Chapter 2

Technetium in Medicine

2

2.1 ^{99m}Technetium Chemistry

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Technetium is an artificial element obtained by the radioactive decay of molybdenum. Element 43, named technetium in 1947, had been discovered in 1937 by Carlo Perrier and Emilio Segrè in a sample obtained from the Berkely Radiation Laboratory (now Lawrence Berkeley National Laboratory) in California (Perrier and Segrè 1937, 1947). By bombarding a molybdenum strip with 8-MeV deuterons in a 37-in. cyclotron, a radioactive molybdenum species (half-life, 65 h) had been obtained which decayed by β-emission to a short-lived isotope (half-life, 6 h) with novel properties, identified as technetium-99m (Segrè and Seaborg 1938).

In 1965, Richards and his collaborators at Brookhaven National Laboratories (N.Y.) have introduced the ^{99}Mo/^{99m}Tc generator for clinical application (Richards 1966). This radionuclide system made technetium-99m available for clinical research and has stimulated the development of the first labeled compounds, which had a considerable impact on radiochemistry and nuclear medicine (Andros et al. 1965; Harper et al. 1966; McAfee et al. 1964a, b; Stern et al. 1965, 1966). In the years to follow, diagnostic nuclear medicine procedures based on ^{99m}Tc pharmaceuticals increased to approximately 85%. The reasons for this rapid growth were the ideal nuclear properties of technetium-99m, its availability worldwide as a radionuclide generator system, and the development of new labeling techniques.

Labeling procedures have been greatly facilitated by kit preparations (Eckelman et al. 1971). Sterile kits for labeling contain the chemical ingredients in lyophilized form are commercially available and used to prepare ^{99m}Tc pharmaceuticals shortly before application to the patient. Manipulation is minimal, since all that needs to be done is adding the ^{99m}Tc activity to the kit. In some cases, heating of the reaction mixture is performed to increase the labeling yield.

^{99m}Tc pharmaceuticals are organ specific and available to delineate blood flow in organs such as the lung (embolism), heart (ischemia/infarction), and brain (perfusion defects); to evaluate the functional state of the thyroid, liver (phagocytic function), kidney, or the hepatobiliary system (acute cholecystitis); and to detect tumor and metastatic growth in bone structures and more specifically, somatostatin-expressing tumors. Accordingly, the demands on chemical structure and biological performance vary considerably and need a sophisticated approach to radiopharmaceutical design.

Research on new molecules has been growing steadily, stimulated by the demand for new medical applications. However, the low concentration of carrier-free ^{99m}Tc (1 Ci $\sim 10^{-9}$ M) in most ^{99m}Tc pharmaceuticals poses difficulties when determining their chemistry. Therefore, structural characterization of new ^{99m}Tc complexes is preferably studied with isotope ^{99}Tc, a long-lived β-emitter ($T_{1/2} = 2.12 \times 10^5$ years), which is commercially available in macroscopic amounts. Analogous ^{99}Tc complexes may be identified using standard analytical techniques such as mass spectrometry, nuclear magnetic resonance (NMR), x-ray crystallography, UV, and elemental analysis.

2.1.1 Technetium Compounds and Their Structures

The knowledge of the chemical properties of technetium has grown over the years, as indicated by review articles and books (Dewanjee 1990; Lever 1995; Nowotnik 1994; Peacock 1966; Schwochau 1983; Steigman and Eckelman 1992). Of particular interest are the Proceedings of the International Symposium on Technetium in Chemistry and Nuclear Medicine, presenting new developments in complex chemistry of technetium and rhenium, with state-of-the-art lectures, listed at the end of this chapter under "Further Reading".

The element technetium belongs to group VIIB of the periodic table, between manganese and rhenium. The atomic radius of technetium is similar to rhenium; thus, many similarities are found in the chemistry between the two elements. The electronic configuration of the neutral atom 43 is described by $[Kr]4d^6 5s^1$, indicating the 4d and 5s orbitals that contribute to several oxidation states. Technetium can exist in eight oxidation states, varying from (VII) to (-I). Considering carrier-free chemistry, the most stable states are (VII), (V), (IV), (III), (I), and 0. Most difficult to stabilize are states (VI), (II), and (-I) (Mazzi 1989).

The highest oxidation state (VII) is occupied by a pertechnetate anion (TcO_4^-) (Fig. 2.1.1), which is eluted from the ^{99}Mo/^{99m}Tc generator. The chemical reactivity of the pertechnetate anion is negligible; it does not bind directly to any ligand. Thus, for the production of ^{99m}Tc pharmaceuticals, reduction to lower oxidation states in the presence of a suitable ligand is a prerequisite for the synthesis of ^{99m}Tc-labeled molecules. During reduction, the ligand stabilizes the lower oxidation state, otherwise, colloidal TcO_2 is formed in aqueous media (Lever 1995; Nowotnik 1994).

An exception is technetium sulfide (Tc_2S_7), known as ^{99m}Tc-sulfur colloid (Stern et al. 1966). Scavenging molecules like phosphinimine ($R_3P=N\text{-}SiMe_3$) have been reported to incorporate TcO_4^-, producing organic molecules containing Tc(VII) (Katti et al. 1993; Singh et al. 1995).

With the exception of ^{99m}Tc colloids, ^{99m}Tc pharmaceuticals used in nuclear medicine are metal complexes, prepared by reducing ^{99m}Tc-pertechnetate to a lower oxidation state. The so-called coordination complexes of technetium (central metal) are formed by means of bonds between technetium acting as Lewis acid, and atoms or functional groups, which act as Lewis bases (they donate electron pairs). Typical ligands for technetium complex formation may have one donor group (monodentate) such as amine, amide, thiol, phosphine, oxime, or isonitrile. With two donor groups, the complex is bidentate; when more than two donor groups from a single molecule bind to one Tc core, is it a chelate (Nowotnik 1994).

The redox potential of TcO_4^-/TcO_2 was found to be +0.738 V, and that of TcO_4^-/Tc, 0.477 V (Mazzi 1989). In the presence of suitable ligands, the redox potentials of the TcO_4^-/Tc complex are dependent upon the stability of the complex itself. It depends on the ligand, in which oxidation state a complex will be stabilized. In presence of oxygen atoms Tc(VI) is not stable, it rather disproportionates to (IV) and (VII). However, if a Tc(VI) complex is very stable, no further reduction is possible. In any case, pertechne-

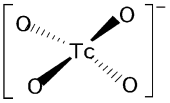

Fig. 2.1.1. Pertechnetate anion

tate is a weak oxidant, certainly weaker than permanganate; in acid medium, it is reduced by weak reductants. In kits, $SnCl_2$ is commonly used as reductant. In certain cases, excess ligand may also act as reductant.

The pertechnetate anion, when reduced in the presence of ligands, usually does not release all the oxygen atoms, leading to complexes in which a TcO^{3+} or a TcO_2^+ core is identified.

Complexes containing a TcO^{3+} core show an octahedral six-coordinated or a square pyramidal five-coordinated spatial configuration; complexes containing a TcO_2^+ core form octahedral six-coordinated complexes. In the presence of suitable ligands, other cores and complexes of lower oxidation states (IV, III, I) may be achieved (Jones and Davison 1982).

2.1.2 Technetium(V) complexes

The majority of ^{99m}Tc pharmaceuticals contain technetium as Tc(V) (Table 2.1.1).

2.1.2.1 Tc-Gluconate

Tc(gluconate) and Tc(glucoheptonate) have been the earliest products of Tc(V) used as radiotracers for renal imaging (De Kieviet 1981; Johannsen and Spies 1988). The compounds were shown to contain a $Tc{=}O^{3+}$ core, but their structures are not completely defined, probably because of more than one stable species at carrier-added level (De Kieviet 1981). However, the x-ray structure of similar Tc complexes with similar ligands (Davison et al. 1987; DePamphilis et al. 1974; Fig. 2.1.2), indirectly supports the formulation as $TcO(Glu)_2^-$, even though the structure of $TcO(Ox)(OxH)^-$ demonstrates the possibility of another species with a $Tc{=}O^{3+}$ core (Abrams et al. 1991).

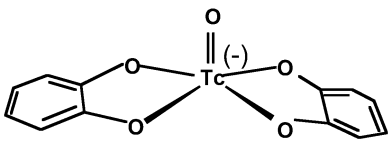

Fig. 2.1.2. $[TcO(cathecol)_2]^-$

These compounds are easily obtained in high yields at no carrier-added level, and they are suitable precursors in the synthesis of new ^{99m}Tc complexes by ligand exchange (or transchelation) (Spies et al. 1980). Other polyhydroxy or hydroxyl acids have been under investigation, such as glycolate, glucarate, tartrate, or citrate, which have been used in transchelation procedures.

Table 2.1.1. Chemical state of ^{99m}Tc-pharmaceuticals in clinical or preclinical use

Compound	Oxidation state core	Geometry	Coordinated number	Charge	Reference
Gluconate	$Tc(V)O_3^+$	Square pyramid	5	–1	Johannsen and Spies 1988
Glucoheptonate	$Tc(V)O_3^+$	Square pyramid	5	–1	De Kieviet 1981
DMSA	TcO_3^+ or Tc(III)	Octahedral	5	0 or –1	Bandoli et al. 1984; Ikeda et al. 1977
Penicillamine	$Tc(V)O_3^+$	Octahedral	6	0	Franklin et al. 1982
EDTA	$Tc(V)O_3^+$	Heptahedral	6	0	Davison and Jones 1982
HMPAO (Ceretec)	$Tc(V)O3^+$	Heptahedral	6	0	Fair et al. 1984
MRP20 (Neuroscint)	$Tc(V)O_3^+$	Heptahedral	6	0	Morgan et al. 1990
DADS	$Tc(V)O_3^+$	Square pyramid	5	0	Davison et al. 1980
DADT	$Tc(V)O_3^+$	Square pyramid	5	0	Watson et al. 1987
ECD (Neurolite)	$Tc(V)O_3^+$	Square pyramid	5	0	Edwards et al. 1990
MAG (MAG_3)	$Tc(V)O_3^+$	Square pyramid	5	0	Nosco et al. 1989
Tetrofosmin (Myoview)	$Tc(V)O_2^+$	Octahedral	5	+1	Kelly et al. 1993
NOEt	$Tc(V)N_2^+$	Octahedral	5	0	Pasqualini et al. 1994
EDTA	Tc(IV) or Tc(III)	Dimeric	7 or 6	0 or –1	Davison and Jones 1982; Burgi et al. 1981
DTPA	Tc(IV) or Tc(III)	Monomeric	?	–1(?)	Gorski and Koch 1970
MDP	Tc(IV)	Monomeric	?	0	Lisbon et al. 1980
HIDA (Choletec)	Tc(III)	Octahedral	6	–1	Loberg and Fields 1978
DMPE	Tc(III)	Octahedral	6	+1	Deutsch et al. 1981
Q12 (Technecard)	Tc(III)	Octahedral	6	+1	Deutsch et al. 1987
BATO (Cardiotec)	Tc(III)	Octahedral	6	0	Bandoli et al. 1982
MIBI (Cardiolite)	Tc(I)	Octahedral	6	+1	Abrams et al. 1983

DMSA dimercaptosuccinic acid, *EDTA* ethylenediaminetetraacetic acid, *HMPAO* hexamethyl propyleneamine oxime, *DADS* *N*,*N*-bis(mercaptoacetyl)ethylenediamine, *DADT* diaminodithiol, *ECD* ethylcysteinate dimer, *MAG_3* mercaptoacetyltriglycine, *NOEt* $Et(OEt)NCS_2$, *DTPA* diethylene triamine pentaacetate, *MDP* methylenediphosphonate, *HIDA* *N*-(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid, *DMPE* 1,2-bis(dimethylphosphino) ethane, *BATO* boronic acid technetium oxime, *MIBI* methoxyisobutyl isocyanide

2.1.2.2 Tc-Dimercaptosuccinic Acid

Two different ^{99m}Tc-dimercaptosuccinic acid (DMSA) complexes are in clinical use, ^{99m}Tc(III)-DMSA with high binding affinity for renal tubuli and ^{99m}Tc(V)-DMSA with tumor affinity.

At acidic pH, at least four ^{99m}Tc-DMSA complexes have been identified in dependence of pH and stannous ion concentration (Ikeda et al. 1977 b). Formation of a ^{99m}Tc(III)-DMSA complex is favored at pH 2.5, using an excess amount of stannous ion (Ikeda et al. 1976). This formulation is used for renal scintigraphy (Ikeda et al. 1977 a). The coordination characteristics of the ^{99m}Tc(III)-DMSA complex have not yet been established.

At an elevated pH (pH 7.5–8.0), a ^{99}Tc-DMSA complex was produced, which accumulated in the skeleton (Johannsen et al. 1979). Further studies performing ligand exchange with Tc(V)-gluconate (Spies et al. 1980) led to the identification of a pentavalent ^{99m}Tc-DMSA complex with two DMSA molecules coordinated to a Tc(V)oxo core (Bandoli et al. 1984) (Fig. 2.1.3).

Pentavalent ^{99m}Tc-DMSA has been evaluated as a soft tumor-imaging agent (Yokoyama et al. 1985).

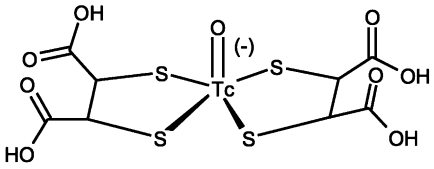

Fig. 2.1.3. Meso-[TcO(dimercaptosuccinic acid)$_2$]$^-$

2.1.2.3 Tc-Penicillamine

Figure 2.1.4 shows the structure of a ^{99}Tc complex with two molecules of penicillamine, confirmed by x-ray crystallography (Franklin et al. 1982). Yet when reduction of ^{99m}Tc-pertechnetate had been performed with $SnCl_2$, a complex with a Tc(IV) oxidation state was reported (Yokoyama et al. 1979).

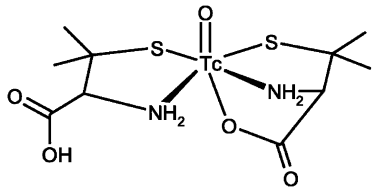

Fig. 2.1.4. [TcO(penicil)$_2$]

2.1.2.4 Tc-Ethylenediaminetetraacetic Acid and Tc-Diethylene Triamine Pentaacetate

Ethylenediamine tetraacetic acid (EDTA) and diethylene triamine pentaacetate (DTPA) are strong coordinating ligands that are administered to reduce in vivo toxicity of heavy metals. Nevertheless, the coordination behavior of EDTA and DTPA ligands with respect to technetium is rather complicated (Steigman et al. 1975).

^{99}Tc-EDTA chemistry studies demonstrated at least two types of stable complexes, one containing a $Tc(V)O_3^+$ core in a hepta-coordinated environment (Davison and Jones 1982), and the other is a complicated dimer in which technetium can be present as Tc(IV) or as Tc(III) (Linder 1986; Noll et al. 1980; Seifert et al. 1982). The crystal structure of a Tc(IV) complex has been reported (Burgi et al. 1981).

The exact structure of the ^{99m}Tc species was not yet found, mainly because at very low concentrations dimerization is very improbable, and in these solutions Tc(IV) or Tc(III) was detected. The participation of tin in the dimer formation cannot be excluded. In any case, the products pass rapidly through the kidneys.

These facts underline the difficulty of defining the chemical species present in the injection solution. To date, no complex with DTPA has been characterized at the ^{99}Tc level.

Undoubtedly, the production of a monomeric species is expected when EDTA and DTPA are used as chelating moiety for monoclonal antibody labeling.

2.1.2.5 Tc-Hexamethyl Propyleneamine Oxime (Ceretec^{TM})

The structure of D,L-TcO (hexamethyl propyleneamine oxime [HMPAO]) is shown in Fig. 2.1.5.

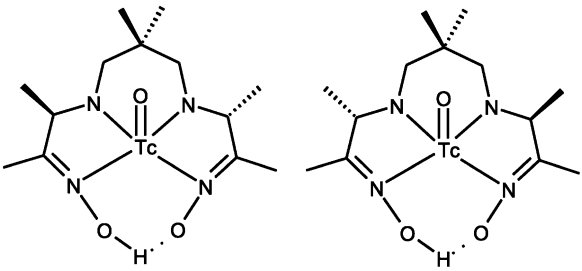

Fig. 2.1.5. D,L-TcO-hexamethyl propyleneamine oxime (HMPAO)

HMPAO is coordinated to a TcO^{3+} core with four nitrogen atoms. Ring closure of the oxime functionalities by hydrogen bonding increases the stability of the lipophilic complex.

^{99m}Tc-D,L-HMPAO was characterized at the ^{99}Tc level (Fair et al. 1984; Jurisson et al. 1987) and is the first neutral ^{99m}Tc complex for brain perfusion imaging (Troutner et al. 1984). The structural configuration has considerable effect on cerebral extraction, the D,L isomers pass the blood–brain barrier (BBB) while the mesoform is excluded (Sharp et al. 1986).

However, lipophilic D,L-HMPAO is easily transformed into a charged complex, which cannot pass the BBB. Once inside the brain, this "secondary" complex is trapped and is released very slowly (Neirinckx et al. 1987). The ^{99m}Tc-HMPAO complex is also used for labeling leukocytes with technetium.

2.1.2.6 Tc-MRP20

MRP20 is one of a series of tetradentate ligands, which incorporate donor sets containing pyrrole, amine, imine, and ketone moieties. The complex is neutral and lipophilic, similar to HMPAO. The chemical structure (Fig 2.1.6.) shows a five coordinated Tc-oxo complex in which the TcO^{3+} core is surrounded in the horizontal plane by the triple deprotonated ligand (Morgan et al. 1990, 1991). Tc-MRP20 was under investigation as a brain perfusion agent.

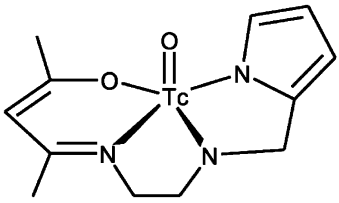

Fig. 2.1.6. Tc-MRP20

2.1.2.7 Tc-*N,N′*-bis(mercaptoacetyl)ethylenediamine and Tc-Diaminodithiol

Tc-*N,N′*-bis(mercaptoacetyl)ethylenediamine (DADS) was introduced as a chelate, based on amide nitrogen and thiolate donor groups (Davison et al. 1979 and 1981). Tetradentate diaminodithiol (DADT) ligands form very stable complexes with oxo-technetium; the introduction of two carbonyl oxygen groups resulted in an overall negative charge. Several DADS-derived ^{99m}Tc complexes have been evaluated as renal agents (Brenner et al. 1984; Fritzberg 1986) (Fig. 2.1.7).

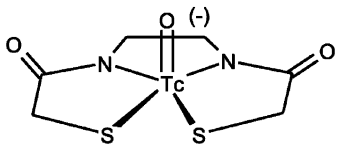

Fig. 2.1.7. Tc-*N,N′*-bis(mercaptoacetyl)ethylenediamine (DADS)

Substitution of the ethylene bridge (center chelate ring) with a carboxylate group produced ^{99m}Tc-CO_2DADS as two stereoisomers (Costello et al. 1983), with one isomer resembling the tubular agent iodohippurate. ^{99m}Tc-CO_2DADS is an important link in the development of the tubular agent ^{99m}Tc-mercaptoacetyltriglycine (MAG_3).

Structural modification of the N_2S_2 ligand has produced several ^{99m}Tc-DADT complexes. An example of substitution of the amine nitrogen (aminoalkyl-DADT) is ^{99m}Tc-

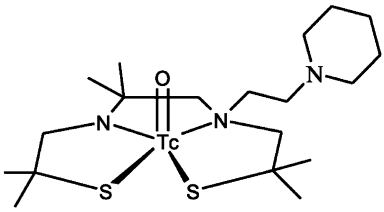

Fig. 2.1.8. TcO-*N*-ethylpiperidinyl-tetradentate diaminodithiol (NEP-DADT)

N-ethylpiperidinyl (NEP)-DADT (Epps et al. 1978). Functionalization of hexamethyl-DADT with a NEP side chain was shown to enhance brain accumulation of neutral, lipophilic ^{99m}Tc-*syn*-NEP-DADT (Lever et al. 1985) (Fig. 2.1.8).

^{99m}Tc-NEP-DADT is an example of systematic derivatization to optimize the structure–biodistribution relationship.

2.1.2.8 Tc-Ethylcysteinate Dimer

The ethylcysteinate dimer (ECD) belongs to the family of neutral, lipophilic tetradentate diaminedithiol ligands. The x-ray structure (Fig. 2.1.9) shows the functionalized ester dimer with a Tc(V)oxo core in a square pyramidal configuration (Watson et al. 1987). In fact, its high-performance liquid chromatography (HPLC) behavior is the same for the ^{99}Tc and ^{99m}Tc-ECD complex (Edwards et al. 1990).

The L,L stereoisomer can cross the BBB and is retained in the brain, presumably due to hydrolysis of the ester function (Leveille et al. 1989). If hydrolysis happens in blood before the molecule has crossed the BBB, the resulting dicarboxylate anion is rapidly excreted by the kidneys. No difference was observed with the monoester monoacid derivatives (Verbruggen et al. 1989 a).

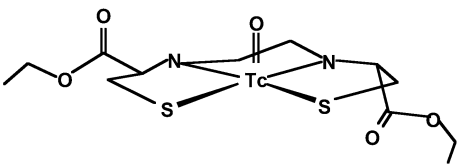

Fig. 2.1.9. Tc(V)O-ECD (ethylcysteinate dimer)

2.1.2.9 Tc-Mercaptoacetyltriglycine

Replacement of one thiolate donor group by a planar amide carrying a carboxylate anion avoids formation of stereoisomers, as observed with ^{99m}Tc-CO_2DADS. MAG_3 is a suitable ligand for producing ^{99m}TcO-MAG_3, a negatively charged complex (Fig. 2.1.10), structurally defined at carrier-added (CA) (Davison et al.1981) and no-carrier added (NCA) (Fritzberg et al. 1986; Verbruggen et al. 1989 b) levels.

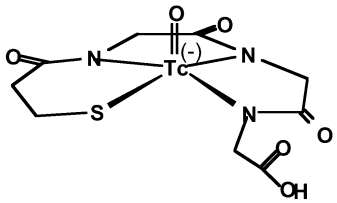

Fig. 2.1.10. Tc(V)O-mercaptoacetyltriglycine^{-}, or Tc(V)O-MAG_3^{-}

Steric arrangement of the carboxylate group in *syn* position with respect to Tc=O is responsible for active tubular secretion (Coveney and Robbins 1987; Fritzberg et al. 1986). A series of positional isomers of ^{99m}Tc-CO_2DADS were synthesized in order to produce MAG_3 as a ligand with suitable biological properties in man (Fritzberg 1986).

2.1.2.10 Tc-Tetrofosmin (P53)

Myoview is a TcO_2^+ complex, obtained by functional derivatization of 1,2-bis(dimethylphosphino)ethane (DMPE) (Kelly et al. 1993). The chemical structure of ^{99m}Tc-tetrofosmin shows four phosphorus atoms of the bidentate diphosphine ligands, arranged in a plane (Fig. 2.1.11). However, tetrofosmin contains four ethoxyethyl groups, which ensure a rapid clearance of activity from the liver. The cationic charge facilitates myocardial uptake.

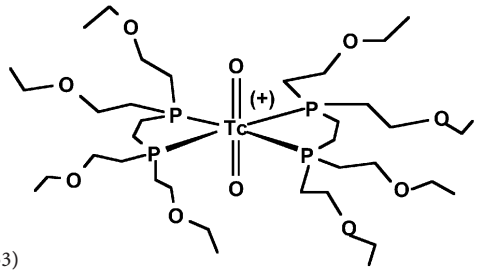

Fig. 2.1.11. $TcO_2P_4^+$ (P53)

Trans-octahedral configuration of the donor atoms has been confirmed by x-ray single crystal analysis of the ^{99}Tc analog. The HPLC behavior is the same for the ^{99}Tc and ^{99m}Tc tetrofosmin complex (Kelly et al. 1993).

Phosphine ligands are interesting coordinating groups because they stabilize complexes with technetium at several oxidation states (from V to I) (Deutsch et al. 1983). They may act as Lewis bases (soft groups stabilizing *trans*-TcO_2^+ core) in the highest technetium oxidation states, and as π-acceptor ligands in the lowest oxidation states in which technetium possesses high electron density. Other π-acceptor ligands are isonitriles, nitrosyl, and carbon monoxide.

Typical DMPE complexes have been reported (Deutsch et al. 1981, 1983). $[Tc^{V}O_2(DMPE)_2]^+$, $[Tc^{III}Cl_2(DMPE)_2]^+$ (Fig. 2.1.12), and $[Tc^{I}DMPE)_3]^+$ were found to be present both at CA and NCA levels, depending on the reaction conditions (Bandoli et al. 1982). As a cationic species, $[Tc^{III}Cl_2(DMPE)_2]^+$ showed myocardial uptake; however, it is species dependent, and there is considerable liver uptake (Deutsch et al. 1989).

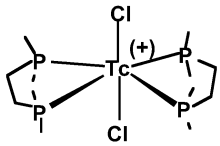

Fig. 2.1.12. $Tc(III)Cl_2(1,2\text{-bis(dimethylphosphino)ethane})_2^+$, or $[Tc(III)Cl_2(DMPE)_2]^+$

The chemistry of Tc(V) complexes offers many possibilities for the synthesis of coordination complexes. In fact, the availability of different central cores can be used to stabilize a considerable number of ligands with very different coordinating properties. The TcO^{3+}, *trans*-TcO_2^+ and *trans*-$XTcO_2^+$ (X = halogenide, O, alcoholate, N groups, etc.) cores are stabilized by various ligands, and the existence of one or the other core is attributed to the arrangement of the coordinating atoms in the horizontal plane perpendicular to Tc=O. Soft atoms stabilize a TcO^{3+} core, while harder ones produce *trans*-$XTcO_2^+$ or *trans*-TcO_2^+ cores (Davison 1983).

For example, a cationic *trans*-TcO_2^+ complex is produced with tetradentate cyclam (Zuckman et al. 1981). $[Tc(V)O_2(Cyclam)]^+$ (Fig. 2.1.13) was investigated as a transchelating compound rather than as a radiopharmaceutical because it has no useful biological properties.

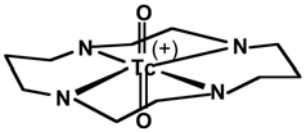

Fig. 2.1.13. TcO_2 (Cyclam)$^+$

2.1.2.11 TcN(NOEt) and Heterocomplexes with Metal–Nitrogen Multiple Bond

Nitrido Tc(V) complexes with a technetium–nitrogen triple bond were introduced by Baldas et al. (1978); structural verification of a stable TcN^{2+} core was also documented (Marchi et al. 1990). Some donors of nitrido nitrogen atom (N^{3-}) to yield the $Tc\equiv N^{2+}$ group have been evaluated; *N*-methyl-*S*-methyl dithiocarbazate $[H_2NN(CH_3)–C(=S)SCH_3]$ in acidic solution was found to be the most efficient ligand (Marchi et al. 1990; Pasqualini et al. 1992). In the presence of $Tc\equiv N^{2+}$, a high variability of the chelating set was observed. In comparison with $Tc=O^{3+}$ cores, softer coordinating atoms produce more stable complexes. Dithiocarbazate seems to produce prereduced intermediary Tc complexes containing a $Tc\equiv N$ core, which undergo facile substitution reactions with the final ligands.

The neutral $[TcN(Et(OEt)NCS_2]$ complex, called TcN(NOEt), was studied as a myocardial agent, demonstrating different biological properties with respect to the monocationic species (Pasqualini et al. 1994). The ^{99}Tc complex with an Et_2NCS_2 ligand was structurally defined (Bolzati et al. 2002) (Fig. 2.1.14), showing a square-pyramidal configuration with two dithiocarbamate groups bound in the equatorial plane and a $Tc\equiv N$ core; the same species is present at the NCA level.

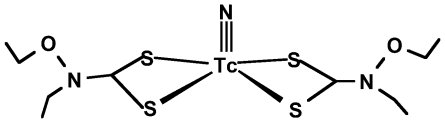

Fig 2.1.14.. The neutral $[TcN(Et(OEt)NCS_2]$, also called Tc-NOEt

2.1.3 Technetium(IV), (III), and (I) complexes

Technetium is stabilized at low oxidation states by suitable ligands such as phosphines, isonitriles, carbon monoxide, and thiourea (Gorski and Koch 1970). Organometallic carbonyl (CO) complexes are interesting precursors for a new class of ^{99m}Tc(I) radiopharmaceuticals (Alberto et al. 2001; Schibli et al. 2000).

As reported previously, EDTA (Burgi et al. 1981) (Fig. 2.1.15) and many other ligands with no π-accepting groups can produce Tc(IV) or Tc(III) stable complexes, because other parameters such as chelating effect and metal–metal bonds contribute to their stabilization. Usually six-coordinated complexes in an octahedral configuration are obtained, but some exceptions are possible. In addition, the complex charge may vary in dependence of the ligand charge, deprotonation of the coordinating group; however, very exceptionally is the net charge more than one, negative, or positive.

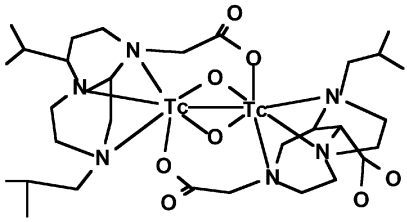

Fig. 2.1.15. Tc-ethylenediaminetetraacetic acid (EDTA)

2.1.3.1 Tc-Diphosphonates

^{99m}Tc-methylenediphosphonate (MDP) and polyphosphate complexes were studied as ^{99}Tc complexes, but only one x-ray structure was obtained with the diphosphonate ligand (Subramanian et al. 1975). ^{99m}Tc-MDP is a polymeric species in which tin is incorporated (Libson et al. 1980) (Fig. 2.1.16). The figure shows primarily the configuration at the central technetium.

Reduction of $^{99}TcO_4^-$ with $NaBH_4$ in the presence of hydroxyethylene diphosphonate (HEDP) produced seven different components detected by HPLC. It was demonstrated that the different species differ in molecular weight, depending on the size of the polymers (Van den Brand et al. 1981). Chemical yield of various components depends on the total technetium concentration, the polymerization reaction following high-order kinetics. As a single component, ^{99m}Tc-MDP and ^{99m}Tc-HMDP localize independently in the inorganic bone matrix. In order to obtain reproducible clinical results, bone agents must be pre-

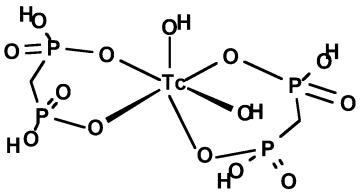

Fig. 2.1.16. Tc-diphosphonate

pared using fresh eluates obtained from a ^{99}Mo/^{99m}Tc generator that is eluted regularly. ^{99m}Tc diphosphonates show high skeletal uptake and are used for bone scintigraphy.

2.1.3.2 Tc-*N*-(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid

N-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid (HIDA) and several ether derivatives have been evaluated as ligands for complexation, producing ^{99m}Tc complexes suitable as hepatobiliary agents. ^{99m}Tc-IDA complexes have a negative charge (Loberg and Fields 1978). Two molecules of ligand are coordinated to one Tc(III)-core (Nunn et al. 1983) (Fig. 2.1.17).

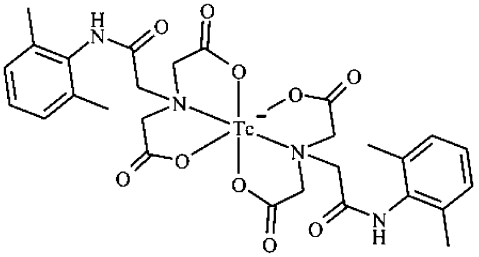

Fig. 2.1.17. Tc-*N*-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid)$_2$ – (HIDA)

2.1.3.3 Tc-Q12

Complexes of the Q series are defined by their structure belonging to the $[Tc^{III}P_2L]^+$ complexes, with polydentate Schiff bases stabilized at the +3 oxidation state by a tertiary phosphine ligand (Deutsch et al. 1987). In fact, ^{99}Tc(V)OCl-L-oxo complexes are easily reduced by a two-electron process to Tc(III). The final Tc(III) compound (Fig. 2.1.18) has an octahedral configuration with the two *trans* phosphines on the apexes and the tetradentate Schiff base on the equatorial plane (Jurisson et al. 1984). Tc-Q12 has a positive charge, the two hydroxyl groups being deprotonated. The ^{99}Tc complex has been prepared in two steps, with an intermediate Tc(V)-oxo complex (Abrams et al. 1982).

These complexes are well modified in the backbone, without decreasing the complex stability. Q12 is the best derivative in the series (Deutsch et al. 1987); however, none of these ligands has been used as a myocardial perfusion agent.

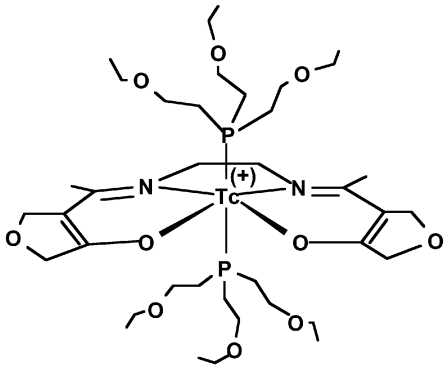

Fig. 2.1.18. Tc(Q12). *L* Equatorial tetracoordinate ligand

2.1.3.4 Tc-Boronic Adducts of Technetium Oximes

Dioxime type ligands can be considered as Schiff base bischelates (Deutsch et al. 1978; Bandoli et al. 1986). The first complexes with oxime ligands were described as monocapped $Tc(dioxime)_3(\mu OH)SnCl_3$ (dioxime = dimethylglyoxime) complexes (Treher et al. 1989). The boronic adducts of technetium oximes – (BATOs) (Fig. 2.1.19) – were well characterized, and some could be used both as myocardial and cerebral perfusion agents. The complexes are neutral; technetium is coordinated to three N-bonded dioxime molecules and to one Cl or Br atom in an axial position (seven covalent bonds).

The three bidentate dioxime groups are joined through covalent B–O bonds to a tetrahedral boron cap derived from an alkyl boronic acid derivative. The six ligating nitrogen atoms form a monocapped distorted trigonal prism. It can be characterized by the geometry of the triangles of nitrogen or oxygen at the capped and uncapped ends of the complex.

Different oximes can be used, but the major structural modifications of the complex are achieved at the boronic side chain (R_1).

One BATO-derived radiopharmaceutical, ^{99m}Tc-teboroxime (Cardiotec), has been available in the United States.

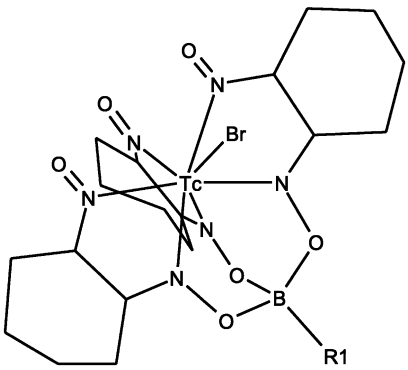

Fig. 2.1.19. Tc-boronic adducts of technetium oxime (BATO)

2.1.3.5 Tc-Methoxyisobutyl Isocyanide

Isonitriles, like carbon monoxide or phosphines, are ligands with high reducing properties together with a high capability of stabilizing low oxidation states. Tertiary butyl isonitrile (TBI) was the first ligand evaluated as a myocardial imaging agent (Holman et al. 1984). The positively charged Tc(I) complex showed high uptake in myocytes; however, clearance from the liver was slow. Introducing the 2-methoxy-derivative had a positive effect on the biodistribution, since the ether is metabolized and cleared faster.

Structural characterization of Tc(I) complexes was performed identifying ^{99m}Tc sestamibi as a complex with six monodentate methoxyisobutyl isocyanide (MIBI) ligands attached symmetrically to a central Tc(I) atom (Abrams et al. 1983; Jones et al. 1985; Fig. 2.1.20).

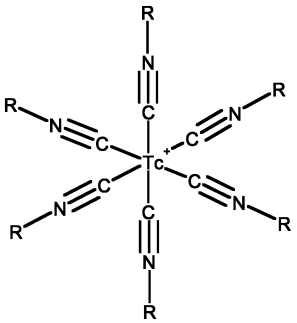

Fig. 2.1.20. Tc-sestamibi. R = 2-methoxyisobutyl

Cationic Tc(I)-hexakis(2-methoxy-isobutyl-isonitrile) tetrafluroroborate is labeled by reacting tetrakis(2-methoxy-isobutyl-isonitrile)-copper(I) tetrafluroroborate adduct with ^{99m}Tc-pertechnetate, using the kit formulation. Heating the reaction vial in a boiling water bath further facilitates formation of ^{99m}Tc(I) sestamibi.

Clinical studies showed high myocardial extraction of ^{99m}Tc sestamibi and fast background clearance (Wackers et al 1989). Redistribution of the lipophilic complex is blocked by intracellular binding.

2.1.4 ^{99m}Tc Labeling

^{99m}Tc chemistry is primarily the chemistry of anionic pertechnetate. This ^{99m}Tc species is eluted from the ^{99}Mo/^{99m}Tc generator with high specific activity as an isotonic solution. Accordingly, ^{99m}Tc chemistry is aqueous solution chemistry in saline suitable to be injected intravenously. Also, ^{99m}Tc chemistry is an NCA chemistry because ^{99m}Tc activity is present in the radiopharmaceutical kit at 10^{-8} to 10^{-9} M.

Direct labeling. Generally, direct labeling is performed by adding ^{99m}Tc eluate in a suitable volume to a sterile kit. The kit contains all chemical components, including a reducing agent. The labeling reaction requires reduction of pertechnetate, which is reacting with the ligand forming the labeled product in high yield (>90%).

Exchange labeling. In a few exceptions, an intermediate ligand complex is formed (MAG_3) that is stabilized by ligand exchange during heating. In the case of MIBI, the kit contains a preformed copper(I) complex, a so-called adduct, which facilitates formation of hexacoordinated ^{99m}Tc(I)-MIBI.

Effect of formulation. Kits contain very low amounts of stannous ion for reduction of ^{99m}Tc-pertechnetate; nevertheless, $SnCl_2$ is usually in high excess. There are several reasons for using stannous salt in excess. Stannous salts are spontaneously oxidized in air. Also, oxidant species in the eluate may have been formed by radiolysis; the amount of Sn(II) available in solution is very low with respect to the total amount of lyophilized $SnCl_2$. In order to assure validity of kits beyond the expiration date, an excess of $SnCl_2$ is used in the kit formulation.

On the other hand, there are cases in which the amount of reductant must be strictly controlled. This is indicated when more than one oxidation state is favored with a certain ligand, or when hydrolysis products interfere with complex stability. This precaution is possible with Sn(II) complexes (Sn-tartrate, Sn-gluconate, Sn-citrate, Sn-EDTA, etc.), which release small amounts of stannous ion into solution. In addition, another reducing agent, including the ligand itself, might be considered.

Formation of colloidal TcO_2 is avoided in the presence of ligand, which competes for the reduced technetium species, producing the labeled ^{99m}Tc pharmaceutical. In the absence of ligand, a mixture of hydrolized, insoluble ^{99m}Tc species, $TcO_2 \cdot nH_2O$, is formed. To increase the rate of coordination, a high amount of the ligand is generally used. The kinetic mechanism of reduction-substitution is rather complicated, and sometimes it depends on the concentration of carrier $^{99m}TcO_4^-$. This is observed when ^{99}Tc carrier in the eluate is increased to CA level.

Kit components. Kit composition is optimized to ensure that the unique ^{99m}Tc-labeled complex is obtained in high yield. Several factors influence the reduction/coordination process; these are primarily the nature and the amounts of reductant and ligand, pH, and temperature. Generally, the rate of complex formation is a good indicator of complex stability, which is essential to avoid increased background activity in vivo. In order to provide a suitable pH environment for the formation of a specific ^{99m}Tc complex, buffers are important components in kit formulations.

Additives include antioxidants, catalysts, accelerators, solubilizing agents, and fillers (Nowotnik 1994).

Antioxidants are added to the formulation in order to increase the stability of the radiopharmaceutical. Antioxidants for ^{99m}Tc complexes that have been used are ascorbic acid (Tofe and Francis 1976), gentisic acid (Tofe et al. 1980), and *p*-aminobenzoic acid (Rimmer 1982).

A *catalyst* might be a ligand, which rapidly forms an intermediary coordination complex such as gluconate, DTPA, and citrate (Davison 1983). Ligand exchange is applied when complex formation with a certain ligand is slow relative to formation of reduced, hydrolized technetium, resulting in a poor radiochemical yield.

Accelerators increase the radiochemical yield and rate of complex formation (Tweedle 1983).

Surfactants might be required to solubilize lipophilic ^{99m}Tc complexes (MIBI) (Bergstein and Subramanyam 1986) and particulate preparations (macroaggregated albumin, microspheres).

Solubility of the product in aqueous solution is indispensable. Equally important is the dissolution of the lyophilized kit contents when ^{99m}Tc eluate is added, in order to assure proper chemistry during the vitally important first few seconds of reconstitution.

Inert fillers are added in order to achieve rapid solubilization of the vial contents through the control of particle size during the lyophilization process. The size of the lyophilizate plug and particle size are controlled by the freeze-dry cycle in kit production. Sodium chloride is added to D,L-HMPAO kits and mannitol to MIBI kits.

Many variables in kit formulation have to be explored during the developmental phase of a new product in addition to the documentation of compatibility with different generator eluates and the shelf-life of the kit.

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Further Reading

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2.2 The Technetium and Rhenium Tricarbonyl Core

R. Schibli

An aqua ion of technetium and rhenium, in analogy to, e.g. $[Cu(OH_2)_6]^{2+}$, would be most convenient for radiolabeling procedures. However, such an aqua ion presumably does not exist or is very unstable. In an effort to solve this dilemma, Alberto and coworkers have designed and developed an organometallic semiaqua ion of the general formula $[M(OH_2)_3(CO)_3]^+$ (M Tc, Re), useful as precursor for the radiolabeling of biomolecules for diagnostic and therapeutic purposes (Alberto et al. 1995, 1998, 1999; Egli et al. 1997). The metal centers are in the oxidation state +1 with a low-spin d^6 electronic configuration. The high electron density is stabilized by three strong π-acceptors (CO) facially arranged. The precursors are water stable and water soluble, and the water molecules readily undergo ligand exchange, whereas the carbonyl ligands are substitution stable.

The precursor $[M(OH_2)_3(CO)_3]^+$ is readily accessible directly from the corresponding sodium permetallate, $Na[MO_4]$ (Alberto et al. 1998; Schibli et al. 2002). The preparation comprises a six-electron reduction and concomitant coordination of three COs. The basis of Tc-99m kit formulation is disodium boronocarbonate (BC), $Na_2[H_3BCO_2]$, which serves as an in situ CO source and at the same time reduces the technetium center (Alberto et al. 2001). The kit is nowadays commercially available under the name Isolink (Mallinckrodt-Tyco Med) for research purposes. BC is stable in aqueous solution and can be lyophilized. The precursor $[^{99m}Tc(OH_2)_3(CO)_3]^+$ can be synthesized in quantitative yield by adding generator eluate to the vial and subsequent heating to 100 °C for 20 min (Alberto et al. 2001). The preparation of the rhenium homologue $[^{188}Re(OH_2)_3(CO)_3]^+$ deviated slightly from technetium, since rhenium is more difficult to reduce and reacts, in general, much slower. Therefore, $H_3B{\cdot}NH_3$ is the reducing agent (eventually in combination with another polymer bound reducing agent), and the reaction must be carried out in the presence of H_3PO_4 at acidic pH (Park et al. 2006; Schibli et al. 2002). This formulation presently excludes an instant kit formulation for $[^{188}Re(OH_2)_3(CO)_3]^+$.

As mentioned above, $[M(OH_2)_3(CO)_3]^+$ can be considered as a normal aqua ion with only three available coordination sites. Substitution of the water molecules with almost any type of chelator (classic/nonclassic) forms kinetically stable coordination compounds. This holds true for mono-, bi-, and tridentate ligands, regardless of their hardness or softness. This behavior also represents the distinct feature of $[M(OH_2)_3(CO)_3]^+$ as compared with other technetium and rhenium metal centers, and is one of its major advantages for the labeling of molecules for imaging and therapeutic purposes.

An enormous variety of mono-, bi-, and tridentate ligand systems comprising different donor atoms or groups have been developed and are still designed and optimized. Bifunctional chelating agents (BFCA) have been readily developed specifically for the purpose of functionalization of biomolecules and subsequent radiolabeling with the $M(CO)_3$ core (Alberto et al. 2004; Alves et al. 2005; Banerjee et al. 2002, 2005 b; Correia et al. 2001; Garcia et al. 2000, 2002; He et al. 2005; Karagiorgou et al. 2005; Lazarova et al. 2005; Lipowska et al. 2004; Mandal et al. 1998; Mundwiler et al. 2005; Schibli et al. 2002; Stephenson et al. 2003; Stichelberger et al. 2003; van Staveren et al. 2004, 2005; Fig. 2.2.1). Bidentate ligands have proven to be very fast coordinating entities, in particular if they are of anionic nature. Tridentate ligands do not display significantly higher thermodynamic stability than bidentate chelates do. However, their reaction rate is

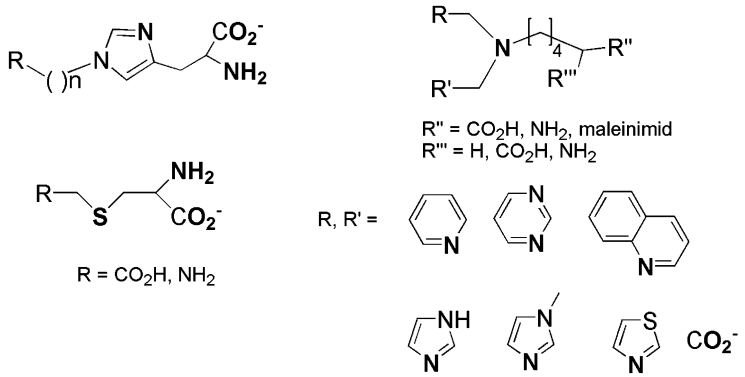

Fig. 2.2.1. Various ligand bifunctional, tridentate chelating systems designed for the coupling to biomolecules and subsequent radiolabeling with the $[M(OH_2)_3(CO)_3]^+$ (coordinative atoms in *boldface*). Single amino acid chelates (SAAC): *R''* CO_2H, *R'''* NH_2

much faster, which becomes the decisive point for radiopharmaceutical application. In that respect, tridentate ligands are favored. In addition, tridentate ligands shield the organometallic metal center from, e.g., in vivo-observed crossreactivity with serum proteins, as observed in the case for complexes of the general formula $[M(OH_2)(L^2)(CO)_3]$ (L^2 bidentate chelate) (Schibli et al. 2000). It has been observed that ^{99m}Tc-tricarbonyl complexes, which are coordinated with a tridentate chelating system, reveal good stability when challenged in human plasma and with excess cysteine, histidine, or glutathione. These complexes show also very good clearance form the blood pool and all tissue and organs when tested in BALB/c mice. In contrast, complexes, which are coordinated in a bidentate fashion, show significant aggregation with plasma proteins in vitro and in vivo. They are significantly retained in the blood and in the organs of excretion such as the liver and the kidneys. These differences may be related to the susceptibility of the third, nonchelating coligand (H_2O) to exchange with more reactive functional groups in vivo, allowing the ^{99m}Tc to be retained in tissues (Pietzsch et al. 2000; Schibli et al. 1999). For both reasons mentioned above, many groups are focusing on the development of novel, potent, tridentate chelates and tridentate BFCAs tailor-made for the $M(CO)_3$ fragment.

Egli et al. (1999) have investigated the ability of amino acids and amino acid fragments to react with the ^{99m}Tc-tricarbonyl core. The most important finding was that histidine reacts quantitatively with the organometallic precursor at very low concentrations (10^{-6} M). In an effort to create novel, bifunctional analogues of histidine, Alberto et al. recently derivatized histidine by introducing various functional groups at the ε-N of the imidazole ring (Alberto et al. 2004; van Staveren et al. 2004). Attachment of these histidine derivatives to the C or N terminus of peptides is an elegant approach, and at the same time liberates both the α-amino group and carboxyl group to participate in tridentate chelation along with the δ-N of the imidazole ring. A similar strategy was applied for *S*-functionalized cysteine BFCAs (van Staveren et al. 2005). Although the amino acid cysteine (and methionine) per se was found to be a rather "slow" coordinating ligand, the situation changed significantly if the sulfur group of cysteine was functionalized.

Valliant and Zubieta developed BFCAs for the $Tc(CO)_3$ core, based on a lysine backbone comprising pyridyl, imidazole, thiolate, carboxylate groups, etc., for the specific purpose of conjugation to small peptides by solid-phase synthetic methods (Banerjee et

al. 2002, 2004, 2005a, b; Stephenson et al. 2003, 2004; Wei et al. 2005). A whole library of such single amino acid chelates (SAAC) derivatives of lysine has been prepared and readily conjugated to small peptides.

The organometallic nature of the $M(CO)_3$ core also allows the introduction and combination with other nonclassic organometallic ligands, such as cyclopentadienes (cp) or cyclopentadienyls (cp^-). Cp^- is one of the smallest ligands with a low molecular weight, but is able to occupy three coordination sites. Complexes of the cymantrene type $[CpM(CO)_3]$ (M Tc, Re) are stable in physiological media (Wenzel 1992; Wenzel et al. 1993, 1994). Cps can also be further derivatized with, e.g., an acetyl group (Bernard et al. 2003) (Fig. 2.2.2). The acetyl group can act as an anchoring group for biomolecules, giving rise to cp biomolecule conjugates. Reaction of such cp derivatives with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ in aqueous media formed the corresponding radiolabeled conjugates in high yields, but at relatively high ligand concentrations (10^{-4} to 10^{-3} M) (Bernard et al. 2003).

Valliant's group has recently built an interesting link between boron neutron capture therapy and diagnostic radiopharmacy (Fig. 2.2.2). The carborane 3-isocyano-1,2-dicarba-*closo*-dodecaborane and functionalized derivatives thereof react with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ under basic conditions quantitatively (Sogbein et al. 2004, 2005a, b). If the carboranes were coupled to targeting biomolecules, this approach would secure first the site-specific delivery of high quantities of boron atoms and second to quantify and to visualize the distribution of boron conjugates, a task that is not easily verified with nonradiolabeled boron compounds. Both types of nonclassic ligand systems, cps, and carboranes became only useful for radiopharmacy because of their reactivity with the novel synthon $[M(OH_2)_3(CO)_3]^+$.

The tricarbonyl technology not only provides new opportunities with respect to the use of "exotic" ligand systems, but it also opens perspectives for novel labeling strategies.

The group at the Paul Scherrer Institute and Plückthun et al. have successfully developed a direct labeling protocol employing $[^{99m}Tc(OH_2)_3(CO)_3]^+$ for scFvs and "mini-antibodies" (bi- and trivalent constructs of scFv) carrying an N- or a C-terminal His-tag (Deyev et al. 2003; Waibel et al. 2000; Willuda et al. 1999, 2001). The method is particu-

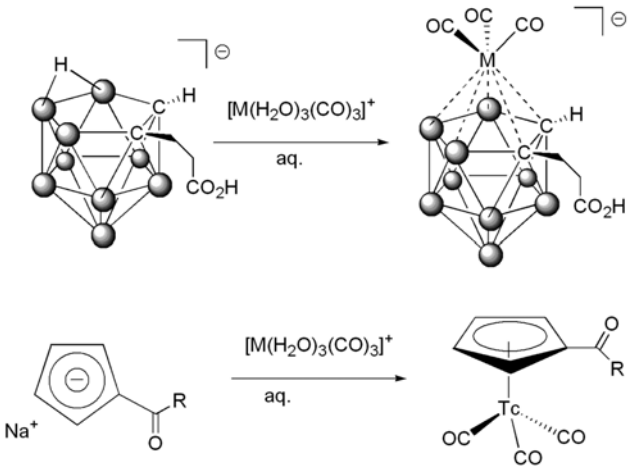

Fig. 2.2.2. Aqueous-base preparation of $M(CO)_3$ complexes comprising nonclassic ligand systems such as functionalized carboranes and cyclopentadienyls

larly elegant and versatile, because His-tags are frequently genetically expressed for ease of purification of the protein on a nickel affinity column. This His-tag can be considered as a multidentate ligand, since two or more imidazoles from histidine can coordinate the metal centre (Fig. 2.2.3). Mixing of such an His-tag protein with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ in buffer at 37 °C for 15 min resulted in > 90 % stable and specific incorporation of the total activity. This gentle procedure allows for the first time the radiolabeling of recombinant proteins "from the shelf", thus, without any chemical modification of the protein structure. The procedure is convenient for the quick and noninvasive evaluation of targeting proteins.

An approach that is particularly interesting for radiolabeling of receptor-targeting radiopharmaceuticals with high specific activity is based on a peculiarity of functionalized aliphatic amines. It was observed that ternary amines involved in the coordination of the $^{99m}Tc(CO)_3$ fragment are cleaved from a solid-phase support during the labeling reaction. It could be shown that metal-assisted cleavage allows the preparation of essentially carrier-free complexes or bioconjugates (Mundwiler et al. 2004). Cleavage occurs exclusively with technetium but not with rhenium. Typical yields of these processes varied between 10 and 50%, relative to the total activity of ^{99m}Tc (Fig. 2.2.4).

Mixed-ligand approaches are well documented for Tc and Re in higher oxidation states. The $M(CO)_3$ fragment allows a similar possibility. As mentioned earlier, the water ligand in complexes of the type $[M(OH_2)(L^2)(CO)_3]$ (L^2 bidentate chelate) is loosely bound and can be exchanged by a potent monodentate ligand (L^1), forming complexes of the general formula $[M(L^1)(L^2)(CO)_3]$. Either L^1 or L^2 can be readily coupled to biomolecules (Mundwiler et al. 2004; Fig. 2.2.5). Several problems have been addressed with this "2+1 approach": (1) the functionalization of biomolecules is minimized or simplified, (2) the resulting complexes/bioconjugates are coordinatively saturated, and (3) further flexibility with respect to the fine-tuning of the physicochemical properties of the radiopharmaceutical is possible.

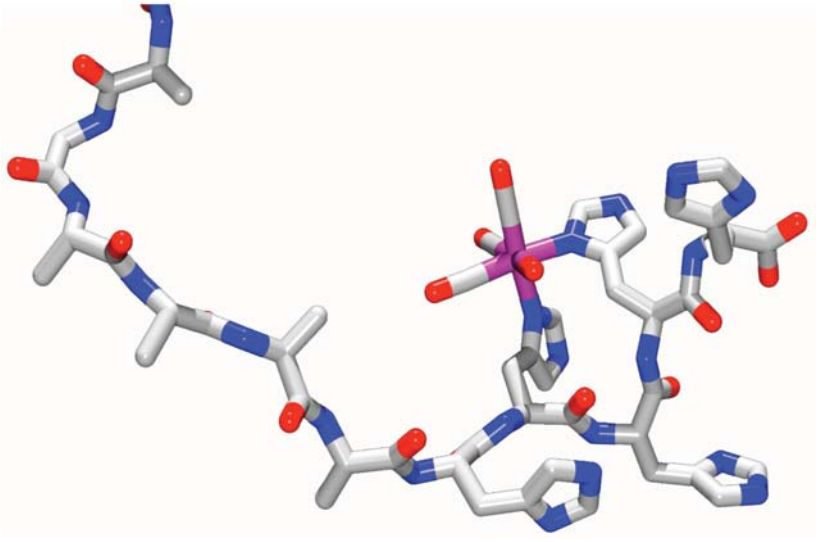

Fig. 2.2.3. Model of the potential coordination of the $M(CO)_3$ core to His_5-tag of a recombinantly produced protein. *Purple* technetium, *bright blue* nitrogen, *red* oxygen, *gray* carbon

Zubieta and coworkers have recently taken advantage of the fluorescent and luminescent properties of organometallic complexes of rhenium (and technetium) comprising certain aromatic ligand systems (Fig. 2.2.6). The nonradioactive $^{nat}Re(CO)_3$ bioconjugates with a formyl peptide receptor-targeting peptide (fMLF), enabeled visualization

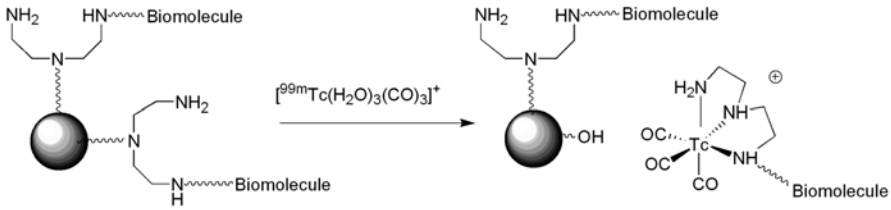

Fig. 2.2.4. $Tc(CO)_3$-assisted cleavage of solid-phase bound biomolecules functionalized with an aliphatic triamine chelate, leading to high specific activity of radiotracer

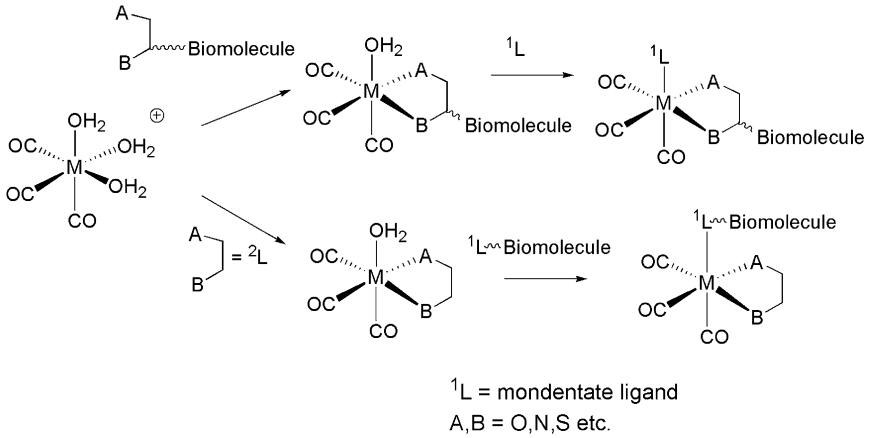

Fig. 2.2.5. Schematic drawing of mixed-ligand approaches using combination of mono- and bidentate ligand systems coupled to biomolecules

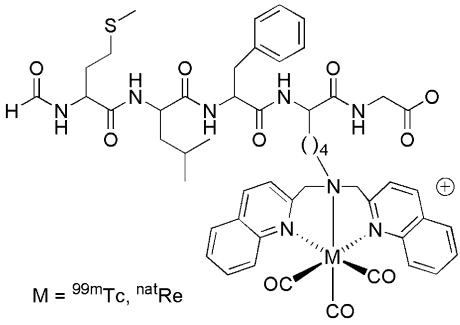

Fig. 2.2.6. Formyl peptide receptor-targeting peptide (fMLF)[(SAACQ-$M(CO)_3)^+$] conjugate useful for in vitro fluorescent microscopy (where M = ^{nat}Re) and in vivo single-photon emission computer tomography (SPECT) (where M = ^{99m}Tc) with isostructural technetium and rhenium complexes

of receptor targeting on the cellular level by means of fluorescent microscopy (Stephenson et al. 2004). The isostructural bioconjugate in the radiolabeled form (with the $^{99m}Tc(CO)_3$ core) allowed the noninvasive detection of corresponding cancer sites in vivo via single-photon emission tomography. Hence, the tricarbonyl technology allows bridging the intrinsic gap between in vitro and in vivo imaging.

2.2.1 Bioconjugates Comprising the $M(CO)_3$ Core

The number of technetium and rhenium tricarbonyl compounds in preclinical evaluation is remarkable. These efforts comprise small molecules as well as macromolecules useful in diagnostic and/or therapeutic nuclear medicine. There are also clinical data available with tumor affine peptides such as neurotensin receptor- and somatostatin receptor-targeting peptides radiolabeled with the $^{99m}Tc(CO)_3$ core.

Dopamine transporter ligand DAT and the 5-HT_{1A} serotonergic receptor ligand WAY100635 have been, and are still, subjects of intense investigation in conjunction with the carbonyl labeling technology (Fig. 2.2.7). WAY100635, has been functionalized with cyclopentadiene and bidentate Schiff-base chelates (Alberto et al. 1999; Arterburn et al. 2003; Bernard et al. 2003; Bigott et al. 2005). The conjugates revealed an IC_{50} value in the low-nanomolar range toward the 5-HT_{1A} receptor. For the preparation of the cp-arylpiperazine derivative, a one-pot, single-step synthesis was described (yields >95%), starting directly from aqueous $[^{99m}TcO_4]^-$, applying the strategy illustrated in Fig. 2.2.2 (Wald et al. 2001). In vitro the receptor affinity and the selectivity of the organometallic derivatives were preserved. However, in vivo the compounds displayed insufficient brain uptake.

Metal carbonyl complexes of steroids have been synthesized by the groups of Johannsen and Katzenellenbogen (Arterburn et al. 2003; Bigott et al. 2005; Luyt et al. 2003; Wust et al. 1998, 1999). Various 17β-progesterone and 7α-estradiole dithioether and cyclopentadiene complexes of technetium/rhenium(I) tricarbonyl have been pre-

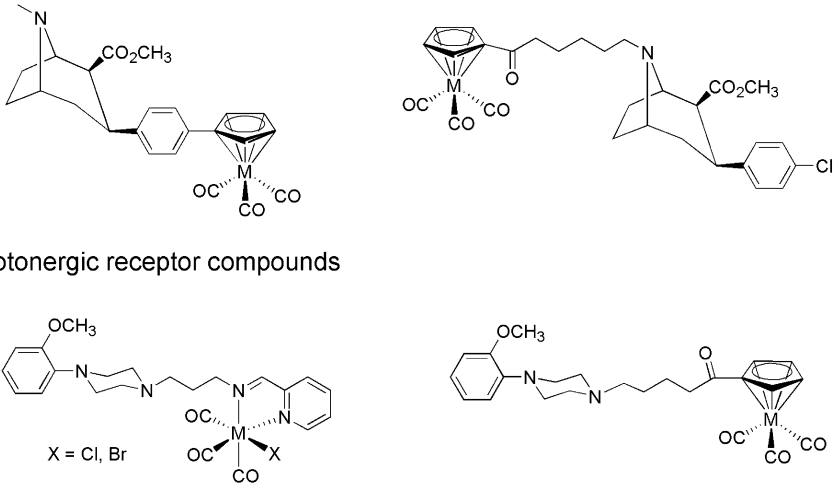

Fig. 2.2.7. Examples of organometallic Tc-99m central nervous system (CNS) complexes

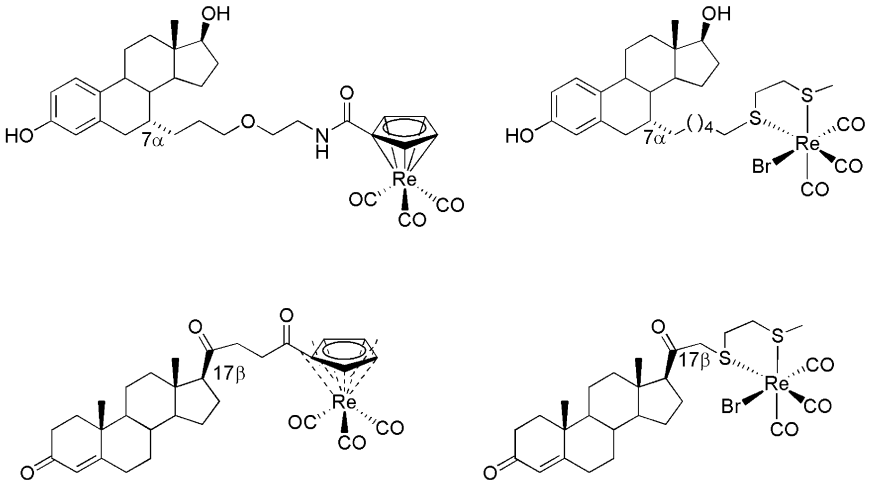

Fig. 2.2.8. Structure of various organometallic steroids for potential radiodiagnostic and radiotherapeutic targeting of progesterone receptor (PR)- and estrogen receptor (ER)-positive cancer

pared and tested (Fig. 2.2.8). The relative binding affinity (RBA) was found to depend on the nature of the spacer between the metal chelate and the steroid moiety. Similar observation and tendencies have been reported for the progestin complexes (Wust et al. 1999). For both examples, the organometallic cyclopentadienyl–tricarbonyl systems were superior to the dithioether-tricarbonyl in terms of RBA for the corresponding receptors. Synthesis and biodistribution studies of the corresponding Tc-99m and even Tc-94m analogues have been performed that suggested limited usefulness of these systems as effective imaging agents for progesterone receptor (PR)- and estrogen receptor (ER)-positive breast cancer.

Schibli and coworkers and other groups have recently published organometallic folate derivatives for targeting α-folate receptor over expressing cancer cells (Müller et al. 2004). Preclinical in vivo single-photon emission computer tomography (SPECT)/CT studies in tumor-bearing mice have revealed almost identical pharmacokinetics for both Tc-99m and the homologous Re-188 folate (Fig. 2.2.9). Based on these in vivo results and results of other organometallic Tc-99m/Re-188-labeled biomolecules (*vide infra*), it is reasonable to propose that for the tricarbonyl technology, the concept of the "matched pair" Tc/Re is indeed valid in various aspects.

Alberto and coworkers have demonstrated that vitamin B_{12}, essential for tumor growth, can be functionalized at several positions and radiolabeled with a $Tc(CO)_3$ core (Kunze et al. 2004; van Staveren et al. 2004). The in vivo assessment of several promising derivatives is currently under investigation.

The most thoroughly studied class of biomolecules that was tested with the tricarbonyl technology was the tumor affine peptides. Peptides reveal biological and pharmacological characteristics (e.g., biological half-life), which are very suited for the imaging and therapy with Tc-99m or Re-188. In fact, peptides have been among the first examples for the efficient labeling with the $M(CO)_3$ core. A number of other peptides have been studied in detail, such as neurotensin, bombesin, octreotide, annexin (Biechlin et al. 2005; Tait et al. 2002), and neuropeptide Y. Neurotensin and stabilized derivatives thereof were derivatized with histidine, either through an amide bond to the carboxylic

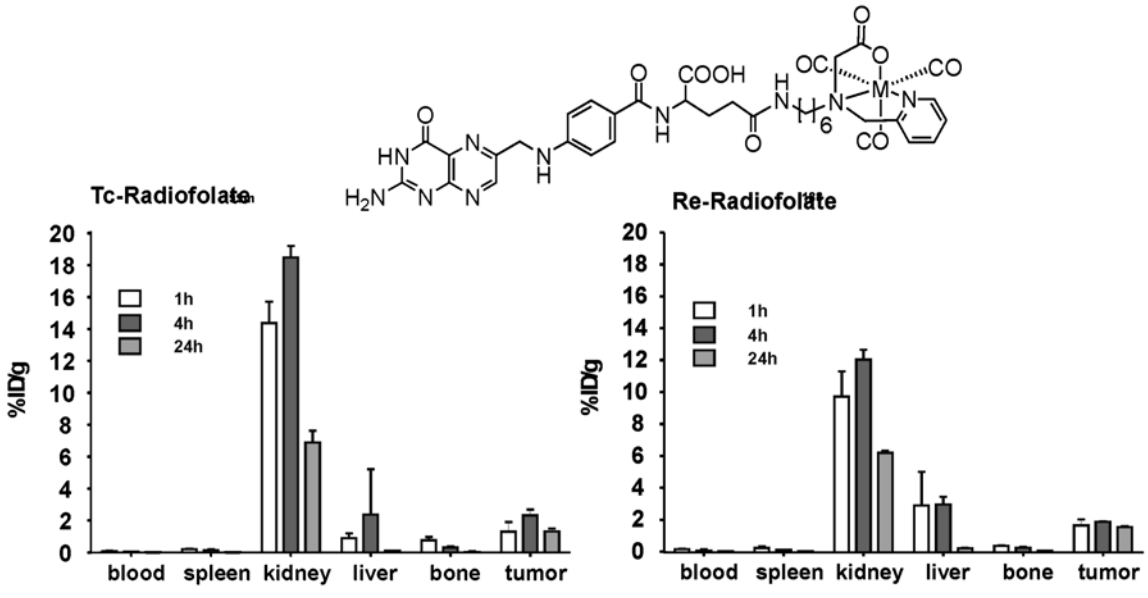

Fig. 2.2.9. Time-dependent biodistribution of novel $^{99m}Tc/^{188}Re$ organometallic folate derivatives in nude mice bearing folate receptor overexpressing KB-cell xenografts

acid to produce a bidentate NN chelator or through alkylation at the N-amino group in order to retain the tripodal coordinating feature (Blauenstein et al. 2004; Bruehlmeier et al. 2002; Egli et al. 1999; Garcia-Garayoa et al. 2001, 2002; Waibel et al. 2000). Biodistribution studies with Tc-99m showed that tridentate ligands are superior to bidentate ones, which is in agreement with the findings and preferences mentioned previously. A phase I clinical study is ongoing with ^{99m}Tc-labeled neurotensin derivatives. Neurotensin analogues with improved pharmacological profiles are currently employed in preclinical therapy studies with $^{188}Re(CO)_3$.

^{0}Tyr^{3}octreotate analogues functionalized with various BFCA have been tested (Marmion et al. 1999). The BFCA gave rise to complexes of different overall charge (+1 to –3). Wester et al. have coupled picoline-aminoacetic acid to a carbohydrated octreotide. The carbohydrate makes the conjugate much more hydrophilic, and an excellent biodistribution in humans was observed (Wester 2003; Wester et al. 2001).

Bombesin was derivatized at the C terminus with bidentate chelators (Smith et al. 2003 a, b). The labeled peptide fully retained the biological activity and was stable in vitro and in vivo. Since the bidentate coordination is not optimal with respect to stability (pharmacokinetics), the coordination sphere of the metal tricarbonyl core has been saturated with a highly hydrophilic phosphine. This additional coordination is an example of the 2+1 approach mentioned in the previous section. The mixed-ligand approach resulted in significantly higher hydrophilicity of the radioconjugates and an improved biodistribution labeled with Tc-99m and also with Re-188 (Smith et al. 2003).

In the case of other receptor avid peptides and proteins, which express an endogenous histidine such as, e.g., bombesin or neuropeptide Y, the pronounced avidity of the tricarbonyl core for histidine can create a problem with unspecific binding (Langer et al. 2001; La Bella et al. 2002 a, b). Prelabeling procedures can circumvent these problems (Langer et al. 2001). However, Garcia et al. could show that a site-specific postlabeling of bombesin is possible by introduction of a potent tridentate ligand such as, e.g., the N_α-Ac-histidine at the N terminus of the peptide (La Bella et al. 2002). As a result, a single, stable species was formed, and unspecific labeling was negligible.

The high efficiency combined with the mild reaction conditions applicable with $[M(OH_2)_3(CO)_3]^+$ is very attractive for radiolabeling of sensitive proteins. This has been recognized by several groups. MUC1 mucin is upregulated and abnormally glycosylated in bladder cancer, and is a promising target for intravesical radioimmunotherapy. The in vivo results in tumor mice have clearly revealed a better retention of immunoreactivity of the $^{188}Re(CO)_3$-labeled monoclonal antibody (mAb) as compared with the 2-mercaptoethanol-reduced-and-Re(V)-labeled mAb (Murray et al. 2001). The surfactant protein B was nonspecifically labeled with $[^{99m}Tc(OH_2)_3(CO)_3]^+$. The highly lipophilic protein has potential in the diagnosis of acute respiratory disease syndrome (Amann et al. 2001). Waibel et al. and Deyev et al. have pioneered the use of site-specific labeling of recombinant proteins via a multi–His-tag (Willuda et al. 2001). The ease of radiolabeling is remarkable and unmet with any other technetium methodology (Deyev et al. 2003).

In conclusion, it is apparent that organometallic compounds are a valuable and realistic alternative for the labeling of biomolecules in, e.g., radiopharmacy. The encouraging results of preclinical and clinical studies with organometallic-labeled tumor affine peptides and vitamins build the scaffold for further investigations. The tricarbonyl technology is the creative precedent of such novel techniques. However, the perspective and potential of organometallic labeling techniques in nuclear medicine will also depend on the success of new compounds for therapeutic use and the availability of ap-

propriate radionuclides. In the future, chemists and radiopharmacists will be equally challenged to exploit the aqueous organometallic chemistry of potential radionuclides to develop novel techniques and compounds for diagnostic and therapeutic application.

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2.3 Technetium Coupled to Biologically Active Molecules

H.-J. Pietzsch, J.-U. Künstler and H. Spies

2.3.1 Introduction

Many ^{99m}Tc pharmaceuticals were designed for the measurement of organ function, based on regional blood flows, ion transport, and cellular retention. Organ specificity is governed by molecular characteristics (e.g., size, shape, charge) and physiological factors.

Primarily, these radiotracers are coordination complexes of technetium leaving either a positive or a negative charge; neutral, lipophilic complexes pass the blood-brain barrier. Organ function is related to regional perfusion (e.g., brain, heart). Hepatocyte function is measured by the excretion of iminodiacetic acid (IDA) derivatives into bile, simulating the active transport of bilirubin. Increased osteogenic activity correlates with increased regional uptake of ^{99m}Tc-diphosphonate complexes in bone structures, delineating tumor and metastatic growth. The functional state of the kidneys as measured by active tubular secretion requires a negatively charged complex with a carboxylate anion.

^{99m}Tc pharmaceuticals based on coordination complexes with functionalized ligands are also known as "Tc essentials"; those concerning labeled particles and macromolecules are called "Tc-tagged" radiopharmaceuticals. A variety of chelating agents have been developed for complex formation with certain oxidation states of technetium, providing the structural requirements for uptake and retention (Schwochau 2000). Examples of Tc essentials are shown in Fig. 2.3.1.

The outstanding interest in the development of novel ^{99m}Tc pharmaceuticals is documented in recent reviews (Hom and Katzenellenbogen 1997; Johannsen and Pietzsch 2002 a; Jurisson and Lydon 1999).

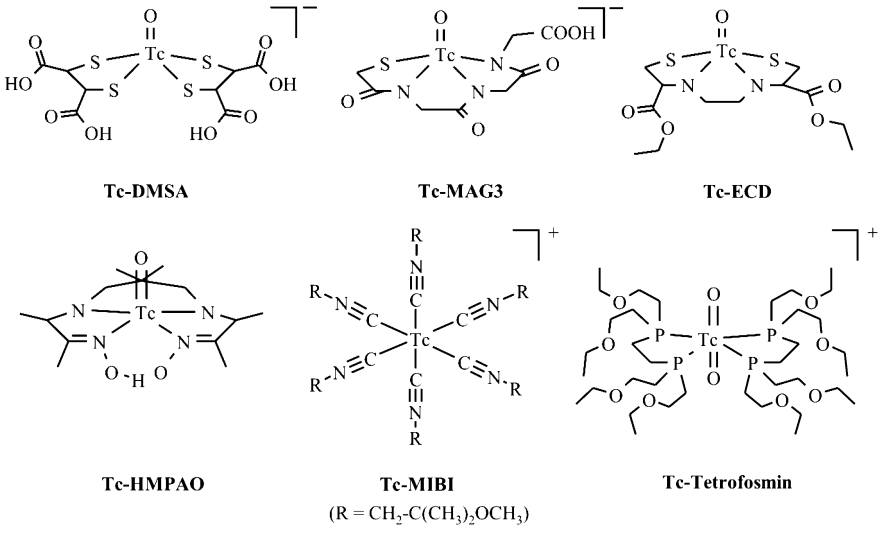

Fig. 2.3.1. "Tc essential" radiopharmaceuticals in clinical use

2.3.1.1 Target-Specific ^{99m}Tc Pharmaceuticals

Besides the merits of coordination complexes for diagnostic imaging, few applications of tumor diagnosis are in clinical use. The need for radiotracers binding specifically to epitopes expressed on tumor cells has grown over the past decade, promoting new labeling techniques, by which technetium is attached to biomolecules.

Direct or random labeling of biologically active molecules with reduced technetium did not produce pharmacologically acceptable radiotracers. Therefore, some known ^{99m}Tc complexes were specifically evaluated as potential chelating units, such as mercaptoacetyltriglycine (MAG_3) and diaminodithiol (DADT).

Prerequisites of an optimal chelator:

- The ligand used as a chelator should not alter the in vivo characteristics of a biomolecule.
- The chelate unit containing technetium should preferably be an integral part of the biomolecule (Johannsen and Pietzsch 2002b).
- The chelating unit should not affect the potency of the biomolecule.

The design of site-specific technetium molecules may complete the quest for the optimal chelator in accordance with the target-specific biomolecule, combining the chemical and biological requirements for tumor imaging.

2.3.2 Factors Affecting In Vivo Performance

Unlike ^{99m}Tc complexes, which are symmetric, small molecules (Schwochau 2000), labeled biomolecules might be asymmetric, integrating building blocks with distinct functions, such as a linker and the technetium chelating unit (Fig. 2.3.2).

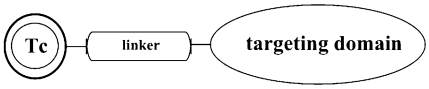

Fig. 2.3.2. Schematic representation of a specific ^{99m}Tc biomolecule

The in vivo distribution of a ^{99m}Tc biomolecule is influenced by its chemistry and by biological factors. The chemical nature of the targeting domain determines uptake and retention in the biological system. Structural integrity includes both chemical and metabolic stability of the labeled conjugate. Thus, the ligand that is used as a chelator, the type of linker, and the biomolecule will determine the bioavailability of the radiotracer.

Biological factors are related to the specific recognition of metal-based molecules, to membrane transport (particularly crossing the blood–brain barrier), clearance from nontarget sites, and high-affinity binding to tumor cell epitopes, permitting imaging and disease assessment (Ballinger 2002).

In Vivo Stability versus Reactivity. Both the chelate unit and the organic moiety may undergo transformations in vivo. In the case of "3+1" mixed-ligand complexes, the

monodentate thiol ligand is exchanged by other SH-containing compounds such as glutathione (Nock et al. 1999) or reacts with proteins (Seifert et al. 2001). In vivo transchelation has been observed with certain ^{99m}Tc complexes (methylenediphosphonate [MDP]/gluconate).

Complexes with robust tetradentate chelate units have shown metabolic degradation, splitting off the whole chelate as observed with ^{99m}Tc-Trodat-1 (Kushner et al. 1999; Mu et al. 1999).

Furthermore, the carbon bond between the linker and the tertiary nitrogen of the coordination shell may break, even during the labeling procedure, as recently reported for ^{99m}Tc tricarbonyl-labeled glucose (Pak and Alberto 2001).

Transport across Cell Membranes. In the body, many interactions of the radiotracer with biological components exist, affecting regional uptake. Transport across membranes and binding affinity have to be verified in suitable models. Specific radiotracers are designed to use transporter-mediated processes for unidirectional uptake; generally, uptake into cells across membranes should be rapid.

Lipophilicity. Lipophilicity of a radiotracer facilitates diffusion across membranes, particularly passing the blood–brain barrier, which is required for brain uptake.

Receptor Binding. Receptor binding is based on high-affinity binding of the radiotracer molecule, which is an antagonist. Since receptor density in the brain is generally in the picomolar range, high specific activity is a prerequisite for receptor ligands.

Displacement Studies. Enzyme-inhibition studies have similar requirements if the target molecule is an enzyme and the radiotracer used for quantification is an inhibitor. An alternate mechanism is based on substrate analogs, like ^{18}F-FDG, which block enzymatic degradation, thus facilitating quantification.

2.3.3 Chelate Units in the Design of Target-Specific ^{99m}Tc Pharmaceuticals

Tc chelates suitable for conjugate formation with biomolecules are derived from Tc in oxidation states V, III, and I. Organometallic carbonyl (CO) complexes serve as precursors for the synthesis of ^{99m}Tc(I) pharmaceuticals (Sect. 2.2).

Oxotechnetium(V) Complexes. The dominant structural element is the oxotechnetium core, TcO^{3+}. The presence of the oxo ligand has a significant effect on the structure and stability of these complexes. DADT-derived ligands have found application in nuclear medicine because of the thiophilic nature of technetium for the thiolate donor group (Schwochau 2000) (Fig. 2.3.3). Tetradentate diaminodithiol (N_2S_2) forms neutral, lipid-soluble technetium complexes – the most prominent is the ethylcysteinate dimer (ECD) – used for the measurement of brain perfusion. The combination of an amine-N or amide-N in an N_2S_2 arrangement (monoamine, monoamine [MAMA]) results in a more polar derivative than the diaminedithiol system. This might be preferable when less lipophilicity is required, e.g., for labeling of proteins. Tripeptides combine donor atoms of different reactivity (e.g., N_3S, N_4). The prototypic N_3S chelator MAG_3 forms

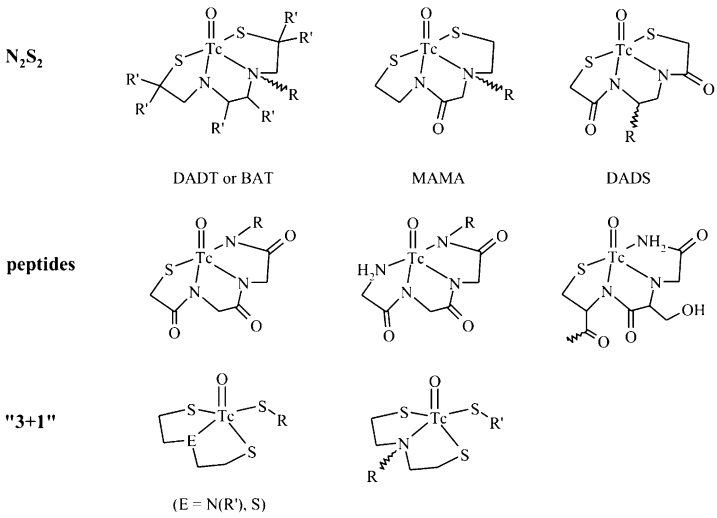

Fig. 2.3.3. Different types of oxotechnetium(V) chelates derived from N,S-ligands for radiotracer design. *R* spacer + targeting domain, *R'* H, alkyl, aryl

the radiopharmaceutical $[TcO(MAG_3)]^-$, which is used for studies of renal tubular function (Fig. 2.3.1).

Mixed-ligand complexes were synthesized in order to reduce the synthetic expenditure necessary for tetradentate compounds. A combination of tridentate (S_3 or NS_2) and monodentate (thiol) ligands is employed in the so-called "3+1" complexes (Spies et al. 1999). While the oxotechnetium/tridentate unit is very stable, exchange of the monodentate ligand has been observed in vivo (Nock et al. 1999; Syhre et al. 1998).

Tc(V) Hydrazino Nicotinamide (HYNIC) Derivatives. The introduction of the Tc(V)-HYNIC system (Schwartz et al. 1991) represents a milestone in the development of Tc-99m radiopharmaceuticals. Particularly, peptides have been labeled with very high specific activity (Edwards et al. 1999a; Harris et al. 1999; Rose et al. 1998). Since the HYNIC linker occupies only one coordination site, coligands such as tricine, ethylenediamine diacetic acid (EDDA), etc., may complete the coordination sphere of the metal (Babich et al. 2000; Edwards et al. 1999b; Ono et al. 2000) (Fig. 2.3.4).

Nitridotechnetium(V) Heterocomplexes. An asymmetric nitridotechnetium(V) heteromoiety has been proposed for radiolabeling bioactive molecules (Bolzati et al. 2000, 2002; Boschi et al. 2001; Pasqualini et al. 1992, 1994; Refosco et al. 2000). The metal fragment $[Tc(N)(PXP)]^{2+}$ can be used as an efficient synthon for the preparation of a series of nitrido heterocomplexes containing bidentate chelators such as dithiocarbamates, dithiocarbazates, cysteine, and dithiolates (Bolzati et al. 2004; Boschi et al. 2005; Tisato et al. 2004) (Fig. 2.3.5).

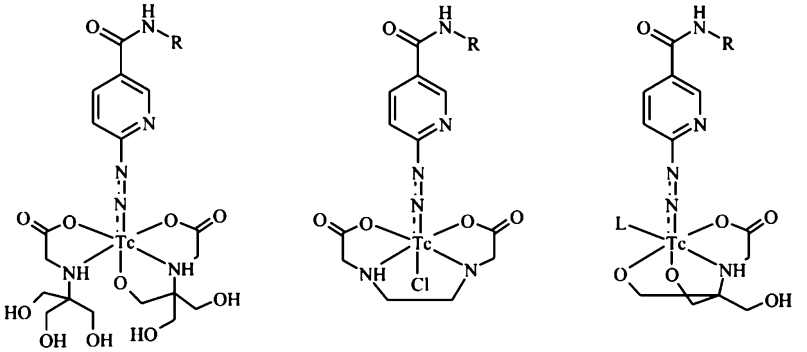

Fig. 2.3.4. Proposed structures of ^{99m}Tc-hydrazino nicotinamide (*HYNIC*) tricine derivatives with various coligands. *R* biomolecule

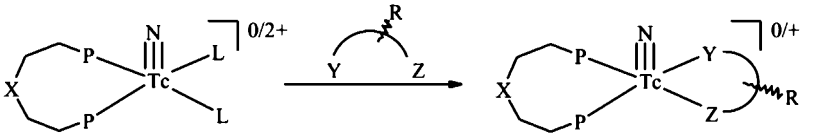

Fig. 2.3.5. Schematic representation of the labeling approach using the novel $[Tc(N)(PXP)]^{2+}$ moiety

Tc(III) Complexes. A novel type of Tc(III) chelate formed by the tripodal chelator 2,2′,2″-nitrilotris(ethanethiol) and a tertiary phosphine or an isocyanide as coligands contains sterically well-shielded oxo-free Tc(III) (Fig. 2.3.6) (Pietzsch et al. 2001a; Seifert et al. 2004; Spies et al. 1999). This moiety fulfils the requirements of a nonpolar building block stable against ligand exchange reactions in vivo.

Another type of neutral Tc(III) complexes derived from the reaction of oxotechnetium(V) "3+1" precursors with tertiary phosphines, namely compounds of the general formula $[M(PR_3)(SES)(SR)]$ (SES = tridentate dithiol ligand; E = S, NR, O), suffers from instability against cysteine and glutathione (Pietzsch et al. 2001b; Seifert et al. 2000). Stability of this class of compounds can be enhanced when a bidentate P,S phosphinothiol ligand is used instead of the monodentate ligand. The resulting "3+2" coordinated Tc(III) mixed-ligand complexes have the general formula $[Tc(SES)(R_2PS)]$ (Pietzsch et al. 2003) (Fig. 2.3.7).

Tc(I) Complexes. The organometallic ligand cyclopentadienyl (cp) offers advantages because of its small size and low molecular weight (Wenzel and Klinge 1994). Stable Re(cp) and Tc(cp) complexes have been prepared that were conjugated to octreotide (Spradau et al. 1999), piperidine (Fig. 2.3.8) (Saidi et al. 2001), tropane (Cesati et al. 2002), and steroid hormones (LeBideau et al. 2001; Mull et al. 2002). However, this approach still suffers from unacceptable reaction conditions for routine use of technetium-99m.

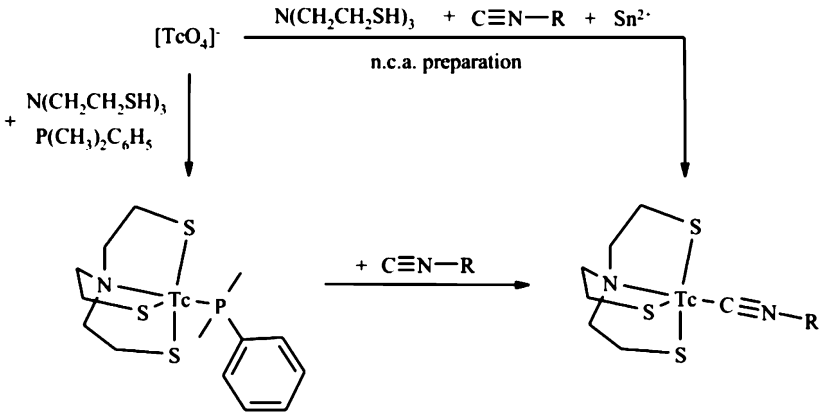

Fig. 2.3.6. Formation of technetium(III) complexes with tetradentate/monodentate coordination

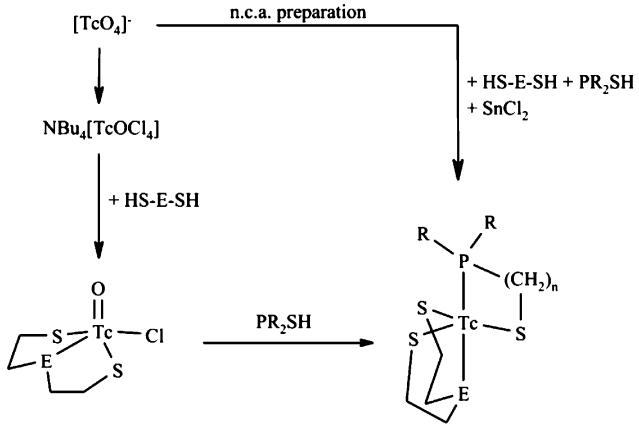

Fig. 2.3.7. Reaction routes to Tc(III) complexes with "3+2" coordination. $E = N(CH_3)$, S

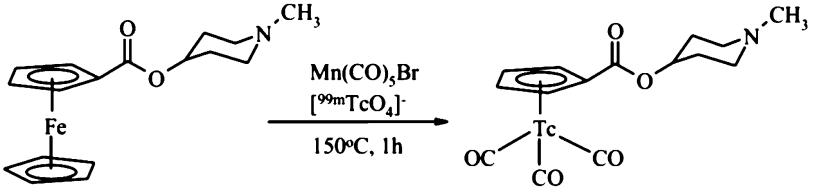

Fig. 2.3.8. Preparation of $^{99m}Tc(CO)_3$ cyclopentadienyl (cp) carboxylate derivative illustrating the double-ligand transfer approach (Saidi 2001; Wenzel 1994)

It has been demonstrated (Wald et al. 2001) that the cyclopentadienyl ligand can be coordinated to $[^{99m}Tc(OH_2)_3(CO)_3]^+$ in water by introducing the electron withdrawing acetyl group in cyclopentadiene to give acetyl-cp.

Technetium(I) chemistry initiated by (Alberto et al. 2001) is greatly facilitated by the available Tc(I)-tricarbonyl synthon. Recent developments and investigations are presented in Sect. 2.2.

2.3.4 Search for Novel Tc Pharmaceuticals

Peptides. Peptides with low molecular weight consisting of 5–15 amino acids have attracted much attention in radiopharmaceutical design because of their low immunogenicity, suitable pharmacokinetic properties, and high binding affinities. They are easier to synthesize and to modify than are larger molecules (Signore 1995). To be suitable as a tumor-imaging peptide, the density of the peptide affine receptor on tumors must be considerably higher than in other regions of the body. The metabolic stability and affinity for the receptor should be high. Many excellent reviews have been published discussing different aspects of technetium radiopharmaceuticals based on peptides (Aloj and Morelli 2004; Eberle et al. 2004; Fichna and Janecka 2003; Giblin et al. 2005; Langer and Beck-Sickinger 2001; Liu 1999; Liu and Edwards 2002; Maecke 2005; Okarvi 2004; Signore et al. 2001). Naturally occurring peptides that can be used for tumor imaging are listed in Table 2.3.1.

Modified derivatives of somatostatine have been synthesized to prolong the biological half-life of native somatostatine. The most important derivative is octreotide (sandostatin), a cyclic peptide with 8 amino acids, unlike the 14 in somatostatine.

The successful use of octreoscan in the diagnosis of somatostatine receptor-positive tumors has intensified the search for improved or new peptide-based agents for imaging thrombi, infection/inflammation, and different tumors.

Among the chelate units used for peptide labeling, the Tc-HYNIC and Tc-tricarbonyl cores have gained importance. A freeze-dried kit formulation for the preparation of ^{99m}Tc-EDDA-HYNIC-D-Phe(1), Tyr(3)-octreotide, another somatostatin analog for tumor diagnosis, has recently been published (von Guggenberg et al. 2004).

Ongoing research on ^{99m}Tc-HYNIC somatostatin analogs has further clarified the effect of labeling methods and peptide sequence on bioperformance (Bangard et al. 2000; Decristoforo and Mather 1999 a, b; Decristoforo et al. 2000). A variety of coligands used for labeling HYNIC-derivatized peptides has been explored, e.g., 2-mercaptopyridines and 2-mercaptopyrimidines (Babich et al. 2001).

Recently described labeled HYNIC-conjugated peptides also involve RGD (Arg-Gly-Asp) peptides targeting the integrin $\alpha_v\beta_3$ (vitronectin) receptor. Tertiary ligand complexes of HYNIC-conjugated peptide, tricine and trisodium triphenylphosphine-3,3′,3″-trisulfonate (TPPTS) have been published (Liu et al. 2001; Su et al. 2002).

Interleukin-8, a chemotactic cytokine involved in activation of neutrophils to areas of infection, can be labeled with ^{99m}Tc-HYNIC with preservation of its leukocyte receptor-binding capacity (Rennen et al. 2001).

After the introduction of the Tc(I) tricarbonyl approach, its application to peptide labeling has been pursued (Egli et al. 1999).

Other chelating frameworks have been studied such as the novel dithia-bisphosphine chelator (Gali et al. 2001), or further employed such as tripeptide N_3S chelators for the

Table 2.3.1. List of naturally occurring peptides that can be used for tumor imaging

Ligand	Selectivity
Somatostatine	Neuroendocrinic tumors, non–Hodgkin's lymphoma,
Derivatives	Melanomas, breast tumors
Alpha-MSH	Melanomas
LHRH	Prostata tumors, breast tumors
VIP/PACAP	SCLC, tumors of colon, stomach, pancreas
RGD	Blood vessels of tumors
CCK-B/gastrine	MTC, SCLC, pancreas tumors, astrocytomes
Neutrotensin	SCLC, colon tumors, exocrinic pancreas tumors
Bombesin/GRP	SCLC, colon tumors, glioblastomas, prostata tumors
Substance P	Glioblastomas, astrocytomas, MTC, breast tumors, Peritoneal blood vessels

SCLC small cell lung carcinoma, *MTC* medullary thyroid cancer

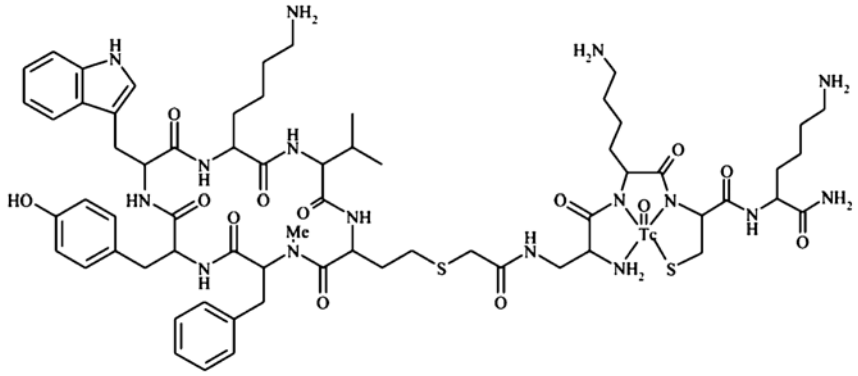

Fig. 2.3.9. Structure of Tc-labeled depreotide (NeoTect^{TM})

inflammation imaging agent ^{99m}Tc-RP128 (Caveliers et al. 2001), a tuftsin receptor-binding peptide (Wong et al. 2001) and melanocortin receptor-1 specific ligands for targeting melanoma (Sharma et al. 2000).

The commercial kit NeoTect^{TM} (Diatide) was designed as a radiopharmaceutical for somatostatin-receptor imaging of lung tumors (Virgolini et al. 1998). It is based on the peptide P829 (depreotide), a structural modification of octreotide, with the technetium binding N_3S sequence diaminopropionic acid-lysine-cysteine built into the molecule (Cyr et al. 1999) (Fig. 2.3.9). This modification is an alternative to octreotide, where the labeling process leads to the reduction of the disulfide bond, resulting in a loss of receptor-binding affinity (Blum et al. 1999; Vallabhajosula et al. 1996).

Novel ^{99m}Tc-based tetra-amine-functionalized [Tyr^3]octreotate analogues (Fig. 2.3.10) have been developed for imaging of somatostatin receptor-positive tumors (Maina et al. 2002; Nikolopoulou et al. 2006).

An intrapatient comparison of ^{99m}Tc-N_4-[Tyr^3]octreotate with ^{99m}Tc-EDDA/HYNIC-[Tyr^3]octreotide showed that ^{99m}Tc-Demotate is a promising agent for somatostatin receptor scintigraphy (Gabriel et al. 2004).

The same open-chain tetra-amine ligand has been conjugated to various bombesin derivatives. First studies in mice showed high and specific accumulation of ^{99m}Tc-De-

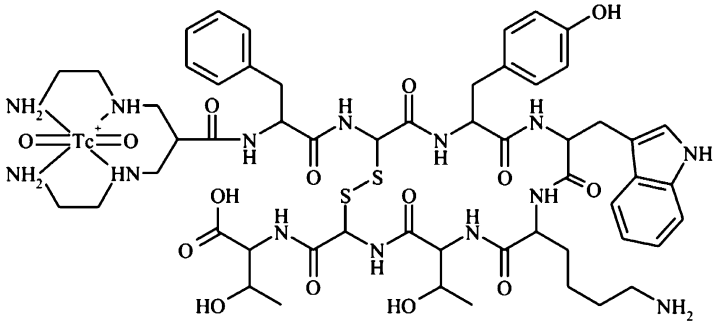

Fig. 2.3.10. Structure of tetra-amine-functionalized ^{99m}Tc-Demotate

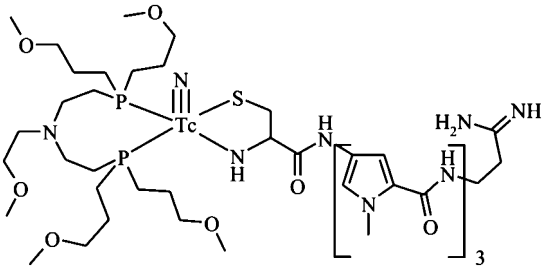

Fig. 2.3.11. Hybrid distamycin-cysteine conjugated with a $[^{99m}Tc(N)(PP)]^{2+}$ fragment

mobesin 1 in gastrin releasing peptide receptor (GRP-R)-positive regions (pancreas, gastrointestinal tract) (Nock 2003).

A new high-affinity technetium-99m-bombesin analogue with low abdominal accumulation has been recently published (Lin et al. 2005).

^{99m}Tc-UBI 29-41, a technetium-99m-labeled peptide derived from ubiquicidine, targets bacterial and fungal infections in experimental animals. Welling et al. reported on the radiochemical and biological features of this radioactive agent and the importance of the amino acid sequence of UBI 29-41 for imaging of infections (Lupetti et al. 2002; Welling et al. 2002, 2005).

An attempt to exploit the chemistry of nitridotechnetium(V) complexes for labeling small biomolecules has been described (Baraldi et al. 2000). The tripyrrole peptide distamycin A, an antibiotic agent that binds to DNA, was functionalized with cysteine to obtain a bidentate ligand, which forms a mixed-ligand complex with a $[^{99m}Tc(N)(PP)]^{2+}$ fragment (Fig. 2.3.11).

Proteins and Antibodies. In the past, considerable work has been focused on the development of ^{99m}Tc-labeled monoclonal antibodies and their fragments. Three main strategies for labeling can be distinguished: direct labeling, bifunctional chelating agent (BFCA)-based prelabeling, and BFCA-based postlabeling.

Among the direct labeling methods, reduction of the antibody by a thiol reagent, such as mercaptoethanol or dithiothreitol, results in high labeling yields (Reilly 1993; Schwarz et al. 1988; Thakur et al. 1991). Table 2.3.2 compiles ^{99m}Tc-labeled antibodies approved as radiopharmaceuticals in the United States and the European Union.

Table 2.3.2. ^{99m}Tc-labeled antibodies approved as radiopharmaceutical (2005)

Drug	Indication	Antibody	Target	^{99m}Tc-binding	Year of approval
Neutrospec	Equivocal signs and appendicitis (infection/ inflammation)	Fanolesomab (IgM, murine)	CD15	Reduced protein	2004 (US)
Humaspect	Colorectal cancer	Votumumab (IgG, human)	CTAA16.88	Reduced protein	1998 (EU)
Leukoscan	Osteomyelitis (infection/ inflammation in bone)	Sulesomab (Fab′, murine)	CEA and NCA90	Reduced protein	1997 (EU)
CEA-Scan	Colorectal cancer	Arcitumomab (Fab′, murine)	CEA	Reduced protein	1996 (US); 1996, withdrawn 2005 (EU)
Verluma	Small cell lung cancer	Nofetumomab (Fab′, murine)	CD20	N_2S_2 chelate	1996 (US)
Tecnemab-K-1	Melanoma	Antimelanoma mAb fragments (Fab′ and F(ab′)$_2$, murine)	HMW-MAA	Reduced protein	1996, withdrawn 2000 (EU)

Sources: pharmacos.eudra.org; www.fda.gov; www.biopharma.com
CEA carcinoembryonic antigen, *CD* cluster of differentiation, *mAb* monoclonal antibody, *CTAA* cytokeratine tumor-associated complex of antigens, *NCA* granulocyte nonspecific crossreacting antigen, *HMW-MAA* high-molecular-weight melanoma-associated antigen

Table 2.3.3. ^{99m}Tc-labeled monoclonal antibodies and antibody fragments for potential application

Antigen	Potential imaging application	^{99m}Tc-binding method	References
CA125	Ovarian cancer	Direct labeling	Kobayashi et al. 1993
CD4	Rheumatoid arthritis	Direct labeling	Kinne et al. 1995; Becker et al. 1990
CD22	Non–Hodgkin's lymphoma	MAG_3	Postema et al. 2003
CD44v6	Head and neck squamous cell carcinoma	MAG_3	Stroomer et al. 2000; Colnot et al. 2003
CD62E (E-Selectin)	Infection/inflammation	Direct labeling	Jamar et al. 2002
EGFR	EGFR-expressing tumors	EC, direct labeling	Schechter et al. 2003; Meenakshi et al. 2003
G250	Renal cell carcinoma	HYNIC, MAG_3, direct labeling	Steffens et al. 1999
MUC1	Bladder cancer, breast cancer	Tricarbonyl, direct labeling	Waibel et al. 1999; Simms et al. 2001
Myosin	Myocardial infraction	Direct labeling	Iwasaki et al. 2001; Taillefer et al. 1995
$P185^{HER-2}$	Breast cancer	Tricarbonyl	Willuda et al. 2001
TAG-72	Adenocarcinomas	HYNIC, introduced SH-group	Goel et al. 2001; Ranadive et al. 1993

CA cancer antigen, *CD* cluster of differentiation, *MUC* mucin, *TAG* tumor-associated glycoprotein, *EGFR* epidermal growth factor receptor, MAG_3 mercaptoacetyltriglycine, *EC* ethylcysteinate, *HYNIC* hydrazino nicotinamide

Table 2.3.4. ^{99m}Tc-labeled proteins (excluding antibodies)

Protein	Imaging application	^{99m}Tc-binding unit	References
Polyclonal IgG	Infection/inflammation, Blood pool	HYNIC, direct labeling	Abrams et al. 1990; Pieri et al. 1991; Claessens et al. 1996; Dams et al. 2000
HSA	Blood pool	HYNIC, MAG_3, direct labeling	Verbeke et al. 1995; Pieri et al. 1991
Annexin V	Apoptotic cells	N_2S_2, HYNIC, MAG_3, EC, tricarbonyl, endogenous peptide sequences, direct labeling	Lahorte et al. 2004; Boersma et al. 2005
Interleukins	Infection/inflammation	HYNIC, N_3S-chelate	Rennen et al. 2001, 2003a; Signore et al. 2004; Chianelli et al. 1997
NGA	Liver disease	Direct labeling	Stadalnik et al. 2001
GSA	Liver disease	DTPA	Kokudo et al. 2003
Aprotinin	Amyloidosis	Direct labeling	Schaadt et al. 2003; Aprile et al. 1995
FGF-1	FGF-1 receptor	HYNIC	Zinn et al. 2000
EGF	EGF-receptor expressing tumors	Introduced thiol group (direct labeling)	Capala et al. 1997
Anaphylatoxin C5a, C5adR	Infection	HYNIC	Rennen et al. 2003b
NAP-2 (CXCL-7)	Infection	HYNIC	Rennen et al. 2004
Ubiquicidin	Infection	Direct labeling	Welling et al. 2000
Lactoferrin	Infection	Direct labeling	Welling et al. 2000
HuS (adapter protein)	Target with a docking tag	HYNIC	Blankenberg et al. 2004

IgG immunoglobulin G, *HSA* human serum albumin, *NGA* galactosyl neoglycoalbumin, *GSA* galactosyl human serum albumin, *FGF-1* acidic fibroblast growth factor, *EGF* epidermal growth factor, *NAP* neutrophil-activating peptide, *HuS* 110–amino acid fragment of human ribonuclease I, *MAG_3* mercaptoacetyltriglycine, *HYNIC* hydrazino nicotinamide, *EC* ethylcysteinate, *DTPA* diethylene triamine pentaacetate

Some further examples for current search in antibody labeling are given by Tang et al. (2005), Francis et al. (2004), and Jeong et al. (2004).

^{99m}Tc-labeled antibodies in experimental evaluation are summarized in Table 2.3.3.

A simple liquid formulation for the preparation of ^{99m}Tc-HYNIC-annexin V has been developed. Biodistribution studies in mice indicated that the target organs were the kidneys (Vanderheyden et al. 2002).

^{99m}Tc-HYNIC annexin V conjugates have been used for detection of apoptotic tumor response in vivo after a single dose of chemotherapy (Mochizuki et al. 2003), and for the evaluation of inflammation and apoptosis in rats with autoimmune myocarditis (Tokita 2003).

A selection of ^{99m}Tc-labeled proteins (excluding antibodies) is summarized in Table 2.3.4.

Oligonucleotides. Small oligonucleotide sequences that are complementary to a small mRNA segment could potentially target any specific mRNA molecule, and be used to image endogenous gene expression at the transcription level (Duatti 2004; Younes et al. 2002). Low in vivo stability continues to be a serious drawback. However, modifications may increase resistance to nucleases (Borkowski and Dinkelborg 2006; Usman and Blatt 2000).

Several authors reported the use of a so-called morpholino (MORF), a commercially available synthetic oligomer for pretargeting application (Liu et al. 2002, 2004). A construct of MAG_3 and cMORF was found to be effective in a mouse tumor model. Biodistribution data indicated high uptake in the tumor and low uptake in the normal tissues (Liu et al. 2002).

Recently, Qin et al. (2005) reported on molecular imaging of atherosclerotic plaques with ^{99m}Tc-labeled antisense oligonucleotides.

A review on recent progress in antisense targeting with radiolabeled DNA derivatives was given by Hnatowich and Nakamura (2004).

Central Nervous System (CNS) Receptor Imaging Agents. The development of ^{99m}Tc-based imaging agents selective for CNS receptors has been an area of considerable research endeavor. Progress has been made in the development of a dopamine transporter (DAT) imaging agent ^{99m}Tc-TRODAT-1 (Kung et al 1997) (Fig. 2.3.12), the development of another DAT ligand, ^{99m}Tc-O(15)O5T (Callahan et al. 2001), and synthesis of ^{99m}Tc complexes with nanomolar in vitro affinity for dopamine (D_1, D_2), serotonin (5-HT_{1A}, 5-HT_{2A}) and muscarinic acetylcholine receptors. The state-of-the-art of technetium-based CNS receptor ligands have been recently reviewed (Johannsen and Pietzsch 2002 a).

Molecular recognition of technetium complexes and their fit into the receptor-binding pocket is achievable. This is indicated by the high in vitro affinities of manifold Tc complexes to the serotonin-5-HT_{1A} receptor in the nanomolar and subnanomolar range (Alberto et al. 1999; Bernard et al. 2003; Bolzati et al. 2003; Boschi et al. 2003; Drews et al. 2002; Heimbold et al. 2002 a, b; Kara 2004; Leon et al. 2002; Papagianopoulou et al. 2002; Saidi et al. 2004; Samnick et al. 2004) (Fig. 2.3.13). Therefore, receptor binding would be high if the ligand would also demonstrate high uptake in brain; however, very low or absent brain uptake is the main issue in the development of receptor-binding imaging agents. A suitable combination of a high receptor affinity with a sufficient brain uptake was achieved only with the DAT ligands.

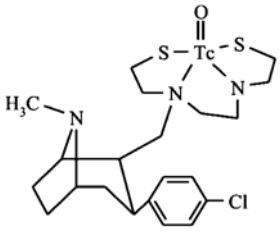

Fig. 2.3.12. Dopamine transporter (DAT) imaging agent ^{99m}Tc-TRODAT-1

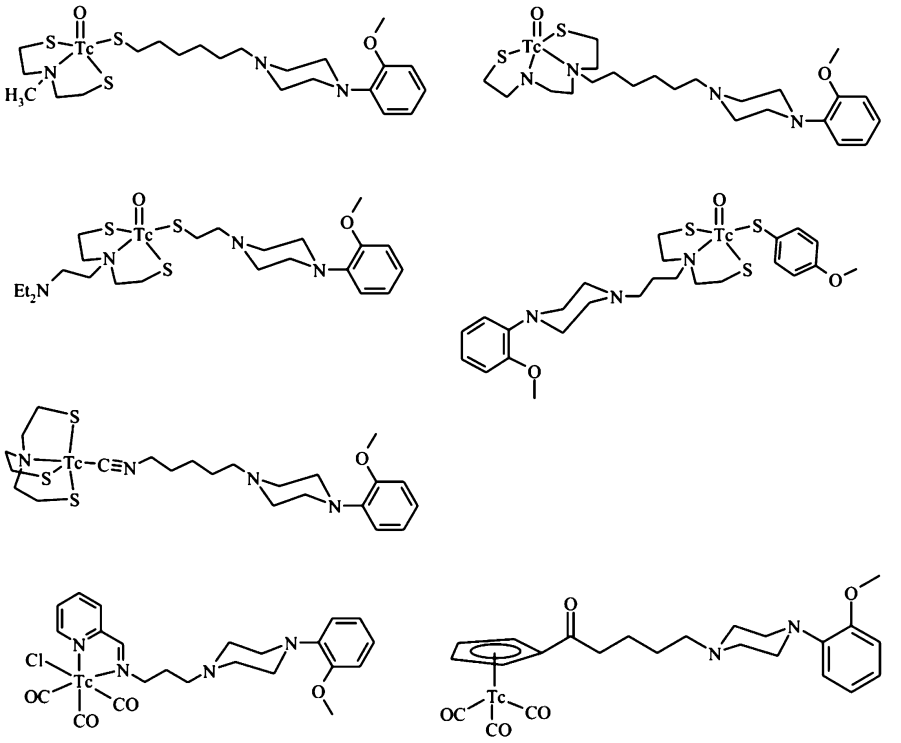

Fig. 2.3.13. ^{99m}Tc receptor ligands with nanomolar and subnanomolar affinities for the 5-HT_{1A} receptor (in vitro)

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