Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 29 July 2013, Revised 25 November 2013, Accepted 10 December 2013 Published online 14 January 2014 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3182

Synthesis and preliminary biological evaluation of the first ^{99m}Tc(I)-specific semi-rigid tridentate ligand based on a click chemistry strategy

Sihem Guizani,[a]* Nadia Malek Saied,[a] Claude Picard,[b,c] Eric Benoist,[b,c]** and Mouldi Saidi[a]

A novel bifunctional chelating agent based on a click chemistry strategy has been synthesized and characterized on the basis of spectroscopic techniques. The metal chelating part of this new class of tridentate N_2O ligand combined a triazole unit and an aromatic ring. This latter semi-rigid framework induced a pre-organization of the chelating cavity, improving the stability of the corresponding metallic complexes (M = ^{99m}Tc, Re). Thus, the ^{99m}Tc(CO)$_3$ complex, obtained with good yield and excellent radiochemical purity (>90%), exhibited a high *in vitro* serum stability. Tissue biodistribution in normal mice showed a rapid clearance, no long-term retention in organs and no *in vivo* reoxidation of technetium-99m, making this compound a promising ^{99m}Tc-chelating system.

Keywords: bifunctional chelating agent; technetium-99m; tissue distribution; click chemistry

Introduction

Among non-invasive imaging modalities, single photon computer tomography and positron emission tomography nuclear imaging techniques are characterized by a nanomolar range sensitivity, which minimizes potential *in vivo* toxicity and allows whole-body imaging. Although numerous radionuclides with suitable decay properties for diagnostic applications have been used for both nuclear techniques, technetium-99m still remains a candidate of choice for nuclear imaging purposes. For example, it is one of the most used single photon computer tomography radioisotopes for labeling bioactive peptides.[1] Additionally, more than 80% of radio-imaging probes are based on ^{99m}Tc-complexes, the extensive use of this radiometal being mainly due to its ideal imaging features (140 keV γ emitter, relatively short half life of 6.02 h) combined with a convenient availability at low cost from the commercial ^{99}Mo/^{99m}Tc generator.[2] Many different bifunctional chelating agents (BCAs) allowing the formation of kinetically inert ^{99m}Tc-complexes and their grafts to a targeting biovector have been developed.[3] The structure of these chelators is largely influenced by the oxidation states of the technetium-99m and also by the nature of its metallic cores. Thus, if the chemistry of Tc(V) species, mainly $[TcO]^{3+}$ and $[TcN]^{2+}$ cores, has firstly dominated the literature,[4] an original organometallic strategy, so-called 'tricarbonyl core' approach, pioneered by Schubiger's team,[5] has gained considerable attention in the last decade. The high chemical inertness and the ease of preparation of the water-soluble and air-stable *fac*-[^{99m}Tc(CO)$_3$(H$_2$O)$_3$]$^+$ core combined with its great availability in the form of a kit formulation (Isolink™ kit) brought new hopes and perspectives concerning the research of efficient ^{99m}Tc(I) complexes for molecular imaging applications.

If a plethora of ligands ranging from bidentate to tridentate species have been investigated for the tricarbonyltechnetium core, in our opinion, the most elegant and promising chemical strategy remains the click-to-chelate approach reported recently by Schibli *et al.*[6] Using the copper(I)-catalyzed alkyne-azide cycloaddition reaction (CuAAC reaction), so-called click chemistry,[7] he showed that this strategy allowed the formation of new technetium-specific chelating systems in a few steps and with high yield, without the need for further purification, and proceeding under friendly conditions, in aqueous media at room temperature. More interestingly, he demonstrated that the 1,2,3-triazole moiety itself could be considered as a promising chelating system for the *fac*-[^{99m}Tc(CO)$_3$]$^+$ core.[6] Therefore, with the great versatility of this synthetic approach,

[a]*Radiopharmaceutical Unit, Centre National des Sciences et Technologies Nucléaires, Sidi Thabet 2020, Tunisia*

[b]*SPCMIB, UMR 5068, CNRS; Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, 118, route de Narbonne, F-31062, Toulouse, France*

[c]*UPS; Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR 5068, Université de Toulouse, 118, route de Narbonne, F-31062, Toulouse, France*

**Correspondence to: Sihem Guizani, Radiopharmaceutical Unit, Centre National des Sciences et Technologies Nucléaires, Sidi Thabet 2020, Tunisia.*
E-mail: sihem.guizani@gmail.com

***Correspondence to: Eric Benoist, UPS; Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, SPCMIB, UMR 5068, Université de Toulouse, 118, route de Narbonne, F-31062 Toulouse, France.*
E-mail: benoist@chimie.ups-tlse.fr

a wide range of ^{99m}Tc(I)-specific click ligands have been reported by several research groups,[8] including ours.[9] Most of tridentate (or polydentate) click chelators have been built on the same model, the chelating cavity being constituted by the 1,2,3-triazole ring and additional metal chelating arms connected at the N1 and C4 positions of the triazole unit. It has been suggested that a combination of an aromatic amine with an aliphatic amine and carboxylate group gave tricarbonyltechnetium complexes, which exhibited high stability in aqueous solutions.[10] Surprisingly, if numerous ^{99m}Tc(I)-specific tridentate ligands based on a triazole framework have been developed, to the best of our knowledge, none of them possess a chelating cavity including a pre-organized moiety. It is well-known that the rigidity of the molecular skeleton reduces the freedom of donor atoms, and this spatial pre-organization favors and stabilizes the chelate ring by an entropic effect and improves the stability of the corresponding metallic complexes.[11] Bearing that in mind, we anticipated that the formation, using a two-steps click-to-chelate strategy, of a semi-rigid chelating system containing an aromatic backbone and the triazole unit, should lead in a few steps and with high yield to the corresponding very stable $Tc(CO)_3$-complex. Even though any semi-rigid ligand has yet been synthesized, the conception of pre-organized chelating systems using a click-to-chelate approach have been recently highlighted for the preparation of new triazole-oxotechnetium complexes.[12] So in this paper, we described a simple, rapid, and convenient synthetic route for the first ^{99m}Tc(I)-specific semi-rigid tridentate click ligand, which incorporates the design features illustrated in Scheme 1; these include the following: (i) a methyl carboxylate arm directly connected at the N1 position of the five-membered triazole ring as potential bioconjugation site and (ii) a N_2O tridentate chelating system combining the 1,2,3-triazole moiety with 2-aminophenol derivative. ^{99m}Tc-radiolabelling of the ligand as well as *in vitro* and *in vivo* behaviors in normal mice of the corresponding ^{99m}Tc-complex will be discussed.

Materials and methods

Chemicals and equipment

All purchased chemicals were of the highest purity commercially available and used without further purification. Reactions were monitored by analytical thin layer chromatography on Merck D.C.-Alufolien Kieselgel 60 F254. Chromatographic purification was conducted using silica gel obtained from Merck. Technetium-99m as sodium pertechnetate [$Na^{99m}TcO_4$] was obtained in physiological saline as commercial ^{99}Mo/^{99m}Tc generator systems. Isolink kit™ for preparation of the ^{99m}Tc-tricarbonyl complex was donated by Mallinckrodt Medical B.V. (the Netherlands). Methyl azidoacetate **1** was prepared as previously described.[13]

^{1}H and ^{13}C-NMR spectra were recorded at 300 (75.5) MHz with a Bruker AC-300 (Bruker Corp., Billerica, MA, USA) spectrometer. Chemical shifts are reported in parts per million, and coupling constants (*J*) are given in Hertz (Hz). Infrared spectra were recorded on a Perkin Elmer FTIR 1725 spectrophotometer (Perkin-Elmer Inc., Waltham, MA, USA) in the range 4000–400 cm^{-1}. Mass spectra (desorption chemical ionization (DCI), positive mode) were obtained on an LCT Premier Waters spectrometer (Waters Corp., MA, USA). Microanalysis was performed by the microanalytical department of the Laboratory of Coordination Chemistry (Toulouse, France). HPLC analysis was performed on an SCL-10Avp SHIMADZU HPLC system coupled to a UV-Absorbance detector from ICS and a Gabi gamma detector from Raytest. Separations were achieved on a reverse phase C-18 column 250 × 4.6-mm (Shim-pack VP-ODS, SHIMADZU) eluted with a binary gradient system at a flow rate of 1 mL/min. Mobile phase A was methanol containing 0.1% trifluoroacetic acid, whereas mobile phase B was water containing 0.1% trifluoroacetic acid. The elution profile was 0–1 min 100% B, followed by a linear gradient to 30% B in 10 min; this composition was held for another 10 min. After a column wash with 95% A for 5 min, the column was re-equilibrated by applying the initial conditions (100% B) for 15 min prior to the next injection.

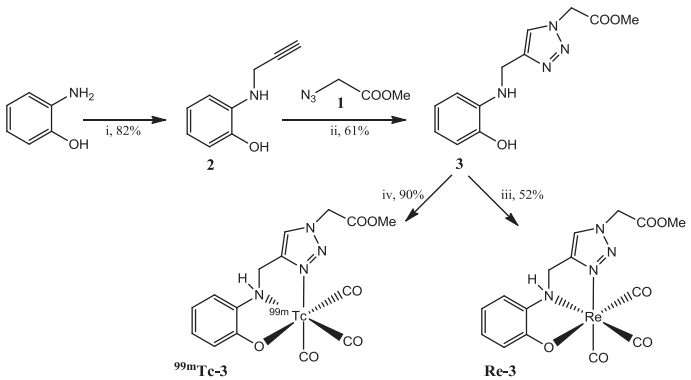
Chemical synthesis

CAUTION : Sodium azide, when inhaled, is highly toxic. Precautions must be taken when weighing the material such as using a powder mask and a Teflon spatula. The azidation reactions should be performed behind a plastic shield because of the potential explosion.

*Synthesis of 2-(prop-2-yn-1-ylamino)phenol (**2**)*

The reagent was prepared following an improved published procedure.[14]

To an ethanolic solution (70 mL) of 2-aminophenol (2.5 g, 23 mmol), propargyl bromide (0.5 mL, 4.5 mmol) was added over a 40-min period. After 4 days at ambient temperature, the solution was concentrated and diethylether (50 mL) was added to the residue. The precipitate was filtered off and the filtrate concentrated



Scheme 1. Synthetic routes of tridentate N_2O ligand and its Re- and ^{99m}Tc-complexes. Reaction conditions: i: propargylbromide, EtOH, r.t., 4 days; ii: **1**, $Cu(OAc)_2.H_2O$, NaAsc, $tBuOH/H_2O$, r.t., one night; iii: $[Re(CO)_5Cl]$, MeOH, 65°, one night; iv: Isolink kit™, 70°, 15 min.

to dryness. Purification by column chromatography on silica gel using pentane/AcOEt (75/25) as eluent gave the title compound as an orange powder (531 mg).

Yield: 82%. ^{1}H NMR ($CDCl_3$): δ (ppm) = 2.25 (t, J = 2.1 Hz, 1H, CH), 3.97 (d, J = 2.1 Hz, 2H, CH_2), 4.60 (br s, 2H, OH + NH), 6.74–6.93 (m, 4H, H_{Ar}); MS (DCI) = 148.1 $[M+H]^+$, 165.1 $[M+NH_4]^+$.

*Synthesis of methyl 2-[4-(2-Hydroxyphenylamino)methyl)-1H-1,2,3-triazol-1-yl]acetate, (**3**)*

Methyl azidoacetate **1** (437 mg, 3.8 mmol), alkyne derivative **2** (527 mg, 3.6 mmol), copper(II) acetate monohydrate (139 mg, 0.7 mmol), and sodium ascorbate (277 mg, 1.4 mmol) were mixed in an equivolumic mixture of water/*tert*-butanol (50 mL) and stirred overnight at room temperature. The resulting green solution was diluted with ethyl acetate (40 mL) and washed twice with saturated Na_2EDTA solution (2 × 40 mL). The aqueous solutions were extracted with ethyl acetate (3 × 20 mL). The organic extracts were combined, dried over Na_2SO_4, and the solvent was taken off under reduce pressure. The crude product was purified by column chromatography on silica gel using AcOEt/pentane (60/40) as eluent. A total of 576 mg of **3** were obtained as a green solid.

Yield: 61%. ^{1}H NMR (CD_3OD): δ (ppm) = 3.77 (s, 3H, CH_3), 4.46 (s, 2H, CH_2), 5.22 (s, 2H, CH_2), 6.53-6.71 (m, 4H, H_{Ar}), 7.85 (s, 1H, H_{ta}); ^{13}C-NMR (CD_3OD): δ (ppm) = 40.7 (CH_3), 52.9 (CH_2), 54.5 (CH_2), 114.0, 116.0, 120.1, 122.4 (CH_{Ar}), 126.7 (CH_{ta}), 139.2, 147.5, 149.5 (Cq), 170.1 (CO); MS (DCI) = 263.2 $[M+H]^+$, 280.2 $[M+NH_4]^+$.

*Synthesis of the tricarbonylrhenium complex, (**Re-3**)*

It was prepared by a substitution route from commercial $[Re(CO)_5Cl]$. A solution of **3** (25 mg, 95 μmol), Et_3N (13.3 μL, 95 μmol), and $[Re(CO)_5Cl]$ (38 mg, 10.5 μmol) in MeOH (3 mL) was stirred overnight at 65 °C. After cooling to room temperature, the solution was concentrated to dryness, and the resulting residue purified by column chromatography on silica gel using CH_2Cl_2/MeOH (95/5) as eluent. A total of 26.5 mg of $Re(CO)_3$ complex were obtained as a green solid.

Yield: 52%. MS (DCI) = 533 $[M+H]^+$, 561 $[M+C_2H_5]^+$; IR (KBr): $\nu_{(NH)}$ = 3343, $\nu_{(C\equiv O)}$ = 2018, 1915, 1884; $\nu_{(C=O)}$ = 1750 cm^{-1}, $C_{15}H_{13}N_4O_6Re$: calcd (found) C, 33.90 (33.95); H, 2.47 (2.25); N, 10.54 (10.30).

Radiochemical synthesis

Preparation of fac-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor

A total of 1000 μL (about 37 MBq) of freshly eluted $^{99m}TcO_4^-$ from a commercial generator were added to the Isolink kit containing sodium tetraborate (2.85 mg), sodium carbonate (7.15 mg), sodium boranocarbonate decahydrate (2.85 mg), and sodium tartrate dihydrate (8.5 mg). The mixture was incubated in boiling water for 30 min. After cooling, the pH was adjusted to 2 with 1 N HCl. Quality control of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor was performed by reverse phase HPLC (C-18 column).

*Synthesis of the technetium-99m complex, (**^{99m}Tc-3**)*

A solution of ligand **3** in methanolic solution (concentration: 1 mg/mL) was added to an aqueous solution of the *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor. After incubation at 70 °C for 15 min, an aliquot of the reaction mixture was analyzed by HPLC to check the complex formation. The labeling efficiency of the mixture was determined by thin layer chromatography on silica gel in ethyl acetate. The ^{99m}Tc-ligand purity and label stability were estimated by HPLC. Then, the resulting Tc-complex was obtained in 90% yield. After RP-HPLC purification (see previous text for elution conditions), complex **^{99m}Tc-3** was obtained with high radiochemical purity (>98%). The retention time of the Tc-complex (14.9 min) is similar to that of the cold rhenium (14.2 min), confirming the chemical identity of **^{99m}Tc-3**.

Partition coefficient determination

Lipophilicity was studied through the partition coefficient between *n*-octanol and phosphate buffer (0.125 M, pH 7.4). In a centrifuge tube containing 500 μL of each phase, 100 μL of the purified ^{99m}Tc complex solution was added, and the mixture was vortexed 1 min at ambient temperature, followed by centrifugation at 5000 rpm for 5 min. A total of 100-μL aliquots of both buffer and organic layers were counted using a NaI well-type gamma counter (OKIDATA, MICROLINE 320). The partition coefficient was calculated using the formula: log P = counts in *n*-octanol/counts in buffer. The reported value represents the average of triplicate measurements.

In vitro stability

The *in vitro* stability of the purified complex **^{99m}Tc-3** was evaluated at different time points using the following procedure: in a borosilicated vial, **^{99m}Tc-3** (100 μL) was added to 1 mL of fresh human serum at 37 °C. Aliquots were withdrawn in duplicate during the incubation at different time intervals till 24 h and treated with 1 mL of acetonitrile to precipitate proteins. After centrifugation (2000 rpm for 5 min), the supernatant was subjected to HPLC using the same system used for quality control. Any increase in the free pertechnetate was considered as the degree of degradation. The complex was found to be stable.

Protein binding assay

The protein binding assay of the purified complex **^{99m}Tc-3** was evaluated at different time points using the following procedure: in a borosilicated vial, **^{99m}Tc-3** (100 μL) was added to 5 mL of fresh human plasma at 37 °C. Aliquots were withdrawn during the incubation at different time intervals till 24 h, and plasma was separated from blood samples by centrifugation (3000 rpm for 4 min); individual fractions were counted in a well counter.

The plasma aliquots were treated with 1 mL of acetonitrile to precipitate proteins. After centrifugation (2000 rpm for 5 min), the activities of both phases (supernatant and precipitate) were measured separately. The reported value represents the average of triplicate measurements.[15]

Biodistribution studies in normal mice

All experiments were carried out following the Tunisian principles of the guide to the care and use of experimental animals. Normal Swiss mice (males, 18–20 g) were obtained from the Pasteur institute of Tunis. Animals were housed for 1 day before the onset of the experiments in our laboratory housing facilities. The radiotracer **^{99m}Tc-3** (100 μL diluted in saline-EtOH (80/20), 3.7 MBq) was injected via a lateral tail vein. At different intervals after injection, the animals (n = 3) were sacrificed by cervical dislocation. Organs of interest, samples of blood, and muscles were collected, weighed, and counted. Bladder and excreted urine were not weighed. The calculation for blood and muscle were based upon measured activity, sample weight,

and body composition data (considering that blood comprises 7% and muscle 43% of body weight). Corrections by different sample geometry were applied when necessary. Results were expressed as percent dose per gram of tissue (%ID g^{-1}).

Results and discussion

Chemistry

The synthesis of the tridentate N_2O ligand **3** was performed in three steps as described in Scheme 1. Methyl azidoacetate **1** was prepared in 63% yield, according to literature protocols.[13] The key step was the introduction of the acetylenic group on the aromatic framework. We used an elegant and rapid route to synthesize the 2-(prop-2-yn-1-ylamino)phenol intermediate **2**.[14] By stirring propargyl bromide with a large excess of 2-aminophenol in absolute ethanol during 4 days, we obtained **2** in 82%. In these conditions, the presence of the hydroxyl group on the aromatic ring did not affect the reaction outcome, and no *O*-alkylation was observed using this procedure. According to our classical copper(I)-catalyzed alkyne-azide cycloaddition conditions,[16] compound **3** was obtained in modest yield through the action of one equivalent of **2** and a slight excess of **1**, in the presence of the Cu(II)/sodium ascorbate catalytic system in *t*BuOH/water solution. The modest yield could be explained considering **3** as a potential copper chelating ligand. The N_2O tridentate ligand is stable at room temperature and does not require any specialized condition for long-term storage.

A ^{99m}Tc-complex being used *in vivo* in very low concentration (10^{-9} M), its structural characterization is generally performed by HPLC-comparison with the corresponding analogous rhenium complex. Therefore, the tricarbonylrhenium complex starting from **3** was prepared in a non-optimized synthesis. By refluxing the ligand with a slight excess of the commercial $[Re(CO)_5Cl]$ precursor in methanol, in the presence of triethylamine as deprotoning agent, **Re-3** was obtained in 52% yield, after silica gel chromatography purification. Spectroscopic data were consistent with the proposed structure of the Re-complex. Briefly, elemental analysis and positive-ion MS spectrum are consistent with a neutral mononuclear complex with a metal-to-ligand ratio of 1:1. Additionally, the IR spectrum revealed firstly, the presence of N–H stretching band of secondary aromatic amine and secondly, three bands in the carbonyl stretching region (2018–1884 cm^{-1}), which is characteristic of a *fac*-octahedral tricarbonyl metal moiety. Moreover, any OH vibrator was observed in the IR spectrum. This proved the tridentate coordination of the metal-tricarbonyl core via the N_2O scaffold, the oxygen being on its anionic form (O^-) whereas the amino group remaining on its neutral NH form. The complex is soluble in all polar organic solvents and is stable to aerial oxidation.

The ^{99m} Tc-radiolabelling was performed by ligand exchange reaction using the freshly prepared *fac*-$[^{99m}Tc(CO)_3\ (H_2O)_3]^+$ intermediate as radiometal precursor, using a previously reported procedure.[17] Several parameters, such as pH, ligand concentration, temperature, and reaction time were optimized to obtain the best radiolabelling yield. Thus, the best labeling protocol involved the reaction of the *fac*-$[^{99m}Tc(CO)_3(H_2O)_3]^+$ core with 1 mL of a solution of **3** at 1 mg/mL concentration, under acidic conditions (pH = 2), at 70 °C during only 15 min. After HPLC purification, the $^{99m}Tc(CO)_3$-complex was obtained in high yield (>90%) and with high radiochemical purity (>98%). By working at lower concentration (radiolabelling of the *fac*-$[^{99m}Tc(CO)_3\ (H_2O)_3]^+$ with 100 µL of a solution of **3** at 1 mg/mL concentration), a very slight decrease of the radiochemical yield was observed (ca. 85%). The chemical identification of the radiochelate was accomplished by HPLC-comparison of its chromatogram with that of the rhenium analogue, as underlined in Figure 1. Similar retention times were observed (14.9 min for **^{99m}Tc-3** vs 14.2 min for **Re-3**), confirming the iso-structurality of both complexes. It is noteworthy that incubation of compound **3** with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ at 37 °C for 15 min resulted in **^{99m}Tc-3** complex in 91% radiochemical yield.

In vitro evaluation

The lipophilicity is an important parameter to predict and interpret the biological activity of a molecule intended to be injected *in vivo*. The lipophilic character of the radiocomplex was assessed by the determination of the partition coefficient (P) in physiological conditions (*n*-octanol/0.1 M phosphate buffer, pH 7.4) and was expressed as log P(oct/buffer). A value of 1.3 was found, indicating that our ^{99m}Tc complex is moderately lipophilic.[18]

The complex showed excellent *in vitro* stability under physiological conditions in human plasma with >99% of the **^{99m}Tc-3** compound remaining intact after 24 h. Consequently, no reoxidation to pertechnetate was observed, even after 24 h of incubation, as indicated by HPLC controls (Figure 2). As expected, the $Tc(CO)_3$ core was highly stabilized by the semi-rigid N_2O tridentate scaffold. The assays with human plasma were also performed to determine the percentage of protein binding. After incubation of the radiotracer in blood samples, plasma was separated from blood samples by centrifugation and treated with acetonitrile to precipitate the proteins. Two collected fractions (supernatant and protein) were measured by well gamma counter. The ^{99m}Tc-complex activity has been found to be higher in plasma fraction compared with the blood cell one. The protein binding study depicted low binding of the complex with blood protein. Only 12% of the complex was bound to serum proteins after 24 h of incubation (Figure 3). The major part of the circulating radioactivity corresponds to

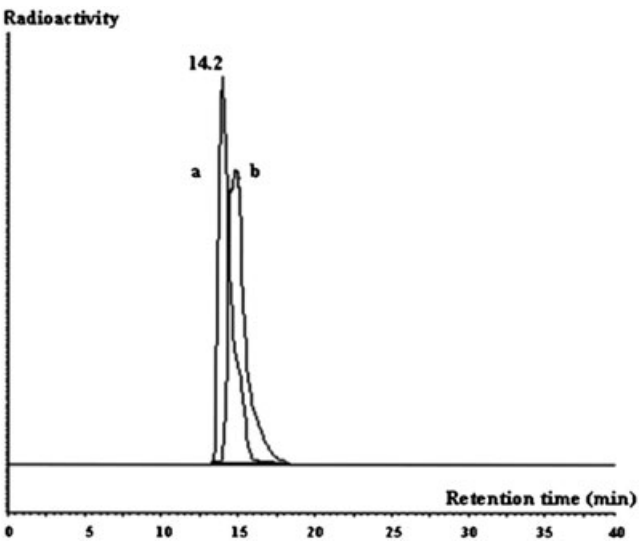


Figure 1. HPLC comparison of rhenium complex **Re-3** (a) and ^{99m}Tc complex **^{99m}Tc-3** (b).

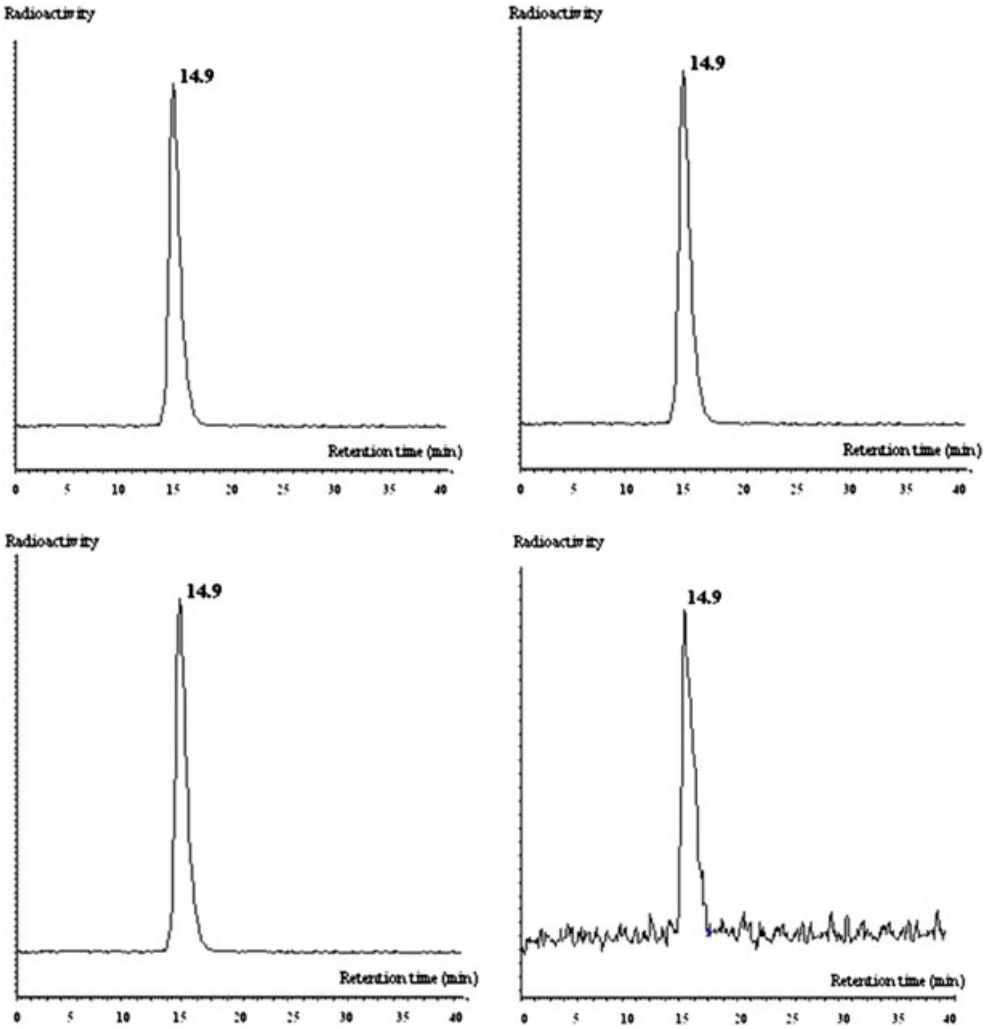


Figure 2. *In vitro* stability study of ^{99m}**Tc-3** by HPLC at 37 °C and at different times: 30 min (up left), 1 h (up right), 2 h (bottom left), and 24 h (bottom right). This figure is available in colour online at wileyonlinelibrary.com/journal/jlc

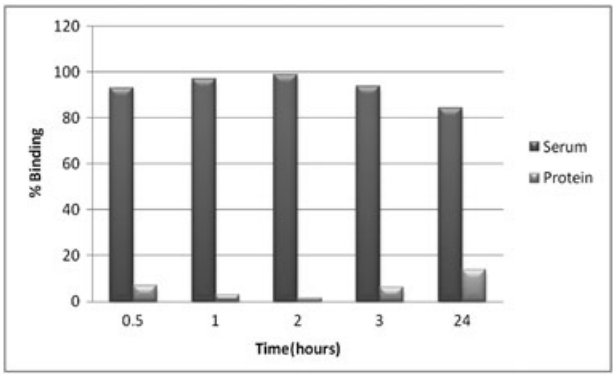


Figure 3. Plasma protein binding study of ^{99m}**Tc-3**.

free complex, which is a favorable feature, in terms of its application as an *in vivo* radioimaging probe.

Biodistribution studies

The biodistribution of ^{99m}**Tc-3** was investigated to (i) confirm its high stability, (ii) assess its ability to clear from nontarget organs such as liver, intestine as well as blood, and (iii) check if its intrinsic chemical and biological properties (molecular weight < 600, neutral species, and moderate lipophilicity) are sufficient to observe a significant brain uptake by passive diffusion. Concerning the latter item, it is noteworthy that a parabolic relationship between lipophilicity and passive diffusion over the brain–blood-barrier with an optimum for neutral molecules having a log P(oct/buffer) between 0.5 and 2.5, was reported.[18]

The radiolabelled technetium compound was administrated separately to healthy male normal Swiss mice. The quantity of radioactivity in selected organs and blood was assessed and the percentage of injected dose per gram of tissue at 2, 5, 30, and 60 min post-injection, was reported in Table 1. The fast clearance of the radiotracer from the bloodstream, reflected in the low blood activity, indicated its high stability against exchange reactions with blood proteins, as expected (0.39 ± 0.04%ID g^{-1} at 60 min). Moreover, no specific uptake or long-term retention in organs or tissues was observed. Only a negligible fraction of the injected radioactivity was retained by the stomach (0.30 ± 0.02% ID g^{-1} at 60 min) and the spleen (0.36 ± 0.08 % ID g^{-1} at 60 min), suggesting minimal *in vivo* oxidation of technetium to pertechnetate or reduction to colloidal technetium species.[19] The higher concentration of radioactivity was measured in liver at all times studied (13.69% ID g^{-1} at 2 min, 12.18% ID g^{-1} at 5 min, 9.89% ID g^{-1} at 30 min,

Table 1. Tissue distribution data of ^{99m}**Tc-3** in normal Swiss mice*

Tissues	2 min	5 min	30 min	60 min
Blood	3.52 ± 0.62	2.21 ± 0.23	1.11 ± 0.07	0.39 ± 0.04
Brain	0.14 ± 0.02	0.07 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
Heart	0.77 ± 0.06	0.58 ± 0.05	0.23 ± 0.04	0.14 ± 0.01
Lungs	2.45 ± 0.81	2.19 ± 0.75	1.84 ± 0.09	0.93 ± 0.03
Liver	13.69 ± 2.15	12.18 ± 1.91	9.89 ± 0.83	5.24 ± 1.22
Spleen	1.64 ± 0.52	1.36 ± 0.42	0.53 ± 0.19	0.36 ± 0.08
Pancreas	1.18 ± 0.19	0.44 ± 0.21	0.15 ± 0.05	0.03 ± 0.01
Kidneys	9.25 ± 1.83	6.69 ± 1.25	4.86 ± 0.87	2.32 ± 0.87
Intestines	7.25 ± 1.45	7.65 ± 0.89	3.68 ± 1.26	3.23 ± 0.75
Muscle	0.42 ± 0.07	0.34 ± 0.03	0.25 ± 0.02	0.11 ± 0.01
Stomach	0.45 ± 0.03	0.42 ± 0.04	0.38 ± 0.03	0.35 ± 0.02

*Values are %ID.g^{-1} ± standard deviation ($n = 3$)

and 5.24% ID g^{-1} at 60 min). Nevertheless, although there is a significant liver uptake at earlier post-injection times, the liver excretion rate is relatively fast. Our compound was mainly excreted through the hepatobiliary pathway, as evidenced by the decreasing activity in liver and intestine. In a lesser extent, a partial excretion through the renal system could be considered. A moderate residual radioactivity and fast clearance associated with the kidneys supports this hypothesis. At least, a very low accumulation of radioactivity in brain was observed at all times studied, indicating that ^{99m}**Tc-3** is not suitable as brain imaging agent despite interesting chemical/biological properties. Conversely, because of its promising *in vivo* behavior (high stability, fast blood clearance, and non-specific binding), the new organometallic scaffold represent an interesting base for the development of targeted diagnostic radiopharmaceuticals.

Conclusion

In summary, a ^{99m}Tc(I)-specific semi-rigid tridentate ligand based on a click chemistry approach was described for the first time. The semi-rigid BCA, whose the metal chelating cavity includes the 1,2,3-triazole moiety with the 2-aminophenol backbone, was formed in three steps with an overall yield of 32%. The corresponding neutral ^{99m}Tc $(CO)_3$ complex, obtained in excellent yield, exhibits a lipophilic character and presents interesting *in vitro* and *in vivo* behaviors. In this context, these promising chemical and biological features—a facile access to the click ligand, which can be grafted to biomolecules through the carboxylate arm, the high stability of the $Tc(CO)_3$-complex, which exhibits rapid clearance and no long-term retention in organs—make this N_2O tridentate ligand a promising BCA for the development of targeted radioimaging probes. Bioconjugation purposes with the *o*-methoxyphenylpiperazine pharmacophore for specific 5-HT_{1A} brain receptor targeting[20] are in progress and will be reported in another paper.

Acknowledgements

The authors thank Mallinckrodt Medical B.V., the Netherlands, for Isolink kit gifts. The authors are also grateful for French Alternative Energies and Atomic Energy Commission (CEA) for the financial support to undertaking a fellowship program with the SPCMIB CNRS Unit (Toulouse).

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] J. D. G. Correia, A. Paulo, P. D. Raposinho, I. Santos, *Dalton Trans.* **2011**, *40*, 6144.
[2] S. Liu, S. Chakraborty, *Dalton Trans.* **2011**, *40*, 6059.
[3] (a) S. Liu, D. S. Edwards, *Chem. Rev.* **1999**, *99*, 2235; (b) S. Liu, *Chem. Soc. Rev.* **2004**, *33*, 445; (c) G. Ribeiro Morais, A. Paulo, I. Santos, *Organometallics* **2012**, *31*, 5693.
[4] (a) J. R. Dilworth, S. J. Parrot, *Chem. Soc. Rev.*, **1998**, *27*, 43; (b) S. Jurisson, J. D. Lydon, *Chem. Rev.* **1999**, *99*, 2205.
[5] (a) R. Alberto, R. Schibli, A. Egli, P. A. Schubiger, *J. Am. Chem. Soc.* **1998**, *120*, 7987; (b) R. Alberto, K. Ortner, N. Wheatley, R. Schibli, P. A. Schubiger, *J. Am. Chem. Soc.* **2001**, *123*, 3135.
[6] T. L. Mindt, H. Struthers, L. Brans, T. Anguelov, C. Schweinsberg, V. Maes, D. Tourwé, R. Schibli, *J. Am. Chem. Soc.* **2006**, *128*, 15096.
[7] (a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004; (b) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.
[8] (a) T. L. Mindt, C. Müller, M. Melis, M. de Jong, R. Schibli, *Bioconjugate Chem.* **2008**, *19*, 1689; (b) H. Struthers, B. Spingler, T. L. Mindt, R. Schibli, *Chem. Eur. J.* **2008**, *14*, 6173; (c) H. Struthers, T. L. Mindt, R. Schibli, *Dalton Trans.* **2010**, *39*, 675; (d) C. A. Kluba, T. L. Mindt, *Molecules* **2013**, *18*, 3206.
[9] (a) C. Camp, S. Dorbes, C. Picard, E. Benoist, *Tetrahedron Lett.* **2008**, *49*, 1979; (b) A. Seridi, M. Wolff, A. Boulay, N. Saffon, Y. Coulais, C. Picard, B. Machura, E. Benoist, *Inorg. Chem. Commun.* **2011**, *14*, 238.
[10] R. Alberto, R. Schibli, R. Waibel, U. Abram, A. P. Schubiger, *Coord. Chem. Rev.* **1999**, *190–192*, 901.
[11] (a) J. Le Gal, E. Benoist, M. Gressier, Y. Coulais, M. Dartiguenave, *Tetrahedron Lett.* **2002**, *43*, 9295; (b) J. Le Gal, L. Latapie, M. Gressier, Y. Coulais, M. Dartiguenave; E. Benoist, *Org. Biomol. Chem.*, **2004**, *2*, 876; (c) J. Le Gal, F. Tisato, G. Bandoli, M. Gressier, J. Jaud, S. Michaud, M. Dartiguenave, E. Benoist, *Dalton Trans.* **2005**, 3800.
[12] O. Martinage, L. Le Clainche, B. Czarny, C. Dugave, *Org. Biomol. Chem.* **2012**, *10*, 6484.
[13] M. Sechi, M. Derudas, R. Dallocchio, A. Dessì, A. Bacchi, L. Sannia, F. Carta, M. Palomba, O. Ragab, C. Chan, R. Shoemaker, S. Sei, R. Dayam, N. Neamati, *J. Med. Chem.* **2004**, *47*, 5298.
[14] A. Manzo, A. Perboni, G. Broggini, M. Rigamonti, *Synthesis* **2011**, *1*, 127.
[15] I. Essouissi, W. Ghali, S. N. Malek, M. Saidi, *Nucl. Med. Biol.* **2010**, *37*, 821.
[16] A. Boulay, A. Seridi, C. Zedde, S. Ladeira, C. Picard, L. Maron, E. Benoist, *Eur. J. Inorg. Chem.* **2010**, 5058.
[17] X. Zhang, P. Zhou, J. Liu, Y. Huang, Y. Lin, Y. Chen, T. Gu, W. Yang, X. Wang, *Appl. Radiat. Isot.* **2007**, *65*, 287.
[18] C. Fernandes, J. D. G. Correia, L. Gano, I. Santos, S. Seifert, R. Syhre, R. Bergmann, H. Spies, *Bioconjugate Chem.* **2005**, *16*, 660.
[19] R. Visentin, M. C. Giron, M. Bello, U. Mazzi, *Nucl. Med. Biol.* **2004**, *31*, 655.
[20] L. Zimmer, D. Le Bars, *J. Label. Compd. Radiopharm.* **2013**, *56*, 105.

