Supplementary information for:

Chemoenzymatic synthesis of acyl coenzyme A substrates enables *in situ* labeling of small molecules and proteins

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General Methods

Chemicals and Solvents: All chemicals were purchased from Acros, Aldrich, Fluka, Oakwood, Alfa Aesar, and TCI America and used as such unless stated otherwise. For flash chromatography technical grade solvents were used without further purification. For reactions technical grade solvents were used without further purification. Deuterated solvents were obtained from Sigma-Aldrich.

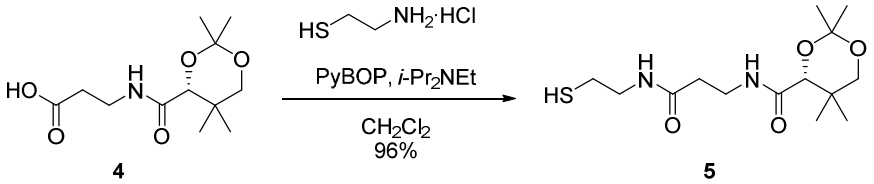
Reactions: All non-aqueous reactions were carried out in dry glassware and open to air. Reactions were magnetically stirred and monitored by TLC unless otherwise stated. Chromatographic purification was performed as flash chromatography (Alfa Aesar silica gel, 60 Å pore size) using the solvents indicated as eluent with 0.3-0.5 bar pressure. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} TLC glass plates and visualized with UV light or stained in ceric ammonium molybdate, potassium permanganate, or ninhydrin solutions. The yields given refer to chromatographically purified and spectroscopically pure compounds unless otherwise stated.

Analysis: ^{1}H- and ^{13}C- NMR spectra were recorded on VARIAN Inova (500 MHz) or BRUKER Avance (600 MHz) spectrometers in the solvents indicated. All signals are reported in ppm with the internal d-chloroform signal at 7.26 ppm or 77.0 ppm or the internal d_4-methanol signal at 3.31 ppm or 49.0 ppm as standard. The data is being reported as (s=singlet, d=doublet, t=triplet, q=quadruplet, m=multiplet or unresolved, br=broad signal, coupling constant(s) in Hz, integration). Preparative HPLC was carried out using a Waters machine with a 600 Controller, 2487 dual wavelength detector, and 600 Pump connected to a Phenomenex Synergi 10µ Hydro- RP 250 x 21 mm column. HPLC-MS was carried out on an Agilent 1100 Series Instrument, or a Maxis Impact Instrument with diode-array and MS detectors on a Phenomenex Luna C18(2) 5µ 100 x 4.6 mm column.

Synthesis of 5–14

Preparation of thiol 5

Thiol **5** has been prepared previously from pantothenic acid.[1]

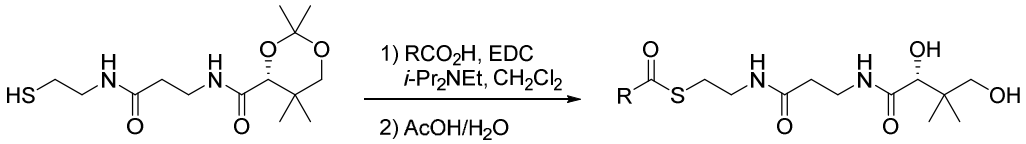


4 **5**

To a solution of acid **4**[2] (100 mg, 0.39 mmol) in CH_2Cl_2 (2 mL) was added cysteamine hydrochloride (88 mg, 0.77 mmol), PyBOP (304 mg, 0.59 mmol) and diisopropylethylamine (0.3 mL, 1.9 mmol). The reaction was allowed to stir for 12 h. The solvent was removed and the residue was subjected to flash column chromatography (hexanes/EtOAc 1:1 → EtOAc) to afford thiol **5** (oil, 119 mg, 0.07 mmol, 96%).

R_f 0.30 (EtOAc); **^{1}H-NMR** (600 MHz, $CDCl_3$): δ 7.01 (bs, 1H, N*H*), 6.57 (bs, 1H, N*H*), 4.03 (s, 1H), 3.64 (d, *J* = 11.8 Hz, 1H), 3.57-3.46 (m, 2H), 3.44-3.33 (m, 2H), 3.24 (d, *J* = 11.7 Hz, 1H), 2.64-2.59 (m, 2H), 2.43 (t, *J* = 6.3 Hz, 2H), 1.42 (s, 3H), 1.38 (s, 3H), 0.98 (s, 3H), 0.93 (s, 3H); **^{13}C-NMR** (150 MHz, $CDCl_3$): δ 171.1, 170.2, 99.0, 77.1, 71.3, 42.4, 36.0, 34.8, 32.9, 29.4, 24.4, 22.0, 18.8, 18.6; **IR** ν_{max} (film)/cm^{-1}: 3313 (bs), 2944, 2872, 1656, 1528, 1461, 1379, 1256, 1223, 1160, 1098; **HRMS** (ESI) *m/z* calculated for $C_{14}H_{26}N_2O_4SNa$ ($[M+Na]^+$) 341.1505, found 341.1511.

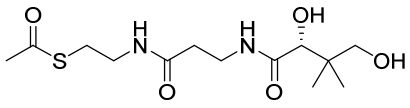
Preparation of compounds 6–14



5 **6-14**

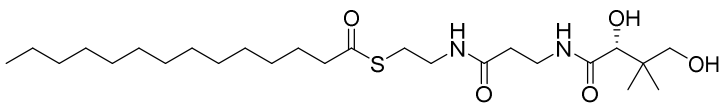
General procedure: A flask was charged with thiol **3** (50 mg, 0.16 mmol), carboxylic acid (0.19 mmol) and EDC (45 mg, 0.24 mmol). CH_2Cl_2 (5 mL) followed by diisopropylethylamine (56 µL, 0.32 mmol) were added. The reaction was allowed to stir for 18 h. The solvent was evaporated and the residue was subjected to flash column chromatography (hexanes/EtOAc 1:1 → EtOAc).

The resulting thioester was dissolved in AcOH/H_2O (2:1, 2 mL) and the mixture was stirred for 5 h. The solvent was evaporated and the residue was subjected to flash column chromatography (CH_2Cl_2/MeOH 20:1) to give the final product.



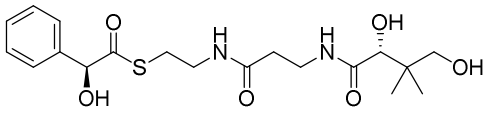
6

(white solid, 42 mg) **R_f** 0.47 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 3.89 (s, 1H), 3.52-3.42 (m, 3H), 3.39 (d, *J* = 10.9 Hz, 1H), 3.34-3.30 (m, 2H), 3.00 (t, *J* = 6.7 Hz, 2H), 2.41 (t, *J* = 6.7 Hz, 2H), 2.33 (s, 3H), 0.92 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD, rotamers, all peaks reported): δ 197.0, 176.0, 173.9, 77.3, 77.3, 70.4, 40.4, 40.0, 36.4, 36.3, 30.5, 29.5, 21.3, 21.3, 20.9, 20.9; **HRMS** (ESI) *m/z* calculated for $C_{13}H_{25}N_2O_5S$ ($[M+H]^+$) 321.1479, found 321.1481.



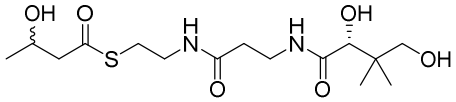
7

(white solid, 51 mg) **R_f** 0.63 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 3.89 (s, 1H), 3.52-3.42 (m, 3H), 3.39 (d, *J* = 11.0 Hz, 1H), 3.35-3.32 (m, 2H), 3.00 (t, *J* = 6.7 Hz, 2H), 2.58 (t, *J* = 7.5 Hz, 2H), 2.41 (t, *J* = 6.7 Hz, 2H), 1.64 (p, *J* = 7.4 Hz, 2H), 1.35-1.26 (m, 20H), 0.92 (s, 6H), 0.90 (t, *J* = 7.0 Hz, 3H); **^{13}C-NMR** (150 MHz, CD_3OD, rotamers, all peaks reported): δ 200.6, 176.0, 173.8, 77.3, 77.3, 70.4, 44.8, 40.4, 40.1, 36.4, 36.3, 33.1, 30.8, 30.8, 30.7, 30.5, 30.5, 30.4, 30.0, 29.1, 26.7, 23.7, 21.4, 21.3, 21.0, 20.9, 14.5, 14.4; **HRMS** (ESI) *m/z* calculated for $C_{25}H_{49}N_2O_5S$ ([M+H]$^+$) 489.3357, found 489.3356.



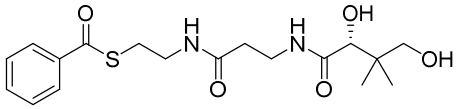
8

8 was further purified by reverse phase HPLC using Phenomenex Synergi 10 µ Hydro-RP 80Å, 250×21.2 mm column operating at room temperature with a mobile phase of water (A) and MeCN (B). A flow rate of 15 mL/min was used with the following elution gradient: 0-3 mins: 5% B, 3-5 min linear gradient to 20% B, 5-20 min: linear gradient to 30% B, 20-25 min: linear gradient to 100% B, 25-27 min: 100% B, 27-29 min: linear gradient to 5% B, 29-30 min: 5% B.
(white solid, 7 mg) **R_f** 0.49 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 7.47 (d, *J* = 7.1, 2H), 7.39-7.35 (m, 2H), 7.35-7.31 (m, 1H), 5.21 (s, 1H), 3.91 (s, 1H), 3.50-3.40 (m, 4H), 3.33-3.30 (m, 2H), 2.99 (m, 2H), 2.36 (t, *J* = 6.5 Hz, 2H), 0.94 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD): δ 204.5, 176.0, 173.9, 140.4, 129.5 (2C), 129.4 (2C), 127.9, 80.7, 77.3, 70.4, 40.3, 40.0, 36.6, 36.3, 28.4, 21.3, 20.9; **HRMS** (ESI) *m/z* calculated for $C_{19}H_{29}N_2O_6S$ ([M+H]$^+$) 413.1741, found 413.1745.



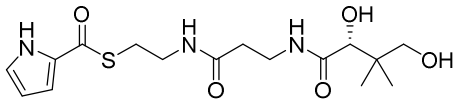
9

(white solid, 12 mg) **R_f** 0.22 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 4.23-4.12 (m, 1H), 3.89 (s, 1H), 3.52-3.42 (m, 3H), 3.39 (d, *J* = 11.0 Hz, 1H), 3.36-3.32 (m, 2H), 3.02 (t, *J* = 6.7 Hz, 2H), 2.73 (dd, *J* = 14.7, 7.7 Hz, 1H), 2.67 (dd, *J* = 14.7, 5.2 Hz, 1H), 2.41 (t, *J* = 6.7 Hz, 2H), 1.20 (d, *J* = 6.3 Hz, 3H), 0.92 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD, mixture of diastereomers and rotamers, all peaks reported): δ 198.6, 176.0, 173.9, 77.3, 77.3, 70.4, 65.8, 65.8, 54.0, 40.4, 40.0, 36.4, 36.3, 29.3, 23.3, 21.3, 21.3, 20.9, 20.9; **HRMS** (ESI) *m/z* calculated for $C_{15}H_{29}N_2O_6S$ ([M+H]$^+$) 365.1741, found 365.1742.



10

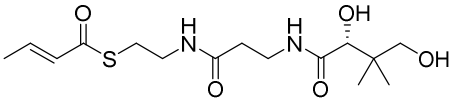
(white solid, 44 mg) **R_f** 0.60 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 7.98-7.95 (m, 2H), 7.64-7.61 (m, 1H), 7.51-7.48 (m, 2H), 3.89 (s, 1H), 3.53-3.43 (m, 5H), 3.39 (d, *J* = 10.9 Hz, 1H), 3.21 (t, *J* = 6.6 Hz, 2H), 2.43 (t, *J* = 6.7 Hz, 2H), 0.92 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD, rotamers, all peaks reported): δ 192.8, 176.0, 174.0, 138.3, 134.8, 129.9 (2C), 128.2 (2C), 77.3, 77.2, 70.4, 40.4, 40.1, 36.4, 36.3, 29.3, 21.3, 20.9 **HRMS** (ESI) *m/z* calculated for $C_{18}H_{27}N_2O_5S$ ([M+H]$^+$) 383.1635, found 383.1654.



11

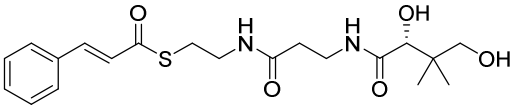
(white solid, 126 mg) **R_f** 0.15 (EtOAc); **^{1}H-NMR** (500 MHz, CD_3OD): δ 7.04 (dd, *J* = 2.5, 1.4 Hz, 1H), 6.98 (dd, *J* = 3.9, 1.4 Hz, 1H), 6.21 (dd, *J* = 3.9, 2.4 Hz, 1H), 3.90 (s, 1H), 3.53-3.44 (m, 3H), 3.41-3.37 (m, 3H), 3.14 (t, *J* = 6.6 Hz, 2H), 2.42 (t, *J* = 6.6 Hz, 2H), 0.92 (s, 6H); **^{13}C-NMR** (125 MHz, CD_3OD): δ 181.9, 175.9, 173.8, 131.0,

125.8, 116.5, 111.1, 77.2, 70.3, 40.7, 40.6, 40.3, 36.3, 28.2, 21.3, 20.9; **HRMS** (ESI) *m/z* calculated for $C_{16}H_{26}N_3O_5S$ ([M+H]$^+$) 372.1588, found 372.1591.



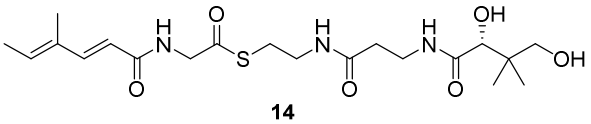
12

(white solid, 23 mg) $\mathbf{R_f}$ 0.53 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 6.95 (dq, *J* = 15.6, 6.9 Hz, 1H), 6.20 (dq, *J* = 15.4, 1.7 Hz, 1H), 3.89 (s, 1H), 3.52-3.42 (m, 3H), 3.39 (d, *J* = 11.0 Hz, 1H), 3.36-3.33 (m, 2H), 3.06 (t, *J* = 6.7 Hz, 2H), 2.41 (t, *J* = 6.7 Hz, 2H), 1.88 (dd, *J* = 6.9, 1.7 Hz, 3H), 0.92 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD, rotamers, all peaks reported): δ 190.9, 176.0, 173.9, 142.9, 142.8, 130.9, 130.9, 77.3, 77.3, 70.4, 40.4, 40.2, 36.4, 36.3, 28.9, 21.3, 21.3, 20.9, 20.9, 18.0, 18.0; **HRMS** (ESI) *m/z* calculated for $C_{15}H_{27}N_2O_5S$ ([M+H]$^+$) 347.1635, found 347.1643.



13

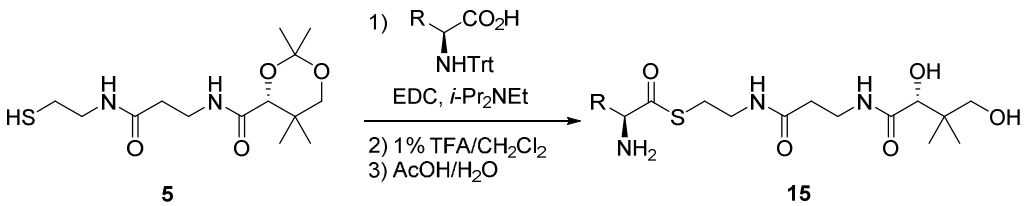
(white solid, 35 mg) $\mathbf{R_f}$ 0.60 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 7.64-7.61 (m, 3H), 7.42-7.39 (m, 3H), 6.86 (d, *J* = 15.9 Hz, 1H), 3.89 (s, 1H), 3.53-3.43 (m, 3H), 3.41-3.38 (m, 3H), 3.14 (t, *J* = 6.7 Hz, 2H), 2.42 (t, *J* = 6.7 Hz, 2H), 0.92 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD, rotamers, all peaks reported): δ 190.9, 176.0, 173.9, 142.1, 142.1, 135.4, 131.8, 130.1 (2C), 129.6 (2C), 125.8, 125.8, 125.7, 77.3, 70.4, 40.4, 40.2, 36.4, 36.4, 29.2, 21.4, 21.3, 21.0, 20.9; **HRMS** (ESI) *m/z* calculated for $C_{20}H_{29}N_2O_5S$ ([M+H]$^+$) 409.1792, found 409.1766.



14

(white solid, 8.6 mg) $\mathbf{R_f}$ 0.25 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 7.25 (d, *J* = 15.4 Hz, 1H), 6.00-6.04 (m, 2H), 4.18 (s, 2H), 3.91 (s, 1H), 3.53-3.44 (m, 3H), 3.41 (d, *J* = 15.5, 1H), 3.37-3.34 (m, 2H), 3.05 (t, *J* = 6.6 Hz, 2H), 2.42 (t, *J* = 6.7 Hz, 2H), 1.87 (m, 6H), 0.94 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD): δ 199.1, 176.0, 174.0, 169.9, 147.8, 136.7, 134.9, 121.4, 118.0, 77.3, 70.4, 40.4, 39.9, 36.4, 36.3, 28.9, 21.3, 20.9, 14.5, 11.9; **HRMS** (ESI) *m/z* calculated for $C_{20}H_{33}N_3O_6SNa$ ([M+Na]$^+$) 466.1982, found 466.1980.

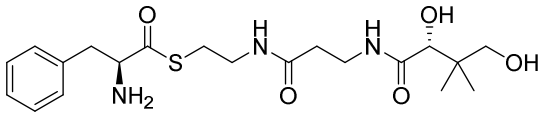
Synthesis of aminoacyl derivative 15



5 **15**

General procedure: A flask was charged with thiol **5** (50 mg, 0.16 mmol), carboxylic acid (0.19 mmol) and EDC (45 mg, 0.24 mmol). CH_2Cl_2 (5 mL) followed by diisopropylethylamine (56 µL, 0.32 mmol) were added. The reaction was allowed to stir for 18 h. The solvent was evaporated and the residue was subjected to flash column chromatography (hexanes/EtOAc 1:1 → EtOAc).

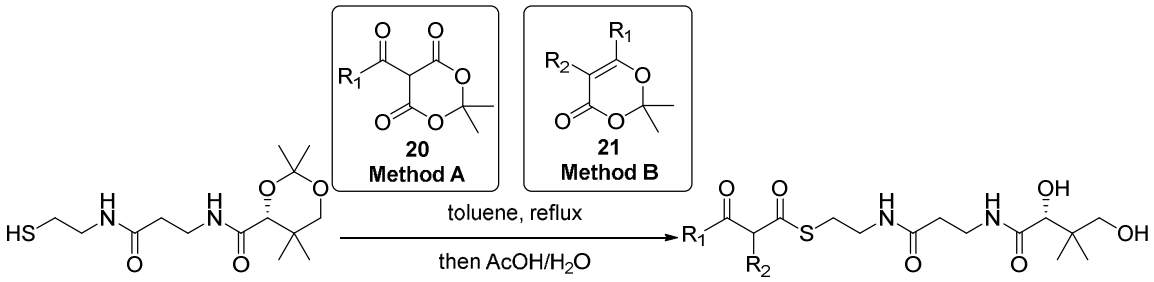
The resulting thioester was dissolved in 1% TFA/CH_2Cl_2 (1 mL) and the mixture was stirred for 1 hour. The solvent was removed under a stream of N_2 and the resulting residue was subjected to flash chromatography (CH_2Cl_2/MeOH 20:1) to yield the free amine. This was then dissolved in AcOH/H_2O (2:1, 2 mL) and the mixture was stirred for 18 h. The solvent was evaporated and the residue was subjected to flash column chromatography (CH_2Cl_2/MeOH 20:1→ CH_2Cl_2/MeOH 9:1→MeOH) to give the final product.



15

(white solid, 8.1 mg) $\mathbf{R_f}$ 0.13 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): 7.35-7.31 (m, 2H), 7.29-7.23 (m, 3H), 3.97-3.95 (m, 1H), 3.92 (s, 1H), 3.55-3.44 (m, 3H), 3.43-3.34 (m, 3H), 3.14 (dd, *J* = 13.8, 6.0 Hz, 1H), 3.04 (t, *J* = 6.7 Hz, 2H), 2.95 (dd, *J* = 13.8, 7.6 Hz, 1H), 2.43 (t, *J* = 6.7 Hz, 2H), 0.94 (s, 6H).; **^{13}C-NMR** (150 MHz, CD_3OD): δ 193.7, 176.1, 173.9, 137.5, 130.5, 129.7, 128.1, 77.3, 70.3, 63.5, 41.3, 40.4, 39.8, 36.4, 36.3, 29.2, 21.3, 21.0; **HRMS** (ESI) *m/z* calculated for $C_{20}H_{32}N_3O_5S$ ($[M+H]^+$) 426.2062, found 426.2058.

Preparation of ketoacyl derivatives 16–18



5 16-18

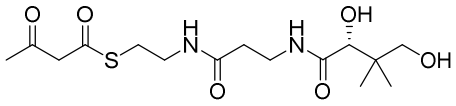
General procedure:

Method A (R_2 = H) To a solution of thiol **5** (50 mg, 0.16 mmol) in toluene (2 mL) was added acyl meldrum's acid **20** (0.32 mmol). The mixture was refluxed for 18 h. The solvent was removed and the residue was purified by flash column chromatography (hexanes/EtOAc 1:1 → EtOAc).

The resulting thioester was dissolved in AcOH/H_2O (2:1, 2 mL) and the mixture was stirred for 5 h. The solvent was evaporated and the residue was subjected to flash column chromatography (CH_2Cl_2/MeOH 20:1) to give the final product.

Method B (R_2 ≠ H) To a solution of thiol **5** (50 mg, 0.16 mmol) in toluene (2 mL) was added ester **21** (0.32 mmol). The mixture was refluxed for 18 h. The solvent was removed and the residue was purified by flash column chromatography (hexanes/EtOAc 1:1 → EtOAc).

The resulting thioester was dissolved in AcOH/H_2O (1:2, 2 mL) and the mixture was stirred for 3 h. The solvent was evaporated and the residue was subjected to flash column chromatography (CH_2Cl_2/MeOH 20:1) to give the final product.



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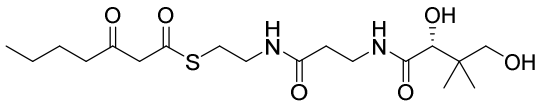
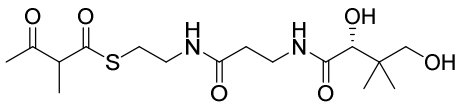
Method A or B. (white solid, 25 mg) R_f 0.40 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 3.89 (s, 1H), 3.52-3.45 (m, 5H), 3.39 (d, *J* = 11.0 Hz, 1H), 3.37-3.35 (m, 2H), 3.35 (s, 2H), 3.06 (t, *J* = 6.6 Hz, 2H), 2.41 (t, *J* = 6.7 Hz, 2H), 2.22 (s, 3H), 0.92 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD, rotamers, all peaks reported): δ 202.5, 193.7, 176.0, 173.9, 77.3, 77.3, 70.4, 40.4, 39.8, 36.4, 36.3, 30.3, 29.7, 21.3, 21.3, 20.9, 20.9; **HRMS** (ESI) *m/z* calculated for $C_{15}H_{27}N_2O_6S$ ([M+H]$^+$) 363.1584, found 363.1580.



17

17 was further purified by reverse phase HPLC using Phenomenex Synergi 10 µ Hydro-RP 80Å, 250×21.2 mm column operating at room temperature with a mobile phase of water (A) and MeCN (B). A flow rate of 15 mL/min was used with the following elution gradient: 0-3 mins: 5% B, 3-23 min: linear gradient to 95% B, 23-28 min: 100% B, 28-30 min: linear gradient to 5% B, 30-35 min: 5% B.

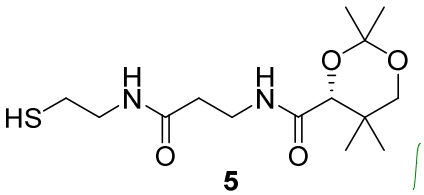
Method A. (white solid, 10 mg) R_f 0.40 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): δ 3.92 (s, 1H), 3.56-3.45 (m, 4H), 3.44-3.37 (m, 3H), 3.08 (t, *J* = 6.6 Hz, 2H), 2.60 (t, *J* = 7.3 Hz, 2H), 2.44 (t, *J* = 6.7 Hz, 2H), 1.63-1.53 (m, 2H), 1.38-1.30 (m, 2H), 1.01-0.88 (m, 11H); **^{13}C-NMR** (150 MHz, CD_3OD, rotamers, all peaks reported): 203.2, 192.3, 174.6, 172.6, 75.9, 69.0, 42.3, 39.0, 38.9, 38.4, 35.0, 34.9, 28.3, 27.0, 25.1, 21.8, 21.7, 19.9, 19.5, 12.7; **HRMS** (ESI) *m/z* calculated for $C_{18}H_{33}N_2O_6S$ ([M+H]$^+$) 405.2054, found 405.2055.



18

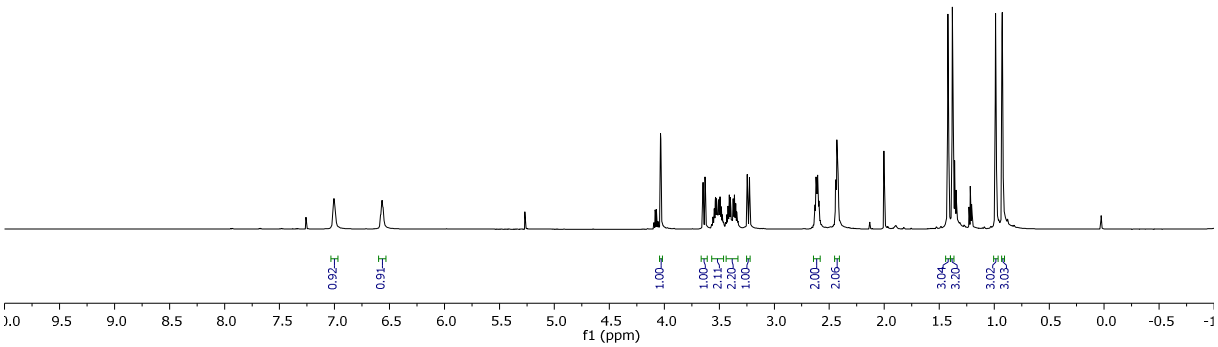
18 was further purified by reverse phase HPLC using Phenomenex Luna 5μ C18(2) 100Å, 250×10 mm column operating at room temperature with a mobile phase of water (A) and MeCN (B). A flow rate of 2.5 mL/min was used with the following elution gradient: 0-5 mins: 15% B, 5-20 min linear gradient to 40% B, 20-22 min: linear gradient to 100% B, 22-27 min: 100% B, 27-28 min: linear gradient to 15% B, 28-30 min: 15% B.
Method B. (white solid, 2.8 mg) $\mathbf{R_f}$ 0.11 (CH_2Cl_2/MeOH 9:1); **^{1}H-NMR** (600 MHz, CD_3OD): 3.92 (s, 1H), 3.55-3.44 (m, 4H), 3.42 (d, *J* = 10.9 Hz, 1H), 3.40-3.36 (m, 2H), 3.09 (t, *J* = 6.3 Hz, 2H), 2.43 (t, *J* = 6.7 Hz, 2H), 2.24 (s, 3H), 1.35 (s, 3H), 0.95 (s, 6H); **^{13}C-NMR** (150 MHz, CD_3OD): δ 203.8, 192.3, 174.6, 172.5, 91.9, 75.9, 68.9, 39.0, 38.3, 35.0, 34.9, 28.2, 27.3, 19.9, 12.3; **HRMS** (ESI) *m/z* calculated for $C_{16}H_{28}N_2O_6SNa$ ($[M+Na]^+$) 399.1560, found 399.1572.

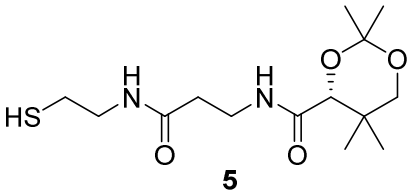
NMR spectroscopic data for 5–18



5

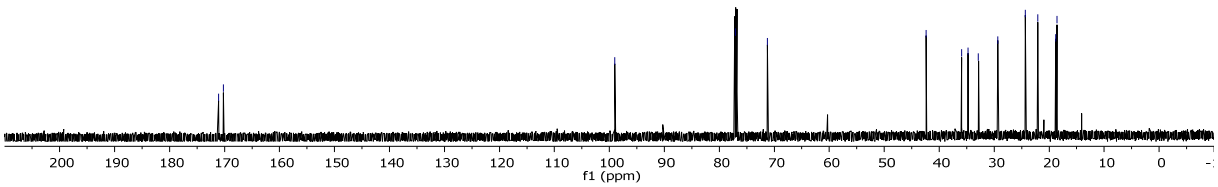
600 MHz ^{1}H
in $CDCl_3$

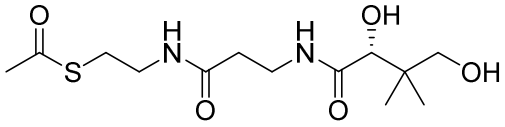




5

150 MHz ^{13}C
in $CDCl_3$

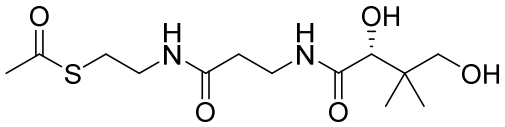




6

600 MHz ^{1}H

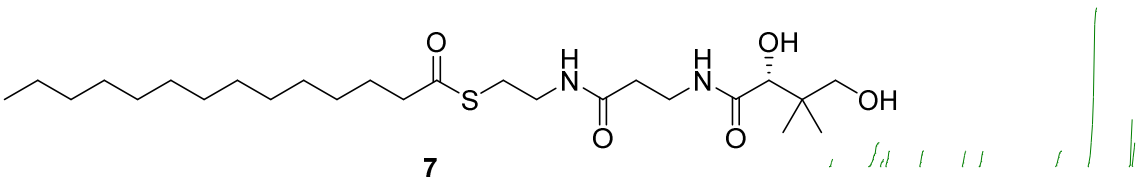
in CD_3OD



6

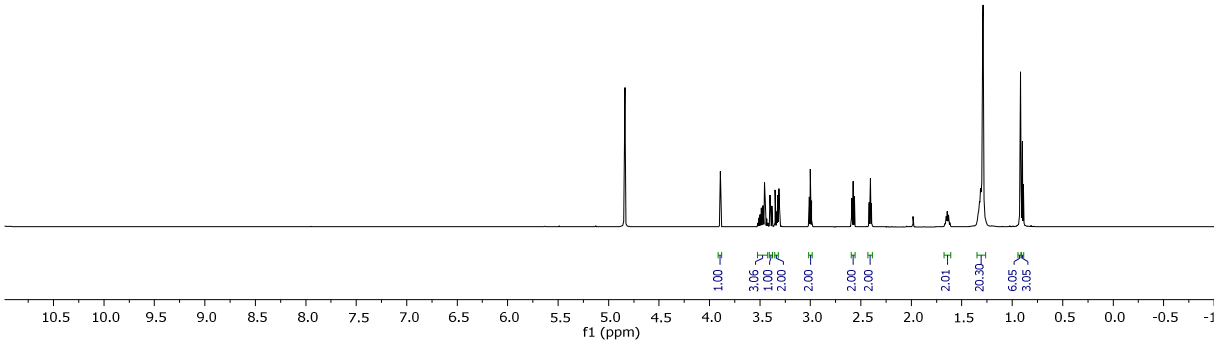
150 MHz ^{13}C

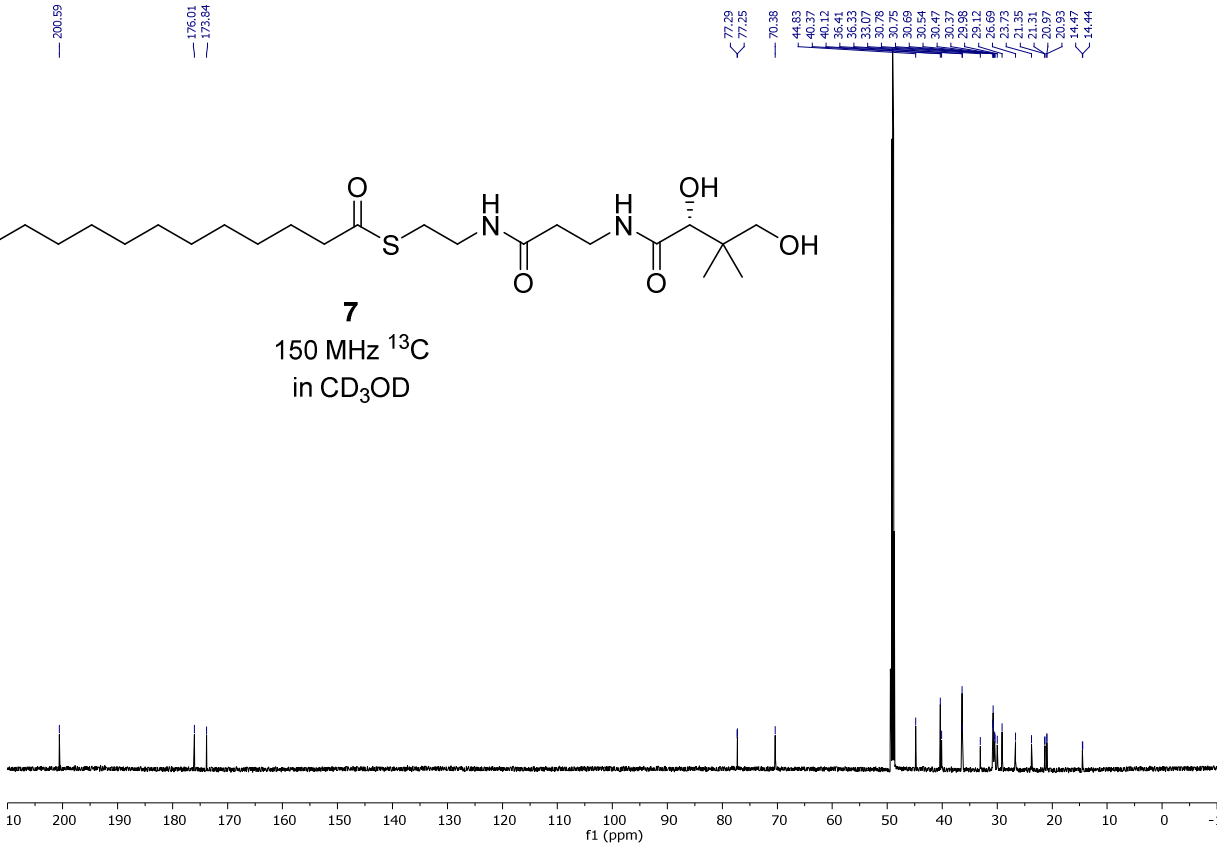
in CD_3OD



7

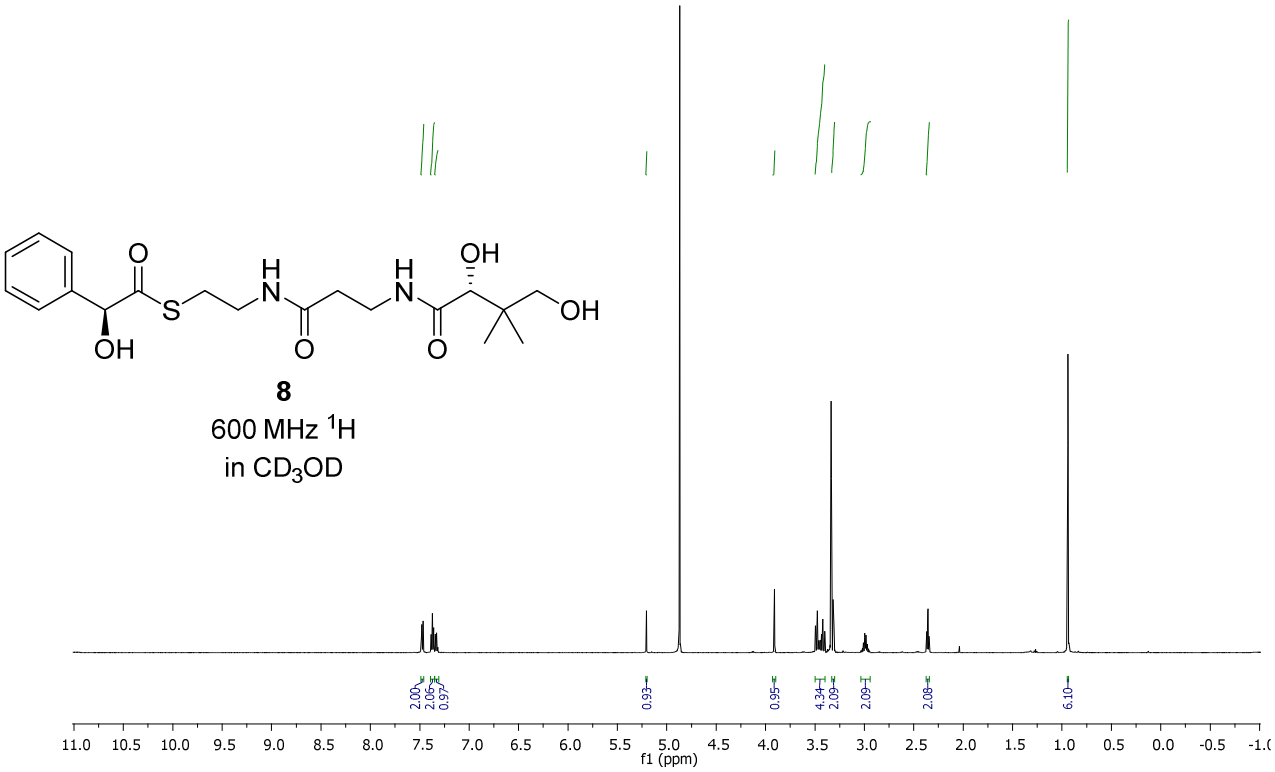
600 MHz ^{1}H
in CD_3OD





7

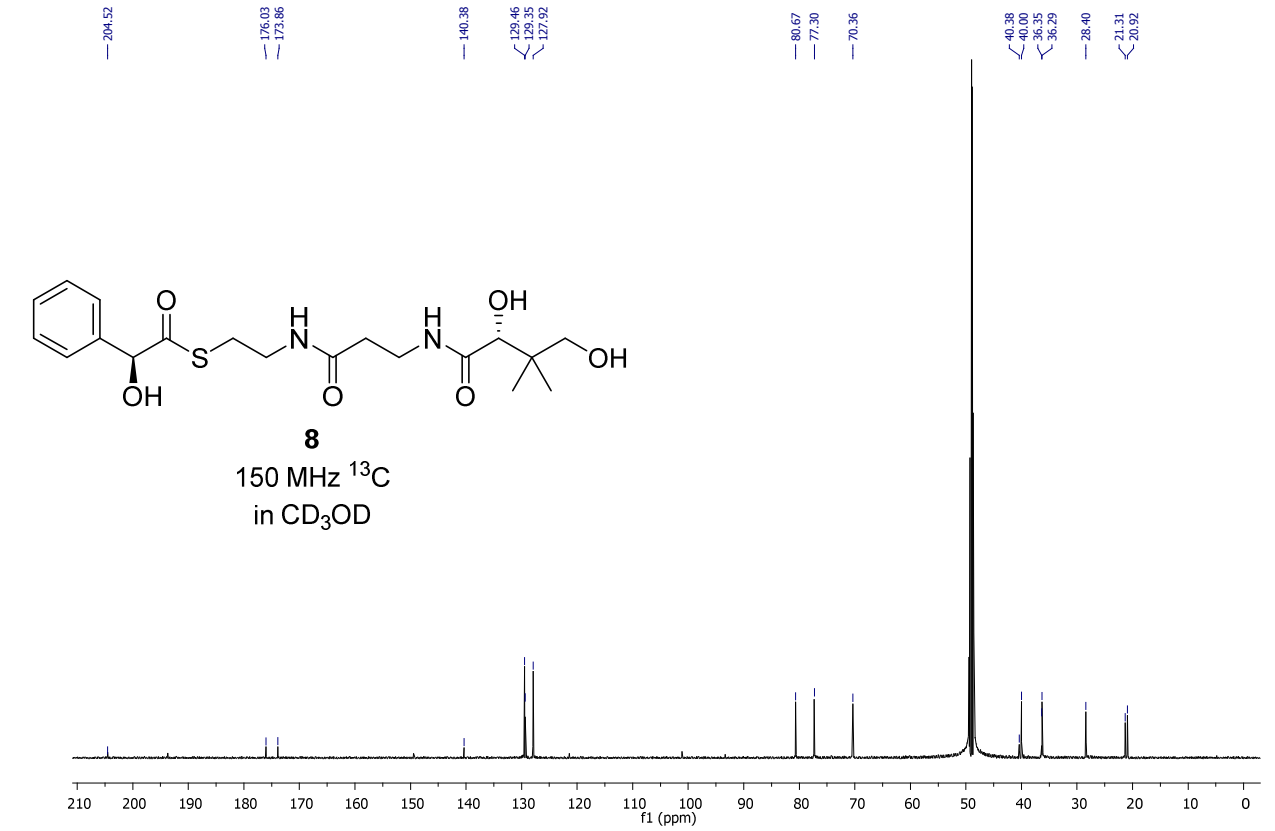
150 MHz ^{13}C
in CD_3OD



8

600 MHz ^{1}H

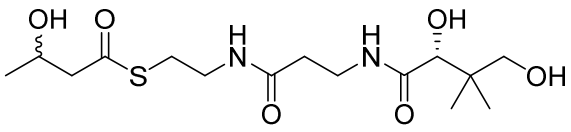
in CD_3OD



8

150 MHz ^{13}C

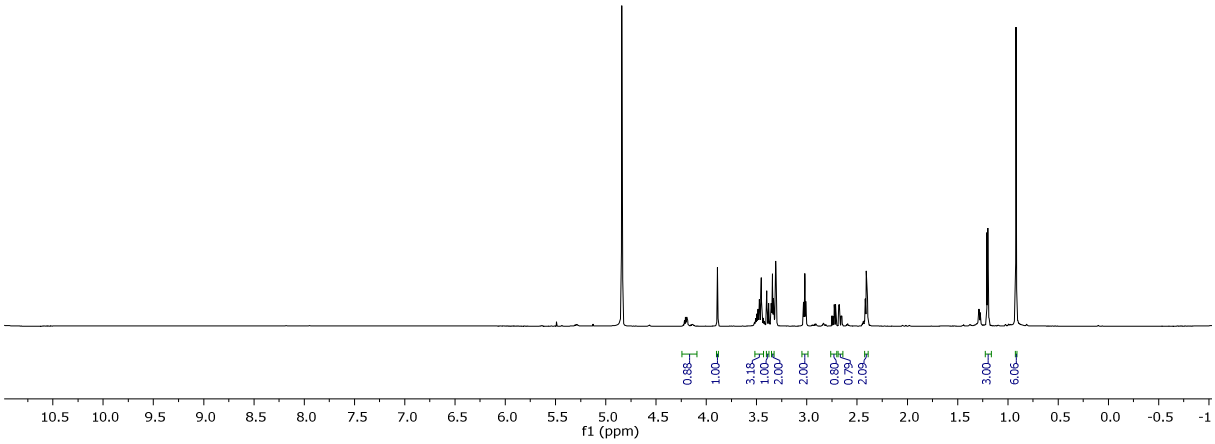
in CD_3OD

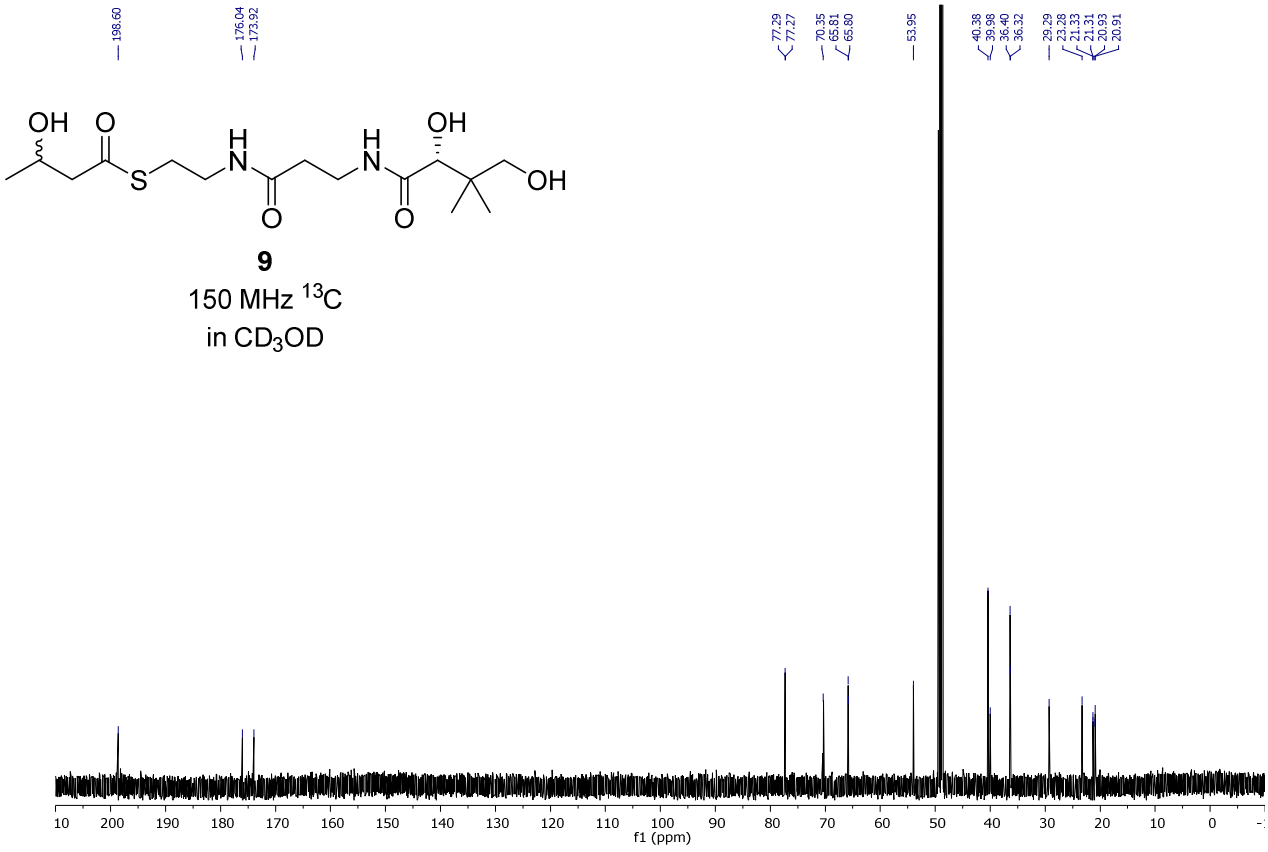


9

600 MHz ^{1}H

in CD_3OD

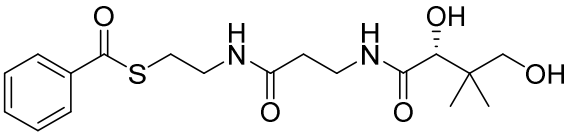




9

150 MHz ^{13}C

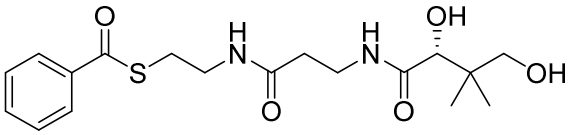
in CD_3OD



10

600 MHz ^{1}H

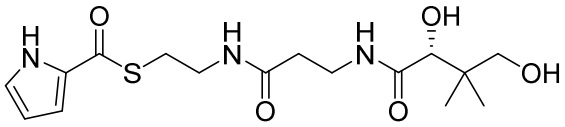
in CD_3OD



10

150 MHz ^{13}C

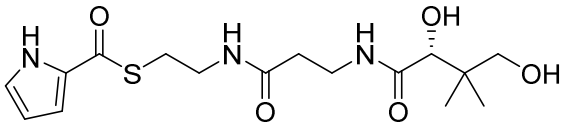
in CD_3OD



11

600 MHz ^{1}H

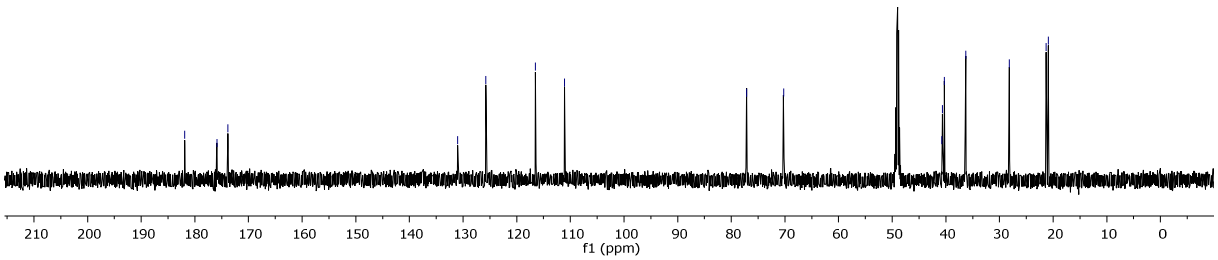
in CD_3OD

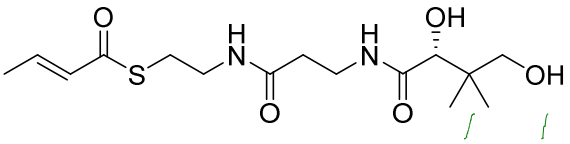


11

150 MHz ^{13}C

in CD_3OD

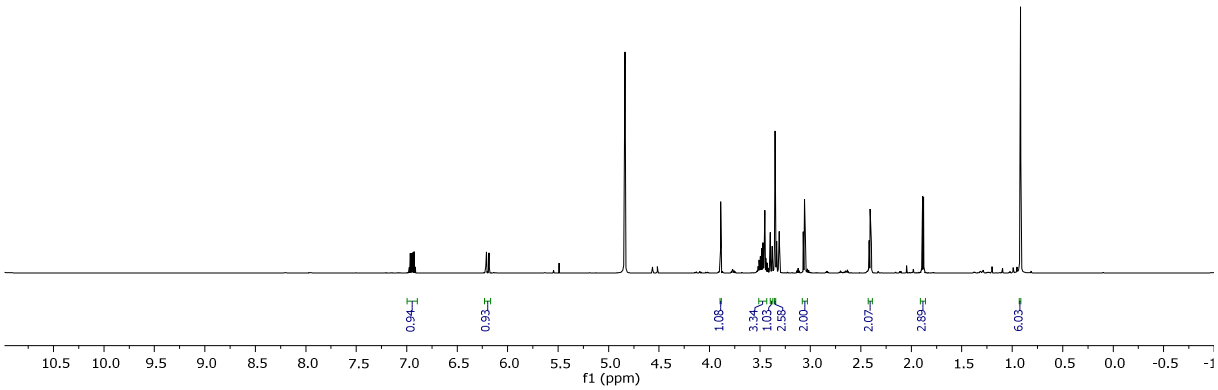


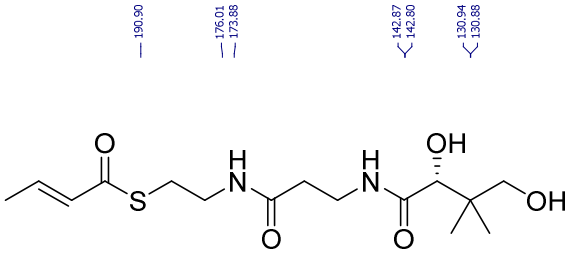


12

600 MHz ^{1}H

in CD_3OD

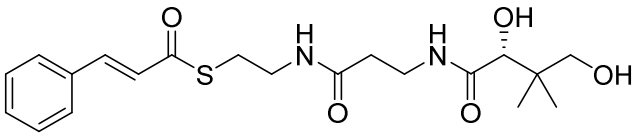




12

600 MHz ^{13}C

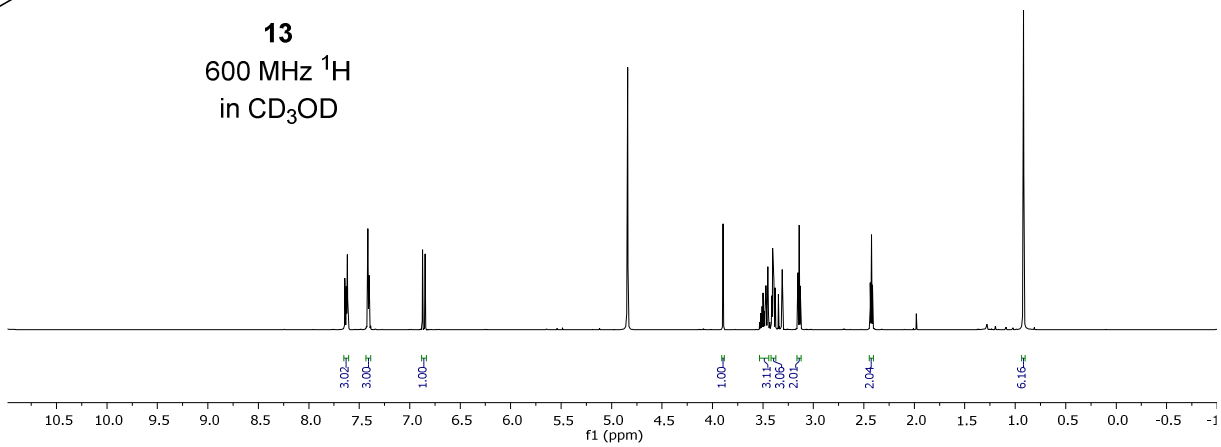
in CD_3OD

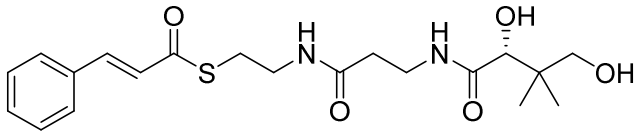


13

600 MHz ^{1}H

in CD_3OD

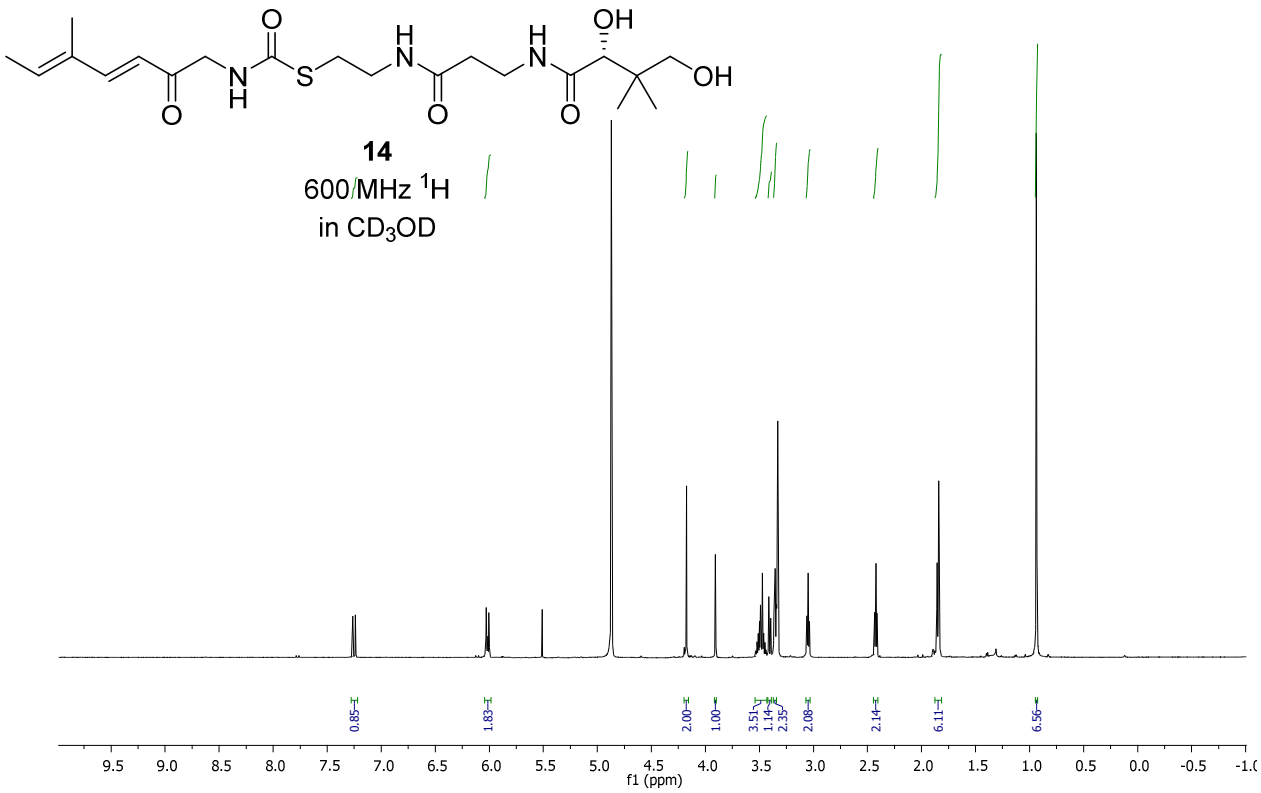


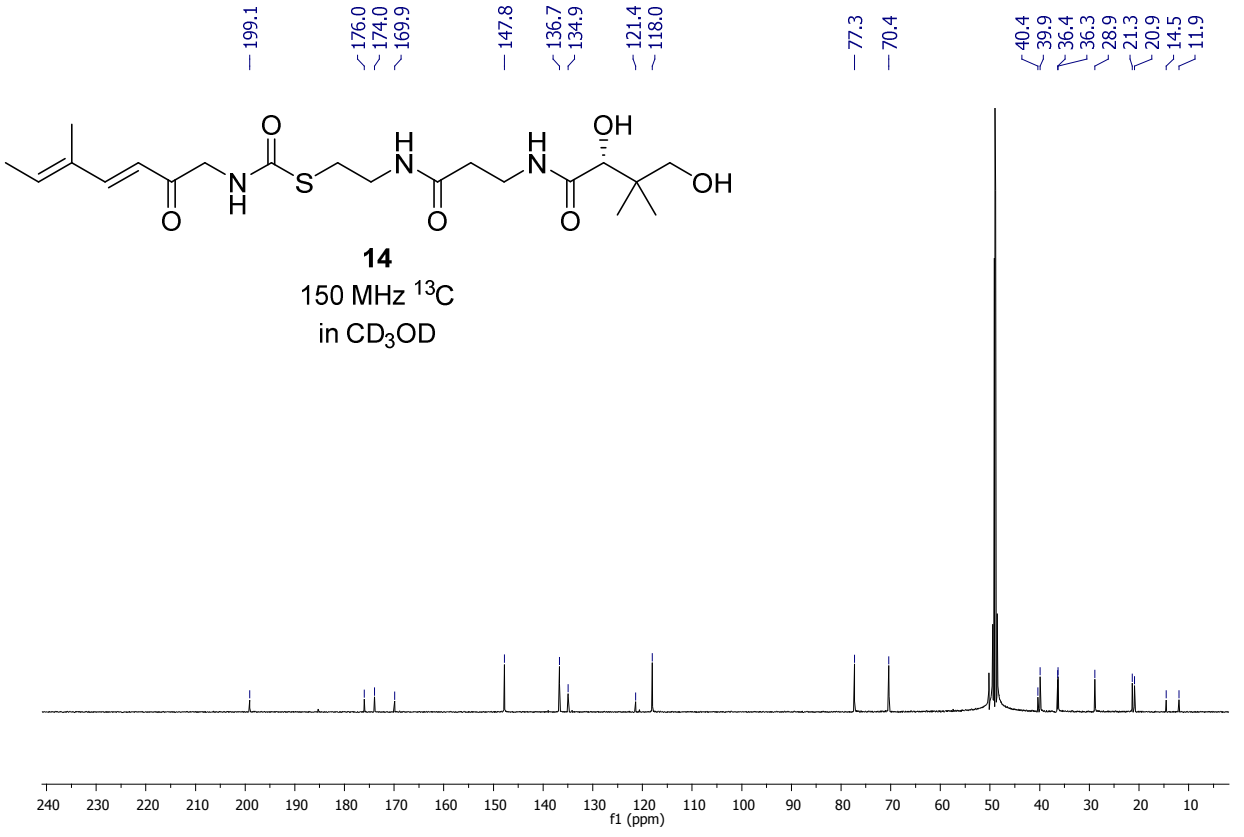


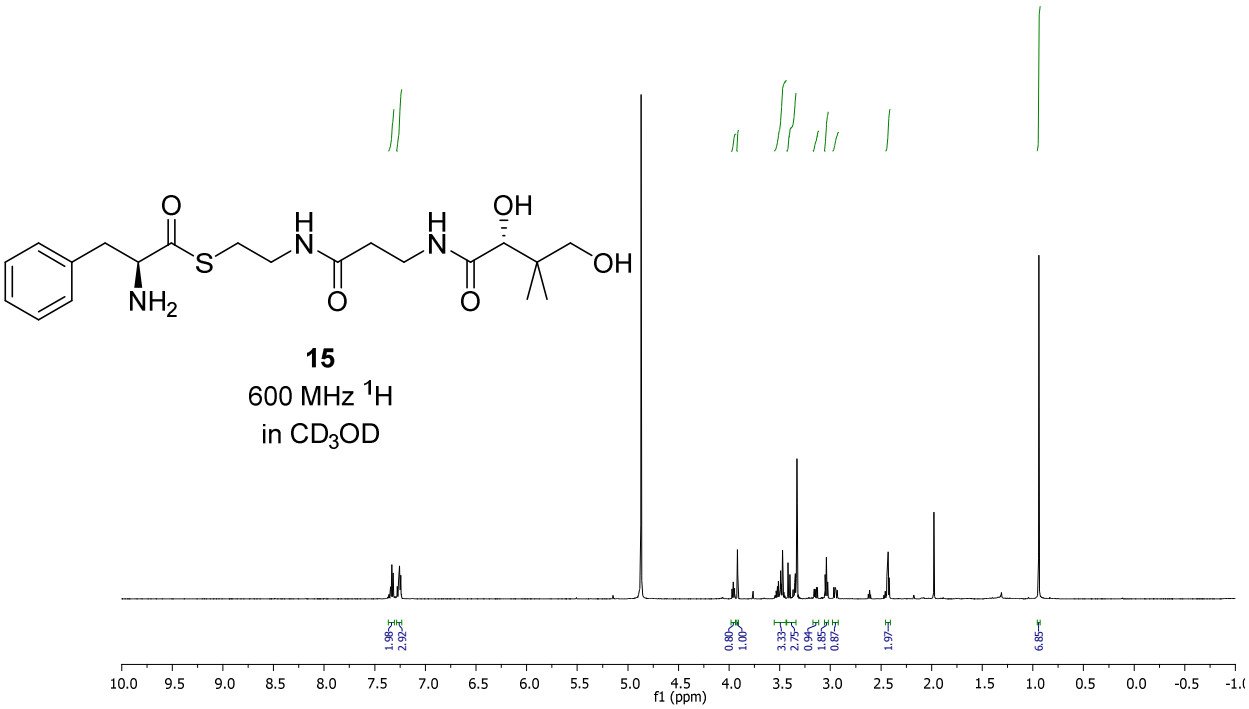
13

150 MHz ^{13}C

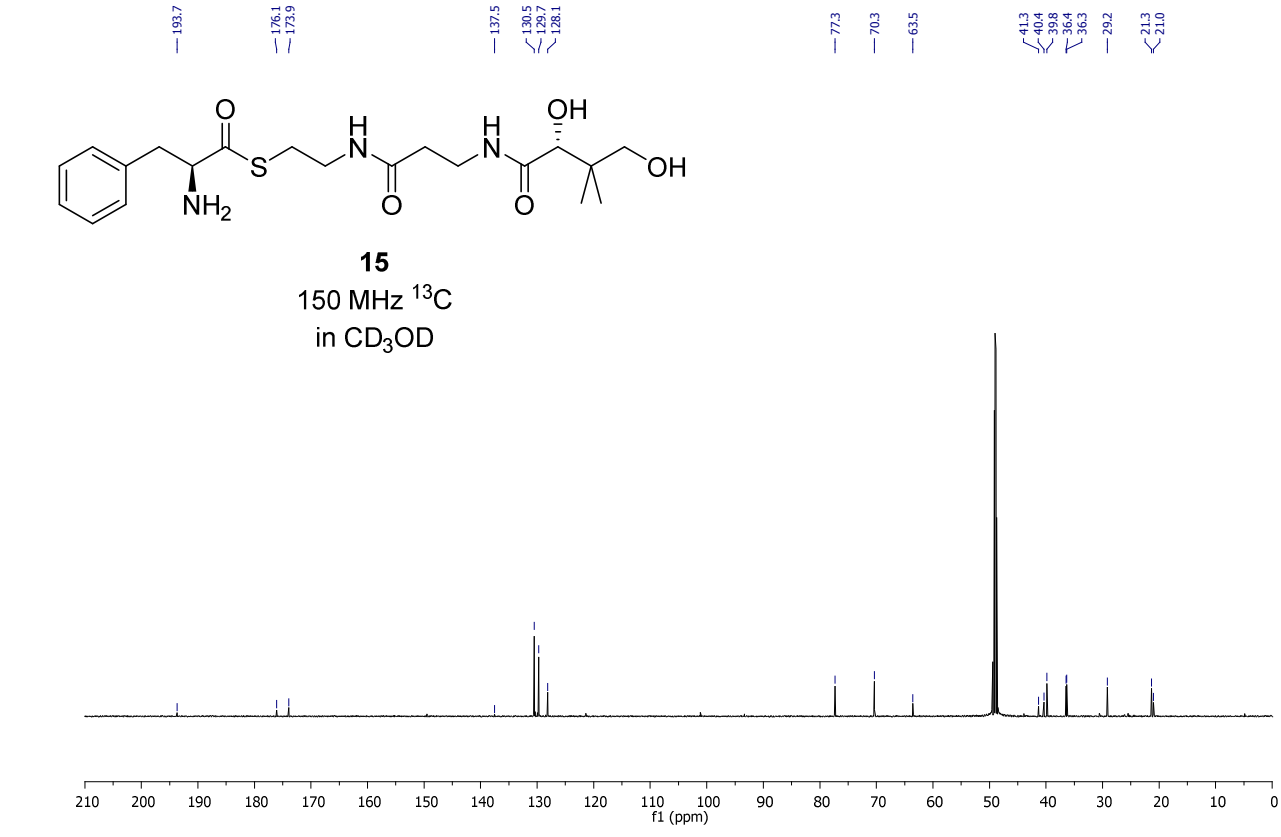
in CD_3OD


14
600 MHz ^{1}H
in CD_3OD
f1 (ppm)

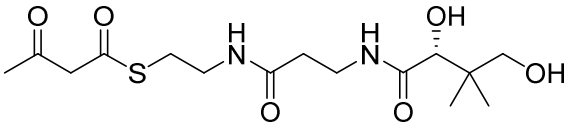

199.1
176.0
174.0
169.9
147.8
136.7
134.9
121.4
118.0
77.3
70.4
40.4
39.9
36.4
36.3
28.9
21.3
20.9
14.5
11.9
14
150 MHz ^{13}C
in CD_3OD
f1 (ppm)



15
600 MHz ^{1}H
in CD_3OD



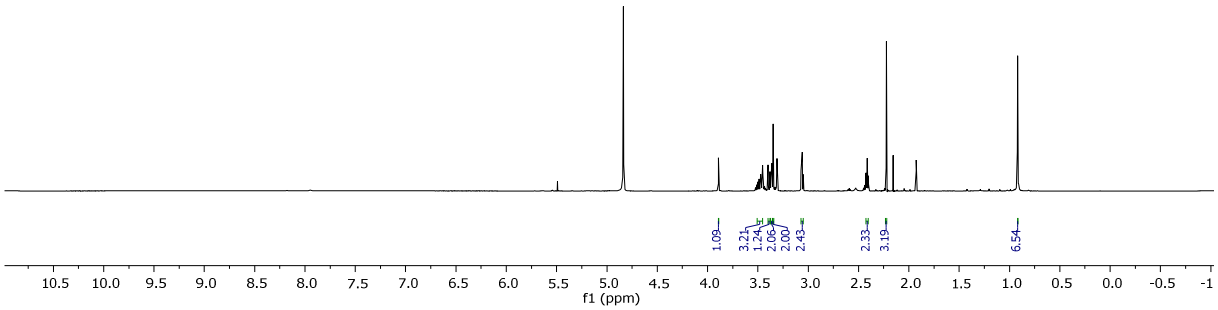
15
150 MHz ^{13}C
in CD_3OD

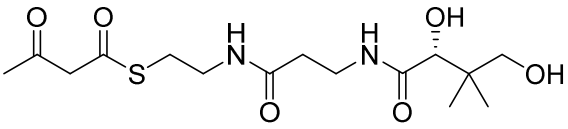


16

600 MHz ^{1}H

in CD_3OD

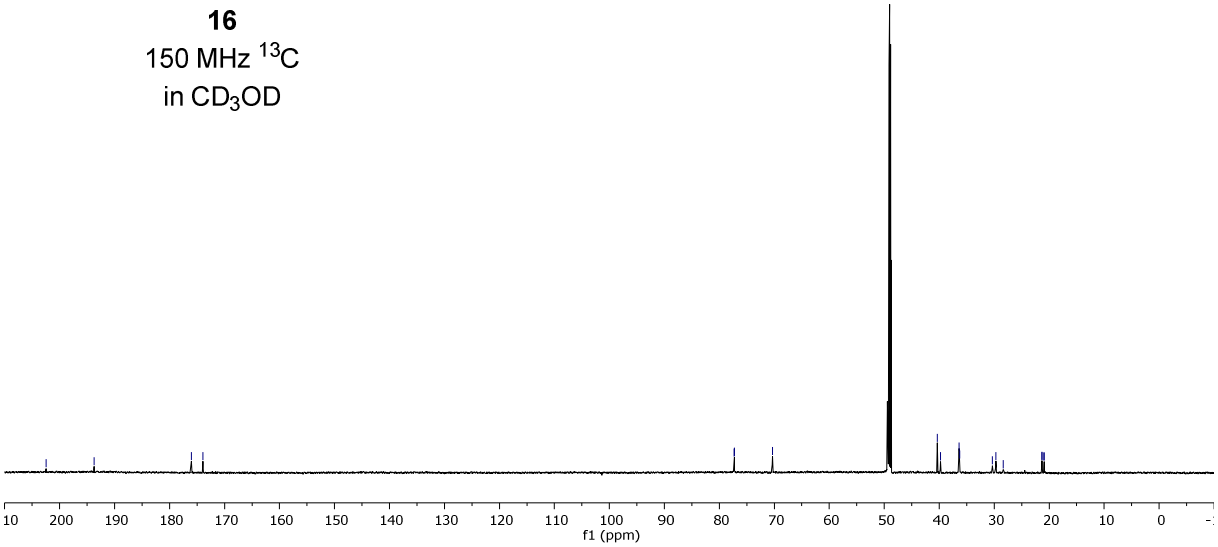


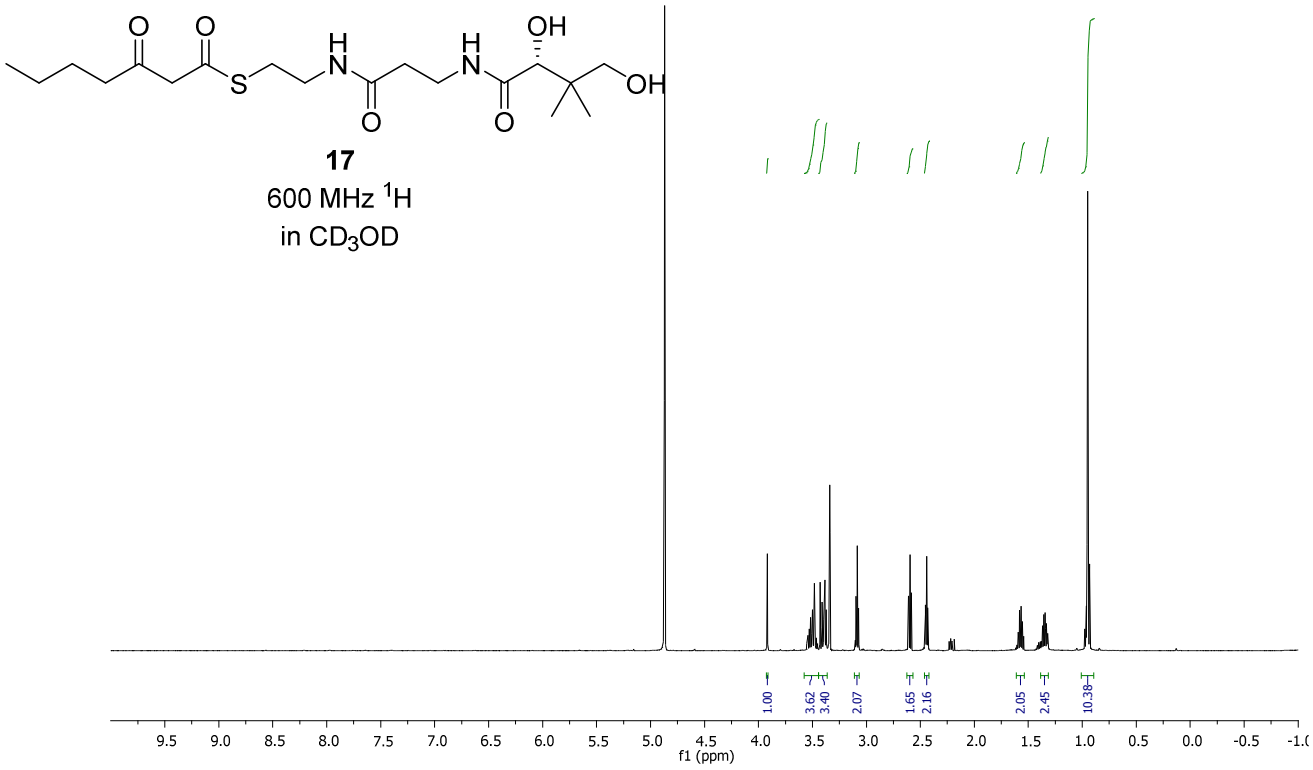


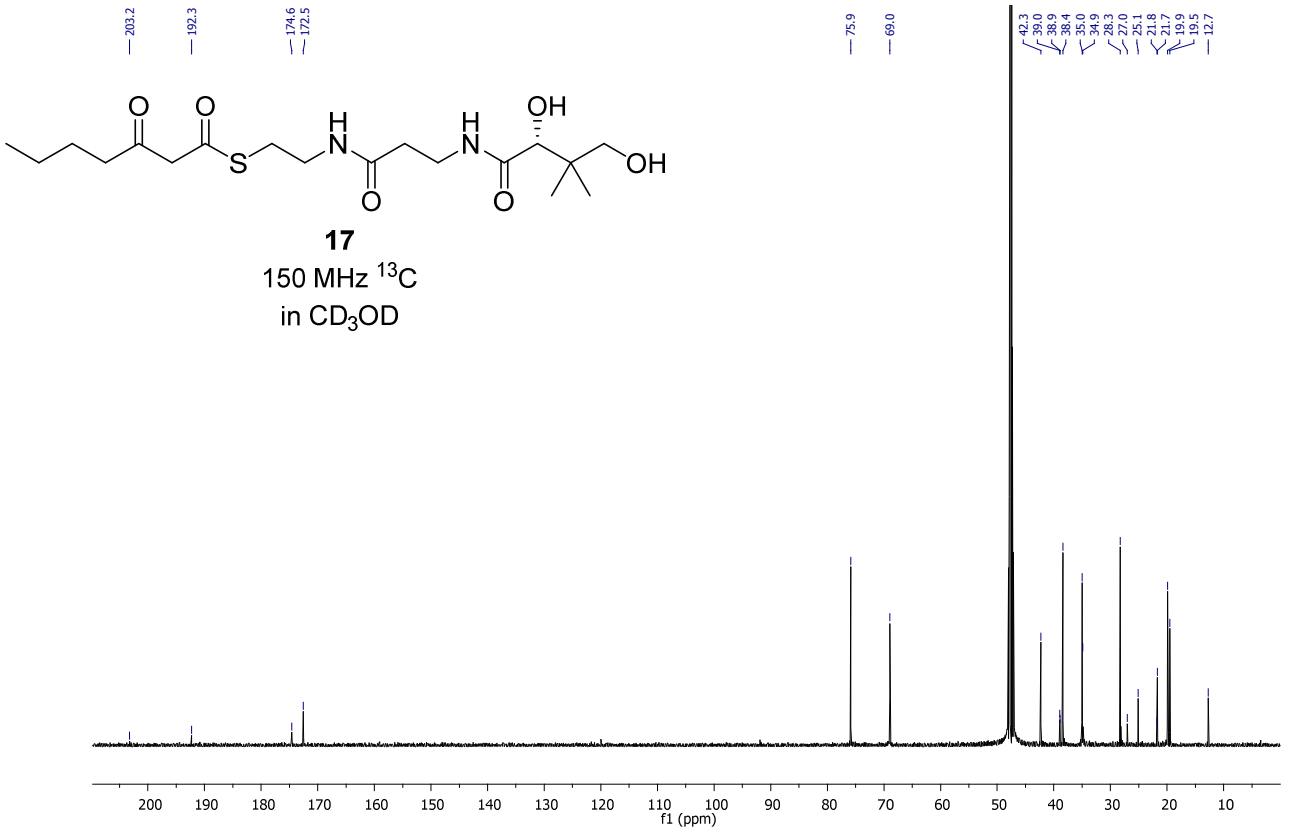
16

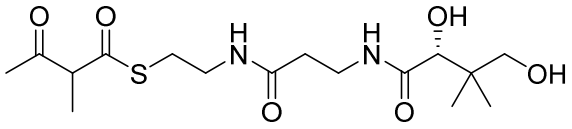
150 MHz ^{13}C

in CD_3OD




17
600 MHz 1H
in CD3OD
1.00
3.62
3.40
2.07
1.65
2.16
2.05
2.45
10.38
f1 (ppm)

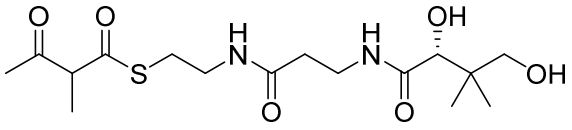

203.2
192.3
174.6
172.5
75.9
69.0
42.3
39.0
38.9
38.4
35.0
34.9
28.3
27.0
25.1
21.8
21.7
19.9
19.5
12.7
17
150 MHz 13C
in CD3OD
f1 (ppm)



18

600 MHz ^{1}H

in CD_3OD



18

150 MHz ^{13}C

in CD_3OD

Cloning and purification of CoaA, CoaD, and CoaE enzymes

CoaA, CoaD and CoaE enzymes were amplified from a colony of *E. coli* BL21(DE3) cells as template using the following primers:

CoaA Fwd NdeI	AAGCAGCCG**CATATG**AGTATAAAAGAGCAAACGTTAATGACG
CoaA Rev XhoI	CTAG**CTCGAG**TTATTTGCGTAGTCTGACCTCTTCTACCGCAT
CoaD Fwd BamHI	CTAG**GGATCC**ATGCAAAAACGGGCGATTTATCCGGGTAC
CoaD Rev XhoI	CTAG**CTCGAG**CTACGCTAACTTCGCCATCAGCGCCTGAT
CoaE Fwd BamHI	CTAG**GGATCC**ATGAGGTATATAGTTGCCTTAACGGGAGGCAT
CoaE Rev XhoI	CTAG**CTCGAG**TTACGGTTTTTCCTGTGAGACAAACTGCGACG

(restriction sites are underlined in bold)

Following PCR amplification, cleanup of PCR product by agarose gel electrophoresis, extraction, and overnight restriction digestion with the appropriate restriction enzymes, the PCR amplicons were ligated to digested pET28b(+) (kan^r) vector (CoaA), or to a previously described pET28MBP (kan^r) vector[3] (CoaD and CoaE). All vectors were checked by restriction digestion and Sanger sequencing.

CoaA, CoaD, and CoaE enzymes were purified using identical protocols. All three plasmids were transformed to *E. coli* BL21Gold(DE3) for overexpression of recombinant proteins. 1 L terrific broth cultures, supplemented with 50 µg/L kanamycin were grown for all three strains at 30 °C. Protein expression was induced by the addition of 0.3 mM IPTG (isopropyl-β-D-1-thiogalactopyranoside) when the optical density (at 600 nm) reached 0.6. The temperature was reduced to 18 °C, and cultures were allowed to grow for an additional 18 h. All subsequent steps were performed at 4 °C or on ice. Cultures were harvested by centrifugation, the supernatant discarded, and the pellet resuspended in 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 10% glycerol buffer, and lysed by sonication. The supernatant was clarified by centrifugation and loaded on to a 5 mL His-Trap Ni-NTA column (GE Biosciences) equilibrated in harvest buffer. The column was extensively washed with 20 mM Tris-HCl (pH 8.0), 1 M NaCl, 30mM imidazole buffer, and eluted by a linear gradient to 20 mM Tris-HCl (pH 8.0), 1 M NaCl, 200 mM imidazole buffer across 20 column volumes. Purity of eluted proteins was checked by SDS-PAGE, and concentrated to 2.5 mL volume using centrifugal filters. The proteins were then buffer exchanged using PD-10 size exclusion columns (GE Biosceinces) to 20 mM Tris-HCl (pH 8.0), 10% glycerol buffer. The protein concentration was measured using the Bradford assay, and all proteins were subsequently stored at -80 °C in 100 µL aliquots. Expression and purification of Sfp has been described previously.[4]

Cloning and purification of ACP

The Mpy15-ACP[5] was cloned using Streptomyces CNQ-418 genomic DNA as PCR template and ligated to pET28b(+) plasmid vector as described above. The primers used for PCR amplification were:

Mpy15 Fwd NdeI	AAGCAGCCG**CATATG**ACCCGCGAAGCAGTCCAGGAACAGTTG
Mpy15 Rev XhoI	CTAG**CTCGAG**CTACTTCGCGGCGGTCTCGCTACGGATGGACA

(restriction sites are underlined in bold)

The plasmid was transformed in to *E. coli* BL21Gold(DE3) for overexpression of recombinant Mpy15 protein. 1 L terrific broth culture, supplemented with 50 µg/L kanamycin were grown at 30 °C, and protein expression was induced as described above. The first step of purification using 5 mL His-Trap Ni-NTA column was also performed as described above. The purified protein was checked by SDS-PAGE, and thrombin was added to a final concentration of 1 unit/mg recombinant protein. Mpy15 was then dialyzed overnight against 20 mM Tris-HCl (pH 8.9), 50 mM KCl buffer at 4 °C. The dialyzed protein was applied to a 5 mL ion exchage Qff column (GE Biosciences) equillibrated in dialysis buffer, and eluted using a linear gradient to 20 mM Tris-HCl (pH 8.9), 1 M KCl buffer. Glycerol was added to the eluted protein to a final concentration of 10% (v/v). Protein concentration was measuered by Bradford assay, and the protein was stored at -80 °C in 500 µL aliquots.

Enzymatic reactions for synthesis of acyl-CoAs and mass spectrometric characterization

6–18 were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 100 mM. To a 100 µL final assay volume, the following components were added in sequence: 50 mM MES-Na (pH 6.5) buffer, 1 mM **6–18** substrates, 1 µM each of CoaA, CoaD, and CoaE enzymes. The reaction was initiated after 2 min incubation at 30 °C by the addition of 9 mM freshly prepared ATP. CoaA, CoaD, and CoaE were diluted in 50 mM HEPES-Na (pH 7.5), 10% glycerol buffer prior to use and mixed together to yield a single enzyme-cocktail. ATP was also dissolved in 50 mM HEPES-Na (pH 7.5), 10% glycerol buffer. This enzyme-cocktail was stable at 4 °C for several months without loss of activity.

The assays were incubated at 30 °C for three hours, and quenched by the addition of equal volume of MeCN + 0.2% formic acid. An immediate white precipitation could be observed. The quenched reactions were vortexed and centrifuged at 16,000×g for 10 min. The assays were then analyzed by HPLC and LC/MS/MS using a Phenomenex Luna C18 5µ 100×4.6 mm column operating at room temperature. HPLC solvents (water and MeCN) were supplemented with 0.1% trifloroacetic acid (TFA). LC/MS/MS solvents were supplemented with 0.1% formic acid. The elution gradient used was as follows:

Flow rate 0.7 mL/min
0-5 mins: 5% B
5- 20 min: linear gradient to 80% B
20-21 min: linear gradient to 100% B
21-26 min: 100% B
26-27 min: linear gradient to 5% B
27-30 min: 5% B

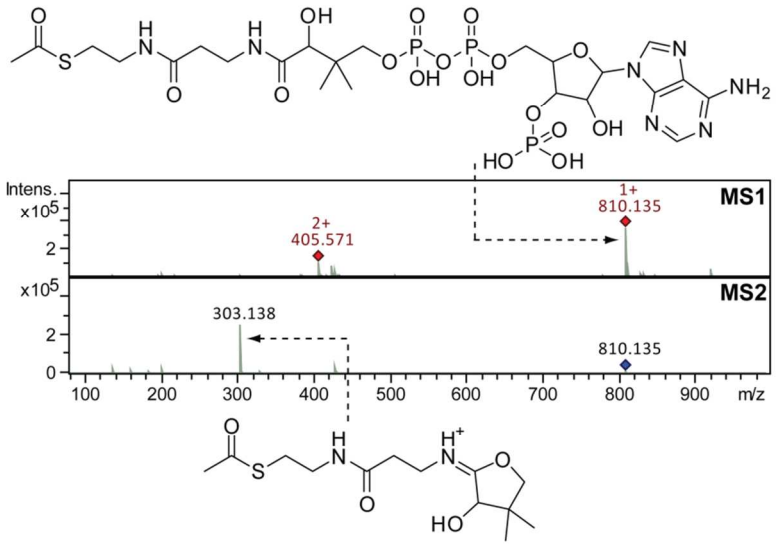


Figure S1: MS1 and MS2 spectra for acyl-CoA product from **6** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (810.13 Da) and $[M+2H]^{2+}$ (405.57 Da) ions are observed that correspond to the molecular formula $C_{23}H_{38}N_7O_{17}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (303.14 Da).

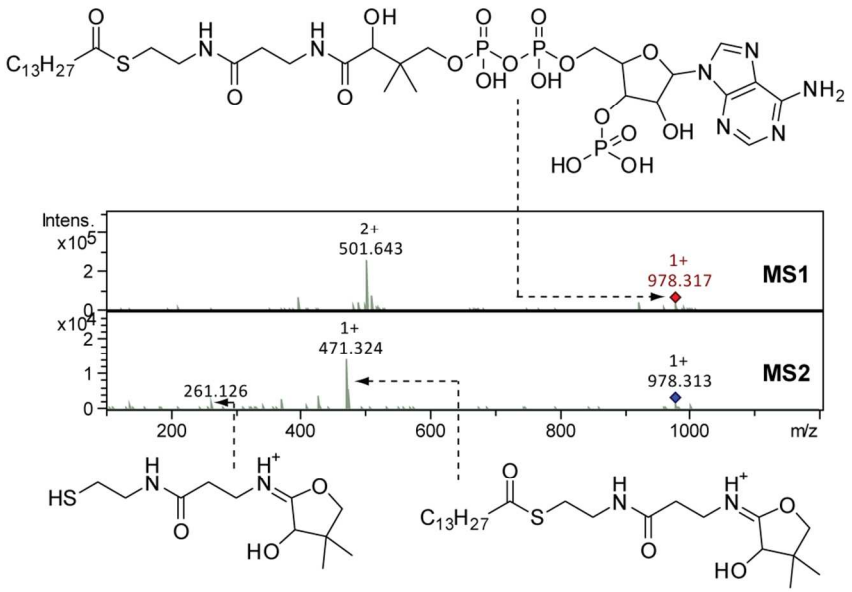


Figure S2: MS1 and MS2 spectra for acyl-CoA product from **7** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (978.32 Da) and $[M+H+Na]^{2+}$ (501.64 Da) ions are observed that correspond to the molecular formula $C_{35}H_{62}N_7O_{17}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (471.32 Da). Note that the long alkyl chain makes limits the ionization of the products.

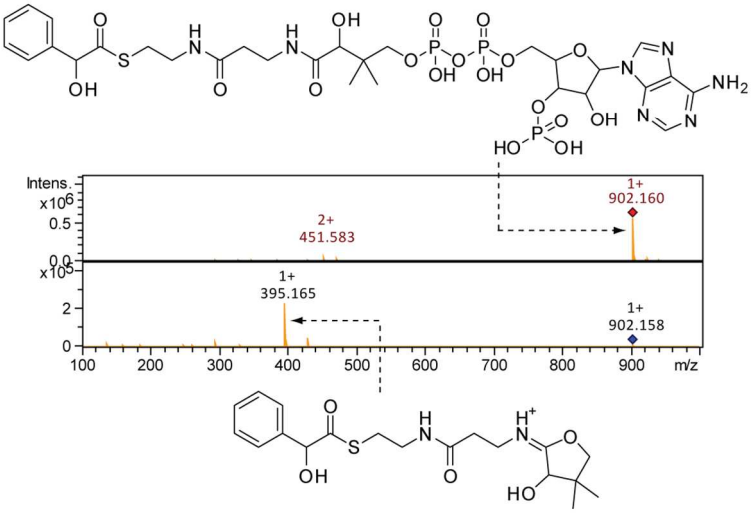


Figure S3: MS1 and MS2 spectra for acyl-CoA product from **8** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (902.16 Da) and $[M+2H]^{2+}$ (451.58 Da) ions are observed that correspond to the molecular formula $C_{29}H_{42}N_7O_{18}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (395.16 Da).

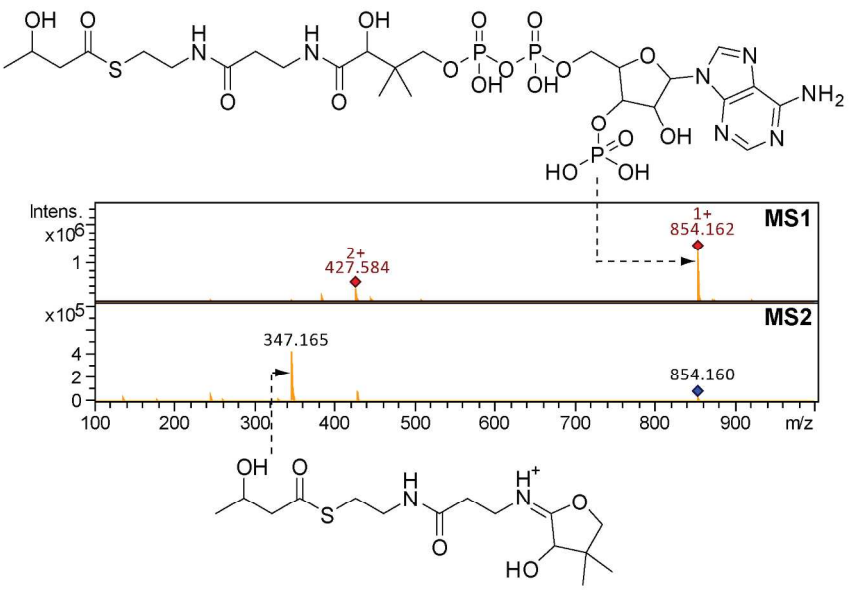


Figure S4: MS1 and MS2 spectra for acyl-CoA product from **9** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (854.16 Da) and $[M+2H]^{2+}$ (427.58 Da) ions are observed that correspond to the molecular formula $C_{25}H_{42}N_7O_{18}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (347.16 Da).

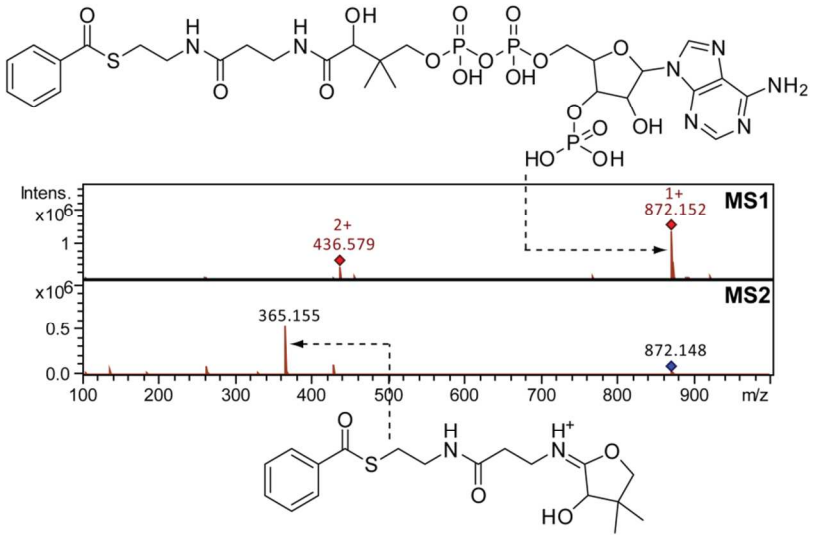


Figure S5: MS1 and MS2 spectra for acyl-CoA product from **10** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (872.15 Da) and $[M+2H]^{2+}$ (436.58 Da) ions are observed that correspond to the molecular formula $C_{28}H_{40}N_7O_{17}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (365.15 Da).

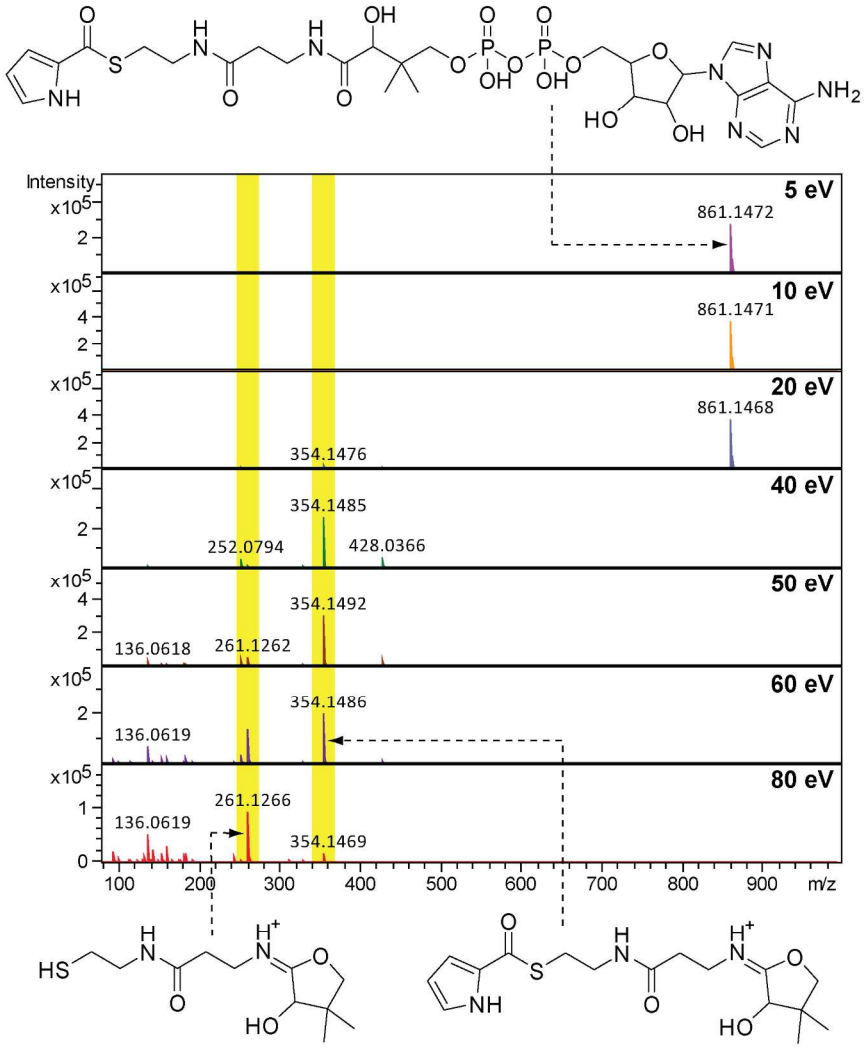


Figure S6: MS1 and MS2 spectra for acyl-CoA product from **11** as the substrate. MS2 spectra for pyrrolyl-*S*-CoA at different fragmentation energies (in eV) are shown. The parent MS1 ion corresponding to pyrrolyl-*S*-CoA (861.14 Da) has been fragmented at increasing energies. At low fragmentation energies, no MS2 product ions can be observed. At intermediate fragmentation energies, the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (354.15 Da) is observed, which further degrades to (cyclo)pantetheine MS2 product ion (261.12 Da) due to the cleavage of the thioester bond.

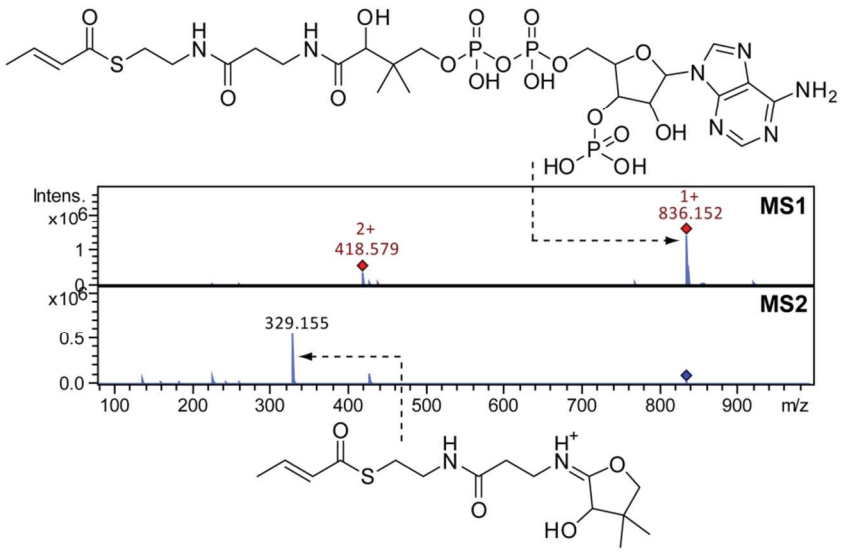


Figure S7: MS1 and MS2 spectra for acyl-CoA product from **12** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (836.15 Da) and $[M+2H]^{2+}$ (418.58 Da) ions are observed that correspond to the molecular formula $C_{25}H_{40}N_7O_{17}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (329.15 Da).

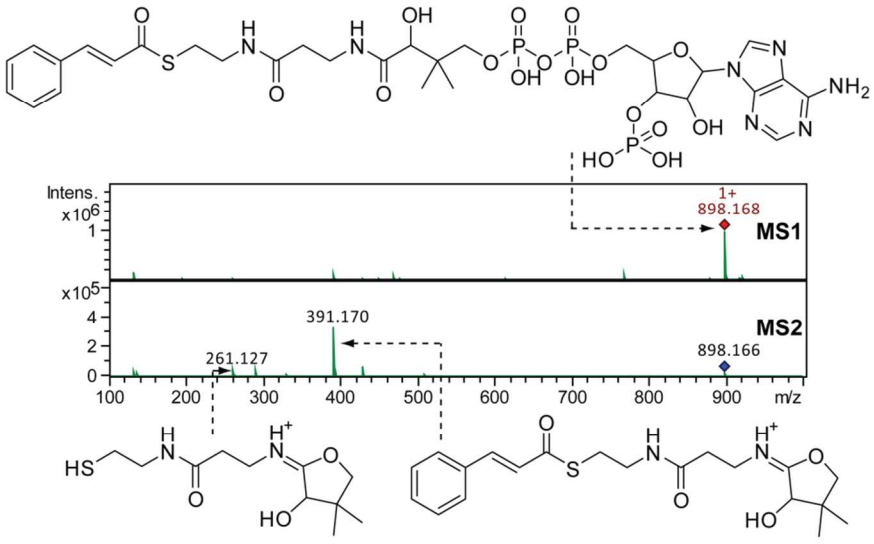


Figure S8: MS1 and MS2 spectra for acyl-CoA product from **13** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (898.17 Da) ion is observed that corresponds to the molecular formula $C_{30}H_{42}N_7O_{17}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (391.17 Da), and the (cyclo)pantetheine MS2 product ion (261.12 Da).

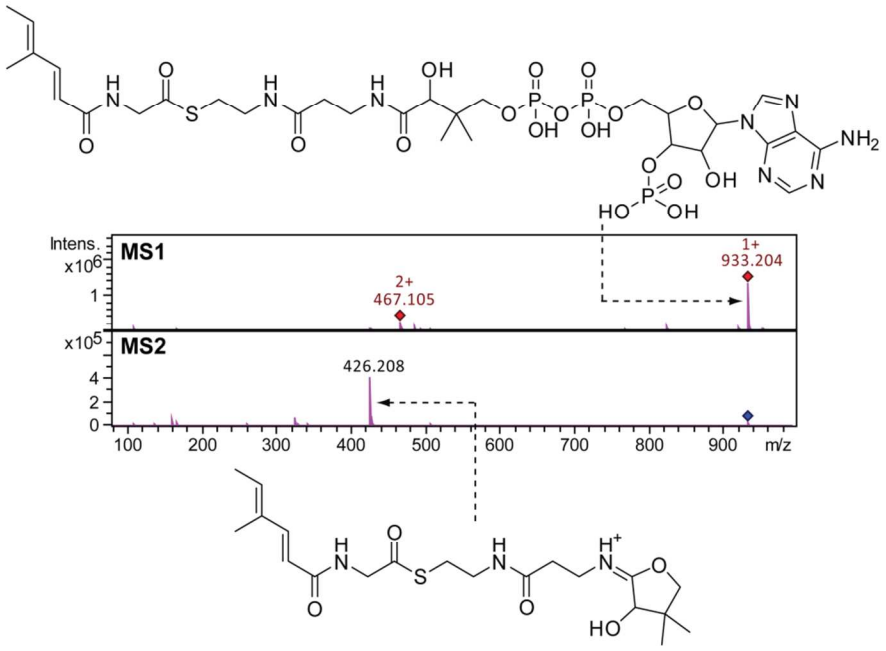


Figure S9: MS1 and MS2 spectra for acyl-CoA product from **14** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (933.20 Da) and $[M+2H]^{2+}$ (467.10 Da) ions are observed that correspond to the molecular formula $C_{30}H_{47}N_8O_{18}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (426.20 Da).

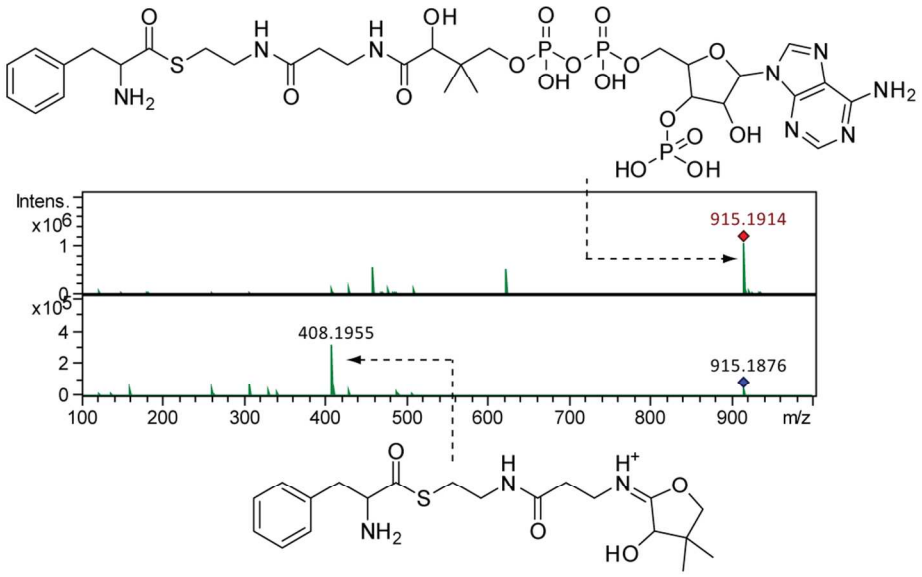


Figure S10: MS1 and MS2 spectra for acyl-CoA product from **15** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (915.19 Da) ion is observed that corresponds to the molecular formula $C_{30}H_{45}N_8O_{17}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (408.19 Da).

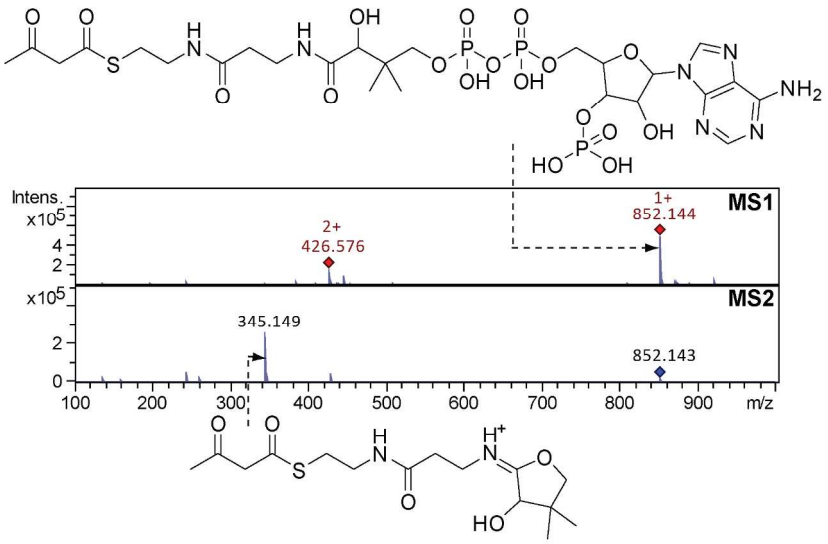


Figure S11: MS1 and MS2 spectra for acyl-CoA product from **16** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (852.14 Da) and $[M+2H]^{2+}$ (426.57 Da) ions are observed that correspond to the molecular formula $C_{25}H_{40}N_7O_{18}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (345.15 Da).

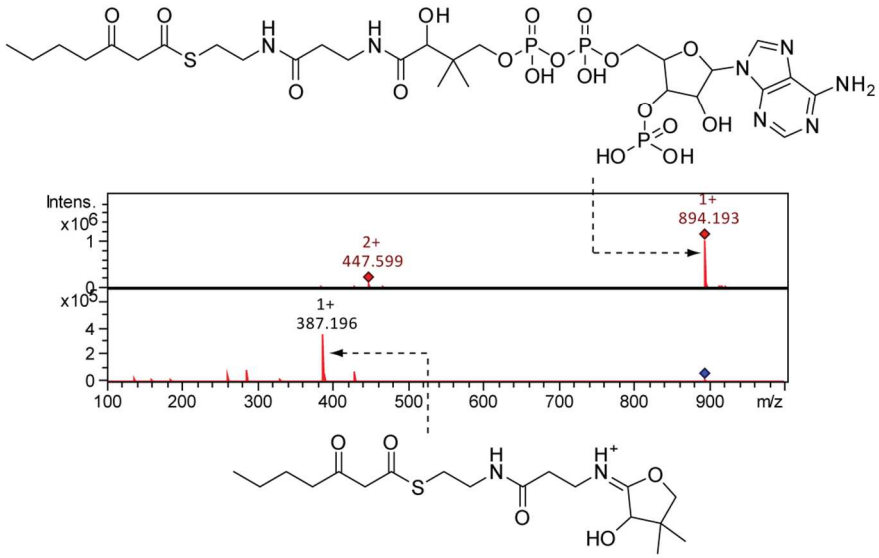


Figure S12: MS1 and MS2 spectra for acyl-CoA product from **17** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (894.19 Da) and $[M+2H]^{2+}$ (447.60 Da) ions are observed that correspond to the molecular formula $C_{28}H_{46}N_7O_{18}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (387.19 Da).

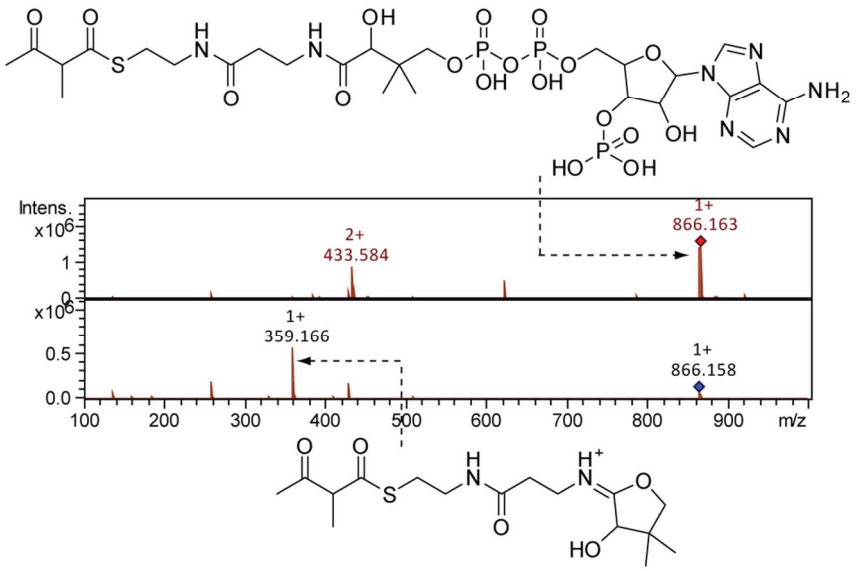


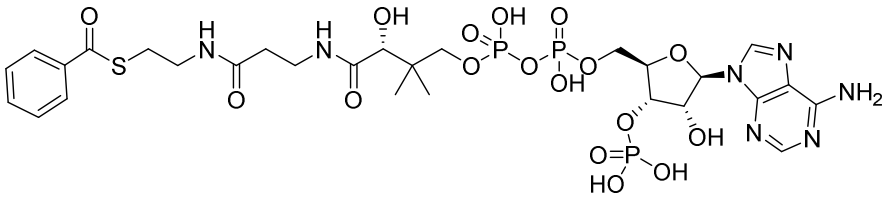
Figure S13: MS1 and MS2 spectra for acyl-CoA product from **18** as the substrate. In the MS1 spectra, the $[M+H]^{1+}$ (866.16 Da) and $[M+2H]^{2+}$ (433.58 Da) ions are observed that correspond to the molecular formula $C_{26}H_{42}N_7O_{18}P_3S$. The $[M+H]^{1+}$ ion generates the characteristic acyl-*S*-(cyclo)pantetheine MS2 product ion (359.16 Da).

Preparative enzymatic synthesis of benzoyl-CoA

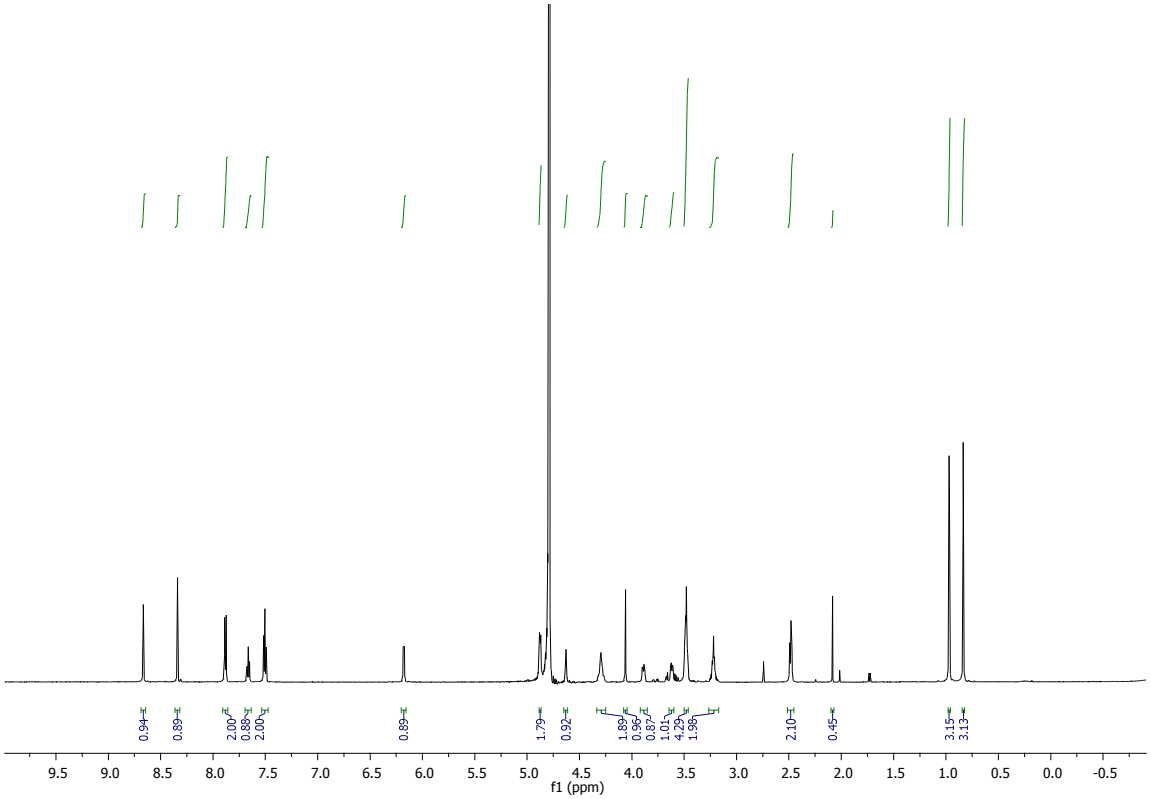
Reactions were set up, as described above, for the preparative scale conversion of **10** (3.8 mg **10** in a 10 mL final reaction volume) to benzoyl-CoA. After 3 h incubation, reaction was quenched with equal volume of MeCN + 0.2% formic acid. Analytical HPLC demonstrated full conversion of **10** to benzoyl-CoA. Precipitate was removed by centrifugation, and the supernatant was frozen and lyophilized to yield a white powder. The powder was dissolved in water, and benzoyl-CoA was purified by reverse phase HPLC using Phenomenex Luna C18 5µ 250×10 mm column operating at room temperature. HPLC solvents (water and MeCN) were supplemented with 0.1% TFA. The elution gradient used was as follows:

Flow rate 3 mL/min
0-5 mins: 5% B
5-15 min: linear gradient to 60% B
15-16 min: linear gradient to 100% B
16-19 min: 100% B
19-20 min: linear gradient to 5% B
20-22 min: 5% B

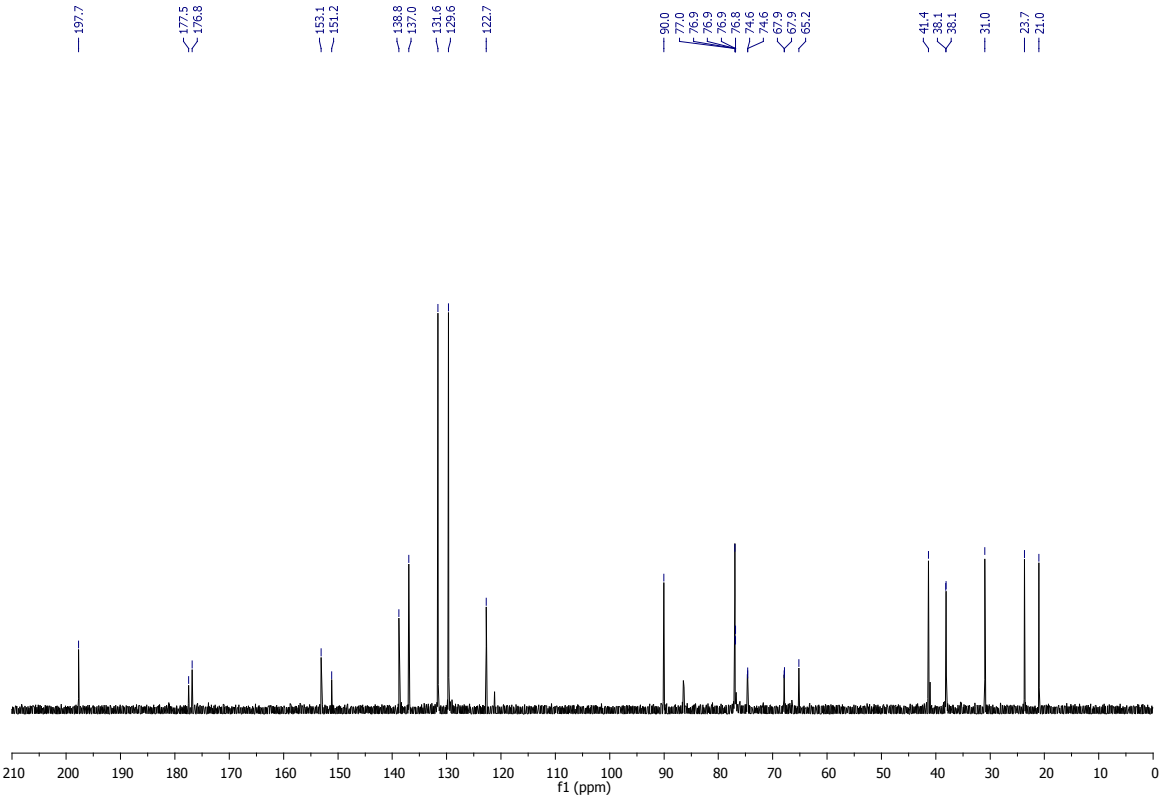
Elution fractions were pooled, frozen and lyophilized to yield a white powder.



^{1}H-NMR (600 MHz, D_2O): δ 8.67 (s, 1H), 8.34 (s, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.66 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 2H), 6.18 (d, *J* = 5.4 Hz, 1H), 4.88 (m, 1H), 4.36, (m, 1H), 4.33-4.26 (m, 2H), 4.06, (s, 1H), 3.89 (dd, *J* = 9.6, 4.4 Hz, 1H), 3.62 (dd, *J* = 9.5, 4.0 Hz, 1H), 3.50 – 3.47 (m, 4H), 3.25-3.17 (m, 2H), 2.38, (t, *J* = 6.4 Hz, 2H), 2.08 (s, 1H), 0.97 (s, 3H), 0.83 (s, 3H) **^{13}C-NMR** (150 MHz, D_2O): δ 197.7, 177.5, 176.8, 153.1, 151.2, 138.8, 137.0, 131.6 (2C), 129.6 (2C), 122.7, 90.0, 77.0, 76.9, 76.9, 76.9, 76.8, 74.6, 74.6, 67.9, 67.9, 65.2, 41.4, 38.1, 38.1, 31.0, 23.7, 21.0.; **HRMS** (ESI) *m/z* calculated for $C_{28}H_{41}N_7O_{17}P_3S$ ($[M+H]^+$) 872.1487, found 872.1520.



^{1}H-NMR spectrum of benzoyl-CoA recorded in D_2O (600 MHz)



^{13}C-NMR spectrum of benzoyl-CoA recorded in D_2O (150 MHz). The spectrum was referenced to the carbon shifts of the pantetheine gem-dimethyl group previously reported for crotonyl-CoA and hexadienoyl-CoA.[6]

***In situ* acetylation of 19**

The following assay components were mixed in order in a 100 µL reaction: 50 mM HEPES-Na (pH 7.0), 1 mM **6**, 0.5 mM **19**, 1 µM each of CoaA, CoaD and CoaE enzymes, and 10 units of CAT (Sigma-Aldrich: C8413). The reaction was initiated after 2 min incubation at 30 °C by the addition of 9 mM freshly prepared ATP.

The reaction was quenched by the addition of equal volume of MeCN + 0.2% formic acid, and the precipitated protein was removed by centrifugation at 14000 rpm for 10 min. 40 µL of the reaction was injected to a reverse phase HPLC Phenomenex Luna 5µ 250×10 mm column operating at room temperature coupled to an ESI-ToF mass spectrometer operating in negative ionization mode. The mobile phase solvents (water and MeCN) were supplemented with 0.1% formic acid (for LC/MS/MS), or with 0.1% TFA (for HPLC, Figure 3). The following elution profile was used at 1 mL/min flow rate:

0 – 4 mins: 5% buffer B
4 – 20 mins: linear gradient to 100% buffer B
20 – 24 mins: 100% buffer B
24 – 25 mins: linear gradient to 5% buffer B

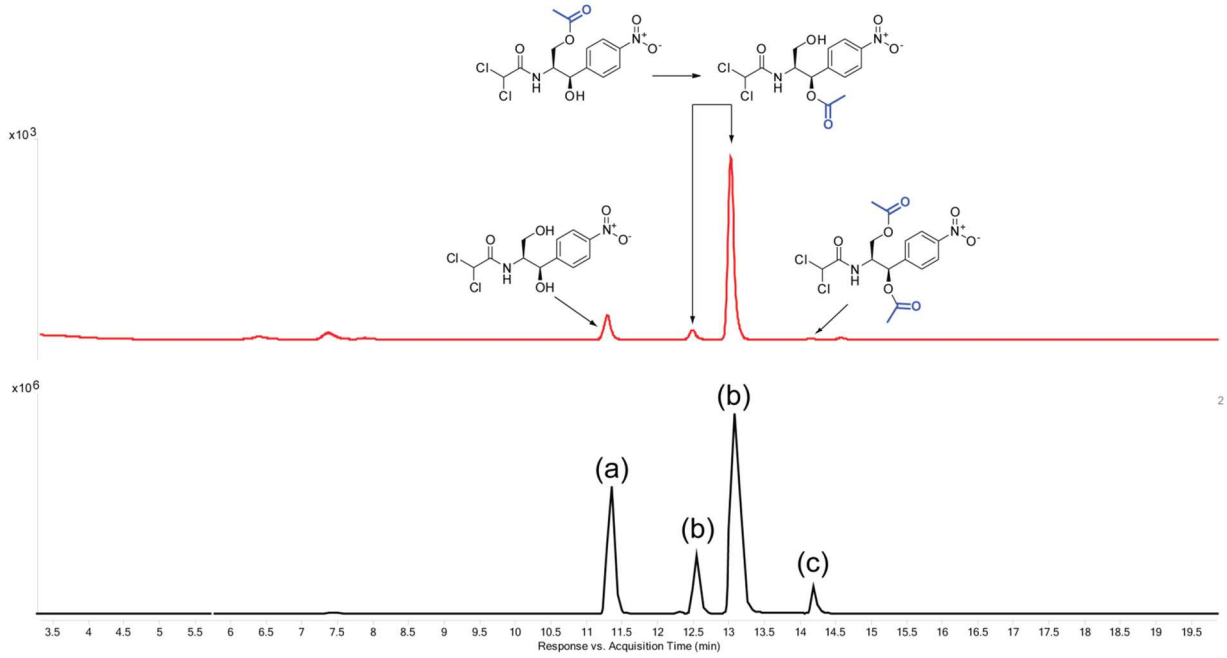


Figure S14: Mass spectrometric characterization of the *in situ* reaction for acetylation of 19. Top panel represents the elution chromatogram monitored by UV-absorbance at 280 nm. The bottom panel represents a combined extracted ion chromatogram (EIC) (calculated within 20 ppm error tolerance) for the following masses:

(a) 321.005 Da (dominant $[M-H]^{1-}$ ion corresponding to chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$)
(b) 363.015 Da (dominant $[M-H]^{1-}$ ion corresponding to monoacetylated chloramphenicol, $C_{13}H_{14}Cl_2N_2O_6$)
(c) 405.026 Da (dominant $[M-H]^{1-}$ ion corresponding to diacetylated chloramphenicol, $C_{15}H_{16}Cl_2N_2O_7$)

Based on the EICs, it can be seen that two monoacetylated isomers of **19** are generated as products of the reaction. This finding is in concert with previous reports describing the acetylation of 3' position of **19**, followed by non-catalytic transfer of the acetyl moiety to the 1' position of **19**. This non-catalytic transfer then affords a second acetyl transfer to occur at the 3' position of **19** to yield diacetylated-**19**, which is also observed as a product of the reaction. Note that the enzyme CoaA has been reported to be strongly inhibited by CoA,[7] which is a product of the coupled reaction after the transfer of the acetyl group from *in situ* generated acetyl-CoA to **19**. In order to overcome this inhibition, a higher yield of products was obtained when the first half reaction was allowed to proceed for 2 h, followed by addition of **19** and CAT for an additional 2 h reaction at 30 °C.

Enzymatic reactions for *in situ* labeling of ACPs and mass spectrometric characterization

The following assay components were mixed in order in a 100 µL reaction: 50 mM HEPES-Na (pH 7.9), 10 mM $MgCl_2$, 1 mM *S*-acyl pantetheine substrates, 1 µM each of CoaA, CoaD, CoaE, and Sfp enzymes, and 250 µM of purified Mpy15-ACP. The reaction was initiated after 2 min incubation at 30 °C by the addition of 9 mM freshly prepared ATP. The reaction was allowed to proceed for 3 h. The reactions were quenched by addition of equal volume of 2% formic acid and the precipitate was removed by centrifugation. The reactions were analyzed by LC/MS/MS and HPLC as described previously.[4]

In each of the Figures S15–S27, the top panel represents the MS1 spectra and the bottom panel represents the MS2 spectra. The parent MS1 ion selected for MS/MS is marked by a red diamond in the MS1 spectra, and by a blue diamond in the MS2 spectra. An envelope of differentially charged peptide ions is observed in the MS1 spectra. By analysis of the isotopic distribution of these differentially charged MS1 ions, ionization states can be assigned that is denoted.

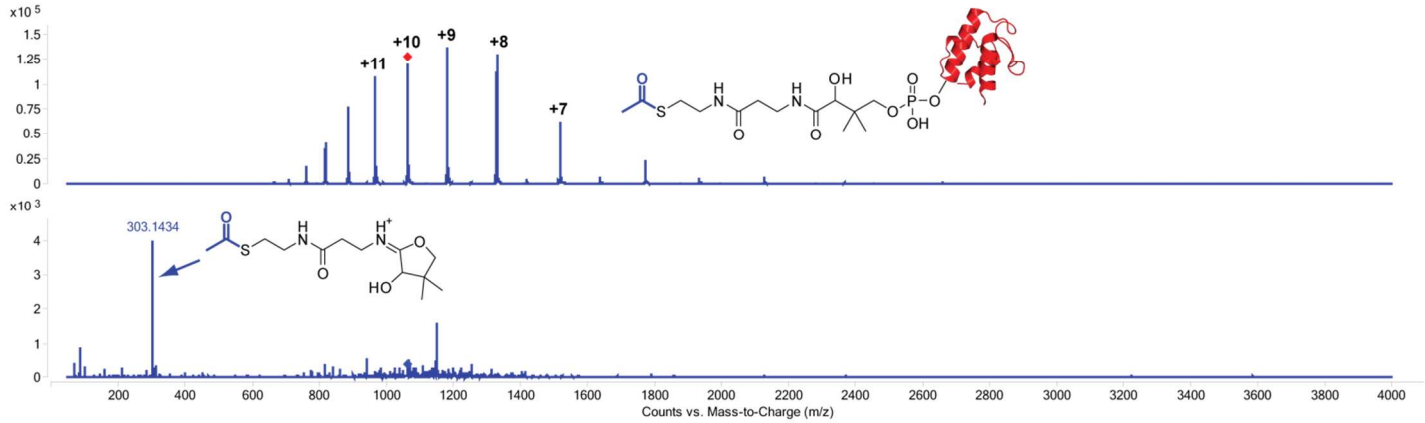


Figure S15: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **6**.

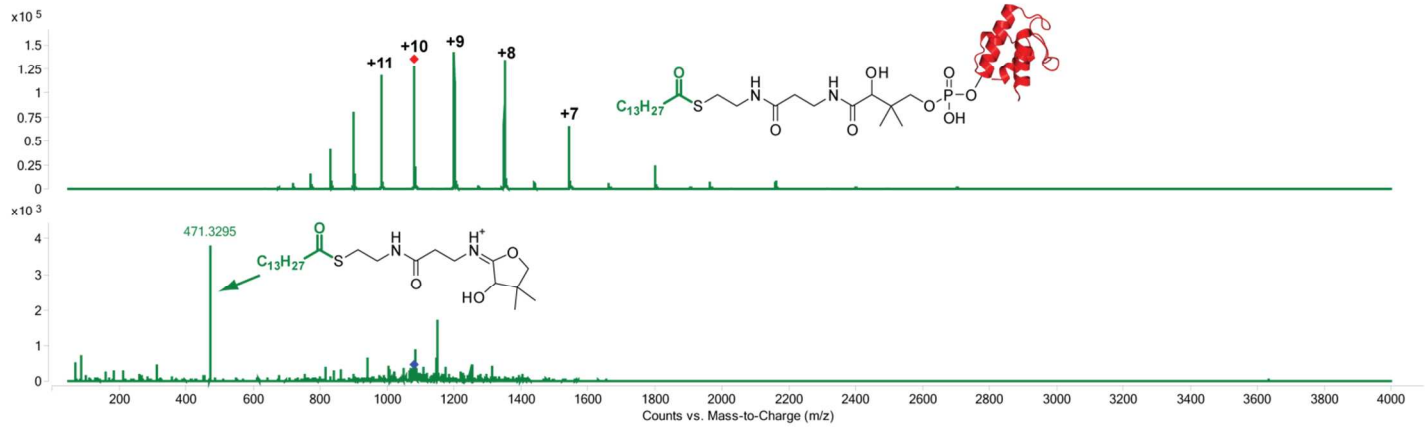


Figure S16: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **7**.

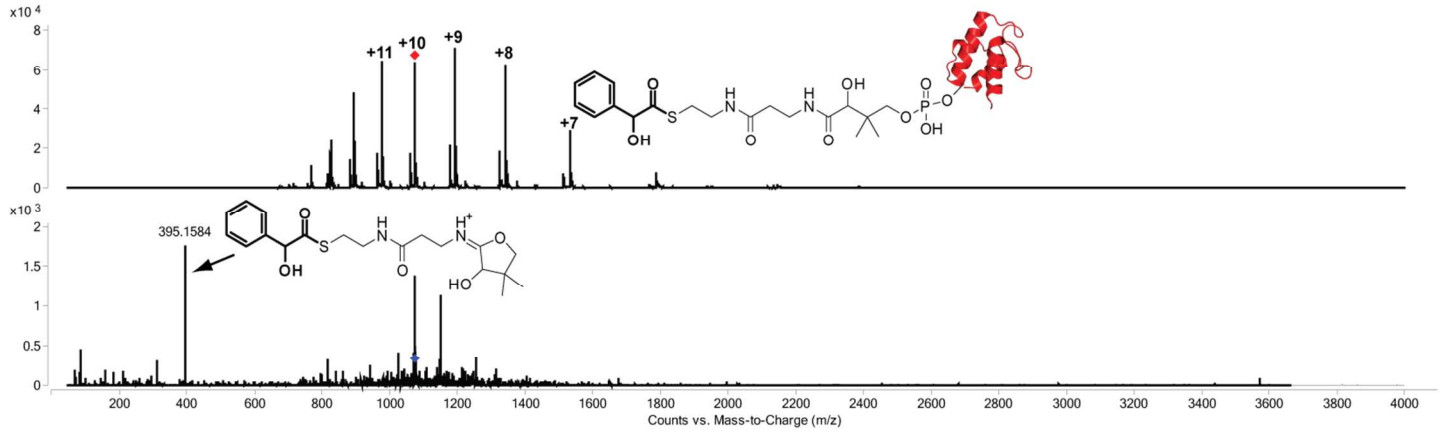


Figure S17: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **8**.

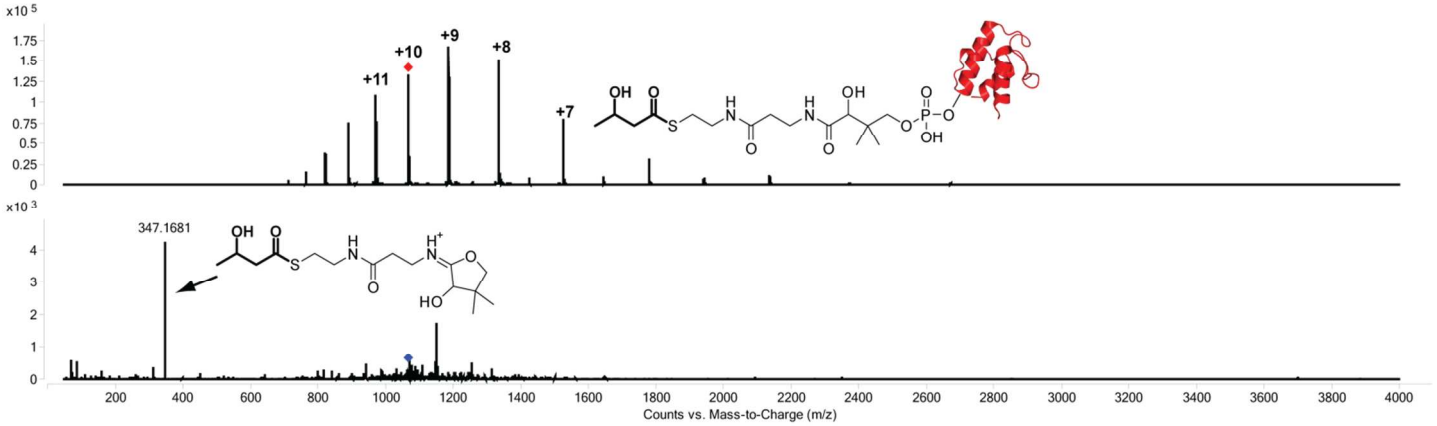


Figure S18: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **9**.

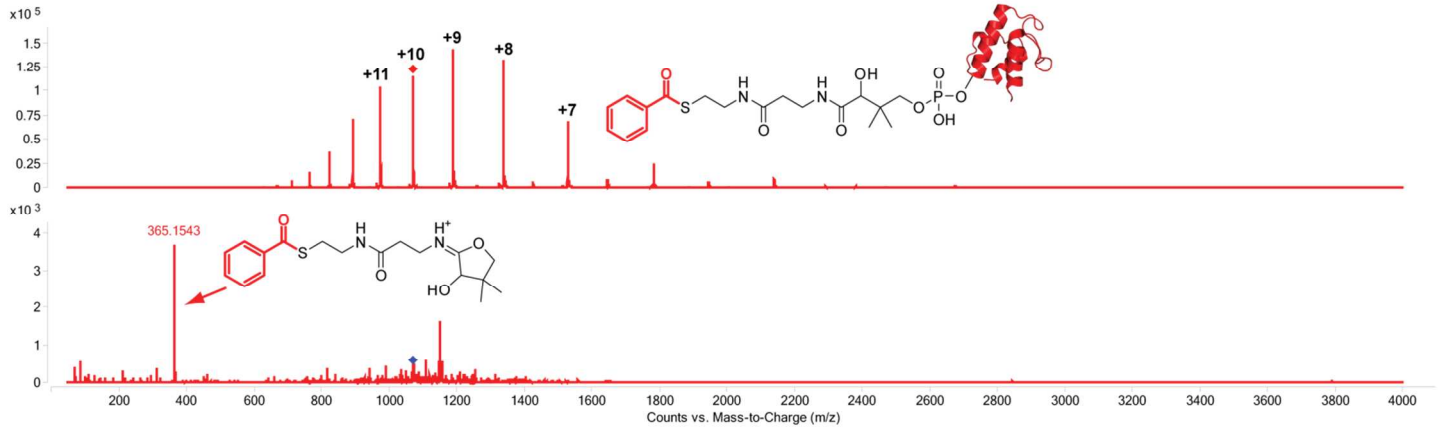


Figure S19: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **10**.

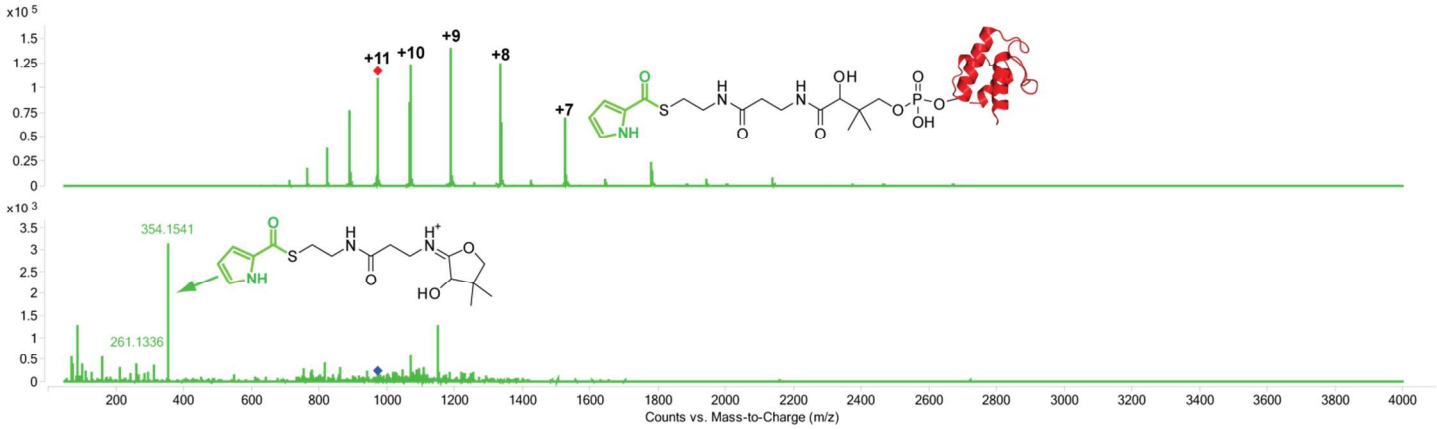


Figure S20: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **11**.

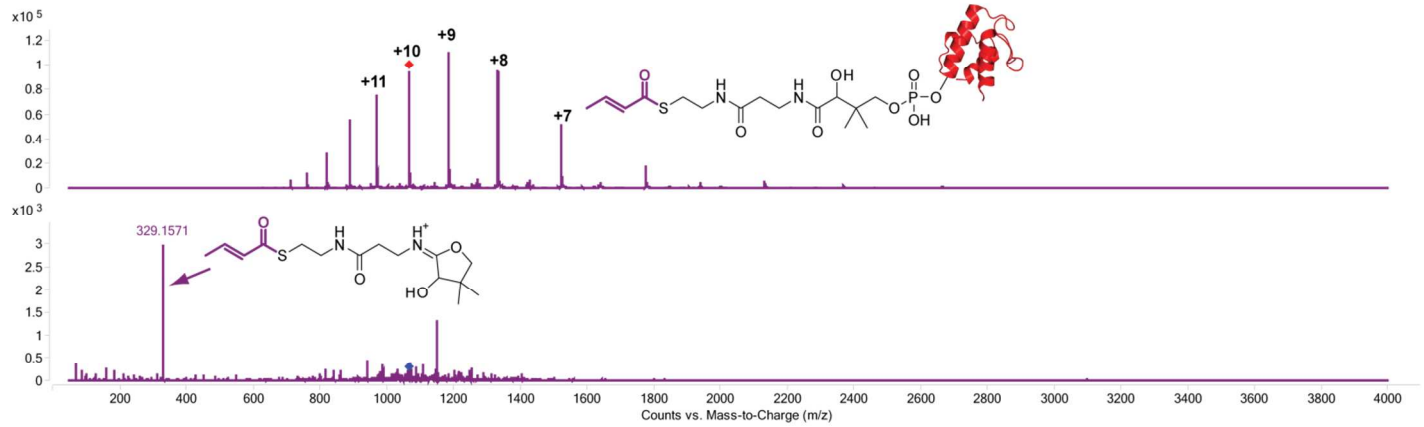


Figure S21: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **12**.

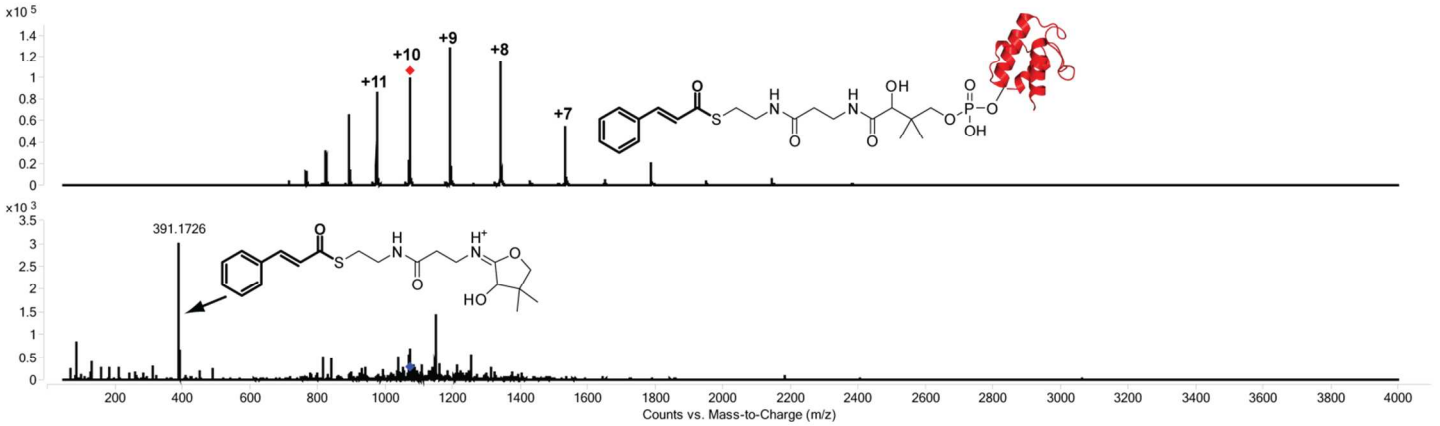


Figure S22: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **13**.

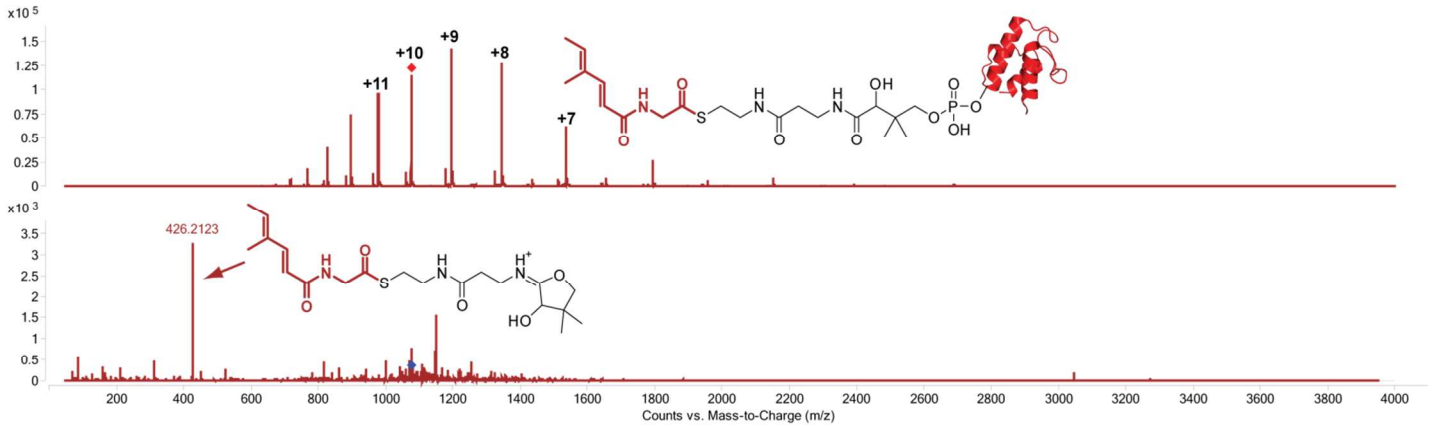


Figure S23: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **14**.

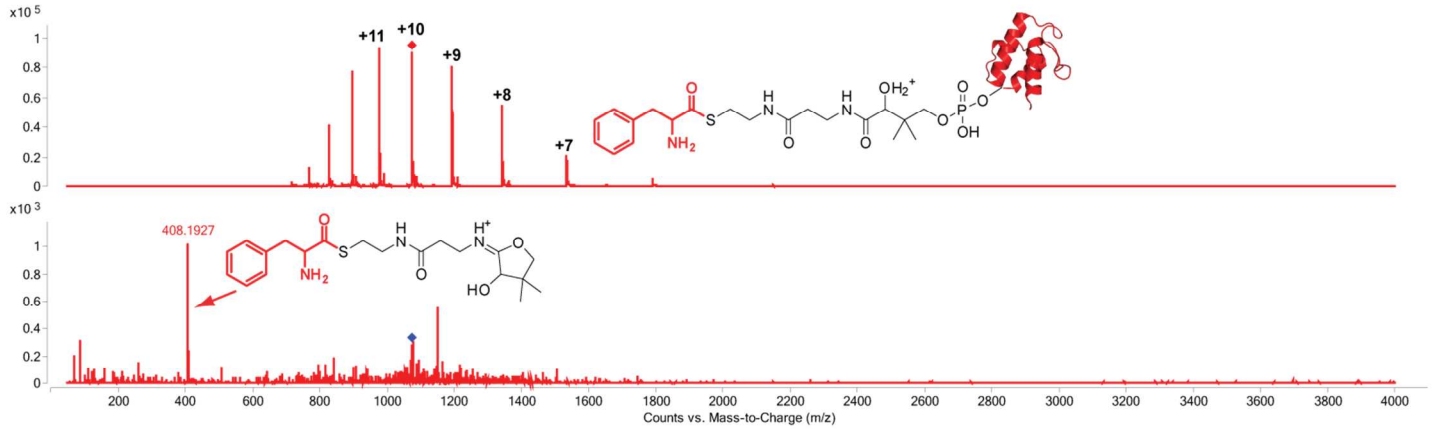


Figure S24: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **15**.

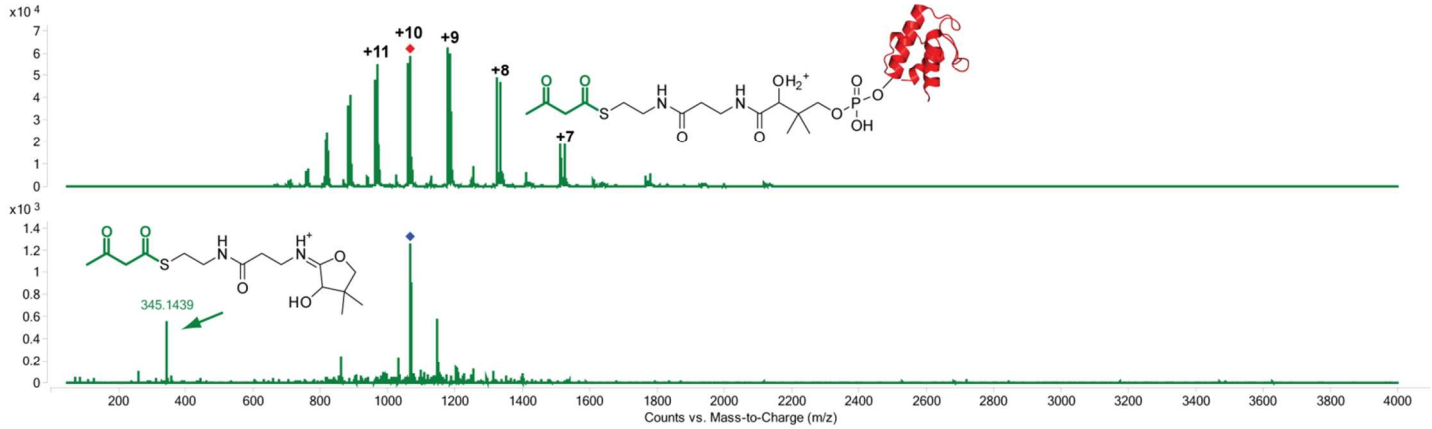


Figure S25: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **16**.

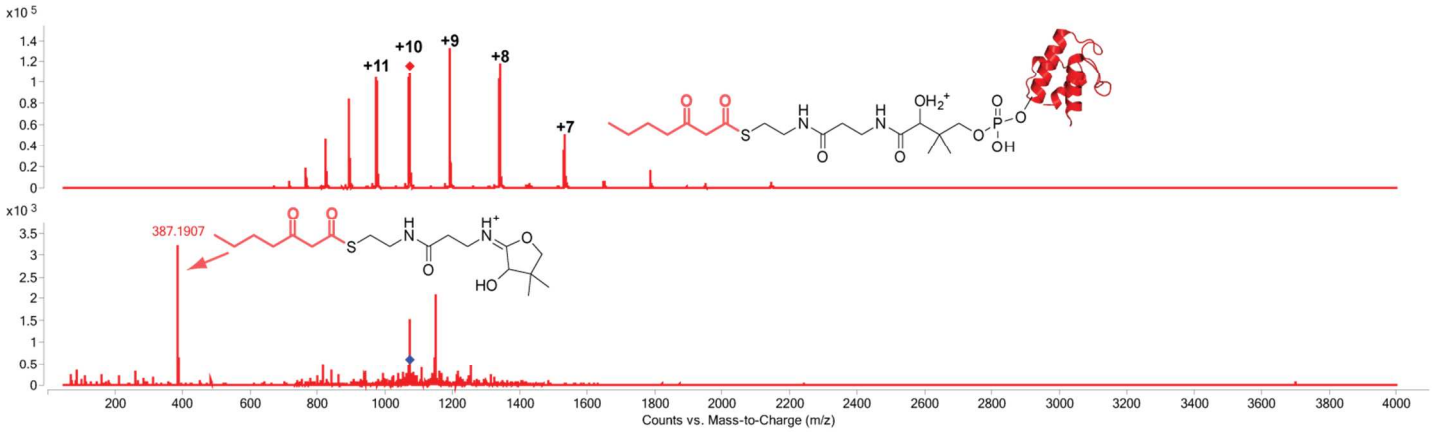


Figure S26: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **17**.

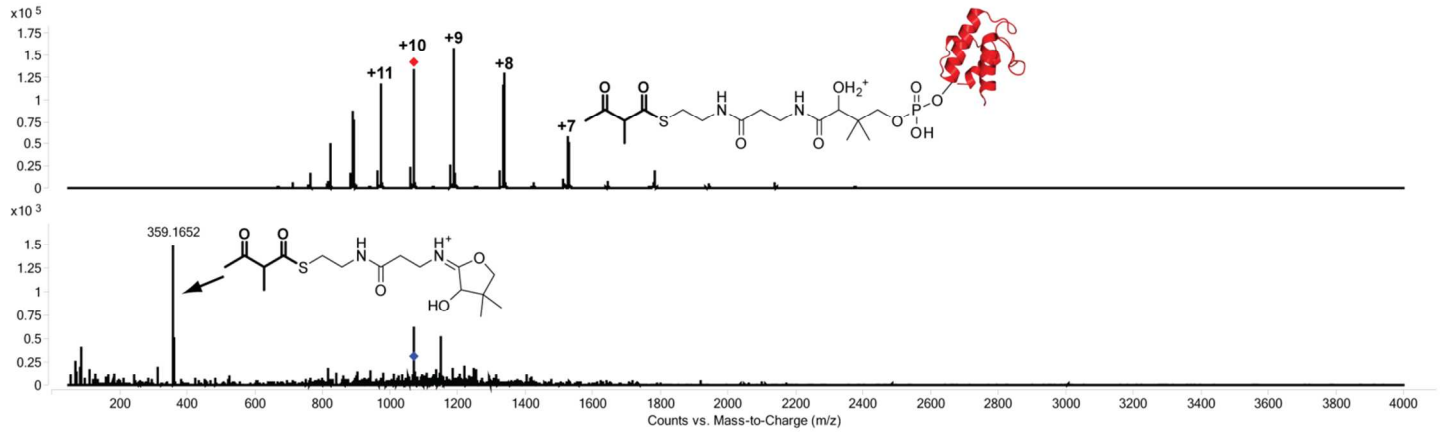


Figure S27: MS1 and MS2 spectra demonstrating Mpy15-ACP acylated with the *S*-acyl phosphopantetheine product corresponding to **18**.

Preparative scale synthesis and purification of acyl-ACPs

Using a reaction scheme as described previously, apo Mpy15-ACP was acylated starting with **11** as a substrate in a 2.5 mL reaction volume. After incubation at 30 °C, the reaction was centrifuged to remove any precipitate, and directly loaded on to a 16/60 Superdex-75 size exclusion chromatography column (GE Biosciences) equilibrated in 20 mM Tris-HCl (pH 8.0) buffer at 4 °C. An isocratic elution at 1 mL/min flow-rate afforded separation of the acyl-ACP from CoaA/D/E and Sfp enzymes, and from residual nucleotidyl substrates and products (Figure S28a). The acylation state of the ACP thus obtained after size exclusion chromatography was verified by comparison of retention time (Figure S28b) and UV-absorbance profile (Figure S28c–e) to mass spectrometrically verified reaction standards of apo-ACP and pyrrolyl-*S*-ACP (see Figure S20).

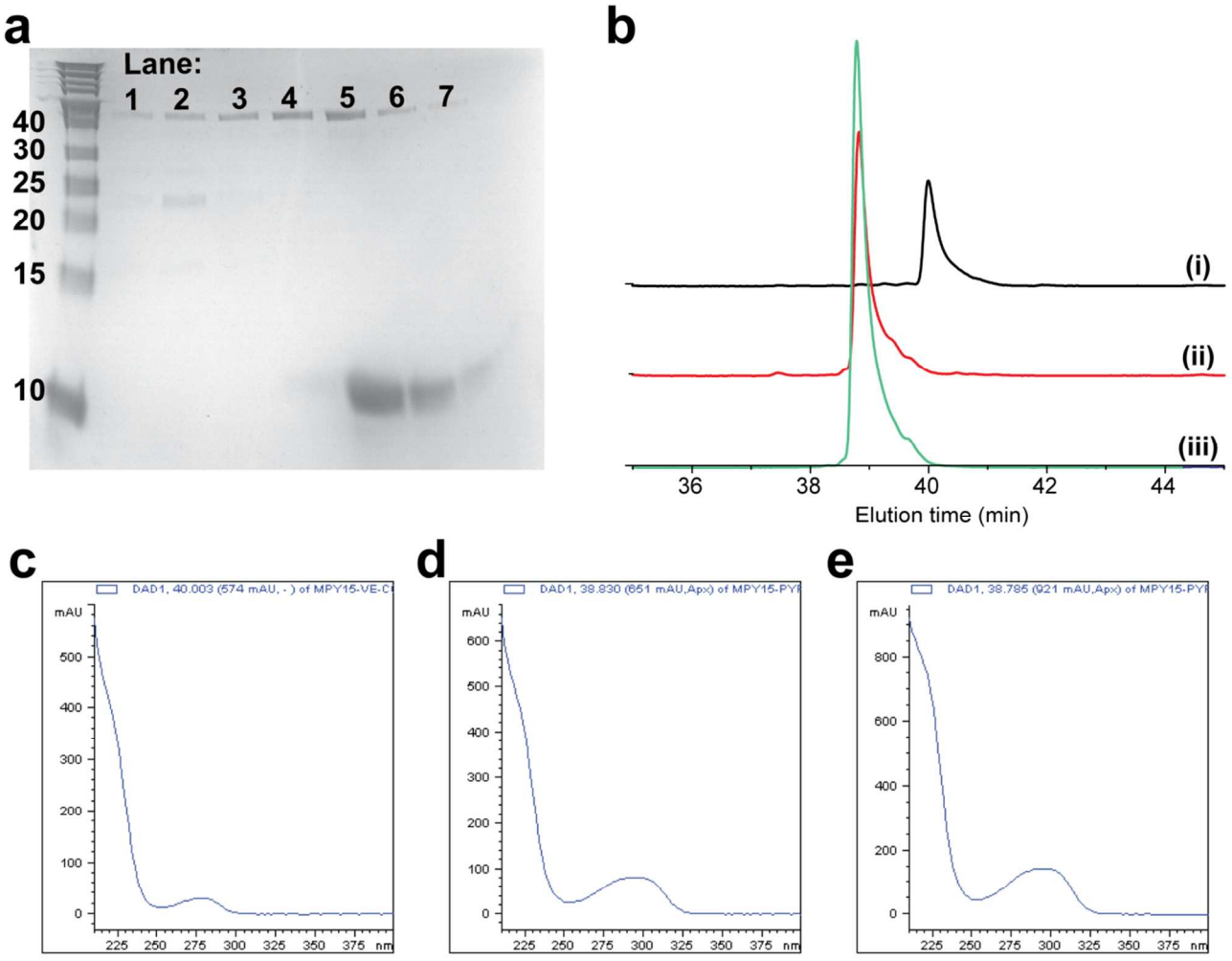


Figure S28: Preparative scale synthesis and purification of acyl-ACPs. (**a**) Tris-tricine SDS-PAGE (Bio-Rad 4563063) demonstrating the size exclusion chromatography separation of high molecular weight CoaA/D/E and Sfp proteins (lanes 1–4) from lower molecular weight acyl-ACP (lanes 5–7) that was generated using **11** as the *S*-acyl pantetheine substrate. Masses (in kDa) corresponding to the protein ladder (Fisher BP3602) are listed on the right. (**b**) Comparison of HPLC retention times of apo-ACP (curve **i**, generated as a negative control with ATP omitted from the reaction), pyrrolyl-*S*-ACP (curve **ii**, reaction product characterized by MS, see Figure S20), and purified pyrrolyl-S-ACP (curve **iii**) after size exclusion chromatography. UV-absorbance profiles for peaks corresponding to (**c**) trace (**i**) in panel b, (**d**) trace (**ii**) in panel b, and (**e**) trace (**iii**) in panel b. By comparison of HPLC retention times and UV-absorbance profiles, it can be deduced that pyrrolyl-*S*-ACP did not undergo deacylation during size exlcusion chromatography and subsequent analysis by HPLC. Pyrrolyl-*S*-ACP at this stage was concentrated by centrifugal filters and stored at -80 °C in small aliquots without degradation or deacylation for extended periods of time.

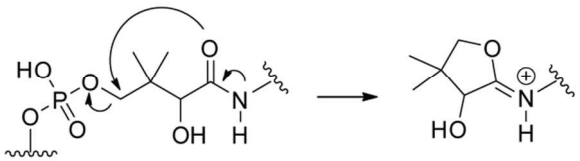


Figure S29: A proposed chemical route for the generation of the observed (cyclo)pantetheine MS2 product ions.

Supplementary References

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