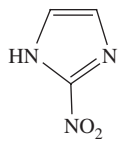
myocardial tissue. Unfortunately the compound showed high liver uptake, which partly obscures the heart in imaging procedures. A more subtle metal centred approach involved modifying the structure of **68** to change the redox potential of the complex and eliminate the need for the nitroimidazole substituent. The inclusion of an additional -CH_2- group in the central chelate ring to give **92** made the complex easier to reduce. Limited human trials showed hypoxic tumor accumulation of **92** in 7 out of 10 patients with lower liver uptake than **91**. These studies illustrate how subtle changes in ligand structure can be exploited to good effect in the development of new radiopharmaceuticals.



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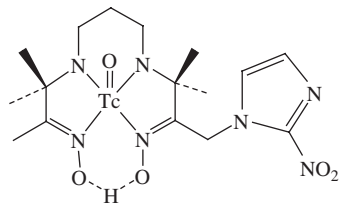
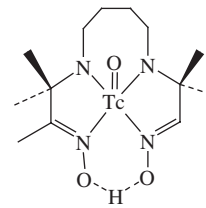


91

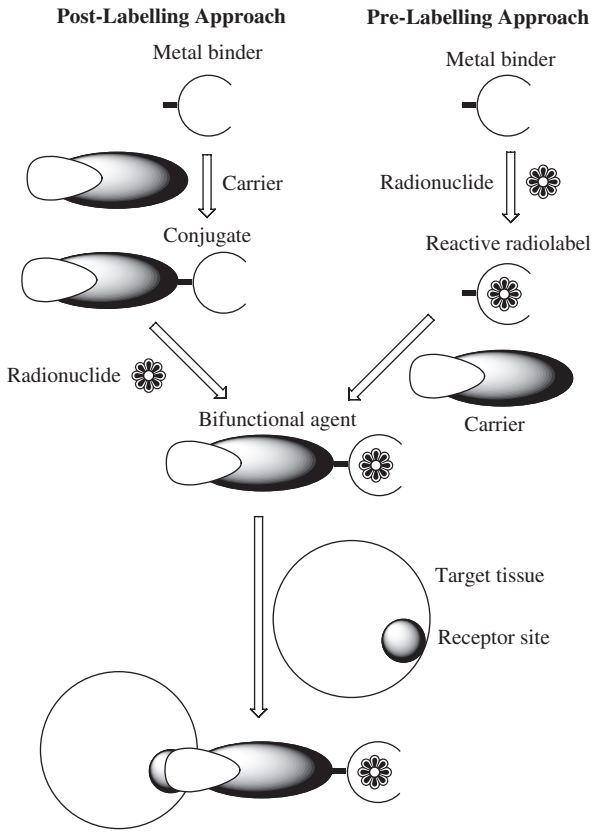


92

3.3.12 Synthetic Approaches to Bifunctional ^{99m}Tc Radiopharmaceuticals

The preceding Sections 3.3.11.2 and 3.3.10.2 contain some examples of what are known as bifunctional radiopharmaceuticals. That is they contain both a biologically active carrier moiety, which selectively binds to a specific receptor *in vivo,* and a radioactive metal ion. In order to form a bifunctional agent it is necessary to covalently attach, to the carrier part of the agent, a metal binding site, which can form a kinetically inert complex with the radionuclide to be used. In principle the radionuclide might be incorporated in the metal binding site before (pre-labelling) or after (post-labelling) its attachment to the carrier (Scheme 12). However, with short-lived nuclides such as ^{99m}Tc, the time required for linking the complexed metal to the carrier, and any subsequent purification step will be very limited and only rapid simple chemical procedures will be acceptable. If the procedure is at all time consuming the post labelling approach will be more appropriate. Thus the ease and efficiency of the chemistry needed to incorporate the metal into the binding site are important considerations in the design of bifunctional agents.

If a bifunctional agent is to be effective, the change in charge and structure resulting from the attachment of the metal and its binding site must not significantly impair the ability of the carrier moiety to recognise and bind to its target receptor. This may place limitations on the type of binding site used and the means by which it is attached to the carrier. Consequently, it is important to allow for flexibility in the design of the binding site. It is not only important that the binding site forms a sufficiently inert complex with the metal ion of choice, its presence must also be compatible with the carrier



Scheme 12

moiety. As an example dtpa anhydride can be an effective reagent for adding a metal binding site to a protein but requires a suitably located functional group, such as a free amine on the protein surface, with which to form a covalent link. In some cases the large size of the attached metal complex might affect the biological activity of the conjugate so that a less bulky binding group may be needed. It is also necessary to consider the charge and lipophilicity of the attached metal complex. The binding of a metal ion of charge 3+ to a binding site with charge 4− would add a charge of 1− on the surface of the carrier. The acceptability of such a change must be considered. Similarly, if the metal complex is more lipophilic or hydrophilic in character than the carrier the effect on the biodistribution of the bifunctional agent will need to be considered. Past experience can be a useful guide and computer modelling offers an increasingly powerful means of streamlining the development of bifunctional agents. However, a positive outcome cannot be predicted with certainty and there remains an element of trial and error in developing successful bifunctional agents. In some cases it may be necessary to change the chemical form of the metal ion or

the binding group. It may, for example, be necessary to change the linking group to one reacting with a different type of functional group found only in a location more remote from the active site of the carrier.

The exceptionally good nuclear properties of ^{99m}Tc make it the usual radionuclide of choice for diagnostic imaging; furthermore the rich chemistry of Tc offers a variety of ways in which ^{99m}Tc might be attached to a biologically active carrier. Thus the development of new highly target specific bifunctional ^{99m}Tc radiopharmaceuticals is a very active area of research where coordination chemistry is being exploited in the design of imaging agents. Historically the Tc(+5) TcO^{3+} centre has been widely used in nuclear medicine and is easily accessible from $[TcO_4]^-$ Not surprisingly therefore, the design of binding groups for TcO^{3+} is an important feature of the approaches being used. However, other Tc(+5) centres are possible which offer lower charges so that TcO_2^+ and TcN^{2+} provide Tc centres with charges +1 and +2, respectively. The 'naked' Tc^+, Tc^{3+} or Tc^{4+} ions offer alternative metal centres. In particular, the Tc(+1) organometallic complexes are of interest following the development of isonitrile complexes for heart imaging and, more recently, the chemistry of the $\{Tc(CO)_3\}^+$ moiety (Section 3.3.4.4). Among the higher oxidation numbers Tc(+6) is found in the TcN^{3+} core of $[TcNCl_4]^-$ (X=Cl, Br) which can be prepared from $[TcO_4]^-$ by reaction with azide, N_3^-, in the presence of excess HX. Other Tc(+6) species tend to be unstable, readily converting to other oxidation states. The highest oxidation state, Tc(+7) is typically encountered in the form of $[TcO_4]^-$ although a few complexes containing Tc(+7) are known. The utilisation of individual Tc oxidation states in the formation of bifunctional ^{99m}Tc-radiopharmaceuticals is considered in a little more detail in subsequent sections.

3.3.12.1 Linking Groups and Labelling Methods

The choice of a suitable binding site for a metal will obviously depend on the nature of the metal ion. Hard metal ions such as Ga^{3+} or In^{3+} can be effectively bound by polydentate amine carboxylates. Closed electron shell ions like these d^{10} systems form kinetically labile complexes so to augment the thermodynamic stability it is helpful to use binding groups which fully saturate the metal ion coordination sphere and block the approach of competitor ligands. Although TcO^{3+} complexes with polyamine carboxylates are also known, the widely used TcO^{3+} centre is more usually found in complexes with 'softer' N and S donor atom systems. The partly filled d-subshell allows some π–donation from amide $>N^-$ or thiolate S^- to the Tc(+5) centre, helping to make the complex more kinetically inert (Section 2.5.2). The presence of large S-donor atoms also helps in this respect by occupying the space around the metal ion more effectively than smaller N or O donor atoms.

The efficiency and ease with which the radionuclide is incorporated into a metal binding site is an important aspect of radiopharmaceutical preparation. If high temperatures, long reaction times or high pH are necessary to incorporate the radionuclide label into the binding site this may limit the clinical

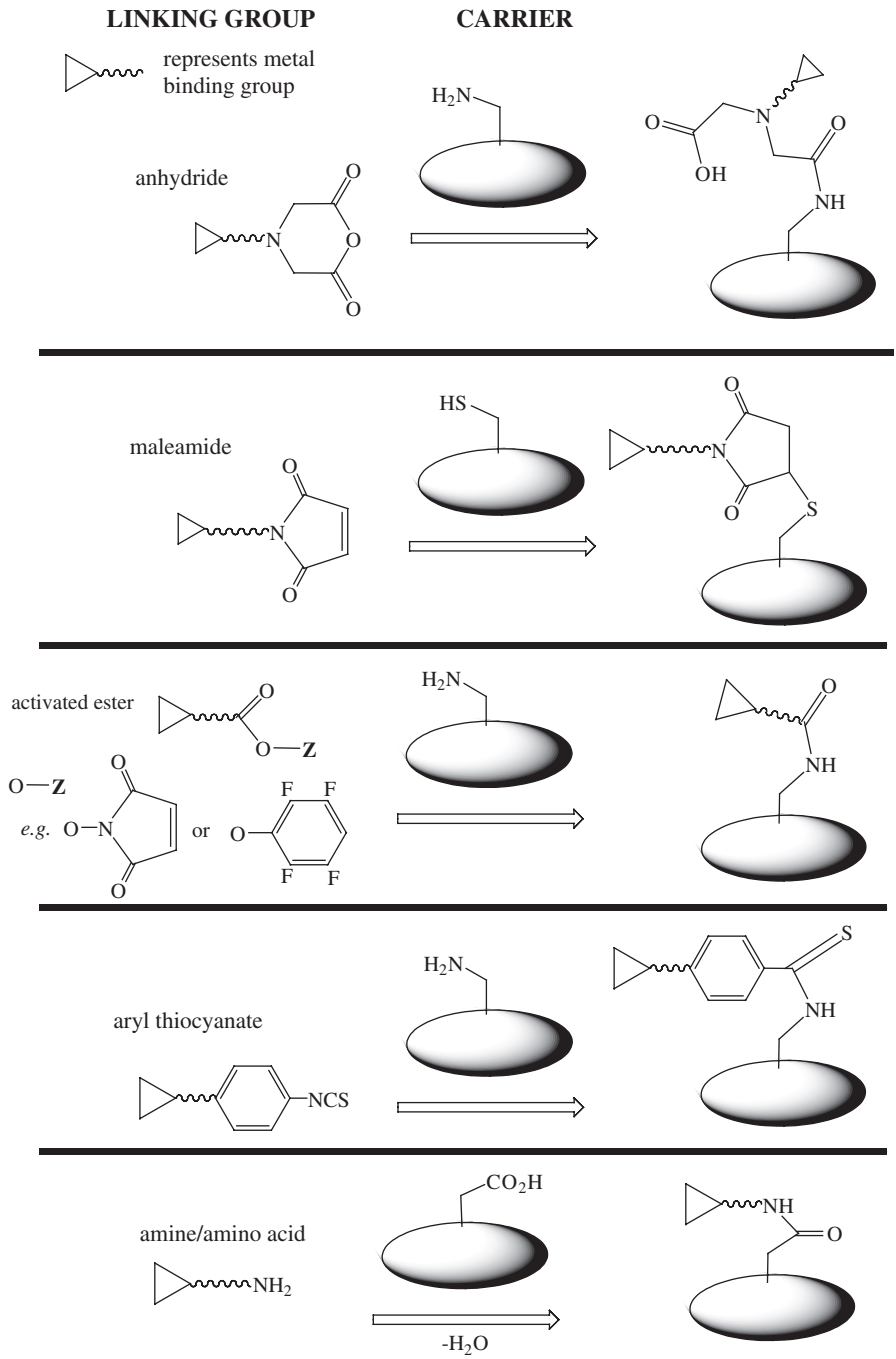
application of the method. If the carrier part of a bifunctional agent contains protein with structurally important disulfide links, any reagent used to reduce $[TcO_4]^-$ must be selected so as not to denature the protein by also reducing the disulfide bonds to dithiol. Such reduction would also create competitor binding sites for Tc leading to a labelled protein which may have undesirable properties *in vivo*.

Any of a variety of methods might be used to link a metal binding group to a biologically active carrier molecule. Perhaps the simplest is to use a substituent atom, such as $-S^-$, on the carrier moiety to bind directly to the metal centre. To be effective this direct monodentate means of attachment requires the formation of a particularly kinetically inert bond to the metal but is being applied in some ^{99m}Tc agents. More often some form of polydentate chelating group will be attached to the carrier by means of a covalent bond and some examples of this have already been mentioned in Section 3.3.11.3. Specifically the use of dtpa anhydride, or an active ester of dtpa, to attach a polyamine carboxylate group and the use of an aryl thiocyanate substituent on dtpa as a means of avoiding the loss of one carboxylate group when forming a link. Some examples of the various types of chemical approach which may be used to connect a metal binding group to a biologically active carrier are shown in Scheme 13. One approach attracting particular interest is the use of short sequences of amino acids designed to provide a suitable metal binding site as in the example of Tc-Depreotide (**86**). The use of a peptide sequence in labelling a protein is attractive in that it merely involves a small extension of the protein structure. This can simplify the chemistry of adding the metal binding sites as methods of extending an amino acid sequence from the carboxylate or amine terminus are well developed. Furthermore it does not add chemically dissimilar substituents to the protein.

It is important that the link joining the carrier and metal binding site is stable under physiological conditions and that the chemistry involved in its formation is simple, efficient and suited to the particular purpose. For example linking strategies which rely on amide bond formation will require a suitable free amine to be located on the surface of the carrier. If required free thiol groups could be created by the reduction of disulfide bonds in proteins but, if the presence of any disulfide in the protein is important for the protein structure and/or function, a reductive approach would not be suitable. Thus careful selection of the linking group is important along with selection of a binding site structure appropriate for the radionuclide used.

3.3.12.2 Labelling with Tc(+1) or Tc(+2)

In the context of radiopharmaceuticals Tc(+1) and Tc(+2) are most clearly associated with heart imaging (Section 3.3.9.1) in the form of the successful Tc(+1) isonitrile complexes $[Tc(CNR)_6]^+$ (*e.g.* **55c**) and the ineffective Tc(+2) chelating diphosphine dihalide complexes $[TcCl_2\{P(R_2)CH_2CH_2P(R_2)\}_2]$ (*e.g.* **23**). The neutral monodentate isonitrile ligand itself would not appear to offer a promising binding group for incorporating ^{99m}Tc in a bifunctional agent. It does not offer additional stability through chelation and Tc(+1) is not


LINKING GROUP
CARRIER
represents metal binding group
anhydride
maleamide
activated ester
e.g.
or
aryl thiocyanate
amine/amino acid
-H2O

Scheme 13