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Communication

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A Magnetic Resonance Imaging Contrast Agent Capable of Detecting Hydrogen Peroxide

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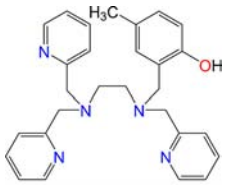
Supporting Information

ABSTRACT: The redox-active ligand *N*-(2-hydroxy-5-methylbenzyl)-*N*,*N*′,*N*′-tris(2-pyridinylmethyl)-1,2-ethanediamine (Hptp1) was prepared and complexed to manganese(II). The isolated $[Mn(Hptp1)(MeCN)]^{2+}$ serves as a magnetic resonance imaging contrast agent, with an r_1 value comparable to those of other mononuclear gadolinium(III) and manganese(II) complexes. The metal and ligand are stable in aerated aqueous solutions, but the addition of H_2O_2 causes the complex to oxidatively couple to itself through a bimolecular reaction involving the phenol groups of two Hptp1 ligands. The binuclear product is less paramagnetic per manganese(II) than its mononuclear precursor, lowering the measured r_1 per manganese(II). The manganese(II) complex with Hptp1 can thereby serve as a sensor for oxidative stress.

Reactive oxygen species (ROSs) have been implicated in a seemingly myriad array of health disorders, including several major cardiovascular and neurological diseases.[1−6] Probing the role of ROSs in these conditions currently depends heavily on indirect methods, such as post-mortem analysis of protein oxidation. Most probes capable of directly detecting ROSs rely on changes in fluorescence or luminescence as a signal.[7−14] Although these probes offer high spatial resolution, the short wavelengths of excitation associated with most fluorophores render them unsuitable for imaging within intact organs or whole bodies. Magnetic resonance imaging (MRI), conversely, uses much longer wavelengths and is often used to visualize softer tissues within patients. In practice, a paramagnetic contrast agent is often administered to shorten the T_1 relaxation time of nearby water protons excited by radio-frequency pulses, thereby improving the signal quality. The ability to increase these relaxation rates is defined as the relaxivity of the contrast agent. Many recently reported contrast agents have been designed to convert into species with different relaxivities upon reaction with a target analyte, such as an enzyme or a metabolite.[15−22] One previously reported target for these sensors is myeloperoxidase (MPO), which is involved in ROS metabolism.[20−22] Another recently developed MRI contrast agent responds to the reductant β-NADH.[23]

In our efforts to develop a MRI contrast agent capable of more directly detecting the oxidative stress associated with ROSs, we prepared the redox-active ligand *N*-(2-hydroxy-5-methylbenzyl)-*N*,*N*′,*N*′-tris(2-pyridinylmethyl)-1,2-ethanediamine (Hptp1; Scheme 1) and its manganese(II) complex. The ligand is prepared in one step from the reaction between 2-hydroxy-5-methylbenzaldehyde and the previously reported *N*,*N*,*N*′-tris(2-pyridinylmethyl)-1,2-ethanediamine.[24] The yield of the Hptp1 ligand is 60% after purification by column chromatography. The subsequent complexation reaction between Hptp1 and $Mn(ClO_4)_2$ in anaerobic acetonitrile (MeCN) provides $[Mn(Hptp1)(MeCN)](ClO_4)_2$ (**1**) in 90% yield.

Scheme 1



Crystalline **1** can be obtained from the slow diffusion of ethyl ether into a MeCN solution of the manganese(II) complex (Figure 1). The crystal structure reveals that the manganese is heptacoordinate, with six donor atoms originating from the Hptp1 ligand. The overall geometry may be best described as a distorted pentagonal bipyramid, with the phenol and one of the

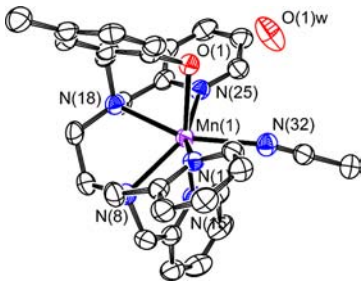


Figure 1. ORTEP representation of $[Mn(Hptp1)(MeCN)]^{2+}$. All hydrogen atoms, both ClO_4^- counteranions, and two solvated MeCN molecules have been omitted for clarity. All thermal ellipsoids are drawn at 50% probability. Further details about the structure are reported in the Supporting Information.

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pyridine rings in the axial positions. The heptacoordination and the metal−ligand bond distances are consistent with a 2+ oxidation state for the manganese. This assignment is corroborated by the light color of the crystals and the 5.8 μ_B magnetic moment measured for the solid.

Unexpectedly, the phenol appears to retain its proton upon coordination, as assessed by the Mn−O and C−O bond lengths and the anion count. The 1.39 Å C−O bond length closely matches the 1.38 Å value for phenol.[25] The phenol oxygen atom is 2.60 Å away from the oxygen atom of an outer-sphere H_2O molecule, suggesting a hydrogen-bonding interaction between the two. This hydrogen bond may stabilize the proton's continued presence on the phenol. Two hydrogen atoms were located on the water, precluding the alternative assignment of an outer-sphere hydroxide anion and a deprotonated ligand ($ptp1^-$).

Solutions of **1** are stable when exposed to air at 25 °C in both MeCN and H_2O. Under these conditions, no discoloration of the samples is observed over 24 h. The aqueous stability of the Hptp1−Mn^{II} bonds is confirmed by ^1H NMR and electron paramagnetic resonance (EPR). No ligand resonances are observed in the spectrum of **1** in D_2O, and the EPR spectrum of **1** in water is distinct from that of $[Mn(H_2O)_6]^{2+}$. log K for the binding of Hptp1 to Mn^{II} was determined from a competitive binding assay with *N*,*N*,*N*′,*N*′-tetrakis(2-pyridinylmethyl)ethylenediamine (log K = 10.3)[26] and was found to be 10.6. A more strongly binding ligand, such as ethylenediaminetetraacetate, will remove Mn^{II} from **1** instantaneously. Our preliminary results suggest that other metal ions do not rapidly substitute for Mn^{II} in the Hptp1 complex. In MeCN, 1 equiv of Fe^{II} does not displace a noticeable amount of Mn^{II} from **1** over the course of 1 h. At 18 h, only 17% of the Mn^{II} has been displaced, as assessed by UV/vis spectroscopy. It should be noted that the 0.10 mM concentration of free iron added at the beginning of this experiment is much higher than biologically relevant levels.

The $[Mn(Hptp1)(MeCN)]^{2+}$ complex is a capable MRI contrast agent, with a measured relaxivity of 4.39 mM^{-1} s^{-1} in buffered aqueous solutions (3 T, 50 mM HEPES, pH 7.00, 25 °C). This r_1 value is relatively high for a mononuclear manganese(II) complex[27−29] but is lower than that measured for $[Mn(H_2O)_6]^{2+}$ under identical conditions.[29] Upon dissolution in water, a molecule of H_2O likely displaces the MeCN, resulting in an aquation number q = 1. We speculate that the observed ability of the coordinated phenol to exchange protons with and hydrogen bond to outer-sphere H_2O molecules may further increase the r_1 value of complex **1** relative to those of other mononuclear manganese(II) complexes.

Upon the addition of H_2O_2 to solutions of **1** in either MeCN or H_2O, their color changes to brown briefly before reverting back to pale yellow. These changes, which finish within seconds, are consistent with the temporary oxidation of the manganese. Mass spectrometric (MS) analysis of the solutions reveals m/z peaks consistent with a binuclear manganese complex. This species was subsequently crystallized from the slow evaporation of an MeCN/MeOH mixture (Figure 2) and identified as $[Mn_2(Hptp1_2)(MeCN)_2](ClO_4)_3$ (**2**). The crystal structure reveals that the binuclear product results from the formation of a novel covalent bond between the 2-position carbon atoms of the phenol groups from two $[Mn(Hptp1)(MeCN)]^{2+}$ ions (Scheme 2). Such oxidative coupling of phenols has been observed in several transition-metal systems,

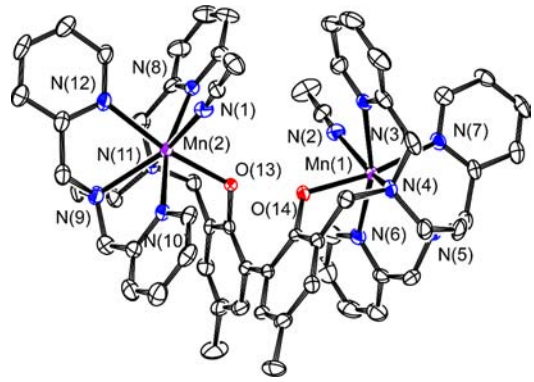
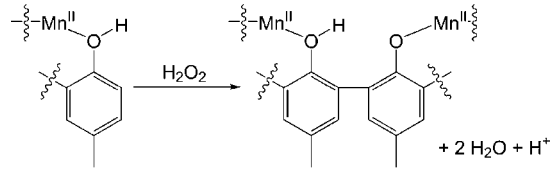


Figure 2. ORTEP representation of $[Mn_2(Hptp1_2)(MeCN)_2]^{3+}$. All hydrogen atoms, ClO_4^- counteranions, and noncoordinated solvent molecules have been omitted for clarity. All thermal ellipsoids are drawn at 50% probability. Additional information about the structure is reported in the Supporting Information.

Scheme 2



including some that contain manganese.[30,31] A gadolinium(III)-containing sensor reported by Weissleder et al. uses similar phenolic coupling chemistry to detect H_2O_2.[22] Weissleder et al.'s probe differs from ours in that it requires MPO to catalyze the coupling. The present manganese(II) complex, conversely, does not need this coanalyte.

The coordination around each manganese ion in **2** is similar to that in the mononuclear precursor. Each manganese ion in $[Mn_2(Hptp1_2)(MeCN)_2]^{3+}$ is heptacoordinate, and the averages of the metal−ligand bond distances for both **1** and **2** are 2.35 Å. These structural observations and the pale-yellow color of the crystals are consistent with a 2+ oxidation state for both metal ions. The bonds between the Mn^{II} ions and the phenol oxygen atoms contract by over 0.2 Å upon oxidation, consistent with deprotonation of at least one of the phenols. The IR spectrum of **2** has a feature at 3420 cm^{-1}, suggesting that the diphenol bridge within the coupled ligand retains a proton. The ligand is therefore best assigned as $Hptp1_2^-$. The C−O bonds in $[Mn_2(Hptp1_2)(MeCN)_2]^{3+}$ are both 1.36 Å, which, although slightly shorter than the 1.39 Å C−O bond in $[Mn(Hptp1)(MeCN)]^{2+}$, are atypical bond lengths for a fully deprotonated phenolate. We therefore believe that the proton in $Hptp1_2^-$ spans the two phenolic oxygen atoms.

When 10 mM H_2O_2 is added to 0.10−1.00 mM solutions of **1** in buffered aqueous solutions (3 T, 50 mM HEPES, pH 7.00, 25 °C), the r_1 per manganese(II) decreases from 4.39 to 3.59 mM^{-1} s^{-1}. As with the mononuclear precursor, H_2O likely exchanges for both inner-sphere MeCN molecules in the binuclear species. The isolated **2** has a magnetic moment of 9.0 μ_B, or 4.5 μ_B per manganese(II). The decrease in the overall paramagnetism associated with the formation of a binuclear product is known to reduce the relaxivity of other transition-metal MRI contrast agents.[32] The r_1 value remains constant over 15 h, suggesting that no further chemical transformations occur after the initial oxidative coupling.

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When H_2O_2 is added to **1** in MeCN containing 2,4-dimethylphenol (DMP), no **2** is observed by MS. DMP serves as a competitive substrate as indicated by the observation of *m*/*z* features consistent with the manganese complex's coupling to DMP. Reasoning that the phenolic residues of tyrosines may also be competent coupling partners, we analyzed the MRI response of **1** in buffered solutions containing 0.10 mM bovine serum albumin (BSA) with and without 10 mM H_2O_2. If the sensor were to oxidatively tether to BSA, the r_1 value would be anticipated to increase because of the slower rate of tumbling.[33] The addition of BSA increases the r_1 values of the reduced and oxidized forms to 5.20 and 4.37 mM^{-1} s^{-1}; these changes are consistent with noncovalent interactions between the proteins and manganese(II) complexes.[32] The ~0.8 mM^{-1} s^{-1} response of the sensor to H_2O_2 is essentially unchanged in the presence of the protein. For BSA, the number of accessible tyrosine residues is apparently not high enough for the potential tyrosine-sensor reactivity to compete with the bimolecular coupling reaction between $[Mn(Hptp1)(MeCN)]^{2+}$ ions. Other peptides, however, may be viable coupling partners.

The manganese-containing MRI contrast agent $[Mn(Hptp1)(MeCN)]^{2+}$ exhibits a predictable and measurable response to H_2O_2. The observed decrease in the relaxivity is correlated to the oxidative coupling of the sensors, which results in a stable binuclear species that is less paramagnetic per manganese(II) than its mononuclear precursor. As such, the sensor provides a novel means of detecting oxidative activity under physiologically relevant conditions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental section, MS, IR, EPR, and crystallographic data for **1** and **2**, and a spectrophotometric study of iron(II)-for-manganese(II) exchange in **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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