Supporting Information

Site-specific DNA Photocleavage by Rhodium Intercalators analyzed by MALDI-TOF Mass Spectrometry

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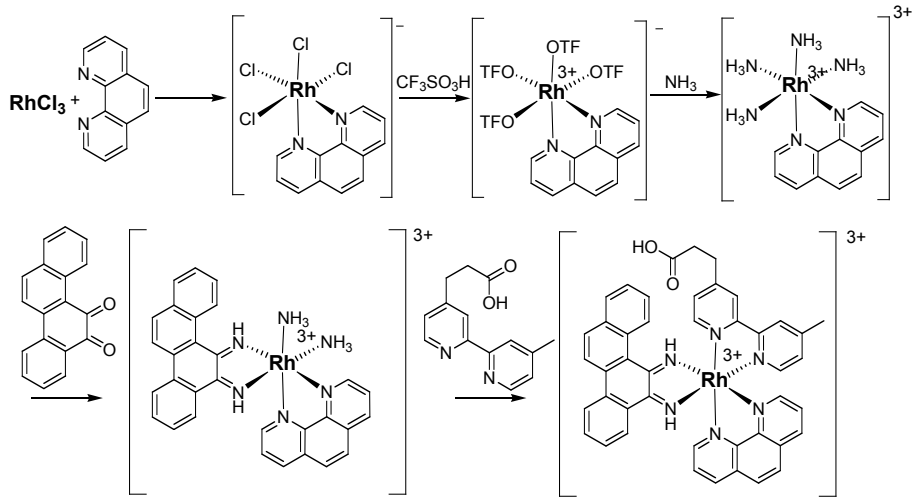
1. Synthesis



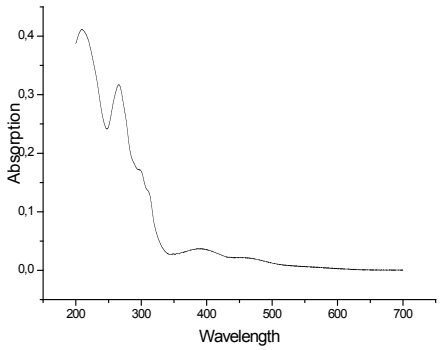
Scheme S1. Synthesis of $[Rh(phen)(chrysi)(bpy3C)]^{3+}$

$[Rh(phen)(chrysi)(NH_3)_2]^{3+}$ was synthesized in four steps from rhodium trichloride, phenantholine and 5,6-chrysenequinone, and purified as described by Schatzschneider and Barton [1]. 4-propionic acid-4′-methyl-2,2′-bipyridine (bpy3C) [2] was then reacted with $[Rh(phen)(chrysi)(NH_3)_2]^{3+}$ in a 50% water, ethanol mixture by refluxing it for 20h. The crude $[Rh(phen)(chrysi)(bpy3C)]^{3+}$ was purified on a Sephadex SP C25 column (eluted with 0.05-0.5M $MgCl_2$) and the product fractions were isolated on a Sep Pak C_{18} Cartridge, washed with copious amounts of water, and then eluted from the cartridge with 50% acetonitrile/water, acidified with 0,1% TFA.

The synthesis of $[Rh(bpy)_2chrysi)]^{3+}$ (**2**) and $[Rh(bpy)(phi)_2]^{3+}$ were as described [3].

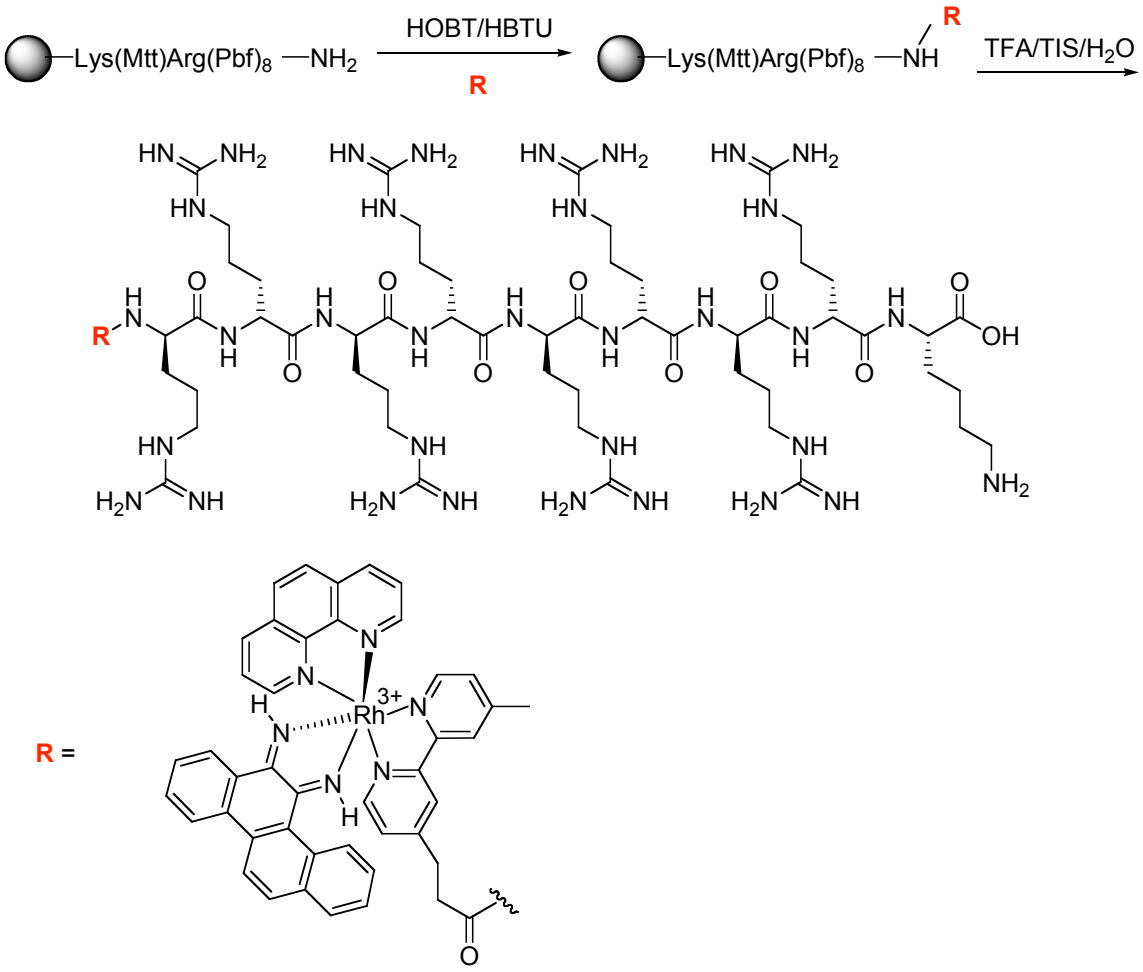
Characterization of [Rh(phen)(chrysi)(bpy3C)]$^{3+}$:
Esi-MS: 779 M^+

UV-Vis:



^{1}H-NMR, D_2O: 8.95 (m, 2H); 8.83 (m, 1H); 8.82 (m, 1H); 8,48 (m, 3H); 8,31 (d, 1 H); 8,27 (s, 1H); 8,21 (s, 1H); 7.99 (m, 2H); 7.78 (m, 3H); 7.53 (m, 2H); 7.41 (m, 1H); 7.31 (m, 1H); 3.17 (m, 1H); 3.00 (m, 1H); 2.75 (m, 1 H); 2.63 (m, 2H); 2.42 (m, 2H); 1.85 (s, 1H)

[1] Schatzschneider, U.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 8630-8631.
[2] Terbrueggen, R. H., Timothy, W. J., Barton, J. K. *Inorg. Chem.* **1998**, *37*, 6874-6883.
[3] Mürner, H.; Jackson, B. A.; Barton, J. K. *Inorg. Chem.* **1998**, *37*, 3007-3012; Pyle, A. M.; Chiang, M.; Barton, J. K. *Inorg. Chem.* **1990**, *29*, 4487.



Scheme S2. Synthesis of the Rh(III) complex modified peptide

Solid phase bound and protected (arginine was protected as its 2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl (Pbf) derivative, lysine as its methyltrityl (Mtt) derivative) peptides were purchased from AnaSpec Inc. (San Jose, CA, USA) The acid-modified metal complex was coupled to the free amine of the peptide by standard HOBT/HBTU activated coupling.The peptides were cleaved from the resin using 95% TFA, 2.5% triisopropylsilane and 2.5% water for 3h at room temperature. In all cases, the final products were obtained in analytical purity using a HP1100 HPLC system fitted with a C18-packed reverse phase column and analyzed by ESI- and Maldi-TOF mass spectrometry. All conjugates employed in this study were used as their corresponding trifluoroacetate salts bearing a full complement of counterions.

Characterization of 1:
Maldi-TOF-MS: M_{calc}: 2157.38 $(M-2H)^+$ M_{found}: 2157.66; M_{calc}: 1977.17 $(M\text{-}Phen\text{-}2H)^+$ M_{found}: 1979.50; M_{calc}: 1901.08 $(M\text{-}Chrysi\text{-}2H)^+$ M_{found}: 1903.48; M_{calc}: 1720.87 $(M\text{-}Chrysi\text{-}Phen\text{-}2H)^+$ M_{found}: 1722.56; M_{calc}: 1620.96 $(M\text{-}Rh\text{-}Chrysi\text{-}Phen+H)^+$ M_{found}: 1621.67
ESI-MS: M_{calc}: 947.83 $(M+6TFA)^{3+}$ M_{found}: 947.62; M_{calc}: 909.83 $(M+5TFA)^{3+}$ M_{found}: 909.62; M_{calc}: 653.62 $(M+H+4TFA)^{4+}$ M_{found}: 654.05; M_{calc}: 625.11 $(M+H-3TFA)^{4+}$ M_{found}: 625.55; M_{calc}: 596.61 $(M+H+2TFA)^{4+}$ M_{found}: 597.05; M_{calc}: 568.1 $(M+H+TFA)^{4+}$ M_{found}: 568.54

2. DNA Photocleavage by MALDI-TOF Mass Spectrometry

The sequences of the DNAs A and B are shown below. The mismatch is highlighted in red. The arrow marks the cleavage position, one base 3′-shifted from the mismatched site. Photocleavage experiments were performed on a 20 µl scale with 2 µM **1** (except for light control experiments LC which were without **1**), 2 µM duplex DNA A:B, 50 mM NaCl, 10 mM NaPi, pH 7, 15 min irradiation with a 1000-W Oriel Hg/Xe arc lamp (Oriel, Stanfort, CT) between 320-440nm wavelength (except for dark control experiments DC which were carried out without irradiation).

Mismatched DNA	Matched DNA
A: 5′-GCG CCG TCG T**C**C ATG TG-3′	C: 5′-GCG CCG TCG TGC ATG TG-3′
B: 3′-CGC GGC AGC A**C**G TAC AC-5′	B: 3′-CGC GGC AGC ACG TAC AC-5′

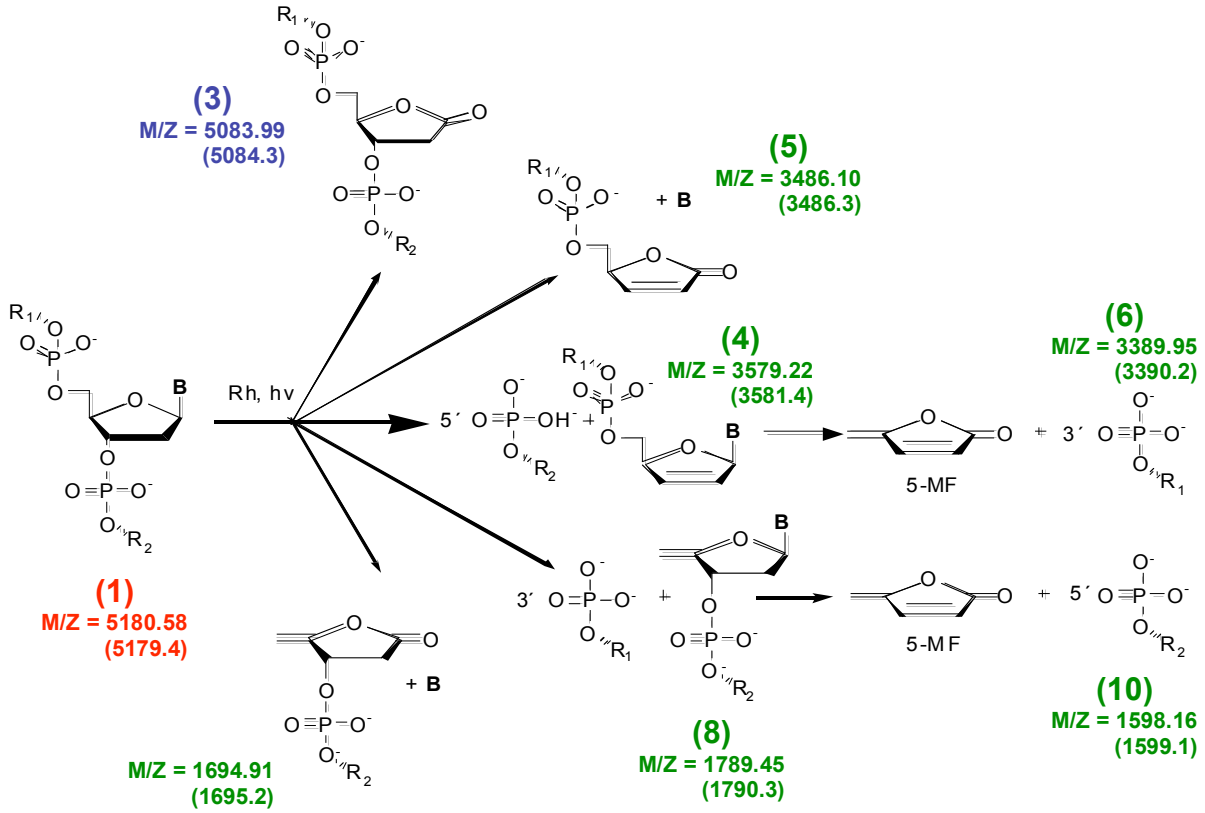


Figure S1. DNA strands used in the photocleavage experiments, marked cleavage site and assigned cleavage products for the mismatch selective cleavage with Rh complexes 1 and 2. Masses given are those found in cleavage with 2 with numbers indicated as in Figure 1. Masses calculated are for the assigned structures. R_1 = 5′-GCG CCG TCG TC-3′; R_2 = 5′-ATG TG-3′, B = Cytosine)

Photocleavage detection by MALDI-TOF mass spectrometry

The photocleavage reaction mixtures were desalted by using the ZipTip procedure. ZipTip C_{18} were equilibrated and the oligonucleotides were bound, washed and eluted in 10µl with acetonitrile/water as described in the procedure for oligonucleotides (Millipore). The oligonucleotides were then dried on a speedvac and redissolved in 1µl water. The MALDI-TOF mass spectra were measured on a PerSeptive Biosystems Voyager-DE Pro instrument. The samples were prepared by the dry droplet method, using 3-hydroxypicolinic acid as matrix. DNA strand B (M/Z = 5166.4) and its double charged species (M/Z = 2583.7) were used as internal standards.

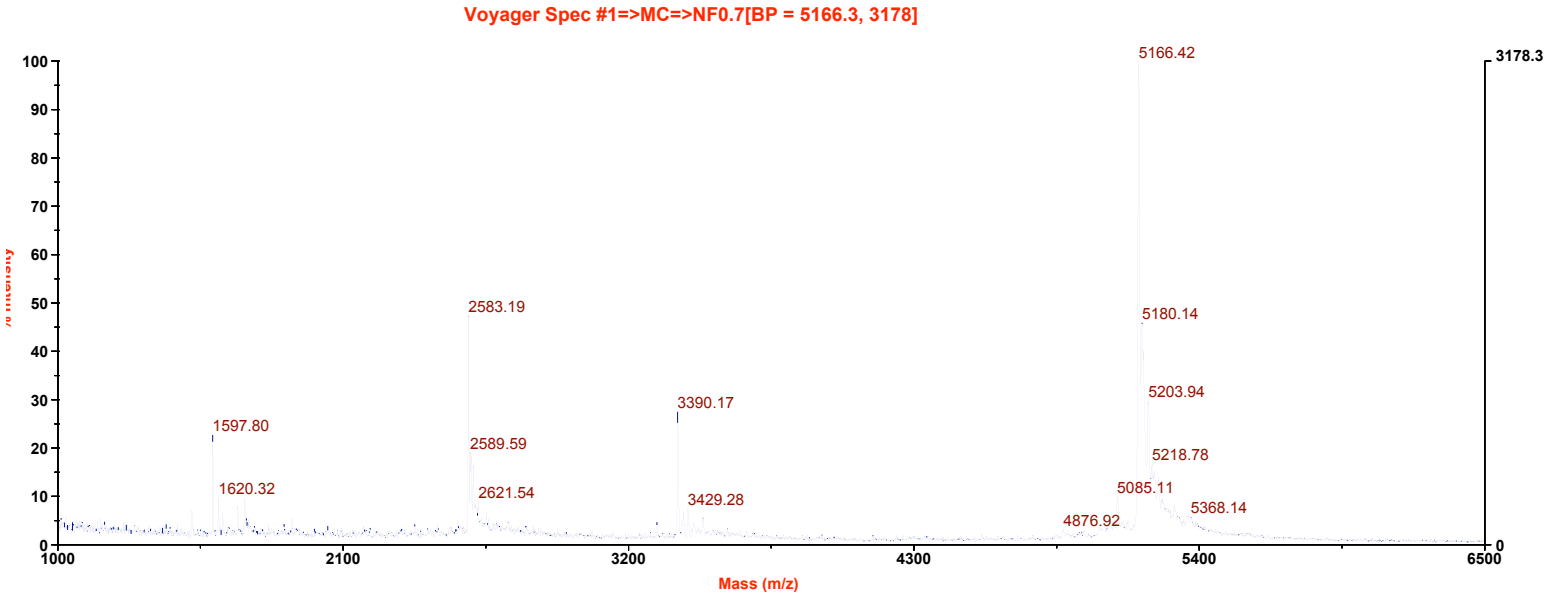


Figure S2. MALDI-TOF mass spectra obtained 48h after photocleavage with 2 µM 1, 2 µM DNA A/B, 50 mM NaCl, 10 mM NaPi, pH 7, 15 min irradiation.

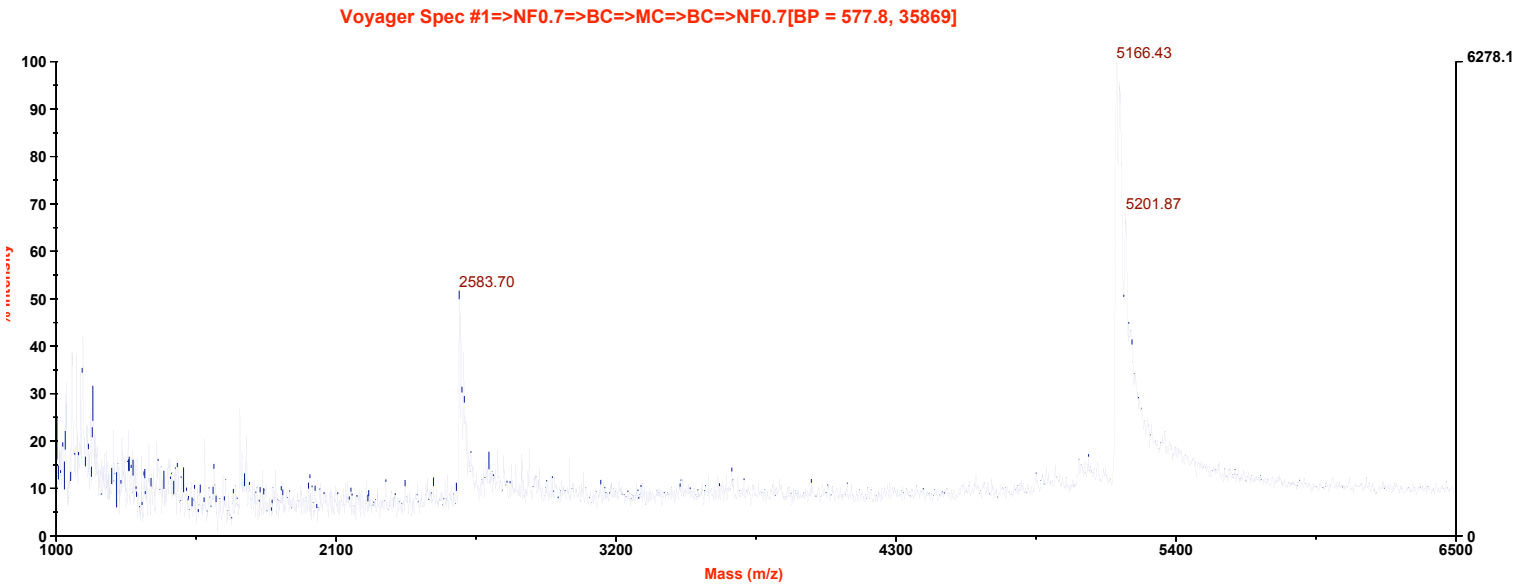


Figure S3. MALDI-TOF mass spectra obtained 48h after photocleavage with 2 µM 1, 2 µM DNA A/B, 50 mM NaCl, 10 mM NaPi, pH 7, but without irradiation. No cleavage products are observed.

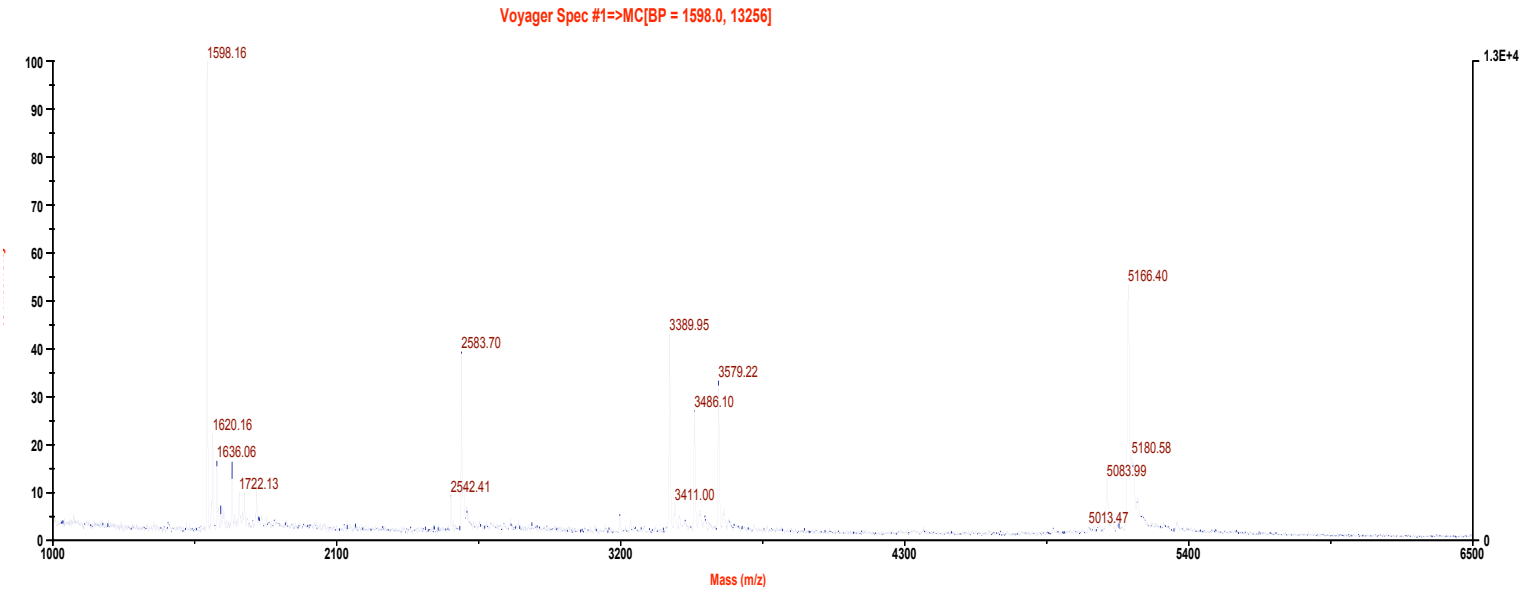


Figure S4: MALDI-TOF mass spectra after photocleavage with 2 µM 2, 4 µM DNA A/B, 50 mM NaCl, 10 mM NaPi, pH 7, 15 min irradiation.

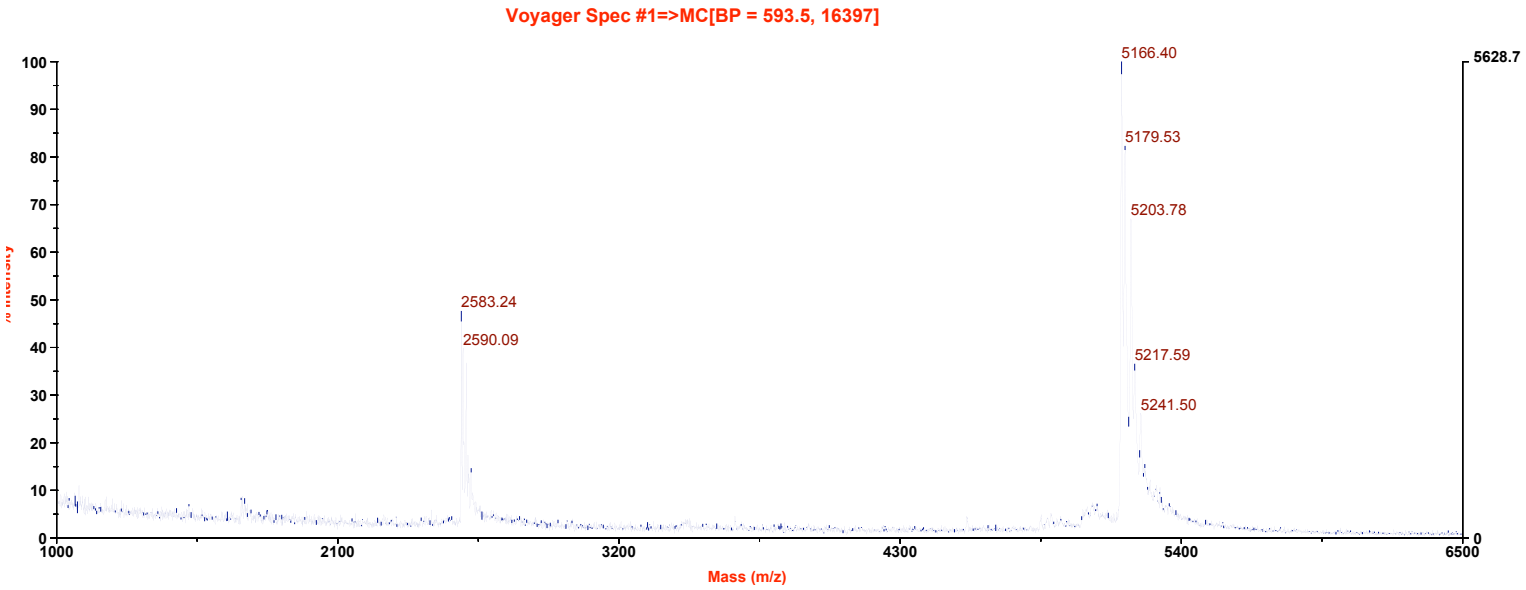


Figure S5. MALDI-TOF mass spectra without 1 or 2 but with irradiation of 2 µM DNA A/B, 50 mM NaCl, 10 mM NaPi, pH 7. With 15 minutes irradiation, no cleavage is observed.

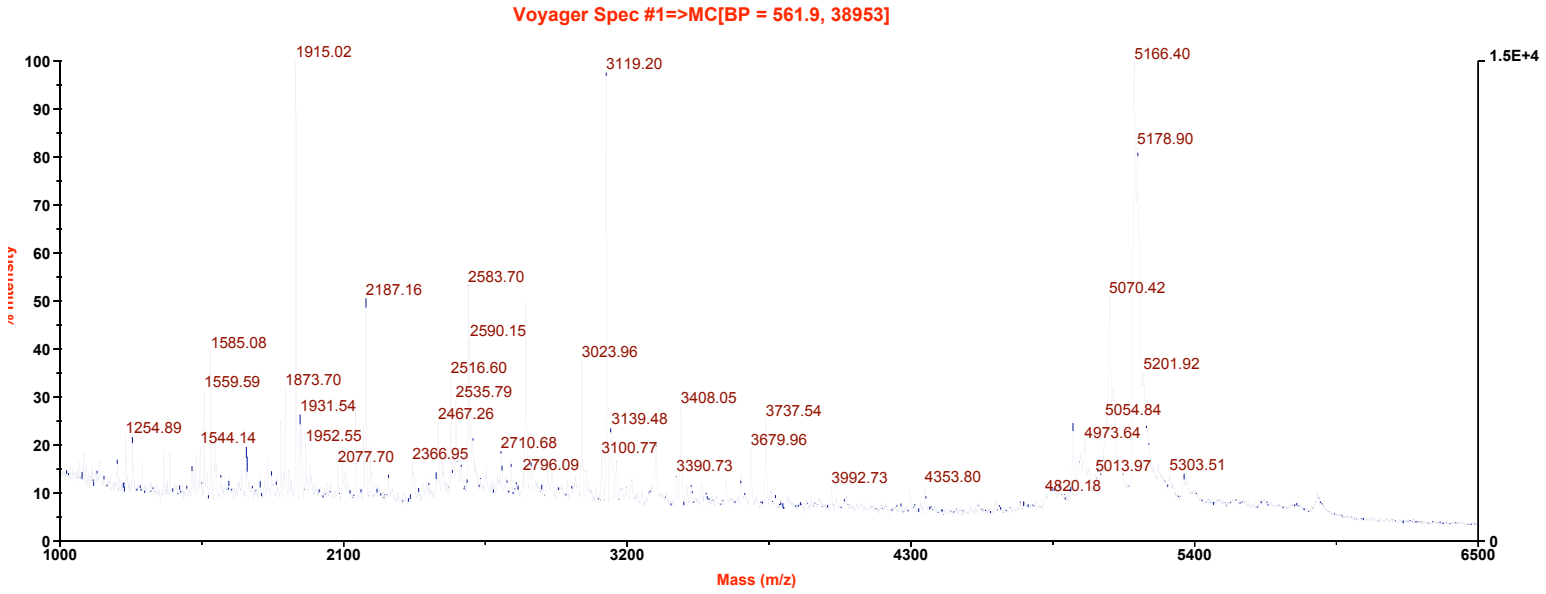


Figure S6. MALDI-TOF mass spectra of photocleavage with 4 µM $[Rh(bpy)(phi)_2]^{3+}$, 4 µM DNA A/B, 50 mM NaCl, 10 mM NaPi, pH 7, 30 min irradiation at 313nm; main products: M/Z = 3119.20: 5´-$(OH)_2$OPO-ACG ACG GCG C-3´; M/Z = 1915.02: 5´-CAC ATG-OPO(OH)OCH_2COOH-3´