

Electronic Supplementary Information

Metal-catalyzed uncaging of DNA-binding agents in living cells

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GENERAL

Synthesis. All reagents were acquired from commercial sources: DMF and TFA were purchased from *Scharlau*, CH₂Cl₂ from *Panreac*, CH₃CN from *Merck*. The rest of reagents were from *Sigma-Aldrich*. Chloro(1,5-cyclooctadiene)(pentamethylcyclopentadienyl)ruthenium(II) (**[Ru]**) was purchased from *Strem Chemicals*.

In addition to TLC analysis, some reactions were monitored by analytical RP-HPLC with an *Agilent 1100* series LC/MS using an *Eclipse XDBC18* (4.6 × 150 mm, 5 μm) analytical column. Compounds were detected by UV absorption (220, 270, 304 and 330 nm) and the standard conditions for analytical RP-HPLC consisted on a linear gradient from 5% to 95% of solvent B for 30 min at a flow rate of 1 mL/min (A: water with 0.1% TFA, B: acetonitrile with 0.1% TFA).

Final products **3**, **©3**, **©5** and **m-©5** were purified on a *Büchi Sepacore* preparative system consisting on a pump manager C-615 with two pump modules C-605 for binary solvent gradients, a fraction collector C-660, and UV Photometer C-635. Purification of **3**, **©3**, and **m-©5** was made using reverse phase conditions with an isocratic regime during the first 5 min at 5% of solvent B, followed by a linear gradient from 5% to 75% of solvent B for 30 min at a flow rate of 30 mL/min (A: water with 0.1% TFA, B: methanol with 0.1% TFA) and in the case of **©5** with an isocratic regime during the first 5 min at 10% of solvent B, followed by a linear gradient from 10% to 95% of solvent B for 30 min at a flow rate of 30 mL/min, using a pre-packed preparative cartridge (150 × 40 mm) with reverse phase RP18 silica gel (*Büchi* catalog #: 54863). Final products **m-©3**, **6**, **©4** and **m-©4** were purified by RP-HPLC with an *Agilent 1100* series equipped with a binary pump system and a UV-visible detector using a *Phenomenex Luna C18 100A* (250 × 10 mm, 5 μm) semipreparative column. Purification was made using reverse phase with an isocratic regime during the first 5 min at 5% of solvent B and then a linear gradients from 5% to 75% of solvent B for 30 min at a flow rate of 30 mL/min (A: water with 0.1% TFA, B: acetonitrile with 0.1% TFA). The fractions containing the products were freeze-dried, and their identity confirmed by ESI⁺-MS and NMR. Compounds were isolated as TFA salts after HPLC purification.

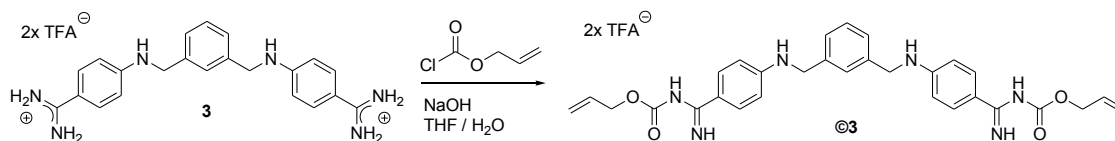
Fluorescence Spectroscopy

Measurements were made at 20 °C with a *Jobin-Yvon Fluoromax-3* coupled to a *Wavelength Electronics LFI-3751* temperature controller, using the following settings: increment: 1.0 nm; integration time, 0.2 s; excitation slit width, 3.0 nm; emission slit width, 6.0 nm. For the experiments with **3**, **©3**, **m-©3** and **6**, the excitation wavelength was set to 329 nm, and the emission spectra were acquired from 345 to 500 nm. For the experiments with DAPI (**4**), **©4** and **m-©4**, the excitation wavelength was set to 360 nm, and the emission spectra were acquired from 380 to 600 nm. For the experiments with ethidium bromide (**5**), the excitation wavelength was set to 545 nm, and the emission spectra were acquired from 555 to 700 nm. For the experiments with **©5** and **m-©5** the excitation wavelength was set to 300 nm, and the emission spectra were acquired from 310 to 590 nm.

Hairpin oligonucleotides were supplied by *Thermo Fischer* and their sequences were: **h-A3·T3**: 5'-GGC AAATTT CAG TTTTT CTG AAATTT GCC-3'; **h-A2·T2**: 5'-GGCG AATT CAGC TTTTT GCTG AATT CGCC-3'; **h-AGA**: 5'-GGCG AGA TTCGC TTTTT GCGAA TCT CGCC-3'; **h-GC**: 5'-GGCA GGCC CAGC TTTTT GCTG GGCC TGCC-3'

SYNTHESIS OF THE PROTECTED DERIVATIVES

Synthesis of ©3



In a round bottom flask containing **3**¹ (365 mg, 0.61 mmol, trifluoroacetic disalt) in a 1:1 mixture of THF/water (6 mL), an aqueous solution of NaOH (1.33 mmol, 270 μ L of a 5 M solution) was added until **3** was completely dissolved. Allyl chloroformate (146 mg, 1.2 mmol) was added dropwise, after 2 hours, the reaction mixture was directly purified on a *Büchi Sepacore* preparative system and the appropriate fractions were collected and freeze-dried to provide ©3 as a white solid (397 mg, 85%).²

¹H NMR (400 MHz, MeOD-*d*₄ δ): 7.62 (d, *J* = 9.2 Hz, 4H), 7.36 (s, 1H), 7.24-7.32 (m, 3H), 6.74 (d, *J* = 9.1 Hz, 4H), 6.04 (tdd, *J* = 17.2, 10.5, 5.9 Hz, 2H), 5.46 (qd, *J* = 17.2, 1.5 Hz, 2H), 5.34 (qd, *J* = 10.5, 1.2 Hz, 2H), 4.82-4.84 (m, 4H), 4.44 (s, 4H).

¹³C NMR (MeOD-*d*₄ δ): 165.9 (C), 156.6 (C), 154.8 (C), 140.4 (C), 132.4 (CH), 132.0 (CH), 130.1 (CH), 127.2 (CH), 127.1 (CH), 120.2 (CH₂), 113.4 (CH), 112.8 (C), 69.2 (CH₂), 47.5 (CH₂).

ESI⁺-MS: [M+H⁺] calc. for C₃₀H₃₃N₆O₄ = 541.2558 found 541.2524; C₃₄H₃₄F₆N₆O₈ (MW 768.6596).

UV (MeCN) λ_{max} (ϵ): 332 nm (33000 M⁻¹cm⁻¹)

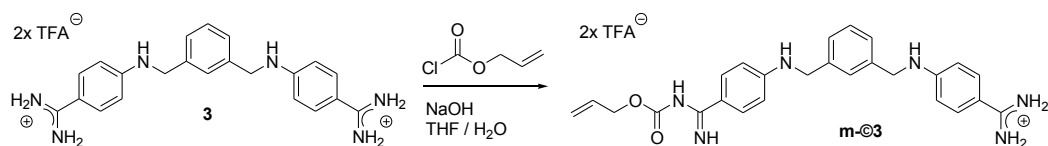
Retention time: 12.7 min.

After one week of storage in the fridge there are no signs of decomposition of product **3**.

¹ The synthesis of **3** is described in ref. 13 of the main manuscript (*Chem. Sci.* **2012**, *3*, 2383-2387)

² The yield was calculated considering the isolation of the products as trifluoroacetic salts (TFA is used for purifications); the same consideration was used for similar products.

Synthesis of m-©3



In a round bottom flask containing **3** (70 mg, 0.12 mmol, trifluoroacetic disalt) in a 1:1 mixture of THF/water (1.2 mL), a solution of NaOH (1.33 mmol, 270 μ L of a 5 M solution in water) was added until **3** was completely dissolved, and then allyl chloroformate (14 mg, 0.12 mmol) was added. After 2 hours the reaction crude obtained after removal of the solvents was purified by RP-HPLC^[1] and freeze-dried to provide **m-©3** (30 mg, 45%) as a white solid.

¹H NMR (400 MHz, MeOD-*d*₄ δ): 7.62 (d, *J* = 9.1 Hz, 2H), 7.57 (d, *J* = 9.0 Hz, 2H), 7.36 (s, 1H), 7.23-7.30 (m, 3H), 6.74 (d, *J* = 9.1 Hz, 2H), 6.69 (d, *J* = 9.0 Hz, 2H), 6.04 (tdd, *J* = 17.2, 10.5, 5.9 Hz, 1H), 5.46 (qd, *J* = 17.2, 1.5 Hz, 1H), 5.34 (ddd, *J* = 10.5, 2.4, 1.2 Hz, 1H), 4.83 (td, *J* = 5.9, 1.3 Hz, 2H, partial overlapping with water signal), 4.44 (s, 2H), 4.41 (s, 2H).

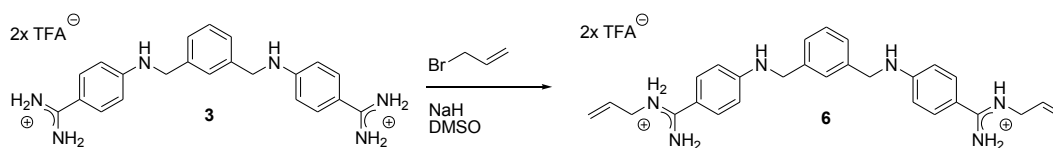
¹³C NMR (MeOD-*d*₄ δ): 167.1 (C), 165.8 (C), 156.6 (C), 155.4 (C), 154.8 (C), 140.8 (C), 140.3 (C), 132.4 (CH), 132.0 (CH), 130.6 (CH), 130.0 (CH), 127.2 (CH), 127.1 (CH), 127.0 (CH), 120.2 (CH₂), 114.3 (C), 113.4 (CH), 113.2 (CH), 112.8 (C), 69.2 (CH₂), 47.6 (CH₂), 47.5 (CH₂).

ESI⁺-MS: [M+H⁺] calc. for C₂₆H₃₀N₆O₂ = 457.2347 found 457.2341; C₃₀H₃₀F₆N₆O₆ (MW 684.5862).

UV (MeCN) λ_{max} (ϵ): 327 nm, (46000 M⁻¹cm⁻¹)

Retention time: 11.4 min.

Synthesis of 6



Bisbenzamidinium **3** (50 mg, 0.083 mmol) was dissolved in 850 μL of DMSO in a round bottom flask; NaH (13 mg, 0.33 mmol) and allyl bromide (22 mg, 0.18 mmol) were added and the resulting mixture stirred for 1 h, until HPLC analysis showed that the desired product was the major component in the reaction mixture. Water (0.5 mL) was added to quench the reaction, and the residue obtained after removal of the solvents was purified by RP-HPLC to provide **6** as (19 mg, 34%) as a white solid.

^1H NMR (400 MHz, *MeOD-d*₄ δ): 7.52 (d, J = 8.9 Hz, 4H), 7.37 (s, 1H), 7.24-7.31 (m, 3H), 6.71 (d, J = 8.9 Hz, 4H), 5.95 (tdd, J = 17.2, 10.3, 5.1 Hz, 2H), 5.32 (ddd, J = 10.4, 2.6, 1.5 Hz, 2H), 5.28 (dt, J = 2.6, 1.5 Hz, 2H), 4.40 (s, 4H), 4.05 (td, J = 5.1, 1.6 Hz, 4H).

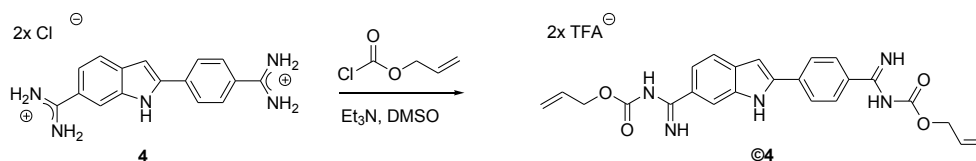
^{13}C NMR (*MeOD-d*₄ δ): 165.1 (C), 155.1 (C), 140.8 (C), 132.2 (CH), 130.4 (CH), 129.9 (CH), 127.1 (CH), 127.0 (CH), 117.9 (CH₂), 115.7 (C), 113.2 (CH), 47.6 (CH₂), 45.7 (CH₂).

ESI⁺-MS: [$\text{M}+\text{H}^+$] calc. for C₂₈H₃₇N₆ = 453.2761 found 453.2760; C₃₂H₃₈F₆N₆O₄ (MW 684.6723).

UV (H₂O) λ_{max} (ϵ): 311 nm, (39000 M⁻¹cm⁻¹).

Retention time: 13.6 min

Synthesis of ©4



DAPI (**4**, 5 mg, 14.3 μmol , dihydrochloride salt) and Et_3N (60 μL , 0.43 mmol) were dissolved in DMSO (285 μL) in an eppendorf tube. Allyl chloroformate (17 mg, 0.143 mmol) was slowly added, and the mixture was shaken overnight. After checking by HPLC-MS that all the starting material was consumed, the reaction crude was concentrated and the residue purified by RP-HPLC. The appropriate fractions were collected and freeze-dried to provide ©4 as a bright yellow solid (7 mg, 75%).

^1H NMR (400 MHz, *MeOD-d₄* δ): 8.16 (d, $J = 8.4$ Hz, 2H) 8.02 (s, 1H), 7.96 (d, $J = 8.4$ Hz, 2H), 7.86 (d, $J = 8.4$ Hz, 1H), 7.50 (dd, $J = 8.4, 1.4$ Hz, 1H), 7.27 (s, 1H), 6.03-6.01 (m, 2H), 5.50 (dd, $J = 17.2, 3.2$ Hz, 2H), 5.37 (dd, $J = 10.2, 4.7$ Hz, 2H), 4.90 (s, 2H, overlapping with water signal) 4.88 (s, 2H, overlapping with water signal).

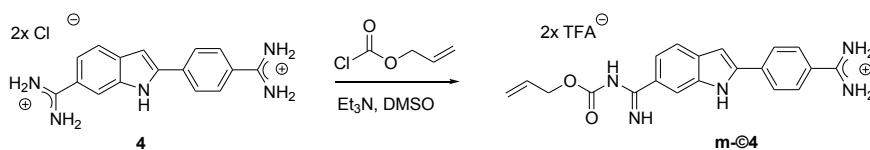
^{13}C NMR (*MeOD-d₄* δ): 168.6 (C), 167.7 (C), 155.2 (C), 154.7 (C), 142.4 (C), 138.8 (C), 138.3 (C) 135.4 (C), 132.4 (CH), 132.3 (CH), 130.7 (CH), 128.6 (C), 127.3 (CH), 122.9 (CH), 121.7 (C), 120.6 (CH), 120.3 (CH₂), 120.2 (CH₂), 114.3 (CH), 103.3 (CH), 69.4 (CH₂), 69.3 (CH₂).

ESI⁺-MS: $[\text{M}+\text{H}^+]$ calc. for $\text{C}_{24}\text{H}_{24}\text{N}_5\text{O}_4 = 446.1750$ found 446.1831; $\text{C}_{28}\text{H}_{25}\text{F}_6\text{N}_5\text{O}_8$ (MW 673.5172).

UV (MeCN) λ_{max} (ϵ): 370 nm, (48000 $\text{M}^{-1}\text{cm}^{-1}$)

Retention time: 10.6 min.

Synthesis of m-④



DAPI (**4**, 5 mg, 14.3 μmol , dihydrochloride salt) and Et₃N (60 μL , 0.428 mmol) were dissolved in DMSO (285 μL) in an eppendorf tube. Allyl chloroformate (13 mg, 0.106 mmol) was slowly added, and mixture was shaken overnight. After checking by HPLC-MS the conversion into the desired product, the reaction crude obtained after concentration was purified by RP-HPLC (gradient: 5% B 5 min, 5% to 75 % B 30 min), and the appropriate fractions were collected, concentrated and freeze-dried to provide the trifluoroacetate salt of **m-④** as a bright-yellow solid (3 mg, 36%).

¹H NMR (400 MHz, MeOD-*d*₄ δ): 8.15 (d, $J = 8.4$ Hz, 2H), 7.98 (s, 1H), 7.95 (d, $J = 8.5$ Hz, 2H), 7.82 (d, $J = 8.4$ Hz, 1H), 7.48 (dd, $J = 8.3, 1.4$ Hz, 1H), 7.24 (s, 1H), 6.07 (dq, $J = 11.4, 11.1, 5.8$ Hz, 1H), 5.49 (dd, $J = 17.2, 1.1$ Hz, 1H), 5.37 (dd, $J = 10.33, 0.70$ Hz, 1H).

The two H from the methylene group are hidden under the water signal.

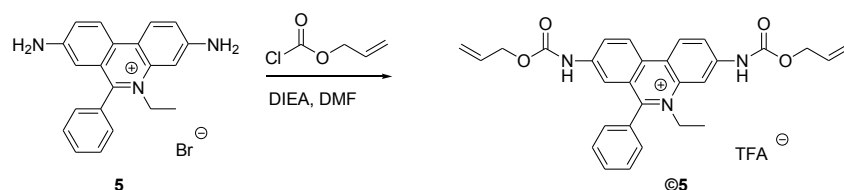
¹³C NMR (MeOD-*d*₄ δ): 169.2 (C), 167.8 (C), 141.5 (C), 139.1 (C), 138.5 (C), 134.6 (C), 132.3 (C), 130.7 (C), 129.9 (C), 127.4 (C), 127.1 (CH), 123.0 (C), 122.7 (CH), 120.2 (CH₂), 119.9 (CH), 113.2 (CH), 103.2 (CH), 69.4 (CH₂).

ESI⁺-MS: [M+H⁺] calc. for C₂₀H₂₀N₅O₂ = 362.1617 found 362.1609; C₂₄H₂₁F₆N₅O₆ (MW 589.4439).

UV (MeCN) λ_{max} (ϵ): 350 NM, (40000 M⁻¹cm⁻¹).

Retention time: 9.1 min.

Synthesis of ©5



Allyl chloroformate (185 mg, 1.52 mmol) was slowly added to a suspension of ethidium bromide (**5**, 80 mg, 0.203 mmol) in DIEA/DMF (4 mL, 0.195 M DIEA). The reaction mixture was stirred overnight, and the crude after concentration was purified on a *Büchi Sepacore* preparative system. The appropriate fractions were collected and freeze-dried to provide ©5 as an orange solid (70 mg, 58%).

¹H NMR (400 MHz, *MeOD-d₄* δ): 8.98 (dd, *J* = 9.2, 2.7 Hz, 1H), 8.90-8.92 (m, 2H), 8.27 (d, *J* = 9.1 Hz, 1H), 7.98 (d, *J* = 9.1 Hz, 1H), 7.79-7.87 (m, 4H), 7.70 (d, *J* = 1.3 Hz, 1H), 7.68 (d, *J* = 1.6 Hz, 1H), 6.05 (tdd, *J* = 16.4, 10.9, 5.5 Hz, 1H), 5.94 (tdd, *J* = 16.2, 10.7, 5.5 Hz, 1H), 5.38 (ddd, *J* = 20.7, 18.2, 1.5 Hz, 2H), 5.25 (ddd, *J* = 29.2, 10.6, 1.4 Hz, 2H), 4.82 (q, partial overlapping with water signal, 2H), 4.75 (dd, *J* = 5.5, 1.3 Hz, 2H), 4.59 (dd, *J* = 5.5, 1.3 Hz, 2H), 1.62 (t, *J* = 7.2 Hz, 3H).

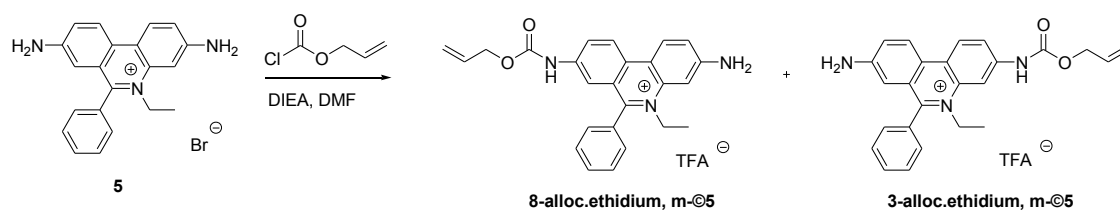
¹³C NMR (*MeOD-d₄* δ): 164.6 (C), 155.4 (C), 155.1 (C), 144.0 (C), 141.6 (C), 135.6 (C), 133.8 (CH), 132.8 (C), 132.7 (CH), 132.3 (C), 130.8 (CH), 130.4 (CH), 129.4 (CH), 127.2 (C), 126.5 (CH), 124.6 (CH), 123.1 (CH), 119.4 (CH), 118.5 (CH₂), 118.2 (CH₂), 107.7 (CH), 67.1 (CH₂), 66.8 (CH₂), 51.7 (CH₂), 14.7 (CH₃). There is an overlapping of the allylic CH and two C.

ESI⁺-MS: [M⁺] calc. for C₂₉H₂₈N₃O₄⁺ = 482.2074 found 482.2071; C₃₁H₂₈F₃N₃O₆ (M.W. 595.5657).

UV (MeCN) λ_{max} (ε): 287 nm, (39000 M⁻¹cm⁻¹).

Retention time: 18.5 min.

Formation of mono-protected ethidium derivatives (m-**5**)



Allyl chloroformate (12 mg, 0.1 mmol) was added to a suspension of ethidium bromide (**5**, 75 mg, 0.192 mmol) in DIEA/DMF (2 mL, 0.195 M DIEA). The reaction mixture was stirred overnight, and the crude obtained after concentration was purified on a *Büchi Sepacore* preparative system. The appropriate fractions were collected and freeze-dried to provide the mixture of the two **m-5** isomers as a purple solid (32 mg, 65%).

ESI⁺-MS: [M^+] calc. for $C_{25}H_{24}N_3O_2 = 398.1861$ found 398.1863; $C_{31}H_{28}F_3N_3O_6$ (MW 511.4924).

UV (MeCN) λ_{max} (ϵ): 288 nm, (63000) $M^{-1}cm^{-1}$

Retention time: 16.1 min.

DNA BINDING STUDIES BY FLUORESCENCE SPECTROSCOPY

DNA binding of ©3 and monoprotected m-©3

To 1 mL of a 0.5 μM solution of ©3 in 20 mM Tris-HCl buffer pH 7.5, 100 mM NaCl, aliquots of a $\approx 450 \mu\text{M}$ stock solution of *h*-A3·T3 were successively added, and the emission spectra were measured after each addition.

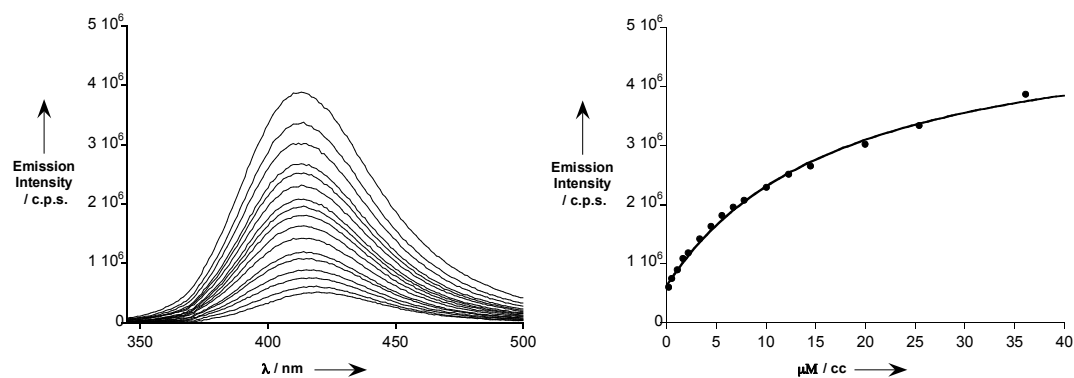


Figure S1: Left: fluorescence emission spectra of ©3 in the presence of successive aliquots of *h*-A3·T3. Right: Plot of the fluorescence emission at 415 nm against the total ds-DNA concentration and best-fit curve to a 1:1 binding mode. Estimated $K_d = 21 \pm 8.2 \mu\text{M}$.

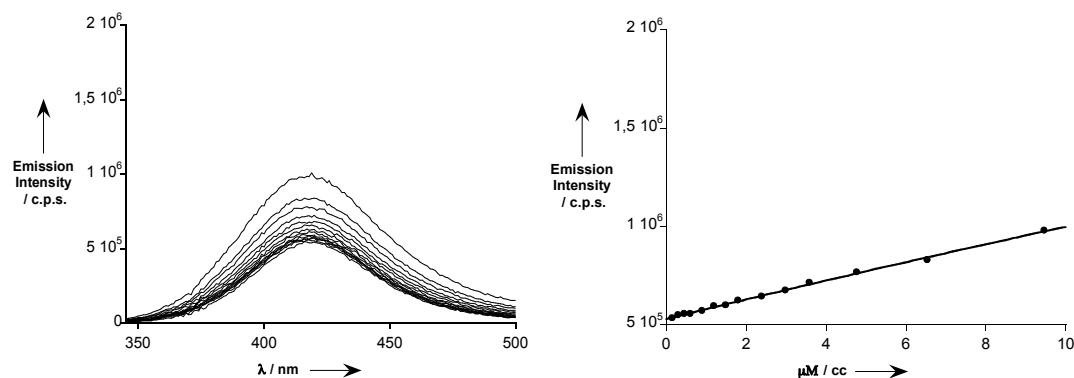


Figure S2: Left: fluorescence emission spectra of ©3 in the presence of successive aliquots of *h*-G·C. Right: Plot of the fluorescence emission at 415 nm against the total ds-DNA concentration.

To 1 mL of a 0.5 μM solution of **m-©3** in 20 mM Tris-HCl buffer pH 7.5, 100 mM NaCl, aliquots of a $\approx 450 \mu\text{M}$ stock solution of *h-A3·T3* were successively added, and the fluorescence spectra were recorded after each addition.

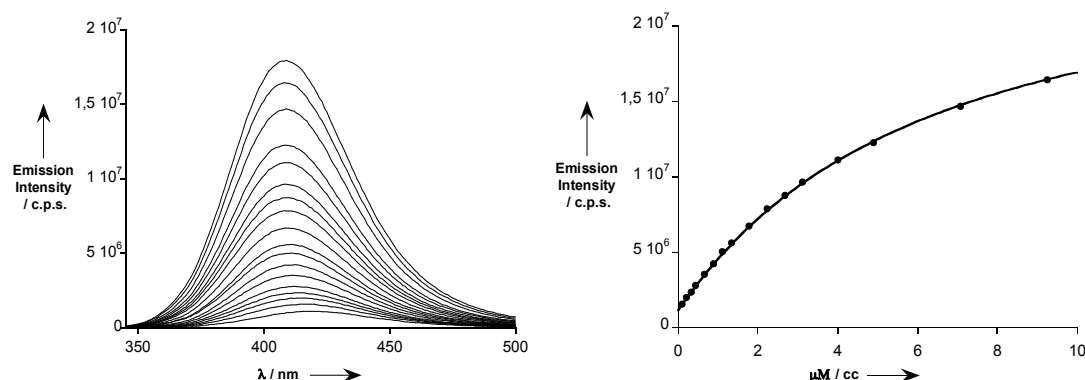


Figure S3: Left: fluorescence emission spectra of **m-©3** in the presence of successive aliquots of *h-A3·T3*. Right: Plot of the fluorescence emission at 410 nm against the total ds-DNA concentration and best-fit curve to a 1:1 binding mode. Estimated $K_d = 6.0 \pm 0.1 \mu\text{M}$.

DNA binding of the diallyl derivative **6**

To 1 mL of a 0.5 μM solution of **6** in Tris-HCl buffer 20 mM pH 7.5, 100 mM NaCl, aliquots of a $\approx 450 \mu\text{M}$ stock solution of *h-A3·T3* were successively added, and the fluorescence spectra was recorded after each addition.

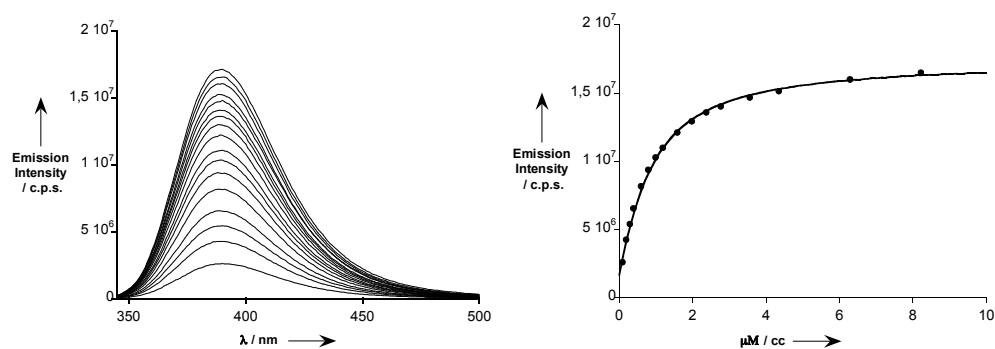


Figure S4: Left: fluorescence emission spectra of **6** in the presence of successive aliquots of *h-A3·T3*. Right: Plot of the fluorescence emission at 389 nm against the total ds-DNA concentration and best-fit curve to a 1:1 binding mode. Estimated $K_d = 0.46 \pm 0.03 \mu\text{M}$.

DNA binding of the DAPI derivatives **©4** and **m-©4**

To 1 mL of a 0.2 μM solution of **©4** in 20 mM Tris-HCl buffer pH 7.5, 100 mM NaCl, aliquots of a $\approx 400 \mu\text{M}$ stock solution of *h-A2·T2* were successively added, and the fluorescence spectra were recorded after each addition.

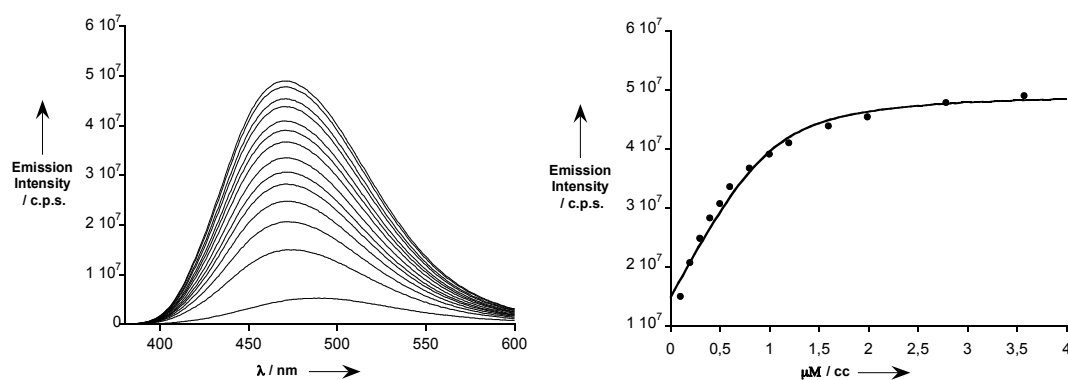


Figure S5: Left: fluorescence emission spectra of **4** in the presence of successive aliquots of *h*-**A2·T2**. Right: Plot of the fluorescence emission at 470 nm against the total ds-DNA concentration and best-fit curve to a 1:1 binding mode. Estimated $K_d = 407.1 \pm 10.5$ nM.

To 1 mL of a 0.2 μM solution of **m-4** in 20 mM Tris-HCl buffer pH 7.5, 100 mM NaCl, aliquots of a ≈ 400 μM stock solution of *h*-**A2·T2** were successively added, and the fluorescence spectra was recorded after each addition.

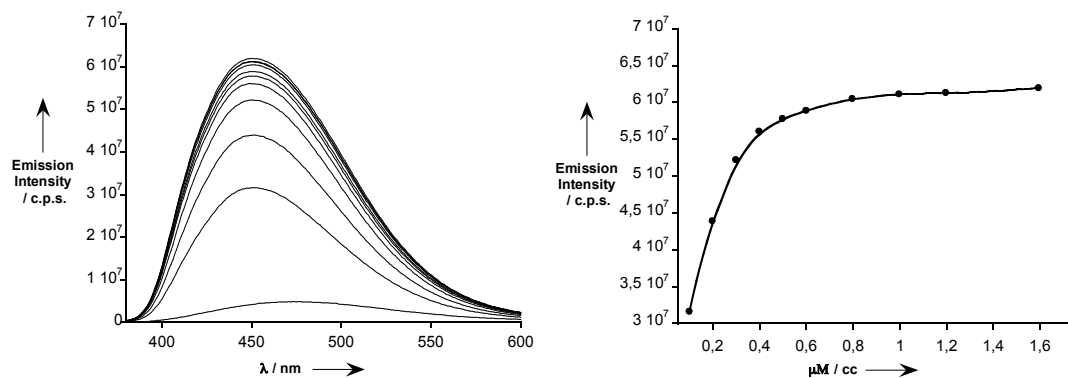


Figure S6: Left: fluorescence emission spectra of **m-4** in the presence of successive aliquots of *h*-**A2·T2**. Right: Plot of the fluorescence emission at 470 nm against the total ds-DNA concentration and best-fit curve to a 1:1 binding mode. Estimated $K_d = 49.1 \pm 5.9$ nM.

DNA binding of ethidium and protected derivatives

To 1 mL of a 1.0 μM solution of **5** in 20 mM Tris-HCl buffer pH 7.5, 100 mM NaCl, aliquots of a ≈ 500 μM stock solution of *h*-**AGA** were successively added, and the fluorescence spectra was recorded after each addition.

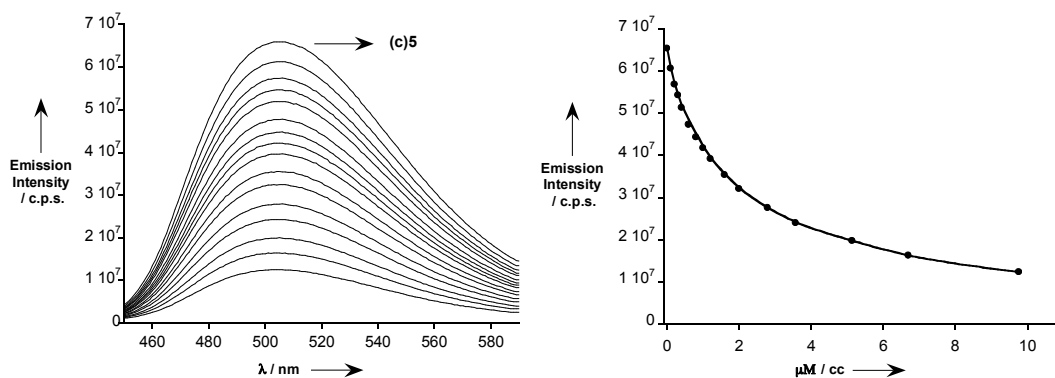


Figure S7: Left: fluorescence emission spectra of ©5 in the presence of successive aliquots of *h*-AGA. Right: Plot of the fluorescence emission at 510 nm against the total ds-DNA concentration and best-fit curve to a 1:1 binding mode. Estimated $K_d \approx 1.8 \mu\text{M}$.

In order to compare the DNA binding ability of the caged derivative ©5 with that of the parent ethidium (5) we also did a direct titration of 5 under the same conditions, which led to an approximately $K_D \approx 0.1 \mu\text{M}$. To 0.1 mL of a 0.25 μM solution of 5 in 20 mM Tris-HCl buffer pH 7.5, 100 mM NaCl, aliquots of a $\approx 100 \mu\text{M}$ stock solution of *h*-AGA were successively added, and the fluorescence spectra was recorded after each addition.

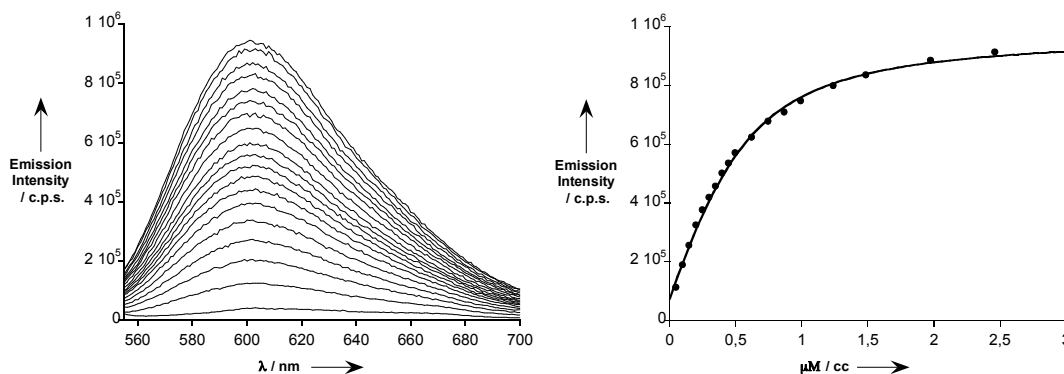


Figure S8: Left: fluorescence emission spectra of 5 in the presence of successive aliquots of *h*-AGA. Right: Plot of the fluorescence emission at 605 nm against the total ds-DNA concentration and best-fit curve to a 1:1 binding mode. Estimated $K_d = 0.10 \pm 0.02 \mu\text{M}$.

We also performed a competition titration with ethidium bromide (5) by adding successive aliquots of a stock solution of ©5 were successively added to 1 mL of a 3 μM solution of 5 in 20 mM Tris-HCl pH 7.5 buffer, 100 mM NaCl in the presence of 1.0 μM of *h*-AGA. Final point is approximately 6 equivalents of ©5.

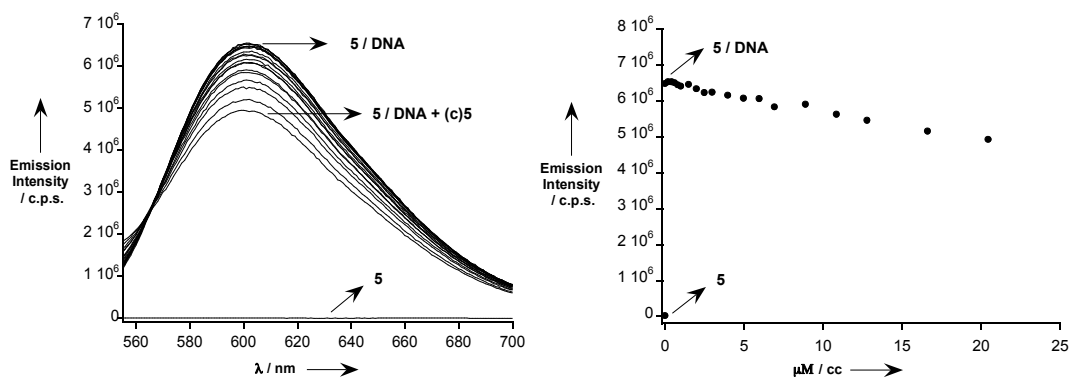


Figure S9: Left: Fluorescence emission spectra of a mixture of **5** and *h*-AGA in the presence of increasing amounts of ©**5**. Right: Emission at 605 nm of the competition titration. The fluorescence emission of **5** in absence of DNA is also indicated as reference.

A similar competition experiment with ethidium bromide was carried out with **m**-©**5**. A stock solution of monoprotected derivatives **m**-©**5** was successively added to 1 mL of a 3 μM solution of **5** in 20 mM Tris-HCl pH 7.5 buffer, 100 mM NaCl, in the presence of 1.0 μM of *h*-AGA. Final point is approximately 6 equivalents of **m**-©**5**.

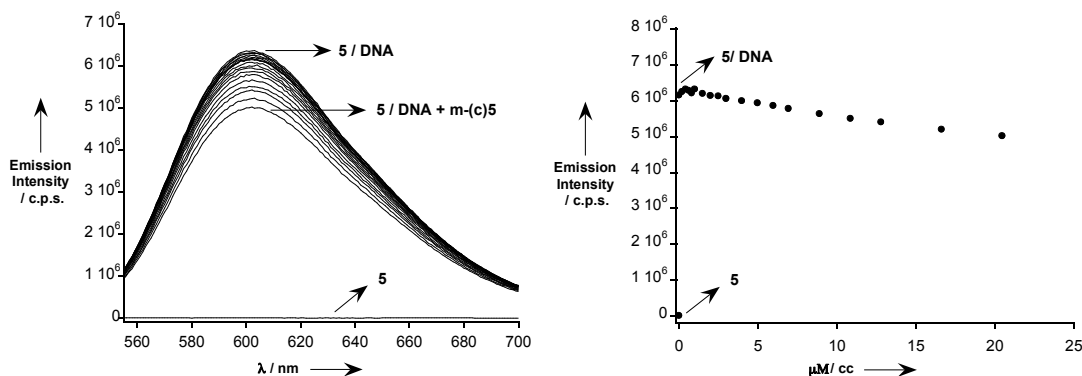


Figure S10: Left: Fluorescence emission spectra of a mixture of **5** and *h*-AGA in the presence of increasing amounts of **m**-©**5**. Right: Emission at 605 nm of the competition titration.

DNA binding of the ruthenium complex

A similar displacement experiment of ethidium bromide was carried out with the ruthenium complex **[Ru]**. A stock solution of **[Ru]** was successively added to 1 mL of a 3 μM solution of **5** in 20 mM Tris-HCl pH 7.5 buffer, 100 mM NaCl in the presence of 1.0 μM of *h*-AGA. Final point is approximately 12 equivalents of the ruthenium complex.

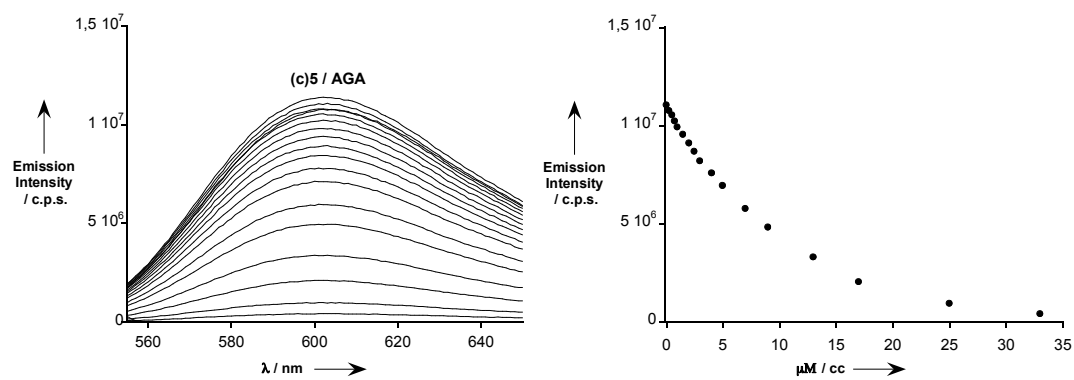
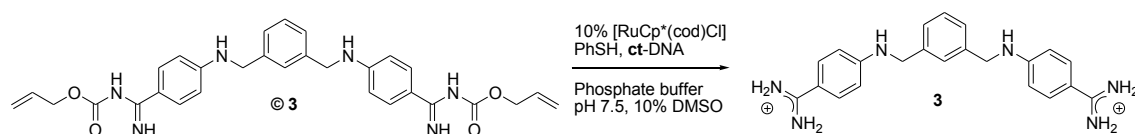


Figure S11: **Left:** Fluorescence emission spectra of a mixture of **5** and *h*-AGA in the presence of increasing amounts of [Ru]. **Right:** Emission at 605 nm of the competition titration.

UNCAGING EXPERIMENTS

Ruthenium-catalyzed deprotection of ©3 in presence of calf-thymus DNA



Diprotected derivative ©3 (10 mg, 0.013 mmol) was dissolved in 146 μL of 100 mM phosphate buffer pH 7.5 in an eppendorf tube, ct-DNA (10 μL , 10 mg/mL). Thiophenol (7 μL , 0.068 mmol) and Cp*Ru(cod)Cl ([Ru], 12 μL , 1.32 μmol) of a 0.11 M fresh DMSO stock solution) were added, and the reaction mixture was shaken for 20 min. The deprotection reaction was monitored by HPLC-MS.

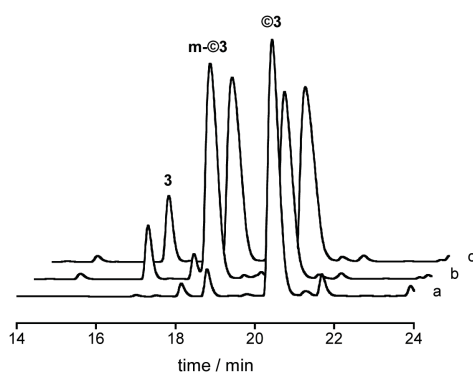
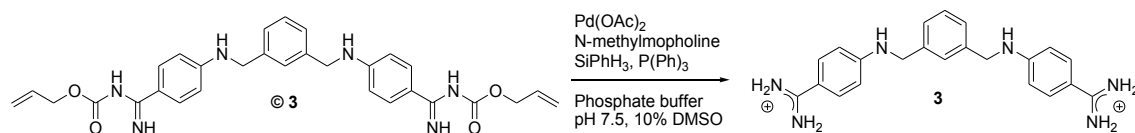


Figure S12: HPLC traces (222 nm detection) of the above reaction: a) ©3 + PhSH; b) ©3 + PhSH + [Ru], $t = 1$ min; c) ©3 + PhSH + [Ru], $t = 20$ min.

Deprotection reaction of ©3 with a Pd catalyst



In an eppendorf tube ©3 (10 mg, 0.013 mmol) was dissolved in 130 μL of 100 mM phosphate buffer pH 7.5. Then 12 μL of a fresh DMSO stock solution of Pd(OAc)₂ (5 mg in 200 μL), *N*-methylmorpholine (14 μL , 0.13 mmol), SiPh₃ (16 μL , 0.13 mmol,) and PPh₃ (5 mg, 0.0195 mmol) were added to the reaction mixture. The reaction was shaken for 5 min, and HPLC-MS revealed that the starting material was cleanly converted into desired product (3). Its identity was confirmed by HPLC-MS.

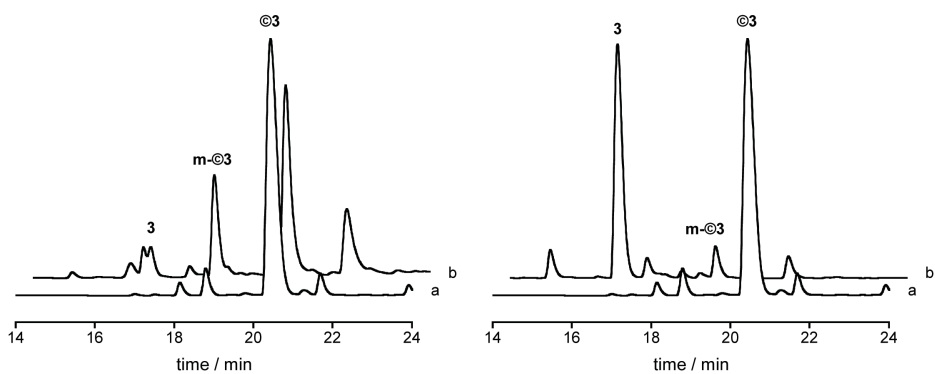
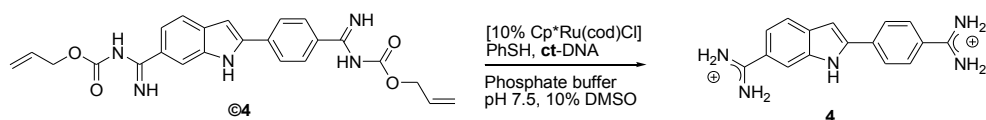


Figure S13: **Left:** HPLC trace (222 nm detection) of **3** deprotection reaction before (a) and after the addition of Pd(OAc)₂ (b). **Right:** HPLC trace (222 nm detection) of **3** deprotection reaction before (a) and after the addition of Pd(OAc)₂, PPh₃, PhSiH₃ and *N*-methylmorpholine (b).

Catalytic uncaging of ©4



Dialloc ©4 (1.3 mg, 1.93 μmol) was dissolved in 75 μL of phosphate buffer 100 mM pH 7.5 in an eppendorf tube. ct-DNA (10 μL of a solution 10 mg/mL), thiophenol (1.3 μL , 12.70 mmol) and Cp*Ru(cod)Cl ([Ru], 4.2 μL , 0.252 μmol) of 0.06 M fresh prepared DMSO stock solutions were added to the mixture. The reaction mixture was shaken for 5 min until HPLC-MS analysis confirmed that almost all the starting material was converted into desired product (4).

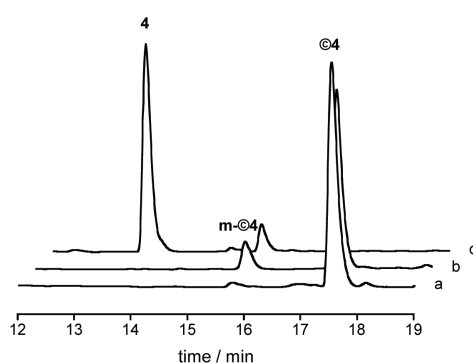
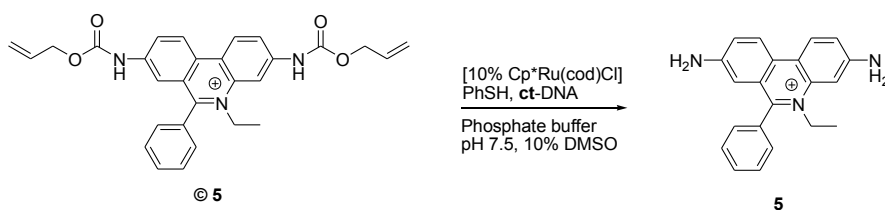


Figure S14: HPLC trace (222 nm) of the deprotection reaction a) initial reaction mixture, before the addition of the ruthenium catalyst; b) reaction mixture after the addition of PhSH and ct-DNA; c) reaction mixture after the addition of the catalyst [Ru], $t = 5$ min.

Catalytic uncaging of ©5



Caged ethidium ©5 (5.5 mg, 9.22 μmol) was dissolved in 200 μL of phosphate buffer 100 mM pH 7.5 in an eppendorf tube. ct-DNA (10 μL of a solution 10 mg/mL), thiophenol (5 μL) and Cp*Ru(cod)Cl ([Ru], 6.8 μL , 1.0 μmol) from a freshly prepared 0.142 M DMSO stock solution) were added to the mixture. The reaction was shaken for 60 min, until HPLC-MS (figure 6, manuscript) analysis confirmed that mostly of the starting material was converted into desired product (5). The reaction was also followed by fluorescence spectroscopy.

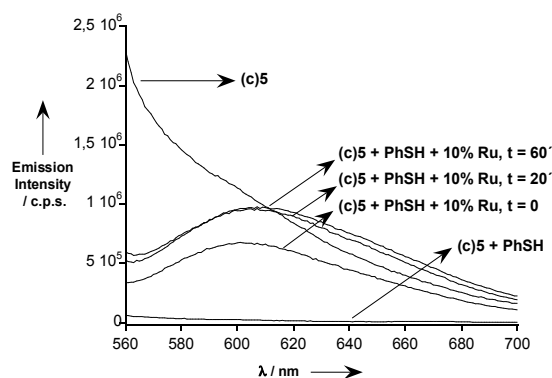


Figure S15: Fluorescence emission spectra of the uncaging reaction of ©5 in the presence of ct-DNA at different reaction times. Samples from the reaction described in the Fig 6 of the main manuscript. The reference curve of ©5 is in the absence of DNA, and therefore we do observe some emission around 600 nm. However the curve of ©5 + PhSH is in the presence of DNA, and therefore we don't observe emission.

MONITORING THE CELLULAR LEAKING OF THE PROBE

CEF cells were incubated in DMEM with ©4 (2.5 μM). Analysis of a sample of the supernatant confirmed the presence of the compound (line b, figure below). The cells we rinsed twice with PBS buffer and incubated in DMEM for another 40 min. Analysis of the new supernatants confirmed a low fluorescence signal (line c). The fluorescence spectrum of the medium was also measured as reference (line a).

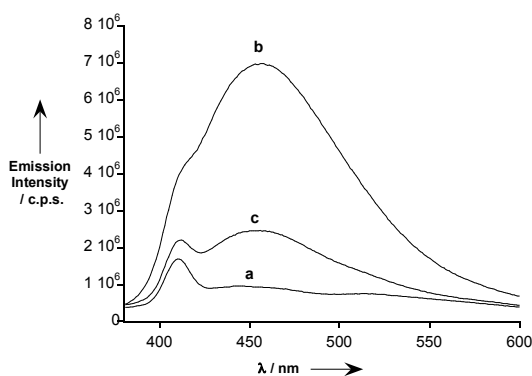


Figure S16: Fluorescence emission spectra of the supernatants: (a) DMEM; (b) supernatant after loading ©4, (c) supernatant after 40 min.

CELL CULTURE EXPERIMENTS

Vero cells were maintained in DMEM (Dulbecco Modified Eagle Medium) containing 10% of FBS (Fetal Bovine Serum). The day before the cellular uptake experiments, cells were seeded in twelve well plates containing glass coverslips (15 mm). Cells were then washed 3 times with PBS and overlaid with 1 mL of fresh DMEM, and no serum is added. Samples with the indicated concentration of the probes were added, and the mixtures incubated for 30 min in an incubator at 37°C; then the medium is removed and the cells are washed with PBS (3 × 1 mL). In the cases we perform a deprotection reaction cells are incubated for 20 min in an incubator at 37 °C with [Ru] and PhSH at the concentration indicated (except in the case of dynamic study with ©4), and further washed with PBS (3 × 1 mL). We used a stock concentration of [Ru] in DMSO of 10 mM.

CEF Primary cultures were prepared from 9- to 10-day old chicken embryos and grown in 199 medium supplemented with 10% tryptose phosphate and 5% calf serum.

Unless otherwise noted, after incubation, and without fixation, the coverslips were mounted on glass slides prior to observation by fluorescence microscopy.

Images were obtained with an Olympus DP-71 digital camera mounted on an Olympus BX51 fluorescence microscope equipped with a built-in Koehler illumination for transmitted light 12 V 100 W halogen bulb Light, and a U-RFL-T power supply unit housing a USH102D 20 V, 100 Watt mercury arc lamp. Images were further processed (cropping, resizing and contrast global contrast and brightness adjustment) with *Adobe Photoshop* (Adobe Systems). All images were taken at ISO 400 sensitivity. The parameters of the fluorescent channels are the following:

- Blue channel: Ultraviolet excitation U-MWU2: excitation filter 360-370 nm, emission filter 420 nm and dichromatic mirror 400 nm.
- Green channel: Blue excitation U-MWB2: excitation filter 460-490 nm, emission filter 520 nm and dichromatic mirror 500 nm.
- Red channel: Green excitation U-MNG2: excitation filter 530-550 nm, emission filter 590 nm and dichromatic mirror 570 nm.

Control, co-staining experiments were performed to clarify the intracellular distribution of the protected dyes, using mitotracker dyes, because this organelle is only in the cytoplasm, and DAPI as a bone fide DNA fluorescent probe.

Cell viability assays

Trypan Blue assay: Cells were washed 3 times with PBS, overlaid with 1 mL of Trypan Blue 2.5% in PBS 1x for 2.5 min and visualized under the microscope.

Propidium iodide assay: Cells were washed 3 times with PBS, overlaid with 1 mL of propidium iodide (20 μM) for 20 min, washed visualized under the fluorescence microscope. (<http://tools.lifetechnologies.com/content/sfs/manuals/mp01304.pdf>)

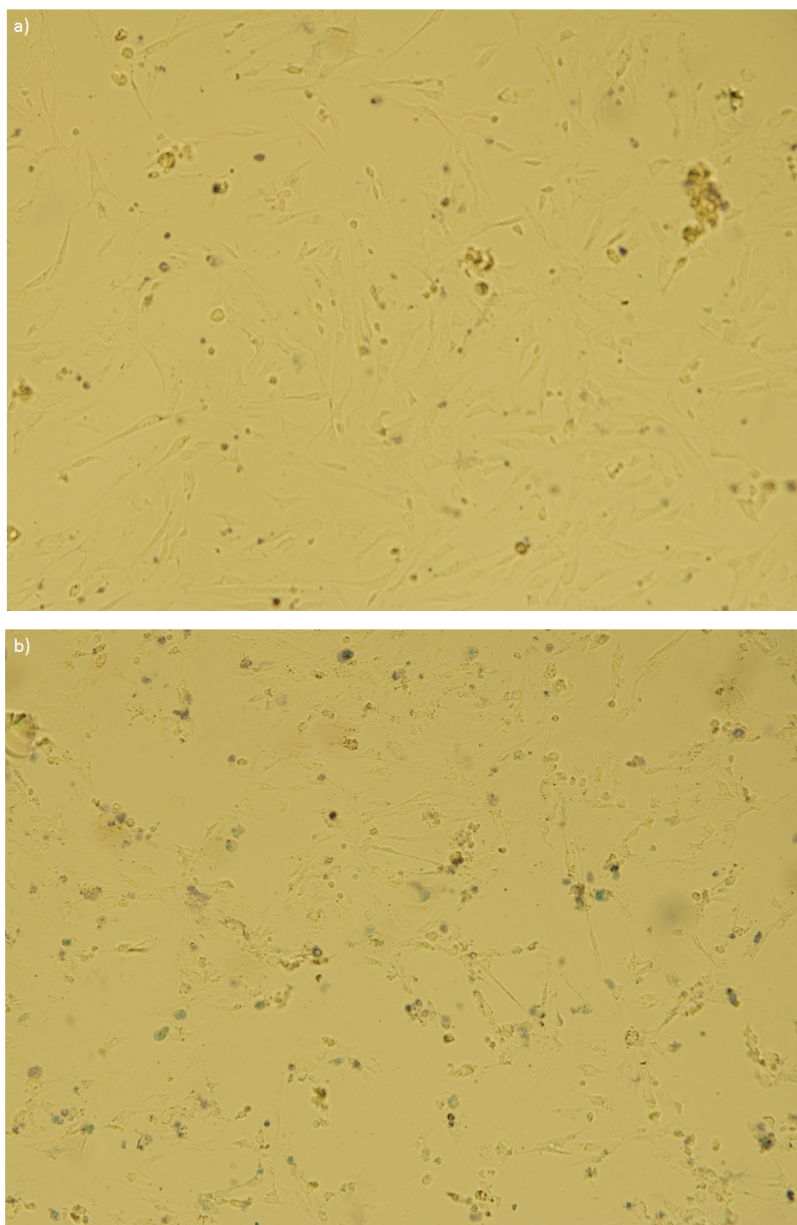


Figure S17: CEF cells incubated with Trypan Blue top) regular cells, bottom) CEF cells incubated with Trypan Blue after being treated with **[Ru]** 20 μM and PhSH 100 μM for 20 min. Dead cells appear as dark blue spots. Pictures were taken at 100X.

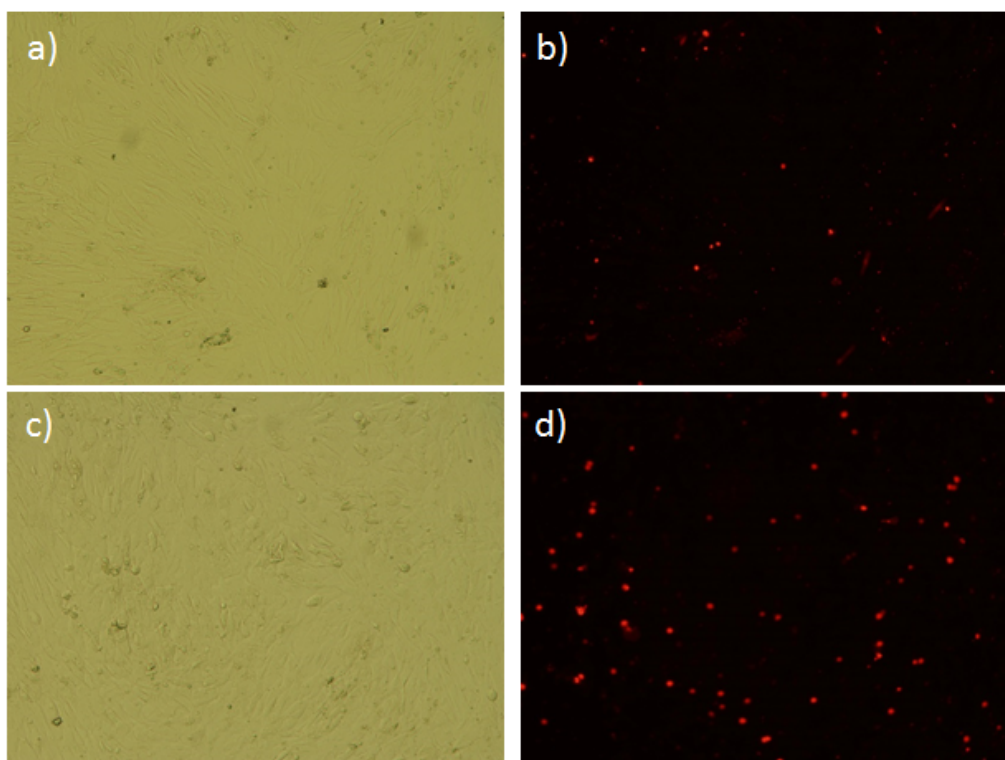


Figure S18: CEF cells stained with Propidium iodide 20 μM . a, b) regular cells; c, d) after incubation with [Ru] 20 μM and PhSH 100 μM . a) Brightfield; b) Red channel (Propidium); c) Brightfield; d) Red channel. Pictures were taken at 400X, ISO 400 and an exposition time for pictures b) and d) of 500 ms.

Control staining experiments

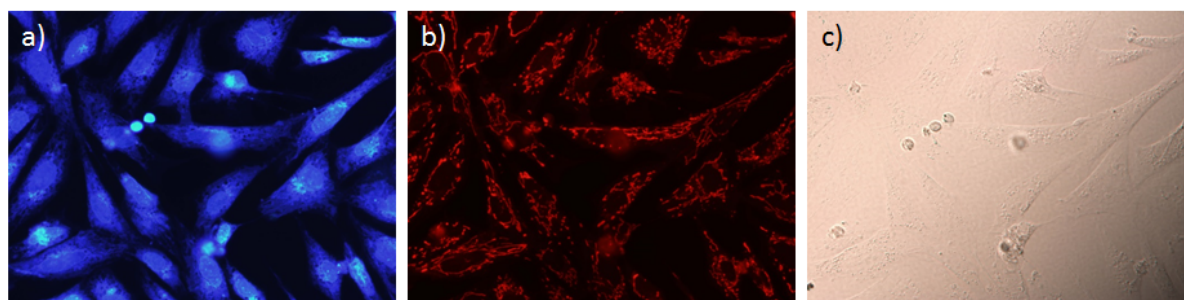


Figure S19: CEF cells treated with ©4 (2.5 μM) and Mitotracker red (500 nM). a) Blue channel (©4 emission) b) Red channel (Mitotracker red emission), c) Brightfield. Pictures were taken at 400X, ISO 400 and an exposition time for picture a) 100 ms and b) 200 ms.

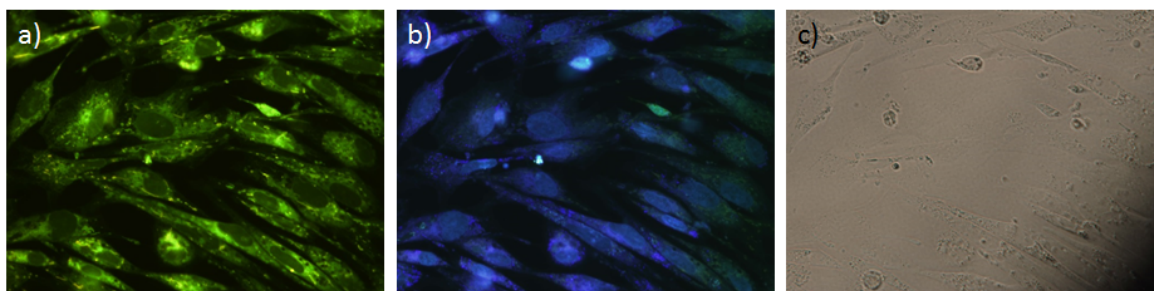


Figure S20: CEF cells incubated with ©5 (10 μM) and DAPI (10 μM). a) Green channel (©5 emission) b) Blue channel (at this excitation wavelength we are also viewing ©5 emission). Pictures were taken at 400X, ISO 400 and an exposition time for pictures a) and b) of 200 ms.

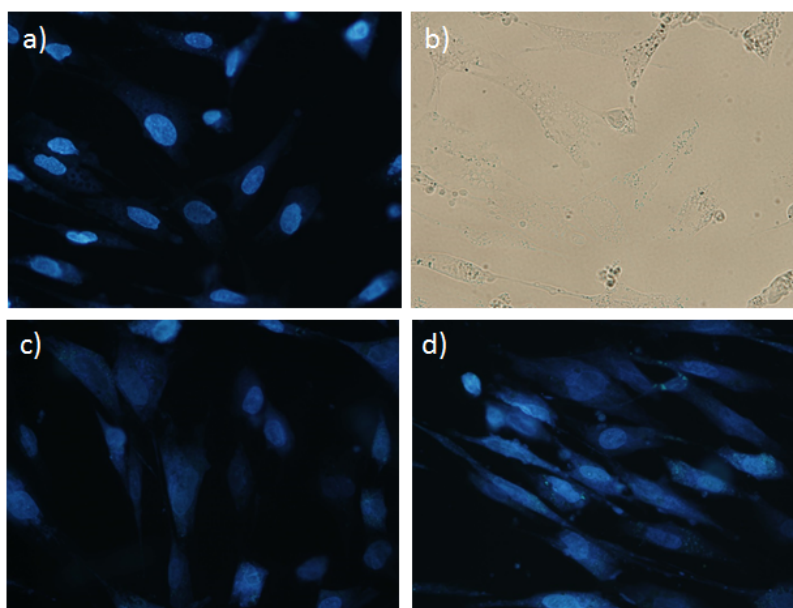


Figure S21: CEF cells incubated with 2.5 μM ©4 and reacted in different conditions for 20 min: a) 2.5 μM [Ru] and 100 μM PhSH; b) brightfield; c) 2.5 μM Ru; d) 100 μM PhSH. Pictures were taken at 400X, ISO 400, and an exposition time for all pictures of 100 ms.

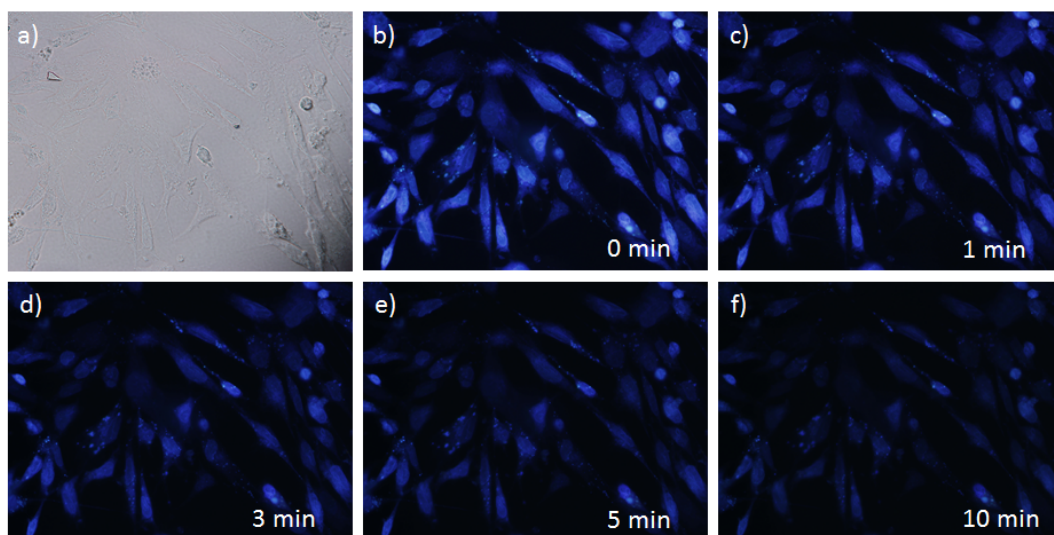


Figure S22: CEF cells incubated with 2.5 μM ©4 and visualized under continuous irradiation at the indicated times: a) Brightfield b) 0 min; c) 1 min; d) 3 min; e) 5 min; f) 10 min. Pictures were taken at 400X, ISO 400 and an exposition time for all pictures of 100 ms.

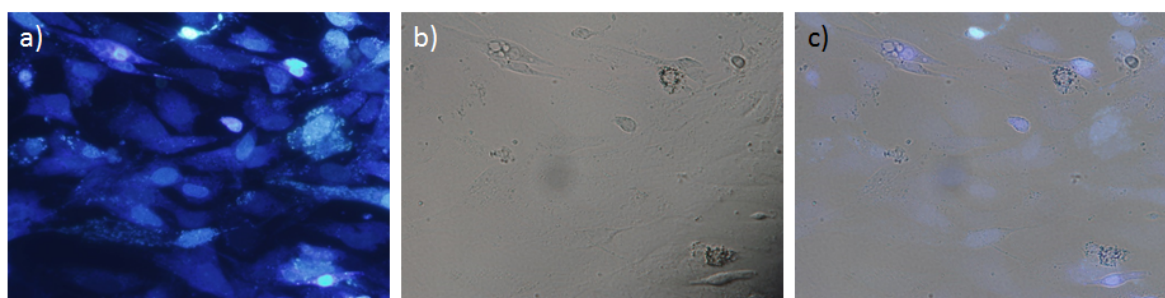


Figure S23: CEF cells incubated with 2.5 μM ©4 a) after 2h 30 min; b) brightfield; c) merged. Pictures were taken at 400X, ISO 400 and an exposition time for picture a) of 100 ms.

UV Spectroscopy

Measurements were done in a *JASCO UV-630* Spectrophotometer coupled with a *PolyScience* thermostat. All the data were recorded at 20 °C with solutions in acetonitrile, using the following settings: band width, 1.5 nm, resolution, 0.2 nm; speed, 400 nm/min; acquisition range, 600-200 nm.

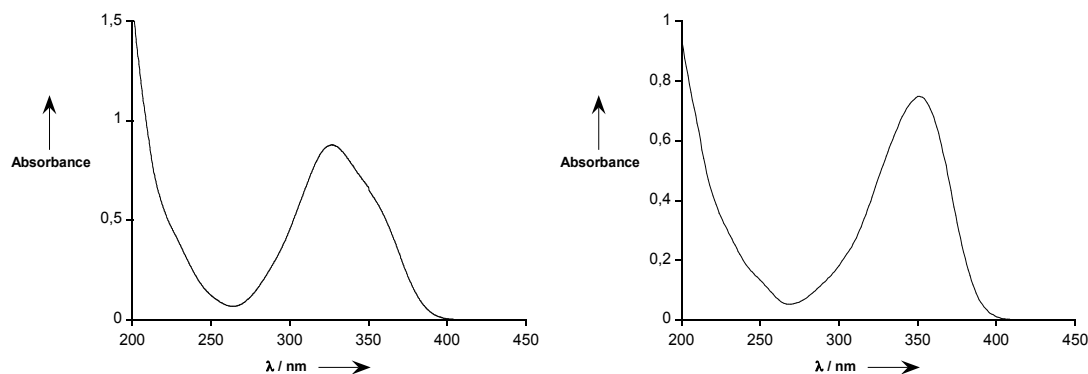


Figure S24: Left: UV spectrum of m-3 (20 μM). Right: UV spectrum of 3 (24 μM).

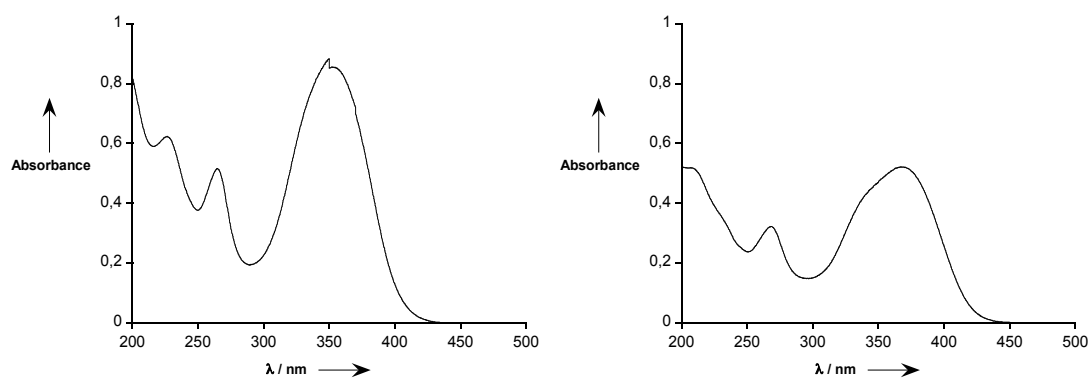


Figure S25: Left: UV spectrum of m-4 (22 μM). Right: UV spectrum of 4 (11 μM).

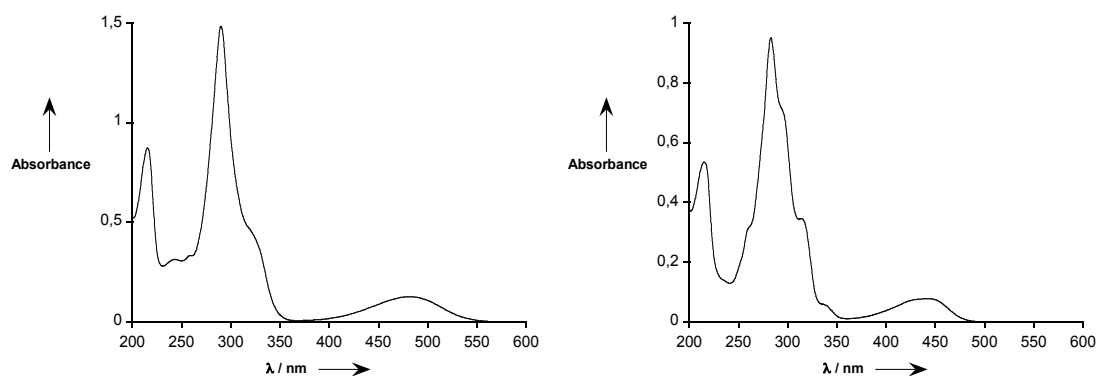


Figure S26: Left: UV spectrum of m-5 (24 μM) Right: UV spectrum of 5 (21 μM).

Curve fitting analysis

The typical equation for a 1:1 binding in which an unlabeled ligand (dsDNA) is added over a fluorescent receptor is described by the following equations, if nonspecific binding is ignored:

$$K_D = \frac{R \times L}{C} \quad (1) \quad C_T = L + C \quad (2)$$

$$R_T = R + C \quad (3) \quad F_T = F_0 + F_C \times C \quad (4)$$

Where R is the concentration of the free receptor in the equilibrium; R_T , total receptor concentration (considered constant throughout the titration); L , concentration of the free ligand in the equilibrium; L_T , total concentration of added ligand (DNA); K_D , dissociation constant of the interaction between the receptor and the ligand; C equilibrium concentration of the ligand-receptor complex; F_T , total observed fluorescence; F_0 , adjustable parameter accounting for the background fluorescence; F_C adjustable parameter for the labeled ligand-receptor complex molar fluorescence. Solving the system for F_T and eliminating R , L , and RL , we obtain the well-known equation 5.

$$F_T = \frac{1}{2} \left(2F_0 + F_C \sqrt{K_D^2 + (C_T - R_T)^2 + 2K_D(L_T + R_T)} \right) \quad (5)$$

If non-specific binding of the probe to the DNA is considered, it is better to use an equation in which the total fluorescence, F_T , takes into account this contribution, assuming that the nonspecific binding under these concentrations is nonsaturable and linearly dependent on the ligand (DNA) concentration ($F_L L_T$)³.

$$F_T = F_0 + F_C \times C + F_L \times L_T \quad (6)$$

The alternative set of equations (1-3, 6) was solved using *Mathematica 6.0.1.0* for *MacOS X (Wolfram Research)*, resulting in equation 7, which was used to fit the experimental data using non-linear regression analysis.

$$F_T = \frac{1}{2} \left(2F_0 + F_C \times K_D + F_C \times F_T + 2F_L \times L_T + F_C \times R_T - F_C \sqrt{K_D^2 + (C_T - R_T)^2 + 2K_D(L_T + R_T)} \right) \quad (7)$$

³ M. H. A. Roehrl, J. Y. Wang, G. Wagner, *Biochemistry* **2004**, *43*, 16056

Biological Activity

Cytotoxicity studies were carried out in the cellular line A2780cis (cis-platinum resistant) and A2780 (human ovarian carcinoma cells). These cells were cultured with growth medium (RPMI 1640 supplemented with 10% FBS (Fetal Bovine Serum) and L-Glutamine 2 mM) in a 95% air and 5% CO₂ atmosphere, at a temperature of 37 °C.

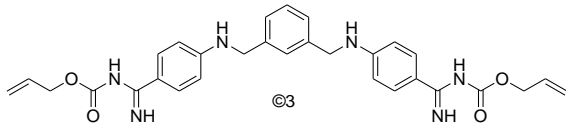
The inhibition of cell growth induced by compounds was evaluated using a system based on cell staining with Sulforhodamine B. Cells were seeded in a 96 well sterile plate at a density of 4000 cells/well in 100 µL of medium and were incubated during 24 hours in growth medium. The compounds, dissolved in DMSO, were added, maintaining the same proportion of DMSO in each well (1%). After 96 hours (37 °C and in a 5% CO₂/95% air atmosphere), cells were seeded in a plate with 50 µL of a 30% trichloroacetic acid solution, by incubation for 60 minutes at 4 °C. After that time, the wells were washed 5 times with distilled water and allowed to dry.

Cell staining was performed with 100 µL of a Sulforhodamine B solution (sulforhodamine 0.4% in a 1% acetic acid solution), stirring the plate at room temperature for 10 min. The excess of dye was removed by 5 washes with acetic acid 0.1% solution and subsequent drying.

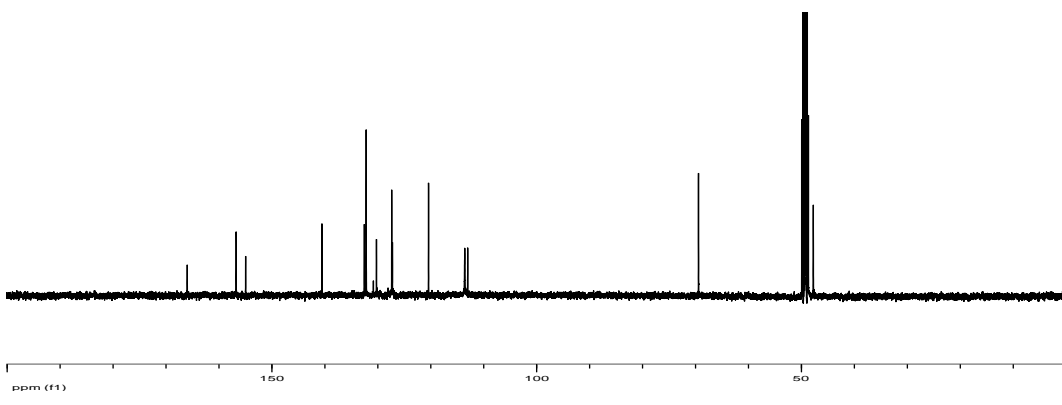
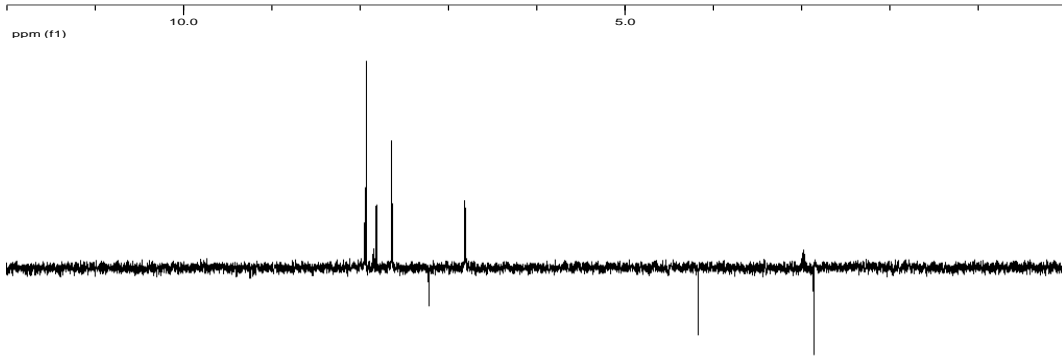
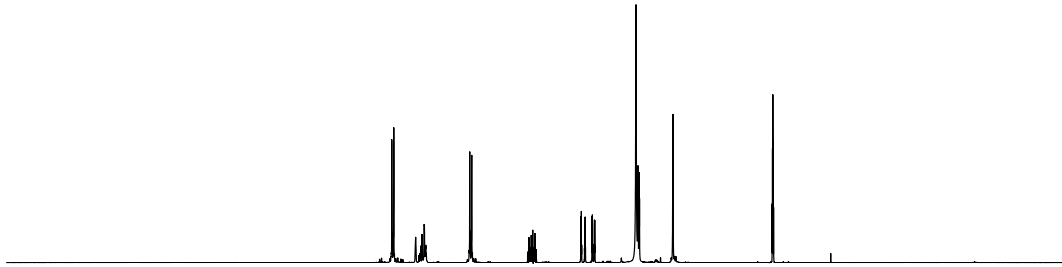
Later 100 µL of Trizma 10mM were added and stirring was maintained for 15 minutes at room temperature, in order to obtain a homogeneous staining in every well. Finally an absorbance reading of the plates at a wavelength of 515 nm was carried out (Tecan Ultra Evolution). Every experiment was carried out with triplicate points.

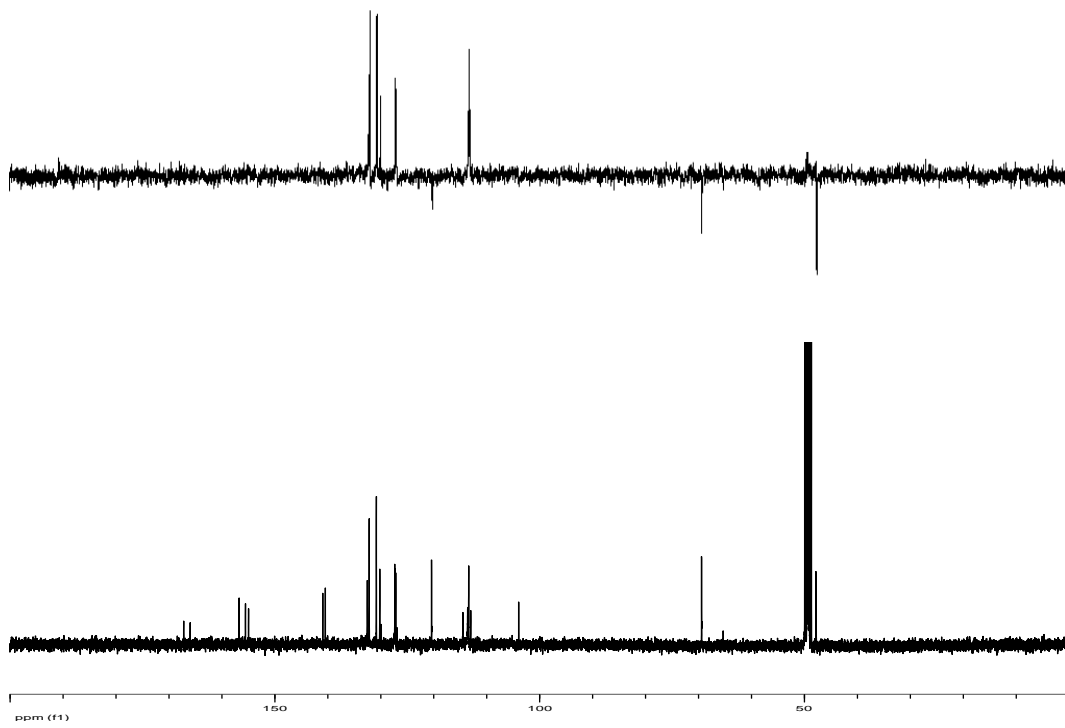
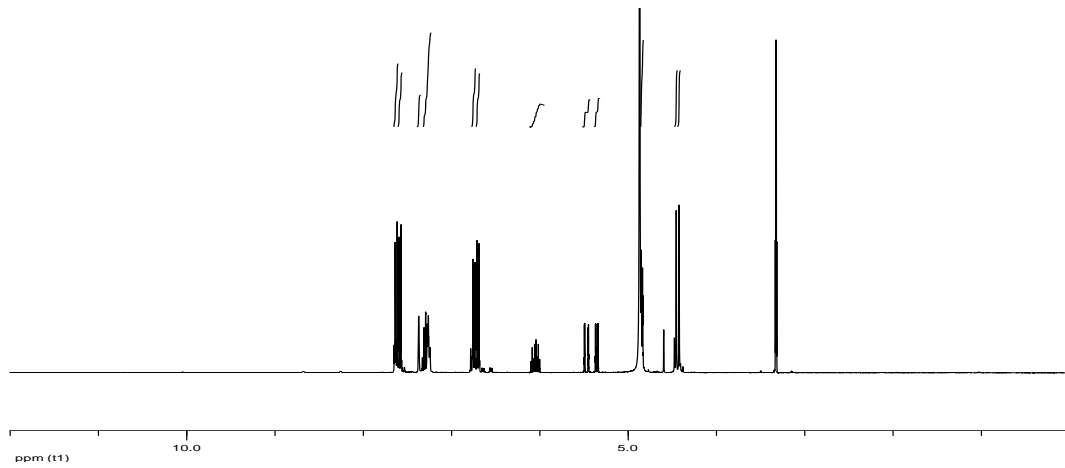
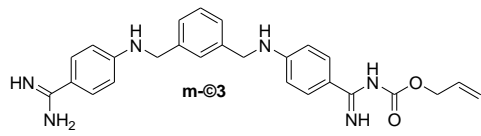
Absorbance measurement range was assessed between 1 point (triplicated) that contained 4000 cells in RPMI 1640 in absence of growth factors (cell concentration is maintained stable), and other point (triplicated) that contained the usual growth medium (which allows to measure the maximum growth at 96h). We also ran control experiments with the compounds dissolved in DMSO, in which was observed a growth inhibition of 8-10 % with respect to a control in which cells were in the usual medium growth.

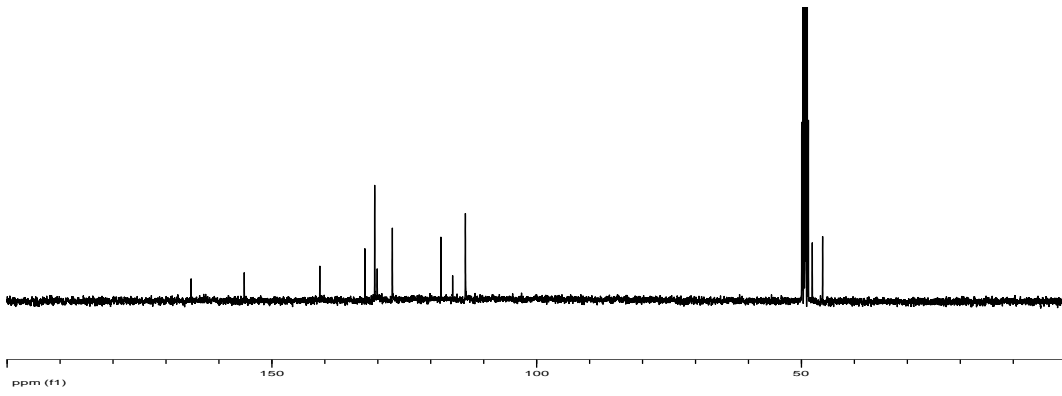
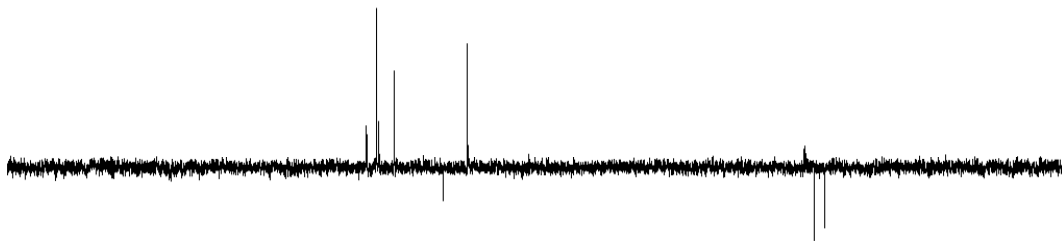
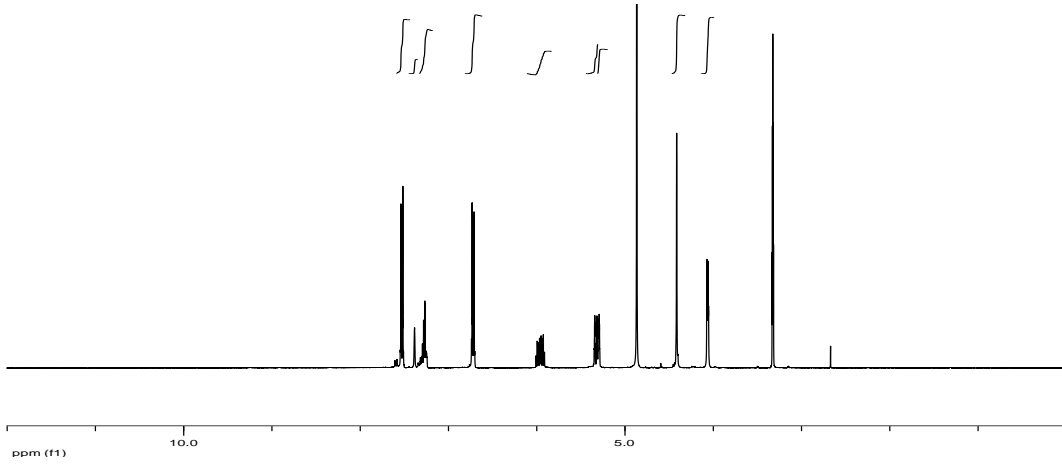
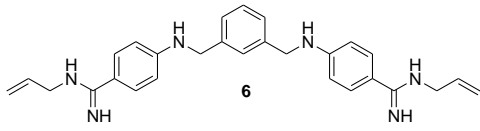
We also tested the cytotoxicity of the ruthenium complex (**[Ru]**) by incubating the cells for 30 min with 20 µM of the complex, washing with growth medium (RPMI 1640 and L-Glutamine 2 mM), and keeping the cells for 96 h. The percentage of the cellular death after 96 hours in A2780 was 0 ±1 in the presence of FBS and 0±2 in its absence.

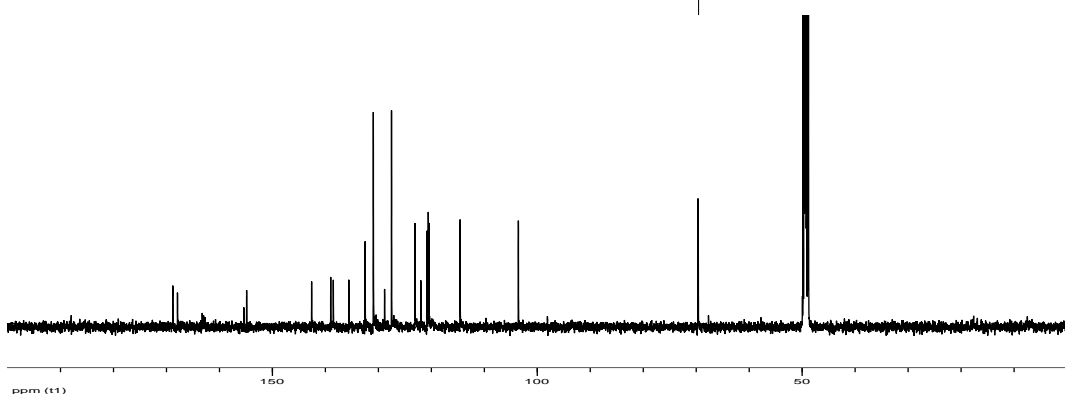
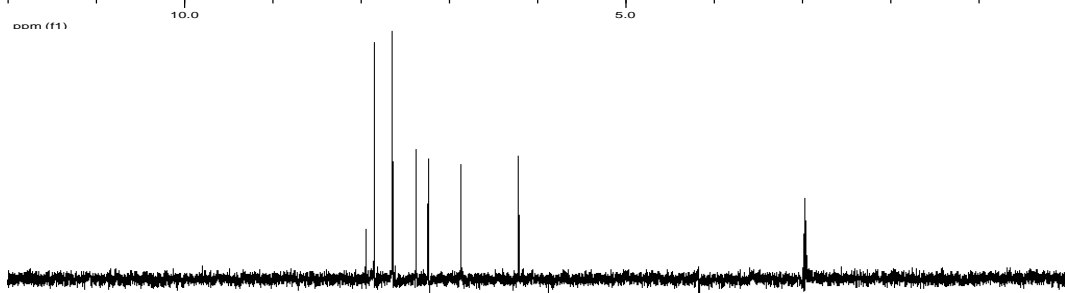
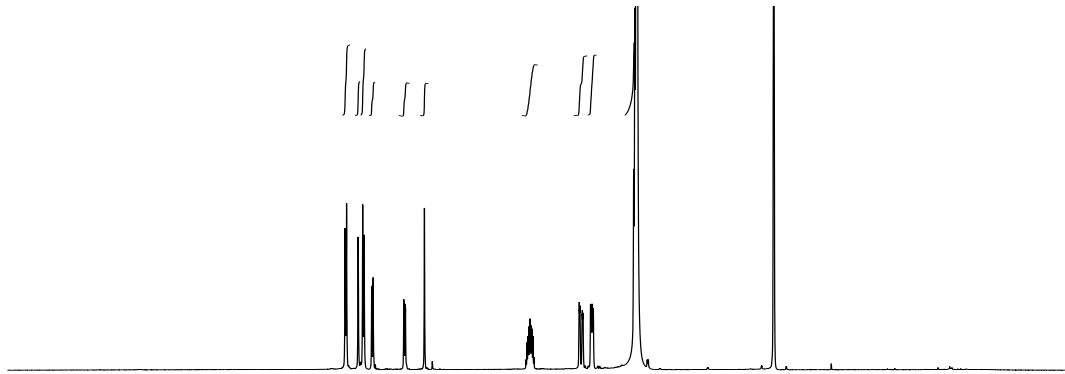
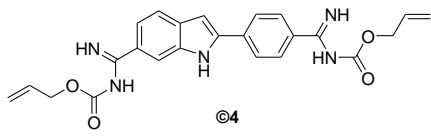


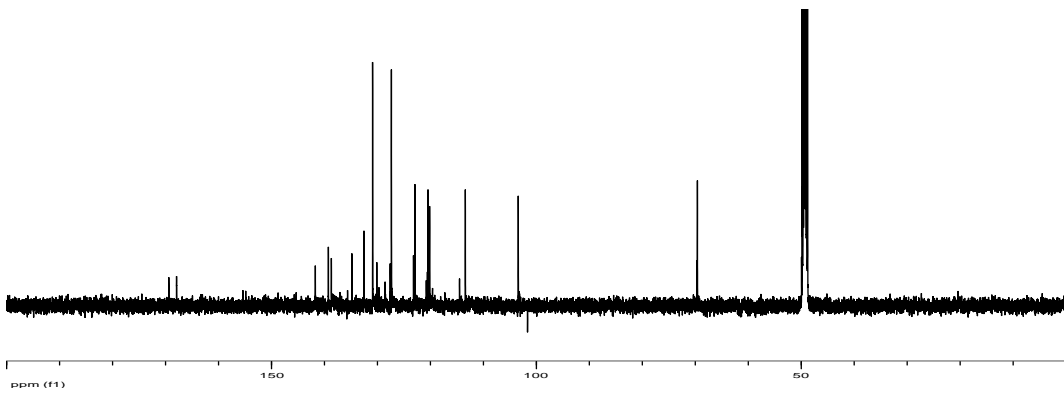
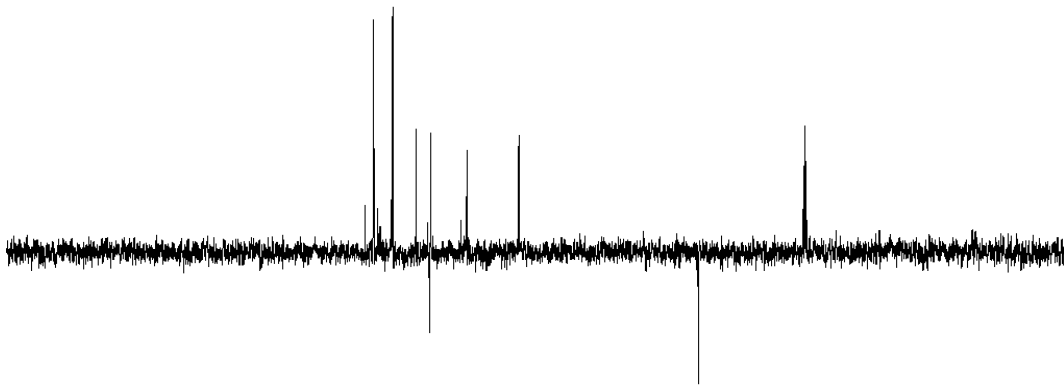
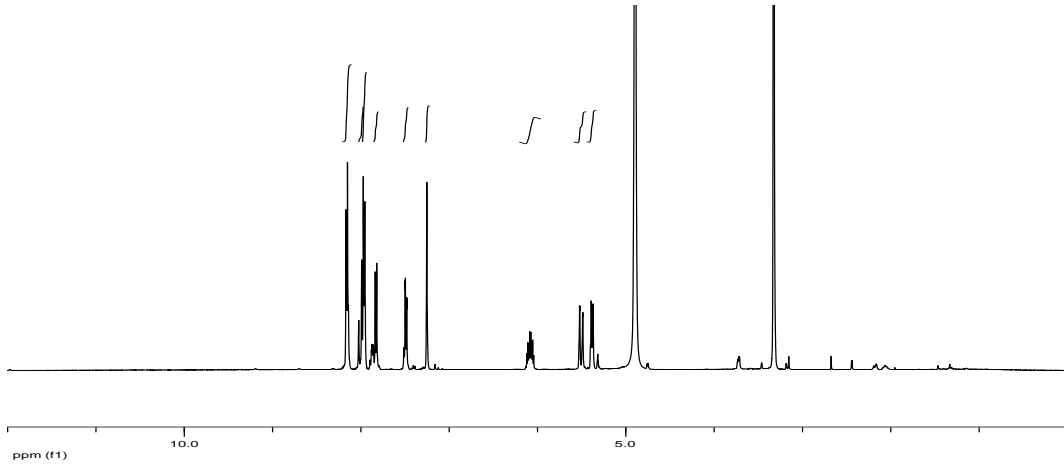
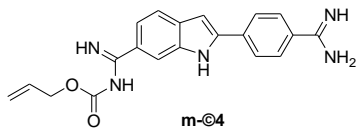
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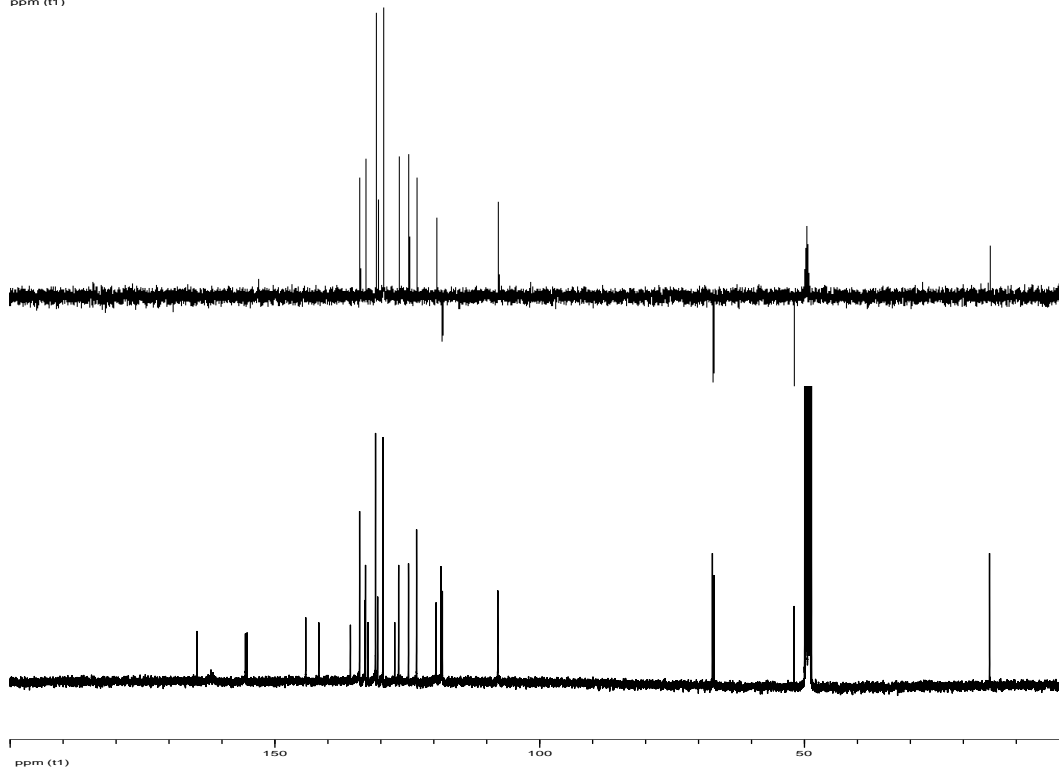
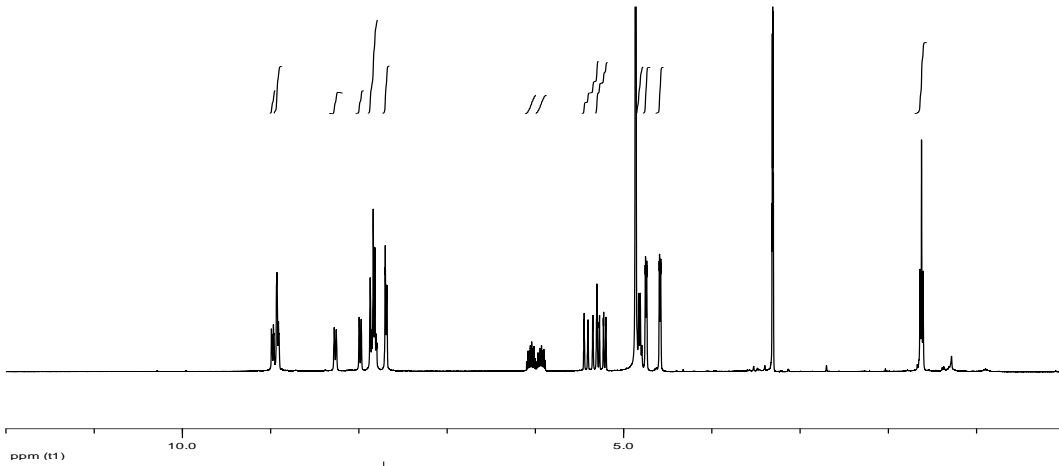
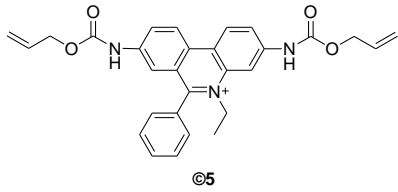


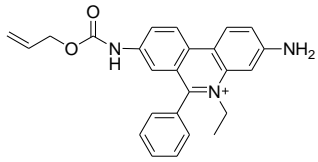




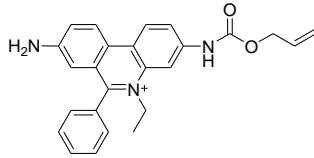




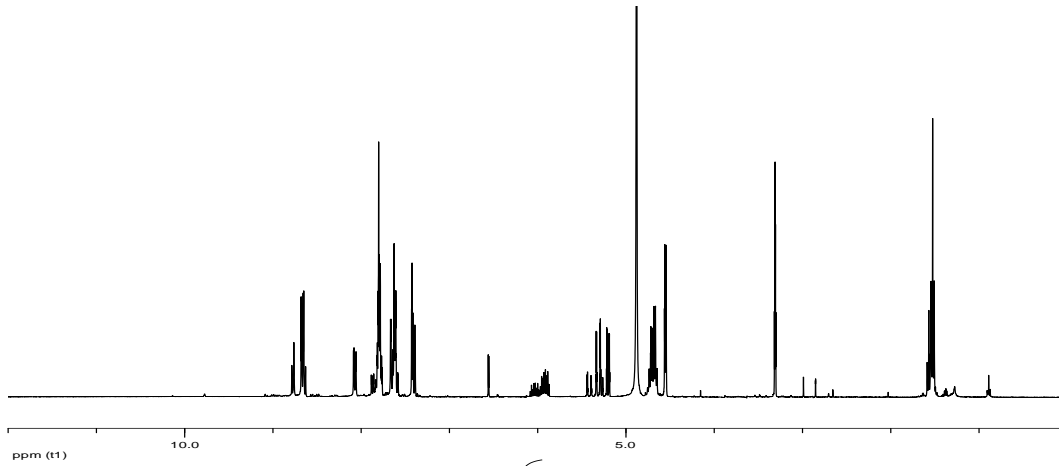




8-alloc-ethidium, m-©5



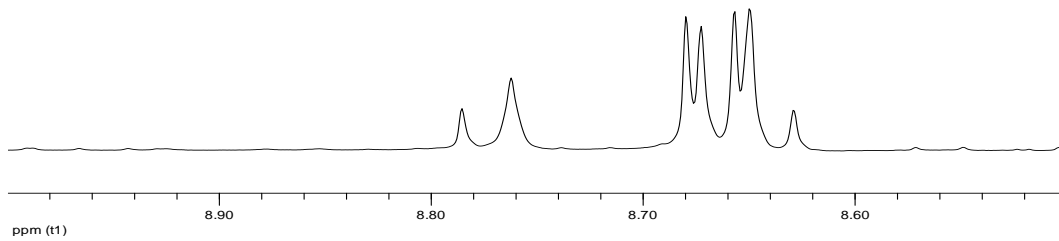
3-alloc-ethidium, m-©5



ppm (t1)

10.0

5.0



ppm (t1)

8.90

8.80

8.70

8.60