Synthesis, DNA binding and photocleavage study of novel anthracene-appended macrocyclic polyamines†

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Two anthracene derivatives appended on cyclen (1,4,7,10-tetraazacyclododecane) moieties were synthesized and characterized. In these new compounds, the anthryl is used as a substitute for the nucleobases of classical PNA backbone, and the cyclen moiety appends on the terminal amino group. The interaction of the compounds with DNA was systematically investigated by absorption, fluorescence, and viscometric titration, DNA melting and gel electrophoresis experiments. From the absorption titration data, bis-anthryl compound **2** can bind to CT DNA with $K_b = 1.21 \times 10^5$ M^{-1} that is 121 times larger than that of mono-anthryl compound **1** ($K_b = 1.00 \times 10^3$ M^{-1}). Through the fluorescence titration data, compound **1** shows distinct CG-selective DNA binding activity. DNA melting and viscometric titration experiments indicate that the binding mode of **2** is a multiple binding mode that involves groove binding and partial intercalation. Compound **2** also shows excellent DNA photocleavage ability, which is much more efficient than the mono-anthryl compound **1**.

Introduction

It is a quite interesting work to investigate the binding and interaction between small molecules and biomolecules, especially DNA.[1] Because of the important functions of DNA in living organisms, studies towards the interactions between small molecules and DNA will be helpful for preventing and curing diseases.[2] Molecules with DNA photocleavage activity have been proved useful in photodynamic therapy (PDT), which is one of the most effective treatment options for anticancer therapies.[3] In PDT drug design, good DNA-binding ability is needed for better cytotoxicity and DNA cleavage activity.[4] Meanwhile, good binding selectivity is needed to obtain the site-specific cleavage ability.[5]

Molecules with multiple binding points always have better DNA-binding ability. Therefore bi-functional or multi-functional molecules with multi-binding positions have been designed and prepared, and some of them have the potential to be effective drugs.[6] It was found that intercalation, groove binding, and outside binding are common DNA binding modes.[7] Drugs containing two intercalation units usually obtain higher DNA-binding affinity *via* a bis-intercalation binding mode,[8] and as a result, the drug residence time is prolonged. Hence, we considered that this principle may be used to design effective DNA cleavage agents. Bis-intercalation is mostly found in a molecule that (i) contains two intercalators; (ii) has a spacer length of more than 10.2 Å; (iii) follows the neighbor exclusion principle.[9] If the spacer is rigid or not long enough, only a mono-intercalation process will happen.[10] In addition, only a few examples violating the neighbor exclusion principle have been reported.[11]

In recent years, some groups have investigated the DNA binding or photocleavage activity of small molecules with fused aromatics, especially with two or more intercalators in their structures. Kumar *et al.* studied the DNA binding affinities of anthracene derivatives with substituents at 9- and/or 10- positions.[6c,12] They found that different substituents influenced the DNA binding mode, binding ability and photocleavage activity of the studied molecules. Furthermore, the structures of the substituents might influence the binding preference (*e.g.* dC-dG over dA-dT). However, their work was limited to the substituent effect of mono-anthracenc derivatives on DNA binding only. Schneider and co-workers synthesized a series of polyamines bearing two or more aromatic rings, and studied their binding affinities with a DNA/RNA model.[13] These compounds were imagined to hold the potential for RNA cleavage. Grant and co-workers synthesized several bi-functional compounds with rigid aromatic spacers,[10,14] and the DNA binding and metal activated photocleavage ability of these compounds were reported. Although great efforts have been devoted to the studies of DNA binding ability of bis-intercalating molecules, less attention was paid to DNA photocleavage agents that work *via* the bis-intercalation mode.

Herein, we designed and synthesized a bis-anthryl compound **2** with multiple peptide band structure backbone (Scheme 1). Cyclen (1,4,7,10-tetraazacyclododecane) moiety was introduced to enhance water solubility and binding ability towards DNA. The DNA binding and photocleavage activity of **2** was compared with the mono-anthryl compound **1** with similar structure (Scheme 1). We found that DNA binding constant of the **2** is 100 times more than that of **1**, and **2** also has greater DNA cleavage ability than **1**. On the other hand, compound **1** shows significant CG-selective DNA binding activity. The bind modes of the

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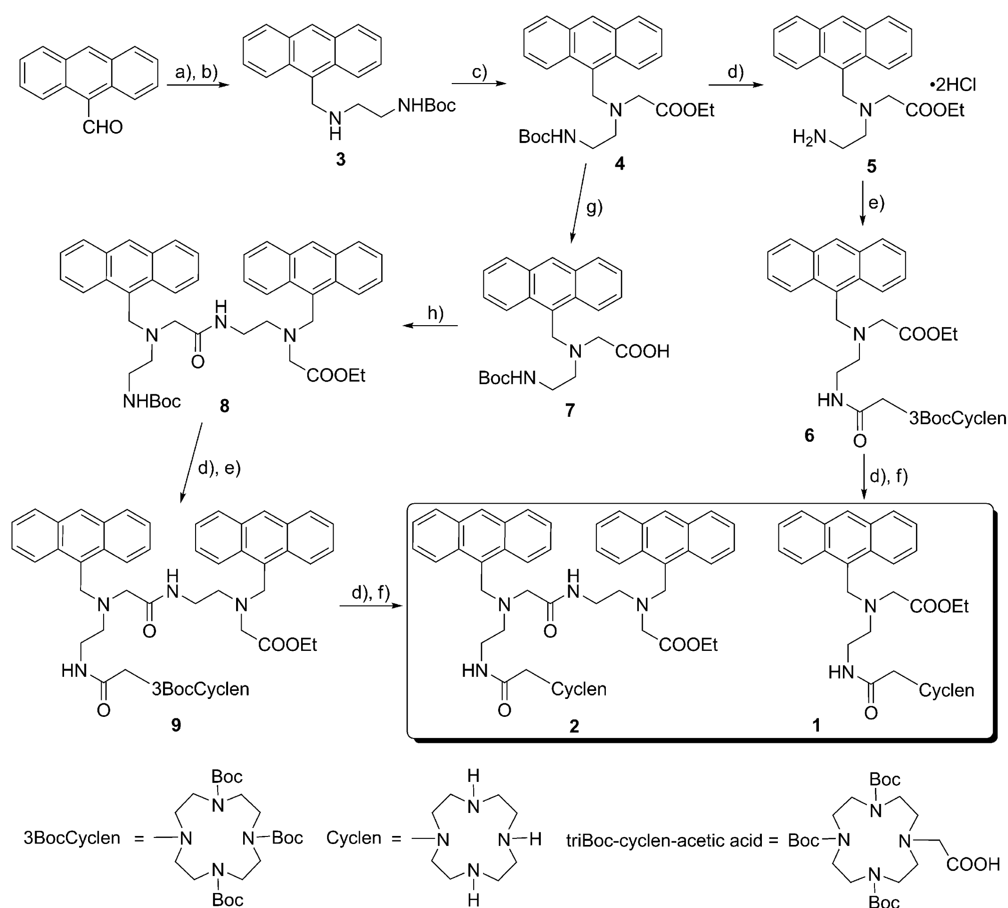

Scheme 1 Synthesis of the title compounds. *Reagents and conditions*: (a) *N*-Boc-1,2-diaminoethane, MeOH, reflux; (b) $NaBH_4$, rt; (c) ethyl bromoacetate, K_2CO_3, EtOAc, reflux; (d) HCl, MeOH; (e) triBoc-cyclen-acetic acid, Et_3N, HOBt, DCC; (f) 5% aqueous $NaHCO_3$; (g) NaOH, MeOH; (h) **5**, Et_3N, HOBt, DCC.

two compounds towards DNA were studied by the methods of absorption titration, DNA melting experiments, and viscometric titration.

Results and discussion

General synthesis

The title compounds were synthesized by the method shown in Scheme 1. As an important intermediate, compound **4** can be obtained from 9-anthraldehyde by adding aminoethyl and carboxylmethyl "arms" successively. Reversal of the sequence led to a low reaction yield and difficult separation of the product. Deprotection of *N*-Boc on **4** by a methanol solution of HCl gives compound **5**, which can be coupled with triBoc-cyclen-acetic acid in the presence of DCC and *N*-hydroxybenzotriazole (HOBt) to form the peptide bond and to yield **6**. The mono-anthryl compound **1** was obtained by the final deprotection of **6**. In another route, saponification of **4** leads to compound **7**, and the reaction between carboxylic acid **7** and amine **5** under the same reaction conditions as those in the preparation of **6** can give the coupling product **8**. The same procedures were used in the preparation from **8** of target compound **2** as those in the preparation from **4** to **1**. The structures of all new compounds were confirmed by ^{1}H NMR, IR and HRMS.

Absorption titration

To measure and compare the DNA-binding constants of mono-anthryl compound **1** and bis-anthryl compound **2**, we studied the absorption titration of these compounds with CT DNA monitored by absorption spectra. As reported,[6c,11,12a,15] the spectra show a decrease in the peak intensity with increasing amounts of CT DNA (Fig. 1). However, the hypochromicity observed here is much weaker than those in the literature. As the hypochromicity was mainly decided by the interaction between the electronic states of the chromophore and nucleobase, stronger hypochromicity always indicates a larger binding strength towards DNA.

We also used the hyprochromicity to calculate K_b of the aromatic compounds. The K_b values of bis-anthryl compound **2** and mono-anthryl compound **1** are 1.21×10^5 M^{-1} and 1.00×10^3 M^{-1}, respectively (Fig. 1 and Figure S1 in Supporting Information). Comparing with similar mono-anthryl compounds reported in the literature,[6c,11,12a] the smaller K_b value of **1** suggested a poor interaction between **1** and DNA basepairs. And the much larger K_b value of **2** suggested multiple binding of the two anthryl groups to the DNA basepairs.

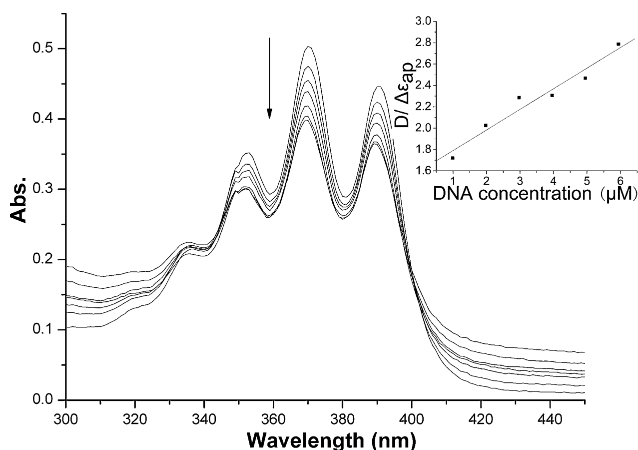

Fig. 1 Absorption spectra of **2** (100 μM) with increasing concentrations of CT DNA (0–6 μM).

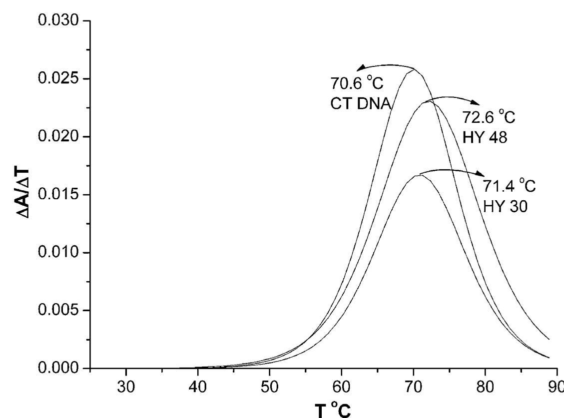

Fig. 2 First derivative plots for the thermal denaturation curves of the DNA duplex.

Fluorescence titration

Fluorescence titration data can be used to calculate the intrinsic binding constant (K_i) and binding site size (n) using the Scatchard equation. Fluorescence titration experiments of **1** with CT DNA, poly(dA-dT)$_9$, and poly(dC-dG)$_9$ were studied (Figure S2 in Supporting Information), and the data are listed in Table 1. The binding constant of **1** towards CG sequences is nearly 10 times over that towards CT DNA and 36 times over that towards AT sequences. Such binding a preference of CG sequences is consistent with the properties of similar anthracene derivatives in the literature.[6c,11] Moreover, the sequence selectivity of **1** is more effective than any other anthracene derivatives ever reported. But the binding site size towards CG sequences was close to that towards AT sequences. Unfortunately, as the fluorescence intensity of bis-anthryl compound **2** always increases under photo irradiation, the sequence selectivity of **2** cannot be studied by fluorescence titration.

DNA melting experiments

DNA melting experiments are an important method for the study of interactions between small molecules and DNA. Strong evidence can be obtained from these experiments for deducing the binding mode of the compounds towards DNA. An increase of the T_m value always indicates that the molecule has bound to DNA and therefore stabilized the DNA duplex especially *via* the classical intercalation mode. The melting curves of CT DNA in the absence and presence of compounds **1** and **2** are given in Fig. 2. The T_m of CT DNA alone was 70.6 °C. After addition of compound **1** or **2**, the T_m value increased to 72.6 °C and 71.4 °C, respectively. The increases of T_m ($\leq$2 °C) are much less than the reported rising extent of 13–14 °C due to intercalation.[16] Therefore, the intercalate binding mode may be strongly ruled out for the interactions between DNA and **1** or **2**. To be beneficial for the comparison of results, we did the experiments under the same conditions as those in literature.[17]

Table 1 K_i and n values for compound **1** from fluorescence titration experiments with various DNA sequences

	K_i	n
CT DNA	8.16×10^4	6
Poly(dC-dG)$_9$	*7.86×10^5*	*10*
Poly(dA-dT)$_9$	2.16×10^4	10

Viscometric titration

Viscometric titration is also used to determine the binding modes of small molecules to DNA.[1] In the three binding modes (intercalation, groove binding and outside binding), only intercalation causes a significant increase of viscosity of DNA solution. This is due to the unwinding of the DNA duplex that leads to the receiving of intercalator into the base pairs and to the increase of DNA length. Groove binding and outside binding do not need to unwind the DNA duplex and therefore keep the DNA length, and as a result, the viscosity of DNA solution does not show significant change.

The results of viscometric titration with anthryl compounds **1** and **2** to CT DNA are shown in Fig. 3. The viscosity of the DNA slightly increased with the addition of **1**. According to the theory of Cohen and Eisenborg, the slope of the titration curve should be close to 1.0 for classical intercalation.[18] Therefore, the slope of 0.05 also rules out the classical intercalation binding of **1** to the DNA duplex. Though the anthracene derivatives usually intercalate into the DNA duplex, groove binding has been reported a few times in the literature.[6c,12c] Together with the above data, we conclude that compound **1** binds to DNA by the groove binding mode. In

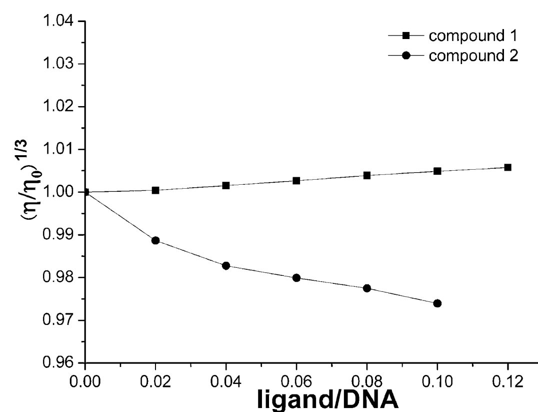

Fig. 3 Results of viscometric titrations in the presence of anthryl compounds.

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typical bis-intercalation, the slope of viscometric titration curve of a bis-intercalator is twice that of the related mono-intercalator.[19] On the contrary, the viscosity of DNA decreased more quickly with the addition of **2**. This decrease might be explained by the reduction of DNA effective length caused by bends or kinks, which usually occur in the interaction between metal ion complexes and DNA *via* the outside binding mode. We speculate that the binding of bis-anthryl compound **2** to DNA happens through partial intercalation by one of its anthryl moiety and groove binding by the other.[20]

DNA photocleavage

The DNA photocleavage ability was studied at room temperature, pH 7.4 in 0.1 M of phosphate buffer. Agarose gel electrophoresis was used to monitor the conversion of supercoiled pUC 19 DNA to nicked forms, and the results are shown in Fig. 4 and 5. The percentages of photocleavage of pUC 19 DNA are shown in Figure S3 (Supporting Information). Like many other photocleavage agents, time-dependent cleavage activity was found by irradiating pUC 19 DNA (Fig. 4a) with compound **2**. The amount of nicked forms of DNA increased with the extension of irradiation time. Almost all the supercoiled DNA converted into nicked forms in 1 h (Fig. 4a, lane 9). In the parallel control reactions, after keeping DNA under irradiation for 1 h, the cleavage was not significant (Fig. 4a, lane 2). Compound **2** also showed concentration-dependent cleavage ability, and supercoiled DNA could be photocleaved almost completely in 45 min in the presence of 47.6 μM of **2** (Fig. 4b). Comparing with mono-anthryl compound **1**, bis-anthryl **2** displays much better cleavage ability towards supercoiled DNA (Fig. 4c). Considering the similar structures of the two anthryl compounds, the large different on cleavage ability might be attributed to the different binding modes between the two anthryl compounds towards DNA.

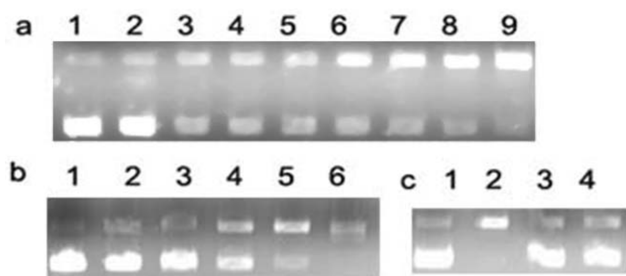

Fig. 4 Light-induced DNA photocleavage. a) Irradiation time effect. Lanes 1 and 2, DNA control in dark and under irradiation respectively; Lanes 3–9, DNA with 23.8 μM of compound **2**, irradiation time: 0, 10, 20, 30, 40, 50, 60 min. b) Concentration effect. Irradiation time: 45 min. Lane 1, DNA control upon irradiation; Lanes 2–6, DNA with 3.0, 6.0, 11.9, 23.8, 47.6 μM of **2**. c) DNA photocleavage by compound **1** and **2**, irradiated for 1 h. Lane 1, DNA control upon irradiation; Lane 2, DNA with 47.6 μM of **2**; Lane 3, DNA with 23.8 μM of **1**; Lane 4, DNA with 47.6 μM of **1**.

DNA photocleavage catalyzed by intercalating agents is usually carried out *via* oxidative mechanisms involving different intermediate reactive species such as hydroxyl radical (•OH) and singlet oxygen ($^1O_2$), which are familiar reactive oxygen species (ROS) that can induce DNA strand breaks. The cleavage mechanism was studied by using scavengers that could inhibit the reactive oxygen species. For example, dimethyl sulfoxide (DMSO) and methanol were used as scavengers of hydroxyl radicals, while sodium azide was used as singlet oxygen scavenger. Plasmid pUC19 DNA was incubated with compound **2** in the presence of sodium azide, methanol and DMSO, respectively, and the results are shown in Fig. 5. It's clear that all three ROS scavengers exhibited inhibition towards DNA cleavage. That is to say, singlet oxygen and hydroxyl radicals act as ROS in DNA cleavage.

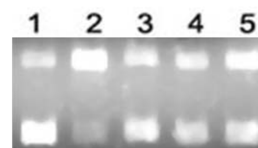

Fig. 5 Effect of "inhibitors" upon irradiations. Lane 1, DNA control; Lane 2, DNA with 23.8 μM of **2**; Lanes 3–5, DNA with 23.8 μM of **2** in the presence of NaN_3 (150.0 mM), MeOH (150.0 mM) and DMSO (150.0 mM), respectively.

Conclusions

In summary, we systematically studied the DNA binding abilities of the synthesized novel mono-anthryl and bis-anthryl compounds by comparing the binding constant, viscometric change and the DNA T_m change. We speculated that the mono-anthryl compound **1** binds to DNA *via* groove binding, while bis-anthryl compound **2** binds to DNA *via* a multiple binding mode that involves groove binding and partial intercalation. This is the fundamental reason for the good binding ability of **2**. The binding constant of compound **2** towards CT DNA is 100-fold larger than that of compound **1**. Compound **2** also shows excellent DNA photocleavage ability, which is much more efficient than the mono-anthryl compound. On the other hand, the binding constant of **1** towards CG sequences is nearly 36 times more that towards AT sequences. This sequence selectivity is more effective than other anthracene derivatives ever reported.

Experimental procedures

Materials and instrumentation

Mass Spectrometer (ESI-MS) and High Resolution Mass Spectrometer (HRMS) data were recorded on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer, respectively. The ^{1}H NMR and ^{13}C NMR spectra were measured on a Brucker AV II-400 MHz spectrometer or a Brucker-300 MHz spectrometer respectively and the δ scale in ppm referenced to residual solvent peaks or internal tetramethylsilane (TMS). Absorption spectra were recorded in phosphate buffer solution on a TU-1901 spectrophotometer or Hitachi U1900 spectrophotometer with a Polyscience temperature controller system (±0.1 °C). Fluorescence spectra were measured at room temperature in air by a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer and corrected for the system response. IR spectra were recorded on an FT-IR 16PC spectrometer. A mercury lamp-light filter assembly (12 W, 360 nm) was used for DNA photocleavage.

All chemicals and reagents were obtained commercially and used without further purification. *N*-*tert*-Butyloxycarbonyl-1,2-diaminoethane[21] and [4,7,10-tris(*tert*-butoxy-carbonyl)-1,4,7,

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10-tetraaza-cyclododecan-1-yl]acetic acid (Scheme 1, triBoc-cyclen-acetic acid) were synthesized according to the literature.[22] CT DNA which was purchased from Sigma was directly dissolved in water at a concentration of 1 mg/mL and stored at 4 °C. The ODNs were purchased from Beijing Sunbiotech Co. Ltd in a page-purified form. The concentrations were determined using the molar extinction coefficients $\varepsilon_{253\,nm}$ = 7400 cm^{-1} M^{-1} and $\varepsilon_{260\,nm}$ = 6000 cm^{-1} M^{-1} for poly(dC-dG)$_9$ and poly(dA-dT)$_9$, respectively. 0.1 M of phosphate buffer was prepared by mixing 1 M of aq. sodium dihydrogen phosphate and 1 M of aq. disodium hydrogen phosphate, then being diluted to 100 mL. Other phosphate buffers were prepared by diluting this solution and adding different amounts of sodium chloride. Storage solutions of compounds were prepared by dissolving different amounts of compounds **1** and **2** in water respectively and stored in the dark.

Preparation of the anthracene derivatives 1–9

[2-((Anthracen-9-ylmethyl)amino)ethyl]carbamic acid *tert*-butyl ester (3). 9-Anthraldehyde (5.0 mmol, 1.0 g) and *N*-*tert*-butyloxycarbonyl-1,2-diaminoethane (5.0 mmol, 0.80 g) were dissolved in methanol (70 mL) and refluxed for 2 h. After cooling to room temperature, $NaBH_4$ (10 mmol, 0.38 g) was added. After refluxing for 2 h, the solvent was removed under reduced pressure. The solid mixture was dissolved in ethyl acetate (150 mL), and washed with saturated $NaHCO_3$ solution (3 × 50 mL). The organic layer was washed with water (30 mL), brine (30 mL) successively and dried over anhydrous Na_2SO_4. After the solvent was removed under reduced pressure, the residue was purified by column chromatography (silica gel, ethyl acetate-petrol ether, 1 : 2, v/v) to yield the product as a light yellow solid **3**. Yield: 69%. Mp: 95–96 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.44 (9H, s, Boc-CH_3), 2.98–3.01 (2H, t, *J* = 11.6 Hz, NH-CH_2), 3.32–3.33 (2H, m, BocNH-CH_2), 4.74 (2H, s, Ar-CH_2), 4.96 (1H, s, Boc-NH), 7.45–7.56 (4H, m, 2-H, 7-H, 3-H, 6-H Anthr), 8.0–8.2 (2H, d, *J* = 8.4 Hz, 4-H, 5-H Anthr), 8.31–8.33 (2H, d, *J* = 8.8 Hz, 1-H, 8-H Anthr), 8.41 (1H, S, 10-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 28.3, 40.1, 45.2, 49.3, 78.9, 123.9, 124.8, 126.0, 127.1, 129.0, 130.1, 131.2, 131.4, 156.0; IR (KBr) ν: 1679, 1525, 1445, 1390, 1366, 1289, 1248, 1167, 1118, 1013, 882, 850, 728, 603, 533 cm^{-1}; MS (ESI): 351.3 [M+H]$^+$.

***N*-[2-((*tert*-Butyloxycarbonyl)amino)ethyl]-*N*-(anthracen-9-ylmethyl) glycine ethyl ester (4).** Compound **3** (3.0 mmol, 1.0 g) was dissolved in ethyl acetate (70 mL), K_2CO_3 (6.0 mmol, 0.80 g) and ethyl bromoacetate (3.3 mmol, 0.36 mL) was added. After refluxing for 24 h, the mixture was filtered. The filtrate was concentrated and purified by column chromatography (silica gel, ethyl acetate-petrol ether, 1 : 5, v/v) to yield the product as a light yellow solid **4**. Yield: 77%. Mp: 85–86 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.22–1.27 (3H, m, OCH_2-CH_3), 1.35 (9H, s, Boc-CH_3), 2.89–2.92 (2H, m, N-CH_2), 3.13–3.15 (2H, m, BocNH-CH_2), 3.35 (2H, s, CO-CH_2), 4.11–4.16 (2H, m, COO-CH_2), 4.82 (2H, s, Ar-CH_2), 4.93 (1H, s, Boc-NH), 7.45–7.55 (4H, m, 2-H, 7-H, 3-H, 6-H Anthr), 8.0–8.02 (2H, d, *J* = 8.8 Hz, 4-H, 5-H Anthr), 8.43–8.48 (3H, t, 1-H, 8-H, 10-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.1, 28.2, 37.8, 50.1, 52.0, 52.9, 60.3, 78.6, 124.5, 124.8, 125.9, 127.8, 128.9, 131.2, 155.8, 171.5; IR (KBr) ν: 1735, 1684, 1650, 1530, 1253, 1160, 1117, 729, 619 cm^{-1}; HR-MS (ESI): Calcd for $C_{26}H_{32}N_2O_4$ [M+Na]$^+$ 459.2260, found 459.1778.

***N*-(2-Aminoethyl)-*N*-(anthracen-9-ylmethyl) glycine ethyl ester dihydrochloride (5).** Compound **4** (2.0 mmol, 0.87 g) was dissolved in methanol (20 mL). An excess of HCl-methanol solution was added. After stirring overnight, the solvent was removed under reduced pressure to obtain the product as a salt. Yield: 99%. Mp: 163–165 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.61–0.64 (3H, m, OCH_2-CH_3), 3.43–3.47 (2H, m, N-CH_2), 3.99 (2H, s, NH_2-CH_2), 4.13 (2H, m, COO-CH_2), 4.61 (2H, s, CO-CH_2), 5.67 (2H, s, Ar-CH_2), 7.49 (2H, s, 2-H, 7-H, Anthr), 7.72–7.73 (2H, d, *J* = 7.6 MHz, 3-H, 6-H Anthr), 8.24 (1H, s, 10-H Anthr), 8.51–8.52 (2H, m, 4-H, 5-H Anthr), 8.86 (2H, s, 1-H, 8-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.9, 34.8, 50.6, 52.8, 56.0, 61.3, 124.7, 125.4, 127.1, 129.1, 129.9, 130.9, 131.6, 168.2; IR (KBr) ν: 3425, 2981, 2916, 1736, 1448, 1212, 735 cm^{-1}; MS (ESI): 337.4 [M-2HCl+H]$^+$.

Mono-anthryl compound 6. A CH_2Cl_2 (50 mL) solution of salt **5** (0.25 mmol, 0.10 g) was added Et_3N (0.63 mmol, 87 μL), triBoc-cyclen-acetic acid (Scheme 1, 0.25 mmol, 0.13 g) and *N*-hydroxybenzotriazole (HOBt, 0.28 mmol, 45 mg). After cooling to 0 °C by using an ice-water bath, DCC (0.30 mmol, 62 mg) was added slowly. The solution was stirred for 1 h at 0 °C and kept stirring at room temperature overnight. The suspension was filtered to remove the urea side product. After removing the solvent under reduced pressure, the mixture was dissolved in ethyl acetate (30 mL) and frozen for 5 h. The urea side product was filtered and the solvent was removed under reduced pressure again. The crude product was purified by column chromatography to yield the pure product as a white solid. Yield: 60%. Mp: 67–69 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.24–1.29 (3H, m, OCH_2-CH_3), 1.43–1.46 (27H, m, Boc-CH_3), 2.63–2.65 (6H, m, N-CH_2, CH_2 cyclen), 2.84 (2H, s, NHCO-CH_2-N), 3.16–3.53 (16H, m, NHCO-CH_2-N, EtOCO-CH_2, CH_2 cyclen), 4.14–4.20 (2H, m, COO-CH_2), 4.81 (2H, s, Ar-CH_2), 6.14 (1H, s, CO-NH), 7.45–7.57 (4H, m, 2-H, 7-H, 3-H, 6-H Anthr), 8.00–8.02 (2H, d, *J* = 8.4 Hz, 4-H, 5-H Anthr), 8.37 (1H, s, 10-H Anthr), 8.48–8.50 (2H, d, *J* = 8.8 Hz, 1-H, 8-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.0, 28.0, 28.3, 28.5, 36.5, 47.4, 48.7, 49.4, 50.2, 51.2, 53.7, 54.8, 57.9, 60.4, 79.1, 79.4, 124.5, 124.8, 126.0, 127.0, 127.9, 129.0, 129.0, 131.2, 131.3, 133.9, 155.3, 155.7, 169.8, 171.5; IR (KBr) ν: 3396, 2975, 2928, 1687, 1458, 1416, 1366, 1249, 1160, 736, 618 cm^{-1}; HR-MS (ESI): Calcd for $C_{65}H_{86}N_8O_{10}$ [M+H]$^+$ 849.5126, found 849.5130.

Mono-anthryl compound 1. Compound **6** (0.17 mmol, 0.15 g) was dissolved in methanol (20 mL). An excess of HCl-methanol solution was added. After stirring overnight, the solvent was removed under reduced pressure to obtain the product as a salt. Then the salt was dissolved in water (20 ml), and washed with CH_2Cl_2 (30 mL). After being basified by saturated $NaHCO_3$ solution, the solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was washed with brine (20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to obtain the product **1**. Yield: 40%. Mp: 42–44 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.26 (3H, m, OCH_2-CH_3), 2.43–2.50 (16H, m, CH_2 cyclen), 2.79–2.82 (2H, m, N-CH_2), 2.88 (2H, s, NHCO-CH_2-N), 3.22–3.23 (2H, m, CONH-CH_2), 3.70 (2H, s, EtOCO-CH_2), 4.20–4.26 (2H, m, COO-CH_2), 4.79 (2H, s, Ar-CH_2), 7.47–7.57 (4H, m, 2-H, 7-H, 3-H, 6-H Anthr), 8.00–8.02 (2H, d, *J* = 8.0 Hz, 4-H, 5-H Anthr), 8.45–8.46 (2H, d, *J* = 6.8 Hz, 1-H, 8-H Anthr), 8.49 (1H, s, 10-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.1, 36.5, 45.6, 45.9, 46.0, 47.2, 50.4, 51.2,

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52.2, 52.7, 53.3, 53.7, 58.9, 60.3, 60.5, 124.5, 124.6, 124.9, 126.0, 127.0, 127.5, 127.8, 128.8, 129.0, 129.4, 131.2, 133.9, 171.3, 171.5; IR (KBr) ν: 3430, 2925, 2853, 1729, 1647, 1449, 1382, 1116, 736, 618 cm^{-1}; HR-MS (ESI): Calcd for $C_{31}H_{44}N_6O_3$ $[M+H]^+$ 549.3553, found 549.3536.

***N*-[2-((*tert*-Butyloxyoxycarbonyl)amino)ethyl]-*N*-(anthracen-9-ylmethyl) glycine (7).** Compound **4** (1.0 mmol, 0.44 g) was dissolved in methanol (20 mL). 2 N NaOH (10 mL) was added. After stirring for 1 h at room temperature, the solvent was removed under reduced pressure. The solution was acidified to pH 3 by 2 N HCl, and then extracted by ethyl acetate (3 × 50 mL). The combined organic layer was washed successively by water (30 mL) and brine (30 mL). After being dried over anhydrous Na_2SO_4, the solvent was removed under reduced pressure to obtain the product **7**. Yield: 80%. Mp: 80–82 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.35 (9H, s, Boc-CH_3), 2.92–2.95 (2H, m, N-CH_2), 3.25 (2H, s, BocNH-CH_2), 3.44 (2H, s, CO-CH_2), 4.86 (2H, s, Ar-CH_2), 7.45–7.57 (4H, m, 2-H, 7-H, 3-H, 6-H Anthr), 8.00–8.02 (2H, d, J = 8.4 Hz, 4-H, 5-H Anthr), 8.36–8.38 (2H, d, J = 8.8 Hz, 1-H, 8-H Anthr), 8.46 (1H, S, 10-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 28.2, 38.2, 49.7, 52.2, 52.8, 59.8, 77.4, 125.0, 125.9, 127.4, 128.9, 129.9, 131.0, 131.1, 155.4, 173.2; IR (KBr) ν: 1716, 1633, 1531, 1363, 1274, 1247, 1165, 892, 737 cm^{-1}; MS (ESI): 407.4 $[M-H]^-$.

5,11-Bis(anthracen-9-ylmethyl)-7-oxo-2,5,8,11-tetraazatridecanedioic acid 1-*tert*-butyl, 13-ethyl ester (8). To a CH_2Cl_2 (50 mL) solution of salt **5** (1.0 mmol, 0.41 g) were added Et_3N (2.5 mmol, 0.35 mL), compound **7** (1.0 mmol, 0.41 g) and *N*-hydroxybenzotriazole (HOBt, 1.1 mmol, 0.18 g). After cooling to 0 °C by using an ice-water bath, DCC (1.2 mmol, 0.18 g) was added slowly. The solution was stirred for 1 h at 0 °C and kept stirring at room temperature overnight. The suspension was filtered to remove the urea side product. After removing the solvent under reduced pressure, the mixture was dissolved in ethyl acetate (30 mL) and frozen for 5 h. The urea side product was filtered and the solvent was removed under reduced pressure again. The crude product was purified by column chromatography to yield the pure product as a white solid. Yield: 70%. Mp: 67.5–69 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.21–1.28 (3H, m, OCH_2-CH_3), 1.35–1.36 (9H, m, Boc-CH_3), 2.67–2.70, 2.75–2.78 (4H, m, N-CH_2), 2.88 (2H, s, CO-CH_2-N), 3.00–3.02 (4H, m, CONH-CH_2), 3.28 (2H, s, EtOCO-CH_2), 4.11–4.18 (2H, m, COO-CH_2), 4.57 (2H, s, Ar-CH_2), 4.72 (2H, s, Ar-CH_2), 6.56 (1H, s, CO-NH), 7.37–7.43 (8H, m, 2-H, 7-H, 3-H, 6-H Anthr), 7.93–7.99 (4H, m, 4-H, 5-H Anthr), 8.30–8.40 (6H, m, 1-H, 8-H, 10-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.0, 28.1, 36.4, 38.0, 49.6, 50.8, 51.9, 52.8, 55.0, 56.1, 60.2, 78.7, 124.2, 124.3, 124.6, 124.7, 125.8, 125.9, 126.9, 127.8, 127.9, 128.6, 128.9, 129.0, 131.0, 131.1, 131.2, 133.8, 155.7, 170.8, 171.5; IR (KBr) ν: 3399, 2935, 1706, 1670, 1516, 1158, 1118, 732, 618 cm^{-1}; HR-MS (ESI): Calcd for $C_{45}H_{50}N_4O_5$ $[M+H]^+$ 727.3859, found 727.3873.

Bis-anthryl compound 9. Compound **8** (1.0 mmol, 0.73 g) was dissolved in methanol (20 mL). An excess of HCl-Methanol solution was added. After stirring overnight, the solvent was removed under reduced pressure to obtain the product as a salt. To a CH_2Cl_2 (50 mL) solution of this salt (0.68 mmol, 0.50 g) were added Et_3N (2.7 mmol, 0.37 mL), triBoc-cyclen-acetic acid (0.68 mmol, 0.36 g) and N-hydroxybenzotriazole (HOBt, 0.75 mmol, 0.12 g). After cooling to 0 °C by using an ice-water bath, DCC (0.82 mmol, 0.17 g) was added slowly. The solution was stirred for 1 h at 0 °C and kept stirring at room temperature overnight. The suspension was filtered to remove the urea side product. After removing the solvent under reduced pressure, the mixture was dissolved in ethyl acetate (30 mL) and frozen for 5 h. The urea side product was filtered and the solvent was removed under reduced pressure again. The crude product was purified by column chromatography to yield the pure product as a white solid. Yield: 50%. Mp: 80–81.5 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.18–1.28 (3H, m, OCH_2-CH_3), 1.41–1.46 (27H, m, Boc-CH_3), 2.62–2.70 (8H, m, N-CH_2, CH_2 cyclen), 2.91 (2H, s, NHCO-CH_2-N), 3.07 (2H, s, CONH-CH_2), 3.18–3.47 (16H, m, NHCO-CH_2-N, EtOCO-CH_2, CONH-CH_2, CH_2 cyclen), 4.09–4.14 (2H, m, COO-CH_2), 4.51 (2H, s, Ar-CH_2), 4.71 (2H, s, Ar-CH_2), 6.58 (1H, s, CO-NH), 7.37–7.45 (8H, m, 2-H, 7-H, 3-H, 6-H Anthr), 7.94–7.96 (4H, m, 4-H, 5-H Anthr), 8.31–8.41 (6H, m, 1-H, 8-H, 10-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.0, 28.3, 28.5, 36.5, 36.6, 47.5, 49.4, 50.0, 50.7, 51.5, 53.4, 56.5, 60.2, 60.3, 79.4, 124.3, 124.5, 124.7, 124.8, 125.9, 126.0, 127.0, 127.8, 127.9, 128.7, 129.0, 129.1, 131.1, 131.2, 133.9, 155.3, 155.9, 170.2, 170.6, 171.5; IR (KBr) ν: 3378, 2974, 1685, 1457, 1415, 1365, 1248, 1161, 735 cm^{-1}; HR-MS (ESI): Calcd for $C_{65}H_{86}N_8O_{10}$ $[M+H]^+$ 1139.6545, found 1139.6543.

Bis-anthryl compound 2. Compound **9** (0.12 mmol, 0.14 g) was dissolved in methanol (20 ml). An excess of HCl-methanol solution was added. After stirring overnight, the solvent was removed under reduced pressure to obtain the product as a salt. Then the salt was dissolved in water (20 mL), and washed with CH_2Cl_2 (30 mL). After being basified by saturated $NaHCO_3$ solution, the solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was washed with brine (20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to obtain the product **2**. Yield: 50%. Mp: 86–88 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.23–1.26 (3H, m, OCH_2-CH_3), 2.64–2.71 (20H, m, N-CH_2, CH_2 cyclen), 3.12 (8H, m, NHCO-CH_2-N, CONH-CH_2), 3.35 (2H, s, EtOCO-CH_2), 4.13 (2H, m, COO-CH_2), 4.43 (2H, s, Ar-CH_2), 4.75 (2H, s, Ar-CH_2), 6.65 (1H, s, CO-NH), 7.44 (8H, m, 2-H, 7-H, 3-H, 6-H Anthr), 7.94 (4H, m, 4-H, 5-H Anthr), 8.32–8.43 (6H, m, 1-H, 8-H, 10-H Anthr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.0, 36.6, 37.1, 45.1, 46.2, 47.2, 48.2, 50.1, 51.0, 51.3, 52.4, 52.7, 53.3, 53.5, 57.4, 59.6, 60.2, 124.4, 124.6, 124.8, 124.9, 125.8, 126.0, 126.9, 127.6, 127.7, 128.9, 129.0, 129.1, 131.0, 131.2, 131.3, 170.8, 171.4, 171.5; IR (KBr) ν: 3402, 2926, 2845, 1732, 1661, 1524, 1448, 1159, 1118, 735 cm^{-1}; HR-MS (ESI): Calcd for $C_{50}H_{60}N_8O_4$ $[M+H]^+$ 839.4972, found 839.4968.

Absorption titration

Phosphate buffer (5.0 mM, pH 7.4), with 50.0 mM NaCl was used in UV spectrophotometric titrations. CT DNA which was purchased from Sigma was used without further purification. The DNA concentration per nucleotide was determined by absorption spectroscopy, using the molar extinction coefficient 6600 M^{-1} cm^{-1} at 260 nm. In absorption titrations, the concentrations of the anthryl compounds were maintained and different amounts of DNA were added. All the experiments were performed at room temperature. The intrinsic binding constant (K_b) was obtained by fitting the data to eqn (1):[11]

$$[\text{DNA}]/(\varepsilon_a - \varepsilon_f) = [\text{DNA}]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

ε_a, ε_b and ε_f are the apparent, bound, and free extinction coefficients respectively. From a plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ *vs.* [DNA], the K_b was obtained from the ratio of the slope to the Y intercept.

Fluorescence titration

The steady-state fluorescence spectroscopic experiments were carried out on a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer with a 1 cm pathlength cuvette. In fluorescence titration experiments, the solution of the ligands was diluted to 10 μM by 5.0 mM phosphate buffer containing 50.0 mM NaCl, then stored in the dark for 1 h before use. Then the stored DNA solution was added and stirred in the dark for 10 min. The recorded fluorescence data were fitted to eqn (2),[23] where C_T is the concentration of the compound added, C_F is the concentration of the free compound, and I_o and I are the fluorescence intensities in the presence and absence of DNA, respectively. P is the ratio of the observed fluorescence quantum yield of the bound compound to the free compound. The value of P was obtained from a plot of I/I_o *vs.* 1/[DNA].

$$C_F = C_T(I/I_o - P)/(1 - P) \quad (2)$$

The concentration of bound compound C_B at any concentration was equal to $C_T - C_F$. A plot of r/C_F *vs.* r, where $r = C_B/[\text{DNA}]$, was constructed according to the modified Scatchard eqn (3) given by McGhee and von Hippel.[24] K_i is the intrinsic binding constant and n is the binding site size in base pairs.

$$r/C_F = K_i(1 - nr)[(1 - nr)/[1 - (n - 1)r]]^{n-1} \quad (3)$$

DNA melting experiments

DNA melting experiments were measured on a Hitachi U1900 spectrophotometer with a Polyscience temperature controller system (±0.1 °C), by monitoring the absorption of CT DNA (0.13 mM) at 260 nm in 5 mM phosphate buffer and 2.0 mM NaCl, pH 7.4, in the absence and presence of anthracene derivatives. The ratio of DNA and compounds was 25:1. The T_m was calculated by plotting temperature *versus* relative $\Delta A/\Delta T$.

Viscometric titration

Viscometric titrations were performed at 37 °C with a Ubblehode viscometer in phosphate buffer (10.0 mM, pH 7.4). CT DNA (0.2 mM bp) was used. The concentrations of anthryl compounds were varied, and the different flow times were measured by stopwatch. The plot of $(\eta/\eta_0)^{1/3}$ *vs.* r was obtained with the data, where η and η_0 are the flow time of anthryl compound bound DNA and DNA respectively, and r is equal to [compound]/[DNA].

Gel electrophoresis experiments

For gel electrophoresis experiments, supercoiled plasmid DNA (pUC 19) (5 μL, 0.025 g/L) in phosphate buffer (100.0 mM, pH 7.4) was treated with the anthryl compounds and diluted with phosphate buffer to a total volume of 17.5 μL. The mixture was irradiated with a 12 W mercury lamp (360 nm). The sample was analyzed by 1% agarose gel containing 1.0 g/ml ethidium bromide. Electrophoresis was carried out at 40 V for 30 min in TAE buffer. Bands were visualized by UV light and photographed followed by the estimation of the intensity of the DNA bands using a Gel Documentation System.

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