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Manganese(III) Complexes of Bis(hydroxyphenyl)dipyrromethenes Are Potent Orally Active Peroxynitrite Scavengers

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Supporting Information

ABSTRACT: We report a new series of biscyclohexano-fused Mn(III) complexes of bis(hydroxyphenyl)dipyrromethenes, **4a**−**c**, as potent and orally active peroxynitrite scavengers. Complexes **4a**−**c** are shown to reduce peroxynitrite through a two-electron mechanism, thereby forming the corresponding Mn(V)O species, which were characterized by UV, NMR, and LC−MS methods. Mn(III) complex **4b** and its strained BODIPY analogue **9b** were analyzed by X-ray crystallography. Finally, complex **4a** is shown to be an orally active and potent analgesic in a model carrageenan-induced hyperalgesia known to be driven by the overproduction of peroxynitrite.

The overproduction of reactive oxygen species (ROS) in vivo is now widely recognized as a key contributor to numerous pathologies.[1] One particularly damaging situation results from the diffusion-controlled radical coupling of the central ROS, superoxide, with nitric oxide to form peroxynitrite.[2] The highly reactive peroxynitrite is a powerful biological oxidant that leaves a trail of dysfunctional oxidized and nitrated proteins, lipids, and nucleotides in its wake.[3] From a pharmacological perspective, peroxynitrite is considered a potent proinflammatory and proapoptotic species that plays a critical role in pain of several etiologies, as demonstrated initially by our team and then by others.[4−6] Accordingly, the discovery of pharmaceutically relevant agents that can effectively decompose peroxynitrite should have significant therapeutic value.[2,3]

As a result of the early discoveries of Groves[7] and Stern,[8] Mn(III) and Fe(III) porphyrins have emerged as important classes of peroxynitrite reductase and isomerase catalysts, respectively (Figure 1A). Elegant mechanistic studies have revealed that the more pharmacologically suitable Mn(III) porphyrins decompose peroxynitrite primarily in a one-electron fashion and require a biological coreductant such as ascorbate to complete the reductase catalytic cycle.[9] One-electron reduction of peroxynitrite produces the potentially damaging nitrogen dioxide radical, which is also thought to undergo rapid reduction by endogenous antioxidants.[5] Thus, if endogenous antioxidants are plentiful, Mn(III) pophryins can fully detoxify peroxynitrite in vivo. From this class, the isomeric Mn(III) tetrakis(*meso*-*N*-alkylpyridinium)porphyrins [e.g., Mn(III)−4-TMPyP^{5+} (**1**)]

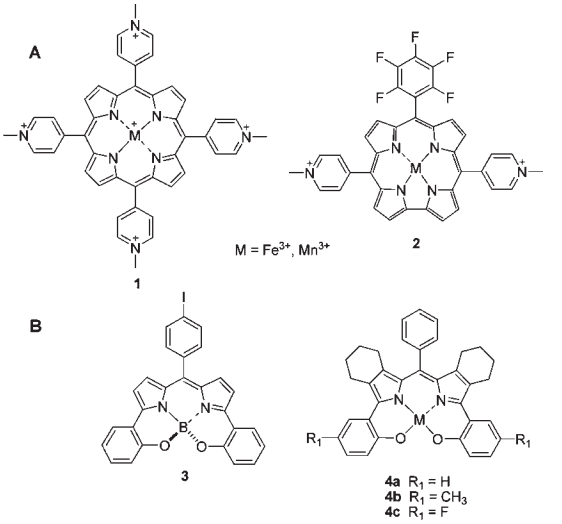

Figure 1. (A) Peroxynitrite decomposition catalysts **1** and **2**. (B) Bis(hydroxyphenyl)dipyrromethene analogues **3** and **4**.

are the most well studied, both as peroxynitrite reductase catalysts and superoxide dismutase (SOD) mimics.[9,10]

Recently, Gross has reported that Mn(III) and Fe(III) corroles are also excellent peroxynitrite decomposition catalysts.[11] Remarkably, the Mn(III) corroles operate through a two-electron cycle, reducing peroxynitrite to nitrite instead of nitrogen dioxide through a novel disproportionation mechanism. The most important finding from that work was that Mn(III) corroles can decompose peroxynitrite in a catalytic fashion [in contrast to Mn(III) porphyrins] and therefore do not require the assistance of endogenous coreductants.

Although Mn(III) porphyrins such as **1** and Mn(III) corrole systems such as **2** have proven to be powerful pharmacological tools in animal studies, where they have demonstrated the benefits of destroying peroxynitrite in vivo,[11−14] they are not optimal as therapeutic candidates. While these types of polycationic complexes have excellent catalytic activities and their high water solubility is useful for laboratory measurements, their corresponding high polarity renders them poorly membrane-soluble.

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More amphiphilic metallocorroles have indeed shown great promise as orally active peroxynitrite decomposition catalysts.[12c] Unfortunately, synthetic methods for accessing polyfunctional corrole systems remain quite challenging, and thus, these systems are not particularly suited for iterative structure−activity relationship (SAR) studies. In view of this, we have been keenly interested in the design, synthesis, and evaluation of new catalyst systems with enhanced druglike properties.

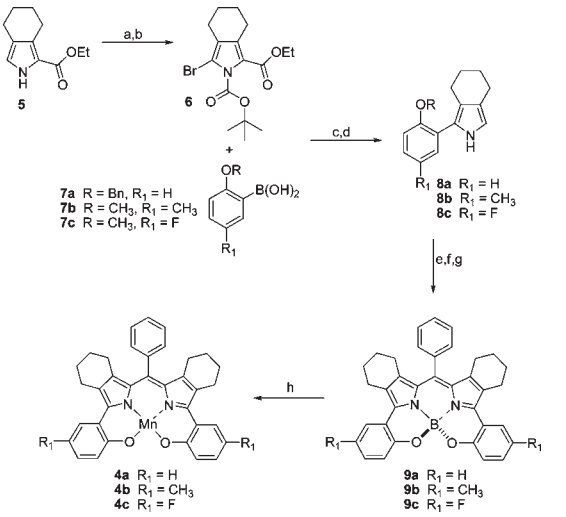
In our search for alternative trianionic ligand systems capable of supporting a Mn(V)O intermediate arising from the two-electron reduction of peroxynitrite [similar to Mn(III) corroles], we were intrigued by the B,O-chelated boron dipyrromethene (BODIPY) dye **3** first reported by Burgess (Figure 1B).[15] This and related dyes are constrained because of the chelate effect and thus have improved fluorescence properties relative to their nonchelated and well-known BODIPY congeners. Not only is the overall "ligand set" in these systems perfect for mimicking the trianionic corrole, but these compounds are also amenable to modular synthesis in good to excellent yields.[16] Here we report the synthesis and evaluation of new biscyclohexano-fused Mn(III) complexes of bis(hydroxyphenyl)dipyrromethenes as potent peroxynitrite scavengers with druglike properties.

The syntheses commenced with readily available tetrahydroisoindole **5**,[17] which was converted to Boc-protected 3-bromotetrahydroisoindole **6** in 95% yield over two steps (Scheme 1). Next, compound **6** underwent smooth Suzuki couplings with a representative set of protected hydroxyphenylboronic acids (**7a**−**c**) in 70−89% yield, and this was followed by a one-pot decarboxylation/deprotection procedure that furnished the corresponding 2-benzyloxy- or 2-methoxyphenyltetrahydroisoindole derivatives **8a**−**c**. Compounds **8a**−**c** were then treated with benzaldehyde and trifluoroacetic acid (TFA) under Lindsey conditions[18] to form the corresponding dipyrromethane derivatives; this was followed by oxidation to the dipyrromethenes (DIPYs) with *p*-chloranil[19] and subsequent phenol ether deprotection with boron tribromide.[19,20] This sequence was carried out without intervening purifications. The boron tribromide deprotection step afforded the crude BODIPY systems **9a**−**c**, which were then directly converted to the Mn(III) complexes **4a**−**c** by reaction with manganese(II) chloride under basic aerobic conditions. The dark-emerald-green complexes **4a**−**c** behave as simple lipophilic "organic" molecules and were thus amenable to purification by flash chromatography on silica gel, which provided analytical materials for characterization and activity studies. The overall yield of this sequence ranged from 31 to 56% (unoptimized) and is therefore quite competitive with porphyrin and corrole syntheses, which require the preparation of functionalized pyrrole and/or dipyrromethane units followed by an often low-yield cyclization/oxidation step.[18]

Magnetic susceptibility measurements confirmed representative complex **4b** to be a high-spin d^4 Mn(III) complex. ^{1}H NMR spectra of **4a** in CD_2Cl_2 revealed broad, shifted peaks indicative of the paramagnetic Mn(III) complex. Oxidation of **4a** with *m*-CPBA in CD_2Cl_2 afforded normal sharpened peaks characteristic of the corresponding diamagnetic low-spin d^2 Mn(V)O complex (Figures S21 and S22 in the Supporting Information).[21]

Treatment of green-colored methanolic solutions of the Mn(III) complexes **4a**−**c** with excess peroxynitrite (in 0.1N NaOH)[22] afforded the corresponding red-colored Mn(V)O intermediates (Figure 2). In methanol solution, the Mn(V)O species persisted for 20−30 min and were stable enough to be confirmed by LC−MS (Figure 2 inset) and UV−vis spectroscopy. The UV−vis spectral changes are analogous to those observed during the oxidative generation and subsequent reduction of Mn(V)O corroles[23] and

Scheme 1. Synthesis of Mn(III) Complexes of Bishydroxyphenyl-DIPYs[a]



[a] Conditions: (a) NBS, THF, 100%; (b) Boc_2O, 4-DMAP, CH_3CN, 95%; (c) 5% $Pd(PPh_3)_4$, Na_2CO_3/H_2O, CH_3OH, toluene, 70−89%; (d) $(CH_2OH)_2$, KOH, 195 °C, 70−85%; (e) PhCHO, cat. TFA, 97−99%; (f) *p*-chloranil, CH_2Cl_2; (g) BBr_3, CH_2Cl_2; (h) $MnCl_2$, $CHCl_3$, CH_3OH, 2,6-lutidine, air, 68−79% for three steps (from f).

in Goldberg's elegant studies of highly functionalized Mn(V)O corrolazines.[21] For all three complexes **4a**−**c**, the oxo species were clearly observable as $[M + H]^+$ species using positive-ion-mode LC−MS, confirming the formal oxidation state of $[Mn(V)O]^{3+}$ (accounting for the trianionic ligand set). Treatment of the methanolic solutions of Mn(V)O species with 5 equiv of ascorbate in phosphate buffer (pH 7.2) resulted in the apparent instantaneous conversion back to the green Mn(III) form, effecting a possible reductase mode of action.

The results shown in Figure 2 are of interest only for identifying the putative two-electron oxidation of **4b** with subsequent study of the Mn(V)O species **10b** by spectroscopic methods. However, to confirm that the complexes can indeed decompose peroxynitrite more rapidly than its spontaneous decomposition at physiological pH, a relevant rapid-throughput in vitro assay was sought.

Complexes **4a**−**c** were therefore assayed for their peroxynitrite decomposition activity by determining their ability to inhibit aryl boronate oxidation.[24] Oxidation of 4-acetylphenylboronic acid to 4-acetylphenol by peroxynitrite is a clean conversion devoid of any observable intermediates, and the second-order rate constant for this reaction has been accurately measured to be $k = 1.6 \times 10^6$ M^{-1} s^{-1} using stopped-flow spectrophotometric methods.[24] Inhibition results for complexes **4a**−**c** and **1** are presented in Table 1. From the percent inhibition values, we calculated the corresponding apparent second-order rate constants for oxidation of the Mn(III) form to the Mn(V)O form for **4a**−**c** and the Mn(IV)O form for **1** at 25 °C in phosphate buffer (pH 7.2). The numbers match up well with the rate constants reported in the literature for the well-known complex **1**.[25] Thus, this assay provides a reliable method for the rapid in vitro measurement of activity toward decomposition of peroxynitrite prior to in vivo studies. Our data reveal that analogues **4a**−**c** have estimated apparent rate constants in the range of those observed for manganese corrole systems.[11] If the resulting Mn(V)O forms are more rapidly reduced back to the Mn(III) resting state by endogenous reductants, the *k* values in Table 1 would be the catalytic rate

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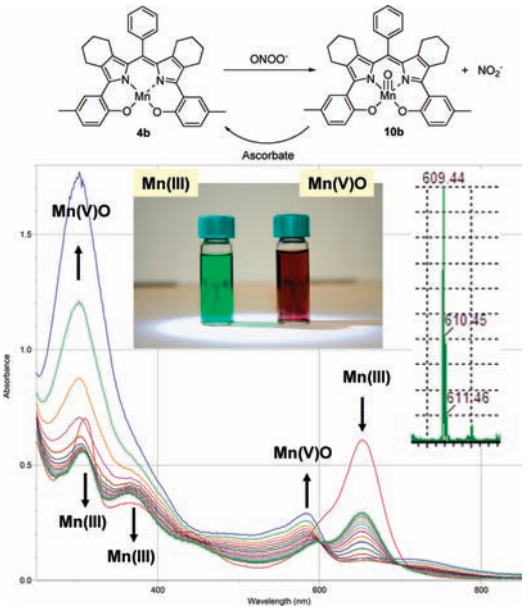


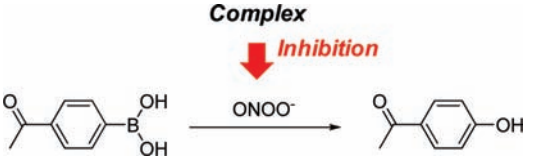
Figure 2. UV−vis spectral changes for **4b** (33 μM, MeOH, 25 °C) after treatment with 0−20 equiv of peroxynitrite (spectra were collected every 2 min; see the Supporting Information). Center inset: samples of **4b** and **10b**. Right inset: LC−MS data ($[M + H]^+$) for **10b**.

constants for reductase-type activity.[9] In that manganese corroles have been shown to possess peroxynitrite decomposition activity without the need for endogenous reductants,[11] complexes **4a**−**c** may also operate via a similar catalytic cycle, as they possess a similar trianionic ligand environment. Future mechanistic studies will address this possible mode of catalysis.

Single-crystal X-ray analysis of **4b** and its BODIPY analogue, the synthetic precursor **9b**, provided very interesting structural information for this new ligand class (Figure 3). The BODIPY system **9b** shows significant distortion of the tetrahedral boron center, similar to that observed for the related non-cyclohexano system.[15] In the case of **9b**, the O−B−O angle is 108.4°, but the N1−B−O2 and N2−B−O1 angles are 114.4 and 113.1°, respectively. The N−B−N angle is pinched inward to 106.5°, most likely in response to the phenyl group that bisects the planar dipyrromethene unit and therefore feels a close steric interaction with the CH_2 groups of the neighboring fused cyclohexano rings. The Mn(III) complex **4b** crystallized with coordinated axial methanol molecules (from the CH_2Cl_2/MeOH solution) to afford a Jahn−Teller-distorted octahedral array around the manganese atom. The phenyldipyrromethene unit in **4b** is displaced upward relative to the equatorial plane, with the coordinating phenolate groups displaced slightly downward. The release in strain associated with the distorted tetrahedron of **9b** most likely drives its direct conversion to **4b** without the need for isolation of the free phenol ligand. The two axial Mn−O(MeOH) bonds are considerably longer than those in similar structures observed for Mn(III) corroles (2.19 Å)[11c] and Mn(III) corrolazines (2.107 Å),[21b] most likely because of the Jahn−Teller effect.[26] The Mn−O3 bond distance is 2.226 Å and the longer Mn−O4 bond distance is 2.342 Å, possibly also influenced by steric interactions with the saddled hydroxyphenyl groups.

In the well-known Hargreaves model,[28] intraplantar injection of carrageenan leads to the time-dependent development of thermal hyperalgesia in rats, an inflammatory response known to be driven by high levels of peroxynitrite flux (Figure 4).[29] This hyperalgesic effect was potently inhibited when **4a** was given by oral gavage

Table 1. Inhibition of Arylboronic Acid Oxidation



catalyst	LogP[a]	% inhibition at 25 °C	k (M^{-1} s^{-1})[b] at 25 °C
4a	+4.18 ± 0.19	32.0 ± 2.40	(7.5 ± 0.6) × 10^5
4b	+4.31 ± 0.02	25.3 ± 2.21	(5.4 ± 0.5) × 10^5
4c	+4.05 ± 0.15	31.5 ± 2.50	(7.3 ± 0.6) × 10^5
1	−4.54 ± 0.16	62.4 ± 0.68	(2.6 ± 0.2) × 10^6, 1.8 × 10^6 [c]

[a] Octanol−water partition coefficients measured using the slow-stir method.[27] [b] Apparent second-order rate constant for the oxidation of complex (1 equiv) by peroxynitrite (1 equiv) estimated from the % inhibition in 100 mM phosphate buffer (pH 7.2) with no secondary antioxidants added. Inhibition data were determined by LC−MS after 1 min of reaction time. [c] Second-order rate constant measured using stopped-flow methods.[25]

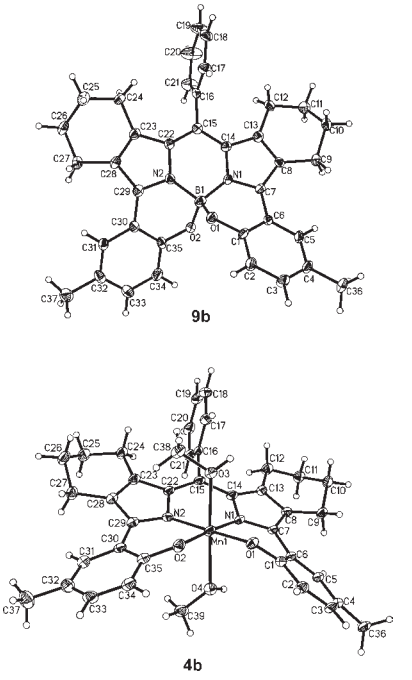


Figure 3. Single-crystal structural analysis of BODIPY analogue **9b** and Mn(III) complex **4b**.

(Figure 4). Nearly 100% inhibition for 2 h was seen, with a substantial inhibitory effect maintained out to 5 h. At the 2 h time point, a similar degree of inhibition was observed with the nonselective COX-1/COX-2 inhibitor ibuprofen at 300 mg/kg (99 ± 5% inhibition, n = 6). Under the same conditions and at 300 mg/kg, acetaminophen or aspirin attenuated hyperalgesia by 20 ± 4% and 50 ± 6% respectively (n = 6, P < 0.05 relative to carrageenan alone at the 2 h time point). Complexes **4b** and **4c** were also shown to possess potent oral activity in this model. All three new complexes have octanol−water partition coefficient (LogP) values in the range of +4, indicating high lipid solubility, while **1** and related catalysts possess highly negative values (Table 1).[30] Both **4b** and **4c** were designed to divert or block the potential metabolic hydroxylation para to the chelated phenol

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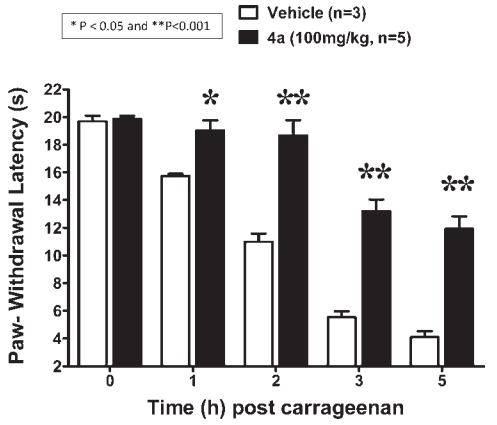

Figure 4. Time-dependent development of carrageenan-induced thermal hyperalgesia in rats ($n = 3$) was blocked by oral administration of **4a** (100 mg/kg, $n = 5$) (n = number of animals). Results are expressed as means ± standard errors of the mean and were analyzed by two-way ANOVA with Bonferroni post hoc tests; ** denotes $P < 0.05$ and ** denotes $P < 0.001$ vs vehicle.

through methyl and fluoro substitution, respectively. Full SAR studies incorporating further electron-donating and -withdrawing functionalities will be the subject of future reports.

In conclusion, the results presented herein demonstrate the modular synthesis of a new class of orally active Mn(III) complexes that function as peroxynitrite scavengers. These studies suggest that this new complex design may afford improved in vivo performance through the two-electron reduction of peroxynitrite to nitrite.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures; characterization data; LC−MS, NMR, and UV−vis data; and crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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