Supporting Information

Amino acid-based H_2S Donors: *N*-thiocarboxyanhydrides that release COS/H_2S with innocuous by-products

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Materials and Methods:

All amino acids, reagents, and solvents were purchased from commercial vendors and used as received unless otherwise noted. NMR spectra were recorded on an Agilent NMR spectrometer (400 MHz), and chemical shifts are reported relative to appropriate internal solvent resonances. H_2S release measurements were performed on an H_2S-sensitive electrochemical probe from World Precision Instruments (WPI). UV-Vis absorbance spectra were recorded on a BioTek Synergy Mx plate reader from BioTek. Carbonic anhydrase (CA) is sourced from bovine erythrocytes and was procured from MP Biomedicals, VWR (product number 0215387925). HT-29 human colon adenocarcinoma cells, MCF-7 estrogen positive human breast cancer cells, and H9C2 rat embryonic cardiomyocytes were obtained from American Type Tissue Collection (ATTC). Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (FBS) (Invitrogen), penicillin (50 U/mL), and streptomycin (50 mg/mL) (Invitrogen) was used to culture cells. CellTiterGlo reagent was purchased from Promega. Cell viability assays were performed by cell counting kit (CCK-8) purchased from Dojindo. 1x PBS (10 mM) was prepared using a standard recipe: 8 g NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 were dissolved in 800 mL of DI water, the pH was adjusted to 7.4 using 1 N NaOH, and the final volume was adjusted to 1L by addition of DI water.

Experimental:

1. H_2S-release measurements

H_2S release measurements via electrochemical probe

20 μL of NTA solution (5 mM) in DMSO was added to 9.73 mL of 1x PBS buffer (pH 7.4) in a 20-mL scintillation vial followed by addition of 250 μL of carbonic anhydrase (CA) (6 μM in 1x PBS buffer). Final concentrations were 10 μM NTA and 150 nM CA. The H_2S-sensitive microelectrode was immediately submerged into the solution, and H_2S release was recorded as output current. The peaking time is referred to as the time where the current output reaches its maximum. A calibration curve was generated as described previously and is shown in Figure S2.[1]

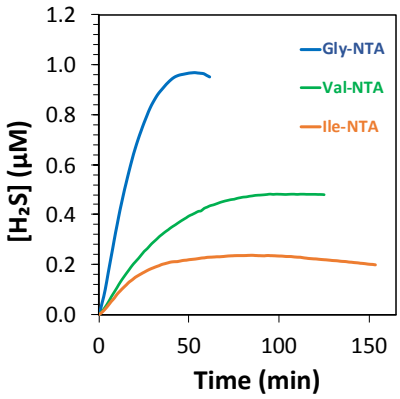


Figure S1: H_2S release profiles for Gly-NTA, Val-NTA, and Ile-NTA measured by an H_2S sensitive microelectrode.

NTA	Peaking time (min)	Peaking concentration (µM)
Gly-NTA	51 ± 6	0.9
Val-NTA	96 ± 1	0.5
Ile-NTA	104 ± 14	0.2

Table S1: H_2S release peaking times and concentrations for C-substituted NTAs. Error bars reflect standard deviation for three experiments (n=3).

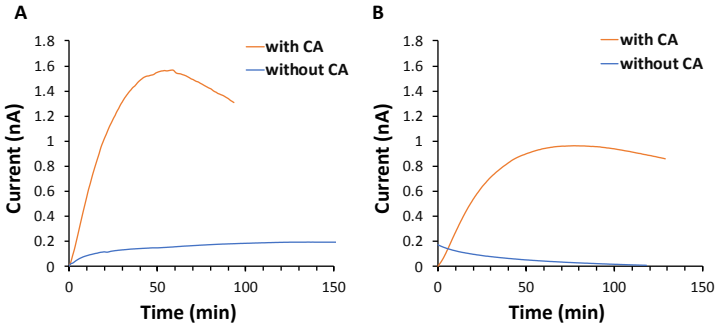


Figure S2: H_2S release profiles for **A)** Gly-NTA and **B)** Leu-NTA in the presence and absence of CA as measured by an H_2S sensitive microelectrode.

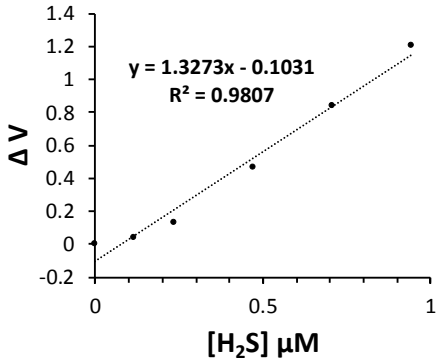


Figure S3: Calibration curve for H_2S selective electrochemical probe. The change in voltage (y-axis), measured after successive additions of Na_2S is plotted against concentration of Na_2S (x-axis) solution in 1x PBS buffer at pH 7.4. The concentration of H_2S is calculated via the equation $[H_2S] = [Na_2S] \Big/ \{1 + \frac{K_a^1}{[H^+]} + \frac{K_a^1 K_a^2}{[H^+]}\}$ where pK_a^1 = 6.89 and pK_a^2 = 19.

H_2S-release measurements via methylene blue assay

20 µL of NTA solution (10 mM) in DMSO was added to 1860 µL of 10 mM PBS buffer containing 100 µL of $Zn(OAc)_2$ solution (40 mM in water) and 20 µL of CA solution (30 µM in 10 mM PBS buffer) in a one-dram vial. The final concentrations in the sample solution were 100 µM NTA, 2 mM $Zn(OAc)_2$, and 300 nM CA. Similarly, a blank solution was also prepared with all the above compounds except for NTAs. At various time points, 100 µL aliquots were removed from sample/blank vials and mixed with equal volumes (100 µL) of $FeCl_3$ (30 mM in 1.2 M HCl) and *N,N'*-dimethyl-*p*-phenylenediamine (20 mM in 7.2 M HCl) solutions. The final solutions were vortexed and allowed to incubate for about 8 h before transferring them into a 96-well plate (280 µL/well). The absorbance spectra in the range from 500–800 nm was collected, and kinetics analysis was carried out after subtracting the absorbance for the blank sample from that of NTA samples at all time points. All experiments were done in triplicate.

NTA	Half-life (h)	k_{obs} (s^{-1})
β-Ala-NTA	1.1 ± 0.1	1.0 x 10^{-2}
Gly-NTA	1.7 ± 0.1	7.0 x 10^{-3}
Ala-NTA	2.2 ± 0.2	5.2 x 10^{-3}
Leu-NTA	4.5 ± 0.5	2.6 x 10^{-3}
Phe-NTA	4.6 ± 0.3	2.5 x 10^{-3}
Pro-NTA	6.9 ± 0.2	1.7 x 10^{-3}
Aib-NTA	10.2 ± 2.0	1.1 x 10^{-3}
Ile-NTA	18.0 ± 1.8	6.4 x 10^{-4}
Val-NTA	20.0 ± 0.2	5.8 x 10^{-4}

Table S2: H_2S release half-lives and rate constants for C-substituted NTAs. Error bars reflect standard deviation for three experiments (n=3).

2. Cell Studies:

Viability studies

Cell Culture

Both cell lines, H9C2 and MCF-7, were cultured in Petri dishes in DMEM supplemented with 10% FBS, penicillin (50 U/mL), and streptomycin (50 mg/mL) in 5% CO_2-air atmosphere at 37 ^oC. Adherent cells were grown to about 90% confluency, culture medium was aspirated, cells were rinsed with PBS (x3) and then single cell suspensions were obtained by trypsinization (0.05% trypsin/EDTA). 1 mL of media was added, cells were spun down at 1000 rpm for 5 minutes, cell pellets were re-suspended in 1 mL of media and an appropriate volume (5 mL) was inoculated into a new Petri dish.

Viability

Adherent cells (5000 cells/well) were seeded in 200 µL/well of DMEM media in a 96-well plate and incubated overnight at 37 ^oC in 5% CO_2 and 90% relative humidity. Media was replaced with 190 µL of fresh DMEM solution and 10 µL of NTA stock solution (2 mM in 5% DMSO in PBS). The final concentration of NTA was 100 µM, and the cells were incubated for 1.5 h. Similarly, control wells were incubated with 10 µL of 5% DMSO in PBS instead of NTA solution. After washing the cells three times with PBS, 190 µL of fresh DMEM (without FBS) was added followed by addition of 10 µL of CCK-8 solution. After incubating the cells for 3 h, the well-plate was analyzed on a plate reader, and the absorbance at 450 nm and 650 nm was recorded (n=3 for each group).

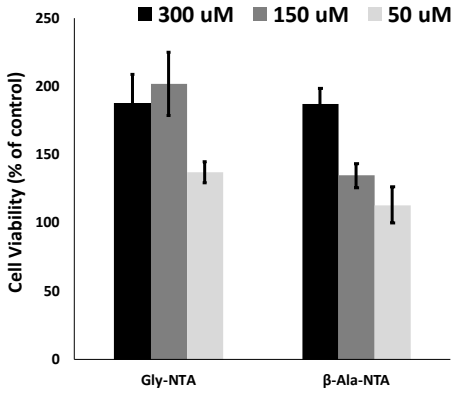


Figure S4: Cell viability of H9C2 cells in 300, 150, and 50 µM concentration of Gly-NTA and β-Ala-NTA relative to an untreated control group. The cells were cultured for 24 h. Error bars represent the average of three experiments (n=3) with 5 replicates per experiment.

Inhibition studies

Cell Culture

Both cell lines, HT-29 and MCF-7, were grown as monolayers in T75 flasks in DMEM, supplemented with 10% fetal calf serum, penicillin (50 U/mL), and streptomycin (50 mg/mL). Adherent cells were grown to about 90% confluency, culture medium was aspirated, cells were rinsed with PBS and then single cell

suspensions were obtained by trypsinization (0.05% trypsin/EDTA). Media (8 mL) was added, cells were spun down at 1000 rpm for 5 min, cell pellets were re-suspended in 10 mL of media, and an appropriate volume was inoculated into a new culture flask.

Compound	IC_{50} µM at 72h	
	MCF-7	HT-29
GYY4137	592 ± 52	474 ± 43
Gly NTA	723 ± 222	1047 ± 55
Ala NTA	857 ± 220	549 ± 61
Leu NTA	308 ± 3	124 ± 13
Val NTA	289 ± 62	135 ± 12

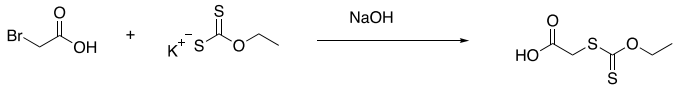
Table S3: IC_{50} values for MCF-7 and HT-29 cells. Results mean ± SEM for 4 independent measurements.

Growth inhibition

Adherent cells (2,000–3,000 cells/well) were seeded in 100 µL of media in a 96-well plate and incubated overnight at 37 °C in 5% CO_2 and 90% relative humidity. Media was replaced with 100 µL of fresh media containing various concentrations of the test compounds. Compounds were dissolved in DMSO, then diluted 100-fold or 20-fold in media to obtain an initial concentration of 1 mM. A 2-fold serial dilution was then done in media. DMSO alone was diluted similarly for controls. The final DMSO concentration was adjusted in all media to 0.5%. After 72 h, 100 µL/well of CellTiterGlo reagent was added according to manufacturer's protocol, and cell viability was measured with a luminometer. IC_{50} values were calculated using SoftMax software.

3. Synthesis and Characterization:

*Synthesis of 2-[(ethoxycarbonothioyl)thio]acetic acid (**XAA**)*



2-[(Ethoxycarbonothioyl)thio]acetic acid (**XAA**) was synthesized according to a modified version of a published procedure.[2] In a 100 mL round bottom flask, potassium ethylxanthogenate (5.00 g, 31 mmol) and NaOH(1.25 g, 31 mmol) were dissolved in DI water (20 mL). The solution was cooled with stirring on an ice bath. Bromoacetic acid (4.30 g, 62 mmol) was dissolved in DI water (15 mL) and added slowly (~1 drop/s) to the reaction mixture via an addition funnel. After complete addition, the reaction mixture was maintained in an ice bath for another 5 min, and then the reaction mixture was allowed to stir as it warmed to room temperature over 24 h. When the TLC (mobile phase 1:4 EtOAc:hexanes) showed complete consumption of starting material, the mixture was acidified with 1 N HCl (aq.) to pH ~ 1. The now cloudy mixture was extracted using CH_2Cl_2 (3 x 15 mL), and the combined extracts were washed with brine (50 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated via rotary evaporation to give a slightly yellow viscous oil. Hexanes (30 mL) was added, and the suspension was stirred on an ice bath to precipitate 2-[(ethoxycarbonothioyl)thio]acetic acid (**XAA,** 4.50 g, 25 mmol, 81% yield) as an off-

white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.66 (q, 2H, CH_2), 3.97 (s, 2H, CH_2), 1.42 (t, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 211.84, 173.87, 70.78, 37.39, 13.51.

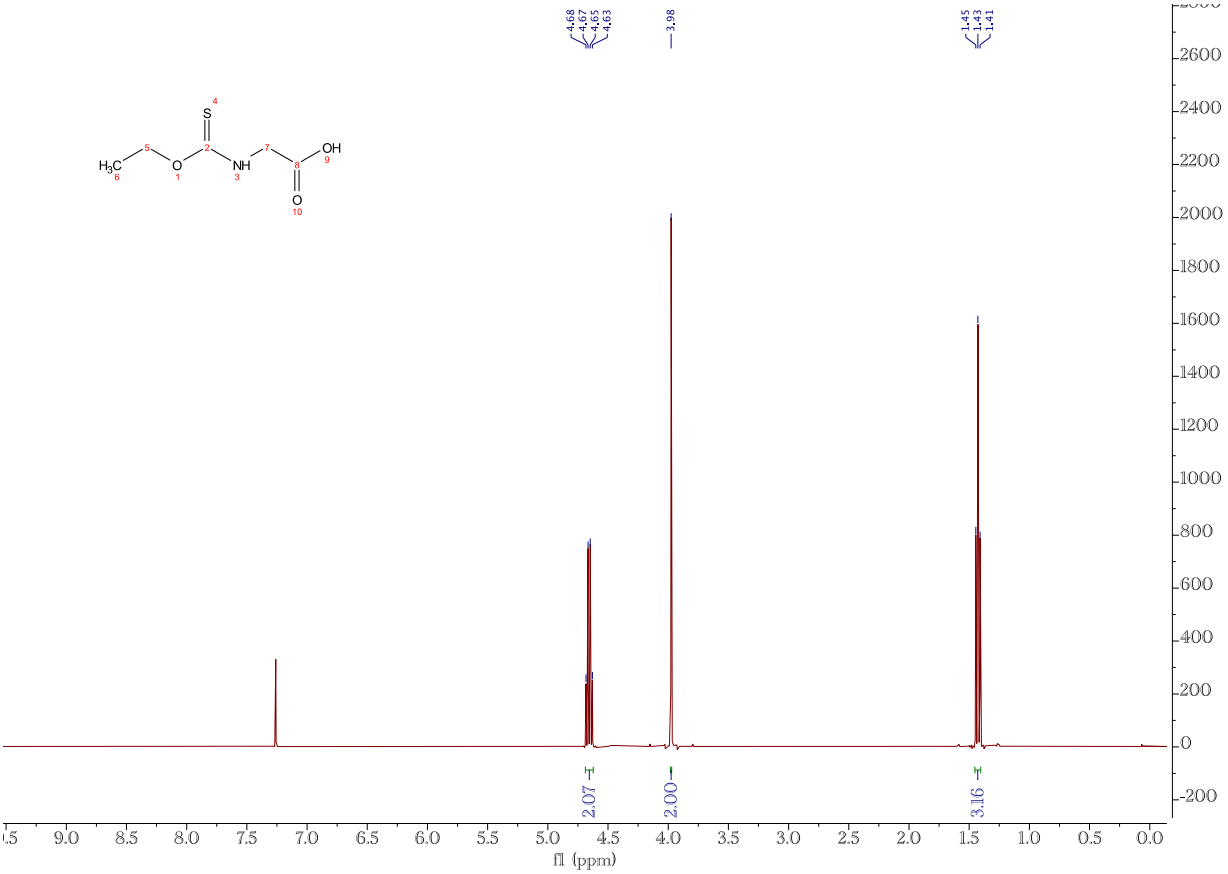


Figure S5: ^{1}H NMR spectrum of 2-[(ethoxycarbonothioyl)thio]acetic acid (**XAA**).

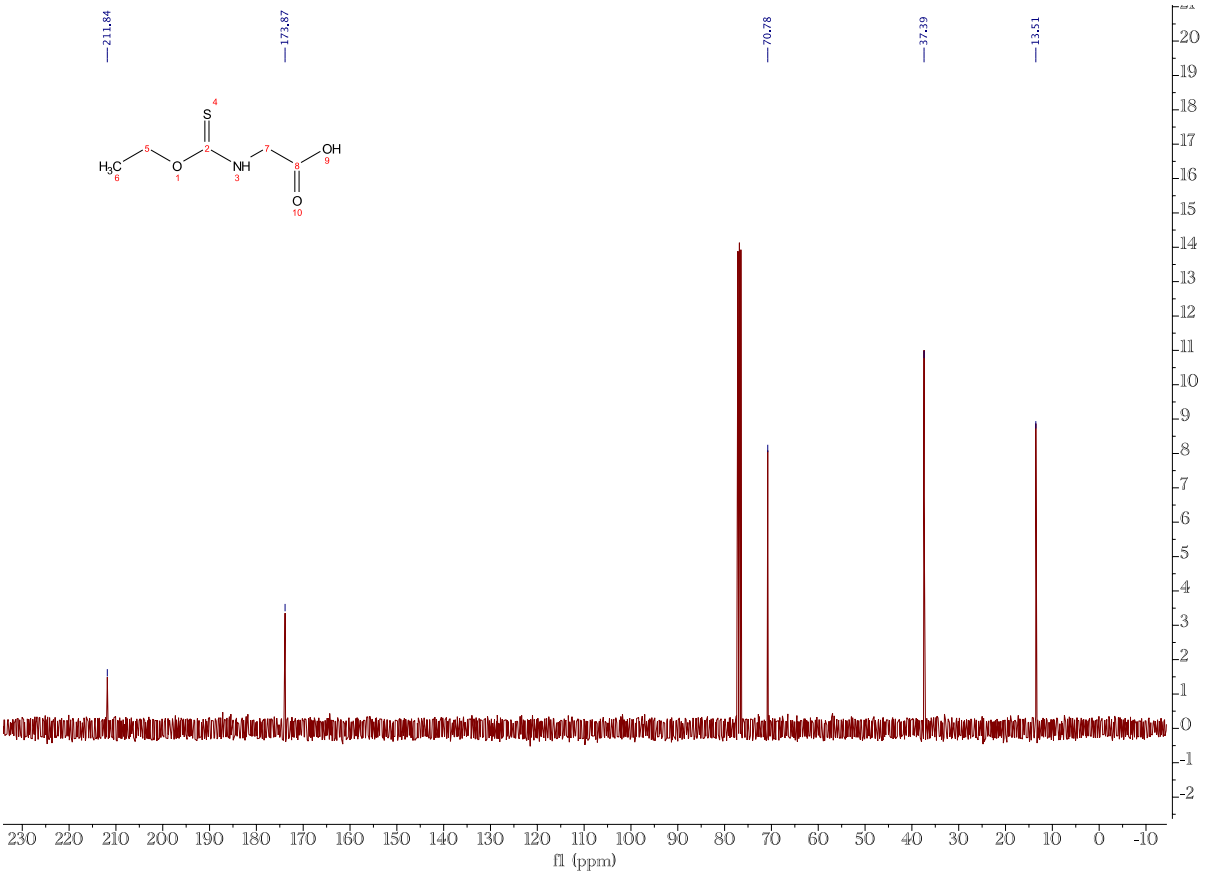
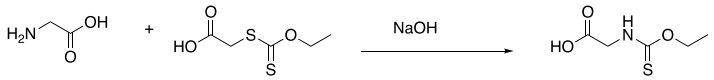


Figure S6: ^{13}C NMR spectrum of 2-[(ethoxycarbonothioyl)thio]acetic acid (**XAA**).

General procedure for the synthesis of thiocarbamate (TC) derivatives

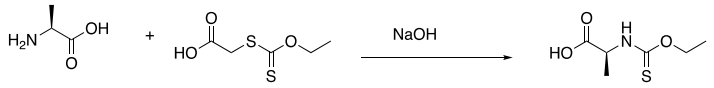
The synthesis of thiocarbamate derivatives was carried out according to a modified version of a published procedure.[3] In general, the desired amino acid (1.0 equiv.) was suspended in a mixture of methanol (0.5-1.0 mL) and 45 wt.% NaOH solution in water (0.7-1.5 mL) to afford a clear solution (volume of methanol and NaOH can be adjusted in case of precipitation). While stirring, a solution of 2-[(ethoxy-carbonothioyl)thio]acetic acid (**XAA**, 0.6–0.8 equiv.) in methanol (0.5-1.0 mL) was added dropwise via syringe over a period of 30 min. During the addition, the reaction mixture was heated to 45 °C in an oil bath and kept there for 24 h. Reaction progress was monitored via TLC (mobile phase 1:4 EtOAc:hexanes) and visualized under UV light (254 nm) and/or with ninhydrin stain (orange-yellow spot). After complete consumption of the XAA starting material, the reaction mixture was cooled to rt and acidified with 1 N HCl (aq.) to pH ~ 1 and extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the resulting yellow oil was either recrystallized from hexanes or directly taken into the next step without any additional purification.

*Synthesis of (ethoxycarbonothioyl)glycine (**TC-1**)*



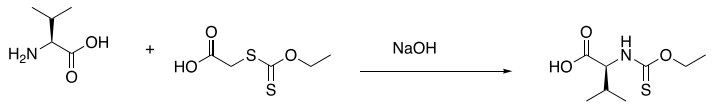
Reagents glycine (2.00 g, 26.64 mmol) and **XAA** (2.88 g, 15.99 mmol) were reacted according to the general procedure described above to yield white solid of (ethoxycarbonothioyl)glycine (**TC-1**, 1.23 g, 7.54 mmol, 47% yield). TC-1 was taken to next step without purification.

*Synthesis of (ethoxycarbonothioyl)-L-alanine (**TC-2**)*



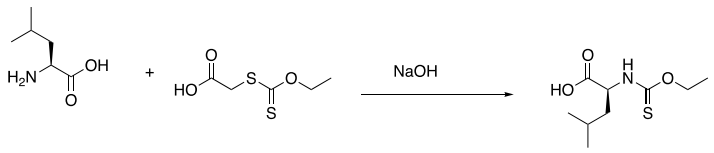
Reagents L-alanine (1.00 g, 11.22 mmol) and **XAA** (1.62 g, 8.98 mmol) were reacted according to general procedure described above to yield a yellow oil that was purified by recrystallization from CH_2Cl_2 and hexanes to yield white solid of (ethoxycarbonothioyl)-L-alanine (**TC-2**, 0.99 g, 5.59 mmol, 62%).

*Synthesis of (ethoxycarbonothioyl)-L-valine (**TC-3**)*



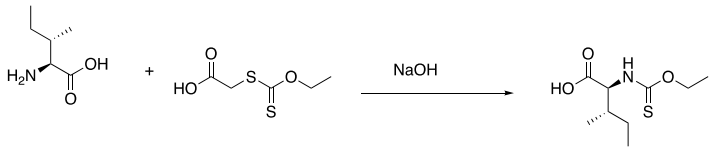
Reagents L-valine (1.50 g, 12.80 mmol) and **XAA** (1.85 g, 10.24 mmol) were reacted according to general procedure described above to yield a yellow oil of (ethoxycarbonothioyl)-L-valine (**TC-3**, 2.03 g, 9.89 mmol, 96% yield). TC-3 was taken to next step without purification.

*Synthesis of (ethoxycarbonothioyl)-L-leucine (**TC-4**)*



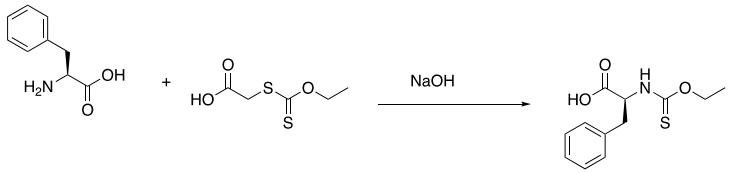
Reagents L-leucine (1.50 g, 11.44 mmol) and **XAA** (1.65 g, 9.15 mmol) were reacted according to general procedure described above to yield (ethoxycarbonothioyl)-L-leucine as white solid (**TC-4**, 1.55 g, 7.07 mmol, 77% yield). TC-4 was taken to next step without purification.

*Synthesis of (ethoxycarbonothioyl)-L-isoleucine (**TC-5**)*



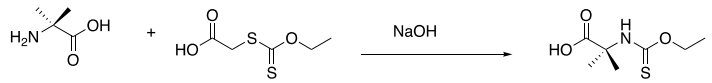
Reagents L-isoleucine (1.00 g, 7.62 mmol) and **XAA** (0.82 g, 4.57 mmol) were reacted according to general procedure described above to yield a highly viscous yellow oil of (ethoxycarbonothioyl)-L-isoleucine (**TC-5**, 0.99 g, 4.51 mmol, 99% yield). TC-5 was taken to next step without purification.

*Synthesis of (ethoxycarbonothioyl)-L-phenylalanine (**TC-6**)*



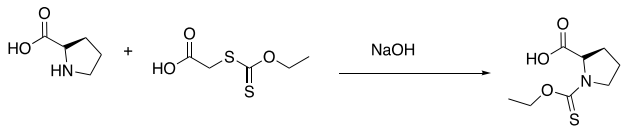
Reagents L-phenylalanine (1.00 g, 6.05 mmol) and **XAA** (0.65 g, 3.63 mmol) were reacted according to general procedure described above to yield (ethoxycarbonothioyl)-L-phenylalanine as white solid (**TC-6**, 0.90 g, 3.55 mmol, 98% yield). TC-6 was taken to next step without purification.

*Synthesis of 2-((ethoxycarbonothioyl)amino)-2-methylpropanoic acid (**TC-7**)*



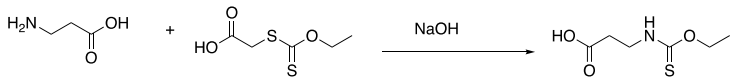
Reagents 2-amino-2-methylpropanoic acid (1.50 g, 14.55 mmol) and **XAA** (1.57 g, 8.73 mmol) were reacted according to general procedure described above to yield a pale yellow solid of 2-((ethoxycarbonothioyl)amino)-2-methylpropanoic acid (**TC-7**, 0.98 g, 5.12 mmol, 59% yield). TC-7 was taken to next step without purification.

*Synthesis of (ethoxycarbonothioyl)-L-proline (**TC-8**)*



Reagents L-proline (1.50 g, 13.03 mmol) and **XAA** (1.41 g, 7.82 mmol) were reacted according to general procedure described above to yield a pale yellow oil of (ethoxycarbonothioyl)-L-proline (**TC-8**, 1.39 g, 6.84 mmol, 87% yield). TC-8 was taken to next step without purification

Synthesis of 3-((ethoxycarbonothioyl)amino)propanoic acid ***(TC-9)***

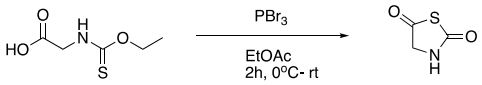


Reagents *β*-alanine (1.00 g, 11.22 mmol) and **XAA** (1.21 g, 6.73 mmol) were reacted according to general procedure described above to yield white crystals of 3-((ethoxycarbonothioyl)amino)propanoic acid (**TC-9**, 0.99 g, 5.59 mmol, 83%). TC-9 was taken to next step without purification.

General procedure for synthesis of NTAs

NTAs were synthesized via a ring-closing reaction of TC derivatives according to a modified version of a published procedure.[3] In general, the desired TC (0.5-3.0 mmol) was dissolved in ethyl acetate (2-3 mL) and cooled on an ice bath. While stirring, a solution of PBr_3 (1.5 equiv.) in ethyl acetate (1 mL) was added via syringe over 20 min. The ice bath was removed, and the reaction mixture was allowed to stir for 1.5 h as it warmed to room temperature. Reactions were monitored by TLC (1:10 EtOAc:hexanes, faintly visible under UV light, 254 nm). After the starting material was consumed, the reaction was quenched and neutralized (pH ~ 7.5) by addition of saturated sodium bicarbonate solution (100 mL), followed by extraction with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated via rotary evaporation to afford the crude NTA product. Purification was carried out by silica gel flash chromatography as described for each specific NTA below.

Synthesis of Gly-NTA



Gly-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with 3-((ethoxycarbonothioyl)amino)propanoic acid (TC-1, 1.0 g, 6.13 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield Gly-NTA as a white solid (380 mg, 3.24 mmol, 53% yield, m.p. 110-111 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.92 (s, 1H, NH), 4.27 (d, ^1J = 1.2 Hz, 2H, CH_2). ^{13}C NMR (400 MHz, $CDCl_3$): δ 195.39, 168.55, 55.86. HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ Calcd for $C_3H_4NO_2S$ 117.9963; Found 117.9962.

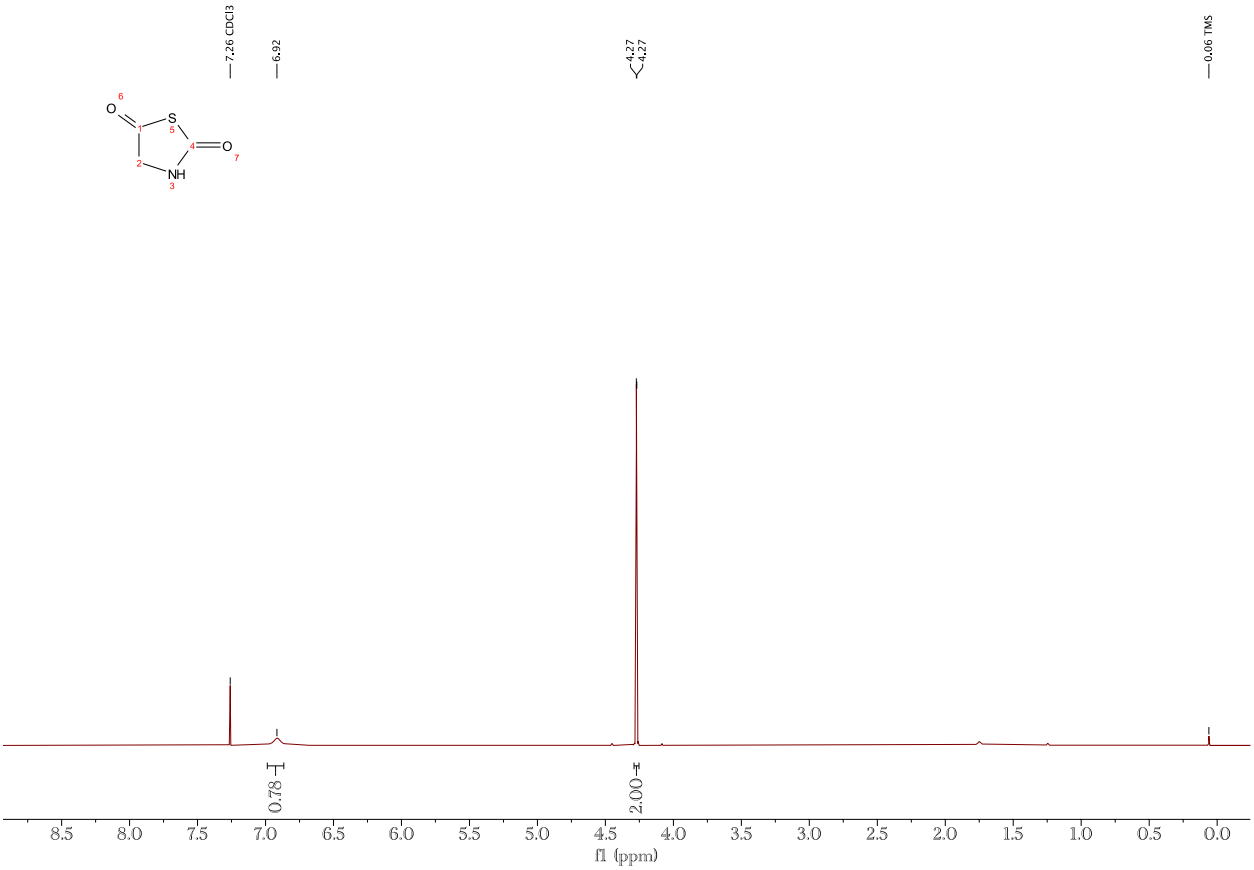


Figure S7: ^{1}H NMR spectrum of **Gly-NTA**

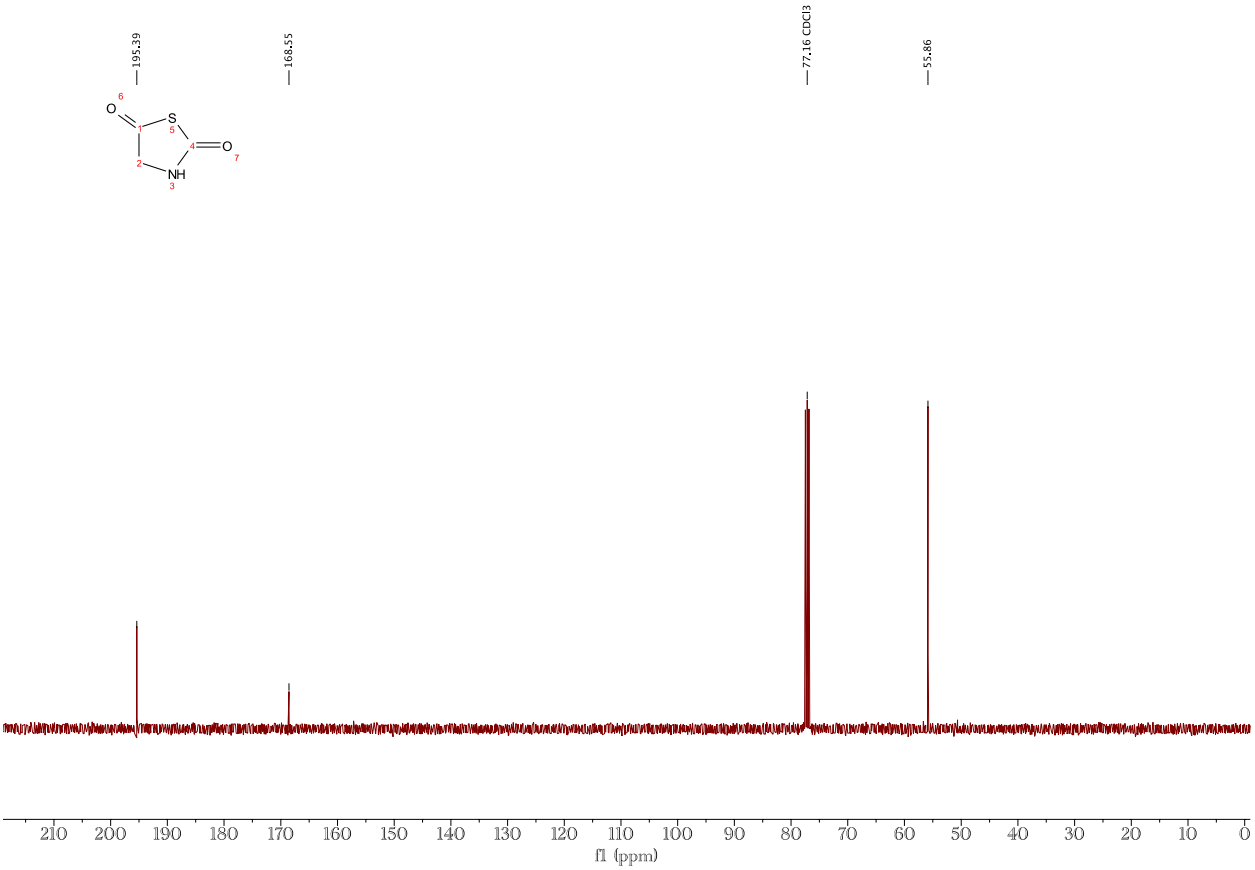
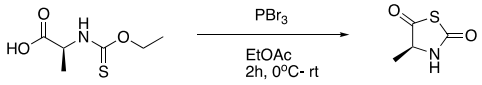


Figure S8: ^{13}C NMR spectrum of **Gly-NTA**

Synthesis of Ala-NTA



Ala-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with (ethoxycarbonothioyl)-L-alanine (TC-2, 1.4 g, 7.90 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield pale yellow powder of Ala-NTA (456 mg, 3.48 mmol, 44% yield, m.p. 89-90 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (s, 1H, NH), 4.40 (dq, ^1J = 1.17 Hz, ^2J = 6.87 Hz, 2H, CH), 1.5 (d, ^1J = 6.87 Hz, 2H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 199.29, 167.81, 63.32, 18.89. HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ Calcd for $C_4H_6NO_2S$ 132.0119; Found 132.0112.

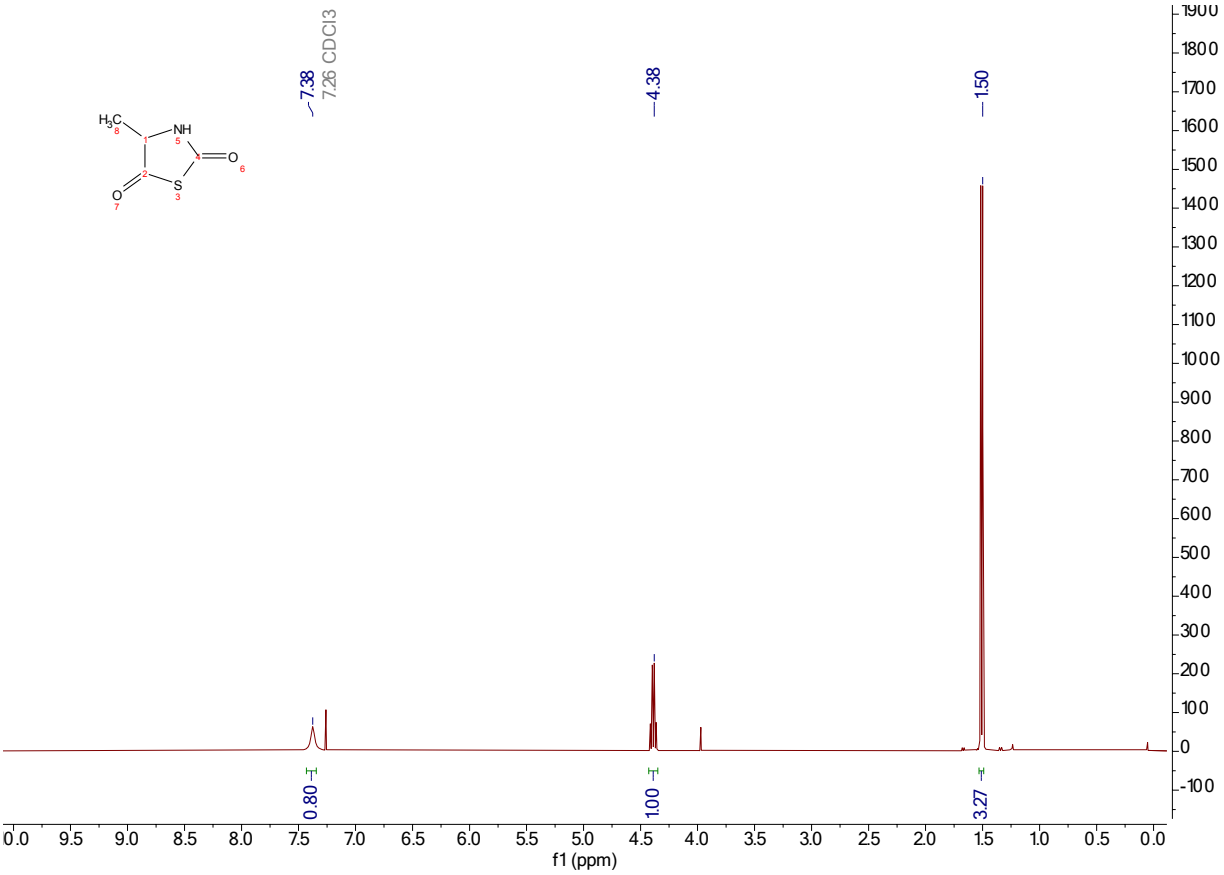


Figure S9: ^{1}H NMR spectrum of Ala-NTA.

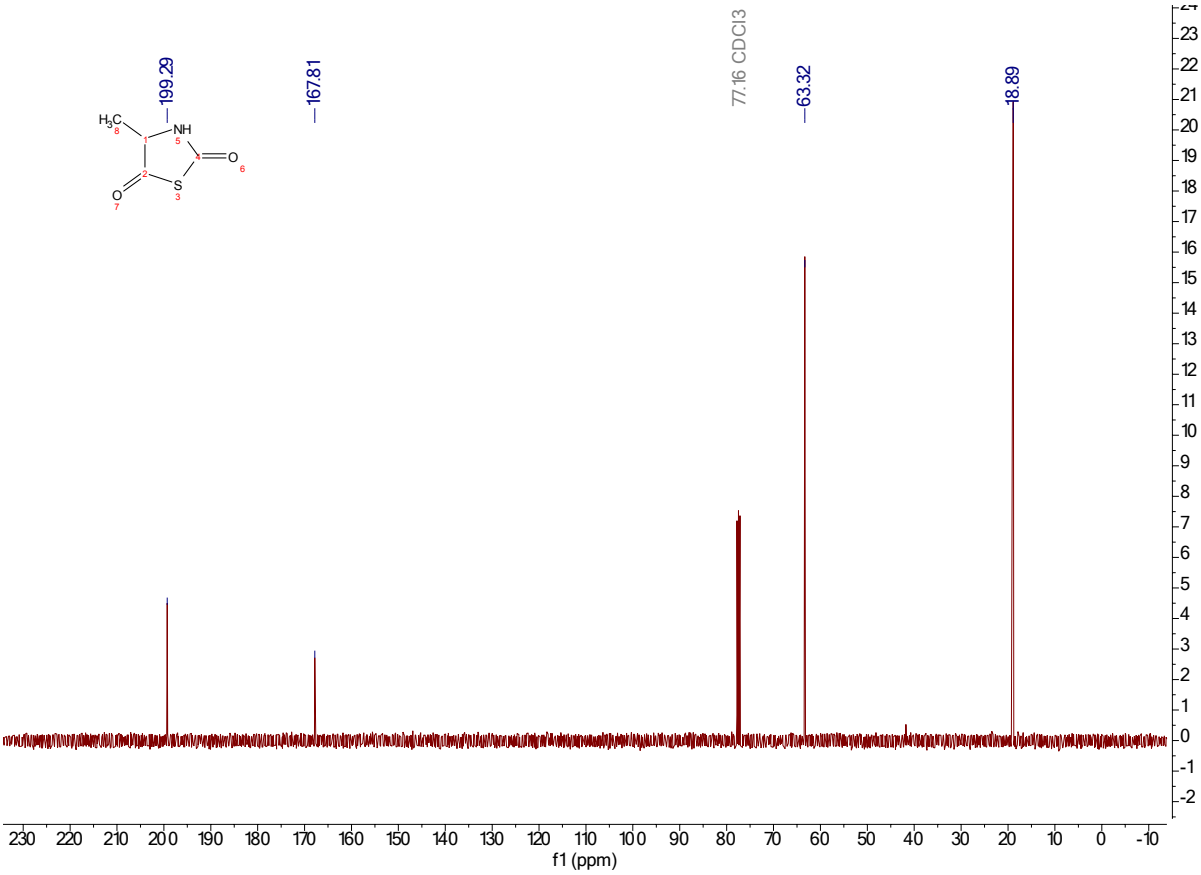
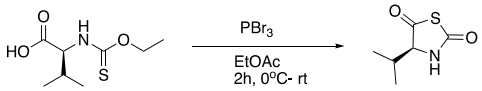


Figure S10: ^{13}C NMR spectrum of Ala-NTA.

Synthesis of Val-NTA



Val-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with (ethoxycarbonothioyl)-L-valine (TC-3, 935 mg, 4.55 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield white powder of Val-NTA (501 mg, 3.15 mmol, 69% yield, m.p. 74-76 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 6.58 (s, 1H, NH), 4.24 (dd, ^1J = 1.26 Hz, ^2J = 3.42 Hz, 1H, CH), 2.36-2.26 (doublet of septet, ^1J = 3.55 Hz, ^2J = 6.71 Hz,1H, CH), 1.09 (d, ^1J = 7.0 Hz, 3H, CH_3), 0.99 (d, ^1J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 198.50, 168.79, 72.40, 31.99, 19.40, 15.81. HRMS (ESI/Q-TOF) m/z: $[M+Na]^+$ Calcd for $C_6H_9NNaO_2S$ 182.0252; Found 182.0245.

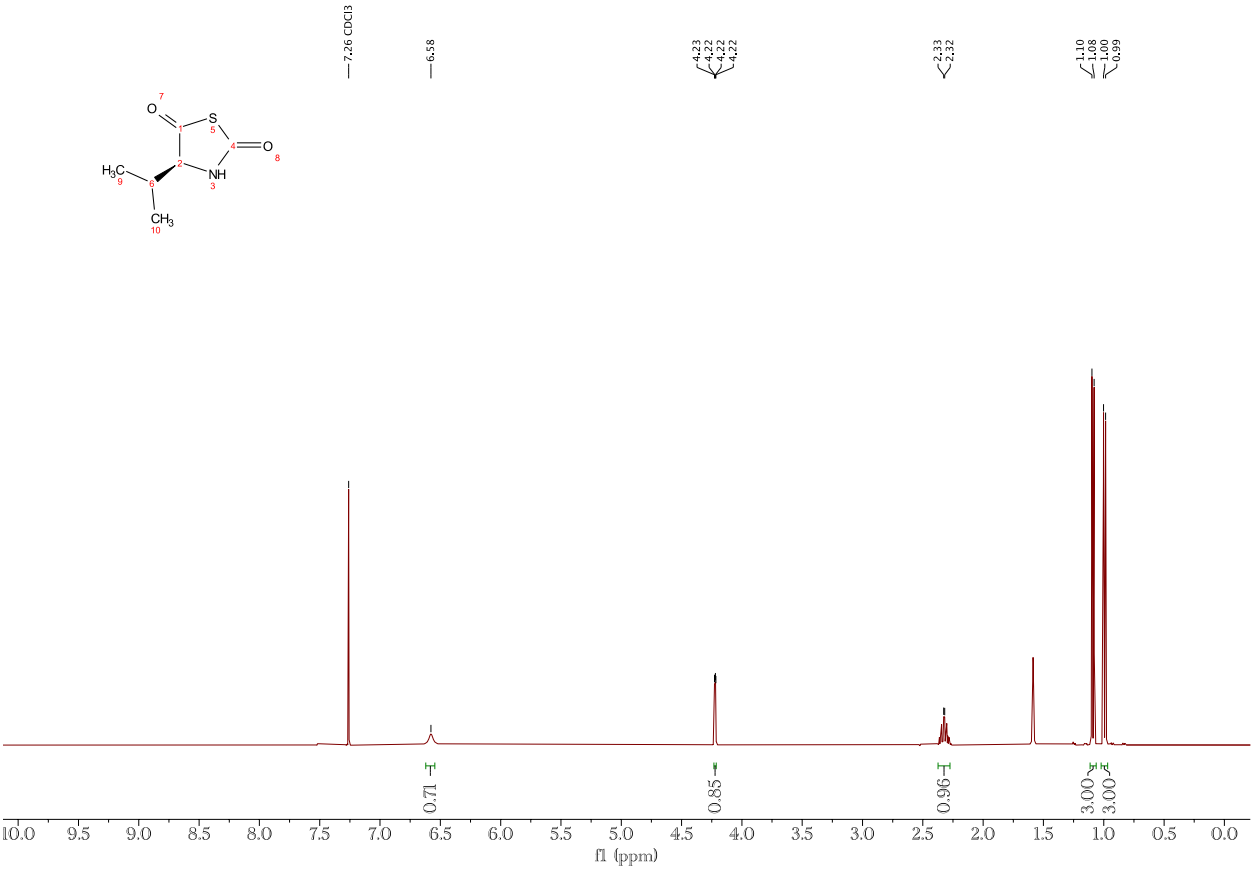


Figure S11: ^{1}H NMR spectrum of Val-NTA.

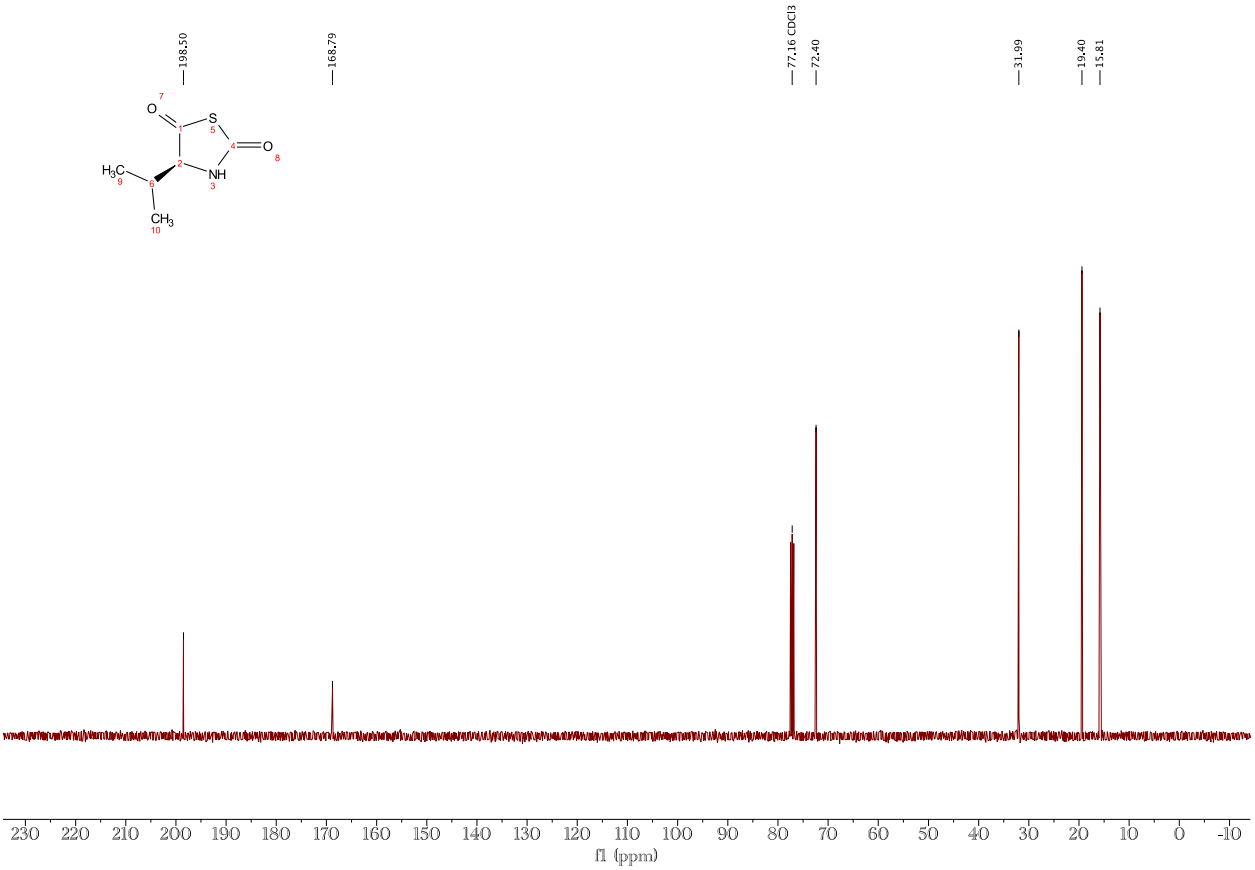
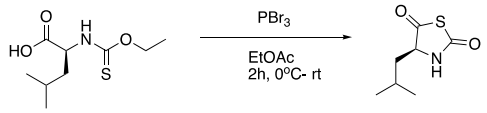


Figure S12: ^{13}C NMR spectrum of Val-NTA.

Synthesis of Leu-NTA



Leu-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with (ethoxycarbonothioyl)-L-leucine (TC-4, 828 mg, 3.78 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield off-white powder of Leu-NTA (397 mg, 2.29 mmol, 61% yield, m.p. 78-80 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 7.59 (s, 1H, NH), 4.33 (dq, ^1J = 1.28 Hz, ^2J = 4.41 Hz, 1H, CH), 1.86-1.78 (m, 1H, CH), 1.77-1.63 (m, 2H, CH_2), 0.98 (dd, ^1J = 6.3 Hz, ^2J = 9.0 Hz, 6H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 198.95, 168.02, 65.88, 42.01, 25.13, 23.33, 21.39. HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ Calcd for $C_7H_{12}NO_2S$ 174.0589; Found 212.0582.

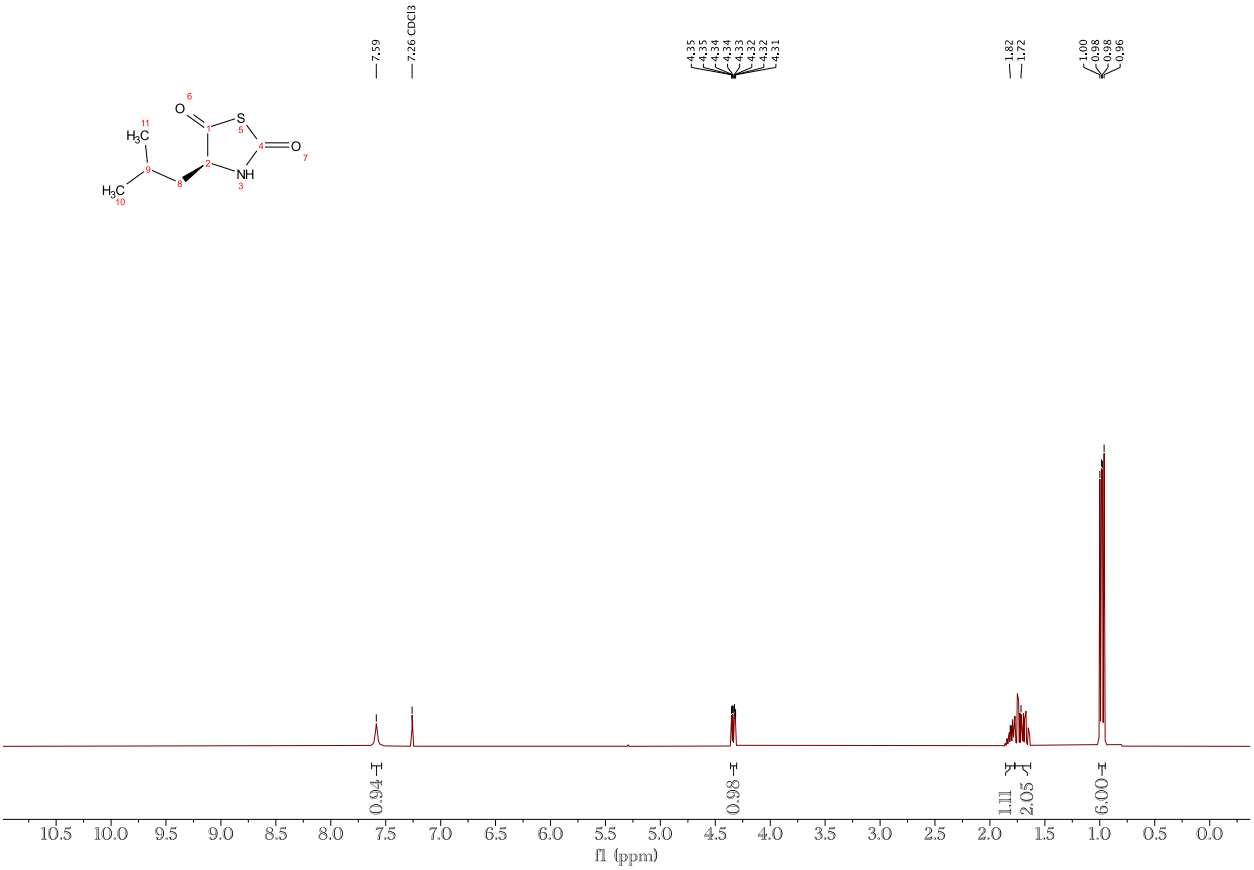


Figure S13: ^{1}H NMR spectrum of Leu-NTA.

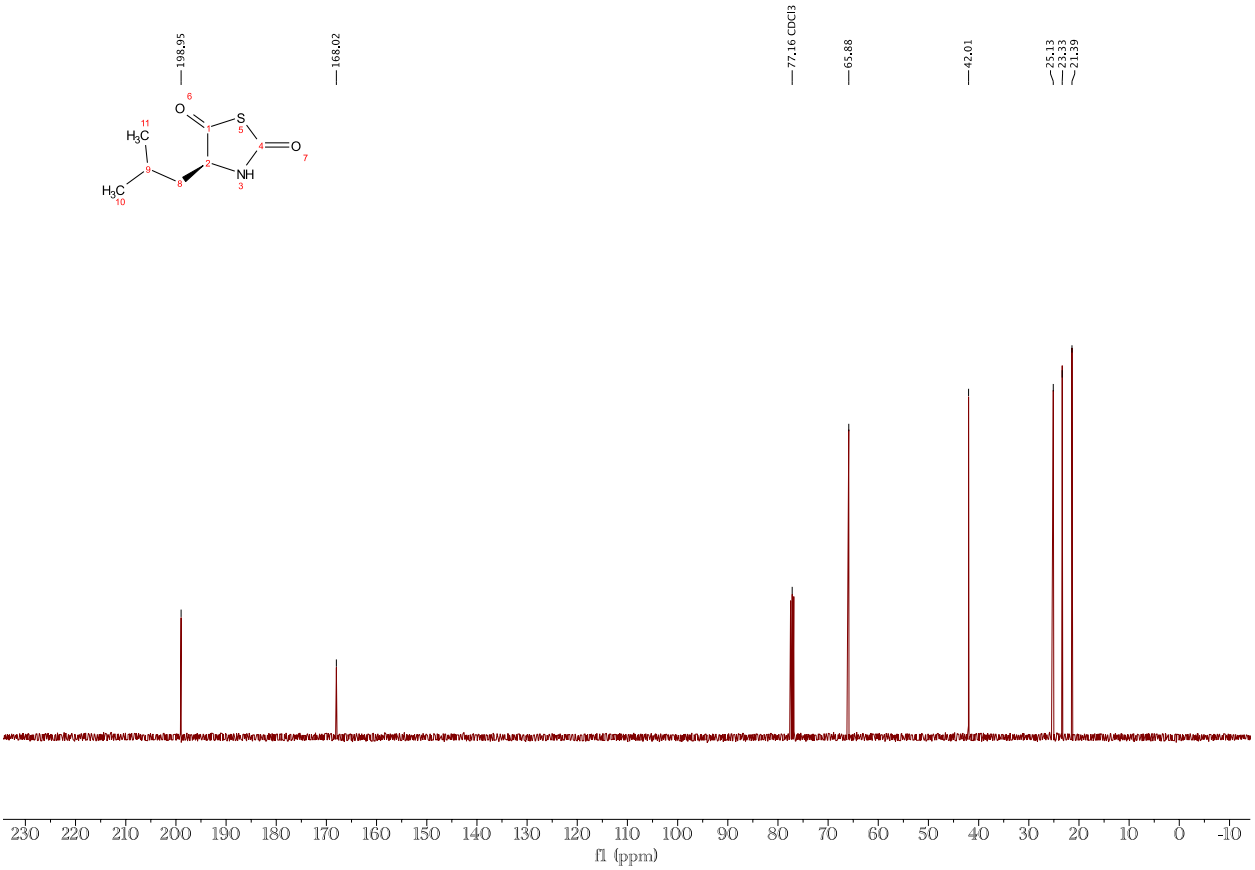
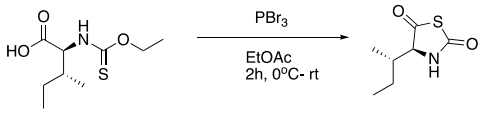


Figure S14: ^{13}C NMR spectrum of Leu-NTA.

Synthesis of Ile-NTA



Ile-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with (ethoxycarbonothioyl)-L-isoleucine (TC-5, 1.0 g, 4.56 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield viscous yellow oil of Ile-NTA (621 mg, 3.58 mmol, 78% yield). ^1H NMR (400 MHz, $CDCl_3$): 7.51 (s, 1H, NH), 4.36 (dd, ^1J = 1.2 Hz, ^2J = 3.0 Hz, 1H, CH), 4.28 (dd, ^1J = 1.2 Hz, ^2J = 3.4 Hz, 1H, CH), 2.13-1.99 (m, 2H, CH), 1.56-1.21 (m, 4H, CH_2), 1.07 (d, ^1J = 7.0 Hz, 3H, CH_3), 0.98 (t, ^1J = 7.3 Hz, 3H, CH_3), 0.94 (d, ^1J = 7.27 Hz, 3H, CH_3), 0.93 (t, ^1J = 7.29 Hz, 3H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 199.16, 198.48, 168.91, 168.67, 72.06, 70.90, 38.71, 38.36, 26.94, 23.69, 15.79, 13.00, 11.85, 11.80. HRMS (ESI/Q-TOF) m/z: $[M+Na]^+$ Calcd for $C_7H_{11}NNaO_2S$ 196.0403; Found 196.0397.

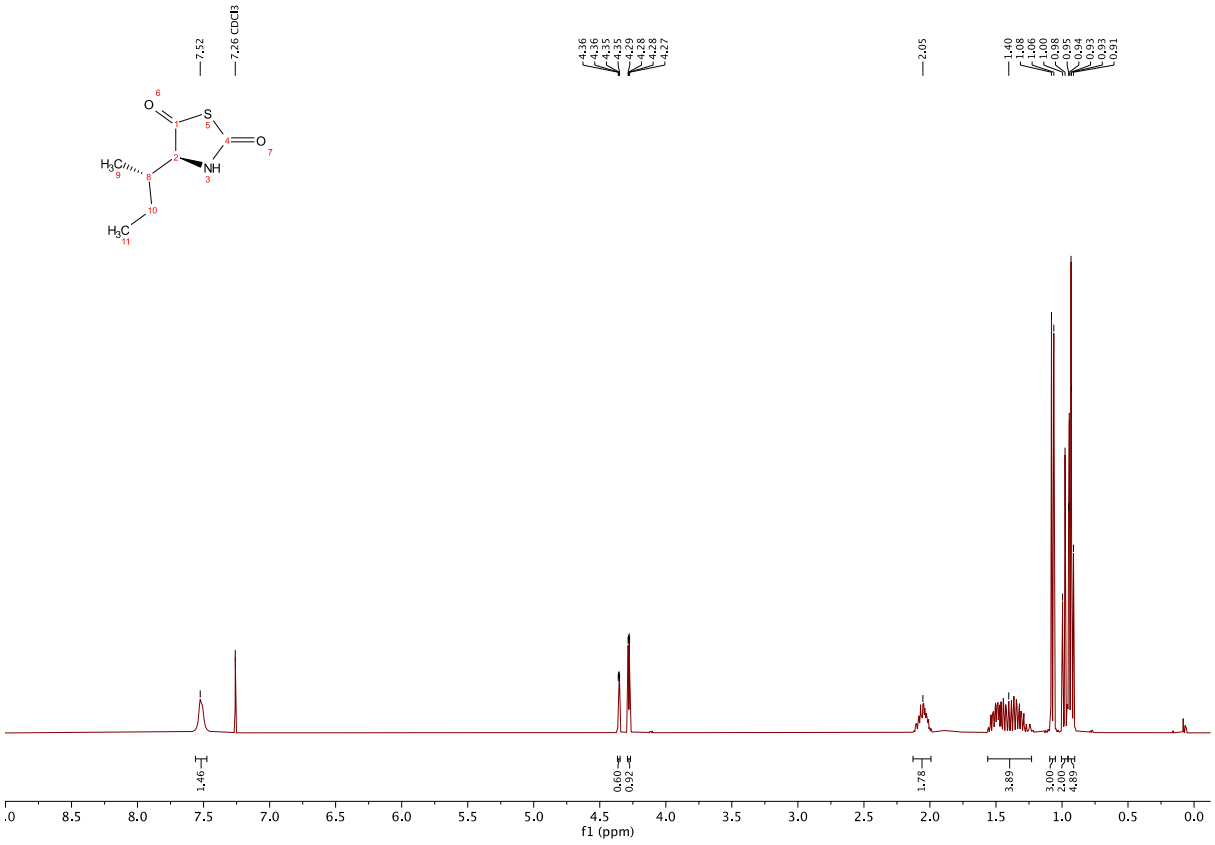


Figure S15: ^{1}H NMR spectrum of Ile-NTA.

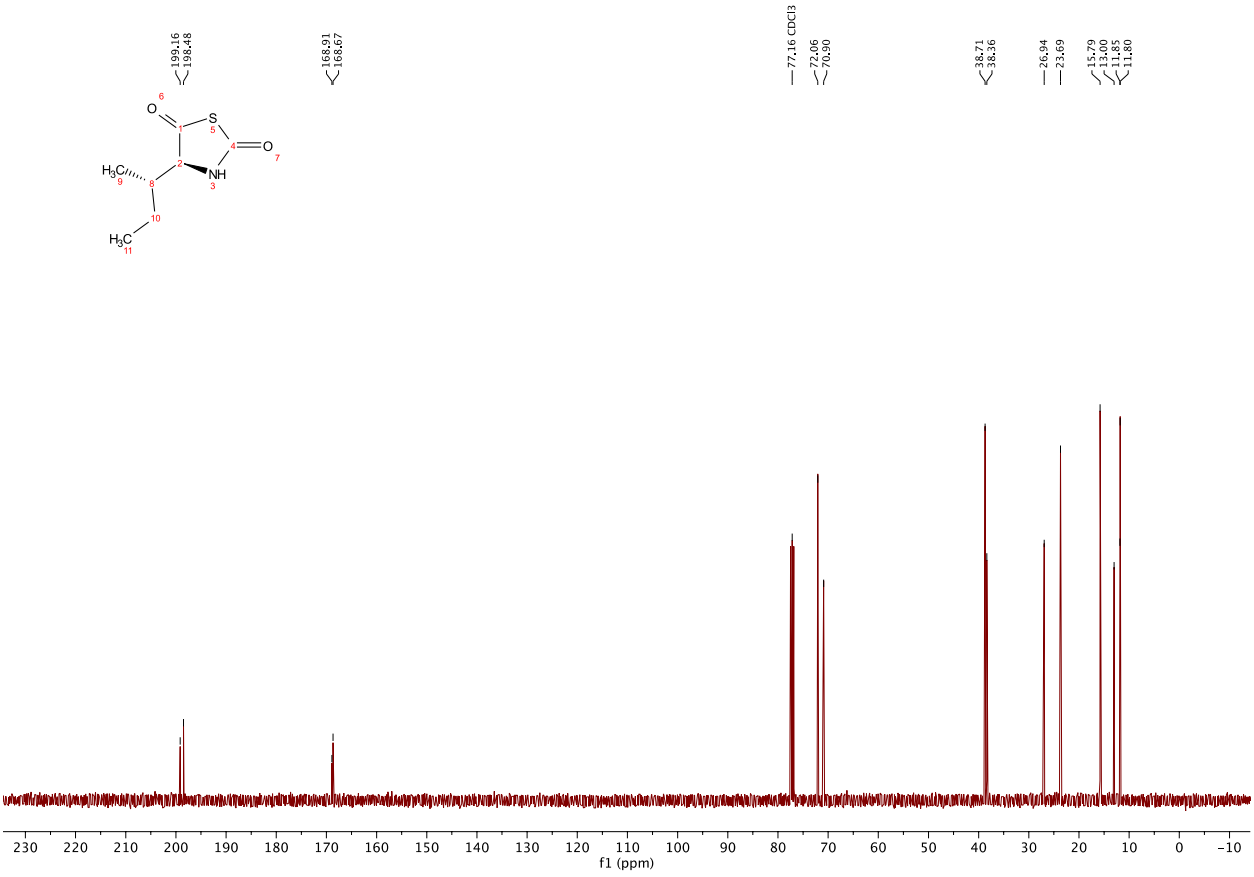
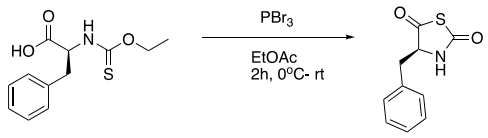


Figure S16: ^{13}C NMR spectrum of Ile-NTA.

Synthesis of Phe-NTA



Phe-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with (ethoxycarbonothioyl)-L-phenylalanine (TC-6, 900 mg, 3.55 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield white powder of Phe-NTA (500 mg, 2.41 mmol, 68% yield, m.p. 108-109 °C). ^1H NMR (400 MHz, DMSO-d^6): δ 9.39 (s, 1H, NH), 7.32-7.22 (m, 3H, Ph-H), 7.18-7.14 (m, 2H, Ph-H), 4.89 (t, ^1J = 4.8 Hz, 1H, CH), 3.05 (dq, ^1J = 4.8 Hz, ^2J = 16.0 Hz, 2H, CH_2). ^{13}C NMR (400 MHz, DMSO-d^6): δ 200.47, 164.87, 134.80, 130.32, 128.63, 127.41, 67.72, 37.67. HRMS (ESI/Q-TOF) m/z: $[M+NH_4]^+$ Calcd for $C_{10}H_{13}N_2O_2S$ 225.0698; Found 225.0690.

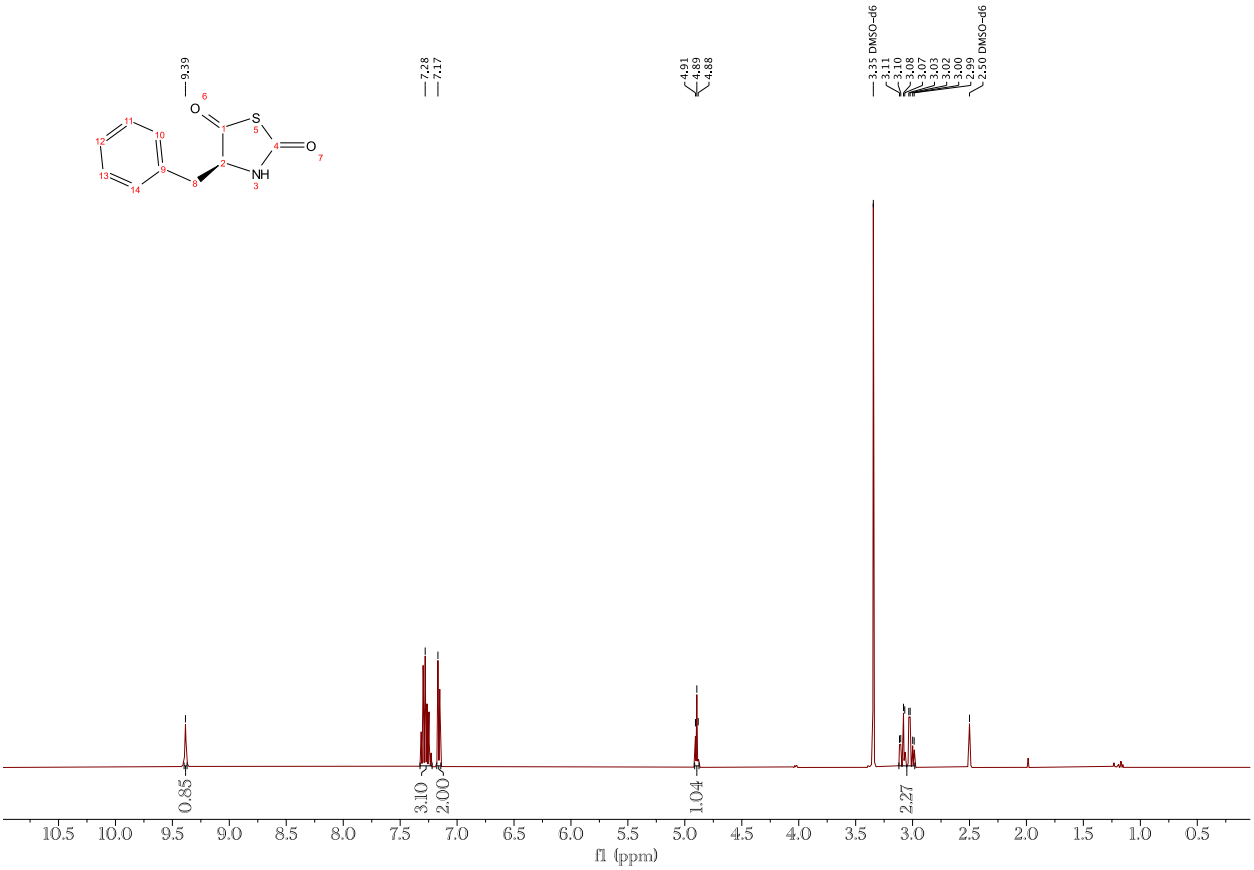


Figure S17: ^{1}H NMR spectrum of Phe-NTA.

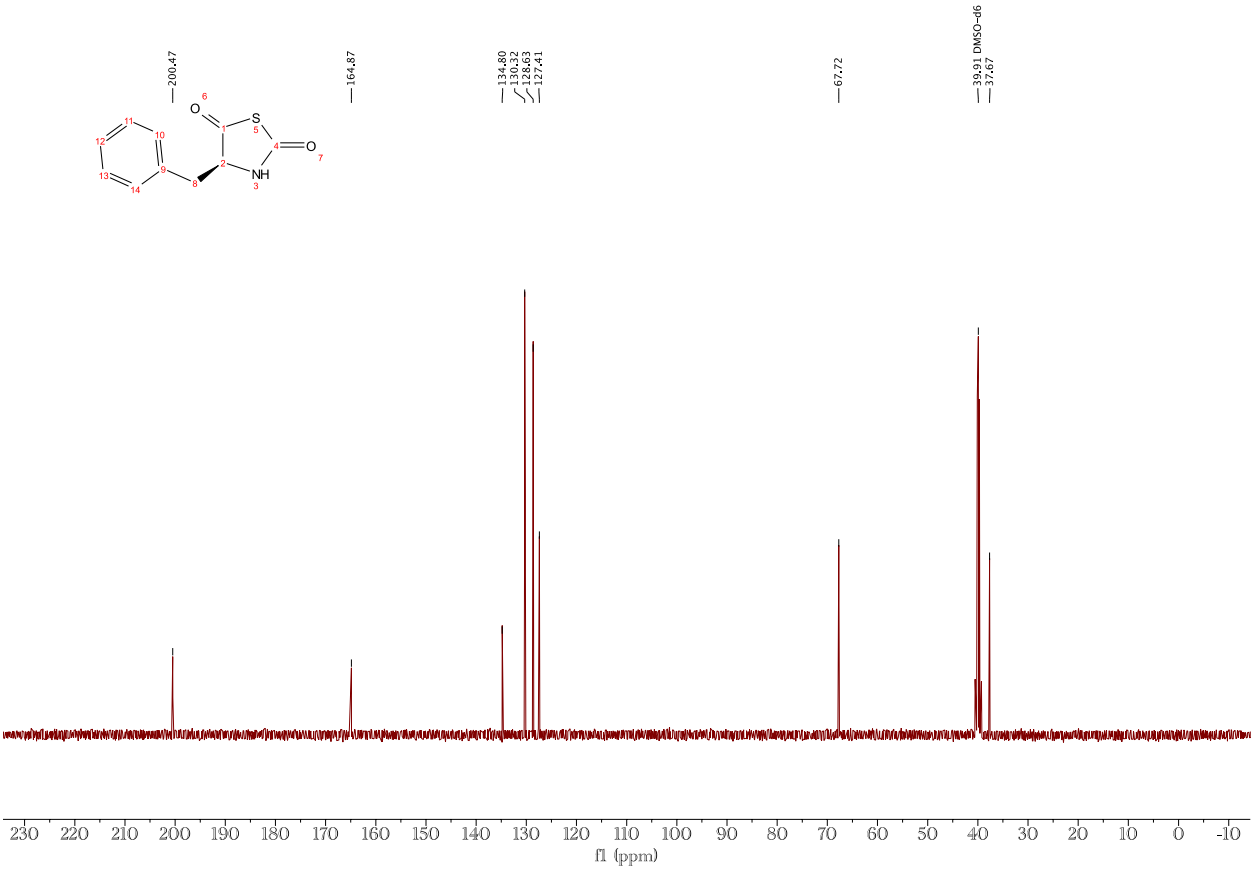
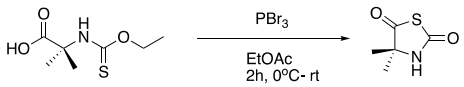


Figure S18: ^{13}C NMR spectrum of Phe-NTA.

Synthesis of Aib-NTA



Aib-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with 2-((ethoxycarbonothioyl)amino)-2-methylpropanoic acid (TC-7, 980 mg, 5.12 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield pale yellow powder of Aib-NTA (380 mg, 2.62 mmol, 51% yield, m.p. 111-113 ^oC).^1H NMR (400 MHz, $CDCl_3$): δ 7.33 (s, 1H, NH), 1.52 (s, 6H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 201.60, 166.11, 69.40, 25.94. HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ Calcd for $C_5H_8NO_2S$ 146.0276; Found 146.0266.

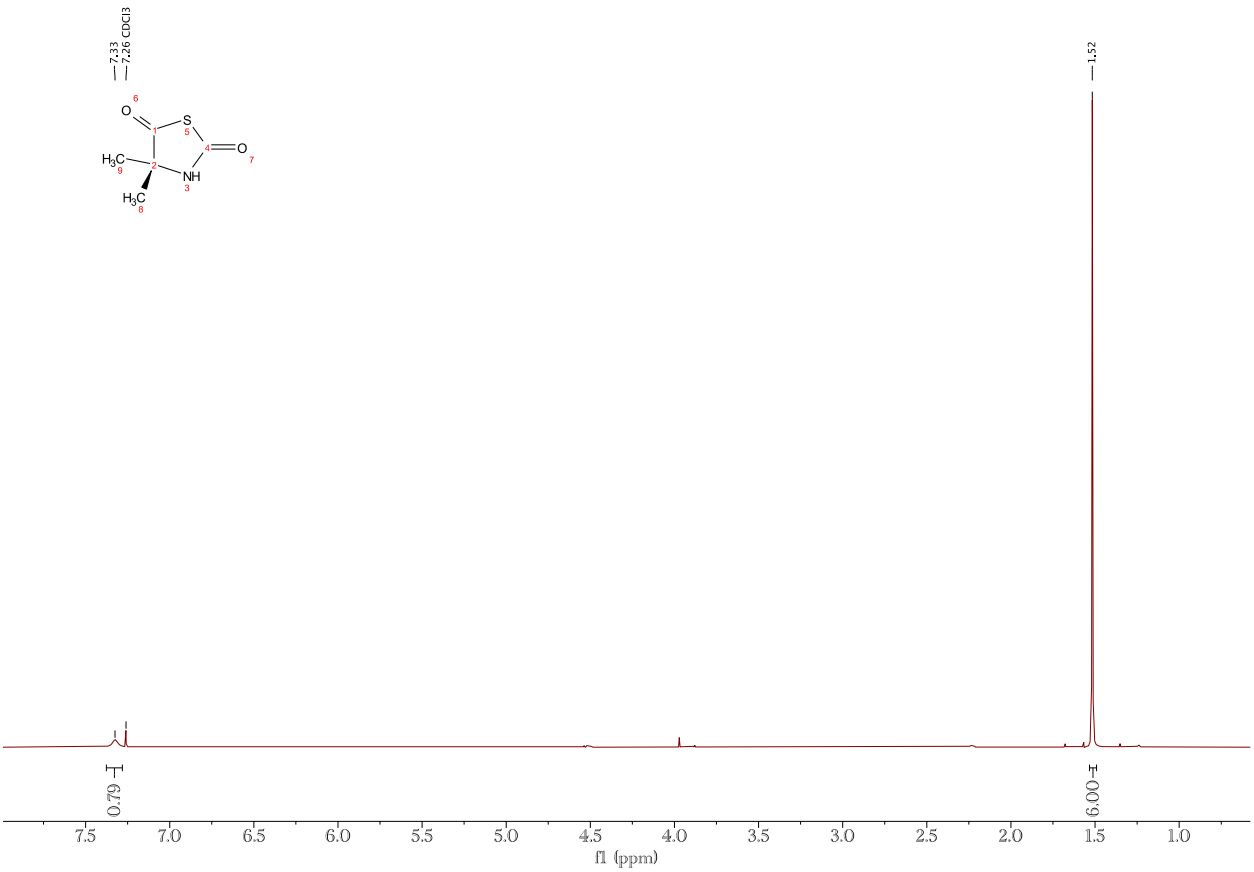


Figure S19: ^{1}H NMR spectrum of Aib-NTA.

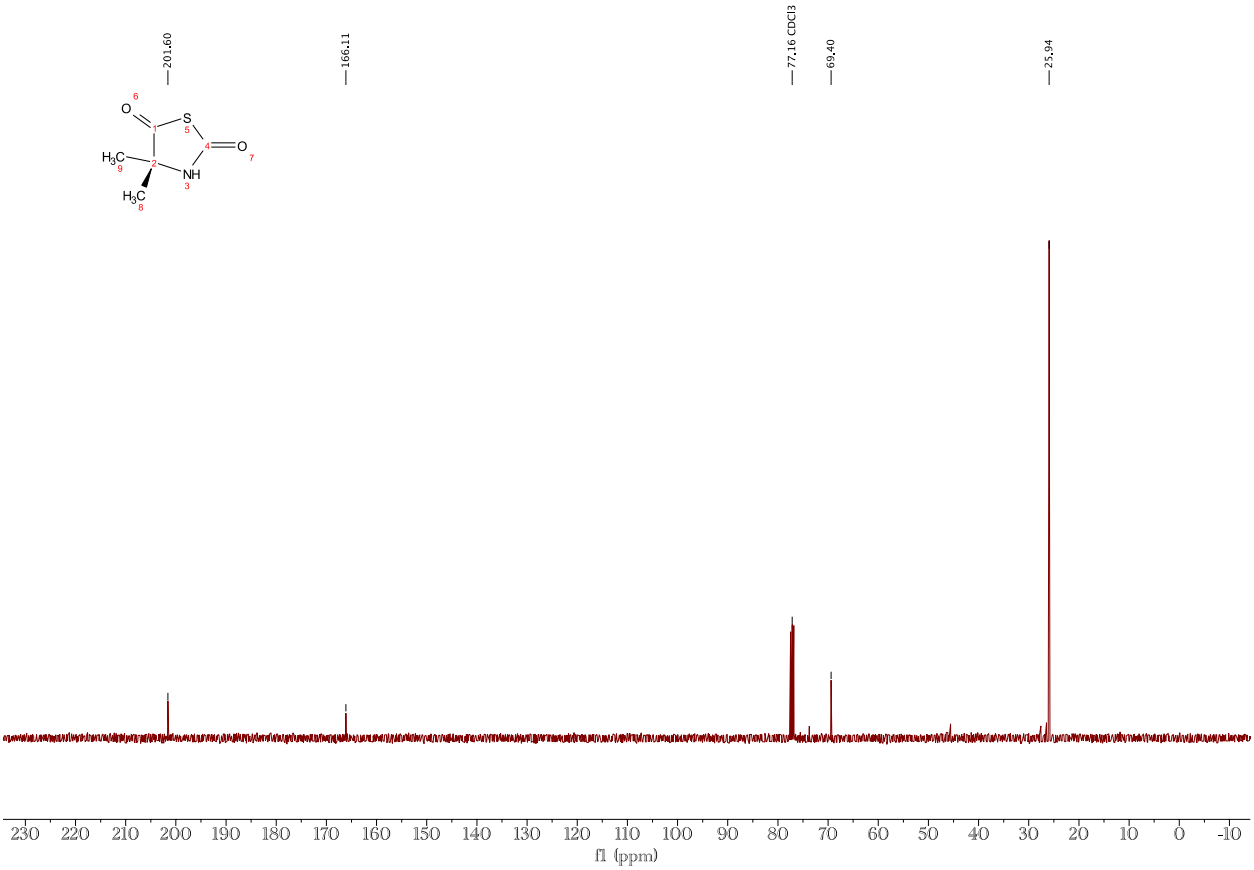
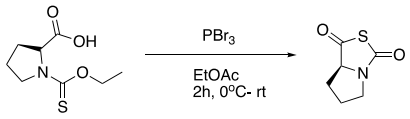


Figure S20: ^{13}C NMR spectrum of Aib-NTA.

Synthesis of Pro-NTA



Pro-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with (ethoxycarbonothioyl)-L-proline (TC-8, 1.39 g, 6.84 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield a pale yellow solid of Pro-NTA (150 mg, 0.95 mmol, 14% yield, m.p. 140-141 °C). ^1H NMR (400 MHz, $CDCl_3$): δ 4.51 (dd, ^1J = 5.6 Hz, ^2J = 10.1 Hz, 1H, CH), 3.89 (dt, ^1J = 8.0 Hz, ^2J = 11.7 Hz, 1H, CH_2), 3.37 (dq, ^1J = 3.7 Hz, ^2J = 9.0 Hz, 1H, CH_2), 2.33-2.21 (m, 2H, CH_2), 2.19-2.03 (m, 1H, CH_2), 1.93-1.82 (m, 1H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.16, 166.27, 73.70, 45.58, 27.60, 26.45.

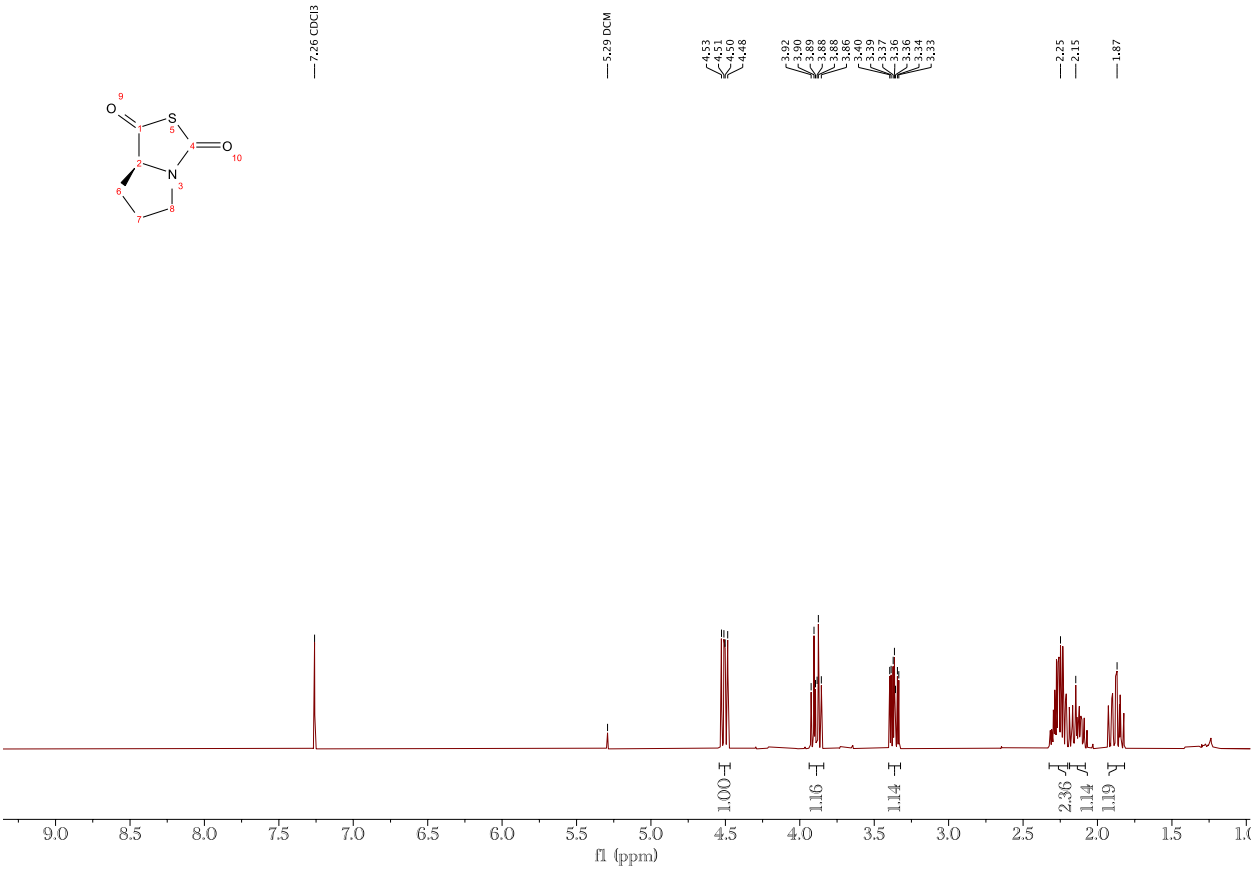


Figure S21: [1]H NMR spectrum of Pro-NTA.

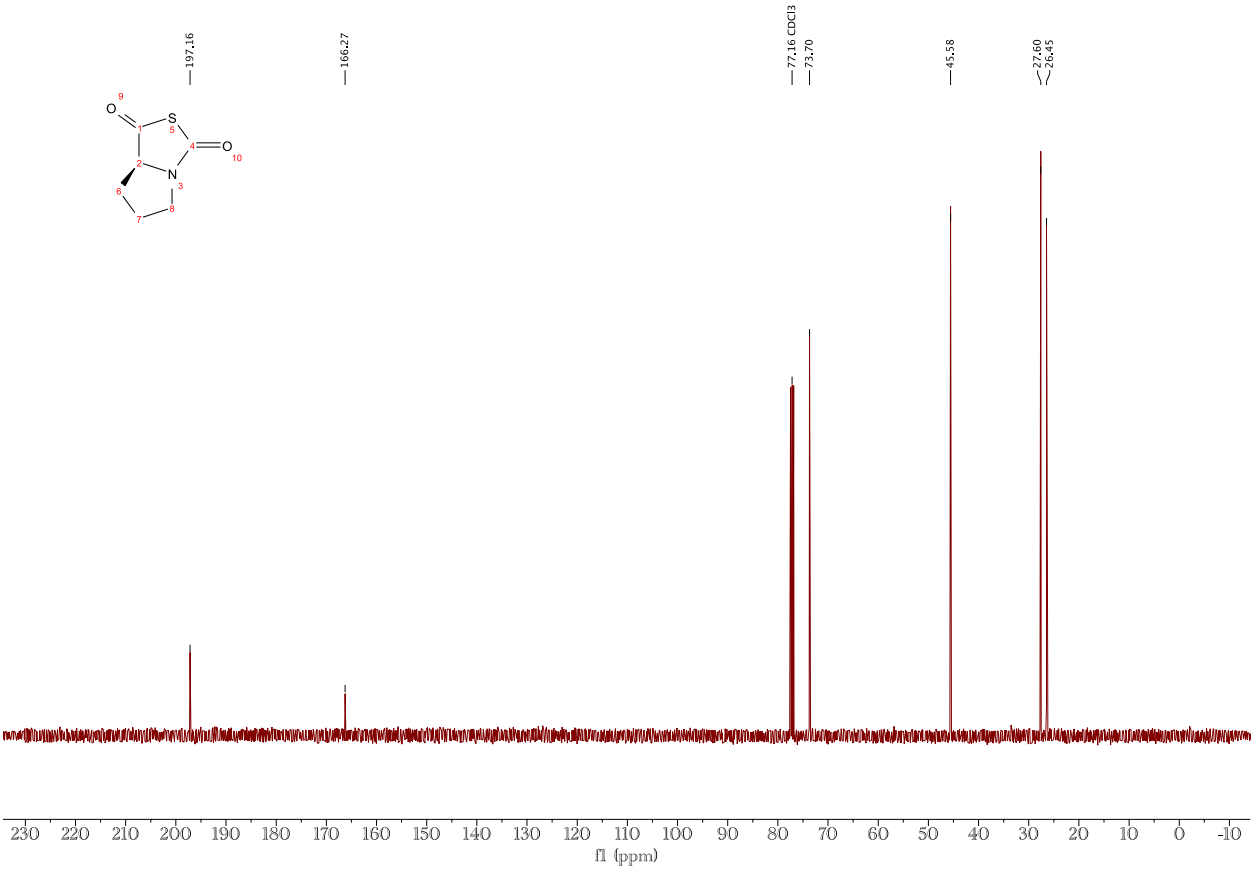
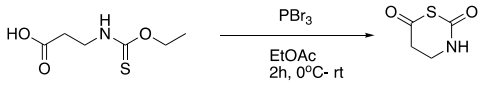


Figure S22: ^{13}C NMR spectrum of Pro-NTA.

Synthesis of β-*Ala-NTA*



β-Ala-NTA was synthesized according to the general procedure described above by reaction of PBr_3 with 3-((ethoxycarbonothioyl)amino)propanoic acid (TC-9, 475 mg, 2.68 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield white powder of β-Ala-NTA (115 mg, 0.88 mmol, 33% yield, m.p. 86-88 °C). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 (s, 1H, NH), 3.59 (q, ^1J = 5.0 Hz, 2H, CH_2), 2.83 (t, ^1J = 6.0 Hz, 2H, CH_2). ^{13}C NMR (400 MHz, $CDCl_3$): δ 195.95, 166.38, 40.12, 37.67. HRMS (ESI/Q-TOF) m/z: $[M+H]^+$ Calcd for $C_4H_5NO_2SH^+$ 132.0119; Found 132.0116.

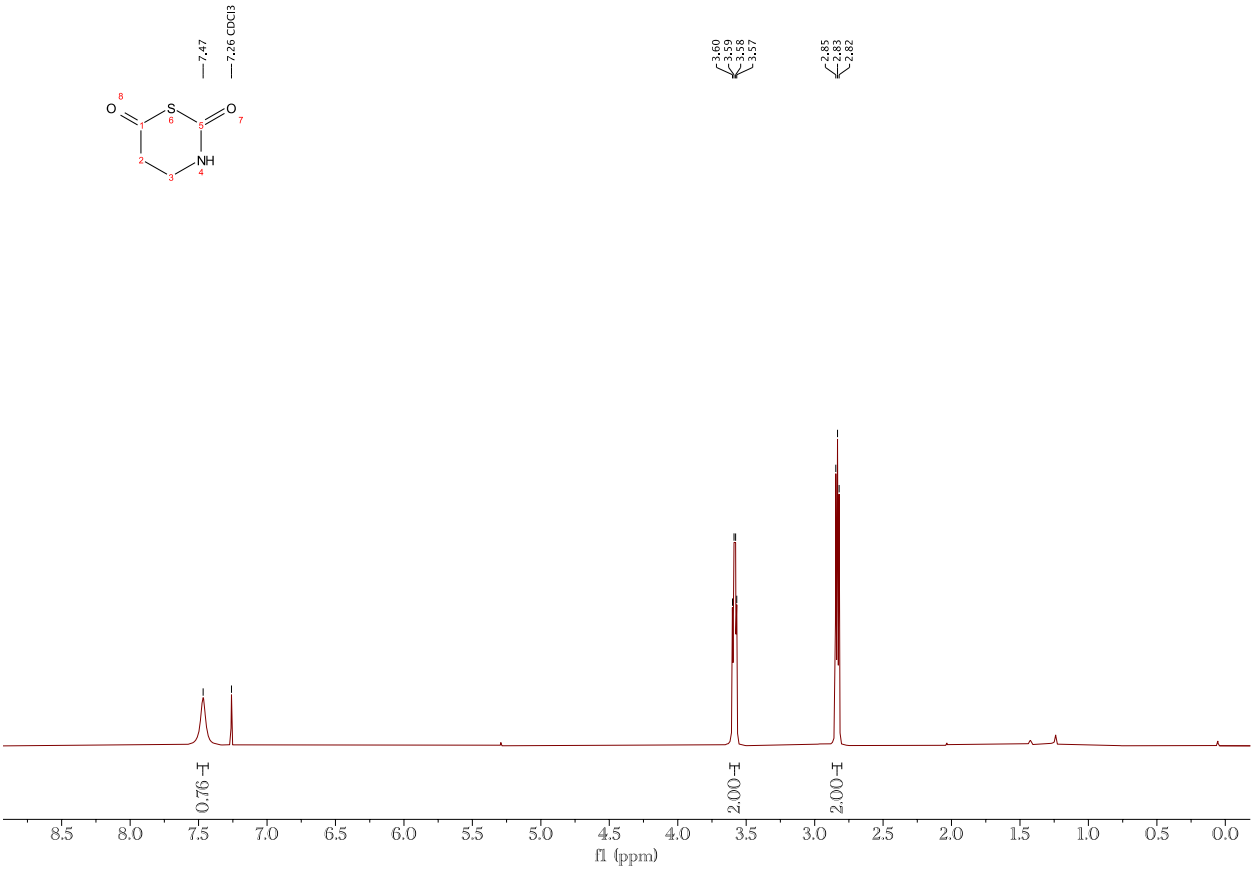


Figure S23: ^{1}H NMR spectrum of β-Ala-NTA.

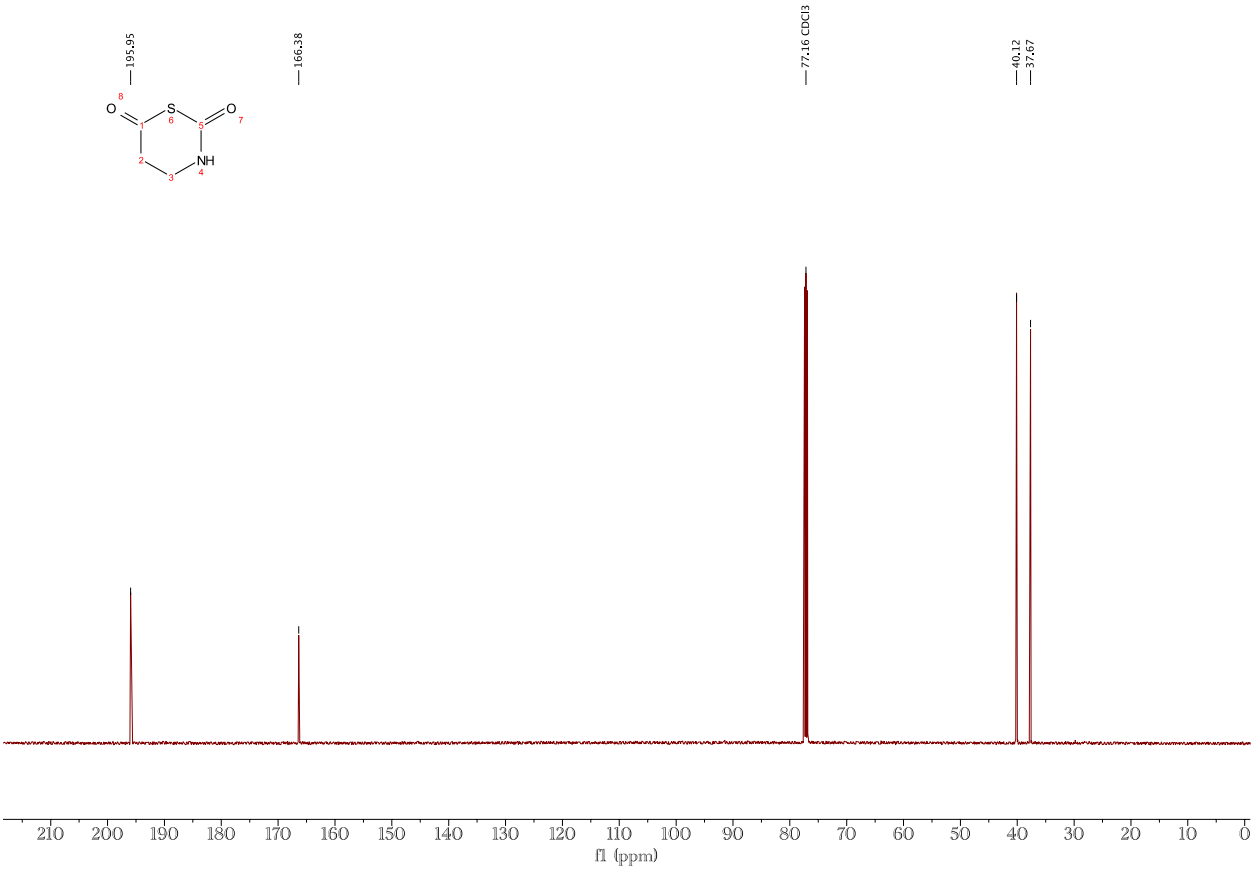
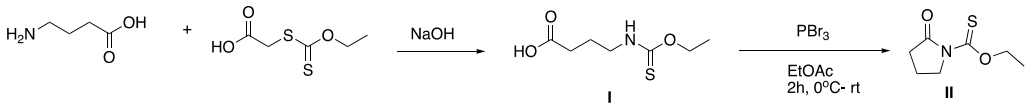


Figure S24: ^{13}C NMR spectrum of β-Ala-NTA.

Synthesis of O-ethyl 2-oxopyrrolidine-1-carbothioate



Reagents 4-aminobutanoic acid (1.1 g, 10.7 mmol) and XAA (8.54 mmol) were reacted according to general procedure described above to yield off-white solid of 4-((ethoxycarbonothioyl)amino)butanoic acid (**I**, 0.60 g, 3.13 mmol, 40 % yield). 4-((ethoxycarbonothioyl)amino)butanoic acid (**I**, 0.590 mg, 3.08 mmol) was then reacted with PBr_3 (0.322 mL, 3.29 mmol). The crude product was purified via flash chromatography (eluted with 100% CH_2Cl_2) to yield pale yellow powder of *O*-Ethyl 2-oxopyrrolidine-1-carbothioate (**II**) (0.247 mg, 1.70 mmol, 55 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.60 (q, ^1J = 7.75 Hz 2H, CH_2), 4.04 (t, ^1J = 7.16 Hz, 2H, CH_2), 2.60 (t, ^1J = 8.14 Hz, 2H, CH_2), 2.01 (quintet, ^1J = 8.14 Hz, 2H, CH_2), 1.41 (t, ^1J = 6.85 Hz, 3H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 188.68, 171.62, 68.69, 51.31, 34.03, 17.28.).

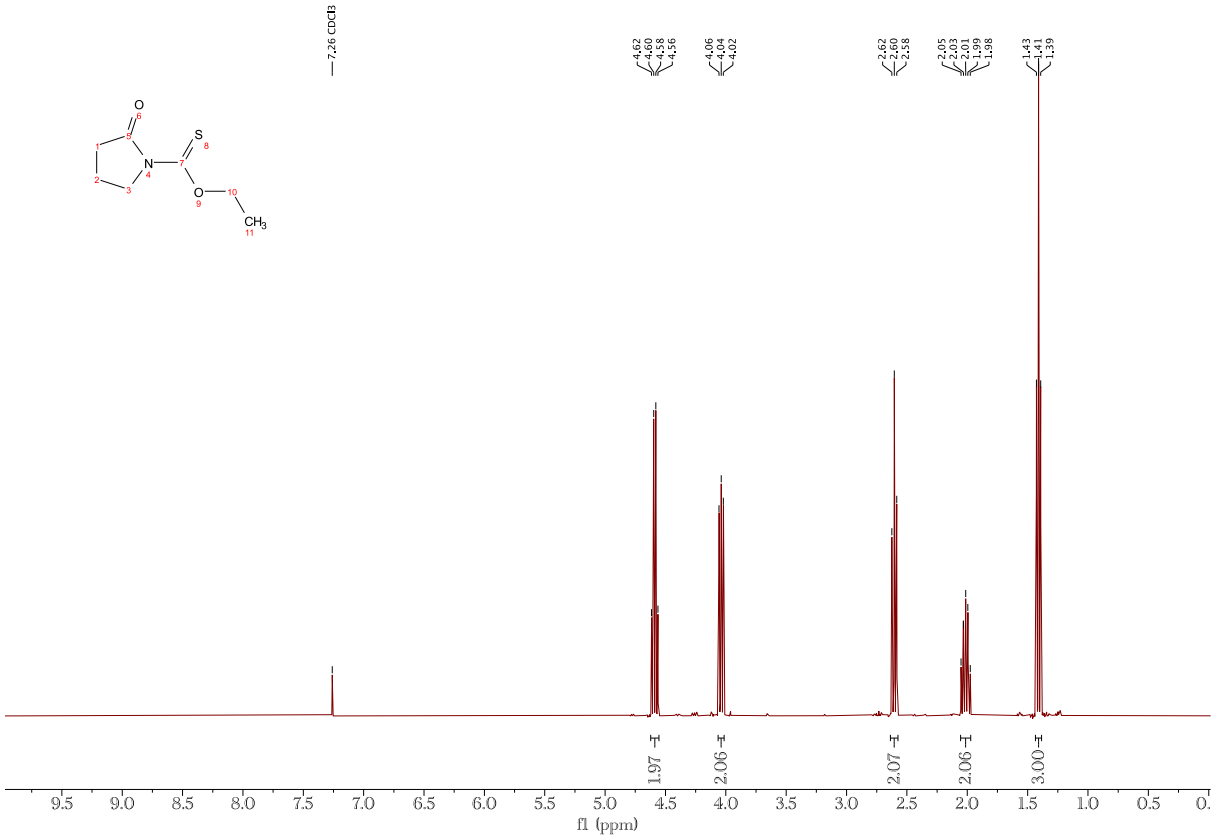


Figure S25: ^{1}H-NMR of *O*-ethyl 2-oxopyrrolidine-1-carbothioate

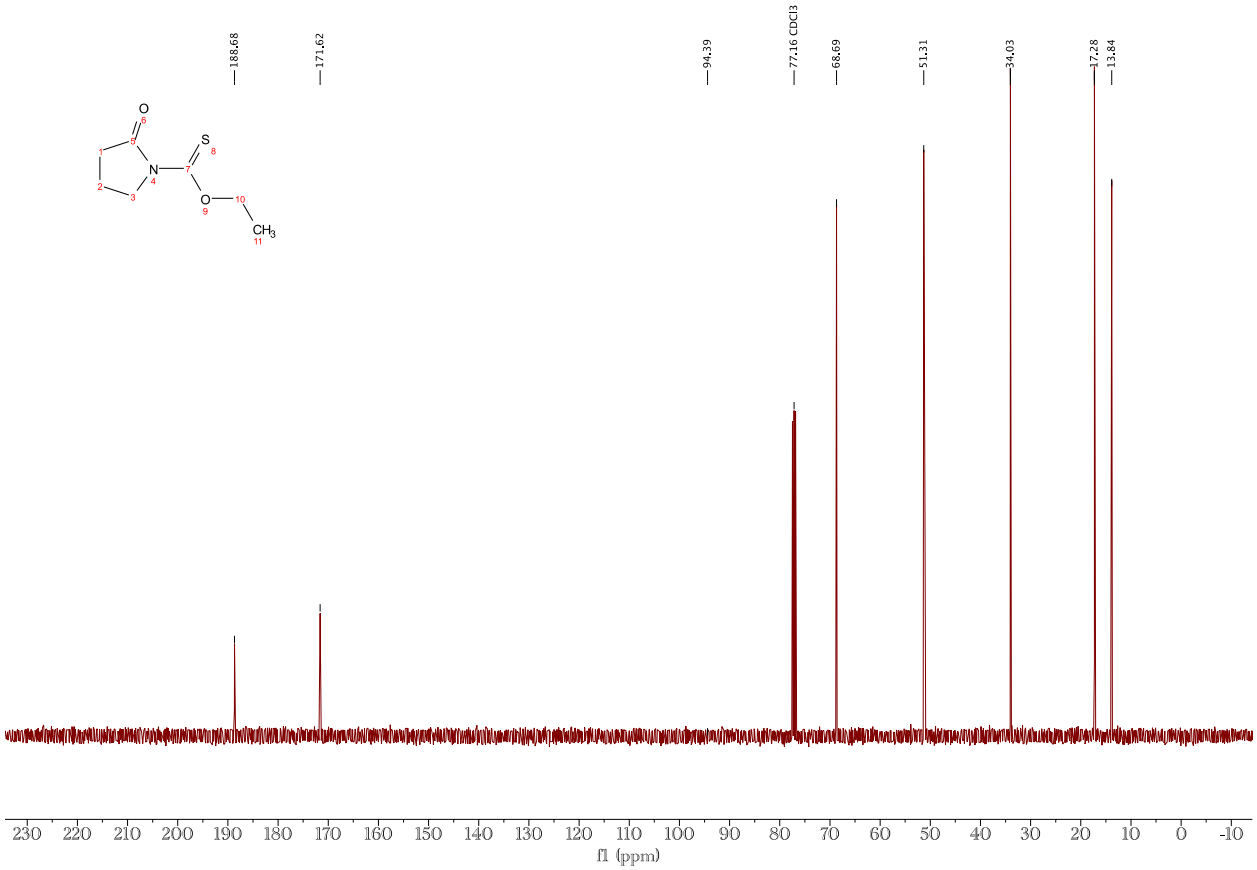


Figure S26: ^{13}C-NMR of *O*-ethyl 2-oxopyrrolidine-1-carbothioate

References

(1) C. R. Powell, K. Kaur, Dillon, K. M., M. Zhou, M. Alaboalirat, J. B. Matson, *ACS Chem. Biol.* 2019, 14, 1129-1134.

(2) J. Cao, D. Siefker, B. A. Chan, T. Yu, L. Lu, M. A. Saputra, F. R. Fronczek, W. Xie, D. Zhang, *ACS Macro Letters* 2017, ***6***, 836-840.

(3) R. Hirschmann, R. S. Dewey, E. F. Schoenewaldt, H. Joshua, W. J. Paleveda, H. Schwam, H. Barkemeyer, B. H. Arison, D. F. Veber, *J. Org. Chem.* 1971, ***36***, 49-59.

(4) S.-J. Zhang, F. Xu, W.-J. Yang, W.-X. Hu, *J. Chem. Crystallogr.,* 2012**,** ***42***, 1182-1189.