

Supplementary Materials: Technetium Nitrido-Peroxo Complexes: An Unexplored Class of Coordination Compounds

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1. PMR Spectra

Proton magnetic resonance spectra (PMR) were carried out using a Bruker Advance 500 (Bruker BioSpin GmbH, Germany in DMSO-*d*₆, at 25°C, using tetramethylsilane (TMS) as reference.

[^{99g}Tc][Tc(N)(O₂)(Pro)]⁻: multiple overlapping peaks in the range 5.2–2.5 ppm (CH and CH₂ ring protons).

[^{99g}Tc][Tc(N)(O₂)(Gly)]⁻: multiplet at 3.60 ppm (CH₂).

[^{99g}Tc][Tc(N)(O₂)(Ala)]⁻: 1.52 ppm (d, CH), (3.78 ppm (q, CH₃).

Proton signals of the [As(C₆H₅)₄]⁺ cation were found in the usual range 7.20–7.30 ppm.

2. ESI-MS Spectra

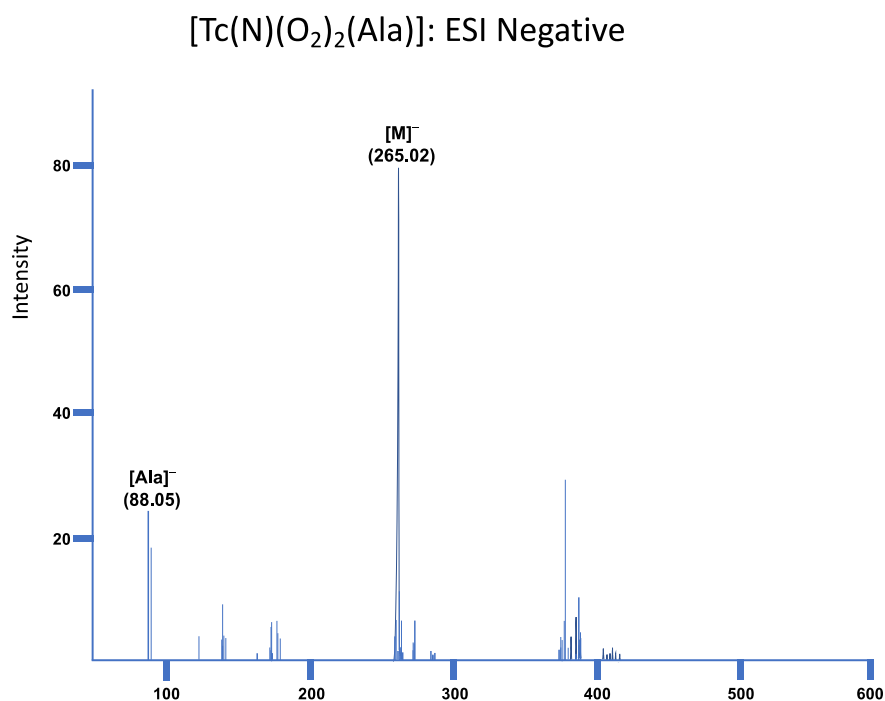


Figure S1. Negative-ion mode ESI-MS of [As(C₆H₅)₄]{[^{99g}Tc]Tc(N)(O₂)₂(Ala)}⁻.

[Tc(N)(O₂)₂(Ala)]: ESI Positive

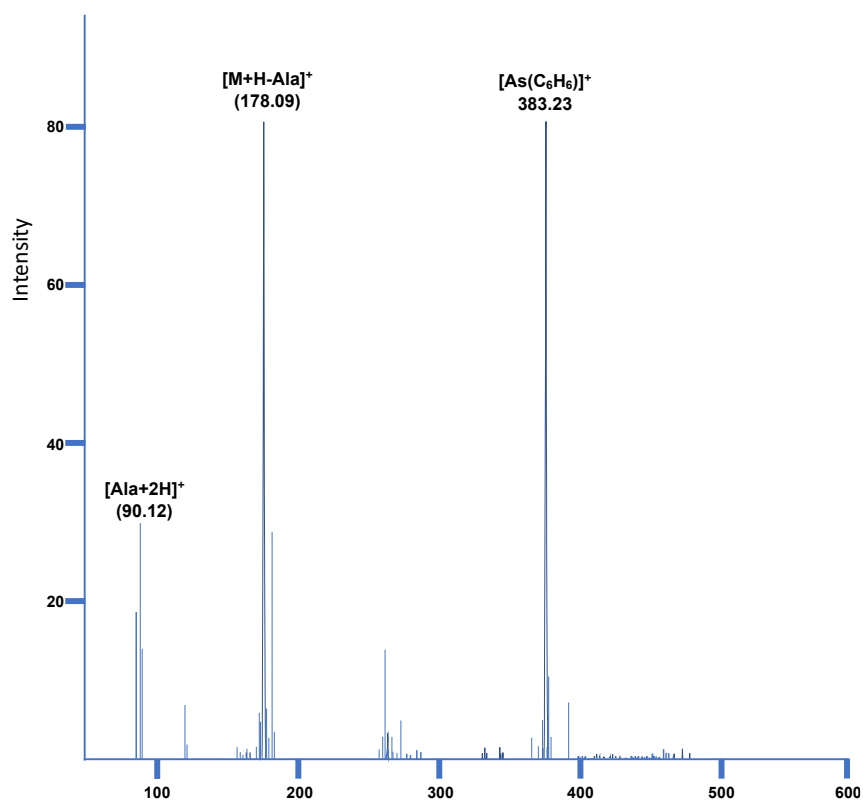


Figure S2. Positive-ion mode ESI-MS of [As(C₆H₅)₄][^{99g}Tc]Tc(N)(O₂)₂(Ala)}.

[Tc(N)(O₂)₂(Pro)]: ESI Negative

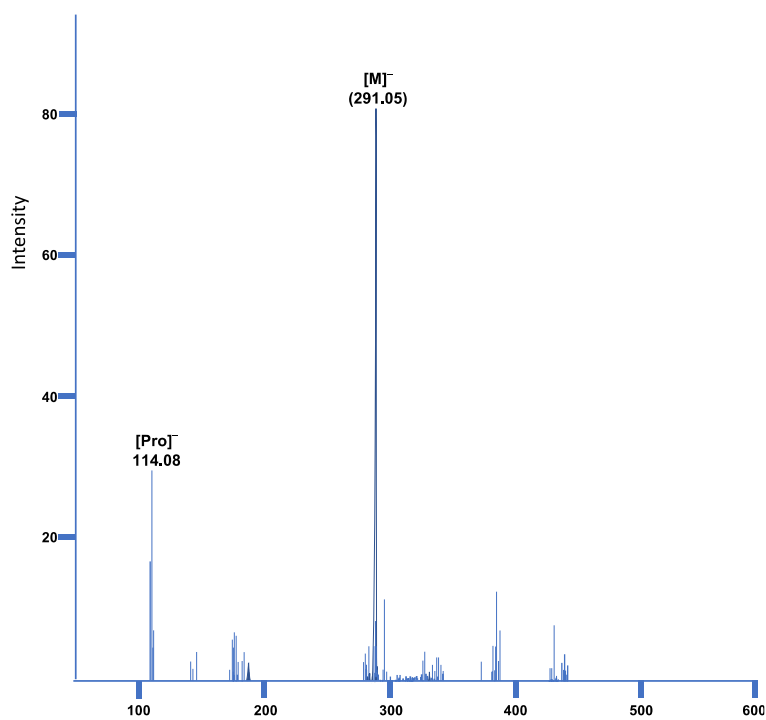


Figure S3. Negative-ion mode ESI-MS of [As(C₆H₅)₄][^{99g}Tc]Tc(N)(O₂)₂(Pro)}.

[Tc(N)(O₂)₂(Pro)]: ESI Positive

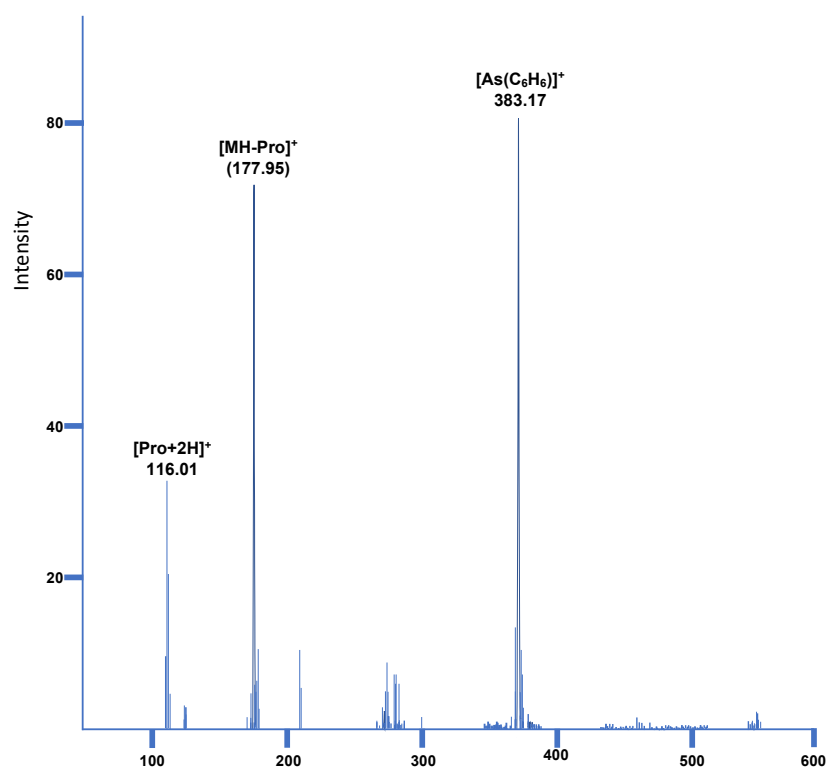


Figure S4. Positive-ion mode ESI-MS of [As(C₆H₅)₄]{^{99g}Tc}Tc(N)(O₂)₂(Pro)}.

[Tc(N)(O₂)₂(Gly)]: ESI Negative

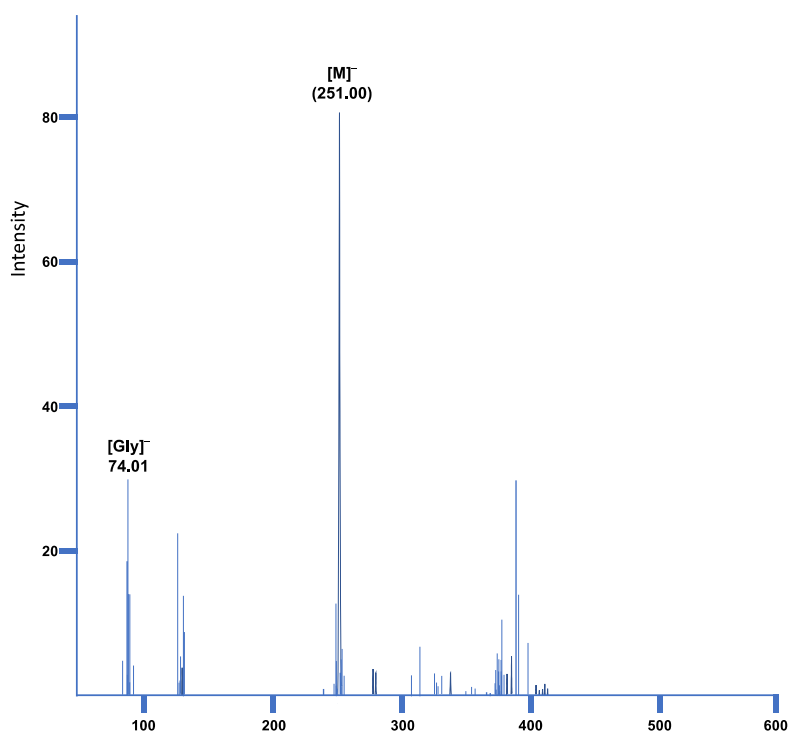


Figure S5. Negative-ion mode ESI-MS of [As(C₆H₅)₄]{^{99g}Tc}Tc(N)(O₂)₂(Gly)}.

[Tc(N)(O₂)₂(Gly)]: ESI Positive

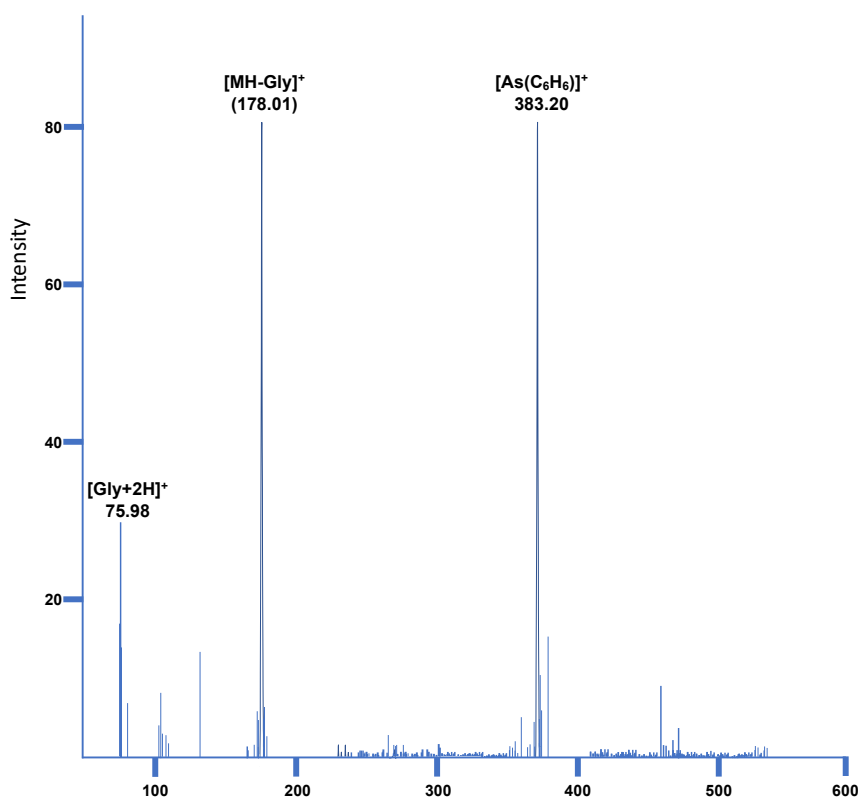


Figure S6. Positive-ion mode ESI-MS of [As(C₆H₅)₄][^{99m}Tc]Tc(N)(O₂)₂(Gly)}.

3. Preparation of ^{99m}Tc Complexes

A generator-eluted, aqueous solution containing [^{99m}Tc][TcO₄]⁻Na (0.400 mL, 50.0 MBq, Drytec™, GE Healthcare) was added to a vial containing *S*-methyl-*N*-methyl-dithiocarbamate (DEDIC = H₃C–NH–NH–C(=S)SCH₃) (15.0 mg) and SnCl₂ (0.8 mg) suspended in 0.100 mL of saline. The vial was kept at room temperature (RT) for 30 min yielding a mixture of ^{99m}Tc-nitrido intermediate complexes. Then, H₂O₂ (8% *w/w*, 20 μL) was added to this mixture, which was left to stand for 10 min at RT.

Freshly prepared samples of this final solution (S) were first flushed with an argon stream passed through a needle and subsequently used for the reactions with the amino acid glycine and with the sodium salt of diethyldithiocarbamate ({DTC = [(CH₃CH₂)₂NC(=S)]⁻}) as described below.

A 0.2-mL aliquot of solution S were mixed with 0.10 mg of glycine and the mixture was gently heated at 40 °C for 10 min.

Similarly, 5.0 mg of the sodium salt of DEDIC and 20 μg of SnCl₂ were added to a 0.2-mL aliquot of solution S and the resulting mixture was heated at 80 °C for 15 min.

4. HPLC and UPLC Chromatography

High-performance liquid chromatography (HPLC) was performed on a Beckman System Gold instrument equipped with a programmable solvent model 126, a sample injection valve 210A, a scanning detector Module 166, and a radioisotope detector model 170. For HPLC analysis of ^{99g/99m}Tc complexes, a reversed-phase Agilent precolumn Zorbax 300SB-C18 (4.6 × 12.5 mm) and a reversed-phase Agilent column Zorbax 300SB-C18 (4.6 × 250 mm) were eluted with a binary gradient with the following mobile phase. A: methanol containing 0.1% trifluoroacetic acid; mobile phase B H₂O containing 0.1% trifluoroacetic acid. The elution gradient was: 0–5 min, 5% A followed by a linear gradient to 95% A in 15 min, at a flow rate of 1.0 mL min⁻¹.

UPLC was performed with an ACQUITY UPLC system, (Waters, Milford, MA) equipped with an autoinjector, UV detector, and gamma flow count detector (Bioscan, Washington DC), on a Waters

ACQUITY UPLC[®] BEH column (1.7 mm × 2.1 0 mm). The Chromeleon chromatography software package was used for data collection. For the analysis of ^{99g/99m}Tc-complexes, the ACQUITY UPLCs BEH column was eluted at 0.5 mL min⁻¹ with a linear gradient from 100% 10 mmol L⁻¹ phosphate buffer (pH = 7.5) to 100% tetrahydrofuran [1].

The UPLC chromatograms of the complexes [^{99g}Tc][Tc(N)(O₂)₂(L)][As(C₆H₅)₄] (L = Gly, Ala, Pro) are reported in Figure S7. The UPLC chromatogram of the mixture resulting from the reaction of solution S and glycine is shown in Figure S8. The UPLC chromatogram of the compound [^{99m}Tc][Tc(N)(DEDCE)₂] is reported in Figure S9.

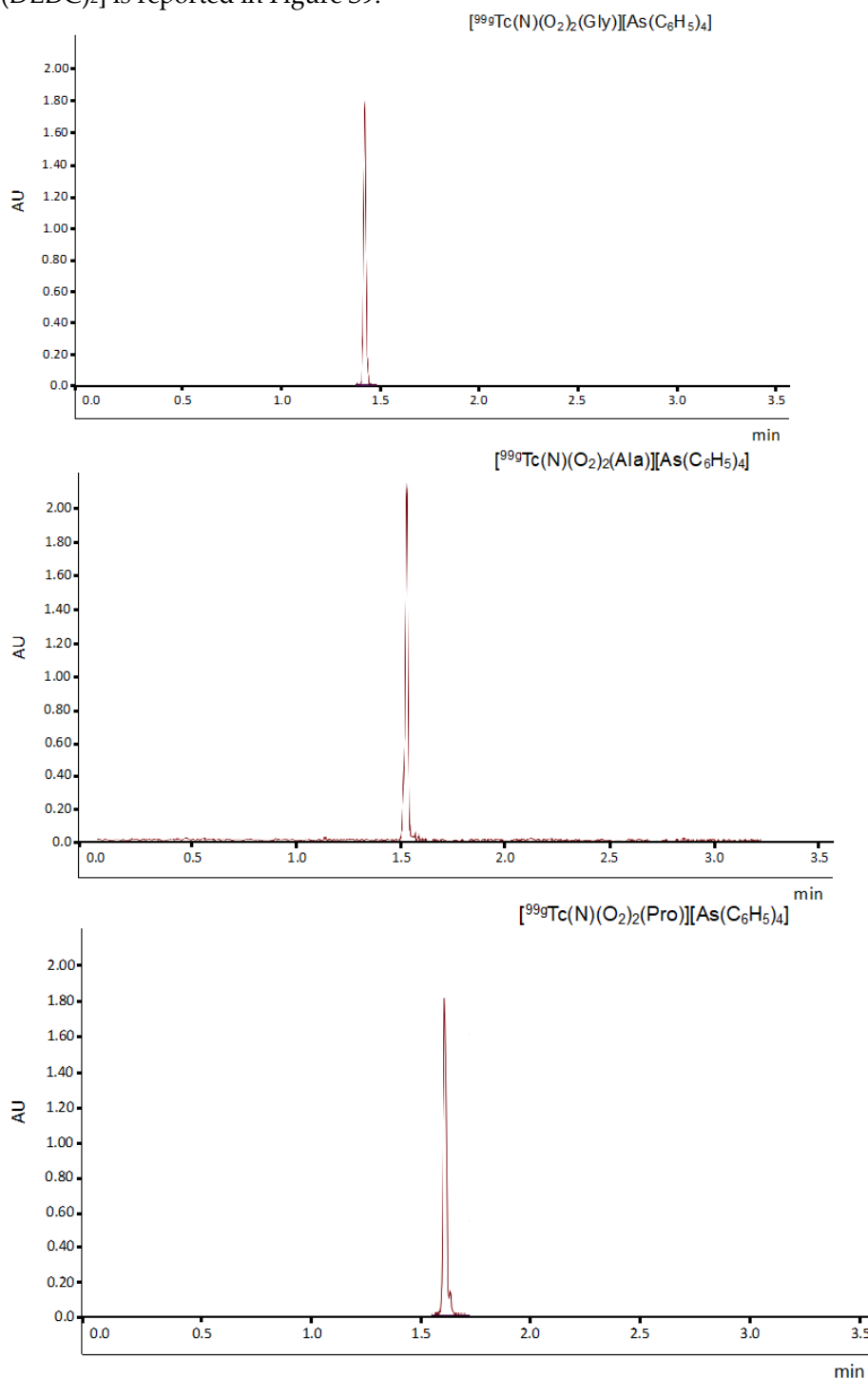


Figure S7. UPLC chromatograms of the novel Tc-99g complexes described in this study.

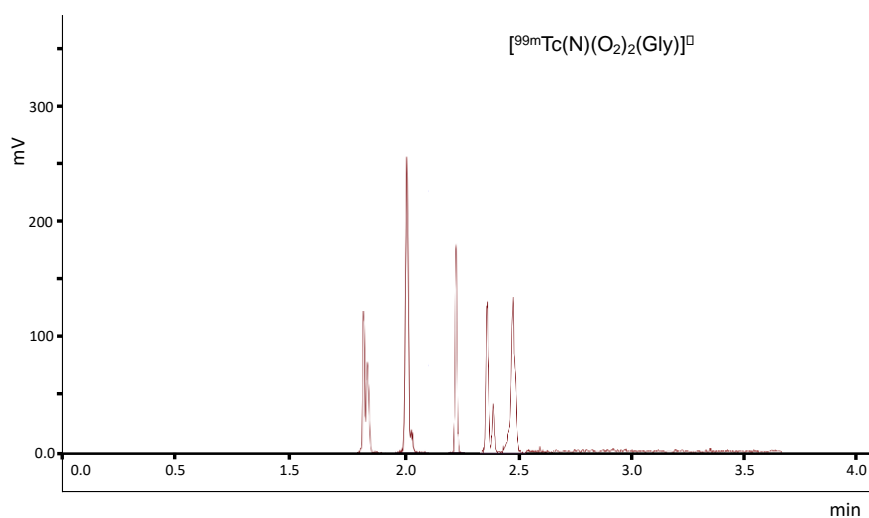


Figure S8. UPLC chromatogram of the mixture resulting from the preparation of the complex $[^{99m}\text{Tc}][\text{Tc}(\text{N})(\text{O}_2)_2(\text{Gly})]^-$.

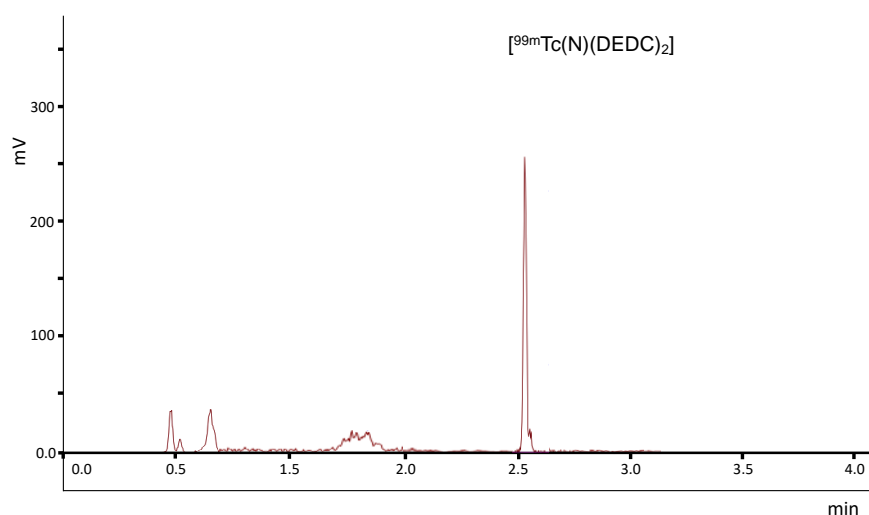


Figure S9. UPLC chromatogram of the complex $[^{99m}\text{Tc}][\text{Tc}(\text{N})(\text{DEDC})_2]$.

5. Antibody labeling

Vials containing 1.0 mg of the lyophilized monoclonal antibody trastuzumab (Herceptin[®]) [2] were reconstituted with an aliquot of the solution containing the precursor $[^{99m}\text{Tc}(\text{N})(\text{O}_2)]$ (52.04–1301.5 MBq) prepared as described in the previous section. The contents were dissolved by gentle swirling and left at room temperature to complete the labeling reaction.

Quality control was carried out by instant thin-layer chromatography (ITLC) on silica gel strips (ITLC-SG, Agilent, Santa Clara, CA) using both 0.9% saline and acetone as mobile phases to detect the presence of $[^{99m}\text{Tc}][\text{TcO}_4]$ that migrated to the solvent front. Then, ITLC-SG strips impregnated with human serum albumin (HSA) (5%) were eluted with a mixture of ethanol: NH_4OH :water (2:1:5 *v/v*) to separate radiocolloids that were retained at the origin while the radiolabeled Mab and pertechnetate moved to the solvent front.

Results of the two combined runs showed that the radiolabeling yield was >90%.

References

1. Kryza, D.; Janier, M. Radio-UHPLC: A tool for rapidly determining the radiochemical purity of technetium-99m radiopharmaceuticals? *Appl. Radiation and Isotopes* **2013**, *78*, 72–76, DOI: 0.1016/j.apradiso.2013.04.005.
2. Stebbing, J., Copson, E., O'Reilly S. Herceptin (trastuzumab) in advanced breast cancer. *Cancer Treat. Rev.* **2000**, *26*, 287–290, DOI: 10.1053/ctrv.2000.0182.