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Chiral Recognition in the Binding of Helicenediamine to Double Strand DNA: Interactions between Low Molecular Weight Helical Compounds and a Helical Polymer

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Abstract—Binding of a helicene, 5,8-bis(aminomethyl)-1,12-dimethylbenzo[*c*]phenanthrene, to calf thymus DNA was studied using UV, CD, and fluorescence spectroscopy as well as calorimetry. The enantiomeric helicenes strongly bound to the double strand DNA possessing the right-handed helical structure. In addition, chiral recognition was observed in the binding, where the (*P*)-helicene with the right-handed helicity formed more stable complex than the (*M*)-helicene with the left-handed helicity. The binding studies of the helicenes and natural nucleosides by ^{1}H NMR spectroscopy also revealed the higher affinity to the (*P*)-helicene. Both monomeric and polymeric nucleic acids thus turned out to favor the (*P*)-helicity.

During our studies on the chemistry of a helicene, 1,12-dimethylbenzo[*c*]phenanthrene, non-bonding interactions of the helical π-electron system turned out to play an important role.[1] Electron-rich and electron-deficient helicenes form charge–transfer (CT) complex in solution, in which a (*P*)-helicene acceptor complexes more strongly with a (*P*)-donor than a (*M*)-donor.[2] A CT complex of the racemic electron-deficient helicene and pyrene crystallizes in the columnar structure, each of which contains a single isomer of the helicene.[3] Dihelicenetriamines form folded structures in the water, and that of the (*M,M*)-isomer turned out to be energetically more stable than the (*P,M*)-isomer.[4] Macrocyclic alkynes containing three helicene moieties aggregate in organic solvents by the π–π interactions, in which the homo-aggregation of the (*P,P,P*)-isomer is stronger than the hetero-aggregation between the (*P,P,P*)-isomer and the (*M,M,M*)-isomer.[5,6] All these results suggest that the same configuration of the helicenes form more stable complex than the enantiomeric helicenes. As a further investigation of the helicene chemistry, we decided to study the non-bonding interactions of a basic helicene derivative, 5,8-bis(aminomethyl)-1,12-dimethylbenzo[*c*]-phenanthrene **1**,[4] and double strand DNA. We were interested to know which the right-handed helical polymer favors, the right-handed or left-handed helicene.[7] Polycyclic aromatic compounds are known to interact with double strand DNA, and chiral recognition have been examined using chiral derivatives of anthracenes or phenanthrenes.[8,9] Such study using chiral helicene, however, was not conducted.

When calf thymus DNA was added to buffered solutions (pH 7) of (*P*)-**1** or (*M*)-**1** containing NaCl and ethylenediaminetetraacetic acid (EDTA), UV and CD spectra changed indicating the complex formation (Figs 1 and 2). Fluorescence spectra exhibited hypochromism in the presence of DNA (Fig. 3), and were used to obtain the binding constants K and the binding site sizes n in base pair employing the McGhee–von Hippel method[10] (Figs 4 and 5): $K=(1.4\pm0.1)\times10^4$ M^{-1}, $n=3.8\pm0.3$ for (*P*)-**1**; $K=(1.2\pm0.1)\times10^4$ M^{-1}, $n=5.9\pm0.2$ for (*M*)-**1**. The binding of (*P*)-**1** to the double strand DNA turned out to be slightly stronger than that of (*M*)-**1**; the right-handed helical DNA favors the right-handed helical molecule (*P*)-**1**.

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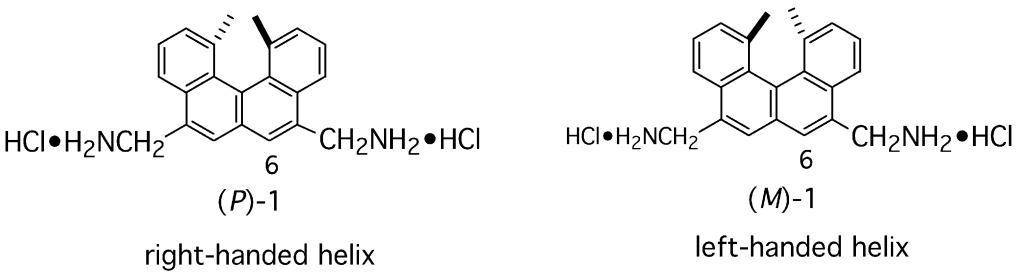


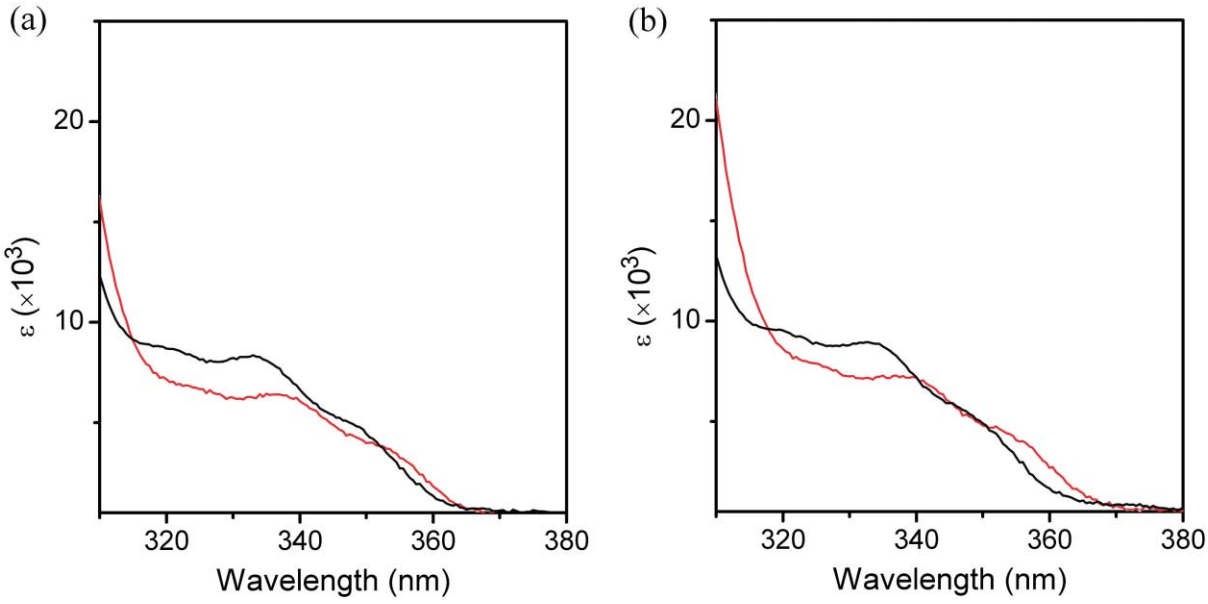


Figure 1. UV spectra of (*P*)-**1** (a) and (*M*)-**1** (b) (0.010 mM, 25 °C) in (4-morpholino)ethanesulfonic acid buffer (1.0 mM, pH 7) containing 100 mM NaCl and 0.01 mM EDTA in the absence (black line) and presence (red line) of calf thymus DNA (2 mM).

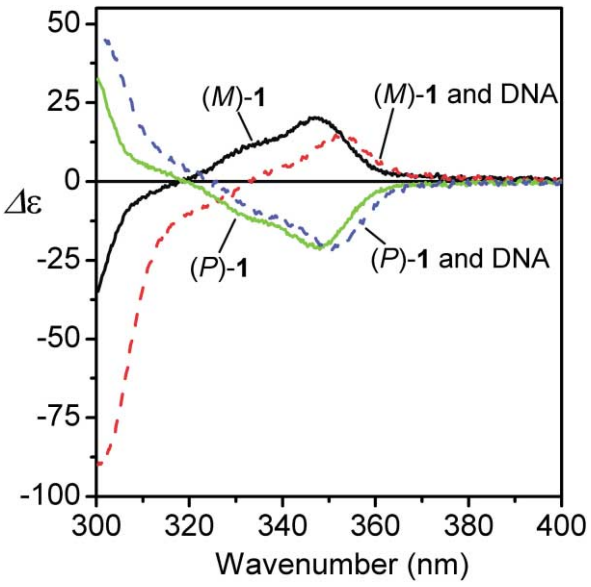


Figure 2. CD spectra of (*P*)-**1** and (*M*)-**1** (0.010 mM, 25 °C) in (4-morpholino)ethanesulfonic acid buffer (1.0 mM, pH 7) containing 100 mM NaCl and 0.01 mM EDTA in the absence (—) and presence (- - -) of calf thymus DNA (1 mM).

Thermochemical investigations on the binding of **1** to calf thymus DNA were conducted using isothermal titration calorimetry (Fig. 6),[11] and were compared with the binding of ethidium bromide, a well-known intercalater (Table 1). Notably, the binding of **1** to the DNA is much stronger than ethidium bromide. In addition, the results of the fluorescence titration experiments were confirmed; the binding of (*P*)-**1** to the DNA is stronger than that of (*M*)-**1**. Although the thermodynamic data indicated the binding of **1** to be enthalpy driven, the chiral recognition was largely affected by the entropy; entropy for (*P*)-**1** binding is positive and that for (*M*)-**1** negative. Such positive entropy was observed in the binding of netropsin to DNA.[11] The chiral recognition thus driven by the entropy difference suggests considerably different binding structures between the enantiomeric **1**.

The chiral recognition phenomenon of the double strand DNA was compared with the binding behaviors of nucleosides and **1**. The binding in D_2O was monitored by the ^1H NMR titration experiments of the racemic (±)-**1** with deoxyribonucleosides (dA, dT, dG, and dC) or ribonucelosides (A, U, G, C) (Fig. 7). In cases other than dC and G, the aromatic peaks of (±)-**1**

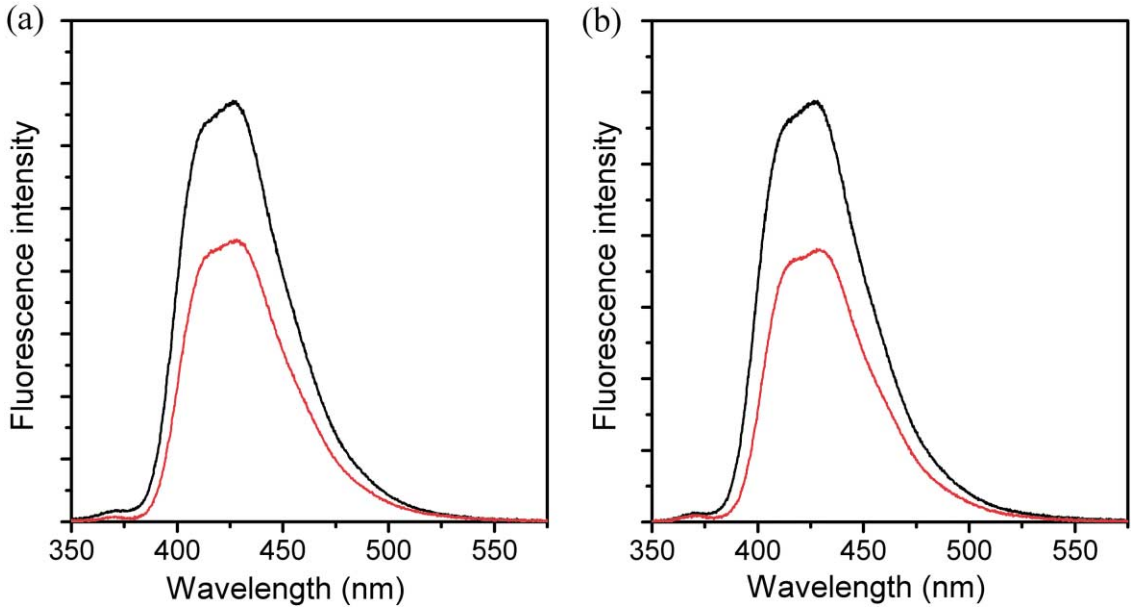


Figure 3. Fluorescence spectra (excitation at 330 nm) of (*P*)-**1** (a) and (*M*)-**1** (b) (0.010 mM, 25 °C) in (4-morpholino)ethanesulfonic acid buffer (1.0 mM, pH 7) containing 100 mM NaCl in the absence (black line) and presence (red line) of calf thymus DNA (2 mM).

separated, and chiral recognition was observed in the complexation (Fig. 7). The binding constants *K* were obtained by the least-square method assuming 1:1 complexation (Fig. 8),[13] and the results are summarized in Table 2. In the complexation of dA, A, or U with **1**, appreciable different *K* were obtained between the enantiomers, and the binding of (*P*)-**1** turned out to be stronger than that of (*M*)-**1**. The differences of the binding constants *K* for dT, dG, and C were marginal with slight preference for (*P*)-**1**. As was the double strand DNA, nucleosides also show higher affinity to the right-handed helical (*P*)-**1**. This is an interesting chiral recognition phenomenon, in which the behaviors of the monomeric and polymeric compounds are clearly related. The chiral recognition in the binding of helicenes was previously reported using HPLC loaded with natural nucleosides,[12] where (*M*)-helicenes eluted fast. The results qualitatively indicate the higher affinity of the (*P*)-isomer to the nucleosides, and are in accordance with our quantitative analyses.

To summarize, double strand DNA forms more stable complex with (*P*)-**1** possessing the right-handed helical array of aromatic ring system rather than (*M*)-**1**. Studies on the DNA base sequence selectivity by the chiral **1** and its oligomeric compounds[4] are now underway.

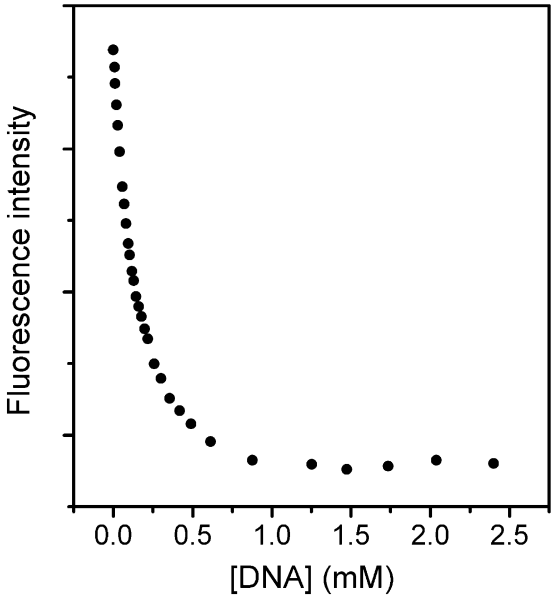


Figure 4. Plots of the fluorescence intensity of (*P*)-**1** (0.010 mM, 25 °C) at 430 nm in (4-morpholino)ethanesulfonic acid buffer (1.0 mM, pH 7) containing 100 mM NaCl against the concentration of calf thymus DNA.

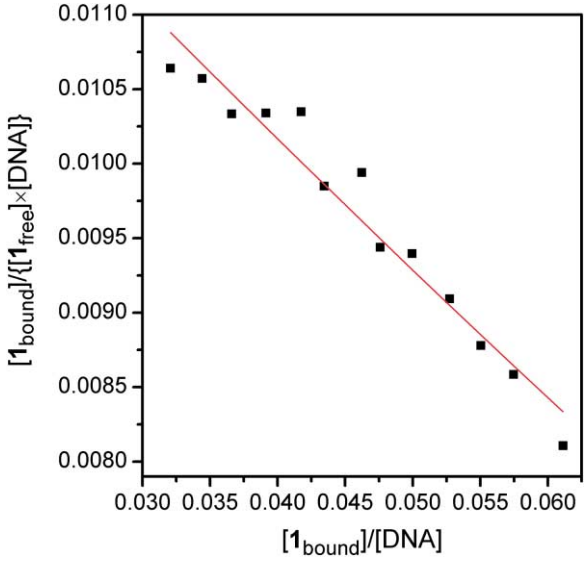


Figure 5. Scatchard plots for the binding of (*P*)-**1** (0.010 mM, 25 °C) to calf thymus DNA in (4-morpholino)ethanesulfonic acid buffer (1.0 mM, pH 7) containing 100 mM NaCl. [$\mathbf{1}_{bound}$] and [$\mathbf{1}_{free}$] mean DNA-bound and free concentrations of **1**, respectively.

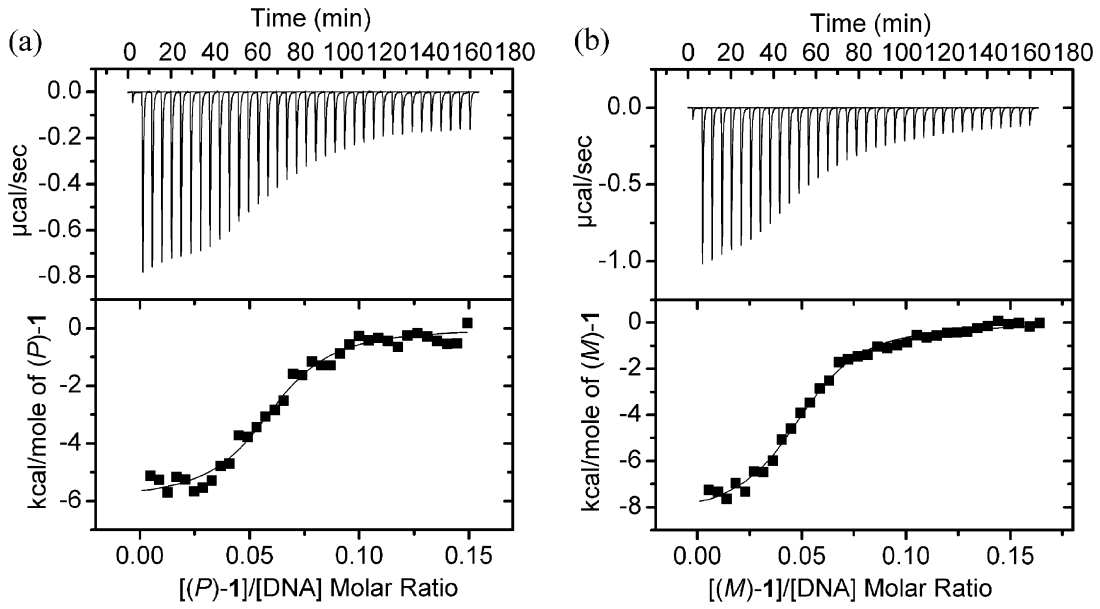


Figure 6. Calorimetric data for (*P*)-**1** (a) or (*M*)-**1** (b) titration of calf thymus DNA. The measurements were made at 25 °C in 20 mM Tris–HCl buffer (pH 7.6) containing 20 mM NaCl.

Table 1. Binding of **1** and ethidium bromide to calf thymus DNA (0.5 mM) in 20 mM Tris–HCl buffer (pH 7.6, 25 °C) containing 20 mM NaCl

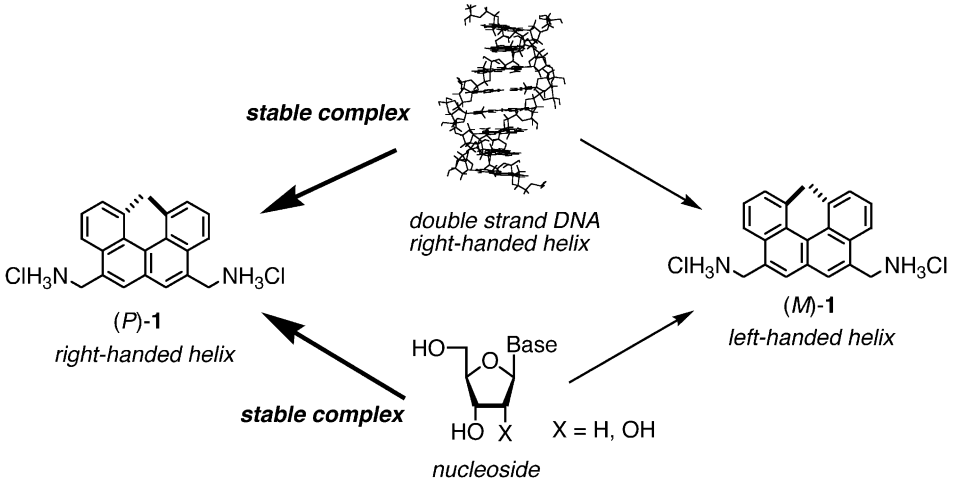
	(*P*)-**1**	(*M*)-**1**	Ethidium bromide
K ($\times 10^5$ M^{-1})	5.7±1.0	3.6±0.5	1.2±0.1
ΔG (kcal/mol)	−7.9±0.1	−7.6±0.1	−6.9±0.1
ΔH (kcal/mol)	−6.0±0.2	−8.1±0.3	−13.9±0.3
ΔS (cal/mol K)	6.3±0.7	−1.9±1.0	−23.4±1.2

Experimental

Calf thymus DNA was purchased from Sigma Chemical Co. (St. Louis, MO), or Amersham Pharmacia Biotech Inc. (Piscataway, NJ), and was used as received. DNA concentrations per base pair were determined by UV spectroscopy using the molar extinction coefficient (6412 M^{-1} cm^{-1} at 260 nm).[14] ^1H NMR spectra in D_2O were recorded on a Varian Mercury (400 MHz), and were referenced to 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (δ 0.00) as an internal standard.

Fluorescence titration experiments

All experiments were performed at 25 °C in 1 mM (4-morpholino)ethanesulfonic acid buffer (pH 7.0) containing 0.01 mM EDTA and 100 mM NaCl. Fluorescence titrations were carried out at a constant concentration (0.010 μM) of **1** and at variable DNA concentrations (0–2.5 mM) with excitation at 330 nm. The binding curves were analyzed by the McGhee–von Hippel method[10] using a fluorescence intensity at 430 nm to obtain the binding constants K and the binding



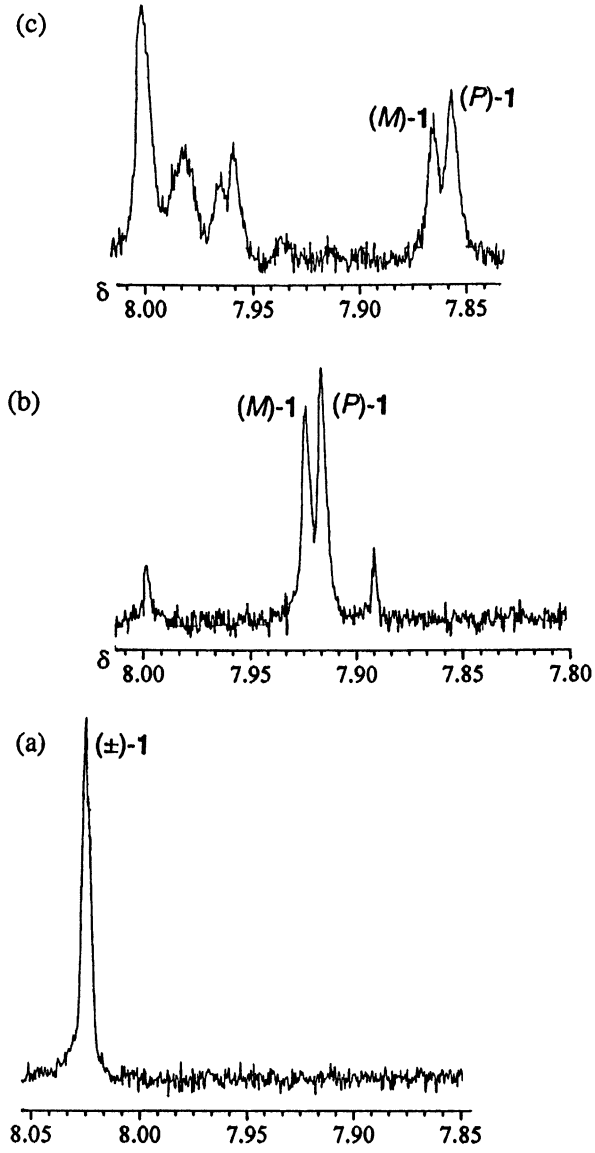


Figure 7. ^{1}H NMR spectra (D_2O, 100 mM phosphate buffer, pD 7.0, 23 °C) of (±)-**1** (1.0 mM) at 6*H* in the absence of dA (a), in the presence of 20 mM dA (b), and 60 mM dA (c).

site sizes *n* values in base pairs. The same experiments were conducted twice.

Isothermal titration calorimetry experiments

Isothermal calorimetry experiments were carried out using a MicroCal VP-ITC calorimeter. All experiments were performed at 25 °C in 20 mM Tris–HCl buffer (pH 7.6) containing 20 mM NaCl. All solutions were degassed before titration. Typically, 0.5 mM samples of DNA in 1.4 mL buffer were titrated by 35 injections of 7.5 μL each of 0.4 mM **1** in the same buffer solution. Heat of dilution was measured by injecting the solution of **1** into a buffer solution, and was used for correction of heat evolved by complexation. Calorimetric data were analyzed using MicroCal ORIGIN software. The same experiments were conducted twice.

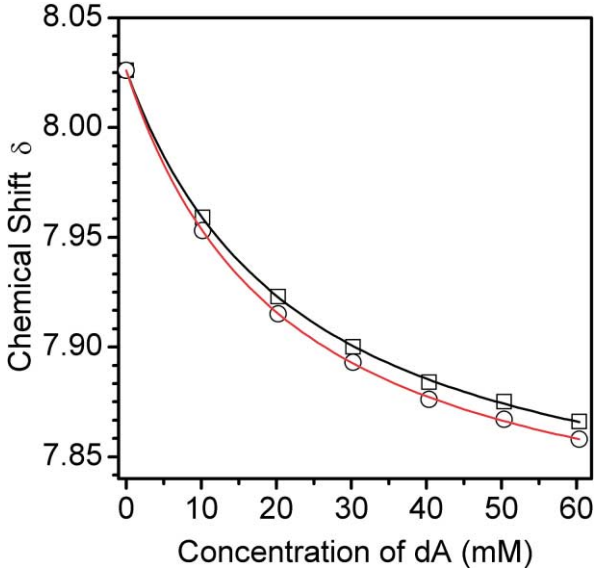


Figure 8. Chemical shifts δ of (*P*)-**1** (○) and (*M*)-**1** (□) (concentration of (±)-**1**, 1.0 mM) at 6*H* on addition of dA in D_2O (100 mM phosphate buffer, pD 7, 23 °C). Lines were obtained by the calculations.

Table 2. Bindig of deoxyribonucleosides or ribonucleosides to **1** (1.0 mM) in D_2O (pD 5.7, 23 °C, 100 mM phosphate buffer) obtained by ^{1}H NMR titration experiments

Nucleoside	*K* (M^{-1})	
	(*P*)-**1**	(*M*)-**1**
dA	48±1	45±1
dT	1.7±0.1	1.6±0.1
dG	44±1	43±2
dC	6.8±1.4	
A	36±1	32±2
U	5.2±0.3	4.5±0.5
G	37±19	
C	7.6±1.7	7.4±1.1

^{1}H NMR titration experiments

^{1}H NMR spectra were measured in pD 5.7 phosphate buffer (93.5 mM NaH_2PO_4 and 6.5 mM Na_2HPO_4 in D_2O) at 23 °C containing 1.0 mM (±)-**1** and variable amounts of nucleosides (0–30 mM). The binding curves were analyzed by the nonlinear least-square method to obtain the binding constants *K*.[13] All the experiments were conducted twice.

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