in aqueous solution.[20−26] The inhibitory effect of **9** on the photoreaction of poly(dT) is also reported.



7 (ZnL^1)
Zn^{2+}-benzylcyclen



8 (Zn_2L^2) *p*-xylyl-bis(Zn^{2+}-cyclen)
9 (Zn_2L^3) *m*-xylyl-bis(Zn^{2+}-cyclen)

Experimental Section

General Information. All reagents and solvents used were of the highest commercial quality and were used without further purification. All aqueous solutions were prepared using deionized and redistilled water. ^{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 in D_2O were used as internal references for ^{1}H and ^{13}C NMR measurements. The pD values in D_2O were corrected for a deuterium isotope effect using pD = [pH-meter reading] + 0.40.

UV spectra were recorded on a Hitachi U-3500 spectrophotometer at 25.0 ± 0.1 °C. Buffer solutions were tris(hydroxymethyl)aminomethane (Tris) and Na_2HPO_4−NaH_2PO_4 (pH 7.6), Na_2CO_3−$NaHCO_3$ (pH 9.3), $NaHCO_3$−NaOH (pH 10.9), and KCl−NaOH (pH 13.6) for photoreaction and HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid, (pK_a = 7.6 at 20 °C) (pH 7.6) for isothermal calorimetric titration. The ionic strength was adjusted to 0.10 with $NaNO_3$.

The zinc(II) complexes **7**,[17] **8**,[16,17] and **9**[19] were synthesized as previously described. The dinucleotides, d(TpT) (**2**) and T[*c*,*s*]T (**3a**),[24−26] were synthesized from 3′-*O*-levulinylthymidine[27] and 5′-*O*-(4,4′-dimethoxytrityl)thymidine 3′-(2′-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (Glen Research) according to Ohtsuka's[27] and Taylor's[28] methods and isolated as an ammonium salt and/or a sodium salt.[17] Poly(dT)$_n$ was purchased from Pharmacia Biotech Inc. (n = approximate averaged length in bases = 221).

Isothermal Calorimetric Titrations.[29] The heats of 1:1 complexation of **2** and **7**−**9** with dT or d(TpT) were recorded on a Calorimetry Science Corp. Isothermal Titration Calorimeter 4200 at 25.0 °C and pH 7.6 (50 mM HEPES buffer with I = 0.10 ($NaNO_3$)). The calorimeter was calibrated by heat (474.7 mJ) of protonation of tris(hydroxymethyl)aminomethane (250 mM, 1.0 mL) by a 10 μL injection of 1.00 mM aqueous HCl at 25.0 °C. The solution (1.0 mL) of **2** or **3a** in 50 mM HEPES was put into a calorimeter cell, to which the solution of **7**, **8**, or **9** in 50 mM HEPES was loaded. In this work, six combinations of guest molecules (dT, **2**, or **3a**) and zinc(II) complexes (**7**, **8**, or **9**) were carried out. The concentration of guests and hosts were as follows: 2.0 mM dT + 38 mM **7**, 1.0 mM **2** + 38 mM **7**, 0.2 mM **2** + 5.0 mM **8**, 0.2 mM **2** + 5.0 mM **9**, 2.0 mM **3a** + 38 mM **7**, and 2.0 mM **3a** + 20 mM **9**. 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 provided by the Calorimetry Sciences Corp.

Photoreactions of d(TpT) 2 and T[*c*,*s*]T 3a. Each sample was prepared in quartz cuvettes (GL Science Inc. Japan, Type S10S-UV-10, 10 mm light path), purged with nitrogen gas for 10 min, and

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irradiated with a high-pressure mercury arc (300 W) through a liquid filter of aqueous $NiSO_4$ (0.3 mM) and aqueous $CoSO_4$ (30 μM)[30] (wavelengths longer than 350 nm were cut off) at 3−5 °C in a cooling water bath. The concentration of **2** was set at 0.2 mM, which was confirmed to be high enough for the complete absorption of the incident light; i.e., the absorbance of the sample solution was >3.5. The average light intensity at 266 nm was 11 J·min^{-1}·cm^{-2}, as measured by chemical actinometry utilizing photohydration of 1,3-dimethyluracil (λ_{max} 266 nm, ϵ 8900 (M^{-1}·cm^{-1})) in H_2O, whose quantum yield had been reported to be (1.24 ± 0.02) × 10^{-2} at 266 nm.[31] The photoreactions were followed by a decrease in UV the absorbance of sample solutions at 266 nm. An aliquot of 0.5 mL was taken from each sample after UV irradiation for a given time and diluted with 2.0 mL of a 0.1 M AcOH−AcONa buffer (pH 4.0 with I = 0.1 ($NaNO_3$)) (for reactions with metal ions such as Ag^+, Hg^{2+}, Ni^{2+}, or Mn^{2+}, the sample mixture was diluted with 10 mM Tris buffer containing 5 mM EDTA with I = 0.1 ($NaNO_3$)). The molar absorption coefficients (ϵ) (M^{-1}·cm^{-1}) of d(TpT) **2** (λ_{max} 267 nm) were 1.8 × 10^4 at pH 7.6 (10 mM Tris with I = 0.1 ($NaNO_3$)), 1.7 × 10^4 at pH 9.3 (10 mM Na_2CO_3−$NaHCO_3$ with I = 0.1 ($NaNO_3$)), and 1.4 × 10^4 at pH 10.9 (10 mM $NaHCO_3$−NaOH with I = 0.1 ($NaNO_3$)) and pH 13.6 (10 mM KCl−NaOH with I = 0.1 ($NaNO_3$)), respectively, at 25 °C. The ϵ values of **5**, **7**, **8**, and **9a** at 266 nm (at pH 7.6 and 25 °C) were ca. 0, 1.2 × 10^2, 2.6 × 10^2, and 2.1 × 10^2 (M^{-1}·cm^{-1}), respectively.

The photoreactions of T[*c*,*s*]T **3a** (0.2 mM) with or without Zn^{2+}−cyclens at pH 7.6 (5 mM Tris with I = 0.1 ($NaNO_3$)) or pH 13.6 (10 mM KCl−NaOH with I = 0.1 ($NaNO_3$)) were conducted using the same high-pressure mercury arc. An aliquot of 0.5 mL was taken from each sample after UV irradiation for a given time and diluted with 2.0 mL of a 0.1 M AcOH−AcONa buffer (pH 4.0 with I = 0.1 ($NaNO_3$)). The UV absorption of T[*c*,*s*]T **3a** occurs at 266 nm with ϵ = 2.4 × 10^2 (M^{-1}·cm^{-1}) at pH 7.6 (lit.[21] ϵ = 2.6 × 10^2 (M^{-1}·cm^{-1}) at 267 nm) and 2.5 × 10^3 (M^{-1}·cm^{-1}) at pH 13.6, respectively, at 25 °C. The ϵ values of **3a** at 265 nm in the presence of 2 equiv of **7** and 1 equiv of **9** at pH 7.6 were almost the same, 4.3 × 10^2 (M^{-1}·cm^{-1}). The reactions were repeated twice or three times, and the experimental deviations were within ±2%.

The photoreactions of poly(dT) (65 μM), with or without **9** at pH 7.6 (5 mM Na_2HPO_4−NaH_2PO_4 buffer with I = 0.1 ($NaNO_3$)), using the same high-pressure mercury arc were carried out at 3−5 °C in the same quartz cuvettes as described above, whose UV absorbances at a given UV exposure time were determined without dilution. Experimental errors are ±3%. The concentration of poly(dT) was determined by using ϵ = 1.86 × 10^4 (M^{-1}·cm^{-1}) at 265 nm for a d(TpT) unit (pH 7.6 (phosphate buffer) at 25 °C).[32]

Photoreactions of 2 and 3a Followed by ^{1}H NMR. The photoreactions of **2** (1 mM) and **3a** (1 mM) were also run in D_2O (with or without 5% acetone-d_6) at 3−5 °C for measuring ^{1}H NMR at 35 °C. The reaction was repeated twice or three times and the avaraged values were calculated. Experimental fluctuations were ±5%.

Results and Discussion

Affinity Constants of d(TpT) 2 with a Monomeric Zn^{2+}−Cyclen 7 and Bis(Zn^{2+}−Cyclen)s 8 and 9. The isothermal calorimetric titration revealed the apparent 1:1 complexation constant of dT with Zn^{2+}−benzylcyclen **7**, log K_{app} (K_{app} = [**7**−dT$^-$]/[**7**$_{free}$][dT$_{free}$] (M^{-1})) to be 3.4 ± 0.1 at pH 7.6 (50 mM HEPES buffer with I = 0.10 ($NaNO_3$)) and 25 °C. We earlier found log K_{app} for dT with Zn^{2+}−cyclen **5** to be 3.1 at pH 7.6 by potentiometric pH titration.[13a] The complexation of **2** with **7** was established to occur in a 1:2 molar ratio, and the log K_{app} for each dT site with **7** was 3.3 ± 0.1 under the same conditions, assuming that the complexation at two imide sites in **2** with **7** occurs independently. The apparent 1:1 complexation constants of **2** with bis(Zn^{2+}−cyclen)s **8** and **9**, log K_{app} (K_{app} = [Zn_2L−d(T$^-$pT$^-$)]/[Zn_2L_{free}][d(TpT)$_{free}$] (M^{-1})), at pH 7.6 and 25 °C were also determined to be a similar value of 6.4 ± 0.1. Thus, the complexes of d(TpT) **2** with bis(Zn^{2+}−cyclen)s (**8** or **9**) are nearly 10^3 times more stable than the complex of each dT site of **2** with **7**. Independent potentiometric pH titration results, which gave the same K_{app} values at pH 7.6, showed that 95% of **2** was bound to **8** or **9** at [**2**] = [**8** or **9**] = 0.2 mM (the concentration employed for the photoreaction) and pH 7.6.[17] Under the same conditions, only 4% of **2** would be in a 1:2 complex with the monomeric **7** at [**2**] = 0.2 mM and [**7**] = 0.4 mM.

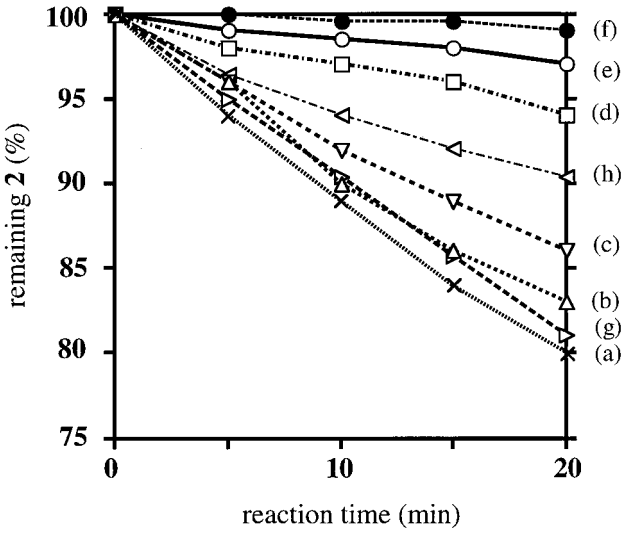


Figure 1. Effect of Zn^{2+}−cyclen complexes and pH on the photoreaction of d(TpT) **2** at 3−5 °C: (a) 0.2 mM **2** at pH 7.6; (b) 0.2 mM **2** + 0.2 mM Zn^{2+}−benzylcyclen **7** at pH 7.6; (c) 0.2 mM **2** + 0.4 mM **7** at pH 7.6; (d) 0.2 mM **2** + 0.2 mM *p*-bis(Zn^{2+}−cyclen) **8** at pH 7.6; (e) 0.2 mM **2** + 0.2 mM *m*-bis(Zn^{2+}−cyclen) **9** at pH 7.6; (f) 0.2 mM **2** + 0.4 mM *m*-bis(Zn^{2+}−cyclen) **9** at pH 7.6; (g) 0.2 mM **2** at pH 9.3; (h) 0.2 mM **2** at pH 10.9.

The Kinetic and Thermodynamic Effects of Zn^{2+}−Cyclens 5, 7, 8, and 9 on Photo[2 + 2]dimerization of d(TpT) 2. The photoreaction of **2** (0.2 mM in 10 mM Tris buffer at pH 7.6 ± 0.1 with I = 0.10 ($NaNO_3$)[33] at 3−5 °C) was simultaneously carried out side by side in the absence, and presence, of various Zn^{2+}−cyclens in the same UV reaction vessel. The initial 20 min reaction, where possible secondary reactions were insignificant, was followed by the decrease in the UV absorbance of sample solutions at 266 nm. Figure 1 summarizes all the results.[34] Curve a is a control reaction whose quantum yield is (1.3 ± 0.2) × 10^{-2} at 266 nm.[35,36] At prolonged irradiation for 3 h, ca. 57% of **2** disappeared, where the photodimerization almost reached an equilibrium with the backward (photosplit-

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

(30) Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*, 2nd ed.; Marcel Dekker: New York, 1993.

(31) Numao, N.; Hamada, T.; Yonemitsu, O. *Tetrahedron Lett.* **1977**, 1661−1664.

(32) Deering, R. A.; Setlow, R. B. *Biochim. Biophys. Acta* **1963**, *68*, 526−534.

(33) We also ran the photoreaction of **2** in Tris buffer, where ionic strength was adjusted with NaCl instead of $NaNO_3$. This may have promoted radical reactions. The results in both conditions were almost the same.

(34) We assume that the effect of photohydration of thymidine on the UV absorption of the reaction mixture is negligible because the quantum yields of photohydration of dT have been reported to be <10^{-3} of those of photo[2 + 2]cycloaddition: Fisher, G. J.; Johns, H. E. *Photochem. Photobiol.* **1973**, *18*, 23−27.

(35) The photoreaction of **2** in D_2O at pD 7.6 ± 0.1 and 3−5 °C was also followed by ^{1}H NMR to check the photoproducts. The ratio of **3a** (*cis-syn*):**3b** (*trans-syn*-I) obtained in the control reaction was 10:1 in the absence of Zn^{2+}−cyclens, as determined from the ratio of Me(5) of **3a** and **3b** (spectra not shown). The formation of a (6−4) photodimer was not detected.

ting) reaction.[20,21] Curves b and c show that the addition of 1 and 2 equiv of monomeric **7** is somewhat effective in reducing the rate of photoreaction of **2**. By contrast, dimeric zinc(II) complexes **8** (curve d) and **9** (curve e) reduced the initial rates of the photoreaction (by 70% and 85% of the control reaction, respectively) far more dramatically than 2 equiv of **7** (curve c) in UV exposure for 20 min. Even after the photoreaction reached the equilibrium, ca. 90% of **2** remained intact. When 2 equiv of **9** (0.4 mM) was used, even stronger inhibition was seen (curve f). The presence of 1 mM $ZnSO_4$ without cyclen ligands did not affect the reaction rate. Silver(I) ion, which was reported to enhance the photodimerization of d(TpT) and d(CpC) sites in DNA,[4a,23] had no effect in this case at $[Ag^+] = 1$ mM. Mercury(II) ion was known to reduce the initial rate of pyrimidine photodimer formation in *Escherichia coli* DNA to one-tenth of the control reaction when $r = [Hg^{2+}]/[\text{base pair}] = 1$.[9] In our experiment, the presence of 1 mM Hg^{2+} ion reduced 34% of the initial rate. This is probably due to the Hg^{2+} complexation to the imide anion of dT, as recently reported by Marzilli et al.[37] Other metal ions, such as Mn^{2+} and Ni^{2+} (both 1 mM), were ineffective in this intramolecular dimerization.[38]

As described in the previous section, d(TpT) **2** forms a 1:1 complex with bis(Zn^{2+}−cyclen) **8** or **9** in ca. 95% at [d(TpT)] = [**8** (or **9**)] = 0.2 mM, pH 7.6, and 25 °C. This means that 95% of d(TpT) is in dianionic form to bind with two zinc(II) cations. At [d(TpT)] = 0.2 mM and [**8** (or **9**)] = 0.4 mM, more than 99% of d(TpT) **2** is in the 1:1 complex. Under the same conditions, **2** (0.2 mM) forms a 1:2 complex with a mono-(Zn^{2+}−cyclen) **7** (0.4 mM) in only 4% yield. To break down the observed kinetic and thermodynamic inhibitory effects by (Zn^{2+}−cyclen)s into the electronic factor (anionic imide formation) and the steric factor, the control reactions without zinc(II) complexes were carried out at pH 9.3 (10 mM Na_2CO_3−$NaHCO_3$) and pH 10.9 (10 mM $NaHCO_3$−NaOH), where 4% and 95% of **2** are in double deprotonated species $\mathbf{2}^{2-}$ (ϵ values ($M^{-1}\cdot cm^{-1}$) of **2** = 1.7×10^4 at pH 9.3 and $\epsilon = 1.4 \times 10^4$ at pH 10.9 at 265 nm), respectively, on the basis of the pK_a values of **2** (pK_1 = 9.5 and pK_2 = 10.2 at 25 °C).[17] Although the photoreaction at pH 9.3 (curve g) was insignificantly different from that at pH 7.6 (curve a), the reaction at pH 10.9 (curve h) exhibited about 50% inhibition compared with the control reaction at pH 7.6, indicating that the enhanced dT-deprotonation effect owing to the complexation of bis(Zn^{2+}−cyclen)s may contribute to almost half of the inhibition.[39] The rest might come from the steric factor by the complexation that keeps two dT moieties far apart. The photoexcited population (at 266 nm) of **2** bound to **9** ($\epsilon = 1.4 \times 10^4$ ($M^{-1}\cdot cm^{-1}$)) was 23% less than that of the uncomplexed **2** ($\epsilon = 1.8 \times 10^4$ ($M^{-1}\cdot cm^{-1}$)) at pH 7.6. The inhibitory effect of **9** on the photodimerization of **2** (85%) was much larger than this value.

Moreover, to make 95% of **2** (0.2 mM) in the dianionic form with a monomeric Zn^{2+}−cyclen, 40 mM Zn^{2+}−cyclen (200 equiv) is theoretically required, assuming that the complexation of two dT sites in **2** with two monomeric Zn^{2+}−cyclen complexes occurs with the apparent affinity constants, log K_{app}, of 3.1−3.4 (vide supra). Thus, we carried out the photoreaction of **2** in the presence of 40 mM **5** at pH 7.6 (50 mM Tris with $I = 0.1$ ($NaNO_3$)) (ϵ values of **10** = 1.7×10^4 ($M^{-1}\cdot cm^{-1}$)). This experiment exhibited 49% reduction of the initial rates, almost overlapping with curve h (the control reaction at pH 10.9). Thus, we concluded that the anionic T^- formation at pH 7.6 is a major cause of the photodimerization inhibition by the monomeric Zn^{2+}−cyclen complex.

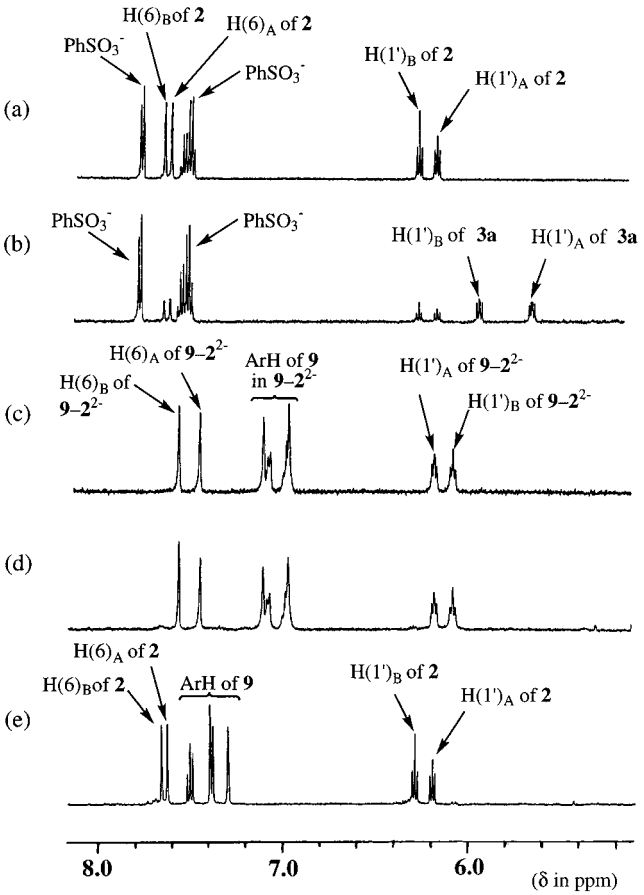


Figure 2. ^{1}H NMR spectra showing the anomeric and aromatic region of **2** (1 mM) in aqueous solution (pD 8.0) containing 5% acetone-d_6: (a) spectrum of **2** before UV irradiation ($PhSO_3^-$ was used as a reference); (b) spectrum of **2** after UV irradiation for 1 h at 3−5 °C; (c) **9**−$\mathbf{2}^{2-}$ complex before UV irradiation; (d) **9**−$\mathbf{2}^{2-}$ complex after UV irradiation for 1 h at 3−5 °C; and (e) spectrum obtained by the addition of DCl/D_2O to (d). For numbering of H(1) protons in **3a**, see Scheme 1. For numbering of other protons, see Scheme 3.

Photodimerization of 2, As Followed by ^{1}H NMR. The 1 h irradiation of **2** (1 mM) in D_2O (pD 8.0 ± 0.1) containing 5% acetone-d_6 (as a sensitizer)[23,24,40] in the absence, and presence, of **9** was followed by ^{1}H NMR (Figure 2). Figure 2a shows the aromatic and anomeric region of **2** before UV exposure without **9** (benzene sulfonate ($PhSO_3^-$) was used as a reference). After UV exposure for 1 h (Figure 2b), 70% of **2** was converted into *cis-syn*-cyclobutanes **3** (**3a** (*cis-syn*):**3b** (*trans-syn*-I) = 93:7, as determined from the ratio of Me(5) of **3a** and **3b** (spectra not shown)). Figure 2c shows H(6) and H(1′) of **2**, and aromatic protons (ArH) of **9** with considerable upfiled shifts in the 1:1 complex **11**. After irradiation for

(36) The reported values of quantum yields of T[*c, s*]T formation from d(TpT) in aqueous solution are (2.0 ± 0.30) × 10^{-2} at 254 nm (ref 20), 1.05 × 10^{-2} at 265 nm (ref 21), and 1.6 × 10^{-2} at 297 nm (ref 22b). The first value is twice that of the reported values in ref 20 on the assumption that excitation of one T ring in **2** is sufficient for photo[2 + 2]cycloaddition of d(TpT).

(37) Kuklenyik, Z.; Marzilli, L. G. *Inorg. Chem.* **1996**, *35*, 5654−5662.

(38) (a) Beukers, R.; Berends, W. *Biochim. Biophys. Acta* **1961**, *49*, 181−189. (b) Fisher, G. J.; Johns, H. E. *Photochem. Photobiol.* **1970**, *11*, 429−444.

(39) Wang et al. have observed that the rates of photodimerization of DNA reach a maxima at ca. pH 8 and decrease at higher pH: Wang, S. Y.; Patrick M. H.; Varghese, A. J.; Rupert, C. S. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 465−472.

(40) Patrick, M. H.; Snow, J. M. *Photochem. Photobiol.* **1977**, *25*, 373−384.

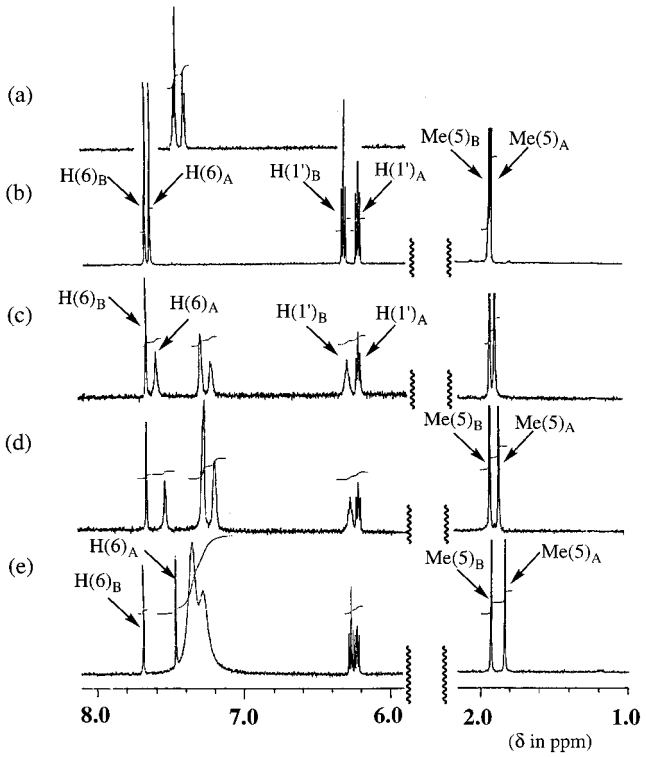


Figure 3. ^{1}H NMR spectral change of **2** (5 mM) on increasing concentration of **7** in D_2O (pD 8.4). The ratio of **2**:**7** = 0:1 (a), 1:0 (b), 1:0.4 (c), 1:1 (d), and 1:4 (e), respectively.

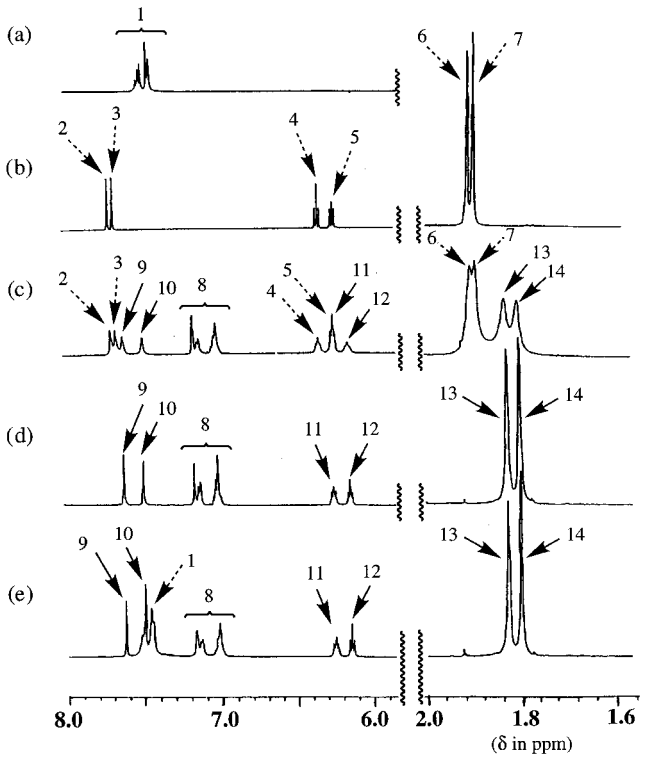


Figure 4. ^{1}H NMR spectral change of **2** (1 mM) on increasing concentration of **9** in D_2O (pD 8.4 ± 0.1). The ratio of **2**:**9** = 0:1 (a), 1:0 (b), 1:0.4 (c), 1:1 (d), and 1:2 (e), respectively. The dashed arrows indicate uncomplexed species, and the plain arrows are complexed species. Peak 1 is aromatic protons (ArH) of **9**. Peaks 2 and 3 are H(6) of dT_B and H(6) of dT_A, peaks 4 and 5 are H(1′) of dT_B and that of dT_A, and peaks 6 and 7 are Me(5) of dT_B and that of dT_A, respectively (for numbering, see Scheme 3). Peak 8 is aromatic protons of **9** in **9**−$\mathbf{2}^{2-}$ complex. Peaks 9, 10, 11, 12, 13, and 14 are assigned to H(6) of dT_B, H(6) of dT_A, H(1′) of dT_B, Me(5) of dT_B and Me(5) of dT_A of $\mathbf{2}^{2-}$ in **9**−$\mathbf{2}^{2-}$ complex, respectively.

1 h, the ^{1}H NMR (Figure 2d) remained almost the same. Figure 2e is the spectrum obtained after decomposition of **11** (by adding DCl/D_2O to Figure 2d), showing almost no peak assignable to the photoproducts **3**. This clearly indicates that **9** protects d(TpT) **2** well (more than 95%) from the photodimerization.[41]

A Labile d(TpT) Complex with 7 vs an Inert d(TpT) Complex with 9. As described above, the prevention of the photodimerization of **2** was partially attributed to the imide anion formation by complexation with Zn^{2+}−cyclens. To help elucidate why the complex with **9** prevented the photodimerization much more effectively than the same anionic complex with **7**, we conducted ^{1}H NMR titration (500 MHz) of **2** with **7** (Figure 3) and **9** (Figure 4) in D_2O (pD 8.4 ± 0.1) at 35 °C. Figure 3a,b shows the aromatic and anomeric region and methyl region of **7** and **2** in free form (both 5 mM), respectively. Figure 3c−e contains ^{1}H NMR spectra of **2** (5 mM) when 2, 5, and 20 mM of **7** were added. As the concentration of **7** was increased, gradual upfield shifts of $H(6)_A$ (= H(6) in dT_A (dT at 5′ site), see Scheme 3), $H(1')_B$ (H(6) in dT_B (dT at 3′ site)), and $Me(5)_A$ (Me(6) in dT_A) occurred (these assignments were made according to Alderfer et al.[24]), which were thus averaged peaks of uncomplexed species and complexed species of **2** and **7**, implying that the displacement of the complex **10** occurred rapidly on the NMR time scale. Namely, the d(TpT) complex with **7** is kinetically labile.

On the other hand, ^{1}H NMR titration of **2** with **9** showed a different complexation pattern (Figure 4). Figure 4a,b shows the ^{1}H NMR spectra of **9** and **2** in free form, respectively (both 1 mM). When 0.4 mM of **9** was added to 1 mM of **2** (Figure 4c), two independent sets of peaks appeared, which were assigned to those for **2** and those for the 1:1 complex of **9** with doubly deprotonated **2** (**9**−$\mathbf{2}^{2-}$). As the concentration of **9** was increased to 1 mM (Figure 4d), the peaks of the complexed species reached maxima, while those of the free **2** disappeared. When 2 mM of **9** was added, aromatic protons of complexed (peak 8) and those of free **9** (peak 1) appeared as completely independent sets of peaks (Figure 4e).[42] These results indicate that the 1:1 complexation of **2** with **9** occurs quantitatively at millimolar order and is inert on the NMR time scale (that is, a lifetime is ca. 10^{-3}−10^{-2} s).[43]

The lifetime of a triplet state of pyrimidines has been estimated to be ca. 10^{-5} s.[3,4a] Therefore, we conclude that two thymine rings in **2** are kept away from each other by complexation with two Zn^{2+}−cyclen moieties spaced by a xylene unit (ca. 10 Å) far enough to prevent close interaction for the photo[2 + 2]cycloaddition via a triplet state. We summarize the inhibitory effect on the photo[2 + 2]cycloaddition by Zn^{2+}−cyclens **5** (or **7**) and **9** in Scheme 4.[44]

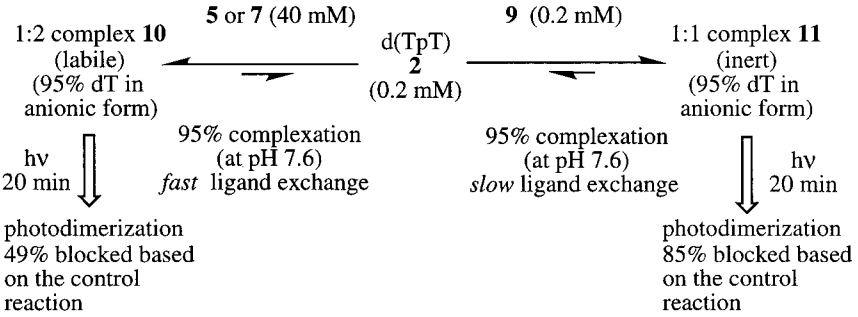
Complexation of T[*c,s*]T 3a with (Zn^{2+}−Cyclen)s. After photodimerization of **2** without Zn^{2+}−cyclen at pH 7.6 for 20

(41) In the presence of dimeric zinc(II) complexes, we could not determine the exact ratio of *cis-syn*- and *trans-syn*-I cyclobutanes, since the peaks of T[*t,s*-I]T were hardly observed in ^{1}H NMR spectra. We suppose that the dimeric zinc(II) complexes prevent photodimerization of d(TpT) via both transition states for *cis-syn*- and *trans-syn*-cyclobutanes.

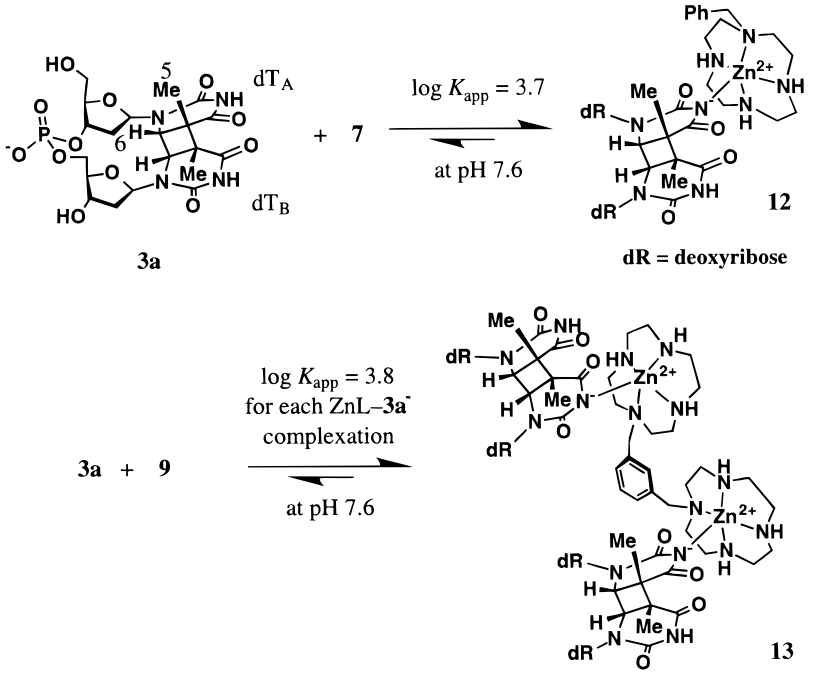
(42) All peaks of ^{1}H NMR spectra of the **9**−doubly deprotonated **2** complex were assigned by COSY, NOE, and ^{31}P−^{1}H HMBC. NOE cross-peaks between Me(5) or H(6) protons of thymine rings and aromatic protons of **9** were not observed. ^{1}H NMR titration of **2** with **8** showed also showed almost the same behavior as that with **9**. However, we were unable to assign all peaks of **8**−doubly deprotonated **2** complex because two H(6) protons of **2** in the complex had the same chemical shift.

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

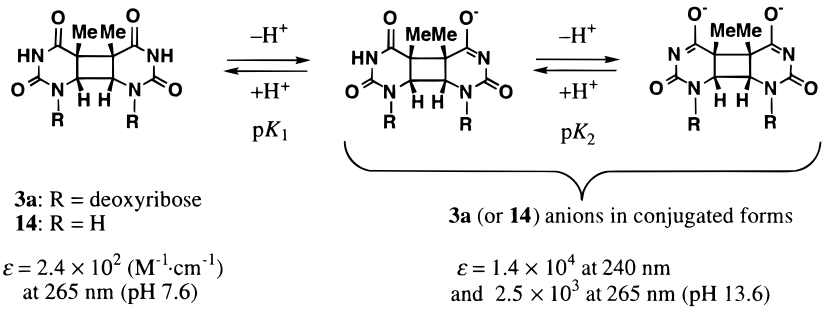
Scheme 4



Scheme 5



Scheme 6



min (20% of **2** was dimerized), 1 equiv (based on the initial amount of **2**) of **9** was added to the reaction mixture, which was then irradiated for another 20 min. This preliminary experiment showed ca. 13% recovery of the UV absorption at 266 nm after **9** was added. The ^{1}H NMR spectrum also indicated the reformation of the starting compound **2**. Thus, we investigated more thoroughly both the interaction of Zn^{2+}−cyclen complexes with a major photodimerization product, *cis-syn*-cyclobutane thymine dimer (T[*c*,*s*]T) (**3a**), and the effects of Zn^{2+}−cyclens on the equilibria for **2** ⇌ **3** (Scheme 1).

The isothermal calorimetric titration of **3a** with **7** led us to conclude that a 1:1 complex **12** was formed (Scheme 5), although we cannot distinguish which imide moiety of dT_A or dT_B is bound to the zinc(II) cation.

One of the reasons why another imide did not bind with the zinc(II) cation is that the second deprotonation of **3a** is extremely difficult compared with the first deprotonation (Scheme 6); e.g., the pK_2 of **14** is 12.45, while its pK_1 is 10.65.[45]

(44) The detailed analysis of complexation of **2** with *p*-bis(Zn^{2+}−cyclen) **8** (ref 17) suggests the presence of a trace of free **2** and monoligated complexes with **8**, in which only one of dT sites in **2** binds to one Zn^{2+} in **8** at pH 7.6. It is not unlikely that the trace photoreaction (in the presence of **8**) occurs through the contaminated free **2** and/or monoligated species, as a reviewer suggested.

(45) Herbert, M. A.; LeBlanc, J. C.; Weinblum, D.; Johns, H. E. *Photochem. Photobiol.* **1969**, *9*, 33−43.

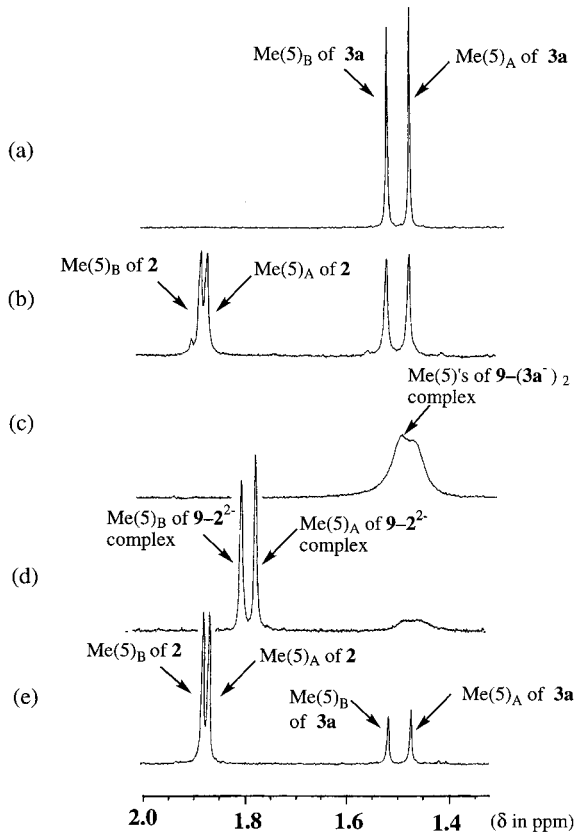


Figure 5. ^{1}H NMR spectra showing methyl region of T[*c*,*s*]T **3a** (1 mM) in D_2O (pD 8.0): (a) spectrum of **3a** before UV irradiation; (b) spectrum of **3a** after UV irradiation for 1 h at 3−5 °C; (c) spectrum of **9**−(**3a**$^-$)$_2$ complex before UV irradiation, (d) spectrum of **9**-(**3a**$^-$)$_2$ complex after UV irradiation for 1 h at 3−5 °C; (e) spectrum obtained by addition of DCl/D_2O to (d).

The apparent 1:1 complexation constant of **3a** with of **7**, log K_{app} (K_{app} = [ZnL^1−**3a**$^-$]/[ZnL$^1_{free}$][**3a**$_{free}$] (M^{-1})), at pH 7.6 ± 0.1 (50 mM HEPES with I = 0.10 ($NaNO_3$)) and 25 °C was 3.7 ± 0.1, which is translated into the fact that 38% of **3a** is complexed with **7** at [**3a**] = [**7**] = 0.2 mM, or 76% of **3a** is complexed with **7** at [**3a**] = 0.2 mM and [**7**] = 0.8 mM (the concentration employed for the photosplitting reaction of **3a**, as described in the following section). The log K_{app} value for the complexation with each Zn^{2+}−cyclen unit in **9** at pH 7.6 ± 0.1 and 25 °C was 3.8 ± 0.1.[46] This value has indicated that 80% of **3a** is in a 2:1 complex with **9** at at [**3a**] = 0.2 mM and [**9**] = 0.4 mM.

The UV spectra of **3a** in the absence of **7** or **9** shifted, as shown in Scheme 6, agreeing with the data by Sztumpf and Shugar.[20] On the contrary, the complexation of **3a** (0.2 mM) with **7** (0.8 mM) or **9** (0.4 mM) at pH 7.6 to **12** or **13** little affected the UV spectrum. We presume that complexes of monodeprotonated **3a** with Zn^{2+}−cyclens (**12** and **13**) possess few conjugated forms as shown in Scheme 5.

The Equilibrium and Kinetic Effect of (Zn^{2+}−Cyclen)s on the Photosplitting of T[*c*,*s*]T 3a. To monitor the backward reaction of the photodimerization, UV irradiation of the separately synthesized **3a** was carried out with the same mercury arc used for the forward photodimerization reaction of **2** in D_2O at pD 8.0 in the absence, and presence, of **9**, which was followed by ^{1}H NMR (Figure 5). Figure 5a shows two singlets corresponding to the two methyl group of **3a**. In the absence of **9**, 54% of the starting **2** was reproduced after 1 h irradiation, as evidenced by reappearance of the two singlets at 1.88 and 1.89 ppm for **2** (Figure 5b). Figure 5c is the ^{1}H NMR spectrum of **3a** in the presence of 1 equiv of **9**. The two methyl peaks are considerably broad, indicating that the imido groups are deprotonated to bind with Zn^{2+}−cyclen moieties. The aromatic protons of **9** and the two anomeric protons of **3a** showed a small downfield shift and appeared as averaged peaks between uncomplexed species and complexed species, suggesting that the complex **13** was kinetically labile as found for **10** (Figure 3). UV irradiation of **13** (1h) afforded Figure 5d, showing a new set of singlets, which were assigned to the two Me(5) peaks of **9**-doubly deprotonated **2** complex. The addition of DCl/D_2O to the reaction mixture gave Figure 5e, which clearly indicated 22% of the remaining **3a** and 78% of the photosplit product **2**.[47]

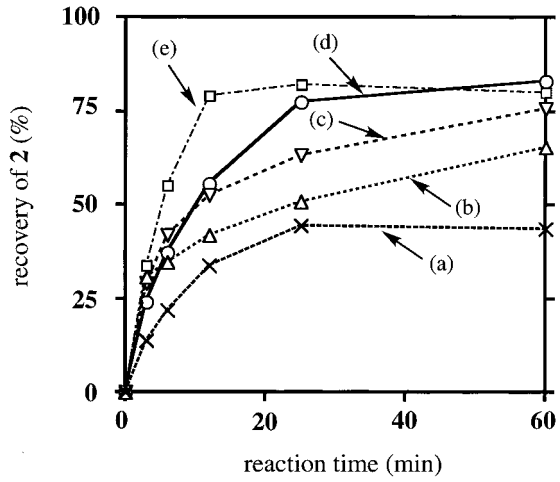


Figure 6. Effect of Zn^{2+}−cyclen complexes and pH on the photosplitting of T[*c*,*s*]T **3a** at 3−5 °C: (a): 0.2 mM **3a** at pH 7.6; (b) 0.2 mM **3a** + 0.2 mM **7** at pH 7.6; (c) 0.2 mM **3a** + 0.8 mM **7** at pH 7.6; (d) 0.2 mM **3a** + 0.4 mM **9** at pH 7.6; (e) 0.2 mM **3a** at pH 13.6.

Figure 6 displays the time course of the reappearance of **2** (0.2 mM) in the absence, and presence, of Zn^{2+}−cyclens **7** (0.2 and 0.8 mM) and **9** (0.4 mM) at pH 7.6 (in 5 mM Tris buffer (pH 7.6 with I = 0.1 ($NaNO_3$)) followed by the increase of UV absorption at 266 nm. Curves b−d display the results of the photosplitting of **3a** (0.2 mM) in the presence of 0.2 mM **7**, 0.8 mM **7**, and 0.4 mM **9**, respectively. These curves demonstrate that the initial rates increased more or less to almost the same extent, 1.7 times faster then the control reaction (curve a).[48] However, the equilibrium ratio of **2**:**3** was much higher than the control; the order of the reappearance of **2** was d > c > b > a. The recovered yield of **2** in the presence of 0.4 mM **9** after 2 h was ca. 90%, which agreed fairly well with the equilibrium ratio attained from the opposite reaction (see the previous section). It was reported that the equilibria ratio of **2**:**3** obtained by irradiation at pH 13 by 254 nm UV light is larger than that at pH 7.[21] The initial rate of the photosplitting of **3a** at pH 13.6 (10 mM KCl−NaOH with I = 0.1 ($NaNO_3$)) (curve e) was somewhat larger than curves b−d and gave almost the same equilibrium point as curve d.

(46) The receptors for *cis-syn*-cyclobutane thymine dimer designed by Hamilton et al. have the affinity constants, log K_{app}, of 2.7−4.3 in $CDCl_3$: Hirst, S. C.; Hamilton, A. D. *Tetrahedron Lett.* **1990**, *31*, 2401−2404.

(47) In the presence of acetone-d_6, the formation of **2** was negligible.

(48) The quantum yields in the photosplitting of **3a** at 254 nm in the absence, and presence, of Zn^{2+}−cyclens at pH 7.6 were determined to be 0.19 and 0.33, respectively, on the basis of the 1,3-dimethyluracil actinometry. The quantum yield at pH 13.6 without Zn^{2+}−cyclens (curve (e)) was 0.48. The typical quantum yields reported in photosplitting of *cis-syn*-thymine dimers by direct photolysis are 0.4−0.7 at 254 nm (at pH 13) (ref 20).

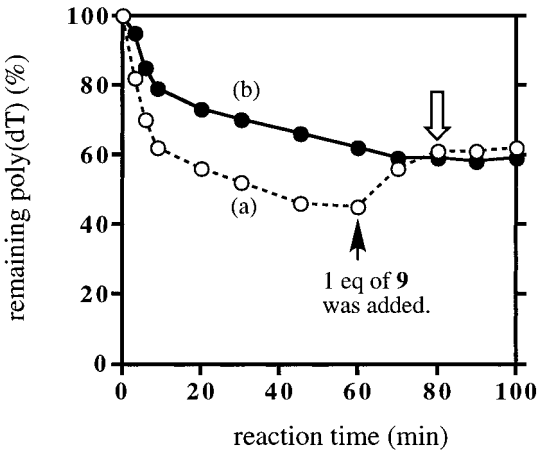


Figure 7. Effect of **9** on the photoreaction of poly(dT) at pH 7.6 (5 mM phosphate buffer with $I = 0.1$ ($NaNO_3$)) and 3−5 °C: (a) 65 μM poly(dT) (base) at pH 7.6; (b) 65 μM poly(dT) (base) + 65 μM **9**.

The Effect of 9 on the Photoreaction of Poly(dT). Finally, we have pursued the inhibitory effect of **9** on the photodimerization of poly(dT)$_n$ (n = approximate averaged length in bases = 221 and [base] = 65 μM) in 5 mM phosphate buffer (pH 7.6 with $I = 0.1$ ($NaNO_3$)) at 3−5 °C spectrophotometrically.[32] The sample solutions of poly(dT)$_n$ in the presence, and absence, of equimolar **9** were exposed to the UV light using the same equipment, and the results were summarized in Figure 7. After the UV irradiation for 60 min, when photodimerization and photosplitting reactions almost reached an equilibrium, the UV absorption decreased by 50% in the absence of **9** (curve a) and 35% (curve b) in the presence of **9**, which indicates that **9** was also effective on the photoreaction of oligonucleotides.

Very interestingly, when we added 1 equiv of **9** to the control reaction after 60 min (indicated by a plain arrow in Figure 7a) and continued the UV irradiation, UV absorption was recovered to the same level as curve b after 20 min (indicated by an open bold arrow). This result strongly indicates that the repair of the photodamaged poly(dT) was promoted by **9**.

Conclusions

The kinetic and thermodynamic effects of Zn^{2+}−cyclen complexes on the photo[2 + 2]cycloaddition of d(TpT) **2** and the photosplitting of T[*c*,*s*]T **3a** were investigated. An ordinary high-pressure mercury arc was used for the present study, which, after being filtered by an aqueous filter solution, emits a wide range (200−300 nm) of UV light. The equilibrium between d(TpT) **2** and its photodimers (**3**, **4**, etc.) is known to shift depending on the UV wavelength; irradiation at shorter wavelengths (ca. 230−240 nm) favors d(TpT), while longer wavelengths (ca. 280−313 nm) favors the photodimers,[20,21,45] Thus, both photoreactions were anticipated in our conditions. Comparison of the initial rates (<20 min) of the photodimerization showed that *p*- and *m*-bis(Zn^{2+}−cyclen) **8** and **9** were far more effective inhibitors than monomeric Zn^{2+}−cyclens. The stronger inhibitory effects by bis(Zn^{2+}−cyclen)s come from the near-quantitative complexation at 10^{-1} mM concentration, as indicated by the apparent 1:1 complexation stability constants K_{app} of ca. 10^6 (M^{-1}) at pH 7.6 (or the dissociation constant of 1 μM), as determined by isothermal calorimetric titration method. The inert nature of the d(TpT) complexes with the bis(Zn^{2+}−cyclen)s works very effectively in keeping the two thymines from joining at the excited states. Another factor in blocking the photodimerization by Zn^{2+}−cyclens is the deprotonation of the adjacent thymines, which are produced by the thermodynamically stable complex formation at pH 7.6. The reverse photosplitting of a major photodimer product T[*c*,*s*]T **3a** back to d(TpT) **2** was also shown, kinetically and thermodynamically, to be favored by the bis(Zn^{2+}−cyclen)s.[49−51] Finally, using poly(dT), we have demonstrated that the photodimerization of the d(TpT) segments was effectively blocked, and the reverse photosplitting of the photodimers was effectively promoted, by the *m*-bis(Zn^{2+}−cyclen) **9**. These results may open a new way to design novel versatile tools for the preparation of oligonucleotides containing photolesions at the specfic site.

Previously, we had found that dimeric zinc(II) complexes effectively disrupt the A−U or A−T hydrogen bonds to unzip the duplex of poly(A)·poly(U) and poly(dA)·poly(dT) by lowering the melting temperatures (T_m).[14] Therefore, the photodimerization of the d(TpT) moiety in double-stranded DNA is likely to be inhibited by the dimeric zinc(II) complexes. These facts, along with the present results, suggest that the bis(Zn^{2+}−cyclen)s may make a new prototype of chemical blocker[52] against DNA photodamage by T−T photodimerization.

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(49) In nature, the photosplitting of cyclobutane thymine dimers is done by DNA photolyases, enzymes that repair cyclobutane-type thymine dimers in damaged DNA. DNA photolyases bind to DNA-containing thymine photodimers and split cyclobutane rings upon excitation by blue light to restore the intact bases (refs 1−4). The photolyases contain chromophores (i.e., $FADH_2$, 5,10-methenyltetrahydrofolate, or 8-hydroxy-5-deazaflavin) to donate one electron to a cyclobutane ring or withdraw one electron from a cyclobutane ring to trigger the cycloreversion (refs 4 and 50).

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