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Effect of the oligo(ethylene glycol) group on the antioxidant activity of manganese salen complexes

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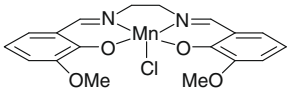
ABSTRACT

The synthesis and antioxidant activity of oligo(ethylene glycol)-modified manganese salen complexes are reported. Their SOD activities were similar and 2- to 3-fold more potent than the standard compound EUK-134. Their catalase-like activity was lower than that of EUK-134 in the initial conversion rate; however, some analogs exhibited a better catalytic turnover number.

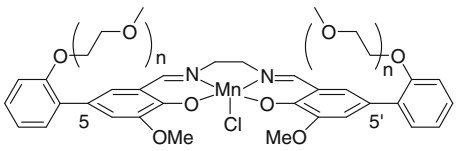
Reactive oxygen species (ROS), such as the superoxide radical anion ($O_2^{-\cdot}$) and hydrogen peroxide (H_2O_2), are inevitably generated from cellular metabolism in aerobic organisms. Under normal circumstances, these ROS are tightly controlled by antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, and by endogenous free radical scavengers.[1] However, an imbalance of pro-oxidants and antioxidants is observed for many diseases and for oxidative stress, and the overproduced ROS have been shown to oxidize various cellular components, including DNA, proteins, and lipids, causing various forms of damage to cells and tissues. Therefore, antioxidant treatments have been studied for a wide variety of disorders, including arthritis,[2] stroke,[3] Parkinson's disease,[4] ALS (Lou Gehrig's disease),[5] cancer,[6] and aging,[7] in which ROS have a significant role. However, due to the difficulties associated with administration and delivery of exogenous antioxidant enzymes, such as SOD and catalase, many different types of small molecules that possess SOD or catalase-like activity have been developed[8] and tested in vivo.[9] Such catalytic mimetics of antioxidant enzymes include manganese(III) and iron(III) porphyrin complexes,[10] manganese(II) complexes of penta-azamacrocycles,[11] manganese(II) complexes centered on tripodal ligands[12] and on a 1,2-ethanediamine,[13] manganese(III) salen complexes,[14] and the tetra-aza[14]annulene-Fe(III) complex.[15] Among them, manganese(III) salen complexes have been reported to have two key antioxidant properties, that is, the catalytic removal of both superoxide radical anions and hydrogen peroxide.[16] The dual activity is advantageous, since the SOD-like activity alone of mimetics would produce hydrogen peroxide, which is cytotoxic either directly or by the formation of highly damaging hydroxyl radicals via the Fenton or the Haber–Weiss reaction.[1]

A number of derivatives of manganese salen complexes have been prepared as SOD/catalase mimetics,[14,17] and some have shown beneficial effects in various disease models[5,18] and in aging.[19] However, we still need to improve the activity and stability under physiological conditions to be useful as a drug, since the activities of salen complexes are lost in a few minutes under the catalase assay conditions.[14]

Here, we describe the synthesis and activities of oligo(ethylene glycol) (OEG) derivatives of manganese bis-(3-methoxysalicylidene)-1,2-ethylenediamine chloride **1** (EUK-134) (Fig. 1). Two aryl



1 (EUK-134)



2a-f : n = 1, 2, 3, 4, 6, 8

Figure 1. Structures of manganese salen complex and its oligo(ethylene glycol) derivatives.

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groups bearing OEG ether linkages at *o*-positions were connected symmetrically to the 5 and 5′ positions of salen complex **1**. We expect that the flexible hanging groups are poised over a salen platform with an increase in chain length, affecting the stability and activity of the Mn complex.

The synthesis of target compounds, **2a–f**, is presented in Scheme 1. First, the commercially available 2-hydroxyphenylboronic acid was coupled to benzyl-protected 5-bromo-2-hydroxy-3-methoxybenzaldehyde (**3**) using Suzuki cross-coupling methodology to produce **4**.[20] The phenol group of the compound **4** was reacted with tosylated mono-, di-, tri-, tetra-, hexa-, and octaethylene glycol monomethyl ethers to furnish **5a–f,** respectively, in 83–90% yields. After the benzyl group was removed from **5a–f**, diimine condensation with ethylene diamine and complex formation with $Mn(OAc)_2$ were carried out simultaneously in EtOH solution. The progress of the reaction was followed by TLC and the oxidized Mn(III) complexes **2a–f** were obtained by air bubbling for 30 min followed by a workup in brine. The purity of each complex was greater than 95%, as assessed by HPLC, and the UV spectra of **2a–f** were almost identical, with λ_{max} at 265 nm.

The manganese complexes **2a–f** were tested for their SOD- and catalase-like activity and the results are given in Table 1.

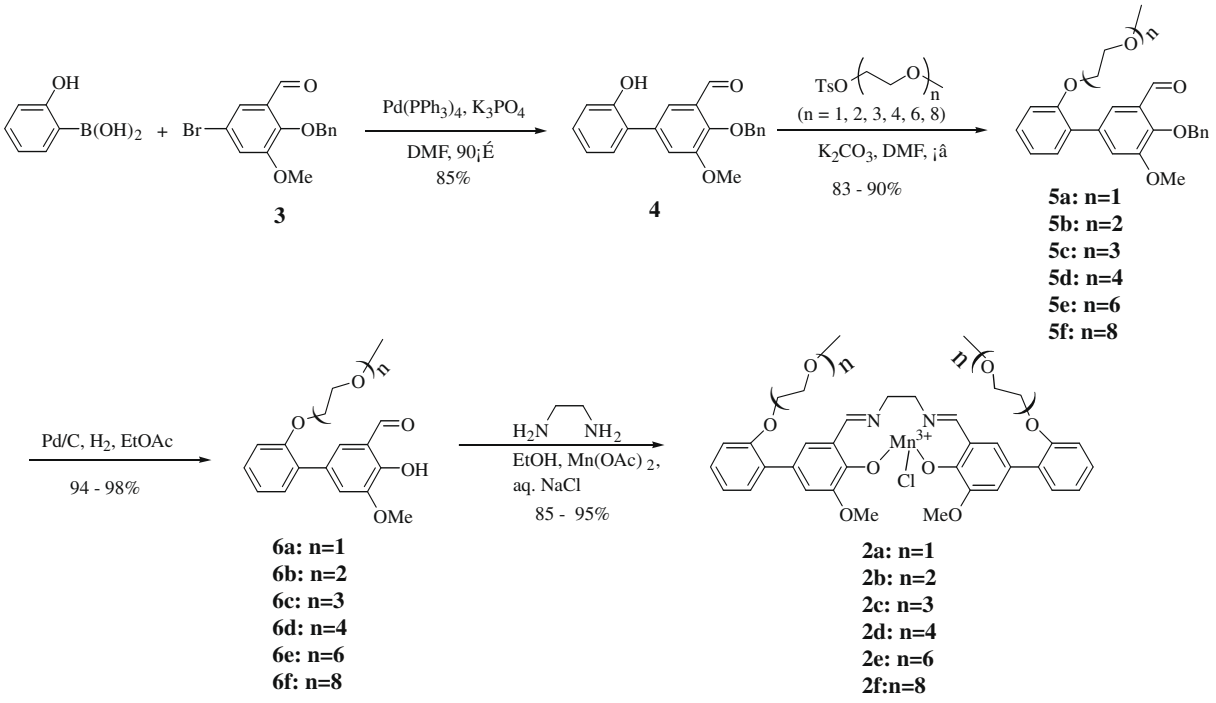
The SOD-like activities of **2a–f** were determined indirectly using cytochrome *c* as an electron acceptor as described by McCord and Freidovich.[21] Ethylenediaminetetraacetic acid (EDTA) was omitted due to artifacts described previously.[22] Superoxide anion was generated by xanthine–xanthine oxidase system and possible interference with the manganese complexes was examined by following the rate of urate formation at 290 nm in the absence of cyt *c*. The complexes did not interfere with reaction of xanthine with xanthine oxidase. The SOD-like activities of the new salen complexes **2a–f** were similar and slightly better than that of EUK-134. It has been reported that similar or even identical SOD activities were obtained for most ring-modified salen–manganese complexes.[14]

The catalase-like activity of the new complexes was determined by monitoring the conversion of hydrogen peroxide to oxygen in phosphate buffered solution (pH 7.4) using a Clark-type polarographic oxygen electrode.[23] Initial rates and maximal concentration of oxygen produced by the salen complexes are given in Table 1. In contrast to the SOD activities of new manganese complexes, their catalase rate and amount of oxygen are considerably influenced by the length of ethylene glycol substituents. For the initial rate of oxygen generation, the triethylene glycol derivative **2c** is the best, though it has a lower level of activity than standard compound **1**. The total amount of oxygen (i.e., catalytic turnover activity) produced by the new complexes is largest for compound **2e**, which is a better catalyst than **1**.

From the time-course of the concentration of molecular oxygen given in Figure 2, the rate of H_2O_2 disproportionation by complex **2e** is slower than that of the standard compound **1** (EUK-134), but the activity lasts for ~2 min, compared to ~1 min for **1**. This result indicates that the hexaethylene glycol group may fluctuate over the salen–Mn plane and stabilize the complex during the catalytic cycle. There are interesting reports that 3-dimensionally fixed H-bonding auxiliary is important for improved catalase-like activity.[17a,24] In our study, the hexaethylene glycol derivative **2e** has improved SOD-like activity and better catalase turnover activity. To observe H_2O_2-complex interaction, a kinetic study was carried out with compound **2e**. From the plot of the initial rate of O_2 formation versus the concentration of H_2O_2, a saturable curve was obtained with K_M = 33 mM (Supplementary data), implying that there is a complex-forming interaction between H_2O_2 and complex **2e**.

Polyethylene glycol modification is often used to obtain desired properties of drugs, such as increased bioavailability and blood circulation time, optimized pharmacokinetics, and decreased immunogenicity.[25] In fact, a cyclic analog of **1**, triethylene glycol linked at positions 3 and 3′, showed greater biological stability, as reflected by its longer plasma half-life.[18i] Therefore, our complexes may have better properties for application in vivo. To establish the activity change of prepared complexes in the presence of competing chelator we determined the catalase-like activity of **1** and **2f** in the presence of EDTA (see Fig. 3).

The activity (maximal oxygen concentration) with the standard complex **1** is reduced to ~45% of that in the absence of EDTA, while that with **2f** is lowered to 60%. This implies that complex **2f** would



Scheme 1. Synthesis of new salen-manganese complexes.

Table 1
SOD- and catalase-like activities for compounds **2a–f**

Compound	SOD activity[a] IC_{50}, μM	Catalase rate[b] μM O_2/min[a]	End point[b] maximal μM O_2
1 (EUK-134)	1.3[c]	164 (±4)	73 (±2)
2a	0.62	85 (±5)	16 (±2)
2b	0.50	97 (±16)	35 (±5)
2c	0.55	115 (±4)	75 (±2)
2d	0.54	100 (±5)	84 (±1)
2e	0.59	84 (±5)	97 (±3)
2f	0.51	64 (±3)	82 (±2)

[a] Values are averages of duplicate determinations at pH 9.8. Standard deviation was about 20%.
[b] Values are means of three experiments; standard deviation is given in parentheses.
[c] Ref. 7b.

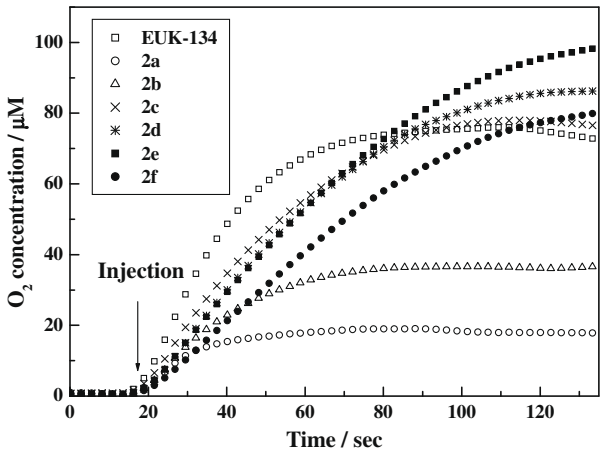


Figure 2. Time-course of catalase-like activity of the manganese salen complexes. The reaction was initiated by addition of the salen complex to the other reagents at the time indicated by the arrow.

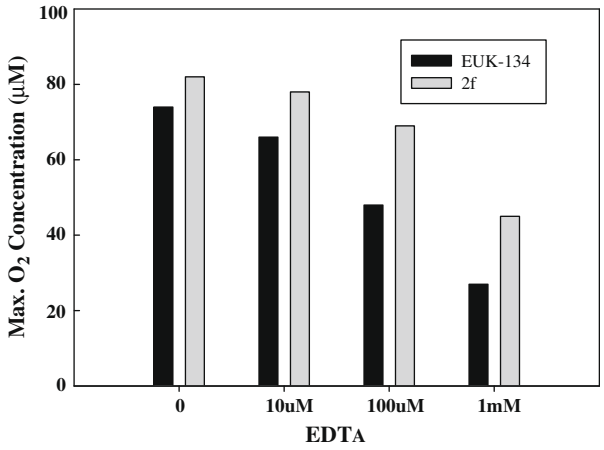


Figure 3. Maximal O_2 concentration of EUK-134 and **2f** in the presence of EDTA. Salen complexes (10 μM) were used in sodium phosphate buffer (pH 7.8).

be a better choice when EDTA is used as a preservative. Another advantage of our complex is possibly its low binding affinity to DNA strands due to the 3-dimensionally located OEG appendage. The planar manganese salen complexes, especially manganese bis(salicylidene)-1,2-ethylenediamine chloride (EUK-8), are known to have pro-oxidant activity in the presence of H_2O_2, damaging free DNA after the intercalation between DNA strands.[26] The ethylene glycol group in **2f** may ameliorate the pro-oxidant activity of planar salen complexes such as EUK-8 by blocking the interaction with DNA.

We determined the peroxidase activity of our complex using 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid (ABTS)[27] as a substrate, since catalase enzymes and manganese salen complexes are known to have peroxidase activity as well.[14] The colorimetric assay with ABTS showed that complex **2a–f** had a range of 1–43% of the activity of **1** (Supplementary data). The peroxidase activities were greatly reduced for both compounds **2a** and **2b** exhibiting little or no activity and were gradually increased with the size of OEG group. This low activity is probably due to the conjugation effect of the phenyl substitution on the salen ring and the activities were enhanced with increasing size of the OEG group on the phenyl substituent, facilitating the access of H_2O_2 and/or ABTS to the active site of the Mn complex. This result is consistent with the kinetic data that associative complex-forming interaction between H_2O_2 and the OEG group.

In summary, we have prepared new 3-dimensionally oriented OEG derivatives of manganese salen complexes and compared their antioxidant activity with one another. The new salen complexes **2a–f** had similar SOD-like activities and were slightly better than the standard compound EUK-134. For the catalase-like activity, **2c** had the best initial conversion rate among the new complexes, while **2e** gave the highest turnover rate. Further investigation of the potential biological applications of these complexes is underway.

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Supplementary data

Experimental procedures, spectral characterization of new compounds are provided. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.12.063.

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