

## Supporting Information for

### Structure-Redox-Relaxivity Relationships for Redox Responsive Manganese-Based Magnetic Resonance Imaging Probes

Eric M. Gale,<sup>‡</sup> Shreya Mukherjee,<sup>‡</sup> Cynthia Liu, Galen Loving, Peter Caravan\*

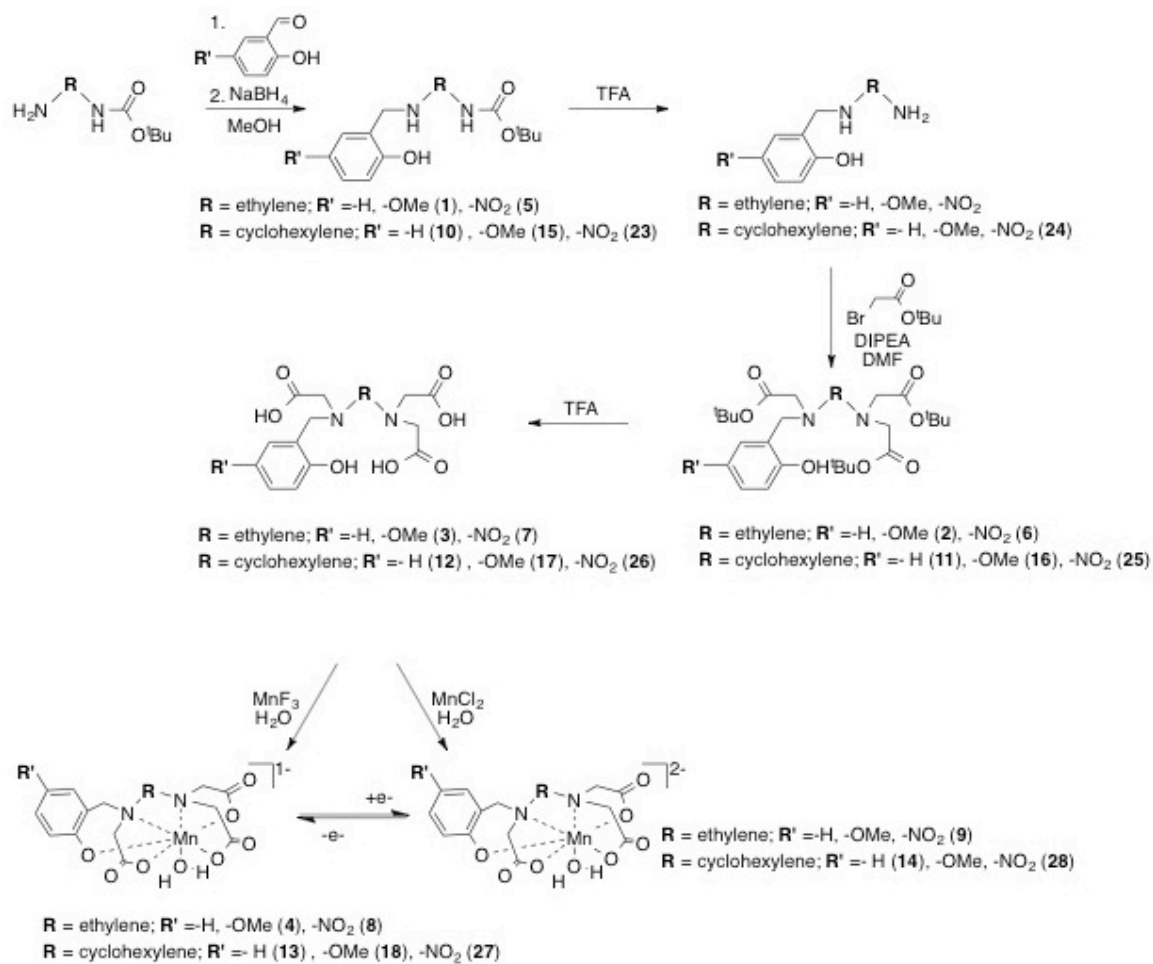
<sup>‡</sup>Authors contributed equally to this manuscript

Athinoula, A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, 149 Thirteenth Street, Charlestown, Massachusetts 02129

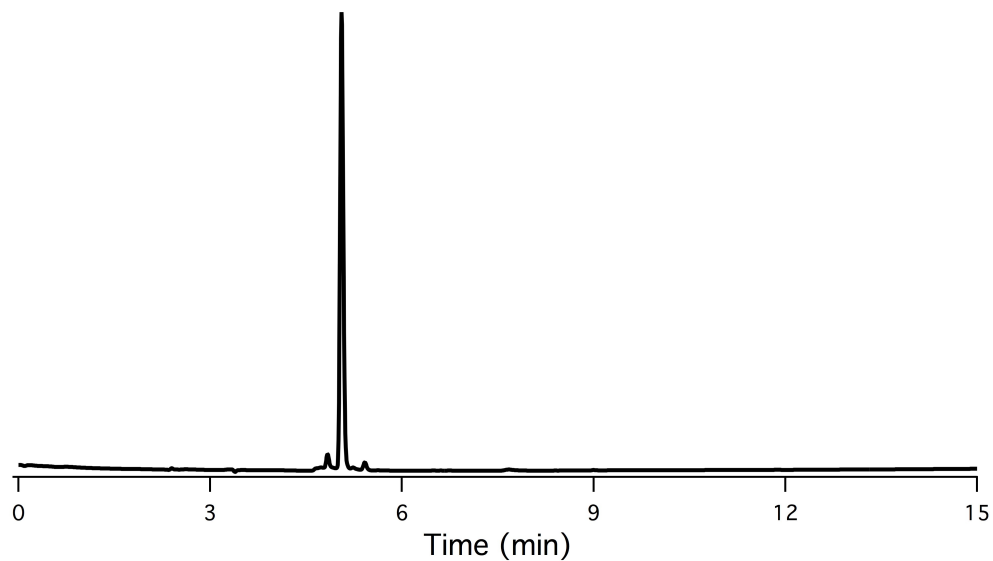
#### Table of Contents

| <u>Content</u>   | <u>Page</u> |
|--|-------------|
| <b>Scheme S1:</b> Ligand synthesis   | S3          |
| <b>Figure S1:</b> LC trace of $[\text{Mn}^{\text{II}}(\text{HBET-OMe})]^{2-}$                        | S4          |
| <b>Figure S2:</b> LC trace of $[\text{Mn}^{\text{II}}(\text{HBET-NO}_2)]^{2-}$                       | S4          |
| <b>Figure S3:</b> LC trace of $[\text{Mn}^{\text{II}}(\text{CyHBET})]^{2-}$                          | S5          |
| <b>Figure S4:</b> LC trace of $[\text{Mn}^{\text{II}}(\text{CyHBET-OMe})]^{2-}$                      | S5          |
| <b>Figure S5:</b> LC trace of $[\text{Mn}^{\text{II}}(\text{CyHBET-NO}_2)]^{2-}$                     | S6          |
| <b>Figure S6:</b> LC trace of $[\text{Mn}^{\text{III}}(\text{HBET-NO}_2)]^{1-}$                      | S6          |
| <b>Figure S7:</b> LC trace of $[\text{Mn}^{\text{III}}(\text{CyHBET})]^{1-}$                         | S7          |
| <b>Figure S8:</b> LC trace of $[\text{Mn}^{\text{III}}(\text{CyBET-NO}_2)]^{1-}$ - pure diastereomer | S7          |
| <b>Figure S9:</b> LC trace of $[\text{Mn}^{\text{III}}(\text{CyBET-NO}_2)]^{1-}$ - pure diastereomer | S8          |
| <b>Figure S10:</b> LC trace of $[\text{Zn}^{\text{II}}(\text{HBET})]^{2-}$                           | S8          |
| <b>Figure S11:</b> LC trace of $[\text{Zn}^{\text{II}}(\text{HBET-OMe})]^{2-}$                       | S9          |
| <b>Figure S12:</b> LC trace of $[\text{Zn}^{\text{II}}(\text{HBET-NO}_2)]^{2-}$                      | S9          |
| <b>Figure S13:</b> LC trace of $[\text{Zn}^{\text{II}}(\text{CyHBET})]^{2-}$                         | S10         |
| <b>Figure S14:</b> LC trace of $[\text{Zn}^{\text{II}}(\text{CyHBET-OMe})]^{2-}$                     | S10         |
| <b>Figure S15:</b> LC trace of $[\text{Zn}^{\text{II}}(\text{CyHBET-NO}_2)]^{2-}$                    | S11         |
| <b>Figure S16:</b> LC trace of reaction of HBET-OMe with $\text{MnF}_3$                              | S11         |
| <b>Figure S17:</b> pH titration profiles of L and 1:1 Mn(II):L for HBET-R'                           | S12         |

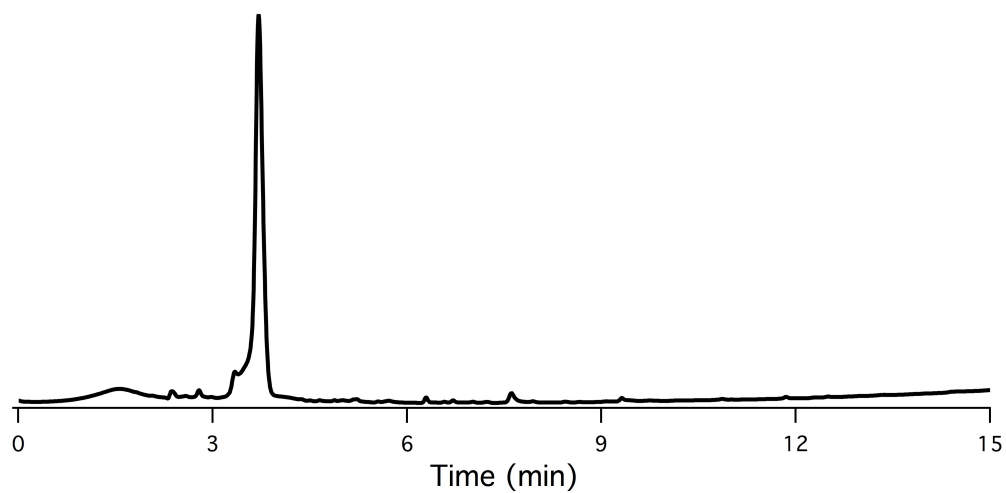
|  |     |
|--|-----|
| <b>Figure S18:</b> pH titration profiles of L and 1:1 Mn(II):L for CyHBET- <b>R'</b>   | S13 |
| <b>Figure S19:</b> pH-distribution diagram for 1:1 Mn:HBET-OMe   | S14 |
| <b>Figure S20:</b> pH-distribution diagram for 1:1 Mn:CyHBET-OMe   | S14 |
| <b>Figure S21:</b> pH-distribution diagram for 1:1 Mn:CyHBET-NO <sub>2</sub>   | S15 |
| <b>Figure S22:</b> UV-vis as function of pH for HBET   | S16 |
| <b>Figure S23:</b> UV-vis as function of pH for HBET-OMe   | S16 |
| <b>Figure S24:</b> UV-vis as function of pH for HBET-NO <sub>2</sub>   | S17 |
| <b>Figure S25:</b> UV-vis as function of pH for CyHBET   | S17 |
| <b>Figure S26:</b> UV-vis as function of pH for CyHBET-OMe   | S18 |
| <b>Figure S27:</b> UV-vis as function of pH for CyHBET-OMe   | S18 |
| <b>Figure S28:</b> UV-vis as function of pH for [Mn <sup>II</sup> (HBET)] <sup>2-</sup>  | S19 |
| <b>Figure S29:</b> UV-vis as function of pH for [Mn <sup>II</sup> (HBET-OMe)] <sup>2-</sup>  | S19 |
| <b>Figure S30:</b> UV-vis as function of pH for [Mn <sup>II</sup> (CyHBET)] <sup>2-</sup>  | S20 |
| <b>Figure S31:</b> UV-vis as function of pH for [Mn <sup>II</sup> (CyHBET-OMe)] <sup>2-</sup>  | S20 |
| <b>Figure S32:</b> UV-vis as function of pH for [Mn <sup>II</sup> (CyHBET-NO <sub>2</sub> )] <sup>2-</sup>   | S21 |
| <b>Figure S33:</b> $r_2^0$ vs T for [Mn <sup>II</sup> (CyHBET- <b>R'</b> )] <sup>2-</sup> complexes  | S22 |
| <b>Figure S34:</b> <sup>17</sup> O $\Delta\omega_p$ vs T of [Mn <sup>II</sup> (HBET- <b>R'</b> )] <sup>2-</sup> at pH 9  | S22 |
| <b>Figure S35:</b> <sup>17</sup> O $\Delta\omega_p$ vs T of [Mn <sup>II</sup> (CyHBET-NO <sub>2</sub> )] <sup>2-</sup> at pH 6   | S23 |
| <b>Figure S36:</b> <sup>17</sup> O $\Delta\omega_p$ vs T of [Mn <sup>II</sup> (CyHBET- <b>R'</b> )] <sup>2-</sup> at pH 9  | S23 |
| <b>Figure S37:</b> Full CV for [Mn <sup>II</sup> (HBET)] <sup>2-</sup> and [Mn <sup>II</sup> (HBET-OMe)] <sup>2-</sup>   | S24 |
| <b>Figure S38:</b> CV data: [Mn <sup>II</sup> /Zn <sup>II</sup> (CyHBET)] <sup>2-</sup>  | S24 |
| <b>Figure S39:</b> CV data: [Mn <sup>II</sup> /Zn <sup>II</sup> (CyHBET-OMe)] <sup>2-</sup>  | S25 |
| <b>Figure S40:</b> CV data: [Mn <sup>II</sup> /Zn <sup>II</sup> (HBET-NO <sub>2</sub> )] <sup>2-</sup> /[Mn <sup>II</sup> /Zn <sup>II</sup> (CyHBET-NO <sub>2</sub> )] <sup>2-</sup> | S25 |
| <b>Figure S41:</b> Kinetics of [Mn <sup>III</sup> (Cy/HBET)] <sup>1-</sup> reduction by L-cys  | S27 |
| <b>Figure S42:</b> Kinetics of [Mn <sup>III</sup> (Cy/HBET-NO <sub>2</sub> )] <sup>1-</sup> reduction by L-cys   | S27 |
| <b>Table S1:</b> $pK_a$ of HL and HML determined through UV-vis spectroscopy   | S28 |



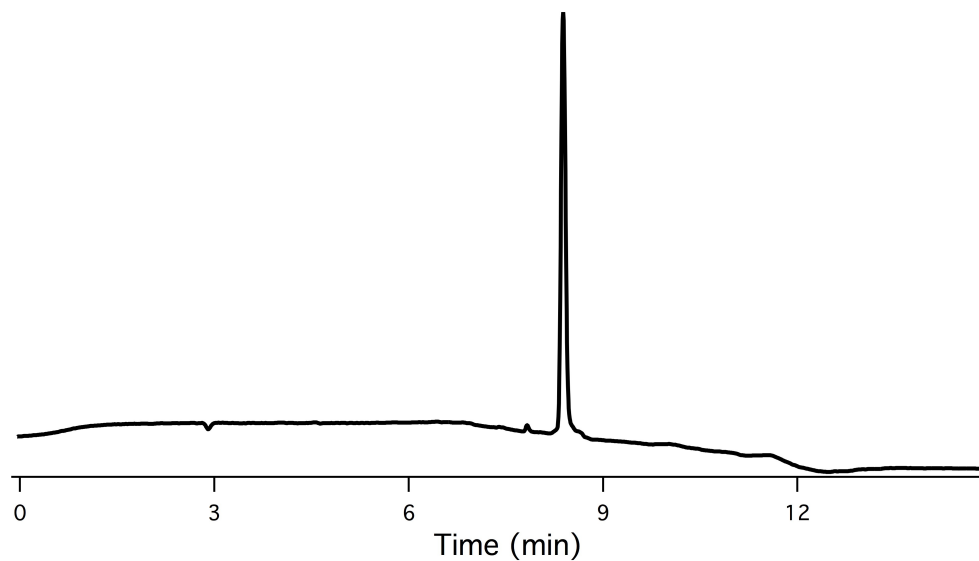
**Scheme S1.** Synthesis of ligands, Mn(II) complexes, and Mn(III) complexes considered in this study.



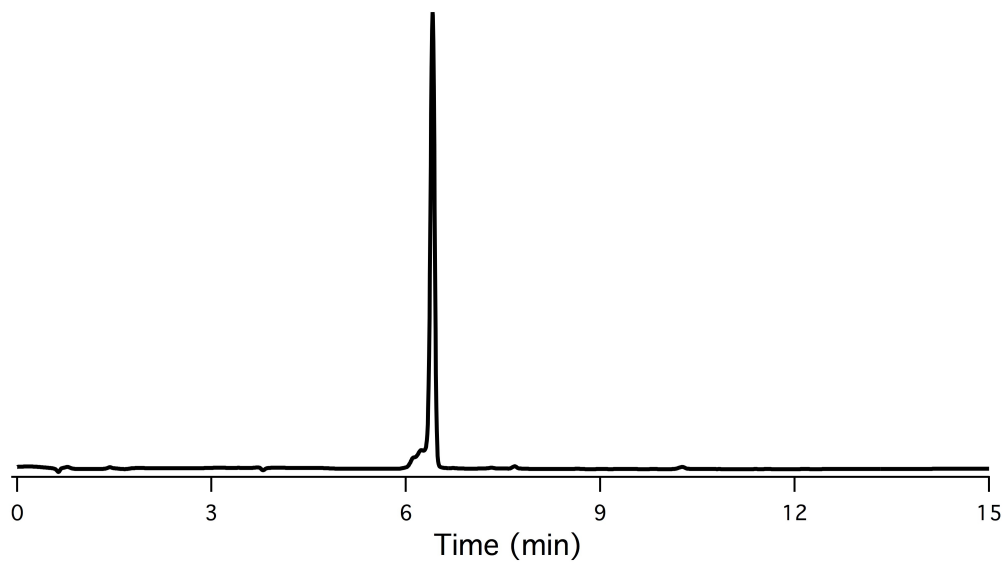
**Figure S1.** LC of  $[\text{Mn}(\text{HBET-OMe})]^{2-}$  detected at 220 nm;  $m/z^+ = 424.1$  eluted with this peak.



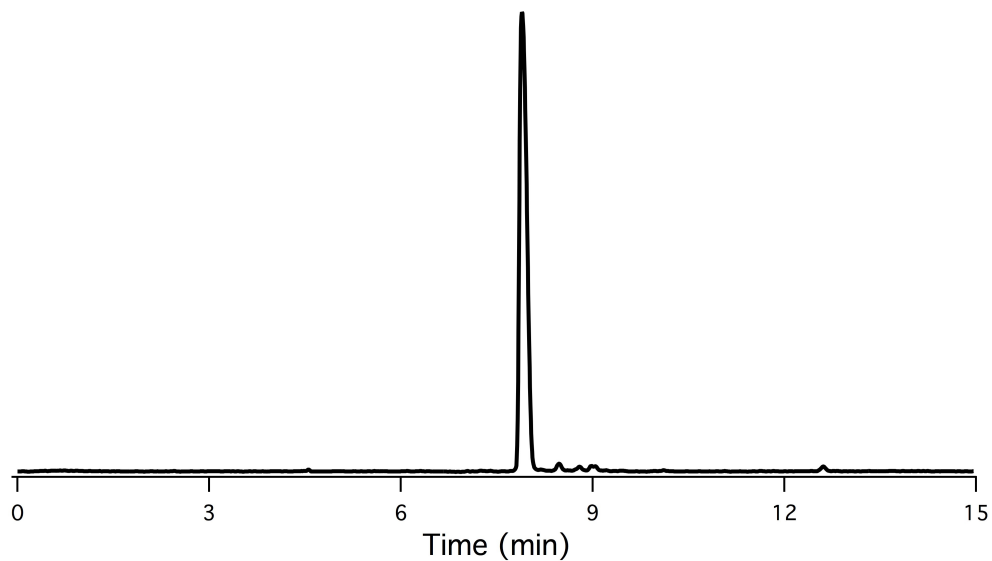
**Figure S2.** LC of  $[\text{Mn}(\text{HBET-NO}_2)]^{2-}$  detected at 254 nm;  $m/z^+ = 439.0$  eluted with this peak.



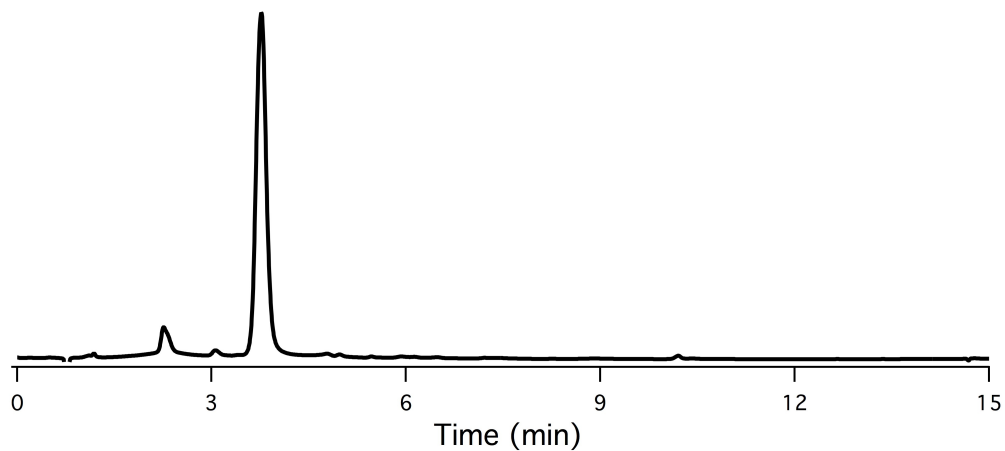
**Figure S3.** LC of  $[\text{Mn}(\text{CyHBET})]^{2-}$  detected at 220 nm;  $m/z^+ = 448.3$  eluted with this peak.



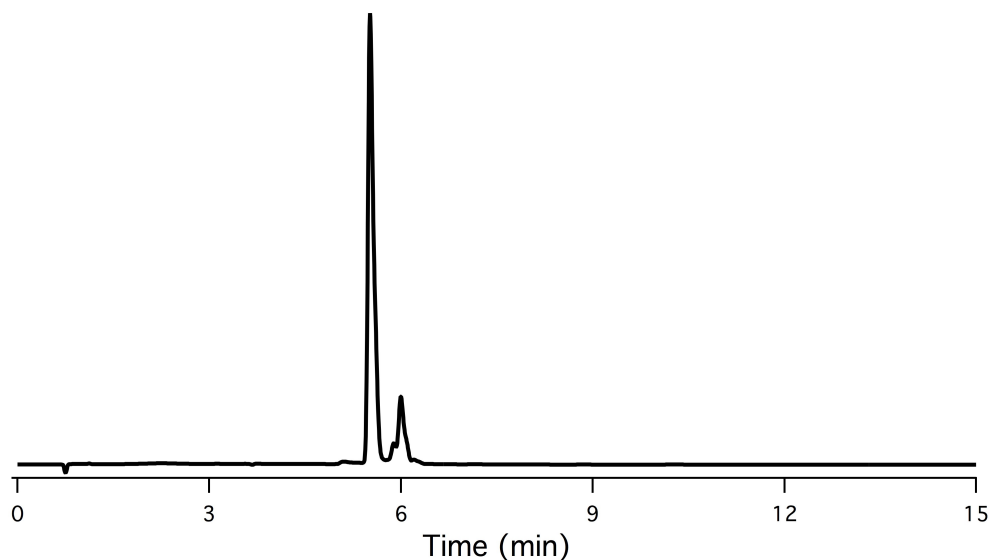
**Figure S4.** LC of  $[\text{Mn}(\text{CyHBET-OMe})]^{2-}$  detected at 220 nm;  $m/z^+ = 478.1$  eluted with this peak.



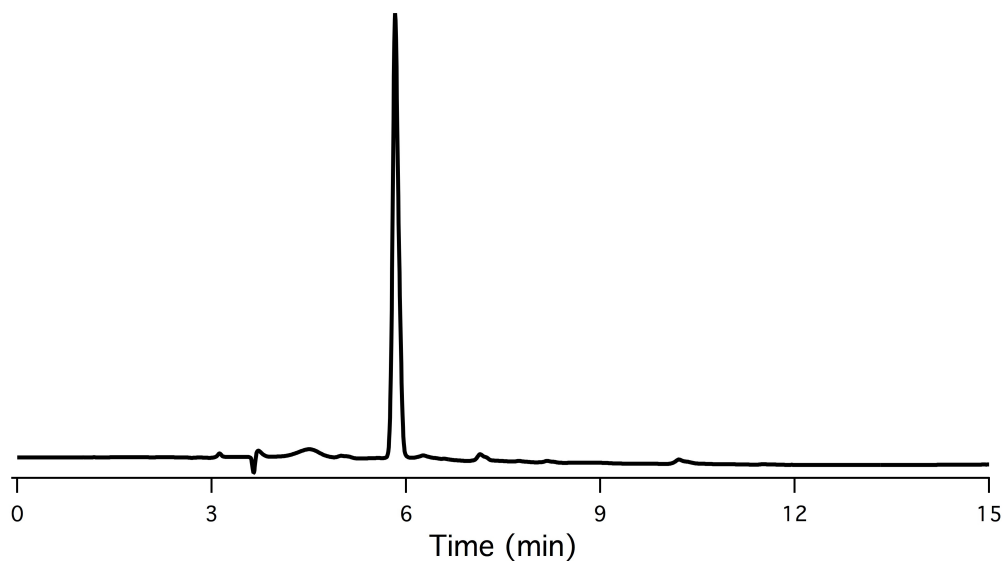
**Figure S5.** LC of  $[\text{Mn}(\text{CyHBET-NO}_2)]^{2-}$  detected at 220 nm;  $m/z^+ = 494.1$  eluted with this peak.



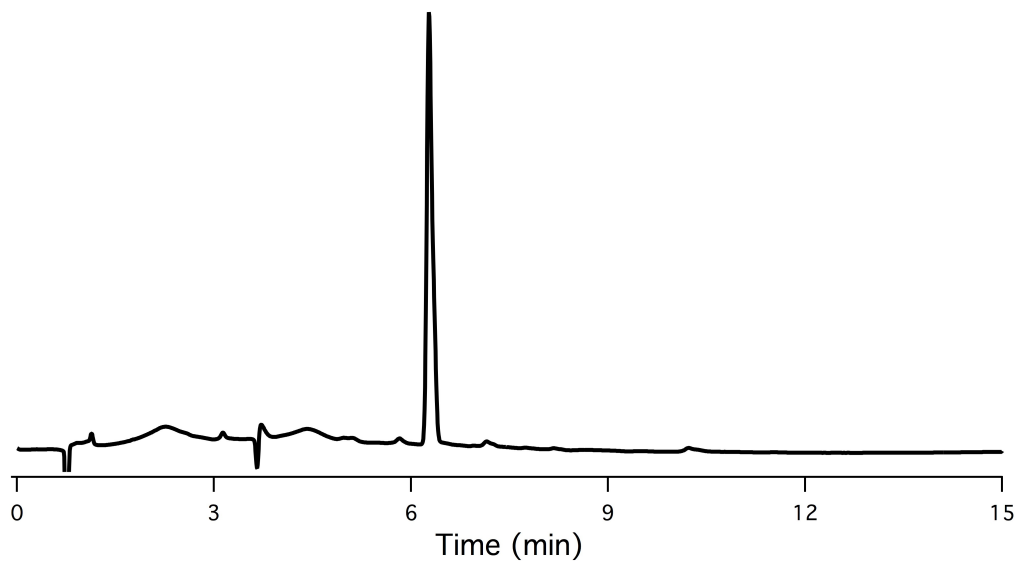
**Figure S6.** LC of  $[\text{Mn}(\text{HBET-NO}_2)]^-$  detected at 280 nm;  $m/z^+ = 438.0$  eluted with this peak.



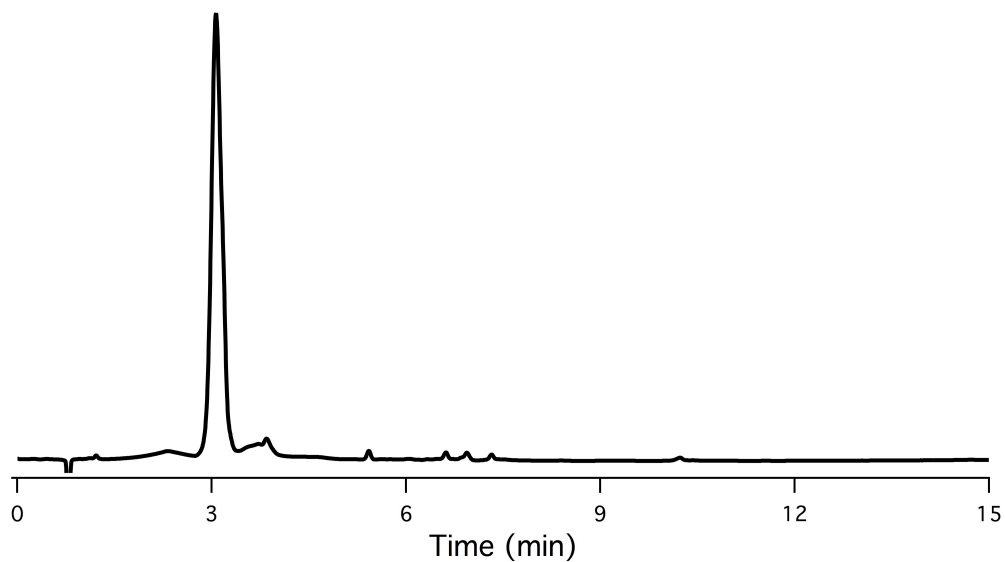
**Figure S7.** LC of  $[\text{Mn}(\text{CyHBET})]^-$  detected at 254 nm showing both diastereomeric forms of this complex;  $m/z^+ = 447.4$  eluted with these peaks.



**Figure S8.** LC of one unique diastereomer of  $[\text{Mn}(\text{CyHBET-NO}_2)]^-$  detected at 254 nm;  $m/z^+ = 493.1$  eluted with this peak.

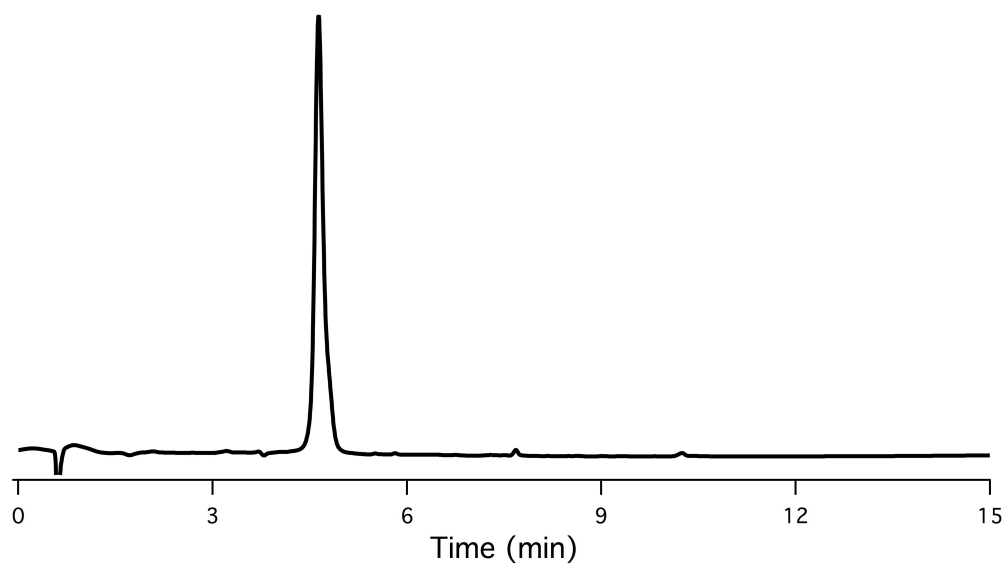


**Figure S9.** LC of the other unique diastereomer of  $[\text{Mn}(\text{CyHBET-NO}_2)]^-$  detected at 254 nm;  $m/z^+ = 493.1$  eluted with this peak.

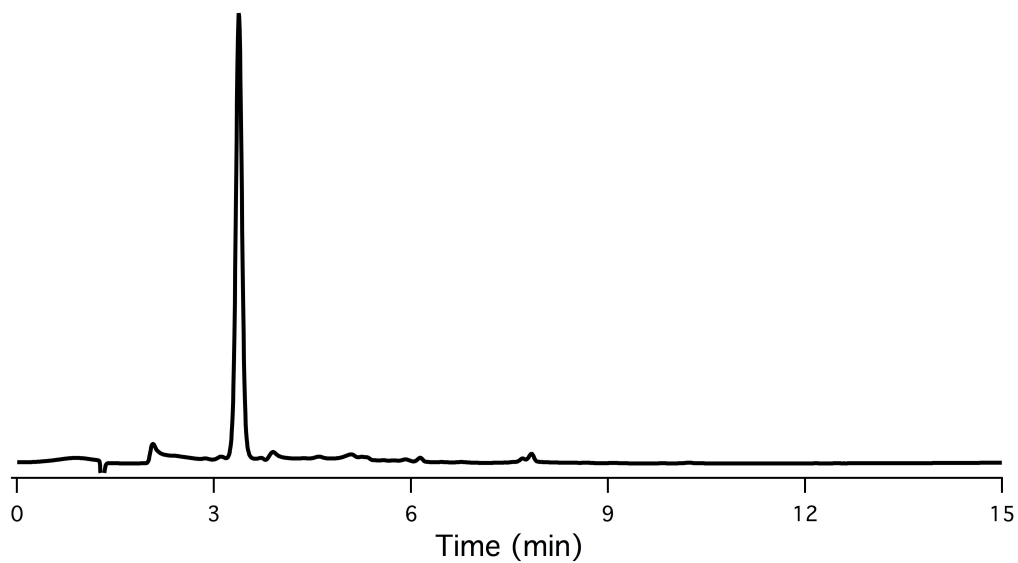


**Figure S10.** LC of  $[\text{Zn}(\text{HBET})]^{2-}$  detected at 280 nm;  $m/z^+ = 403.1$  eluted with this peak.

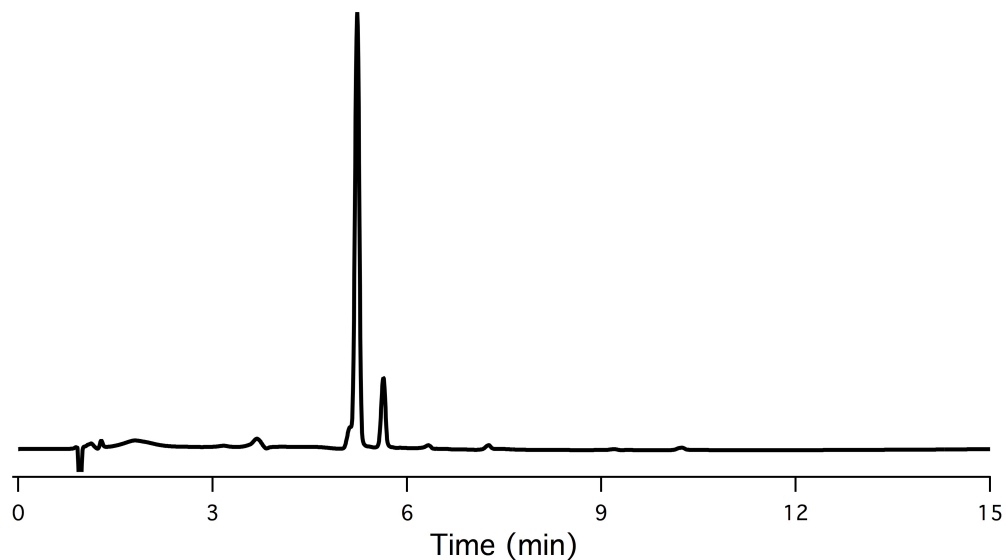




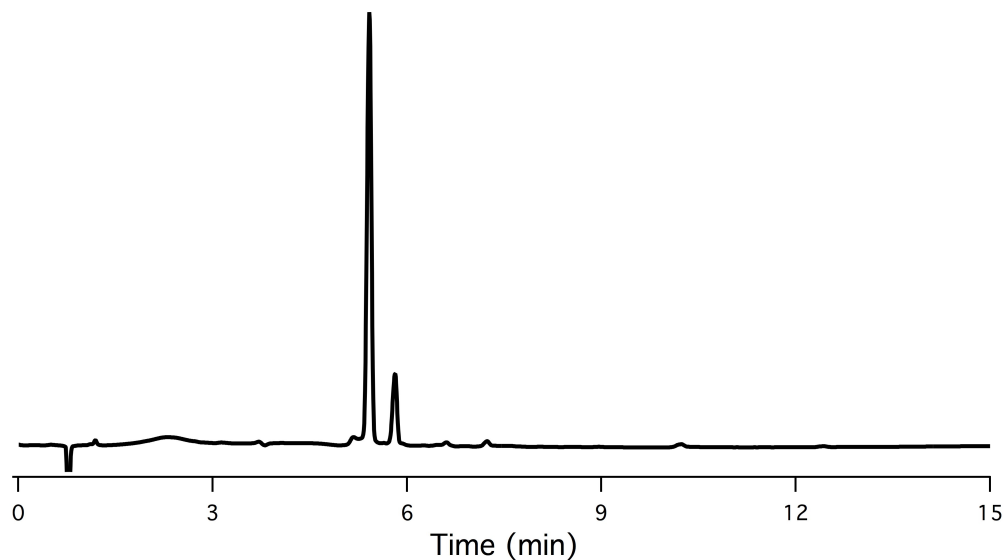
**Figure S11.** LC of  $[\text{Zn}(\text{HBET-OMe})]^{2-}$  detected at 280 nm;  $m/z^+ = 431.1$  eluted with this peak.



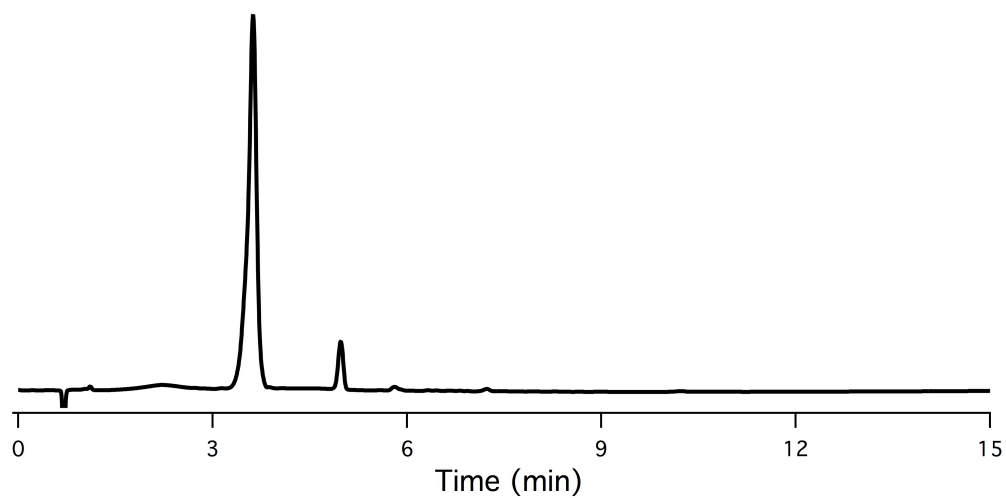
**Figure S12.** LC of  $[\text{Zn}(\text{HBET-NO}_2)]^{2-}$  detected at 280 nm;  $m/z^+ = 448.0$  eluted with this peak.



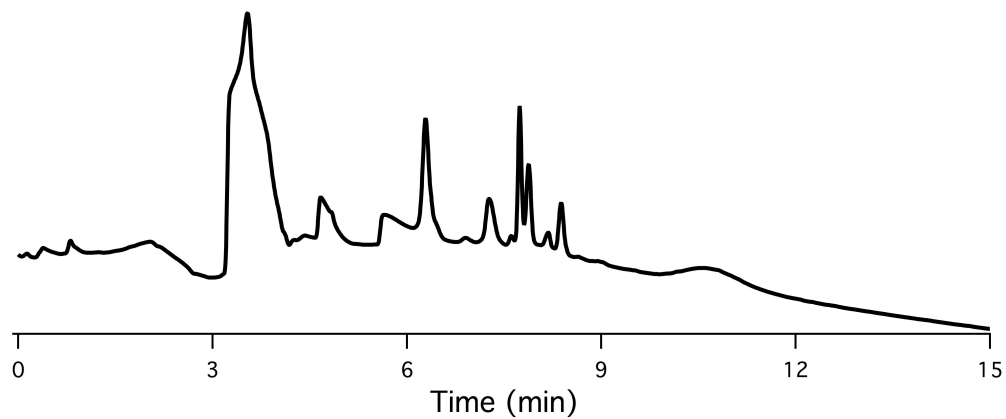
**Figure S13.** LC of  $[\text{Zn}(\text{CyHBET})]^{2-}$  detected at 280 nm showing both diastereomeric forms of this complex;  $m/z^+ = 457.1$  eluted with these peaks.



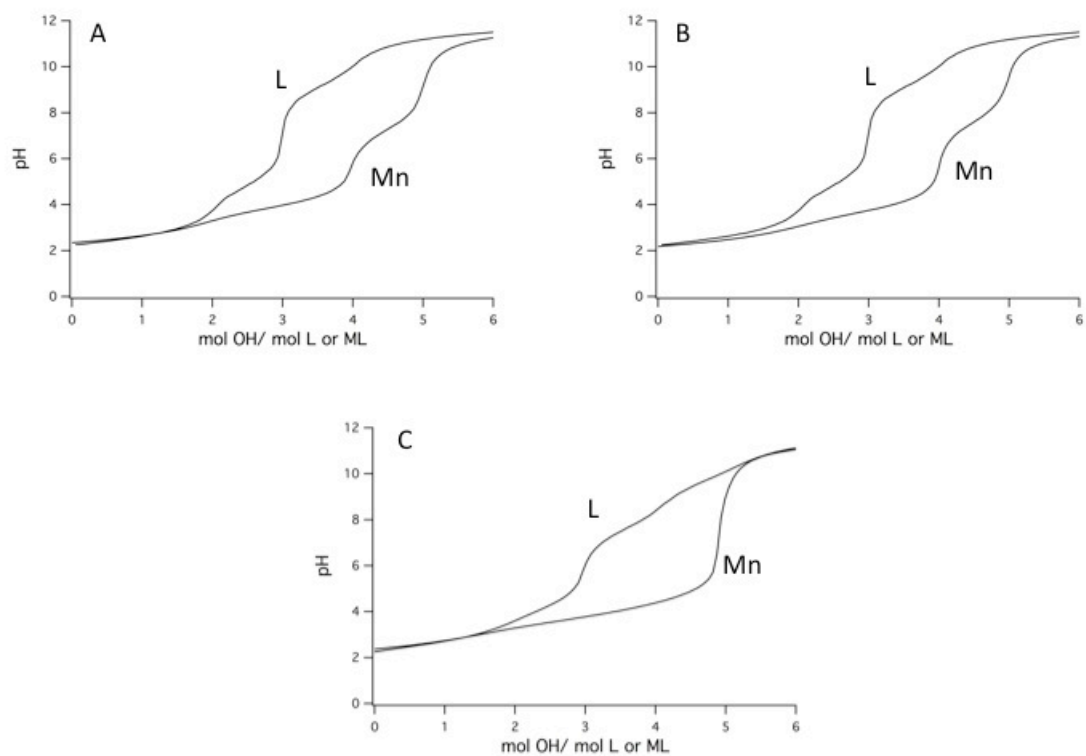
**Figure S14.** LC of  $[\text{Zn}(\text{CyHBET-OMe})]^{2-}$  detected at 280 nm showing both diastereomeric forms of this complex;  $m/z^+ = 487.1$  eluted with these peaks.



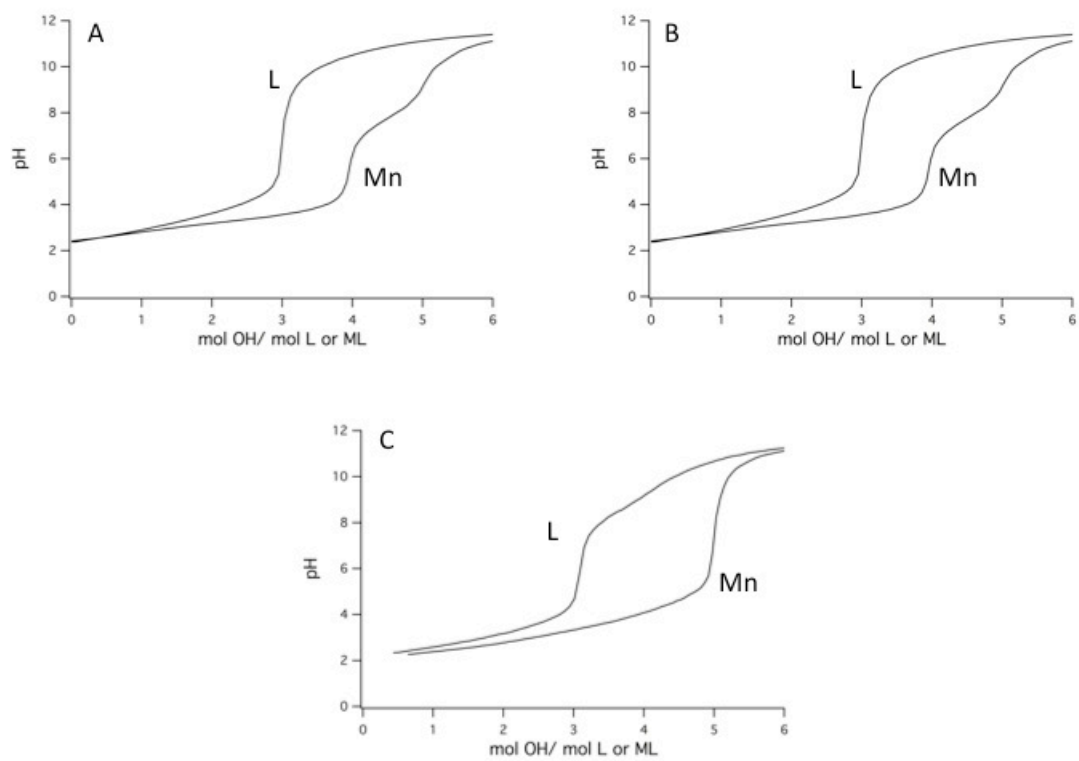
**Figure S15.** LC of  $[\text{Zn}(\text{CyHBET-NO}_2)]^{2-}$  detected at 254 nm showing both diastereomeric forms of this complex;  $m/z^+ = 502.1$  eluted with these peaks.



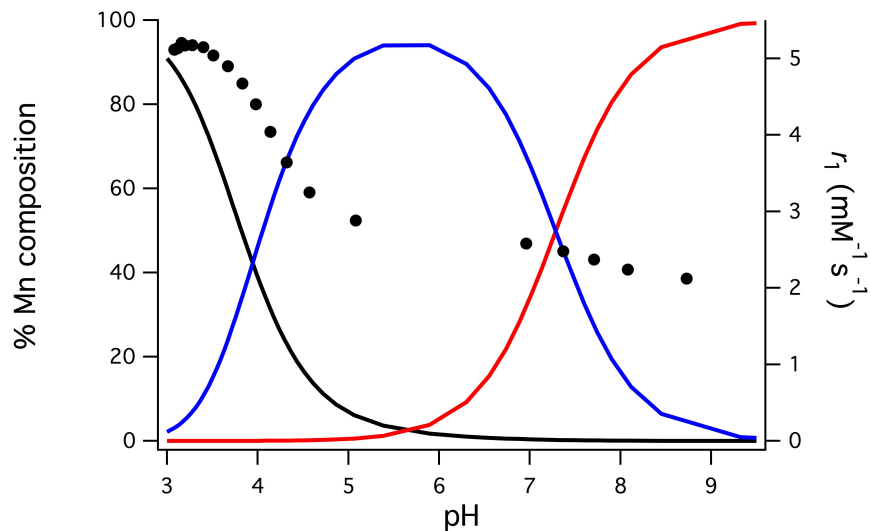
**Figure S16.** LC of crude reaction mixture of 1:1 HBET-OMe: $\text{MnF}_3$  at 220 nm detection.



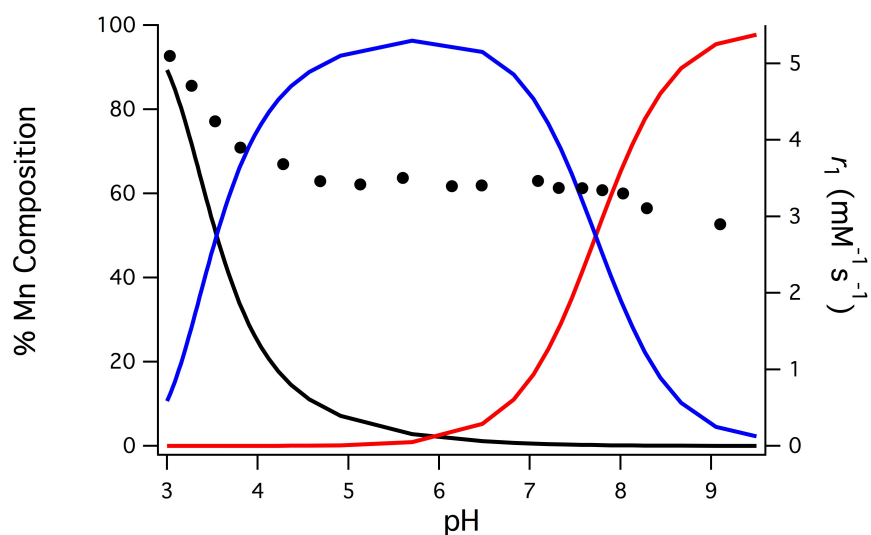
**Figure S17.** pH profiles of L and 1:1 Mn(II):L + 1 mol equiv. TFA. (A) HBET, (B) HBET-OMe, and (C) HBET-NO<sub>2</sub> (25 °C, *I* = 0.1 M NaCl).



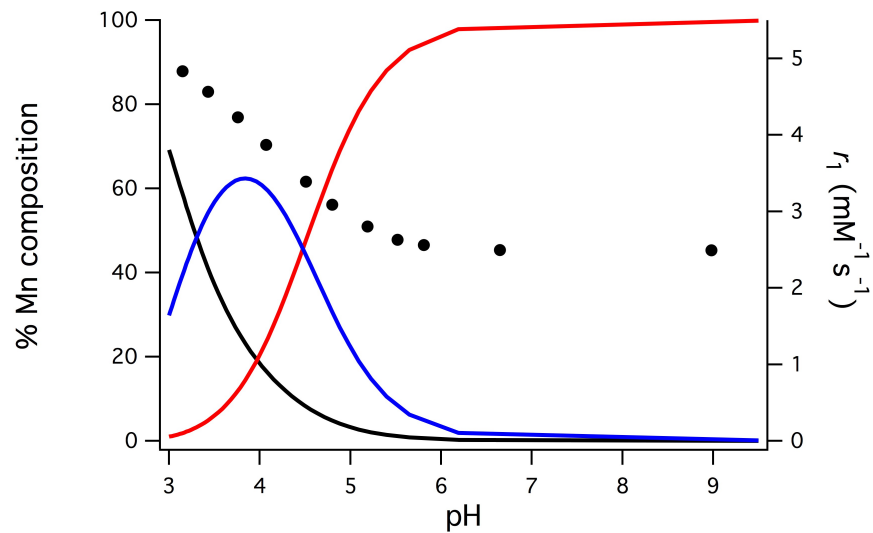
**Figure S18.** pH profiles of L and 1:1 Mn(II):L + 1 mol equiv. TFA. (A) CyHBET, (B) CyHBET-OMe, and (C) CyHBET-NO<sub>2</sub> (25 °C, *I* = 0.1 M NaCl).



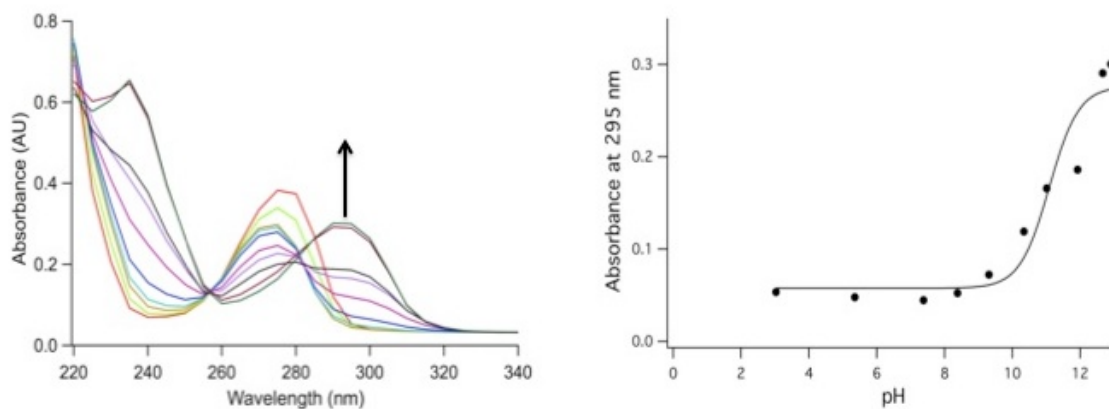
**Figure S19.** Distribution diagram for 1:1 Mn(II):HBET-OMe mixture. ML, HML and free Mn are depicted by red, blue and black traces, respectively ( $[\text{Mn}] = [\text{L}] = 1 \text{ mM}$ ,  $25 \text{ }^\circ\text{C}$ ,  $I = 0.1 \text{ M NaCl}$ );  $r_1$  ( $37 \text{ }^\circ\text{C}$ ,  $1.4 \text{ T}$ ) is overlaid in black dots.



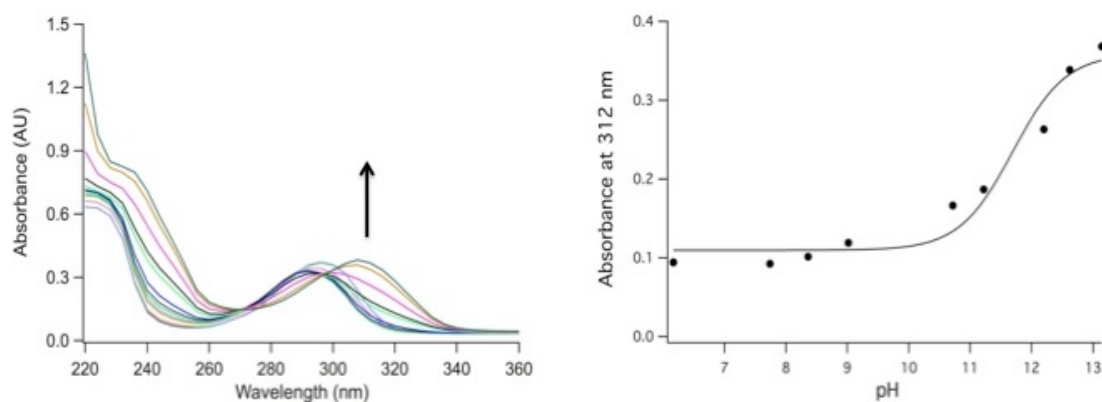
**Figure S20.** Distribution diagram for 1:1 Mn(II):CyHBET-OMe mixture. ML, HML and free Mn are depicted by red, blue and black traces, respectively ( $[\text{Mn}] = [\text{L}] = 1 \text{ mM}$ ,  $25 \text{ }^\circ\text{C}$ ,  $I = 0.1 \text{ M NaCl}$ );  $r_1$  ( $37 \text{ }^\circ\text{C}$ ,  $1.4 \text{ T}$ ) is overlaid in black dots.



**Figure S21.** Distribution diagram for 1:1 Mn(II):CyHBET-NO<sub>2</sub> mixture. ML, HML and free Mn are depicted by red, blue and black traces, respectively ( $[\text{Mn}] = [\text{L}] = 1 \text{ mM}$ ,  $25 \text{ }^\circ\text{C}$ ,  $I = 0.1 \text{ M NaCl}$ );  $r_1$  ( $37 \text{ }^\circ\text{C}$ ,  $1.4 \text{ T}$ ) is overlaid in black dots.

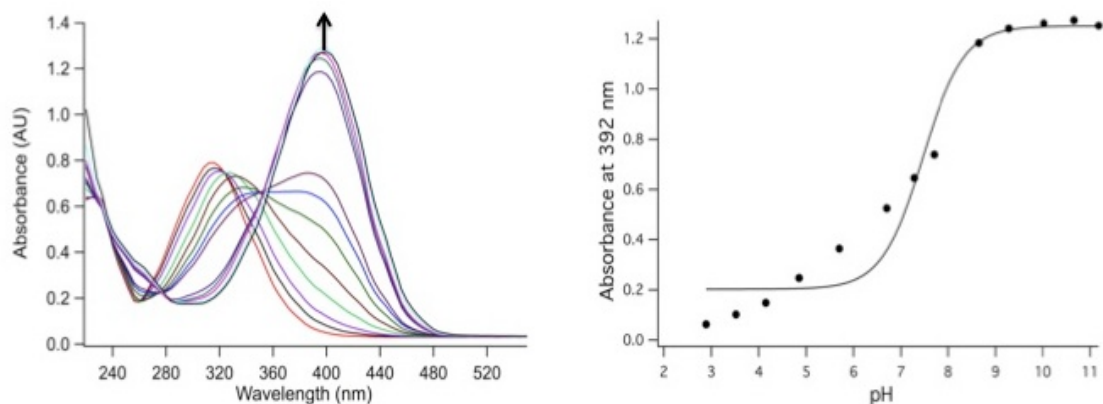


**Figure S22.** Left: UV-vis spectrum of HBET shown as a function of pH. Arrow indicates increase in absorbance at 295 nm with increasing pH. Right: Absorbance at 295 nm as a function of pH. Solid line represents the fit to the data.

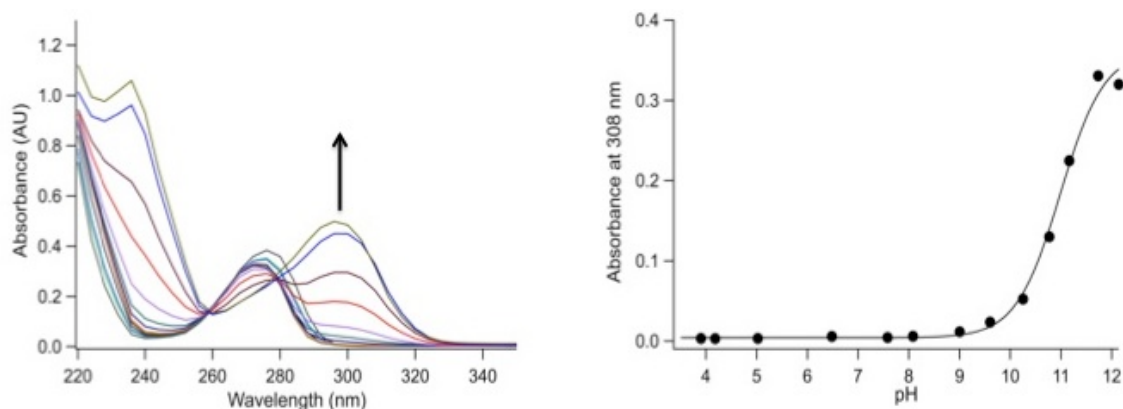


**Figure S23.** Left: UV-vis spectrum of HBET-OMe shown as a function of pH. Arrow indicates increase in absorbance at 312 nm with increasing pH. Right: Absorbance at 312 nm as a function of pH. Solid line represents the fit to the data.

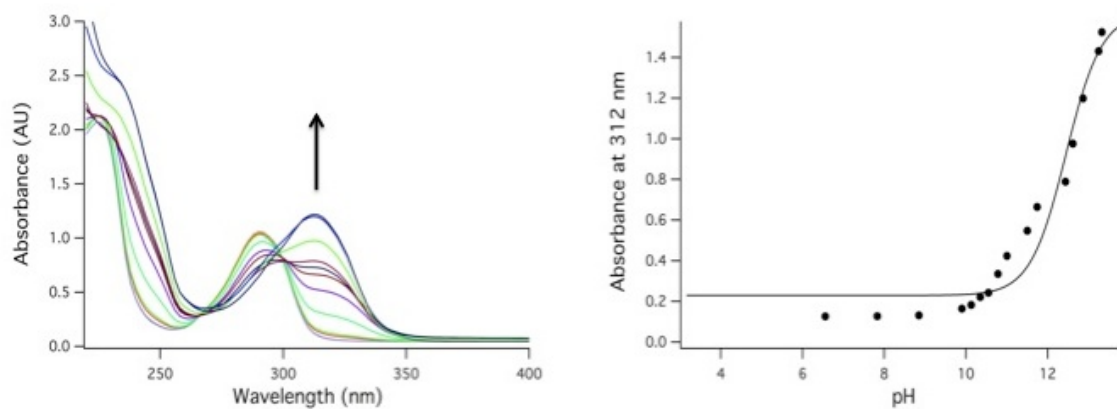




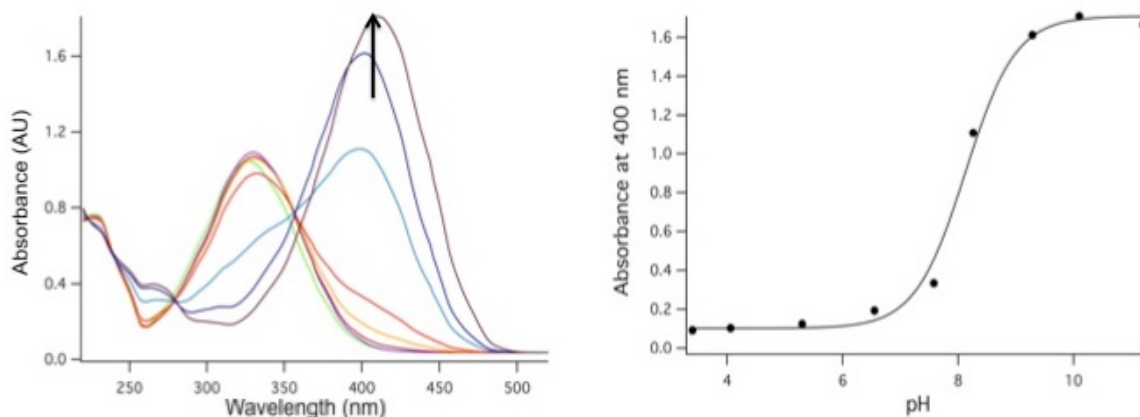
**Figure S24.** Left: UV-vis spectrum of HBET-NO<sub>2</sub> shown as a function of pH. Arrow indicates increase in absorbance at 410 nm with increasing pH. Right: Absorbance at 410 nm as a function of pH. Solid line represents the fit to the data.



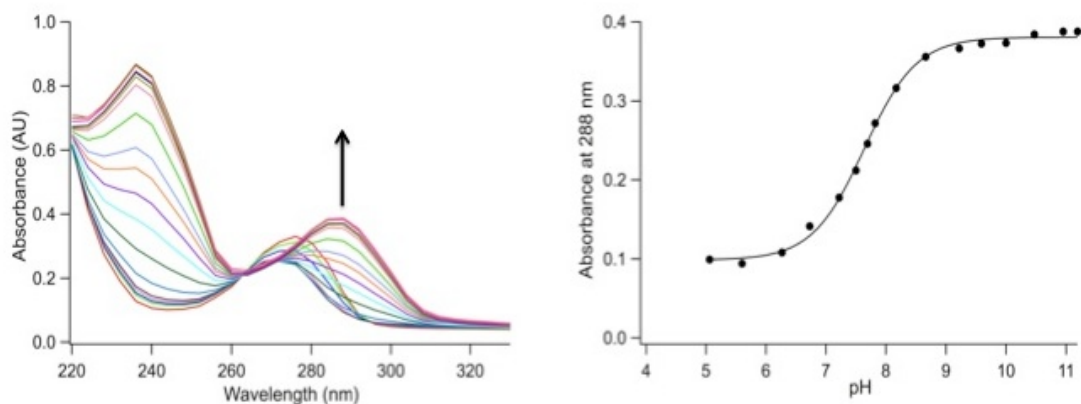
**Figure S25.** Left: UV-vis spectrum of CyHBET shown as a function of pH. Arrow indicates increase in absorbance at 308 nm with increasing pH. Right: Absorbance at 308 nm as a function of pH. Solid line represents the fit to the data.



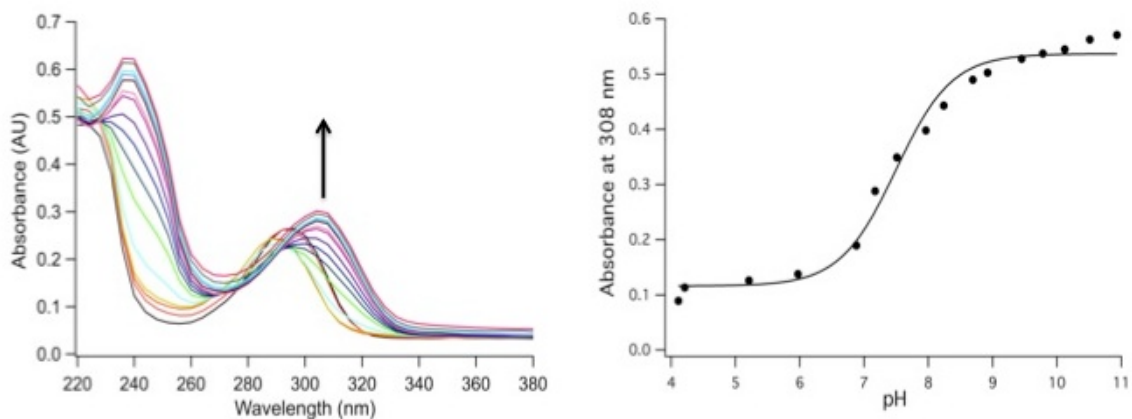
**Figure S26.** Left: UV-vis spectrum of CyHBET-OMe shown as a function of pH. Arrow indicates increase in absorbance at 312 nm with increasing pH. Right: Absorbance at 312 nm as a function of pH. Solid line represents the fit to the data.



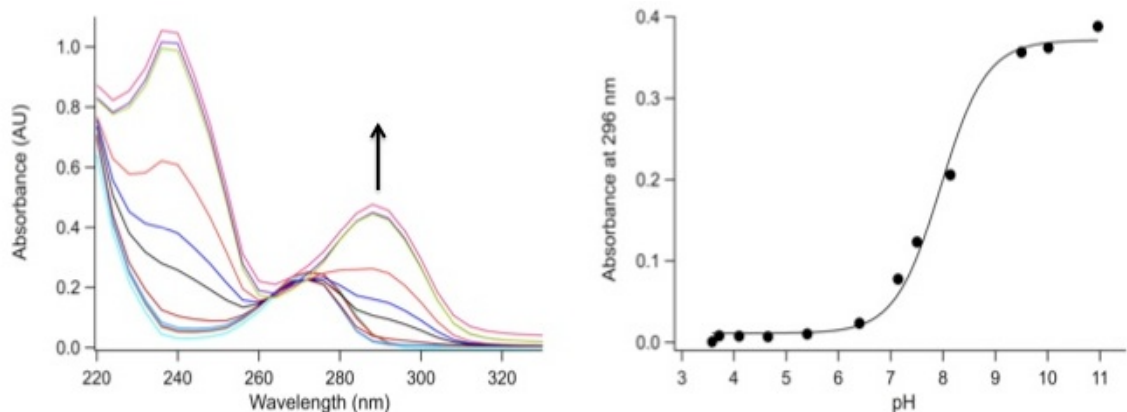
**Figure S27.** Left: UV-vis spectrum of CyHBET-NO<sub>2</sub> shown as a function of pH. Arrow indicates increase in absorbance at 400 nm with increasing pH. Right: Absorbance at 400 nm as a function of pH. Solid line represents the fit to the data.



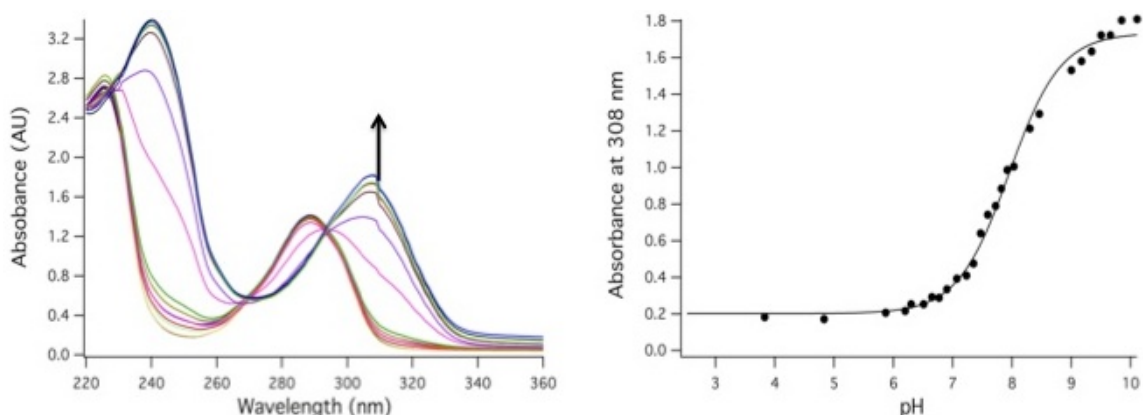
**Figure S28.** Left: UV-vis spectrum of  $[\text{Mn}(\text{HBET})]^{2-}$  shown as a function of pH. Arrow indicates increase in absorbance at 288 nm with increasing pH. Right: Absorbance at 288 nm as a function of pH. Solid line represents the fit to the data.



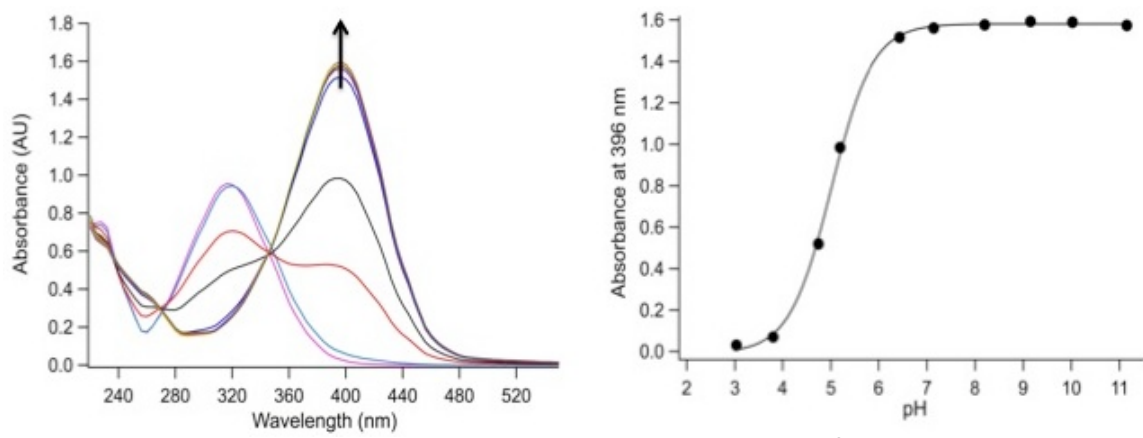
**Figure S29.** Left: UV-vis spectrum of  $[\text{Mn}(\text{HBET-OMe})]^{2-}$  shown as a function of pH. Arrow indicates increase in absorbance at 308 nm with increasing pH. Right: Absorbance at 308 nm as a function of pH. Solid line represents fit to the data.



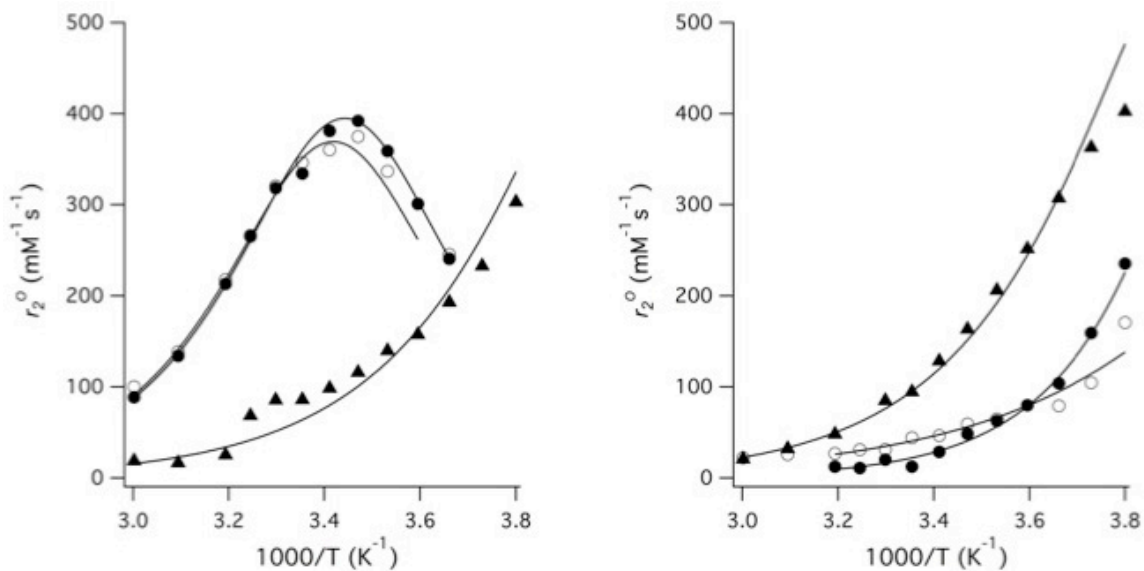
**Figure S30.** Left: UV-vis spectrum of  $[\text{Mn}(\text{CyHBET})]^{2-}$  shown as a function of pH. Arrow indicates increase in absorbance at 296 nm with increasing pH. Right: Absorbance at 296 nm as a function of pH. Solid line represents fit to the data.



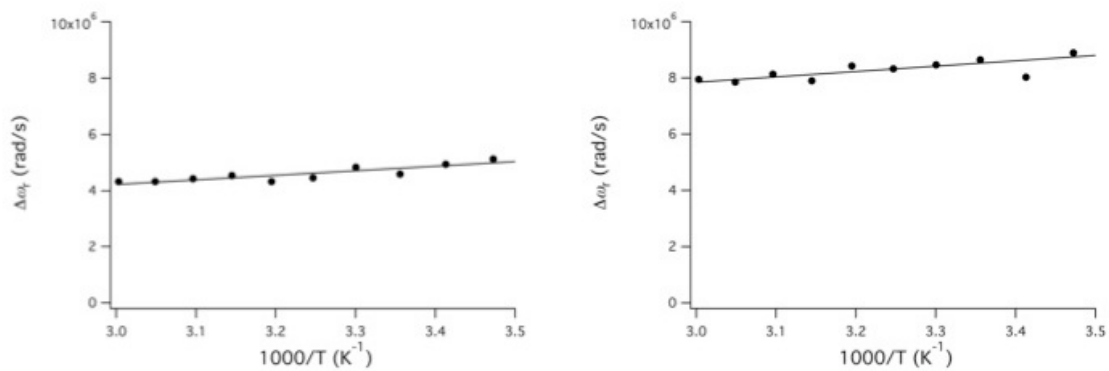
**Figure S31.** Left: UV-vis spectrum of  $[\text{Mn}(\text{CyHBET-OMe})]^{2-}$  shown as a function of pH. Arrow indicates increase in absorbance at 308 nm with increasing pH. Right: Absorbance at 296 nm as a function of pH. Solid line represents fit to the data.



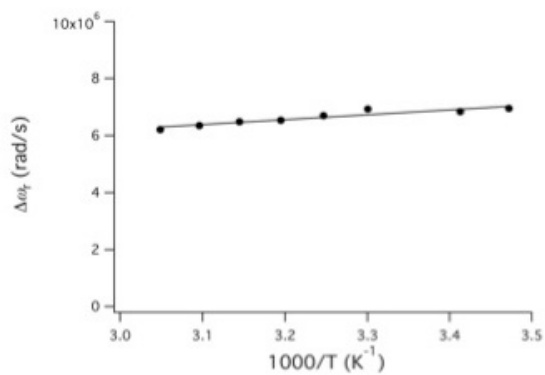
**Figure S32.** Left: UV-vis spectrum of  $[\text{Mn}(\text{CyHBET-NO}_2)]^{2-}$  shown as a function of pH. Arrow indicates increase in absorbance at 396 nm with increasing pH. Right: Absorbance at 396 nm as a function of pH. Solid line represents fit to the data.



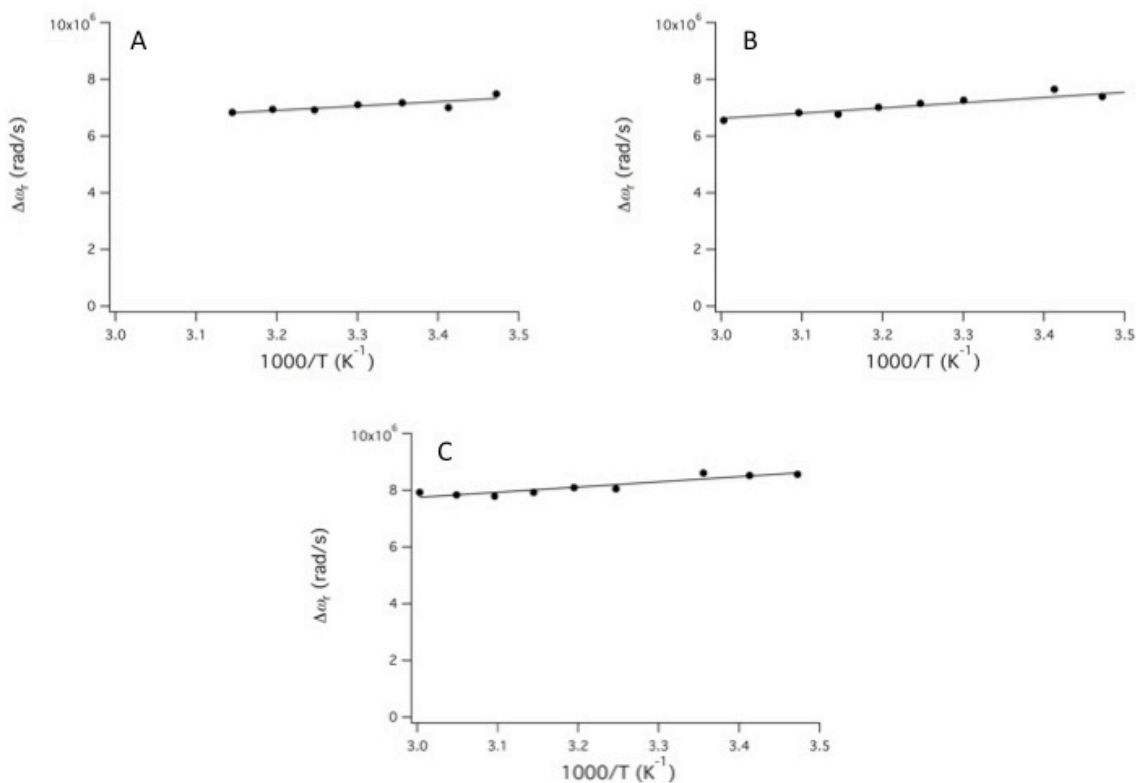
**Figure S33.** Plot of  $r_2^0$  as a function of temperature at pH 6 (left) and pH 9 (right) for  $[\text{Mn}^{\text{II}}(\text{CyHBET})]^{2-}$  (filled circles),  $[\text{Mn}^{\text{II}}(\text{CyHBET-OMe})]^{2-}$  (open circles), and  $[\text{Mn}^{\text{II}}(\text{CyHBET-NO}_2)]^{2-}$  (triangles).



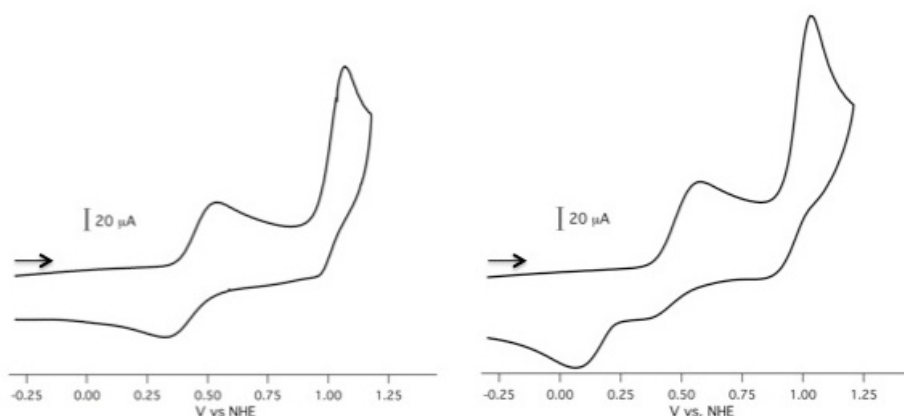
**Figure S34.** Reduced  $\text{H}_2^{17}\text{O}$  chemical shift in the presence of  $[\text{Mn}^{\text{II}}(\text{HBET})]^{2-}$  (left) and  $[\text{Mn}^{\text{II}}(\text{HBET-OMe})]^{2-}$  (right) at pH 9.



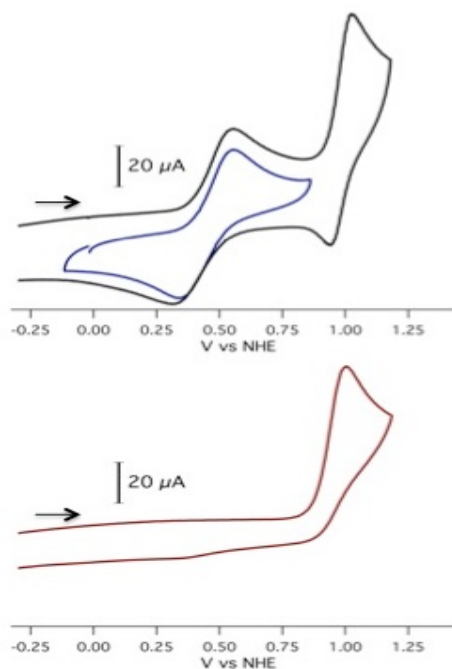
**Figure S35.** Reduced H<sub>2</sub><sup>17</sup>O chemical shift in the presence of [Mn<sup>II</sup>(CyHBET-NO<sub>2</sub>)]<sup>2-</sup> at pH 6.



**Figure S36.** Reduced H<sub>2</sub><sup>17</sup>O chemical shift in the presence of (A) [Mn<sup>II</sup>(CyHBET)]<sup>2-</sup>, (B) [Mn<sup>II</sup>(CyHBET-OMe)]<sup>2-</sup>, and (C) [Mn<sup>II</sup>(CyHBET-NO<sub>2</sub>)]<sup>2-</sup> at pH 9.

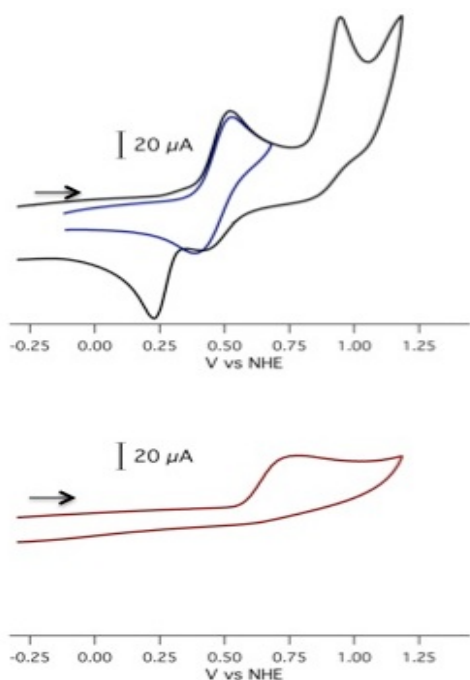


**Figure S37.** CV of  $[\text{Mn}^{\text{II/III}}(\text{HBET})]^{2-/1-}$  (left) and  $[\text{Mn}^{\text{II/III}}(\text{HBET-OMe})]^{2-/1-}$  (right) between -0.3 to 1.2 V. GC working electrode, Pt counter electrode, pH 7.4 with 0.5 M  $\text{KNO}_3$  as supporting electrolyte, scan rate: 100 mV/s. Arrows indicate the position from which the scans were initiated.

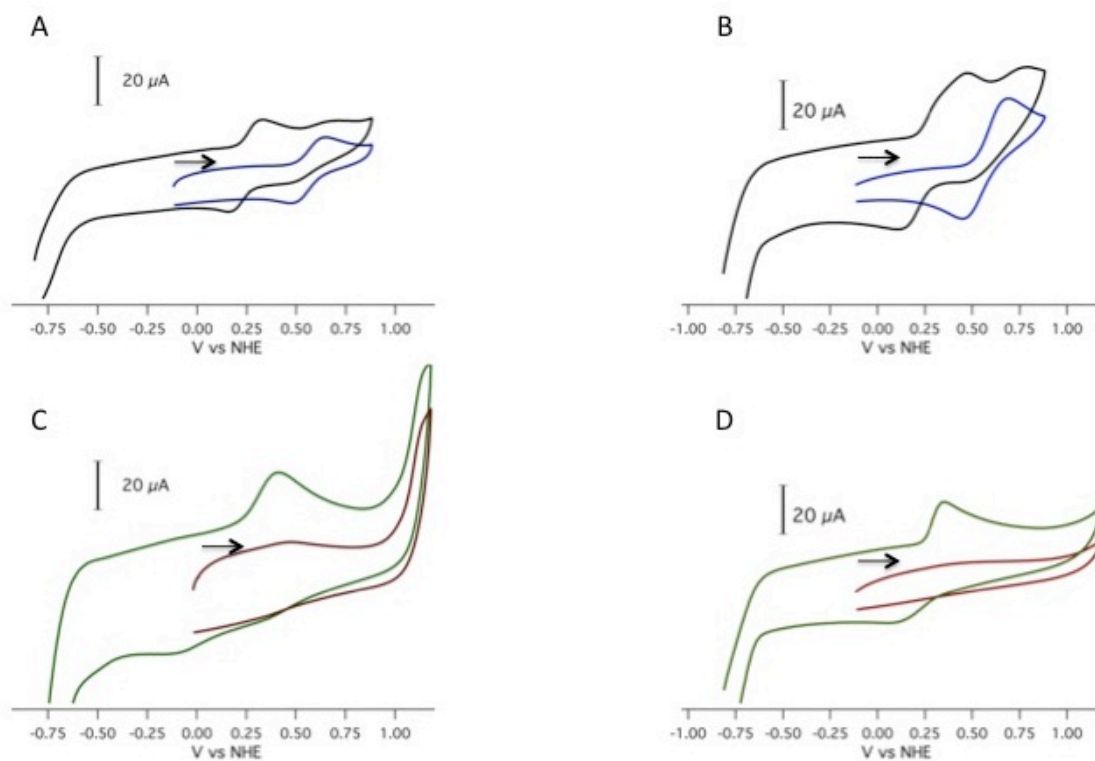


**Figure S38.** Top: CV of  $[\text{Mn}^{\text{II/III}}(\text{CyHBET})]^{2-/1-}$  between -0.3 to 0.7 V (blue) or -0.3 to 1.2V (black). Bottom: CV of  $[\text{Zn}(\text{CyHBET})]^{2-}$ . 5 mM complex, GC working electrode, Pt counter electrode, pH 7.4 w/ 0.5 M  $\text{KNO}_3$  as supporting electrolyte, scan rate: 100 mV/s. Arrows indicate the position from which the scans were initiated.

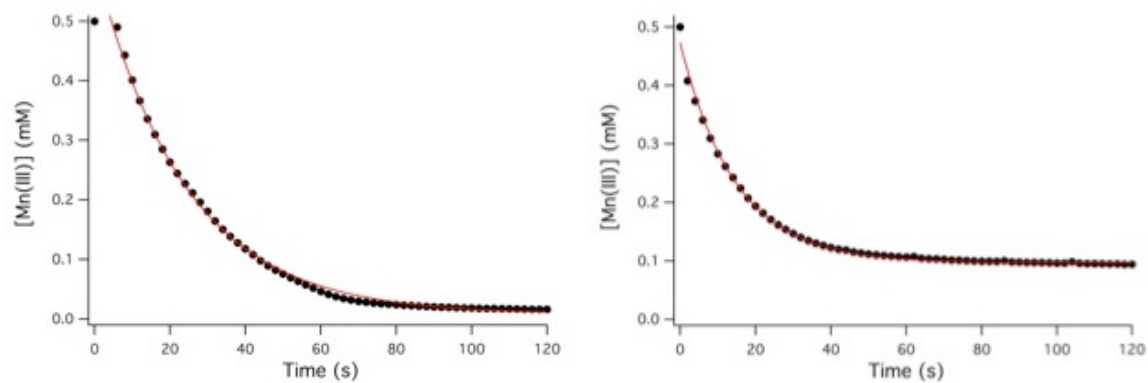




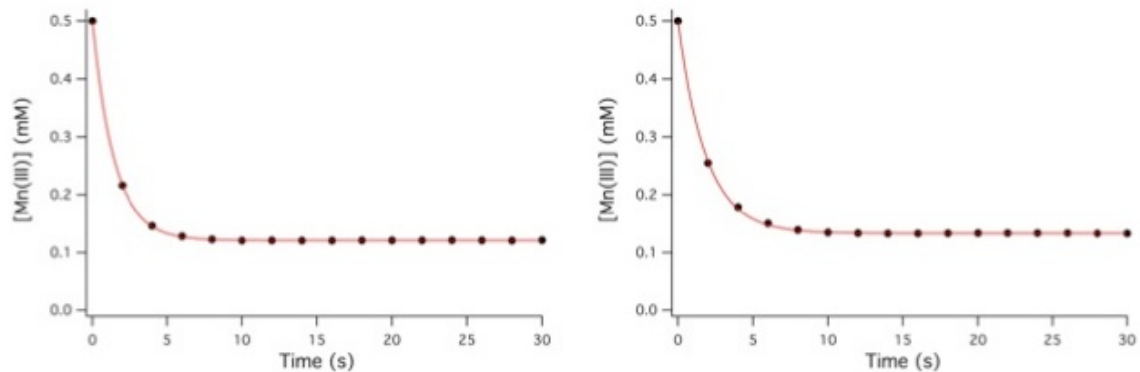
**Figure S39.** Top: CV of  $[\text{Mn}^{\text{II/III}}(\text{CyHBET-OMe})]^{2-/1-}$  between -0.3 to 0.7 V (blue) or -0.3 to 1.2 V (black). Bottom: CV of corresponding  $[\text{Zn}(\text{CyHBET-OMe})]^{2-}$  complex. 5 mM complex, GC working electrode, Pt counter electrode, pH 7.4 with 0.5 M  $\text{KNO}_3$  as supporting electrolyte, scan rate: 100 mV/s. Arrows indicate the position from which the scans were initiated.



**Figure S40.** A: CV of  $[\text{Mn}^{\text{II/III}}(\text{HBET-NO}_2)]^{2-/1-}$  scanning from -0.2 to 0.7 V (blue) or -0.3 to 1.2 V (black). B: CV of  $[\text{Mn}^{\text{II/III}}(\text{CyHBET-NO}_2)]^{2-/1-}$  scanning from -0.2 to 0.7 V (blue) or 1.2 V (black). C: CV of  $[\text{Zn}(\text{HBET-NO}_2)]^{2-}$ . D: CV of  $[\text{Zn}(\text{CyHBET-NO}_2)]^{2-}$ . 5 mM complex, GC working electrode, Pt counter electrode, pH 7.4 with 0.5 M  $\text{KNO}_3$  as supporting electrolyte, scan rate: 100 mV/s. Arrows indicate the position from which the scans were initiated.



**Figure S41.** Conversion of 0.5 mM Mn(III) to Mn(II) in the presence of 10 mM cysteine in pH 7.4 Tris buffer. Left: [Mn<sup>III</sup>(HBET)]<sup>-</sup>. Right: [Mn<sup>III</sup>(CyHBET)]<sup>-</sup>.



**Figure S42.** Conversion of 0.5 mM Mn(III) to Mn(II) in the presence of 10 mM cysteine in pH 7.4 Tris buffer. Left: [Mn<sup>III</sup>(HBET-NO<sub>2</sub>)]<sup>-</sup>. Right: [Mn<sup>III</sup>(CyHBET-NO<sub>2</sub>)]<sup>-</sup>.

| Ligand                 | p <i>K</i> <sub>a</sub> | Complex                                     | p <i>K</i> <sub>a</sub> |
|------------------------|-------------------------|---|-------------------------|
| HBET                   | 11.09                   | [Mn(HBET)] <sup>2-</sup>                    | 7.64                    |
| HBET-OMe               | 11.69                   | [Mn(HBET-OMe)] <sup>2-</sup>                | 7.91                    |
| HBET-NO <sub>2</sub>   | 7.46                    | [Mn(HBET-NO <sub>2</sub> )] <sup>2-</sup>   | 4.84                    |
| CyHBET                 | 11.37                   | [Mn(CyHBET)] <sup>2-</sup>                  | 7.95                    |
| CyHBET-OMe             | 12.48                   | [Mn(CyHBET-OMe)] <sup>2-</sup>              | 7.95                    |
| CyHBET-NO <sub>2</sub> | 8.12                    | [Mn(CyHBET-NO <sub>2</sub> )] <sup>2-</sup> | 4.87                    |

**Table S1. Phenol p*K*<sub>a</sub> Values Determined by UV-vis Spectroscopy.**