

containing such s-block and p-block metals. One therapeutic application in which coordination chemistry is playing a major rôle is in the development of new drugs for the treatment of cancer. These exploit the particular properties of metals such as platinum to produce a therapeutic effect. As a measure of the importance of this type of compound, the estimated global market in platinum cancer drugs in 2000 was estimated to be about US\$1 billion. The particular reactivity or electron transfer properties of specific metals also find application in the treatment of rheumatoid arthritis, diabetes and problems associated with the regulation of bloodflow through the vascular system. Radioactive metals also have potential uses in therapy and, although the application of coordination chemistry in this field is not yet highly developed, there is growing interest in radiotherapy using internal sources of radiation in the form of radiopharmaceuticals.

4.2 Chelation Therapy

4.2.1 Metal Sequestration

The term ‘chelation therapy’ usually refers to the use of proligands as drugs to treat disorders resulting from the presence of unwanted metal ions arising from intoxication or disease. To be effective, the proligand must complex and sequester the target metal ion then promote its excretion and removal from the body in complexed form without impairing the normal biochemistry of metals *in vivo*. Consequently the proligand used must be selective for the target metal ion so as not to remove other biologically important metals. In cases where a biologically essential metal such as iron or copper is the target, the sequestering agent must not compete with any natural binding sites for the target metal to the extent that it compromises normal function and health. However, where non-essential toxic metals are involved the sequestering agent may need to compete with natural metal binding sites to prevent uptake of the target metal ion. As the name implies, the compounds used in chelation therapy exploit the chelate effect (Section 2.7.3) to sequester the target metal ion. It is also important that the electronic properties of the ligand and the metal are matched depending upon whether the metal is hard, soft or intermediate in character (Section 2.7.2).

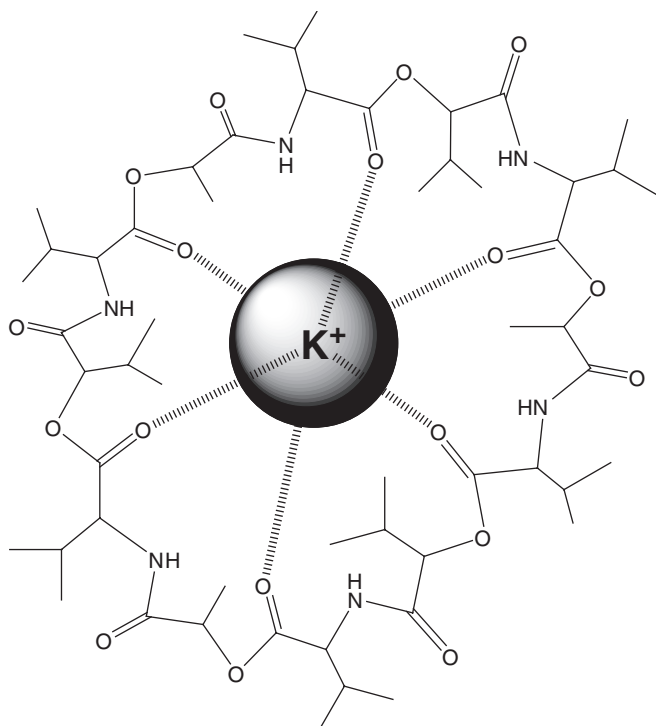
Chelation therapy is particularly relevant to two diseases, Wilson disease which leads to excess copper levels in the body and thalassaemia which is treated with repeated blood transfusions leading to iron overload in patients. In suitable cases polydentate proligands can also be used to treat cases of toxic metal poisoning and a particular example of ligand design is provided by the development of sequestering agents for plutonium. The design of proligands for use in chelation therapy has to meet several challenges. Not only the proligand must be of low toxicity and show strong and highly selective binding to the target metal, it must also be neutral in its effect on the biological activity of essential metals. As an example iron is able to undergo electron transfer reactions involving the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple. These can lead to the formation of reactive free radicals which can be toxic. Under natural conditions these reactions are controlled by the

coordination environments of iron *in vivo*. However if the wrong type of ligand were used to sequester iron, its electron transfer properties could be promoted by complex formation and cause oxidative stress. The lipophilicity of the metal complex will also be dependent on the proligand design, affecting the ability of the metal to penetrate cell membranes and the proportions removed through the different excretion pathways. However, increased lipophilicity can also lead to increased brain uptake of the sequestered metal and, for oral treatments, increased uptake in the gut. Careful design of the proligand structure is thus important in achieving the required selectivity and pharmacokinetics.

4.2.2 Macrocyclic Antibiotics

A broader view of the concept of chelation therapy might include the use of drugs to sequester metal ions in order to disrupt an unwanted biochemical process. An example of this is provided by the use of metal binding agents for the Group 1 metals Na^+ and K^+ as antibiotics. In this case the function of the drug is not to promote the excretion of the target metal ion, but rather to interfere with its use by bacteria and so produce an antibacterial effect. Antibiotics which work in this way include the macrocyclic antibiotics nonactin and valinomycin. Nonactin is a member of a family of antibiotics based on naturally occurring metal ion binding agents known as the macrotetrolides. These compounds are capable of selectively forming complexes with K^+ and transporting it through cell membranes. In this way they change the permeability of the membrane to K^+ disrupting oxidative phosphorylation and inhibiting the processing of some proteins. Valinomycin acts similarly but is structurally different from nonactin.

The Group 1 metal ions are classified as hard ions and form their most stable complexes with hard ligand donor atoms, particularly the oxygen of water so that the metal ions are strongly hydrophilic. In aqueous media conventional proligands containing nitrogen or oxygen donors bound within organic molecules are ineffective competitors for K^+ or Na^+ ions against coordinated water. However, organic compounds such as nonactin or valinomycin can exploit the macrocyclic effect (Section 2.7.3) by creating an oxygen lined cavity capable of encapsulating the hydrophilic K^+ ion. The ligand structure surrounds the hydrophilic K^+ ion with a lipophilic exterior (*e.g.* valinomycin Figure 1) solubilising it in a hydrophobic environment such as a lipid bilayer membrane. In achieving this nonactin and valinomycin exploit the principles of coordination chemistry demonstrated by a group of compounds of simpler macrocyclic structure known as crown ethers. The crown ethers are macrocyclic polyethers in which the size of the ring and number of oxygen atoms may be varied, examples being provided by 15-crown-5, **1**, dibenzo-18-crown-6, **2** and 24-crown-8, **3**, where the first number in the name refers to the number of atoms in the macrocyclic ring, and the second the number of oxygen atoms. These compounds show an unusual affinity for Group 1 metal ions and have the remarkable ability to solubilise their salts in non-polar organic solvents. As an example dibenzo-18-crown-6, **2**, renders KMnO_4 soluble in benzene by complexing the K^+ ion to produce the benzene soluble compound $\{\text{K}^+(\mathbf{2})\}\{\text{MnO}_4^-\}$.



K^+ Valinomycin

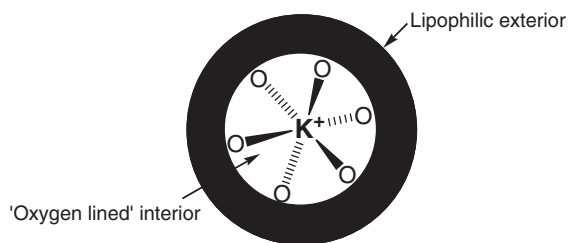
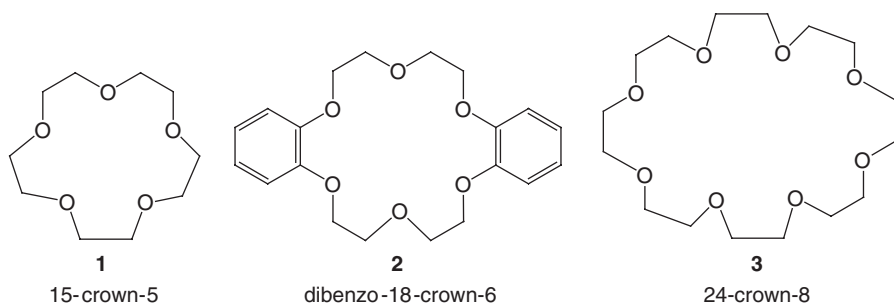


Figure 1 The binding of valinomycin to K^+ (top) and its 'cartoon' representation (bottom)



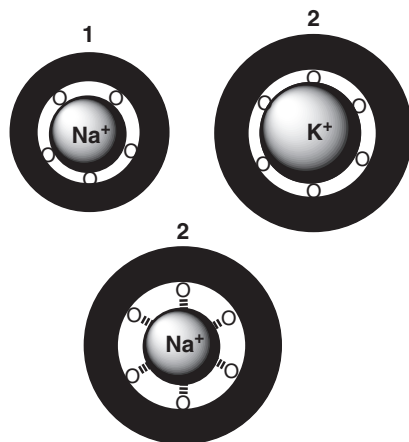


Figure 2 Cartoons showing how the larger **2** ligand is a better fit for K^+ than Na^+ which is best fit by **1**

A further important feature of the crown ethers is their selectivity for specific metal ions. This arises from the well-defined size of the macrocyclic cavity which can discriminate between Group 1 metals according to their ionic radii. Thus in 1:1 ligand/metal complexes the size of the cavity in **1** allows an optimum interaction between the Na^+ ion and the polyether oxygens. In the case of the larger K^+ ion the larger cavity of 18-crown-6 (or the dibenzo-derivative **2** in the example shown) provides the best fit, optimising the strength of the metal-ligand interaction (Figure 2). The structure of valinomycin is optimised for K^+ binding as is that of nonactin. However, the nonactin molecule binds to K^+ through eight oxygen atoms in an approximately cubic 8-coordinate arrangement by folding around the K^+ ion, adopting the shape of a tennis ball seam. Valinomycin folds to offer approximately octahedral 6-coordinate to K^+ (Figure 1).

4.2.3 Metal Intoxication

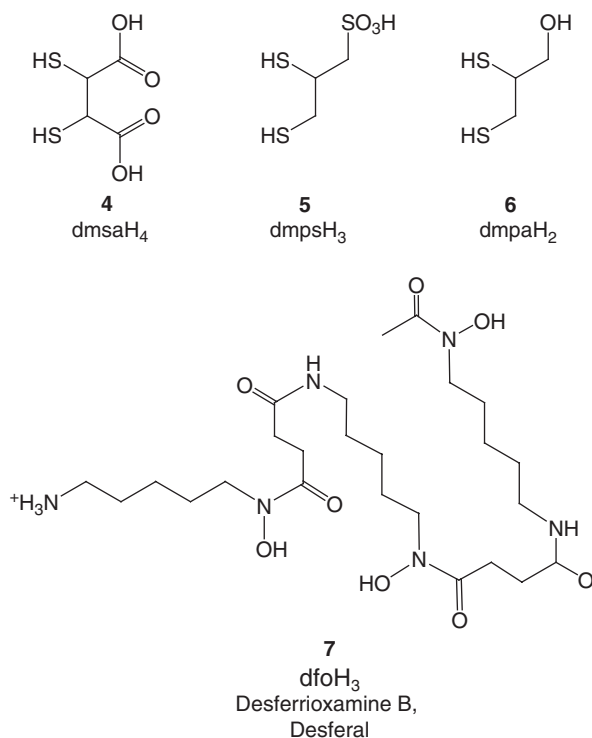
4.2.3.1 Historical Development

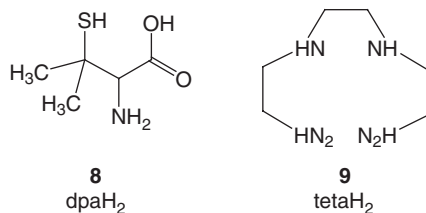
The use of chelation therapy to treat metal intoxication was developed during the 20th century, initially to moderate the toxicity of arsenic compounds used to treat syphilis. The use of citrate for the treatment of lead poisoning was also investigated but met with only limited success. Subsequently the polyamine carboxylic acids ethylenediaminetetra-acetic acid (see Chapter 3, **4**, $edtaH_4$)¹ and, more effectively, diethylenetriaminepenta-acetic acid (see Chapter 3, **3a**, $dtpaH_5$) were used to treat lead intoxication and for the decorporation of radionuclides. These two compounds are poorly absorbed by the gastrointestinal tract and are best administered by slow intravenous infusion. They

¹See footnote 1 in Section 3.2.1.7 regarding the use of upper and lower case abbreviations for ligands/proligands.

distribute into extracellular regions and are rapidly excreted in urine. Toxic effects can arise from their ability to sequester essential metal ions such as Ca^{2+} and Zn^{2+} but these can be moderated by the use of $\text{CaNa}_2(\text{edta})$, $\text{Ca}_2(\text{edta})$, $\text{Zn}_2(\text{edta})$ or $\text{ZnNa}_3(\text{dtpa})$ instead of simple sodium salts. As an example, for a given dose, $\text{Ca}_2(\text{edta})$ is around 20 times less toxic than $\text{Na}_4(\text{edta})$ and, in turn, $\text{Zn}_2(\text{edta})$ is around 10 times less toxic than $\text{Ca}_2(\text{edta})$.

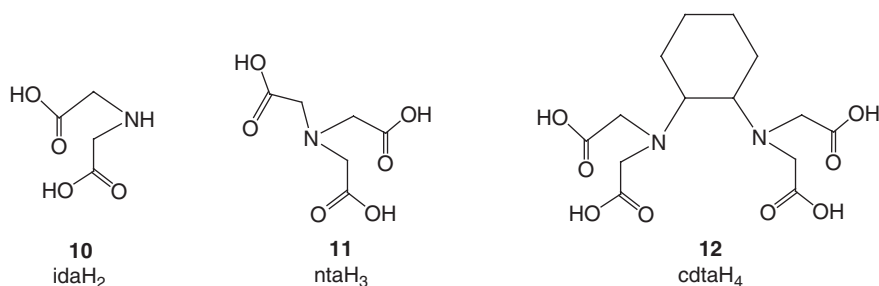
In the 1950s *meso*-2,3-dimercaptosuccinic acid (**4**, DMSA, dmsaH_4) and *d,l*-2,3-dimercaptopropane-1-sulfonic acid (**5**, dmpsH_3) came into use as metal sequestering agents and are registered drugs in the USA and Germany, respectively. These superseded the less biologically stable and more toxic compound British Anti Lewisite, 2,3-dimercaptopropanol (**6**, dmpaH_2), developed in World War II as an antidote to the chemical weapon dichlorovinylarsine (Lewisite). In addition to their low toxicity compared to dmpaH_2 , dmsaH_4 and dmpsH_3 can be taken orally while dmpaH_2 must be injected and has unpleasant side effects. However, dmpaH_2 appears to be distributed into intracellular spaces whereas dmsaH_4 and dmpsH_3 are primarily extracellular in distribution, so to some extent the compounds address different biological compartments. All are excreted in urine and, in humans, the whole blood and urinary excretion half times of dmsaH_3 are less than 4 h and, for dmpsH_3 , rather longer at 9–10 h. Sequestering agents specific to iron and copper were also developed to treat diseases which give rise to toxic effects resulting from imbalances of these metals. These include desferrioxamine (**7**, DFO, dfoH_3), *d*-penicillamine (**8**, dpaH_2) and 2,2,2-tet (**9**, tetaH_2 also known as TETA). These are discussed in more detail in Sections 4.2.4 and 4.2.5.





4.2.3.2 Choice of Ligand

The polyamine carboxylic acids exploit the chelate effect (Section 2.7.3) in forming stable complexes through the formation of multiple chelate rings. The increase in complex stability with increasing number of chelate rings can be seen in the stability constant (β_1) variation for the series of Cd²⁺ complexes with iminodiacetic acid (**10**, idaH₂), $\beta_1 = 10^{5.7}$; nitrilotriacetic acid (**11**, ntaH₃), $\beta_1 = 10^{9.8}$; edtaH₄, $\beta_1 = 10^{16}$ and dtpaH₅, $\beta_1 = 10^{19}$. The match between the size of the chelate ring and the size of the metal ion, together with the ability of the ligand to fold around the metal to optimise metal-ligand interactions, are also important considerations. This is illustrated by the equilibrium constant (K) values for the reaction in which two tridentate *N*-methylimidodiacetate, $\{\text{CH}_3\text{N}(\text{CH}_2\text{CO}_2)_2\}^{2-}$, ligands on Cd²⁺ are displaced by a single hexadentate $\{(\text{O}_2\text{CCH}_2)_2\text{N}(\text{CH}_2)_n\text{N}(\text{CH}_2\text{CO}_2)_2\}^{4-}$ ligand in which the size of the central $-(\text{CH}_2)_n-$ link is varied. As the chelate ring size increases with increasing n , the value of K decreases from the value for a 5-membered chelate ring at $n = 2$ as follows: $K = 10^{7.2}$ ($n = 2$, edta⁴⁻), $10^{2.4}$ ($n = 3$), 0.1 ($n = 4$) and 0.025 ($n = 5$). Thus as the central chelate ring size increases the ligand structure becomes less well adapted to a 6-coordinate geometry and the hexadentate ligand becomes less competitive with two separate tridentate $\{\text{CH}_3\text{N}(\text{CH}_2\text{CO}_2)_2\}^{2-}$ ligands.



The rigidity of the ligand structure can also be controlled by ligand design. In edta⁴⁻ rotation about the C–C bonds in the central $-\text{CH}_2\text{CH}_2-$ group allows the ligand to adopt many structural arrangements which are not suitable for complete ligand binding to a single metal ion. A more rigid structure arises if the $-\text{CH}_2\text{CH}_2-$ group is replaced by a 1,2-cyclohexyl group as in cdtaH₄, **12**. Here the amino carboxylate groups are constrained to adopt a structure where the N atoms occupy locations more nearly suited for metal ion coordination. This can improve the stability of the complex formed through preorganisation of the ligand (Sections 2.7.3 and 3.2.1.7).

In the polyamine carboxylate ligands the amine groups are best suited to binding borderline metals while the carboxylate groups are best suited to binding hard character metal ions, although they are also effective in binding to borderline metals. The dithiol ligands dmsaH₄ and dmsh₃ are particularly suited to binding soft metal ions (Section 2.7.2) such as Cd²⁺, Hg²⁺ and Pb²⁺ but, again, are also able to form complexes with a variety of metals in the borderline category (Table 7, Chapter 2). Some examples of the application of different sequestering agents to various toxic metals follow. The range of metals for which information is available is somewhat limited and, although quite a lot of animal data has been collected, data obtained clinically on human subjects is mostly confined to a few industrially important metals.

4.2.3.3 *Some Applications of Chelation Therapy to Metal Intoxication*

4.2.3.3.1 Aluminium. Al toxicity is typically treated by dfoH₃ infusion, although the similarity in charge and radius of Al³⁺ and Fe³⁺ ions has led to the consideration of other iron chelators (Section 4.2.4) for Al detoxification.

4.2.3.3.2 Antimony. Antimony is a component of 'tartar emetic' and its compounds are used to treat Schistosomiasis by intravenous infusion. Human cases of antimony poisoning have been treated with some success using dmpaH₂, dmsaH₄ or dmsh₃. Animal studies suggest that dmsaH₄ should be the proligand of choice for this element.

4.2.3.3.3 Arsenic. Historically dmpaH₂ or dpaH₂ have been used to treat arsenic poisoning. However animal studies indicate that dmsaH₄ or dmsh₃ would be more effective. Human data for the latter two compounds are limited but they appear to have been used with some success.

4.2.3.3.4 Bismuth. Bismuth compounds have been used in the past for the treatment of syphilis and are used today to treat gastric disorders. In humans mixed results have been obtained using dmpaH₂ but there is evidence that dmsh₃ offers an effective treatment.

4.2.3.3.5 Cadmium. Cadmium is widely used in a variety of applications and the treatment of Cd intoxication by chelation therapy has attracted some interest. However, toxicity issues can be a problem with novel bespoke chelating agents. It has been suggested that oral dmsa combined with parenteral CaNa₃dtpa might be the best approach.

4.2.3.3.6 Cobalt. Animal studies suggest that edtaH₄ or dtpaH₅ would be more effective than dmsaH₄ but information is limited.

4.2.3.3.7 Copper. Animal studies have shown that dmpaH₂, dpaH₂, 2,2,2-tet and dmsaH₄ can promote copper excretion. Human cases of copper poisoning have been treated using dmpaH₂ and dpaH₂ but it was not clear whether this, or the other supportive treatment, was responsible for the recovery. In China

dmsaH₄ has been used for many years to treat copper intoxication arising from Wilson disease (Section 4.2.5).

4.2.3.3.8 Gold. Historically dmpaH₂ and dpaH₂ have been used to treat gold intoxication but animal studies suggest dmsaH₄ and dmphsH₃ could also be effective and are less toxic than dmpaH₂.

4.2.3.3.9 Iron. The American Association of Poison Control Centers surveillance system indicates that around 20,000 cases of iron intoxication in children arise each year, mainly involving vitamin and mineral supplements. Chelation therapy with dfoH₃ appears to offer no benefit in mild cases. Fatalities continue to arise from acute iron poisoning due to the ingestion of iron salts, for example in concentrated iron supplements and, in these acute cases intravenous infusion of dfoH₃ can be an effective treatment. Iron overload in thalassaemia sufferers has traditionally been treated using dfoH₃ infusion but other oral chelators are coming into clinical use (Section 4.2.4).

4.2.3.3.10 Lead. Even though lead is no longer used in all of these applications, its widespread use in car batteries, buildings, paints, petrol antiknock agents and water pipes make it an important target for chelation therapy. Epidemiological evidence that environmental lead poses a risk of cognitive impairment in children has been a major driver for the application of chelation therapy and, in 1991, dmsaH₄ was licensed in the USA as an oral treatment for children with blood lead levels above 450 µg l⁻¹. Animal studies suggest that dmsaH₄ has advantages over dmpaH₂, dpaH₂ and edtaH₄ which have also been used to chelate lead. In particular dmpaH₂ and edtaH₄ form toxic complexes with iron so interfering with the simultaneous use of iron therapy to treat lead-induced iron deficiency in cases of lead poisoning.

4.2.3.3.11 Manganese. Animal studies showed increased urinary excretion of Mn when dtpaH₅ or, less effectively, edtaH₄ were administered. Parenterally administered Mn was not mobilised by dmsaH₄ in rats. In chronic human intoxication by Mn neither edtaH₄ nor dmsaH₄ relieved the symptoms but edtaH₄ did increase Mn excretion in urine while dmsaH₄ was ineffective.

4.2.3.3.12 Mercury. As with lead mercury has a long history of human toxicity incidents and the methyl mercurials are particularly dangerous. A variety of chelators including dmpaH₂, dpaH₂, dmsaH₄ and dmphsH₃ have been applied to the problem and dmpaH₂ followed by oral dpaH₂ has been a traditional approach. However, dmpaH₂ increases brain deposition of CH₃Hg⁺ and is unsuited for methylmercury poisoning. A significant amount of animal and human data has now accumulated and suggests that dmsaH₄ and dmphsH₃ should be the agents of choice for treating mercury poisoning. Chronic intoxication by inorganic mercury would seem to be best treated with dmphsH₃ while dmsaH₄ seems to be the more effective for cases of organic mercury poisoning.

4.2.3.3.13 Nickel. Dermatitis induced by exposure to soluble nickel compounds probably represents the primary incidence of toxic effects from nickel. Creams containing edtaH₄, diethyldithiocarbamate [edt⁻, (C₂H₅)₂NC(=S)S⁻]

or dimethylglyoxime [dmgH_2 , $\text{CH}_3\text{C}(=\text{NOH})\text{C}(=\text{NOH})\text{CH}_3$] have been found to reduce the response of nickel allergic subjects to nickel salts in patch tests but effects on skin penetration by Ni^{2+} were variable.

4.2.3.3.14 Platinum. The introduction of platinum anticancer drugs has promoted interest in Pt^{2+} chelators to moderate the toxic side effects of these drugs. Reduced nephrotoxicity and increased biliary excretion have been found when using ed^- and, in animal studies, dmsaH_4 increased urinary excretion of Pt. The use of Amifostine, a phosphorylated aminothiol compound, for this purpose is mentioned in Section 4.3.2.3.1. Some people have a severe allergic reaction to Pt^{2+} compounds such as $\text{K}_2[\text{PtCl}_4]$ and must avoid any contact with such materials.

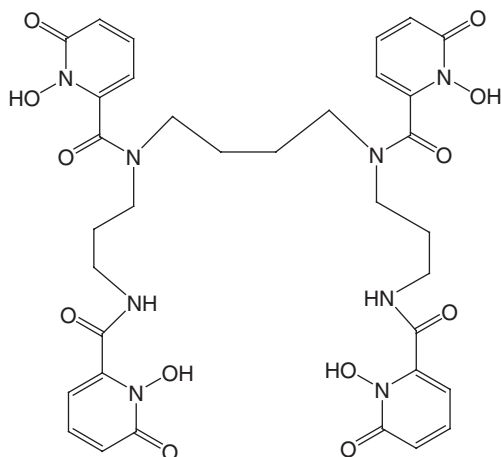
4.2.3.3.15 Plutonium. A particular example of the application of ligand design to toxic metal sequestration is provided by the development of agents for the decorporation of radiotoxic plutonium. As an f-block actinide element $\text{Pu}(+4)$ is oxophilic forming its strongest complexes with hard donors. The chemistry and biological behaviour of Pu^{4+} shows some similarities to that of Fe^{3+} but the larger ionic radius of the Pu^{4+} ion can accommodate 8-coordination. The typical choice of sequestering agent for an ion of this type would be dtpa^{5-} which offers the prospect of 8-coordination within a pentanegative multichelating ligand. In an attempt to improve on this agent various other polydentate ligands designed to encapsulate the Pu^{4+} ion were investigated. In mice the so-called LICAM proligands containing four 1,2-dihydroxyaryl [derived from 1,2-dihydroxybenzene or catechol, 1,2-(HO) $_2\text{C}_6\text{H}_4$] chelating groups could produce similar urinary excretion of injected ^{238}Pu to CaNa_3dtpa Table 1, but were limited in their effectiveness by the weak acidity of the aryl OH groups. Under physiological conditions of near neutral pH the aryl OH groups remain largely protonated and, in effect, H^+ competes for the aryl- O^- groups. Better total excretion could be obtained with proligands containing more acidic hydroxamate and *N*-hydroxypyridinonecarboxylate chelating groups, exemplified by 3,4,3-lihopo H_4 , **13**, and dfohopo H_4 , **14**. Although these

Table 1 Excretion of injected ^{238}Pu from mice following chelation therapy^a

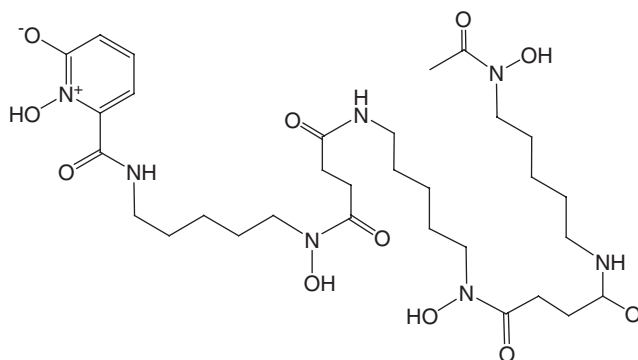
Sequestering Agent (30 $\mu\text{mol kg}^{-1}$ ip)	% injected $^{238}\text{Pu}(+4)$ citrate excreted via		
	Urine	Gut (Faeces and GI tract contents)	Total
CaNa_3dtpa	63	7	69
LICAM (Carboxylic acid derivative)	49	22	71
LICAM (Sulfonic acid derivative)	62	2.4	64.4
Lihopo	24	57	81
Fe^{3+} -lihopo complex	40	46	86
Dfohopo	19	68	87

^a Data from D.L. White, P.W. Durbin, N. Jeung, and K.N. Raymond, *J. Med. Chem.*, 1988, **31**, 11–18.

compounds produced lower urinary excretion than CaNa_3dtpa a much larger proportion of the Pu was excreted *via* the gut.



13
lihopoH₄



14
dfohopoH₄

4.2.3.3.16 Thallium. Although banned in many countries thallium salts were once used in rodenticides among other applications. The superficial similarity between Tl^+ and K^+ has led to the administration of K^+ salts to moderate Tl^+ toxicity and promote its urinary excretion. Prussian blue, particularly the ammonium salt $\text{NH}_4\text{Fe}[\text{Fe}(\text{CN})_6]$, appears to be an effective treatment for $\text{Tl}(+1)$ poisoning but acts as an ion exchange reagent rather than a chelator. It is of low toxicity, retained within the gut and so promotes faecal elimination of Tl^+ through its exchange with NH_4^+ .

4.2.3.3.17 Zinc. Zinc is widely used, particularly as an anticorrosion coating and in skin creams and ointments. Cases of zinc poisoning have been treated with dmpaH_2 or edtaH_4 and it is known that edtaH_4 and, more

efficiently, dtpaH_5 mobilise Zn in humans. Studies of parenteral Zn intoxication in rodents suggested that edtaH_4 , cdtaH_4 and dtpaH_5 are the most effective treatments but dmsaH_4 also reduced acute mortality. A higher chelator/zinc ratio was needed to produce an effect with dmpsH_3 .

4.2.3.3.18 Conclusions. Apart from cases resulting from disease, acute metal intoxication is comparatively rare nowadays. An effective panel of well established chelating agents is available to treat metal intoxication, including edtaH_4 , dtpaH_5 , dmsaH_4 , dmpsH_3 and dfoH_3 . No doubt more effective sequestering agents could be developed for specific metals but increasing controls of environmental and occupational exposure to toxic levels of metals reduces the economic incentive to pursue research of this type. However, the search for new agents to address metal intoxication resulting from diseases or their treatment offers an important avenue for research into chelation therapy agents.

4.2.4 Thalassaemia–Iron

Thalassaemia is a genetic disease, which affects a significant number of the babies born each year worldwide. In 1990 of the order of 100,000 of babies were said to be seriously affected by thalassaemia. The number of gene carriers for the disease at that time was said to be around 100 million, widely distributed across the globe but particularly in areas affected by malaria. The genetic disorder is characterised by a reduced rate of synthesis of hemoglobin protein and is particularly acute for those having two defective genes (homozygotes) for hemoglobin. Sufferers of this more severe form of beta-thalassaemia, that is thalassaemia-major or Cooley's anaemia, usually die within one year of birth if left untreated. However, those having only one defective gene (heterozygotes) for hemoglobin show increased resistance to malaria. In humans the malaria parasite spends part of its life cycle in the red blood cells and, in the heterozygotes, the lifetime of these cells is reduced and development of the malaria parasite is impaired. Unfortunately, there is a one in four chance that two heterozygote parents will have a homozygote offspring.

The life expectancy of a thalassaemia-major sufferer can be extended to around 20 years by repeated regular blood transfusions. However, each pint of blood contains around 200 mg of Fe compared to a normal daily requirement of 1–5 mg. As there is no efficient excretion pathway for this excess iron, over a period of about 10 years a transfusion dependent thalassaemia patient might accumulate over 50 g of iron. This causes damage to the heart, liver and endocrine system and produces the toxic effects of iron overload. Removal of the excess iron by chelation therapy can substantially extend the life expectancy of transfusion dependent thalassaemia sufferers.

Polyamine carboxylates such as edta^{4-} might appear to offer an obvious approach to the treatment of iron overload by chelation therapy. However, these compounds are insufficiently selective for iron and remove other metals such as calcium or zinc. In fact the first chelating agent to be widely used to

treat iron overload in thalassaemia patients was the naturally occurring fungal siderophore desferrioxamine B (**7**, dfoH₃). This compound is a potent iron scavenger in nature and is highly selective for iron having much lower affinities for copper, zinc, calcium and manganese. The three hydroxamate groups provide strong binding sites for iron and the structure of the molecule allows it wrap around Fe³⁺ offering an essentially octahedral 6-coordinate environment. Desferrioxamine B is used clinically under the name Desferal, is water soluble, not strongly bound to protein in plasma and shows an extracellular distribution. The disadvantages are that it is expensive to prepare, can have side effects and is unsuitable for oral use. The hydroxamate groups are sensitive to acid in the stomach and the compound is poorly absorbed in the gastrointestinal tract. As a consequence administration by intravenous infusion over a period of hours is necessary several times a week. The complexed iron is excreted in the urine and *via* bile through the gut, the renal excretion being bimodal with the half time for the slower excretion being around 6 h. The compound is not well tolerated by all patients and a range of side effects have been found in long-term therapy. These include allergic reactions and renal, pulmonary and neurological effects. More recently a new agent has been obtained by chemically attaching dfoH₃ to a modified starch polymer. Like Desferal the resulting compound shows a high affinity and specificity for Fe³⁺ but it does not cause the acute toxic effects. Promising results have been obtained from Phase I clinical trials of this preparation and it may offer a means of extending the efficacy of the dfoH₃ chelation approach.

Despite the undoubted success of dfoH₃ in treating iron overload, the expense of this chelator, the need for its intravenous administration and unacceptable side effects in some patients provide strong driving forces to develop an alternative oral treatment. The design of such new agents might be informed by consideration of the structures of other naturally occurring siderophores secreted by micro-organisms to scavenge iron. Apart from the hydroxamate moiety found in dfoH₃, the 1,2-dihydroxybenzene (catechol) unit found in the cyclic trimer siderophore enterobactin (**15**) offers a possible alternative approach to the sequestration of iron. Unfortunately catechol derivatives tend to be poorly absorbed in the gut and prone to oxidation so do not offer a viable oral alternative to dfoH₃. A structure which incorporates the structural features of both catechol and hydroxamate is provided by 1-hydroxy-pyridin-2-one (**16**) derivatives. Such compounds show a high affinity for iron ($\log\beta_3 = 27$ for **16**) and are relatively stable to acid. Higher binding constants are possible using 1-alkyl-3-hydroxy-pyridin-2-one (**17**, $\log\beta_3 = 32$) derivatives or, even better, 1,2 dialkyl-3-hydroxy-pyridin-4-one (**18**, $\log\beta_3 = 37$) derivatives. The 3-hydroxy-pyridin-4-ones show high selectivity for iron over copper and zinc compared to other chelating agents (Table 2) and are neutral ($pK_{a1} = 3.6$, $pK_{a2} = 9.9$) over the physiologically important pH range of 5.0–9.0. In solution under these conditions the 3:1 complex of **18** with iron predominates. The solid-state structure of the iron complex with **18** ($R^1 = C_2H_5$, $R^2 = CH_3$) reveals a distorted octahedral 6-coordinate geometry around the Fe³⁺ ion as expected. In the biological context the 1-alkyl-3-hydroxy-pyridin-2-ones do not remove

Table 2 Selected stability constants ($\log \beta$) of metal complexes

Metal ion	Proligand and $\log \beta^a$			
	18^b	DFO- B^c	Catechol d	edta H_4
Fe $^{3+}$	37 ($\log \beta_3$)	31	40 ($\log \beta_3$)	25
Cu $^{2+}$	17 ($\log \beta_2$)	14	25 ($\log \beta_2$)	18
Zn $^{2+}$	12.5 ($\log \beta_2$)	11	17 ($\log \beta_2$)	16
Ca $^{2+}$	4.5 ($\log \beta_2$)	2.5		11
Mg $^{2+}$	7 ($\log \beta_2$)	4	6 ($\log \beta_2$)	9

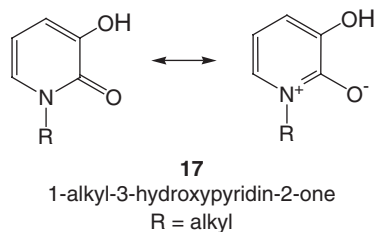
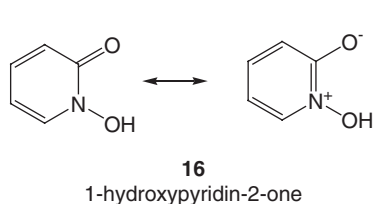
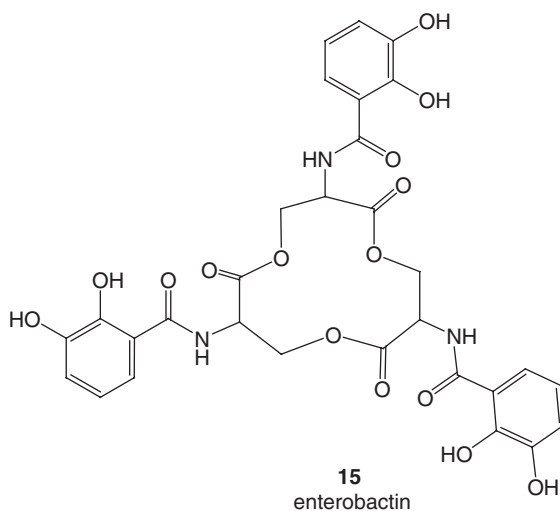
^a As $\log \beta_1$ unless otherwise specified

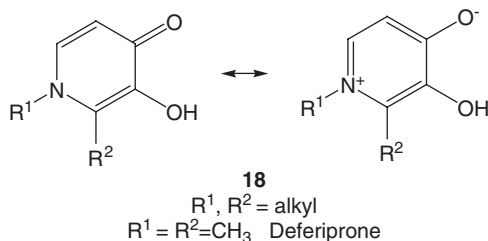
^b 3-hydroxypyridin-4-one.

^c Desferrioxamine B.

^d 1,2-dihydroxybenzene.

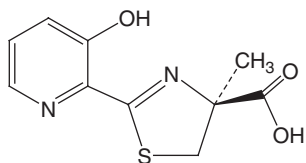
iron from hemoglobin or cytochromes but do compete effectively with the weaker albumin binding sites in blood. They show a similar affinity for iron to the iron transport protein transferrin so can reduce iron levels without greatly affecting essential iron uptake in bone marrow. In contrast to dfoH₃, which is too large for a molecule to enter channels in the structure of the iron storage protein ferritin, the 1,2 dialkyl-3-hydroxy-pyridin-4-ones can enter the structure and mobilise stored iron.



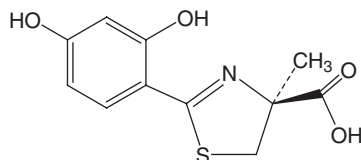


The dimethyl derivative **18** ($R^1 = R^2 = \text{CH}_3$) has undergone clinical trials under the name Deferiprone (L1) with promising results from several countries. However, the compound is not universally effective with discontinuation rates of 20–40% in some trials. There is also a higher risk of agranulocytosis which may restrict its use to patients for whom treatment with desferrioxamine is unsuitable. In 1999, Deferiprone was licensed in Europe for use with patients in whom treatment with dfoH_3 proves inadequate, and is now in use in around 50 countries. The possibility of a synergistic effect from using dfoH_3 and Deferiprone together has been considered and there is increasing evidence to suggest that this may be the case. Combined treatment may provide therapeutically beneficial results in that using lower doses of Deferiprone may reduce the occurrence or severity of side effects while requiring fewer dfoH_3 infusions each week. In addition recent studies indicate that the long-term use of Deferiprone can markedly improve cardiac function.

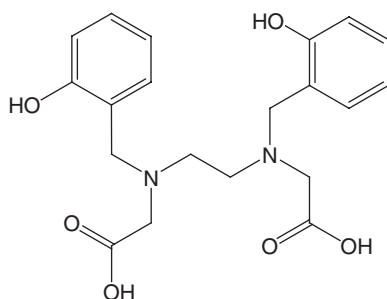
In order to minimise the ability of $\text{Fe}^{3+/2+}$ to enter into unwanted oxidation/reduction reactions it is important to fully saturate the iron coordination sphere to prevent the approach of reactive substrates. Increasing the size of the sequestering ligand and moving to tridentate or hexadentate, rather than didentate, chelation may offer improvements over the hydroxypyridinones. Examples of tridentate sequestering agents for iron include the siderophore Desferrithiocin (DFT, **19**, dftH_2) originally isolated from *Streptomyces antibioticus*. Animal studies showed this to be an orally available agent which can reduce acute iron overload. However there were side effects raising doubts over its suitability for long-term use in humans. A number of analogues of Desferrithiocin are now being investigated with a view to identifying less toxic alternatives. Among these, GT56-252 (**20**) has entered clinical trials with promising results. An example of the development of a hexadentate sequestering agent is provided by HBED (**21**, hbedH_4). This compound has a high affinity for Fe^{3+} and forms a 1:1 complex with good selectivity. Despite promising results in rodents it is insufficiently active in humans when given orally. However, in animal studies the monosodium salt NahbedH_3 appeared to be about twice as efficient as dfoH_3 in promoting iron excretion when delivered parenterally. The compound did not show significant systemic toxicity or have detrimental effects on blood pressure or heart rate. If the compound is similarly well tolerated in humans it may offer an alternative to dfoH_3 for treating acute iron poisoning or chronic iron overload, although parenteral administration would still be necessary. Since hbedH_4 is chemically different from dfoH_3 , it may offer an important alternative for patients allergic to dfoH_3 .



19
Desferrithiocin

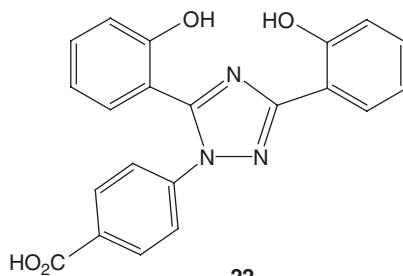


20
GT56-252



21
HBED, hbedH₄

Most notable among the variety of other iron chelators being investigated is the tridentate chelator deferasirox (**22**, ICL670). In clinical trials involving over 1000 patients this compound was well tolerated and produced a dose dependent excretion of iron almost entirely *via* faeces. In patients with thalassaemia and sickle cell disease, as well as other rare anaemias, who were receiving blood transfusions, the iron burden could be maintained or reduced using doses of 20–30 mg kg⁻¹ day⁻¹. The drug has the great advantage that it can be administered orally and only once daily at that. Approval in the USA for the worldwide use of deferasirox under the trade name Exjade[®] was granted in November 2005.



22
Deferasirox
ICL670

4.2.5 Wilson Disease–Copper

Wilson disease is a genetic disorder of gene ATP7B on chromosome 13 which affects around 1 out of every 30,000 members of the population. It is an

autosomal recessive condition so that a person must inherit an altered gene from each parent if they are to develop the disease. Those with only one altered gene do not develop the disease but remain carriers who may pass the disease gene on to their offspring. The ATP7B gene is associated with the synthesis of a P-type ATP-ase used in copper transport and is expressed mainly in the liver, kidney and placenta. In Wilson disease sufferers, this genetic disorder leads to excess copper accumulating in the liver. Defective biliary excretion of copper may play an important role in the process and the build up of copper in the cytosol of hepatocytes eventually leads to necrosis. This results in the release of copper into the bloodstream where it can damage erythrocyte membranes. It also delivers excess copper to other organs, particularly the brain, leading to more widespread damage including neurological symptoms.

Copper is an essential trace metal required, for example, by enzymes such as oxidoreductases and mono-oxygenases. Most people obtain more than enough copper to meet their needs from their diet and, in order to maintain normal copper levels, the excess copper must be excreted. Ingested copper is absorbed through the wall of the intestine to enter the bloodstream as exchangeable Cu^{2+} bound to albumin and low molecular weight amino acid complexes. Reduction to Cu^+ is thought to occur in a membrane reductase before entry to the cell *via* a membrane Cu^+ transporter. Once inside the cell it is thought that most of the copper is bound to glutathione (GSH) as Cu^+ . The copper is also processed by various so-called 'chaperone proteins' which mediate its transport to proteins such as cytochrome oxidase and superoxide dismutase, as well as to copper transporting ATP-ases. One of the recipient sites for copper is the Wilson ATP-ase which has been found to bind six Cu^+ ions in the N terminal region. The defective function of this protein in Wilson disease sufferers leads to inadequate excretion of copper and its accumulation in the liver. Chelation therapy can be used to create a negative copper balance thereby preventing the development of symptoms in presymptomatic patients or relieving the symptoms where the effects of the disease are more fully developed. Since chelation therapy cannot address the underlying cause of the illness it must be continued throughout the lifetime of the subject. This places demands on the toxicity of the chelating agent used and toxicity levels acceptable for short-term treatment of acute copper poisoning would not necessarily be acceptable for long-term treatment of Wilson disease.

Under physiological conditions copper can be present either of two oxidation states, $\text{Cu}(+2)$ or $\text{Cu}(+1)$. The $d^9 \text{Cu}^{2+}$ ion has a small Crystal Field Stabilisation Energy and is most usually found with coordination number 6 in distorted octahedral geometries or with coordination number 4 in square planar geometries. It is borderline in character typically forming complexes with ligands containing N or O donor atoms. In contrast $d^{10} \text{Cu}^+$ has no Crystal Field Stabilisation Energy and is normally found with coordination number 4 in near tetrahedral geometries. It is a soft metal ion which is very readily oxidised and most stable when bound to soft donor atoms such as phosphorus or sulfur. One approach to the removal of extracellular copper would be to sequester it in ligands which can effect the reduction of Cu^{2+}

releasing it from serum protein or amino acid binding sites and complexing it as Cu^+ in a form which can be excreted. Certainly thiol containing proligands have been used with good effect to sequester copper in treatment of Wilson disease. Initially dmpaH_2 (**6**) was used but, as this compound required intramuscular injection, it was superseded in the 1950s by oral treatment using *D*-penicillamine (**8**, dpaH_2) known as Cuprimine or Depen. A reductive chelation mechanism has been proposed in which dpaH_2 reduces protein bound copper Cu^{2+} by converting it to Cu^+ which is sequestered by the dpaH_2 . This produces a marked increase in urinary excretion of copper which then decreases to more normal levels over a period of months. Removal of excess copper from the liver is a much slower process and elevated levels may persist for years after the start of treatment. Toxicity is a major limitation on the long-term use of dpaH_2 and around one third of patients are hypersensitive to the drug. Also the condition of patients with neurological symptoms can become worse in around half the cases treated with dpaH_2 . However, in China oral dmsaH_4 (**4**) has been used for over 30 years to good effect, even in treating the later stages of the disease.

The problems with dpaH_2 intolerance in a significant proportion of patients has led to the use of alternative sequestering agents. The linear tetramine 2,2,2-tet (**9**) also known as trien, trientine or Syprine was also found to be effective in competing for protein bound Cu^{2+} and promoting urinary excretion. However, this drug does not appear to mobilise copper accumulated in the liver. An important alternative to chelation therapy in the treatment of Wilson disease is the use of zinc acetate (Galzin[®]) which has lower toxicity than dpaH_2 . Zinc induces the metal binding protein metallothionein in the intestinal cells and this binds copper from the gut blocking its absorption. The copper is held in the intestinal cells until they are shed into the gut as normal and passed out by faecal excretion.

More recently there has been experimental interest in the use of tetrathiomolybdate, $[\text{MoS}_4]^{2-}$, which can induce copper deficiency in ruminants. This compound seems to block copper absorption in the intestine increasing faecal excretion. It also seems to convert absorbed copper to a form not readily accumulated by the liver. The treatment does not appear to cause neurological deterioration, unlike dpaH_2 , and may offer an alternative treatment for patients intolerant to all other approaches. However, $[\text{MoS}_4]^{2-}$ is known to have toxic effects on the skeletal system of growing animals and toxicity issues may limit its utility.

4.3 Cancer Therapy

4.3.1 Metal Complexes in Cancer Therapy

Compounds of many different metals, and representing a variety of differing structural types, have shown activity against tumor cells in animal models or tissue cultures. However, so far only compounds of platinum have attained any real importance in terms of clinical usage. The serendipitous discovery that