Supporting Information

Starter Unit Flexibility for Engineered Product Synthesis by the Non-Reducing Polyketide Synthase PksA

Callie R Huitt-Roehl, Eric A. Hill, Martina M. Adams, Anna L. Vagstad, Jesse W. Li and Craig A. Townsend*

Department of Chemistry, Johns Hopkins University, 3400 N. Charles Street, Baltimore, Maryland 21218

Contents

Cloning and mutagenesis

All plasmids used in this study are summarized in Table S1. Cloning of plasmids to express PksA-SAT-KS-MAT (pENKA4) and PT-ACP (pEPTACP), as well as a series of active site mutants of PksA-SAT-KS-MAT (see table) has been described previously(*1, 2*). Additional mutants and PksA-SAT-KS-MAT-PT-ACP were cloned for this study using standard methods. DNA manipulations were carried out in *E. coli* BL21(DE3). PksA-SAT-KS-MAT-PT-ACP was amplified from a pET-28a vector containing full-length PksA cDNA using pksA5-NheI and ACP-3-NotI primers. The resulting PCR product was ligated into pET28a at NheI and NotI sites to give pEPksA-SKMPA. For the SAT active site mutants, overlap extension PCR was used to introduce specific point mutations at Cys117. These fragments were amplified from pENKA4 using the primers listed in Table S2. PCR products were ligated into pET-28a at NcoI and NotI sites to give the plasmids listed in Table S1. All expression constructs were confirmed by automated sequencing (Johns Hopkins University Synthesis and Sequencing Facility, Baltimore MD).

Table S1

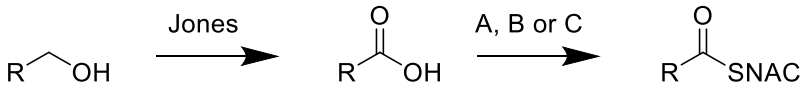
Plasmid	Protein	Tag	Mol. Wt (kDa)	Reference
pENKA4	SAT-KS-MAT	*C*-His_6	144	(*1*)
pEPT-ACP	PT-ACP	*C*-His_6	59	(*1*)
pENKA4-C117A	SAT^0-KS-MAT	*C*-His_6	144	(*2*)
pENKA4-C117S	SAT^0-KS-MAT	*C*-His_6	144	This study
pENKA4-C117G	SAT^0-KS-MAT	*C*-His_6	144	This study
pENKA4-C117N	SAT^0-KS-MAT	*C*-His_6	144	This study
pEPksA-SKMPA	SAT-KS-MAT-PT-ACP	*N*-His_6 & *C*-His_6	201	This study

Table S2

Primer	Sequence 5' to 3'
NT5.1	gatccatggctcaatcaaggcaactc
MAT3.4	gagtgcggccgcggatggacggt
C117S-5'	gtgctgggattcagcatgggttccttg
C117S-3'	caaggaacccatgctgaatcccagcac
C117G-5'	gtgctgggattcggcaggggttccttg
C117G-3'	caaggaacccatgccgaatcccagcac
C117N-5'	cgtgctgggattcaacatgggttccttggcc
C117N-3'	caaggaacccatgttgaatcccagcac
pksA5-NheI	gctagcatggctcaatcaaggc
ACP-3-NotI	gtaagcggccgcctagccagcatccccgcttc

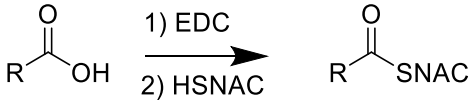
Synthesis of acyl-SNAC substrates

All reactions, unless otherwise stated, were performed under a positive N_2 or Ar atmosphere, in anhydrous, freshly distilled solvents. Commercially available compounds were purchased from Sigma-Aldrich or Alfa-Aesar and were used without further purification. All ^{1}H and ^{13}C-NMR spectra were acquired on a 300 MHz or 400 MHz Bruker spectrometer and are reported in parts per million (δ) referenced against a TMS standard or residual solvent peak. All NMR solvents were purchased from Cambridge Isotope Laboratories, Inc. Column chromatography was carried out on Silica Gel 60 (Sorbent Technologies, 200 x 400 mesh). Exact masses were determined on an Analytical VG-70SE Magnetic Sector Mass Spectrometer at the Johns Hopkins University Chemistry Department Mass Spectrometry Facility. ESI-MS spectra were measured at the Mass spectrometry facility of Old Dominion University in Norfolk, VA on a Bruker Apex-Qe Qh-FTMS instrument.



To a room temperature solution of the alcohol in acetone (200 mM), Jones reagent was added dropwise until the red color persisted. After 15 min, the reaction was quenched with isopropanol, filtered, concentrated, dissolved (5% HCl, CH_2Cl_2), separated, dried and concentrated to afford the desired carboxylic acid (~90%).

Method A:

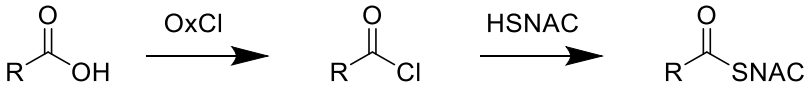


To a room temperature suspension of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (1 eq.) in CH_2Cl_2, under N_2, Et_3N (1 eq.) was added. The carboxylic acid (1 eq.) was added dropwise followed by the addition of 4-(dimethylamino)pyridine (0.1 eq.). After 10 min. *N*-acetylcysteamine (1 eq.) was added dropwise. The resulting solution was allowed to react for 12-14 h then poured into a saturated solution of NH_4Cl and extracted with CH_2Cl_2 (3×). The combined organic phases were washed with H_2O and brine, dried (Na_2SO_4), concentrated and purified by column chromatography to afford the corresponding thioester.

Method B:

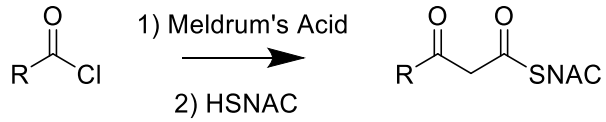
A room temperature solution of carboxylic acid (1 eq.) and CDI (1.4 eq.) in dry THF were allowed to react for 10 min. A solution of *N*-acetylcysteamine (1.1 eq.) was added and the reaction was allowed to stir at room temperature for 12-14 h. The solvent was removed under vacuum and the residue was redissolved in 10 mL of EtOAc, washed (10 ml of brine), dried (Na_2SO_4), concentrated and purified by column chromatography to furnish the respective SNAC-derivative.

Method C:

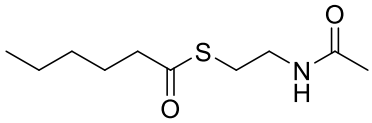


To a 0 °C solution of the carboxylic acid (1 equiv) and DMF (cat) in CH_2Cl_2 (150 mM), oxalyl chloride (1.1 equiv) was added slowly. The ice bath was removed and the mixture was allowed to warm to room temperature over 2 h, then concentrated *in vacuo* and used immediately without further purification. To a 0 °C solution of *N*-acetylcysteamine (1.0 equiv) and DIPEA (1.1 equiv) in CH_2Cl_2 (350 mM), a 0 °C solution of the acid chloride (1.0 equiv) in CH_2Cl_2 (500 mM) was added slowly, and the mixture was allowed to warm to room temperature over 12 h. The resulting light orange solution was washed (NH_4Cl, sat, 1x, $CuSO_4$, sat, 1x, NH_4Cl, sat, 3x), filtered through a plug of silica gel, concentrated and recrystallized or purified by column chromatography.

Method D:

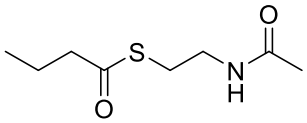


To a 0 °C solution of Meldrum's acid (1.0 equiv) and pyridine (1.0 equiv) in CH_2Cl_2 (350 mM), a 0 °C solution of the acid chloride (1.0 equiv) in CH_2Cl_2 (350 mM) was added. The resulting red solution was allowed to react at 0 °C for one hour, then warm to room temperature for 2 h. The mixture was concentrated *in vacuo*, then dissolved in toluene (200 mM). *N*-acetylcysteamine (1.0 equiv) was added and the red solution was heated to reflux for 2 h, cooled, washed (NH_4Cl, sat, 3x, $CuSO_4$, sat, 2x), dried, concentrated and recrystallized to afford the desired β-keto thioester.



***S*-(2-acetamidoethyl) hexanethioate (5)**

Watanabe, CMH, Wilson, D, Linz, JE and Townsend, CA. *Chemistry & Biology* **1996**, *3* (6), 463-469.

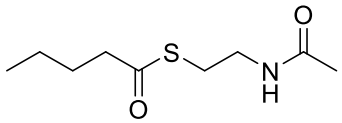


***S*-(2-acetamidoethyl) butanethioate (6)**

Method B

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.17 (br s, 1H), 3.38 (app q, J = 6.3, 2H), 2.98 (t, J = 6.5, 2H), 2.51 (t, J = 7.4, 2H), 1.92 (s, 3H), 1.65 (sext, J = 7.4, 2H), 0.91 (t, J = 7.4, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 200.0, 170.4, 46.0, 39.7, 28.4, 23.2, 19.2, 13.5. HR-ESI-MS: 212.0706 ([*M* +

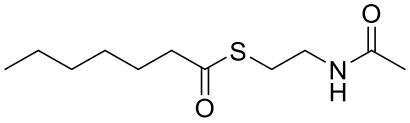
Na]$^+$, ($C_8H_{15}NO_2S$)Na^+; calc. 212.0716). Spectral data matched that reported by J. Patel et al. *Tetrahedron* **1998**, 54, 15927-15936.



***S*-(2-acetamidoethyl) pentanethioate (7)**

Method A

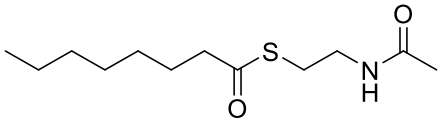
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.48 (br s, 1H), 3.32 (app q, J = 6.3 Hz, 2H), 2.94 (t, J = 6.6 Hz, 2H), 2.49 (t, J = 7.2 Hz, 2H), 1.89 (s, 3H), 1.57 (pent, J = 7.2 Hz, 2H), 1.25 (hex, J = 7.2 Hz, 2H), 0.83 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.1, 170.5, 43.8, 39.7, 28.4, 27.7, 23.1, 22.07, 13.7. HR-ESI-MS calculated exact mass for $C_9H_{18}NO_2S$: 204.1058, found: 204.1062 $[M+H]^+$. Spectral data matched that reported by Piasecki, SK, Taylor, CA, Detelich, JF, Liu, J, Zheng, J, Komsoukaniants, A, Siegel, DR and Keatinge-Clay, AT. *Chem. Biol.* **2011**, 18, 1331-1340.



***S*-(2-acetamidoethyl) heptanethioate (8)**

Method A

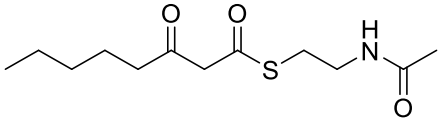
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.07 (br s, 1H), 3.66 (app q, J = 6.8 Hz, 2H), 2.98 (t, J = 6.8 Hz, 2H), 2.53 (t, J = 7.6 Hz, 2H), 1.93 (s, 3H), 1.61 (pent, J = 7.6 Hz, 2H), 1.25 (br m, 6H), 0.84 (t, J = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 200.4, 170.4, 44.3, 39.9, 31.5, 28.7, 28.6, 25.9, 23.3, 22.6, 14.1. HR-ESI-MS: 254.1181 ([M + Na]$^+$, $C_{11}H_{21}NO_2SNa^+$; calc. 254.1185). Spectral data matched that reported by Prasad, G, Borketey, LS, Lin, T and Schnarr, NA. *Org. Biomol. Chem.* **2012**, 10, 6717–6723.



***S*-(2-acetamidoethyl) octanethioate (9)**

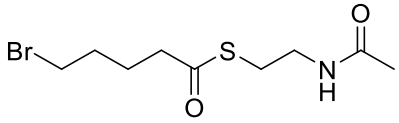
Method C

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.89 (br s, 1H), 3.46 (app q, J = 6.2 Hz, 2H), 3.09 (t, J = 6.3 Hz, 2H), 2.52 (t, J = 7.4 Hz, 2H), 1.97 (s, 3H), 1.59 (pent, J = 7.3 Hz, 2H), 1.34–1.27 (m, 8H), 0.89 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 202.5, 192.6, 170.5, 57.3, 43.6, 39.4, 31.3, 29.4, 23.3, 23.3, 22.5, 14.01. HRFAB *m/z* calculated exact mass for $C_{12}H_{24}NO_2S$ 246.1527, found 246.1525 $[M+H]^+$. Spectral data matched that reported by Tse, ML, Watts, RE and Khosla, C. *Biochemistry* **2007**, 46, 3385-3393.



***S*-(2-acetamidoethyl) 3-oxooctanethioate (10)**

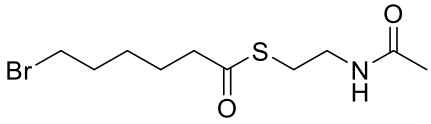
Brobst, SW and Townsend, CA. *Canadian Journal of Chemistry*, **1994**, *72* (1), 200-207.



***S*-(2-acetamidoethyl) 5-bromopentanethioate (11)**

Method B: 76% of a colorless oil.

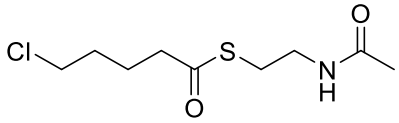
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.05 (br s, 1H), 3.36 (m, 4H), 2.98 (t, *J* = 8.0 Hz, 2H), 2.55 (t, *J* = 8.0 Hz, 2H), 1.92 (s, 3H), 1.82 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.3, 170.4, 42.9, 39.6, 32.8, 31.7, 28.5, 24.1, 23.2. HRFAB *m/z* calculated exact mass for $C_9H_{17}NO_2S^{79}Br$ 282.0163, found 282.01662 $[M+H]^+$ (^{79}Br); calculated exact mass for $C_9H_{17}NO_2S^{81}Br$ 284.0142, found 284.0146 $[M+H]^+$ (^{81}Br). See Figure S32 for NMR spectra.



***S*-(2-acetamidoethyl) 6-bromohexanethioate (12)**

Method B: 38% of a colorless oil.

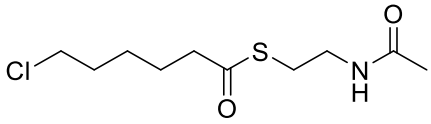
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.88 (br s, 1H), 3.39 (app q, *J* = 6.2 Hz, 2H), 3.34 (t, *J* = 6.7 Hz, 2H), 2.99 (t, *J* = 6.7 Hz, 2H), 2.55 (t, *J* = 6.7 Hz, 2H), 1.92 (s, 3H), 1.83 (m, 2H), 1.67 (m, 2H), 1.44 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.6, 170.2, 43.7, 39.6, 33.3, 32.3, 28.5, 27.4, 24.7, 23.2. HRFAB *m/z* calculated exact mass for $C_{10}H_{19}NO_2S^{79}Br$ 296.0319, found 296.0324 $[M+H]^+$ (^{79}Br); calculated exact mass for $C_{10}H_{19}NO_2S^{81}Br$ 298.0299, found 298.0305 $[M+H]^+$ (^{81}Br). See Figure S33 for NMR spectra



***S*-(2-acetamidoethyl) 5-chloropentanethioate (13)**

Method B: 99% of a colorless oil.

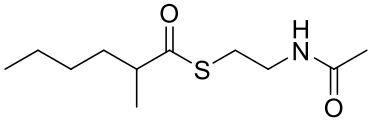
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (br s, 1H), 3.51 (m, 2H), 3.41 (app q, *J* = 6.2 Hz, 2H), 3.01 (t, *J* = 6.6 Hz, 2H), 2.58 (m, 2H), 1.92 (s, 3H), 1.80 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.3, 170.2, 44.2, 43.1, 39.6, 31.6, 28.6, 23.2, 22.8. HRFAB *m/z* calculated exact mass for $C_9H_{17}NO_2S^{35}Cl$ 238.0668, found 238.0676 $[M+H]^+$ (^{35}Cl); calculated exact mass for $C_9H_{17}NO_2S^{37}Cl$ 240.0639, found 240.0639 $[M+H]^+$ (^{37}Cl). See Figure S34 for NMR spectra



***S*-(2-acetamidoethyl) 6-chlorohexanethioate (14)**

Method B: 97% of a colorless oil.

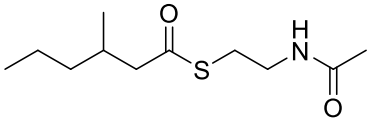
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (br s, 1H), 3.49 (t, *J* = 6.6 Hz, 2H), 3.39 (app q, *J* = 6.2 Hz, 2H), 3.00 (t, *J* = 6.6 Hz, 2H), 2.57 (t, *J* = 7.4 Hz, 2H), 1.93 (s, 3H), 1.80 – 1.60 (m, 4H), 1.45 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.8, 170.4, 44.7, 43.8, 39.7, 32.1, 28.5, 26.1, 25.4, 22.9. HRFAB *m/z* calculated exact mass for $C_{10}H_{19}NO_2S^{35}Cl$ 252.0825, found 252.0823 $[M+H]^+$ (^{35}Cl); calculated exact mass for $C_{10}H_{19}NO_2S^{37}Cl$ 254.0796, found 254.0794 $[M+H]^+$ (^{37}Cl). See Figure S35 for NMR spectra



***S*-(2-acetamidoethyl) 2-methylhexanethioate (15)**

Method A: 87 % of a colorless oil

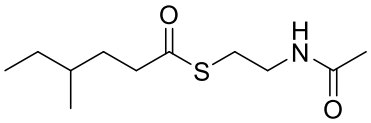
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.84 (br s, 1H), 3.43 (app q, J = 6.1 Hz, 2H), 3.02 (t, J = 6.4 Hz, 2H), 2.64 (hex, J = 6.8 Hz, 1H), 1.96 (s, 3H), 1.70 (m, 1H), 1.42 (m, 1H), 1.29 (m, 4H), 1.11 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 204.9, 170.4, 48.9, 40.1, 34.0, 29.5, 28.3, 23.4, 22.8, 17.9, 14.1. HR-ESI-MS: 254.1182 ([M + Na]$^+$, $C_{11}H_{21}NO_2SNa^+$; calc. 254.1185). See Figure S36 for NMR spectra



***S*-(2-acetamidoethyl) 3-methylhexanethioate (16)**

Method C: 95% of a colorless oil.

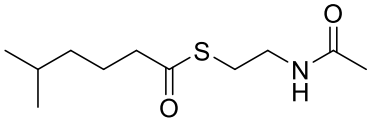
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.87 (br s, 1H), 3.44 (app q, J = 6.1 Hz, 2H), 3.02 (t, J = 6.6 Hz, 2H), 2.58 (m, 2H), 1.98 (s, 3H), 1.71 (m, 1H), 1.46 (m, 1H), 1.35 (m, 2H), 1.16 (m, 1H), 0.87 (t, J = 7.2 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 200.0, 170.4, 51.4, 39.9, 38.8, 30.9, 28.4, 23.2, 19.9, 19.5, 14.1. HRFAB *m/z* calculated exact mass for $C_{11}H_{21}NO_2S$ 232.1371, found 232.1369 [M+H]$^+$. See Figure S37 for NMR spectra



***S*-(2-acetamidoethyl) 4-methylhexanethioate (17)**

Method C: 86% of a colorless oil.

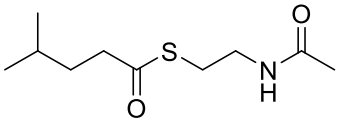
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.00 (br s, 1H), 3.41 (app q, J = 6.0 Hz, 2H), 3.00 (t, J = 6.4 Hz, 2H), 2.64 (q, J = 6.8 Hz, 1H), 2.00 (s, 3H), 1.67 (m, 1H), 1.42 (m, 1H), 1.25 (m, 3H), 1.11 (d, J = 6.8 Hz, 3H), 0.86 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 200.5, 170.5, 42.0, 39.9, 33.9, 32.1, 29.1, 28.4, 23.1, 18.8, 11.2. HRFAB *m/z* calculated exact mass for $C_{11}H_{21}NO_2S$ 232.1371, found 232.1366 [M+H]$^+$. See Figure S38 for NMR spectra



***S*-(2-acetamidoethyl) 5-methylhexanethioate (18)**

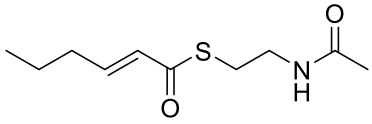
Method B: 100% of a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.94 (br s, 1H), 3.38 (app q, J = 6.2 Hz, 2H), 2.98 (t, J = 6.6 Hz, 2H), 2.53 (app.t, J = 15.2, 7.5 Hz, 2H), 1.93 (s, 3H), 1.62 (m, 2H), 1.51 (m, 1H), 1.17 (m, 2H), 0.84 (d, J = 6.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 200.2, 170.3, 44.3, 39.7, 38.1, 28.4, 23.5, 24.1, 23.1, 22.0. HRFAB *m/z* calculated exact mass for $C_{11}H_{22}NO_2S$ 232.1371, found 232.1377 [M+H]$^+$. See Figure S39 for NMR spectra

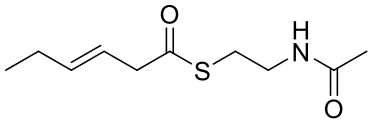


***S*-(2-acetamidoethyl) 4-methylpentanethioate (19)**

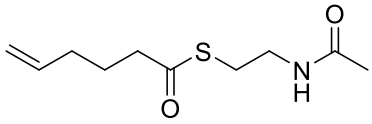
Method B: 100% of a colorless oil.
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.94 (br s, 1H), 3.38 (app q, *J* = 6.2 Hz, 2H), 2.97 (t, *J* = 6.6 Hz, 2H), 2.53 (app t, *J* = 7.4 Hz, 2H), 1.91 (s, 3H), 1.51 (m, 3H), 0.85 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 200.4, 170.3, 42.3, 39.7, 34.4, 28.4, 27.6, 23.1, 22.2. HRFAB *m/z* calculated exact mass for $C_{10}H_{20}NO_2S$: 218.1215, found: 218.1215 $[M+H]^+$. See Figure S40 for NMR spectra



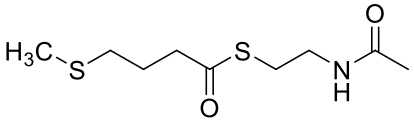
***S*-(2-acetamidoethyl) (*E*)-hex-2-enethioate (20)**
Method A
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.92 (dt, *J* = 15.1, 7.3 Hz, 1H), 6.12 (d, *J* = 15.5 Hz, 1H), 5.97 (br s, 1H), 3.44 (app q, *J* = 6.4 Hz, 2H), 3.08 (t, *J* = 6.4 Hz, 2H), 2.18 (app q, *J* = 7.2 Hz, 2H), 1.96 (s, 3H), 1.50 (pent, *J* = 7.4 Hz, 2H), 1.72 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 190.6, 170.5, 146.7, 128.57, 47.7, 40.0, 34.4, 28.4, 23.4, 21.3. HR-ESI-MS: 238.0866 ([*M* + Na]$^+$, $C_{10}H_{17}NO_2SNa^+$; calc. 238.0872). Spectral data matched that reported by Tang, MC, He, H, Zhang, F and Tang, G. *ACS Catal.* **2013**, 3, 444−447.



***S*-(2-acetamidoethyl) (*E*)-hex-3-enethioate (21)**
Method C
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.90 (br s, 1H), 5.65 (m, 1H), 5.48 (m, 1H), 3.43 (app q, *J* = 6.3 Hz, 2H), 3.24 (ddd, *J* = 1.0, 4.4, 6.8 Hz, 1H), 3.02, (t, *J* = 6.4 Hz, 2H), 2.07 (m, 2H), 1.96 (s, 3H), 1.00 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 198.9, 170.4, 138.3, 119.8, 47.6, 39.7, 28.5, 25.6, 23.2, 13.3. HRFAB *m/z* calculated exact mass for $C_{10}H_{17}NO_2S$: 216.1058, found: 216.1062 $[M+H]^+$. Spectral data matched that reported by Gay, DC, Spear, PJ and Keatinge-Clay, AT. *ACS Chem. Biol.* **2014**, 9, 2374-2381.

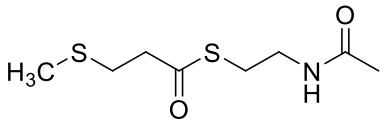


***S*-(2-acetamidoethyl) hex-5-enethioate (22)**
Method A: 24% of a colorless oil
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.13 (br s, 1H), 5.72 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 5.02–4.95 (m, 2H), 3.38 (app q, *J* = 6.3 Hz, 2H), 2.99 (t, *J* = 6.3 Hz, 2H), 2.55 (t, *J* = 7.5 Hz, 2H), 2.05 (q, *J* = 6.9, CH_2), 1.93 (s, 3H), 1.72 (pent, *J* = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 199.4, 170.5, 137.2, 115.5, 43.1, 39.4, 32.6, 28.3, 24.50, 23.0. HR-ESI-MS calculated exact mass for $C_{10}H_{18}NO_2S$: 216.1058, found: 216.1059 $[M+H]^+$. See Figure S41 for NMR spectra



***S*-(2-acetamidoethyl) 4-(methylthio)butanethioate (23)**
Method A: 30% of a colorless oil

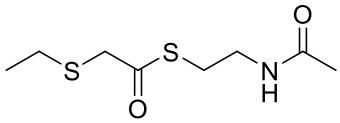
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.89 (br s, 1H), 3.42 (app q, J = 6.3 Hz, 2H), 3.04 (t, J = 6.3 Hz, 2H), 2.75 (t, J = 6.9 Hz, 2H), 2.44 (t, J = 6.9 Hz, 2H), 2.03 (s, 3H), 1.97 (m, 2H), 1.96 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.3, 170.4, 42.7, 39.6, 33.3, 28.6, 24.6, 23.4, 15.4. HR-ESI-MS: 258.0589 ([M + Na]$^+$, $C_9H_{17}NO_2S_2Na^+$; calc. 258.0593). See Figure S42 for NMR spectra



***S*-(2-acetamidoethyl) 3-(methylthio)propanethioate (24)**

Method A: 27% of a colorless oil

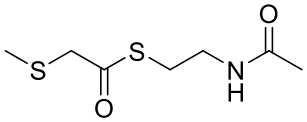
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.29 (br s, 1H), 3.36 (app q, J = 6.3 Hz, 2H), 2.99 (t, J = 6.5 Hz, 2H), 2.81 (AA'BB', $J \approx$ 7.1, 1.9 Hz, 2H), 2.73 (AA'BB', $J \approx$ 7.1, 1.9 Hz, 2H), 2.06 (s, 3H), 1.91 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.9, 170.4, 43.4, 39.3, 29.3, 28.5, 23.1, 15.5. HR-ESI-MS: 244.0431 ([M + Na]$^+$, $C_8H_{15}NO_2S_2Na^+$; calc. 244.0436). See Figure S43 for NMR spectra



***S*-(2-acetamidoethyl) 2-(ethylthio)ethanethioate (25)**

Method A: colorless oil

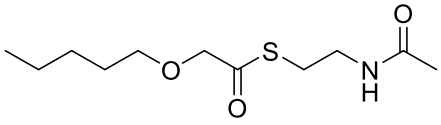
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.89 (br s, 1H), 3.45 (app q, J = 6.4 Hz, 2H), 3.43 (s, 2H), 3.06 (t, J = 6.4 Hz, 2H), 2.64 (dq, J = 0.8, 7.2 Hz, 2H), 1.97 (s, 3H), 1.27 (dt, J = 0.8, 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.4, 170.3, 41.9, 39.5, 29.1, 27.0, 23.2, 14.1. HR-ESI-MS calculated exact mass for $C_8H_{16}NO_2S_2$: 222.0623, found: 222.0622 $[M+H]^+$. See Figure S44 for NMR spectra



***S*-(2-acetamidoethyl) 2-(methylthio)ethanethioate (26)**

Method A: colorless oil

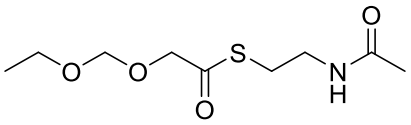
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.98 (br s, 1H), 3.44 (app q, J = 6.3 Hz, 2H), 3.38 (s, 2H), 3.06 (t, J = 6.3 Hz, 2H), 2.19 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.0, 170.4, 44.2, 39.4, 29.1, 23.2, 16.5. HR-ESI-MS calculated exact mass for $C_7H_{14}NO_2S_2$: 208.0466, found: 208.0464 $[M+H]^+$. See Figure S45 for NMR spectra



***S*-(2-acetamidoethyl) 2-(pentyloxy)ethanethioate (27)**

Method A: 61% of a colorless oil

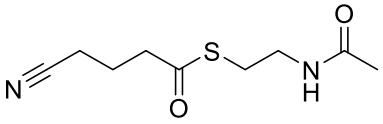
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.04 (br s, 1H), 4.10 (s, 2H), 3.54 (t, J = 6.9 Hz, 2H), 3.41 (app q, J = 6.4 Hz, 2H), 3.03 (t, J = 6.4 Hz, 2H), 1.95 (s, 3H), 1.63 (p, J = 6.9 Hz, 2H), 1.35 (m, 4H), 0.90 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.0, 170.4, 75.8, 72.9, 39.5, 29.3, 28.1, 27.4, 23.2, 22.5, 14.0. HR-ESI-MS: 270.1132 ([M + Na]$^+$, $C_{11}H_{21}NO_3SNa^+$; calc. 270.1134). See Figure S46 for NMR spectra



***S*-(2-acetamidoethyl) 2-(ethoxymethoxy)ethanethioate (28)**

Method A colorless oil

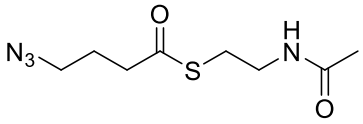
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.15 (br s, 1H), 4.73 (s, 2H), 4.22 (s, 2H), 3.63 (q, *J* = 7.0 Hz, 2H), 3.40 (app q, *J* = 6.4 Hz, 2H), 3.03 (t, *J* = 6.4 Hz, 2H), 1.94 (s, 3H), 1.19 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.7, 170.5, 95.3, 71.9, 64.1, 39.4, 27.5, 23.1, 15.0. HR-ESI-MS calculated exact mass for $C_9H_{18}NO_4S$: 236.0957, found: 236.0966 $[M+H]^+$. See Figure S47 for NMR spectra



***S*-(2-acetamidoethyl) 4-cyanobutanethioate (29)**

Method A: 60% of a colorless oil

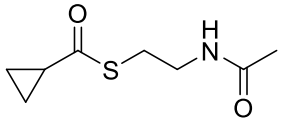
^{1}H NMR (300 MHz, $CDCl_3$): δ 5.95 (br s, 1H), 3.42 (app q, *J* = 6.0 Hz, 2H), 3.01 (t, *J* = 6.0 Hz, 2H), 2.71 (t, *J* = 7.2 Hz, 2H), 2.52 (t, *J* = 6.9 Hz, 2H), 1.95 (pent, *J* = 7.2 Hz, 2H), 1.95 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.1, 170.4, 118.9, 42.0, 39.4, 28.9, 23.3, 21.2, 16.3. HR-ESI-MS calculated exact mass for $C_9H_{15}N_2O_2S$: 215.0854, found: 215.0856 $[M+H]^+$. See Figure S48 for NMR spectra



***S*-(2-acetamidoethyl) 4-azidobutanethioate (30)**

Method A: 72% of a colorless oil

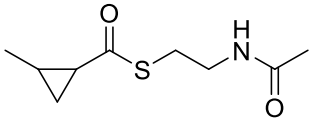
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.17 (br s, 1H), 3.39 (app q, *J* = 6.3 Hz, 2H), 3.32 (t, *J* = 6.6 Hz, 2H), 3.00 (t, *J* = 6.3 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 1.93 (s, 3H), 1.88 (pent, *J* = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.6, 170.5, 50.4, 40.8, 39.4, 28.6, 24.7, 23.1. HR-ESI-MS: 253.0735 ($[M + Na]^+$, $C_8H_{14}N_4O_2SNa^+$; calc. 253.0711). See Figure S49 for NMR spectra



***S*-(2-acetamidoethyl) cyclopropanecarbothioate (31)**

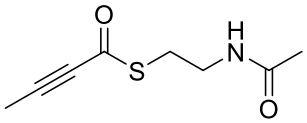
Method C

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.08 (br s, 1H), 3.41 (app q, *J* = 6.1 Hz, 2H), 3.03 (t, *J* = 6.4 Hz, 2H), 2.02 (sym m, 1H), 1.95 (s, 3H), 1.15 (sym m, 2H), 1.35 (m, 2H), 0.97 (sym m, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 199.9, 170.3, 39.8, 31.1, 28.5, 22.8, 23.2, 22.7, 11.1. HR-ESI-MS calculated exact mass for $C_8H_{14}NO_2S$: 188.0745, found: 188.0751 $[M+H]^+$. Spectral data matched that reported by Lee, MS, Qin, G, Nakanishi, K, Zagorski, MG. *JACS* **1989**, 111, 6234-6241.

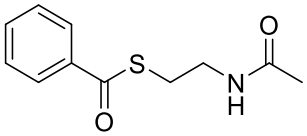


***S*-(2-acetamidoethyl) 2-methylcyclopropane-1-carbothioate (32)**

Method C: 72% of a colorless oil
As a 3:1 mixture of diastereomers. Major: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.99 (br s, 1H), 3.42 (app q, J = 6.0 Hz, 2H), 3.02 (t, J = 6.8 Hz, 2H), 1.95 (s, 3H), 1.76 (sym m, 1H), 1.55 (b m, 1H), 1.35 (m, 1H), 1.13 (d, J = 6.0 Hz, 3H), 0.82 (sym m, 1H). Minor: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.99 (br s, 1H), 3.42 (app q, J = 6.0 Hz, 2H), 3.06 (m, 2H), 2.10 (b m, 1H) 1.95 (s, 3H), 1.48 (b m, 1H), 1.14 (d, J = 6.0 Hz, 3H), 1.11 (m, 1H), 1.10 (m, 1H). Major: ^{13}C-NMR (101 MHz, $CDCl_3$): δ 199.2, 170.3, 39.9, 31.4, 28.5, 23.2, 20.4, 19.6, 17.9. Minor: ^{13}C-NMR (101 MHz, $CDCl_3$): δ 197.7, 170.3, 40.1, 28.6, 28.3, 23.2, 19.6, 16.3, 11.9. HR-ESI-MS calculated exact mass for $C_9H_{16}NO_2S$: 202.0902, found: 202.0910 $[M+H]^+$. See Figure S50 for NMR spectra



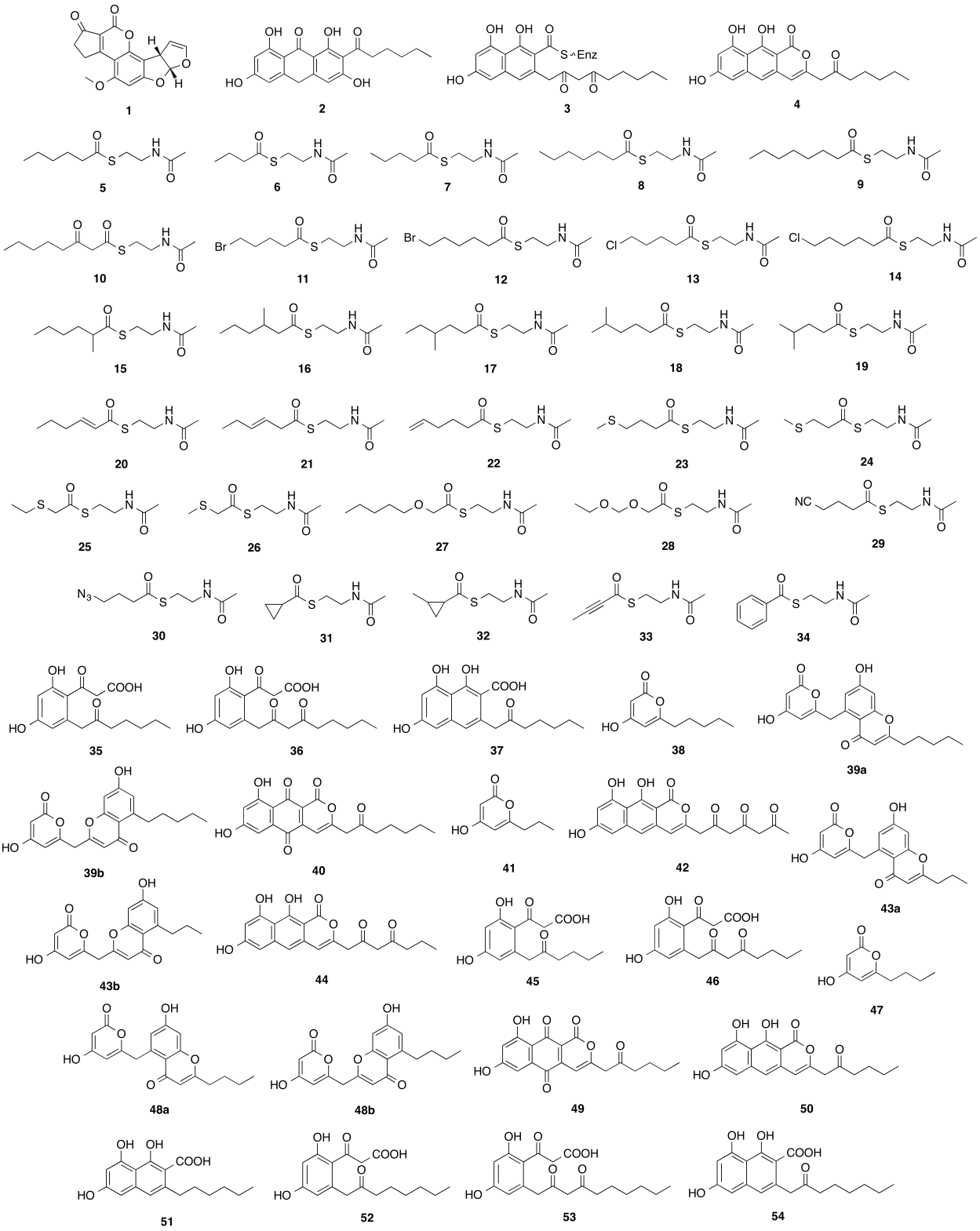
***S*-(2-acetamidoethyl) but-2-ynethioate (33)**
Method A: colorless prisms
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.01 (br s, 1H), 3.45 (app q, J = 6.3 Hz, 2H), 3.10 (t, J = 6.3 Hz, 2H), 2.05 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.6, 170.4, 92.5, 77.9, 39.3, 29.3, 23.2, 4.3. HR-ESI-MS calculated exact mass for $C_8H_{12}NO_2S$: 186.0589, found: 186.0585 $[M+H]^+$. Melting point: 59 °C. See Figure S51 for NMR spectra

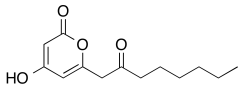


***S*-(2-acetamidoethyl) benzothioate (34)**
Method C
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, J = 4.9 Hz, 2H), 7.58 (d, J = 3.8 Hz, 1H), 7.46 (dd, J = 4.7, 3.8 Hz, 2H), 6.01 (br s, 1H), 3.51 (app q, $J \approx$ 6.0 Hz, 2H), 3.23 (t, J = 6.0 Hz, 2H), 1.97 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.4, 170.5, 136.8, 133.8, 128.8, 127.4, 39.8, 28.7, 23.4. HR-ESI-MS: 246.0554 ($[M + Na]^+$, $C_{11}H_{13}NO_2SNa^+$; calc. 246.0559). Spectral data matched that reported by Prasad, G, Borketey, LS, Lin, T and Schnarr, NA. *Org. Biomol. Chem.* **2012**, 10, 6717–6723.

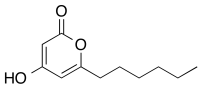
References

1. Crawford, J. M., Thomas, P. M., Scheerer, J. R., Vagstad, A. L., Kelleher, N. L., and Townsend, C. A. (2008) Deconstruction of iterative multidomain polyketide synthase function, *Science 320*, 243-246.
2. Vagstad, A. L., Bumpus, S. B., Belecki, K., Kelleher, N. L., and Townsend, C. A. (2012) Interrogation of global active site occupancy of a fungal iterative polyketide synthase reveals strategies for maintaining biosynthetic fidelity, *J Am Chem Soc 134*, 6865-6877.

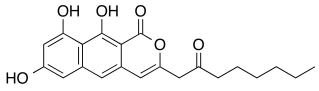

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39a
39b
40
41
42
43a
43b
44
45
46
47
48a
48b
49
50
51
52
53
54



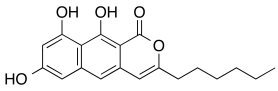
55



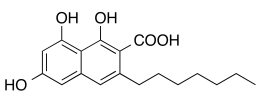
56



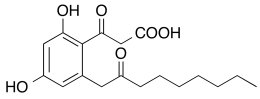
57



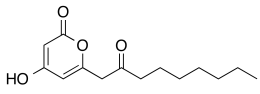
58



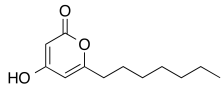
59



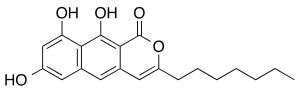
60



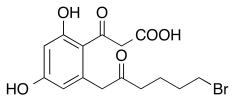
61



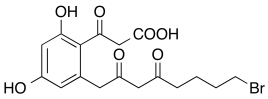
62



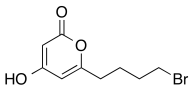
63



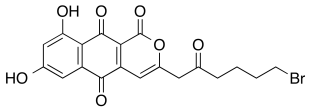
64



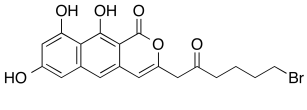
65



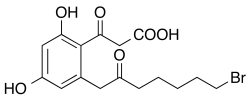
66



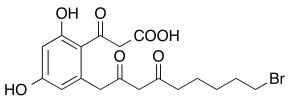
67



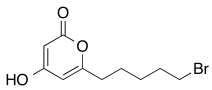
68



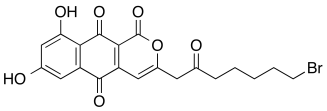
69



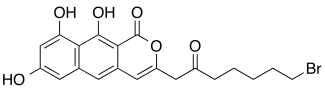
70



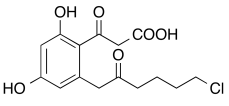
71



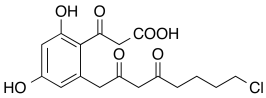
72



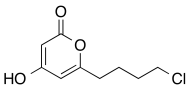
73



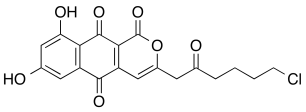
74



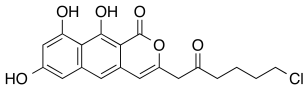
75



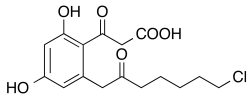
76



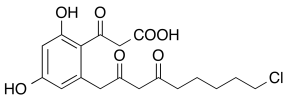
77



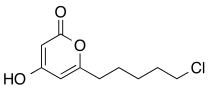
78



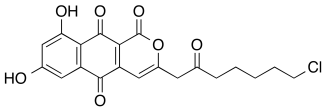
79



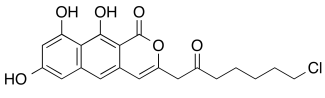
80



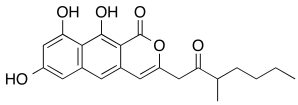
81



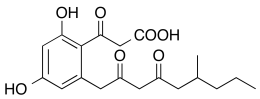
82



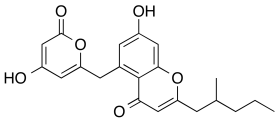
83



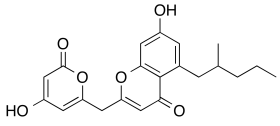
84



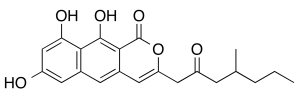
85



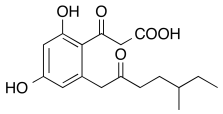
86a



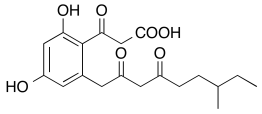
86b



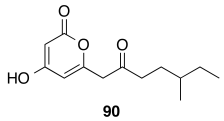
87



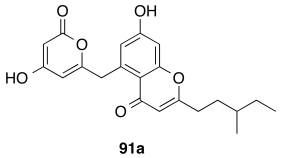
88



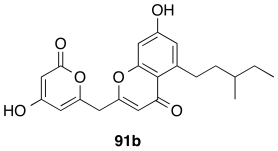
89



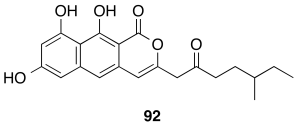
90



91a



91b



92

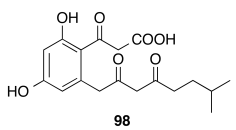
93

94

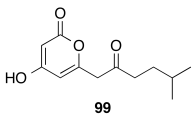
95

96

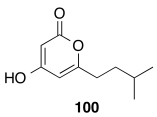
97



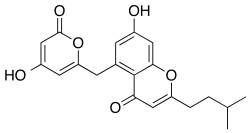
98



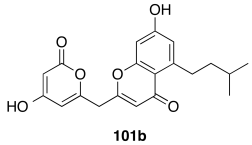
99



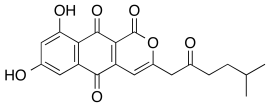
100



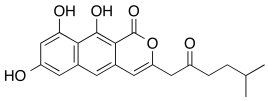
101a



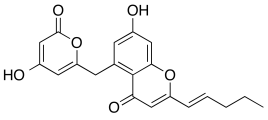
101b



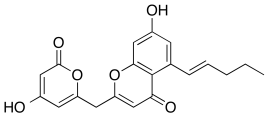
102



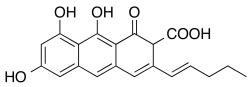
103



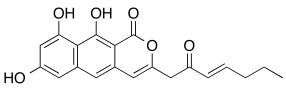
104a



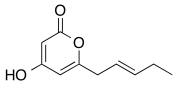
104b



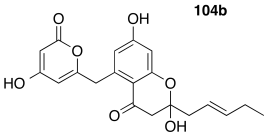
105



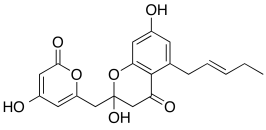
106



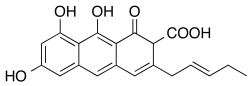
107



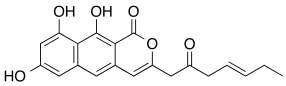
108a



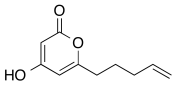
108b



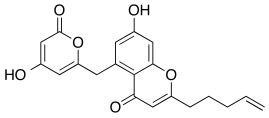
109



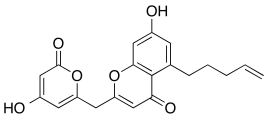
110



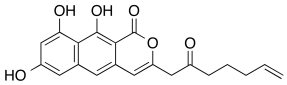
111



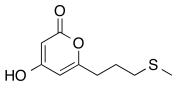
112a



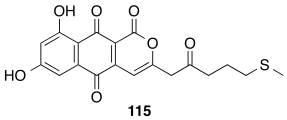
112b



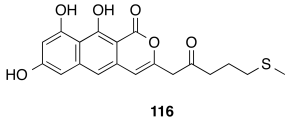
113



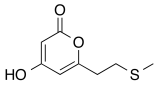
114



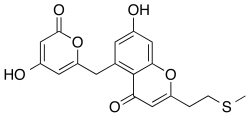
115



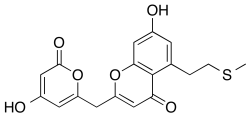
116



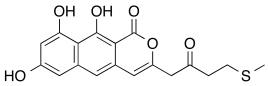
117



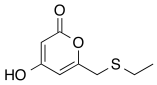
118a



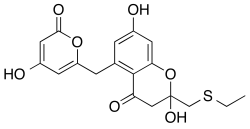
118b



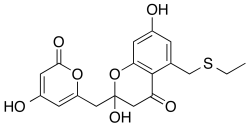
119



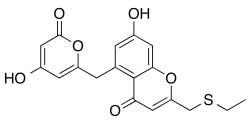
120



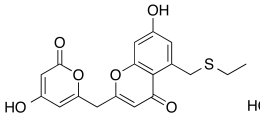
121a



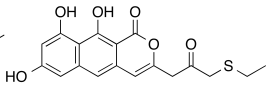
121b



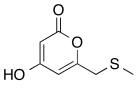
122a



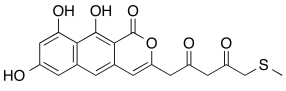
122b



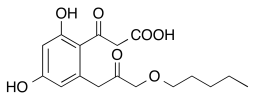
123



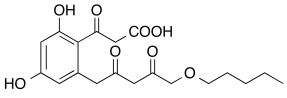
124



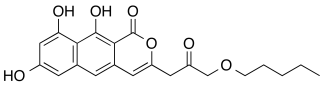
125



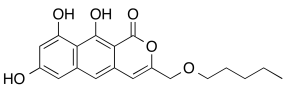
126



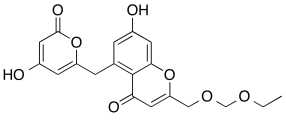
127



128



129



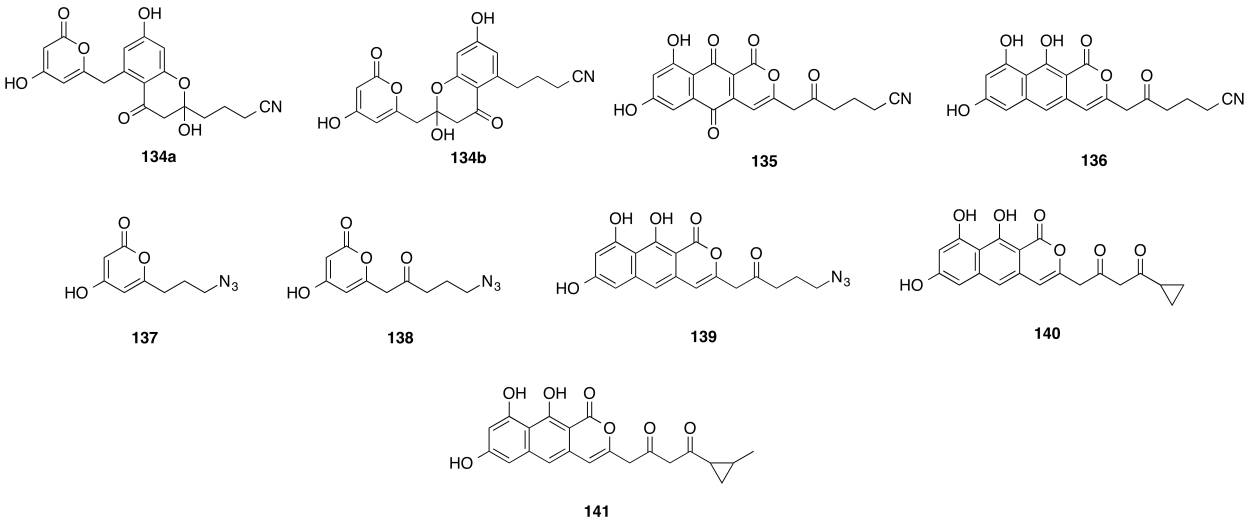
130a

130b

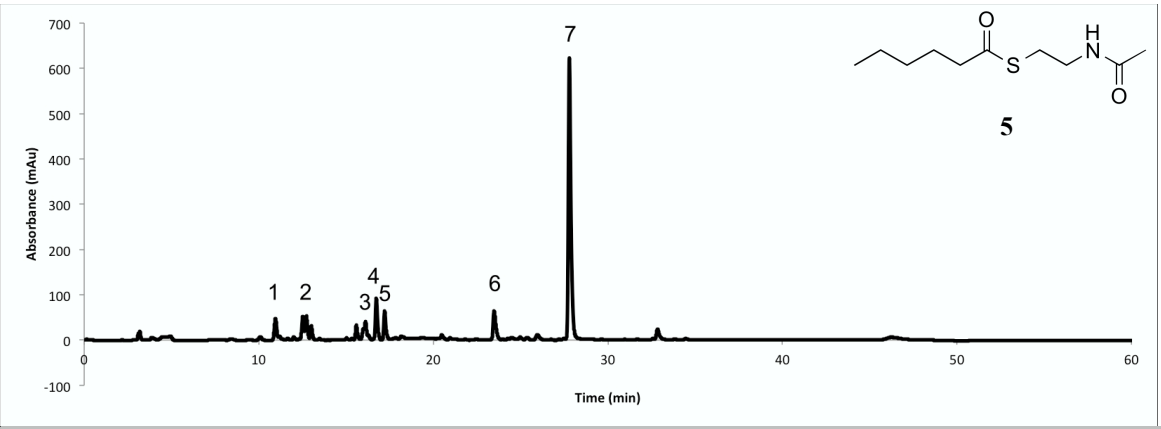
131

132

133

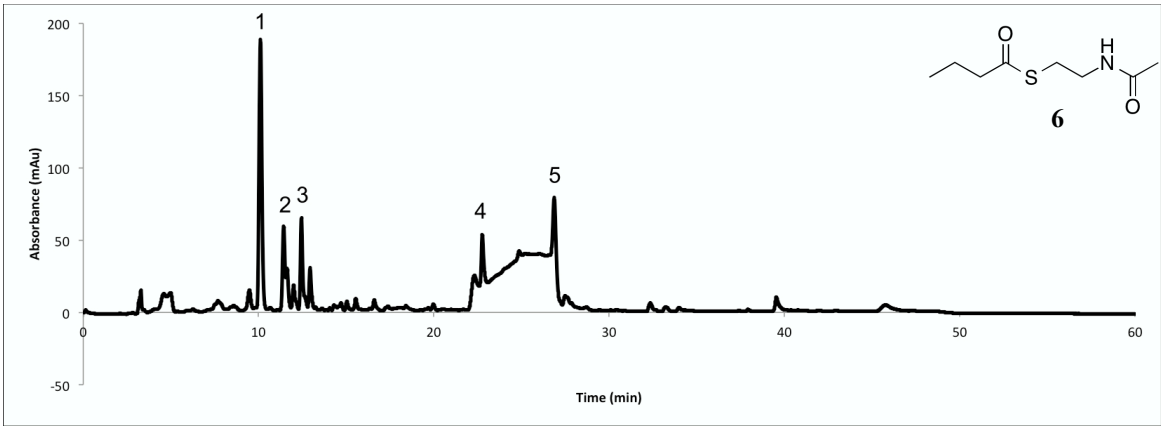


Scheme S1. Full structures of all starter units assayed in this paper and the resulting enzymatic products. See following figures (Figures S1-S31) for HPLC and HR-ESI-MS characterization of enzymatic products **35-141**.



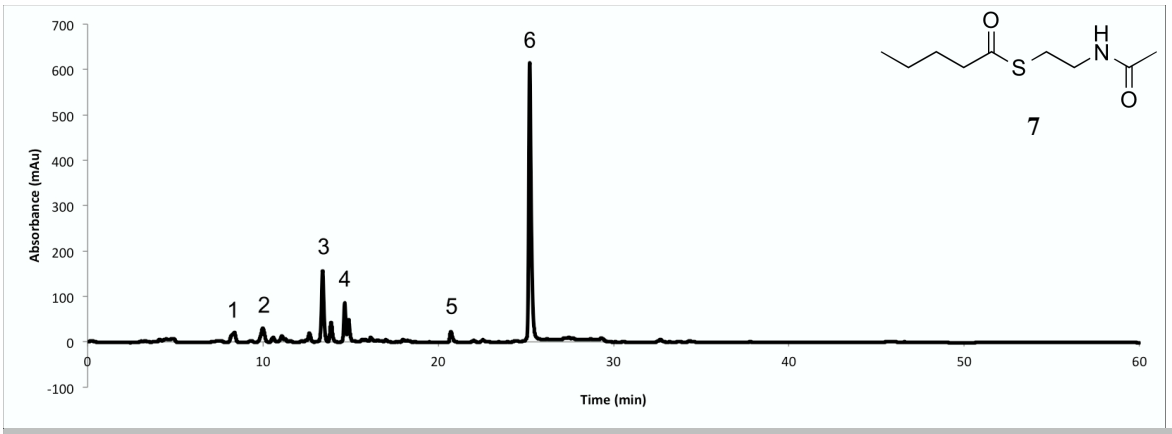
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	35	B	C_5H_{11}	260, 288	309.1342	Hex+5Mal	$-H_2O$	$+H_2O$	$C_{16}H_{20}O_6$
2	36	B	$CH_2COC_5H_{11}$	262, 290	351.1434	Hex+6Mal	$-H_2O$	$+H_2O$	$C_{18}H_{22}O_7$
3	37	A	$CH_2COC_5H_{11}$	220, 288	333.1337	Hex+6Mal	$-2H_2O$	$+H_2O$	$C_{18}H_{20}O_6$
4	38	C	C_5H_{11}	220, 286	183.1027	Hex+2Mal	-	O-C	$C_{10}H_{14}O_3$
5	39a,b	D/E	C_5H_{11}	224, 278, 306	357.1342	Hex+7Mal	$-H_2O$	O-C	$C_{20}H_{20}O_6$
6	40	F	$CH_2COC_5H_{11}$	248, 270, 340, 468	371.1124	Hex+7Mal	$-2H_2O$	O-C, [O]	$C_{20}H_{18}O_7$
7	4	G	$CH_2COC_5H_{11}$	270, 280, 390	357.1332	Hex+7Mal	$-2H_2O$	O-C	$C_{20}H_{20}O_6$

Figure S1. Product profile for starter unit **5** reaction (*S*-(2-acetamidoethyl) hexanethioate)



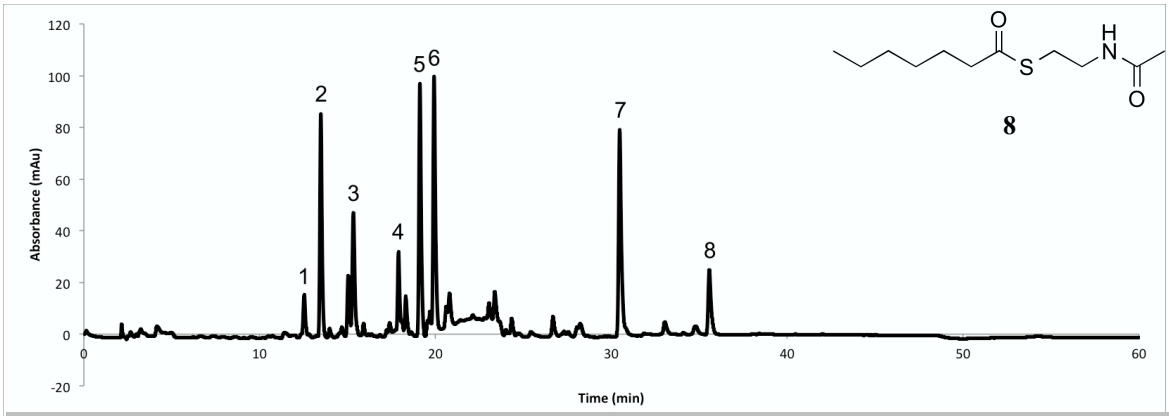
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release	Mol. Formula
1	41	C	C_3H_7	232, 284	155.0712	But+2Mal	-	O-C	$C_8H_{10}O_5$
2	42	G	$CH_2COCH_2COCH_2COCH_3$	270, 280, 390	385.0917	10Mal	$-2H_2O$	O-C	$C_{20}H_{16}O_8$
3	43a,b	D/E	C_3H_7	224, 278, 306	329.1027	But+7Mal	$-2H_2O$	O-C	$C_{18}H_{16}O_6$
4	44	G	$CH_2COCH_2COC_3H_7$	270, 280, 390	371.1130	But+8Mal	$-2H_2O$	O-C	$C_{20}H_{18}O_7$
5	44	G	$CH_2COCH_2COC_3H_7$	270, 280, 390	371.1128	But+8Mal	$-2H_2O$	O-C	$C_{20}H_{18}O_7$

Figure S2. Product profile for starter unit **6** reaction (*S*-(2-acetamidoethyl) butanethioate).



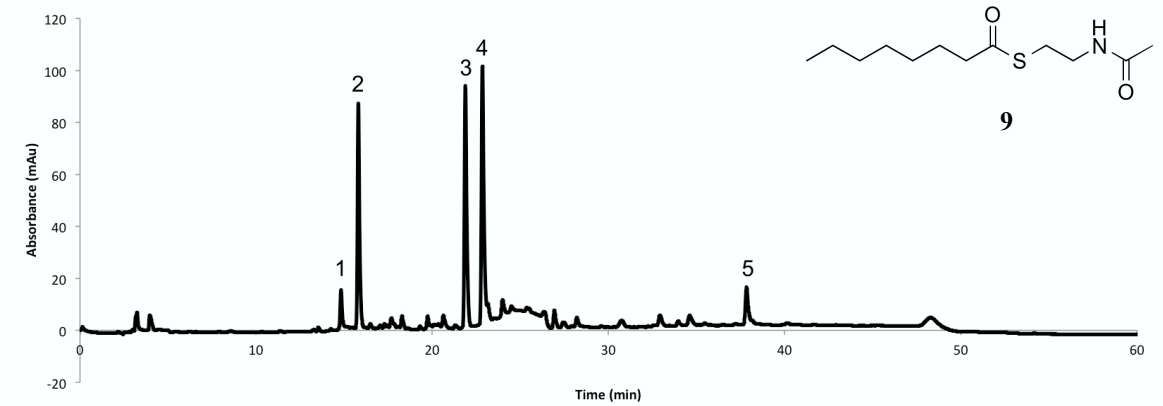
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	45	B	C_4H_9	260, 288	295.1184	Pent+5Mal	$-H_2O$	$+ H_2O$	$C_{15}H_{18}O_6$
2	46	B	$CH_2COC_4H_9$	262, 288	337.1285	Pent+6Mal	$-H_2O$	$+ H_2O$	$C_{17}H_{20}O_7$
3	47	C	C_4H_9	220, 286	169.0872	Pent+2Mal	-	O-C	$C_9H_{12}O_3$
4	48a,b	D/E	C_4H_9	224, 278, 306	343.1178	Pent+7Mal	$-2H_2O$	O-C	$C_{19}H_{18}O_6$
5	49	F	$CH_2COC_4H_9$	246, 270, 336, 468	357.0974	Pent+7Mal	$-2H_2O$	O-C, [O]	$C_{19}H_{16}O_7$
6	50	G	$CH_2COC_4H_9$	272, 280, 390	343.1174	Pent+7Mal	$-2H_2O$	O-C	$C_{19}H_{18}O_6$

Figure S3. Product profile for starter unit **7** reaction (*S*-(2-acetamidoethyl) pentanethioate).



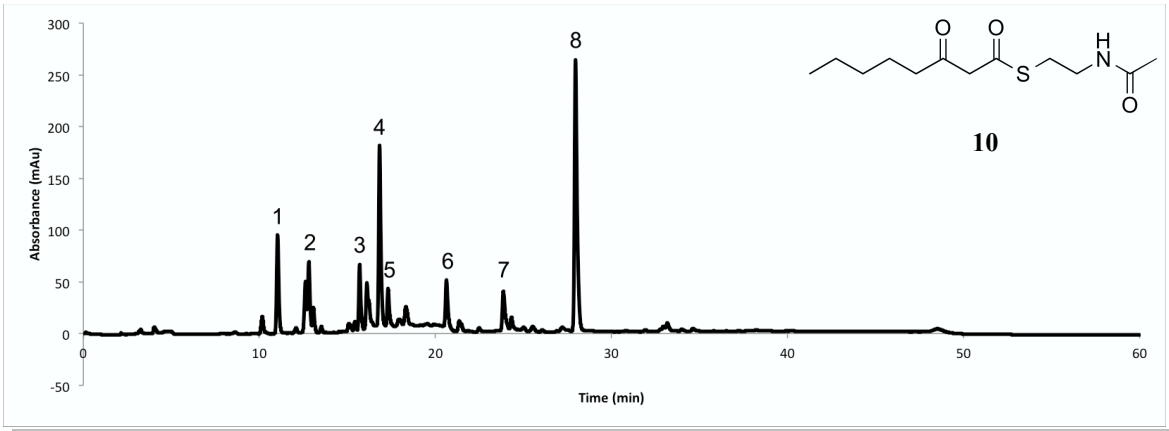
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	51	A	C_6H_{13}	262, 288	305.1391	Hept+5Mal	$-2H_2O$	$+H_2O$	$C_{17}H_{20}O_5$
2	52	B	C_6H_{13}	260, 288	323.1494	Hept+5Mal	$-H_2O$	$+H_2O$	$C_{17}H_{22}O_6$
3	53	B	$CH_2COC_6H_{13}$	262, 288	365.1602	Hept+6Mal	$-H_2O$	$+H_2O$	$C_{19}H_{24}O_7$
4	54	A	$CH_2COC_6H_{13}$	222, 288	347.1497	Hept+6Mal	$-2H_2O$	$+H_2O$	$C_{19}H_{22}O_6$
5	55	C	$CH_2COC_6H_{13}$	224, 286	239.1288	Hept+3Mal	-	O-C	$C_{13}H_{18}O_4$
6	56	C	C_6H_{13}	220, 286	197.1183	Hept+2Mal	-	O-C	$C_{11}H_{16}O_3$
7	57	G	$CH_2COC_6H_{13}$	270, 280, 390	371.1493	Hept+7Mal	$-2H_2O$	O-C	$C_{21}H_{22}O_6$
8	58	G	C_6H_{13}	270, 280, 390	329.1403	Hept+6Mal	$-2H_2O$	O-C	$C_{19}H_{20}O_5$

Figure S4. Product profile for starter unit **8** reaction (*S*-(2-acetamidoethyl) heptanethioate).



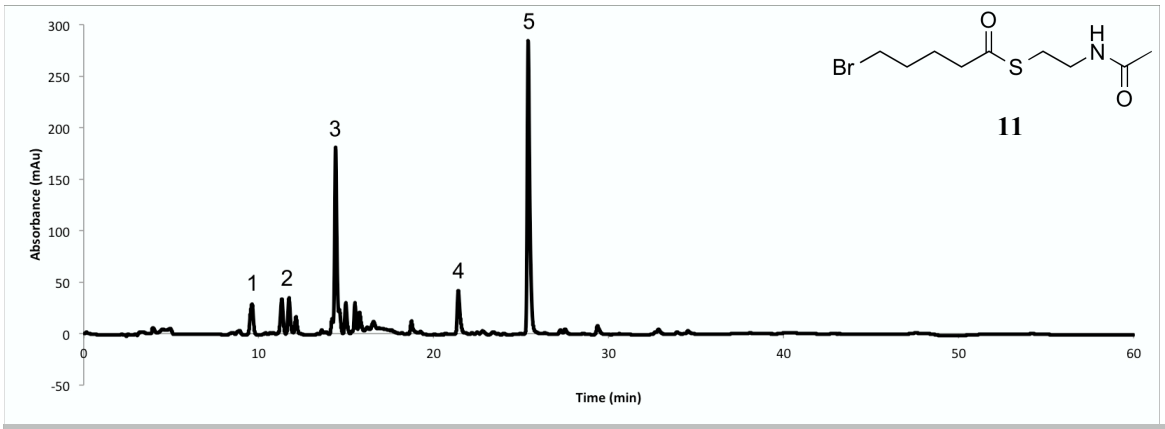
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	59	A	C_7H_{15}	262, 288	319.1548	Oct+5Mal	$-2H_2O$	$+H_2O$	$C_{18}H_{22}O_5$
2	60	B	C_7H_{15}	260, 288	337.1651	Oct+5Mal	$-H_2O$	$+H_2O$	$C_{18}H_{24}O_6$
3	61	C	$CH_2COC_7H_{15}$	220, 286	253.1443	Oct+3Mal	-	O-C	$C_{14}H_{20}O_4$
4	62	C	C_7H_{15}	220, 286	211.1339	Oct+2Mal	-	O-C	$C_{12}H_{18}O_3$
5	63	G	C_7H_{15}	270, 280, 390	343.1538	Oct+6Mal	$-H_2O$	O-C	$C_{20}H_{22}O_5$

Figure S5. Product profile for starter unit **9** reaction (*S*-(2-acetamidoethyl) octanethioate).



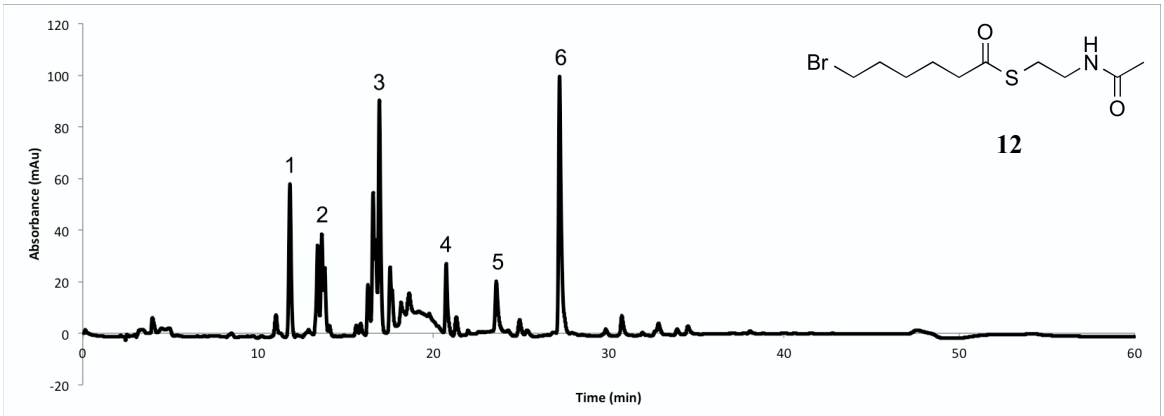
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	35	B	C_5H_{11}	260, 288	309.1342	Oct+4Mal	$-H_2O$	$+H_2O$	$C_{16}H_{20}O_6$
2	36	B	$CH_2COC_5H_{11}$	262, 288	351.1435	Oct+5Mal	$-H_2O$	$+H_2O$	$C_{18}H_{22}O_7$
3	37	A	$CH_2COC_5H_{11}$	220, 288	333.1336	Oct+5Mal	$-2H_2O$	$+H_2O$	$C_{18}H_{20}O_6$
4	38	C	C_5H_{11}	218, 286	183.1025	Oct+Mal	-	O-C	$C_{10}H_{14}O_3$
5	39a,b	D/E	C_5H_{11}	224, 278, 310	357.1337	Oct+6Mal	$-2H_2O$	O-C	$C_{20}H_{20}O_6$
6				234, 248, 280, 304	333.1341	Oct+5Mal			$C_{18}H_{20}O_6$
7	40	F	$CH_2COC_5H_{11}$	246, 264, 340, 468	371.1127	Oct+6Mal	$-2H_2O$	O-C, [O]	$C_{20}H_{18}O_7$
8	4	G	$CH_2COC_5H_{11}$	270, 280, 390	357.1332	Oct+6Mal	$-2H_2O$	O-C	$C_{20}H_{20}O_6$

Figure S6. Product profile for starter unit **10** reaction (*S*-(2-acetamidoethyl) 3-oxooctanethioate).



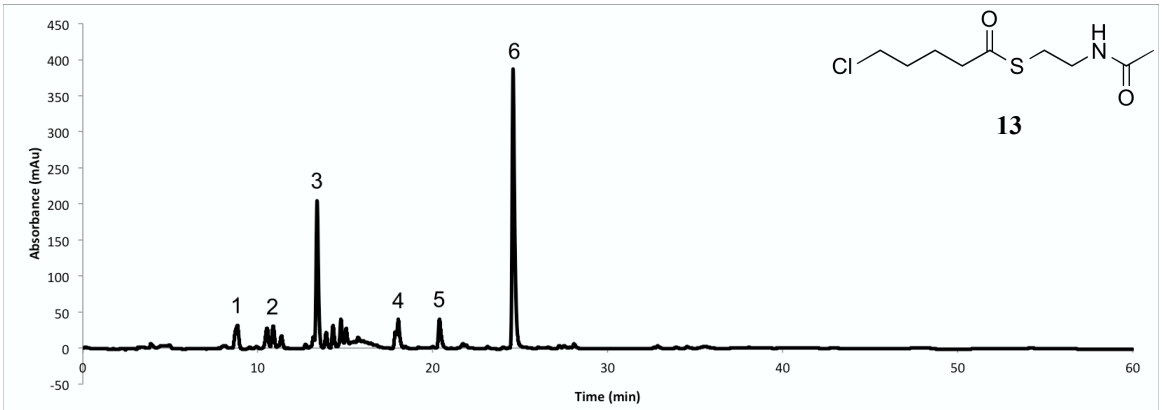
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	64	B	C_4H_8Br	262, 290	373.0296	Br-Pent+5Mal	-H_2O	+H_2O	$C_{15}H_{17}BrO_6$
2	65	B	$CH_2COC_4H_8Br$	262, 290	415.0380	Br-Pent+6Mal	-H_2O	+H_2O	$C_{17}H_{19}BrO_7$
3	66	C	C_4H_8Br	220, 286	246.9974	Br-Pent+2Mal	-	O-C	$C_9H_{11}BrO_3$
4	67	F	$CH_2COC_4H_8Br$	246, 268, 338, 468	435.0079	Br-Pent+7Mal	-2H_2O	O-C, [O]	$C_{19}H_{15}BrO_7$
5	68	G	$CH_2COC_4H_8Br$	272, 280, 388	421.0273	Br-Pent+7Mal	-2H_2O	O-C	$C_{19}H_{17}BrO_6$

Figure S7. Product profile for starter unit **11** reaction (*S*-(2-acetamidoethyl) 5-bromopentanethioate).



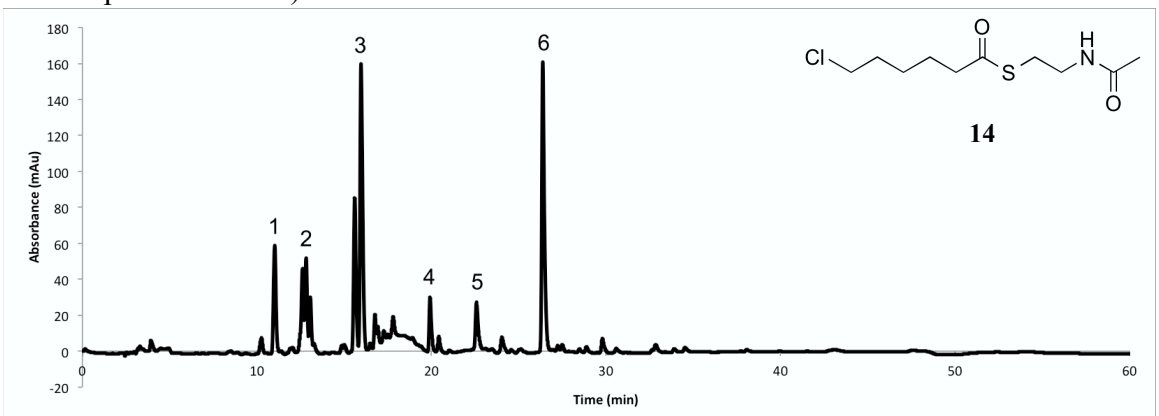
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	69	B	$C_5H_{10}Br$	260, 288	387.0440	Br-Hex+5Mal	-H_2O	+H_2O	$C_{16}H_{19}BrO_6$
2	70	B	$CH_2COC_5H_{10}Br$	262, 288	429.0569	Br-Hex+6Mal	-H_2O	+H_2O	$C_{18}H_{21}BrO_7$
3	71	C	$C_5H_{10}Br$	220, 286	261.0121	Br-Hex+2Mal	-	O-C	$C_{10}H_{13}BrO_3$
4				212, 234, 278, 302	411.0431	Br-Hex+6Mal			$C_{18}H_{19}BrO_6$
5	72	F	$CH_2COC_5H_{10}Br$	246, 264, 336, 468	449.0241	Br-Hex+7Mal	-2H_2O	O-C, [O]	$C_{20}H_{17}BrO_7$
6	73	G	$CH_2COC_5H_{10}Br$	272, 280, 390	435.0427	Br-Hex+7Mal	-2H_2O	O-C	$C_{20}H_{19}BrO_6$

Figure S8. Product profile for starter unit **12** reaction (*S*-(2-acetamidoethyl) 6-bromohexanethioate).



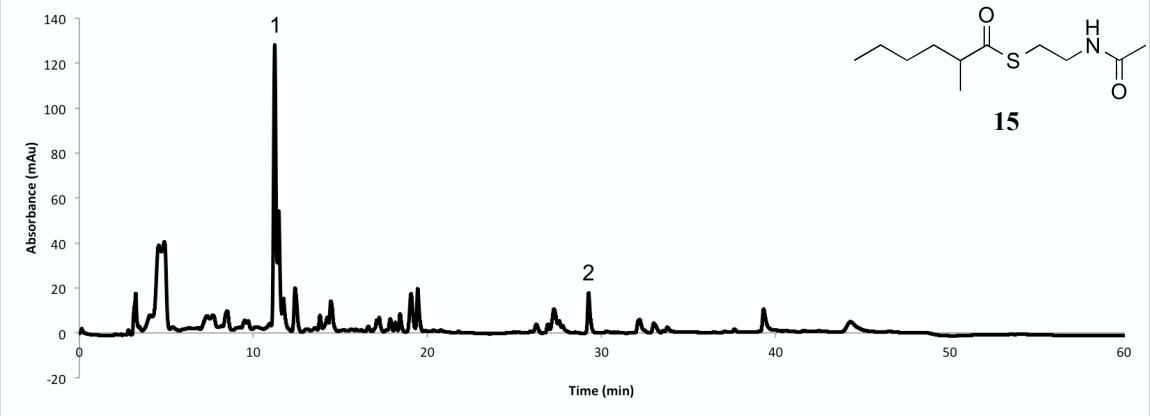
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	74	B	C_4H_8Cl	262, 288	329.0812	Cl-Pent+5Mal	-H_2O	+H_2O	$C_{15}H_{17}ClO_6$
2	75	B	$CH_2COC_4H_8Cl$	262, 288	371.0872	Cl-Pent+6Mal	-H_2O	+H_2O	$C_{17}H_{19}ClO_7$
3	76	C	C_4H_8Cl	220, 286	203.0479	Cl-Pent+2Mal	-	O-C	$C_9H_{11}ClO_3$
4				218, 286	245.0481				$C_{11}H_{13}ClO_4$
5	77	F	$CH_2COC_4H_8Cl$	246, 264, 338, 468	391.0569	Cl-Pent+7Mal	-2H_2O	O-C, [O]	$C_{19}H_{15}ClO_7$
6	78	G	$CH_2COC_4H_8Cl$	272, 280, 390	377.0784	Cl-Pent+7Mal	-2H_2O	O-C	$C_{19}H_{17}ClO_6$

Figure S9. Product profile for starter unit **13** reaction (*S*-(2-acetamidoethyl) 5-chloropentanethioate).



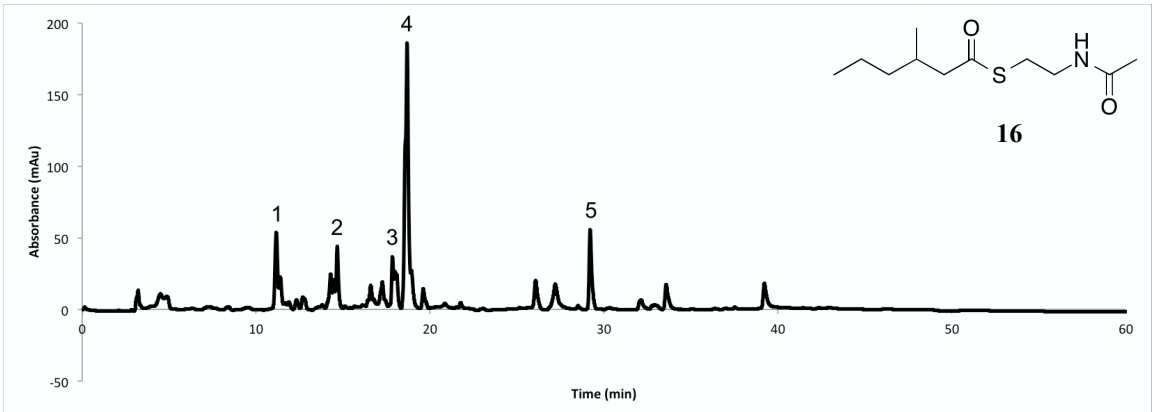
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	79	B	$C_5H_{10}Cl$	260, 288	343.0923	Cl-Hex+5Mal	-H_2O	+H_2O	$C_{16}H_{19}ClO_6$
2	80	B	$CH_2COC_5H_{10}Cl$	262, 288	385.1041	Cl-Hex+6Mal	-H_2O	+H_2O	$C_{18}H_{21}ClO_7$
3	81	C	$C_5H_{10}Cl$	220, 286	217.0627	Cl-Hex+2Mal	-	O-C	$C_{10}H_{13}ClO_3$
4				218, 234, 278, 300	367.0952	Cl-Hex+6Mal			$C_{18}H_{19}ClO_6$
5	82	F	$CH_2COC_5H_{10}Cl$	246, 270, 330, 468	405.0741	Cl-Hex+7Mal	-2H_2O	O-C, [O]	$C_{20}H_{17}ClO_7$
6	83	G	$CH_2COC_5H_{10}Cl$	270, 280, 390	391.0914	Cl-Hex+7Mal	-2H_2O	O-C	$C_{20}H_{19}ClO_6$

Figure S10. Product profile for starter unit **14** reaction (*S*-(2-acetamidoethyl) 6-chlorohexanethioate).



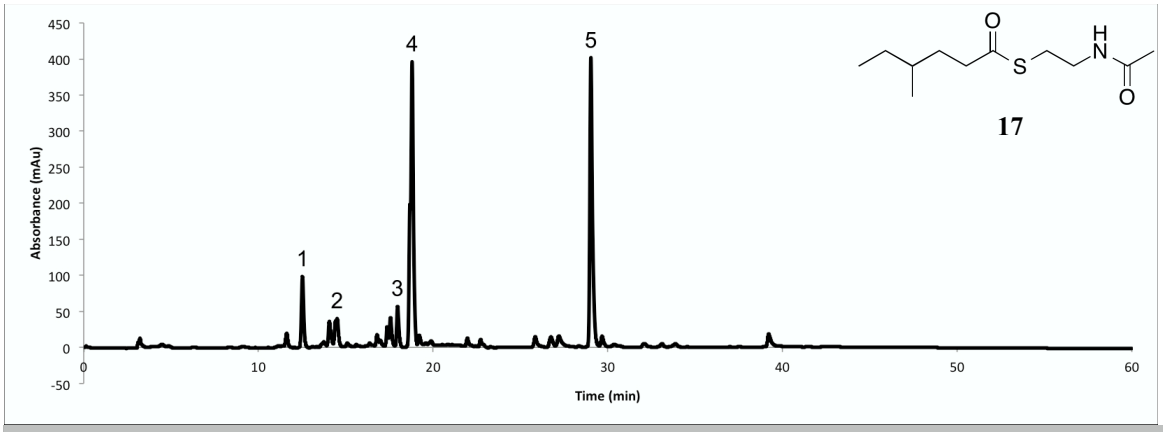
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	42	G	$CH_2COCH_2COCH_2COCH_3$	272, 280, 390	385.0911	10Mal	$-2H_2O$	O-C	$C_{20}H_{16}O_8$
2	84	G	$CH_2COCH(CH_3)C_4H_9$	270, 280, 390	371.1487	Me-Hex+7Mal	$-2H_2O$	O-C	$C_{21}H_{22}O_6$

Figure S11. Product profile for starter unit **15** reaction (*S*-(2-acetamidoethyl) 2-methylhexanethioate).



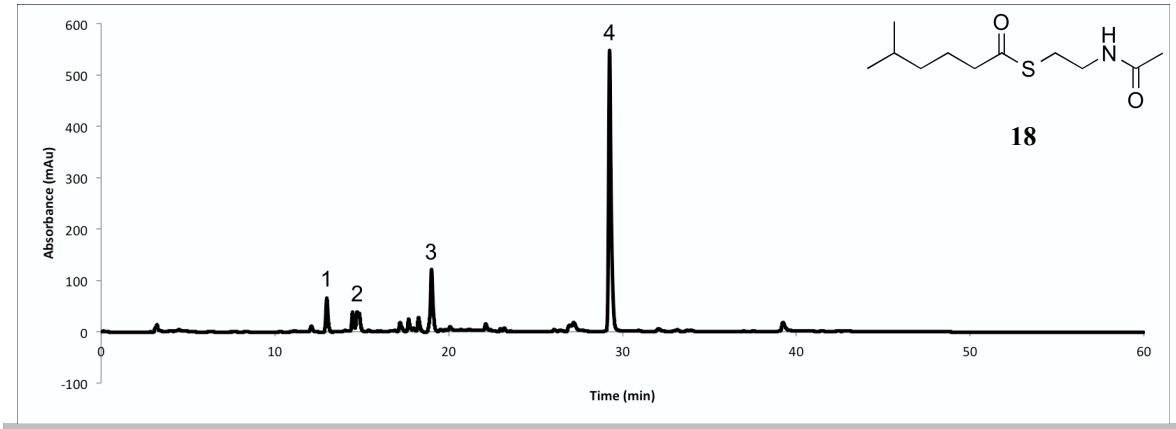
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	42	G	$CH_2COCH_2COCH_2COCH_3$	270, 280, 390	385.0905	10Mal	$-2H_2O$	O-C	$C_{20}H_{16}O_8$
2	85	B	$CH_2COCH_2CH(CH_3)C_3H_7$	266, 288	365.1596	Me-Hex+6Mal	$-H_2O$	$+H_2O$	$C_{19}H_{24}O_7$
3				240, 288, 336	197.1178				$C_{11}H_{16}O_3$
4	86a,b	D/E	$CH_2CH(CH_3)C_3H_7$	224, 278, 310	371.1493	Me-Hex+7Mal	$-2H_2O$	O-C	$C_{21}H_{22}O_6$
5	87	G	CH_2CO $CH_2CH(CH_3)C_3H_7$	270, 280, 390	371.1478	Me-Hex+7Mal	$-2H_2O$	O-C	$C_{21}H_{22}O_6$

Figure S12. Product profile for starter unit **16** reaction (*S*-(2-acetamidoethyl) 3-methylhexanethioate).



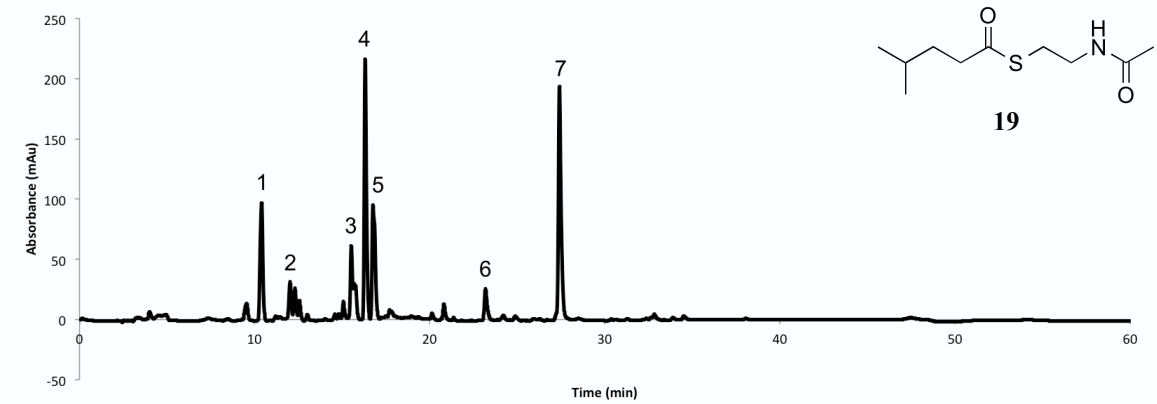
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	88	B	$C_2H_4CH(CH_3)C_2H_5$	260, 288	323.1493	Me-Hex+5Mal	$-H_2O$	$+H_2O$	$C_{17}H_{22}O_6$
2	89	B	$CH_2CO C_2H_4CH(CH_3)C_2H_5$	262, 288	365.1594	Me-Hex+6Mal	$-H_2O$	$+H_2O$	$C_{19}H_{24}O_7$
3	90	C	$CH_2COC_2H_4CH(CH_3)C_2H_5$	220, 284	239.1281	Me-Hex+3Mal	-	O-C	$C_{13}H_{18}O_4$
4	91a,b	D/E	$C_2H_4CH(CH_3)C_2H_5$	224, 278, 310	371.1492	Me-Hex+7Mal	$-2H_2O$	O-C	$C_{21}H_{22}O_6$
5	92	G	$CH_2COC_2H_4CH(CH_3)C_2H_5$	270, 280, 390	371.1486	Me-Hex+7Mal	$-2H_2O$	O-C	$C_{21}H_{22}O_6$

Figure S13. Product profile for starter unit **17** reaction (*S*-(2-acetamidoethyl) 4-methylhexanethioate).



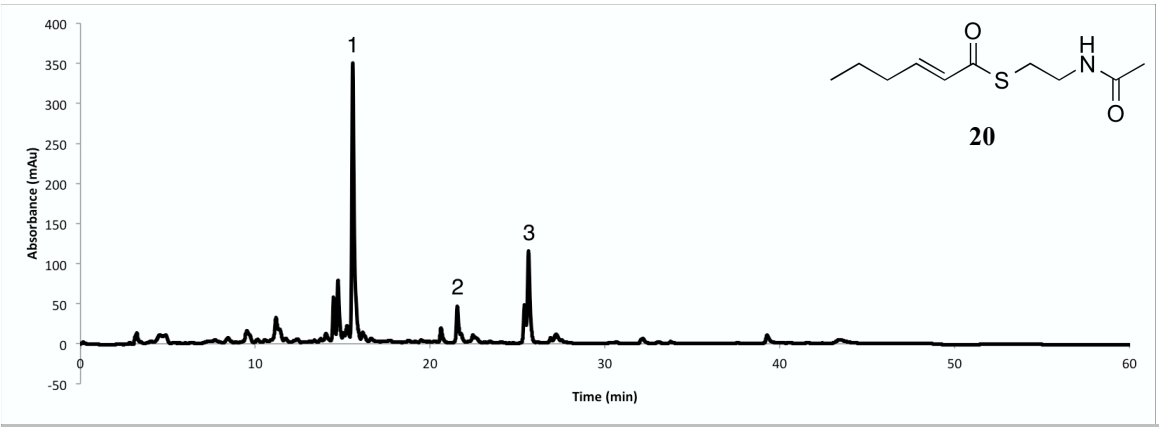
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	93	B	$C_3H_6C(CH_3)_2$	260, 288	323.1488	Me-Hex+5Mal	$-H_2O$	$+H_2O$	$C_{17}H_{22}O_6$
2	94	B	$CH_2COC_3H_6C(CH_3)_2$	266, 288	365.1590	Me-Hex+6Mal	$-H_2O$	$+H_2O$	$C_{19}H_{24}O_7$
4	95	C	$C_3H_6C(CH_3)_2$	224, 284	197.1179	Me-Hex+2Mal	-	O-C	$C_{11}H_{16}O_3$
5	96	G	$CH_2COC_3H_6C(CH_3)_2$	270, 280, 390	371.1487	Me-Hex+7Mal	$-2H_2O$	O-C	$C_{21}H_{22}O_6$

Figure S14. Product profile for starter unit **18** reaction (*S*-(2-acetamidoethyl) 5-methylhexanethioate).



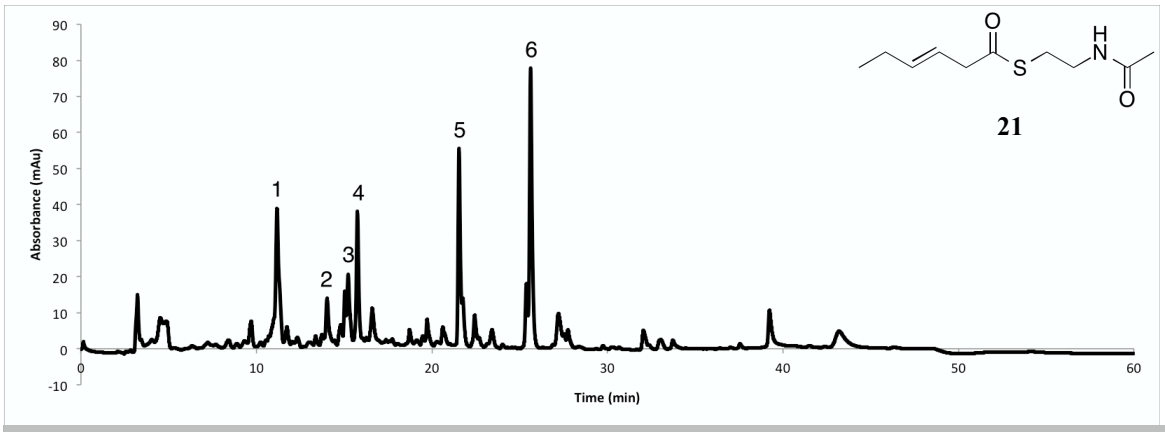
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	97	B	$C_2H_4C(CH_3)_2$	260, 288	309.1338	Me-Pent+5Mal	$-H_2O$	$+H_2O$	$C_{16}H_{20}O_6$
2	98	B	$CH_2COC_2H_4C(CH_3)_2$	262, 288	351.1435	Me-Pent+6Mal	$-H_2O$	$+H_2O$	$C_{18}H_{22}O_7$
3	99	C	$CH_2COC_2H_4C(CH_3)_2$	220, 284	225.1127	Me-Pent+3Mal	-	O-C	$C_{12}H_{16}O_4$
4	100	C	$C_2H_4C(CH_3)_2$	220, 286	183.1024	Me-Pent+2Mal	-	O-C	$C_{10}H_{14}O_3$
5	101a,b	D/E	$C_2H_4C(CH_3)_2$	232, 278, 304	357.1339	Me-Pent+7Mal	$-2H_2O$	O-C	$C_{20}H_{20}O_6$
6	102	F	$CH_2COC_2H_4C(CH_3)_2$	246, 270, 330, 468	371.1121	Me-Pent+7Mal	$-2H_2O$	O-C, [O]	$C_{20}H_{18}O_7$
7	103	G	$CH_2COC_2H_4C(CH_3)_2$	270, 280, 390	357.1349	Me-Pent+7Mal	$-2H_2O$	O-C	$C_{20}H_{20}O_6$

Figure S15. Product profile for starter unit **19** reaction (*S*-(2-acetamidoethyl) 4-methylpentanethioate).



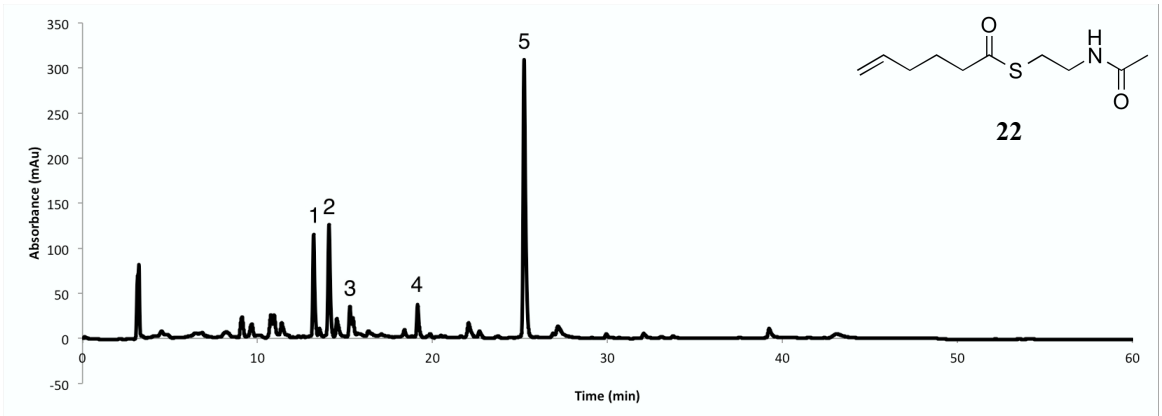
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	104a,b	D/E	$CHCHC_3H_7$	226, 262	355.1176	Hex+7Mal	$-2H_2O$	O-C	$C_{20}H_{18}O_6$
2	105	H	$CHCHC_3H_7$	222, 264, 304, 374	355.1179	Hex+7Mal	$-2H_2O$	$+H_2O$, C-C	$C_{20}H_{18}O_6$
3	106	G	$CH_2COCHCHC_3H_7$	270, 280, 390	355.1184	Hex+7Mal	$-2H_2O$	O-C	$C_{20}H_{18}O_6$

Figure S16. Product profile for starter unit **20** reaction (*S*-(2-acetamidoethyl) (*E*)-hex-2-enethioate).



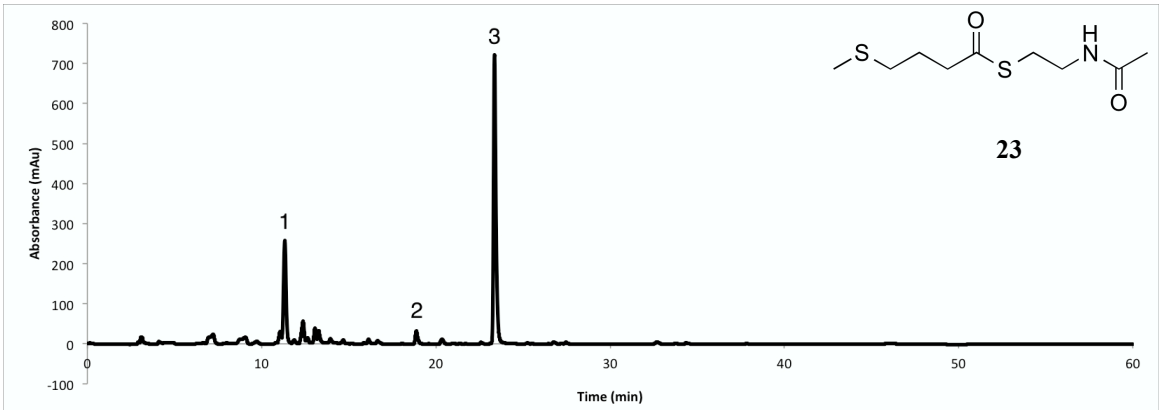
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1				218, 268, 382	349.1281	Hex+6Mal			$C_{18}H_{20}O_7$
2				228, 284	331.1187	Hex+6Mal			$C_{18}H_{18}O_6$
3	107	C	$CH_2CHCHC_2H_5$	220, 288	181.0874	Hex+2Mal	-	O-C	$C_{10}H_{12}O_3$
4	108a,b	D/E*	$CH_2CHCHC_2H_5$	224, 280	373.1280	Hex+7Mal	$-H_2O$	O-C	$C_{20}H_{20}O_7$
5	109	H	$CH_2CHCHC_2H_5$	222, 264, 304, 374	355.1182	Hex+7Mal	$-2H_2O$	C-C	$C_{20}H_{18}O_6$
6	110	G	$CH_2COCH_2CHCHC_2H_5$	270, 280, 390	355.1183	Hex+7Mal	$-2H_2O$	O-C	$C_{20}H_{18}O_6$

Figure S17. Product profile for starter unit **21** reaction (*S*-(2-acetamidoethyl) (*E*)-hex-3-enethioate). *108a,b contain hydrated versions of cores D, E



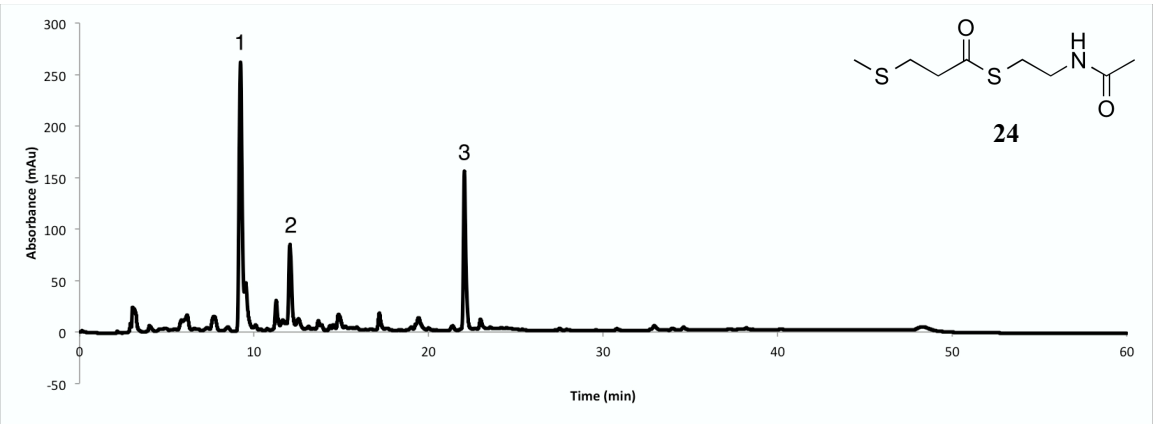
Peak	Compound	Core Structure	R	UV max	[M+H]$^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1				272, 280, 390	490.1537				?
2	111	C	$C_3H_6CHCH_2$	220, 286	181.0865	Hex+2Mal	-	O-C	$C_{10}H_{12}O_3$
3	112a,b	D/E	$C_3H_6CHCH_2$	224, 278, 310	355.1177	Hex+7Mal	$-2H_2O$	O-C	$C_{20}H_{18}O_6$
4				270, 280, 390	331.1183	Hex+6Mal			$C_{18}H_{18}O_6$
5	113	G	$CH_2COC_3H_6CHCH_2$	270, 280, 390	355.1182	Hex+7Mal	$-2H_2O$	C-O	$C_{20}H_{18}O_6$

Figure S18. Product profile for starter unit **22** reaction (*S*-(2-acetamidoethyl) hex-5-enethioate).



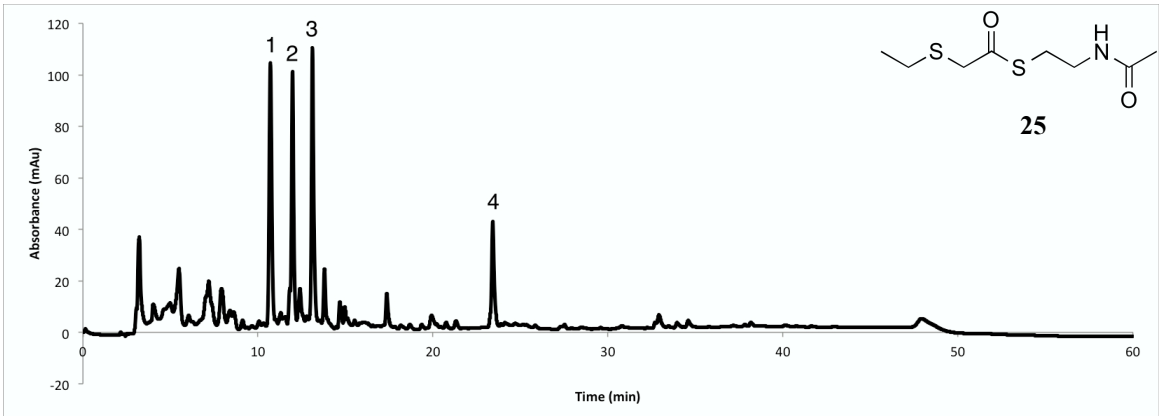
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	114	C	$C_3H_6SCH_3$	220, 286	201.0591	'Hex'+2Mal	-	O-C	$C_9H_{12}O_3S$
2	115	F	$CH_2COC_3H_6SCH_3$	246, 270, 330, 468	389.0688	'Hex'+7Mal	$-2H_2O$	O-C, [O]	$C_{19}H_{16}O_7S$
3	116	G	$CH_2COC_3H_6SCH_3$	270, 280, 390	375.0899	'Hex'+7Mal	$-2H_2O$	O-C	$C_{19}H_{18}O_6S$

Figure S19. Product profile for starter unit **23** reaction (*S*-(2-acetamidoethyl) 4-(methylthio)butanethioate).



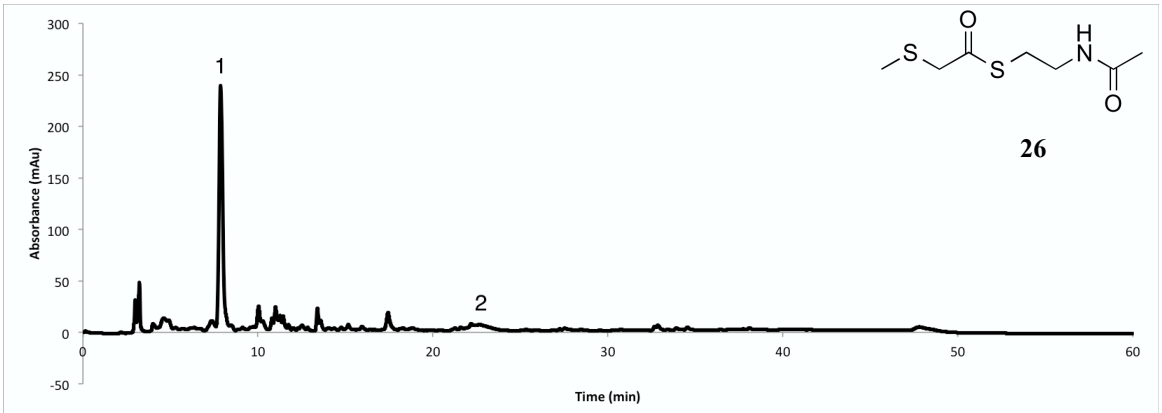
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	117	C	$C_2H_4SCH_3$	220, 286	187.0428	'Pent'+2Mal	-	O-C	$C_8H_{10}O_3S$
2	118a,b	D/E	$C_2H_4SCH_3$	224, 280, 388	361.0740	'Pent'+7Mal	$-2H_2O$	O-C	$C_{18}H_{16}O_6S$
3	119	G	$CH_2COC_2H_4SCH_3$	270, 280, 390	361.0744	'Pent'+7Mal	$-2H_2O$	O-C	$C_{18}H_{16}O_6S$

Figure S20. Product profile for starter unit **24** reaction (*S*-(2-acetamidoethyl) 3-(methylthio)propanethioate).



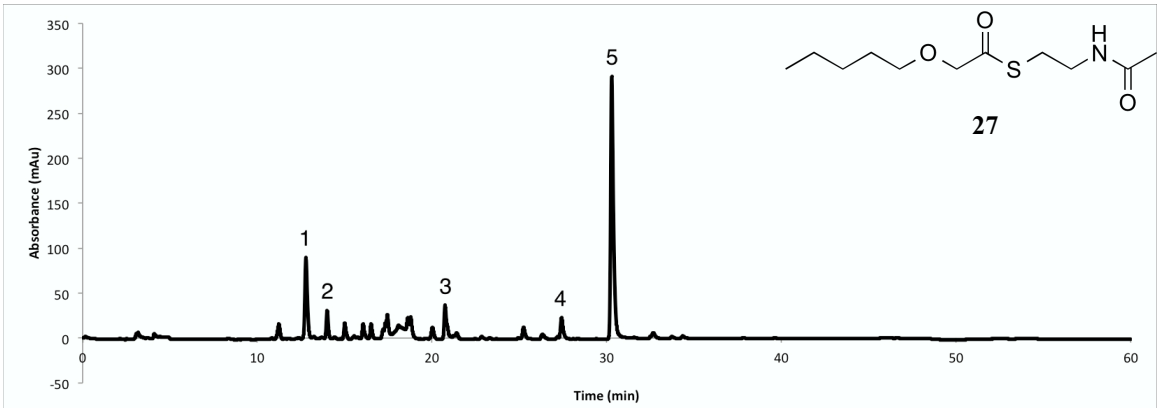
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	120	C	$CH_2SC_2H_5$	222, 288	187.0433	'Pent'+2Mal	-	O-C	$C_8H_{10}O_3S$
2	121a,b	D/E*	$CH_2SC_2H_5$	224, 282	379.0844	'Pent'+7Mal	$-H_2O$	O-C	$C_{18}H_{18}O_7S$
3	122a,b	D/E	$CH_2SC_2H_5$	224, 278	361.0744	'Pent'+7Mal	$-2H_2O$	O-C	$C_{18}H_{16}O_6S$
4	123	G	$CH_2COCH_2SC_2H_5$	270, 280, 390	361.0744	'Pent'+7Mal	$-2H_2O$	O-C	$C_{18}H_{16}O_6S$

Figure S21. Product profile for starter unit **25** reaction (*S*-(2-acetamidoethyl) 2-(ethylthio)ethanethioate).
*129a,b are hydrated versions of 130a,b



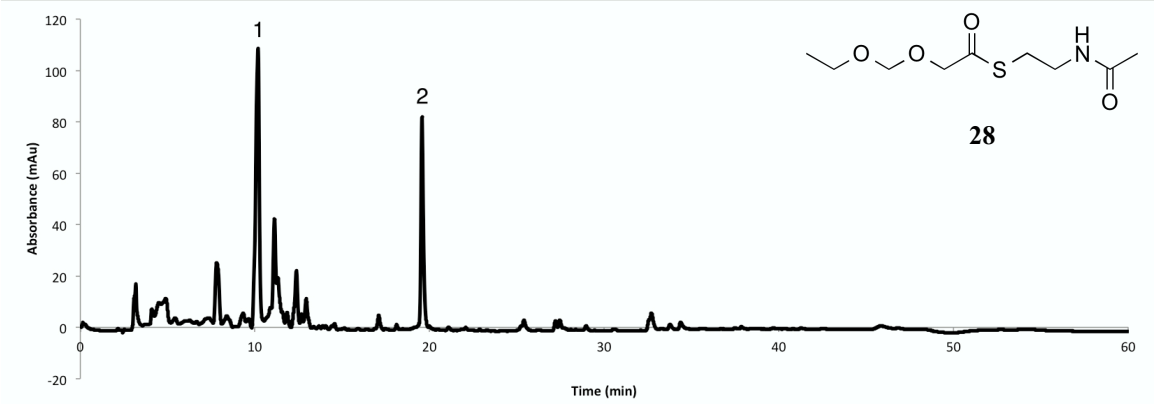
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	124	C	CH_2SCH_3	236, 288	173.0273	'But'+2Mal	-	O-C	$C_7H_8O_3S$
2	125	G	$CH_2COCH_2COCH_2SCH_3$	272, 280, 390	389.0678	'But'+8Mal	$-2H_2O$	O-C	$C_{19}H_{16}O_7S$

Figure S22. Product profile for starter unit **26** reaction (*S*-(2-acetamidoethyl) 2-(methylthio)ethanethioate).



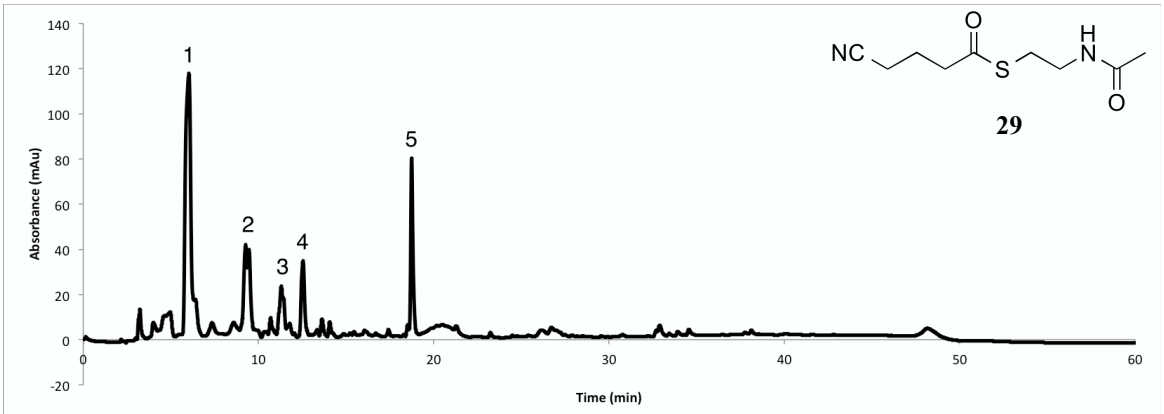
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	126	B	$CH_2OC_5H_{11}$	262, 288	339.1443	'Oct'+5Mal	$-H_2O$	$+H_2O$	$C_{17}H_{22}O_7$
2	127	B	$CH_2COCH_2OC_5H_{11}$	262, 288	381.1544	'Oct'+6Mal	$-H_2O$	$+H_2O$	$C_{19}H_{24}O_8$
3				234, 278, 300	363.1441	'Oct'+6Mal			$C_{19}H_{22}O_7$
4	128	G	$CH_2COCH_2OC_5H_{11}$	270, 280, 390	387.1476	'Oct'+7Mal	$-2H_2O$	O-C	$C_{21}H_{22}O_7$
5	129	G	$CH_2OC_5H_{11}$	270, 280, 390	345.1339	'Oct'+6Mal	$-2H_2O$	O-C	$C_{19}H_{20}O_6$

Figure S23. Product profile for starter unit **27** reaction (*S*-(2-acetamidoethyl) 2-(pentyloxy)ethanethioate).



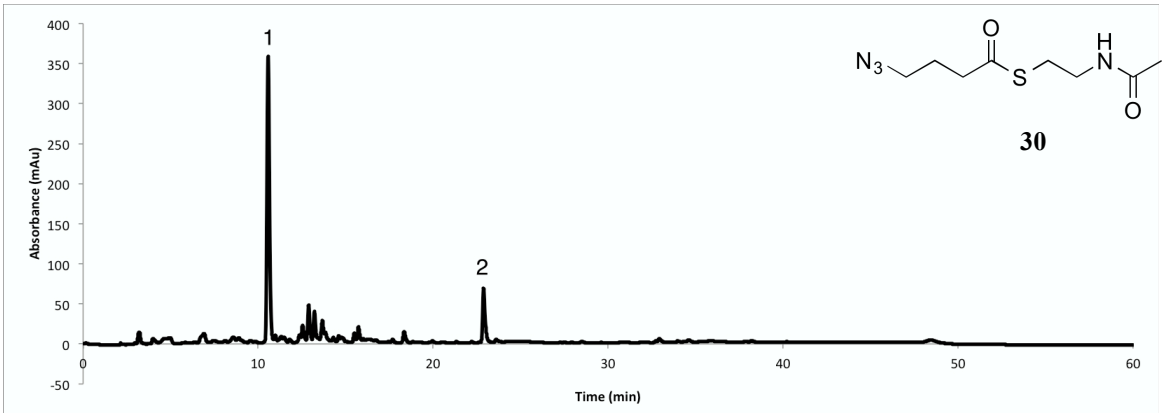
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	130a,b	D/E	$CH_2OCH_2OC_2H_5$	232, 280	375.1074	'Hept'+7Mal	$-2H_2O$	O-C	$C_{19}H_{18}O_8$
2	131	G	CH_2CO $CH_2OCH_2OC_2H_5$	270, 280, 390	375.1076	'Hept'+7Mal	$-2H_2O$	O-C	$C_{19}H_{18}O_8$

Figure S24. Product profile for starter unit **28** reaction (*S*-(2-acetamidoethyl) 2-(ethoxymethoxy)ethanethioate).



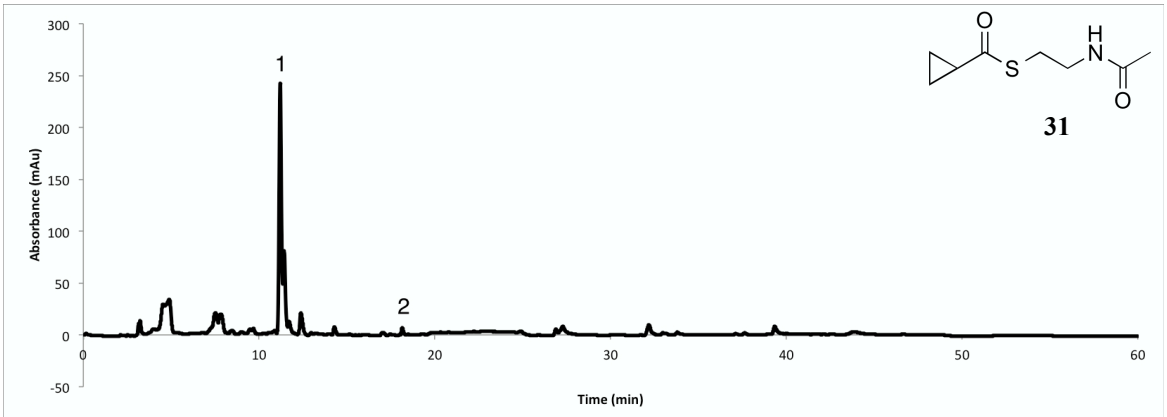
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	132	C	C_3H_6CN	220, 284	180.0661	Cyanobut+2Mal	-	O-C	$C_9H_9NO_3$
	133	C	$CH_2COC_3H_6CN$	220, 284	222.0766	Cyanobut+3Mal	-	O-C	$C_{11}H_{11}NO_4$
2	134a,b	D/E*	C_3H_6CN	224, 280	372.1071	Cyanobut+7Mal	$-H_2O$	O-C	$C_{19}H_{17}NO_7$
3	135	F	$CH_2COC_3H_6CN$	226, 270, 390	368.0763	Cyanobut+7Mal	$-2H_2O$	O-C, [O]	$C_{19}H_{13}NO_7$
4				234, 266, 350	396.1095	Cyanobut+8Mal			$C_{21}H_{17}NO_7$
5	136	G	$CH_2COC_3H_6CN$	270, 280, 390	354.0964	Cyanobut+7Mal	$-2H_2O$	O-C	$C_{19}H_{15}NO_6$

Figure S25. Product profile for starter unit **29** reaction (*S*-(2-acetamidoethyl) 4-cyanobutanethioate).
*142a,b contain hydrated versions of cores D, E



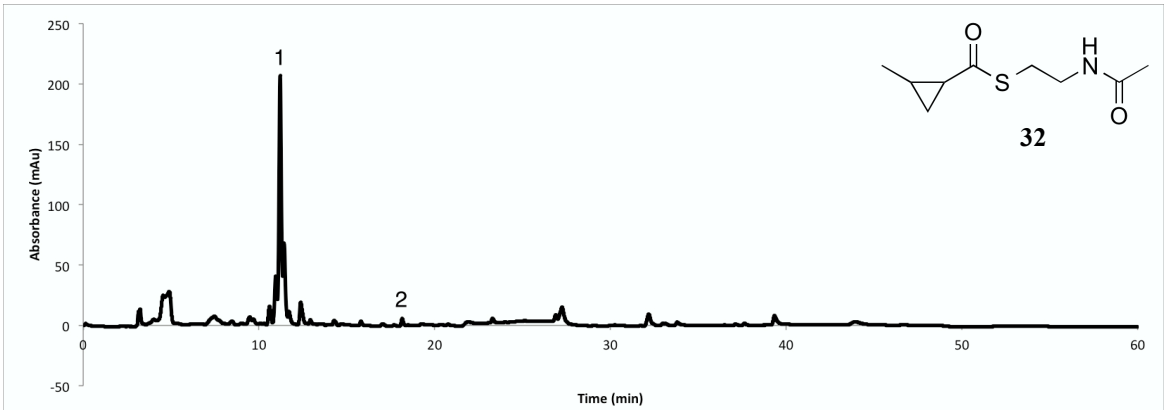
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	137	C	$C_3H_6N_3$	220, 284	196.0718	Azidobut+2Mal	-	O-C	$C_8H_9N_3O_3$
	138	C	$CH_2COC_3H_6N_3$	220, 284	238.0417	Azidobut+3Mal	-	O-C	$C_{10}H_{11}N_3O_4$
2	139	G	$CH_2COC_3H_6N_3$	270, 280, 390	370.1022	Azidobut+7Mal	$-2H_2O$	O-C	$C_{18}H_{15}N_3O_6$

Figure S26. Product profile for starter unit **30** reaction (*S*-(2-acetamidoethyl) 4-azidobutanethioate).



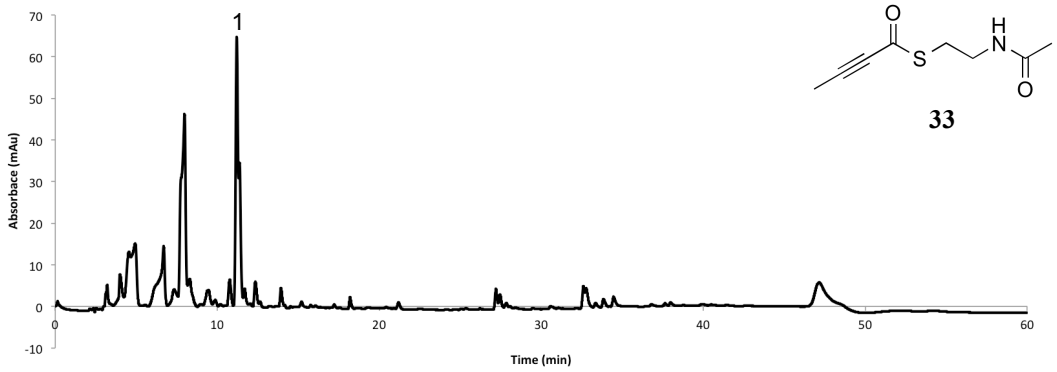
Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	42	G	$CH_2COCH_2COCH_2COCH_3$	270, 280, 390	385.0923	10Mal	$-2H_2O$	O-C	$C_{20}H_{16}O_8$
2	140	G	$CH_2COCH_2COC_3H_5$	280, 390	369.0964	+8Mal	$-2H_2O$	O-C	$C_{20}H_{16}O_7$

Figure S27. Product profile for starter unit **31** reaction (*S*-(2-acetamidoethyl) cyclopropanecarbothioate).



Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	42	G	$CH_2COCH_2COCH_2COCH_3$	270, 280, 390	385.0921	10Mal	$-2H_2O$	O-C	$C_{20}H_{16}O_8$
2	141	G	$CH_2COCH_2COC_3H_4CH_3$	280, 390	383.1123	+8Mal	$-2H_2O$	O-C	$C_{21}H_{18}O_7$

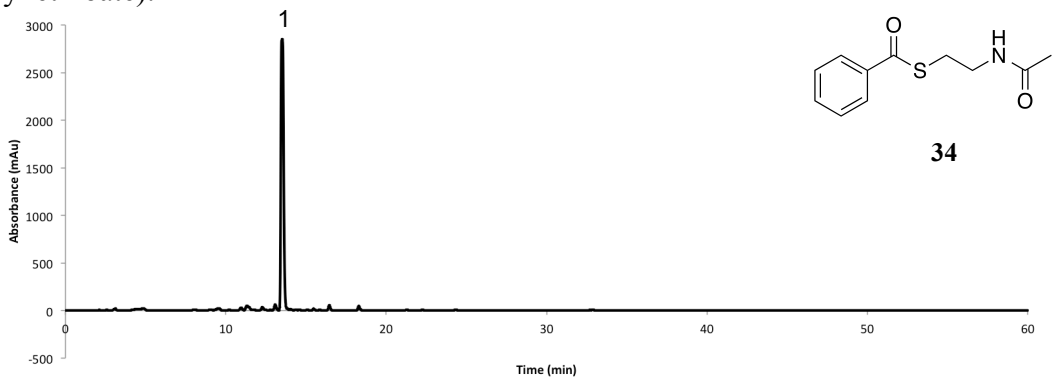
Figure S28. Product profile for starter unit **32** reaction (*S*-(2-acetamidoethyl) 2-methylcyclopropane-1-carbothioate).



33

Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	42	G	$CH_2COCH_2COCH_2COCH_3$	270, 280, 390	385.0914	10Mal	$-2H_2O$	O-C	$C_{20}H_{16}O_8$

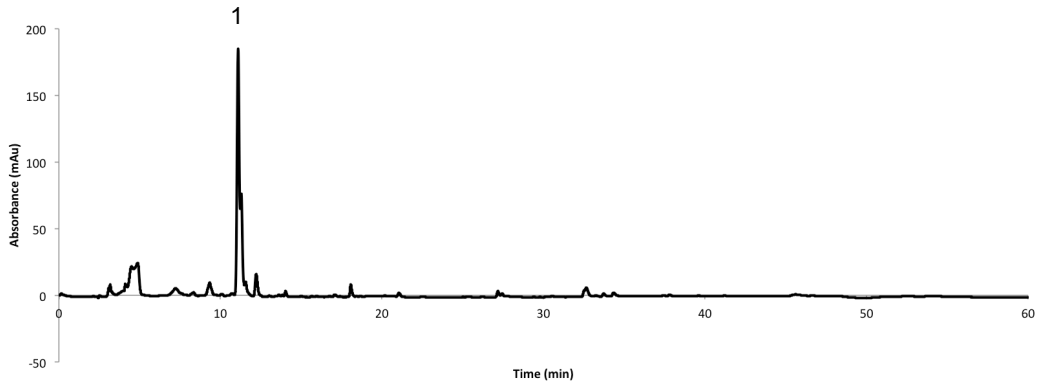
Figure S29. Product profile for starter unit **33** reaction (*S*-(2-acetamidoethyl) but-2-ynethioate).



34

Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	34	N/A		245, 274	224.0749				$C_{11}H_{13}NO_2S$

Figure S30. Product profile for starter unit **34** reaction (*S*-(2-acetamidoethyl) benzothioate).



Peak	Compound	Core Structure	R	UV max	$[M+H]^+$	Chain Length	Dehydration	Release, Other	Mol. Formula
1	42	G	$CH_2COCH_2COCH_2COCH_3$	270, 280, 390	385.0912	10Mal	$-2H_2O$	O-C	$C_{20}H_{16}O_8$

Figure S31. Product profile for control reaction containing only malonyl-SNAC.

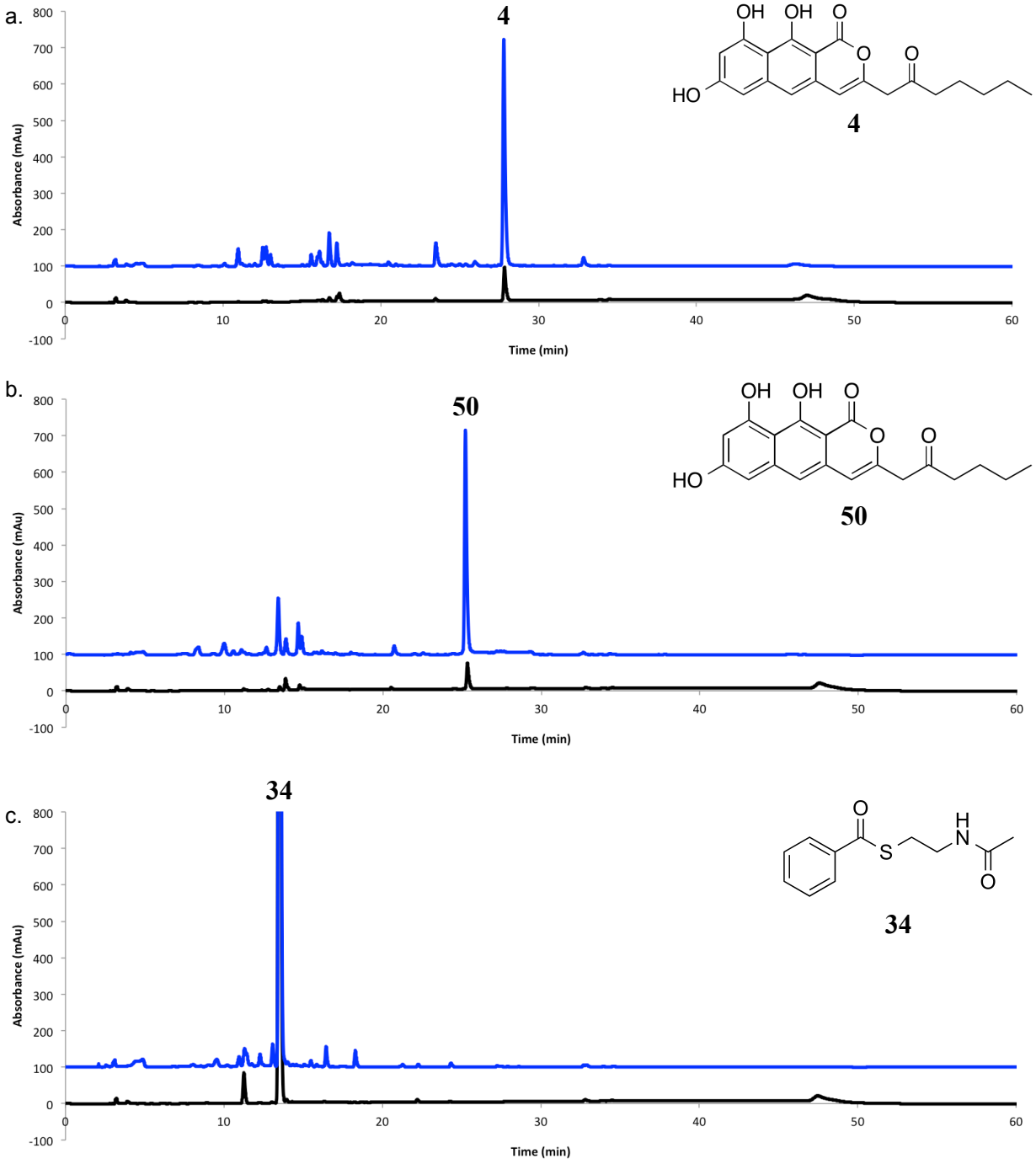


Figure S32. Comparison of intact pentadomain (black) and dissected two-part (blue) reactions. (A) Reaction of starter unit **5** (*S*-(2-acetamidoethyl) hexanethioate) (B) Reaction of starter unit **7** (*S*-(2-acetamidoethyl) pentanethioate). (C) Reaction of starter unit **34** (*S*-(2-acetamidoethyl) benzothioate).

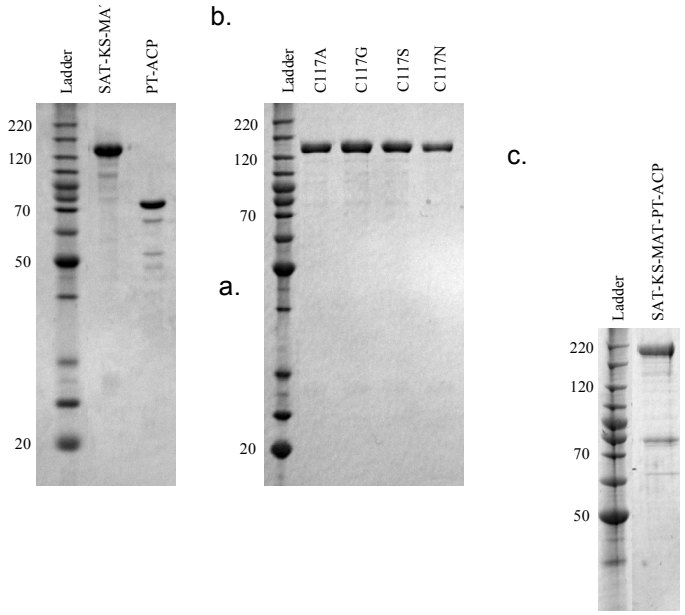


Figure S33. Purified proteins used in this study separated by SDS-PAGE and stained with Coomassie Blue. BenchMark Protein Ladder (Invitrogen) was used as a molecular weight standard (indicated in kDa). (A) 12% SDS-PAGE of wild-type PksA SAT-KS-MAT and PT-ACP. (B) 12% SDS-PAGE of PksA SAT-KS-MAT mutants. (C) 12% SDS-PAGE of PksA-SAT-KS-MAT-PT-ACP

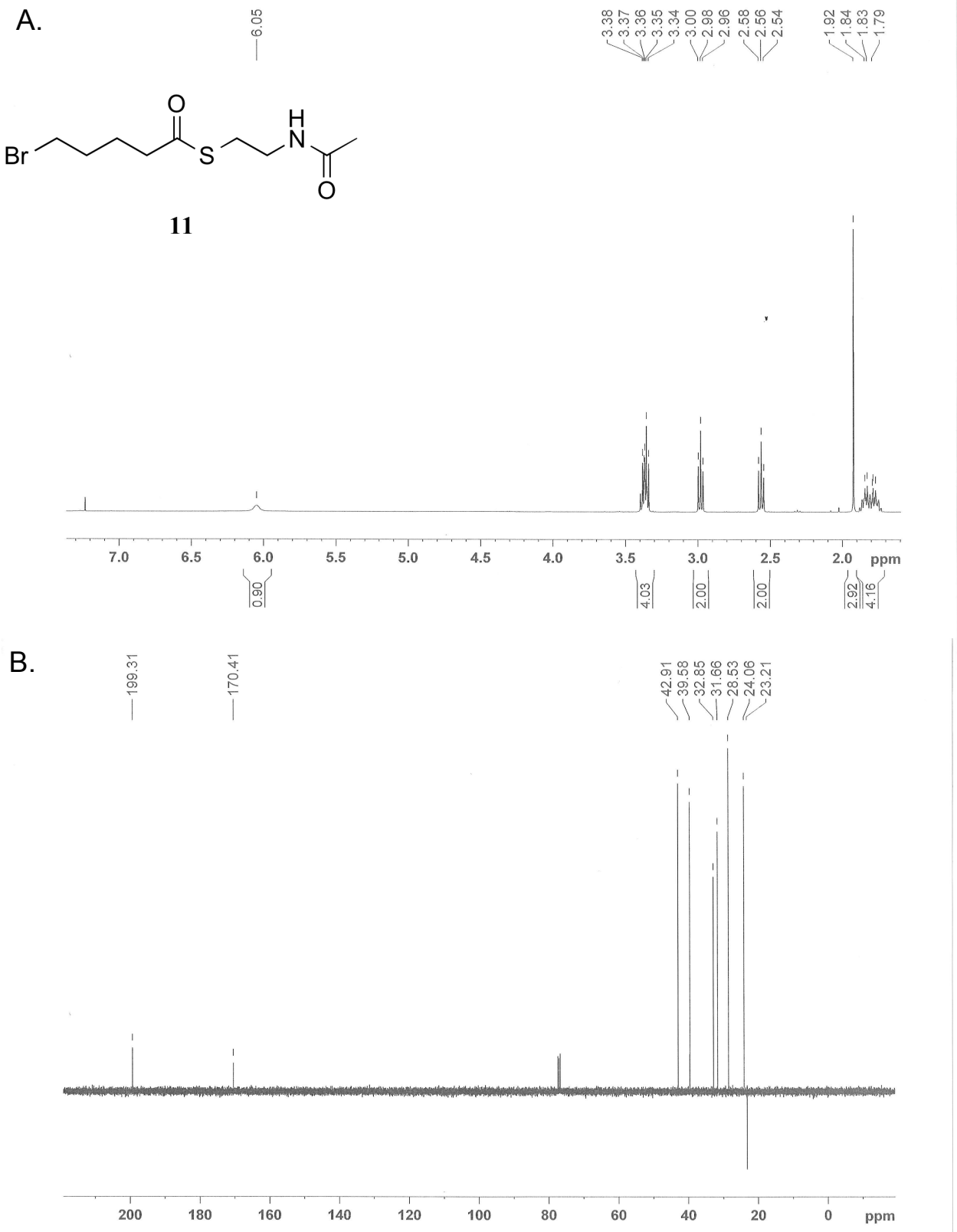


Figure S34. NMR spectra of *S*-(2-acetamidoethyl) 5-bromopentanethioate (**11**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

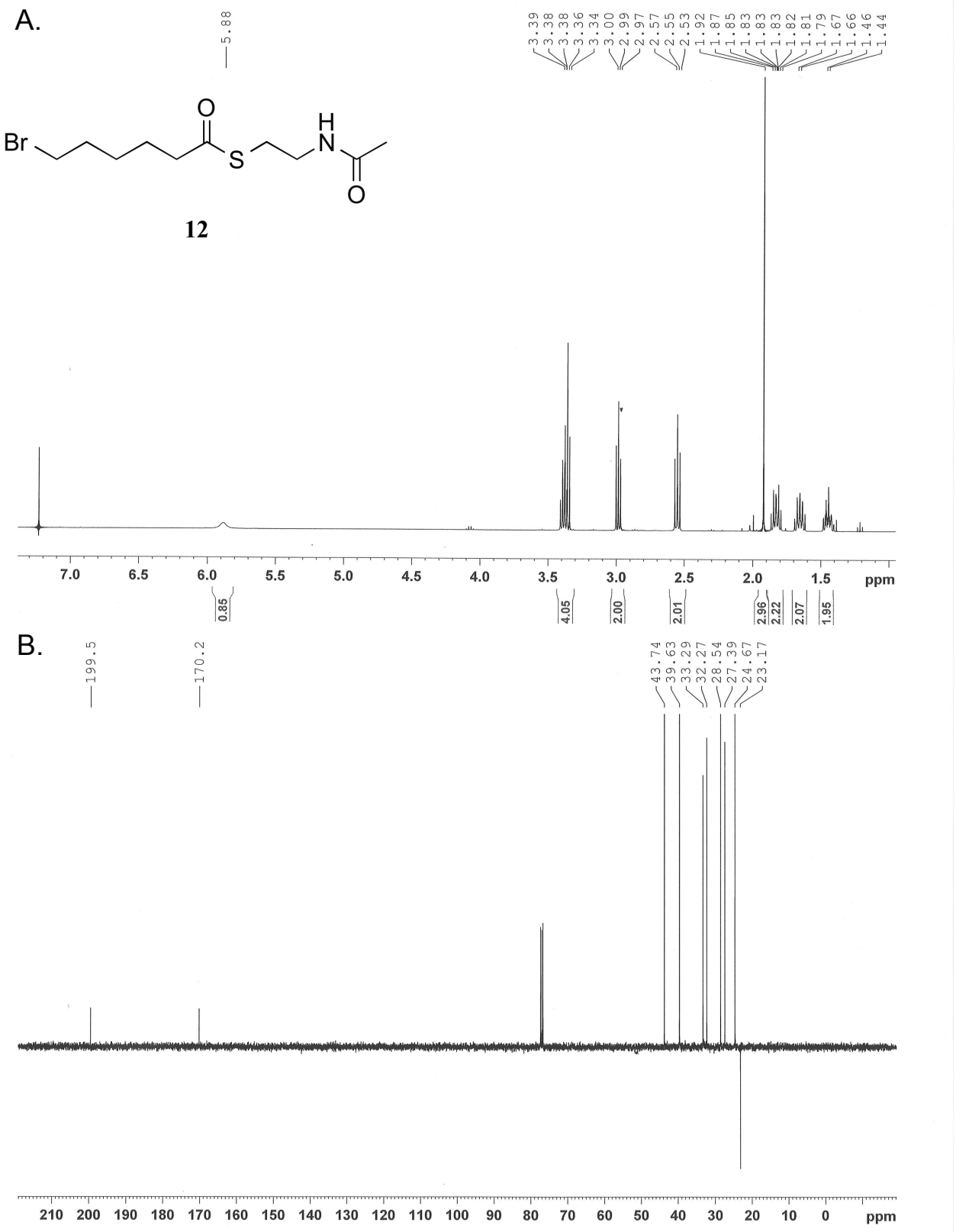


Figure S35. NMR spectra of *S*-(2-acetamidoethyl) 6-bromohexanethioate (**12**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

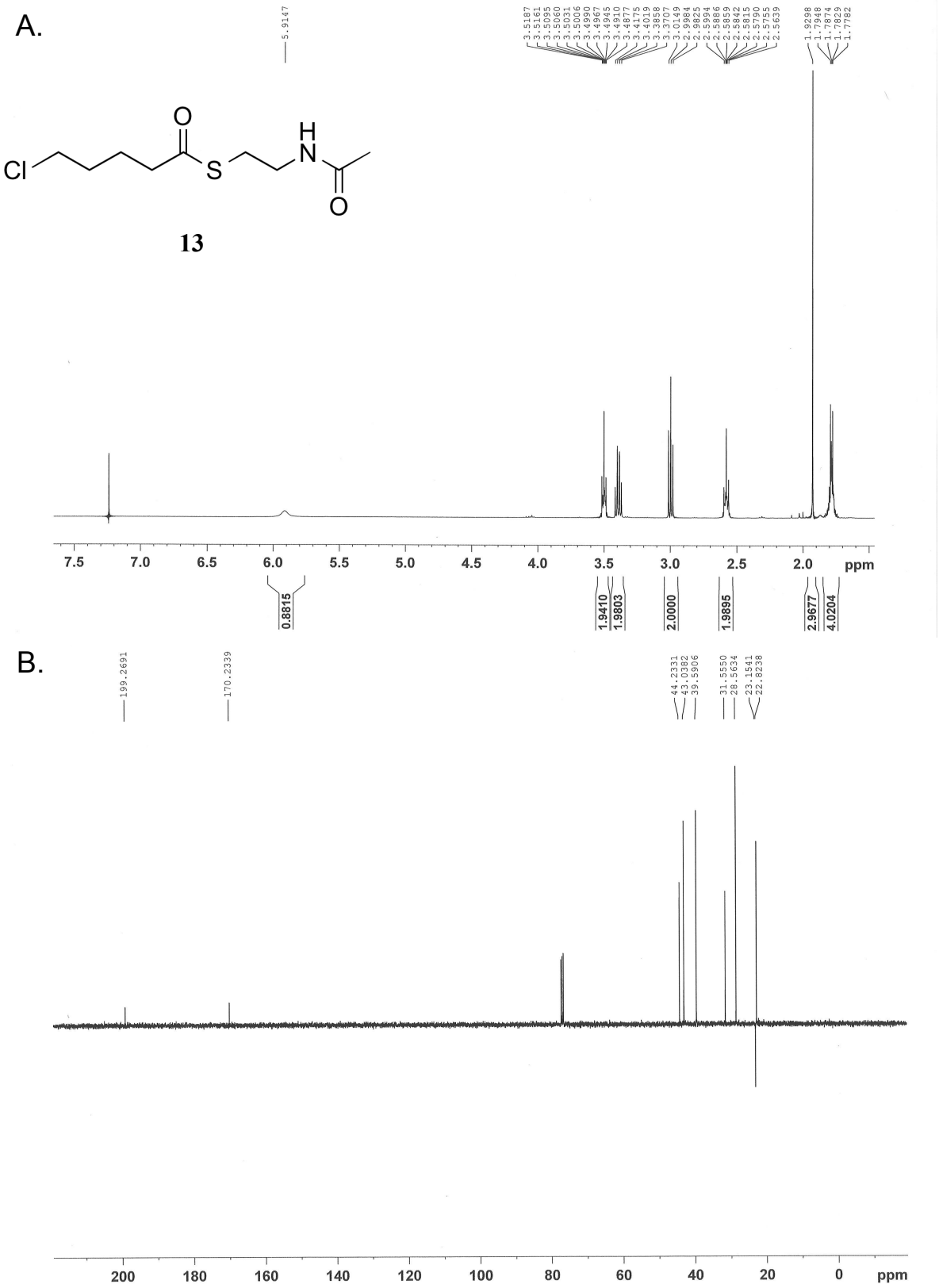


Figure S36. NMR spectra of *S*-(2-acetamidoethyl) 5-chloropentanethioate (**13**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

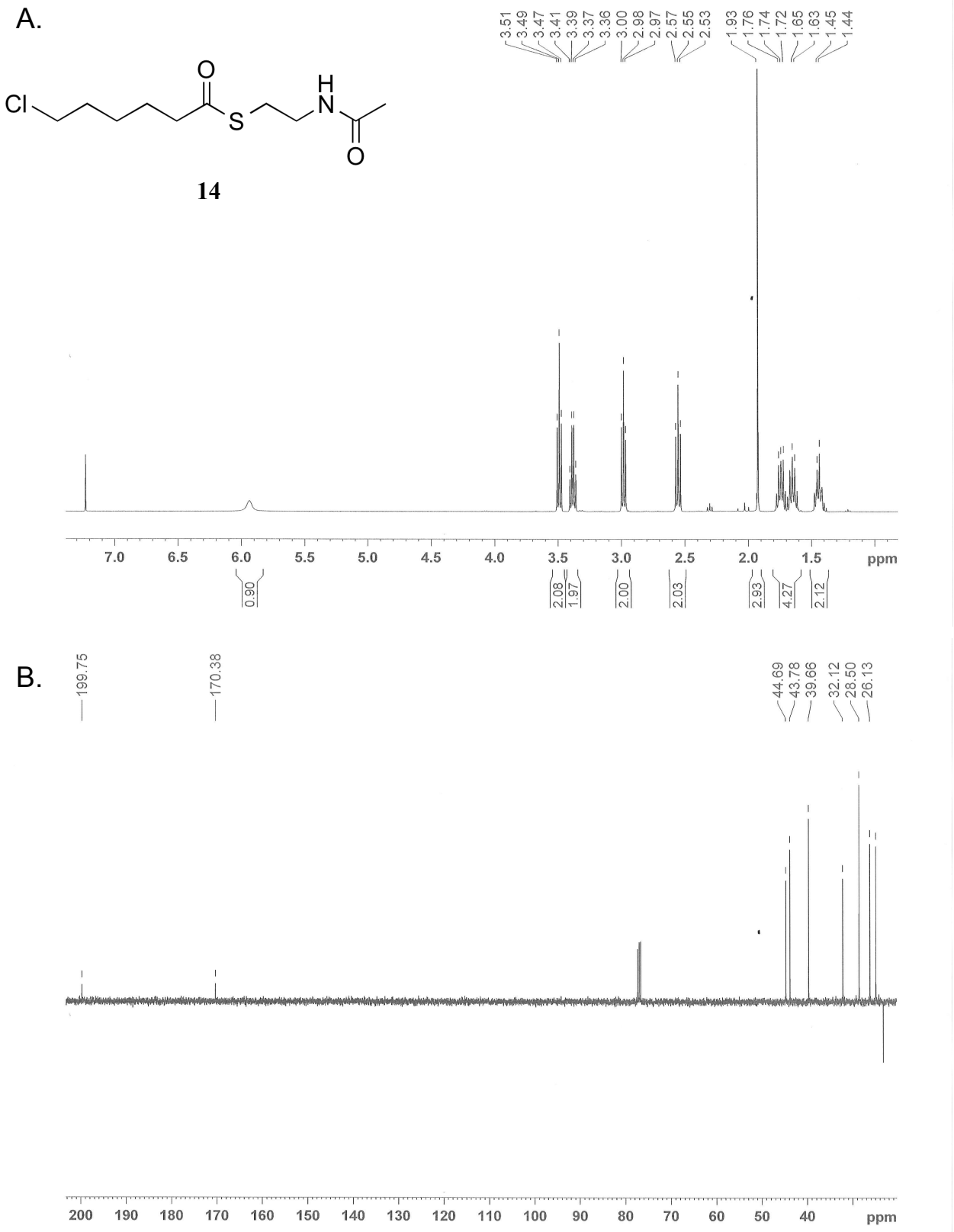


Figure S37. NMR spectra of *S*-(2-acetamidoethyl) 6-chlorohexanethioate (**14**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

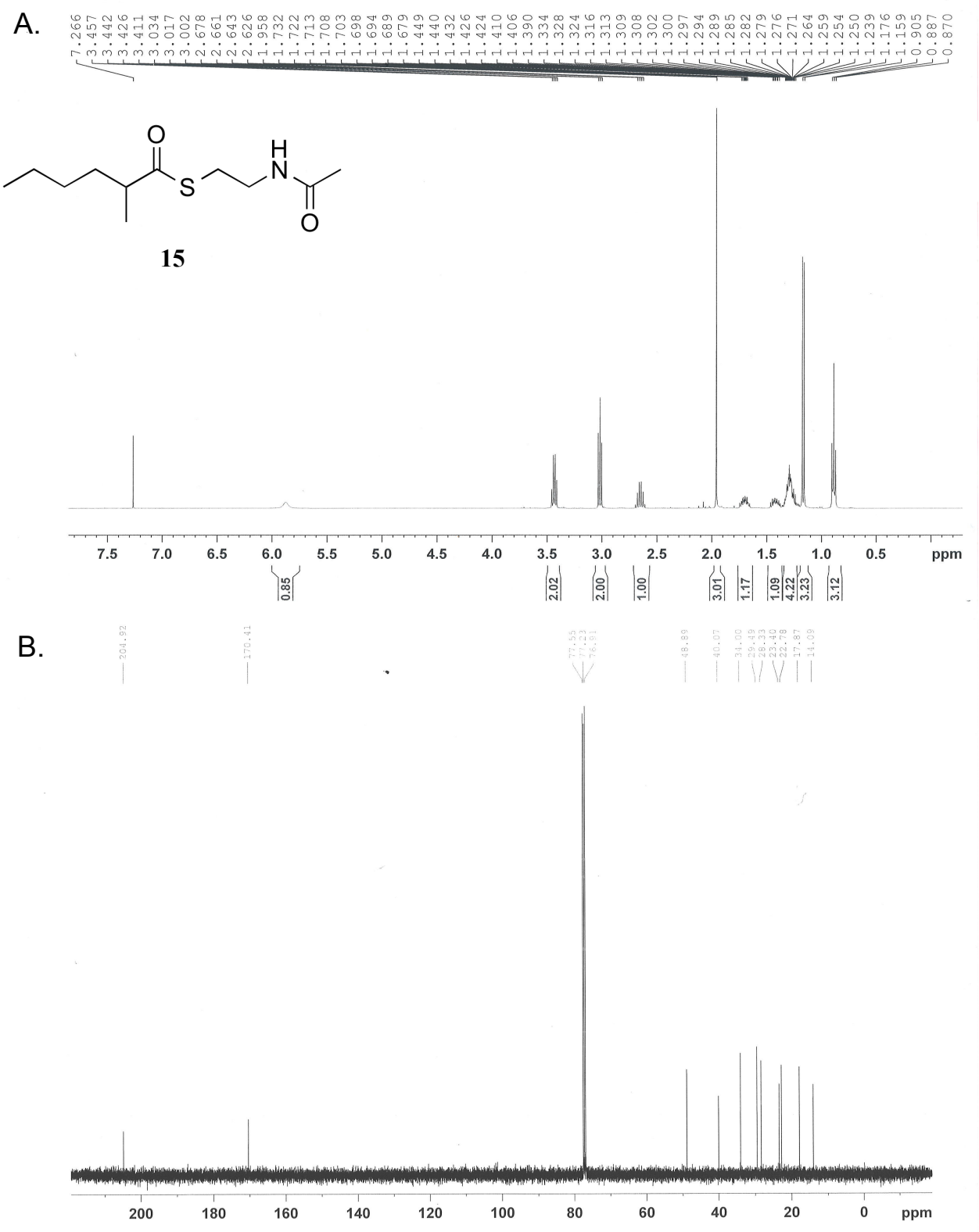


Figure S38. NMR spectra of *S*-(2-acetamidoethyl) 2-methylhexanethioate (**15**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

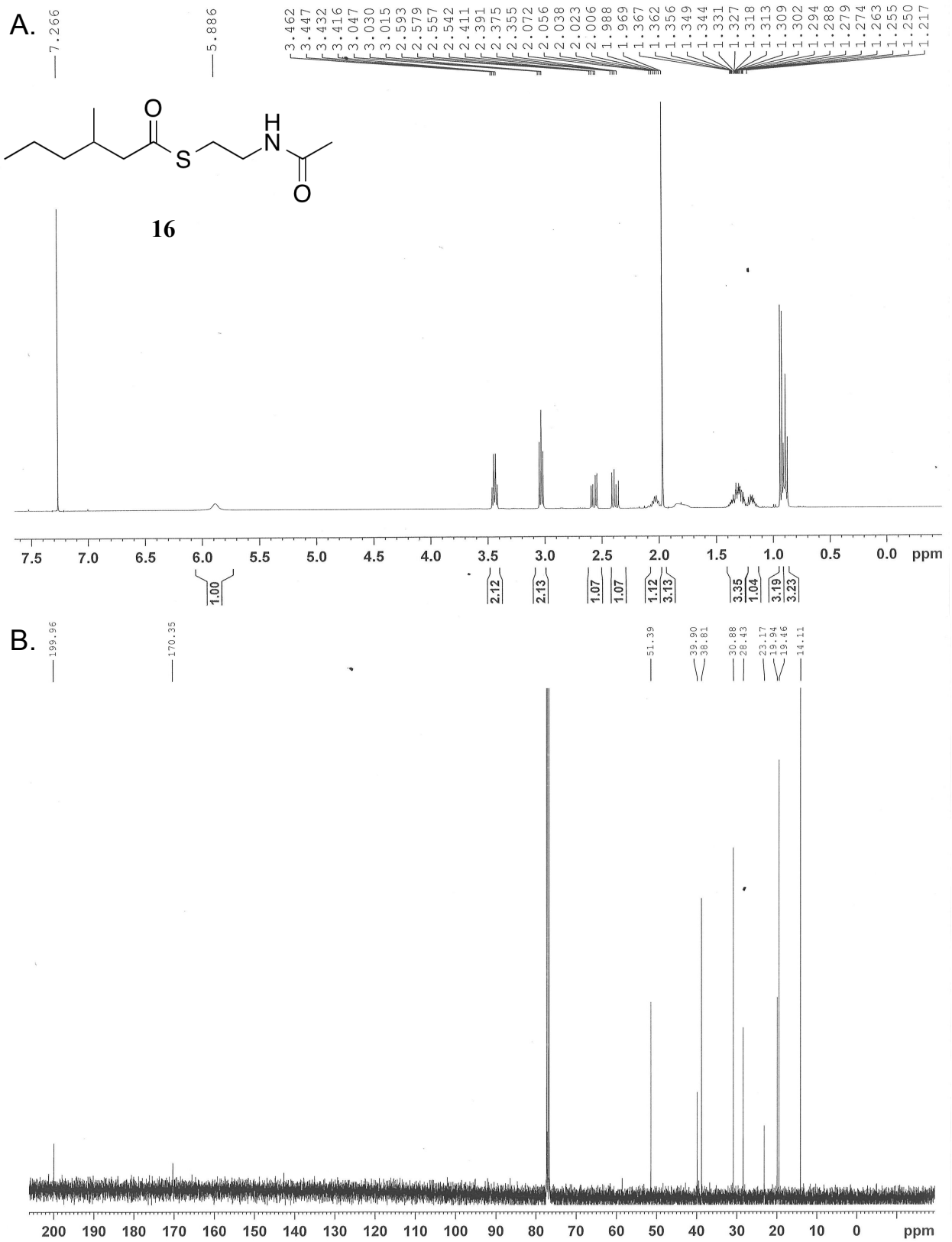


Figure S39. NMR spectra of *S*-(2-acetamidoethyl) 3-methylhexanethioate (**16**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

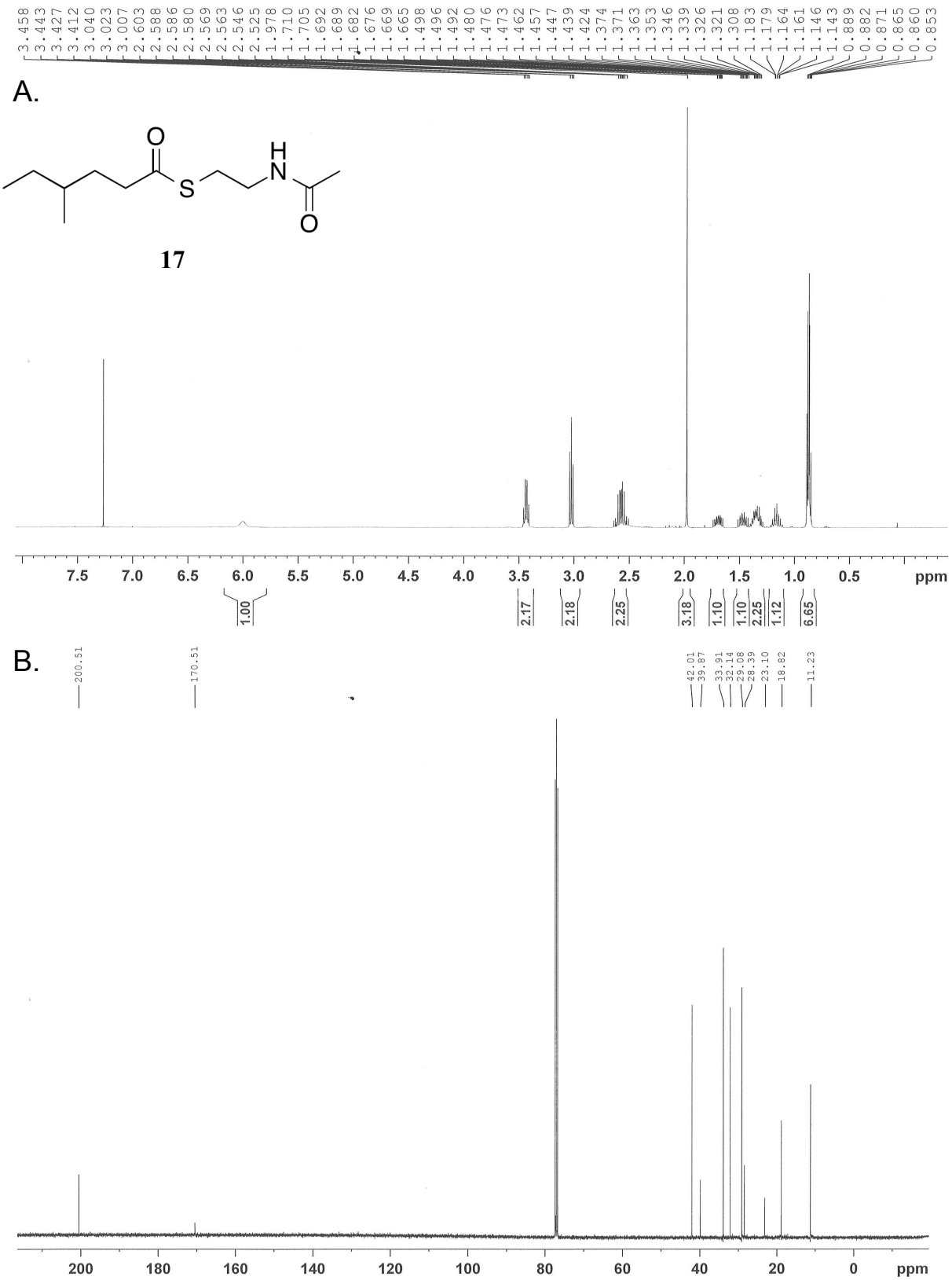


Figure S40. NMR spectra of *S*-(2-acetamidoethyl) 4-methylhexanethioate (**17**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

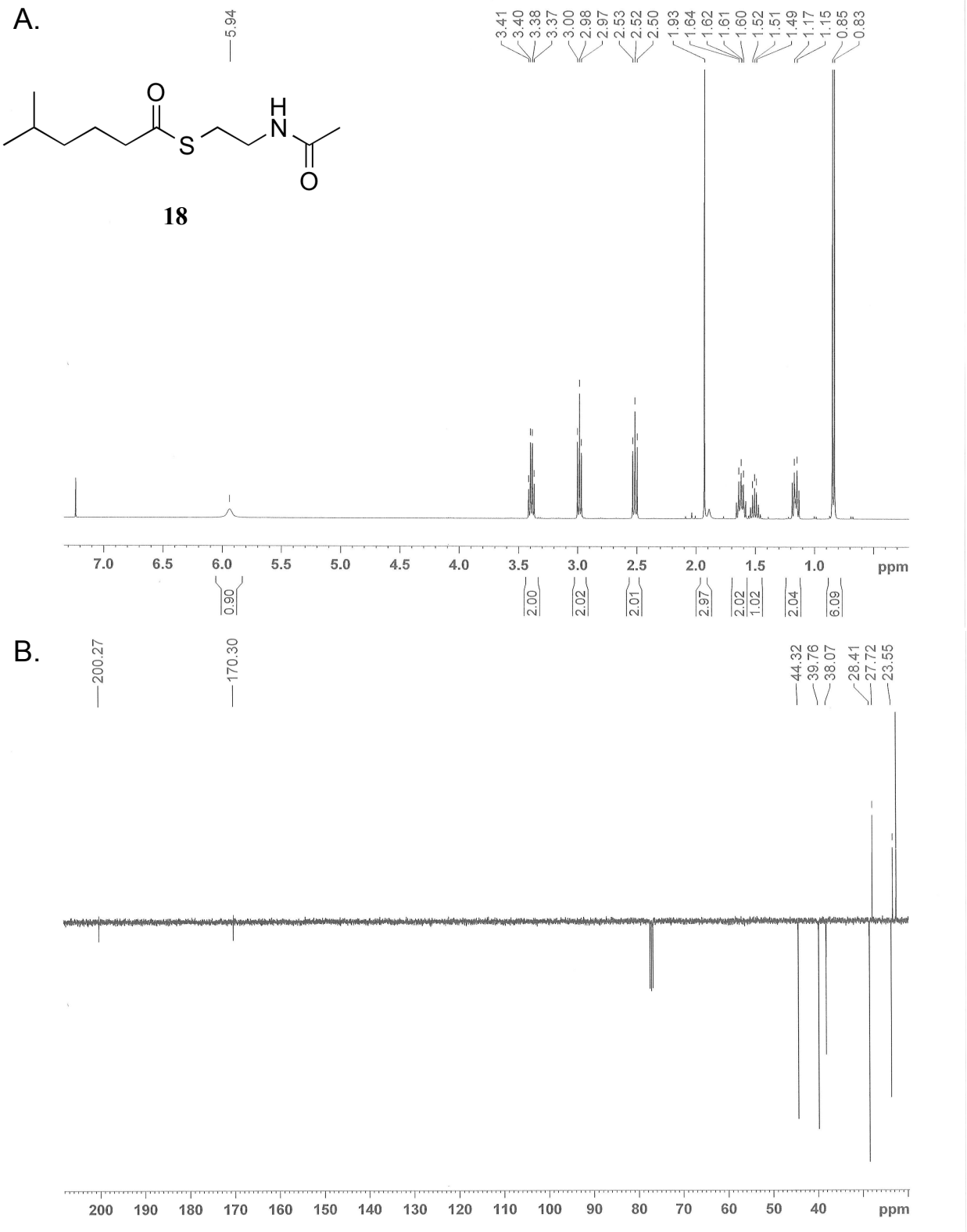


Figure S41. NMR spectra of *S*-(2-acetamidoethyl) 5-methylhexanethioate (**18**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

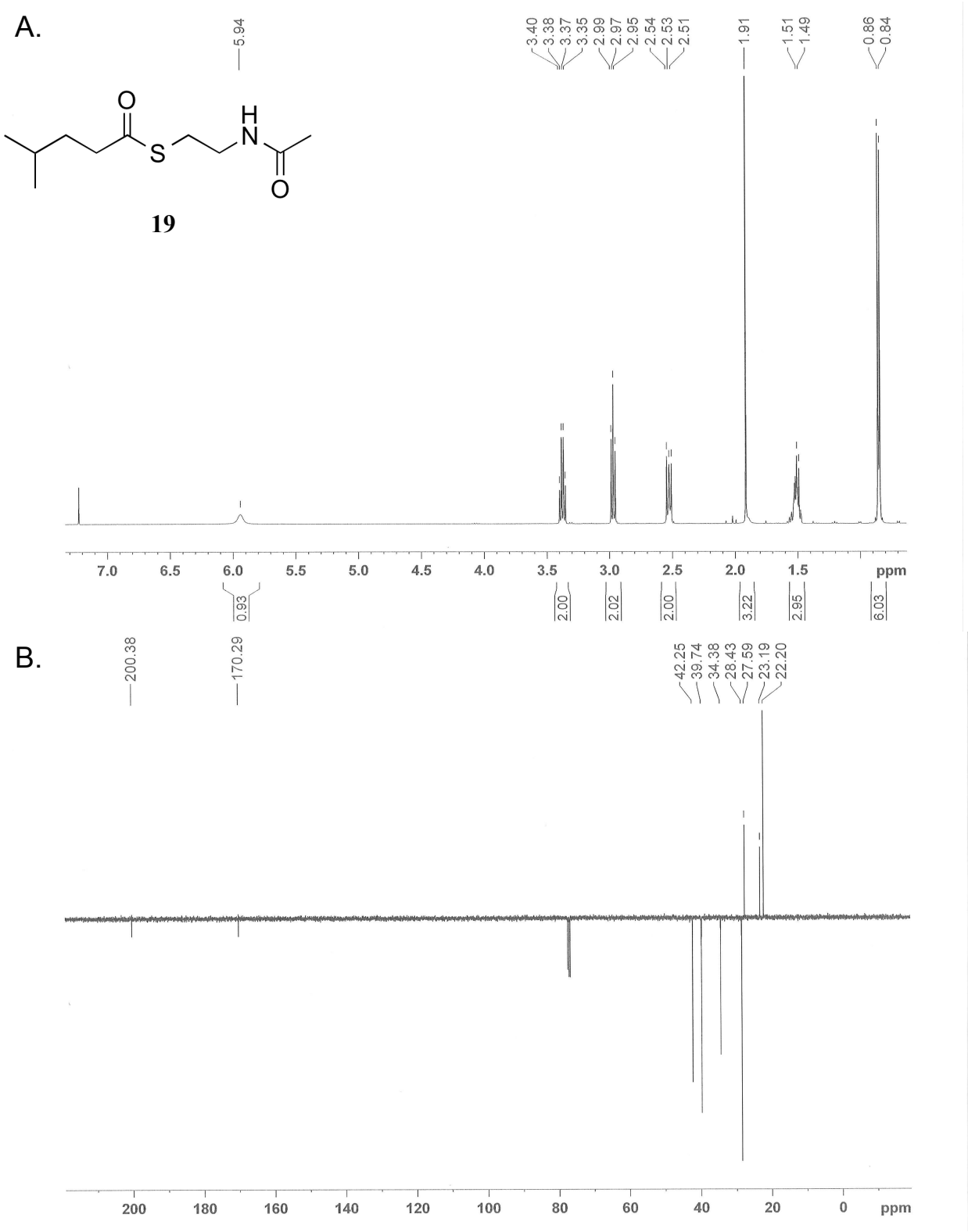


Figure S42. NMR spectra of *S*-(2-acetamidoethyl) 4-methylpentanethioate (**19**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

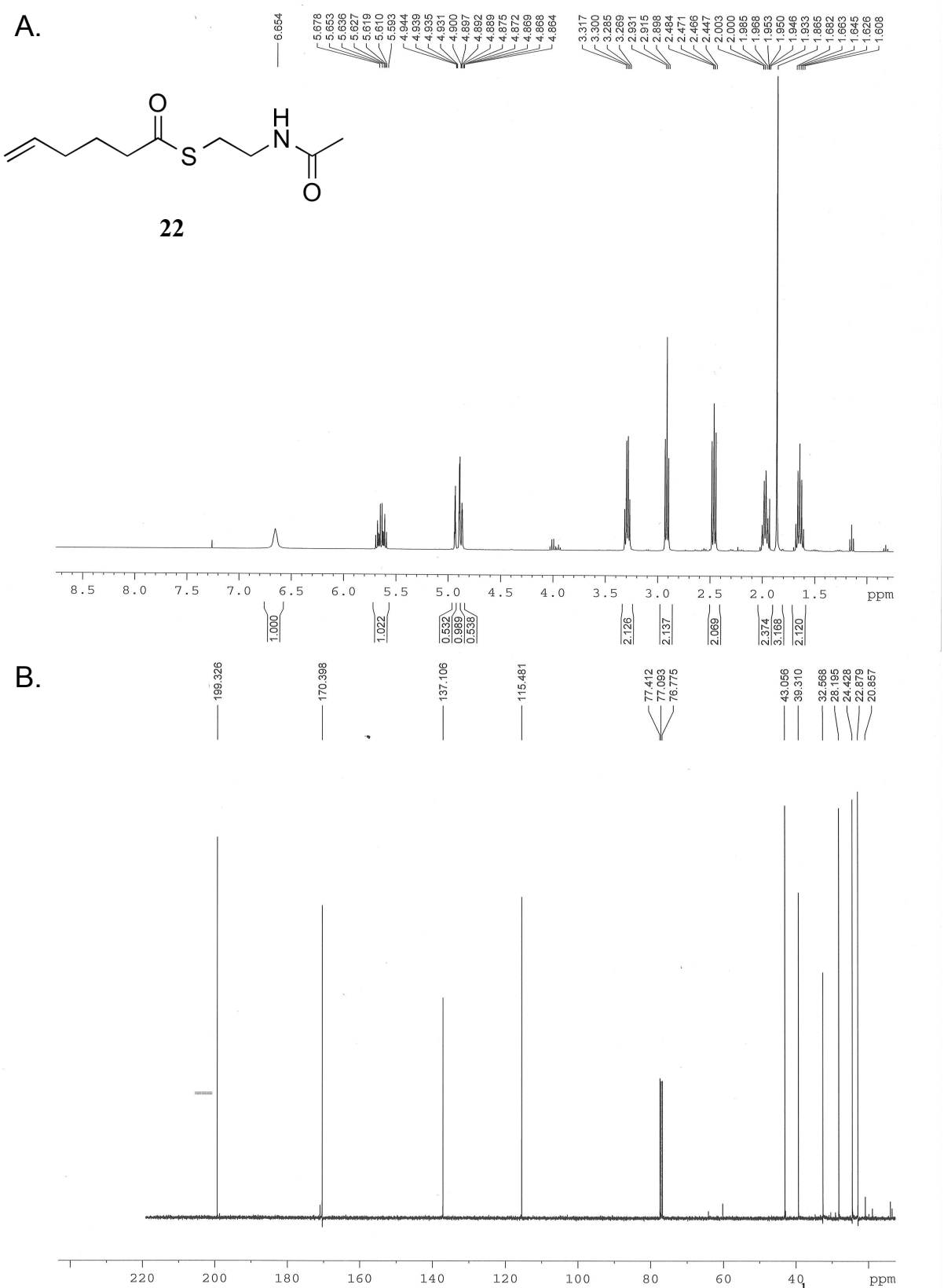


Figure S43. NMR spectra of *S*-(2-acetamidoethyl) hex-5-enethioate (**22**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

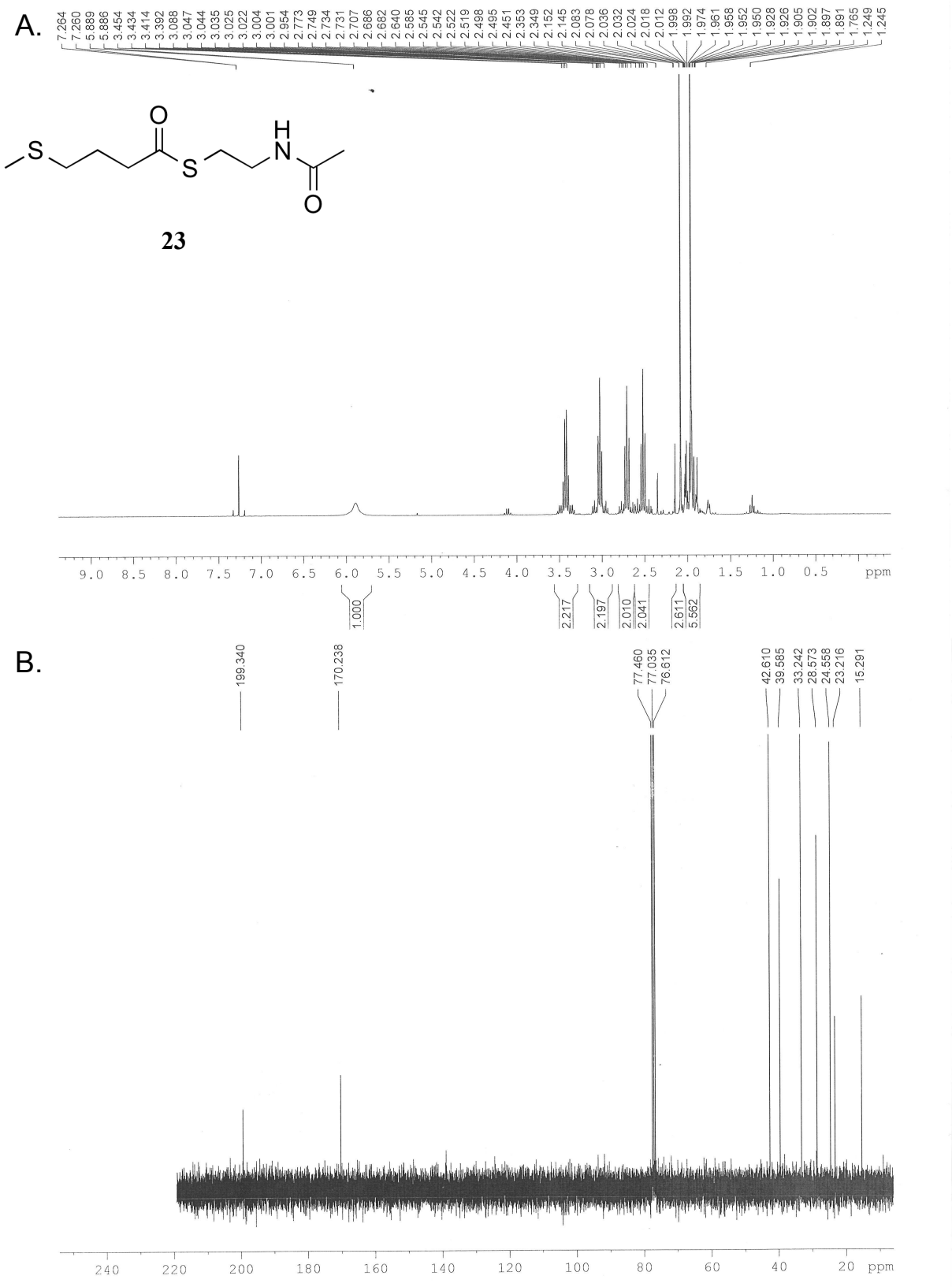


Figure S44. NMR spectra of *S*-(2-acetamidoethyl) 4-(methylthiol)butanethioate (**23**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

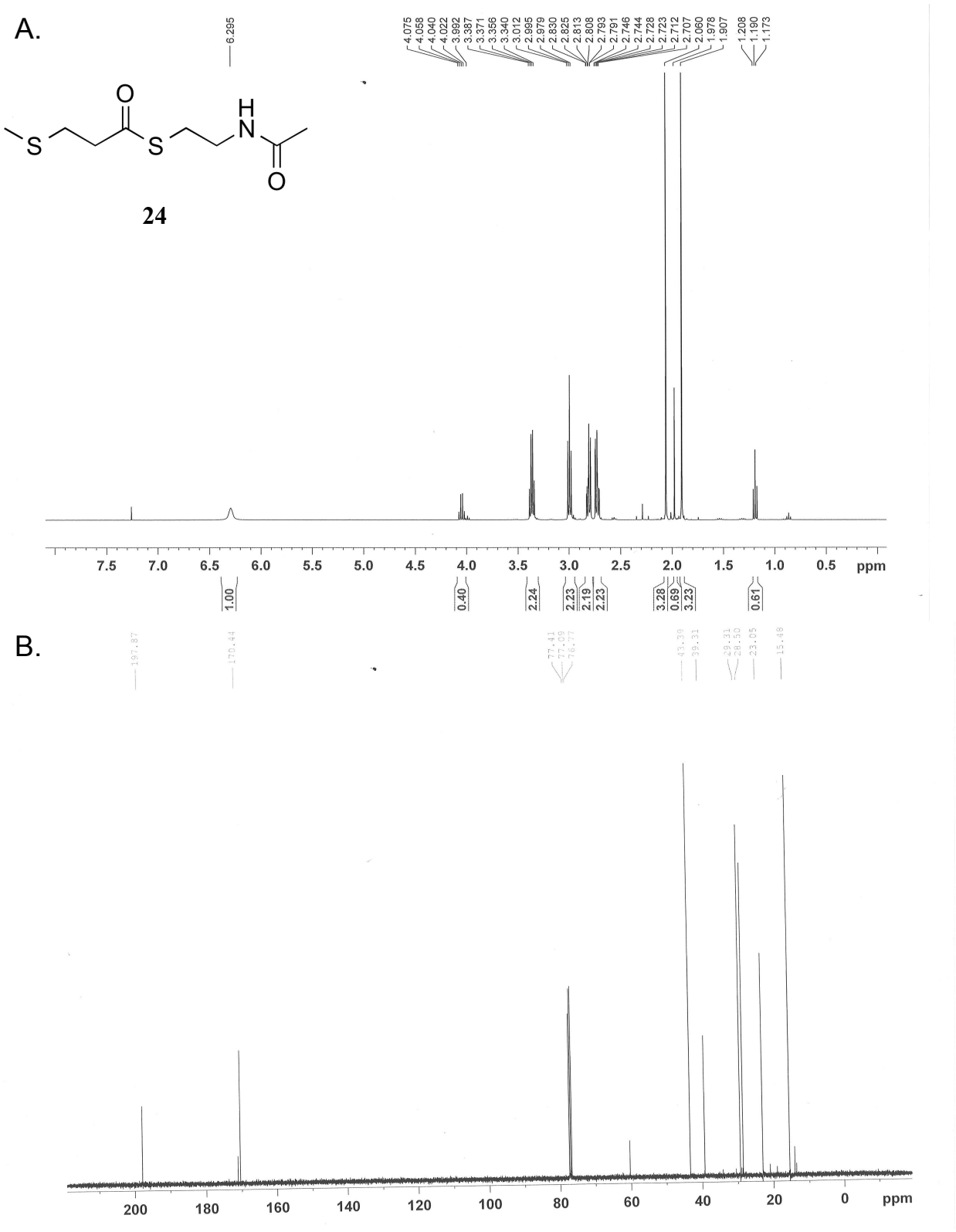


Figure S45. NMR spectra of *S*-(2-acetamidoethyl) 3-(methylthio)propanethioate (**24**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

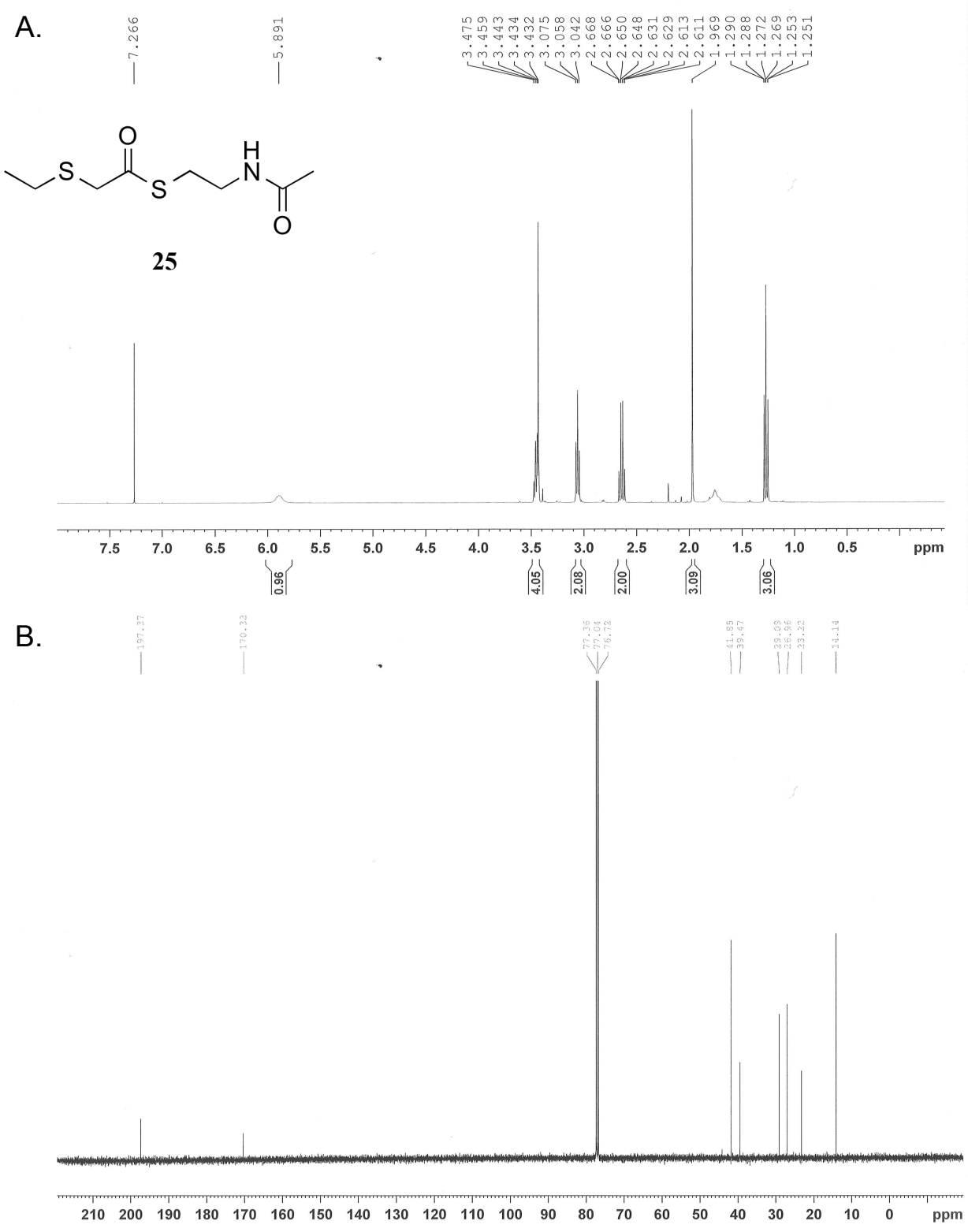


Figure S46. NMR spectra of *S*-(2-acetamidoethyl) 2-(ethylthio)ethanethioate (**25**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

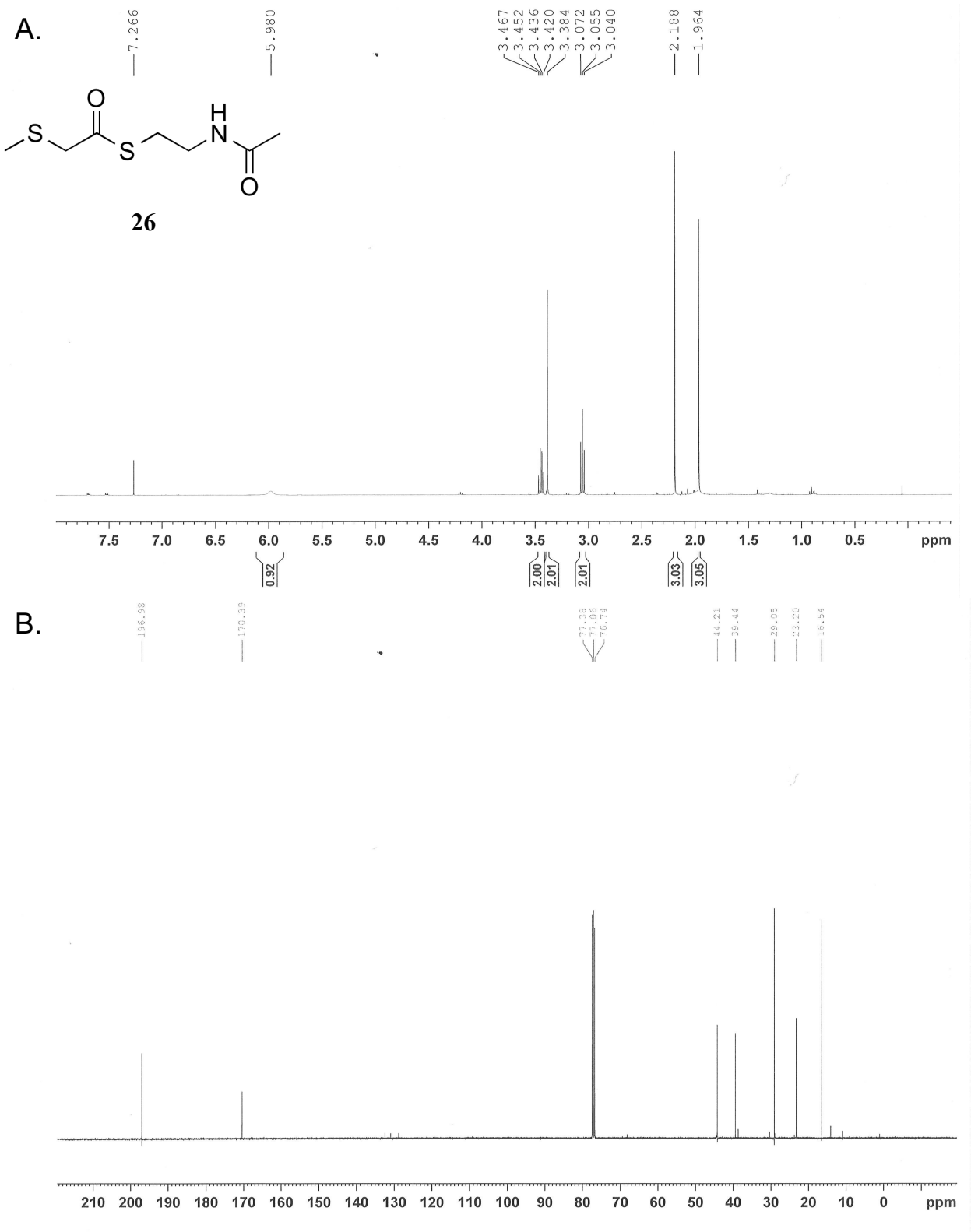


Figure S47. NMR spectra of *S*-(2-acetamidoethyl) 2-(methylthio)ethanethioate (**26**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

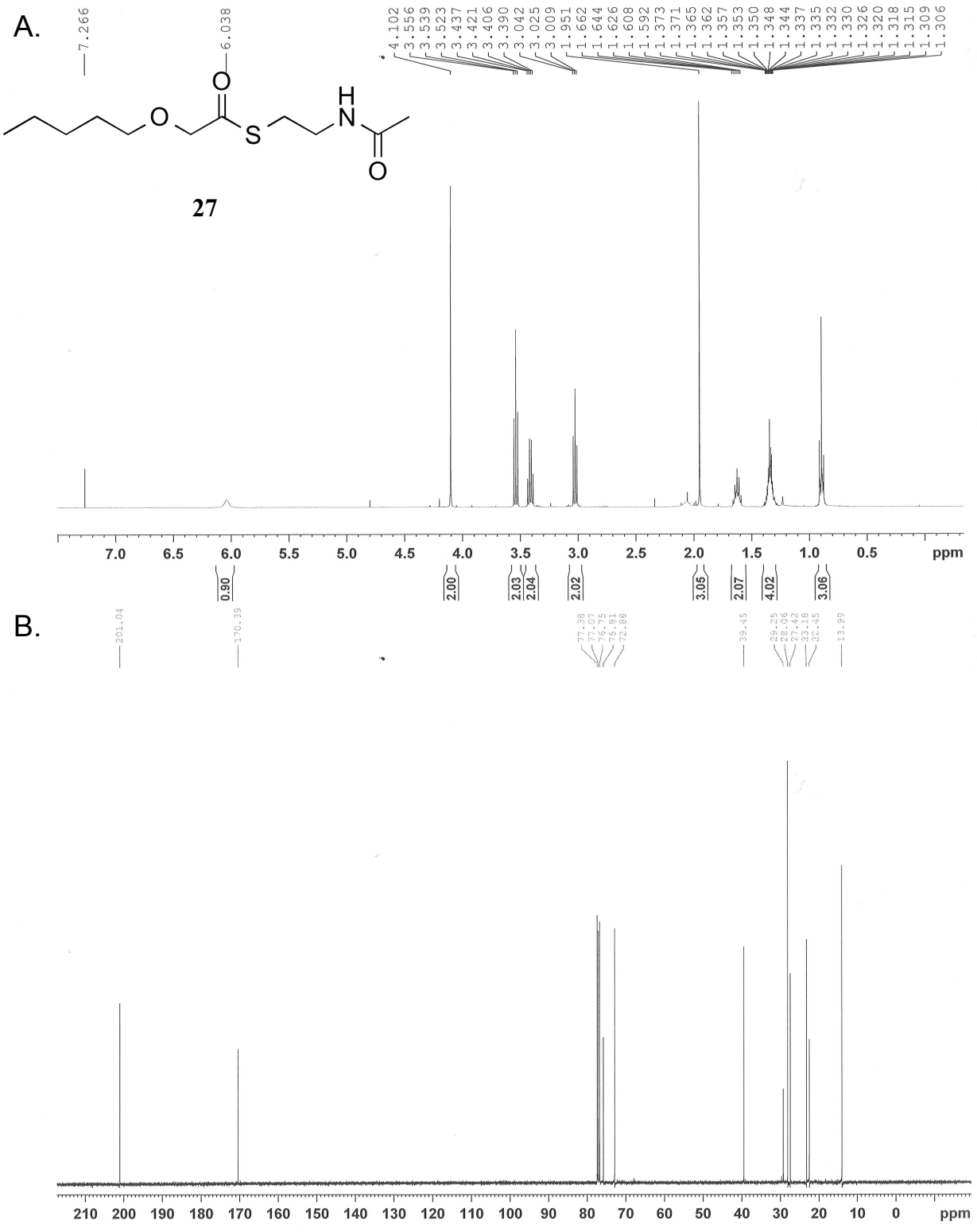


Figure S48. NMR spectra of *S*-(2-acetamidoethyl) 2-(pentyloxy)ethanethioate (**27**) (A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

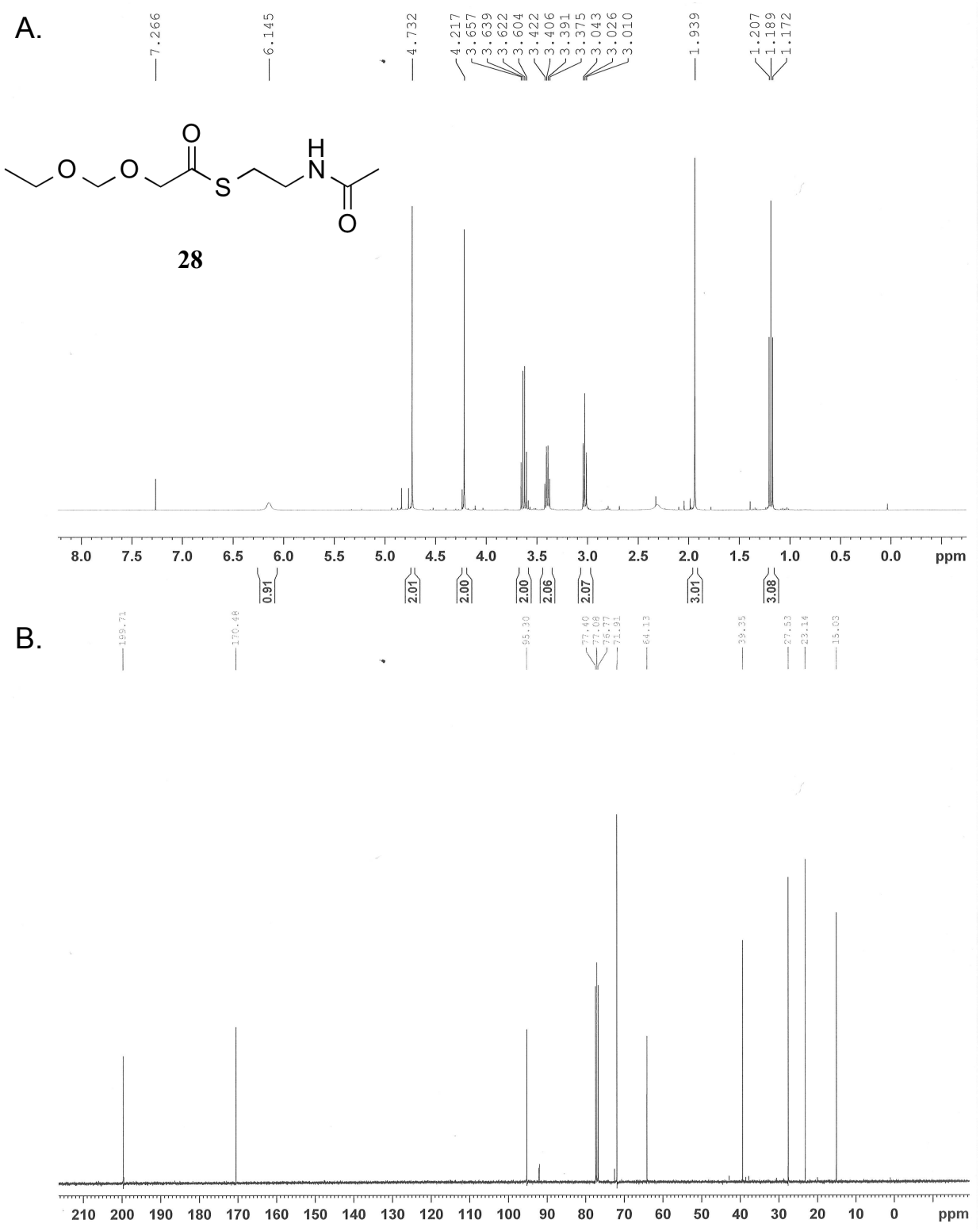


Figure S49. NMR spectra of *S*-(2-acetamidoethyl) 2-(ethoxymethoxy)ethanethioate (**28**) (A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

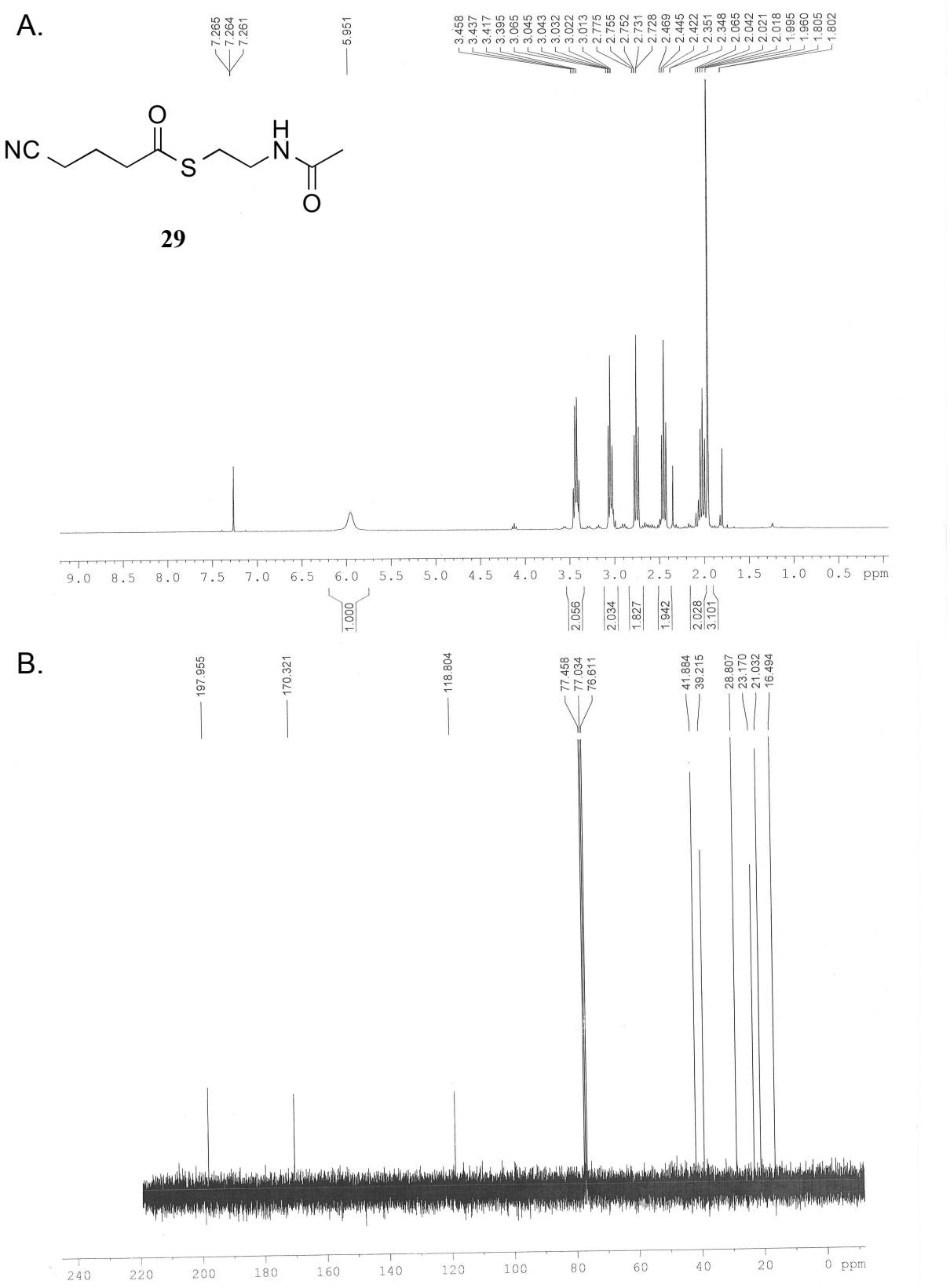


Figure S50. NMR spectra of *S*-(2-acetamidoethyl) 4-cyanobutanethioate (**29**) (A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

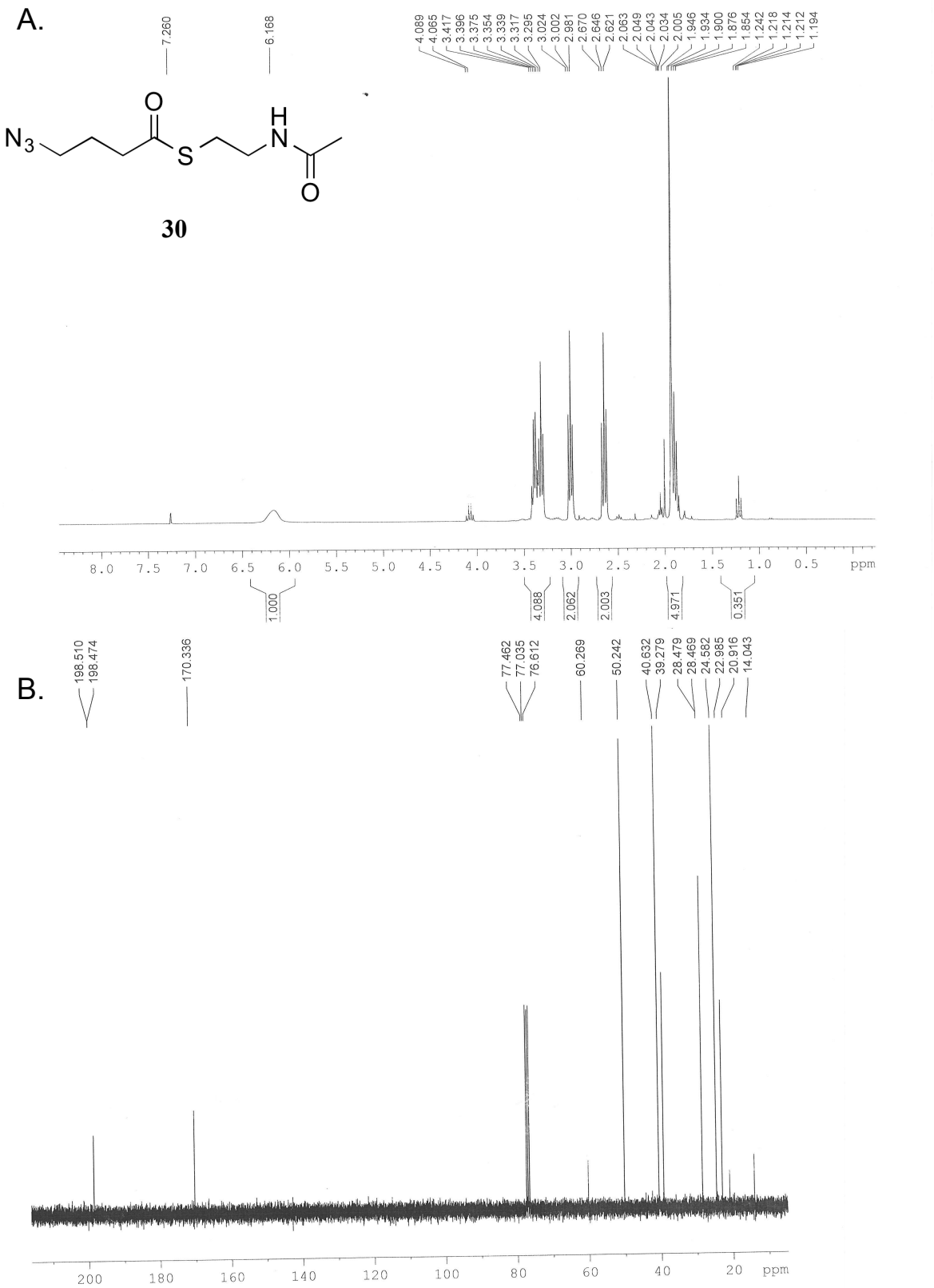
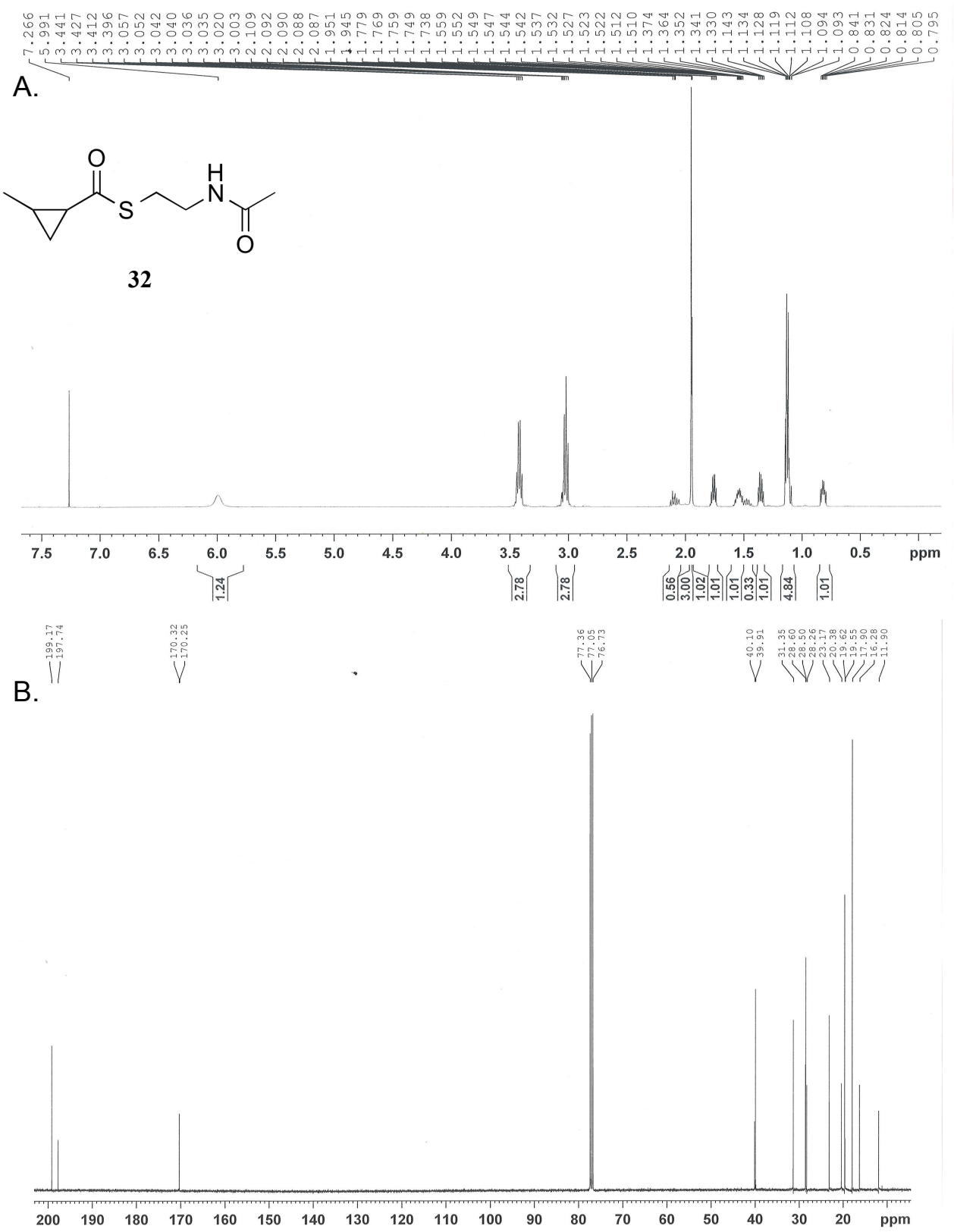


Figure S51. NMR spectra of *S*-(2-acetamidoethyl) 4-azidobutanethioate (**30**) (A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum



32

Figure S52. NMR spectra of *S*-(2-acetamidoethyl) 2-methylcyclopropane-1-carbothioate (**32**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum

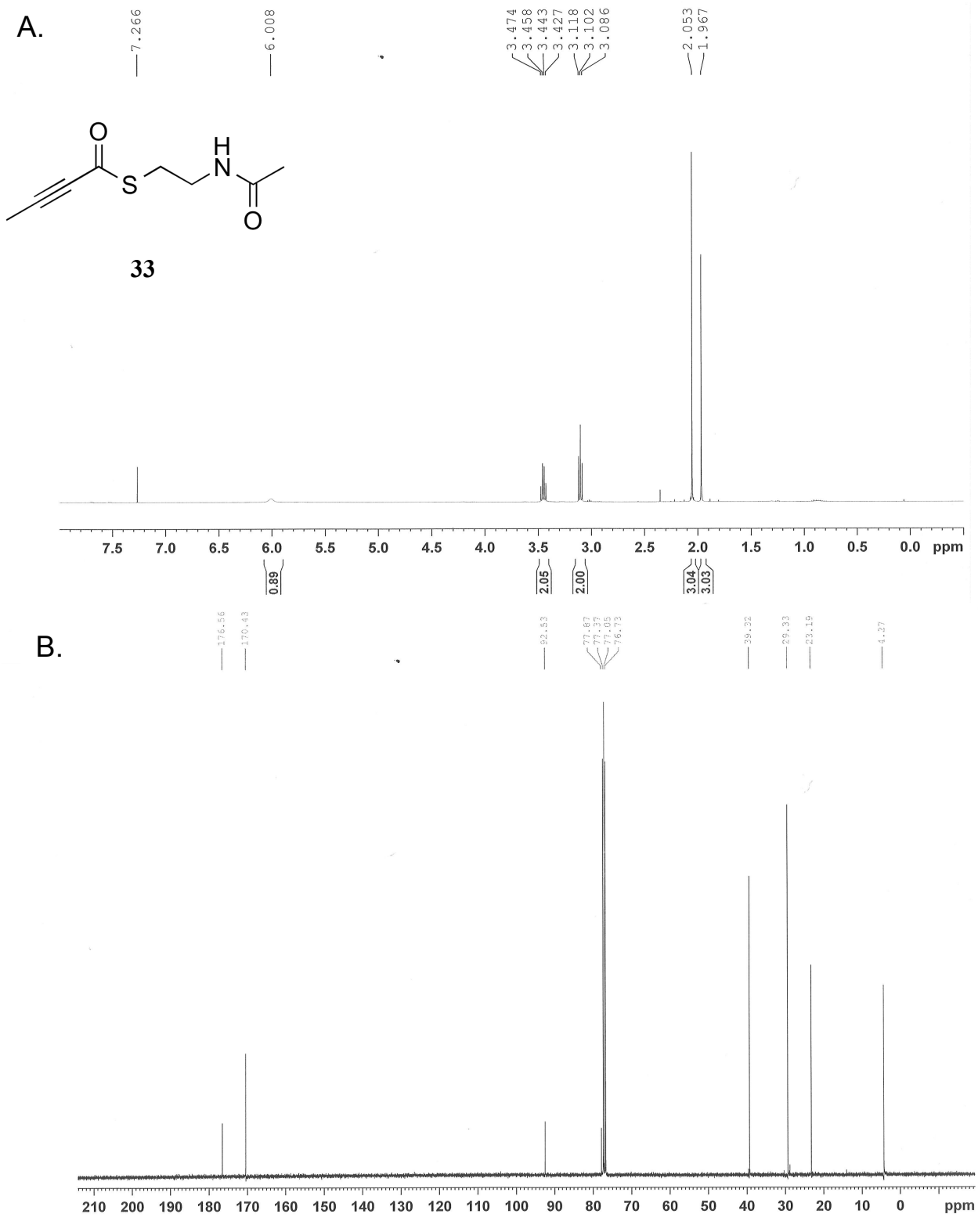


Figure S53. NMR spectra of *S*-(2-acetamidoethyl) but-2-ynethioate (**33**) A) ^{1}H NMR spectrum B) ^{13}C NMR spectrum