Supporting Information for

Investigation of fungal iterative polyketide synthase functions using partially assembled intermediates

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1. Protein purification

1.1. Routine purification method for Hpm3, Hpm8

The expression plasmids, pZH126 and pZH74[1] harboring the Hpm3 and Hpm8 genes respectively were transformed into *Saccharomyces cerevisiae* strain BJ5464-NpgA[1] for expression. 1 L yeast cultures were grown at 28 °C in YPD media with 1% dextrose for 72 hours. The cells were harvested by centrifugation (3273g rpm, 15 minutes, 4 °C), resuspended in 25mL lysis buffer (50 mM NaH_2PO_4, pH = 8.0, 0.15 M NaCl, 10 mM imidazole) and lysed with sonication on ice (sonicate for 1 minute, then cool for 1 minute. Repeat 9 - 10 times). Cellular debris was removed by centrifugation (17000 g, 1 hour, 4 °C). Ni-NTA agarose resin was added to the supernatant (2 mL/L of culture) and the solution was shaken (LABQUAKE Shaker, 22 to 70 reversals/min) at 4 °C for at least 6 hours. The protein/resin mixture was loaded into a gravity flow column and proteins were purified with increasing concentration of imidazole in Buffer A (50 mM Tris-HCl, pH = 7.9, 2 mM EDTA, 2 mM DTT). Purified proteins were concentrated and exchanged into Buffer A + 20% sucrose, then concentrated, aliquoted and flash frozen. Protein concentrations were determined using the Bradford dye-binding assay (Biorad). Yields of Hpm8 and Hpm3 are 1.5 mg/L and 2.0 mg/L, respectively, in a typical purification process.

1.2. Optimized purification method for Hpm3 and Hpm8

The expression plasmids, pZH126 and pZH74[1] harboring the Hpm3 and Hpm8 genes respectively were transformed into *Saccharomyces cerevisiae* strain BJ5464-NpgA[1] for expression. Yeast cultures (1 L) were grown at 28 °C in YPD media with 1% dextrose for 72 hours. The cells were harvested by centrifugation (3723 g, 15 minutes, 4 °C), resuspended in 25mL lysis buffer (50 mM NaH_2PO_4, pH = 8.0, 0.15 M NaCl, 10 mM imidazole) and lysed with sonication on ice (sonicate for 1 minute, then cool for 1 minute. Repeat for 9 to 10 times). Cellular debris was removed by centrifugation (17000 g, 1 hour, 4 °C) and the supernatant was collected into a falcon tube. The supernatant was poured into a column charged with 5 cm height of Ni-NTA agarose resin. The desired proteins will slowly bind to the resin during flow through the column. The binding process was repeated at least three times to ensure optimized binding (This binding process usually takes up to 3 hours). The proteins were purified with increasing concentration of imidazole in buffer A (50 mM Tris-HCl, pH = 7.9, 2 mM EDTA, no DTT). Purified proteins were concentrated and buffered exchanged into Buffer A + 20% sucrose, concentrated, aliquoted and flash frozen. Protein concentrations were determined using the Bradford dye-binding assay (Biorad). Yields of Hpm8 and Hpm3 are 10.0 mg/L and 12.0 mg/L, respectively, in a typical purification process. Approximately 600 mg Hpm8 (from 60 L) and Hpm3 (from 50 L) were purified and used for the *in vitro* assays.

2. *In vitro* assays and purification of ^{13}C labeled-DHZ

In a 100 µL reaction volume, 10 μM of Hpm8 was incubated with 0.2 mM ^{13}C labeled substrates in 100 mM phosphate buffer (pH 7.5) for 15 minutes at rt. The system was supplied with 10 μM of Hpm3, 4 mM NADPH, 0.4 mM malonyl-CoA. After 1 h incubation, the system was supplied with 0.4 mM malonyl-CoA at 1 h intervals (9 times) until the final concentration of malonyl-CoA reached 4 mM. An additional 0.2 mM ^{13}C labeled substrate was supplied to the system every 2 h until the final concentration of ^{13}C labeled substrates nominally reached 0.8 mM. For large scale preparations, 300 reactions were conducted in parallel and combined before extraction with organic solvents.

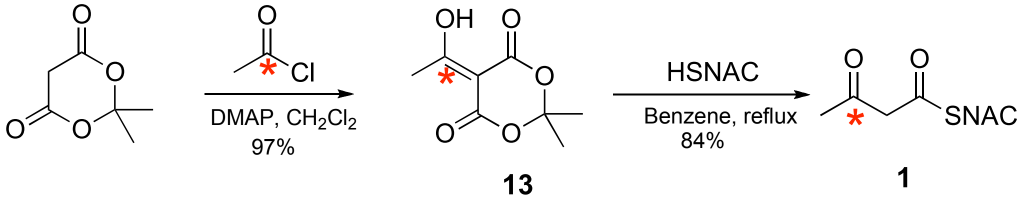
The reactions were quenched and extracted once with 99% EtOAC (EtOAc)/ 1% acetic acid (AcOH). The resultant organic extracts were evaporated to dryness, redissolved in methanol, and then analyzed by LC-MS. LC-MS was conducted with a Shimadzu 2010EV Liquid Chromatography Mass Spectrometer by using both positive and negative electrospray ionization, and a Phenomenex Luna 510 µ 2.0 × 100 mm C18 reverse-phase column. Samples were separated on a linear gradient of 5 to 95% CH_3CN (vol/vol) in H_2O supplemented with 0.05% (vol/vol) formic acid at a flow rate of 0.1 mL/min by HPLC. HPLC was conducted with a Phenomenex Luna 5 µ 10 × 250 mm C18 reverse-phase column.

3. Organic synthesis

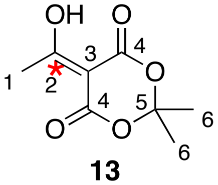
General Synthetic Procedures. All reactions involving air or moisture sensitive reactants were conducted under a positive pressure of dry argon. All solvents and chemicals were reagent grade and used as supplied unless otherwise stated. For anhydrous reactions, solvents were dried according to the procedures detailed in Perrin and Armarego[2]. Removal of solvent was performed under reduced pressure, below 40 °C, using a Büchi rotary evaporator. Labeled ^{13}C-acetyl chloride and acrylic acid were purchased from *Cambridge Isotope Laboratories*. All other chemical reagents were purchased from *Sigma-Aldrich Chemical Company*. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC). Analytical TLC was done on glass plates (5 × 1.5 cm) precoated (0.25 mm) with silica gel (normal SiO_2, Merck 60 F254). Compounds were visualized by exposure to UV light and by dipping the plates in 1% $Ce(SO_4)_2{\bullet}4H_2O$ 2.5% $(NH_4)Mo_7O_{24}{\bullet}4H_2O$ in 10% H_2SO_4 followed by heating on a hot plate. Flash chromatography was performed on silica gel (EM Science, 60Å, 230-400 mesh).

Spectroscopic Analyses. Nuclear magnetic resonance (NMR) spectra were obtained on Varian Inova 500 MHz and 600 MHz spectrometers. ^{1}H NMR chemical shifts are reported in parts per million (ppm) using the residual proton resonance of solvents as reference: $CDCl_3$ δ 7.26, CD_2Cl_2 δ 5.32, and CD_3OD δ 3.30. ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ δ 77.0, CD_2Cl_2 δ 53.8, and CD_3OD δ 49.0. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR spectrometer. Film

Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra were recorded on a Kratos IMS-50 (high resolution, electron impact ionization (EI)), and a ZabSpec IsoMass VG (high resolution. Electrospray (ES)).



Scheme 1: Synthesis of diketide **1**

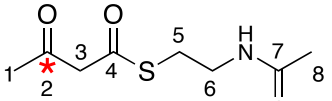


13

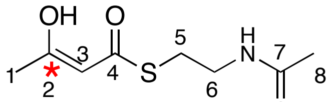
The known compound **13**[3] was synthesized by a modified procedure.

13: To a stirred solution of Meldrum's acid (1.82 g, 12.7 mmol) in dry CH_2Cl_2 (20 mL) was added dimethylaminopyridine (3.09 g, 25.3 mmol) at 0 °C under Ar. The reaction mixture was stirred for 5 min, and then [1-^{13}C]-acetyl chloride (1.00 g, 12.7 mmol) was added. The resulting yellow solution was stirred at 0 °C for another 30 min, and then warmed to 23 °C for another 30 min of stirring. About 10 mL of 1 N HCl was added. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were washed with 1 N HCl (10 mL), brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* to give **13** (2.28 g, 97% yield) as a yellow solid. IR ($CHCl_3$, cast film) 3200 - 2200, 3012, 2986, 2942, 2885, 1732, 1670, 1552 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.69 (d, 3H, $J_{1H\text{-}13C}$ = 6.2 Hz, H-1), 1.75 (s, 6H, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.6 (enriched), 170.2, 160.5, 104.9, 91.8 (d,

$J_{13C\text{-}13C}$ = 69.8 Hz), 26.8, 23.5 (d, $J_{13C\text{-}13C}$ = 45.4 Hz); HRMS (ES) *m/z* calculated for $C_7[^{13}C]H_{10}O_5Na$ 210.0454, found 210.0452 $[M+Na]^+$.



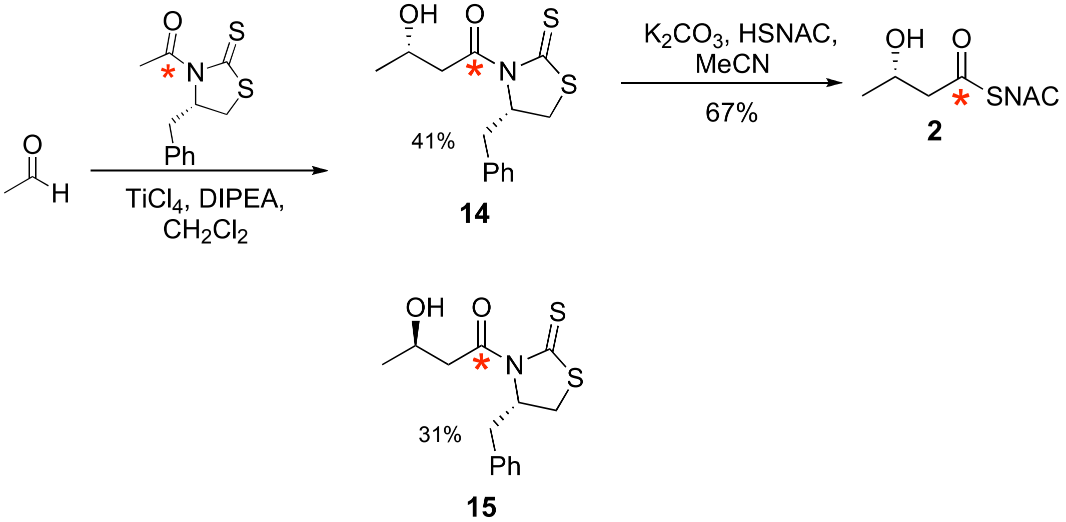
1-keto



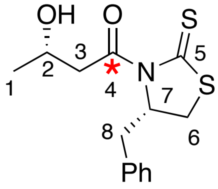
1-enol

The known compound (unlabeled) **1**[4] was synthesized by a literature procedure.

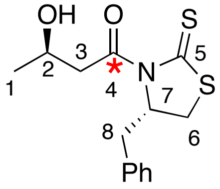
1: 2.03 g, white solid, 84% yield, keto:enol = 4.5:1. IR ($CHCl_3$, cast film) 3291, 3076, 2932, 1722, 1665, 1548 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.02 (br s, 1H, N<u>H</u>), 5.45 (d, 0.20H, $J_{1H\text{-}13C}$ = 4.0 Hz, enol-H-3), 3.69 (d, 1.80H, $J_{1H\text{-}13C}$ = 6.1 Hz, keto-H-3), 3.45 (m, 2H, H-6), 3.05 (m, 2H, H-5), 2.25 (d, $J_{1H\text{-}13C}$ = 6.1 Hz, H-1), 3.05 (m, 2H, H-5), 1.96 (s, 3H, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.8 (enriched), 194.1, 192.2, 173.9 (enriched), 170.5, 170.3, 99.8 (d, $J_{13C\text{-}13C}$ = 69.7 Hz), 57.9 (d, $J_{13C\text{-}13C}$ = 36.7 Hz), 39.9, 39.2, 30.3 (d, $J_{13C\text{-}13C}$ = 42.6 Hz), 29.2, 27.8, 23.2, 23.1, 21.2; HRMS (ES) *m/z* calculated for $C_7[^{13}C]H_{13}NSO_3Na$ 227.0542, found 227.0538 $[M+Na]^+$.



Scheme 2: Synthesis of diketide **2**



14



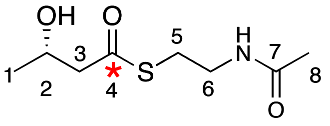
15

To a stirred solution of (*S*)-4-benzyl-*N*-acetyl-1,3-thiazolidine-2-thione (252 mg, 1.00 mmol) in dry CH_2Cl_2 (10 mL) was added $TiCl_4$ (1.0 M solution in CH_2Cl_2, 1.10 mL, 1.10 mmol) at 0 °C under Ar. The reaction mixture was stirred for 5 min and then cooled to -78 °C. A solution of diisopropylethylamine (DIPEA) (129 mg, 1.00 mmol) in CH_2Cl_2 (2 mL) was added. The reaction mixture was stirred at -78 °C for 2 h. A solution of acetaldehyde (0.50 mL, 8.90 mmol) was added to the reaction mixture, which was then stirred for 15 min at -78 °C. The reaction was quenched by the addition of 10 mL saturated ammonium chloride. The layers were separated, and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (1:6 EtOAc/hexanes) to give two diastereomers **14** (120 mg, 41% yield) and **15** (90 mg, 31% yield) as yellow oils.

14: IR ($CHCl_3$, cast film) 3433, 2966, 2925, 1693 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.25 (m, 5H, Ph), 5.40 (ddd, 1H, J = 10.6, 7.3, 4.0 Hz, H-7), 4.32 (m, 1H, H-2), 3.64 (ddd, 1H, J = 17.7, 3.0 Hz, $J_{1H\text{-}13C}$ = 7.0 Hz, H-3), 3.40 (ddd, 1H, J = 11.6, 7.2, 0.8 Hz, H-6), 3.22 (dd, 1H, J = 13.2, 3.9 Hz, H-8), 3.13 (ddd, 1H, J = 17.7, 9.2 Hz, $J_{1H\text{-}13C}$ = 6.0 Hz, H-3), 3.05 (dd, 1H, J = 13.2, 10.4 Hz, H-8), 2.89 (d, 1H, J = 11.6 Hz, H-6), 1.27 (d, 3H, J = 6.3 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.4 (d, $J_{13C\text{-}13C}$ = 3.4 Hz), 173.1(enriched), 136.4, 129.4, 128.9, 127.3, 68.3, 64.1(d, $J_{13C\text{-}13C}$ = 2.1 Hz), 47.3(d, $J_{13C\text{-}13C}$ = 49.6 Hz), 36.8, 32.1, 22.3 (d, $J_{13C\text{-}13C}$ = 5.4 Hz); α_D^{25} = 123 (c = 0.280, $CHCl_3$);

HRMS (ES) *m/z* calculated for $C_{13}[^{13}C]H_{17}NS_2O_2Na$ 319.0626, found 319.0627 $[M+Na]^+$.

15: IR ($CHCl_3$, cast film) 3429, 2970, 2929, 1651cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.25 (m, 5H, Ph), 5.40 (ddd, 1H, J = 10.6, 7.1, 4.0 Hz, H-7), 4.24 (m, 1H, H-2), 3.45 (ddd, 1H, J = 17.7, 9.2 Hz, $J_{1H\text{-}13C}$ = 6.4 Hz, H-3), 3.40 (dd, 1H, J = 11.4, 7.2 Hz, H-6), 3.32 (ddd, 1H, J = 17.6, 2.7 Hz, $J_{1H\text{-}13C}$ = 6.6 Hz, H-3), 3.22 (dd, 1H, J = 13.2, 3.8 Hz, H-8), 3.05 (dd, 1H, J = 13.2, 10.4 Hz, H-8), 2.89 (d, 1H, J = 11.5 Hz, H-6), 1.27 (d, 3H, J = 6.3 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.4 ((d, $J_{13C\text{-}13C}$ = 3.4 Hz), 173.6 (enriched), 136.3, 129.4, 128.9, 127.3, 68.2, 64.6 (d, $J_{13C\text{-}13C}$ = 2.0 Hz), 47.0 (d, $J_{13C\text{-}13C}$ = 49.7 Hz), 36.8, 32.1, 22.3 (d, $J_{13C\text{-}13C}$ = 5.1 Hz); α_D^{25} = 153.76 (c = 0.380, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{13}[^{13}C]H_{17}NS_2O_2Na$ 319.0626, found 319.0624 $[M+Na]^+$.

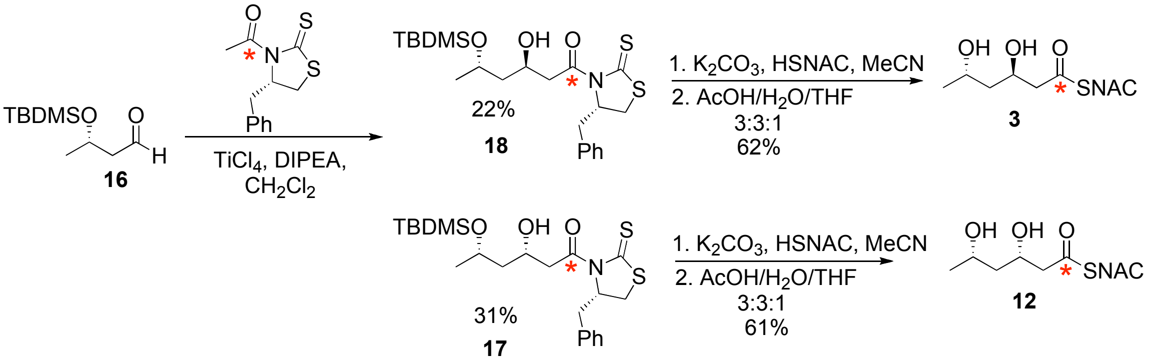


2

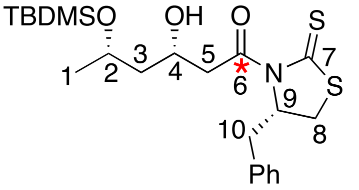
The known compound (unlabeled) **2**[5] was synthesized by a different procedure.

2: To a stirred solution of **14** (105 mg, 0.355 mmol) in 5 mL MeCN was added K_2CO_3 (188 mg, 1.24 mmol) and *N*-acetylcysteamine (50.8 mg, 0.426 mmol). The reaction mixture was stirred until the yellow color disappeared (about 5 min). The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (EtOAc) to give **2** (49.0 mg, 67% yield) as a white solid. IR ($CHCl_3$, cast film) 3296, 3087, 2970, 2929, 1653, 1658, 1553 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.32 (s, 1H, N<u>H</u>), 4.22 (m, 1H, H-2), 3.39 (m, 2H, H-6), 3.23 (br s, 1H, O<u>H</u>), 3.03 (m, 2H, H-5), 2.68 (dd, 2H, J = 5.7 Hz, $J_{1H\text{-}13C}$ = 5.7 Hz, H-3), 1.96 (s, 3H, H-8), 1.20 (d, 3H, J = 6.3 Hz, H-

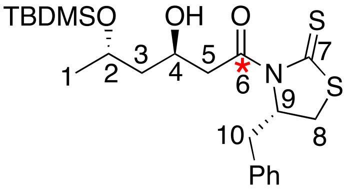
1); ^{13}C NMR (125 MHz, CDCl$_3$) δ 199.1(enriched), 170.8, 64.9 (d, $J_{13C\text{-}13C}$ = 2.3 Hz), 52.2 (d, $J_{13C\text{-}13C}$ = 44.3 Hz), 39.2, 28.7, 23.1, 22.7 (d, $J_{13C\text{-}13C}$ = 4.6 Hz); α_D^{25} = 31.49 (c = 0.200, CHCl$_3$); HRMS (ES) *m/z* calculated for C$_7$[^{13}C]H$_{15}$NSO$_3$Na 229.0698, found 229.0696 [M+Na]$^+$.



Scheme 3: Synthesis of triketides **3, 12**



17

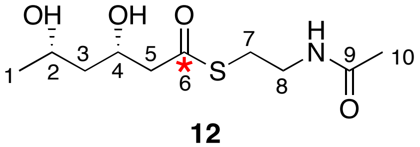


18

Compounds **17** and **18** were synthesized from **16**[5] by the method used for synthesis of **14** and **15**.

17: 139.0 mg, yellow oil, 31% yield. IR (CHCl$_3$, cast film) 3489, 2955, 2928, 1647 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37 – 7.25 (m, 5H, Ph), 5.40 (m, 1H, H-9), 4.35 (m, 1H, H-4), 4.12 (m, 1H, H-2), 3.50 (ddd, 1H, *J* = 17.6, 3.4 Hz, $J_{1H\text{-}13C}$ = 7.2 Hz, H-5), 3.48 (m, 1H, O$\underline{H}$), 3.38 (ddd, 1H, *J* = 11.4, 7.1, 0.8 Hz, H-8), 3.27 (ddd, 1H, *J* = 17.6, 8.6 Hz, $J_{1H\text{-}13C}$ = 6.2 Hz, H-5), 3.23 (dd, 1H, *J* = 13.3, 3.8 Hz, 10), 3.04 (dd, 1H, *J* = 13.2, 10.6 Hz, H-10), 2.88 (d, 1H, *J* = 11.5 Hz, H-8), 1.73 (m, 1H, H-3), 1.62 (ddd, 1H, *J* = 14.0, 4.5, 3.5 Hz, H-3), 1.20 (d, 3H, *J* = 6.1 Hz, H-1), 0.89 (s, 9H, Si-C(C$\underline{H}_3$)$_3$), 0.087 (s, 3H, SiC$\underline{H}_3$), 0.082 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 201.2 (d, $J_{13C\text{-}13C}$ = 3.4 Hz), 172.4 (enriched), 136.5, 129.4, 128.9, 127.2, 68.6 , 68.4, 67.0 (d, $J_{13C\text{-}13C}$ = 1.6 Hz), 46.3 (d, $J_{13C\text{-}13C}$ = 50.8 Hz), 45.5 (d, $J_{13C\text{-}13C}$ = 4.1 Hz), 36.7, 32.0 (d, $J_{13C\text{-}13C}$ = 1.3 Hz), 25.8, 24.2, 18.0, -3.97, -4.72; α_D^{25} = 253.80 (c = 0.130, CHCl$_3$); HRMS (ES) *m/z* calculated for $C_{22}[^{13}C]H_{35}NO_2S_2SiNa$ 477.1756, found 477.1750 [M+Na]$^+$.

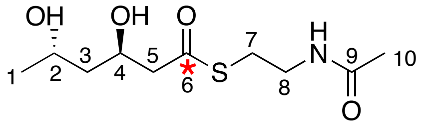
18: 100 mg, yellow oil, 22% yield. IR (CHCl$_3$, cast film) 3433, 2966, 2925, 1693 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37 – 7.25 (m, 5H, Ph), 5.40 (ddd, 1H, *J* = 10.6, 7.3, 4.0 Hz, H-9), 4.37 (m, 1H, H-4), 4.18 (m, 1H, H-2), 3.56 (d, 1H, *J* = 2.8 Hz, O$\underline{H}$), 3.48 (m, 1H, H-5), 3.38 (m, 1H, H-8), 3.34 (m, 1H, H-5), 3.23 (dd, 1H, *J* = 13.3, 2.8 Hz, 10), 3.03 (dd, 1H, *J* = 13.0, 10.5 Hz, H-10), 2.88 (d, 1H, *J* = 11.5 Hz, H-8), 1.68 (ddd, 1H, *J* = 13.7, 9.8, 2.9 Hz, H-3), 1.54 (ddd, 1H, *J* = 14.0, 7.4, 2.3 Hz, H-3), 1.21 (d, 3H, *J* = 6.2 Hz, H-1), 0.88 (s, 9H, Si-C(C$\underline{H}_3$)$_3$), 0.087 (s, 3H, SiC$\underline{H}_3$), 0.082 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 202.9 (d, $J_{13C\text{-}13C}$ = 3.1 Hz), 173.1 (enriched), 136.5, 129.4, 128.9, 127.2, 68.3 (d, $J_{13C\text{-}13C}$ = 3.1 Hz), 66.2, 65.1 (d, $J_{13C\text{-}13C}$ = 1.8 Hz), 46.3 (d, $J_{13C\text{-}13C}$ = 50.6 Hz), 44.9 (d, $J_{13C\text{-}13C}$ = 4.4 Hz), 36.7, 31.9 (d, $J_{13C\text{-}13C}$ = 2.1 Hz), 25.8, 23.6, 18.0, -4.45, -4.86; α_D^{25} = 95.8 (c = 0.400, CHCl$_3$); HRMS (ES) *m/z* calculated for $C_{22}[^{13}C]H_{35}NO_2S_2SiNa$ 477.1756, found 477.1746 [M+Na]$^+$.



12

The known compound (unlabeled) **12**^{5} was synthesized by a modified procedure.

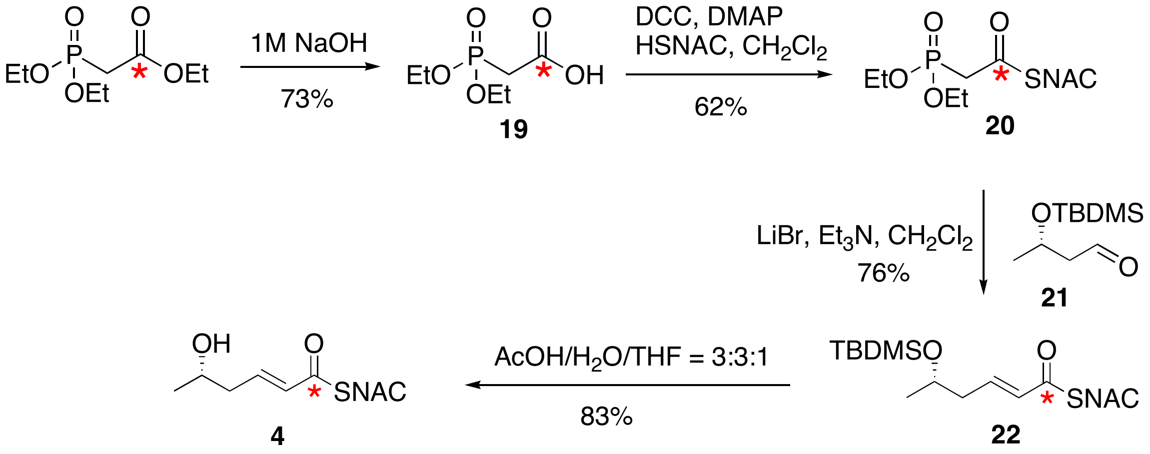
12: To a stirred solution of **17** (6.00 mg, 132 μmol) in 0.5 mL MeCN was added K$_2$CO$_3$ (3.00 mg, 198 μmol) and *N*-acetylcysteamine (HSNAC) (2.40 mg, 198 μmol). The reaction mixture was stirred until the yellow color disappeared (about 5 min). The reaction was quenched by the addition of 1 mL saturated ammonium chloride. The layers were separated and the aqueous layer was extracted with EtOAc (3x2 mL). The combined organic layers were washed with brine (5 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. The residue was dissolved in 1 mL of a solution of 3:3:1 AcOH/H_2O/THF. The resulting solution was stirred at 25 °C for 2 h. The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (9:1 EtOAc/MeOH) to give **12** (2.00 mg, 61% yield) as a white solid. IR and optical rotation data were indistinguishable from that previously reported.**[4]** ^{1}H NMR (500 MHz, CD_2Cl_2) δ 5.80 (br s, 1H, N$\underline{\text{H}}$), 4.30 (m, 1H, H-4), 4.13 (m, 1H, H-2), 3.46 – 3.36 (m, 2H, H-8), 3.10 – 2.95 (m, 2H, H-7), 2.72 - 2.65 (m, 2H, H-5), 1.96 (s, 3H, H-10), 1.55 (m, 2H, H-3), 1.23 (d, 3H, J = 6.2, H-1); ^{13}C NMR (125 MHz, CD_2Cl_2) δ 199.0 (enriched), 170.2, 69.9 (d, $J_{13C\text{-}13C}$ = 2.3 Hz), 68.6, 51.9 (d, $J_{13C\text{-}13C}$ = 45.6 Hz), 44.6 (d, $J_{13C\text{-}13C}$ = 4.1 Hz), 39.2, 29.5, 24.1, 23.3; HRMS (ES) *m/z* calculated for $C_9[^{13}C]H_{19}NSO_4Na$ 273.0961, found 273.0955 $[M+Na]^+$.



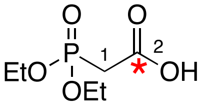
3

The known compound (unlabeled) **3**[5] was synthesized from **18** by the method for synthesizing **12**. IR and optical rotation data were indistinguishable from that previously reported. [4]

3: 4.00 mg, white solid, 62% yield. ^{1}H NMR (500 MHz, CD_2Cl_2) δ 5.90 (br s, 1H, N$\underline{\text{H}}$), 4.40 (m, 1H, H-4), 4.13 (m, 1H, H-2), 3.46 – 3.36 (m, 2H, H-8), 3.39 (d, 1H, J = 6.3 Hz, O$\underline{\text{H}}$), 3.10 – 2.90 (m, 2H, H-7), 2.70 -2.60 (m, 2H, H-5), 2.27 (d, 1H, J = 4.5 Hz, O$\underline{\text{H}}$), 1.96 (s, 3H, H-10), 1.65 – 1.55 (m, 2H, H-3), 1.23 (dd, 3H, J = 6.3, $J_{1H\text{-}13C}$ = 0.8 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.2 (enriched), 170.5, 66.9 (d, $J_{13C\text{-}13C}$ = 2.1 Hz), 65.4, 51.6 (d, $J_{13C\text{-}13C}$ = 45.9 Hz), 44.1 (d, $J_{13C\text{-}13C}$ = 3.9 Hz), 39.2, 29.5, 23.8, 23.3; HRMS (ES) *m/z* calculated for $C_9[^{13}C]H_{19}NSO_4Na$ 273.0961, found 273.0955 $[M+Na]^+$.



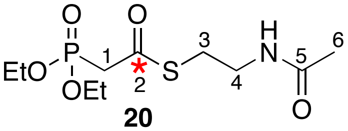
Scheme 4: Synthesis of triketide **4**



19

The known compound (unlabeled) **19**[6] was synthesized by a literature method.

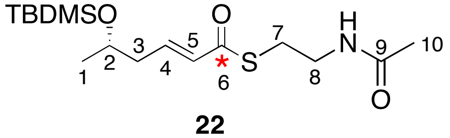
19: To a stirred solution of 1M NaOH (3.6 mL), ethyl (diethoxyphosphono)-[1-^{13}C]-acetate (0.630 mL, 3.20 mmol) was added dropwise. After 3 hours at room temperature, ethanol produced in the reaction was removed under reduced pressure. The remaining aqueous solution was acidified to pH = 1 using concentrated HCl and extracted with EtOAC (3x5 mL). The combined organic extracts were washed with brine (10 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressured to give **19** as a colourless liquid (450 mg, 73% yield). IR ($CHCl_3$, cast film) 2987, 1689, 1224 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.48 (br s, 1H, COO$\underline{H}$), 4.22-4.15 (m, 4H, $OC\underline{H}_2CH_3$), 2.98 (dd, 2H, $J_{1H\text{-}13C}$ = 7.3 Hz, $J_{1H\text{-}31P}$ = 21.8 Hz, H-1), 1.34 (t, 6H, J = 7.1 Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.8 (enriched, d, $J_{13C\text{-}31P}$ = 5.4 Hz), 63.2 (d, $J_{13C\text{-}31P}$ = 6.5 Hz), 34.2 ppm (dd, $J_{13C\text{-}31P}$ = 134.5 Hz, $J_{13C\text{-}13C}$ = 55.2 Hz), 16.3; HRMS calculated for $C_5[^{13}C]H_{12}O_5P$ 196.0461, Found 196.0464 $[M\text{-}H]^-$.



20

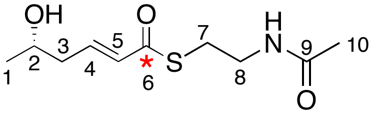
The known compound (unlabeled) **20**[1] was synthesized by a literature method.

20: In a flame-dried flask under argon, diethylphosphonoacetic acid (330 mg, 1.70 mmol) was dissolved in 10 mL of CH_2Cl_2 and cooled to 0 °C. Dimethylaminopyridine (20.0 mg, 0.163 mmol) and dicyclohexylcarbodiimide (DCC) (385 mg, 1.85 mmol) were added, followed by an excess of *N*-acetylcysteamine (0.500 mL, 4.70 mmol). The reaction mixture was stirred at 0 °C for 30 min, then warmed to room temperature and stirred for an additional 22 hours. The solvent was removed under reduced pressure, and the crude reaction mixture was redissolved in EtOAC/EtO_2 = 1:1 (10 mL) and filtered to remove precipitated dicyclohexylurea. The filtrate was concentrated under reduced pressure, and then purified by flash column chromatography (EtOAc to 10% MeOH/EtOAc) to give **20** as a white solid (315 mg, 62% yield). IR ($CHCl_3$, cast film) 3294, 2983, 1648, 1548, 1255 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.65 (br s, 1H, N$\underline{H}$), 4.20 (m, 4H, $OC\underline{H}_2CH_3$), 3.45 (dt, 2H, J = 6.2, 6.2 Hz, H-4), 3.22 (dd, 2H, $J_{1H\text{-}31P}$ = 21.2 Hz, $J_{1H\text{-}13C}$ = 6.4 Hz, H-1), 3.09 (2H, dt, $J = 6.5$ Hz, $J_{1H\text{-}13C} = 5.6$ Hz, H-3), 2.00 (s, 3H, H-6), 1.34 (t, 6H, $J = 7.1$ Hz, $OCH_2C\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.5 (enriched, d, $J_{13C\text{-}31P} = 7.0$ Hz), 170.5, 62.9 (d, $J_{13C\text{-}31P} = 6.2$ Hz), 43.0 (dd, $J_{13C\text{-}31P} = 131.0$ Hz, $J_{13C\text{-}13C} = 44.7$ Hz), 39.0, 29.5, 23.1, 16.3 (d, $J_{13C\text{-}31P} = 6.2$ Hz); HRMS (ES) *m/z* calculated for $C_9[^{13}C]H_{21}NO_5PS$ 299.0906, found 299.0909 $[M+H]^+$.



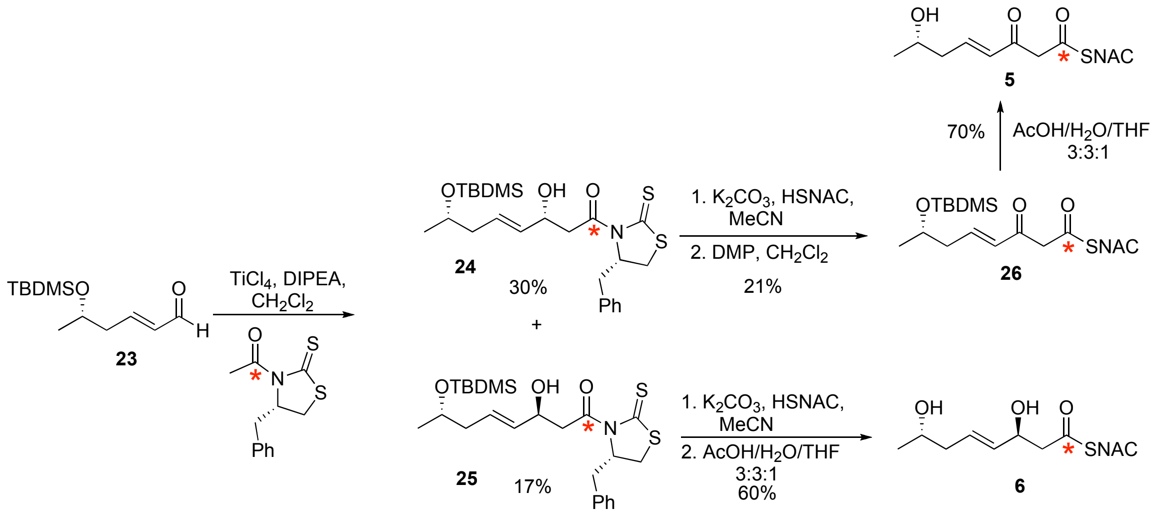
22

22: To a stirred solution of LiBr (252 mg, 3.00 mmol) in dry THF, compound **20** (280 mg, 0.950 mmol) was added. The solution was stirred for 10 min at 25 °C, followed by the addition of Et_3N (0.400 mL, 3.00 mmol). The solution was stirred for an additional 10 min and then aldehyde **21**[7] (404 mg) in 2 mL dry THF was added. After 12 hours at room temperature, the reaction was quenched by the addition of 15 mL of water, and the resulting solution was extracted with EtOAc (3x10 mL). The combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (1:1 EtOAc/hexanes to 2:1 EtOAc/hexanes) to give **22** as a yellow oil (241 mg, 76 % yield). IR ($CHCl_3$, cast film) 3289, 2956, 2929, 1651, 1619, 1552, 1462; ^1H NMR (500 MHz, $CDCl_3$) δ 6.94 (dddd, 1H, $J = 15.6, 7.3, 7.3$ Hz, $J_{1H\text{-}13C} = 7.3$ Hz, H-4), 6.14 (ddt, 1H, $J = 15.6, 1.3$ Hz, $J_{1H\text{-}13C} = 6.1$ Hz, H-5), 6.1 (br s, 1H, N$\underline{H}$), 3.95 (sextet, 1H, $J = 6.1$ Hz, H-2), 3.46 (dt, 2H, $J = 6.1, 6.1$ Hz, H-8), 3.10 (dt, 2H, $J = 6.6, 4.9$ Hz, H-7), 2.32 (t, 2H, $J = 6.6$ Hz, H-3), 1.97 (s, 3H, H-10), 1.17 (d, 3H, $J = 6.1$ Hz, H-1), 0.88 (s, 9H, $Si\text{-}C(C\underline{H}_3)_3$), 0.05 (s, 3H, $Si\text{-}C\underline{H}_3$), 0.04 (s, 3H, $Si\text{-}C\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.3 (enriched), 170.4, 143.6, 130.1 (d, $J_{13C\text{-}13C} = 61.4$ Hz), 67.5, 42.5 (d, $J_{13C\text{-}13C} = 6.7$ Hz), 39.9, 28.2, 25.8, 23.9, 23.1, 18.0, -4.80; α_D^{25} = 7.88 (c = 0.510, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{15}[^{13}C]H_{32}NO_3SSi$ 347.19, found 347.1896 $[M+H]^+$.

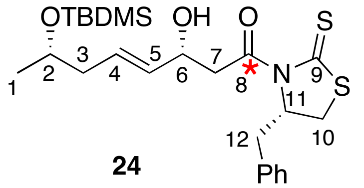


4

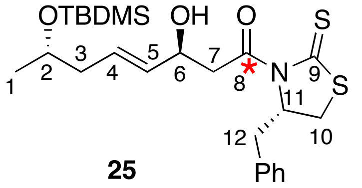
4: To a solution of AcOH/H_2O/THF (3:3:1, 7 mL) was added compound **22** (225 mg, 0.650 mmol). The mixture was stirred at room temperature for 19 hours, after which the volatile components were removed under high vacuum. The residue was diluted with 5 mL water, and the solution was extracted with EtOAc (3x5 mL). The combined organic portions were washed with brine (20 mL) and dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **4** as a white solid (125 mg, 83%). IR (cast film) 3295, 3086, 2968, 2930, 1650, 1617, 1553 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.92 (dddd, 1H, *J* =15.4, 7.3, 7.3, $J_{1H\text{-}13C}$ = 7.30, H-4), 6.20 (ddt, 1H, *J* = 15.4, 1.5 Hz, $J_{1H\text{-}13C}$ = 6.10 Hz, H-5), 6.00 (br s, 1H, N$\underline{H}$), 3.96 (sextet, 1H, *J* = 6.10 Hz, H-2), 3.46 (dt, 2H, *J* = 6.1, 6.1 Hz, H-8), 3.10 (dt, 2H, $J_{1H\text{-}13C}$ = 5.6 Hz, *J* = 4.9 Hz, H-7), 2.34 (t, 2H, *J* = 7.4 Hz, H-3), 1.94 (s, 3H, H-10), 1.22 (d, 3H, *J* = 6.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.3 (enriched), 170.6, 142.6, 130.5 (d, $J_{13C\text{-}13C}$ = 61.3 Hz), 66.6, 41.8 (d, $J_{13C\text{-}13C}$ = 6.7 Hz), 39.7, 28.3, 23.4, 23.2; α_D^{25} = 0.0944 (c = 0.780, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_9[^{13}C]H_{18}NO_3S$ 233.1035, found 233.1034 $[M+H]^+$.



Scheme 5: Synthesis of tetraketides **5**, **6**



24

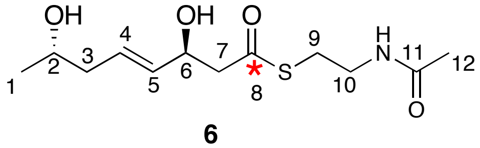


25

Compounds **24** and **25** were synthesized from **11**[5] by the method used for synthesis of **14** and **15**.

24: 143 mg, yellow oil, 30% yield. IR (CHCl$_3$, cast film) 3440, 2955, 2927, 2855, 1654 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37 – 7.25 (m, 5H, Ph), 5.76 (dtd, 1H, J = 15.4, 7.2, 1.1 Hz, H-4), 5.59 (ddt, 1H, J = 15.4, 6.3, 1.3 Hz, H-5), 5.39 (m, 1H, H-11), 4.69 (m, 1H, H-6), 3.85 (AB$_2$X$_3$, 1H, J = 6.1 Hz, H-2), 3.62 (ddd, 1H, J = 17.6, 2.8 Hz, $J_{1H\text{-}13C}$ = 7.0 Hz, H-7), 3.40 (ddd, 1H, J = 11.5, 7.2, 1.0 Hz, H-10), 3.30 (ddd, 1H, J = 17.6, 9.2 Hz, $J_{1H\text{-}13C}$ = 6.0 Hz, H-7), 3.23 (dd, 1H, J = 13.2, 3.9 Hz, H-12), 3.05 (dd, 1H, J = 13.3, 10.5 Hz, H-12), 2.91 (d, 1H, J = 11.6 Hz, H-10), 2.23 – 2.10 (m, 2H, H-3), 1.15 (d, 1H, J = 6.0 Hz, H-1) 0.88 (s, 9H, Si-C(C$\underline{H}_3$)$_3$), 0.05 (s, 6H, SiC$\underline{H}_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 201.4 ((d, $J_{13C\text{-}13C}$ = 3.1 Hz), 172.6 (enriched), 136.5, 132.7 (d, $J_{13C\text{-}13C}$ = 4.6 Hz), 129.4, 129.3, 128.9, 127.3, 68.6 (d, $J_{13C\text{-}13C}$ = 2.1 Hz),, 68.4, 68.3, 45.7 (d, $J_{13C\text{-}13C}$ = 50.3 Hz), 42.6, 36.8, 32.1 (d, $J_{13C\text{-}13C}$ = 1.8 Hz), 25.9, 23.5, 18.2, - 4.42, - 4.56; α_D^{25} = 106.53 (c = 0.800, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{24}[^{13}C]H_{37}NO_2S_2SiNa$ 503.1912, found 503.1902 $[M+Na]^+$.

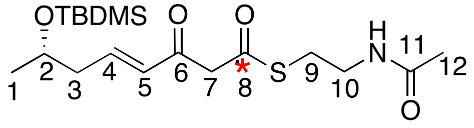
25: 82 mg, yellow oil, 17% yield. IR ($CHCl_3$, cast film) 3438, 2955, 2927, 2855, 1654 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.25 (m, 5H, Ph), 5.75 (dtd, 1H, *J* = 15.4, 7.1, 1.1 Hz, H-4), 5.57 (ddt, 1H, *J* = 15.4, 6.2, 1.2 Hz, H-5), 4.58 (m, 1H, H-6), 3.83 (AB_2X_3, 1H, *J* = 6.1 Hz, H-2), 3.62 (ddd, 1H, *J* = 17.3, 9.0 Hz, $J_{1H\text{-}13C}$ = 6.2 Hz, H-7), 3.42- 3.32 (m, 2H, H-10, H-7), 3.23 (dd, 1H, *J* = 13.3, 3.9 Hz, H-12), 3.05 (dd, 1H, *J* = 13.3, 10.4 Hz, H-12), 3.00 (d, 1H, *J* = 7.3 Hz, O$\underline{H}$), 2.91 (d, 1H, *J* = 11.5 Hz, H-10), 2.19 (m, 2H, H-3), 1.12 (d, 1H, *J* = 6.1 Hz, H-1) 0.88 (s, 9H, Si-C($C\underline{H}_3$)$_3$), 0.05 (s, 6H, $SiC\underline{H}_3$); 201.4 ((d, $J_{13C\text{-}13C}$ = 3.1 Hz), 173.1 (enriched), 136.4, 132.7 (d, $J_{13C\text{-}13C}$ = 4.9 Hz), 129.5, 129.3, 129.0, 127.3, 69.2 (d, $J_{13C\text{-}13C}$ = 1.6 Hz),, 68.4, 68.3, 45.5 (d, $J_{13C\text{-}13C}$ = 50.3 Hz), 42.6, 36.8, 32.0 (d, $J_{13C\text{-}13C}$ = 1.8 Hz), 25.9, 23.6, 18.2, - 4.42, - 4.58; α_D^{25} = 72 .9 (c = 1.100, $CHCl_3$); HRMS (ES) *m/z* calculated for C_{24} $[^{13}C]H_{37}NO_2S_2SiNa$ 503.1912, found 503.1903 $[M+Na]^+$.



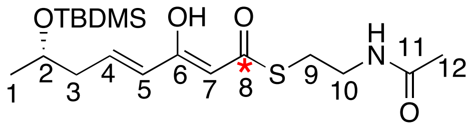
6

The known compound (unlabeled) **6**[5] was synthesized from **25** by the method used for synthesis of **12**. IR and optical rotation data were indistinguishable from that previously reported.

6: 8.00 mg, white solid, 60% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.00 (s, 1H, N<u>H</u>), 5.75 (m, 1H, H-4), 5.58 (ddt, 1H, J = 15.5, 6.3, 1.1 Hz, H-5), 4.56 (m, 1H, H-6), 3.82 (m, 1H, H-2), 3.44 (m, 2H, H-10), 3.04 (m, 2H, H-9), 2.80 (m, 2H, H-7), 2.30 – 2.10 (m, 2H, H-3), 1.97 (s, 3H, H-12), 1.19 (d, 3H, J = 6.2 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.5 (enriched), 170.6, 134.1 (d, $J_{13C\text{-}13C}$ = 3.3 Hz), 128.7, 69.5 (d, $J_{13C\text{-}13C}$ = 1.8 Hz), 67.0, 51.0 (d, $J_{13C\text{-}13C}$ = 45.7 Hz), 42.0, 39.2, 29.1, 23.2, 23.0; HRMS (ES) *m/z* calculated for $C_{11}[^{13}C]H_{21}NSO_4Na$ 299.1117, found 299.1111 $[M+Na]^+$.



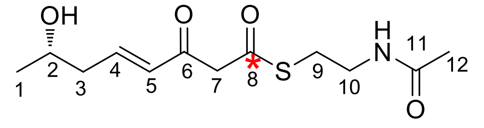
26-keto



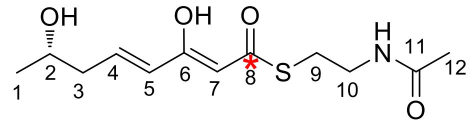
26-enol

The known compound (unlabeled) **26**[5] was synthesized by a modified procedure.

26: To a stirred solution of **24** (160 mg, 0.340 mmol) in 5 mL MeCN were added K_2CO_3 (78.6 mg, 0.510 mmol) and *N*-acetylcysteamine (48.6 mg, 0.408 mmol). The reaction mixture was stirred until the yellow color disappeared (about 5 min). The reaction was quenched by the addition of 5 mL saturated ammonium chloride. The layers were separated and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo*. To the resulting residue was added 10 mL CH_2Cl_2 and Dess-Martin periodinane (92.0 mg, 0.216 mmol). The resulting solution was stirred at 0 °C for 1 h. The reaction was quenched by the addition of 10 mL of 1:1 10% $Na_2S_2O_3$: saturated aqueous $NaHCO_3$. The layers were separated and the aqueous layer was extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (EtOAc) to give **26** (28.0 mg, 21% yield, keto:enol = 2:3) as a colorless oil. IR and optical rotation data were indistinguishable from that previously reported.[4] ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (m, 0.4H, keto-H-4), 6.76 (m, 0.6H, enol-H-4), 6.16 (dt, 0.4H, *J* = 15.9, 1.4 Hz, keto-H-5), 6.14 (br s, 1H, N$\underline{H}$), 5.74 (m, 0.6H, enol-H-5), 5.43 (d, 0.6H, $J_{1H\text{-}13C}$ = 4.5 Hz, enol-H-7), 3.98 – 3.89 (m, 1H, H-2), 3.86 (d, 0.8H, $J_{1H\text{-}13C}$ = 6.3 Hz, keto-H-7), 3.48 (m, 2H, H-10), 3.11 (m, 2H, H-9), 2.42 – 2.30 (m, 2H, H-3), 1.97 (s, 3H, H-12), 1.25 (d, 1.2H, *J* = 6.2 Hz, keto-H-1), 1.23 (d, 1.8H, *J* = 6.2 Hz, enol-H-1), 0.88 (m, 9H, Si-C(C$\underline{H}_3$)$_3$), 0.04 (m, 6H, SiC$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.8 (enriched), 192.6 (enriched), 191.4, 170.6, 170.5, 167.3, 147.1, 139.6, 131.3, 126.6 (d, $J_{13C\text{-}13C}$ = 5.7 Hz), 100.3 (d, $J_{13C\text{-}13C}$ = 63.8 Hz), 67.8, 67.4, 54.8 (d, $J_{13C\text{-}13C}$ = 46.3 Hz), 43.1, 42.7, 40.0, 39.2, 29.6, 27.9, 23.8, 23.2, 23.1, 23.3, 18.1, 18.0, - 4.47, - 4.56, - 4.80, -4.81; HRMS (ES) *m/z* calculated for $C_{18}[^{13}C]H_{33}NSSiO_4Na$ 411.1828, found 411.1820 $[M+Na]^+$.



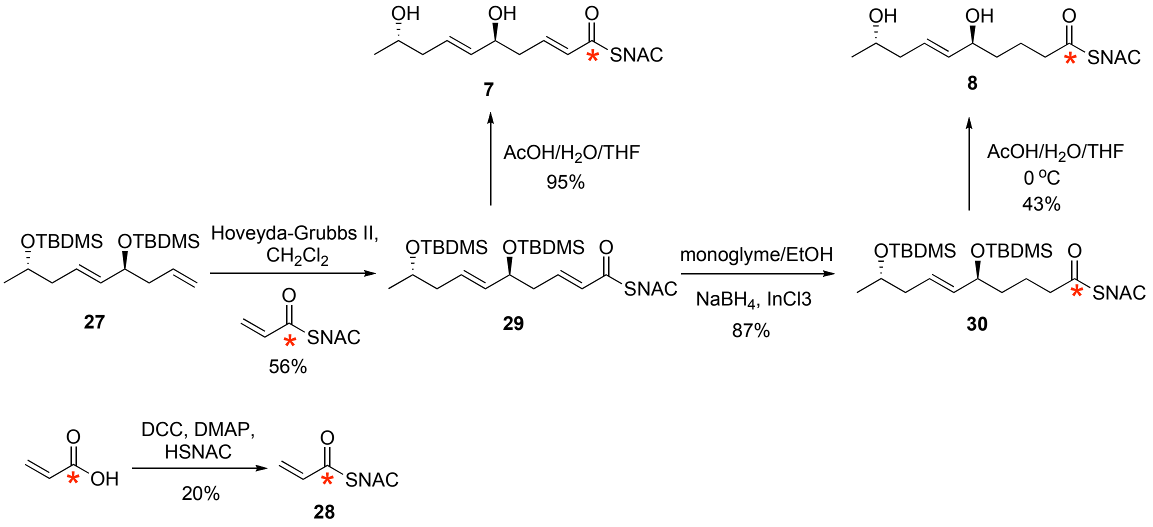
5-keto



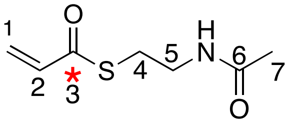
5-enol

The known compound (unlabeled) **5**[5] was synthesized by a modified procedure.

5: To compound **26** (4.00 mg, 10.4 μmol) was added 0.5 mL AcOH/H_2O/THF = 3:3:1 solution. The solution was stirred at 25 °C for 4 h. The solvent was removed *in vacuo,* and the residue was purified using flash column chromatography (EtOAc) to give **5** (2.00 mg, keto:enol = 2:3, 70% yield) as a colorless oil. IR and optical rotation data were indistinguishable from that previously reported[4]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (m, 0.4H, keto-H-4), 6.78 (m, 0.6H, enol-H-4), 6.22 (m, 0.4H, keto-H-5), 5.90 (br s, 1H, N$\underline{H}$), 5.82 (m, 0.6H, enol-H-5), 5.46 (d, 0.6H, $J_{1H\text{-}13C}$ = 4.5 Hz, enol-H-7), 3.95 (m, 1H, H-2), 3.86 (d, 0.8H, $J_{1H\text{-}13C}$ = 6.4 Hz keto-H-7), 3.48 (m, 2H, H-10), 3.09 (m, 2H, H-9), 2.40 (m, 2H, H-3), 1.97 (s, 3H, H-12), 1.25 (d, 1.2H, J = 6.2 Hz, keto-H-1), 1.23 (d, 1.8H, J = 6.2 Hz, enol-H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.8 (enriched), 192.6(enriched), 191.4, 170.6, 170.5, 166.8, 147.1, 139.6, 131.8, 126.6 (d, $J_{13C\text{-}13C}$ = 5.6 Hz), 100.3 (d, $J_{13C\text{-}13C}$ = 63.5 Hz), 67.1, 66.8, 55.1(d, $J_{13C\text{-}13C}$ = 45.6 Hz),, 42.6, 42.3, 40.0, 39.2, 29.5, 28.2, 23.6, 23.4, 23.4, 21.3; HRMS (ES) *m/z* calculated for $C_{11}[^{13}C]H_{19}NSO_4Na$ 297.0961, found 297.0955 $[M+Na]^+$.

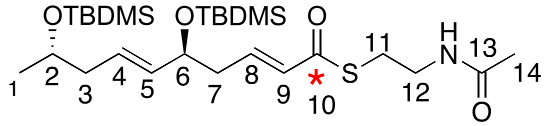


Scheme 6: Synthesis of pentaketides **7, 8**



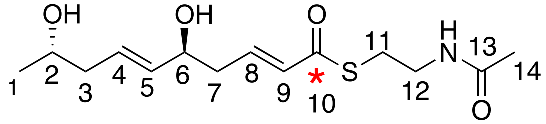
28

28: To a stirred solution of ^{13}C labeled acrylic acid (72.1 mg, 1.00 mmol) in 5 mL CH_2Cl_2 was added dimethylaminopyridine (12.2 mg, 0.100 mmol) at 0 °C. Then DCC (227 mg, 1.10 mmol), *N*-acetylcysteamine (HSNAC) (119 mg, 1.00 mmol) were added to the solution sequentially. The mixture was stirred at 0 °C for 30 min, then warmed to 25 °C and stirred for a further 12 h. The solvent was removed *in vacuo* and then 10 ml 1:1 EtOAc/Et_2O was added. The precipitated dicyclohexylurea (DCU) was removed by filtration; the filtrate was concentrated and then purified using flash column chromatography (10:1 EtOAc/MeOH) to give **28** (36.0 mg, 20% yield) as a colorless oil. IR ($CHCl_3$, cast film) 3285, 3074, 2976, 2933, 1670, 1613, 1549 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.36 (m, 2H, H-1), 6.10 (br s, 1H, N<u>H</u>), 5.76 (ddd, 1H, J = 14.9, 1.3 Hz, $J_{1H\text{-}13C}$ = 9.9 Hz, H-2), 3.48 (q, 2H, J = 6.3 Hz, H-5), 3.13 (td, 2H, J = 6.5 Hz, $J_{1H\text{-}13C}$ = 4.8 Hz, H-4), 1.98 (s, 3H, H-7); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.6 (enriched), 171.8, 134.7 (d, $J_{13C\text{-}13C}$ = 60.1 Hz), 127.0, 39.5, 28.3 (d, $J_{13C\text{-}13C}$ = 0.9 Hz), 23.2; HRMS (ES) *m/z* calculated for $C_{13}[^{13}C]H_{23}NO_4SNa$ 325.1274, found 325.1272 $[M+Na]^+$.



29

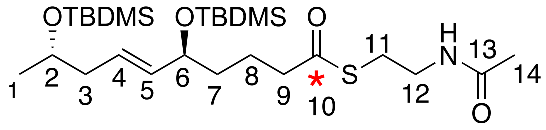
29: To a stirred solution of **27**^{5} (115 mg, 0.300 mmol) in 6 mL dry CH_2Cl_2 were sequentially added Hoveyda-Grubbs II catalyst (12.5 mg, 19.9 μmol) and **18** (35.0 mg, 0.202 mmol). The solution was stirred at 25 °C for 18 h. The solvent was removed under vacuum, and the residue was purified by column chromatography on silica gel using a 3:1 = EtOAc/hexanes eluent to afford **29** as a colorless oil (61.0 mg, 56% yield). IR ($CHCl_3$, cast film) 3289, 3079, 2955, 2929, 2895, 2856, 1728, 1665, 1635, 1548 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.90 (m, 1H, H-8), 6.13 (ddt, 1H, J = 15.5, 1.3 Hz, $J_{1H\text{-}13C}$ = 6.0 Hz, H-9), 5.96 (br s, 1H, N<u>H</u>), 5.60 (m, 1H, H-4), 5.42 (ddt, 1H, J = 15.4, 6.4, 1.3 Hz, H-5), 4.20 (q, 1H, J = 6.1 Hz, H-6), 3.80 (AB_2X_3, 1H, J = 6.0 Hz, H-2), 3.44 (q, 2H, J = 6.2 Hz, H-12), 3.06 (m, 2H, H-11), 2.36 (t, 2H, *J* = 6.8 Hz, H-7), 2.20 – 2.10 (m, 2H, H-3), 1.95 (s, 3H, H-14), 1.09 (d, 3H, *J* = 6.1 Hz, H-1), 0.88 (s, 18H, Si-C(C<u>H</u>$_3$)$_3$), 0.06 (s, 6H, SiC<u>H</u>$_3$), 0.05 (s, 3H, SiC<u>H</u>$_3$), 0.03 (s, 3H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 190.2 (enriched), 170.2, 143.2, 134.5, 130.2 (d, $J_{13C\text{-}13C}$ = 61.6 Hz), 127.7, 72.3, 68.4, 42.5, 41.5 (d, $J_{13C\text{-}13C}$ = 6.7 Hz), 39.9, 28.2, 25.9, 25.9, 23.4, 23.2, 18.2, 18.1, - 4.22, - 4.43, - 4.67, - 4.78; α_D^{25} = -11.9 (c = 0.180, CHCl$_3$); HRMS (ES) *m/z* calculated for $C_{25}[^{13}C]H_{52}NO_4SSi_2Na$ 553.3003, found 553.3002 [M+Na]$^+$.



7

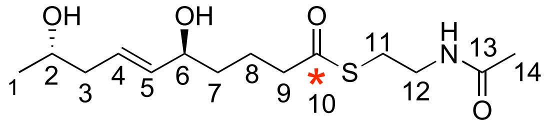
Compound **7** was synthesized from **29** by the method used for synthesis of **5**.

7: 6.00 mg, white solid, 95% yield. IR (CHCl$_3$, cast film) 3299, 3089, 2965, 2929, 1649, 1618, 1553 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.90 (m, 1H, H-8), 6.19 (m, 1H, H-9), 6.02 (br s, 1H, N<u>H</u>), 5.73 (m, 1H, H-4), 5.58 (m, 1H, H-5), 4.20 (q, 1H, *J* = 6.3 Hz, H-6), 3.80 (AB$_2$X$_3$, 1H, *J* = 6.1 Hz, H-2), 3.44 (m, 2H, H-12), 3.09 (q, 2H, *J* = 5.8 Hz, H-11), 2.46 (q, 2H, *J* = 5.9 Hz, H-7), 2.30 – 2.10 (m, 2H, H-3), 1.96 (s, 3H, H-14), 1.19 (d, 3H, *J* = 6.2 Hz, H-1); ^{13}C NMR (125 MHz, CDCl$_3$) δ 190.2 (enriched), 170.5, 141.9, 135.1, 130.6 (d, $J_{13C\text{-}13C}$ = 61.6 Hz), 128.7, 71.3, 67.2, 41.9, 39.9 (d, $J_{13C\text{-}13C}$ = 6.2 Hz), 39.6, 28.5, 23.2, 23.0; α_D^{25} = 2.20 (c = 0.150, CHCl$_3$); HRMS (ES) *m/z* calculated for $C_{13}[^{13}C]H_{23}NO_4SNa$ 325.1274, found 325.1272 [M+Na]$^+$.



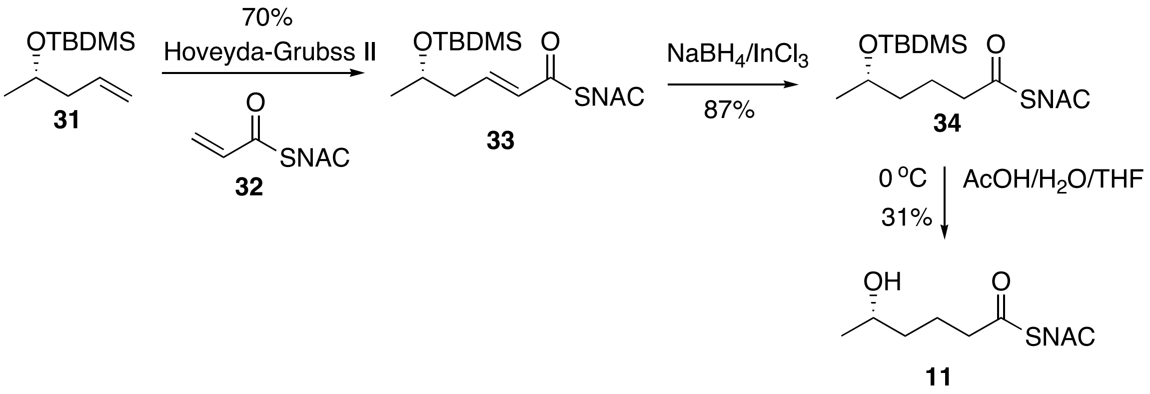
30

30: To 0.5 mL of 1:1 = monoglyme:EtOH solution was added $InCl_3$ (1.50 mg, 6.75 μmol) and $NaBH_4$ (2.00 mg, 50.0 μmol). The resulting solution was stirred at 25 °C for 5 min. A solution of **29** (8.00 mg, 15.0 μmol) in 0.5 mL of 1:1 = monoglyme:EtOH was added, and the resulting solution was stirred at 25 °C for another 3 h. The reaction was quenched by the addition of 2 mL saturated ammonium chloride. The layers were separated and the aqueous layer was extracted with EtOAc (3x2 mL). The combined organic layers were washed with brine (5 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* to give **30** (7.00 mg, 87% yield) as a colorless oil. IR ($CHCl_3$, cast film) 3286, 3079, 2955, 2929, 2896, 2856, 1654, 1553 cm^{-1}; 1H NMR (500 MHz, $CDCl_3$) δ 5.80 (br s, 1H, N$\underline{H}$), 5.55 (dtd, 1H, J = 15.3, 7.2, 0.7 Hz, H-4), 5.39 (ddt, 1H, J = 15.4, 6.5, 1.2 Hz, H-5), 4.05 (q, 1H, J = 6.2 Hz, H-6), 3.80 (AB_2X_3, 1H, J = 6.0 Hz, H-2), 3.44 (q, 2H, J = 6.2 Hz, H-12), 3.02 (m, 2H, H-11), 2.58 (td, 2H, J = 7.4 Hz, $J_{1H\text{-}13C}$ = 5.9 Hz, Hz, H-9), 2.20 – 2.10 (m, 2H, H-3), 1.96 (s, 3H, H-14), 1.76 – 1.61 (m, 2H, H-7), 1.53 – 1.42 (m, 2H, H-8), 1.10 (d, 3H, J = 6.1 Hz, H-1), 0.88 (s, 18H, Si-C(C$\underline{H}_3$)$_3$), 0.06 (s, 6H, SiC$\underline{H}_3$), 0.05 (s, 3H, SiC$\underline{H}_3$), 0.03 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (125 MHz, CD_2Cl_2) δ 199.9 (enriched), 170.1, 135.7, 127.3, 73.4, 68.9, 44.3 (d, $J_{13C\text{-}13C}$ = 45.9 Hz), 42.9, 39.9, 37.8 (d, $J_{13C\text{-}13C}$ = 3.6 Hz), 28.8, 26.0, 26.0, 23.5, 23.3, 21.8 (d, $J_{13C\text{-}13C}$ = 2.0 Hz), 18.4, 18.3, - 4.02, - 4.40, - 4.61, - 4.67; α_D^{25} = -1.46 (c = 0.130, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{25}[^{13}C]H_{53}NO_4SSi_2Na$ 555.3153, found 555.3160 $[M+Na]^+$.

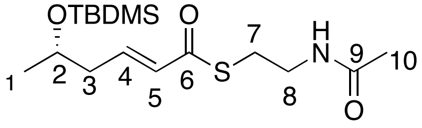


8

8: To compound **30** (7.00 mg, 13.1 μmol) was added 2 mL AcOH/H_2O/THF =2:3:1 solution at 0 °C. The resulting solution was stirred at 0 °C for 8 h. The solvent was removed *in vacuo* and the residue was purified using flash column chromatography (first elute with EtOAc, then with EtOAc/MeOH = 10:1) to give **8** (1.70 mg, 43% yield) as a colorless oil. IR (CH_2Cl_2, cast film) 3293, 3089, 2964, 2928, 2865, 1653, 1554 cm^{-1}; 1H NMR (500 MHz, CD_2Cl_2) δ 5.80 (br s, 1H, N<u>H</u>), 5.65 (dddd, 1H, *J* = 15.4, 7.6, 6.6, 0.9 Hz, H-4), 5.56 (ddt, 1H, *J* = 15.4, 6.6, 1.2 Hz, H-5), 4.05 (q, 1H, *J* = 6.5 Hz, H-6), 3.80 (m, 1H, H-2), 3.32 (q, 2H, *J* = 6.3 Hz, H-12), 3.00 (m, 2H, H-11), 2.59 (td, 2H, *J* = 7.3 Hz, J_{1H-13C} = 5.9 Hz, Hz, H-9), 2.20 – 2.10 (m, 2H, H-3), 1.89 (s, 3H, H-14), 1.76 – 1.61 (m, 2H, H-7), 1.53 – 1.42 (m, 2H, H-8), 1.15 (d, 3H, *J* = 6.2 Hz, H-1), 0.88 (s, 18H, Si-$C(C\underline{H}_3)_3$), 0.06 (s, 6H, $SiC\underline{H}_3$), 0.05 (s, 3H, $SiC\underline{H}_3$), 0.03 (s, 3H, $SiC\underline{H}_3$); ^{13}C NMR (125 MHz, CD_2Cl_2) δ 199.8 (enriched), 171.2, 136.4, 128.1, 72.5, 67.4, 44.3 (d, $J_{13C-13C}$ = 45.6 Hz), 42.4, 39.7, 36.5 (d, $J_{13C-13C}$ = 3.3 Hz), 28.9, 23.3, 23.1, 22.0 (d, $J_{13C-13C}$ = 2.1 Hz; HRMS (ES) *m/z* calculated for $C_{13}[^{13}C]H_{25}NO_4SNa$ 327.1430, found 327.1428 $[M+Na]^+$.



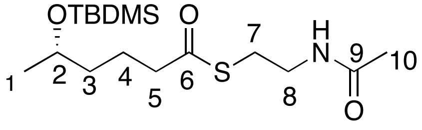
Scheme 7: Synthesis of triketide **11**



33

Compound **33** was synthesized from **31**[1] and **32** by the method used for synthesis of **29**.

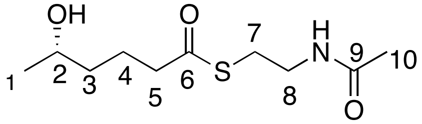
33: 84.0 mg, colorless oil, 70% yield. IR ($CHCl_3$, cast film) 3286, 3076, 2955, 2929, 2895, 2857, 1662, 1551 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.91 (dt, 1H, *J* = 15.4, 7.6 Hz, H-4), 6.12 (dt, 1H, *J* = 15.4, 1.4 Hz, H-5), 6.00 (br s, 1H, N$\underline{H}$), 3.92 (AB_2X_3, 1H, *J* = 6.0 Hz, H-2), 3.44 (q, 2H, *J* = 6.2 Hz, H-8), 3.07 (t, 2H, *J* = 6.4 Hz, H-7), 2.30 (ddd, 2H, *J* = 7.4, 5.9, 1.37 Hz, H-3), 1.98 (s, 3H, H-10), 1.09 (d, 3H, *J* = 6.1 Hz, H-1), 0.92 (s, 9H, Si-C(C$\underline{H}_3$)$_3$), 0.03 (s, 3H, SiC$\underline{H}_3$), 0.02 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.2, 170.3, 143.5, 130.2, 67.4, 42.4, 39.8, 28.2, 25.8, 23.9, 23.2, 18.0, - 4.50, - 4.79; α_D^{25} = 2.00 (c = 0.320, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{16}H_{32}NO_3SSiNa$ 368.1686, found 368.1686 $[M+Na]^+$.



34

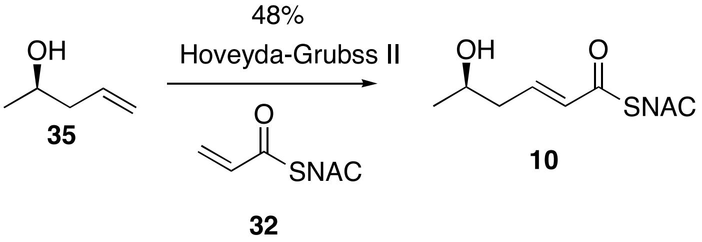
Compound **34** was synthesized from **33** by the method used for synthesis of **30**.

34: 8.00 mg, colorless oil, 87% yield. IR ($CHCl_3$, cast film) 3290, 3080, 2955, 2929, 2895, 2857, 1693, 1655, 1554 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.82 (br s, 1H, N$\underline{H}$), 3.77 (AB_2X_3, 1H, *J* = 6.0 Hz, H-2), 3.42 (q, 2H, *J* = 6.2 Hz, H-8), 3.02 (t, 2H, *J* = 6.4 Hz, H-7), 2.58 (t, 2H, *J* = 7.5, Hz, H-3), 1.95 (s, 3H, H-10), 1.80 – 1.60 (m, 2H, 3), 1.38 – 1.45 (m, 2H, 4), 1.11 (d, 3H, *J* = 6.1 Hz, H-1), 0.92 (s, 9H, Si-C(C$\underline{H}_3$)$_3$), 0.03 (s, 3H, SiC<u>H</u>3), 0.02 (s, 3H, SiC<u>H</u>3); ^{13}C NMR (125 MHz, CDCl$_3$) δ 200.1, 170.2, 68.1, 44.1, 39.8, 38.7, 28.5, 25.9, 23.8, 23.3, 21.9, 18.1, - 4.32, - 4.69; α_D^{25} = 10.2 (c = 0.320, CHCl$_3$); HRMS (ES) *m/z* calculated for $C_{16}H_{33}NO_3SSiNa$ 370.1843, found 370.1839 [M+Na]$^+$.

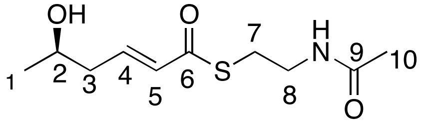


11

11: To compound **34** (8.00 mg, 23.0 μmol) was added 2 mL AcOH/H$_2$O/THF =2:3:1 solution at 0 °C. The solution was stirred at 0 °C for 4 h. The solvent was removed *in vacuo,* and the residue was purified using flash column chromatography (first elute with EtOAc/Hexane = 1:1, then with EtOAc) to give **11** (2.00 mg, 31% yield) as a colorless oil. IR (CH$_2$Cl$_2$, cast film) 3288, 3084, 2964, 2930, 2869, 1690, 1657, 1554 cm^{-1}; ^{1}H NMR (500 MHz, CD$_2$Cl$_2$) δ 5.80 (br s, 1H, N<u>H</u>), 3.76 (AB$_2$X$_3$, 1H, *J* = 6.2 Hz, H-2), 3.39 (q, 2H, *J* = 6.3 Hz, H-8), 3.00 (t, 2H, *J* = 6.5 Hz, H-7), 2.60 (t, 2H, *J* = 7.3, Hz, H-3), 1.90 (s, 3H, H-10), 1.80 – 1.65 (m, 2H, 3), 1.45 - 1.40 (m, 2H, 4), 1.11 (d, 3H, *J* = 6.1 Hz, H-1), 0.92 (s, 9H, Si-C(C<u>H</u>$_3$)$_3$), 0.03 (s, 3H, SiC<u>H</u>$_3$), 0.02 (s, 3H, SiC<u>H</u>$_3$); ^{13}C NMR (125 MHz, CD$_2$Cl$_2$) δ 199.9, 170.2, 67.7, 44.2, 39.7, 38.6, 28.9, 23.7, 23.3, 22.2; α_D^{25} = 10.0 (c = 0.080, CH$_2$Cl$_2$); HRMS (ES) *m/z* calculated for $C_{10}H_{19}NO_3SNa$ 256.0978, found 256.0975 [M+Na]$^+$.



Scheme 7: Synthesis of triketide **27**



10

The known compound **10**[5] was synthesized from **35** and **32** by the method used for synthesis of **29**.

10: 22.0 mg, brown oil, 48% yield. IR ($CHCl_3$, cast film) 3297, 3086, 2969, 2931, 2857, 1660,1635, 1551 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.93(dt, 1H, *J* = 15.4, 7.5 Hz, H-4), 6.20 (dt, 1H, *J* = 15.4, 1.4 Hz, H-5), 5.95 (brs, 1H, N$\underline{H}$), 3.99 (AB_2X_3, 1H, *J* = 6.1 Hz, H-2), 3.44 (q, 2H, *J* = 6.2 Hz, H-8), 3.09 (t, 2H, *J* = 6.4 Hz, H-7), 2.38 (m, 2H, H-3), 1.96 (s, 3H, H-10), 1.25 (d, 3H, *J* = 6.2 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.2, 170.3, 142.3, 130.6, 66.7, 41.8, 39.8, 28.4, 23.5, 23.3; α_D^{25} = - 8.69 (c = 0.230, $CHCl_3$); HRMS (ES) *m/z* calculated for $C_{10}H_1NO_3SNa$ 254.0821, found 254.0818 $[M+Na]^+$.

4. Supplementary figures

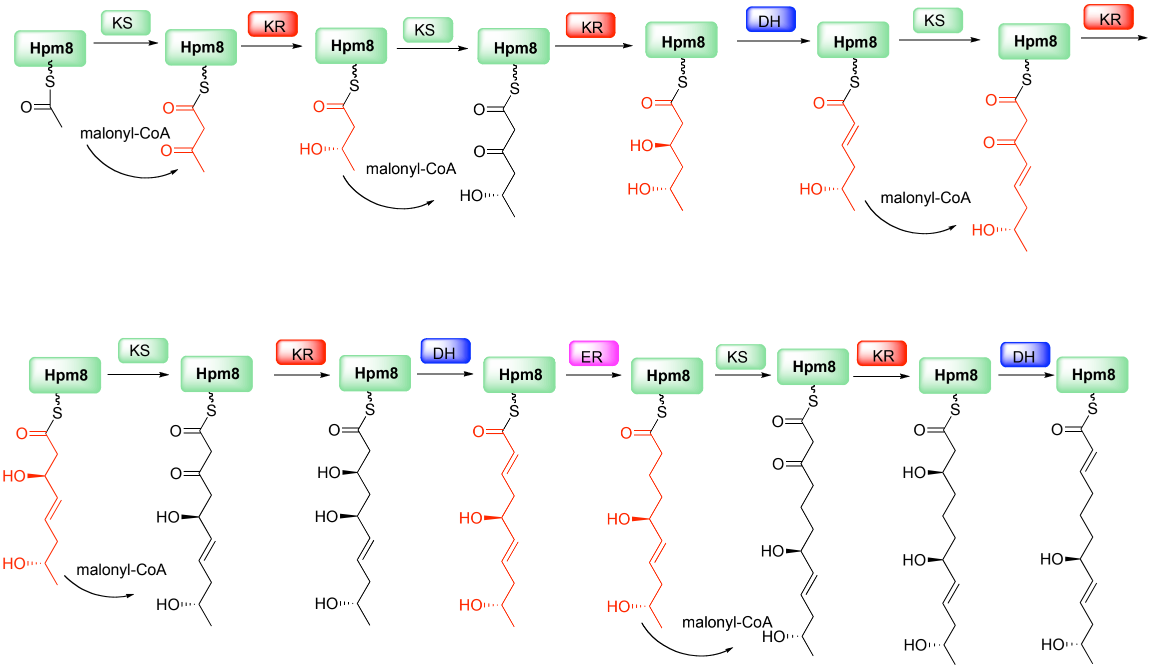


Figure S1. Fourteen ACP-tethered intermediates en route to the hexaketide (red structures were synthesized as ^{13}C labeled SNAC thioesters)

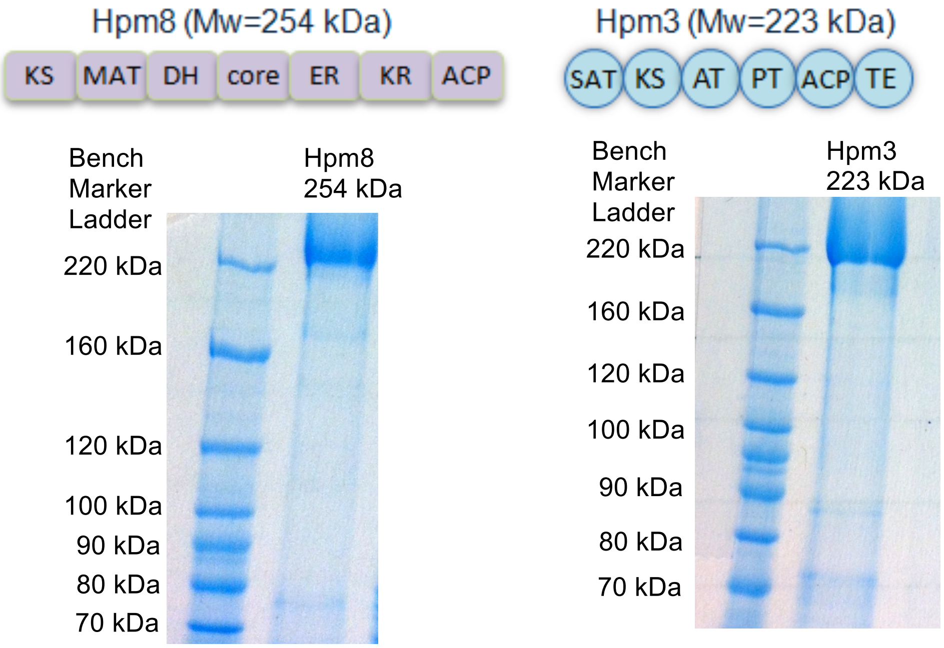


Figure S2: SDS-PAGE of the purified of Hpm8 and Hpm3 from *S. cerevisiae* strain BJ5464-NpgA

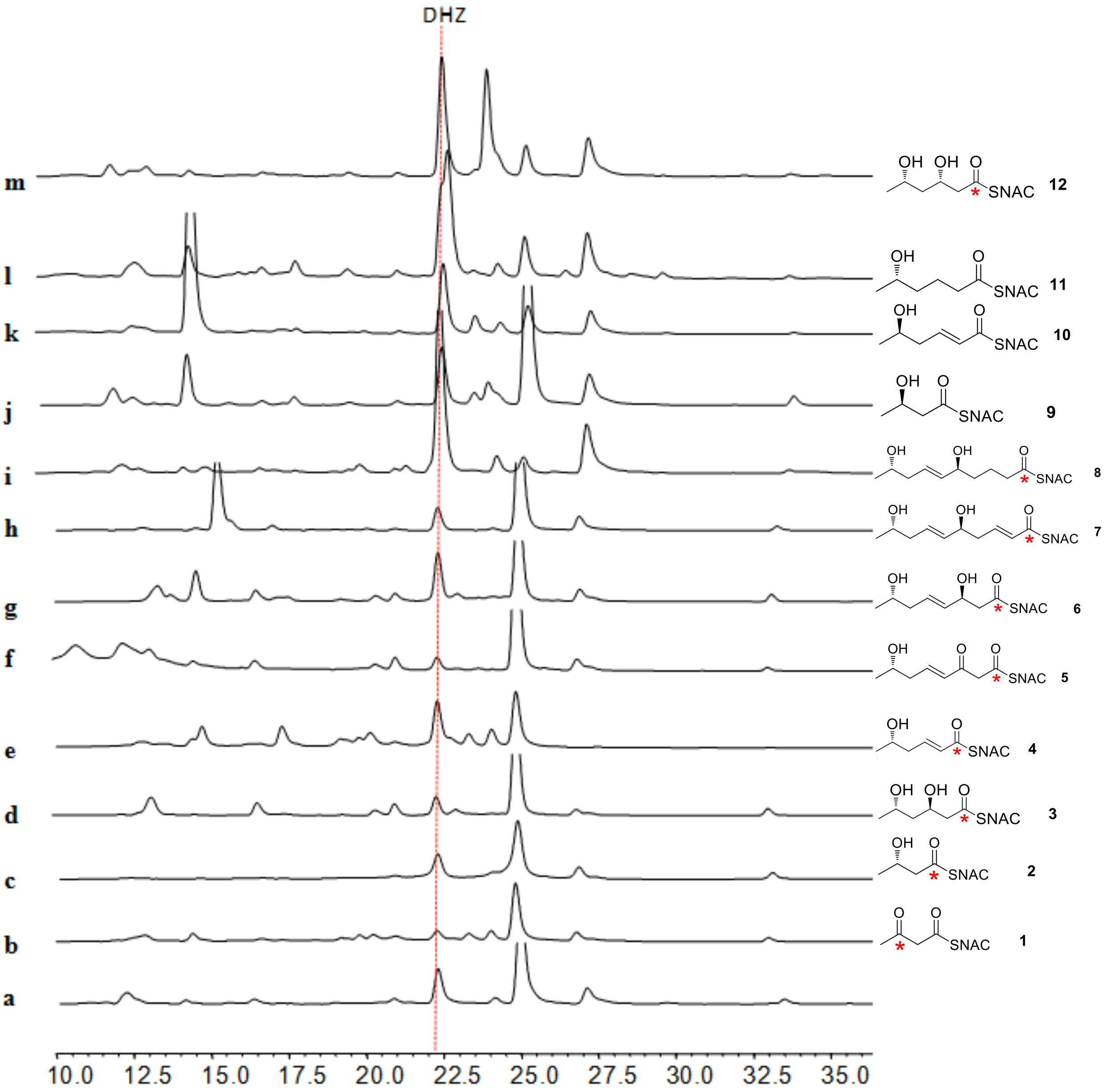


Figure S3: HPLC analysis of DHZ or analogs synthesized by (a) Hpm8 and Hpm3, (b-m) Hpm8, Hpm3, and substrates **1-12**. In all reactions, 4mM NADPH and 4mM malonyl-CoA were added to PBS buffer.

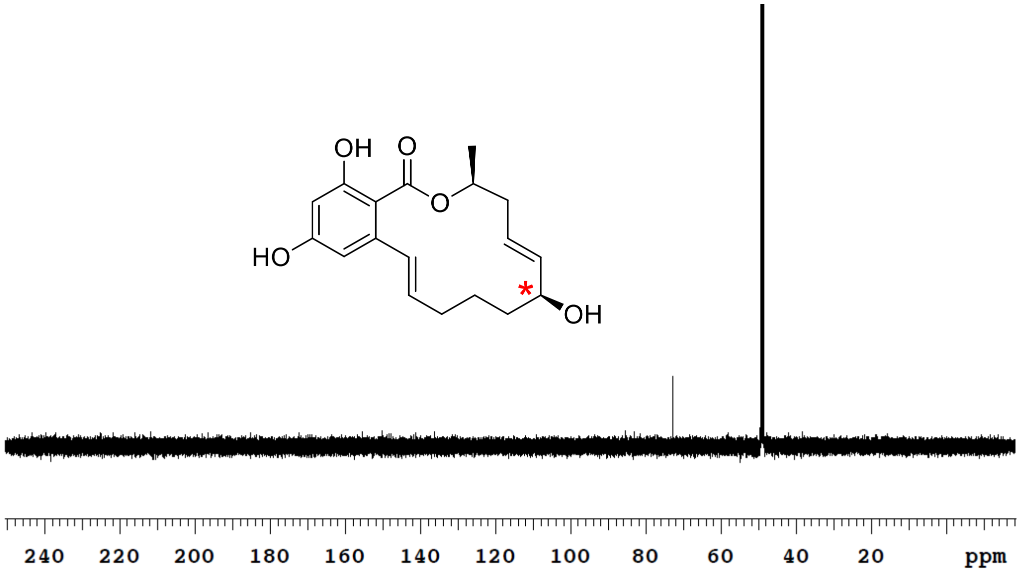


Figure S4: ^{13}C NMR spectra for ^{13}C labeled-DHZ

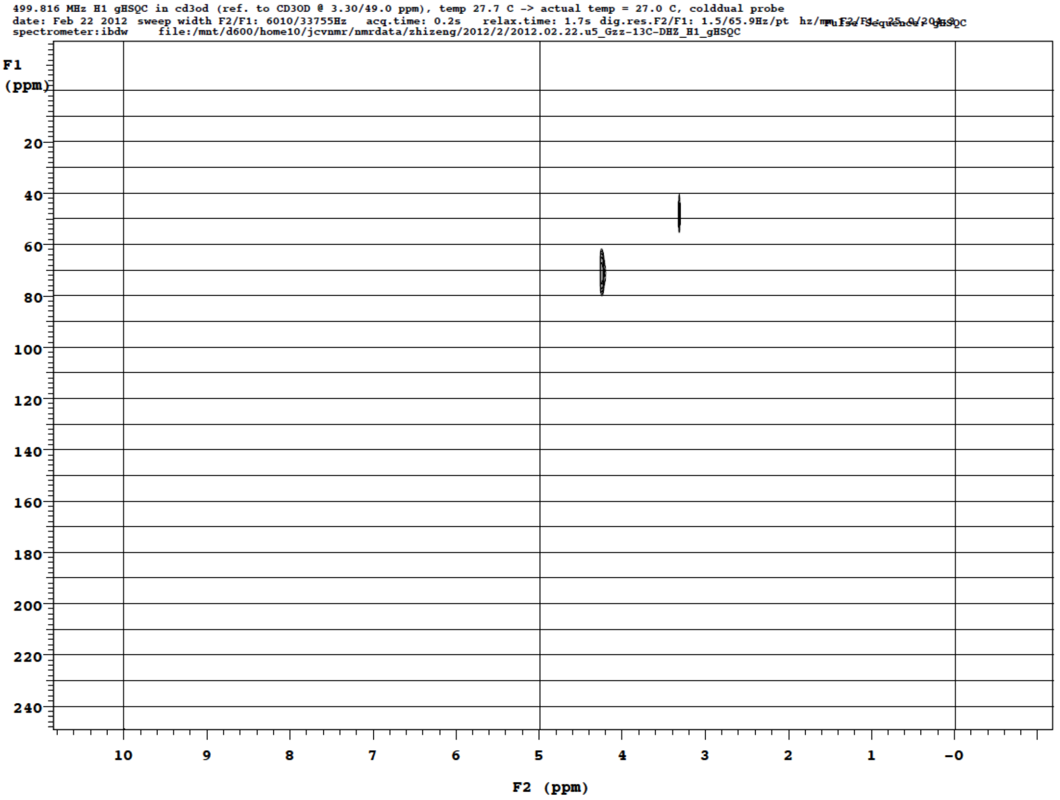


Figure S5: **HSQC spectra for ^{13}C labeled-DHZ**

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