Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

$[Mn_2(bpmp)]^{3+}$ complex as an artificial peroxidase and its applications in colorimetric pyrophosphate sensing and cascade-type pyrophosphatase assay

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Determination of optimal pH condition of ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system

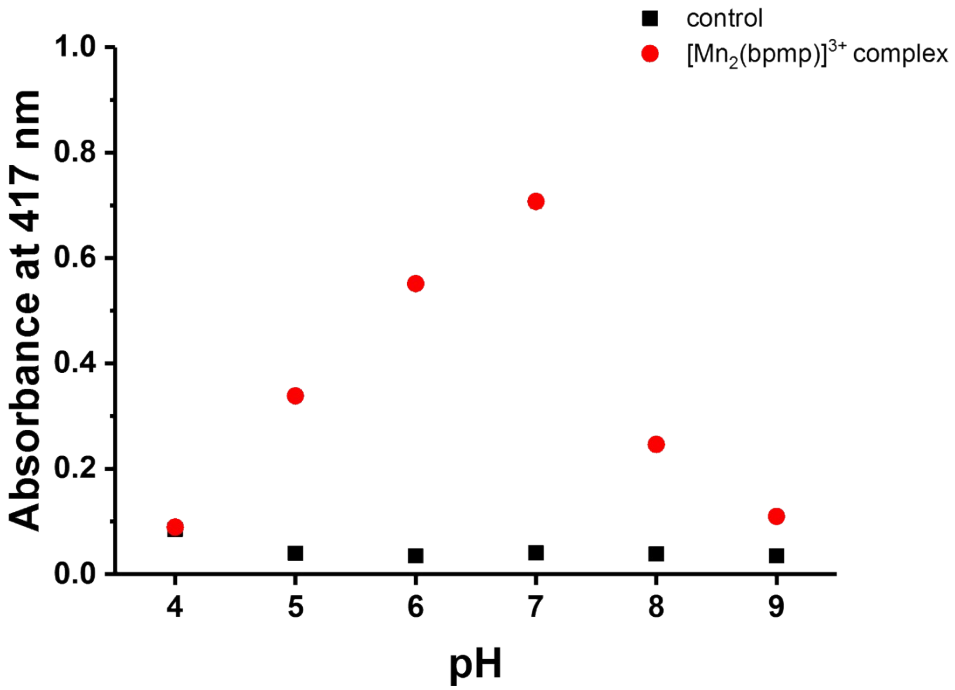


Fig. S1 Absorbance of ABTS (1 mM) in a various pH buffer solution containing $[Mn_2(bpmp)]^{3+}$ complex (2 µM) and H_2O_2 (10 mM) at 417 nm: pH 4 - 5 (acetate, 20 mM), pH 6 (citrate, 20 mM), and pH 7 – 9 (Tris, 20 mM).

Determination of optimal conditions of ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system for application

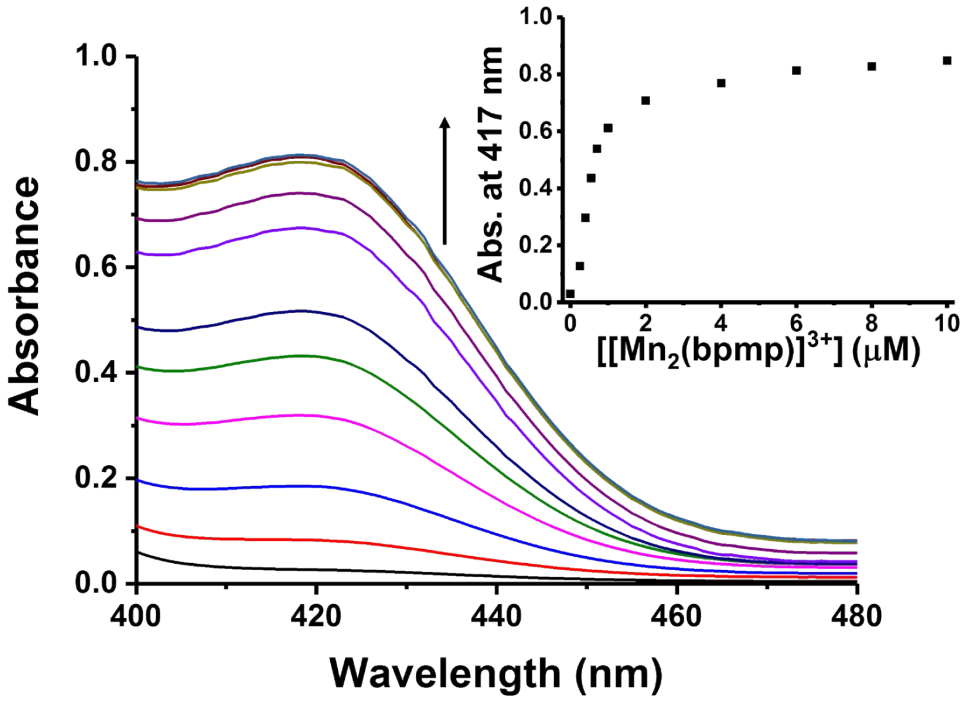


Fig. S2 Absorbance changes of ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system in various concentrations of $[Mn_2(bpmp)]^{3+}$ complex (from 0 to 10 μM) at 2 min. Inset: Plot of absorbance at 417 nm *versus* $[Mn_2(bpmp)]^{3+}$ complex concentrations at 2 min. [ABTS] = 1 mM and [H_2O_2] = 10 mM in a buffer solution (Tris, 20 mM, pH 7.0).

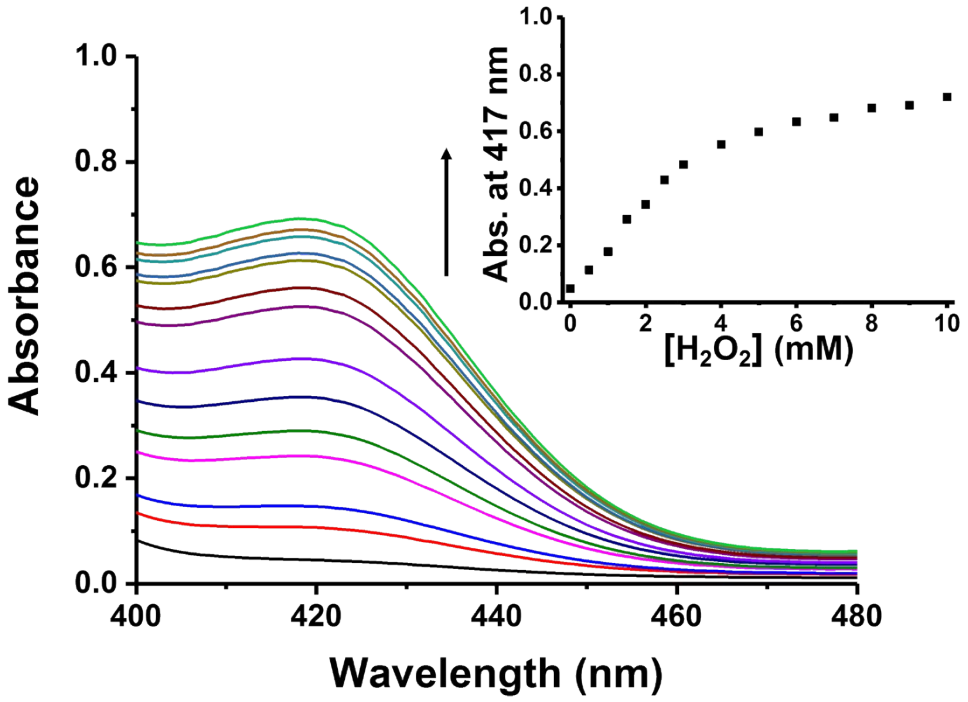


Fig. S3 Absorbance changes of ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system in various concentrations of H_2O_2 (from 0 to 10 mM) at 2 min. Inset: Plot of absorbance at 417 nm *versus* H_2O_2 concentrations at 2 min. [ABTS] = 1 mM and $[[Mn_2(bpmp)]^{3+}]$ = 2 μM in a buffer solution (Tris, 20 mM, pH 7.0).

Determination of detection limit for PPi in the ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system

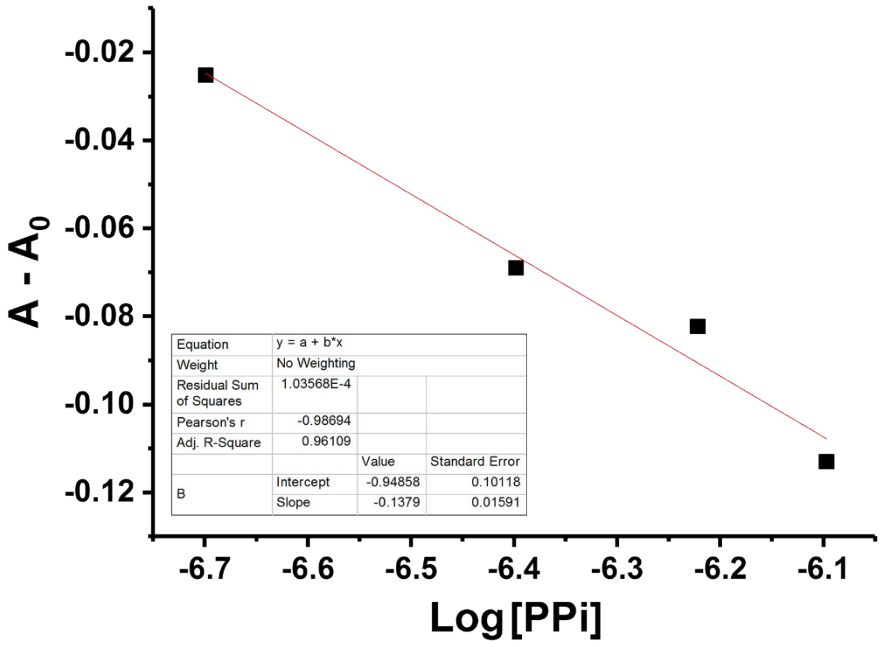


Fig. S4 Plot of the absorbance at 417 nm against the logarithm of PPi concentrations.

The limit of detection (LOD) for PPi was obtained from the low concentration range of PPi (0 ~ 0.8 µM) in the PPi titration (see Fig. 3). As shown in Fig. S4, a linear function was obtained when the x-axis is log[PPi] and the y-axis is A - A_0. The LOD was estimated from the x-intercept of this function.

A_0 is the absorbance in the absence of PPi.

Intercept = - 0.949

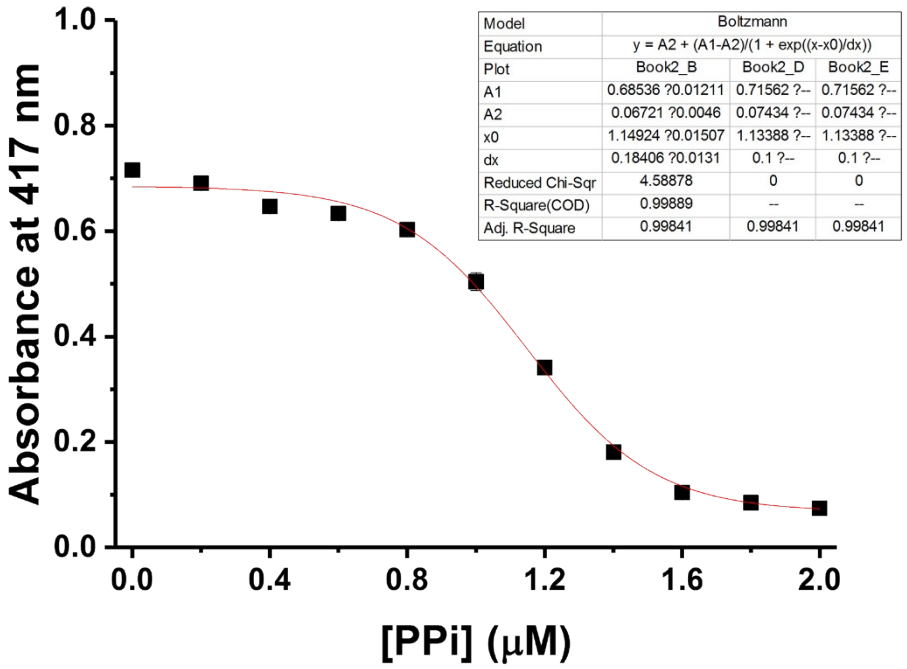
Slope = - 0.138

R^2 = 0.961

$A - A_0 = - 0.138 \times \text{Log[PPi]} - 0.949$

LOD = 0.133 µM = 133 nM

Calibration curve of PPi titration



Model	Boltzmann		
Equation	y = A2 + (A1-A2)/(1 + exp((x-x0)/dx))		
Plot	Book2_B	Book2_D	Book2_E
A1	0.68536 ?0.01211	0.71562 ?--	0.71562 ?--
A2	0.06721 ?0.0046	0.07434 ?--	0.07434 ?--
x0	1.14924 ?0.01507	1.13388 ?--	1.13388 ?--
dx	0.18406 ?0.0131	0.1 ?--	0.1 ?--
Reduced Chi-Sqr	4.58878	0	0
R-Square(COD)	0.99889	--	--
Adj. R-Square	0.99841	0.99841	0.99841

Fig. S5 Calibration curve of PPi titration in the ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system.

A_1 = 0.685

A_2 = 0.067

x_0 = 1.149

dx = 0.184

R^2 = 0.998

y = 0.067 + 0.618 x [1+e^{(x-1.149)/0.184}]

ATP titration using the ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system

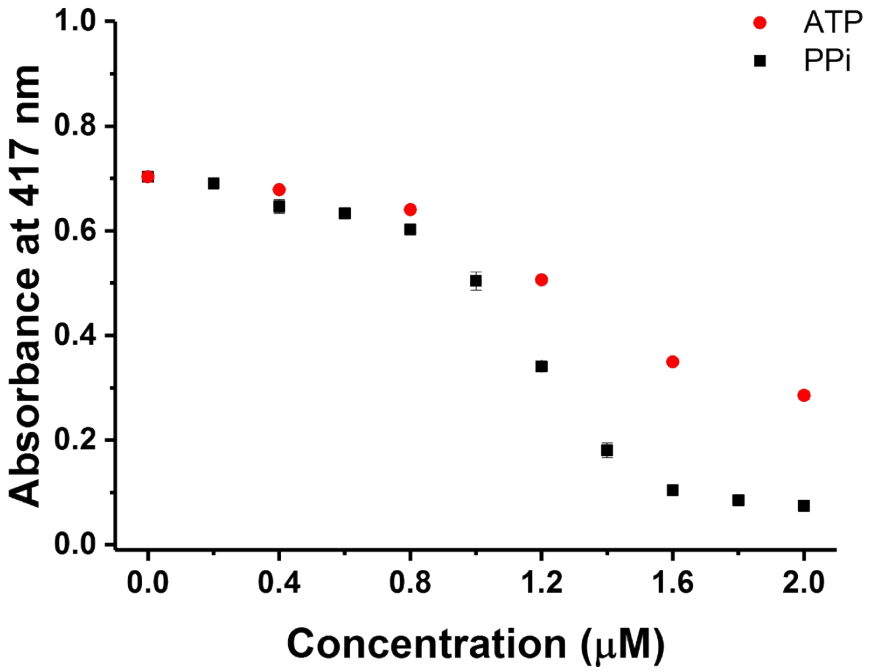


Fig. S6 Absorbance changes of ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system in the presence of various concentrations of ATP and PPi (from 0.0 to 2.0 μM) at 2 min. [ABTS] = 1 mM, [H_2O_2] = 10 mM, and [$[Mn_2(bpmp)]^{3+}$] = 2 μM in a buffer solution (Tris, 20 mM, pH 7.0).

Determination of detection limit for PPase in the ABTS/H_2O_2/$[Mn_2(bpmp)]^{3+}$ system

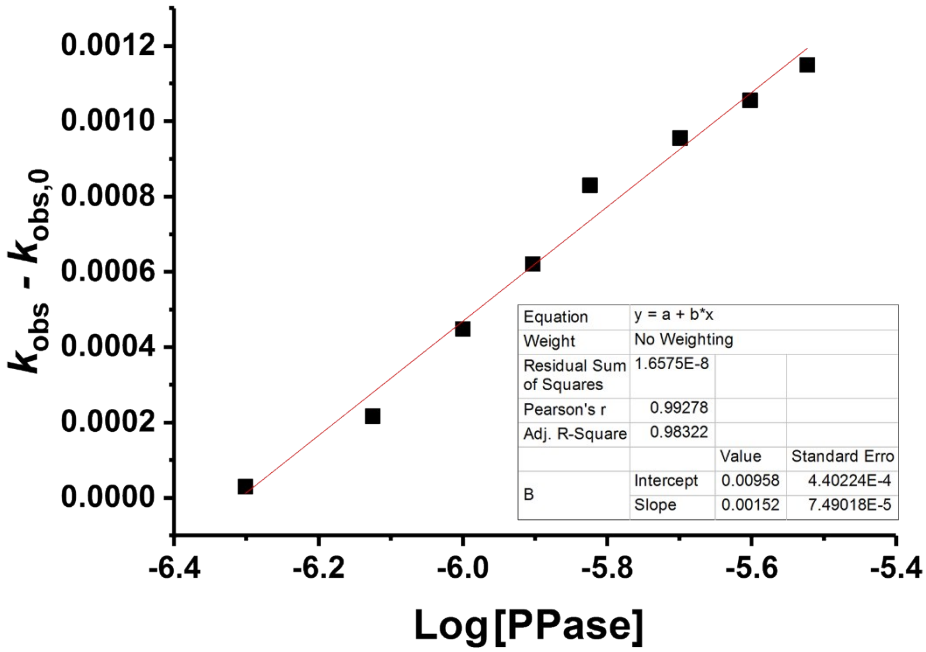


Fig. S7 Plot of the absorbance at 417 nm against the logarithm of PPase concentrations.

The LOD for PPase was obtained from the low concentration range of PPi (0 ~ 3 U/mL) in the PPase titration (see Fig. 5). As shown in Fig. S6, a linear function was obtained when the x-axis is log[PPase] and the y-axis is k_{obs} - $k_{obs,0}$. The LOD was estimated from the x-intercept of this function.

$k_{obs,0}$ is the k_{obs} value in the absence of PPase.

Intercept = 0.00958

Slope = 0.00152

R^2 = 0.98322

k_{obs} - $k_{obs,0}$ = 0.00152 x Log[PPase] – 0.00958

LOD = 0.49816 U/mL

NMR data

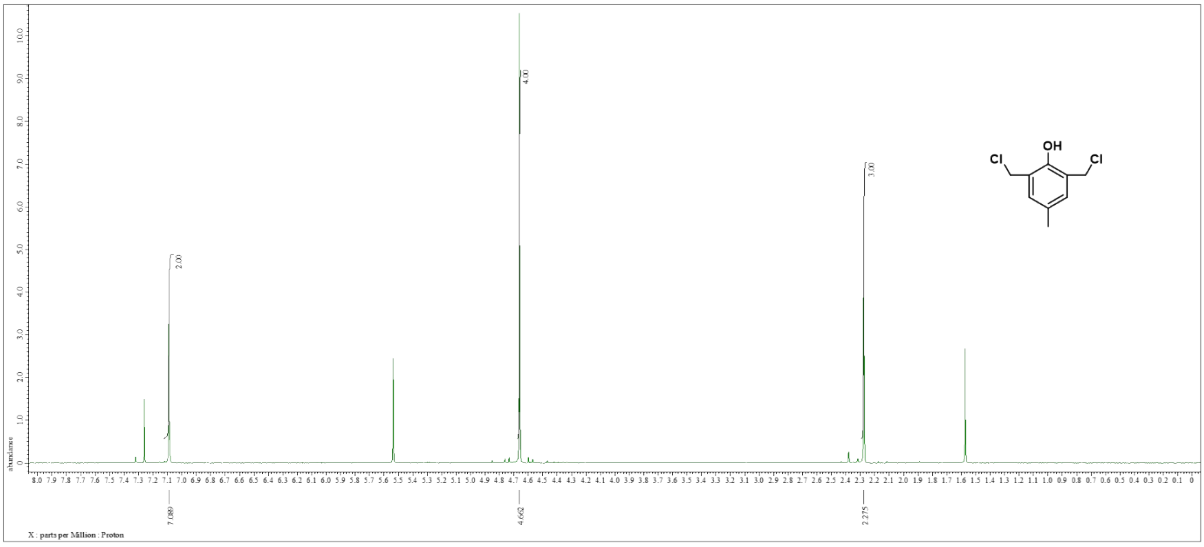


Fig. S8 ^{1}H NMR spectrum (400 MHz) of 2,6-Bis(chloromethyl)-4-methoxyphenol in $CDCl_3$

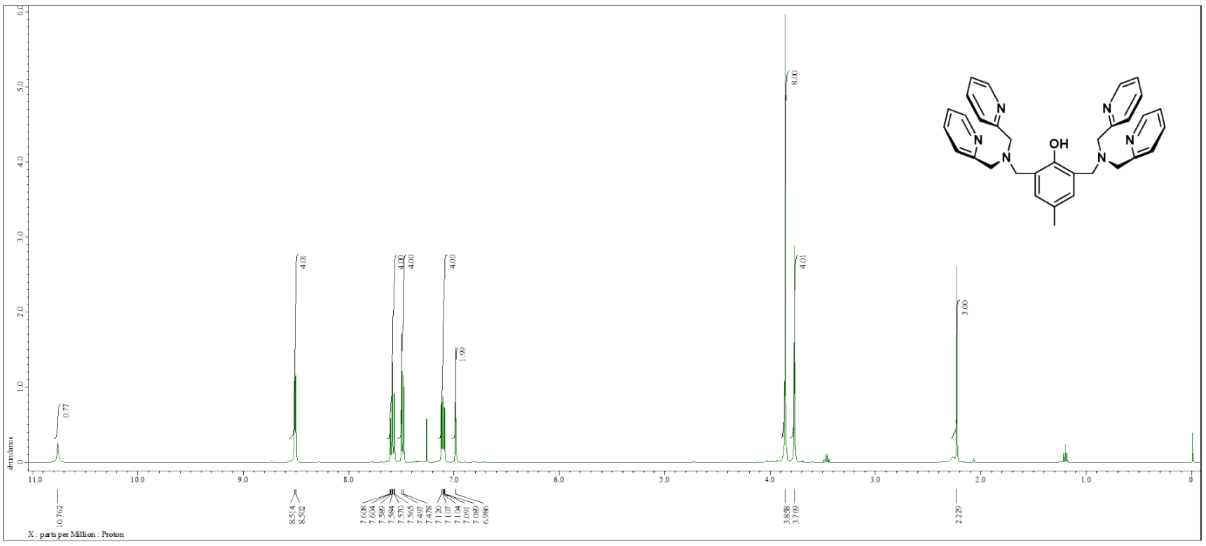


Fig. S9 ^{1}H NMR spectrum (400 MHz) of H-bpmp in $CDCl_3$

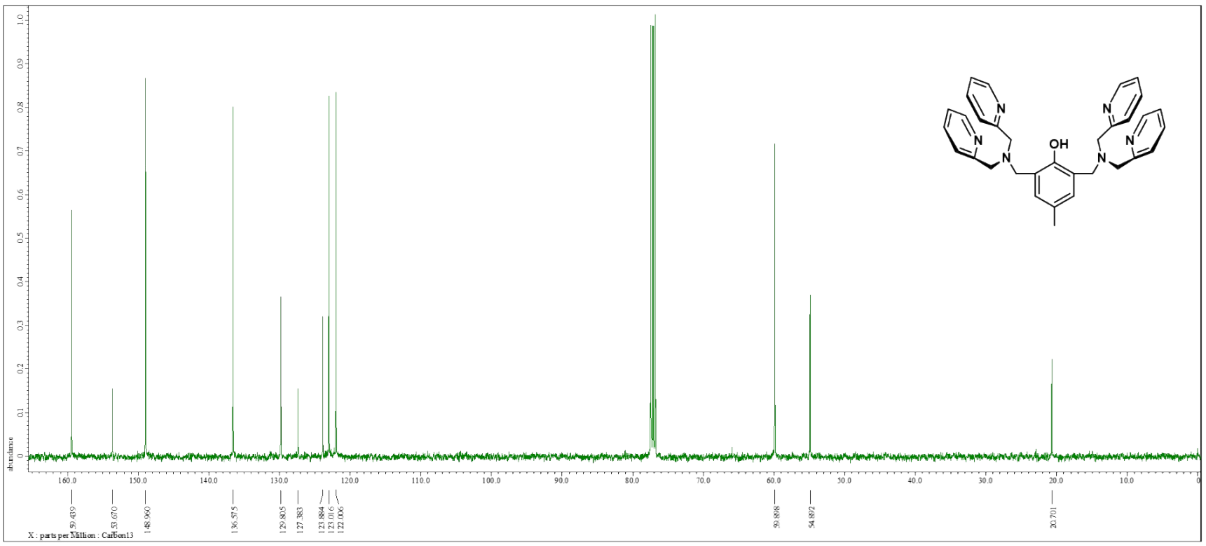


Fig. S10 ^{13}C NMR spectrum (100 MHz) of H-bpmp in $CDCl_3$

Mass data

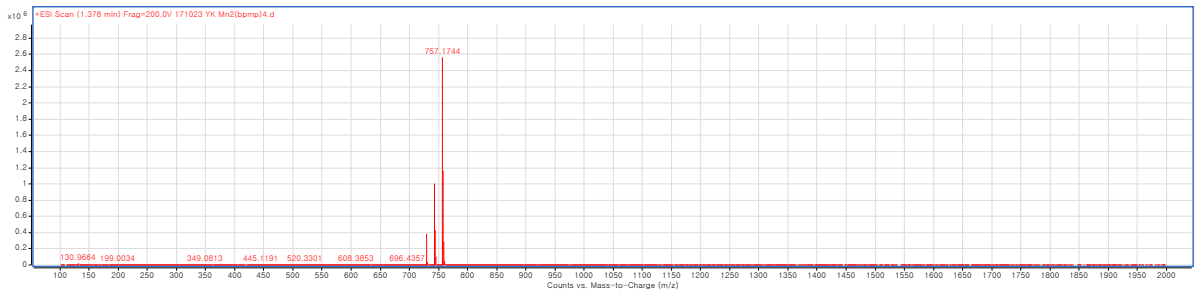


Fig. S11 Mass spectrum of $[Mn_2(bpmp)(OAc)_2](ClO_4)$ complex in acetonitrile.

IR data

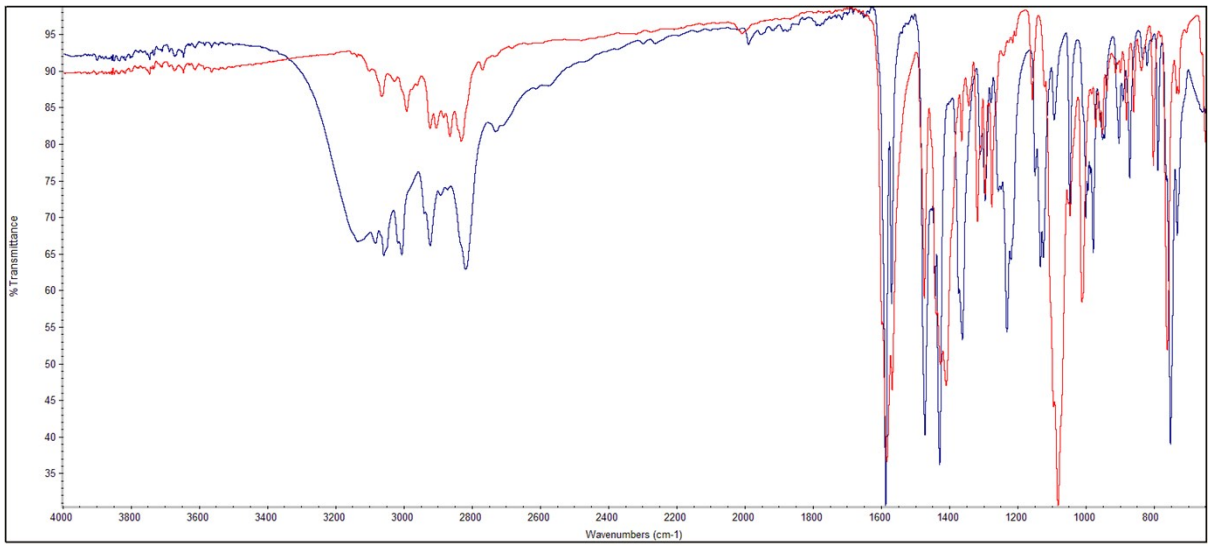


Fig. S12 IR spectra of H-bpmp (blue line) and $[Mn_2(bpmp)(OAc)_2](ClO_4)$ complex (red line).