Synthesis and DNA cleavage activity of triazacrown-anthraquinone conjugates†

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1,4,7-Triazacyclononane (TACN), with DNA cleaving ability, was appended to anthraquinone *via* different spacers to construct the new compounds 1,8-[2,2′-(1,4,7-triazacyclonon)diethoxy] anthracene-9,10-dione hydrochloride (**1**) and 1,8-[2,2′-(1,4,7-triazacyclonon)dihexyloxy] anthracene-9,10-dione hydrochloride (**2**) as new agents for metal-free DNA cleavage. Fluorescence and CD spectroscopic studies suggest an intercalating DNA binding mode, and the apparent DNA binding constants of **1** and **2** are 3.93×10^7 and 6.07×10^7 M^{-1}, respectively. Compound **2**, bearing the longer spacer, exhibits the higher DNA binding ability. The apparent initial first-order rate constant (k_{obs}) of DNA cleavage promoted by **1** and **2** (0.05 mM) in physiological media are 0.077 ± 0.0028 and 0.123 ± 0.0027 h^{-1}, respectively. The 51-fold and 82-fold rate accelerations over parent TACN (the k_{obs} is 0.0015 ± 0.00003 h^{-1} (0.05 mM) under the same conditions) are due to the anthraquinone moiety of compounds **1** and **2** intercalating into the DNA base pairs *via* stacking interactions. ESI-MS analysis of the dinucleotide cleavage promoted by **1** and **2**, and radical scavenger inhibition studies suggest that the cleavage process is a hydrolytic mechanism.

Introduction

DNA recognition, binding, cleavage and modification, or cross-linking by small molecules, have attracted extensive interest due to their potential applications in the fields of molecular biological technology and drug development.[1] The transition metal complexes of macrocyclic polyamines as efficient cleaving agents of nucleic acids have been widely investigated.[2] However, their medicinal application is still limited due to the distinct toxicity of the free radicals generated in the oxidative cleaving processes of these metal complexes,[3] such as copper complexes. Hence, it is of great significance to develop small organic molecules to promote the cleavage of nucleic acids *via* a non-oxidative pathway.

Recently, metal-free DNA cleaving reagents have been put forward by Göbel and co-workers.[4] These compounds are thought of as safer agents for cleaving the P–O bond of phosphodiesters in nucleic acids, showing clinical potential. Many small organic molecules, such as guanidinium derivatives,[4,5] cyclodextrin derivatives,[6] dipeptides[7] and especially macrocyclic polyamines,[8] have also been used as cleaving agents of nucleic acids. The macrocyclic polyamines reported by Liang and co-workers, such as 1,7-dimethyl-1,4,7,10-tetraazacyclododecane (DMC), can cleave DNA *via* the hydrolysis pathway in physiological media (37 °C, pH 7.2) without the presence of metal ions.[8] Recently, we also developed a new artificial DNA cleaving agent *via* combining the triazacrown ether 1,4,7-triazacyclononane (TACN) with guanidinoethyl and hydroxyethyl side arms. The promoted kinetic data k_{max} of DNA cleavage *via* transphosphorylation disclosed *via* Michaelis–Menten fitting is ~ 0.160 h^{-1}, giving a 10^7-fold rate acceleration over uncatalyzed DNA.[9] This is a successful example of an azacrown ether derivative as an artificial nuclease for cleaving the phosphodiester bond of DNA *via* non-redox "metal-free catalysis". However, compared with natural nucleases such as staphylococal nuclease (SNase, the rate acceleration for the hydrolysis of the phosphodiester of DNA is up to 10^{16}-fold),[10] the efficiency of DNA cleavage promoted by the azacrown ether derivatives is still very low.

The anthraquinone group, as a fine intercalator of DNA, has been frequently adopted in certain anticancer drugs, such as doxorubicin, anthracyclines, mitoxantrone and anthrapyrazoles.[11] Teilla, Boseggia and co-workers reported that the compound formed by conjugating the *cis*,*cis*-triaminocyclohexane-Zn^{2+} complex (cleaving moiety) with anthraquinone (intercalating moiety) *via* an alkyl spacer led to a 15-fold increase in DNA cleavage efficiency when compared with the *cis*,*cis*-triamino-cyclohexane-Zn^{2+} complex without the anthraquinone moiety.[12] Yu and co-workers also reported that macrocyclic polyamine bis-anthracene conjugates showed higher DNA binding and photocleaving abilities than their corresponding mono-anthracene conjugates.[13a] All these reports suggest that the conjugation of the intercalating group to a normal DNA cleaving agent is an efficient strategy to develop DNA cleaving agents of enhanced DNA cleavage rate.

In this work, we report the design and synthesis of two triazacrown-anthraquinone conjugates linked by bis-alkyl spacers,

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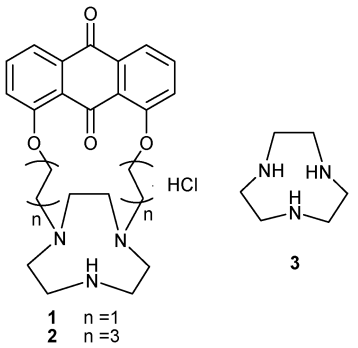


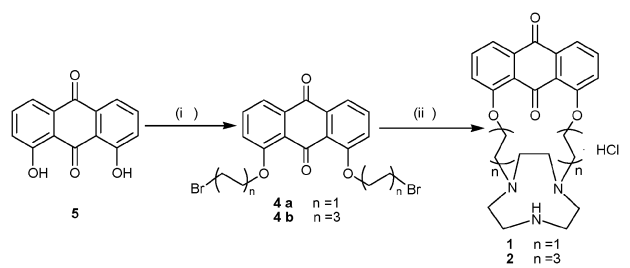
Scheme 1 Chemical structures of compounds **1**, **2** and **3**.

1,8-[2,2′-(1,4,7-triazacyclonon)diethoxy] anthracene-9,10-dione (**1**) and 1,8-[2,2′-(1,4,7-triazacyclonon)dihexyloxy] anthracene-9,10-dione (**2**) (Scheme 1). In these two compounds, the TACN moiety and the anthraquinone moiety are employed as DNA cleaving group and intercalating group, respectively. Moreover, the length of the alkyl spacers involved in the two compounds is adjusted to altering their DNA binding and cleaving ability. Besides the calf thymus DNA binding behavior of the two compounds investigated by fluorescence and circular dichroism (CD) spectroscopy, the plasmid pUC 19 DNA cleaving behavior of the two compounds is assessed *via* agarose gel electrophoresis. For comparison, the DNA cleavage activity of the parent TACN (Scheme 1) is studied as well.

Results and discussion

Synthesis of compounds 1 and 2

Synthesis of target compounds **1** and **2** were achieved *via* a two-step reaction from 1,8-dihydroxyanthracene-9,10-dione (**5**) (Scheme 2). Therefore, reacting **5** with 1,2-dibromoethane or 1,6-dibromohexane in the presence of base afforded compounds **4a** or **4b**. The crude compounds were purified by silica gel column chromatography using petroleum ether–dichloromethane (v/v = 1 : 1) as eluent and recrystallized from EtOAc. Purified compounds **4** were transformed into **1** or **2** *via* nucleophilic substitution by TACN in anhydrous chloroform in the presence of base. ^{1}H NMR, ^{13}C NMR and ESI-MS analytical characterization was carried out for compounds **1**, **2** and all intermediates (Fig. S1–S12, ESI†). In the ^{13}C NMR spectra of compounds **1** and **2**, the signals at δ 183.1, 186.2 and 182.2, 183.5 are assigned to the signals of the carbonyl carbon atoms. In the ESI mass spectra, the signals at m/z 422.17 and 444.25 can be assigned as $(M - Cl^-)^+$ (calc. 422.20) and $(M - HCl + Na)^+$ (calc. 444.18) of **1**, respectively. The signals at m/z 534.33 and 556.33 correspond to $(M - Cl^-)^+$ (calc. 534.33) and $(M - HCl + Na)^+$ (calc. 556.32) of **2**, respectively.



Scheme 2 Synthesis of compounds **1** and **2**. Reagents and conditions: (i) 1,2-dibromoethane or 1,6-dibromohexane, K_2CO_3, N_2, 110 °C, 72 h. Yield: 55% for **4a** and 80% for **4b**; (ii) (1) 1,4,7-triazacyclonon, K_2CO_3, anhydrous chloroform, reflux, 4 h; (2) r.t., ethanol, conc. HCl. Yield: 30% for **1** and 24% for **2**.

DNA binding assays

The DNA binding of compounds **1** and **2** is essential for DNA cleavage. Therefore, the DNA binding behaviors of **1** and **2** to calf thymus DNA (CT-DNA) have been studied by using fluorescence and CD spectroscopy.

Fluorescence spectroscopic studies. The CT-DNA binding ability of the two compounds was studied by evaluating the fluorescence emission intensity of the ethidium bromide (EB)-DNA system upon the addition of the two compounds, respectively. Normally the emission intensity of the EB-DNA system undergoes a distinct reduction when the intercalated EB is replaced by the investigated compounds. The fluorescence quenching effect of EB bound to DNA induced by the addition of **2** and **1** is shown in Fig. 1 and Fig. S13 (ESI†), respectively, according to the fluorescence intensity at 604 nm (λ_{ex} = 530 nm) of EB in the bound form. Under the same conditions, the fluorescence of compounds **1** and **2** is very weak when measured in the DNA system without EB (Fig. S24†).

The relative binding propensity to CT-DNA of the two compounds was determined by fitting the emission intensity to the classical Stern–Volmer equation

$$I_0/I = 1 + Kr \quad (1)$$

where I_0 and I are the fluorescence intensities in the absence and the presence of the quencher, respectively, K is the linear Stern–Volmer quenching constant, dependent on the ratio of r_{bE} (the ratio of $[EB]_{bound}$ to [DNA]), and r is the ratio of $[quencher]_{total}$ to [DNA].[14] Fig. 2 shows the plot of I_0/I *vs.* [quencher]/[DNA], and the quenching constant K is given by

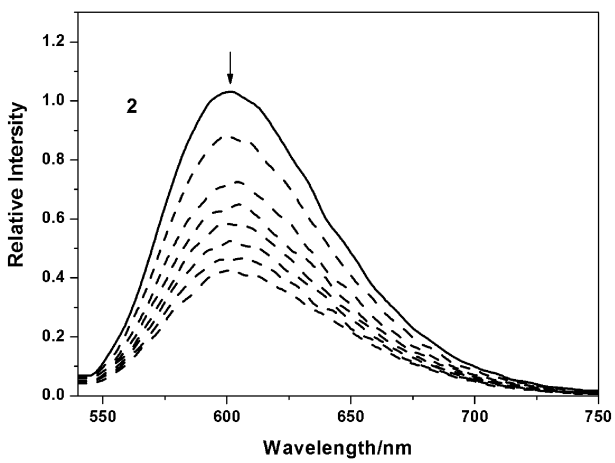


Fig. 1 Emission spectra of EB bound to DNA in the absence (—) and presence (---) of **2** (r = 0, 0.05, 0.07, 0.10, 0.13, 0.15, 0.21, 0.23, from top to bottom), r = [compound]/[CT-DNA], [CT-DNA] = 0.039 mM, [EB] = 3.9 μM, λ_{ex} = 530 nm.

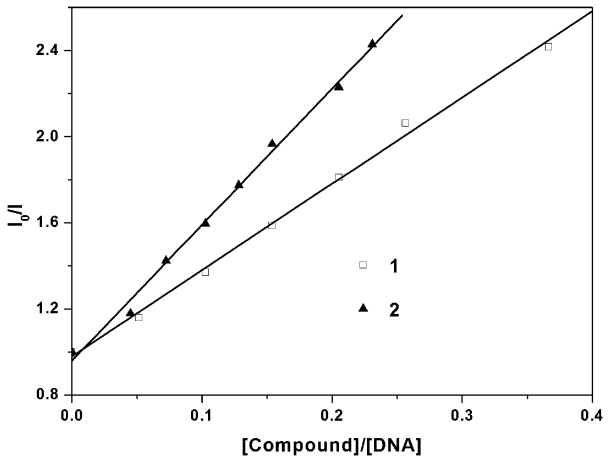


Fig. 2 Stern–Volmer quenching plots of EB bound to DNA by **2** (▲) and **1** (□), The experiments were conducted by adding 0–1.43 μM **2** or **1** to the EB-bound CT-DNA solution in 5 mM Tris-HCl buffer (pH 7.0).

the gradient of the slope. The quenching constant K values obtained for **1** and **2** are 4.01 ± 0.11 and 6.32 ± 0.18, respectively, much greater than that observed for TACN (0.053 ± 0.010).[2a] These results demonstrate that anthraquinone-based compounds **1** and **2** possess much better DNA binding abilities than the free macrocyclic polyamine, TACN. Moreover, compound **2** exhibits a slightly higher affinity to DNA than compound **1**.

The apparent binding constant (K_{app}) is also calculated from the equation

$$K_{EB}[EB] = K_{app}[compound] \quad (2)$$

where the compound concentration is the value at a 50% reduction of the fluorescence intensity of EB and K_{EB} = 1.0×10^7 M^{-1} ([EB] = 3.9 μM).[15a] The K_{app} values for **1** and **2** are 3.93×10^7 and 6.07×10^7 M^{-1}, respectively. The significantly high K_{app} value for the two compounds might be correlated to the presence of anthraquinone rings

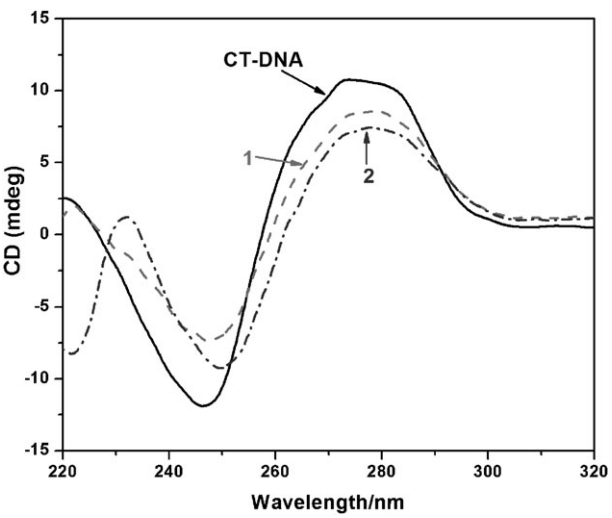


Fig. 3 CD spectra of CT-DNA (0.129 mM) in black and the interaction with **1** and **2** at ratio of [compound]/[DNA] = 0.4. All the spectra were recorded in 5 mM Tris-HCl buffer at pH 7.0.

in their chemical structures. Normally, the binding constants can be adopted to evaluate the DNA-binding modes: a value above 10^6 M^{-1} is an indication of intercalation (ethidium and daunomycin bind DNA with an affinity over 10^6 M^{-1}), while values in the range 10^4–10^6 M^{-1} imply the groove binding mode.[16] Therefore the DNA binding of compounds **1** and **2** could be accomplished *via* the intercalation binding mode. In fact, the anthraquinone rings are normal in reported DNA intercalating agents.[12,15b–g] Besides, according to the fluorescence changes, both conjugates **1** and **2** exhibit almost equal affinity for the ethidium bromide (EB)-DNA system. This is because both have the same intercalating group, anthraquinone rings, which play a fundamental role in the intercalation.

The apparent binding constant (K_{app}) of **1** and **2** to calf thymus DNA in the presence of various concentrations of sodium chloride was investigated in the EB-DNA system in 5 mM Tris-HCl buffer (pH 7.0) at room temperature. The K_{app} changing with the ionic strength of the solvent medium expressed as a total positive ion concentration, [M^+], is shown in Table S1 (ESI†). The effect of ionic strength on the apparent binding constant (K_{app}) is small at low concentrations of sodium chloride, which implies that the compounds bind to DNA mainly by intercalation rather than by electrostatic interactions. The apparent binding constant decreased gradually as the ionic strength increased when the concentration of sodium chloride was more than 10 mM. The addition of a relatively high concentration of Na^+ resulted in a decrease in the negative charge density of DNA. The DNA double helix in solution becomes tighter and it becomes difficult for compounds intercalating to DNA.[13b]

Circular dichroism (CD) studies. CD spectroscopy is a useful technique to monitor the conformation alteration of nucleic acids upon complex formation, since the positive band due to base stacking (275 nm) and the negative band due to right-handed helicity (248 nm) are quite sensitive to the interaction between DNA and small molecules.[17] The simple groove binding mode and electrostatic interactions between small molecules and DNA normally induce little or no perturbation of the base stacking and helicity bands, while the intercalation mode enhances the intensities of both bands *via* stabilizing the right-handed B conformation of CT-DNA, just as observed for the classical intercalator methylene blue.[18]

In the CD spectrum of CT-DNA treated with **1** or **2** ([compound]/[DNA] = 0.4 : 1) (Fig. 3), the positive band (~ 275 nm) of CT-DNA decreases in intensity upon the addition of **1** or **2**, while the negative band (~ 247 nm) undergoes an obvious reduction (Fig. 3). Moreover, a bathochromic shift to 250 nm of the negative band is observed upon the addition of **2**. The results suggest that **1** and **2** could unwind the DNA helix and reduce the helicity possibly due to the partial intercalation of **1** or **2** into the DNA base-pairs.[19] The larger decrement in the CD bands induced by **2** than **1** implies that **2** is more effective in perturbing the secondary structure of DNA at the same concentration because compound **2** has a longer spacer than **1**, resulting in a stronger interaction between the triazacrown subunit and the phosphate backbone.[12]

From the results of the fluorescence and CD spectroscopic studies, it is concluded that compound **2** binds more strongly to DNA than **1**, and the DNA binding constants of **1** and **2** indicate that these compounds bind with DNA through the intercalation binding mode.

DNA cleavage activity

The cleavage reaction of supercoiled pUC 19 DNA (0.025 mM bp) promoted by **1** and **2** was investigated in the dark at 37 °C in 50 mM Tris-HCl buffer with a total volume of 15 μL. The results indicated that supercoiled DNA (Form I) could be degraded to relaxed circular DNA (Form II) in the presence of **1** and **2**.

pH-dependence of the promoted DNA cleavage. Incubation of supercoiled pUC 19 DNA with compounds **1** or **2** for 16 h at 37 °C resulted in a different extent of DNA cleavage, depending on the pH of the buffer. Fig. S14 (the agarose gel electrophoretograms, ESI†) shows the decrease in supercoiled pUC 19 DNA and the appearance of relaxed DNA. Fig. 4 presents the pH-dependence profile, which indicates that the DNA cleavage ability of **2** is stronger than that of **1**, and the optimal pH for DNA cleavage in the presence of **1** or **2** in 50 mM Tris-HCl at 37 °C is pH 7.0–7.5. This accords with the result reported by Liang and co-workers.[8] They found that macrocyclic polyamines such as 1,7-dimethyl-1,4,7,10-tetraazacyclododecane (DMC) can cleave DNA *via* the hydrolysis pathway and that the optimal pH for DNA cleavage is 7.25. Therefore, pH 7.25 was chosen for the following agarose gel electrophoresis assays.

Concentration dependence assays of DNA cleavage promoted by 1 and 2. Reactions that lead to the formation of relaxed circular DNA from supercoiled DNA over various concentrations of **1** or **2** (6.7–40 μM) and constant DNA concentration (25 μM, bp) were carried out for 16.0 h at 37 °C (the agarose gel electrophoretograms are shown in Fig. S15, ESI†). Under the experimental conditions, form II was observed, even at low

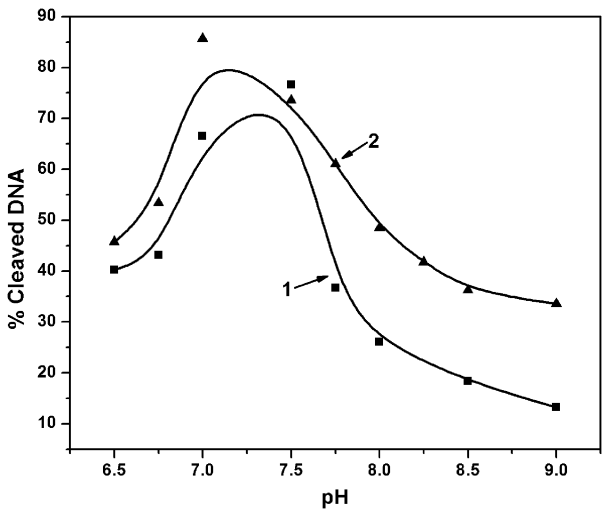


Fig. 4 pH-dependent profile for pUC DNA (0.025 mM bp) cleavage promoted by 0.033 mM **1** (■) and **2** (▲) in different pH buffers. The incubation time was 16 h at 37 °C.

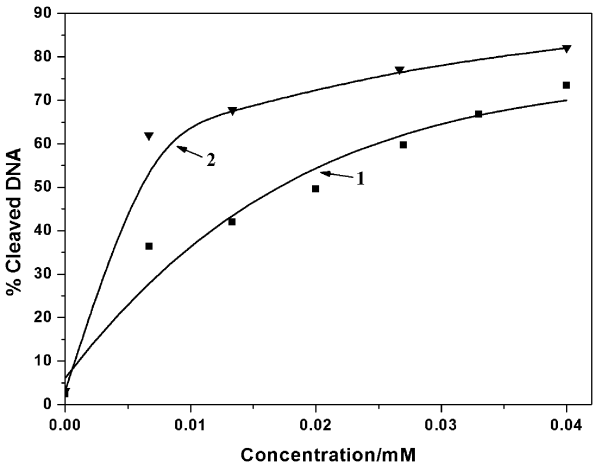


Fig. 5 Plot of % cleaved DNA *vs.* different concentrations of **1** (■) and **2** (▼). The reactions were carried out at 37 °C in 50 mM Tris-HCl buffer (pH 7.25).

concentrations, and no other forms were observed. Fig. 5 shows the plots of % cleaved DNA *vs.* different concentrations of **1** and **2**, which indicates that the DNA cleavage activities of **1** and **2** are increased with an increase in their concentrations. However, further increases of the concentrations of **1** and **2** (larger than 0.05 mM) could lead to the formation of relatively high molecular weight DNA–**1** (or –**2**) complexes. Consequently DNA is stuck in the well (the agarose gel electrophoretogram is shown in Fig. S16, ESI†).

For comparison, the DNA-cleaving behaviors of compounds **4a** and **4b** (0.027 mM), lacking TACN, were also investigated. It was found that the promotion of DNA cleavage induced by these two compounds is very minor (Table S2, ESI†), similar to the result reported by Teilla, Boseggia and co-workers.[12]

Kinetic assays. The kinetics of pUC 19 DNA degradation have been studied. Fig. 6a shows the agarose gel electrophoretogram of the supercoiled plasmid DNA cleavage promoted by **2** (0.04 mM) in Tris-HCl buffer (pH 7.25) at 37 °C into nicked forms. The rate of conversion of form I to form II increases with the increase reaction time. The time course plot (Fig. 6b) indicates that the extent of supercoiled DNA cleavage varies exponentially with the reaction time, giving pseudo first-order kinetics (the first-order rate constant is given by the gradient of the slope) with an apparent initial first-order rate constant (k_{obs}) of 0.113 ± 0.0071 h^{-1}. Then, the apparent initial first-order rate constants of DNA cleavage reactions promoted by a series of various concentrations of **2** and **1** under the similar conditions, as described above, are summarized in Table S2 (ESI†). Fig. 7 shows the kinetics profiles of the supercoiled DNA cleavage at various concentrations of **2** and **1**. The k_{obs} promoted by **1** and **2** (0.05 mM) in physiological media are 0.077 ± 0.0028 and 0.123 ± 0.0027 h^{-1}, respectively (Table S3, ESI†).

As a comparison, the DNA-cleaving behavior of parent TACN, without the anthracenedione moiety, was also investigated under the same conditions, and showed a low cleavage activity (k_{obs} was 0.0015 ± 0.00003 h^{-1} (0.05 mM) in Tris-HCl/NaCl buffer at 37 °C) (Fig. S18 and Table S4, ESI†).

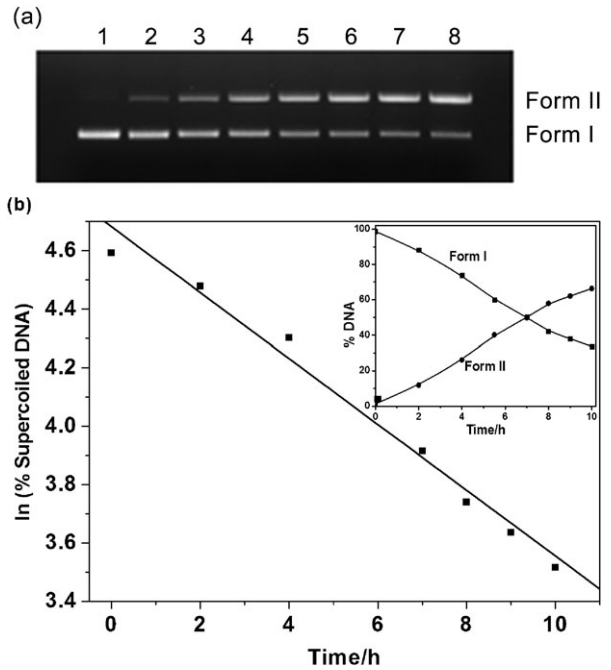


Fig. 6 Time course of pUC19 DNA (0.025 mM bp) cleavage promoted by **2** (0.040 mM). (a) Agarose gel (1%) of the time-variable reaction products: lanes 1–8, reaction times of 0, 2.00, 4.00, 5.50, 7.00, 8.00, 9.00 and 10.00 h, respectively. (b) Plot of ln (% supercoiled DNA) *vs.* reaction time. The inset of Fig. 6b is the plot of % DNA *vs.* time. The reactions were carried out at 37 °C in 50 mM Tris-HCl/10 mM NaCl buffer (pH 7.25).

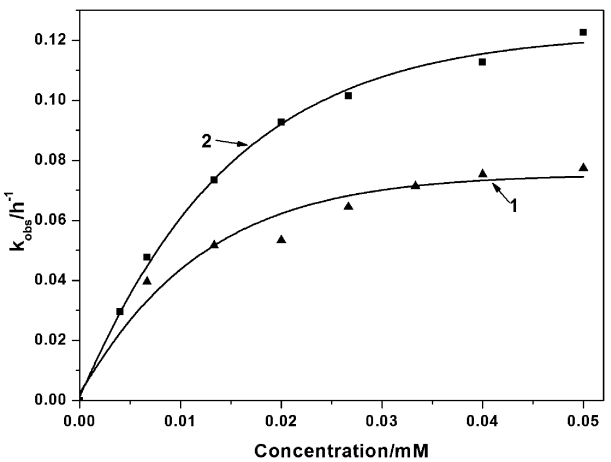


Fig. 7 Kinetics plot of k_{obs} *vs.* various concentrations of **2** (■) and **1** (▲).

The cleavage efficiency of compounds **1** and **2** gave 51- and 82-fold rate accelerations over **3**, respectively, which indicates that the anthraquinone moiety effectively increases the DNA cleavage activity.

Furthermore, kinetic data for DNA cleavage promoted by **1** and **2** show that **2**, with C6 spacers, has a rate acceleration over **1**, with C2 spacers. In the two compounds, the TACN moiety and the anthraquinone moiety are employed as DNA cleaving group and intercalating group, respectively. Moreover, the length of the alkyl spacers involved in the two compounds is adjusted to alter their DNA binding and cleaving abilities. After intercalation of the anthraquinone moiety, the spacer must be free to fold in such a way as to position the TACN moiety close to a phosphate group, and too-short a spacer may prevent correct folding. This accords with the result reported by Teilla, Boseggia and co-workers.[12] They investigated a series of conjugates, a *cis*,*cis*-triamino-cyclohexane (TACH) Zn^{2+} complex and an anthraquinone intercalator linked by alkyl spacers of different lengths, and demonstrated that the length and flexibility of the spacer played a fundamental role in the interaction between the catalytic subunit and the phosphate backbone.

Studies on mechanism of DNA cleavage

The anthracenedione moiety is known to undergo redox processes, which could directly produce cytotoxic effects.[20] To verify if reactive oxygen species (ROS) are, at least in part, responsible for the cleavage of DNA promoted by compounds **1** and **2**, reactions were carried out in the presence of typical scavengers for singlet oxygen (NaN_3, 10 mM), for superoxide (KI, 10 mM) and for hydroxyl radicals (1 mM DMSO and 1 mM *t*-BuOH) (Fig. S19 and Table S5, ESI†).[21a] Fig. 8 and Fig. S20† show that there is no evident inhibition effect on the DNA cleavage in the presence of all the scavengers (NaN_3, DMSO, *t*-BuOH and KI), which rules out the involvement of these reactive oxygen species, at least in a free and diffusible form. Therefore, DNA cleavage promoted by compounds **1** and **2** might occur by a non-oxidative pathway.

Mechanistic profiles of DNA cleavage by **1** and **2** were also evaluated in the presence of excess EDTA to scavenge adventitious transition metal ions (Fig. S19(c) and Table S6, ESI†). The inhibition of DNA cleavage was very small, which rules out the involvement of adventitious transition metal ions.

To further study the pathway of DNA cleavage promoted by **1** and **2**, dinucleotide adenylyl(3′-5′)phosphoadenine (ApA) was used as the nucleic acid mimic. ApA (0.10 mM) and **1**

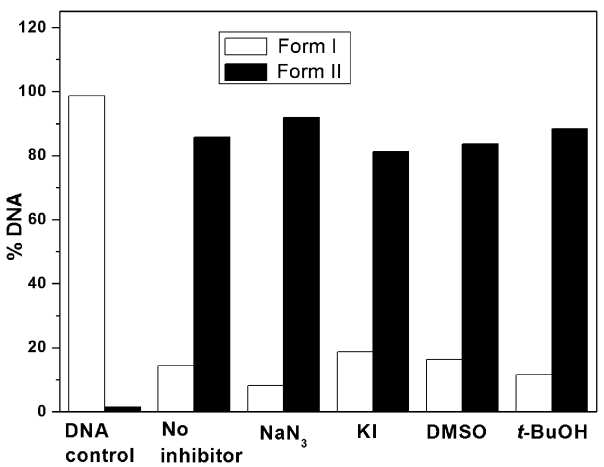


Fig. 8 Histogram representing of pUC19 plasmid DNA (0.025 mM bp) cleaved by **2** (0.04 mM) in the presence of standard radical scavengers for singlet oxygen (NaN_3, 10 mM), for superoxide (KI, 10 mM) and for hydroxyl radicals (1 mM DMSO and 1 mM *t*-BuOH), incubated for 18 h at 37 °C in pH 7.25 buffer (50 mM Tris-HCl/10 mM NaCl) (the agarose gel electrophoretogram is shown in Fig. S19(a), ESI†).

(0.05 mM) or **2** (0.05 mM) were dissolved in de-ionized water (1 : 1), and after 16 h equilibration time at 37 °C, ESI-MS analysis was carried out. In the ESI-MS spectrum of ApA treated with **2** (Fig. S21(a), ESI†), the signals at *m*/*z* 268.08 and 345.99 show the ApA cleavage products A (adenosine) ($[A + H]^+$, calc. 268.10) and AMP (adenosine monophosphate) ($[AMP - H]^-$, calc. 346.06); no sign of ApA was found. Under the same conditions, the ESI-MS spectrum of ApA treated with **1** (Fig. S21(b), ESI†) showed not only the signal of A and AMP, but also signs of ApA ($[ApA- H]^-$; *m*/*z* 595.17, calc. *m*/*z* 595.14). As a control experiment, an ESI-MS analysis of ApA alone was also carried out under the same conditions (Fig. S21(c), ESI†), showing only the signal of ApA and no sign of A or AMP. The generation of adenosine and AMP indicates that the phosphodiester bonds of ApA were cleaved by **1** or **2** *via* the hydrolysis pathway.[21b] Therefore, similar to ApA, the hydrolysis pathway is the likely mechanism for DNA cleavage promoted by **1** and **2**.

Conclusions

In conclusion, 1,8-[2,2′-(1,4,7-triazacyclonon)diethoxy]anthracene-9,10-dione hydrochloride (**1**) and 1,8-[2,2′-(1,4,7-triazacyclonon)-dihexyloxy]anthracene-9,10-dione hydrochloride (**2**) as metal-free DNA cleaving reagents were prepared. Fluorescence and CD spectroscopic studies suggest an intercalating DNA binding mode. Kinetic data of DNA cleavage promoted by **1** (0.05 mM) and **2** (0.05 mM) under physiological conditions give k_{obs} of 0.077 ± 0.0028 and 0.123 ± 0.0027 h^{-1}, respectively, which shows that **1** and **2** give 51- and 82-fold rate accelerations over parent TACN, respectively, due to the anthraquinone moiety of these compounds intercalating into the DNA base-pairs *via* stacking interactions. Furthermore, **2**, with C6 spacers, has a rate acceleration over **1**, with C2 spacers. ESI-MS analyses and radical scavenger inhibition studies suggest that the DNA cleavage promoted by **1** and **2** in physiological conditions is by a hydrolytic mechanism.

Experimental

Materials

All reagents and chemicals, purchased from commercial sources, were of analytical grade and used without further purification. pUC 19 plasmid DNA was purchased from TaKaRa Biotechnology (Dalian) Co. Ltd., and the purity was checked by agarose gel electrophoresis and their concentration was determined by UV spectroscopy using the extinction coefficient appropriate for double-stranded DNA (1.0 OD_{260} = 50 μg mL^{-1}). Agarose was from Oxoid Limited of Basingstoke (UK). Ethidium bromide (EB) was from Amresco. Inc., and tris(hydroxymethyl)aminomethane (Tris-Base) was from Robiot Co. Ltd. Dinucleotide (ApA) was purchased from Sigma-Aldrich. Bromophenol blue, glycerol and ethyl diamine tetraacetic acid (EDTA) were commercially available. De-ionized water was obtained by an ionized column from double distilled water. All solvents were purified by standard procedures. All aqueous solutions were prepared from de-ionized water.

The stock solution of CT-DNA (stored at 4 °C and used for not more than 2 d) was prepared in 5 mM Tris-HCl/10 mM NaCl in water, pH 7.0. The concentration of the CT-DNA was determined according to its absorption intensity at 260 nm with a known molar extinction coefficient value of 6600 M^{-1} cm^{-1}. The ratio of the UV absorbance at 260 and 280 nm, A_{260}/A_{280} = 1.8–1.9, indicated that the DNA was sufficiently free of protein.[22]

Apparatus

^1H NMR and ^{13}C NMR data were recorded on a Brucker AM 300 spectrometer (Germany). Mass spectra were obtained on an electrospray mass spectrometer (LCQ, Finnigan). Elemental analyses (C, H, N) were performed on a VARIO EL instrument. The pH value was confirmed by ORION868 pH meter with an Ag/AgCl electrode as the reference electrode in saturated KCl solution at room temperature. Agarose gel electrophoresis was conducted with a DYY-5 electrophoresis apparatus. Bands were visualized by UV light and photographed using a DigiDoc-It gel imaging and documentation system (version 1.1.23, UVP, Inc. Unpland, CA). The intensity of the DNA bands was estimated by TotalLab image analysis software (version 2.01).

Synthesis of the compounds

1,4,7-triazacyclononane (3). 1,4,7-Triazacyclononane (TACN) was prepared according to the reported procedure.[23] ^1H NMR (300 MHz, $CDCl_3$) δ: 2.00 (s, 3H, 3 × NH), 2.79 (s, 12H, 6 × NCH_2); ^{13}C NMR (300 MHz, D_2O) δ: 41.2 (CH_2).

1,8-Bis(2-bromoethoxy)anthraquinone (4a). 1,8-Dihydroxy-anthraquinone (2.00 g, 8.3 mmol), anhydrous potassium carbonate (14.00 g, 101.3 mmol) were added to 1,2-dibromoethane (40 mL, 464 mmol) and the mixture stirred at 110 °C under an atmosphere of dry N_2. A color change from orange to deep purple was observed. After 72 h, the reaction mixture was cooled and filtered, and the residue was washed with $CHCl_3$ (3 × 10 mL). All of the organic layers were merged and the solvent was removed under vacuum. Column chromatography (silica gel, petrol ether-dichloromethane, 1 : 1, v/v) followed by recrystallization from EtOAc afforded **4a** (2.09 g, 55%) as long orange needles. mp 154–156 °C; ^1H NMR (300 MHz, $CDCl_3$) δ: 3.78 (t, *J* = 6.7 Hz, 4H, 2 × CH_2Br), 4.44 (t, *J* = 6.7 Hz, 4H, 2 × OCH_2), 7.33 (d, *J* = 8.2 Hz, 2H, 2 × ArH), 7.61–7.67 (m, 2H, 2 × ArH), 7.91 (d, *J* = 7.7 Hz, 2H, 2 × ArH); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 28.8 (CH_2Br), 70.4 (OCH_2), 120.5 (Ar C), 121.6 (Ar C), 125.2 (Ar C), 133.9 (Ar C), 134.9 (Ar C), 157.9 (Ar C), 182.0 (C═O), 183.5 (C═O); Anal. calc. for $C_{18}H_{14}Br_2O_4$: C, 47.61; H, 3.11. Found: C, 47.51; H, 3.13%.

1,8-Bis(6-bromohexyloxy)anthraquinone (4b). Compound **4b** was synthesized in a similar procedure to compound **4a** using 1,8-dihydroxyanthraquinone (2.00 g, 8.3 mmol), anhydrous potassium carbonate (10.35 g, 74.9 mmol), 1,6-dibromohexane (40 mL, 260 mmol). A orange solid (3.77 g, 80%) was obtained. mp 62–63 °C; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.53–1.69 (m, 8H, 4 × CH_2), 1.89–1.99 (m, 8H, 4 × CH_2), 3.45 (t, *J* = 6.8 Hz, 4H, 2 × CH_2Br), 4.14 (t, *J* = 6.4 Hz, 4H, 2 × OCH_2), 7.28 (d, 2H, 2 × ArH), 7.57–7.63 (m, 2H, 2 × ArH),

7.82 (d, J = 7.7 Hz, 2H, 2 × ArH); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 25.1 (CH_2), 27.8 (CH_2), 28.9 (CH_2), 32.7 (CH_2), 34.0 (CH_2Br), 69.4 (OCH_2), 118.8 (Ar C), 119.4 (Ar C), 124.5 (Ar C), 133.6 (Ar C), 134.8 (Ar C), 158.7 (Ar C), 182.0 (C═O), 184.1 (C═O); Anal. calc. for $C_{26}H_{30}Br_2O_4$: C, 55.14; H, 5.34. Found: C, 55.11; H, 5.36%.

1,8-[2,2′-(1,4,7-triazacyclonon)diethoxy] anthracene-9,10-dione hydrochloride (1). Compound **3** (0.42 g, 3.3 mmol) was dissolved in 5 mL dry chloroform, and to this solution was added 6.70 g of K_2CO_3 (6.70 g, 48.5 mmol). A dry chloroform (15 mL) solution of 1,8-bis(2-bromoethoxy)anthraquinone (0.30 g, 0.66 mmol) was then added dropwise with stirring to this suspension. The mixture was refluxed for 4 h, cooled to room temperature and then filtered and washed with chloroform. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in ethanol (5 mL) and standing for 4 h after concentrated HCl (10 drops) was added dropwise. The mixture was then filtered and the crude product was dissolved in water (30 mL), and the pH adjusted to 11 using a 1 M sodium hydroxide solution. The desired product was extracted into chloroform (30 mL × 3). The organic layer was then dried over sodium sulphate and evaporated to dryness *in vacuo*. Ethanol (5 mL) and concentrated HCl (2 mL) was added. Followed by filtration, the desired product **1** was obtained (90 mg, 30%). ^{1}H NMR (300 MHz, D_2O) δ: 3.38–3.58 (m, 16H, 8 × NCH_2), 4.36–4.38 (m, 4H, 2 × OCH_2), 7.31–7.34 (m, 2H, 2 × ArH), 7.52–7.59 (m, 4H, 4 × ArH); ^{13}C NMR (300 MHz, D_2O) δ: 42.7 (CH_2NH), 48.1 (NCH_2), 52.3 (NCH_2), 58.6 (NCH_2), 66.9 (OCH_2), 121.4 (Ar C), 123.9 (Ar C), 125.5 (Ar C), 133.0 (Ar C), 136.3 (Ar C), 157.8 (Ar C), 183.1 (C═O), 186.2 (C═O); ESI-MS m/z 422.17 (M − Cl^-. $C_{24}H_{28}N_3O_4$ requires 422.20), 444.18. Anal. calc. for $C_{24}H_{28}N_3O_4Cl$: C, 62.95; H, 6.16; N, 9.18. Found: C, 63.05; H, 6.18; N 9.15%.

1,8-[2,2′-(1,4,7-triazacyclonon)dihexyloxy]anthracene-9,10-dione hydrochloride (2). Compound **2** was synthesized in a similar procedure to compound **1** using 1,4,7-triazacyclononane (**3**) (0.42 g, 3.3 mmol), potassium carbonate (6.70 g, 48.5 mmol) and 1,8-bis(6-bromohexyloxy)anthraquinone (0.35 g, 0.62 mmol). A tan solid (85.0 mg, 24%) was obtained. ^{1}H NMR (300 MHz, D_2O) δ: 1.11–1.63 (m, 16H, 8 × CH_2), 2.78–3.34 (m, 16H, 8 × NCH_2), 3.83 (m, 4H, 2 × OCH_2), 7.03–7.29 (m, 6H, 6 × ArH); ^{13}C NMR (300 MHz, D_2O) δ: 25.5 (CH_2), 26.4 (CH_2), 28.3 (CH_2), 35.3 (CH_2), 42.0 (CH_2NH), 44.5 (NCH_2), 48.7 (NCH_2), 57.2 (NCH_2), 62.5 (NCH_2), 69.3 (OCH_2), 118.1 (Ar C), 119.7 (Ar C), 122.7 (Ar C), 133.4 (Ar C), 134.1 (Ar C), 158.2 (Ar C), 182.2 (C═O), 183.5 (C═O); ESI-MS m/z 534.33 (M-Cl^-. $C_{32}H_{44}N_3O_4$ requires 534.33), 556.33. Anal. calc. for $C_{32}H_{44}N_3O_4Cl$: C, 67.41; H, 7.78; N, 7.37. Found: C, 67.22; H, 7.81; N 7.41%.

Photophysical properties of the compounds

UV-visible spectra were measured on a Perkin-Elmer Lambda 25 UV-vis spectrometer using a 1 cm path length UV cell at room temperature. There are four absorption bands in the UV-visible absorption spectra of the compounds (Fig. S22, ESI†), and the absorption peaks are at 195, 220, 256 and 393 nm for **1**, and 195, 222, 257 and 403 nm for **2**, respectively. The weak absorption bands at about 400 nm are caused by the carbonyl groups. The molar extinction coefficients of compounds **1** and **2** at about 400 nm are 5.59 × 10^3 and 5.14 × 10^3 M^{-1} cm^{-1}, respectively. Fig. S23 (ESI†) shows the emission spectra of compounds **1** and **2** excited at 430 nm, where the maximum emission peaks are at about 592 nm.

Fluorescence measurements

The fluorescent spectral studies were performed by measurements of the emission intensity of ethidium bromide (EB) on an AMINCO Bowman Series 2 luminescence spectrometer. The experiments were carried out by adding 0–1.43 μM **1** or **2** into the EB-bound CT-DNA (3.9 μM) solution in 5 mM Tris-HCl buffer (pH 7.0). The measured fluorescence was normalized to 100% relative fluorescence.

Circular dichroism measurements

All CD spectroscopic studies were carried out with a continuous flow of nitrogen purging the polarimeter, and the measurements were performed at room temperature with 1 cm pathway cells. The CD spectra were run from 320∼220 nm at a speed of 20 nm min^{-1} and the buffer background was subtracted automatically. Data were recorded at an interval of 0.1 nm. The CD spectrum of CT-DNA alone (129 μM) was recorded as the control experiment.

Agarose gel electrophoresis assays

The plasmid DNA cleavage experiments were performed using pUC19 DNA in Tris-HCl buffer. Reactions were performed by incubating DNA (0.025 mM bp) at 37 °C in a total volume of 15 μL in the dark for the indicated time. All reactions were quenched by loading buffer (3.5 μL) (30 mM EDTA, 0.05% (w/v) glycerol, 36% (v/v) bromophenol blue). Agarose gel electrophoresis was carried out on a 1% agarose gel in 0.5 × TAE (Tris-acetate–EDTA) buffer containing 1 μg mL^{-1} EB at 80 V for 1.5 h. The resolved bands were visualized with a UV transilluminator and quantified using TotalLab 2.01 software. The supercoiled plasmid DNA values were corrected by a factor of 1.3 on the basis of average literature estimates of lowered binding of EB to this structure.[24]

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