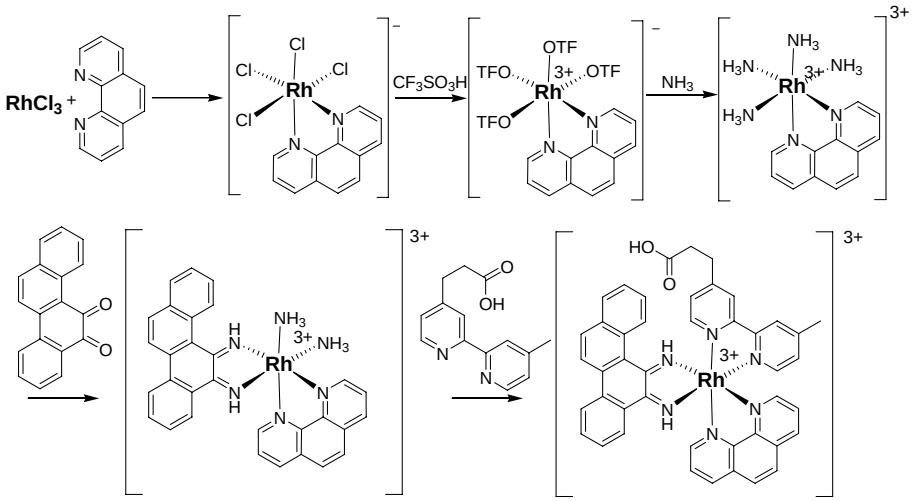
Supporting information

Metal complex synthesis

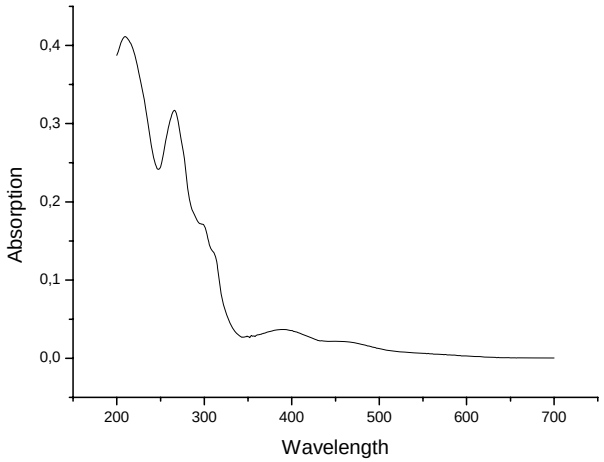


Scheme 1: Synthesis of the carboxylic acid modified Rh(III) complex

$[Rh(phen)(chrysi)(NH_3)_2]^{3+}$ was synthesized in four steps from rhodium trichloride, phenantholine and 5,6-chrysenequinone, and purified as described by Mürner, Jackson and Barton [1]. Propionic acid modified bipyridine 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 (eluated 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.

Characterization:

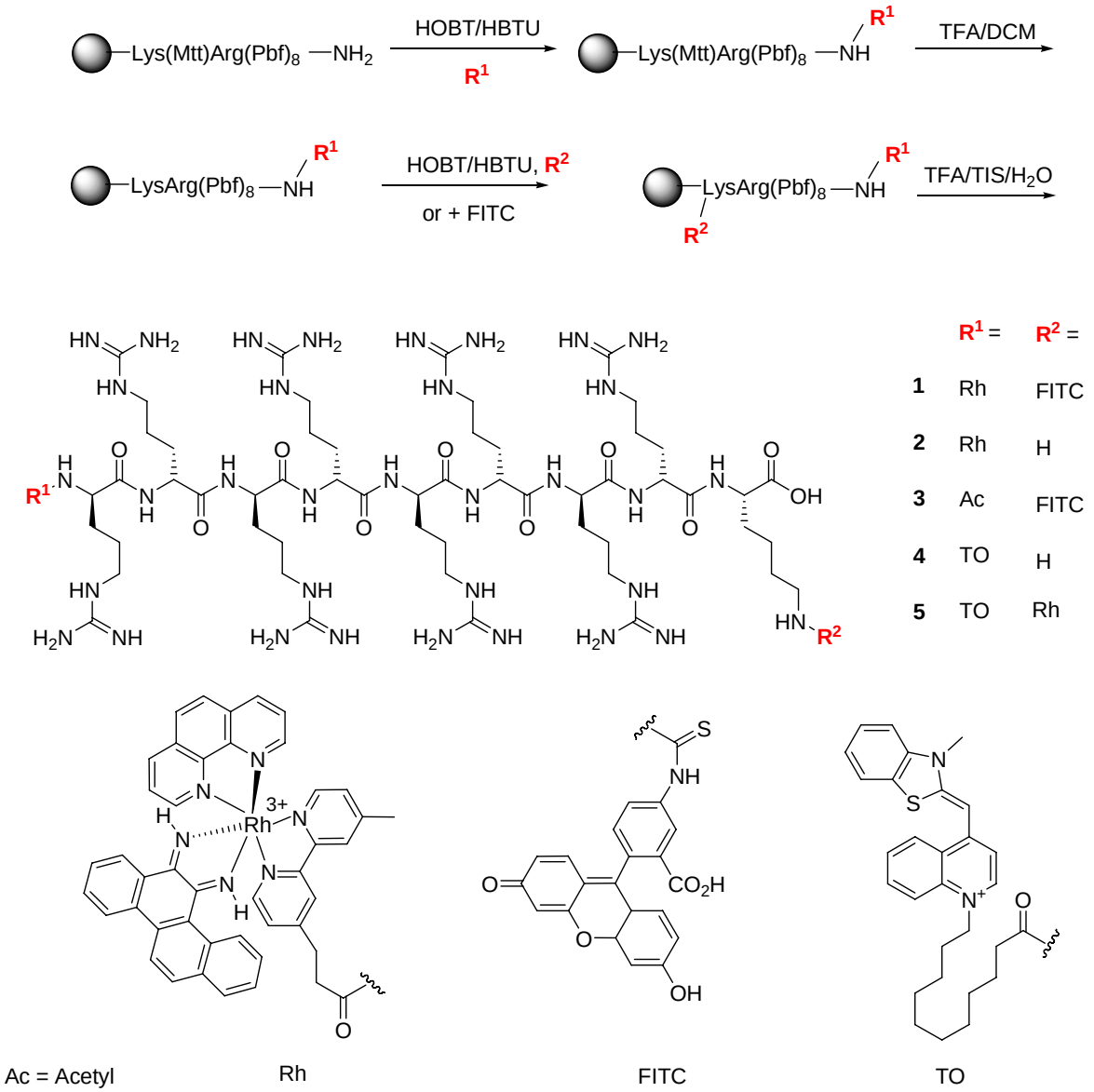
Esi-MS: M_{calc}: 779.6 $(M-2H)^+$ M_{found}: 778.9 $^+$
UV-Vis:



[1] H. Mürner, B. A, Jackson, J. K. Barton, *Inorg. Chem.* **1998**, *37*, 3007-3012

^{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)

Coupling of the carboxylic acid modified metal complex to the peptide and addition of the fluorophores

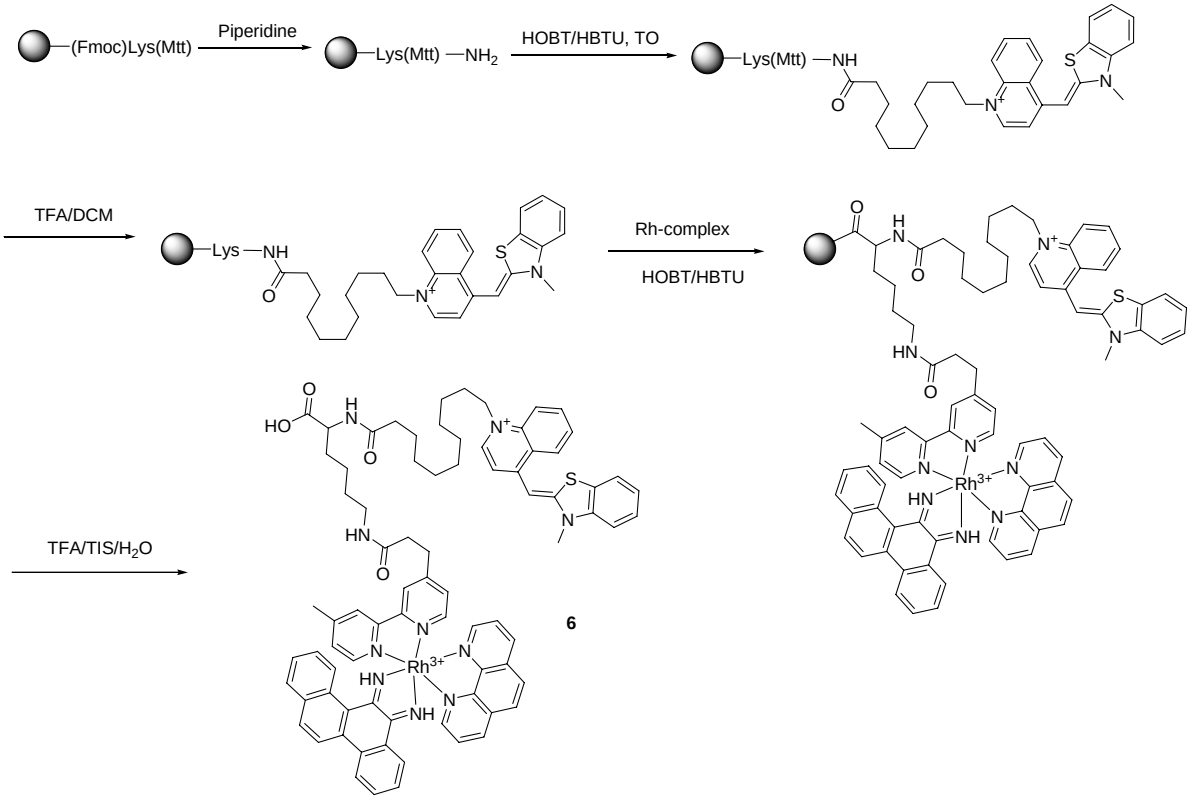


Scheme 2: Synthesis of the Rh(III) complex and fluorophore modified peptides

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. The acid modified metal complex or the acid modified fluorophores were coupled to the free amines of the peptide by standard HOBT/HBTU activated coupling reactions. The MTT-group was selectively deprotected by treatment with 3% TFA in dichloromethane for 10 minutes. The free amino group could then be acylated as described above, or treated with fluoresceinisothiocyanate in dimethylformamide. The peptides were cleaved from the resin using 95% trifluoroacetic acid (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/or Maldi-Tof mass spectrometry. All conjugates employed in this study were used as their corresponding trifluoroacetate salts bearing a full complement of counterions.

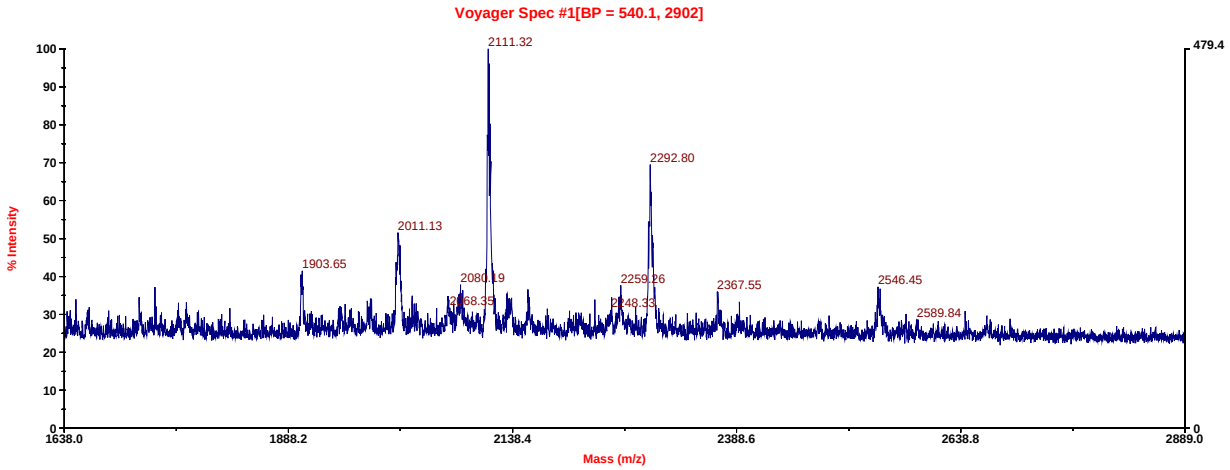
Synthesis of Compound 6



Solid phase bound and protected lysine (methyltrityl (Mtt) and 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group) amino acid was purchased from novabiochem. The acid modified metal complex or the acid modified thiazole orange were coupled to the free amines of the peptide by standard HOBT/HBTU activated coupling reactions. The MTT-group was selectively deprotected by treatment with 3% TFA in dichloromethane for 10 minutes. The free amino group could then be acylated as described above. The compound was cleaved from the resin using 95% trifluoroacetic acid (TFA), 2.5% triisopropylsilane and 2.5% water for 3h at room temperature. The final products was 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. The conjugate employed in this study was used as its corresponding trifluoroacetate salt bearing a full complement of counterions.

Characterizations:

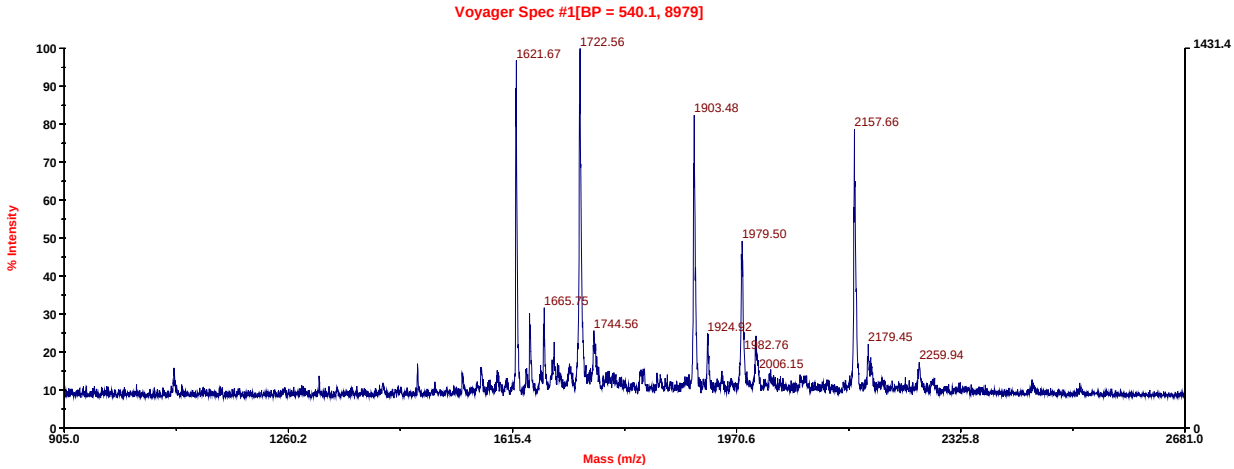
Compound 1:



Maldi-Tof-MS: M_{calc}: 2546.76$(M-2H)^+$ M_{found}: 2546.45; M_{calc}: 2366.55$(M\text{-}Phen\text{-}2H)^+$ M_{found}: 2367.55; M_{calc}: 2290.46$(M\text{-}Chrysi\text{-}2H)^+$ M_{found}: 2292.80; M_{calc}: 2110.25$(M\text{-}Chrysi\text{-}Phen\text{-}2H)^+$ M_{found}: 2111.32; M_{calc}: 2010.34$(M\text{-}Rh\text{-}Chrysi\text{-}Phen+H)^+$ M_{found}: 2011.13

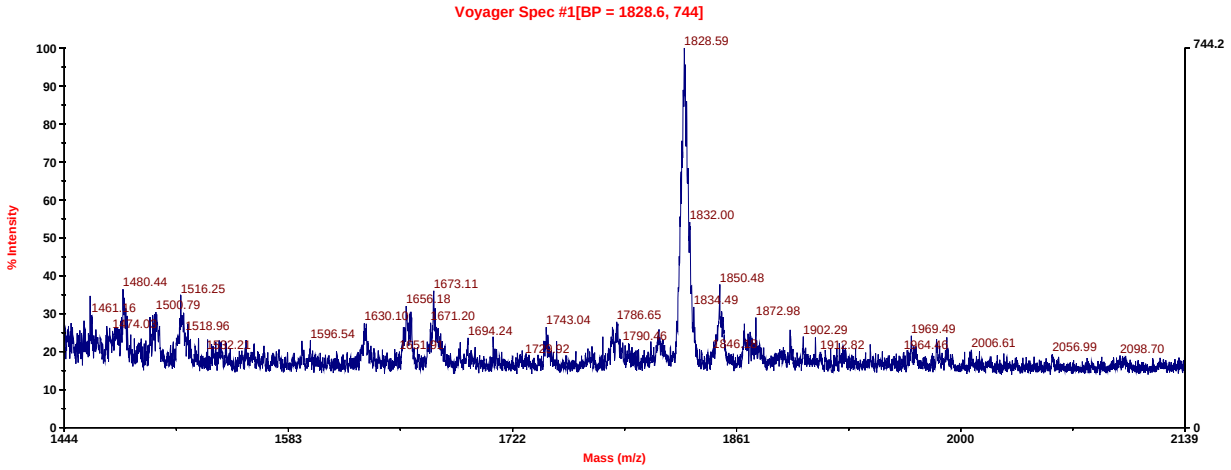
ESI-MS: M_{calc}: 637.44 $(M+4H)^{4+}$ M_{found}: 637.33; M_{calc}: 666.20 $(M+3H+TFA)^{4+}$ M_{found}: 665.83; M_{calc}: 693.95 $(M+2H+2TFA)^{4+}$ M_{found}: 694.34; M_{calc}: 722.46 $(M+H+3TFA)^{4+}$ M_{found}: 722.84; M_{calc}: 510.35 $(M+5H)^{5+}$ M_{found}: 510.04

Compound 2:



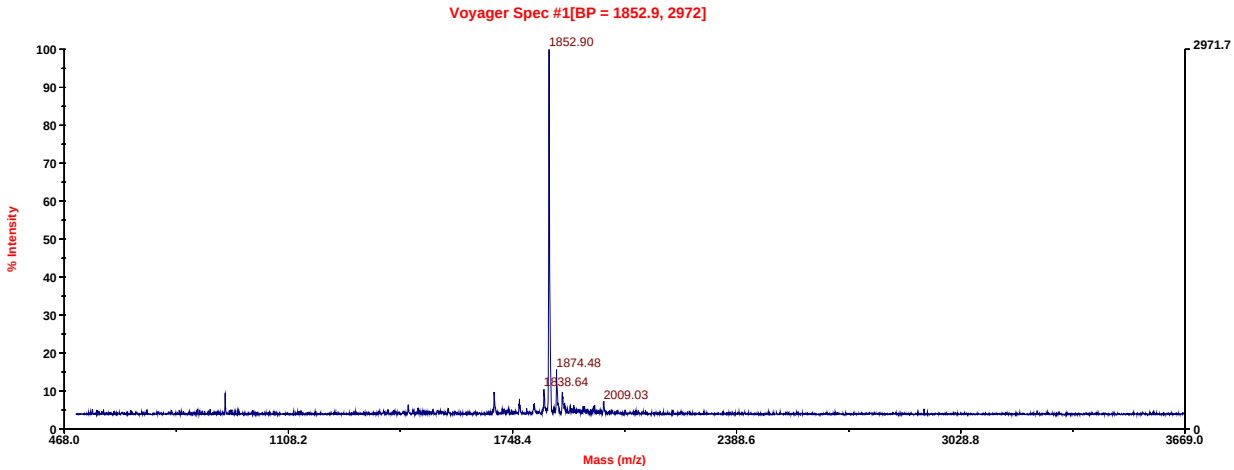
Maldi-Tof-MS: M_{calc}: M_{calc}: 2157.38 $(M-2H)^+$ M_{found}: 2157.66; M_{calc}: 1977.17 $(M\text{-}Phen\text{-}2H)^+$ M_{found}: 1979.50; M_{calc}: 1901.08 $(M\text{-}Chrysi\text{-}2H)^+$ M_{found}: 1903.48; M_{calc}: 1720.87 $(M\text{-}Chrysi\text{-}Phen\text{-}2H)^+$ M_{found}: 1722.56; M_{calc}: 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 $(M+H\text{-}3TFA)^{4+}$ 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

Compound 3:



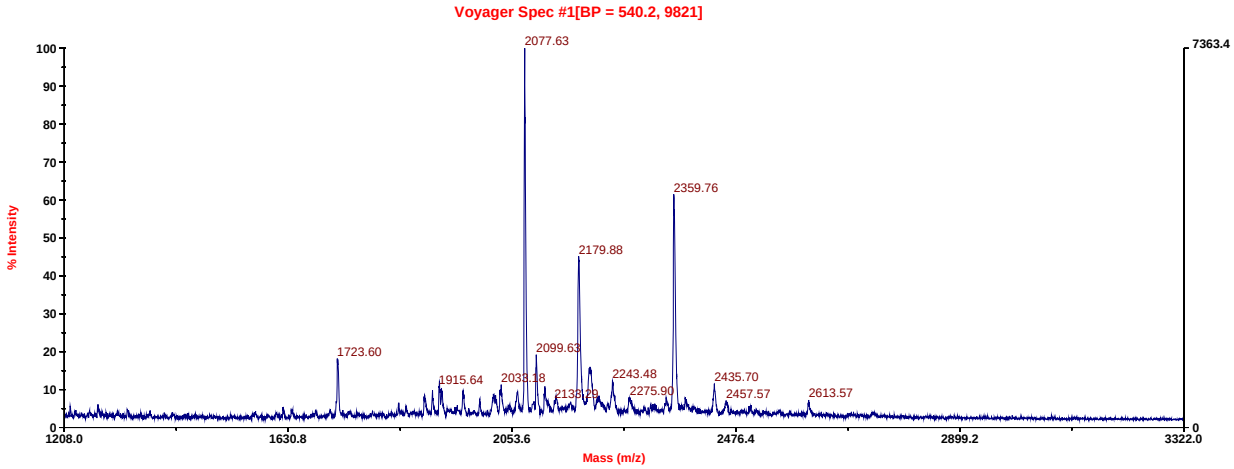
Maldi-Tof MS: M_{calc}: $(M+H^+)$ 1828.07; M_{found}: 1828.59

Compound 4:



Maldi-Tof-MS: M_{calc}: 1853.93 $(M+H^+)$ M_{found}: 1852.90

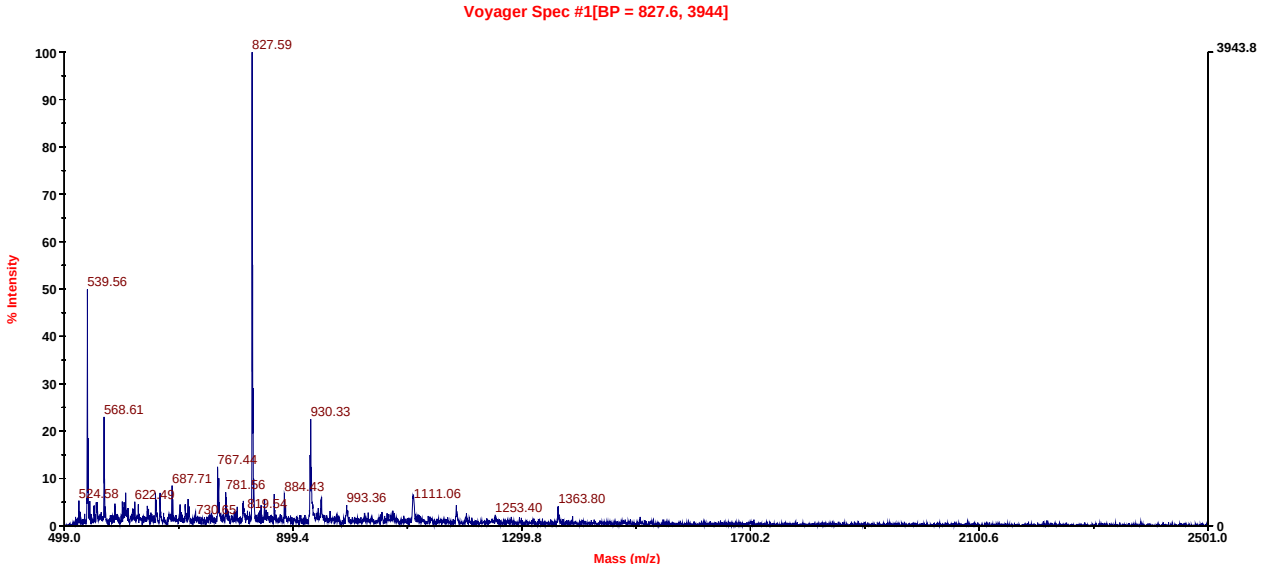
Compound 5:



Maldi-Tof-MS: M_{calc}: 2614.62 $(M\text{-}2H)^+$ M_{found}: 2613.57; M_{calc}: 2358.32 $(M\text{-Chrysi-}2H)^+$ M_{found}: 2359.76; M_{calc}: 2178.11 $(M\text{-Chrysi-Phen-}2H)^+$ M_{found}: 2179.88; M_{calc}: 2078.20 $(M\text{-Rh-Chrysi-Phen+}H)^+$ M_{found}: 2077.63

ESI-MS: M_{calc}: 796.93 $(M+5TFA+H)^{4+}$ M_{found}: 796.35; M_{calc}: 768.43 $(M+4TFA+H)^{4+}$ M_{found}: 767.85; M_{calc}: 739.92 $(M+3TFA+H)^{4+}$ M_{found}: 739.35; M_{calc}: 711.42 $(M+2TFA+H)^{4+}$ M_{found}: 710.85; M_{calc}: 682.91 $(M+TFA+H)^{4+}$ M_{found}: 683.63

Compound 6:

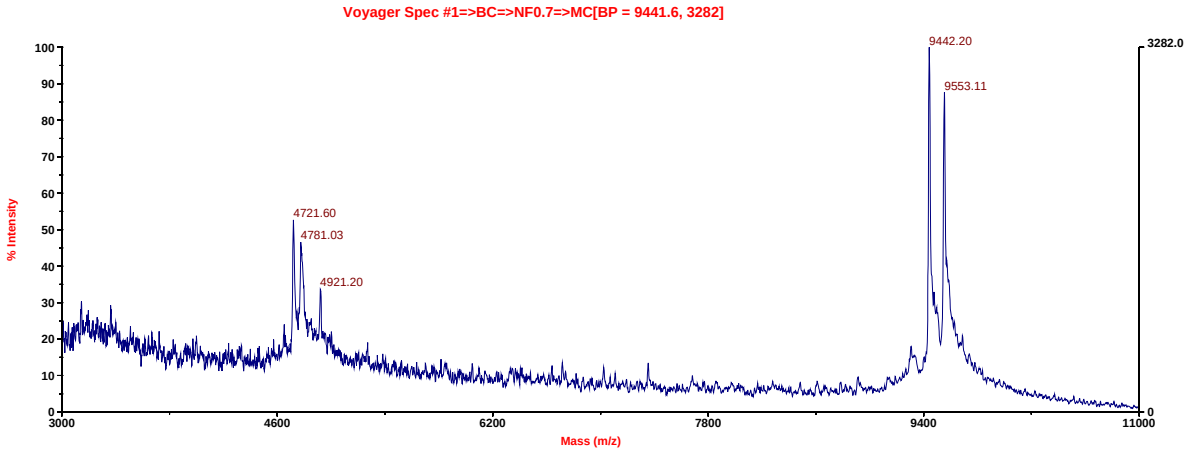


Maldi-TOF-MS: 1363.8=M^+; 1111.06=$(M\text{-Chrysi})^+$; 930.33=$(M\text{-Chrysi-Phen})^+$; 827.59=$(M\text{-Chrysi-Phen-Rh})^+$; 539.56=$(RhChrysiPhen)^+$

ESI-MS: 455.1 (M^{3+}); 682.6 $(M\text{-}H^+)^{2+}$

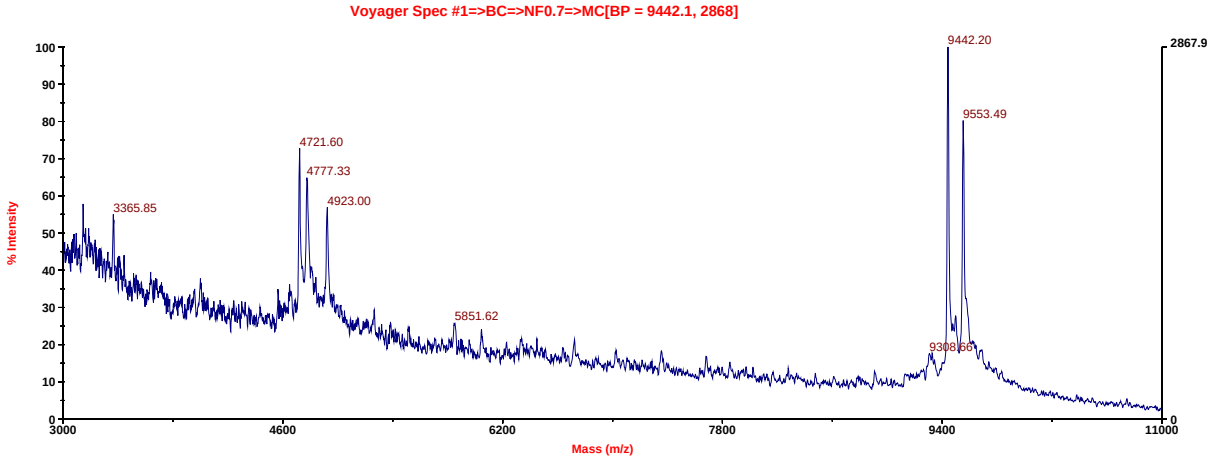
Control experiments for the MALDI-TOF analysis of the photocleavage reaction

Irradiation without 1 (light control, LC)



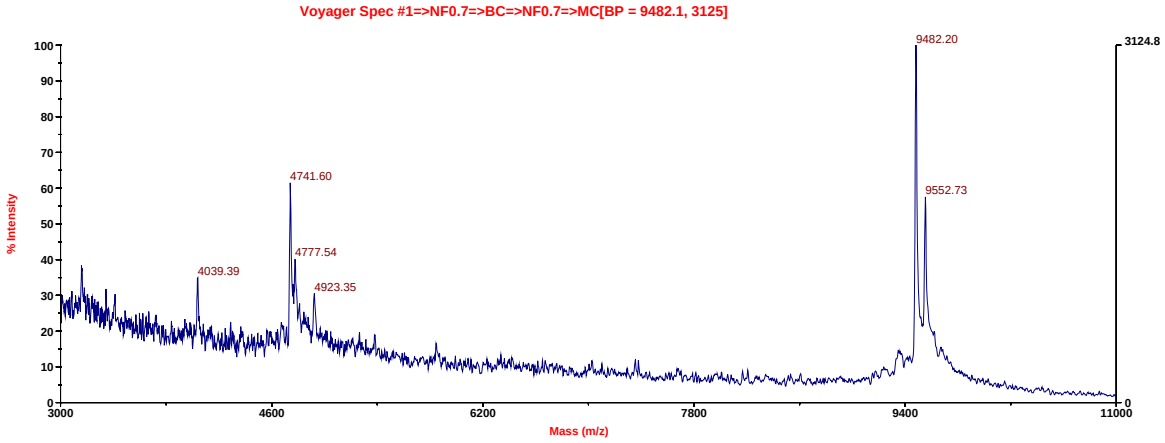
1 µM mismatched DNA AB, 50 mM NaCl, 10 mM phosphate buffer pH 7, 15 min irradiation.

Compound 1 and mismatched DNA without irradiation (dark control, DC)



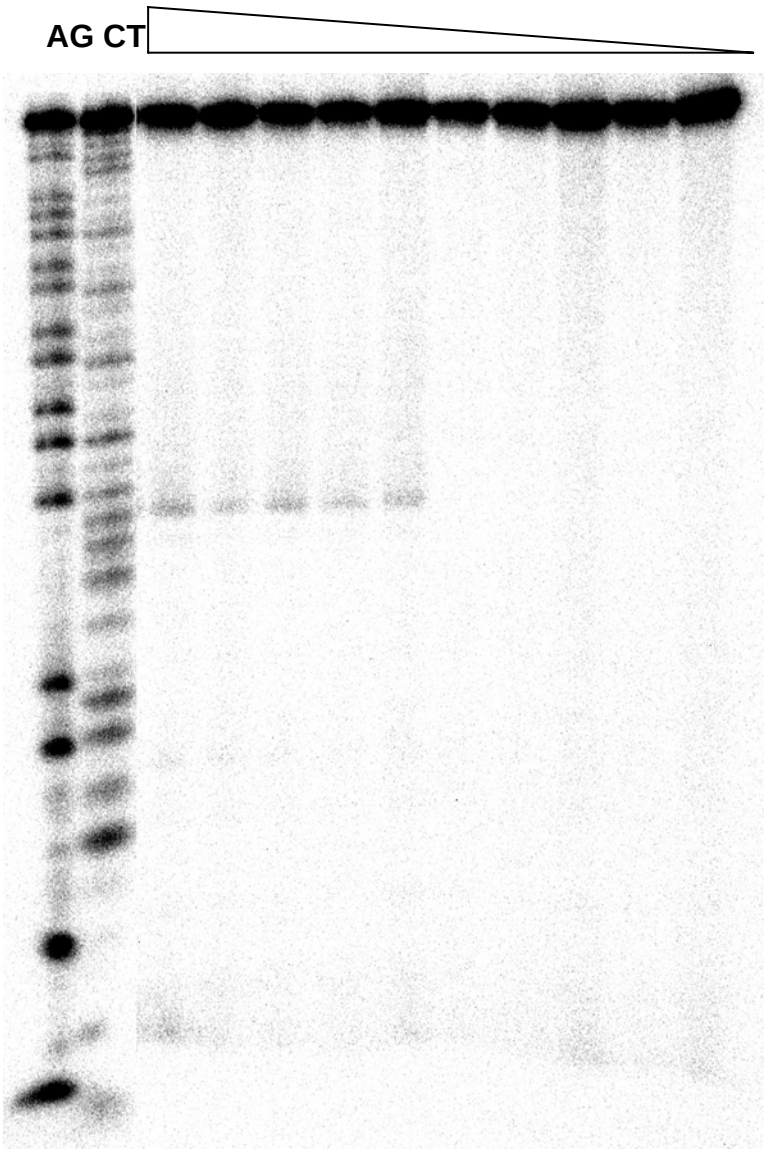
1 µM Rh-(Arg)8-FITC (1), 1 µM mismatched DNA AB, 50 mM NaCl, 10 mM phosphate buffer pH 7, without irradiation.

Compound 1, matched DNA



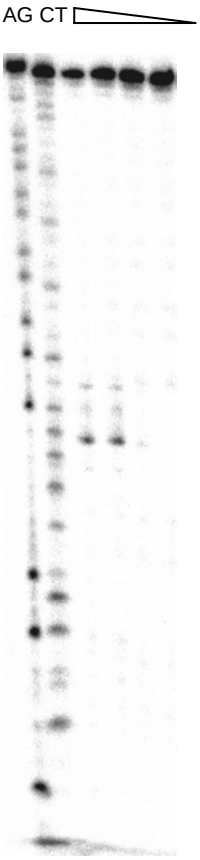
1 µM Rh-(Arg)8-FITC (1), 1 µM matched DNA AC, 50 mM NaCl, 10 mM phosphate buffer pH 7, 15 min irradiation.

Photocleavage experiments of 1 with mismatched DNA AB; DNA A was ^{32}P-labeled; analysis by PAGE



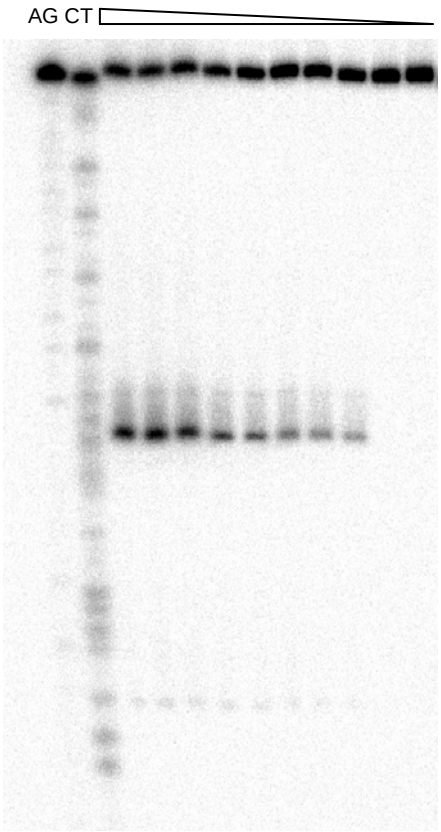
0,005-0,75 μM ***1,*** *0,005-0,75 μM mismatched DNA AB, 50 mM NaCl, 10 mM phosphate buffer pH 7, 15 min irradiation.*

Photocleavage of **5** with mismatched DNA AB; DNA A was ^{32}P-labeled; analysis by PAGE



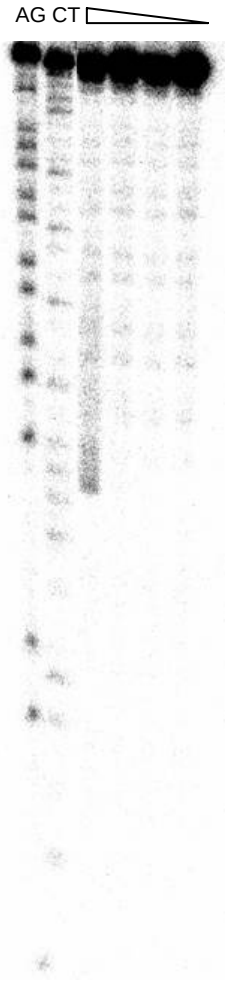
0,001-1 μM DNAs and **5***, 50 mM NaCl, 10 mM phosphate buffer pH 7, 15 minutes irradiation.*

Photocleavage of **2** with mismatched DNA AB; DNA A was ^{32}P-labeled; analysis by PAGE



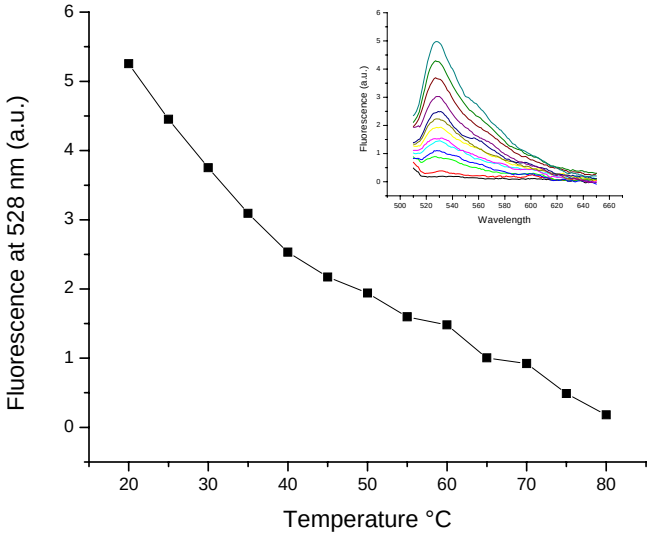
*0,005-1 μM mismatch DNA and **2**, 50 mM NaCl, 10 mM phosphate buffer pH 7, 15 min irradiation.*

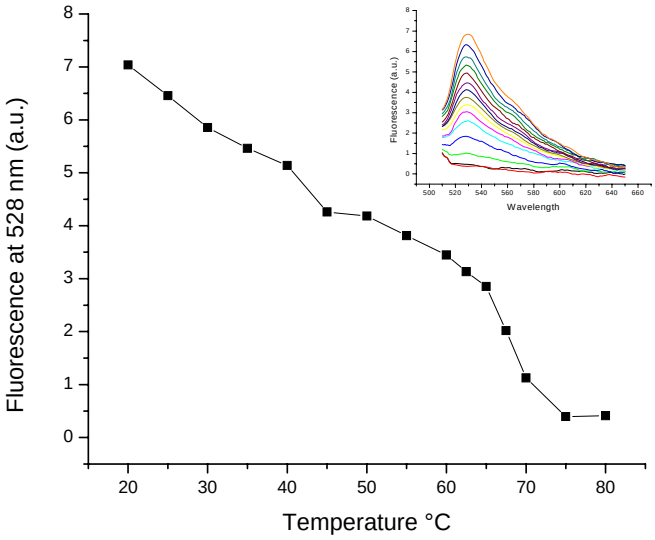
Photocleavage of **6** with mismatched DNA AB; DNA A was ^{32}P-labeled; analysis by PAGE



*0,01-0,5 μM DNAs, **6**, 50 mM NaCl, 10 mM phosphate buffer pH 7, 15 min irradiation.*

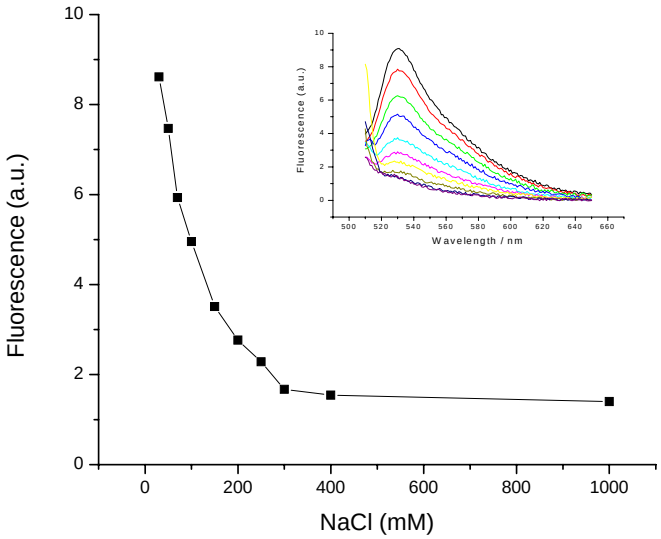
Temperature dependence of fluorescence of compound 5 to matched (left, DNA AC) and mismatched (right, DNA AB) DNA

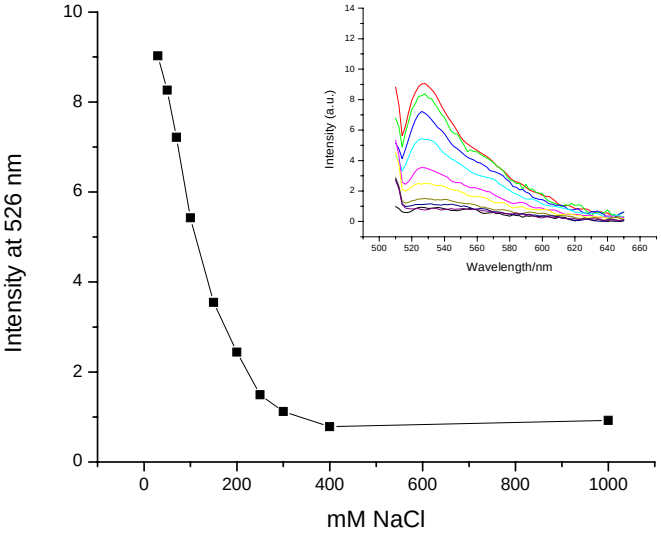




0,1 μM **5***, 0,1 μM DNAs, 50 mM NaCl, 10 mM phosphate buffer pH 7, excitation wavelength: 500 nm.*

Salt concentration dependence of fluorescence of 5 with match (left) and mismatch (right) DNA





0,1 μM **5***, 0,1 μM DNAs, 30-1000 mM NaCl, phosphate buffer pH 7, excitation wavelength: 500 nm.*