carbonic acid H_2CO_3 which exists in equilibrium with bicarbonate, HCO_3^-. The enzyme carbonic anhydrase catalyses the hydration of CO_2 to H_2CO_3 facilitating its dissolution and allowing its efficient transport in dissolved form from the point of production to the lungs where it is expelled. A similar dissolution mechanism is not available for NO and O_2 and these gases are far less soluble in water than CO_2. It would be difficult to properly regulate the distribution and availability of NO and O_2 by managing them in the form of dissolved gases. Instead NO is produced where needed through the enzymatic oxidation of arginine and O_2 is transported or stored through binding to the iron centres in the metalloproteins hemoglobin and myoglobin.

Nitric oxide has a variety of functions *in vivo* and is typically produced where needed by members of a group of enzymes known as Nitric Oxide Synthase (NOS) enzymes. One function of NO is as a neuromodulator, for example in the hippocampus of the brain, where it is implicated in the development of short-term memory. Another important function of NO is to regulate the dilation of the blood vessels in the cardiovascular system. Excessive production of NO in response to infection or injury can result in arterial expansion and may ultimately lead to cardiovascular collapse. In order to maintain acceptable cardiovascular function under such conditions, it is important for the availability of NO to be controlled efficiently. This can be achieved using inhibitors for the enzymes producing NO *in vivo* but NO scavenging by suitable metal complexes offers another possible treatment.

Dioxygen supports cellular respiration in mammals and is transported throughout the body by the cardiovascular system. In the bloodstream O_2 is carried on the iron containing protein hemoglobin to its point of use. The ultimate product of dioxygen reduction is water but a proportion of the O_2 is converted to the reactive superoxide, O_2^-, ion. Normally O_2^- is rapidly removed by a SuperOxide Dismutase (SOD) enzyme to prevent damage arising from unwanted reactions of O_2^-. However, there can be occasions where O_2^- regulation is compromised, for example during reperfusion following acute myocardial infarct or stroke. Superoxide has also been implicated in the development of arthritis and neurological disorders such as Alzheimer's and Parkinson's diseases. If occasions arise when a patient's normal capacity for managing levels of O_2^- or NO is compromised, pharmaceutical intervention may be beneficial. The ability of certain d-block metal ions to react with small molecules such as O_2^- or NO, and to undergo electron transfer reactions, offers an important opportunity to develop metal containing pharmaceutical agents which address these issues.

4.6.2 Superoxide Dismutase Mimics

During normal respiration O_2 is consumed in living mammalian tissues but the biochemical processes involved may also produce superoxide, O_2^- or, in more acidic media, HO_2. Both of these are very reactive species capable of damaging DNA and initiating the auto-oxidation of membrane lipids and so are potentially damaging to tissues. Superoxide can also react with NO to produce highly reactive peroxynitrite, $O{=}NO{-}O^-$ which is also very damaging to tissues.

Protection from the destructive effects of superoxide is achieved *in vivo* by the rapid conversion (dismutation) of O_2^- into O_2 and H_2O_2 according to Equation (12).

$$2O_2^- + 2H^+ = O_2 + H_2O_2 \tag{12}$$

This reaction is catalysed by the SOD group of enzymes which exploit the electron transfer properties of certain metal ions. Examples are known with Mn, Fe, Ni or both Cu and Zn at the active site. The dioxygen produced in the reaction is, in effect, recycled while the peroxide is rapidly consumed *in vivo* by peroxidase enzymes carrying out biochemically controlled oxidations. Many forms of SOD enzymes are found in animal and plant species. In humans there are two main types. The enzyme found in extracellular spaces contains both Cu and Zn at the active site, while that found in mitochondria contains Mn. SOD enzymes containing Fe or Ni are also known to occur but in other organisms.

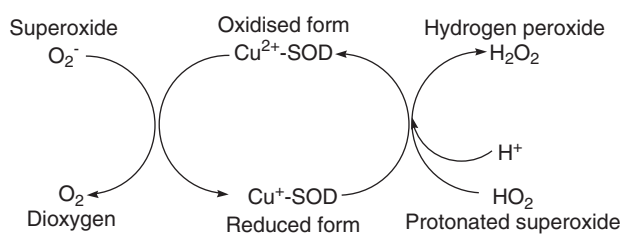
The human SOD in plasma and extracellular spaces effects the dismutation of O_2^- to O_2 and O_2^{2-} through successive electron transfers involving the copper centre according to Equations (13) and (14) (Scheme 12).

$$O_2^- + Cu^{2+}(SOD) \rightarrow O_2 + Cu^+(SOD) \tag{13}$$

$$HO_2 + Cu^+(SOD) + H^+ \rightarrow Cu^{2+}(SOD) + H_2O_2 \tag{14}$$

The d^{10} Zn^{2+} ion cannot undergo electron transfer reactions but has structural and electronic effects on the Cu^{2+} site. In the first reaction the Cu^{2+} centre is reduced to Cu^+ by O_2^- which is converted to O_2. In the second reaction the Cu^+ formed reduces a second O_2^- to O_2^{2-} reforming Cu^{2+}. These reactions are very fast *in vivo* so that the rate of removal of superoxide is essentially determined by the rate at which O_2^- can diffuse to the enzyme. Mitochondrial SOD employs a similar catalytic cycle involving Mn^{3+} and Mn^{2+} while iron containing SOD similarly utilises a cycle involving Fe^{3+} and Fe^{2+}. SOD enzymes containing Fe or Mn as the reactive metal react with O_2^- a little more slowly than the Cu/Zn enzyme.

In some cases of disease or trauma the production of O_2^- exceeds the capacity of the available SOD to carry out dismutations. This can result in tissue damage



Scheme 12

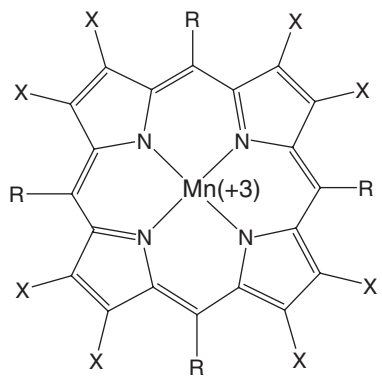
through processes such as the oxidation of lipid membranes and the site selective oxidation of DNA. In such cases clinical administration of natural SOD enzyme might be used to alleviate the problem. However, the use of synthetic low molecular weight SOD mimics as pharmaceutical agents to dismute excess O_2^- may offer some advantages over the use of the SOD enzyme itself. The human enzyme preparations may induce immunological responses, are stable in blood for only short periods, are unsuitable for oral administration and are expensive to produce. Synthetic SOD mimics should produce no immunological responses, be more stable in blood and they might be expected to have better access to intercellular spaces and show better permeability into cells. It is also possible that compounds could be developed which would be suitable for oral administration. These possibilities have driven a large body of research into synthetic SOD mimics containing metal ions for potential use in pharmaceutical products.

The selection of a suitable metal for use in a SOD mimic has focused on those found in the naturally occurring SOD enzymes, particularly Cu, Fe and Mn. Free aquated Cu^{2+} ions are a very effective catalysts for O_2^- dismutation but would rapidly bind to serum proteins and other species *in vivo*. Similar problems arise with free aquated Fe^{2+}, Fe^{3+} or Mn^{2+} ions. There also particular toxicity issues with Cu^{2+}, Fe^{2+} and Fe^{3+} in that they can react with peroxide, a product of O_2^- dismutation, leading to the formation of reactive hydroxyl radicals which contribute to toxicity. In order to develop a SOD mimic for clinical use it is necessary to incorporate the metal in a suitable complex which has the required catalytic effect while showing low toxicity combined with acceptable biodistribution and pharmokinetics. The known structure of the Cu/Zn SOD enzyme might be thought a good starting point for the design of a SOD mimic containing Cu^{2+}. However, the structure of the Cu-Zn enzyme is elaborate and any attempt to reproduce the active site structure introduces an unwelcome level of complexity to the design of a simple synthetic SOD mimic. A variety of simpler copper complexes have been investigated as potential SOD mimics but evaluation of their catalytic performance has been complicated by the fact that aquated Cu^{2+} ions alone are very effective catalysts for O_2^- dismutation. Consequently it is often difficult to be sure that the activity arises from the complex not traces of free aquated Cu^{2+}. Iron complexes have also been investigated but slower dismutation kinetics and toxicity issues have limited their suitability. The metal emerging as the most promising for clinical use is Mn. The $Mn^{3+/2+}$ system is less prone to form hydroxyl radicals and has lower toxicity in the free aquated form.

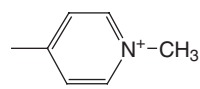
In order to be of pharmaceutical value the metal complex should be relatively easy to prepare, very stable so as not to release the metal ion and produce unwanted toxicity and, importantly, the ligand system chosen needs to be resistant to attack by O_2^-, O_2 or HO_2^-. As with all metallodrugs, the biodistribution and pharmokinetic properties of the complex also need to be acceptable. Since high spin Mn^{2+} is a d^5 ion, weak field Mn^{2+} complexes would have no CFSE and be more prone to dissociation than strong field complexes.

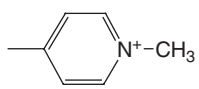
The additional stability conferred by polydentate macrocyclic ligands is thus an important consideration for catalytic cycles involving Mn^{2+}.

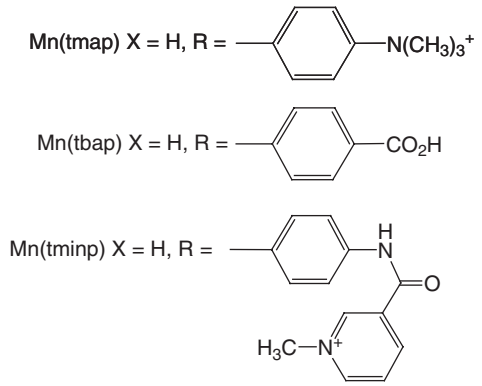
A variety of ligand types have been investigated in the search for a suitable Mn based SOD mimic. Complexes containing macrocyclic porphyrin ligands **136** have been found to show catalytic properties. The complex Mn(tmpyp) catalysed O_2^- dismutation with an apparent rate constant of $10^7\ M^{-1}\ s^{-1}$ at pH 7.6 ± 0.2 and 21 °C while Mn(Br_4tmpyp) is said to have a SOD activity of about 12% that of the Mn enzyme itself. Patents have been taken out for the therapeutic use of some of these compounds but there are limitations on the suitability of this class of compound. Under oxidising conditions porphyrin complexes of this type are prone to the formation of dimers through the formation of O^{2-} or OH^- bridges. These dimers are not active SOD catalysts. However, dimer formation could be suppressed by incorporating bulky substituents which prevent the close approach of two molecules necessary for dimer formation. Although the Mn(+3) complexes appear quite stable in competition experiments with $edta^{4-}$ this is not always true of the Mn^{2+} complexes which appear in the catalytic cycle. Furthermore the porphyrin ligands can be prone to oxidative degradation in some cases. In addition to these chemical issues there are problems with toxicity. Porphyrin complexes can act as DNA intercalators, and can show phototoxicity or hepatotoxicity. Thus, although Mn(+3) porphyrin complexes do represent potential SOD mimics in that they have the necessary catalytic activity, to obtain a suitable therapeutic agent it is necessary to overcome the toxicity and reactivity issues associated with porphyrin complexes. This presents a substantial challenge in ligand design.



136

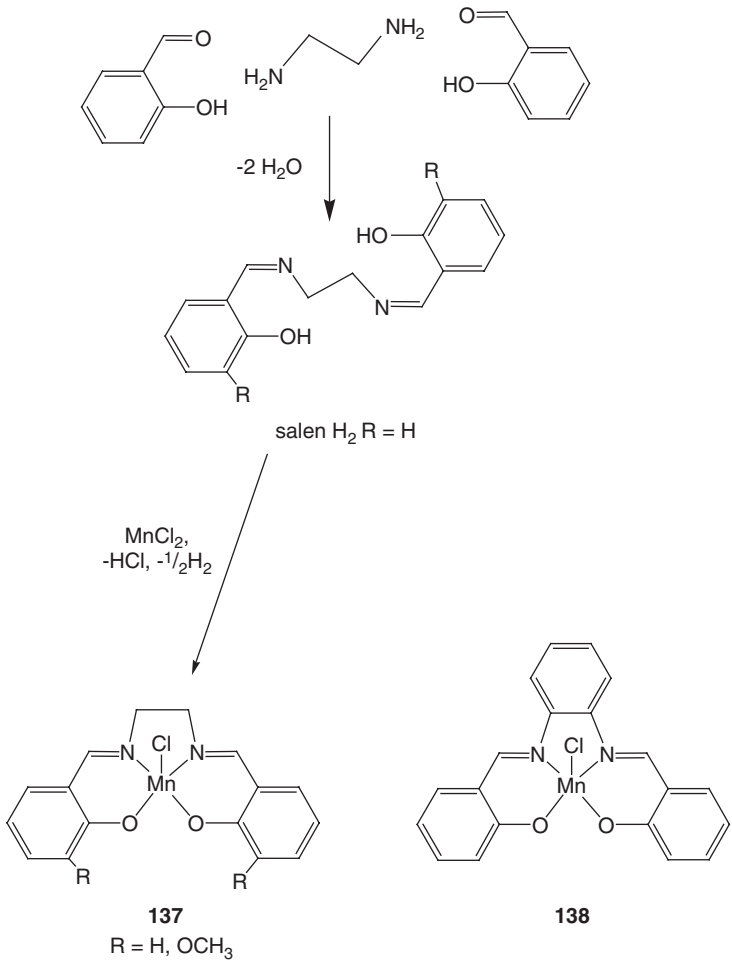
Mn(tmpyp) X = H, R = 

Mn(Br_4tmpyp) X = Br, R = 



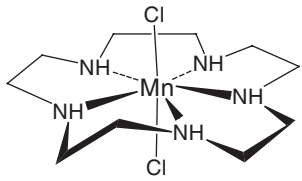
Another group of Mn complexes which have been investigated as SOD mimics contain what are known as 'salen' type ligands. These ligands are obtained from the condensation reactions of a hydroxybenzaldehyde and 1,2-diamino ethane to produce a tetradentate proligand,which forms a complex containing Mn^{3+} (Scheme 13). The structures and properties of the complexes may be varied by adding substituents to the ligand framework and through modification of the hydroxybenzaldehyde or diamine precursors (**137**, **138**, Scheme 13). Patents covering the therapeutic use of such compounds have been taken out by Eukarion Inc. and their development undertaken in collaboration with Glaxo-Wellcome. Promising results were obtained in animal models for oxidative stress including reperfusion injury and neurological disorders. However, kinetic studies reveal relatively low catalytic activity for these compounds, the rate for **137** (R═H) being less than 8×10^5 M^{-1} s^{-1} at pH 8. Furthermore the compounds do not appear to be genuine SOD mimics but rather their effect appears to arise from a more complicated mechanism which may not be catalytic in nature but may involve reactions with peroxide. Similarly, studies of $\{Fe(salen)\}^+$ reduction by O_2^- show that the reaction is too slow for the iron complex to be a competent SOD mimic. In addition to the uncertainties over the mechanism of action salen complexes also show low water solubility, which is problematic for clinical applications.

The most promising types of Mn^{2+} complex for use in SOD mimetic applications are the polyazamacrocyclic Mn(+2) complexes exemplified by **139**. In the solid state this complex contains 7-coordinate Mn^{2+} with a *trans* arrangement of the Cl^- ligands. It is quite thermodynamically stable with $\log K = 10.7$ at pH 7.4 and is also kinetically stable. Stopped flow kinetic analysis showed that the complex catalysed O_2^- dismutation with a rate of 4×10^7 M^{-1} s^{-1} at pH 7.4 in the presence of $>100{:}1$ excess $[O_2^-]/[\mathbf{139}]$. The complex is an effective anti-inflammatory agent *in vivo* and was found to block cardiac reperfusion injury in a canine model. Investigations of the effects of ligand structure on the catalysis of O_2^- dismutation have shown that adding

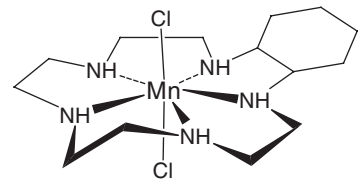


Scheme 13

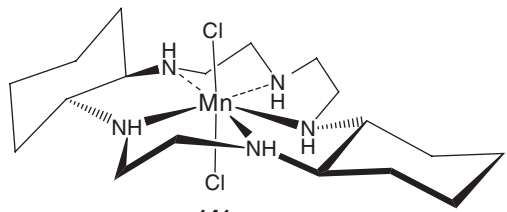
substituents to the nitrogen donor atoms destroys the catalytic activity. However, adding substituents on the carbon atoms of the macrocycle can increase chemical stability. Adding one fused cyclohexane ring to give **140** results in a 2-fold improvement in kinetic stability, increases the thermodynamic stability to $\log K = 11.6$ and increases the catalytic rate to 9.1×10^7 M^{-1} s^{-1} at pH 7.4. Further improvements were achieved by adding a second fused cyclohexanyl ring to give **141**, the all *R*-isomer having $\log K = 13.3$ and a catalytic rate of 1.2×10^8 M^{-1} s^{-1} at pH 7.4. However, the structural effects of substituents can be quite subtle and the *R,R,S,S*-isomer of **141**, despite having similar stability, has almost no catalytic activity for O_2^- dismutation. Addition of two further methyl substituents to **141** further improves the thermodynamic and kinetic stability of the complex but reduces slightly the catalytic rate for O_2^- dismutation.



139



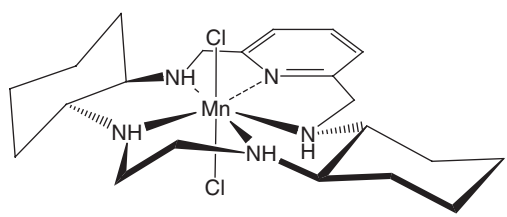
140



141

Mechanistic studies indicate that the O_2^- dismutation reaction with these Mn(+2) complexes involves two pathways, a minor inner sphere pathway and a faster outer sphere pathway. Both require oxidation of Mn(+2) to Mn(+3) and appear to involve a 6-coordinate intermediate the formation of which involves folding of the macrocyclic ligand. Molecular mechanics calculation show that the all *R*-isomer of **141** favours the folded geometry while the *R,R,S,S*-isomer is constrained to a planar arrangement favouring 7-coordination. In this way the substantial difference in reactivity between the two isomers can be explained.

The all *R*-complex **141** inhibits ischemic and reperfusion injury in anaesthetised cats and further elaboration of the ligand through the incorporation of a pyridyl ring into the structure has provided the SOD mimic M40403 (**142**). This compound has been developed by Metaphore Pharmaceuticals Inc. and clinical trials undertaken. Favourable results were obtained in a Phase II clinical trial of the enhanced analgesic effects of combining M40403 with morphine for post-operative pain relief from dental operations. A similar trial of this combination approach was conducted on a bunionectomy post-operative pain model and a further trial is in progress to study the relief of moderate to severe pain in cancer patients. The development of these SOD mimics demonstrates how it can be possible to design small molecules to mimic the function of much larger enzyme systems with the goal of obtaining metal complexes with therapeutic applications. It also illustrates the importance of ligand design in the formulation of such therapeutic agents.



142
M40403