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Supporting information

An analyte-triggered artificial peroxidase system based on dimanganese complex for a versatile enzyme assay

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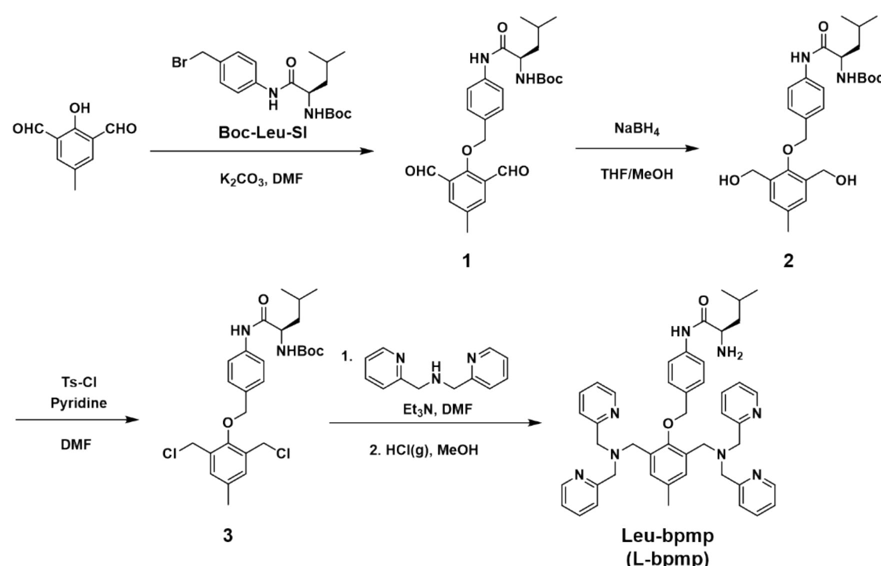
1. Materials and instruments

All chemical reagents were purchased from commercial suppliers (Sigma-Aldrich, Tokyo Chemical Industry, and Alfa Aesar) and used without further purification. LAP (microsomal from porcine kidney), GGT (from equine kidney), other enzymes, and human serum were purchased from Sigma-Aldrich. Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 (^{13}C) NMR spectra were obtained using a Zeol (400 MHz) NMR spectrometer. Chemical shifts are reported as δ (ppm) values relative to chloroform ($CDCl_3$, δ 7.260) and methanol (CD_3OD, δ 3.31), and the coupling constants are reported in Hz. High-resolution mass spectroscopy (HRMS) data were obtained using a Bruker Impact II ESI-Q/TOF (quadrupole/time-of-flight). A Waters preparative high-performance liquid chromatography (HPLC) system (Waters 2489 ultraviolet/visible (UV/vis) detector, Waters 2545 Quaternary HPLC pump, and Waters fraction collector III) with a C_{18} column (SunFire C_{18}, 19 × 150 mm, 5 μm) was used for purification. All UV/vis spectra were recorded using an Agilent Cary 8454 UV/vis spectrophotometer, and all fluorescence spectra were recorded using an Agilent Cary Eclipse fluorescence spectrophotometer. Analytical HPLC was conducted using a Waters reversed-phase HPLC system on a C_{18} column (SunFire C_{18}, 4.6 × 250 mm, 5 μm) at 40 °C.

2. Synthesis of caged bpmp ligand

Conjugates of self-immolative linkers and Boc-protected amino acids (**Boc-Leu-SI** and **Boc-Glu-OtBu-SI**) were prepared, according to previously reported methods.[S1-S3]



Scheme S1. Synthesis of Leu-bpmp (L-bpmp).

*tert-butyl (R)-(1-((4-((2,6-diformyl-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**1**)* To a solution of 2-hydroxy-5-methyl-1,3-benzenedicarboxaldehyde (2.13 g, 13 mmol) and K_2CO_3 (3.59 g, 26 mmol) in dimethylformamide (DMF) 25 mL, **Boc-Leu-SI** (5.19 g, 13 mmol) was added. The reaction mixture was stirred at room temperature for 12 h. After the completion of the

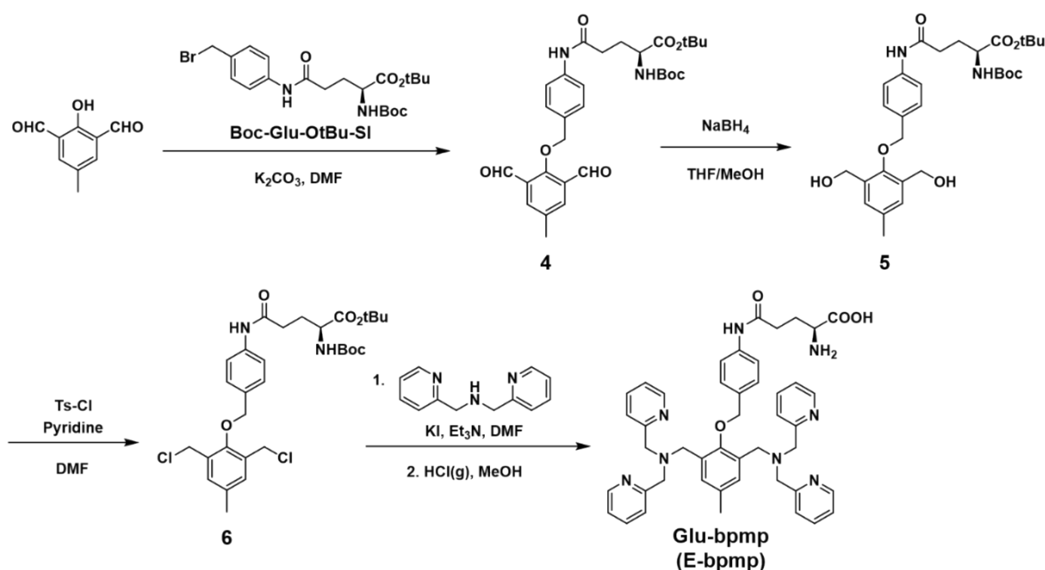
reaction, the reaction mixture was diluted with H_2O and extracted with dichloromethane (DCM). The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in vacuo. The crude was purified by flash column chromatography with n-hexane:ethyl acetate (EtOAc) (3:1) on silica to afford **1** (5.02 g, 80%) as colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 10.20 (s, 2H), 8.78 (br s, 1H), 7.87 (s, 2H), 7.51 (d, J = 8.2 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 5.12 (d, J = 7.3 Hz, 1H), 5.05 (s, 2H), 4.30 (br s, 1H), 2.40 (s, 3H), 1.71-1.78 (m, 2H), 1.60 (q, J = 8.9 Hz, 1H), 1.44 (s, 9H), 0.96 (dd, J = 8.2, 6.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.0, 171.2, 161.4, 156.5, 138.8, 135.5, 135.1, 130.2, 129.8, 119.8, 81.1, 80.8, 54.1, 41.0, 28.7, 24. 7, 22.8, 21.8, 20.7. HRMS (ESI) $[M+Na]^+$ calcd 505.2309, found 505.2308.

*tert-butyl (R)-(1-((4-((2,6-bis(hydroxymethyl)-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**2**)* To a solution of **1** (4.82 g, 10 mmol) in tetrahydrofuran (THF) 50 mL and methanol (MeOH) 15 mL, $NaBH_4$ (1.13 g, 30 mmol) was added in three portions. The reaction mixture was stirred at room temperature for 4 h. After the completion of the reaction, the reaction mixture was quenched with H_2O and extracted with DCM. The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in vacuo to give a **2** (4.86 g, quantitative yield) as colorless oil. The product was used without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 9.29 (br s, 1H), 7.44 (d, J = 8.2 Hz, 2H), 7.18 (d, J = 7.3 Hz, 2H), 7.07 (s, 2H), 5.59 (d, J = 7.3 Hz, 1H), 4.72 (br s, 2H), 4.55 (s, 4H), 4.43 (m, 1H), 2.85 (br s, 2H), 2.24 (s, 3H), 1.77 (td, J = 12.9, 6.3 Hz, 1H), 1.66 (t, J = 6.2 Hz, 2H), 1.41 (s, 9H), 0.96 (t, J = 6.9 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.0, 156.7, 152.3, 138.2, 134.3, 133.7, 132.6, 129.5, 128.8, 120.2, 80.6, 60.6, 54.0, 41.5, 28.5, 24.9, 23.0, 22.0, 21.0. HRMS (ESI) $[M+Na]^+$ calcd 509.2622, found 509.2621.

*tert-butyl (R)-(1-((4-((2,6-bis(chloromethyl)-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**3**)*[S4] To a solution of **2** (4.87 g, 10 mmol) in DMF 25 mL, the mixture of *p*-toluenesulfonyl chloride (Ts-Cl, 11.05 g, 58 mmol) and pyridine (4.68 mL, 58 mmol) in DMF 10 mL was added at 0 °C. The reaction mixture was stirred at 0 °C to room temperature for 4 h. After the completion of the reaction, the reaction mixture was diluted with H_2O and extracted with EA. The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in vacuo. The crude was purified by flash column chromatography with $CHCl_3$:EA (30:1) on silica to afford **3** (1.85 g, 35%) as colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 8.74 (br s, 1H), 7.56 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 7.8 Hz, 2H), 7.21 (s, 2H), 5.15 (d, J = 5.5 Hz, 1H), 4.99 (s, 2H), 4.57 (s, 4H), 4.33 (m, 1H), 2.33 (s, 3H), 1.76 (s, 2H), 1.57-1.66 (m, 1H), 1.46 (s, 9H), 0.97 (t, J = 6.4 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 156.5, 153.0, 138.2, 135.5, 134.9, 132.5, 131.4, 129.0, 120.0, 80.8, 54.00, 41.1, 40.8, 28.5, 25.1, 23.1, 22.0, 20.8. HRMS (ESI) $[M+Na]^+$ calcd 545.1944, found 545.1943.

*(R)-2-amino-N-(4-((2,6-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-methylphenoxy)methyl)phenyl)-4-methylpentanamide (**Leu-bpmp, L-bpmp**)* To a solution of **3** (1.83 g, 3.5 mmol) in DMF 10 mL, the mixture of bis(2-pyridyl)methylamine (1.23 mL, 6.8 mmol) and Et_3N (1.97 mL, 14 mmol) in DMF 10 mL

was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C to room temperature for 48 h. After the completion of the reaction, the reaction mixture was diluted with H_2O and extracted with DCM. The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in vacuo. The crude was pre-purified by flash column chromatography with acetone on silica to afford intermediate mixture as beige solid (1.63 g, 55%). To a solution of intermediate (0.26 g, 0.3 mmol) in MeOH 10 mL, HCl gas was added at 0 °C until the starting material was completely consumed. After the completion of the reaction, excess of diethyl ether was added to the reaction mixture until no further precipitation occurred. The precipitate was filtered and dissolved in H_2O, and pH was adjusted to pH 8 with 1 M NaOH solution. Then, the mixture was extracted with DCM. The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in vacuo to give a **Leu-bpmp** (0.135 g, 60%) as purple oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.59 (br s, 1H), 8.47 (d, J = 4.6 Hz, 4H), 7.51-7.59 (m, 10H), 7.32 (s, 2H), 7.24 (d, J = 8.2 Hz, 2H), 7.08 (t, J = 5.7 Hz, 4H), 4.66 (s, 2H), 3.79 (s, 8H), 3.71 (s, 4H), 3.53 (m, 1H), 2.32 (s, 3H), 1.76-1.88 (m, 2H), 1.42-1.49 (m, 1H), 0.99 (dd, J = 10.8, 6.2 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 159.8, 154.1, 149.0, 137.7, 136.5, 133.6, 132.9, 131.9, 129.9, 128.5, 123.0, 121.8, 119.5, 75.7, 60.3, 54.1, 52.7, 44.0, 25.1, 23.6, 21.4. HRMS (ESI) $[M+Na]^+$ calcd 771.4105, found 771.4107.



Scheme S2. Synthesis of Glu-bpmp (E-bpmp).

tert-butyl N^2*-(tert-butoxycarbonyl)-N*^{5}*-(4-((2,6-diformyl-4-methylphenoxy)methyl)phenyl)-L-glutaminate (**4**)* To a solution of 2-hydroxy-5-methyl-1,3-benzenedicarboxaldehyde (0.84 g, 5.1 mmol) and K_2CO_3 (1.27 g, 9.2 mmol) in DMF 8 mL, **Boc-Glu-OtBu-SI** (2.17 g, 4.6 mmol) was added. The reaction mixture was stirred at room temperature for 15 h. After the completion of the reaction, the reaction mixture was diluted with H_2O and extracted with DCM. The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in vacuo. The crude was purified by column chromatography with n-hexane:EA (3:2) on silica to afford **4** (1.63 g, 64%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.20

(s, 2H), 9.05 (br s, 1H), 7.85 (s, 2H), 7.62 (d, J = 7.8 Hz, 2H), 7.23 (d, J = 8.2 Hz, 2H), 5.41 (d, J = 6.9 Hz, 1H), 5.07 (s, 2H), 4.18 (m, 1H), 2.43 (t, J = 6.6 Hz, 2H), 2.38 (s, 3H), 2.19-2.27 (m, 1H), 1.84-1.90 (m, 1H), 1.43 (d, J = 2.3 Hz, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.9, 171.4, 170.6, 161.9, 156.8, 135.5, 135.1, 130.5, 129.9, 129.5, 120.0, 83.0, 81.4, 80.7, 54.1, 53.1, 34.5, 30.9, 28.5, 28.0, 20.7. HRMS (ESI) $[M+Na]^+$ calcd 577.2520, found 577.2511.

*tert-butyl N^5-(4-((2,6-bis(hydroxymethyl)-4-methylphenoxy)methyl)phenyl)-N^2-(tert-butoxycarbonyl)-L-glutaminate (**5**)* To a solution of 4 (2.11 g, 3.8 mmol) in THF 30 mL and MeOH 5 mL, $NaBH_4$ (0.43 g, 11.4 mmol) was added in three portions. The reaction mixture was stirred at room temperature for 10 min. After the completion of the reaction, the reaction mixture was quenched with H_2O and organic solvent was removed by vacuum evaporation. Then, the reaction mixture was extracted with EA and the organic layer was washed with brine. The organic layer dried over Na_2SO_4, filtered, and evaporated in vacuo to give a **5** (2.13 g, quantitative yield) as colorless oil. The product was used without further purification. ^{1}H NMR (400 MHz, CD_3OD) δ 10.20 (s, 2H), 9.05 (br s, 1H), 7.85 (s, 2H), 7.62 (d, J = 7.8 Hz, 2H), 7.23 (d, J = 8.2 Hz, 2H), 5.41 (d, J = 6.9 Hz, 1H), 5.07 (s, 2H), 4.18 (m, 1H), 2.43 (t, J = 6.6 Hz, 2H), 2.38 (s, 3H), 2.19-2.27 (m, 1H), 1.84-1.90 (m, 1H), 1.43 (d, J = 2.3 Hz, 18H). ^{13}C NMR (100 MHz, CD_3OD): δ 188.9, 171.4, 170.6, 161.9, 156.8, 135.5, 135.1, 130.5, 129.9, 129.5, 120.0, 83.0, 81.4, 80.7, 54.1, 53.1, 34.5, 30.9, 28.5, 28.0, 20.7. HRMS (ESI) $[M + Na]^+$ calcd 581.2833, found 581.2836.

*tert-butyl N^5-(4-((2,6-bis(chloromethyl)-4-methylphenoxy)methyl)phenyl)-N^2-(tert-butoxycarbonyl)-L-glutaminate (**6**)*[S4] To a solution of 5 (0.28 g, 0.5 mmol) in DMF 2 mL, the mixture of Ts-Cl (0.57 g, 3 mmol) and pyridine (0.24 mL, 3 mmol) in DMF 1 mL was added at 0 °C. The reaction mixture was stirred at 0 °C to room temperature for 2 h. After the completion of the reaction, the reaction mixture was diluted with H_2O and extracted with DCM. The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in vacuo. The crude was purified by flash column chromatography with $CHCl_3$:EA (30:1) on silica to afford **6** (0.223 g, 75%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (br s, 1H), 7.69 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 8.5 Hz, 2H), 7.23 (s, 2H), 5.38 (d, J = 8.5 Hz, 1H), 5.04 (s, 2H), 4.61 (s, 4H), 4.21-4.26 (m, 1H), 2.44-2.48 (m, 2H), 2.34 (s, 3H), 2.25-2.32 (m, 1H), 1.81-1.90 (m, 1H), 1.48 (s, 9H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 170.8, 156.7, 153.0, 138.8, 134.9, 132. 6, 132.3 131.4 128.9, 119.9,83.0, 80.5, 77.1, 53.3, 41.3, 34.6, 30.9, 28.5, 28.1, 20.8. HRMS (ESI) $[M+Na]^+$ calcd 617.2155, found 617.2150.

*N^5-(4-((2,6-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-methylphenoxy)methyl)phenyl)-L-glutamine (**Glu-bpmp**, **E-bpmp**)* To a solution of 6 (0.595 g, 1 mmol) and KI (0.083 g, 0.5 mmol) in DMF 2 mL, the mixture of bis(2-pyridyl)methylamine (0.35 mL, 1.95 mmol) and Et_3N (0.56 mL, 4 mmol) in DMF 2 mL was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C to room temperature for 22 h. After the completion of the reaction, the reaction mixture was diluted with H_2O and extracted with DCM. The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in

vacuo. The crude was pre-purified by flash column chromatography with acetone on silica to afford intermediate mixture as orange oil (0.399 g, 43%). To a solution of intermediate (0.184 g, 0.2 mmol) in MeOH 5 mL, HCl gas was added at 0 °C until the starting material was completely consumed. After the completion of the reaction, excess of diethyl ether was added to the reaction mixture until no further precipitation occurred. The precipitate was filtered and dissolved in H_2O, and pH was adjusted to pH 8 with 1 M NaOH solution. Then, the mixture was extracted with DCM. The organic layer was washed with brine, and dried over Na_2SO_4, filtered, and evaporated in vacuo to give a **Glu-bpmp** (0.125 g, 80%) as yellow oil. A second purification by preparative HPLC can afford colorless oil after freeze-drying. ^{1}H NMR (400 MHz, CD_3OD) δ 8.59 (d, J = 4.6 Hz, 4H), 7.95 (t, J = 7.8 Hz, 4H), 7.47-7.53 (m, 10H), 7.33 (s, 2H), 7.17 (d, J = 8.7 Hz, 2H), 4.74 (s, 2H), 4.30 (s, 8H), 4.16 (s, 4H), 4.12 (t, J = 6.6 Hz, 1H), 2.72-2.75 (m, 2H), 2.23-2.38 (m, 5H). ^{13}C NMR (100 MHz, CD_3OD): δ 172.4, 171.5, 155. 6, 154.2, 147.2, 142.6, 139.9, 136.6, 134.7, 132.9, 130.11, 128.6, 126.2, 125.9, 121.4, 78.2, 58.2, 54.9, 53.5, 33.0, 27.01, 20.6. HRMS (ESI) $[M+H]^+$ calcd 765.3871, found 765.3869.

3. General procedure for monitoring peroxidase-like activity change in caged Mn_2(bpmp)

Stock solutions of bpmp ligands including H-bpmp, **L-bpmp**, and **E-bpmp**, and that of the peroxidase substrates, Amplex red and TMB, were prepared in dimethyl sulfoxide (DMSO). Other stock solutions of $Mn(CH_3COO)_2 \cdot 4H_2O$, H_2O_2, and the target enzymes were prepared in distilled H_2O. For a spectroscopic analysis of peroxidase-like activity, the sample solution containing the bpmp ligands, Mn^{2+}, substrate, and H_2O_2, with or without target enzymes was prepared in a buffer solution (pH 7.4 sodium phosphate buffer (SPB) 20 mM) at the desired final concentration. For the LAP assay, the final concentration was fixed at **L-bpmp** (10 μM), Mn^{2+} (20 μM), substrate (100 μM for Amplex red and 500 μM for TMB), and H_2O_2 (200 μM) in an aqueous solution (10% DMSO, pH 7.4 SPB 20 mM). For the GGT assay, the final concentration was fixed at **E-bpmp** (50 μM), Mn^{2+} (100 μM), substrate (500 μM for TMB), and H_2O_2 (1 mM), in an aqueous solution (5% DMSO, pH 7.4 SPB 20 mM). After rapid mixing of the solution, it was transferred to a quartz cuvette. All spectroscopic measurements were performed at 37 °C.

4. Mechanism study

The sample solution containing each bpmp ligand (50 μM) in the absence and presence of target enzymes (200 U/L) was prepared in an aqueous solution (5% DMSO, pH 7.4 SPB 20 mM) and incubated at 37 °C for 1 h. An equal volume of MeOH was added to the sample solution to quench the enzymatic reaction and centrifuged for 30 min at 13,000 rpm. The supernatant was used for the HPLC analysis.

5. Selectivity evaluation

A sample solution containing target enzymes or other hydrolytic enzymes (alkaline phosphatase from *Escherichia coli*, carboxylesterase from porcine liver, acetylcholinesterase from *Electrophorus electricus*, butyrylcholinesterase from equine serum, chymotrypsin from bovine pancreas, trypsin from

bovine pancreas, lipase from porcine pancreas, and β-galactosidase from *Escherichia coli*) was prepared. For the LAP assay, the final concentration of the enzyme substances was fixed at 25 U/L of LAP and 50 U/L of other enzymes. For the GGT assay, the final concentration was fixed at 100 U/L of LAP and others at 150 U/L. To confirm the interference from other bioanalytes including metal ions (K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Cu^{2+} of perchlorate salt) and biomolecules (glucose, GSH, Cys, Hcy), a sample solution containing bioanalytes was prepared. The UV/vis spectral change in the sample solution was recorded after 1 h incubation at 37 °C.

6. Determination of IC_{50} Values

Stock solutions of inhibitors including bestatin and acivicin, were prepared in distilled H_2O. The solution containing the desired concentration of the inhibitor and target enzyme (LAP or GGT, 100 U/L) was prepared in a buffer solution (pH 7.4, SPB 20 mM) and preincubated at 37 °C before use. After 1 h of incubation, the sample solution was prepared by adding each bpmp ligand, Mn^{2+}, the substrate, and H_2O_2. After rapidly mixing the solution, it was transferred to a quartz cuvette. All spectroscopic measurements were performed at 37 °C. k_{obs} was calculated based on the spectral change in the sample solution for 30 min, and the inhibition efficiency (%) was determined based on the difference in k_{obs} in the absence and presence of inhibitors. Thereafter, the IC_{50} values were calculated by nonlinear regression analysis using the Origin 2019 software.

7. Determination of LAP activity in human serum

Human serum was purchased from Sigma-Aldrich and ultrafiltered. Afterward, the sample solution with or without the diluted human serum (now containing 2.5% human serum) was prepared, and the fluorescence intensity (FI) at 586 nm was recorded for 1 h at 37 °C. The LAP activity was calculated based on the calibration curve, as shown in Figure S1. For comparison, a commercial colorimetric LAP substrate, L-leucine-naphthylamide (LNA), was utilized as a standard substrate. For the LAP assay using LNA, a stock solution of LNA was prepared in distilled H_2O, and that of *p*-(dimethylamino)-cinnamaldehyde (PDAC) was prepared in ethanol (EtOH). The sample solution containing LNA (400 μM) with or without LAP in an aqueous solution (pH 7.4 SPB 20 mM) was prepared and incubated for 50 min at 37 °C. An equal volume of a 0.2 M HCl solution was added to quench the enzymatic reaction, followed by the addition of an equal volume of a 4 mM PDAC solution. The mixture was incubated for 30 min at 37 °C for colorization. The LAP activity in the same human serum sample was calculated based on the calibration curve, as shown in Figure S5.

8. Supplementary figures and table

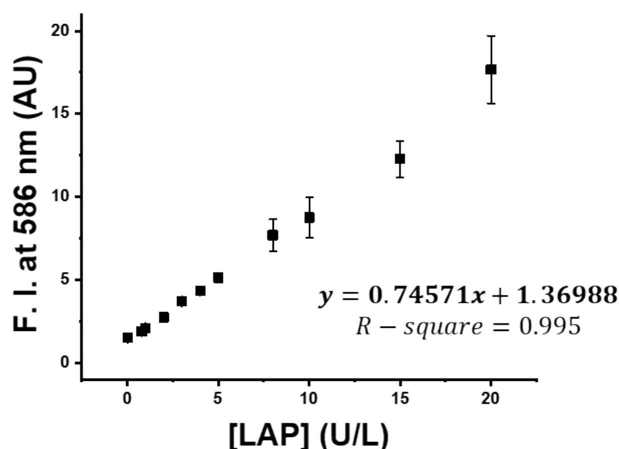


Figure S1. Plot of fluorescence intensity at 586 nm of assay solution containing **L-bpmp** (10 μM) with Mn^{2+} (20 μM), Amplex red (100 μM), H_2O_2 (200 μM) and various concentration of LAP in an aqueous solution (10% DMSO, pH 7.4 sodium phosphate buffer (SPB) 20 mM) at 37 °C after 60 min, the detection limit was calculated to be 0.72 U/L based on 3σ/slope.

Table S1. Comparison of our method using **L-bpmp** with other assays for LAP.

Probe	Detection method	Detection limit	Reference
DCM−Leu (dicyanomethylene-4H-pyran derivative)	Ratiometric fluorescence change	46 ng/mL	S5
BODIPY-C-Leu	Fluorescence enhancement	41.9 ng/mL	S6
HCAL (NIR hemicyanine derivative)	Fluorescence enhancement	0.19 U/L	S7
DPA-TPE-Leu (tetraphenylethylene derivative)	Fluorescence enhancement	0.16 U/L (=8.9 ng/mL),	S8
CHMC-M-Leu (chlorohydroxylmerocyanine derivative)	Fluorescence enhancement	50 ng/mL	S9
Bis-*s*-*s′*-[(*s*)-2-amino-*N*-(3-thiophenyl)-Leu] (b-(*s*)-ANT-Leu)	Surface-enhanced Raman spectroscopy (SERS) spectral change	0.16 U/L	S10
HCHMC-S-Leu (chlorohydroxylmerocyanine derivative)	Ratiometric fluorescence change	42.2 ng/mL	S11
Caged Mn_2(bpmp) with L-bpmp	Tunable according to the type of peroxidase substrate (Fluorescence enhancement for Amplex red, colorimetric change for TMB)	0.72 U/L (=41.7 ng/mL, using Amplex red)	***This work***

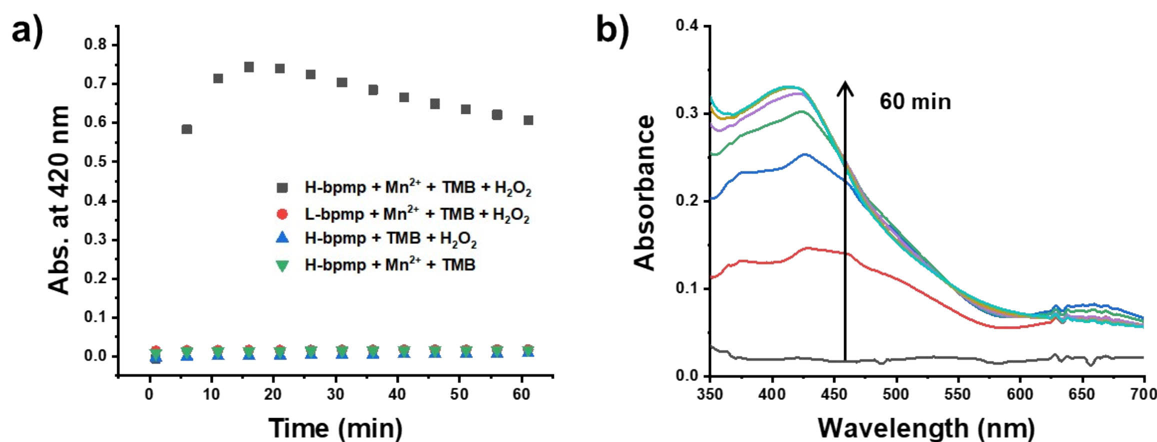


Figure S2. a) Time-dependent absorbance change of TMB substrate depending on peroxidase-like activity of H-bpmp and **L-bpmp** (10 μM) in absence and presence of Mn^{2+} (20 μM), TMB (500 μM), H_2O_2 (200 μM) in aqueous solution (10% DMSO, pH 7.4 SPB 20 mM) at 37 ºC, b) UV/vis spectra of assay solution containing **L-bpmp** with Mn^{2+}, TMB, H_2O_2 in presence of LAP (100 U/L) at 37 °C.

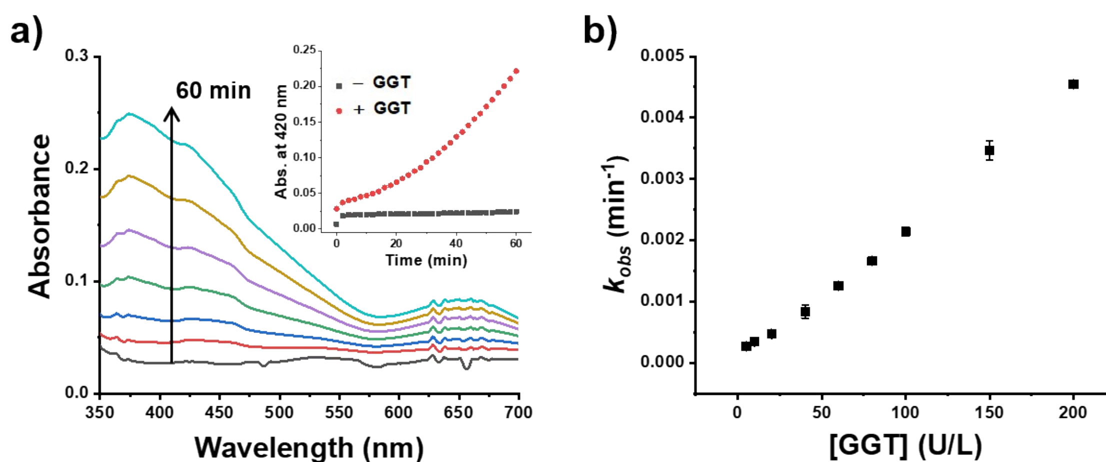


Figure S3. a) Time-dependent UV/vis spectral change in assay solution containing **E-bpmp** (50 μM) with Mn^{2+} (100 μM), TMB (500 μM), and H_2O_2 (1 mM) in the absence and presence of GGT (100 U/L) in an aqueous solution (5% DMSO, pH 7.4 SPB 20 mM) at 37 °C, b) Plot of the rate of absorbance change (k_{obs}) versus the concentration of GGT.

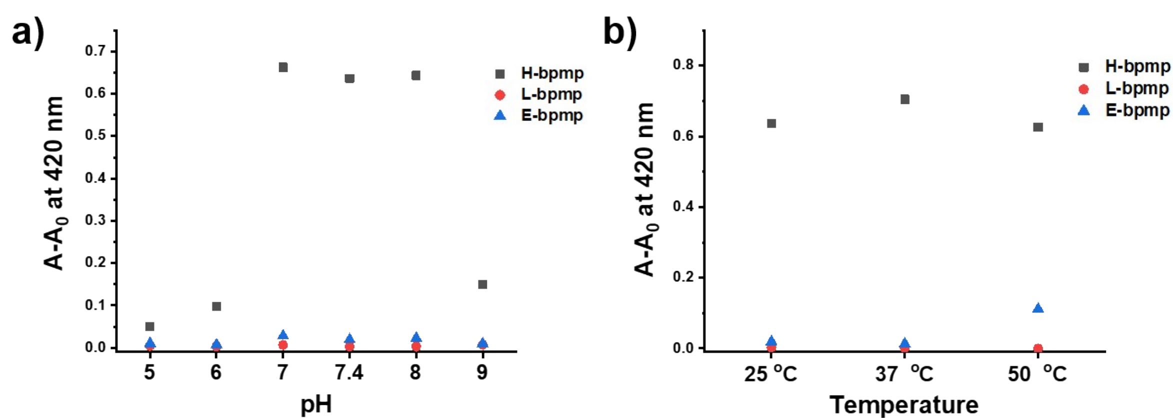


Figure S4. Effect of pH and temperature on peroxidase-like activity of caged Mn_2(bpmp) containing caged bpmp ligands (**L-bpmp**, 10 μM; **E-bpmp**, 50 μM) with Mn^{2+} (2 equiv.), TMB (0.5 mM), H_2O_2 (20 equiv.) and raw Mn_2(bpmp) containing H-bpmp (10 μM) with Mn^{2+} (2 equiv.), TMB (0.5 mM), H_2O_2 (20 equiv.), A_0: initial absorbance at 420 nm, A: absorbance at 420 nm after 1 h.

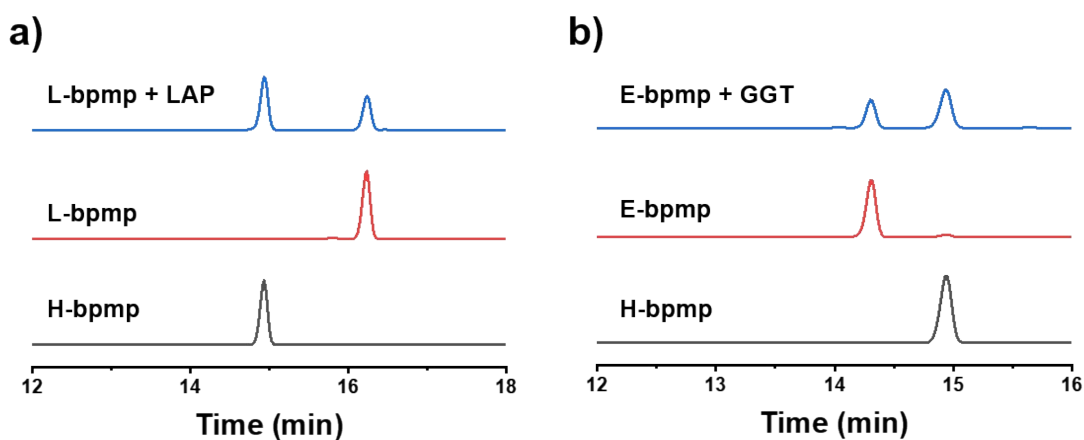


Figure S5. Chromatogram of H-bpmp, caged bpmp (**L-bpmp** and **E-bpmp**, respectively), and caged bpmp with the target enzymes, a) LAP and b) GGT in aqueous solutions.

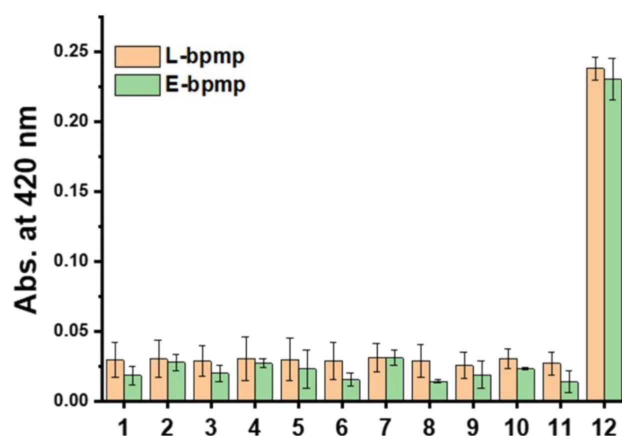


Figure S6. Change in absorbance at 420 nm in the assay solution containing caged bpmp ligands (**L-bpmp**, 10 μM; **E-bpmp**, 50 μM) with Mn^{2+} (2 equiv.), TMB (0.5 mM), H_2O_2 (20 equiv.) upon addition of various bioanalytes: (1) blank; (2) K^+ (1 mM); (3) Ca^{2+} (500 μM); (4) Mg^{2+} (500 μM); (5) Zn^{2+} (100 μM); (6) Fe^{3+} (10 μM); (7) Cu^{2+} (10 μM); (8) glucose (1 mM); (9) GSH (100 μM); (10) Cys (100 μM); (11) Hcy (100 μM); (12) target enzymes.

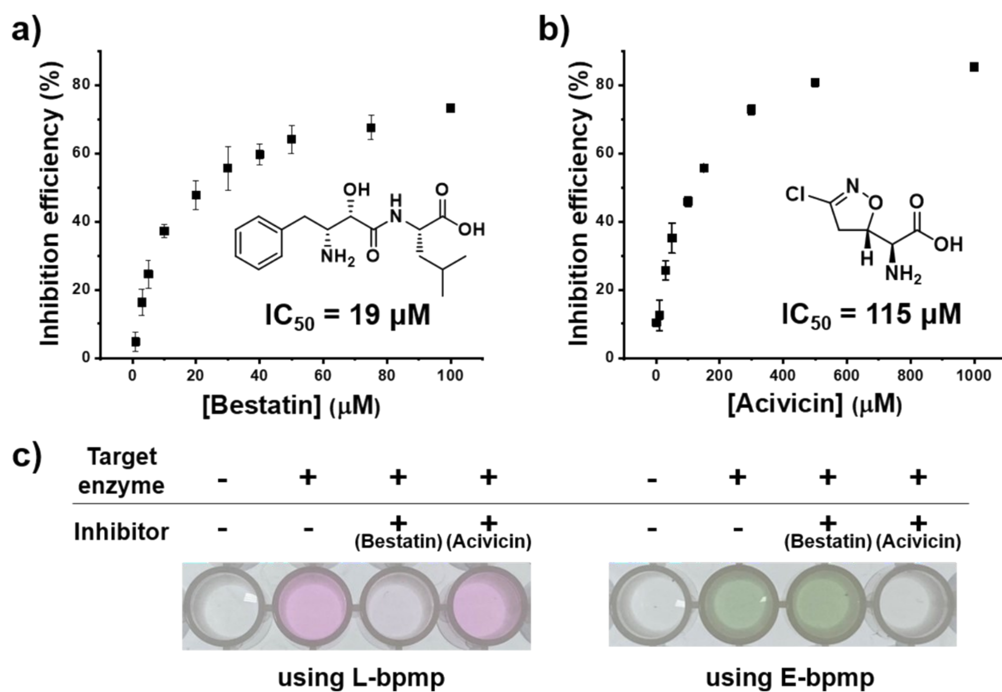


Figure S7. Plot of the inhibition efficiency of a) bestatin for LAP (100 U/L) and b) acivicin for GGT (100 U/L) versus various concentrations of inhibitors. c) Photograph showing the change in peroxidase-like activity in the absence and presence of inhibitors; Amplex red and TMB were used as substrates for the LAP and GGT assays, respectively.

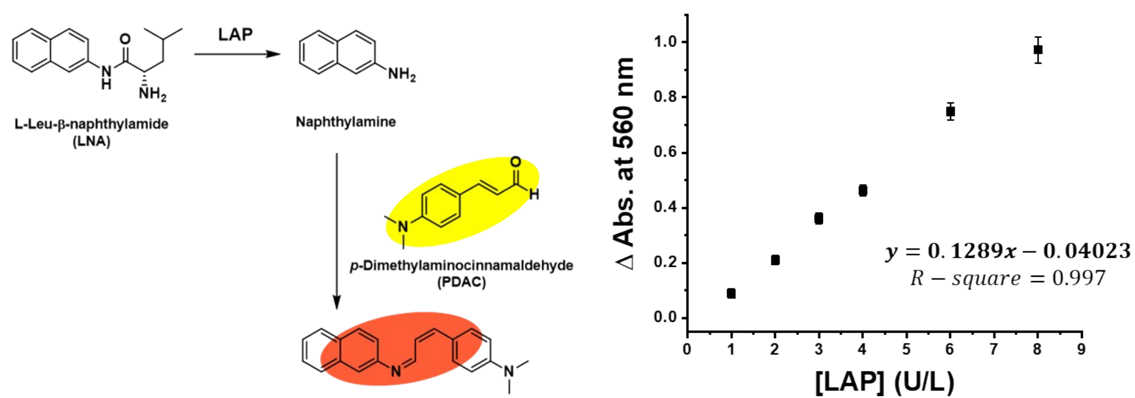


Figure S8. Schematic illustration of standard method using commercial LAP substrate, L-Leu-β-naphthylamide (left), Plot of the change in absorbance versus various LAP concentration (right).

Table S2. The recovery test using LAP-spiked human serum samples.

	Added	Found	Recovery (%)
LAP activity in Human serum from Sigma Aldrich (U/L)	0	60.7 ± 3.2	
	100	158.8 ± 5.7	98
	200	275.3 ± 15.6	107
	300	379.0 ± 13.2	106

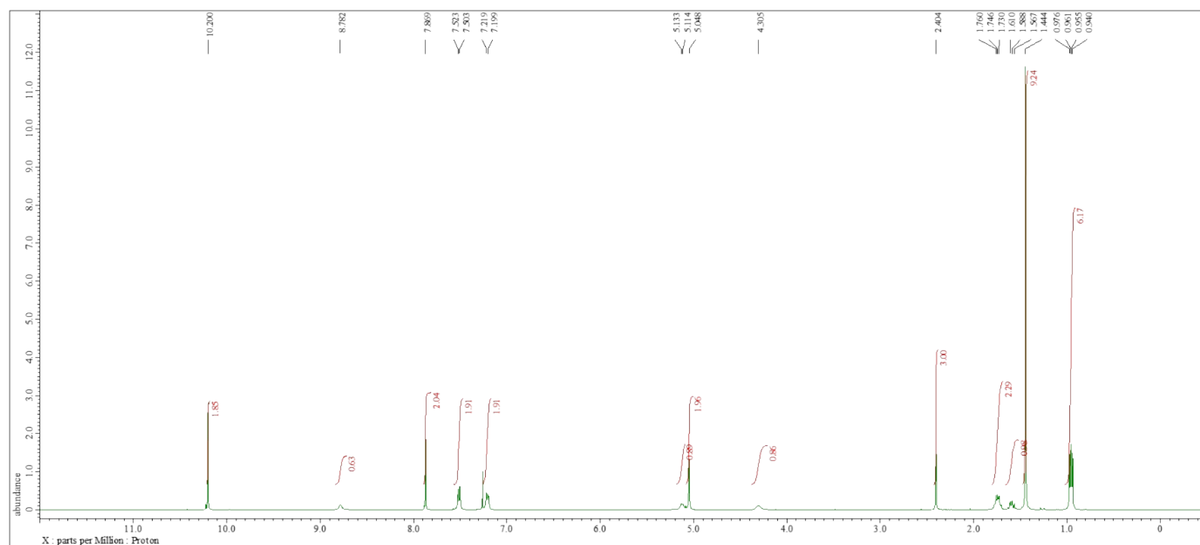


Figure S9. ^{1}H NMR spectrum of *tert*-butyl (*R*)-(1-((4-((2,6-diformyl-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**1**) in $CDCl_3$.

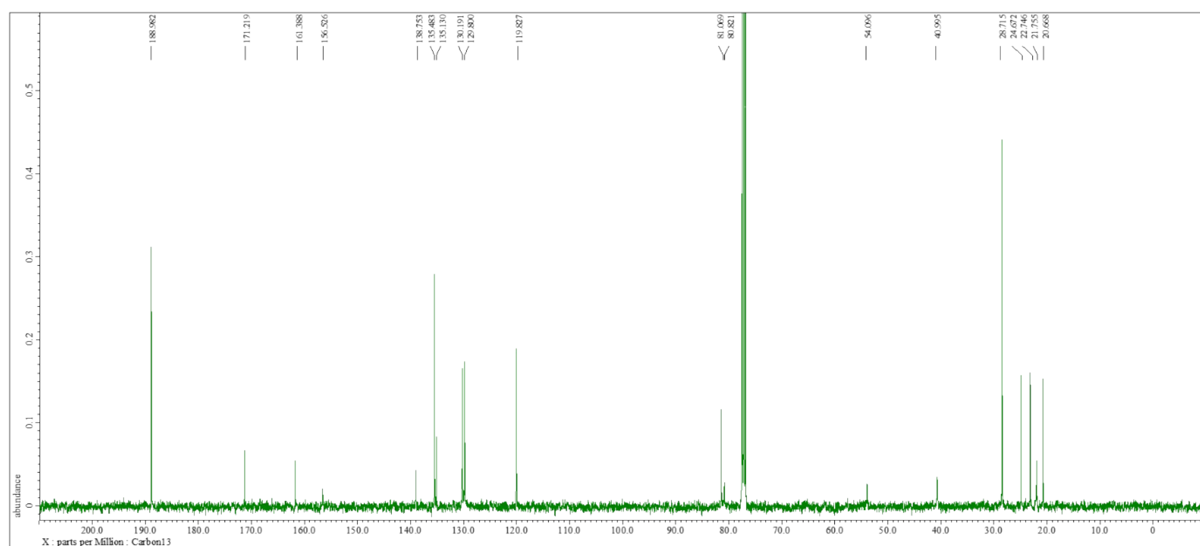


Figure S10. ^{13}C NMR spectrum of *tert*-butyl (*R*)-(1-((4-((2,6-diformyl-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**1**) in $CDCl_3$.

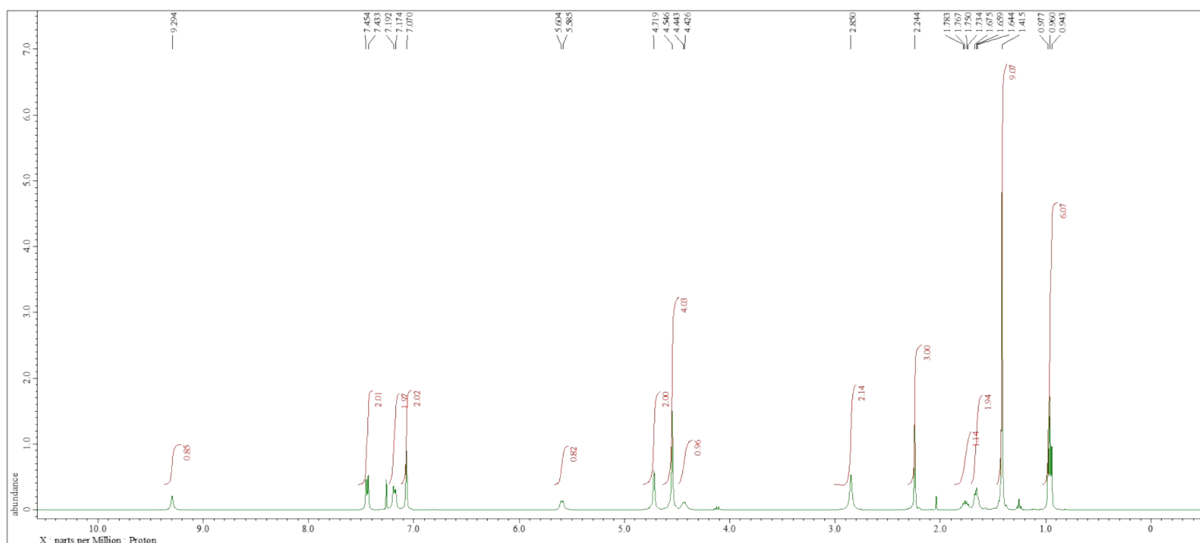


Figure S11. ^{1}H NMR spectrum of *tert*-butyl (*R*)-(1-((4-((2,6-bis(hydroxymethyl)-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**2**) in $CDCl_3$.

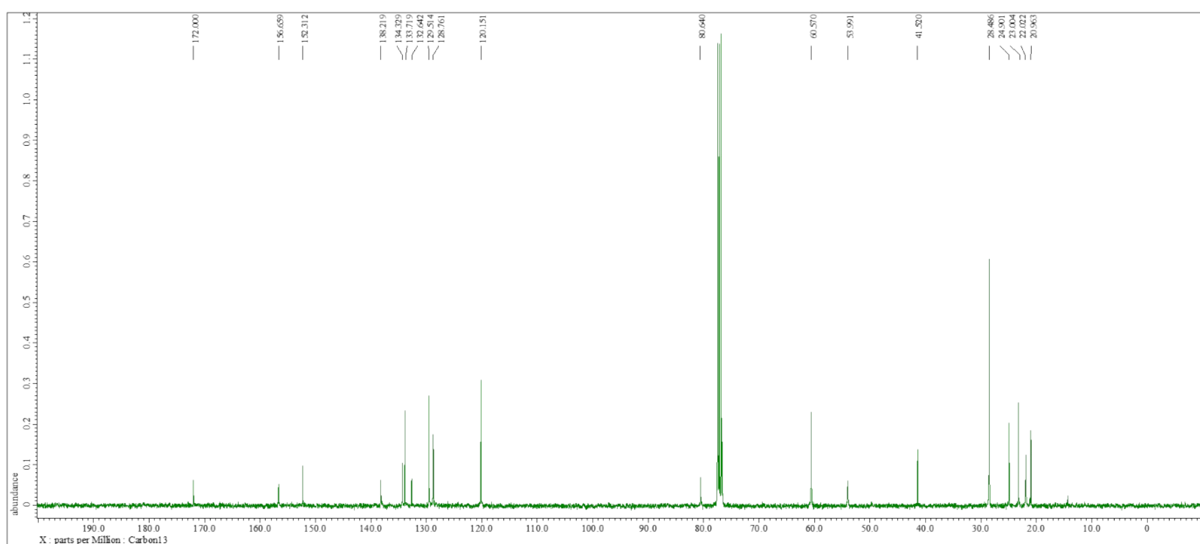


Figure S12. ^{13}C NMR spectrum of *tert*-butyl (*R*)-(1-((4-((2,6-bis(hydroxymethyl)-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**2**) in $CDCl_3$.

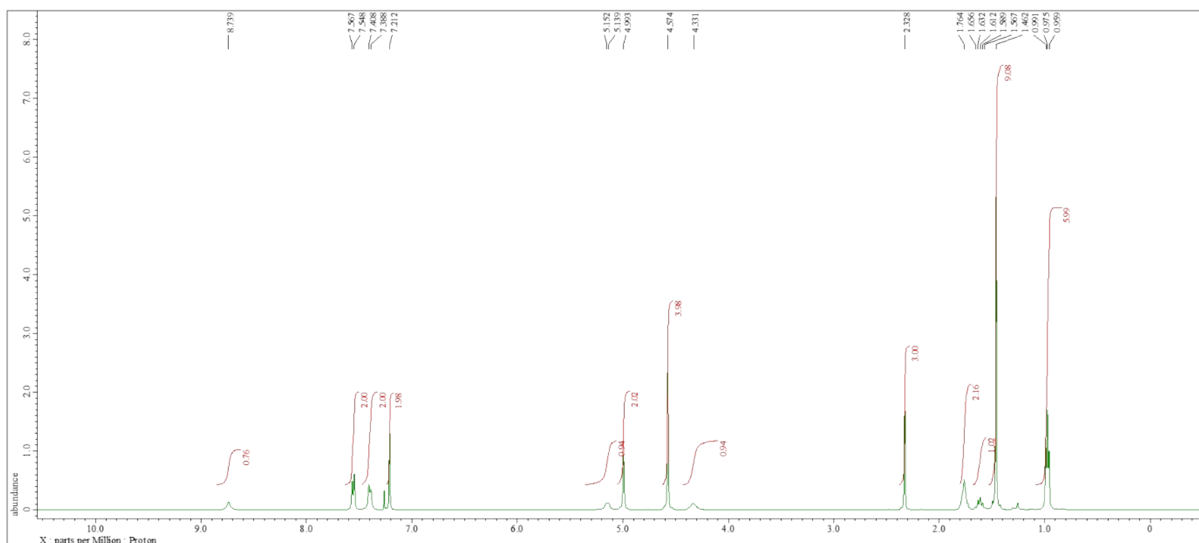


Figure S13. ^{1}H NMR spectrum of *tert*-butyl (*R*)-(1-((4-((2,6-bis(chloromethyl)-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**3**) in $CDCl_3$.

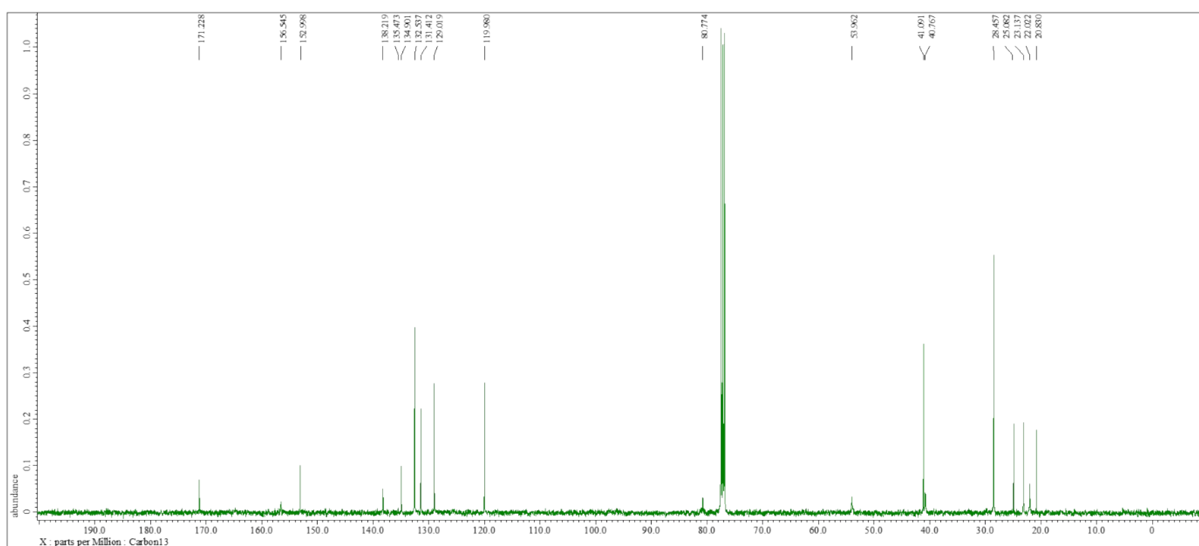


Figure S14. ^{13}C NMR spectrum of *tert*-butyl (*R*)-(1-((4-((2,6-bis(chloromethyl)-4-methylphenoxy)methyl)phenyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (**3**) in $CDCl_3$.

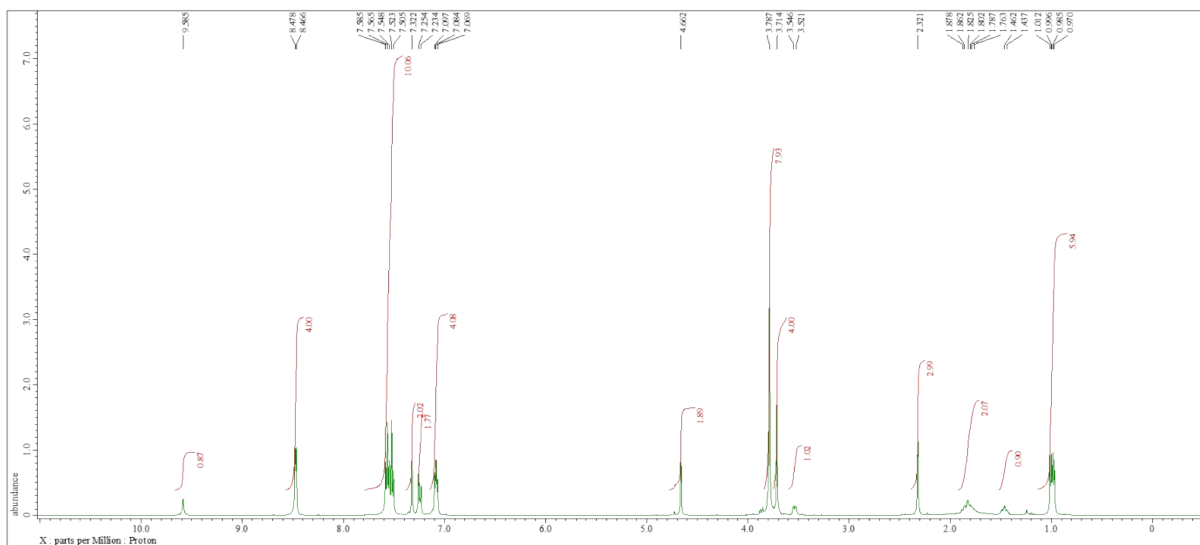


Figure S15. ^{1}H NMR spectrum of (*R*)-2-amino-*N*-(4-((2,6-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-methylphenoxy)methyl)phenyl)-4-methylpentanamide (**Leu-bpmp**, **L-bpmp**) in $CDCl_3$.

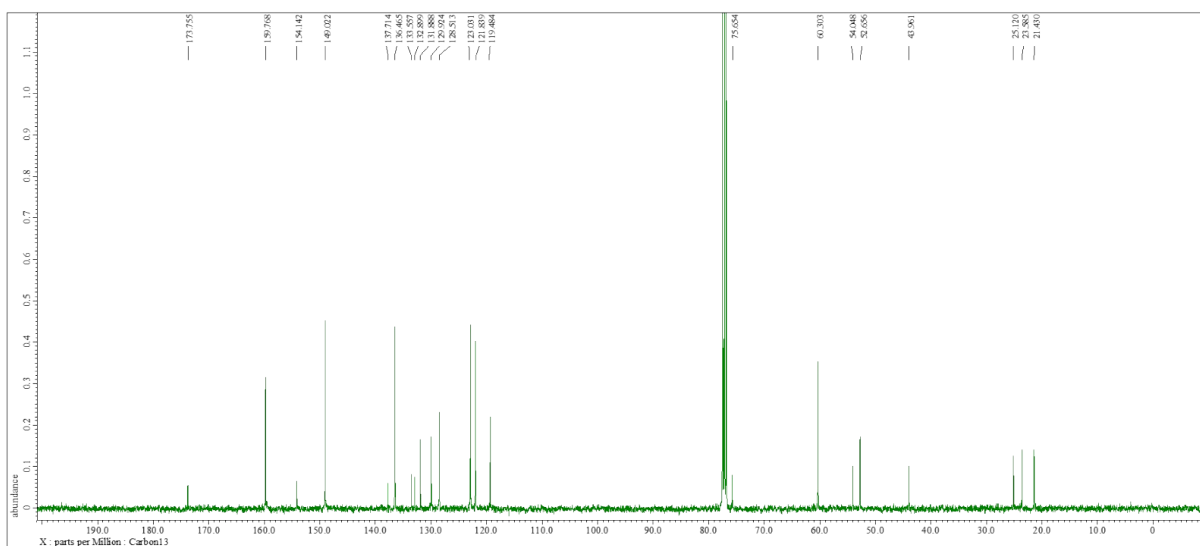


Figure S16. ^{13}C NMR spectrum of (*R*)-2-amino-*N*-(4-((2,6-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-methylphenoxy)methyl)phenyl)-4-methylpentanamide (**Leu-bpmp**, **L-bpmp**) in $CDCl_3$.

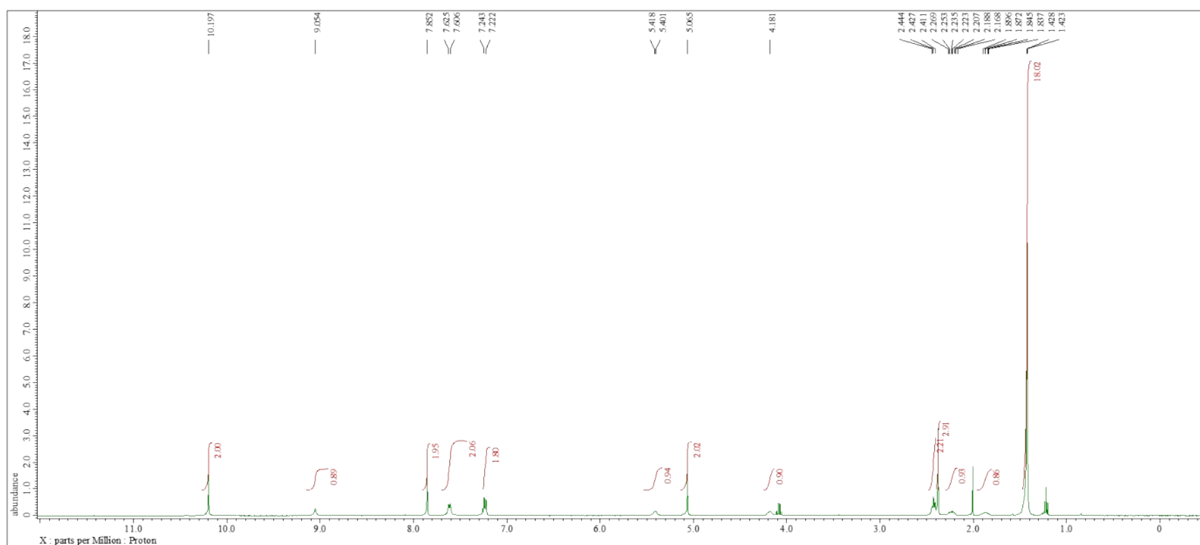


Figure S17. ^{1}H NMR spectrum of *tert*-butyl *N*^{2}-(*tert*-butoxycarbonyl)-*N*^{5}-(4-((2,6-diformyl-4-methylphenoxy)methyl)phenyl)-*L*-glutaminate (**4**) in $CDCl_3$.

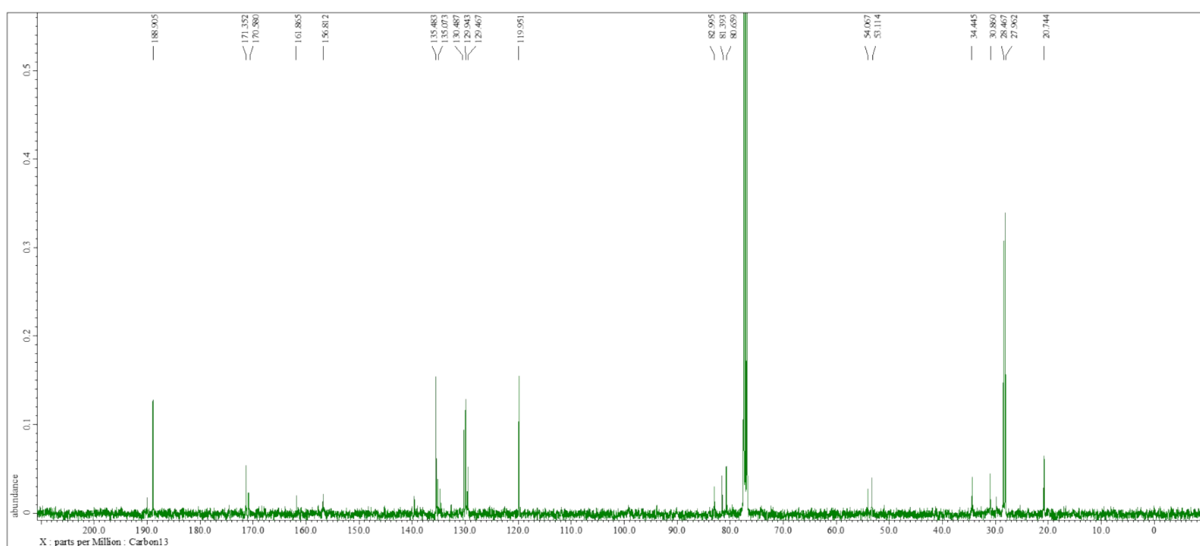


Figure S18. ^{13}C NMR spectrum of *tert*-butyl *N*^{2}-(*tert*-butoxycarbonyl)-*N*^{5}-(4-((2,6-diformyl-4-methylphenoxy)methyl)phenyl)-*L*-glutaminate (**4**) in $CDCl_3$.

Figure S19. ^{1}H NMR spectrum of *tert*-butyl N^5-(4-((2,6-bis(hydroxymethyl)-4-methylphenoxy)methyl)phenyl)-N^2-(*tert*-butoxycarbonyl)-*L*-glutaminate (**5**) in CD_3OD.

Figure S20. ^{13}C NMR spectrum of *tert*-butyl N^5-(4-((2,6-bis(hydroxymethyl)-4-methylphenoxy)methyl)phenyl)-N^2-(*tert*-butoxycarbonyl)-*L*-glutaminate (**5**) in CD_3OD.

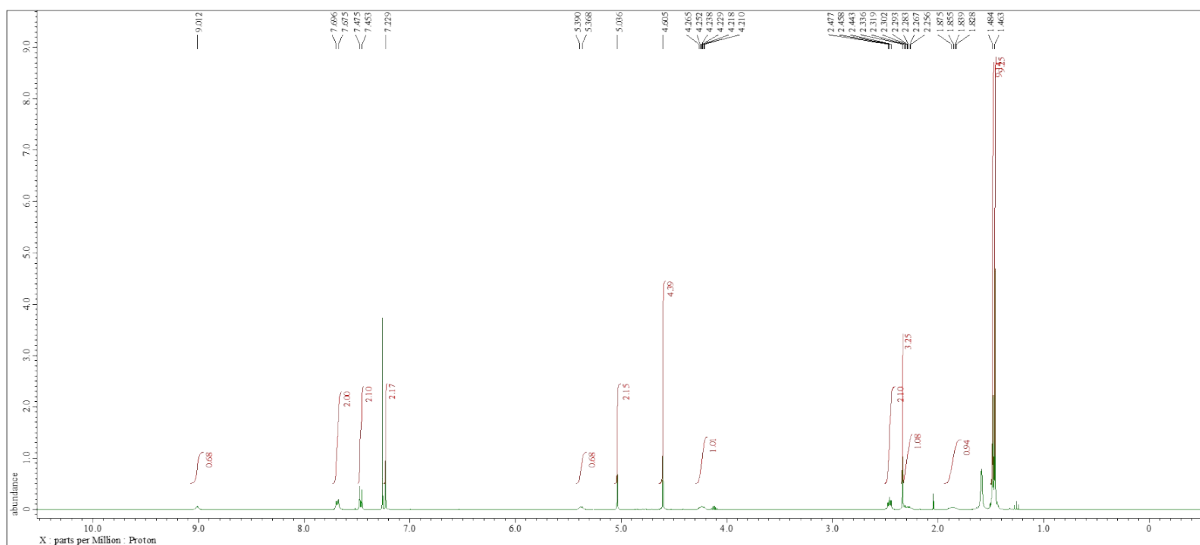


Figure S21. ^{1}H NMR spectrum of *tert*-butyl N^{5}-(4-((2,6-bis(chloromethyl)-4-methylphenoxy)methyl)phenyl)-N^{2}-(tert-butoxycarbonyl)-*L*-glutaminate (**6**) in $CDCl_3$.

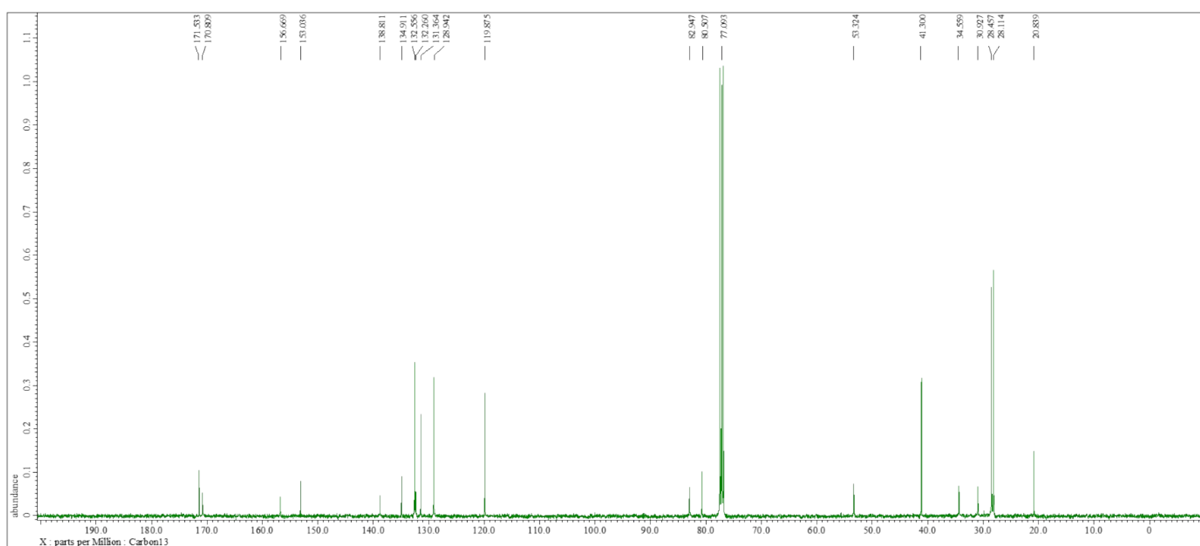


Figure S22. ^{13}C NMR spectrum of *tert*-butyl N^{5}-(4-((2,6-bis(chloromethyl)-4-methylphenoxy)methyl)phenyl)-N^{2}-(tert-butoxycarbonyl)-*L*-glutaminate (**6**) in $CDCl_3$.

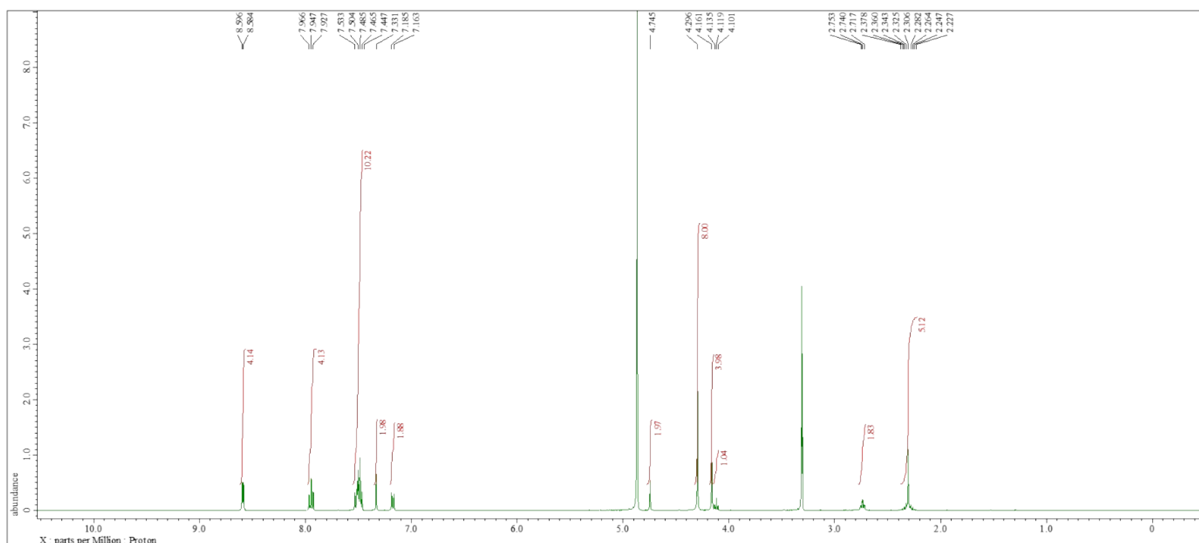


Figure S23. ^{1}H NMR spectrum of *N*^{5}-(4-((2,6-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-methylphenoxy)methyl)phenyl)-*L*-glutamine (**Glu-bpmp, E-bpmp**) in CD_3OD.

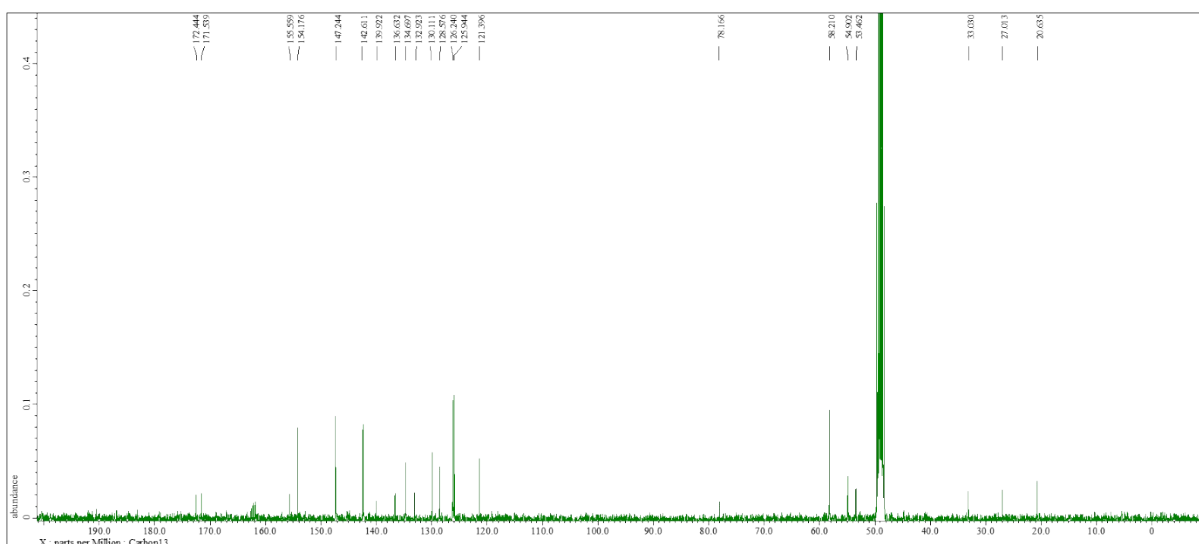


Figure S24. ^{13}C NMR spectrum of *N*^{5}-(4-((2,6-bis((bis(pyridin-2-ylmethyl)amino)methyl)-4-methylphenoxy)methyl)phenyl)-*L*-glutamine (**Glu-bpmp, E-bpmp**) in CD_3OD.

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