Electronic Supplementary Information (ESI) for

Chelation of [^{111}In]In^{3+} with the Dual-Size-Selective Macrocycles Py-macrodipa and Py$_2$-macrodipa

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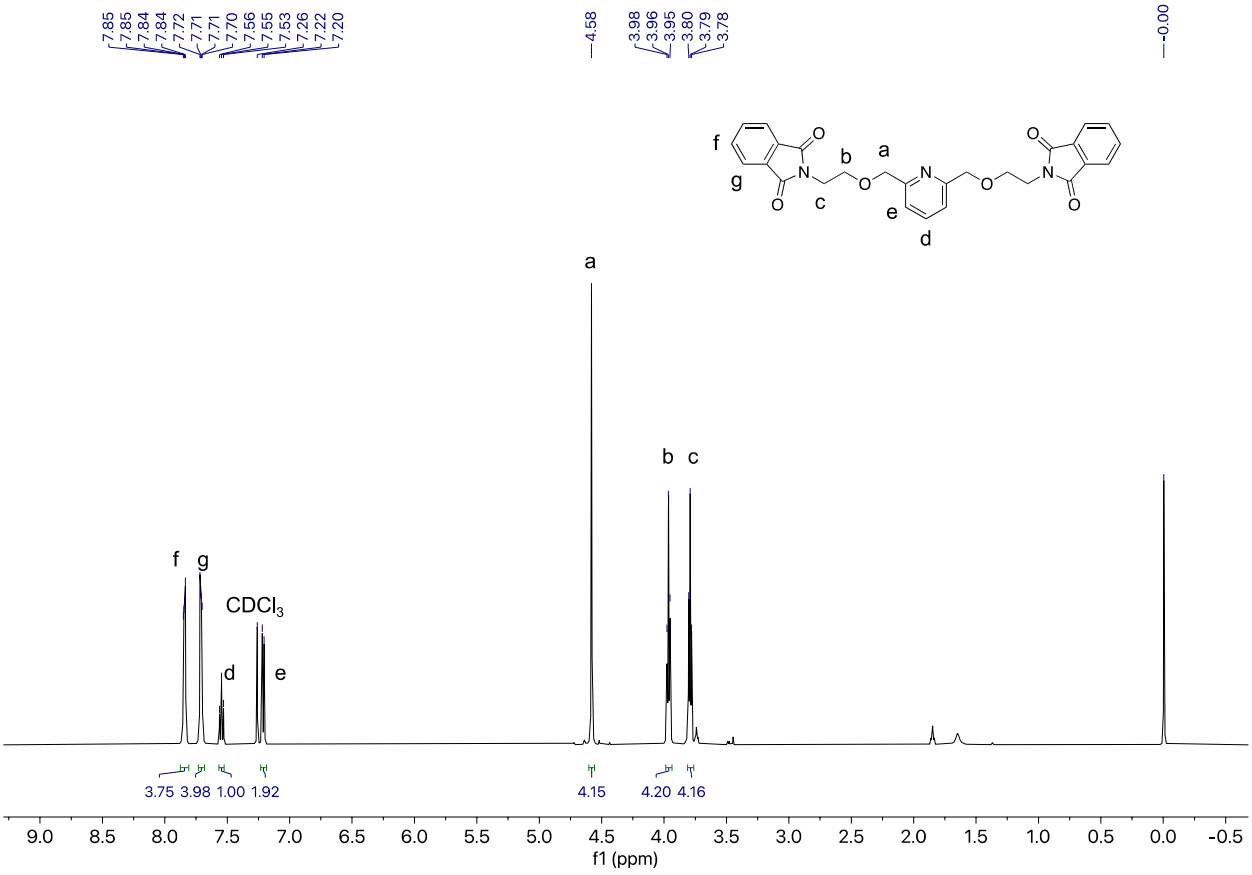

Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) spectrum of **5.**

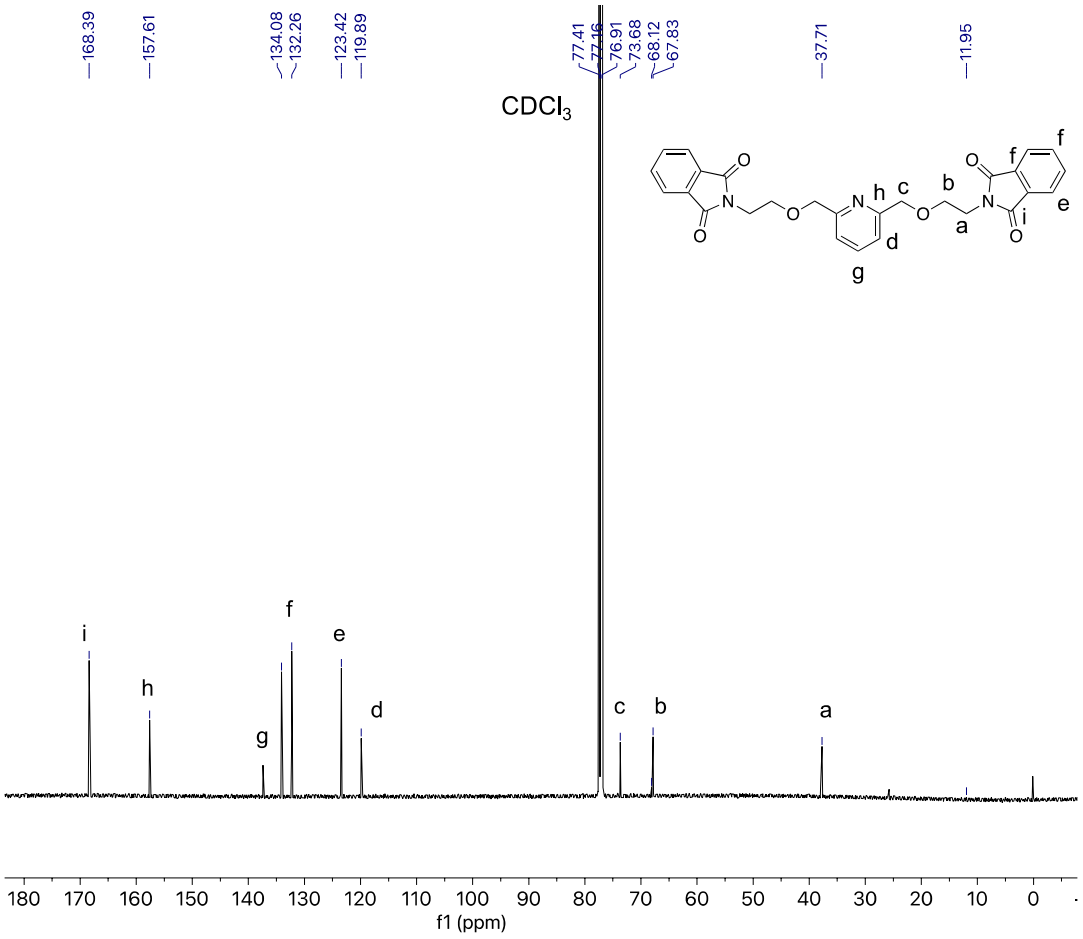

Figure S2. $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$, 25 °C) spectrum of **5.**

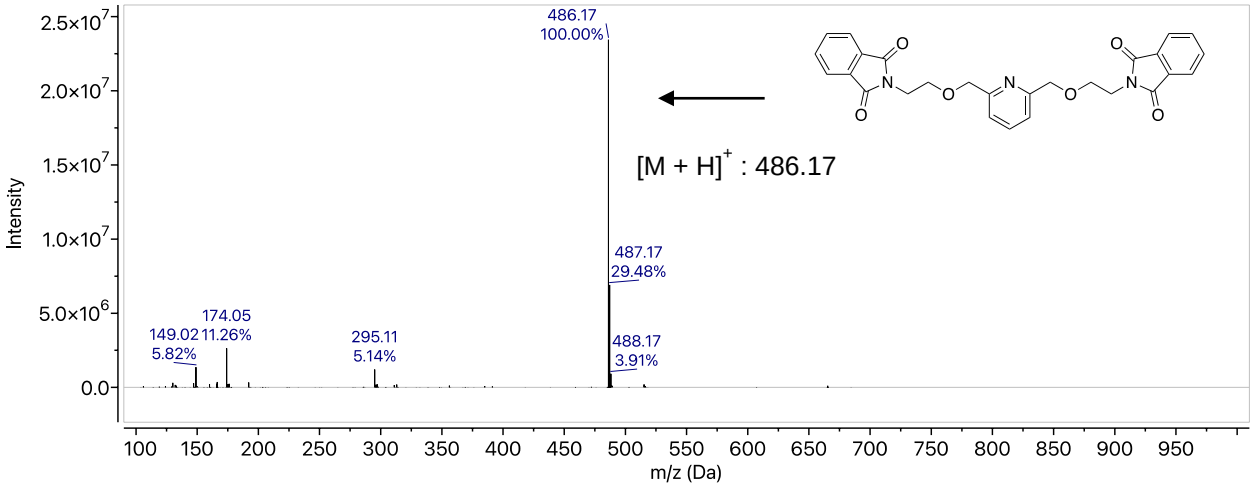

Figure S3. DART-MS of **5**. Chloroform was used as the solvent.

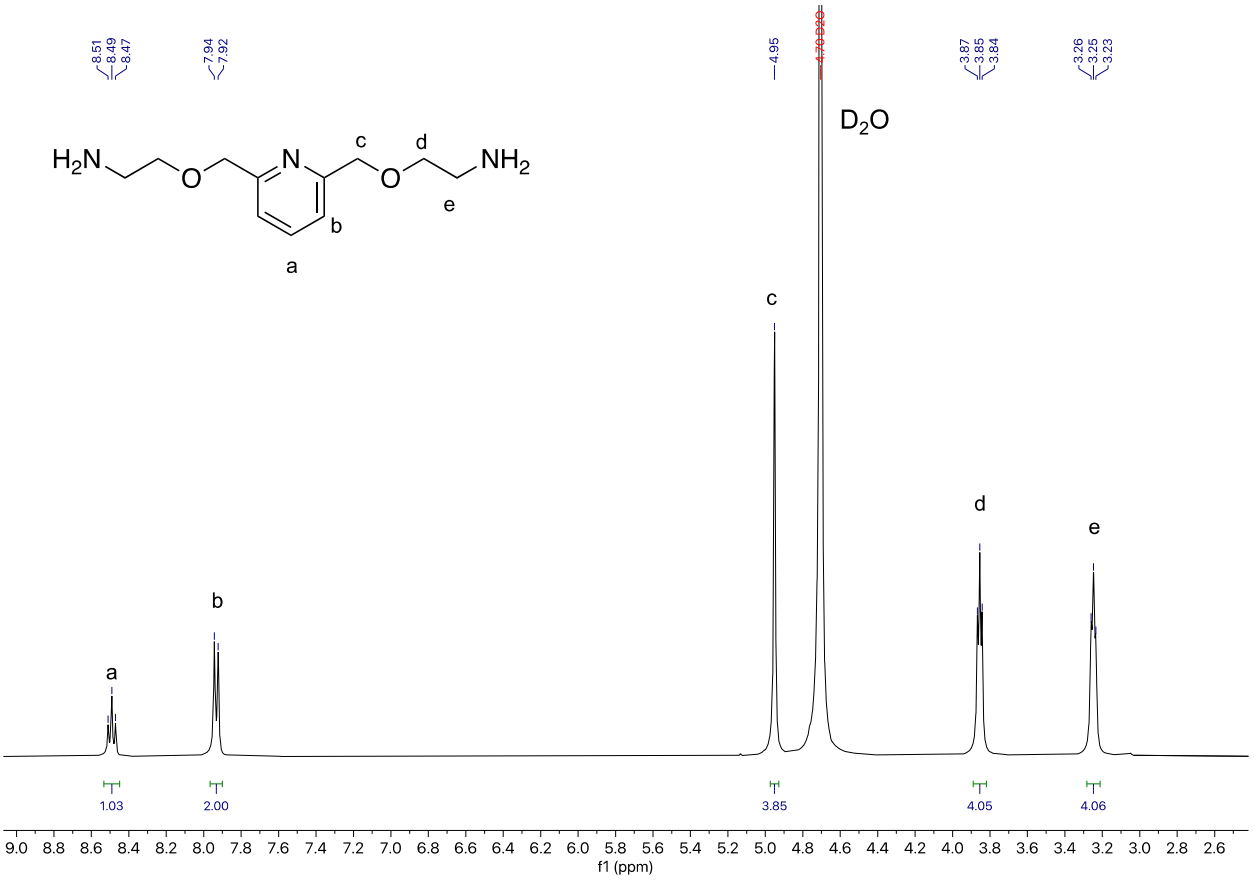

Figure S4. ^{1}H NMR (500 MHz, D_2O, 25 °C) spectrum of **6**.

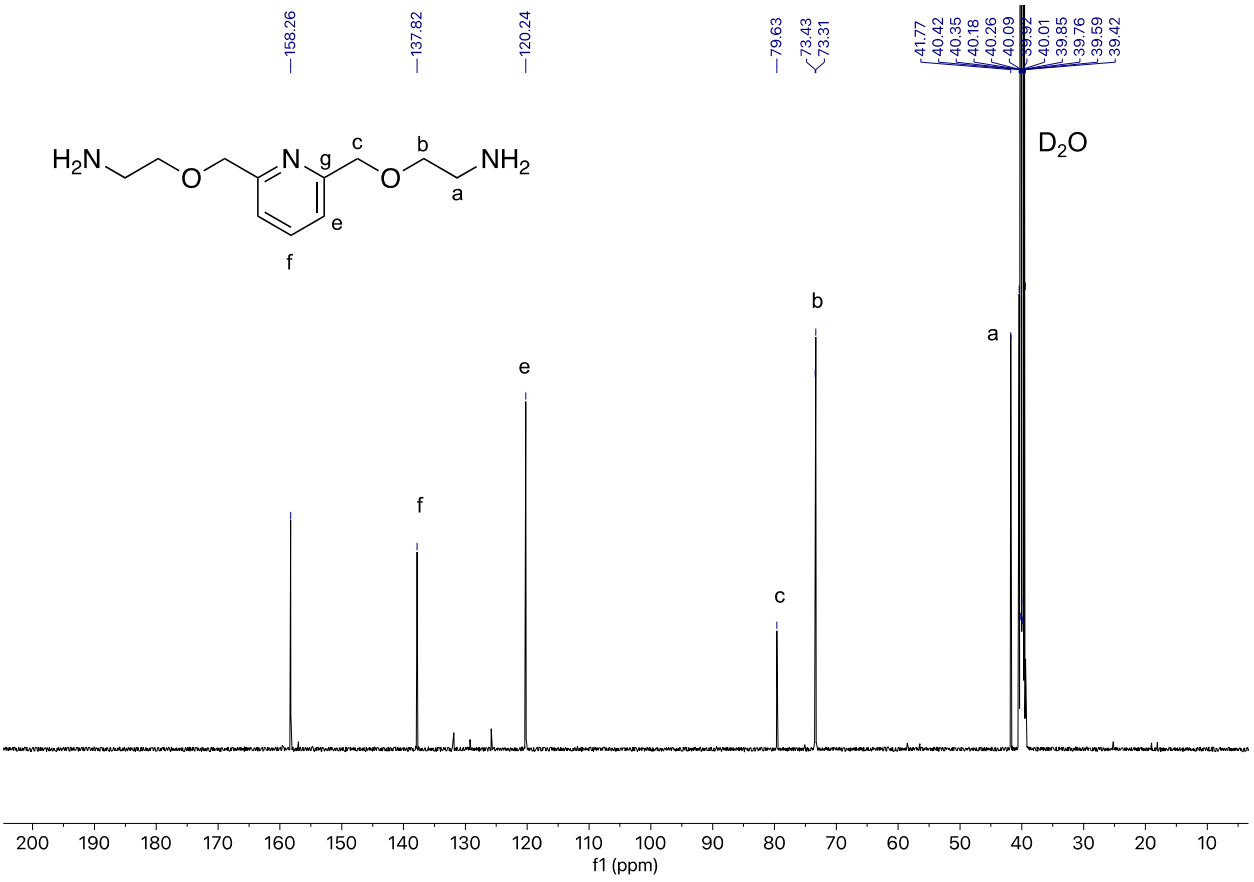

Figure S5. $^{13}C\{^{1}H\}$ NMR (126 MHz, DMSO-d_6, 25 °C) spectrum of **6**.

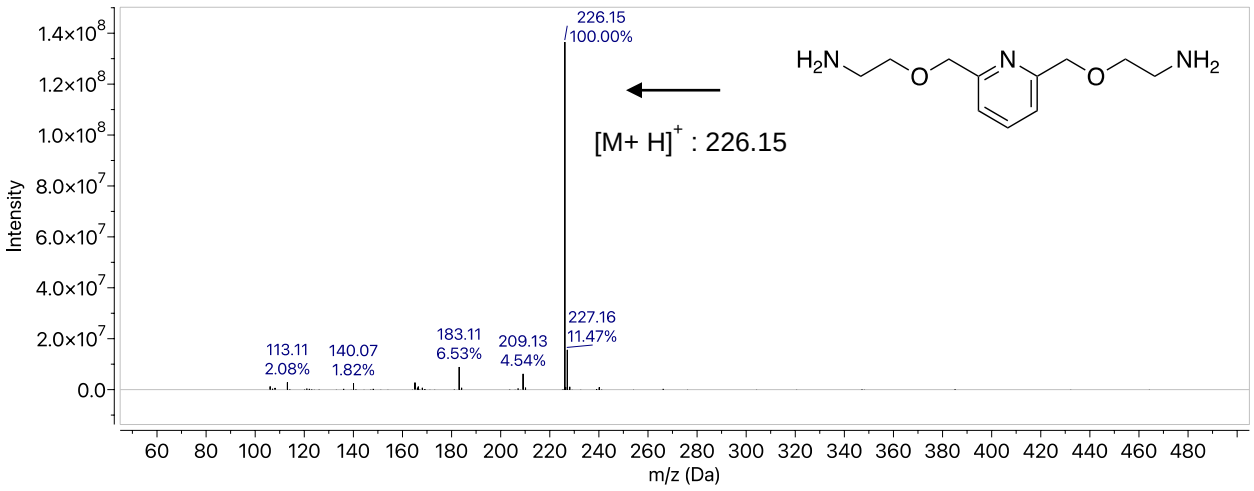

Figure S6. DART-MS of **6**. Water was used as the solvent.

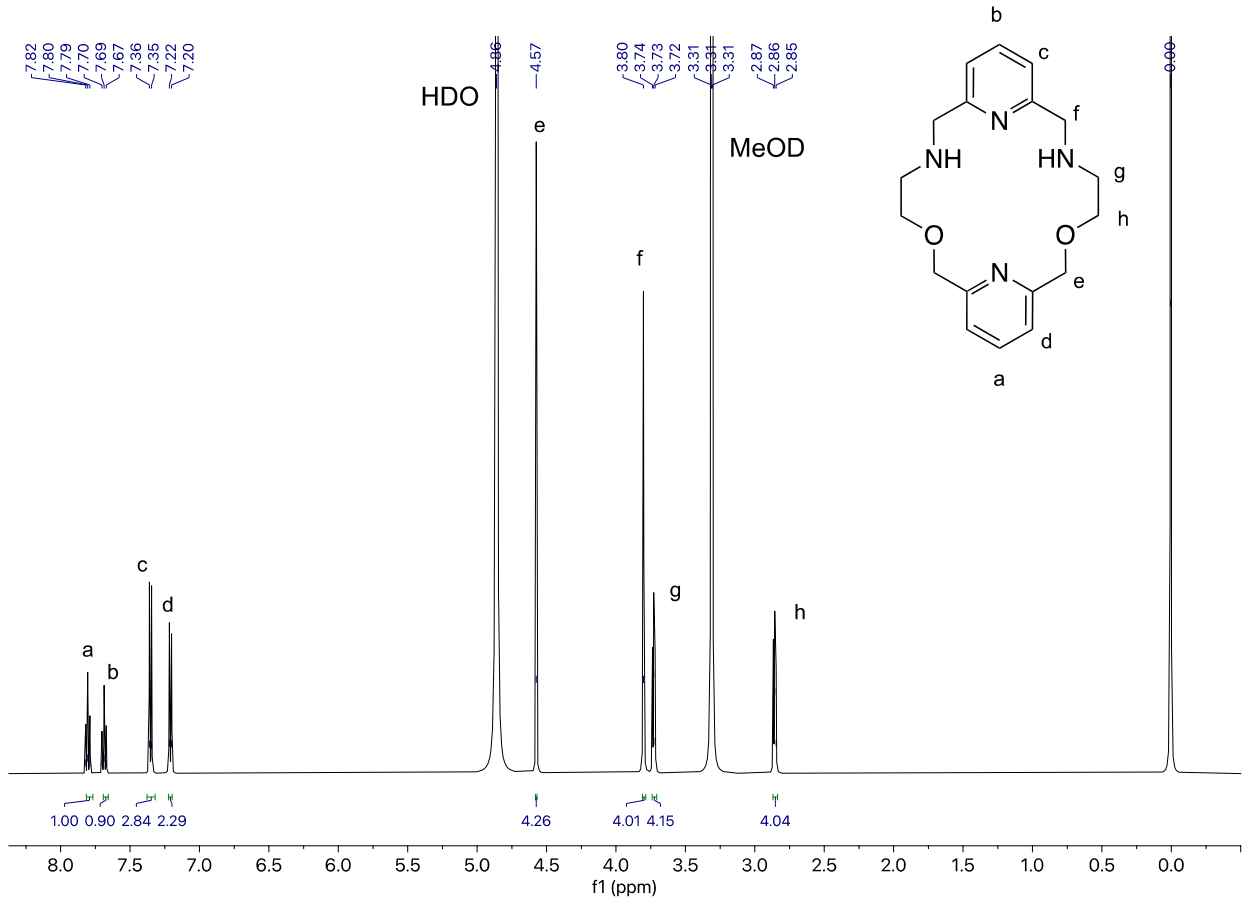

Figure S7. ^{1}H NMR (500 MHz, CD_3OD, 25 °C) spectrum of **4**.

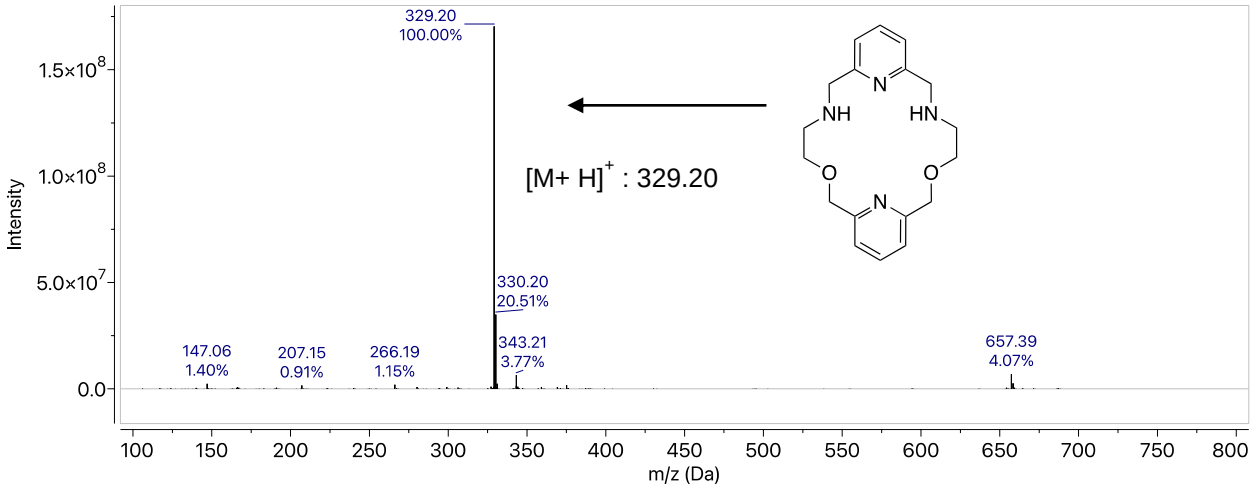

Figure S8. DART-MS of **4**. Methanol was used as the solvent.

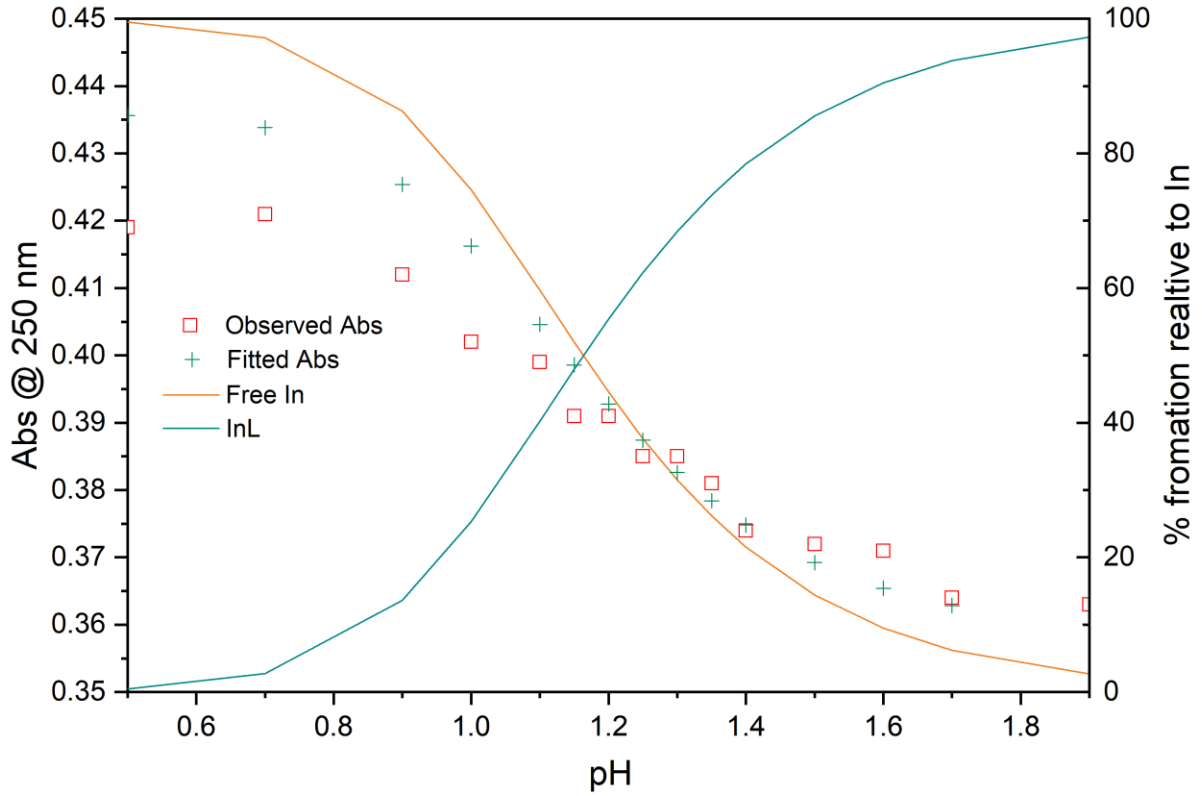

Figure S9. Representative UV–Vis spectrophotometric titration of In^{3+}-py-macrodipa system. $c_{In} = c_{py\text{-}macordipa} = 8 \times 10^{-5}$ M. Data fitting at 250 nm (where the most significant spectral change occurs) and speciation distribution over the titration pH range (0.1 M KCl, 25 °C).

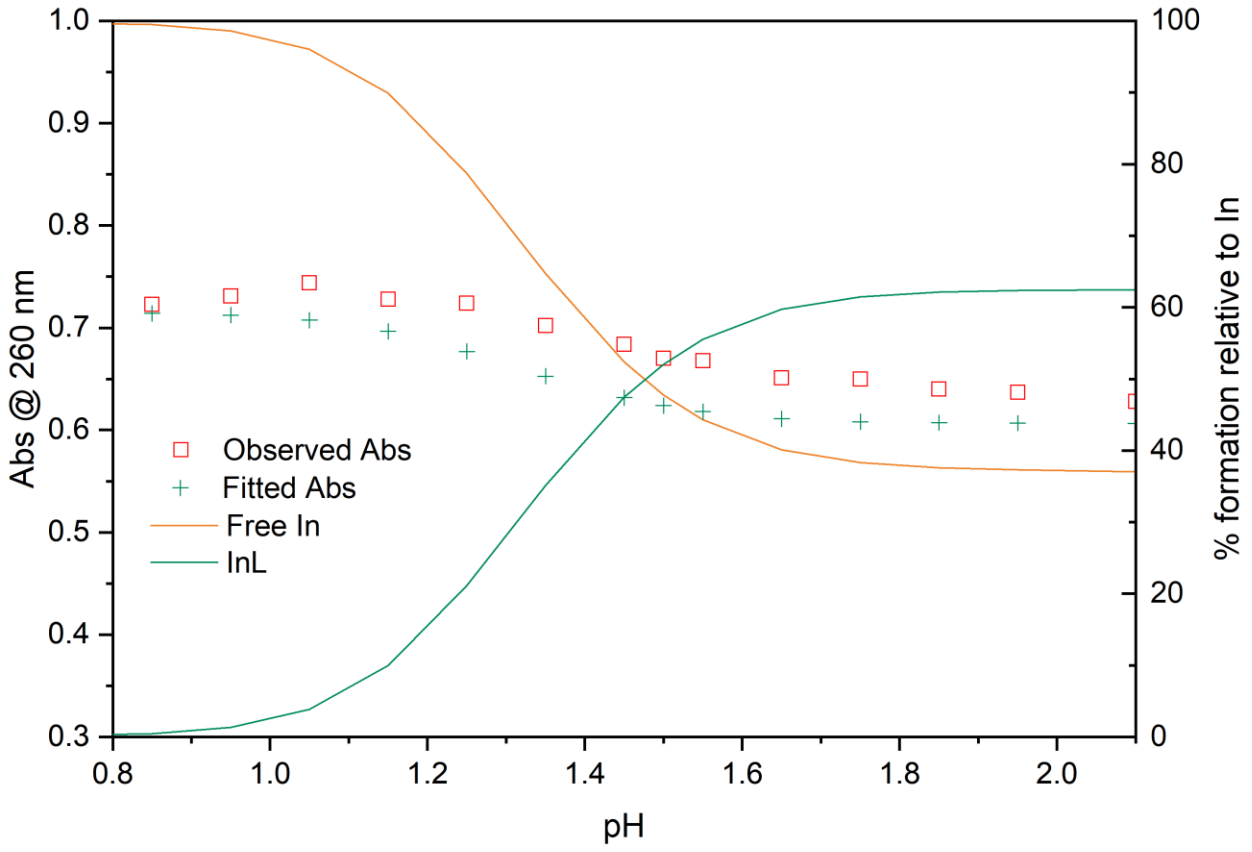

Figure S10. Representative UV–Vis spectrophotometric titration of In^{3+}-py-macrodipa system. $c_{In} = c_{py2\text{-}macordipa} = 5 \times 10^{-5}$ M. Data fitting at 260 nm (where the most significant spectral change occurs) and speciation distribution over the titration pH range (0.1 M KCl, 25 °C).

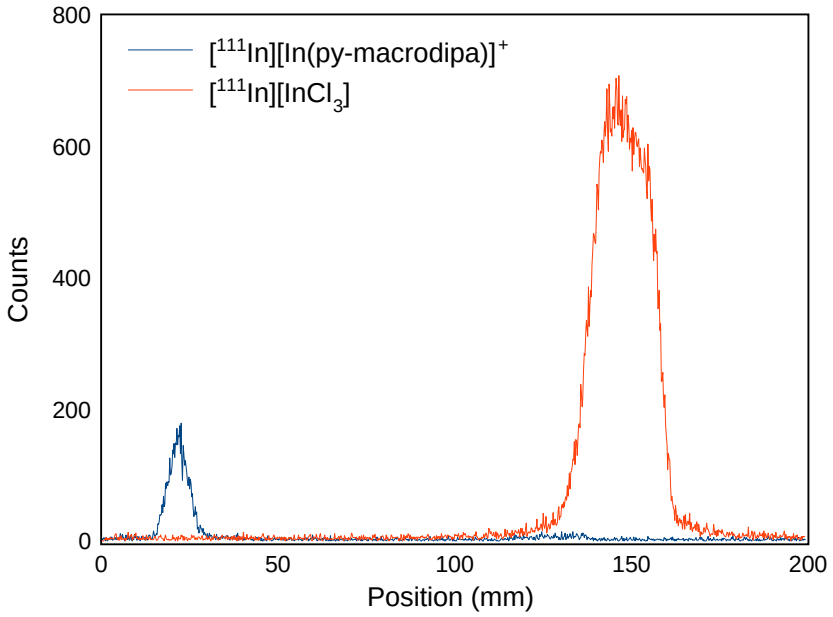

Figure S11. Representative radio-TLC of $[^{111}In][In(py\text{-}macrodipa)]^+$ compared to radio-TLC of $[^{111}In]InCl_3$. Radiolabeling was performed with 100 µM of py-macrodipa at 25 °C for 5 min, iTLC-SG eluted with 50 mM EDTA (pH 5.5).

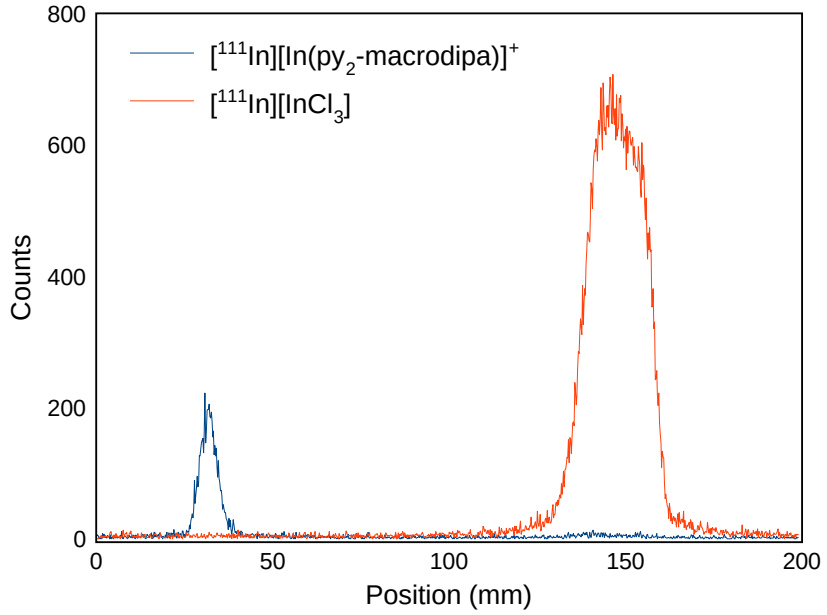

Figure S12. Representative radio-TLC of $[^{111}In][In(py_2\text{-}macrodipa)]^+$ compared to radio-TLC of $[^{111}In]InCl_3$. Radiolabeling was performed with 100 µM of py_2-macrodipa at 25 °C for 5 min, iTLC-SG eluted with 50 mM EDTA (pH 5.5).

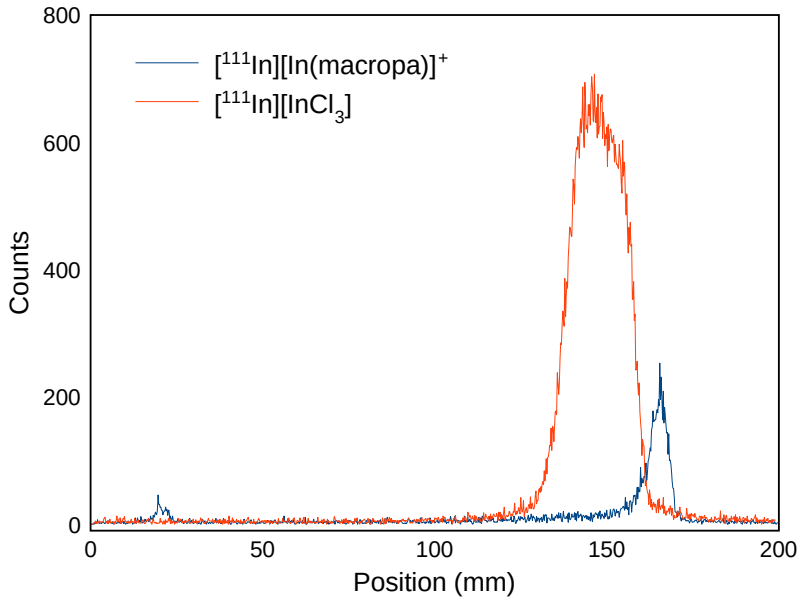

Figure S13. Representative radio-TLC of $[^{111}In][In(macropa)]^+$ compared to radio-TLC of $[^{111}In]InCl_3$. Radiolabeling was performed with 1 mM of macropa at 25 °C for 5 min, iTLC-SG eluted with 50 mM EDTA (pH 5.5).

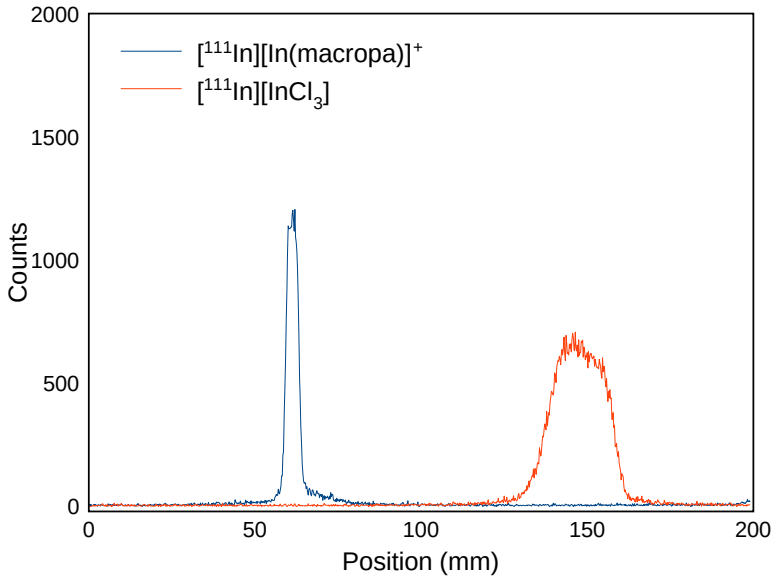

Figure S14. Representative radio-TLC of $[^{111}In][In(macropa)]^+$ compared to radio-TLC of $[^{111}In][InCl_3]$. Radiolabeling was performed with 4 mM of macropa at 25 °C for 60 min, iTLC-SG eluted with 50 mM EDTA (pH 5.5).

Table S1. Radiochemical Conversions of [^{111}In]In^{3+} with Py-macrodipa and Py_2-macrodipa at Different Ligand Concentrations. (pH 5.5 ammonium acetate buffer; 25 °C; 5 min)[a]

	RCC (%)	
Ligand Concentration (M)	**Py-macrodipa**	**Py_2-macrodipa**
1.0×10^{-3}	94.5 ± 1.8	94.6 ± 2.6
1.0×10^{-4}	94.4 ± 2.5	95.4 ± 1.7
1.0×10^{-5}	94.4 ± 2.2	95.5 ± 2.0
1.0×10^{-6}	93.9 ± 2.1	92.9 ± 2.4
1.0×10^{-7}	15.2 ± 1.4	25.3 ± 3.8
1.0×10^{-8}	11.6 ± 0.3	19.0 ± 0.7

[a] Measurements were carried out in triplicate and the error is the standard deviation of these replicates.

Table S2. Stability of [^{111}In][In(py-macrodipa)$^+$ and [^{111}In][In(py_2-marodipa)]$^+$ in Human Serum at 37 °C[a]

	% Complex Intact	
Time (h)	**Py-macrodipa**	**Py_2-macrodipa**
1	98.6 ± 0.2	99.1 ± 0.1
18	96.7 ± 2.1	97.3 ± 0.6
30	94.6 ± 3.5	98.2 ± 1.0
42	96.8 ± 4.6	98.6 ± 0.5
54	95.9 ± 3.4	97.7 ± 1.4
66	91.0 ± 7.8	97.1 ± 0.3
78	95.4 ± 5.9	97.1 ± 1.9
90	92.0 ± 6.4	92.9 ± 2.2
102	95.8 ± 1.2	95.4 ± 3.6
114	96.8 ± 1.0	98.6 ± 1.2
126	97.3 ± 3.5	94.2 ± 3.3
138	94.8 ± 4.1	98.4 ± 1.8

[a] Measurements were carried out in triplicate and the error is the standard deviation of these replicates.

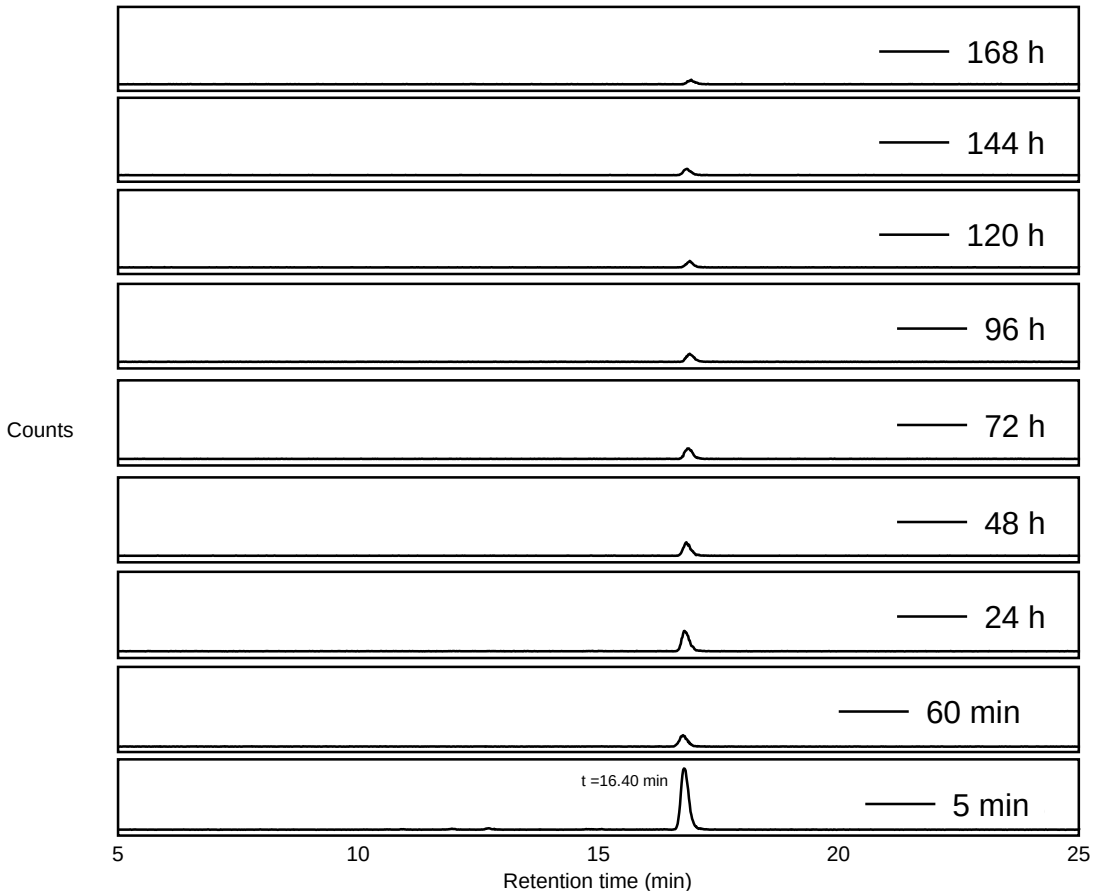

Figure S15. Representative radio-HPLC of $[^{111}In][In(py\text{-}macrodipa)]^+$ in human serum at 37 °C at each time point. The $[^{111}In][In(py\text{-}macrodipa)]^+$ complex was eluted with 0 to 90% B linear gradient 20 min (A: H_2O + 0.1% TFA, B: MeOH).

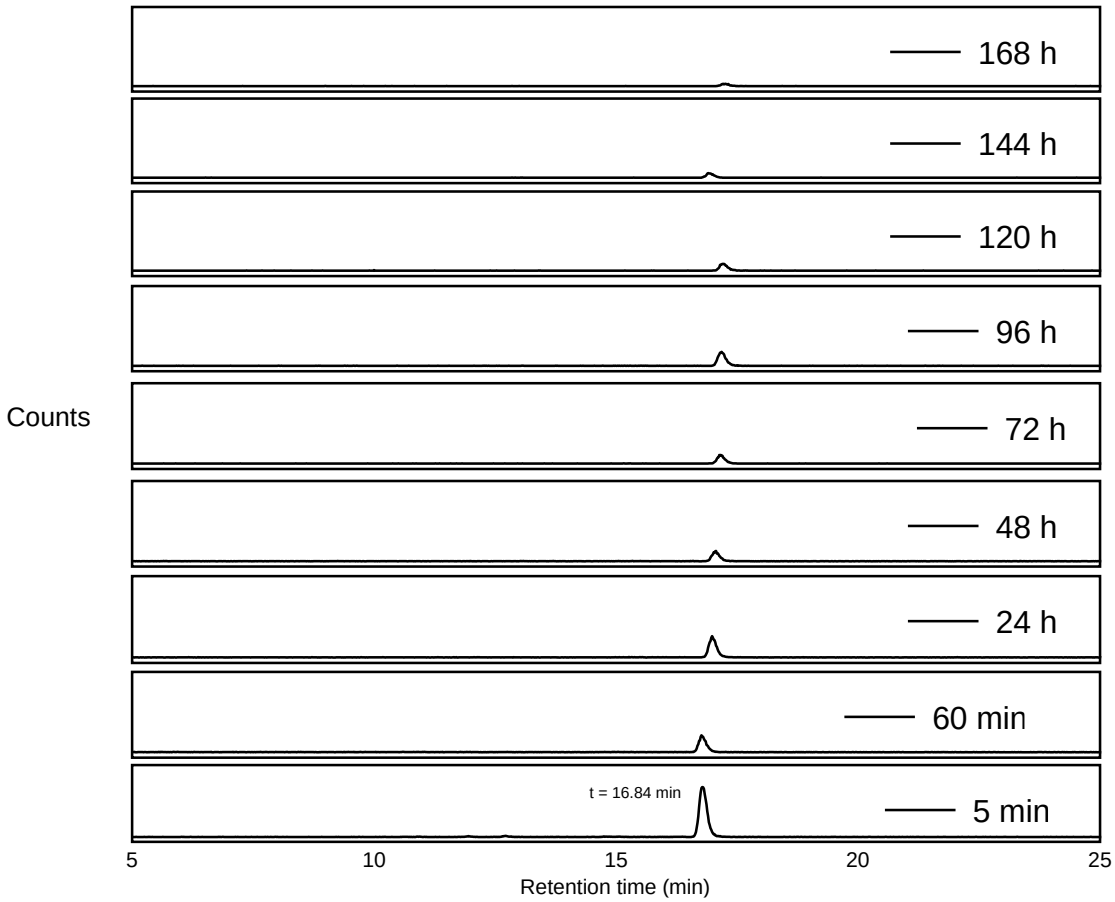

Figure S16. Representative radio-HPLC of $[^{111}In][In(py_2\text{-macrodipa})]^+$ in human serum at 37 °C at each time point. The $[^{111}In][In(py_2\text{-macrodipa})]^+$ complex was eluted with 0 to 90% B linear gradient 20 min (A: H_2O + 0.1% TFA, B: MeOH).

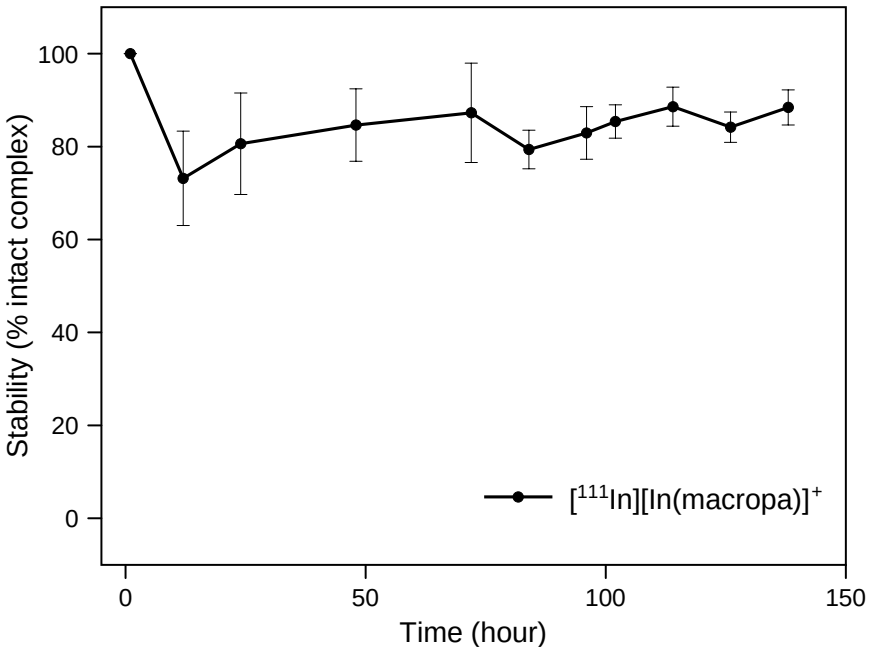

Figure S17. Human serum stability of $[^{111}In][In(macropa)]^+$, prepared from a 4 mM solution of macropa, in human serum at 37 °C.

Table S3. In Vivo biodistribution of $[^{111}In]InCl_3$, $[^{111}In][In(macropa)]^+$, $[^{111}In][In(py\text{-}macrodipa)]^+$, and $[^{111}In][In(py_2\text{-}macrodipa)]^+$. Values for each time point are given as percent injected activity per gram (% IA/g).

$[^{111}In]InCl_3$									
Organ	0.5h	SD	N	2h	SD	N	24h	SD	N
Blood	11.13107	2.387162	3	10.99167	2.248077	4	1.539148	0.382377	4
Heart	3.560892	1.424547	4	4.265713	1.552329	4	1.605656	0.283035	4
Lungs	7.568539	2.37174	4	8.971456	1.380778	4	2.172134	0.467755	4
Liver	3.948502	1.321652	4	5.339713	0.931532	4	6.374404	1.075833	4
Kidneys	26.30511	7.142211	4	22.21027	1.907116	4	18.81397	3.571606	4
Spleen	1.501223	0.384365	4	2.151759	0.34837	4	2.475891	0.523328	4
Stomach	1.457286	0.239089	4	1.551429	0.304916	4	1.486169	0.432787	4
SI	1.915439	0.537025	4	2.525415	0.24857	4	3.526138	1.28484	4
LI	1.62573	0.337636	4	1.731226	0.093102	4	2.888496	0.95843	4
Skin	1.929875	0.286741	4	3.03683	0.7356	4	3.316639	1.747006	4
Muscle	1.269709	0.175996	4	1.555392	0.328284	4	1.090269	0.130789	4
Fat	0.645009	0.067731	4	0.73238	0.150988	4	1.448041	0.240477	4
Tibia	3.730426	0.805453	4	4.426114	0.983589	4	5.544644	1.344146	4

Calvaria	6.207776	2.774584	4	5.743241	2.183205	4	4.829896	1.645127	4
Brain	0.388945	0.107009	4	0.453189	0.136824	4	0.329639	0.143389	4
[^{111}In][In(macropa)]$^{+}$									
Organ	0.5h	SD	N	2h	SD	N	24h	SD	N
Blood	4.782224	2.914834	4	4.094154	0.904548	4	0.878577	0.268915	4
Heart	1.558579	0.829127	4	1.421515	0.630122	4	0.9374	0.253781	4
Lungs	2.763457	1.134809	4	2.990757	0.341958	4	1.431556	0.385342	4
Liver	15.19309	5.041646	4	12.91125	2.031918	4	14.57441	1.334882	4
Kidneys	20.17845	8.143421	4	16.838	1.912519	4	12.78058	1.49039	4
Spleen	3.078484	1.032171	4	2.943872	1.025778	4	4.88973	0.924699	4
Stomach	1.048924	0.684695	4	0.752056	0.214279	4	0.599637	0.114436	4
SI	1.302555	0.523919	4	1.055602	0.269007	4	1.777868	0.612654	4
LI	0.787561	0.278362	4	0.564742	0.126833	4	1.395949	0.183911	4
Skin	2.422796	1.36334	4	1.604344	0.252224	4	1.397086	0.674619	4
Muscle	0.855188	0.453229	4	0.786775	0.111746	4	0.613638	0.275787	4
Fat	0.316589	0.163447	4	0.343115	0.103872	4	0.373918	0.174359	4
Tibia	2.559857	1.258309	4	2.109007	0.549312	4	5.020104	2.332986	4
Calvaria	1.95803	0.768036	4	5.811043	5.047601	4	3.248906	1.590549	4
Brain	0.193591	0.102604	4	0.328824	0.232935	4	0.29827	0.192681	4
[^{111}In][In(py-macrodipa)]$^{+}$									
Organ	0.5h	SD	N	2h	SD	N	24h	SD	N
Blood	1.400774	0.723207	4	0.594822	0.106937	4	0.114847	0.008651	4
Heart	0.355246	0.071371	4	0.252819	0.027638	4	0.109244	0.016135	4
Lungs	0.84843	0.135726	4	0.579747	0.118124	4	0.212841	0.032395	4
Liver	1.708602	0.408891	4	1.52174	0.269168	4	1.212741	0.081314	4
Kidneys	4.183916	0.858547	4	2.454069	0.426451	4	1.715354	0.406197	4
Spleen	0.230208	0.042861	4	0.209191	0.029657	4	0.228522	0.025462	4
Stomach	0.219969	0.114095	4	0.240923	0.074726	4	0.204004	0.09544	4
SI	1.608592	0.48418	4	0.474462	0.157343	4	0.325727	0.050428	4
LI	0.22966	0.022873	4	0.389954	0.084001	3	0.655123	0.216229	4
Skin	0.57443	0.310589	4	0.356216	0.148534	4	0.260106	0.085732	4
Muscle	0.209054	0.063685	4	0.153686	0.027049	4	0.089358	0.04378	4
Fat	0.100041	0.045277	4	0.056719	0.016277	4	0.061684	0.038679	4
Tibia	0.342032	0.022672	4	0.236833	0.080962	4	0.326742	0.032326	4
Calvaria	0.88553	0.731249	4	0.258182	0.126479	4	0.194959	0.065245	4
Brain	0.065461	0.016188	4	0.038077	0.002702	4	0.022074	0.002256	4
[^{111}In][In(py$_2$-macrodipa)]$^{+}$									
Organ	0.5h	SD	N	2h	SD	N	24h	SD	N

Blood	1.2221	0.25207	4	0.738129	0.093919	4	0.112384	0.040665	4
Heart	0.330945	0.033477	4	0.273234	0.016921	4	0.097447	0.040542	4
Lungs	0.935808	0.138492	4	0.588315	0.047808	4	0.149852	0.061284	4
Liver	2.287751	0.945966	4	1.215243	0.141374	4	1.372107	0.564503	4
Kidneys	4.572923	0.832515	4	2.143948	0.39301	4	1.452306	0.398568	4
Spleen	0.251381	0.05831	4	0.201484	0.014043	4	0.167861	0.051657	4
Stomach	0.396485	0.338435	4	0.238631	0.062347	4	0.10076	0.054667	4
SI	2.657816	1.07697	3	0.529708	0.060434	4	0.206904	0.098133	4
LI	0.238404	0.025538	4	0.604338	0.341423	4	0.434948	0.162334	4
Skin	0.986358	0.289192	4	0.349595	0.092482	4	0.254275	0.103598	4
Muscle	0.233684	0.039382	4	0.120034	0.026868	4	0.087325	0.04271	4
Fat	0.10147	0.038766	4	0.062716	0.023519	4	0.047849	0.024926	4
Tibia	0.441128	0.112989	4	0.27799	0.028405	4	0.372908	0.156774	4
Calvaria	0.455986	0.310202	4	0.449871	0.160244	4	0.247592	0.094029	4
Brain	0.23701	0.368349	4	0.129971	0.184326	4	0.01973	0.006846	4

DFT Calculation Supplementary Data

We herein list all energy outputs of our computations in this work. Their corresponding geometry outputs (.xyz) are included in a compressed file (.zip) as an independent ESI file.

(1) In–py-macrodipa complex, Conformation A

Zero-point correction= 0.619717 (Hartree/Particle)
Thermal correction to Energy= 0.654514
Thermal correction to Enthalpy= 0.655458
Thermal correction to Gibbs Free Energy= 0.556568
Sum of electronic and zero-point Energies= –1925.979569
Sum of electronic and thermal Energies= –1925.944771
Sum of electronic and thermal Enthalpies= –1925.943827
Sum of electronic and thermal Free Energies= –1926.042718

SMD method:
E(ωB97XD) = -1926.68381750

(2) In–py-macrodipa complex, Conformation B without H_2O

Zero-point correction= 0.618445 (Hartree/Particle)
Thermal correction to Energy= 0.653934
Thermal correction to Enthalpy= 0.654878
Thermal correction to Gibbs Free Energy= 0.553760
Sum of electronic and zero-point Energies= –1925.965301
Sum of electronic and thermal Energies= –1925.929812
Sum of electronic and thermal Enthalpies= –1925.928867
Sum of electronic and thermal Free Energies= –1926.029985

SMD method:
E(ωB97XD) = –1926.68750206

(3) In–py-macrodipa complex, Conformation B with H_2O

Zero-point correction= 0.645745 (Hartree/Particle)
Thermal correction to Energy= 0.682813
Thermal correction to Enthalpy= 0.683758
Thermal correction to Gibbs Free Energy= 0.579899
Sum of electronic and zero-point Energies= –2002.406091
Sum of electronic and thermal Energies= –2002.369023
Sum of electronic and thermal Enthalpies= –2002.368079
Sum of electronic and thermal Free Energies= –2002.471937

SMD method:
E(ωB97XD) = –2003.14303801

(4) In–py_2-macrodipa complex, Conformation A

Zero-point correction= 0.625731 (Hartree/Particle)

Thermal correction to Energy= 0.662191
Thermal correction to Enthalpy= 0.663135
Thermal correction to Gibbs Free Energy= 0.558961
Sum of electronic and zero-point Energies= –2019.190013
Sum of electronic and thermal Energies= –2019.153552
Sum of electronic and thermal Enthalpies= –2019.152608
Sum of electronic and thermal Free Energies= –2019.256782

SMD method:
E(ωB97XD) = –2019.90344501

(4) In–py_2-macrodipa complex, Conformation B with H_2O

Zero-point correction= 0.653076 (Hartree/Particle)
Thermal correction to Energy= 0.690976
Thermal correction to Enthalpy= 0.691921
Thermal correction to Gibbs Free Energy= 0.586259
Sum of electronic and zero-point Energies= –2095.625773
Sum of electronic and thermal Energies= –2095.587873
Sum of electronic and thermal Enthalpies= –2095.586929
Sum of electronic and thermal Free Energies= –2095.692590

SMD method:
E(ωB97XD) = –2096.37041781

(5) In–py_2-macrodipa complex, Conformation B without H_2O

Zero-point correction= 0.624846 (Hartree/Particle)
Thermal correction to Energy= 0.661379
Thermal correction to Enthalpy= 0.662324
Thermal correction to Gibbs Free Energy= 0.558857
Sum of electronic and zero-point Energies= –2019.182320
Sum of electronic and thermal Energies= –2019.145787
Sum of electronic and thermal Enthalpies= –2019.144842
Sum of electronic and thermal Free Energies= –2019.248309

SMD method:
E(ωB97XD) = –2019.9111516