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Review

The preparation of substitution-inert ^{99}Tc metal-fragments: Promising candidates for the design of new ^{99m}Tc radiopharmaceuticals

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Contents

Abstract

^{99}Tc mixed-ligand complexes displaying characteristic substitution-inert metal-fragments are emerging as alternative platforms in the design of potential ^{99m}Tc radiopharmaceuticals. Starting from the already well-known $[Tc(CO)_3]^+$ moiety, this review describes recent efforts in the coordination chemistry of technetium aiming at the exploitation of the metal-fragment strategy. Examples include Tc^{III} '4 + 1' and Tc^{III} 'SSS' systems, along with a description of the Tc^{V}(N)(PNP) system and the transfer of this technology to other cores (M=NPh and M=O).

Keywords: Technetium radiopharmaceuticals; Tc(III) complexes; Nitrido-Tc(V) complexes; Aminodiphosphine

Abbreviations: 2-MPP, (2-methoxyphenyl)piperazine; 9S3, 1,4,7-trithiacyclononane; BFCA, bifunctional chelate agent; BLYP, Becke–Lee–Yang–Parr; bt, benzenethiolate(1−); CNR, isocyanide or isonitrile; DBODC, *N*,*N*′-bis(ethoxyethyl)dithiocarbamate(1−); DEDC, *N*,*N*′-diethyldithiocarbamate(1−); DFT, density-functional theory; dmpe, dimethylphosphinoethane; DTC, dithiocarbamate(1−); ECD, ethylenediyl-cysteine, diethyl ester(3−); EDTA, ethylenediamine tetraacetate(4−); HYNIC, 6-hydrazino-nicotinamide; HYPY, 2-hydrazino-pyridine; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; MAG3, mercaptoacetyltriglycinate(4−); NS_3, nitrilotris(ethanethiolate) (3−); PnAO, propyleneamineoxime; PNHP, bis[2-(diphenylphosphino)ethyl]amine; PNP, aminodiphosphine; PNP5, bis[2-(dimethoxypropylphosphino)ethyl]ethoxyethylamine; PNP7, bis[(2-diphenylphosphino)propyl]methoxyethylamine; PN(R)P, substituted aminodiphosphine; POOP, 1,8-bis(diphenylphosphino)-3,6-dioxaoctane; POP, bis[2-(diphenylphosphino)ethyl]ether; PXP, heterodiphosphines; SAAC, single amino acid analogue chelate; SPECT, single photon emission computerized tomography; SSS, 'super six sulphur'; TDT, 3-thiapentane 1,5-dithiolate(2−); TPPTS, trisodium triphenylphosphine 3,3′,3″-trisulphonate; tricine, *N*-[tris(hydroxymethyl)methyl]glycine; TTOD, 5,8,11,14-tetrathiaoctadecane

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1. Introduction

Single photon emission computerized tomography (SPECT) continues to play a prominent role in diagnosis of diseases since the introduction of the ^{99}Mo/^{99m}Tc generator in 1960s [1]. ^{99m}Tc possesses almost ideal nuclear properties: (i) 6 h half life, which is sufficiently long for any radiopharmaceutical preparation and in vivo accumulation in the target tissue, and yet short enough to minimize radiation dose delivered to the patient and (ii) nearly pure 140 keV γ-emission, close to optimal for imaging with commercial gamma cameras. These nuclear properties are combined with easy availability of the ^{99}Mo/^{99m}Tc generator, moderate cost of the isotope and reasonably simple reconstitution of the radiopharmaceutical starting from eluted pertechnetate with a series of kits provided by several pharmaceutical companies.

The possibility to study systematically the chemistry of the metal utilizing the long-lived ^{99}Tc isotope ($E_\beta = 292$ keV, half life $= 2.12 \times 10^5$ yr) offers the additional advantages to elucidate the molecular structure of the injected ^{99m}Tc agents, and eventually to finely tune the biological properties of these radiopharmaceuticals. Efforts in this direction have produced a number of 'Tc-essential' radiopharmaceuticals (whose biodistribution depends primarily on their physico-chemical properties such as charge, size and lipophilicity), which are currently utilized in the clinical practice as perfusion tracers [2]. More recently, 'target-specific' radiopharmaceuticals (whose tissue localization is driven by specific receptor interactions) represent a remarkable opportunity in the imaging of cancer cells [3], and of the central nervous system [4].

A number of recent, excellent review articles cover the main aspects of technetium chemistry and radiopharmaceuticals developed in the past 20 years [5–15], and the readers are addressed to these contributions to achieve an extensive knowledge of the argument. In particular: (i) volume 5 of the Comprehensive Coordination Chemistry II contains an exhaustive description of basic technetium chemistry [5], (ii) four consecutive surveys illustrate the X-ray molecular structures of technetium complexes so far reported in the literature [6–9], and (iii) a special thematic issue of Chemical Review entitled 'Medicinal Inorganic Chemistry' comprises two articles describing 'essential' [2] and 'target-specific' [4] ^{99m}Tc radiopharmaceuticals, respectively. Moreover, three very recent contributions analyze (i) the role of coordination chemistry in the development of target-specific radiopharmaceuticals [10], (ii) new directions in the coordination chemistry of technetium [11], with particular emphasis to three molecular platforms comprising the mercaptoacetyltriglycine (MAG_3) bifunctional chelates, the hydrazino-nicotinamide (HYNIC) system and the single amino acid analogue chelate (SAAC) coupled with the tricarbonyl synthon, and (iii) the development of ^{99m}Tc radiopharmaceuticals containing a terminal metal–nitrido multiple bond [12].

The metal-fragment strategy involves the construction of a substitution-inert moiety comprising the metal (or a metal core) and a suitably tailored polydentate ligand. The role of the latter is to seek the metal (or the metal core), and to promote labilization of the remaining coordinating groups, which are selectively substituted by other, different chelate ligands in the final step. Starting from the well-known $[Tc(CO)_3]^+$ synthon, which represents a clear, illustrative example of the metal-fragment approach, the present review focuses on new and less known systems based on the same concept. Examples of this strategy include Tc^{III} '4 + 1' and Tc^{III} 'SSS' systems, along with the Tc^{V}(N)(PNP) structure and the transfer of this technology to related cores (M=NPh and M=O).

2. Background: the extensive investigation on the $[TcO]^{3+}$ core

The $[TcO]^{3+}$ has been the most investigated core in the development of ^{99m}Tc radiopharmaceuticals due to the availability of labile $[^{99m}TcO]^{n+}$ mixtures from eluted pertechnetate, from which stabilization of the core has been successfully achieved using several polydentate frameworks. Among these chelate ligands, $N_xS_{(4-x)}$ skeletons including N_2S_2 monoaminomonoamidodithiols [16], N_3S triamidomonothiol [17] and N_4 propylene amineoxime [18] have been used for the synthesis of ^{99m}Tc radiopharmaceuticals currently utilized in the clinical practice to image the cerebral tissue and the kidney district (^{99m}TcO(ECD), Neurolite®; $[^{99m}TcO(MAG_3)]^-$, Technescan®; ^{99m}TcO(PnAO), Ceretec®). In these cases each tetra-dentate ligand coordinates equatorially the $[TcO]^{3+}$ group giving five-coordinate, distorted square-pyramidal geometries.

The easy access to stable square-pyramidal configurations prompted several research groups to design and investigate $^{99m}TcO(N_xS_{(4-x)})$ agents incorporating biomolecules, peptides, monoclonal antibodies, etc. for targeted imaging. In the so called bifunctional approach, the unsubstituted tetradentate ligand able to bond tightly the metal is derivatized with suitable, external anchor functions (COOH, NH_2, NCS) able, in turn, to conjugate the relevant biomolecule. This biological fragment is in charge of driving the radiolabeled agent to receptors, transporters and enzymes [19].

Incorporation of a biomolecule into a tetradentate framework often requires complicated multi-step reactions and, once coordinated, such substituted chelates usually give rise to diastereoisomeric mixtures which might affect the biological properties of the resulting agent.

Attempts to optimize the $TcO(N_xS_{(4-x)})$ chemistry have produced poor results. Neither changing the coordinating atom set from $N_xS_{(4-x)}$ to N_xP_y [20] and S_xP_y [21] nor trying to adjust the polydentate ligand gave better results. For example, in the so called '3 + 1' system [22], which comprises a tridentate aminodithiolate and a monodentate thiolate functions around the $[TcO]^{3+}$ core, any advantage to make the system more flexible and to incorporate the biomolecule into the monothiol is minimized by the lack of stability of the overall system. In fact, the monothiolate ligand undergoes substitution reactions in vivo in the presence of challenging competitors such as cysteine and/or glutathione [23]. An interesting step ahead in the stabilization of the mono–oxo '3 + 1' system is represented by the introduction of a phosphorus donor in the tridentate ligand (Fig. 1) [24].

The resulting compounds have shown improved stability toward transchelation reactions at 'carrier free' level [25].

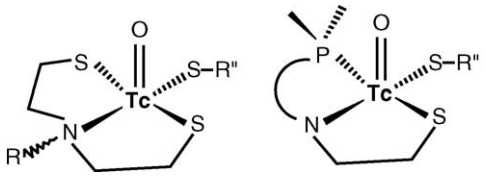

Fig. 1. From substitution-labile oxo-Tc(V) '3 + 1' [22,23] to substitution-inert oxo-Tc(V) '3 + 1' species [24].

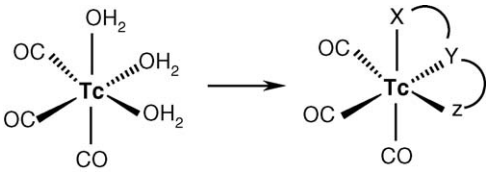

Fig. 3. The Tc-tricarbonyl metal-fragment. Labile water molecules are replaced by a variety of chelate ligands [33–35].

Other stereochemistries (see Fig. 2) including a mono–oxo '3 + 2' combination of a tridentate model SNX-dipeptides (X = S, N, O) with a bidentate PO-phosphinophenolate [26], another '3 + 1 + 1' arrangement including a histidine model dipeptide and halides [27], a '4 + 1' combination of a N_2O_2-diazophosphinate with an halide [28], and a rare seven-coordinate pentagonal bipyramidal system comprising a N_4O_2-hexadentate bis-aminopyridylphenolate or bis-aminopyridylcarboxylate ligand [29] have been proposed for rhenium, but no examples with the second-row congener technetium have appeared in the literature so far.

3. Other chemical platforms for ^{99m}Tc radiopharmaceuticals

The huge effort dedicated to the development of oxo containing radiopharmaceuticals has left little room for different chemical strategies for many years, even if the discovery of the organometallic hexakis-isonitrile Tc(I) agent ^{99m}Tc-sestamibi [30] as a heart perfusion tracer indicated that other, lower oxidation states were easily accessible, and unpredictable ligands could be employed to stabilize the metal in vivo.

Two new approaches are receiving significant attention recently. The first system is based on the replacement of the oxo for the HYNIC core [11,31], which allows straightforward conjugation of several biomolecules to its skeleton. Although ^{99m}Tc complexes of HYNIC are chemically robust, their chemical structures are still not as well defined as those containing the oxo core, and poorly identified at the tracer level [11]. Molecular structures shown by model HYPY ^{99}Tc and Re compounds show both linear and chelate arrangement of the HYPY unit, along with a metal/HYPY ratio of 1:1 or 1:2. At tracer level, the coordination of π-acceptor ligands, either phosphines or pyridines, contributes to the overall stabilization of the Tc/HYNIC core, as demonstrated by series of hydrophilic [^{99m}Tc(HYNIC)(tricine)(TPPTS)] and [^{99m}Tc(HYNIC)(tricine)(pyridine)] compounds [32].

The second platform, the $[Tc(CO)_3]^+$ synthon, has been already described comprehensively in other reviews [5,14]. We just wish to point out that the tricarbonyl moiety constitutes a clear, illustrative example of the metal-fragment strategy. One-pot reduction of aqueous pertechnetate in the presence of boranocarbonate gives an excellent yield of the water-soluble, water-stable technetium carbonyl complex $[^{99m}Tc(CO)_3(OH_2)_3]^+$ [33–35]. As shown in Fig. 3, the molecular structure of this aqua ion displays an octahedral geometry, the carbonyl groups adopting invariably a facial configuration. Hence, each CO faces a trans coordinated water molecule.

While the $[Tc(CO)_3]^+$ metal-fragment remains intact, water molecules are efficiently replaced by bidentate or tridentate ligands counting hard and soft donors. The kinetic stability of the *fac*-$[Tc(CO)_3]^+$ moiety arises from its d^6 low spin configuration. A large number of mixed complexes have been prepared including simple coordinating groups [36] and more sophisticated peptide conjugated chelates [37]. Moreover, the carbonyl groups appear to confer a lipophilic character to the synthon. The hydro/lipophilic balance of the overall molecule can be efficiently tuned by changing the properties of the co-ligand(s).

4. New platforms based on the metal-fragment strategy

4.1. The $Tc^{III}(NS_3)$ metal-fragment (Fig. 4)

Starting from the concept that mixed ligand approach may facilitate smooth tuning of the ligand properties, Spies and coworkers proposed to change the coordination of the mono–oxo core operated by a tetra-dentate framework with a more flexible '3 + 1' combination [22]. As mentioned above, the instability of this system was presumably due to a combination of effects associated with the coordination vacancy exhibited by five-coordinated compounds and the intrinsic tendency of the

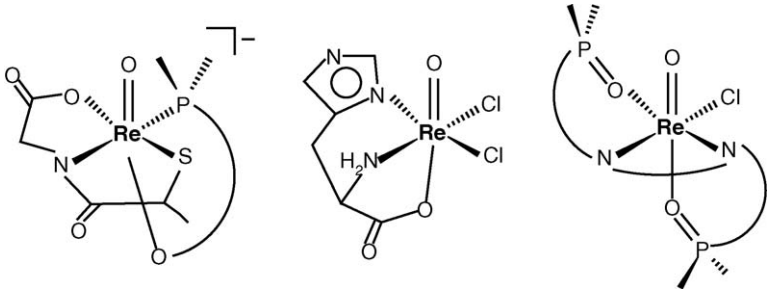

Fig. 2. Other stereochemistries including the mono–oxo $[Re = O]^{3+}$ core [26–28].

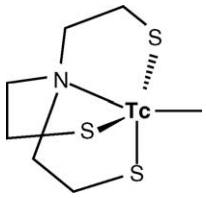

Fig. 4. The $Tc^{III}(NS_3)$ metal-fragment.

metal to reach lower oxidation states. A consequent rational design involved reduction of mono–oxo Tc(V) species to Tc(III) by abstraction of the oxo core and coordination of monodentate phosphines or chelating phosphinothiolate ligands in the '3 + 1 + 1' and '3 + 2' systems [38,39], respectively (see Fig. 5).

The presence of the characteristic tridentate aminodithiolate ligand was not sufficient to stabilize the '3 + 1 + 1' Tc(III) system. Although the '3 + 2' configuration appears much more stable, future developments are not easily foreseen due to the complexity in setting up reliable procedures for the derivatization of the phosphinothiolate backbone. The observation that all these compounds invariably display a trigonal bipyramidal (tbp) geometry with the trigonal plane filled by thiolate donors has led to the use of a specifically tbp tailored, 'umbrella' shaped NS_3-nitrilotris(ethanethiol) ligand. In this case, all three thiolate groups coordinates on the trigonal plane, and the amine at the apex of the bipyramid affording a substitution-inert $Tc(NS_3)$ metal-fragment, to which a further monodentate phosphine or isocyanide (CNR) ligand can be added [40].

Model ^{99}Tc '4 + 1' phosphine complexes outlined in Fig. 6 are prepared from pertechnetate in the presence of H_3NS_3 and PMe_2Ph. Treatment of $^{99}Tc(NS_3)(PMe_2Ph)$ with isocyanides allows to replace labile phosphine with the relevant CNR group [41].

'Carrier free' $^{99m}Tc(NS_3)(CNR)$ complexes are instead prepared using a simpler one-step procedure starting from $[^{99m}TcO_4]^-$ with stannous chloride as reducing agent, in the presence of propylene glycol, H_3NS_3 and the relevant isocyanide. The high yield formation of the $^{99m}Tc(NS_3)(CNR)$ mixed complex obtained by contemporary addition of H_3NS_3 and CNR ligands proves that the reduction of pertechnetate is accompanied with coordination of the NS_3 chelate giving the $Tc(NS_3)$ metal-fragment, followed by ligation of the isocyanide. Otherwise, octahedral isocyanide rich complexes such as $[Tc(CNR)_6]^+$ and/or $Tc(NS_3)(CNR)_2$ would be recovered. $^{99m}Tc(NS_3)(CNR)$ compounds are stable toward transchelation reactions with glutathione. Moreover, both sides of the molecule can be conveniently functionalized to tune its hydrophilic/lipophilic character and/or to introduce biomolecules for receptor-binding activity. Hence, one carboxylic group is introduced in the NS_3 framework to increase the hydrophilicity of the system and/or for covalent connection with biomolecules via a peptidic bond. The same strategy applies to the isocyanide side [42], as illustrated in Fig. 7.

The use of EDTA and Cu(I)-isocyanide complexes as isocyanide source allow the transfer of this chemistry to 'carrier free' Re, through the formation of the ^{188}Re(III)-EDTA intermediate species, which further reacts with H_3NS_3 and isocyanides to afford substitution-inert $^{188}Re(NS_3)(CNR)$ agents designed for therapeutic purposes [43].

4.2. The Tc^{III} 'SSS' metal-fragment (Fig. 8)

As shown for the family of '4 + 1' complexes described above, thiolate sulphur atoms represent well suited donors able to stabilize the Tc(III) ion, mostly within a trigonal

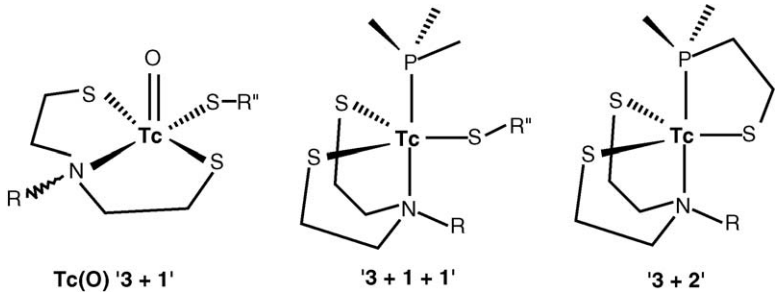

Fig. 5. From oxo-Tc(V) '3 + 1' [22] to reduced Tc(III) '3 + 1 + 1' [38] and '3 + 2' complexes [39].

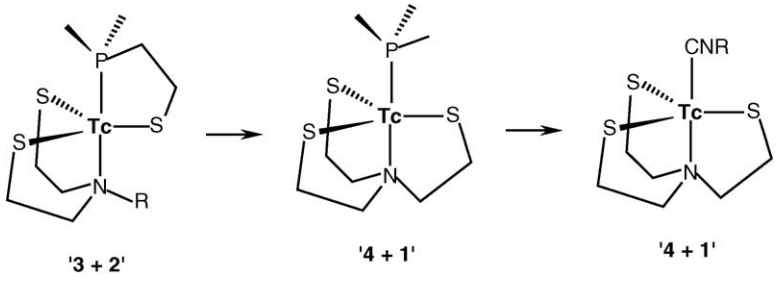

Fig. 6. From Tc(III) '3 + 2' [39] to Tc(III) '4 + 1' [40,41] complexes.

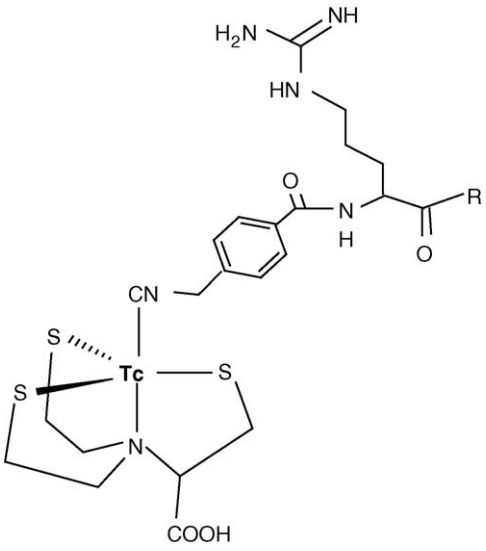

Fig. 7. Versatility of the Tc(III) '4 + 1' system: tuning of the lipophilicity onto the NS_3 framework and incorporation of a biomolecule onto the isocyanide side [42].

bipyramidal environment. Introduction of different types of sulphur donors makes accessible enlarged coordination spheres (6 versus 5) and other geometries, as illustrated by the mixed thiolate/tetrathioether, distorted octahedral complex *cis*-$[Tc(bt)_2(TTOD)]^+$ [44,45], or the mixed dithiolato/trithioether, trigonal prismatic complex $[Tc(TDT)(9S3)]^+$ [46,47] (see Fig. 9).

The mixing of thiolate and thioether sulphur donors generates different metal–S interactions (Tc–S in the 2.26–2.46 Å range) and rich redox chemistries. These features are more marked in the Tc^{III} 'SSS' system.

Reactions of dithiobenzoates $(PhCS_2)^-$ with 'carrier free' pertechnetate or perrhenate give unexpected mixed ligand complexes after metal induced oxidation of the ligand and formation of sulphur enriched trithioperoxybenzoate $(PhCS_3)^-$ adducts. As established with model ^{99}Tc and Re derivatives, the resulting six-coordinate, trigonal prismatic complexes $M^{III}(PhCS_3)_2(PhCS_2)$ contain two oxidised dithiobenzoate and

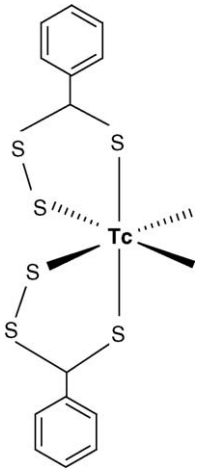

Fig. 8. The $[Tc^{III}('SSS')]^+$ metal-fragment.

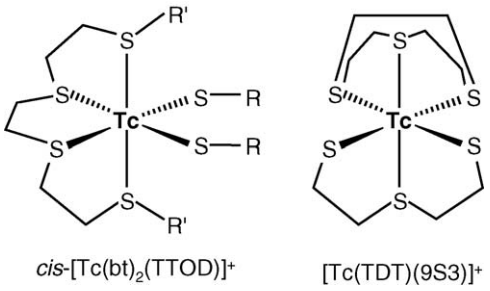

Fig. 9. Cationic 'sulphur rich' Tc(III) molecules [44,46].

one dithiobenzoate unit [48,49]. Such surprising reaction is confirmed using a series of substituted dithiobenzoates [50]. In these molecules, the whole coordination is achieved using three different types of sulphur atoms. As a consequence, the Tc–S distances cover a very wide range. For example, in the prototype complex $Tc(PhCS_3)_2(PhCS_2)$ (Fig. 10), short covalent metal–thiolato type interactions (ca. 2.23 Å), coordinative metal–thiourea type bonds (ca. 2.35 Å), and long metal–arenethiolato (ca. 2.50 Å) are observed.

The result of this ligand combination is that the two perthiobenzoate fragments are tightly bonded to the metal giving a substitution-inert $[Tc(PhCS_3)_2]^+$ metal-fragment, in which the peculiar arrangement of two trans axial π-acceptor thiourea type sulphurs and two *cis*-positioned, π-donor thiolate type sulphurs induce labilization of the third, unique dithiobenzoate ligand. Better nucleophiles such as dithiocarbamates smoothly substitute the third ligand producing another series of more stable mixed compounds of the type $Tc(PhCS_3)_2(DEDC)$ (Fig. 10).

The concomitant presence of several sulphur atoms and phenyl groups imparts a lipophilic character to these molecules, a property which can be profitably used for applications in nuclear medicine. For example, the $^{99m}Tc(PhCS_3)_2(PhCS_2)$ compound has shown high yield and selective in vitro labelling of lymphocytes in whole blood [51]. This agent is under investigation as a potential radiopharmaceutical in the differentiation of infection versus inflammation. After efficient labelling with lipiodol, the mixture containing the $^{188}Re(PhCS_3)_2(PhCS_2)$ analogue has shown elevated uptake and retention in the hepatic tissue of animal models suitable for the treatment of hepatocellular carcinoma in vivo [52].

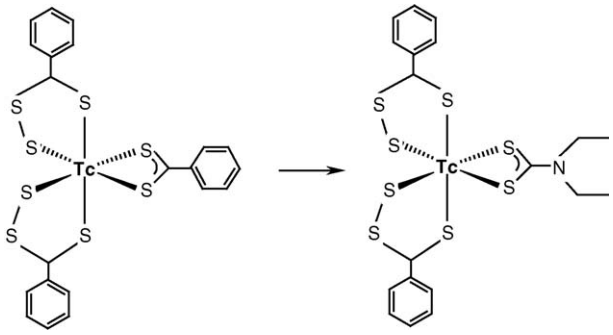

Fig. 10. Substitution of labile dithiobenzoate with dithiocarbamate in the $[Tc^{III}('SSS')]^+$ system [50].

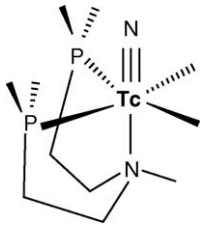

Fig. 11. The *fac*-$[Tc^{V}(N)(PNP)]^{2+}$ metal-fragment.

The opportunity to introduce a second ligand after the construction of the $[Tc(PhCS_3)_2]^+$ metal-fragment [50], opens the possibility to design bidentate ligands conjugated to appropriate biomolecules, peptides, etc. for receptor targeting. This option is still on the paper, but functionalization of dithiocarbamates for the above purposes represents a promising approach.

4.3. The metal-fragment including a core: fac-$[Tc^{V}N(PNP)]^{2+}$ (Fig. 11)

In spite of the unproductive results obtained with the mono–oxo '3 + 1' system, the idea to clamp a [Tc(E)] core (E = O, N, NR, NNR, etc.) with an appropriate chelate ligand (L) in order to stabilise a [Tc(E)(L)] synthon having additional coordination vacancies remains a promising alternative approach in the design of new Tc molecular platforms.

The extensive efforts dedicated to the mono–oxo core suggested to shift the attention on the alternative, rich class of terminal metal–nitrogen multiple bonds. Among these cores, the simplest [Tc to N] group was considered a good starting point for several reasons: (a) studies devoted to the elucidation of the molecular structure of nitrido containing technetium compounds were and still remain rare [6–9], (b) nitrido Tc-species reveal a strong reluctance to undergo redox processes [53], as it is observed in some cases with oxo-complexes, and (c) an efficient method for the synthesis of [^{99m}Tc≡N] was recently introduced [54].

In this connection, a very recent survey by Duatti and coworkers summarises the development of ^{99m}Tc and ^{188}Re radiopharmaceuticals containing a terminal metal–nitrido multiple bond for diagnosis and therapy [12]. Among the agents under evaluation as potential radiopharmaceuticals, the cationic $[TcN(DBODC)(PNP5)]^+$ represents a significant example of the use of the metal-fragment strategy. As outlined in Fig. 12, this mixed complex is composed by a substitution-inert *fac*-$[TcN(PNP)]^{2+}$ moiety to which a bidentate dithiocarbamate ligand is attached.

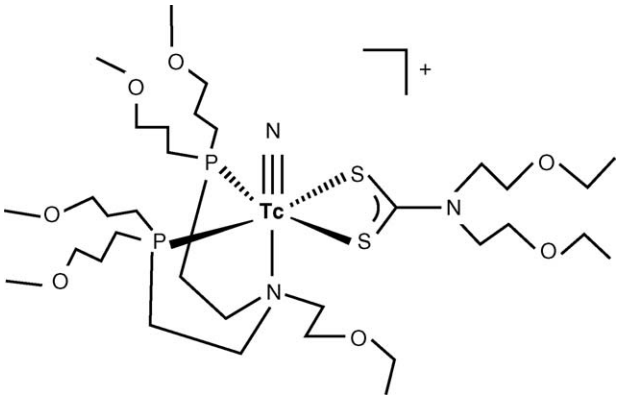

Fig. 12. The molecular structure of *fac*-$[TcN(DBODC)(PNP5)]^+$ [61].

It has to be pointed out that this compound shows a rather unusual pseudo-octahedral geometry along with a reciprocal *cis*-arrangement of diphosphine phosphorus [55]. Both evidences are somewhat in contrast with the behaviour usually exhibited by nitrido containing compounds, which commonly assume five-coordinated (either square-pyramidal or trigonal-bipyramidal) arrangements and place P donors, where available, in a reciprocal *trans* configuration [12].

In the $[TcN(DBODC)(PNP5)]^+$ complex, the mixing of π-donor S atoms with π-acceptor P atoms ensures an appropriate electronic balance around the [Tc≡N] core and this event contributes to the overall robustness of the system, which is further stabilised by the additional metal–N contact arising from the diphosphine heteroatom. The presence of this heteroatom and the length of the diphosphine bridging chain are crucial factors [56] in determining both the stereochemistry of the metal-fragment and its substitution chemistry, as it appears from the following analysis.

Treatment of labile $[TcNCl_4]^-$ or $[TcNCl_2(PPh_3)_2]$ precursors with diphosphines incorporating only methylene groups in the chain ($R_2P(CH_2)_nPR_2$), give rise to bis-substituted diphosphine complexes (e.g. $[TcNCl(dmpe)_2]^+$; Fig. 13(a)) if a short chain (n = 2,3) is comprised between the P donors [57], but afford intractable oily mixtures, from which the isolation of pure and defined species is not possible, when longer chains (n = 5,6) are utilized [58]. On the contrary, the insertion of an heteroatom

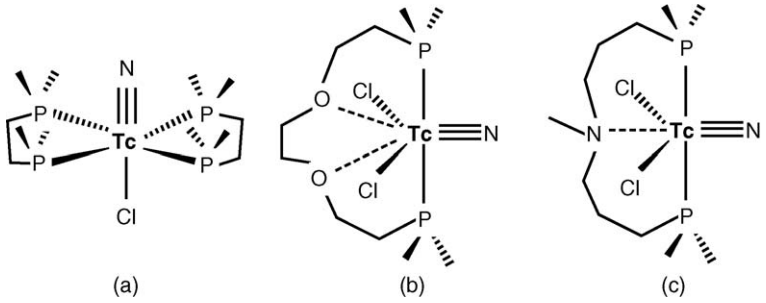

Fig. 13. Molecular structures of nitrido complexes containing diphosphines with different bridging chains [57,58].

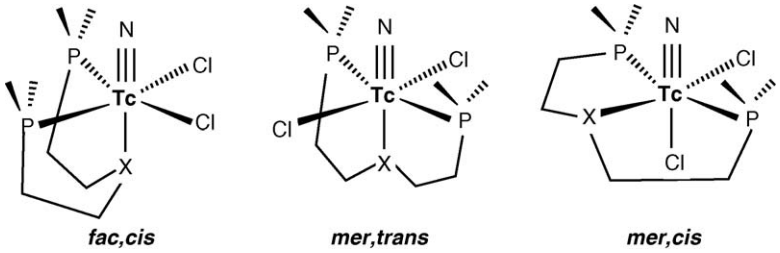

Fig. 14. Stereochemistries exhibited by nitrido compounds containing tridentate PXP-heterodiphosphines [56].

in the diphosphine bridge allows the isolation of stable mono-substituted diphosphine complexes, as sketched in Fig. 13(b) and (c).

Diphosphines with more than five atoms (including the heteroatom) in the bridging chain display the expected tendency of nitrido containing species to place the P donors in a reciprocal *trans* configuration (Fig. 13(b) and (c)) [59], the PXP ligand thereby adopting a meridional configuration. Much less straightforward is the behaviour shown by diphosphines incorporating only five members in the chain. In this case three stereochemical arrangements are achievable: *fac*, *cis*; *mer*, *trans*; *mer*, *cis* (Fig. 14).

The choice of the arrangement depends primarily on the nature of the diphosphine heteroatom X, and often isomeric equilibria are observed in the solution state. Hence, when X = O the formation of *mer*,*trans*-[$TcNCl_2$(POP)] is favoured [59], when X = NH *mer*,*cis*-[$TcNCl_2$(PN(H)P)] complex is collected [56], and when X = S or N(R) *fac*,*cis*-[$TcNCl_2$(PXP)] compounds are preferentially formed [55,56]. Only *fac*,*cis*-[$TcNCl_2$(PXP)] species undergo exchange reactions, by substitution of electron depleted and geometrically prone halide donors, with bidentate nucleophiles.

The facial–meridional isomerization of [$TcNCl_2$(POP)] and [$TcNCl_2$(PN(R)P)] compounds was investigated by means of DFT calculations [55]. These studies, while confirming that the *mer*,*trans* arrangement is energetically favoured for the POP complex, indicate that the facial PN(R)P coordination shown by [$TcNCl_2$(PN(R)P)] grants the minimization of the steric constraints of the amino-diphosphine bridging chain and increases the efficiency of the N–metal interaction. In addition, the HOMO of the *fac*,*cis*-[$TcNCl_2$(PN(R)P)] complex has a large participation of the Cl p states, whereas no significant contribution from the P orbitals is present. This means that the Tc–Cl bonds should be more labile than the Tc–P ones. This statement is in complete agreement with the synthetic evidence that halides are easily substituted, whereas [TcN(PN(R)P)] remains untouched.

'Carrier free' preparation of mixed [^{99m}TcN(DTC)(PN(R)P)]$^+$ species is performed by simultaneous mixing of the relevant aminodiphosphine and dithiocarbamate to a mixture containing the pre-reduced [^{99m}TcN] group [60]. The quantitative formation of the [^{99m}TcN(DTC)(PN(R)P)]$^+$ mixed complex without detectable formation of the corresponding symmetric bis-substituted [^{99m}TcN$(DTC)_2$] and/or [^{99m}TcN$(PN(R)P)_2]^{2+}$ allows to delineate a possible reaction pathway starting from generator-eluted pertechnetate.

Generation of the [^{99m}TcN] core is performed by action of dihydrazine onto the pertechnetate ion [12]. Since the nitride ligand is one of the strongest known donor system, the Tc≡N group becomes electron-rich and efficiently (kinetically) reacts with ligands having π-acceptor atoms (PN(R)P) to delocalise its electron density via P p orbitals. The peculiar steric (five atoms in the diphosphine chain) and electronic (lone pair available at the N donor) properties of the PN(R)P ligand dictates the facial coordination, leaving two *cis*-positioned labile sites, geometrically prone for substitution. The *fac*-[^{99m}TcN(PN(R)P)]$^{2+}$ moiety is now a strong electrophilic metal-fragment which selectively reacts with mono-anionic and di-anionic bidentate ligands having soft π-donor coordinating atoms to afford mixed complexes of the type [^{99m}TcN(DTC)(PN(R)P)]$^+$. Bidentate ligands include [S,S]$^-$ dithiocarbamates [61], [S^-,S^-] dithiolates [62], [NH_2,S^-] or alternatively [O^-,S^-] cysteine derivatives [55,63,64]. This peculiar chemistry promotes high specific activity labelling procedures. In fact, the cysteine bifunctional chelating system containing [NH_2,S^-] and [O^-,S^-] donors can coordinate the [^{99m}TcN(PN(R)P)]$^{2+}$ metal-fragment with a specific activity up to 70 GBq/μmol, thus representing suitable BFCA for the labelling of peptides and biomolecules (e.g. biotin, 2-MPP (2-methoxyphenylpiperazine)) [63,64].

The presence of the chiral alpha aminoacid C in the cysteine backbone determines the formation of two diastereoisomers upon coordination onto the [TcN(PN(R)P)] moiety, depending on the orientation (syn or anti) of the cysteine pendant group towards the Tc≡N unit. In the case of O,S-cysteine biotinylated or 2-MPP compounds the relative abundance of the two forms is typically 15:85 at 'carrier free' level and 40:60 at macroscopic level for the syn:anti isomers, respectively. The anti-isomer is invariably the thermodynamically more favoured species, and contemporary exhibits the highest affinity to the receptor site [63]. Both the length and type of the spacer incorporated in the bifunctional biotinylated cysteine ligand has a major influence on the retention of the affinity of the biotinylated agent for avidin. For example, when the biotin carboxylic function is directly conjugated to the cysteine amine group, almost complete loss of affinity of the biotinylated agent (Fig. 15(a)) is observed, indicating that the metal-fragment strongly affects the biotin avidin interaction. As illustrated in Fig. 15(b), only the insertion of aliphatic spacers including a sequence of five members keeps biotin sufficiently away from the metal centre, thereby allowing retention of the bioactivity [65]. This evidence indicates that the choice of the spacer plays a crucial role in determining the bioaffinity of any molecular fragment appended

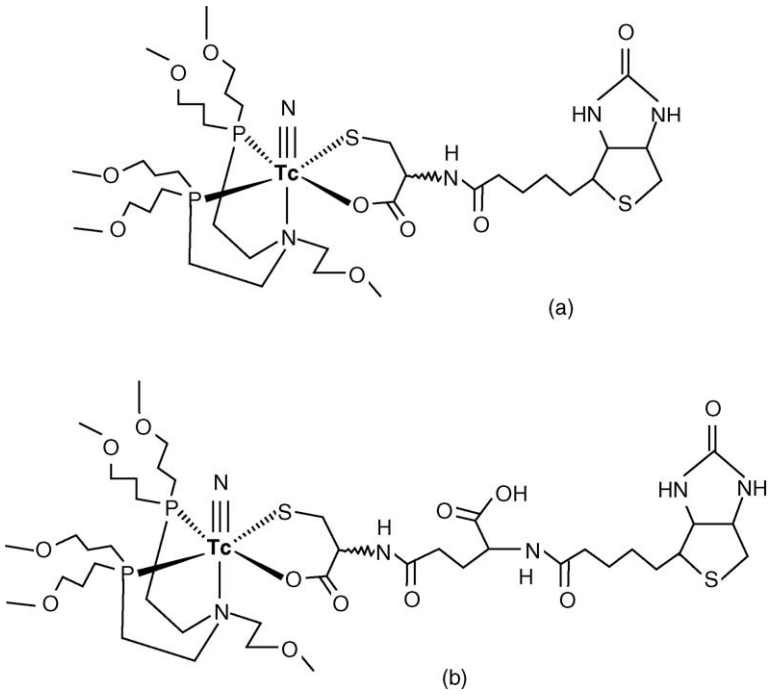

Fig. 15. Molecular structure of TcN(PNP)(O,S-cys-biotin) (a) and TcN(PNP)(O,S-cys-glu-biotin) (b) [65].

to O,S-cysteine based BFCA coupled to the $[TcN(PNP)]^{2+}$ metal-fragment.

In addition, nitrido containing mixed species are extremely flexible in the sense that the hydrophilic/lipophilic balance of the overall molecule can be controlled by varying not only the nature of the 'second' ligand, as in the case of the $[Tc(CO)_3]$ moiety, but also the nature of the aminodiphosphine substituents both at distal P and at central N donors.

4.4. Transfer of the metal-fragment approach to other cores

The encouraging results obtained in the case of the *fac*-$[TcN(PN(R)P)]^{2+}$ moiety prompted us to apply a similar metal-fragment strategy to other cores. Since each core possesses distinct electronic and steric requirements, direct transfer of the above detailed nitrido chemistry, for example, to mono–oxo or mono–imido species is not predictable. According to recent investigations in the field of density-functional theory (DFT) [66], quantum chemistry permits inorganic chemists to predict and explain the properties of transition–metal complexes at the ab initio level.

Consequently, DFT studies on a series of labile precursors containing the nitrido, imido and oxo technetium cores have been performed in order to gain information on the stability of the complexes as a function of the core and, eventually, to achieve deeper insight on their substitution chemistry.

The metal-based energy levels of a series of complexes, as obtained from DFT calculations using the ADF code and the

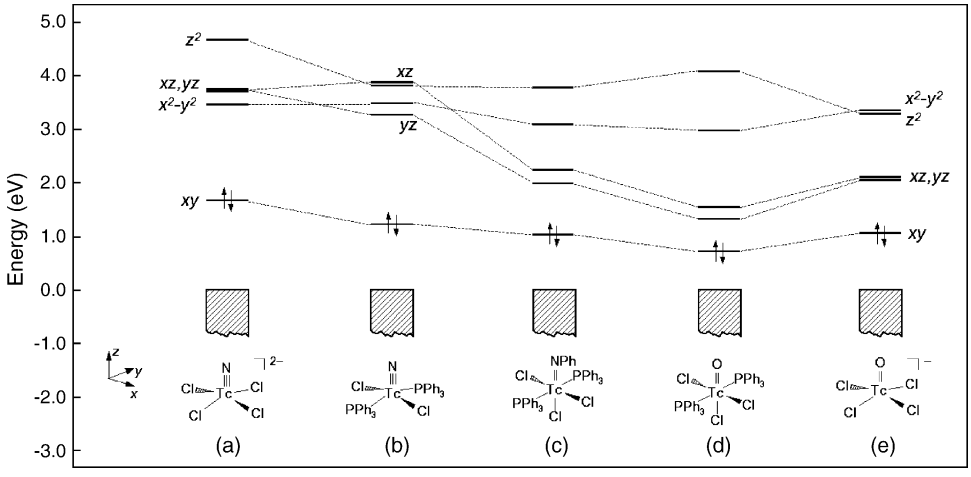

Fig. 16. One-electron levels of the investigated series of Tc complexes. The boxes indicate the energy region spanned by the ligand-based non-bonding and antibonding combinations.

BLYP functional [67] is reported in Fig. 16. The complexes have been oriented in order to (approximately) keep the Tc–P bonds in the *yz* plane. The energy scales have been shifted to align the levels of the highest occupied ligand-localized molecular orbital (dashed boxes in Fig. 16). This is always found to be a non-bonding combination centered on the chlorine atoms.

An inspection of Fig. 16 shows that whereas for all the complexes the highest occupied MO (HOMO) is the antibonding d_{xy} the energy ordering of the other empty d^* orbitals depends on the particular complex. Furthermore, the lowest unoccupied MO (LUMO) levels span a much wider energy range with respect to the HOMO ones. This makes the HOMO–LUMO gap change considerably along the series of complexes. The factors which tune this gap, which ultimately drive the reactivity of these precursors complexes, are here analysed.

(i) *Strength of the apical Tc–E multiple bond*: substantially, this factor consists in the replacement of a formal Tc≡E triple bond with a formal Tc=E double bond, viz. (a) → (e). The effect of a weaker Tc–E π-bond is the stabilization of the metal-based orbitals representing the antibonding partners of that interaction, i.e. d_{xz} and d_{yz}.

(ii) *Presence of π-acid ligands (PPh3)*: when Cl^- is replaced by PPh_3, viz. (a) → (b), the d_π levels are split, as a consequence of the π-acid properties of the PPh_3 ligand, which acts on d_{yz}, but not on d_{xz}. This is evident by inspecting the shapes of the d_{yz} reported in Fig. 17. The net effect is a marked stabilization of the d_{yz} level on passing from $[TcNCl_4]^{2-}$ to $TcNCl_2(PPh_3)_2$.

(iii) *Number of ligands*: the introduction of the axial Cl^- ligand, viz. (e) → (d), causes a structural change from a square-pyramidal to an octahedral arrangement, and has a two-fold effect. First there is the obvious *destabilization* of the d_{z^2} orbital (directly involved with the new bond). Then, there is a *stabilization* of the d_{xz} and d_{yz} orbitals.

Because the d_{yz} orbital is the LUMO in all the small-gap complexes, and its energy is mainly influenced by the first two of the above described factors, we can conclude that the *reactivity* is mainly enhanced by weakening the bond with the apical ligand and by introducing π-acid ligands in the basal/equatorial plane.

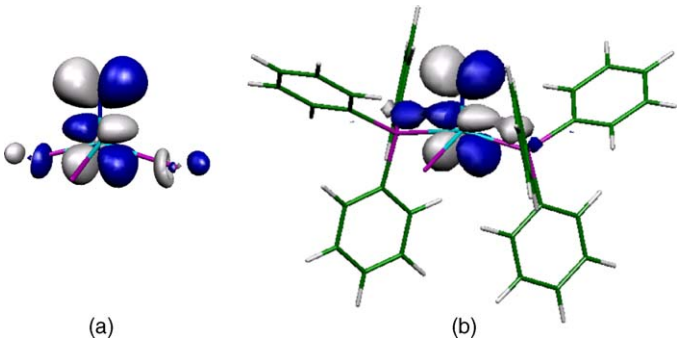

Fig. 17. Isodensity maps of the d_{yz} antibonding components for: (a) $[TcNCl_4]^{2-}$ and (b) $TcNCl_2(PPh_3)$.

Moving now to a more general comparison between the complexes, it is interesting to note that the nature of the frontier orbitals of $TcNCl_2(PPh_3)_2$ and $Tc(NPh)Cl_3(PPh_3)_2$ is the same, but the electronic structures of these complexes, and notably the HOMO–LUMO gaps, are rather dissimilar.

It is also apparent that $TcOCl_3(PPh_3)_2$ has the smallest HOMO–LUMO gap (∼0.6 eV). This indicates high reactivity of $TcOCl_3(PPh_3)_2$, in particular towards nucleophiles (see the low LUMO energy), thus explaining why all the efforts to synthesize this complex have failed so far. Further comparison of the electronic structures of $Tc(NPh)Cl_3(PPh_3)_2$ and $TcOCl_3(PPh_3)_2$ reveals that the two compounds exhibit qualitative similarity, in tune with the fact that they are identical on the basis of the factors indicated above. However, $TcOCl_3(PPh_3)_2$ displays a *less* stable d_{z^2} and more stable d_{xz}, d_{yz} orbitals. This indicates that the phenylimido group is a worse σ-donor and a better π-donor compared with the oxo one, and it ultimately suggests that the Tc=NPh bond has a partial triple bond character. This interpretation is corroborated by the value of the Tc–N–C angle (optimized 175.9° versus experimental 171.6°), and it is in agreement with well-known literature evidence [68].

4.4.1. The $[Tc^V(NPh)(PNP)]^{3+}$ metal-fragment (Fig. 18)

According to the identical nature exhibited by frontier orbitals of $TcNCl_2(PPh_3)_2$ and $Tc(NPh)Cl_3(PPh_3)_2$ precursors we decided to use the same PNP framework to explore the possibility for the obtainment of a phenylimido containing metal-fragment. Slight, but significant variations in the route of formation of a substitution-inert metal-fragment were observed, compared to the straightforward preparation of the nitride containing *fac*-$[TcN(PN(R)P)]^{2+}$ moiety. Such a different reactivity is likely in agreement with the diverse HOMO–LUMO gaps observed in $TcNCl_2(PPh_3)_2$ and $Tc(NPh)Cl_3(PPh_3)_2$ precursors. In the phenylimido case, more than one PNP stereochemistries were observed. *Mer*,*cis* is the preferred configuration, as established by the X-ray crystal structure determination of prototype complexes $[Tc(NPh)Cl_2(PN(H)P)]^+$ and $[Re(NPh)Cl_2(PN(Me)P)]^+$ in the solid state [69]. This form is in equilibrium with the *fac*,*cis*-form in solution. This equilibrium is the key step for further substitution of halides with bidentate ligands because only *fac*,*cis*-arrangements promote the formation mixed-chelate phenylimido complexes of the type *fac*,*cis*-$[M(NPh)(O,O)(PN(R)P)]^+$ (O,O = 1,2-ethaneglycolate, cathecolate). Another point which deserves attention is the nature of the donor atoms of the bidentate ligand. While *fac*-$[M(N)(PN(R)P)]^{2+}$ are stabilised mostly by π-donor S, the corresponding *fac*-$[M(NPh)(PN(R)P)]^{3+}$ moiety prefer harder O-based donors, in accordance with the higher hard character of the $[M(NPh)]^{3+}$ core compared to that exhibited by the $[M(N)]^{2+}$ one.

Synthesis of mixed phenylimido complexes of the type *fac*,*cis*-$[M(NPh)(O,O)(PN(R)P)]^+$ can be performed using a one-step procedure starting from $M(NPh)Cl_3(PPh_3)_2$ at room temperature. Since the 'non-carrier added' synthesis of $^{188}Re(NPh)Cl_3(PPh_3)_2$ is already known from the literature [70], this method might constitute a good start for the preparation of stable ^{188}Re mixed-ligand compounds. Derivatives containing the $[M(NPh)(PNP)]^{3+}$ moiety are, in principle, extremely

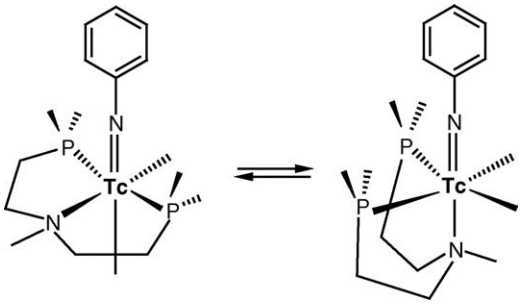

Fig. 18. The $[Tc^{V}(NPh)(PNP)]^{3+}$ metal-fragment. Equilibrium between *mer*,*cis*- and *fac*,*cis*-isomers [69].

flexible. In fact, the introduction of biologically active fragments onto the O,O-glycolate or O,O-cathecolate frameworks or onto *para*-substituted phenylhydrazines [71] are feasible, thus constituting the basis for the design of target-specific radiopharmaceuticals.

4.4.2. A metal-fragment including the oxo core?

Scrutiny of the molecular orbital splitting diagram of isoelectronic d^2 systems $[TcNCl_4]^{2-}$ and $[TcOCl_4]^{-}$ extended to ligand-localised MOs [72] reveals that the mono–oxo core gives rise to MO which reside at lower energy compared to the corresponding ones in nitrido species. In particular, the HOMO–LUMO energy gap is markedly reduced in the oxo complex, indicating that filling of the LUMO orbital with consequent reduction to d^4 Tc(III) species in the presence of nucleophilic agents is strongly favoured for oxo-complexes. This statement, entirely based on theoretical evidences, is in perfect agreement with the observation that oxo-Tc(V) are reduced to Tc(III) species in the presence of tertiary phosphines [73], through abstraction of the oxo group and formation of the corresponding phosphineoxide.

On these bases, formation of metal-fragments of the type $[TcO(PNP)]^{3+}$, which resemble those just described for isoelectronic nitrido and phenylimido species, appears to be quite unlikely. In fact, treatment of $[TcOCl_4]^{-}$ or $[TcO_4]^{-}$ precursors with PNP ligands proceed through abstraction of the oxo group(s) and isolation of low valent Tc(III) complexes of the type *fac*-$[TcCl_3(PNP)]$ and/or *mer*-$[TcCl_3(PNP)]$, depending on the length of the chain interposed between the P donors [74].

The observation that low energy molecular orbitals (those predominantly halide-localised) are shifted toward lower energies in the $[TcOCl_4]^{-}$ complex compared to the corresponding orbitals in $[TcNCl_4]^{2-}$, suggests one should use different donor

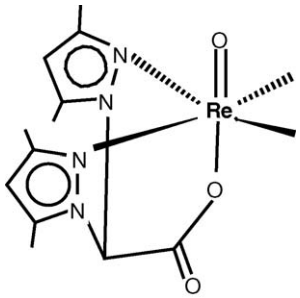

Fig. 19. The $[Re^{V}(O)(NON)]^{2+}$ metal-fragment.

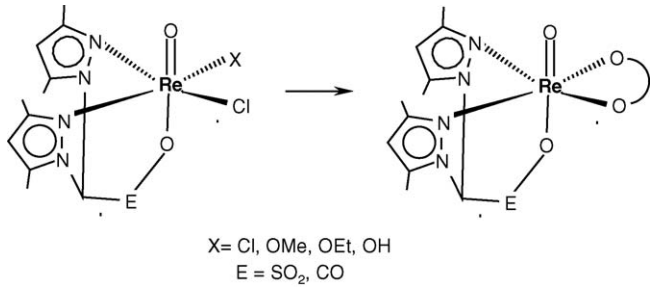

Fig. 20. Reactivity of the $[Re^{V}(O)(NON)]^{2+}$ metal-fragment [76].

atoms to stabilise the mono–oxo core. Inspection of the frequency of occurrence of various donor atoms in structurally authenticated mono–oxo technetium complexes [8,9] offers a comprehensive view of the system, and indicates that oxygen is the preferred donor for the site *trans* to the mono–oxo group, and nitrogen, followed by sulphur and oxygen, are the preferred donors for the equatorial sites.

Heteroscorpionates such as polypyrazolylacetate and polypyrazolylsulphonate ligands [75] possess proper stereochemical arrangement to fill facially the coordination sphere of the mono–oxo core and a suitable NON donor atom set to match the requirements suggested above (Fig. 19).

Coordination chemistry with ^{99}Tc is under progress, whereas model rhenium compounds are already available [76]. In these Re(O)(NON)(X)Cl intermediate compounds (see Fig. 20), facial κ^3-NON coordination is confirmed in a series of distorted octahedral species in which the acetate (or sulphonate) group is located *trans* to the Re=O linkage and the pyrazolyl nitrogens accommodate on the equatorial plane. Halide(s) and or alcoholate groups complete the octahedral sphere.

The latter monodentate groups are exchanged with bidentate glycolate only when substituted 3,5-methylpyrazolyl rings are inserted in the scorpionate framework. The increasing donor ability of the 3,5-dimethylpyrazolyl nitrogens compared to that of the unsubstituted pyrazols provides the correct electronic balance to enhance the overall stability of the facially arranged $[Re(O)(NON)]^{2+}$ moiety and, at the same time, contributes to the labilization of *trans* coordinated halide or alcoholate groups. The nature of the scorpionate tail (either acetate or sulphonate) has a negligible effect on the reactivity and on the stereochemistry of the substitution processes. However, carboxylate containing heteroscorpionates usually give cleaner reactions [76].

5. Conclusion and perspectives

Chemical platforms based on the construction of substitution-inert metal-fragments suitable for the development of new ^{99m}Tc radiopharmaceuticals have been summarised. The synthetic strategy involves, in the first step, clamping of the metal, either a naked Tc(III) ion or a Tc(V) = E unit (E = N, NPh, O) including a distinctive terminal group, with an appropriately arranged polydentate ligand. Peculiar stereochemical arrangements dictated by the denticity of the ligand ensure both the substitution inertness of the metal-fragment and the contemporary labilization of the remaining groups, which are selectively

exchanged with a second, different chelate ligand in the final step. This multi-step reaction pathway allows control of the hydrophilic/lipophilic balance of the tracer by incorporation of various substituents at the donor atoms of the ancillary ligand, and conjugation of biomolecules into the second chelate ligand. This approach has been successfully applied to low valent Tc(III) systems via formation of the [Tc(NS_3)] and [Tc 'SSS'] metal-fragments and to high valent nitrido and phenylimido containing [TcN(PNP)] and [Tc(NPh)(PNP)] moieties. Extension of this approach to the oxo core requires substitution of PNP-aminodiphosphines ancillary ligands with harder NON-bis-pyrazolylacetate ligands. Application of the same concept to additional metal-fragments containing Tc nitrogen multiple bonds, e.g. linear diazenido, chelated hydrazido, isodiazene may be feasible by controlling the nature of the donor atoms of the ancillary co-ligand. This might be useful to improve knowledge on the coordination chemistry of related HYNIC containing compounds, the coordination chemistry of which is still poorly understood.

Application of the metal-fragment technology could be extended, in principle, to other, selected radioactive isotopes (e.g. rhenium, copper, rhodium, gold, etc.) for which basic coordination chemistry studies have already demonstrated the possibility to generate mixed coordination spheres.

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