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Manganese Salen Complexes with Acid–Base Catalytic Auxiliary: Functional Mimetics of Catalase

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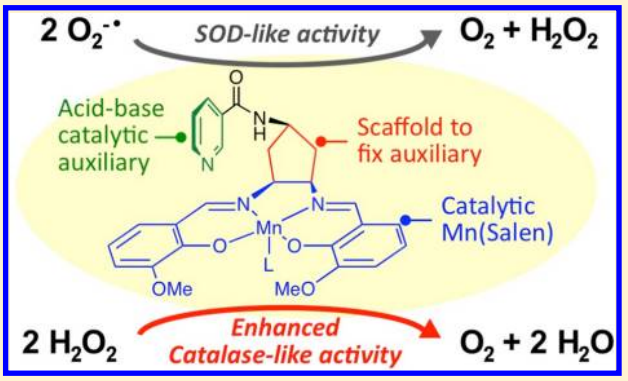
Supporting Information

ABSTRACT: Antioxidant therapies have been considered for a wide variety of disorders associated with oxidative stress, and synthetic catalytic scavengers of reactive oxygen species would be clinically superior to stoichiometric ones. Among them, salen–manganese complexes (Mn(Salen)) seem promising, because they exhibit dual functions, i.e. superoxide dismutase- and catalase-mimetic activities. We have been developing enzyme-mimetic Mn(Salen) complexes bearing a functional group that enhances their catalytic activity. Here, we describe the design and synthesis of novel Mn(Salen) complexes with general acid–base catalytic functionality, inspired by the reaction mechanism of catalase. As expected, these Mn(Salen) complexes showed superior catalase-like activity and selectivity, while retaining moderate SOD-like activity. An unsubstituted pyridyl group worked well as a functionality to promote catalase-like activity. The introduced functionality did not alter the redox potential suggesting that the auxiliary-modified complex acted as an acid–base catalyst analogous to catalase. We believe that our approach provides a new design principle for sophisticated catalyst design. Further, the compounds described here appear to be good candidates for use in antioxidant therapy.



INTRODUCTION

Reactive oxygen species (ROS), represented by hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^•$), and superoxide ($O_2^{-•}$), are naturally formed during biological metabolic reactions under aerobic conditions. Overproduction of ROS often causes cell injury through oxidation of cellular components, such as lipids, proteins, and nucleic acids.[1,2] These pathological events have important roles in aging and also in many disorders, including cancer, rheumatoid arthritis, stroke, cardiac infarction, Parkinson's disease, and Alzheimer's disease.[3−7] ROS have also been reported to be modulators of a number of cell signaling pathways.[8,9]

Superoxide dismutase (SOD) and catalase are metalloproteins that catalyze dismutation reactions, which detoxify $O_2^{-•}$ and H_2O_2, respectively, as follows:

$$\text{SOD: } 2O_2^{-•} \rightarrow O_2 + H_2O_2$$

$$\text{Catalase: } 2H_2O_2 \rightarrow O_2 + 2H_2O$$

Oxidative stress is traditionally defined as an imbalance between ROS production and antioxidant defense against ROS. A consequence of oxidative stress is an increase in the formation of oxidized cellular macromolecules. Thus, antioxidant therapies have been considered for a wide variety of disorders associated with oxidative stress, as well as for reducing the injurious effects of radiation exposure. Indeed, many therapeutic strategies using antioxidants to decrease oxidative stress and restore redox balance have already shown promising in vivo results.[10−13]

There are three main classes of ROS scavengers, namely antioxidant enzymes, stoichiometric antioxidants, and synthetic catalytic antioxidants. Attempts to use antioxidant enzymes, such as SOD and catalase as therapeutic agents have met with some success in animals but not in humans.[14] Pharmacokinetic issues, including difficulty of delivery, short half-life in the blood and immunogenic responses, are major obstacles to the use of enzymes in humans. Stoichiometric antioxidants, which react with ROS to form less toxic species, must be administered in high doses to produce a sufficient antioxidant effect, because of their low rates of reaction with ROS and their limited ability to be recycled endogenously. Compounds in this class include naturally occurring vitamins C and E, trolox (water-soluble derivative of vitamin E), curcumin, and flavonoids.[12] From a clinical point of view, synthetic catalytic scavengers of ROS would be superior to the other classes of agents because they can in principle overcome the above disadvantages.

Catalytic synthetic scavengers of ROS react rapidly with ROS to form less-reactive species. During the course of the reaction, the synthetic molecules are not consumed but are regenerated. The majority of catalytic antioxidants are designed with redox-active metal centers that catalyze the dismutation reaction of $O_2^{-•}$ and/or H_2O_2 through a mechanism that is similar to the

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mode of action of the active-site metals of SOD and catalase. Several synthetic catalytic antioxidants have been reported, and most of them are metal complexes containing transition metals such as iron, manganese, or copper (manganese is most common).[10−12] These molecules include metal complexes of porphyrins (ex. AEOL10150), Salen (ex. EUK-113, EUK-134) and cyclic polyamines (ex. M40403), as illustrated in Figure 1.[15−18] Whereas manganese complexes of polyamines scavenge

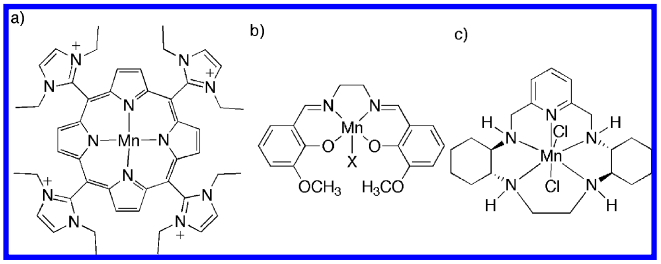

Figure 1. Representative catalytic antioxidants. (a) AEOL10150, (b) EUK-113 (X = OAc), EUK-134 (X = Cl), (c) M40403.

only $O_2^{-\bullet}$ (SOD-mimetic activity), porphyrin−manganese complexes and Salen−manganese complexes (Mn(Salen)) detoxify both $O_2^{-\bullet}$ and H_2O_2 (SOD- and catalase-mimetic activities). Such dual functions are advantageous for therapeutics, because the products of SOD- and catalase-like reactions are completely nontoxic (O_2 and H_2O), whereas the product of SOD-like reaction (H_2O_2) remains toxic. Mn(Salen) complexes seem a particularly attractive platform, because they have low molecular weight, which is advantageous for orally active drugs, and their synthesis is relatively simple and straightforward.[19] The efficacy of Mn(Salen) complexes has been demonstrated in many animal models of human diseases.[11,20] In addition to oxidative stress, cells may be exposed to nitrosative stresses from certain nitrogen oxides, and in this connection, Mn(Salen) complexes were also reported to convert reactive nitric oxide ($^{\bullet}NO$) and peroxynitrite ($ONOO^-$) to benign species.[21]

Thus, highly active Mn(Salen) complexes are of interest from the standpoint of both sophisticated catalyst design and development of clinical drugs to treat ROS-associated diseases. To design such complexes, our approach was to increase the catalase-like activity of Mn(Salen) by arranging an appropriate auxiliary proximal to the Mn atom to create an environment resembling the interior of the active site of an enzyme. We have already reported catalase-mimetic Mn(Salen) complexes with an auxiliary that is three-dimensionally fixed by a cyclopentane ring fused to the Salen structure, and a ureido group was effective for enhancing catalase-like activity in neutral aqueous media under near-physiological conditions.[22]

Now, we report the design, synthesis, and evaluation of novel Mn(Salen) complexes in which a general acid−base catalyst auxiliary is introduced proximal to the Mn atom to create an environment resembling the interior of the active site of catalase aiming at enhanced H_2O_2 dismutation rate and increased substrate selectivity. The molecular design was inspired by the structure and reaction mechanism of catalase. The effectiveness of the auxiliary was evaluated in terms of changes of catalase-like, peroxidase-like, and SOD-like activities.

RESULTS AND DISCUSSION

Molecular Design. We previously designed and synthesized auxiliary-fixed Mn(Salen) complexes and found that a ureido group significantly enhanced the catalase-like activity, probably due to its H_2O_2 recognition ability (**1**, Figure 2). Urea

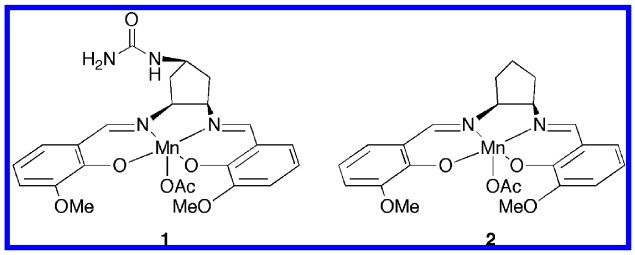

Figure 2. Chemical structure of Mn(Salen) **1** with a ureido group auxiliary, and **2** without the auxiliary.[22]

forms a stable complex with H_2O_2 via two hydrogen bonds. As a parent Mn(Salen) structure, we focused on EUK-113 (Figure 1) because of its simple structure and relatively strong SOD- and catalase-like activities.[16,19,20] Our strategy to fix the auxiliary at an appropriate position over the Mn(Salen) plane was to introduce a rigid bicyclo[3,3,0]octane structure such that the cyclopentane ring holds the auxiliary fixed on the syn side of Mn(Salen). The presence of the expected cis−syn form and highly planar Mn(Salen) moiety was confirmed by single crystal X-ray diffraction analysis of **1** confirming that the cyclopentane unit is an appropriate scaffold for auxiliary incorporation.[22] In addition to **1**, we synthesized complex **2** as a conformational control molecule without an auxiliary,[22] and this was also used in the present study.

To design a new auxiliary, we considered the putative catalase-like reaction mechanism of Mn(Salen) complexes (Figure 3) and the reported mechanism of catalase (Figure

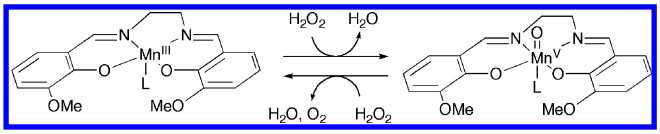

Figure 3. Putative catalase-like reaction mechanism of Mn(Salen) complexes.

4). Theoretical studies of the mechanism of Mn(Salen) have been reported by Abanskin et al.[23,24] The reaction is considered to involve two steps, as shown in Figure 3, that is, high-valent oxo-Mn(Salen) formation via O−O bond cleavage of HOO−Mn(Salen), followed by oxo-Mn(Salen) deoxygenation with generation of an oxygen molecule. In the case of catalase, a histidine residue in the active site works as general acid−base catalyst to accelerate the formation of high-valent iron intermediate, known as compound I (part a of Figure 4).[25−27] As shown in part b of Figure 4, compound I is putatively reduced by H_2O_2 via a His-mediated mechanism that involves the distal His acting as an acid−base catalyst to mediate transfer of a proton, although the precise mechanism is still controversial.[26,28−31] The apparent mechanistic similarity between the Mn(Salen) and catalase reactions prompted us to incorporate a general acid−base catalyst moiety close to the active site of Mn(Salen). Functionalities such as imidazole or pyridine would work as general acid−base catalysts assisting both high-valent intermediate formation and H_2O_2 oxidation. Further, high-valent oxo-Mn(Salen) can potentially oxidize various substrates besides H_2O_2, but incorporation of an acid−base catalyst moiety should promote selective oxidation of H_2O_2. If the formed high-valent intermediate selectively

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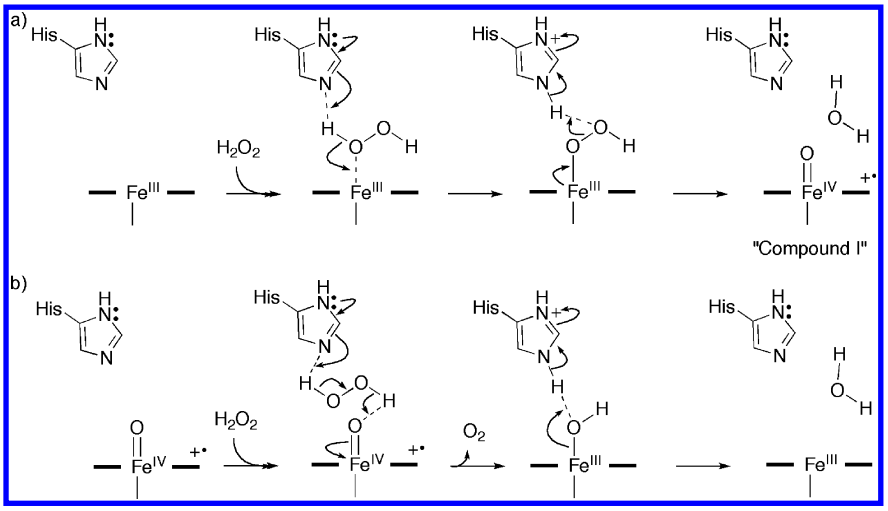

Figure 4. Role of general acid–base catalyst moiety in the proposed mechanism of catalase. (a) formation of high-valent iron intermediate, compound I, (b) ionic mechanism of H_2O_2 oxidation.

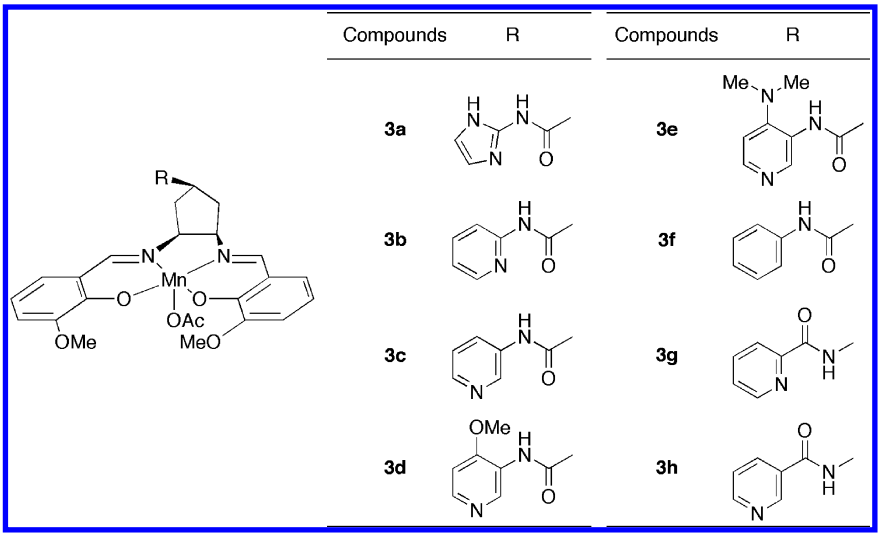

Figure 5. Designed Mn(Salen) complexes.

oxidizes H_2O_2, prooxidant activity of Mn(Salen) would be suppressed.

The designed Mn(Salen) complexes are illustrated in Figure 5. These complexes contain an imidazolyl or a pyridyl functionality, which is expected to provide acid–base catalytic activity. Compound **3f**, which contains a phenyl group, was used as a control compound to identify the effect of an aromatic moiety.

Synthesis. Compounds **3a–f** were synthesized according to part a of Scheme 1. Carboxylic acid **4**, which was synthesized according to our previously reported protocol,[22] was condensed with the appropriate amine to give amides **5a–f**. Amines used in the preparation of **5a–c** and **5f** were commercial products; amines for the preparation of **5d** and **5e** were synthesized according to parts b and c of Scheme 1, respectively. Diazides **5a–f** were reduced by catalytic hydrogenation followed by condensation with *o*-vanillin to afford Salen ligands **6a–f**. Metal insertion into **6a–f** gave the desired Mn(Salen) complexes **3a–f**. Compounds **3g–h** were synthesized according to part d of Scheme 1. After conversion of carboxylic acid **4** to amine **12** via Curtius rearrangement, similar protocols to Scheme 1a were employed. In this case, diazides **5g–h** were reduced by means of the Staudinger reaction.

Catalase-Like Activity. The catalase-like activities of the prepared complexes, along with EUK-113, **1**, and **2**, were determined (Table 1). The concentration of molecular oxygen, which was formed from H_2O_2 by the reaction of the Mn(Salen) complexes, was monitored in real time as described in the Experimental Section.[19] Initial rates and maximal amounts of oxygen formation are shown in Table 1, both of which are useful parameters to compare catalase-like activities.[19,22] The latter provides an indication of the total number of substrate turnovers completed prior to inactivation of Mn(Salen). As previously reported by Doctrow et al.[16,19] and by us,[22] Mn(Salen) complexes are inactivated under the catalase assay conditions. In our case, each complex was bleached within 2 min from the start of the reaction and oxygen evolution stopped accompanied by catalyst decomposition as shown in Figure S1 of the Supporting Information. No catalyst precipitation was observed at the end of the reaction. The mechanism of inactivation may involve peroxidative decomposition, but currently unknown. The relative instability of

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Scheme 1. Synthesis of Mn(Salen); Reagent and conditions: (a) condensation, Experimental Section for reaction details; (b) Pd−C, H_2, MeOH; (c) *o*-vanillin, MeOH; (d) $Mn(OAc)_2{\cdot}4H_2O$, MeOH; (e) KNO_3, H_2SO_4; (f) MeI, K_2CO_3, DMF, 70 °C; (g) 10% Pd−C, H_2, MeOH; (h) HNO_3, H_2SO_4, H_2O; (i) $SnCl_2$, MeOH, 50 °C; (j) $(COCl)_2$, CH_2Cl_2, reflux; (k) NaN_3, *n*-Bu_4NCl, CH_2Cl_2/H_2O; (l) toluene, 100 °C; (m) NaOH, H_2O; (n) α-picolinic acid (5g) or nicotinic acid (5h), 2-chloro-1-methylpyridinium iodide, TEA, CH_2Cl_2; (o) PPh_3, THF/H_2O, 60 °C; (p) *o*-vanillin, MeOH; (q) $Mn(OAc)_2{\cdot}4H_2O$, MeOH

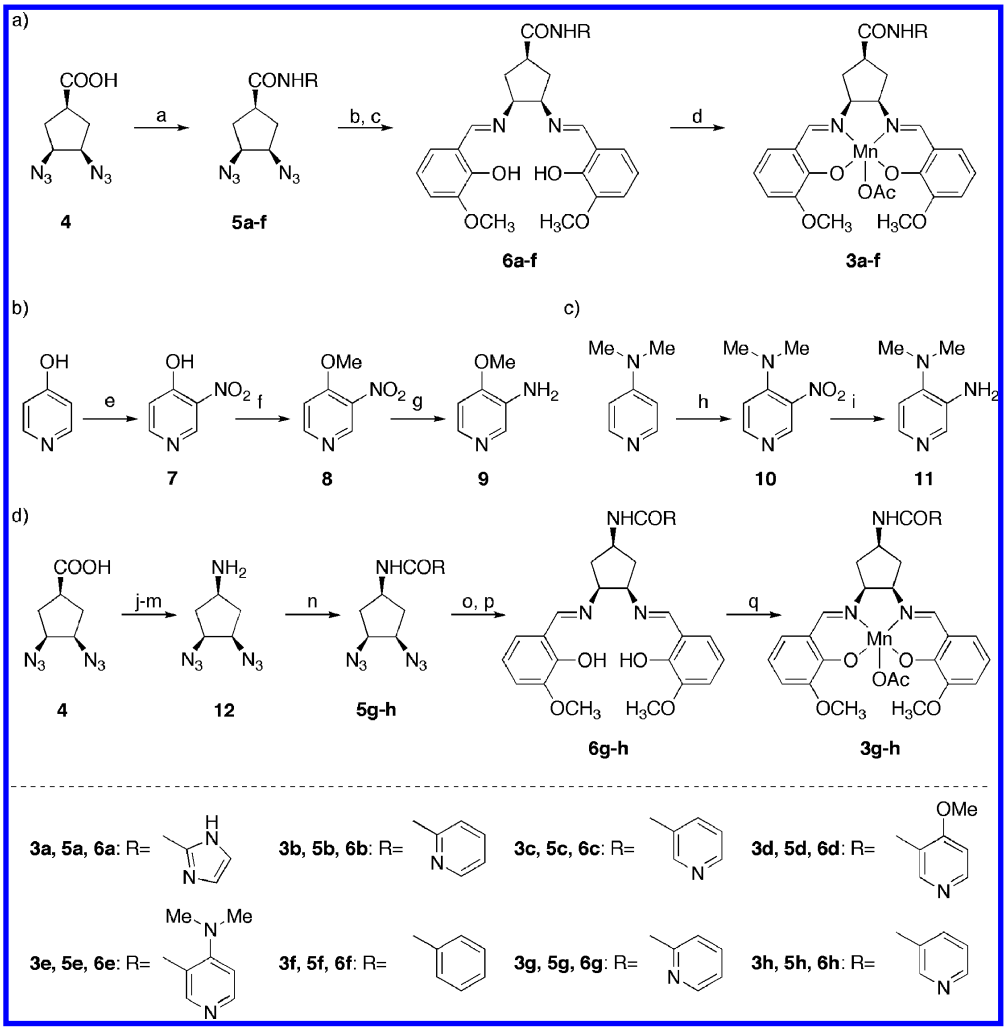

Table 1. Catalase-Like Activities of Mn(Salen) Complexes[a]

compounds	initial rate (μM O_2/min)	end point (maximal μM O_2)
EUK-113	97[b]	52[b]
1	238[b]	122[b]
2	110[b]	82[b]
3a	83	36
3b	268	143
3c	290	144
3d	105	52
3e	76	47
3f	96	42
3g	180	135
3h	281	167

[a]Catalase-like activities were determined by real-time monitoring of molecular oxygen generation, as described in the Experimental Section. [b]Although the general tendency is similar, these data are different from previously reported values,[22] probably due to differences in the experimental setup.

Mn(Salen) complexes would explain that turnover numbers remain 10−15 times.

Although the activity of imidazolyl-substituted Mn(Salen) **3a** was lower than that of parent EUK-113, the complexes bearing an unsubstituted pyridyl group (**3b**, **3c**, **3g**, **3h**) showed higher activities than EUK-113. The activity of phenyl-substituted Mn(Salen) **3f** was lower than that of the parent EUK-113 suggesting that the general acid−base catalytic nature of the pyridyl group is important to enhance the catalase-like activity. Compound **3g** exhibited lower activity than the other pyridyl derivatives (**3b**, **3c**, **3h**). The reason for this might be that the pyridyl group of **3g** shows lower basicity than the other pyridyl derivatives. To evaluate the effect of basicity, we introduced an electron-donating-group-substituted pyridyl ring into Mn(Salen) (**3d**, **3e**). The activities of these compounds, however, were considerably lower than that of Mn(Salen) bearing an unsubstituted pyridyl group. This result might be due to modification of the conformation of the pyridyl group to a less favorable form by the electron-donating groups.

There is a report that a modified Mn(Salen)-type catalase mimic with a carboxy functionality as an auxiliary showed greatly enhanced catalase-like activity in CH_2Cl_2/methanol.[32] In contrast, we found that a basic functional group, such as a pyridyl group, enhanced the catalase-like activity, whereas a

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carboxy group had a negative effect, in agreement with a previous report.[22] These different results probably reflect differences of auxiliary position and solvent system. DFT study by Abashkin et al. showed that no energetic advantages were found for the assisted proton-transfer mechanism with participation of an ancillary water molecule as a base on the catalase-like reaction of Mn(Salen).[23,24] However, water molecule is much less basic than pyridine or imidazole, and the water as a base is not fixed at an appropriate position intramolecularly. Their result derived from calculation would not be directly applied to our case.

Peroxidase-Like Activity. Mn(Salen) complexes are known to exhibit peroxidase activity, which is consistent with catalase-type function. Catalase is well-known to perform both peroxidative and catalatic reactions.[33] In the putative catalytic cycle of hydrogen peroxide dismutation by Mn(Salen), a high-valent oxo-Mn species is presumed to be generated as an active intermediate (Figure 3). Oxo−Mn(Salen) species generally oxidize not only H_2O_2 but also other oxido-labile compounds. Therefore, we examined the oxidation of various external substrates (peroxidase-like activity) by Mn(Salen) complexes using a colorimetric assay with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a substrate.[19] The results indicated that Mn(Salen) complexes have broad peroxidase substrate specificity.

Peroxidase-mimetic activities of the Mn(Salen) complexes are summarized in Table 2. The complexes with higher catalase-like activity also tended to show increased peroxidase-like activity (**1**, **3b**, **3c**, **3h**). Surprisingly, however, their peroxidase-like activities were comparable to, or even lower than, that of EUK-113, whereas significant enhancement of catalase-like activity was observed. This result indicated that Mn(Salen) with an unsubstituted pyridyl or a ureido group reacts more selectively with H_2O_2 than does EUK-113, and this may result in an increase of the catalase-like activity end point.

Table 2. Peroxidase-Like Activities of Mn(Salen) Complexes[a]

compound	initial rate (μM ABTS/min)
EUK-113	15.8
1	14.8
2	8.3
3a	6.5
3b	13.5
3c	14.7
3d	7.3
3e	8.1
3f	6.9
3g	9.6
3h	17.9

[a]Peroxidase-like activities were evaluated by means of ABTS oxidation assay as described in the Experimental Section.

The reaction of Mn(III) with O═Mn(V) could form Mn(IV)−O−Mn(IV) (μ-oxo dimer). However, the catalase-like reaction and the peroxidase-like reaction using ABTS were found to obey pseudofirst-order kinetics on varying Mn(Salen) concentration apparently in the case of complexes **1** and **2** (parts a and b of Figure S2 of the Supporting Information). These results are thought to support that the catalyst form is not changed during the reaction.

Cyclic Voltammetry. Introduction of an acid−base catalytic auxiliary clearly enhanced catalase-like activity and reduced peroxidase-like activity. This behavior is expected to be due to the general acid−base catalytic nature of the auxiliary, but there is also a possibility that the auxiliary may directly affect the electronic environment of Mn. Thus, the redox potentials of selected Mn(Salen) complexes were examined; the complexes with an unsubstituted pyridyl group were chosen, since they showed strong catalase-like activity. The measurement was conducted in DMSO because of the limited solubility of Mn(Salen). The voltammograms showed one cathodic peak and one anodic peak in the scan range from −1.5 to 0 V representing a quasi-reversible couple for all derivatives (Supporting Information). Table 3 shows the redox potentials of Mn(Salen) complexes with an unsubstituted pyridyl group and EUK-113. All the compounds showed rather similar redox potentials in the range from −0.68 to −0.63 V. The redox potentials were independent of the catalase-like activities suggesting that the auxiliary-mediated enhancement of the catalase-like activities of Mn(Salen) complexes was due to the function of the auxiliary as an acid−base catalyst.

Table 3. Redox Potentials of Mn(Salen) Complexes[a]

compound	$E_{1/2}$ (V)
EUK-113	−0.68
2	−0.68
3b	−0.65
3c	−0.65
3g	−0.66
3h	−0.63

[a]Cyclic voltammograms were measured in DMSO (the Experimental Section).

SOD-Like Activity. EUK-113 is known to have both catalase-like and SOD-like activities.[19] Although the molecular design strategy of compounds **3a**−**3h** was aimed at improvement of the catalase-like activity and selectivity, the effect of the auxiliary on SOD-like activity is also of interest. A certain level of SOD-like activity would make these compounds more promising as drug candidates for antioxidant therapy. M40403-type catalytic SOD mimetics with an auxiliary over the plane of the Mn complex have been reported, and the pyridyl auxiliary had a positive effect on their SOD-like activity.[34] Therefore, SOD-like activities of our complexes were evaluated by use of the standard cytochrome c method,[35,36] which is based on inhibition of cytochrome c reduction by $O_2^{-\bullet}$ generated in a xanthine/xanthine oxidase system. The IC_{50} values of our Mn(Salen) complexes are shown in Table 4. All the complexes showed SOD-like activities similar to, or even more potent than, that of EUK-113 suggesting that these compounds, especially **3b** and **3c**, are superior to EUK-113 as drug candidates for antioxidant therapy.

CONCLUSIONS

We designed and synthesized novel Mn(Salen) complexes equipped with a general acid−base catalytic functionality, inspired by the reaction mechanism of catalase. As expected, these Mn(Salen) complexes showed superior catalase-like activity and selectivity while retaining moderate SOD-like activity. An unsubstituted pyridyl group worked well as an auxiliary to promote catalase-like activity. The redox potential of Mn(Salen) was not affected by the introduced group

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Table 4. SOD-Like Activities of Mn(Salen) Complexes[a]

compound	IC_{50} (nM)
EUK-113	130
1	80
2	110
3a	146
3b	86
3c	87
3d	132
3e	112
3f	44
3g	111
3h	135

[a]SOD-like activities were measured in 50 mM potassium phosphate buffer (pH 7.4) as described in the Experimental Section.

suggesting that the auxiliary-modified complex works as an acid−base catalyst with functionality mimicking that of catalase, in accordance with the design strategy. We believe that our approach provides a new design principle for sophisticated catalyst design. Further, the compounds described here appear to be good candidates for use in antioxidant therapy.

EXPERIMENTAL SECTION

General. All reagents and solvents were of the highest commercial quality and were used without further purification unless otherwise noted. Thin layer chromatography (TLC) was performed on Merck precoated plates (silica 60 F_{254}, 0.25 mm) and bands were visualized by fluorescence quenching or stained with cerium molybdate. ^{1}H NMR spectra were recorded on a JEOL GSX-400 at 400 MHz. ^{13}C NMR spectra were recorded on a JEOL JNM-LA 500 at 125 MHz. Chemical shifts are reported in δ value (ppm) relative to tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on JASCO FT/IR-680 Fourier-transform infrared spectrophotometer. Fast atom bombardment mass spectroscopy (FAB-MS) was done with a JEOL JMS-LCMATE or JEOL JMS-700 mass spectrometer using 3-nitrobenzyl alcohol as the matrix. Electron ionization mass spectra (EI-MS) and high-resolution mass spectra (HRMS) were obtained with a JEOL JMS-SX102A. Column chromatography was performed on BW-300 (Fuji Silysia Chemical Ltd.). UV−vis spectra were recorded on a JASCO U-550 UV/vis spectrophotometer. Cyclic voltammograms were recorded on ALS/CHI Electrochemical Analyzer Model 700A.

Synthesis of (1*R*,3*R*,4*S*)-3,4-Diazido-*N*-(1*H*-imidazol-2-yl)-cyclopentanecarboxamide (5a). To a solution of **4** (497 mg, 2.53 mmol) in DMF (5 mL) were added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (732 mg, 3.82 mmol), 1-hydroxybenzotriazole (HOBt) (587 mg, 3.83 mmol), and triethylamine (3.5 mL, 25.1 mmol). The mixture was stirred at room temperature for 30 min, and then 2-aminoimidazole sulfate (791 mg, 2.99 mmol) was added to the solution and stirring was continued at room temperature for 9 h. The solvent was removed under reduced pressure and the residue was diluted with sat. $NaHCO_3$ (50 mL). The aqueous mixture was extracted with CH_2Cl_2 (50 mL × 3). The combined organic solution was dried over Na_2SO_4 and concentrated. The residue was purified by recrystallization (CH_2Cl_2/*n*-hexane) to give **5a** as a white crystalline solid (60.1 mg, 23.0 μmol, 9%): IR (KBr): ν 1625, 2108, 2790, 3360 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.32 (dd, 4H, J = 6.3, 8.5 Hz), 3.08 (quin, 1H, J = 8.5 Hz), 3.93−3.98 (m, 2H), 6.79 (s, 2H), 10.90 (brs, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 32.1, 40.3, 63.8, 143.2, 159.5, 172.9; MS (EI): m/z 261 (M); HRMS (EI) calcd for $C_9H_{11}N_9O$ 261.1087 (M), found: 261.1092.

Synthesis of (1*R*,3*S*,4*R*)-3,4-Diazido-*N*-(pyridin-2-yl)-cyclopentanecarboxamide (5b). Triethylamine (800 μL), 2-aminopyridine (271 mg, 2.88 mmol), and 2-chloro-1-methylpyridinium iodide (734 mg, 2.87 mmol) were added to a solution of **4** (467 mg, 2.38 mmol) in CH_2Cl_2 (4 mL). The reaction mixture was stirred at room temperature for 16 h, then diluted with H_2O (50 mL), and extracted with CH_2Cl_2 (50 mL × 3). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 4/1; v/v) to give **5b** (261 mg, 959 μmol, 40%) as a yellow oil: IR (neat): ν 3303, 2104, 1696 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.05−2.12 (m, 2H), 2.26−2.33 (m, 2H), 3.07−3.14 (m, 1H), 4.11 (quin, 2H, J = 4.4 Hz), 7.10 (ddd, 1H, J = 0.98, 4.9, 7.2 Hz), 7.75 (ddd, 1H, J = 1.6, 7.2, 8.8 Hz), 8.20 (brd, 1H, J = 8.55 Hz), 8.29 (ddd, 1H, J = 0.98, 1.6, 4.9 Hz), 9.19 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 32.5, 41.4, 64.2, 114.6, 120.1, 138.7, 147.4, 151.5, 173.5; MS (FAB, NBA): m/z 273 (M+H); HRMS (EI) calcd for $C_{11}H_{12}N_8O$ 272.1134 (M), found: 272.1130.

Synthesis of (1*R*,3*S*,4*R*)-3,4-Diazido-*N*-(pyridin-3-yl)-cyclopentanecarboxamide (5c). To a solution of **4** (47.7 mg, 243 μmol) in DMF (2 mL) and triethylamine (110 μL) were added 3-aminopyridine (27.0 mg, 287 μmol) and O-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluoroborate (TBTU) (118 mg, 368 μmol). The reaction mixture was stirred at 60 °C for 5 h, then diluted with sat. $NaHCO_3$ (10 mL), and extracted with *n*-hexane/AcOEt (1/1; v/v) (10 mL × 3). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 1/4; v/v) to give **5c** (32.7 mg, 120 μmol, 49%) as a brown oil: IR (KBr): ν 1673, 2102, 2955 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.96−2.03 (m, 2H), 2.11−2.17 (m, 2H), 3.13−3.20 (m, 1H), 4.25−4.30 (m, 2H), 7.33 (dd, 1H, J = 4.9, 9.0 Hz), 8.01 (brd, 1H, J = 9.0 Hz), 8.26 (brd, 1H, J = 4.9 Hz), 8.73 (brs, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 33.5, 42.3, 66.0, 125.3, 129.0, 137.6, 141.7, 145.1, 176.1; MS (FAB, NBA): m/z 273 (M+H); HRMS (FAB, NBA) calcd for $C_{11}H_{13}N_8O$ 273.1213 (M), found: 273.1214.

Synthesis of 3-Nitropyridin-4-ol (7).[37] To conc. H_2SO_4 (5 mL) was added 4-hydroxypyridine (1.02 g, 10.6 mmol) in portions, followed by slow addition of KNO_3 (2.17 g, 21.5 mmol). The resulting mixture was heated to 100 °C for 3 h, then cooled to 0 °C and neutralized with NH_4OH. The precipitate was collected by filtration and dried under reduced pressure to give **7** as a yellow solid (660 mg, 4.71 mmol, 44%): ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.45 (d, 1H, J = 7.6 Hz), 7.76 (d, 1H, J = 7.6 Hz), 8.77 (s, 1H).

Synthesis of 4-Methoxy-3-nitropyridine (8).[37] To a solution of **7** (312 mg, 2.24 mmol) in DMF (5 mL) were added K_2CO_3 (472 mg, 3.42 mmol) and iodomethane (160 μL, 2.56 mmol). The reaction mixture was stirred at 70 °C for 10 h, then concentrated, and the residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 8/92; v/v) to give **8** as a yellow solid (328 mg, 2.14 mmol, 96%): ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.76 (s, 3H), 6.49 (d, 1H, J = 7.8 Hz), 7.75 (dd, 1H, J = 2.2, 7.8 Hz), 8.91 (d, 1H, J = 2.2 Hz); MS (EI) m/z 154 (M).

Synthesis of 4-Methoxypyridin-3-amine (9).[38] To a solution of **8** (277 mg, 1.81 mmol) in MeOH (10 mL) was added 10% Pd/C (30.4 mg). The reaction mixture was stirred at room temperature for 1 h under hydrogen, then filtered through a pad of Celite and concentrated to give **9** as a brown solid (243 mg, 1.96 mmol, quant): ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.59 (s, 3H), 4.63 (brs, 2H), 5.92 (d, 1H, J = 7.1 Hz), 7.02 (d, 1H, J = 2.3 Hz), 7.32 (dd, 1H, J = 2.3, 7.1 Hz).

Synthesis of (1*R*,3*R*,4*S*)-3,4-Diazido-*N*-(4-methoxypyridin-3-yl)cyclopentanecarboxamide (5d). To a solution of **4** (450 mg, 2.29 mmol) in CH_2Cl_2 (10 mL) were added molecular sieves 4A and triethylamine (319 μL, 2.29 mmol). The reaction mixture was stirred at room temperature under argon for 1 h, then 2-chloro-1-methylpyridinium iodide (643 mg, 2.52 mmol) was added and stirring was continued at 0 °C under argon for 3 h. To this solution, 4-methoxypyridine **9** (389 mg, 3.14 mmol) was added and stirring was continued at 0 °C for 5 h. The reaction mixture was filtered through a pad of Celite and the residue was diluted with 2 M NaOH (50 mL), and extracted with CH_2Cl_2 (50 mL x 3). The combined organic solution was washed with brine (50 mL), dried over Na_2SO_4, concentrated and purified by GPC to give **5d** (224 mg, 742 μmol,

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32%) as a white solid: IR (KBr): ν 1632, 2097, 2940, 3235 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.17−2.26 (m, 4H), 2.99 (quin, 1H, J = 8.5 Hz), 3.68 (s, 3H), 3.86−3.90 (m, 2H), 6.38 (d, 1H, J = 7.3 Hz), 7.24 (dd, 1H, J = 2.2, 7.3 Hz), 8.78 (d, 1H, J = 2.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 32.0, 40.9, 44.5, 63.6, 113.7, 128.1, 129.7, 138.2, 169.4, 172.1; MS (EI): m/z 302 (M); HRMS (EI) calcd for $C_{12}H_{14}N_8O_2$ 302.1240 (M), found: 302.1239.

Synthesis of *N*,*N*-Dimethyl-3-nitropyridin-4-amine (10).[39] To a solution of *N*,*N*-dimethyl-4-aminopyridine (1.00 g, 8.19 mmol) in conc. H_2SO_4 (10 mL) was added dropwise 60% HNO_3 (750 μL) in conc. H_2SO_4 (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 5 h, then neutralized with Na_2CO_3 and extracted with $CHCl_3$ (100 mL × 3). The combined organic layers were passed through a short silica gel column to give **10** as a yellow oil (1.10 g, 6.57 mmol, 80%): ^1H NMR ($CDCl_3$, 400 MHz): δ 3.00 (s, 6H), 6.78 (d, 1H, J = 6.1 Hz), 8.28 (d, 1H, J = 6.1 Hz), 8.78 (s, 1H); MS (EI): m/z 167 (M).

Synthesis of N^4,N^4-Dimethylpyridine-3,4-diamine (11).[40] To a solution of **10** (584 mg, 3.49 mmol) in MeOH (10 mL) was added tin(II) chloride dihydrate (3.94 g, 17.4 mmol). The reaction mixture was stirred at 50 °C for 7 h, then the solvent was removed under reduced pressure and the residue was diluted with 2 M NaOH (50 mL). The resulting aqueous mixture was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layers were dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 4/96, v/v) to give **11** as a brown solid (194 mg, 1.41 mmol, 40%): ^1H NMR ($CDCl_3$, 400 MHz): δ 2.74 (s, 6H), 3.68 (brs, 2H), 6.79 (d, 1H, J = 5.4 Hz), 7.97 (d, 2H, J = 5.4 Hz); MS (EI): m/z 137 (M).

Synthesis of (1*R*,3*R*,4*S*)-3,4-Diazido-*N*-(4-(dimethylamino)-pyridin-3-yl)cyclopentanecarboxamide (5e). To a solution of **4** (239 mg, 1.22 mmol) in CH_2Cl_2 (7 mL) were added molecular sieves 4A and triethylamine (170 μL, 1.22 mmol). The reaction mixture was stirred at room temperature under argon for 1 h, then 2-chloro-1-methylpyridinium iodide (344 mg, 1.35 mmol) was added and stirring was continued at −20 °C under argon for 2 h. To the resulting solution, 4-dimethylaminopyridine (301 mg, 2.47 mmol) and N^4,N^4-dimethylpyridine-3,4-diamine **11** (222 mg, 1.62 mmol) were added and stirring was continued at the same temperature for 3 h. The reaction mixture was filtered through a pad of Celite and the residue was diluted with 1 M NaOH (50 mL). The resulting aqueous solution was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by GPC to give **5e** (112 mg, 355 μmol, 29%) as a white solid; IR (KBr): ν 1680, 2100, 2895, 2944, 3161 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.21−2.28 (m, 2H), 2.33−2.40 (m, 2H), 2.76 (s, 6H), 2.98−3.07 (m, 1H), 3.95−4.00 (m, 2H), 6.92 (d, 1H, J = 5.6 Hz), 8.23 (d, 1H, J = 5.6 Hz), 8.29 (brs, 1H), 9.14 (s,1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 32.4, 41.8, 42.8, 64.0, 113.4, 127.6, 143.9, 146.2, 150.9, 171.8; MS (EI) m/z 315 (M); HRMS (EI) calcd for $C_{13}H_{17}N_9O$ 315.1557(M), found: 315.1551.

Synthesis of (1*R*,3*S*,4*R*)-3,4-Diazido-*N*-phenylcyclopentane-carboxamide (5f). To a solution of **4** (92.2 mg, 470 μmol) in DMF (10 mL) were added aniline (60 μL, 658 μmol), HOBt (87.5 mg, 571 μmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (109 mg, 570 μmol) at 0 °C. The reaction mixture was stirred at the same temperature for 5 h, then the solvent was removed under reduced pressure and the residue was diluted with 1 N HCl (10 mL). The resulting aqueous mixture was extracted with CH_2Cl_2 (10 mL × 3). The combined organic layers were washed with sat. $NaHCO_3$ (10 mL) and brine (10 mL), and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 7/3, v/v) to give **5f** (93.5 mg, 345 μmol, 75%) as a colorless oil: IR (neat): ν 694, 758, 1768, 2094, 2941, 3066, 3305 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.17−2.24 (m, 2H), 2.27−2.34 (m, 2H), 2.85−2.93 (m, 1H), 3.92 (brt, 2H, J = 4.8 Hz), 7.11 (brt, 1H, J = 7.7 Hz), 7.31 (brt, 2H, J = 7.7 Hz), 7.50 (brd, 2H, J = 7.7 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 32.4, 41.8, 63.9, 120.1, 124.5, 128.9, 137.7, 172.2; MS (FAB, NBA): m/z 272 (M+H); HRMS (EI): m/z calcd for $C_{12}H_{13}N_7O$ 271.1182 (M), found 271.1184.

Synthesis of (1*R*,3*R*,4*S*)-3,4-Diazidocyclopentanamine (12). To a solution of **4** (2.03 g, 10.4 mmol) in CH_2Cl_2 (50 mL) was added oxalyl chloride (1.33 mL, 15.5 mmol). The reaction mixture was refluxed under argon for 5 h, and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (10 mL). To this solution, tetra-*n*-butylammonium chloride (30.0 mg, 108 μmol) was added. The solution was cooled to 0 °C, and then sodium azide (1.01 g, 15.5 mmol) in H_2O (5 mL) was carefully added dropwise. The reaction mixture was stirred at 0 °C for 14 h and then diluted with CH_2Cl_2 (50 mL), washed with H_2O (100 mL × 3) and brine (100 mL) and dried over Na_2SO_4. The organic layer was concentrated and the residue was added dropwise to toluene (30 mL). The resulting solution was heated to 100 °C, stirred at the same temperature for 1 h, then cooled to room temperature and added dropwise to 2.5 N NaOH (100 mL). The resulting solution was stirred at the same temperature for 18 h, then acidified to pH 1 with 2 M HCl, and washed with CH_2Cl_2 (50 mL × 3). The aqueous layer was basified to pH 14 with 2 M NaOH, and extracted with CH_2Cl_2 (100 mL × 3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated to give **12** (1.07 g, 6.40 mmol, 62%) as a pale brown oil: IR (neat): ν 1591, 2106, 2939, 3359 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.61−1.67 (m, 2H), 2.29−2.37 (m, 2H), 3.37−3.44 (m, 1H), 3.80−3.85 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 39.2, 48.8, 63.7; MS (FAB, NBA): m/z 168 (M+H); HRMS (FAB, NBA): m/z calcd for $C_5H_{10}N_7$ 168.0998 (M+H), found 168.0994.

Synthesis of *N*-((1*R*,3*R*,4*S*)-3,4-Diazidocyclopentyl)-Picolinamide (5g). To a solution of **12** (108 mg, 645 μmol) in CH_2Cl_2 (5 mL) were added triethylamine (270 μL, 1.95 mmol), 2-chloro-1-methylpyridinium iodide (247 mg, 968 μmol) and α-picolinic acid (95.4 mg, 775 μmol). The reaction mixture was stirred at room temperature under argon for 40 h, then diluted with sat. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (10 mL × 3). The combined organic layers were concentrated and the residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 6/4, v/v) and GPC to give **5g** (126 mg, 462 μmol, 72%) as a yellow oil: IR (neat): ν 1523, 1687, 2083, 2980, 3057, 3369 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.87 (brdt, 2H, J = 5.4, 14 Hz), 2.46−2.53 (m, 2H), 3.94−3.98 (m, 2H), 4.64 (ddt, 1H, J = 5.4, 8.4 Hz), 7.44 (ddd, 1H, J = 1.0, 4.8, 7.8 Hz), 7.85 (dt, 1H, J = 1.7, 7.8 Hz), 8.17 (dt, 1H, J = 1.0, 7.8 Hz), 8.32 (brd, 1H, J = 8.4 Hz), 8.58 (ddd, 1H, J = 1.0, 1.7, 4.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 36.4, 45.7, 63.6, 122.2, 126.2, 137.3, 148.2, 149.4, 163.6; MS (FAB, NBA): m/z 273 (M+H); HRMS (EI) calcd for $C_{11}H_{12}N_8O$ 272.1134 (M), found: 272.1141.

Synthesis of *N*-((1*R*,3*R*,4*S*)-3,4-Diazidocyclopentyl)-nicotinamide (5h). To a solution of **12** (106 mg, 636 μmol) in CH_2Cl_2 (5 mL) were added triethylamine (270 μL, 1.95 mmol), 2-chloro-1-methylpyridinium iodide (267 mg, 1.04 mmol), and nicotinoic acid (94.7 mg, 769 μmol). The reaction mixture was stirred at room temperature under argon for 40 h, then diluted with sat. $NaHCO_3$ (20 mL) and extracted with CH_2Cl_2 (10 mL × 3). The combined organic layers were concentrated and the residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 3/7; v/v) and GPC to give **5h** (96.5 mg, 354 μmol, 56%) as a white solid: IR (KBr): ν 1560, 1637, 2113, 2976, 3081, 3228 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.85 (ddd, 2H, J = 4.4, 5.2, 15 Hz), 2.44−2.52 (m, 2H), 3.98−4.02 (m, 2H), 4.64−4.73 (m, 1H), 6.84 (brd, 1H, J = 7.6 Hz), 7.39 (ddd, 1H, J = 0.7, 4.9, 7.9 Hz), 8.10 (brdt, 1H, J = 2.0, 7.9 Hz), 8.73 (dd, 1H, J = 1.2, 4.8 Hz), 8.98 (d, 1H, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 36.6, 46.9, 63.9, 123.5, 129.8, 135.0, 148.0, 152.3, 164.8; MS (FAB, NBA): m/z 273 (M+H); HRMS (FAB, NBA) calcd for $C_{11}H_{13}N_8O$ 273.1213 (M), found: 273.1212.

Preparation of Salen Ligand: General Method A. To a solution of diazide in MeOH (0.05−0.1 M) was added 10% palladium on carbon (10% w/w). The reaction mixture was stirred under hydrogen at room temperature until the reduction was completed (monitored by TLC), then filtered through a pad of Celite. The filtrate was concentrated to give the desired diamine. A mixture of this diamine (1 equiv) and *o*-vanillin (2 equiv) in MeOH (0.05−0.1 M) was stirred at room temperature. The precipitate formed during the reaction was collected by filtration to give the desired Salen ligand.

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Preparation of Salen Ligand: General Method B. To a solution of diazide in THF/H_2O (10/1; v/v) (0.02−0.3 M) was added triphenylphosphine (2.4 equiv). The reaction mixture was stirred at 60 °C until the reduction was completed (monitored by TLC), then diluted with 1 M HCl and washed with CH_2Cl_2. The aqueous layer was concentrated and the residue was purified by cation exchange column chromatography to give the desired diamine. A mixture of diamine (1 equiv) and *o*-vanillin (2 equiv) in MeOH (0.02−0.6 M) was stirred at room temperature. The reaction mixture was concentrated and purified by silica gel column chromatography or GPC to give the desired Salen ligand.

Synthesis of 6a. Compound **6a** was prepared from **5a** (27.8 mg, 106 μmol) according to general method A as a yellow solid (32.4 mg, 67.9 μmol, 64%). This compound was used for the next step without further purification: ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.17−2.24 (m, 2H), 2.38−2.45 (m, 2H), 3.20−3.27 (m, 1H), 3.73 (s, 6H), 4.04−4.09 (m, 2H), 6.71 (s, 2H), 6.79 (t, 2H, *J* = 7.9 Hz), 7.00 (d, 4H, *J* = 7.9 Hz), 8.54 (s, 2H), 12.38 (brs, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 35.3, 40.7, 55.5, 71.4, 114.5, 117.8, 118.3, 123.2, 141.0, 147.8, 151.0, 165.4, 172.8; MS (EI): *m*/*z* 477 (M); HRMS (EI) calcd for $C_{25}H_{27}N_5O_5$ 477.2012 (M); found: 477.2004.

Synthesis of 6b. Compound **6b** was prepared from **5b** (261 mg, 959 μmol) according to general method A as a yellow solid (350 mg, 716 μmol, 75%). This compound was used for the next step without further purification: IR (KBr): ν 3464, 3327, 1688, 1631 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.33−2.40 (m, 2H), 2.46−2.52 (m, 2H), 3.35−3.43 (m, 1H), 3.87 (s, 6H), 4.16 (quin, 2H, *J* = 4.5 Hz), 6.79 (t, 2H, *J* = 7.9 Hz), 6.87 (dd, 2H, *J* = 1.6, 7.9 Hz), 6.90 (dd, 2H, *J* = 1.6, 7.9 Hz), 7.05 (ddd, 1H, *J* = 0.99, 4.9, 7.2 Hz), 7.71 (ddd, 1H, *J* = 1.6, 7.2, 8.7 Hz), 7.93 (s, 1H), 8.20 (brd, 1H, *J* = 8.7 Hz), 8.29 (ddd, 1H, *J* = 0.99, 1.6, 4.9 Hz), 8.39 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 35.4, 42.8, 56.1, 72.7, 114.0, 114.2, 118.1, 118.5, 120.0, 123.2, 138.4, 147.9, 148.3, 151.3, 151.3, 165.3, 174.3; MS (FAB, NBA): *m*/*z* 490 (M+H); HRMS (FAB, NBA) calcd for $C_{27}H_{29}N_4O_5$ 489.2138 (M+1); found: 489.2147.

Synthesis of 6c. Compound **6c** was prepared from **5c** (119 mg, 437 μmol) according to general method A as a yellow solid (129 mg, 264 μmol, 60%). This compound was used for the next step without further purification: IR (KBr): ν 1254, 1628, 1692, 2936, 3342 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.13−2.20 (m, 2H), 2.33−2.39 (m, 2H), 3.00 (brs, 1H), 3.47−3.51 (m, 1H), 3.75 (s, 6H), 3.45−3.53 (m, 2H), 6.81 (t, 2H, *J* = 7.9 Hz), 7.01 (dd, 2H, *J* = 1.3, 7.9 Hz), 7.03 (dd, 2H, *J* = 1.3, 7.9 Hz), 7.34 (dd, 1H, *J* = 4.7, 8.3 Hz), 8.07 (ddd, 1H, *J* = 1.5, 2.5, 8.3 Hz), 8.26 (dd, 1H, *J* = 1.5, 4.7 Hz), 8.61 (s, 2H), 8.77 (d, 1H, *J* = 2.5 Hz), 10.3 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 35.4, 41.4, 55.7, 71.4, 115.2, 117.4, 118.4, 123.0, 123.1, 135.6, 140.7, 140.8, 147.7, 151.1, 165.4, 165.5, 173.9; MS (FAB, NBA): *m*/*z* 489 (M+H); HRMS (FAB, NBA) calcd for $C_{27}H_{29}N_4O_5$ 489.2138 (M+1), found: 489.2130.

Synthesis of 6d. Compound **6d** was prepared from **5d** (194 mg, 640 μmol) according to general method B as a yellow solid (75.0 mg, 144 μmol, 23%). This compound was used for the next step without further purification: IR (KBr): ν 1251, 1271, 1568, 1631, 2938, 3310, 3521 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.40−2.55 (m, 4H), 3.20−3.28 (m, 1H), 3.73 (s, 3H), 3.84 (s, 6H), 4.03−4.08 (m, 2H), 6.42 (d, 1H, *J* = 7.2 Hz), 6.76 (t, 2H, *J* = 7.8 Hz), 6.86 (t, 4H, *J* = 7.8 Hz), 7.24 (dd, 1H, *J* = 2.0, 7.2 Hz), 8.33 (s, 2H), 8.50 (s, 1H), 8.89 (d, 1H, *J* = 2.0 Hz), 13.19 (brs, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 34.4, 42.3, 44.6, 56.0, 72.2, 113.7, 114.1, 117.9, 118.6, 123.4, 128.3, 130.0, 138.1, 148.3, 151.3, 164.7, 169.6, 172.7; MS (EI): *m*/*z* 518 (M); HRMS (EI) calcd for $C_{28}H_{30}N_4O_6$ 518.2165 (M), found: 518.2163

Synthesis of 6e. Compound **6e** was prepared from **5e** (112 mg, 355 μmol) according to general method B as a yellow solid (26.8 mg, 50.4 μmol, 14%). This compound was used for the next step without further purification: IR (KBr): ν 1253, 1627, 1679, 2938, 3253, 3368 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.52−2.53 (m, 4H), 2.75 (s, 6H), 3.31−3.34 (m, 1H), 3.82 (s, 6H), 4.08 (brs, 2H), 6.76 (t, 2H, *J* = 7.9 Hz), 6.84−6.87 (m, 5H), 8.14 (s, 1H), 8.22 (d, 1H, *J* = 5.2 Hz), 8.35 (s, 2H), 9.13 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 34.7, 34.9, 42.4, 56.0, 71.8, 112.5, 114.3, 117.6, 117.8, 118.4, 123.3, 143.9, 144.8, 148.2, 151.6, 152.3, 164.9, 172.5; MS (FAB, NBA): *m*/*z* 532 (M+1); HRMS (FAB, NBA) calcd for $C_{29}H_{34}N_5O_5$ 532.2560 (M+1); found: 532.2553.

Synthesis of 6f. Compound **6f** was prepared from **5f** (61.7 mg, 227 μmol) according to general method A and purified by preparative TLC. Crude Salen ligand **6f** was further purified by recrystallization from $CHCl_3$ as yellow needles (24.4 mg, 50.0 μmol, 22%): IR (KBr): ν 253, 1530, 1627, 1674, 2937, 3310, 3444 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz): δ 2.39−2.53 (m, 4H), 3.24−3.26 (m, 1H), 3.77 (s, 6H), 4.09−4.11 (m, 2H), 6.73 (t, 2H, *J* = 7.9 Hz), 6.91 (dd, 2H, *J* = 1.3, 7.9 Hz), 6.94 (dd, 2H, *J* = 1.3, 7.9 Hz), 7.06 (brt, 1H, *J* = 7.7 Hz), 7.28 (brt, 2H, *J* = 7.7 Hz), 7.58 (brd, 2H, *J* = 7.7 Hz), 8.42 (s, 2H); ^{13}C NMR (CD_3OD, 125 MHz): δ 36.5, 43.7, 56.5, 73.0, 115.9, 118.8, 119.6, 121.3, 124.9, 1124.9, 129.7, 149.9, 154.5, 159.3, 166.7, 175.2; MS (FAB, NBA): *m*/*z* 488 (M+H); HRMS (FAB, NBA) calcd for $C_{28}H_{30}N_3O_5$ 488.2185 (M+1), found: 488.2191.

Synthesis of 6g. Compound **6g** was prepared from **5g** (112 mg, 410 μmol) according to general method B as a yellow solid (47.7 mg, 97.6 μmol, 24%). This compound was used for the next step without further purification: IR (neat): ν 1254, 1632, 1669, 2936, 3368 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.04−2.12 (m, 2H), 2.70−2.77 (m, 2H), 3.85 (s, 1H), 4.03−4.08 (m, 2H), 4.79−4.89 (m, 1H), 6.78 (t, 2H, *J* = 7.8 Hz), 6.86 (dd, 2H, *J* = 1.7, 7.8 Hz), 6.88 (dd, 2H, *J* = 1.7, 7.8 Hz), 7.40 (ddd, 1H, *J* = 1.2, 4.9, 7.8 Hz), 7.82 (dt, 1H, *J* = 1.7, 7.8 Hz), 8.15 (dt, 1H, *J* = 1.2, 7.8 Hz), 8.32−8.35 (m, 3H), 8.56 (ddd, 1H, *J* = 1.2, 1.7, 4.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 38.7, 46.9, 56.0, 71.1, 114.2, 118.0, 118.4, 121.9, 123.2, 126.1, 137.1, 148.2, 149.4, 151.2, 152.6, 164.1, 164.9; MS (FAB,NBA): *m*/*z* 489 (M+1); HRMS (EI) calcd for $C_{27}H_{29}N_4O_5$ 488.2060 (M), found: 488.2056.

Synthesis of 6h. Compound **6h** was prepared from **5h** (48.2 mg, 177 μmol) according to general method B as a yellow solid (26.0 mg, 53.2 μmol, 30%). This compound was used for the next step without further purification: IR (KBr): ν 1254, 1626, 1649, 2956, 3378 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.06−2.10 (m, 2H), 2.69−2.76 (m, 2H), 3.84 (s, 6H), 4.01−4.04 (m, 2H), 4.86−4.95 (m, 1H), 6.77−6.81 (m, 3H), 6.87 (dd, 2H, *J* = 1.5, 8.0 Hz), 6.90 (dd, 2H, *J* = 1.5, 8.0 Hz), 7.30 (dd, 1H, *J* = 4.9, 7.8 Hz), 8.15 (dt, 1H, *J* = 2.0, 7.8 Hz), 8.38 (s, 2H), 8.68 (dd, 1H, *J* = 1.5, 4.9 Hz), 9.06 (d, 1H, *J* = 2.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 39.4, 47.7, 56.0, 71.5, 114.4, 117.8, 118.4, 119.5, 123.2, 129.6, 134.6, 148.2, 148.7, 151.6, 152.1, 165.2, 165.3; MS (FAB, NBA): *m*/*z* 489 (M+H); HRMS (EI) calcd for $C_{27}H_{29}N_4O_5$ 488.2060 (M), found: 488.2055

Preparation of Mn(Salen) Complex: General Method C. To a solution of Salen ligand (1 equiv) in MeOH (0.04−0.13 M) was added manganese(II) acetate tetrahydrate (1 equiv). The reaction mixture was stirred at room temperature for 1−2 h, then concentrated, and the residue was thoroughly washed with acetone to give the desired Mn(Salen) complex.

Synthesis of 3a. Compound **3a** was prepared from **6a** (62.0 mg, 130 μmol) according to general method C as a brown solid (69.7 mg, 118 μmol, 91%): IR (KBr): ν 1253, 1551, 1612, 2938, 3412 cm^{-1}; MS (FAB, NBA): *m*/*z* 530 (M-OAc); Anal. Calcd for $C_{27}H_{28}MnN_5O_7\cdot 4H_2O$: C, 49.02; H, 5.49; N, 10.59. Found: C, 49.32, H, 5.49, N, 10.25.

Synthesis of 3b. Compound **3b** was prepared from **6b** (336 mg, 687 μmol) according to general method C as a brown solid (357 mg, 594 μmol, 86%): IR (KBr): ν 3414, 1687, 1550 cm^{-1}; MS (FAB, NBA): *m*/*z* 541 (M-OAc); Anal. Calcd for $C_{29}H_{29}MnN_4O_7\cdot 2H_2O$: C, 54.72; H, 5.23; N, 8.80. Found: C, 54.40, H, 5.15, N, 8.81.

Synthesis of 3c. Compound **3c** was prepared from **6c** (41.8 mg, 85.6 μmol) according to general method C as a brown solid (47.0 mg, 78.3 μmol, 91%): IR (KBr): ν 1254, 1551, 1616, 2937, 3420 cm^{-1}; FAB-MS (NBA): *m*/*z* 541 (M-OAc); Anal. Calcd for $C_{29}H_{29}MnN_4O_7\cdot 2H_2O$: C, 54.72; H,5.23; N, 8.80. Found: C, 54.78; H, 5.30; N, 8.85.

Synthesis of 3d. Compoud **3d** was prepared from **6d** (60.6 mg, 117 μmol) according to general method C as a brown solid (49.0 mg, 77.7 μmol, 66%): IR (KBr): ν 1224, 1252, 1551, 1612, 2939, 3418 cm^{-1}; MS (FAB, NBA): *m*/*z* 571 (M-OAc); Anal. Calcd for

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$C_{30}H_{31}MnN_4O_8 \cdot 5H_2O$: C, 50.00; H, 5.73; N, 7.77. Found: C, 50.01; H, 5.64; N, 7.57.

Synthesis of 3e. Compound **3e** was prepared from **6e** (26.4 mg, 49.7 μmol) according to general method C as a brown solid (27.4 mg, 42.6 μmol): IR (KBr): ν 1253, 1551, 1609, 2937, 3418 cm^{-1}; MS (FAB, NBA): *m/z* 584 (M-OAc) Anal. Calcd for $C_{39}H_{29}MnN_4O_7 \cdot AcOH \cdot 2H_2O$: C, 53.45; H, 5.35; N, 8.04. Found: C, 53.43; H, 5.08; N, 8.44.

Synthesis of 3f. Compound **3f** was prepared from **6f** (17.1 mg, 35.1 μmol) according to general method C as a brown solid (20.9 mg, 34.9 μmol, 38%): IR (KBr): ν 1251, 1553, 1614, 2956, 3436 cm^{-1}; MS (FAB, NBA): *m/z* 540 (M-OAc); HRMS (FAB, NBA) calcd for $C_{28}H_{27}MnN_3O_5$ 540.1331 (M-OAc); found: 540.1327. HPLC data: part a of Figure S6 of the Supporting Information.

Synthesis of 3g. Compound **3g** was prepared from **6g** (21.4 mg, 43.8 μmol) according to general method C as a brown solid (10.1 mg, 16.8 μmol, 38%): IR (KBr): ν 1253, 1550, 1613, 2927, 3435 cm^{-1}; MS (FAB, NBA): *m/z* 541 (M-OAc); HRMS (FAB, NBA) calcd for $C_{27}H_{26}MnN_4O_5$ 541.1284 (M-OAc); found: 541.1292. HPLC data: part b of Figure S6 of the Supporting Information.

Synthesis of 3h. Compound **3h** was prepared from **6h** (20.9 mg, 42.8 μmol) according to general method C as a brown solid (16.6 mg, 27.6 μmol, 65%): IR (KBr): ν 1252, 1615, 1645 cm^{-1}; FAB-MS (NBA): *m/z* 541 (M-OAc); HRMS (FAB, NBA) calcd for $C_{27}H_{26}MnN_4O_5$ 541.1284 (M-OAc); found: 541.1279. HPLC data: part c of Figure S6 of the Supporting Information.

Measurement of Catalase-Like Activity. The catalase-like activity of the salen−manganese complexes, that is, catalytic activity of H_2O_2 dismutation, was measured by use of an assay described by Doctrow et al.[19] with some modifications.[22] The activity was determined by monitoring the concentration of molecular oxygen generated from hydrogen peroxide using a Clark-type polarographic oxygen electrode. To the solution of Mn(Salen) complex (10 μM) in 50 mM sodium phosphate buffer (pH 7.4) was added 10 mM (final concentration) H_2O_2. The reaction mixture was maintained at 25 ± 0.2 °C under argon. Initial rates were calculated by linear regression using the data from 0 to 5 s of the reaction.

Measurement of SOD-Like Activity. SOD-like activity of salen−manganese complexes was measured by using the cytochrome c assay originally described by McCord and Fridovich[35] with some modifications.[19,36] Catalysis of the dismutation of $O_2^{-\bullet}$ was measured by using the xanthine/xanthine oxidase system as the source of $O_2^{-\bullet}$ and cytochrome c^{III} as the indicating scavenger of $O_2^{-\bullet}$. Rates of reduction of cytochrome c were followed by measuring absorption at 550 nm. SOD-like activity was evaluated in terms of inhibition of cytochrome c reduction by $O_2^{-\bullet}$ generated from the xanthine/xanthine oxidase system. The assay was performed in 50 mM potassium phosphate buffer (pH 7.4) at 25 ± 0.2 °C. The reaction mixture contained 1.0 mM cytochrome c^{III}, 1200 units/mL catalase, 50 mM xanthine, and sufficient xanthine oxidase to produce a rate of reduction of cytochrome c^{III} at 550 nm of 0.025 absorbance unit per min. Omission of ethylenediaminetetraacetic acid (EDTA) was critical, because EDTA appears to generate artifacts.[41] The IC_{50} values of Mn(Salen) complexes were determined from concentration-dependent plots performed at 4 different concentrations.

Measurement of peroxidase-like activity. Peroxidase-like activity was evaluated by monitoring ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) oxidation spectrophotometrically, as described by Doctrow et al.[19] with some modifications. Assay mixtures consisted of 50 mM sodium phosphate (pH 7.4), 0.5 mM ABTS, 0.2 mM H_2O_2, and 10 μM Mn(Salen) complex. Assays were conducted at 25 ± 0.2 °C. ABTS oxidation was estimated using an $\Delta\varepsilon_{740}$ of 20 300 $M^{-1}cm^{-1}$.[19]

Cyclic Voltammetry. A platinum electrode was used as the working electrode and all potentials were recorded versus Ag/AgCl as a reference electrode. A solution of 1.0 mM Mn(Salen) complex in anhydrous DMSO containing 0.1 M tetra-*n*-butylammonium perchlorate as a supporting electrolyte was prepared, and purged with argon prior to the experiment.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Cyclic voltammograms of Mn(Salen)s (EUK-113, compounds **2**, **3b**, **3c**, **3g**, **3h**). 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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