

# Supporting Information

for

## Acyl-group specificity of AHL synthases involved in quorum-sensing in *Roseobacter* group bacteria

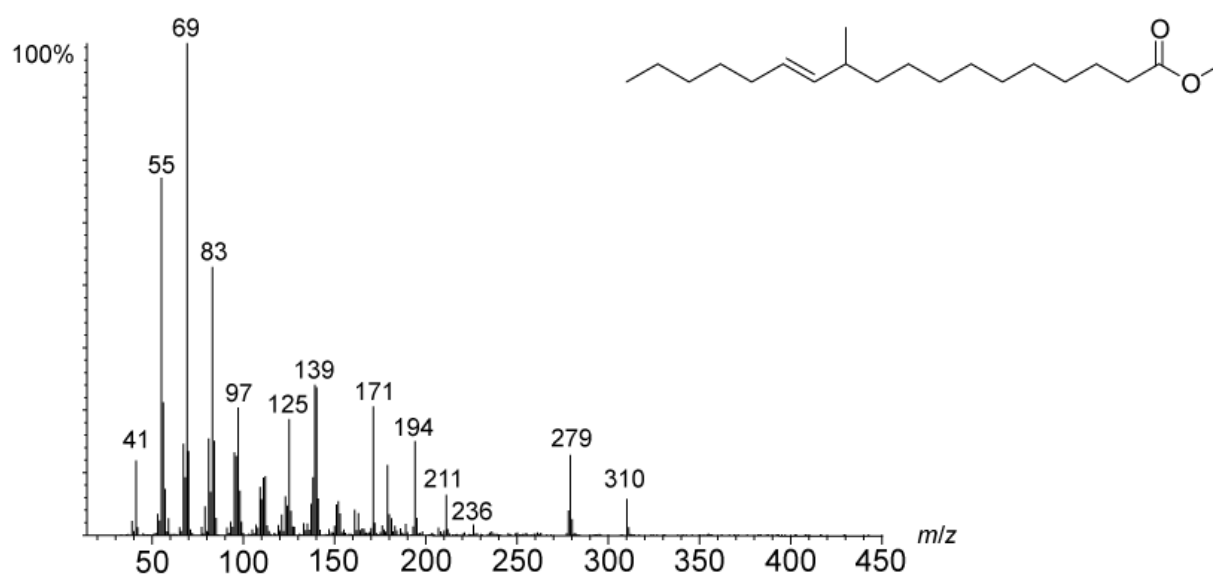
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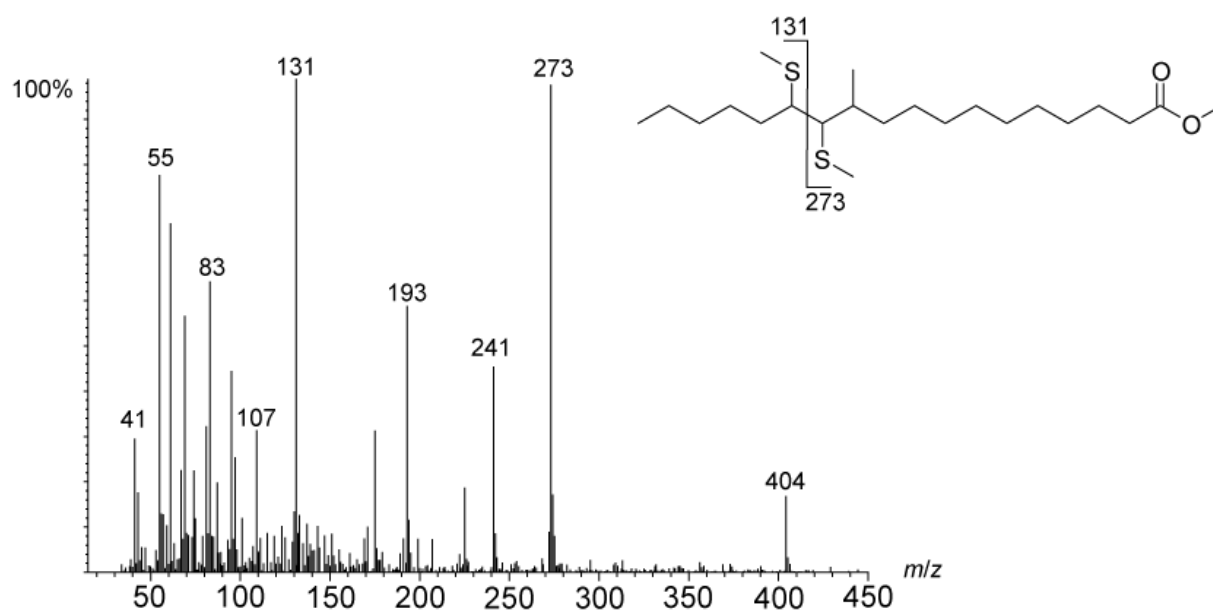
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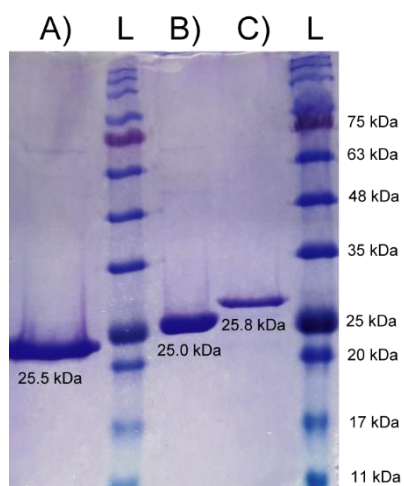
**Experimental, mass spectra, SDS page and NMR spectra**



**Figure S1:** EI-mass spectrum of methyl (*E*)-11-methyloctadec-12-enoate.



**Figure S2:** EI-mass spectrum of the dimethyl disulfide adduct of methyl (*E*)-11-methyloctadec-12-enoate.



**Figure S3:** SDS-PAGE analysis of obtained elution fractions of A) Pgal<sub>2</sub>, B) LuxI<sub>1</sub>, and C) LuxI<sub>2</sub>. Calculated molecular weights of the recombinant proteins are shown.

## Experimental

**General conditions:** Chemicals were obtained from Sigma-Aldrich, Carl Roth or from Acros Organics and were used without further purification. Solvents were purified by distillation and dried according to standard procedures. Moisture and/or oxygen-sensitive reactions were carried out under a nitrogen atmosphere in vacuum-heated flasks with dried solvents. Thin layer chromatography (SiO<sub>2</sub>, TLC) was performed on 0.20 mm Macherey-Nagel silica gel plates (Polygram SIL G/UV254), and column chromatography was performed with Merck silica gel 60 (0.040–0.063 mm) by using standard flash chromatographic methods. NMR spectra were recorded on Bruker DRX-400 (400 MHz), AV III-400 (400 MHz), AV III-HD500 (500 MHz) or AV II-600 (600 MHz) spectrometers and were referenced against TMS ( $\delta = 0.00$  ppm), CDCl<sub>3</sub> ( $\delta = 7.26$  ppm), and CD<sub>3</sub>OD ( $\delta = 3.32$  ppm) for <sup>1</sup>H NMR and CDCl<sub>3</sub> ( $\delta = 77.01$  ppm) and CD<sub>3</sub>OD ( $\delta = 49.0$  ppm) for <sup>13</sup>C NMR experiments. GC/MS analyses of extracts were carried out on an Agilent GC 7890A gas chromatograph connected to a 5975C mass-selective detector (Agilent). Synthetic samples were analyzed on an HP GC 6890 system connected to a HP 5973 mass selective detector fitted with a HP-5

MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.22 μm film; Hewlett-Packard). Conditions were as follows: carrier gas (He): 1.2 mL min<sup>-1</sup>; injection volume: 1 mL; injector: 250 °C; transfer line: 300 °C, EI 70 eV. The gas chromatograph was programmed as follows: 50 °C (5 min isothermal), increasing with 5 °C min<sup>-1</sup> to 320 °C, and operated in splitless mode for extracts and 50 °C (5 min isothermal), increasing with 10 °C min<sup>-1</sup> to 320 °C in split mode (20:1) for synthetic compounds. Gas chromatographic retention indices, *I*, were determined from a homologous series of *n*-alkanes. HPLC/MS analyses were carried out on a Thermo Fisher Accela LC System connected to an LTQ XL mass spectrometer (Thermo Scientific) operated in the ESI positive mode and fitted with a RP-C18 column (150 mm length, 2.1 mm diameter, Agilent). HRMS analyses were carried out on a Thermo Fisher linear iontrap coupled with a LTQ-Orbitrap mass spectrometer in the ESI positive mode. ESI measurements were performed by direct infusion mode using a custom made microspray device mounted on a Proxeon nanospray ion source. All solvents used were of LC/MS grade.

**Strains and culture conditions:** *Phaeobacter inhibens* DSM17395 and *Dinoroseobacter shibae* DFL-12 were obtained from the DSMZ collection (Braunschweig, Germany) and cultured in marine broth medium (MB) at 28 °C.

**XAD extracts:** Bacterial cultures (100 mL) were grown for three days in marine broth medium containing Soxhlet precleaned Amberlite XAD-16 (2 g). The adsorbent was separated from the culture by filtration and extracted three times with CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O (10:1) [1]. The combined organic phases were dried with MgSO<sub>4</sub> and the solvent was removed under reduced pressure. The extract was redissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 μL) and analyzed by GC/MS.

**Fatty acid methyl esters:** A few colonies of bacteria were transferred with a loop from an agar plate or a cell pellet, obtained by centrifugation from a well-grown liquid culture, to a 1 mL GC vial filled with 10  $\mu$ L distilled water. The bacteria were directly lysed by adding 30  $\mu$ L methanolic trimethylsulfonium hydroxide (TMSH) solution (0.25 M) that concomitantly converted bound and free fatty acids to FAMES [2]. The mixture was dried in a nitrogen stream and the residue was dissolved in 200  $\mu$ L diethyl ether/methanol (10:1). This solution was analyzed by a GC/MS.

**Determination of double bond positions:** FAME samples were dissolved in 50  $\mu$ L dichloromethane (DCM) and 50  $\mu$ L of dimethyl disulfide in a 1 mL vial, and 5  $\mu$ L of 5% ethereal iodine solution was added. The closed vial was kept for a day in a heating block at 60 °C. The cooled mixture was diluted with 200  $\mu$ L of DCM. The mixture was shaken with 200  $\mu$ L of aqueous sodium sulfite solution to reduce iodine. After separation of the phases, the organic phase was dried with  $MgSO_4$ , filtered and the solvent was evaporated in a gentle stream of nitrogen. The residue was redissolved in DCM and analyzed by GC/MS [1,3,4].

**Gene cloning:** Genomic DNA was isolated from liquid cultures of *P. inhibens* DSM17395 and *D. shibae* DFL-12 in half strength MB medium grown for 7 days using a standard protocol based on phenol/chloroform/isoamyl alcohol extraction [5]. Using these templates, the target AHL synthase genes *pgal<sub>2</sub>*, *luxI<sub>1</sub>* and *luxI<sub>2</sub>* (for accession numbers, see Table S1) were amplified by PCR using Phusion DNA polymerase (New England Biolabs, Ipswich, MA, USA) and the primers without homology arms listed in Table 5 (PCR conditions: initial denaturation 95 °C for 5 min; 30 cycles 95 °C for 15 s, annealing 68 °C for 30 s, 72 °C for 30 s; final elongation 72 °C for 5 min). The obtained fragments were further elongated in a second PCR

reaction using the corresponding primers harboring homology arms (Table S2) with the reaction conditions described above. These fragments were added to linearized yeast-*E. coli* shuttle vector pYE-express [6] (HindIII and EcoRI digested) and used for homologous recombination in yeast by the LiOAc/SS DNA/PEG protocol [7].

Transformed yeast colonies were grown on SM-URA medium to isolate the product plasmids by Plasmid Miniprep II kit (Zymo Research, Irvine, USA). The obtained plasmid mixtures were shuttled to *E. coli* BL21(DE3) cells by electroporation. The transformed cells were grown on selective LB agar medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, 18 g/L agar, pH 7.2) supplied with kanamycin (50 µg/mL) to pick single colonies, which were grown in liquid LB medium (50 µg/mL kanamycin) at 37 °C. Isolated plasmids were checked for correct insertion of the target gene by analytical digest and sequencing of the positive samples.

**Table S1:** AHL synthases from *P. inhibens* DSM17395 and *D. shibae* DFL-12.

Organism	Abbreviation <sup>a</sup>	Locus tag	Accession nr.	Alt. accession nr.
<i>P. inhibens</i> DSM17395	Pgal <sub>1</sub>	PGA1_c03890	WP_014879070	ZP_02146368, AFO90123
<i>P. inhibens</i> DSM17395	<b>Pgal<sub>2</sub></b>	PGA1_c07680	WP_014879372	AFO90496
<i>D. shibae</i> DFL-12	<b>LuxI<sub>1</sub></b>	Dshi_0312	WP_012176991	ABV92061
<i>D. shibae</i> DFL-12	<b>LuxI<sub>2</sub></b>	Dshi_2851	WP_012179512	ABV94584
<i>D. shibae</i> DFL-12	LuxI <sub>3</sub>	Dshi_4152	WP_012187430	ABV95863

<sup>a</sup>Enzymes investigated in this work are shown in bold. Abbreviations are used in analogy to previous work [1,8,9].



by ultra-sonication on ice (50% power, 5 × 30 s, 4 °C) and the cell debris was removed by centrifugation (5500g, 10 min, 4 °C) to yield the soluble protein fraction, which was applied to Ni<sup>2+</sup>-NTA affinity chromatography using Ni-NTA superflow (Qiagen, Venlo, Netherlands). Washing was done by binding buffer (3 × 2 mL) and the target protein was isolated by elution buffer (2 × 1 mL; 50 mM TRIS-HCl, 500 mM imidazole, 20% glycerol, pH 8.0, 4 °C). The obtained protein solution was analyzed by SDS-PAGE for the target protein size (Figure S3) and used for incubation experiments.

**Incubation experiments with recombinant AHL synthases:** For substrate testing, each synthetic substrate analogue (1 μmol) was dissolved in DMSO (30 μL) and diluted by binding buffer (470 μL). After adding glycerol (50 μL), an AHL synthase elution fraction (250 μL) was supplied to the mixture and the reaction was started by adding a solution of *S*-adenosylmethionine chloride dihydrochloride (SAM, 0.67 mg, 1 μmol, in 200 μL H<sub>2</sub>O). The reaction was incubated for 5 h at 28 °C before it was extracted with DCM (200 μL). The extract was washed with water (200 μL), dried with MgSO<sub>4</sub>, filtered and analyzed by GC/MS for the target AHLs.

For the competition experiments, a solution of the mixed substrate analogues **10a–h** or **10a–i**, **11a–c** and **12** (0.1 μmol each, in 50 μL DMSO) was added to binding buffer (450 mL) and glycerol (50 mL), before AHL synthase elution fraction (250 mL) and finally a solution of SAM (1.1 mg, 1.6 μmol, in 200 μL H<sub>2</sub>O) were added. The reaction was incubated, extracted and analyzed as described above.



### **(R)-N-Pantothenoylcysteamine acetonide (8)**

Calcium (*R*)-pantothenate (5 g, 10.49 mmol), *p*-toluenesulfonic acid monohydrate (4.79 g, 25.18 mmol), 3 Å molecular sieves (5 g) and 250 mL HPLC grade acetone were stirred overnight at room temperature in a round-bottomed flask. The suspension was filtered through Celite, washed three times with 100 mL acetone and the solvent was evaporated. The oil was redissolved in 200 mL EtOAc, washed two times with 100 mL brine and dried with Na<sub>2</sub>SO<sub>4</sub>. Most of the solvent was removed and hexane was added slowly to form a white solid. The solid was dried in high vacuum and used in the next step without further purification.

The freshly prepared ketal **7** (5.06 g, 19.51 mmol) was dissolved in 100 mL dry THF, treated with 1,1'-carbonyldiimidazole (CDI) (4.75 g, 29.27 mmol) and stirred for one hour at room temperature. Cysteamine hydrochloride (3.38 g, 29.27 mmol) was added to this solution and the mixture stirred at room temperature overnight. THF was removed in vacuo and the residue was redissolved in DCM. The organic phase was washed with 50 mL sat. NH<sub>4</sub>Cl solution and with 50 mL brine, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by flash chromatography on silica (EtOAc/pentane 70:30 to 100% EtOAc) to get the thiol as a white solid (6.1 g, 19.15 mmol, 98%) [10].

R<sub>f</sub> = 0.2 (EtOAc/pentane 70:30); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 7.04 (br s, 1H), 6.49 (br s, 1H), 4.08 (s, 1H), 3.69 (d, *J* = 11.8 Hz, 1H), 3.63-3.51 (m, 2H), 3.51-3.36 (m, 2H), 3.29 (d, *J* = 11.8 Hz, 1H), 2.69-2.63 (m, 2H), 2.48 (t, *J* = 6.3 Hz, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.04 (s, 3H), 0.97 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ = 171.1, 170.2, 99.1, 77.1, 71.4, 42.4, 36.1, 34.8, 32.9, 29.4, 24.5, 22.1, 18.9, 18.7; MS (70 eV, EI): *m/z* (%): 59 (100), 143 (97), 260 (69), 88 (69), 206 (55), 43 (50), 55 (32), 245 (27), 184 (25), 303 (24), 318 [M<sup>+</sup>].

### **(Z)-7-Tetradecenoic acid**

To a solution of 7-tetradecynoic acid (0.22 g, 0.98 mmol) and methanol (5 mL) Lindlar's catalyst (0.02 g) was added. The mixture was stirred for 20 minutes at room temperature under a hydrogen atmosphere. The catalyst was removed by filtration through a short pad of silica and the solvent was evaporated. The product was obtained as colorless oil without the need of further purification (205 mg, 0.90 mmol, 92%) [11]. All analytical data were in agreement with those previously reported [4].

### **S-Acyl-N-pantothenoyl acetonide derivatives (9)**

To a stirred solution of an acid in dry DCM, *p*-dimethylaminopyridine (DMAP) and **8** were added. *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC·HCl) was added at 0 °C, the solution was stirred for 5 minutes at 0 °C and overnight at room temperature [12]. 1N HCl was added to the reaction mixture followed by extraction with DCM three times. The combined organic phases were washed with sat. NaHCO<sub>3</sub> sol. and brine, dried with MgSO<sub>4</sub>, filtered and concentrated to give an oil.

### **(R)-S-Butanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (435 mg, 1.12 mmol, 99%)  $R_f = 0.3$  (EtOAc/pentane 6:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta = 7.03$  (br s, 1H), 6.24 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.5$  Hz, 1H), 3.62-3.48 (m, 2H), 3.48-3.38 (m, 2H), 3.28 (d,  $J = 11.8$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.57-2.53 (m, 2H), 2.43 (t,  $J = 6.3$  Hz, 2H), 1.74-1.65 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.26 (t,  $J = 7.0$  Hz, 3H), 1.04 (s, 3H), 0.97 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta = 199.7, 171.1, 170.1, 99.1, 77.1, 71.4, 45.9, 39.6, 35.9, 34.8, 33.0, 29.4, 28.4, 22.1, 19.1, 18.9, 18.7, 13.4$ ; (+)-ESI MS  $m/z$ : 389.3 [M+H]<sup>+</sup>, 411.3 [M+Na]<sup>+</sup>.

**(R)-S-Hexanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (354 mg, 0.85 mmol, 98%)  $R_f = 0.4$  (EtOAc/pentane 6:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.03$  (br s, 1H), 6.20 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.8$  Hz, 1H), 3.61-3.53 (m, 2H), 3.51-3.38 (m, 2H), 3.28 (d,  $J = 11.8$  Hz, 1H), 3.01 (t,  $J = 6.3$  Hz, 2H), 2.56 (t,  $J = 7.5$  Hz, 2H), 2.42 (t,  $J = 6.3$  Hz, 2H), 1.70-1.62 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.35-1.29 (m, 2H), 1.28-1.24 (m, 2H), 1.04 (s, 3H), 0.97 (s, 3H), 0.89 (t,  $J = 7.0$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.8, 171.1, 170.1, 99.1, 77.2, 71.4, 44.1, 39.6, 35.9, 34.8, 33.0, 31.1, 29.5, 28.4, 25.3, 22.3, 22.1, 18.9, 18.7, 13.8$ ; (+)-ESI MS  $m/z$ : 417.3  $[\text{M}+\text{H}]^+$ , 439.3  $[\text{M}+\text{Na}]^+$ .

**(R)-S-Octanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (315 mg, 0.70 mmol, 99%)  $R_f = 0.4$  (EtOAc/pentane 6:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.03$  (br s, 1H), 6.13 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.8$  Hz, 1H), 3.61-3.50 (m, 2H), 3.50-3.38 (m, 2H), 3.29 (d,  $J = 11.8$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.5$  Hz, 2H), 2.42 (t,  $J = 6.3$  Hz, 2H), 1.69-1.62 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.30-1.24 (m, 8H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.9, 171.1, 170.1, 99.1, 77.2, 71.5, 44.1, 39.6, 36.0, 34.8, 33.0, 31.6, 29.5, 28.9, 28.9, 28.4, 25.6, 22.6, 22.1, 18.9, 18.7, 14.0$ ; (+)-ESI MS  $m/z$ : 445.3  $[\text{M}+\text{H}]^+$ , 467.3  $[\text{M}+\text{Na}]^+$ .

**(R)-S-Decanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (224 mg, 0.47 mmol, 86%)  $R_f = 0.4$  (EtOAc/pentane 6:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.03$  (br s, 1H), 6.13 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.8$  Hz, 1H), 3.61-3.50 (m, 2H), 3.50-3.38 (m, 2H), 3.29 (d,  $J = 11.8$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.3$  Hz, 2H), 2.42 (t,  $J = 6.3$  Hz, 2H), 1.70-1.61 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.33-1.24 (m, 12H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J =$

6.5 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.9, 171.1, 170.1, 99.1, 77.2, 71.5, 44.1, 39.6, 36.0, 34.8, 33.0, 31.8, 29.5, 29.3, 29.2, 29.2, 28.9, 28.4, 25.6, 22.6, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$ : 473.3  $[\text{M}+\text{H}]^+$ , 495.3  $[\text{M}+\text{Na}]^+$ .

**(R)-S-Dodecanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (250 mg, 0.49 mmol, 98%)  $R_f = 0.4$  (EtOAc/pentane 6:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.03$  (br s, 1H), 6.18 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.5$  Hz, 1H), 3.61-3.53 (m, 2H), 3.51-3.39 (m, 2H), 3.28 (d,  $J = 11.8$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.5$  Hz, 2H), 2.42 (t,  $J = 6.3$  Hz, 2H), 1.68-1.61 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.33-1.24 (m, 16H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.9, 171.1, 170.1, 99.1, 77.1, 71.4, 44.1, 39.6, 35.9, 34.8, 33.0, 31.9, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 28.4, 25.6, 22.7, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$ : 501.3  $[\text{M}+\text{H}]^+$ , 523.3  $[\text{M}+\text{Na}]^+$ .

**(R)-S-Tetradecanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (227 mg, 0.43 mmol, 98%)  $R_f = 0.4$  (EtOAc/pentane 5:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.03$  (br s, 1H), 6.13 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.5$  Hz, 1H), 3.64-3.50 (m, 2H), 3.50-3.38 (m, 2H), 3.28 (d,  $J = 11.8$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.5$  Hz, 2H), 2.42 (t,  $J = 6.3$  Hz, 2H), 1.69-1.61 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.33-1.24 (m, 20H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.5$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.9, 171.1, 170.1, 99.1, 77.2, 71.5, 44.2, 39.6, 36.0, 34.8, 33.0, 31.9, 29.7, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 28.4, 25.6, 22.7, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$ : 529.3  $[\text{M}+\text{H}]^+$ , 551.4  $[\text{M}+\text{Na}]^+$ .

**(R)-S-Hexadecanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (211 mg, 0.38 mmol, 97%)  $R_f = 0.4$  (EtOAc/pentane 4:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.02$  (br s, 1H), 6.11 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.5$  Hz, 1H), 3.64-3.51 (m, 2H), 3.50-3.36 (m, 2H), 3.28 (d,  $J = 11.5$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.5$  Hz, 2H), 2.42 (t,  $J = 6.1$  Hz, 2H), 1.69-1.61 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.34-1.24 (m, 24H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.5$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.9, 171.1, 170.1, 99.1, 77.2, 71.5, 44.2, 39.6, 36.0, 34.8, 33.0, 31.9, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.4, 29.4, 29.2, 29.0, 28.4, 25.6, 22.7, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$ : 557.3  $[\text{M}+\text{H}]^+$ , 579.5  $[\text{M}+\text{Na}]^+$ .

**(R)-S-Octadecanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (198 mg, 0.34 mmol, 97%)  $R_f = 0.3$  (EtOAc/pentane 4:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.02$  (br s, 1H), 6.10 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.3$  Hz, 1H), 3.61-3.51 (m, 2H), 3.50-3.39 (m, 2H), 3.28 (d,  $J = 11.5$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.3$  Hz, 2H), 2.42 (t,  $J = 6.3$  Hz, 2H), 1.69-1.60 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.32-1.24 (m, 28H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.9, 171.1, 170.1, 99.1, 77.2, 71.5, 44.2, 39.6, 36.0, 34.8, 33.0, 31.9, 29.7, 29.7, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.2, 29.0, 28.5, 25.6, 22.7, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$ : 585.3  $[\text{M}+\text{H}]^+$ , 607.5  $[\text{M}+\text{Na}]^+$ .

**(R)-S-Icosanoyl-N-pantothenoylcysteamine acetonide**

Colorless oil (134 mg, 0.22 mmol, 67%)  $R_f = 0.3$  (EtOAc/pentane 2:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.03$  (br s, 1H), 6.17 (br s, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.5$  Hz, 1H), 3.61-3.51 (m, 2H), 3.50-3.40 (m, 2H), 3.28 (d,  $J = 11.5$  Hz, 1H), 3.01 (t,

$J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.5$  Hz, 2H), 2.42 (t,  $J = 6.1$  Hz, 2H), 1.69-1.61 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.32-1.25 (m, 32H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.9, 171.1, 170.1, 99.1, 77.2, 71.4, 44.2, 39.6, 39.5, 36.0, 35.9, 34.8, 33.0, 31.9, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 28.9, 28.4, 28.4, 25.6, 22.7, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$  613.4  $[\text{M}+\text{H}]^+$ , 635.6  $[\text{M}+\text{Na}]^+$ .

**(R)-S-(3-Hydroxydecanoyl)-N-pantothenoylcysteamine acetonide**

Colorless oil (47 mg, 0.10 mmol, 76%)  $R_f = 0.3$  (EtOAc);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.01$  (br s, 1H), 6.4 (br s, 1H), 4.13-4.08 (m, 1H), 4.08 (s, 1H), 3.69 (d,  $J = 11.5$  Hz, 1H), 3.59-3.50 (m, 2H), 3.49-3.34 (m, 2H), 3.29 (d,  $J = 11.8$  Hz, 1H), 3.16-3.08 (m, 1H), 3.04-2.96 (m, 1H), 2.75-2.65 (m, 2H), 2.44-2.40 (m, 2H), 1.62-1.50 (m, 2H), 1.47 (s, 3H), 1.42 (s, 3H), 1.34-1.23 (m, 10H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.2, 171.1, 170.5, 99.1, 77.2, 71.4, 69.0, 51.2, 39.1, 37.1, 36.3, 35.2, 33.0, 31.8, 29.5, 29.4, 29.2, 28.7, 25.5, 22.6, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$  489.2  $[\text{M}+\text{H}]^+$ .

**(R)-S-((Z)-7-Tetradecenoyl)-N-pantothenoylcysteamine acetonide**

Colorless oil (251 mg, 0.48 mmol, 55%)  $R_f = 0.3$  (EtOAc/pentane 4:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.03$  (br s, 1H), 6.15 (br s, 1H), 5.39-5.33 (m, 2H), 4.08 (s, 1H), 3.69 (d,  $J = 11.8$  Hz, 1H), 3.61-3.50 (m, 2H), 3.48-3.40 (m, 2H), 3.28 (d,  $J = 11.8$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.5$  Hz, 2H), 2.42 (t,  $J = 6.3$  Hz, 2H), 2.04-1.94 (m, 4H), 1.71-1.64 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.35-1.24 (m, 12H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.5$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.8, 171.1, 170.1, 129.8, 129.3, 99.1, 77.1, 71.4, 44.1, 39.6, 36.0, 34.8, 33.0, 32.3, 31.7, 29.7, 29.6,$

29.1, 28.8, 28.6, 28.4, 27.2, 25.5, 22.6, 22.1, 18.9, 18.7, 14.1; (+)-ESI MS  $m/z$ : 527.1  
[M+H]<sup>+</sup>.

**(R)-S-((Z)-9-Hexadecenoyl)-N-pantothenoylcysteamine acetonide**

Colorless oil (193 mg, 0.35 mmol, 88%)  $R_f$  = 0.4 (EtOAc/pentane 4:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 7.03 (br s, 1H), 6.17 (br s, 1H), 5.39-5.33 (m, 2H), 4.08 (s, 1H), 3.69 (d,  $J$  = 11.8 Hz, 1H), 3.61-3.49 (m, 2H), 3.48-3.38 (m, 2H), 3.28 (d,  $J$  = 11.8 Hz, 1H), 3.01 (t,  $J$  = 6.5 Hz, 2H), 2.56 (t,  $J$  = 7.5 Hz, 2H), 2.42 (t,  $J$  = 6.3 Hz, 2H), 2.05-1.94 (m, 4H), 1.75-1.61 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.34-1.24 (m, 16H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J$  = 6.8 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 199.8, 171.1, 170.1, 130.0, 129.7, 99.1, 77.1, 71.4, 44.1, 39.6, 36.0, 34.8, 33.0, 31.8, 29.7, 29.7, 29.5, 29.1, 29.0, 29.0, 28.9, 28.4, 27.2, 27.1, 25.6, 22.6, 22.1, 18.9, 18.7, 14.1; (+)-ESI MS  $m/z$ : 555.5 [M+H]<sup>+</sup>.

**(R)-S-((Z)-9-Octadecenoyl)-N-pantothenoylcysteamine acetonide**

Colorless oil (199 mg, 0.34 mmol, 97%)  $R_f$  = 0.3 (EtOAc/pentane 4:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 7.03 (br s, 1H), 6.18 (br s, 1H), 5.37-5.31 (m, 2H), 4.08 (s, 1H), 3.69 (d,  $J$  = 11.5 Hz, 1H), 3.61-3.49 (m, 2H), 3.48-3.38 (m, 2H), 3.28 (d,  $J$  = 11.5 Hz, 1H), 3.01 (t,  $J$  = 6.5 Hz, 2H), 2.56 (t,  $J$  = 7.5 Hz, 2H), 2.43 (t,  $J$  = 6.3 Hz, 2H), 2.05-1.98 (m, 4H), 1.69-1.61 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.34-1.24 (m, 20H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J$  = 6.8 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 199.8, 171.1, 170.1, 130.0, 129.7, 99.1, 77.1, 71.4, 44.1, 39.6, 36.0, 34.8, 33.0, 31.8, 29.7, 29.7, 29.5, 29.5, 29.3, 29.3, 29.1, 29.0, 29.0, 28.4, 27.2, 27.1, 25.6, 22.7, 22.1, 18.9, 18.7, 14.1; (+)-ESI MS  $m/z$ : 583.2 [M+H]<sup>+</sup>.

**(R)-S-((Z)-11-Octadecenoyl)-N-pantothenoylcysteamine acetonide**

Colorless oil (197 mg, 0.34 mmol, 97%)  $R_f = 0.3$  (EtOAc/pentane 4:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.02$  (br s, 1H), 6.10 (br s, 1H), 5.39-5.33 (m, 2H), 4.08 (s, 1H), 3.69 (d,  $J = 11.5$  Hz, 1H), 3.62-3.50 (m, 2H), 3.49-3.38 (m, 2H), 3.28 (d,  $J = 11.8$  Hz, 1H), 3.01 (t,  $J = 6.5$  Hz, 2H), 2.56 (t,  $J = 7.5$  Hz, 2H), 2.42 (t,  $J = 6.1$  Hz, 2H), 2.05-1.94 (m, 4H), 1.68-1.61 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.35-1.24 (m, 20H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 199.8, 171.1, 170.1, 130.0, 129.8, 99.1, 77.2, 71.5, 44.2, 39.6, 36.0, 34.8, 33.0, 31.8, 29.7, 29.6, 29.5, 29.5, 29.4, 29.2, 29.0, 29.0, 28.8, 28.5, 27.2, 27.2, 25.6, 22.7, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$ : 583.5  $[\text{M}+\text{H}]^+$ .

**(R)-S-((2E,11Z)-2,11-Octadecadienoyl)-N-pantothenoylcysteamine acetonide**

Colorless oil (118 mg, 0.20 mmol, 67%)  $R_f = 0.3$  (EtOAc/pentane 2:1);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta = 7.04$  (br s, 1H), 6.93 (dt,  $J = 15.6, 7.0$  Hz, 1H), 6.13 (br s, 1H), 6.1 (t,  $J = 1.5$  Hz, 1H), 5.39-5.31 (m, 2H), 4.08 (s, 1H), 3.69 (d,  $J = 11.8$  Hz, 1H), 3.62-3.51 (m, 2H), 3.49-3.41 (m, 2H), 3.28 (d,  $J = 11.5$  Hz, 1H), 3.08 (t,  $J = 6.4$  Hz, 2H), 2.42 (t,  $J = 6.1$  Hz, 2H), 2.23-2.17 (m, 2H), 2.06-1.99 (m, 4H), 1.46 (s, 3H), 1.42 (s, 3H), 1.40-1.23 (m, 18H), 1.04 (s, 3H), 0.97 (s, 3H), 0.88 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 190.1, 171.2, 170.1, 146.8, 130.0, 129.7, 128.2, 99.1, 77.1, 71.4, 39.7, 35.9, 34.8, 33.0, 32.2, 31.8, 29.7, 29.7, 29.5, 29.2, 29.1, 29.1, 29.0, 28.2, 27.9, 27.2, 27.1, 22.7, 22.1, 18.9, 18.7, 14.1$ ; (+)-ESI MS  $m/z$ : 581.0  $[\text{M}+\text{H}]^+$ .



### **(R)-S-Acyl-N-pantothenoylcysteamines (10–13)**

The thioesters **9** were dissolved in AcOH/H<sub>2</sub>O 2:1 (2 mL) and stirred for 5 h at room temperature. The solvent was evaporated in vacuo and the crude product was used for incubation experiments without further purification [13].

### **(R)-S-Butanoyl-N-pantothenoylcysteamine (10a)**

Crude product (18.9 mg, 0.05 mmol, 99%); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ = 3.88 (s, 1H), 3.51-3.33 (m, 6H), 3.00 (t, *J* = 6.7 Hz, 2H), 2.56 (t, *J* = 7.5 Hz, 2H), 2.40 (t, *J* = 6.7 Hz, 2H), 1.67 (sxt, *J* = 7.4 Hz, 2H), 0.95 (t, *J* = 7.3 Hz, 3H), 0.91 (s, 6H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ = 200.6, 176.1, 173.9, 77.3, 70.4, 46.7, 40.4, 40.1, 36.4, 36.3, 29.1, 21.3, 20.9, 20.2, 13.8; HRMS (ESI +) *m/z*: 371.1612 [M+Na]<sup>+</sup>.

### **(R)-S-Hexanoyl-N-pantothenoylcysteamine (10b)**

Crude product (15.4 mg, 0.04 mmol, 99%); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ = 3.88 (s, 1H), 3.53-3.33 m, 6H), 2.99 (t, *J* = 6.8 Hz, 2H), 2.57 (t, *J* = 7.4 Hz, 2H), 2.40 (t, *J* = 6.7 Hz, 2H), 1.65 (quin, *J* = 7.2 Hz, 2H), 1.36-1.29 (m, 4H), 0.91, (s, 6H), 0.91 (t, *J* = 7.02 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ = 200.7, 176.1, 173.9, 77.3, 70.4, 44.8, 40.4, 40.1, 36.4, 36.4, 32.2, 29.1, 26.4, 23.4, 21.4, 21.0, 14.2; HRMS (ESI +) *m/z*: 399.1922 [M+Na]<sup>+</sup>.

### **(R)-S-Octanoyl-N-pantothenoylcysteamine (10c)**

Crude product (7.9 mg, 0.01 mmol, 99%); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ = 3.88 (s, 1H), 3.52-3.33 (m, 6H), 2.99 (t, *J* = 6.7 Hz, 2H), 2.57 (t, *J* = 7.5 Hz, 2H), 2.40 (t, *J* = 6.7 Hz, 2H), 1.64 (quin, *J* = 7.3 Hz, 2H), 1.35-1.25 (m, 8H), 0.91 (s, 6H), 0.90 (t, *J* = 7.02 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ = 200.7, 176.1, 173.9, 77.3, 70.4,

44.9, 40.4, 40.1, 36.4, 36.4, 32.8, 30.1, 30.0, 29.1, 26.7, 23.7, 21.4, 20.9, 14.4;  
HRMS (ESI +)  $m/z$ : 427.2239 [M+Na]<sup>+</sup>.

**(R)-S-Decanoyl-N-pantothenoylcysteamine (10d)**

Crude product (12.7 mg, 0.02 mmol, 99%); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ= 3.88 (s, 1H), 3.52-3.37 (m, 6H), 2.99 (t,  $J$  = 6.7 Hz, 2H), 2.57 (t,  $J$  = 7.5 Hz, 2H), 2.40 (t,  $J$  = 6.7 Hz, 2H), 1.64 (quin,  $J$  = 7.2 Hz, 2H), 1.35-1.22 (m, 12H), 0.91 (s, 6H), 0.90 (t,  $J$  = 7.02 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ= 200.7, 176.1, 173.9, 77.3, 70.4, 44.9, 40.4, 40.2, 36.4, 36.4, 33.1, 30.5, 30.4, 30.4, 30.0, 29.1, 26.7, 23.8, 21.4, 21.0, 14.5;  
HRMS (ESI +)  $m/z$ : 433.2733 [M+H]<sup>+</sup>, 455.2554 [M+Na]<sup>+</sup>.

**(R)-S-Dodecanoyl-N-pantothenoylcysteamine (10e)**

Crude product (16 mg, 0.03 mmol, 99%); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ= 3.88 (s, 1H), 3.52-3.37 (m, 6H), 2.99 (t,  $J$  = 6.7 Hz, 2H), 2.57 (t,  $J$  = 7.3 Hz, 2H), 2.40 (t,  $J$  = 6.7 Hz, 2H), 1.64 (quin,  $J$  = 7.2 Hz, 2H), 1.34-1.27 (m, 16H), 0.91 (s, 6H), 0.90 (t,  $J$  = 7.0 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ= 200.7, 176.1, 173.9, 77.3, 70.4, 44.9, 40.4, 40.2, 36.4, 36.4, 33.1, 30.7, 30.7, 30.6, 30.5, 30.4, 30.0, 29.2, 26.7, 23.8, 21.4, 21.0, 14.5; HRMS (ESI +)  $m/z$ : 483.2868 [M+Na]<sup>+</sup>.

**(R)-S-Tetradecanoyl-N-pantothenoylcysteamine (10f)**

Crude product (6.1 mg, 0.01 mmol, 98%); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ= 3.88 (s, 1H), 3.52-3.33 (m, 6H), 2.99 (t,  $J$  = 6.7 Hz, 2H), 2.57 (t,  $J$  = 7.3 Hz, 2H), 2.40 (t,  $J$  = 6.7 Hz, 2H), 1.64 (quin,  $J$  = 7.2 Hz, 2H), 1.36-1.26 (m, 20H), 0.91 (s, 6H), 0.90 (t,  $J$  = 7.3 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ= 200.7, 176.1, 173.9, 77.3, 70.4, 44.9,

40.4, 40.1, 36.4, 36.4, 33.1, 30.8, 30.8, 30.8, 30.7, 30.6, 30.5, 30.4, 30.0, 29.1, 26.7, 23.8, 21.4, 20.9, 14.5; HRMS (ESI +)  $m/z$ : 511.3175 [M+Na]<sup>+</sup>.

**(R)-S-Hexadecanoyl-N-pantothenoylcysteamine (10g)**

Crude product (5.8 mg, 0.01 mmol, 99%); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ= 3.88 (s, 1H), 3.52-3.33 (m, 6H), 2.99 (t,  $J$  = 6.7 Hz, 2H), 2.57 (t,  $J$  = 7.3 Hz, 2H), 2.40 (t,  $J$  = 6.7 Hz, 2H), 1.64 (quin,  $J$  = 7.2 Hz, 2H), 1.33-1.25 (m, 24H), 0.91 (s, 6H), 0.90 (t,  $J$  = 7.0 Hz, 3H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ= 200.7, 176.1, 173.9, 77.3, 70.4, 44.9, 40.4, 40.1, 36.4, 36.4, 33.1, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.7, 30.6, 30.5, 30.4, 30.0, 29.1, 26.7, 23.8, 21.4, 21.0, 14.5; HRMS (ESI +)  $m/z$ : 539.3489 [M+Na]<sup>+</sup>.

**(R)-S-Octadecanoyl-N-pantothenoylcysteamine (10h)**

Crude product (3.4 mg, 0.006 mmol, 99%); <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD): δ= 3.88 (s, 1H), 3.51-3.32 (m, 6H), 2.99 (t,  $J$  = 6.8 Hz, 2H), 2.57 (t,  $J$  = 7.4 Hz, 2H), 2.40 (t,  $J$  = 6.7 Hz, 2H), 1.64 (quin,  $J$  = 7.3 Hz, 2H), 1.33-1.28 (m, 28H), 0.91 (s, 6H), 0.90 (t,  $J$  = 7.1 Hz, 3H); <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD): δ= 200.7, 176.1, 173.9, 77.3, 70.4, 44.9, 40.4, 40.1, 36.4, 36.4, 33.1, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.7, 30.6, 30.5, 30.4, 30.0, 29.1, 26.7, 23.8, 21.4, 20.9, 14.5; HRMS (ESI +)  $m/z$ : 567.3802 [M+Na]<sup>+</sup>.

**(R)-S-Icosanoyl-N-pantothenoylcysteamine (10i)**

Crude product (21.2 mg, 0.03 mmol, 98%); <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD): δ= 3.88 (s, 1H), 3.51-3.33 (m, 6H), 2.99 (t,  $J$  = 6.1 Hz, 2H), 2.57 (t,  $J$  = 7.0 Hz, 2H), 2.39 (t,  $J$  = 6.7 Hz, 2H), 1.64 (quin,  $J$  = 7.2 Hz, 2H), 1.34-1.28 (m, 32H), 0.91 (s, 6H), 0.89 (t,  $J$  = 7.1 Hz, 3H); <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD): δ= 200.5, 176.1, 173.9, 77.3, 70.4, 44.9, 44.4, 40.2, 36.4, 36.3, 34.0, 33.1, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.8, 30.7, 30.6,

30.5, 30.4, 30.0, 29.8, 29.2, 26.7, 23.8, 21.4, 21.0, 14.5; HRMS (ESI +)  $m/z$ : 595.4117 [M+Na]<sup>+</sup>.

**(R)-S-(3-Hydroxydecanoyl)-N-pantothenoylcysteamine (12)**

Crude product (12.8 mg, 0.02 mmol, 99%); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ = 4.04-3.99 (m, 1H), 3.89 (s, 1H), 3.52-3.32 (m, 6H), 3.02 (t,  $J = 6.7$  Hz, 2H), 2.73-2.65 (m, 2H), 2.40 (t,  $J = 6.6$  Hz, 2H), 1.47-1.44 (m, 2H), 1.36-1.28 (m, 10H), 0.92 (s, 6H), 0.90 (t,  $J = 7.0$  Hz, 3H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ = 198.9, 176.1, 174.0, 77.3, 70.4, 69.6, 52.7, 40.4, 40.0, 38.2, 36.4, 36.4, 33.0, 30.6, 30.4, 29.3, 26.6, 23.7, 21.3, 21.0, 14.5; HRMS (ESI +)  $m/z$ : 471.2505 [M+Na]<sup>+</sup>.

**(R)-S-((Z)-7-Tetradecenoyl)-N-pantothenoylcysteamine (11a)**

Crude product (15.6 mg, 0.02 mmol, 99%); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ = 5.42-5.32 (m, 2H), 3.89 (s, 1H), 3.52-3.33 (m, 6H), 2.99 (t,  $J = 6.7$  Hz, 2H), 2.59-2.55 (m, 2H), 2.40 (t,  $J = 6.7$  Hz, 2H), 1.67-1.62 (m, 4H), 1.37-1.29 (m, 14H), 0.91 (s, 6H), 0.90 (t,  $J = 7.02$  Hz, 3H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ = 200.6, 176.1, 173.9, 131.8, 131.2, 77.3, 70.4, 44.8, 40.4, 40.1, 36.4, 36.4, 33.0, 30.8, 30.4, 29.9, 29.4, 29.1, 28.2, 27.9, 26.6, 23.7, 21.3, 21.0, 14.5; HRMS (ESI +)  $m/z$ : 509.3019 [M+Na]<sup>+</sup>.

**(R)-S-((Z)-9-Hexadecenoyl)-N-pantothenoylcysteamine (11b)**

Crude product (191.6 mg, 0.35 mmol, 98 %); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ = 5.34 (t,  $J = 4.9$  Hz, 2H), 3.88 (s, 1H), 3.51-3.33 (m, 6H), 2.99 (t,  $J = 6.8$  Hz, 2H), 2.57 (t,  $J = 7.3$  Hz, 2H), 2.40 (t,  $J = 6.7$  Hz, 2H), 2.05-1.97 (m, 4H), 1.64 (quin,  $J = 6.8$  Hz, 2H), 1.38-1.27 (m, 16 H), 0.91 (s, 6H), 0.90 (t,  $J = 7.3$  Hz, 3H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ = 200.6, 176.1, 173.9, 131.5, 130.8, 77.3, 70.4, 44.8, 40.4, 40.1, 36.4,

36.4, 33.0, 30.9, 30.8, 30.2, 30.1, 30.1, 30.0, 29.1, 28.2, 28.1, 26.7, 23.7, 21.4, 21.0, 14.5; HRMS (ESI +)  $m/z$ : 537.3334 [M+Na]<sup>+</sup>.

**(R)-S-((Z)-9-Octadecenoyl)-N-pantothenoylcysteamine (11c)**

Crude product (16.2 mg, 0.03 mmol, 99%); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ = 5.34 (t,  $J$  = 4.6 Hz, 2H), 3.88 (s, 1H), 3.53-3.33 (m, 6H), 2.99 (t,  $J$  = 6.7 Hz, 2H), 2.57 (t,  $J$  = 7.3 Hz, 2H), 2.40 (t,  $J$  = 6.5 Hz, 2H), 2.12-1.97 (m, 4H), 1.64 (quin,  $J$  = 6.8 Hz, 2H), 1.43-1.22 (m, 20H), 0.91 (s, 6H), 0.90 (t,  $J$  = 7.0 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ = 200.6, 176.1, 173.9, 130.9, 130.8, 77.3, 70.4, 44.9, 40.4, 40.2, 36.5, 36.4, 33.1, 30.9, 30.8, 30.6, 30.5, 30.4, 30.3, 30.1, 30.0, 29.2, 28.2, 28.1, 26.7, 23.8, 21.4, 21.0, 14.5; HRMS (ESI +)  $m/z$ : 565.3652 [M+Na]<sup>+</sup>.

**(R)-S-((Z)-11-Octadecenoyl)-N-pantothenoylcysteamine (11d)**

Crude product (4.4 mg, 0.008 mmol, 99%); <sup>1</sup>H NMR (600 MHz, CD<sub>3</sub>OD): δ = 5.34 (t,  $J$  = 4.9 Hz, 2H), 3.88 (s, 1H), 3.50-3.33 (m, 6H), 2.99 (t,  $J$  = 6.7 Hz, 2H), 2.57 (t,  $J$  = 7.4 Hz, 2H), 2.40 (t,  $J$  = 6.7 Hz, 2H), 2.09-1.96 (m, 4H), 1.64 (quin,  $J$  = 7.1 Hz, 2H), 1.45-1.29 (m, 20H), 0.91 (s, 6H), 0.90 (t,  $J$  = 7.2 Hz, 3H); <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD): δ = 200.7, 176.1, 173.9, 130.9, 130.8, 77.3, 70.4, 44.9, 40.4, 40.2, 36.4, 36.4, 33.1, 30.9, 30.8, 30.6, 30.5, 30.4, 30.3, 30.1, 30.0, 29.9, 29.1, 28.1, 26.7, 23.8, 21.4, 20.9, 14.5; HRMS (ESI +)  $m/z$ : 565.3647 [M+Na]<sup>+</sup>.

**(R)-S-((2E,11Z)-2,11-Octadecadienoyl)-N-pantothenoylcysteamine (13)**

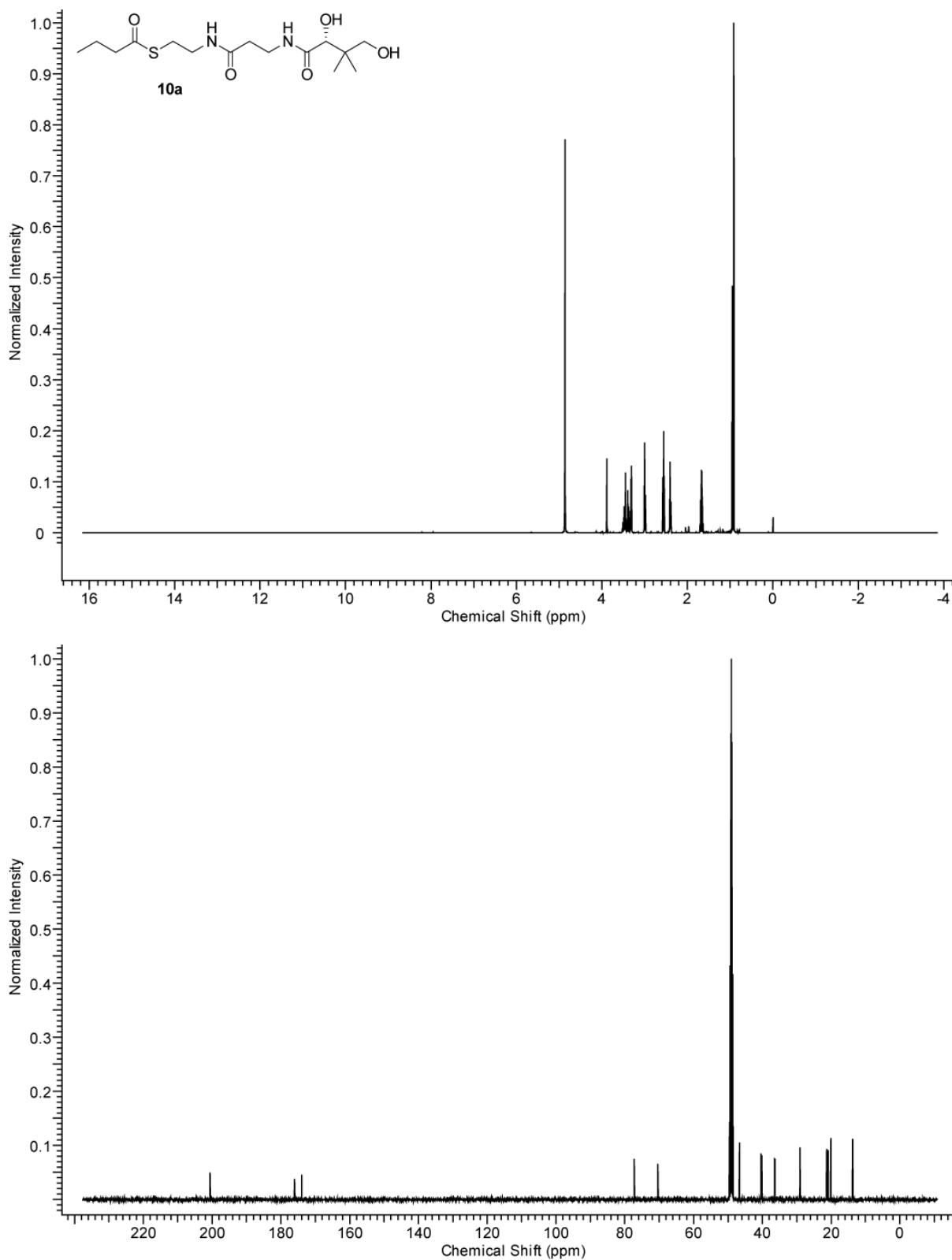
Crude product (72.6 mg, 0.13 mmol, 93%); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ = 6.92 (dt,  $J$  = 15.3, 7.0 Hz, 1H), 6.16 (d,  $J$  = 15.3 Hz, 1H), 5.34 (t,  $J$  = 4.9 Hz, 2H), 3.88 (s, 1H), 3.51-3.33 (m, 6H), 3.06 (t,  $J$  = 6.8 Hz, 2H), 2.40 (t,  $J$  = 6.7 Hz, 2H), 2.22 (q,  $J$  = 7.1 Hz, 2H), 2.07-2.01 (m, 4H), 1.49-1.29 (m, 18H), 0.91 (s, 6H), 0.90 (t,  $J$  = 6.8 Hz,

3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta = 191.1, 175.3, 173.9, 147.6, 130.9, 130.8, 129.6, 77.3, 70.4, 40.4, 40.2, 36.4, 36.4, 33.2, 33.0, 30.9, 30.8, 30.4, 30.3, 30.2, 30.1, 29.2, 28.9, 28.2, 28.1, 23.8, 21.4, 21.0, 14.5$ ; HRMS (ESI +)  $m/z$ : 541.3671  $[\text{M}+\text{H}]^+$ , 563.3491  $[\text{M}+\text{Na}]^+$ .

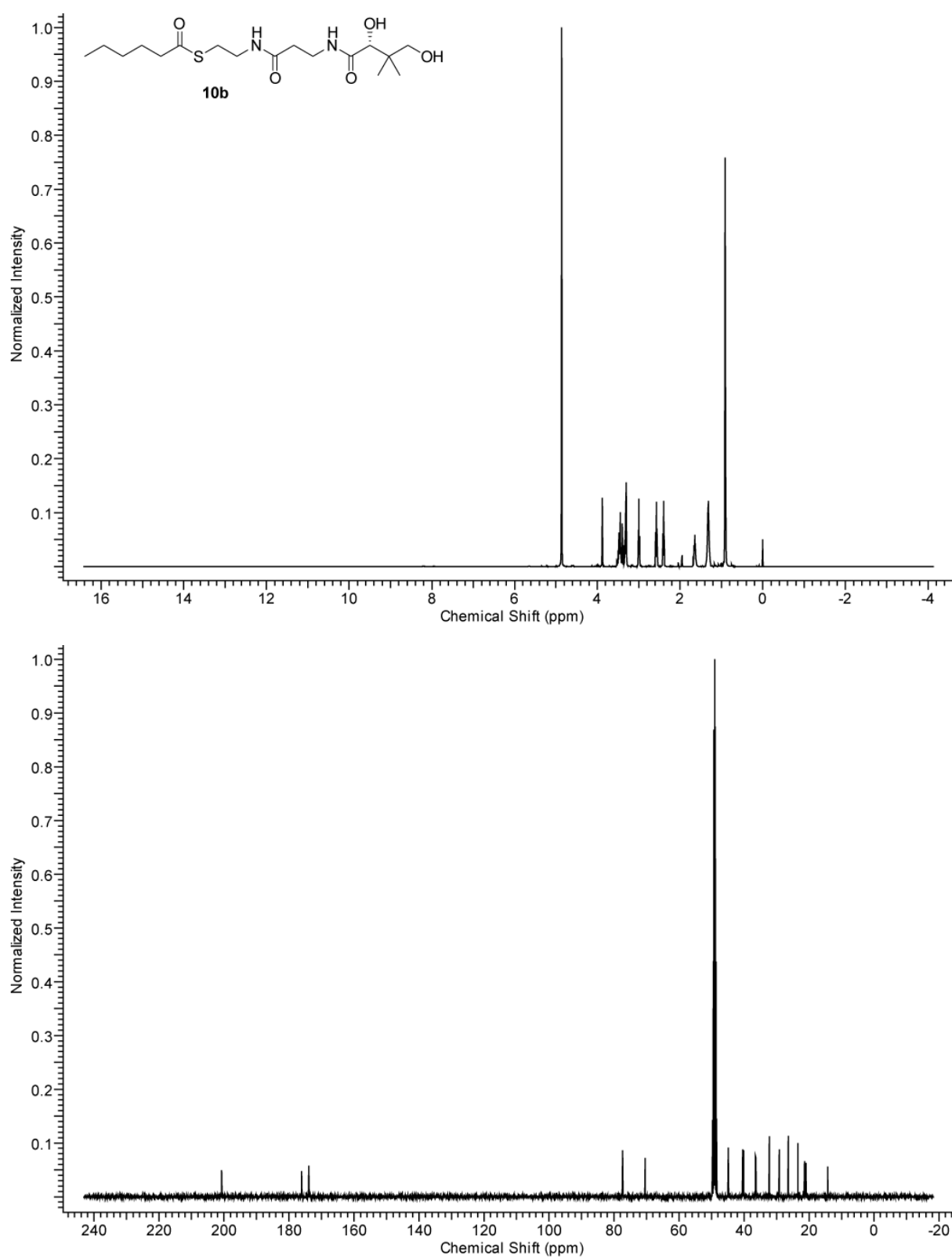
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Moore, B. S. *Org. Lett.* **2015**, *17*, 4452–4455.

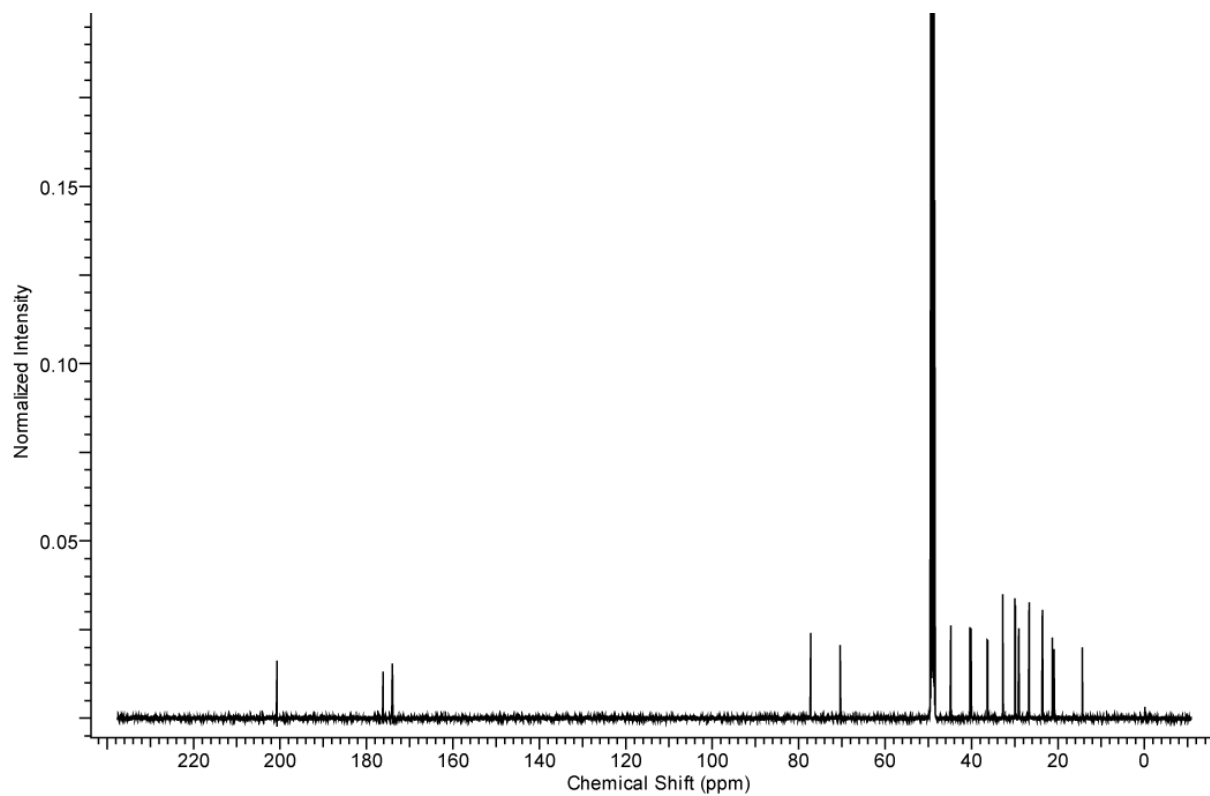
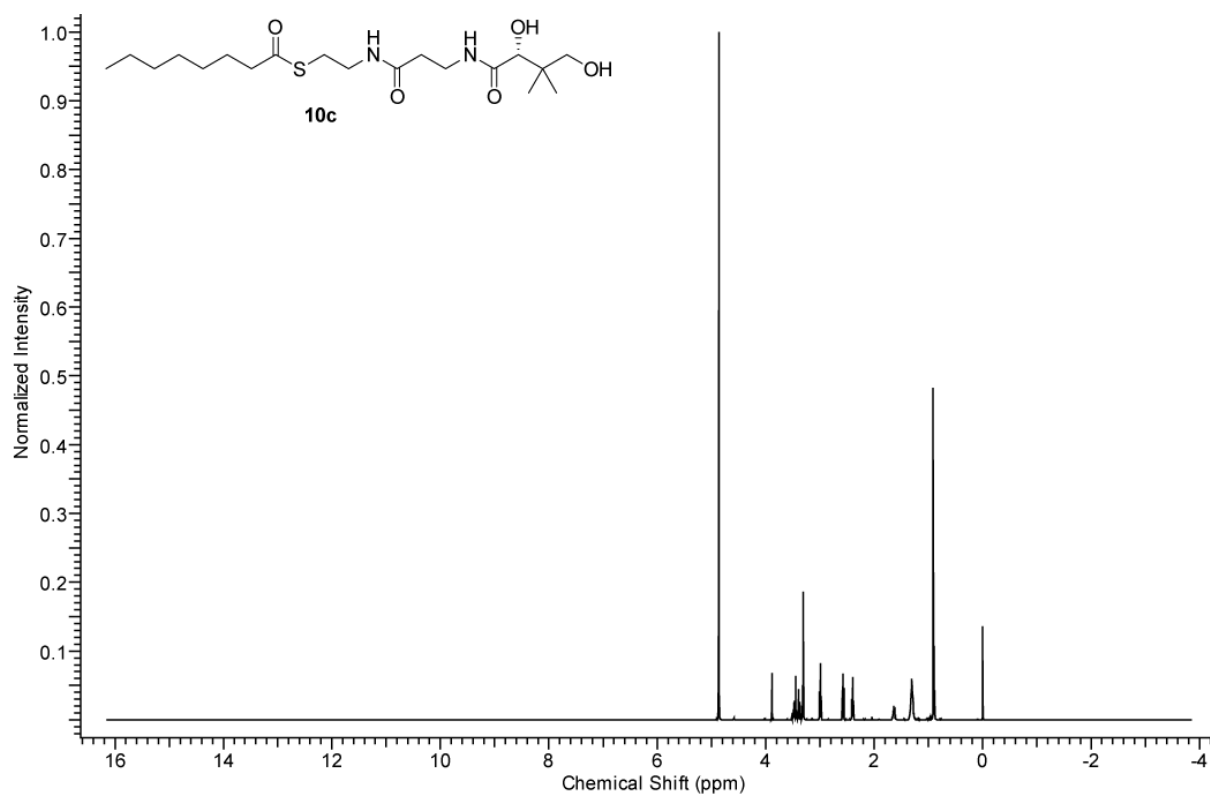


**Figure S4:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-butanoyl-*N*-pantothenoylcysteamine (**10a**).

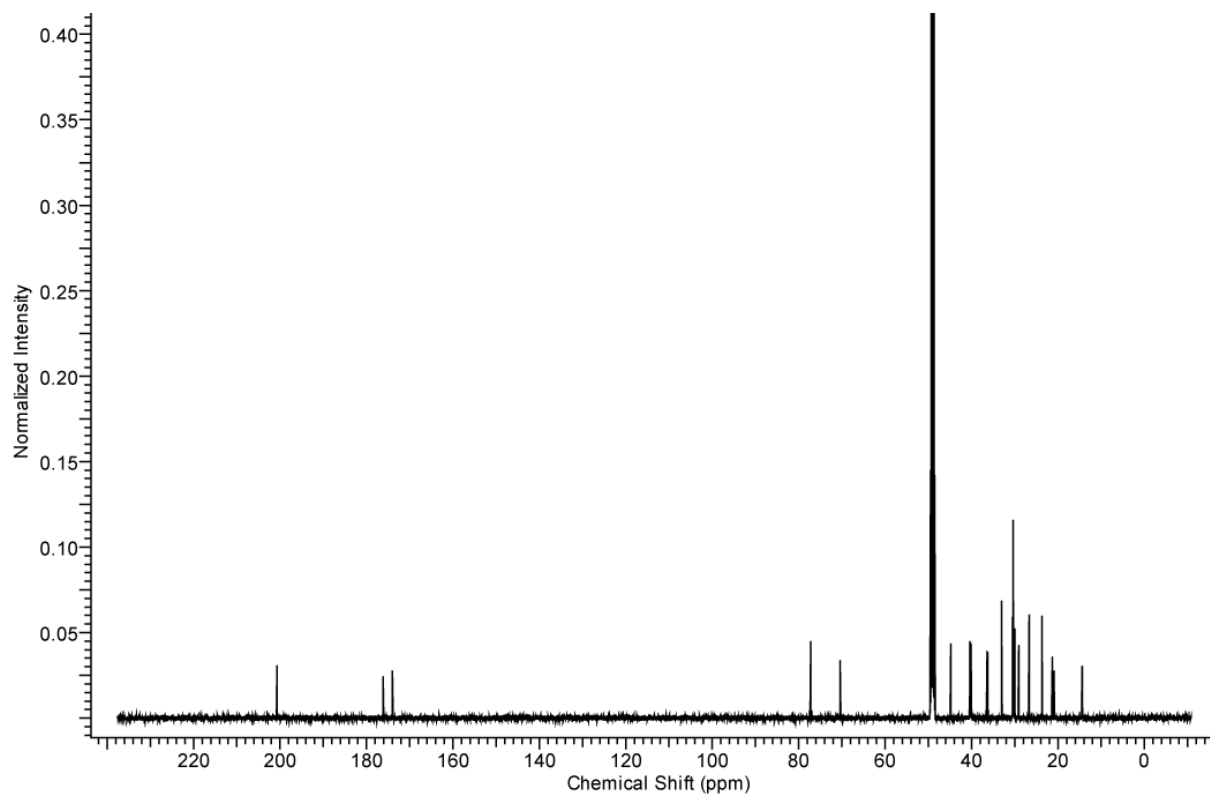
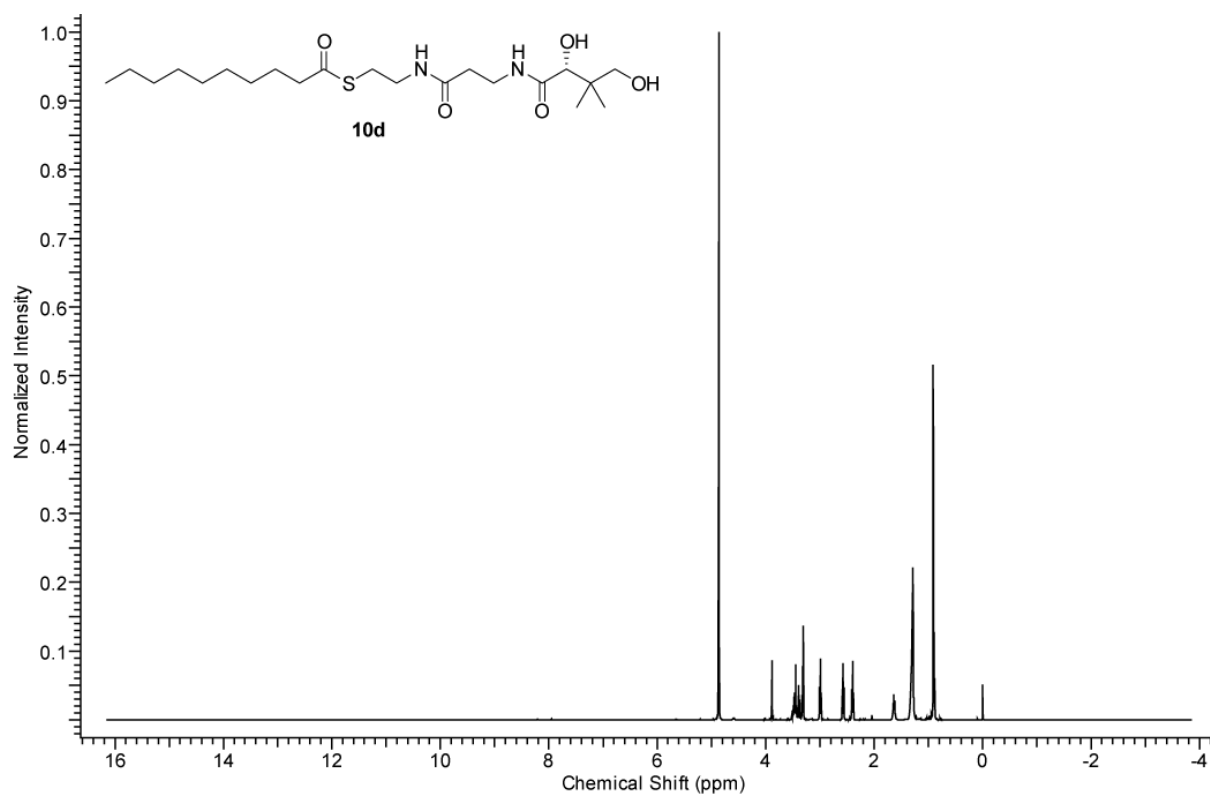


**Figure S5:**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum of S-hexanoyl-N-pantothenoylcysteamine (**10b**).

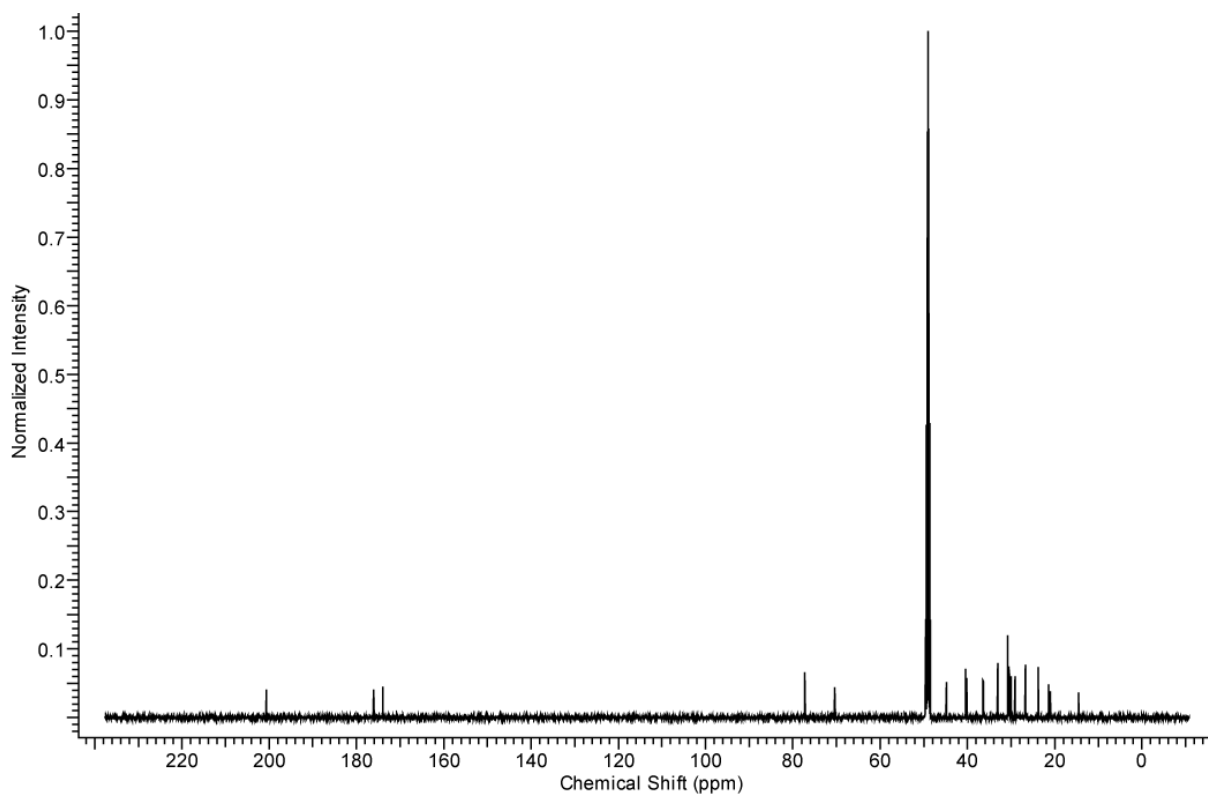
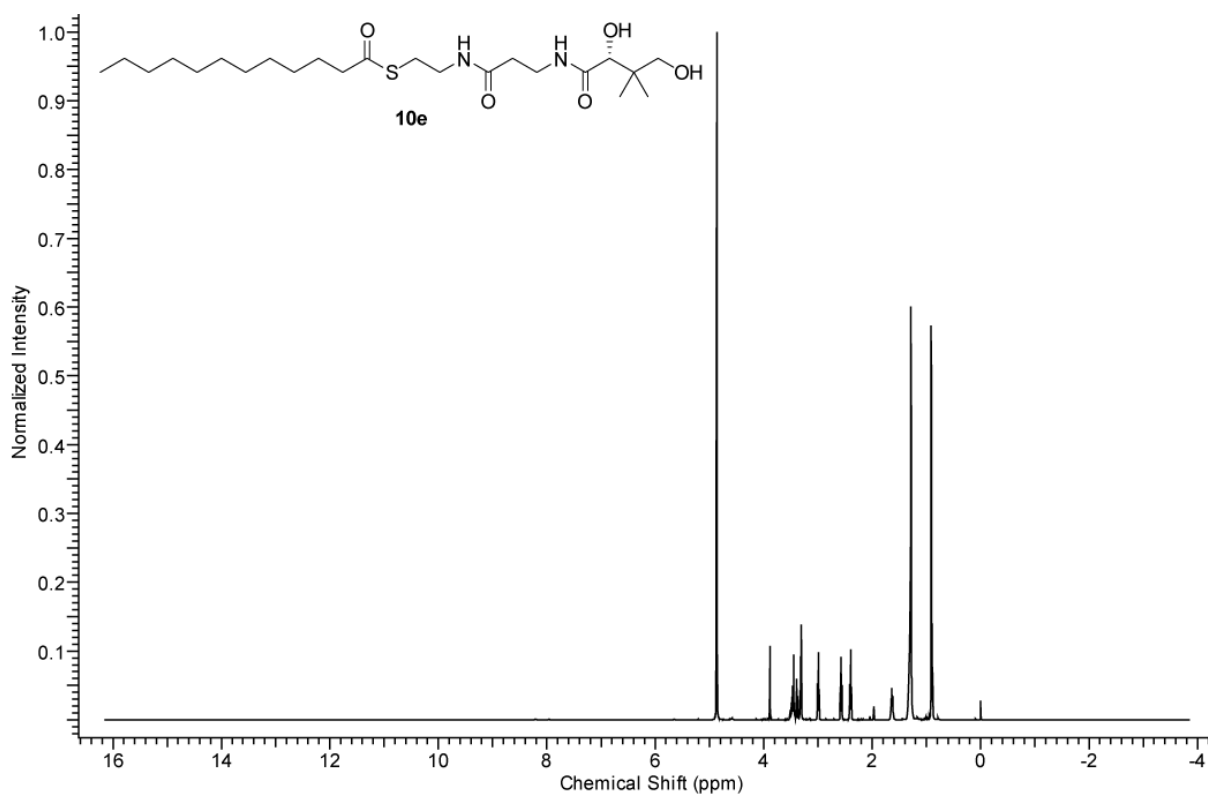




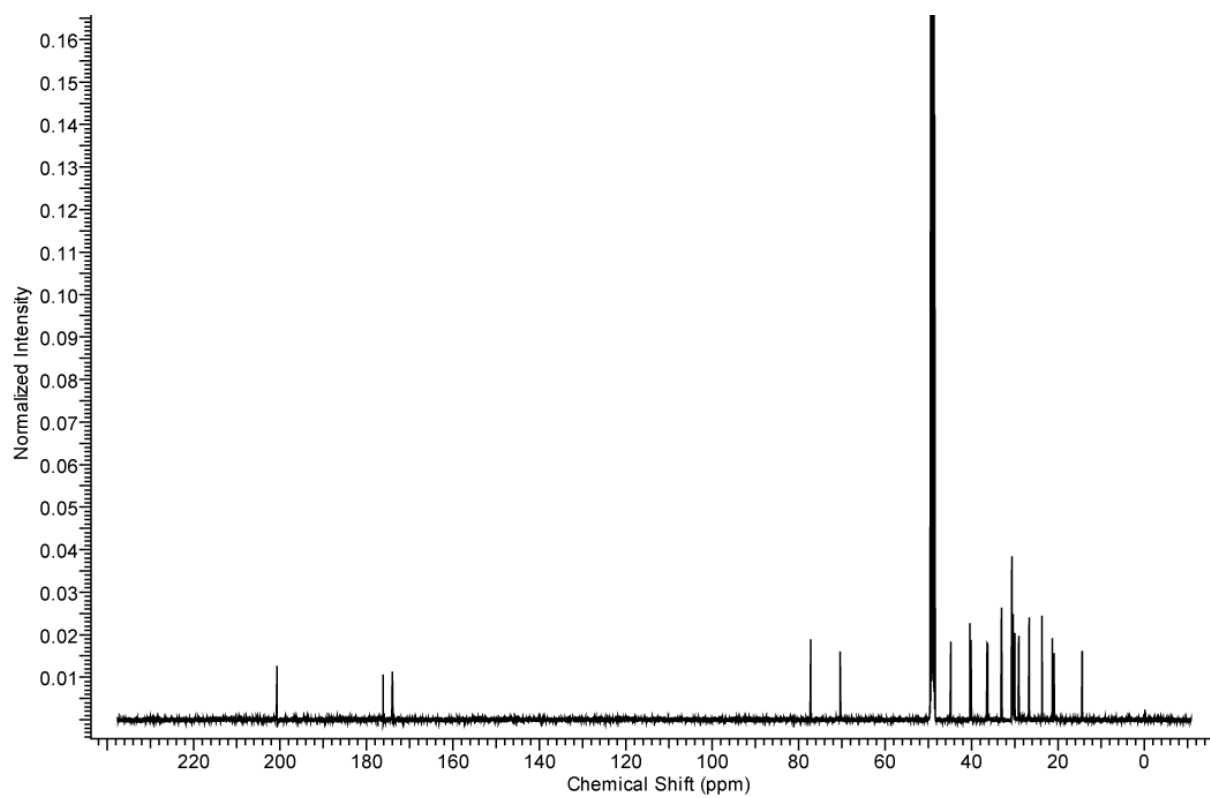
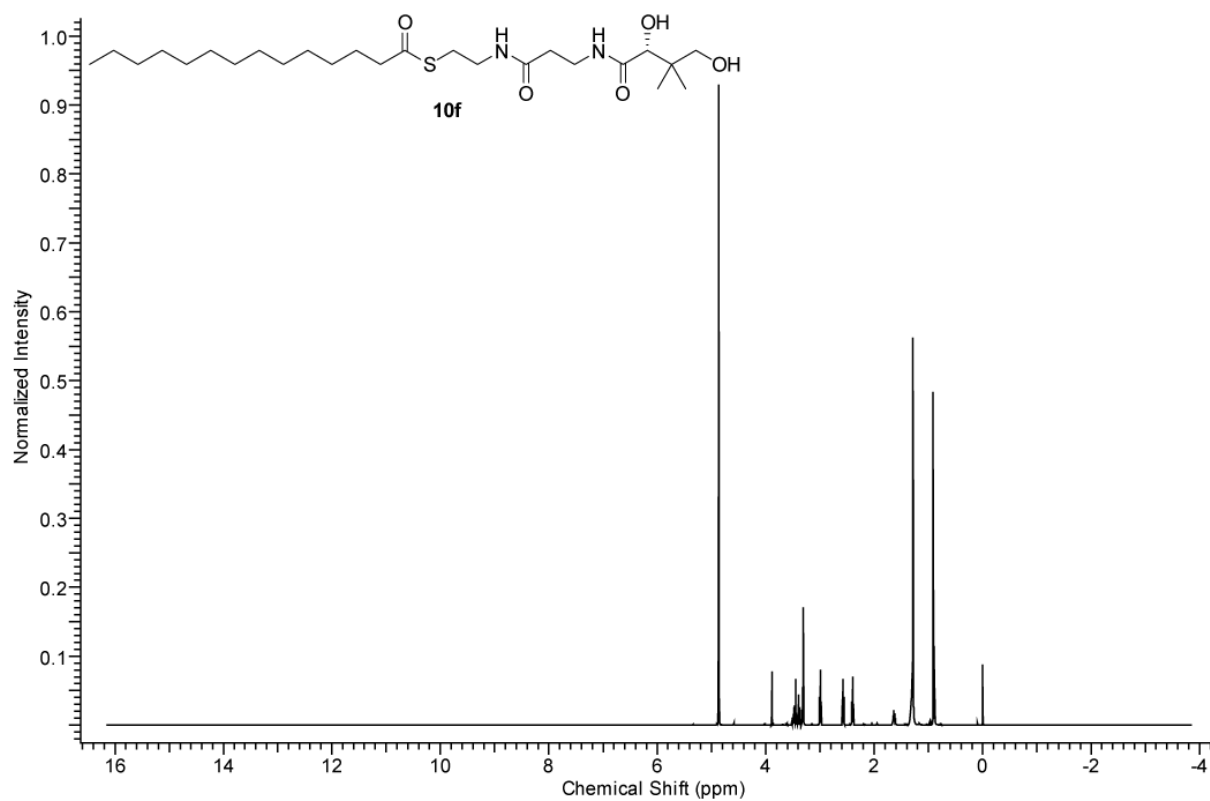
**Figure S6:**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum of *S*-octanoyl-*N*-pantothenoylcysteamine (**10c**).



**Figure S7:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-decanoyl-*N*-pantothenoylcysteamine (**10d**).

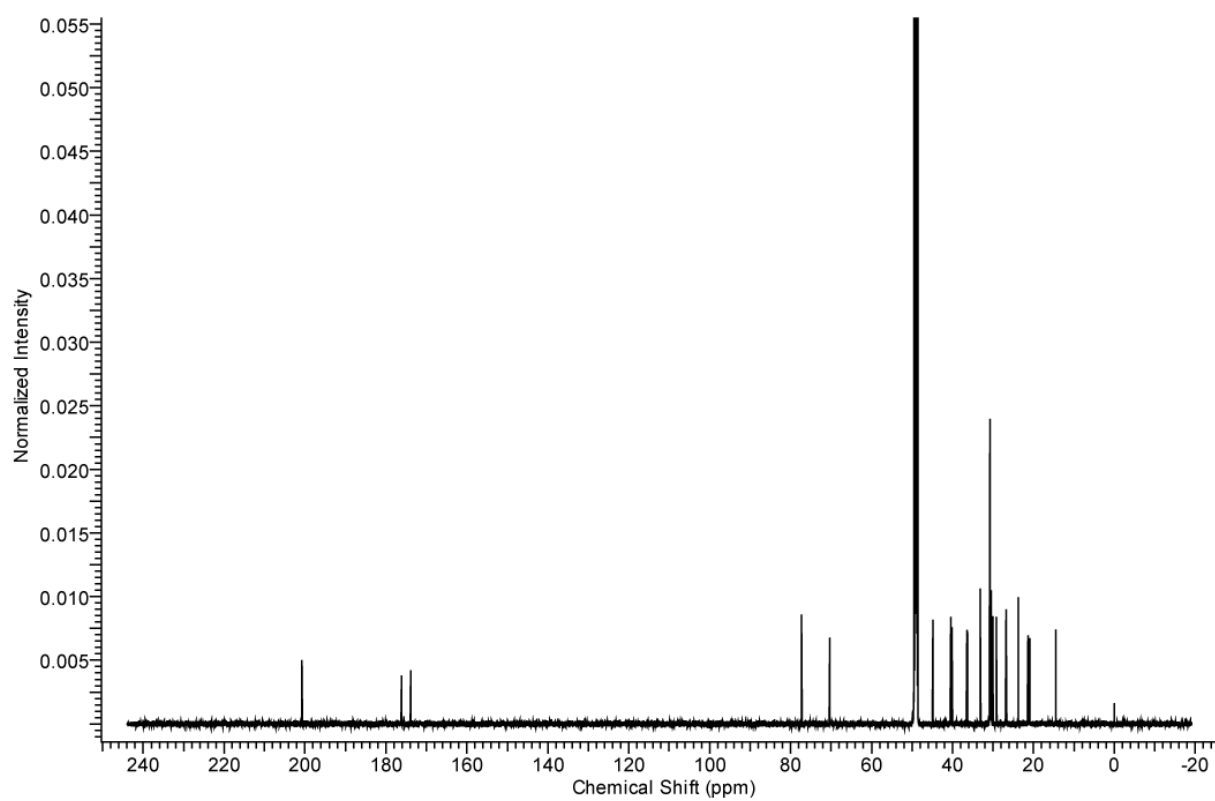
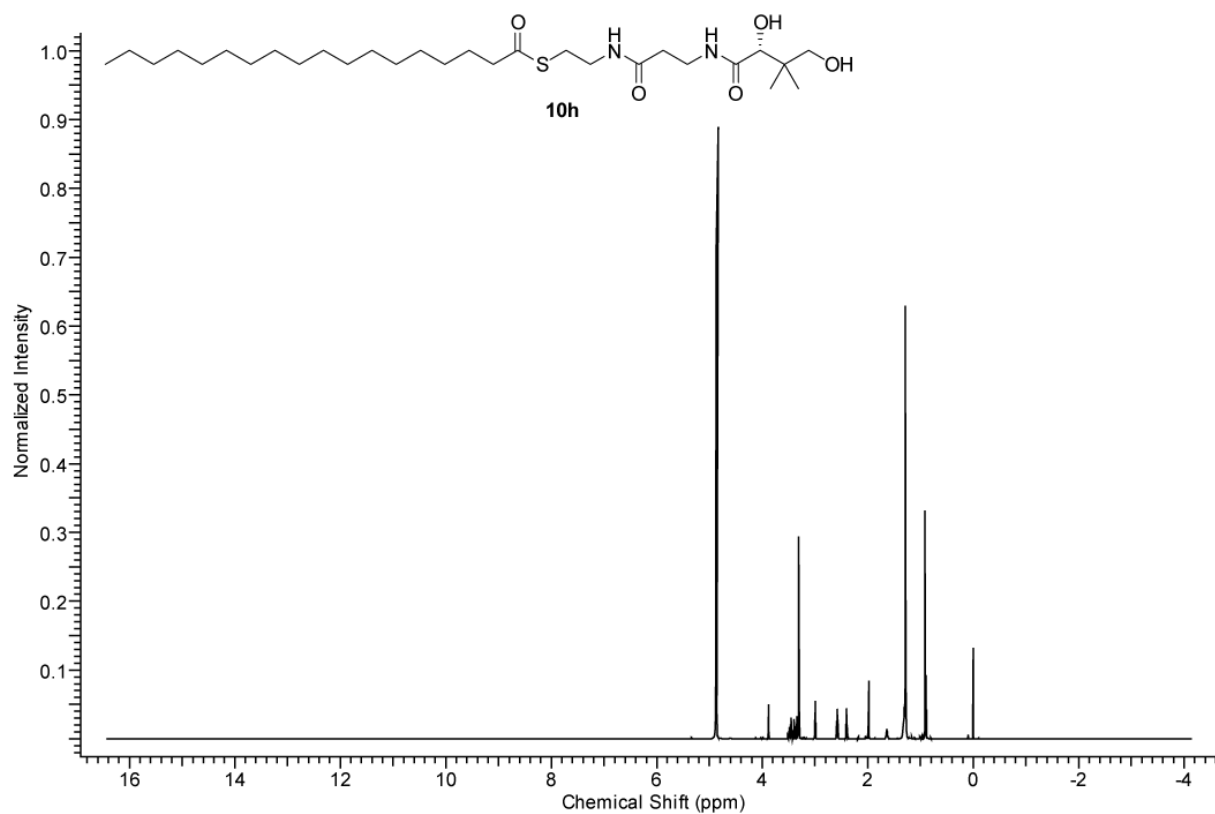


**Figure S8:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-dodecanoyl-*N*-pantothenoylcysteamine (**10e**).

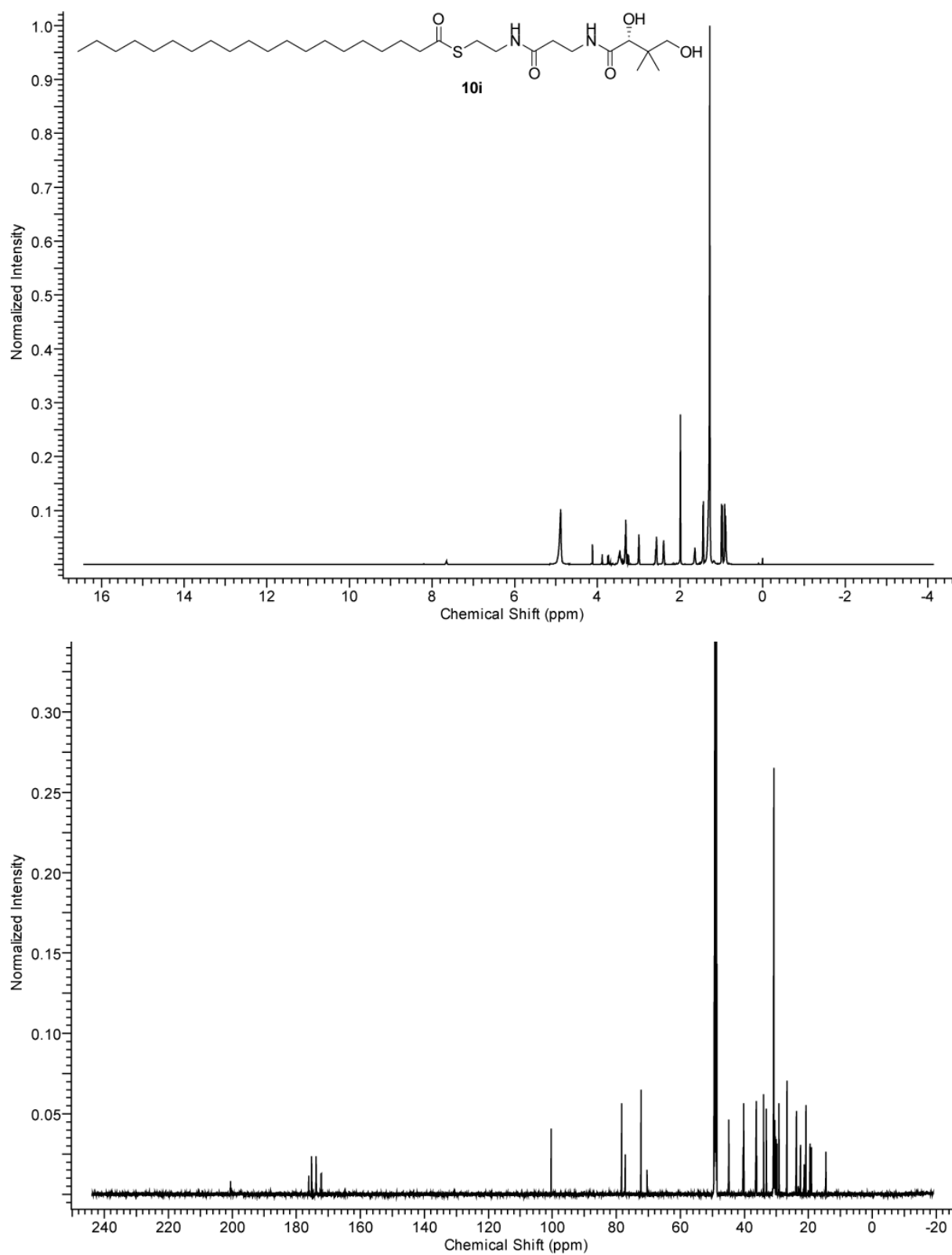


**Figure S9:**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum of *S*-tetradecanoyl-*N*-pantothenoylcysteamine (**10f**).

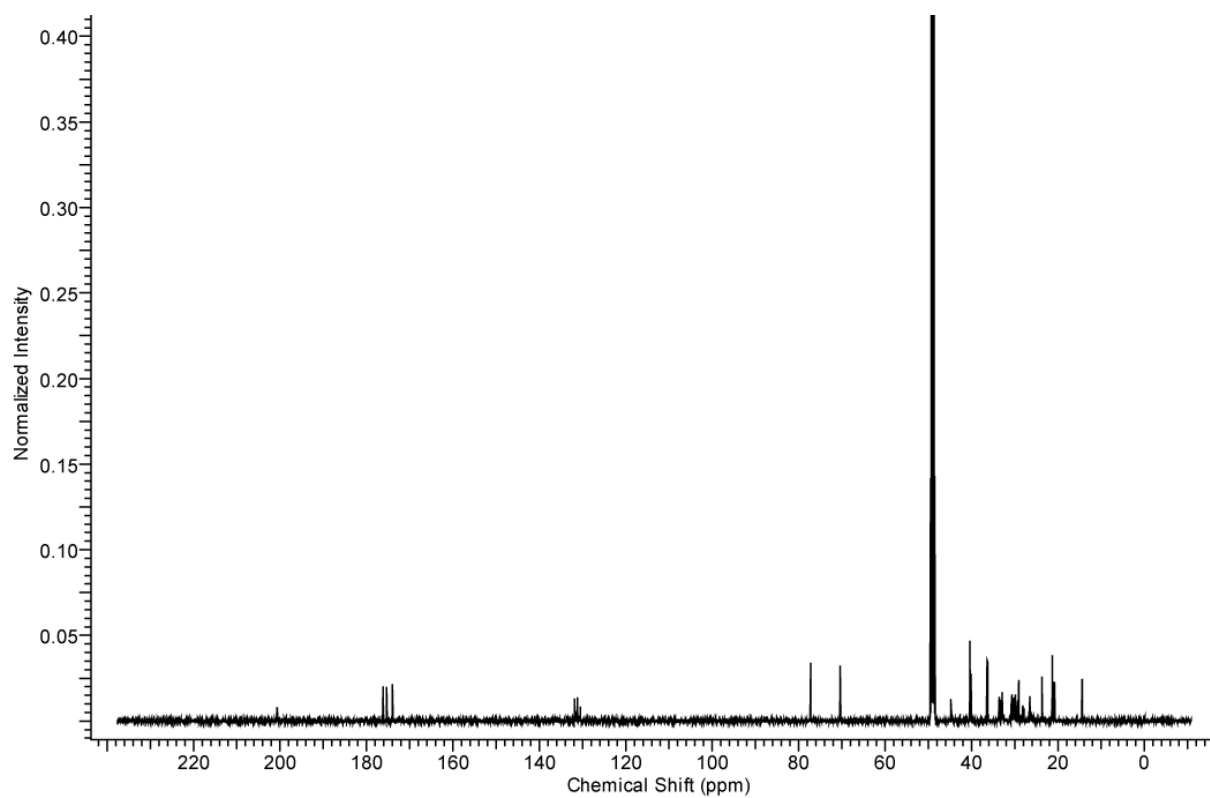
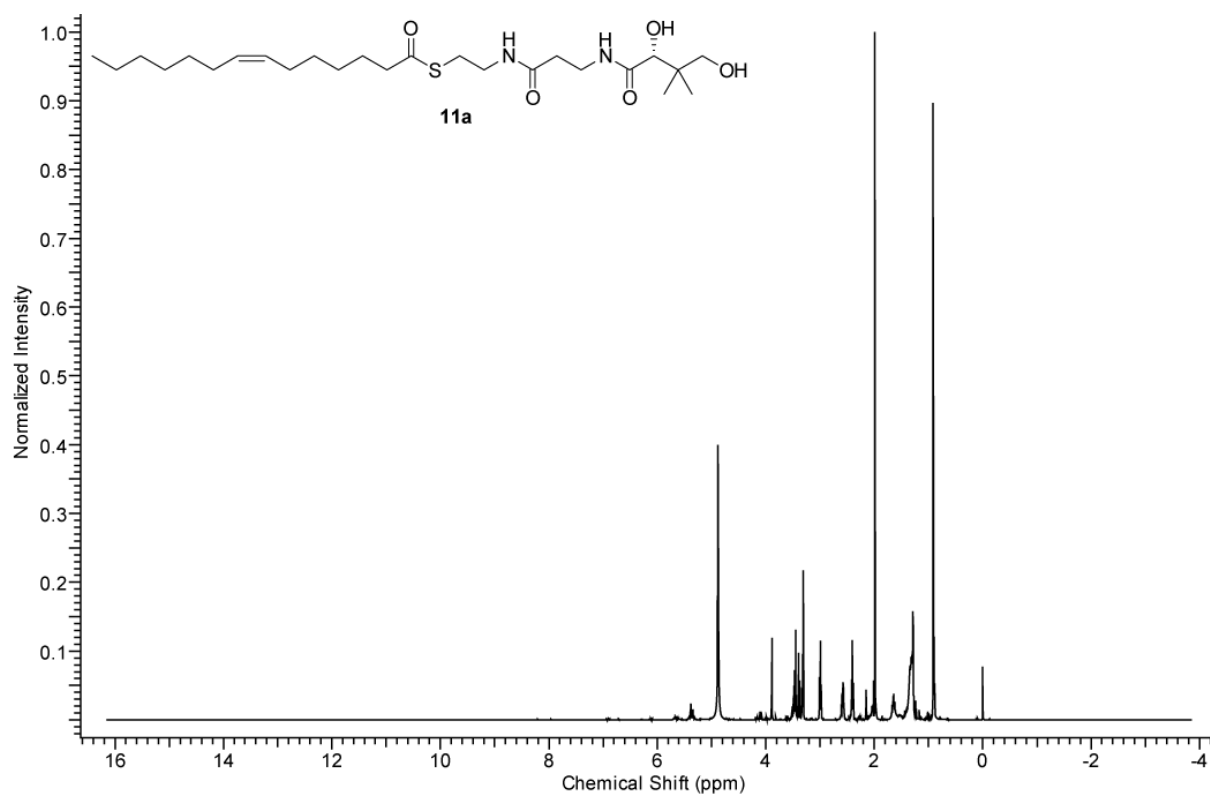




**Figure S11:**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum of *S*-octadecanoyl-*N*-pantothenoylcysteamine (**10h**).

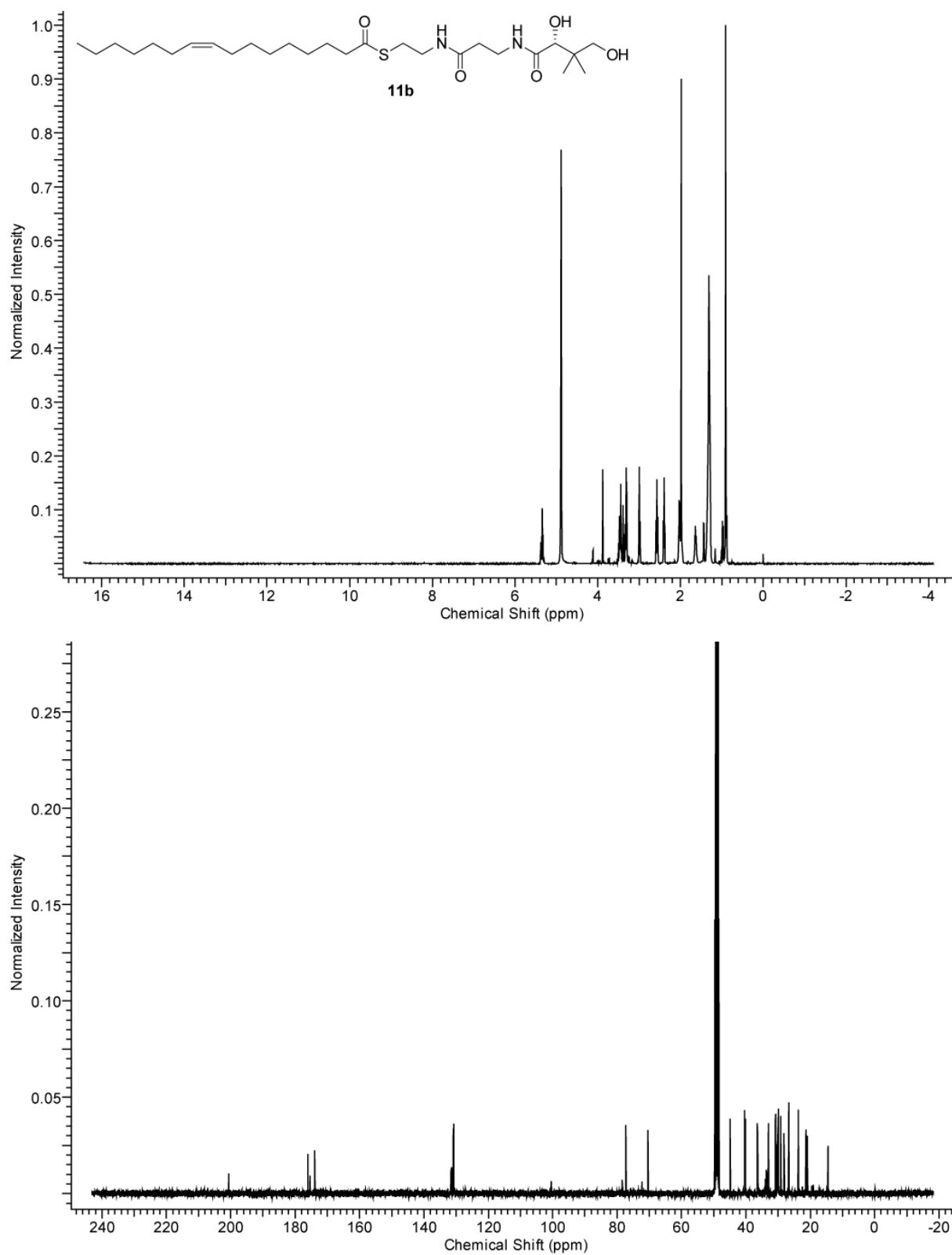


**Figure S12:**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum of S-icosanoyl-N-pantothenoylcysteamine (**10i**).

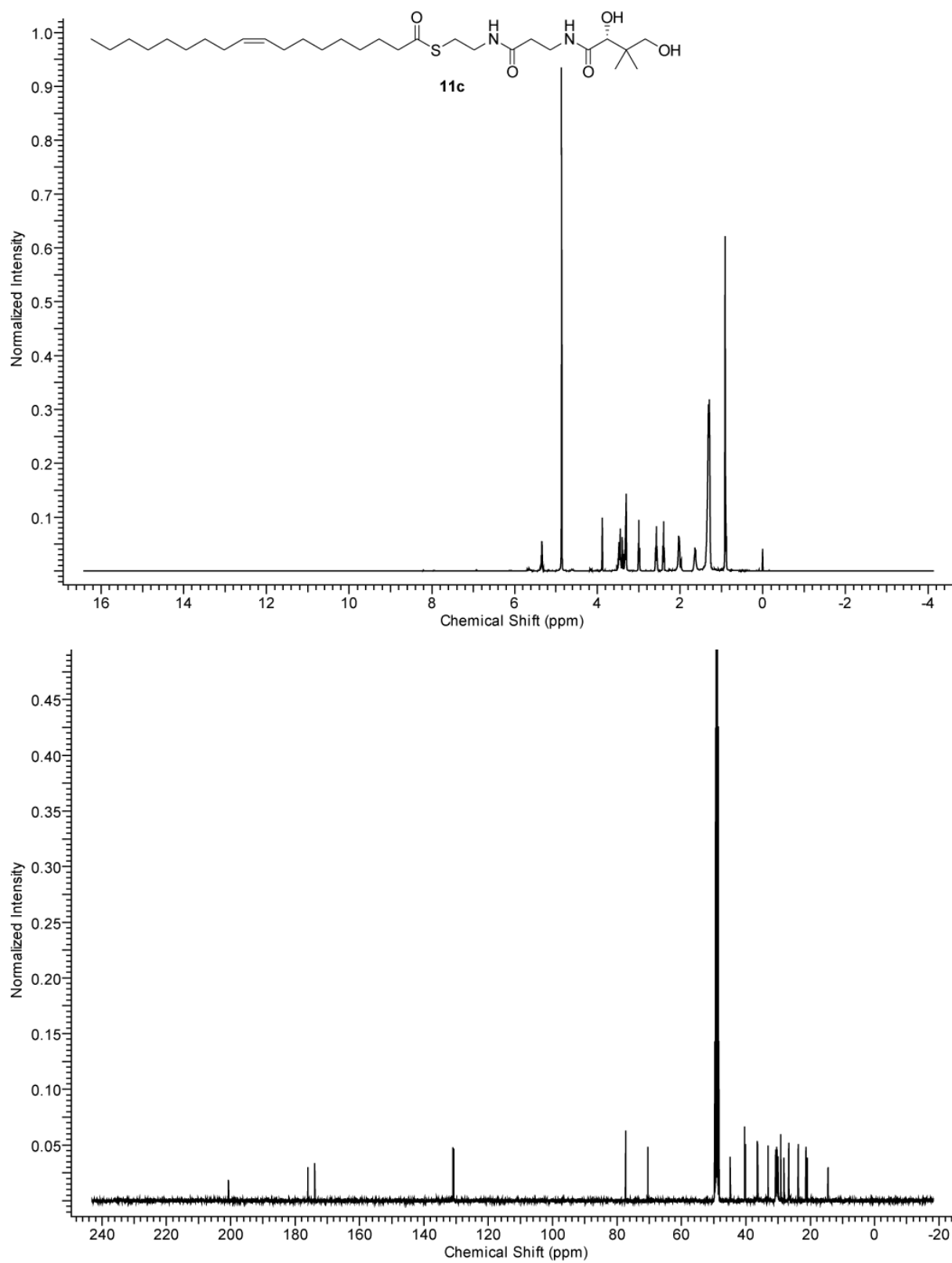


**Figure S13:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-((*Z*)-7-tetradecenoyl)-*N*-pantothenoylcysteamine (**11a**).

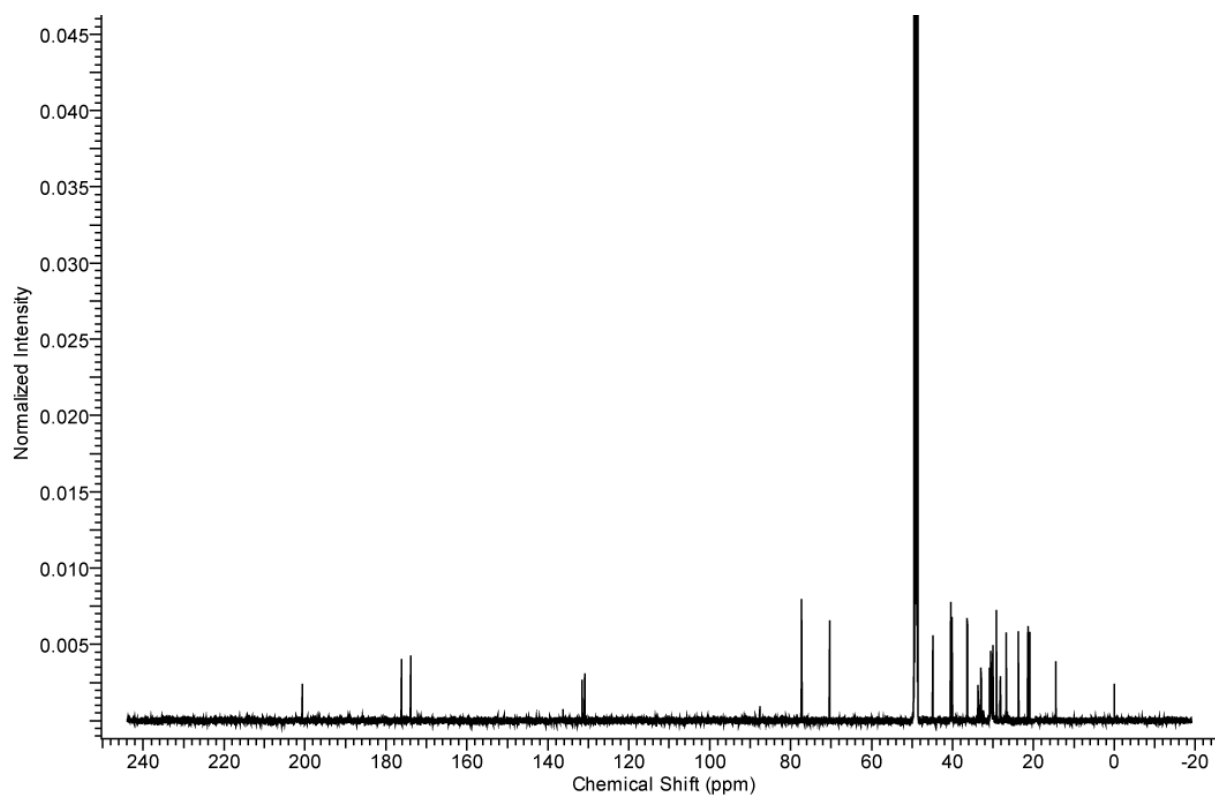
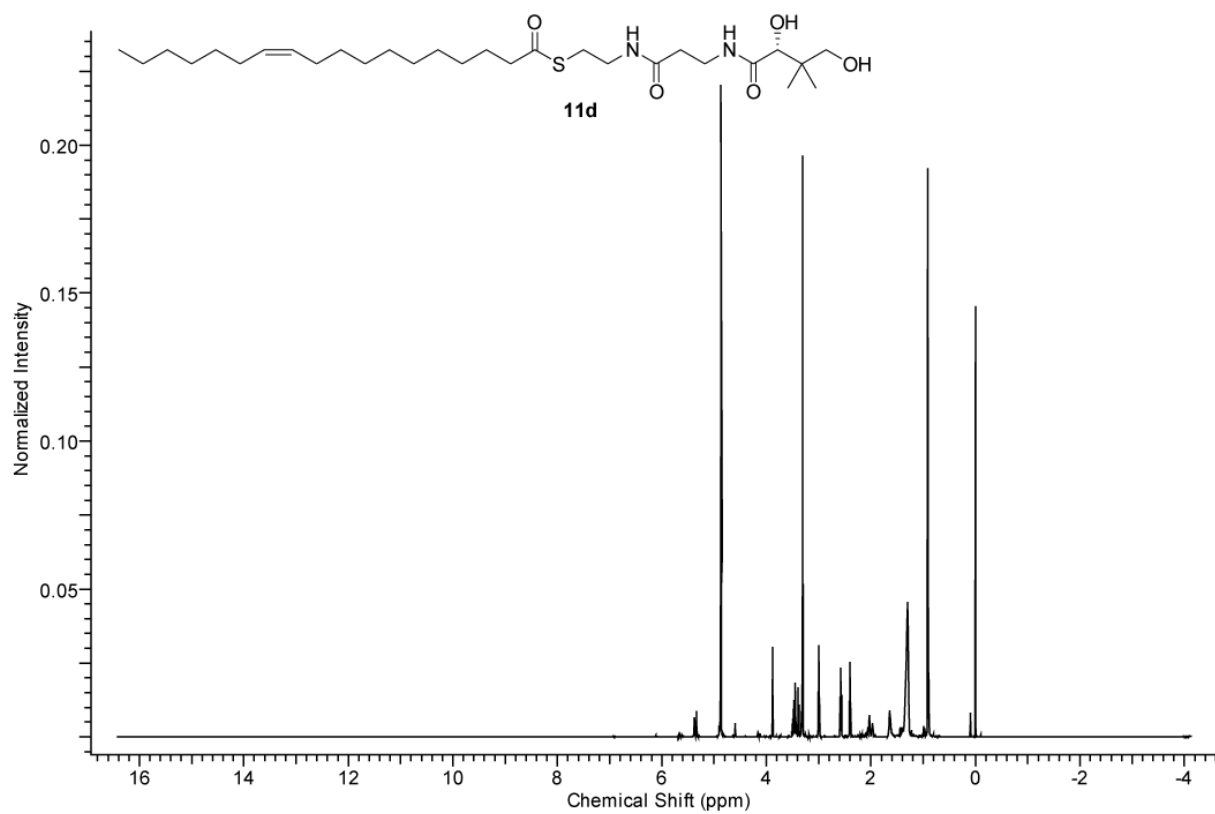




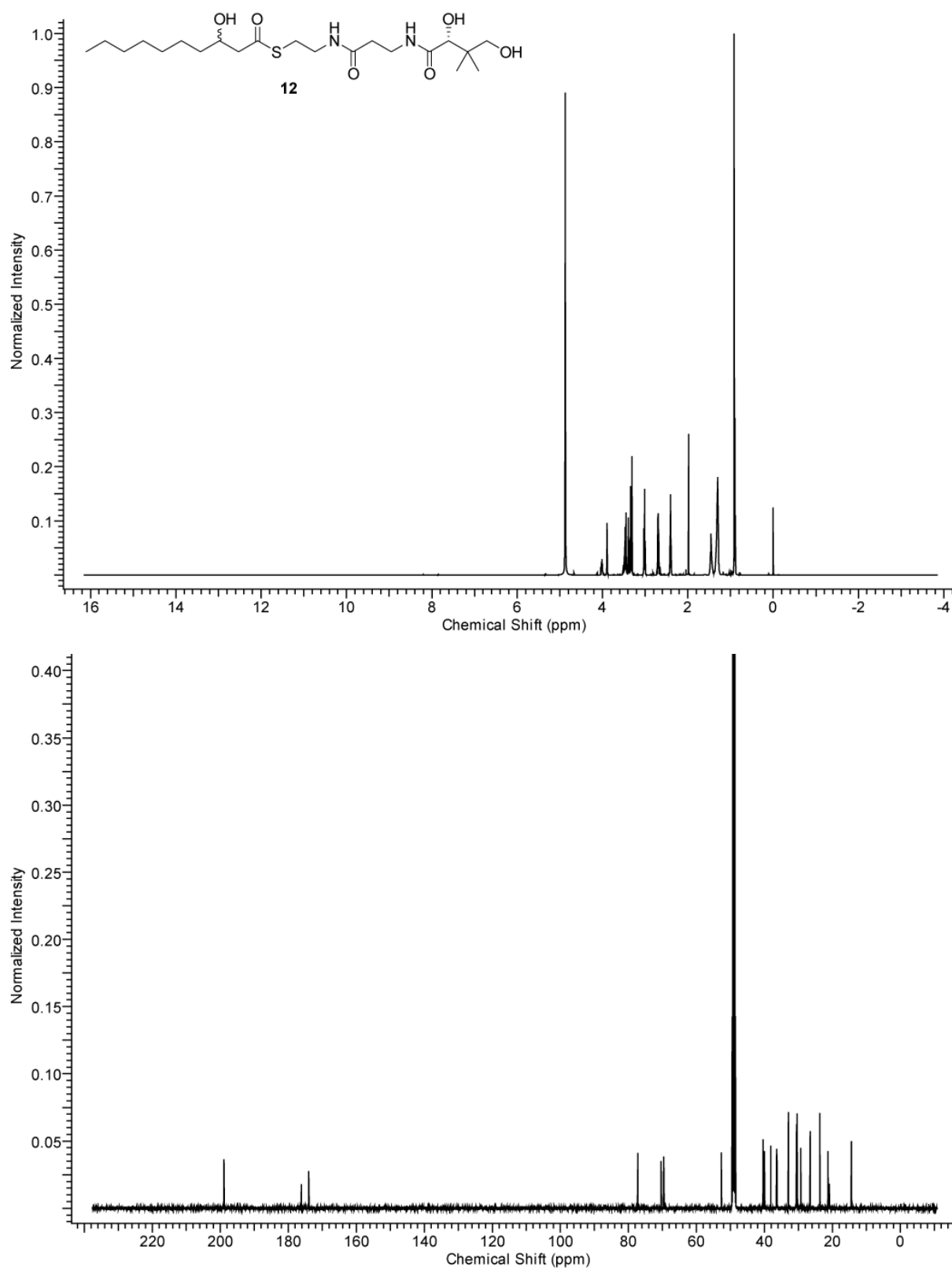
**Figure S14:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-((*Z*-9-hexadecenoyl)-*N*-pantothenoylcysteamine (**11b**).



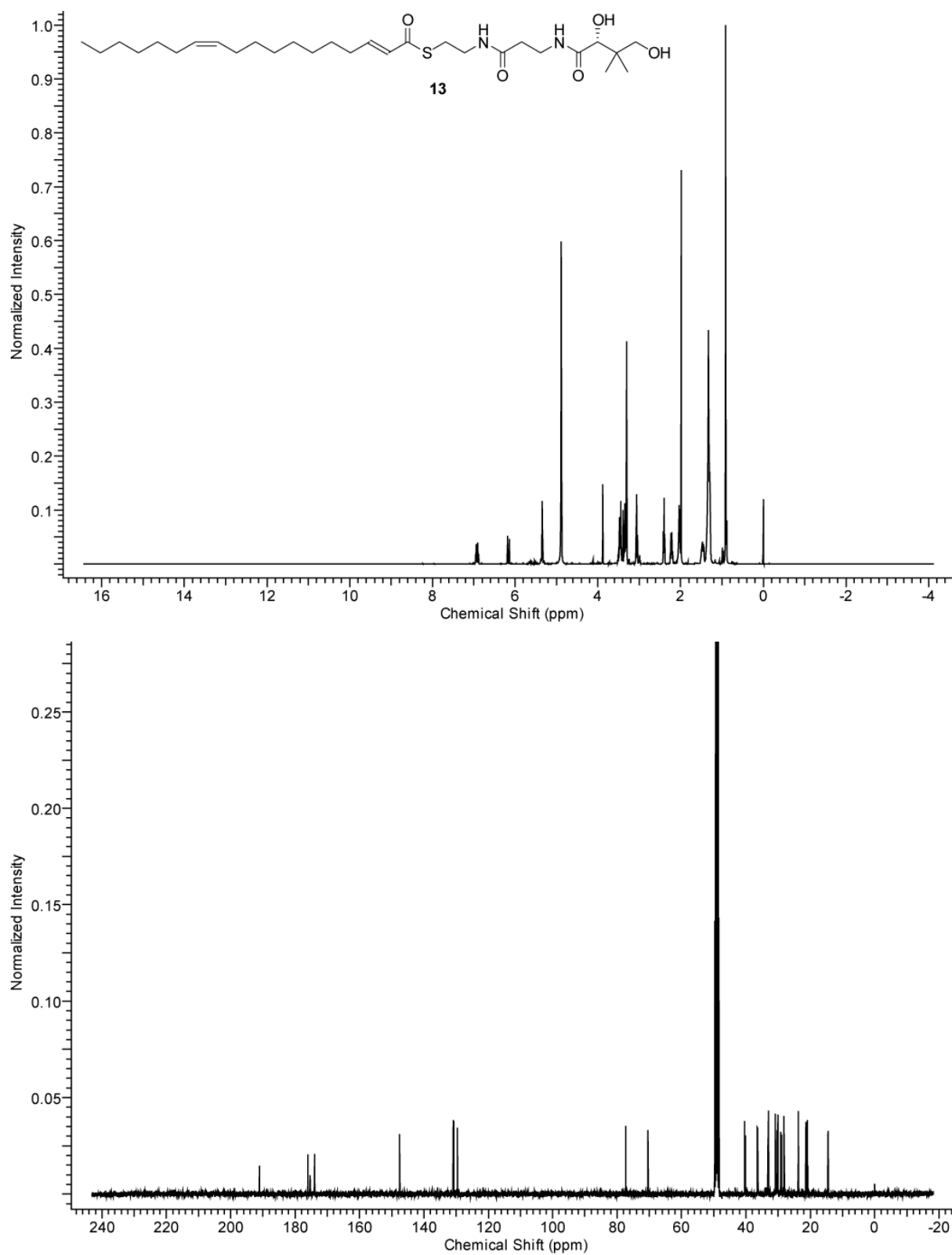
**Figure S15:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-((*Z*)-9-octadecenoyl)-*N*-pantothenoylcysteamine (**11c**).



**Figure S16:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-((*Z*)-11-octadecenoyl)-*N*-pantothenoylcysteamine (**11d**).



**Figure S17:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-(3-hydroxydecanoyl)-*N*-pantothenoylcysteamine (**12**).



**Figure S18:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectrum of *S*-((2*E*,11*Z*)-2,11-octadecadienoyl)-*N*-pantothenoylcysteamine (**13**).