

Supporting Information

A Bone-Seeking *Trans*-Cyclooctene for Pretargeting and Bioorthogonal Chemistry:

A Proof of Concept Study Using ^{99m}Tc - and ^{177}Lu -Labeled Tetrazines.

Authors

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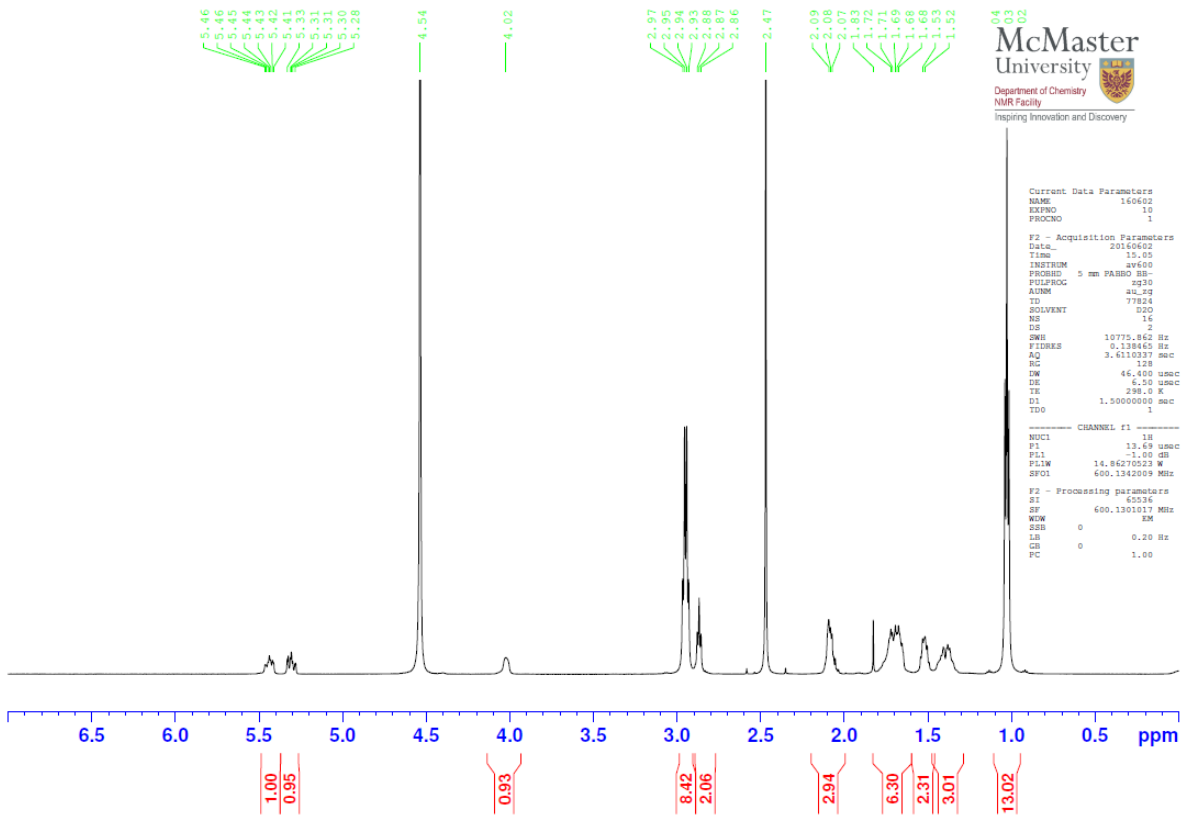


Figure S1. ^1H NMR spectrum (D_2O , 600 MHz) of TCO-BP (**2**).

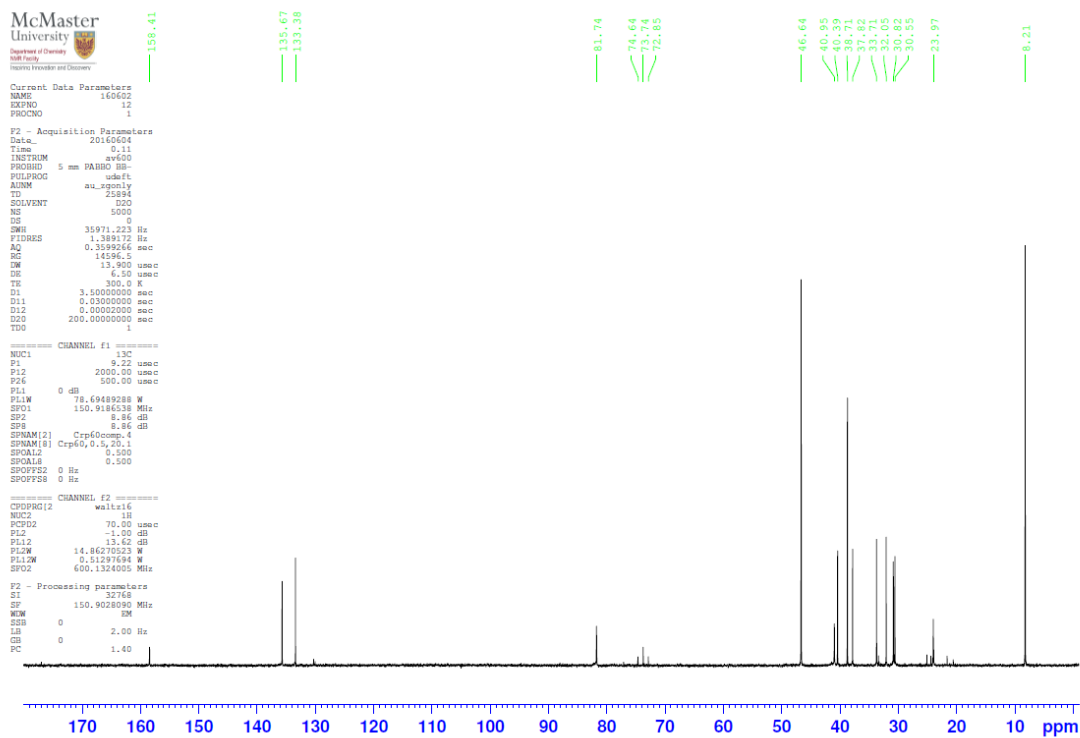


Figure S2. ^{13}C NMR spectrum (D_2O , 150 MHz) of **2**.

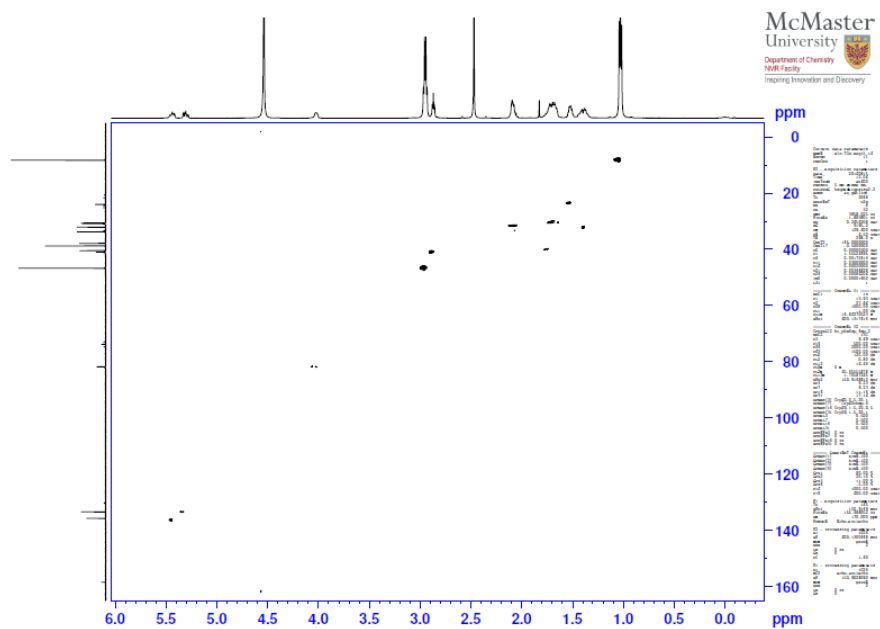


Figure S3. HSQC of **2** (600 MHz, CDCl_3).

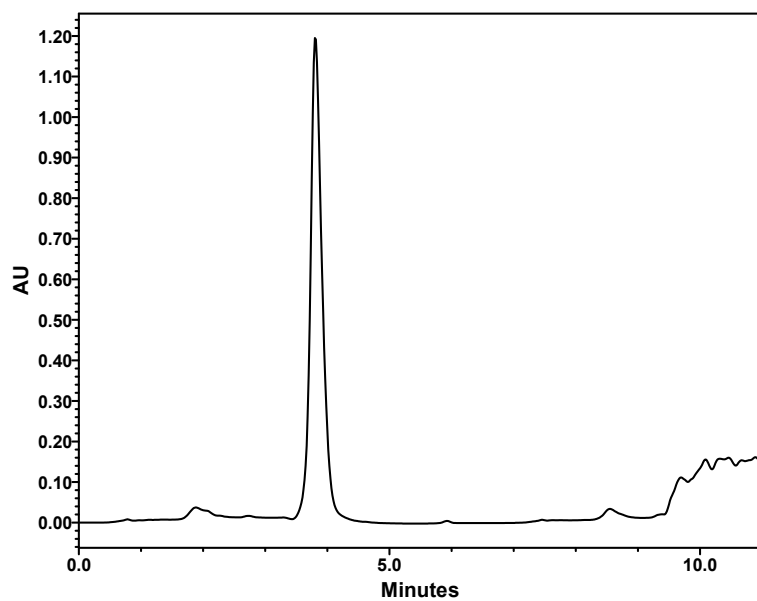


Figure S4. UV HPLC trace (UV 220 nm) of **2**.

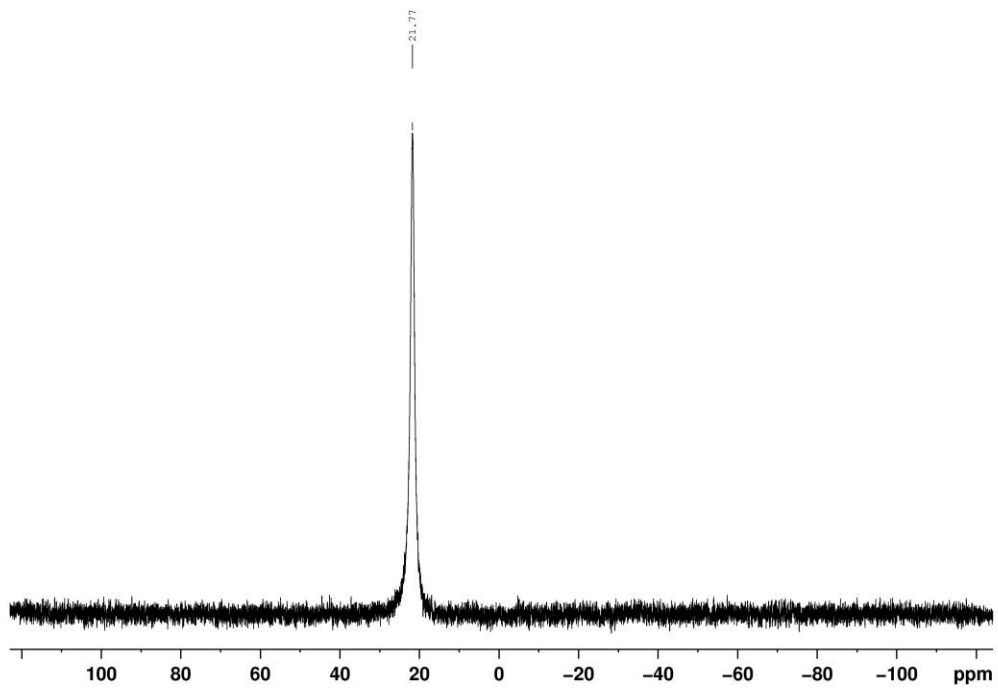


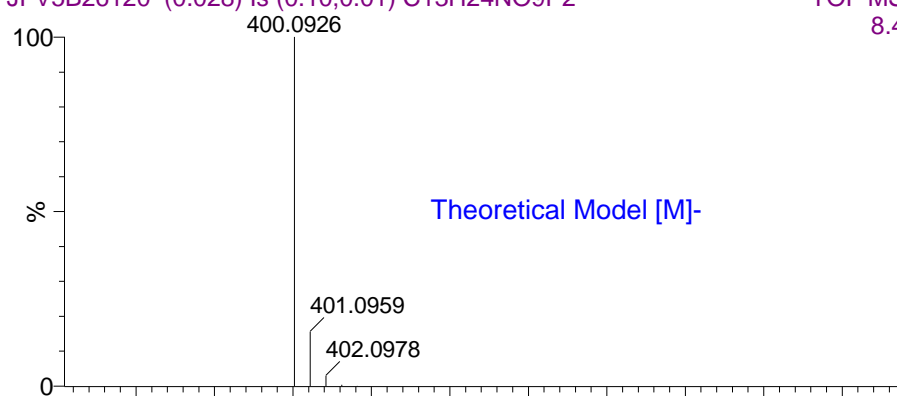
Figure S5. $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum (D_2O , 242 MHz) of **2**.

R179HP

JFV5B26120 (0.028) Is (0.10,0.01) C₁₃H₂₄NO₉P₂

03-May-2016

TOF MS ES-
8.42e12



JFV5B26120 975 (18.464) AM (Cen,4, 80.00, Ar,6500.0,734.01,0.80); Sb (99,10.00); S
6.11e4

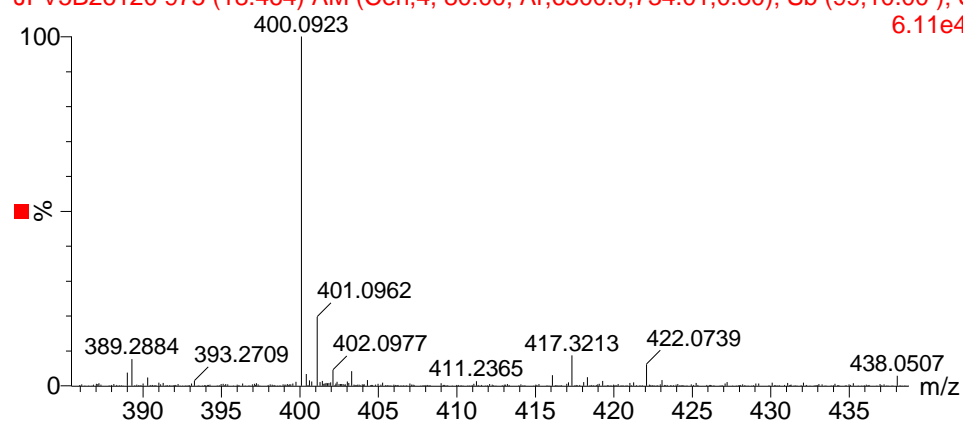


Figure S6. HRMS (TOF-MS) of 2.

UV/Vis kinetic studies

The reaction between 4-(1,2,4,5-tetrazin-3-yl)phenylmethanamine hydrochloride and **2** was conducted at 25 ± 0.5 °C in a 1-mL UV cuvette, and monitored by UV-Vis spectroscopy monitoring at 515 nm. The substrates were dissolved separately in 1:2 v/v MeOH-saline and spectra acquired every 0.1 seconds over one minute. The kinetics were measured in triplicate for three different concentrations of **2** (5.7×10^{-3} , 7.0×10^{-3} , 9.0×10^{-3} M) against the tetrazine (0.5×10^{-3} M). The K_{obs} , for each run was determined from a plot of absorbance versus time (Graphpad Prism 5). The average K_{obs} obtained from experiments was determined in triplicate and plotted against the concentration of **2** to determine the second order rate constant.

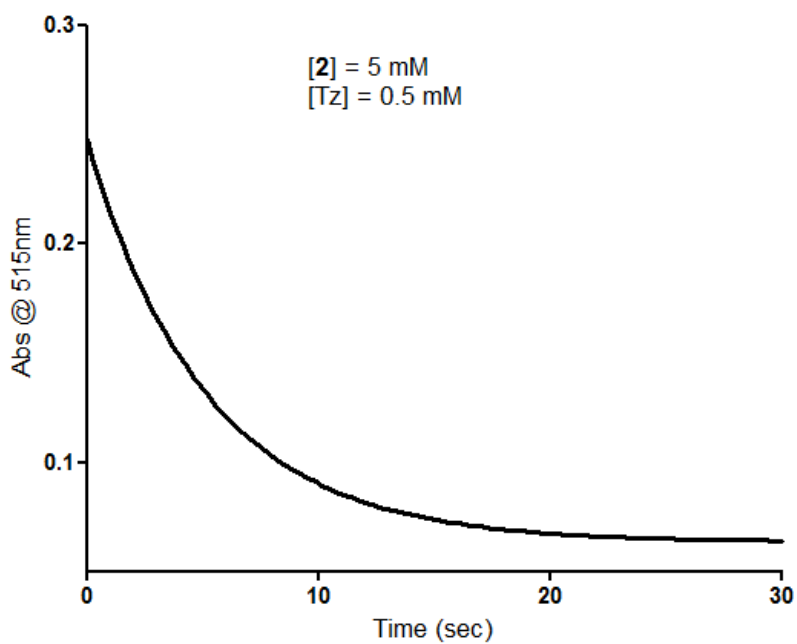


Figure S7. Plot of absorption at 515 nm versus time for the reaction of **2** with 4-(1,2,4,5-tetrazin-3-yl)phenyl)methanamine hydrochloride in 1:2 MeOH/saline.

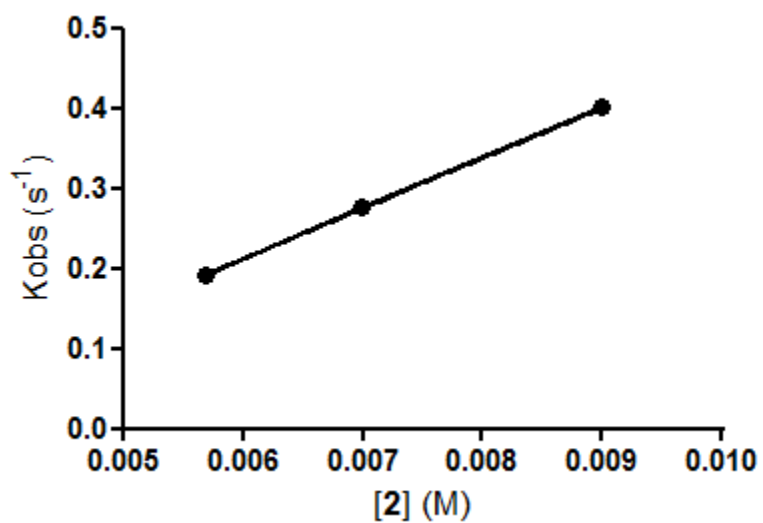


Figure S8. Plot of K_{obs} vs. the concentration of TCO-BP **2**.

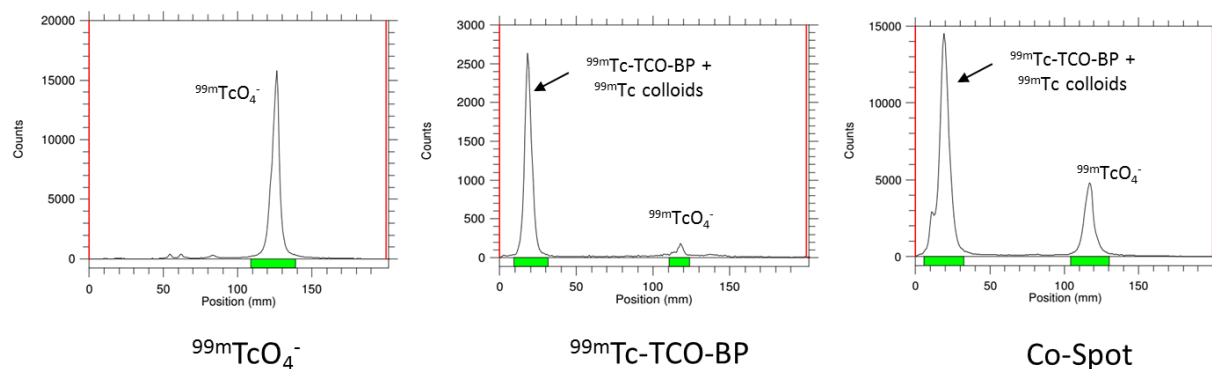


Figure S9. RadioTLC of $^{99m}\text{TcO}_4^-$, [^{99m}Tc]-TCO-BP (**3**) and a co-spot using acetone as the eluent. In acetone, both **3** and ^{99m}Tc colloidal impurities remain on the baseline, while $^{99m}\text{TcO}_4^-$ travels with the solvent front. TLC were performed using iTLC-SG glass microfiber chromatography paper (Agilent Technologies, SGI0001) plates and visualized on a Bioscan AR-2000 Imaging Scanner.

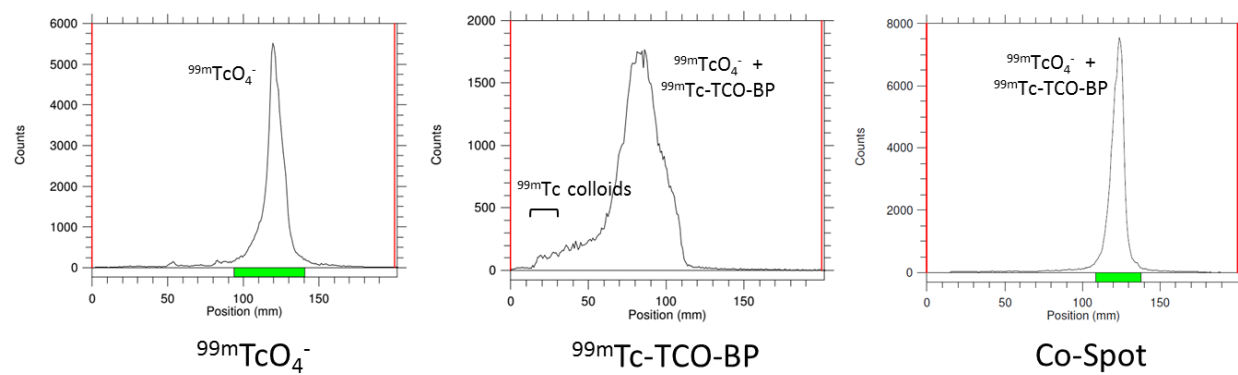


Figure S10. RadioTLC of $^{99m}\text{TcO}_4^-$, [^{99m}Tc]-TCO-BP (**3**) and a co-spot using distilled water as the eluent. In distilled water, $^{99m}\text{TcO}_4^-$ and [^{99m}Tc]-TCO-BP (**3**) travel with the solvent front, while ^{99m}Tc colloidal impurities remain on the baseline. TLCs were performed using iTLC-SG glass microfiber chromatography paper (Agilent Technologies, SGI0001) plates and visualized on a Bioscan AR-2000 Imaging Scanner.

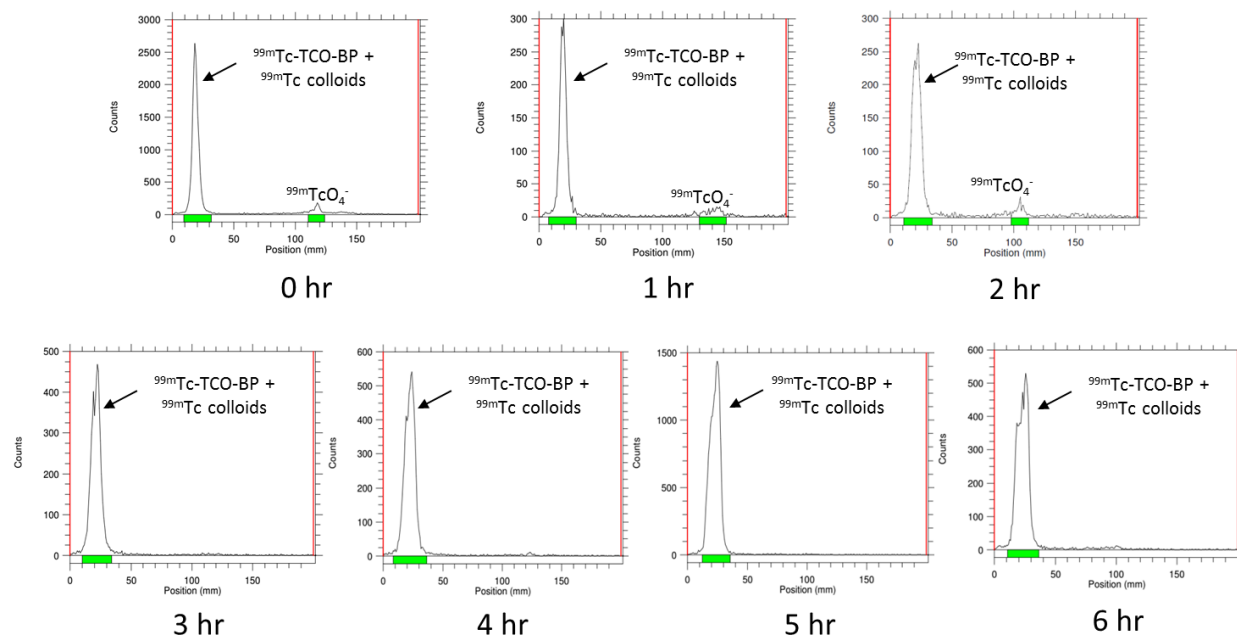


Figure S11. Stability of $[^{99m}\text{Tc}]\text{-TCO-BP (3)}$ in saline at $37\text{ }^\circ\text{C}$ at the indicated time points, as assessed by Radio-TLC with acetone as the eluent. TLCs were performed using iTLC-SG glass microfiber chromatography paper (Agilent Technologies, SGI0001) plates and visualized on a Bioscan AR-2000 Imaging Scanner.

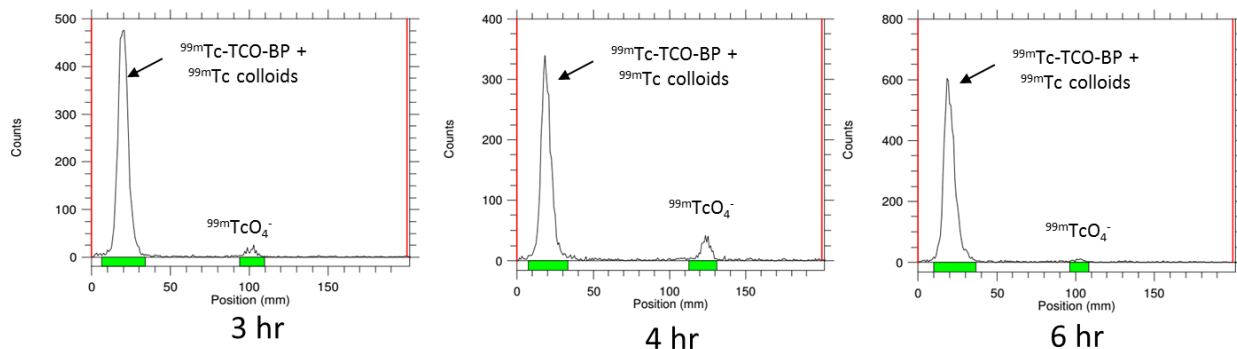


Figure S12. Stability of $[^{99m}\text{Tc}]\text{-TCO-BP (3)}$ in plasma at $37\text{ }^\circ\text{C}$ at the indicated time points, as assessed by Radio-TLC with acetone as the eluent. TLCs were performed using iTLC-SG glass microfiber chromatography paper (Agilent Technologies, SGI0001) plates and visualized on a Bioscan AR-2000 Imaging Scanner.

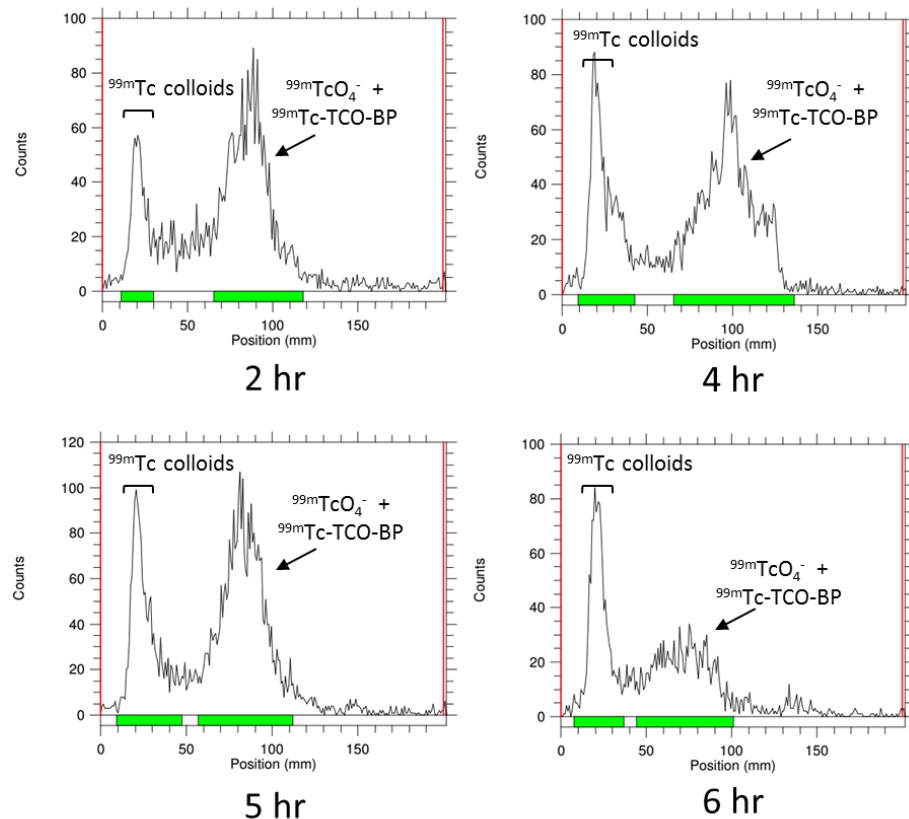


Figure S13. Stability of [^{99m}Tc]-TCO-BP (**3**) in plasma at 37 °C at the indicated time points, as assessed by Radio-TLC with distilled water as the eluent. A broad peak (arrow) indicates co-migration of ^{99m}Tc with **3**, whereas ^{99m}Tc colloidal impurities accumulate at the baseline. TLCs were performed using iTLC-SG glass microfiber chromatography paper (Agilent Technologies, SGI0001) plates and visualized on a Bioscan AR-2000 Imaging Scanner.

%ID/g	n=3		n=3		n=3	
	1h		4h		6h	
Organs	avg	SEM	avg	SEM	avg	SEM
Blood	0.78	0.05	0.06	0.02	0.03	0.00
Adipose	0.36	0.19	0.15	0.07	0.08	0.01
Adrenals	3.14	0.59	0.91	0.36	1.21	0.16
Bone (arm + shoulder)	8.56	0.35	6.55	1.08	7.77	0.20
Bone (leg + knee)	14.29	0.51	12.36	0.48	12.49	0.29
Brain	0.03	0.00	0.03	0.02	0.02	0.00
Gall Bladder	1.59	0.59	0.96	0.38	1.43	0.34
Heart	1.04	0.12	0.29	0.13	0.46	0.03
Kidneys	42.54	1.60	22.10	5.75	35.46	1.86
Lg Intestine + Caecum	0.26	0.03	2.35	1.86	1.04	0.05
Liver	6.52	0.54	1.99	1.25	3.66	0.26
Lungs	11.02	3.57	12.10	6.46	18.22	3.08
Pancreas	1.09	0.12	0.19	0.11	0.30	0.08
Skeletal Muscle	0.28	0.03	0.18	0.08	0.12	0.05
Sm Intestine	0.80	0.13	0.31	0.08	0.27	0.05
Spleen	3.36	0.48	2.14	0.94	3.36	0.43
Stomach	3.30	0.50	1.25	0.38	1.09	0.16
Thyroid/Trachea	2.80	0.22	2.05	0.24	2.24	0.37
Urine + Bladder	231.61	86.63	281.35	102.89	68.20	51.21

Table S1. Biodistribution data for **3** expressed as percent injected dose per gram (%ID/g) and percent injected dose per organ (%ID/g). Data were taken at the indicated timepoints and studies were performed in Balb/c mice.

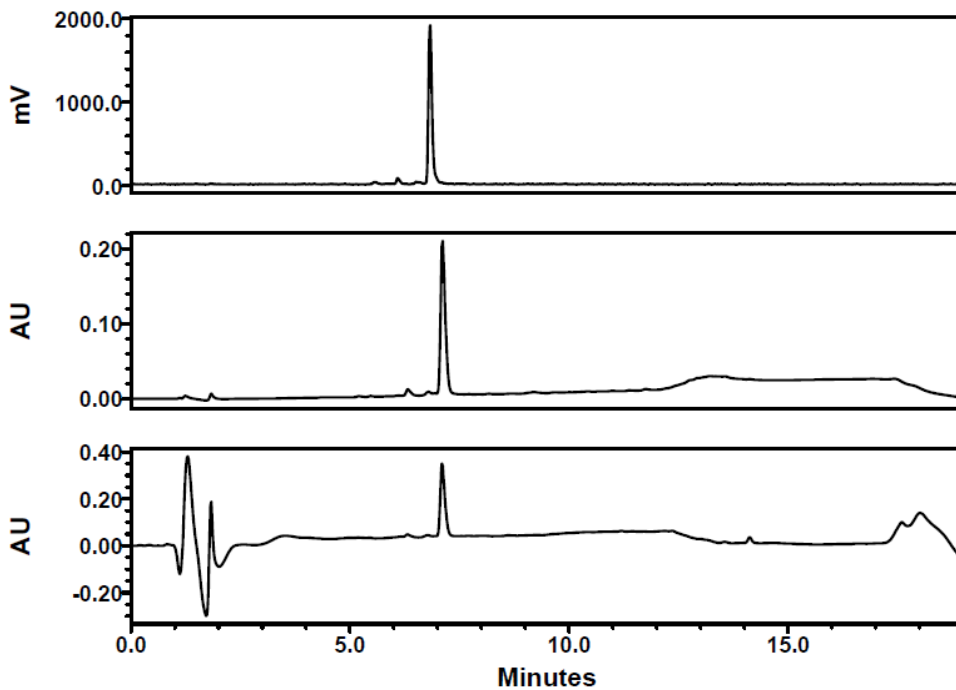


Figure S14. HPLC trace of ^{177}Lu -Tz (**5**). Top: gamma; middle: UV 254 nm; bottom: UV 214 nm.

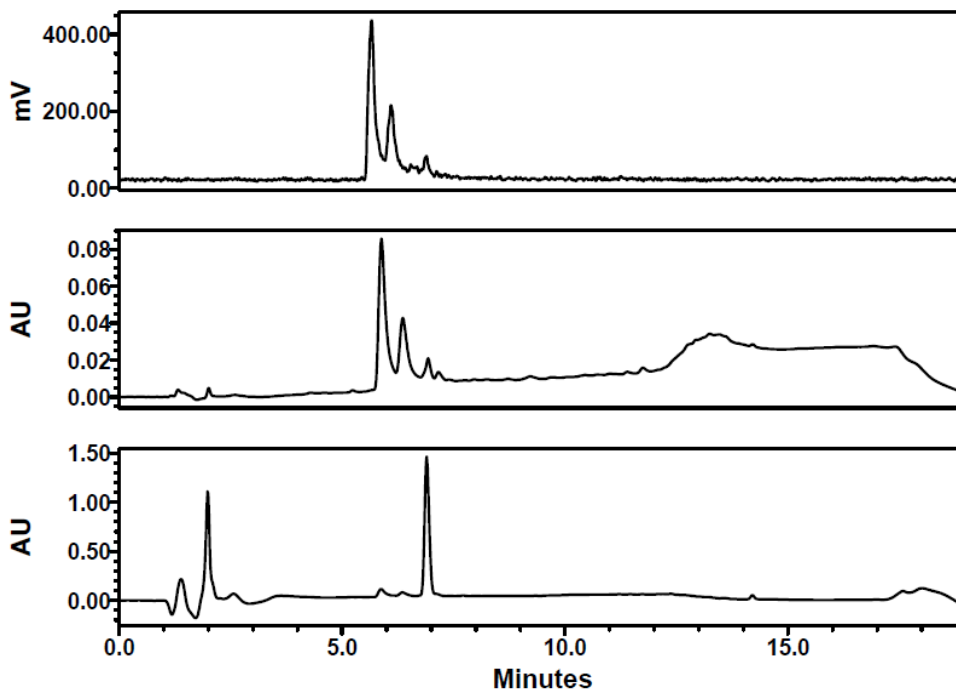


Figure S15. HPLC trace of ^{177}Lu -Tz (**5**) following treatment with TCO-BP (**2**). Top: gamma; middle: UV 254 nm; bottom: UV 214 nm.

%ID/g	n=3		n=3	
	Pretargeting		Active targeting	
Organs	avg	avg	avg	SEM
Blood	0.79	0.33	0.07	0.00
Gall Bladder	1.38	0.69	1.63	0.79
Kidneys	2.44	0.10	2.09	0.07
Knee	15.50	1.24	16.45	2.27
Liver	1.99	0.73	0.52	0.01
Shoulder	9.10	0.76	8.39	1.83
Sm & Lg Intestine	0.31	0.00	0.41	0.12
Stomach	0.25	0.02	0.12	0.02
Thyroid/Trachea	1.24	0.07	1.55	0.17

%ID/O	n=3		n=3	
	Pretargeting		Active targeting	
Organs	avg	avg	avg	avg
Blood	0.62	0.25	0.06	0.00
Gall Bladder	0.02	0.01	0.01	0.00
Kidneys	0.50	0.01	0.43	0.01
Knee	27.82	3.40	29.88	4.11
Liver	1.65	0.58	0.40	0.01
Shoulder	16.34	2.05	15.14	3.18
Sm & Lg Intestine	0.38	0.01	0.55	0.15
Stomach	0.04	0.00	0.02	0.00
Thyroid/Trachea	0.02	0.00	0.02	0.00

Table S2. Biodistribution data expressed as (Top) percent injected dose per gram (%ID/g) and (Bottom) percent injected dose per organ (%ID/O) for **5**, when combined with **2** prior to injection (Active targeting) or following injection of **2**, 1 h prior to administration of **5** (Pretargeting). Data were taken at 6 h post-injection of the labeled compound and studies were performed in Balb/c mice.

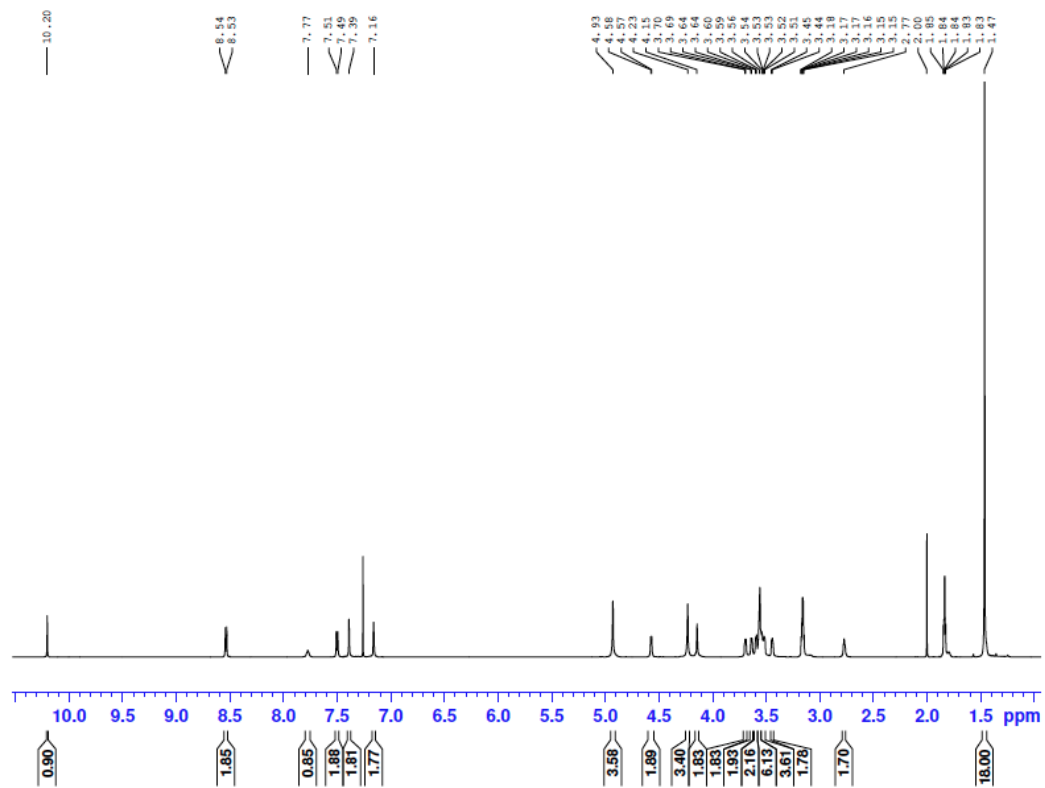


Figure S16. ^1H NMR (600 MHz, CDCl_3) of **6**.

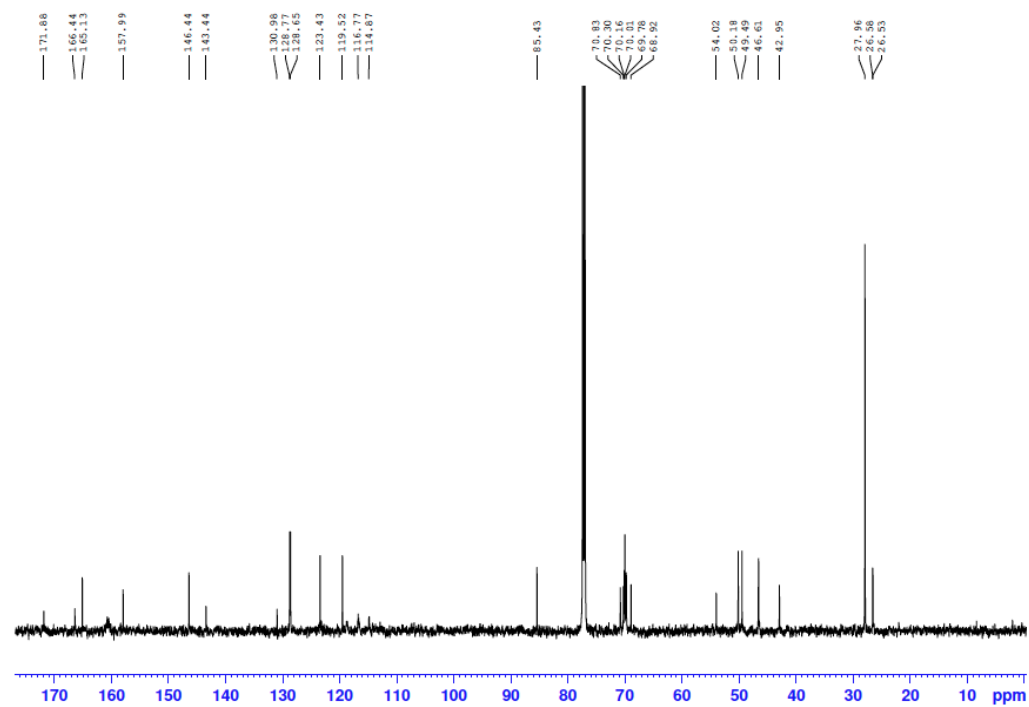


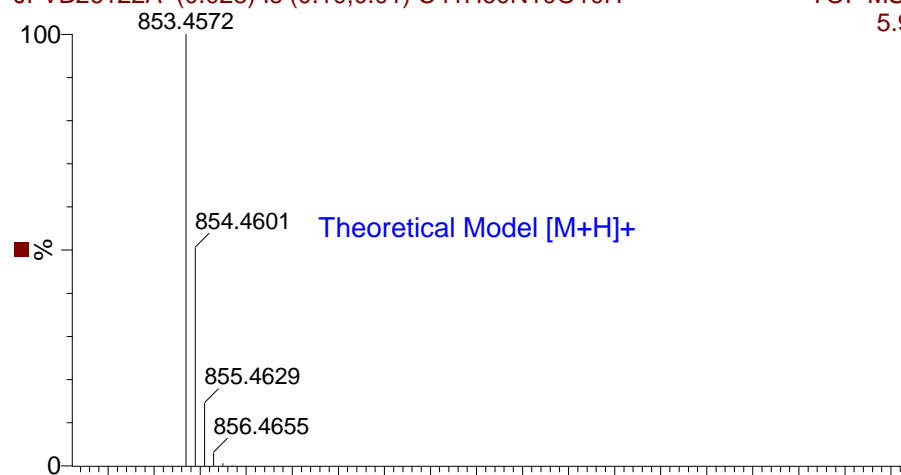
Figure S17. ^{13}C NMR (150 MHz, CDCl_3) of **6**.

PEG5-TZ

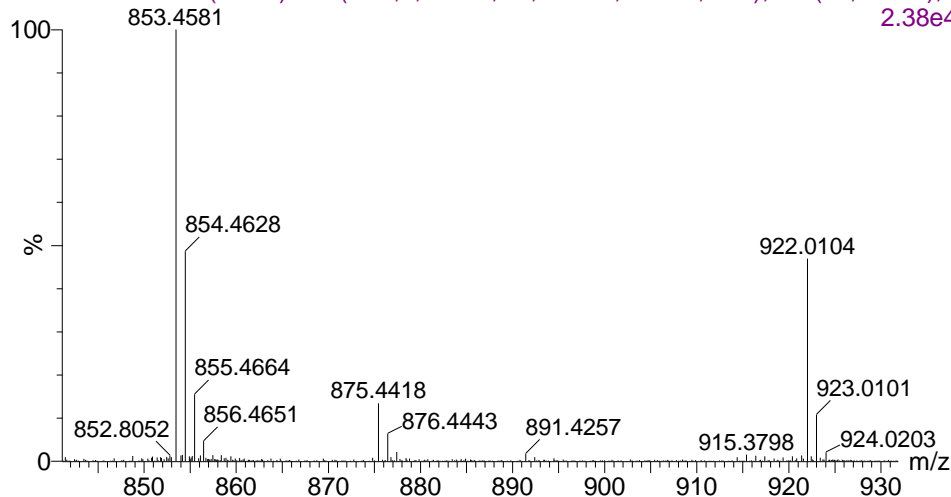
JFVB26122A (0.028) Is (0.10,0.01) C₄₁H₆₀N₁₀O₁₀H

03-May-2016

TOF MS ES+
5.93e12



JFVB26122A 362 (6.852) AM (Cen,4, 80.00, Ar,6500.0,922.01,0.80); Sb (99,10.00) ; S
2.38e4



Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 300.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Min/Max Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
853.4581	853.4572	0.9	1.0	11.0	1	C ₄₂ H ₆₇ N ₃ O ₁₅
	853.4572	0.9	1.0	16.5	3	C ₄₁ H ₆₁ N ₁₀ O ₁₀
	853.4599	-1.8	-2.1	15.5	6	C ₄₅ H ₆₅ N ₄ O ₁₂
	853.4559	2.2	2.6	11.5	2	C ₄₀ H ₆₅ N ₆ O ₁₄
	853.4612	-3.1	-3.7	20.5	8	C ₄₆ H ₆₁ N ₈ O ₈
	853.4612	-3.1	-3.7	15.0	7	C ₄₇ H ₆₇ N ₁₀ O ₁₃
	853.4545	3.6	4.2	12.0	4	C ₃₈ H ₆₃ N ₉ O ₁₃

Figure S18. HRMS (TOF-MS) of **6**.

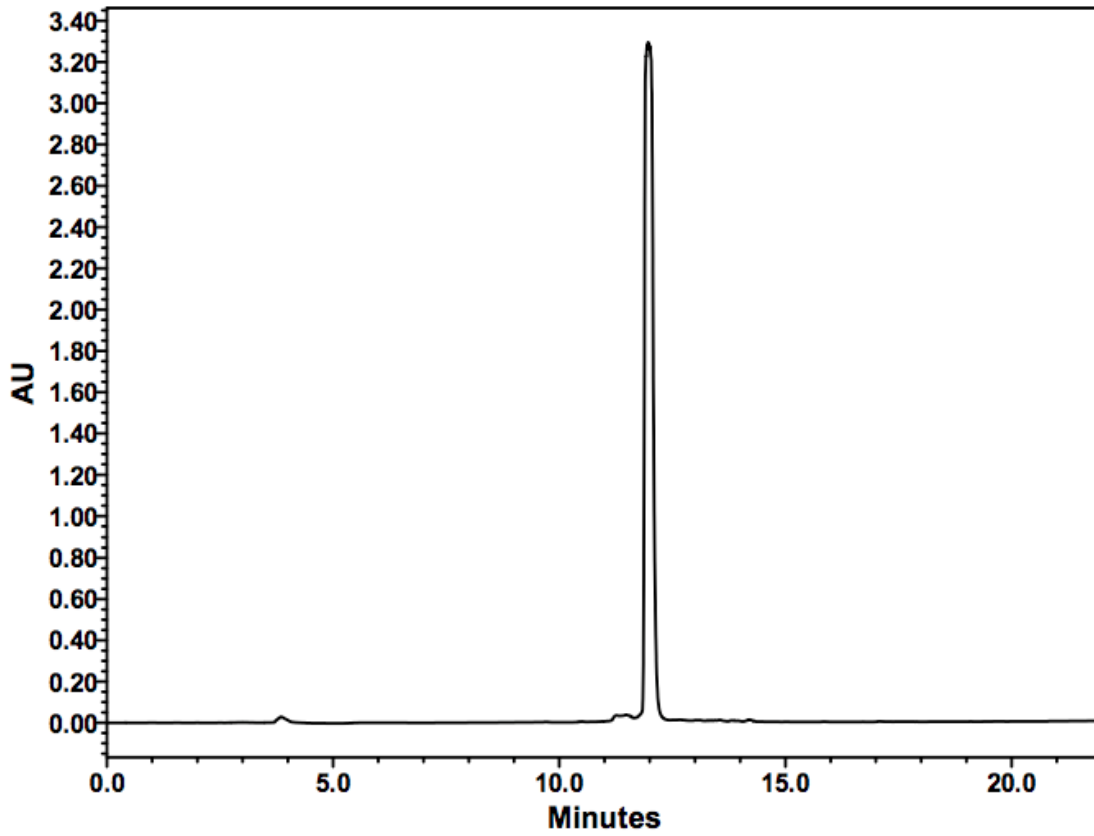


Figure S19. UV HPLC trace of **6** (UV 254 nm).

%ID/g	n=3		n=3	
	Pretargeting		Active targeting	
Organs	avg	SEM	avg	SEM
Blood	0.77	0.07	0.82	0.05
Gall Bladder	91.46	17.09	44.96	21.84
Kidneys	12.33	0.74	12.56	0.45
Knee	20.07	4.91	21.48	4.82
Liver	1.85	0.17	1.82	0.12
Shoulder	16.16	4.84	15.95	5.16
Sm & Lg Intestine	8.26	0.58	11.00	1.42
Stomach	1.12	0.30	3.88	1.47
Thyroid/Trachea	2.45	0.83	1.50	0.04

%ID/O	n=3		n=3	
	Pretarget		Active targeting	
Organs	avg	SEM	avg	SEM
Blood	0.61	0.05	0.72	0.06
Gall Bladder	0.39	0.20	0.29	0.14
Kidneys	2.37	0.15	2.75	0.14
Knee	34.95	8.21	39.99	7.97
Liver	1.49	0.09	1.74	0.04
Shoulder	27.91	8.05	29.83	9.06
Sm & Lg Intestine	9.52	0.39	15.63	0.96
Stomach	0.22	0.02	0.79	0.25
Thyroid/Trachea	0.02	0.00	0.02	0.00

Table S3. Biodistribution data expressed as (top) percent injected dose per gram (%ID/g) and (bottom) percent injected dose per organ (%ID/O) for **7** when combined with **2** prior to injection (Active targeting) or following injection of **2**, 1 h prior to administration of **7** (Pretargeting). Data were taken at 6 h post-injection of the labeled compound and studies were performed in Balb/c mice.

%ID/g Organs	n=3	
	avg	SEM
Blood	0.70	0.28
Gall Bladder	32.52	7.33
Kidneys	16.09	6.99
Knee	9.55	1.88
Liver	1.35	0.45
Shoulder	6.98	2.38
Sm & Lg Intestine	8.96	1.87
Stomach	0.21	0.04
Thyroid/Trachea	1.64	0.38

%ID/O Organs	n=3	
	avg	SEM
Blood	0.78	0.32
Gall Bladder	0.32	0.12
Kidneys	4.72	2.18
Knee	22.81	4.64
Liver	1.54	0.65
Shoulder	16.87	6.03
Sm & Lg Intestine	12.01	2.18
Stomach	0.08	0.02
Thyroid/Trachea	0.02	0.00

Table S4. Biodistribution data expressed as (top) percent injected dose per gram (%ID/g) and (bottom) percent injected dose per organ (%ID/O) for pretargeting with injection of **2**, 12 h prior to administration of **7**. Data were taken at 6 h post-injection of the labeled compound and studies were performed in Balb/c mice.

%ID/g	n=3		n=3	
	30 min		60 min	
Organs	avg	SEM	avg	SEM
Blood	1.03	0.07	0.76	0.05
Adipose	0.05	0.00	0.16	0.12
Adrenals	6.61	6.36	0.32	0.12
Bone	0.14	0.01	0.11	0.01
Brain	0.03	0.00	0.04	0.02
Gall Bladder	1061.52	319.89	398.67	141.73
Heart	0.20	0.01	0.16	0.01
Kidneys	0.55	0.06	0.49	0.04
Lg Intestine + Caecum	0.05	0.01	1.27	1.22
Liver	1.22	0.21	1.05	0.21
Lungs	0.75	0.05	0.76	0.06
Pancreas	0.11	0.03	0.20	0.11
Skeletal Muscle	0.08	0.02	0.07	0.01
Sm Intestine	39.65	3.68	43.28	2.24
Spleen	0.18	0.03	0.18	0.05
Stomach	0.71	0.17	0.91	0.08
Thyroid/Trachea	0.29	0.03	0.31	0.07
Urine + Bladder	5.89	1.67	6.09	3.45

Table S5. Biodistribution data expressed as percent injected dose per gram (%ID/g) for **7** in Balb/c mice.

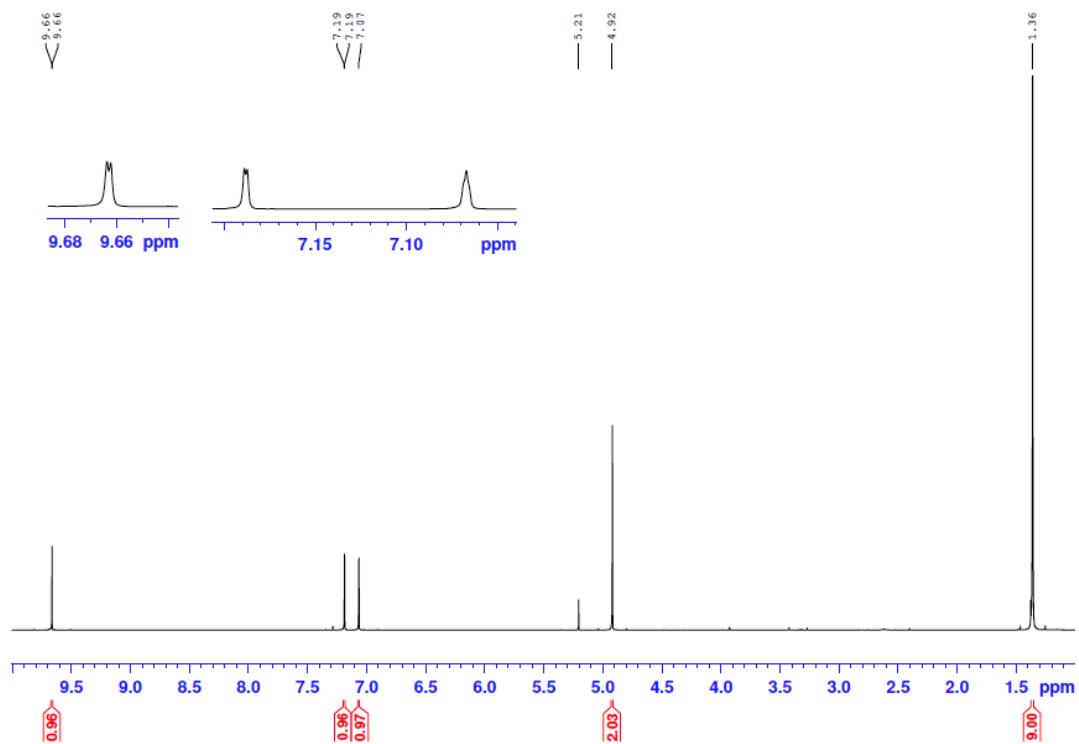


Figure S20. ^1H NMR (600 MHz, CDCl_3) of *tert*-butyl 2-(2-formyl-1*H*-imidazol-1-yl)acetate (**8**).

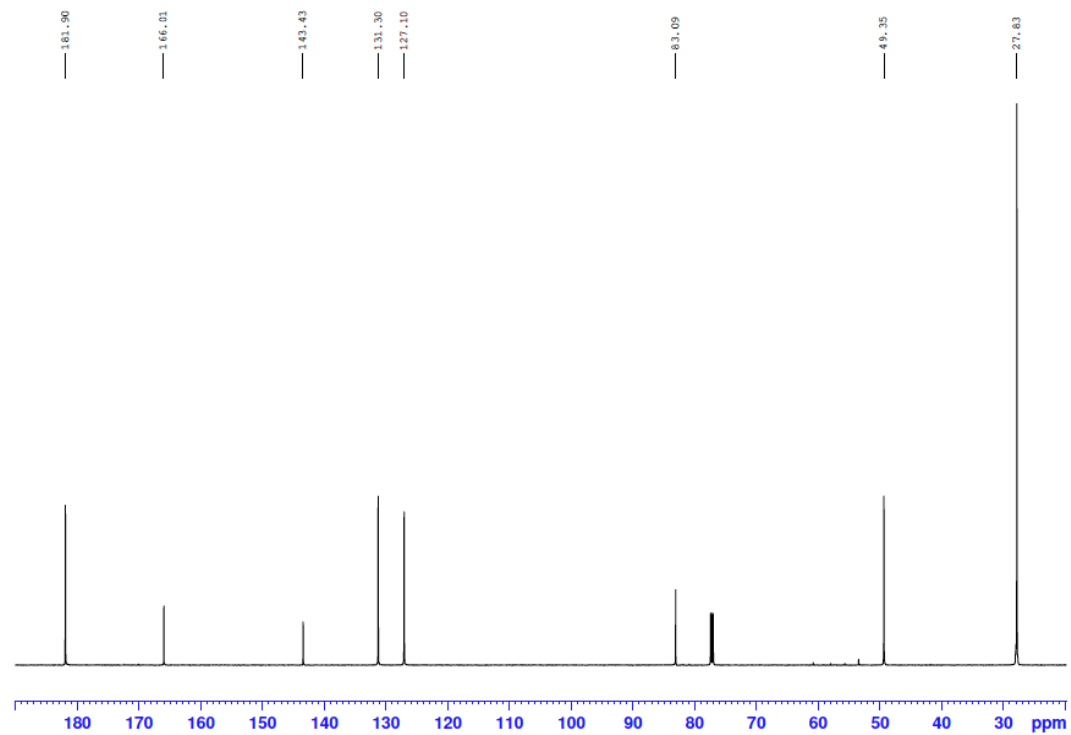


Figure S21. ^{13}C NMR (150 MHz, CDCl_3) of *tert*-butyl 2-(2-formyl-1*H*-imidazol-1-yl)acetate (**8**).

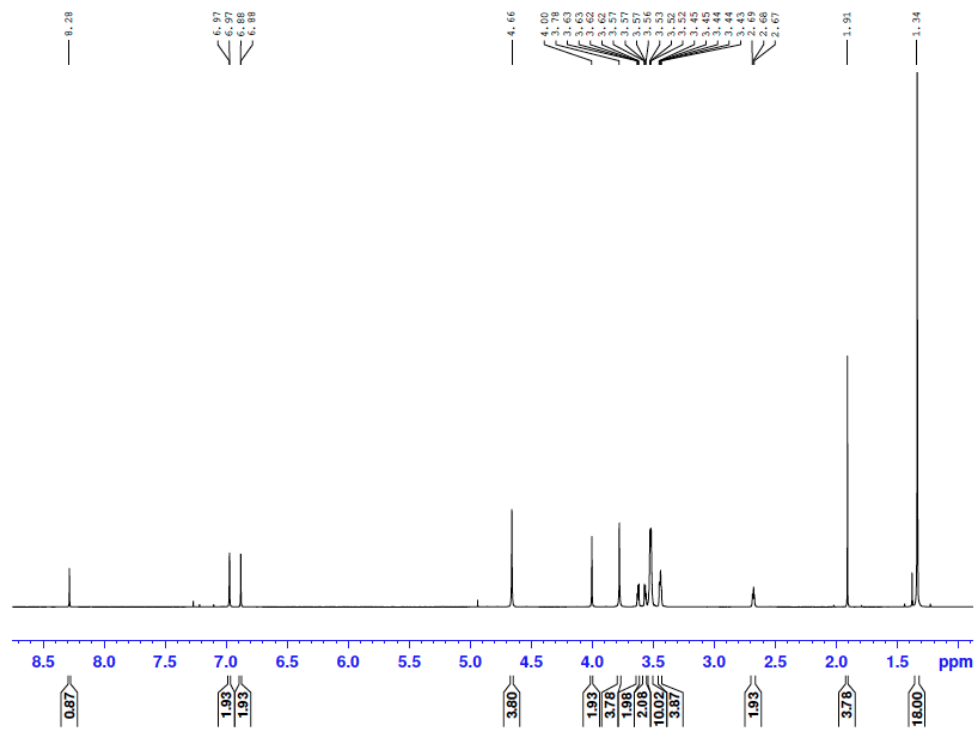


Figure S22. ^1H NMR (600 MHz, CDCl_3) of **9**.

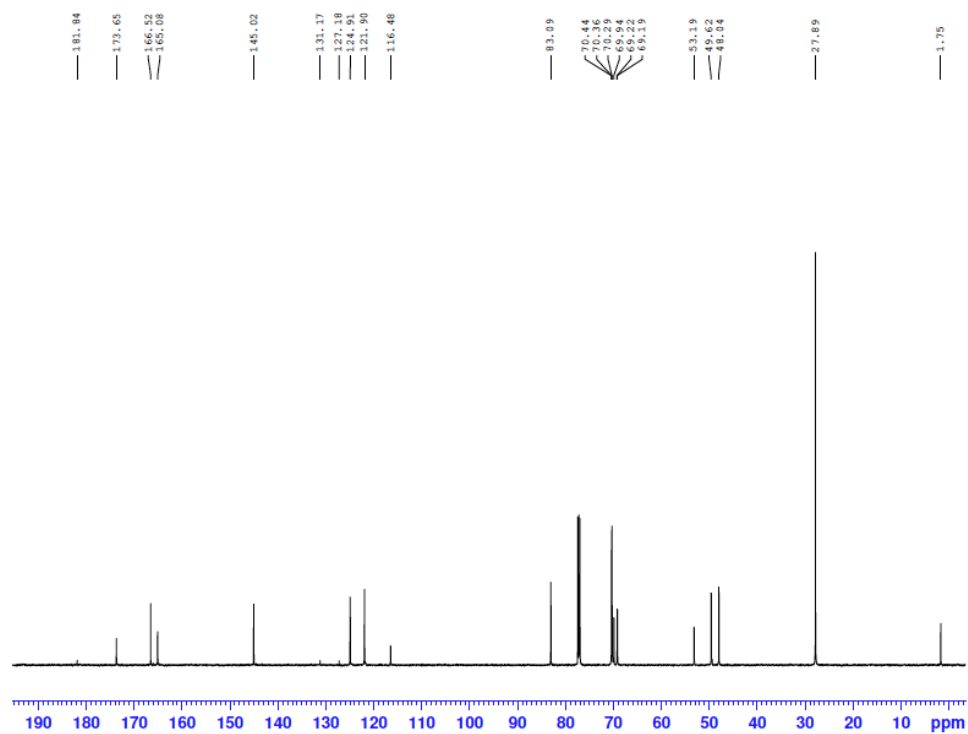


Figure S23. ^{13}C NMR (150 MHz, CDCl_3) of **9**.