Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science

SUPPORTING INFORMATION

Light-induced tryptophan radical generation in a click modular assembly of sensitiser-tryptophan residue

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Synthesis of Ruthenium-Tryptophan (Ru-Trp)

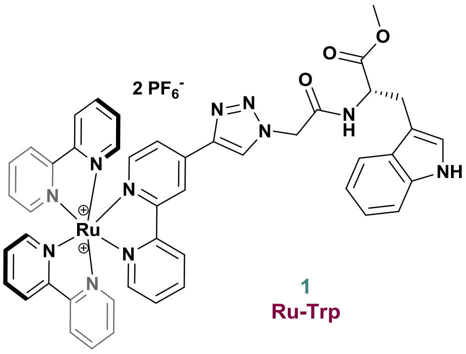
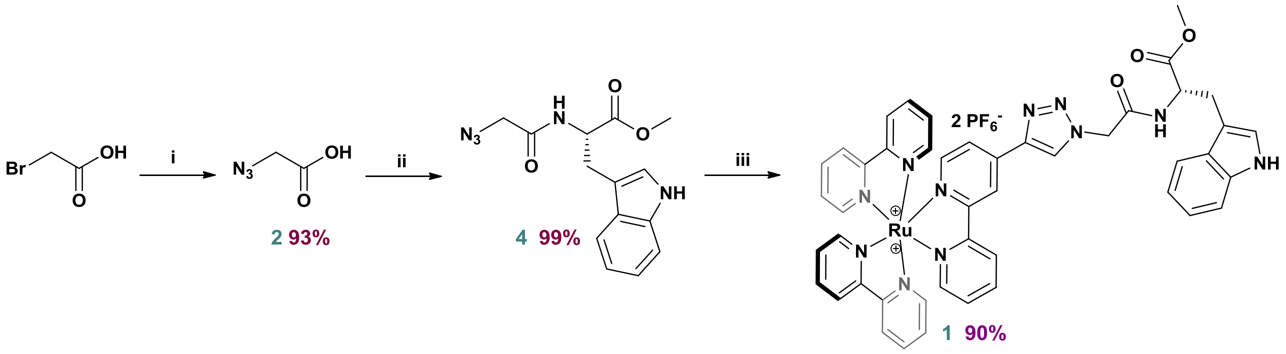


Chart 1 : Structures of the complex **Ru-Trp**.



Scheme 1 : *Reagents and Conditions*: (i) NaN_3, H_2O (93%);[1,2] (ii) (**3**) HBTU, DMAP, DIPEA, CH_3CN (99%);[2] (iii) (**5**),[3] $CuSO_4.5H_2O$ (15 mol%), sodium ascorbate (45 mol%), CH_2Cl_2/H_2O (1:1) (90%).

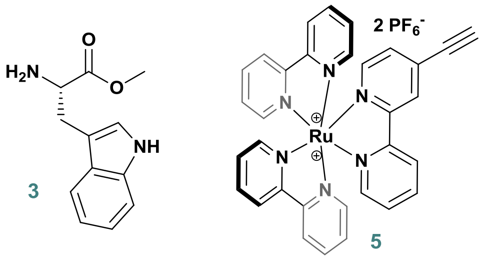
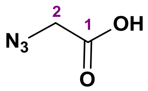


Chart 2 : Structures of the compounds (**3**) and (**5**)[3]

2-Azidoacetic acid (2):[1,2]



To sodium azide (3.74 g, 57.6 mmol) in H_2O (25 mL) was added bromoacetic acid (1.60 g, 11.5 mmol). The reaction mixture was stirred at room temperature overnight, acidified to pH 1 with 32% hydrochloric acid and extracted with EtOAc (3 × 75 mL). The combined organic extracts were washed with saturated aqueous NaCl solution then dried ($MgSO_4$), filtered and concentrated under reduced pressure to give 1.08g (10.6 mmol, 93%) of 2-azidoactic acid (**2**) as a colourless oil.

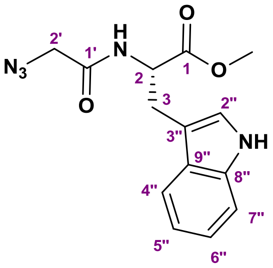
2-Azidoacetic acid (2): $C_2H_3N_3O_2$.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.98 (br s, 1H, COO**H**); 3.96 (s, 2H, **H-2**).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.7 (**C-1**); 50.2 (**C-2**).

MS (ESI$^-$): *m/z* 100.0 [M-H]$^-$. **HRMS (ESI$^-$)**: calculated for $C_2H_2N_3O_2$ 100.0152, found 100.0149.

(S)-Methyl 2-(2’’-azidoacetamido)-3-(1*H*-indol-3’’-yl)propanoate (4):[2]



To a solution of 2-azidoacetic acid (**2**) (0.53 g, 2.10 mmol, 1.0 eq.) and L-tryptophan methyl ester hydrochloride (**3**) (0.21 g, 2.10 mmol, 1.0 eq.) in anhydrous CH_3CN (52.0 mL) were added HBTU (0.79 g, 2.10 mmol, 1.0 eq.), DMAP (51.0 mg, 0.42 mmol, 0.2 eq.) and *N*,*N*-diisopropylethylamine (1.1 mL, 0.81 g, 6.29 mmol, 3.0 eq.). The reaction mixture was stirred at room temperature under argon atmosphere for 12 hours and concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with 10% citric acid (2 × 40 mL), 5% $NaHCO_3$ (2 × 40mL) and brine (1 × 40 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using silica gel (cyclohexane/EtOAc, 1:1) to give 0.62 g (2.07 mmol, 99%) of the desired amide (**4**) as colourless glue.

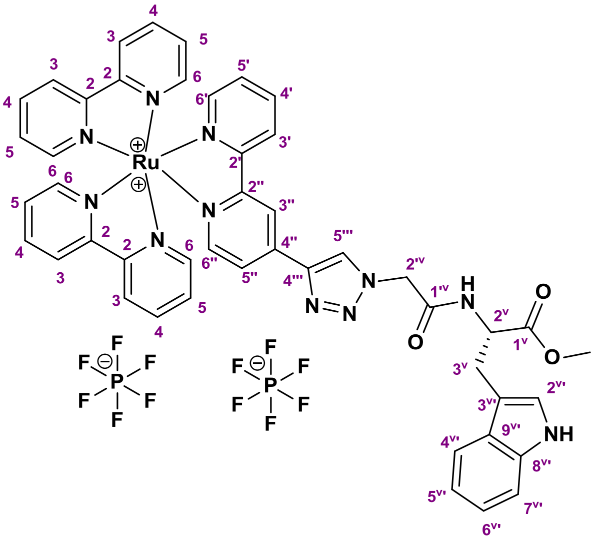
(S)-Methyl 2-(2’’-azidoacetamido)-3-(1*H*-indol-3’’-yl)propanoate (4): $C_{14}H_{15}N_5O_3$.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.15 (br s, 1H, N**H$_{Ar}$**); 7.51 (ddd, 1H, $^{3}J_{4'',5''}$ 7.9, $^{4}J_{4'',6''}$ 1.1, $^{5}J_{4'',7''}$ 0.9 Hz, **H-4’’**); 7.35 (ddd, 1H, $^{3}J_{7'',6''}$ 8.1, $^{4}J_{7'',5''}$ 1.1, $^{5}J_{7'',4''}$ 0.9 Hz, **H-7’’**); 7.19 (ddd, 1H, $^{3}J_{6'',7''}$ 8.1, $^{3}J_{6'',5''}$ 7.0, $^{4}J_{6'',4''}$ 1.1 Hz, **H-6’’**); 7.12 (ddd, 1H, $^{3}J_{5'',4''}$ 7.9, $^{3}J_{5'',6''}$ 7.0, $^{4}J_{5'',7''}$ 1.1 Hz, **H-5’’**); 6.98 (d, 1H, $^{3}J_{2'',NH}$ 2.4 Hz, **H-2’’**); 6.75 (br d, 1H, $^{3}J_{NH,2}$ 8.0 Hz, CON**H**); 4.92 (dt, 1H, $^{3}J_{2,NH}$ 8.0, $^{3}J_{2,3}$ 5.5 Hz, **H-2**); 3.90 (s, 2H, 2 **H-2’**); 3.69 (s, 3H, OC**H$_3$**); 3.33 (d, 2H, $^{3}J_{3,2}$ 5.5 Hz, 2 **H-3**).

^{13}C NMR (100 MHz, CDCl$_3$) δ: 171.9 (**C-1**); 166.6 (**C-1’**); 136.3 (**C-8’’**); 127.7 (**C-9’’**); 122.9 (**C-2’’**); 122.6 (**C-6’’**); 120.0 (**C-5’’**); 118.6 (**C-4’’**); 111.5 (**C-7’’**); 110.0 (**C-3’’**); 53.1 (**C-2**); 52.7 (**C-2’**, O**C**H$_3$); 27.8 (**C-3**).

MS (ESI^{+}): *m/z* 324.1 [M+Na]$^{+}$. **HRMS (ESI^{+})**: calculated for $C_{14}H_{15}N_5NaO_3$ 324.1067, found 324.1063.

(Bipyridine)$_2$-(bipyridine-triazol-tryptophan) ruthenium hexafluorophosphate (1):



Ruthenium complex (**5**) (151.3 mg, 0.17 mmol, 1.0 eq.) was suspended in CH$_2$Cl$_2$ (8.6 mL) under an argon atmosphere. (S)-Methyl 2-(2’’-azidoacetamido)-3-(1*H*-indol-3’’-yl)propanoate (**4**) (77.4 mg, 0.26 mmol, 1.5 eq.) were added, followed by successive addition of water (8.6 mL), sodium ascorbate (15.3 mg, 0.08 mmol, 0.45 eq.) and copper sulfate pentahydrate (6.4 mg, 0.03 mmol, 0.15 eq.). After 20 hours of stirring at room temperature, the reaction mixture was concentrated under vacuum. The crude solid was partially dissolved in a mixture of CH$_2$Cl$_2$/EtOAc then filtered through an alumina plug. The excess of azido derivative was removed by washing with CH$_2$Cl$_2$ and EtOAc. Then the desired ruthenium complex (**1**) was recovered by dissolving the crude solid and washing the alumina plug with CH_3CN and a solution of ammonium hexafluorophosphate in CH_3OH. The solvents were evaporated and the residue was dissolved in a minimum amount of CH_2Cl_2, then added drop wise to a large volume of pentane. The formed precipitate was filtered, washed several times with H_2O then Et_2O and dried under vacuum to yield 181.5 mg (0.15 mmol, 90%) of the desired hexafluorophosphate ruthenium complex (**1**) as a orange/red solid.

(Bipyridine)$_2$-(bipyridine-triazol-tryptophan) ruthenium hexafluorophosphate (1):

$C_{46}H_{39}F_{12}N_{11}O_3$P2Ru.

^{1}H NMR (400 MHz, CD$_3$CN) δ: 9.20 (br s, 1H, N**H**); 8.88 (d, 1H, ^4J 1.6 Hz, **H-3’’**); 8.65 (d, 1H, ^3J 8.0 Hz, **H-3’)**; 8.54-8.47 (m, 4H, 4 **H-3**); 8.42 (br s, 1H, **H-5’’’**); 8.11-8.02 (m, 5H, 4 **H-4**, **H-4’**); 7.82-7.71 (m, 5H, 4 **H-6**, **H-6’**); 7.79 (dd, 1H, ^3J 6.0, ^4J 1.6 Hz, **H-5’’**); 7.73 (d, 1H, ^3J 6.0 Hz,**H-6’’**); 7.50 (br d, 1H, ^3J 7.8 Hz, **H-4^{v}’**); 7.44-7.36 (m, 5H, 4 **H-5**, **H-5’**); 7.36 (br d, 1H, ^3J 8.0 Hz, **H-7^{v}’**); 7.12 (br d, 1H, ^3J 7.4 Hz, CON**H**); 7.12 (br d, 1H, ^3J 2.4 Hz, **H-2^{v}’**); 7.09 (br dd, 1H, ^3J 8.0, ^3J 6.8 Hz, **H-6^{v}’**); 7.04 (br dd, 1H, ^3J 7.8, ^3J 6.8 Hz, **H-5^{v}’**); 5.18 (d, 1H, ^2J 16.6 Hz, **H-2’^{v}a**); 5.13 (d, 1H, ^2J 16.6 Hz, **H-2’^{v}b**); 4.75 (ddd, 1H, ^3J 7.4, ^3J 6.7, ^3J 5.7 Hz, **H-2^{v})**; 3.66 (s, 3H, OC**H$_3$**); 3.29 (dd, 1H, ^2J 14.6, ^3J 5.7 Hz, **H-3^{v}a**); 3.22 (dd, 1H, ^2J 14.6, ^3J 6.7 Hz, **H-3^{v}a**).

^{13}C NMR (100 MHz, CD$_3$CN) δ: 173.0 (**C-1^{v}**); 166.3 (**C-1’^{v}**); 158.9 (**C-2’’**); 158.3 (4 **C-2**, **C-2’**); 153.3 (**C-6’’**); 153.1 (4 **C-6**); 153.0 (**C-6’**); 144.4 (**C-4’’’**); 141.2 (**C-4’’**); 139.2 (4 **C-4**, **C-4’**); 137.7 (**C-8^{v}’**); 129.1 (**C-5’**); 128.9 (4 **C-5**); 128.8 (**C-9^{v}’**); 126.9 (**C-5’’’**); 125.8 (**C-3’**); 125.6 (4 **C-3**); 125.3 (**C-2^{v}’**); 124.3 (**C-5’’**); 123.0 (**C-6^{v}’**); 121.4 (**C-3’’**); 120.5 (**C-5^{v}’**); 119.6 (**C-4^{v}’**); 112.8 (**C-7^{v}’**); 110.6 (**C-3^{v}’**); 54.9 (**C-2^{v}**); 53.5 (**C-2’^{v}**); 53.3 (O**C**H$_3$); 28.5 (**C-3^{v}**).

MS (ESI$^+$): *m/z* 1040.2 $[M+PF_6]^+$. **HRMS (ESI$^+$)**: calculated for $C_{46}H_{39}F_6N_{11}O_3PRu$ 1040.1929, found 1040.1904; calculated for $C_{46}H_{39}N_{11}O_3Ru$ 447.6141, found 447.6140.

References:

1. I. Choi, Y.-K. Kim, D.-H. Min, S. Lee and W.-S. Yeo, *Journal of the American Chemical Society*, 2011, **133**, 16718-16721.
2. M. Yu, J. R. Price, P. Jensen, C. J. Lovitt, T. Shelper, S. Duffy, L. C. Windus, V. M. Avery, P. J. Rutledge and M. H. Todd, *Inorganic Chemistry*, 2011, **50**, 12823-12835.
3. A. Baron, C. Herrero, A. Quaranta, M.-F. Charlot, W. Leibl, B. Vauzeilles and A. Aukauloo, *Inorganic Chemistry*, 2012, **51**, 5985-5987.

Experimental Section

Electrochemistry

Cyclic voltammetry and differential pulse voltammetry were performed using a CH Instruments potentiostat and a three-electrode system under argon environment A glassy carbon electrode was used as the working electrode and a platinum wire as counter electrode. The reference electrode was Ag/AgCl containing a 0.1 M AgCl:$AgNO_3$ solution. The working electrode was polished with 3 µm diamond paste before each measurement. All mentioned potentials in acetonitrile are with ferrocene as a reference measured before the experiment while ferri/ferrocyanide was used as reference in water. Supporting electrolytes were 100 mM tetrabutyl ammonium hexafluoro phosphate (in acetonitrile) and 500 mM KCl (in water).

EPR

X-band EPR measurements were obtained using a Bruker Elexsys E 500 EPR spectrometer with an Oxford ESR9 liquid helium flow cryostat using a gold-chromel thermocouple directly below the sample position. A 120 µL mixture of complex **Ru-Trp** (500 µM) and 4-Nitrobenzenediazonium tetrafluoroborate (25 mM) were transferred to an EPR tube which was rapidly submerged in an ethanol/liquid nitrogen bath at ~ -100°C. The EPR tube was pumped to vacuum and purged with helium 4-5 times in order to remove oxygen from the sample, and finally cooled to 77 K into liquid nitrogen prior to its transfer into the EPR spectrometer.

For sample illumination with monochromatic light we used a ThorLabs 450 nm LED. 10 second illuminations were performed with the sample in the EPR tube (prepared as described above) at room temperature. This time was sufficient to observe a change in colour in the sample. After illumination, the sample was rapidly immersed into liquid nitrogen before being transferred to the EPR spectrometer.

Photophysics

Ground state absorption spectra were measured with a Specord spectrophotometer using 1cm quartz cells. Time-resolved flash photolysis experiments were performed using an Edinburgh instruments LP 920 Flash Photolysis Spectrometer system equipped with a Continuum Surelite OPO operated at 460 nm for sample excitation. A 450 W Xenon arc lamp is used as source for the probe light for absorption measurements and detection was either by a PMT or a water-cooled ICCD camera (Andor). The presented transient absorption and emission traces were typically the average of 50 measurements. Time-resolved spectra were averages of 20 measurements. Samples were purged for 10 min with argon prior to each experiment.

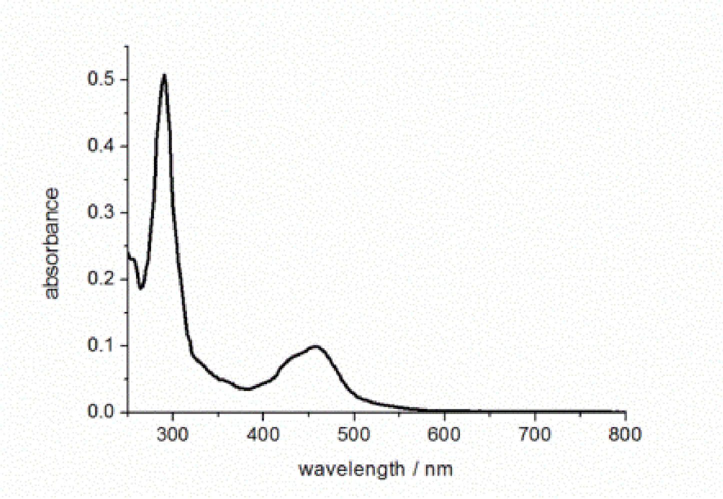


Fig. S1 UV/vis absorption spectrum of **Ru-Trp** in acetonitrile.

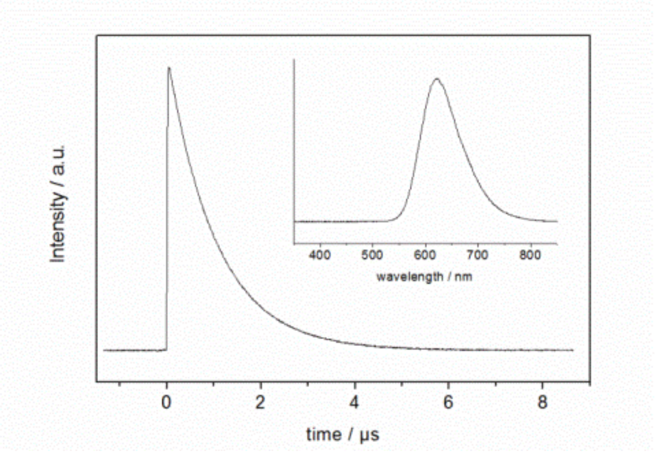


Fig. S2 Emission kinetics at 620 nm of **Ru-Trp** in argon-saturated acetonitrile. Inset: Emission spectrum at 100 ns. Excitation was done with a nanosecond laser flash at 460 nm.