Synthesis and photophysics of a fullerene-triquinoxaline ensemble

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In this contribution, we report the synthesis and photophysical characterization of a molecular conjugate, in which C_{60} is covalently attached to a triquinoxaline-based cavitand through a pyrrolidine ring. Comparative experiments performed by using suitable model compounds demonstrate negligible interactions between the quinoxaline chromophores and the fullerene centre in the ground state. On the other hand, fluorescence emission and excitation spectra provide evidence for the occurrence of efficient photoinduced singlet–singlet energy transfer from the quinoxaline moiety to the C_{60} core. Laser flash photolysis experiments show that such an intramolecular process precludes the population of the lowest triplet state of the quinoxaline. By way of contrast, the lowest triplet state of the fullerene is effectively populated and is capable of sensitizing the formation of singlet oxygen in high yield, as unambiguously demonstrated by its typical infrared phosphorescence, detected by using time-resolved luminescence apparatus. The fullerene-quinoxaline conjugate exhibits photoinduced DNA-cleaving activity, as confirmed by preliminary photocleavage experiments carried out with a pBR322 supercoiled plasmid.

Introduction

The remarkable electron and energy acceptor properties of fullerenes have made them very attractive for participating in photoinduced electron and energy transfer processes.[1–4] The consolidation of versatile synthetic protocols addressed to the chemical modification of the surface of these ball-shaped molecules[5,6] has led to simple and sophisticated multicomponent architectures in which organic and inorganic components are appropriately linked to the fullerene core. A variety of molecular systems with appealing characteristics and potential applications in material science, especially in the field of solar energy conversion, have been reported over the years.[7–16] In addition, fullerene and its derivatives have also intriguing prospective in biomedical chemistry.[17,18] This is mainly due to their possibility of undergoing light-mediated redox reactions with biological substrates and to effectively generate singlet oxygen, $^1O_2$ ($^1\Delta_g$) in high yields as a product of excited triplet state quenching by molecular oxygen.[19,20] These features suggest, for example, the potential use of fullerenes as DNA photocleaving agents that can trigger DNA breakage *via* type I (electron transfer mediated) and type II (singlet oxygen mediated) photosensitization mechanisms.[21–24] With this aim in mind, covalent functionalization of the fullerene sphere with suitable chromogenic units capable of transferring their excitation energy to the fullerene centre and exhibiting intercalating activity has proven a viable strategy to enhance the light harvesting properties and effectiveness of DNA photocleavage.[25,26]

Quinoxalines are naturally occurring heterocycles that possess biological and pharmaceutical activity.[27–30] They are also good DNA intercalators[31,32] and fundamental building blocks for resorcinarene-derived cavitands.[33] Furthermore, quinoxalines exhibit interesting responses to light excitation. In fact, they generate excited triplet states in high yields[34] and undergo photoinduced electron transfer with a variety of substrates.[35]

On the basis of these considerations, in this paper we considered it of interest to synthesize a novel molecular ensemble integrating a [60]fullerene (C_{60}) and a triquinoxaline-based cavitand within the same molecular skeleton, and to gain insights into its photophysical properties by combining steady-state and time-resolved spectroscopic techniques.

Results and discussion

The molecular conjugate object of the present work, **1**, was synthesized in one step starting from the monoformyl cavitand **1a**, C_{60} and sarcosine (Scheme 1).

In particular, to a toluene solution of **1a**, C_{60} and sarcosine were added, and the resulting mixture was refluxed for 48 h under a nitrogen flux to give **1** in 5% yield.

MacroModel 8.6, MM2* calculations gave an energy-minimized structure of **1**, in which the quinoxaline moieties are opened in a "kite conformation"[33] (Fig. 1). Such a structure was also supported by the typical chemical shifts of the methine hydrogens, in the range 4–5 ppm, observed in the ^{1}H NMR spectrum.

Fig. 2 shows the steady-state electronic absorption spectrum of **1**. It shows a main absorption in the UV region with maxima at 317 and 329, and a less intense absorption in the visible region extending beyond 700 nm. These spectral features match fairly well the profile obtained by summing the spectra of the suitable model compounds **2** and **3** (b and c in Fig. 2), accounting for only weak interactions between the quinoxaline chromophores and the fullerene moiety in the ground state.

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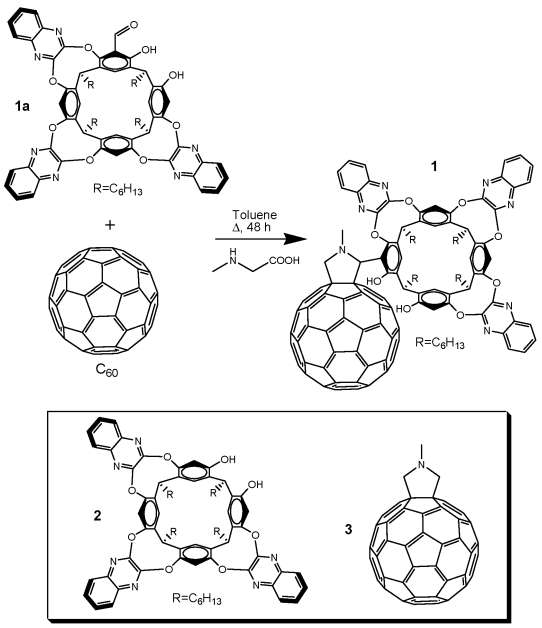

Scheme 1 Synthesis of the fullerene-triquinoxaline ensemble **1**, and the molecular structures of model compounds **2** and **3**.

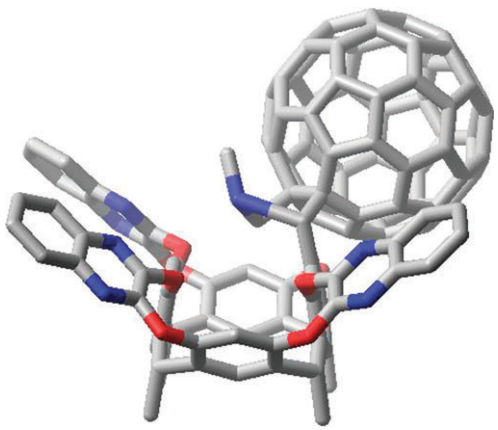

Fig. 1 The energy-minimized structure of **1** (MacroModel 8.6, MM2*, hydrogens and aliphatic chains are omitted for clarity).

Quinoxaline and fullerene chromophores exhibit characteristic fluorescence in completely different regions of the UV-vis spectral window.[36,37] Therefore, emission spectroscopy is a powerful tool that can be employed to obtain information on the interaction between the two units of **1** in their excited states. Fig. 3 shows the fluorescence emission spectra of optically matched solutions of **1**, and the model compounds **2** and **3**, obtained at λ_{exc} = 320 nm. Under such conditions, *ca.* 33% of the incident light is absorbed by the triquinoxaline fragment of compound **1**. Nevertheless, the characteristic fluorescence band of the quinoxaline fluorophore (λ_{max} = 415 nm) is totally quenched, whereas the typical fluorescence band characteristic of the fulleropyrrolidine fluorogenic centre (λ_{max} = 715 nm) is detected. Interestingly, the fluorescence quantum yield of this fluorophore in the case of **1** and model compound **3** are identical ($\Phi_F = 1.1 \times 10^{-3}$), although in the former only 66% of the incident light is absorbed by the fullerene fragment.

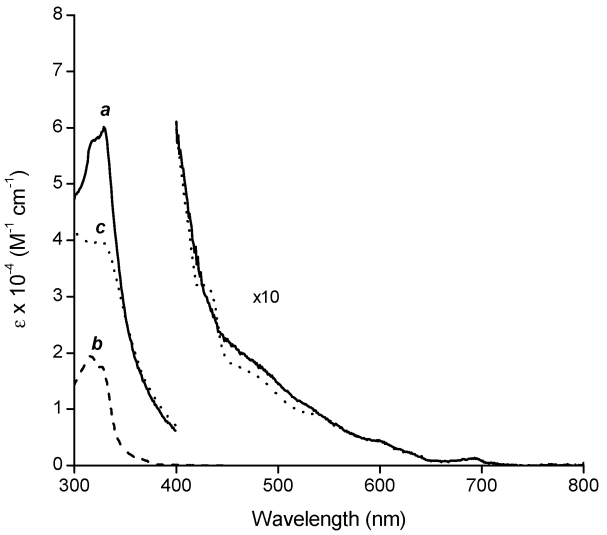

Fig. 2 Absorption spectra of **1** (a) and the model compounds **2** (b) and **3** (c) in toluene at 298 K; the portion above 400 nm is multiplied by a factor 10, for sake of clarity.

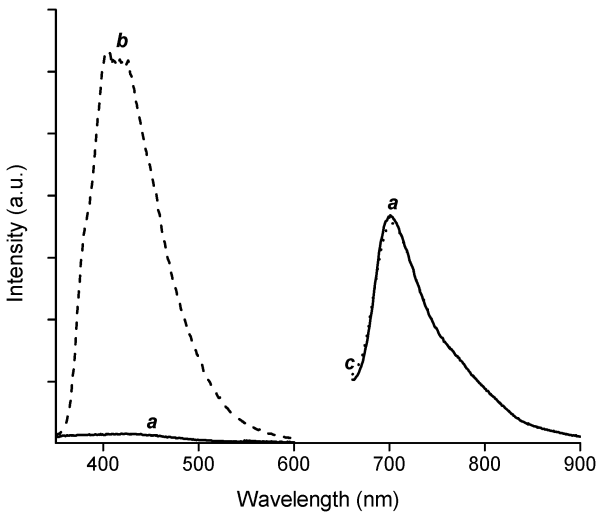

Fig. 3 Fluorescence emission spectra of **1** (a), and the model compounds **2** (b) and **3** (c) in toluene at 298 K (λ_{exc} = 320 nm). Above 660 nm, the sensitivity of the spectrofluorimeter is increased by 5 orders of magnitude due to the weakness of the fulleropyrrolidine fluorescence compared to that of the quinoxaline.

Furthermore, the excitation spectrum of **1**, taken at λ_{max} = 715 nm (data not shown), matches fairly well the absorption profile illustrated in Fig. 2, showing characteristic peaks of the quinoxaline chromophore at *ca.* 317 and 330 nm, respectively.

These results are fully consistent with the occurrence of a quantitative, thermodynamically favored, photoinduced singlet–singlet energy transfer from the quinoxaline unit to the fullerene moiety in compound **1**. This conjugate indeed presents two indispensable pre-requisites for this energy transfer to occur, that is: (i) the lowest excited singlet state of the quinoxaline (~3.2 eV)[38] is higher than that of the fullerene (~1.7 eV)[39] and (ii) the emission spectrum of the donor considerably overlaps the absorption of the acceptor. Note that, even when

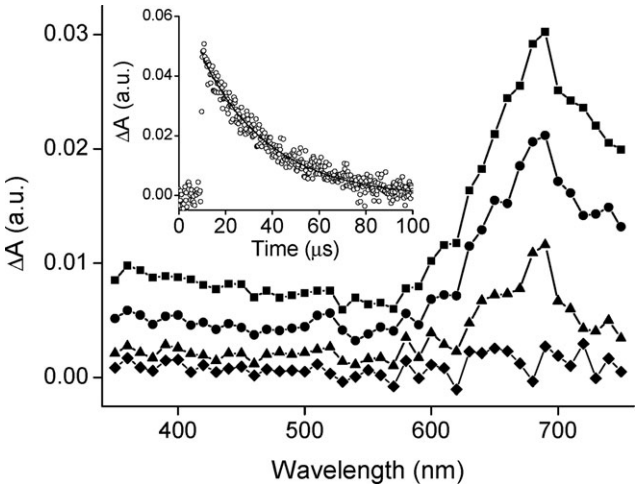

Fig. 4 Transient absorption spectra observed upon 266 nm laser excitation of an Ar-saturated CH_2Cl_2 solution of **1** recorded (■) 0.1 μs, (●) 10 μs, (▲) 30 μs and (◆) 80 μs after the laser pulse. $E_{532} \sim 12$ mJ pulse^{-1}. Each point was obtained by the signal average of 10 traces. The inset shows the decay trace monitored at 690 nm and the related first-order fitting.

the excitation is selectively addressed to the fullerene moiety ($\lambda_{exc} = 530$ nm), the fluorescence quantum yield of **1** and model compound **3** are identical, ruling out the occurrence of photoinduced electron transfer between the quinoxaline and fullerene units.

It is well known that the lowest excited singlet state of both quinoxaline and fullerene derivatives are converted to the lowest triplet state by intersystem crossing.[34,19] These excited states, having energy positions at ~2.6[35] and 1.5 eV,[19] respectively, exhibit intense absorption bands in completely different spectral regions and possess lifetimes falling in the microsecond time regime. Therefore, laser flash photolysis with nanosecond time resolution is a powerful tool for obtaining spectroscopic and kinetic features of excited triplets of both the chromophoric components of **1**. Fig. 4 shows the transient absorption spectra obtained upon 266 nm laser excitation of **1** in an Ar-saturated solution, recorded at different delay times with respect to the initial laser pulse. The spectrum taken at 0.1 μs shows a main band with a maximum at 690 nm that decays with first-order kinetics with a lifetime $\tau \sim 30$ μs (inset Fig. 4) and is effectively quenched by molecular oxygen with a bimolecular rate constant of 1.3×10^9 M^{-1} s^{-1}. This spectroscopic and kinetic behaviour is fairly similar to that displayed by model fulleropyrrolidine **3** under the same experimental conditions and is in excellent agreement with that recently reported for fullerene pyrazine dyads.[25] Therefore, we can safely attribute the observed transient spectrum to the triplet–triplet absorption of the fullerene unit. Note that the time evolution of the absorption showed in Fig. 4 reveals that no new transient is formed concurrent with the triplet decay, ruling out any possible reaction of this species with the quinoxaline appendage. On the other hand, despite the absorption by the triquinoxaline fragment of *ca.* 25% of the incident light under these experimental conditions, we did not observe the typical bands of the quinoxaline triplet state, which are expected to be at *ca.* 360 and 430 nm.[35] This result accounts well for the quantitative singlet–singlet photoinduced energy transfer discussed above, which, analogously to the suppression of fluorescence (see Fig. 2), precludes intersystem crossing to the triplet state of the quinoxaline.

In order to investigate whether the triquinoxaline cavitand affects the quantum yield of the population of the fullerene triplet state in compound **1**, we carried out laser power intensity dependence experiments by setting the excitation wavelength of the laser at 532 nm. Fig. 5 shows the top ΔA of the triplet of **1** and, for comparison, of model compound **3**. The linear behaviour observed is typical of a one-photon process, such as the generation of the lowest triplet state. The slope of each set of data points is proportional to the product $\Phi_T \times \varepsilon_{T-T}$, where Φ_T and ε_{T-T} are the quantum yield of the triplet state and its molar absorption coefficient, respectively. By taking into account that all solutions are optically matched at the excitation wavelength and that large changes in the ε_{T-T} between the two samples are fairly unlikely, the band profiles being substantially unchanged (*vide supra*), the Φ_T for compound **1** ratio may be directly estimated by the slopes (π) of the straight lines of the plots in Fig. 5 *via* the simple equation:

$$\Phi_T^{(1)} = \Phi_T^{(3)} \pi^{(1)}/\pi^{(3)}$$

The data illustrated in Fig. 5 clearly demonstrate that the presence of the quinoxaline-based appendage does not affect the population of the fullerene triplet state, whose Φ_T value is therefore ~0.9, analogous to what is reported in the literature for fulleropyrrolidine derivatives.[40]

The validity of the above assumptions is supported by the time resolved experiments addressed to evaluate the capability of **1** to photogenerate $^1O_2(^1\Delta_g)$, described below.

As shown earlier, the triplet state of the fullerene is effectively quenched by oxygen with a bimolecular rate constant very similar to those reported for pristine fullerenes and monofunctionalized fullerene derivatives,[41] suggesting that

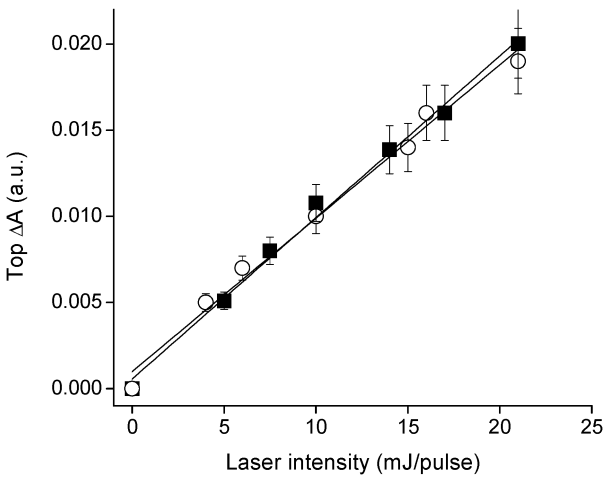

Fig. 5 Laser intensity dependence of the absorbance changes, ΔA, taken 0.1 μs after 532 nm laser pulse excitation of optically matched Ar-saturated toluene solutions of **1** (○) and **3** (■). Each point represents signal average of 10 traces.

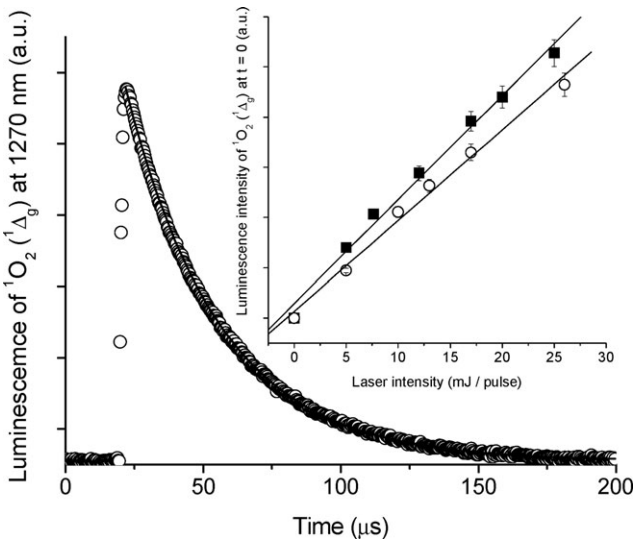

Fig. 6 A representative decay trace of $^1O_2(^1\Delta_g)$ monitored at 1270 nm, observed upon 532 nm laser excitation of an air-equilibrated toluene solution of **1**, and related first-order fitting. The inset shows the luminescence intensity at zero time as a function of the laser intensity in the case of optically matched toluene solutions of **1** (○) and C_{60} (■). Each point represents the signal average of 10 traces.

the fullerene core of **1** can generate $^1O_2(^1\Delta_g)$ quite efficiently through the energy transfer process:

$$^3C_{60}^* + {}^3O_2 \rightarrow C_{60} + {}^1O_2(^1\Delta_g)$$

Time-resolved near-infrared luminescence with a microsecond time resolution is the most suitable technique to unequivocally demonstrate the generation of $^1O_2(^1\Delta_g)$. This species, in fact, exhibits a typical luminescence signal with a maximum at 1.27 μm and decays in the microsecond timescale[42] (see the Experimental section for details). Fig. 6 shows a representative luminescence decay trace of $^1O_2(^1\Delta_g)$, observed upon the 532 nm laser excitation of **1** in an air-equilibrated toluene solution, confirming the photosensitization of this transient species. We then examined the quantum yield of singlet oxygen (Φ_Δ). To this end, the luminescence intensity at zero time of the time-dependent signal obtained in the case of optically matched solutions of **1** and C_{60} ($\Phi_\Delta = 1$) was plotted as a function of the laser intensity (see inset Fig. 6). A value of $\Phi_\Delta \sim 0.9$ for **1** was directly obtained from the different slopes π of the straight lines of each set of data points *via* the simple equation:

$$\Phi_\Delta^{(1)} = \Phi_\Delta^{(C_{60})}\, \pi^{(1)}/\pi^{(C_{60})}$$

Note that the fraction of triplet that generates $^1O_2(^1\Delta_g)$, $S_\Delta = \Phi_\Delta/\Phi_T$ is ~1, is in excellent agreement to what is expected for fullerene derivatives.[20]

As illustrated in the Introduction section, quinoxalines are excellent DNA binders, and fullerenes exhibit DNA photocleaving activity. Therefore, we considered it to be of interest to carry out some preliminary experiments addressed at exploring the potential ability of compound **1** to bind DNA and to promote its photoinduced cleavage.

Conjugate **1** is not soluble in aqueous solution. On the other hand, it is fairly soluble in the presence of DNA, as demonstrated by the appearance of the weak but characteristic

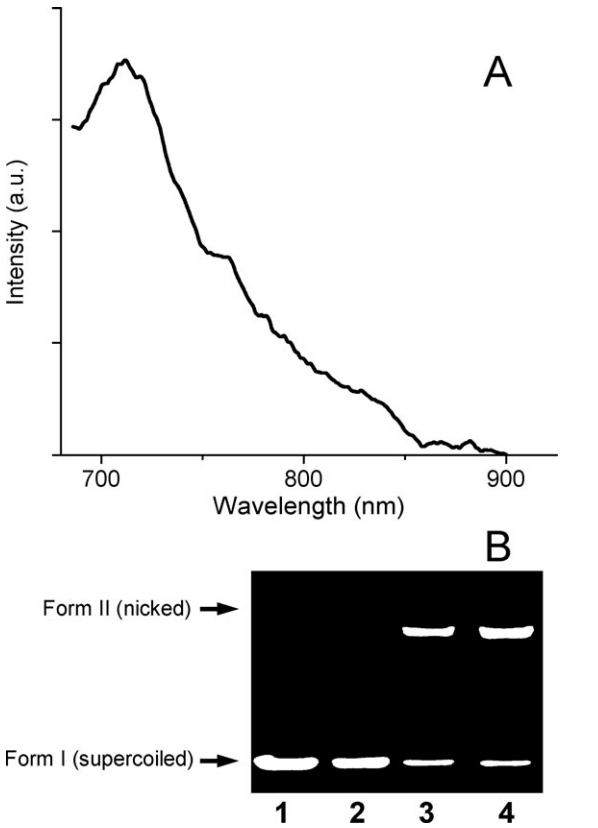

Fig. 7 (A) Fluorescence emission spectra of **1** in PBS 10 mM, pH 7.4 in the presence of ct-DNA 15 mM; λ_{exc} = 530 nm. (B) Agarose gel electrophoretic patterns of pBR322 plasmid DNA under aerobic conditions. Line 1: no reagent irradiated for 60 min; line 2–4: 3 μM of **1** incubated in the dark for 60 min and irradiated for 40 and 60 min, respectively.

fluorescence emission of the fullerene component at *ca.* 715 nm (Fig. 7A). Control experiments carried out with model compound **3** showed this latter compound to be totally insoluble in the presence of the same amount of DNA. These results suggest that quinoxaline chromophores play a key role in encouraging the binding of **1** to the biopolymer. Therefore, we examined the photodriven cleavage ability of **1** towards pBR322 supercoiled DNA. The results, reported in Fig. 7B, clearly show that compound **1** does not display any DNA-cleaving activity in the dark (line 2). In contrast, the conjugate exhibits a distinct DNA cleaving activity upon light irradiation, as confirmed by the significant conversion (higher than 50%) of the supercoiled DNA (form I) to the nicked DNA (form II) (lines 3 and 4).

Conclusions

A novel molecular ensemble in which the fullerene cage is covalently linked to a triquinoxaline-based cavitand has been prepared and its photophysical behaviour studied by combining steady-state and time-resolved spectroscopic techniques. As pictorially sketched in the energy level of Fig. 8, we have demonstrated that the integration of three quinoxaline moieties within the same molecular skeleton improves the light harvesting efficiency of the fullerene centre. The light energy absorbed by

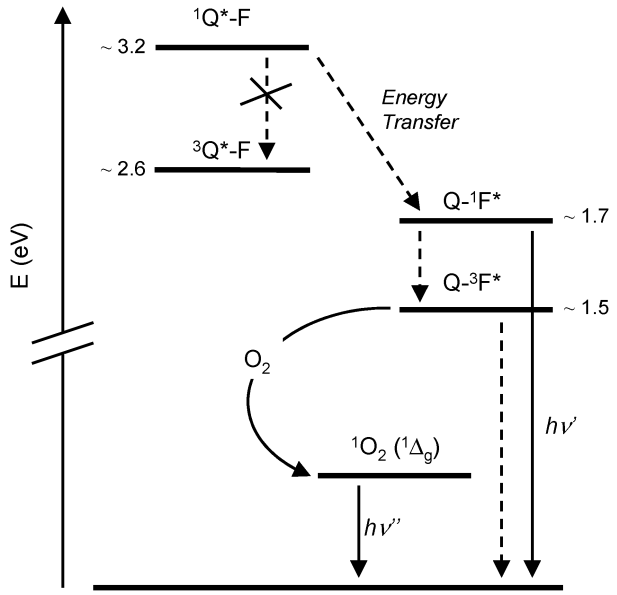

Fig. 8 The energy level diagram for **1**. The quinoxaline and fullerene units are indicated with the letters **Q** and **F**, respectively.

the triquinoxaline antennas is in fact effectively transferred to the fullerene through a photoinduced singlet–singlet energy transfer, which precludes the population of the lowest triplet state of the quinoxaline. In contrast, the features of the lowest triplet of the fullerene component are retained, as demonstrated by its effective population and its capability to sensitize the photogeneration of $^1O_2(^1\Delta_g)$ with a very high quantum yield, analogously to other fullerene derivatives.

Preliminary investigations carried out in the presence of DNA showed that compound **1** binds a polynucleotide and promotes its cleavage through an exclusively light-triggered process. These results will encourage the undertaking of further studies directed at gaining insights into the binding mode and towards clarifying the molecular mechanism of DNA photodamage.

Experimental section

General

Sonicated calf thymus DNA (phenol extracted, lyophilized, average size 2000 bases, range 200–6000 bases) was obtained from Pharmacia (Milan). The concentration of DNA in base pair ($[ct\text{-}DNA]_{bp}$) was determined spectrophotometrically. Supercoiled pBR322 DNA (sc-DNA), form I, molecular weight 2.9×10^6 Da, 4365 base pairs was obtained from Pharmacia (Milano, Italy). The percentage of relaxed form II of pBR322 was less than 12% in the starting material and no linear form III was detected (the data were obtained from densitometric analysis of agarose gel electrophoresis). Water was purified through a Millipore Milli-Q system. The experiments in the presence of DNA were performed in phosphate buffered saline (PBS) (pH 7.4) consisted of a 10 mM phosphate buffer added with 0.05 M NaCl. The pH of solution was measured with a glass electrode. All other chemicals were reagent grade.

All chemicals were of reagent grade and used without further purification. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The NMR experiments were carried out at 27 °C on a Varian UNITY Inova 500 MHz spectrometer (^{1}H NMR at 499.88 MHz, ^{13}C NMR at 125.7 MHz in $CDCl_3$) equipped with pulse field gradient module (Z axis) and a tunable 5 mm Varian inverse detection probe (ID-PFG). The chemical shifts (ppm) were referenced to TMS. ESI mass spectra were obtained by employing an ES-MS Thermo-Finnigan LCQ-DECA spectrometer equipped with an ion trap analyzer.

Synthesis

Compound 1a. Compound **1a** was obtained according to an unpublished protocol developed in our lab.[43] ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.68 (1H, s, OH), 10.18 (1H, s, CHO), 8.35 (1H, s, ArH *ortho* OH), 8.01 (1H, d, J = 7.5 Hz, ArH quinoxaline), 7.93 (1H, d, J = 7.5 Hz, ArH quinoxaline), 7.88 (1H, d, J = 8.0 Hz, ArH quinoxaline), 7.84 (1H, d, J = 8.0 Hz, ArH quinoxaline), 7.78 (1H, d, J = 7.5 Hz, ArH quinoxaline), 7.74 (1H, s, ArH), 7.69 (2H, d, J = 8.0 Hz, ArH quinoxaline), 7.50–7.64 (6H, m, ArH quinoxaline), 7.44 (1H, s, ArH), 7.39 (1H, s, ArH), 7.34 (1H, s, ArH), 7.24 (1H, s, ArH), 7.13 (1H, s, ArH), 5.63 (1H, t, J = 8.0 Hz, CH methine), 5.54 (1H, t, J = 8.0 Hz, CH methine), 4.71 (4H, m, $CH_2(CH_2)_3CH_3$), 4.31 (1H, t, J = 8.0 Hz, CH methine), 4.12 (1H, t, J = 8.0 Hz, CH methine), 3.62 (2H, m, $CH_2(CH_2)_3CH_3$), 2.30 (4H, m, $CH_2(CH_2)_3CH_3$), 1.54–1.62 (31H, m, $CH_2(CH_2)_3CH_3$), 1.33–1.39 (4H, m, $CH_2(CH_2)_3CH_3$), 0.89 (6H, t, J = 6.5 Hz, $CH_2(CH_2)_3CH_3$), 0.74 (3H, t, J = 6.5 Hz, $CH_2(CH_2)_3CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.2, 156.5, 155.8, 153.0, 152.65, 152.61, 152.3, 152.2, 152.0, 151.7, 148.9, 147.4, 147.1, 139.8, 139.7, 139.6, 138.4, 138.3, 136.7, 136.2, 136.1, 135.1, 133.1, 132.2, 129.5, 129.3, 129.2, 129.0, 128.9, 128.1, 128.0, 127.9, 127.7, 127.6, 127.3, 127.2, 127.0, 126.7, 126.4, 126.3, 124.4, 123.3, 122.8, 121.8, 118.9, 117.9, 111.6, 110.0, 34.7, 34.3, 34.0, 33.5, 33.2, 32.9, 32.5, 32.3, 31.88, 31.82, 31.7, 31.6, 29.4, 29.3, 29.2, 29.0, 27.9, 27.8, 27.7, 27.6, 22.65, 22.60, 22.5, 22.4, 14.1, 14.06, 14.00, 13.8. ESI-MS m/z 1277 $[MH + (C_2H_5OH)]^+$. Anal. calc. for $C_{77}H_{78}N_6O_9$: C, 75.10; H, 6.38; N, 6.82. Found: C, 75.04 H, 6.37; N, 6.76.

Compound 1. In a round bottom flash 60 mg of monoformyl cavitand (**1a**) (0.0487 mmol) was dissolved in 20 mL of toluene under nitrogen atmosphere. Then 17.5 mg of fullerene (0.0243 mmol) and 4.34 mg of sarcosine (0.0487 mmol) were added to the solution and the mixture was refluxed for 48 h under nitrogen.[44] The solvent was removed under reduced pressure, and the residue was chromatographated on silica gel (toluene : EtOAc 95 : 5) to give 4.6 mg of **1** (yield 5%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (1H, s, ArH *ortho* OH), 8.06 (1H, d, J = 8.0 Hz, ArH quinoxaline), 7.97 (1H, d, J = 8.0 Hz, ArH quinoxaline), 7.94 (1H, s, ArH), 7.90 (1H, d, J = 8.0 Hz, ArH quinoxaline), 7.82 (2H, d, J = 8.0 Hz, ArH quinoxaline), 7.78 (1H, s, ArH), 7.73 (1H, d, J = 8.0 Hz, ArH quinoxaline), 7.57–7.69 (6H, m, ArH quinoxaline), 7.55 (2H, s, ArH), 7.53 (1H, d, J = 8.0 Hz, ArH quinoxaline), 7.40 (1H, s, ArH), 7.37 (1H, s, ArH), 7.32 (1H, s, ArH), 5.70

(1H, t, J = 8.0 Hz, CH methine), 5.63 (1H, t, J = 8.0 Hz, 1H, CH methine), 4.70 (2H, m, $CH_2(CH_2)_3CH_3$), 4.68 (1H, d, J = 13.0 Hz, CH_2 sarcosine), 4.64 (1H, s, CH sarcosine), 4.37 (1H, t, J = 8.0 Hz, CH methine), 4.14 (1H, d, J = 13.0 Hz, CH_2 sarcosine), 2.40 (9H, m, $CH_2(CH_2)_3CH_3$), 1.54–1.62 (31H, m, $CH_2(CH_2)_3CH_3$), 1.33–1.39 (4H, m, $CH_2(CH_2)_3CH_3$), 0.89 (6H, t, J = 6.5 Hz, $CH_2(CH_2)_3CH_3$), 0.74 (3H, t, J = 6.5 Hz, $CH_2(CH_2)_3CH_3$); ESI-MS m/z 2004 $[M + Na]^+$. Anal. calc. for $C_{139}H_{85}N_7O_8$: C, 84.26; H, 4.32; N, 4.95. Found: C, 84.11; H, 4.29; N, 4.88.

Compound 2. Compound **2** was prepared according to literature:[33] a 250 mL round-bottom flask was charged with 355 mg (0.28 mmol) of tetraquinoxaline cavitand[45] and 857 mg (5.64 mmol) of CsF in 100 mL of dry DMF. The mixture was heated to 80 °C in an oil-bath and 31.7 mg (0.288 mmol) of catechol was added. The reaction turned light green immediately. The reaction was followed by TLC on silica gel employing a 95 : 5 CH_2Cl_2–EtOAc v/v mixture as eluent. After 1 h the reaction was complete and quenched by pouring into 500 mL of ice-cold brine. The resulting precipitate was collected, washed with water (3 × 100 mL) and air-dried. Purification by chromatography (silica gel, hexane : EtOAc 70 : 30) afforded triquinoxaline cavitand **2**, 180 mg (60%). m.p. > 225 °C dec. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (2H, s, Ar–H *ortho* to OH), 7.92 (2H, d of d, J = 1.0 Hz, 8.0 Hz, ArH quinoxaline), 7.86 (2H, bs, OH), 7.81 (2H, m, ArH quinoxaline opposite to OH), 7.66 (2H, d of d, J = 1.0 Hz, 8.0 Hz, ArH quinoxaline), 7.54 (2H, t or d of t, J = 7.0 Hz, ArH quinoxaline), 7.49–7.43 (4H, m, AA′BB′, ArH quinoxaline), 7.28 (2H, s, Ar–H *meta* to OH), 7.13 (2H, s, Ar–H), 7.08 (2H, s, Ar–H), 5.58 (1H, t, J = 8.0 Hz, CH methine between OH), 5.50 (2H, t, J = 8.0 Hz, CH methine), 4.25 (1H, t, J = 8.0 Hz, CH methine opposite to OH), 2.29–2.16 (8H, m, $CH_2(CH_2)_3CH_3$), 1.46–1.24 (32H, m, $CH_2(CH_2)_3CH_3$), 0.93 (12H, t, J = 6.5 Hz, $CH_2(CH_2)_3CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.6, 152.49, 152.48, 152.44, 152.3, 151.3, 139.64, 139.63, 139.4, 136.2, 135.6, 130.4, 130.3, 129.2, 128.9, 128.8, 128.6, 127.8, 127.7, 127.5, 123.7, 123.2, 118.6, 110.3, 34.4, 34.1, 33.7, 33.6, 32.6, 32.3, 31.87, 31.86, 31.82, 29.6, 29.3, 29.2, 27.9, 27.8, 22.67, 22.65, 14.05, 14.04. ESI-MS m/z 1202 $[M + H]^+$. Anal. calc. for $C_{76}H_{78}N_6O_8$: C, 75.84; H, 6.53; N, 6.98. Found: C, 75.80; H, 6.47; N, 6.91.

Compound 3. Compound **3** was prepared according to literature.[44] In a round bottom flask, 50 mg of fullerene (0.0694 mmol) was dissolved in 20 mL of toluene under nitrogen atmosphere. Then 10.4 mg of paraformaldehyde (0.347 mmol) and 12.36 mg of sarcosine (0.13 mmol) was added to the solution and the mixture was refluxed for 3 h under nitrogen. The solvent was removed under reduced pressure, and the residue was chromatographated on silica gel (toluene : EtOAc 95 : 5) to give 4.6 mg of **3** (yield 35%). ^{1}H NMR (500 MHz, $CDCl_3/CCl_4$) δ 4.41 (4H, s, CH_2), 3.01 (3H, s, NCH_3).

Photophysical measurements

Absorption and emission spectra were recorded with a Jasco V-560 spectrophotometer and a Spex Fluorolog-2 (mod. F-111) spectrofluorimeter equipped with a double monochromator, respectively. For the determination of the fluorescence quantum yields, an argon-deaerated toluene solution of C_{60} was used as a standard (Φ_F = 3.2 × 10^{-4}).[46]

Laser flash photolysis setup. The sample was excited with either the fourth (266 nm) or the second (532 nm) harmonic of a Nd–YAG Continuum Surelite II-10 laser (6 ns FWHM). The measurements were carried out with a 10 × 10 mm^2 quartz cell with a 3 mL capacity. The excited samples were analyzed by a Luzchem Research mLFP-111 apparatus with an orthogonal pump/probe configuration. The probe source was a ceramic xenon lamp coupled to quartz fiber-optical cables. The laser pulse and the mLFP-111 system were synchronized by a Tektronix TDS 3032 digitizer, operating in pre-trigger mode. The signals from a compact Hamamatsu photomultiplier were initially captured by the digitizer and then transferred to a personal computer, controlled by Luzchem Research software operating in the National Instruments LabView 5.1 environment. The sample temperature was 295 ± 2 K. The energy of the laser pulse was measured at each shot with a SPHD25 Scientech pyroelectric meter.

Singlet oxygen measurements. Photogeneration of singlet oxygen upon 532 nm laser excitation was monitored by luminescence measurements in air-saturated solutions. The near-IR luminescence of singlet oxygen at 1.27 μm resulting from the forbidden transition $^3\Sigma_g^- \leftarrow {}^1\Delta_g$, was probed orthogonally to the exciting beam with a pre-amplified (low impedance) Ge-photodiode (Hamamatsu EI-P, 300 ns resolution) maintained at −196 °C and coupled to a long-pass silicon filter (> 1.1 μm) and an interference filter (1.27 μm). The temporal profile of the luminescence was fitted to a single-exponential decay function. The luminescence at initial time was extrapolated from the curve fitting.

DNA photocleavage experiments. The samples containing pBR322 DNA with or without **1** were prepared in a final volume of 18 μL, placed in Eppendorf tubes and irradiated in a Rayonet photochemical reactor equipped with "black light" phosphor lamps with an emission in the 310–390 nm range with a maximum at 350 nm. The fluence rate at the irradiation position was about 800 μW cm^{-2}. A "merry-go-round" irradiation apparatus was used to ensure that all the samples received equal radiation. The incident photon flux on solution in the Eppendorf tubes (0.2 cm of optical path), used for the experiments was ca 10^{16} quanta sec^{-1}.

Following irradiation, 4 μL of a mixture composed of 0.22% (w/v) bromophenol blue, 40% (w/v) sucrose, 0.1 mM EDTA pH 8, and sodium lauryl sulfate (0.5% w/V), were added to the samples. 18 μL of each sample (0.4 μg DNA) were loaded onto 1% agarose gel of 5 mm thickness (up to 22 wells). The electrophoretic analysis was performed in tris-borate-EDTA buffer with a Pharmacia horizontal apparatus (GNA-200). The power supply was set at 40 V for 15 h at 25 °C. Following electrophoresis, the gel was stained with ethidium bromide (1 μg mL^{-1}) for 30 min and rinsed with a $MgCl_2$ solution (10 mM) for 20 min.

DNA forms were detected by excitation of ethidium bromide fluorescence on a 300 nm UV transilluminator

(Pharmacia). Quantitation of bands was achieved by microdensitometry of the negative produced from the gel photograph using a Beckman DU 650 spectrophotometer equipped with gel scan accessory.

The fraction of sc-DNA after electrophoresis was calculated using the following equation:

$$\text{sc-DNA} = \frac{\text{Area}_{sc}}{\text{Area}_{sc} + \text{Area}_{cl}/1.66}$$

where Area_{sc} and Area_{cl} are, respectively, the percentages of sc-DNA (Forms I) and cleaved DNA (Forms II), which are both detected through densitometric analysis of fluorescent gels. The correct proportions of Forms I, and II in each sample were calculated by using the coefficient 1.66 for the lower efficiency of ethidium bromide in binding to the Form I DNA with respect to Form II.[47]

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