Supporting Information

Stereochemical Structure Activity Relationship Studies (S-SAR) of Tetrahydrolipstatin

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Contents

Section A. Biological assays procedures

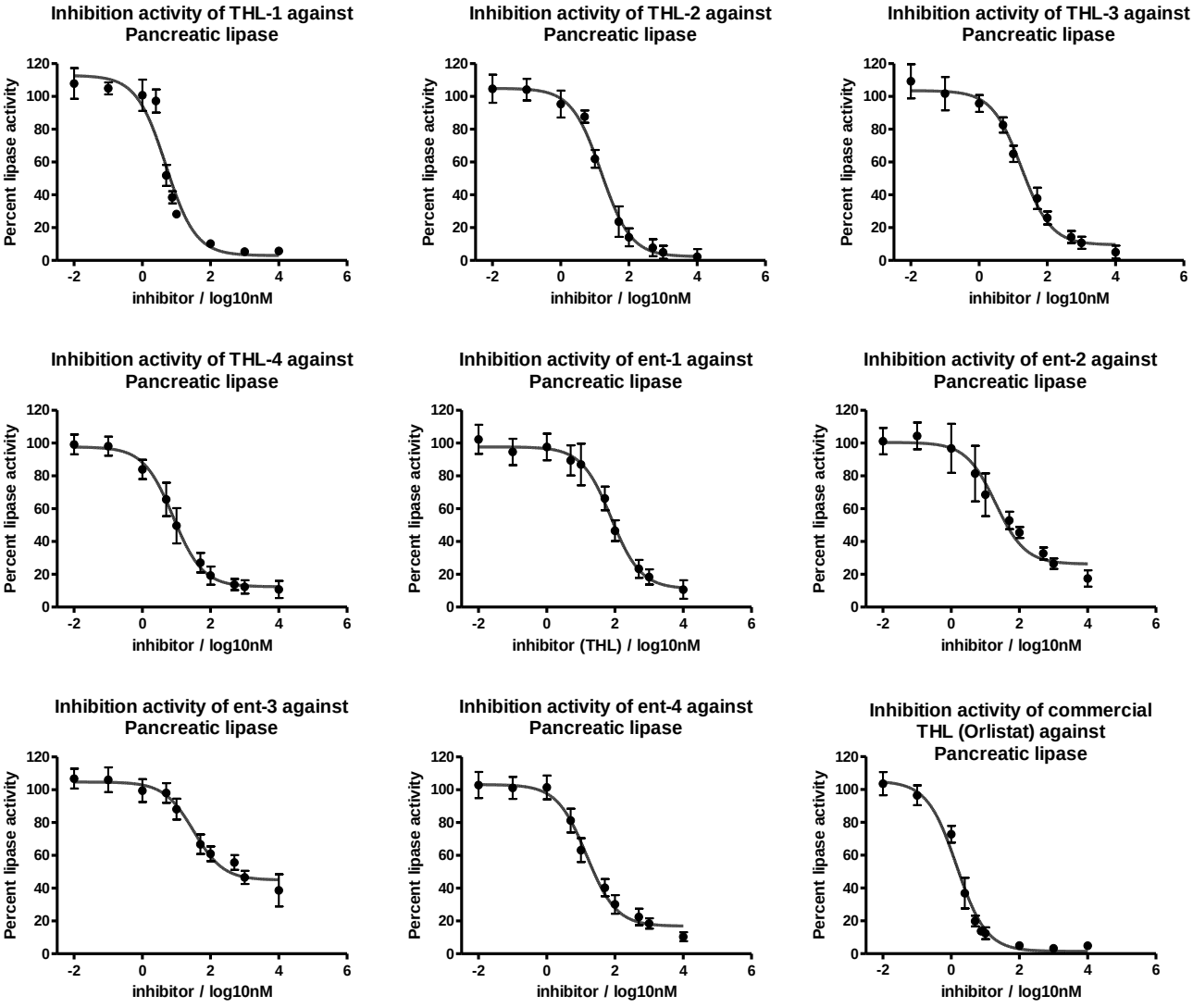
Porcine pancreatic lipase (PPL) inhibition IC_{50} of all the THL and stereoisomers and commercial THL

Porcine pancreatic lipase (Type II, Sigma 3126), tetrahydrolipstatin (THL, CAS 96829-58-2, Sigma O4139, ≥98%), reporter compound 4-nitrophenol butyrate (*p*-NPB, CAS 2635-84-9, Sigma N9876, ≥98%,), and hydrolysis product standard *p*-nitrophenol (CAS 100-02-7) were obtained from Sigma-Aldrich. The stock 10 mM solutions of 4-nitrophenol butyrate (*p*NPB) reporter compound and 4-nitrophenol positive control standard in DMSO were freshly prepared before each assay. All solvent ratios are by volume. All assay data were obtained as the mean of triplicate experiments of n = 3 and are reported as the percentage of controls. IC_{50} determinations utilized Graphpad Prism software. All IC_{50} data are actually apparent IC_{50} as all inhibitors in this investigation undergo covalent reactions in the active site of the hydrolytic pancreatic lipase enzyme.

Assay of inhibition of porcine pancreatic lipase (PPL) enzyme. Absorbance at 400 nm of 200 uL final volumes were performed in 96-well polypropylene plates. The experiment performed in triplicate wells for a single compound. Assay buffer final concentrations consisted of 90:10 water/DMSO containing 50 mM HEPES pH 7.5, 10 mM $CaCl_2$, with inhibitor, enzyme, and reporter compound as appropriate. Following addition of inhibitor solutions in pure DMSO to appropriate wells, the pancreatic lipase stock solution was prepared immediately before use. The concentrations of PL enzyme used was 5 ug (per 200 uL well). Weighed porcine pancreatic lipase was dissolved by hand mixing in 90:10 buffer/DMSO to the 0.5 mg/mL stock concentration, low speed centrifugation removed suspended particles, and the homogeneous enzyme solution was decanted to be added to appropriate wells as 10 μL aliquots by multipipettor. The covered 96-well plates were then gently swirled on a Heidolph unimax rotary mixer/incubator mixer for 20 min at 25 °C during enzyme inactivation. During this time, the reporter compound solution was prepared immediately before use. Reporter compound pNPB stock concentration of 5.0 mM was made up in 0.1 volumes of DMSO, with addition of the 0.9 volume of buffer and vortex mixing just before loading the multipipettor. After the reporter compound was added into all appropriate wells at a final concentration of 0.25 mM, the plates were

loaded into the Biotek reader (pre-equilibrated to 25 °C). Absorbance data were corrected for absorbance of negative control (no enzyme) and also by subtraction of any absorption at 400 nm due to porcine pancreatic lipase (PPL) enzyme. Plots of absorbance (400 nm) vs time were linear for at least 30 min, at which point about 10% of available substrate had been consumed.

The identities of the THL in this figure still have not been corrected to correspond with THL1-4 and ent-1 to ent-4 of this manuscript



2. Km (Michaelis-Menten constant) experiment procedure:

Km was measured using the same assay as the one for porcine pancreatic lipase inhibition. Assay buffer final concentrations consisted of 90:10 water/DMSO containing 50 mM HEPES pH 7.5, 10 mM $CaCl_2$,

with enzyme, and reporter compound as appropriate. Lipase stock solution was prepared immediately before use. The concentrations of PL enzyme used was 5 ug (per 200 uL well). The reporter compound (pNPB) solution which was the substrate for kinetic measurement was prepared immediately before use. Reporter compound pNPB stock solution of various concentrations (2.5 mM, 5 mM, 10 mM, 15 mM) were made up in 0.9 volumes of DMSO, with addition of the 0.1 volume of water and vortex mixing just before loading the multipipettor. Weighed porcine pancreatic lipase was dissolved by hand mixing in 90:10 buffer/DMSO to the 0.5 mg/mL stock concentration, low speed centrifugation removed suspended particles, and the homogeneous enzyme solution was decanted to be added to appropriate wells as 10 uL aliquots by multipipettor. The enzyme solution was added after the reporter compound was added into all appropriate wells at a final concentration of 0.125 mM, 0.25 mM, 0.5 mM 0.75 mM.. The plates were loaded into the Biotek reader (pre-equilibrated to 25 °C). Absorbance data were corrected for absorbance of negative control (no enzyme) and also by subtraction of any absorption at 400 nm due to PL enzyme. The velocity (V) of hydrolysis was determined using absorbance of 30 min and 15 min.

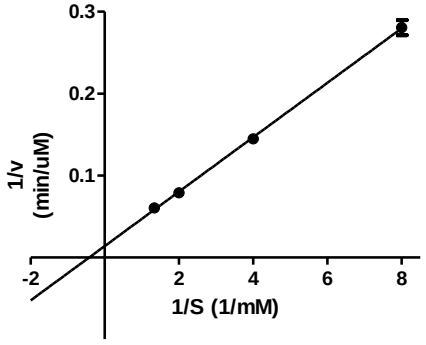


Figure Lineweaver-Burke Plot

Km = -1/(-0.4151) = 2.41 mM

3. Cytotoxicity evaluation of eight diastereomers using MTT assay.

Human breast cancer cell line MCF-7, MDA-MB-231 and human large-cell lung carcinoma line H460 were obtained from the American Type Culture Collection (Manassas, VA). The cells MCF-7 and MDA-MB-231 were cultured in Dulbecco's Modification of Eagle's Medium (DMEM, 1X) supplemented with 10% fetal bovine serum (FBS), 1% l-glutamine and 1% penicillin-streptomycin solution. H460 was cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 1% l-glutamine and 1% penicillin-streptomycin solution. The cell cultures were maintained in a humidified

incubator with 5% CO_2 at 37 °C. Cells were subcultured at preconfluent densities using a solution containing 0.25% trypsin and 0.5 mM EDTA (Invitrogen). The cell line was grown in 96-well plates at a density of 5×10^3 cell/well for 12 to 16 h. Drugs were pre-dissolved in dimethyl sulfoxide (DMSO). The resulting solutions were diluted with relative culture medium by 100 times, and added to the wells in increasing concentration by 10 times dilution, with untreated wells as controls. After treatment, cells were incubated for a period of 72 h, and subjected to a solution of 3-(4,5-dimethylthylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL, 10 μL/well) in deionized water. After incubation at 37 °C for 4 h, the culture medium was removed, and the cells were suspended in DMSO. The viability was measured by a Gen5 Fluorescence Reader at 570 nm. The dose-dependent experiment was performed in 2 replicate wells of each compound for a single concentration with 3 independent experiments (N = 6). IC_{50} values (calculated as the dose necessary to cause a 50% reduction in cell viability compared to untreated control.

Table 3. Percent inhibition of cell lines at 100 uM dose of drug

Cpd	H460	MDA-MB-	MCF-7
1	0	15	38
2	25	27	68
3	19	35	24
4	10	18	24
***ent*-1**	17	21	22
***ent*-2**	15	12	10
***ent*-3**	11	13	20
***ent*-4**	6	14	11
CPT	76	72	63

Section B: : Synthetic experimental procedures

General Information

^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 or 500 MHz spectrometer. Chemical shifts were reported relative to internal tetramethylsilane (δ 0.00 ppm) or $CDCl_3$ (δ 7.26 ppm) for ^{1}H NMR and $CDCl_3$ (δ 77.2 ppm) for ^{13}C NMR. Infrared (IR) spectra were obtained on a FT-IR spectrometer by preparing neat sample on potassium bromide plates. Optical rotations were measured with a digital polarimeter in the solvent specified. Melting points were determined with a standard melting point apparatus. Flash column chromatography was performed on 60-200 or 230-400 mesh silica gel. Preparative HPLC was performed on a Haisil 100 silica column (5 μm, 250 × 10 mm) with ethyl acetate and hexanes as mobile phase. Analytical thin-layer chromatography was performed with precoated glass-backed plates and visualized by quenching of fluorescence and by charring after treatment with *p*-anisaldehyde or potassium permanganate stain. Retention factors (R_f) were obtained by elution in the stated solvent ratios. Diethyl ether, tetrahydrofuran, methylene dichloride and triethylamine were dried by passing through activated alumina column with argon gas pressure. Commercial reagents were used without purification unless otherwise noted. Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon/nitrogen using oven- or flame-dried glassware and standard syringe/septa techniques. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained using α-cyano-4-hydroxycinnamic acid (CCA) as the matrix on a MALDI-TOF mass spectrometer.

For carbonylation, anhydrous tetrahydrofuran was purchased from Fischer and purified by passing through two neutral alumina-packed columns and a third column packed with activated 4Å molecular sieves under nitrogen pressure. Tetrahydrofuran was degassed by three freeze-pump-thaw cycles prior to use. All other solvents were reagent grade or better and used as received. Commercial reagents were used without purification unless otherwise noted. Carbon monoxide (Airgas, 99.99% minimum purity) was used as received. Bis(tetrahydrofuran)-*meso*-tetra(4-chlorophenyl)porphyrinato aluminum tetracarbonyl cobaltate, $[ClTPPAl]^{+}[Co(CO)_4]^{-}$, was synthesized as previously reported.[1] All carbonylation reactions were performed in a custom-designed and -fabricated, six-chamber, stainless

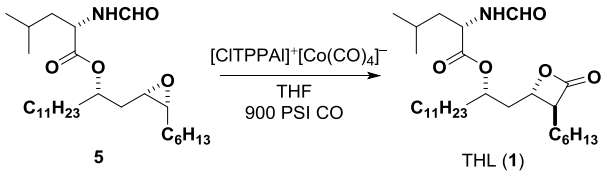
1 Rowley, J. M.; Lobkovsky, E. B.; Coates, G. W. *J. Am. Chem. Soc.* **2007**, *129*, 4948–4960.

steel, high-pressure reactor, which accommodated six 4- or 8-mL glass vials, that has been described previously.[2,3] Because carbon monoxide is a highly toxic gas, all carbonylation reactions were performed in a well-ventilated fume hood equipped with a CO sensor.

General Procedure for Carbonylation of Epoxides

In a nitrogen glove box, a 4 mL glass vial equipped with a Teflon-coated magnetic stir bar was charged with the appropriate amount of $[ClTPPAl]^+[Co(CO)_4]^-$ and tetrahydrofuran. The vial was placed in the glove box freezer at –30 °C along with a custom-made six-well high-pressure reactor (see Section A: General Information) to cool for 30 minutes. (In the absence of CO, isomerization of the epoxide to ketone products[4] can be minimized by keeping the temperature of the reactor below 0 °C.) The appropriate amount of epoxide (also cooled at –30 °C for 30 minutes) was then added to the vial. After adding a cap with a Teflon-coated septum pierced by a 20 G needle (to prevent the reaction solvent from refluxing into the reactor chamber), the vial was placed quickly into the reactor. Subsequently, the reactor was sealed, taken out of the glove box, placed in a well-ventilated hood, and pressurized with carbon monoxide (900 psi). The reactor was then heated to 50 °C and the reaction mixture stirred for the specified time. The reactor was cooled on dry ice for 10 minutes and carefully vented in a fume hood. The crude reaction mixture was concentrated under reduced pressure and then purified via flash column chromatography or preparative HPLC.

(*S*)-1-((2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl formyl-L-leucinate (1)[5]



The general procedure for carbonylation of epoxides was followed using **5** (69.8 mg, 0.149 mmol), $[ClTPPAl]^+[Co(CO)_4]^-$ (3.4 mg, 0.0031 mmol, 2.1 mol %), and tetrahydrofuran (0.30 mL) for 24 hours.

2 Getzler, Y. D. Y. L.; Kundnani, V.; Lobkovsky, E. B.; Coates, G. W. *J. Am. Chem. Soc.* **2004**, *126*, 6842–6843.

3 Schmidt, J. A. R.; Mahadevan, V.; Getzler, Y. D. Y. L.; Coates, G. W. *Org. Lett.* **2004**, *6*, 373–376.

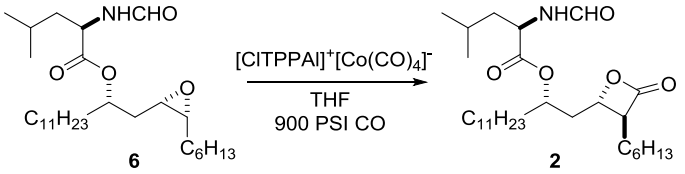
4 Church, T. L.; Getzler, Y. D. Y. L.; Coates, G. W. *J. Am. Chem. Soc.* **2006**, *128*, 10125–10133.

5 Lipstatins exist as a ~20:1 mixture of formamide rotamers. Because of the low concentration of the minor rotamer, the shifts of the minor isomer are not reported.

The crude residue was purified by preparative HPLC to afford **1** (59.4 mg, 80%) as a colorless oil.

Data for **1**: R_f = 0.27 (30% EtOAc in hexanes); $[\alpha]_D^{23}$ = –33.7 ($CHCl_3$, *c* = 0.48); IR (neat) 3380, 2926, 2854, 1823, 1738, 1666, 1553, 1467, 1378, 1253, 1187, 1123 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 5.91 (d, *J* = 8.0 Hz, 1H), 5.05–5.00 (m, 1H), 4.69 (ddd, *J* = 9.0, 9.0, 5.0 Hz, 1H), 4.29 (ddd, *J* = 7.5, 5.0, 5.0 Hz, 1H), 3.22 (ddd, *J* = 7.5, 7.5, 4.0 Hz, 1H), 2.17 (ddd, *J* = 15.0, 7.5, 7.5 Hz, 1H), 2.00 (ddd, *J* = 15.0, 4.5, 4.5 Hz, 1H), 1.84–1.78 (m, 1H), 1.77–1.63 (m, 4H), 1.61–1.53 (m, 2H), 1.47–1.41 (m, 1H), 1.32–1.24 (m, 25H), 0.972 (d, *J* = 6.5 Hz, 3H), 0.967 (d, *J* = 6.5 Hz, 3H), 0.885 (t, *J* = 6.5 Hz, 3H), 0.878 (t, *J* = 7.0, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.92, 170.75, 160.60, 74.76, 72.76, 57.03, 49.60, 41.56, 38.70, 34.05, 31.89, 31.47, 29.60 (2C), 29.53, 29.42, 29.33, 29.29, 28.96, 27.61, 26.70, 25.09, 24.88, 22.87, 22.68, 22.51, 21.73, 14.12, 14.01 ($CDCl_3$, δ 77.0 ppm, for comparison with existing papers); MALDI-TOF/CCA-HRMS calcd for $C_{29}H_{53}NO_5Na$ $[M + Na]^+$: 518.3816, found 518.3814.

(*S*)-1-((2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl formyl-D-leucinate (2)[5]

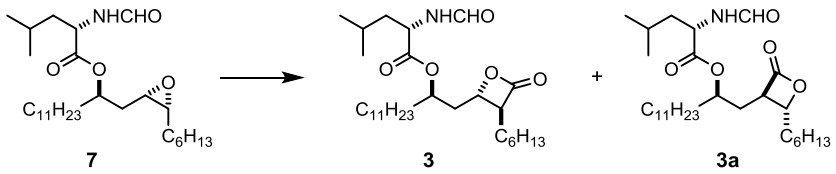


The general procedure for carbonylation of epoxides was followed using **6** (20.0 mg, 0.0428 mmol), $[ClTPPAl]^+[Co(CO)_4]^-$ (2.6 mg, 0.0024 mmol, 5.6 mol %) and tetrahydrofuran (0.25) mL for 3 days. The crude residue was purified by flash column chromatography (50% Et_2O in hexanes) on silica gel (30 mL) to afford **2** (16.3 mg, 77%) as a colorless oil.

Data for **2**: R_f = 0.27 (30% EtOAc in hexanes); $[\alpha]_D^{22}$ = –5.3 ($CHCl_3$, *c* = 0.17); IR (neat) 3325, 2957, 2927, 2855, 1824, 1740, 1685, 1511, 1467, 1379, 1252, 1188, 1125 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 5.87 (d, *J* = 8.0 Hz, 1H), 5.03 (dddd, *J* = 7.5, 7.5, 5.0, 5.0 Hz, 1H), 4.66 (ddd, *J* = 8.5, 8.5, 5.5 Hz, 1H), 4.35 (ddd, *J* = 8.0, 5.0, 4.0 Hz, 1H), 3.22 (ddd, *J* = 7.5, 7.5, 4.5 Hz, 1H), 2.18 (ddd, *J* = 15.0, 7.5, 7.5 Hz, 1H), 2.02 (ddd, *J* = 15.0, 5.0, 4.5 Hz, 1H), 1.84–1.77 (m, 1H), 1.76–1.71 (m, 1H), 1.70–1.64 (m, 3H), 1.60–1.53 (m, 2H), 1.47–1.41 (m, 1H), 1.38–1.24 (m, 25H); 0.970 (d, *J* = 6.5 Hz, 3H), 0.967 (d, *J* = 6.5 Hz, 3H), 0.882 (t, *J* = 7.0 Hz, 3H), 0.879 (t, *J* = 7.0, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.2, 160.8, 74.8, 72.8, 57.2, 49.8, 41.6, 38.7, 34.0, 32.1, 31.6, 29.8 (2C), 29.7, 29.6, 29.5, 29.4, 29.2, 27.8, 26.9, 25.4, 25.0, 23.0, 22.9, 22.7, 22.0, 14.3, 14.2; MALDI-TOF/CCA-HRMS

calcd for $C_{29}H_{53}NO_5Na$ [M + Na]$^+$: 518.3816, found 518.3813.

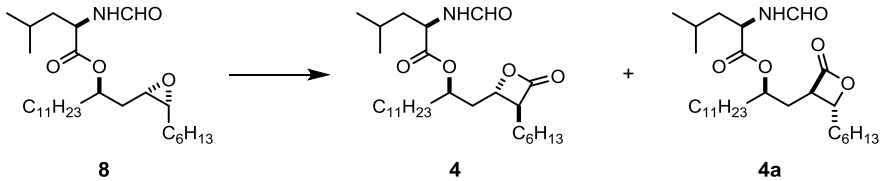
(*R*)-1-((2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl formyl-L-leucinate (3)[5]



The general procedure for carbonylation of epoxides was followed using **7** (61.6 mg, 0.132 mmol), [ClTPPAl]$^+$[Co(CO)$_4$]$^-$ (5.8 mg, 0.0053 mmol, 4.0 mol %) and tetrahydrofuran (0.30) mL for 24 hours. The crude residue was purified by preparative HPLC to afford **3** (46.5 mg, 72%, regioselectivity **3**:**3a** = 6:1; the ratio determined by ^{1}H NMR) as a colorless oil.

Data for **3**: R_f = 0.27 (30% EtOAc in hexanes); $[\alpha]_D^{21}$ = –21.5 ($CHCl_3$, *c* = 0.36); IR (neat) 3353, 2925, 2855, 1822, 1736, 1685, 1461, 1380, 1272, 1187, 1125 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 5.92 (d, *J* = 8.0 Hz, 1H), 5.04 (dddd, *J* = 7.0, 7.0, 5.5, 5.5 Hz, 1H), 4.68 (ddd, *J* = 8.5, 8.5, 5.0 Hz, 1H), 4.29 (ddd, *J* = 9.0, 4.5, 4.5 Hz, 1H), 3.22 (ddd, *J* = 7.5, 7.5, 4.5 Hz, 1H), 2.10–2.00 (m, 2H), 1.85–1.78 (m, 1H), 1.77–1.53 (m, 6H), 1.49–1.42 (m, 1H), 1.38–1.24 (m, 25H), 0.976 (d, *J* = 6.5 Hz, 3H), 0.963 (d, *J* = 6.5 Hz, 3H), 0.89–0.87 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 171.1, 160.8, 74.5, 72.7, 56.9, 49.7, 41.9, 39.3, 34.4, 32.1, 31.7, 29.8 (2C), 29.7, 29.6, 29.5, 29.4, 29.1, 27.8, 27.0, 25.2, 25.0, 23.0, 22.9, 22.7, 22.1, 14.3, 14.2; MALDI-TOF/CCA-HRMS calcd for $C_{29}H_{53}NO_5Na$ [M + Na]$^+$: 518.3816, found 518.3798.

(*R*)-1-((2*S*,3*S*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl formyl-D-leucinate (4)[5]



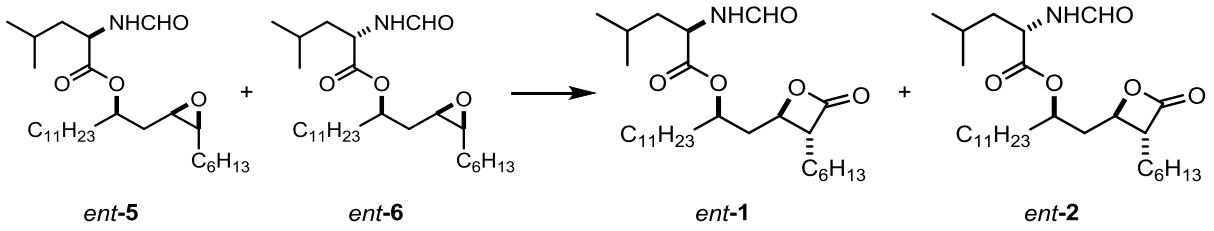
The general procedure for carbonylation of epoxides was followed using **8** (71.1 mg, 0.152 mmol), [ClTPPAl]$^+$[Co(CO)$_4$]$^-$ (6.7 mg, 0.0061 mmol, 4.0 mol %) and tetrahydrofuran (0.30) mL for 24 hours. The crude residue was purified by preparative HPLC to afford **4** (58.2 mg, 77%, regioselectivity **4**:**4a** = 5:1; the ratio determined by ^{1}H NMR) as a colorless oil.

Data for **4**: R_f = 0.32 (30% EtOAc in hexanes); $[\alpha]_D^{21}$ = –12.3 ($CHCl_3$, *c* = 0.32); IR (neat) 3324, 2926,

2856, 1823, 1736, 1684, 1461, 1256, 1189, 1123 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 5.90 (d, *J* = 9.0 Hz, 1H), 5.04–4.99 (m, 1H), 4.71 (ddd, *J* = 8.5, 8.5, 4.5 Hz, 1H), 4.26 (ddd, *J* = 8.5, 4.5, 4.5 Hz, 1H), 3.23 (ddd, *J* = 7.0, 7.0, 4.0 Hz, 1H), 2.09–2.00 (m, 2H), 1.86–1.79 (m, 1H), 1.76–1.63 (m, 4H), 1.61–1.54 (m, 2H), 1.47–1.41 (m, 1H), 1.38–1.24 (m, 25H), 0.976 (d, *J* = 6.0 Hz, 3H), 0.964 (d, *J* = 6.0 Hz, 3H), 0.887 (t, *J* = 7.0 Hz, 3H), 0.879 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 170.9, 160.7, 74.4, 72.8, 56.8, 49.8, 41.9, 39.1, 34.2, 32.1, 31.7, 29.8 (2C), 29.7, 29.6, 29.53, 29.51, 29.1, 27.9, 27.0, 25.2, 25.1, 23.0, 22.9, 22.7, 22.1, 14.3, 14.2; MALDI-TOF/CCA-HRMS calcd for $C_{29}H_{53}NO_5Na$ $[M + Na]^+$: 518.3816, found 518.3843.

(*R*)-1-((2*R*,3*R*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl formyl-D-leucinate (*ent*-1)[5]

(*R*)-1-((2*R*,3*R*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl formyl-L-leucinate (*ent*-2)[5]



ent-**5** *ent*-**6** *ent*-**1** *ent*-**2**

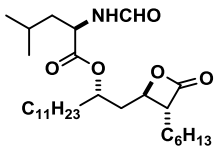
The general procedure for carbonylation of epoxides was followed using 102.7 mg (0.2196 mmol) (*R*)-1-((2*R*,3*S*)-3-hexyloxiran-2-yl)tridecan-2-yl formyl-D,L-leucinate, 12.8 mg (0.0117 mmol, 5.33 mol %) [ClTPPAl][$Co(CO)_4$], and 0.50 mL tetrahydrofuran for 3 days. The crude residue was purified by flash column chromatography (50% Et_2O in hexanes) on silica gel (30 mL) to afford a mixture of *ent*-**1** and *ent*-**2** (90.0 mg, 82%) as a colorless oil. The mixture of *ent*-**1** and *ent*-**2** was separated with HPLC.

Data for *ent*-**1**: R_f = 0.27 (30% EtOAc in hexanes); $[\alpha]_D^{21}$ = +22.3 ($CHCl_3$, *c* = 0.45); IR (neat) 3304, 2956, 2926, 2855, 1823, 1739, 1671, 1523, 1467, 1381, 1252, 1193, 1124 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 5.91 (d, *J* = 8.5 Hz, 1H), 5.05–5.00 (m, 1H), 4.69 (ddd, *J* = 9.0, 9.0, 5.0 Hz, 1H), 4.29 (ddd, *J* = 7.5, 5.0, 5.0 Hz, 1H), 3.22 (ddd, *J* = 7.5, 7.5, 4.0 Hz, 1H), 2.17 (ddd, *J* = 15.0, 7.5, 7.5 Hz,

1H), 2.00 (ddd, *J* = 15.0, 4.5, 4.5 Hz, 1H), 1.84–1.78 (m, 1H), 1.77–1.63 (m, 4H), 1.61–1.53 (m, 2H), 1.47–1.41 (m, 1H), 1.32–1.24 (m, 25H), 0.973 (d, *J* = 6.5 Hz, 3H), 0.965 (d, *J* = 6.5 Hz, 3H), 0.884 (t, *J* = 6.5 Hz, 3H), 0.877 (t, *J* = 7.0, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 170.8, 160.6, 74.8, 72.8, 57.0, 49.6, 41.6, 38.7, 34.1, 31.89, 31.5, 29.6 (2C), 29.5, 29.4, 29.3, 29.3, 29.0, 27.6, 26.7, 25.1, 24.9, 22.9, 22.7, 22.5, 21.7, 14.1, 14.0 ($CDCl_3$, δ 77.0 ppm); MALDI-TOF/CCA-HRMS calcd for $C_{29}H_{53}NO_5Na$ $[M + Na]^+$: 518.3816, found 518.3817.

Data for *ent*-**2**: R_f = 0.27 (30% EtOAc in hexanes); $[\alpha]_D^{22}$ = +4.4 ($CHCl_3$, *c* = 0.81); IR (neat) 3307, 2926, 2855, 1824, 1740, 1687, 1523, 1467, 1380, 1251, 1190, 1125 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H), 5.92 (d, *J* = 8.5 Hz, 1H), 5.02 (dddd, *J* = 7.5, 7.5, 5.0, 5.0 Hz, 1H), 4.66 (ddd, *J* = 8.5, 8.5, 5.5 Hz, 1H), 4.35 (ddd, *J* = 8.0, 5.0, 4.0 Hz, 1H), 3.22 (ddd, *J* = 7.5, 7.5, 4.5 Hz, 1H), 2.17 (ddd, *J* = 15.0, 7.5, 7.5 Hz, 1H), 2.01 (ddd, *J* = 15.0, 5.0, 4.5 Hz, 1H), 1.84–1.77 (m, 1H), 1.76–1.71 (m, 1H), 1.70–1.64 (m, 3H), 1.60–1.53 (m, 2H), 1.47–1.41 (m, 1H), 1.38–1.24 (m, 25H), 0.970 (d, *J* = 6.5 Hz, 3H), 0.967 (d, *J* = 6.5 Hz, 3H), 0.882 (t, *J* = 7.0 Hz, 3H), 0.879 (t, *J* = 7.0, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.2, 160.8, 74.8, 72.7, 57.1, 49.8, 41.6, 38.7, 34.0, 32.1, 31.6, 29.8 (2C), 29.7, 29.6, 29.5, 29.4, 29.1, 27.8, 26.8, 25.4, 25.0, 23.0, 22.9, 22.7, 22.0, 14.3, 14.2; MALDI-TOF/CCA-HRMS calcd for $C_{29}H_{53}NO_5Na$ $[M + Na]^+$: 518.3816, found 518.3813.

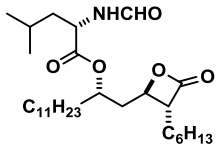
(*S*)-1-((2*R*,3*R*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl formyl-D-leucinate (*ent*-3)[5]



Data for *ent*-**3**: R_f = 0.27 (30% EtOAc in hexanes); $[\alpha]_D^{21}$ = +23.5 ($CHCl_3$, *c* = 0.61); IR (neat) 3353, 2926, 2857, 1822, 1739, 1684, 1462, 1380, 1251, 1189, 1124 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 5.92 (d, *J* = 8.5 Hz, 1H), 5.04 (dddd, *J* = 7.5, 7.5, 5.0, 5.0 Hz, 1H), 4.68 (ddd, *J* = 8.5, 8.5, 5.0

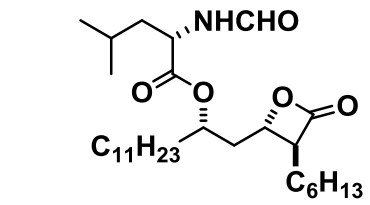
Hz, 1H), 4.29 (ddd, *J* = 9.0, 4.5, 4.5 Hz, 1H), 3.22 (ddd, *J* = 7.5, 7.5, 4.5 Hz, 1H), 2.10–2.00 (m, 2H), 1.85–1.78 (m, 1H), 1.77–1.53 (m, 6H), 1.49–1.42 (m, 1H), 1.38–1.24 (m, 25H), 0.975 (d, *J* = 6.0 Hz, 3H), 0.965 (d, *J* = 6.5 Hz, 3H), 0.89–0.87 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 171.1, 160.8, 74.5, 72.7, 56.9, 49.7, 41.9, 39.3, 34.4, 32.1, 31.7, 29.8 (2C), 29.7, 29.6, 29.5, 29.4, 29.1, 27.8, 27.0, 25.2, 25.1, 23.0, 22.9, 22.7, 22.1, 14.3, 14.2; MALDI-TOF/CCA-HRMS calcd for $C_{29}H_{53}NO_5Na$ [M + Na]$^+$: 518.3816, found 518.3792.

(*S*)-1-((2*R*,3*R*)-3-Hexyl-4-oxooxetan-2-yl)tridecan-2-yl formyl-L-leucinate (*ent*-4)[5]



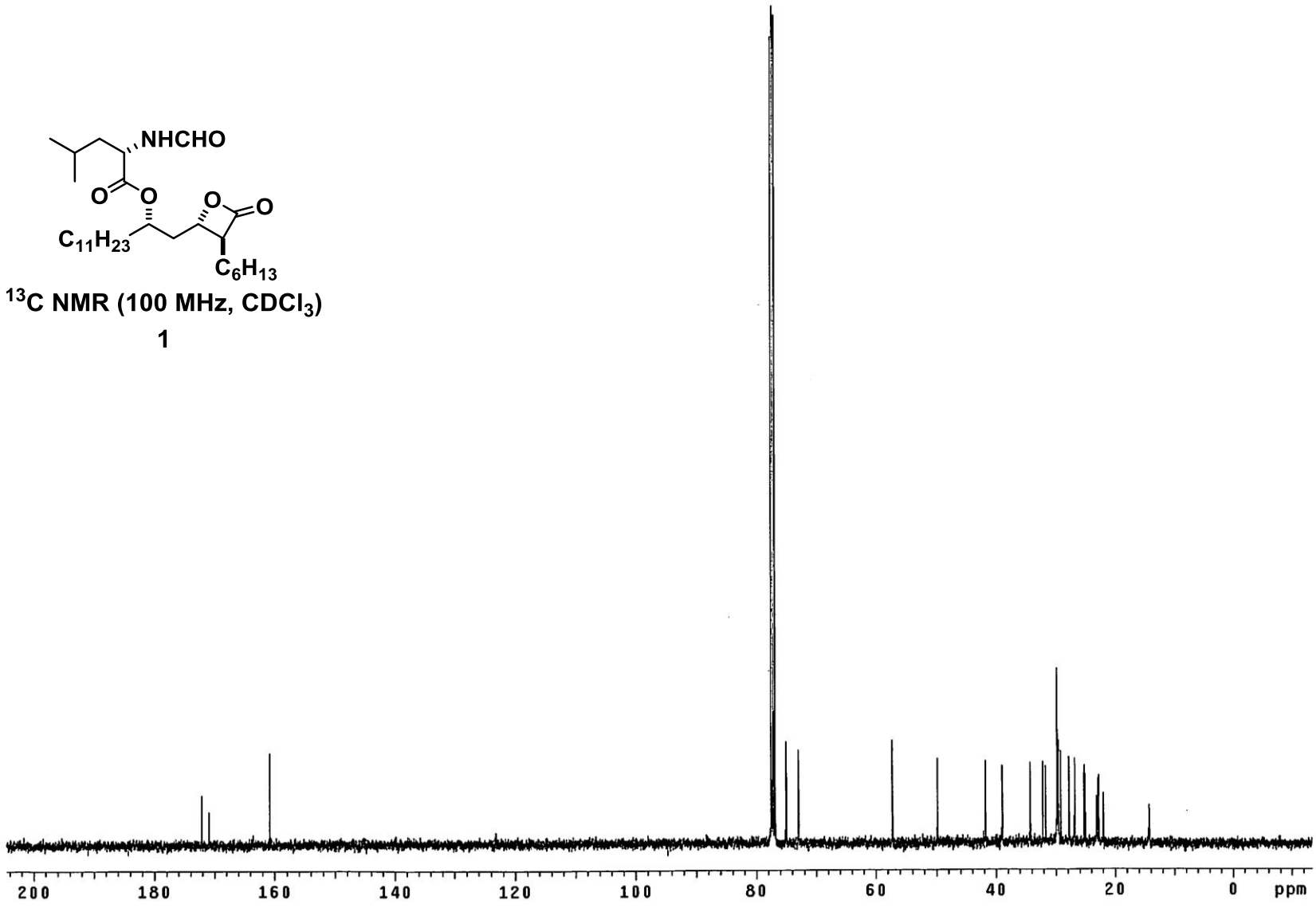
Data for *ent*-**4**: R_f = 0.27 (30% EtOAc in hexanes); $[\alpha]_D^{21}$ = +12.6 ($CHCl_3$, *c* = 0.55); IR (neat) 3311, 2926, 2857, 1823, 1739, 1673, 1521, 1462, 1380, 1251, 1193, 1122 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 5.94 (d, *J* = 8.5 Hz, 1H), 5.01(ddd, *J* = 7.0, 7.0, 5.5, 5.5 , 1H), 4.71 (ddd, *J* = 9.0, 9.0, 4.5 Hz, 1H), 4.26 (ddd, *J* = 8.5, 4.5, 4.5 Hz, 1H), 3.24 (ddd, *J* = 7.0, 7.0, 4.0 Hz, 1H), 2.09–2.00 (m, 2H), 1.86–1.79 (m, 1H), 1.76–1.63 (m, 4H), 1.61–1.54 (m, 2H), 1.47–1.41 (m, 1H), 1.38–1.24 (m, 25H), 0.972 (d, *J* = 6.0 Hz, 3H), 0.960 (d, *J* = 6.0 Hz, 3H), 0.883 (t, *J* = 7.0 Hz, 3H), 0.875 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 170.9, 160.8, 74.4, 72.7, 56.8, 49.8, 41.9, 39.1, 34.3, 32.1, 31.7, 29.8 (2C), 29.7, 29.6, 29.52, 29.50, 29.1, 27.9, 27.0, 25.2, 25.1, 23.0, 22.9, 22.7, 22.1, 14.3, 14.2; MALDI-TOF/CCA-HRMS calcd for $C_{29}H_{53}NO_5Na$ [M + Na]$^+$: 518.3816, found 518.3801.

Section C: ^{1}H NMR, ^{13}C NMR spectra



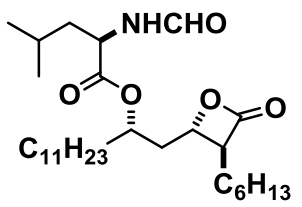
^{1}H NMR (500 MHz, $CDCl_3$)

1



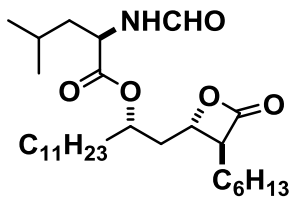
^{13}C NMR (100 MHz, $CDCl_3$)

1



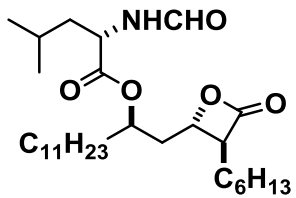
^{1}H NMR (500 MHz, $CDCl_3$)

2



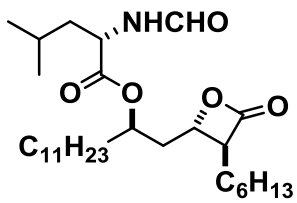
^{13}C NMR (100 MHz, $CDCl_3$)

2



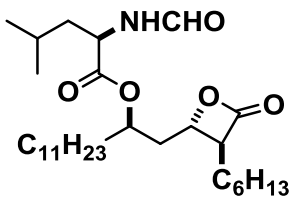
^{1}H NMR (500 MHz, $CDCl_3$)

3



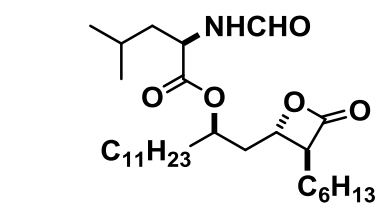
^{13}C NMR (100 MHz, $CDCl_3$)

3



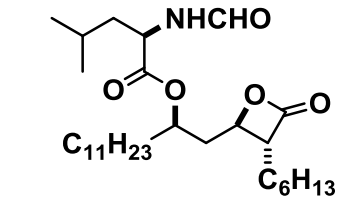
^{1}H NMR (500 MHz, $CDCl_3$)

4



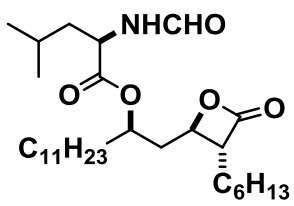
^{13}C NMR (100 MHz, $CDCl_3$)

4



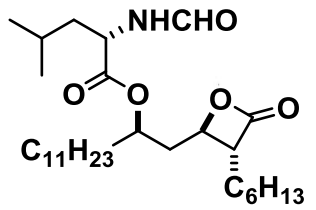
^{1}H NMR (400 MHz, $CDCl_3$)

***ent*-1**



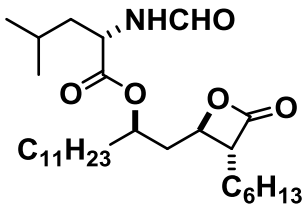
^{13}C NMR (100 MHz, $CDCl_3$)

***ent*-1**



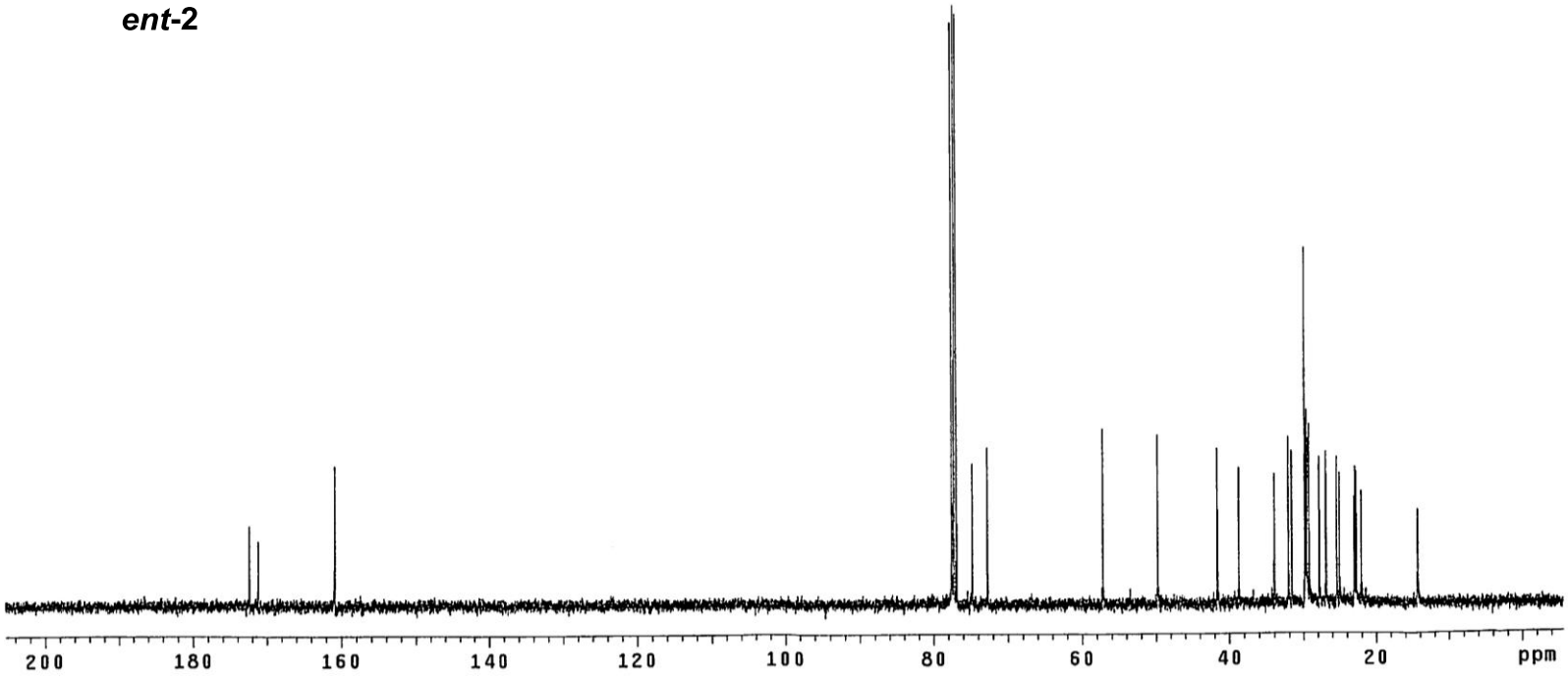
^{1}H NMR (400 MHz, $CDCl_3$)

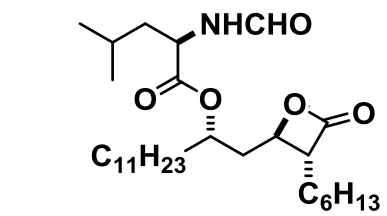
***ent*-2**



^{13}C NMR (100 MHz, $CDCl_3$)

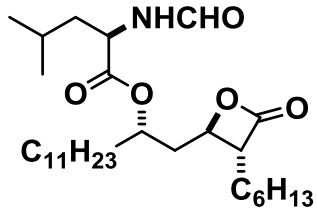
***ent*-2**





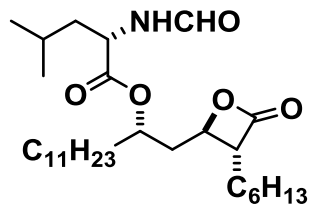
^{1}H NMR (500 MHz, $CDCl_3$)

***ent*-3**



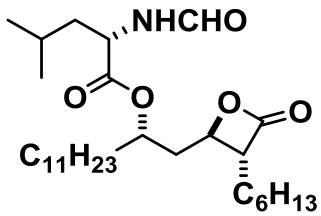
^{13}C NMR (100 MHz, $CDCl_3$)

***ent*-3**



^{1}H NMR (500 MHz, $CDCl_3$)

***ent*-4**



^{13}C NMR (100 MHz, $CDCl_3$)

***ent*-4**