Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information for

Novel carbazole-based two-photon photosensitizer for efficient DNA photocleavage in anaerobic condition using near-infrared light

Yong-Chao Zheng,[a,d] Mei-Ling Zheng,[a*] Ke Li, [b,d] Shu Chen,[a] Zhen-Sheng Zhao,[a] Xue-Song Wang[b] and Xuan-Ming Duan[a,c*]

[a] Laboratory of Organic NanoPhotonics and Key Laboratory of Functional Crystals and Laser Technology, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, No.29 Zhongguancun East Road, Beijing 100190, P. R. China

[b] Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, No.29 Zhongguancun East Road, Beijing 100190, P. R. China

[c] Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, No.266 Fangzheng Ave, Shuitu technology development zone, Beibei District, Chongqing 400714, P. R. China

[d] University of Chinese Academy of Sciences, No.29 Zhongguancun East Road, Beijing 100190, P. R. China

Phone: +86-10-82543596, Fax: +86-10-82543597

E-mail address: zhengmeiling@mail.ipc.ac.cn, xmduan@mail.ipc.ac.cn

Contents

1. Materials and Instruments

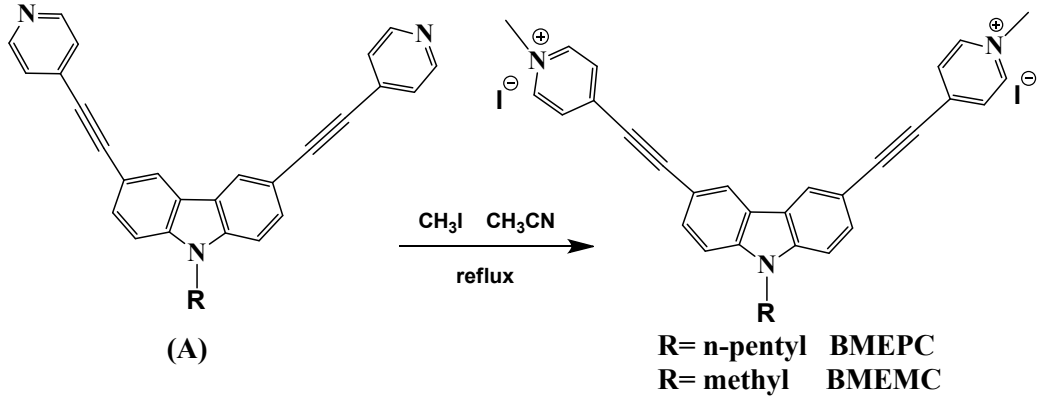
All the chemicals and solvents were obtained from Beijing Chemical Reagent Company and used without further purification. TE buffer (pH = 8.0) was purchased from HEOWNS (China). Calf thymus DNA (CT-DNA) gel loading buffer, tris-hydroxymethylaminomethane (Tris base), ethylenediaminotetraacetic acid (EDTA), superoxide dismutase (SOD) and mannitol were purchased from Sigma Co. (USA). The supercoiled pBR322 plasmid DNA was purchased from TaKaRa Biotechnology Company.

CT-DNA solution was obtained by dispersing the desired amount of CT-DNA in TE buffer solution and stirring overnight at temperature below 4 °C. The concentration of ct-DNA was estimated by measuring the UV absorbance at 260 nm (ε=6600 $M^{-1}\cdot cm^{-1}$).

^1H NMR spectra were recorded on a Varian Jemini-300/Brucker AV 400 spectrometer using $CDCl_3$ or DMSO-d6 as a solvent and all shifts are referred to tetramethylsilane (TMS). The chemical shift (s = singlet, d = doublet, t = triplet, m = multiplet) is shown in ppm. High resolution ESI mass spectra (HRMS) were measured on Q Exactive Mass Spectrometer (Thermo Fisher, USA). UV-Vis spectra were recorded at the concentration of 1×10^{-5} M on a Hitachi U-3900 spectrophotometer. One-photon excited fluorescence (OPEF) spectra were performed at the concentration of 2.5×10^{-6} M using a Hitachi F-4600 spectrometer. The EPR spectra were obtained on a Bruker ESP-300E spectrometer at 9.75 GHz, X-band with 100 Hz field modulation, using DMPO (5,5-dimethyl-1-pyrroline-N-oxide) as a spin trapping agent. Samples at N_2 atmosphere were injected quantitatively into specialized quartz capillaries, and then illuminated in the cavity of the EPR spectrometer with a mercury lamp (λ > 400 nm). All measurements were carried out at room temperature.

2. Synthesis and characterizations of BMEPC and BMEMC

The intermediate products (A) were synthetized according to our previous work.[1] The synthesis procedure of **BMEPC** and **BMEMC** is shown as follows: A mixture of compound **A** (0.39 mmol) and iodomethane (0.3 mL) in acetonitrile (10 mL) was refluxed overnight. The reaction mixture was cooled to rt and filtered. The filtered solid was washed with ethanol. After filtration and recrystallization using methanol, **BMEPC** and **BMEMC** were obtained as yellow solids.

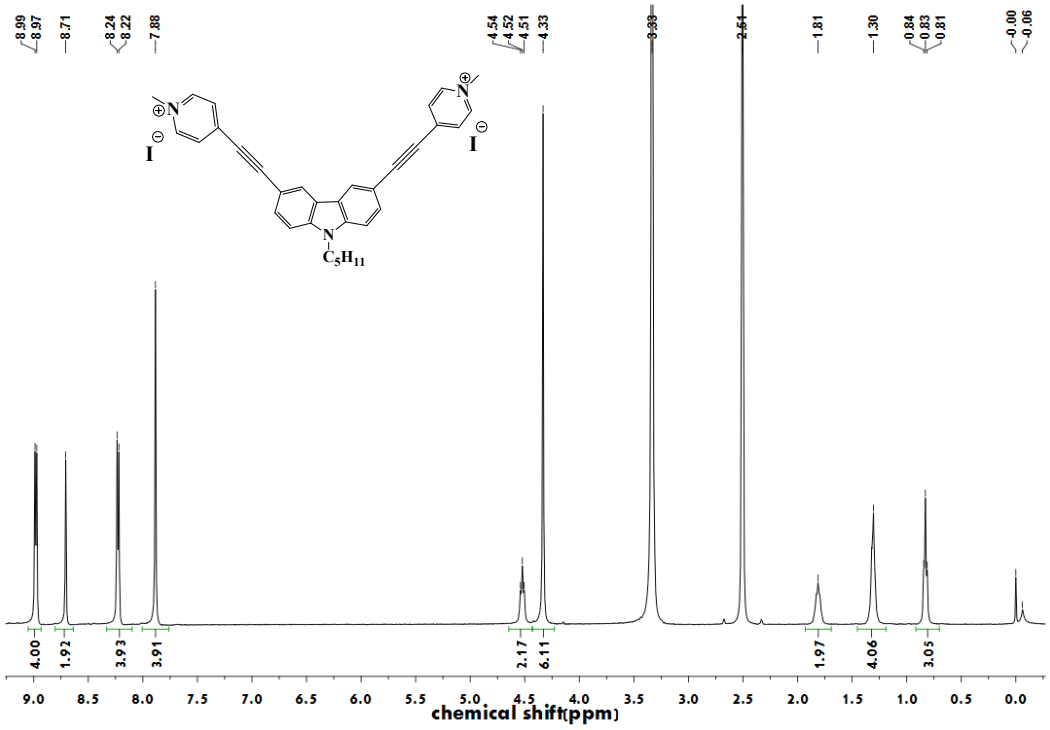


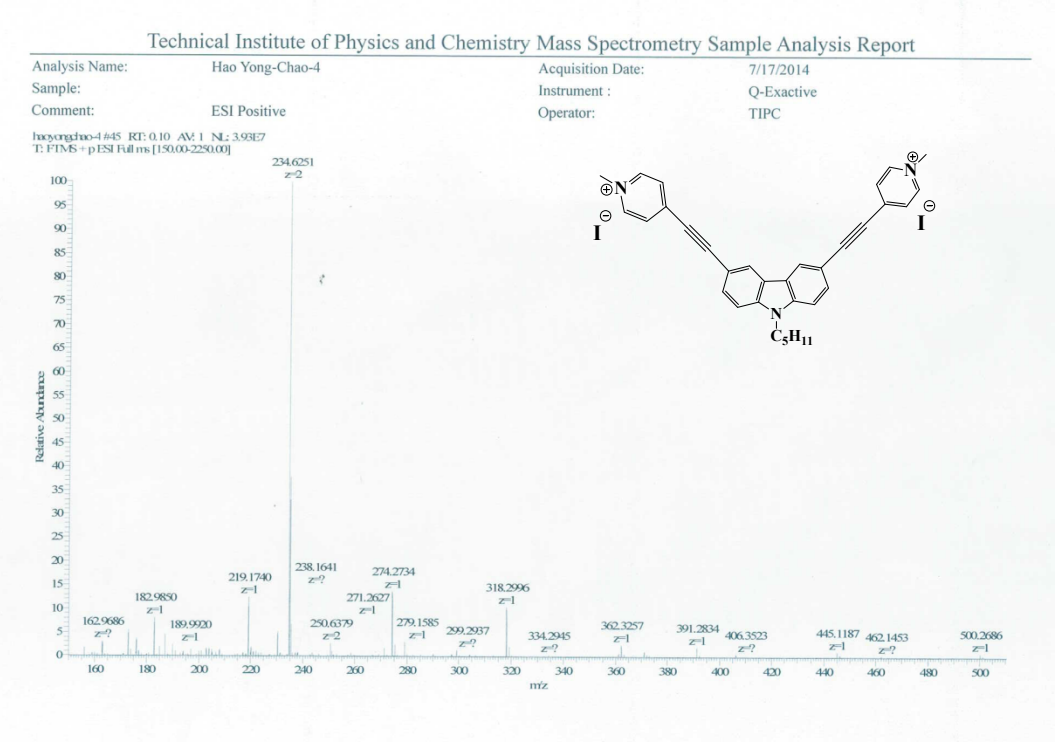
Scheme S1 Synthesis route of **BMEPC** and **BMEMC**.

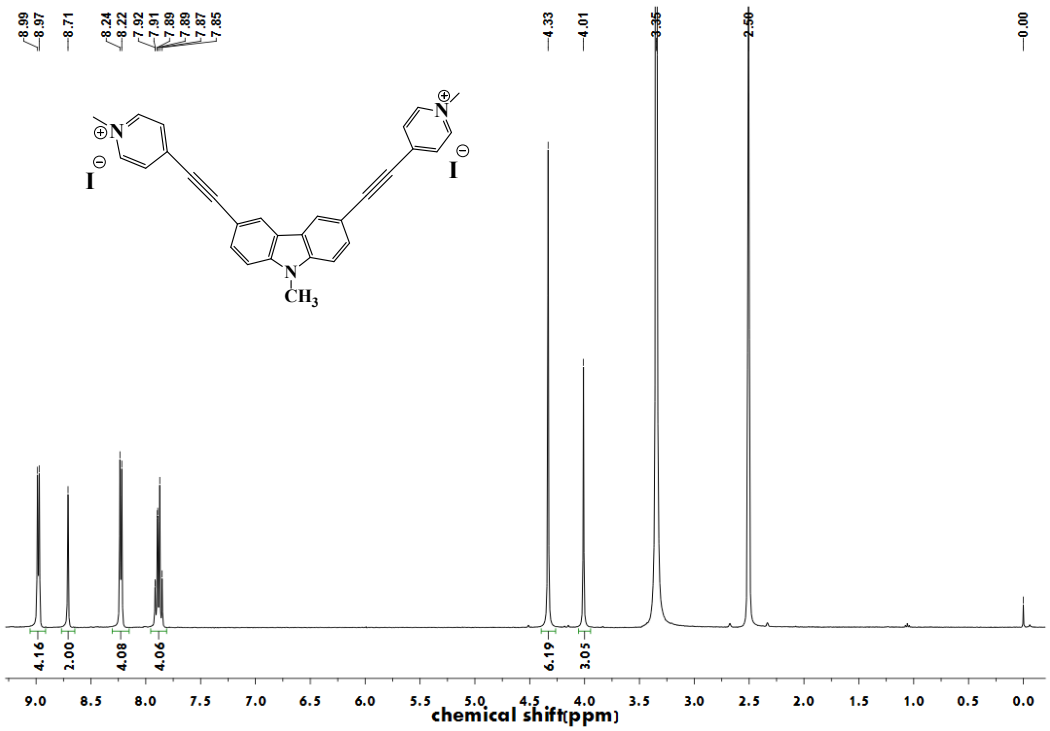
3,6-bis[2-(1-methylpyridinium)ethynyl]-9-pentyl-carbazole diiodide (BMEPC) Yield: 87.5%. ^1H NMR (400 MHz, DMSO-d6, ppm): 0.83 (t, 3 H), 1.30 (m, 4 H), 1.81 (t, 3 H, *J* = 6.8 Hz), 4.33 (s, 6 H), 4.52 (t, 2 H, *J* = 6.8 Hz), 7.88 (s, 4 H), 8.23 (d, 4 H, *J* = 6.8 Hz), 8.71 (s, 2 H), 8.98 (d, 4 H, *J* = 6.8 Hz).

MS (HR-ESI): m/z Calcd.: for $C_{33}H_{31}I_2N_3$ 234.6253 $[M-2I]^{2+}$; Found: 234.6251.

3,6-bis[2-(1-methylpyridinium)ethynyl]-9-methyl-carbazole diiodide (BMEMC) Yield: 85.3%. ^1H NMR (400 MHz, DMSO-d6, ppm): 4.01 (s, 3 H), 4.33 (s, 6 H), 7.89 (dd, 4 H, *J* = 8.8 Hz), 8.23 (d, 4 H, *J* = 6.8 Hz), 8.71 (s, 2 H), 8.98 (d, 4 H, *J* = 6.8 Hz). MS (HR-ESI): m/z Calcd.: for $C_{29}H_{23}I_2N_3$ 206.5940 $[M-2I]^{2+}$; Found: 206.5938.







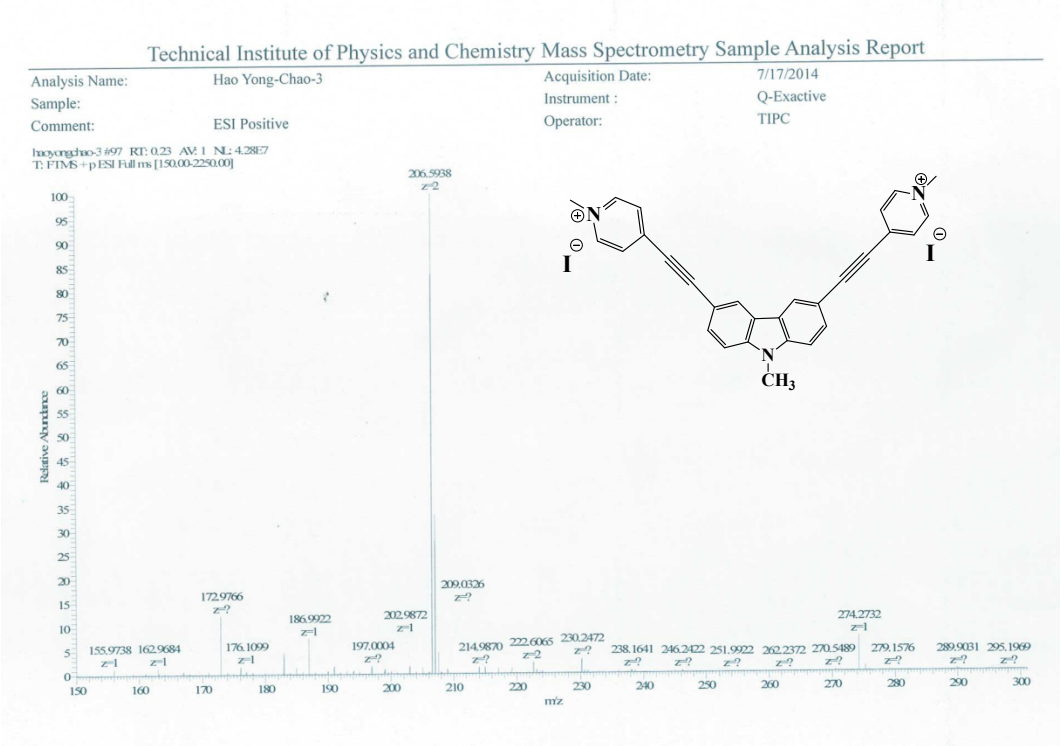


Fig. S1 ^{1}HNMR spectrum and HR-ESI MS spectrum of **BMEPC** and **BMEMC**.

3. Two-photon properties

The TPA cross sections were measured by nonlinear transmission measurement method.[2] The solutions of **BMEPC** and **BMEMC** at the concentration of 2 × 10^{-3} M in DMSO were used. The laser beam was divided into two parts. One was used as the intensity reference and monitored by 5020PE laser power and energy meter (Genetec.). The other was used for transmittance measurement, the laser beam was focused by passing through lens (f = 150 mm) and a quartz cell with a sample thickness of 1 cm.[3] The transmitted laser beam from the sample cell was then detected by the same power meter as used for reference monitoring. The peak intensity of the incident pulses at the focal point, I_0, ranged from 20 to 100 GW/cm^2. The nonlinear absorption coefficient β is obtained from Eq. (1):

$$T_i = \frac{ln(1 + I_0 L\beta)}{I_0 L\beta}$$

where T_i is transmissivity, I_0 is input light intensity, and L is the thickness of the cuvette with sample solution. T_i can be obtained from Eq. (2):

$$T_i = \frac{I_i}{I_0}$$

where I_i is light intensity after passing through the sample cell. The δ_{TPA} of sample is calculated by Eq. (3):

$$\delta_{TPA} = \left(\frac{h\nu\beta}{N_A}\right) \times d \times 10^{-3}$$

where N_A is the Avogadro constant, d is the concentration of compounds in solution, h is the Planck constant, and ν is the frequency of the incident laser beam.

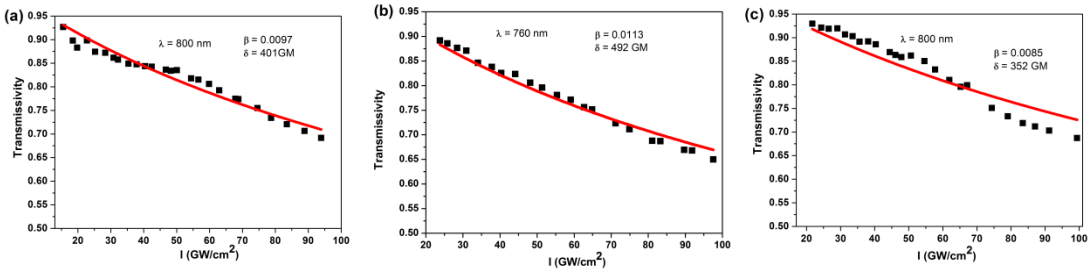


Fig. S2 The plot of transmissivity dependence on light intensity for **BMEPC** at (a) 800 nm, **BMEMC** at (b) 760 nm and (b) 800 nm. Squares denote the experimental value of transmissivity; solid lines denote the theoretical fitting line.

4. Absorption titration

The absorption spectra of **BMEPC** and **BMEMC** in the present of CT-DNA were performed at rt using a Hitachi U-3900 spectrophotometer by conventional quartz cells of 1 cm path. The CT-DNA with concentration ranged from 0 to 70 μM was titrated into 10 μM solutions of **BMEPC** and **BMEMC** in TE buffer. Each absorption spectrum was taken after stirring for 10 min.

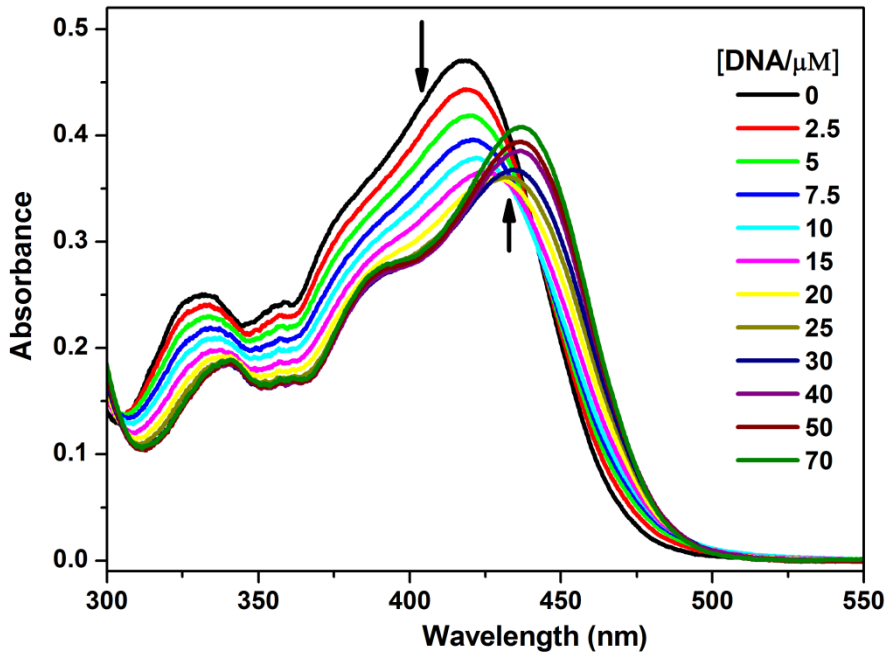


Fig. S3 The absorption spectra of **BMEMC** (10 μM) with the addition of CT-DNA (0-70 μM) in TE buffer.

5. Fluorescence titration to determine the binding constants

Fluorescence spectra were measured at rt using a Hitachi F-4600 spectrometer by quartz cells of 1 cm path. CT-DNA was added to the solutions of the **BMEPC** and **BMEMC** (1 μM) in TE buffer , leading to a concentration from 0 to 100 μM. Each fluorescence spectrum was taken after 10 min incubation. Then, the corresponding fluorescence spectra were measured (ex 410 nm, ex/em 5 nm/5 nm). The binding constants were derived from nonlinear curve fitting, using the following equation: [4]

$$F - F_0 = [(F_m - F_0)/2C_0]\{C_{DNA}/n + C_0 + 1/K_b - [(C_{DNA}/n + C_0 + 1/K_b)^2 - 4C_{DNA} \times C_0/n]^{1/2}\}$$

where C_{DNA} and C_0 are the concentrations of DNA and drugs, respectively; n represents the average number of binding sites of ligand to per DNA structure; F, F_0, and F_m represent the fluorescence intensities of the sample, **BMEPC** or **BMEMC** alone, and the intensity when drugs are totally bound, respectively.

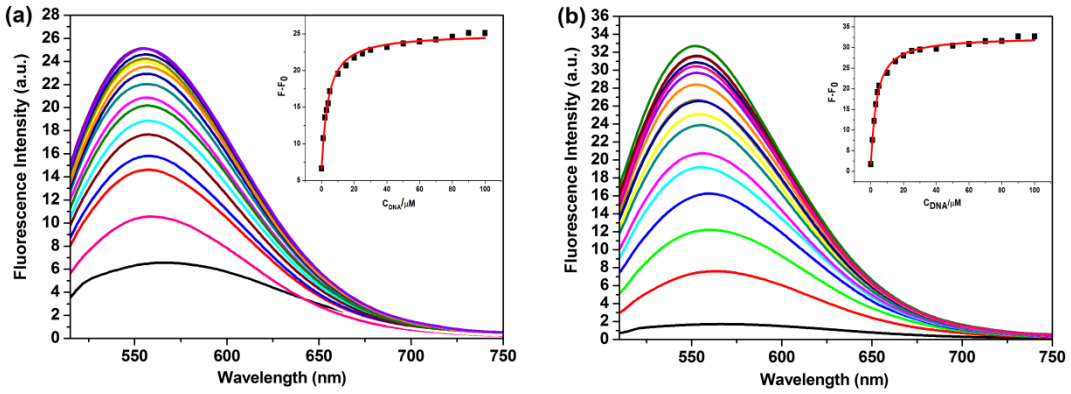


Fig. S4 Fluorescence spectra of (**a) BMEPC** (1 μM) and (b) **BMEMC** (1 μM) with different concentrations of CT-DNA from 0 to 100 μM. The insets show the fluorescence enhancement during titration and the results of K_b by non-linear fitting.

6. Agarose gel electrophoretic DNA photocleavage

DNA photocleavage abilities of **BMEPC** and **BMEMC** were evaluated by supercoiled pBR322 plasmid DNA as target. The mixture of 50 μL supercoiled pBR322 DNA (31 μM in base pair) in PBS buffer (pH 7.4) and 0.25 μL examined **BMEPC** or **BMEMC** in DMSO was irradiated under an Oriel 91192 Solar Simulator with a glass filter to cut off the light below 400 nm or 800 nm femtosecond (fs) laser pulses. After irradiation, 20 μL of gel loading buffer was added. The sample was then subjected to agarose gel (1 %) electrophoresis (Tris/acetic acid/EDTA buffer, pH 8.0) at 80 V for about 1.5 h. The gel was stained with 1 mg/L EB for 1 h, and then analyzed with a Gel Doc XR system (Bio-Rad).

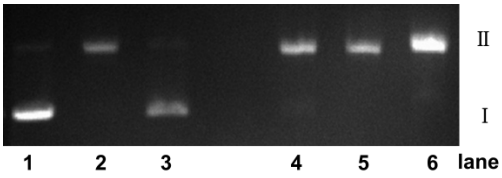


Fig. S5 Agarose gel electrophoresis patterns of the photocleaved supercoiled pBR322 DNA (31 μM in base pair) by **BMEPC (**20 μM) upon 800 nm femtosecond (fs) laser pulses for 35 min in air-saturated Tris/CH_3COOH/EDTA buffer (pH = 7.4). Lane 1, DNA alone (in dark); lane 2, DNA + **BMEPC** + irradiation; lane 3, DNA + **BMEPC** + NaN_3 (50 mM); lane 4, DNA + **BMEPC** + SOD (1000 U/mL); lane 5, DNA + **BMEPC** + mannitol (50 mM); lane 6, DNA + **BMEPC** in N_2 atmosphere. Form I and II denote supercoiled circular and nicked circular forms, respectively.

7. Electrochemical properties

Redox potentials were measured on an EG&G model 283 potentiostat/galvanostat in a three-electrode cell with a microdisc Pt working electrode, a Pt wire counter electrode, and a saturated calomel electrode (SCE) reference electrode. The cyclic voltammetry was conducted at a scan rate of 1.5 V/s in N_2-saturated, anhydrous DMSO containing 0.1 M tetra-n-butylammonium hexafluorophosphate as the supporting electrolyte.

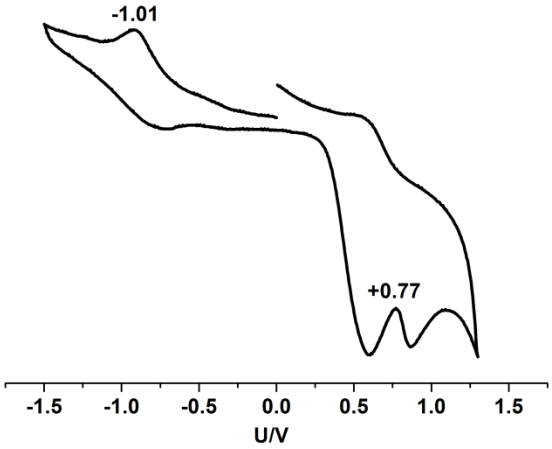


Fig. S6 Cyclic voltammograms of **BMEPC** in DMSO Vs SCE.

Guanine, which exhibits the lowest redox potential of one-electron oxidation (1.24 V vs SCE in acetonitrile), is the most easily oxidized DNA bases. Thus, the Gibbs free energy (ΔG) of the electron transfer was roughly estimated using the following Rehm–Weller equation:[5]

$$\Delta G = \left(E^{+} - E^{-}\right) - E(S_1)$$

where E (S1) is the S1 energy estimated from the fluorescence maximum (591 nm), E^{+} is the oxidation potential of the guanine (1.24 V vs SCE), and E^{-} is the reduction potential of **BMEPC** (−1.01 V vs SCE). The estimated value of ΔG (+0.15 eV) indicates it is impossible for electron transfer from guanine to the photoexcited **BMEPC** in terms of energy.[6] Moreover, the excited state lifetime of **BMEPC** is too short to be estimated by a LP-920 nanosecond laser flash photolysis setup (Edinburgh), excluding the possibility of electron transfer process from guanine.

8. References

1 J.-F. Xing, W.-Q. Chen, J. Gu, X.-Z. Dong, N. Takeyasu, T. Tanaka, X.-M. Duan and S. Kawata, *J. Mater. Chem.*, 2007, **17**, 1433.

2 C. Xu and W. W. Webb, *J. Opt. Soc. Am. B*, 1996, **13**, 481.

3 J.-F. Xing, W.-Q. Chen, X.-Z. Dong, T. Tanaka, X.-Y. Fang, X.-M. Duan and S. Kawata, *J. Photochem. Photobiol., A*, 2007, **189**, 398.

4 H. J. Y. Schneider, A. K., Principles and methods in supramolecular chemistry, John Wiley, New York, 2000, pp. 137-143.

5 D. Rehm and A. Weller, *Isr. J. Chem.*, 1970, **8**, 259.

6 K. Hirakawa, K. Ota, J. Hirayama, S. Oikawa and S. Kawanishi, *Chem. Res. Toxicol.*, 2014, **27**, 649.