

Supplementary Information

Synthesis and preliminary evaluation of cardiac imaging with [⁶⁸Ga]Ga-NOTA-CTP in normal and infarcted CD1 mice

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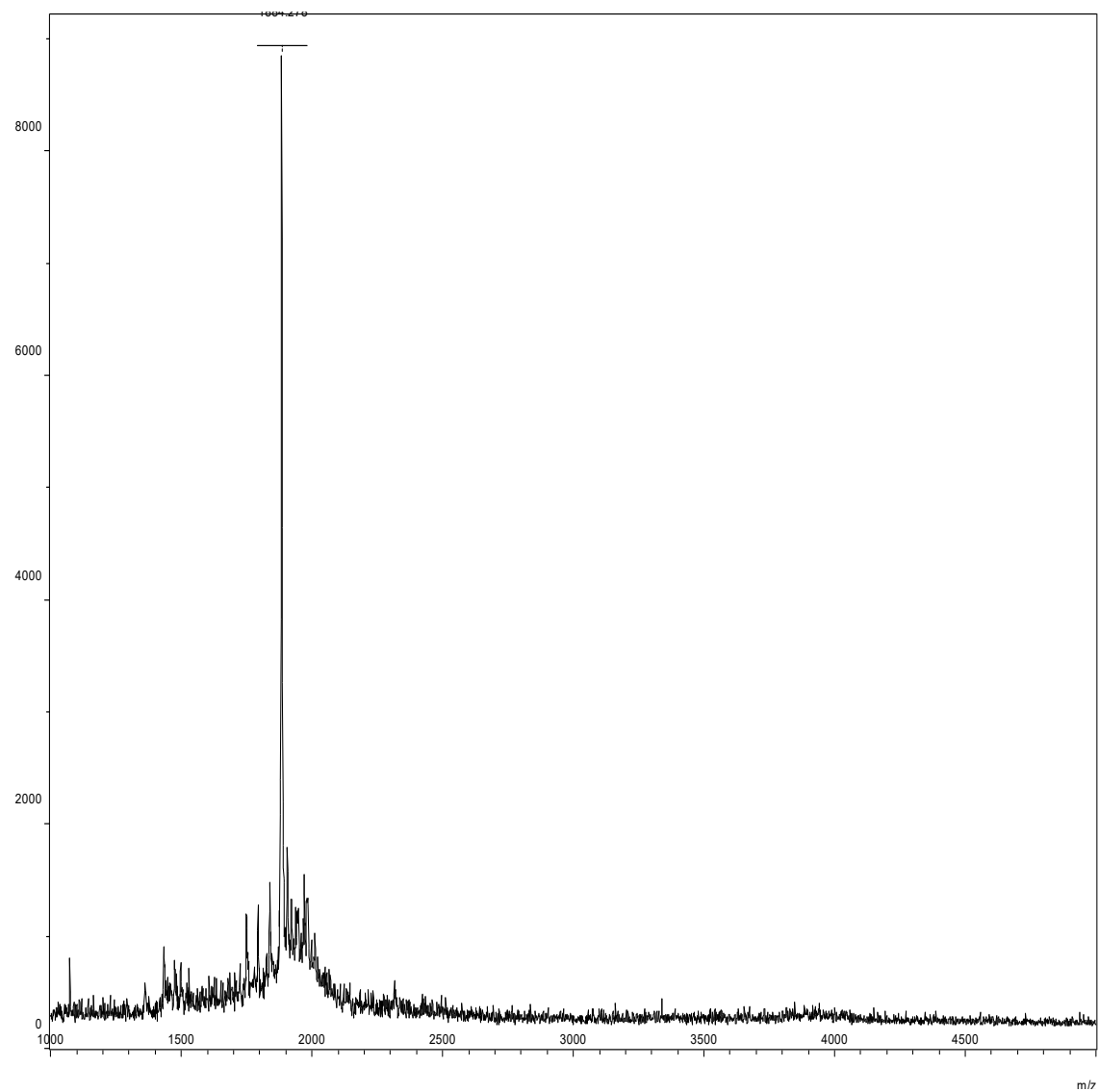
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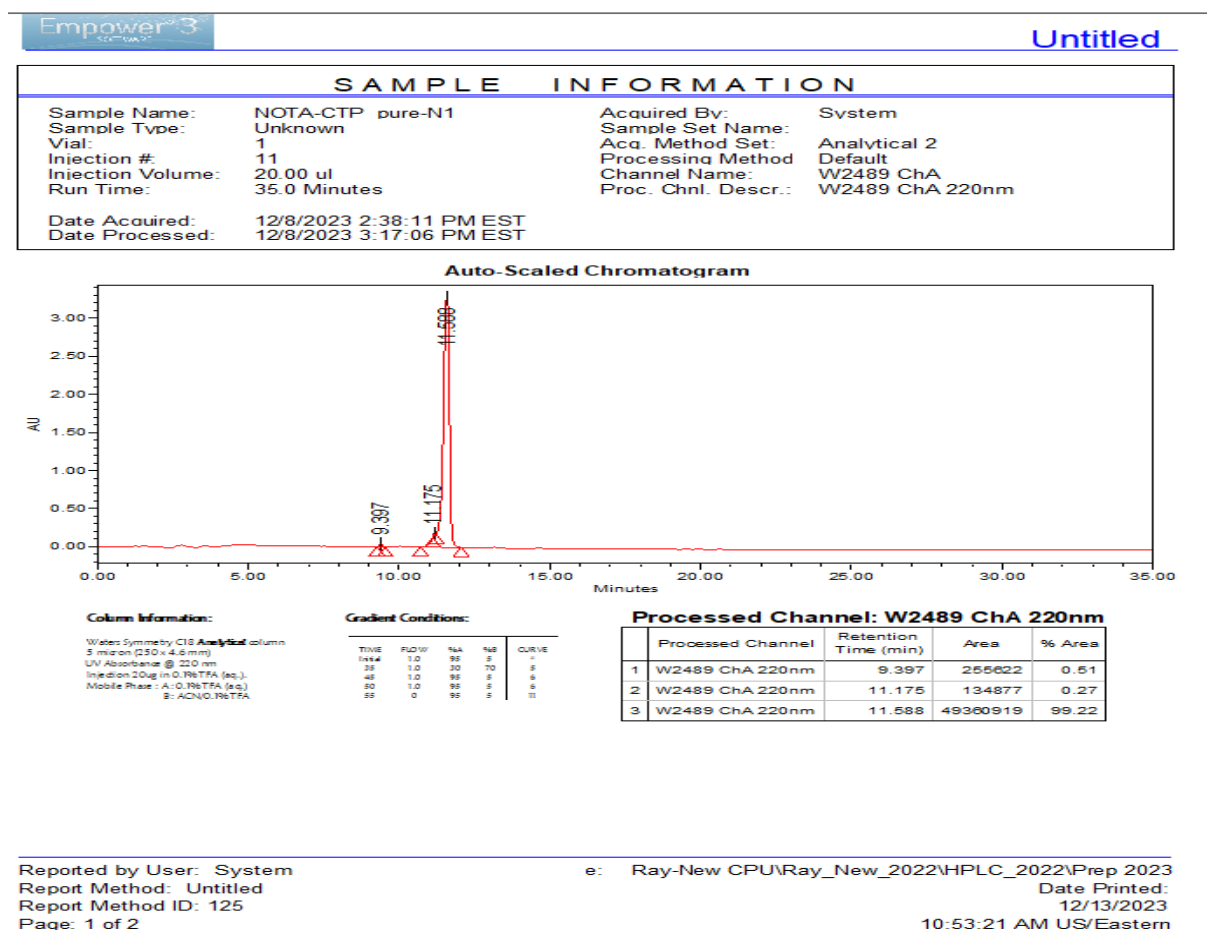
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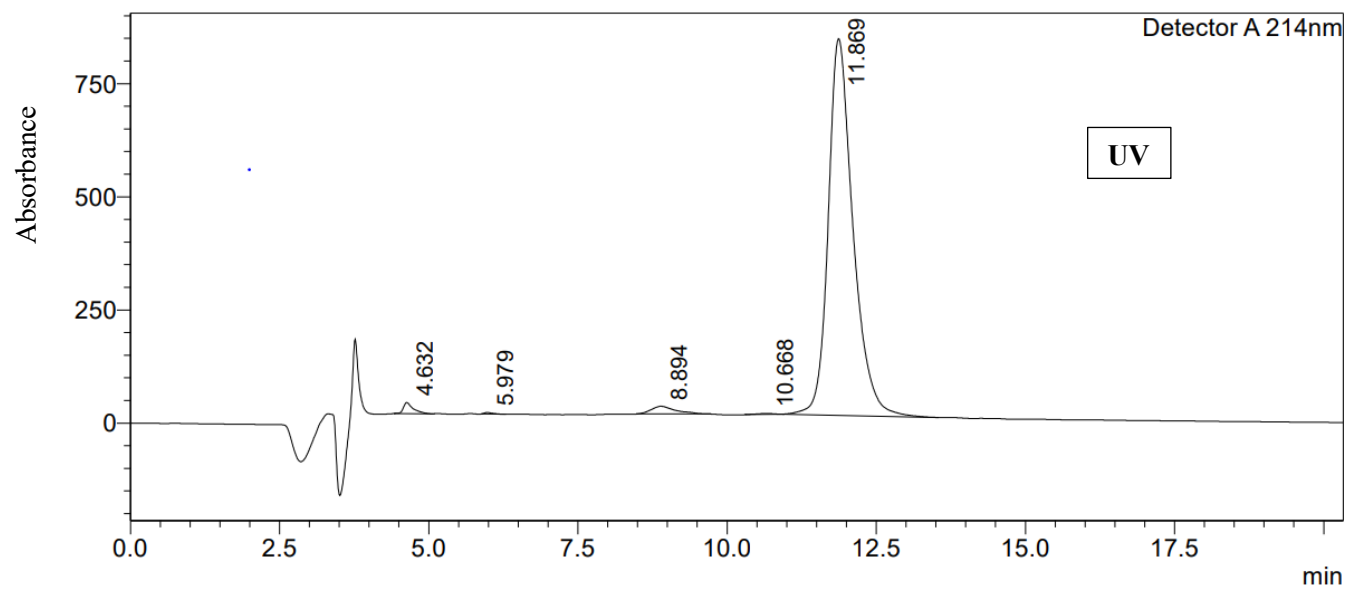
Supplementary Figure S1 Mass Spectrum (MALDI-TOF) of NOTA-CTP provided by Peptide & Peptoid Synthesis Facility, University of Pittsburgh.



Supplementary Figure S2: Representative HPLC UV trace of NOTA-CTP provided by Peptide & Peptoid Synthesis Facility, University of Pittsburgh.

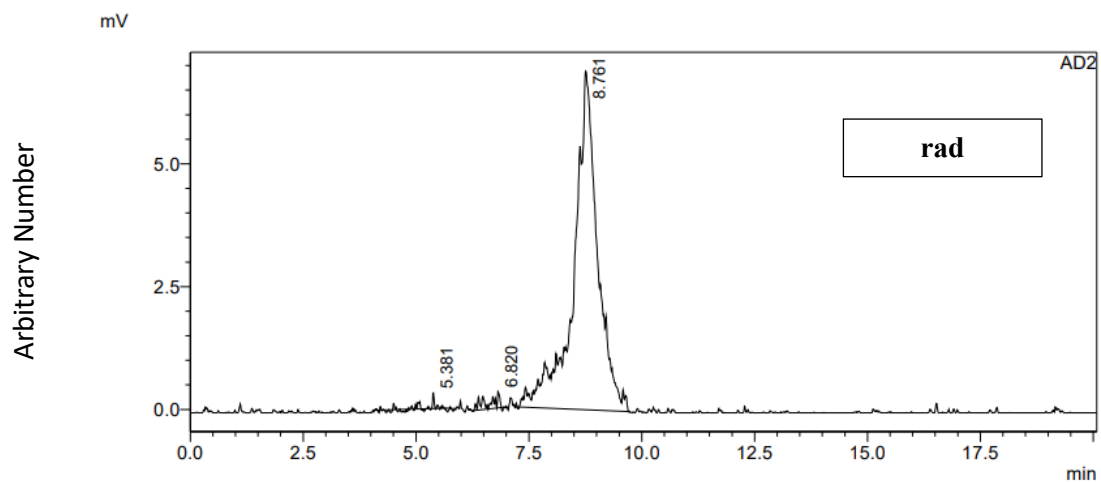
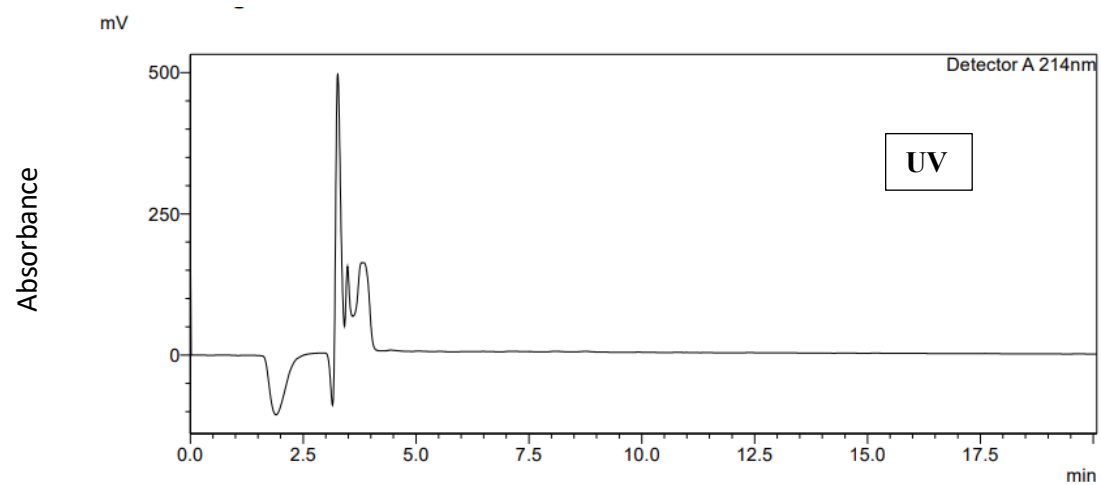


Supplementary Figure S3: Representative HPLC UV trace of NOTA-CTP



Peak#	Name	Ret. Time	Area	Height	Area%
1		4.632	276306	24326	1.109
2		5.979	32961	3420	0.132
3		8.894	491073	17097	1.971
4		10.668	38664	2069	0.155
5		11.869	24073610	832100	96.632
Total			24912615	879012	100.000

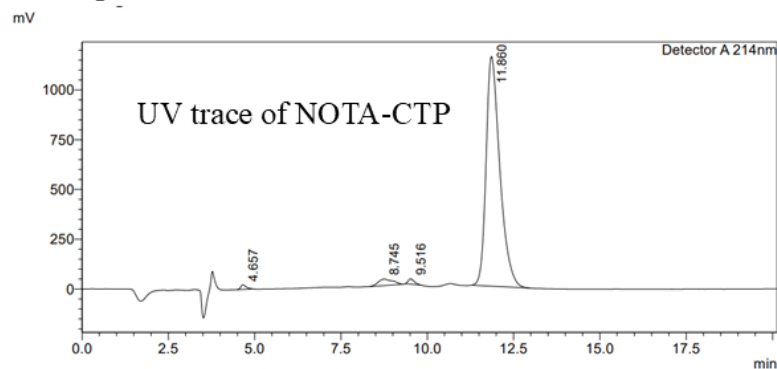
Supplementary Figure S4: Representative rad-HPLC of [^{68}Ga]Ga-NOTA-CTP after HPLC semipreparative purification (r.t.=8.76 min)



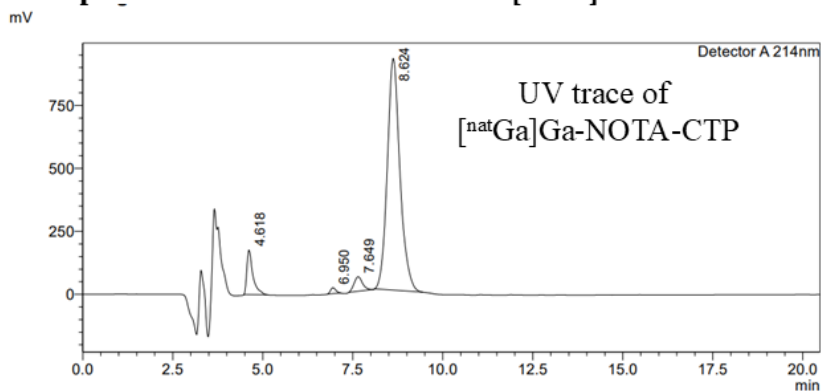
AD2					
Peak#	Name	Ret. Time	Area	Height	Area%
1		5.381	923	316	0.356
2		6.820	4676	335	1.804
3		8.761	253575	6888	97.840
Total			259174	7539	100.000

Supplementary Figure S5: Compilation of HPLC Chromatograms depicting the difference in the retention time between the non-labelled NOTA-CTP peptide and the labelled NOTA-CTP peptide **A:** Representative HPLC UV trace of NOTA-CTP with a retention time of 11.86 min. **B:** Representative HPLC UV trace of [^{nat}Ga]Ga-NOTA-CTP with a retention time of 8.62 min. **C:** Representative rad-HPLC of [^{68}Ga]Ga-NOTA-CTP after HPLC semipreparative purification (r.t.=8.76 min)

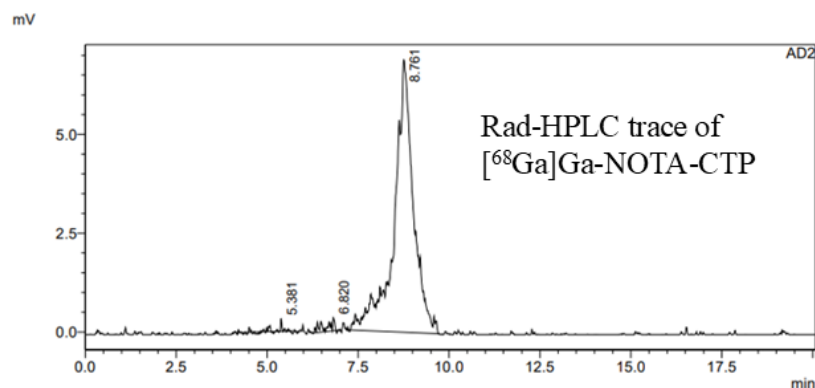
A: Representative HPLC UV trace of NOTA-CTP



B: Representative HPLC UV trace of [^{nat}Ga]Ga-NOTA-CTP



C: Representative rad-HPLC of [^{68}Ga]Ga-NOTA-CTP after HPLC semipreparative purification

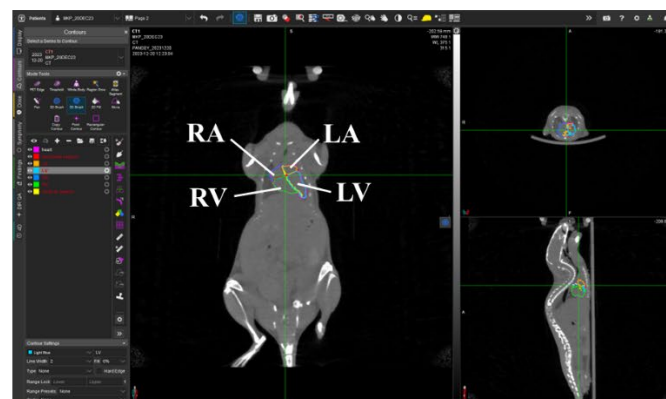


Supplementary Figure S6 Graphical representation of the region of interest (ROI) drawn in the heart to delineate the whole heart, atrium, and ventricles on representative coronal, sagittal, and transverse CT images of CD1 mouse.

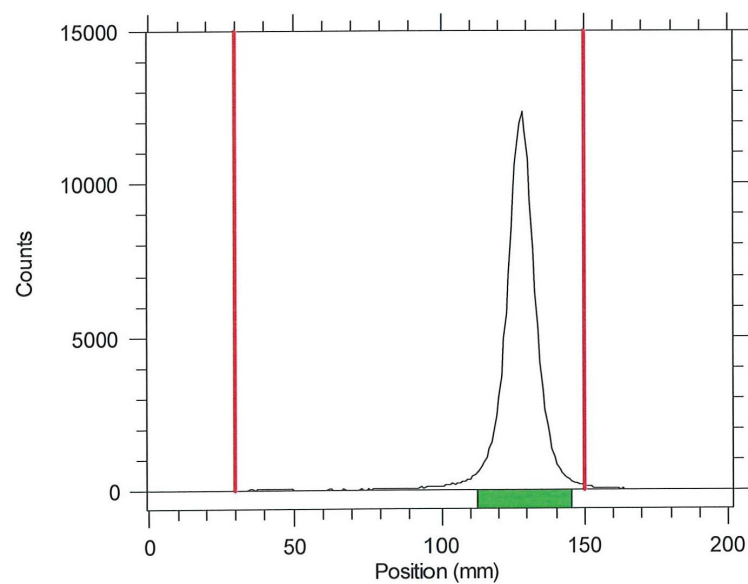
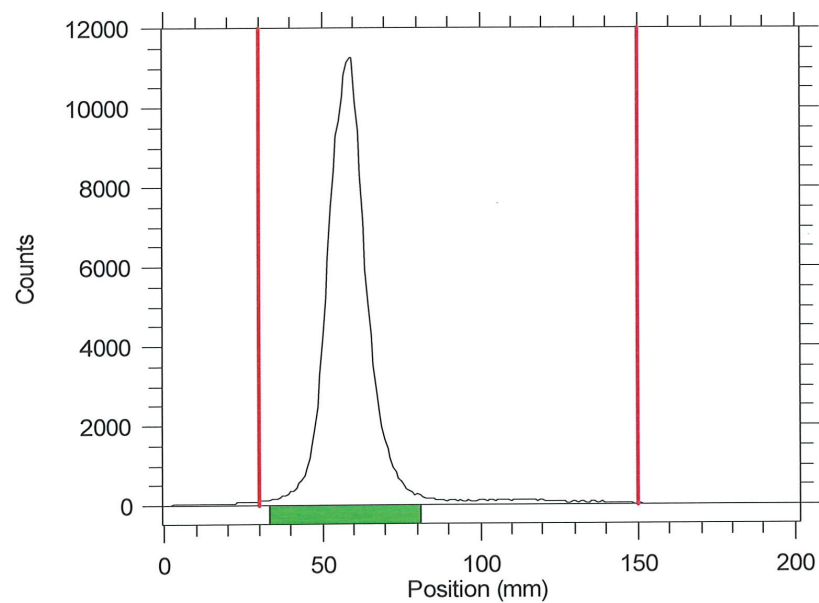
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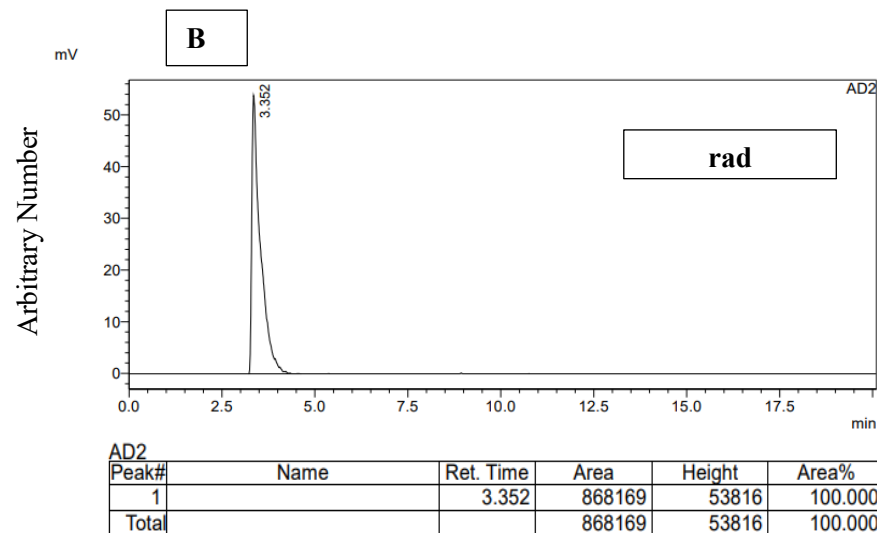
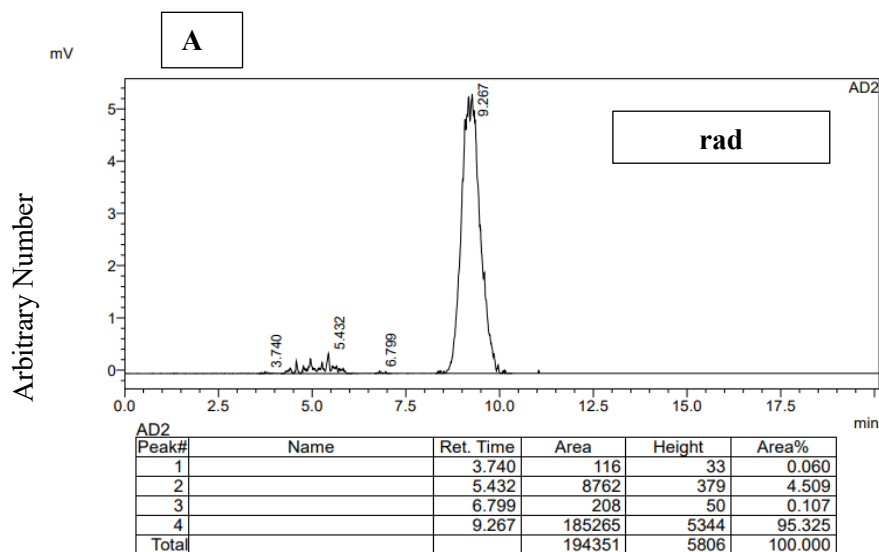
B



Supplementary Figure S7: Representative rad-TLCs of [^{68}Ga]Ga-NOTA-CTP and [^{68}Ga]GaCl₃



Supplementary Figure S8: Representative rad-HPLC of the $[^{68}\text{Ga}]\text{Ga-NOTA-CTP}$ stability analysis in the final formulation. A) rad-HPLC chromatogram of the $[^{68}\text{Ga}]\text{Ga-NOTA-CTP}$ at 60 min post formulation showing the formation of degradation products with retention time between 4-6 minutes. B) rad-HPLC chromatogram of the $[^{68}\text{Ga}]\text{GaCl}_3$ solution showing a retention time of 3.35 minutes.



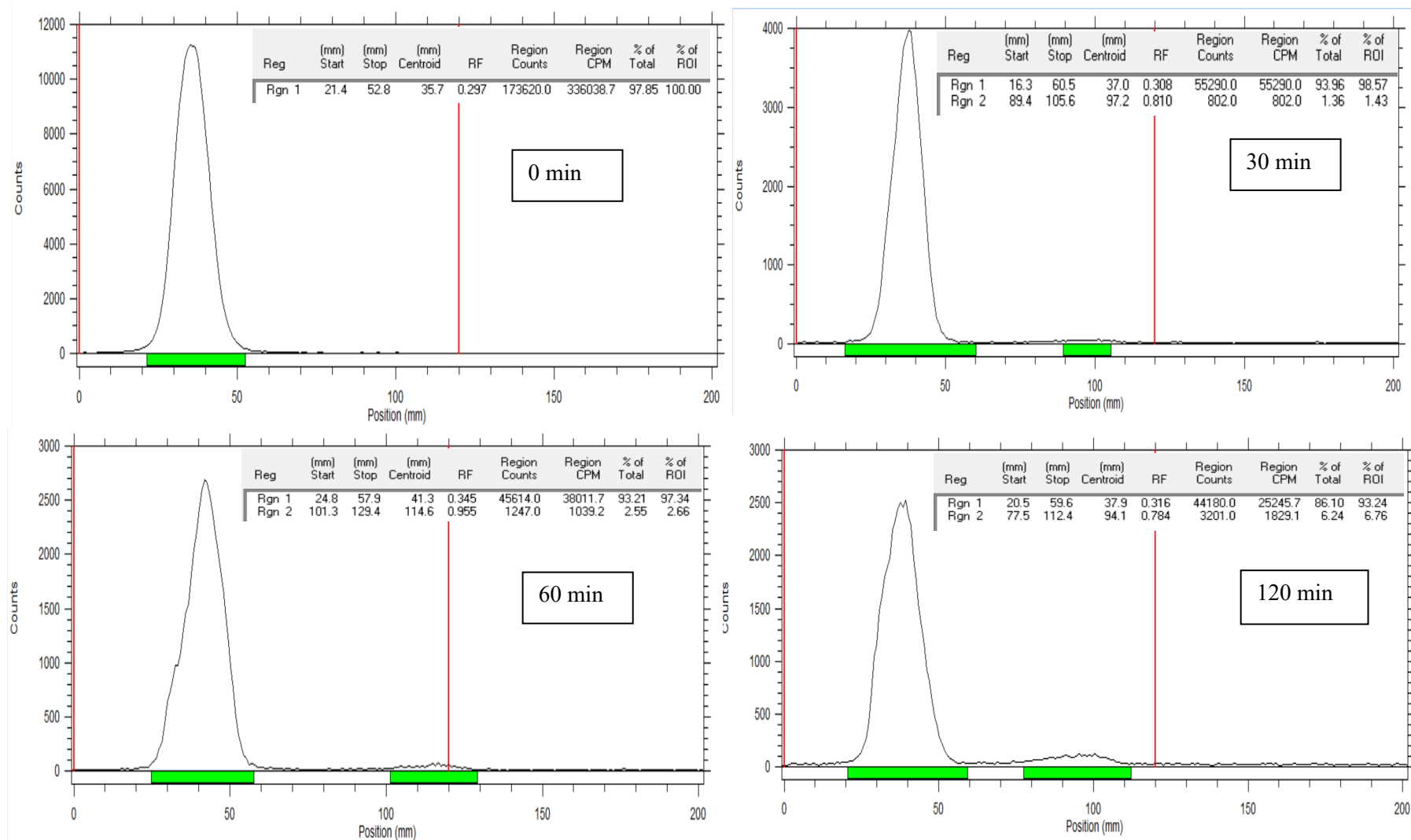
Supplementary Table S1: Stability studies of [⁶⁸Ga]Ga-NOTA-CTP in final formulation solution over time.

Time(min)	Stability in formulation				Mean ± SD
0	99.36	100	97.90	97.84	98.78 ± 1.08
20	99.71	-	96.90	94.43	97.01 ± 2.64
40	-	99.70	96	92.80	96.17 ± 3.45
60	98.83	99.88	95.32	92.62	96.66 ± 3.33
80	-	99.31	92.90	92.21	94.81 ± 3.92
100	99.96	98.86	90.50	-	96.44 ± 5.17
120	-	96.50	89.31	91.75	92.52 ± 3.66

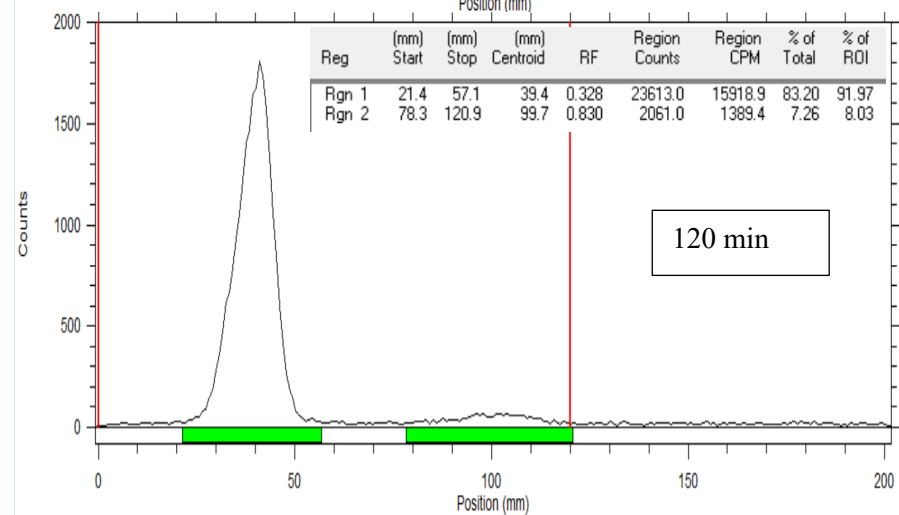
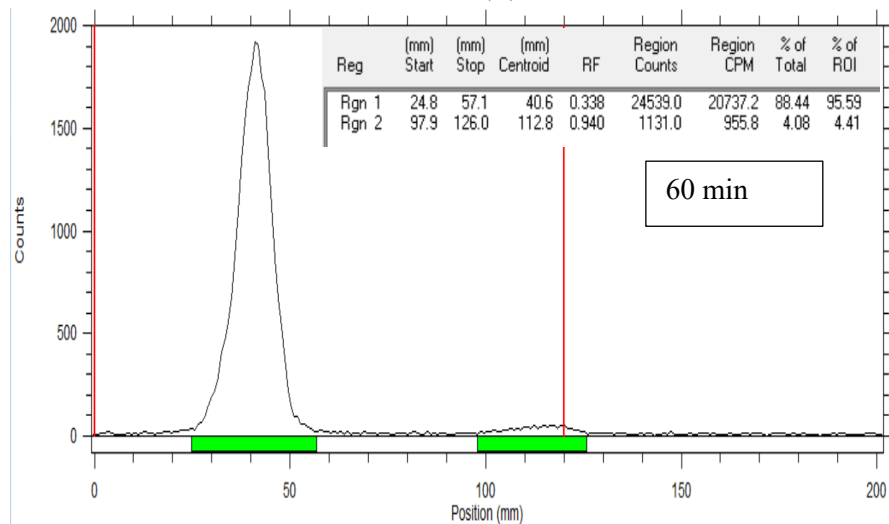
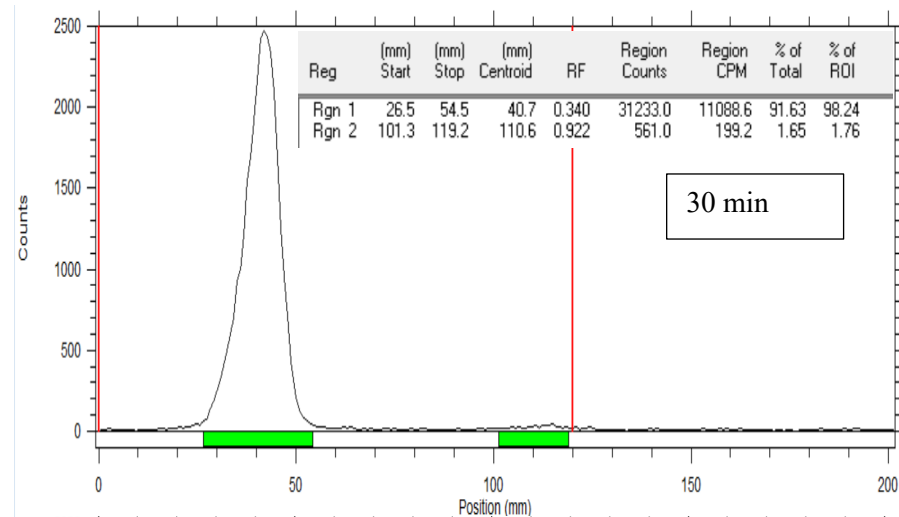
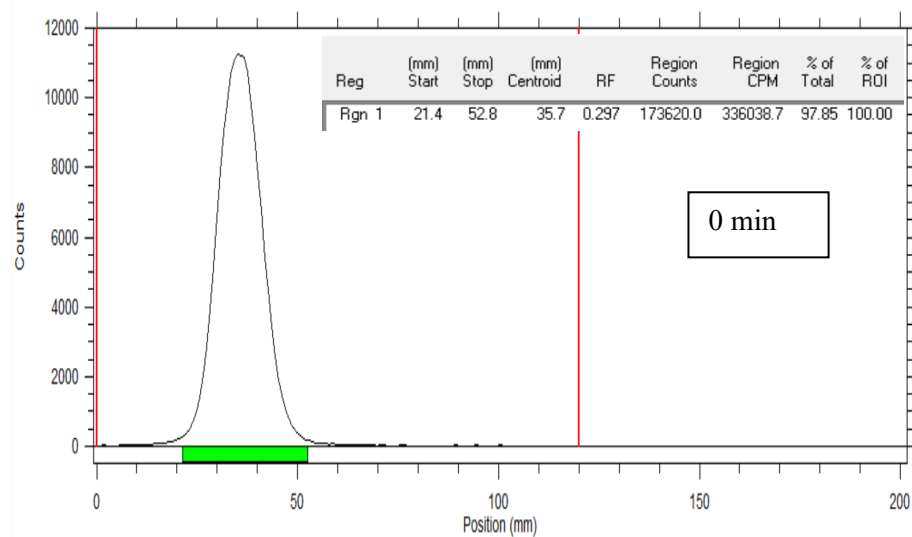
Supplementary Table S2: Stability studies of [⁶⁸Ga]Ga-NOTA-CTP in mouse and human sera over time.

Time(min)	Human serum				Mean ± SD	Mice serum						Mean ± SD
0	98.80	100	100	100	99.70 ± 0.52	98.80	100	100	100	100	98.37	99.53 ± 0.68
30	98.96	98.57	95.91	99.36	98.20 ± 1.35	98.36	98.70	98.24	99.41	99.20	93.48	97.90 ± 2.02
60	98.00	97.34	98.16	98.32	97.96 ± 0.37	96.48	97.48	95.59	98.04	98.01	92.40	96.33 ± 1.96
120	93.57	93.24	93.46	95.66	93.98 ± 0.98	92.33	93.53	91.97	95.23	96.17	92.08	93.55 ± 1.62

Supplementary Figure S9: Representative rad-TLCs of [^{68}Ga]Ga-NOTA-CTP stability studies in human serum over time.



Supplementary Figure S10: Representative rad-TLCs of [^{68}Ga]Ga-NOTA-CTP stability studies in mouse serum over time



Supplementary Table S3A: Uptake of [^{68}Ga]Ga-NOTA-CTP in whole heart and ventricles in healthy and infarcted CD1 mice, assessed by PET/CT imaging. LV = left ventricle and RV = right ventricle

Time point (min)	Healthy Heart			Infarcted Heart		
	SUV Heart (Average $\pm$ SD, n=5)	SUV LV (Average $\pm$ SD, n=5)	SUV RV (Average $\pm$ SD, n=5)	SUV Heart (Average $\pm$ SD, n=5)	SUV LV (Average $\pm$ SD, n=5)	SUV RV (Average $\pm$ SD, n=5)
1	1.21 $\pm$ 0.27	1.17 $\pm$ 0.21	1.22 $\pm$ 0.23	1.38 $\pm$ 0.58	1.45 $\pm$ 0.65	1.41 $\pm$ 0.61
2	1.08 $\pm$ 0.26	1.05 $\pm$ 0.20	1.09 $\pm$ 0.19	1.26 $\pm$ 0.57	1.32 $\pm$ 0.64	1.29 $\pm$ 0.60
3	1.00 $\pm$ 0.28	0.93 $\pm$ 0.20	0.99 $\pm$ 0.19	1.16 $\pm$ 0.57	1.19 $\pm$ 0.62	1.21 $\pm$ 0.62
4	0.92 $\pm$ 0.26	0.83 $\pm$ 0.13	0.91 $\pm$ 0.14	1.08 $\pm$ 0.57	1.11 $\pm$ 0.62	1.14 $\pm$ 0.61
5	0.86 $\pm$ 0.28	0.76 $\pm$ 0.12	0.84 $\pm$ 0.13	1.01 $\pm$ 0.55	1.02 $\pm$ 0.59	1.07 $\pm$ 0.58
6	0.78 $\pm$ 0.25	0.69 $\pm$ 0.08	0.78 $\pm$ 0.12	0.96 $\pm$ 0.55	0.98 $\pm$ 0.58	1.05 $\pm$ 0.60
7	0.74 $\pm$ 0.25	0.66 $\pm$ 0.09	0.74 $\pm$ 0.08	0.91 $\pm$ 0.55	0.92 $\pm$ 0.56	1.01 $\pm$ 0.61
8	0.69 $\pm$ 0.24	0.59 $\pm$ 0.10	0.70 $\pm$ 0.06	0.86 $\pm$ 0.53	0.86 $\pm$ 0.53	0.96 $\pm$ 0.60
9	0.65 $\pm$ 0.22	0.58 $\pm$ 0.09	0.65 $\pm$ 0.09	0.83 $\pm$ 0.50	0.83 $\pm$ 0.50	0.92 $\pm$ 0.56
10	0.62 $\pm$ 0.21	0.55 $\pm$ 0.08*	0.65 $\pm$ 0.07	0.80 $\pm$ 0.50	0.80 $\pm$ 0.51	0.90 $\pm$ 0.55
15	0.55 $\pm$ 0.22	0.47 $\pm$ 0.05*	0.58 $\pm$ 0.04	0.67 $\pm$ 0.47	0.66 $\pm$ 0.45	0.77 $\pm$ 0.54
20	0.48 $\pm$ 0.23	0.40 $\pm$ 0.05*	0.51 $\pm$ 0.05	0.55 $\pm$ 0.39	0.54 $\pm$ 0.37	0.65 $\pm$ 0.46
25	0.43 $\pm$ 0.23	0.34 $\pm$ 0.05*	0.46 $\pm$ 0.06	0.48 $\pm$ 0.32	0.46 $\pm$ 0.29	0.58 $\pm$ 0.39
30	0.40 $\pm$ 0.22	0.31 $\pm$ 0.05*	0.43 $\pm$ 0.06	0.44 $\pm$ 0.27	0.41 $\pm$ 0.24	0.54 $\pm$ 0.35

*P value < 0.05 LV vs RV in healthy heart

Supplementary Table S3B: Uptake of [^{68}Ga]Ga-NOTA-CTP in left and right ventricles normalized to whole heart in healthy and infarcted CD1 mice, assessed by PET/CT imaging. LV/H= left ventricle/whole heart and RV/H = right ventricle/whole heart.

	Healthy mice		Infarcted mice	
Time point (min)	SUV Ratio LV/Whole Heart (Average $\pm$ SD, n=5)	SUV Ratio RV/Whole Heart (Average $\pm$ SD, n=5)	SUV Ratio LV/Whole Heart (Average $\pm$ SD, n=5)	SUV Ratio RV/Whole Heart (Average $\pm$ SD, n=5)
1	1.00 $\pm$ 0.12	1.03 $\pm$ 0.07	1.01 $\pm$ 0.02	1.02 $\pm$ 0.06
2	1.01 $\pm$ 0.12	1.05 $\pm$ 0.07	1.01 $\pm$ 0.01	1.02 $\pm$ 0.06
3	0.98 $\pm$ 0.10	1.06 $\pm$ 0.07	0.99 $\pm$ 0.02	1.03 $\pm$ 0.05
4	0.98 $\pm$ 0.10*	1.08 $\pm$ 0.07	0.99 $\pm$ 0.03 [#]	1.05 $\pm$ 0.03
5	0.97 $\pm$ 0.10*	1.07 $\pm$ 0.07	0.98 $\pm$ 0.02 [#]	1.07 $\pm$ 0.04
6	0.98 $\pm$ 0.09*	1.10 $\pm$ 0.07	1.00 $\pm$ 0.03 [#]	1.09 $\pm$ 0.04
7	1.00 $\pm$ 0.09*	1.12 $\pm$ 0.07	1.01 $\pm$ 0.02 [#]	1.10 $\pm$ 0.04
8	0.97 $\pm$ 0.06*	1.15 $\pm$ 0.10	1.00 $\pm$ 0.01 [#]	1.11 $\pm$ 0.02
9	1.01 $\pm$ 0.08*	1.12 $\pm$ 0.04	1.00 $\pm$ 0.04 [#]	1.11 $\pm$ 0.05
10	1.01 $\pm$ 0.08*	1.19 $\pm$ 0.07	1.01 $\pm$ 0.02 [#]	1.13 $\pm$ 0.06
15	0.99 $\pm$ 0.06*	1.23 $\pm$ 0.09	1.04 $\pm$ 0.06 [#]	1.14 $\pm$ 0.04
20	1.01 $\pm$ 0.06*	1.30 $\pm$ 0.13	1.04 $\pm$ 0.06 [#]	1.16 $\pm$ 0.08 [†]
25	1.01 $\pm$ 0.05*	1.36 $\pm$ 0.16	1.05 $\pm$ 0.10 [#]	1.20 $\pm$ 0.11
30	1.00 $\pm$ 0.04*	1.39 $\pm$ 0.17	1.04 $\pm$ 0.07 [#]	1.22 $\pm$ 0.14

*P value < 0.05 LV/H vs RV/H in healthy heart; [#]P value < 0.05 LV/H vs RV/H in infarcted heart; [†]P value < 0.05 RV/H in healthy heart vs RV/H in infarcted heart.