A Mismatch-Selective Bifunctional Rhodium-Oregon Green Conjugate: A Fluorescent Probe for Mismatched DNA

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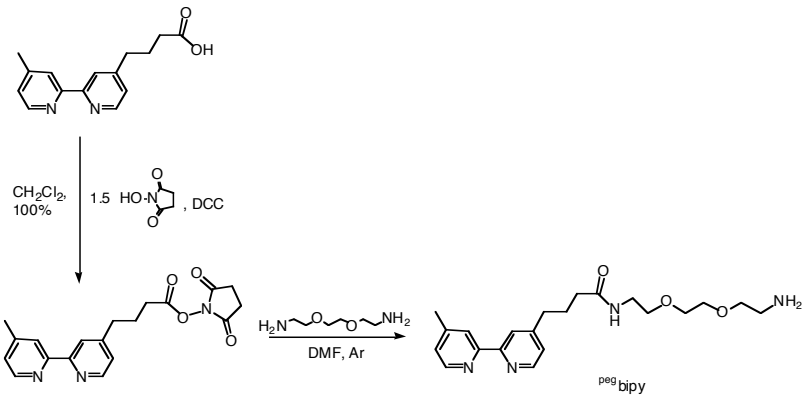
Supporting Information

General Synthesis: $RhCl_3$ was purchased from Pressure Chemicals and used as received. Oregon Green 514™ succinimidyl ester was purchased from Molecular Probes (Invitrogen), stored at -20 C, and used as received. Unless otherwise noted, all non-aqueous solvents were purchased from Fluka and stored under argon and over molecular sieves. All water used was purified using the MilliQ water purification system. All other starting materials were purchased from Aldrich Chemical Company and used as received. ^{1}H NMR were performed on a 300 MHz Varian Spectrometer at room temperature using solvent residual signal as a reference to TMS. ESI mass spectrometry was performed at the Protein/Peptide Microanalytical Laboratory (California Institute of Technology). UV-Vis spectra were taken on a Beckman DU7400 spectrophotometer, and extinction coefficients were determined using ICP-MS.

Ligand Synthesis: The peg-linker modified bipyridine linker was synthesized in two steps (Supplementary Figure 1) from 4-(4'-methyl-[2,2']bipyridinyl-4-yl)-butyric acid prepared according to the published procedure in Della Ciana, L.; Hamachi, I. Meyer, T.J. *JOC* **1989**, 54, 1731-1725.

Supplementary Figure 1



4-(4'-methyl-[2,2']bipyridinyl-4-yl)-butyric acid succinimidyl ester: 27 mg of DCC (.129 mmol) and 14 mg N-hydroxysuccinimide (.129 mmol) were added to 30 mg (.117 mmol) of 4-(4'-methyl-[2,2']bipyridinyl-4-yl)-butyric acid (prepared according to the published procedure in Della Ciana, L.; Hamachi, I. Meyer, T.J. *JOC* **1989**, 54, 1731-1725.) in CH_2Cl_2 and stirred at room temperature for 2 hours. After 2 hours, the reaction mixture was filtered and concentrated in vacuo. The pure final product was obtained as a clear oil after column chromatography (SiO_2, EtOAc, Hex). ^{1}H NMR: 8.55 (dd, 4H), 8.26 (d, 2H), 7.21 (d, 2H), 2.86 (m, 6H), 2.68 (t, 2H), 2.46 (s, 3H), 2.171 (t, 2H). ESI-MS: m/z = 354.

***N*-(2-[2-(2-aminoethoxy)-ethoxy]-ethyl)-4-(4'-methyl-[2,2']bipyridinyl-4-yl)-butyramide (peg-bpy):** 100 mg 4-(4'-methyl-[2,2']bipyridinyl-4-yl)-butyric acid succinimidyl ester was dissolved in 3 mL DMF, and added to a solution of 2 mL (excess) of 2,2'(ethylenedioxy)bis(ethylamine) in 1 mL DMF. After two hours, .05 mL DIEA was added to ensure deprotonation. The reaction mixture was stirred for 16 hours at room temperature. After 16 hours, the reaction mixture was concentrated in vacuo, taken up in CH_2Cl_2, extracted 2X with a saturated sodium bicarbonate solution, dried over $MgSO_4$, filtered, and re-concentrated in vacuo. The final product was obtained pure as a clear oil without column chromatography. ^{1}H NMR: 8.5 (m, 2H), 8,26 (s, 2H), 7.14 (t, 2H), 3,5-3.3 (m, 10H), 3.9-3.6 (m, 4H), 2.43 (s, 3H), 2.25-2.15 (m, 2H), 2.15-2.05 (m, 2H). ESI-MS: 387.

Conjugate Synthesis: As stated earlier, the metallointercalator moiety was synthesized via the sequential addition of phenanthroline, chrysenequinone, and peg-modified bypyridine to $RhCl_3$ according to the procedure published in Petitjean, A.; Barton, J.K. *J. Am. Chem. Soc.* **2004,** 126, 14728-14729. However, the peg-linker modified bipyridine was substituted for the alklyamino-bipyridine ligand from that publication.

Metallointercalator/Oregon Green Coupling: 5 mg Oregon Green 514 succinimidyl ester was dissolved in DMF and added to a solution of 5 mg of **2**. After two hours of stirring, .5 mL DIEA were added, and the resultant reaction mixture was allowed to stir under argon overnight. After 16 hours, 4 ml $H_{2=}O$ were added to the reaction mixture, and the resultant solution was loaded onto a Waters Sep-Pak, washed with water, and eluted with 1:1:.001 (H_2O:MeCN:TFA). The solution was then frozen with liquid nitrogen and lyophilized. The desired final product, **1**, was purified by preparative HPLC using a gradient of 99.9:.1 (water:TFA) to 99.9:1 (acetonitrile:TFA) over the course of 80 minutes. ESI-MS: m/z = 709, 1418. UV-Vis: 302 nm (ε=54,800); 313 nm (ε=44,600); 519 (ε=78,000).