

A Macrocyclic Ligand Framework That Improves Both the Stability and T_1 -Weighted MRI Response of Quinol-Containing H_2O_2 Sensors

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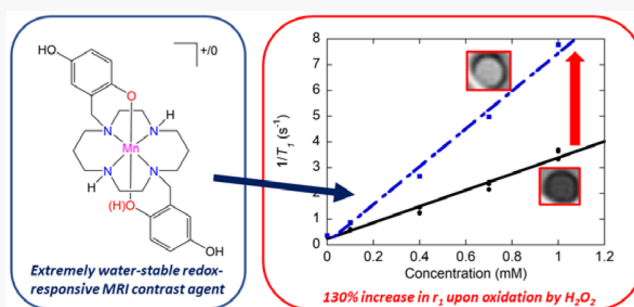


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ABSTRACT: Previously prepared Mn(II)- and quinol-containing magnetic resonance imaging (MRI) contrast agent sensors for H_2O_2 relied on linear polydentate ligands to keep the redox-activatable quinols in close proximity to the manganese. Although these provide positive T_1 -weighted relaxivity responses to H_2O_2 that result from oxidation of the quinol groups to p -quinones, these reactions weaken the binding affinity of the ligands, promoting dissociation of Mn(II) from the contrast agent in aqueous solution. Here, we report a new ligand, 1,8-bis(2,5-dihydroxybenzyl)-1,4,8,11-tetraazacyclotetradecane, that consists of two quinols covalently tethered to a cyclam macrocycle. The macrocycle provides stronger thermodynamic and kinetic barriers for metal-ion dissociation in both the reduced and oxidized forms of the ligand. The Mn(II) complex reacts with H_2O_2 to produce a more highly aquated Mn(II) species that exhibits a 130% greater r_1 , quadrupling the percentile response of our next best sensor. With a large excess of H_2O_2 , there is a noticeable induction period before quinol oxidation and r_1 enhancement occurs. Further investigation reveals that, under such conditions, catalase activity initially outcompetes ligand oxidation, with the latter occurring only after most of the H_2O_2 has been depleted.



INTRODUCTION

The overproduction of reactive oxygen species (ROS), such as H_2O_2 and O_2^- , has been implicated in a wide array of pathologies, including a host of neurological and cardiovascular health conditions.^{1–5} Given the possible roles of ROS in disease, our research group has strived to develop molecular probes that can be used to noninvasively monitor ROS concentrations in physiological environments. Such sensors could potentially distinguish pathologies with similar clinical symptoms and better inform preventative and ameliorative therapies. To this end, our laboratory has reported a series of complexes that respond to H_2O_2 with changes in their T_1 -weighted relaxivity (r_1).^{6–9}

Recent sensors from our laboratory have consisted of Mn(II) ions complexed to quinol-containing polydentate ligands (Scheme 1).^{7–9} Positive r_1 responses to H_2O_2 are observed with N_4O_2 or N_5O coordination spheres, where the nitrogen donors come from either neutral pyridine or amine groups.^{7,8} With a weakly anionic coordination sphere, the Mn(III/II) reduction potential is high enough to discourage oxidation of the metal to the less paramagnetic 3+ oxidation state. Terminal oxidants, such as H_2O_2 , instead preferentially oxidize the ligand, differentiating our sensors from redox-responsive magnetic resonance imaging (MRI) contrast agents reported by Gale, Caravan, and others.^{10–12} Oxidation of the

quinol portions of the ligands to p -quinones is accompanied by an increase in r_1 . Water molecules are proposed to displace p -quinones from the metal center, enhancing r_1 through better aquation of Mn(II).⁸ Having a second quinol in the coordination sphere generally improves the r_1 response to H_2O_2 . The relaxivities of $[Mn(H_2qp1)(MeCN)]^{2+}$ (MeCN = acetonitrile) and $[Mn(H_4qp2)Br_2]$ (**3**) increase by 10% and 30%, respectively, upon oxidation by excess H_2O_2 .^{7,8}

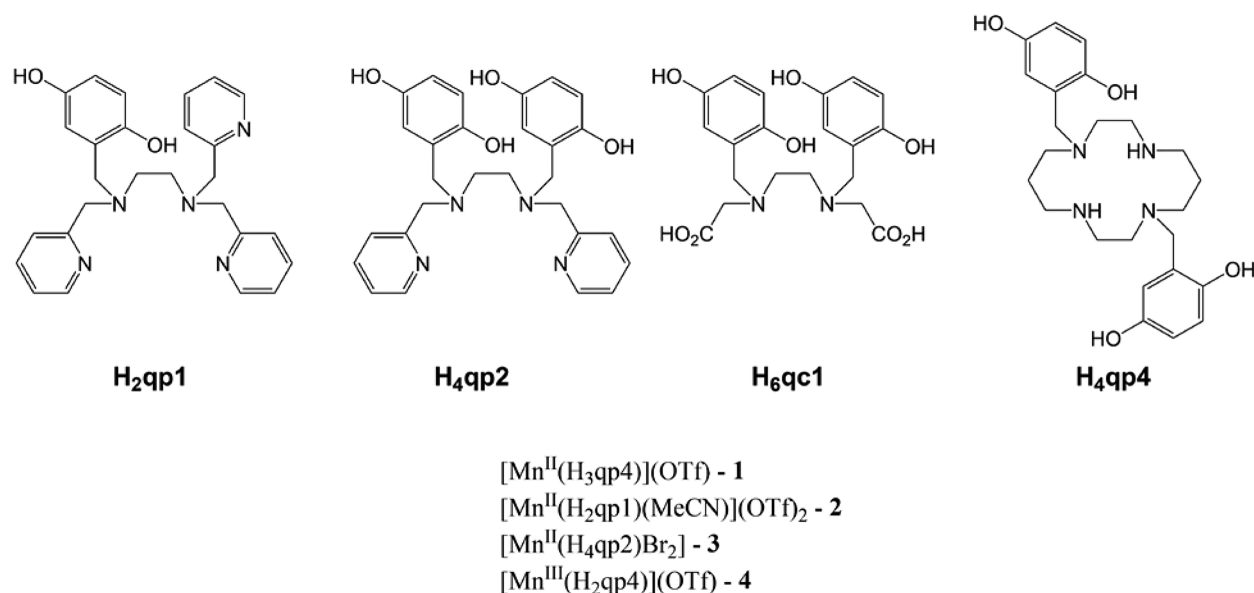
This strategy necessarily relies on a weakening of the ligand's affinity for Mn(II), and in the case of **3**, a substantial portion of the metal ion is likely released upon oxidation by H_2O_2 .⁸ Anionic groups and macrocycles are frequently used to stabilize Gd(III)- and Mn(II)-containing MRI contrast agents.^{13,14} We had previously replaced the H_4qp2 pyridines with carboxylic acids in order to stabilize the Mn(II) complexes; the carboxylic acids entirely deprotonate to carboxylates at pH 7. Although the more anionic H_3qc1^{3-} and H_2qc1^{4-} forms of the H_6qc1 ligand do indeed allow it to

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Scheme 1. Molecular Structures of the Quinol-Containing Polydentate Ligands and Compositions of the Coordination Complexes Mentioned in This Paper



coordinate more tightly to the dicationic metal center, the additional negative charges in the coordination sphere render the manganese more susceptible to oxidation. Oxidation of the metal center to the less paramagnetic Mn(III) eliminates the r_1 response of this complex to H_2O_2 .⁹ A Mn(II) complex with a fluorinated tetradentate ligand with nearly identical chelating groups likewise gets readily oxidized to a lower-relaxivity Mn(III) species upon reaction with excess H_2O_2 .¹⁵

The other strategy to discourage metal release would be to incorporate a macrocycle into the ligand framework to stabilize the resultant transition-metal complexes both thermodynamically and kinetically.¹⁶ Here, we synthesize a new macrocyclic ligand, 1,8-bis(2,5-dihydroxybenzyl)-1,4,8,11-tetraazacyclotetradecane ($\text{H}_4\text{qp4}$), and its complex with Mn(II). We find that the redox reactivity of the Mn(II) compound is similar to those of the Mn(II) complexes with $\text{H}_2\text{qp1}$ and $\text{H}_4\text{qp2}$ in that (1) the reactivity with air is slight and (2) the reactivity with H_2O_2 primarily oxidizes the ligand rather than the metal ion. In addition to the anticipated stabilization of the Mn(II) complexes with the quinol and *p*-quinone forms of the ligand, inclusion of the macrocycle improves the maximal MRI response, with r_1 increasing by 130% upon oxidation by H_2O_2 .

EXPERIMENTAL SECTION

Materials. All chemicals and solvents were purchased from Sigma-Aldrich and used as received unless otherwise noted. All deuterated solvents were bought from Cambridge Isotopes. Diethyl ether and methanol (MeOH) were bought from Fisher. Methylene chloride (CH_2Cl_2) was purchased from Mallinckrodt Baker.

Instrumentation. All ^1H and ^{13}C NMR spectra were recorded on either a 400 MHz or a 600 MHz AV Bruker NMR spectrometer. All reported NMR resonance peak frequencies were referenced to internal standards. ^{17}O NMR data were collected on a Bruker AVANCE DRX 400WB spectrometer with a superconducting wide-bore magnet operating at a 54.24 MHz resonance frequency and a 9.4 T magnetic induction. A Varian Cary 50 spectrophotometer was used to collect optical data, which were then processed using software from the WinUV Analysis Suite. Electron paramagnetic resonance (EPR) spectra were collected using a Bruker EMX-6/1 X-band EPR spectrometer operated in the perpendicular mode and subsequently

analyzed with the program *EasySpin*. All EPR samples were run as frozen solutions in quartz tubes. We used a Johnson Matthey magnetic susceptibility balance (model MK I#7967) to measure the magnetic moments of solid samples of the metal complexes and estimated the diamagnetic component of the susceptibility using Pascal's constants.¹⁷ Cyclic voltammetry (CV) was performed under N_2 at 294 K with an Epsilon electrochemistry workstation (Bioanalytical System, Inc.). The working, auxiliary, and reference electrodes were gold, platinum wire, and silver/silver(I) chloride, respectively. High-resolution mass spectrometry (HR-MS) data were collected at the Mass Spectrometer Center at Auburn University on a Bruker microflex LT matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer via direct probe analysis operated in the positive-ion mode. Solid samples of the Mn(II) complex were dried, stored under N_2 , and sent to Atlantic Microlabs (Norcross, GA) for elemental analysis.

X-ray Crystallography. Crystallographic data for $(\text{H}_4\text{qp4})(\text{OTf})_2$ and the oxidized product $[\text{Mn}^{\text{III}}(\text{H}_2\text{qp4})](\text{OTf})$ were collected using a Bruker D8 VENTURE κ -geometry diffractometer system equipped with an Incoatec $\text{I}\mu\text{S}$ 3.0 microfocus sealed tube and a multilayer mirror monochromator ($\text{Mo K}\alpha$, $\lambda = 0.71073 \text{ \AA}$). Diffraction data were integrated with the Bruker SAINT software package using a narrow-frame algorithm. Data were corrected for absorption effects using the Multiscan method (SADABS). The structure was solved and refined using the Bruker SHELXTL software package. Selected crystallographic data are presented in the Supporting Information and Table 1.

Potentiometric Titrations. The aqueous speciations of $\text{H}_4\text{qp4}$ and its Mn(II) complex were assessed using a METROHM 765 Dosimat with a jacketed, airtight glass titration vessel. A Fisher Scientific Accumet Research AR15 pH meter was used to monitor the pH of the sample solutions during the titrations. The electrode was calibrated before each titration using commercially available standard solutions buffered to pH 4.0, 7.0, and 10.0. All samples were purged with argon prior to analysis and subsequently analyzed under an argon atmosphere at 25 °C to prevent carbonate contamination. All solution samples were prepared in solutions of 100 mM KCl in deionized Millipore water. The titrations investigating metal/ligand speciation were run with solutions that contained a 1:1 molar mixture of the ligand and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$. Carbonate-free solutions of 0.10 M KOH and 0.10 M HCl were prepared using argon-saturated deionized Millipore water. The titration data were analyzed and fit to speciation models using the *Hyperquad2006* program.¹⁸

Table 1. Selected Crystallographic Data for $[\text{H}_6\text{qp4}](\text{OTf})_2$ and **4**

parameter	$[\text{H}_6\text{qp4}](\text{OTf})_2$	$[\text{Mn}(\text{H}_2\text{qp4})](\text{OTf})\cdot\text{CH}_2\text{Cl}_2$ (4)
formula	$\text{C}_{26}\text{H}_{38}\text{F}_6\text{N}_4\text{O}_{10}\text{S}_2$	$\text{C}_{26}\text{H}_{35}\text{Cl}_2\text{F}_3\text{MnN}_4\text{O}_7\text{S}$
MW	744.72	730.48
cryst syst	triclinic	monoclinic
space group	$P\bar{1}$	$P12_1/m1$
<i>a</i> (Å)	9.2212(3)	8.3460(2)
<i>b</i> (Å)	10.0950(4)	19.4089(5)
<i>c</i> (Å)	10.4689(3)	9.9382(3)
α (deg)	107.310(1)	90
β (deg)	111.234(1)	108.9230(10)
γ (deg)	104.783(1)	90
<i>V</i> (Å ³)	791.83(5)	1522.85(7)
<i>Z</i>	1	2
cryst color	colorless	pale red
<i>T</i> (K)	110	110
reflns collected	63351	36113
unique reflns	5270	3586
<i>R</i> 1(<i>F</i>) [<i>I</i> > 2σ(<i>I</i>)] ^a	0.0492	0.0259
w <i>R</i> 2(<i>F</i> ²) (all data) ^a	0.1415	0.0837

$$^a R1 = \sum ||F_o| - |F_c|| / \sum |F_o|; wR2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)]^{1/2}.$$

High-Pressure Liquid Chromatography (HPLC). HPLC was performed with UV detection at 254 nm using an Agilent 1100 series apparatus and an Agilent Zorbax SB-C18 column (4.6 × 150 mm; 5 μm pore size). The following eluents were used: (A) 99.9% water with 0.1% trifluoroacetic acid (TFA); (B) 99.9% MeCN with 0.1% TFA. The following method was used: gradient = 90% A and 10% B to 100% B over 20 min, flow rate = 0.20 mL/min, injection volume = 25.0 μL, and column temperature = 37.0 °C. Before each run, the HPLC instrument was flushed with eluent 100% A to 100% B over 16 min with a flow rate of 0.49 mL/min and an injection volume of 25.0 μL.

Measurement of the Aquation Numbers (*q*). The *q* values were calculated from the maximum ¹⁷O transverse relaxivity, *r*_{2max}[°] and the equation *q* = *r*_{2max}[°]/510. Gale, Zhu, and Caravan previously used this relationship to estimate the inner-sphere hydration state of Mn(II) in coordination complexes.¹⁹ Relaxation rates were measured both for aqueous solutions containing Mn(II) complexes and for metal-free solutions buffered to pH 7.4. The line widths at half-height of the signal were determined by a deconvolution procedure on the real part of the Fourier-transformed spectra with a Lorentzian shape function in the data analysis module of Bruker *Topspin* 1.3 software. The measurements were performed with a commercial 5 mm Bruker broadband probe thermostated with a Bruker B-VT 3000 variable-temperature unit. Samples were prepared by adding a solution of solid dissolved in a minimal amount of MeCN to an aqueous solution containing either 60 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) or 60 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffered to pH 7.4. ¹⁷O-labeled water (10%; D-Chem Ltd., Tel Aviv, Israel) was added to these solutions, resulting in a total enrichment of 1% ¹⁷O in the studied samples. The resultant mixtures contained either 6.0 or 2.5 mM of the Mn(II) complex. The 2.5 mM sample was oxidized by 15 equiv of H₂O₂ for 15 min prior to data acquisition. The temperature dependence of the ¹⁷O-line broadening was studied from 274.2 to 338.2 K.

MRI. All MRI data were collected at the Auburn University MRI Research Center on a Siemens Verio open-bore 3-T MRI clinical scanner. A 15-channel knee coil was used to simultaneously image 12–15 samples. The imaging procedure was identical with those used for similar studies from our laboratory.^{6–9,20} An inversion–recovery (IR) sequence was used that featured a nonselective adiabatic inversion pulse followed by a slice-selective gradient-recalled-echo (GRE) readout after a delay period corresponding to the inversion time (TI).^{21,22} The GRE was a saturation readout, such that only one

line of *k* space was acquired per repetition time (TR), in order to maximize both the signal strength and accuracy of the *T*₁ estimates. The specific imaging parameters were as follows: TR was set to 10 s, TI was varied from 10 to 2600 ms over 20 steps, the echo time (TE) was set to 2.75 ms, the flip angle equaled 90°, averages = 1, slice thickness = 10 mm, field of view = 64 × 64 mm, and matrix = 64 × 64, resulting in a pixel size of 1.0 × 1.0 × 10.0 mm. All samples were run in 50 mM solutions of HEPES in water, buffered to pH 7.0 and kept at 22 °C. The manganese content was systematically varied from 0.10 to 1.00 mM. The inverses of the *T*₁ values from two separate batches of contrast agent were plotted versus the concentration of Mn(II) to obtain *r*₁ values.

MRI Data Analysis. Image analysis was performed using custom *Matlab* programs (Mathworks, Natick, MA). The initial TI = 4.8 ms image was used as a baseline to determine circular region of interest (ROI) boundaries for each sample; from these, the mean pixel magnitudes for each ROI were calculated. For each of the 36 subsequent TI images, the same ROI boundaries were applied, and the mean pixel magnitude calculations were repeated. This gave consistent ROI spatial definitions and a corresponding time course of magnitudes for each of the samples over all of the TI time points. Each sample's complex phase was used to correct the magnitude polarity to produce a complete exponential *T*₁ IR curve. The Nelder–Mead simplex algorithm²³ was applied to each sample's exponential curve to estimate its corresponding *T*₁ value.

Preliminary Analysis of the Catalase Activity. In order to assess the ability of the Mn(II) complex to catalyze the degradation of H₂O₂, we reacted 100 nM **1** with 10 mM H₂O₂ in a 100 mM phosphate solution buffered to pH 7.0 and monitored the absorbance at 240 nm over time. H₂O₂ has a molar extinction coefficient of 39.4 M^{−1} cm^{−1} at this wavelength.²⁴ The consumption of H₂O₂ was evaluated using a Shimadzu UV-1601 spectrophotometer.

Synthesis. *1,8-Bis(2,5-dihydroxybenzyl)-1,4,8,11-tetraazacyclotetradecane (H₄qp4).* 1,4,8,11-Tetracyclotetradecane (cyclam; 1.00 g, 4.99 mmol) and 2,5-dihydroxybenzaldehyde (1.37 g, 9.91 mmol) were combined in 15 mL of dry MeOH. The mixture was heated at reflux for 4 h under N₂. The reaction mixture was then cooled to 0 °C with an ice bath. Once the temperature reached 0 °C, 20 mL of additional dry MeOH and NaBH₄/Al₂O₃ (10 wt %, 0.83 g, ~0.02 mol) were gradually added to the solution. The resultant solution was heated at reflux for 6 h under N₂ and then cooled to 0 °C. The residual reductant was titrated with 1 M HCl until the solution reached pH 8, depositing the crude product as a solid, which was then collected via filtration. The solid was dissolved in acetone and filtered. The acetone was rotavaped to yield the product as a yellow powder (842 mg, 38% yield). Typical yields range from 38 to 42%. ¹H NMR (400 MHz, DMSO-*d*₆, 297 K): δ 8.55 (s, 2H), 6.97 (s, 2H), 6.50–6.56 (m, 4H), 6.45 (s, 2H), 3.16 (s, 4H), 2.45–2.59 (m, overlap with the solvent peak), 2.28 (s, 4H), 1.69 (s, 4H), 1.14 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆, 297 K): δ 149.98, 149.17, 124.98, 118.02, 117.10, 115.48, 53.80, 53.11, 50.50, 49.26, 46.38, 24.71. MS (ESI). Calcd for MH⁺: *m/z* 445.2815. Found: *m/z* 445.2821.

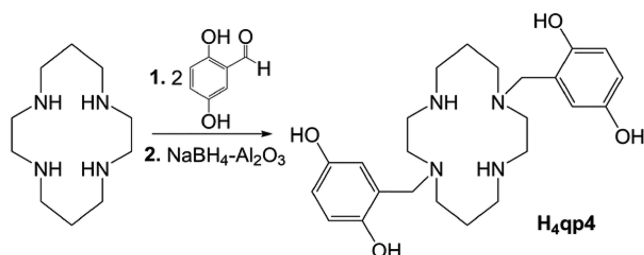
*[1,8-Bis(2,5-dihydroxybenzyl)-1,4,8,11-tetraazacyclotetradecane]manganese(II) triflate ([Mn(H₃qp4)](OTf), **1**).* H₄qp4 (500 mg, 1.12 mmol) and Mn(OTf)₂ (397 mg, 1.12 mmol) were dissolved in 5 mL of dried 1:1 MeCN/tetrahydrofuran (THF) under N₂. The mixture was stirred at 60 °C for 48 h; over this time, a green solid precipitated from the solution. The crude product was collected via filtration and washed with cold MeCN to yield the product as a green powder (577 mg, 76% yield). Typical yields range from 70 to 75%. MS (ESI). Calcd for [Mn(H₂qp4)]⁺: *m/z* 497.1961. Found: *m/z* 497.1931. Calcd for [Mn(H₂qp4)(OTf)]⁺: *m/z* 646.1481. Found: *m/z* 646.1470. Solid-state magnetic susceptibility (294 K): μ_{eff} = 5.6 μ_B. Optical spectroscopy (MeCN, 294 K): 300 nm (6800 M^{−1} cm^{−1}), 388 nm (3500 M^{−1} cm^{−1}). IR (cm^{−1}): 3282 (m), 3069 (w), 2852 (w), 1611 (w), 1511 (m), 1483 (m), 1361 (m), 1279 (s), 1191 (s), 1238 (s), 1212 (s), 1180 (s), 1150 (s), 1090 (m), 1060 (m), 1026 (s), 992 (m), 916 (m), 868 (m), 815 (s), 751 (m), 631 (s), 572 (m), 510 (m).

Elem. anal. Calcd for $C_{25}H_{35}N_4O_7F_3S_1Mn \cdot 1.5H_2O$ (powder): C, 44.51; H, 5.67; N, 8.30. Found: C, 44.27; H, 5.14; N, 8.09.

RESULTS

Synthesis and Nonaqueous Characterization. The H_4qp4 ligand is synthesized in one step from cyclam, 2 equiv of 2,5-dihydroxybenzaldehyde, and excess $NaBH_4/Al_2O_3$ (Scheme 2). The synthesis was inspired by that used to

Scheme 2. Synthesis of H_4qp4



prepare 1,8-bis(2-hydroxybenzyl)-1,4,8,11-tetraazacyclotetradecane ($H_2bcyclamb$), which features phenols in place of the quinol groups.²⁵ The preparation of H_4qp4 is complicated by the sensitivity of the bisaminal intermediate to air, necessitating that the addition of the quinols be done in a one-pot reaction rather than over two discrete steps. The one-pot reaction has the unexpected benefit of modestly improving the yield of H_4qp4 (38%); the overall yield of $H_2bcyclamb$ was 28%. The purity and identity of H_4qp4 were confirmed by NMR and HR-MS. We also crystallized the triflate acid salt of the ligand, $[H_6qp4](OTf)_2$, from MeOH (Figure 1).

A Mn(II) complex with H_4qp4 , $[Mn(H_3qp4)](OTf)$ (**1**), can be prepared by refluxing the ligand and $Mn(OTf)_2$ in 1:1 MeCN/THF for 2 days. The complexation reaction requires a much higher temperature and a much longer reaction time than the syntheses of $[Mn(H_2qp1)(MeCN)](OTf)_2$ (**2**) and **3**.^{7,8} The incorporation of metal ions into macrocycles often requires such measures. Complex **1** differs from previously isolated Mn(II) complexes with polydentate quinol-containing ligands in that it features a deprotonated quinol. Elemental analysis of powdered samples of **1** indicates that there is a single triflate per manganese. Both the EPR and magnetic susceptibility measurements indicate that the metal ion is high-

spin Mn(II). Because there is only one counteranion, this thereby necessitates a 1− charge on the polydentate ligand (H_3qp4^-). The presence of a quinolate is further supported by the presence of two bands in the UV/vis spectrum of the isolated product in MeCN (Figure S6). The feature at 304 nm is consistent with a neutral quinol; bands at similar energies are the sole UV/vis features observed above 250 nm for both **2** and **3** in MeCN.^{7,8} The additional band at 388 nm has an energy that is more consistent with a quinolate group; these have been observed for the H_2qp1 and H_4qp2 complexes in water.

Complex **1** is stable to air in the solid and solution states for prolonged periods of times. Samples of **1** in MeCN display negligible changes to their UV/vis features over a 12 h exposure to air (Figure S6). If solutions of **1** in either aqueous or organic solvents are kept under air for 1–2 weeks, the compound does eventually oxidize to $[Mn^{III}(H_2qp4)](OTf)$ (**4**), where H_2qp4^{2-} is the doubly deprotonated form of the ligand.

Structures of $[H_6qp4](OTf)_2$ and **4.** The crystal structure of the doubly protonated H_4qp4 ligand is shown in Figure 1A. The protonation state of the ligand was deduced from the 1:2 ligand/triflate ratio of the solid. Each additional proton bridges the two nitrogen atoms from a 1,2-ethanediamine portion of the macrocycle. One of the oxygen atoms from the nearest quinol is centered over each 1,2-ethanediamine moiety, with nearly equal distances between the oxygen atom and each nitrogen atom (2.94 and 2.95 Å).

Attempts to crystallize **1** have thus far been unsuccessful, but we crystallized **4** by slowly diffusing CH_2Cl_2 into a saturated solution of **1** in MeCN under air over 2 weeks (Figure 1). The reddish color of the crystals suggests that the manganese has been oxidized to either the 3+ or 4+ oxidation state. Single-crystal X-ray diffraction data unambiguously assign the metal center as Mn(III). The Mn–N and Mn–O bonds for **4** average 2.16 and 1.89 Å, respectively. Typical bonds between Mn(II) and neutral nitrogen donors are longer, ranging from 2.2 to 2.3 Å, and bonds between Mn(II) and even anionic oxygen donors usually exceed 2.0 Å.^{6–8,20,26–28} The Mn–N and Mn–O bond distances observed for **4** are instead more consistent with a Mn(III) ion bound to neutral nitrogen donors and anionic oxygen donors.²⁶ Additionally, the coordination complex displays a rhombic $[2+2+2]$ Jahn–Teller distortion, with pairs of short [Mn–O(1) and Mn–

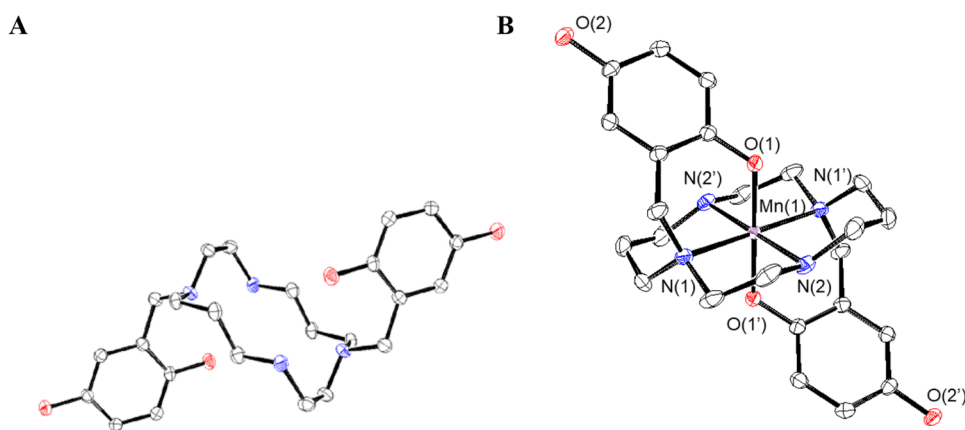


Figure 1. ORTEP representations of (A) $[H_6qp4]^{2+}$ and (B) $[Mn(H_2qp4)]^+$ (**4**). All hydrogen atoms, solvent molecules, and counteranions have been removed for clarity. All ellipsoids depict 50% probability.

Table 2. pMn, log K_{ML} , and p K_a Values Determined by Potentiometric Titration at 25 °C

p K_{L1} ^a	3.50 (±0.05)	log $K_{ML}[Mn(H_2qp4)]^c$	20.85
p K_{L2} ^a	7.70 (±0.05)	log $K_{ML}[Mn(H_3qp4)]^+^c$	18.22
p K_{L3} ^a	8.80 (±0.05)	log $K_{ML}[Mn(H_4qp4)]^{2+}^c$	14.52
p K_{L4} ^a	10.02 (±0.05)	pMn (pH 7.4) ^d	9.81
p $K_a[Mn(H_4qp4)]^{2+}^b$	5.09 (±0.05)		
p $K_a[Mn(H_3qp4)]^+^b$	7.39 (±0.05)		

^aLigand p K_a values: $K_{L1} = [H_5qp4^+][H^+]/[H_6qp4^{2+}]$; $K_{L2} = [H_4qp4][H^+]/[H_5qp4^+]$; $K_{L3} = [H_3qp4^-][H^+]/[H_4qp4]$; $K_{L4} = [H_2qp4^{2-}][H^+]/[H_3qp4^-]$. ^bMetal complex p K_a values: $K_a[Mn(H_4qp4)]^{2+} = [Mn(H_3qp4)]^+[H^+]/[Mn(H_4qp4)]^{2+}$; $K_a[Mn(H_3qp4)]^+ = [Mn(H_2qp4)][H^+]/[Mn(H_3qp4)]^+$. ^cMetal complex K_{ML} values: $K_{ML}[Mn(H_2qp4)] = [Mn(H_2qp4)]/([Mn(II)][H_2qp4^{2-}])$; $K_{ML}[Mn(H_3qp4)]^+ = [Mn(H_3qp4)]^+/([Mn(II)][H_3qp4^-])$; $K_{ML}[Mn(H_4qp4)]^{2+} = [Mn(H_4qp4)]^{2+}/([Mn(II)][H_4qp4])$. ^dpMn = $-\log [Mn(II)]_{free}$ calculated for $[Mn(II)] = 1.0$ mM, $[H_4qp4] = 1.0$ mM, 298 K, pH 7.4.

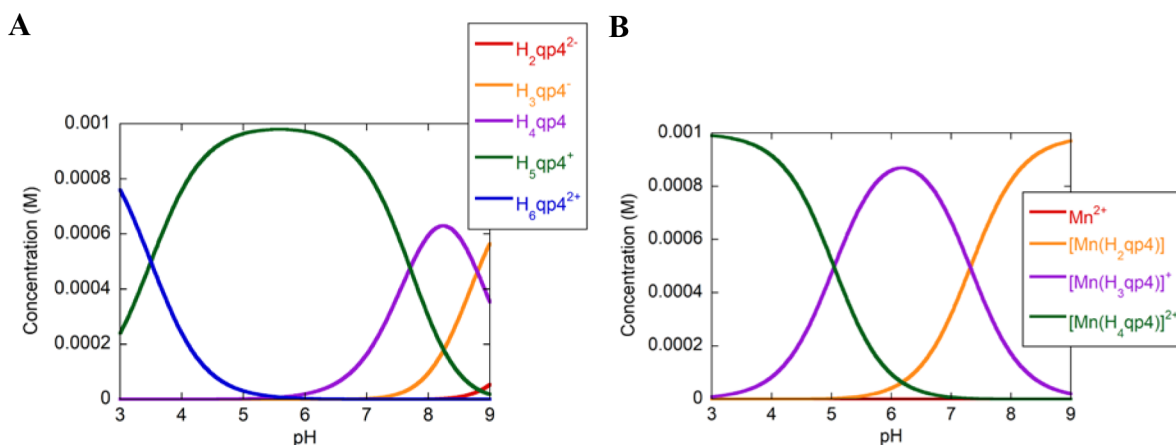


Figure 2. Predicted speciation as a function of the pH for (A) 1.0 mM H_4qp4 and (B) a 1:1 mixture of H_4qp4 and $MnCl_2$ in a 100 mM KCl solution.

O(1')), intermediate [Mn–N(2) and Mn–N(2')], and long [Mn–N(1) and Mn–N(1')] metal–ligand bonds.^{26,29} Such distortions would be anticipated for a high-spin d^4 electronic configuration but not for a d^3 metal ion. The metal center is therefore more likely to be Mn(III) than Mn(IV).

Stability and Speciation of 1 in Water. The speciation of the free H_4qp4 ligand was investigated from pH 3 to 9 (Figure S7). The best-fitting model to the potentiometric pH titration data is comprised of four ionization events corresponding to p K_a values of 3.5, 7.7, 8.8, and 10.0 (Figure S8 and Tables S1 and 2). Cyclam by itself is quadruply protonated under extremely acidic conditions, with two of the protons being retained from pH 2 to 10.³⁰ The H_4qp4 ligand appears to behave similarly, and the species at pH 3 is assigned as H_6qp4^{2+} . The ligand exists primarily as H_5qp4^+ at pH 7.0, with a considerable amount of H_4qp4 (Figure 2A). Traces of doubly deprotonated H_2qp4^{2-} , which would feature two quinolates, are seen at the basic end point of the titration.

The inclusion of a macrocycle into the ligand framework greatly stabilizes **1** in water relative to previously prepared contrast agents from our laboratory. The stability and speciation of **1** were assessed using potentiometric pH titration data acquired from pH 3 to 9. Our best-fitting model for the data suggests that there is negligible Mn(II) release from the ligand even at pH 3 (Figure S9, Figure 2B). The pMn at pH 7.4 with 1.0 mM of Mn(II) and ligand is calculated to be 9.81. Two ionization events are observed between pH 3.0 and 9.0. The associated p K_a values of 5.09 and 7.39 are consistent with the sequential deprotonation of two Mn(II)-bound quinols as the solution is made more basic.^{8,12,31} The calculated log K_{ML} values for Mn(II) bound to H_4qp4 , H_3qp4^- , and H_2qp4^{2-} all

exceed 14, with the binding affinity becoming stronger as the ligand deprotonates to more anionic forms.

The stability of the Mn(II)- H_4qp4 complex in water is confirmed by HPLC (Figures S10 and S11). The H_4qp4 ligand and **1** each give rise to single LC peaks with distinct retention times. Partial deprotonation of the metal-bound quinols in **1** at pH 7.00 is supported by UV/vis measurements. The spectrum of **1** in a 50 mM HEPES solution buffered to pH 7.00 displays an intense band at 388 nm that is absent in the spectrum for metal-free H_4qp4 under the same conditions (Figure S12). The energy of this new band is consistent with a phenolate or quinolate group.⁸ The assignment of the 5.09 and 7.39 p K_a values to deprotonation of the metal-bound quinols is also supported by a parallel spectrophotometric pH titration (Figure S13). The UV/vis spectrum of **1** changes markedly and continually as the pH rises from 4 to 9. Because Mn(II) does not generally support charge-transfer or d-d bands, these changes can be assigned to sequential deprotonation of the ligand's two quinols.

Complex **1** was electrochemically characterized by CV in an aqueous 50 mM phosphate solution buffered to pH 7.2. An irreversible feature with $E_{pa} = 1.25$ V versus Ag/AgCl was observed and assigned to the oxidation of the metal to Mn(III). In addition, we also detect a redox feature with $E_{1/2} = 100$ mV versus Ag/AgCl (295 mV vs NHE; Figure S14). The separation between the anodic and cathodic peaks (ΔE) is approximately 260 mV. Because redox processes with similar $E_{1/2}$ values were found for manganese and zinc complexes with polydentate quinol-containing ligands, we tentatively assign the 100 mV versus Ag/AgCl feature to the oxidation and reduction of the ligand rather than the manganese.^{7,8,32} The ΔE value for

the 100 mV redox event is larger than the 230 mV value measured for **3**; we attribute the poor reversibility of both features to the more extensive acid/base chemistry associated with having two, rather than one, quinol/quinolate groups in these coordination complexes.

The ability of **1** to interact with water was studied using variable-temperature ^{17}O NMR using the methodology pioneered by Gale et al. (Figure 3).¹⁹ The results at pH 7.4

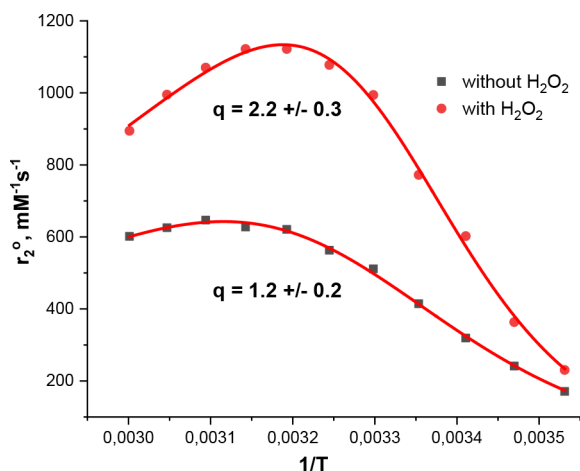


Figure 3. Plots of r_2^{O} as a function of the temperature for **1** before and after oxidation by H_2O_2 . Experimental conditions for the preactivated sensor (black squares): $[\text{I}] = 6.0 \text{ mM}$ in 60 mM HEPES buffered to pH 7.4 and 10% (v/v) of 10% $^{17}\text{OH}_2$, and $B = 9.4 \text{ T}$. Experimental conditions for the activated sensor (red circles): $[\text{I}] = 2.5 \text{ mM}$ in 60 mM MOPS buffered to pH 7.4 with 15 equiv of H_2O_2 and 10% (v/v) of 10% $^{17}\text{OH}_2$, and $B = 9.4 \text{ T}$. We began to acquire NMR data 15 min after the start of the oxidation reaction.

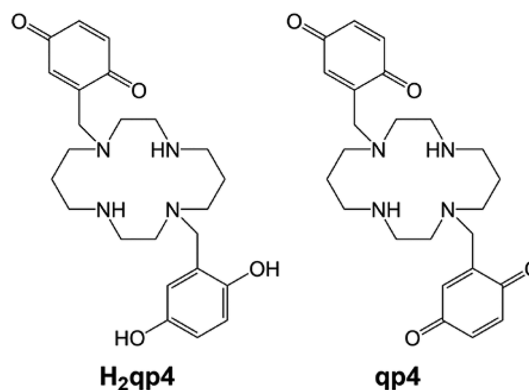
are consistent with $q = 1.2$ (Table 3). When **1** is dissolved in pH 7.4 water, the predominant species is therefore $[\text{Mn}(\text{H}_3\text{qp4})(\text{H}_2\text{O})]^+$, with the next most prevalent species being $[\text{Mn}(\text{H}_2\text{qp4})(\text{H}_2\text{O})]$. The calculated rate constant for water exchange at 298 K is $1.7 \times 10^7 \text{ s}^{-1}$, which is at the slower end of the range typically seen for Mn(II) complexes.^{8,19,34–40} $\Delta S^\ddagger$ is highly positive, consistent with a dissociate mechanism for water exchange at the metal center.

Stability of 1 to Air and Adventitious Metal Ions. Complex **1** does not display any noticeable short-term reactivity to air. The UV/vis spectrum of **1** in MeCN does not appreciably change over the course of a 12 h exposure to air (Figure S6). Both **2** and **3**, conversely, appear to oxidize slightly (5–10%) to manganese(II) *p*-quinone complexes under the same conditions. O_2 does eventually oxidize **1**, with the Mn(III)-containing **4** depositing over 1–2 weeks (Figure 1B).

Complex **1** differs from **2** and **3** in that it strongly resists metal-ion exchange. The reaction between 0.1 mM $\text{Fe}(\text{ClO}_4)_2$

and 0.1 mM **1** in either MeCN or buffered water does not yield UV/vis-detectable quantities of $[\text{Fe}(\text{H}_3\text{qp4})]^+$, even at 18 h (Figure S15). The changes to the UV/vis spectrum of **1** over this time are negligible. Complex **2**, conversely, slowly exchanges Fe(II) for Mn(II) in MeCN, with approximately 10% of the Mn(II) being displaced by an equimolar amount of Fe(II) by 15 h.⁷ Complex **3** is the most susceptible of the three manganese(II) quinol complexes to metal-ion displacement, and 80% of its Mn(II) is displaced by an equimolar amount of Fe(II) by 3 h.⁸ Both the $\text{H}_2\text{qp1}$ and $\text{H}_4\text{qp2}$ complexes with Mn(II) react readily with Zn(II), with the strong diamagnetic ^1H NMR features of the Zn(II)- $\text{H}_2\text{qp1}$ and Zn(II)- $\text{H}_4\text{qp2}$ complexes appearing within 2 h.^{7,8} The reactions between 20 mM $\text{Zn}(\text{ClO}_4)_2$ and 10 mM **1** in CD_3CN or D_2O , however, fail to dislodge Mn(II) from the ligand, as assessed by ^1H NMR (Figure S16). The ^1H NMR spectra of the reactions are featureless aside from solvent peaks even 24 h after the introduction of the Zn(II). When 20 mM H_2O_2 is added to a mixture of 20 mM $\text{Zn}(\text{ClO}_4)_2$ and 10 mM **1**, Mn(II) likewise remains in the oxidized forms of the ligand ($\text{H}_2\text{qp4}$ and qp4 , Scheme 3), as assessed by ^1H NMR.

Scheme 3. Oxidized Forms of the $\text{H}_4\text{qp4}$ Ligand



Reactivity between 1 and H_2O_2 . Upon reaction with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), the quinols in **1** appear to be partially oxidized to *p*-quinones, as assessed by MS, UV/vis, and IR (Scheme 3 and Figures S17–S19). A new IR feature at 1656 cm^{-1} provides strong evidence for formation of the C=O bonds associated with *p*-quinone.

When **1** reacts with a large 10 mM excess of H_2O_2 , MS reveals m/z features similar to those seen for oxidation of the Mn(II) complex by DDQ, suggesting that the quinols are likewise converted to *p*-quinones by this oxidant (Figure S20). Further investigation reveals that the speed of the reaction between **1** and excess H_2O_2 is significantly slower than analogous reactions with **2** and **3**.^{7,8} Curiously, the changes to the optical spectra during the first 30 min are slight, consistent with an induction period (Figure 4A). The reaction then

Table 3. Water-Exchange Activation Parameters Obtained for Quinol-Containing Mn(II) Complexes **1–3**

parameter	1 (pH 7.4)	1 (pH 7.4) after oxidation	2 (pH 7.4) ^a	3 (pH 7.0) ^b
q	1.2 (± 0.2)	2.2 (± 0.3)	0.7 (± 0.2)	0.9 (± 0.2)
$k_{\text{ex}}^{298} (\text{s}^{-1})$	$1.7 (\pm 0.1) \times 10^7$	$1.6 (\pm 0.4) \times 10^7$	$6.9 (\pm 0.6) \times 10^7$	$4.9 (\pm 1.4) \times 10^6$
$\Delta H^\ddagger (\text{kJ mol}^{-1})$	45 (± 2)	50 (± 9)	24.2 (± 1.0)	22.0 (± 1.7)
$\Delta S^\ddagger (\text{J K}^{-1} \text{mol}^{-1})$	43 (± 6)	62 (± 11)	−13.1 (± 3.4)	−41 (± 2)

^aFrom ref 33. ^bFrom ref 8.

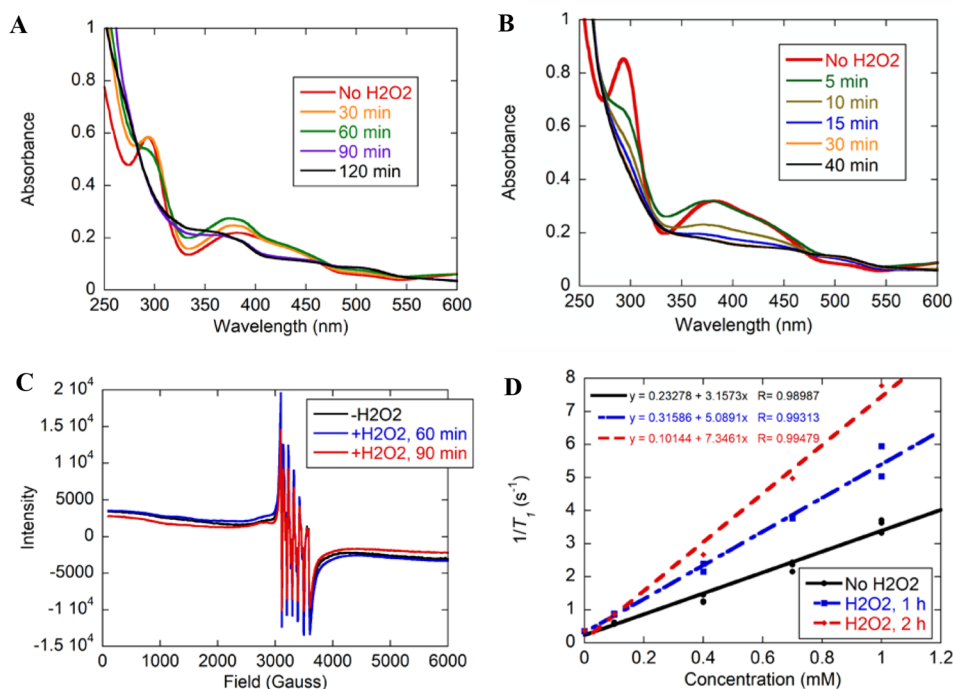


Figure 4. Response of **1** to H₂O₂. (A) UV/vis spectra acquired during a reaction between 0.07 mM **1** and 10 mM H₂O₂ in 50 mM HEPES buffered to pH 7.00 at 298 K. (B) UV/vis spectra acquired during a reaction between 0.10 mM **1** and 0.60 mM H₂O₂ in 50 mM HEPES buffered to pH 7.00 at 298 K. (C) X-band EPR spectra of 1.0 mM solutions of **1** in 50 mM HEPES buffered to pH 7.00 in the absence and presence of 10 mM H₂O₂. The reactions between **1** and H₂O₂ proceeded for 60 min (blue) and 90 min (red) before the samples were frozen and analyzed at 77 K. (D) Plots of 1/T₁ versus Mn(II) concentration for **1** in the presence (blue, red) and absence (black) of 10 mM H₂O₂. All samples were run in 298 K aqueous solutions containing 50 mM HEPES buffered to pH 7.00, using a 3 T field provided by a clinical MRI scanner. All samples were prepared under air. The oxidized samples were prepared by directly adding H₂O₂ to solutions of **1** in aqueous solutions buffered to pH 7.0. Two sets of oxidation reactions were allowed to proceed for 60 min at 298 K before T₁ was measured (blue). A third set of oxidation reactions was allowed to proceed for 120 min before data acquisition. The data were fit to the indicated linear equations. The y intercepts were within error of 1/T₁ measurements associated with two control samples that contained no Mn(II): pure water (0.35 s⁻¹) and 50 mM HEPES buffer (0.34 s⁻¹).

accelerates and finishes by 90 min. The most noticeable optical changes are the disappearance of the peaks at 293 and 388 nm; features with similar energies have been observed for **2** and **3** and assigned to intraligand transitions for the quinol and quinolate moieties.^{7,8} When a smaller amount of H₂O₂ (0.6 mM) is added to **1**, the quinol peak decreases immediately, with the reaction completing in 20 min (Figure 4B), indicating that the induction period only occurs when H₂O₂ is present in a large excess.

When the reaction between 1.0 mM **1** and 10 mM H₂O₂ in a pH 7.0 HEPES buffer is monitored by EPR, the spectrum taken before the oxidant was added looks nearly identical with those taken at 60 and 90 min, suggesting that the manganese largely remains in the 2+ oxidation state throughout the reaction (Figure 4C). As with prior quinol-containing H₂O₂ sensors from our laboratory, not all of the quinols are oxidized to *p*-quinones by excess H₂O₂.^{7,8} The Mn(II) exists as a mixture of unreacted **1**, [Mn(Hqp4)]⁺, and [Mn(qp4)]²⁺, where Hqp4⁻ and qp4 correspond to the mono-*p*-quinone/quinolate and di-*p*-quinone oxidized forms of the ligand. Oxidation of the quinols is potentially reversible. When **1** is oxidized by H₂O₂ in MeOH, we can regenerate the H₃qp4⁻ complex by subsequently adding sodium dithionite (Figure S21).

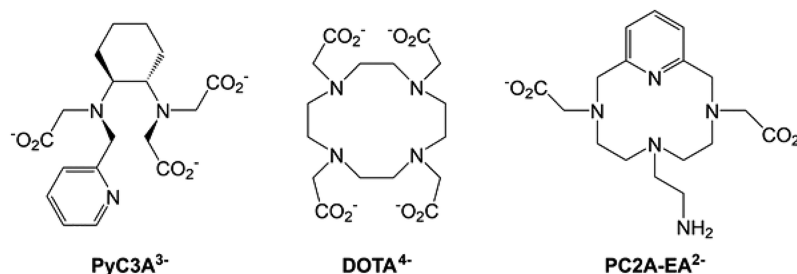
Upon oxidation by H₂O₂, the aquation number of the Mn(II) center in **1** increases by approximately one water molecule, resulting in an average of 2.2 water molecules binding to each metal center (Figure 3 and Table 3). The rate

of water exchange does not significantly change, with the rate constant being identical within error with that measured before oxidation. ΔS[‡] for water exchange remains highly positive after the reaction with H₂O₂, suggesting that this process still occurs through a dissociative pathway.

MRI Properties. The relaxivities of complex **1** before and during its reaction with H₂O₂ were assessed using a 3 T MRI scanner (Figure 4D). T₁ values were measured for aqueous solutions containing 0–1.0 mM **1** and 50 mM HEPES buffered to pH 7.00. For each concentration of **1**, an oxidized sample was also prepared by adding 10 mM H₂O₂; the large excess of oxidant was used to ensure as full a turn-on as possible at the time points and to facilitate comparisons to manganese-containing MRI contrast agents that were previously prepared and characterized by our laboratory.^{6–9,20} The T₁ values for the H₂O₂-containing solutions were measured 1 and 2 h after the reagents were initially mixed. Full series of data were collected for two independently prepared batches of **1** in order to confirm that the results were replicable.

Relaxivities (r₁) were obtained from the slopes of plots of 1/T₁ versus manganese concentration. Prior to oxidation by excess H₂O₂, r₁ was 3.16 mM⁻¹ s⁻¹. The data taken for samples kept under air for 1 and 2 h overlay well, suggesting that the short-term reactivity with air is slight. After oxidation by H₂O₂, the relaxivity rises, reaching 5.09 mM⁻¹ s⁻¹ by 1 h and peaking at 7.35 mM⁻¹ s⁻¹, as assessed by measurements taken at 2 h (Figure 4D). The pH dependence of the relaxivity was investigated from pH 3 to 9 (Figure S22). The r₁ values were

Scheme 4. Other Mn(II)-Binding Ligands Used in MRI Contrast Agents



measured for a 0.50 mM sample of **1**. r_1 is highest at pH 3 and lowest at pH 7. As the pH increases, r_1 drops from pH 3 to 4, remains approximately constant from pH 4 to 6.5, decreases from pH 6.5 to 7, and then rises slightly from pH 7 to 9.

DISCUSSION

Both H₄qp4 and **1** were prepared in straightforward manners using techniques slightly modified from previously successful procedures. The synthesis for a closely related cyclam derivative with two phenols appended to two of the amines was done in two distinct steps,²⁵ but the heightened air sensitivity of the quinols under basic conditions led us to explore and eventually adopt a one-pot reaction. Once the ligand was prepared, the installation of Mn(II) into the macrocycle proceeded cleanly using protocols commonly used for other macrocycle complexation reactions.^{41–44} Unexpectedly, Mn(II) is bound to the singly deprotonated ligand H₃qp4[−], rather than neutral H₄qp4, in the solid isolated from precipitation of the Mn(II) complex from organic solvents. Analogous complexes with H₂qp1 and H₄qp2 (Scheme 1), **2** and **3**, contain exclusively quinols rather than quinolates.^{7,8} We speculate that residual metal-free H₄qp4 may serve as the base that deprotonates the metal-bound ligand. Although we have not isolated the protonated ligand byproduct(s), we feel that this is a likely explanation for deprotonation of the Mn(II) complex because of the ligand's high affinity for protons and the ~75% yield of **1**, which would provide enough residual ligand to serve as a base.

As anticipated, **1** contains a high-spin Mn(II) metal center, as evidenced by EPR, UV/vis, and solid-state magnetic susceptibility data. The UV/vis features correspond to intraligand transitions for the quinol and quinolate groups; these provide convenient spectroscopic signatures to follow the oxidation state of the ligand.^{7–9}

Complex **1** is much more thermodynamically stable in water than the previously characterized **3**, which has a similar coordination sphere around the metal center in aqueous solution.⁸ The pMn value measured at pH 7.4 with 1.0 mM concentrations of the ligand and Mn(II) is 9.81, which represents over 4 orders of magnitude of improvement over the 5.36 value measured for **3**.⁸ The gains in stability can be attributed largely to macrocyclic effects because H₄qp2 and H₄qp4 provide similar coordination spheres: four neutral nitrogen donors in addition to the two quinols/quinolates. Complex **1** is also more stable than **2**, which has a pMn of 7.25 under our standard conditions.³³

The stability of **1** also exceeds those of most other reported mononuclear Mn(II)-containing MRI contrast agents.^{9,14,37,39,40,45–48} The Gale and Caravan groups have recently reported a series of linear ligands with multiple carboxylate donors that coordinate tightly to Mn(II).^{37,46} One

representative example, PyC3A^{3−}, forms a Mn(II) complex with a log K_{ML} of 14.14 (Scheme 4).³⁷ The H₆qc1 ligand (Scheme 1) was inspired by this work, and the triply deprotonated H₃qc1^{3−} forms a Mn(II) complex with a log K_{ML} of 15.59.⁹ The log K_{ML} values for the Mn(II) complexes with H₂qp4^{2−} and H₃qp4[−] both exceed 18, demonstrating that the macrocycle can stabilize Mn(II) complexes more efficiently than more highly anionic linear ligands. The DOTA^{4−} ligand represents a combination of these strategies in that it is comprised of four carboxylates tethered to a cyclen framework (Scheme 4). The log K_{ML} value for its Mn(II) complex (19.89) is higher than the 18.22 value for H₃qp4[−] but less than the 20.85 value measured for H₂qp4^{2−}.^{47,48} The 19.01 log K_{ML} value for the Mn(II) complex with the macrocyclic PC2A-EA^{2−} (Scheme 4) is also similar to that of **1**.⁴⁰

Inclusion of the cyclam into the ligand also endows **1** with a high level of kinetic stability as assessed by metal competition experiments. Once in the macrocycle, Mn(II) cannot be readily displaced by either Fe(II) or Zn(II), two of the most common transition-metal ions in biology. Complexes **2** and **3**, conversely, gradually react with equimolar amounts of free Fe(II) and quickly react with Zn(II). The inertness of **1** toward metal-ion exchange compares well to those of other Mn(II)-containing MRI contrast agents with macrocyclic organic components.^{14,40,44} The kinetic stability extends to Mn(II) complexes with oxidized forms of the H₄qp4 ligand. Oxidation by H₂O₂ yields a mixture of Mn(II) complexes with the monoquinol/monoquinone and diquinone ligands H₂qp4 and qp4 (Scheme 3). ¹H NMR analysis of the reaction between these oxidized complexes and Zn(II) reveals that Zn(II) does not noticeably displace Mn(II). The oxidized forms of complex **3**, conversely, exchange Zn(II) for Mn(II) under these conditions, leading to intense diamagnetic peaks when visualized by NMR.⁸

Like **2** and **3**, **1** is a redox-active T_1 -weighted MRI contrast agent. Curiously, the preoxidation relaxivity of **1** (3.15 mM^{−1} s^{−1}) is much lower than that of **3** (5.46 mM^{−1} s^{−1}), despite the two compounds having similar coordination spheres and acid/base behavior. Both r_1 values are the weighted averages of the relaxivities of the individual Mn(II) species present at pH 7.00: [Mn(H₃qp4)(H₂O)]⁺ and [Mn(H₂qp4)(H₂O)] in the case of **1**. The average aquation number measured for **1** is slightly higher than that of **3**, but this would be anticipated to raise rather than lower the r_1 . The pK_a values for the two Mn(II)-bound quinols in **1** and **3** are also similar: 5.82 and 7.14 for **3** versus 5.09 and 7.39 for **1**. The higher pK_a value in each pair would be anticipated to modulate the rate of proton transfer.⁴⁹ The slightly more basic quinolate in **1** could slow the rate of proton transfer between the manganese(II) quinols and the bulk water enough to substantially reduce its contribution to the measured r_1 , but further studies are needed to fully

elucidate that possible relationship. The rate constants for water exchange at 298 K differ the most, with the k_{ex} value measured for **3** ($4.9 \times 10^6 \text{ s}^{-1}$) being approximately a third of that of **1** ($1.7 \times 10^7 \text{ s}^{-1}$).⁸ Complex **1**, unlike **3**, appears to exchange water molecules through a dissociative mechanism, as evidenced by the highly positive entropy of activation (Table 3).

The relaxivity measured for nonoxidized **1**, like that of **3**, is higher under acidic conditions (Figure S22).⁸ This may be consistent with water molecules displacing the quinol portions of the ligand from the metal. Alternatively, protonation of the quinolate groups under more acidic conditions may increase the relaxivity by enabling more extensive exchange with the protons from the bulk water. As the pH becomes more basic, the metal-bound quinols deprotonate to anionic quinolates that can outcompete water for coordination sites on the metal center.

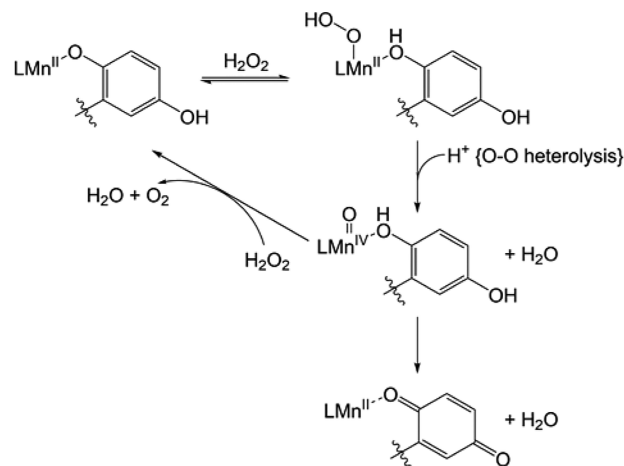
In addition to the higher stability, the inclusion of cyclam in the ligand framework has the nonintuitive benefit of improving the relaxivity response to H_2O_2 . The 130% increase in r_1 is approximately quadruple the percentile increase observed for **3**.⁸ Complex **3** rapidly destabilizes as the pH drops below 7.0 because of the protonation of manganese(II) quinolates, and it is reasonable to assume that oxidation of the quinols to *p*-quinones likewise weakens the binding affinity of the ligand enough to trigger release of the Mn(II). The H_2O_2 -enhanced r_1 for **3**, however, cannot be attributed to release of the metal. The r_1 of $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}$ was measured independently ($5.26 \text{ mM}^{-1} \text{ s}^{-1}$) and found to be approximately equal to that of the preactivated form of **3** ($5.46 \text{ mM}^{-1} \text{ s}^{-1}$).^{8,20} Although one may expect $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}$ to have a higher r_1 because of its higher aquation number, this is not observed. Proton exchange between water molecules and the Mn(II)-bound quinols/quinolates would be anticipated to markedly increase the r_1 values of **1–3** relative to $q = 1$ systems that lack metal-bound hydroxyl groups from their organic ligands.⁴⁹ Counter-intuitively, Mn(II)-ion release from the oxidized forms of **H₄qp2** may actually curtail the response of **3** to H_2O_2 .

If **1** were to release free Mn(II) upon oxidation, we would anticipate an increase in r_1 due to the lower starting value, but this mechanism is not consistent with our data. Our NMR measurements indicate that the oxidation of **1** by H_2O_2 does not release significant amounts of Mn(II). Further, the maximum r_1 exceeds that of $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}$. We instead believe that the increase in relaxivity results from the formation of more highly aquated Mn(II) species with the oxidized forms of the ligand. Because the H_2O_2 reaction does not make either $[\text{Mn}(\text{Hqp4})(\text{H}_2\text{O})_x]^+$ or $[\text{Mn}(\text{qp4})(\text{H}_2\text{O})_x]^{2+}$ cleanly, we unfortunately cannot measure the individual r_1 values of these two species and ascertain their contributions to the overall relaxivity. Oxidation by H_2O_2 does improve the aquation of the metal center, as evidenced by variable-temperature ^{17}O NMR measurements (Table 3 and Figure 3). On average, the metal centers coordinate one additional water molecule after oxidation.

Complex **1** differs substantially from **2** and **3** in that its response to H_2O_2 depends on the manner in which the oxidant is administered. When a large excess of H_2O_2 is added to **1** in a single portion, oxidation of the quinols occurs after an induction period. The oxidation by 10 mM H_2O_2 takes approximately 90 min to complete, as assessed by changes to both the relaxivity and UV/vis spectrum. When a smaller portion of H_2O_2 is added, the quinols are oxidized more

quickly and without a noticeable induction period. On the basis of these observations, we propose that the initial reaction between H_2O_2 and **1** generates an oxidant that can react either unimolecularly to oxidize one of the quinols to *p*-quinone or bimolecularly with another equiv of H_2O_2 to yield O_2 and regenerate **1** (Scheme 5). In high concentrations of H_2O_2 , the

Scheme 5. Proposed Competing Catalase and Quinol Oxidation Pathways



second pathway dominates, and the manganese complex primarily acts as a catalase mimic. Complexes **2** and **3**, conversely, quickly react with excess H_2O_2 to yield manganese(II) *p*-quinone complexes, and these reactions do not feature induction periods. We speculate that efficient oxidation of quinols to *para*-quinones may require that the quinols be *cis* to the H_2O_2 -derived ligand in a transient higher-valent intermediate, which we depict as a manganese(IV) oxo complex in Scheme 5. If the macrocycle coordinates the manganese in a square-planar fashion as it does to the Mn(III) in **4** (Figure 1B), an incoming H_2O_2 will likely displace a quinol/quinolate, forcing the H_2O_2 -derived ligand to be *trans* to the remaining metal-bound quinol/quinolate. This would hinder intramolecular oxidation and enable bimolecular reactions with additional equivalents of H_2O_2 to proceed. A similar hindrance of intramolecular quinol oxidation may also explain the reaction between **1** and O_2 . The product of this reaction is **4** (Figure 1B) rather than a Mn(II) complex with a *p*-quinone-containing ligand, the latter of which might be anticipated to be more thermodynamically stable based on the electrochemistry (Figure S14).

Preliminary data suggest that **1** can indeed catalyze the degradation of H_2O_2 . When reactions between 100 nM **1** and 10 mM H_2O_2 are followed spectrophotometrically, the absorbance of the H_2O_2 peak at 240 nm decreases quickly (Figure S23). From these data, the initial rate, $v_0/[1]_T$, was estimated to be $6.6 (\pm 2.3) \times 10^3 \text{ s}^{-1}$.

Biological environments generally provide low but rapidly replenishing concentrations of H_2O_2 that are closer to 0.6 mM than 10 mM.^{50–52} We therefore anticipate that **1** would provide a fast r_1 response to physiologically generated H_2O_2 that is more similar to that displayed in Figure 4B than in Figure 4A. Even with this in mind, it is uncertain whether the sensor will respond quickly enough to an oxidant to activate before circulating out of an area with elevated levels of H_2O_2 . Other challenges exist in translating probes such as **1** to the

clinic. The nonratiometric response of **1**, for instance, makes it difficult to distinguish regions under oxidative stress from sites that merely accumulate more of the preactivated sensor. Although **2** and **3** seem to be reasonably tolerated by cells,^{7,8} the potential toxicity of **1** also needs to be considered and assessed.

CONCLUSIONS

A Mn(II) complex with a cyclam macrocycle derivatized with two quinols is a highly water-stable MRI contrast agent that displays a positive r_1 response to H_2O_2 , one of the most prevalent ROS in biology. The percentile relaxivity response is approximately four times that of a Mn(II) complex with a linear ligand that provides a similar set of donor atoms: two quinols/quinolates and four neutral nitrogen donors. In addition to boosting the thermodynamic stability, the macrocycle also provides a kinetic barrier for metal-ion dissociation, and the Mn(II) complex appears to retain the metal after oxidation of the quinols to more poorly metal-binding *p*-quinones. The greater stability of the current complex addresses a key concern about other quinol-containing MRI contrast agent sensors that were previously prepared by our laboratory and should smooth the pathway toward using such complexes to monitor biologically relevant oxidative stress.

The reactivity with H_2O_2 appears to proceed through two competing pathways. With low H_2O_2 levels, intramolecular oxidation of the quinols to *p*-quinones occurs. This process appears to be slow relative to analogous reactions seen with manganese complexes with linear quinol-containing ligands. With high—and physiologically unrealistic— H_2O_2 concentrations, catalase activity is observed. Under such conditions, quinol oxidation and the concomitant increase in r_1 only occur after much of H_2O_2 has been consumed.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.inorgchem.1c00896>.

NMR, IR, UV/vis, HPLC, and MS data for H_4qp4 and **1**, pH titration data for H_4qp4 and **1**, metal-exchange data, spectroscopic and MS data for the reaction between **1** and DDQ, variable-temperature r_1 measurements, and preliminary kinetic data for H_2O_2 degradation (PDF)

Accession Codes

CCDC 2071437 and 2071438 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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