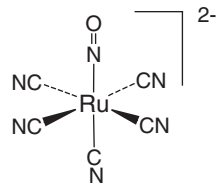
4.6.3 Nitric Oxide Management

Nitric oxide is important in a variety of physiological processes. Examples are provided by its action as a neuromodulator in the hippocampus of the brain, its production by macrophages to attack bacteria, fungi or tumor cells as part of the immunological response and its action as a vasodilator in the cardiovascular system. *In vivo* NO is produced by NOS enzymes through the oxidation of arginine to citrulline and NO. Endothelial, neuronal and immunological classes of NOS are known. These enzymes act to incorporate oxygen from O_2 into the NO and citrulline formed from the oxidation of argenine. *In vivo* NO has toxic effects, probably through its binding to intracellular iron or iron in non heme proteins and, although this may be beneficial in the immunological context, excessive NO production also has deleterious effects. One of these is to cause vasodilation and, in toxic-shock syndrome, excess NO production in macrophages produces arterial expansion which, in the extreme, can lead to cardiovascular collapse. Metal complexes which can absorb NO may thus play a rôle in treating toxic shock. Conversely it is sometimes necessary to stimulate vasodilation, to reduce blood pressure for example, and in such cases a metal complex capable of releasing NO under appropriate conditions would be of pharmacological value.

The complex used clinically to treat hypertension is sodium nitroprusside, $Na_2[Fe(CN)_5(NO)].2H_2O$ available as NITROPRESS® or NITROPRUSSIN. This compound is most often used because its effect is almost instantaneous and careful adjustment of the dose can allow a smooth reduction in blood pressure. However, because of its very rapid action, it is important that patients are carefully monitored and doses regulated properly to avoid irreversible ischemic injuries. Another potential problem with the use of $[Fe(CN)_5(NO)]^{2-}$ is the release of cyanide from the complex in conjunction with NO. If excessive doses are used it is possible for toxic levels of cyanide to be reached. The usual range of dose rates used is 0.5–10 pg kg^{-1} min^{-1} and at dose rates of <2 pg kg^{-1} min^{-1} cyanide toxicity is not usually an issue. However, infusion at the maximum dose rate for more than a few minutes might lead to unacceptable cyanide levels.

The release of NO from $[Fe(CN)_5(NO)]^{2-}$ (**143**) follows reduction of the complex *in vivo*. The bonding in $[Fe(CN)_5(NO)]^{2-}$ is complicated by the 'non-innocent' behaviour of the NO ligand. The complex can be thought of as containing a d^6 Fe(+2) ion bound to NO^+ acting as a 2-electron ligand in similar manner to CO. Calculations suggest that reduction of the complex adds an electron to an orbital which has both NO antibonding π^* and metal t_{2g} character. In the synergic bonding model (Section 2.5.2) addition of an electron to the NO antibonding π^* orbitals opposes back bonding from the metal to the NO weakening the Fe–N(nitric oxide) bond through reducing the π-interaction while leaving the σ-interaction intact. The resulting increase in electron density at the metal labilises the CN^- ligand *trans* to the NO. When this *trans*-CN^- dissociates to form a 5-coordinate complex the metal d_{z^2} orbital decreases in energy and becomes partially populated at the expense of the NO π^* orbitals. Since d_{z^2} contributes to the e_g^* orbital which is σ^*-antibonding with respect to

the metal-ligand bonding, this has the effect of labilising all of the ligands leading to the release of NO and CN^-. The action of nitroprusside depends upon the subtle interplay between the π-acceptor properties of the NO^+ ligand, the π-donor properties of the metal ion and the lability of NO, which is, in effect, formed from coordinated NO^+ by reduction of the complex.



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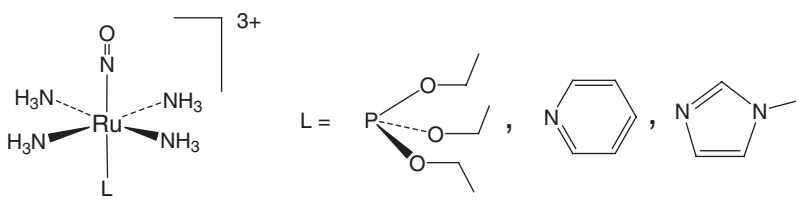
It would clearly be advantageous to develop a metal nitrosyl complex which could act as a source of NO *in vivo* without the associated toxicity effects due to cyanide. Attempts to identify such compounds have focused on Ru complexes. This metal lies immediately below Fe in the Periodic Table, it has a well established chemistry involving the NO ligand, its complexes tend to be more kinetically inert than those of Fe and it its compounds tend to have low toxicity. A simple cyanide free counterpart to $[Fe(CN)_5(NO)]^{2-}$ is provided by the complex $[trans\text{-}Ru(NH_3)_4(L)(NO)]^{3+}$ (**144**) in which L is a ligand chosen to optimise the lability of the *trans*-NO. Examples of L are provided by $P(OC_2H_5)_3$ or aromatic nitrogen heterocycles such as pyridine or *N*-methyl imidazole. Such complexes do show vasodilator properties so, for example, $[Ru(NH_3)_4\{P(OC_2H_5)_3\}(NO)]^{3+}$ is easily reduced to $[Ru(NH_3)_4\{P(OC_2H_5)_3\}(NO)]^{2+}$ which undergoes a hydration reaction to form $[Ru(NH_3)_4\{P(OC_2H_5)_3\}(H_2O)]^{2+}$ releasing NO. In a similar manner to $[Fe(CN)_5(NO)]^{2-}$, in an ionic bonding model, $[trans\text{-}Ru(NH_3)_4(L)(NO)]^{3+}$ could be thought to contain NO^+ coordinated to d^6 Ru(+2). Unfortunately the π-acceptor properties of $P(OC_2H_5)_3$ compete with *trans*-NO^+ ligand for electron density on the metal ion making it more susceptible to attack by OH^-. This leads to the formation of coordinated nitrite, NO_2^-, which readily dissociates to leave the Ru(+2) complex $[Ru(NH_3)_4\{P(OC_2H_5)_3\}(H_2O)]^{2+}$ but without NO evolution (Equation (15)).

$$[Ru(NH_3)_4\{P(OC_2H_5)_3\}(NO)]^{2+} + 2OH^- \rightarrow [Ru(NH_3)_4\{P(OC_2H_5)_3\}(H_2O)]^{2+} + NO_2^- \quad (15)$$



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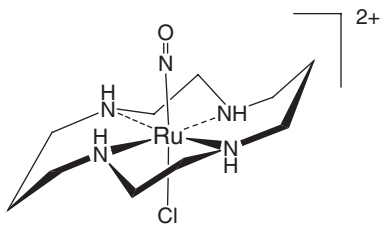
This problem can be alleviated by replacing the $P(OC_2H_5)_3$ligand with Cl^- which does not have π-acceptor properties. In addition the ammonia ligands can be replaced by the macrocyclic ligand cyclam to give a more robust platform for carrying NO in the form of $[Ru(cyclam)Cl(NO)]^{2+}$(**145**). Following reduction, this complex dissociates NO more slowly than $[Fe(CN)_5(NO)]^{2-}$ and produces a hypotensive effect in Wistar rats lasting some 20 times longer. After reduction the complex releases NO with a rate of 2.2×10^{-3} s^{-1} at 35 °C with no associated cyanide release.



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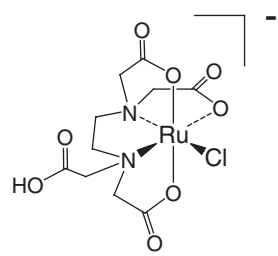

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The presence of the neutral cyclam ligand in $[Ru(cyclam)Cl(NO)]^{2+}$ leads to the formation of a positively charged complex in which π-donation from the metal centre to the NO ligand is inhibited. By increasing the negative charge on the ligand set around the metal centre to promote back donation to coordinated NO, it is possible to strengthen the binding of NO in the complex. This provides a basis for the design of an NO scavenger to treat toxic shock arising from high NO levels in the bloodstream. Polyamine carboxylate ligands offer one approach in that they possess multiple negative charges and confer high thermodynamic stability on their metal complexes through a multiple chelate effect. The negatively charged carboxylate groups help polarise the molecule to encourage π-back donation to π-acceptor ligands such as NO^+. The water soluble Ru(+3) ethylenediaminetetra-acetic acid ($edtaH_4$) complex $[Ru(edtaH)Cl]^-$ (**146**) has been found to act as an NO scavenger and to inhibit the vasodilation effects of NO-releasing agents. In this chloro-complex one carboxylic acid group of the $edtaH^{3-}$ remains uncoordinated so that the Ru(+3) ion is 6-coordinate. In aqueous media the complex is easily hydrated and an equilibrium is established between $[Ru(edtaH)Cl]^-$ and $[Ru(edtaH)(H_2O)]$. The latter complex reacts with NO in a second order reaction (rate = 2×10^{-7} M^{-1} s^{-1} at pH 7.4, 7 °C) to give [Ru(edtaH)(NO)] which can be thought of as containing Ru(+2), NO^+ and $edtaH^{3-}$. Acid dissociation of the pendant carboxylate affords the negatively charged ion $[Ru(edta)(NO)]^-$. Tests in rats used to model toxic shock syndrome showed that treatment with K[Ru(edtaH)Cl] reduced the time taken to return to normal blood pressure from *ca.* 26 to 9 h. These results demonstrate the potential for using Ru complexes to regulate NO levels *in vivo*. Careful selection of the ligands used allows the properties of the complexes to be modified to suit NO scavenging or NO releasing behaviour. These properties have potential applications in the

treatment of toxic shock, the formulation of vasodilators and in treatments for neurological disorders arising from imbalances in NO levels.

4.7 Therapeutic Radiopharmaceuticals

4.7.1 Radiation Therapy

Conventional radiotherapy procedures involving external radiation sources play a vital rôle in the treatment of some forms of cancer. Typically the subject will be moved around in a narrow beam of radiation so that the cancerous region always lies within in the beam but other tissue is only transiently irradiated. In this way the whole body radiation is minimised while giving a therapeutic cytotoxic dose to the diseased tissues. Unfortunately this approach cannot be applied when the cancer is dispersed or metastatic. Radiopharmaceuticals provide a potential means of selectively targeting diseased tissue for irradiation through internal processes. These accumulate the radioactive agent in the diseased tissue where it can deliver a cytotoxic radiation dose. This general approach to managing the *in vivo* distribution of radionuclides is well established in non-invasive diagnostic medicine (Section 3.3). However, the use of cytotoxic radiopharmaceuticals in therapy is much more challenging because of the necessarily high radiotoxicity of the doses used.

In order to be acceptable for clinical use a therapeutic radiopharmaceutical must accumulate in the target tissue with high selectivity and at a rate which does not lead to unacceptable non-target tissue radiation doses. The agent must also be rapidly cleared from non-target tissues and excreted. It is also important that the products of chemical degradation and radioactive decay of the agent are of low toxicity and/or rapidly cleared form the body. These demands place restrictions on the nature of the radionuclide used in terms of its half life and decay products as well as on the biodistribution and pharmokinetic behaviour of the radiopharmaceutical agent itself. The energy and type of radiation emitted by the radionuclide, together with its cost, availability and chemistry, will also have a bearing on its acceptability for clinical use. The types of radiation emitted by radionuclides are summarised in Section 3.3.2.1 and radionuclides which emit either α or β-particles are of potential use in therapeutic applications. However, most clinical studies have focused on β-emitters and regulatory approval has not yet encompassed any α-emitting radionuclides.

4.7.2 α-Emitters

Radionuclides which emit α-particles are of potential interest in situations where very short range (<0.1 mm) cytotoxic effects are sought. As an example irradiation of cancerous bone surfaces to control pain while not irradiating the blood forming bone marrow could be of interest. However, few α-emitters have suitable half lives for therapy or can be obtained in sufficient quantity and radiochemical purity. The two which have attracted most interest are ^{211}At