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Mimicking the reduced, oxidized and azide inhibited form of manganese superoxide dismutase by mononuclear Mn compounds utilizing tridentate ligands

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Dedicated to Professor Helmut Werner on the occasion of his 70th birthday

Abstract

The manganese complexes $[Mn^{II}(Hbmimpm)_2(NO_3)](NO_3)\cdot Et_2O$ **(1)**, $[Mn^{III}(bmimpm)_2(OAc)]\cdot 2CH_2Cl_2$ **(2)**, and $[Mn^{III}(bmiapm)_2(OAc)]\cdot MeOH\cdot H_2O\cdot CH_2Cl_2$ **(3)** containing the new ligands Bis(1-methylimidazol-2-yl)-(4-methoxyphen-1-yl)methanol (Hbmimpm) and Bis[(1-methylimidazol-2-yl)](2-aminophenyl)methanol (Hbmiapm) were synthesized. They are good structural models for the reduced (**1**) and oxidized (**2**, **3**) form of manganese superoxide dismutase. All complexes were characterized by spectroscopic methods and X-ray structure analysis. Compounds **1** and **2** crystallize in the monoclinic space group $P2_1/c$ whereas complex **3** crystallizes in the monoclinic space group $P2_1/n$. The coordination sphere around the manganese cores is distorted octahedral with two corresponding tridentate ligands representing the protein ligands and one nitrate (**1**) or acetate (**2**, **3**) ion occupying two *cis* positions. Similar to the enzyme the Mn(III) complex **2** reacts with sodium azide. The obtained microcrystalline azide adduct was characterized by UV–Vis and IR spectroscopy.

Keywords: Superoxide dismutase; Tridentate ligands; Mononuclear manganese complexes; Crystal structures

1. Introduction

Superoxide dismutases catalyze the disproportionation of superoxide into oxygen and hydrogen peroxide (see Scheme 1):

Superoxide is the one electron reduced form of oxygen. It is a byproduct of O_2 metabolism in the cell [1–4]. Furthermore the appearance of superoxide anions is discussed together with oxidative damage in cells. Therefore superoxide dismutases play an important role to protect cells from oxidative damage. They are present in several organisms [5,6]. Besides the manganese containing superoxide dismutases from *Thermus thermophilus* [7], *Escherichia coli* [8] and human mitochondria [9–12] also Fe [13–20], Cu/Zn [21,22], and Ni SOD [23] are known.

The manganese containing SOD from the bacterium *T. thermophilus* is a homotetramer with one Mn^{II} ion per subunit. In the active site the central manganese(II) is coordinated by three histidine residues, one aspartate and a hydroxide ion in a trigonal bipyramidal fashion [7]. The equatorial plane is formed by two histidine residues and one aspartate whereas the third histidine residue and the hydroxide ion are found in axial positions.

In addition the azide inhibited form of Mn SOD of *T. thermophilus* is available [7]. Upon binding of azide, the coordination number increases from five to six. Additionally the bond between the oxygen's aspartate and the central manganese ion is elongated by 0.45 Å [24]. During the catalytic cycle the manganese ion changes its oxidation state between +II and +III [25].

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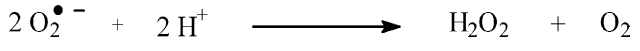
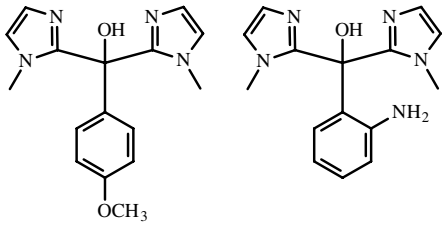


Scheme 1. Disproportionation of superoxide.



Hb mimpm Hb miapm

Scheme 2. Utilized tridentate ligands Hbmimpm and Hbmiapm.

Up to now three different classes of functional manganese SOD mimics are known. Pharmaceutical interest is focused on [Mn^{III}(salen)]-, [Mn^{III}(porphyrin)]- and [Mn^{II}([15]aneN_5)] complexes where salen is salicylidenethylendiimine, and [15]aneN_5 is pentaazacyclopentadecane [26]. Although none of these compounds are structural mimics of Mn SODs, the catalytic activity of these complexes places them within 10–100 orders of magnitude of the three characterized enzymes. A derivate of [Mn^{II}([15]aneN_5)] exceeding the enzyme activity has been developed using molecular modelling methods [27]. In general, model compounds for Mn SODs are of high interest in terms of synzymes (synthetic enzymes) [28] degrading toxic superoxide radical anions.

In this paper we report the synthesis, characterization and spectroscopic investigations of one model compound for the reduced form and two complexes for the oxidized form of Mn SOD using the ligands Bis(1-methylimidazol-2-yl)-(4-methoxyphen-1-yl)methanol (Hbmimpm) and Bis[(1-methylimidazol-2-yl)](2-aminophenyl)methanol (Hbmiapm) (see Scheme 2).

Furthermore a solution of **2** was treated with sodium azide. The reaction was monitored by UV–Vis spectroscopy and the resulting powder has been characterized by IR spectroscopy.

2. Experimental

2.1. Materials

All chemicals were of reagent grade and used as received.

2.2. Physical measurements

Elemental analyses were performed on a Perkin–Elmer 2400 Series 2 analyser, an Elementar Vario EL III analyser and a Heraeus CHN-O-Rapid analyser. IR spectra were recorded on a Perkin–Elmer Spectrum GX FT-IR spectrometer and a Bruker IFS 48 spectrometer in the range of 4000–400 cm^{-1}. Samples were prepared as KBr disks. UV–Vis spectra were measured at 25 °C in acetonitrile on a Hewlett-Packard 8453 diode array spectrometer using quartz cuvettes (1 cm). ^1H and ^{13}C NMR spectra were recorded on a Bruker WH 300 spectrometer.

2.3. Synthesis of the ligands

2.3.1. Synthesis of Bis(1-methylimidazol-2-yl)(4-methoxyphen-1-yl)methanol (Hbmimpm)

2.3.1.1. 4-Methoxybenzoicacid ethylester. Sulfuric acid (10 mL) was added dropwise to an ice cooled suspension of *p*-anisic acid (7.6 g, 50 mmol) in ethanol (150 mL). The resulting colorless solution was refluxed for 4 h. After the mixture was cooled to room temperature the solvent was evaporated off. The remaining highly viscous oil was added into the fivefold amount of an ice/water mixture. The resulting white emulsion was extracted three times with diethyl ether (50 mL) to isolate the crude product. The combined organic layers were washed twice with saturated potassium carbonate solution (25 mL) and twice with water (25 mL). The solvent was removed under vacuum after drying with magnesium sulfate to yield a colorless oil. Yield: 6.8 g, 75%. *Anal*. Calc. for $C_{10}H_{12}O_3$: C: 66.64; H: 6.72; Found: C: 67.02; H: 6.86%. ^1H NMR ($CDCl_3$) δ (ppm): 1.34 (t, 3H), 3.85 (s, 3H), 4.34 (q, 2H), 6.92 (d, 2H), 8.00 (d, 2H).

2.3.1.2. Hbmimpm Bis(1-methylimidazol-2-yl)(4-methoxyphen-1-yl)methanol. A solution of *N*-methylimidazole (6 mL, 75.4 mmol) in diethyl ether (abs., 200 mL) under argon atmosphere was cooled to −78 °C (suspension) and treated with 1.6 M *n*-butyllithium (55 mL, 75.4 mmol) and warmed to 0 °C to complete deprotonation. Afterwards it was cooled down again to −78 °C and 4-methoxybenzoicacid ethylester (6.8 g, 38 mmol) was added dropwise. The mixture was stirred for 12 h at 0 °C and quenched with water (50 mL). The resulting emulsion was extracted twice with diethyl ether (30 mL) and four times with chloroform (30 mL). The combined organic layers were dried over magnesium sulfate and the solvent was evaporated off. The remaining colorless oil was stored at −20 °C over night yielding a crude white product which was recrystallized from chloroform. Yield: 4.5 g, 40%. *Anal*. Calc. for $C_{16}H_{18}N_4O_2$: C: 64.41; H: 6.08; N: 18.78; Found: C: 64.61; H: 6.13; N: 18.44%. ^1H NMR ($CDCl_3$) δ (ppm): 3.38 (s, 6H), 3.77 (s, 3H), 6.82 (d, 2H), 6.84 (d, 2H), 6.93 (d, 2H), 6.98 (d, 2H). ^{13}C NMR ($CDCl_3$) δ (ppm): 34.7 (N–CH_3), 55.3 (O–CH_3), 77.2 (OH–C), 113.6 (phenyl–C), 123.4 (phe-

nyl–C), 125.9 (imidazole–C), 128.7 (imidazole–C), 134.2 (phenyl–C), 148.6 (imidazole–C), 159.5 (phenyl–C).

2.3.2. Synthesis of Bis[(1-methylimidazol-2-yl)](2-aminophenyl)methanol (Hbmiapm)

A solution of *N*-methylimidazole (12.5 mL, 158 mmol) in diethyl ether (abs., 300 mL) under argon atmosphere was cooled to −78 °C to yield a white suspension. 1.6 M *n*-butyllithium (100 mL, 160 mmol) was added carefully and the reaction mixture was allowed to warm up to 0 °C to complete deprotonation. Afterwards it was cooled down again to −78 °C and methyl-anthranilate (10.2 ml, 79 mmol) was added dropwise. The mixture was allow to warm up to 0 °C and stirred for 12 h at 0 °C. The reaction was quenched with water (50 mL) and the resulting emulsion was extracted twice with diethyl ether (40 mL) and five times with chloroform (30 mL). The combined organic layers were dried over magnesium sulfate and the solvent was removed under vacuum. The remaining colorless oil was stored at −20 °C over night yielding a crude yellow product which was washed with methanol/pentane. Yield: 4.6 g, 20%. *Anal.* Calc. for $C_{15}H_{17}N_5O$: C: 63.59; H: 6.05; N: 24.72; Found: C: 62.61; H: 6.28; N: 23.99%. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.44 (s, 6H), 4.24 (s, 2H), 6.27 (d, 1H), 6.60 (d, 1H), 6.64 (d, 1H), 6.88 (d, 2H), 6.91 (d, 2H), 7.11 (d, 1H). ^{13}C NMR ($CDCl_3$) δ (ppm): 34.9 (N–CH_3), 76.1 (OH–C), 117.6 (phenyl–C), 117.9 (phenyl–C), 123.4 (phenyl–C), 124.0 (imidazole–C), 126.1 (imidazole–C), 128.4 (phenyl–C), 129.8 (phenyl–C), 146.2 (phenyl–C), 159.5 (phenyl–C).

2.4. Synthesis of [Mn(Hbmimpm)$_2$(NO$_3$)](NO$_3$)·Et$_2$O (**1**)

The compound was prepared by reacting Hbmimpm (119 mg, 0.4 mmol) with $Mn(NO_3)_2 \cdot 4H_2O$ (50 mg, 0.2 mmol) in ethanol (10 mL) in the presence of acetic acid (0.5 mL). The solution was stirred for 1 h. Vapor diffusion of diethyl ether into the filtered solution yielded rhombic colorless crystals suitable for X-ray diffraction. Yield: 135 mg, 79% (with solvent molecule). M.p. 225 °C (decomposition). *Anal.* Calc. for $C_{32}H_{36}N_{10}MnO_{10}$ (without solvent molecule): C: 49.55; H: 4.68; N: 18.06; Found: C: 49.76; H: 4.83; N: 17.79%. IR (KBr): 3128 (m), 2961 (m), 2838 (w), 1608 (s), 1511 (s), 1496 (s), 1384 (vs), 1306 (s), 1283 (s), 1252 (vs), 1163 (m), 1033 (m), 902 (m), 761 (m), 733(w), 689 (w).

2.5. Synthesis of [Mn(bmimpm)$_2$(OAc)]·2CH$_2$Cl$_2$ (**2**) and reaction of **2** with sodium azide

2.5.1. Synthesis of [Mn(bmimpm)$_2$(OAc)]·2CH$_2$Cl$_2$ (**2**)

The compound was prepared by reacting Hbmimpm (119 mg, 0.4 mmol) with $Mn(OAc)_2 \cdot 4H_2O$ (49 mg, 0.2 mmol) in dichloromethane (10 mL) in the presence of triethylamine (0.25 mL). The solution was stirred for 2 h and filtered. The purple filtrate was layered with the same amount of *n*-hexane and stored at −5 °C to yield purple needle shaped crystals suitable for X-ray diffraction. Yield: 102 mg, 58%. M.p. 295 °C (decomposition). *Anal.* Calc. for $C_{36}H_{41}N_8Cl_4MnO_6$: C: 49.22; H: 4.70; N: 12.75; Found: C: 49.45; H: 4.77; N: 12.83%. IR (KBr): 3130 (m), 2935 (m), 2835 (w), 1607 (s), 1585 (s), 1509 (vs), 1487 (s), 1387 (m), 1250 (vs), 1157 (s), 1026 (vs), 831 (m), 753 (s), 704 (w), 680 (w).

2.5.2. Reaction of **2** with sodium azide

Caution! *Azide salts are potentially explosive. Only small quantities of these compounds should be prepared and suitable precautionary measures should be taken when they are handled.*

Microcrystalline powder of an azide complex of **2** was obtained by reacting equivalent amounts of **2** and sodium azide in dichloromethane. The reaction mixtures were layered with *n*-hexane and stored at −20 °C. After two days the resulting powder was filtered off and dried under vacuum. Attempts to recrystallize the powder to obtain crystals suitable for X-ray diffraction did not succeed. IR (KBr): 3128 (m), 2931 (m), 2839 (w), 2053 (vs), 1606 (s), 1584 (s), 1509 (vs), 1247 (m), 1062 (s), 832 (s), 750 (m), 708 (w), 695 (w).

2.6. Synthesis of [Mn(bmiapm)$_2$(OAc)]·MeOH·H$_2$O·CH$_2$Cl$_2$ (**3**)

The compound was prepared by reacting Hbmiapm (113 mg, 0.4 mmol) with $Mn(OAc)_2 \cdot 4H_2O$ (49 mg, 0.2 mmol) in a 1:4 mixture of methanol:dichloromethane (5 mL). The solution was stirred over night and filtered. The filtrate was layered with the same amount of *n*-hexane to yield brown crystals suitable for X-ray diffraction. Yield: 45 mg, 32%. M.p. 263 °C. *Anal.* Calc. for $C_{34}H_{43}N_{10}MnO_6Cl_2$: C: 50.19; H: 5.33; N: 17.22; Found: C: 50.43; H: 5.18; N: 17.61%. IR (KBr): 3113 (m), 2947 (m), 2850 (w), 1618 (s), 1577 (s), 1547 (s), 1456 (vs), 1386 (m), 1205 (w), 1159 (s), 1011 (vs), 849 (m), 735 (s), 715 (s), 683 (w).

2.7. Crystallography

The unit cell data and diffraction intensities of compounds **1** and **2** were collected on a Bruker-AXS SMART APEX CCD diffraction system using Mo Kα radiation (graphite monochromated, $\lambda = 0.71073$ Å), whereas the unit cell data and diffraction intensities of compound **3** were collected on a Bruker-AXS SMART

Table 1
Crystal data and X-ray experimental parameters for complexes **1**, **2**, and **3**

	1	**2**	**3**
Formula	$C_{36}H_{46}N_{10}$ MnO_{11}	$C_{36}H_{41}N_8Cl_4MnO_6$	$C_{34}H_{43}N_{10}Cl_2MnO_6$
M (g mol^{-1})	849.77	878.51	813.62
Crystal system	monoclinic	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/c$	$P2_1/n$
Crystal dimensions (mm^3)	$0.50 \times 0.15 \times 0.14$	$0.42 \times 0.12 \times 0.12$	$0.22 \times 0.21 \times 0.18$
Wavelength (Å)	Mo Kα ($\lambda = 0.71073$)	Mo Kα ($\lambda = 0.71073$)	Cu Kα ($\lambda = 1.54178$)
μ (mm^{-1})	0.399	0.629	8.386
T (K)	123(2)	158(2)	150(2)
a (Å)	7.421(1)	17.506(4)	9.151(1)
b (Å)	26.954(5)	16.229(3)	18.659(1)
c (Å)	20.119(4)	15.622(3)	22.495(1)
β (°)	91.55(3)	110.75(3)	93.43(1)
Volume (Å^3)	4023(1)	4150(2)	3833(1)
Z	4	4	4
D_{calc} (g cm^{-3})	1.403	1.406	1.392
Data collection range (°)	$2.5 \leqslant 2\theta \leqslant 56.5$	$2.5 \leqslant 2\theta \leqslant 52.8$	$6.2 \leqslant 2\theta \leqslant 143.1$
Reflections measured	41,524	38,832	21,949
Independent reflections	9965 [$R_{int} = 0.0646$]	8490 [$R_{int} = 0.0972$]	7145 [$R_{int} = 0.0338$]
Reflections observed [$I > 2\sigma(I)$]	4490	5486	5627
Variables	499	496	483
$R_1[I > 2\sigma(I)]$[a]	0.0568	0.0543	0.0481
$wR_2[I > 2\sigma(I)]$[b]	0.1489	0.1466	0.1396
Goodness-of-fit[c]	0.843	1.059	1.076

[a] $R_1 = \sum ||F_{obs}| - |F_{calc}|| / \sum |F_{obs}|$.
[b] $wR_2 = \left\{ \sum [w(F_{obs}^2 - F_{calc}^2)^2] \Big/ \sum [w(F_{obs}^2)^2] \right\}^{1/2}$.
[c] $\mathrm{GOF} = \left\{ \sum [w(F_{obs}^2 - F_{calc}^2)^2] \Big/ n_{data} - n_{vari} \right\}^{1/2}$.

6000 CCD diffraction system using Cu Kα radiation ($\lambda = 1.54178$ Å). The crystal structure of **1** was solved by a Patterson synthesis, the crystal structures of **2** and **3** were solved with direct methods using the program system SHELXS 97 [29]. All non-hydrogen atoms were taken from a series of full-matrix least-squares refinement cycles based on F^2 with the SHELXL 97 program followed by difference Fourier synthesis [30]. All hydrogen atoms were placed at calculated positions and allowed to ride on their corresponding carbon atoms with isotropic thermal parameters for the methyl protons 1.5 times the value for U_{eq} of the bonding atom and all other hydrogen atoms 1.2 times the value for U_{eq} of the bonding atom. All non-hydrogen atoms were refined anisotropically, accept one methanol solvent molecule in the crystal structure of **3**. Further crystal data and experimental parameters are listed in Table 1.

2.8. Spectrophotometric titration

A 2×10^{-3} M solution of **2** in acetonitrile was treated with 0.2, 0.5, 0.7, 1.0, 2.0, and 3.0 equivalents of sodium azide, solved in a 3:2 mixture of acetonitrile and methanol. The formation of the azide complex was monitored by UV–Vis spectroscopy at ambient temperature (25 °C).

3. Results and discussion

The crystallographic analysis of manganese containing SOD from *T. thermophilus* has revealed the coordination of three histidine ligands to the central manganese ion. The tridentate ligands in this paper were designed to mimic this coordination environment. A close structural and electronical analogy to histidine is ensured using methylimidazole moieties as donor groups. Therefore we have synthesised the two novel tridentate ligands Hbmimpm and Hbmiapm. Both compounds share two methylimidazole units and one alcoholate groups as potential donor function. Due to the rigid structure two different donor sets can be provided. As the crystal structure analyses show manganese in higher oxidation state (d^4 ion) is stabilized by the coordination of a deprotonated alcoxo group and one methylimidazole moiety, whereas in the Mn(II) compound both methylimidazole moieties of the corresponding ligand are bonded to the metal center. In each complex the central manganese ion is surrounded by two tridentate ligands and one labile bidentate nitrate or acetate, resulting in an N_4O_2 (**1**) or N_2O_4 (**2**, **3**) donor set, respectively.

Though some iron and one copper complex are known with derivatives of these ligands none of them has been described as model compounds for superoxide dismutases [31–34].

Table 2
Selected bond lengths and angles in complexes **1–3**

	1	2	3
Selected bond lengths (Å)			
Mn(1)–O(1)	2.303(2)	2.125(2)	2.013(2)
Mn(1)–O(2)	2.343(3)	2.333(2)	2.513(2)
Mn(1)–O(3)		1.874(2)	1.875(2)
Mn(1)–O(4)/O(5)[a]		1.869(2)	1.877(2)
Mn(1)–N(1)	2.158(3)	2.100(2)	2.134(2)
Mn(1)–N(3)	2.173(3)		
Mn(1)–N(5)	2.173(3)	2.045(2)	2.030(2)
Mn(1)–N(7)	2.155(3)		
Selected angles (°)			
O(1)–Mn(1)–O(2)	55.6(1)	58.2(1)	56.9(1)
O(1)–Mn(1)–N_{trans}	155.1(1)	154.3(1)	156.7(1)
O(2)–Mn(1)–N_{trans}	160.0(1)	154.1(1)	151.2(1)
O(3)–Mn(1)–N(1)		81.2(1)	80.3(1)
O(3)–Mn(1)–O(4)/O(5)[a]		177.0(1)	173.7(1)
N(1)–Mn(1)–N(3)	83.2(1)		
N(3)–Mn(1)–N(5)	174.0(1)		
N(1)–Mn(1)–N(5)/N(7)[a]	101.4(1)/ 98.6(1)	106.9(1)	106.3(1)
O(4)/O(5)[a]–Mn(1)–N(1)		98.1(1)	102.0(1)

[a] The atom labels depend on the ligand cf. Figs. 1–3.

3.1. Crystal structures of $[Mn(Hbmimpm)_2(NO_3)](NO_3)\cdot Et_2O$ (**1**), $[Mn(bmimpm)_2(OAc)]\cdot 2CH_2Cl_2$ (**2**), and $[Mn(bmiapm)_2(OAc)]\cdot MeOH\cdot H_2O\cdot CH_2Cl_2$ (**3**)

All compounds crystallize in the monoclinic space system with four complex molecules per unit cell. Selected bond lengths and angles are summarized in Table 2.

The coordination environment around the Mn(II) center in compound **1** can be described as distorted octahedral where nitrogen atoms from two ligands of Hbmimpm occupy four coordination sites. Two oxygen atoms from a coordinating nitrate ion complete the coordination sphere resulting in an N_4O_2 donor set (see Fig. 1). O(1) and O(2) of the nitrate ion are within a distance of 2.303(2) Å and 2.343(3) Å, respectively, to Mn(1). This asymmetric coordination is due to a hydrogen bond between O(2) and a protonated alcoholate function O(4)′ of an adjacent complex molecule. The distance between the hydrogen exchanging oxygen atoms O(2) and O(4)′ is 3.036(4) Å. Within the scope of error margins both nitrogen atoms opposing the coordinating nitrate are within the same distance to Mn(1). Bond length of 2.158(3) Å for Mn(1)–N(1) and 2.155(3) Å for Mn(1)–N(7) have been obtained. The remaining two *trans* standing methylimidazole moieties are bonded to Mn(1) in the accurate distance of 2.173(3) Å for both Mn(1)–N(3) and Mn(1)–N(5). The distortion of the octahedral coordination environment is further manifested in the O(1)–Mn(1)–O(2) angle of 55.6(1)° which differs significantly from 90°. Additionally, the average angle of *trans* standing donor atoms also differs within a value of 163° from 180°.

The crystal structures of **2** and **3** show high similarity to each other (see Figs. 2 and 3). Therefore the structure of Mn(III) compound **2** is described in detail as representative for both. The corresponding bond lengths and angles of complex **3** can be found in Table 2. The manganese(III) core in the model compound adopts a distorted octahedral coordination. The N_2O_4 donor set

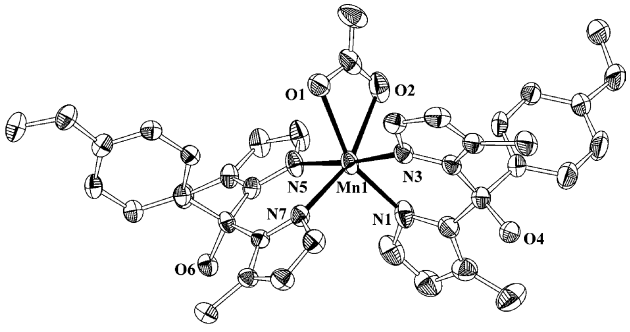


Fig. 1. Crystal structures of the cation in **1** showing 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

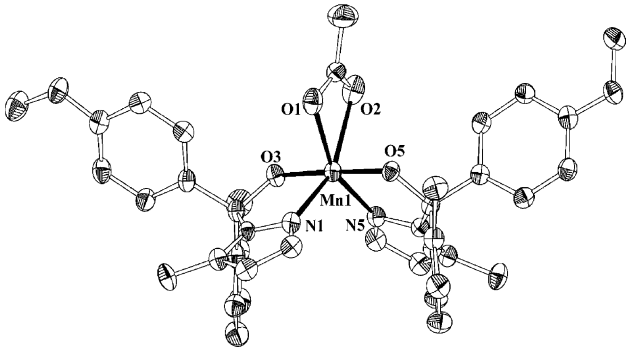


Fig. 2. Crystal structures of the neutral Mn(III) complex in **2** showing 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

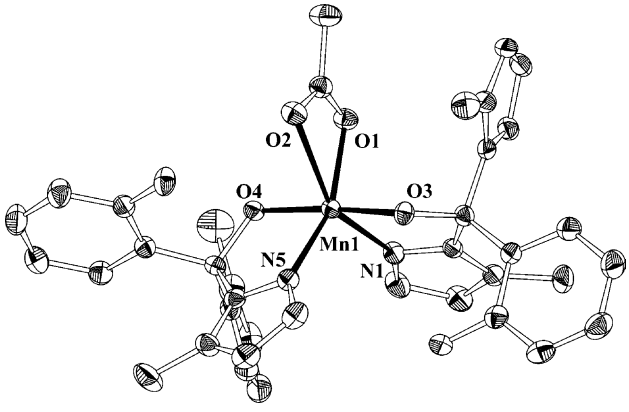


Fig. 3. Crystal structures of the neutral Mn(III) complex in **3** showing 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

consists of the tridentate ligand and one chelating acetate binding in a bidentate fashion. The short bond lengths of 1.874(2) Å for Mn(1)–O(3) and 1.869(2) Å for Mn(1)–O(5) are attributed to a rare Jahn–Teller compression along the manganese–alkoxide axis O(3)–Mn(1)–O(5), arising from the d^4 configuration of the metal center. All bond lengths in the equatorial plane exhibit larger values. The distances for Mn(1)–N(1) and Mn(1)–N(5) are within the expected range with values of 2.100(2) Å and 2.045(2) Å, respectively. It is noteworthy, that the bond lengths for the manganese–acetate oxygen bonds with values of 2.125(2) Å for Mn(1)–O(1) and 2.333(2) Å differ significantly from each other. Due to this asymmetric binding mode the manganese can also be described as *pseudo* five-coordinated. Analogous to Mn(II) compound **1** an apparent distortion of the octahedral coordination environment is further clarified in the O(1)–Mn(1)–O(2) angle of 58.2(1)° which differs significantly from 90°.

3.2. Spectroscopic investigations

The UV–Vis spectrum of **1** shows no bands due to the ^6S ground state of the Mn^{II} center. The optical spectra of **2** and **3** are almost identical. In acetonitrile bands originate from d–d-transitions at 413 nm (409 M^{-1} cm^{-1}) and 546 nm (354 M^{-1} cm^{-1}) for **2** and 409 nm (428 M^{-1} cm^{-1}) and 524 nm (314 M^{-1} cm^{-1}) for **3**. The spectrum of **2** is included in Fig. 4.

Analogous to the enzyme compound **2** reacts with sodium azide. To investigate this reaction UV–Vis experiments have been performed. Fig. 4 shows the corresponding UV–Vis spectra. To exclude solvent effects like reaction of the complex with methanol UV–Vis experiments with blank samples have been performed. The solution of compound **2** has been treated with corresponding amounts of methanol/acetonitrile mixture (2:3). No changes to the original spectrum of **2** have been obtained.

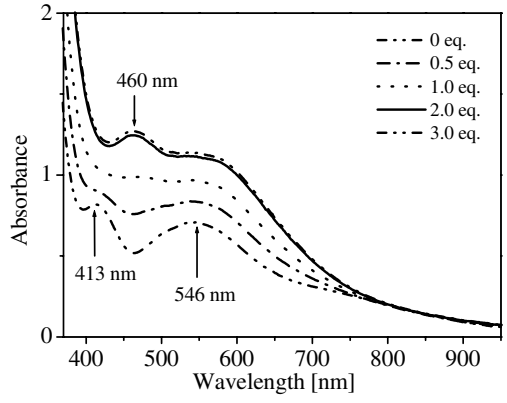


Fig. 4. Spectrophotometric titration of **2** with sodium azide. The UV–Vis data is summarized in Table 3.

Table 3
UV–Vis data for complexes **2, 3** and azide complex of **2**

Complex	λ_{max} (nm) (ε (M^{-1} cm^{-1}))
2	413 (409), 546 (354)
3	409 (428), 524 (314)
2 + 2 eq. azide	460 (623), 543 (557)

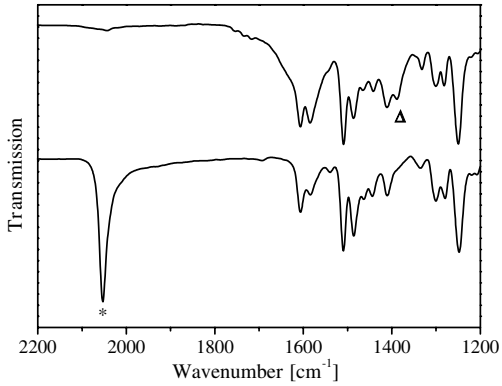


Fig. 5. IR-Spectrum of **2** (top) and its azide adduct (bottom) (2200–1200 cm^{-1}).

The reaction of **2** with sodium azide in acetonitrile/methanol solution revealed the formation of a band at 460 nm ($\varepsilon = 623$ M^{-1} cm^{-1}). It reaches a maximum after the addition of two equivalents of sodium azide. Further addition of azide does not lead to any increase of the band. It can be concluded that the coordinated acetate ion in **2** is replaced in solution by azide (Table 3).

The reaction product was further investigated by IR spectroscopy. Complex **2** was dissolved in dichloromethane and treated with sodium azide. The IR spectrum of the obtained microcrystalline powder reveals a strong band at 2053 cm^{-1} typical for the asymmetric stretching frequency of coordinated azide (see * in Fig. 5). Furthermore no band occurs around 1380 cm^{-1} indicating the loss of coordinated acetate (see Δ in Fig. 5). The remaining spectrum shows high similarity to the IR spectrum of complex **2**, suggesting that no further structural changes have taken place. Fig. 5 gives a comparison of the IR spectra of both compounds.

The obtained manganese compounds **1**–**3** will be compared with [Mn(ntb)(Hsal)](ClO_4) [35]. In this structural and functional model complex for Mn SOD the central Mn(II) is surrounded by an enzyme analogous N_4O donor set. It consists of four nitrogen donor atoms from the tripodal ligand tris(2-benzimidazolylmethyl)amine (ntb) and a monodentate binding carboxylate group from salicylate (Hsal). The benzimidazole moieties in ntb mimic the coordination of histidine. The coordinated salicylate can be regarded as a structural equivalent to aspartate in the active site of Mn SOD. All

benzimidazole–manganese bond lengths in Mn(ntb)(Hsal)](ClO$_4$) are almost within the same distance as the obtained methylimidazole–manganese bond lengths in **1**. It is noteworthy, that the coordinated oxygen of Hsal is within a distance of 2.035(3) Å to the central manganese. This is remarkably short for an oxygen–Mn(II) bond length and even shorter than the oxygen–Mn(III) bond lengths in **2** and **3**. Whereas acetate binds in **2** and **3** in an asymmetric bidentate fashion, this can be excluded for the carboxylate group of Hsal. This is manifested in the large oxygen–manganese distance of 3.181(8) Å for the second oxygen atom in Hsal. In addition, the superoxide dismutase activity of Mn(ntb)(Hsal)](ClO$_4$) was examined indirectly using the nitro blue tetrazolium (nbt) assay. The complex showed an IC_{50} value of 0.70 μM (concentration of the complex which exerts activity equal to one unit of that of native SOD) indicating that it is a potent superoxide dismutase mimic.

4. Conclusion

With the tridentate ligands Hbmimpm and Hbmiapm three compounds have been crystallographically and spectroscopically characterized that are structural models for the reduced and oxidized active site of manganese superoxide dismutase. In complex **1** three of the four methylimidazole moieties coordinated to the central Mn(II) ion mimic all three histidine residues in the active site of reduced MnSOD. The oxygen donors aspartate and hydroxide are rendered by a coordinated nitrate ion. Compounds **2** and **3** are structural models for the oxidized Mn(III) form of MnSOD. The aspartate residue in the active site is mimicked by an acetate coordinated to the central Mn(III) ion. Two methylimidazole moieties of the corresponding ligand Hbmimpm and Hbmiapm, respectively, mimic the coordination of histidine.

Mn(III) compound **2** can react with sodium azide yielding an azide adduct. It has been characterized by IR and UV–Vis spectroscopy indicating the loss of acetate and the coordination of azide.

5. Supplementary material

Further details of the crystal structure investigation have been deposited at the Cambridge Cyrystallographic Data Centre under the depository number CCDC 217791, 217792, and 217793. Copies of this information can be obtained free of charge from The Directors, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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