Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information for: Trivalent Metal Complex Geometry of the Substrate Governs Cathepsin B Enzymatic Cleavage Rate.

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General methods. All starting materials were purchased from Acros Organics, Sigma Aldrich, Macrocyclics or TCI America and used without further purification. *NMR spectra* (^{1}H, ^{13}C, HSQC, HMBC) were collected on a 700 MHz AdvanceIII Bruker instrument at 25 °C and processed using TopSpin 3.5pl7. Chemical shifts are reported as parts per million (ppm). *Mass spectrometry:* low-resolution electrospray ionization (ESI) mass spectrometry was carried out at the Stony Brook University Institute for Chemical Biology and Drug Discovery (ICB&DD) Mass Spectrometry Facility. *High Performance Liquid Chromatography* (*HPLC):* Preparative HPLC was carried out using a Shimadzu HPLC-20AR equipped with a Binary Gradient, pump, UV-Vis detector, manual injector on a Phenomenex Luna C18 column (250 mm×21.2 mm, 100 Å, AXIA packed). Method A (preparative purification method): A = 0.1% TFA in water, B = 0.1% TFA in MeCN. Gradient: 0-5 min: 95% A. 5-24 min: 5−95% B gradient. Method B (preparative purification method): A = 10^{-2} M ammonium formate in water, B = 10% 10^{-2} M ammonium formate in water, 90% MeCN. Gradient: 0-5 min: 95% A. 5-24 min: 5−95% B gradient. CombiFlash purifications were performed on RediSep RF Gold C18 column (30G, 100 Å) using method C (A = 0.1% TFA in water, B = 0.1% TFA in MeCN. Gradient: 0-3 min: 95% A. 3-18 min: 5−95% B gradient. 18-22 min: 95% B. Flow rate: 35 mL/min. UV detection at 254 and 280 nm). HPLC characterizations were performed on the Shimadzu HPLC-20AR on a Gemini-NX C18 column (100 mm×3 mm, 110 Å, AXIA packed) using method D (A = 0.1% TFA in water, B = 0.1% TFA in MeCN. Gradient: 0-5 min: 95% A. 5-23 min: 5−95% B gradient. 23-26 min: 95% B, 0.8 mL/min). *ICP-OES* was carried out using an Agilent 5110 ICP-OES. A 10-point standard with respect to each metal was used and fits were found to be with R^2 value of 0.9999. *FT-IR Spectroscopy:* Infrared spectra were collected on a Bruker Vertex 80 spectrometer equipped with a liquid nitrogen cooled MCT detector. The samples were lyophilized and resuspended in D_2O (Cambridge Isotope Labs) prior to measurement. Samples were injected into a fixed 50 μM CaF2 cell and 128 scans/spectra were acquired and the spectrum of D_2O acquired under the same conditions was subtracted from each spectrum.

Ionic radius vs. coordination number of metal complexes.

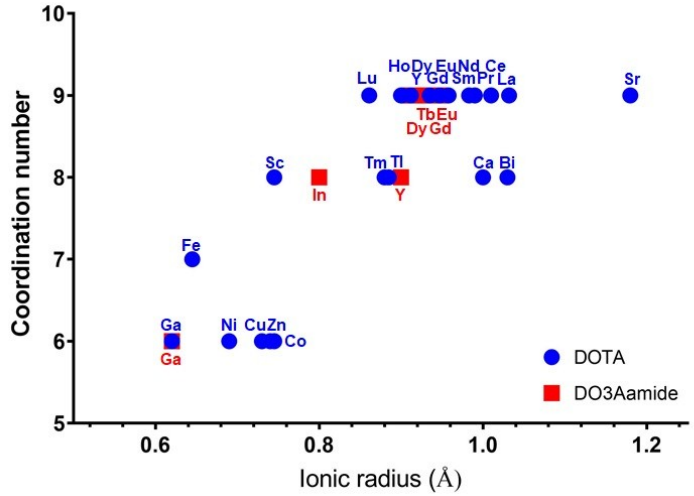


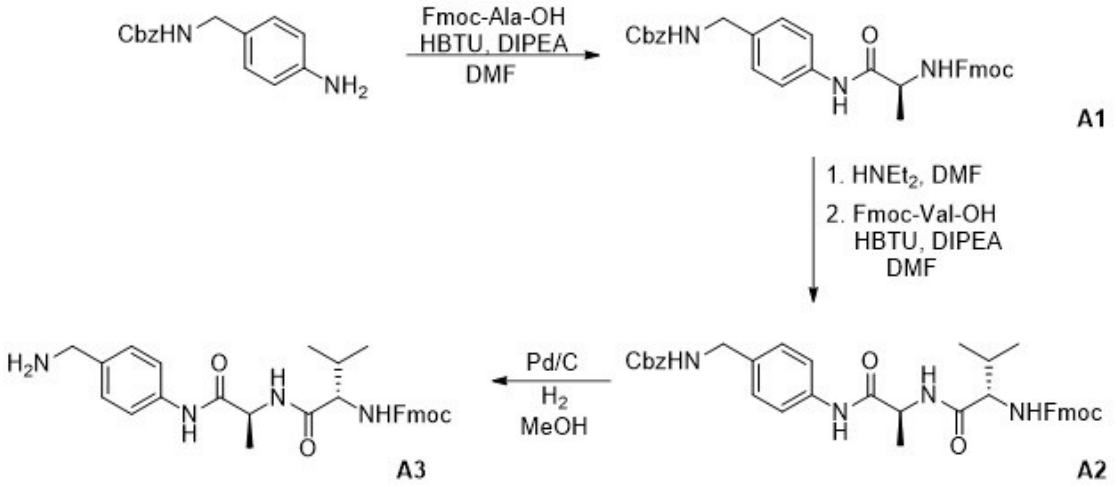
Figure S1. Plot of ionic radius vs coordination number of metal complexes of DOTA or DOTA derivatives.

Table S1. Crystal structures of M-DOTA and M-DO3Aamide complexes reported in literature.

Chelator	Metal	Coordination number	Reference
DOTA	Ga(III)	6	1, 2
	Co(II)	6	2
	Tm(III)	8	3
	Pr(III)	9	
	Nd(III)	9	
	Ce(III)	9	
	Dy(III)	9	
	Sc(III)	8	
	Ca(II)	8	4
	Bi(II)	8	5
	Fe(III)	7	6
	Gd(III)	9	
	Y(III)	9	6,7
	Ni(II)	6	8
	Cu(II)	6	
	Zn(II)	6	9
	Lu(III)	9	10
	Ho(III)	9	11
	Eu(III)	9	12
	Sr(III)	9	13

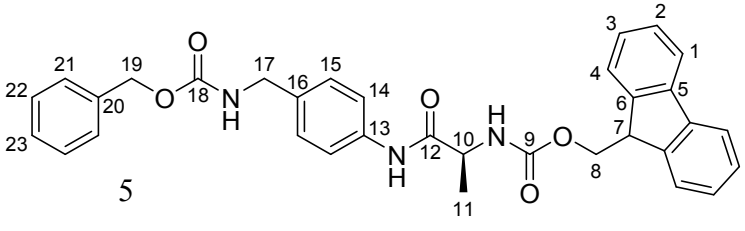
	La(III)	9	14
DO3A-aminoanilide	In(III)	8	15
DO3A-phthalate	Tb(III)	9	16
DO3A-propionamide	Gd(III)	9	17
DO3A-sulfonamide	Gd(III)	9	18
DO3A-d-PheNH_2	Ga(III)	6	19
	Y(III)	8	19
DO3A-MeAlk	Eu(III)	9	20
DO3A-carbostyril	Dy(III)	9	21

Synthesis of bifunctional chelators 2-4



Scheme S1. Synthesis of the cleavable aniline-Ala-Val-OH peptide linker.

Benzyl (4-aminobenzyl)carbamate was prepared according to literature method.[22] Fmoc-L-alanine (60 mg, 0.2 mmol) and HBTU (74 mg, 0.2



mmol) were dissolved in 5 mL dry DMF and stirred for 5 minutes. 50 mg of benzyl (4-aminobenzyl)carbamate (0.2 mmol) was dissolved in 5 mL of dry DMF and added to the above solution, followed by the addition of 67 µL of DIPEA (0.4 mmol). The reaction mixture was left to stir overnight. Upon completion as confirmed by LC-MS, the mixture was filtered and the filtrate was purified using preparative HPLC (method A), with the product eluting at 24.49 minutes. The solvent was removed in vacuo to yield **A1** as white solid (24.3 mg, 0.04 mmol, 22% yield). This reaction can be performed at a gram-scale, but due to low solubility, a different purification method should be used when working with large amounts. After removing DMF in vacuo, 8 mL of H_2O was added to the reaction flask, and the brick-red mixture was agitated using a sonicator, upon which rapid precipitation was observed. The contents were centrifuged, supernatant was decanted, and the precipitates were washed with 2x8 mL of H_2O. Afterwards, the precipitates were washed with a 50:50 solution of H_2O:MeCN, until the precipitates are white.

ESI-MS calcd. for $C_{33}H_{31}N_3O_5$: 549.23. Found: 550.2 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 700 MHz): δ 7.76 (d, 2H, H^1), 7.58 (t, 2H, H^3), 7.45 (d, 2H, H^{14}), 7.39 (t, 2H, H^2), 7.36 (m, 4H, H^4, H^{22}), 7.32 (m, 1H, H^{23}), 7.29 (m, 2H, H^{21}), 7.24 (d, 2H, H^{15}), 5.13 (s, 2H, H^{19}), 4.48 (m, 2H, H^8), 4.35 (m, 3H, H^{10}, H^{17}), 4.22 (t, 1H, H^7), 1.46 (d, 3H, H^{11}).

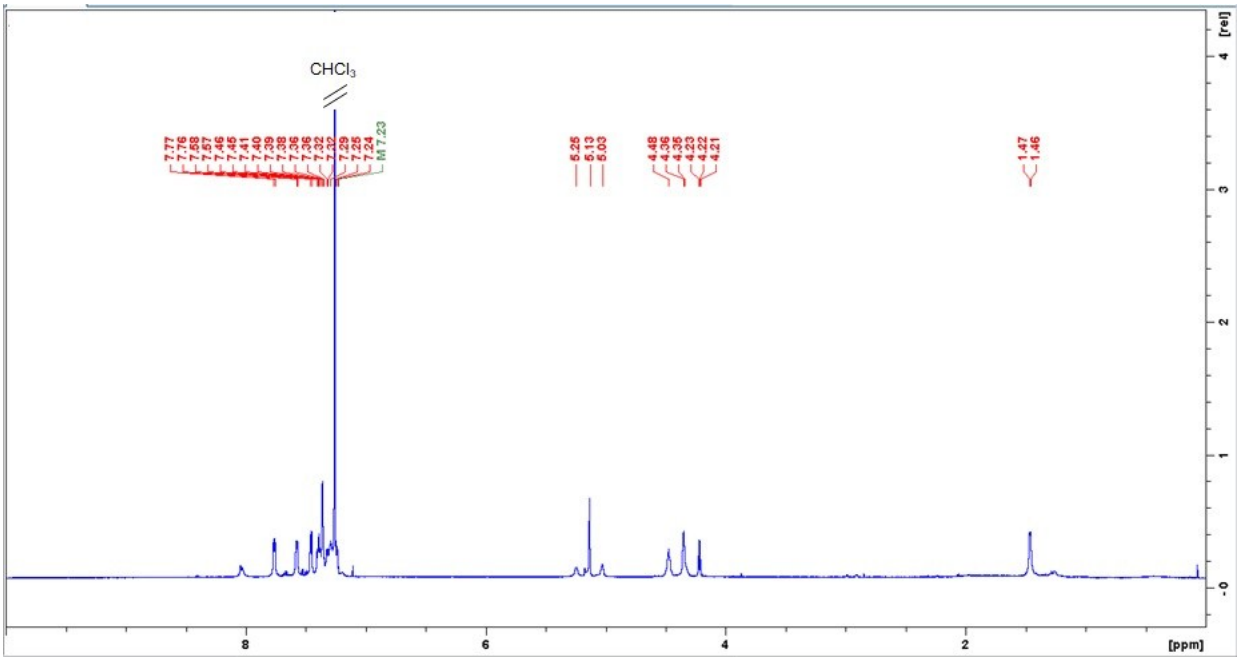
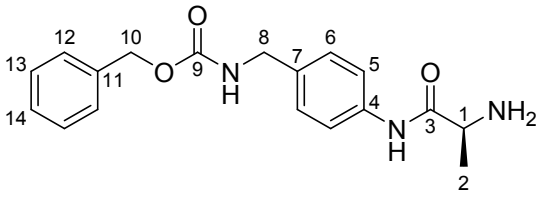


Figure S2. ^{1}H NMR spectrum of **A1** in $CDCl_3$.

A1 (20.0 mg, 0.036 mmol) and diethylamine (DEA, 56 µL, 0.54 mmol) were dissolved in 1 mL of DMF, and the solution was stirred at room temperature for 2 h. The solvent was then removed in vacuo, and the product was purified using method C. The product elutes at 40% organic media, and upon removal of solvent, **A2** is obtained as white solid (10.8 mg, 92% yield).



ESI-MS calcd. for $[C_{18}H_{21}N_3O_3]$: 327.16. Found: 328.1 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 700 MHz): δ 7.46 (d, 2H, H^5), 7.39 (m, 2H, H^{13}), 7.32 (m, 1H, H^{14}), 7.28 (m, 2H, H^{12}), 7.21 (d, 2H, H^6), 5.12 (s, 2H, H^{10}), 4.34 (m, 3H, H^1, H^8), 1.46 (d, 3H, H^2).

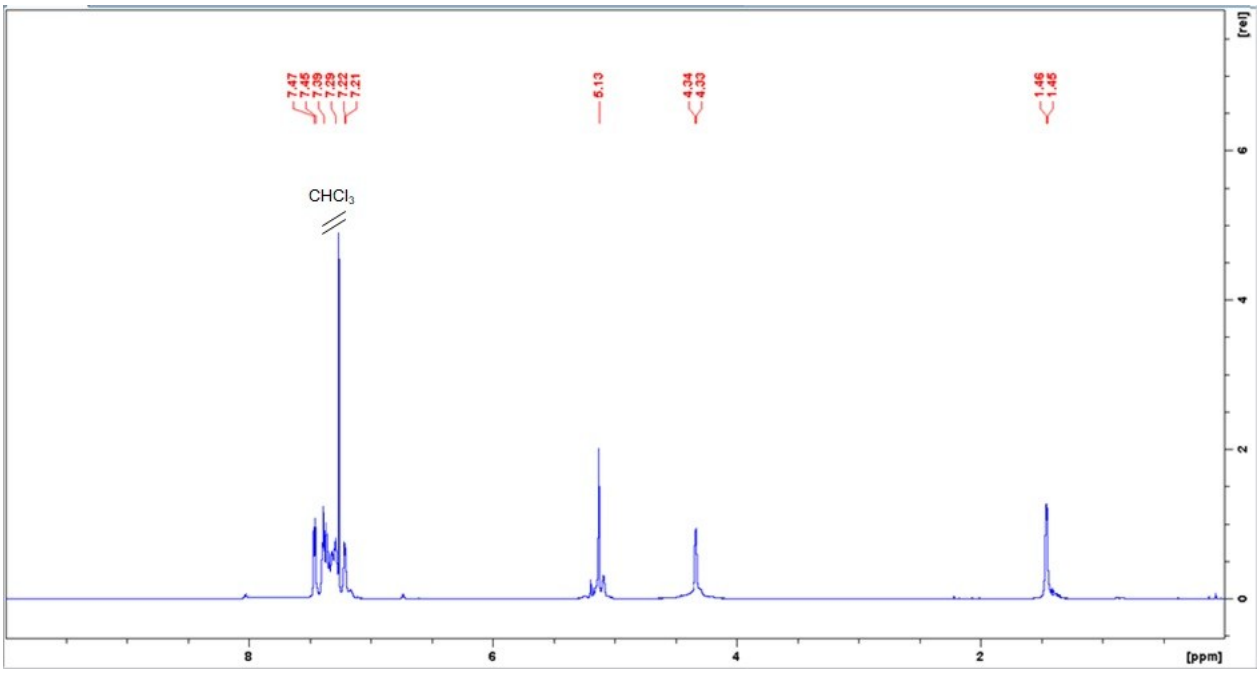
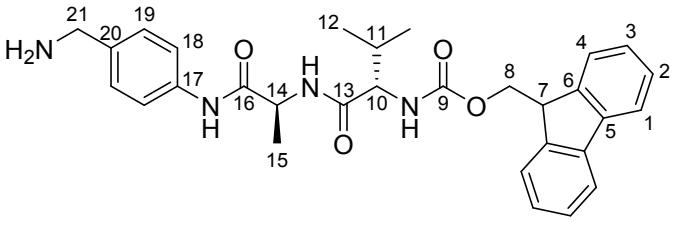


Figure S3. ^{1}H NMR spectrum of **A2** in $CDCl_3$.

Fmoc-l-valine (60.0 mg, 0.17 mmol) and HBTU (68 mg, 0.17 mmol) were dissolved in 2 mL of dry DMF and stirred for 10 min. **A2** (58.5 mg, 0.18 mmol) was dissolved in 1 mL of dry DMF and added to the above solution, followed by 60 µL of DIPEA (0.34 mmol). The reaction mixture was stirred at room temperature overnight. Upon completion of reaction as determined from LC-MS, the solvent was removed in vacuo, and the product was purified using method C,



eluting at 75% organic media. Upon removal of solvent, the Cbz-protected aniline-Ala-Val-Fmoc is obtained as white solid, with small amounts of TFA salt (113.0 mg, 0.17 mmol).

ESI-MS calcd. for $[C_{38}H_{40}N_4O_6]$: 648.29. Found: 649.3 $[M+H]^+$.

Cbz-protected aniline-Ala-Val-Fmoc is not readily soluble in common deuterated solvents, and therefore no NMR characterization was performed. The Cbz-protected molecule was dissolved in 10 mL of THF:MeOH (1:1), and 10 mg of Pd/C were added to this solution. After stirring under H_2 atmosphere for 4 h, the solution was filtered, and solvent removed in vacuo to afford **A3** as a colorless solid (80.6 mg, 0.16 mmol, some TFA salt present).

ESI-MS calcd. for $[C_{30}H_{34}N_4O_4]$: 514.26. Found: 515.3 $[M+H]^+$.

^{1}H NMR (CD_3OD, 700 MHz): δ 7.79 (d, 2H, H^1), 7.66 (m, 2H, H^3), 7.39 (m, 4H, H^2,H^{18}), 7.30 (m, 4H, H^4, H^{19}), 4.45 (m, 2H, H^8), 4.37 (m, 2H, H^{10}, H^{14}), 4.22 (t, 1H, H^7), 4.05 (s, 2H, H^{21}), 2.07 (m, 1H, H^{11}), 1.44 (d, 3H, H^{15}). 0.97 (m, 6H, H^{12}).

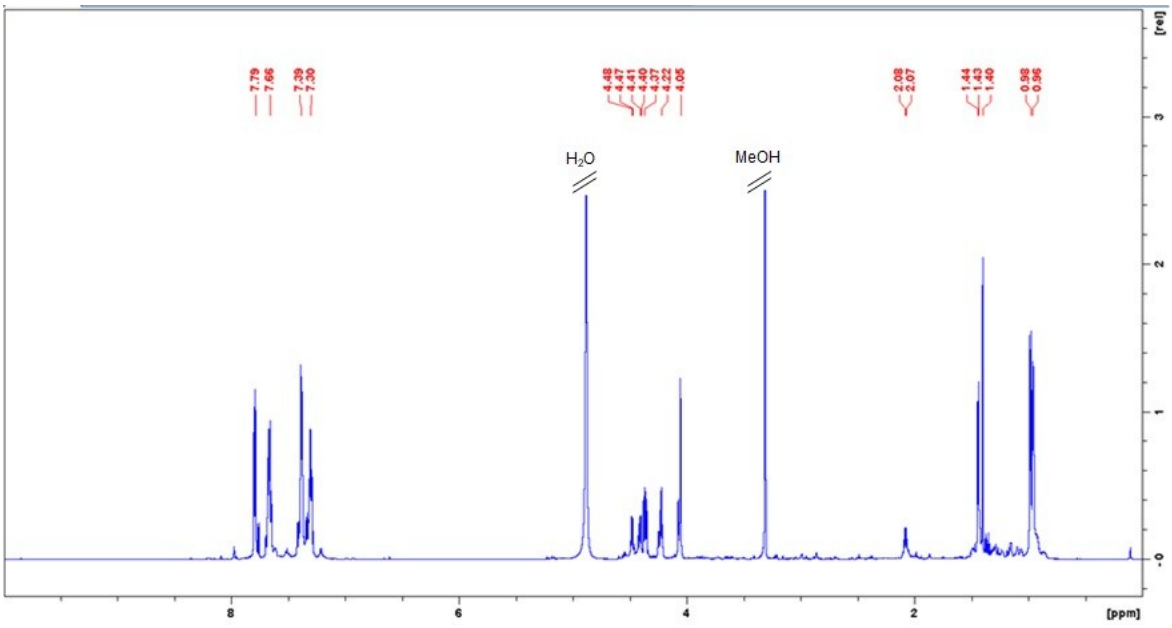
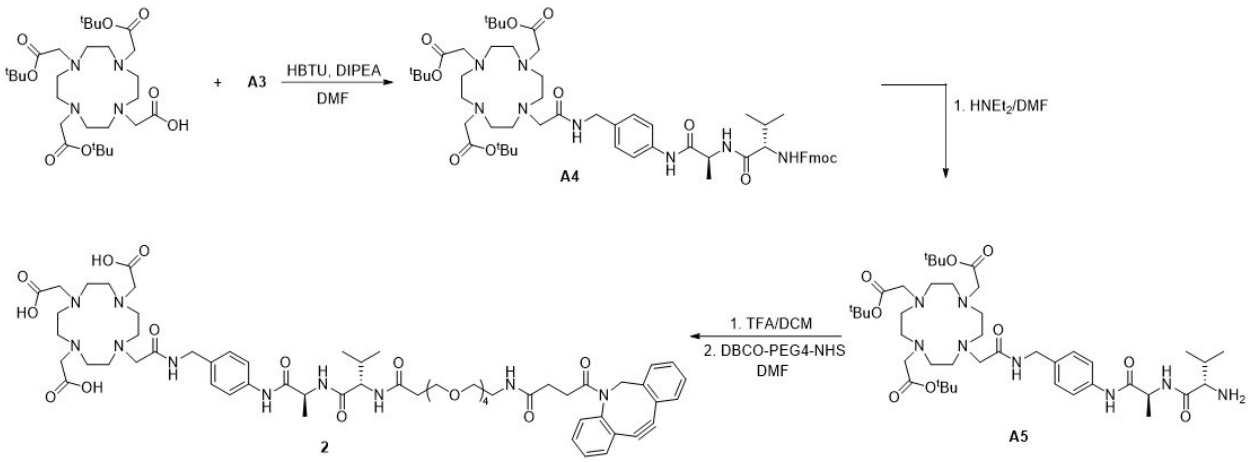
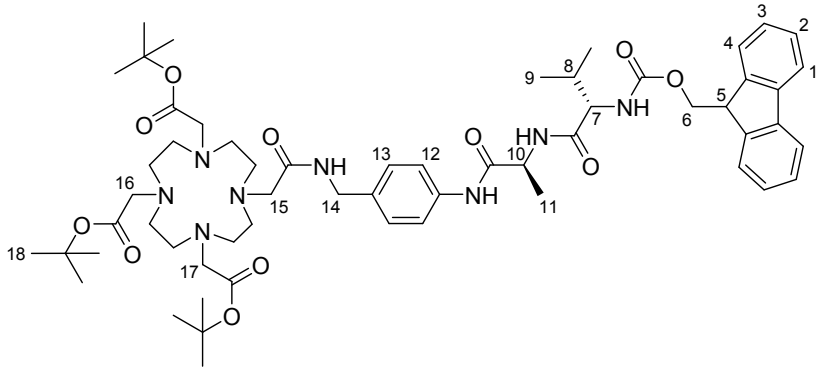


Figure S4. ^{1}H NMR spectrum of **A3** in CD_3OD.



Scheme S2. Synthesis of the cleavable conjugate **2**.



DOTA-tri-t-Bu-ester (22.0 mg, 0.04 mmol) and HBTU (16.2 mg, 0.04 mmol) were dissolved in dry DMF and stirred for 20 minutes. **A3** (20.0 mg, 0.04 mmol) was then added to the solution, followed by 13 µL of DIPEA (0.08 mmol). The reaction mixture was left to stir overnight at room temperature. Upon completion, as determined from LC-MS, the solvent was removed in vacuo, and the product was purified using method A, eluting at 25.21 min. The solvent was removed in vacuo, and **A4** is obtained as colorless solid (19.2 mg, 0.018 mmol, 45% yield).

ESI-MS calcd. for $[C_{58}H_{84}N_8O_{11}]$: 1068.63. Found: 1069.7 $[M+H]^+$.

^{1}H NMR (CD_3OD, 500 MHz): δ 7.80 (d, 2H, H^1), 7.67 (m, 2H, H^3), 7.55 (m, 2H, H^{12}), 7.40 (m, 2H, H^2), 7.32 (m, 2H, H^4), 7.24 (m, 2H, H^{13}), 4.49 (m, 2H, H^6), 4.41 (m, 3H, H^5, H^{14}), 4.32 (m, 1H, H^{10}), 4.24 (m, 1H, H^7), 3.79-2.92 (m, 16H, H^{cyclen}), 3.54 (br. s, 8H, $H^{15,16,17}$), 2.09 (m, 1H, H^8), 1.45 (s, 27H, H^{18}), 1.30 (s, 3H, H^{11}), 0.98 (m, 6H, H^9).

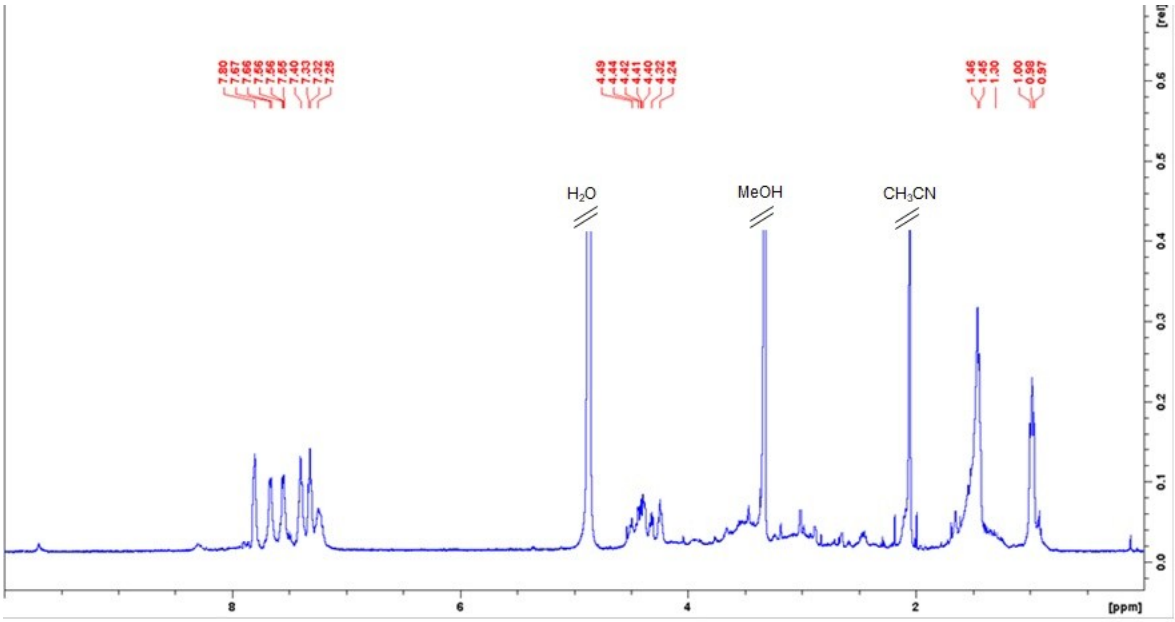
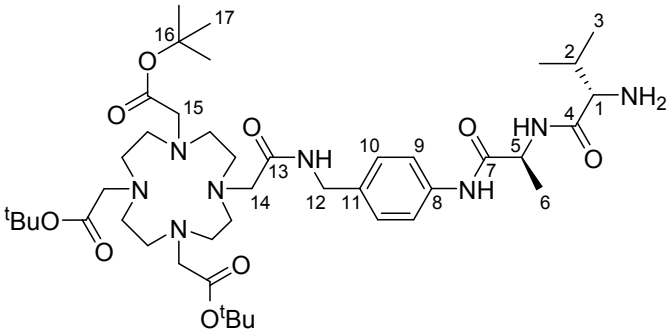


Figure S5. ^{1}H NMR spectrum of **A4** in CD_3OD.

A4 (22.3 mg, 0.02 mmol) and $HNEt_2$ (38 µL, 0.4 mmol) are dissolved in 1 mL of dry DMF and stirred for 4h at room temperature. The solvent is removed in vacuo, and **A5** is purified using method C eluting at 40% organic media (14.2 mg, 0.017 mmol, 84% yield).



ESI-MS calcd. for $[C_{43}H_{74}N_8O_9]$: 846.56. Found: 847.6 $[M+H]^+$.

^{1}H NMR ($CDCl_3$, 700 MHz): δ 7.43 (m, 2H, H^{9}), 7.34 (m, 2H, H^{10}), 4.53 (m, 2H, H^{12}), 4.33 (m, 1H, H^{5}), 4.24 (m, 1H, H^{1}), 3.79-2.92 (m, 16H, H^{cyclen}), 3.51 (s, 8H, H^{14}, H^{15}), 2.03 (m, 1H, H^{2}), 1.47 (s, 27H, H^{17}), 1.28 (s, 3H, H^{6}), 0.91 (m, 6H, H^{3}).

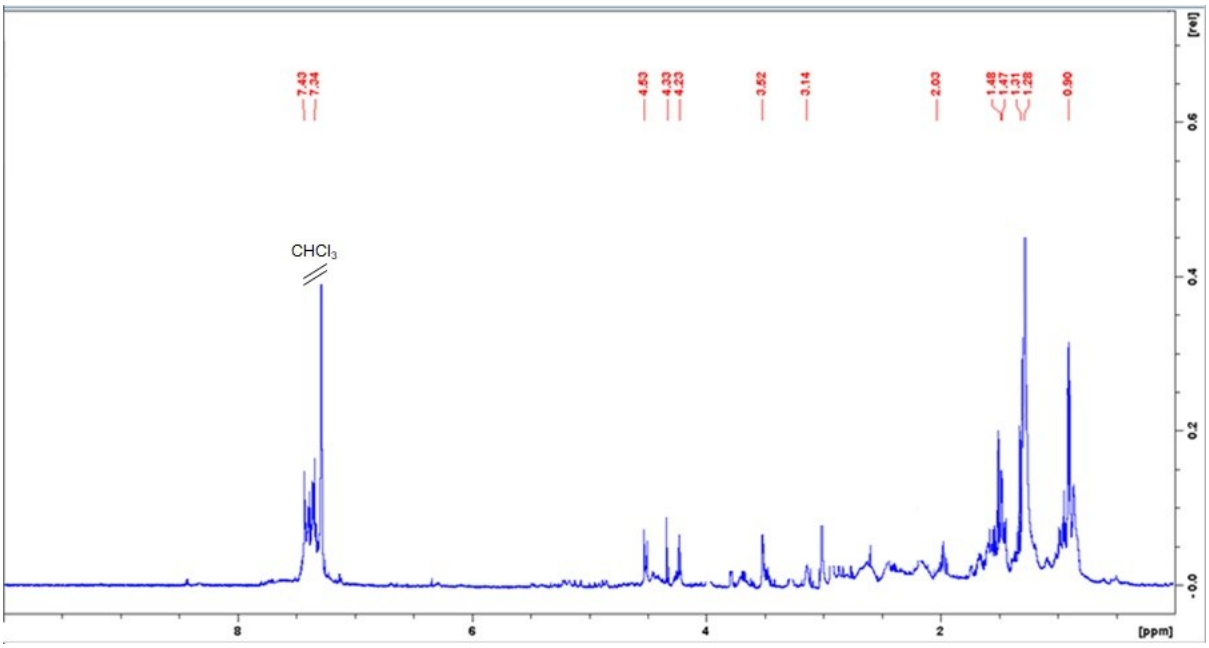
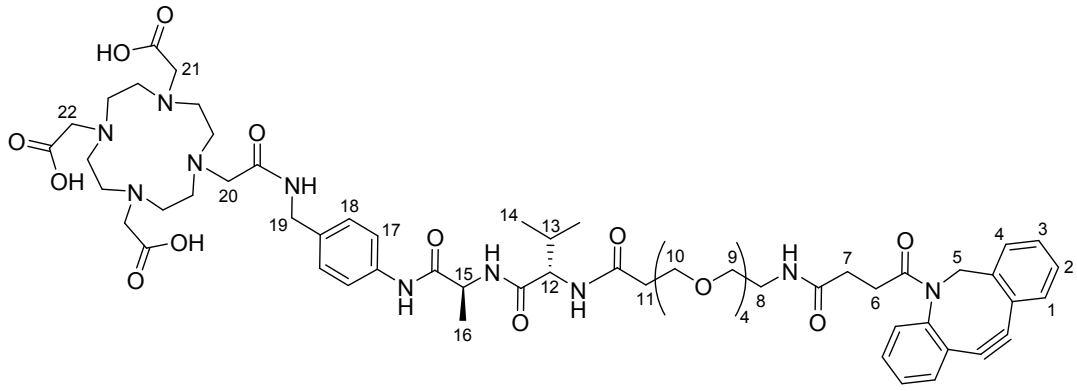


Figure S6. ^{1}H NMR spectrum of **A5** in $CDCl_3$.



To a vial containing **A5** (4 mg, 6 µmol) and DBCO-PEG4-NHS ester (4 mg, 6 µmol) dissolved in 1 mL of DMF, DIPEA (2 µL, 12 µmol) is added, and the reaction mixture is stirred overnight at room temperature. Upon completion of reaction as monitored via LC-MS, the product is purified using method B (Rt = 16.3 min). The solvent is removed in vacuo to yield **2** as white solid (1.2 mg, 1.0 µmol, 16% yield).

ESI-MS calcd. for $[C_{61}H_{84}N_{10}O_{16}]$: 1212.61. Found: 1213.7 $[M+H]^+$.

^{1}H NMR (D_2O, 700 MHz): δ 7.38-7.53 (m, 10H, H^{1}-H^{4}, H^{17}), 7.34 (m, 2H, H^{18}), 5.17 (s, 2H, H^{5}), 4.53 (m, 2H, H^{19}), 4.33 (m, 1H, H^{15}), 4.24 (m, 1H, H^{12}), 3.70 (s, 8H, H^{20}-H^{22}), 3.54-3.68 (m, 20H, H^{8}, H^{9}, H^{10}, H^{cyclen}), 3.23-3.49 (m, 6H, H^{cyclen}), 3.05-3.20 (m, 4H, H^{cyclen}), 2.59 (m, 2H, H^{11}), 2.43-

2.53 (m, 4H, H^6, H^7), 2.24-2.42 (m, 4H, H^{cyclen}), 2.05 (m, 1H, H^{13}), 1.30 (s, 3H, H^{16}), 0.89 (m, 6H, H^{14}).

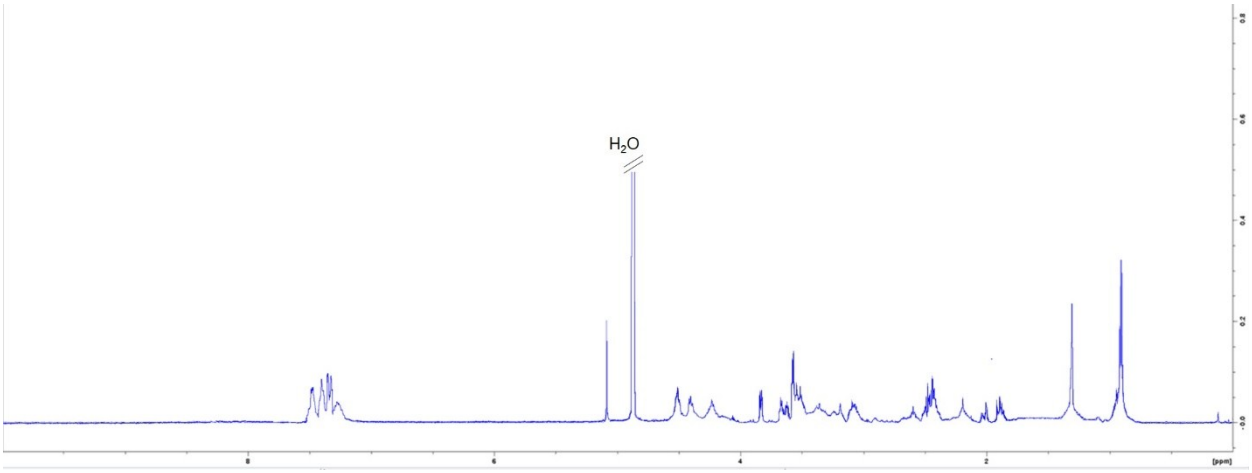


Figure S7. ^{1}H NMR spectrum of **2** in CD_3OD.

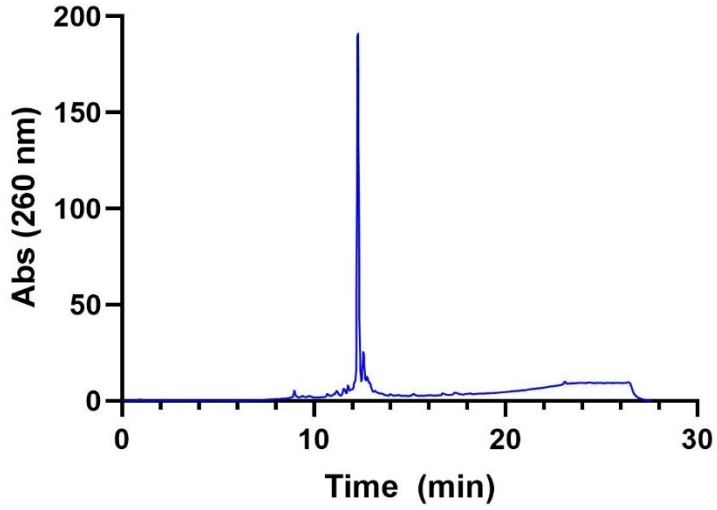
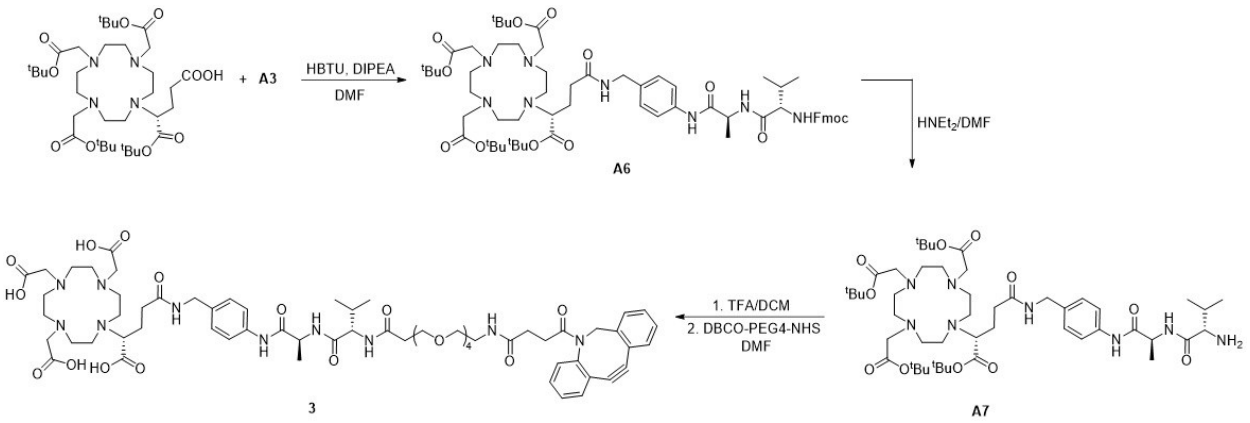
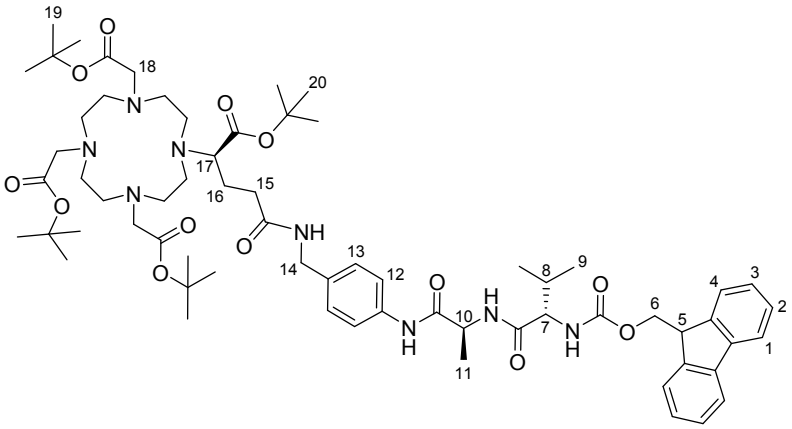


Figure S8. HPLC spectrum of **2**. Rt (method D) = 12.17 min.



Scheme 3. Synthesis of the cleavable conjugate **3**.



(*R*)-*tert*-Bu$_4$-DOTAGA was prepared according to literature procedure.[23] (*R*)-*tert*-Bu$_4$-DOTAGA (68.0 mg, 0.1 mmol) and HBTU (41.0 mg, 0.11 mmol) were dissolved in 2 mL DMF and stirred for 30 minutes at room temperature. **A3** (50.0 mg, 0.1 mmol) was dissolved in 1 mL of DMF and added to the above solution, followed by the addition of 67 µL (0.4 mmol) of DIPEA. The reaction was stirred overnight at room temperature. Upon completion of reaction as observed by LC-MS, the solvent was removed in vacuo, and the product was purified using method C, eluting at 80% organic media. Upon removal of solvent, **A6** is obtained as a white solid (22.3 mg, 0.02 mmol, 20% yield).

ESI-MS calcd. for [$C_{65}H_{96}N_8O_{13}$]: 1196.71. Found: 1197.8 $[M+H]^+$.

^{1}H NMR (CD_3OD, 500 MHz): δ 7.80 (d, 2H, H^1), 7.67 (t, 2H, H^3), 7.55 (m, 2H, H^{12}), 7.40 (t, 2H, H^2), 7.32 (m, 2H, H^4), 7.25 (m, 2H, H^{13}), 4.40 (m, 6H, H^5, H^6, H^{10}, H^{14}), 4.24 (m, 1H, H^7), 4.16-2.92 (m, 18H, H^{15}, H^{cyclen}), 3.94 (m, 1H, H^{17}), 3.82 (s, 6H, H^{18}), 2.10 (m, 2H, H^{16}), 2.08 (m, 1H, H^8), 1.51 (m, overlapping signals, 39H, H^{11}, H^{19}, H^{20}), 0.99 (m, 6H, H^9).

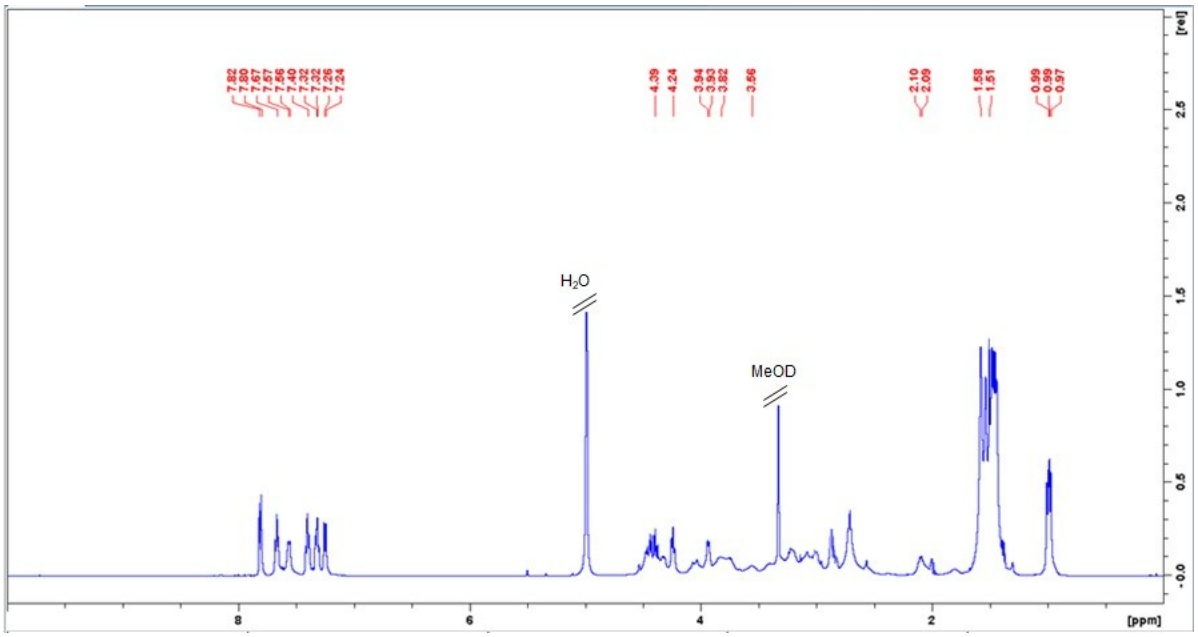
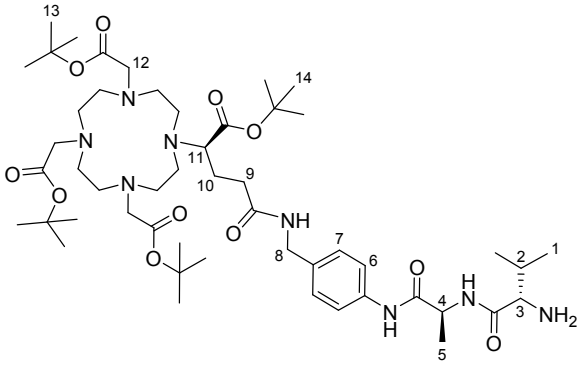


Figure S9. ^{1}H NMR spectrum of **A6** in CD_3OD.

A6 (22.3 mg, 0.02 mmol) and $HNEt_2$ (38 µL, 0.4 mmol) were dissolved in 1 mL of DMF and stirred at room temperature for 4 hours. The solvent was removed in vacuo, and the product was purified using method C, eluting at 80% organic media. The solvent was removed and **S7** was obtained as a white solid (17.3 mg, 0.02 mmol, 89% yield).



ESI-MS calcd. for $[C_{50}H_{86}N_8O_{11}]$: 974.64. Found: 975.5 $[M+H]^+$.

^{1}H NMR (CD_3OD, 700 MHz): δ 7.55 (m, 2H, H^6), 7.25 (m, 2H, H^7), 4.37 (m, 3H, H^4, H^8), 4.26 (m, 1H, H^3), 4.12-3.01 (m, 19H, H^9, H^{11}, H^{cyclen}), 3.46 (s, 6H, H^{12}), 2.10 (m, 1H, H^{10}), 1.81 (m, 1H, H^{10}), 1.55 (s, 39H, H^5, H^{13}, H^{14}), 1.09 (m, 6H, H^9).

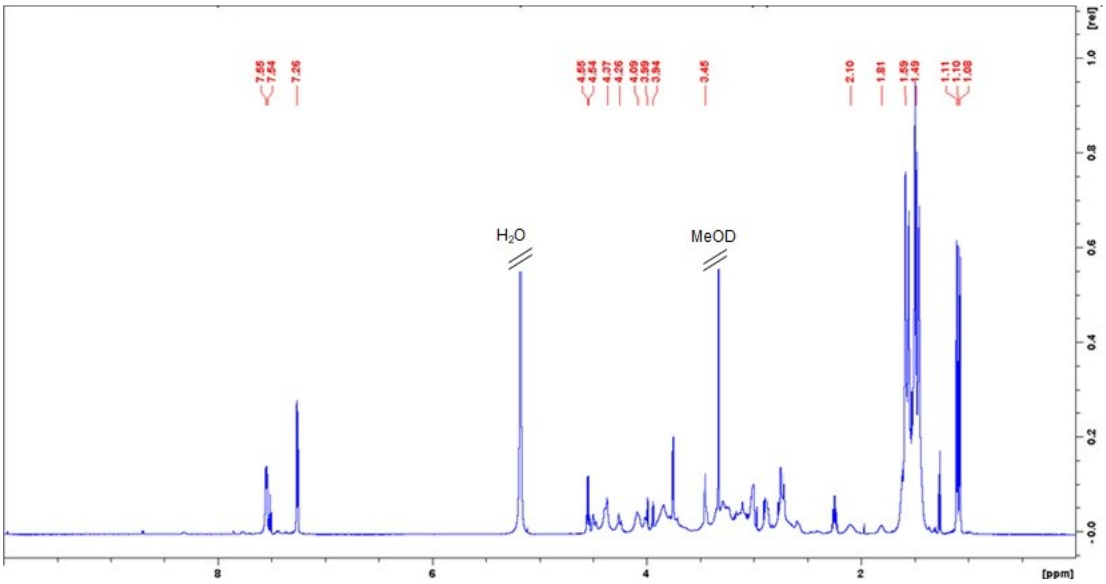
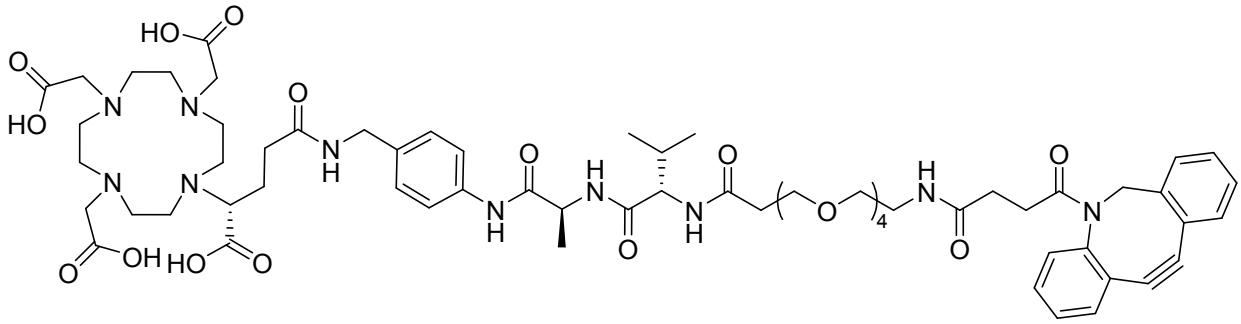


Figure S10. ^{1}H NMR spectrum of **A7** in CD_3OD.



A7 (10.3 mg, 0.01 mmol) was dissolved in 1 mL of DCM and 2 mL of TFA. The reaction mixture was stirred at room temperature for 10 h, then the solvent was removed in vacuo to yield fully deprotected DOTAGA-aniline-Ala-Val as a white powder. This substrate was then dissolved in 1 mL of DMF with DBCO-PEG4-NHS ester (7 mg, 0.01 mmol) and DIPEA (7 µL, 0.04 mmol) and stirred overnight at room temperature. Upon completion of reaction as monitored via LC-MS, the product is purified using method B (Rt = 14.4 min). The solvent is removed in vacuo to yield **3** as white solid (1.2 mg, 1.0 µmol, 10% yield).

ESI-MS calcd. for $[C_{64}H_{88}N_{10}O_{18}]$: 1284.63. Found: 1285.7 $[M+H]^+$.

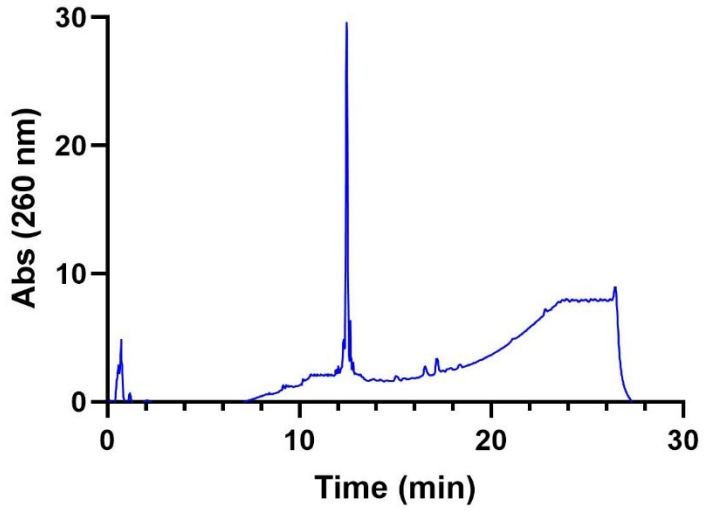
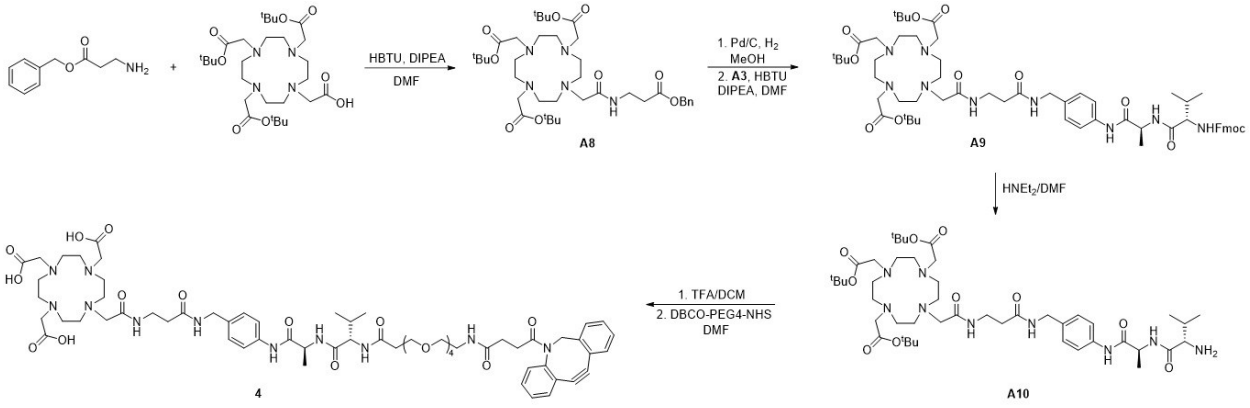
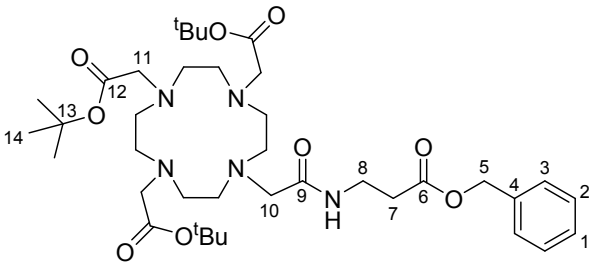


Figure S11. HPLC spectrum of **3**. Rt (Method D): 12.45 min.



Scheme 4. Synthesis of the cleavable conjugate **4**.

DOTA-tri-tertbutyl-ester (150 mg, 0.26 mmol) and HBTU (100 mg, 0.26 mmol) were dissolved in 3 mL of DMF and stirred at room temperature for 20 minutes. O-benzyl-β-Alanine (43.0 mg, 0.24 mmol) was prepared according to literature procedures,[24] and added to the above solution, followed by the addition of DIPEA (90 µL, 0.5 mmol). The reaction mixture was stirred overnight at room temperature, then the product was purified using method C, eluting at 85% organic media. The solvent was removed in vacuo and **A8** was obtained as a brown oil (78.5 mg, 0.11 mmol, 45% yield).



ESI-MS calcd. for [$C_{38}H_{63}N_5O_9$]: 733.46. Found: 734.5 $[M+H]^+$.

^{1}H NMR (CD_3OD, 700 MHz): δ 7.37 (m, 5H, H^1, H^2, H^3), 5.15 (H^5), 3.53 (br. s, 8H, H^{10}, H^{11}), 4.13-2.83 (m, 8H, H^{cyclen}), 3.29 (t, 2H, H^8), 2.79 (t, 2H, H^7), 1.51 (s, 27H, H^{14}).

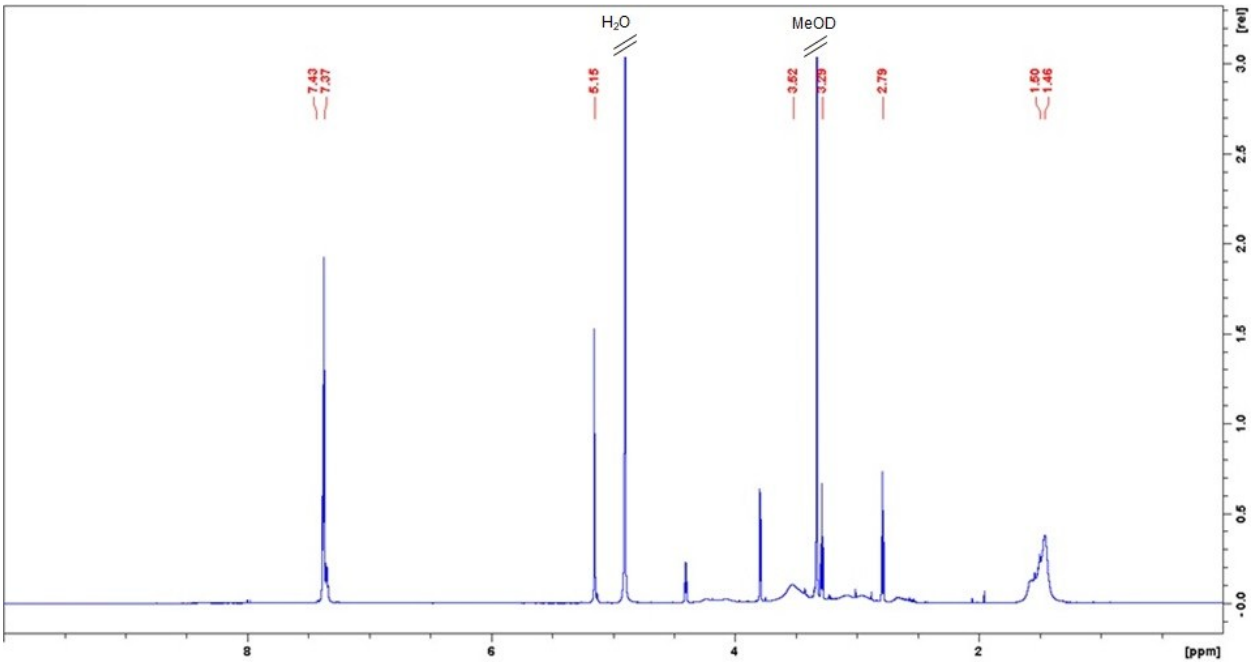
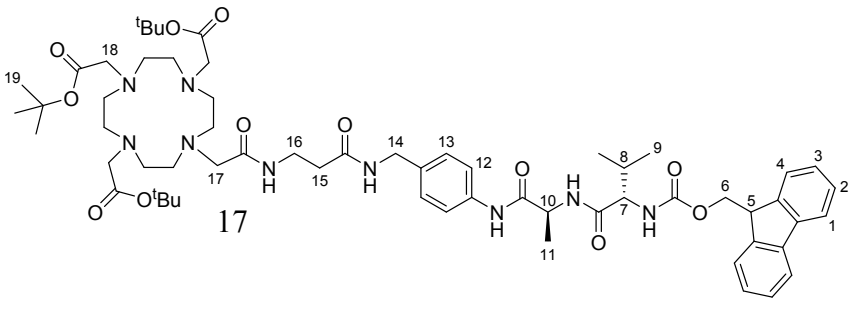


Figure S12. ^{1}H NMR spectrum of **A8** in CD_3OD.

To a solution of **A8** (30.0 mg, 0.04 mmol) dissolved in MeOH (2 mL), a suspension of Pd/C catalyst (10% w/w, 3.0 mg) in MeOH (4 mL)



was added. The flask was evacuated, charged with H_2 (1 atm), and then stirred for 3 h, until no more starting material was detected according to LC-MS (ESI-MS calcd. for $[C_{31}H_{57}N_5O_9]$: 643.42. Found: 644.5 $[M+H]^+$). The reaction mixture was filtered, and the volatiles were removed from the filtrate to afford the debenzylated material as a white powder. The debenzylated molecule and HBTU (14 mg, 0.04 mmol) were dissolved in 3 mL DMF and stirred at room temperature for 20 minutes. **A3** (22 mg, 0.04 mmol) and DIPEA (26 µL, 0.15 mmol) were added to the above solution, and the reaction mixture was stirred overnight at room temperature. The solvent was removed in vacuo, and the product was purified using method A, eluting at 17.7 minutes. The solvent was once again removed in vacuo to afford **A9** as a white powder (18.9 mg, 0.02 mmol, 41% yield).

ESI-MS calcd. for $[C_{61}H_{89}N_9O_{12}]$: 1139.66. Found: 1140.6 $[M+H]^+$.

^{1}H NMR (CD_3OD, 700 MHz): δ 7.82 (d, 2H, H^1), 7.68 (t, 2H, H^3), 7.56 (d, 2H, H^{12}), 7.41 (m, 2H, H^2), 7.33 (m, 2H, H^4), 7.26 (d, 2H, H^{13}), 4.46 (m, 2H, H^6), 4.41 (m, 2H, H^7, H^{10}), 4.33 (s, 2H, H^{14}), 4.26 (t, 1H, H^5), 3.53 (br. s, 8H, H^{17}, H^{18}), 4.13-2.83 (m, 8H, H^{cyclen}), 3.29 (t, 2H, H^{16}), 2.59 (m, 2H, H^{15}), 2.11 (m, 1H, H^8), 1.51 (s, 30H, H^{11}, H^{19}), 0.99 (dd, 6H, H^9).

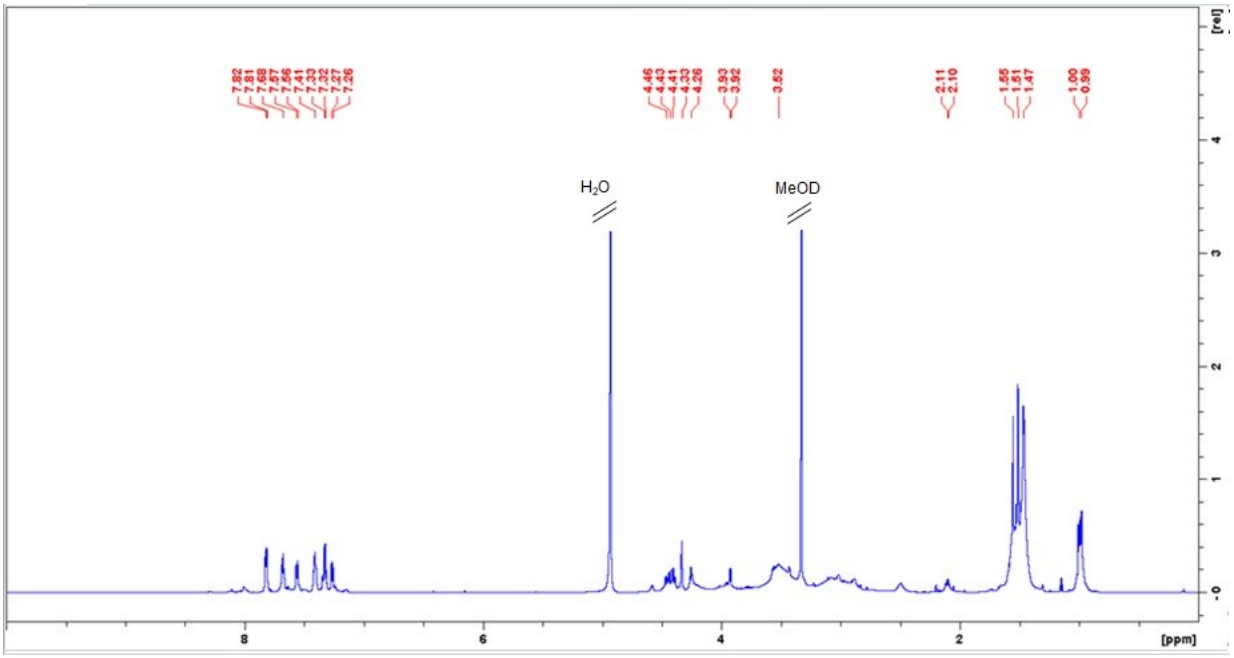
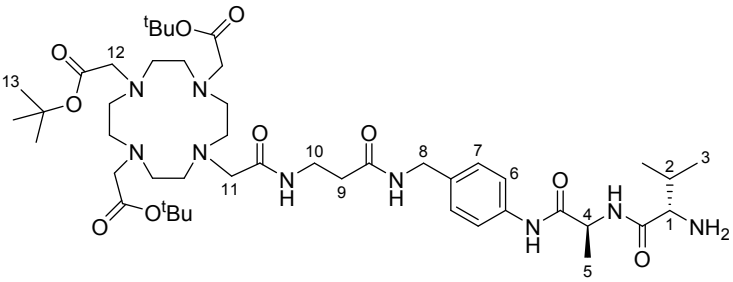


Figure S13. ^{1}H NMR spectrum of **A9** in CD_3OD.

To a solution of **A9** (18.5 mg, 0.01 mmol) dissolved in 2 mL of DMF, $HNEt_2$ (34 µL, 0.3 mmol) and the solution was stirred at room temperature for 3h. The solvent was removed in vacuo, and the deprotected product was purified using method C,



eluting at 80% organic media. The solvent was removed in vacuo, and **A10** was obtained as a white powder (6.4 mg, 0.007 mmol, 44% yield).

ESI-MS calcd. for [$C_{46}H_{79}N_9O_{10}$]: 917.59. Found: 918.7 $[M+H]^+$.

^{1}H NMR (CD_3OD, 700 MHz): δ 7.54 (d, 2H, H^6), 7.26 (d, 2H, H^7), 4.60 (br. s, 1H, H^1), 4.55 (q, 1H, H^4), 4.35 (s, 2H, H^8), 3.53 (br. s, 8H, H^{11}, H^{12}), 4.13-2.83 (m, 8H, H^{cyclen}), 3.29 (t, 2H, H^{10}), 2.57 (t, 2H, H^9), 2.23 (m, 1H, H^2), 1.51 (s, 30H, H^5, H^{13}), 1.09 (dd, 6H, H^3).

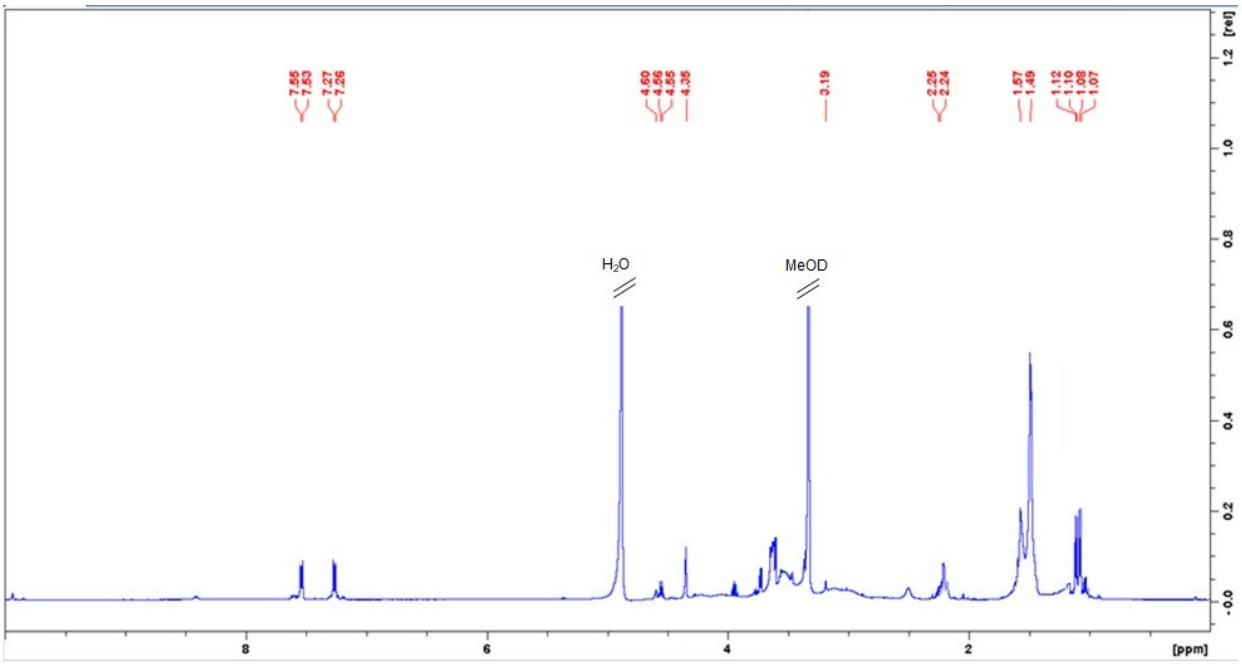
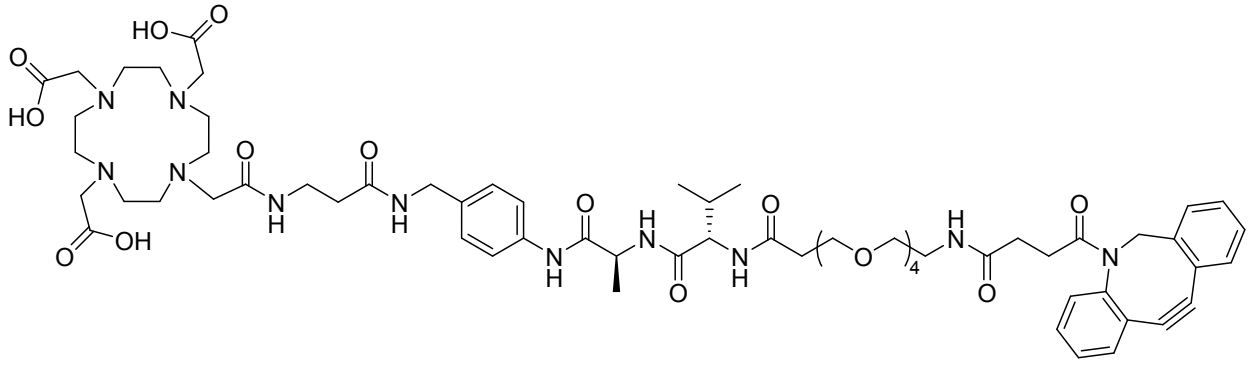


Figure S14. ^{1}H NMR spectrum of **A10** in CD_3OD.



A10 (6.4 mg, 0.007 mmol) was dissolved in 1 mL of DCM and 2 mL of TFA. The reaction mixture was stirred at room temperature for 10 h, then the solvent was removed in vacuo to yield fully deprotected DO3A-β-Ala-aniline-Ala-Val as a white powder. This substrate was then dissolved in 1 mL of DMF with DBCO-PEG4-NHS ester (3 mg, 0.005 mmol) and DIPEA (4 µL, 0.02 mmol)

and stirred overnight at room temperature. Upon completion of reaction as monitored via LC-MS, the product is purified using method B (Rt = 16.1 min). The solvent is removed in vacuo to yield **4** as white solid (1.4 mg, 1.0 μmol, 22% yield).

ESI-MS calcd. for $[C_{64}H_{89}N_{11}O_{17}]$: 1283.64. Found: 1284.8 $[M+H]^+$.

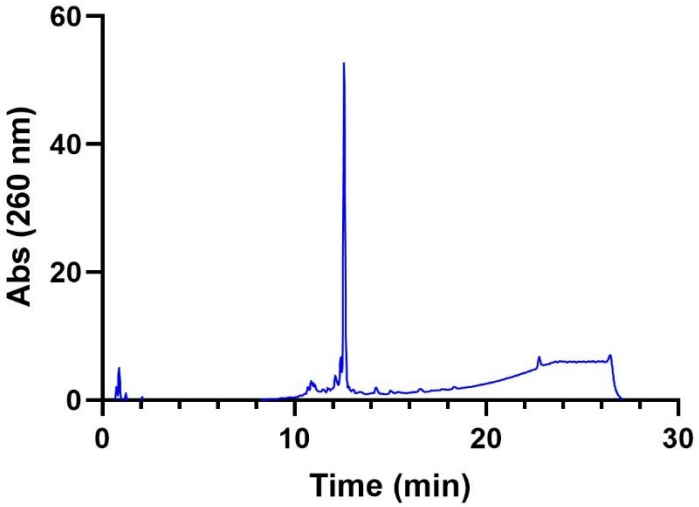
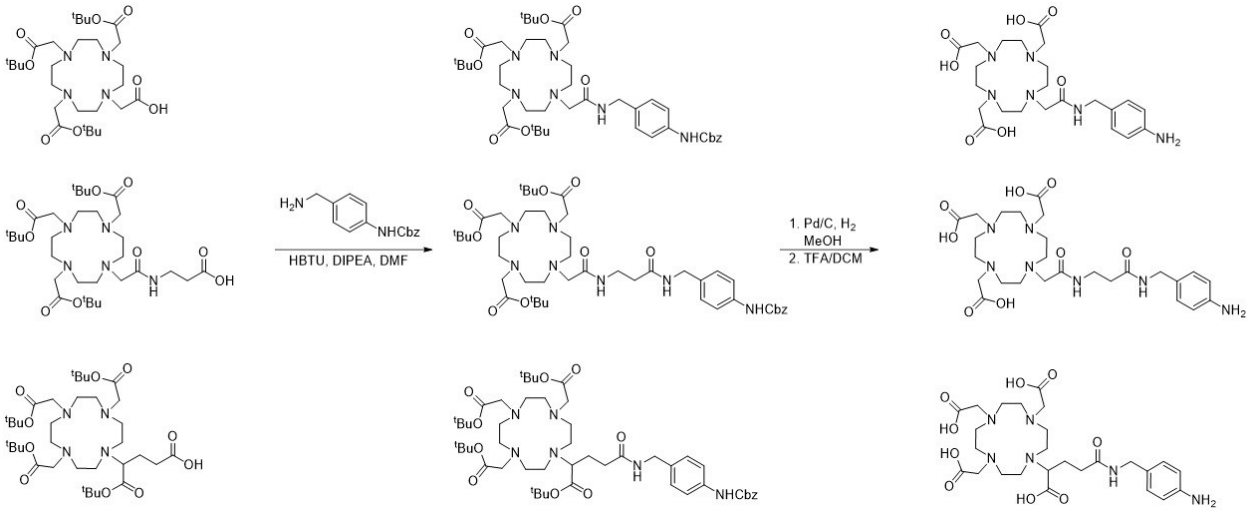
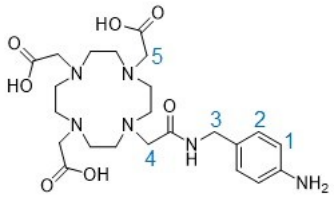


Figure S15. HPLC spectrum of **4**. Rt (Method D): 12.58.

Synthesis of model compounds 5-7.



Scheme S5. Synthesis of model compounds **5-7**.

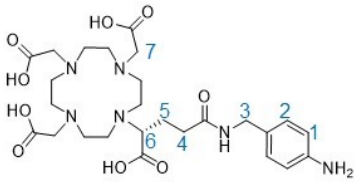


DOTA-tri-t-Bu-ester (92.5 mg, 0.16 mmol) and HBTU (61 mg, 0.16 mmol) were dissolved in 3 mL of dry DMF and stirred for 15 minutes at room temperature. 4-(Aminomethyl)-*N*-(benzyloxycarbonyl)phenylamine (41.0 mg, 0.16 mmol) was prepared according to literature[25] and added to the reaction vessel, followed by DIPEA (150 µL, 0.87 mmol, 5.4 eq). The solution was stirred overnight, and the solvent removed in vacuo. Tert-butyl 2-[4-({[(4-{[(benzyloxy)carbonyl]amino}phenyl)methyl]carbamoyl}methyl)-7,10-bis[2-(tert-butoxy)-2-oxoethyl]-1,4,7,10-tetraazacyclododecan-1-yl]acetate was purified using method A (R_t = 19.6 min) and the solvent removed to afford white powder (84.0 mg, 0.10 mmol, 64.8% yield). The intermediate was then dissolved in 10 mL methanol and 6 mg of Pd/C (10% w/w) in a suspension of methanol (3 mL) were added to this solution. After stirring under H_2 atmosphere for 4 h, the solution was filtered, and solvent removed in vacuo to afford tert-butyl 2-[4-({[(4-aminophenyl)methyl]carbamoyl}methyl)-7,10-bis[2-(tert-butoxy)-2-oxoethyl]-1,4,7,10-tetraazacyclododecan-1-yl]acetate as a white powder. This powder was subsequently dissolved in 3 mL of TFA/DCM (1:1) solution and stirred at 40 °C for 2 hours to afford the fully deprotected 2-[4-({[(4-aminophenyl)methyl]carbamoyl}methyl)-7,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid as a colorless solid (43.2 mg, 0.09 mmol, 85% yield).

ESI-MS calcd. for $[C_{23}H_{36}N_6O_7]$: 508.26. Found: 509.3 $[M+H]^+$, 531.3 $[M+Na]^+$.

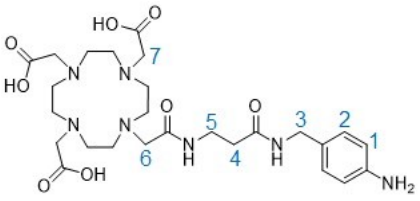
^{1}H NMR (D_2O, 700 MHz): δ 7.37 (m, 0.4H, H^2), 7.24 (m, 0.6H, H^2), 7.19 (m, 1H, H^2), 7.04 (m, 0.4H, H^1), 6.81 (m, 1.6H, H^1), 4.27 (m, 2H, H^3), 3.73 (s, 2H, H^4), 2.84-3.96 (m, 16H, H^{cyclen}), 2.73

(s, 6H, H^5). Some complexation with Na(I), from the $NaOH_{(aq)}$ used to raise the pH following TFA-mediated deprotection, results in peaks at 6.81, 7.19 and 7.24 ppm.



(*R*)-*tert*-Bu_4-DOTAGA (93.6 mg, 0.13 mmol) and HBTU (52.0 mg, 0.13 mmol) were dissolved in 3 mL of dry DMF and stirred for 15 minutes at room temperature. 4-(Aminomethyl)-*N*-(benzyloxycarbonyl)phenylamine (34.0 mg, 0.13 mmol) was added to the reaction vessel, followed by DIPEA (78 µL, 0.45 mmol, 3.5 eq). The solution was stirred overnight, and the solvent removed in vacuo. Upon purification using method D, tert-butyl 4-{[(4-{[(benzyloxy)carbonyl]amino}phenyl)methyl]carbamoyl}-2-{4,7,10-tris[2-(tert-butoxy)-2-oxoethyl]-1,4,7,10-tetraazacyclododecan-1-yl}butanoate was quantitatively obtained as a white powder (121.1 mg, 0.13 mmol). The intermediate was then dissolved in 20 mL of methanol, and 12 mg of Pd/C catalyst (10% w/w) were added to this solution. After stirring under H_2 atmosphere for 4 h, the solution was filtered and solvent removed in vacuo to afford tert-butyl 4-{[(4-aminophenyl)methyl]carbamoyl}-2-{4,7,10-tris[2-(tert-butoxy)-2-oxoethyl]-1,4,7,10-tetraazacyclododecan-1-yl}butanoate as a white solid (94.8 mg, 0.12 mg). This white solid was dissolved in 3 mL of TFA/DCM (1:1) solution and stirred at 40 °C for 2 hours to afford the fully deprotected 4-{[(4-aminophenyl)methyl]carbamoyl}-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]butanoic acid as a colorless solid (64.4 mg, 0.11 mmol).

ESI-MS calcd. for $[C_{26}H_{40}N_6O_9]$: 580.29. Found: 581.3 $[M+H]^+$.



^{1}H NMR (D_2O, 700 MHz): δ 7.42 (d, 2H, H^2), 7.37 (d, 2H, H^1), 4.39 (s, 2H, H^3), 3.89 (m, 1H, H^6), 3.03-3.85 (m, 16H, H^{cyclen}), 2.70 (s, 6H, H^7), 2.59 (m, 2H, H^4), 2.01 (m, 2H, H^5). To a solution of **S8** (60.0 mg, 0.08 mmol) dissolved in MeOH (4 mL), a suspension of Pd/C catalyst (10% w/w, 6.0 mg) in MeOH (4 mL) was added. The flask was evacuated, charged with H_2 (1 atm), and then stirred for 3 h, until no more starting material was detected according to LC-MS. The reaction mixture was filtered, and the solvent was removed from the filtrate to afford the debenzylated material as a white powder. The debenzylated molecule and HBTU (28 mg, 0.08 mmol) were dissolved in 3 mL DMF and stirred at room temperature for 20 minutes. 4-(Aminomethyl)-*N*-(benzyloxycarbonyl)phenylamine (21.0 mg, 0.08 mmol) was added to this solution, followed by DIPEA (55 µL, 0.32 mmol, 4 eq), and the solution was stirred overnight at room temperature. The solvent was removed in vacuo, and the fully protected tert-butyl 2-(4-{[(2-{[(4-{[(benzyloxy)carbonyl]amino}phenyl)methyl]carbamoyl}ethyl)carbamoyl]methyl}-7,10-bis[2-(tert-butoxy)-2-oxoethyl]-1,4,7,10-tetraazacyclododecan-1-yl)acetate was purified using method D (elute at 65% organic media). Smaller batches can be purified using method A (Rt = 18.7 min). The solvent was removed in vacuo to afford a white powder (52.7 mg, 0.06 mmol, 75% yield). This intermediate was then dissolved in 5 mL of methanol, to which a suspension of Pd/C catalyst (10% w/w, 6.0 mg) in MeOH (2 mL) was added. After stirring under H_2 atmosphere for 4

h, the solution was filtered, and solvent removed in vacuo to afford tert-butyl 2-(4-{[(2-{[(4-aminophenyl)methyl]carbamoyl}ethyl)carbamoyl]methyl}-7,10-bis[2-(tert-butoxy)-2-oxoethyl]-1,4,7,10-tetraazacyclododecan-1-yl)acetate as a white powder. This powder was subsequently dissolved in 3 mL of TFA/DCM (1:1) solution and stirred at 40 °C for 2 hours to afford the fully deprotected 2-(4-{[(2-{[(4-aminophenyl)methyl]carbamoyl}ethyl)carbamoyl]methyl}-7,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetic acid as a colorless solid (23.6 mg, 0.04 mmol, 68% yield).

ESI-MS calcd. for $[C_{26}H_{41}N_7O_8]$: 579.30. Found: 580.4 $[M+H]^+$.

^{1}H NMR (D_2O, 700 MHz): δ 7.42 (m, 2H, H^2), 7.36 (m, 2H, H^1), 4.28 (s, 2H, H^3), 3.93 (m, 2H, H^5), 3.03-3.85 (m, 16H, H^{cyclen}), 2.63 (s, 6H, H^7), 2.57 (s, 2H, H^6), 2.51 (m, 2H, H^4).

Metal Complexation

Aqueous metal solutions (45 mM) were prepared from $ScCl_3 \cdot 6H_2O$, $Ga(NO_3)_3 \cdot 6H_2O$, $Y(NO_3)_3 \cdot 6H_2O$, $InCl_3$, $LaCl_3 \cdot 6H_2O$ and $LuCl_3 \cdot 6H_2O$. To a solution of **1**, **2**, **3** or **4** (0.05 mg, 0.04 μmol) dissolved in 25 μL of H_2O, 5 μL of each metal solution (0.2 mmol) were added, and the pH was adjusted to 6.0 with 1M NaOH. The complexed molecule was characterized with ESI-MS and HPLC (method D). Conjugates with Y^{3+}, La^{3+} and Lu^{3+} ions were incubated at 37°C for 2 h to achieve full complexation. The metal complexes were then purified via Waters Sep-Pak C-18 Plus Short cartridge to remove excess metal ions.

Sc-**1** ESI-MS calcd. for $[C_{51}H_{71}N_8O_{14}Sc]$: 1064.46. Found: 533.4 $[M+2H]^{2+}$. Rt = 11.93 min.

Ga-**1** ESI-MS calcd. for $[C_{51}H_{71}N_8O_{14}Ga]$: 1088.43. Found: 545.2 $[M+2H]^{2+}$. Rt = 11.92 min

Y-**1** ESI-MS calcd. for $[C_{51}H_{71}N_8O_{14}Y]$: 1108.41. Found: 1109.4 $[M+H]^+$. Rt = 11.94 min

In-**1** ESI-MS calcd. for $[C_{51}H_{71}N_8O_{146}In]$: 1134.41. Found: 1135.2 $[M+H]^+$. Rt = 11.89 min.

La-**1** ESI-MS calcd. for $[C_{51}H_{71}N_8O_{14}La]$: 1158.42. Found: 580.2 $[M+2H]^{2+}$. Rt = 11.93 min

Lu-**1** ESI-MS calcd. for $[C_{51}H_{71}N_8O_{14}Lu]$: 1194.45. Found: 598.2 $[M+2H]^{2+}$. Rt = 11.94 min.

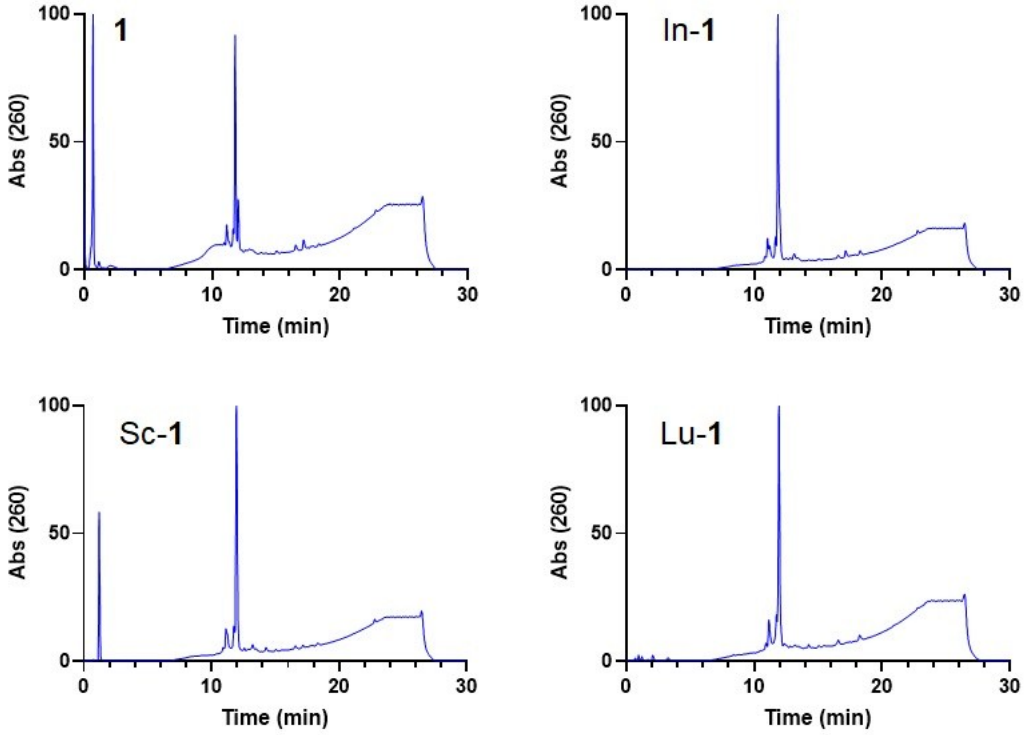


Figure S16. HPLC traces of M-**1**.

Sc-**2** ESI-MS calcd. for [$C_{61}H_{81}N_{10}O_{16}Sc$]: 1254.54. Found: 628.4 $[M+2H]^{2+}$. Rt = 12.40 min.

Ga-**2** ESI-MS calcd. for [$C_{61}H_{81}N_{10}O_{16}Ga$]: 1278.51. Found: 1279.6 $[M+H]^{+}$. Rt = 12.40 min.

Y-**2** ESI-MS calcd. for [$C_{61}H_{81}N_{10}O_{16}Y$]: 1298.49. Found: 650.6 $[M+2H]^{2+}$. Rt = 12.37 min.

In-**2** ESI-MS calcd. for [$C_{61}H_{81}N_{10}O_{16}In$]: 1324.49. Found: 1325.7 $[M+H]^{+}$. Rt = 12.32 min.

La-**2** ESI-MS calcd. for [$C_{61}H_{81}N_{10}O_{16}La$]:1348.49. Found: 675.3 $[M+2H]^{2+}$. Rt = 12.41 min

Lu-**2** ESI-MS calcd. for [$C_{61}H_{81}N_{10}O_{16}Lu$]: 1384.52. Found: 462.6 $[M+3H]^{3+}$, 693.4 $[M+2H]^{2+}$. Rt = 12.42 min.

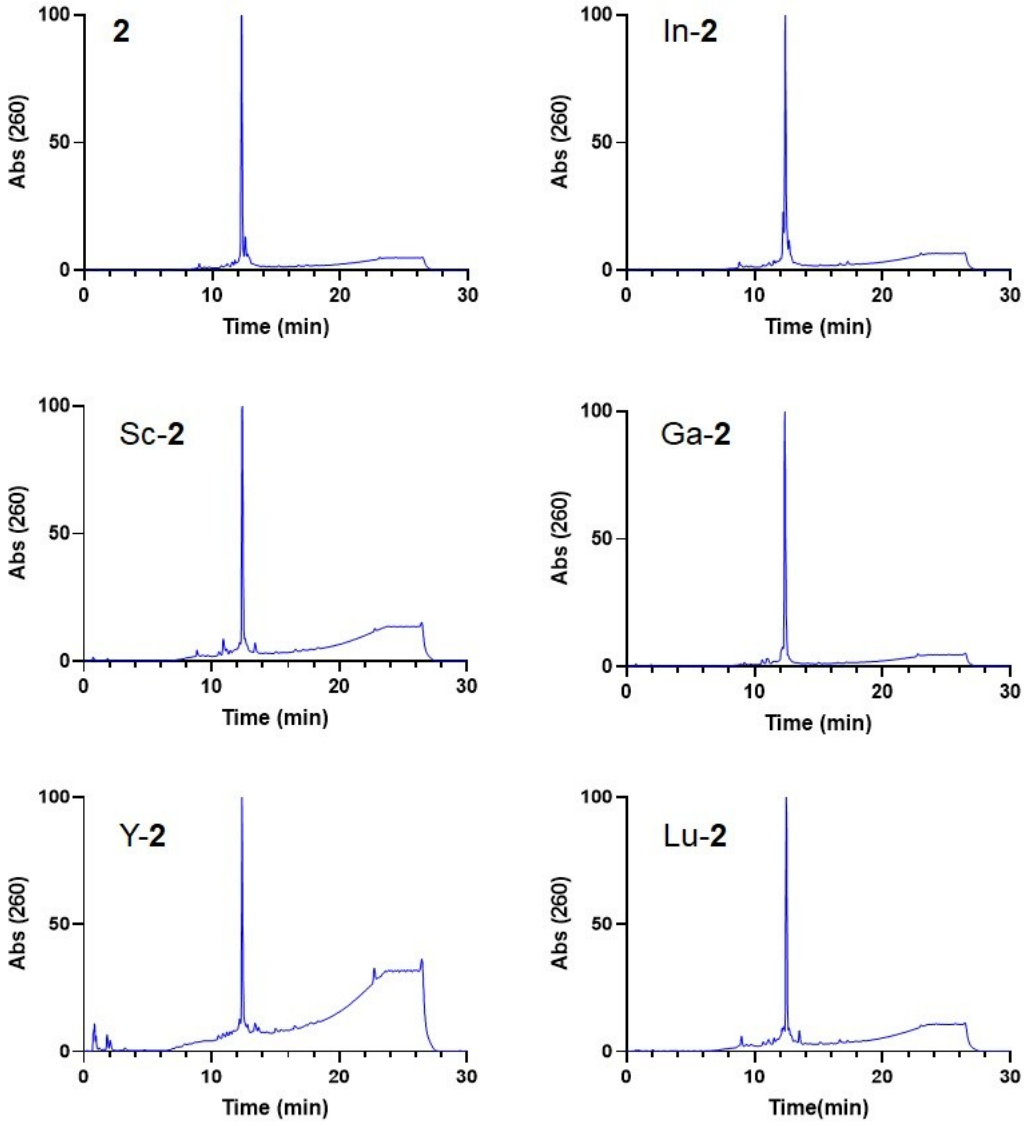


Figure S17. HPLC traces of M-**2**.

Sc-**3** ESI-MS calcd. for [$C_{64}H_{85}N_{10}O_{18}Sc$]: 1326.56. Found: 1325.5 [M-H]$^-$. Rt = 12.60 min.

Ga-**3** ESI-MS calcd. for [$C_{64}H_{85}N_{10}O_{18}Ga$]: 1350.53. Found: 1349.3 [M-H]$^-$. Rt = 12.63 min.

Y-**3** ESI-MS calcd. for [$C_{64}H_{85}N_{10}O_{18}Y$]: 1370.51. Found: 1369.5 [M-H]$^-$. Rt = 12.60 min.

In-**3** ESI-MS calcd. for [$C_{64}H_{85}N_{10}O_{18}In$]: 1396.51. Found: 1395.4 [M-H]$^-$. Rt = 12.63 min.

La-**3** ESI-MS calcd. for [$C_{61}H_{81}N_{10}O_{16}La$]: 1420.51. Found: 1419.6 [M-H]$^-$ Rt = 12.68 min.

Lu-**3** ESI-MS calcd. for [$C_{61}H_{81}N_{10}O_{16}Lu$]: 1456.55. Found: 1455.6 [M-H]$^-$. Rt = 12.60 min.

Sc-**4** ESI-MS calcd. for [$C_{64}H_{86}N_{11}O_{17}Sc$]: 1325.58. Found: 1327.2 $[M+H]^+$. Rt = 12.70 min.

Ga-**4** ESI-MS calcd. for [$C_{64}H_{86}N_{11}O_{17}Ga$]: 1349.55. Found: 1350.6 $[M+H]^+$. Rt = 12.75 min.

Y-**4** ESI-MS calcd. for [$C_{64}H_{86}N_{11}O_{17}Y$]: 1369.53. Found: 1370.6 $[M+H]^+$. Rt = 12.77 min.

In-**4** ESI-MS calcd. for [$C_{64}H_{86}N_{11}O_{17}In$]: 1395.52. Found: 1396.7 $[M+H]^+$. Rt = 12.73 min.

La-**4** ESI-MS calcd. for [$C_{64}H_{86}N_{11}O_{17}La$]: 1419.53. Found: 1420.6 $[M+H]^+$. Rt = 12.72 min.

Lu-**4** ESI-MS calcd. for [$C_{64}H_{86}N_{11}O_{17}Lu$]: 1455.56. Found: 1456.8 $[M+H]^+$. Rt = 12.73 min.

Sc-**5** ESI-MS calcd. for [$C_{23}H_{33}N_6O_7Sc$]: 550.20. Found: 551.1 $[M+H]^+$.

Ga-**5** ESI-MS calcd. for [$C_{23}H_{33}N_6O_7Ga$]: 574.17. Found: 575.3 $[M+H]^+$.

Y-**5** ESI-MS calcd. for [$C_{23}H_{33}N_6O_7Y$]: 594.15. Found: 595.2 $[M+H]^+$.

In-**5** ESI-MS calcd. for [$C_{23}H_{33}N_6O_7In$]: 620.14. Found: 621.2 $[M+H]^+$.

La-**5** ESI-MS calcd. for [$C_{23}H_{33}N_6O_7La$]: 644.15. Found: 645.3 $[M+H]^+$.

Lu-**5** ESI-MS calcd. for [$C_{23}H_{33}N_6O_7Lu$]: 680.18. Found: 681.3 $[M+H]^+$.

Sc-**6** ESI-MS calcd. for [$C_{26}H_{37}N_6O_9Sc$]: 622.22. Found: 621.1 $[M-H]^-$.

Ga-**6** ESI-MS calcd. for [$C_{26}H_{37}N_6O_9Ga$]: 646.19. Found: 645.2 $[M-H]^-$.

Y-**6** ESI-MS calcd. for [$C_{26}H_{37}N_6O_9Y$]: 666.17. Found: 665.0 $[M-H]^-$.

In-**6** ESI-MS calcd. for [$C_{26}H_{37}N_6O_9In$]: 692.17. Found: 691.0 $[M-H]^-$.

La-**6** ESI-MS calcd. for [$C_{26}H_{37}N_6O_9La$]: 716.19. Found: 715.0 $[M-H]^-$.

Lu-**6** ESI-MS calcd. for [$C_{26}H_{37}N_6O_9Lu$]: 752.20. Found: 751.2 $[M-H]^-$.

Sc-**7** ESI-MS calcd. for [$C_{26}H_{38}N_7O_8Sc$]: 621.23. Found: 622.2 $[M+H]^+$.

Ga-**7** ESI-MS calcd. for [$C_{26}H_{38}N_7O_8Ga$]: 645.20. Found: 646.3 $[M+H]^+$.

Y-**7** ESI-MS calcd. for [$C_{26}H_{38}N_7O_8Y$]: 665.18. Found: 666.3 $[M+H]^+$.

In-**7** ESI-MS calcd. for [$C_{26}H_{38}N_7O_8In$]: 691.18. Found: 692.2 $[M+H]^+$.

La-**7** ESI-MS calcd. for [$C_{26}H_{38}N_7O_8La$]: 715.18. Found: 716.2 $[M+H]^+$.

Lu-**7** ESI-MS calcd. for [$C_{26}H_{38}N_7O_8Lu$]: 751.22. Found: 752.2 $[M+H]^+$.

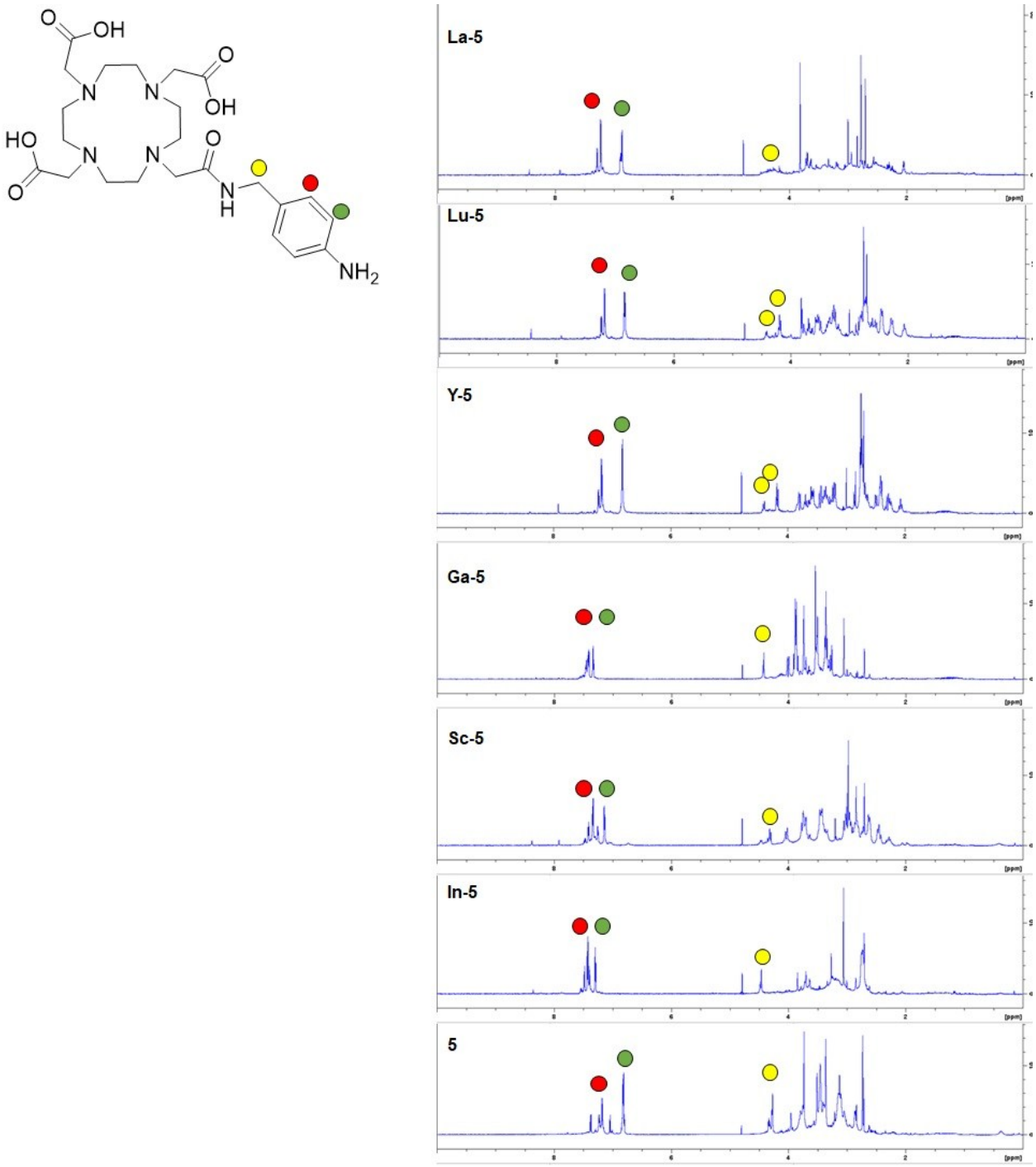


Figure S18. ^{1}H NMR spectra of the metal complexes of **5**, in D_2O.

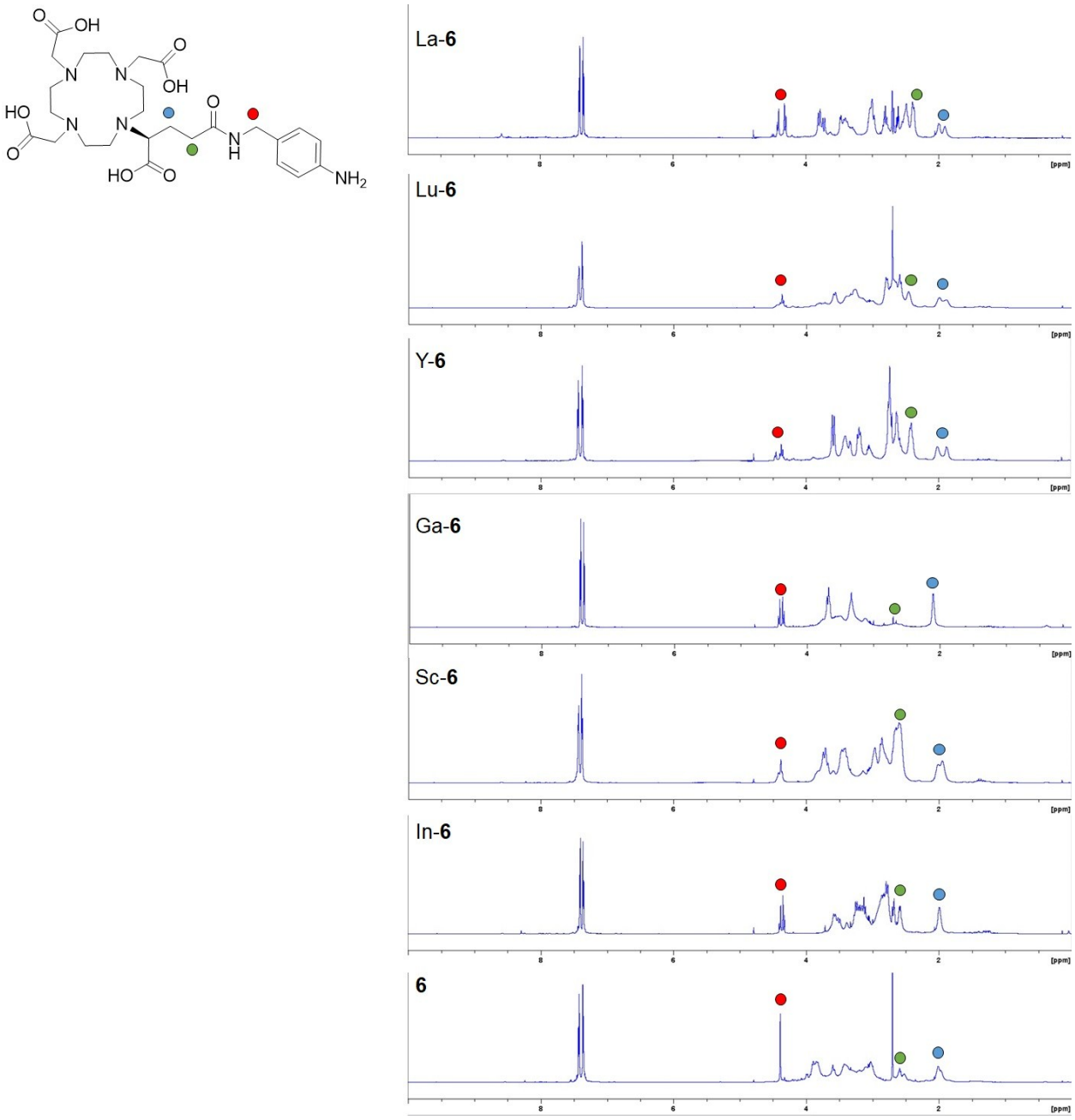


Figure S19. ^{1}H NMR spectra of the metal complexes of **6**, in D_2O

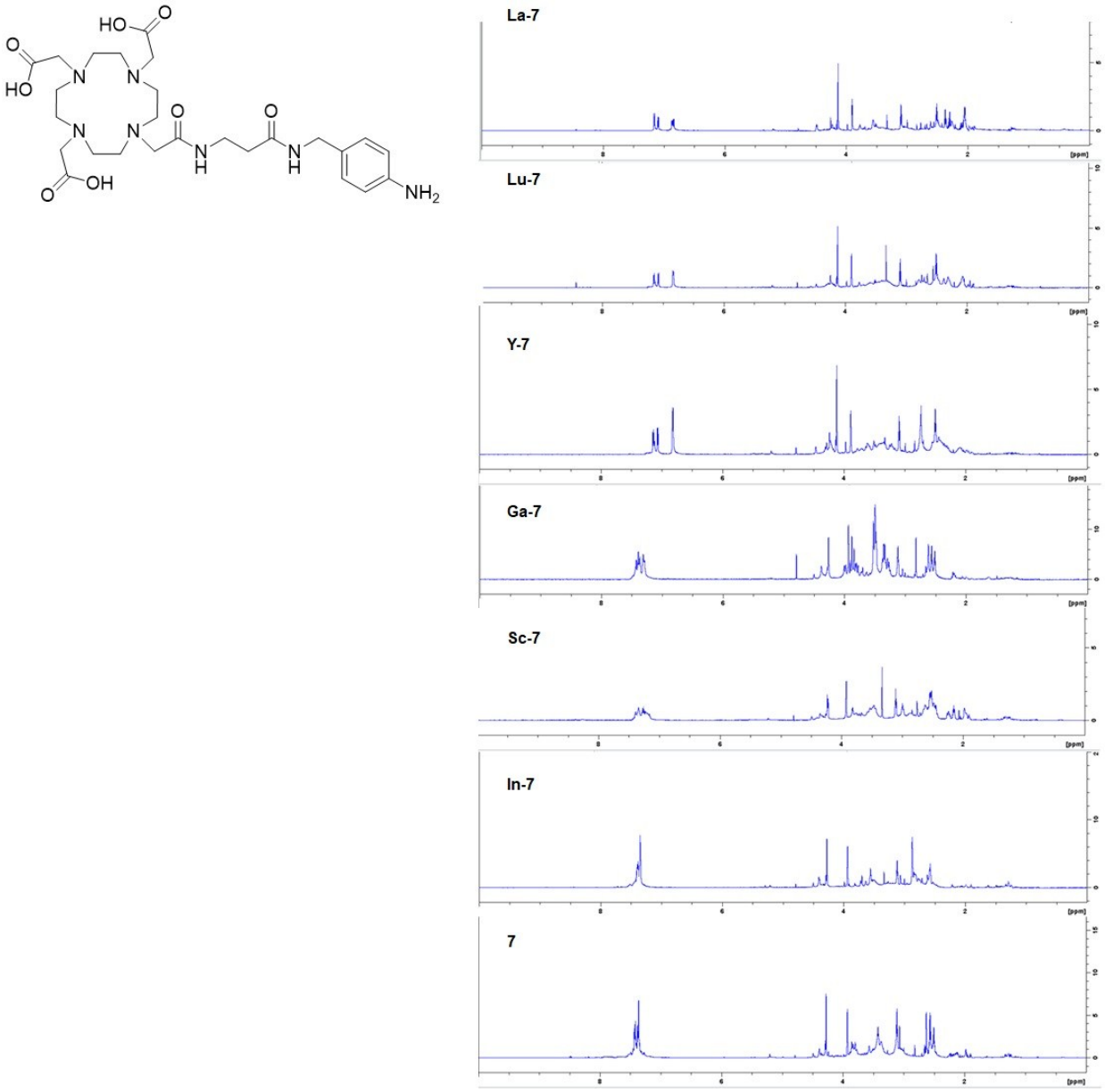


Figure S20. ^{1}H NMR spectra of the metal complexes of **7**, in D_2O

Solution state FT-IR characterization

Samples for FT-IR characterization were prepared by dissolving 3-5 mg of each chelator or metal complex in 0.5 mL of D_2O, for a final concentration of 10-15 mM.

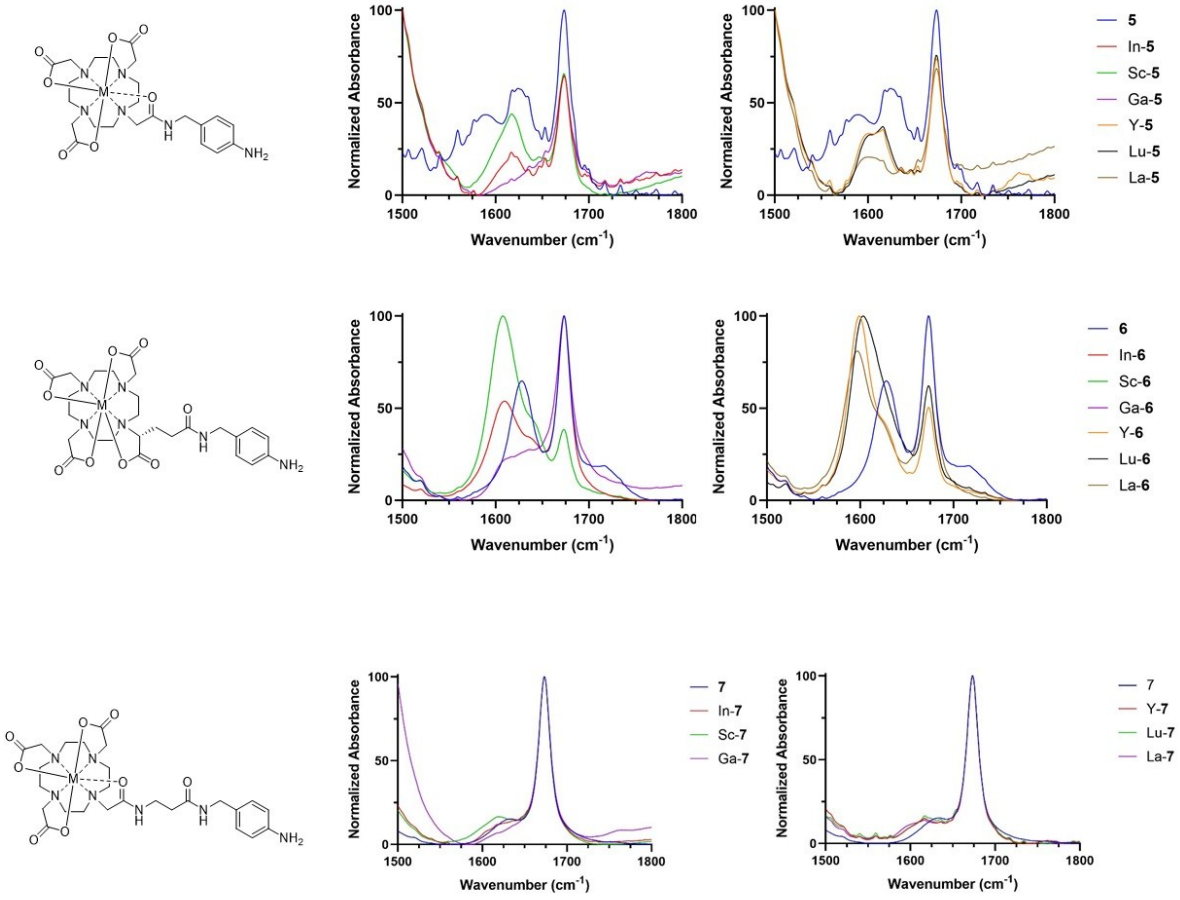


Figure S21. Solution FT-IR spectra of metal complexes of **5-7**, in D_2O.

Table S2. FT-IR bands of metal complexes of **5-7**.

Compound	λ (cm^{-1})		Compound	λ (cm^{-1})		Compound	λ (cm^{-1})	
5	1674	1625	**6**	1673	1609	**7**	1673	1634
In-**5**		1617	In-**6**		1638, 1607	In-**7**		1617
Sc-**5**			Sc-**6**		1638, 1609	Sc-**7**		
Ga-**5**		-	Ga-**6**		1604	Ga-**7**		-
Y-**5**		1617, 1600	Y-**6**		1628, 1599	Y-**7**		1617
Lu-**5**			Lu-**6**		1628, 1597	Lu-**7**		
La-**5**			La-**6**		1628	La-**7**		

In vitro Cathepsin B mediated peptide cleaving of small molecule

The concentration of metal complexes of **1-4** were determined via ICP-OES analysis.

Cathepsin B from bovine spleen (3.22 mg, ≥10 units/mg protein) was dissolved in 1 mL of 25 mM NaOAc/1 mM EDTA buffer (pH 5.0). A 6 µL aliquot (20 µg) of this stock solution was activated with 12 µL of 30 mM DTT/15 mM EDTA (pH 5.0) solution at room temperature for 15 minutes. To this solution, 40 µL of 25 mM NaOAc/1 mM EDTA (pH 5.0, preincubated at 37°C) was added, followed by the addition of 0.01 µmol of either free or metal complexed bifunctional chelators **1-4** in 50 µL of H_2O, resulting in an approximate enzyme-to-substrate ratio of 1:100. The samples were incubated at 37°C, and 8 µL aliquots were removed at various timepoints for HPLC analysis (method D).

Table S3. Approximate time required to cleave half of the substrate concentration ($t_{1/2}$) at enzyme-to-substrate ratio of 1:100.

Substrate	$T_{1/2}$ (h)	Substrate	$T_{1/2}$ (h)	Substrate	$T_{1/2}$ (h)
2	0.5	**3**	3	**4**	<0.5
In-**2**	<1	In-**3**	<1	In-**4**	
Sc-**2**	7	Sc-**3**	9	Sc-**4**	
Ga-**2**	n.d.	Ga-**3**	n.d.	Ga-**4**	
Y-**2**		Y-**3**	<1	Y-**4**	
Lu-**2**		Lu-**3**	12	Lu-**4**	
La-**2**		La-**3**	36	La-**4**	

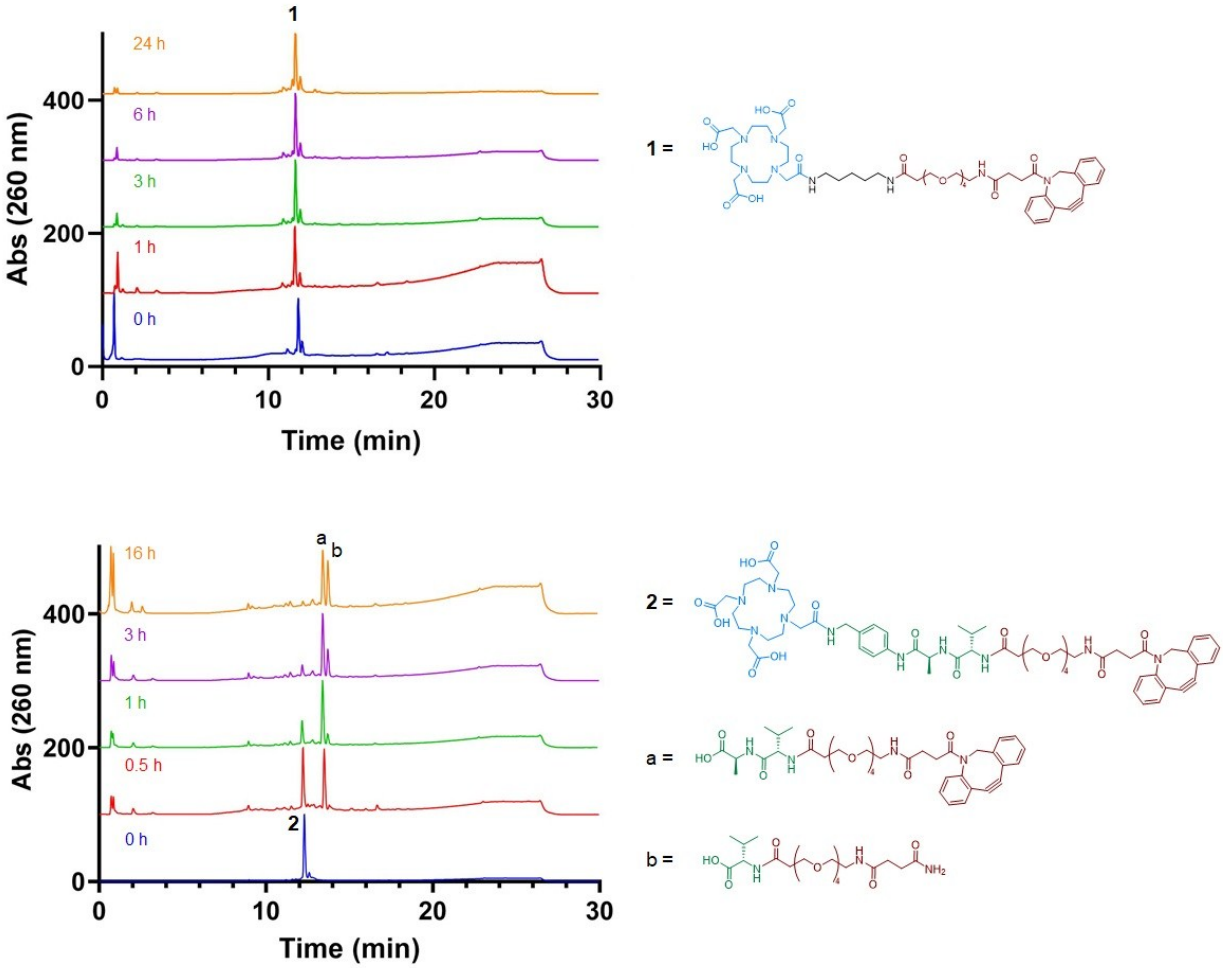


Figure S22. Cathepsin B mediated cleavage of the free ligands **1** (top) and **2** (bottom) monitored over time via HPLC.

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