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Synthesis of a novel intercalator based on 2,2′-binaphthalene bearing dimethylammonium groups

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Abstract—A novel type of DNA intercalator, 8,8′-bis(dimethylaminomethyl)-2,2′-binaphthalene (**1**) based on 2,2′-binaphthalene skeleton, was prepared via homocoupling of 7-bromo-1-methylnaphthalene as a key step. The binding ability of **1** for calf thymus (CT) DNA was evaluated by UV–vis and fluorescence spectroscopic titrations and the melting temperature of CT DNA. The apparent association constant of **1** with CT DNA was larger than that of ethidium bromide (EB).

Intercalation by small organic molecules to DNA has widely been studied due to application for medicinal use.[1] In general, planar and fused heterocyclic aromatic compounds bearing cationic side arms or cationic quaternary nitrogen in the aromatic ring such as EB which is used for staining of DNA, are employed as an intercalator. Aromatic hydrocarbons, such as naphthalene and anthracene derivatives bearing cationic side chains, have also been reported as intercalators.[2] Recently, Wilson and Strekowski have reported a series of intercalators bearing plural heterocyclic aromatic rings connected with a single bond to interact with duplex and triplex of DNA.[3] By connection with a freely rotatable single bond, two aromatic rings are expected to show dynamic structural change through interaction with DNA. In such a case, large spectral change would be observed. In addition, relatively flexible structure of the intercalator would have large solubility in aqueous buffer solution and high binding ability due to induced fit through interaction into the twisted forms of base pairs. However, no intercalators, which consist of two aromatic hydrocarbons such as naphthalene moieties connected with a single bond, have been reported. We have developed 2,2′-binaphthalene bearing two thiourea groups at 8- and 8′-positions as an anion receptor.[4] The skeleton of 2,2′-binaphthalene would be suitable for the intercalator due to the reasons mentioned above. In this communication, we show the synthesis of a novel intercalator **1**, 2,2′-binaphthalene bearing dimethylaminomethyl groups at 8- and 8′-positions, and its intercalation properties to CT DNA.

Synthetic route of **1** is illustrated in Figure 1. Synthesis of 7-bromo-1-methylnaphthalene (**2**) was carried out by slightly modified procedures according to the literature from bromobenzene in five steps.[5] Homocoupling of **2** by a catalytic amount of nickel(II) chloride with a stoichiometric amount of zinc as a reducing metal in the presence of 2,2′-bipyridine and triphenylphosphine in *N*,*N*-dimethylacetamide at 70 °C[6] gave 8,8′-dimethyl-2,2′-binaphthalene (**3**) in 75% yield which is a versatile intermediate for construction of various 2,2′-binaphthalene derivatives. After bromination of the methyl groups of **3**, the target compound **1** was obtained by

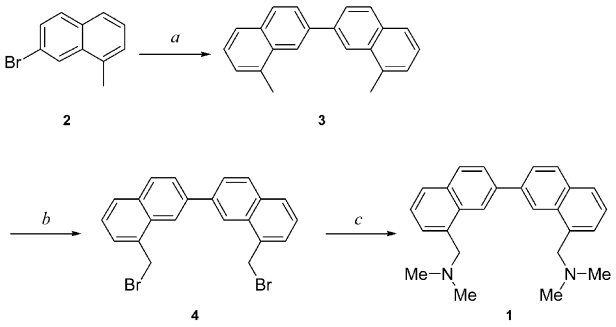

Figure 1. Synthesis of **1**. Reagents and conditions: (a) Zn, $NiCl_2$ (cat.), bpy, PPh_3, DMAc, 60 °C, 75%; (b) NBS, AIBN (cat.), CCl_4, reflux, 76%; (c) dimethylamine, DMF, rt, 43%.

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substitution with dimethylamine in *N*,*N*-dimethylformamide in 43% yield. The product was characterized by ^{1}H NMR spectroscopy, ESI-MS and elemental analysis.[7] UV–vis spectrum of **1** in buffer (10 mM Tris–HCl, pH 7.2, 20 mM NaCl) showed λ_{max} at 310 (log $\varepsilon = 4.18$), 257 (4.86), and 215 nm (4.54).

The binding of **1** with CT DNA was studied by UV–vis and fluorescence spectroscopic titrations. UV–vis spectral changes of **1** upon the addition of CT DNA are shown in Figure 2. A large bathochromic shift (ca. 35 nm shift of the maximum) through an isosbestic point at 319 nm was observed, suggesting that the twisted conformation of **1** in free form changes to the more planar structure on complexation with CT DNA. Similar spectral changes are observed in the case of 2,2′-bipyridine on complex formation with metal ions.[8] The apparent association constant was calculated to be 1.9 10^5 M^{-1} by the half reciprocal plot according to the literature[9] as shown in Figure 3. Two amino groups of **1** are protonated to form ammonium groups at pH 7.2 diminishing a photo-induced electron transfer process from benzylic nitrogen atoms. In fact, **1** showed strong fluorescence. Figure 4 shows the fluorescence response of **1** excited at 319 nm (isosbestic point determined by UV–vis spectroscopy) upon the addition of CT DNA. Increasing the amount of CT DNA, quenching of the fluorescence of **1** was observed and F/F_0 reached to ca. 0.1. The similar quenching was also reported in the case of 9-aminomethylanthracene.[2b] Figure 5 shows Scatchard plots of fluorescence titrations of **1** and EB with CT DNA analyzed by McGhee and von Hippel models.[10] The intrinsic association constants (K_i) and exclusive parameters in base pairs (n) for **1** and EB were determined to be 8.0×10^5 and 3.6×10^5 M^{-1}, and 2.0 and 2.2,[11] respectively by nonlinear least-square fittings. It should be noted that apparent association constant of structurally related 2-aminomethylnaphthalene which has only one naphthalene moiety for CT DNA was reported to be 2.2×10^3 M^{-1} ,[2c] indicating that two naphthalene moieties of **1** play an important role for intercalation to DNA double helix.

Displacement of DNA-bound EB by a competitive intercalator is a qualitative measurement of the DNA affinity. The C_{50} value was defined as the concentration of the competitive intercalator required for a 50% reduction of the fluorescence of EB ($\lambda_{ex} = 520$ nm and $\lambda_{em} = 600$ nm) with [EB] = 2 μM and [CT DNA] = 1 μM bp. The C_{50} value of **1** was determined to be 7.2 μM.

Intercalation of small molecules into double helix of DNA is known to increase the melting temperature of DNA.[12] As shown in Figure 6a, the melting temperatures (T_m) of CT DNA (50 μM) in the absence and the presence (5.0 μM) of **1** were 75.0 and 81.2 °C,

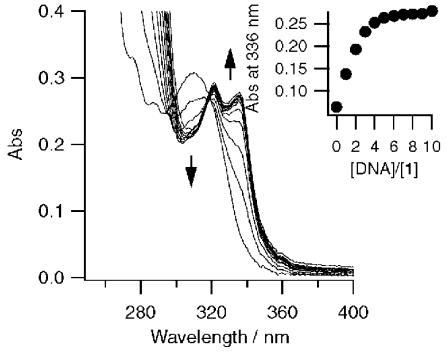

Figure 2. UV–vis spectral changes of **1** upon the addition of CT DNA. [**1**] = 2.0×10^{-5} M, [CT DNA] = 0–2.0×10^{-4} M bp in 10 mM Tris–HCl (pH 7.2) with 20 mM NaCl at 25 °C. Inset: Absorbance changes of **1** at 336 nm upon the addition of CT DNA.

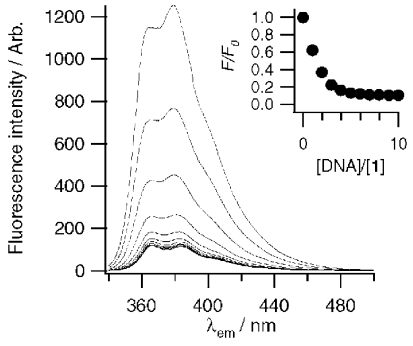

Figure 4. Fluorescence spectral changes of **1** upon the addition of CT DNA. [**1**] = 1.0×10^{-5} M, [CT DNA] = 0–1.0×10^{-4} M bp in 10 mM Tris–HCl (pH 7.2) with 20 mM NaCl at 25 °C. $\lambda_{ex} = 319$ nm. Inset: Changes of fluorescence intensity of **1** ($\lambda_{em} = 379$ nm) upon the addition of CT DNA.

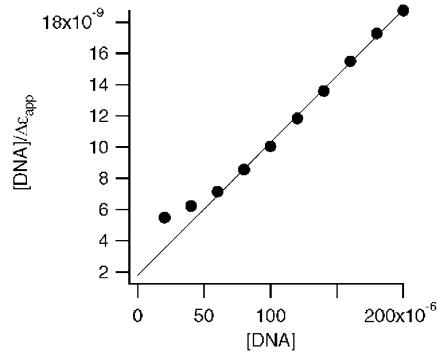

Figure 3. A half-reciprocal plot of **1** binding with CT DNA as determined from the UV–vis titration.

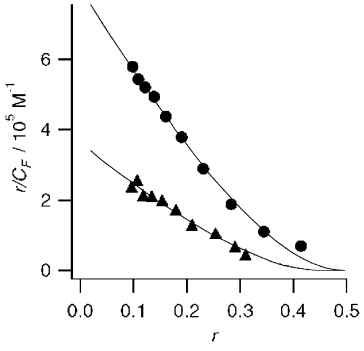

Figure 5. Scatchard plots of the fluorescence titration of **1** (filled circles, conditions are shown in the caption of Fig. 4) and ethidium bromide (filled triangles, [ethidium bromide] = 1.0×10^{-5} M, $\lambda_{ex} = 520$ nm and $\lambda_{em} = 600$ nm. Other conditions were the same as for **1**).

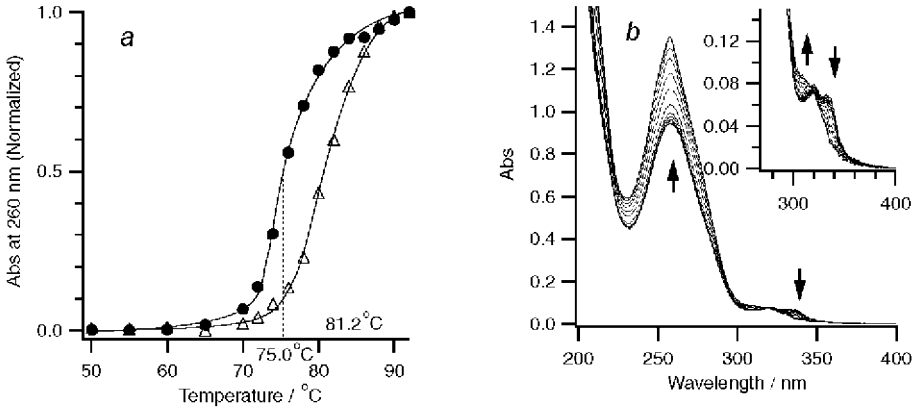

Figure 6. The normalized changes of absorbance at 260 nm of CT DNA on heating in the presence (open triangles) and the absence (filled circles) of **1** (*a*) and spectral changes of CT DNA on heating in the presence of **1** (*b*). [CT DNA] = 5.0×10^{-5} M bp and [**1**] = 5.0×10^{-6} M in 10 mM Tris–HCl (pH 7.2) with 20 mM NaCl at 25 °C. Inset: enlarged spectra of **1** upon the addition of CT DNA.

respectively. The large increase in the melting temperature in the presence of **1** obviously shows the increased stability of the double helix of CT DNA. It should be noted that increasing of the temperature of CT DNA in the presence of **1** caused a hypsochromic shift of the absorbance around 335 nm as similar to the free **1** due to release of **1** as shown in Figure 6b. These results clearly support interaction of **1** into the double helix DNA.

In summary, we have demonstrated that 2,2′-binaphthalene derivative **1** is an effective intercalator for DNA. We believe the skeleton would be useful for construction of various types of compounds by introduction of functional groups. The apparent association constant of **1** is in the order of 10^5 M^{-1} for CT DNA. Functionalization of **1** and detailed studies including selectivity of DNA sequences are in progress in our laboratory.

Acknowledgements

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7. Selected data for **1**: Viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (s, 12H, CH_3), 3.91 (s, 4H, CH_2), 7.42 (t, 2H, 6-CH, J = 7.2 Hz), 7.45 (d, 2H, 7-CH, J = 7.2 Hz), 7.82 (d, 2H, 5-CH, J = 7.2 Hz), 7.89 (dd, 2H, 3-CH, J_1 = 8.4, J_2 = 1.5 Hz), 7.97 (d, 2H, 4-CH, J = 8.4 Hz), 8.62 (s, 2H, 1-CH). Anal. calcd for $C_{26}H_{28}N_2\cdot0.5H_2O$: C, 82.72; H, 7.74; N, 7.42. Found: C, 82.63; H, 7.68; N, 7.28. ESI-MS (positive ion mode): calcd for $[C_{26}H_{28}N_2+H]^+$ m/z 368.23; found: 368.3.
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