

Supporting Information

Preparation of tetrazine-containing [2+1] complexes of $^{99\text{m}}\text{Tc}$ and
in vivo targeting using bioorthogonal inverse electron demand

Diels-Alder chemistry

Abdolreza Yazdani, Nancy Janzen, Shannon Czorny, Robert Ungard, Tanya Miladinovic,

Gurmit Singh, and John F. Valliant

Table of Contents

Item	Description	Page
1.	¹ H NMR spectrum of 5 . (Figure S1)	S3
2.	¹³ C NMR spectrum of 5 . (Figure S2)	S4
3.	¹ H- ¹³ C HSQC NMR spectrum and HRMS of 5 . (Figures S3, S4)	S5
4.	¹ H NMR spectrum of 9a . (Figure S5)	S6
5.	¹³ C NMR spectrum and HRMS of 9a . (Figure S6)	S7
6.	HRMS and IR spectrum of 9a . (Figures S7, S8)	S8
7.	¹ H NMR spectrum of 10a . (Figure S9)	S9
8.	¹³ C NMR spectrum and HRMS of 10a . (Figure S10)	S10
9.	HRMS and IR spectrum of 10a . (Figures S11, S12)	S11
10.	HPLC chromatograms (UV and γ) of 9a with 9b . (Figure S13)	S12
11.	Stability of 9b with 2 mM cysteine, 0.5 and 1 h; γ -HPLC (Figures S14, S15)	S13
12.	Stability of 9b with 2 mM cysteine, 2 and 3 h; γ -HPLC (Figures S16, S17)	S14
13.	Stability of 9b with 2 mM cysteine, 6 h; γ -HPLC (Figure S18)	S15
14.	Stability of 9b with 2 mM histidine, 0.5 and 1 h; γ -HPLC (Figures S19, S20)	S16
15.	Stability of 9b with 2 mM histidine, 2 and 3 h; γ -HPLC (Figures S21, S22)	S17
16.	Stability of 9b with 2 mM histidine, 6 h; γ -HPLC (Figure S23)	S18
17.	HPLC chromatograms (UV and γ) of 10a with 10b . (Figure S24)	S19
18.	Stability of 10b with 2 mM cysteine, 0.5 and 1 h; γ -HPLC (Figures S25, S26)	S20
19.	Stability of 10b with 2 mM cysteine, 2 and 3 h; γ -HPLC (Figures S27, S28)	S21
20.	Stability of 10b with 2 mM cysteine, 6 h; γ -HPLC (Figure S29)	S22
21.	Stability of 10b with 2 mM histidine, 0.5 and 1 h; γ -HPLC (Figures S30, S31)	S23
22.	Stability of 10b with 2 mM histidine, 2 and 3 h; γ -HPLC (Figures S32, S33)	S24
23.	Stability of 10b with 2 mM histidine, 6 h; γ -HPLC (Figure S34)	S25
24.	Biodistribution data (%ID/organ) for TCO-BP administered 1 h prior to 10b . (Figure S35)	S26
25.	Absorption and emission spectra for compounds 9a and 10a , with and without TCO-OH. (Figures S36, S37)	S27
26.	Reaction kinetics between 9b and TCO-OH. Plot of k_{obs} vs. the concentration of TCO-OH, fitted using linear regression. (Figure S38)	S28
28.	Quantitative comparison of SPECT image signals from knee joints of tumor-bearing and non-tumor-bearing mice. (Figure S39)	S29
27.	Tabulated biodistribution data (% ID/ gram and %ID/organ) for TCO-BP administered 1 h prior to 10b . (Table S1)	S30-S31

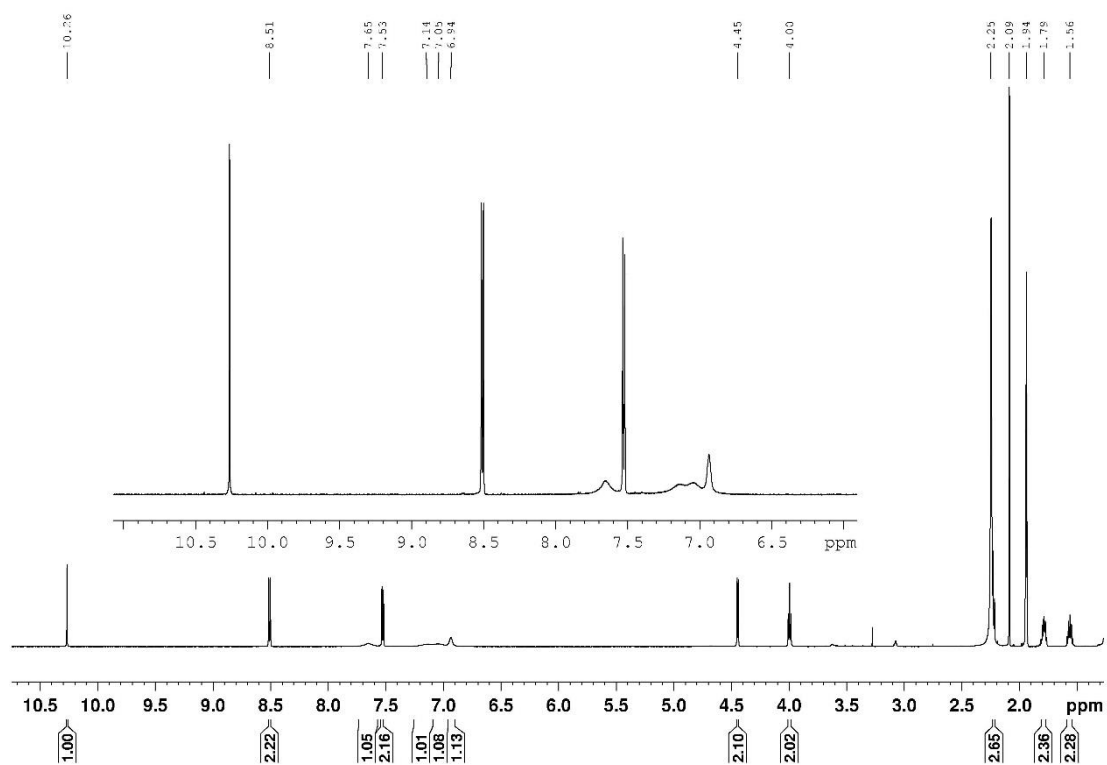


Figure S1. ^1H NMR spectrum (CD_3CN , 600 MHz) of 5.

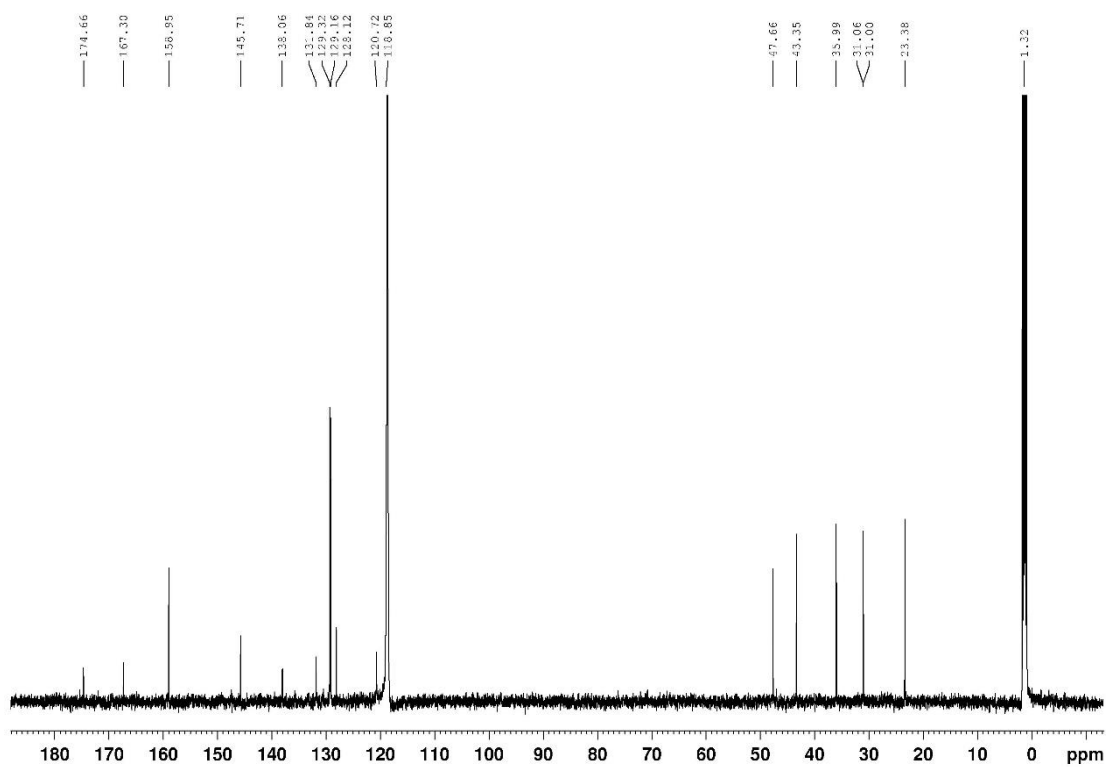


Figure S2. ¹³C NMR spectrum (CD₃CN, 150 MHz) of 5.

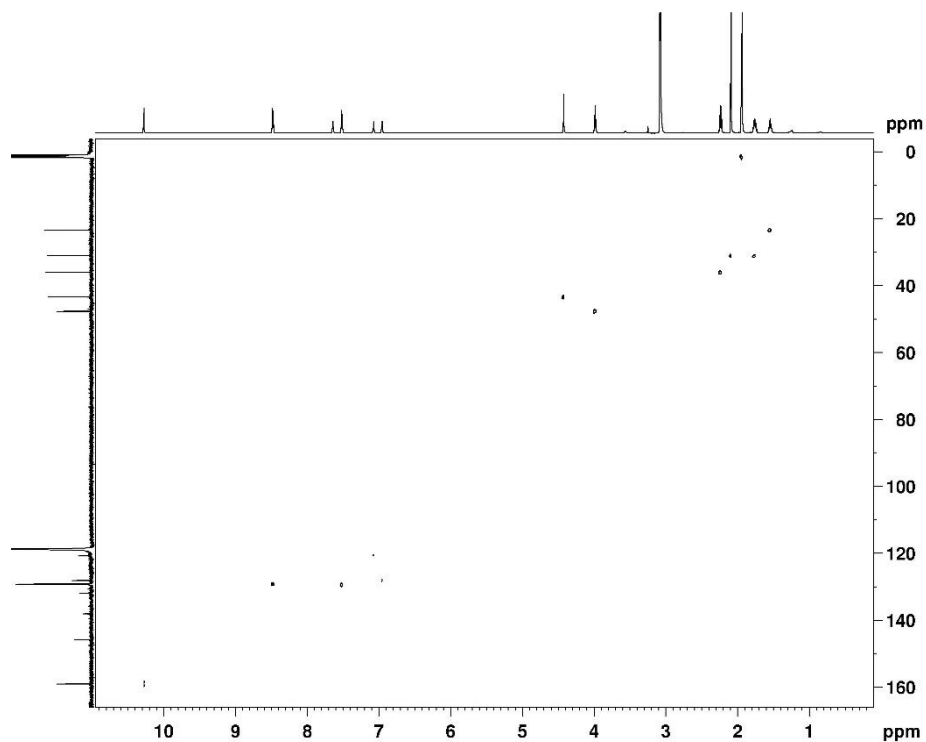


Figure S3. ^1H - ^{13}C HSQC NMR spectrum (CD_3CN) of 5.

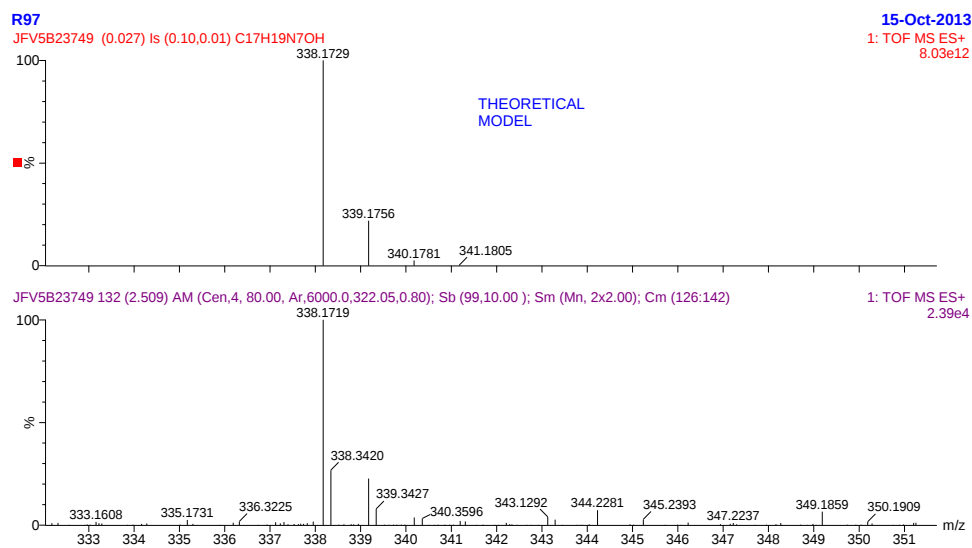


Figure S4. HRMS of 5.

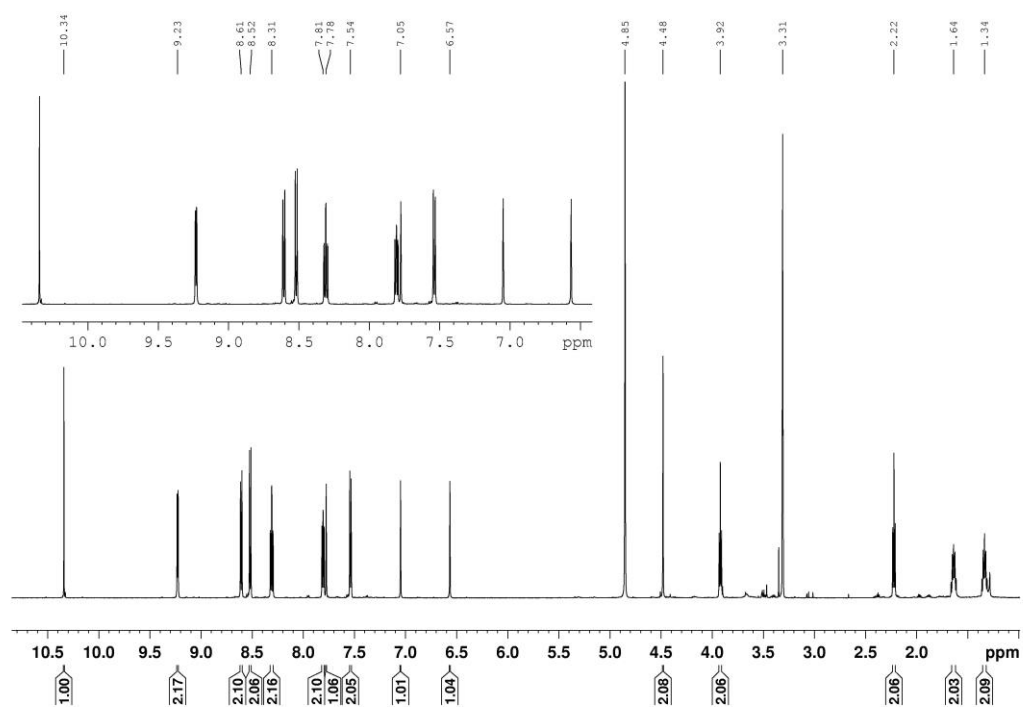


Figure S5. ^1H NMR spectrum (MeOD , 600 MHz) of **9a**.

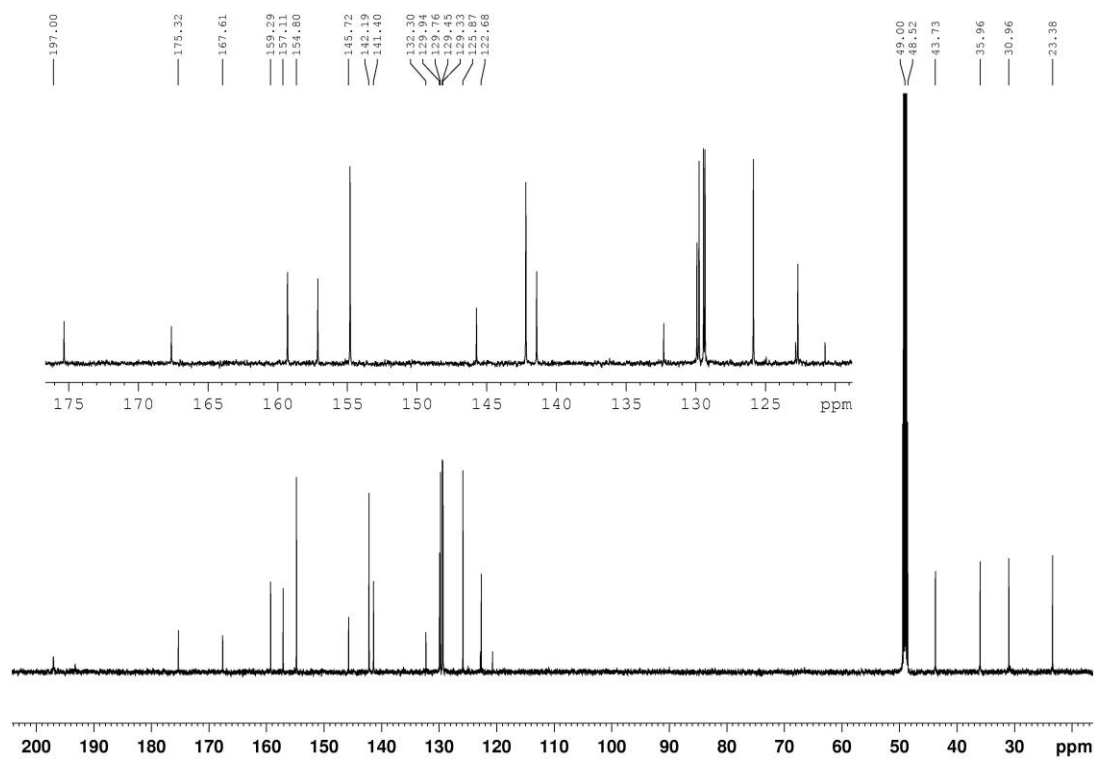


Figure S6. ^{13}C NMR spectrum (MeOD , 150 MHz) of **9a**.

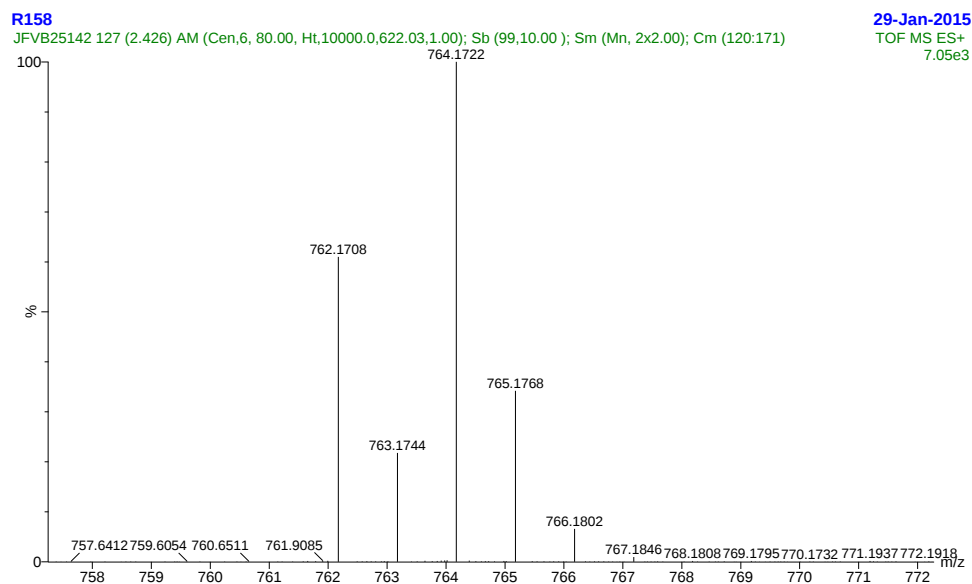


Figure S7. HRMS of 9a.

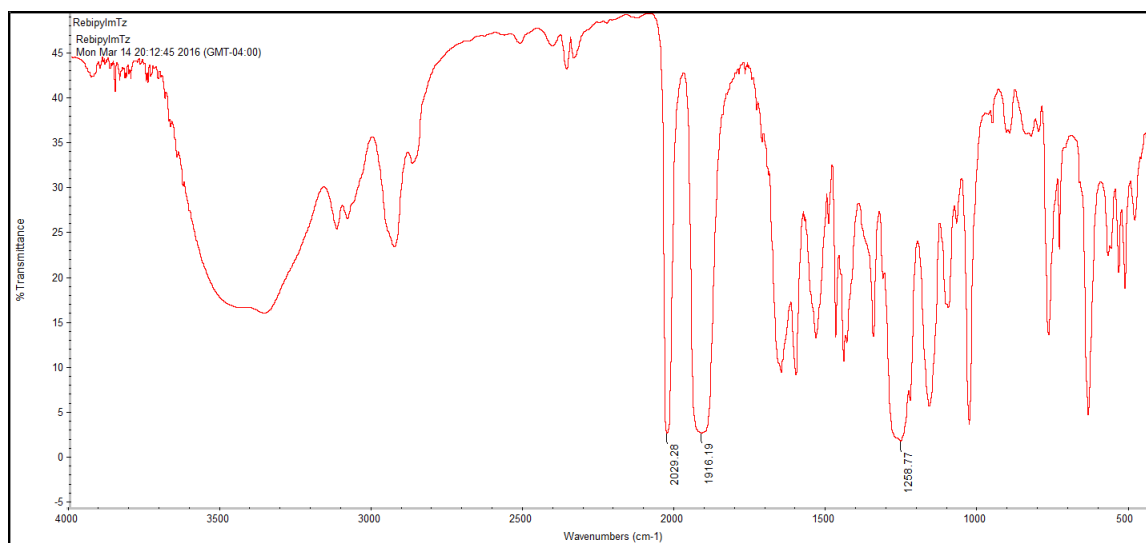


Figure S8. IR spectrum of 9a.

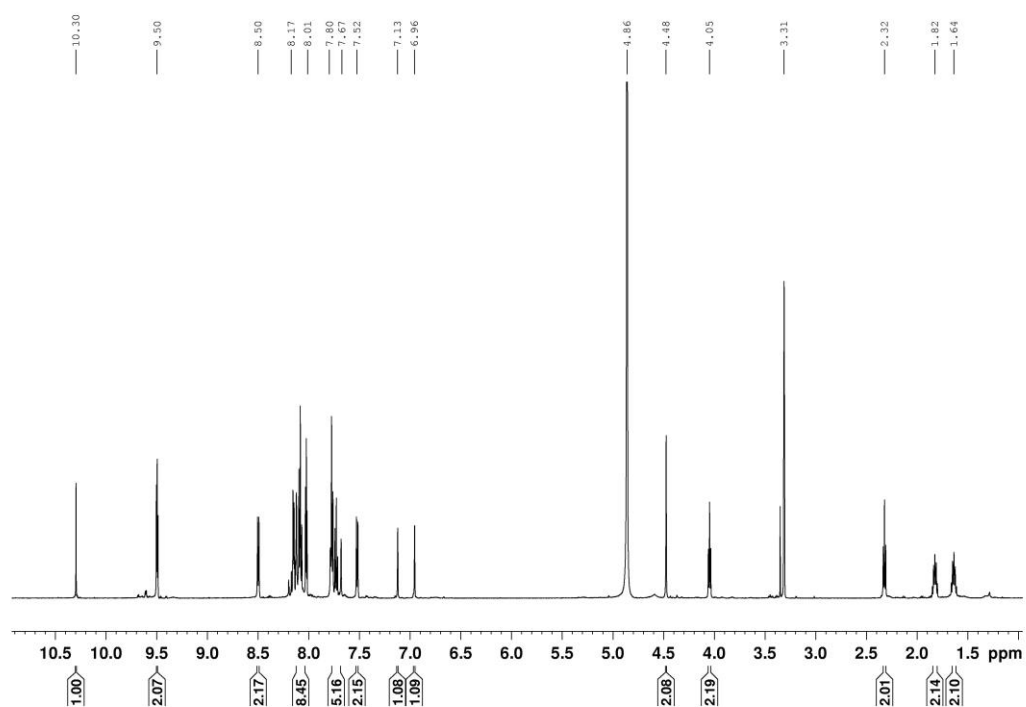


Figure S9. ^1H NMR spectrum (MeOD , 600 MHz) of **10a**.

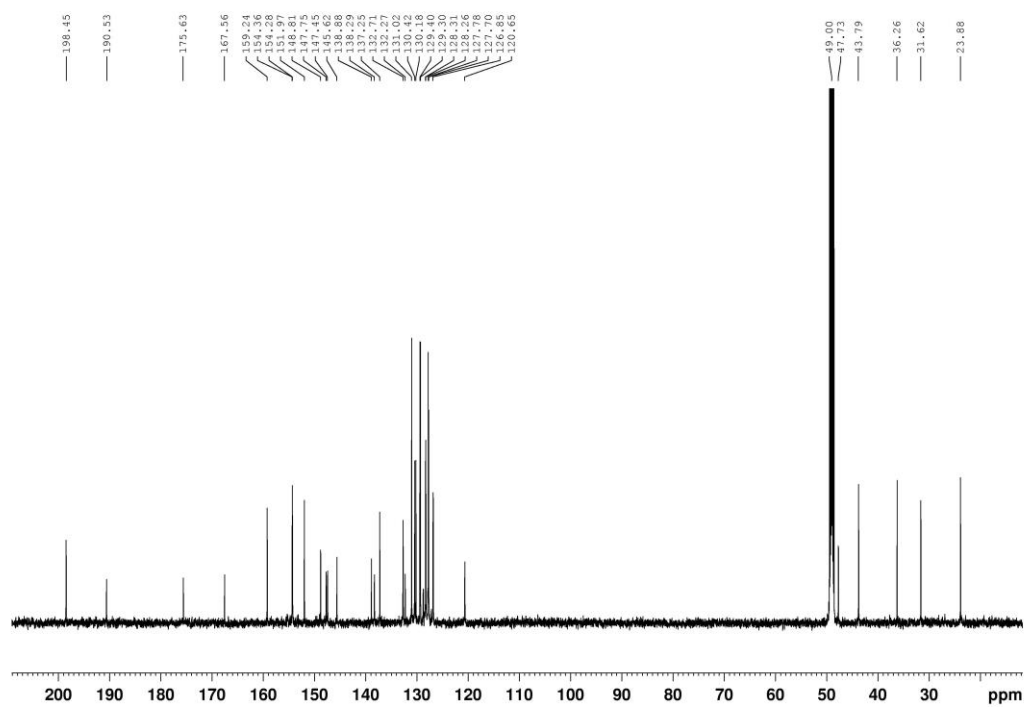


Figure S10. ^{13}C NMR spectrum (MeOD, 150 MHz) of **10a**.

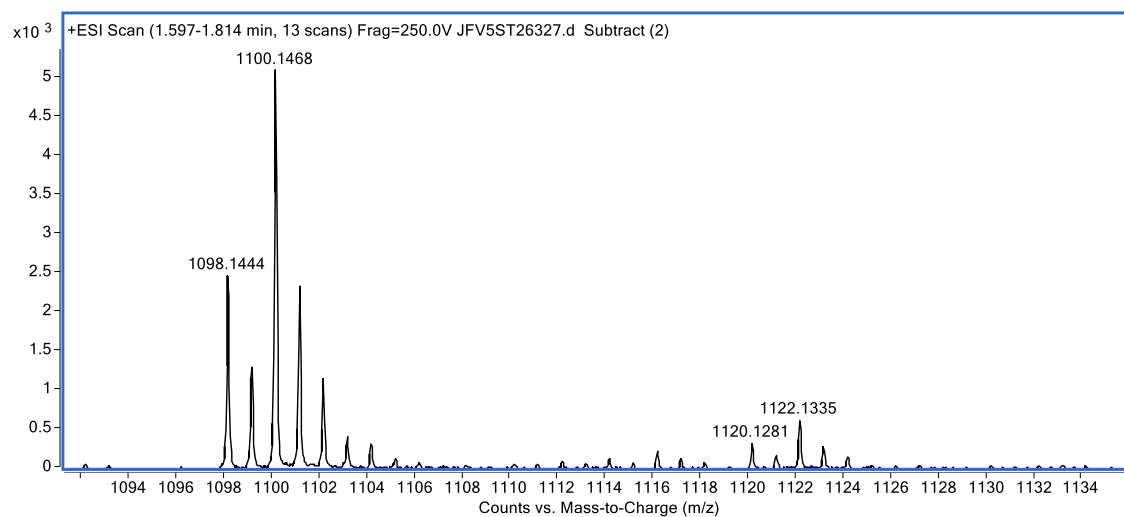


Figure S11. HRMS of **10a**.

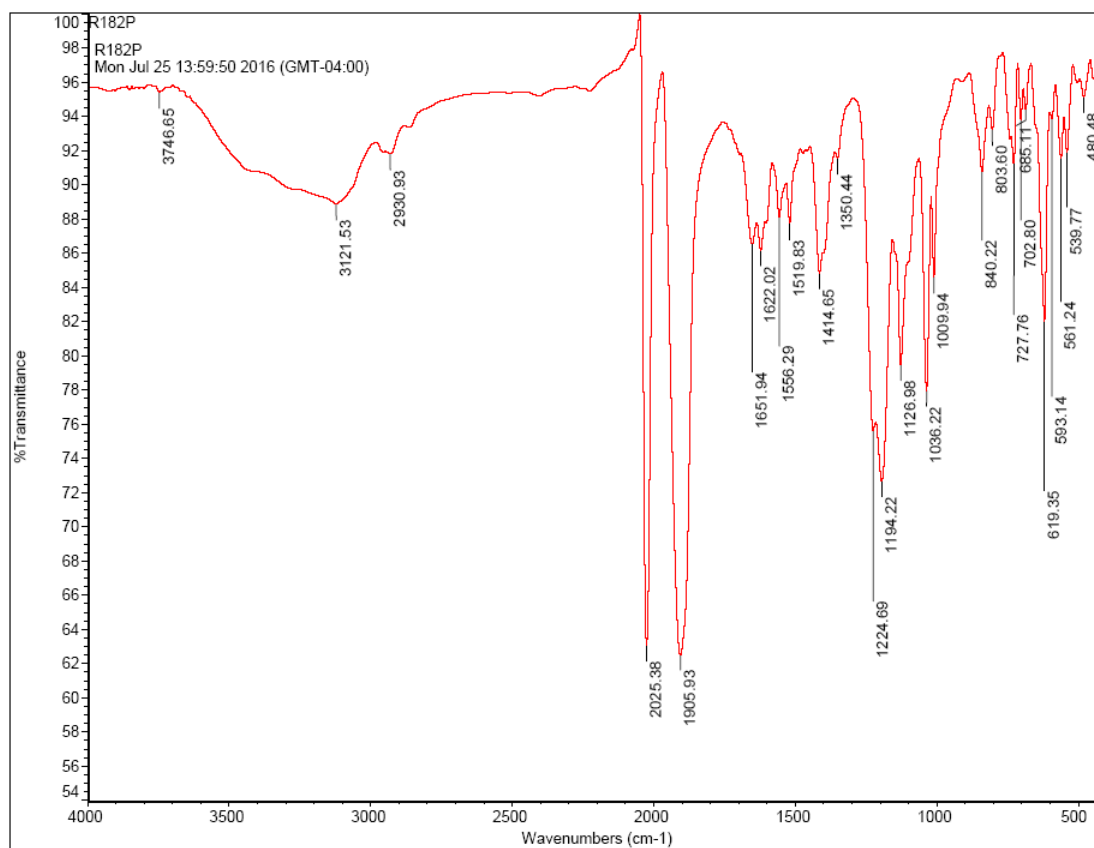


Figure S12. IR spectrum of **10a**.

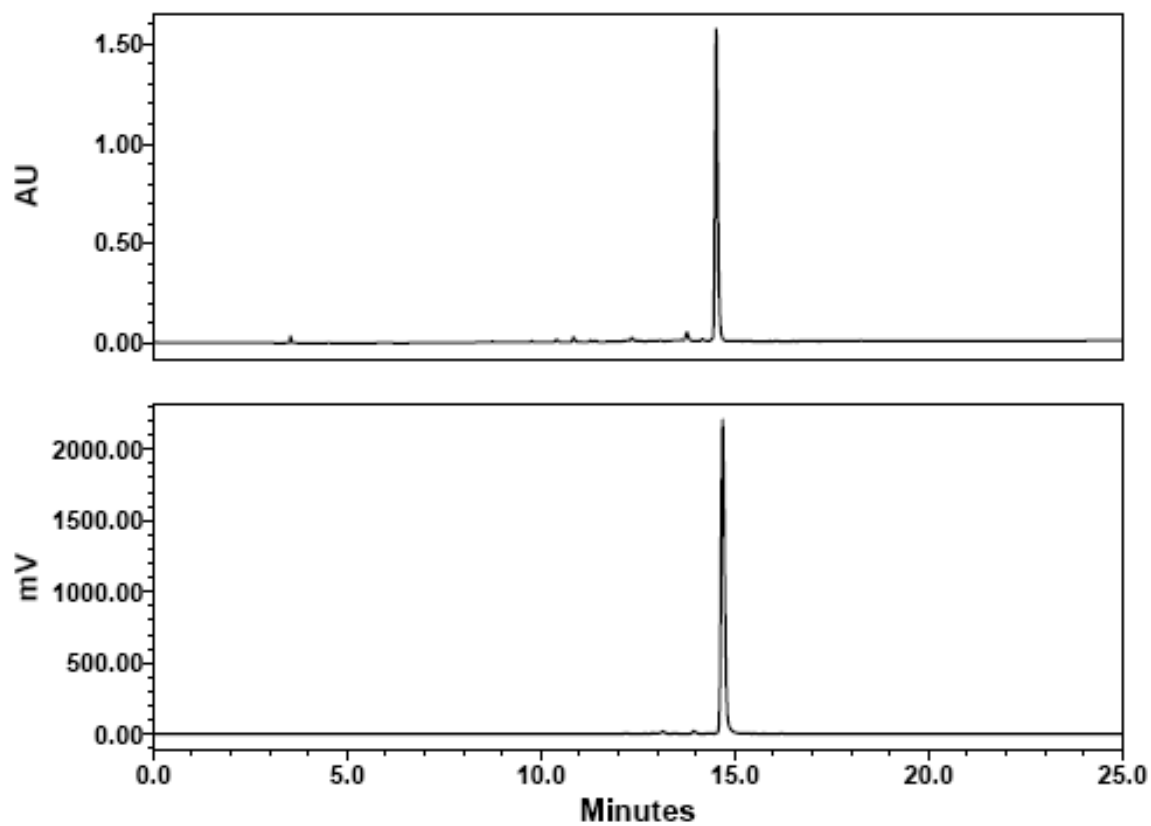


Figure S13. HPLC chromatograms (UV, top and γ , bottom) of $[\text{Re}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9a** co-injected with **9b**.

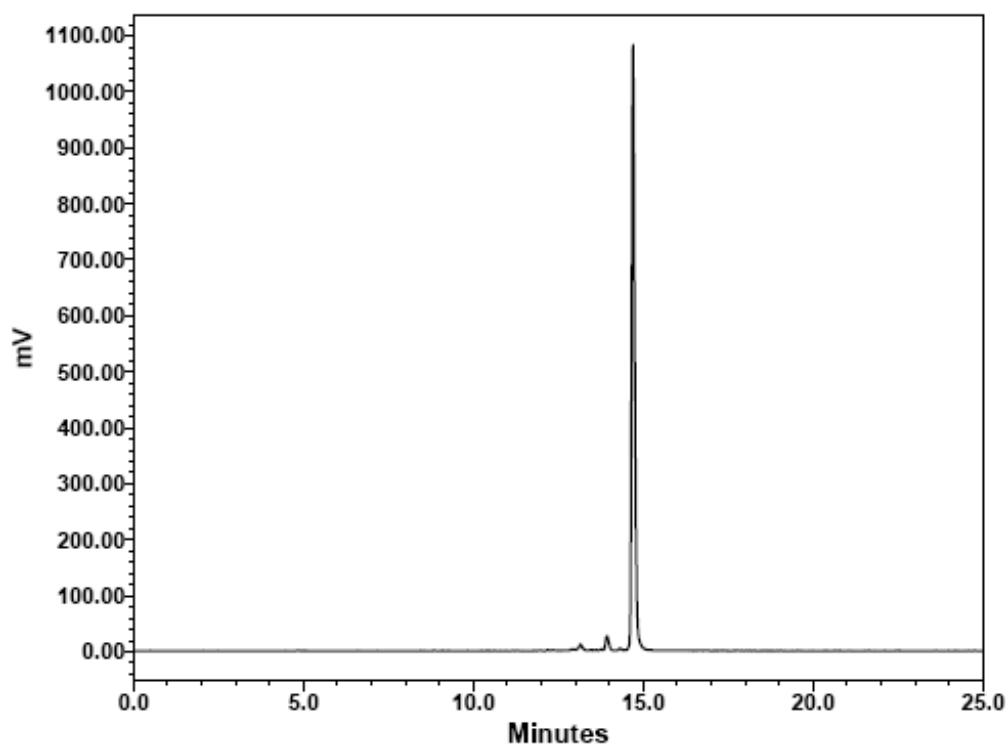


Figure S14. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 0.5 h following cysteine challenge (2 mM).

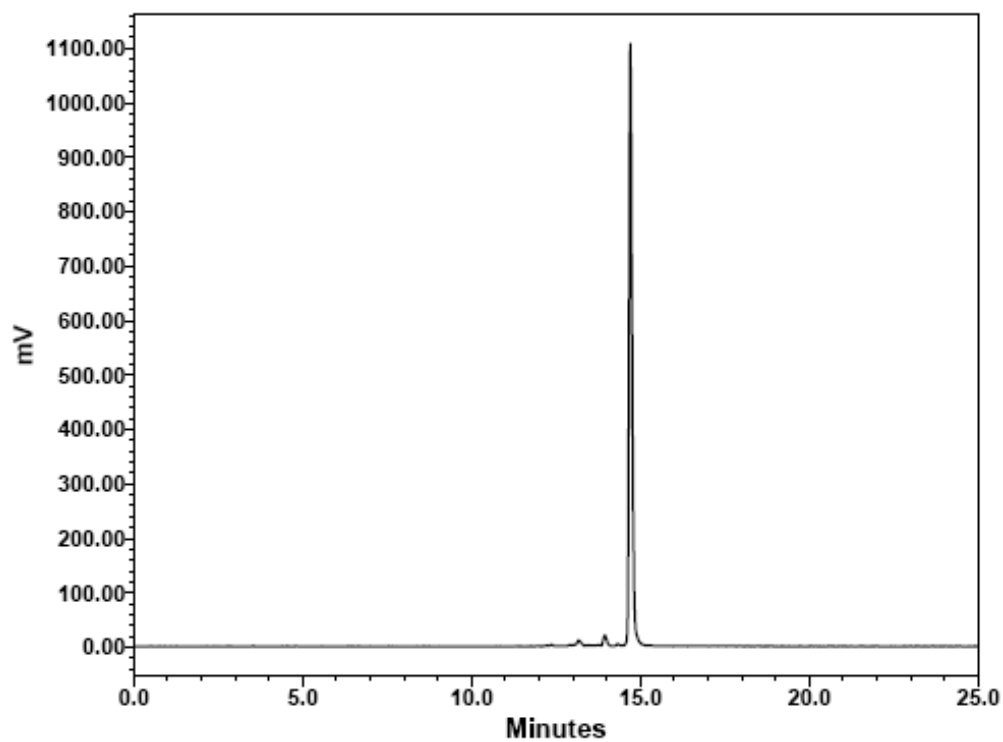


Figure S15. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 1 h following cysteine challenge (2 mM).

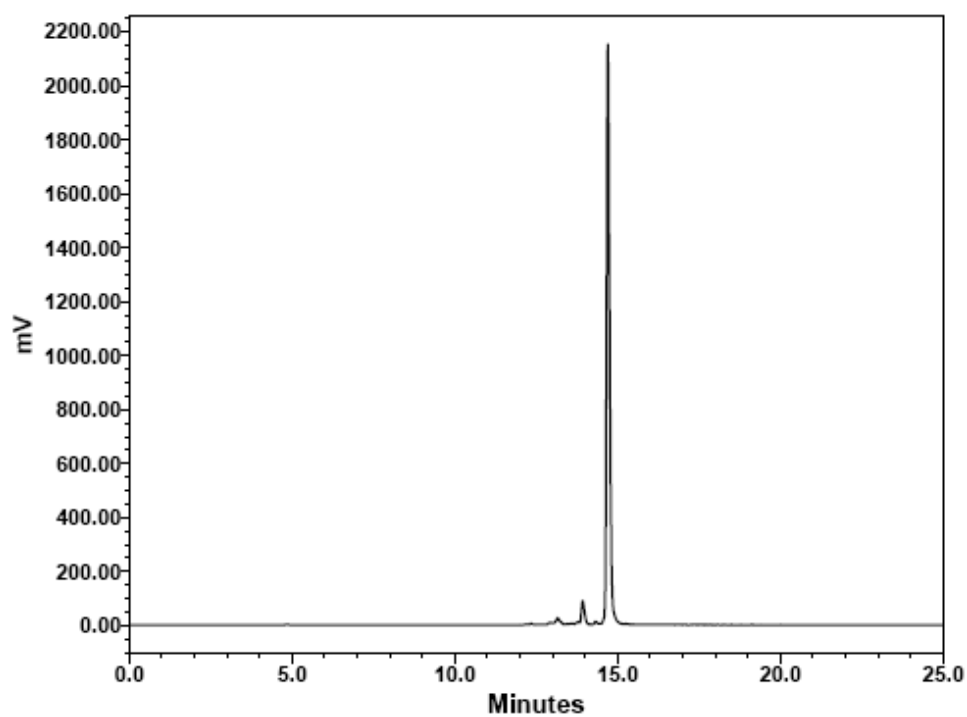


Figure S16. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 2 h following cysteine challenge (2 mM).

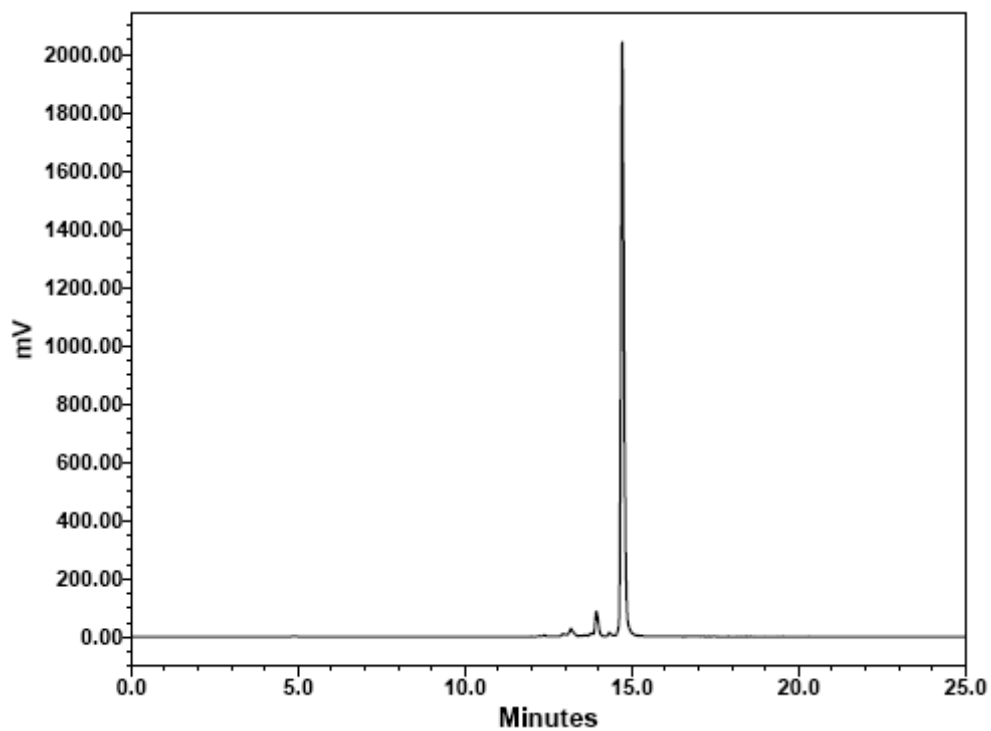


Figure S17. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 3 h following cysteine challenge (2 mM).

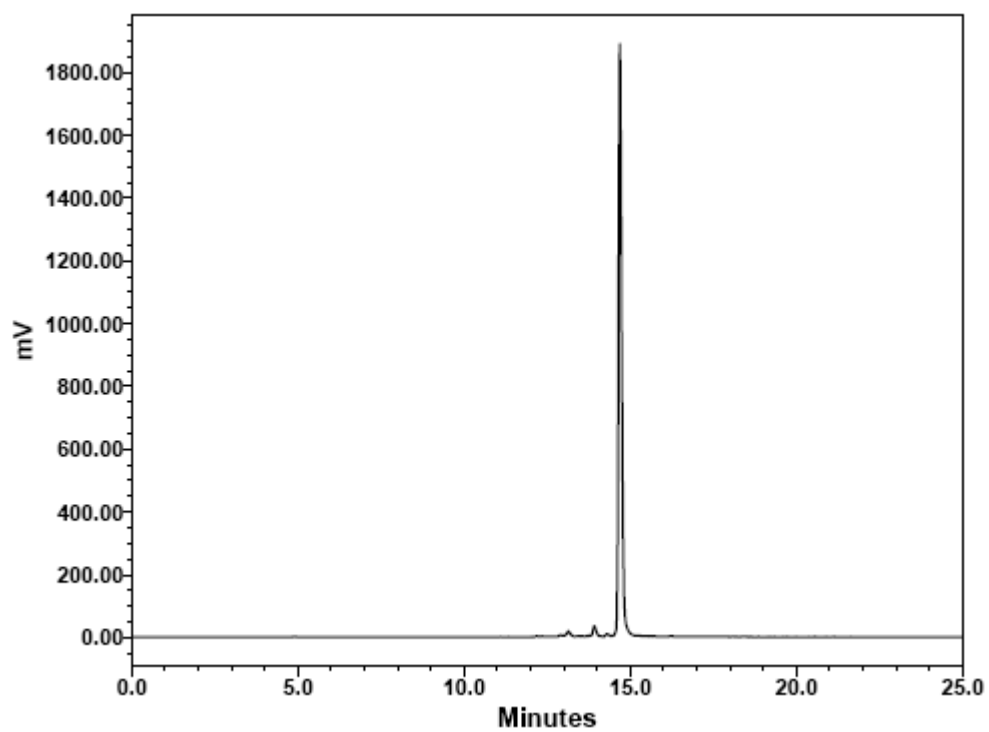


Figure S18. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 6 h following cysteine challenge (2 mM).

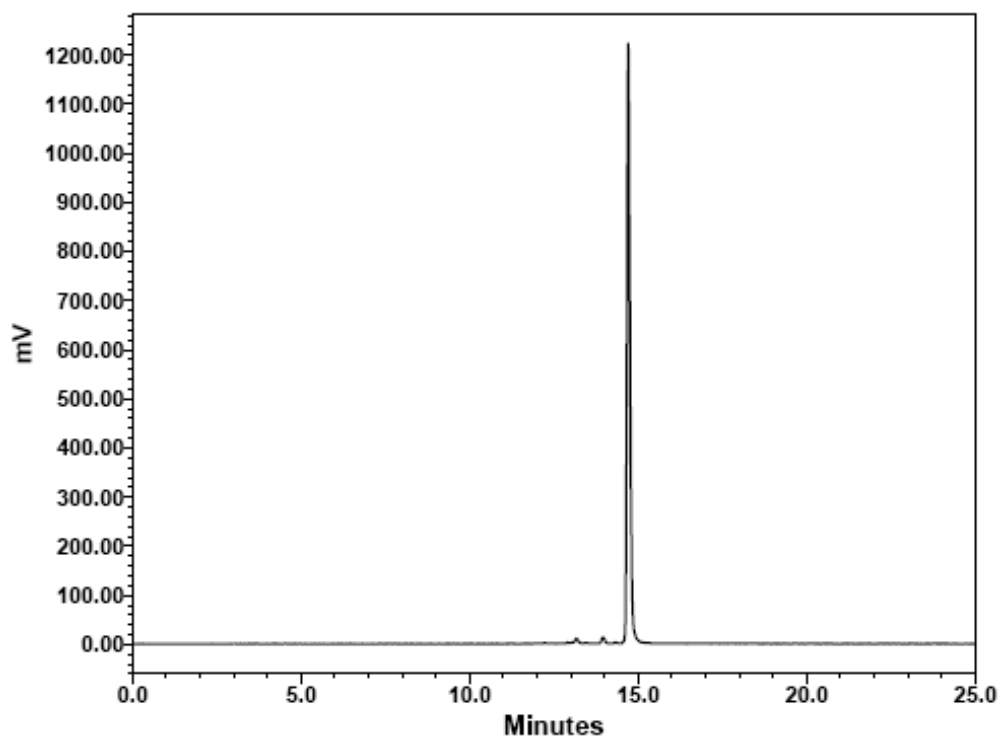


Figure S19. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 0.5 h following histidine challenge (2 mM).

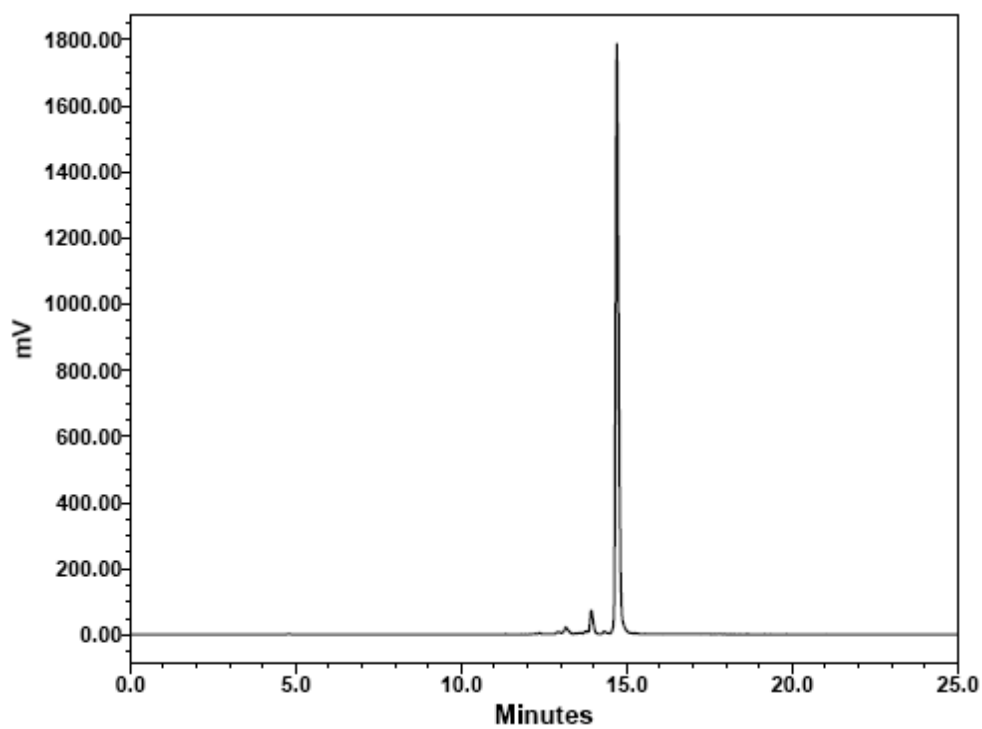


Figure S20. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 1 h following histidine challenge (2 mM).

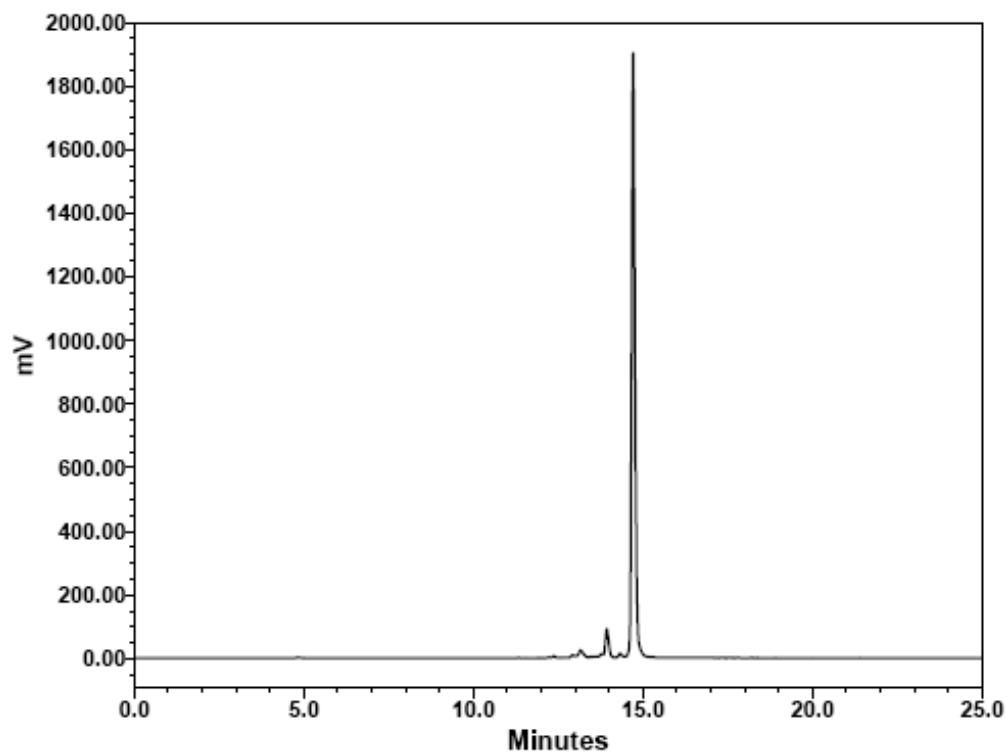


Figure S21. γ -HPLC chromatogram of $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 2 h following histidine challenge (2 mM).

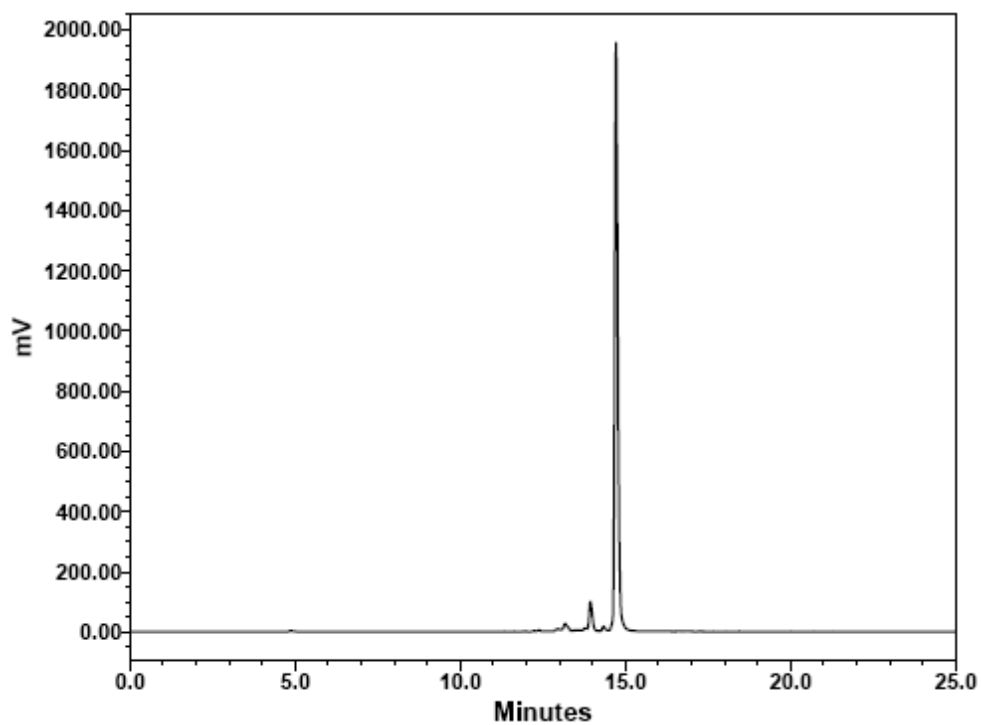


Figure S22. γ -HPLC chromatogram of $[\text{}^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 3 h following histidine challenge (2 mM).

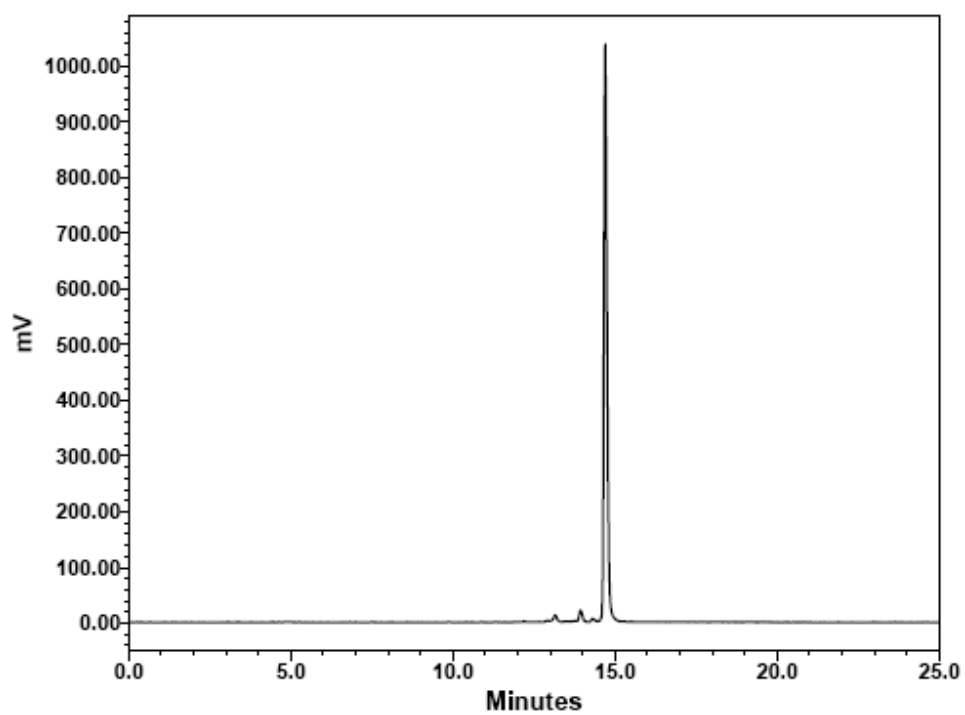


Figure S23. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{bipy})(\text{ImTz})]^+$ **9b** at 6 h following histidine challenge (2 mM).

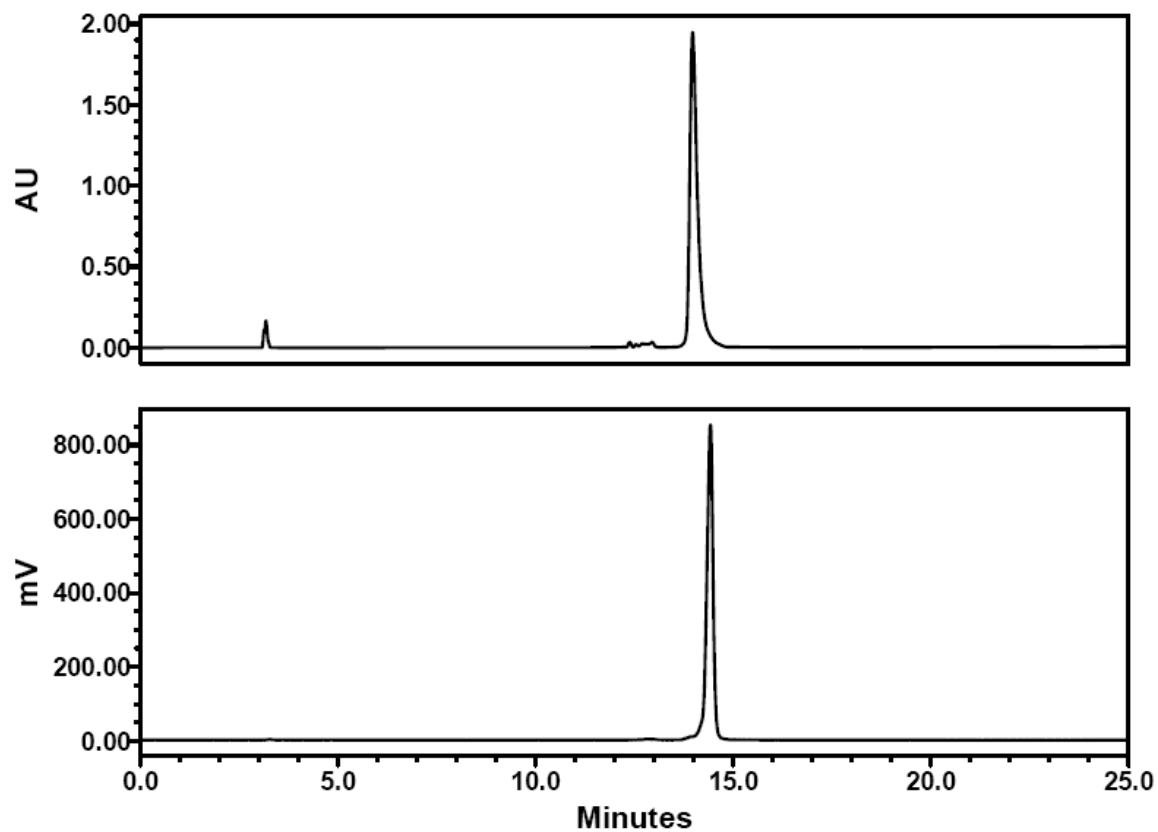


Figure S24. HPLC chromatograms (UV, top and γ , bottom) of $[\text{Re}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10a** co-injected with **10b**.

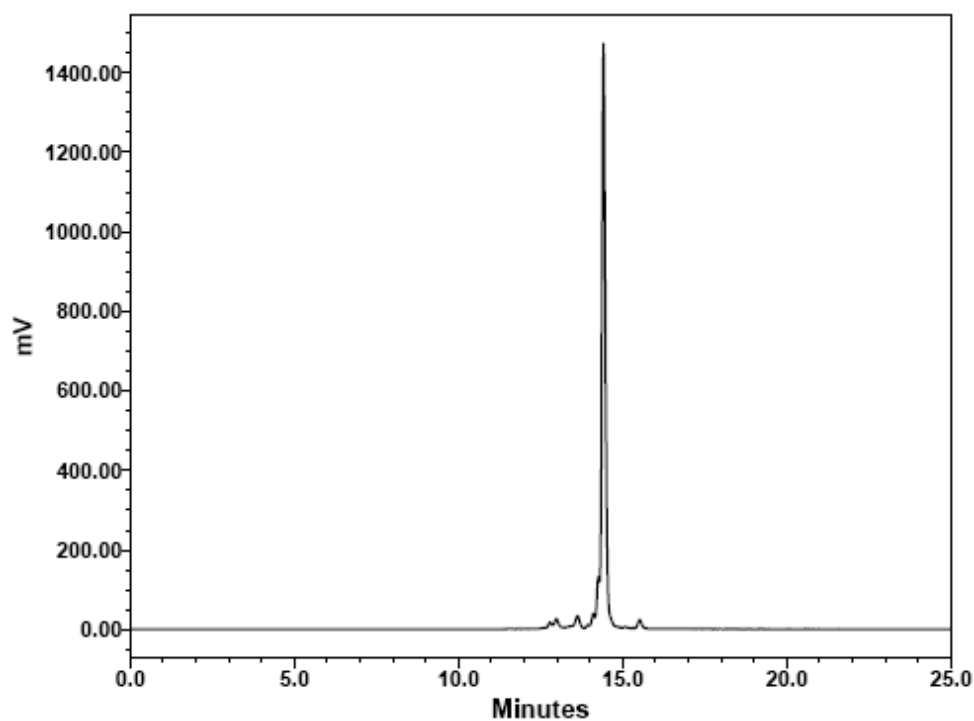


Figure S25. γ -HPLC chromatogram of $[^{99m}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 0.5 h following cysteine challenge (2 mM).

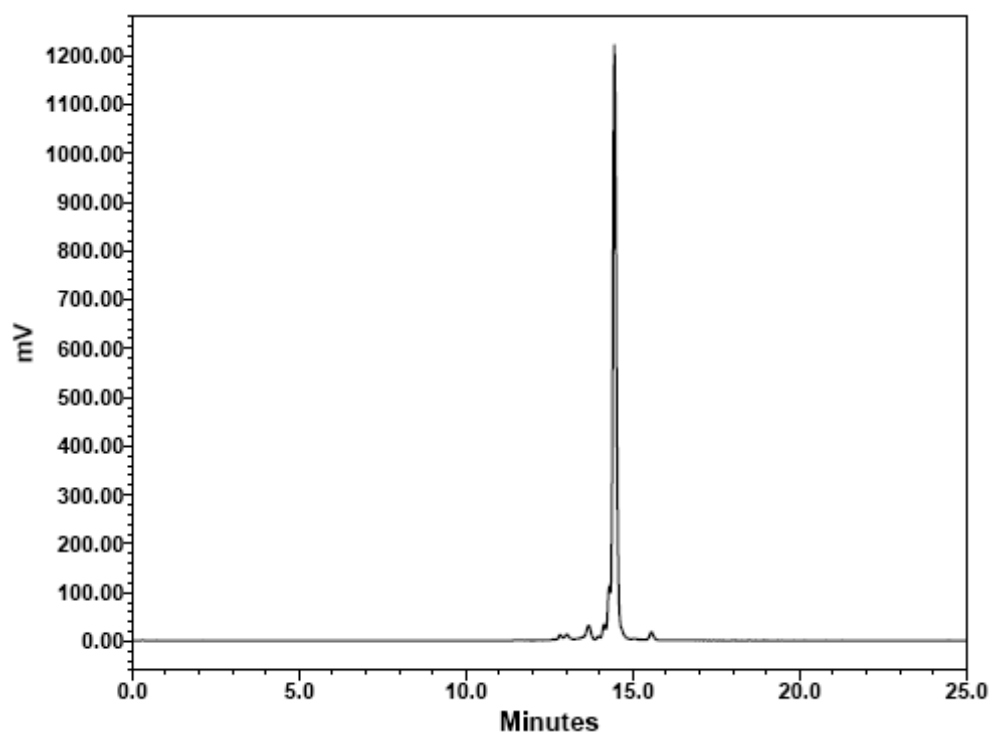


Figure S26. γ -HPLC chromatogram of $[^{99m}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 1 h following cysteine challenge (2 mM).

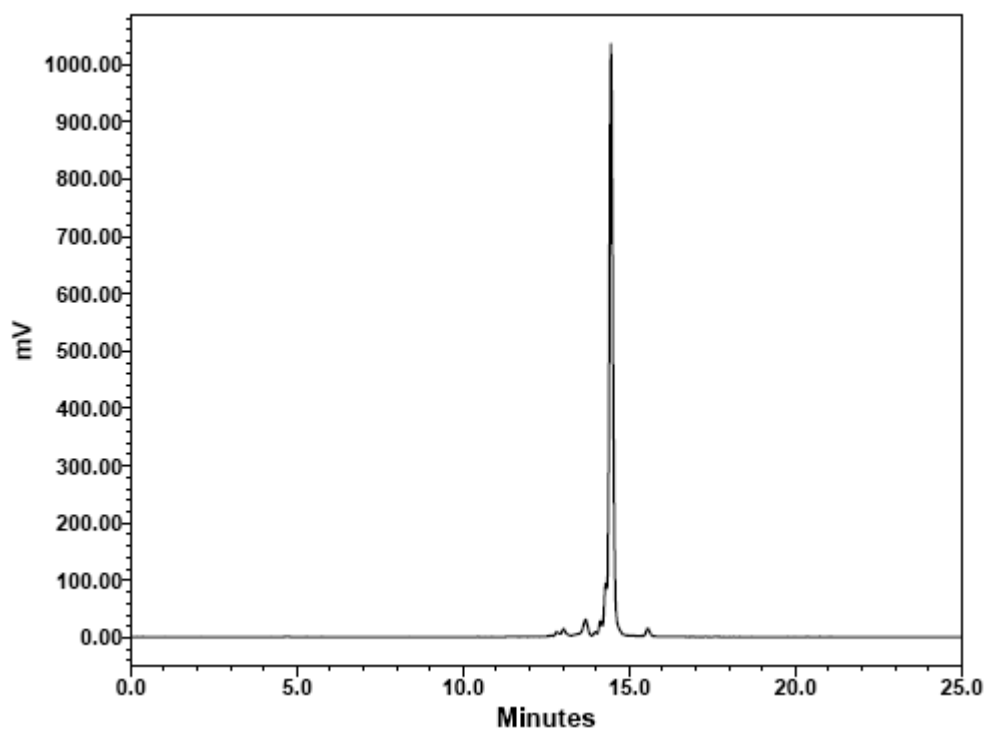


Figure S27. γ -HPLC chromatogram of $[^{99m}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 2 h following cysteine challenge (2 mM).

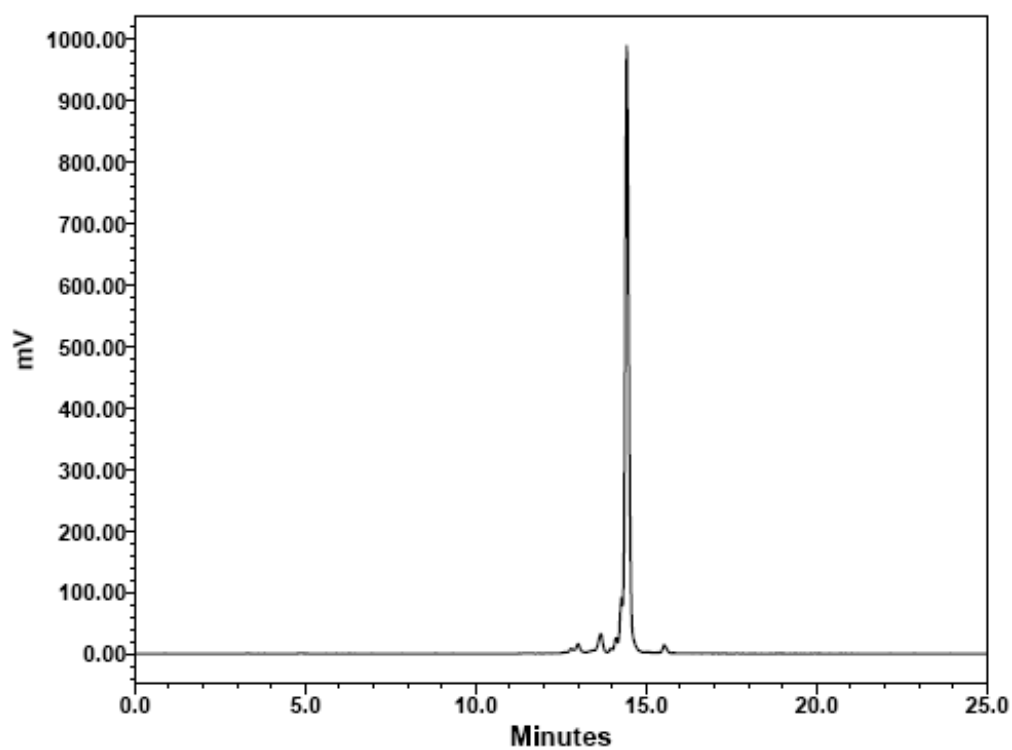


Figure S28. γ -HPLC chromatogram of $[^{99m}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 3 h following cysteine challenge (2 mM).

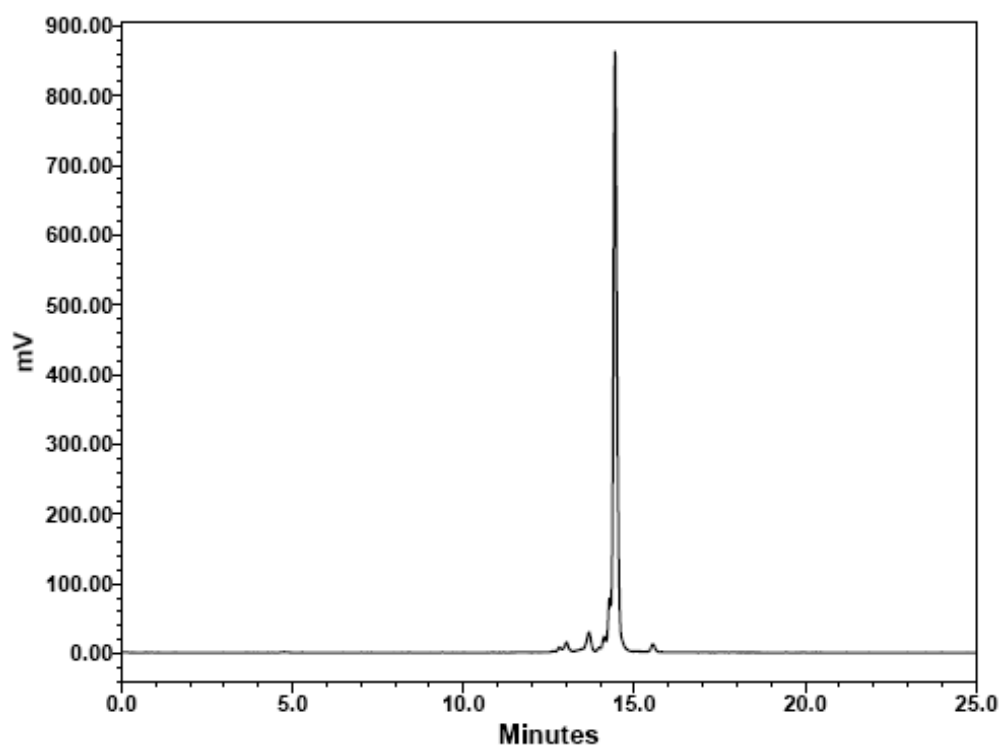


Figure S29. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 6 h following cysteine challenge (2 mM).

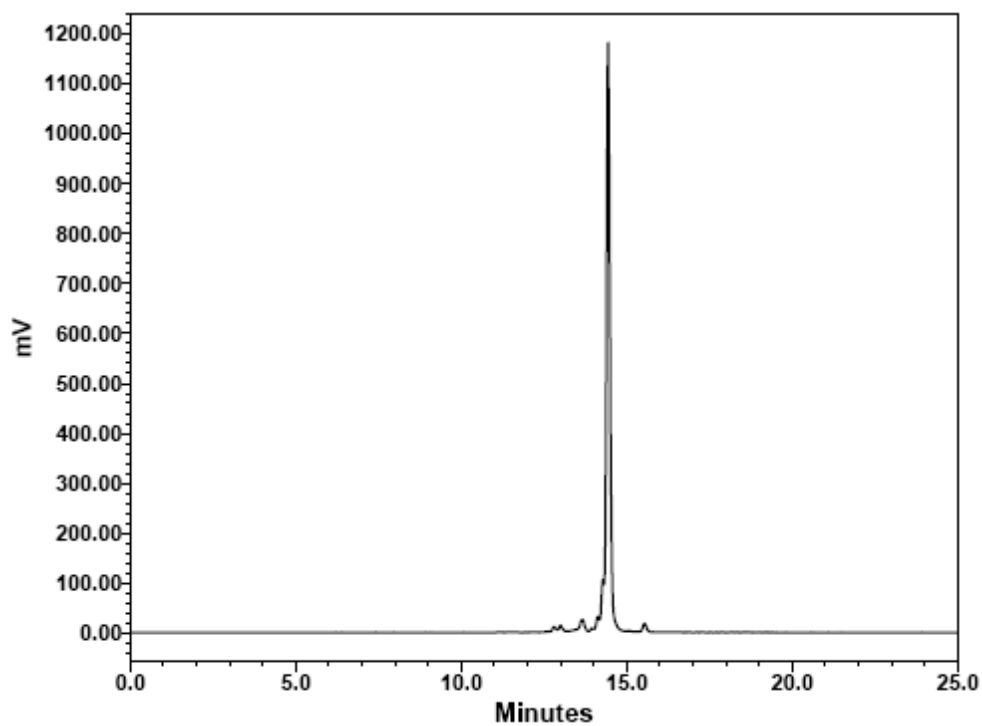


Figure S30. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 0.5 h following histidine challenge (2 mM).

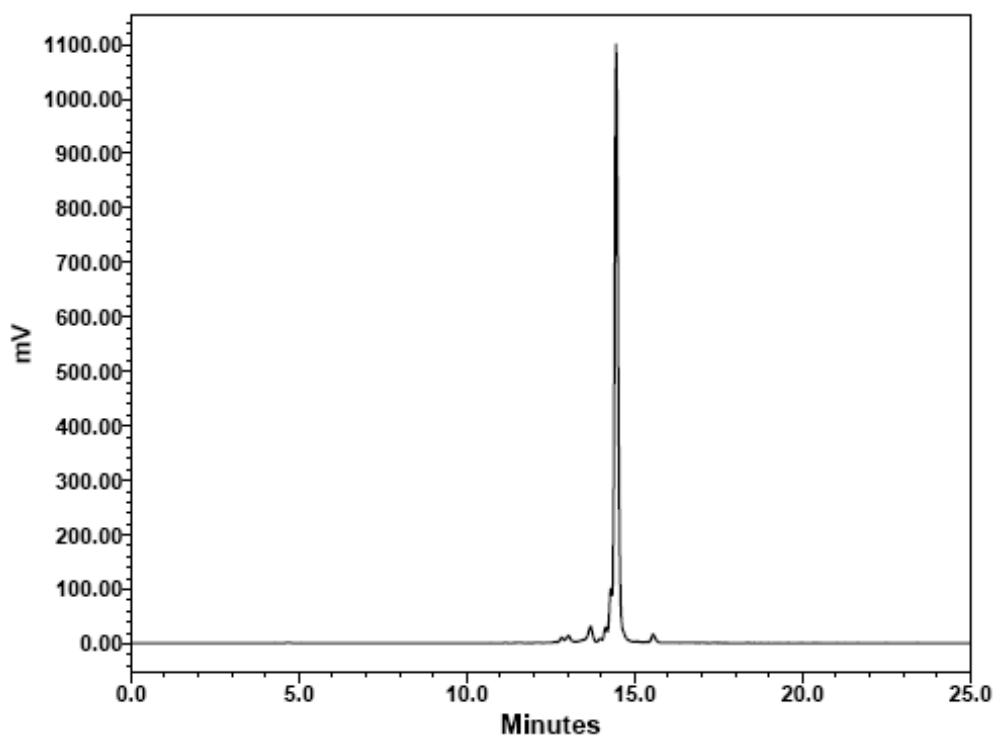


Figure S31. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 1 h following histidine challenge (2 mM).

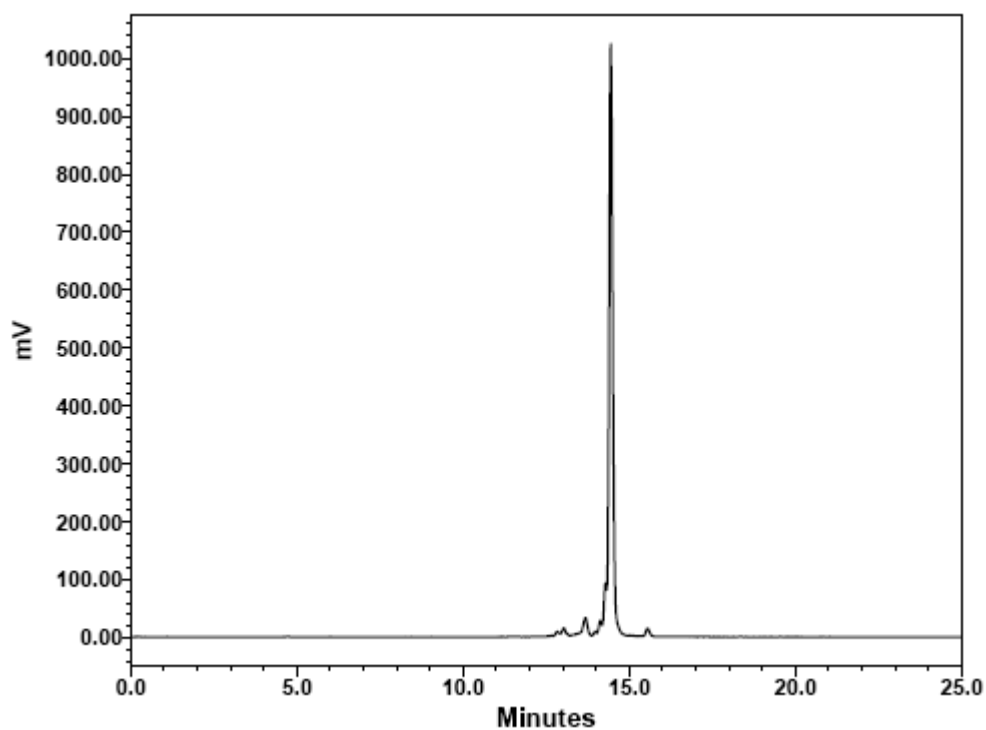


Figure S32. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 2 h following histidine challenge (2 mM).

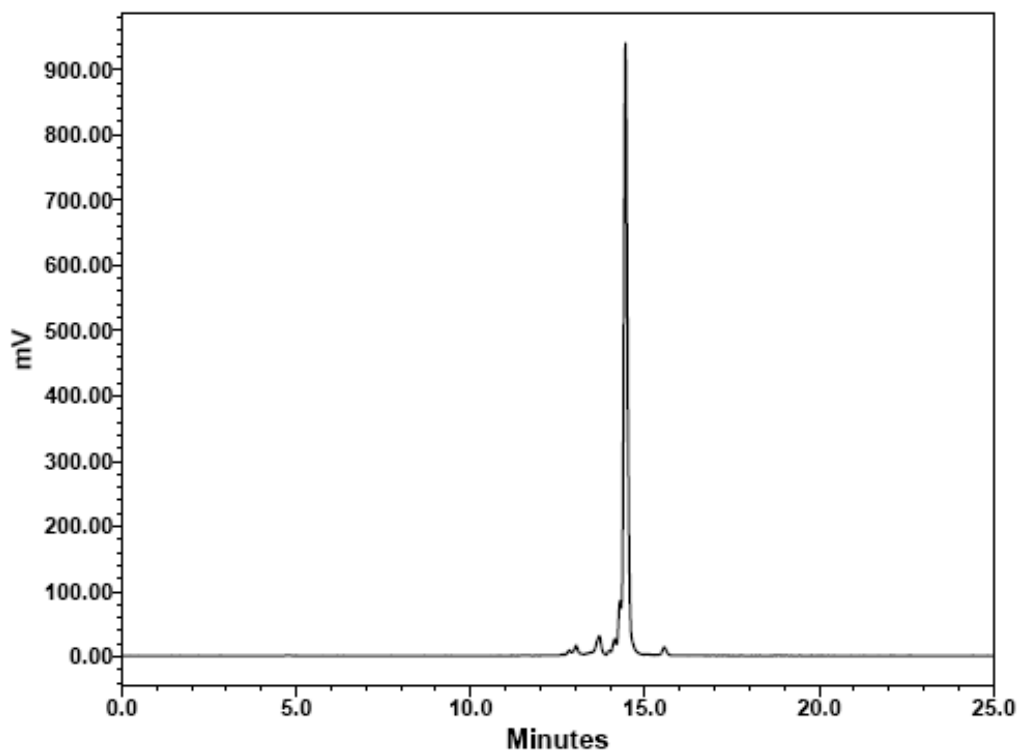


Figure S33. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 3 h following histidine challenge (2 mM).

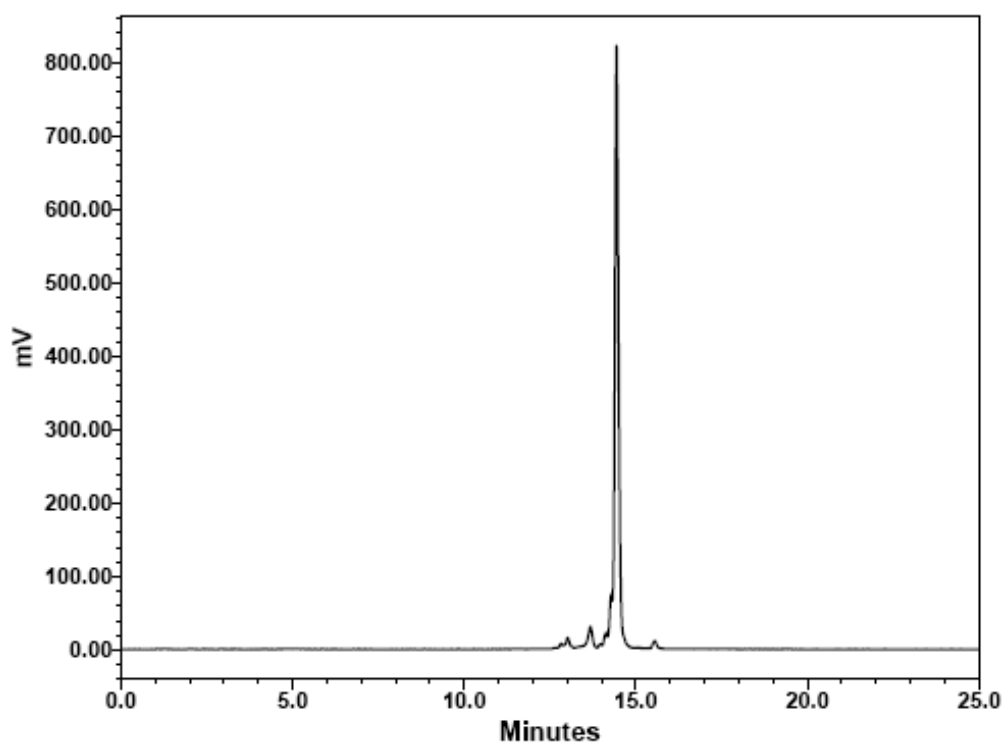


Figure S34. γ -HPLC chromatogram of $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{BPS})(\text{ImTz})]^+$ **10b** at 6 h following histidine challenge (2 mM).

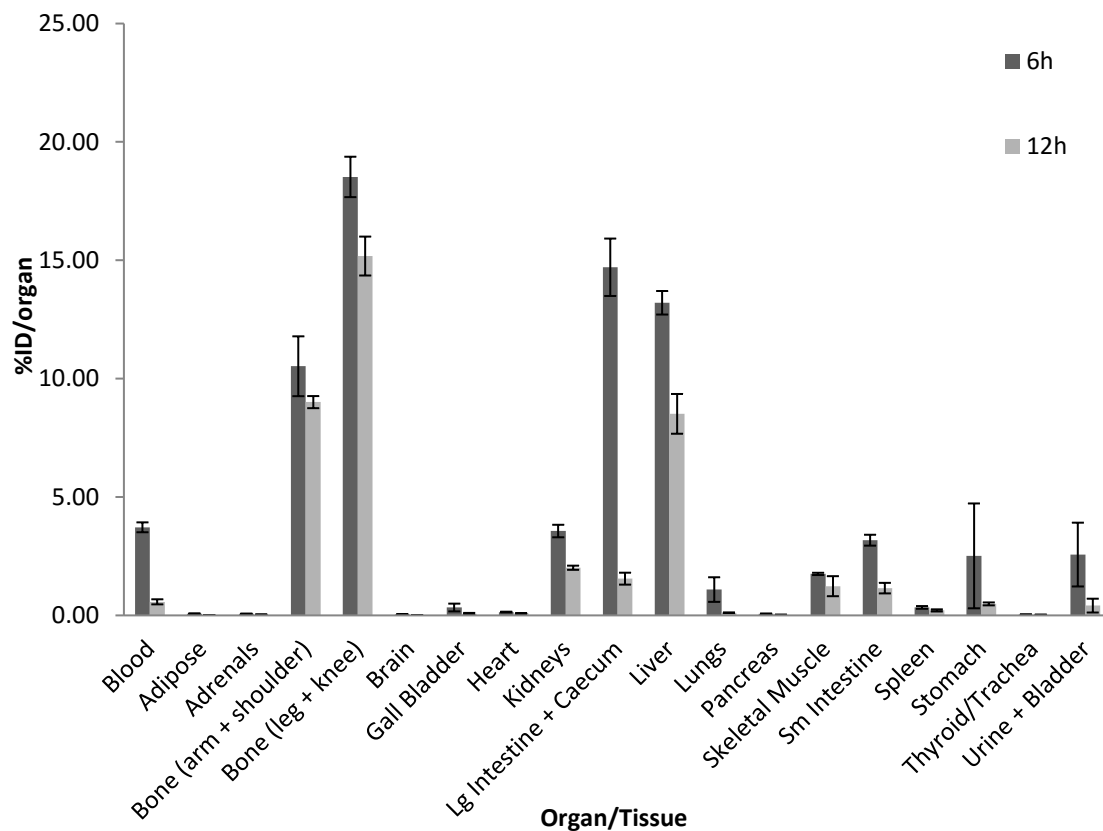


Figure. S35. Biodistribution data of select fluids and tissues after pretargeting with 20 mg/kg of TCO-BP administered 1 h prior to **10b**. Experiments were performed using Balb/c mice (n = 3 per time point) with tissues collected at 6 h and 12 h post administration of the labeled compound. Data are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

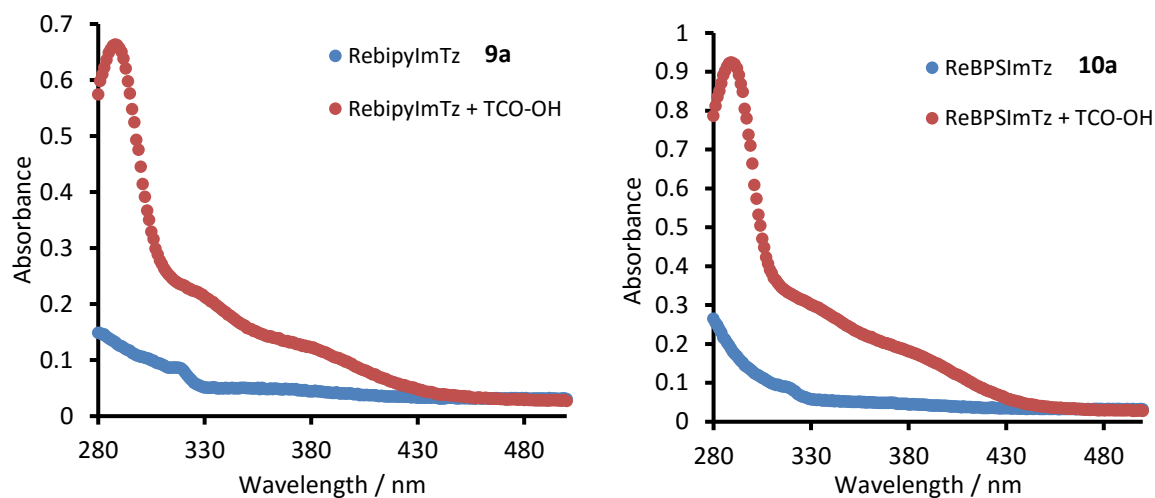


Figure S36. Absorption spectra for compounds **9a** and **10a** (50 μM in MeOH) in the absence (blue) and presence of 500 μM (red) TCO-OH.

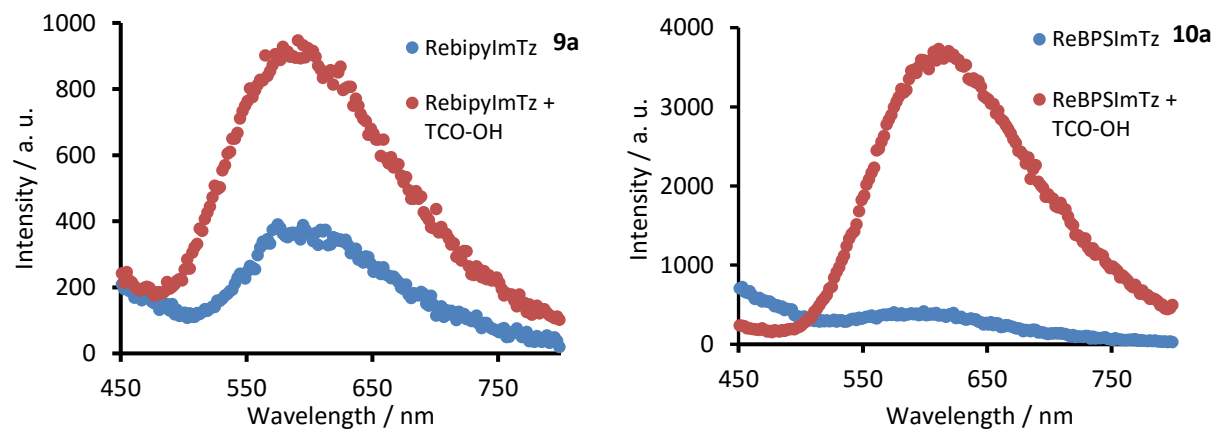


Figure S37. Emission spectra for compounds **9a** and **10a** (50 μM in MeOH) in the absence (blue) and presence of 500 μM (red) TCO-OH.

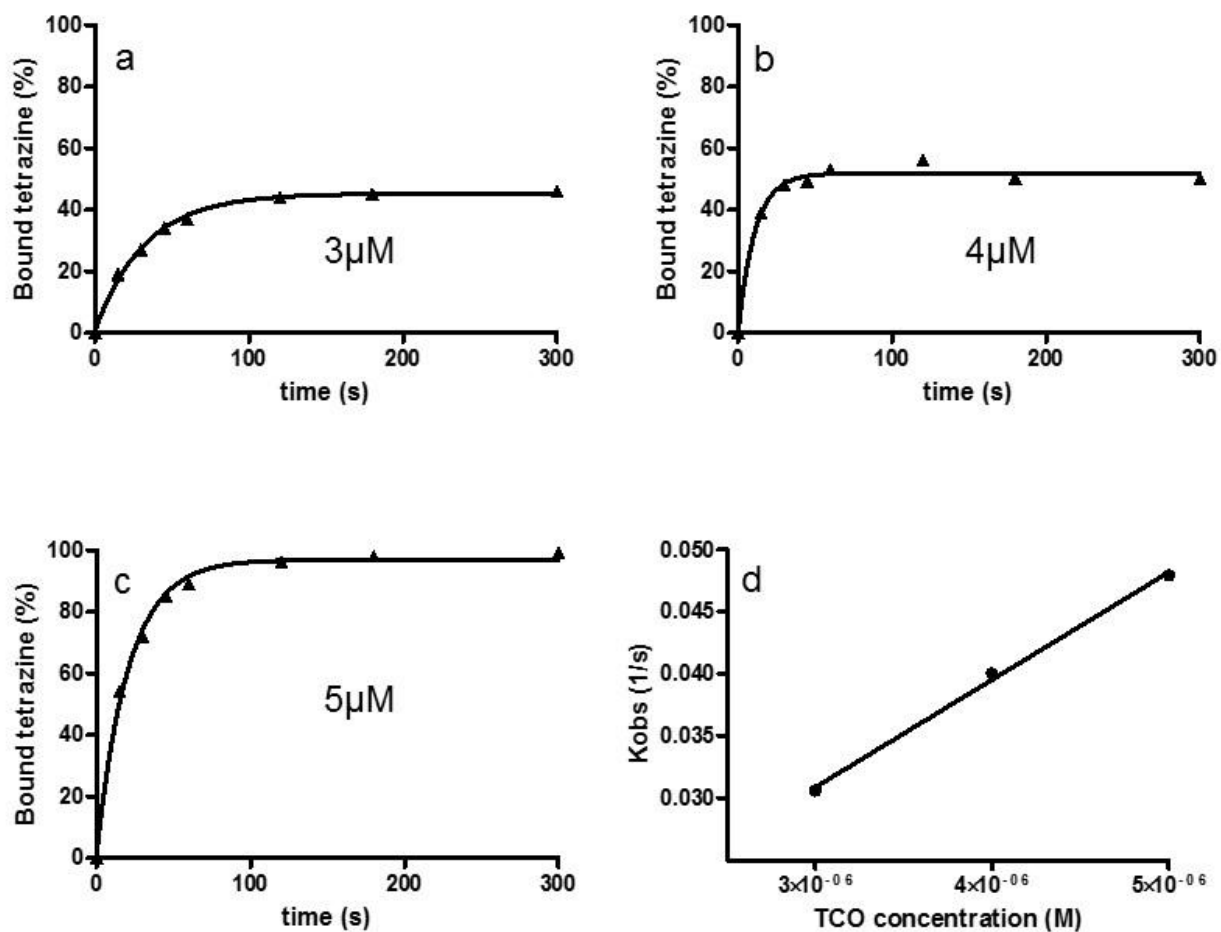


Figure S38. Reaction kinetics between **9b** and TCO-OH: Reaction of **9b** with a) TCO-OH (3 μM), b) TCO-OH (4 μM), c) TCO-OH (5 μM) vs. time, and fitted to a first-order exponential. A pseudo first-order rate constant (k_{obs}) was determined from the fit. d) Plot of k_{obs} vs. the concentration of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

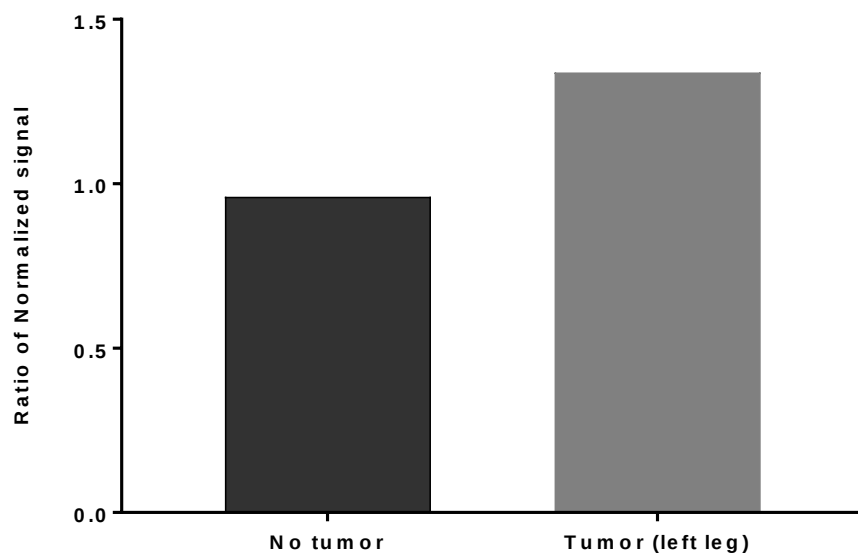


Figure S39. Quantitative comparison of SPECT image signals from knee joints of tumor-bearing and non-tumor-bearing mice. SPECT image signals were calculated as described in Experimental section, based on region of interest (ROI) defined around knee joints. Data are expressed as ratio of left to right 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		n=3	
	6h		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		n=3	
	6h		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