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6,6′-Bis(2-hydroxyphenyl)-2,2′-bipyridine Manganese(III) Complexes: A Novel Series of Superoxide Dismutase and Catalase Mimetics

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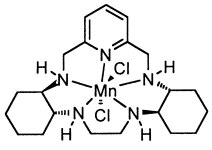
Abstract—A series of novel manganese(III) complexes is described based on a 6,6′-bis(2-hydroxyphenyl)-2,2′-bipyridine template. These complexes show superoxide dismutase and catalase activity. The effect of the aromatic substitution pattern on the SAR is described.

Introduction

Oxidative stress[1] is an in vivo phenomenon associated with an imbalance between the rate of production of oxidant species (such as superoxide and hydrogen peroxide) and the rate of their removal by endogenous antioxidants [e.g., the enzymes superoxide dismutase (SOD) and catalase (CAT)]. Typically, excess oxidants cause damage to proteins and lipids leading to cell death. A range of pathophysiological conditions has been associated with oxidative stress. For example, the pathology of Alzheimer's disease shows markers of oxidative stress,[2] and tissue damage in cardiac ischemic/reperfusion injury is known to be, in part, caused by oxidative damage.[3]

Synthetic antioxidants, which scavenge excess oxidants, are rational targets for drug design. However, stoichiometric antioxidants based on endogenous systems, for example vitamin E derivatives, have proven disappointing in the clinical setting.[4] Renewed interest in the area has recently been stimulated by reports of catalytic antioxidants as novel therapeutics based on their ability to sequester oxidants at a much higher kinetic rate than stoichiometric antioxidants. For example, investigators from Monsanto, and presently at Metaphore, have published extensively on the penta-aza series of manganese(II) SOD mimetics,[5] culminating on the recent disclosure of a potential drug candidate M40403.[6] The salen manganese(III) complexes, for example EUK-134, are combined SOD and CAT mimetics,[7] possibly offering a therapeutic advantage arising from their multiple mechanism of action. EUK-134 has been shown to be active in animal models of stroke,[8] cardiac ischemia,[9] kidney reperfusion injury[10] and seizure-induced neuronal



M40403

EUK-134

Figure 1. Catalytic antioxidants.

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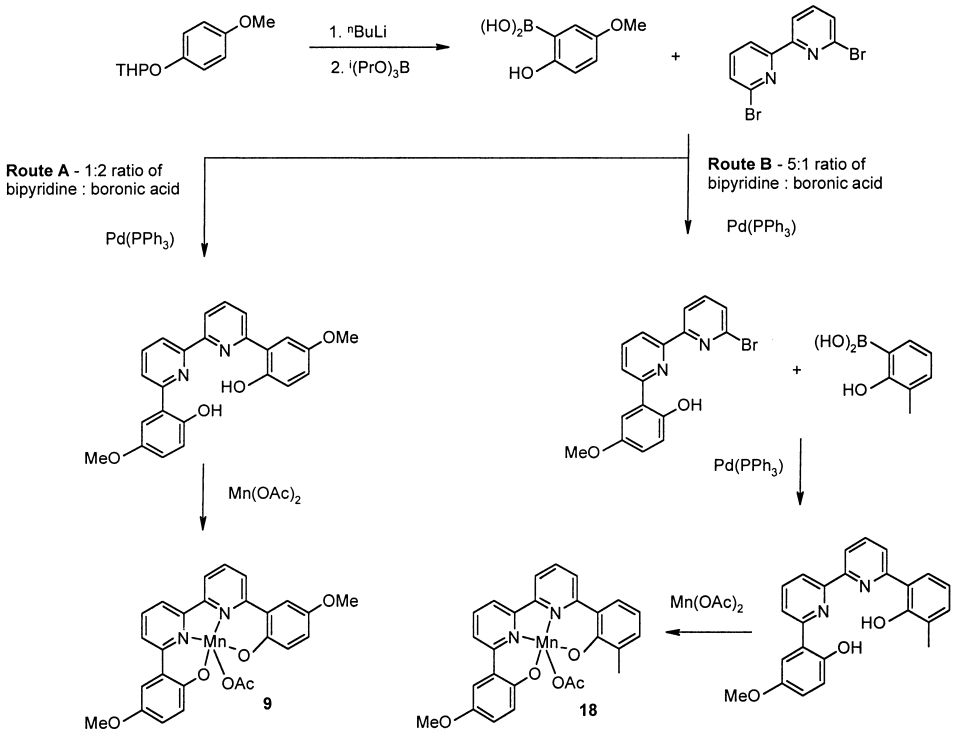
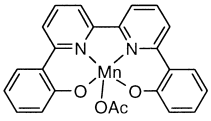


Scheme 1. The synthesis of symmetrical and unsymmetrical manganese complexes.

death.[11] Recently, EUK-134 was shown to enhance the lifespan of the nematode worm *C. elegans*.[12]



As part of a Medicinal Chemistry programme we were interested in the bipyridine analogues of the salen ligand. Herein we disclose some initial results including the synthesis, antioxidant activity and SAR of the 6,6′-bis(2-hydroxyphenyl)-2,2′-bipyridine series.

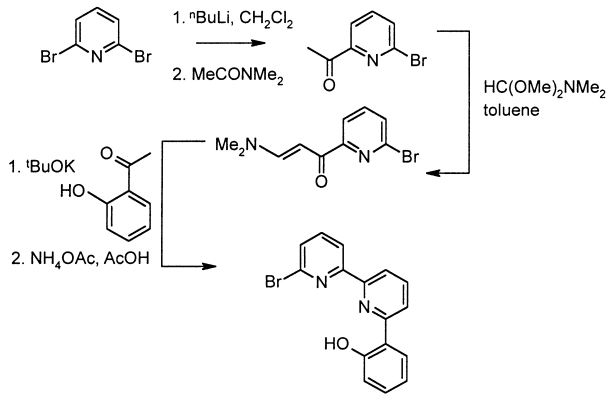
Chemistry

Synthesis of the 6,6′-bis(2-hydroxyphenyl)-2,2′-bipyridine template[13] is illustrated in Scheme 1. THP-protected phenols[14] were subject to *ortho*-directed metalation[15] followed by quenching with tri-isopropylborate. Symmetrically substituted complexes were synthesised by Suzuki-type palladium catalysed couplings of the resulting boronic acids (2 equiv) with commercially available 6,6′-dibromo-2,2′-bipyridine (route A) to furnish the required ligands.

Unsymmetrical ligands were accessed via mono-coupled intermediates (route B), which were isolated by chromatography from the Suzuki reaction of 5 equiv of boronic acid and 1 equiv of bromopyridine. An alternative synthesis of the key unsymmetrical intermediate is shown in Scheme 2.[16] Reaction of the bromopyridine with a second boronic acid gave the unsymmetrically substituted ligands. In each case complexation with manganese(II) acetate tetrahydrate with in situ air oxidation afforded the manganese(III) complexes in high yield.[17]

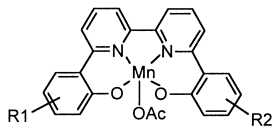
Antioxidant Activities

Two of the best characterised species that contribute to oxidative stress are superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Superoxide is produced in vivo by leakage from the respiration pathway and by the oxidative burst of neutrophils. Hydrogen peroxide is the product of superoxide dismutase and damages cells through production of the highly damaging hydroxyl radical via Fenton chemistry. In order to assay for SOD-like and CAT-like activity, the manganese complexes were



Scheme 2. Alternative route to unsymmetrical intermediate.

Table 1. SOD and CAT activity of symmetrically substituted bipyridine manganese complexes[a]



Compounds	R1	R2	SOD activity (Units/mM)	CAT activity
EUK-134	—	—	650	0.25
1	H	H	215	0.13
2	3-Me	3′-Me	101	0.07
3	4-Me	4′-Me	163	0.20
4	5-Me	5′-Me	189	0.01
5	3,5-diMe	3′,5′diMe	250	0.07
6	3-F	3′-F	255	0.07
7	5-F	5′-F	209	0.08
8	3-MeO	3′-MeO	203	0.13
9	5-MeO	5′-MeO	137	0.20
10	6-MeO	6′-MeO	155	0.08
11	3-EtO	3′-EtO	206	0.13
12	3-MeO,5-Me	3′-MeO,5′-Me	250	0.07

[a]SOD activity is expressed in Units/mM of complex where 1 unit = one International Unit of SOD activity. CAT activity is expressed as number of mmol of H_2O_2 degraded/min/mmol of complex. SOD and CAT activities are the average of three determinations with standard deviations $< 10\%$.

evaluated using standard assays adapted for a 96-well plate format. The superoxide dismutase assay[18] measures the rate at which the complex scavenges superoxide. Data are expressed in Units/mM. This is normalised data representing the number of International Units of SOD activity per millimolar of complex. The catalase assay[19] measures millimoles of hydrogen peroxide removed per minute per millimole of compound.

Results and Discussion

The SOD and CAT data for symmetrically substituted analogues of the 6,6′-bis(2-hydroxyphenyl)-2,2′-bipyridinemanganese(III) complexes is represented in Table 1. The unsubstituted 6,6′-bis(2-hydroxyphenyl)-2,2′-bipyridine **1** has significant SOD and CAT activity but is less potent than EUK-134. Methyl substitution is detrimental to activity, although the 4-methyl analogue **3** is a slightly more potent CAT mimetic than unsubstituted analogue **1**. A 3-fluorine substituent shows an increased SOD activity but reduced CAT. 3- or 5-Methoxy substituents (**8** and **9**) have good CAT activity; in contrast a 6-MeO substituent has reduced activity (**10**).

Although these analogues were not as potent as we had hoped, we were encouraged to explore the template further. In order to fine-tune the substituent effects we proceeded to synthesise unsymmetrically substituted analogues. The antioxidant activity is presented in Table 2. The effects of the most beneficial substituents from the symmetrical series were probed with one ring unsubstituted. The rank order of activity for both SOD and CAT was 5-MeO > 3-MeO > 4-Me (**15**, **14** and **13** respectively). The same rank order was observed for catalase activity on addition of a 3-Me substitution (**16**, **17** and **18**) but not for SOD activity. For the first time we observed significantly higher catalase activity than EUK-134 with 5-MeO analogues **15** and **18**. With a 4-Me substituent on one ring, the 5-MeO analogue **20** is again the most active catalase mimetic. Compounds **27**–**29** confirm that the 5-MeO substituent is optimal for catalase activity. In particular, the 3,4-dimethyl analogue **28** is the most potent compound in this series for both SOD and CAT activity.

Conclusions

A novel series of combined SOD and CAT mimetics has been discovered based on 6,6′-bis(2-hydroxyphenyl)-2,2′-bipyridine. This series includes compounds with improved catalase activity over that of the manganese complex EUK-134. Further characterisation, including in vivo activity, will be reported shortly.

Table 2. SOD and CAT activity of unsymmetrically substituted bipyridine manganese complexes (for generic structure, refer to Table 1)[a]

Compounds	R1	R2	SOD activity (Units/mM)	CAT activity
13	H	4-Me	86	0.09
14	H	3-MeO	138	0.28
15	H	5-MeO	159	0.59
16	3-Me	4-Me	177	0.09
17	3-Me	3-MeO	183	0.18
18	3-Me	5-MeO	97	0.46
19	4-Me	3-MeO	184	0.30
20	4-Me	5-MeO	130	0.40
21	4-Me	3-F	123	0.08
22	4-Me	3,4-diMe	149	0.11
23	4-Me	5-Me	116	0.18
24	3-MeO	5-MeO	124	0.31
25	3-MeO	3,4-diMe	145	0.11
26	3-MeO	4-MeO,5-Me	142	0.27
27	5-MeO	5-F	162	0.49
28	5-MeO	3,4-diMe	259	0.67
29	5-MeO	4-MeO,5-Me	173	0.40
30	5-MeO	5-Me	193	0.63

[a]See Table 1 footnote for explanation of SOD/CAT activity.

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17. The structure of all ligands was confirmed by ^{1}H NMR spectroscopy. Purity (>98%) was assessed by UV integration of LC–MS peaks. Manganese(III) complexes were routinely isolated in quantitative yield. The identity of complexes was determined by observation of a $(M-OAc)^+$ ion in the mass spectrum. Purity (>98%) was assessed by UV integration of LC–MS peaks corresponding to the $(M-OAc)^+$ ion. The only impurity observed was a small amount of ligand (<1%). Importantly, uncomplexed ligand showed no significant activity in the antioxidant assays. Selected data for 6,6′-bis(2-hydroxyphenyl)-2,2′-bipyridinemanganese acetate: mass spectrum: 393 $(M-OAc)^+$. ^{1}H NMR: δ_H (MeOD) includes: −42.5 (2H, br), −25 (2H, br), −20 (2H, br), −11.5 (2H, br), −1 (2H, br), 12 (2H, br). For a detailed discussion of the ^{1}H NMR spectrum of manganese(III) complexes see: Ciringh, Y.; Gordon-Wylie, S. W.; Norman, R. E.; Clark, G. R.; Weitraub, S. T.; Horwitz, C. P. *Inorg. Chem.* **1997**, *36*, 4968.
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19. Adapted from Galati, H. J. *Clin. Chem. Clin. Biochem.* **1979**, *17*, 1. The catalase assay measures the ability to remove hydrogen peroxide from solution in the absence of hydrogen donors after 1 h. After this time any remaining peroxide was removed by introduction to the mixture of high concentrations of horseradish peroxidase and the hydrogen donor 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). This rapid reaction produces peroxide-concentration-dependent product with a maximal absorbance at 405 nm. Assays were carried out in 96-well plate. Compounds were solubilised in DMF at 10 mM. DMF concentrations in the assay never exceeded 1% and were controlled for in assay plates. Hydrogen peroxide (100 mM) was incubated with various concentrations of compound in 0.05 M Tris–HCl pH 7.4 at 25 °C for 60 min. After incubation, 0.1 volumes of horseradish peroxidase (Sigma P-6140) 1 U/mL and ABTS 3 mM were added. The resultant reaction was allowed to go to completion by incubation at 25 °C for 60 min. Plates were then read for absorbance at 405 nm. A standard curve for absorbance against known hydrogen peroxide concentration was constructed. Absorbance data for wells containing compound were interpolated against this curve using Excel (Microsoft Corp.) and Xlfit (ID Business Solutions). This analysis gives the amount of hydrogen peroxide removed per minute per mmol of compound.