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PAPER

Synthesis and biological evaluation of a new triazole–oxotechnetium complex†

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A new triazole oxotechnetium chelating agent was synthesized *via* a 'Click-to-Chelate' strategy. *In vivo* evaluation of the corresponding ^{99m}Tc complex shows that the tracer exhibits very interesting properties for molecular imaging.

Introduction

Technetium (^{99m}Tc)-based tracers are employed in 85% of routine anatomic and functional radiodiagnostic exams carried out in hospitals. Many technetium chelates have been reported in the literature and have permitted the emergence of a wide variety of tracers for molecular imaging. In particular, chelates containing an oxotechnetium(V) core are the most readily accessible complexes.[1]

Technetium can be coordinated by heteroatoms and electron donating chemical groups to form stable complexes.[2] On the one hand, some tetradentate oxotechnetium(V) complexes containing either a pyridyl (compound **1**) or an imidazoyl group (compound **2**) have shown satisfactory stabilities *in vivo* (Fig. 1).[3]

On the other hand, the study of Tc(I) chelates containing a heterocycle such as imidazole[4] and pyrazole[5] has led to the development of Tc(I)–triazole derivatives that can be obtained through a click-chemistry strategy called 'Click to Chelate'.[6]

This latter application has opened new perspectives in terms of combinatorial chemistry and biological applications.[7] To the best of our knowledge, no data regarding the complexation of the oxotechnetium(V) core by triazole-containing tetradentate chelating agents could be found in the literature. The complexation of the oxorhenium core (a chemical analog of oxotechnetium) by benzotriazole-containing bidentate ligands was also reported by Machura and co-workers.[8] All these results suggested that the conjugated triazole ring is able to participate in the stabilization of the oxotechnetium core as well. Therefore, we investigated the potential of triazole to chelate the oxotechnetium core for the preparation of biologically stable tetradentate ^{99m}Tc complexes (Fig. 1).

Based on previous works,[3] we chose to build candidate chelating agents around a glycine central moiety bearing both a triazole motif and a thiol group at each terminus. In fact, the thiol group is known to facilitate oxotechnetium coordination and consequently is found in many ^{99m}Tc complexes that exhibit very high *in vivo* stabilities such as reference compounds **3** and **4** (Fig. 1).[1] On the other hand, the triazole group offers a unique potential for the development of new tracers through combinatorial chemistry techniques.[6,7]

In this study we investigated the potential of new oxotechnetium tetradentate chelating agents usable as novel tracers for *in vivo* molecular imaging.

Results and discussion

Among the wide variety of oxotechnetium complexes, the N_3S chelating motif offers a unique potential in terms of possible arrangements and substitutions.[1] We explored four possible combinations including either a thioalkyl or a thiophenyl moiety together with a terminal triazole group (Scheme 1). Compounds **7** and **13** feature the most adaptable structures that might facilitate oxotechnetium coordination. Compound **14** was anticipated to promote rigidity of the TcO^{3+} square pyramidal base and to enhance complex stability due to the combination of the 1,2,3-triazole-4-carboxyl moiety with the 1-aminothiophenyl group.[9] Compound **8** was expected to have an intermediary rigidity. In this study, we used a simple and relatively inert benzyl moiety as the triazole substituent. Compounds **7**, **8**, **13** and **14** were synthesized from acetylenic precursors using the Huisgen [3 + 2] cyclodipolar addition[7] through *in situ* azide substitution[10] followed by acidolysis of the *S*-trityl group (Scheme 1). The final products were purified by RP-HPLC and directly used for $^{99m}TcO^{3+}$ chelation.

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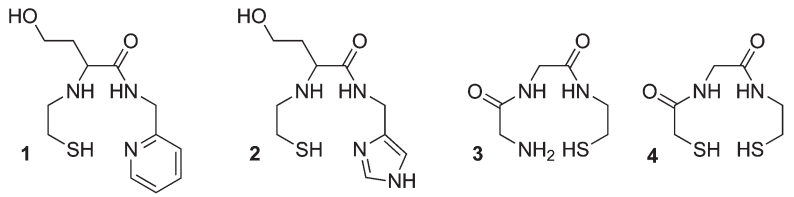
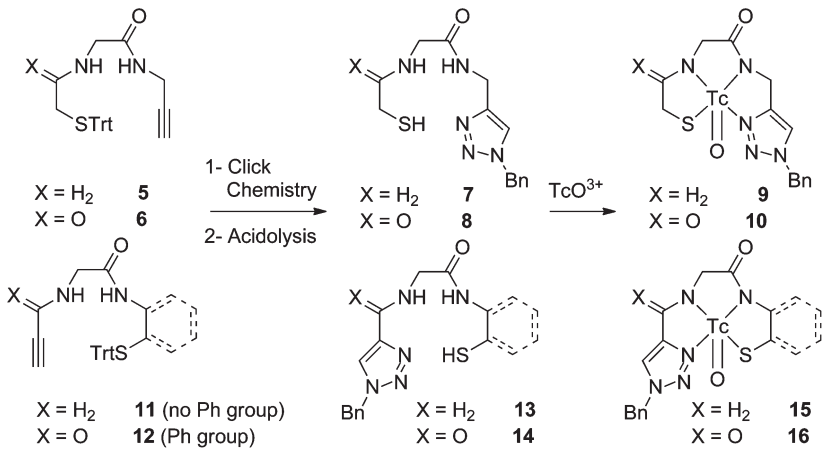
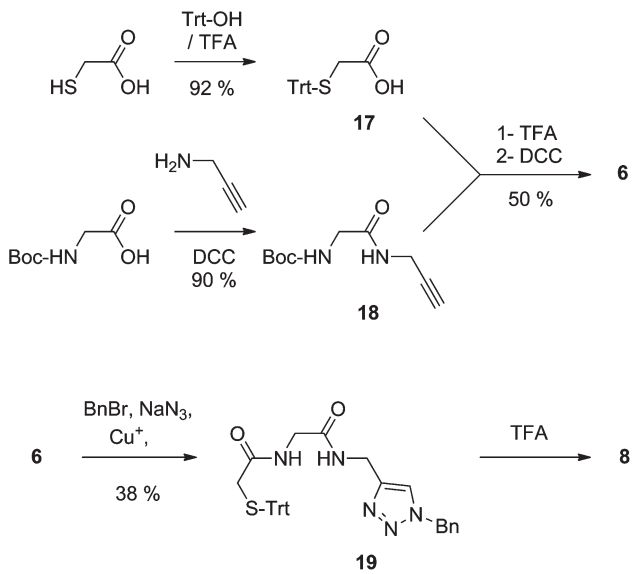


Fig. 1 Chemical structures of tetradentate oxotechnetium chelating agents **1**–**4**.



Scheme 1 General synthetic sketch of triazole-based chelating agents and corresponding Tc complexes starting from acetylenic precursors. Compounds **12**, **14** and **16** contain a phenyl group, which was replaced with a more flexible ethyl chain in compounds **11**, **13** and **15**.



Scheme 2 Example of synthesis of acetylenic precursor **6** and triazole-containing tetradentate chelating agent **8**.

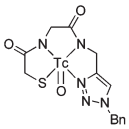
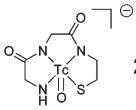
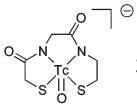
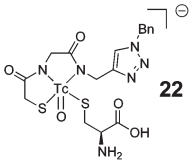
The acetylenic precursors **5**, **6** and **12** were prepared by classical chemical procedures using very simple reactants. As an example, compound **6** was obtained from mercaptoacetic acid and *N-tert*-butyloxycarbonylglycine as depicted in Scheme 2 in 41% overall yield. Copper-catalyzed Huisgen [3 + 2] cyclodipolar addition of benzyl azide, generated *in situ* from benzyl bromide and sodium azide, followed by acidolysis led to the tetradentate chelating agent **8**.[10] Precursor **11** as well as reference compounds **3** and **4** were prepared by a classical stepwise solid-phase synthesis using a 4-methoxytrityl-cysteamine resin.[11]

Complexation of the four Tc-chelating agents was carried out at room temperature which enabled discrimination of compounds that do not coordinate the TcO^{3+} core efficiently. In three cases, a complex mixture of radiolabelled species was eluted by RP-HPLC. The presence of a massive peak around 5.0 min suggested that most of the reduced technetium remained in a non-complexed form. Increasing temperature did not improve the Tc coordination for these compounds. Conversely, complex **10** was repeatedly obtained in quantitative yields (radio-HPLC) and high purity (Table 1) from chelating agent **8** (Scheme 1).

The stability of complex **10** in mice plasma was investigated. For this purpose, the complex was incubated at 37 °C and aliquots were analyzed by RP-HPLC after varying periods of time. As shown in Table 1 and Fig. 2, the oxotechnetium complex **10** exhibited a stability in fresh plasma that is equivalent to that of complex **21** with about 90% of the initial complex remaining after one ^{99m}Tc half-life (6 h). In the same conditions, reference complex **20** was more sensitive to plasma constituents.

In order to evaluate the behaviour of the oxotechnetium complex **10** *in vivo*, we injected a solution of this complex into the tails of a set of four healthy female BalbC mice that were sacrificed after 2 h. Three mice were used for tissue distribution studies and the fourth one served for imaging. We observed a very low accumulation of radioactivity in tissues, particularly in liver and intestine (<5%), suggesting that, after 2 h, tracer **10** is

Table 1 RP-HPLC retention times, ES/MS, purities and stabilities of technetium complexes **10**, **20** and **21** in mice plasma at 37 °C; RP-HPLC retention time and ES/MS of complex **22**

Ligand	Complex	t_R (min)[a]	m/z[b] (MALDI)	Purity[a,c] (%)	Stability[a,d] (%)
8	 **10**	17.4	432.0[b]	98	90
3	 **20**	11.1	—	98	60
4	 **21**	14.5	—	99	90
8 + Cys	 **22**	8.9	534.0[e]	—	—

[a] ^{99m}Tc complexes. [b] ^{99g}Tc complexes. [c] Radiochemical purity (RP-HPLC). [d] After incubation for 6 h in mice plasma at 37 °C. [e] MH^+–H_2O.

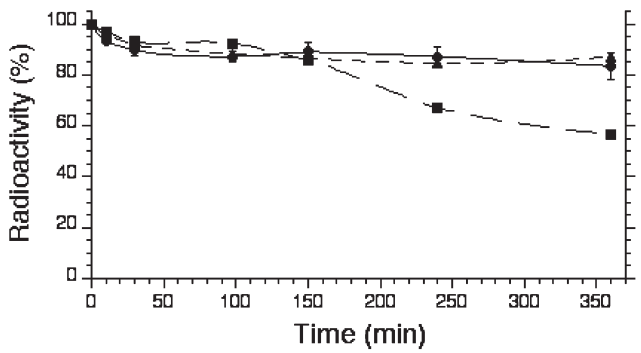


Fig. 2 Time-dependent evolution of complex **10** (%) in BalbC female mice plasma (● complex **10**, ■ complex **20**, ▲ complex **21**).

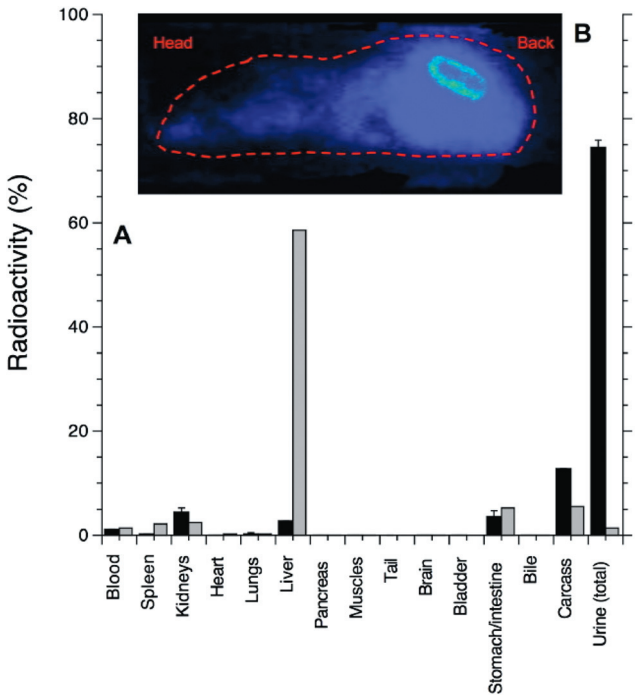


Fig. 3 Tissues and fluids accumulation of complex **10** (black) and TcO^{3+} (grey) injected in to female BalbC mice (22–25 g). The planar β-imaging of complex **10** injected in to a BalbC mouse (longitudinal section) using the minor ^{99m}Tc β-emission (E = 2 keV) showed a partial accumulation of tracer in the kidney. A very low level of radioactivity was detected in other tissues (inset B).

not metabolized through the gastro-intestinal system (Fig. 3A). Very low radioactivity levels in blood and strong accumulation in urine showed a massive clearance from the circulating milieu and suggests a rapid excretion through the renal pathway. A low but significant residual radioactivity associated with the kidneys also supports this hypothesis. Conversely, in the same conditions, non-complexed oxotechnetium (TcO^{3+}) as well as the Tc complex obtained from compound **1**[3] mainly accumulated in liver.

A high-resolution beta-imaging experiment using the minor 2 keV β^- radiations associated with the ^{99m}Tc radioactive decay was preferred to SPECT for these preliminary studies due to higher resolution of the former technique. The image (Fig. 3B) clearly shows a slight concentration of radioactivity in the cortex of kidneys that is consistent with a predominant renal excretion of the tracer. A very low and apparently non-specific accumulation of tracer was observed throughout the whole body.

In order to determine the behaviour of tracer **10** *in vivo*, we investigated whether a tracer demetallation process[12] or a partial metabolization were taking place during the renal excretion. For this purpose, we injected two supplementary mice with compound **10**. After 2 h, their urine was collected and analyzed by

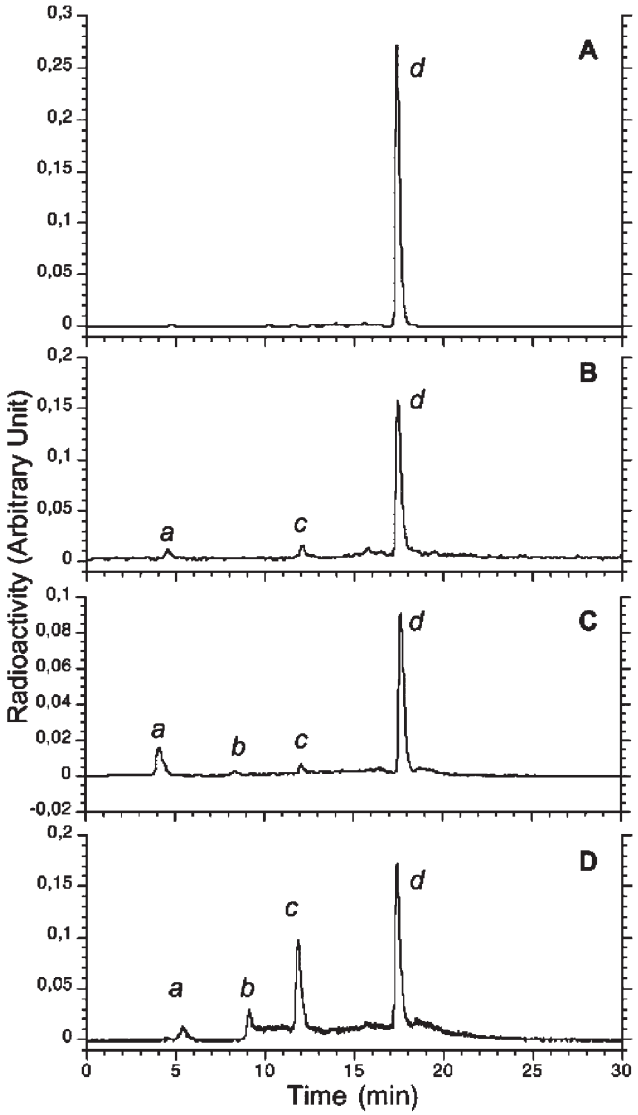


Fig. 4 RP-HPLC radiochromatograms of pure complex **10** (A), complex **10** incubated in mice plasma for 2 h at 37 °C (B), complex **10** incubated in mice urine for 2 h at 37 °C (C), and complex **10** recovered from the urine 2 h after i.v. injection into BalbC mice (D).

radio-RP-HPLC. The tracer was recovered as a mixture of peaks *d* (60%) at 17.4 min (see Fig. 4D, reference tracer 17.4 min in Fig. 4A–4C) and peaks *b* and *c* at 9.0 (7%) and 11.9 min (33%) respectively. In these experiments, peak *a* was identified as non-complexed technetium.

In order to identify the side products *b* and *c*, the tracer was incubated for 30 min in fresh mice urine and the incubate was analyzed by RP-HPLC. In this experiment, the tracer was prepared from a mixture of ^{99m}Tc–^{99g}Tc to enable the identification of the products by mass spectrometry. Peaks *b*, *c* and *d* were isolated and analyzed by MALDI/TOF mass spectrometry. The tracer (peak *d*) was unambiguously identified (*m*/*z* 432.0). Despite a rise in tracer concentration from the nanomolar to the micromolar range, the same compounds were detected, though compound *b* was obtained in a lower amount.

Determination of the structure of the 2 side-products was more difficult. The elution time of peak *b* at 9.0 min suggested a transmetallation by more hydrophilic endogenous thiols such as glutathione (GSH), cysteine or β-cysteine; a tracer metabolization process that is frequently reported in the literature.[13] In particular, GSH conjugation is known to participate in the activation and metabolization of *cis*-platin derivatives.[14] The RP-HPLC elution-time of a reference obtained upon tracer/cysteine challenge (t_R = 9.0 min) suggests that peak *b* (t_R = 9.0 min) might be the chimeric tracer–cysteine adduct **22** (Table 1). Conversely, MALDI-MS analysis of peak *c*, which elutes at 11.9 min, gave two major signals (*m*/*z* 713.3 and 743.3) that could not be identified by MS/MS fragmentation experiments. This compound was also observed at lower levels after prolonged incubation of tracer **10** in mice plasma. This minor transformation could be related to the binding of oxotechnetium to a plasma constituent but not to the loading of the complex by a blood protein, in particular serum albumin (for example through serum albumin Cys^{34}–Tc binding),[12] since no corresponding signal could be detected. A possible conjugation to GSH with either a partial or total dissociation of complex **10** was also examined. However, isolated compound *c* corresponds neither to the symmetric [bis-GS–TcO] complex (in a native or a partially proteolyzed form) nor to the chimeric [GS–TcO–**8**] complex.[14]

A possible metabolization of the tracer by blood erythrocytes, as previously reported,[3] was suspected. This hypothesis was confirmed by incubation of complex **10** in fresh mice blood for 2 h at 37 °C: peak *c* (11.9 min) was detected as the major product. However, this apparent sensitivity to erythrocytes was far lower after injection in mice models. Moreover, as shown by previous *in vivo* experiments, a specific tracer is usually brought to its target in a few minutes through the vasculature, whereas the residual radioactivity is excreted. For these reasons, the structure of the unknown ^{99m}Tc-labelled metabolite *c* was not further investigated.

All these results strongly suggest that complex **10** is stable for at least 2 h after intravenous injection in mice and exhibits an interesting tissue distribution as a non-targeted tracer. In particular, it has a very low metabolization through the hepatic pathway.

Conclusions

We identified compound **8** as a new chelating motif that complexes the oxotechnetium core quantitatively at room temperature to give the corresponding complex **10**. The high stability of this technetium complex in mice plasma for 6 h prompted us to inject it intravenously in to a set of healthy BalbC mice in order to investigate its behaviour *in vivo*. As shown from tissue distribution and beta-imaging experiments, the tracer led to a remarkably low non-specific residual labelling of tissues. Although it is difficult to draw conclusions regarding the general stability of triazole-containing SN_3 TcO^{3+} complexes, the predominant urinary excretion pathway, confirmed by the slight labelling of kidneys displayed by imaging slides, encourages us to investigate further the potential of such imaging agents. In particular, the triazole moiety, readily accessible from the versatile click-chemistry strategy, offers many developments for the optimization of new tracers by the 'Click-to-Chelate-oxotechnetium' combinatorial chemistry approach.

Experimental

General

Chemical reagents and solvents were purchased from Sigma-Aldrich, VWR, Fluka or SDS and were of the highest purity available. Amino acids and resins were from Novabiochem. $[^{99m}TcO_4]^-$ was eluted as a physiological saline solution from commercially available $^{99}Mo/^{99m}Tc$ generator system (ELUMATIC III, CIS bio international). Analytical RP-HPLC purifications were achieved on a Prostar Varian chromatography system coupled to a Varian 335 diode array detector. RP-HPLC analyses were carried out on a Varian Pursuit C18 analytical column (250 × 4.6 mm, 5 µm) protected by an analytical Security Guard (Phenomenex). The chromatography system was coupled to a gamma detector (radioflow monitor) HERM LB500 (Berthold). Preparative RP-HPLC purifications were done using a Knauer purification system made of 2 LAPrep P310 pump modules coupled to a LAPrep P311 UV detector and a Varian Pursuit C18 preparative column (250 × 21.2 mm, 5 µm) or a Varian Pursuit C18 semipreparative column (250 × 10.0 mm, 5 µm). ^1H and ^{13}C NMR spectra were recorded on Bruker AVANCE 250 spectrometer; δ and J are reported in ppm relative to TMS and Hz, respectively. Infrared spectra were recorded on a JASCO FTIR-410 spectrometer. ES/MS analysis were carried out on a Quattro Micro apparatus or on a Platform LCZ (Micromass). MALDI TOF MS were done using an Applied Biosystems 4800 MALDI TOF/TOF analyzer. High-Resolution Mass Spectrometry (HRMS) was performed on a Q-tof apparatus (Micromass). Radioactivity was counted on a Medisystem MEDI 404 gamma counter. Beta-imaging was performed on a Biospace 200 beta-imager.

2-(Tritylthio)acetic acid 17. Triphenylmethanol (5.2 g, 20.0 mmol) dissolved in TFA (60 mL) was treated with mercaptoacetic acid (1.39 mL, 20.0 mmol, 1.0 equiv.) for 3 h at room temperature under an atmosphere of argon. After removal of TFA *in vacuo*, toluene was chased (×3) over the orange solid to give compound **17** as a white amorphous solid (6.12 g, 18.3 mmol, 92%) which was identical to the literature data:[15] ^1H NMR ($CDCl_3$, 250 MHz): δ 7.31 (m, 9H, Trt), 3.05 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 176.0 (CO), 143.9 (C, Ph), 129.7 (C, *meta* Trt), 128.2 (C, *ortho* Trt), 127.1 (C, *para*, Trt), 67.3 (C, Trt), 34.6 (CH_2); ES/MS (negative mode) 333.3 ($M - H^+$).

***tert*-Butyl (2-oxo-2-(prop-2-yn-1-ylamino)ethyl)carbamate 18.** Boc-glycine (875 mg, 5.0 mmol) was treated successively with DIPEA (2.61 mL, 15.0 mmol, 3.0 equiv.), propargylamine (0.320 mL, 5.0 mmol, 1.0 equiv.) and DCC (1.238 g, 6.0 mmol, 1.2 eq.) in DCM (25 mL) overnight at room temperature. After filtration of the precipitate, the filtrate was washed with 10% citric acid (×2) and brine (×2). The organic layer was dried over sodium sulfate and, after filtration, the resulting oil was purified by silica gel flash chromatography (eluent hexane–AcOEt 1 : 1) to give compound **18** (950 mg, 4.48 mmol, 90%): ^1H NMR ($CDCl_3$, 250 MHz): δ 6.41 (s, 1H, N–CO), 5.30 (s, 1H, N–CO), 4.08 (dd, J = 5.2, J' = 2.5, 2H, CH_2–C≡C), 3.82 (d, J = 5,8, 2H, CH_2–CO), 2.24 (t, J = 2.5, 1H, C≡CH), 1.46 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 169.3 (CO), 79.28 (C, Boc), 77.36 (C, alkyne), 71.89 (CH, alkyne), 29.23 (N–*C*H_2–C≡CH), 28.43 (CH_3, Boc); IR (KBr): 2140, 1728, 1641; ES/MS (positive mode): 212.2 (MH^+, 100%).

***N*-(2-Oxo-2-(prop-2-yn-1-ylamino)ethyl)-2-(tritylthio) acetamide 6.** Compound **18** (950 mg, 4.5 mmol) was treated with DCM–TFA 2 : 1 (30 mL) for 1.5 h at 0 °C. After evaporation of the solvent, toluene was chased on the product (×3). After drying *in vacuo*, the residue was added to a mixture of compound **17** (1.503 g, 4.5 mmol, 1.0 equiv.), DIPEA (4.7 mL, 27.0 mmol, 6.0 equiv.) and DCC (1.114 g, 5.4 mmol, 1.2 equiv.) in DCM (20 mL) and the mixture was stirred overnight at room temperature. After filtration of the precipitate, the filtrate was washed with 10% citric acid (×3) and brine (×3) and the organic layer was dried over sodium sulfate. After removal of the solvent under reduced pressure, the resulting oil was purified by silica gel flash chromatography (eluent: hexane–AcOEt 4 : 6) to give compound **6** as a brown solid (0.963 g, 2.25 mmol, 50%); ^1H NMR ($CDCl_3$, 250 MHz): δ 7.27 (m, 15H, Trt), 3.99 (s, 2H, CH_2–C≡C), 3.59 (s, 2H, N–CH_2–CO), 3.17 (s, 2H, S–CH_2–CO), 2.15 (s, 1H, C≡CH); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 169.1 + 168.0 (2 CO), 143.8 (C, Trt), 129.4 + 128.2 + 128.0 (3 CH, Trt), 77.2 (*C*≡CH), 71.8 (C≡*C*H), 43.5 (CH_2–N), 35.5 (CH_2–S), 29.1 (*C*H_2–C≡C); IR (KBr): 2140, 1687, 1654; ES/MS (positive mode) 429.1 ($M + H^+$); Elem. anal. calcd for $C_{26}H_{24}N_2O_2S$ C, 72.87; H, 5.64; N, 6.54; S, 7.48; found C, 72.83; H, 5.67; N, 6.53; S, 7.45%.

***N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(2-(tritylthio) acetamido)acetamide 19.** Compound **6** (214 mg, 0.5 mmol) dissolved in DMSO–water (9 : 1) was treated successively with benzyl bromide (59.4 µL, 0.5 mmol, 1.0 equiv.), L-proline (11.5 mg, 0.1 mmol, 0.2 equiv.), sodium carbonate (63.6 mg, 0.6 mmol, 1.2 equiv.), sodium ascorbate (50 µL of a 1 M aqueous solution), $CuSO_4 \cdot 5H_2O$ (25 µL of a 1 M aqueous solution), and sodium azide (39.0 mg, 0.6 mmol, 1.2 equiv.). After the solution color has turned green, the mixture was stirred overnight at 65 °C under argon. The resulting brown mixture was poured into iced water and stirred for 30 min. Extraction with ethyl acetate and reduction of the volume of the organic layer under reduced pressure gave compound **19** as a brown amorphous solid (107 mg, 0.19 mmol, 38%): ^1H NMR ($CDCl_3$, 250 MHz): δ 7.45 (s, 1H, CH–N–N=N), 7.39 (m, 2H, NH–CO), 7.25 (m, 5H, CH Ar), 5.50 (s, 2H, CH_2Ph), 4.51 (d, J = 5.5, 2H, CH_2–C≡C), 3.95 (d, J = 5.5, 2H, N–CH_2–CO), 3.27 (d, J = 9.0, 2H, S–CH_2–CO); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 169.01 168.0 (CO, amides), 143.7 (C, triazole), 129.4–127.8 (Ar CH, Trt + Ph), 127.0 (CH, triazole), 71.6 (C, Trt), 60.3 (CH_2, Bn), 35.4 (*C*H_2-C=C), 33.8 (N–*C*H_2–CO), 29.0 (S–CH_2–CO); IR (KBr): 1691, 1648, 1601, 1248–1229; ES/MS (positive mode) *m/z* 562.2 ($M + H^+$, 54%), 584.2 ($M + Na^+$, 100%), 600.1 ($M + K^+$, 21%); Elem. anal. calcd for $C_{33}H_{31}N_5O_2S$ C, 70.56; H, 5.56; N, 12.47; S, 5.71; found C, 70.54; H, 5.60; N, 12.41; S, 5.69%.

***N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(2-mercapto acetamido)acetamide 8.** Compound **19** was treated with TFA–TIPS–water 95 : 2.5 : 2.5 for 2 h at room temperature. After removal of the solvent *in vacuo*, the product was dried under reduced pressure and dissolved in water–ACN 4 : 1. The resulting

solution was purified by semi-preparative RP-HPLC on a semi-preparative column Varian Pursuit C18, linear gradient 250 × 10 mm, linear gradient (100% of 0.1% aqueous TFA to 100% of ACN containing 0.1% TFA) in 30 min at 3.0 mL min^{-1} (t_R = 17.4 min) to give compound **8** (44 mg): analytical RP-HPLC t_R = 14.6 min, purity 99%; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.45 (s, 1H, CH–N–N=N), 7.39 (m, 2H, NH–CO), 7.25 (m, 5H, CH Ar), 5.50 (s, 2H, CH_2Ph), 4.51 (d, J = 5.5, 2H, CH_2–C≡C), 3.95 (d, J = 5.5, 2H, N–CH_2–CO), 3.27 (d, J = 9.0, 2H, S–CH_2–CO); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 168.5 + 167.6 (CO), 145.1 (C-N_3), 144.0, 129.1, 128.8, 128.1 (CH Ar),123.0 (CH–N_3), 65.9 (CH_2 Bn), 40.0 (CH_2, Gly); IR (KBr): 1684, 1664, 1651, 1224; ES/MS (positive mode) 320.1 (M + H^+, 100%), 342.1 (M + Na^+, 2.6%), 358.0 (M + K^+, 3.6%); HRMS calcd for $C_{14}H_{17}N_5O_2S$, 319.1103; found, 320.1114.

General protocol for $^{99m}TcO^{3+}$ preparation and complexation. An aliquot (20 µL, 62.5 nmol) of a 1 g L^{-1} solution of ligand was successively treated with a 1 g L^{-1} solution of stannous chloride (35 µL, 184.4 nmol, 3 equiv.), 0.1 M aqueous sodium hydroxide (70 µL) and a freshly eluted solution of sodium tetraoxotechnetate (75 µL) in water (80 mL). After 5 min at room temperature, the reaction was quenched by addition of 1 M aqueous hydrogen chloride (7 µL). The complex was analyzed by RP-HPLC using an analytical column Varian Pursuit C18 250 × 4.6 mm, linear gradient (100% of 0.1% aqueous TFA to 100% of ACN containing 0.1% TFA) in 30 min at 1.0 mL min^{-1}.

Preparative synthesis of ^{99g}Tc labelled complex 10. Compound **8** (5 mg, 15.6 µmol) dissolved in degassed water (25 mL) was successively treated with a solution (1 mg mL^{-1}) of stannous chloride (8.75 mL, 46.0 µmol, 3.0 equiv.), 0.1 M sodium hydroxide (17.5 mL) and a 7.5 mM aqueous solution of $Na^{99g}TcO_4$ (2.1 mL). The mixture was stirred for 5 min at room temperature (light yellow color). The solution was neutralized by addition of 1 M hydrogen chloride (1.75 mL). Purification of complex **10** by semi-preparative RP-HPLC on a semi-preparative column Varian Pursuit C18 250 × 10 mm, linear gradient (100% of 0.1% aqueous TFA to 100% of ACN containing 0.1% TFA) in 30 min at 3.0 mL min^{-1} (t_R = 19.8 min) afforded 1.9 mg of pure complex (4.4 µmol, 28%): ^{1}H NMR ($CDCl_3$, 250 MHz): δ 7.72 (s, 1H, NH triazole), 7.44–7.50 (m, 5H, Ph), 5.71–5.86 (m, 2H, CH_2 Bn), 5.32–5.39 (d, J = 18 Hz, 1H), 4.65–4.86 (m, 3H), 4.10 (AB, J_{AB} = 55.3 Hz, J = 17 Hz, 2H, CH_2); IR (KBr): 1655, 1638, 978; ES/MS (positive mode): 431.9 (M + H^+, 100%), 453.9 (M + Na^+, 7.2%), 469.9 (M + K^+, 1.8%).

Time-dependent stability studies of complex 10 in mice plasma. All experiments were carried out in Eppendorf tubes 'Low bind'. The solution of complex (50 µL, 4.92 MBq) was added to fresh mice plasma (450 µL) at 37 °C (t = 0 min). Aliquots are treated with 10% TFA (150 µL). After centrifugation for 5 min, the solution was isolated and radioactivity was measured. Solution (50 µL) was diluted with water (150 µL) and analyzed by analytical RP-HPLC.

General protocol for *in vivo* studies. Four female BalbC mice (22–25 g, Charles River Laboratories, Lyon, France) were handled according to a protocol that has been approved by the French Animal Care and Use Committee (Décret 87-848, 87-10-19). Mice were injected intravenously with a solution of complex **16** (caudal injection of typically 100 µL, 8 MBq) previously filtrated on Sep-Pak Light QMA cartridge (Waters). Another mouse was injected with the solution of oxotechnetium alone (neutralized and filtrated as reported above) as a reference. After 2 h, animals were anesthetized with 2% isoflurane.

Beta-imaging studies. One mouse was anesthetized and then immersed in an −80 °C mixture of dry ice and isopentane. After blocking body in mounting medium, tissue sections (20 µm) were made at −20 °C with a slicing microtome (LEICA Microsystems, France). Sections were kept at room temperature for 2 h in the presence of silica gel to ensure complete drying. The quantitative determination of the radioactivity (^{99m}Tc 2 keV emission) in dried tissue sections and imaging were carried out using a β-imager (Biospace Lab, Paris, France) on 3 different sensitivities.

Biodistribution studies. After 2 h, three mice (injected with complex **10**) and one mouse (injected with the solution of oxotechnetium) were sacrificed by exsanguination after anesthesia. Organs were removed then immediately weighed and counted with a MEDI 404 gamma counter (Medisystem, France). The blood and urine were removed and counted too.

Urine analysis. Aliquots of mice urine (200 µL) collected 2 h after injection of complex **10** were acidified with 0.1 M HCl and analyzed by analytical RP-HPLC as reported above. The chromatogram was compared to references.

Incubation of complex 10 in mice urine. Aliquots of complex **10** (^{99m}Tc labelled enriched with ^{99g}Tc) were incubated at 37 °C in fresh mice urine for 30 min. After acidification, the urine was analyzed by analytical RP-HPLC. Peaks were isolated, solution volumes were concentrated and analyzed by MALDI TOF/TOF spectrometry.

Complex 10/cysteine challenge. ^{99g}Tc-labelled complex **10** was treated with 10 equivalent of cysteine in a pH 7.2 HEPES buffer for 2 h at 37 °C. The mixture was analyzed by RP-HPLC and MALDI TOF/TOF spectrometry.

Incubation of complex 10 in complete blood. A solution of TriaS–^{99m}Tc (50 µL) was incubated for 2 h at 37 °C in fresh complete blood (450 µL). After centrifugation for 15 min at 1600 rpm, the clear solution was treated with 10% TFA. After centrifugation for 5 min at 1600 rpm, an aliquot of 50 µL diluted in water (150 µL) was analyzed by analytical radio-HPLC.

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