Supporting Information for:
DESIGN OF MORE POWERFUL IRON-TAML PEROXIDASE ENZYME MIMICS
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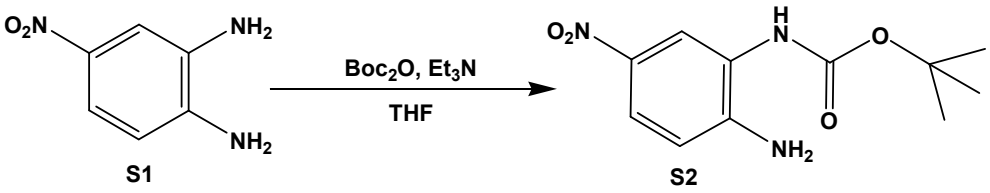
EXPERIMENTAL DETAILS

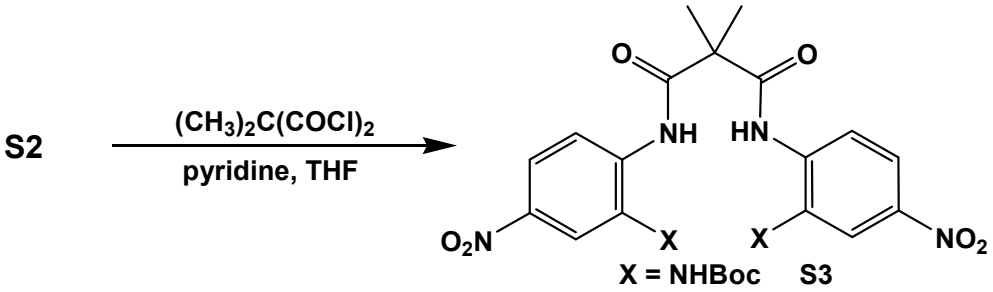
1. Instrumentation. Spectrophotometric measurements were carried out on Hewlett-Packard Diode Array spectrophotometers (models 8452A and 8453) equipped with a thermostatted cell holder and automatic 8-cell positioner. ^{1}H NMR data were collected at 300 K with a Bruker Avance 300 operating at 300 MHz using DMSO-d_6 with chemical shifts (δ) referenced to the residual proton DMSO peak at δ = 2.5. Elemental analysis was performed by Midwest Microlabs, LLC.

2. Materials. Fe^{III}-TAML complexes were synthesized at Carnegie Mellon University by published methods, or as described here.[1, 2] All reagents and solvents were at least ACS reagent grade in quality and were obtained from commercial sources. They were used as received, or if noted, after purification as described elsewhere.[3]

3. Synthesis of 2e includes (*a*) the protection of one amino group of the 1,2-diaminobenzene derivative, (*b*) the double amination of dimethyl malonyl dichloride, (*c*) acid-catalyzed deprotection, and (*d*) macrocyclization with oxalyl dichloride followed by (e) the base-assisted insertion of iron.

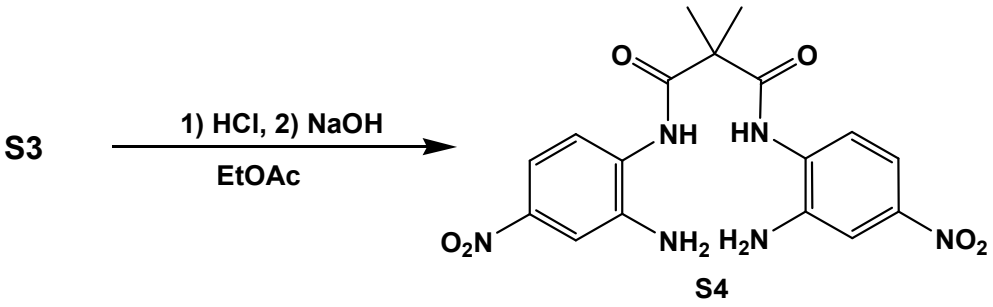


Step a: Synthesis of *(2-Amino-5-nitrophenyl)carbamic acid tert-butyl ester***.**
S1 (98%, Acros) was dissolved in ethanol and the solution was filtered through a glass frit. The solvent was evaporated and the solid was then recrystallized from hot water by aqueous Soxhlet extraction to yield deep red needles. Purified **S1** (2.5 g, 16 mmol) was dissolved in distilled THF (75 mL) in a 250 mL round bottom flask (RBF). HPLC grade Et_3N (0.95 eq, 1.54 g, 2.12 mL, 15.2 mmol) was added. Di-*t*-Butyl-dicarbonate (Boc_2O) (0.95 eq, 3.32 g, 15.2 mmol) was dissolved in distilled THF (25 mL) and added by dropping funnel to the rapidly stirred solution at a rate of *ca.* 1 drop/second. The reaction was performed under argon and allowed to proceed overnight. The solvent was removed on a rotary evaporator with gentle warming to yield a viscous oil. Sonication in heptane or diethyl ether converted the oil to a brown solid. Recrystallization from ethanol/heptane yields the product **S2** as greenish-gold microcrystals. Yield 82%. ^{1}H NMR: 8.5 (s, 1H, br), 8.3 (m, 1H), 7.8 (m, 1H), 6.75 (m , 1H), 6.45 (s, 2H, br), 1.49 (s, 9H,). Anal. Calcd. (Found) for $C_{11}H_{15}N_3O_4$: C 52.17 (52.21), H 5.97 (5.94), N 16.59 (16.28) %.



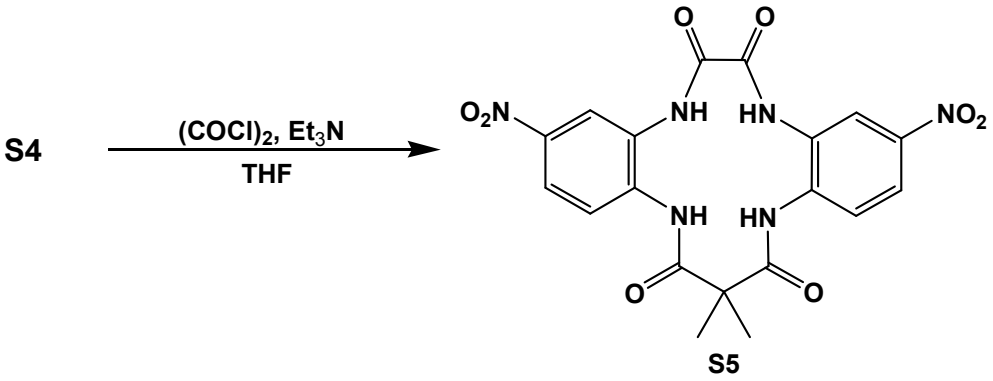
Step b: Synthesis of *N,N'-Bis-(2-carbamic acid tert-butyl ester-4-nitrophenyl)-2,2-dimethylmalonamide*.

S2 (0.7 g, 2.76 mmol) was placed into a 100 mL RBF under argon. Distilled THF (30 mL) and anhydrous pyridine (1.25 eq, 273 mg, 3.45 mmol) were added. Dimethylmalonyl dichloride (0.5 eq, 233 mg, 1.38 mmol) in distilled THF (10 mL) was added by dropping funnel to the reaction flask at a rate of *ca.* 1 drop/s and the mixture was stirred overnight. A white precipitate of pyridinium chloride formed. The colorless product **S3** was obtained by evaporating the mother liquor after filtration of the solids. It can be used as is or recrystallized from ethanol/heptane. Yield 85%. ^{1}H NMR: 9.7 (s, 2H, br), 9.1 (s, 2H, br), 8.4 (m, 2H), 8.0 (m, 2H), 7.9 (m, 2H), 1.6 (s, 6H), 1.4 (s, 18H).



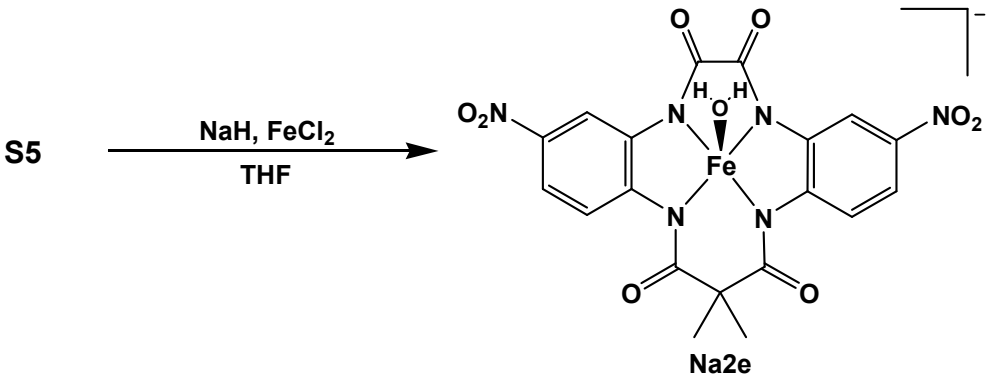
Step c: Synthesis of *N,N'-Bis-(2-amino-4-nitrophenyl)-2,2-dimethylmalonamide.*

S3 (0.5 g, 0.83 mmol) was dissolved in rapidly stirred EtOAc (50 mL) in a 500 mL RBF and conc. HCl (15 mL) was added slowly to give a biphasic reaction mixture (*ca.* 3 M HCl in the volume of the biphasic mixture). After 5–7 min. of stirring, the acid was neutralized with ice cold aqueous NaOH and the pH of the aqueous layer was adjusted to 10.5. The organic layer turned first red and then bright yellow. The mixture was extracted with dichloromethane (4 × 50 mL) until the organic extracts became colorless. The organic layers were combined, dried, and the solvent was removed to yield **S4** as a deep yellow solid. The solid was sonicated in heptane and filtered. Yield 90%. ^{1}H NMR: 9.3 (s, 2H, br), 7.6 (m, 2H), 7.45 (m, 2H), 7.35 (m 2H), 5.5 (s, 4H, br), 1.62 (s, 6H).



Step d: Synthesis of *15,15-Dimethyl-3,10-dinitro-5,8,13,17-tetrahydro-5,8,13,17-tetraaza-dibenzo[a,g]cyclotridecene-6,7,14,16-tetraone.*

S4 (0.1 g, 0.25 mmol) was added to distilled THF (125 mL) with Et_3N (2.1 eq, 53 mg, 0.073 mL, 0.53 mmol) in a 250 mL RBF under argon. Oxalyl dichloride (2 M in dichloromethane, 1 eq, 0.125 mL, 0.25 mmol) was diluted with THF (25 mL) and added by dropping funnel slowly to the RBF, with stirring, at a rate of 1 drop/s. A white precipitate formed. The reaction was stirred overnight. Filtration of the solids yielded a mixture of $Et_3N{\cdot}HCl$ and **S5.** The $Et_3N{\cdot}HCl$ was washed away with water. The remaining wet, off-white solid was suspended in acetonitrile (10 mL) and isopropyl alcohol (10 mL) and then recollected as a chalky solid. Yield 55%. ^{1}H NMR: 10.35 (s, 2H, br), 10.15 (s, 2H, br), 8.4 (m, 2H), 8.2 (m, 2H), 7.6 (m, 2H), 1.6 (b, 6H). Dissolution of approximately 20 mg **S5** in approximately 15 mL acetone, followed by slow evaporation yielded an off-white solid in low yield for elemental analysis. Anal. Calcd. (Found) for $C_{19}H_{16}N_6O_8((CH_3)_2CO,(H_2O)_2)$: C 48.91 (49.07), H 4.2 (4.01), N 16.69 (16.60) %.

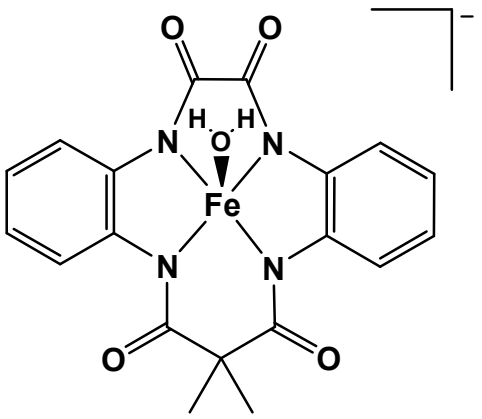


Step e: Metallation of **S5** to yield Na**2e.**

S5 (130 mg, 0.29 mmol) was mixed with distilled THF (50 mL) under argon and NaH (60% NaH in mineral oil, 4.4 eq, 51 mg, 1.276 mmol) was added rapidly to the suspension. The reaction mixture turned yellow as the ligand **S5** dissolved in a matter of 15 min. $FeCl_2$ (1.25 eq, 46 mg, 0.363 mmol) was added as a solid with positive argon flow coming from the reaction flask. After 12 h, the reaction mixture was opened to air and became brown. The mixture was filtered and the resulting filtrate was poured into heptane (200 mL). The precipitate was filtered to yield crude Na**2e.** Yield 45%. ESI-

MS: 508.0 m/z, $[(M-H_2O)]^-$. Purification of Na**2e** involved dissolving it in approximately 1 mL water, filtrating it through a 0.2 μm filter onto a C18 silica column, and eluting with a solvent gradient of methanol-water (0-25% methanol). The orange band was collected and the eluting solvent was removed by rotary evaporation at 35 °C. Isopropanol was added sequentially during the evaporation process until the solvent was *ca.* 100% isopropanol. The isopropanol was removed completely to yield Na**2e as** a red-orange solid.

Step f: Cation metathesis of Na**2e** to yield $[PPh_4]$**2e** or [PNP]**2e**.
The Na^+ cation was metathetically replaced by either the tetraphenylphosphonium ($[PPh_4^+]$) or bis(triphenylphosphoranylidene)ammonium ($[PNP^+]$) cation by dissolving 3-5 mg of Na**2e** in water (2 mL). Excess PPh_4Cl (*ca*. 40 mg) dissolved in water (3-5 mL) or excess PNPCl (*ca*. 80 mg) dissolved in 1:1 methanol:water (3-5 mL) was added dropwise to the solution of Na**2e** on stirring until no more precipitate formed. The red/orange precipitate was collected by filtration and recrystallized from methanol/water. Crystals of $[PPh_4]$**2e** were obtained by slow evaporation of methanol from water. Anal. Calcd. (Found) for $[PPh_4]$**2e**$\cdot(CH_3OH)_2\cdot H_2O$, $C_{45}H_{42}N_6PO_{11}Fe$: C 58.14 (57.97), H 4.55 (3.96), N 9.04 (8.61) %. X-ray quality crystals of [PNP]**2e**$\cdot(CH_3OH)_2$ were grown similarly. Compound **2d** was synthesized similarly and its anion is shown in Scheme 1S below.

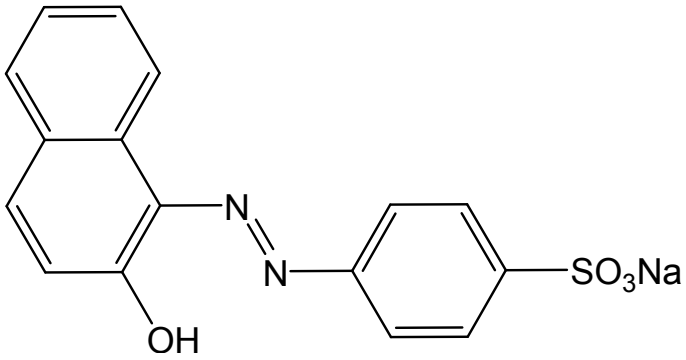


Scheme 1S. The anion of compound **2d.**

4. X-ray details: $C_{36}H_{30}NP_2\cdot C_{20}H_{16}FeN_6O_9\cdot CH_4O$, M_r=1110.8, monoclinic, $P2_1/c$, Z=4, 11942 independent reflections, 699 L.S. parameters, R_1=0.049 (8921 observed reflections), wR_2=0.107 (all reflections), S=1.09, difference Fourier map peak/hole 0.46/–0.43.

5. Kinetic Studies of Orange II by H_2O_2 catalyzed by 1b and 2e. Stock solutions of **2e**, **1b,** Orange II, and hydrogen peroxide were prepared in HPLC grade water of 1×10^{-4} M, 1×10^{-4} M, 3×10^{-4} M, and *ca.* 0.013 M, respectively. Solutions of hydrogen peroxide were standardized daily by measuring the absorbance at 230 nm (ε = 72.8 M^{-1} cm^{-1}).[4] In a polymethylmethacrylate UV-vis cuvette, aliquots of these solutions (except hydrogen peroxide) were measured into an appropriate amount of 0.01 M phosphate

buffer at a pH ranging from 6.5 up to 11 (using a Corning 220 pH meter). Hydrogen peroxide was added to initiate the reaction— [**2e**] or [**1b**] was 2×10^{-7} M, [Orange II] was varied from 3×10^{-6} to 1.2×10^{4} M, [H_2O_2] was varied from 5×10^{-5} to 1.5×10^{-3} M. The cell compartment was thermostatted at 25 °C. Initial rates of Orange II oxidation were calculated using the pH dependent extinction coefficients for Orange II of 18,100 M^{-1} cm^{-1}, 18,500 M^{-1} cm^{-1}, and 20,000 M^{-1} cm^{-1} at 485 nm at pH 7, 9, and 11, respectively. All initial rates were measured in triplicate. Calculations of the initial rates and of the rate constants k_I and k_{II} were performed using a Sigma Plot 2007 package (v 10.0).



Scheme 2S. Structure of the Orange II dye.

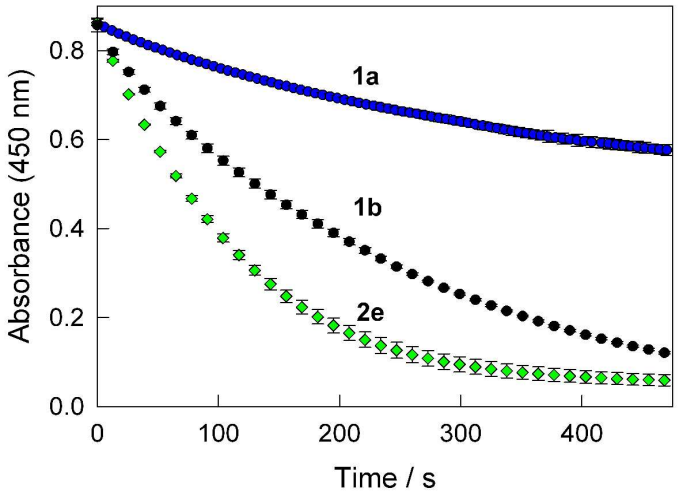


Figure 1S. *A:* Comparative Orange II bleaching by H_2O_2 at pH 7 for **1a**, **1b**, and **2e** at [Fe^{III}–TAML] = 2×10^{-7} M, [H_2O_2] = 0.0015 M, [Orange II] = 4×10^{-5} M, 0.01 M phosphate buffer, 25 ºC.

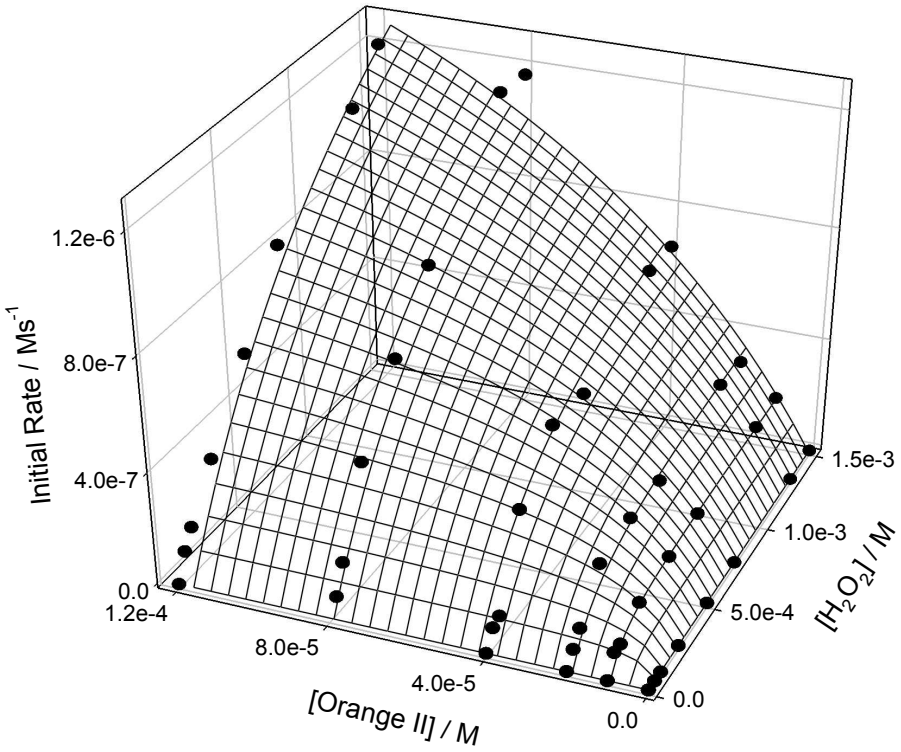


Figure 2S. 3D plot showing the initial rates of **1b** catalyzed bleaching of Orange II by H_2O_2 as a function of [H_2O_2] and [Orange II] at pH 11 with [**1b**] = 2 x 10^{-7} M, 0.01 M phosphate, 25 °C. Each data point is the mean value of three determinations. The mesh was calculated using eq 1 and the rate constants in Table 1.

Supplemental Information References

1) Ghosh, A. PhD. Dissertation, Carnegie Mellon, Pittsburgh, 2004.
2) Sen Gupta, S. PhD. Dissertation, CMU, Pittsburgh, 2002.
3) Armarego, W.L.F., Chai, C.L.L. *Purification of Laboratory Chemicals, 5th ed.* Elsevier Science: Burlington, MA, 2003.
4) George, P. *Biochem. J.*, **1953**, 54, 267.