

# Synthesis, metal complexation and biological evaluation of a novel semi-rigid bifunctional chelating agent for $^{99m}\text{Tc}$ labelling

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## Abstract

A novel bifunctional chelating agent bearing an aromatic ring has been synthesised and characterised. This ligand formed well-defined oxorhenium complexes. The analogous  $^{99m}\text{TcO}$ -complex was obtained in an excellent yield with high radiochemical purity (>95%). The biodistribution of the  $^{99m}\text{Tc}$ -complex after intravenous injection studied in normal rats showed that the activity was excreted mainly via renal-urinary pathway indicating its use for labelling peptides with  $^{99m}\text{Tc}$ .

## Graphical abstract

In this paper, we report a convenient synthesis of a novel semi-rigid bifunctional chelating agent, their complexes with rhenium (V) and the in vivo behaviour in normal rats of the corresponding  $^{99m}\text{TcO}$ -complex.

**Keywords:** Radiopharmaceuticals; Technetium-99m; Rhenium; Bifunctional chelating agent; Metallic complexes

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## 1. Introduction

There is a great current interest in the use of bifunctional chelating agents (BFCAs) in labelling of peptides and proteins with technetium-99m and rhenium-186/188 for the development of target-specific radioimaging/radiotherapeutic agents, respectively.<sup>1</sup> The technetium-99m is the most important radionuclide in diagnostic nuclear medicine due to its ideal nuclear properties ( $T_{1/2} = 6$  h, 140 KeV  $\gamma$  emitter, convenient availability from a commercial generator), while rhenium-186 ( $T_{1/2} = 3.78$  days,  $\beta^-$  (91%) and  $\gamma$  (9%) emitter) and rhenium-188 ( $T_{1/2} = 16.98$  h,  $\beta^-$  (85%) and  $\gamma$  (15%) emitter) are two of the most promising radionuclides for radiotherapy.<sup>1</sup> and <sup>2</sup> Typically, BFCAs are chelating ligands comprising a donor atom set to strongly coordinate the metal radioisotope and a functional group for attachment to a bio-targeting vector. The ability of a BFCA to avoid *in vivo* transchelation or *in vivo* dissociation of the radionuclide is crucial. So, an ideal BFCA is one which is able to form a stable and inert complex in high yield, under mild conditions, without the formation of isomers. However, although several designs have been used in practice with success, BFCAs for technetium and rhenium that meet all these criteria do not exist yet. Consequently, it is of high priority to develop novel, high stability chelators for these two nuclides for subsequent use in diagnostic and therapy, respectively.

It is well known that a semi-rigid structure provides a significant increase for the stability of their complexes.<sup>3</sup> Most of the semi-rigid polydentate ligands are based on a cycloalkyl ring<sup>4</sup> (cyclohexane, cyclopentane...) instead of a benzene skeleton.<sup>5</sup> To our knowledge, to include an aromatic cycle in the chelate ring compared to the cycloalkyl moiety presents two advantages: its ease of functionalisation, which is an essential feature for coupling the ligand to the target molecule, and the fact that this ligand system does not lead to the formation of *syn/anti* isomers.

In connection with our interest in the preparation of new substitution-inert technetium and rhenium compounds, we have reported, in previous works,<sup>6</sup> and <sup>7</sup> the synthesis of a new family of nonfunctionalised semi-rigid Ph-XN<sub>2</sub>S-type ligands (X = O, N or S) characterised by the presence of an aromatic cycle in the framework to favour and stabilise the chelate ring of the corresponding complexes by an entropic effect. The reactivity of these ligands with technetium-99m and the stability of the corresponding radiocomplexes were also investigated. These ligands produced unique and highly stable mono oxotechnetium complexes in the following order for the *ortho* substituent: OH > NH<sub>2</sub> > Strt.<sup>7</sup>

In order to label biomolecules with technetium-99m or rhenium-186/188 via this kind of ligand, we decided to develop a novel BFCA derived from our best candidate Ph-ON<sub>2</sub>S. The functionalisation of multidentate ligands often needs time-consuming multistep syntheses. So, the aim of this work was to produce an easily synthesised BFCA derived from Ph-ON<sub>2</sub>S and to characterise its suitability to complex Tc and Re. In this paper, we report a simple and convenient route of such a ligand, their complexes with rhenium (V) and the *in vivo* behaviour in normal rats

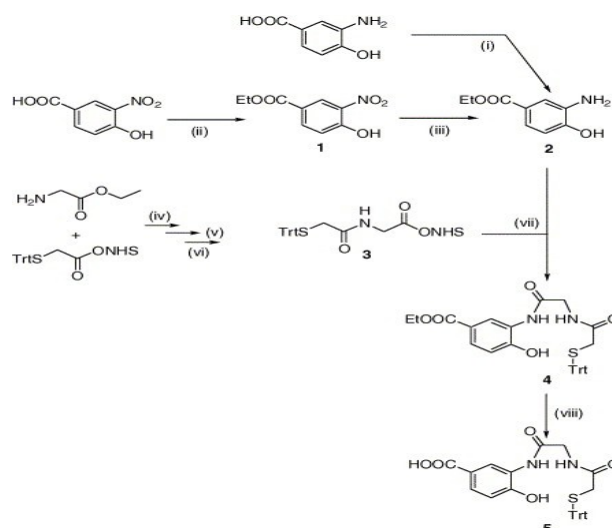
of the corresponding  $^{99m}\text{TcO}$ -complex.

## 2. Results and discussion

### 2.1. BFCA synthesis

To link to the target molecule, we selected the carbonyl group since many biomolecules contain amino groups available for amide bond formation. The synthesis of compound **5** was performed in seven steps as outlined in Scheme 1.

Scheme 1. Synthetic route of ligand **5**. Reagents and conditions: (i)  $\text{HCl}_g$ , EtOH, 40%; (ii)  $\text{HCl}_g$ , EtOH, 95%; (iii)  $\text{H}_2$ , Pd/C, MeOH, 92%; (iv)  $\text{Et}_3\text{N}$ , THF/DMF, rt, 98%; (v) NaOH 1 M, MeOH, 50 °C then HCl 6 M, 99%; (vi) NHS/DCC, THF, rt, 85%; (vii) **3**, DMAP, 60°,  $\text{CH}_3\text{CN}$ , 92%; (viii) NaOH 1 M, MeOH, 75° then HCl 3 M, 63%.



The first key step was the preparation of ethyl 3-amino-4-hydroxybenzoate **2**. Papachristou and coll.<sup>8</sup> proposed a one-step synthesis for the structural isomer ethyl 4-amino-3-hydroxybenzoate by refluxing 4-amino-3-hydroxybenzoic acid in ethanol in the presence of HCl. Anyway, neither yield nor experimental or spectroscopic details were given. Despite the apparent simplicity of this method, compound **2** has never been obtained from commercially available 3-amino-4-hydroxybenzoic acid, in this way, with satisfactory yields (40% maximum). So, an alternative pathway was investigated to prepare this compound in excellent yield. The synthesis of **2** involved a two-step reaction procedure. The first step consisted in protection of the carboxylic acid function of 4-hydroxy-3-nitrobenzoic acid by a classical acid esterification reaction in ethanol, giving **1**. The second step consisted in reduction of the nitro function of **1** by a palladium on charcoal-promoted hydrogenation reaction in MeOH. The synthesis of precursor **2** was achieved with a global yield of 87%.

Precursor **3** was prepared in three steps from a previously described protocol.<sup>7</sup> After an amide coupling reaction between ethyl glycinate and *N*-hydroxysuccinimide-

(triphenylmethylthio)ethanoate<sup>9</sup> in the presence of Et<sub>3</sub>N in a THF/DMF mixture, basic hydrolysis of the ethyl ester function followed by the activation of the acid function with NHS-DCC afforded the precursor **3** in 82% overall yield. The aromatic core **2** reacted with intermediate **3** in the presence of DMAP in acetonitrile to give **4** in 92% yield.

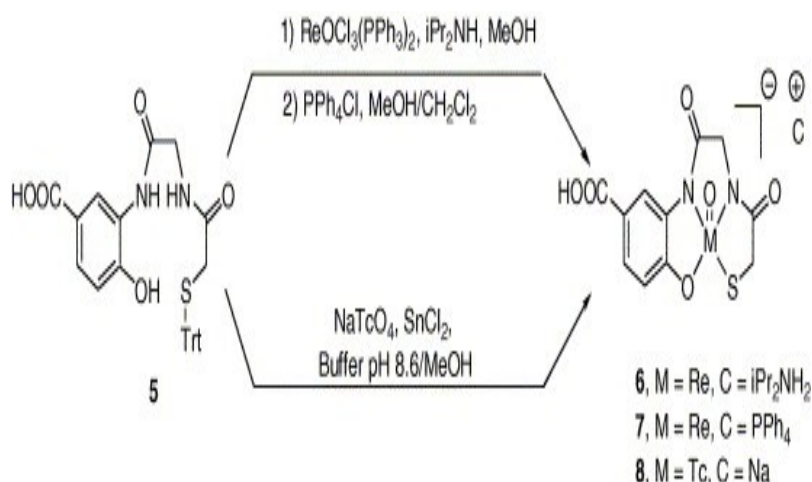
The final step consisted in a basic hydrolysis reaction. Several classical saponification conditions<sup>10</sup> (KOH or NaOH in alcohol at rt) were attempted to obtain the carboxylic acid **5**, but without success. Recently, Ziessel reported an alternative method to hydrolyse similar ethyl esters (ethyl aryl esters bearing amide functions on the aromatic core) using a large excess of NaOH (50 equiv) in a mixture of water and THF at reflux.<sup>11</sup> According to this methodology, we have performed the saponification by heating compound **4** in the presence of an excess of NaOH using methanol or THF/MeOH mixture as a solvent. The progress of the reaction, followed by TLC, showed that a temperature  $\geq 75$  °C is required. Under this temperature, the saponification only occurred partially. After 4 h at 75 °C in methanol, all the starting material **4** was consumed. Controlled acidification then provided the expected acid **5**, which was isolated pure by column chromatography with an acceptable yield (63%). Analytical studies indicate, without ambiguity, formation of the expected carboxylic acid **5**. However, we have noticed the formation of by-products as responsible for the significant decrease of the isolated yield. 2-(Triphenylmethylthio)ethanoic acid, resulting in the break of the non-aromatic amide bond of compound **4** during saponification step, was isolated in 10% yield and clearly identified by proton NMR.

The BFCA **5** was performed in seven steps with an overall yield of 42%, is stable at room temperature and does not require any particular condition for long-term storage.

## 2.2. Rhenium chemistry

The oxorhenium complex **6** was synthesised in good yield using a ligand exchange reaction with our ligand **5** and a slight excess of labile ReO(PPh<sub>3</sub>)<sub>2</sub>Cl<sub>3</sub><sup>12</sup> in the presence of diisopropylamine as deprotonating agent (Scheme 2). In these conditions, the cleavage of trityl group was accomplished during the coordination of the ligand to the ReO<sup>3+</sup> core and this is in agreement with the acidic contribution of the metal in the mechanism of sulfur detritylation.<sup>13</sup> The presence of diisopropylammonium chloride as a persistent contaminant, even after column chromatography purification, suggested the replacement of the counteranion with larger PPh<sub>4</sub><sup>+</sup>. The exchange reactions were performed in dichloromethane/methanol solutions at room temperature. Under these conditions, analytically pure complex **7** was obtained in 69% yield (Scheme 2).

Scheme 2. Preparation of the oxorhenium and oxotechnetium complexes.



These two oxorhenium complexes were unambiguously identified by conventional analytical techniques and their spectroscopic characteristics were similar to those obtained for nonfunctionalised complexes,<sup>7</sup> that is, the complexes exhibit a distorted square pyramidal geometry with yl-oxygen in the apical position. Briefly, IR spectra showed an intense band attributable to the  $\text{Re}=\text{O}$  stretching vibration<sup>14</sup> at 957 and 960  $\text{cm}^{-1}$  for complexes **6** and **7**, respectively. Methylene protons of the tetradentate ligand framework were found to be diastereotopic (AB pattern) with coupling constants in the range of 17–18.5 Hz. According to the literature,<sup>13</sup> we assigned the downfield signal of both AB patterns to the *endo* protons (*syn* to the  $\text{Re}=\text{O}$  group) and the upfield signals to the *exo* ones (*anti* to the  $\text{Re}=\text{O}$  group). Finally, negative-ion electrospray of each complex presents two prominent ion peaks with an isotope distribution pattern consistent with the monomeric anion.

As expected, this structural study confirmed the presence of a unique isomer with respect to the carbonyl function. Moreover, the carboxyl group does not coordinate to the rhenium core and thus it could be used for the coupling to a biomolecule.

### 2.3. <sup>99m</sup>Tc radiolabelling and biodistribution in rats

Trityl deprotection and <sup>99m</sup>Tc-labelling of the ligand **5** were performed in situ in a methanol/buffer solution, pH 8.6 (1/4: v/v), by direct reduction of sodium pertechnetate in the presence of an excess of tin chloride at 40 °C during 30 min (Scheme 2). Under these mild conditions, the labelling reaction was quantitative and only one negatively charged <sup>99m</sup>TcO- product was observed. Purification of <sup>99m</sup>TcO-complex **8** was accomplished by C-18 reverse-phase HPLC and resulted in only one component with an excellent radiochemical purity (>95%).

Preliminary biological evaluation of HPLC-isolated complex **8** was performed in healthy rats at 5 and 30 min pi. Complex **8** showed a reasonably high liver uptake (14% ID at 5 min post-injection), but was preferentially eliminated via the renal-urinary excretion route, as revealed by the 37% ID in urine at 30 min post-injection (Table 1), indicating the hydrophilic character of the radiocomplex. The higher concentration of radioactivity was measured in kidneys at all times studied (9.74, 2.89% ID/g).

Table 1.

Biodistribution data (% of ID/organ)<sup>a</sup> of the <sup>99m</sup>TcO-complex **8** in normal rats at different time points (pi)

	5 min	30 min
Blood	29.54 ± 0.09	6.55 ± 0.45
Brain	0.06 ± 0.01	0.02 ± 0.01
Heart	0.41 ± 0.03	0.13 ± 0.01
Intestine	4.53 ± 0.17	15.74 ± 2.12
Kidneys	25.29 ± 2.48	7.26 ± 2.34
Liver	13.91 ± 1.37	4.7 ± 0.83
Lung	0.79 ± 0.12	0.37 ± 0.04
Pancreas	1.17 ± 0.17	0.57 ± 0.08
Spleen	0.20 ± 0.05	0.05 ± 0.01
Stomach	0.58 ± 0.20	0.51 ± 0.28
Urine	3.07 ± 1.18	37.15 ± 10.42

<sup>a</sup> Values represent means ± SD ( $n = 3$ ).

The rapid clearance of this compound from the bloodstream, reflected in the low blood activity, indicated its high stability against exchange reactions with blood proteins and no specific uptake or long-term retention in organs or tissues. The biodistribution studies also indicated that little, if any, in vivo decomposition of complex **8** occurred. The minimal activity accumulation in the stomach ( $0.51 \pm 0.28\%$  ID/organ at 30 min) and in spleen ( $0.05 \pm 0.01\%$  ID/organ at 30 min) indicated that  $^{99m}\text{TcO}_4^-$  was not produced in relevant amount during biodistribution.<sup>15</sup> The observed stability provides important evidence that this ligand system is capable of stabilising  $^{99m}\text{Tc}$  even under in vivo conditions.

### **3. Conclusion**

In short, the synthetic protocol described herein provides an easy access to an original BFCA including an aromatic cycle in the chelate ring, in seven steps with an overall yield of 42%, the rate-limiting step being the final hydrolysis step. This compound produced well-defined ReO complexes. Moreover, this BFCA formed a stable and inert  $^{99m}\text{TcO}$ -complex in high yield, under mild conditions, without the formation of isomers. In vivo study demonstrates the high stability of the radiocomplex and shows that this chelate is efficiently cleared to a great extent from the bloodstream via the renal-urinary excretion route and to a less extent via the hepatobiliary pathway. No specific uptake or retention in the kidneys or other organs was observed. All these significant results justify its use for labelling peptides with  $^{99m}\text{Tc}$ . Development of new target-specific radioimaging agents with this new kind of semi-rigid BCFA is currently underway and the results will be disclosed in the near future.

## 4. Experimental

All chemicals were of the highest purity commercially available and all solvents were freshly distilled by standard methods before use. Column chromatography was carried out using 'gravity' silica (Merck). Rhenium(VII) oxide, purchased from Aldrich Chem. Co, was converted to  $\text{ReOCl}_3(\text{PPh}_3)_2$  according to published protocols.<sup>12</sup> The intermediate **3** has been prepared as described previously.<sup>7</sup> NMR spectra were recorded on a Bruker AC 250 (62.896 MHz for  $^{13}\text{C}$  and 250.133 MHz for  $^1\text{H}$ ) or 400 (100.63 MHz for  $^{13}\text{C}$  and 400.133 MHz for  $^1\text{H}$ ). Chemical shifts are indicated in  $\delta$  values (ppm) downfield from internal TMS, and coupling constants ( $J$ ) are given in Hertz (Hz). Infrared spectra were recorded as KBr pellets on a Bruker Vector 22 spectrophotometer in the range 4000–400  $\text{cm}^{-1}$ . Negative electrospray or DCI-Mass spectra were obtained on a NERMAG R 10-10 mass spectrometer. Microanalysis was performed by the microanalytical department of the Ecole Nationale Supérieure de Chimie de Toulouse. HPLC analysis and purification were achieved on a Waters 600E gradient chromatography with a Waters Lambda Max UV detector, a SAIP radioactivity detector and an ICS dual integrator for effluent monitoring and a Satisfaction RP18AB column (5  $\mu\text{m}$ , 125  $\times$  4.6 mm) using  $\text{MeOH-H}_2\text{O-TFA}$ : 55/45/0.1 as eluent (flow rate of 1 mL/min).

### 4.1. Ethyl 4-hydroxy-3-nitrobenzoate **1**

A solution of 4-hydroxy-3-nitrobenzoic acid (5.00 g, 27.3 mmol) in freshly distilled ethanol (150 mL) was saturated in  $\text{HCl}_g$ . The mixture was stirred at room temperature overnight and then the solvent was removed under reduced pressure to give **1** as a yellow powder (5.48 g, 95%).

$^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  (ppm): 1.40 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_3$ ), 4.38 (q, 2H,  $J = 7.1$  Hz,  $\text{OCH}_2$ ), 7.20 (d, 1H,  $J = 8.8$  Hz,  $\text{H}_{\text{Ar}}$ ), 8.22 (dd, 1H,  $J = 2.1$  and 8.8 Hz,  $\text{H}_{\text{Ar}}$ ), 8.80 (d, 1H,  $J = 2.1$  Hz,  $\text{H}_{\text{Ar}}$ ), 10.88 (s, 1H, OH);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (62.9 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  (ppm): 14.3 ( $\text{CH}_3$ ), 61.7 ( $\text{OCH}_2$ ), 120.3, 127.3, 137.8 ( $3\text{CH}_{\text{Ar}}$ ), 123.2, 133.2, 158.1 ( $3\text{C}_{\text{Ar}}$ ), 164.4 (CO); MS (DCI/ $\text{NH}_3$ ): 229 [ $\text{M}+\text{NH}_4^+$ ].

### 4.2. Ethyl 3-amino-4-hydroxybenzoate **2**

Catalytic hydrogenation of **1** (5.00 g, 23.7 mmol) in methanol (250 mL) over 10% Pd/C (20% w/w, 1.25 g) was carried out at atmospheric pressure. The mixture was stirred for 2 h, then the catalyst was filtered off through Celite and the solvent was removed under reduced pressure to give **2** as a grey solid (3.92 g, 92%).

$^1\text{H}$  NMR (250 MHz, MeOD)  $\delta_{\text{H}}$  (ppm): 1.33 (t, 3H,  $J = 7.1$  Hz,  $\text{CH}_3$ ), 4.27 (q, 2H,  $J = 7.1$  Hz,  $\text{OCH}_2$ ), 6.72 (d, 1H,  $J = 8.3$  Hz,  $\text{H}_{\text{Ar}}$ ), 7.30 (dd, 1H,  $J = 2.1$  and 8.3 Hz,  $\text{H}_{\text{Ar}}$ ), 7.40 (d,  $J = 2.1$  Hz,  $\text{H}_{\text{Ar}}$ );  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (62.9 MHz, MeOD)  $\delta_{\text{C}}$  (ppm): 14.9 ( $\text{CH}_3$ ), 61.6 ( $\text{OCH}_2$ ), 114.7, 118.0, 122.5 ( $3\text{CH}_{\text{Ar}}$ ), 122.9, 136.4, 151.3 ( $3\text{C}_{\text{Ar}}$ ), 168.8 (CO); MS (DCI/ $\text{NH}_3$ ): 182 [ $\text{M}+\text{H}^+$ ], 199 [ $\text{M}+\text{NH}_4^+$ ].

### 4.3. Ethyl 3{2-[(triphenylmethylsulfanyl)methylcarbonylamino]ethanamido}-4-hydroxybenzoate 4

To a solution of **2** (0.50 g, 2.76 mmol) and **3** (1.35 g, 2.76 mmol) in acetonitrile (40 mL) was added DMAP (0.34 g, 2.76 mmol). The solution was heated at 60 °C, under nitrogen, for 3 h. After cooling, the product was left to precipitate overnight at -18 °C. After filtration, the precipitate was washed with cold acetonitrile and then purified by column chromatography on silica gel (CHCl<sub>3</sub> then CHCl<sub>3</sub>-MeOH 95/5) to afford **4** as a white powder (1.41 g, 92%).

<sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ<sub>H</sub> (ppm): 1.28 (t, 3H, *J* = 7.1 Hz, CH<sub>3</sub>), 2.87 (s, 2H, CH<sub>2</sub>S), 3.86 (d, 2H, *J* = 5.6 Hz, CH<sub>2</sub>N), 4.25 (q, 2H, *J* = 7.1 Hz, OCH<sub>2</sub>), 6.94 (d, 1H, *J* = 8.3 Hz, H<sub>Ar</sub>), 7.30 (m, 15H, H<sub>Ar Trr</sub>), 7.58 (dd, 1H, *J* = 1.8 and 8.3 Hz, H<sub>Ar</sub>), 8.35 (s, 1H, NH), 8.53 (d, 1H, *J* = 1.8 Hz, H<sub>Ar</sub>), 9.25 (s, 1H, NH), 10.87 (s, 1H, OH); <sup>13</sup>C {<sup>1</sup>H} NMR (100.6 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> (ppm): 14.6 (CH<sub>3</sub>), 35.7 (CH<sub>2</sub>S), 44.6 (CH<sub>2</sub>N), 61.2 (OCH<sub>2</sub>), 68.3 (C<sub>Trr</sub>), 118.6 (CH<sub>Ar</sub>), 122.6 (C<sub>Ar</sub>), 124.1 (CH<sub>Ar</sub>), 125.5 (C<sub>Ar</sub>), 128.6 (CH<sub>Ar</sub>), 127.4, 128.5, 129.6 (15CH<sub>Ar Trr</sub>), 143.9 (3C<sub>Ar Trr</sub>), 152.6 (C<sub>Ar</sub>), 166.5, 168.3, 170.3 (3CO); MS (DCI/NH<sub>3</sub>): 555 [M+H<sup>+</sup>], 572 [M+NH<sub>4</sub><sup>+</sup>].

### 4.4. 3-{2-[(triphenylmethylsulfanyl)methylcarbonylamino]ethanamido}-4-hydroxybenzoic acid 5

To a solution of **4** (2.00 g, 3.61 mmol) in MeOH (180 mL) was added a solution of 1 N NaOH (140 mL). After 4 h at 75 °C, the mixture was cooled and 3 N HCl was added until the solution became acidic. The solution was concentrated (to eliminate the MeOH) and then extracted with AcOEt (4 × 60 mL). The organic layer was separated, dried over sodium sulfate, filtered off and concentrated to dryness under reduce pressure. The residue was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>-AcOEt 6/4 then CH<sub>2</sub>Cl<sub>2</sub>-MeOH 95/5) to give **5** as a white powder (1.20 g, 63%).

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ<sub>H</sub> (ppm): 2.89 (s, 2H, CH<sub>2</sub>S), 3.85 (d, 2H, *J* = 5.4 Hz, CH<sub>2</sub>N), 6.92 (d, 1H, *J* = 8.4 Hz, H<sub>Ar</sub>), 7.33 (m, 16H, H<sub>Ar</sub> + 15H<sub>Ar Trr</sub>), 7.55 (d, 1H, *J* = 8.4 Hz, H<sub>Ar</sub>), 8.35 (s, 1H, NH), 8.49 (s, 1H, NH), 9.23 (s, 1H, OH); <sup>13</sup>C {<sup>1</sup>H} NMR (100.6 MHz, DMSO-*d*<sub>6</sub>) δ<sub>C</sub> (ppm): 36.5 (CH<sub>2</sub>S), 43.9 (CH<sub>2</sub>N), 66.7 (C<sub>Trr</sub>), 115.4 (CH<sub>Ar</sub>), 121.9 (C<sub>Ar</sub>), 123.5 (CH<sub>Ar</sub>), 126.4 (C<sub>Ar</sub>), 127.1 (CH<sub>Ar</sub>), 127.5, 128.8, 129.8 (15CH<sub>Ar Trr</sub>), 144.7 (3C<sub>Ar Trr</sub>), 151.1 (C<sub>Ar</sub>), 168.2, 168.6 (3CO); MS (DCI/NH<sub>3</sub>): 527 [M+H<sup>+</sup>], 544 [M+NH<sub>4</sub><sup>+</sup>]; IR (KBr): ν<sub>C=O</sub> = 1683, 1652, 1602 cm<sup>-1</sup>; elemental analysis found C, 68.37; H, 5.05; N, 4.96% C<sub>30</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S requires C, 68.42; H, 4.98; N, 5.32%.

### 4.5. [iPrNH<sub>2</sub>][ReO{(COOH)Ph-ON<sub>2</sub>S}] 6

To **5** (157.8 mg, 0.3 mmol) and diisopropylamine (1.68 mL, 0.12 mmol) dissolved in dry methanol (40 mL) was added ReOCl<sub>3</sub>(PPh<sub>3</sub>)<sub>2</sub> (324.5 mg, 0.39 mmol). After refluxing for 4 h, the solution was cooled, filtered and then evaporated to dryness. The residue was purified by column chromatography on silica gel (eluent: CHCl<sub>3</sub>-MeOH: 95/15 then 90/10) to yield the complex **6** as a dark red powder (155 mg, 88%).

<sup>1</sup>H RMN (400 MHz, MeOD): δ<sub>H</sub> = 1.29 (m, 12H, CH<sub>3</sub>), 3.45 (m, 2H, CHN), 3.75 (d, 1H,



$J = 17.2$  Hz, CH<sub>2</sub>S), 4.08 (d, 1H,  $J = 17.2$  Hz, CH<sub>2</sub>S), 4.49 (d, 1H,  $J = 18.4$  Hz, CH<sub>2</sub>N), 5.44 (d, 1H,  $J = 18.4$  Hz, CH<sub>2</sub>N), 7.04 (d, 1H,  $J = 8.2$  Hz, H<sub>Ar</sub>), 6.66 (dd, 1H,  $J = 8.2$  and 1.8 Hz, H<sub>Ar</sub>), 8.90 (m, 1H, H<sub>Ar</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (100.6 MHz, MeOD):  $\delta_C = 8.0$  (6CH<sub>3</sub>), 39.9 (CH<sub>2</sub>S), 46.8 (2CHN), 60.6 (CH<sub>2</sub>N), 114.5, 119.5 (2CH<sub>Ar</sub>), 121.4 (C<sub>Ar</sub>), 126.1 (CH<sub>Ar</sub>), 140.2, 169.4 (2 C<sub>Ar</sub>), 174.1, 187.2, 194.2 (3CO); MS (ES<sup>-</sup>),  $m/z$  (%): 481 (60), 483 (100) [M<sup>-</sup>]; IR (KBr):  $\nu_{\text{Re}=\text{O}} = 960$  cm<sup>-1</sup>.

#### 4.6. [PPh<sub>4</sub>][ReO{(COOH)Ph-ON<sub>2</sub>S}] 7

Compound **6** (128.5 mg, 0.22 mmol) and tetraphenylphosphonium chloride (89.2 mg, 0.24 mmol) were dissolved in a mixture of MeOH (15 mL) and CH<sub>2</sub>Cl<sub>2</sub> (15 mL). After 30 min under stirring, the solvent was removed and the crude mixture was purified by column chromatography on silica gel (eluent: CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 95/5 then 90/10) to yield the complex **7** as an orange powder (125.0 mg, yield = 69%).

<sup>1</sup>H RMN (400 MHz, CDCl<sub>3</sub>):  $\delta_H = 3.75$  (d, 1H,  $J = 17.1$  Hz, CH<sub>2</sub>S), 4.05 (d, 1H,  $J = 17.1$  Hz, CH<sub>2</sub>S), 4.41 (d, 1H,  $J = 18.3$  Hz, CH<sub>2</sub>N), 5.39 (d, 1H,  $J = 18.3$  Hz, CH<sub>2</sub>N), 6.97 (d, 1H,  $J = 8.3$  Hz, H<sub>Ar</sub>), 7.51 (m, 9H, H<sub>Ar</sub> + H<sub>Ar PPh4</sub>), 7.68 (m, 8H, H<sub>Ar PPh4</sub>), 7.82 (m, 4H, H<sub>Ar PPh4</sub>), 8.83 (m, 1H, H<sub>Ar</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (100.6 MHz, CDCl<sub>3</sub>):  $\delta_C = 40.1$  (CH<sub>2</sub>S), 61.0 (CH<sub>2</sub>N), 116.9, 118.0, 119.9 (3CH<sub>Ar</sub>), 115.2 (4C<sub>Ar PPh4</sub>), 130.5, 130.7, 134.2, 134.3, 135.7, 135.8 (20CH<sub>Ar PPh4</sub>), 141.0, 171.5 (2C<sub>Ar</sub>), 175.3, 186.5, 193.3 (3CO); MS (ES<sup>-</sup>):  $m/z$  (%) 481 (60), 483 (100) [M<sup>-</sup>]; IR (KBr):  $\nu_{\text{C}=\text{O}} = 1644, 1635, 1610$  cm<sup>-1</sup>,  $\nu_{\text{Re}=\text{O}} = 957$  cm<sup>-1</sup>. Found: C, 51.19; H, 3.52; N, 3.24; C<sub>35</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>PreS requires C, 51.15; H, 3.43; N, 3.41.

#### 4.7. <sup>99m</sup>Tc labelling

Ligand **5** (100  $\mu$ L of a freshly prepared stock methanolic solution of 1 mg/mL of **5**) was added to a buffer solution pH = 8.6 (200  $\mu$ L). Successively were added SnCl<sub>2</sub>·2H<sub>2</sub>O (75  $\mu$ L of a freshly prepared solution of 9 mg of SnCl<sub>2</sub>·2H<sub>2</sub>O in a mixture of methanol (4 mL) and HCl concentrated (0.5  $\mu$ L)) and <sup>99m</sup>Tc-pertechnetate solution generator eluate (100  $\mu$ L, 74 MBq). The vial was sealed with a Teflon-lined cap and the mixture was heated at 40 °C for 30 minutes. After cooling, the resulting complex was analysed and purified with the HPLC system described before. The retention time of **8** was 3.88 min.

#### 4.8. Biodistribution in healthy rats

All experiments were carried out in compliance with French laws relating to the conduct of animal experimentation.

Before being used in the animal studies, purified complex **8** solution was filtered through a 0.22  $\mu$ m sterile filter (Millipore<sup>®</sup>) and diluted with sterile saline solutions. Male Wistar rats, about 400 g and anaesthetised with Nesdonal<sup>®</sup>, were sacrificed 5 and 30 min post-injection (pi) ( $n = 3$ ) after an intra-jugular injection of 300  $\mu$ L of the diluted tracer solution. The organs of interest (liver, spleen, heart, lungs, kidneys and brain) were dissected, weighed and their radioactivity was

measured in a Packard autogamma counter. Results, expressed as percentage of injected dose per organ (% ID/organ), are summarized in [Table 1](#). The percentage radioactivity in the blood was calculated assuming that the whole-blood volume was 7% of the body weight.

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