Enhanced catalase-like activity of manganese salen complexes in water: effect of a three-dimensionally fixed auxiliary†

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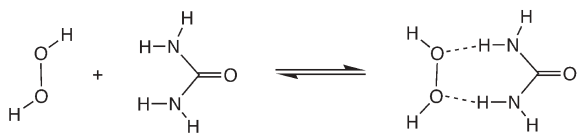
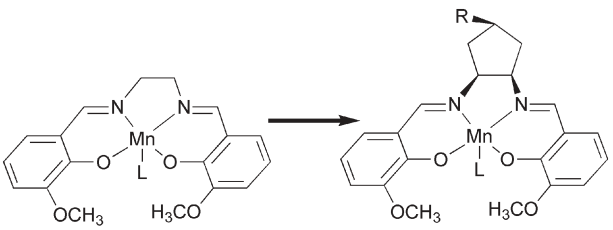
A new Mn(Salen) complex bearing an ureido group as an auxiliary that is three-dimensionally fixed by a cyclopentane ring fused to the Salen structure was developed. This compound exhibited considerably higher catalase-like activity than the original Mn(Salen), *i.e.*, the cyclopentane-fused Mn(Salen) without the auxiliary, under near-physiological conditions.

Reactive oxygen species (ROS), generated from mitochondria, macrophages and *via* enzymatic reactions, often cause cell injury and have been implicated in a number of diseases as well as aging.[1] Antioxidant therapies have been considered for a wide variety of disorders associated with oxidative stress. Synthetic catalytic scavengers of ROS would be clinically superior to stoichiometric ones.[2] Among them, Salen–manganese complexes (Mn(Salen)) seem promising, because they exhibit dual functions, *i.e.* superoxide dismutase- and catalase-mimetic activities, and the reaction mechanism is thought to resemble that of catalase.[3] Design of highly active Mn(Salen) complexes is of interest from the standpoint of both sophisticated catalyst design and development of candidate clinical drugs for ROS-associated diseases. 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. There have been a few reports showing that an intramolecularly attached carboxy group accelerates the catalytic oxidative activity of metalloporphyrins with hydrogen peroxide.[4,5] Here, we report catalase-mimetic Mn(Salen) complexes of a new type, with an auxiliary that is three-dimensionally fixed over the Salen plane.



Auxiliary candidates were designed based on the putative catalase-like reaction mechanism (Fig. 1) of Salen complexes.[3,6,7] The reaction is considered to involve a step of high-valent oxo-Mn(Salen) formation *via* the O–O bond cleavage of HOO–Mn(Salen) (Step A), and a step of two-electron oxidation of H_2O_2 with the active intermediate (Step B). We initially chose an ureido group as the auxiliary for accepting hydrogen peroxide (H_2O_2). Urea forms a stable complex with H_2O_2 *via* two hydrogen bonds.[8] Effective conversion of H_2O_2 into O_2 and H_2O can be expected, if the proximal ureido group recognizes and efficiently supplies H_2O_2 to the active center. Selectivity is an important property, because if the formed active intermediate selectively oxidizes H_2O_2, pro-oxidant activity of Mn(Salen) can be suppressed. We also chose a carboxy group as a candidate auxiliary, expecting acid catalysis to assist the O–O bond cleavage of HOO–Mn(Salen).[7] Several other functionalities were also selected to examine the range of effects of such an auxiliary. Our strategy to fix the auxiliary at an appropriate position over the Mn(Salen) plane is to introduce a rigid bicyclo[3,3,0]octane structure such that the cyclopentane ring held the auxiliary fixed at the *syn* side to Mn(Salen) (**2a–2f**). Methoxy groups on compound **2** were essential for an efficient reaction;[3] the presence of an electron-donating group on the salen ligand is thought to stabilize the high-valent manganese-oxo intermediate and/or to facilitate heterolytic cleavage of the O–O bond.[7]



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2a: R=CO_2Et
2b: R=CO_2H
2c: R=CH_2OH
2d: R=$NHCONH_2$
2e: R=$CONH_2$
2f: R=H

The designed urea-bearing Mn(Salen) complex was prepared as shown in Scheme 1. Nucleophilic ring opening of the *trans*-epoxide **3**[9] by azide was followed by mesylation of the hydroxyl group and S_N2 substitution by azide to give ethyl *cis-syn*-3,4-diazidocyclopentane carboxylate **5**. Hydrolysis of the obtained **5** gave **6**. Compound **6** was converted into the urea **7** *via* Curtius rearrangement. The urea-bearing diazide **7** was reduced by catalytic hydrogenation, then condensed with *o*-vaniline to afford the urea-bearing, cyclopentane-fused Salen ligand **8d**. Finally,

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† Electronic supplementary information (ESI) available: Synthetic details for all new compounds, kinetic study of catalase-like activity of **2d**, and crystal structure of **2d**. See DOI: 10.1039/b608846e

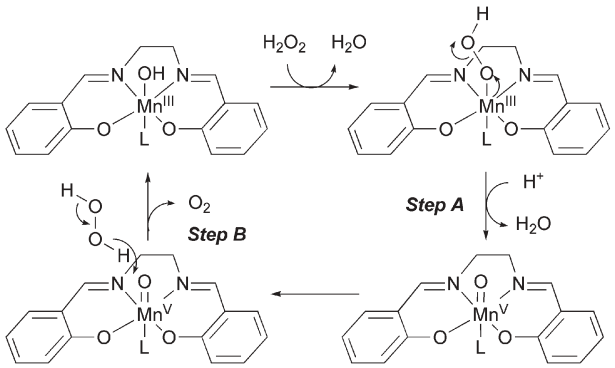
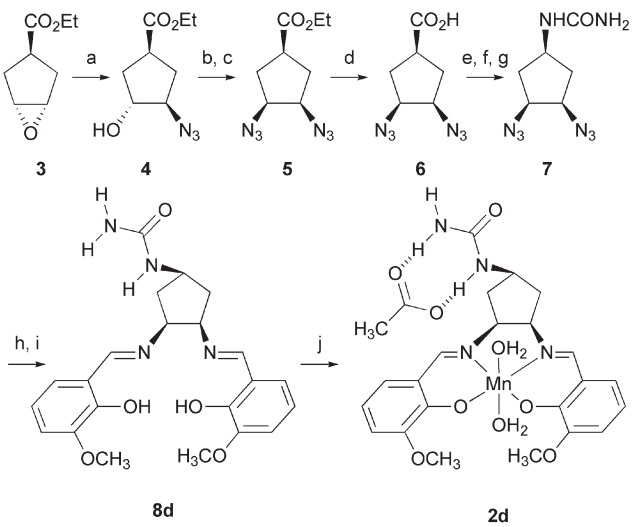


Fig. 1 Putative catalase-like reaction mechanism of Mn(Salen) complexes.

metal insertion into **8d** afforded the Mn(Salen) **2d**. The carboxy group-pendant Mn(Salen) **2b** was also obtained from **6** in the same manner. Other substituent-bearing Mn(Salen) complexes, **2a**, **2c**, **2e**, **2f**, were prepared analogously.†

The full molecular geometry of **2d** was elucidated by single-crystal X-ray diffraction analysis, and the ORTEP diagram is given in Fig. 2, showing the expected *cis-syn* form with two water molecules as axial ligands, and pairing of the ureido group with an acetate anion.‡ This result also shows that the Mn(Salen) moiety is highly planar.

Catalase-like activities of the prepared complexes were determined in aqueous phosphate buffer solution (pH 7.4) by real-time monitoring of molecular oxygen concentration according to the method of Doctrow *et al* (Fig. 3).[3] Our results and representative results taken from Doctrow *et al.*[3] are summarized in Table 1. Cyclopentane-fused Mn(Salen) without auxiliary **2f** had two fold higher activity than simple Mn(Salen) **1**. The presence of a hydroxymethyl group (**2c**) or an ethoxycarbonyl group (**2a**) on the cyclopentane ring had no effect on the activity of cyclopentane-fused



Scheme 1 Reagents and conditions: (a) NaN_3, NH_4Cl, H_2O/EtOH, reflux, 97%; (b) MsCl, Pyridine, CH_2Cl_2, rt; (c) NaN_3, Pyridine, DMF–H_2O, 130 °C, 73% (2 steps); (d) KOH, H_2O–EtOH, rt, 98%; (e) $(COCl)_2$, CH_2Cl_2, reflux; (f) NaN_3, $Bu_4N^+Cl^-$, H_2O–CH_2Cl_2, 0 °C; (g) NH_3, CH_2Cl_2, reflux, 78% (3 steps); (h) Pd/C/H_2, MeOH, rt; (i) *o*-vanillin, MeOH, rt, 72% (2 steps); (j) $Mn(OAc)_2{\cdot}4H_2O$, MeOH, rt, 91%.

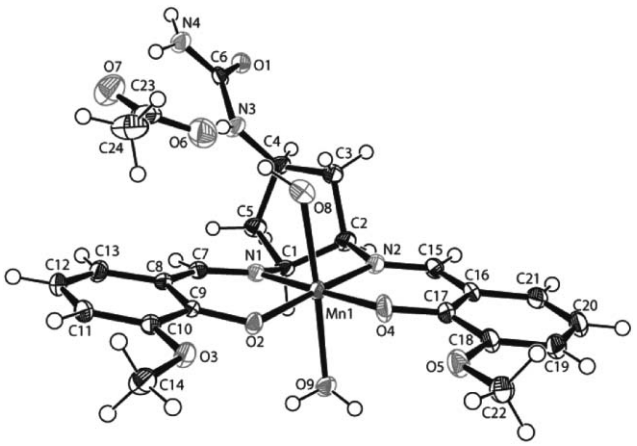


Fig. 2 ORTEP diagram of complex **2d**. Only one H atom is shown on water oxygen O8 as the other could not be located unequivocally. Thermal ellipsoids are drawn at 50% probability.

Mn(Salen). In contrast, ureido group-attached Mn(Salen) **2d** exhibited considerably higher activity than **2f**. This result clearly indicates that the neutral ureido group accelerates the catalase-like activity of cyclopentane-fused Mn(Salen). In order to confirm the effectiveness of the proximal ureido group, urea (up to 0.8 M) was added to the assay media of **2d**. Only slight decrease of the catalytic activity was observed, which suggests that the proximal ureido group contributed specifically to the activity enhancement.

Surprisingly, however, carboxy group-bearing Mn(Salen) **2b** was found to have much lower activity than the control compounds **2f**, **2a** and even **1**. There is a report concerning a modified Mn(Salophen)-type catalase mimic based on a similar concept to ours,[7] in which the use of carboxy group as an auxiliary greatly enhanced the catalase-like activity in MeOH–CH_2Cl_2 solvent. At first, we speculated that the low activity of **2b** could be

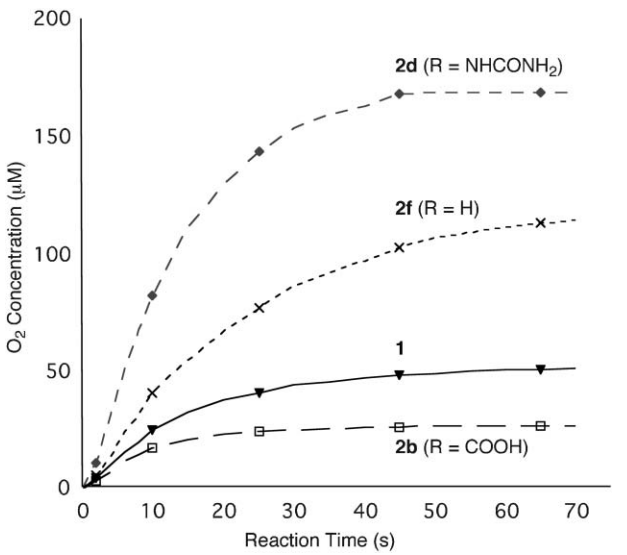
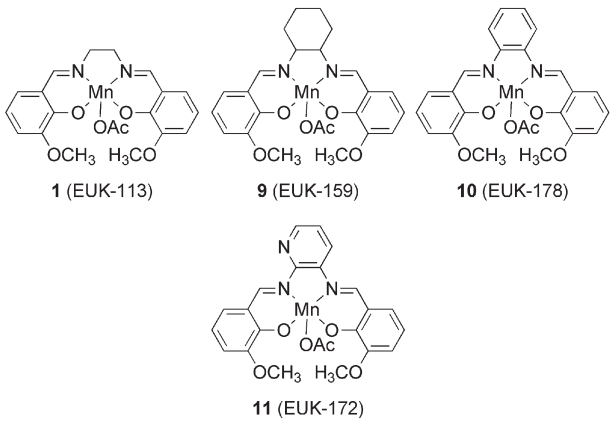


Fig. 3 Time course of catalase-like reaction. Catalase-like activity was examined by monitoring the conversion of H_2O_2 to O_2 using a Clark type polarographic oxygen electrode. To a solution of Mn(Salen) (10 μM) in 50 mM sodium phosphate buffer (pH 7.4), 10 mM (final concentration) H_2O_2 was added at 25 °C under Ar.

Table 1 Catalase-like activity of Mn(Salen) complexes

Compound	R	Initial Rate (μM O_2 min^{-1})	O_2 Concentration (Maximal μM O_2)
1 (EUK-113)	—	159 (260)[b]	52 (79)[b]
2a	CO_2Et	215	113
2b	CO_2H	119	27
2c	CH_2OH	190	113
2d	$NHCONH_2$	525	169
2e	$CONH_2$	190	49
2f	H	243	115
9 (EUK-159)	—	—[a] (251)[b]	—[a] (91)[b]
10 (EUK-178)	—	—[a] (814)[b]	—[a] (230)[b]
11 (EUK-172)	—	—[a] (1073)[b]	—[a] (300)[b]
Catalase (bovine liver; 29 units mL^{-1})	—	—[a] ($\sim$0.33 mM min^{-1})[b]	—



1 (EUK-113) **9** (EUK-159) **10** (EUK-178)

11 (EUK-172)

[a] Not measured in this study. [b] Data from ref. 3.

explained in terms of repulsive interaction between the carboxylate anion and H_2O_2. However, experiments carried out at pH 5.8 (phosphate buffer) and pH 3.0 (glycine buffer) showed that low pH did not enhance the catalytic activity of **2b**. This result suggests that carboxylate anion form is not primarily responsible for the low activity of **2b**. Hydrated carboxy group might have repulsive interaction with H_2O_2, or water of which hydrogen makes hydrogen bond to the carboxylic acid oxygen(s) of **2b** could repel H_2O_2; but further investigation would be needed to confirm this.

Although direct comparison of the data reported by Doctrow *et al.*[3] and by us is difficult (the assay conditions are different; we used neutral buffer (pH 7.4), while they used basic buffer (pH 8.1)), the catalase-like activity of **2d** seems comparable to those of the most active Mn(Salen) complexes currently known.

The H_2O_2–ureido group interaction was difficult to observe directly by NMR or other conventional methods. Therefore, a kinetic approach was adopted; a plot of initial rate of O_2 formation versus H_2O_2 concentration for compound **2d** showed a saturable curve, which was consistent with a Michaelis–Menten model.† This result suggests that the ureido group may form a complex with H_2O_2, like an enzyme-substrate complex. Complex **2d** is a rare example of a synthetic catalyst for the disproportionation of H_2O_2 that shows saturation kinetics at high substrate concentrations.[10]

In summary, we present a new strategy for enhancing the activity of Mn(Salen) complexes in neutral aqueous media under near-physiological conditions. Only the ureido group was effective on H_2O_2 decomposition among the functional groups examined, probably due to its H_2O_2 recognition ability. Our strategy should be applicable for the design of other highly active catalytic scavengers of active oxygen species for biological applications. Further investigation is in progress in our laboratory.

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Notes and references

‡ Crystal data for **2d**: $C_{24}H_{31}MnN_4O_9$, M = 574.46, a = 13.8291(11), b = 9.3189(8), c = 21.4664(18)Å, α = 90°, β = 108.220(1)°, γ = 90°, U = 2627.7(4) Å^3, T = 90 K, space group P $2_1/n$, Z = 4, μ(Mo-Kα) = 0.561 mm^{-1}, 12381 reflections collected, 5866 unique (R_{int} = 0.0273), R_1 = 0.0813 [$I > 2\sigma(I)$], wR_2 = 0.2742 (all data). CCDC 611674. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b608846e

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