CHEMISTRY OF LIGHT INTERACTION
CHEMPHOTOCHEM
ACROSS THE WHOLE SPECTRUM

Supporting Information

Intramolecular Photogeneration of a Tyrosine Radical in a Designed Protein

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Materials and Methods

Synthesis:

All chemicals with the exception of propargyl maleimide (Kerafast) were acquired from Sigma-Aldrich or a subsidiary and used without prior purification, unless noted. Steps were based on published literature methods.[1,2]

2,2'-Bipyridine-N-oxide (1)

To a solution of 2,2'-bipyridyl (3.0 g, 0.0188 mol, 1.0 eq.) in trifluoroacetic acid (15.0 mL, 21.0 g, 0.50 mol, 10 eq.) was added 30% hydrogen peroxide (3.0 mL, 0.95 g, 0.075 mol, 1.5 eq.). After stirring at room temperature for 4 h, the reaction mixture was neutralized by addition of aqueous 6N NaOH, then extracted with dichloromethane (4 x 50 mL). The combined organic layers were washed with aqueous saturated NaCl, dried over Na_2SO_4, filtered and concentrated to afford a colourless oil, which solidified under vacuum to a white solid (1.71g, 53%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.90 (d, *J* = 8.1 Hz, 1H), 8.74 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.33 (d, *J* = 6.4 Hz, 1H), 8.19 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.84 (td, *J* = 7.9, 1.8 Hz, 1H), 7.41 – 7.33 (m, 2H), 7.29 (dd, *J* = 6.8, 2.1 Hz, 1H). ESI+ m/z=194.9 $[M+Na]^+$ calculated 195.05344.

4'-Nitro-2,2'-bipyridine-N'-oxide (2)

2,2'-Bipyridine-N'-oxide (**1**) (3.0 g, 17.0 mmol, 1 eq.) was dissolved in concentrated sulphuric acid under stirring. sodium nitrate (0.93 g, 18.7 mmol, 1.1 eq.) was added slowly and heated to 100°C overnight. Once cooled, the solution was poured into ice (150 g) and neutralized in an ice-bath to pH 8 using 6N NaOH. The light yellow precipitate was filtered and washed with water. The solid was dissolved in methylene chloride, water was added and the mixture was extensively extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate, filtered and concentrated to yield 0.8 g (7.0 mmol, 37 %) of 4'-nitro-2,2'-bipyridine-N'-oxide as a beige solid. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.17 (d, *J* = 3.3 Hz, 1H), 8.89 (d, *J* = 8.1 Hz, 1H), 8.80 (d, *J* = 4.7 Hz, 1H), 8.36 (d, *J* = 7.2 Hz, 1H), 8.07 (dd, *J* = 7.2, 3.3 Hz, 1H), 7.88 (td, *J* = 7.9, 1.7 Hz, 1H), 7.47 – 7.41 (m, 1H). *HRMS:* calculated 218.0560, found 218.0565 for $C_{10}H_8N_3O_3$. Calculated 240.0380, found 240.0381 for $C_{10}H_8N_3O_3Na$.

4'-Azido-2,2'-bipyridine-N-oxide (3)

4’-Nitro-2,2’-bipyridine-N’-oxide (**2**) (0.8 g, 3.68 mmol, 1.0 eq.) and sodium azide (0.86 g, 13.25 mmol, 3.6 eq.) were suspended in anhydrous DMF (50 mL) and heated at 80°C for 48 hours under an argon atmosphere. After evaporation of the solvent, water was added and the mixture was extensively extracted with methylene chloride. The combined organic layers were dried over sodium sulfate, filtered and concentrated. The reaction mixture was then purified by flash chromatography on silica using methanol:dichloromethane (5:95) as the eluent. The desired product was the second fraction to elute from the column, which was a cakey, yellow solid (0.5 g, 64%). *HRMS*: calculated 236.0543, found 236.0541 for $C_{10}H_7N_5ONa$.

4’-Azido-2,2’-bipyridine (4)

4’-Azido-2,2’-bipyridine-N-oxide (**3)** (0.5 g, 2.36 mmol, 1.0 eq) was dissolved in dry dichloromethane (40 mL) and the solution was cooled to 0°C. Phosphorous tribromide (7.1 mL, 7.1 mmol, 3 eq) was added carefully. The reaction was allowed to stir under Ar(g) for 1 hour and then heated to reflux overnight. The solution was poured into ice and neutralized with 6N NaOH. The mixture was extensively extracted with CH_2Cl_2. The combined organic layers were dried over sodium sulfate, filtered and concentrated to yield 0.35 g (72.4 %). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.73 – 8.66 (m, 1H), 8.58 (d, *J* = 5.3 Hz, 1H), 8.40 (dt, *J* = 7.9, 1.1 Hz, 1H), 8.15 (d, *J* = 2.3 Hz, 1H), 7.83 (td, *J* = 7.7, 1.8 Hz, 1H), 7.34 (ddd, *J* = 7.4, 4.8, 1.2 Hz, 1H), 6.94 (dd, *J* = 5.4, 2.3 Hz, 1H). *ESI*+ m/z =220.1 $[M+Na]^+$ calculated 220.05991.

Bpymal: (1-((1-([2,2'-bipyridin]-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl)-1*H*-pyrrole-2,5-dione)

4’-Azido-2,2’-bipyridine (**5**) (100 mg, 0.51 mmol, 1.0 eq.) was suspended in CH_2Cl_2 under an argon atmosphere. Propargyl maleimide (69 mg, 1.0 eq.) was added, followed by successive addition of water, sodium ascorbate (100 mg, 1 eq.) and copper sulfate pentahydrate (127 mg, 1 eq.). The reaction mixture was diluted with CH_2Cl_2/H_2O (1:1) and extensively extracted with CH_2Cl_2. The organic layers were combined and washed with water, dried over sodium sulfate, filtered and concentrated to yield 90 mg of **bpymal** as a dark, orange oil (53%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.82 (d, *J* = 5.3 Hz, 1H), 8.71 (d, *J* = 4.6 Hz, 1H), 8.68 (d, *J* = 2.4 Hz, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 8.26 (s, 1H), 7.94 – 7.81 (m, 2H), 7.42 – 7.36 (m, 1H), 6.78 (s, 1H), 4.95 (s, 2H). *HRMS* calculated 333.1095 found 333.1098 for $C_{17}H_{13}N_6O_2$. Calculated 355.0914, found 355.0897 for $C_{17}H_{13}N_6O_2Na$.

Rubpymal:

$Ru(bpy)_2Cl_2$ (Sigma Aldrich) (120 mg, 0.25 mmol, 1.0 eq.) was reacted with silver nitrate (84 mg, 0.50 mmol, 2.0 eq.) in methanol (17.0 mL) for 3 hours at room temperature under an argon atmosphere. The suspension was filtered in order to remove the silver salt, and the filtrate was added to **bpymal** (90 mg, 0.28 mmol, 1.2 eq.). The solution was heated at reflux in the dark overnight under an argon atmosphere. The reaction mixture was allowed to go to room temperature and the solvent was evaporated. The remaining solid was re-dissolved in a minimum amount of methanol, and the desired compound was precipitated by drop wise addition of a saturated aqueous solution of ammonium hexafluorophosphate. The precipitate was filtered and dried under vacuum to yield 180 mg (0.17 mmol, 70 %) of the desired hexafluorophosphate ruthenium complex as a red solid and used without further purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.26 (s, 1H), 9.14 (s, 1H), 9.03 (d, J = 8.3 Hz, 1H), 8.97 – 8.80 (m, 5H), 8.25 (t, J = 8.1 Hz, 1H), 8.22 – 8.15 (m, 4H), 8.10 (d, J = 6.4 Hz, 1H), 7.86 (d, J = 5.9 Hz, 2H), 7.82 – 7.72 (m, 4H), 7.65 – 7.47 (m, 5H), 7.15 (s, 2H), 4.82 (s, 2H). ESI+ m/z= 891.2 $[M+PF_6]^+$ calculated 891.10818.

Peptide Labeling:

α_3DH_3-GSGC (sequence: MGS WAEFKQR LAAIKTR HQAL GG SEAEHAAFEKE IAAFESE LQAY KGKG NPE VEALRKE AAAIRDE HQA**Y** RHN GSGC) was prepared in phosphate buffer, pH 7.0. After the concentration was determined, 1.5 eq of Ru-bpymal in DMSO was added and allowed to react for 30 min. The peptide was purified via HPLC and the mass confirmed via QTOF-MS. Calculated average mass: 9060.97 m/z. Found, deconvoluted: 9058.3 m/z.

UV-visible and Transient Spectroscopy:

Samples were dissolved in 20 mM acetate-phosphate-borate (APB) buffer with 140 mM KCl for ionic strength. The pH was adjusted with concentrated HCl or NaOH. Absorption spectra were measured in a Specord spectrophotometer with 1 cm quartz cells. For subsequent transient absorption measurements, the absorption of the MLCT of Ru-bpy at 460 nm was measured and used to calculate the concentration of **α_3DH_3-Rubpymal**.

For transient absorption kinetics and spectral measurements in the time range 10 ns to 1 ms we used an Edinburgh Instruments LP920 Flash Photolysis Spectrometer system that incorporated a Continuum Surelite OPO for sample excitation (~7 ns pulse duration). The OPO was pumped by a Continuum Q-switched Nd:YAG laser operating at 355 nm. The LP920 system uses a 450 W Xenon arc lamp as the probe for the transient absorption kinetics measurements. Detection of the signal was performed either by a PMT or a water-cooled ICCD camera. The presented transient absorption spectra were typically the average of 20-50 measurements.

Kinetics analysis:

Transient absorption spectra in the presence of $[Ru^{III}(NH_3)_6]^{3+}$ were reconstructed after global fit analysis using

$$A_\lambda(t) = \Delta\varepsilon_Q[Q] + \Delta\varepsilon_{ox}[ox] \quad (1)$$

Where $\Delta\varepsilon_Q$< 300 M^{-1} cm^{-1} for $[Ru^{III}(NH_3)_6]^{3+}$, $\Delta\varepsilon_{ox}$ is the changes from the initially formed Ru^{III} to the final oxidized species, and

$$[ox](t) = \frac{Q_0}{1+ k_{rec}Q_0 t} e^{-k_{iet}t} \quad (2)$$

Is the time evolution of the initially oxidized Ru^{III}, which can either recombine with $[Ru^{II}(NH_3)_6]^{2+}$ or oxidized Y_{70}. Q_0= 5.3 µM was obtained by the fit in agreement with the initial ΔA, k_{rec} and k_{iet}, obtained by the fit, are respectively 0.4 x 10^9 s^{-1} and 3.3 x 10^5 s^{-1} and the charge recombination rate k_{rec} Q_0 is 3.6 x 10^3 s^{-1}.

EPR Spectroscopy:

X-band EPR spectra were recorded on a Bruker ELEXSYS 500 spectrometer equipped with a Bruker ER4119HS X band resonator, an Oxford Instrument continuous flow ESR 900cryostat and a temperature control system. The EPR tubes were put through 3 cycles of vacuum/helium in order to purge oxygen from the samples before being illuminated. For sample illumination with monochromatic light, we used a Thorlabs high power LED operating at 455 nm. Illuminations were performed with the sample in the EPR tube (see below for conditions). After illumination, the sample was rapidly transferred to a ~ -100°C ethanol bath for ~1-2 minutes, and then into liquid nitrogen before being transferred to the EPR spectrometer.

Sample conditions: 0.17 mM of α_3DH_3-**Rubpymal**, 7.2 mM $Co^{II}(NH_3)_5$ in 20 mM acetate-phosphate-borate buffer with 140 mM KCl for ionic strength at pH 5.5.

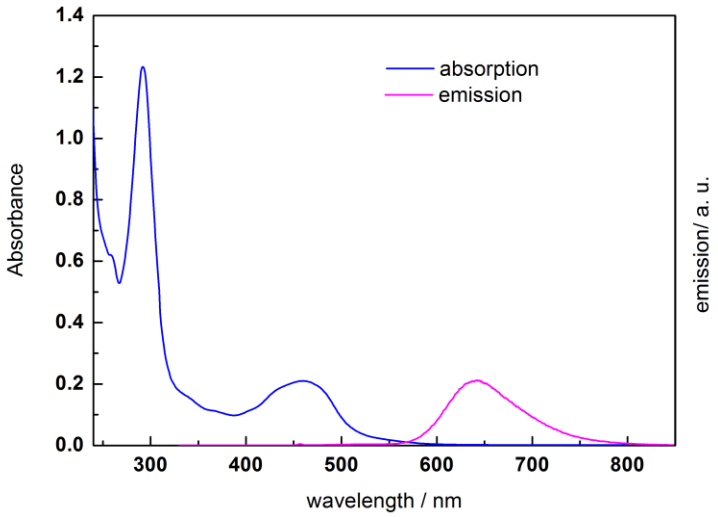


Figure S1. Steady-state absorption (blue,) and emission (magenta,) spectra in aqueous solution at pH 5 of **α_3DH_3-Rubpymal**. Emission spectrum has been taken upon laser pulse at 460 nm (integration time 10 ns)

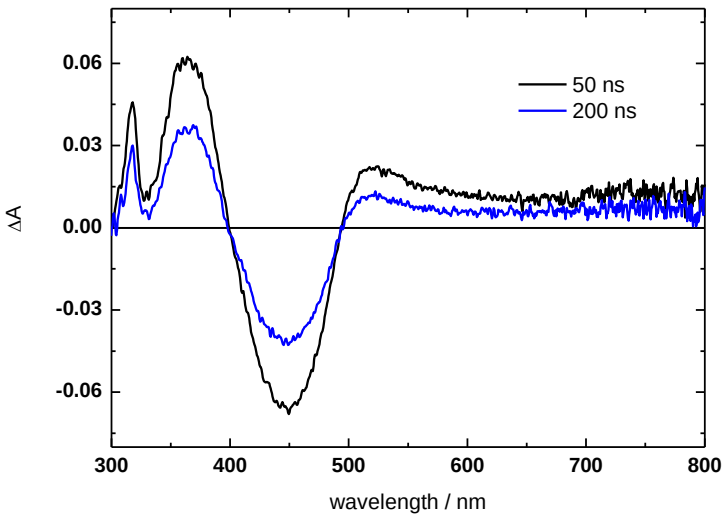
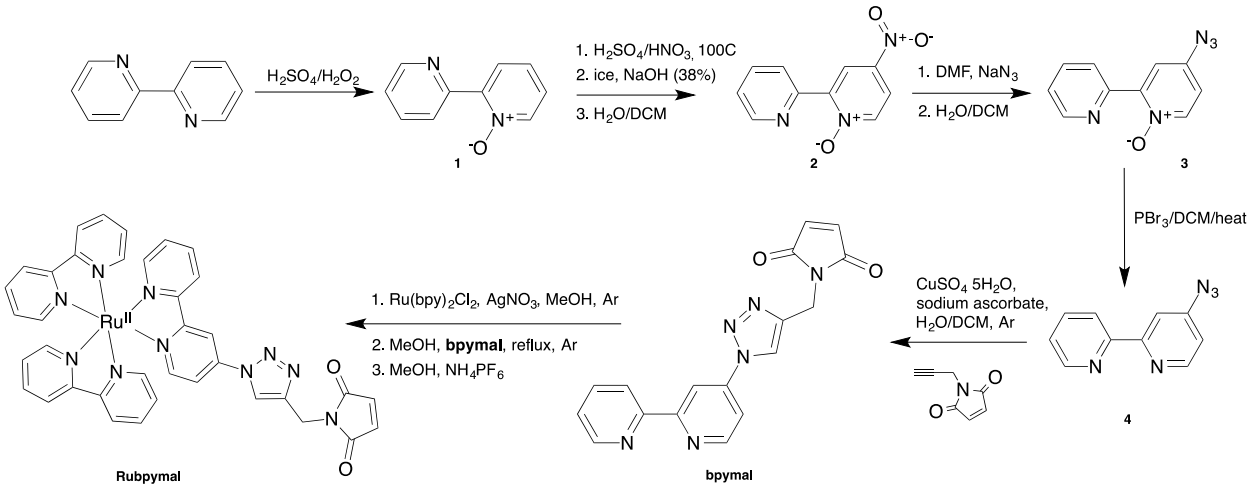


Figure S2. Differential absorption spectra of **α_3DH_3-Rubpymal** in aqueous solution, pH 5 at 50 ns (black) and 200 ns (blue) after laser pulse (integration time 10 ns). Excitation wavelength 460 nm, laser energy ~5mJ. OD_{460}= 0.38. Bleaching at 450 nm is due to loss of Ru(II) and formation of excited state Ru*$(bpy)_3$ and subsequently, Ru(III)$(bpy)_3$.



Scheme S1. Synthesis of **Rubpymal**

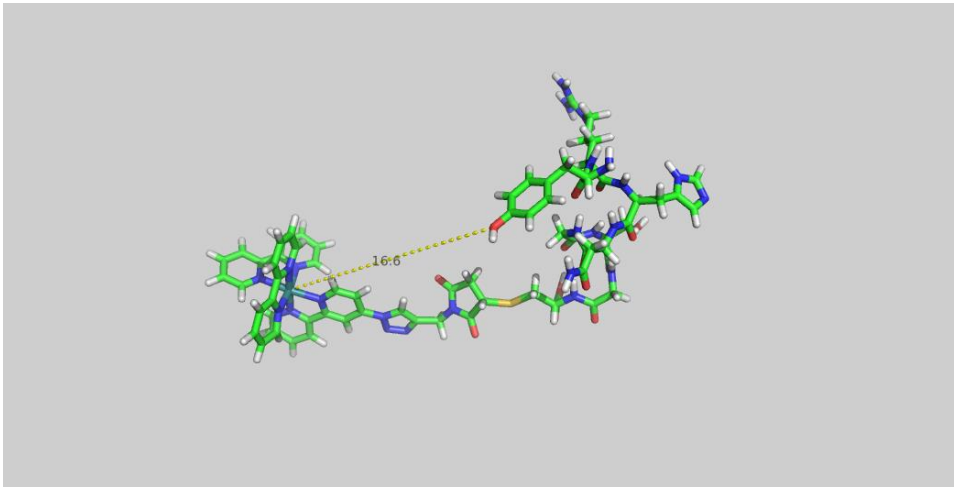
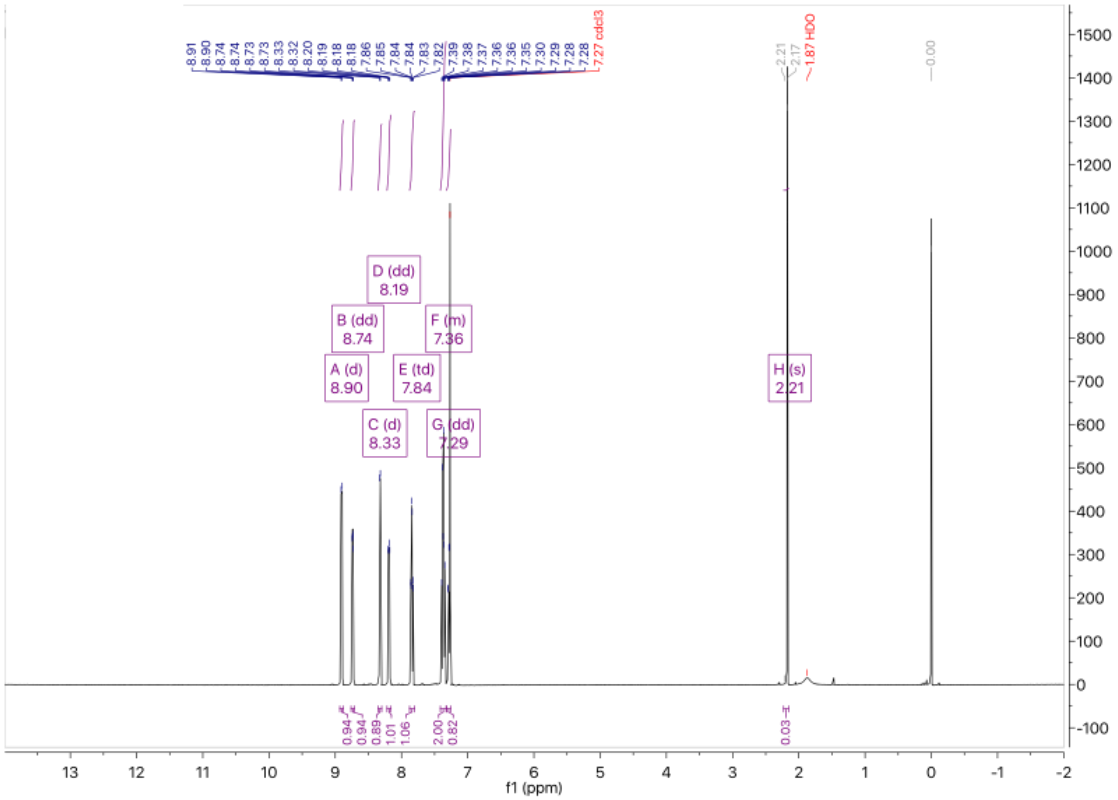
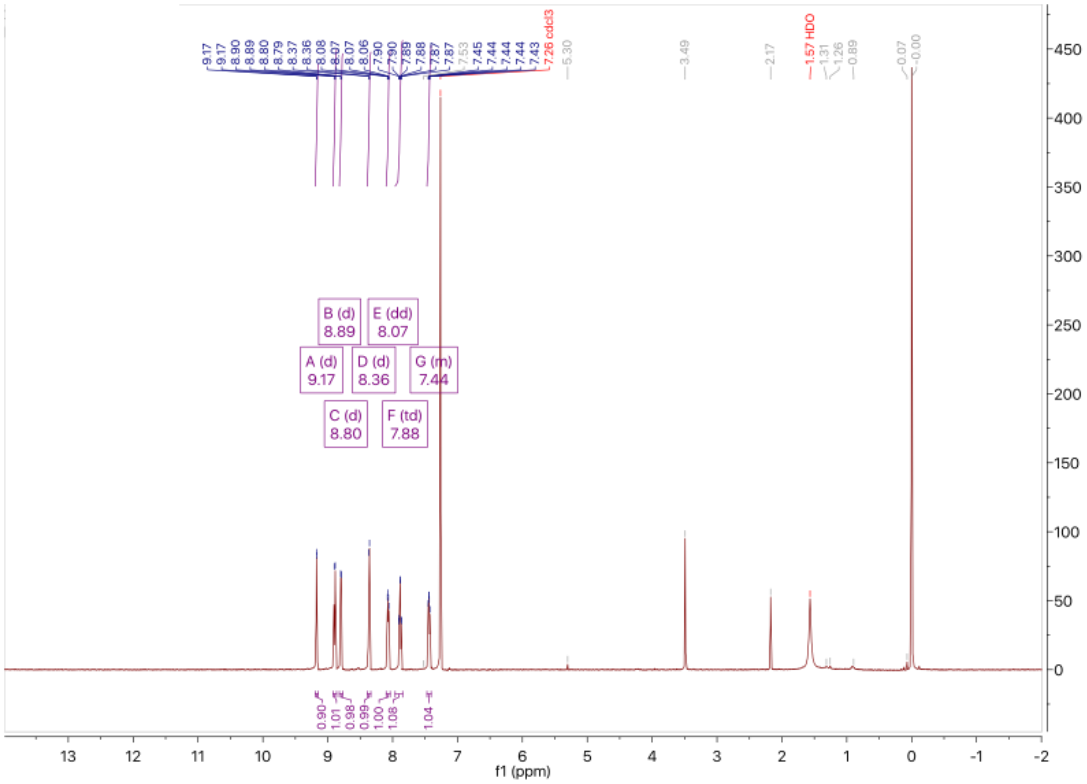


Figure S3. Model of **Rubpymal** bound to last eight residues of α_3DH_3-GSGC. $Ru(bpy)_3$ is derived from crystal structure (CCDC: 1173907) and the rest of the structure was built and optimized in Avogadro.[3]

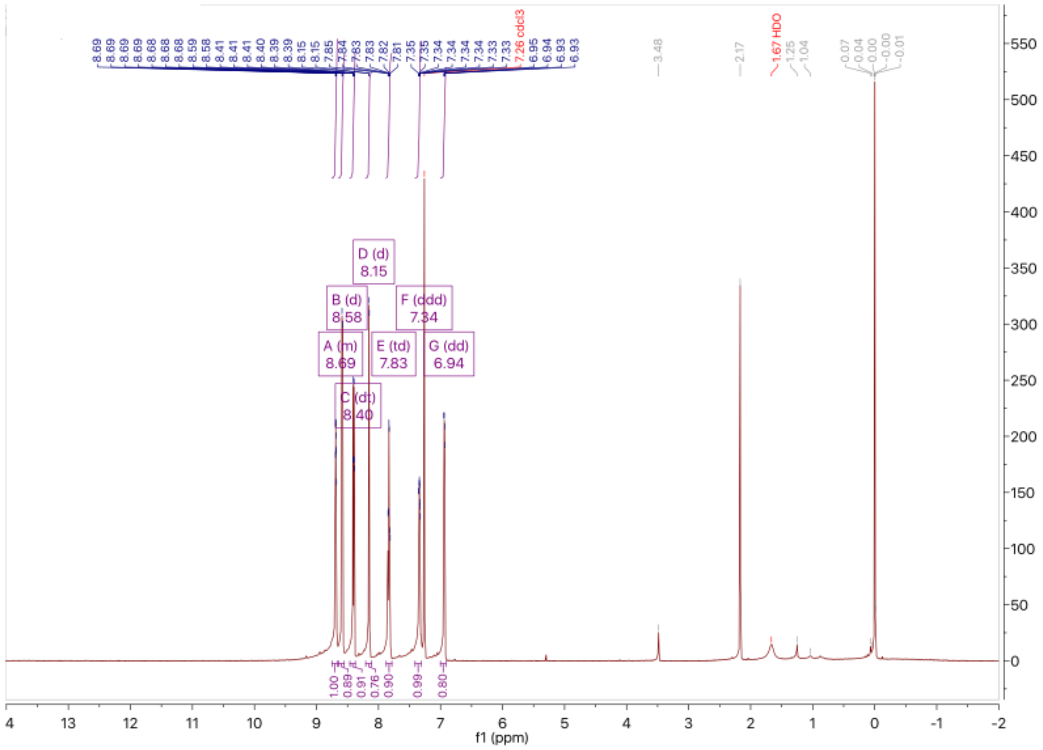
NMR SPECTRA: **2,2'-Bipyridine-N-oxide**



4’-Nitro-2,2’-bipyridine-N’-oxide



4’-Azido-2,2’-bipyridine-N-oxide



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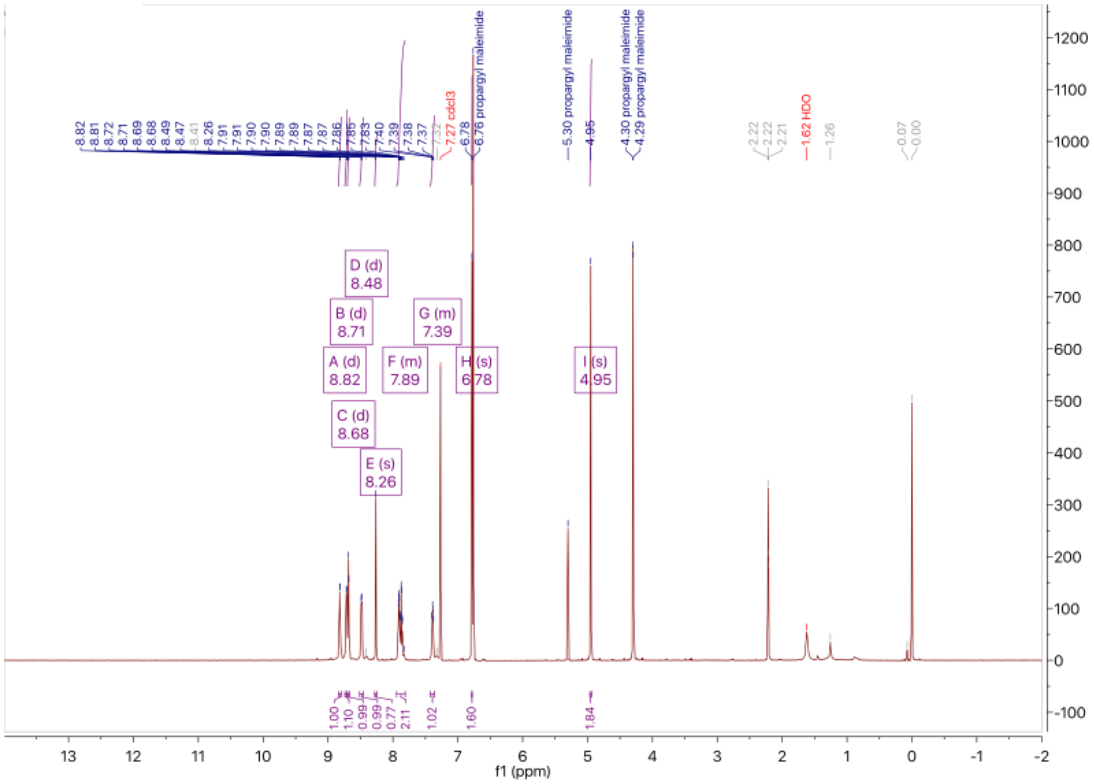
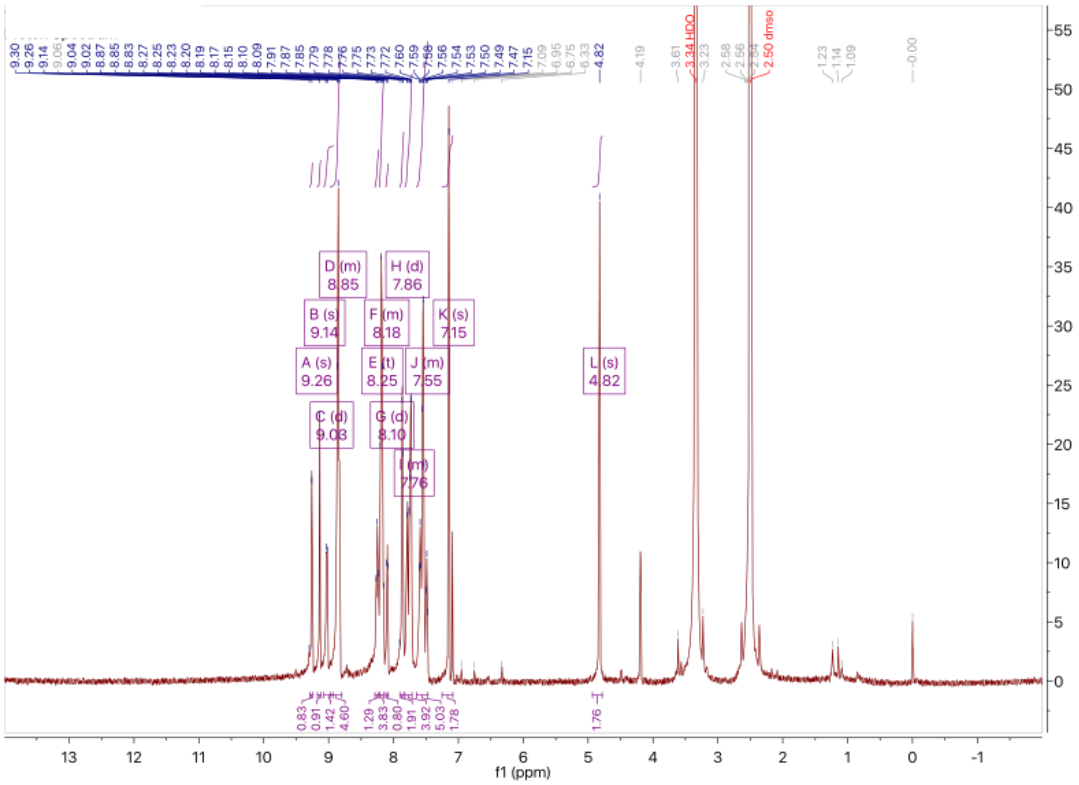


Rubpymal:



[1] A. Baron, C. Herrero, A. Quaranta, M.-F. Charlot, W. Leibl, B. Vauzeilles, A. Aukauloo, *Inorg. Chem.* **2012**, *51*, 5985–5987.

[2] A. Baron, C. Herrero, A. Quaranta, M.-F. Charlot, W. Leibl, B. Vauzeilles, A. Aukauloo, *Chem. Commun.* **2011**, *47*, 11011–11013.

[3] M. D. Hanwell, D. E. Curtis, D. C. Lonie, T. Vandermeersch, E. Zurek, G. R. Hutchison, *J Cheminf* **2012**, *4*, 17–33.