Synthesis and Characterisation of [Re(CO)$_3$(SS)(P)] Complexes: A [2+1] Concept for ^{99m}Tc- and ^{188}Re-Radiopharmaceutical Applications

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A new (SS)(P) coordination set based on the concurrent use of a dithiocarbamate and a functionalised phosphane is reported. The (SS)(P) fashion is achieved by applying the [2+1] mixed-ligand concept and makes it possible to obtain stable rhenium tricarbonyl complexes without employing tripodal bifunctional chelating agents (BFCAs). The coordination chemistry of the simple sodium *N*,*N*-dimethyldithiocarbamate and new functionalised phosphanes **L1**–**L3** [**L1** = $Ph_2PCH_2CH_2C(O)OCH_3$, **L2** = $Ph_2PCH_2CH_2C(O)NHCH_2C(O)OCH_3$, **L3** = $Ph_2PCH_2CH_2C(O)NHCH_2CH_2C(O)OCH_3$] was studied by reaction with the rhenium complex $[NEt_4]_2[fac\text{-}Re(CO_3)Br_3]$. The rhenium tricarbonyl core was found to be stabilised by the SS chelation of dithiocarbamate and by the P coordination of the phosphane. Two synthetic pathways leading to the complexes [*fac*-Re(CO)$_3$(L)(MDTC)] (L = **L1**, **L2**, **L3**; MDTC = *N*,*N*-dimethyldithiocarbamate) are described. The structural characterisation of the isolated complexes by spectroscopic methods is reported, including X-ray crystallographic analysis for rhenium complexes with ligands **L1** and **L3**. The homologous Tc-99m compounds with the same (SS)(P) coordination set were prepared at tracer level with the **L2** and **L3** ligands, and characterised by HPLC methods.

Introduction

The most important goal of radiopharmacy is the application of radiolabelled compounds in medicine for non-invasive diagnostic and therapeutic purposes. Technetium-99m is a γ-emitter with optimal characteristics that is widely employed in nuclear medicine for imaging and is readily available as pertechnetate from the ^{99}Mo/^{99m}Tc generator.[1] Rhenium-188 appears to be promising as a candidate for the treatment of neoplasia for its β^- emission and is available as perrhenate from a commercially available ^{188}W/^{188}Re generator, proposed by Oak Ridge National Laboratory (USA).[2]

For the development of site-directed diagnostic and therapeutic radiopharmaceuticals, a biomolecule (hormones, peptides) with high affinity for a receptor target should be labelled to deliver the radionuclide in a specific body region.[3] However, labelling a biomolecule might give an important alteration of its biological properties due to the link with transition metals such as Tc or Re. Hence, the most successfully employed procedure at present is the "bifunctional approach". This consists of a chelating system (bifunctional chelating agent, BFCA) that is able to stabilise and keep the metal far from the target molecule.[4]

In recent years, Tc and Re radiopharmaceutical chemistry with the cation *fac*-$[M(H_2O)_3(CO)_3]^+$ has been intensely stimulated by the fundamental contributions of Alberto, Schibli and Schubiger with the development of novel and simple methods for preparing the aqua ions *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ and *fac*-$[^{188}Re(H_2O)_3(CO)_3]^+$.[5] The interest in the design of radiopharmaceuticals with carbonyl ligands results from their high thermodynamic stability, small size, kinetic inertia and in vivo stability.[6] Furthermore, the $[M(CO)_3]^+$ moiety allows the use of a wide range of ligand systems, therefore many different chelating sets have been tested.[7]

In this context, several research groups are currently looking for an "ideal building block" with an optimised in vitro and in vivo behaviour. In the last few years, these complexation studies have been principally focused on the development of tridentate BFCAs to obtain a tripodal mode of coordination (Scheme 1). Nevertheless, tricoordination can also be achieved by using a mixture of two ligands, one bidentate L_2 and one monodentate L_1 to produce an $[M(L_2)(L_1\text{-Biomol})(CO)_3]$ complex by means of a [2+1] mixed-ligand approach (Scheme 1).[8] A recent paper has reported the use of this "[2+1]" approach to prepare new *fac*-M(CO)$_3$ compounds for radiopharmaceutical use from a dithiocarbamate and a monodentate isocyanide ligand.[9]

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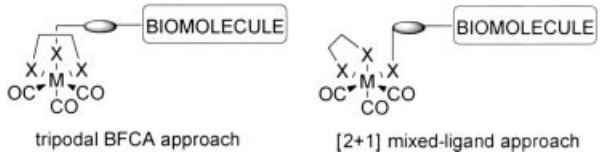


Scheme 1. Two different approaches for the stabilisation of a tricarbonylrhenium or -technetium core (M = Re/Tc).

Here, we report an investigation where we propose a suitable chelating system for the stabilisation of the *fac*-[M(CO)$_3$]$^+$ moiety by the [2+1] mixed-ligand approach, based on the simple *N*,*N*-dimethyldithiocarbamate (MDTC) as bidentate ligand and a functionalised phosphane as monodentate ligand. The phosphane acts as a bifunctional agent because it contains an anchor group that is useful for the conjugation with the biological vector, while MDTC acts as a co-ligand as it is able to neutralise and stabilise the metal system.

Phosphane ligands are well known for their capability to form very stable and inert bonds with organometallic fragments. Succinimidyl 3-diphenylphosphanylpropionate was selected as the BFCA because it can be simultaneously linked to a metal centre and to a biomolecule (Scheme 2).[10,11]

To evaluate the viability of this new [2+1] mixed-ligand approach we synthesised phosphane derivatives with aminoacid methyl esters following the above reaction. These ligands can simulate the presence of a conjugated targeting biomolecule and test the potential application of the new [2+1] mixed-ligand system in the presence of a biomolecule anchored to the monodentate ligand.

The coordination chemistry of the phosphane derivatives and sodium MDTC was studied by reaction with "cold" [NEt$_4$]$_2$[Re(CO$_3$)Br$_3$].[12] Rhenium complexes are structural models of technetium complexes due to the lanthanide contraction that ensures that the analogous complexes display very similar coordination parameters and physical properties.[13]

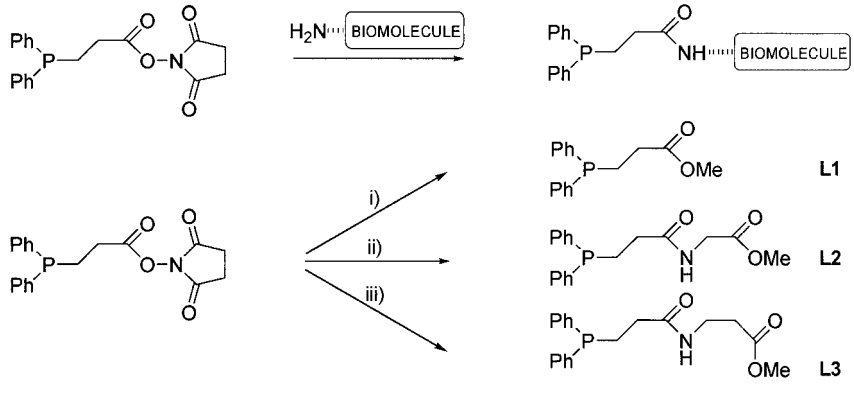
This study presents the syntheses and structural characterisation of three phosphane ligands and the [2+1] rhenium complexes obtained by combining the prepared phosphanes and sodium MDTC with the [Re(CO)$_3$]$^+$ moiety.

Results and Discussion

Synthesis of the Ligands

The synthesis and characterisation of the phosphane ester ligand **L1** has been reported previously.[14,15] We obtained the same compound using an alternative preparation starting from succinimidyl 3-diphenylphosphanylpropionate. The new functionalised phosphanes **L2** and **L3** were prepared by reaction of the same activated ester with the amino acid derivatives glycine methyl ester (**L2**) and β-alanine methyl ester (**L3**; Scheme 2) using modifications of the published procedures.[10,16] All these results are consistent with the ability of succinimidyl 3-diphenylphosphanylpropionate to act as a synthon for preparing phosphane-derivatised compounds. All ligands were obtained in good yields (70–75%) after purification by flash column chromatography as pale-yellow pure oils (air-sensitive compounds) and were characterised by NMR (^{1}H, ^{13}C, ^{31}P) and FT-IR spectroscopy and ESI mass spectrometry.

The collected data are consistent with the proposed structures and can be compared with the characterisation reported for similar ligands.[11,16] The ^{31}P NMR spectra of **L1**–**L3** show a sole singlet in the range δ = –13.9/–14.4 ppm characteristic of a ligand containing a diphenylphosphane group. The formation of the amide bond in **L2** and **L3** is evidenced by the IR spectra, which show a strong band near 1650 cm^{-1}, and by the proton signals of the amide group observed at δ = 6.12 (**L2**) and 6.03 ppm (**L3**) in the ^{1}H NMR spectra. The presence of a methyl ester group in **L1**–**L3** was evidenced in the IR spectra by a strong absorption between 1738–1752 cm^{-1} and by a singlet in the ^{1}H NMR spectra at around δ = 3.65 ppm. The ^{1}H and ^{13}C NMR



Scheme 2. General syntheses for biomolecule-functionalised phosphanes and synthesis of the phosphane ligands **L1**–**L3**.

spectra display the signals of all organic groups involved in ligands **L1**–**L3**. Finally, the identities of **L1**–**L3** were confirmed by the positive ion ESI mass spectra, which display an intense signal assigned to the $[M + H]^+$ cation.

Complexation Studies

The great capability of phosphorus(III) to form stable complexes with transition metals in low oxidation states is well known, and coordination studies employing phosphanes with the Re^I tricarbonyl core have been carried out and reported in the literature.[17–20] Benak et al. have demonstrated that the reaction between $[Re(CO)_5Cl]$ and a diphenylphosphane ester affords the mononuclear tricarbonyl complexes *fac*-$[Re(CO)_3ClL_2]$ (L = phosphane) with the metal centre stabilised by the two phosphorus ligands.[18] Santos et al. have explored the coordination ability of tridentate hetero-functionalised diphenylphosphanes and obtained mainly PO or PN bicoordinated complexes.[19,20] In both cases tricoordination is generally not achieved, and the last position of the metal coordination sphere is occupied by a halogen. Furthermore, the formation of different isomers is often observed when bidentate ligands are employed.[21]

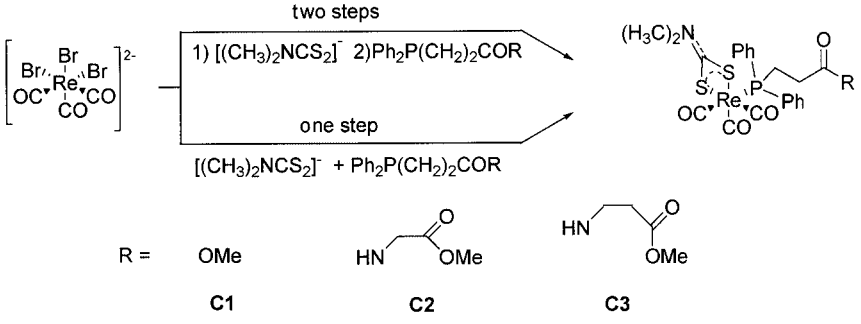
The use of sodium MDTC and diphenylphosphane in the [2+1] strategy has the potential advantage of producing monomeric and neutral tricoordinate complexes. Only one phosphane coordinates to rhenium in a monodentate fashion to produce minimal steric impact between the aliphatic chain and the metal sphere.

The rhenium(I) tricarbonyl complexes **C1**–**C3** were prepared in two different ways, as shown in Scheme 3. In the two-step pathway, or "indirect synthesis", complexes **C1**–**C3** were formed from an intermediate dithiocarbamate complex. This complex was synthesised in the first step by simple addition of sodium MDTC to a solution of the rhenium tricarbonyl precursor $[Re(CO)_3Br_3]^{2-}$ in methanol. The resulting product was not characterised; however, we suppose that a neutral soluble monomeric SS-bicoordinated species is formed, as reported by Gorshkov et al. for similar dithiocarbamates with the $[Re(CO)_3]^+$ moiety.[9] The simple substitution of a coordinated methanol by a monodentate phosphane ligand (**L1**–**L3**) blocks the coordination vacancy and leads to the target complexes (**C1**–**C3**) in good yields after purification by flash chromatography. The solid compounds are remarkably inert and stable to air and moisture, even in solution (organic solvents). No decomposition was observed after many weeks.

The presence of the *fac*-$[Re(CO)_3]$ fragment in complexes **C1**–**C3** was revealed by the three characteristic CO stretching bands (**C1**: 2016, 1921 and 1890 cm^{-1}) observed in the IR spectra.[18–21] This technique also supplies useful information about the coordination of the dithiocarbamate ligand, since a single, strong band at 1522–1526 cm^{-1} was observed, which was assigned to the C–N stretching of a symmetrical bidentate dithiocarbamate.[22] The C=O stretching vibrations of the ester and amide groups are similar to those of the free ligand, and were accorded to the exclusion of these groups from the participation in the metal sphere.

The ^{31}P NMR spectra of complexes **C1**–**C3** show the complete absence of the corresponding free ligand, and display a sole signal in the range δ = 5–8 ppm, thus confirming the absence of isomers. A significant downfield shift with respect to the resonance of the free ligands (δ = –14 to –16 ppm) was observed, in agreement with the formation of a phosphorus–metal bond. The coordination of the phosphane ligand is also evidenced by the ^{13}C NMR spectra, which show an important increase of the $^1J_{P,C}$ and $^2J_{P,C}$ couplings of the methylene groups with respect to the values observed in the free ligands. These data are in accordance with literature reports.[11,17,18] Furthermore, the simultaneous presence of MDTC and phosphane ligands with the rhenium tricarbonyl core is also consistent with the ^1H NMR, ^{13}C NMR and ESI-MS data, supporting the (SS)(P) coordination to the fragment *fac*-$[Re(CO)_3]^+$.

Single crystals of complexes **C1** and **C3** were obtained by recrystallisation and their structures were determined by X-ray methods. Perspective views of both molecules are displayed in Figures 1 and 2, respectively. Selected bond lengths and angles are collected in Table 1. The simultaneous coordination of the chelated dithiocarbamate and the phosphane to the *fac*-$[Re(CO)_3]^+$ fragment confirms the proposed structure and therefore the formation of the target [2+1] mixed-ligand rhenium tricarbonyl complexes. The coordination geometry around the metal atom can be described as a slightly distorted octahedron. The principal distortion comes from the bite angle of the chelated dithiocarbamate ligand, which forces the S–Re–S angles to be near 70°, a common value in other metal complexes with chelated dithiocarbamates.[23] The almost identical Re–S dis-



Scheme 3. Syntheses of the complexes **C1**–**C3** by the one-step and two-step approaches.

tances indicate that the dithiocarbamate ligand links to rhenium in a symmetrical bidentate mode in both complexes. The SCS plane of the dithiocarbamate is nearly coincident with the SReS plane, with S42–Re–S41 and S41–C43–S42 angles of 2.2(4)° and 4.7(5)° for **C1** and **C3**, respectively Consequently, the metal atom, the dithiocarbamate and the two carbonyl ligands *trans* to sulfurs lie in a slightly distorted plane. The third carbonyl and the Re–P bond are arranged almost perpendicular to this plane, and the Re–P distances for both **C1** and **C3** are in the range observed for other diphenylphosphanyl–metal complexes. It should be pointed out that the ester and amido groups of the functionalised phosphanes are located away from the metal atom. This result is relevant with regard to radiopharmaceutical applications since the amino acid fragment linked to the phosphane is not affected by the coordination to the metal. Another interesting point concerns the similar Re–C distances observed in the *fac*-Re(CO)$_3$ fragments despite the different electronic characteristics of the ligands located in the *trans* position (dithiocarbamate or phosphane). The Re–C distances of carbonyl ligands *trans* to phosphane are comparable with the carbonyls *trans* to dithiocarbamate both for **C3** and **C1**. This fact could be related to the capability of phosphane and dithiocarbamate ligands to form similar strong bonds with rhenium and to the observed experimental stability and kinetic inertia of the studied complexes.[22]

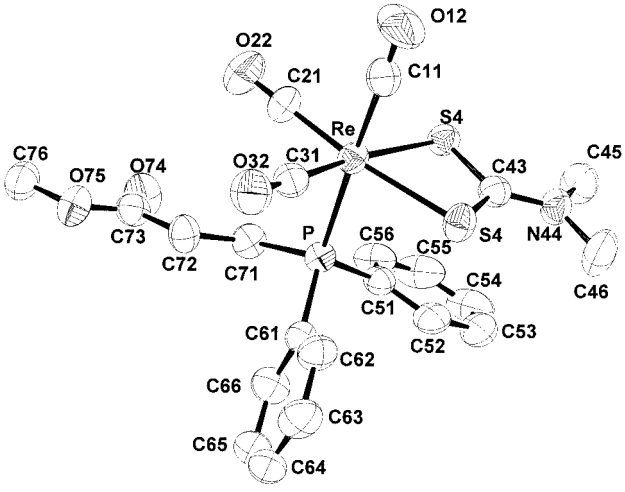


Figure 1. ORTEP diagram of **C1** showing 50% probability ellipsoids (hydrogen atoms omitted for clarity).

The one-step synthesis of complexes **C1**–**C3** by direct reaction between the rhenium precursor [Re(CO)$_3$Br$_3$]$^{2-}$ and an equimolecular mixture of dithiocarbamate and phosphane was studied in order to evaluate whether the characterised complexes are the main products by this pathway (Scheme 3). This reaction can be regarded as a test at macroscopic concentrations of the viability for future development of a [2+1] mixed-ligand kit for radiopharmaceutical applications.[24] Complexes **C1**–**C3** were obtained in good yields (68–75%) by this direct pathway and they display identical spectroscopic and analytical parameters to

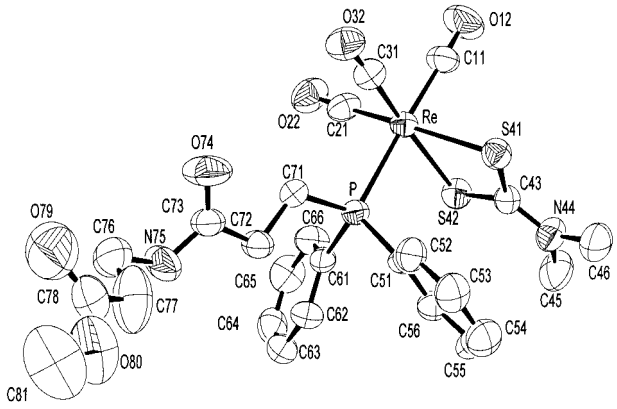


Figure 2. ORTEP diagram of **C3** showing 50% probability ellipsoids (hydrogen atoms omitted for clarity).

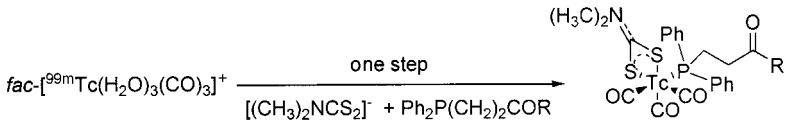
Table 1. Selected bond lengths [Å] and angles [°] for **C1** and **C3**.

	C1	**C3**
Re–P	2.495(2)	2.492(2)
Re–C11	1.911(8)	1.943(10)
Re–C21	1.929(6)	1.905(10)
Re–C31	1.898(6)	1.913(9)
Re–S41	2.508(2)	2.516(2)
Re–S42	2.511(2)	2.507(2)
S41–C43	1.715(6)	1.733(8)
S42–C43	1.718(6)	1.710(8)
C43–N44	1.327(7)	1.313(10)
S41–Re–S42	70.18(5)	70.00(7)
P–Re–S41	88.63(5)	88.86(7)
P–Re–S42	88.16(5)	84.08(7)
C11–Re–C21	89.1(3)	87.6(4)
C21–Re–C31	89.8(3)	89.0(4)
C11–Re–C31	89.0(3)	88.5(4)
C11–Re–P	177.5(2)	178.1(3)
S41–Re–C31	100.3(2)	99.9(3)
S42–Re–C21	99.8(2)	101.3(3)
S41–C43–S42	114.3(4)	113.6(5)

the complexes synthesised by the two-step method. On comparing the two methods, the yields seem to be independent of the reaction path, but in the second case the product is obtained in a simple one-step reaction. In the reaction with a ligand mixture, the main product was still the tricoordinate monomeric neutral complex. The absence of significant quantities of phosphane complexes or dithiocarbamate complexes (or bidentate complexes in general) as by-products is a relevant result since it could be related to the peculiar thermodynamic stability of the tripodal (SS)(P) donor set for the metal tricarbonyl core.

Labelling Studies with ^{99m}Tc

The preparations of the homologous radioactive complexes were studied under no-carrier-added conditions by ligand substitution with the precursor *fac*-[^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ and checked by HPLC analysis. The labile water ligands can easily be replaced by more stable and inert ligands. This reaction has been explored with DMTC[9] and phosphane ligands.[20,25,26]



R = $HNCH_2COOCH_3$ (**C4**), $HN(CH_2)_2COOCH_3$ (**C5**)

Scheme 4. Syntheses of the radio complexes **C4** and **C5** by the one-step approach.

We used the representative ligands **L2** and **L3** to simulate the presence of a biomolecule conjugated to the phosphorus atom of the phosphane (Scheme 4). The one-step synthesis was used to evaluate the (SS)(P) approach because it is the more attractive method for preparing radiopharmaceuticals. So, we added the dithiocarbamate and the phosphane ligand at the same time. The lipophilic character of diphenylphosphanes justified the use of ethanol to prepare the mixed-ligand solution. HPLC analysis showed that the addiction of the ligand mixture to the precursor *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ led to the formation of a major compound in both cases. The retention times between the two ^{99m}Tc compounds (13.27 min for **C4** and 13.44 min for **C5**) and the analogous Re complexes (13.39 and 13.54 min for **C2** and **C3**, respectively) are almost identical (Figure 3). These results are consistent with the structure of the $Re(CO)_3$(SS)(P) coordination system for the radio complexes **C4** and **C5**.

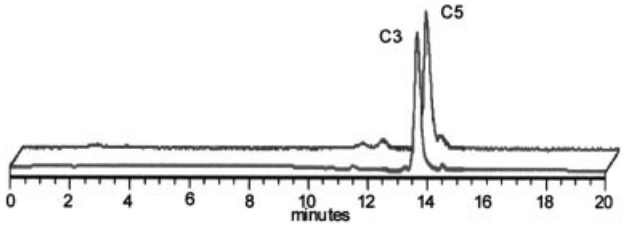


Figure 3. HPLC UV trace of the rhenium complex **C3** and γ-trace of the radioactive ^{99m}Tc complex **C5**.

The labelling reactions were also studied under different reaction conditions (pH temperature, reaction time, reagents' concentration and ratio) in order to obtain the optimised protocols. Reaction of a 4:1 mixture of MDTC and phosphane ligands at 80 °C for 60 min was found to give the best results in terms of yields.

To confirm the [2+1] coordination set, the precursor was reacted under the same conditions, but with only dithiocarbamate or with only phosphane ligands in the reaction mixture. The labelling mixtures were analysed by HPLC to determine the retention times of the products. DMTC reacts with the tricarbonyl radioactive moiety to give a unique peak at 14.21 min, while the reaction with the phosphane ligands gave two peaks at 10.98/11.57 min (**L2**) and 11.38/12.12 min (**L3**). These results support the formation of (SS)(P) coordination to $^{99m}Tc(CO)_3^+$ as the main product because these signals were observed as minor products in the reaction with the simultaneous addition of the dithiocarbamate and the phosphane ligand.

Conclusions

The coordinating chelating system (SS)(P) appears to be promising for a future medical application since complexes with the $[Re(CO)_3]^+$ moiety can easily be prepared in a one-step synthesis (useful in the design of a commercial kit), with good yields, and the synthesised products are very stable. The achieved tricoordination presented in this paper is very versatile and offers the possibility to link a wide range of target molecules to the monodentate phosphane. Furthermore, the chemistry of dithiocarbamates is well understood, and it is relatively easy to synthesise derivatives with different residues to modulate the hydro- or lipophilic characteristics of the desired complexes. Indeed, this [2+1] integrated approach with a symmetric bidentate chelating agent also avoids the formation of stereoisomers, which are often detected when using non-symmetric bidentate ligands or tridentate BFCA. The isomer formation influences the specific receptor binding because of the different metal–ligand orientation and may produce labelled species with different affinity for the same target, principally for small biomolecules.[27] From the perspective of developing a new drug, single and chemically stable products are essential for FDA approval.[8a]

Tc-99m labelling studies using the one-step method have confirmed the capability to produce (SS)(P) radio complexes. The next step will be to test the stability and kinetic inertia of the prepared compounds in order to evaluate their application for the formulation of new radiopharmaceuticals.

We conclude that the present [2+1] complex fashion could be a useful building block in the design and development of new target-specific ^{99m}Tc and $^{186/188}Re$ radiopharmaceuticals based on the tricarbonyl concept.

Experimental Section

Materials and Analytical Methods: All reactions were performed under nitrogen using standard Schlenk tube techniques. Solvents were degassed and tested for peroxides prior to use. All chemicals and solvents were reagent grade and were used without further purification. Infrared spectra were recorded with a Perkin–Elmer 1710 FT spectrometer as KBr pellets. The NMR spectra were recorded by the Servei de Ressonància Magnètica Nuclear de la Universitat Autònoma de Barcelona with a Bruker AM400 instrument. The ^{31}P chemical shifts are reported in ppm up-field from external 85% H_3PO_4. The ^1H and ^{13}C chemical shifts are expressed in ppm up-field from TMS.

Succinimidyl 3-diphenylphosphanylpropionate was provided by Argus Spechem S.a.s. (Prato, Italy) and the precursor $[NEt_4]_2[Re(CO_3)Br_3]$ was prepared by published procedures.[12] Microanalyses were performed at the Servei d'Anàlisi Química de la Universitat Autònoma de Barcelona. Electrospray ionisation mass spectra (ESI-MS) were obtained with an Applied Mariner System 5220 mass spectrometer (PerSeptive Biosystems Inc., Framingham, MA) in the positive-ion mode after dissolving the samples in acetonitrile (10^{-3} M).

The diffraction measurements were recorded by the X-ray Diffraction Service of the Universitat Autònoma de Barcelona.

Analytical thin-layer chromatography (TLC) was performed on Merck (Darmstadt, Germany) silica gel 60-F_{254} plates using the following elution systems: (a): *n*-hexane/EtOAc (3:2); (b): *n*-hexane/AcOEt (1:3); visualisation was accomplished by UV detection at 254 nm. Flash chromatography was carried out using silica gel 230–400 Mesh (Merck, Darmstadt, Germany) according to the method of Still.[28]

RP-HPLC analyses were performed on a Waters chromatography system (Waters, Milford, MA) controlled with a binary Waters 510 programmable gradient pump of the Department of Pharmaceutical Sciences, University of Padova. The analyses were monitored with a Waters 486 tunable absorbance detector set at 215 nm and a Bioscan-γ-detector B-FC 3200 (Milano, Italy). A Hamilton C18 reverse-phase column (10 μm, 250×4.6 mm, Alltech Italia, Sedriano, Milano) was used and the solvents were water with 0.1% trifluoroacetic acid (A) and acetonitrile with 0.05% trifluoroacetic acid (B). The HPLC gradient system started at 50% A/50% B, which was maintained for 5 min, rising to 100% B in 5 min with a linear gradient. It was maintained at a maximum of B for 8 min before returning to initial conditions in 2 min. The flow rate was 1 mL min^{-1}.

$Na^{99m}TcO_4$ was eluted from a commercial Drytec Sorin ^{99}Mo/^{99m}Tc Nycomed Amersham Sorin generator (Saluggia, Vercelli, Italy) using a 0.9% saline solution. The radioactive precursor *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ was prepared with an IsoLink kit purchased from Mallinckrodt Med. B.V. (Pettern, Netherland).

All manipulations were carried out as approved for low-level radioactivity use.

Methyl 3-Diphenylphosphanylpropionate (L1): A solution of succinimidyl 3-diphenylphosphanylpropionate (1.41 mmol) in methanol (20 mL) was added dropwise to a stirred solution of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP, 1.41 mmol) in the same solvent (20 mL). The mixture was left stirring for 6 h at room temperature, then the solvent was removed under vacuum. The oily residue containing product **L1** was purified by silica gel flash column chromatography (a) and concentrated to obtain a pale-yellow oil. Yield: 268 mg (70%). TLC (a): R_f = 0.8. HPLC: R_t = 14.63 min. FT-IR (KBr): $\tilde{\nu}$ = 1744 cm^{-1} (C=O, ester). ^{1}H NMR ($CDCl_3$): δ = 2.35–2.41 (m, 4 H, PCH_2CH_2), 3.64 (s, 3 H, OCH_3), 7.32–7.43 (m, 10 H, ArH) ppm. ^{13}C NMR ($CDCl_3$, except phenyl resonances): δ = 22.7 (d, $^1J_{C,P}$ = 12.5 Hz, P*C*H$_2$), 30.3 (d, $^2J_{C,P}$ = 19.2 Hz, PCH$_2$*C*H$_2$), 52.5 (s, OCH$_3$), 173.2 (d, $^3J_{C,P}$ = 15.3 Hz, *C*OOMe) ppm. ^{31}P NMR ($CDCl_3$): δ = –14.4 ppm. ESI-MS: *m*/*z* = 273 [M + H]$^+$.

Methyl [3-(Diphenylphosphanyl)propionylamino]acetate (L2): Triethylamine (2.39 mmol) was added to a suspension of glycine methyl ester hydrochloride (1.59 mmol) in anhydrous CH_2Cl_2 (50 mL). The solution was stirred for 30 min and then succinimidyl 3-(diphenylphosphanyl)propionate (1.59 mmol) was added. The mixture was stirred for 3 h and the solvent was then removed under vacuum. The oily crude containing product **L2** was purified by silica gel flash column chromatography (b) and concentrated to obtain a pale-yellow oil. Yield: 368 mg (70%). TLC (b): R_f = 0.8. HPLC: R_t = 9.90 min. FT-IR (KBr): $\tilde{\nu}$ = 1752 cm^{-1} (C=O, ester), 1654 (C=O, amide). ^{1}H NMR ($CDCl_3$): δ = 2.31–2.41 (m, 4 H, PCH_2CH_2), 3.72 (s, 3 H, OCH$_3$), 3.98 (d, $^3J_{CH,NH}$ = 6.3 Hz, 2 H, -NHCH_2), 6.12 (br., 1 H, NH), 7.30–7.45 (m, 10 H, ArH) ppm. ^{13}C NMR ($CDCl_3$, except phenyl resonances): δ = 23.3 (d, $^1J_{C,P}$ = 10.6 Hz, P*C*H$_2$), 32.5 (d, $^2J_{C,P}$ = 17.3 Hz, PCH$_2$*C*H$_2$), 41.5 (s, *C*H$_2$COOMe), 52.5 (s, OCH$_3$), 170.3 (s, *C*OOMe), 171.4 (d, $^3J_{C,P}$ = 13.4 Hz, *C*ONH) ppm. ^{31}P NMR ($CDCl_3$): δ = –13.9 ppm. ESI-MS: *m*/*z* = 330 [M + H]$^+$

Methyl [3-(Diphenylphosphanyl)propionylamino]propionate (L3): Triethylamine (2.68 mmol) was added to a suspension of β-alanine methyl ester hydrochloride (1.79 mmol) in anhydrous CH_2Cl_2 (50 mL). The resulting solution was stirred for 30 min and succinimidyl 3-(diphenylphosphanyl)propionate (1.79 mmol) was added. The mixture was stirred for 4 h and the solvent was then removed under vacuum. The oily crude residue containing product **L3** was purified by silica gel flash column chromatography (b) and concentrated to obtain a pale-yellow oil. Yield: 442 mg (72%). TLC (b): R_f = 0.75. HPLC: R_t = 9.98 min. FT-IR (KBr): $\tilde{\nu}$ = 1738 cm^{-1} (C=O, ester), 1652 (C=O, amide). ^{1}H NMR ($CDCl_3$): δ = 2.15–2.23 (m, 2 H, PCH_2), 2.32–2.35 (m, 2 H, PCH$_2$CH_2), 2.43 (t, ^3J = 6.3 Hz, 2 H, CH_2COOMe), 3.35–3.41 (dt, ^3J = 6.3, $^3J_{CH,NH}$ = 6.0 Hz, 2 H, -NHCH_2), 3.59 (s, 3 H, OCH$_3$), 6.03 (t, $^3J_{CH,NH}$ = 6.0 Hz, 1 H, NH), 7.22–7.36 (m, 10 H, ArH) ppm. ^{13}C NMR ($CDCl_3$, except phenyl resonances): δ = 23.1 (d, $^1J_{C,P}$ = 11.5 Hz, P*C*H$_2$), 32.4 (d, $^2J_{C,P}$ = 18.2 Hz, PCH$_2$*C*H$_2$), 33.5 (s, *C*H$_2$COOMe), 34.7 (s, HN*C*H$_2$), 51.5 (s, OCH$_3$), 171.9 (d, $^3J_{C,P}$ = 13.2 Hz, CONH), 172.1 (s, 1C, *C*OOMe) ppm. ^{31}P NMR ($CDCl_3$): δ = –14.3 ppm. ESI-MS: *m*/*z* = 344 [M + H]$^+$.

Synthesis of Rhenium Complexes. Two-Step Method: A solution of sodium *N*,*N*-dimethyldithiocarbamate (0.35 mmol) dissolved in methanol (20 mL) was added dropwise to a solution of rhenium tricarbonyl precursor $[NEt_4]_2[Re(CO_3)Br_3]$ (0.35 mmol) in the same solvent (20 mL). The obtained mixture was stirred for 30 min. A methanolic solution (20 mL) of phosphane ligand (0.35 mmol) was then added dropwise to the resulting pale yellow-green solution, and stirred for 90 min. The solvent was removed under vacuum and the oily residue containing the complex was purified by silica gel flash column chromatography (elution system a for **C1** and b for **C2** and **C3**). The solvents were then evaporated under vacuum. The obtained oil was taken up with 5 mL of Et_2O and then 50 mL of *n*-hexane was added to precipitate the product. The white powder was filtered off and dried under vacuum.

One-Step Method: A solution of *N*,*N*-dimethyldithiocarbamate (0.28 mmol) and phosphane ligand (0.28 mmol) dissolved in methanol (40 mL) was added dropwise to a solution of rhenium tricarbonyl precursor $[NEt_4]_2[Re(CO_3)Br_3]$ (0.28 mmol) in the same solvent (20 mL). The obtained mixture was stirred for 60 min and the solvent was removed under vacuum. The crude product containing the complex was purified by silica gel flash column chromatography (elution system a for **C1** and b for **C2** and **C3**). The solvents were evaporated under vacuum. The obtained oil was taken up with 5 mL of Et_2O and then 50 mL of *n*-hexane was added to precipitate the product. The white solid was filtered off and dried under vacuum.

[Re(CO)$_3$(L1)(*N*,*N*-dimethyldithiocarbamate)] (C1): Two-step method: Yield: 172 mg (76%). One-step method: Yield: 132 mg (73%). TLC (a): R_f = 0.4. HPLC: R_t = 14.94 min. FT-IR (KBr): $\tilde{\nu}$ = 2016 cm^{-1}, 1921, 1890 (C≡O), 1733 (C=O, ester), 1523 (C–N,

S_2CNMe_2). ^1H NMR ($CDCl_3$): δ = 2.31–2.39 (m, 2 H, PCH_2), 2.86 [s, 6 H, N(CH_3)$_2$], 2.89–2.97 (m, 2 H, PCH_2CH_2), 3.61 (s, 3 H, OCH_3), 7.26–7.55 (m, 10 H, ArH) ppm. ^{13}C NMR ($CDCl_3$, except phenyl resonances): δ = 22.7 (d, $^1J_{C,P}$ = 28.7 Hz, PCH_2), 30.1 (d, $^2J_{C,P}$ = 43.3 Hz, PCH_2CH_2), 38.2 [s, N(CH_3)$_2$], 51.6 (s, OCH_3), 172.6 (d, $^3J_{C,P}$ = 16.4 Hz, *C*OOMe), 190.1–193.6 [m, Re(CO)$_3$], 206.6 (s, NCS_2) ppm. ^{31}P NMR ($CDCl_3$): δ = 5.2 ppm. $C_{22}H_{23}NO_5PReS_2$: calcd. C 39.77, H 3.50, N 2.11, S 9.68; found C 39.66, H 3.65, N 2.08, S 9.42. ESI-MS: *m*/*z* = 664 [M + H]$^+$.

[Re(CO)$_3$(L2)(*N*,*N*-dimethyldithiocarbamate)] (C2): Two-step method: Yield: 165 mg (68%). One-step method: Yield: 146 mg (75%). TLC (b): R_f = 0.5. HPLC: R_t = 13.39 min. FT-IR (KBr): $\tilde{\nu}$ = 2016 cm^{-1}, 1919, 1890 (C≡O), 1750 (C=O, ester), 1654 (C=O, amide) 1522 (C–N, S_2CNMe_2). ^1H NMR ($CDCl_3$): δ = 2.25–2.33 (m, 2 H, PCH_2), 2.85 [s, 6 H, N(CH_3)$_2$], 2.94–3.02 (m, 2 H, PCH_2CH_2), 3.74 (s, 3 H, OCH_3), 3.96 (d, $^3J_{CH,NH}$ = 5.1 Hz, 2 H, -NHCH_2), 5.88 (br., 1 H, NH), 7.37–7.55 (m, 10 H, ArH) ppm. ^{13}C NMR ($CDCl_3$, except phenyl resonances): δ = 24.1 (d, $^1J_{C,P}$ = 28.7 Hz, PCH_2), 30.2 (d, $^2J_{C,P}$ = 43.0 Hz, PCH_2CH_2), 38.2 [s, N(CH_3)$_2$], 41.4 (s, CH_2COOMe), 52.4 (s, OCH_3), 170.16 (s, *C*O-OMe), 171.48 (d, $^3J_{C,P}$ = 14.7 Hz, CONH), 189.2–193.3 [m, Re(CO)$_3$], 212.4 (s, NCS_2) ppm. ^{31}P NMR ($CDCl_3$): δ = 6.7 ppm. $C_{24}H_{26}N_2O_6PReS_2$: calcd. C 40.05, H 3.64, N 3.89, S 8.91; found C 39.97, H 3.75, N 3.65, S 8.62. ESI-MS: *m*/*z* = 721 [M + H]$^+$.

[Re(CO)$_3$(L3)(*N*,*N*-dimethyldithiocarbamate)] (C3): Two-step method: Yield: 192 mg (77%). One-step method: Yield: 147 mg (73%). TLC (b): R_f = 0.5. HPLC: R_t = 13.54 min. FT-IR (KBr): $\tilde{\nu}$ = 2015 cm^{-1}, 1920, 1889 (C≡O), 1734 (C=O, ester), 1652 (C=O, amide) 1526 (C–N, S_2CNMe_2). ^1H NMR ($CDCl_3$,): δ = 2.20–2.27 (m, 2 H, PCH_2CH_2), 2.40 (t, ^3J = 6.1 Hz, 2 H, Ala-CH_2-COOMe), 2.85 [s, 6 H, N(CH_3)$_2$], 2.93–3.00 (m, 2 H, PCH_2CH_2), 3.42–3.49 (dt, ^3J = 6.3, $^3J_{CH,NH}$ = 5.0 Hz, 2 H, Ala-NH-CH_2), 3.70 (s, 3 H, OCH_3), 5.97 (t, $^3J_{CH,NH}$ = 5.0 Hz, 1 H, NH), 7.41–7.57 (m, 10 H, ArH) ppm. ^{13}C NMR ($CDCl_3$, except phenyl resonances): δ = 24.3 (d, $^1J_{C,P}$ = 28.8 Hz, PCH_2), 30.0 (d, $^2J_{C,P}$ = 47.0 Hz, PCH_2CH_2), 31.9 (s, CH_2COOMe), 34.9 (s, HNCH_2), 38.4 [s, N(CH_3)$_2$], 51.5 (s, OCH_3), 171.5 (d, $^3J_{C,P}$ = 14.4 Hz, CONH), 172.5 (s, *C*OOMe), 189.2–193.4 [m, Re(CO)$_3$], 212.2 (s, NCS_2) ppm. ^{31}P NMR ($CDCl_3$): δ = 7.7 ppm. $C_{25}H_{28}N_2O_6PReS_2$: calcd. C 40.92, H 3.85, N 3.82, S 8.74; found C 40.87, H 3.93, N 3.71, S 8.70. ESI-MS: *m*/*z* = 735 [M + H]$^+$.

Synthesis of Technetium-99m Complexes. General Procedure: Technetium-99m labelling was performed by addiction of 15 μL of an ethanolic solution of *N*,*N*-dimethyldithiocarbamate (0.8 mM, 0.012 μmol) and phosphane ligand (0.2 mM, 0.003 μmol) to 85 μL of a freshly prepared solution of *fac*-[^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ (15 MBq), pH 7.4. The mixture was incubated at 80 °C and stirred for 1 h. Analysis by HPLC equipped with a γ-radiometric detector showed a main peak for all experiments. Data are expressed as means of ±SD of three experiments.

Comparative *fac*-[^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ labelling reactions with the only presence of *N*,*N*-dimethyldithiocarbamate or phosphane ligand were carried out under the same conditions and analysed by HPLC.

[^{99m}Tc(CO)$_3$(L2)(*N*,*N*-dimethyldithiocarbamate)] (C4): HPLC: R_t = 13.27 min. Labelling yield = 83 ± 1.7%. (HPLC: R_t [^{99m}Tc(CO)$_3$ with DMTC] = 14.29 min, R_t [^{99m}Tc(CO)$_3$ with L2] = 10.98 min, 11.57 min).

[^{99m}Tc(CO)$_3$(L3)(*N*,*N*-dimethyldithiocarbamate)] (C5): HPLC: R_t = 13.44 min. Labelling yield = 83 ± 1.7%. (HPLC: R_t [^{99m}Tc(CO)$_3$ with DMTC] = 14.29 min, R_t [^{99m}Tc(CO)$_3$ with L3] = 11.38 min, 12.12 min).

X-ray Crystallography: Colourless single crystals were obtained from methanol at –30 °C (**C1**) or by slow diffusion of *n*-hexane into a solution of the compound in ethyl acetate at room temperature (**C3**). Crystal data and selected information on data collection and structure determination are given in Table 2. Data were collected on a Bruker SMART CCD area-detector diffractometer at room temperature. Graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å) was used. Lorentz-polarisation and absorption corrections were applied using Bruker SAINT and SADABS software. The structures were solved by direct methods and refined by full-matrix least-squares on F^2 for all unique measured data with weighting $w^{-1} = \sigma^2(F_o^2) + (aP)^2 + bP$, where $P = (F_o^2 + 2F_c^2)/3$.[29] Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included with riding model constraints and isotropic displace-

Table 2. Crystal data and structure refinement parameters for **C1** and **C3**.

	C1	**C3**
Empirical formula	$C_{22}H_{23}NO_5PReS_2$	$C_{25}H_{28}N_2O_6PReS_2$
Formula mass	662.70	733.78
Crystal system	monoclinic	monoclinic
Space group	*C*2/*c*	$P2_1/c$
a [Å]	22.6340(16)	10.6473(8)
b [Å]	10.6284(7)	29.191(2)
c [Å]	21.2346(14)	9.1776(7)
β [°]	103.212(1)	92.566 (2)
V [Å^3]	4973.1(6)	2849.6(4)
Z	8	4
$\rho_{calcd.}$ [Mg m^{-3}]	1.770	1.710
Absorption coefficient [mm^{-1}]	5.152	4.508
F(000)	2592	1448
Crystal size [mm]	0.22 × 0.18 × 0.08	0.22 × 0.10 × 0.05
Theta range for data collection	1.85 to 28.26	1.40 to 28.22
Reflections collected/unique	15196/5601 (R_{int} = 0.0445)	17896/6380 (R_{int} = 0.0560)
Max. and min. transmission	0.66 and 0.32	0.80 and 0.37
Data/restraints/parameters	5601/12/292	6380/12/337
Goodness-of-fit on F^2	1.056	1.068
$R(F)$, $R_w(F^2)$ [$I > 2\sigma(I)$]	0.0457, 0.0899	0.0603, 0.1120
$R(F)$, $R_w(F^2)$ (all data)	0.0636, 0.0960	0.0989, 0.1275

ment parameters 1.5 (methyl H) or 1.2 (the rest) times the U_{eq} values of the corresponding carbon or nitrogen atoms.
CCDC-258371 (for **C1**) and -258372 (for **C3**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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