Electronic Supplementary Material (ESI) for Chemical Communications.

General Chemoenzymatic Route to Two-Stereocenter Triketides

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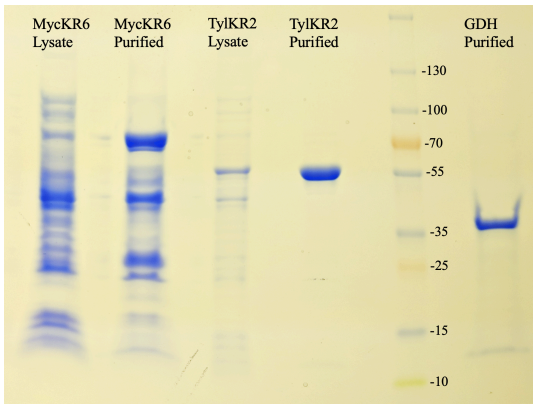
Protein preparation

The expression plasmids for MycKR6, TylKR2, and GDH as well as the purification of GDH have been previously described[1]. To harvest large quantities of KRs for the biocatalytic reactions, ammonium sulfate precipitations were performed. After growing 6 L of transformed *E. coli* BL21(DE3) in LB supplemented with 50 mg/L kanamycin to OD_{600}=0.6, cells were harvested through centrifugation (4000 x g, 10 min). They were then resuspended in 75 mL lysis buffer [30 mM HEPES, 500 mM NaCl, 5% (v/v) glycerol, pH 7.0], sonicated, and centrifuged (30,000 x g, 30 min) to obtain the cell lysate. Ammonium sulfate was slowly added to the lysate while stirring at 4 °C until the solution was 65% saturated. The mixture was centrifuged (30,000 x g for 30 min) to yield protein pellets that were stored at -80 °C.



SDS-PAGE analysis of overexpressed enzymes
(lysate was employed, but Ni-NTA purified enzymes are presented
to show where KR is in the lysate).

Equipment and settings for characterization

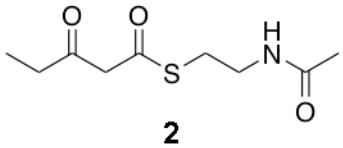
NMR MR 400 MHz Agilent and NMR VNMRS 600 MHz Agilent

HPLC/MS analysis for high resolution masses of pure compounds:
Agilent Technologies 6530 Accurate-Mass Q-TOF, Direct Inject, Jet Stream Ion Source ESI, in pos/neg modes.

HPLC/UV analysis of triketides:
Beckman Coulter HPLC system with a 20 µL loop, detection at 230 nm.
Daicel Chiralcel OC-H, 4.6 mm x 250 mm, 5 µm.
Isocratic, 7% ethanol, 93% hexanes at 0.8 mL/min.

HPLC/MS analysis of **3a**, **3b**, and triketides:
Agilent 1260 Infinity II HPLC with an Agilent 6230 TOF ESI instrument, pos/neg modes
CHIRALPAK®IF-3 column, 4.6 mm × 250 mm, 3µm; column temperature 30°C.
Isocratic, 88% water, 12% acetonitrile at 0.8 mL/min.

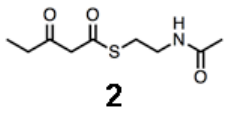
Synthesis of **2**



2

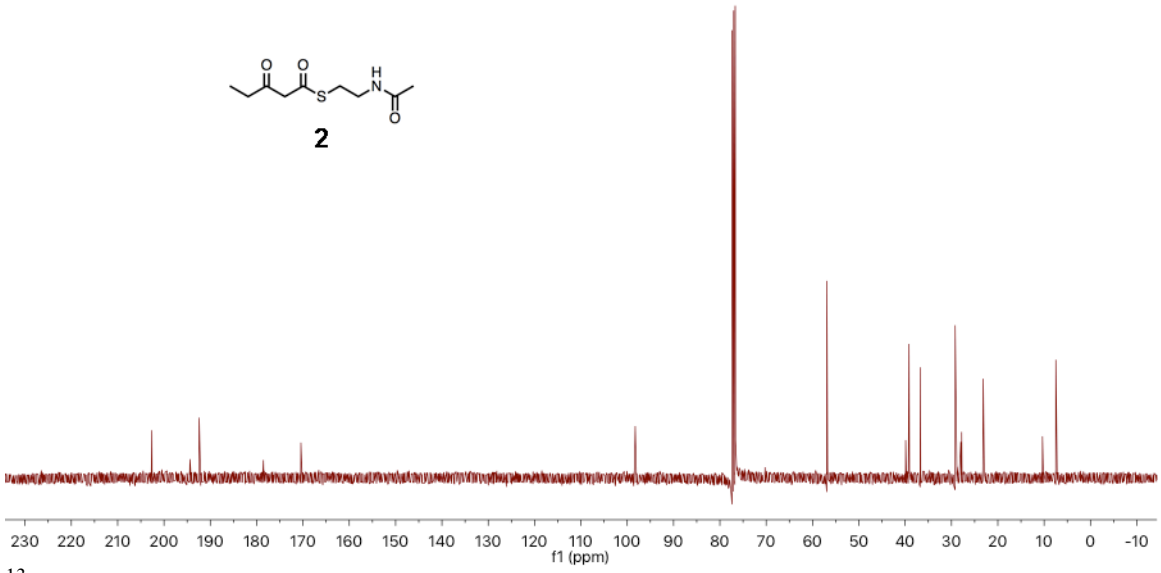
Meldrum's acid (7.2 g, 50 mmol) was dissolved in 80 mL dry DCM at 0 °C, and pyridine (8 mL, 100 mmol) was added to the solution. Propionyl chloride (4.35 mL, 50 mmol) was then supplied dropwise over 15 min. The color of the system gradually turned dark orange. The reaction was allowed to warm to 22 °C (r.t.) and stirred overnight. After that, the reaction was washed with 6 x 50 mL 0.1 M HCl to remove pyridine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Crude **1** (7.6 g, 76%) was obtained as dark orange crystals and used directly in the next step.

1 (16.8 g, 200 mmol) was dissolved in 150 mL dry toluene, and *N*-acetylcysteamine (NAC, 9.5 g, 119 mmol) was added. The reaction was refluxed at 115 °C for 5 h. The reaction was then cooled to 22 °C, and the solvent was removed through reduced pressure. Purification of the residue through $CuSO_4$-impregnated silica gel gave **2** (11.1 g, 61%) as a light yellow to dark brown solid. Column conditions used for half of the crude product: 6 x 11 cm; 600 mL (2:1 hexanes:EtOAc), 600 mL (1:1 hexanes:EtOAc), followed by pure EtOAc until all of the product eluted.



2

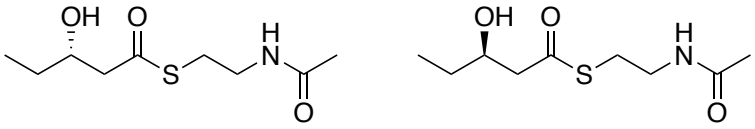
^{1}H NMR (400 MHz, chloroform-*d*) δ 5.97 (s, 1H), 3.69 (s, 2H), 3.45 (m, 2H), 3.08 (t, J = 6.0 Hz, 2H), 2.56 (q, J = 7.3 Hz, 2H), 1.96 (s, 3H), 1.10 (t, J = 7.2 Hz, 3H).



^{13}C NMR (101 MHz, chloroform-*d*) δ 202.61, 192.40, 170.45, 56.91, 39.17, 36.73, 29.20, 23.19, 7.48.

HRESIMS of **2** *m/z* 218.0850 $[M+H]^+$ (218.0851 calculated for $C_9H_{16}NO_3S$).

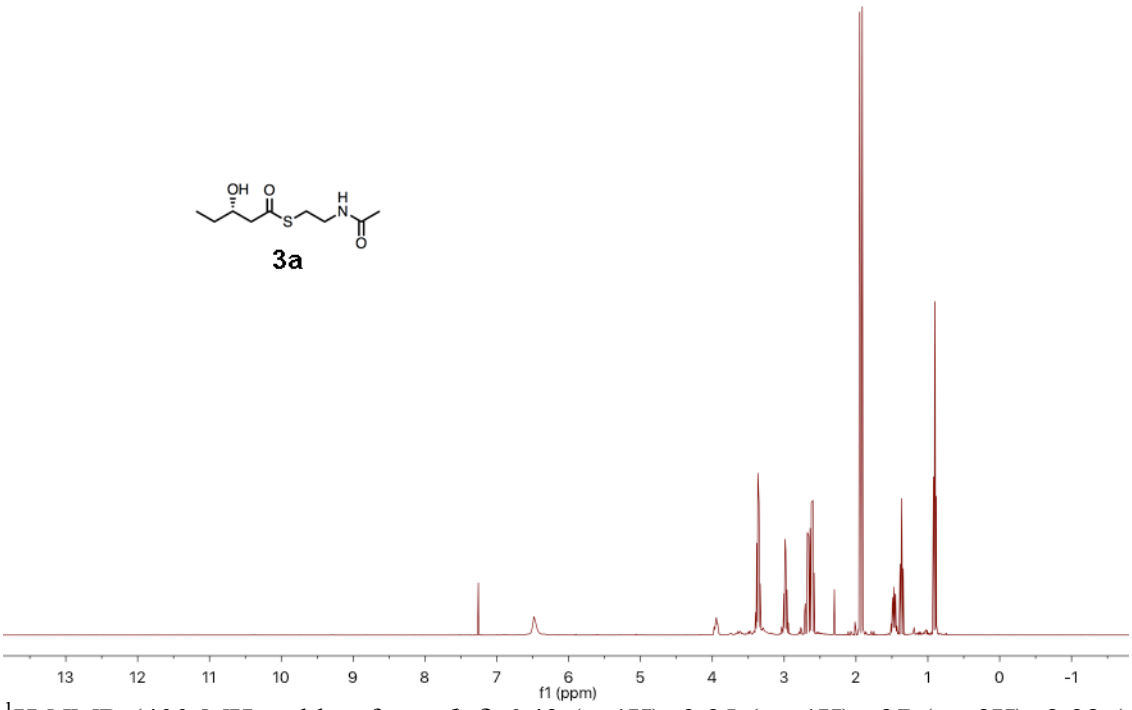
Syntheses of **3a** and **3b**



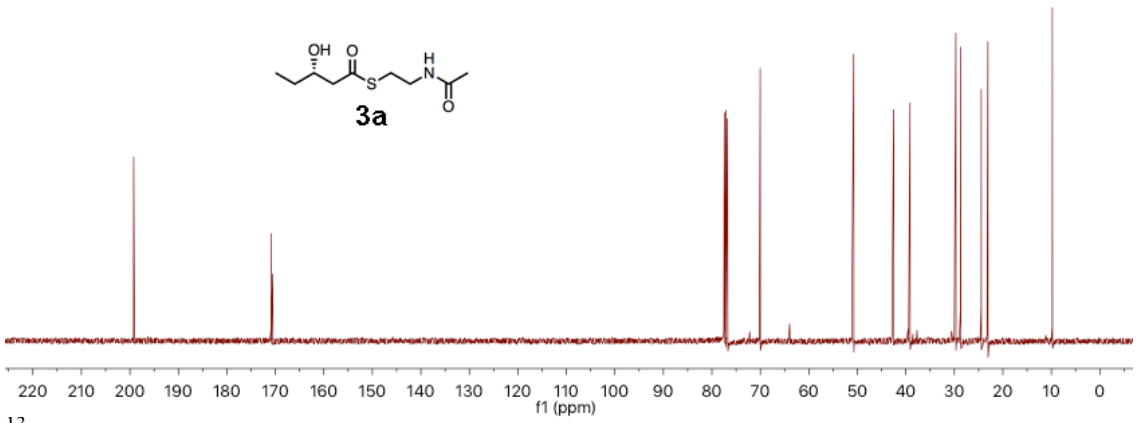
3a **3b**

Synthesis of **3a**

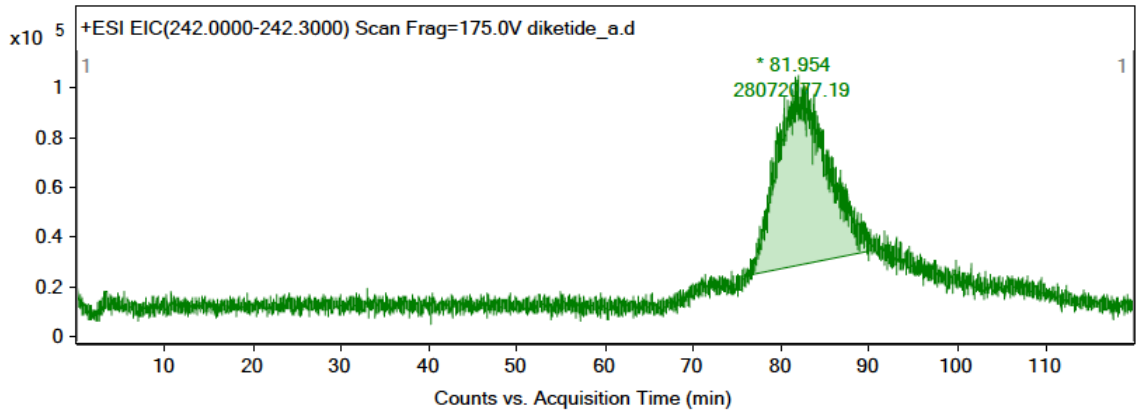
2 (4.0 g, 18.4 mmol) was combined with 120 mL water, 144 mL 1 M HEPES (pH 7.7), 9.6 mL 5 M NaCl solution, 80 mL 2 M D-glucose, 320 µL 150 mM $NADP^+$, 240 µL 20 mg/mL GDH, and 80 mL MycKR6 lysate (~3 mg/mL). The reaction was stirred at 22 °C overnight or until judged complete by LC/MS. To prevent emulsification, heat was applied, and denatured enzyme was separated by centrifugation. After that, the reaction was extracted with 2 L EtOAc, which was dried over Na_2SO_4. Solvent was removed by reduced pressure to give crude **3a** (2.6 g, 65%) as an odorless, yellow oil without further separation.



^{1}H NMR (400 MHz, chloroform-*d*) δ 6.49 (s, 1H), 3.95 (m, 1H), .37 (m, 2H), 2.98 (m, 2H), 2.64 (m, 2H), 1.92 (s, 3H), 1.47 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H).



^{13}C NMR (101 MHz, chloroform-*d*) δ 199.12, 170.76, 69.92, 50.66, 39.04, 29.59, 28.62, 22.98, 9.69.

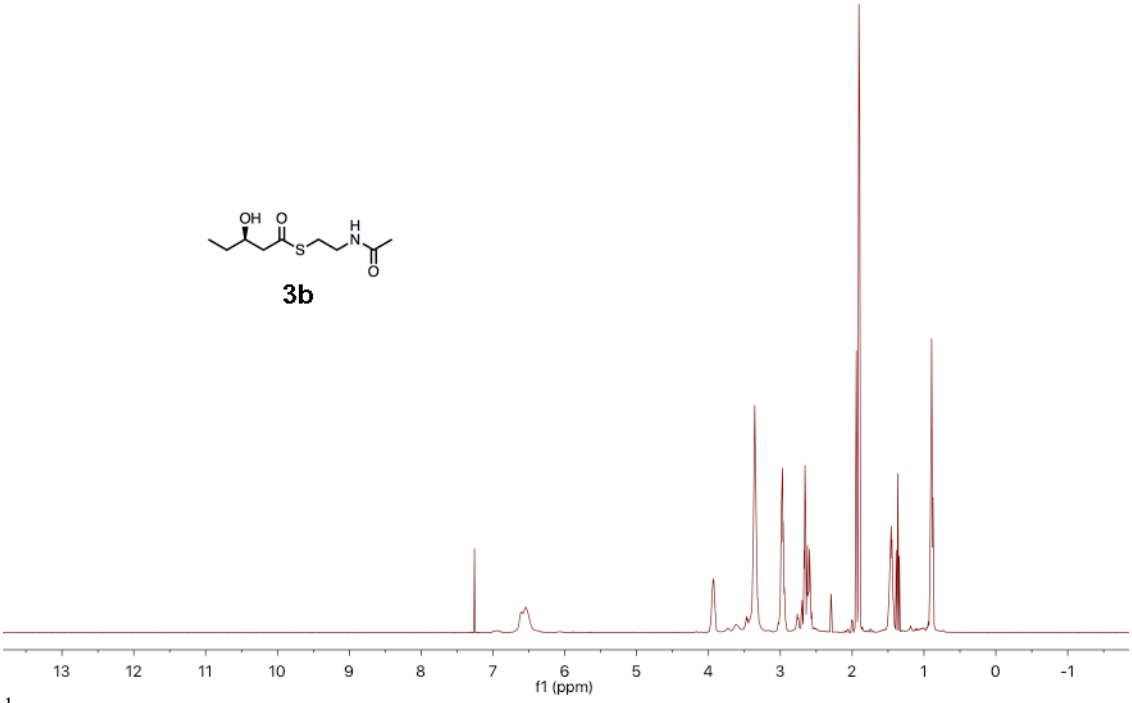


Extracted-ion chromatogram

HRESIMS of **3a** *m/z* 242.0819 $[M+Na]^+$ (242.0827 calculated for $C_9H_{17}O_3NSNa$).

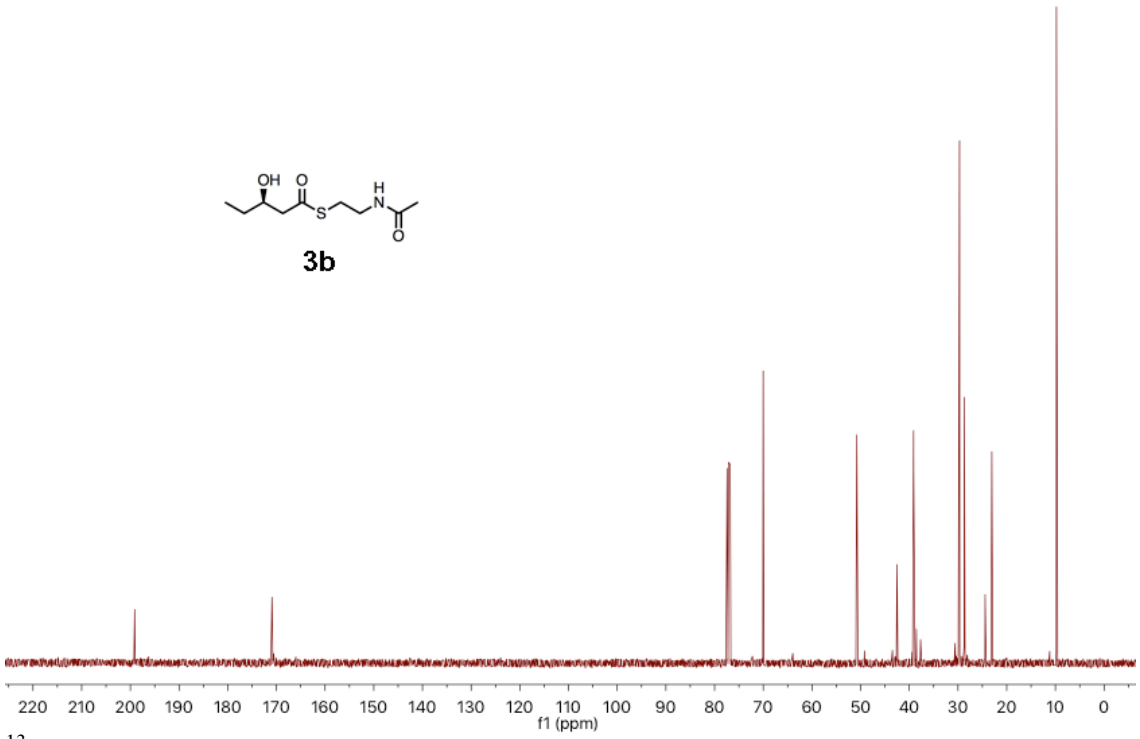
Synthesis of **3b**

2 (4.0 g, 18.4 mmol) was combined with 120 mL water, 144 mL 1M HEPES (pH 7.7), 9.6 mL 5 M NaCl solution, 80 mL 2 M D-glucose, 320 µL 150 mM $NADP^+$, 240 µL 20 mg/ml GDH, and 80 mL TylKR2 lysate (~3 mg/mL). The reaction was stirred at 22 °C overnight or until judged complete by LC/MS. To prevent emulsification, heat was applied, and denatured enzyme was separated by centrifugation. After that, the reaction was extracted with 2 L EtOAc, which was dried over Na_2SO_4. Solvent was removed by reduced pressure to give crude **3b** (2.3 g, 58%) as an odorless, yellow oil without further separation.

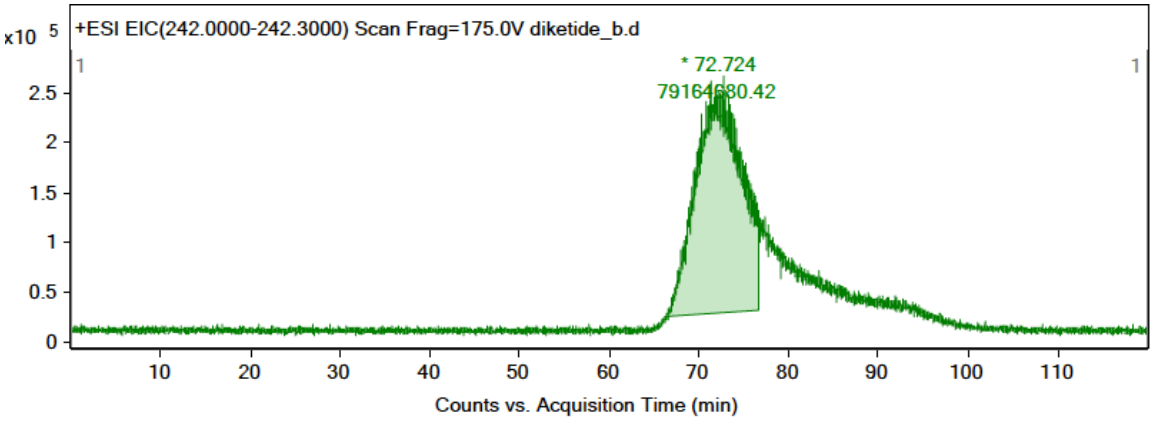


3b

^{1}H NMR (400 MHz, chloroform-*d*) δ 6.55 (s, 1H), 3.93 (m, 1H), 3.35 (m, 2H), 2.97 (m, 2H), 2.63 (m, 2H), 1.91 (s, 3H), 1.46 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H)

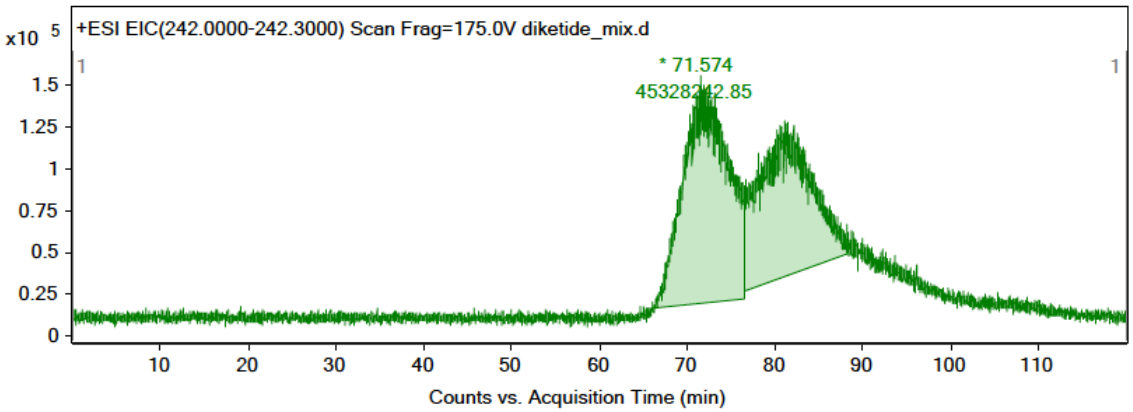


^{13}C NMR (101 MHz, chloroform-*d*) δ 199.03, 170.80, 69.90, 50.67, 39.01, 29.58, 28.59, 22.96, 9.68.



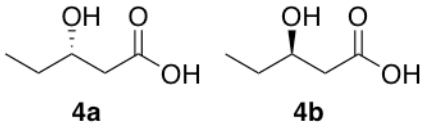
Extracted-ion chromatogram

HRESIMS *m/z* 242.0820 $[M+Na]^+$ (242.0827 calculated for $C_9H_{17}O_3NSNa$).



Extracted-ion chromatogram for a mixture of **3a** and **3b**

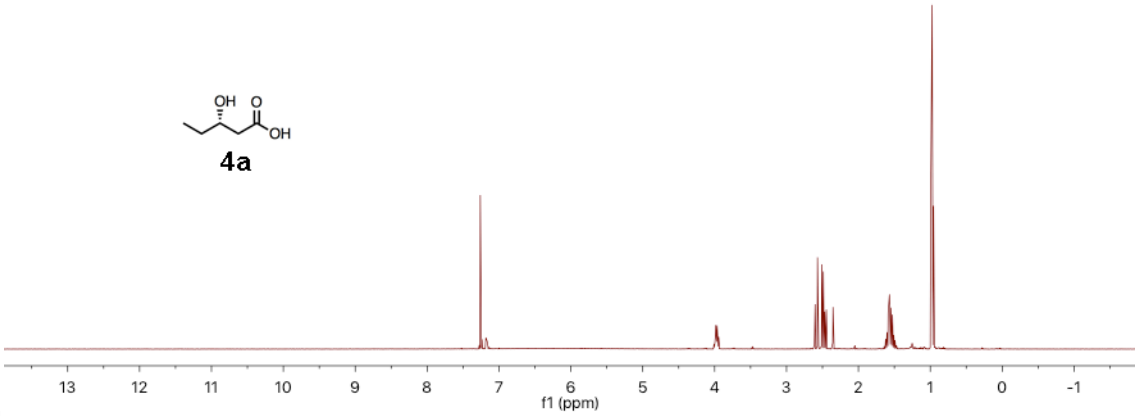
Syntheses of **4a** and **4b**



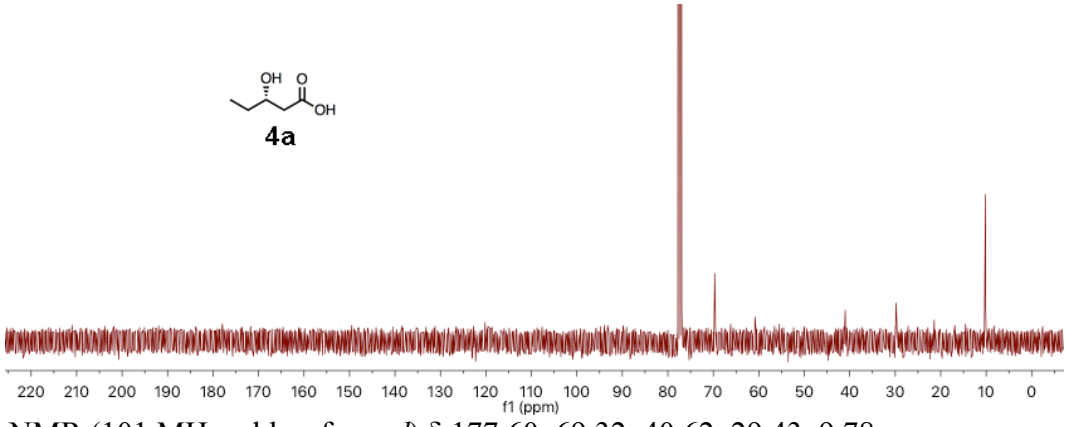
4a **4b**

Synthesis of **4a**

3a (2.5 g, 11.4 mmol) was dissolved in 60 mL 5.0 M NaOH and stirred at 80 °C overnight. The solution was adjusted to pH = 9 and washed with 3 x 50 mL EtOAc. Next, it was acidified to pH = 1 with concentrated HCl and extracted with EtOAc (5 x 100 mL). The organic phase was dried over Na_2SO_4, and the solvent was twice co-evaporated with 20 mL toluene through reduced pressure to give crude **4a** (630 mg, 51%) as a dark oil without further purification.



^{1}H NMR (400 MHz, chloroform-*d*) δ 3.97 (m, 1H), 2.59 (dd, *J* = 16.0, 3.1 Hz, 1H), 2.48 (dd, *J* = 16.3, 8.9 Hz, 1H), 1.56 (m, 2H), 0.97 (t, *J* = 7.5 Hz, 3H).

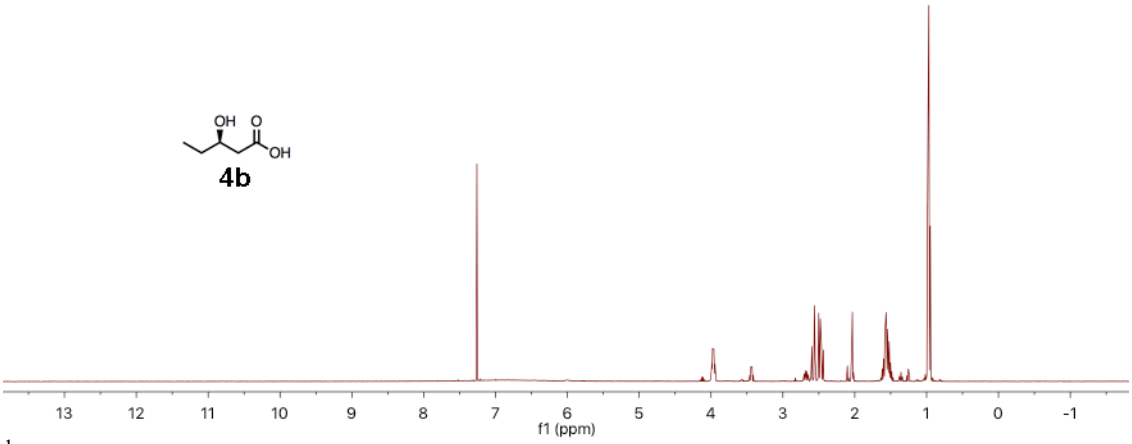


^{13}C NMR (101 MHz, chloroform-*d*) δ 177.60, 69.32, 40.62, 29.43, 9.78.

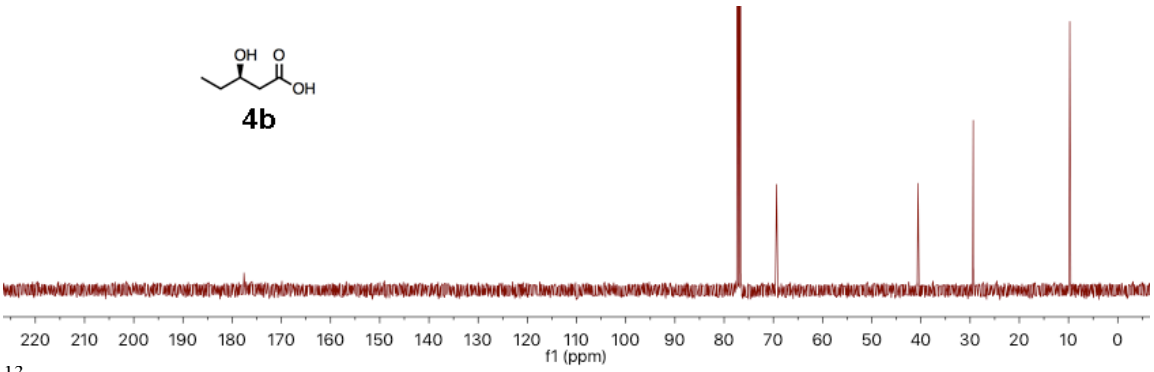
HRESIMS of **4a** *m/z* 117.0557 $[M-H]^-$ (117.0552 calculated for $C_5H_9O_3$).

Synthesis of **4b**

3b (2.6 g, 11.6 mmol) was dissolved in 60 mL of 5 M NaOH and stirred at 80 °C overnight. The solution was adjusted to pH = 9 and washed with 3 x 50 mL EtOAc. Next, it was adjusted to pH = 1 with concentrated HCl and extracted with 5 x100 mL EtOAc. The organic phase was dried over Na_2SO_4, and the solvent was twice co-evaporated with 20 mL toluene under reduced pressure to give crude **4b** (820 mg, 59%) as a dark oil without further purification.



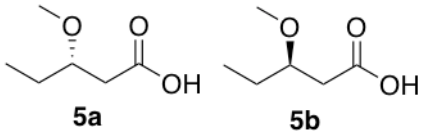
^{1}H NMR (400 MHz, chloroform-*d*) δ 3.97 (m, 1H), 2.58 (dd, *J* = 16.7, 3.1 Hz, 1H), 2.41 (dd, *J* = 16.7, 8.9 Hz, 2H), 1.55 (m, 2H), 0.97 (t, *J* = 7.4 Hz, 3H).



^{13}C NMR (101 MHz, chloroform-*d*) δ 177.60, 69.34, 40.60, 29.38, 9.76.

HRESIMS of **4b** *m/z* 117.0556 $[M-H]^-$ (117.0552 calculated for $C_5H_9O_3$).

Syntheses of **5a** and **5b**

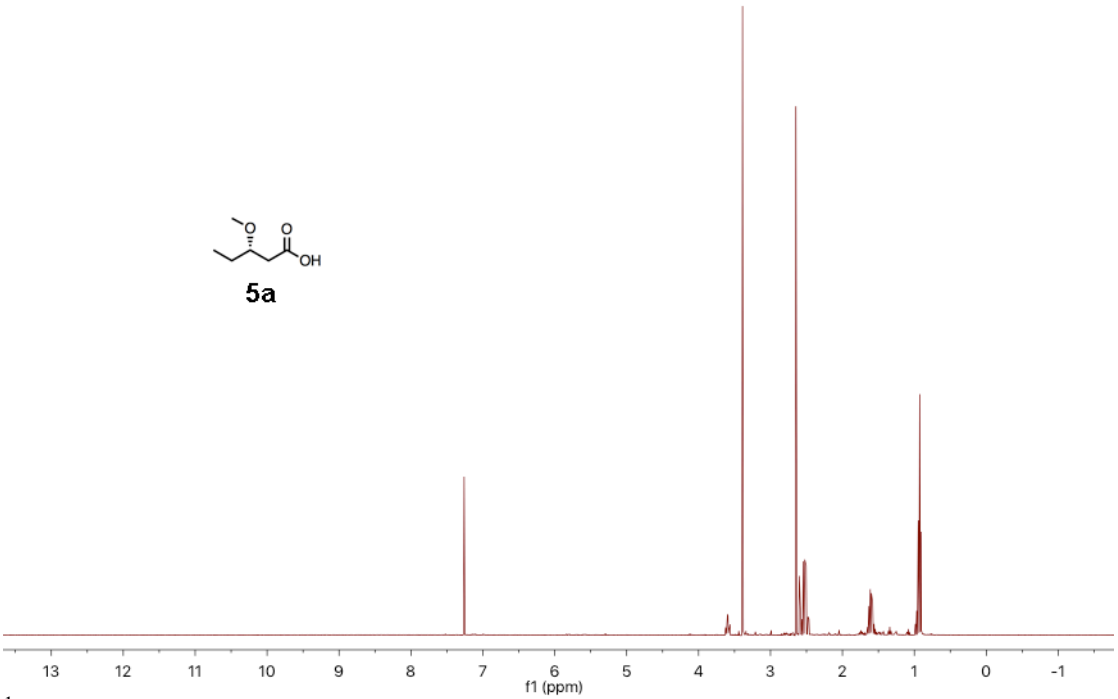


5a **5b**

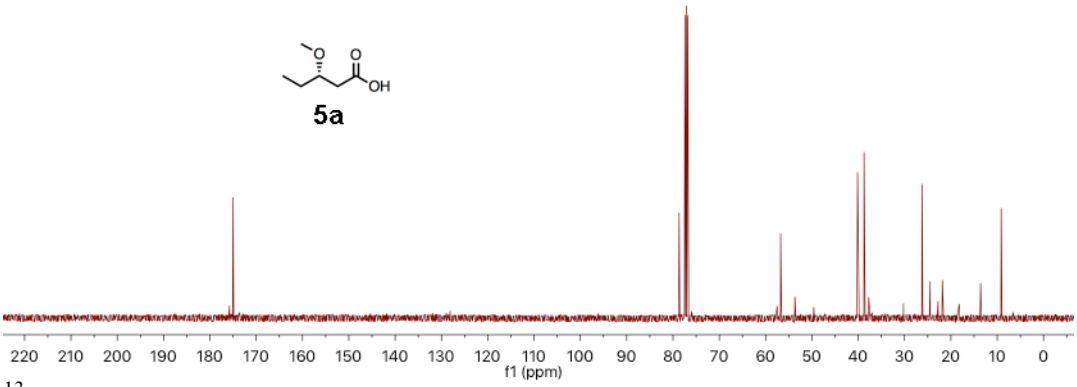
This procedure is a modification of a published protocol[2].

Synthesis of **5a**

5.8 mL 2.5 M *n*-butyllithium in hexanes (14.5 mmol) was carefully dripped into 7.0 mL dry DMSO under argon. The reaction was stirred for 40 min to form DMSO lithium base. **4a** (567 mg, 4.80 mmol) was dissolved in 4.0 mL DMSO, and this solution was slowly added to the reaction and stirred for 2 h. Then 0.71 mL MeI (1.67 g, 11.34 mmol) was added, and the reaction was stirred at 22 °C overnight. Next, 15 mL water was added to quench the reaction, and the mixture was washed with 4 x 50 mL diethyl ether. The solution was adjusted to pH = 1 and extracted with 6 x 50 mL diethyl ether, which was dried over Na_2SO_4. The solvent was passed through $CuSO_4$-packed silica gel and removed under reduced pressure to give crude **5a** (462 mg, 73%) as a dark oil without further purification.



^{1}H NMR (400 MHz, chloroform-*d*) δ 3.59 (m, 1H), 3.39 (s, 3H), 2.55 (dd, J = 15.4, 7.3 Hz, 1H), 2.49 (dd, J = 15.4, 5.1 Hz, 1H), 1.60 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H).

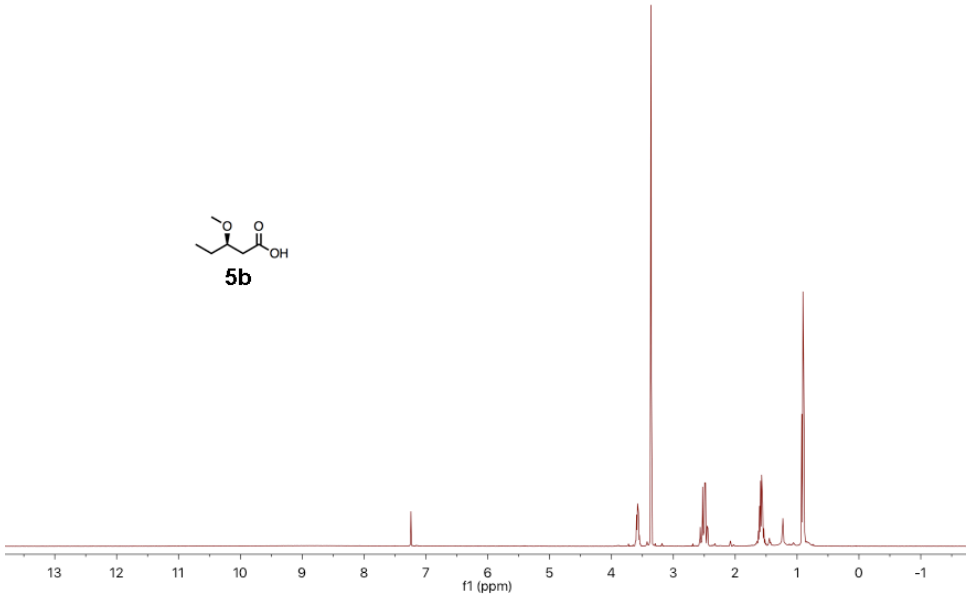


^{13}C NMR (101 MHz, chloroform-*d*) δ 174.88, 78.56, 56.64, 38.64, 26.09, 9.02.

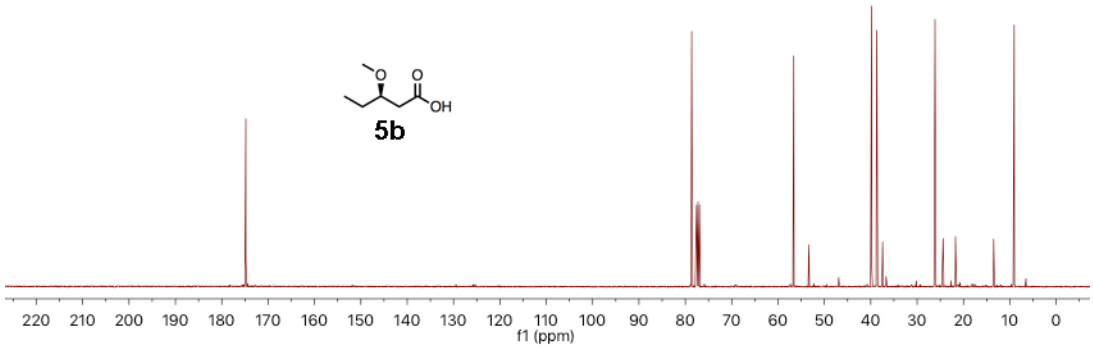
HRESIMS of **5a** *m/z* 131.0717 $[M-H]^-$ (131.0708 calculated for $C_6H_{11}O_3$).

Synthesis of **5b**

7.8 mL 2.5 M *n*-butyllithium in hexanes (19.5 mmol) was carefully dripped into 10 mL dry DMSO under argon. The reaction was stirred for 40 min to form DMSO lithium base. **4b** (850 mg, 7.19 mmol) was dissolved in 6.0 mL DMSO, and this solution was slowly added to the reaction and stirred for 2 h. Then 1.1 mL MeI (1.67 g, 17.26 mmol) was added, and the reaction was stirred at 22 °C overnight. Next, 20 mL water was added to quench the reaction. The mixture was washed with 4 x 50 mL diethyl ether, adjusted to pH = 1, and extracted with 5 x 50 mL diethyl ether. The organic layer was dried over Na_2SO_4. The solvent was passed through $CuSO_4$-packed silica gel and then removed by reduced pressure to give crude **5b** (740 mg, 78%) as a dark oil without further purification. While not necessary, traces of DMSO can be removed from **5a** and **5b** through flash chromatography (column conditions: 3 x 5 cm; 300 mL 10:1 hexanes:EtOAc and then 5:1 hexanes:EtOAc until all the product elutes).



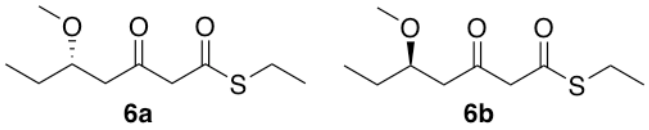
^{1}H NMR (400 MHz, chloroform-*d*) δ 3.59 (m, 1H), 3.38 (s, 3H), 2.54 (dd, *J* = 15.4, 7.3 Hz, 1H), 2.47 (dd, *J* = 15.4, 5.2 Hz, 1H), 1.61 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H).



^{13}C NMR (101 MHz, chloroform-*d*) δ 174.54, 78.36, 56.35, 38.43, 25.87, 8.79.

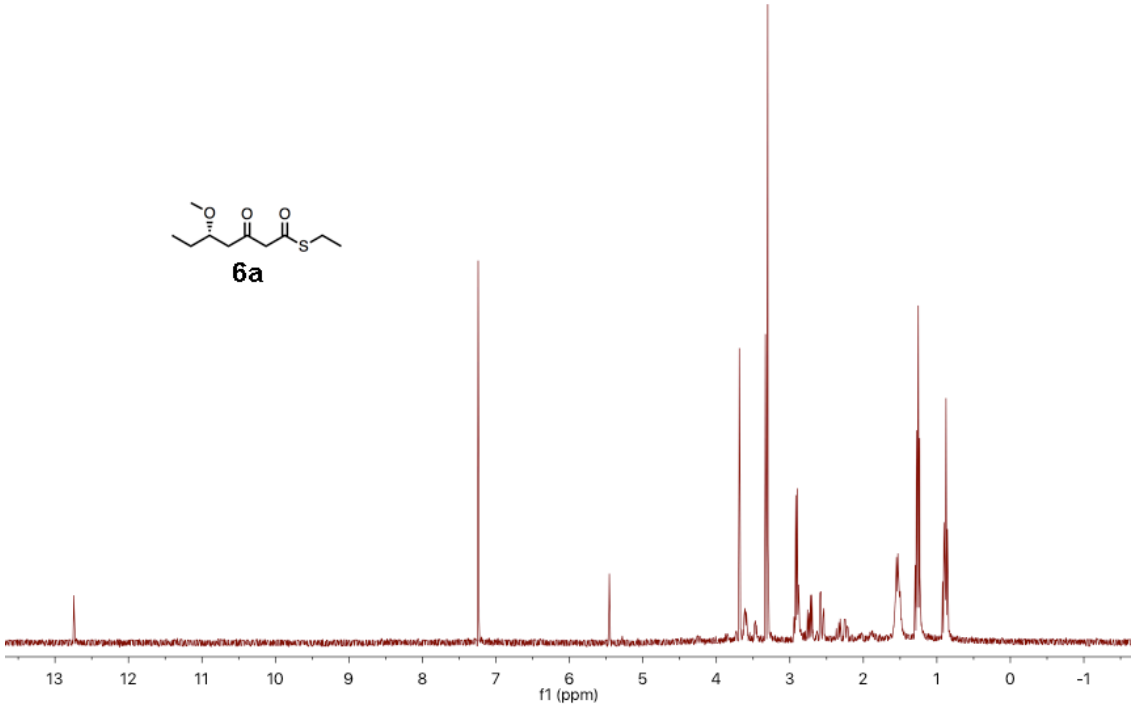
HRESIMS *m/z* 131.0712 $[M-H]^-$ (131.0708 calculated for $C_6H_{11}O_3$).

Syntheses of **6a** and **6b**

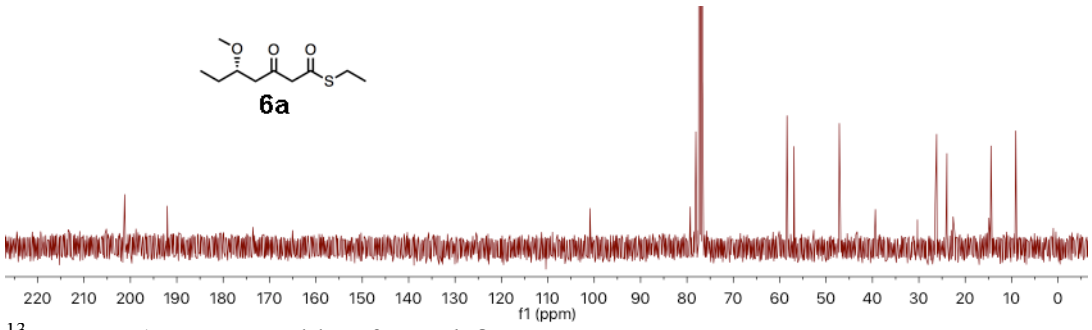


Synthesis of **6a**

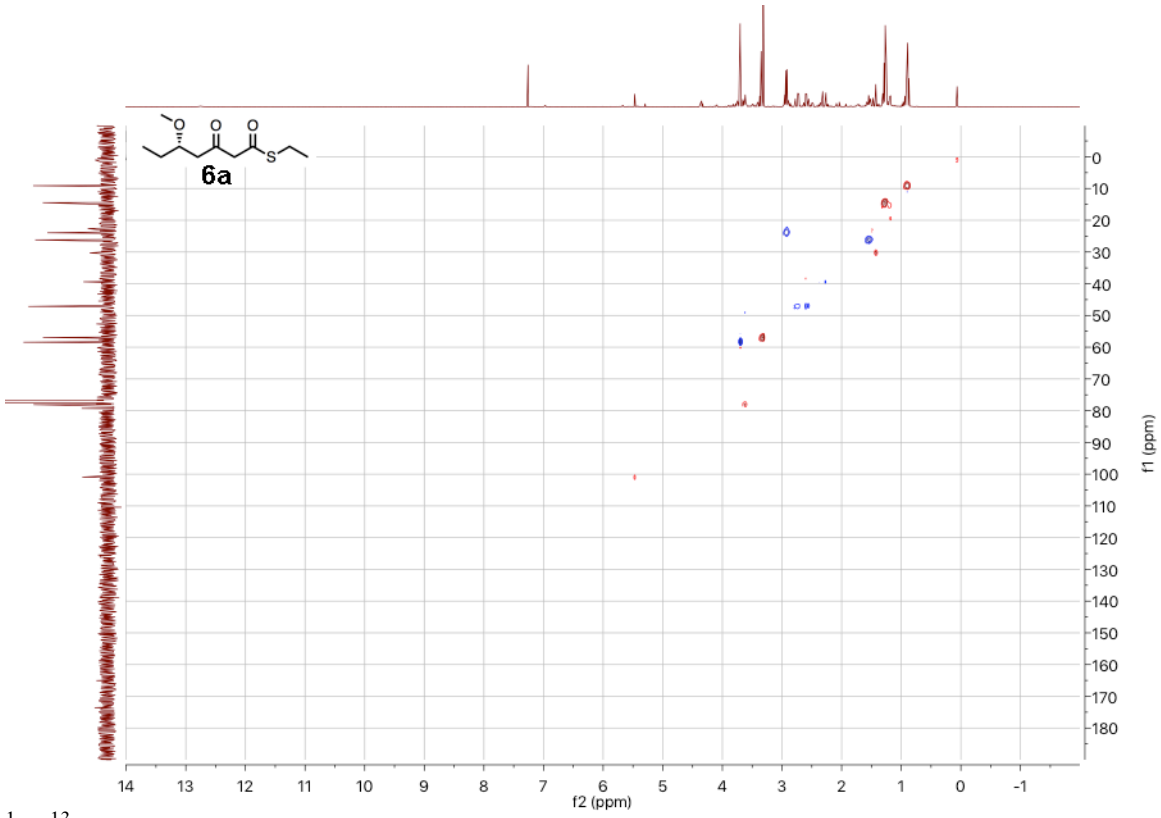
This protocol is modified from a reported procedure[3]. Portion A: 1,1'-Carbonyldiimidazole (CDI) (1.16 g, 7.18 mmol) and **5a** (863 mg, 6.53 mmol) were carefully dissolved in 30 mL anhydrous THF. The reaction was stirred for 6 h at 22 °C. Portion B: $Mg(OEt)_2$ (411 mg, 3.59 mmol) and malonyl ethanethiol thioester (1.06 g, 7.17 mmol) were dissolved in 15 mL anhydrous THF, and the reaction was kept stirring for 3 h at 22 °C. Portions A and B were then combined and stirred overnight at 22 °C. The reaction was quenched with 100 mL 0.5 M HCl and the aqueous layer was extracted with 3 x 100 mL diethyl ether. The organic layer was washed with saturated $NaHCO_3$ and dried over Na_2SO_4. The solvent was carefully removed through reduced pressure without heat. Purification of the residue by column chromatography gave **6a** (633 mg, 35%) as a reddish liquid. Column condition: 3x12 cm. Eluent: 300 mL (50:1 hexanes:EtOAc), 200 mL (40:1 hexanes:EtOAc) and then around 200 mL (20:1 hexanes:EtOAc). Column chromatography is usually unnecessary.



^{1}H NMR (400 MHz, chloroform-*d*) δ 3.70 (s, 2H), 3.63 (m, 1H), 3.32 (s, 3H), 2.93 (q, J = 7.5 Hz, 2H), 2.75 (dd, J = 16.5, 7.6 Hz, 2H), 2.58 (dd, J = 15.6, 4.6 Hz, 2H), 1.54 (m, 2H), 1.27 (t, J = 7.5 Hz, 3H), 0.89 (t, J = 7.5 Hz, 3H).



^{13}C NMR (101 MHz, chloroform-*d*) δ 201.24, 192.06, 78.09, 58.43, 56.93, 47.15, 26.17, 23.95, 14.45, 9.10.



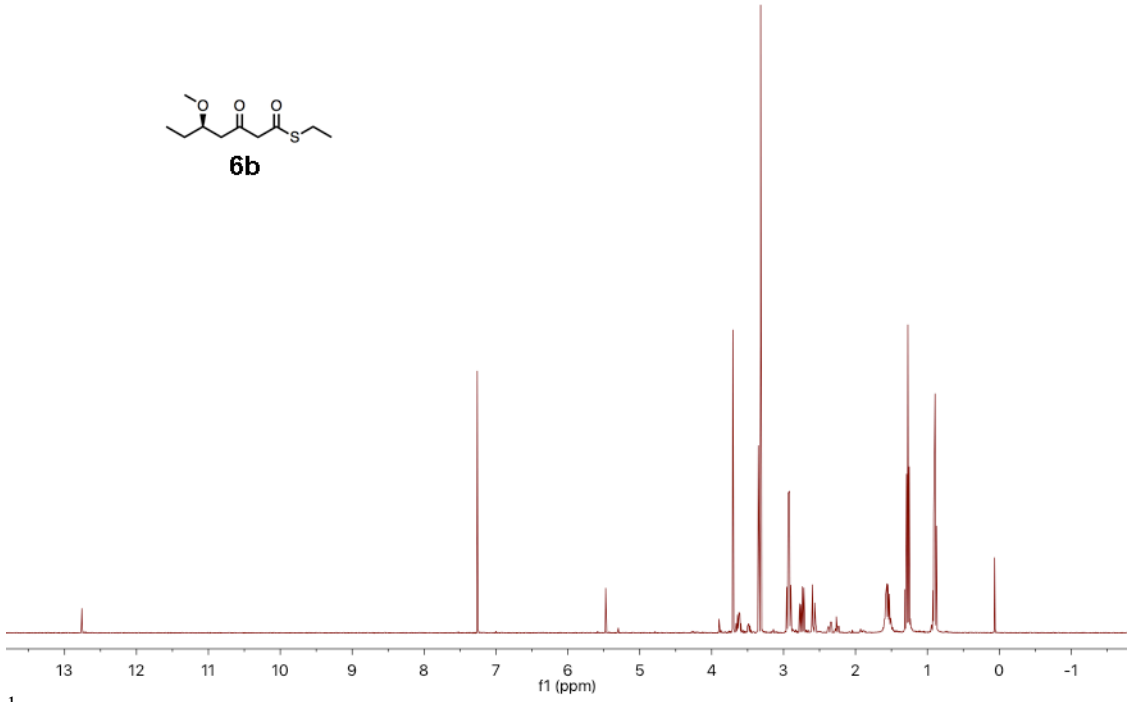
^{1}H-^{13}C HSQC

HRESIMS of **6a** *m/z* 241.0874 $[M+Na]^+$ (241.0874 calculated for $C_{10}H_{18}O_3SNa$).

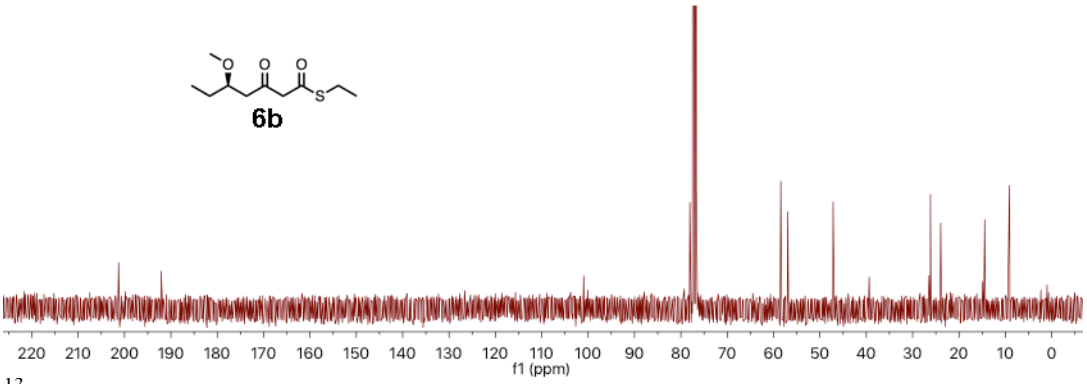
Synthesis of **6b**

Portion A: CDI (597 mg, 3.68 mmol) and **5b** (442 mg, 3.34 mmol) were carefully dissolved in anhydrous 15 mL THF. The reaction was kept stirring for 5 h at 22 °C. Portion B: $Mg(OEt)_2$ (221 mg, 1.84 mmol) and malonyl ethanethiol thioester (545 mg, 3.68 mmol) were dissolved in 7 mL anhydrous THF, the reaction was kept stirring for 3 h at 22 °C. Portion A and Portion B were combined and kept stirring overnight at 22 °C.

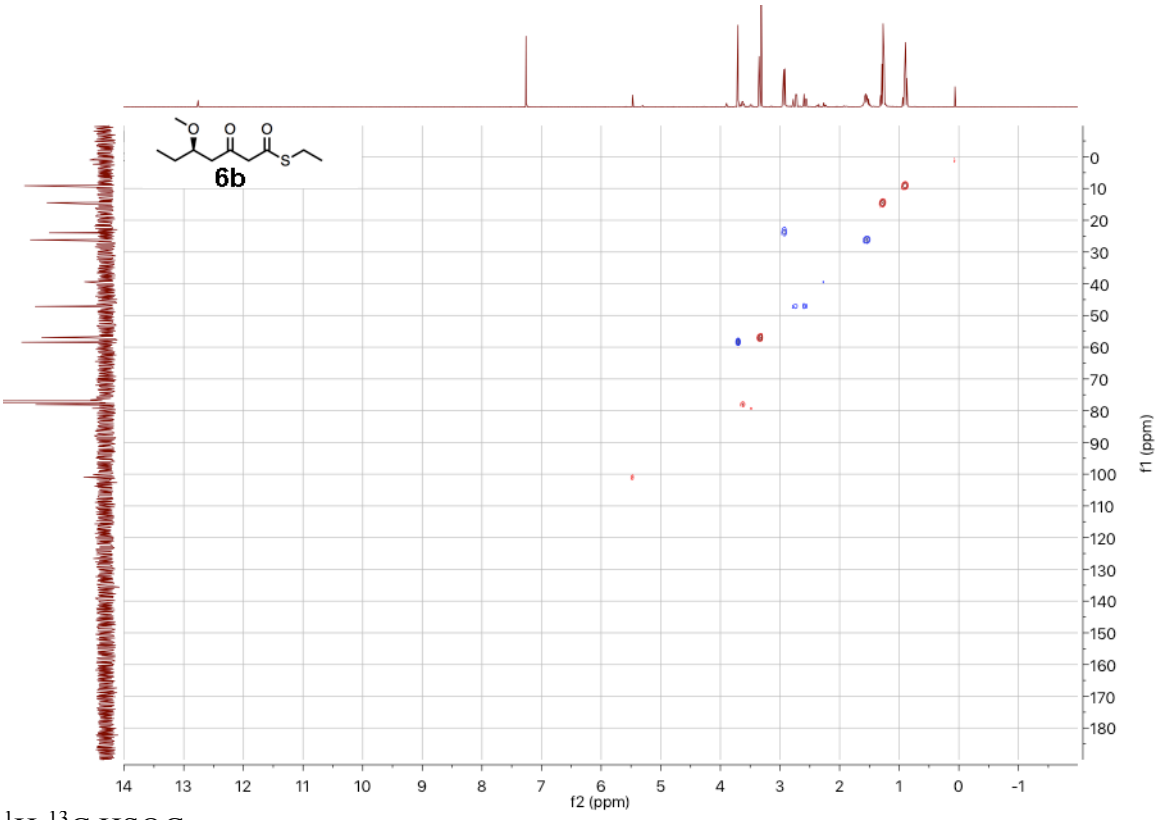
After that, the reaction was quenched with 50 mL 0.5 M HCl and partitioned by 50 mL diethyl ether. The aqueous layer was extracted by diethyl ether 50 mL twice and 25 mL once. The organic layer was washed with saturated $NaHCO_3$ and dried over Na_2SO_4. The solvent was carefully removed by reduced pressure without heat. Purification of the residue via column chromatography gave **6b** (251 mg, 34%) as a reddish liquid. Column condition: 1x12 cm. Eluent: 50:1 hexanes:EtOAc (chromatography is usually unnecessary).



^{1}H NMR (400 MHz, chloroform-*d*) δ 3.70 (s, 2H), 3.63 (m, 1H), 3.33 (s, 3H), 2.93 (q, J = 7.5 Hz, 2H), 2.75 (dd, J = 15.5, 7.7 Hz, 1H), 2.58 (dd, J = 15.9, 4.7 Hz, 1H), 1.54 (m, 2H), 1.27 (t, J = 7.6 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H).



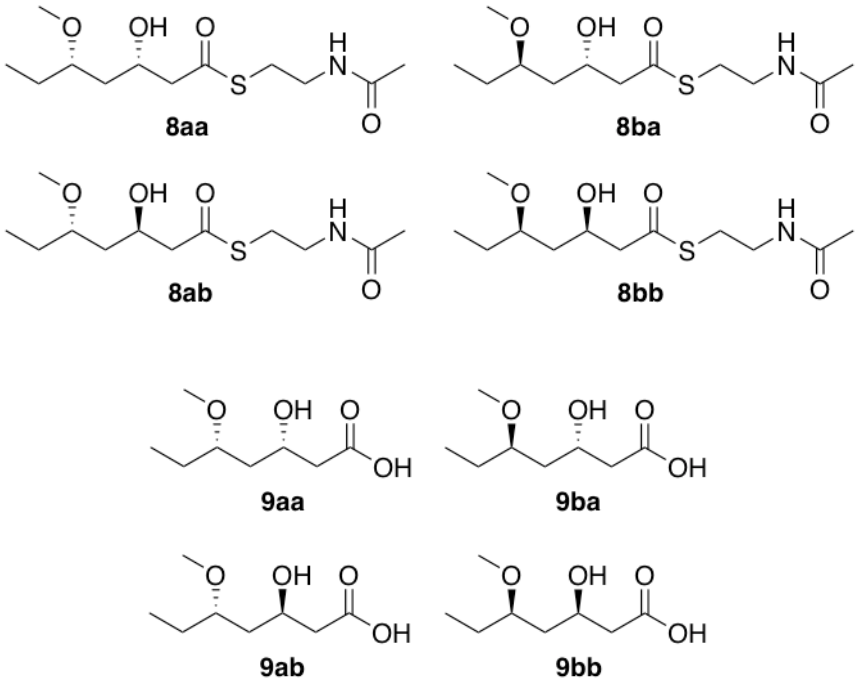
^{13}C NMR (101 MHz, chloroform-*d*) δ 201.27, 192.08, 78.07, 58.44, 56.94, 47.16, 26.16, 23.96, 14.46, 9.11.



^{1}H-^{13}C HSQC

HRESIMS of **6b** *m/z* 241.0872 $[M+Na]^+$ (241.0874 calculated for $C_{10}H_{18}O_3SNa$).

Syntheses of **8aa** & **9aa**, **8ab** & **9ab**, **8ba** & **9ba**, and **8bb & 9bb**



Synthesis of **8aa & 9aa**

6a (110 mg, 0.389 mmol) was added to a solution containing 500 µL NAC (~10 eq.) 10 mL water, and 12 mL of 1 M HEPES (pH 8.5). The thiol-thioester exchange was performed over 2 h at 22 °C, and then the pH was adjusted to 7.7 with concentrated HCl. 800 µL of 5 M NaCl, 6 mL of 2.0 M D-glucose, 160 µL of 0.15 M $NADP^+$, 180 µL of GDH (15 mg/mL), and 30 mL of MycKR6 lysate (3 mg/mL) were then consecutively added to the reaction. The reaction was kept stirring at 22 °C overnight or until it was done as monitored by LC/MS. Heat was applied by microwave, and the aggregated enzyme was separated by centrifugation. After that, the reaction was extracted by 450 mL EtOAc. The extract was dried over Na_2SO_4, and the solvent was removed by reduced pressure. Formation of **8aa** and its stereoisomers could be analyzed by chiral chromatography. 10 mL of 5 M NaOH was added to the residue, and the reaction was heated to 80 °C overnight. After that, the reaction was cooled down and washed with 2 x 50 mL EtOAc. The pH was then adjusted to 1, and the reaction was extracted with 3 x 50 mL EtOAc. The extract was dried over Na_2SO_4. The solvent was passed through a plug of $CuSO_4$-impregrated silica gel and co-evaporated with toluene under reduced pressure to give crude **9aa** (50 mg, 57%) as a dark oil.

HRESIMS *m/z* 300.1247 $[M+Na]^+$ (300.1245 calculated for $C_{12}H_{23}NO_4SNa$).

Stereoisomer purity determined through complementary chiral chromatographies shown in Figure S1: >90%

Synthesis of **8ab & 9ab**

6a (55 mg, 0.195 mmol) was added to a solution containing 250 μL NAC (~10 eq.), 5 mL water and 6 mL of 1 M HEPES (pH 8.5). The thiol-thioester exchange was performed over 2 h at 22 °C, and then the pH was adjusted to 7.7 with concentrated HCl. 800 μL of 5 M NaCl, 6 mL of 2.0 M D-glucose, 160 μL of 0.15 M $NADP^+$, 180 μL of GDH (15 mg/ml), and 30 mL of TylKR2 lysate (3 mg/mL) were then consecutively added to the reaction. The reaction was kept stirring at 22 °C for 2 d or until it was done monitored by LC/MS. Heat was applied by microwave, and the aggregated enzyme was separated by centrifugation. After that, the reaction was extracted by 450 mL EtOAc. The extract was dried over Na_2SO_4, and the solvent was removed by reduced pressure. Formation of **8ab** and its stereoisomers could be analyzed by chiral chromatography. 10 mL of 5 M NaOH was added to the residue, and the reaction was heated to 80 °C overnight. After that, the reaction was cooled down and washed with 2 x 50 mL EtOAc. The pH was then adjusted to 1, and the reaction was extracted with 3 x 50 mL EtOAc. The extract was dried over Na_2SO_4. The solvent was passed through a plug of $CuSO_4$-impregrated silica gel and co-evaporated with toluene under reduced pressure to give crude **9ab** (16 mg, 36%) as a dark oil.

HRESIMS *m/z* 300.1245 $[M+Na]^+$ (300.1246 calculated for $C_{12}H_{23}NO_4SNa$).

Stereoisomer purity determined through complementary chiral chromatographies shown in Figure S1: >90%

Synthesis of **8ba & 9ba**

6b (20 mg, 0.092 mmol) was added to a solution containing 91 μL NAC (~10 eq.), 1.82 mL water, and 2.2 mL of 1 M HEPES (pH 8.5). The thiol-thioester exchange was performed over 2 h at 22 °C, and then the pH was adjusted to 7.7 with concentrated HCl. 291 μL of 5 M NaCl, 2.2 mL of 2.0 M D-glucose, 58 μL of 0.15 M $NADP^+$, 110 μL of GDH (15 mg/mL), and 20 mg MycKR6 precipitate were then consecutively added to the reaction. The reaction was kept stirring at 22 °C overnight or until it was done monitored by LC/MS. Formation of **8ba** could be observed by HPLC through an EtOAc extraction. Heat was applied by microwave, and the aggregated enzyme was separated by centrifugation. After that, the reaction was extracted by 450 mL EtOAc. The extract was dried over Na_2SO_4, and the solvent was removed by reduced pressure. Formation of **8ba** and its stereoisomers could be analyzed by chiral chromatography. 10 mL of 5 M NaOH was added to the residue, and the reaction was heated to 80 °C overnight. After that, the reaction was cooled down and washed with 2 x 50 mL EtOAc. The pH was then adjusted to 1, and the reaction was extracted with 3 x 50 mL EtOAc. The solvent was passed through a plug of $CuSO_4$-impregrated silica gel and co-evaporated with toluene under reduced pressure to give crude **9ba** (7 mg, 44%) as a dark oil.

HRESIMS *m/z* 300.1228 $[M+Na]^+$ (300.1246 calculated for $C_{12}H_{23}NO_4SNa$).

Stereoisomer purity determined through complementary chiral chromatographies shown in Figure S1: >90%

Synthesis of **8bb & 9bb**

6b (106 mg, 0.486 mmol) was added to a solution containing 500 μL NAC (~10 eq.), 10 mL water, and 12 mL of 1 M HEPES (pH 8.5). The thiol-thioester exchange was performed over 2 h at 22 °C, and then the pH was adjusted to 7.7 with concentrated HCl. 800 μL of 5 M NaCl, 6 mL of 2.0 M D-glucose, 160 μL of 0.15 M $NADP^+$, 180 μL GDH (15 mg/mL), and 1.0 g of TylKR2 precipitate were then consecutively added to the reaction. The reaction was kept stirring at 22 °C overnight or until it was complete, as monitored by LC/MS. Heat was applied by microwave, and the aggregated enzyme was separated by centrifugation. After that, the reaction was extracted by 450 mL EtOAc. The extract was dried over Na_2SO_4, and the solvent was removed by reduced pressure. Formation of **8bb** and its stereoisomers could be analyzed by chiral chromatography. 10 mL of 5 M NaOH solution was added to the residue, and the reaction was heated to 80 °C overnight. After that, the reaction was cooled and washed with 2 x 50 mL EtOAc. The pH was then adjusted to 1, and the reaction was extracted with 3 x 50 mL EtOAc. The extract was dried over Na_2SO_4. The solvent was passed through a plug of $CuSO_4$-impregrated silica gel and co-evaporated with toluene under reduced pressure to give crude **9bb** (22 mg, 24%) as a dark oil.

HRESIMS *m/z* 300.1242 $[M+Na]^+$ (300.1246 calculated for $C_{12}H_{23}NO_4SNa$).

Stereoisomer purity determined through complementary chiral chromatographies shown in Figure S1: >90%

In converting β-ketotriketides **6a** and **6b** to β-hydroxytriketides **8aa**, **8ab**, **8ba**, and **8bb**, the purity of final compounds depended on the reaction conditions. Greater enzyme concentrations and shorter reaction times minimized the formation of byproducts. When byproducts were substantial, detection by mass spectrometry was preferable to UV absorbance.

Chiral chromatographic analysis of chemoenzymatically-generated triketides

Two complementary, chiral chromatography systems were used to determine which stereoisomers were generated in the chemoenzymatic syntheses of triketides **8aa**, **8ab**, **8ba**, and **8bb** - an OC-H column (normal phase) coupled with a UV detector and an IF3 column (reversed phase) coupled with a time-of-flight (TOF) mass spectrometer. The *anti*-products, **8ab** & **8ba**, eluted before the *syn*-products, **8aa** & **8bb**, in both chromatographies. While the *anti*-products could not be resolved from one another on the OC-H column, they could be on the IF3 column, and while the *syn*-products could not be resolved from one another on the IF3 column, they could be on the OC-H column. For all LC/MS-TOF studies, 3-oxohexanoyl-*S*-NAC, **14**, was supplied as an internal standard (synthesized the same as **2**, except with the use of butyryl chloride). While the extracted ion chromatogram of the triketide targets is shown in Figure S1, the extracted ion chromatograms for **14** is shown in Figure S2.

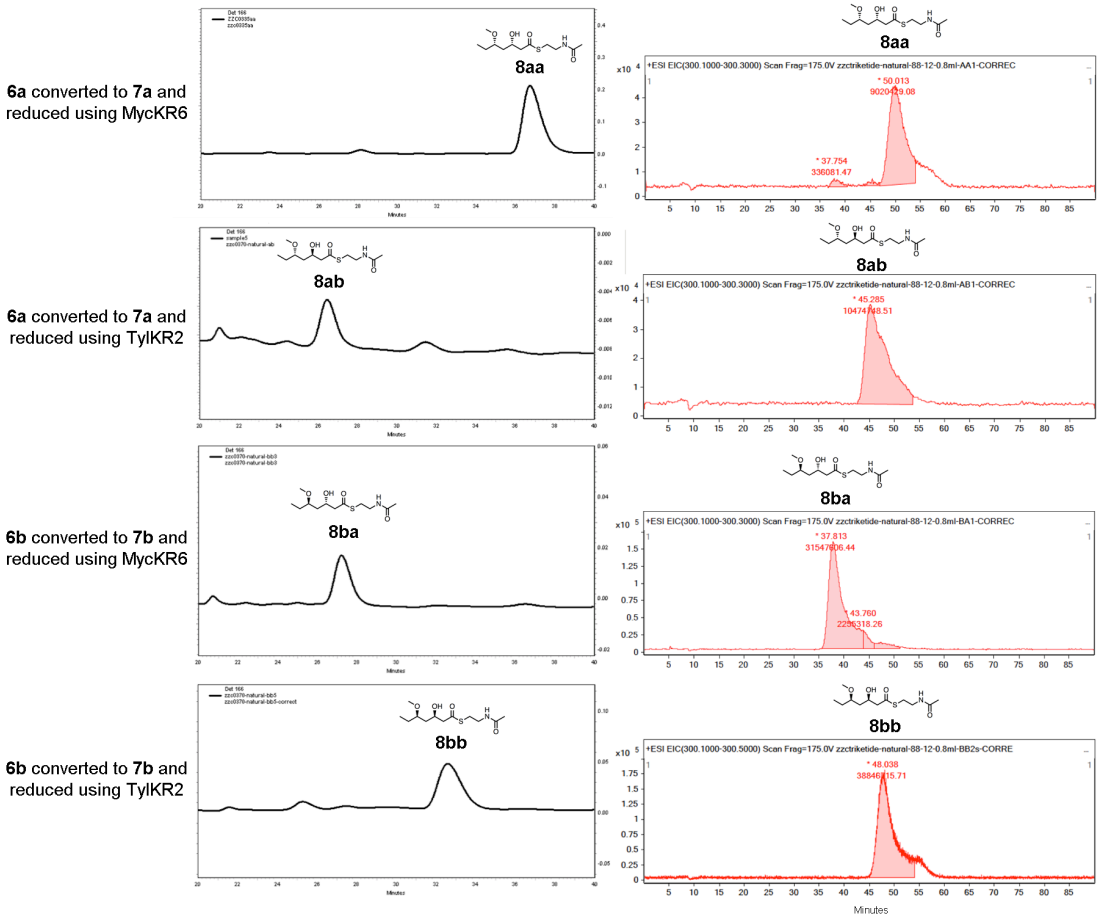
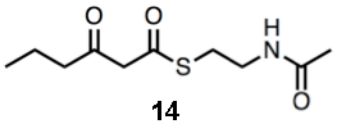


Figure S1. Normal phase HPLC/UV (OC-H column) and reverse phase HPLC/MS (IF3 column) analysis of chemoenzymatically-generated **8aa**, **8ab**, **8ba**, and **8bb**.



14

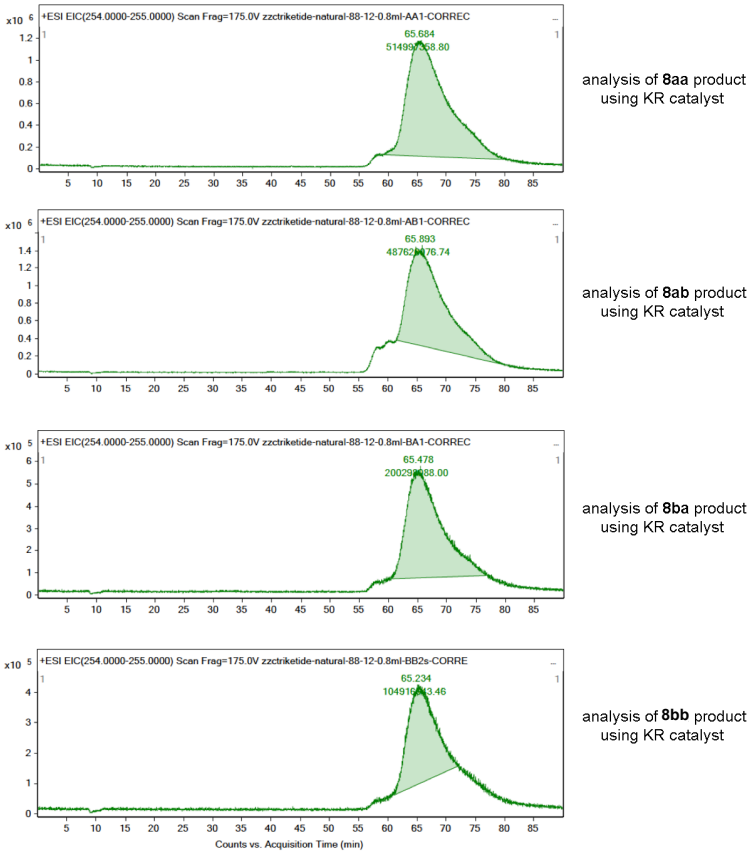
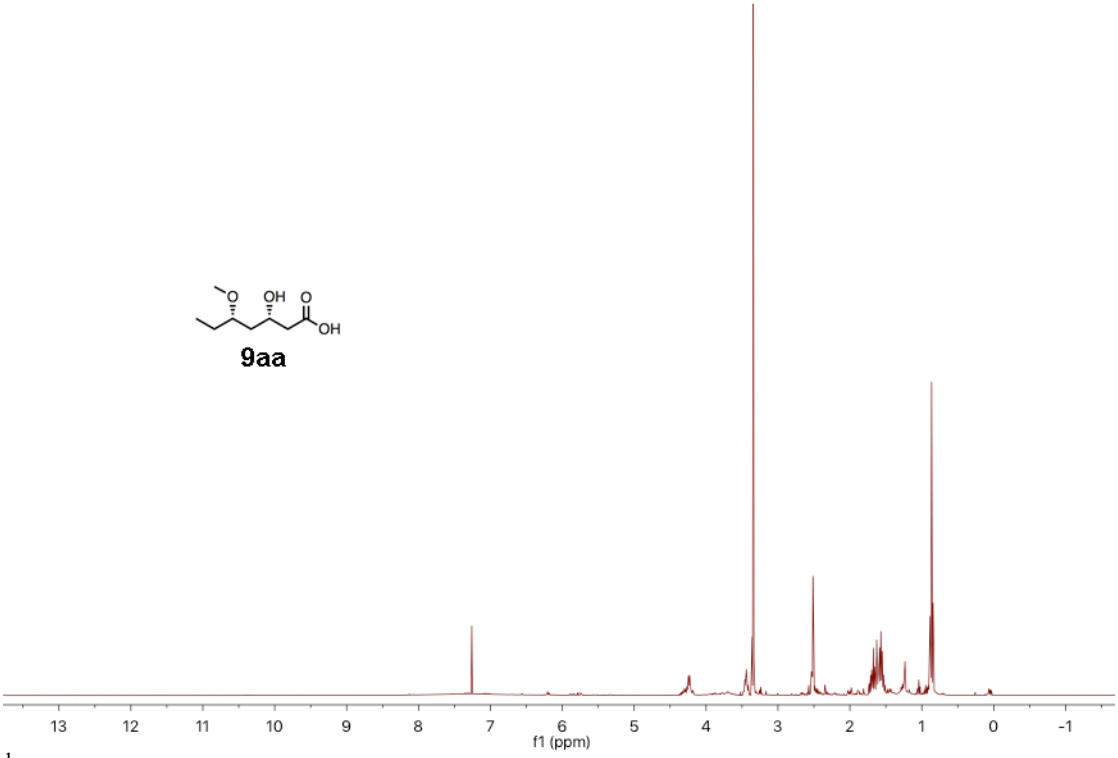


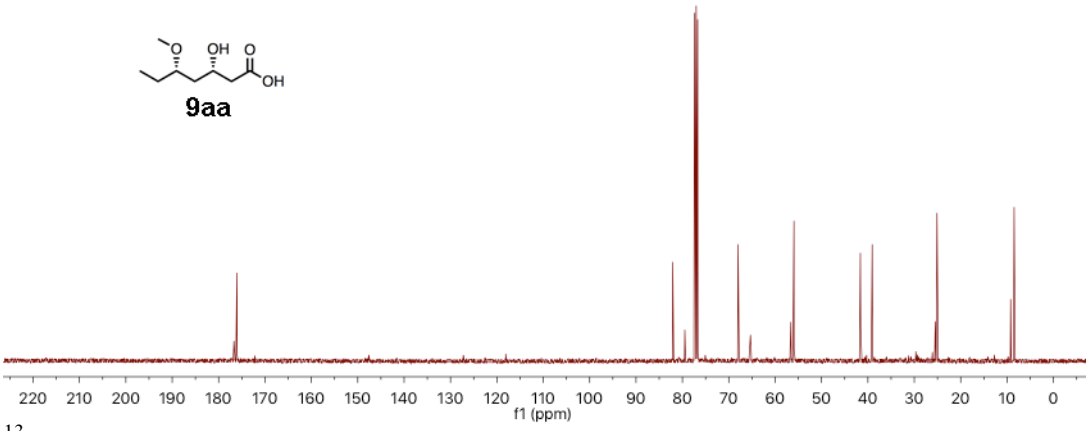
Figure S2. Extracted-ion chromatograms of internal standard **14** from each of the HPLC/MS runs in Figure S1.

Target	% of **8aa**	% of **8ab**	% of **8ba**	% of **8bb**
8aa	91%	3%	4%	2%
8ab	3%	96%	0%	1%
8ba	3%	5%	90%	2%
8bb	1%	1%	2%	96%

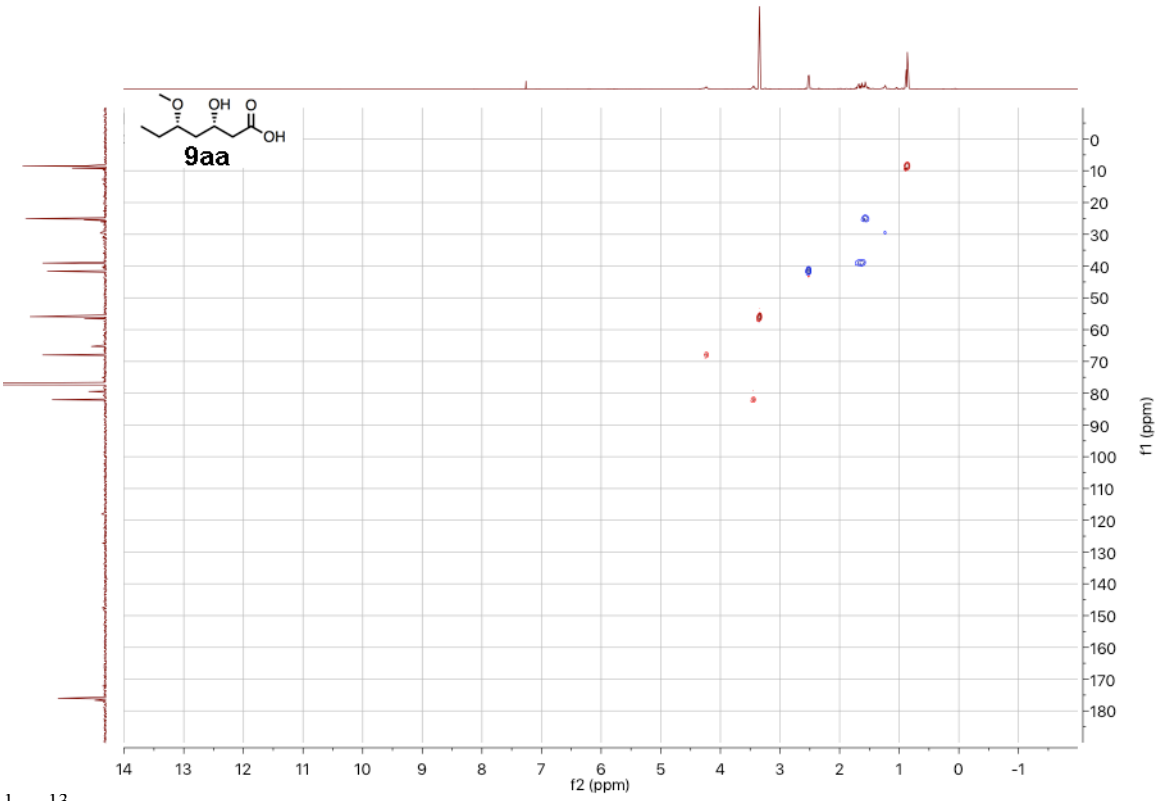
Table S1. Stereoisomeric compositions for targeted triketides. Percentages were obtained by comparing the peak integrations from the chromatograms in Figure S1.



^{1}H NMR (400 MHz, chloroform-*d*) δ 4.24 (m, 1H), 3.44 (m, 1H), 3.34 (s, 3H), 2.52 (m, 2H), 1.61 (m, 4H), 0.86 (t, *J* = 7.6 Hz, 3H).

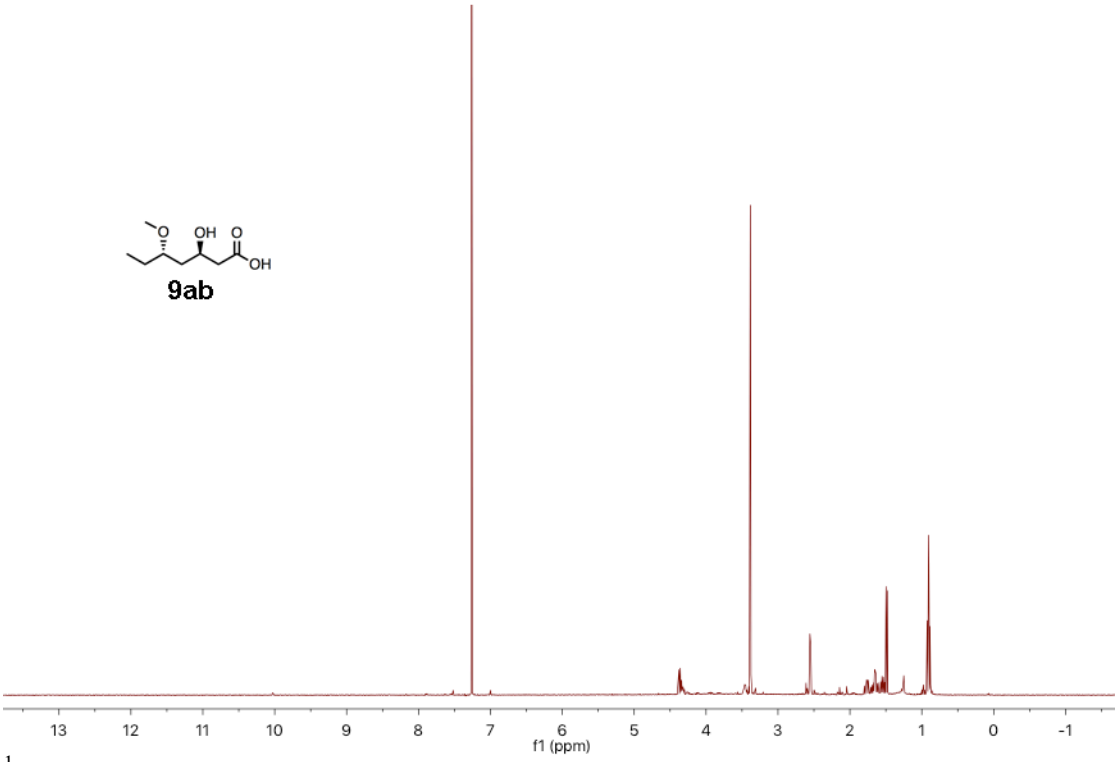


^{13}C NMR (101 MHz, chloroform-*d*) δ 176.01, 82.06, 67.96, 55.91, 41.56, 39.04, 25.07, 8.44.

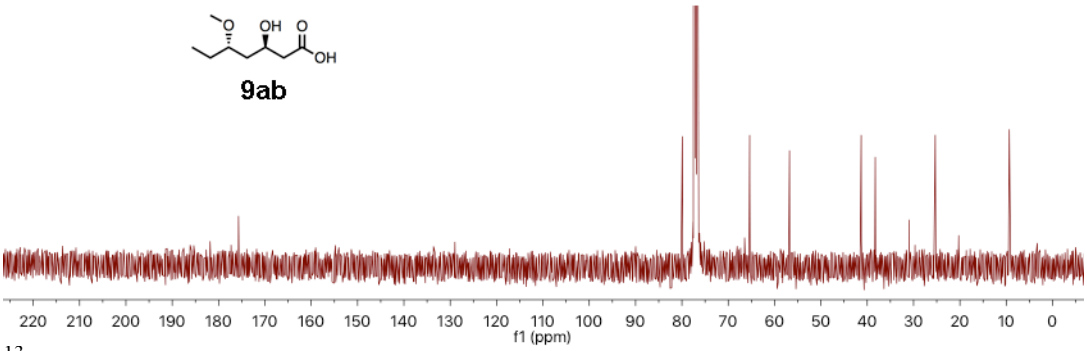


^{1}H-^{13}C HSQC

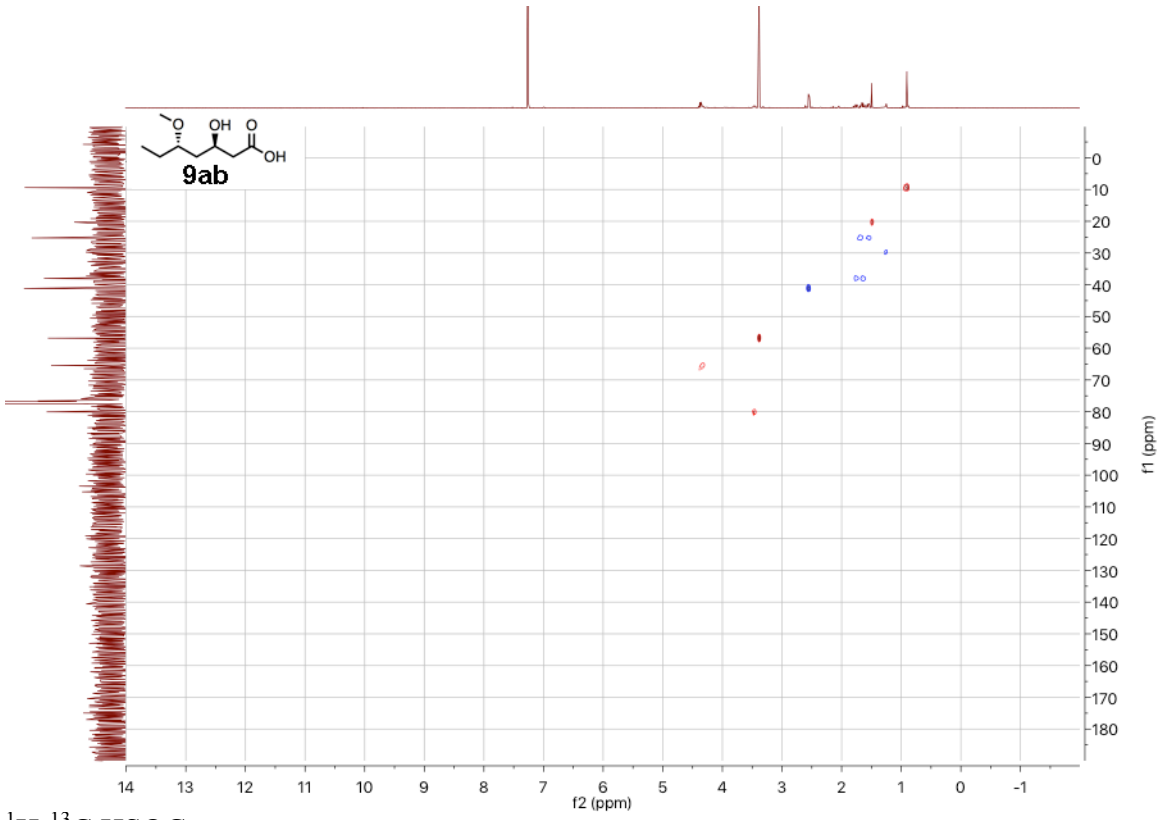
HRESIMS of **9aa** *m/z* 175.0977 $[M-H]^-$ (175.0970 calculated for $C_8H_{15}O_4$).



^{1}H NMR (400 MHz, chloroform-*d*) δ 4.32 (m, 1H), 3.45 (m, 1H), 3.39 (s, 3H), 2.55 (m, 2H), 1.65 (m, 4H), 0.91 (t, J = 7.5 Hz, 3H).

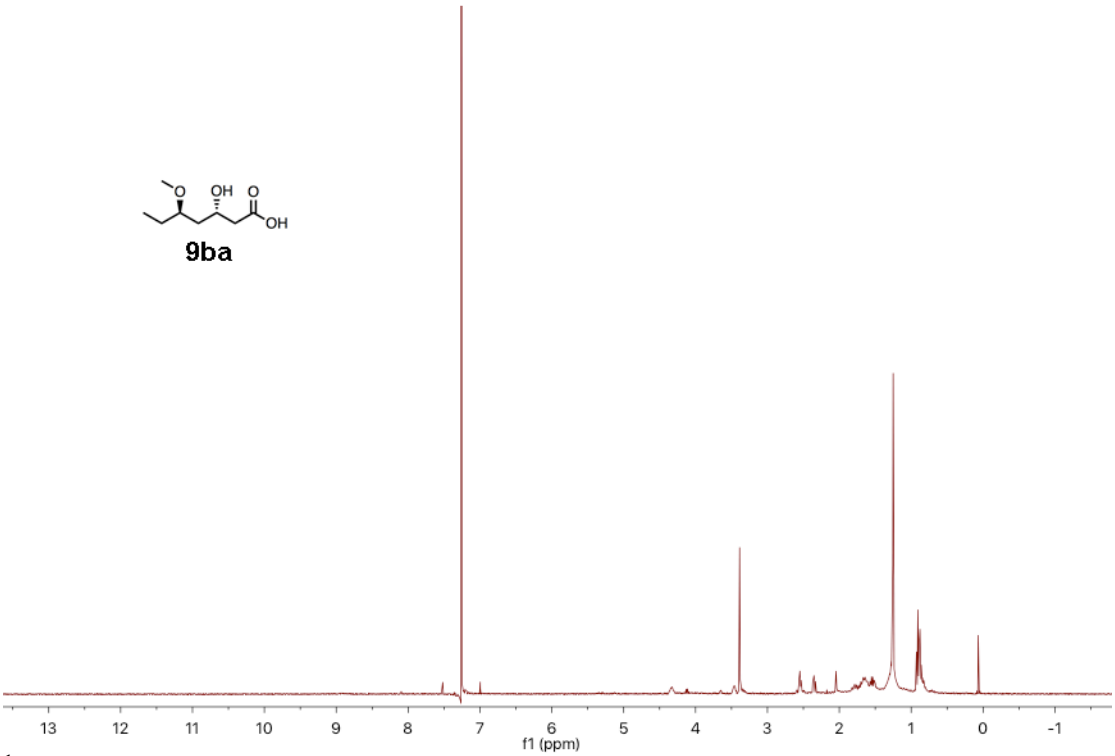


^{13}C NMR (101 MHz, chloroform-*d*) δ 175.71, 79.92, 65.37, 56.78, 41.31, 38.27, 25.33, 9.34.

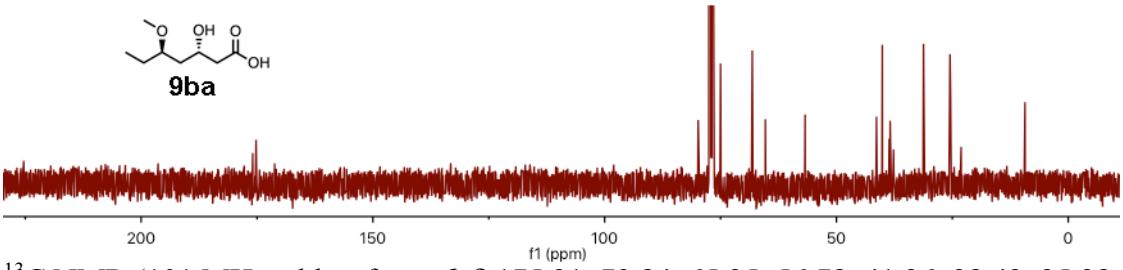


^{1}H-^{13}C HSQC

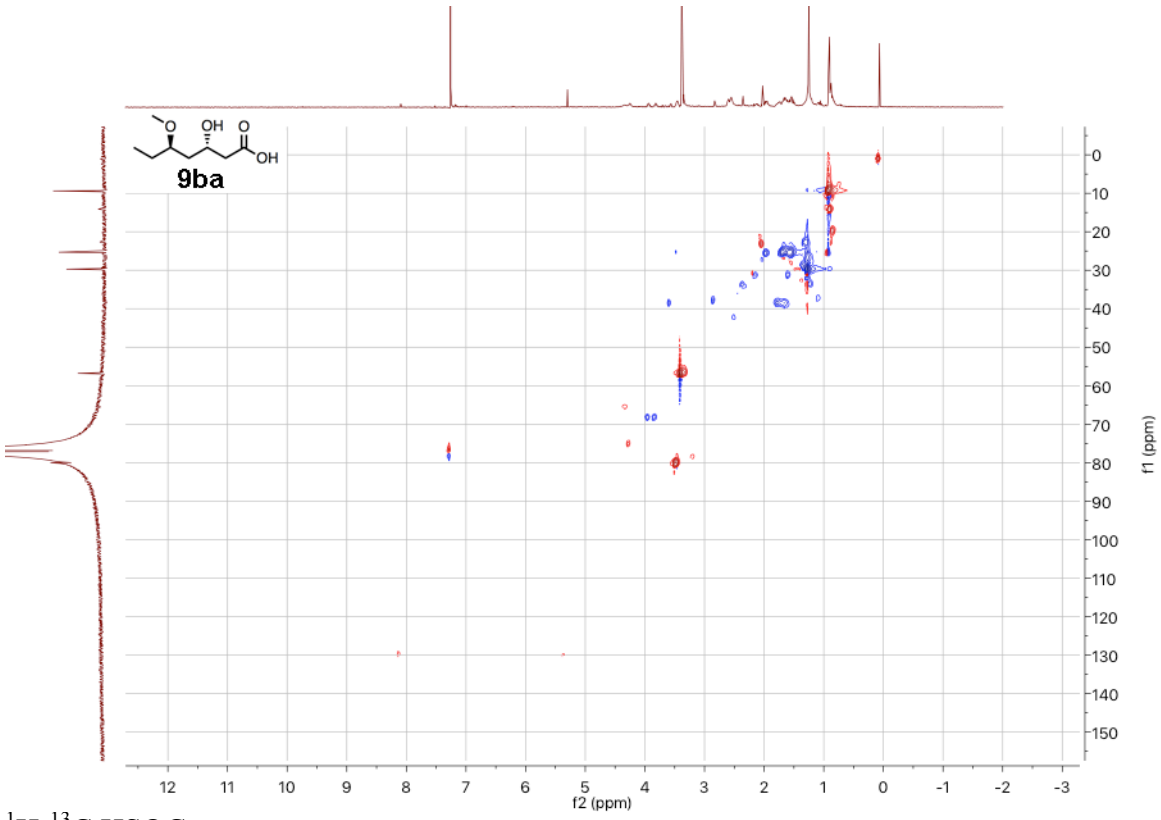
HRESIMS of **9ab** *m/z* 175.0980 $[M-H]^-$ (175.0970 calculated for $C_8H_{15}O_4$).



^{1}H NMR (400 MHz, chloroform-*d*) δ 4.33 (m, 1H), 3.47 (m, 1H), 3.39 (s, 3H), 2.54 (m, 2H), 1.65 (m, 4H), 0.91 (t, J = 7.6 Hz, 3H).

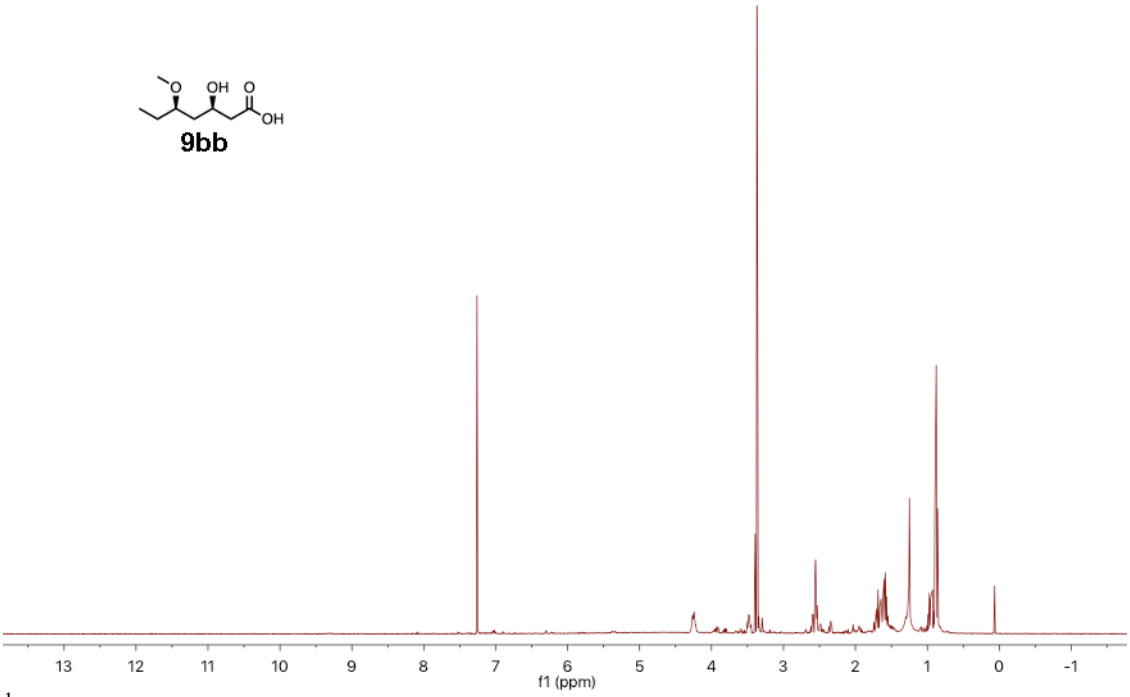


^{13}C NMR (101 MHz, chloroform-*d*) δ 175.21, 79.84, 65.35, 56.78, 41.36, 38.42, 25.38, 9.32.

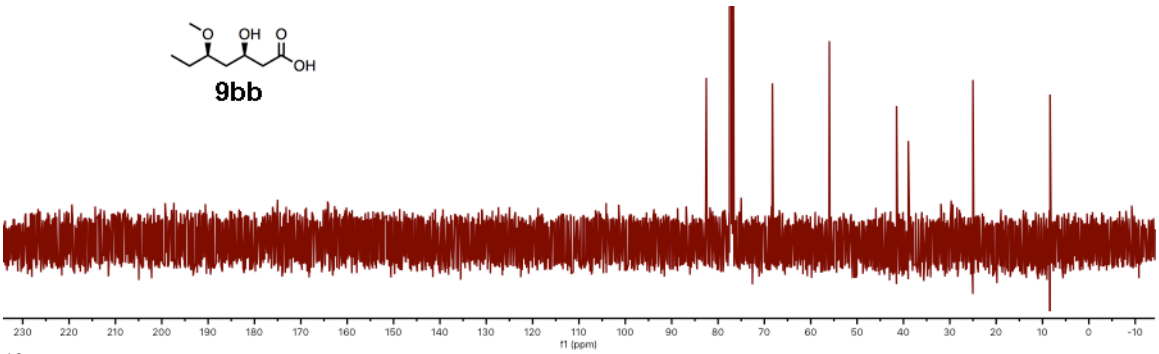


^{1}H-^{13}C HSQC

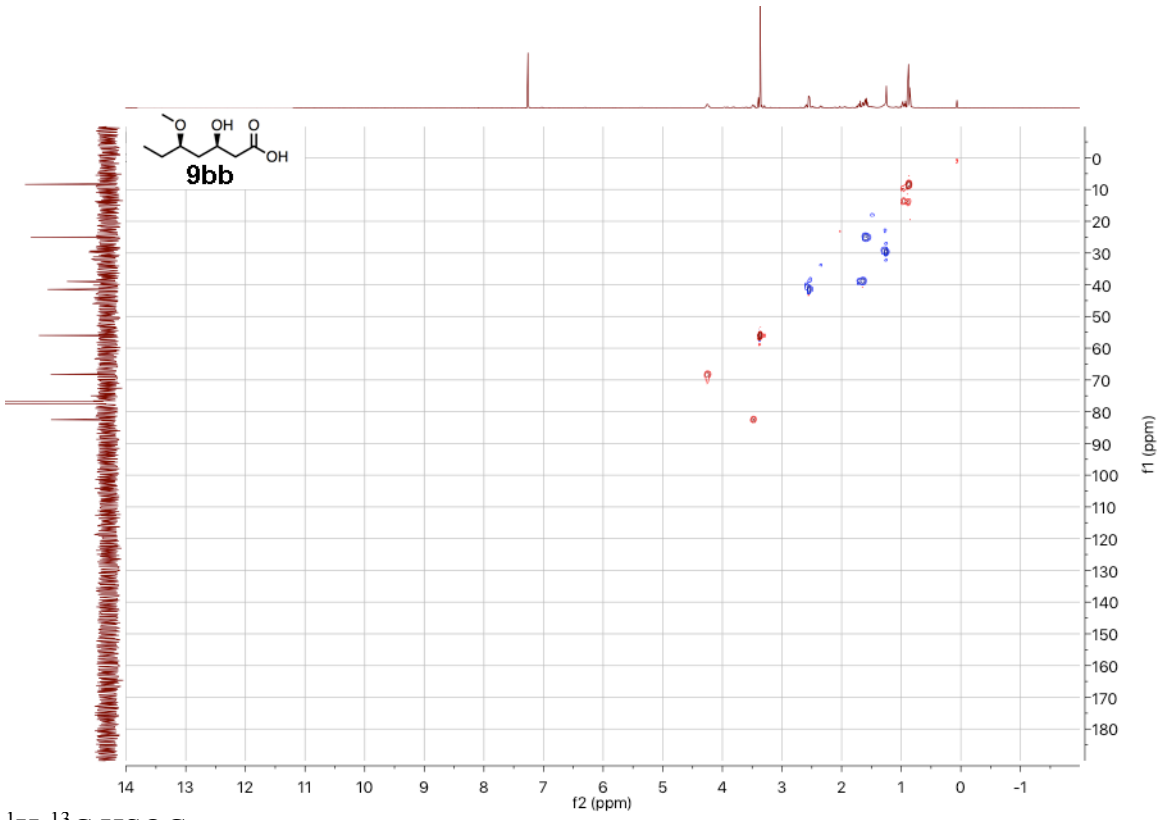
HRESIMS of **9ba** *m/z* 175.0974 $[M-H]^-$ (175.0970 calculated for $C_8H_{15}O_4$).



^{1}H NMR (400 MHz, chloroform-*d*) δ 4.24 (m, 1H), 3.48 (m, 1H), 3.38 (s, 3H), 2.55 (m, 2H), 1.62 (m, 4H), 0.88 (t, J = 7.5 Hz, 3H).



^{13}C NMR (101 MHz, chloroform-*d*) δ 175.01, 82.52, 68.25, 55.97, 41.48, 38.95, 25.02, 8.38.

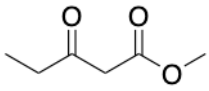


^{1}H-^{13}C HSQC

HRESIMS of **9bb** *m/z* 175.0970 $[M-H]^-$ (175.0970 calculated for $C_8H_{15}O_4$).

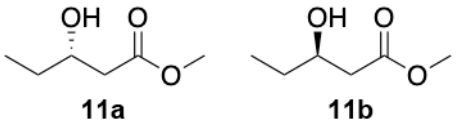
Synthesis of chemical standards through asymmetric hydrogenation

Synthesis of **11a** and **11b**

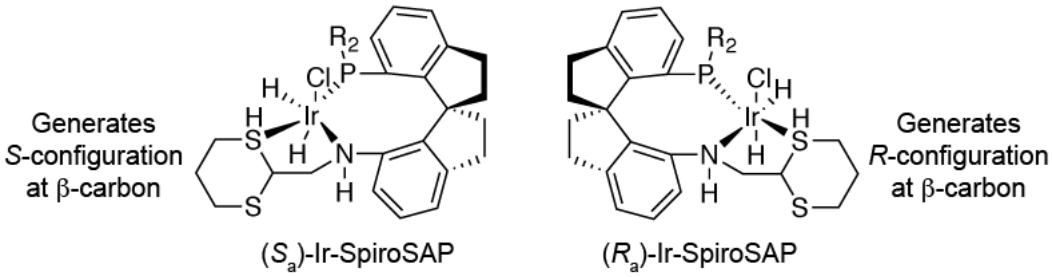


10

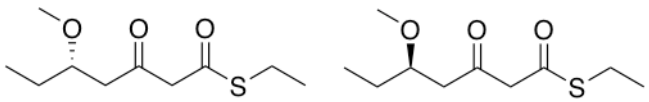
Commercially available **10** was quantitatively converted to **11a** or **11b** using (S_a)-Ir-SpiroSAP or (R_a)-Ir-SpiroSAP, respectively, in a Parr® Series 4760 Pressure Vessel following Zhou and coworkers[4-5].



11a **11b**



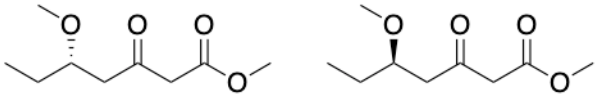
Route to **6a** and **6b**



6a **6b**

Hydrolysis of **11a** and **11b** to **4a** and **4b** was performed using the same procedure as described in the chemoenzymatic route for converting **3a** and **3b** to **4a** and **4b**. Methylation and extension of **4a** and **4b** to obtain **6a** and **6b** was also performed as described in the chemoenzymatic route.

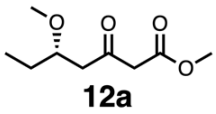
Synthesis of **12a** and **12b**



12a **12b**

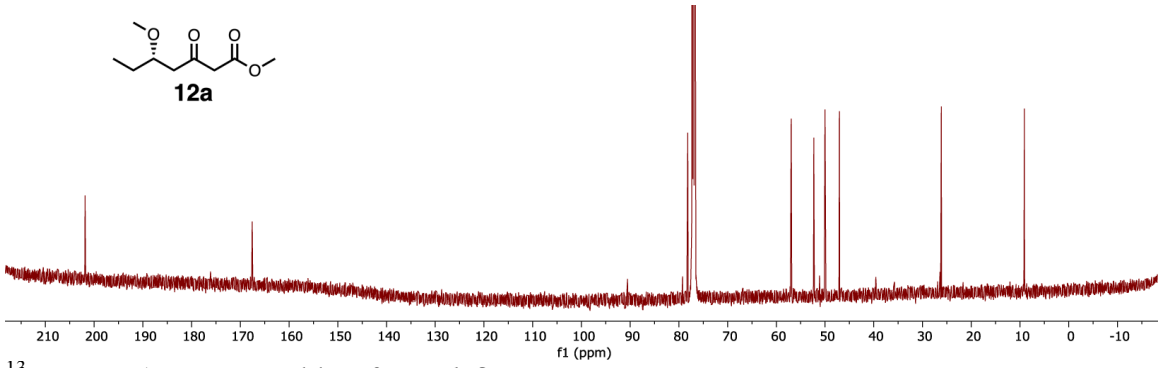
Synthesis of **12a**

To 800 mg **6a** (366 mmol) dissolved in 40 mL anhydrous methanol 2.0 g sodium methoxide (3.70 mol) was slowly added, turning the solution yellowish. The reaction was monitored for 6-9 h by TLC. When the reaction was complete, methanol was removed and aqueous hydrochloric acid was added to make a neutral solution. The EtOAc extraction (3 x 50 mL) was dried over Na_2SO_4. Evaporation of the organic solvent and purification of the residue by column chromatography gave **12a** (186 mg, 27%) as a yellowish liquid. Column: 3x13 cm. Eluent, 50:1 hexanes:EtOAc, 40:1 hexanes:EtOAc, 30:1 hexanes:EtOAc, 10:1 hexanes:EtOAc, and then 1:1 hexanes:EtOAc until all of the product eluted.

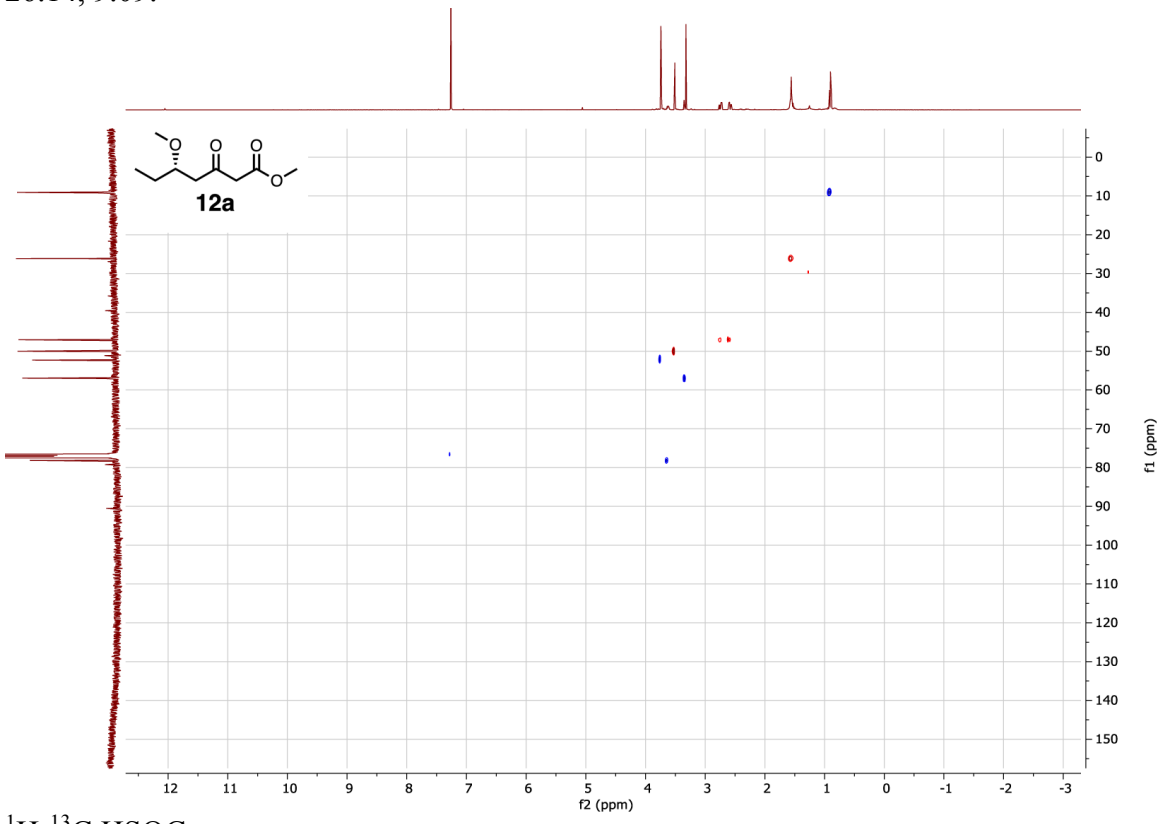


12a

^{1}H NMR (500 MHz, chloroform-*d*) δ 3.74 (s, 3H), 3.62 (m, 1H), 3.51 (s, 2H), 3.32 (s, 3H), 2.74 (dd, *J* = 14.1, 7.8 Hz, 1H), 2.58 (dd, *J* = 15.9, 4.5 Hz, 1H), 1.56 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H).



^{13}C NMR (126 MHz, chloroform-*d*) δ 201.86, 167.58, 78.18, 56.95, 52.31, 50.02, 47.06, 26.14, 9.09.

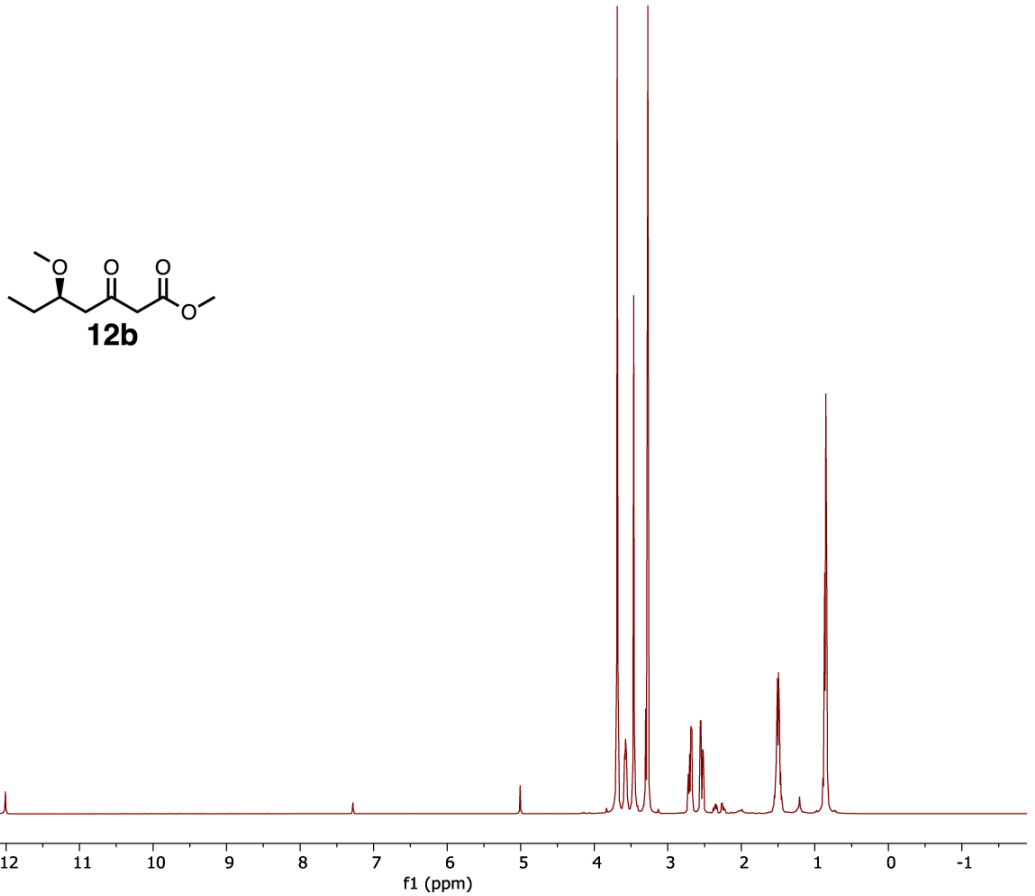


^{1}H-^{13}C HSQC

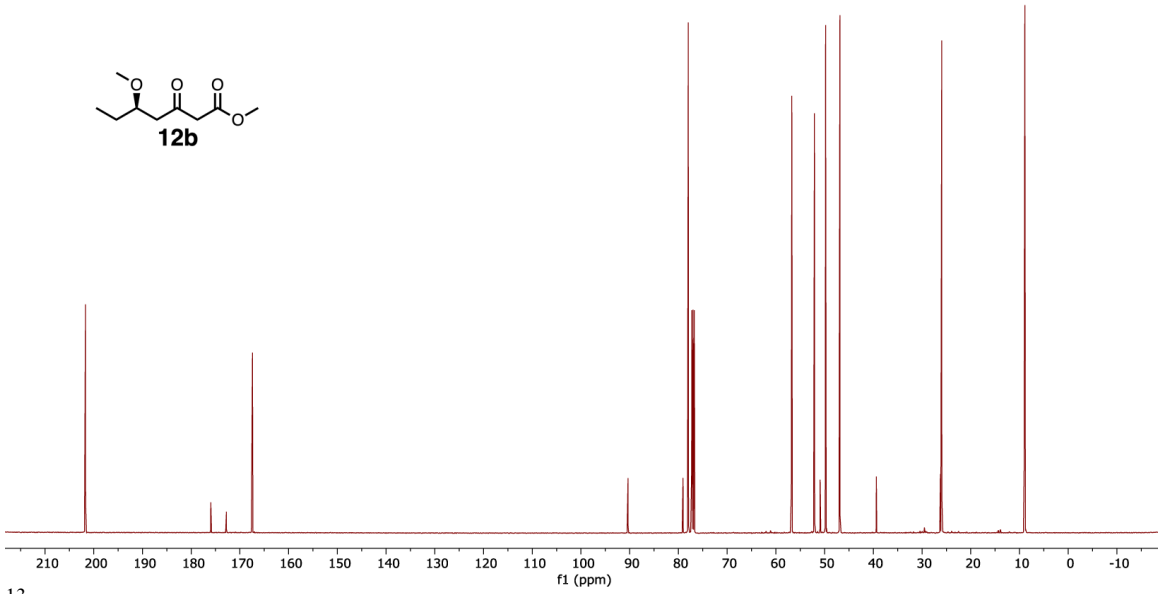
HRESIMS of **12a** *m/z* 211.09359 $[M+Na]^+$ (211.0941 calculated for $C_9H_{16}O_4Na$).

Synthesis of **12b**

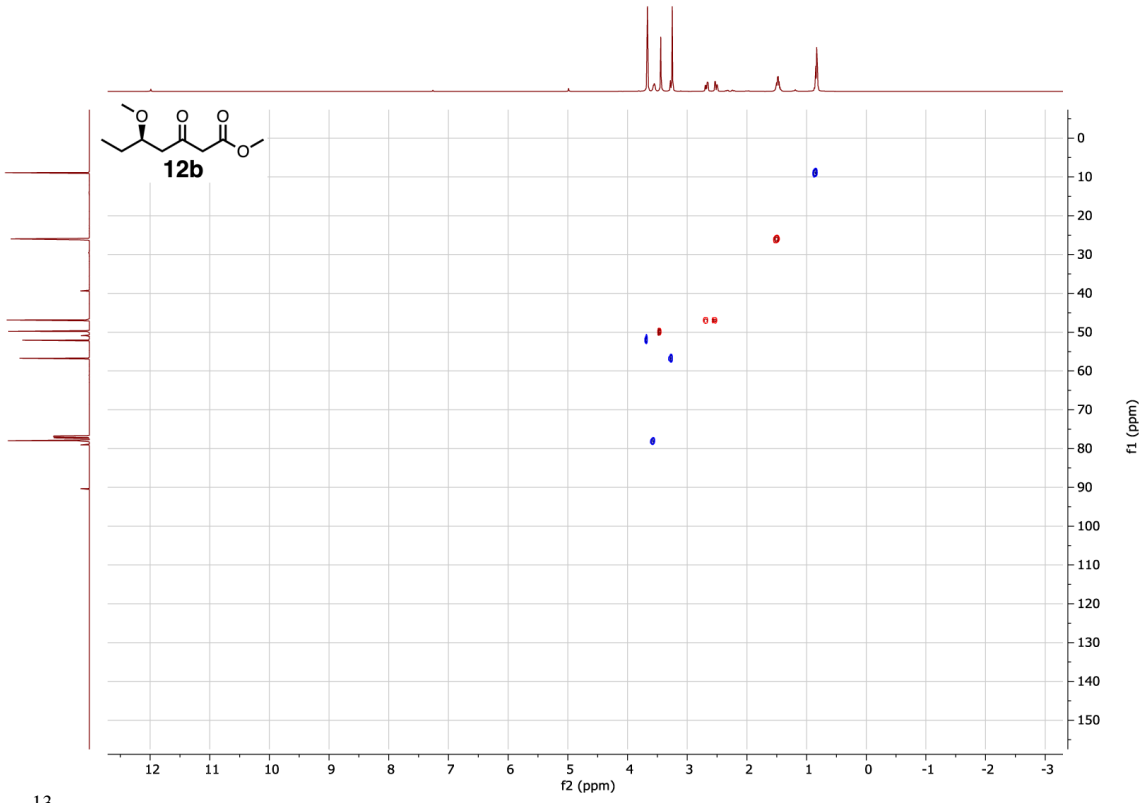
To 292 mg **6b** (134 mmol) dissolved in 20 mL anhydrous methanol 756 mg sodium methoxide (1.40 mol) was slowly added, turning the solution yellowish. The reaction was monitored for 6-9 h by TLC. When the reaction was complete, methanol was removed and aqueous hydrochloric acid was added to make a neutral solution. The EtOAc extraction (3 x 50 mL) was dried over Na_2SO_4. Evaporation of the organic solvent and purification of the residue by column chromatography gave **12b** (41 mg, 16%) as a yellowish liquid. Column: 1x10 cm. Eluent, 50:1 hexanes:EtOAc, 40:1 hexanes:EtOAc, 30:1 hexanes:EtOAc, and then 1:1 hexanes:EtOAc until all of the product eluted.



^{1}H NMR (500 MHz, chloroform-*d*) δ 3.67 (s, 3H), 3.55 (m, 1H), 3.44 (s, 2H), 3.25 (s, 3H), 2.67 (dd, *J* = 15.0, 7.8 Hz, 1H), 2.52 (dd, *J* = 16.1, 4.3 Hz, 2H), 1.50 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H).



^{13}C NMR (126 MHz, chloroform-*d*) δ 201.69, 167.41, 77.97, 56.72, 52.08, 49.79, 46.86, 25.96, 8.90.



^{1}H-^{13}C HSQC

HRESIMS of **12b** *m/z* 211.0937 $[M+Na]^+$ (211.0941 calculated for $C_9H_{16}O_4Na$).

Synthesis of **9aa**, **9ab**, **9ba**, and **9bb** standards

Triketides **12a** and **12b** were asymmetrically reduced using (S_a)-Ir-SpiroSAP or (R_a)-Ir-SpiroSAP in a Parr® Series 4760 Pressure Vessel following Zhou and coworkers[4-5].

Synthesis of **9aa**

In 2 mL anhydrous MeOH, 95 mg **12a**, 6 mg (S_a)-Ir-SpiroSAP, and 6 mg t-BuOK were dissolved. The reaction was quickly relocated in the hydrogenation vessel and pressurized to 100 atm. After 2 d (monitoring the reaction by NMR), the reaction was depressurized, and the solvent was removed. Then 5 mL 5M NaOH was added to the residue, and the solution was kept at 60 ^{o}C overnight. Next, the aqueous solution was washed (3 x 10 mL EtOAc), adjusted to pH = 0, and extracted (3 x 50 mL EtOAc). The organic layers were dried with Na_2SO_4 and filtered through a $CuSO_4$-impregnated silica gel column. Finally, the filtrate was co-evaporated with toluene to give **9aa** as a sticky, yellow liquid. (70 mg, 77% yield)

Synthesis of **9ab**

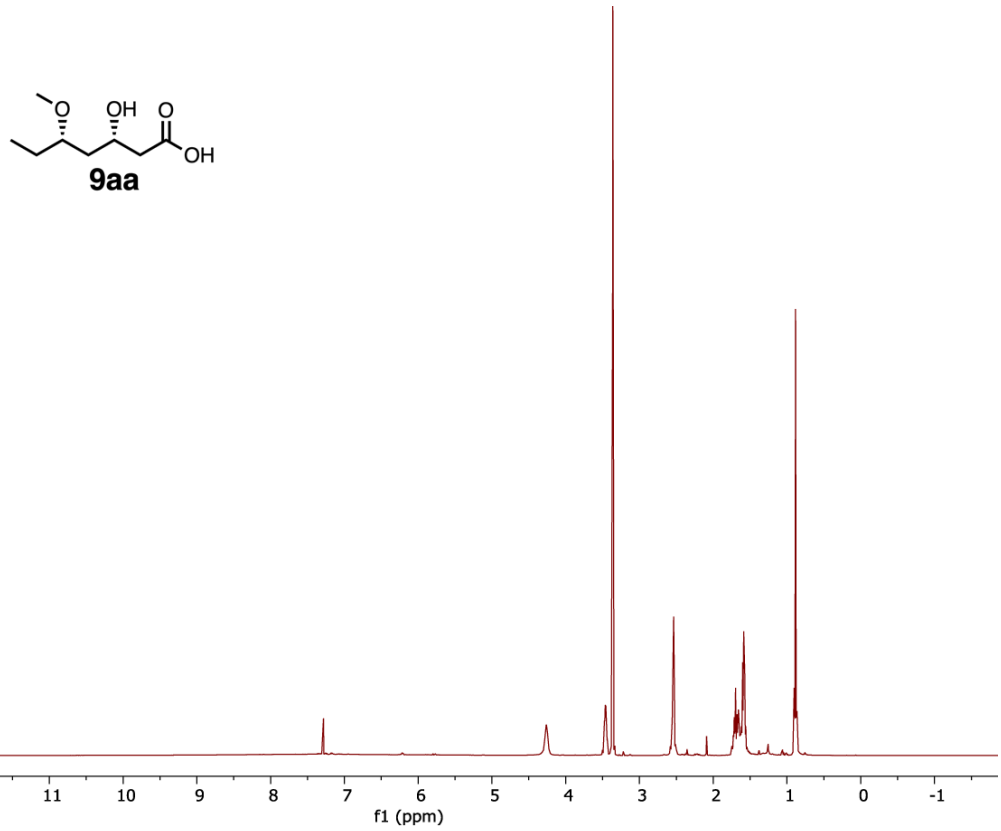
In 2 mL anhydrous MeOH, 91 mg **12a**, 6 mg (R_a)-Ir-SpiroSAP, and 6 mg t-BuOK were dissolved. The reaction was quickly relocated in the hydrogenation vessel and pressurized to 100 atm. After 2 d (monitoring the reaction by NMR), the reaction was depressurized, and the solvent was removed. Then 5 mL 5M NaOH was added to the residue, and the solution was kept at 60 ^{o}C overnight. Next, the aqueous solution was washed (3 x 10 mL EtOAc), adjusted to pH = 0, and extracted (3 x 50 mL EtOAc). The organic layers were dried with Na_2SO_4 and filtered through a $CuSO_4$-impregnated silica gel column. Finally, the filtrate was co-evaporated with toluene to give **9ab** as a sticky, yellow liquid. (83 mg, 97% yield)

Synthesis of **9ba**

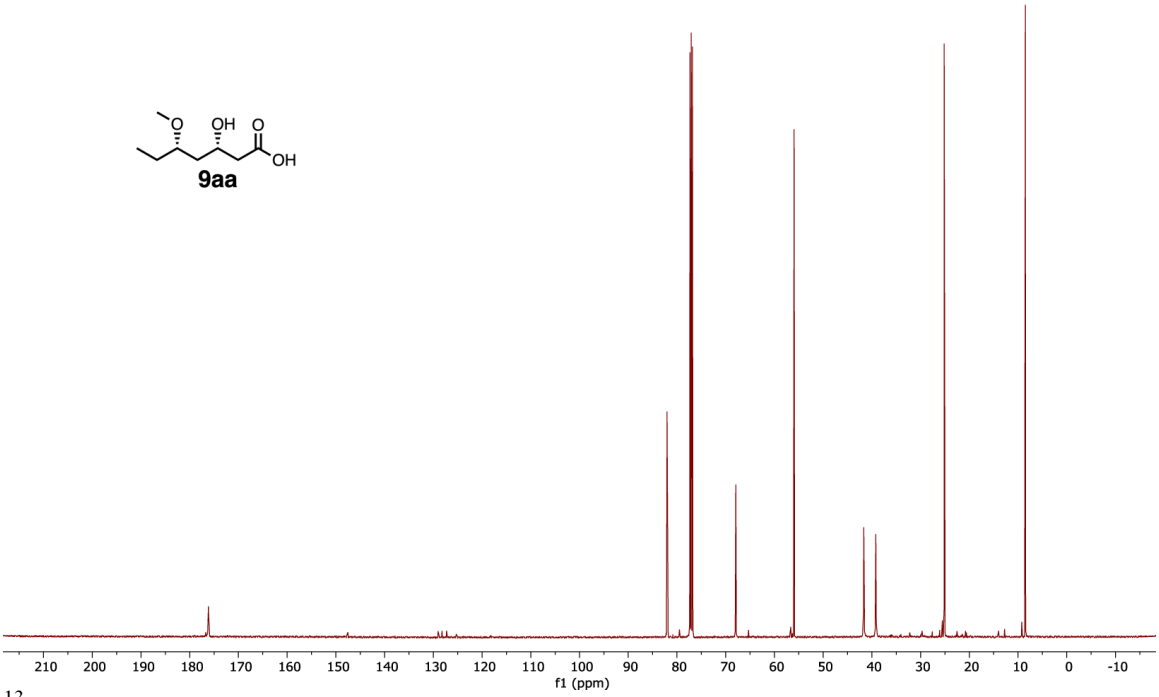
In 1 mL anhydrous MeOH, 41 mg **12b**, 2 mg (S_a)-Ir-SpiroSAP, and 2 mg t-BuOK were dissolved. The reaction was quickly relocated in the hydrogenation vessel and pressurized to 100 atm. After 2 d (monitoring the reaction by NMR), the reaction was depressurized, and the solvent was removed. Then 5 mL 5M NaOH was added to the residue, and the solution was kept at 60 ^{o}C overnight. Next, the aqueous solution was washed (3 x 10 mL EtOAc), adjusted to pH = 0, and extracted (3 x 50 mL EtOAc). The organic layers were dried with Na_2SO_4 and filtered through a $CuSO_4$-impregnated silica gel column. Finally, the filtrate was co-evaporated with toluene to give **9ba** as a sticky, yellow liquid. (32 mg, 83% yield)

Synthesis of **9bb**

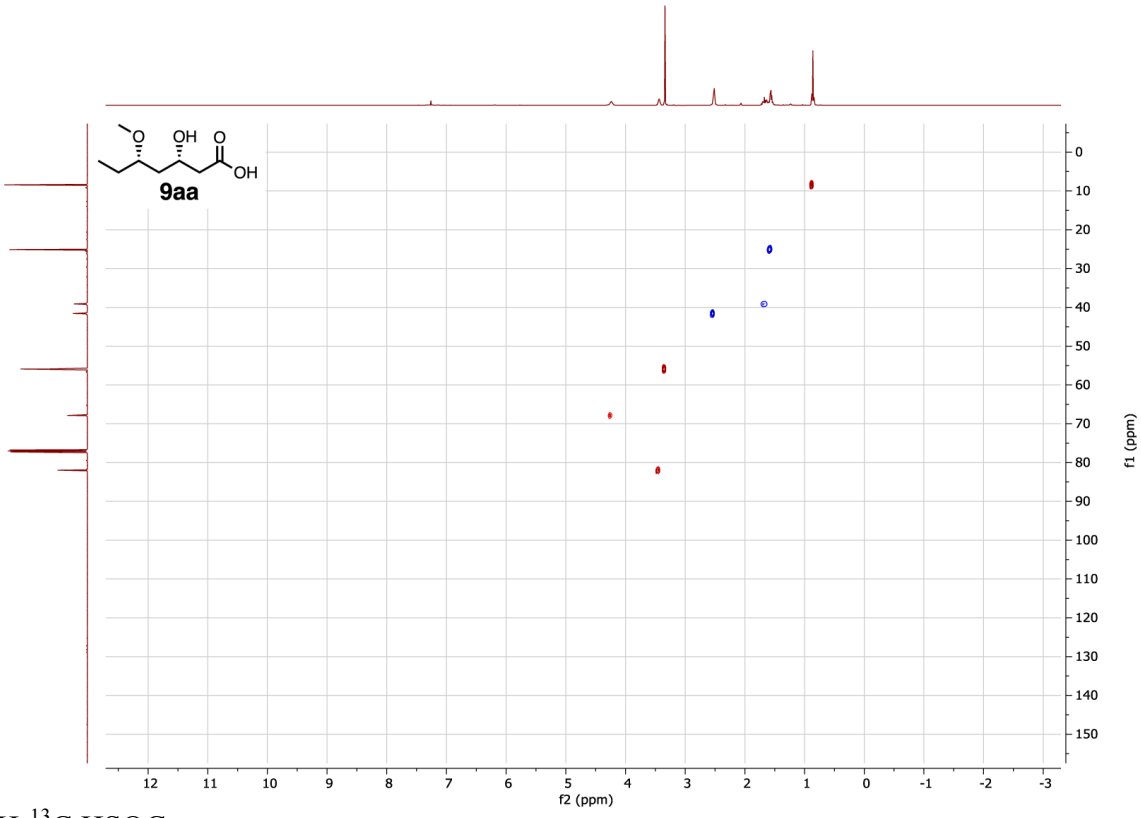
In 2 mL anhydrous MeOH, 57 mg **12b**, 3 mg (R_a)-Ir-SpiroSAP, and 3 mg t-BuOK were dissolved. The reaction was quickly relocated in the hydrogenation vessel and pressurized to 100 atm. After 2 d (monitoring the reaction by NMR), the reaction was depressurized, and the solvent was removed. Then 5 mL 5M NaOH was added to the residue, and the solution was kept at 60 ^{o}C overnight. Next, the aqueous solution was washed (3 x 10 mL EtOAc), adjusted to pH = 0, and extracted (3 x 50 mL EtOAc). The organic layers were dried with Na_2SO_4 and filtered through a $CuSO_4$-impregnated silica gel column. Finally, the filtrate was co-evaporated with toluene to give **9bb** as a sticky, yellow liquid. (46 mg, 86% yield)



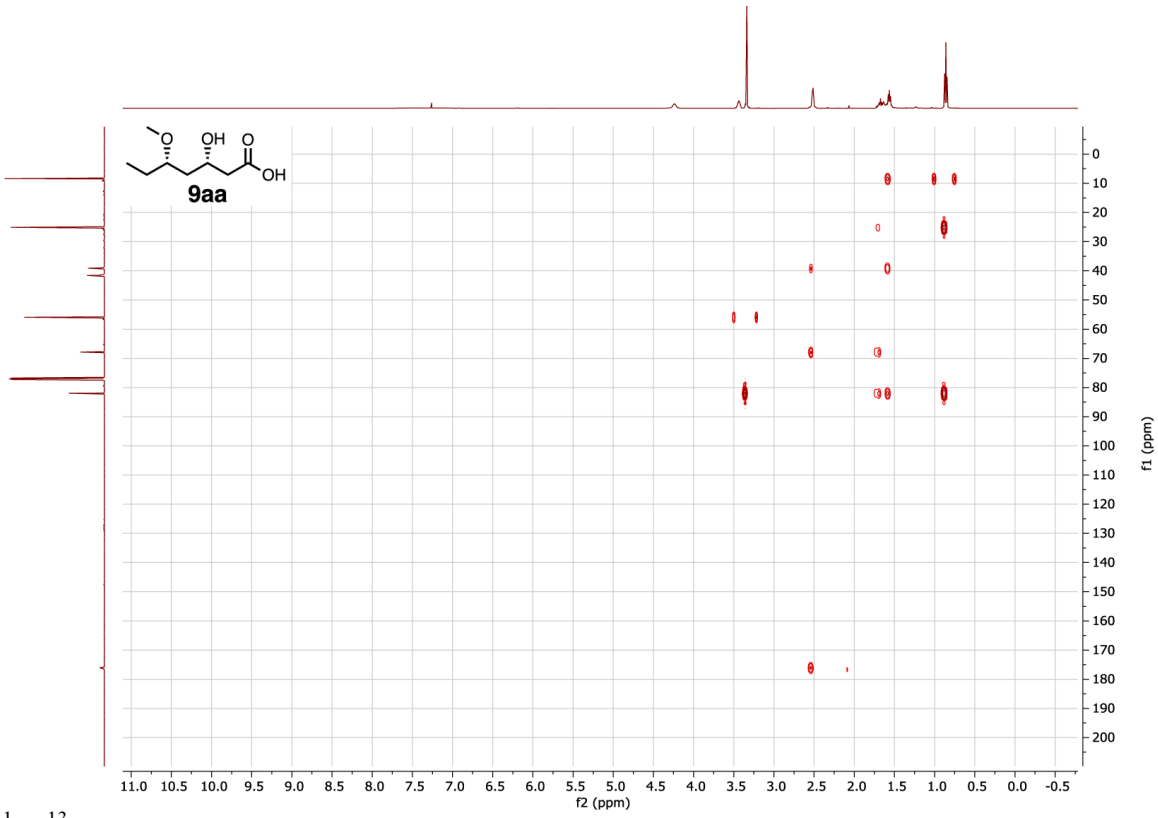
^{1}H NMR (500 MHz, chloroform-*d*) δ 4.24 (s, 1H), 3.44 (m, 1H), 3.34 (s, 3H), 2.52 (m, 2H), 1.61 (m, 4H), 0.86 (t, J = 7.5 Hz, 3H).



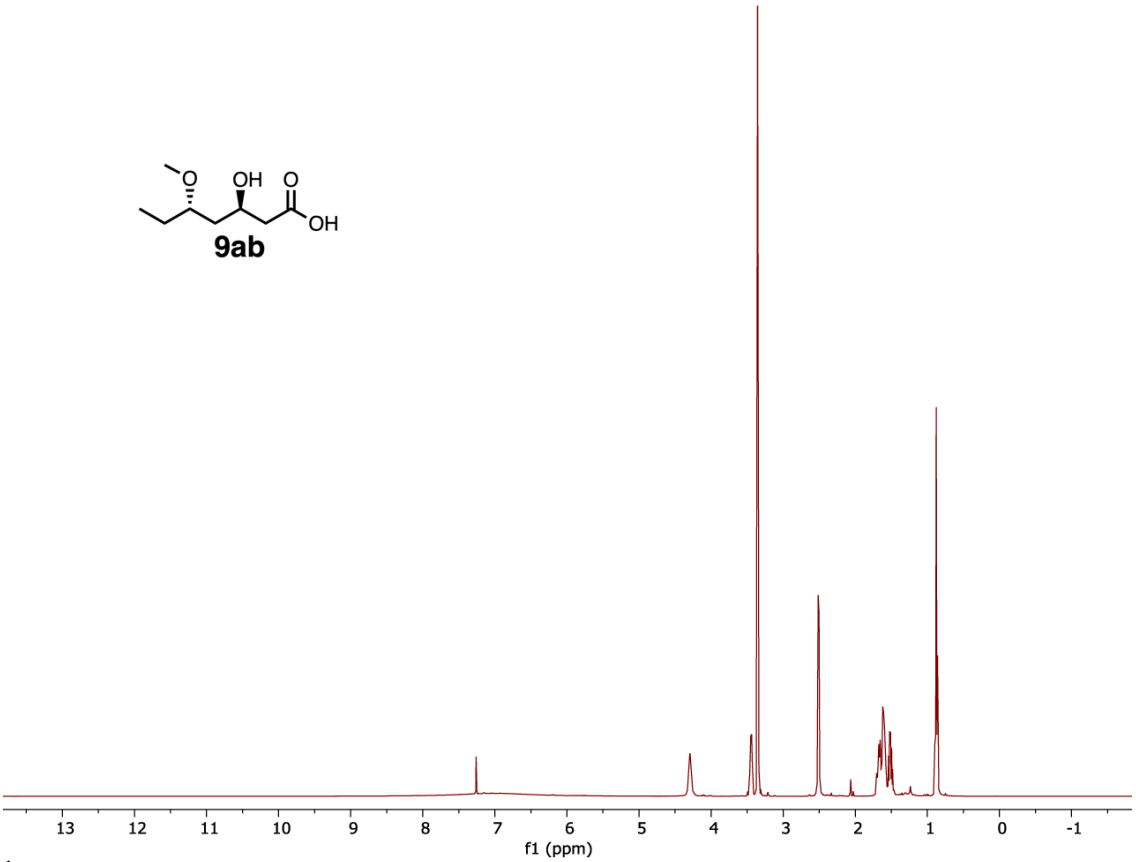
^{13}C NMR (126 MHz, chloroform-*d*) δ 176.64, 81.98, 67.85, 55.90, 41.59, 39.12, 25.09, 8.44.



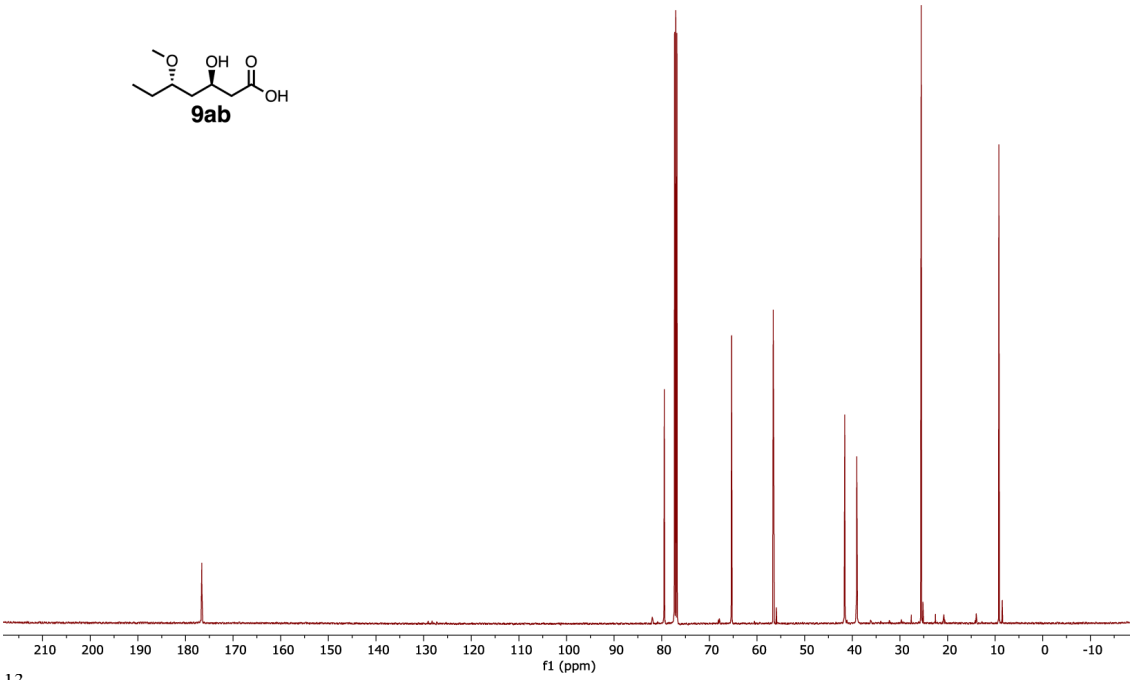
^{1}H-^{13}C HSQC



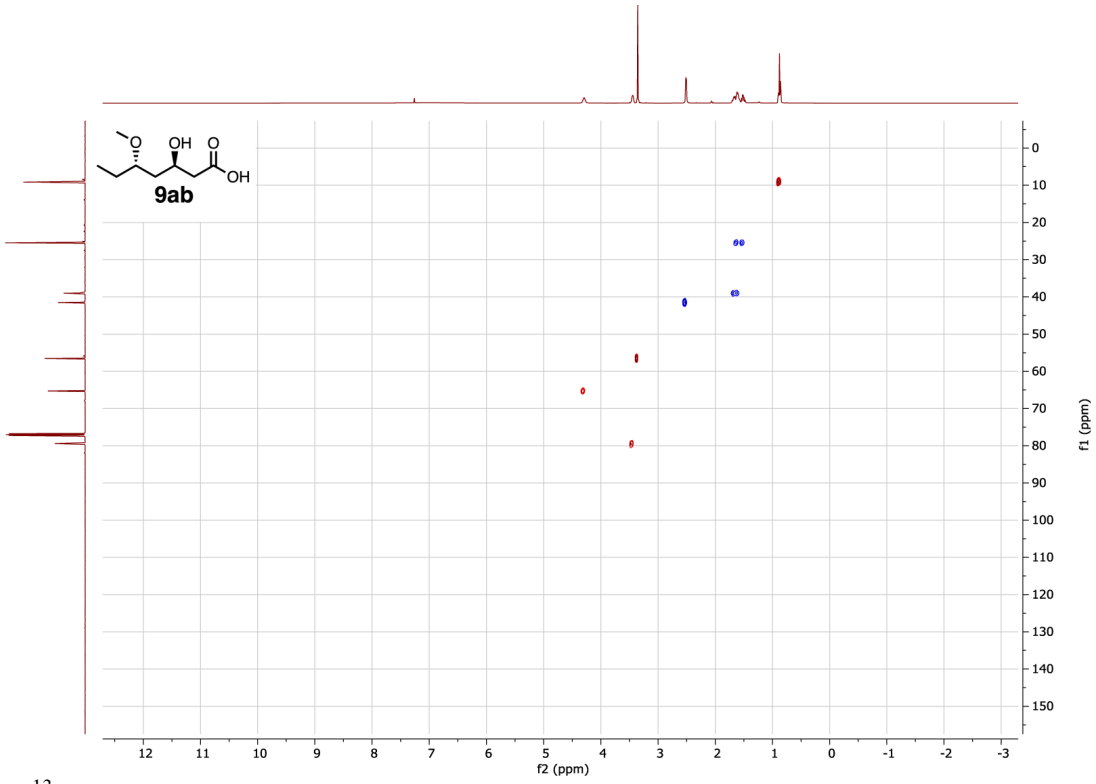
^{1}H-^{13}C HMBC



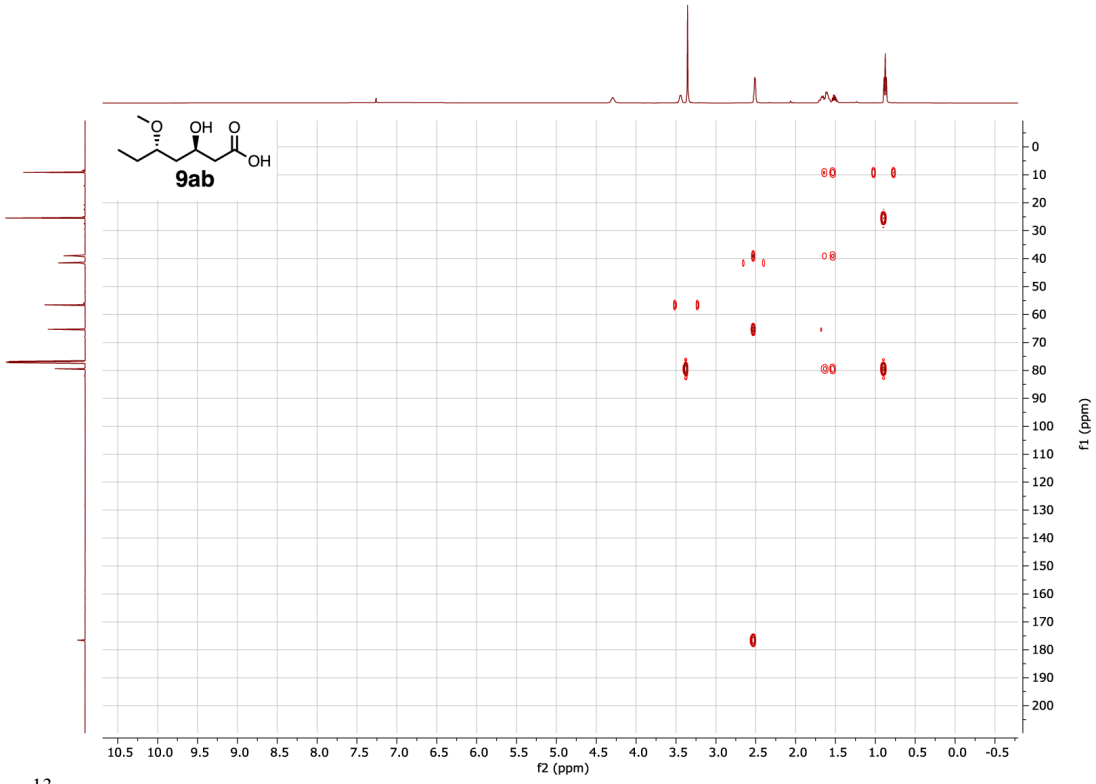
^{1}H NMR (500 MHz, chloroform-*d*) δ 4.30 (m, 1H), 3.44 (m, 1H), 3.36 (s, 3H), 2.51 (m, 2H), 1.65 (m, 4H), 0.88 (t, *J* = 7.5 Hz, 3H).



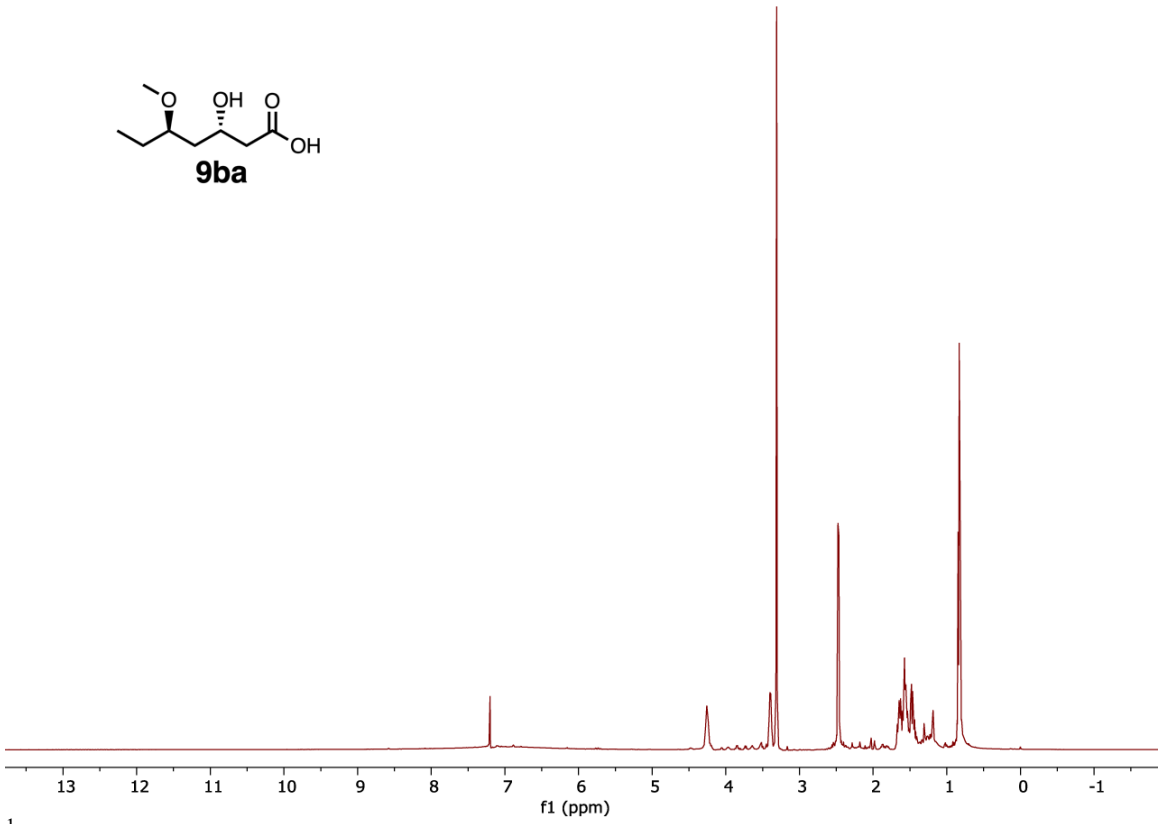
^{13}C NMR (126 MHz, chloroform-*d*) δ 176.53, 79.43, 65.29, 56.55, 41.52, 39.00, 25.47, 9.16.



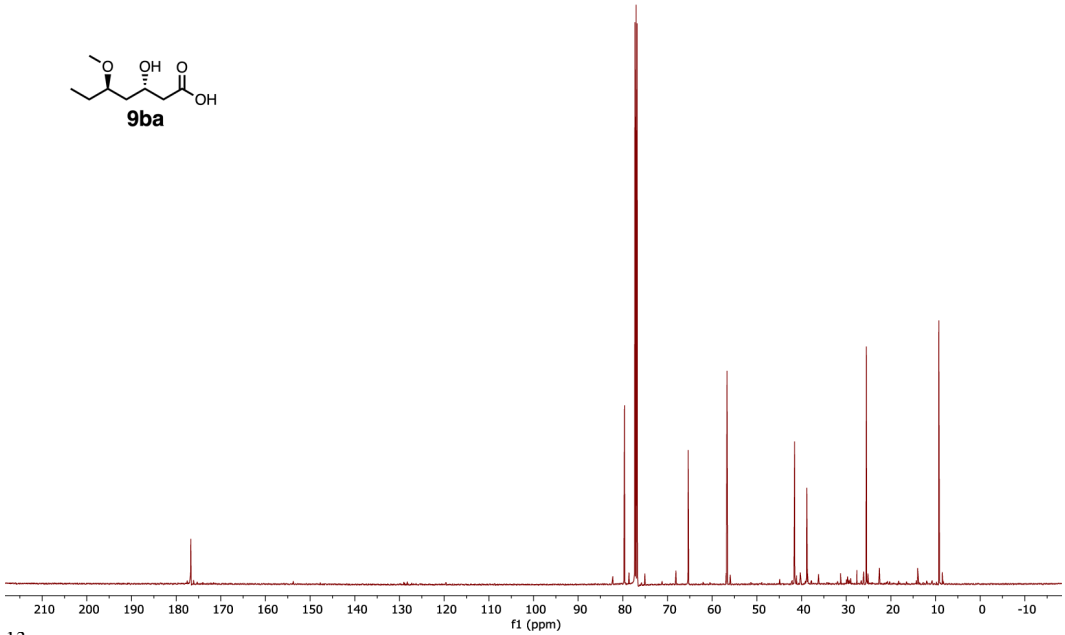
^{1}H-^{13}C HSQC



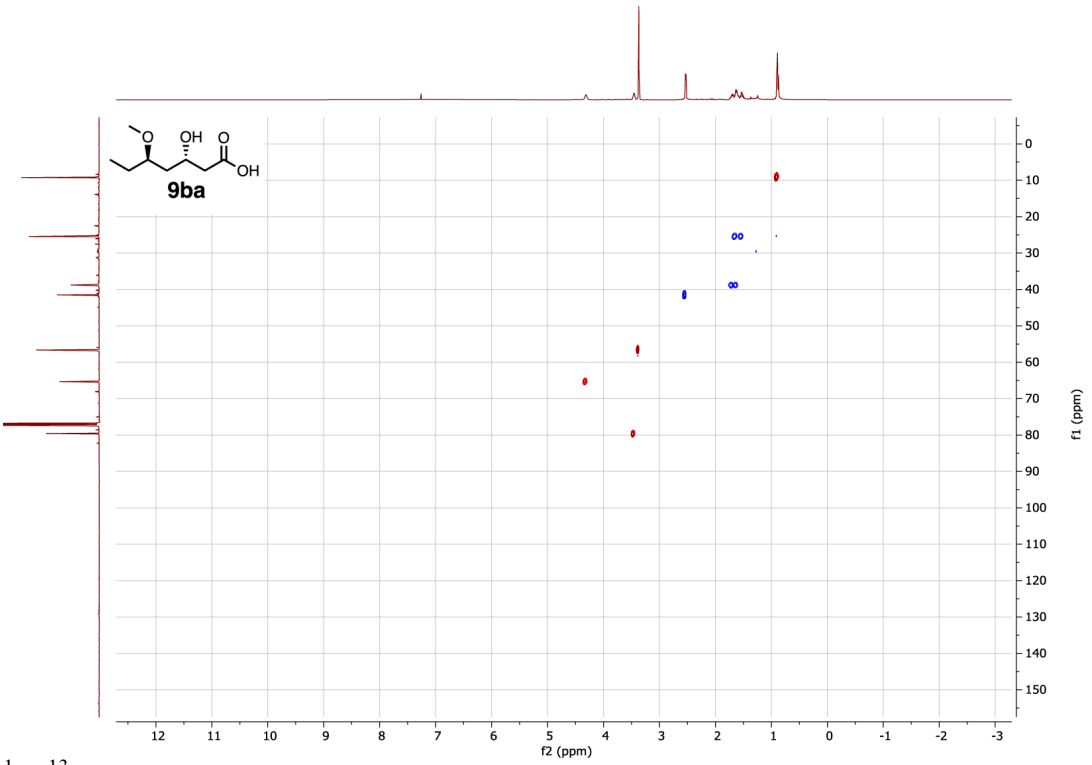
^{1}H-^{13}C HMBC



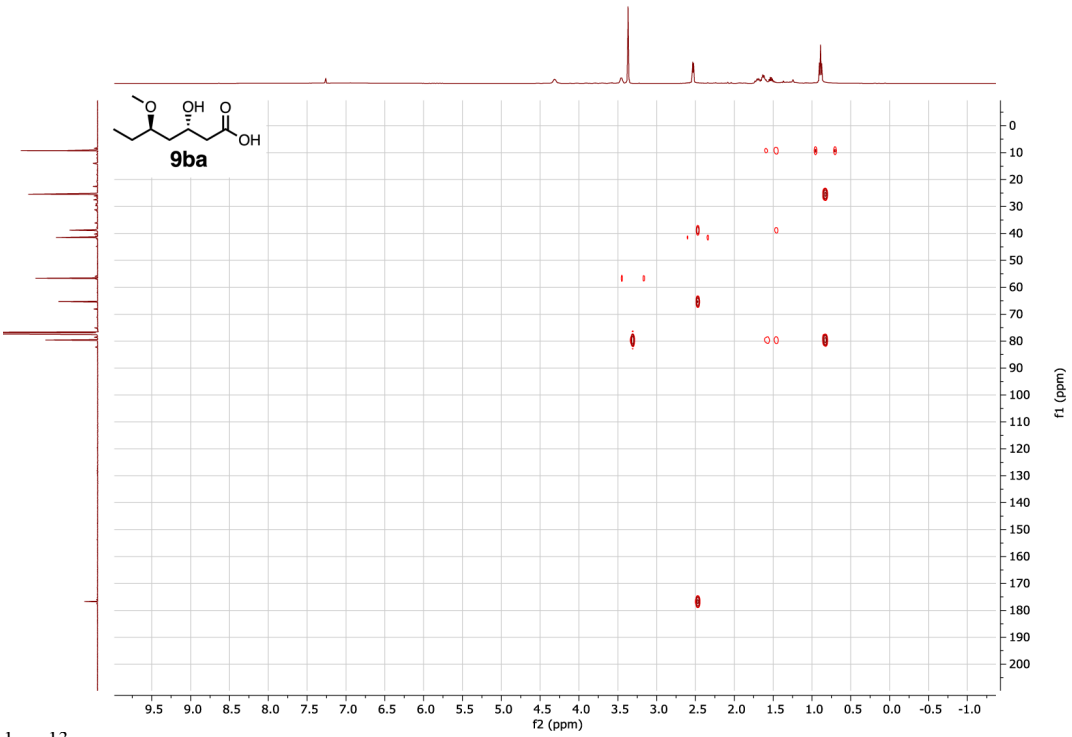
^{1}H NMR (500 MHz, chloroform-*d*) δ 4.32 (m, 1H), 3.46 (m, 1H), 3.37 (s, 3H), 2.53 (m, 2H), 1.63 (m, 4H), 0.89 (t, J = 7.5 Hz, 3H).



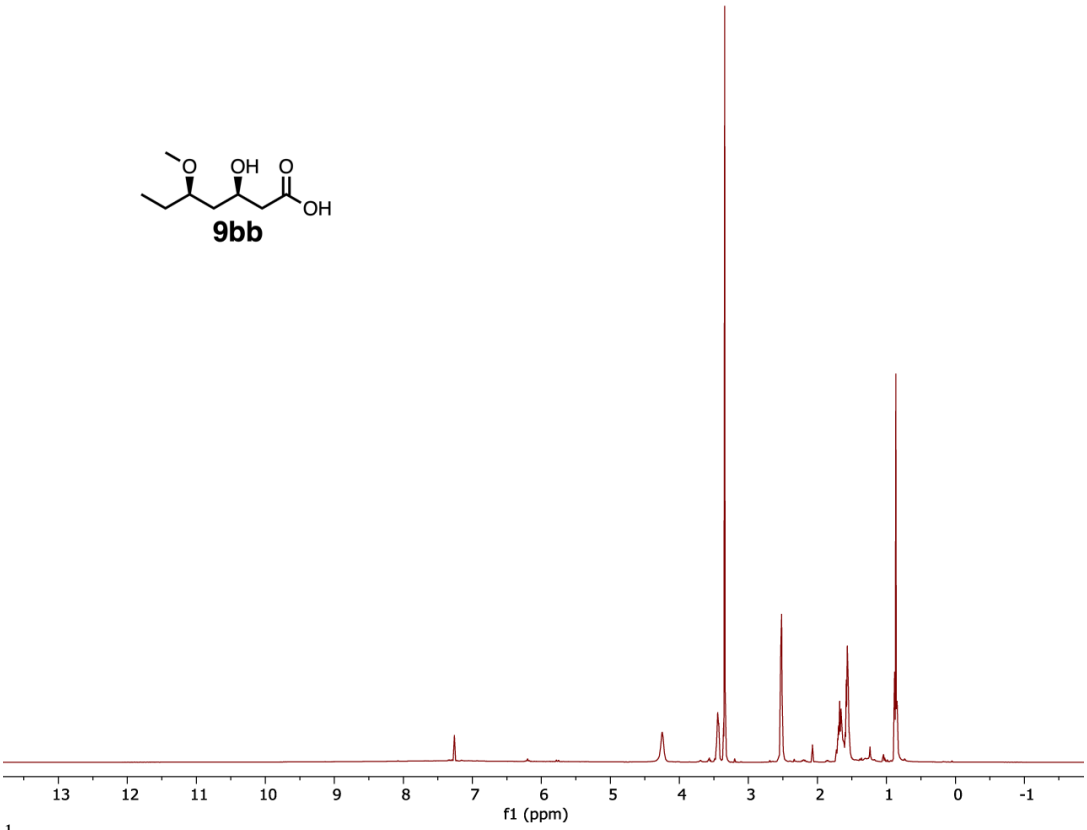
^{13}C NMR (126 MHz, chloroform-*d*) δ 176.69, 79.59, 65.33, 56.63, 41.50, 38.78, 25.44, 9.23.



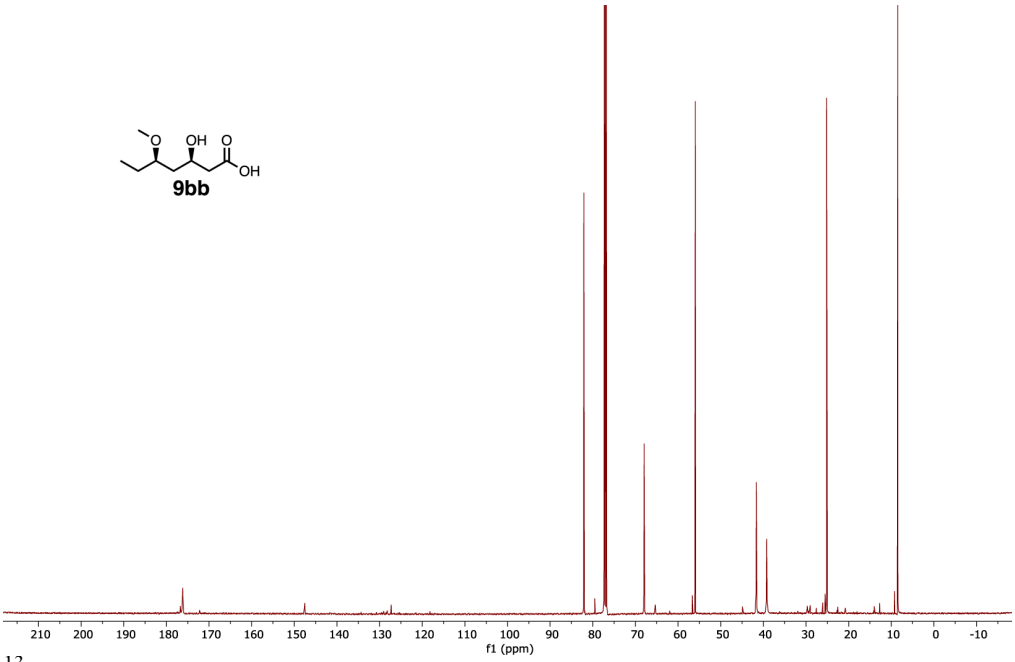
^{1}H-^{13}C HSQC



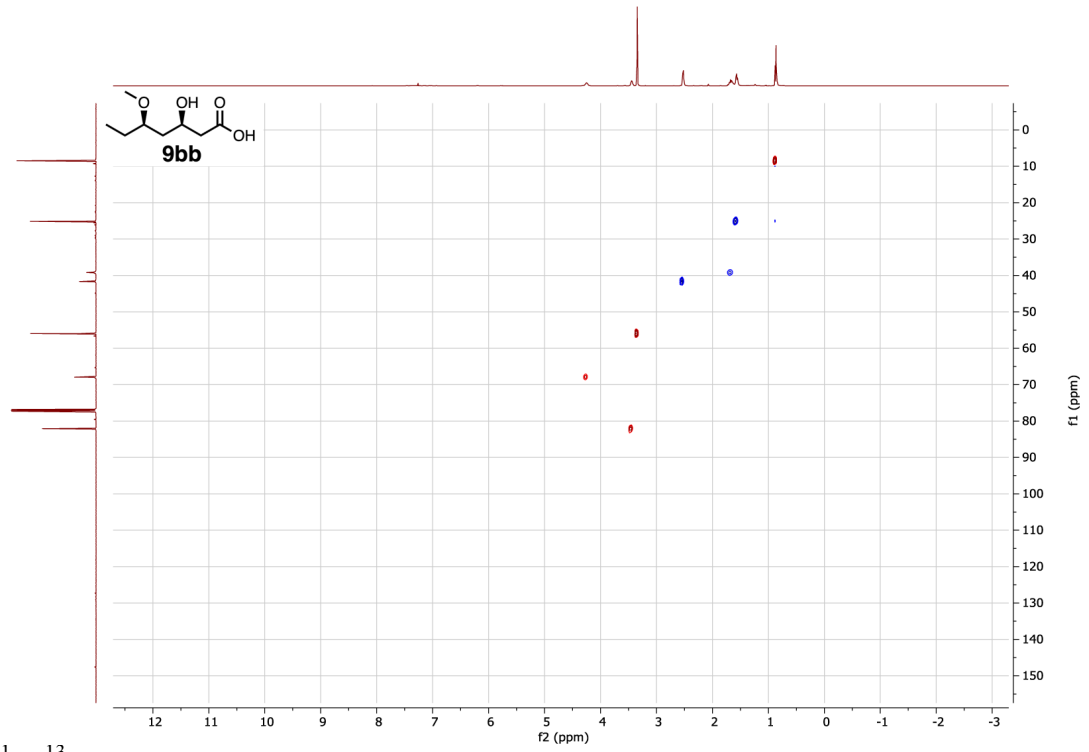
^{1}H-^{13}C HMBC



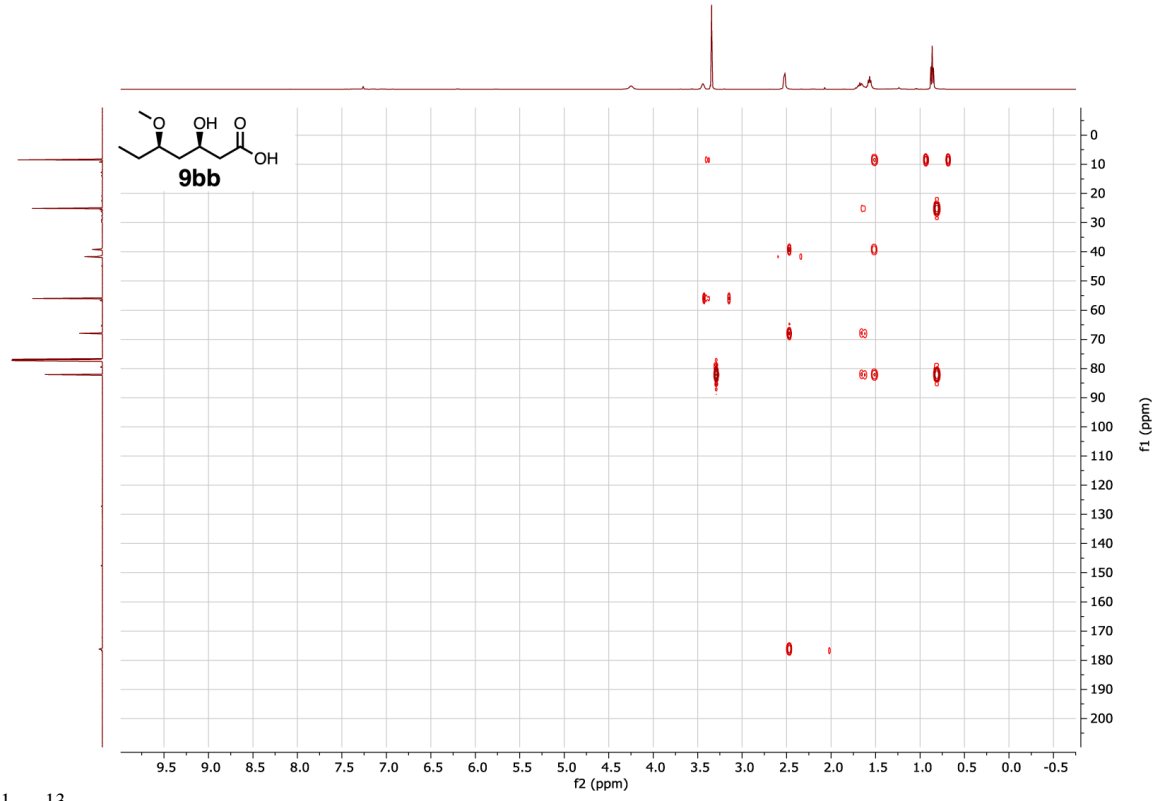
^{1}H NMR (500 MHz, chloroform-*d*) δ 4.25 (m, 1H), 3.44 (m, 1H), 3.34 (s, 3H), 2.52 (m, 2H), 1.61 (m, 4H), 0.86 (t, *J* = 7.5 Hz, 3H).



^{13}C NMR (126 MHz, chloroform-*d*) δ 176.68, 82.04, 67.89, 55.91, 41.60, 39.16, 25.10, 8.44.



^{1}H-^{13}C HSQC

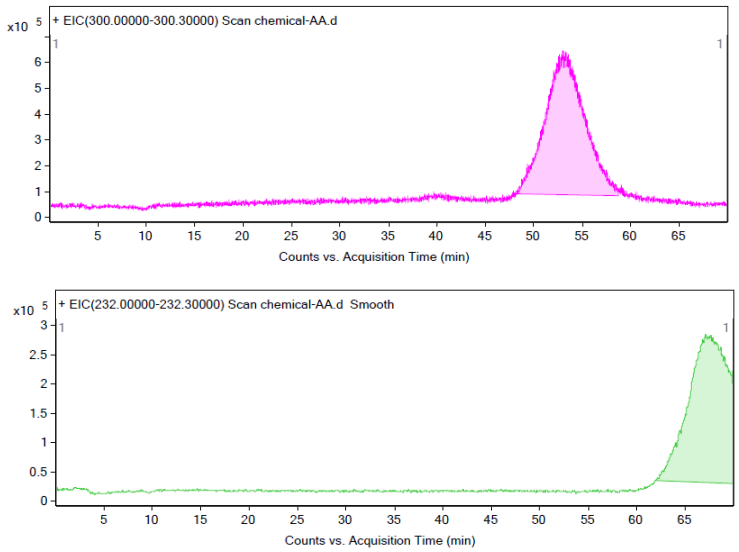


^{1}H-^{13}C HMBC

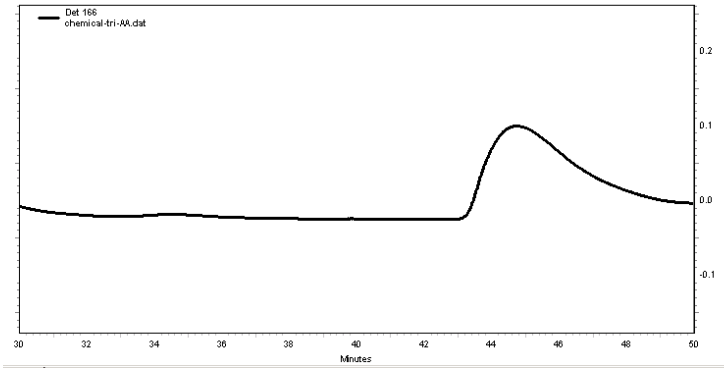
Synthesis of **8aa, 8ab, 8ba, 8bb** standards

Synthesis of **8aa**

70 mg **9aa** was dissolved in 3 mL anhydrous THF. Next, 24 mg DMAP, 176 mg EDC-HCl, and 250 mg NAC were added. The slurry was stirred overnight at 22 ^{o}C and then quenched with 1 M HCl. The aqueous layer was extracted with EtOAc and dried over Na_2SO_4. The organic phase was passed through a $CuSO_4$-packed silica gel and evaporated under reduced pressure. The residue was analyzed by reverse phase HPLC/MS (IF3 column, 0.8 mL/min 12% acetonitrile in water) and normal phase HPLC/UV (OC-H column, 0.8 mL/min, 7% ethanol in hexanes).



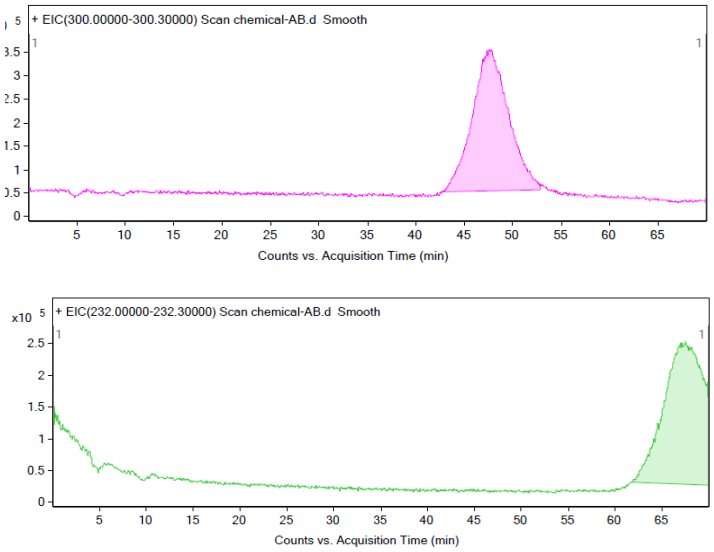
Extracted-ion chromatograms of **8aa** (top) and internal standard **14** (bottom) on IF3 column



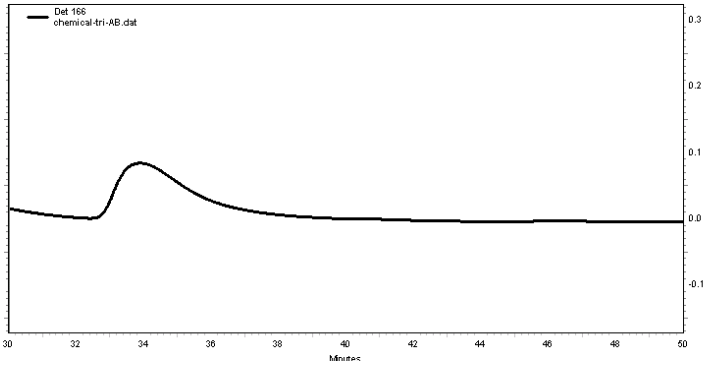
8aa on OC-H column

Synthesis of **8ab**

83 mg **9ab** was dissolved in 3 mL anhydrous THF. Next, 24 mg DMAP, 207 mg EDC-HCl, and 280 mg NAC were added. The slurry was stirred overnight at 22 ^{o}C and then quenched with 1 M HCl. The aqueous layer was extracted with EtOAc and dried over Na_2SO_4. The organic phase was passed through $CuSO_4$-impregnated silica gel and evaporated under reduced pressure. The residue was analyzed by reverse phase HPLC/MS (IF3 column, 0.8 mL/min 12% acetonitrile in water) and normal phase HPLC/UV (OC-H column, 0.8 mL/min, 7% ethanol in hexanes).



Extracted-ion chromatograms of **8ab** (top) and internal standard **14** (bottom) on IF3 column

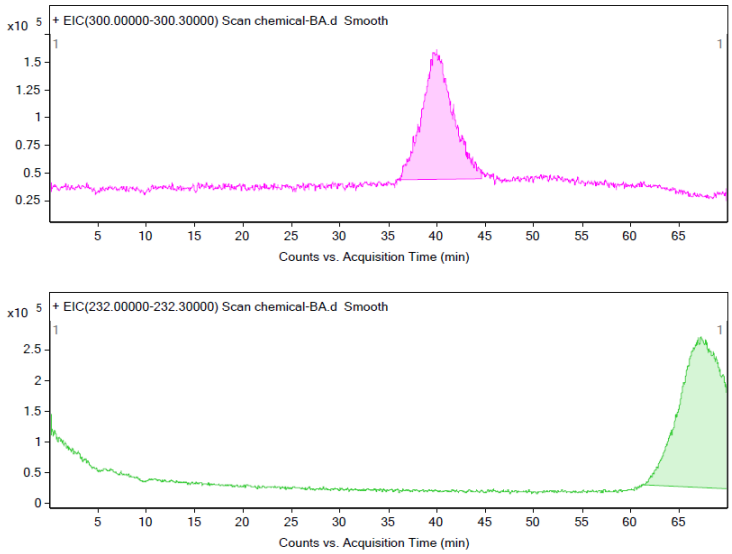


8ab on OC-H column

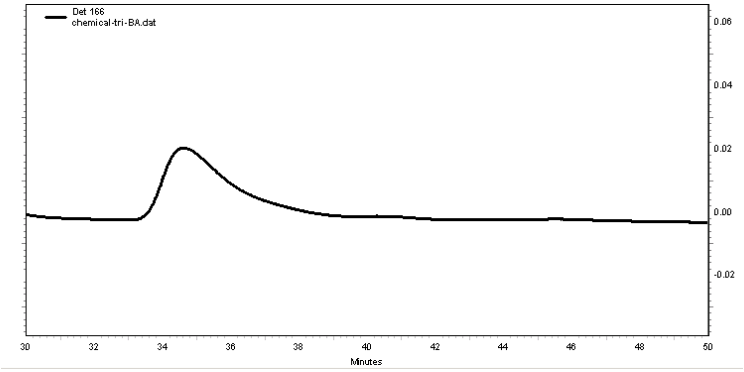
Synthesis of **8ba**

83 mg **9ba** was dissolved in 3 mL anhydrous THF. Next, 11 mg DMAP, 80 mg EDC-HCl, and 110 mg NAC were added. The slurry was stirred overnight and then quenched

with 1 M HCl. The aqueous layer was extracted with EtOAc and dried over Na_2SO_4. The organic phase was passed through a $CuSO_4$-packed silica gel and evaporated under reduced pressure. The residue was analyzed by reverse phase HPLC/MS (IF3 column, 0.8 mL/min 12% acetonitrile in water) and normal phase HPLC/UV (OC-H column, 0.8 mL/min, 7% ethanol in hexanes).



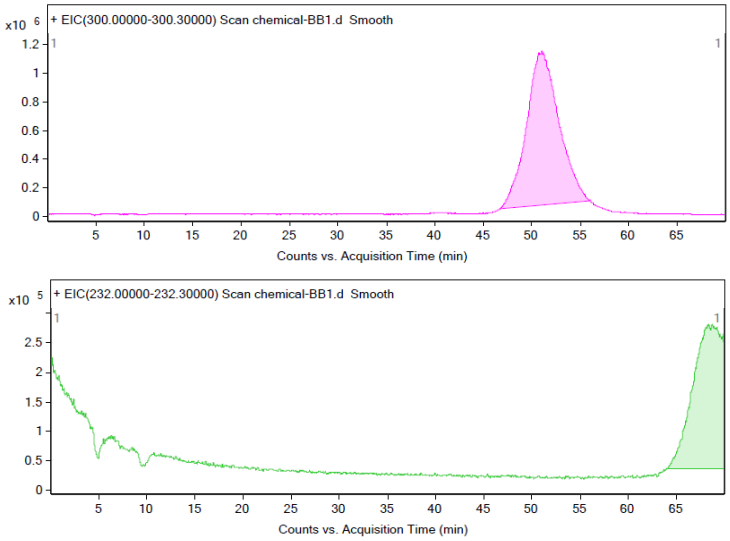
Extracted-ion chromatograms of **8ba** (top) and internal standard **14** (bottom) on IF3 column



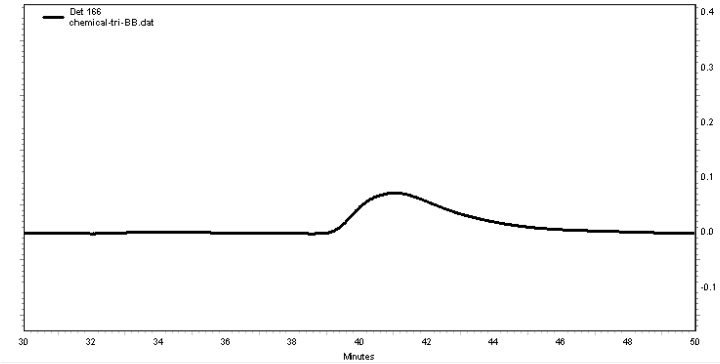
8ba on OC-H column

Synthesis of **8bb**

46 mg **9bb** was dissolved in 3 mL anhydrous THF. Next, 16 mg DMAP, 115 mg EDC-HCl, and 160 mg NAC were added. The slurry was stirred overnight and then quenched with 1 M HCl. The aqueous layer was extracted with EtOAc and dried over Na_2SO_4. The organic phase was passed through a $CuSO_4$-packed silica gel and evaporated under reduced pressure. The residue was analyzed by reverse phase HPLC/MS (IF3 column, 0.8 mL/min 12% acetonitrile in water) and normal phase HPLC/UV (OC-H column, 0.8 mL/min, 7% ethanol in hexanes).



Extracted-ion chromatograms of **8bb** (top) and internal standard **14** (bottom) on IF3 column



8bb on OC-H column

Comparison of chemoenzymatically-synthesized **8aa**, **8ab**, **8ba**, and **8bb** with standards

Retention times of triketides relative to **14** on IF3 column

	chemoenzymatic	standard
8aa	-15.7 min	-14.1 min
8ab	-20.6 min	-19.7 min
8ba	-27.7 min	-27.3 min
8bb	-17.2 min	-17.7 min

Retention times of triketides relative to **8ab** on OC-H column

	chemoenzymatic	standard
8aa	10.4 min	10.8 min
8ab	0.0 min	0.0 min
8ba	0.6 min	0.6 min
8bb	6.0 min	5.6 min

Table S2. Relative retention times on IF3 and OC-H columns, comparing chemoenzymatic products with standards (relative to **14** and **8ab**, respectively)

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(5) Che, W.; Wen, D. Y. C.; Zhu, S. F.; Zhou, Q. L., Enantioselective Total Synthesis of (-)-Doliculide Using Catalytic Asymmetric Hydrogenations. *Helv. Chim. Acta* **2019,** *102*.