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Synthesis, DNA intercalation and europium(III)-triggered DNA photocleavage by a bis-proflavine succinamide conjugate

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Dedicated to Professor Dr. José L. Soto on the occasion of his retirement

Abstract—We describe the synthesis of a bis-proflavine derivative containing a succinamide linking chain. Levels of pUC19 plasmid DNA photocleavage by this compound are significantly enhanced in the presence of Eu^{3+} (350 nm, 22 °C, pH 7.0). UV–visible spectrophotometric studies of the ligand with calf thymus DNA show bathochromic-shifts and hypochromicity in the major absorption bands of the bis-proflavine derivative. Viscosimetric analysis of the helical extension of sonicated calf thymus DNA agrees with a monofunctional intercalation process.

The design and synthesis of new artificial systems to promote efficient DNA cleavage under physiological conditions is a topic of widespread interest. Compounds able to mimic the function of natural endonucleases are of great importance due to their potential applications in biotechnology, molecular biology and gene therapy. Thus, different types of chemical nucleases operating through hydrolytic or oxidative pathways have been synthesized in an effort to obtain more efficient and selective DNA and RNA cleaving agents.[1] Among the different approaches, the use of lanthanide ions and their complexes has aroused considerable interest because of their remarkably high effectiveness as catalysts for hydrolysis of the stable phosphodiester bond. (The DNA fragments generated can be easily reattached, representing a significant advantage of lanthanides over oxidative DNA cleaving agents.) Their unique behaviour is the result of their high oxidation states and charge densities, along with their ability to form complexes with high coordination numbers and rapid ligand exchange rates.[2] In recent years, different systems exploiting the catalytic effects of lanthanide ions and their complexes have been described.[3–6] An important goal is the obtention of selective hydrolysis, which has lead to the design of metallic complexes with appended oligonucleotides to target DNA cleavage to specific sequences.[7,8]

Although hydrolytic cleavage promoted by lanthanide ions and complexes has received considerable attention, references to the use of these metallonucleases as DNA photocleavers are scarce, with only a few examples appearing in the literature. In 1994, Komiyama[9] reported efficient DNA photocleavage by macrocyclic lanthanide complexes in the absence of molecular oxygen. Afterwards, Magda[10] described the cleavage of polymer DNA and RNA by lutetium(III) and europium(III) photosensitive texaphyrin systems. These systems were the first examples of oligonucleotide-directed DNA photocleavage with irradiation above 700 nm.

In this paper, we describe the synthesis of a bis-proflavine derivative containing a succinamide linking chain (**1**). This compound (Scheme 1) was designed to target

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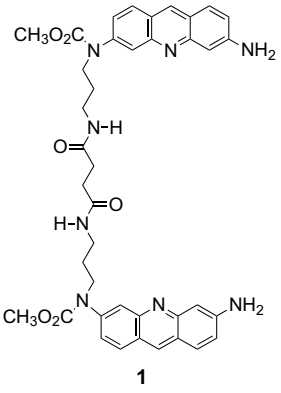


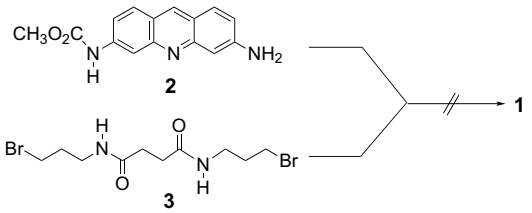
Scheme 1.

DNA in a bifunctional fashion, incorporating in the same molecule the proflavine chromophore, as a photoactive intercalating agent, and a succinamide ligand as a metal coordinating center. Preliminary investigations of ligand/DNA interactions are reported in terms of UV–visible and viscosimetric binding studies. We also show that irradiation of 50 μM of compound **1** in the presence of europium(III) effectively introduces single-stranded and double-stranded nicks into pUC19 plasmid DNA (350 nm, 22 °C, pH 7.0).

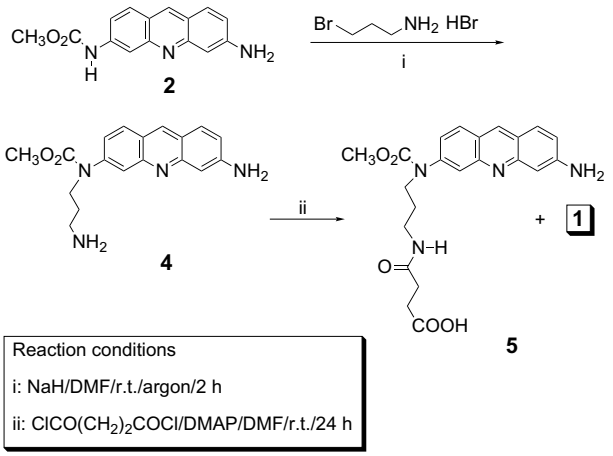
Our first approach to the synthesis of compound **1** consisted of the reaction of (6-aminoacridin-3-yl)-carbamic acid methyl ester (**2**)[11] and N,N′-bis-(3-bromopropyl)succinamide (**3**),[12] using DMF as solvent and sodium hydride as a base (Scheme 2). Unfortunately, this reaction did not afford bis-proflavine derivative **1**.

We then attempted an alternative step-by-step route (Scheme 3) in which (6-aminoacridin-3-yl)-(3-aminopropyl)carbamic acid methyl ester (**4**)[13] was synthesized from (6-aminoacridin-3-yl)carbamic acid methyl ester (**2**) and 3-bromopropylamine. The following step involved the reaction of **4** with succinyl chloride in DMF as solvent and 4-(dimethylamino)pyridine (DMAP) as a catalyst. Using this approach, the reaction afforded a mixture of the carboxylic acid **5**[14] (47% yield) and bis-proflavine **1**, the latter with a very low yield (<5%).

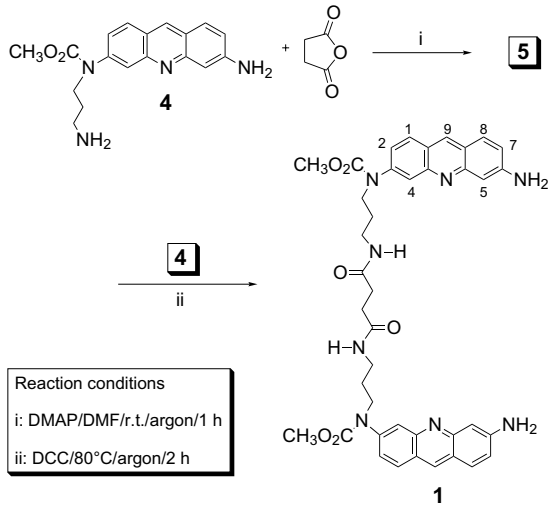
A final modification of the procedure (Scheme 4) allowed for a facile, one-pot synthesis of **1** from proflavine derivative **4**. Thus, by stirring a mixture of **4** with 1 equiv of succinic anhydride and catalytic amounts of DMAP for 1 h, the acid **5** was obtained. Then,



Scheme 2.



Scheme 3.



Scheme 4.

dicyclohexylcarbodiimide (1.3 equiv) and **4** (1 equiv) were added to the reaction and heated at 80 °C for 2 h. Subsequent work-up afforded pure bis-proflavine **1**.[15]

DNA intercalation produces bathochromic wavelength shifts and depressed absorption (hypochromic effect) in the UV–visible spectra of most if not all intercalators. The binding of **1** to DNA was therefore studied spectrophotometrically. As expected, we found that the major visible bands of **1** were red-shifted in the presence of calf thymus DNA ($\Delta\lambda = 22$ and 17 nm for the absorption bands at 375 and 455 nm, respectively) relative to the free-compound and displayed hypochromicity ($H = 18$ and 10 for the 375 and 455 nm bands, respectively; Fig. 1). H is percent hypochromicity [$H = (1 - \varepsilon_{\text{bound}}/\varepsilon_{\text{free}}) \times 100$].

We next studied the change in DNA viscosity caused by lengthening and unwinding of the DNA helix by the intercalated complex. Figure 2 represents a plot of the relative increase in DNA contour length (L/L_0) versus r, where r is the molar ratio of **1** to DNA nucleotides. The contour length in the presence of **1** (L) and the contour

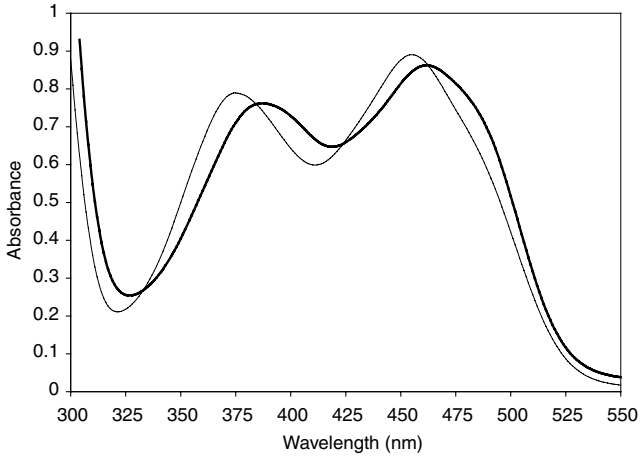


Figure 1. Effect of calf thymus DNA on the UV spectrum of **1** in 10 mM sodium phosphate buffer pH 5.7. The thin line—is the spectrum of free ligand at a concentration of 6.9×10^{-6} M, while the bold line—represents the spectrum of the same concentration of **1** in the presence of 8.4×10^{-5} M DNA (nucleotide molarity).

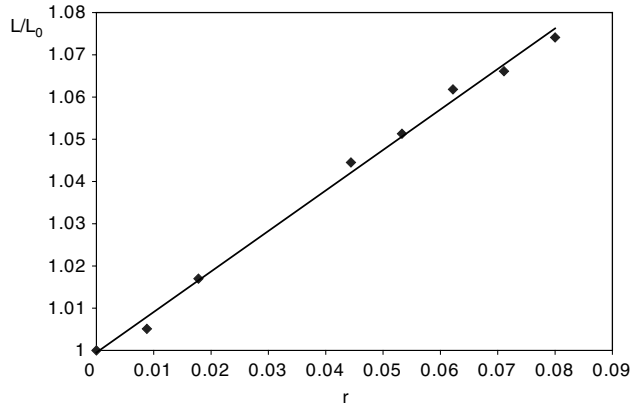


Figure 2. Relative length increase L/L_0 of DNA as a function of the molar ratio of **1** to DNA nucleotides r. The contour lengths in the presence (L) or absence (L_0) of the compound **1** were calculated from viscosity measurements on sonicated calf thymus DNA. Experiments were conducted in 50 mM Tris–HCl buffer containing 15 mM NaCl at pH = 7.5.

length of free DNA (L_0) were calculated from viscosity measurements of sonicated calf thymus DNA. The slope of the L/L_0 plot (0.96) falls within the range expected for monofunctional intercalators.

It has been previously established that, irrespective of chemical environment, the lowest energy conformation of N,N-dimethylsuccinamide is a folded, seven-membered hydrogen-bonded ring.[16] This folding process induces a shortening of succinamide chain length, which may account for the behaviour of **1** as a monointercalant.

DNA photocleavage by compound **1** in the absence and presence of Eu^{3+} (1:1 stoichiometric ratio) was assessed by monitoring the conversion of supercoiled pUC19 plasmid DNA (Form I) to its nicked (Form II) and linear (Form III) forms. In Figure 3, DNA cleavage

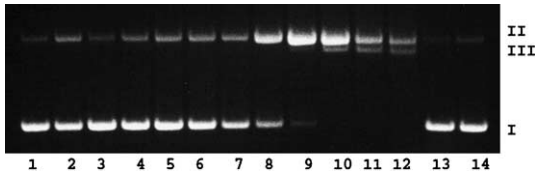


Figure 3. Photocleavage of 38 μM bp pUC19 plasmid DNA by 50 μM **1** in the absence and presence of 50 μM $EuCl_3$ (10 mM HEPES buffer, pH 7.0). (In lanes 8–12 and 14, compound **1** was pre-equilibrated in the dark with a 1:1 molar ratio of $EuCl_3$ for 1 h prior to its addition to DNA.) Reactions were equilibrated in the dark (1 h, 22 °C), and then irradiated with 13 RPR–3500 Å 24 W Rayonet lamps. Cleaved plasmid was electrophoresed on a 1% agarose gel stained with ethidium bromide (0.5 μg/mL). Lane 1: DNA control irradiated for 25 min; lane 2: 50 μM $EuCl_3$ irradiated for 25 min; lanes 3–7: 50 μM compound **1** irradiated for 5, 10, 15, 20, 25 min, respectively; lanes 8–12: 50 μM compound **1**+50 μM $EuCl_3$ irradiated for 5, 10, 15, 20, 25 min, respectively; lane 13: 50 μM compound **1** in the dark for 25 min; lane 14: 50 μM compound **1**+50 μM $EuCl_3$ in the dark for 25 min.

products produced by irradiation of 50 μM of compound **1** and by 50 μM of the metallic complex are resolved on a 1.0% agarose gel. After a 1 h equilibration (22 °C, pH 7.0), reactions were irradiated at 350 nm at intervals from 5 to 25 min under aerobic conditions in a Rayonet Photochemical Reactor. Visual inspection of the gel shows that compound **1** exhibits moderate levels of DNA photocleavage at all time points (lanes 3–7). However, in samples that contain both compound **1** and Eu^{3+}, very significant enhancements in cleavage are observed (lanes 8–12) and linear DNA is obtained after only 15 min of irradiation (lane 10). The interaction between compound **1** and the metal appears to be synergistic, as the photocleavage induced by the resulting complex (lanes 8–12) exceeds the theoretical yields that would be produced by the addition of europium(III)-induced cleavage in lane 2 to compound **1**-induced cleavage in lanes 3–7. (Essentially no reactivity was observed in the two dark controls; lanes 13 and 14.)

In conclusion, we have described the synthesis of a new bis-proflavine derivative that interacts with calf thymus DNA through intercalation. Because the succinamide linking chain of this compound is a coordinating center for metal ions, DNA photocleavage by compound **1** is dramatically enhanced in the presence of Eu^{3+} (350 nm, 22 °C, pH 7.0). This conjugate constitutes a good example of the use of a lanthanide to optimize the photocleaving activity of an organic ligand. Thus, this system might be useful as a starting point for the development of new, photoactive metallonucleases.

Acknowledgements

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12. N,N′-bis(3-bromopropyl)succinamide (**3**) was obtained from 3-bromopropylamine hydrobromide (4 equiv) and succinyl chloride in dry dichloromethane with triethylamine (4 equiv) as base. The reaction was carried out at 0 °C and then allowed to stand at room temperature for 4 h. The precipitate thus obtained was filtered and washed with water; mp 102–104 °C. Yield: 35%. ^{1}H NMR ($CDCl_3$) δ: 2.01–2.14 (m, 4H, CH_2–*CH_2*–CH_2), 2.57 (s, 4H, HNCO*CH_2*), 3.36–3.45 (m, 8H, *CH_2*–CH_2–*CH_2*), 6.64 (br s, 2H, NH).
13. (6-Aminoacridin-3-yl)-(3-aminopropyl)carbamic acid methyl ester (**4**). The crude product obtained after evaporation of the solvent was purified by flash column chromatography using silica gel as adsorbent and acetone–triethylamine (9/1, v/v) as eluent. Yield: 37%; mp 218–220 °C. ^{1}H NMR (DMSO-d_6) δ: 1.77–1.83 (m, 2H, CH_2–*CH_2*–CH_2), 2.83 (t, 2H, $J = 7.7$ Hz, NH_2–*CH_2*), 3.62 (s, 3H, CO_2CH_3), 3.83 (t, 2H, $J = 7.1$ Hz, CH_3O_2C–N–*CH_2*), 6.17 (br s, 2H, NH_2), 6.88 (br s, 1H, H-5 acridine), 7.08 (dd, 1H, $J = 9.1$, 1.8 Hz, H-7 acridine), 7.28 (dd, 1H, $J = 9.1$, 1.8 Hz, H-2 acridine), 7.65 (br s, 2H, NH_2), 7.74 (d, 1H, $J = 1.8$ Hz, H-4 acridine), 7.81 (d, 1H, $J = 9.1$ Hz, H-8 acridine), 7.94 (d, 1H, $J = 9.1$ Hz, H-1 acridine), 8.66 (s, 1H, H-9 acridine). MS (EI, 70 eV) *m/z* 324 (M^+, 1%), 245 (41), 236 (44), 223 (36), 209 (100), 182 (47).
14. N-{3-[(6-Aminoacridin-3-yl)methoxycarbonylamino]propyl} succinamic acid (**5**). The crude product obtained after concentration of the reaction mixture to dryness was purified by flash column chromatography using silica gel as adsorbent and acetone–methanol–triethylamine (4/2/1, v/v/v) as eluent. Yield: 47%; mp 182–184 °C (dec.). ^{1}H NMR (DMSO-d_6) δ: 1.61–1.66 (m, 2H, CH_2–*CH_2*–CH_2), 2.24 (part A of a A_2B_2 system, $J = 6.2$ Hz, *CH_2*–CH_2), 2.38 (part B of a A_2B_2 system, CH_2–*CH_2*), 2.98–3.05 (m, 2H, NH–*CH_2*), 3.61 (s, 3H, CO_2CH_3), 3.76 (t, 2H, $J = 7.5$ Hz, CH_3O_2C–N–*CH_2*), 6.2 (br s, 2H, NH_2), 6.88 (d, 1H, $J = 2.2$ Hz, H-5 acridine), 7.06 (dd, 1H, $J = 9.1$, 2.2 Hz, H-7 acridine), 7.28 (dd, 1H, $J = 9.1$, 2.2 Hz, H-2 acridine), 7.68 (d, 1H, $J = 2.2$ Hz, H-4 acridine), 7.79–7.85 (m, 2H, H-8 acridine and N–H), 7.91 (d, 1H, $J = 9.1$ Hz, H-1 acridine), 8.66 (s, 1H, H-9 acridine). MS (EI, 70 eV) *m/z* 423 (M^+-1, 1%), 406 (14), 363 (12), 348 (19), 294 (13), 280 (13), 248 (18), 236 (33), 222 (100).
15. N,N′-Bis-{3-[(6-aminoacridin-3-yl)methoxycarbonylamino] propyl}succinamide (**1**). The crude product obtained after evaporation of the solvent was purified by flash column chromatography using silica gel as adsorbent and acetone–methanol–triethylamine (17/1/2, v/v/v) as eluent. Yield: 58%; mp 128–130 °C. ^{1}H NMR (DMSO-d_6) δ: 1.59–1.66 (m, 4H, CH_2–*CH_2*–CH_2), 2.21 (s, 4H, *CH_2*–*CH_2*), 2.98–3.05 (m, 4H, NH–*CH_2*), 3.62 (s, 6H, CO_2CH_3), 3.75 (t, 4H, $J = 7.3$ Hz, CH_3O_2C–N–*CH_2*), 6.7 (br s, 4H, NH_2), 6.87 (d, 2H, $J = 1.8$ Hz, H-5 acridine), 7.1 (dd, 2H, $J = 9.1$, 1.8 Hz, H-7 acridine), 7.34 (dd, 2H, $J = 9.1$, 1.8 Hz, H-2 acridine), 7.74 (d, 2H, $J = 1.8$ Hz, H-4 acridine), 7.8 (t, 2H, $J = 5.7$ Hz, N–H), 7.85 (d, 2H, $J = 9.1$ Hz, H-8 acridine), 7.97 (d, 2H, $J = 9.1$ Hz, H-1 acridine), 8.78 (s, 2H, H-9 acridine). FAB MS *m/z* 731 (M^++1, 32%), 730 (M^+, 4), 307 (23), 236 (15), 154 (100), 136 (84).
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