Supplementary Information

Metal coordination, and metal-ligand redox non-innocence, modulates allosteric C-N bond homolysis in an N-benzyl tetrazine

Cooper W. Johnston, Travis R. Schwantje, Michael J. Ferguson, Robert McDonald, and Robin G. Hicks

Department of Chemistry, The University of Victoria, Victoria V8W 3V6, B.C., Canada.

Department of Chemistry, The University of Alberta, Edmonton T6G 2G2, Alta, Canada.

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Experimental Details

General Considerations

1,5-diisopropyl-3-pyridin-2-yl-6-oxoverdazyl (1), [1,5-diisopropyl-3-(2-pyridyl)-6-oxoverdazyl] bis(hexafluorocacetylacetonato)ruthenium (1Ru), and 1-benzyloxy-2,2,6,6-tetramethylpiperidine (2) were synthesized using literature procedures. All reagents were purchased from Aldrich and used as received without further purification unless otherwise stated. DCM and tert-butylbenzene were purified according to literature.

1H, 13C, and 19F NMR spectra were recorded on Bruker AC300 (300MHz), AC360 (360MHz), and AC500 (500MHz) instruments. FT-IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer as pressed KBr discs. UV-Vis spectra were recorded on a Perkin-Elmer Lambda 1050 spectrometer. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses were performed by Canadian Microanalytical Services Ltd., Delta, British Columbia, Canada. Mass spectrometry data was collected an ESI-Orbitrap by Ori Granot (University of Victoria).

5-benzyl-2,4-diisopropyl-6-(pyridin-2-yl)-4,5-dihydro-1,2,4,5-tetrazin-3(2H)-one (Bn-1)

Benzyl bromide (0.15mL, 1.2mmol) and 1 (287mg, 1.10mmol) were dissolved in dry, degassed CH2Cl2 (40mL) added. Mn2(CO)10 (218mg, 0.56mmol) was added and the reaction flask was placed into a light box containing 6 full spectrum light bulbs and fitted with a condenser making sure to exclude air. The reaction was stirred in the light box for 24h after which a dark coloured solution was obtained. The reaction was removed from the light box and DBU (0.33mL, 2.2mmol) was added. After 2h, the solvent was removed in vacuo and the resulting black solid was stirred with Et2O (70mL) for 1h. The Et2O was collected and this process was repeated another 3 times. The Et2O was combined with H2O (50mL) and extracted with 10% HCl (5x20mL). The organic layer was discarded. The aqueous layer was rendered basic using 2M NaOH solution until pH 14 was reached and was extracted with Et2O (4X100mL). The organic layer was washed with H2O until a neutral pH was reached and then dried over MgSO4 overnight. The solvent was evaporated yielding a light tan product (220mg, 57%). 1H NMR (500MHz, DCM-d2, 250K): δ 8.71 (d, 1H, J = 8 Hz), 7.99 (d, 1H, J = 8 Hz), 7.79 (dt, 1H, J = 2, 8 Hz), 7.36 (ddd, 1H, J = 1, 5, 7 Hz), 7.28-7.20 (m, 3H), 7.15-7.10 (m, 2H), 4.32 (sept, 1H, J = 7 Hz), 4.32 (d, 1H, J = 15 Hz), 4.00 (sept, 1H, J = 7 Hz), 1.34 (d, 3H, J = 7 Hz), 1.34 (d, 3H, J = 7 Hz), 1.03 (d, 3H, J = 7 Hz), 0.65 (d, 3H, J = 7 Hz) ppm. 13C NMR (500MHz, DCM-d2, 250K): δ 156.5, 149.8, 149.7, 147.3, 136.8, 135.8, 129.5, 128.3, 128.0, 124.3, 122.8, 59.6, 52.6, 47.1, 20.8, 20.0, 19.7, 19.6 ppm. FT-IR (KBr): 3050 (w), 3032 (w), 2977 (m), 2963 (w), 2873 (w), 1765 (s), 1607 (m), 1582 (m), 1566 (m), 1474 (m), 1456 (m), 1435 (m), 1381 (s), 1361 (s), 1338 (m), 1317 (m), 1200 (m), 1180 (m), 1138 (m), 1043 (m), 966 (w), 826 (w), 790 (m), 747 (m), 706 (s), 670 (m), 608 (m) cm⁻¹. UV-Vis (MeCN): λmax 258nm (ε = 1.0x10⁴ L·mol⁻¹·cm⁻¹), 332nm (ε = 4.8x10³ L·mol⁻¹·cm⁻¹). MS (ESI): m/z LR-MS 352 (M+H+, 100%), HR-MS: calcd. 352.2132, expct. 352.2130 (M+H+, 100%). MP: 85-88°C (decomposition).
Benzyl bromide (0.22mL, 0.176mmol) and 1Ru (137mg, 0.176mmol) were dissolved in dry CH\textsubscript{2}Cl\textsubscript{2} (40mL) under an argon atmosphere. The solution was sparged with Ar for 30min. Mn\textsubscript{2}(CO)\textsubscript{10} (35mg, 0.090mmol) was added under Ar and the solution was stirred for 24h in a light box containing 6 full spectrum light bulbs and fitted with a condenser while making sure to exclude air. Solvent was then removed in vacuo. Column chromatography of the dark red solid (SiO\textsubscript{2}, DCM:hexanes, 1:1) yielded a major red fraction. The solvent was removed in vacuo and the product was dissolved in a minimal amount of hexanes and left to evaporate. Dark red crystals were obtained (130mg, 85%) which were suitable for X-ray crystallography. 

$^{1}$H NMR (300MHz, DCM-d\textsubscript{2}, 298K): \(\delta\) 8.36 (mult, 2H), 7.60-7.71 (mult, 4H), 7.13-7.27 (mult, 12H), 6.23 (s, 1H), 6.22 (s, 1H), 6.06 (s, 1H), 4.29-4.49 (mult, 7H), 3.44 (septet, 1H, J = 7 Hz), 1.46 (d, 3H, J = 8 Hz), 1.45 (d, 3H, J = 8 Hz), 1.32 (d, 3H, J = 7 Hz), 1.20 (d, 3H, J = 7 Hz), 1.06 (d, 3H, J = 7 Hz), 1.02 (d, 3H, J = 7 Hz), 1.02 (d, 3H, J = 7 Hz), 0.94 (d, 3H, J = 7 Hz) ppm. 

$^{13}$C NMR (360MHz, DCM-d\textsubscript{2}, 298K): \(\delta\) 175.2 (q, \(^2\)J\textsubscript{CF} = 34 Hz), 174.6 (q, \(^2\)J\textsubscript{CF} = 34 Hz), 173.7 (q, \(^2\)J\textsubscript{CF} = 34 Hz), 173.5 (q, \(^2\)J\textsubscript{CF} = 34 Hz), 172.4 (q, \(^2\)J\textsubscript{CF} = 34 Hz), 171.3 (q, \(^2\)J\textsubscript{CF} = 34 Hz), 170.2 (q, \(^2\)J\textsubscript{CF} = 34 Hz), 170.2 (q, \(^2\)J\textsubscript{CF} = 34 Hz), 155.2, 155.1, 154.6, 154.2, 152.9, 151.6, 151.5, 151.5, 151.5, 136.4, 136.2, 133.9, 133.7, 131.0, 130.8, 129.3, 129.1, 125.0, 125.0, 124.5, 117.6 (q, \(^1\)J\textsubscript{CF} = 283 Hz), 117.5 (q, \(^1\)J\textsubscript{CF} = 283 Hz), 117.4 (q, \(^1\)J\textsubscript{CF} = 283 Hz), 116.9 (q, \(^1\)J\textsubscript{CF} = 283 Hz), 116.8 (q, \(^1\)J\textsubscript{CF} = 283 Hz), 116.5 (q, \(^1\)J\textsubscript{CF} = 283 Hz), 116.3 (q, \(^1\)J\textsubscript{CF} = 283 Hz), 92.8, 92.4, 92.2, 62.0, 61.8, 56.9, 56.2, 51.9, 51.6, 21.6, 21.0, 20.7, 20.3, 19.3, 19.1 ppm. 

$^{19}$F NMR (360MHz, DCM-d\textsubscript{2}, 298K): \(\delta\) -74.86 (s, 3F), -74.94 (s, 3F), -75.10 (s, 3F), -75.22 (s, 3F), -75.24 (s, 3F), -75.34 (s, 6F), -75.39 (s, 3F) ppm. FT-IR (KBr): 2983 (w), 2940 (w), 1679 (s), 1570 (m), 1543 (m), 1514 (m), 1477 (m), 1402 (m), 1389 (m), 1333 (m), 1262 (s), 1205 (s), 1150 (s), 1094 (m), 1059 (m), 944 (w), 801 (w), 789 (w), 754 (m), 694 (m), 614 (w) cm\textsuperscript{-1}. UV-Vis (MeCN): \(\lambda_{\text{max}}\) 290nm (\(\epsilon = 2.5\times10^4\) L·mol\textsuperscript{-1}·cm\textsuperscript{-1}), 337nm (\(\epsilon = 6.4\times10^3\) L·mol\textsuperscript{-1}·cm\textsuperscript{-1}), 495nm (\(\epsilon = 1.2\times10^4\) L·mol\textsuperscript{-1}·cm\textsuperscript{-1}). MS (ESI): \(m/z\) LR-MS 867 (M\textsuperscript{+}, 100%), HR-MS: theor 867.08640, expt 867.08665 (M\textsuperscript{+}, 100%). Anal. Calc. for C\textsubscript{30}H\textsubscript{27}N\textsubscript{5}O\textsubscript{5}F\textsubscript{12}Ru: C, 41.58; H, 3.14; N, 8.08. Found: C, 41.42; H, 3.20; N, 8.18. MP: 138-140°C (decomposition begins ca. 60°C).
Figure S1. $^1$H NMR of Bn-1 in DCM-$d_2$.

Figure S2. $^{13}$C NMR of Bn-1 in DCM-$d_2$. 

NMR Spectra
Figure S3. $^1$H NMR Bn-1Ru in DCM-$d_2$.

Figure S4. $^1$H NMR of Bn-1Ru in DCM-$d_2$ (Downfield).
Figure S5. $^1$H NMR of Bn-1Ru in DCM-d$_2$ (Midfield).

Figure S6. $^1$H NMR of Bn-1Ru in DCM-d$_2$ (Upfield).
Figure S7. $^{13}$C NMR of Bn-1Ru in DCM-$d_2$. 
**Figure S8.** $^{13}$C NMR of Bn-1Ru in DCM-$d_2$ (COF$_3$).

**Figure S9.** $^{13}$C NMR of Bn-1Ru in DCM-$d_2$ (CF$_3$).
Figure S10. $^{19}$F NMR of Bn-1Ru in DCM-$d_2$. 
## Crystallographic Data

**Table S1.** Crystallographic data of Bn-1 and Bn-1Ru.

<table>
<thead>
<tr>
<th></th>
<th>Bn-1</th>
<th>Bn-1Ru</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical Formula</strong></td>
<td>C(<em>{20})H(</em>{25})N(_{5})O</td>
<td>C(<em>{30})H(</em>{27})F(<em>{12})N(</em>{5})O(_{5})Ru</td>
</tr>
<tr>
<td><strong>Formula wt. (g/mol)</strong></td>
<td>351.45</td>
<td>866.64</td>
</tr>
<tr>
<td><strong>T (K)</strong></td>
<td>173</td>
<td>173</td>
</tr>
<tr>
<td><strong>Wavelength (Å)</strong></td>
<td>0.71073</td>
<td>1.54178</td>
</tr>
<tr>
<td><strong>Crystal system</strong></td>
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<td>monoclinic</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>(P2_1/c)</td>
<td>(P2_1/n)</td>
</tr>
<tr>
<td><strong>a (Å)</strong></td>
<td>10.0802 (8)</td>
<td>20.7821 (4)</td>
</tr>
<tr>
<td><strong>b (Å)</strong></td>
<td>9.8139 (8)</td>
<td>7.89150 (10)</td>
</tr>
<tr>
<td><strong>c (Å)</strong></td>
<td>19.8569 (15)</td>
<td>23.1429 (4)</td>
</tr>
<tr>
<td><strong>α(°)</strong></td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td><strong>β(°)</strong></td>
<td>90.1493 (9)</td>
<td>115.7100 (10)</td>
</tr>
<tr>
<td><strong>γ(°)</strong></td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td><strong>V (Å(^3))</strong></td>
<td>1964.4 (3)</td>
<td>3419.73 (10)</td>
</tr>
<tr>
<td><strong>Z</strong></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>μ (mm(^{-1}))</strong></td>
<td>0.077</td>
<td>4.746</td>
</tr>
<tr>
<td><strong>(ρ_{calc}) (g/cm(^3))</strong></td>
<td>1.188</td>
<td>1.683</td>
</tr>
<tr>
<td><strong>Data collected</strong></td>
<td>17156</td>
<td>21899</td>
</tr>
<tr>
<td><strong>Unique data</strong></td>
<td>4513</td>
<td>5950</td>
</tr>
<tr>
<td><strong>parameters</strong></td>
<td>236</td>
<td>491</td>
</tr>
<tr>
<td><strong>g.o.f.</strong></td>
<td>1.048</td>
<td>1.054</td>
</tr>
<tr>
<td><strong>R_1</strong></td>
<td>0.0410</td>
<td>0.0288</td>
</tr>
<tr>
<td><strong>wR_2</strong></td>
<td>0.1125</td>
<td>0.0764</td>
</tr>
<tr>
<td><strong>CCDC#</strong></td>
<td>1010822</td>
<td>1010823</td>
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</table>
**Bond Homolysis**

*Preliminary Testing*

The stability of the radical species (1 and 1Ru) was evaluated at 120°C (**Figure S11**). Each compound was heated in solution for the time indicated on the Figure. There is no appreciable change in the visible or near IR regions indicating the radical-based compounds are thermally stable under the bond homolysis experimental conditions which generate them.

**Figure S11.** Investigation of the thermal stability of 1 and 1Ru at 120°C.
**Scheme S1** shows the major species involved in the thermal decomposition of Bn-1 in the presence of excess TEMPO; a possible (minor) species missing from the scheme is bibenzyl (from the coupling of two benzyl radicals).

![Scheme S1](image)

**Scheme S1**. Decomposition reaction of Bn-1 in the presence of excess TEMPO.

The deconvolution method used for the determination of the concentration of Bn-1 and 1 required that the molar extinction coefficients of each species to be determined experimentally. **Figure S12** shows the molar extinction coefficients of the various species present in the decomposition of Bn-1. **Table S2** provides a summary of molar extinction coefficients of each compound at the λ_max of the other compounds in the range of 300-850 nm (2 does not have a λ_max in this range).

![Figure S12](image)

**Figure S12**. Molar extinction coefficients of the compounds involved in the C-N bond homolysis of Bn-1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wavelength (nm)</th>
<th>310</th>
<th>338</th>
<th>409</th>
<th>469</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td></td>
<td>0.292</td>
<td>0.110</td>
<td>0.483</td>
<td>0.325</td>
</tr>
<tr>
<td>Bibenzyl</td>
<td></td>
<td>152</td>
<td>0.626</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bn-1</td>
<td></td>
<td>3.13x10^3</td>
<td>4.94x10^3</td>
<td>36.9</td>
<td>3.69</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>399</td>
<td>123</td>
<td>2.00x10^3</td>
<td>4.58x10^2</td>
</tr>
<tr>
<td>TEMPO</td>
<td></td>
<td>29.0</td>
<td>1.47</td>
<td>5.15</td>
<td>10.8</td>
</tr>
</tbody>
</table>

**Table S2**. Summary of the ε values at the λ_max of each compound.
Scheme S2 shows the major species involved in the thermal decomposition of Bn-1Ru in the presence of excess TEMPO.

![Scheme S2](image)

**Scheme S2.** Decomposition reaction of Bn-1Ru in the presence of TEMPO.

The deconvolution method used for the determination of the concentration of Bn-1Ru and 1Ru required that the molar extinction coefficients of each species to be determined experimentally. Figure S13 shows the molar extinction coefficients of the various species present in the decomposition of Bn-1Ru. Table S3 provides a summary of molar extinction coefficients of each compound at the $\lambda_{\text{max}}$ of the other compounds in the range of 300-1300 nm (2 does not have a $\lambda_{\text{max}}$ in this range).

![Graph](image)

**Figure S13.** Molar extinction coefficients of the compounds involved in the C-N bond homolysis of Bn-1Ru.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>310 451 469 498</td>
</tr>
<tr>
<td>2</td>
<td>9.20 0.789 0.706 0.678</td>
</tr>
<tr>
<td>Bibenzyl</td>
<td>162 -0.266 -0.318 -0.322</td>
</tr>
<tr>
<td>1Ru</td>
<td>6.93x10^3 9.74x10^3 7.80x10^3 3.58x10^3</td>
</tr>
<tr>
<td>TEMPO</td>
<td>31.0 10.0 10.8 9.54</td>
</tr>
<tr>
<td>Bn-1Ru</td>
<td>1.49x10^4 7.00x10^3 8.10x10^3 1.02x10^4</td>
</tr>
</tbody>
</table>

**Table S3.** Summary of the $\varepsilon$ values at the $\lambda_{\text{max}}$ of each compound.
Procedure
A 50 mL sample containing tetrazine (Bn-1 or Bn-1Ru) and TEMPO were combined in tert-butylbenzene such that the tetrazine concentration was ~1.0x10^{-4} M for Bn-1 and ~8.0x10^{-5} M for Bn-1Ru while the TEMPO concentration was ~5.00x10^{-3} M (≥50-fold excess of scavenger thus ensuring a zeroth order dependence on the concentration of TEMPO5,6). The mixture was degassed and placed under an argon atmosphere by three freeze-pump-thaw cycles. The reaction mixture was placed in a temperature controlled oil bath and upon reaching the desired temperature, the initial aliquot was extracted, immediately quenched in liquid nitrogen, and stored in a freezer (-20 °C) until the aliquots were ran on a UV-Vis instrument; subsequent aliquots were taken at various times to allow for ~10% decomposition between each aliquot collection. A minimum of three trials at three different temperatures were performed for each tetrazine analyzed.

Deconvolution Method
The method of deconvolution is based on the assumption that the Beer-Lambert law is applicable to each species present in solution. The experimental absorbance (A_{exp}) at a wavelength corresponds to sum of the absorbances due to all i-th species (A_i) at the corresponding wavelength and is expressed as a sum of contributions of all species in solution according to the Eq. 1:

\[ A_{\text{exp}} = \sum_{i=1}^{n} A_i = \sum_{i=1}^{n} \varepsilon_i \cdot c_i \quad (1) \]

where \( \varepsilon_i \) represents the molar extinction coefficient and \( c_i \) represents the concentration of the i-th species in solution. As the values of \( \varepsilon_i \) were previously determined experimentally and \( A_{\text{exp}} \) is the measured absorbance, the determination of \( c_i \) is possible from the system of linear Eq. 1 which requires the use of a matrix algorithm to perform the deconvolution of the individual species7,8. The matrix algorithm used the experimental absorbance values at the \( \lambda_{\text{max}} \) of each species in its calculation; compound 2 was not included since its \( \varepsilon \) was negligible (compared to the other species) at the wavelengths of interest (>300 nm).

The absorbance values of the linear combination spectrum (A_{lc}) is compared to the absorbance values of the experimental spectrum (A_{exp}) according to Eq. 2 and a plot of this data is shown in Figure S16 and Figure S22 for Bn-1 and Bn-1Ru, respectively.

\[ \% \text{Comparison} = \frac{A_{\text{lc}}}{A_{\text{exp}}} \times 100 \quad (2) \]

Using the calculations described above, the concentration of the tetrazine (Bn-1 or Bn-1Ru) and verdazyl (1 or 1Ru) can be determined. If the [TEMPO] is sufficiently high relative to the [tetrazine] then the rate of reaction is first-order with respect to the [tetrazine] and it will be represented by Eq. 3. Integration of the first-order rate gives Eq. 4 and a plot of the data over the course of the experiment allows for the determination of the dissociation rate constant \( (k_d) \). A plot of the Arrhenius equation Eq. 5 using the data collected for the various temperatures allows for the determination of the activation energy \( (E_a) \) and pre-exponential factor \( (A) \).

\[ \text{rate} = -k_d \cdot [\text{tetrazine}] \quad (3) \]
\[
\ln\left(\frac{[\text{tetrazine}]_t}{[\text{tetrazine}]_0}\right) = -k_d \cdot t \quad \text{(4)}
\]

\[
\ln (k_d) = -\frac{E_a}{R} \cdot \frac{1}{T} + \ln(A) \quad \text{(5)}
\]

Figures S14-S19 show the data collected for the decomposition of Bn-1. Figures S20-S25 show similar data for the decomposition of Bn-1Ru. Figure S26 shows the Arrhenius plots of Bn-1 and Bn-1Ru on one graph.
Figure S14. UV-Vis spectra obtained for the decomposition of Bn-1 conducted at 150°C.

Figure S15. Deconvolution of UV-Vis absorption spectrum at t = 1702 s.
Figure S16. % Comparison between the absorbance values of the linear combination spectrum and the experimental spectrum at t = 1702 s.

Figure S17. Plot of the change in concentration of Bn-1 ○, 1 ●, and (Bn-1+1) ▲ in solution.
Figure S18. First-order plots of the decomposition of Bn-1 at (a) 130°C, (b) 140°C, and (c) 150°C. Individual trials are represented by ♦, ▪, ▲, and ● and the equation for the average line is shown.

Figure S19. Arrhenius plot of Bn-1 ▪.
Figure S20. UV-Vis spectra obtained for the decomposition of Bn-1Ru conducted at 120°C.

Figure S21. Deconvolution of UV-Vis absorption spectrum at t = 1791 s.
Figure S22. % Comparison between the absorbance values of the linear combination spectrum and the experimental spectrum at \( t = 1791 \) s.

Figure S23. Plot of the change in concentration of Bn-1Ru ○, 1Ru ●, and (Bn-1Ru+1Ru) ▲ in solution.
**Figure S24.** First-order plots of the decomposition of Bn-1Ru at (a) 110 °C, (b) 120 °C, and (c) 130 °C. Individual trials are represented by ♦, *, and ▲ and the equation for the average line is shown.

**Figure S25.** Arrhenius plot of Bn-1Ru ○.
Figure S26. Arrhenius plot of Bn-1 • and Bn-1Ru ○.
References


