Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Synthesis of Ramariolide Natural Products and Discovery of their Targets in Mycobacteria

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General: All reactions with air- or moisture-sensitive substances were carried out using standard SCHLENK techniques with Argon (Ar 4.6) as inert gas. Unless indicated otherwise, glass equipment was dried under high vacuum (10^{-3} mbar) at 500–600 °C using a heat gun. All chemical reagents and solvents were of reagent grade or of higher purity and used without further purification as obtained from commercial sources. Analytical thin-layer chromatography (TLC) was performed on MERCK silica gel 60 F_{254} glass-baked plates, which were analyzed by fluorescence detection with UV-light (λ = 254 nm, 366 nm, [UV]), as well as exposure to $KMnO_4$ staining reagent and subsequent heat treatment. Reverse-phase HPLC analysis was performed on a WATERS 2695 separation module, equipped with a WATERS PDA 2996 and a WATERS XBridge C18 column (3.5 mm, 4.6 x 100 mm, flow = 1.2 mL/min). For preparative scale RP-HPLC separation a WATERS 2545 quarternary gradient module in combination with a WATERS PDA 2998 and a WATERS XBridge C18 (5.0 µm, 30 x 150 mm, flow = 50 mL/min) column or a YMC Triart C18 (3.5 µm, 10 x 250 mm, flow = 10 mL/min) column was used. The mobile phase for elution consisted of a gradient mixture of 0.1 % (v/v) TFA in water (buffer A, HPLC grade) and 0.1 % (v/v) TFA in FLUKA acetonitrile (buffer B, HPLC grade). ^{1}H- and ^{13}C-NMR spectra were recorded with BRUKER 250, BRUKER Avance 360 and Avance 500 spectrometers. Chemical shifts (δ) were referred to the residual proton and carbon signal of the deuterated solvent ($CDCl_3$: 7.26 ppm, C_6D_6: 7.16 ppm, CD_2Cl_2: 5.32 ppm). Coupling constants (*J*) were reported in Hertz (Hz) and multiplicity is reported as follows: s – singulet, d – doublet, t – triplet, q – quartet, m – multiplet, br – broad. High-resolution mass spectra (HRMS) were obtained on a THERMO Scientific LTQ-FT Ultra by electronspray inonization (ESI-MS) or atmospheric-pressure chemical ionization (APCI-MS). Specific optical rotation spectra were obtained on a PERKIN-ELMER polarimeter 241 MC using a 1 dm cuvette at λ = 589 nm (Na-D-line) at RT. Substances were used in g/100 mL concentrated solutions. Optical rotations are annotated in 10^{-1} ° cm^2 g^{-1}. For SDS gel electrophoresis, fluorescence was recorded in a FUDJIFILM LAS-4000 luminescent image analyzer with a FUJINON VRF43LMD3 lens and a 575DF20 filter. All proteomics experiments were performed on Orbitrap Fusion Tribird mass spectrometer or LTQ Orbitrap XL mass spectrometer.

1. Supplementary Figures

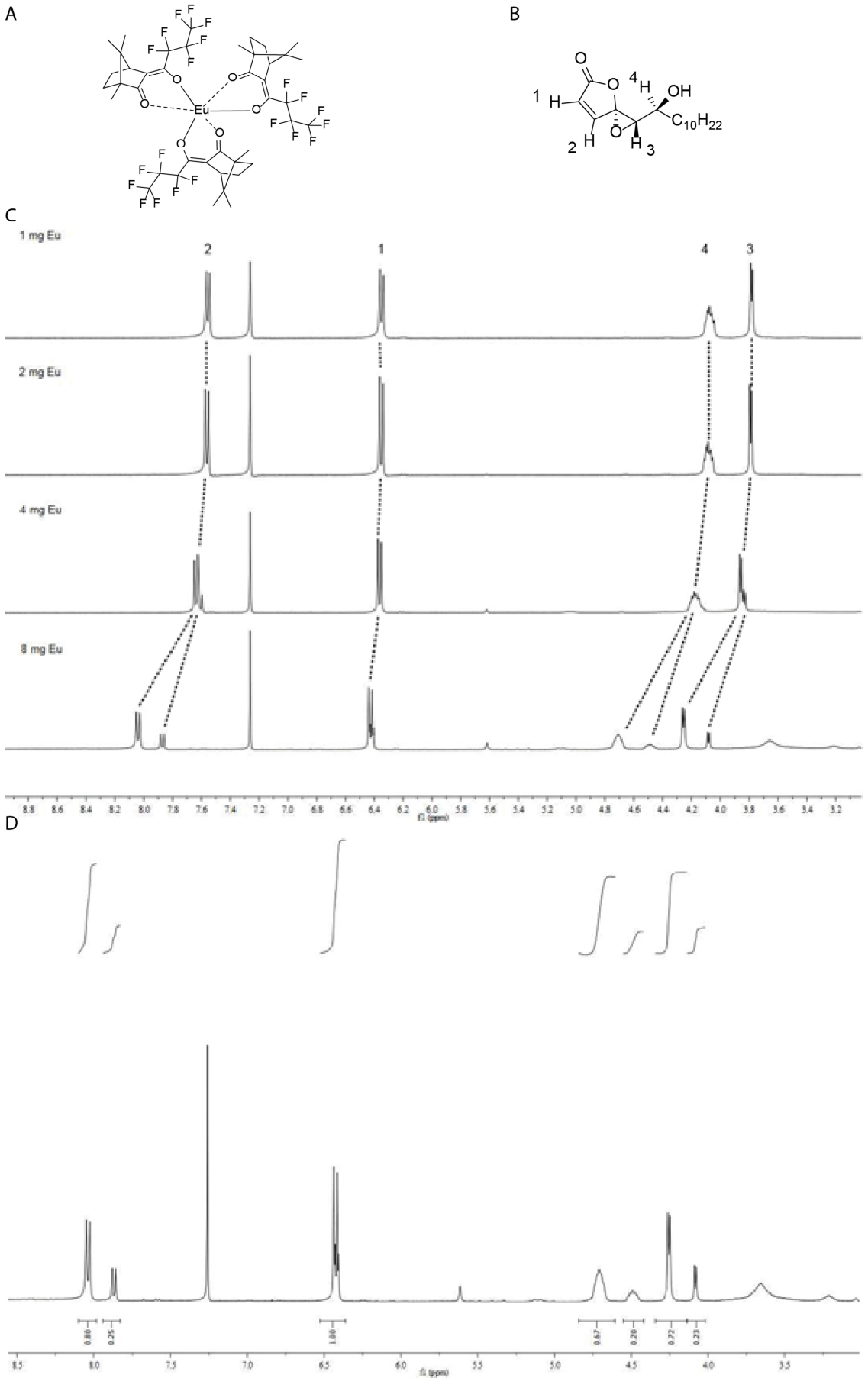
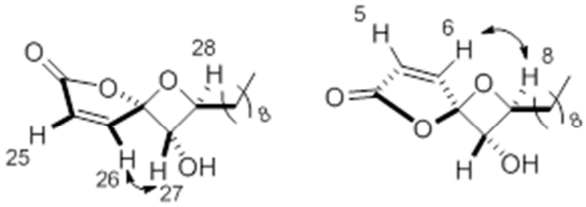


Figure S1: Determination of enantiomeric excess of (–)-ramariolide A by. **A.** Molecular structure of europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate) = Eu. **B.** Assignment of (–)-ramariolide A proton signals above 3.00 ppm in ^{1}H NMR ($CDCl_3$). **C.** ^{1}H NMR titration by subsequent addition of Eu to (–)-ramariolide A. **D.** Enlargement of ^{1}H NMR of enantiomeric mixture after addition of 8 mg Eu. Integration of individual signals gives an ee estimate of 50-55% of (–)-ramariolide A.

To a solution of excessive (–)-ramariolide A of an enantiomeric mixture in $CDCl_3$ were added subsequent amounts of europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate) indicated on each spectrum. Coordination of the chiral europium-salt to the oxygen rich head group of the molecule induced a diastereomeric environment and a downfield shift of specific signals of which enantiomeric excess could be determined by integration of individual signals:



2 **15**

Proton assignment of relevant signals for determination of epimeric compounds **2** and **15**.

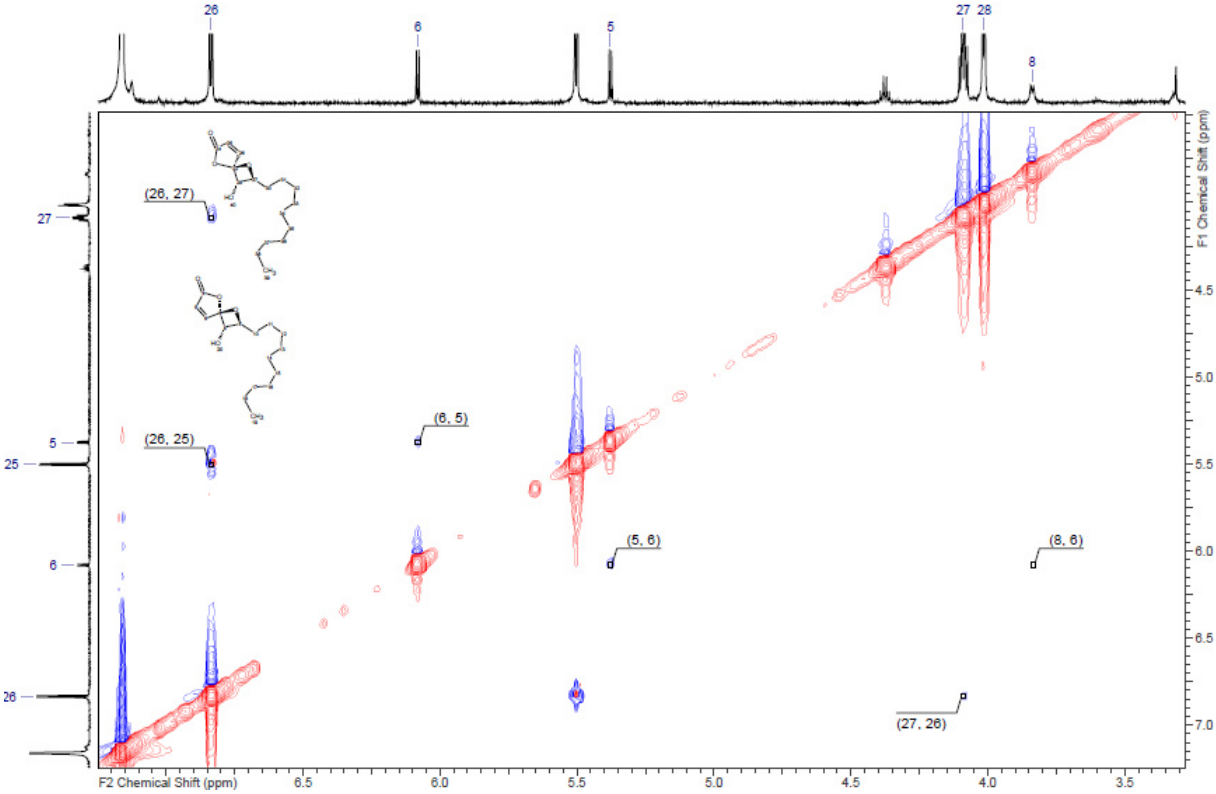


Figure S2: 2D NOE-couplings of epimeric spirocycles **2** and **15** in C_6H_6.

A diasteromeric mixture of compounds **2** and **15** was used to show proton coupling. While coupling of signals 25 to 26 and 5 to 6 were also obtained by COSY experiments, NOE showed clear indication of a coupling of signal 26 to 27 and 6 to 8.

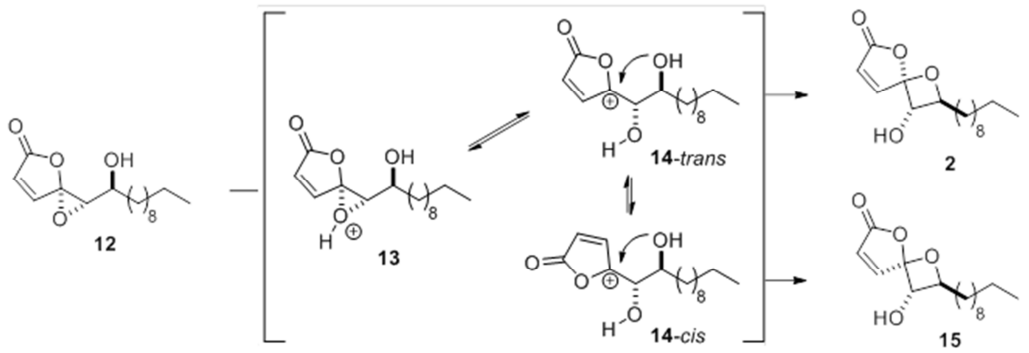


Figure S3: Proposed mechanism for the Brønsted acid-mediated rearrangement of **12**.

Having the formation of the epimeric spirocycles confirmed by NOE data, it was proposed that upon protonation of the epoxide-oxygen (**13**) the oxirane ring opens to the stabilized cation (**14**-*trans*) and close the oxetane ring by an attack of the β-hydroxy group to give **2** or rotation of the butenolide ring to the *cis*-configured rotamer and closing to the epimeric spirocycle (**15**).

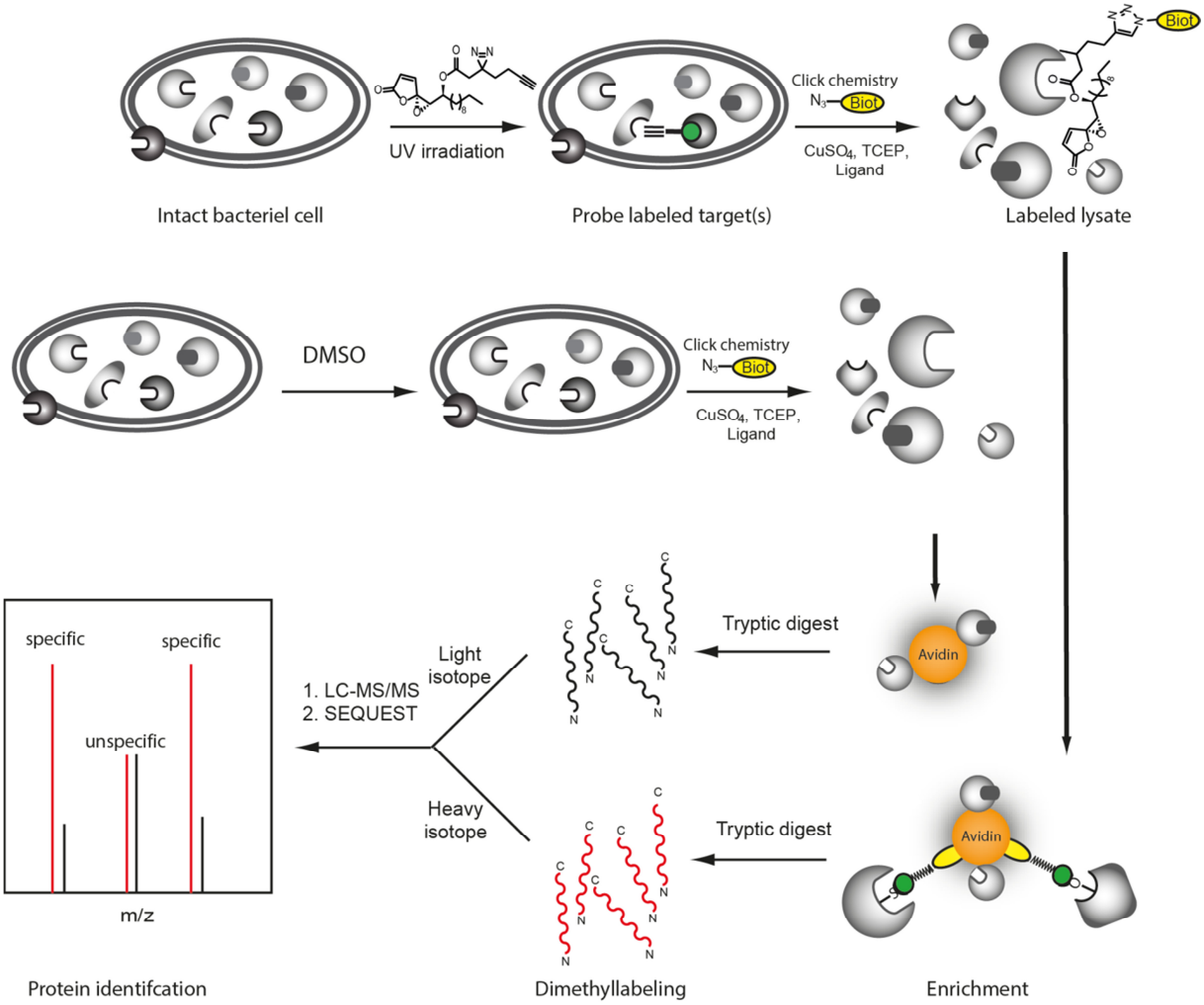


Figure S4: Schematic representation of gel–free activity based protein profiling.

Cells are either incubated with ramariolide UV-probe or DMSO for 1h and irradiated to form covalent binding. Afterwards cells are lysed and clicked to biotin azide and proteins are enriched on avidin beads. Tryptic digest was performed to release free peptides from beads, which were subsequently isotope labeled using dimethyl labeling. Modified peptides of probe and control trials were combined and measures by LC-MS/MS.

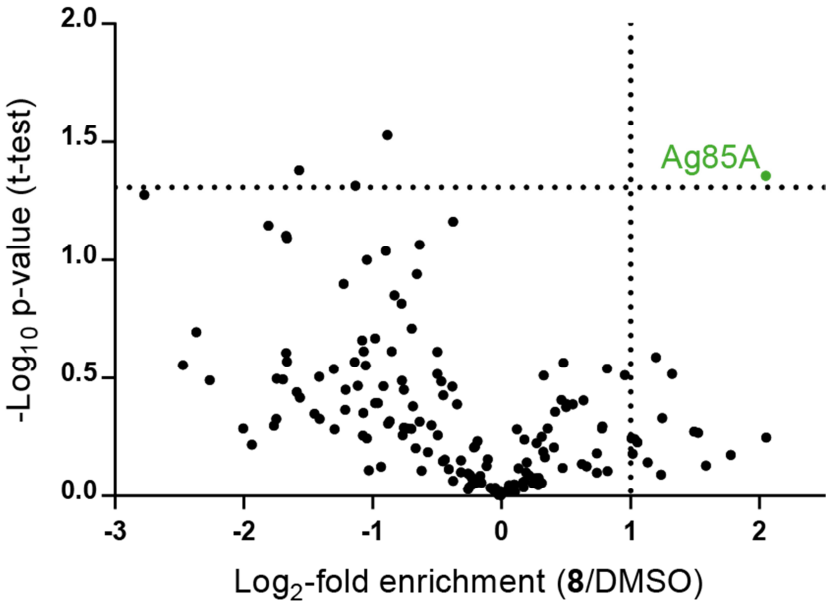


Figure S5: Scatter plot of gel-free ABPP dimethyl labeling experiments with 30 µM **8** vs. DMSO in *M. smegmatis* mc^{2}155 insoluble fraction after UV-irradiation. Green dots depict protein hits non-essential for bacterial growth. Criteria: $\log_2$-fold enrichment > 1 and p-value < 0.05. Data derived from three biological replicates with technical triplicates calculated using a two-sided one sample Student's *t*-test with Perseus.

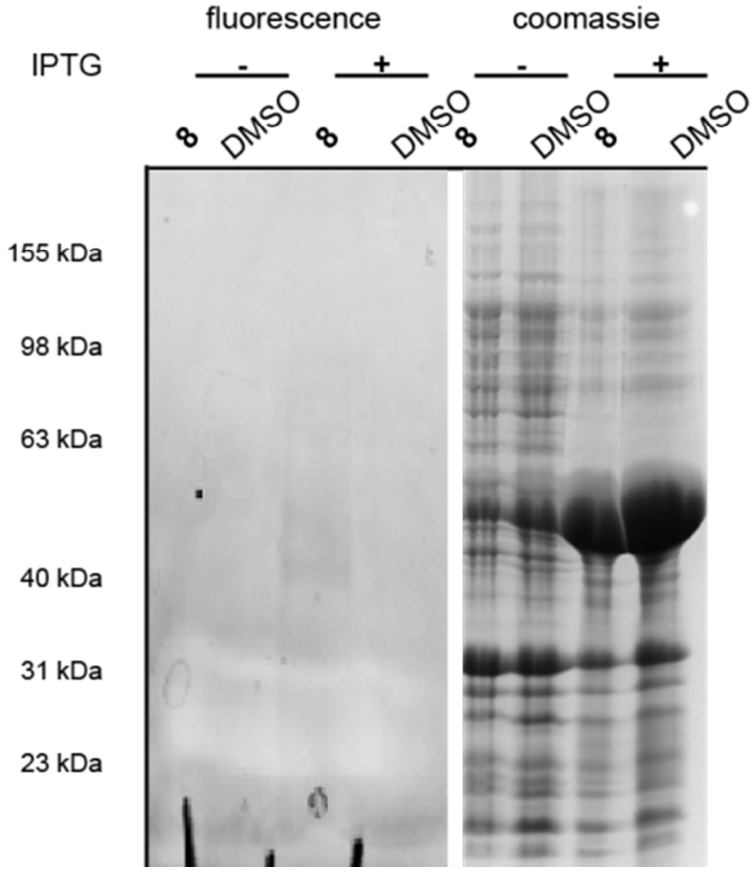


Figure S6: Gel-based APBB of mycobacterial ClpX overexpressed in *E. coli* using 30 µM **8** without UV-irradiation. Fluorescence scan after incubation in the dark and application of click-chemistry using rhodamine-azide. "+" indicates induction of protein expression by addition of IPTG; "–" represents absence of inducer. IPTG = isopropyl-β-D-thiogalactopyranosid.

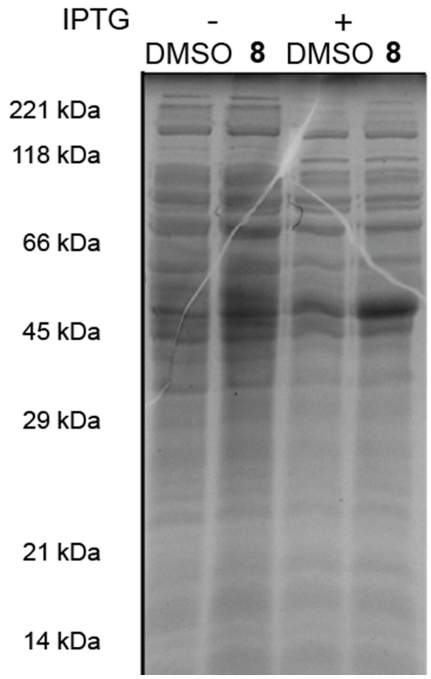


Figure S7: Coomassie stain of mycobacterial Ask overexpressed in *E. coli* (Vgl. Fig. 2). "+" indicates induction of protein expression by addition of IPTG; "-" represents absence of inducer. IPTG = isopropyl-β-D-thiogalactopyranosid.

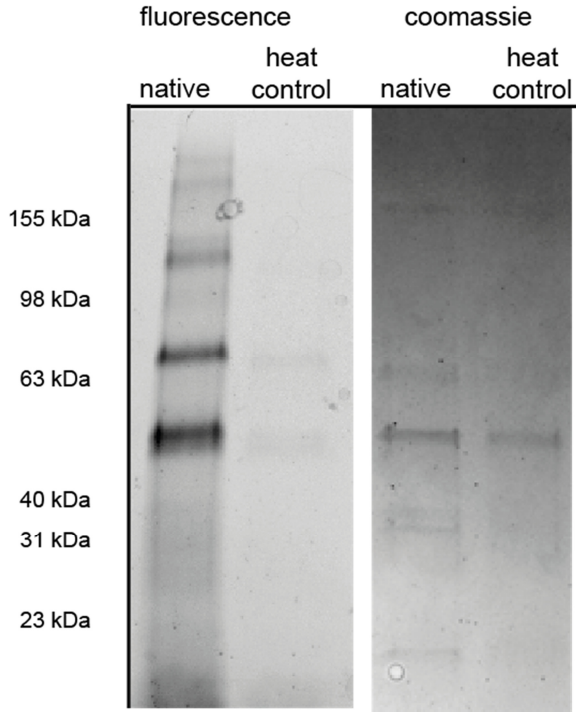
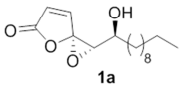
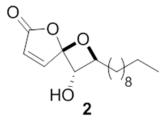
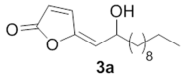
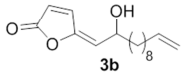
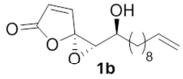
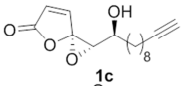
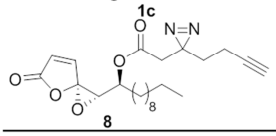


Figure S8: *In-vitro* labeling of purified mycobacterial Ask using 30 µM **8** without UV-irradiation under native and heat denatured conditions. Fluorescence scan after incubation in the dark and application of click-chemistry using rhodamine-azide.

Table S1: Overview of MICs in various bacterial strains.

compound	*S. aureus* USA 300	*L. monocytogenes* EGD-e	*M. smegmatis* mc(2) 155	*M tuberculosis* H37Rv	*E. faecalis* V583	*P. aeruginosa* PAO1
 **1a**	10 µM	3 µM	30 µM	25 µM	30 µM	> 100 µM
 **2**	30 µM	-	30 µM	-	-	> 100 µM
 **3a**	30 µM	30 µM	100 µM	-	100 µM	> 100 µM
 **3b**	30 µM	-	100 µM	-	-	> 100 µM
 **1b**	30 µM	-	30 µM	-	-	> 100 µM
 **1c**	> 100 µM	-	> 100 µM	-	-	> 100 µM
 **8**	10 µM	-	30 µM	-	-	> 100 µM

Minimal inhibitory concentration of each compound was determined as described in Methods section in 96-well plate. Growth was detected by optical density or resazurin assay. Not determined MICs are labeled "–".

Table S2: Protein hits without essential function for bacterial growth in mycobacteria.

entry	Gene name	Uniprot accession number
1	Msmeg_1028	A0QR89
2	Msmeg_1305	A0QS07
3	Msmeg_0309	A0QP86
4	Msmeg_0067	A0QNJ7
5	devR	A0R2V2
6	suhB	A0QW04
7	gspA	A0QUZ5
8	Msmeg_4686	A0R1A8
9	Msmeg_4272	A0R061

Entries 1-4 were found for UV-irradiated enrichment; 5-9 were found for enrichment without UV-irradiation.

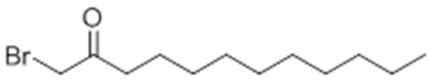
2. Chemical Synthesis

2.1. Synthetic procedures

General procedure for α-bromo ketones[1, 2]

Carboxylic acid (1.00 eq) was dissolved in CH_2Cl_2 (0.5 M) and DMF (0.05 eq) was added dropwise at rt. Oxalyl chloride (1.50 eq) was added dropwise in portions while gas formation was observed. The reaction mixture was stirred for 3 h and concentrated under reduced pressure. The crude residue was dissolved in MeCN (0.5 M) and cooled to 0 °C. TMS diazomethane (1.10 eq, 2 M in Et_2O) was slowly added dropwise. The reaction was stirred for 16 h and warmed to rt. Solvents were removed *in-vacuo* and the remaining residue was dissolved in Et_2O (0.4 M) at rt. Hydrogen bromide (10.0 eq, 37% in H_2O) was slowly added and gas formation could be observed. The reaction mixture was heated under reflux for 3 h and stopped by addition of one volume of saturated $NaHCO_3$ solution. After separation of phases the aqueous layer was extracted with EtOAc (3 times 50 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness under reduced pressure. The crude residue was applied to flash chromatography. The product was isolated as a white wax.

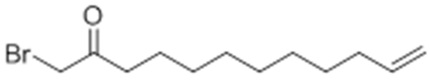
1-Bromododecon-2-one (**5a**)



Chemical Formula: $C_{12}H_{23}BrO$
Exact Mass: 262,0932

Material: undecanoic acid (4.06 g, 21.8 mmol), oxalyl chloride (2.8 mL, 4.15 g, 32.7 mmol), TMS diazomethane (12 mL, 24 mmol), HBr (14.4 mL, 218 mmol).
Yield: 96% (5.46 g, 20.8 mmol).
Purification: flash chromatography – hexan/EtOAc = 98/2.
R_f = 0.32 (Pentane/Et_2O = 98/2).
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 3.88 (s, 2 H), 2.64 (t, *J* = 7.4 Hz, 2 H), 1.66-1.56 (m, 2 H), 1.35-1.18 (m, 14 H), 0.87 (t, *J* = 6.6 Hz, 3 H).
^{13}C-NMR (91 MHz, $CDCl_3$): δ [ppm] = 202.5, 40.0, 34.4, 32.0, 29.6, 29.5, 29.47, 29.45, 29.2, 24.0, 22.8, 14.3.
HRMS (APCI): calc. for $C_{12}H_{24}BrO$ $[M+H]^+$: 263.1005; found: 263.1008.

1-Bromododec-11-en-2-one (**5b**)



Chemical Formula: $C_{12}H_{21}BrO$
Exact Mass: 260,0776

Material: 10-undecenoic acid (3.04 g, 15 mmol), oxalyl chloride (1.93 mL, 2.86 g, 22.5 mmol) TMS diazomethane (8.25 mL, 16.5 mmol), HBr (9.90 mL, 150 mmol).
Yield: 94% (3.68 g, 14.1 mmol).
Purification: flash chromatography – hexan/EtOAc = 98/2

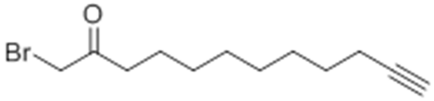
***R*$_f$** = 0.34 (Pentane/Et_2O = 98/2).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 5.81 (ddt, *J* = 6.7, 10.1, 16.9 Hz, 1 H), 5.03- 4.89 (m, 2 H), 3.88 (s, 2H), 2.64 (t, *J* = 7.4 Hz, 2 H), 2.08-1.98 (m, 2 H), 1.63-1.55 (m, 2 H), 1.39-1.27 (m, 10 H).

^{13}C-NMR (76 MHz, $CDCl_3$): δ [ppm] = 202.4, 139.3, 114.3, 40.0, 34.5, 33.9, 29.4, 29.3, 29.2, 29.0, 24.0, 23.7.

HRMS (APCI): calc. for $C_{12}H_{22}BrO$ $[M+H]^+$: 261.0849; found: 261.0841.

1-Bromododec-11-yn-2-one (**5c**)



Chemical Formula: $C_{12}H_{19}BrO$
Molecular Weight: 259,19

Material: 10-undecynoic acid (5.00 g, 27.4 mmol), oxalyl chloride (3.53 mL, 5.20 g, 41.1 mmol) TMS diazomethane (15.1 mL, 30.2 mmol), HBr (18.1 mL, 274 mmol).

Yield: 92% (6.52 g, 25.2 mmol).

***R*$_f$** = 0.35 (Pentane/Et_2O = 98/2).

Purification: flash chromatography – hexan/EtOAc = 98/2.

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 3.87 (s, 2 H), 2.64 (t, *J* = 7.4 Hz, 2 H), 2.17 (td, *J* = 2.6, 7.0 Hz, 2 H), 1.93 (t, *J* = 2.6 Hz, 1 H), 1.68-1.44 (m, 4 H), 1.45-1.20 (m, 8 H).

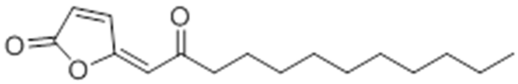
^{13}C-NMR (63 MHz, $CDCl_3$): δ [ppm] = 202.3, 84.8, 68.2, 39.9, 34.4, 29.3, 29.1, 29.0, 28.8, 28.6, 24.0, 18.5.

HRMS (APCI): calc. for $C_{12}H_{20}BrO$ $[M+H]^+$: 259.0692; found: 259.0691.

General procedure for 5-(2-Oxododecylidene)furan-2(*5H*)-ones[3-5]

α-Bromo ketone (1.00 eq) was dissolved in $CHCl_3$ (0.2 M) at rt and PPh_3 (1.10 eq) was added. The clear solution turned turbid within 30 min and was stirred for 16 h. Solvents were removed under reduced pressure and the residue was dissolved in MeOH/H_2O (v/v = 4/1, 0.4 M). KOH (10 eq, 5 M in H_2O) was added at rt. The reaction mixture immediately turned into a yellow suspension, which was rigorously stirred for 3 h. After separation of layers the aqueous phase was extracted with CH_2Cl_2 (3 times 40 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness under reduced pressure. The waxy residue was dissolved in toluene (0.02 M) and maleic anhydride (1.20 eq) was added at rt. The reaction mixture was heated to 110 °C and refluxed for 16 h. The reaction was allowed to cool down to rt and solvents were removed *in-vacuo*. The residue was applied to flash chromatography to yield the corresponding (*E*)- and (*Z*)- constituted olefins as white solids.

(*E*)-5-(2-Oxododecylidene)furan-2(*5H*)-one (**6a**)



Chemical Formula: $C_{16}H_{24}O_3$
Exact Mass: 264,17

Material: 1-bromododecon-2-one (5.46 g, 20.8 mmol), triphenyl phosphine (6.0 g, 22.9 mmol), maleic anhydride (2.47 g, 25.0 mmol).

Yield: 45% (2.49 g, 9.42 mmol).

R_f = 0.22 (hexane/EtOAc = 92/8).

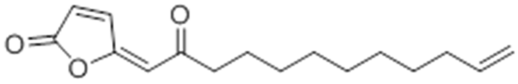
Purification: precipitation (two times), flash chromatography: hexane/EtOAc = 92/8.

^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 8.32 (dd, *J* = 0.7, 5.6 Hz, 1 H), 6.47 (dd, *J* = 1.7, 5.6 Hz, 1 H), 6.24 (dd, *J* = 0.7, 1.7 Hz, 1 H), 2.58 (t, *J* = 7.4 Hz, 2 H), 1.67-1.55 (m, 2 H), 1.34-1.20 (m, 14 H), 0.87 (t, *J* = 6.8 Hz, 3 H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 200.1, 168.2, 158.7, 143.0, 125.2, 107.9, 45.3, 32.0, 29.7, 29.6, 29.5, 29.4, 29.2, 24.1, 22.8, 14.3.

HRMS (ESI): calc. for $C_{16}H_{25}O_3$ $[M+H]^+$: 267.1798; found: 267.1796.

(*E*)-5-(2-Oxododec-11-en-ylidene)furan-2(*5H*)-one (**6b**)



Chemical Formula: $C_{16}H_{22}O_3$
Exact Mass: 262,16

Material: 1-bromododec-11-en-2-one (3.68 g, 14.1 mmol), triphenyl phosphine (4.07 g, 15.5 mmol), maleic anhydride (1.67 g, 16.9 mmol).

Yield: 42% (1.56 g, 5.95 mmol)

R_f = 0.26 (hexane/EtOAc = 90/10).

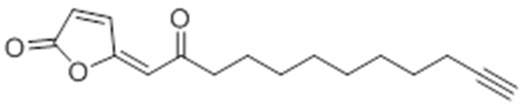
Purification: precipitation (two times), flash chromatography: hexane/EtOAc = 90/10.

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.32 (dd, *J* = 0.7, 5.6 Hz, 1 H), 6.47 (dd, *J* = 1.7, 5.6 Hz, 1 H), 6.24 (dd, *J* = 0.7, 1.7 Hz, 1 H), 5.81 (ddt, *J* = 6.7, 10.1, 17.0 Hz, 2 H), 5.04-4.88 (m, 1 H), 2.60 (t, *J* = 7.4 Hz, 2 H), 2.09-1.98 (m, 2 H), 1.67-1.55 (m, 2 H), 1.34-1.20 (m, 12 H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 200.0, 168.2, 158.7, 143.0, 139.3, 125.2, 114.3, 107.9, 45.3, 33.9, 29.5, 29.4, 29.2, 29.2, 29.0, 24.1.

HRMS (ESI): calc. for $C_{16}H_{23}O_3$ $[M+H]^+$: 263.1642; found: 263.1644.

(*E*)-5-(2-Oxododec-11-yn-ylidene)furan-2(*5H*)-one (**6c**)



Chemical Formula: $C_{16}H_{20}O_3$
Exact Mass: 260,14

Material: 1-bromododec-11-yn-2-one (6.52 g, 25.2 mmol), triphenyl phosphine (7.27 g, 27.7 mmol), maleic anhydride (3.0 g, 30.2 mmol).

Yield: 37% (2.39 g, 9.2 mmol)

R_f = 0.29 (hexane/EtOAc = 90/10).

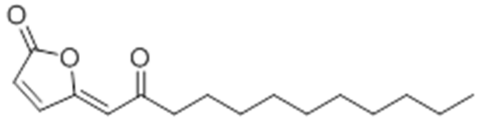
Purification: precipitation (two times), flash chromatography: hexane/EtOAc = 90/10.

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.32 (dd, *J* = 0.7, 5.6 Hz, 1 H), 6.47 (dd, *J* = 1.7, 5.6 Hz, 1 H), 6.23 (dd, *J* = 0.7, 1.7 Hz, 1 H), 2.58 (t, *J* = 7.4 Hz, 2 H), 2.18 (dt, *J* = 2.6 Hz, 7.1, 2 H), 1.93 (t, *J* = 2.6 Hz, 1 H), 1.68-1.60 (m, 2 H), 1.55-1.49 (m, 4 H), 1.43-1.34 (m, 2 H) , 1.34-1.20 (m, 6 H).

^{13}C-NMR (90.6 MHz, $CDCl_3$): δ [ppm] = 199.9, 168.2, 158.8, 143.0, 125.2, 107.9, 83.7, 68.3, 45.3, 29.4, 29.2, 29.0, 28.8, 28.6, 24.1, 18.5.

HRMS (ESI): calc. for $C_{16}H_{21}O_3$ $[M+H]^+$: 261.1485; found: 261.1485.

(*Z*)-5-(2-Oxododecylidene)furan-2(*5H*)-one (**7a**)



Chemical Formula: $C_{16}H_{24}O_3$
Exact Mass: 264,17

Material: 1-bromododecon-2-one (5.46 g, 20.8 mmol), triphenyl phosphine (6.0 g, 22.9 mmol), maleic anhydride (2.47 g, 25.0 mmol).

Yield: 15% (819 mg, 3.1 mmol).

R_f = 0.30 (hexane/EtOAc = 70/30).

Purification: precipitation (two times), flash chromatography: hexane/EtOAc = 70/30.

^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.48 (d, *J* = 5.6 Hz, 1 H), 6.42 (d, *J* = 5.6 Hz, 1 H), 5.56 (s, 1 H), 2.87 (t, *J* = 7.3 Hz, 2 H), 1.66-1.58 (m, 2 H), 1.34-1.20 (m, 14 H), 0.86 (t, *J* = 7.1 Hz, 3 H).

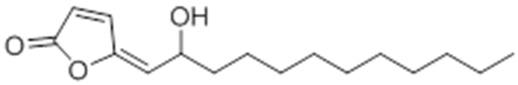
^{13}C-NMR (75 MHz, $CDCl_3$): δ [ppm] = 199.2, 168.0, 154.4, 145.8, 123.4, 110.7, 43.6, 32.0, 29.7, 29.6, 29.6, 29.5, 29.3, 24.0, 22.8, 14.3.

HRMS (ESI): calc. for $C_{16}H_{25}O_3$ $[M+H]^+$: 265.1798; found: 265.1796.

General procedure for 5-(2-Hydoxydodecylidene)furan-2(*5H*)-ones[6]

5-(2-Oxododecylidene)furan-2(*5H*)-one (1.00 eq) was dissolved in THF (1.0 M) at rt. $NaBH_3CN$ (5.00 eq, 2 M in THF) was added at rt and the reaction mixture was stirred for 1 h. The reaction was stopped by addition of HCl (15 mL, 2 M) and stirred for 1 h. The solution was diluted with EtOAc and after separation of layers the aqueous phase was extracted with EtOAc (3 times 15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in-vacuo*. The remaining residue was applied to flash chromatography to yield the desired alcohols as white waxes.

(±)-Ramariolide C (**3a**)



Chemical Formula: $C_{16}H_{26}O_3$
Exact Mass: 266,19

Material: (*E*)-5-(2-oxododecylidene)furan-2(*5H*)-one (560 mg, 2.12 mmol), sodium cyanoborohydride solution (5.3 mL,10.6 mmol).

Yield: 92% (521 mg, 1.96 mmol).

R_f = 0.24 (hexane/EtOAc = 70/30).

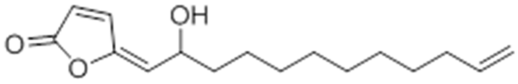
Purification: flash chromatography: hexane/EtOAc = 70/30.

^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.80 (d, *J* = 5.6 Hz, 1 H), 6.24 (dd, *J* = 1.7, 5.6 Hz, 1 H), 5.75 (dd, *J* = 1.7, 8.1 Hz, 1 H), 4.52 (dt, *J* = 6.5, 8.1 Hz, 1 H), 1.80 (bs, 1 H), 1.76-1.66 (m, 1 H), 1.66-1.57 (m, 1 H), 1.36-1.20 (m, 14 H), 0.87 (t, *J* = 6.8 Hz, 3 H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 169.5, 150.6, 140.8, 121.2, 117.6, 68.4, 38.1, 32.0, 29.7, 29.7, 29.6, 29.5, 29.4, 25.4, 22.8, 14.3.

HRMS (ESI): calc. for $C_{16}H_{27}O_3$ $[M+H]^+$: 267.1955; found: 267.1952.

(±)-Ramariolide D (**3b**)



Chemical Formula: $C_{16}H_{24}O_3$
Exact Mass: 264,17

Material: (*E*)-5-(2-oxododec-11-en-ylidene)furan-2(*5H*)-one (420 mg, 1.60 mmol), sodium cyanoborohydride solution (4.0 mL, 8.00 mmol).

Yield: 82% (346 mg, 1.31 mmol).

R_f = 0.29 (hexane/EtOAc = 65/35).

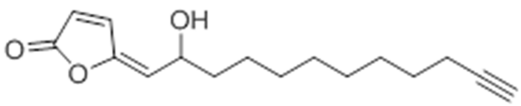
Purification: flash chromatography: hexane/EtOAc = 65/35.

^{1}H-NMR (500 MHz, C_6D_6): δ [ppm] = 6.98 (dd, *J* = 0.6, 5.6 Hz, 1 H), 5.81 (ddt, *J* = 6.7, 10.2, 17.0 Hz, 1 H), 5.57 (dd, *J* = 1.8, 5.6 Hz, 1 H), 5.36 (dd, *J* = 1.8, 7.9 Hz, 1 H), 5.06 (dq, *J* = 1.7, 17.0 Hz, 1 H), 5.01 (dt, *J* = 1.6, 10.2 Hz, 1 H), 3.94-3.88 (m, 1 H), 2.07-1.97 (m, 2 H), 1.38-1.31 (m, 4 H), 1.31-1.08 (m, 10 H).

^{13}C-NMR (126 MHz, C_6D_6): δ [ppm] = 167.9, 149.8, 139.3, 138.4, 120.2, 115.7, 113.7, 67.2, 37.4, 33.3, 29.2, 29.1, 29.1, 28.8, 28.6, 24.8.

HRMS (ESI): calc. for $C_{16}H_{25}O_3$ $[M+H]^+$: 265.1798; found: 265.1798.

(±)-(*E*)-5-(2-Hydroxyododec-11-yn-ylidene)furan-2(*5H*)-one (**3c**)



Chemical Formula: $C_{16}H_{22}O_3$
Exact Mass: 262,16

Material: (*E*)-5-(2-oxododec-11-yn-ylidene)furan-2(*5H*)-one (390 mg, 1.50 mmol), sodium cyanoborohydride solution (3.75 mL, 7.50 mmol).

Yield: 72% (284 mg, 1.08 mmol)

R_f = 0.27 (hexane/EtOAc = 65/35).

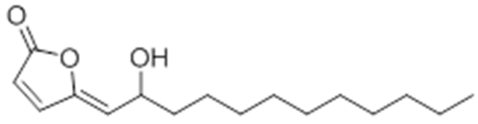
Purification: flash chromatography: hexane/EtOAc = 65/35.

^{1}H-NMR (250 MHz, $CDCl_3$): δ [ppm] = 7.80 (dd, *J* = 0.7, 5.6 Hz, 1 H), 6.25 (dd, *J* = 1.8, 5.6 Hz, 1 H), 5.75 (ddd, *J* = 0.7, 1.8, 8.2 Hz, 1 H), 4.52 (dt, *J* = 6.5, 8.2 Hz, 1 H), 2.18 (td, *J* = 2.6, 6.9 Hz, 2 H), 1.94 (t, *J* = 2.6 Hz, 1 H), 1.78-1.43 (m, 4 H), 1.43-1.18 (m, 10 H).

^{13}C-NMR (91 MHz, $CDCl_3$): δ [ppm] = 169.4, 150.7, 140.8, 121.3, 117.5, 84.9, 68.4, 68.3, 38.1, 29.5, 29.1, 28.8, 28.6, 25.4, 18.5.

HRMS (ESI): calc. for $C_{16}H_{23}O_3$ $[M+H]^+$: 263.1642; found: 263.1642.

(±)-(*Z*)-5-(2-Hydroxydodecylidene)furan-2(*5H*)-one (**9**)



Chemical Formula: $C_{16}H_{26}O_3$
Exact Mass: 266,19

Material: (*Z*)-5-(2-oxododecylidene)furan-2(*5H*)-one (490 mg, 1.85 mmol), sodium cyanoborohydride solution (4.63 mL, 9.25 mmol).

Yield: 94% (462 mg, 1.74 mmol)

R_f = 0.22 (hexane/EtOAc = 70/30).

Purification: flash chromatography: hexane/EtOAc = 70/30.

^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.37 (d, *J* = 5.4 Hz, 1 H), 6.22 (d, *J* = 5.4 Hz, 1 H), 5.32 (d, *J* = 8.4 Hz, 1 H), 4.82-4.76 (m, 1 H), 1.92 (bs, 1 H), 1.74-1.69 (m, 1 H), 1.63-1.54 (m, 1 H), 1.36-1.21 (m, 14 H), 0.87 (t, *J* = 6.8 Hz, 3 H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 169.5, 149.0, 144.0, 120.5, 118.5, 67.2, 37.2, 32.0, 29.7, 29.7, 29.7, 29.6, 29.5, 25.3, 22.8, 14.3.

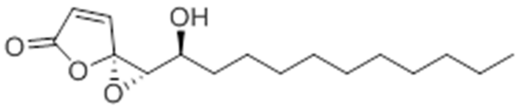
HRMS (ESI): calc. for $C_{16}H_{27}O_3$ $[M+H]^+$: 267.1955; found: 267.1961.

General procedure for 1-Hydroxyundecyl)-1,4-dioxaspiro[2.4]hept-6-en-5-ones[7]

(*E*)-5-(2-Hydoxydodecylidene)furan-2(*5H*)-one (1.00 eq) was dissolved in CH_2Cl_2 (0.5 M) at –32 °C. Titatnium-(IV)-isopropoxide (1.20 eq) and (+)-DIPT (1.40 eq) were added and the reaction mixture was stirred for 30 min before *t*-BuO_2H (4.50 eq, 5.5 M in hexane) was added. The reaction was allowed to warm to rt under stirring for 16 h. The reaction was stopped by addition of one volume of saturated potassium tartrate solution, freezing and re-thawing. After separation of layers the aqueous phase was extracted with CH_2Cl_2 (3 times 10 mL) and the organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness under reduced pressure. The remaining residue was applied to flash chromatography followed by RP-HPLC to yield *anti*- and *syn*-diastereomer as a white wax. The *anti*-diastereomer of ramariolide A was applied to optical rotation spectroscopy and the *syn*-diasteromer was not analyzed in more detail. Enantiomeric excess

(%ee) was determined by NMR-titration using europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate).

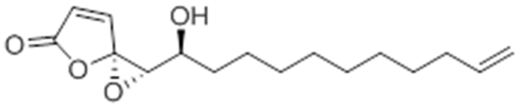
(–)-Ramariolide A (**1a**)



Chemical Formula: $C_{16}H_{26}O_4$
Exact Mass: 282,18

Material: (±)-ramariolide C (100 mg, 0.38 mmol), titatnium-(IV)-isopropoxide (67 µL, 128 mg, 0.46 mmol) (+)-diisopropyl tartrate (55 µL, 122 mg, 0.52 mmol), *tert*-butyl peroxide (310 µL, 1.72 mmol).
Yield: 35% (37 mg, 0.13 mmol/d.r. anti/syn = 87/13, ee = ~55 %).
R_f = 0.31 (hexane/EtOAc = 70/30).
Purification: flash chromatography: hexane/EtOAc = 70/30, RP-HPLC (preparative setup, method: gradient 2% B → 98% B over 10 min): t_R = 9.53 min.
^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.55 (d, *J* = 5.7 Hz, 1 H), 6.35 (d, *J* = 5.7 Hz, 1 H), 4.08 (ddd, *J* = 3.2, 4.4, 7.7 Hz, 1 H), 3.78 (d, *J* = 3.2 Hz, 1 H), 1.93 (bs, 1 H), 1.70-1.56 (m, 2 H), 1.56-1.43 (m, 2 H), 1.35-1.20 (m, 14 H), 0.87 (t, *J* = 6.8 Hz, 3 H).
^{13}C-NMR (101 MHz, $CDCl_3$): δ [ppm] = 168.5, 149.2, 126.4, 89.9, 67.6, 64.9, 33.7, 32.0, 29.7, 29.7, 29.6, 29.6, 29.4, 25.3, 22.8, 14.3.
HRMS (ESI): calc. for $C_{16}H_{25}O_4$ $[M-H]^-$: 281.1758; found: 281.1763.
$[\alpha]^{25}_D$ -76.7 (*c* 5.4, MeOH); (literature: **$[\alpha]^{25}_D$** -42 (*c* 5.4, MeOH)).

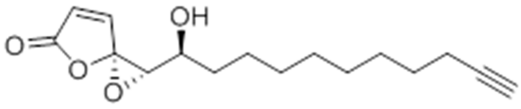
(2*S*,3*S*)-2-((*S*)-1-Hydroxyundec-10-en-1-yl)-1,4-dioxaspiro[2.4]hept-6-en-5-one (**1b**)



Chemical Formula: $C_{16}H_{24}O_4$
Exact Mass: 280,17

Material: (±)-ramariolide D (60 mg, 0.23 mmol), titatnium-(IV)-isopropoxide (80 µL, 77 mg, 0.27 mmol) (+)-diisopropyl tartrate (60 µL, 72 mg, 0.35 mmol), *tert*-butyl peroxide (20 µL, 1.1 mmol).
Yield: 27% (17.3 mg, 0.062 mmol).
R_f = 0.30 (hexane/EtOAc = 70/30).
Purification: flash chromatography: hexane/EtOAc = 70/30, RP-HPLC (analytical setup, method: gradient 2% B → 98% B over 10 min): t_R = 9.48 min.
^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.44 (d, *J* = 5.7 Hz, 1 H), 6.39 (d, *J* = 5.7 Hz, 1 H), 5.81 (ddt, *J* = 6.7, 10.2, 17.0 Hz, 1 H), 4.99 (dt, *J* = 1.8, 17.0 Hz, 1 H), 4.95-4.88 (m, 1 H), 3.89-3.82 (m, 1 H), 3.78 (d, *J* = 4.0 Hz, 1 H), 2.04 (q, *J* = 7.0, 7.2 Hz, 2 H), 1.74-1.58 (m, 4 H), 1.37-1.23 (m, 10 H).
^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 162.2, 148.5, 139.3, 126.9, 114.3, 110.1, 90.4, 68.4, 65.5, 34.9, 33.9, 29.5, 29.5, 29.2, 29.0, 25.1.
HRMS (ESI): calc. for $C_{16}H_{25}O_4$ $[M+H]^+$: 281.1747; found: 281.1748.

(2*S*,3*S*)-2-((*S*)-1-Hydroxyundec-10-yn-1-yl)-1,4-dioxaspiro[2.4]hept-6-en-5-one (**1c**)



Chemical Formula: $C_{16}H_{22}O_4$
Exact Mass: 278,15

Material: (±)-(*E*)-5-(2-hydroxyododec-11-yn-ylidene)furan-2(*5H*)-one (100 mg, 0.38 mmol), titatnium-(IV)-isopropoxide (140 µL, 130 mg, 0.46 mmol) (+)-diisopropyl tartrate (110 µL, 125 mg, 0.53 mmol), *tert*-butyl peroxide (310 µL, 1.7 mmol).

Yield: 13% (17.5 mg, 0.063 mmol).

R_f = 0.30 (hexane/EtOAc = 70/30).

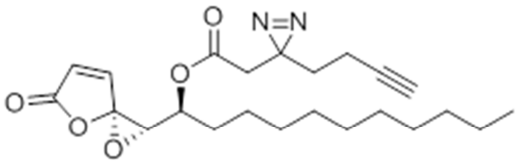
Purification: flash chromatography: hexane/EtOAc = 70/30, RP-HPLC (analytical setup, method: gradient 2% B → 98% B over 10 min): t_R = 9.59 min.

^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.56 (d, *J* = 5.7 Hz, 1 H), 6.36 (d, *J* = 5.7 Hz, 1 H), 4.09 (ddd, *J* = 3.1, 4.3, 7.7 Hz, 1 H), 3.79 (d, *J* = 3.1 Hz, 1 H), 2.18 (td, *J* = 2.6, 7.1 Hz, 2 H), 1.94 (t, *J* = 2.6 Hz, 1 H), 1.67-1.58 (m, 2 H), 1.53-1.44 (m, 4 H), 1.42-1.21 (m, 8 H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 168.6, 149.2, 126.4, 110.1, 89.9, 84.9, 68.3, 67.5, 64.9, 33.6, 29.5, 29.1, 28.8, 28.5, 25.3, 18.5.

HRMS (ESI): calc. for $C_{16}H_{23}O_4$ $[M+H]^+$: 279.1591; found: 279.1591.

(*S*)-1-((2*S*,3*S*)-5-Oxo-1,4-dioxaspiro[2.4.]hept-6-en-2-yl)undecyl 2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)acetate (**8**)



Chemical Formula: $C_{23}H_{32}N_2O_5$
Exact Mass: 416,23

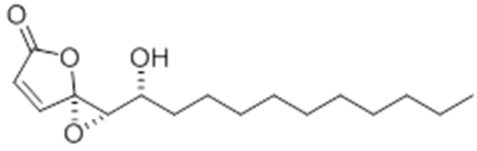
2-(3-(But-3-yn-1-yl)-3H-diazirin-3-yl)acetic acid (45 mg, 0.29 mmol, 1.10 eq) was dissolved in CH_2Cl_2 (1.0 M) at rt. DMF (10 µL ,0.013 mmol, 0.05 eq) and oxalyl chloride (27 µL, 40 mg, 0.31 mmol, 1.20 eq) were added dropwise and the reaction mixture was stirred for 2 h at rt. Solvents were removed under reduced pressure and the remaining residue was dissolved in CH_2Cl_2 (1.0 M). Ramariolide A (74 mg, 0.26 mmol, 1.00 eq) was dissolved in CH_2Cl_2 (1.0 M) and pyridine (14 µL, 13 mg, 0.78 mmol, 3.00 eq) was added and cooled to 0 °C. The solution of activated acid was added dropwise to the reaction mixture and a deep orange color change was observed. The reaction was allowed to warm to rt and monitored by TLC. After 2 h no further change could be observed and the reaction was stopped by addition of saturated NH_4Cl solution. After separation of phases the aqueous layer was extracted with CH_2Cl_2 (3 times 10 mL). Combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness under reduced pressure. The remaining residue was applied to flash chromatography (hexane/EtOAc = 95/5 → 90/10) to give the desired product as a yellow oil.

Yield: 24% (19 mg, 0.132 mmol).

R_f = 0.28 (hexane/EtOAc = 90/10).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.37 (d, *J* = 5.7 Hz, 1 H), 6.38 (d, *J* = 5.7 Hz, 1 H), 4.80 (ddd, *J* = 4.6, 7.1, 8.3 Hz, 1 H), 3.72 (d, *J* = 7.1 Hz, 1 H), 2.48 (td, *J* = 1.9, 7.3 Hz, 2 H), 2.25 (td, *J* = 2.6, 6.9 Hz, 2 H), 1.97 (t, *J* = 2.6 Hz, 1 H), 1.87-1.76 (m, 4 H), 1.34-1.21 (m, 14 H), 0.88 (t, *J* = 6.9 Hz, 3 H).
^{13}C-NMR (76 MHz, $CDCl_3$): δ [ppm] = 172.4, 152.4, 148.2, 126.6, 90.1, 82.8, 69.9, 69.9, 69.6, 62.1, 41.3, 32.7, 32.5, 32.0, 31.6, 29.7, 29.6, 29.4, 24.8, 23.5, 22.8, 17.8, 14.3.
HRMS (ESI): calc. for $C_{23}H_{33}N_2O_5$ $[M+H]^+$: 417.2384; found: 417.2388.

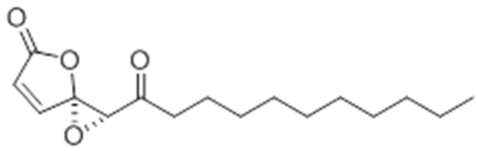
(2*S,3*R**)-2-((*R**)-1-Hydroxyundecyl)-1,4-dioxaspiro[2.4]hept-6-en-5-one** (**10**)



Chemical Formula: $C_{16}H_{26}O_4$
Exact Mass: 282,18

(*Z*)-5-(2-Hydroxydodecylidene)furan-2(*5H*)-one (576 mg, 2.16 mmol) was dissolved in CH_2Cl_2 (21 mL) and 3-Chloroperoxybenzoic acid (560 mg, 3.24 mmol) was added at rt. The reaction was monitored by TLC. After 3 h complete conversion of starting material was observed. The reaction was stopped by addition of saturated $NaHCO_3$ solution (5 mL) and layers were separated. The aqueous phase was extracted with EtOAc (3 times 10 mL) and organic phases were combined, washed with brine, dried over $MgSO_4$ and filtered. The filtrate was evaporated to dryness under reduced pressure and the remaining residue was applied to flash chromatography (H/EtOAc = 80/20 → 70/30). The desired product (585 mg, 2.07 mmol, 96%) was obtained as a white wax.
R_f = 0.38 (hexane/EtOAc = 70/30).
^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.56 (d, *J* = 5.7 Hz, 1 H), 6.36 (d, *J* = 5.7 Hz, 1 H), 4.09 (ddd, *J* = 3.2, 4.4, 7.7 Hz, 1 H), 3.79 (d, *J* = 3.2 Hz, 1 H), 1.94 (bs, 1 H), 1.69-1.43 (m, 4 H), 1.43-1.21 (m, 14 H), 0.88 (t, *J* = 6.8 Hz, 3 H).
^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 168.6, 149.2, 126.4, 89.9, 67.5, 64.9, 33.6, 32.0, 29.7, 29.7, 29.6, 29.6, 29.5, 25.3, 22.8, 14.3.
HRMS (ESI): calc. for $C_{16}H_{27}O_4$ $[M+H]^+$: 283.1904; found: 283.1911.

(2*S,3*R**)-2-Undecanoyl-1,4-dioxaspiro[2.4]hept-6-en-5-one** (**11**)



Chemical Formula: $C_{16}H_{24}O_4$
Exact Mass: 280,17

(2*S**,3*R**)-2-((*R**)-1-Hydroxyundecyl)-1,4-dioxaspiro[2.4]hept-6-en-5-one (464 mg, 1.65 mmol) was dissolved in CH_2Cl_2 (15 mL) at rt. DMP (773 mg, 1.82 mmol) are added to the reaction mixture. The reaction was monitored by TLC and after 2 h complete conversion was observed. The solvents were removed under reduced pressure and the remaining residue was applied to flash

chromatography (hexane/EtOAc = 80/20) to yield 95% of the desired product (437 mg, 1.56 mmol) as a white powder.

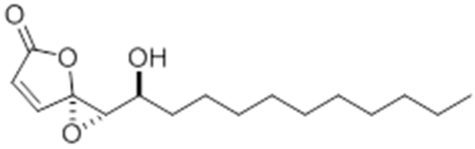
R_f = 0.27 (hexane/EtOAc = 80/20).

^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm] = 7.11 (d, *J* = 5.6 Hz, 1 H), 6.48 (d, *J* = 5.6 Hz, 1 H), 3.84 (s, 1 H), 2.67 (td, *J* = 3.0, 7.2 Hz, 2 H), 1.68-1.53 (m, 2 H), 1.36-1.22 (m, 14 H), 0.87 (t, *J* = 6.7 Hz, 3 H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 203.3, 167.4, 148.3, 127.6, 89.2, 62.7, 39.2, 32.0, 29.7, 29.6, 29.5, 29.4, 29.1, 22.8, 22.7, 14.3.

HRMS (ESI): calc. for $C_{16}H_{25}O_4$ $[M+H]^+$: 281.1747; found: 281.1754.

(2*S,3*R**)-2-((*S**)-1-Hydroxyundecyl)-1,4-dioxaspiro[2.4]hept-6-en-5-one** (**12**)



Chemical Formula: $C_{16}H_{26}O_4$
Exact Mass: 282,1831

(2*S**,3*R**)-2-Undecanoyl-1,4-dioxaspiro[2.4]hept-6-en-5-one (313 mg, 1.12 mmol) was dissolved in THF (20 mL) at rt. $Zn(BH_4)_2$ (1.12 mL, 5 M in THF) was added dropwise via syringe at 0 °C. The cloudy mixture was allowed to warm to rt and monitored by TLC. After 2 h complete conversion was observed and the reaction was stopped by addition of HCl (5 mL, 2 M). The mixture was diluted with EtOAc and after separation of layers the aqueous phase was extracted with EtOAc (3 times 15 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated to dryness *in-vacuo*. The remaining residue was applied to flash chromatograpy (hexane/EtOAc = 75/25 → 70/30). The desired product (146 mg, 0.52 mmol) could be isolated in 46% yield as a white wax.

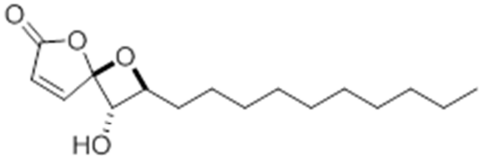
R_f = 0.34 (hexane/EtOAc = 70/30).

^{1}H-NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.14 (d, *J* = 5.6 Hz, 1 H), 6.4 (d, *J* = 5.6, 1 H), 3.90 (td, *J* = 4.5, 8.0 Hz, 1 H), 3.36 (d, *J* = 7.7 Hz, 1 H), 1.92 (bs, 1 H), 1.83-1.73 (m, 1 H), 1.73-1.64 (m, 1 H), 1.63-1.49 (m, 1 H), 1.48-1.39 (m, 1 H), 1.38-1.20 (m, 14 H), 0.88 (t, *J* = 6.9 Hz, 3 H).

^{13}C-NMR (126 MHz, $CDCl_3$): δ [ppm] = 168.6, 149.8, 126.4, 90.3, 69.6, 64.2, 34.9, 32.1, 29.7, 29.7, 29.7, 29.6, 29.5, 24.9, 22.8, 14.3.

HRMS (ESI): calc. for $C_{16}H_{27}O_4$ $[M+H]^+$: 283.1904; found: 283.1901.

(±)-Ramariolide B (**2**)



Chemical Formula: $C_{16}H_{26}O_4$
Exact Mass: 282,1831

(2*S**,3*R**)-2-((*S**)-1-Hydroxyundecyl)-1,4-dioxaspiro[2.4]hept-6-en-5-one (50 mg, 177 µmol) was dissolved in CH_2Cl_2 (10 mL) at rt. (±)-CSA (41 mg, 177 µmol) was added at rt and the reaction

was monitored by TLC. Over a period of 3 h a color change from light yellow, orange to deep red could be observed until complete conversion of starting material. The reaction was stopped by addition of saturated $NaHCO_3$ solution. After separation of layers the aqueous phase was extracted with CH_2Cl_2 (3 times 10 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The remaining residue was applied to flash chromatography (hexane/EtOAc = 75/25 → 70/30). Ramariolide B could be obtained as a white wax (12 mg, 43 µmol, 25%).

R_f = 0.33 (hexane/EtOAc = 70/30).

^{1}H-NMR (500 MHz, C_6H_6): δ [ppm] = 6.08 (d, *J* = 5.6 Hz, 1 H), 5.38 (d, *J* = 5.6 Hz, 1 H), 4.37 (q, *J* = 6.7 Hz, 1 H), 3.84 (dd, *J* = 6.1, 12:2 Hz, 1 H), 2.29 (d, *J* = 12.2 Hz, 1 H), 1.54-1.45 (m, 1 H), 1.43-1.38 (m, 1 H), 1.38-1.21 (m, 14 H), 0.93 (t, *J* = 6.8 Hz, 1 H).

^{13}C-NMR (126 MHz, C_6H_6): δ [ppm] = 168.2, 148.8, 124.4, 112.8, 90.4, 74.1, 34.4, 32.2, 29.9, 29.8, 29.7, 29.6, 29.5, 24.5, 23.0, 14.2.

HRMS (ESI): calc. for $C_{16}H_{25}O_4$ $[M+H]^+$: 283.1904; found: 283.1904.

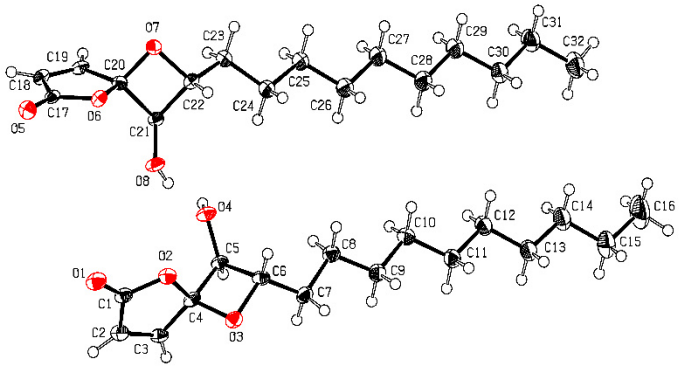
2.2. Single-crystal X-ray structure determinations

2.2.1.Compound 2 (CCDC 1508943)

A clear colorless plate-like specimen of $C_{16}H_{26}O_4$, approximate dimensions 0.028 mm x 0.349 mm x 0.816 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured on a Bruker D8 Venture TXS system equipped with a Helios optic monochromator and a Mo TXS rotating anode (λ = 0.71073 Å).

A total of 6269 frames were collected. The total exposure time was 17.16 hours. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using a triclinic unit cell yielded a total of 91055 reflections to a maximum θ angle of 26.37° (0.80 Å resolution), of which 6584 were independent (average redundancy 13.830, completeness = 99.4%, R_{int} = 6.59%, R_{sig} = 2.77%) and 5571 (84.61%) were greater than $2\sigma(F^2)$. The final cell constants of a = 5.3710(12) Å, b = 10.283(2) Å, c = 29.398(7) Å, α = 87.277(8)°, β = 84.938(8)°, γ = 89.485(8)°, volume = 1615.5(6) $Å^3$, are based upon the refinement of the XYZ-centroids of 121 reflections above 20 σ(I) with 8.564° < 2θ < 45.11°. Data were corrected for absorption effects using the Multi-Scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.856. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.6380 and 0.7454.

The structure was solved and refined using the Bruker SHELXTL Software Package in conjunction with SHELXLE, using the space group P -1, with Z = 4 for the formula unit, $C_{16}H_{26}O_4$. The final anisotropic full-matrix least-squares refinement on F^2 with 369 variables converged at R1 = 5.20%, for the observed data and wR2 = 11.37% for all data. The goodness-of-fit was 1.134. The largest peak in the final difference electron density synthesis was 0.285 $e^-/Å^3$ and the largest hole was -0.344 $e^-/Å^3$ with an RMS deviation of 0.052 $e^-/Å^3$. On the basis of the final model, the calculated density was 1.161 g/cm^3 and F(000), 616 e^-.



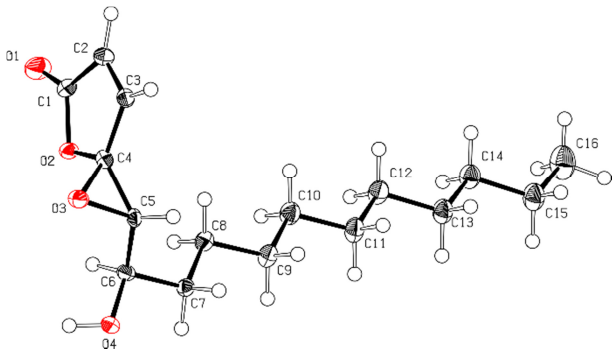
Identification code	LehJo3 AP9012-100	
Chemical formula	$C_{16}H_{26}O_4$	
Formula weight	282.37	
Wavelength	0.71073 Å	
Crystal size	0.028 x 0.349 x 0.816 mm	
Crystal habit	clear colourless plate	
Crystal system	triclinic	
Space group	P -1	
Unit cell dimensions	a = 5.3710(12) Å	α = 87.277(8)°
	b = 10.283(2) Å	β = 84.938(8)°
	c = 29.398(7) Å	γ = 89.485(8)°
Volume	1615.5(6) $Å^3$	
Z	4	
Density (calculated)	1.161 g/cm^3	
Absorption coefficient	0.082 mm^{-1}	
F(000)	616	
Diffractometer	Bruker D8 Venture TXS	
Radiation source	TXS rotating anode, Mo	
Theta range for data collection	2.37 to 26.37°	
Index ranges	-6<=h<=6, -12<=k<=12, -36<=l<=36	
Reflections collected	91055	
Independent reflections	6584 [R(int) = 0.0659]	
Coverage of independent reflections	99.4%	
Absorption correction	Multi-Scan	
Max. and min. transmission	0.7454 and 0.6380	
Structure solution technique	direct methods	
Structure solution program	SHELXS97 (Sheldrick, 1997)	
Refinement method	Full-matrix least-squares on F2	
Refinement program	SHELXL2014 (Sheldrick, 2014), SHELXLE (Huebschle, 2011)	
Function minimized	Σ w(Fo2 - Fc2)2	
Data / restraints / parameters	6584 / 0 / 369	
Goodness-of-fit on F2	1.134	
Δ/σmax	0.001	
Final R indices	5571 data; I>2σ(I)	R1 = 0.0520, wR2 = 0.1079
	all data	R1 = 0.0643, wR2 = 0.1137
Weighting scheme	$W=1/[\Sigma^2(FO^2)+(0.0317P)^2+0.9794P]$ WHERE $P=(FO^2+2FC^2)/3$	
Largest diff. peak and hole	0.285 and -0.344 $eÅ^{-3}$	
R.M.S. deviation from mean	0.052 $eÅ^{-3}$	

2.2.2.Compound 10 (CCDC 1508942)

A clear light yellow block-like specimen of $C_{16}H_{26}O_4$, approximate dimensions 0.120 mm x 0.170 mm x 0.320 mm, was used for the X-ray crystallographic analysis. The X-ray intensity data were measured on a Bruker Kappa APEX II CCD system equipped with a MONTEL mirror monochromator and a Mo FR591 rotating anode (λ = 0.71073 Å).

A total of 2811 frames were collected. The total exposure time was 62.46 hours. The frames were integrated with the Bruker SAINT software package using a narrow-frame algorithm. The integration of the data using an orthorhombic unit cell yielded a total of 14742 reflections to a maximum θ angle of 25.37° (0.83 Å resolution), of which 2921 were independent (average redundancy 5.047, completeness = 99.8%, R_{int} = 3.56%, R_{sig} = 3.12%) and 2621 (89.73%) were greater than $2\sigma(F^2)$. The final cell constants of a = 5.3751(3) Å, b = 7.6377(4) Å, c = 38.647(2) Å, volume = 1586.59(15) $Å^3$, are based upon the refinement of the XYZ-centroids of 6465 reflections above 20 σ(I) with 6.201° < 2θ < 50.69°. Data were corrected for absorption effects using the multi-scan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.840. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.9737 and 0.9898.

The structure was solved and refined using the Bruker SHELXTL Software Package in conjunction with SHELXLE, using the space group P 21 21 21, with Z = 4 for the formula unit, $C_{16}H_{26}O_4$. The final anisotropic full-matrix least-squares refinement on F^2 with 186 variables converged at R1 = 3.11%, for the observed data and wR2 = 6.03% for all data. The goodness-of-fit was 1.038. The largest peak in the final difference electron density synthesis was 0.132 $e^-/Å^3$ and the largest hole was -0.126 $e^-/Å^3$ with an RMS deviation of 0.029 $e^-/Å^3$. On the basis of the final model, the calculated density was 1.182 g/cm^3 and F(000), 616 e^-.



Identification code	LehJo1 AP6106-123	
Chemical formula	$C_{16}H_{26}O_4$	
Formula weight	282.37	
Temperature	123(2) K	
Wavelength	0.71073 Å	
Crystal size	0.120 x 0.170 x 0.320 mm	
Crystal habit	clear light yellow block	
Crystal system	orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 5.3751(3) Å	α = 90°
	b = 7.6377(4) Å	β = 90°
	c = 38.647(2) Å	γ = 90°
Volume	1586.59(15) $Å^3$	
Z	4	
Density (calculated)	1.182 g/cm^3	
Absorption coefficient	0.083 mm^{-1}	
F(000)	616	
Diffractometer	Bruker Kappa APEX II CCD	
Radiation source	FR591 rotating anode, Mo	
Theta range for data collection	2.11 to 25.37°	
Index ranges	-6<=h<=6, -9<=k<=9, -46<=l<=46	
Reflections collected	14742	
Independent reflections	2921 [R(int) = 0.0356]	
Coverage of independent reflections	99.8%	
Absorption correction	multi-scan	
Max. and min. transmission	0.9898 and 0.9737	
Structure solution technique	direct methods	
Structure solution program	SHELXS-97 (Sheldrick, 1990)	
Refinement method	Full-matrix least-squares on F^2	
Refinement program	SHELXL-97 (Sheldrick, 1997)	
Function minimized	$\Sigma w(F_o^2 - F_c^2)^2$	
Data / restraints / parameters	2921 / 0 / 186	
Goodness-of-fit on F^2	1.038	
Δ/σ_{max}	0.001	
Final R indices	2621 data; I>2σ(I)	R1 = 0.0311, wR2 = 0.0585
	all data	R1 = 0.0375, wR2 = 0.0603
Weighting scheme	$w=1/[\sigma^2(F_o^2)+(0.0227P)^2+0.2403P]$ where $P=(F_o^2+2F_c^2)/3$	
Absolute structure parameter	-0.3(8)	
Largest diff. peak and hole	0.132 and -0.126 $eÅ^{-3}$	
R.M.S. deviation from mean	0.029 $eÅ^{-3}$	

3. Biochemical Procedures

3.1. Bacterial strains and media

Mycobacterium smegmatis mc^2155 stain was gratefully gifted by Prof. EJ Rubin (Harvard School of Public Health) and generally cultivated in 7H9 media (7H9, for per liter: 4.7 g 7H9 powder, 2 mL glycerol, 2.5 mL 20% Tween 80, 5 g BSA (fraction V), 2 g dextrose, 850 mg NaCl, 3 mg catalase, sterile filter) at 37 °C with agitation. *Mycobacterium tuberculosis* H37Rv was generally cultivated in 7H9-OADC media (7H9 medium supplemented with 25 mg/L oleic acid) at 37 °C with agitation.
Recombinant proteins were expressed in different *Escherichia coli* strains cultivated in Luria-Bertani (LB) media (LB, for per liter: 10 g digested casein, 5 g yeast extract, 5 g NaCl, adjust pH to 7.5, autoclave) at 37 °C.

Overnight cultures

5 mL of the strain specific media for cultivation were inoculated with 5 µL of the desired bacterial cryostock (1:1,000) with a sterile tip in a culture tube. The culture was incubated for 16 h at 200 rpm in an Innova incubator shaker. Cultures were always prepared freshly to avoid genetiv variations and sterile controls (medium not containing bacteria) were added each time.

Strain construction

Molecular methods were carried out according to the manufacter's instructions. Kits for the isolation of plasmids and purification of PCR products were purchased from Suedlabor (Gauting, Germany). Enzymes were purchased from New England BioLabs (Frankfurt, Germany) and Fermentas (St. Leon-Rot, Germany). A N-terminal His_6 affinity tagged Ask construct was cloned in a pDONR201 (Invitrogen) vector and then in a pET300 expression vector via the GATEWAY cloning system using the primers shown below and genomic DNA from *M. smegmatis* mc^2155.

Construct: N-His6-attB1-ask-Stop-attB2

Primer1: ggggacaagtttgtacaaaaaagcaggctttGCGCTCGTCGTACAGAAATAC

Primer2: ggggaccactttgtacaagaaagctgggtgTCAGCGCCCCGTTCCCG

3.2. Minimal inhibitory concentration (MIC) determination

Compound-mediated growth inhibition was carried out using 96-well plates. A culture of stationary phase growing bacteria was diluted to a final OD_{600} = 0.001 in fresh media. 100 µL of diluted bacteria was added to 100 µL of two fold inhibitory concentration of compound to be tested and diluted two fold in each well. 96-well plates were incubated at 37 °C under a water impermeable membrane. To determine the growth of mycobacteria 100 µL of a 0.02 % resazurin solution was added 7 days for *M. tuberculosis*. A color change from purple to pink within 2 to 4 days indicated viable cells while purple colored wells suggested no bacterial growth.

3.3. Activity based protein profiling (ABPP) experiments

Bacterial strains were cultivated under defined growth conditions to exponential phase. OD_{600} was measured on Novaspec Plus visible spectrophotometer. Cultures were collected in a 50 mL falcon tube, pelletized at 6,000 rpm for 5 min at 4 °C and washed with PBS. The pellet was then resuspended in PBS to a theoretical final OD_{600} = 40.

Preparative gel-free ABPP using dimethyl labeling

1000 µL of bacterial suspension at OD_{600} = 40 were incubated with **1a** (final concentration = 100 µM) for competition experiments or with DMSO for enrichment at RT for 30 min and then incubated sequentially with the natural product derived UV-probe **8** (final concentration = 30 µM) at RT for 1 h. One sample was consistently treated with DMSO as control. For identification of non-covalent targets samples were diluted with 5 mL PBS, distributed on 6-well plates and irradiated with UV-light at 365 nm (Phillips TL-D BLB UV-lamps) under cooling of cool packs for 10 min. Cells were pelletized at 6,000 rpm for 5 min and washed with PBS two times before finally resuspended in 1000 µL PBS. Samples were sonicated (duty cycle: 50; outpout 80 %; 20 sec) at 4 °C under four repetitions, centrifuged at 15,000 rpm and 4 °C to give 800 µL of the soluble fraction (supernatant) and insoluble fraction (pellet), which was resuspended in 800 µL PBS.

Experiments were conducted in technical triplicates with the same bacterial suspension in parallel while biological triplicates were performed with bacteria harvested on different days. All samples of each fraction were treated with 120 µL gel-free ABPP Mix (40 µL Biotin-PEG_3-N_3 (Jena Bioscience, CLK-AZ104P4-100; 10 mM in DMSO), 20 µL fresh TCEP (50 mM in ddH_2O), 60 µL TBTA Ligand (1.667 mM in 80 % tBuOH and 20 % DMSO)). The final concentrations were 233 µM Biotin-PEG_3-N_3, 581 µM TCEP and 58.2 µM TBTA Ligand. The lysates were mixed by vortexing and 20 µL $CuSO_4$ solution (50 mM in ddH_2O) were added. The lysates were mixed by vortexing again and incubated for 1h at RT in the dark. After the click-reaction the lysates were transferred to 15 mL falcon tubes and 8 mL of cold acetone (-80°C, MS grade) were added. Proteins were precipitated over night at -80°C.

The precipitated proteins were thawed on ice, pelletized (16900 xG, 15 min, 4°C) and supernatant was disposed. Falcon tubes were stored on ice during the following washing procedure: The proteins were washed two times with 1 mL cold methanol (-80°C). Resuspension was achieved by sonication (15 sec at 10 % intensity) and proteins were pelletized via centrifugation (16900 xG, 10 min, 4°C). Only MS grade water was used for the following procedures. After two washing steps supernatant was disposed and the pellet was resuspended in 500 µL 0.2 % SDS in PBS at RT by sonication (15 sec at 10 % intensity). Avidin beads were thawed on ice and resuspended by carefully inverting. Then 50 µL of bead suspension were transferred into Protein LoBind Eppendorf tubes using wide bore pipette tips and washed three times with 1 mL 0.2 % SDS in PBS (resuspension: carefully inverting 10 times, pelleting: 400 xG, 3 min, RT). 500 µL protein solution from the 15 mL falcon tubes were transferred to the Protein LoBind Eppendorf tubes with washed avidin beads and incubated under continuous inverting (20 rpm, 1 h, RT). Beads were washed 3 times with 1 mL 0.2 % SDS in PBS, 2 times with 1 mL 6 M urea in water and 3 times with 1 mL PBS (resuspension: carefully inverting 20 times, pelleting: 400 xG, 3 min, RT).

The beads were resuspended in 200 µl denaturation buffer (7 M urea, 2 M thiourea in 20 mM pH 7.5 HEPES buffer). Proteins were reduced through addition of dithiothreitol (DTT, 1 M, 0.2 µL), the tubes were mixed by vortexing shortly and incubated in a thermomixer (450 rpm, 45 min, RT). Then 2-iodoacetamide (IAA, 550 mM, 2 µL) was added for alkylation, the tubes were mixed by vortexing shortly and incubated in a thermomixer (450 rpm, 30 min, RT, in the dark). Remaining IAA was quenched by the addition of dithiothreitol (DTT, 1 M, 0.8 µL). The tubes were shortly mixed by vortexing and incubated in a thermomixer (450 rpm, 30 min, RT). LysC (0.5 µg/µL, Wako) was thawed on ice and 1µL was added to each microcentrifuge tube, the tubes were shortly mixed by vortexing and incubated in a thermomixer (450 rpm, 2 h, RT, in the dark). TEAB solution (600 µL, 50 mM in water) and then trypsin (1.5 µL, 0.5 µg/µL in 50 mM acetic acid, Promega) were added and tubes were shortly vortexed after each addition. The reaction was incubated in a thermomixer (450 rpm, 13-15 h, 37 °C). The digest was stopped by adding 6 µL formic acid (FA) and vortexing. After centrifugation (100 xG, 1 min, RT), the supernatant was transferred to a new Protein LoBind Eppendorf tube. FA (50 µL, aqueous 0.1 % solution) was added to the beads and after vortexing and centrifugation (100 xG, 1 min, RT) the supernatant was added to the supernatant collected before. Again FA (50 µL, aqueous 0.1 % solution) was added to the beads and after vortexing and centrifugation (16200 xG, 3 min, RT) the supernatant was transferred to the combined supernatants.

50 mg SepPak C18 columns (Waters) were equilibrated by gravity flow with 1mL acetonitrile, 1 mL elution buffer (80% ACN, 0.5% FA) and 3 mL aqueous 0.5% FA solution. Subsequently the samples were loaded by gravity flow, washed with 5 mL aqueous 0.5 % FA solution and labeled with 5 mL of the respective dimethyl labeling solution. The following solutions were used: light (L): 30 mM $NaBH_3CN$, 0.2 % CH_2O, 10 mM NaH_2PO_4, 35 mM Na_2HPO_4, pH 7.5; medium (M): 30 mM $NaBH_3CN$, 0.2 % CD_2O, 10 mM NaH_2PO_4, 35 mM Na_2HPO_4, pH 7.5; heavy (H): 30 mM $NaBHD_3CN$, 0.2 % $^{13}CD_2O$, 10 mM NaH_2PO_4, 35 mM Na_2HPO_4, pH 7.5. Labeled peptides were eluted into new 2.0 mL Protein LoBind Eppendorf tubes using two times 250 µL elution buffer. The eluates were lyophilized and stored at -20°C.

Prior to MS measurement the samples were dissolved in 30 µL 1 % FA by pipetting up and down, vortexing and sonication for 15 min (brief centrifugation after each step). Differentially labeled samples were mixed. 0.45 µm centrifugal filter units (VWR) were equilibrated with two times 500 µL water, 500 µL 0.05 N NaOH and two times 500 µL 1 % FA (centrifugation: 16200 xG, 1 min, RT). Reconstituted and mixed peptide samples were filtered through the equilibrated filters (centrifugation: 16200 xG, 2 min, RT). Samples were analyzed via HPLC-MS/MS using an UltiMate 3000 nano HPLC system (Dionex, Sunnyvale, California, USA) equipped with Acclaim C18 PepMap100 75 µm ID x 2 cm trap and Acclaim C18 PepMap RSLC, 75 µM ID x 15 cm separation columns coupled to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Samples were loaded on the trap and washed for 10 min with 0.1 % formic acid, then transferred to the analytical column and separated using a 120 min gradient from 3 % to 25 % acetonitrile in 0.1 % formic acid and 5 % dimethyl sulfoxide (at 200 nL/min flow rate). Orbitrap Fusion was operated in a 3 second top speed data dependent mode. Full scan acquisition was performed in the orbitrap at a resolution of 120000 and an ion target of 4E5 in a scan range of 300 – 1700 m/z. Monoisotopic precursor selection as well as dynamic exclusion for 60 s were enabled. Precursors with charge states of 2 – 7 and

intensities greater than 5E3 were selected for fragmentation. Isolation was performed in the quadrupole using a window of 1.6 m/z. Precursors were collected to a target of 1E2 for a maximum injection time of 250 with “inject ions for all available parallelizable time” enabled. Fragments were generated using higher-energy collisional dissociation (HCD) and detected in the ion trap at a rapid scan rate. Internal calibration was performed using the ion signal of fluoranthene cations (EASY-ETD/IC source)

Peptide and protein identifications were performed using MaxQuant 1.5.3.8 software with Andromeda as search engine using following parameters: Carbamidomethylation of cysteines as fixed and oxidation of methionine as well as acetylation of N-termini as dynamic modifications, trypsin/P as the proteolytic enzyme, 4.5 ppm for precursor mass tolerance (main search ppm) and 0.5 Da for fragment mass tolerance (ITMS MS/MS tolerance). Searches were done against the Uniprot database for *M smegmatis* mc^2 155. Quantification was performed using dimethyl labeling with the following settings: light: DimethLys0, DimethNter0; medium: DimethLys4, DimethNter4 and heavy: DimethLys8, DimethNter8. Variable modifications were included for quantification. The I = L and requantify options were used. Identification was done with at least 2 unique peptides and quantification only with unique peptides.

Statistical analysis was performed with Perseus 1.5.1.6. Putative contaminants, reverse peptides and peptides only identified by site were omitted from further processing. Dimethyllabeling ratios were $\log_2(x)$ transformed and z-score normalized. The average values of technical replicates were calculated and $-\log_{10}$(p-values) were obtained by a two sided one sample t-test over three biological replicates for standard ABPP with DMSO control or competition experiments.

Proteins were plotted as ($\log_2$-)ratio regarding DMSO or natural product against statistical significance ($-\log_{10}$(p-value)). For UV-irradiated analyses proteins enriched by a factor > 2 with a p-value of 0.05 or below are considered as hits. For covalent analyses cut-offs for enrichment were increased to an enrichment factor of 3 or greater.

3.4. Recombinant expression and *in-situ* labeling of proteins in *E.coli*

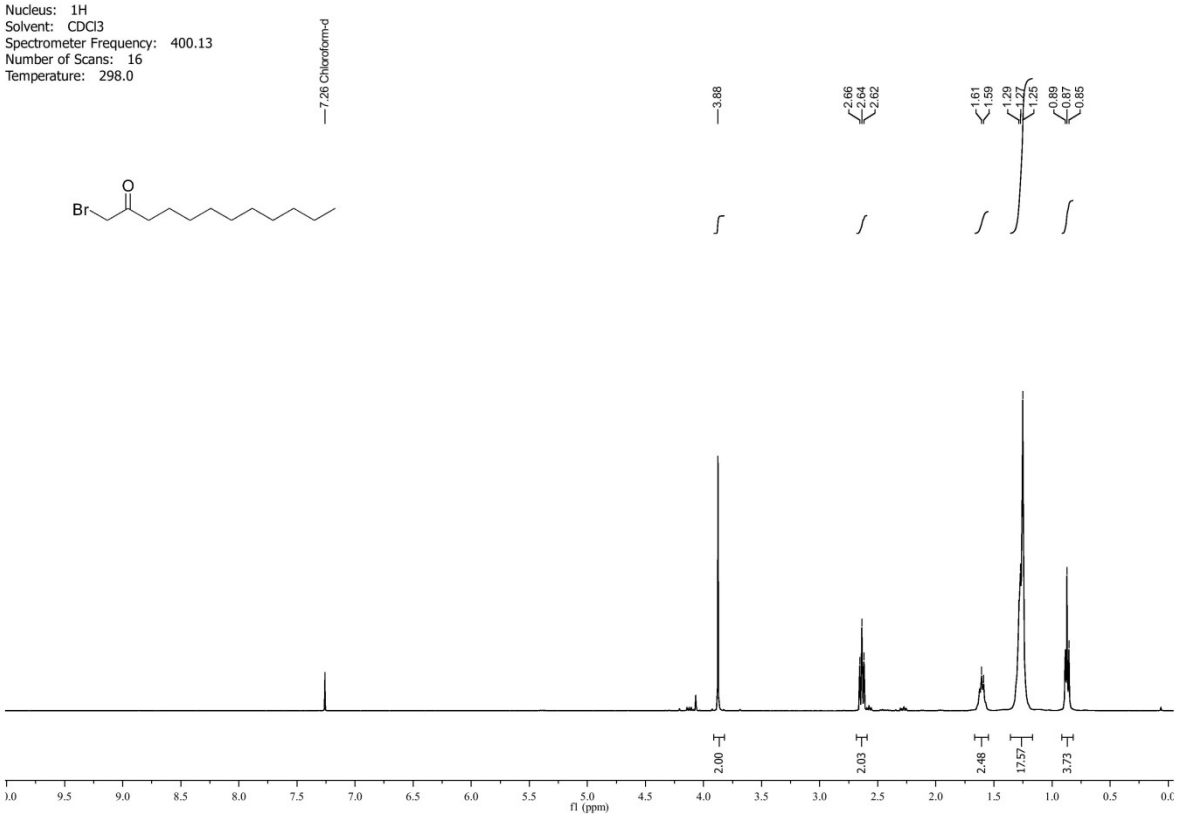
For recombinant protein expression LB medium were inoculated with of overnight culture of *E. coli* BL21 (1:100) carrying the expression vector at 37 °C. Expression was induced at an OD_{600} of 0.6 by addition of isopropyl-β-D-thiogalactopyranosid (IPTG; final concentration: 0.20 mM) and carried out 20 h at 16°C. After centrifugation (5 min, 6200 xG, 4°C) and removal of the supernatant bacteria were resuspended in PBS to get an OD_{600} of 40. To 1 mL of this suspension in a microcentrifuge tube 30 µL of **8** in DMSO (or just DMSO as a control) were added. After 30 min incubation at RT in the dark the microcentrifuge tube was again mixed by vortexing and incubated for another 30 min at RT in the dark. After centrifugation (6200 xG, 2 min, 4°C) the supernatant was removed and the pellets were stored at -80°C.

Pellets were resuspended in 1 mL PBS (4°C) and transferred to a ’Precellys Glass/Ceramic Kit SK38 2.0 mL’ tube. Tubes were cooled on ice for about 5 min or longer and cells were lysed with the Precellys Homogeniser using two times lysis program 3 (5400 rpm, run number: 1, run time: 20 sec,

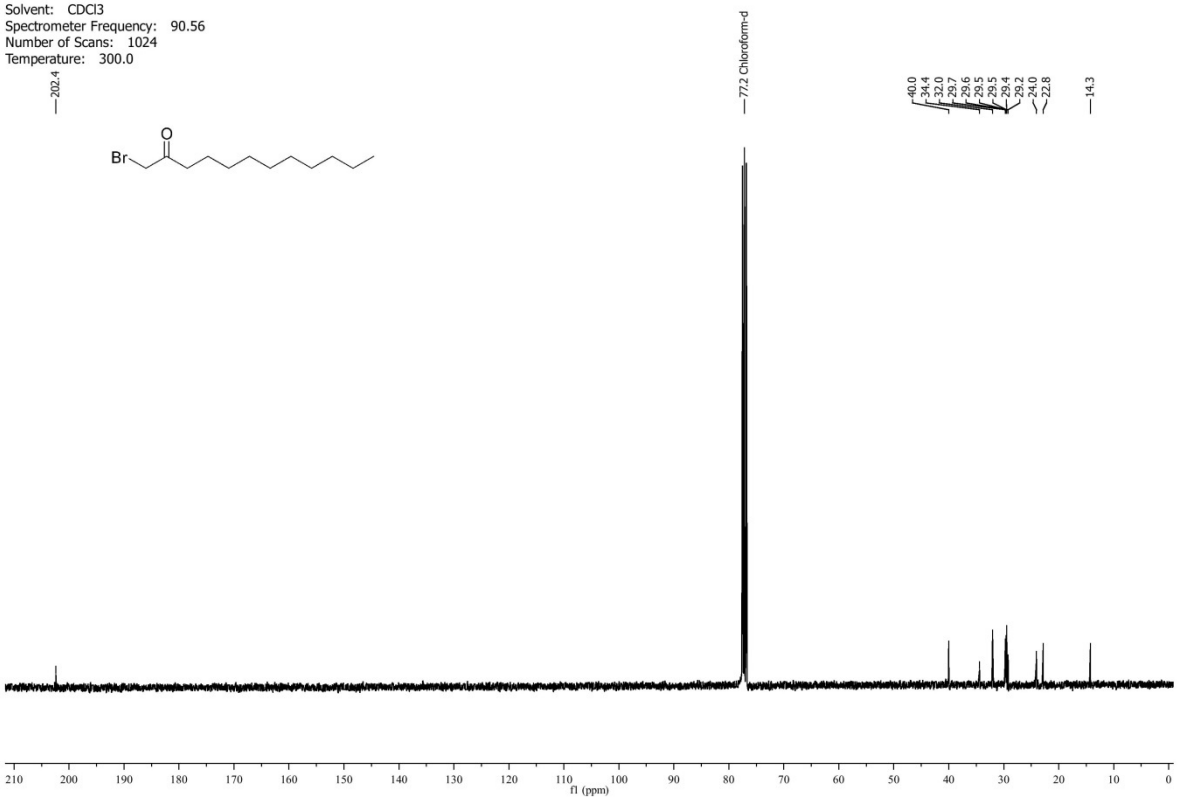
pause: 5 sec). After each lysis run the tubes were cooled on ice for 5 min. The ball mill tubes were centrifuged (16200 xG, 10 min, 4°C) and 86 µL of supernatant were transferred to new 1.5 mL microcentrifuge tubes and treated with 10 µL gel-based ABPP Mix (2 µL RhN_3 (Tetramethylrhodamine (TAMRA) Azide (Tetramethylrhodamine 5-Carboxamido-(6-Azidohexanyl)), 5-isomer (life technologies, T10182); 5 mM in DMSO), 2 µL fresh TCEP (50 mM in ddH_2O), 6 µL TBTA Ligand (1.667 mM in 80 % tBuOH and 20 % DMSO)). The final concentrations were: 100 µM RhN_3, 1.0 mM TCEP and 100 µM TBTA Ligand. The lysates were mixed by vortexing and 2 µL $CuSO_4$ solution (50 mM in ddH_2O) were added. The lysates were again mixed by vortexing and incubated for 1h at RT in the dark. Then 80 µL 2x Laemmli Sample Buffer were added, samples were mixed in a thermomixer (300 rpm, 3 min, 96°C) and analyzed via SDS PAGE (10 % agarose gel (PEQLAB Biotechnologie GmbH, Erlangen, PerfectBlue Dual Gel System, the gel was prepared according to the manual), 3.5 h, 300 V, 8 µL fluorescent protein standard) and fluorescence imaging (GE Healthcare, ImageQuant LAS-4000). After fluorescence scanning the gel was coomassie stained.

4. Appendix

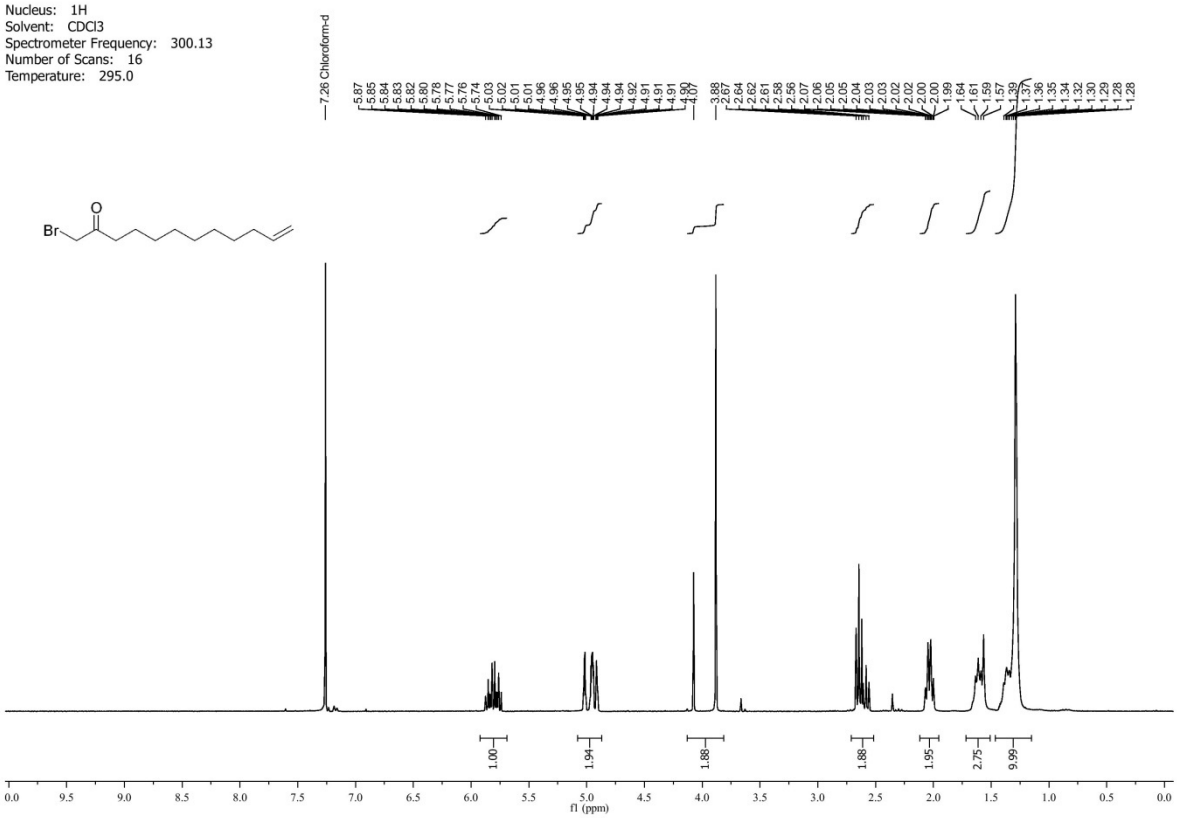
Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 400.13
Number of Scans: 16
Temperature: 298.0



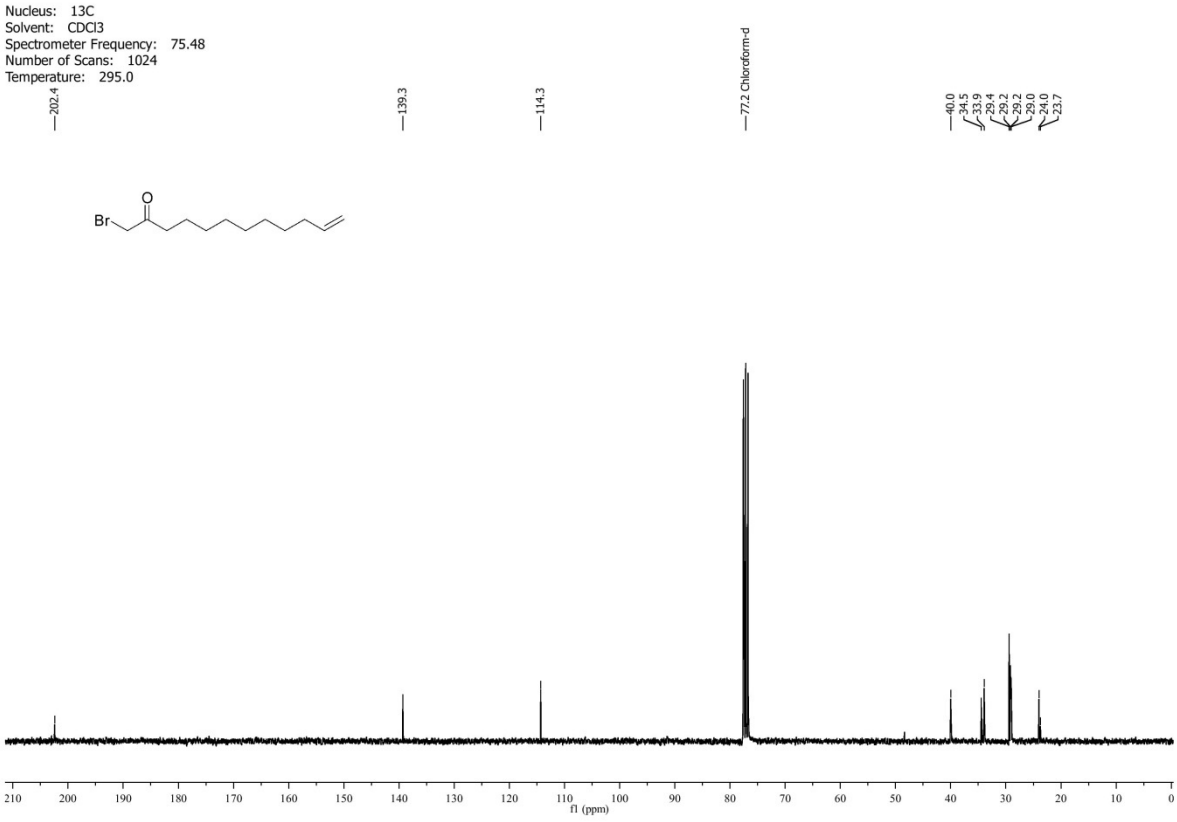
Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 90.56
Number of Scans: 1024
Temperature: 300.0



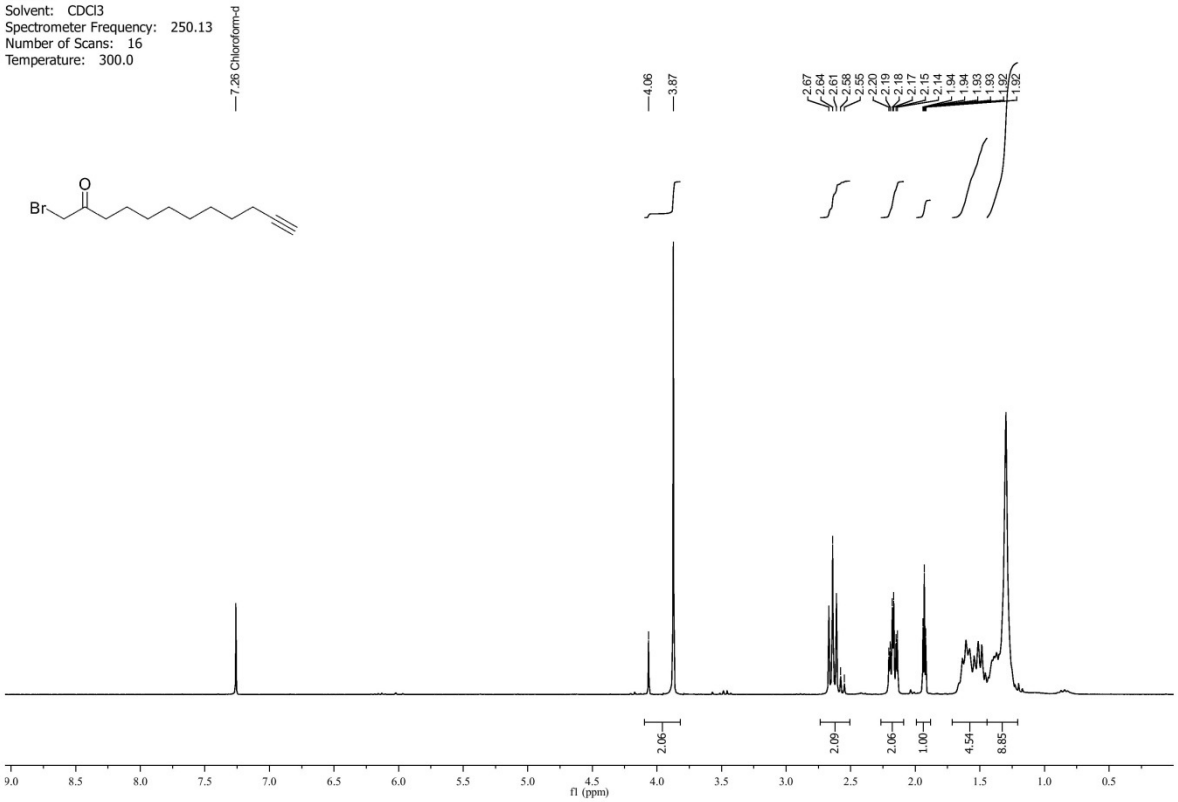
Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 300.13
Number of Scans: 16
Temperature: 295.0



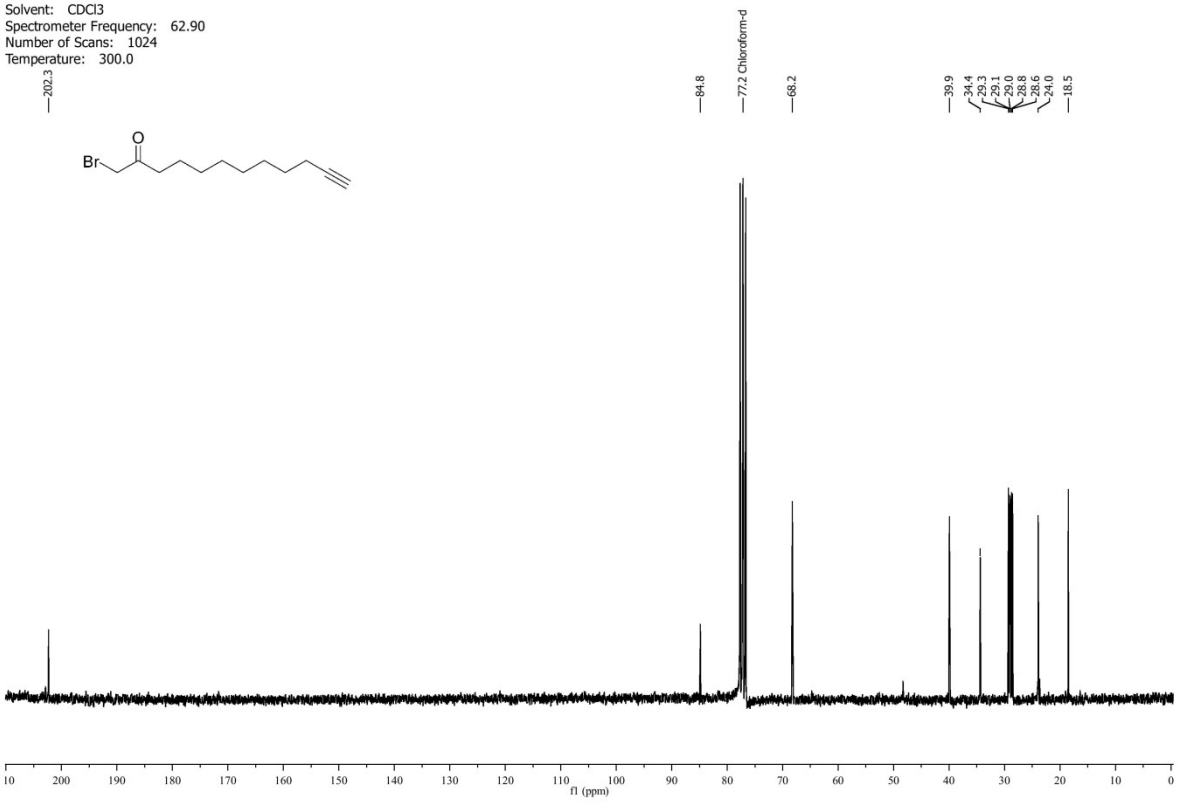
Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 75.48
Number of Scans: 1024
Temperature: 295.0



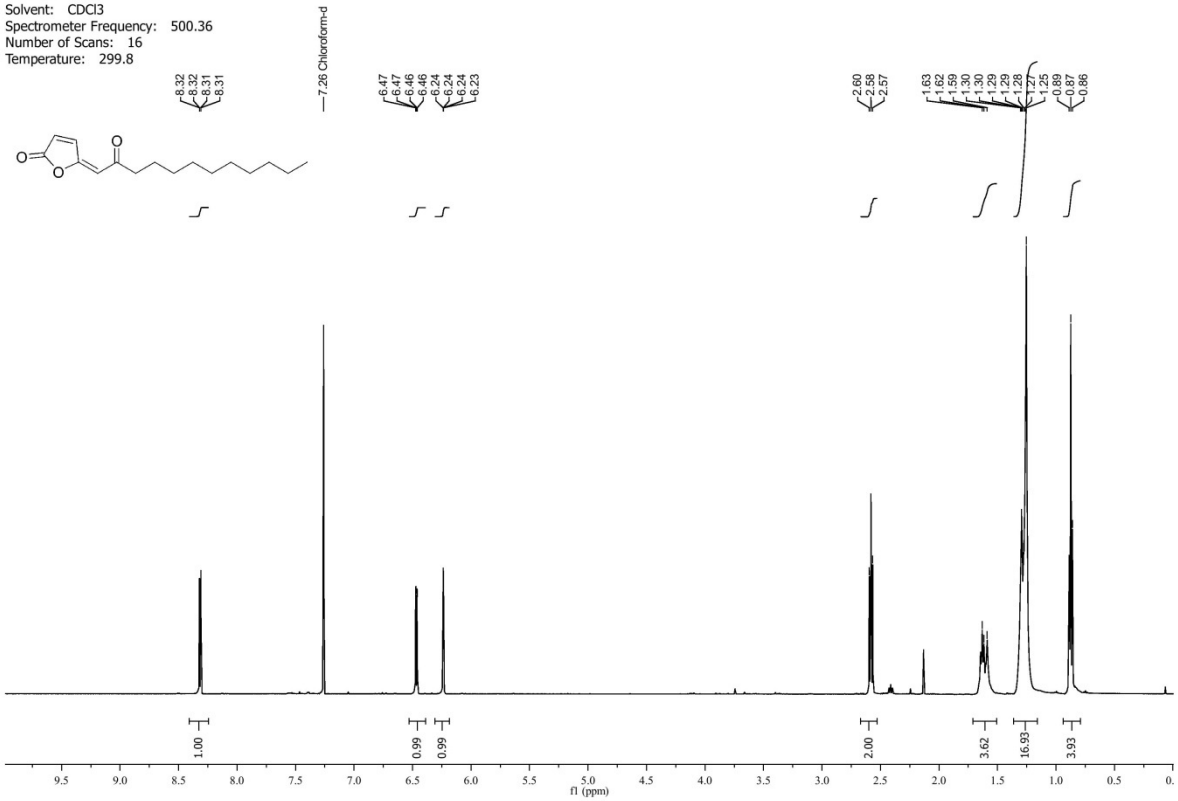
Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 250.13
Number of Scans: 16
Temperature: 300.0



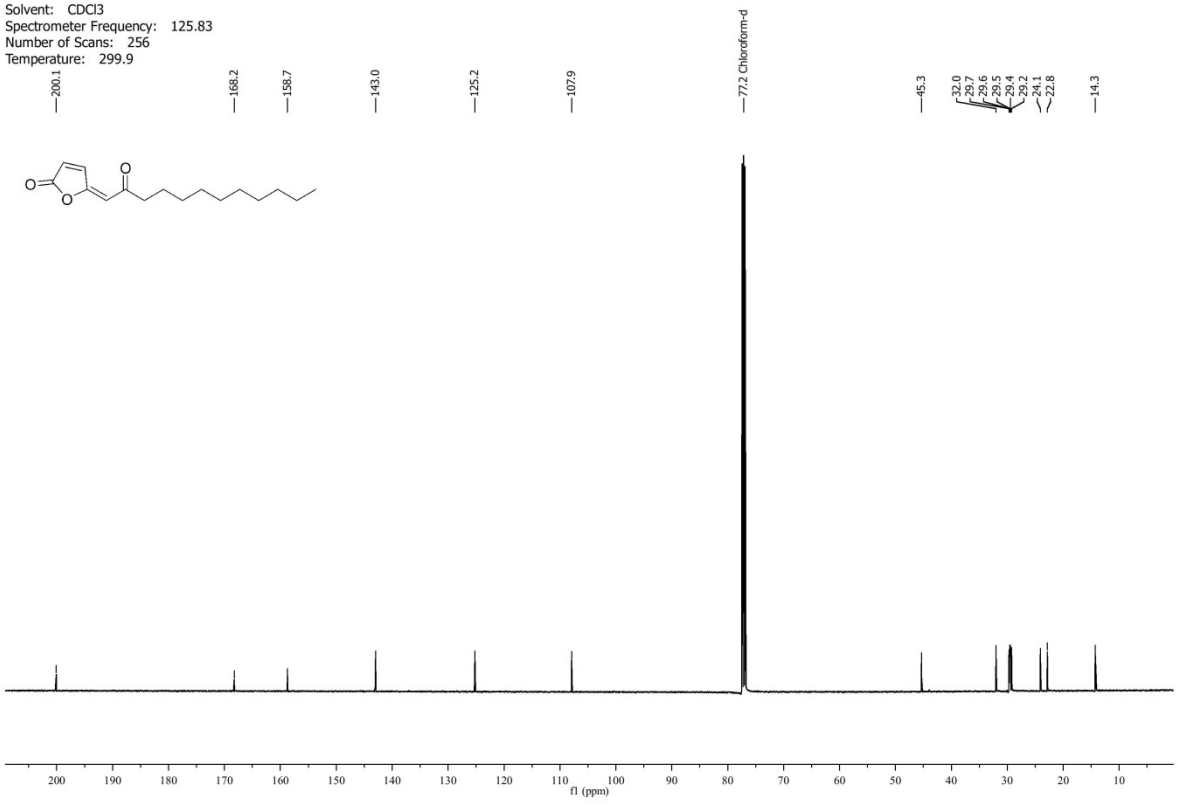
Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 62.90
Number of Scans: 1024
Temperature: 300.0

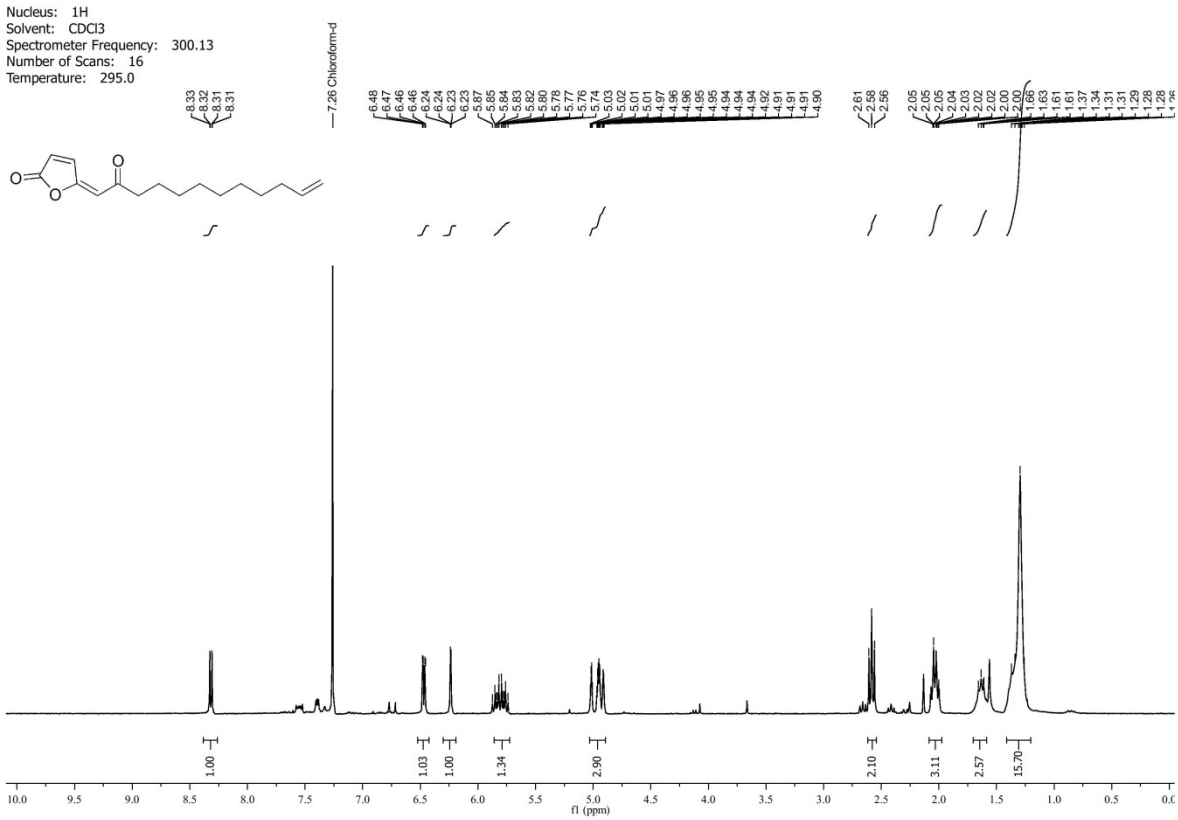


Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 500.36
Number of Scans: 16
Temperature: 299.8

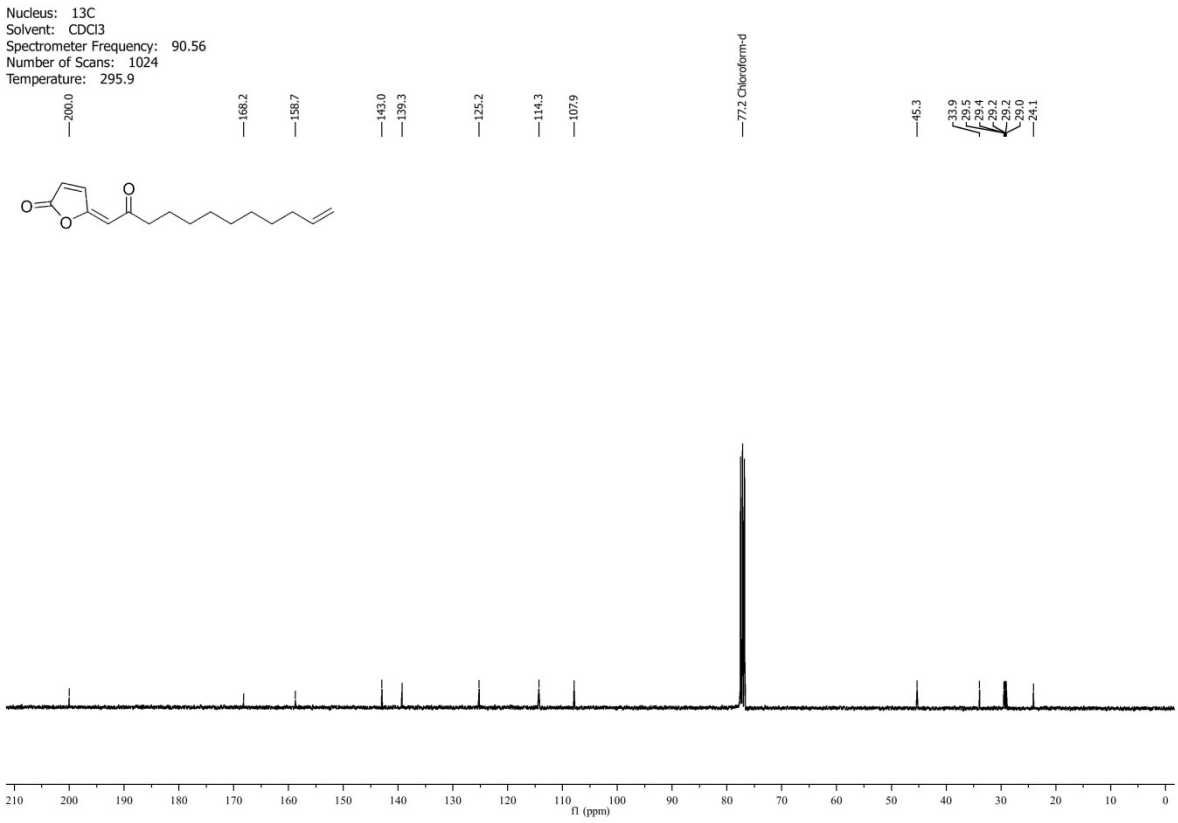


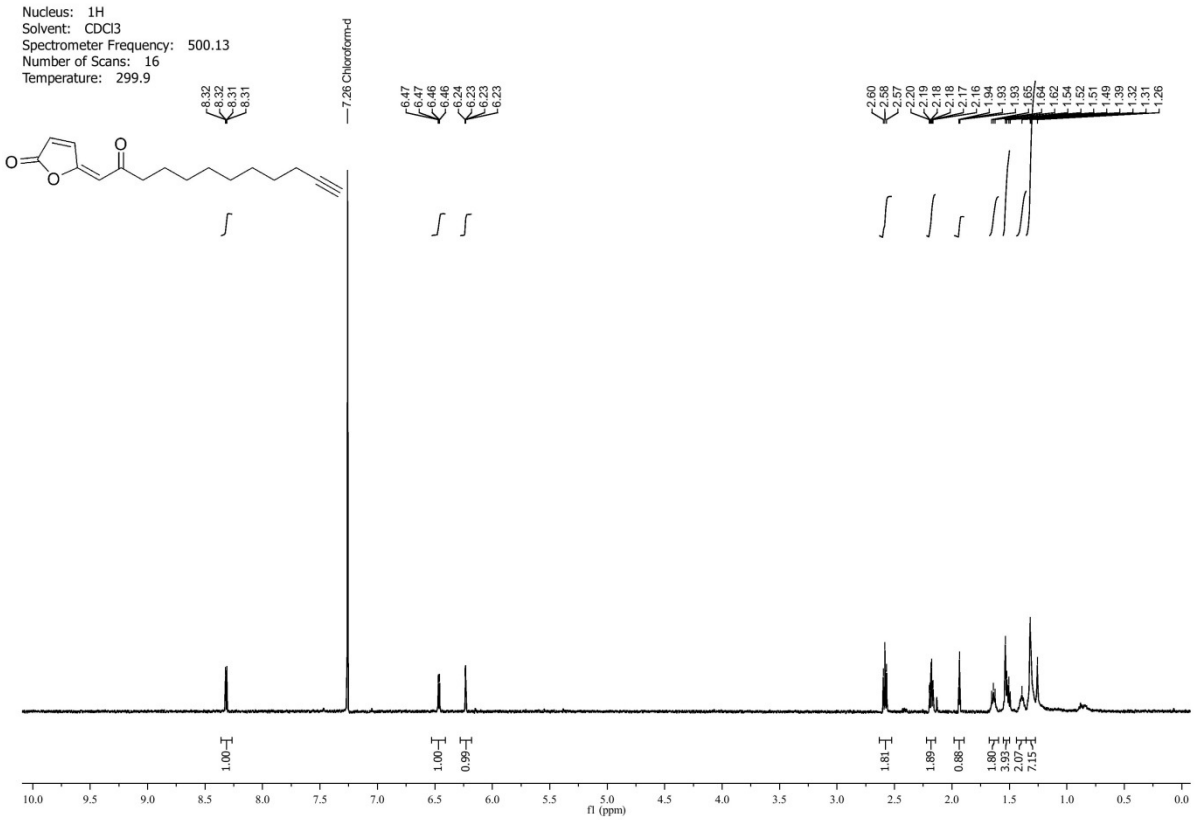
Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 125.83
Number of Scans: 256
Temperature: 299.9

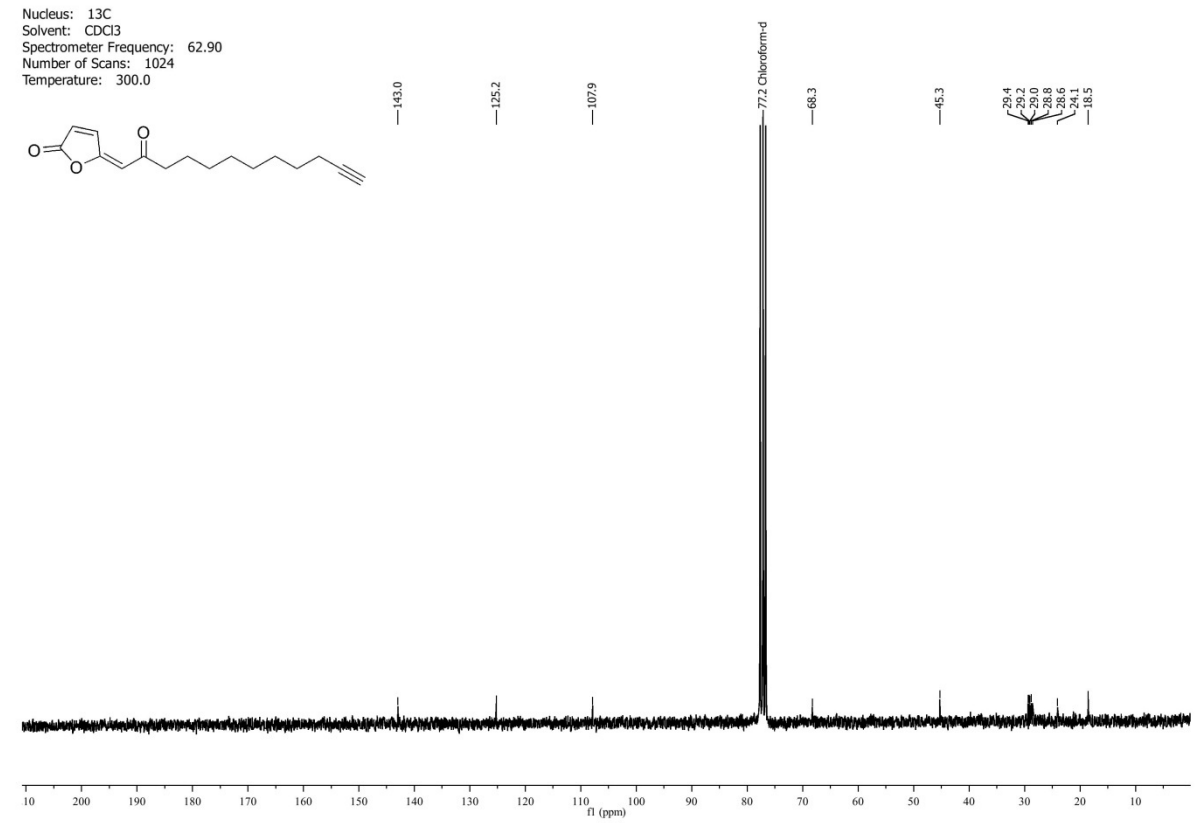


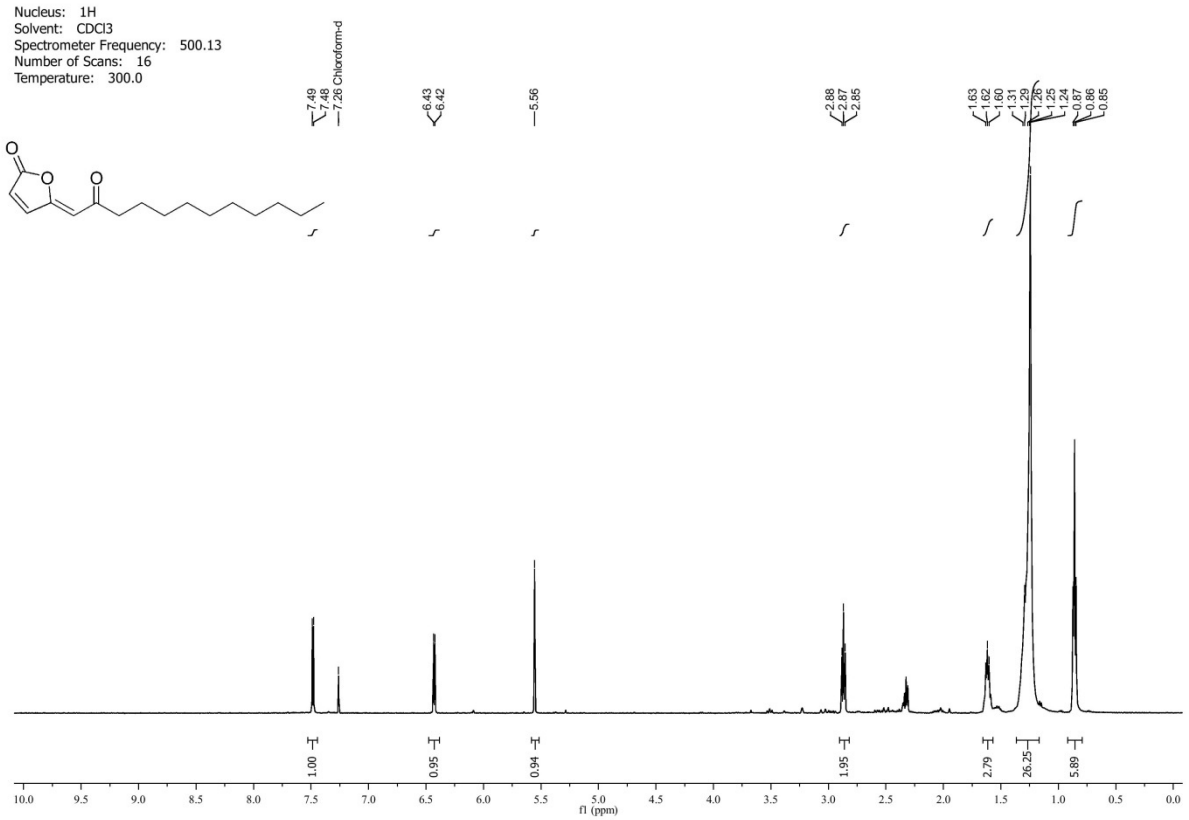


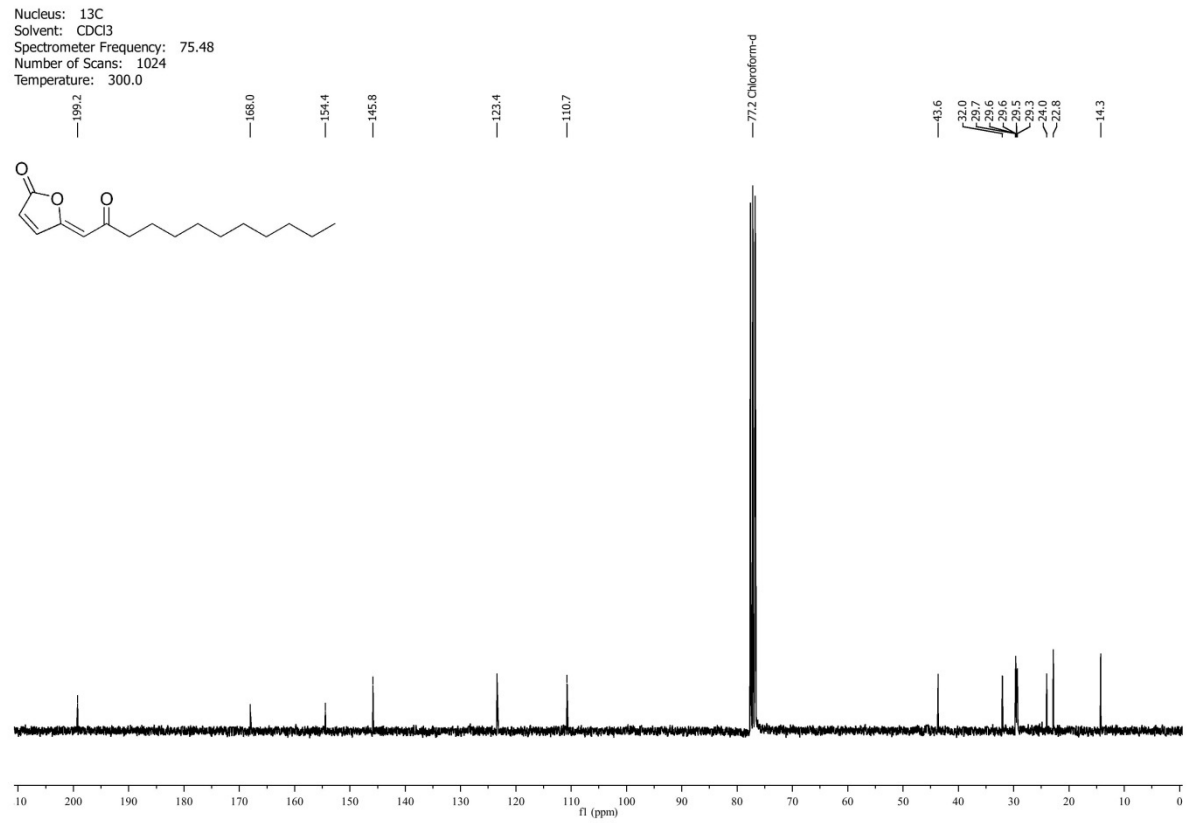
Impurities at around 7.5 ppm trace back to PPh_3



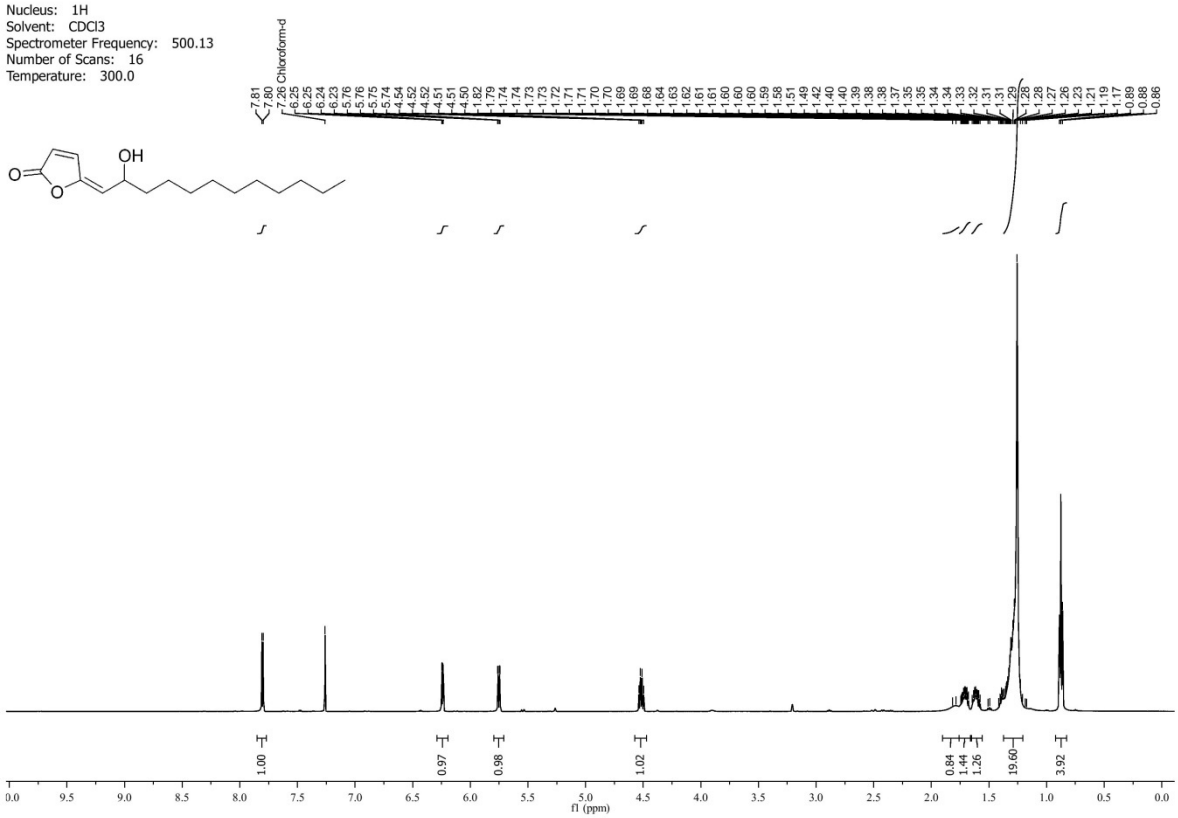

Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 500.13
Number of Scans: 16
Temperature: 299.9
7.26 Chloroform-d
f1 (ppm)


Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 62.90
Number of Scans: 1024
Temperature: 300.0
77.2 Chloroform-d
f1 (ppm)

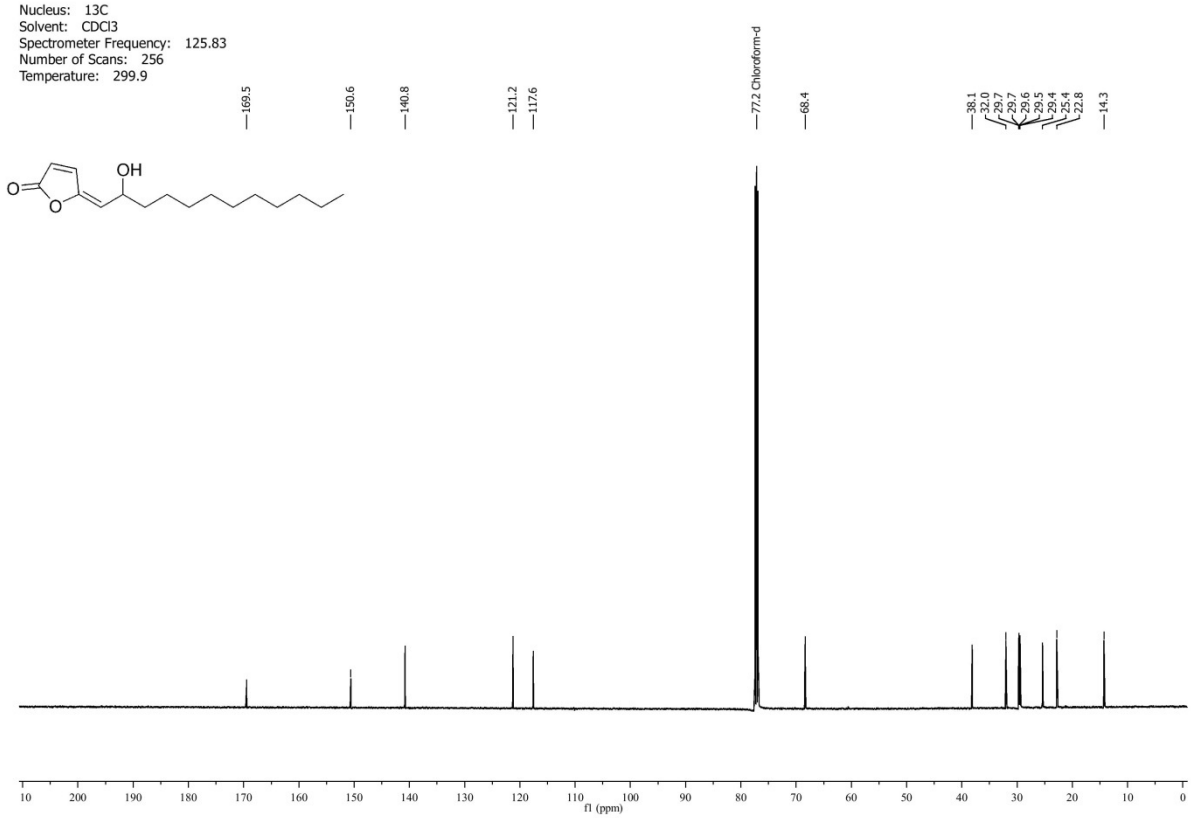

Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 500.13
Number of Scans: 16
Temperature: 300.0
7.49
7.48
7.26 Chloroform-d
6.43
6.42
5.56
2.88
2.87
2.85
1.63
1.62
1.60
1.31
1.29
1.26
1.25
1.24
0.87
0.86
0.85
1.00
0.95
0.94
1.95
2.79
26.25
5.89
f1 (ppm)

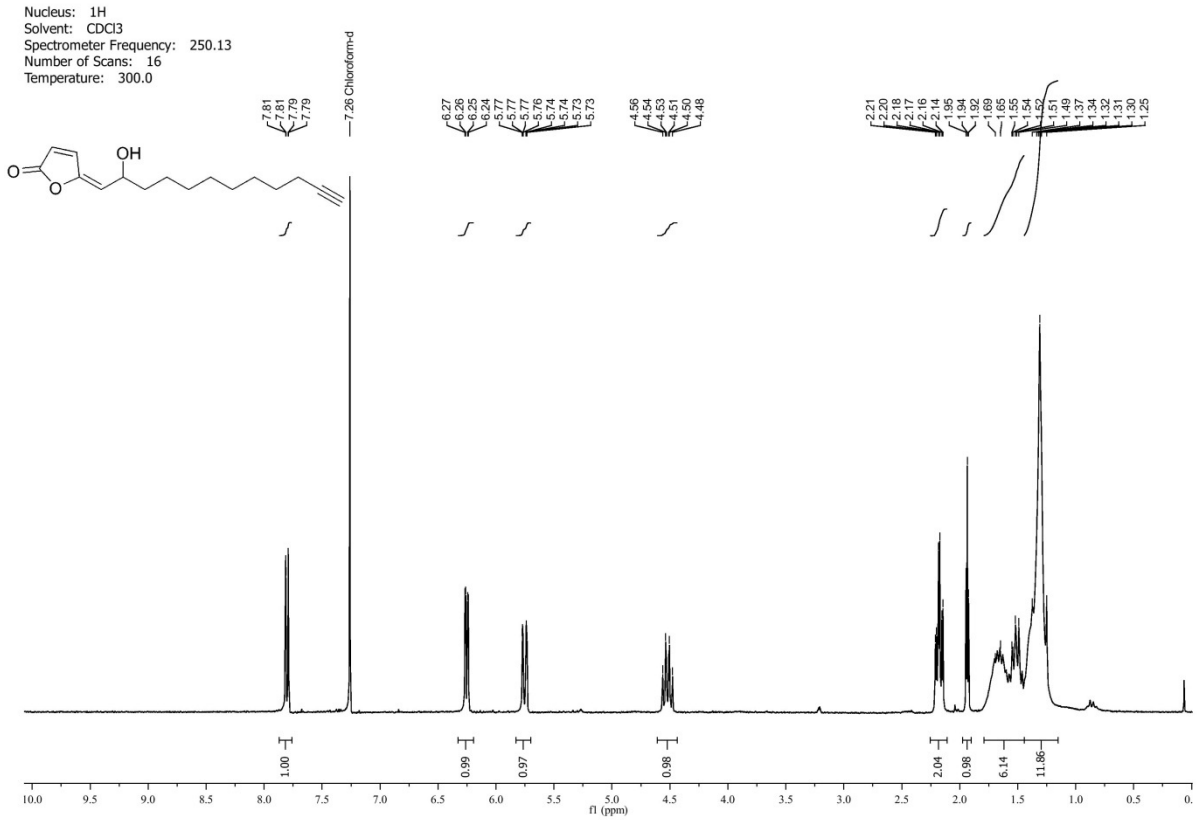

Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 75.48
Number of Scans: 1024
Temperature: 300.0
199.2
168.0
154.4
145.8
123.4
110.7
77.2 Chloroform-d
43.6
32.0
29.7
29.6
29.6
29.5
29.3
24.0
22.8
14.3
f1 (ppm)

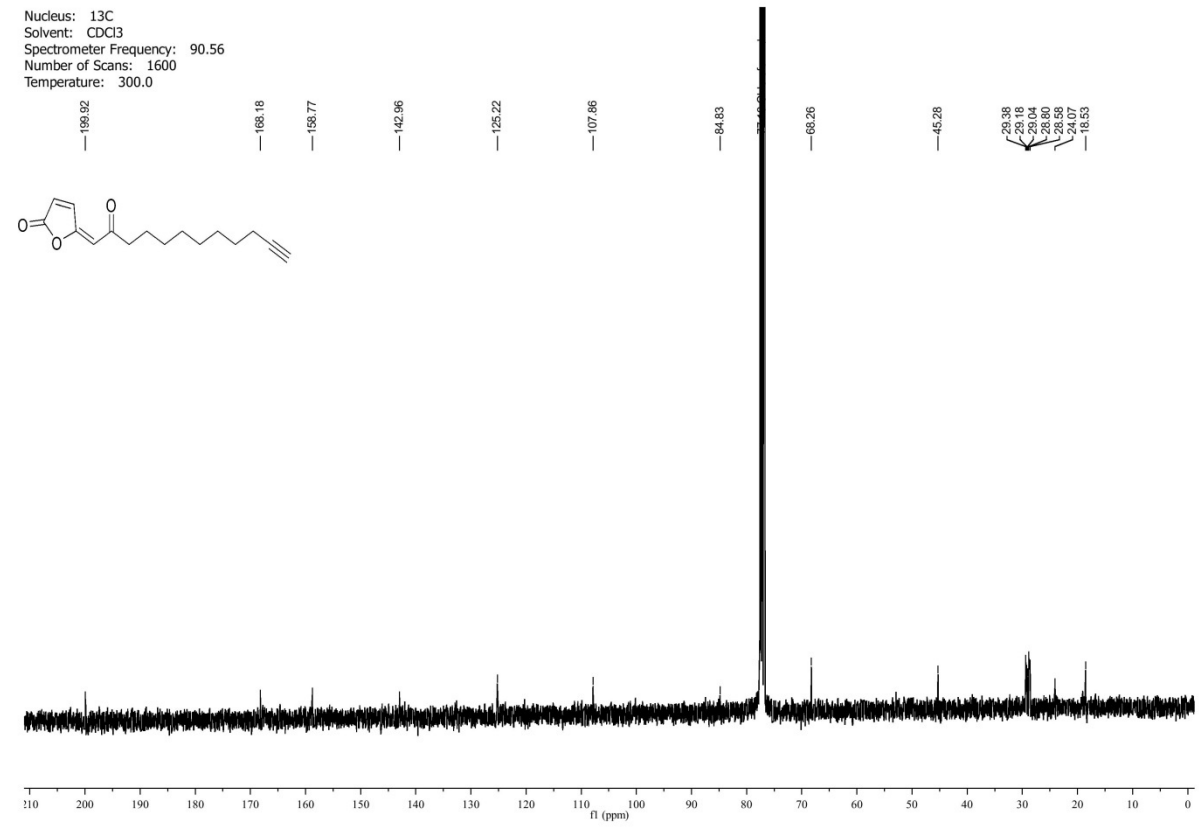
Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 500.13
Number of Scans: 16
Temperature: 300.0



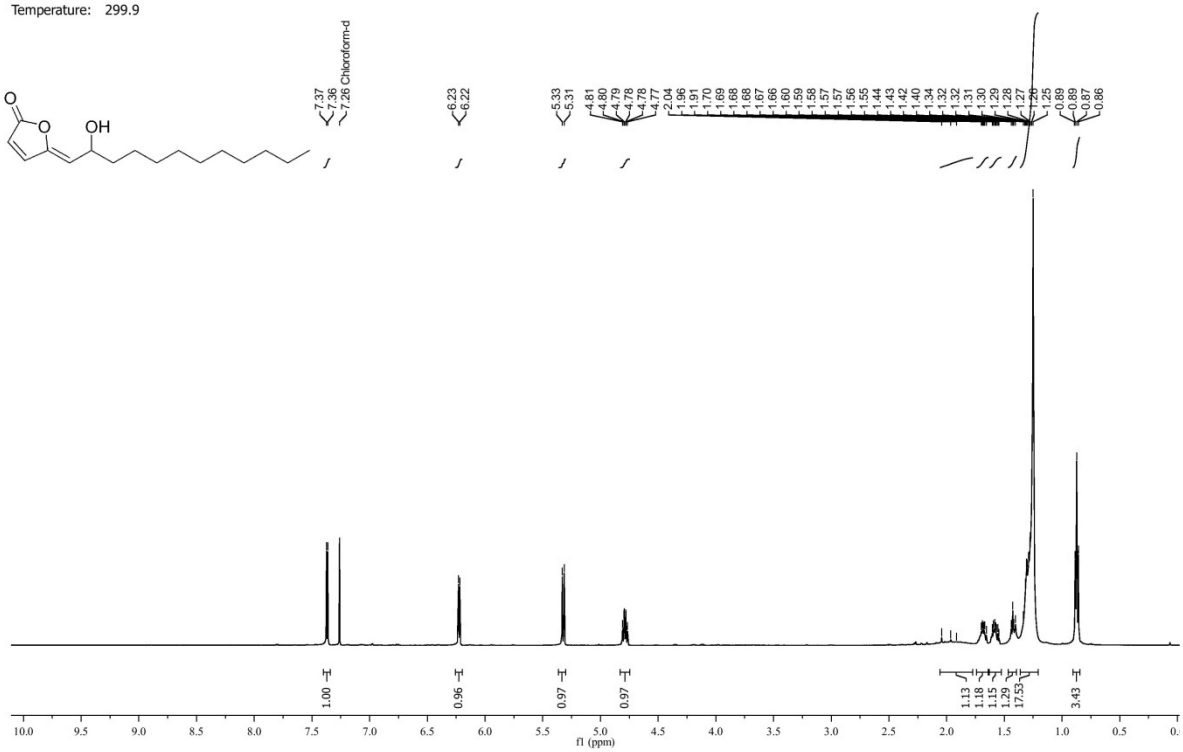
Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 125.83
Number of Scans: 256
Temperature: 299.9




Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 250.13
Number of Scans: 16
Temperature: 300.0
7.81
7.81
7.79
7.79
7.26 Chloroform-d
6.27
6.26
6.25
6.24
5.77
5.77
5.77
5.76
5.74
5.74
5.73
5.73
4.56
4.54
4.53
4.51
4.50
4.48
2.21
2.20
2.18
2.17
2.16
2.14
1.95
1.94
1.92
1.69
1.65
1.55
1.54
1.52
1.51
1.49
1.37
1.34
1.32
1.31
1.30
1.25
OH
O
O
1.00
0.99
0.97
0.98
2.04
0.98
6.14
11.86
f1 (ppm)


Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 90.56
Number of Scans: 1600
Temperature: 300.0
199.92
168.18
158.77
142.96
125.22
107.86
84.83
77.48
68.26
45.28
29.38
29.18
29.04
28.80
28.58
24.07
18.53
O
O
O
f1 (ppm)

Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 500.36
Number of Scans: 16
Temperature: 299.9



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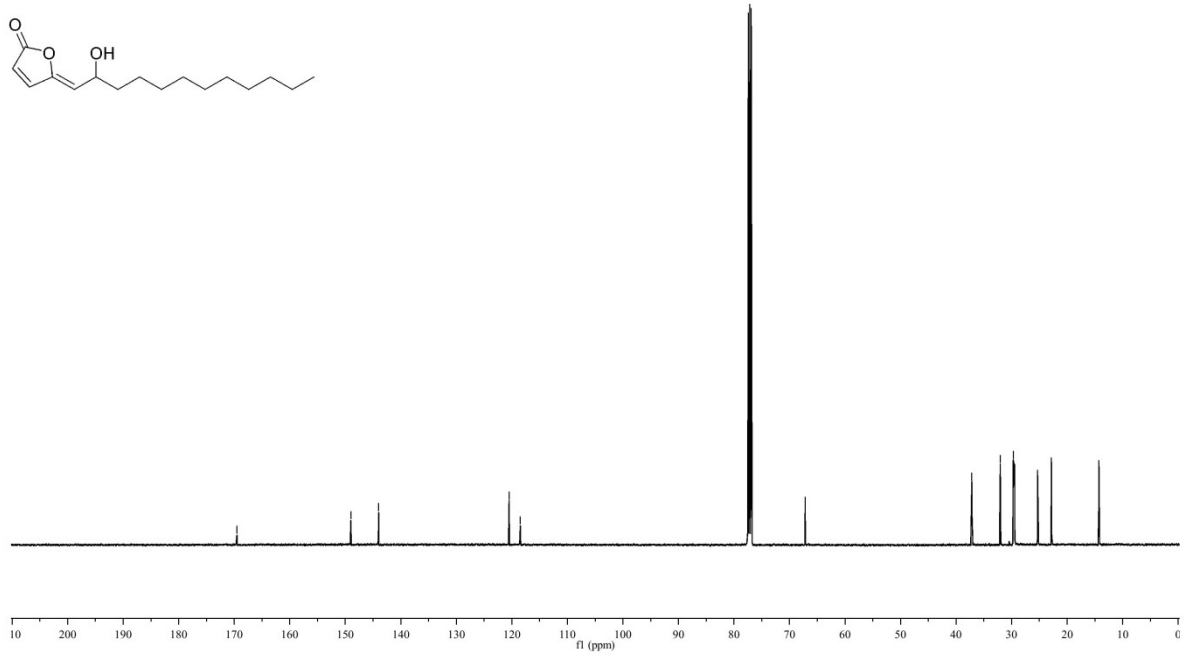
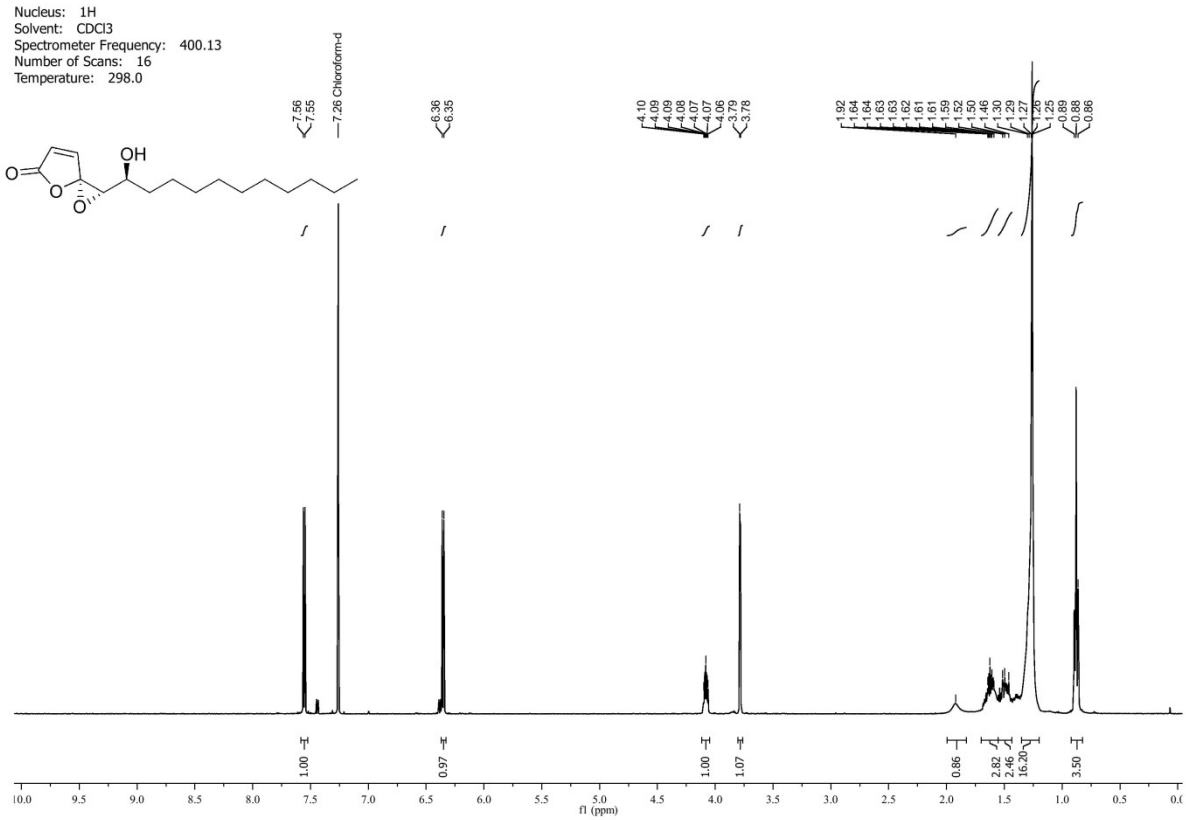
Temperature: 299.8




Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 400.13
Number of Scans: 16
Temperature: 298.0
7.56
7.55
7.26 Chloroform-d
6.36
6.35
4.10
4.09
4.09
4.08
4.07
4.07
4.06
3.79
3.78
1.92
1.64
1.64
1.63
1.63
1.62
1.61
1.61
1.59
1.52
1.50
1.46
1.30
1.29
1.27
1.26
1.25
0.89
0.88
0.86
OH
O
O
O
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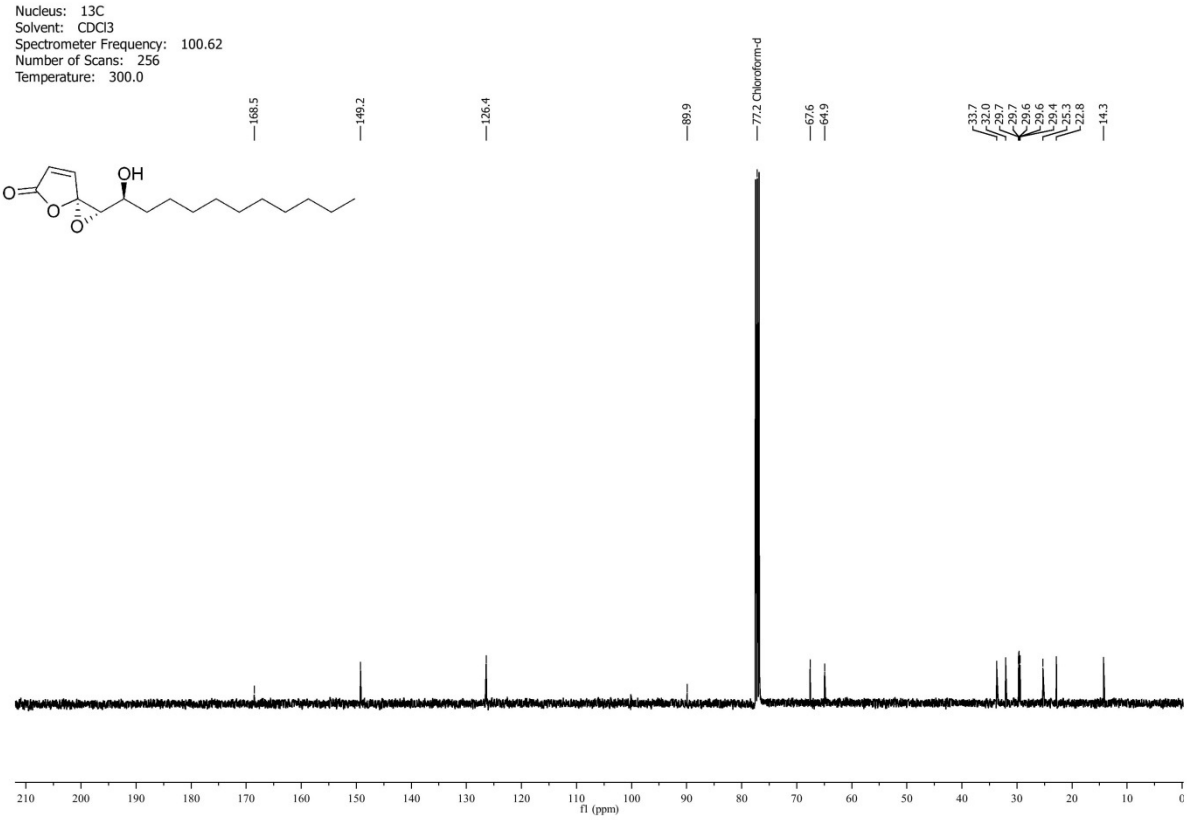
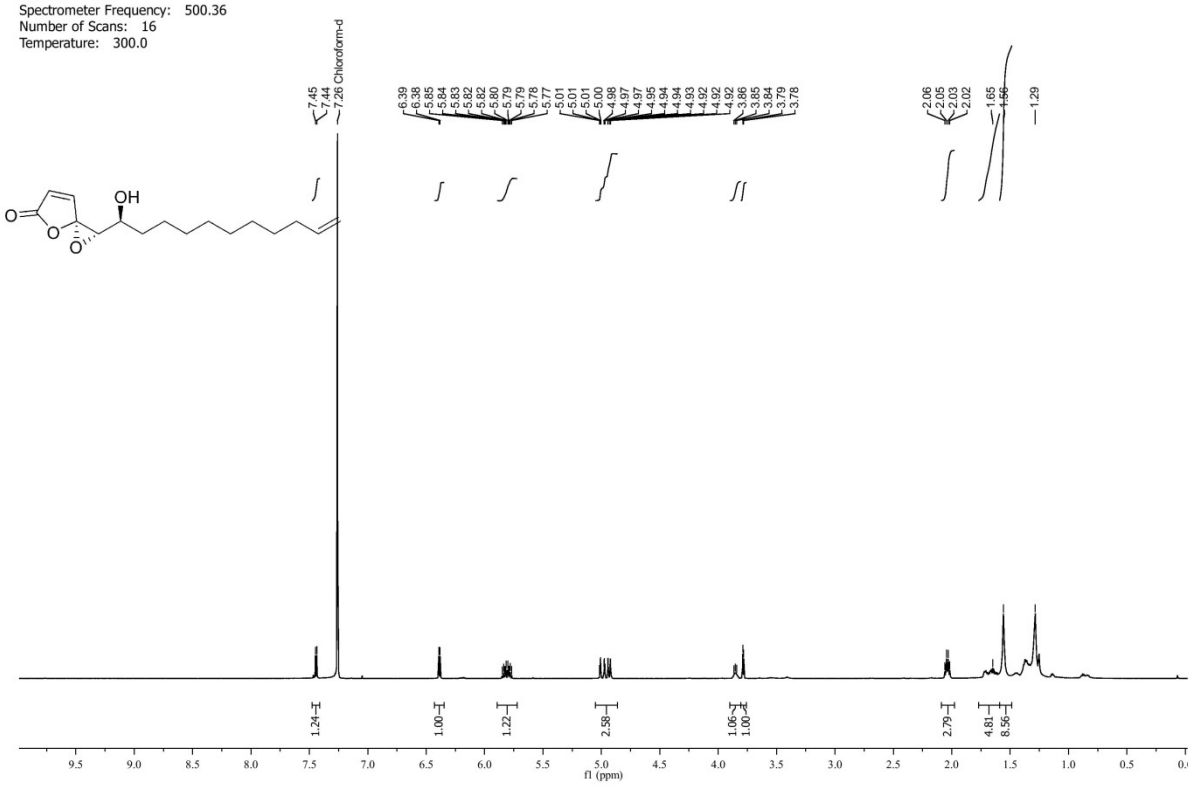
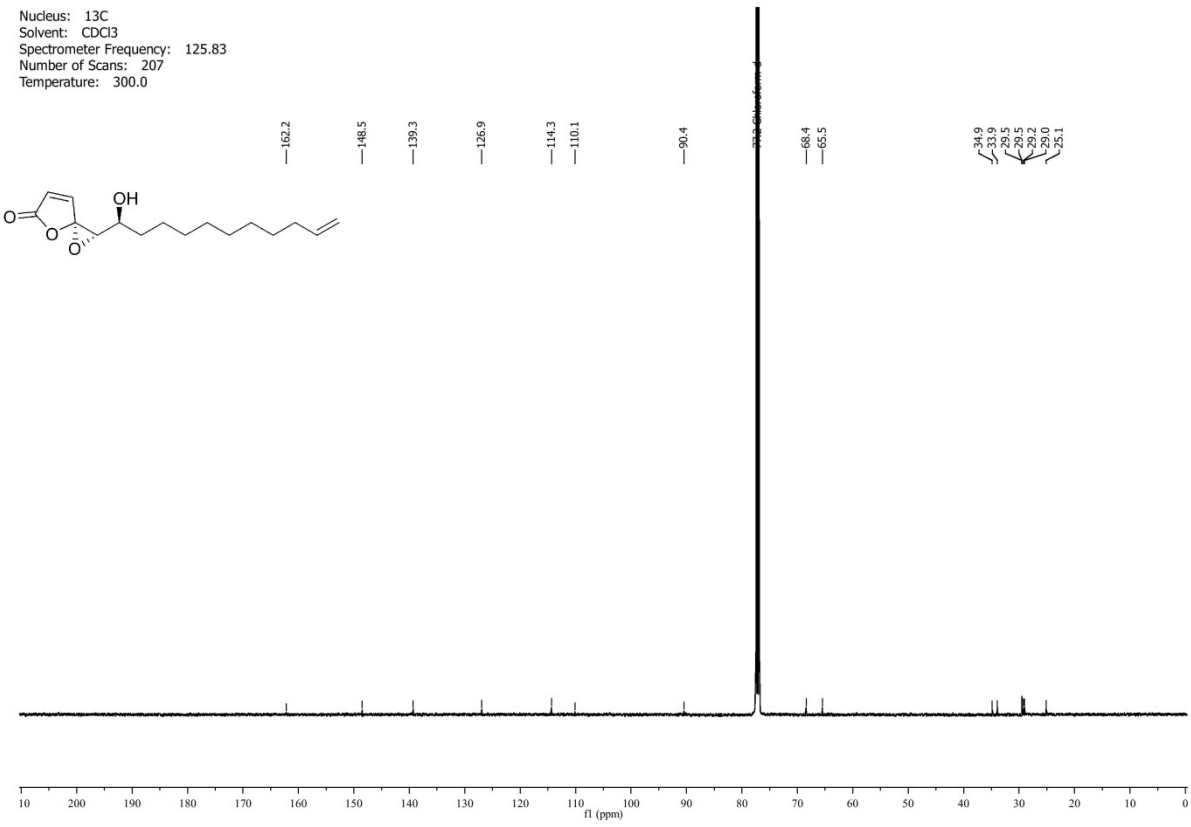
0.97
1.00
1.07
0.86
2.82
2.46
16.20
3.50
f1 (ppm)


Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 100.62
Number of Scans: 256
Temperature: 300.0
168.5
149.2
126.4
89.9
77.2 Chloroform-d
67.6
64.9
33.7
32.0
29.7
29.7
29.6
29.6
29.4
25.3
22.8
14.3
OH
O
O
O
f1 (ppm)

Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 500.36
Number of Scans: 16
Temperature: 300.0



Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 125.83
Number of Scans: 207
Temperature: 300.0



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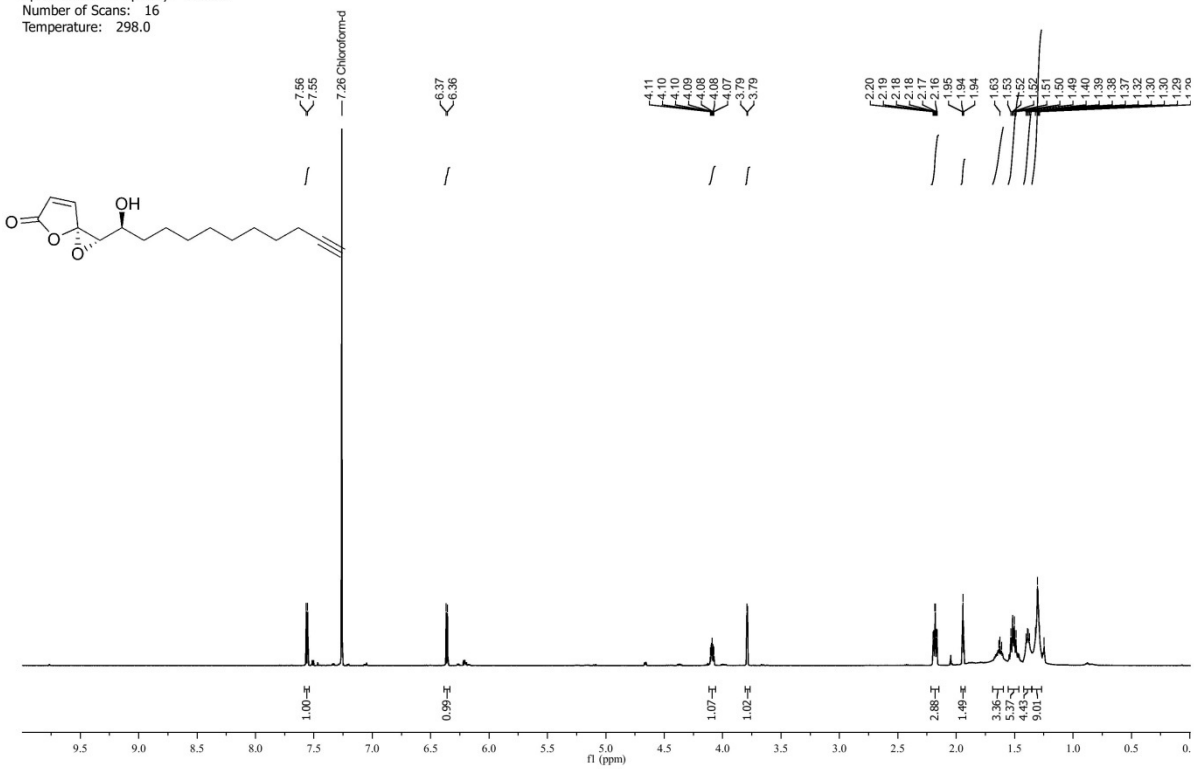
Spectrometer Frequency: 500.36
Number of Scans: 16
Temperature: 298.0



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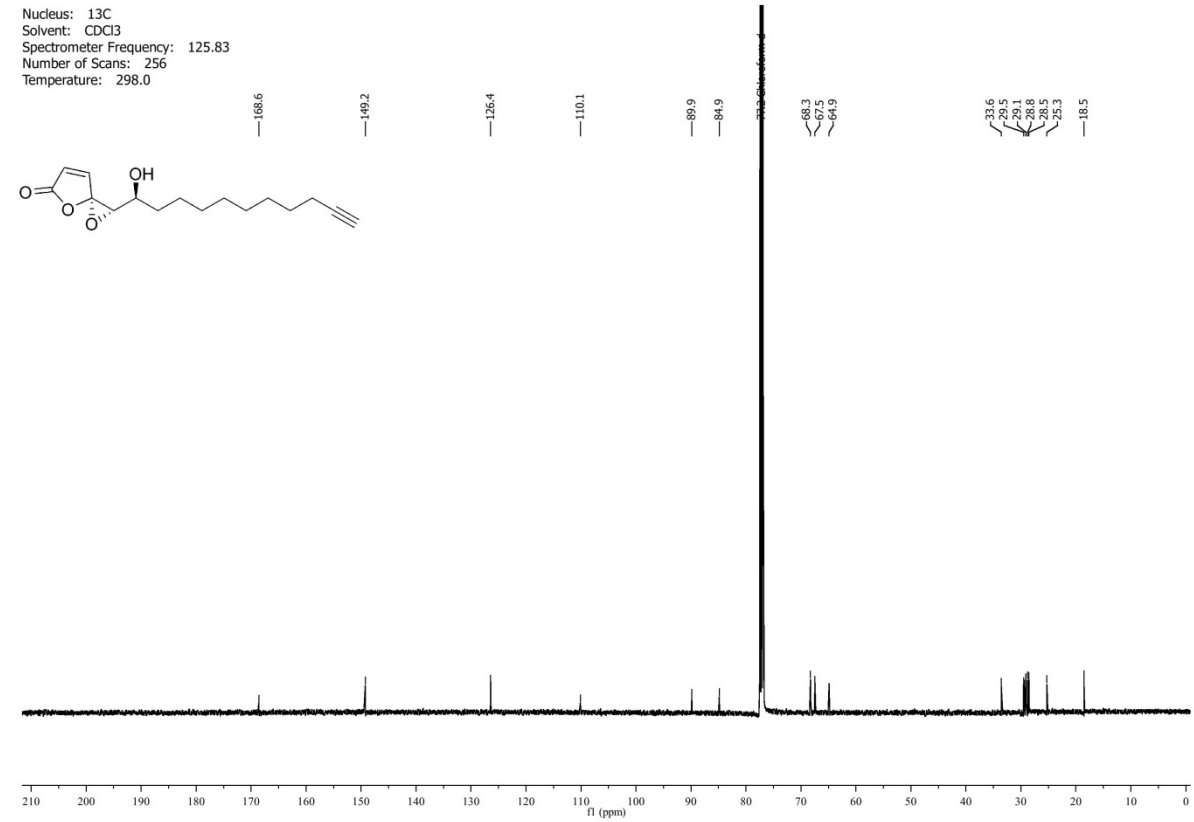
Spectrometer Frequency: 125.83
Number of Scans: 256
Temperature: 298.0



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Solvent: CDCl3
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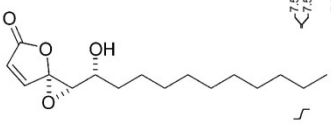
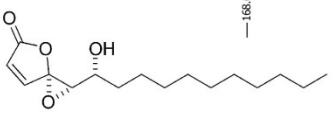
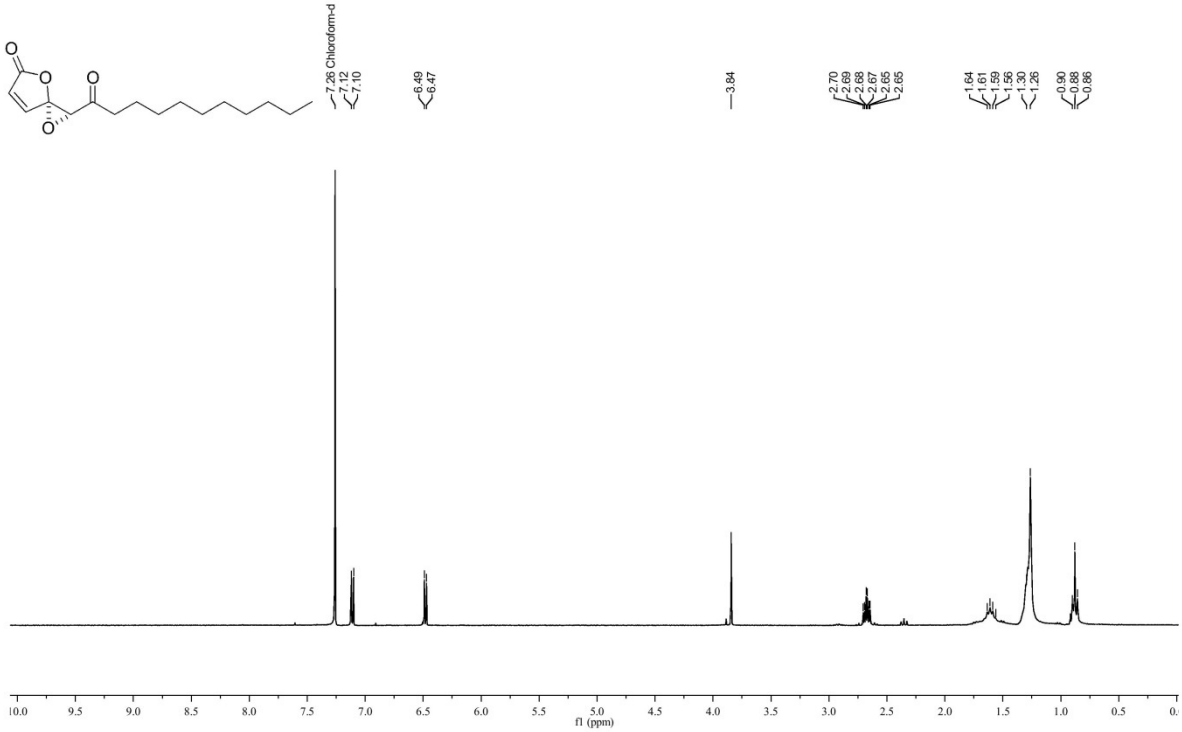
Number of Scans: 16
Temperature: 300.0



Nucleus: 13C
Solvent: CDCl3
Spectrometer Frequency: 125.83
Number of Scans: 512
Temperature: 300.0



Nucleus: 1H
Solvent: CDCl3
Spectrometer Frequency: 300.13
Number of Scans: 16
Temperature: 298.0



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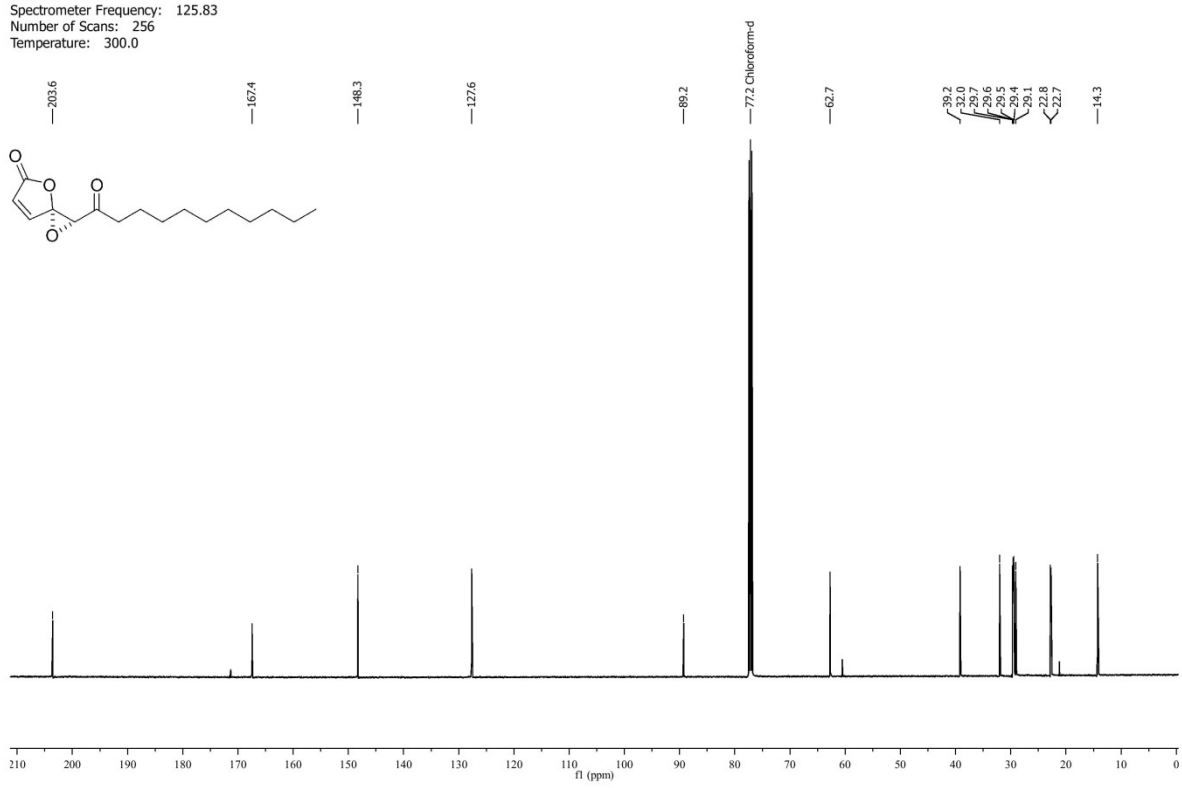
Solvent: CDCl3
Spectrometer Frequency: 125.83
Number of Scans: 256
Temperature: 300.0



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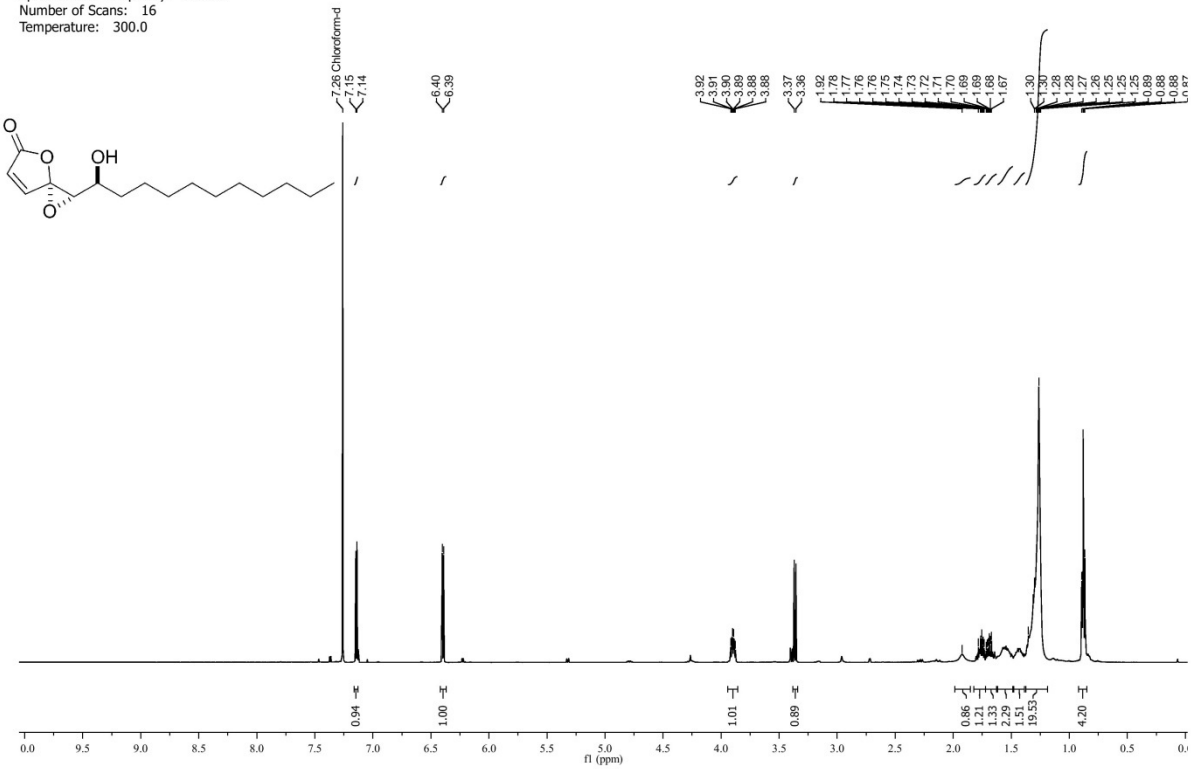
Temperature: 300.0



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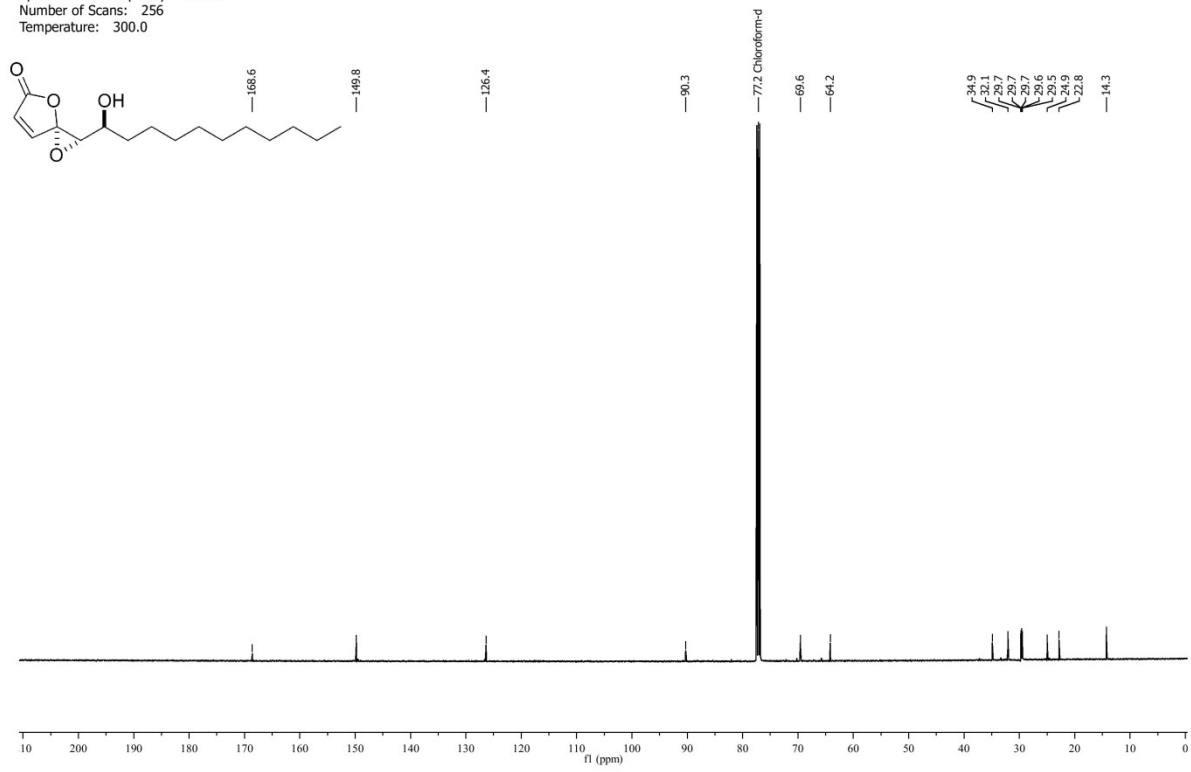
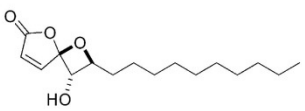
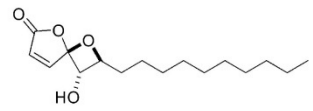
Solvent: CDCl3
Spectrometer Frequency: 125.83
Number of Scans: 256
Temperature: 300.0



Nucleus: 1H
Solvent: C6D6
Spectrometer Frequency: 500.13
Number of Scans: 16
Temperature: 298.0



Nucleus: 13C
Solvent: C6D6
Spectrometer Frequency: 125.83
Number of Scans: 256
Temperature: 299.9



5. References

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2. L. Wang, C. Cherian, S. K. Desmoulin, L. Polin, Y. Deng, J. Wu, Z. Hou, K. White, J. Kushner, L. H. Matherly and A. Gangjee, *Journal of medicinal chemistry*, 2010, **53**, 1306-1318.
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