SUPPORTING INFORMATION

Ultrasonic-Assisted Solid-Phase Peptide Synthesis of DOTA-TATE and DOTA-*linker*-TATE Derivatives as a Simple and Low-Cost Method for the Facile Synthesis of Chelator-Peptide Conjugates

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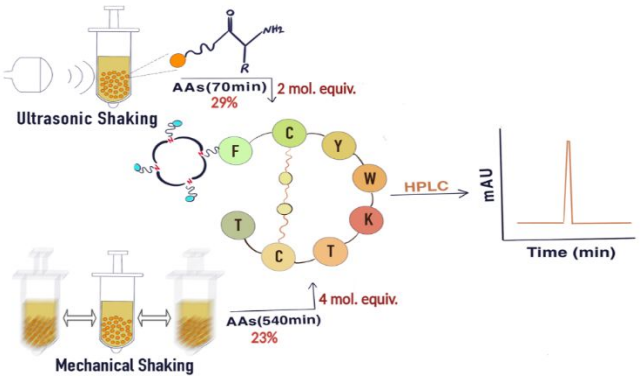

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1. General Synthesis of DOTA-TATE using Mechanical Agitation and I_2 for cyclization

Table S1: Sequence of amino acid coupling reactions with their optimized coupling times and agents, as performed with 50 mg pre-loaded with 1st amino acid Wang resin (Capacity = 0.8 g/mol), 780 µL DMF, and 100 µL DIEA, with manual agitation (shaking) at ambient temperature.

Coupling Step #	Amino Acid	Amino Acid Weight (mg)	Peptide Coupling Agent	Coupling Time (min)
1	Fmoc-Cys(Acm)-OH	66	HBTU	40
2	Fmoc-Thr(*t*-Bu)-OH	64	HBTU	40
3	Fmoc-Lys(Boc)-OH	75	HATU	120
4	Fmoc-D-Trp(Boc)-OH	84	HBTU	100
5	Fmoc-Tyr(*t*-Bu)-OH	74	HBTU	40
6	Fmoc-Cys(Acm)-OH	66	HBTU	40
7	Fmoc-D-Phe-OH	62	HBTU	40
8	DOTA-tri (tBu-ester)	92	HBTU	120

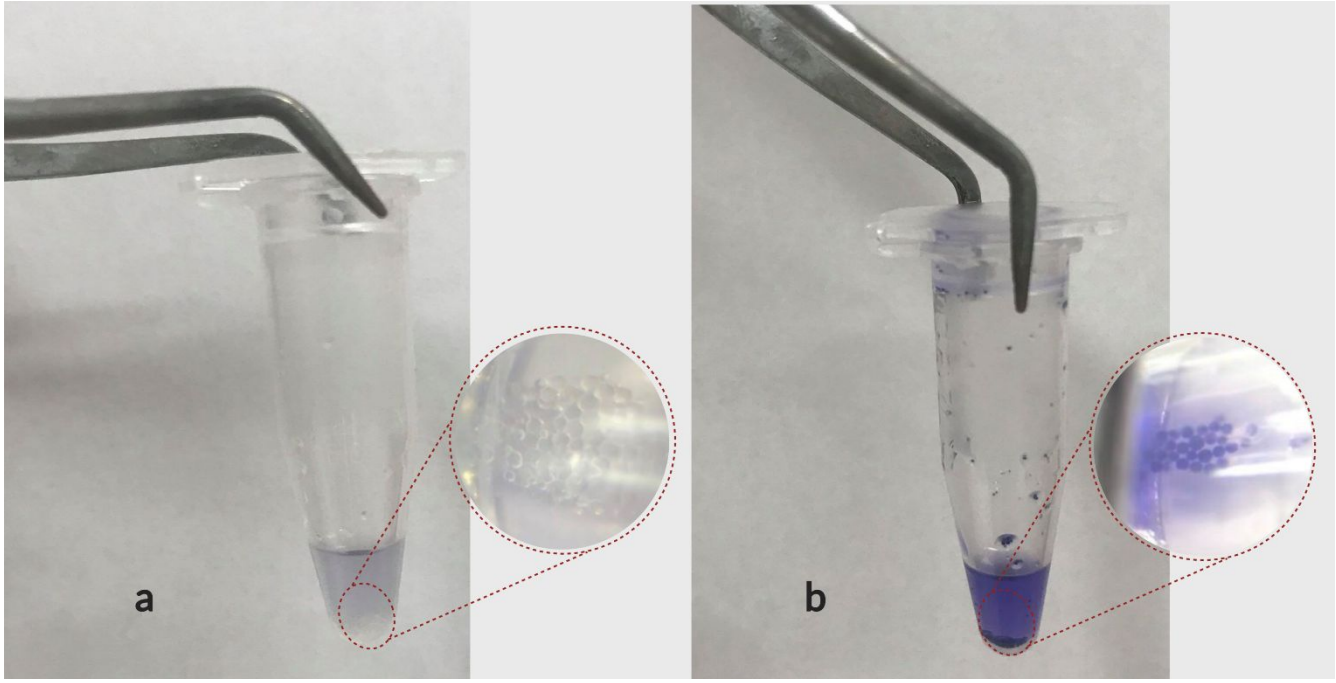

Figure S1: Qualitative color-based Kaiser test, transparent or pale yellow beads indicate successful coupling (**a**), blue to violet beads indicate the presence of free amine and the coupling failed (**b**).

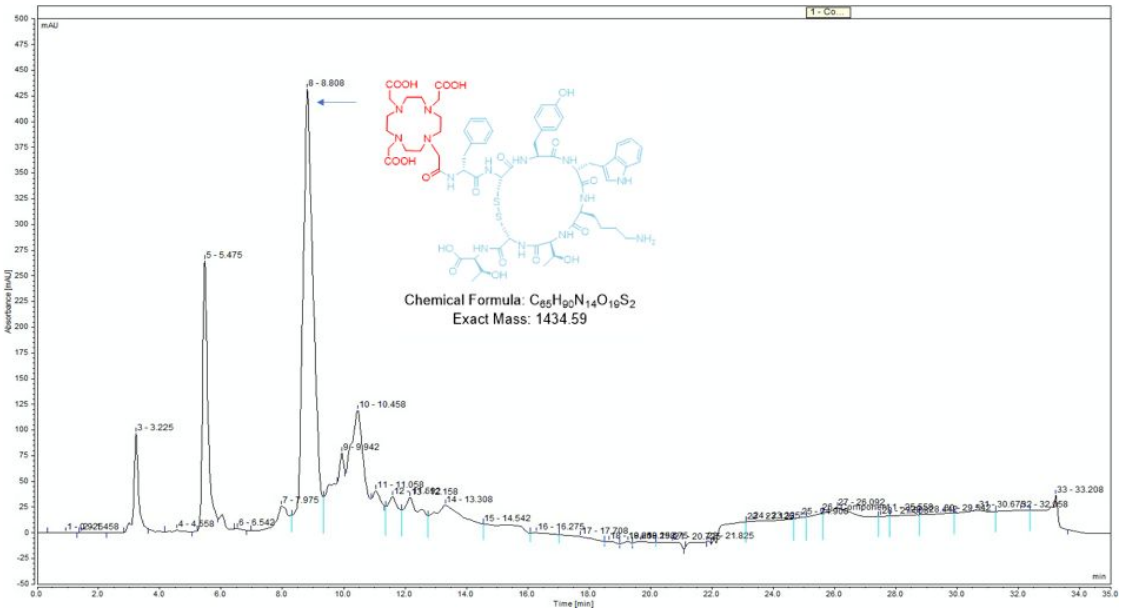

Figure S2: Semi-Prep HPLC UV/Vis trace of crude DOTA-TATE before purification using I_2 for cyclization.

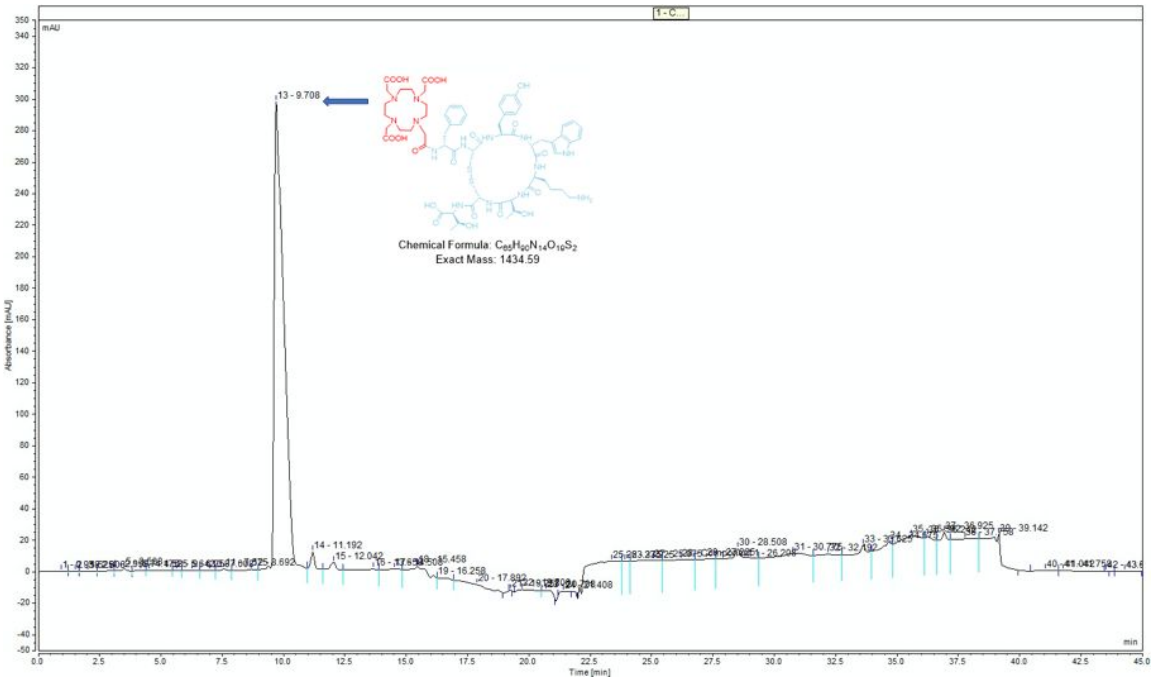

Figure S3: Analytical HPLC UV/Vis trace of purified DOTA-TATE using I_2 for cyclization.

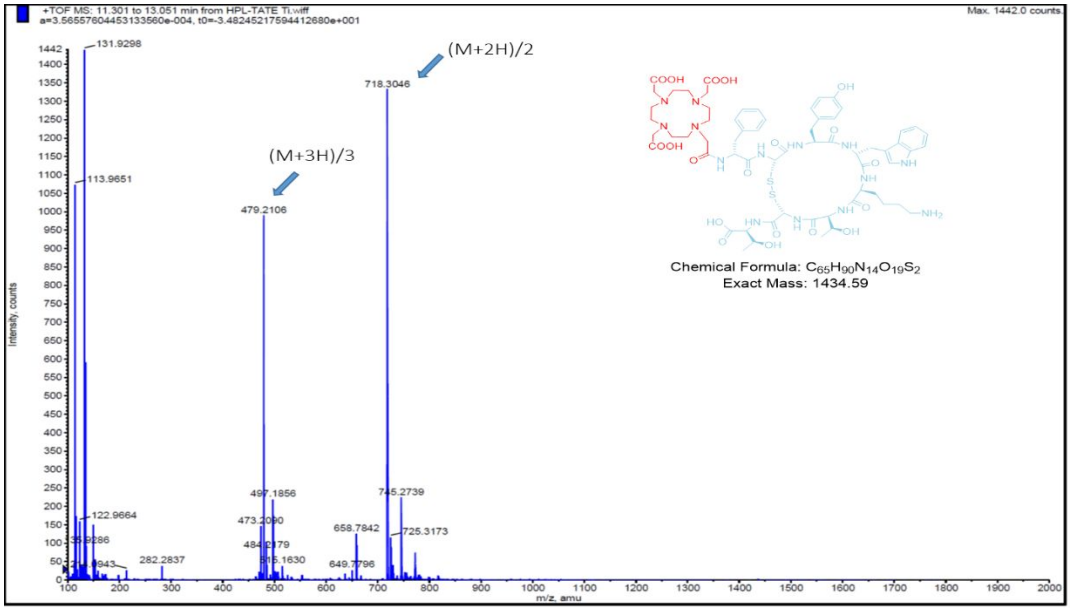

Figure S4*:* ESI-MS spectrum of pure DOTA-TATE using I_2 for cyclization.

2. Cyclization using thallium (III) Oxidation.

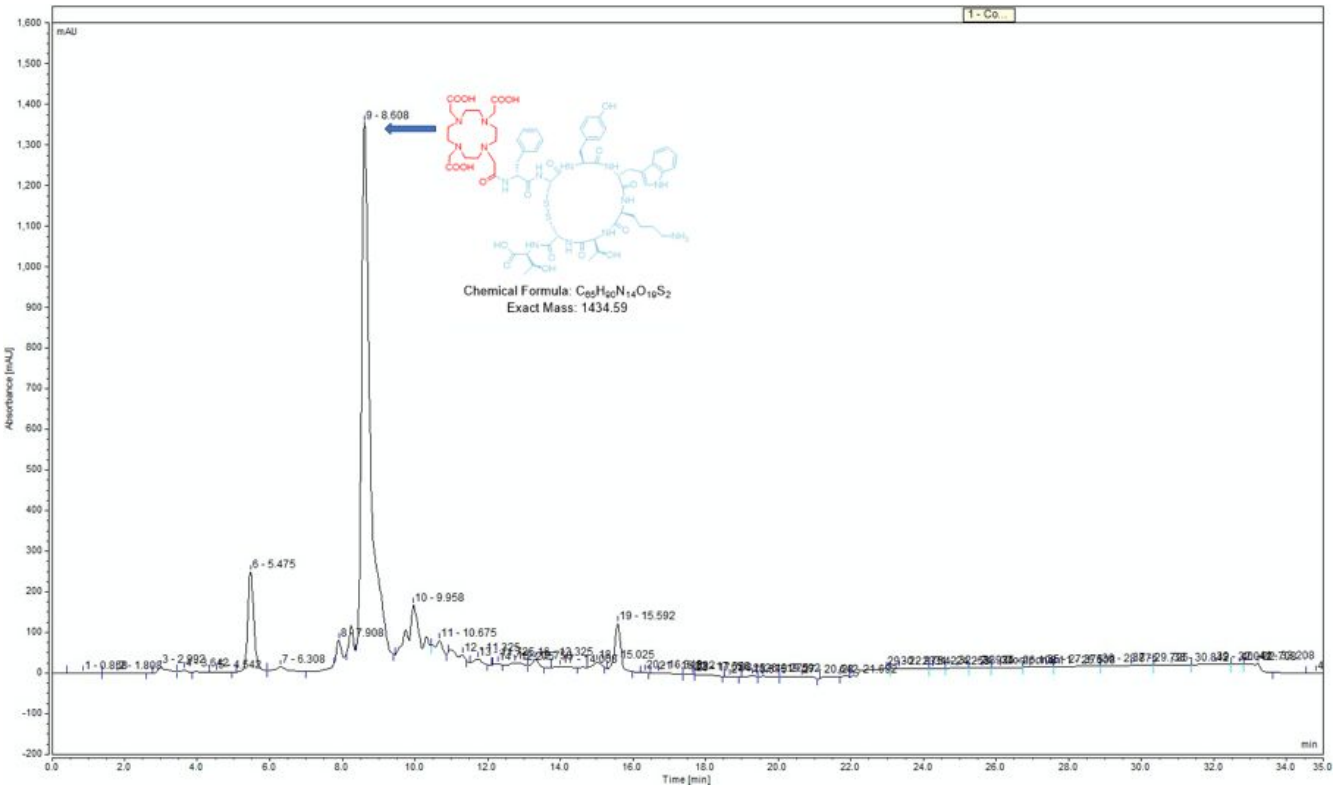

Figure S5: Semi-Prep HPLC UV/Vis trace of crude DOTA-TATE before purification using $Tl(CF_3CO_2)_3$ for cyclization.

3. Cyclization using Dimethyl Sulfoxide Oxidation.

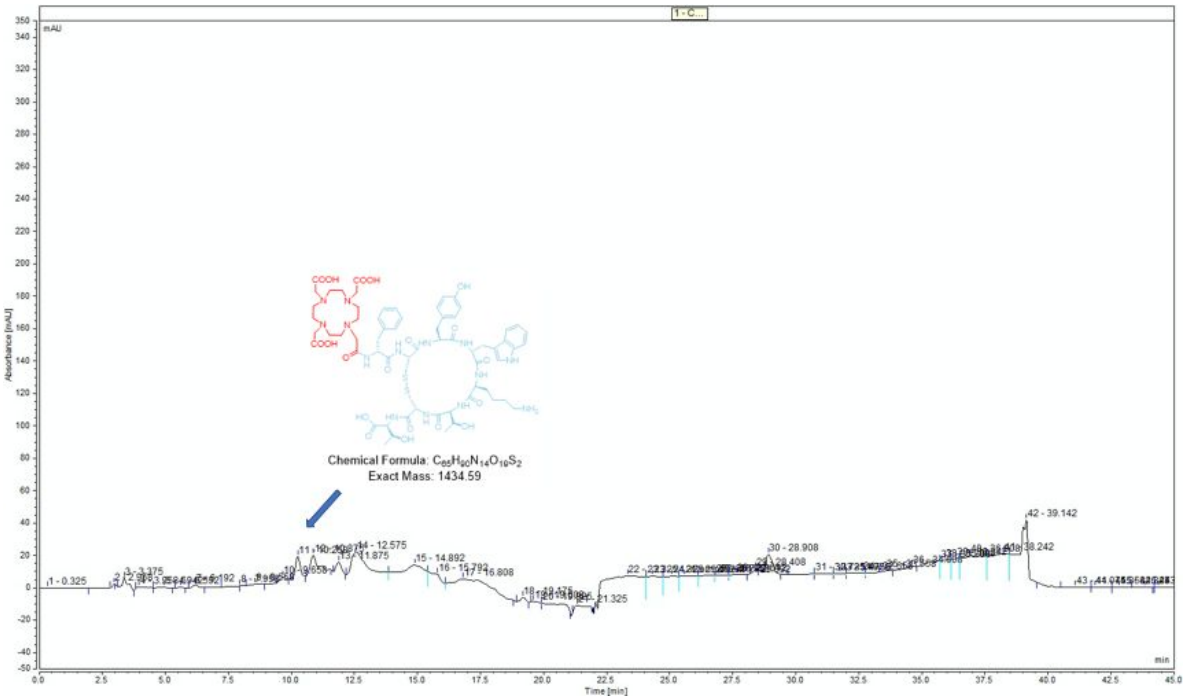

Figure S6: Semi-Prep HPLC UV/Vis trace of crude DOTA-TATE before purification using DMSO for cyclization.

4. Hydrogen Peroxide Oxidation.

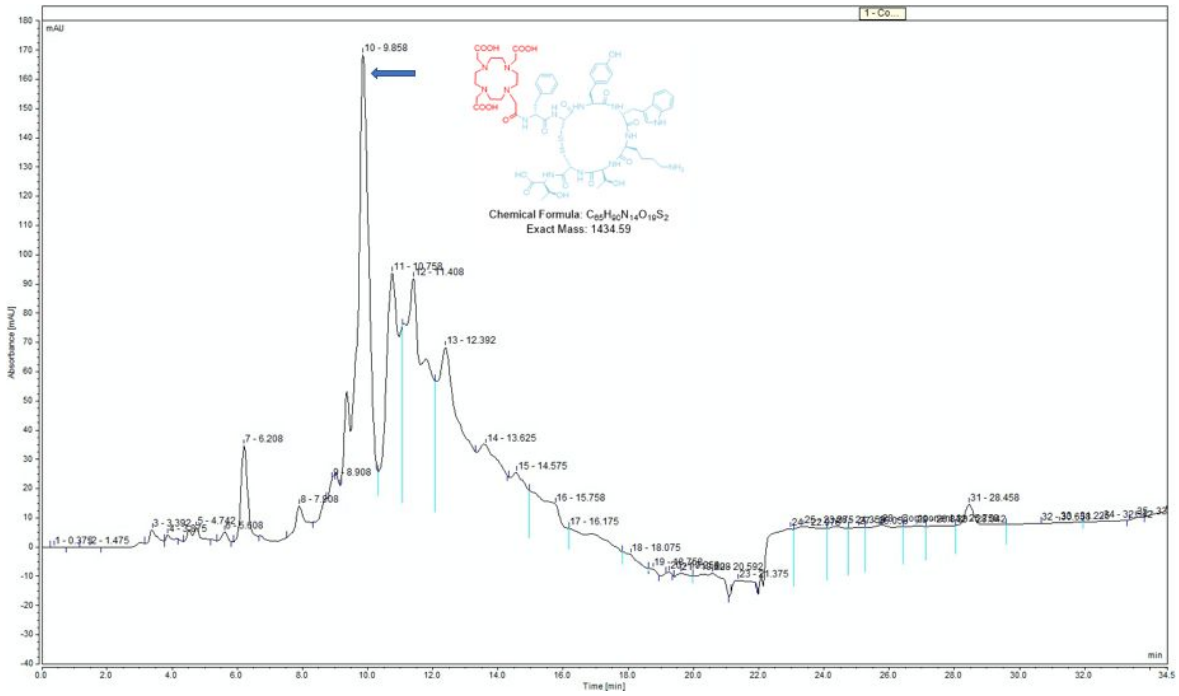

Figure S7: Semi-Prep HPLC UV/Vis trace of crude DOTA-TATE before purification using H_2O_2 for cyclization.

5. General Synthesis of DOTA-TATE Using Ultrasound Approach.

Table S2: Sequence of amino acid coupling reactions with their optimized coupling times and agents, as performed with 50 mg Wang resin (Capacity = 0.8 g/mol), 780 µL DMF, and 100 µL DIEA, with 4 molar equivalents of reagents for each step and ultrasonic agitation at 25-30 degrees Celsius.

Coupling Step #	Amino Acid	Amino Acid weight (mg)	Peptide Coupling Agent	Coupling Time (min)
1	Fmoc-Cys(Acm)-OH	66	HBTU	5
2	Fmoc-Thr(*t*-Bu)-OH	64	HBTU	5
3	Fmoc-Lys(boc)-OH	75	HBTU	5
4	Fmoc-D-Trp(boc)-OH	84	HBTU	10
5	Fmoc-Tyr(*t*-Bu)-OH	74	HBTU	10
6	Fmoc-Cys(Acm)-OH	66	HBTU	10
7	Fmoc-D-Phe-OH	62	HBTU	10
8	DOTA-tri (tBu-ester)	92	HBTU	15

Table S3: Sequence of amino acid coupling reactions with their optimized coupling times and agents, as performed with 50 mg Wang resin (Capacity = 0.8 g/mol), 399 µL DMF, and 65 µL DIEA, with 2 molar equivalents of reagents for each step ultrasonic agitation at 25-30 degrees Celsius.

Coupling Step #	Amino Acid	Amino Acid weight (mg)	Peptide Coupling Agent	Coupling Time (min)
1	Fmoc-Cys(Acm)-OH	33	HBTU	5
2	Fmoc-Thr(*t*-Bu)-OH	32	HBTU	5
3	Fmoc-Lys(boc)-OH	38	HBTU	5
4	Fmoc-D-Trp(boc)-OH	42	HBTU	10
5	Fmoc-Tyr(*t*-Bu)-OH	37	HBTU	10
6	Fmoc-Cys(Acm)-OH	33	HBTU	10
7	Fmoc-D-Phe-OH	31	HBTU	10
8	DOTA-tri (tBu-ester)	46	HBTU	15

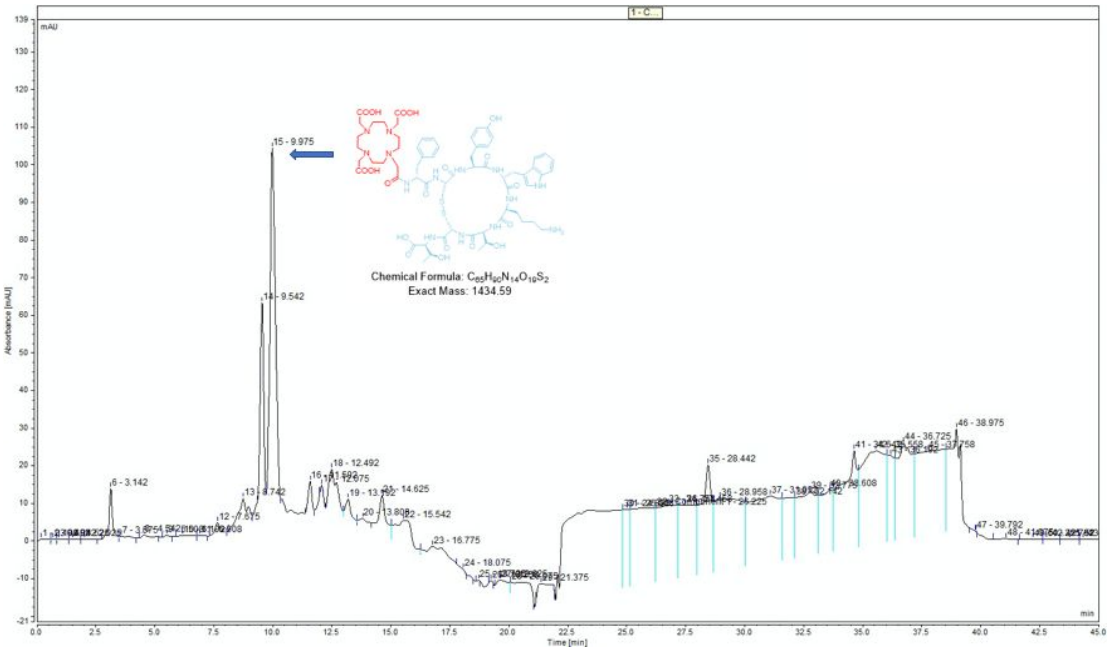

Figure S8: Semi-Prep HPLC UV/Vis trace of crude DOTA-TATE before purification using ultrasonic agitation and I_2 for cyclization.

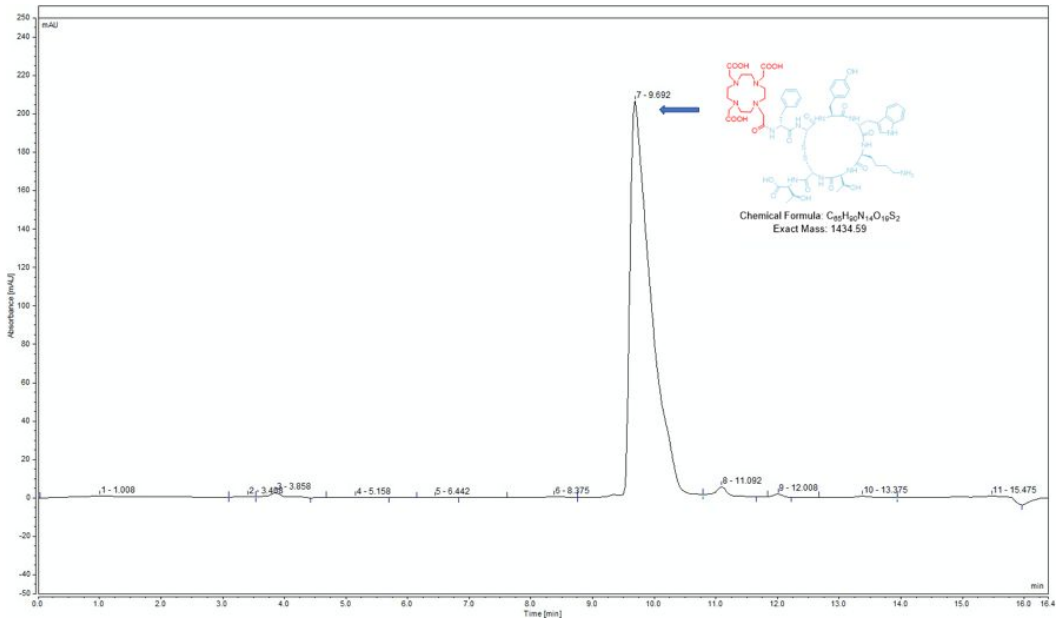

Figure S9: Analytical HPLC UV/Vis trace of pure DOTA-TATE using ultrasonic agitation and I_2 for cyclization.

6. Total Synthesis of Fmoc-NH-PEG$_2$-COOH

2-(2-(dibenzylamino)ethoxy)ethanol (P1): Synthesis was carried out according to a literature procedure with minor modifications.[1] Both of 2-(aminoethoxy)ethanol (1.0 g, 9.5 mmol) and potassium carbonate (3.3 g, 23.7 mmol) were stirred in acetonitrile (50 mL) at room temperature, followed by addition of benzyl bromide (2.7 mL, 1.9 mmol), and were then stirred for 20 hours at 50 °C. Potassium carbonate was removed by filtration and the filtrate was reduced to a solid residue using a rotary evaporator under *vacuo*. The solid residue was dissolved in hydrochloric acid (0.1 M) and washed with ethyl acetate (2 × 10 mL). The aqueous layer was basified with sodium hydroxide (1 M) and extracted with dichloromethane (4 × 10 mL). The solvent was dried ($MgSO_4$) and removed under *vacuo*, to give **P1**, (2.3 g, 85%).^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 2.70 (t, 2H, *J*=5.9 Hz),

3.49 (t, 2H, J=4.7 Hz), 3.59 (t, 2H, J=5.9 Hz), 3.60-3.70 (m, 6H), 7.24-7.40 (m, 10H). ^{13}C NMR (CDCl3) δ(ppm): 52.97, 59.00, 61.89, 69.64, 72.10, 127.02, 128.29, 128.92, 139.40. LRMS-ESI *m/z* calcd for $[C_{18}H_{23}NO_2 + H]^+$: 286.1, found: 285.9.

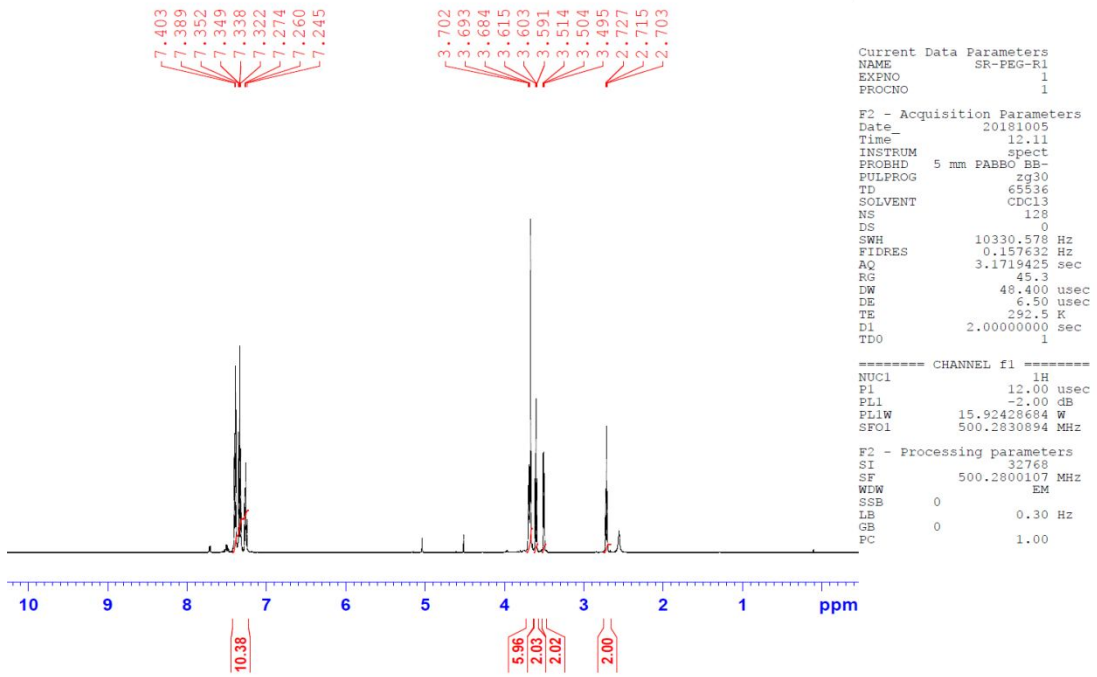

Figure S10: ^{1}H NMR spectrum of **P1** in $CDCl_3$.

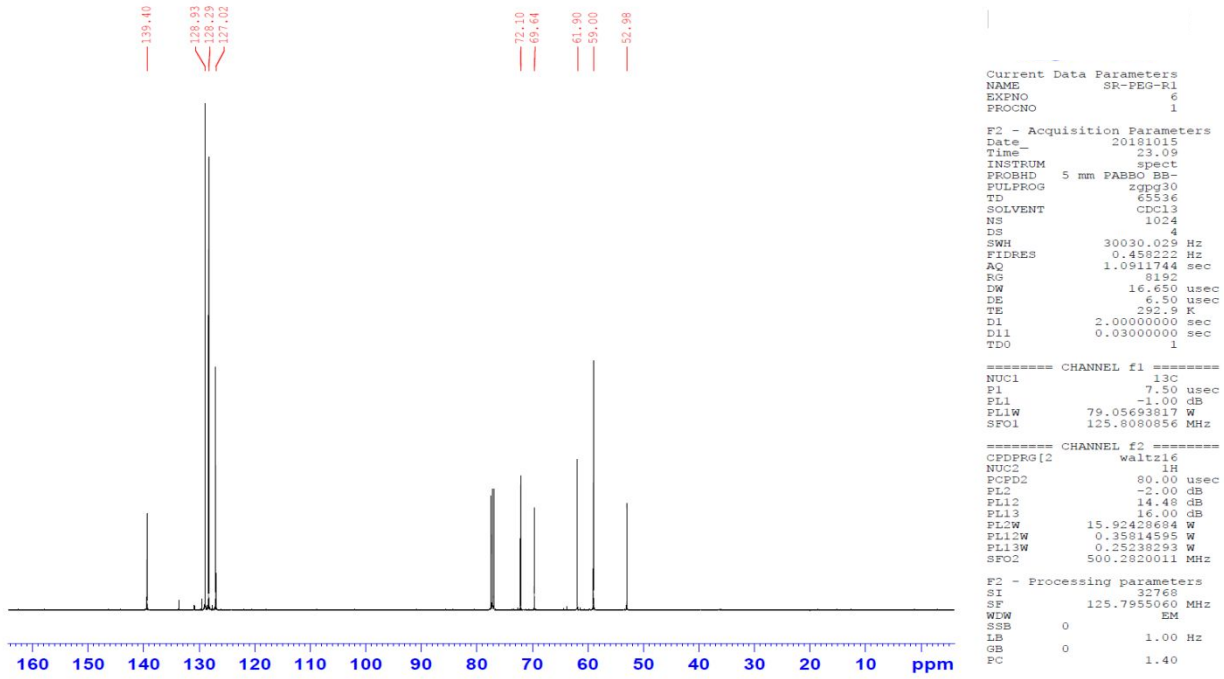

Figure S11: ^{13}C NMR Spectrum of **P1** in $CDCl_3$.

2-(2-(2-(dibenzylamino)ethoxy)ethoxy)acetic acid (P2): In a Schlenk flask, NaH (1.2 g, 28.8 mmol; 60% dispersion in oil) was washed twice with 15 mL hexane under N_2 then dry THF (14 mL) was added at 0 °C. This was followed by addition of **P1** (2.05 g, 7.2 mmol) dropwise and kept stirring at 0 °C for a half an hour. Alpha bromo acetic acid (1.5 g, 10.8 mmol) was added dropwise and the mixture was refluxed overnight. Carefully, 1 mL of H_2O was added dropwise and the reaction was left stirring for 5 minutes at room temperature, then extra H_2O (20 mL) was added. The aqueous layer was washed with a mixture of hexane/diethyl ether 1:1, (2 × 15 mL). The aqueous solution was acidified to pH 2-3 with hydrochloric acid (1 M) and washed with diethyl ether (3 × 10 mL), then neutralized to pH 6-7 with NaOH (1 M). Solid sodium chloride was added and the mixture was extracted with DCM (5 × 150 mL). The solvent was dried and removed under *vacuo*, the crude product **P2** was used in the next reaction without further purification (1.2 g, 61%). ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 2.85 (t, 2H, J = 5.6 Hz), 3.54 (t, 2H, J =

5.6 Hz), 3.66-3.68 (m, 4H), 3.93 (s, 4H), 4.08 (s, 2H), 7.27-7.33 (m, 10H), 11.4 (s, 1H). ^{13}C NMR ($CDCl_3$) δ(ppm): 51.33, 53.50, 58.18, 68.13, 69.60, 70.16, 70.46, 127.98, 128.56, 129.94, 135.53, 174.42. LRMS-ESI *m/z* calcd for $[C_{20}H_{25}NO_4 + H]^+$:344.18, found: 344.1.

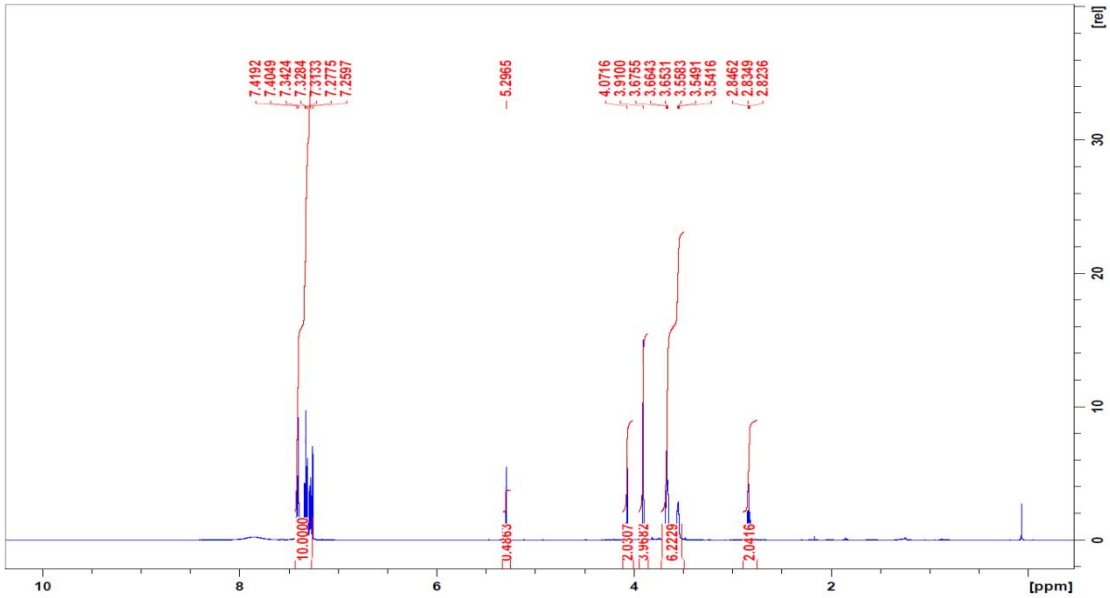

Figure S12: ^{1}H NMR spectrum of **P2** in $CDCl_3$.

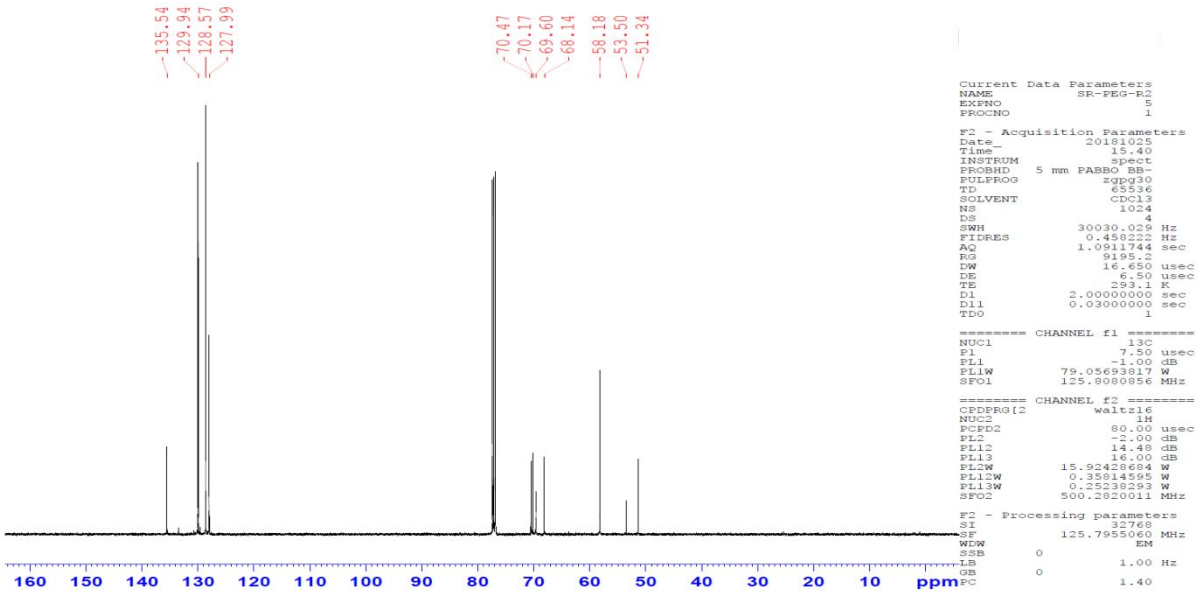

Figure S13: ^{13}C NMR spectrum of **P2** in $CDCl_3$.

2-(2-(2-aminoethoxy)ethoxy)acetic acid (P3): Both of **P2** (1.14 g, 3.31 mmol) and palladium (280 mg; 10% Pd/C) were dissolved in methanol (20 mL). The mixture was transferred in to a hydrogenator and stirred under H_2 at 30 psi for 12 hours. The completion of the reaction was confirmed by TLC and the catalyst was removed by filtration through celite powder. The solvent was removed under *vacuo* and the residue was triturated with cold diethyl ether (20 mL) to give **P3** (453 mg, 85%). ^{1}H NMR (500 MHz, MeOD) δ(ppm): 3.10 (t, 2H, *J*=5.1 Hz), 3.62 (t, 2H, *J*= 6.1 Hz), 3.69 (t, 2H, *J*= 6.1 Hz), 3.73 (t, 2H, *J*= 5.1 Hz), 3.88 (s, 2H). LRMS-ESI *m/z* calcd for $[C_6H_{13}NO_4 + H]^+$:164.09, found: 164.1.

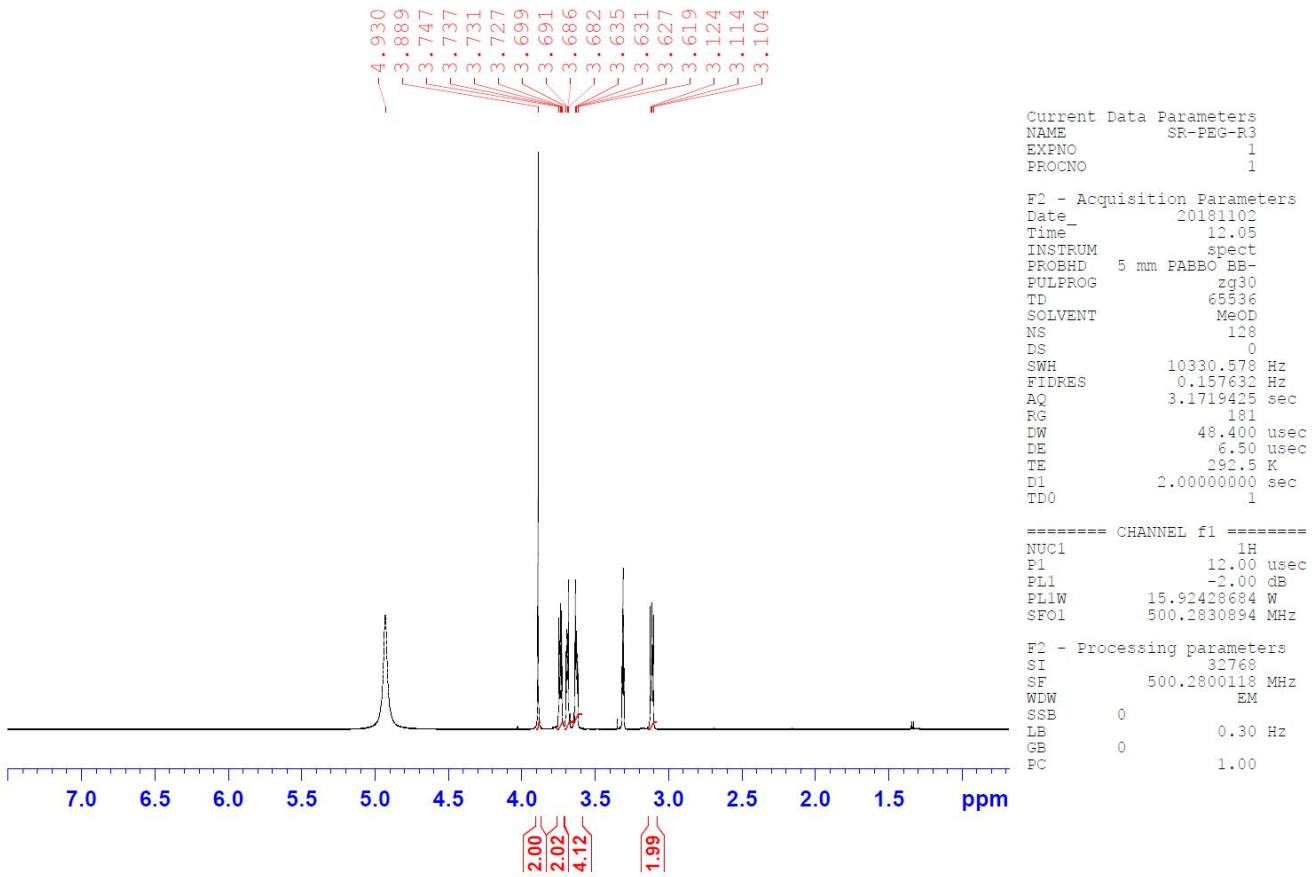

Figure S14: ^{1}H NMR Spectrum of **P3** in MeOD.

1-(9H-fluoren-9-yl)-3-oxo-2,7,10-trioxa-4-azadodecan-12-oic acid (P4): A mixture of **P3** (450 mg, 2.75 mmol) and potassium carbonate (763 mg, 5.52 mmol) were stirred for 10 minutes in H_2O (10 mL). This was followed by addition of Fmoc-*N*-hydroxysuccinimide ester (1.03 g, 3.05 mmol) and stirred at 40 °C for 16 hours. The pH should be adjusted to 9 before washing with diethyl ether (2 × 5 mL). The aqueous solution was acidified (pH ~2) by HCl (1 M) then extracted with DCM (5 × 5 mL). The organic solvent was removed and the solid residue was recrystallized from acetonitrile to obtain **P4** (511.4 mg, 51%). ^{1}H NMR (500 MHz, MeOD) δ(ppm): 3.28 (t, 2H, *J* =5.4 Hz), 3.51 (t, 2H, *J*=5.4 Hz), 3.60-3.62 (m, 2H), 3.67-3.68 (m, 2H), 4.11 (s, 2H), 4.08 (t, 1H, *J*=6.8 Hz), 4.33 (d, 2H, *J*=6.9 Hz), 7.28-7.78 (m, 10H). ^{13}C NMR (CDCl3) δ(ppm): 41.69, 67.67, 69.02, 70.91, 71.28, 71.75, 120.9, 126.17, 128.12,

128.74, 142.57, 145.31, 158.89, 174.02 . LRMS-ESI *m/z* calcd for $[C_{21}H_{23}NO_6 + Na]^+$:408.1, found: 407.9.

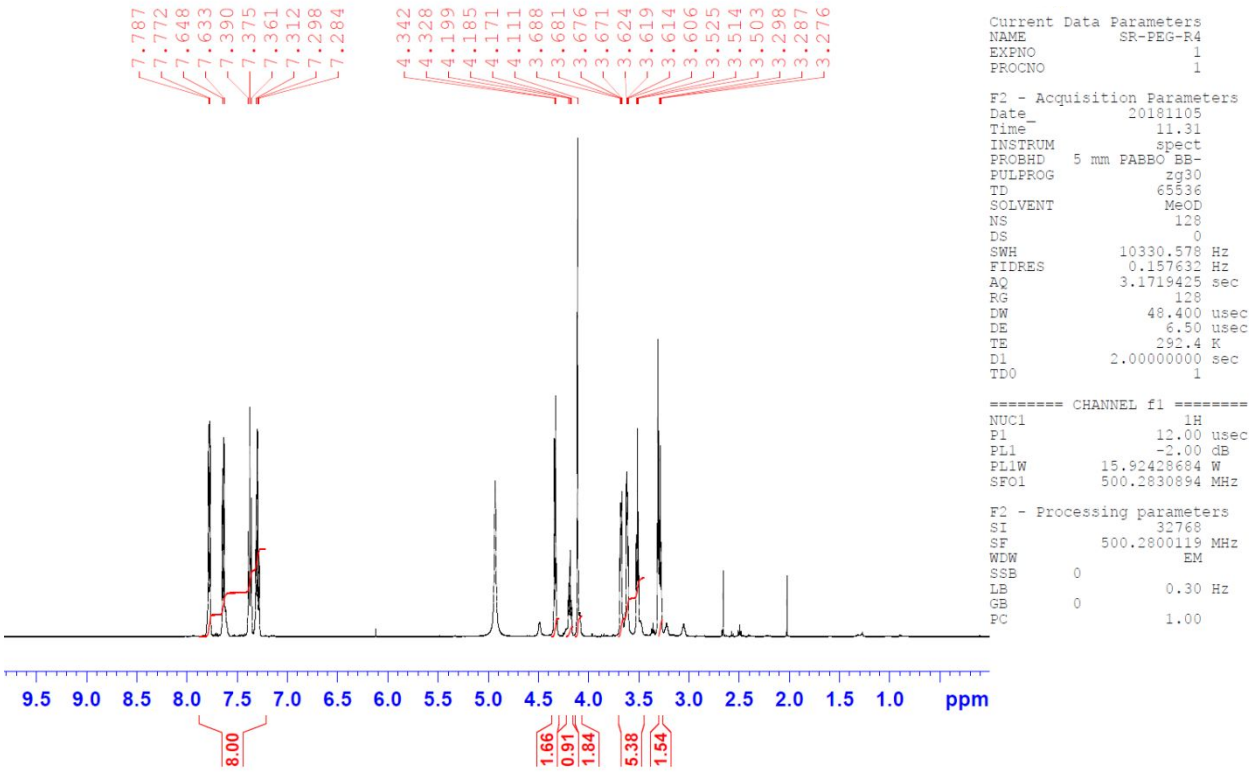

Figure S15: ^{1}H NMR Spectrum of **P4** in MeOD.

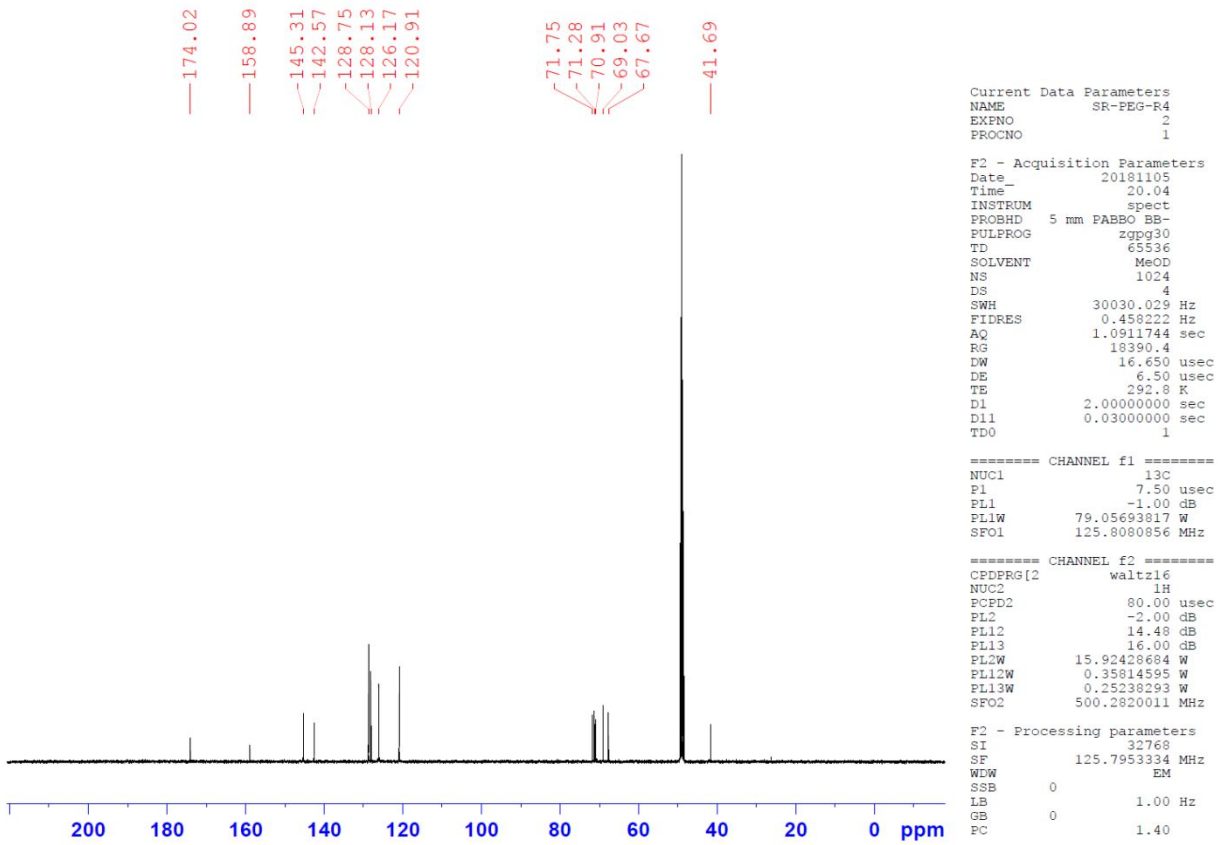

Figure S16: ^{13}C NMR spectrum of **P4** in MeOD.

7. Synthesis of DOTA−tris(tert-butyl)-COOH.

Benzyl-2-bromoacetate (D1): Synthesis was carried out according to a literature procedure.[2] A solution of benzyl alcohol (0.39 g, 3.6 mmol), *p*-toluenesulfonic acid (0.007 g, 0.036 mmol), and bromoacetic acid (0.50 g, 3.6 mmol) in toluene (20 mL) was stirred at 120 °C for 24 hours with continuous H_2O removal by using a Dean-Stark trap. The solvent was removed and the residue was purified using silica chromatography (Hexane: EtOA gradient 10%-50% EtOA) to give (**D1**) as a yellow oil (0.452 g, 55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 3.87 (s, 2H), 5.21 (s, 2H), 7.35-7.39 (m, 5H). ^{13}C NMR (CDCl3) δ(ppm): 25.19, 67.97, 128.4, 128.6, 128.7, 135.0, 167.1. HRMS-ESI *m/z* calcd for $[C_9H_9BrO_2+ H]^+$: 228.9864, found: 228.9830.

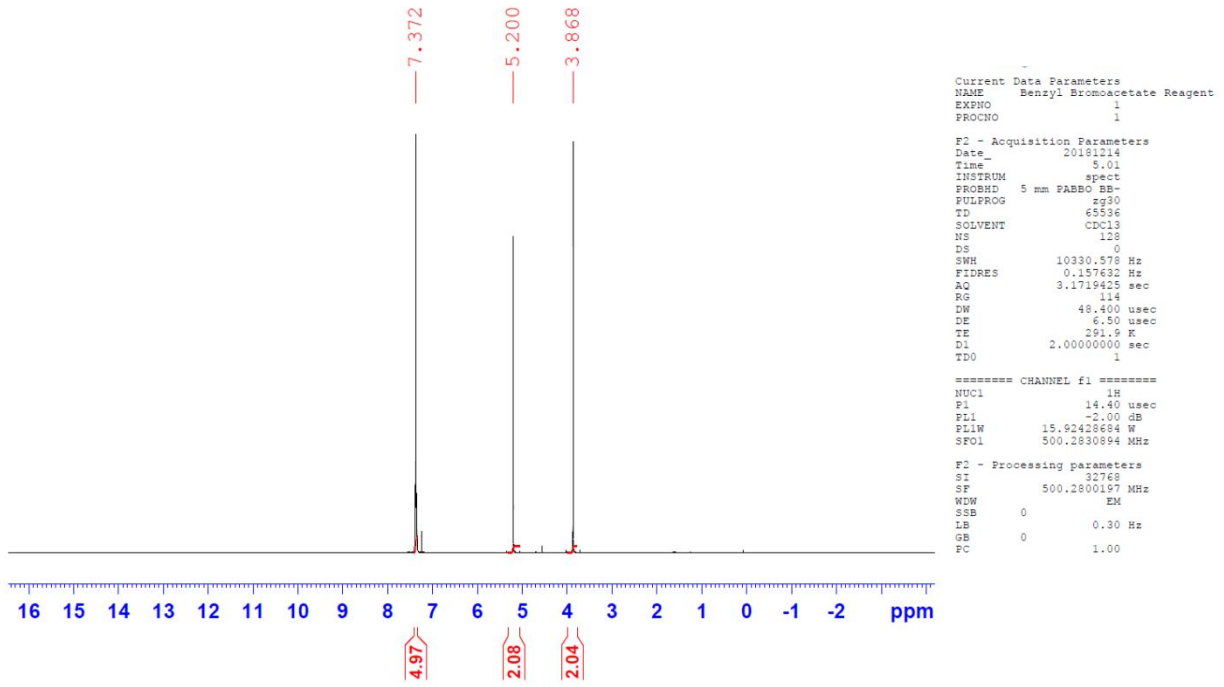

Figure S17: ^{1}H NMR Spectrum of **D1** in $CDCl_3$.

Tri-tert-butyl-2,2',2''-(10-(2-(benzyloxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetate (D2): To a commercially available 1,4,7,10-tetraazacyclododecane-1,4,7-tris(t-butyl acetate) (**DO3A**, Macrocyclics) (0.100 g, 0.194 mmol), both of benzyl-2-bromoacetate (**D1**) (0.0470 g, 0.204 mmol) and potassium carbonate (0.0540 g, 0.388 mmol) were added with anhydrous CH_3CN (10 mL) and stirred for 12 hours at room temperature. After 12 hours, the solvent was removed by rotary evaporation under *vacuo* and the solid residue was purified immediately with silica chromatography (DCM: MeOH 90:10%), to give (**D2**) as yellow oil (0.104 g, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 1.42 (s, 27H),2.12-3.40 (b, 24H), 5.08 (s, 2H), 7.26-732 (m, 5H). ^{13}C NMR (CDCl3) δ(ppm): 27.76, 50.28, 54.86, 55.58, 66.71, 66.79, 81.89, 128.0, 128.3, 128.5, 134.9, 172.1, 173.4. HRMS-ESI *m/z* calcd for $[C_{35}H_{58}N_4O_8+ H]^+$: 663.4333, found: 662.4267.

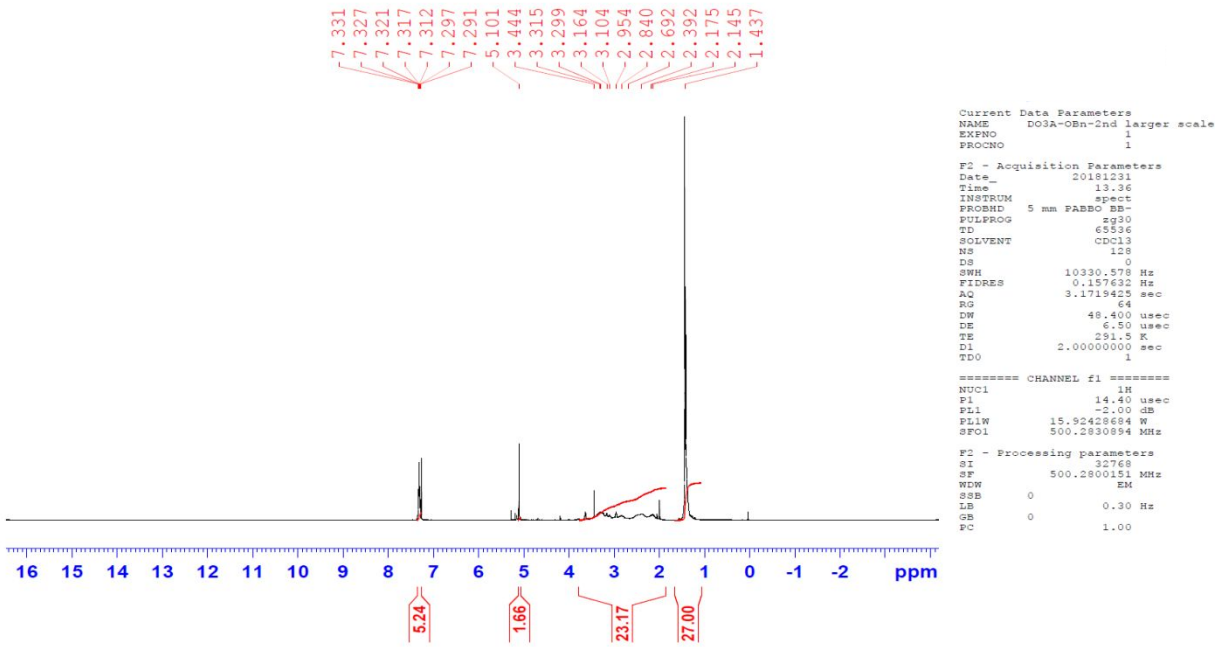

Figure S18: ^{1}H NMR Spectrum of **D2** in $CDCl_3$.

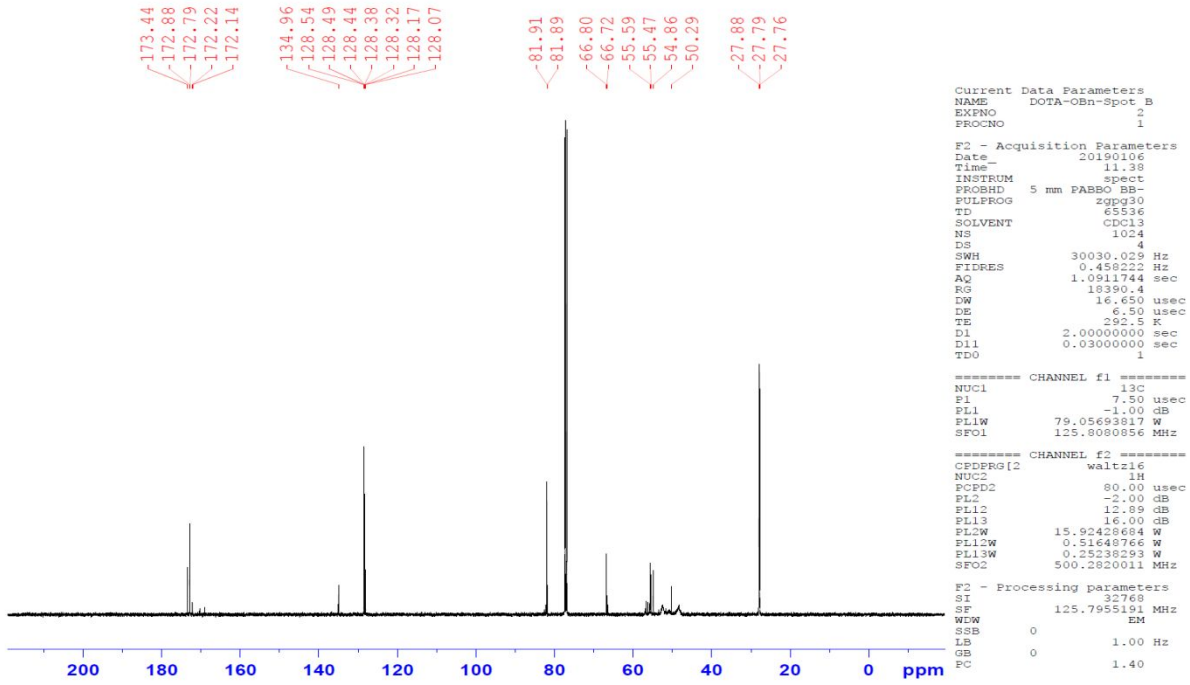

Figure S19: ^{13}C NMR spectrum of **D2** in $CDCl_3$.

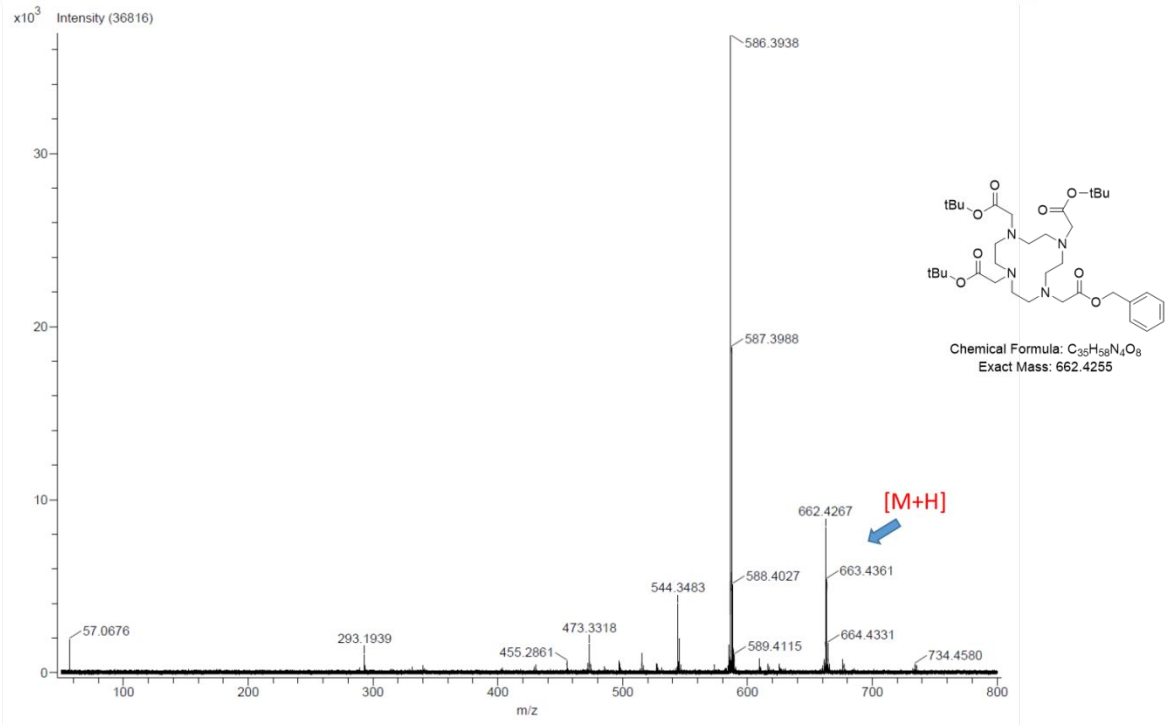

Figure S20: HR ESI-MS spectrum of **D2** in $CDCl_3$.

2-(4,7,10-Tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetic acid (D3): Both of **D2** (0.100 g, 0.151 mmol) and palladium (0.016 g; 10% Pd/C) were dissolved in methanol (10 mL). The mixture was transferred in to a hydrogenator and stirred under H_2 at 30 psi for 5 hours. The completion of the reaction was confirmed by TLC and the catalyst was removed by filtration through celite powder. The solvent was removed by rotary evaporation under *vacuo* and the residue was purified immediately using silica chromatography (DCM: MeOH 90:10%) to give **D3** as a pale yellow solid (0.080 g, 91 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 1.43 (s, 27H), 2.02 (s, 2H), 2.78 (s, 8H), 3.04 (s, 4H), 3.31 (s, 4H), 3.39 (s, 2H), 3.62 (s, 2H), 3.72 (s, 2H). ^{13}C NMR (CDCl3) δ(ppm): 28.19, 48.57, 50.31, 53.48, 55.77, 56.86, 81.87, 163.26, 167.30, 170.00, 170.73. HRMS-ESI *m/z* calcd for $[C_{28}H_{52}N_4O_8 + H]^+$: 573.3863, found:573.3840.

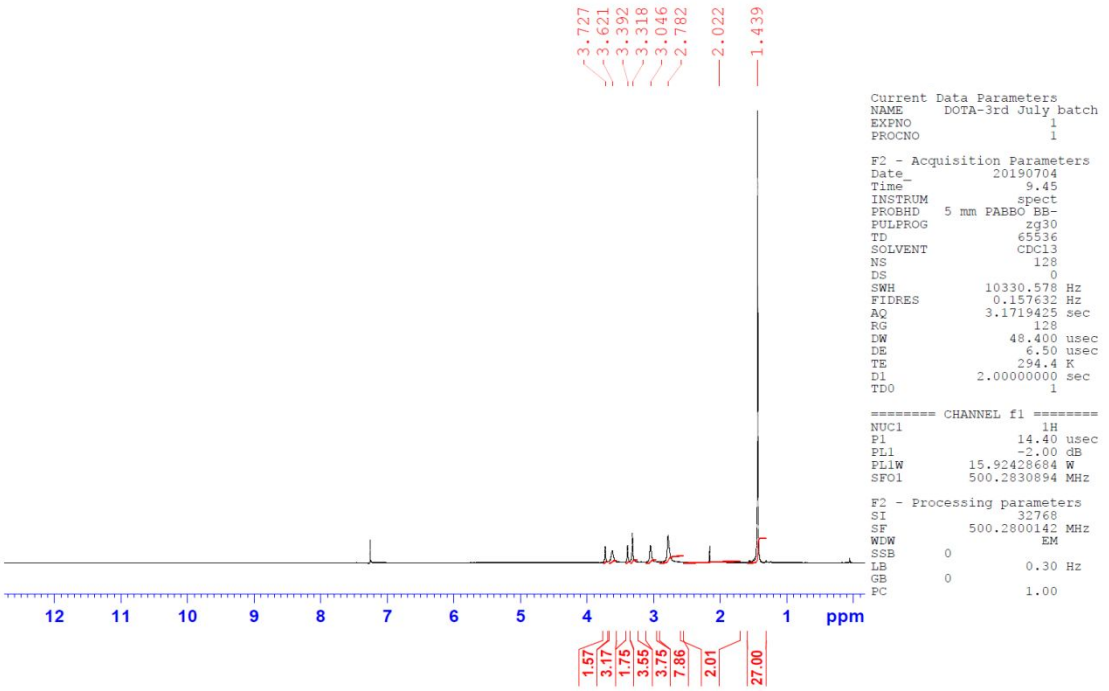

Figure S21: ^{1}H NMR Spectrum of **D3** in $CDCl_3$.

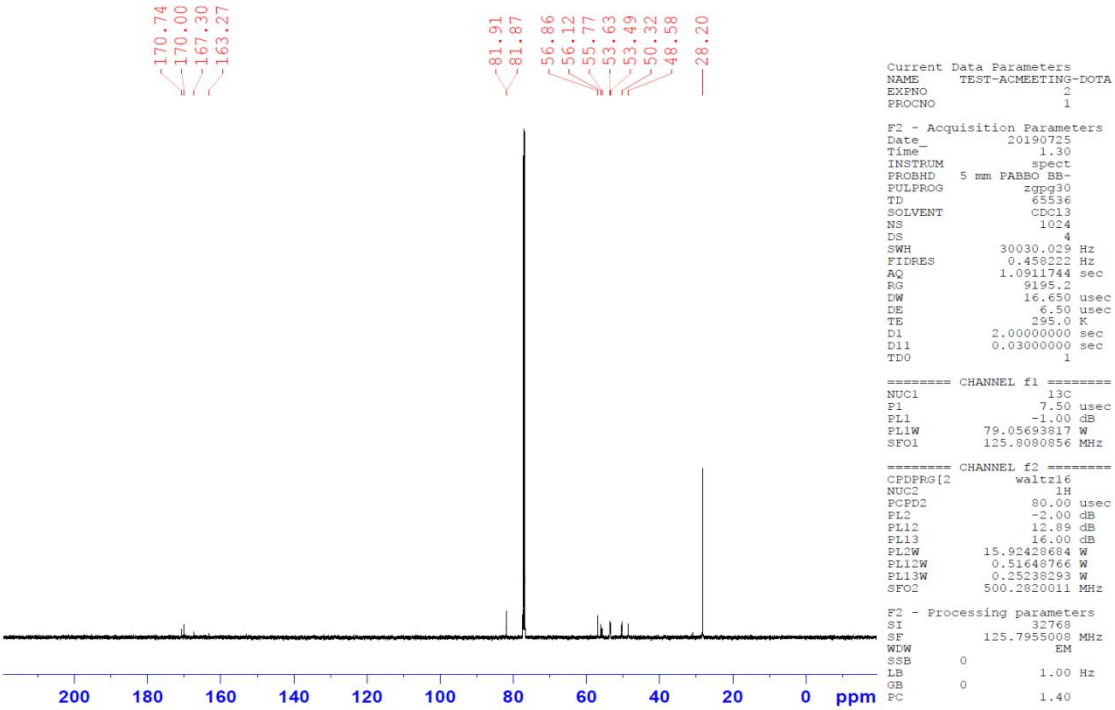

Figure S22: ^{13}C NMR spectrum of **D3** in $CDCl_3$.

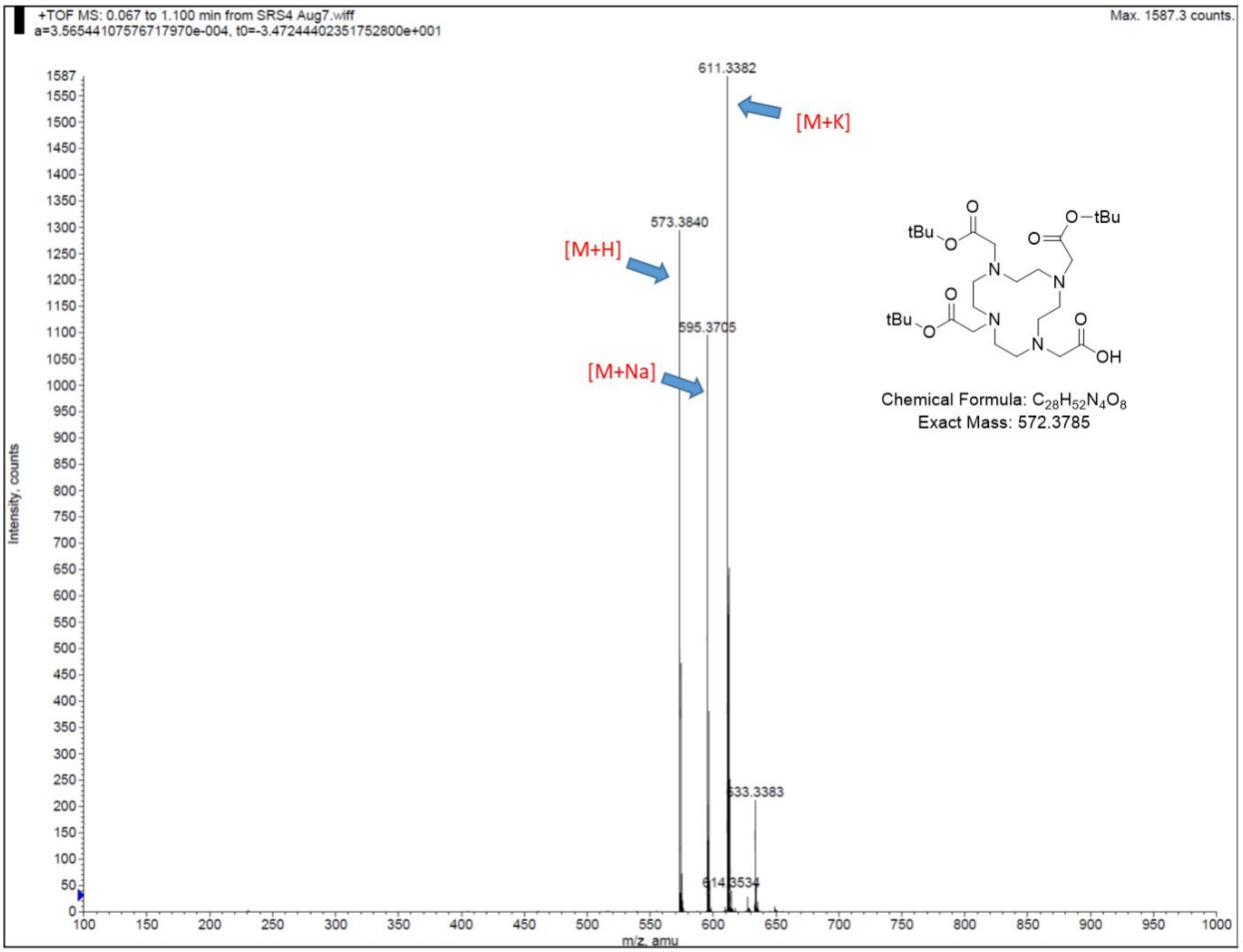

Figure S23: HR ESI-MS spectrum of **D3** in $CDCl_3$.

8. Radiolabeling of DOTA-TATE with [^{68}Ga]Ga^{3+}.

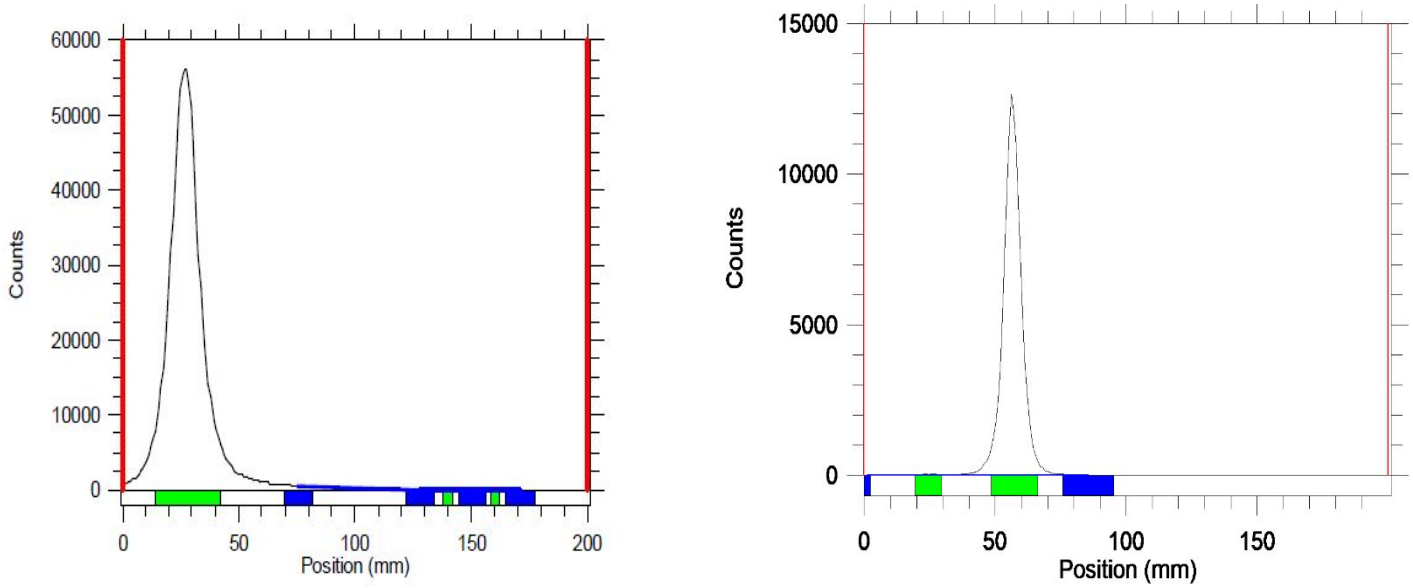

Figure S24: Radio-iTLC profile of [^{68}Ga]Ga^{3+} (left) and [^{68}Ga]Ga-DOTA-TATE (right) eluted with 1.0 M ammonium acetate and methanol (1:1) mobile phase.

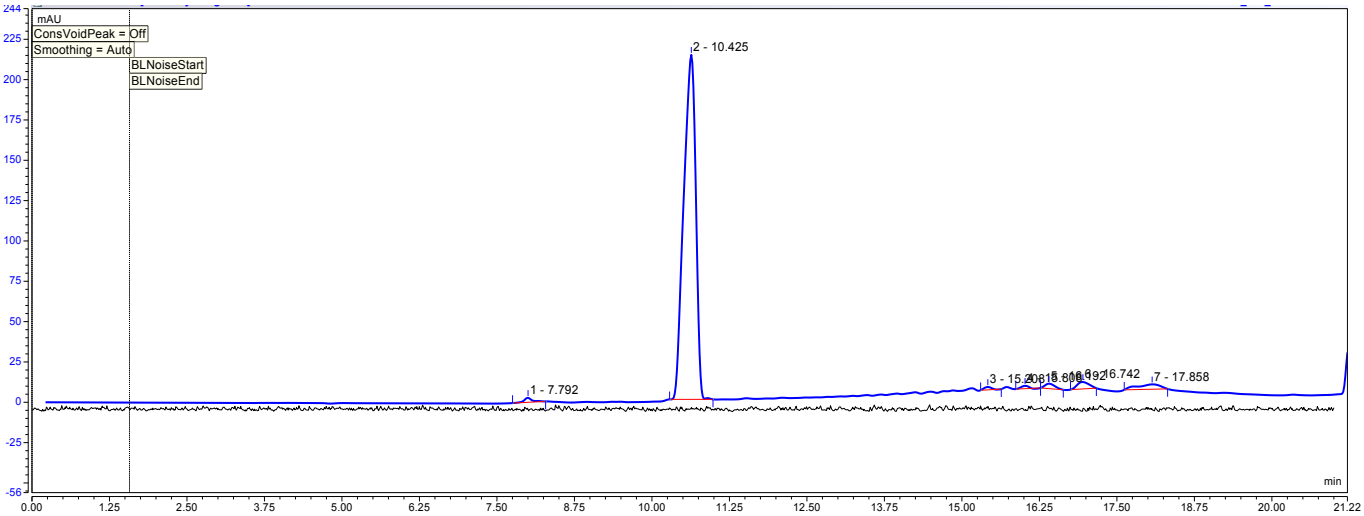

Figure S25: Reverse phase C-18 HPLC UV/Vis chromatogram for non-radiolabeled DOTA-TATE, R_T =10.42 min, Purity > 95%.

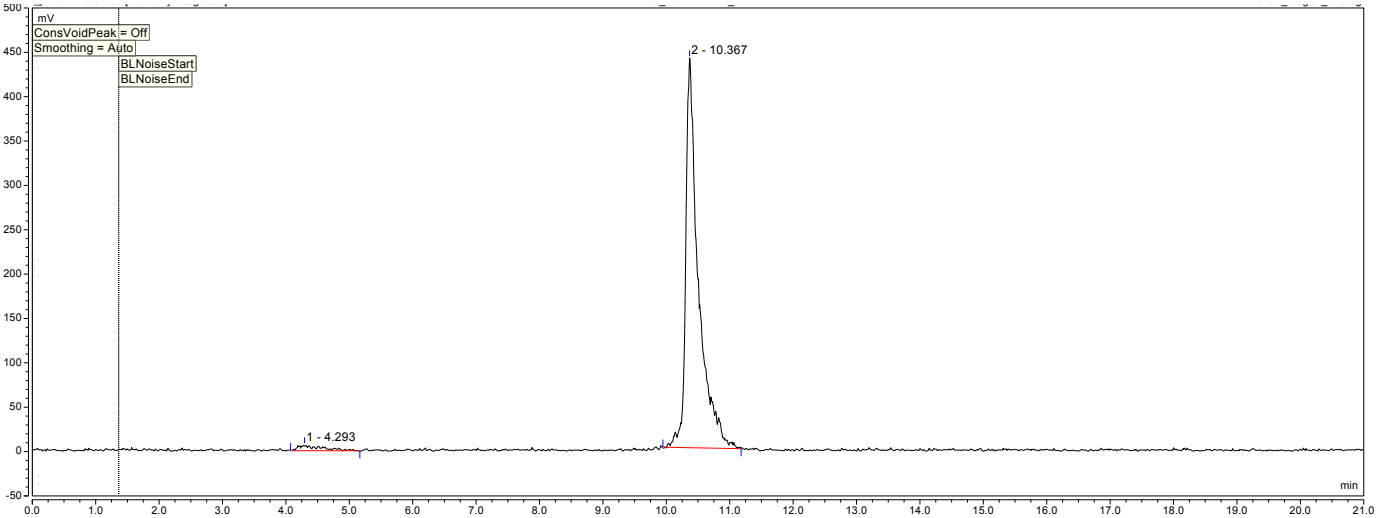

Figure S26: Radio-HPLC chromatogram for radiolabeled [^{68}Ga]Ga-DOTA-TATE, R_T =10.37 min, RCP > 95%.

References

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