Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Preparation of tetrazine-containing [2+1] complexes of ^{99m}Tc and *in vivo* targeting using bioorthogonal inverse electron cemand Diels-Alder chemistry

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Table of Contents

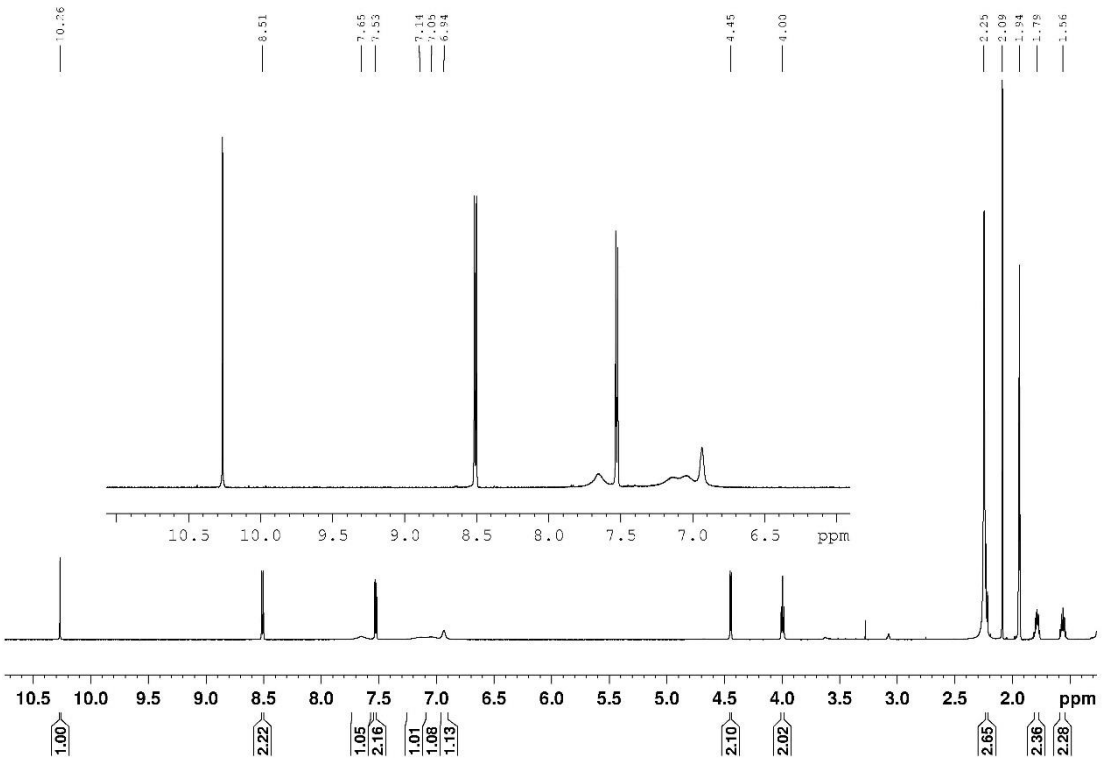

Figure S1. ^{1}H NMR spectrum (CD_3CN, 600 MHz) of **5**.

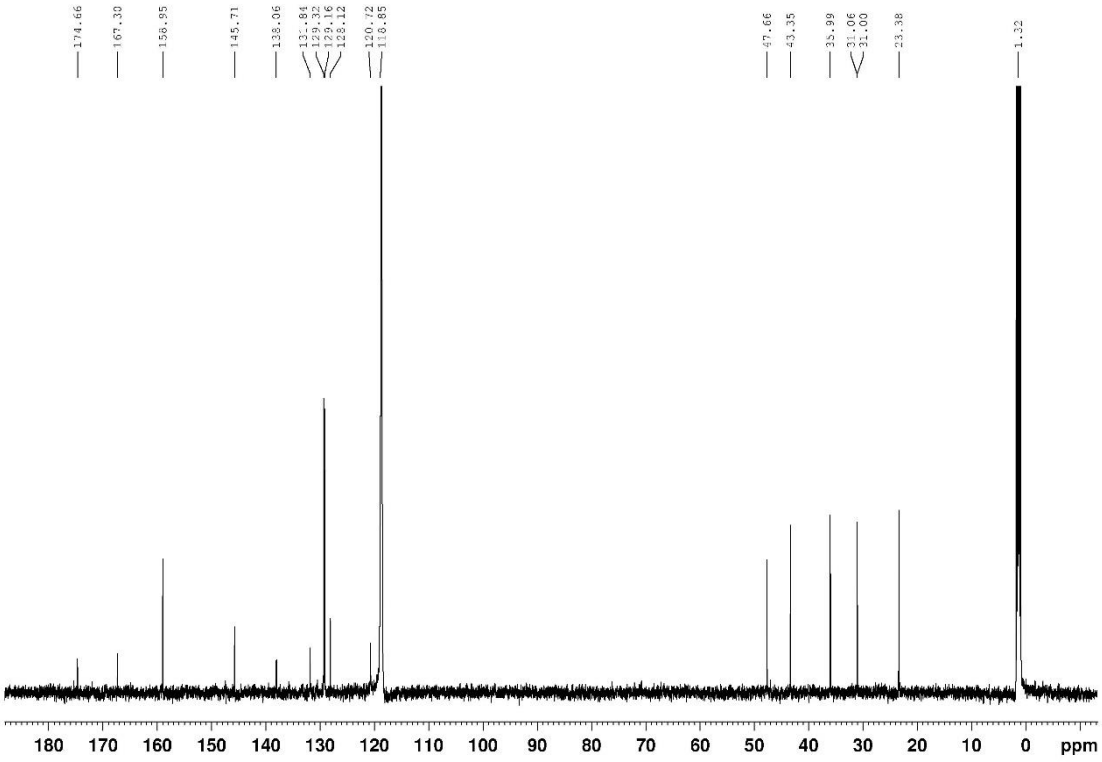

Figure S2. ^{13}C NMR spectrum (CD_3CN, 150 MHz) of **5**.

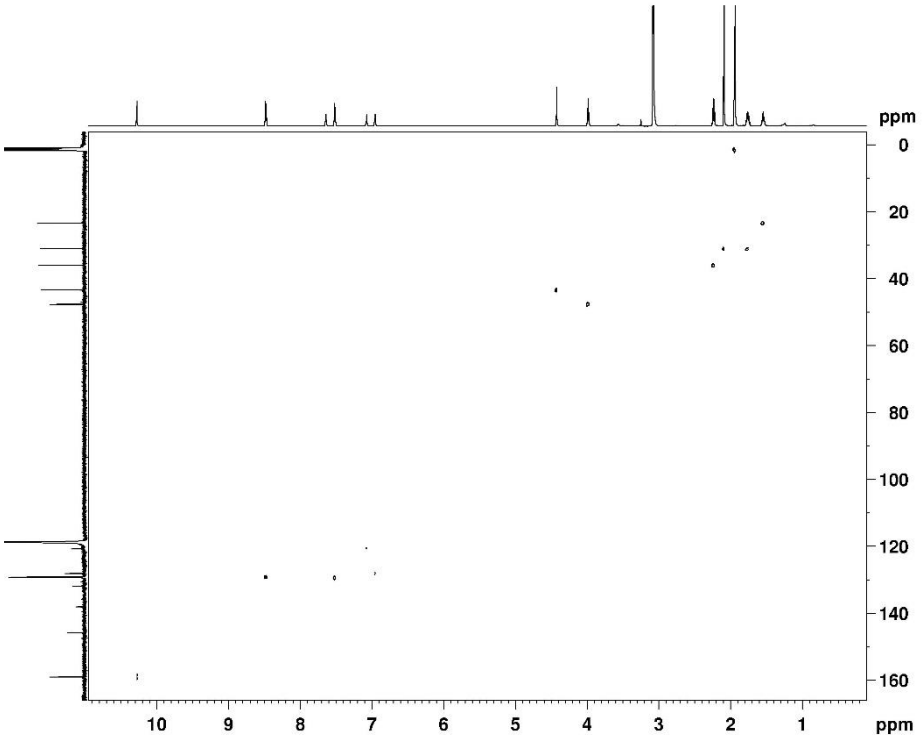

Figure S3. ^{1}H-^{13}C HSQC NMR spectrum (CD_3CN) of **5.**

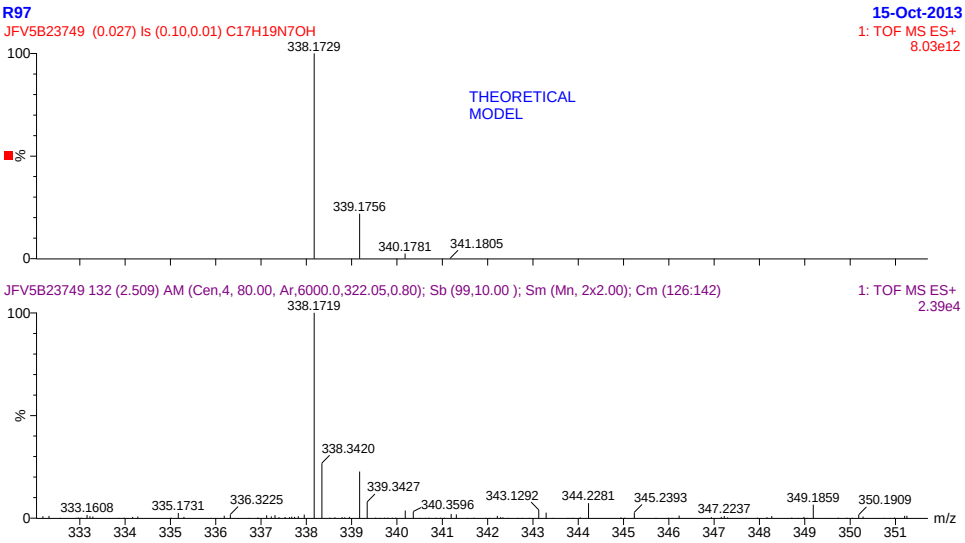

Figure S4. HRMS of **5**.

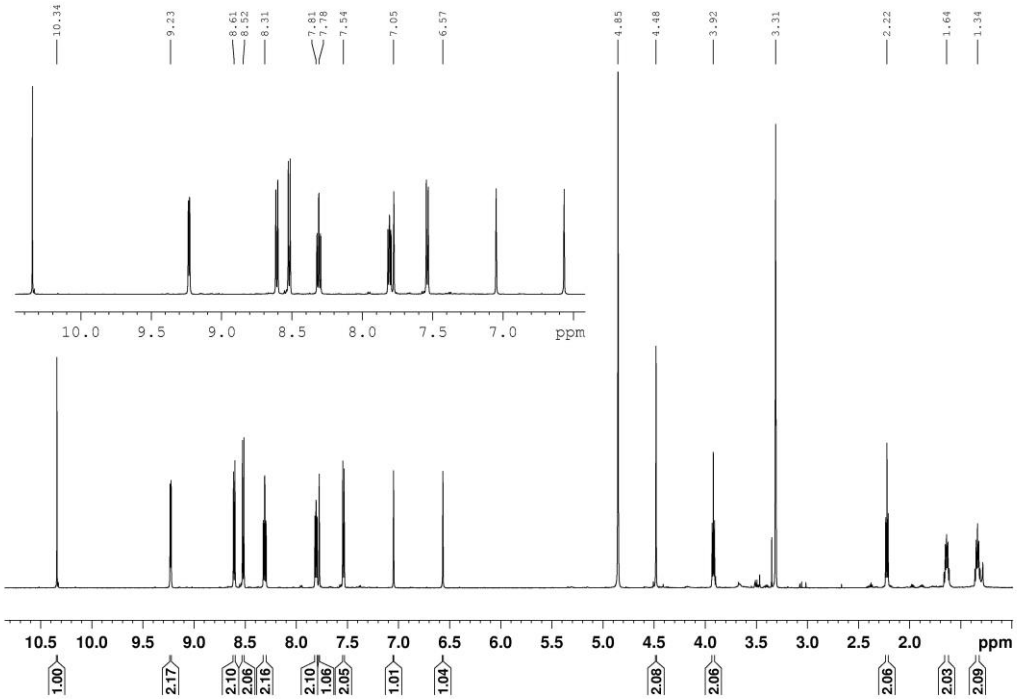

Figure S5. ^{1}H NMR spectrum (MeOD, 600 MHz) of **9a**.

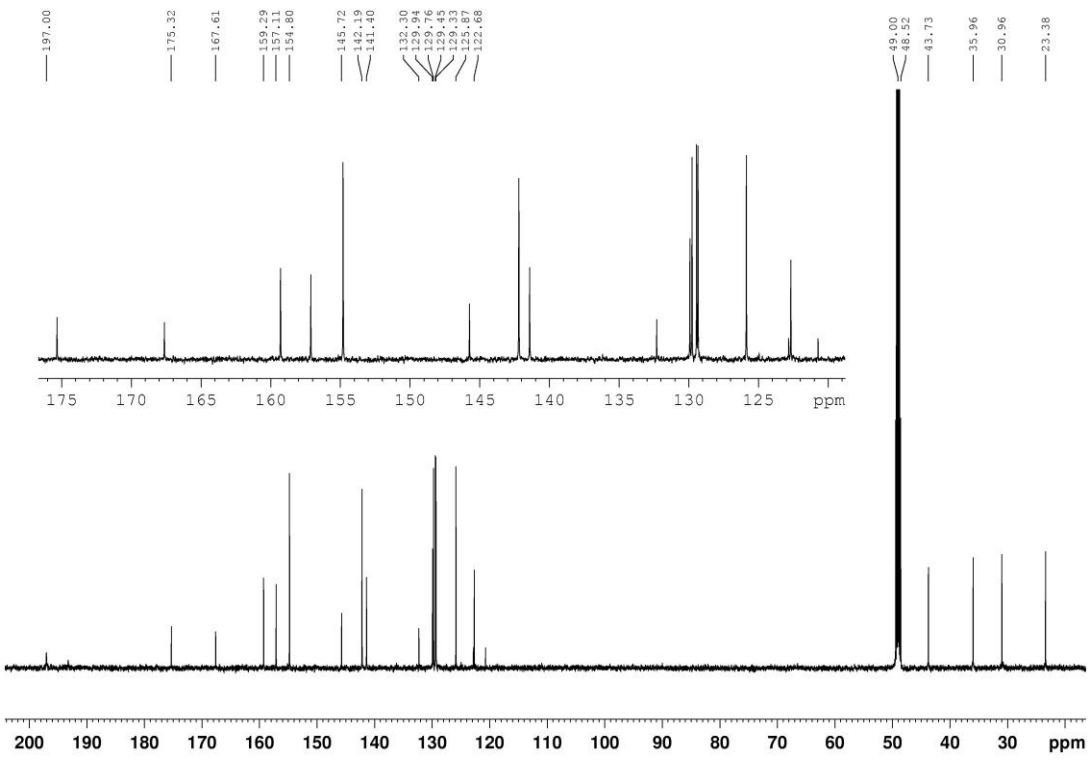

Figure S6. ^{13}C NMR spectrum (MeOD, 150 MHz) of **9a**.

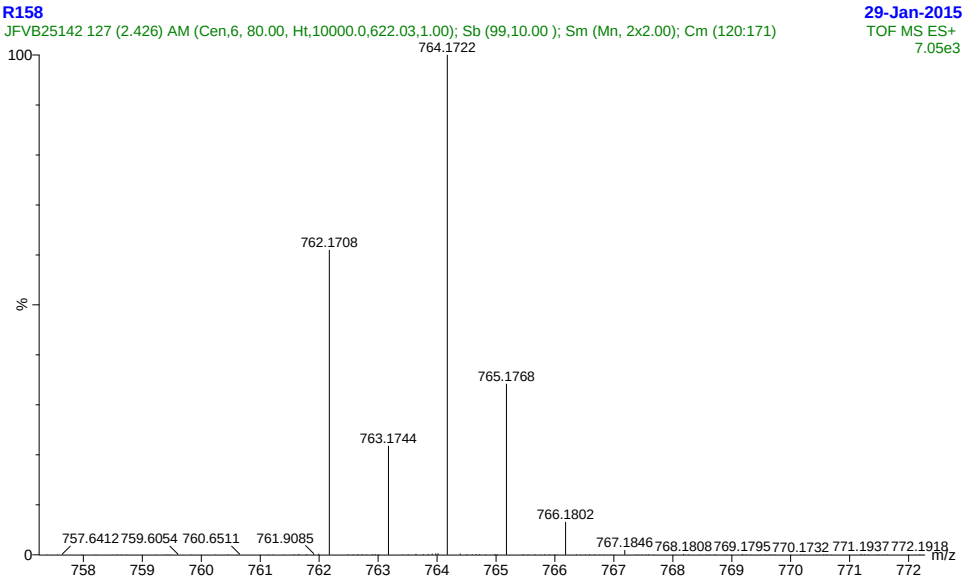

Figure S7. HRMS of **9a**.

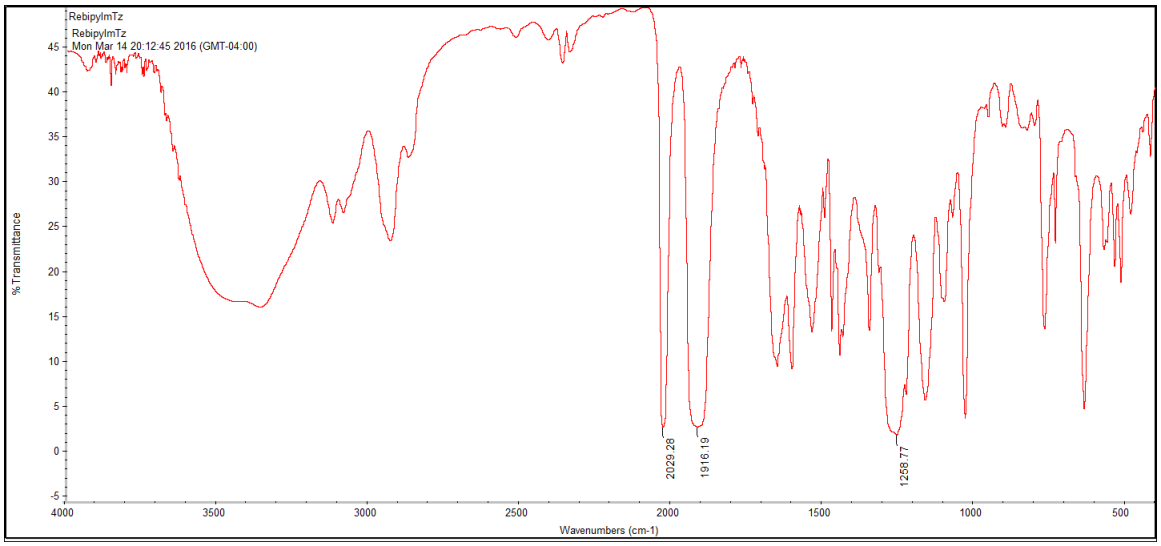

Figure S8. IR spectrum of **9a**.

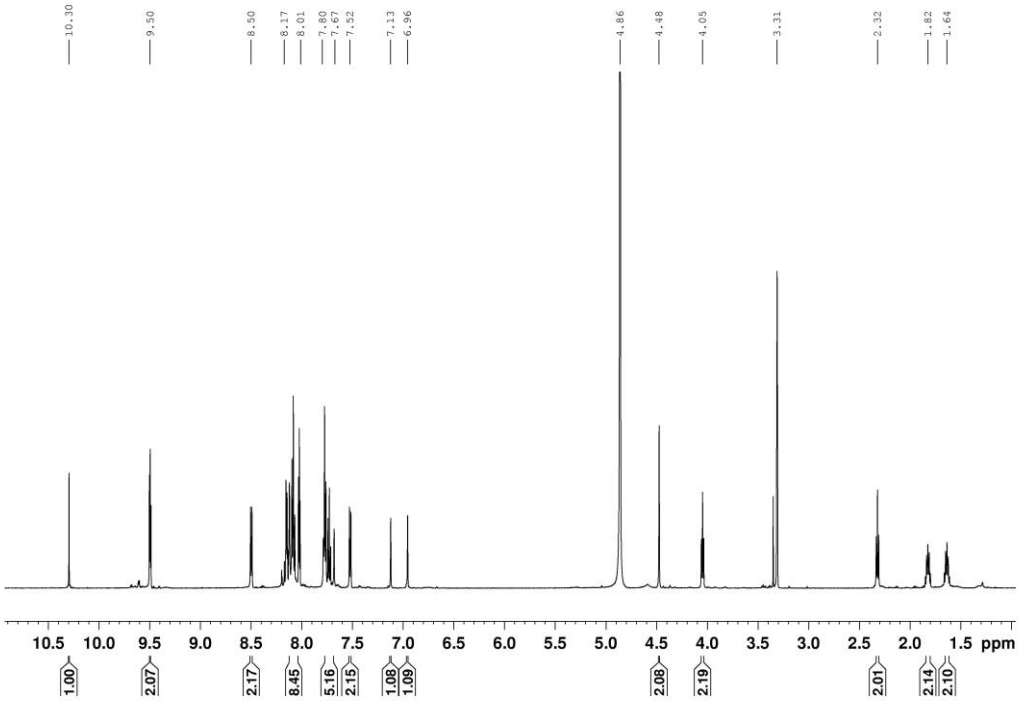

Figure S9. ^{1}H NMR spectrum (MeOD, 600 MHz) of **10a**.

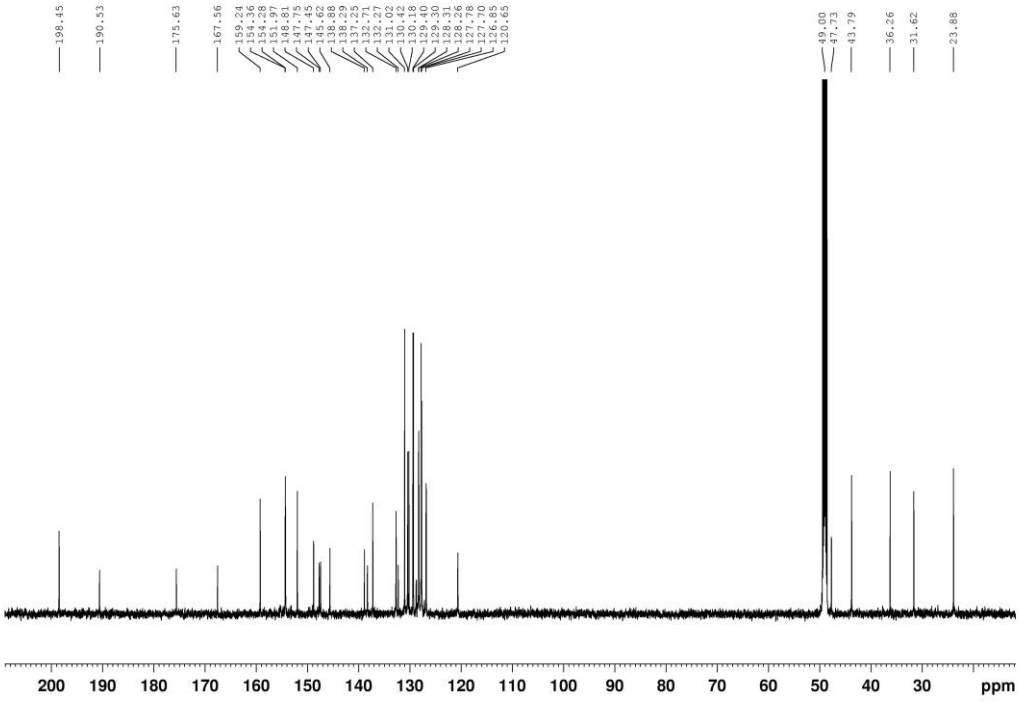

Figure S10. ^{13}C NMR spectrum (MeOD, 150 MHz) of **10a**.

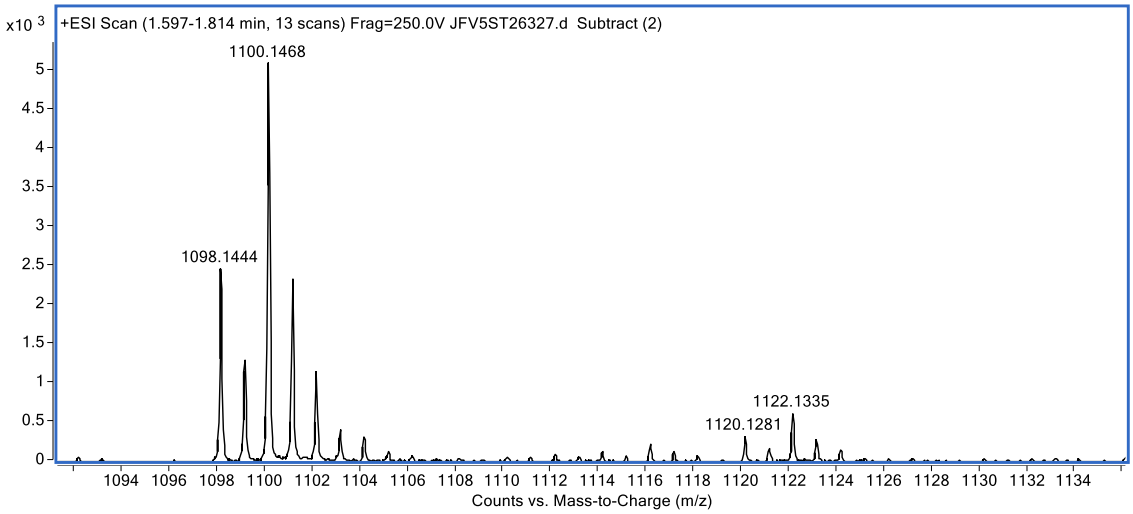

Figure S11. HRMS of **10a**.

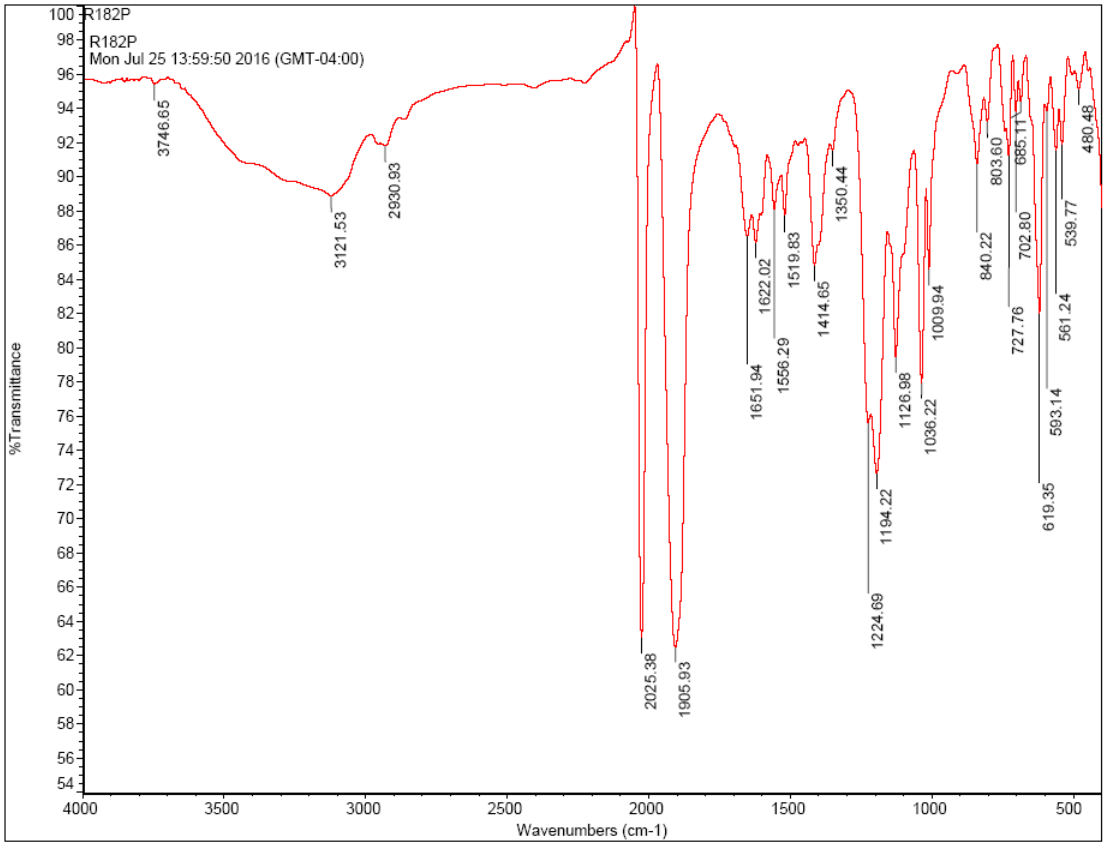

Figure S12. IR spectrum of **10a**.

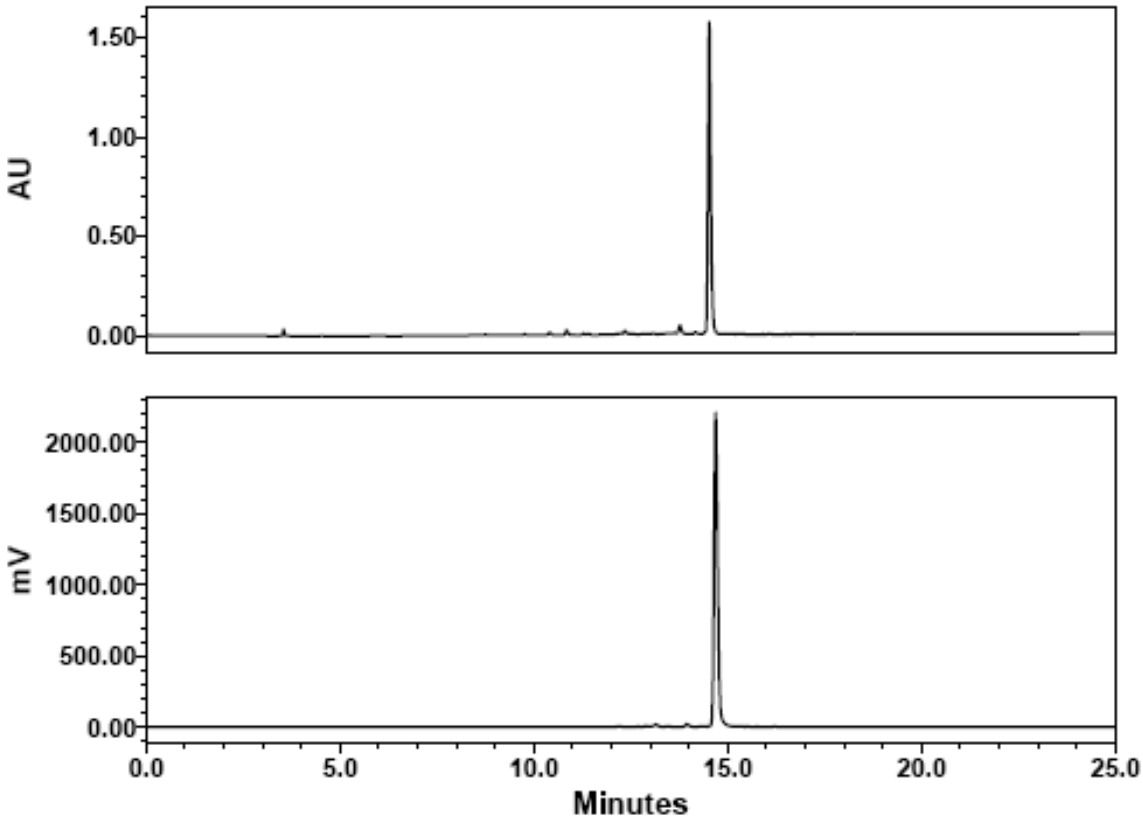

Figure S13. HPLC chromatograms (UV, top and γ, bottom) of $[Re(CO)_3(bipy)(ImTz)]^+$ **9a** co-injected with **9b**.

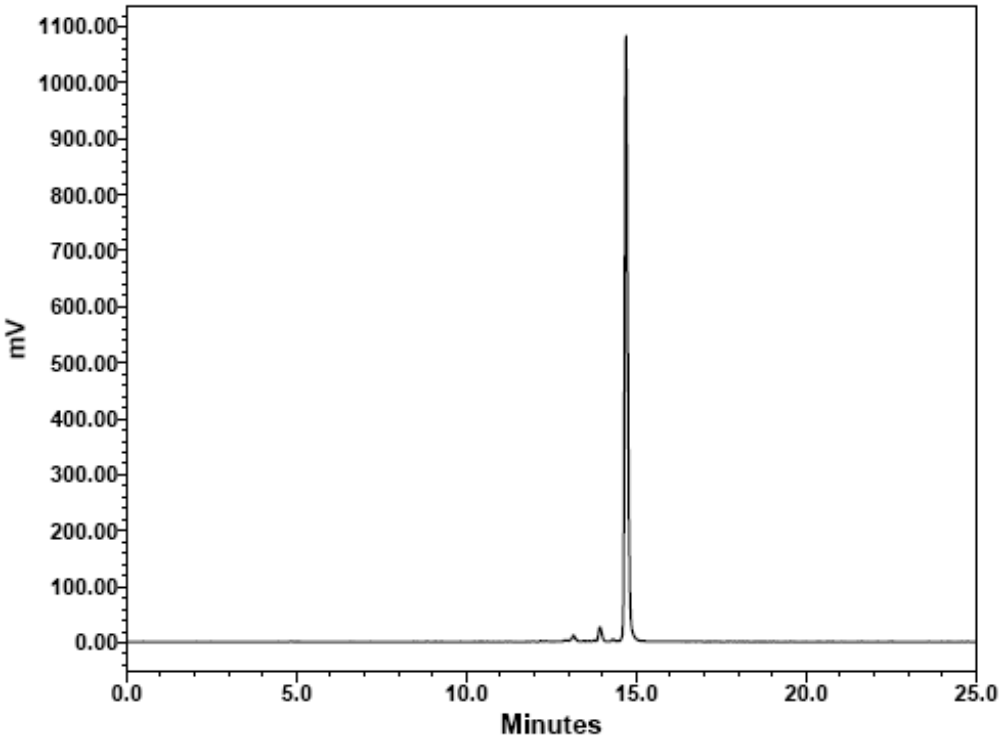

Figure S14. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 0.5 h following cysteine challenge (2 mM).

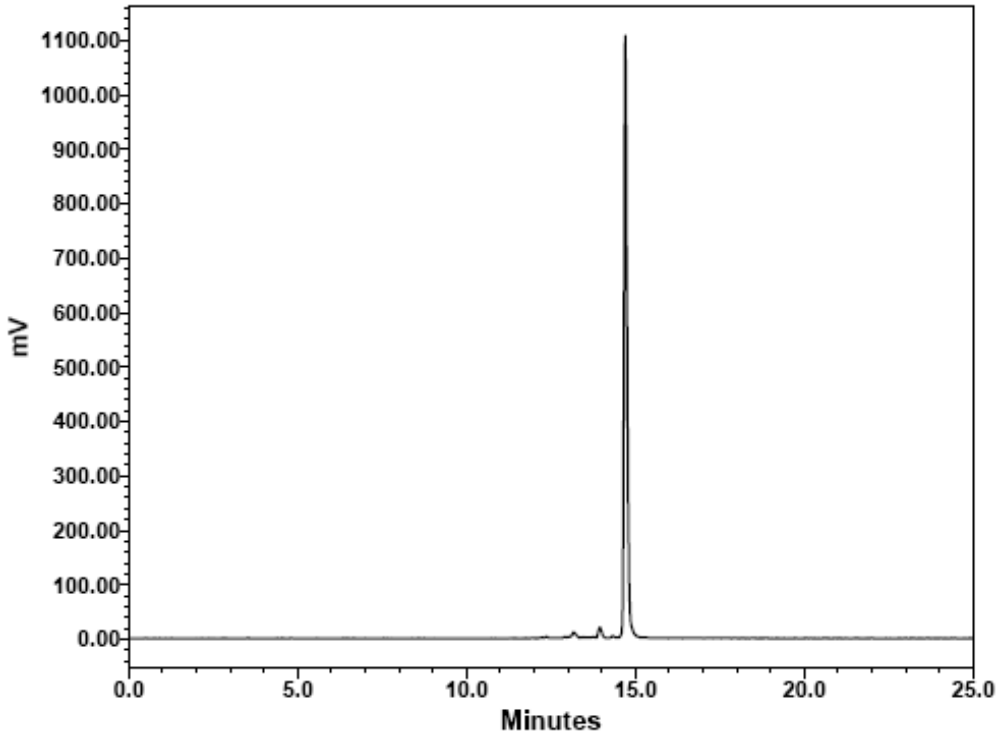

Figure S15. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 1 h following cysteine challenge (2 mM).

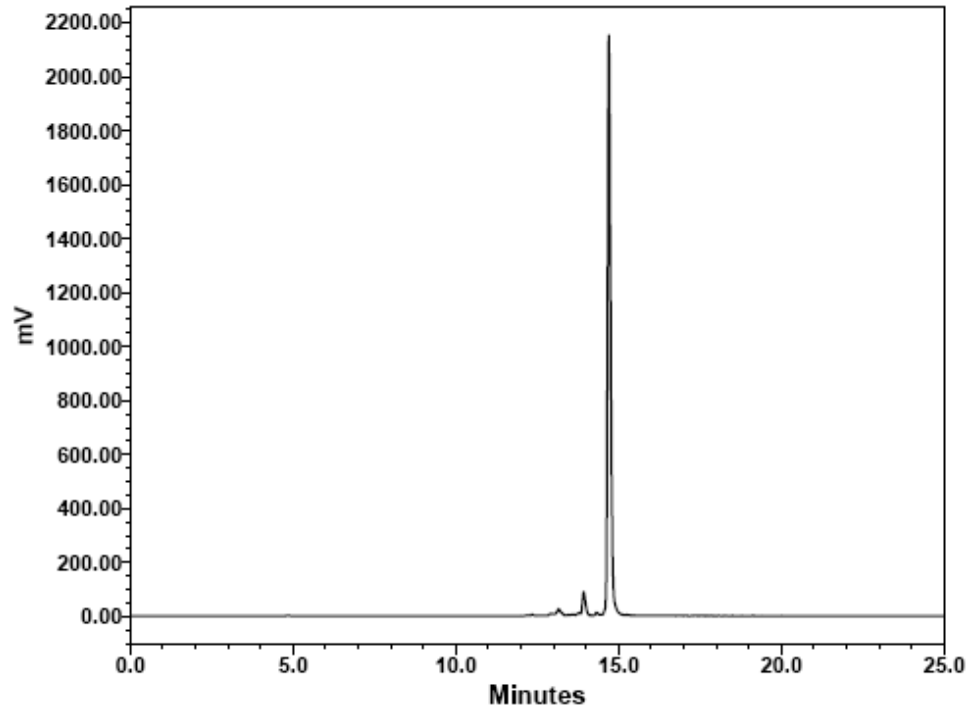

Figure S16. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 2 h following cysteine challenge (2 mM).

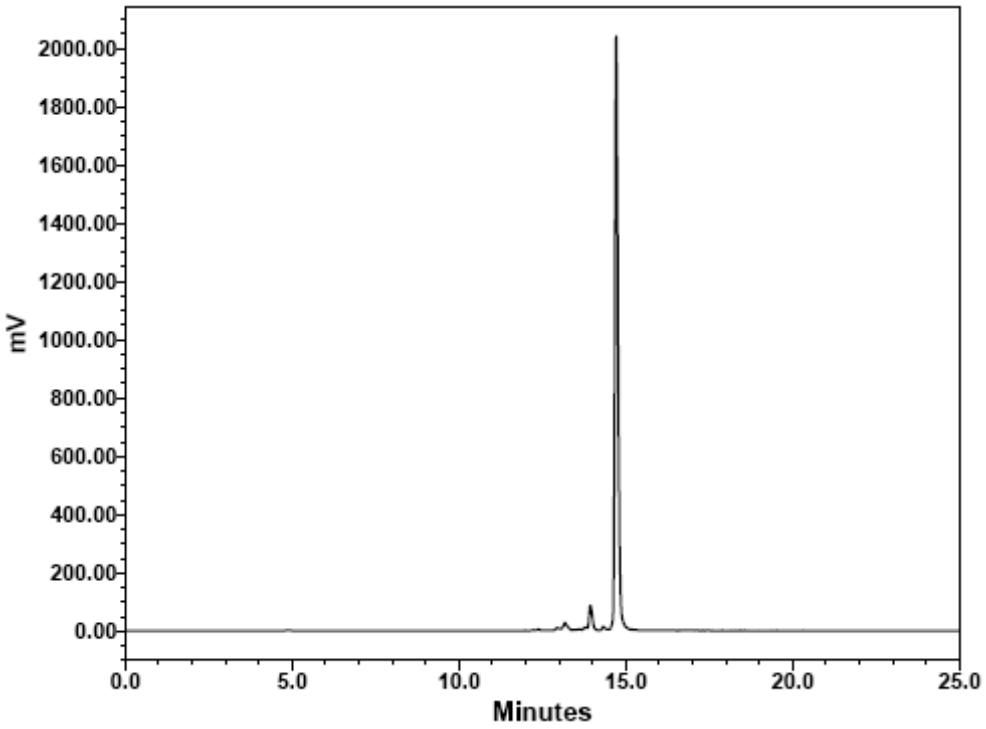

Figure S17. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 3 h following cysteine challenge (2 mM).

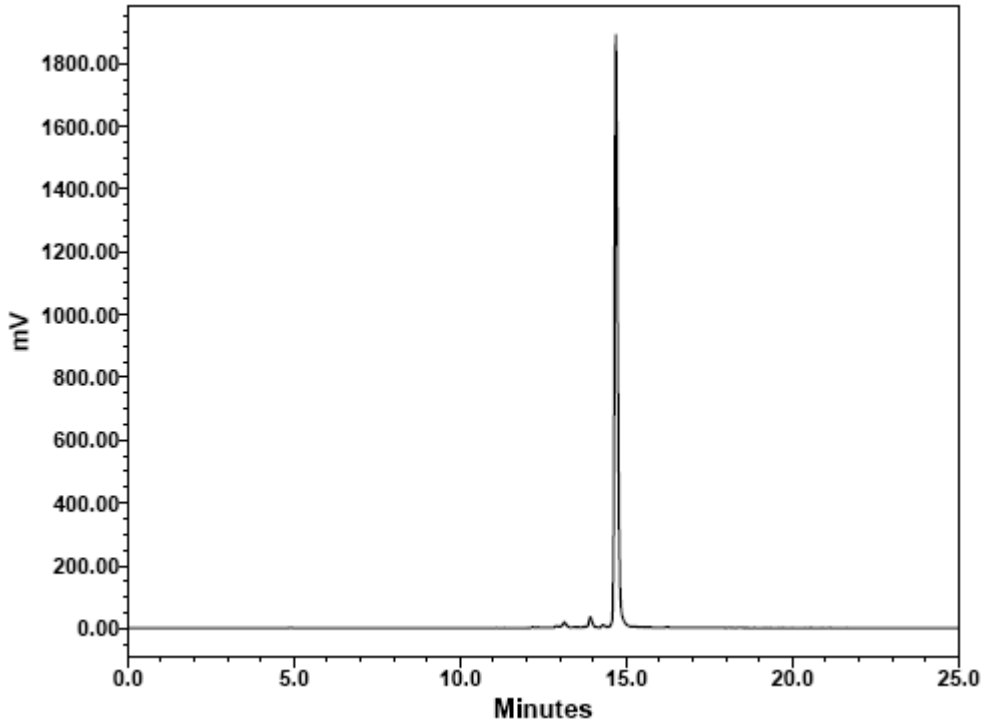

Figure S18. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 6 h following cysteine challenge (2 mM).

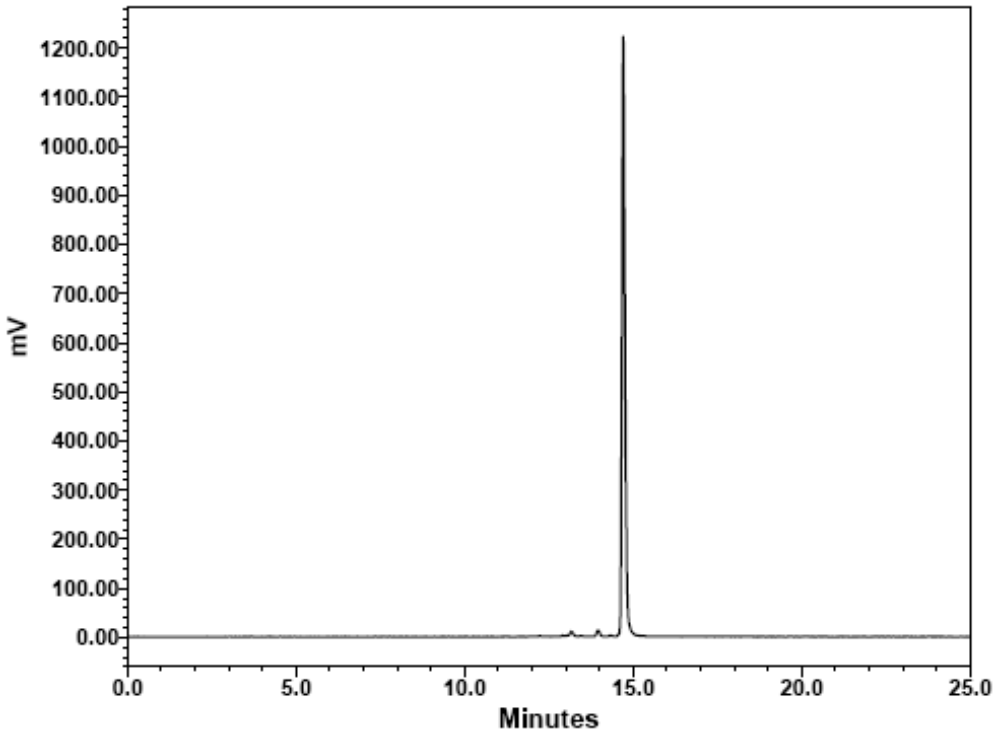

Figure S19. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 0.5 h following histidine challenge (2 mM).

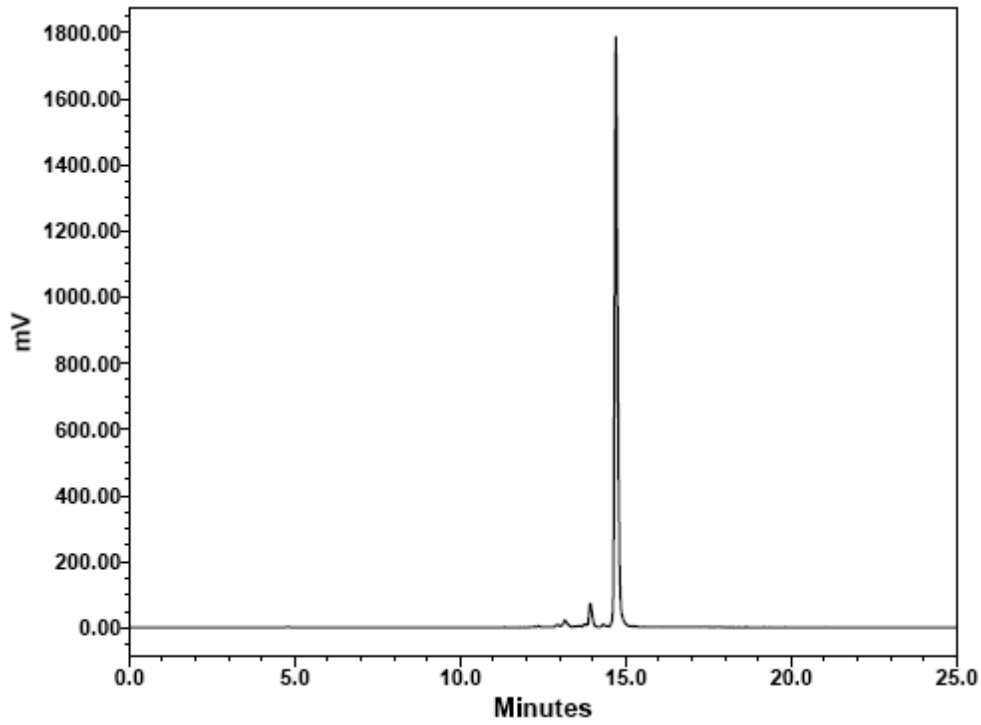

Figure S20. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 1 h following histidine challenge (2 mM).

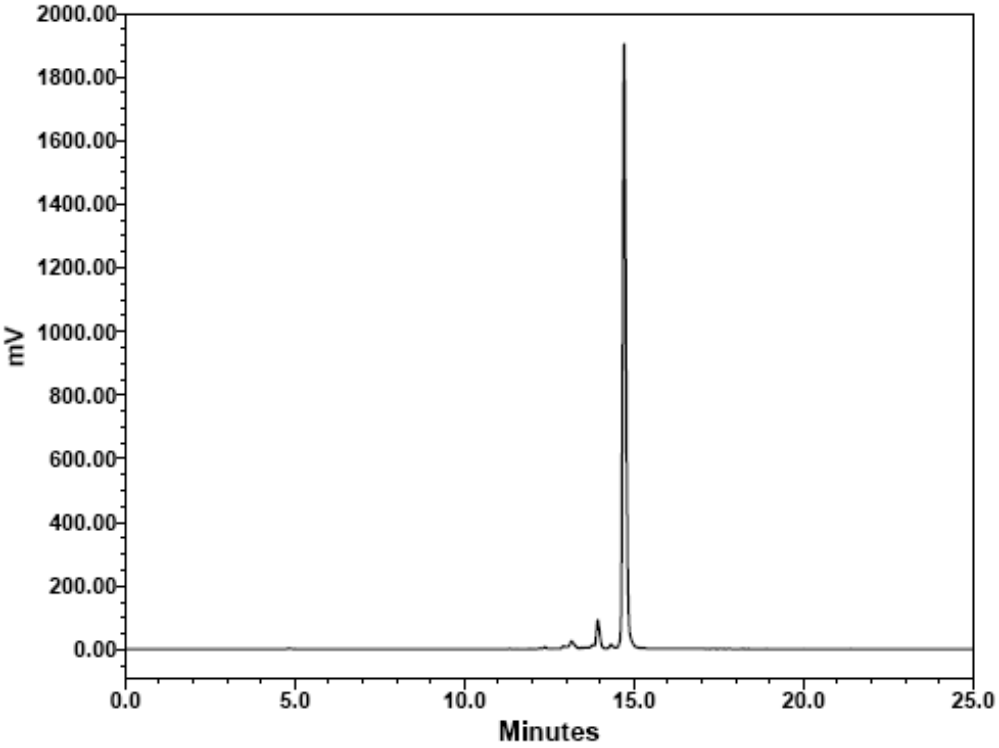

Figure S21. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 2 h following histidine challenge (2 mM).

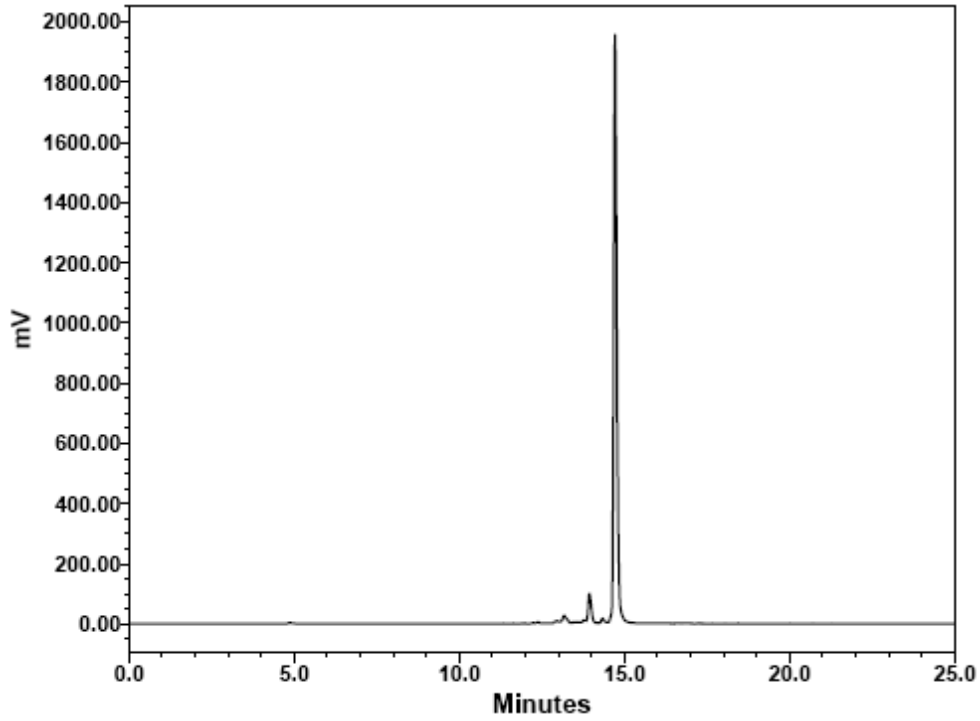

Figure S22. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 3 h following histidine challenge (2 mM).

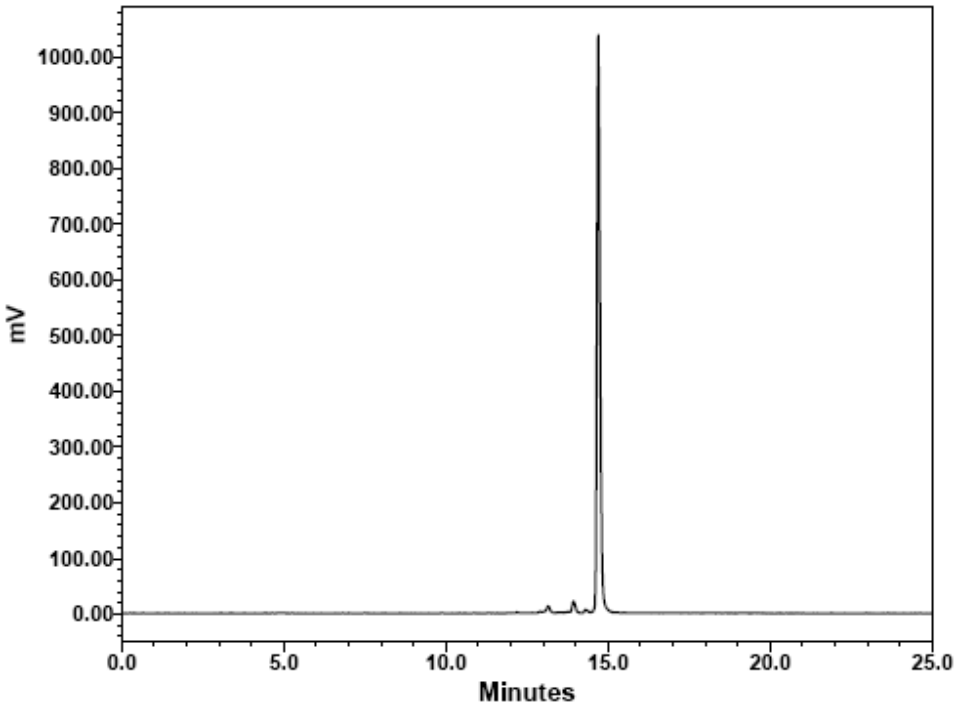

Figure S23. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(bipy)(ImTz)]^+$ **9b** at 6 h following histidine challenge (2 mM).

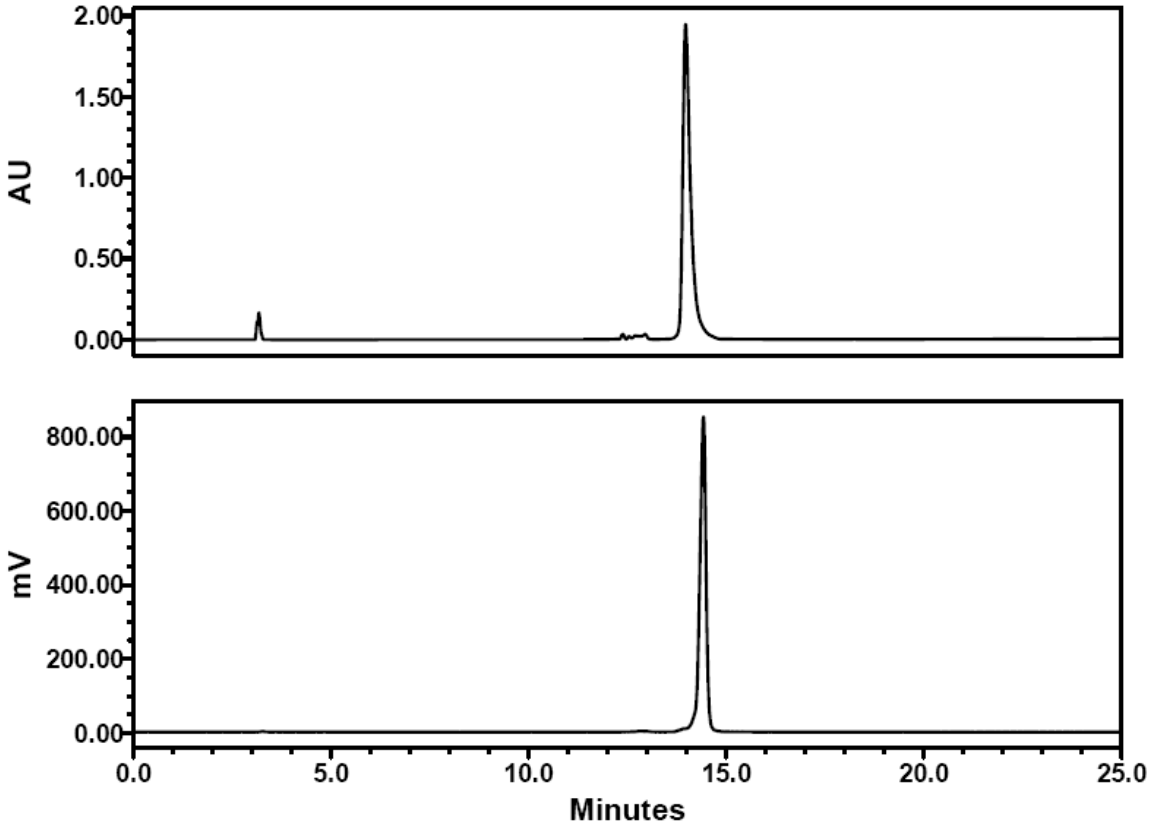

Figure S24. HPLC chromatograms (UV, top and γ, bottom) of $[Re(CO)_3(BPS)(ImTz)]^+$ **10a** co-injected with **10b**.

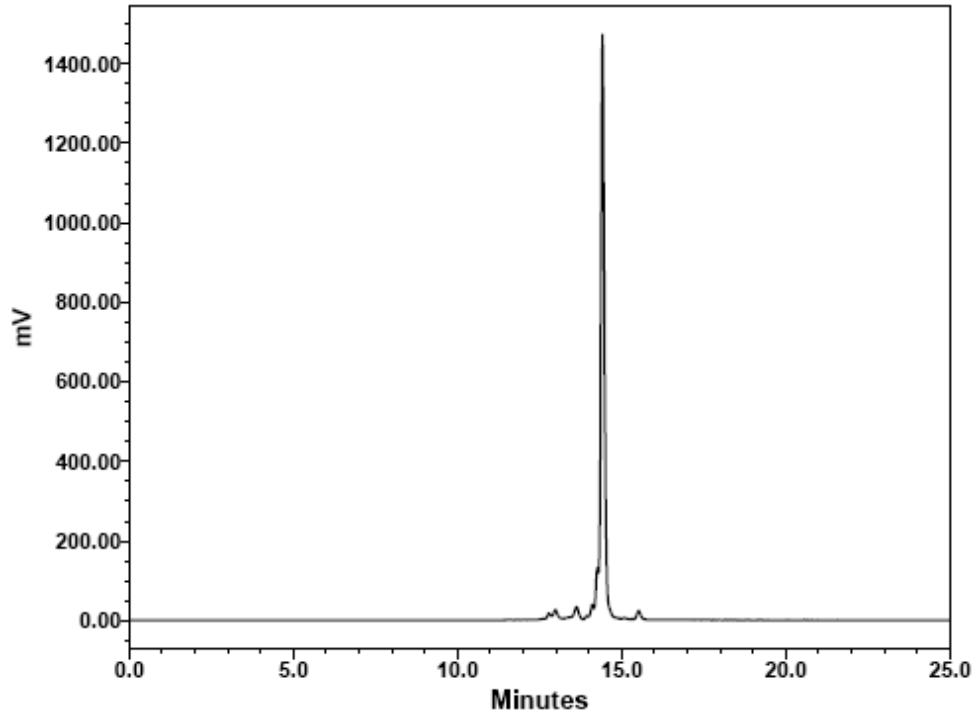

Figure S25. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 0.5 h following cysteine challenge (2 mM).

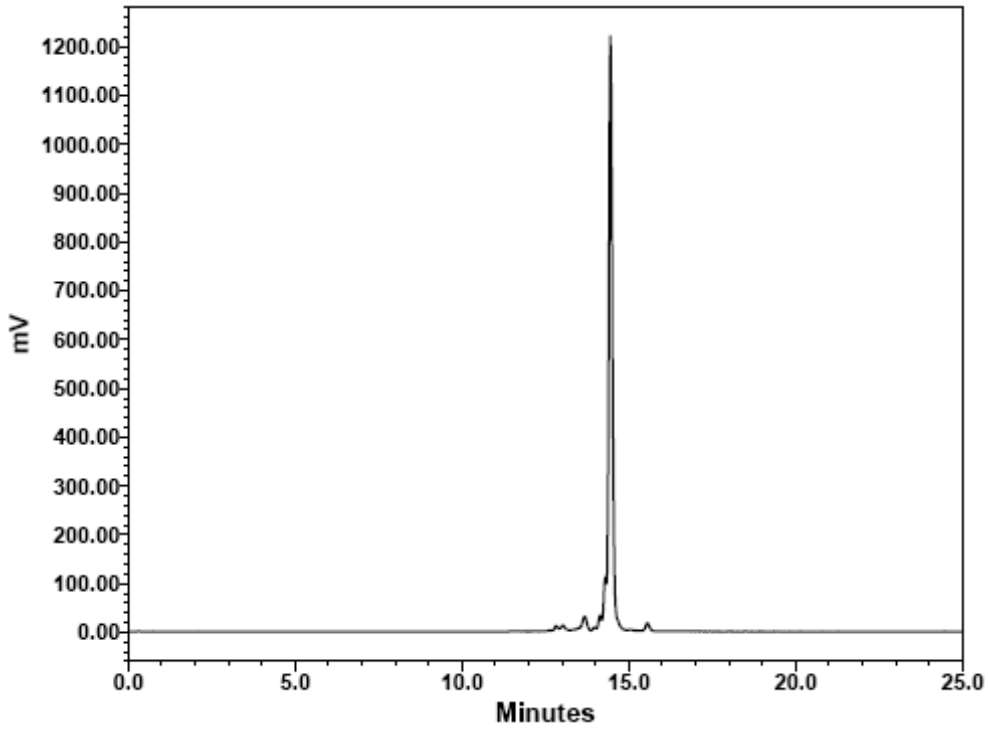

Figure S26. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 1 h following cysteine challenge (2 mM).

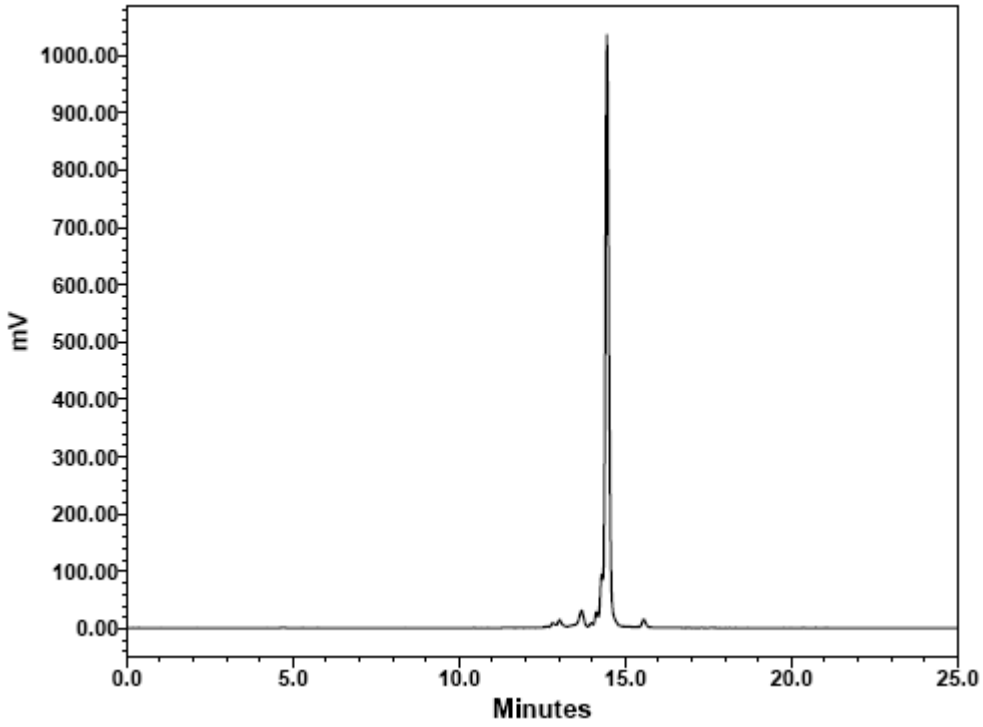

Figure S27. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 2 h following cysteine challenge (2 mM).

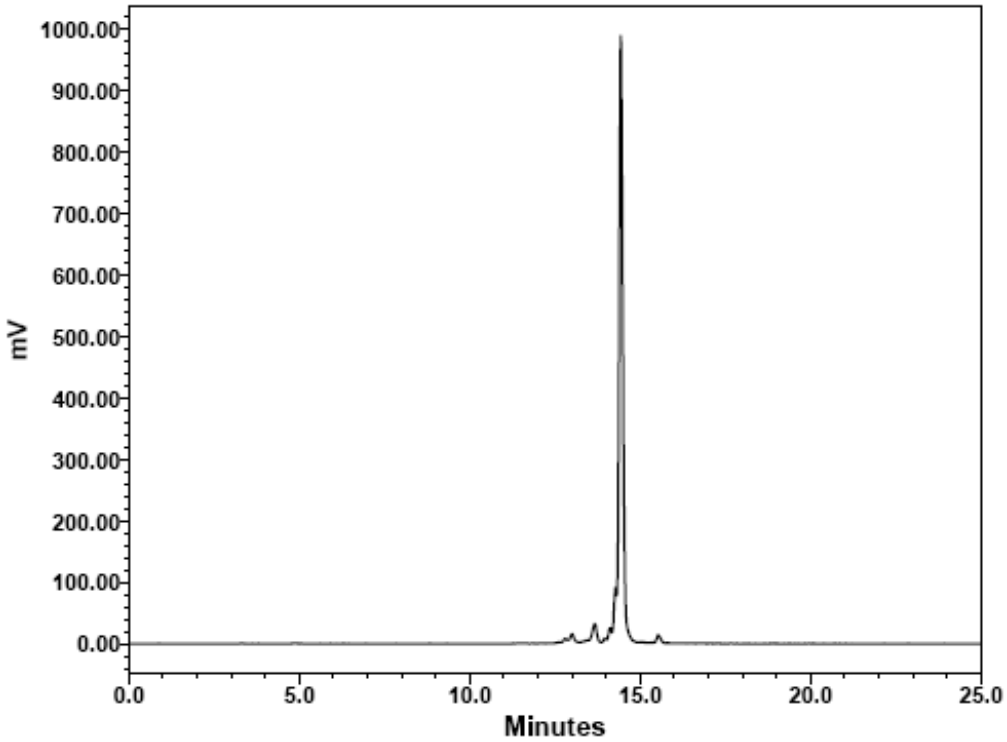

Figure S28. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 3 h following cysteine challenge (2 mM).

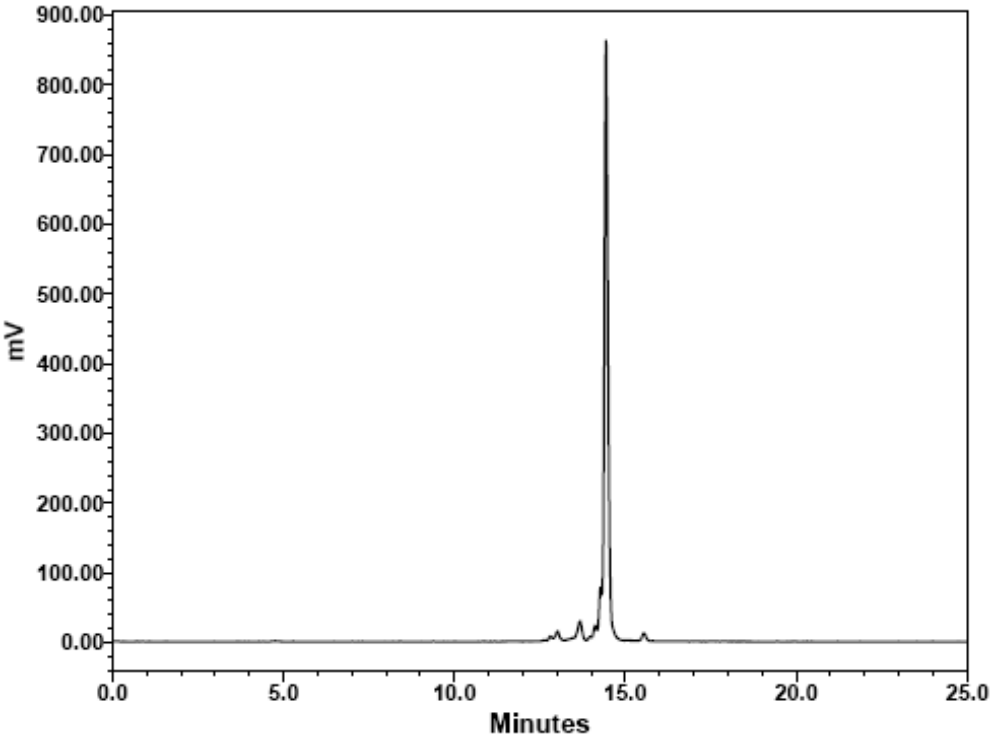

Figure S29. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 6 h following cysteine challenge (2 mM).

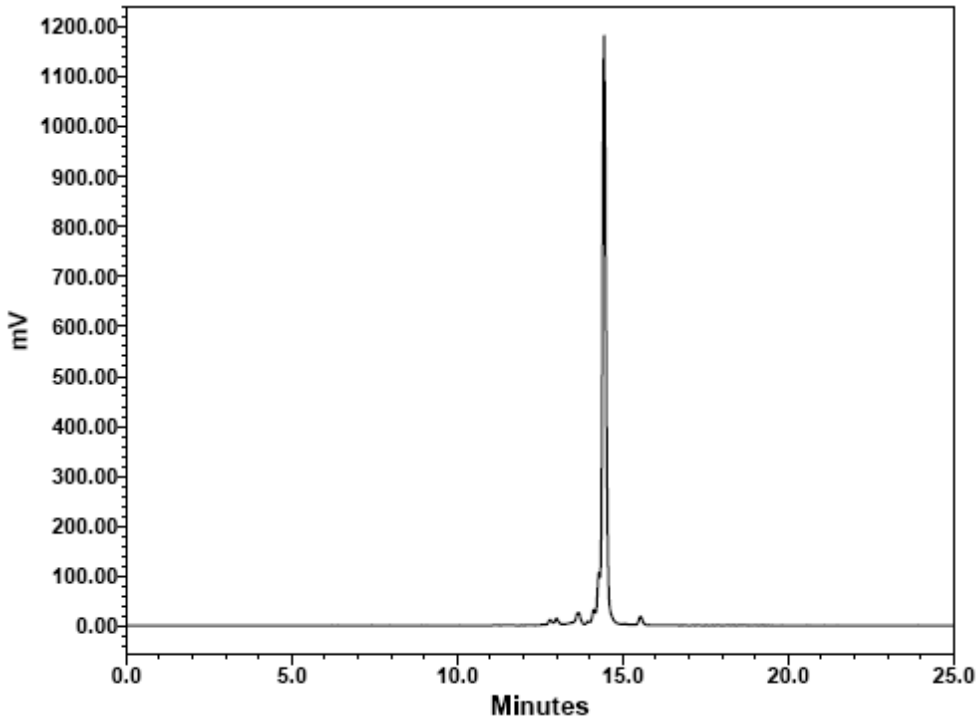

Figure S30. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 0.5 h following histidine challenge (2 mM).

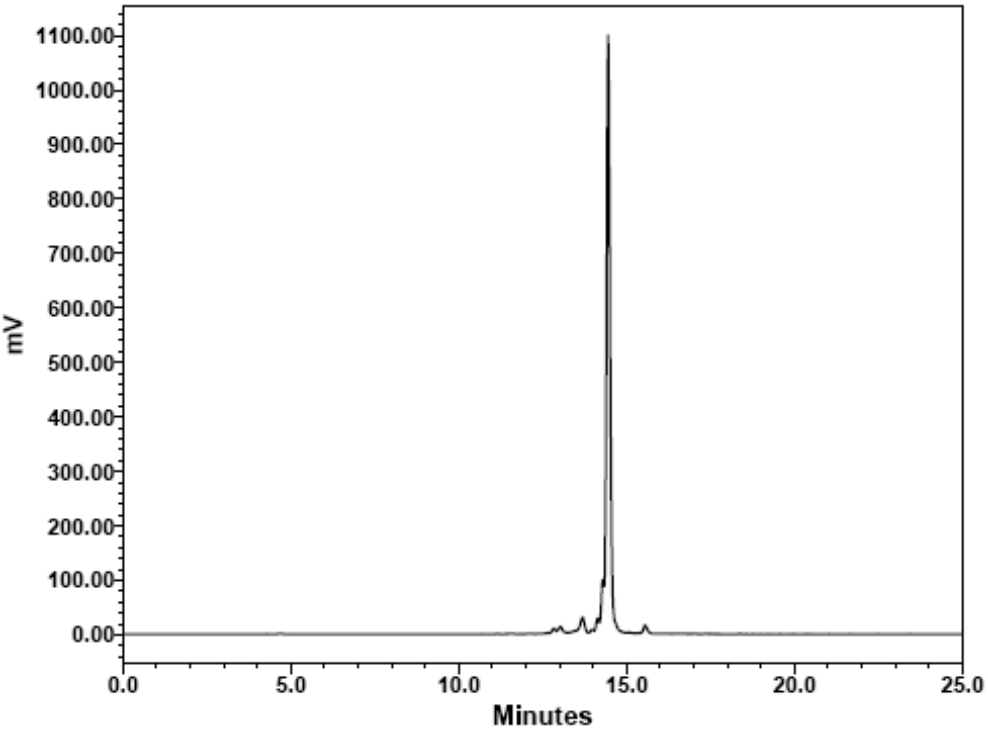

Figure S31. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 1 h following histidine challenge (2 mM).

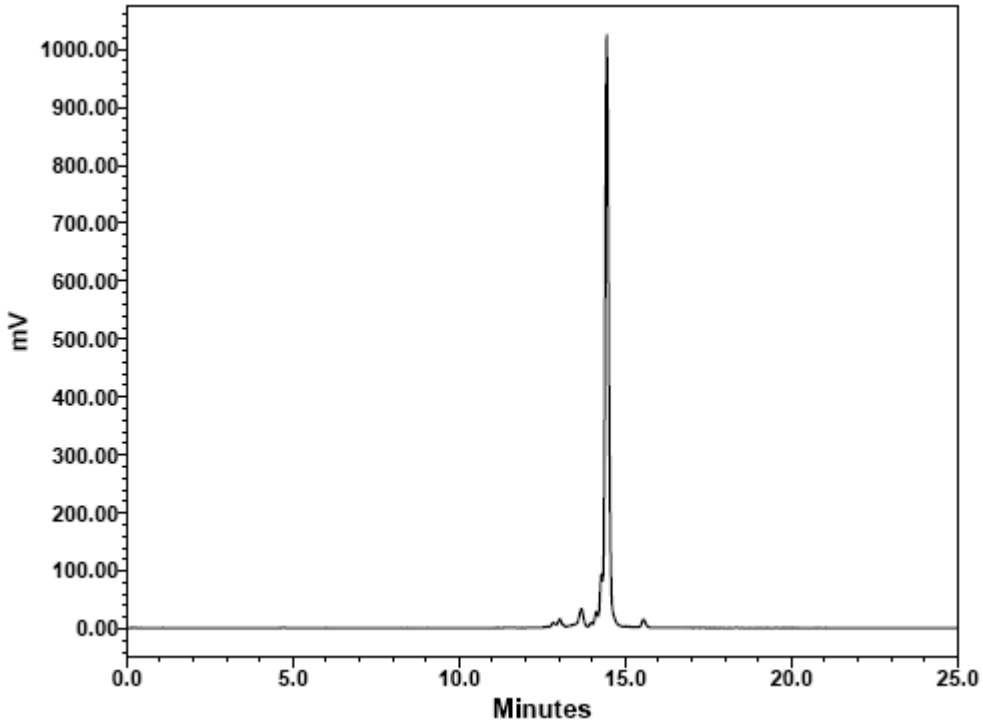

Figure S32. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 2 h following histidine challenge (2 mM).

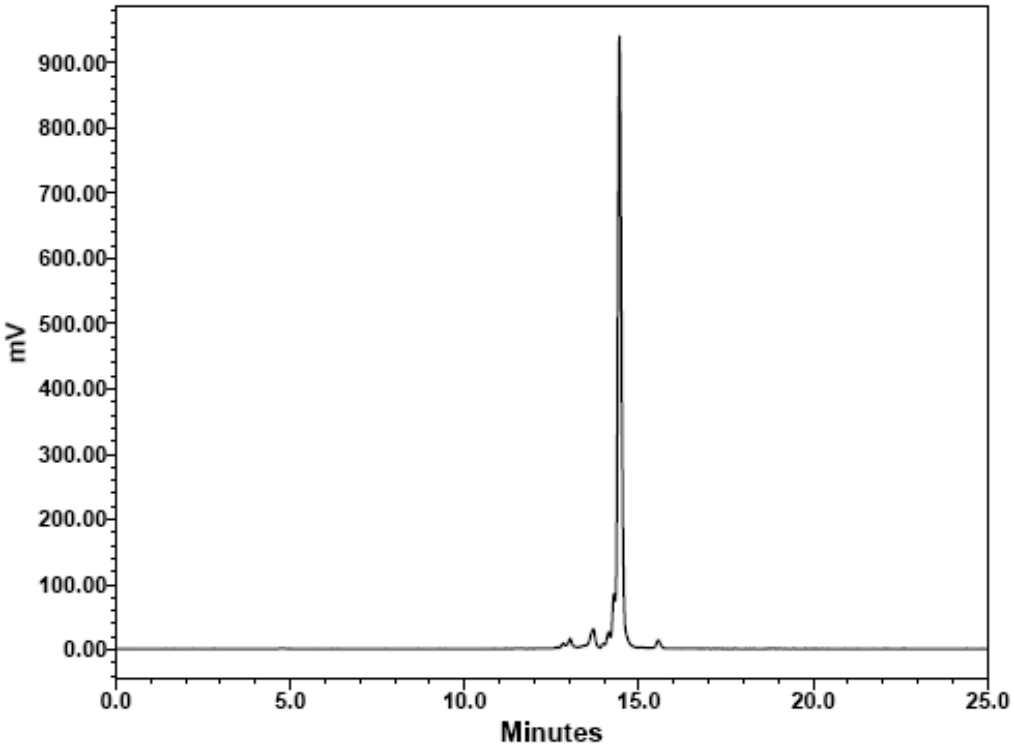

Figure S33. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 3 h following histidine challenge (2 mM).

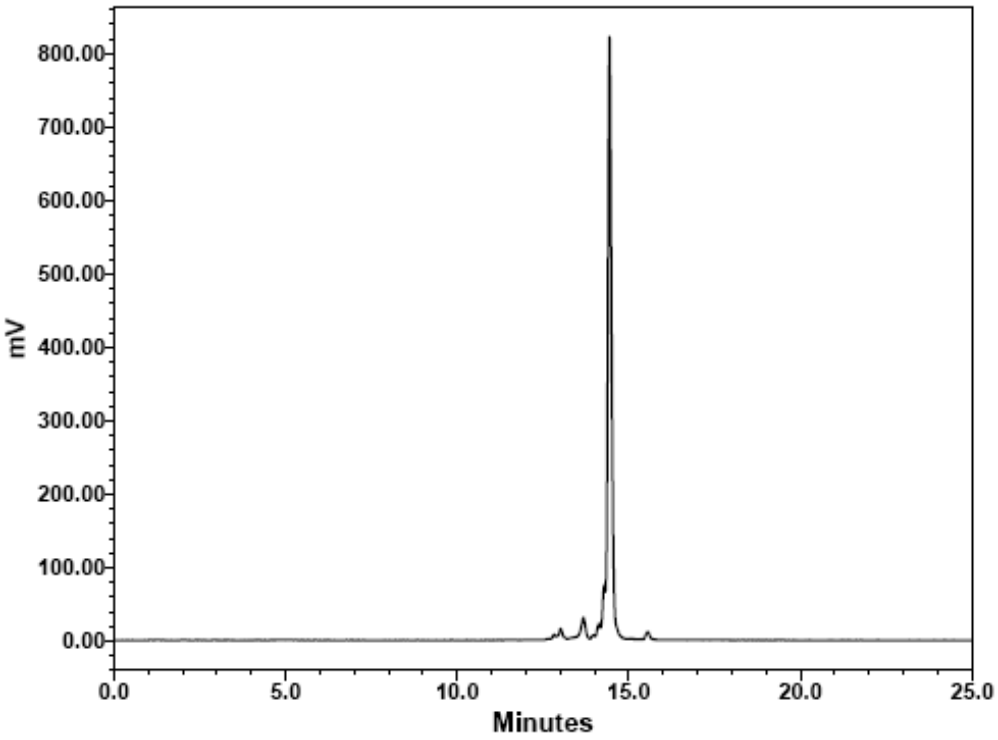

Figure S34. γ-HPLC chromatogram of $[^{99m}Tc(CO)_3(BPS)(ImTz)]^+$ **10b** at 6 h following histidine challenge (2 mM).

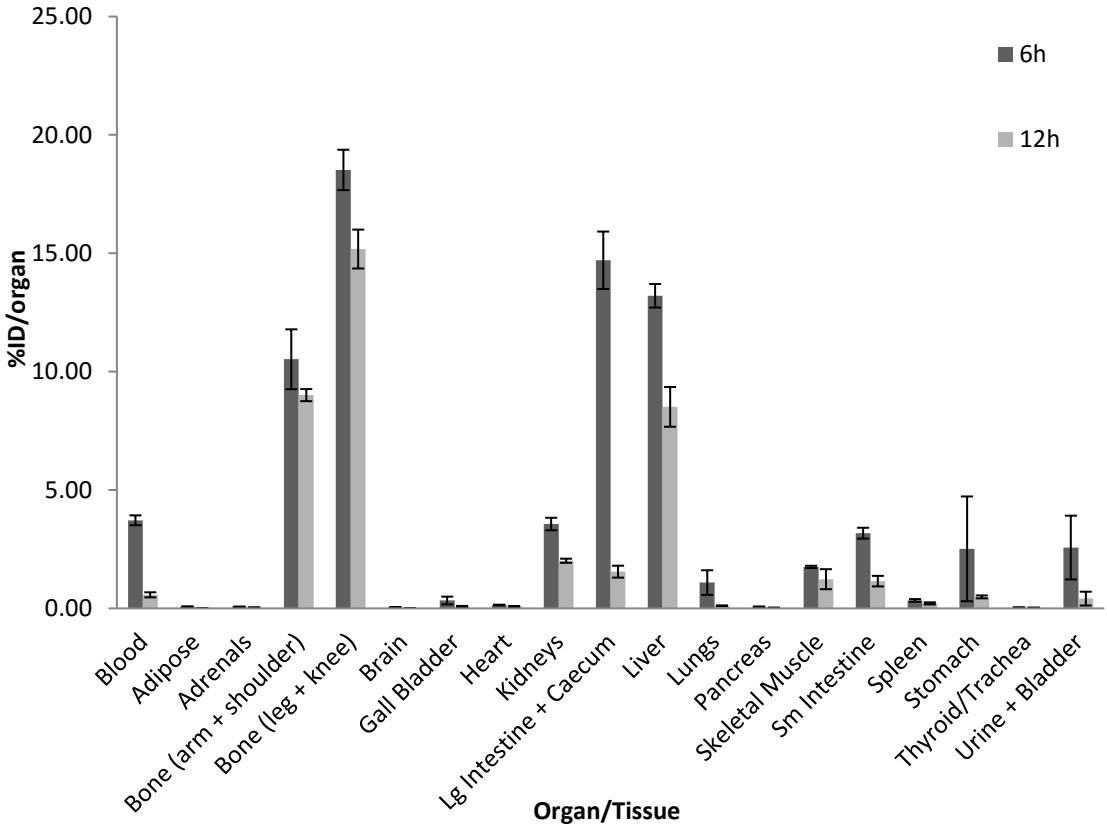

Figure. S35. Biodistribution data of select fluids and tissues after pretargeting with 20 mg/kg of TCO-BP administered 1 h prior to **10b**. Experiments were performed using Balb/c mice (n = 3 per time point) with tissues collected at 6 h and 12 h post administration of the labeled compound. Data are expressed as mean percent injected dose per organ (%ID/organ) ± SEM.

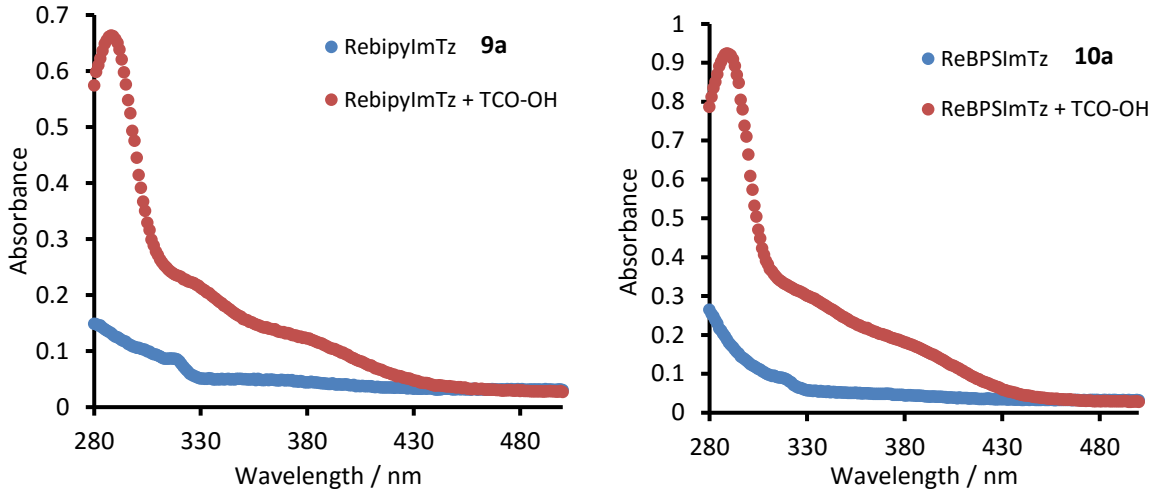

Figure S36. Absorption spectra for compounds **9a** and **10a** (50 µM in MeOH) in the absence (blue) and presence of 500 µM (red) TCO-OH.

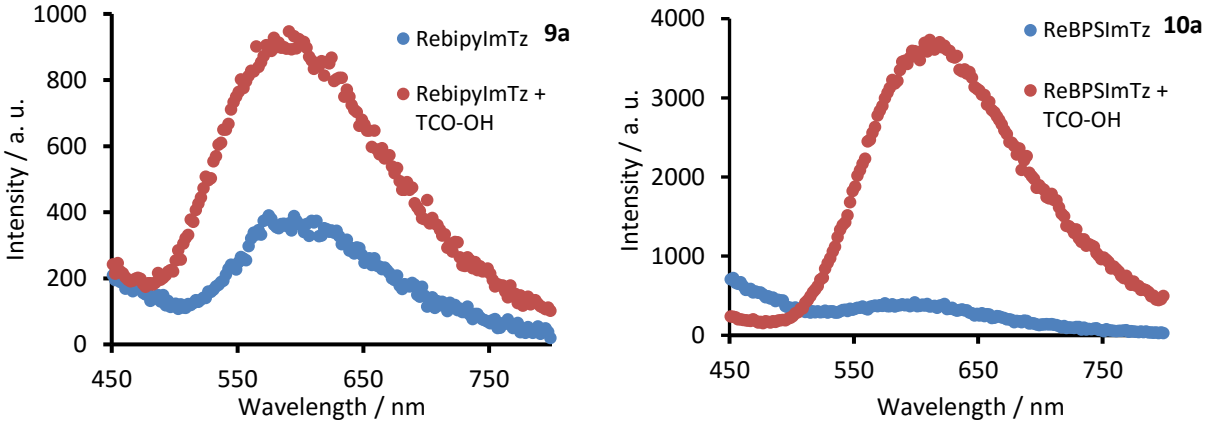

Figure S37. Emission spectra for compounds **9a** and **10a** (50 µM in MeOH) in the absence (blue) and presence of 500 µM (red) TCO-OH.

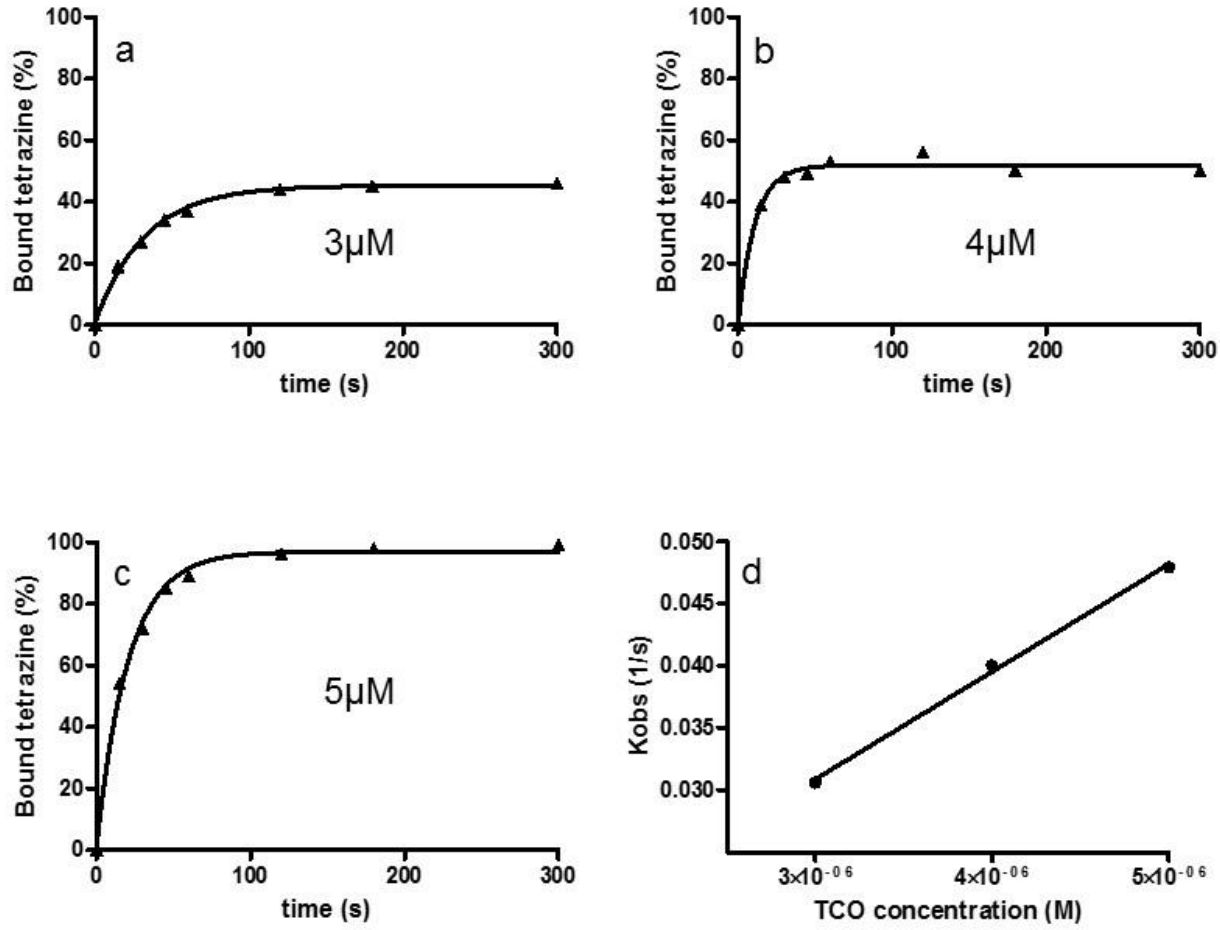

Figure S38. Reaction kinetics between **9b** and TCO-OH: Reaction of **9b** with a) TCO-OH (3 μM), b) TCO-OH (4 μM), c) TCO-OH (5 μM) vs. time, and fitted to a first-order exponential. A pseudo first-order rate constant (k_{obs}) was determined from the fit. d) Plot of k_{obs} vs. the concentration of TCO-OH, fitted using linear regression.

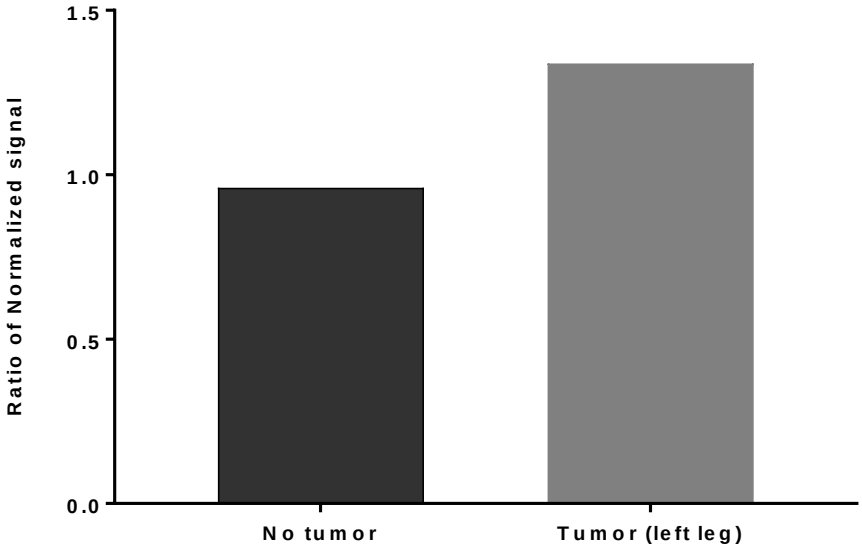

Figure S39. Quantitative comparison of SPECT image signals from knee joints of tumor-bearing and non-tumor-bearing mice. SPECT image signals were calculated as described in Experimental section, based on region of interest (ROI) defined around knee joints. Data are expressed as ratio of left to right knee image signals from mice with no tumor or with tumor (left knee).

Table S1. Biodistribution data expressed as (top) percent injected dose per gram (%ID/g) and (bottom) percent injected dose per organ (%ID/o) for select fluids and tissues after pretargeting with 20 mg/kg of TCO-BP administered 1 h prior to **10b**. Experiments were performed using Balb/c mice (n = 3 per time point) with tissues collected at 6 h and 12 h post administration of the labeled compound.

%ID/g

	n=3 6h		n=3 12h	
Organs	avg	SEM	avg	SEM
Blood	4.06	0.16	0.62	0.10
Adipose	1.04	0.31	0.15	0.02
Adrenals	4.96	0.65	2.56	0.74
Bone (arm + shoulder)	5.28	0.67	4.51	0.08
Bone (leg + knee)	9.27	0.32	7.62	0.49
Brain	0.10	0.02	0.02	0.00
Gall Bladder	54.32	5.45	7.94	2.75
Heart	1.53	0.12	0.86	0.05
Kidneys	13.43	0.33	7.76	0.95
Lg Intestine + Caecum	27.70	4.63	2.95	0.68
Liver	12.95	0.63	8.09	0.67
Lungs	7.54	3.25	0.89	0.13
Pancreas	0.45	0.07	0.19	0.01
Skeletal Muscle	0.24	0.00	0.17	0.06
Sm Intestine	3.34	0.22	1.20	0.28
Spleen	3.21	0.40	2.34	0.49
Stomach	9.09	7.92	1.58	0.45
Thyroid/Trachea	2.16	0.19	1.76	0.06
Urine + Bladder	23.58	6.67	3.01	0.19

%ID/o

	n=3 6h		n=3 12h	
Organs	avg	SEM	avg	SEM
Blood	3.72	0.21	0.57	0.11
Adipose	0.07	0.02	0.01	0.00
Adrenals	0.07	0.01	0.03	0.01
Bone (arm + shoulder)	10.52	1.26	9.01	0.26
Bone (leg + knee)	18.52	0.85	15.18	0.82
Brain	0.04	0.01	0.01	0.00
Gall Bladder	0.33	0.16	0.07	0.03
Heart	0.14	0.02	0.08	0.01
Kidneys	3.56	0.26	2.01	0.09

Lg Intestine + Caecum	14.71	1.21	1.55	0.25
Liver	13.21	0.50	8.51	0.84
Lungs	1.09	0.52	0.11	0.01
Pancreas	0.06	0.02	0.03	0.00
Skeletal Muscle	1.75	0.05	1.23	0.42
Sm Intestine	3.18	0.23	1.15	0.22
Spleen	0.34	0.06	0.21	0.05
Stomach	2.51	2.21	0.49	0.06
Thyroid/Trachea	0.03	0.00	0.03	0.00
Urine + Bladder	2.57	1.35	0.41	0.29