Bioorg. Med. Chem. Lett. 37 (2021) 127840

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Bioconjugated technetium carbonyls by transmetalation reaction with zinc derivatives

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ARTICLE INFO

Keywords:
Technetium
Carbonyl
Radiopharmaceutical
Transmetallation
Rhenium

ABSTRACT

The transmetalation reaction between zinc dithiocarbamates functionalized with organic groups and the cation *fac*-[^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ has been studied as a new strategy to bind biomolecules to this radionuclide for preparing radiopharmaceuticals with high molar activity. All complexes were obtained in high yields by heating at moderate temperatures and without subsequent purification. The chemical identity was ascertained by HPLC comparison with the homologous rhenium complexes. Stability studies in cysteine solution and serum have shown a good stability of the coordination set *fac*-[^{99m}Tc(CO)$_3$(SS)(P)]. Preliminary biological studies of the radiocomplex functionalized with D-(+)-glucosamine with carcinoma cells have been performed.

Molecular imaging methods are important in medicine because they provide information about the biological processes *in vivo*. Molecular imaging can be applied for noninvasive monitoring of spatial-temporal distribution of molecular or cellular processes, for early disease detection and real-time monitoring of therapeutic responses.[1,2] Amongst the molecular imaging techniques, radionuclide imaging is one of the modalities able to provide functional information about the disease and track biochemical processes *in vivo*. Radionuclide imaging can maps the biodistribution of radiopharmaceuticals using two imaging methods, according to the emission properties of the radionuclide used: Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT).[2,3]. A radiopharmaceutical is a radioactive compound that can be applied as imaging or therapeutic agent in nuclear medicine.[2,3]

Tc-99m radionuclide is the most widely used for SPECT imaging because it is easily accessible from the ^{99}Mo/^{99m}Tc generators, and it exhibits very suitable emission properties, such as detectable gamma rays with a photon energy of 140 keV and an appropriate half-life (≈ 6 h).[3–6] The number of publications with organometallic radiopharmaceuticals has increased significantly during the last 20 years due to the development of simple and convenient preparations of the aqua ions *fac*-[^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ and *fac*-[^{188}Re(H$_2$O)$_3$(CO)$_3$]$^+$. These cations are suitable organometallic synthons for the preparation of new radiopharmaceuticals.[7–11] The water ligands are labile and they can be replaced by other ligands, leading to new rhenium and technetium radiopharmaceuticals with carbonyl ligands, which exhibit high thermodynamic stability, kinetic inertia and high *in vivo* stability. The above-mentioned properties make these cations ideal organometallic precursors for the preparation of bioconjugated compounds by the labeling of biologically active molecules.

One point that should be noted about labeling reactions with Tc-99m, is that due to the fact that the concentration of the radioactive metal in the labeling reactions is very low (10^{-6} – 10^{-8} M),[4–6] it is common to find that the concentration of the derivatized biomolecule used in the reaction is several orders of magnitude higher than the concentration of the radioactive metal.[12] Therefore, to avoid saturation of the receptor site, the concentration of the unlabeled derivatized biomolecule should be as low as possible (Fig. 1). Labeled biomolecules must have a very high molar activity (defined as the ratio between the radioactivity of a labeled biomolecule and the total amount of the biomolecule in moles[13,14]).

We proposed an approach based on a transmetalation reaction as an alternative labeling procedure that could help to achieve a high labeling yield with an extremely low concentration of unlabeled derivatized biomolecule in the medium.[15]

Transmetalation is an essential reaction in organometallic chemistry,

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https://doi.org/10.1016/j.bmcl.2021.127840
Received 5 November 2020; Received in revised form 20 January 2021; Accepted 26 January 2021
Available online 5 February 2021

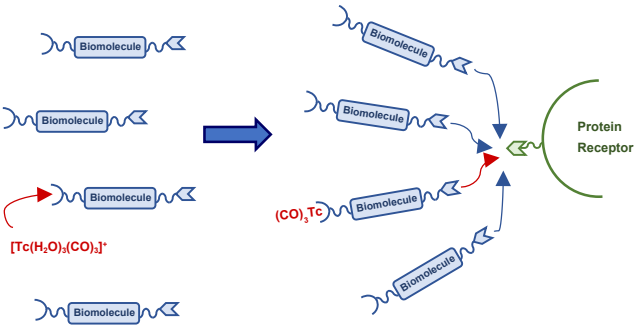
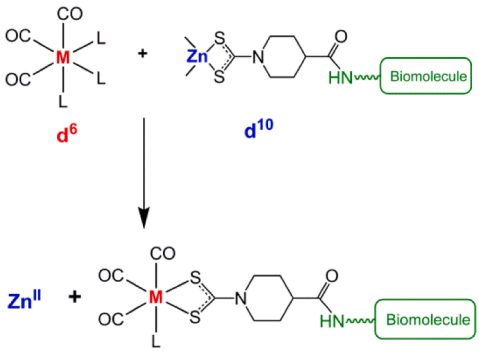
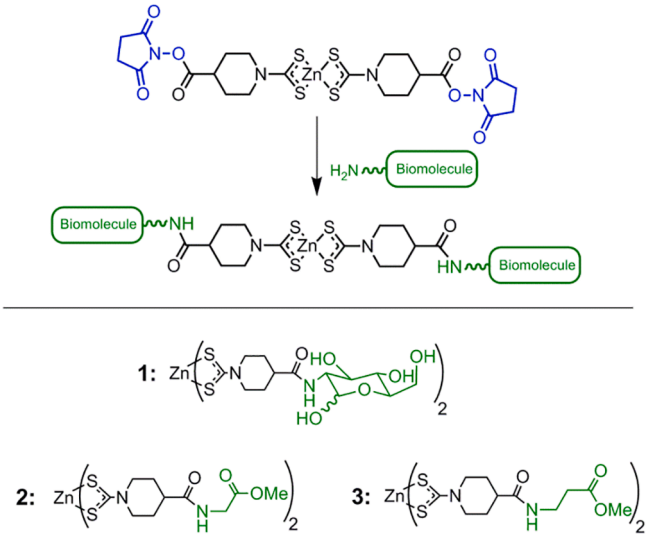


Fig. 1. Schematic representation of the competition between labeled and unlabeled molecules towards the receptor sites.

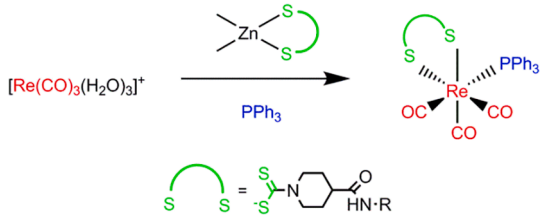


Scheme 1. Representation of the strategy for obtaining of potential radiopharmaceuticals based on transmetallation reaction.

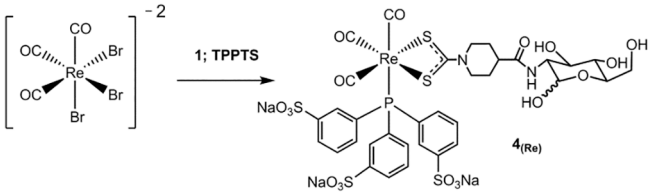


Scheme 2. Preparation of bioconjugated zinc complexes **1**–**3**.

which has been used since the beginnings to prepare new compounds by the transfer of one ligand between two metals. It is still as an important reaction in some fields, such as the preparation of *N*-heterocyclic carbenes (NHCs).[16] Our proposal is to use a transmetalation reaction between zinc complexes of the derivatized biomolecules, which, in general, show very low solubilities in water, with the radioactive metal to obtain high labeling yield in a medium with a very low concentration of the derivatized biomolecule. This transmetalation reaction is thermodynamically favorable owing to the higher stability of bonds with the {M(CO)$_3$} fragment (Scheme 1), where M is Tc or Re in the oxidation state + 1 (d^6 configuration).[15] A recent publication has applied this idea to the preparation of diaminedithiol radiopharmaceuticals.[17]



Scheme 3. [2 + 1] Transmetalation reaction with rhenium.



Scheme 4. Synthesis of **4$_{(Re)}$** complex.

Reactions with Tc-99m for radiopharmaceutical uses should be performed in aqueous media, because this radiometal is prepared in saline solution and Zn-dithiocarbamates are a useful family of compounds for our purposes. As mentioned above, they exhibit low solubilities in water and give rise to transmetalation reactions with transition metal atoms.[18] In the previous paper,[15] we reported that the succinimidyl ester derivative of a zinc(II) dithiocarbamate of isonipecotic acid (top, Scheme 2) is a valuable synthon for preparing bioconjugated Zn compounds (in fact, a posterior publication[19] has shown the versatility and utility of this complex in the synthesis of bioconjugated transition metals). Thus, compounds **1**–**3** were reported (see scheme 2) and the viability of the transmetalation reaction between zinc and rhenium was demonstrated.[15] However, the transmetalation reaction in aqueous medium was only studied with rhenium in a concentration nearly two orders of magnitude higher than the metal concentration commonly used in the preparation of Tc-99m radiopharmaceuticals. In the present work, we have undertaken the study of the feasibility of the transmetalation reaction between the previously reported zinc complexes and Tc-99m in water medium but using the low Tc-99m concentration obtained from ^{99}Mo/^{99m}Tc generators, commonly used for the preparation of Tc-99m radiopharmaceuticals. Although the chemistry of technetium and rhenium shows many similar aspects, there are also notable differences as the stability of the oxidation states and the kinetics of the substitution reactions,[20,21] which force to design different approaches to obtain the homologous technetium complexes.

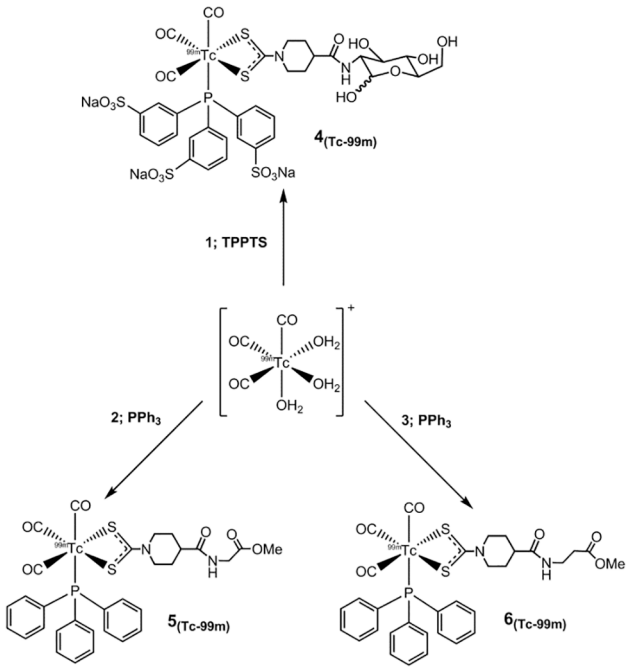
Furthermore, preliminary biological studies with one of the new reported Tc-99m complexes have been performed in order to study the viability of the hypothesis that this approach could lead to radiopharmaceuticals with an improved molar activity.

Part of this work was previously communicated[22] and a recently published study[23] has shown that this transmetallation reaction can be applied to the preparation of metallosurfactants.

In a previous publication[15] we reported the synthesis of [2 + 1] *fac*-[Re(CO)$_3$(SS)(PPh$_3$)] complexes by means of the transmetalation reaction using a bidentate ligand (SS = dithiocarbamate) and a monodentate ligand (PPh$_3$) (Scheme 3).

In the present work, we want to increase the versatility of our approach using other useful monodentate ligands from a radiopharmaceutical point of view, such as sodium triphenylphosphine trisulfonate (TPPTS). Consequently, the following model rhenium complex with this monodentate ligand was synthetized and characterized (Scheme 4).

The use of glucosamine as delivery agent of Tc-99m or other metals



Scheme 5. Synthesis of Tc-99 m complexes (complexes $\mathbf{5_{(Re)}}$ and $\mathbf{6_{(Re)}}$ were previously reported[15]).

has been widely studied since ^{18}F-FDG was discovered to be a very efficient radiopharmaceutical for myocardial perfusion or tumor detection.[24–29] For the design of the rhenium homologue of the potential Tc-99m radiopharmaceutical, TPPTS was chosen as a monodentate ligand due to its water solubility and because it increases the hydrophilicity of the complex, favoring its excretion by the renal-urinary system.[30–32] The product was prepared by heating to reflux a methanol solution of $[NEt_4]_2[ReBr_3(CO)_3]$ and complex **1**, following by the addition of TPPTS to obtain the expected rhenium complex. The final product was obtained with a moderate yield (56%). Two signals at 18.8 and 18.5 (minor) ppm in ^{31}P NMR spectra certainly confirmed that the [2 + 1] reaction took place. This phenomenon was previously observed in similar compounds with PPh_3 as a monodentate ligand.[15] The fact of observing two peaks was explained by the presence of two isomers in solution of the piperazine ring (axial, equatorial) due to the non-free rotation of the C—N bond of the dithiocarbamate group.[15] Spectroscopic (IR, ^{1}H and ^{13}C NMR) and spectrometric (HRMS) data, as well as elemental analysis, were consistent with the proposed structure. The RP-HPLC analysis showed a peak at the retention time of 11.7 min (using method 3, see Supplementary Data), a piece of data that will be useful to characterize the homologous Tc-99m complex.

All reactions with Tc-99m (Scheme 5) were performed under experimental conditions similar to those commonly used in the preparation of Tc-99m radiopharmaceuticals. Thus, in all cases, reactions are conducted by adding a suspension of an small amount of the appropriate zinc complex (**1**–**3**, ~1mg)[15] and monodentate ligand to an aqueous solution of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ and subsequent heating at moderate temperatures (45–65 °C) for a short time (~25 min). After cooling down, the suspension is centrifuged and the supernatant is ready to be analyzed in biological studies as a potential radiopharmaceutical. It should be highlighted that reaction temperatures and the short reaction times make this procedure compatible with the usual preparation methods of Tc-99m radiopharmaceuticals. In addition, the fact that the synthesis is carried out under moderate temperatures makes possible to apply this method to a wide range of biomolecules that are denatured at higher temperatures.

The Tc-99m concentration in these preparations is so low that is not possible to use standard methods to characterize the radiocomplexes.

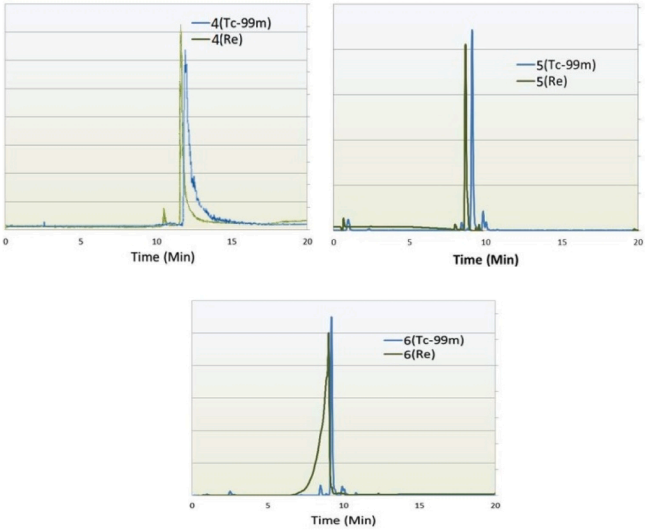


Fig. 2. Chromatograms of each Tc-99m complex and their corresponding homologous rhenium complex.

Table 1
Results of the solubility of each zinc complex obtained by ICP-OES analysis. Data are expressed in ppm and molarities are shown in parenthesis.

Zn complex	Solubility
1	11 ($1.3\cdot10^{-5}$M)
2	3.1 ($5.0\cdot10^{-6}$M)[15]
3	0.66 ($1.0\cdot10^{-6}$M)[15]

Thus, the Tc-99m complexes are characterized by the usual methods based on comparing the HPLC retention times of the technetium complex with its homologue of rhenium that has been previously fully characterized.[4–6,33] Fig. 2 shows chromatograms for each Tc-99m radiocomplex and their rhenium homologue. As can be seen, the similar retention times between rhenium and technetium complexes confirm that the radiosynthesis took place successfully. The small differences between the UV-trace (rhenium complexes) and the gamma-trace (Tc-99m complexes) in Fig. 2 are a consequence of the different times that the sample needs to pass through the two detectors and also to the different concentration of rhenium and technetium complexes in solution. A relevant point is the yields obtained for all the radiosynthesis performed by transmetalation with zinc complexes studied in this work, which are values that have been obtained without any kind of purification. Values of 95–98% were achieved for complexes $\mathbf{4_{(Tc\text{-}99m)}}$(98%), $\mathbf{5_{(Tc\text{-}99m)}}$(95%), and $\mathbf{6_{(Tc\text{-}99m)}}$(95%). These data are consistent with the potential application of the transmetalation reaction in the preparation of Tc-99m radiopharmaceuticals.

As has been pointed out in the introduction, the objective of our approach is to achieve high chemical yields in the transmetalation reaction using zinc complexes with a solubility as low as possible, in order to improve the molar activity of the radiopharmaceutical.

Table 1 shows the water solubilities of the zinc complexes that have been measured from aqueous saturated solution of the zinc compounds by inductively coupled plasma optical emission spectrometry (ICP-OES). These data confirm our hypothesis that the transmetalation reaction can lead to the preparation of the Tc-99m complexes in high yields, in a reaction medium with a very low concentration of the zinc complex. Consequently, if in the previous work[15] it was only possible to verify that the transmetalltion reaction was possible with rhenium, the present study has confirmed that this approach is potentially useful for the preparation of radiopharmaceuticals with high molar activity.

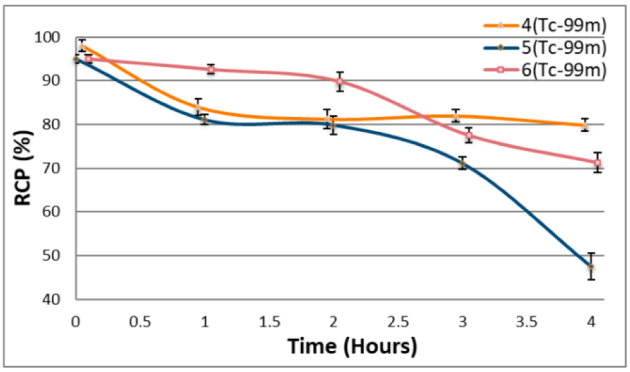


Fig. 3. Stability study of complexes $\mathbf{4}_{(Tc\text{-}99m)}$, $\mathbf{5}_{(Tc\text{-}99m)}$ and $\mathbf{6}_{(Tc\text{-}99m)}$ after incubation with cysteine amino acid (RCP = Radiochemical Purity; mean ± std. dev.; n = 3).

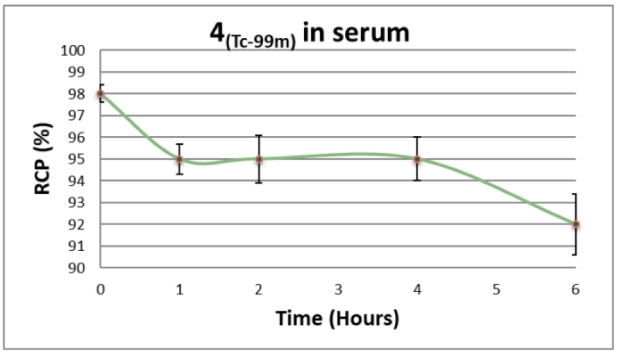


Fig. 4. Stability study of complex $\mathbf{4}_{(Tc\text{-}99m)}$ in mice serum. (RCP = Radiochemical Purity; mean ± std. dev.; n = 3).

A first approach to evaluate the *in vivo* stability of the potential radiopharmaceuticals was performed by incubation of these compounds in presence of cysteine, since it is one of the amino acids present in the blood with high coordination ability. Therefore, radiocompounds that remain unaltered after some hours of incubation in solution with an excess of cysteine, demonstrated a good stability against to the ligand substitution reactions.

The radiocomplexes $\mathbf{4}_{(Tc\text{-}99m)}$, $\mathbf{5}_{(Tc\text{-}99m)}$ and $\mathbf{6}_{(Tc\text{-}99m)}$ were added to a solution of cysteine (10^{-3}M; molar ratio cysteine/radiocomplex was 10^3–10^5) in phosphate buffered saline (PBS) for 4 h at 37 °C. Aliquots were withdrawn at 1, 2, 3 and 4 h, and analyzed with RP-HPLC in order to know the radiochemical purity (RCP).[34] Results are shown in Fig. 3, evidencing that the coordinative set [^{99m}Tc(CO)$_3$(SS)(P)] shows a remarkable stability in cysteine medium in the first three hours. It should be highlighted that $\mathbf{4}_{(Tc\text{-}99m)}$, which is one of the most interesting radiocomplex from a radiopharmaceutical perspective, is the most stable compound after four hours.

Serum stability studies can provide additional information about whether the studied radiocomplexes would remain unaltered in physiological conditions.[35,36] This experiment was only performed for complex $\mathbf{4}_{(Tc\text{-}99m)}$ because it is the most attractive radiocompound from the radiopharmaceutical point of view, since it is bioconjugated with a biologically active molecule and it is reasonably stable in cysteine medium. Thus, for this experiment, compound $\mathbf{4}_{(Tc\text{-}99m)}$ was added to fresh mouse serum and incubated at 37 °C for 6 h. At time points 1, 2, 4 and 6 h, aliquots were removed and the proteins were precipitated with acetonitrile. The RCP of each aliquot was measured by RP-HPLC. The results showed that the radiocomplex remains almost unaltered for 4 h in serum (Fig. 4). Hence, we can conclude that the radiocomplex studied may be used for further *in vitro* and *in vivo* experiments.

As mentioned above, one of our aims was to perform preliminary

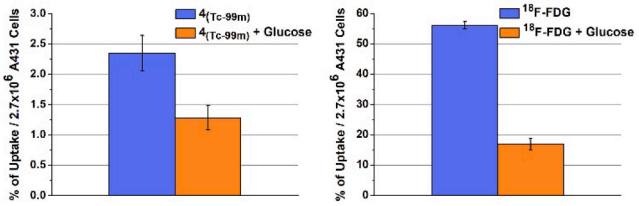


Fig. 5. Results of binding and blocking assay for $\mathbf{4}_{(Tc\text{-}99m)}$ and ^{18}F-FDG (mean ± std. dev.; n = 3).

biological studies to demonstrate that this approach could be used to produce radiopharmaceuticals with an improved molar activity. The radiocompound $\mathbf{4}_{(Tc\text{-}99m)}$ was used for *in vitro* studies because it showed good stability in cysteine and serum, in addition it could be transported into the cell via the GLUT transporters due to its glucose similar structure. Since the GLUT-1 transporters are overexpressed in cancer, we choose to evaluate the radiolabeled compound in the human epidermoid carcinoma cell line, A431.[37–38] To evaluate the transport of the potential radiopharmaceutical by GLUT-1, cell uptake experiments were carried out using the $\mathbf{4}_{(Tc\text{-}99m)}$ radiocompound, both with and without 3 mM glucose (competitor biomolecule) in parallel. This provides a measure of the efficiency and specificity of the transport, as the free glucose should saturate the uptake mechanism since the concentration is about 4 orders of magnitude higher than that of the $\mathbf{4}_{(Tc\text{-}99m)}$ complex. Thus, if uptake is occurring via GLUT-1, a significant drop in the radioactivity uptake should occur on addition of glucose. The results are shown in Fig. 5, and are compared with results obtained using ^{18}F-FDG (the most commonly-used radiopharmaceutical for targeting the GLUT-1 transporter) as the radiotracer.[39–40]

It is clear that the absolute value of the uptake is much higher for ^{18}F-FDG than $\mathbf{4}_{(Tc\text{-}99m)}$, which is not an unexpected result since ^{18}F-FDG is a small molecule that shows very efficient transport via GLUT-1.[41] Nevertheless, similar to ^{18}F-FDG, $\mathbf{4}_{(Tc\text{-}99m)}$ showed a statistically significant drop in uptake in the presence of non-radioactive glucose, suggesting that our complex could undergoes moderate uptake into cancer cells via the GLUT-1 transporter. However, further *in vitro* studies need to be done to clearly demonstrate that $\mathbf{4}_{(Tc\text{-}99m)}$ uptake is due to a specific mechanism regulated by the GLUT-1 transporter.

It should be highlighted that our results are in accordance with previously reported data in the literature, because all ^{99m}Tc- glucosamine bioconjugates reported have unfortunately shown low uptake values in tumor cells[24–29,42–43]. In this case the $\mathbf{4}_{(Tc\text{-}99m)}$ low uptake could be due to the fact that the bioactive moiety is too close to the metal center, causing steric hindrance. Thus, probably the linker should be larger and less rigid in order to promote efficient transport, decreasing steric hindrance. This slight modification would be compatible with our method, and relatively easy to implement. Moreover, taking into account that our approach does not require any purification steps, it is worth mentioning that, as far as we know, using this approach we have

Table 2
Biodistribution results of complex $\mathbf{4}_{(Tc\text{-}99m)}$ in mice. Data are presented as a mean from 3 animals ± SEM.

Organs and tissues	Amount of tracer uptake (%ID/g)
Tumor	0.07 ± 0.02
Intestine	20.8 ± 2.9
Pancreas	0.024 ± 0.005
Spleen	0.036 ± 0.006
Kidney	0.8 ± 0.1
Liver	0.6 ± 0.3
Heart	0.039 ± 0.003
Lung	0.09 ± 0.02
Blood	0.097 ± 0.012
Muscle	0.019 ± 0.006
Tail	0.15 ± 0.01

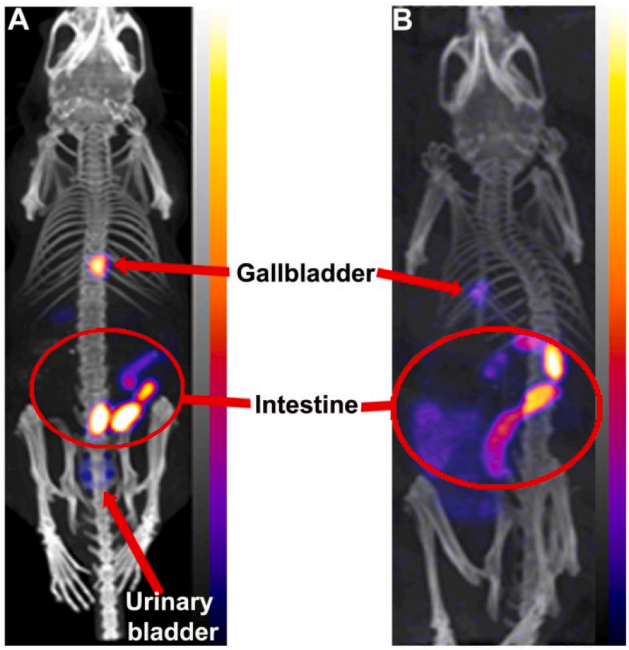


Fig. 6. SPECT/CT image of a mouse that has been previously injected complex $\mathbf{4}_{(Tc\text{-}99m)}$. One hour post-injection (A) and four hours post-injection (B).

been able to reach better uptake values compared with other previously reported Tc-99m tricarbonyl compounds bioconjugated with glucosamine.[24–29,42–43]

Three female athymic mice (6 weeks old) bearing a subcutaneous tumor (A431 cells) on the right flank were used for biodistribution studies. The animals were euthanized 4 h after injection of the $\mathbf{4}_{(Tc\text{-}99m)}$ complex and the main organs were harvested and weighed. The activity of each organ was measured using a gamma counter. Given the activity injected and the concentration of **1**, we have calculated that the molar activity in this experiment was 7.1×10^2 MBq/nmol. This value is one of the highest value of molar activity reported so far for a Tc-99m complexes linked to biomolecules.[44] The radioactivities measured in the individual tissues were expressed as the percentage of the total injected radioactivity, per gram of tissue (Table 2). It should be highlighted that the radiocomplex was highly accumulated in the intestines, liver and kidneys. In general terms, these results are in agreement with previously reported data for complexes that contain the $^{99m}Tc(CO)_3$ core.[45–47] The lipophilic core $\{Tc(CO)_3\}^+$ promotes the hepatobiliary excretion of the radiocompound, which explains the high activity in intestines and liver. The incorporation of the coordinated TPPTS into the molecule increases its hydrophilic character. However, the hepatobiliary clearance remains the main excretion route.

Although the accumulation in the tumor is three times higher than muscle, it is too low to be considered as a good diagnostic agent, and this result is consistent with the *in vitro* studies with cells. However, we should keep in mind that this low accumulation in the tumor can be a consequence of the molecular structure and, as discussed above, it can be improved in future by structural changes such as the increase of the linker between the biomolecule and the metallic group.

A SPECT/CT image was collected from one of three treated mice at one and four hours post injection in order to evaluate the pharmacokinetics of the radiocompound. The images (Figure 6) are matched with the previous biodistribution studies and they illustrate the main hepatobiliary clearance of the radiocompound. SPECT/CT imaging at 1 h and 4 h shows high activity in gallbladder and intestines. No significant uptake was observed in the tumor site, in agreement with the biodistribution studies reported in the above section.

The main conclusion of this work is that it has been possible to carry out a transmetalation reaction that allows the easy and straightforward bioconjugation of tricarbonyl technetium complexes with potentially high molar activity without any purification step. To the best of our knowledge, the reported value of 7.1×10^2 MBq/nmol is significantly higher than previously reported data for technetium carbonyl complexes linked to biomolecules. Despite the low water solubility of the zinc precursors (1–10 ppm), the reaction is so thermodynamically favored that it takes place with a very high yield in the presence of a phosphine ligand, which is important in order to tune the physico-chemical properties of the potential radiopharmaceutical. In addition, the reaction conditions of the transmetalation reaction are mild, which makes it possible to apply this approach to other biomolecules that cannot be stable in harsher reaction conditions.

Stability studies in cysteine and serum media have provided evidence of the stability of the $[^{99m}Tc(CO)_3(SS)(P)]$ coordination set in biological medium, which makes its application viable for radiopharmaceutical purposes.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgments

This work was supported by Spanish MINECO-FEDER grants (BIO2015-67358-C2-2-P and CTQ2015-70371-REDT) and by Generalitat de Catalunya (2017SGR-795).

We thank to Dr. Ramon Barnadas (Departament de Bioquímica, UAB) for valuable helpful in the revision of the manuscript and to Dr. Imma Ponte (Departament de Bioquímica, UAB) for allowing us to use the laboratory for handling radioactive substances.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127840.

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