**Supporting Information**

# Stereodivergent, chemoenzymatic synthesis of azaphilone natural products

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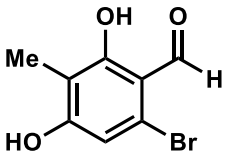
**Part I. Substrate Synthesis**

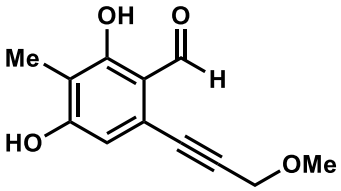
All reagents were used as received unless otherwise noted. Reactions were carried out under a nitrogen atmosphere using standard Schlenck techniques unless otherwise noted. Solvents were degassed and dried over aluminum columns on an MBraun solvent system (Innovative Technology, inc., Model PS-00-3). Reactions were monitored by thin layer chromatography using Millipore 60 $F_{254}$ precoated silica TLC plates (0.25 mm) that were visualized using UV, *p*-anisaldehyde, CAM, DNP, or bromocresol stain. Flash column chromatography was performed using Machery-Nagel 60 µm (230-400 mesh) silica gel. All compounds purified by column chromatography were sufficiently pure for use in further experiments unless otherwise indicated. $^{1}H$ and $^{13}C$ NMR spectra were obtained in $CDCl_3$ at rt (25 °C), unless otherwise noted, on Varian 400 MHz or Varian 600 MHz spectrometers. Chemical shifts of $^{1}H$ NMR spectra were recorded in parts per million (ppm) on the δ scale. High resolution electrospray mass spectra were obtained on an Agilent HPLC-TOF at the University of Michigan Life Sciences Institute.

**List of reagents prepared or purified:**

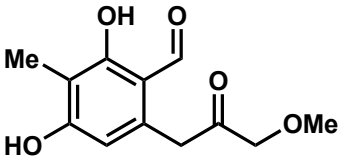
**2-Iodoxybenzoic Acid (IBX)** was synthesized according to the procedure described by Sputore *et al*.[1]
**Trifluoroacetic acid** and **acetic anhydride** were distilled prior to use.




**6-Bromo-2,4-dihydroxy-3-methylbenzaldehyde (S1)**
Prepared as previously reported by Baker Dockrey *et al*.[2]

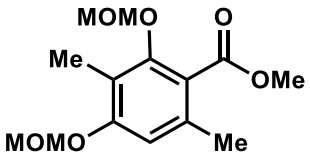



**2,4-Dihydroxy-6-(3-methoxyprop-1-yn-1-yl)-3-methylbenzaldehyde (S2)**
Aryl bromide **S1** (150 mg, 0.65 mmol), $PdCl_2(PPh_3)_2$ (23 mg, 0.033 mmol, 0.050 equiv), CuI (12 mg, 0.065 mmol, 0.10 equiv) was stirred in 4.8 mL of anhydrous DMF in a flame-dried round bottom flask equipped with a stir bar. $Et_3N$ (0.30 mL, 2.2 mmol, 3.3 equiv) was added and the mixture was sparged with $N_2$ for 15 min before methyl propargyl ether (0.11 mL, 1.3 mmol, 2.0 equiv) was added. The resulting mixture was heated to 60 °C for 14 h. The reaction mixture was cooled to rt, diluted with water (2.0 mL), and acidified with 1 M HCl (4.0 mL). The mixture was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with water and brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to afford a dark brown solid. Purification on silica gel (10-20% EtOAc in hexanes) afforded 135 mg (94% yield) of **S2** as a tan solid. **$^{1}H$ NMR** (400 MHz, $CD_3OD$) δ 10.04 (s, 1H), 6.47 (s, 1H), 4.33 (s, 2H), 3.41 (s, 3H), 1.98 (s, 3H). All spectra obtained were consistent with literature values.[3]



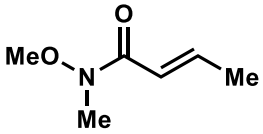

**2,4-dihydroxy-6-(3-methoxy-2-oxopropyl)-3-methylbenzaldehyde (S3)**
To a mixture of **S2** (20 mg, 0.091 mmol, 1.0 equiv) and $Au(OAc)_3$ (1.7 mg, 0.0046 mmol, 0.050 equiv) in DCE (1.0 mL) was added TFA (0.091 mL, 1.2 mmoL, 13 equiv) in a flame-dried vial under $N_2$. The resulting mixture

was stirred for 1 h at rt before 10 mL of 20% $H_2O$/MeCN was added. The mixture was stirred for 12 h at rt before it was diluted with water (1.0 mL) and extracted with EtOAc (3 x 5.0 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure to afford a dark brown solid. Purification on silica gel (80% EtOAc in hexanes) afforded 20 mg (92% yield) of **S3** as a light tan solid. **$^1$H NMR** (400 MHz, $CD_3OD$) δ 9.82 (s, 1H), 6.23 (s, 1H), 4.18 (s, 2H), 4.02 (s, 2H), 3.39 (s, 3H), 2.00 (s, 3H);**$^{13}$C NMR** (150 MHz, $CD_3OD$) δ 205.8, 193.4, 163.7, 163.0, 137.0, 112.3, 110.2, 110.1, 76.4, 58.1, 41.0, 5.9; **HR-ESI-MS**: *m/z* calculated for $C_{12}H_{15}O_5$ $[M+H]^+$: 239.2465, found: 239.2465; **IR** (thin film): 2921, 2827, 1721, 1614, 1503 $cm^{-1}$; **MP**: 127-129 °C.



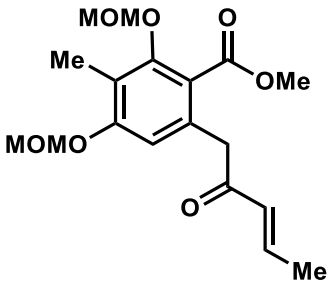

**Methyl 2,4-bis(methoxymethoxy)-3,6-dimethylbenzoate (S4)**

Methyl atratate (13 g, 66 mmol, 1.0 equiv) in THF (650 mL) was cooled to 0 °C and NaH (60%, 7.9 g, 200 mmol, 3.0 equiv) was added portionwise. MOMCl[4] (15 mL, 200 mmol, 3.0 equiv) was slowly added to the resulting mixture. The solution was warmed to rt and stirred for 5 h before it was cooled to 0 °C and quenched with $NH_4Cl$ (500 mL, saturated aq.). The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 500 mL). The combined organic layers were washed with $NaHCO_3$, dried over $Na_2SO_4$ and concentrated under reduced pressure to afford a yellow oil. Purification on silica gel (0-20% EtOAc in hexanes) afforded 15 g of the ester as a colorless oil (90% yield). **$^1$H NMR** (300 MHz, $CDCl_3$): δ 6.72 (s, 1H), 5.19 (s, 2H), 4.96 (s, 2H), 3.89 (s, 3H), 3.54 (s, 3H), 3.47 (s, 3H), 2.28 (s, 3H), 2.15 (s, 3H). All spectra obtained were consistent with literature values.[5]




**(*E*)-N-methoxy-N-methylbut-2-enamide (S5)**

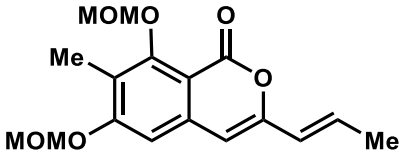
Crotonic acid (10 g, 120 mmol, 1.0 equiv) was dissolved in oxalyl chloride (12 mL, 140 mmol, 1.2 equiv) and stirred at 70 °C for 1 h. The resulting acyl chloride was distilled at 124 °C, then added to a solution of N,O-dimethylhydroxylamine hydrochloride (10 g, 100 mmol, 0.90 equiv) in DCM (190 mL). The mixture was cooled to 0 °C before pyridine (21 mL, 260 mmol, 2.2 equiv) was added slowly. The mixture was stirred at 0 °C for 30 min, then allowed to warm to rt for 30 min before it was diluted with 1 M HCl (150 mL) and extracted $Et_2O$ (3 x 150 mL). The combined organic layers were washed with brine (300 mL), dried over $MgSO_4$, and concentrated to afford a red oil. Purification on silica gel (60-80% $Et_2O$ in hexanes) afforded 12 g (82% yield) of the title compound as a pale yellow oil. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 6.98 (m, 1H), 6.41 (d, J = 15.4 Hz, 1H), 3.69 (s, 3H), 3.23 (s, 3H), 1.90 (d, J = 8.6 Hz, 3H). All spectra obtained were consistent with literature values.[6]



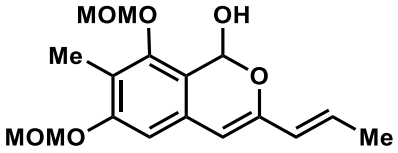

**Methyl (*E*)-2,4-bis(methoxymethoxy)-3-methyl-6-(2-oxopent-3-en-1-yl)benzoate (S6)**

Diisopropylamine (9.1 mL, 65 mmol, 1.1 equiv) in THF (300 mL) was stirred at -78 °C. *n*-BuLi (2.5 M in hexane, 26 mL, 65 mmol, 1.1 equiv) was slowly added. The resulting mixture was warmed to 0 °C and stirred for 15 min before it was cooled to -78 °C and a solution of ester **S5** (15 g, 20 mmol, 1.0 equiv) in THF (50 mL) was added. The resulting mixture was stirred at -78 °C for 15 min before a solution cooled to -78 °C of amide **S6** (9.2 g, 71

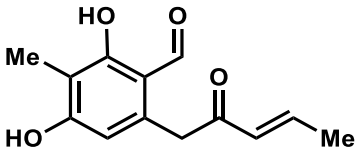
mmol, 1.2 equiv) in THF (25 mL) was added by cannula. The mixture was stirred for 1 h at -78 °C and was then acidified with 1 M HCl (aq. 100 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 x 400 mL). The combined organic layers were washed with brine (400 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure to afford a yellow oil. Purification on silica gel (5-15% EtOAc in hexanes) afforded 4.5 g of an inseparable mixture of the title compound and remaining amide **S5**. This mixture was carried forward without further purification.




**(*E*)-6,8-Bis(methoxymethoxy)-7-methyl-3-(prop-1-en-1-yl)-1H-isochromen-1-one (S7)**
NaH (170 mg, 4.50 mmol, 1.05 equiv, 60%, dispersion in mineral oil) was stirred in THF (290 mL) at -20 °C. Enone **S7** (1.5 g, 4.3 mmol, 1.0 equiv) in THF (50 mL) was slowly added to the suspension. *t*-BuOH (10 µL) was added and the solution was stirred for 1 h at 20 °C. The reaction was cooled to 0 °C and quenched with EtOAc (30 mL), followed by addition of a saturated aqueous solution of $NH_4Cl$ (40 mL). The mixture was then allowed to warm to rt. The layers were separated, and the aqueous layer extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (400 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure to afford a white solid. Purification on silica gel (0-20% EtOAc in hexanes) afforded 1.3 g of the lactone **S8** as a white crystalline solid (22% yield over 2 steps). **$^1$H NMR** (400 MHz, $CDCl_3$): δ 6.78 (s, 1H), 6.59 (m, 1H), 6.12 (s, 1H), 5.99 (dt, J = 15.7, 1.7 Hz, 1H), 5.28 (s, 2H), 5.16 (s, 2H), 3.64 (s, 3H), 3.49 (s, 3H), 2.27 (s, 3H), 1.89 (d, J = 6.9 Hz, 3H); **$^{13}$C NMR** (150 MHz, $CDCl_3$) δ 161.0, 159.2, 158.9, 152.2, 139.1, 131.6, 122.9, 122.3, 107.4, 105.1, 103.7, 101.6, 94.2, 57.7, 56.4, 18.3, 9.9; **HR-ESI-MS**: *m/z* calculated for $C_{17}H_{21}O_6$ $[M+H]^+$: 321.1333, found: 321.1335; **IR** (thin film): 2934, 2832, 1715, 1658, 1598 $cm^{-1}$**; MP**: 124-126 °C.

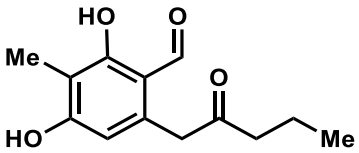



**(*E*)-6,8-bis(methoxymethoxy)-7-methyl-3-(prop-1-en-1-yl)-1H-isochromen-1-ol (S8)**
Lactone **S7** (1.0 g, 2.6 mmol, 1.0 equiv) in THF (13 mL) was stirred at -78 °C. DIBALH (0.48 mL, 2.7 mmol, 1.1 equiv) was added dropwise via syringe. The resulting solution was allowed to stir at -78 °C for 30 min, and then the reaction was quenched by the addition of EtOAc (1.0 mL) and Rochelle's salt (saturated, 4.0 mL). The resulting mixture was stirred for 1 h at rt. The crude mixture was extracted with EtOAc (3 x 50 mL), and the combined organic layers were washed brine (20 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification on silica gel (0-20% EtOAc in hexanes) afforded 730 mg of the lactol **S8** as a colorless oil (89% yield). **$^1$H NMR** (400 MHz, $CDCl_3$): δ 6.63 (s, 1H), 6.56 (d, J = 5.8 Hz, 1H), 6.33 (m, 1H), 5.97 (dd, J = 15.3, 2.1 Hz, 1H), 5.78 (s, 1H), 5.16 (s, 2H), 5.01 (s, 2H), 3.60 (s, 3H), 3.45 (s, 3H), 2.15 (s, 3H), 1.84 (m, 3H); **$^{13}$C NMR** (100 MHz, $CDCl_3$) δ 156.7, 153.0, 148.7, 129.1, 128.3, 126.0, 119.0, 116.4, 105.8, 102.0, 99.9, 94.4, 89.0, 57.5, 56.0, 18.2, 9.9; **HR-ESI-MS**: *m/z* calculated for $C_{17}H_{23}O_6$ $[M+H]^+$: 323.1489, found: 323.1489**; IR** (thin film): 3357, 2930, 2823, 1604, 1447 $cm^{-1}$; **MP**: 62-65 °C.

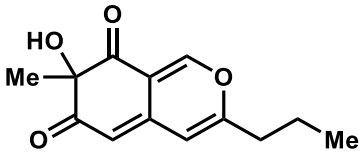



**(*E*)-2,4-Dihydroxy-3-methyl-6-(2-oxopent-3-en-1-yl)benzaldehyde (19)**
Lactol **S8** (300 mg, 0.933 mmol) in MeCN and water (7:1, 0.04 M) was stirred at rt. $LiBF_4$ (630 mg, 6.7 mmol, 7.2 equiv) was added and the resulting mixture was stirred at 70 °C for 3 h. The reaction mixture was cooled to rt and quenched by the addition of water (15 mL). The mixture was diluted with EtOAc (30 mL) and the layers were

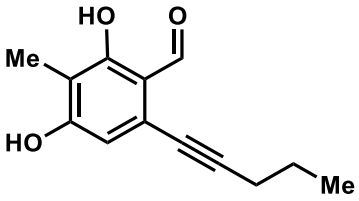
separated. The aqueous layer was extracted with EtOAc (3 x 30 mL) and the combined organic layers were washed brine (40 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification on silica gel (10-40% EtOAc in hexanes) afforded 200 mg (90% yield) of the title compound as a tan crystalline solid. **$^1$H NMR** (600 MHz, $CD_3OD$) δ 9.77 (s, 1H), 7.06 (m, 1H), 6.25 (d, J = 1.8 Hz, 1H), 6.22 (s, 1H), 4.12 (s, 2H), 2.00 (s, 3H), 1.92 (d, J = 5.2 Hz, 3H); **$^{13}$C NMR** (150 MHz, $CD_3OD$) δ 197.5, 193.4, 163.7, 163.0, 144.8, 138.0, 130.3, 112.3, 110.3, 109.9, 42.5, 17.0, 5.8; **HR-ESI-MS**: *m/z* calculated for $C_{13}H_{15}O_4$ $[M+H]^+$: 235.0965, found: 235.0947**; IR** (thin film): 3231, 2928, 2824, 2409, 1614 $cm^{-1}$; **MP**: 139-142 °C.




**2,4-Dihydroxy-3-methyl-6-(2-oxopentyl)benzaldehyde (S9)**
Prepared as previously reported by Baker Dockrey *et al*.[2]




**7-hydroxy-7-methyl-3-propyl-6H-isochromene-6,8(7H)-dione (S10)**
Prepared as previously reported by Baker Dockrey *et al*.[2]




**2,4-Dihydroxy-3-methyl-6-(pent-1-yn-1-yl)benzaldehyde (S11)**
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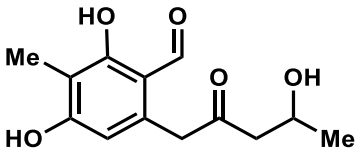


**2,4-Dihydroxy-6-(4-hydroxy-2-oxopentyl)-3-methylbenzaldehyde (S12)**
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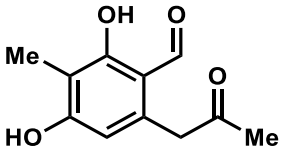
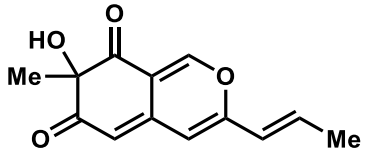
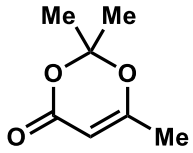



**2,4-dihydroxy-3-methyl-6-(2-oxopropyl)benzaldehyde (21)**
Prepared as previously reported by Baker Dockrey *et al*.[2]



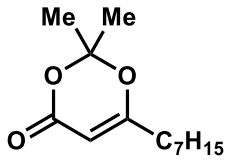
**(*E*)-7-hydroxy-7-methyl-3-(prop-1-en-1-yl)-6*H*-isochromene-6,8(7*H*)-dione (18)**

**19** (20 mg, 0.085 mmol, 1.0 equiv) and $Au(OAc)_3$ (1.7 mg, 0.0043 mmol, 0.05 equiv) in DCE (0.94 mL) were added to a flame-dried vial. The mixture was stirred at rt for 30 min before IBX (28 mg, 0.10 mmol, 1.1 equiv), TBAI (19 mg, 0.051 mmol, 0.60 equiv), and TFA (0.094 mL, 10% total volume) were added. The mixture was stirred for an additional 40 min before the reaction was quenched with 5 drops of a saturated $Na_2S_2O_3$ solution. The mixture was filtered through a plug of ceilte, which washed with DCM, before the solution was concentrated under reduced pressure. Purification by preparative TLC with 2.5% MeOH in DCM yielded 4.7 mg **18** (22% yield) of the title compound as an orange oil. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 6.59 (m, 1H), 6.10 (s, 1H), 6.01 (d, J = 15.6 Hz, 1H), 5.58 (s, 1H), 1.94 (d, J = 7.0 Hz, 3H), 1.55 (s, 3H). All spectra obtained were consistent with reported values.[7]



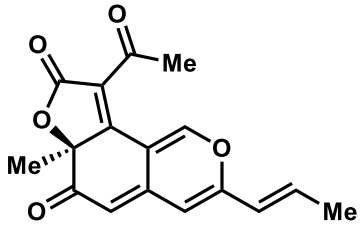
**2,2,6-trimethyl-4H-1,3-dioxin-4-one (20)**

Prepared as reported previously by Fuse *et al*.[8] 1.04 g (73% yield) of the title compound was obtained as a yellow oil. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.24 (s, 1H), 1.98 (s, 3H), 1.68 (s, 6H). All spectra obtained were consistent with literature values.[8]



**6-heptyl-2,2-dimethyl-4H-1,3-dioxin-4-one (23)**

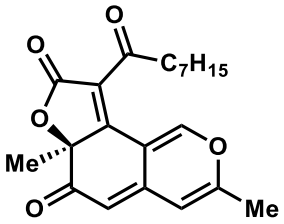
Prepared as reported previously by Franck and coworkers.[5] 356 mg (26% yield over 3 steps) of the title compound was obtained as a yellow oil and taken on crude without further purification.



**trichoflectin (17)**

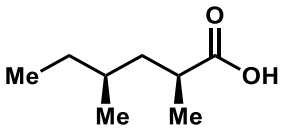
To a solution of **18** (5.5 mg, 0.023 mmol, 1.0 equiv) and dioxinone **20** (5 mg, 0.035 mmol, 1.5 equiv) in toluene (0.64 mL) in a flame dried vial under $N_2$ was added mol sieves. The mixture was stirred at rt for 10 min and then heated to 110 °C. After 1 h, $Et_3N$ (0.0064 mL, 0.046 mmol, 2.0 equiv) was added. The mixture was stirred for an additional hour at 110 °C before it was cooled to room temperature and quenched with 1 M HCl (1.0 mL). The mixture was extracted with EtOAc (3 x 2.0 mL). The organic layers were combined, washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification by preparative HPLC yielded 7.2 mg of **17** (>99% yield) as a yellow oil. **$^1$H NMR** (600 MHz, $CDCl_3$) δ 8.82 (s, 1H), 6.63 (m, 1H), 6.06 (s, 1H), 6.01 (d, J = 13.8 Hz, 1H), 5.35 (d, J = 1.2 Hz, 1H), 2.60 (s, 3H), 1.95 (d, J = 5.2 Hz, 3H), 1.69 (s, 3H); **$^{13}$C NMR** (150 MHz, $CDCl_3$) δ 194.5, 190.0, 168.2, 165.6, 155.2, 153.1, 144.0, 136.3, 123.3, 122.3, 110.9, 107.5, 105.8, 87.6, 30.1,

29.7, 26.3, 18.7; **HR-ESI-MS**: *m/z* calculated for $C_{17}H_{15}O_5$ $[M+H]^+$: 299.0914, found: 299.0922; **IR** (thin film): 2921, 2827, 1721, 1614, 1503 $cm^{-1}$; $[\alpha]_D$ +34 ° (*c* 0.1, $CHCl_3$). All spectra obtained were consistent with literature values.[9]



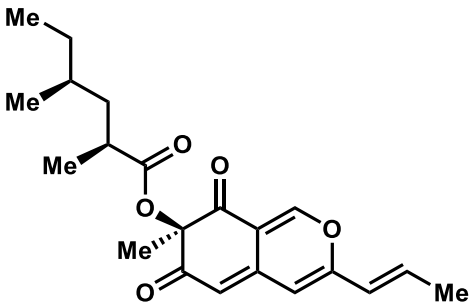

**deflectin-1a (24)**

To a solution of **22** (4.0 mg, 0.019 mmol, 1.0 equiv) and dioxinone **23** (6.6 mg, 0.029 mmol, 1.5 equiv) in toluene (0.55 mL) in a flame dried vial under $N_2$ was added mol sieves. The mixture was stirred at rt for 10 min and then was heated to 110 °C. After 1 h, $Et_3N$ (53 µL, 0.038 mmol, 2.0 equiv) was added. The mixture was stirred for an additional hour at 110 °C before it was cooled to room temperature and quenched with 1 M HCl (1.0 mL). The mixture was extracted with EtOAc (3 x 2.0 mL) and the combined organic layers were washed with (5.0 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification on silica gel (20% EtOAc in hexanes) yielded 5.9 mg of **24** (87% yield) as a yellow oil. **$^1$H NMR** (599 MHz, $CDCl_3$) δ 8.77 (s, 1H), 6.08 (s, 1H), 5.28 (s, 1H), 3.16 (m, 1H), 2.83 (m, 1H), 2.20 (s, 3H), 1.68 (s, 3H), 1.62 (m, 2H), 1.55 (s, 3H), 1.27 (m, 8H), 0.87 (m, 3H); **$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 197.3, 190.3, 168.1, 165.2, 158.7, 153.3, 144.1, 123.6, 111.1, 108.4, 104.9, 87.6, 42.1, 31.6, 29.0, 29.0, 26.2, 23.4, 22.6, 19.4, 14.1; **HR-ESI-MS**: *m/z* calculated for $C_{21}H_{25}O_5$ $[M+H]^+$: 357.1697**,** found: 357.1754; **IR** (thin film): 2924, 1763, 1684, 1642 1540 $cm^{-1}$; $[\alpha]_D$ +88 ° (*c* 0.1, EtOAc). All spectra obtained were consistent with literature values.[10]




**(2S,4S)-2,4-dimethylhexanoic acid (S13)**

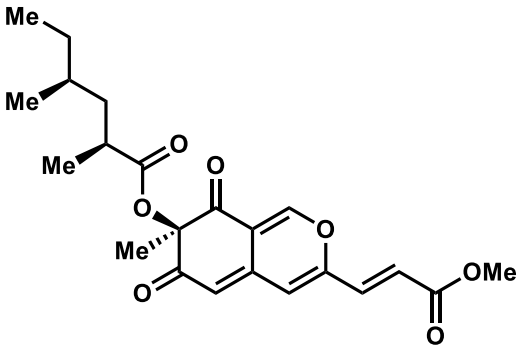
Prepared as reported previously by Myers *et al*.[11] 278 mg (32% yield over 3 steps) of the title compound was obtained as a white crystalline solid. **$^1$H NMR** (599 MHz, $CDCl_3$) δ 2.56 (m, 1H), 1.72 (m, 1H), 1.39 (m, 1H), 1.32 (m, 1H), 1.17 (d, J = 7.0 Hz, 3H), 1.13 (m, 2H), 0.88 (d, J = 6.6 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H). All spectra obtained were consistent with literature values.[11]




**(R)-7-methyl-6,8-dioxo-3-((E)-prop-1-en-1-yl)-7,8-dihydro-6H-isochromen-7-yl (2S,4S)-2,4-dimethylhexanoate (26)**

To a solution of carboxylic acid **S13** (4.5 mg, 0.031 mmol, 1.1 equiv) in THF (0.2 mL) was added 2,4,6-trichlorobenzoyl chloride (0.0048mL, 0.031 mmol, 1.1 equiv) and $Et_3N$ (0.0043 mL, 0.031 mmol, 1.1 equiv). The mixture was stirred at rt for 30 min before it was filtered through celite and concentrated. The resulting clear oil was dissolved in toluene (0.8 mL) and added to **18** (6.5 mg, 0.028 mmol, 1 equiv) and DMAP (10.3 mg, 0.084 mmol, 3 equiv). This mixture was stirred at 110 °C for 1 h before it was cooled to rt, acidified with a half-saturated

solution of $NH_4Cl$ (1 mL), and extracted with EtOAc. The organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. Purification on silica gel (10-30% EtOAc in hexanes) provided 5 mg (50% yield) of the title compound as an orange oil. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 6.55 (m, 1H), 6.07 (s, 1H), 6.00 (d, J = 15.0 Hz, 1H), 5.57 (s, 1H), 2.70 (m, 1H), 1.93 (d, J = 6.9 Hz, 3H), 1.77 (m, 1H), 1.53 (s, 3H), 1.34 (m, 2H), 1.19 (d, J = 6.9 Hz, 3H), 1.13 (m, 2H), 0.89 (m, 6H). **$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 193.2, 192.7, 176.3, 155.3, 153.4, 142.6, 135.3, 122.4, 114.9, 108.5, 107.8, 83.8, 40.8, 36.2, 31.8, 29.4, 22.1, 19.1, 18.6, 17.6, 11.1; **HR-ESI-MS**: *m/z* calculated for $C_{21}H_{27}O_5$ $[M+H]^+$: 359.1853, found: 359.1858; **IR** (thin film): 2925, 2853, 1717, 1634, 1453 $cm^{-1}$.




**(*R*)-3-((E)-3-methoxy-3-oxoprop-1-en-1-yl)-7-methyl-6,8-dioxo-7,8-dihydro-6H-isochromen-7-yl (2S,4S)-2,4-dimethylhexanoate (28)**

To a solution of **26** (5.5 mg, 0.015 mmol, 1 equiv) and methyl acrylate (5.4 μL, 0.060 mmol, 4.0 equiv) in degassed DCM (0.6 mL) was added a solution of Grubbs Catalyst 2$^{nd}$ generation (2.5 mg, 0.0030 mmol, 0.20 equiv) in degassed DCM (0.5 mL). The mixture was stirred at 45 °C for 4 h before it was cooled to rt and concentrated. Purification on silica gel (10-35% EtOAc in hexanes) afforded 2.6 mg (48% yield) of the title compound as a yellow glass. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.13 (d, J = 15.6 Hz, 1H), 6.52 (d, J = 15.6 Hz, 1H), 6.45 (s, 1H), 5.69 (s, 1H), 3.82 (s, 3H), 2.69 (m, 1H), 1.75 (m, 1H), 1.53 (s, 3H), 1.29 (m, 2H), 1.19 (d, J = 6.8 3H), 1.13 (m, 2H), 0.89 (m, 6H). **$^{13}$C NMR** (201 MHz, $CDCl_3$) δ 192.8, 192.5, 165.8, 153.2, 153.1, 152.7, 140.7, 133.5, 124.0, 115.8, 114.9, 110.5, 83.8, 52.3, 40.8, 36.2, 31.8, 29.5, 21.9, 19.1, 17.6, 11.1; **HR-ESI-MS**: *m/z* calculated for $C_{22}H_{27}O_6$: 403.1751, found: 403.1728; **IR** (thin film): 2926, 2853, 1718, 1630, 1454 $cm^{-1}$. $[\alpha]_D$ -12.2 ° (*c* 0.1, $CHCl_3$). All spectra obtained were consistent with literature values.[12]

**Part II. Plasmids and Proteins**

**Plasmids:** The plasmid encoding *azaH* (G3XMC2.1), in a modified pET28 vector to afford protein with both C- and N-terminal 6 x His-tags, was a generous gift from Professor Yi Tang at the University of California, Los Angeles.[19] The plasmid encoding *afoD* was synthesized by GeneArt and cloned into a pET21a vector. The plasmids encoding for *fdmo1-7* were synthesized by Twist Bioscience and cloned into a pET28a vector.

Non-optimized *azaH* Sequence

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AzaH Protein Sequence

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Codon-Optimized *afoD* Sequence

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AfoD Protein Sequence

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Codon-Optimized *fdmo1* Sequence

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Codon-Optimized *fdmo2* Sequence

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Codon-Optimized *fdmo5* Sequence

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Codon-Optimized *fdmo6* Sequence

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Codon-Optimized *fdmo7* Sequence

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GATGTGGATGCGATGGTGGCGGATGCGCTGGGCAAACTGAAAGCGAAACTGAGCGAAAAAAAA

**Protein overexpression and purification:** Plasmids containing the genes of interest were transformed using standard heat-shock protocols into chemically competent *E. coli* into BL21(DE3) cells. Overexpression of AfoD was achieved in 500 mL 4% glycerol (v/v) Terrific Broth (TB) in 2.8 L flasks. 500 mL portions of media were inoculated with 5 mL overnight culture prepared from a single colony in Luria Broth (LB) and 100 μg/mL ampicillin (Gold Biotechnology). Cultures were grown at 37 °C and 250 rpm until the optical density at 600 nm reached 0.8. The cultures were then cooled to 18 °C for 1 h and protein expression was induced with 0.1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG, Gold Biotechnology). Expression continued at 20 °C overnight (approx. 18 h) at 200 rpm. The typical yield for one 500 mL culture was ~15 g cell pellet. Overexpression of AzaH followed the same protocol as described above, except 1 L cultures were grown in 2.8 L flasks and kanamycin was used at 50 μg/mL (Gold Biotechnology) in place of ampicillin. The typical yield for one 1 L culture was ~30 g cell pellet.

**General purification procedure:** 25-30 g of cell pellet was resuspended in 100 mL of lysis buffer containing 50 mM Tris HCl pH 7.4, 300 mM NaCl, 10 mM imidazole, and 10% glycerol. Protease inhibitors were added to lysis

buffer of AzaH only and consisted of 1 mM phenylmethane sulfonyl fluoride (v), 0.1 mg/mL benzamidine HCl, 0.5 mg/mL leupeptin, and 0.5 mg/mL pepstatin. Approximatley 1 mg/mL lysozyme was added to resuspended cells that were then incubated on a rocker at 4 °C for 30 min. Cells were lysed by passing the total cell lysate through an Avestin pressure homogenizer at 15000 psi. The total lysate was centrifuged at 40,000 x g for 30 min and the supernatant was filtered through a 0.45 μm filter. The crude cell lysate was loaded onto a 5 mL HisTrap HP column (General Electric) on an ÄKTA Pure FPLC system (General Electric) at a flow rate of 2.5 mL/min. Buffer A = the lysis buffer listed above, and Buffer B = 50 mM Tris HCl pH 7.4, 300 mM NaCl, 10% glycerol, and 400 mM imidazole. The column was washed with 25 mM imidazole (6.3% Buffer B) for 6 CV and eluted in a gradient to 100% Buffer B over 8 CV. Fractions containing AfoD or AzaH were visibly yellow and pooled for desalting on a PD10 desalting column. Average yields: 100 mg from 1 L AfoD, 20 mg from 1 L AzaH. Molecular weights including 6xHis-tags for each protein were estimated by the ProtParam tool on the Expasy server to be 49.0 kDa for AfoD and 47.6 kDa for AzaH. These molecular weights are consistent with the mass of proteins bands observed by SDS-PAGE analysis (Figure S1). The purified proteins were aliquoted into 0.6 mL tubes and frozen in liquid nitrogen before long-term storage at -80 °C.

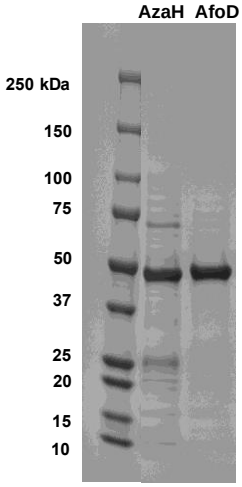



**Figure S1.** Purified AzaH and AfoD. Approximately 5 µL of 1.25 µM each protein was loaded onto an MiniPROTEAN TGX Precast 4-15% SDS-PAGE gel (Bio-Rad). The gel was stained with Quick Coomassie stain (Anatrace) and visualized with the Azure Gel Imaging System. The relative apparent masses are consistent with the predicted estimates.

**Determination of flavin incorporation and extinction coefficients:** Samples of each protein were diluted to 10 µM in 1 mL using dialysis buffer for UV-vis analysis using a disposable poly(methyl 2-methylpropenoate) cuvette. The absorbance spectrum for each protein was taken from 300 nm to 700 nm in 2 nm increments (blue traces in Figure S2). A 20 µL aliquot of fresh 10% sodium dodecyl sulfate (w/v) was added to each 1 mL solution and mixed. Samples were incubated at room temperature for 10 min before reading the absorbance spectra again under the same conditions (red traces in Figure S2). The absorbance at 450 nm for the denatured enzymes and the extinction coefficient of free FAD (11300 $M^{-1}$ $cm^{-1}$) was used to calculate the concentration of FAD in each protein sample using Beer's law. The typical FAD incorporation was 82% for AzaH, 81% for AfoD. Extinction coefficients were calculated using the concentrations of free flavin obtained and the absorbance at 450 nm of the native enzymes. At 450 nm, the extinction coefficients of the proteins are 17490 $M^{-1}$ $cm^{-1}$ for AzaH, 6,870 $M^{-1}$ $cm^{-1}$ for AfoD.

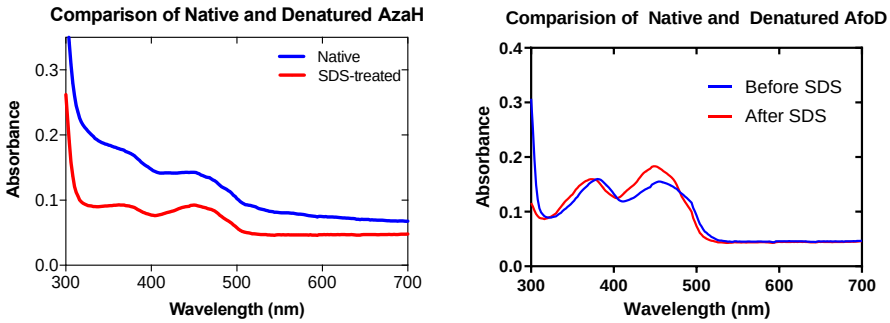



**Figure S2.** Native enzyme absorbance spectra compared to denatured enzyme absorbance spectra exposing free FAD to solution.

## Part III. Generation of AfoD Y118F variant

### General Considerations

E. coli cloning strains DH5α (Invitrogen) were used for DNA propagation. Phusion HF polymerase was purchased from New England BioLabs. All primers were purchased from Integrated DNA Technologies (IDT) ddH2O was sourced from a MilliQ Biocel water purification unit from Millipore.

### Site-directed mutagenesis

Table S1. Primer Sequences

| Variant | Plasmid ID | Oligo sequence |
|---|---|---|
| **AfoD Y118F** | AfoD_Y118F_P1 | GAAAAGAACATGGCATTTTGGGCATGTC |

The AfoD(Y118F) substitution was generated by site-directed mutagenesis on pET151-afoD(WT). 25 µL PCR reaction mixture contained 5 µL of 5X Phusion HF buffer, 1 ng/µL WT parent plasmid, 0.5 µM of primer, 200 µM dNTPs, 0.5 U µL-1 Phusion HF. Amplification was accomplished with the following PCR protocol: 95 °C for 0:30 s, (95 °C 0:30 95 °C for 0:30 s (-0.5 °C/cycle), 72 °C 0:30/kb) for 12 cycles, (95 °C for 0:30 s, 65 °C for 0:30 s, 72 °C 0:30/kb) for 20 cycles with a final extension of 72 °C for 10:00 min. This was followed by a 10 µL digestion containing 1 µL of NEB CutSmart buffer, 8 µL of PCR mixture and 20 units of DpnI. The reaction was incubated at 37 °C for 3 h and transformed into chemically competent E. coli DH5α cells.

### Protein Expression and Purification

**Protein overexpression**: AfoD(Y118F) plasmid was transformed into E. coli strain BL21(DE3). 500 mL of Terrific Broth (TB) containing 100 µg mL-1 ampicillin was inoculated with 5 mL overnight culture prepared from a single colony in Luria Broth (LB) and 100 µg mL-1 ampicillin. The culture was grown at 37 °C and 250 rpm for 4 h. The culture was then cooled to 20 °C for 1 h at 200 rpm, and protein expression was induced with 0.1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) and expressed at 20 °C for 18 h at 200 rpm. After overnight expression, cultures were centrifuged at 13,881 x g for 30 min. Cell pellets from overexpression were stored at -80 °C for long-term storage.

**General purification procedure:** Cell pellets from overexpression were resuspended in 40 mL of lysis buffer (50 mM Tris:HCl pH 7.8, 300 NaCl, 10 mM imidazole, and 10% (v/v) glycerol) with 0.1 mg mL-1 lysozyme, 0.05 mg mL-1 DNase, and 0.1 mM flavin adenine dinucleotide (FAD), incubated on a rocker at 4 °C for 45 min, and lysed by sonication. Insoluble material was removed by centrifugation (46,413 x g for 30 min). The cell pellet was resuspended in 40 mL lysis buffer (50 mM Tris:HCl pH 7.8, 300 NaCl, 10 mM imidazole, 10% (v/v) glycerol) with 0.1 mg mL-1 lysozyme, 0.05 mg mL-1 DNase, and 0.1 mM FAD, incubated on a rocker at 4 °C for 45 min, lysed by sonication and cleared by centrifugation (46,413 x g for 30 min). The supernatant was incubated with Ni-NTA on a rocker for 2 h at 4 °C, followed by purification by gravity using a 25-50 mM gradient with increments of 5 mM imidazole. Protein was eluted with 100% elution buffer (50 Mm Tris:HCl pH 7.8, 300 mM NaCl, 400 mM imidazole, 10% (v/v) glycerol). Concentrated protein was desalted over a PD-10 desalting column (GE Healthcare). The protein was concentrated further using a 30 kDa centrifugal concentrator at 4,000 x g, 4 °C. Concentrated protein was divided into 100 µL aliquots, frozen in liquid nitrogen and stored at -80 °C.

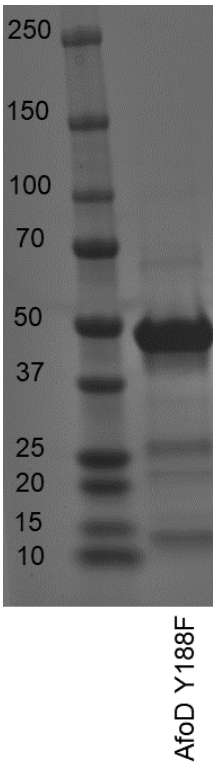



**Figure S3**. AfoD(Y118F) SDS-PAGE gel. The gel was stained with Quick Coomassie stain (Anatrace) and visualized with the Azure Gel Imaging System

**Part IV. Sequence Similarity Network (SSN)**

**Figure S4.** SSN of flavin-dependent monooxygenases created using web tools originating from the Enzyme Function Initiative (EFI). [14-16]

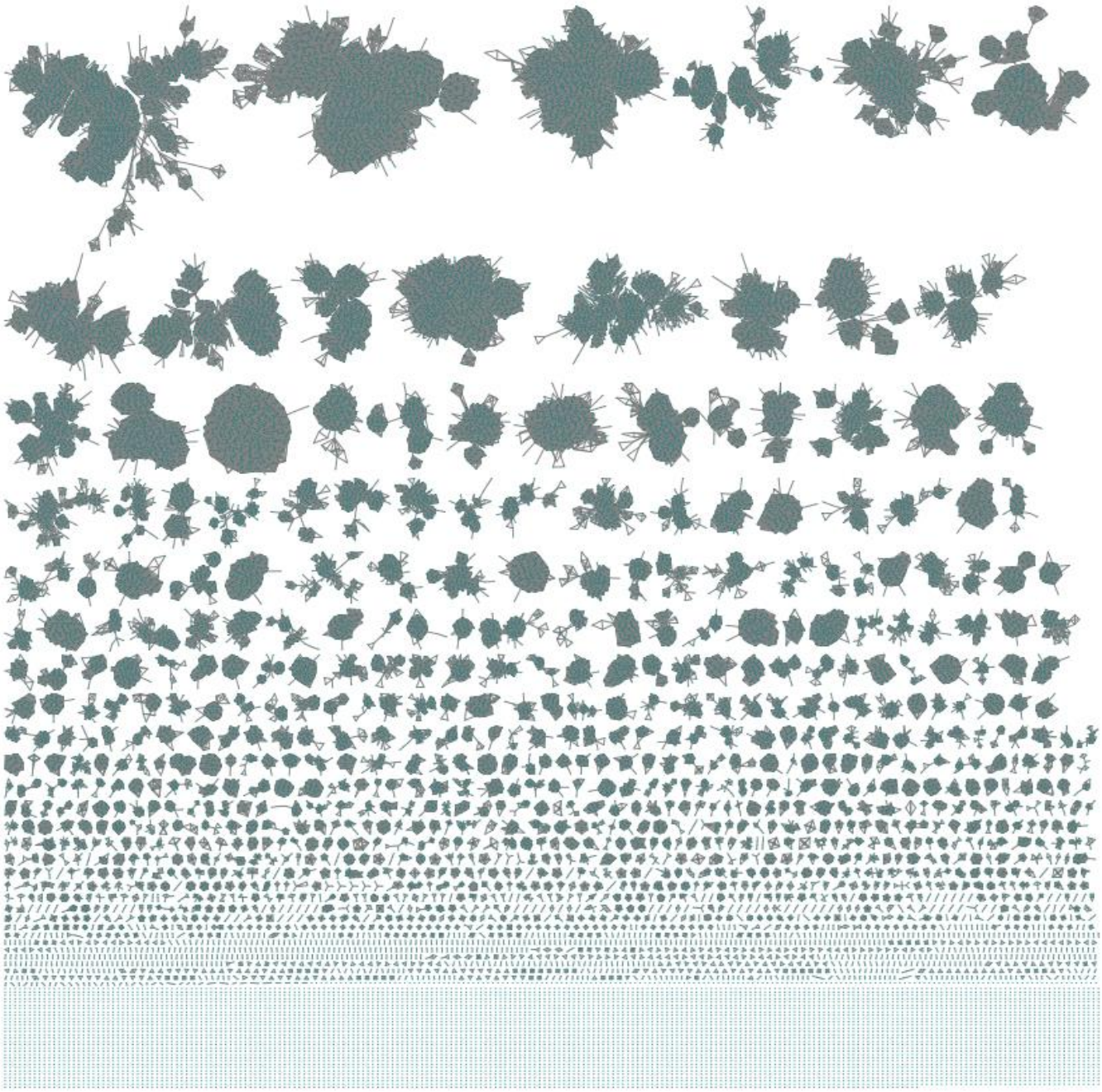
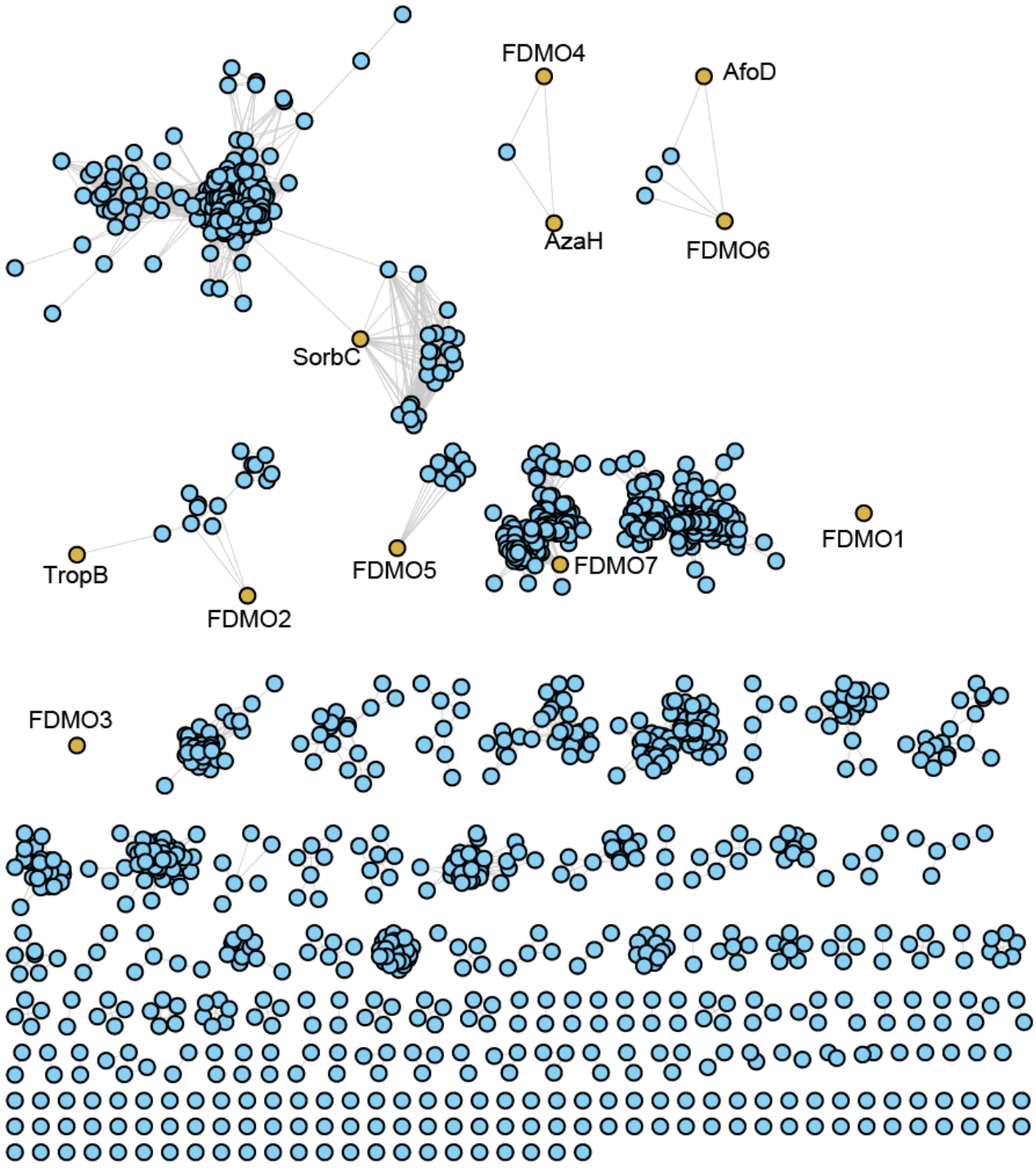


**Figure S5.** Sequence similarity network with E value of 150.[14-16]



## Part V. FDMO Sequence Alignment


**Figure S6**. AfoD cluster sequence alignment with other sequences within its cluster Y118 and F237 are highlighted. Alignment is colored by conservation.

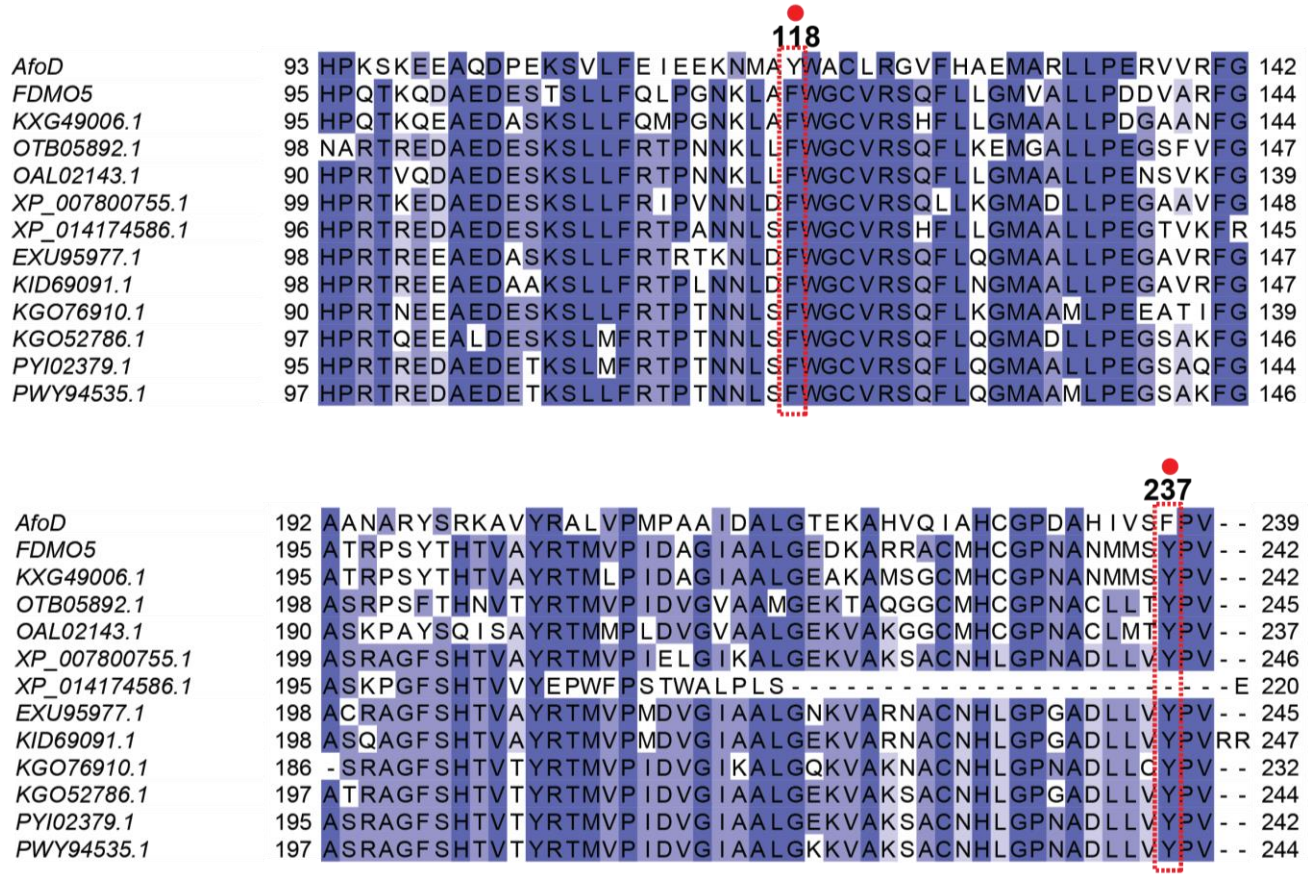



**Figure S7**. FDMO5 cluster sequence alignment with other sequences within its cluster Y118 and F237 are highlighted (From AfoD). Alignment is colored by conservation.

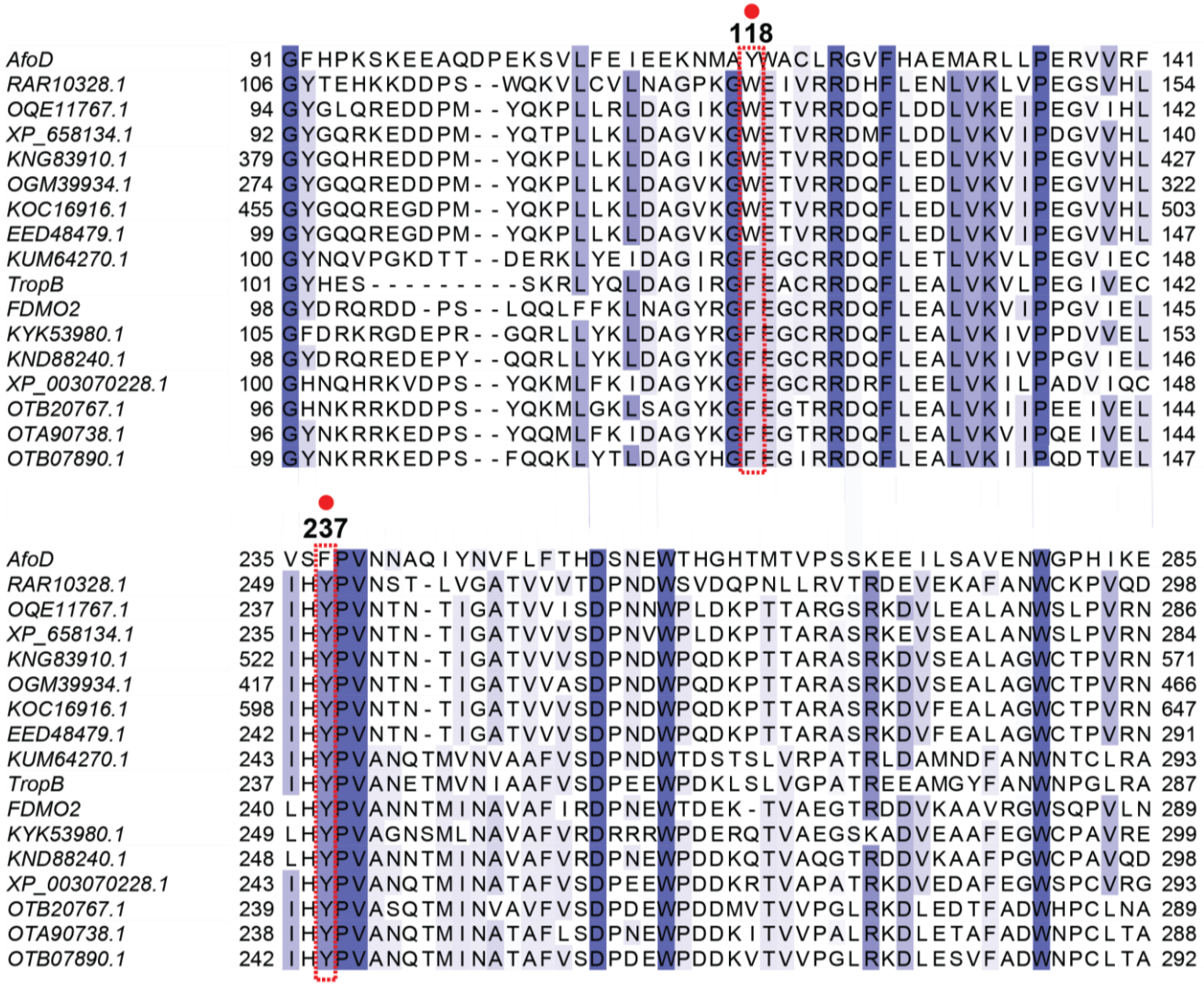
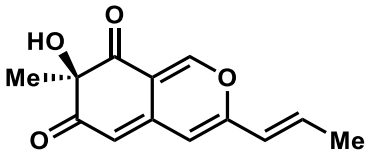



**Figure S8**. TropB cluster sequence alignment with other sequences within its cluster Y118 and F237 are highlighted (From AfoD). Alignment is colored by conservation.

**Part VI. Biocatalytic Reactions**

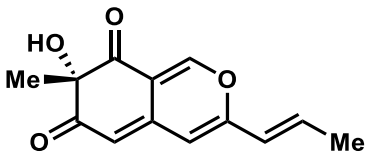
**Stock solutions:** Stock solutions of each substrate (50 mM) were prepared by dissolving the substrate in DMSO (analytical grade). Stock solutions of $NADP^+$ (100 mM) and glucose-6-phosphate (G6P, 500mM) were stored at -20 °C. Aliquots of each flavin-dependent enzyme and glucose-6-phosphate dehydrogenase (G6PDH, 100 U/mL) were stored at -80 °C. Analytical-scale reactions: Each reaction contained 25 µL 100 mM potassium phosphate buffer, pH 8.0, 2.5 mM substrate (2.5 µL of a 50 mM stock solution in DMSO), 5-20 µM flavin-dependent monooxygenase, 5 mM G6P (0.5 µL, 500 mM), 1 mM NADP+ (0.5 µL, 100 mM), 1 U/mL G6P-DH (0.5 µL, 100 U/mL), and Milli-Q water to a final volume of 50 µL. The reaction was carried out at 30 °C for 1 h and quenched by addition of 75 µL acetonitrile with 25 mM pentamethylbenzene as an internal standard. Precipitated biomolecules were pelleted by centrifugation (16,000 x g, 12 min). The supernatant was analyzed by UPLC-DAD and conversion obtained by comparison to calibration curves of each substrate. The subsequent liquid chromatography PDA spectrometry (UPLC) analysis was performed on a Waters Aquity H-Class UPLC-PDA using a Phenomenex Kinetex 1.7 µm C18, 2.1x150 mm column under the following conditions: Method A: mobile phase (A = deionized water + 0.1% formic acid, B = acetonitrile + 0.1% formic acid), 5% to 100% B over 1.5 min, 100% B for 1.0 min; flow rate, 0.5 mL/min; Method B: mobile phase (A = deionized water + 0.1% formic acid, B = acetonitrile + 0.1% formic acid), 5% to 100% B over 2 min, 100% B for 1 min; flow rate, 0.5 mL/min. Based on calibration curves of the starting materials, the percent conversion of the substrate to dearomatized product was calculated with AUCsubstrate/AUCinternal standard at 270 nm. All reactions were performed and analyzed in triplicate.

**General procedure for *in vitro* preparative-scale reactions**: Preparative–scale enzymatic reactions were conducted on 20 mg of each substrate under the following conditions: 5-20 µM flavin-dependent monooxygenase, 2.5 mM substrate, 1 mM NADP+, 1 U/mL G6PDH, and 5 mM G6P for NADPH generation in reaction buffer (50 mM potassium phosphate buffer, pH 8.0). The reaction mixture was added to a 50 mL Erlenmeyer flask and incubated at 30 °C with 100 rpm shaking. After 2 h, a 50 µL aliquot was removed and processed in an identical manner to the analytical-scale reactions described above to determine substrate conversion. The remaining reaction mixture was diluted with acetone (2 x total reaction volume). Precipitated biomolecules were pelleted by centrifugation (4,000 x g, 12 min). Isolation procedure: The supernatant was concentrated under reduced pressure to a final volume of approximately 2 mL. The resulting mixture was filtered through a 0.22 µm filter and purified by preparative HPLC using a Phenomenex Kinetex 5 µm C18, 150 x 21.2 mm column under the following conditions: mobile phase A = deionized water + 0.1% formic acid and B = acetonitrile + 0.1% formic acid; method = 5% to 100% B over 13 min, 100% B for 4 min; flow rate, 15 mL/min.




**(*R,E*)-7-hydroxy-7-methyl-3-(prop-1-en-1-yl)-6H-isochromene-6,8(7H)-dione ((*R*)-18)**

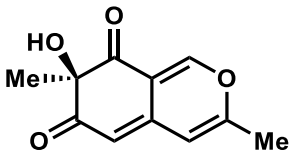
The title compound was synthesized using AzaH according to the general procedure for milligram-scale *in vitro* enzymatic oxidative dearomatization and isolated using the general isolation method. Purification by preparative HPLC afforded 9.6 mg (96% yield) of the title compound as a yellow oil. **$^1$H NMR** (400 MHz, $CDl_3$) δ 7.89 (s, 1H), 6.59 (m, 1H), 6.10 (s, 1H), 6.01 (d, J = 15.6 Hz, 1H), 5.57 (s, 1H), 2.62 (s, 2H), 1.94 (d, J = 7.0 Hz, 3H), 1.55 (s, 3H). All spectra obtained were consistent with reported values.[7]




**(*S,E*)-7-hydroxy-7-methyl-3-(prop-1-en-1-yl)-6H-isochromene-6,8(7H)-dione ((*S*)-18)**

The title compound was synthesized using AfoD according to the general procedure for milligram-scale *in vitro* enzymatic oxidative dearomatization and isolated using the general isolation method. Purification by preparative

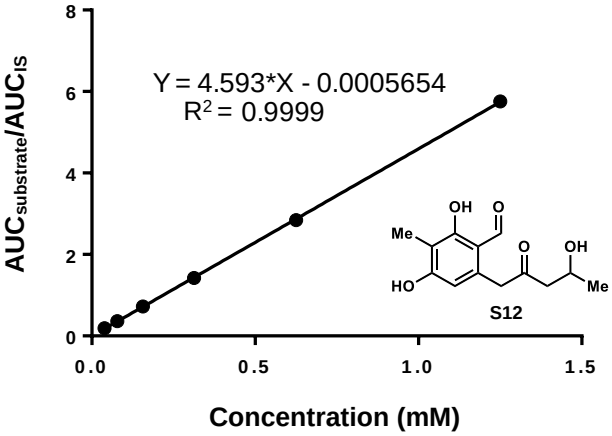
HPLC afforded 4 mg (83% yield) of the title compound as a yellow oil. **$^1$H NMR** (400 MHz, $CDl_3$) δ 7.89 (s, 1H), 6.59 (m, 1H), 6.10 (s, 1H), 6.01 (d, J = 15.6 Hz, 1H), 5.57 (s, 1H), 2.62 (s, 2H), 1.94 (d, J = 7.0 Hz, 3H), 1.55 (s, 3H). All spectra obtained were consistent with reported values.[7]

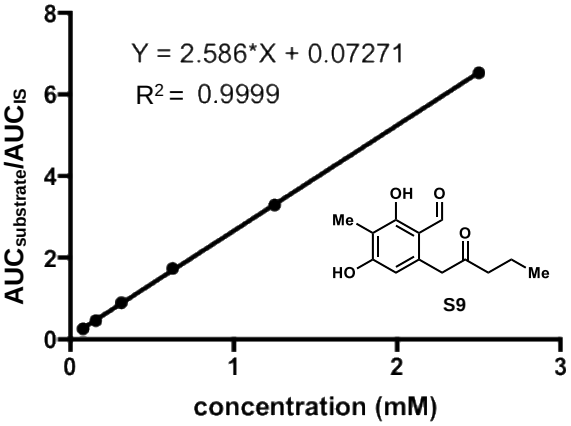


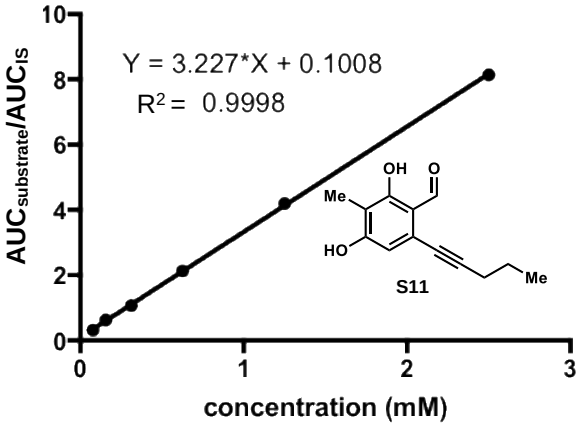

**(*R*)-7-hydroxy-3,7-dimethyl-6H-isochromene-6,8(7H)-dione (22)**

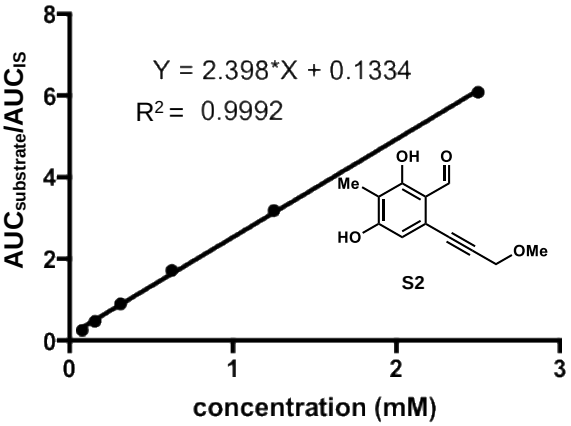
The title compound was synthesized using AzaH according to the general procedure for milligram-scale *in vitro* enzymatic oxidative dearomatization and isolated using the general isolation method. Purification by preparative HPLC afforded 9.5 mg (95% yield) of the title compound as a yellow oil. **$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.26 (s, 2H), 6.13 (s, 1H), 5.51 (s, 1H), 2.20 (s, 3H), 1.55 (s, 3H). All spectra obtained were consistent with reported values.[13]

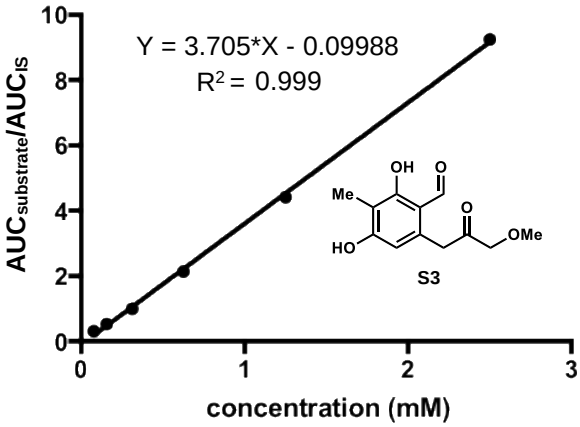
## Part VII. Substrate Calibration Curves

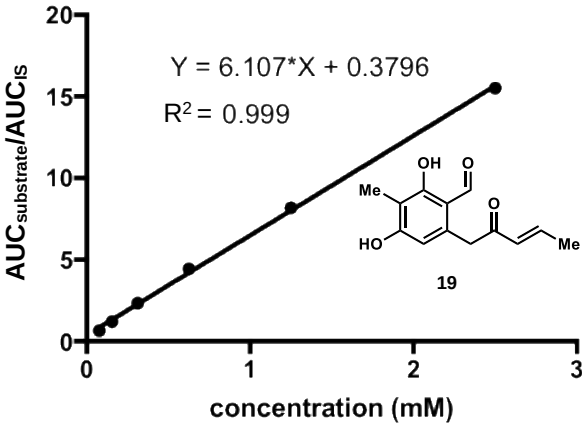


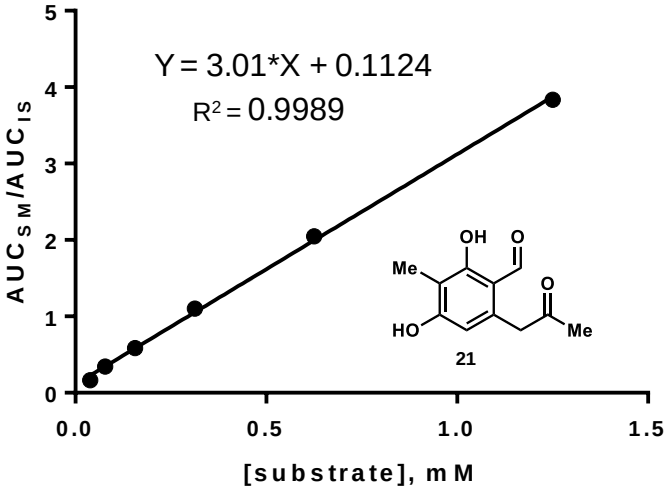

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R2 = 0.9989
AUCSM/AUCIS
[substrate], mM
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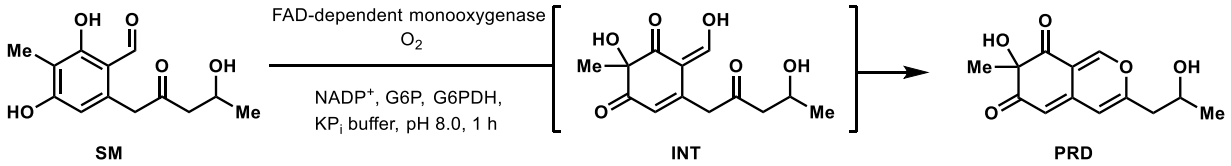
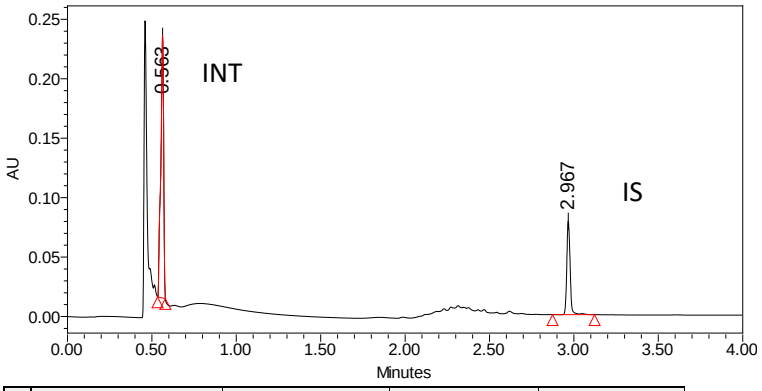
0.5
1.0
1.5
0
1
2
3
4
5
OH
O
Me
O
HO
Me
21

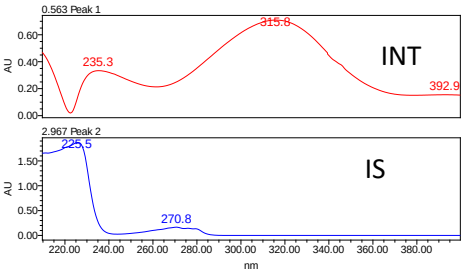
## Part VIII. UPLC Traces of Biotransformations

**Figure S9.** Oxidative dearomatization of **S12** by AfoD and AzaH. PDA traces of enzymatic reaction and control reaction. (Table 1, entry 2). SM = starting material, INT = intermediate, PRD = product, IS = internal standard. The anionic form of the intermediate elutes near the solvent front.




### With AzaH




IS

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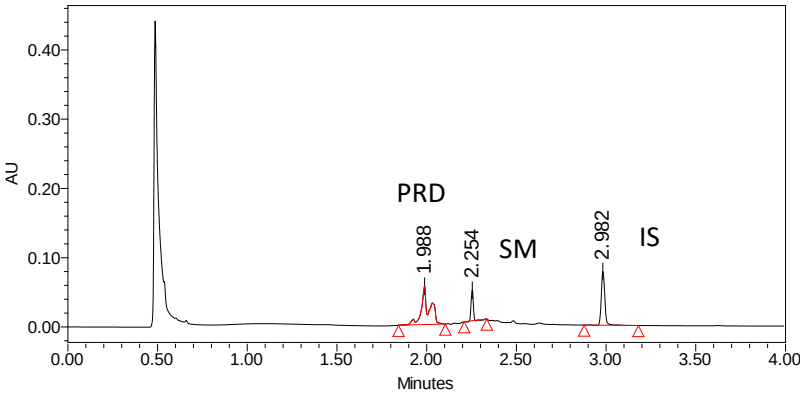
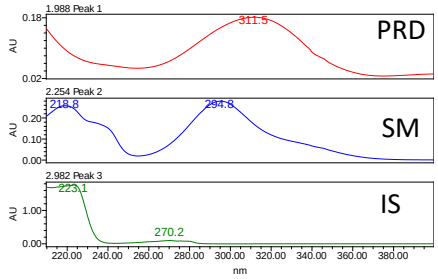
### With AfoD

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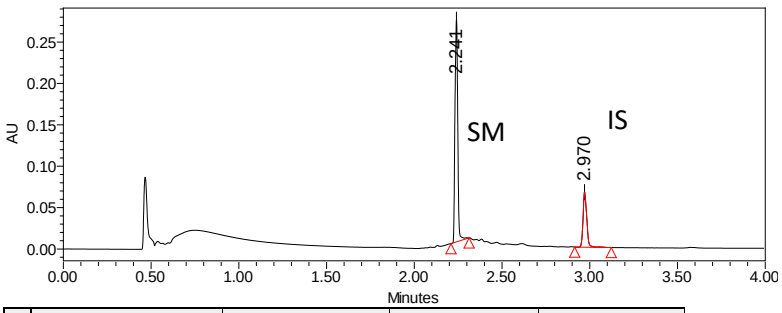
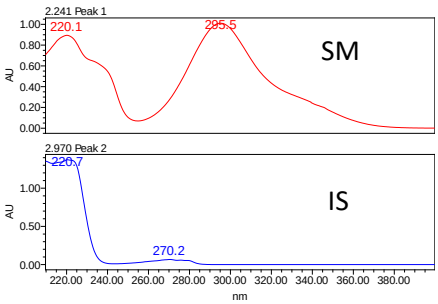
|---|---|---|---|---|
| 1 | 1.988 | 174634 | 54.03 | 55311 |
| 2 | 2.254 | 41199 | 12.75 | 44106 |
| 3 | 2.982 | 107399 | 33.23 | 77715 |

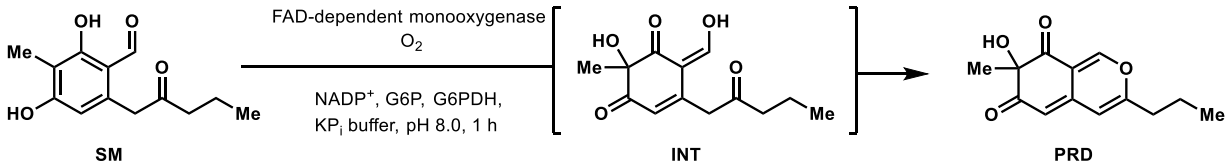
### NEC



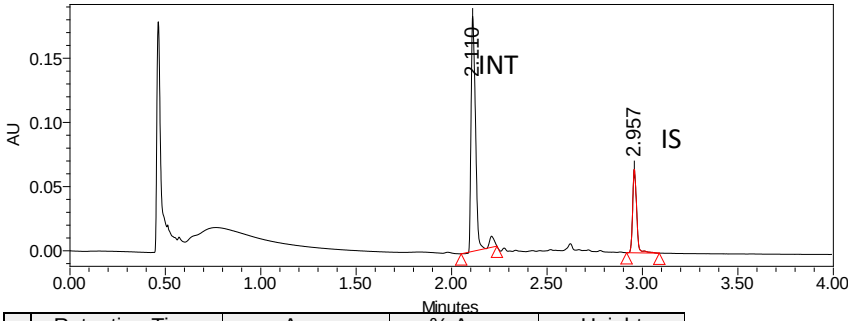



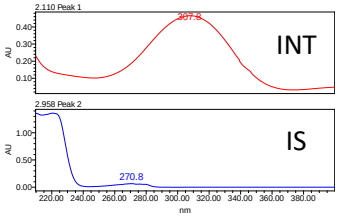

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.241 | 292664 | 74.92 | 268642 |
| 2 | 2.970 | 97969 | 25.08 | 66721 |

**Figure S10.** Oxidative dearomatization of **S9** by AfoD and AzaH. PDA traces of enzymatic reaction and control reaction (Table 1, entry 1).



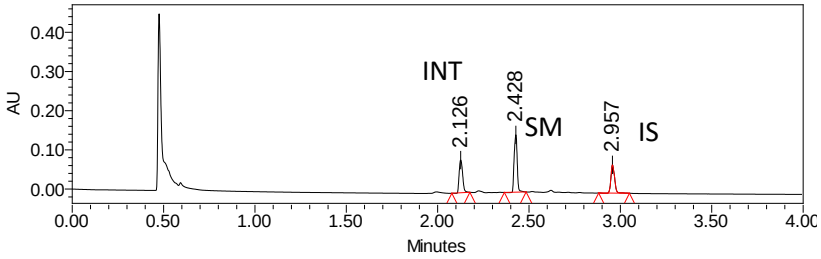

## With AzaH

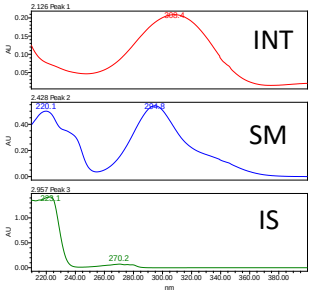



| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.110 | 305704 | 76.28 | 183131 |
| 2 | 2.957 | 95087 | 23.72 | 65154 |

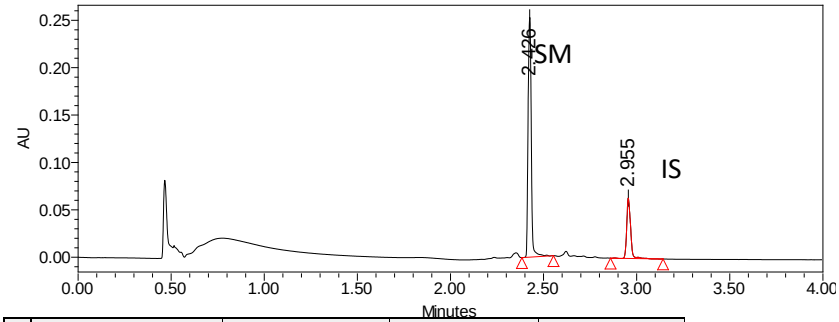
## With AfoD

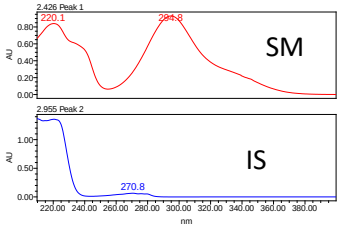



| 9 | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.126 | 111917 | 29.11 | 83638 |
| 2 | 2.428 | 171664 | 44.65 | 146387 |
| 3 | 2.957 | 100880 | 26.24 | 72085 |

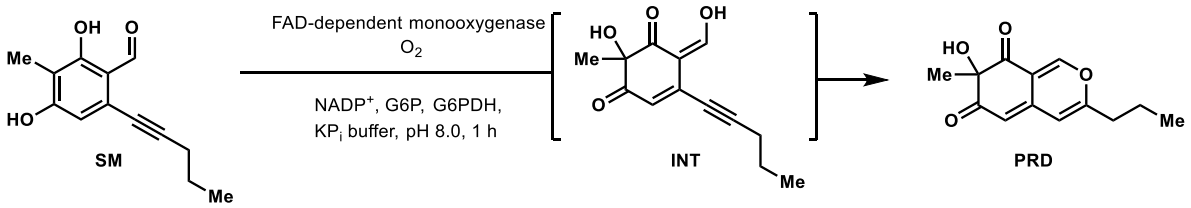
## NEC



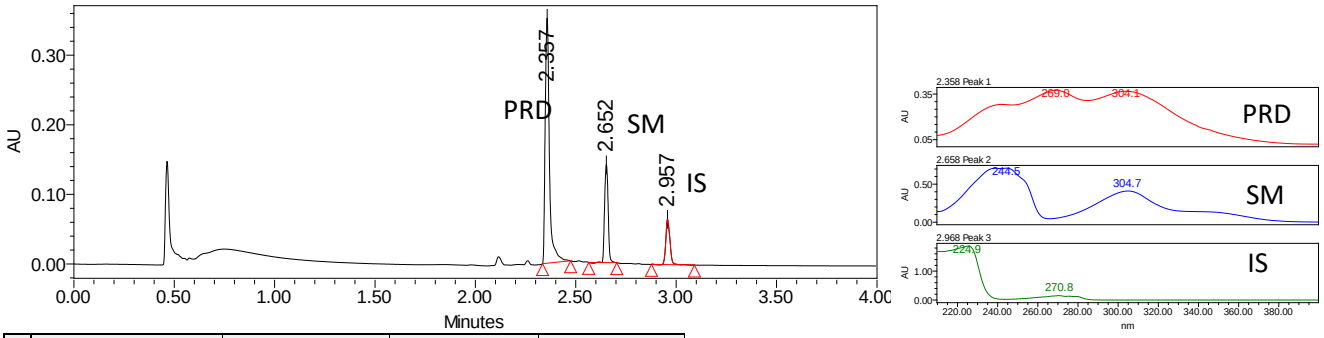




| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.426 | 301558 | 76.61 | 252996 |
| 2 | 2.955 | 92051 | 23.39 | 63755 |

**Figure S11.** Oxidative dearomatization of **S11** by AfoD and AzaH. PDA traces of enzymatic reaction and control reaction. (Table 1, entry 3).

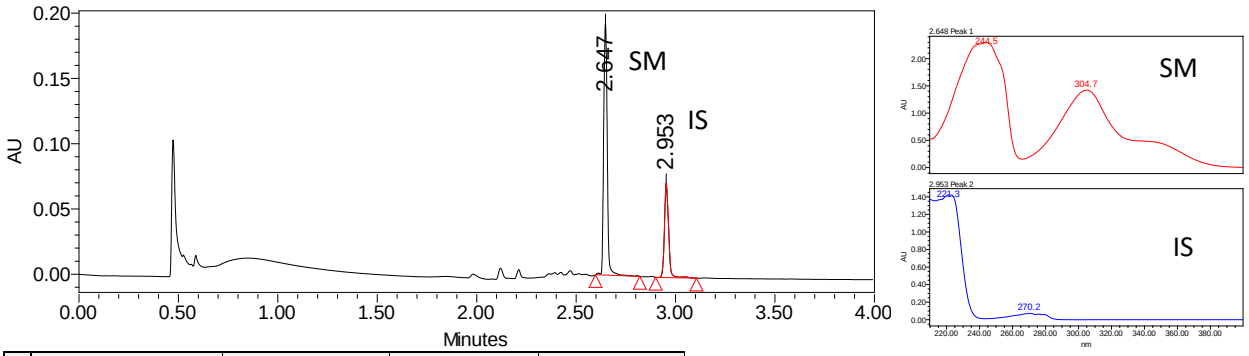



## With AzaH



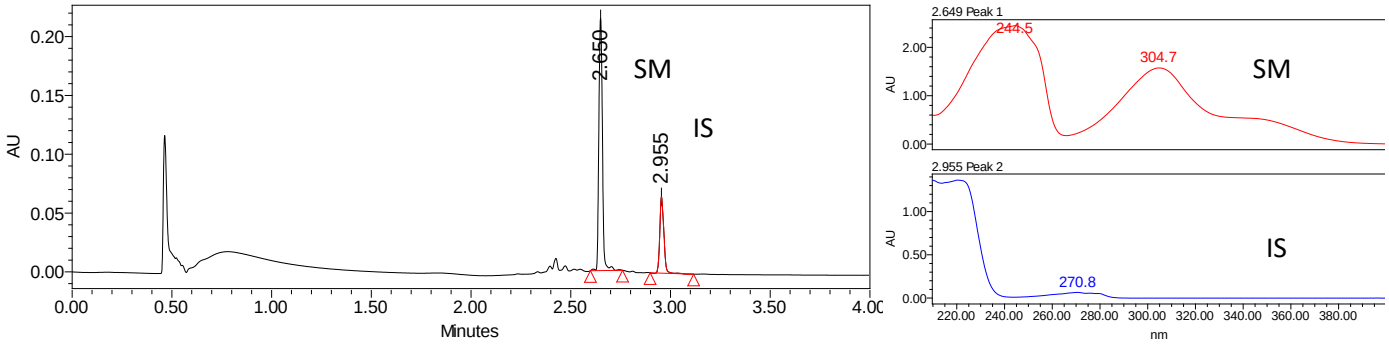

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.357 | 517469 | 65.70 | 351986 |
| 2 | 2.652 | 176051 | 22.35 | 140720 |
| 3 | 2.957 | 94126 | 11.95 | 65187 |

## With AfoD



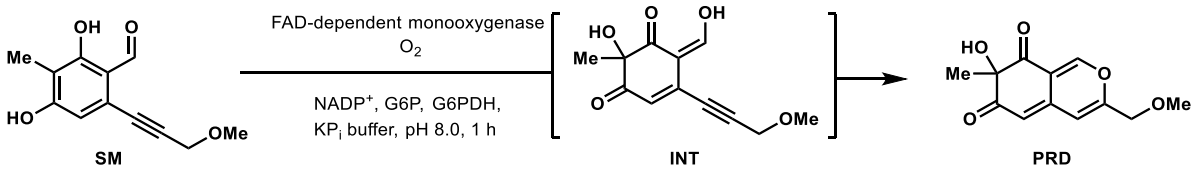

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.647 | 240563 | 69.66 | 192549 |
| 2 | 2.953 | 104788 | 30.34 | 72317 |

## NEC

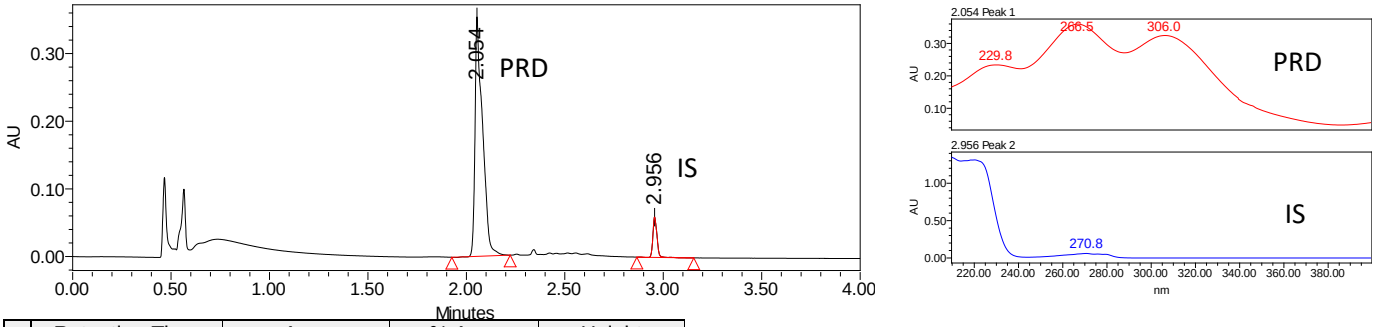



| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.650 | 266100 | 74.22 | 214826 |
| 2 | 2.955 | 92442 | 25.78 | 64989 |

**Figure S12.** Oxidative dearomatization of **S2** by AfoD and AzaH. PDA traces of enzymatic reaction and control reaction. (Table 1, entry 4).

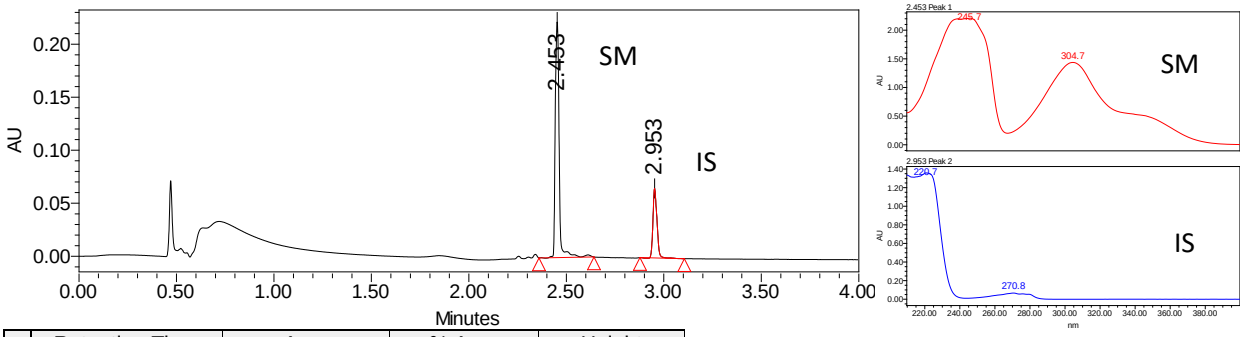



## With AzaH



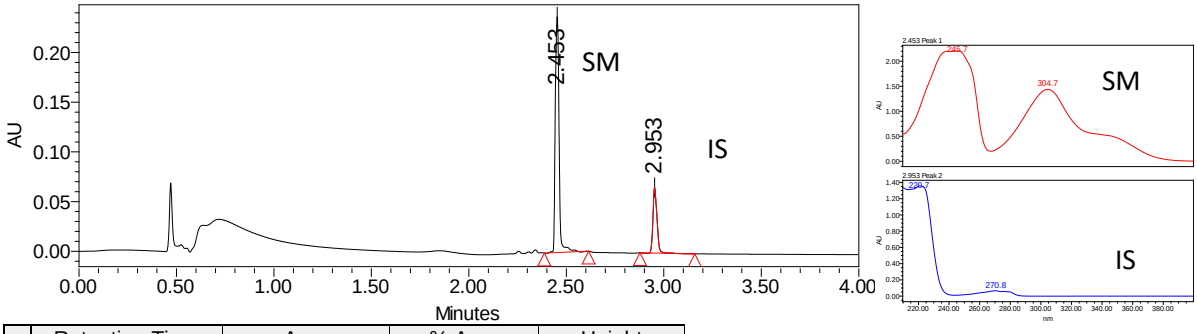

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.054 | 944560 | 91.63 | 354150 |
| 2 | 2.956 | 86293 | 8.37 | 59688 |

## With AfoD



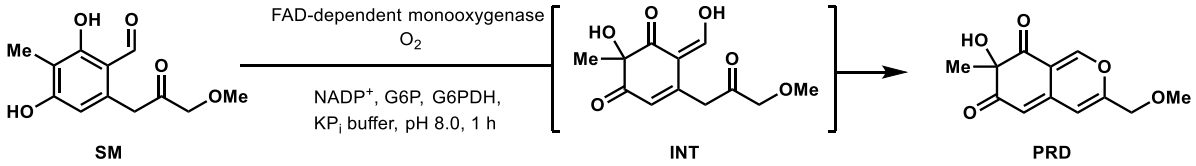

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.453 | 309943 | 76.49 | 222375 |
| 2 | 2.953 | 95246 | 23.51 | 65655 |

## NEC

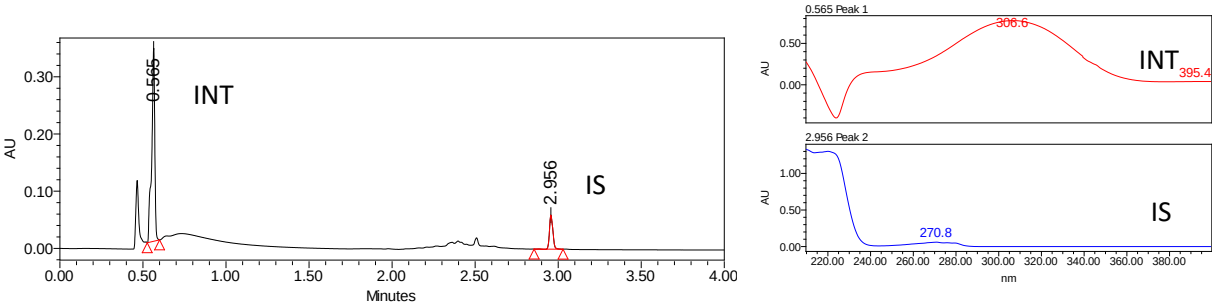



| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.453 | 321740 | 77.09 | 237334 |
| 2 | 2.953 | 95619 | 22.91 | 65898 |

**Figure S13.** Oxidative dearomatization of **S3** by AfoD and AzaH. PDA traces of enzymatic reaction and control reaction. (Table 1, entry 5).

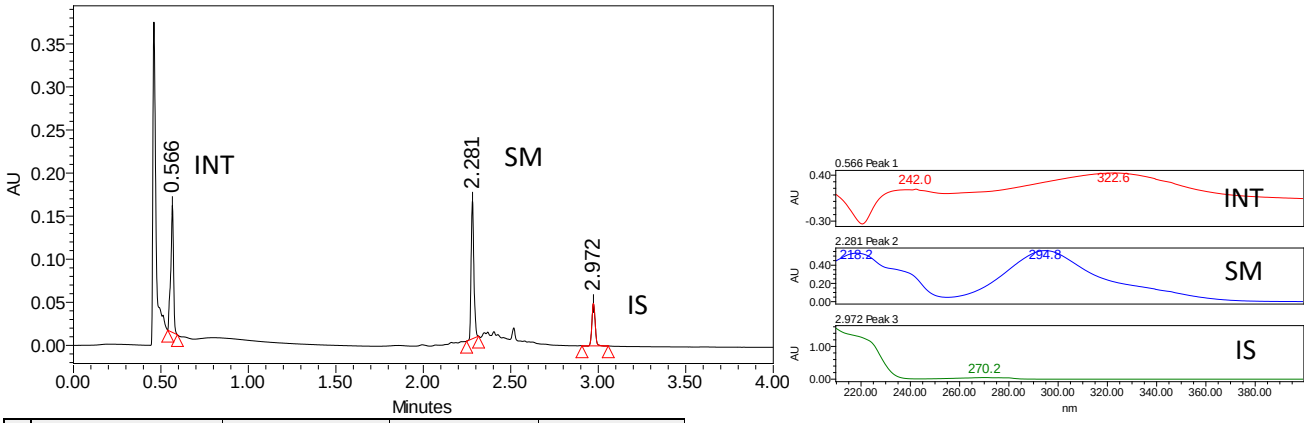



## With AzaH



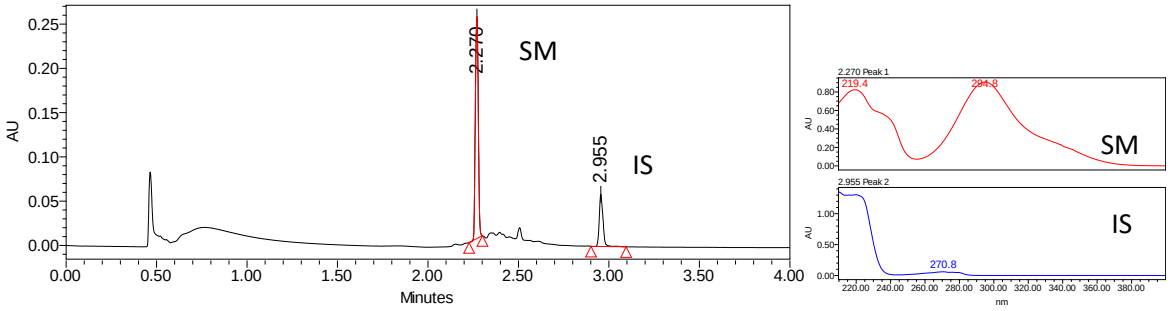

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 0.565 | 434990 | 83.72 | 338239 |
| 2 | 2.956 | 84593 | 16.28 | 59668 |

## With AfoD



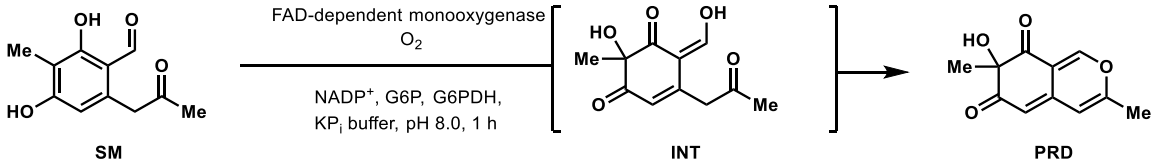

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 0.572 | 113995 | 30.42 | 89263 |
| 2 | 2.271 | 157150 | 41.94 | 133701 |
| 3 | 2.953 | 103585 | 27.64 | 72398 |

## NEC

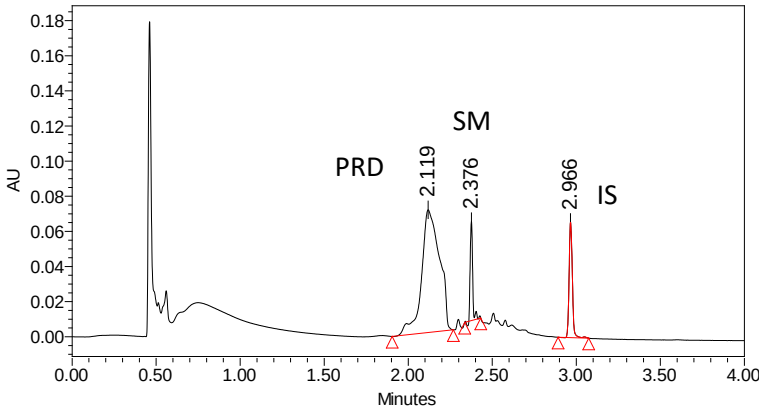


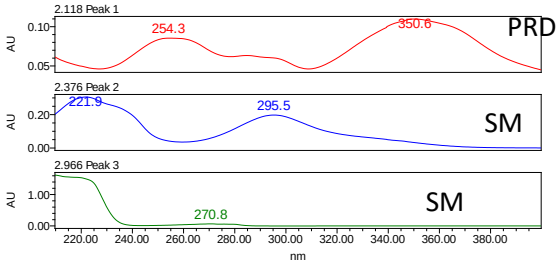

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.270 | 284197 | 77.11 | 250475 |
| 2 | 2.955 | 84347 | 22.89 | 58999 |

**Figure S14.** Oxidative dearomatization of **21** by AfoD and AzaH. PDA traces of enzymatic reaction and control reaction. (Table 1, entry 7).



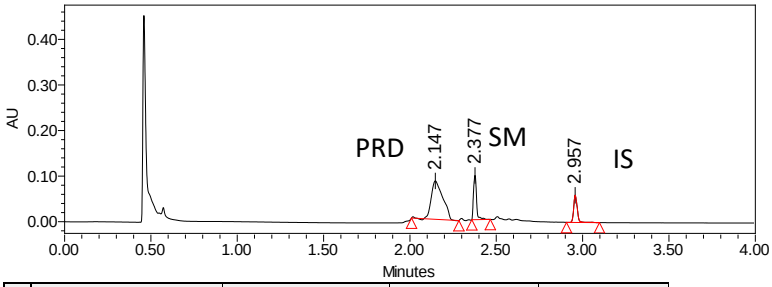

## With AzaH

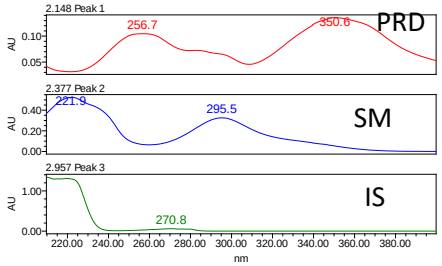



| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.119 | 519413 | 77.67 | 69861 |
| 2 | 2.376 | 60100 | 8.99 | 56370 |
| 3 | 2.966 | 89232 | 13.34 | 65670 |

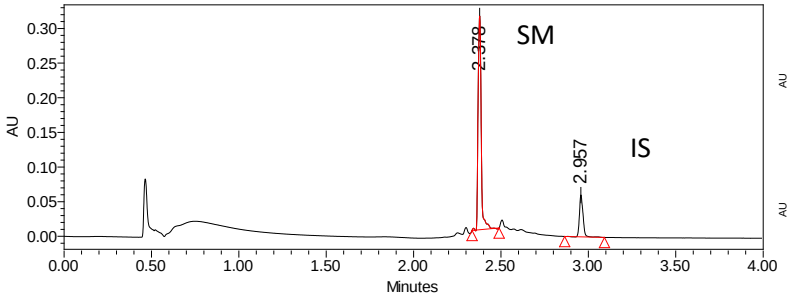
## With AfoD

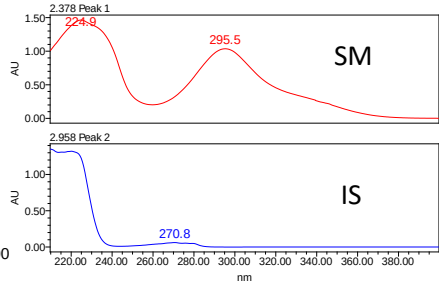



| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.147 | 398043 | 65.96 | 84339 |
| 2 | 2.377 | 119827 | 19.86 | 97170 |
| 3 | 2.957 | 85588 | 14.18 | 59788 |

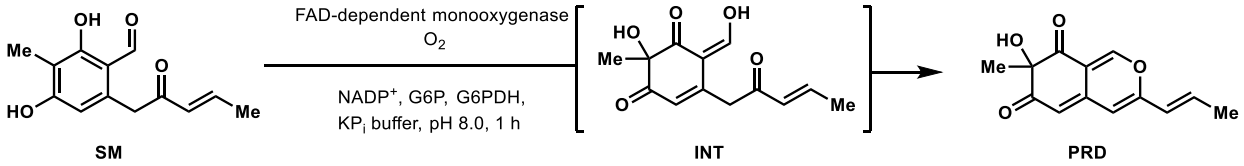
## NEC



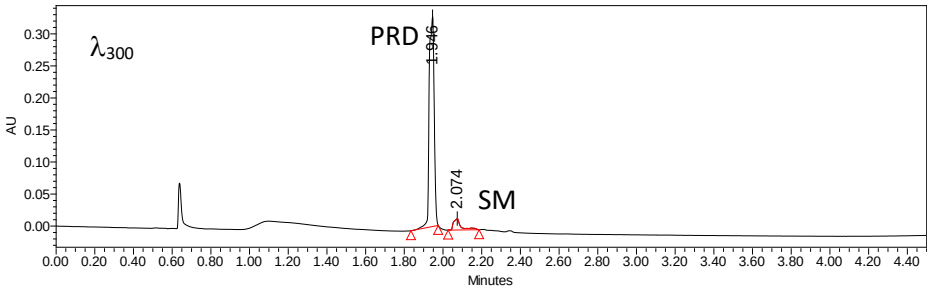



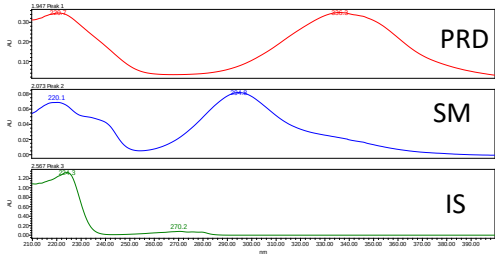

| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.378 | 383633 | 81.55 | 308926 |
| 2 | 2.957 | 86811 | 18.45 | 60584 |

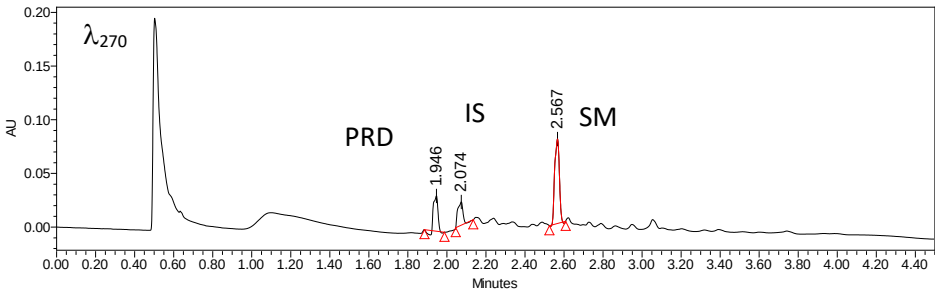
**Figure S15.** Oxidative dearomatization of **19** by AfoD and AzaH. PDA traces of enzymatic reaction and control reaction. (Table 1, entry 6). Two wavelengths are shown to visualize product.




## With AzaH

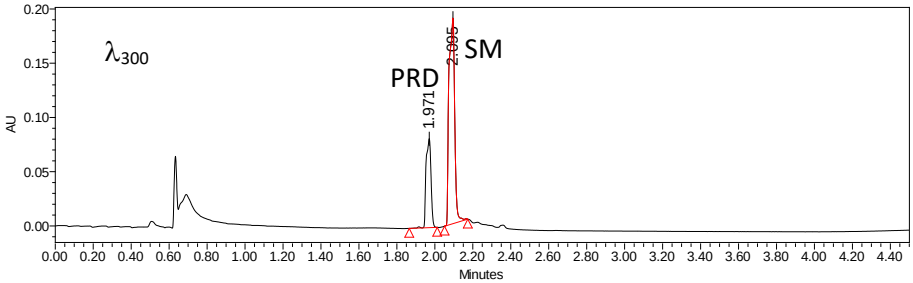


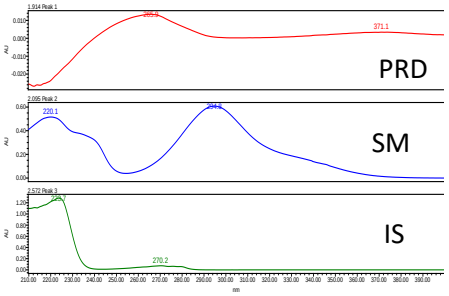



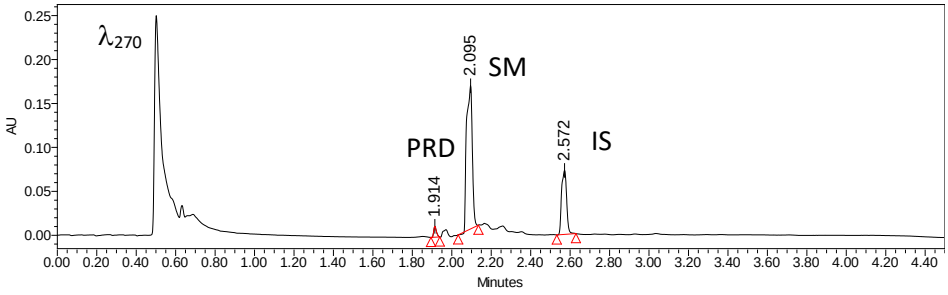




| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 1.946 | 59575 | 25.80 | 32920 |
| 2 | 2.074 | 40004 | 17.33 | 21701 |
| 3 | 2.567 | 131297 | 56.87 | 78806 |

## With AfoD

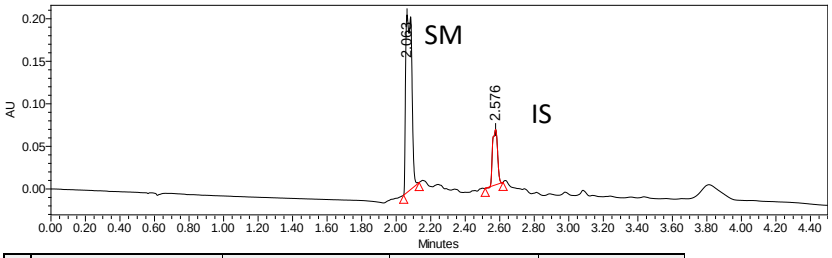


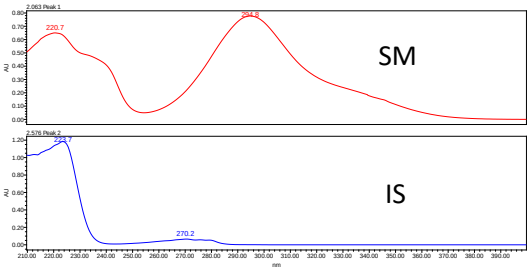




| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 1.946 | 59575 | 25.80 | 32920 |
| 2 | 2.074 | 40004 | 17.33 | 21701 |
| 3 | 2.567 | 131297 | 56.87 | 78806 |

## NEC



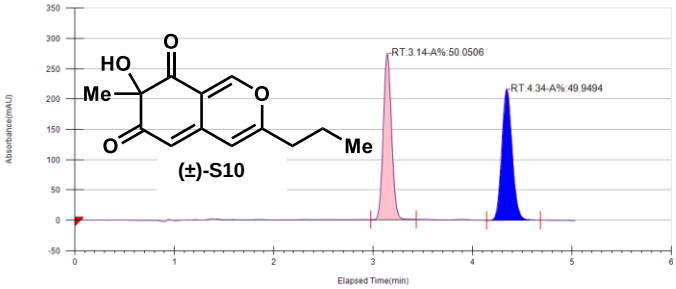




| | Retention Time | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.063 | 507851 | 79.50 | 208715 |
| 2 | 2.576 | 130976 | 20.50 | 65081 |

## Part IX. Determination of Enantiomeric Excess

**Figure S16.** PDA traces of racemic **S10** obtained from an IBX-mediated oxidative dearomatization, (*S*)-**S10** obtained from AfoD-mediated oxidative dearomatization, (*R*)-**S10** obtained from AzaH-mediated oxidative dearomatization, and **S10** obtained from AfoD Y118F mediated oxidative dearomatization (CHIRALPAK® AD-H, 30%, $CO_2$, 3.5 mL/min).

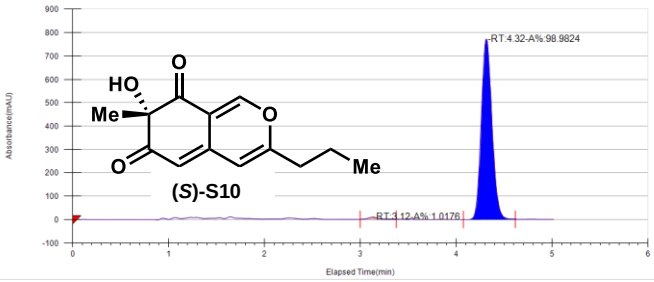
**Racemic standard**




| Instrument method | Inj. Vol. | Solvent | Column | Sample | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 5 | iPrOH | AD-H Chiral Analytical | sbdIV-087-rac | 40 | 3.5 | 30 | 120 |

| Peak No | % Area | Ret. Time | Cap Factor |
|---|---|---|---|
| 1 | 50.0506 | 3.14 min | 0 |
| 2 | 49.9494 | 4.34 min | 0 |

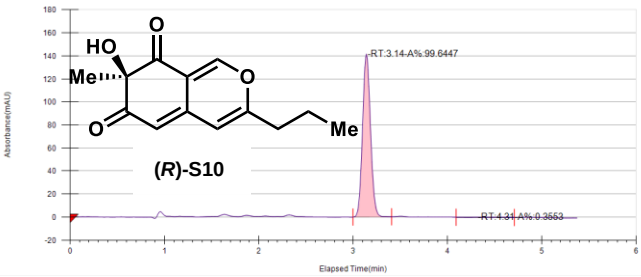
**AfoD Reaction**




| Instrument method | Inj. Vol. | Solvent | Column | Sample | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 5 | iPrOH | AD-H Chiral Analytical | sbdIV-087-AfoD | 40 | 3.5 | 30 | 120 |

| Peak No | % Area | Ret. Time | Cap Factor |
|---|---|---|---|
| 1 | 1.0176 | 3.12 min | 0 |
| 2 | 98.9824 | 4.32 min | 0 |

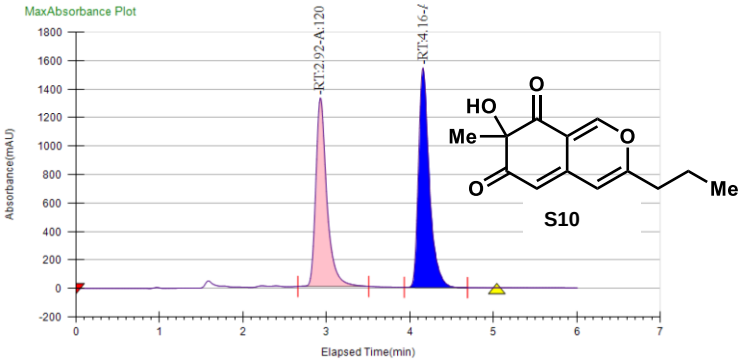
**AzaH Reaction**




| Instrument method | Inj. Vol. | Solvent | Column | Sample | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 5 | iPrOH | AD-H Chiral Analytical | sbdIV-087-rac | 40 | 3.5 | 30 | 120 |

| Peak No | % Area | Ret. Time | Cap Factor |
|---|---|---|---|
| 1 | 99.6447 | 3.14 min | 0 |
| 2 | 0.3553 | 4.31 min | 0 |

**AfoD Y118F reaction**

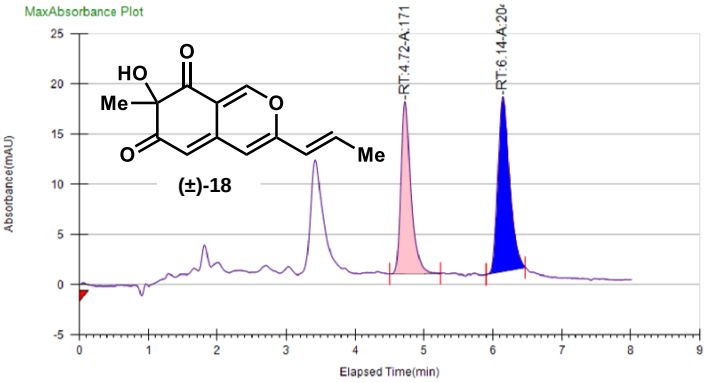



| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. (C) | Flow (g/min) | % Modifier | Pressure (Bar) |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 6 uL | Isopropanol | AD-H | ARB-V-071 AfoD_1 | 12A | 40 | 3.5 | 30 | 120 |

| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 46.8333 | 12090.3845 | 2.92 min | 1322.9644 | 0 |
| 2 | 53.1667 | 13725.3924 | 4.16 min | 1541.5059 | 0 |

**Figure S17.** PDA traces of racemic **18** obtained from a 1:1 mixture of the compound generated using AzaH and AfoD, (*S*)-**18** obtained from AfoD-mediated oxidative dearomatization, (*R*)-**18** obtained from AzaH-mediated oxidative dearomatization (CHIRALPAK® AD-H, 30%, $CO_2$, 3.5 mL/min).

**Racemic standard**



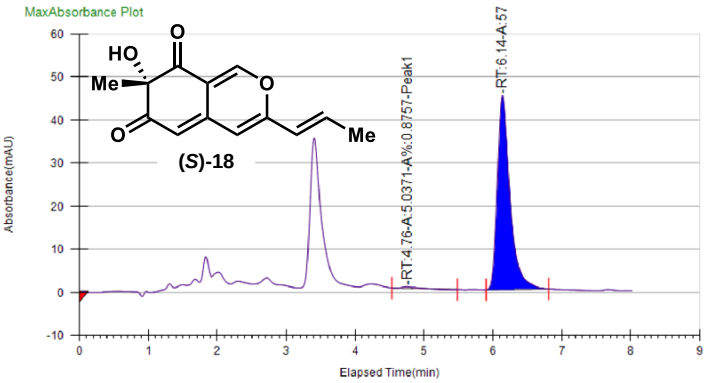

**Run Information**

| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 5 | iPrOH | AD-H Chiral Analytical | Rac_bicycle | 11A | 40 | 3.5 | 30 | 120 |

**Peak Information**

| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 45.5935 | 171.6805 | 4.72 min | 17.1822 | 0 |
| 2 | 54.4065 | 204.8656 | 6.14 min | 17.4111 | 0 |

**AfoD reaction**



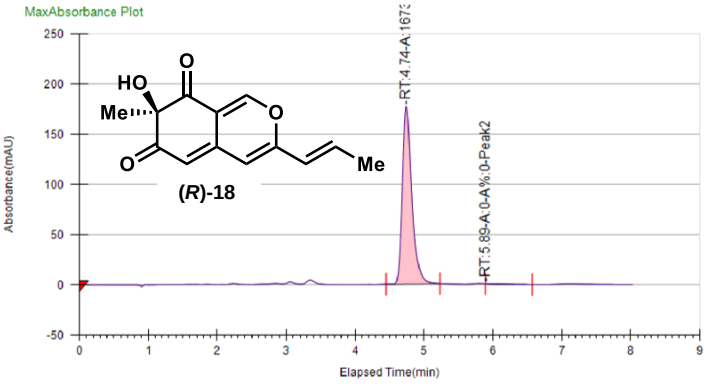

**Run Information**

| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 5 | iPrOH | AD-H Chiral Analytical | AfoD_bicycle | 13A | 40 | 3.5 | 30 | 120 |

**Peak Information**

| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 0.8757 | 5.0371 | 4.76 min | 0.3701 | 0 |
| 2 | 99.1243 | 570.1434 | 6.14 min | 45.0677 | 0 |

**AzaH reaction**




**Run Information**

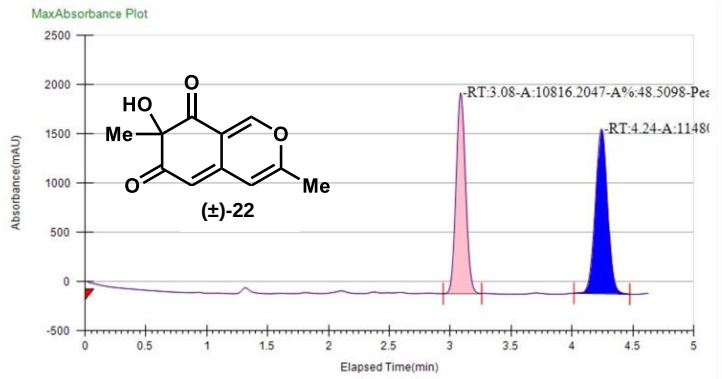
| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 5 | iPrOH | AD-H Chiral Analytical | AzaH_bicycle | 12A | 40 | 3.5 | 30 | 120 |

**Peak Information**

| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 100 | 1673.5999 | 4.74 min | 176.9264 | 0 |
| 2 | 0 | 0 | 5.89 min | 0 | 0 |

**Figure S18.** PDA traces of racemic **22** obtained from an IBX-mediated oxidative dearomatization, (*S*)-**22** obtained from AfoD-mediated oxidative dearomatization, (*R*)-**22** obtained from AzaH-mediated oxidative dearomatization (CHIRALPAK® AD-H, 30%, $CO_2$, 3.5 mL/min).

**Racemic standard**



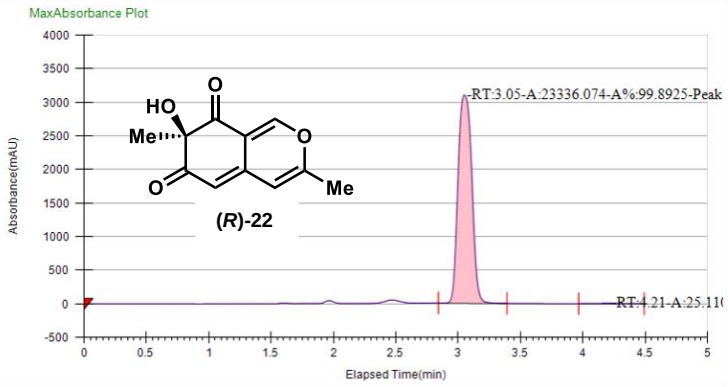

**Run Information**

| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 5 | iPrOH | AD-H Chiral Analytical | sbdIV-174_30% | 12A | 40 | 3.5 | 30 | 120 |

**Peak Information**

| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 48.5098 | 10816.2047 | 3.08 min | 2038.3863 | 3082.3 |
| 2 | 51.4902 | 11480.7275 | 4.24 min | 1675.0946 | 4240.6167 |

**AzaH reaction**



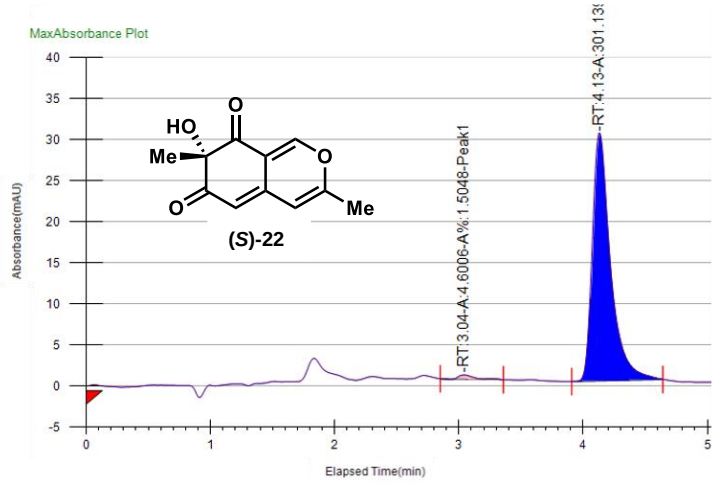

**Run Information**

| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 10 | iPrOH | AD-H Chiral Analytical | sbdIV-108_30iPrOH | 12A | 40 | 3.5 | 30 | 120 |

**Peak Information**

| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 99.8925 | 23336.074 | 3.05 min | 3103.1264 | 3049.0167 |
| 2 | 0.1075 | 25.1102 | 4.21 min | 2.823 | 4207.35 |

**AfoD reaction**



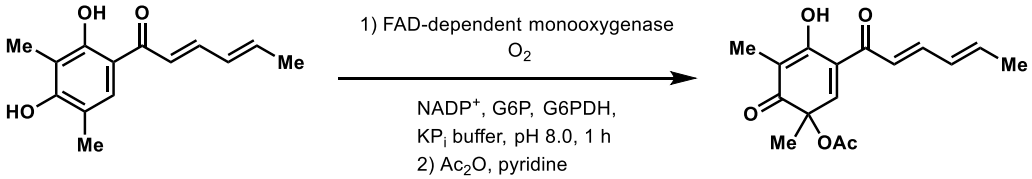

**Run Information**

| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_30%_300-330 | 10 | iPrOH | AD-H Chiral Analytical | JBP-3-169-AfoD | 13A | 40 | 3.5 | 30 | 120 |

**Peak Information**

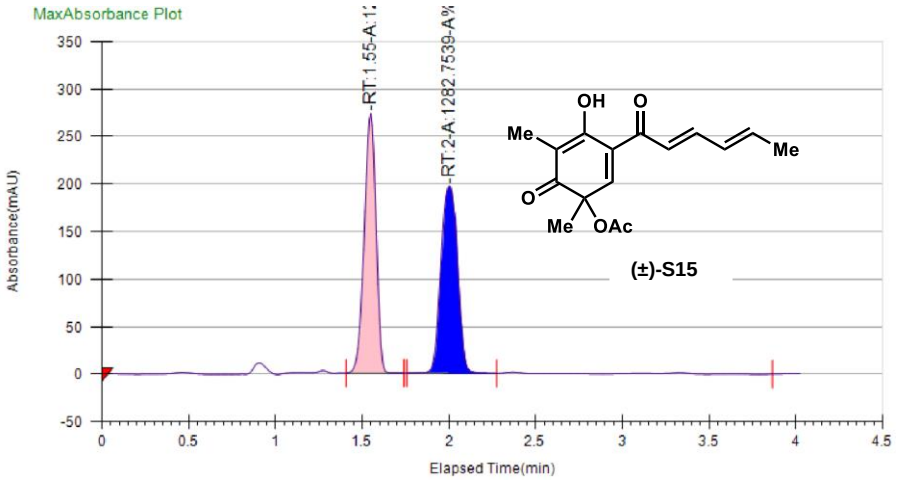
| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 1.5048 | 4.6006 | 3.04 min | 0.51 | 0 |
| 2 | 98.4952 | 301.1399 | 4.13 min | 30.1492 | 0 |

**Figure S19.** PDA traces of racemic **17** obtained from an IBX-mediated oxidative dearomatization and (*S*)-**17** obtained from FDMO7-mediated oxidative dearomatization (CHIRALPAK® AD-H, 30%, $CO_2$, 3.5 mL/min).




**S14** **S15**

**Racemic standard**



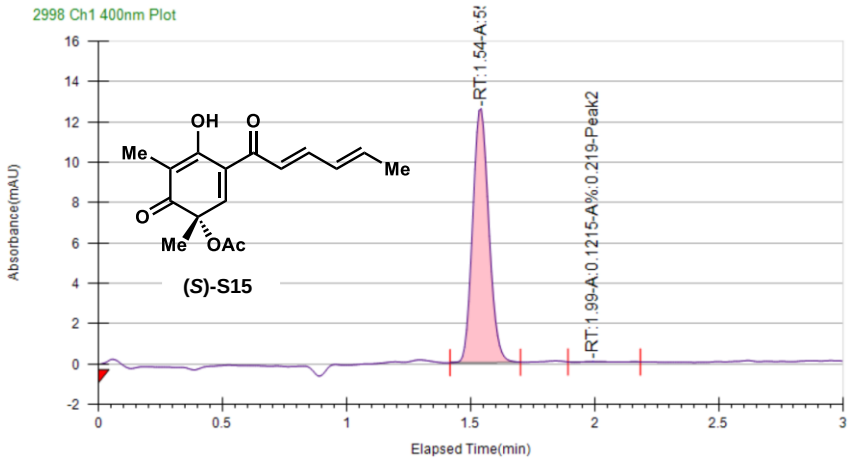

**Run Information**

| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_10% | 10 | iPrOH | AD-H Chiral Analytical | sbdIV-003_ADH | 15A | 40 | 3.5 | 10 | 120 |

**Peak Information**

| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 49.5405 | 1259.3926 | 1.55 min | 273.3204 | 1548.9833 |
| 2 | 50.4595 | 1282.7539 | 2 min | 197.3901 | 1998.9833 |

**FDMO-7 reaction**



**Run Information**

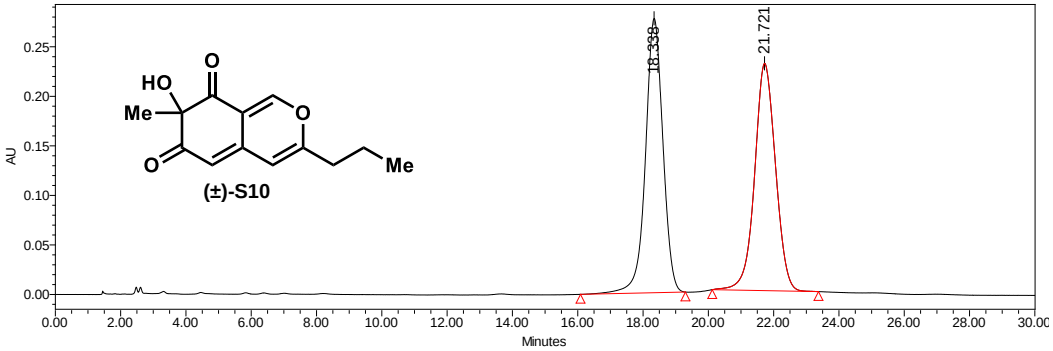
| Instrument Method | Inj. Vol. | Solvent | Column | Sample | Well Location | Temp. | Flow | % Modifier | Pressure |
|---|---|---|---|---|---|---|---|---|---|
| AD-H_10% | 1 | iPrOH | AD-H Chiral Analytical | sbdVIII-120 | 11A | 40 | 3.5 | 10 | 120 |

**Peak Information**

| Peak No | % Area | Area | Ret. Time | Height | Cap. Factor |
|---|---|---|---|---|---|
| 1 | 99.781 | 55.3487 | 1.54 min | 12.5858 | 0 |
| 2 | 0.219 | 0.1215 | 1.99 min | 0.0203 | 0 |

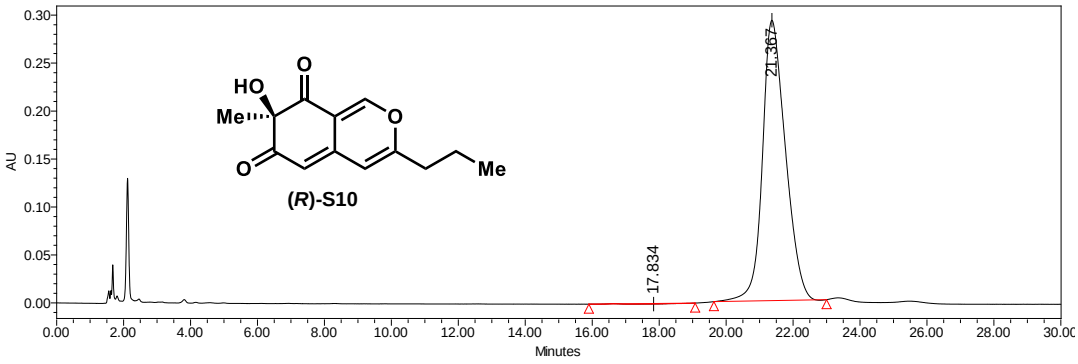
**Figure S20.** PDA traces of racemic **S10** obtained from an IBX-mediated oxidative dearomatization, (*R*)-**S10** obtained from FDMO-2 and FDMO-5-mediated oxidative dearomatization, and (*S*)-**S10** obtained from FDMO-6-mediated oxidative dearomatization (Phenomenex Lux Cellulose, 25% MeCN, 75% $H_2O$, 1 mL/min).

**Racemic standard**



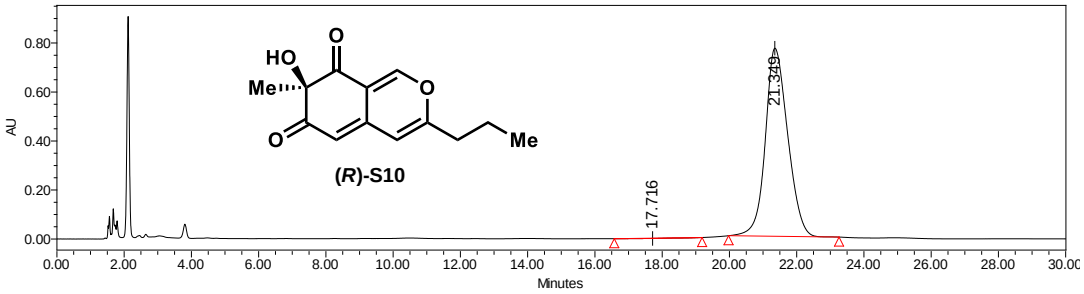

| | Name | Retention Time | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | | 18.341 | 13429370 | 50.10 | 362068 |
| 2 | | 21.724 | 13377480 | 49.90 | 300570 |

**FDMO-2 reaction**



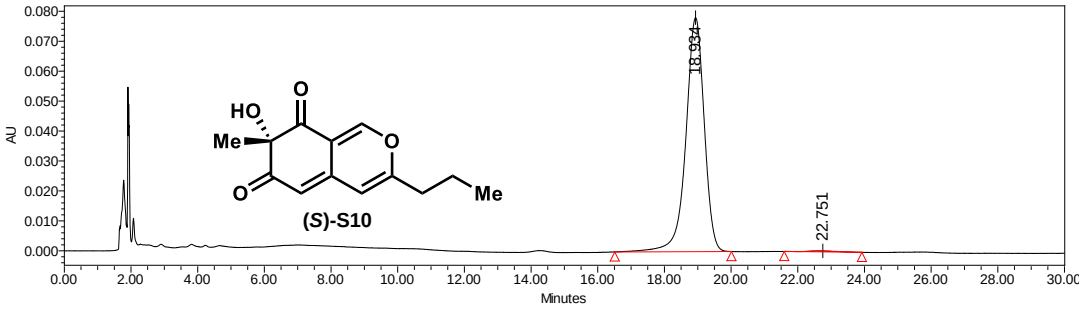

| | Name | Retention Time | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | | 17.860 | 229964 | 0.45 | -2129 |
| 2 | | 21.365 | 51102440 | 99.55 | 1059976 |

**FDMO-5 reaction**



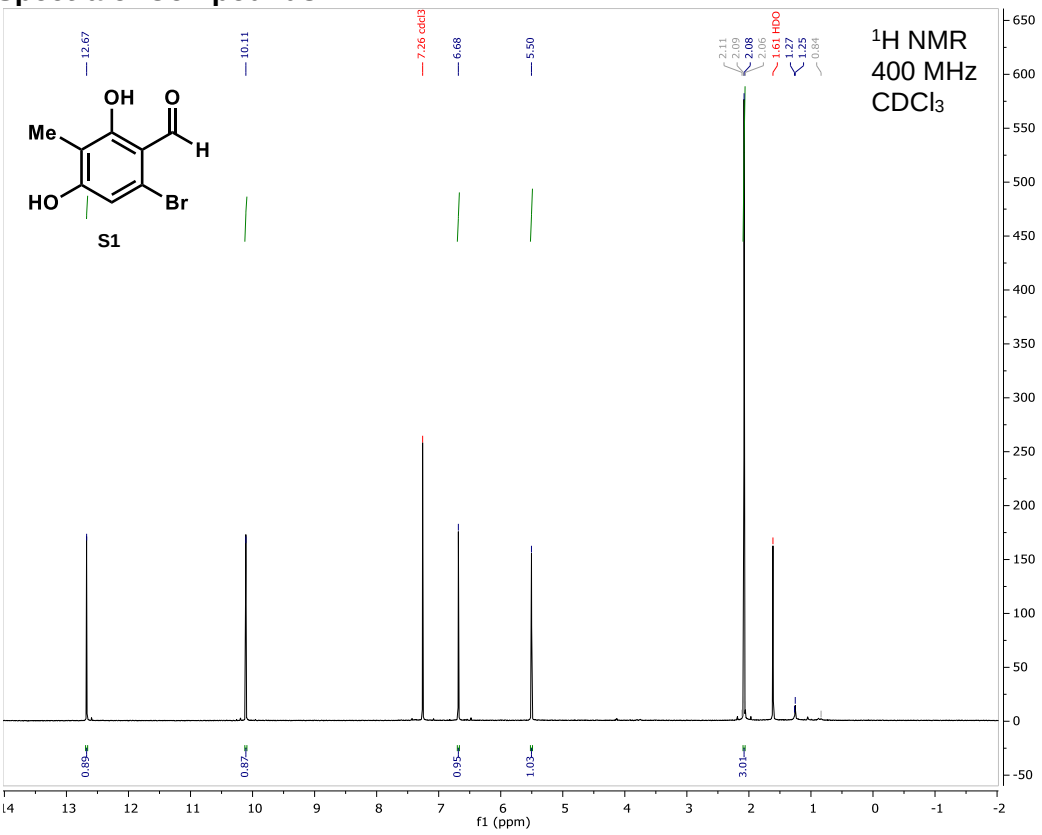

| | Name | Retention Time | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | | 18.829 | 149446 | 0.42 | -2017 |
| 2 | | 21.349 | 35753101 | 99.58 | 769344 |

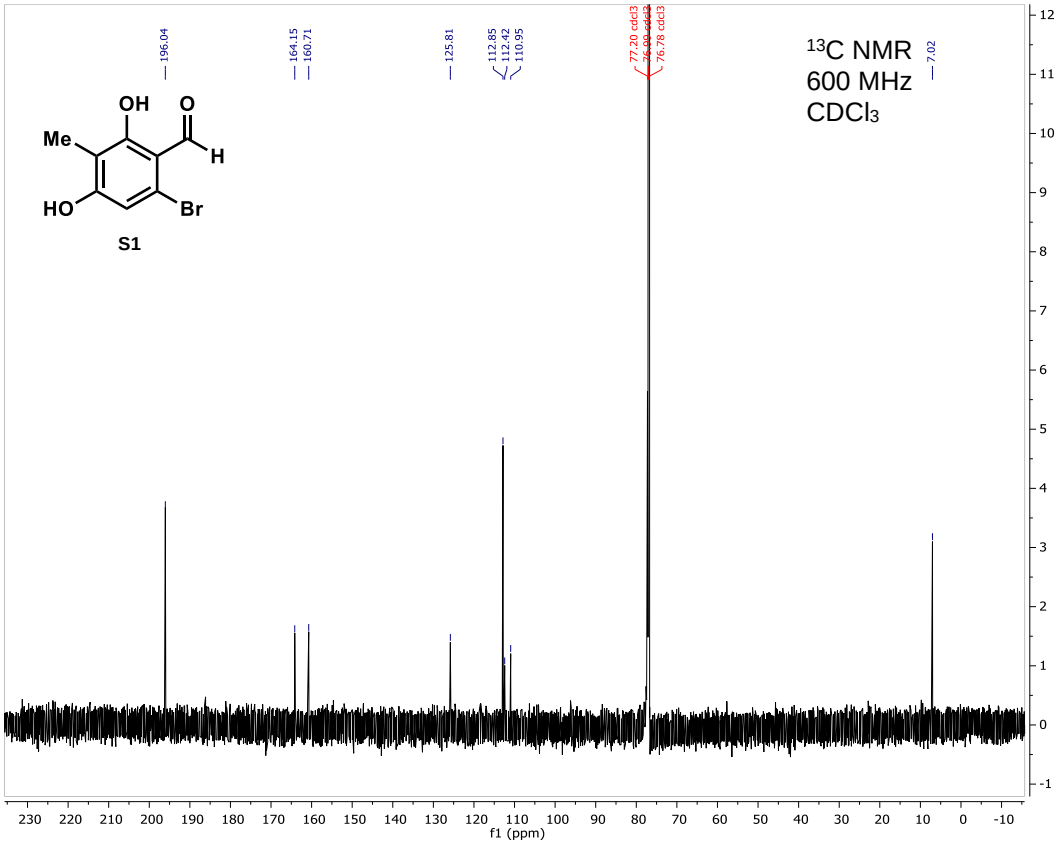
**FDMO-6 reaction**

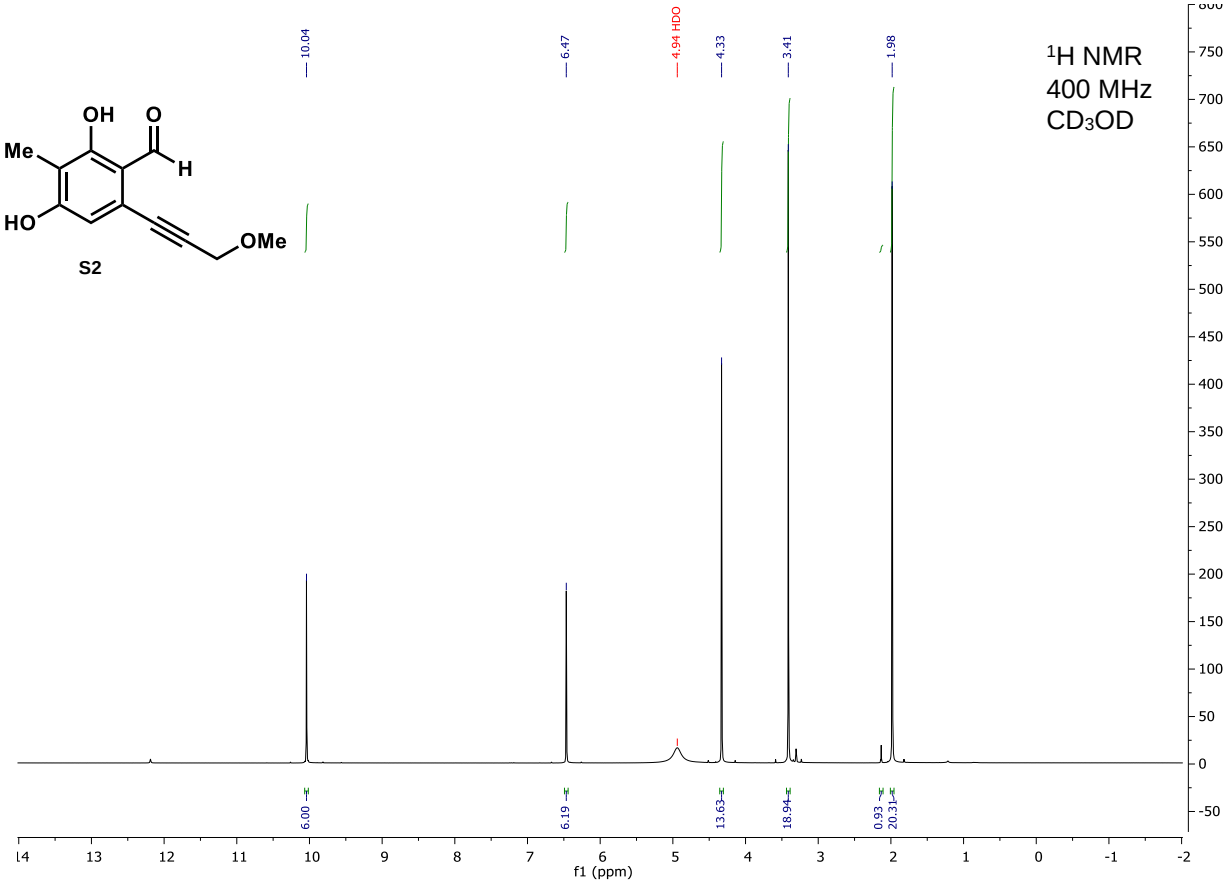


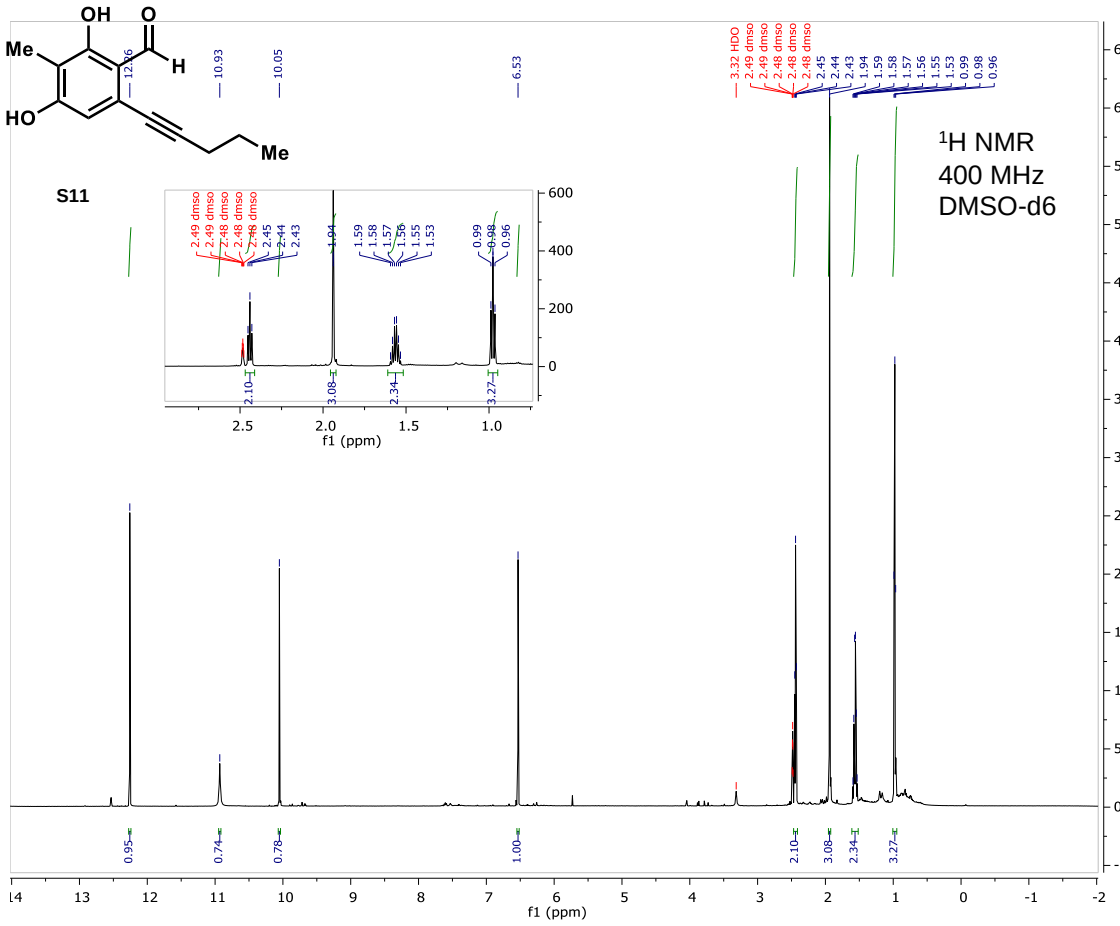

| | Name | Retention Time | Area | % Area | Height |
|---|---|---|---|---|---|
| 1 | | 18.934 | 3146288 | 99.83 | 83349 |
| 2 | | 22.126 | 5282 | 0.17 | -105 |

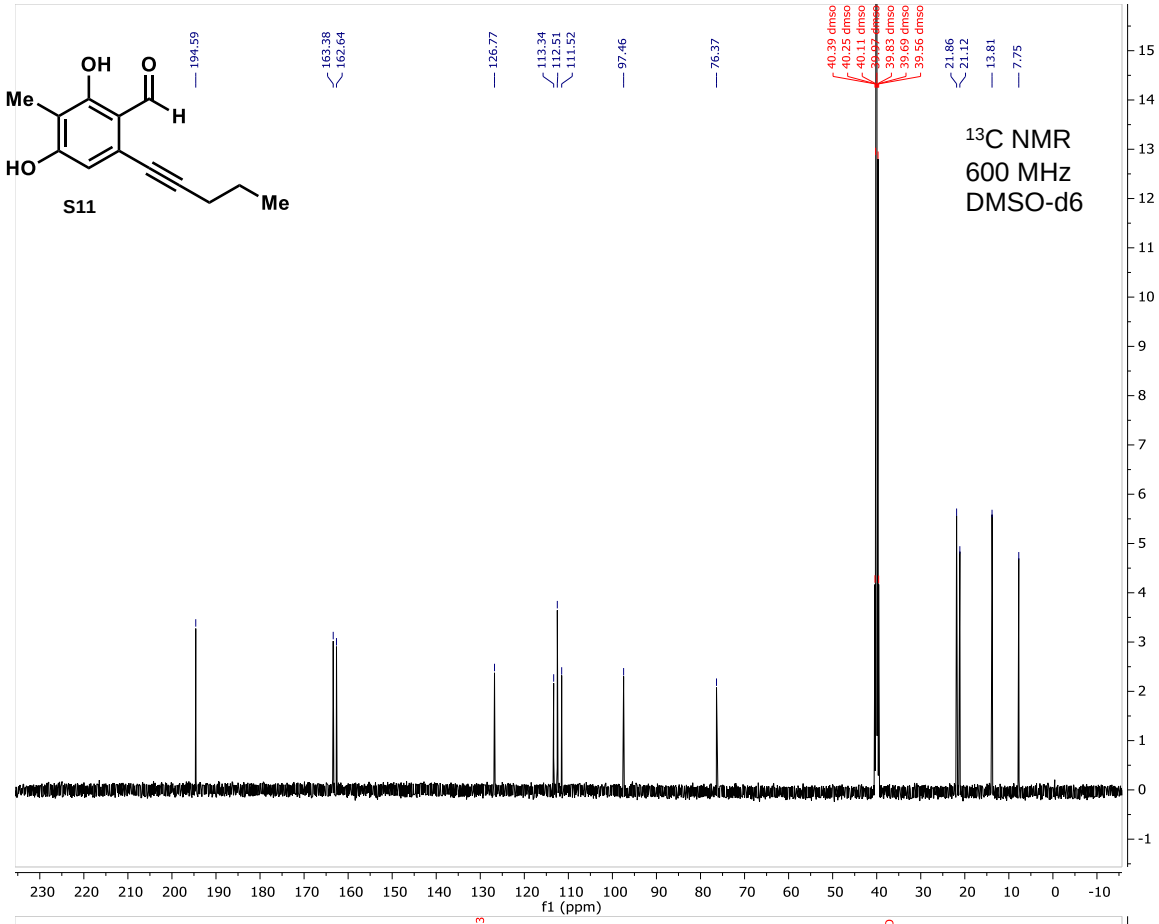
## Part X. NMR Spectra of Compounds

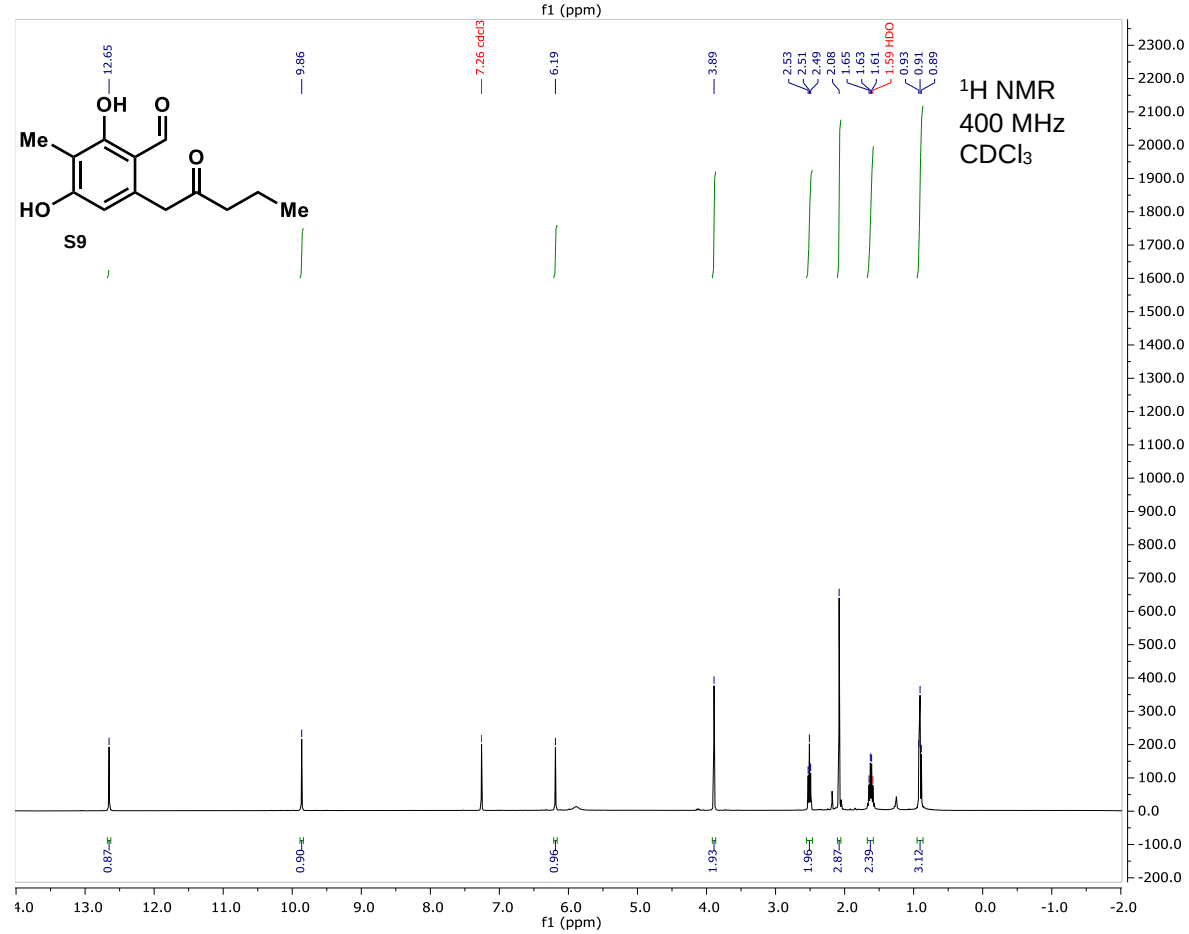


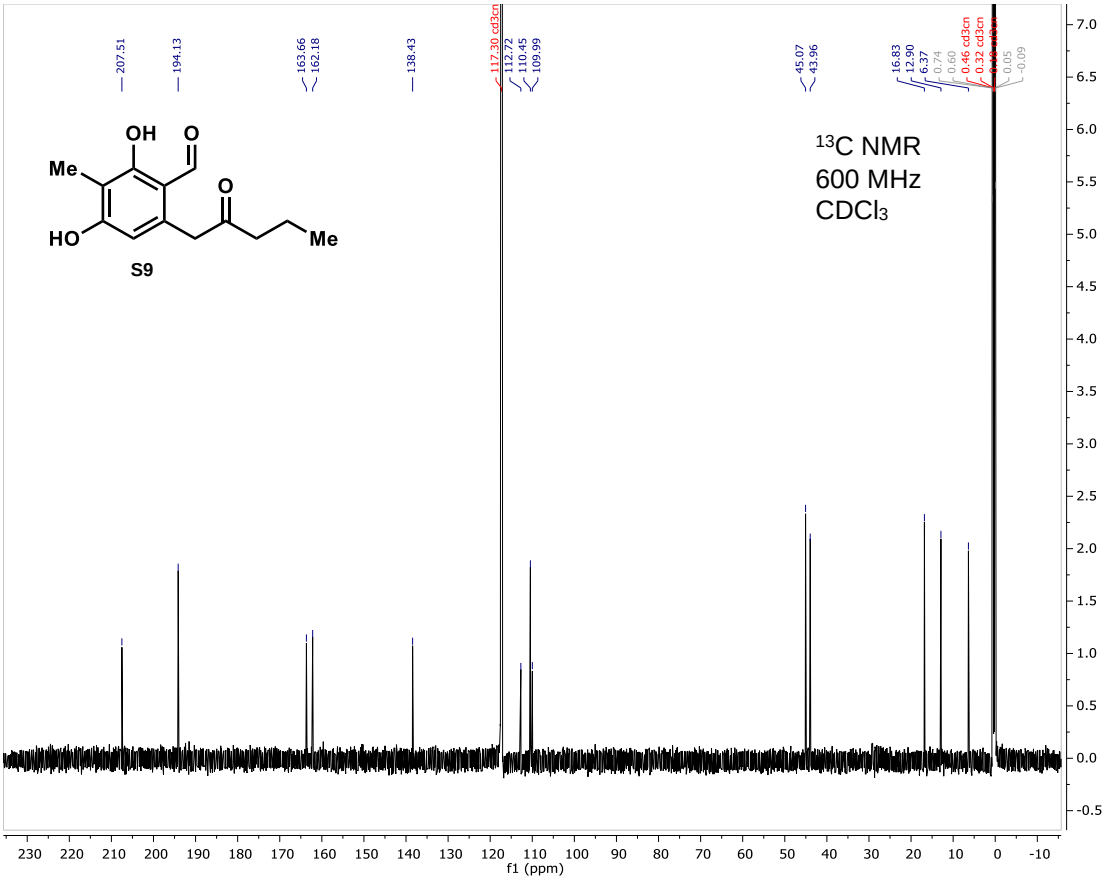



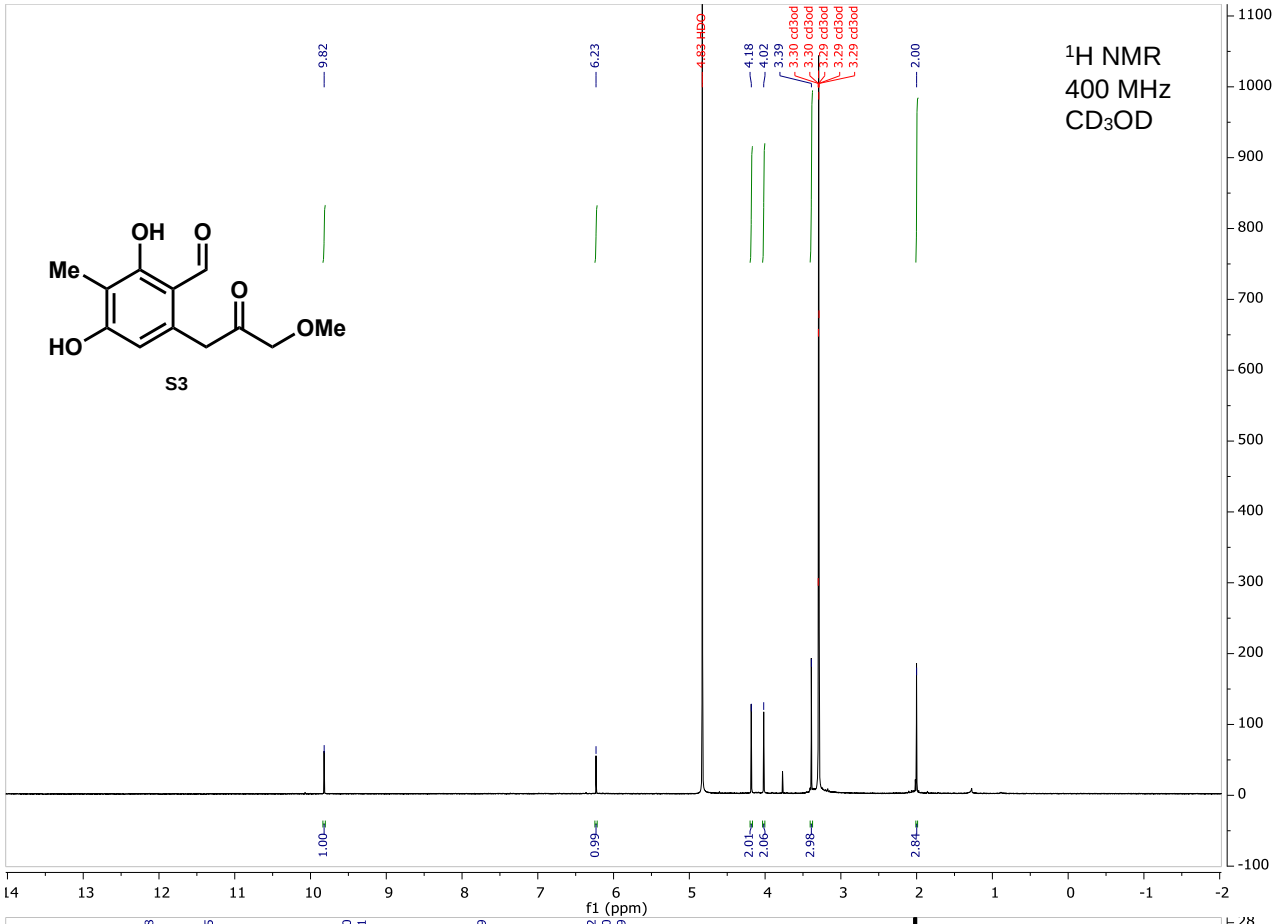

OH
O
Me
H
HO
OMe
S2
$^1$H NMR
400 MHz
$CD_3OD$
10.04
6.47
4.94 HDO
4.33
3.41
1.98
6.00
6.19
13.63
18.94
0.93
20.31
f1 (ppm)

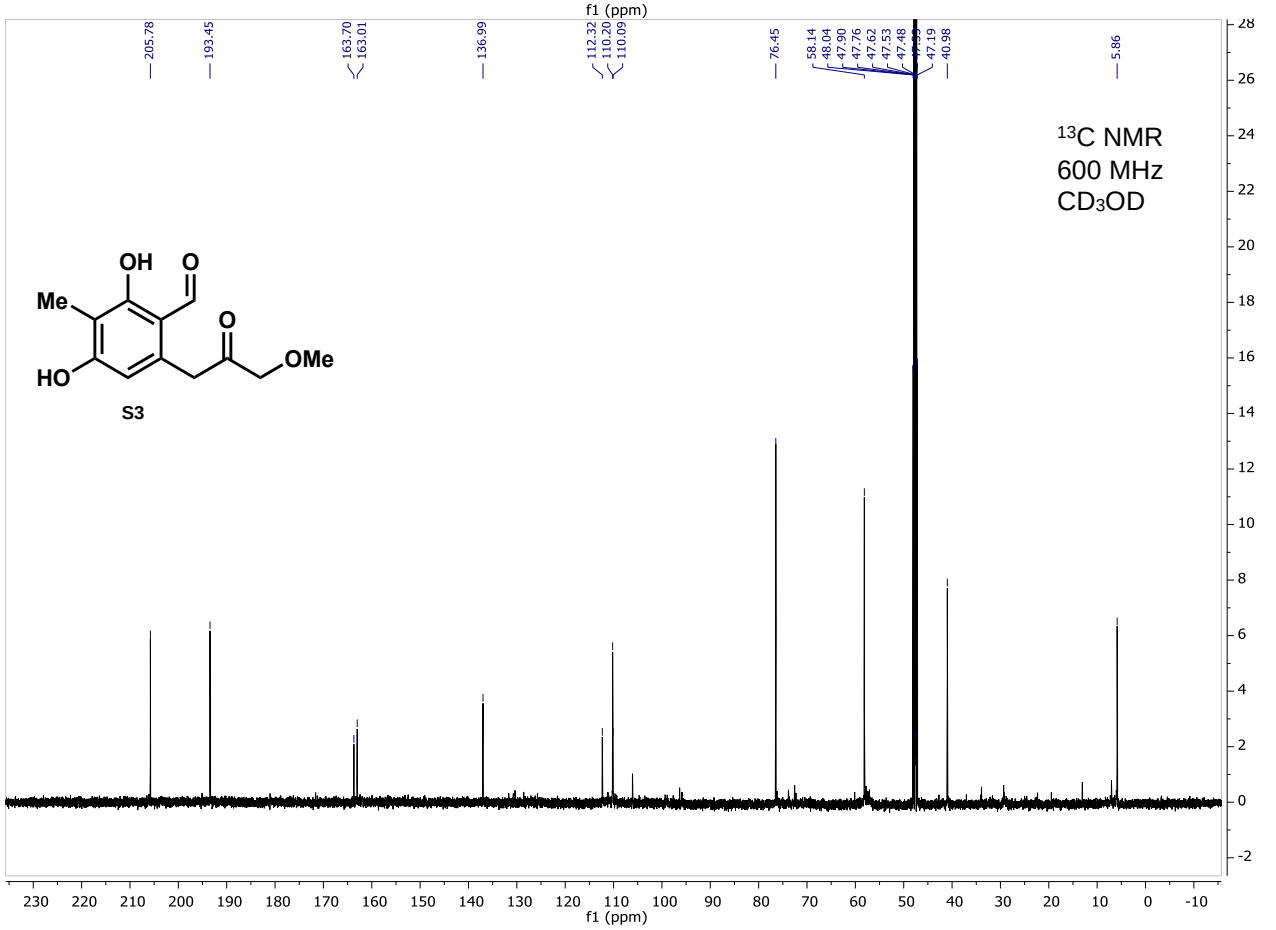

OH
O
Me
H
HO
Me
S11
12.46
10.93
10.05
6.53
3.32 HDO
2.49 dmso
2.48 dmso
2.45
2.44
2.43
1.94
1.59
1.58
1.57
1.56
1.55
1.53
0.99
0.98
0.96
$^1$H NMR
400 MHz
DMSO-d6
2.10
3.08
2.34
3.27
0.95
0.74
0.78
1.00
f1 (ppm)

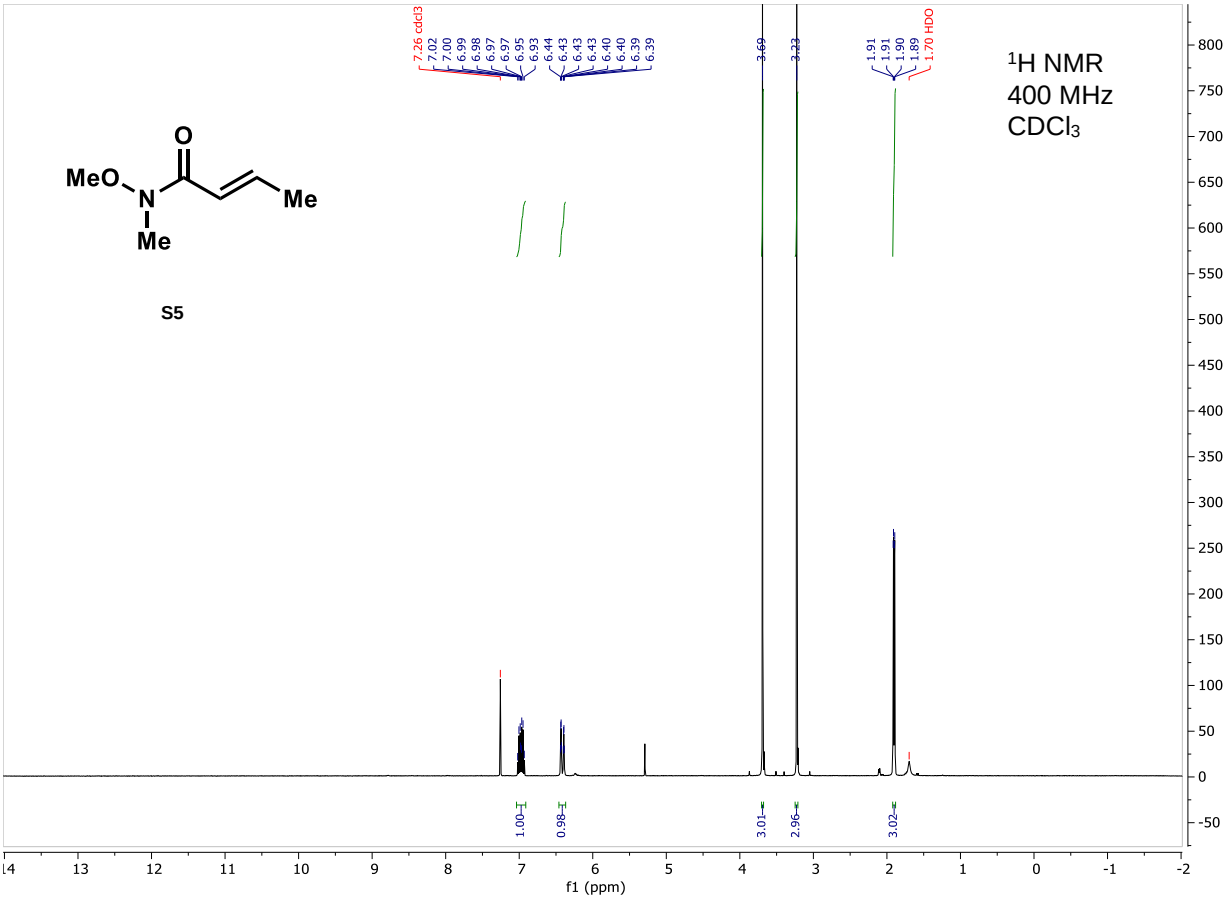

OH
O
Me
H
HO
Me
S11
194.59
163.38
162.64
126.77
113.34
112.51
111.52
97.46
76.37
40.39 dmso
40.25 dmso
40.11 dmso
39.97 dmso
39.83 dmso
39.69 dmso
39.56 dmso
21.86
21.12
13.81
7.75
13C NMR
600 MHz
DMSO-d6
f1 (ppm)


OH
O
Me
O
HO
Me
S9
12.65
9.86
7.26 cdcl3
6.19
3.89
2.53
2.51
2.49
2.08
1.65
1.63
1.61
1.59 HDO
0.93
0.91
0.89
1H NMR
400 MHz
CDCl3
0.87
0.90
0.96
1.93
1.96
2.87
2.39
3.12
f1 (ppm)

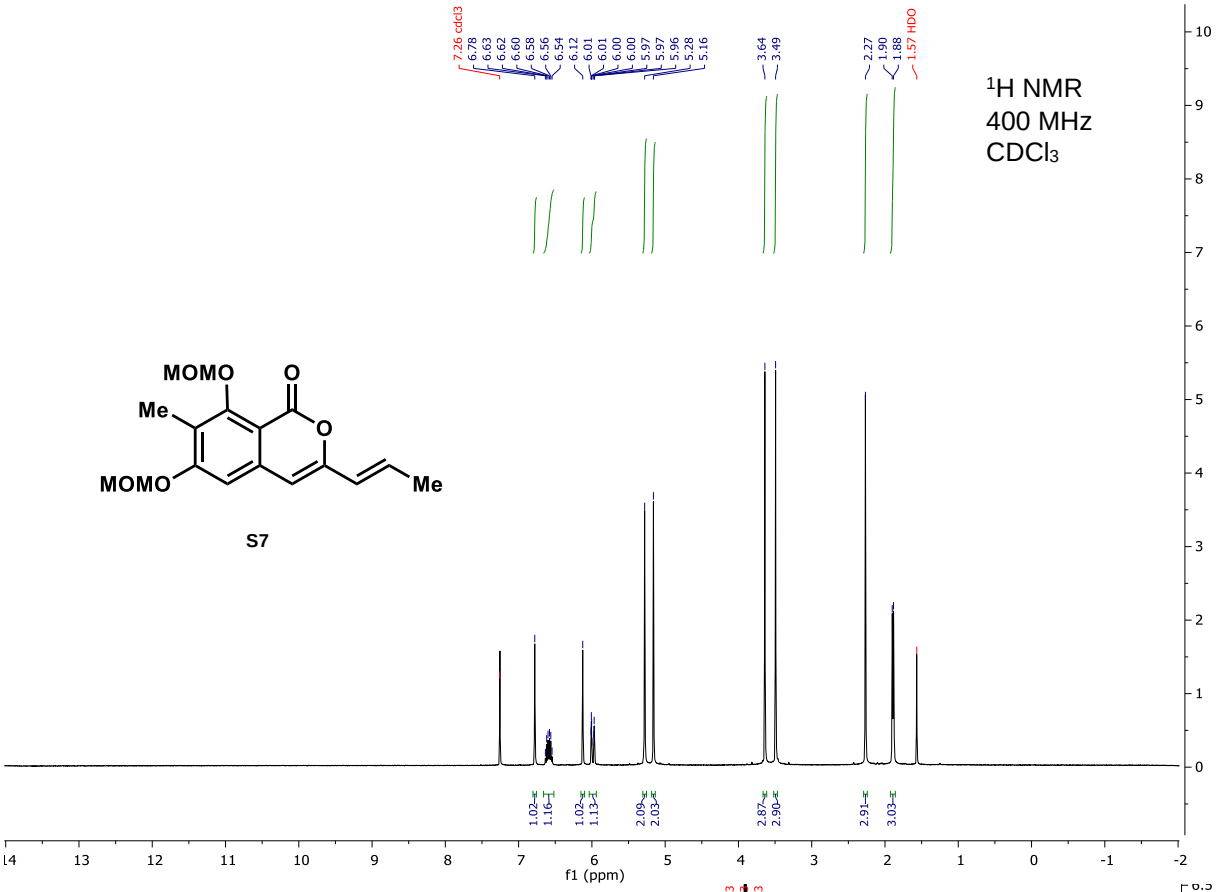

207.51
194.13
163.66
162.18
138.43
117.30 cd3cn
112.72
110.45
109.99
45.07
43.96
16.83
12.90
6.37
0.74
0.60
0.46 cd3cn
0.32 cd3cn
0.05
-0.09
OH
O
Me
O
HO
Me
S9
13C NMR
600 MHz
CDCl3
f1 (ppm)

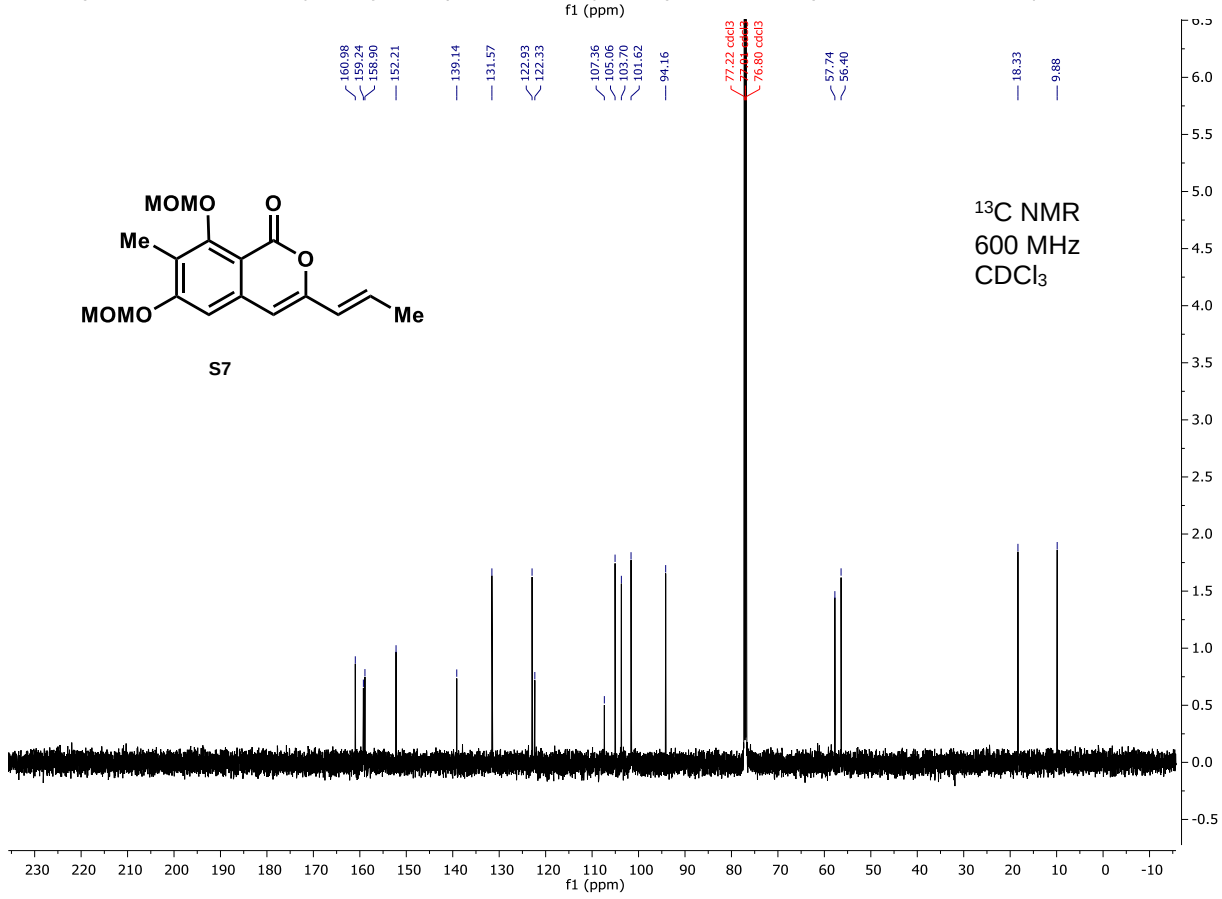

$^1$H NMR
400 MHz
$CD_3OD$
OH
O
Me
O
HO
OMe
S3
9.82
6.23
4.83 HDO
4.18
4.02
3.39
3.30 cd3od
3.30 cd3od
3.29 cd3od
3.29 cd3od
3.29 cd3od
2.00
1.00
0.99
2.01
2.06
2.98
2.84
f1 (ppm)

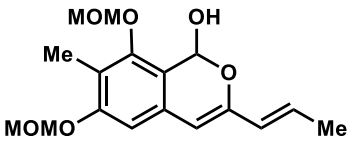

$^{13}$C NMR
600 MHz
$CD_3OD$
OH
O
Me
O
HO
OMe
S3
205.78
193.45
163.70
163.01
136.99
112.32
110.20
110.09
76.45
58.14
48.04
47.90
47.76
47.62
47.53
47.48
47.34
47.19
40.98
5.86
f1 (ppm)


7.26 cdcl3
7.02
7.00
6.99
6.98
6.97
6.97
6.95
6.93
6.44
6.43
6.43
6.43
6.40
6.40
6.39
6.39
3.69
3.23
1.91
1.91
1.90
1.89
1.70 HDO
1H NMR
400 MHz
CDCl3
MeO
N
Me
O
Me
1.00
0.98
3.01
2.96
3.02
f1 (ppm)

S5


7.26 cdcl3
6.78
6.63
6.62
6.60
6.58
6.56
6.54
6.12
6.01
6.01
6.00
6.00
5.97
5.97
5.96
5.28
5.16
3.64
3.49
2.27
1.90
1.88
1.57 HDO
1H NMR
400 MHz
CDCl3
MOMO
O
Me
O
MOMO
Me
S7
1.02
1.16
1.02
1.13
2.09
2.03
2.87
2.90
2.91
3.03
f1 (ppm)

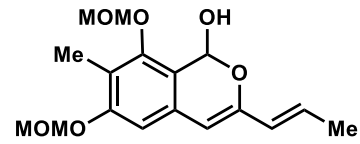

160.98
159.24
158.90
152.21
139.14
131.57
122.93
122.33
107.36
105.06
103.70
101.62
94.16
77.22 cdcl3
76.80 cdcl3
57.74
56.40
18.33
9.88
13C NMR
600 MHz
CDCl3
MOMO
O
Me
O
MOMO
Me
S7
f1 (ppm)




S8

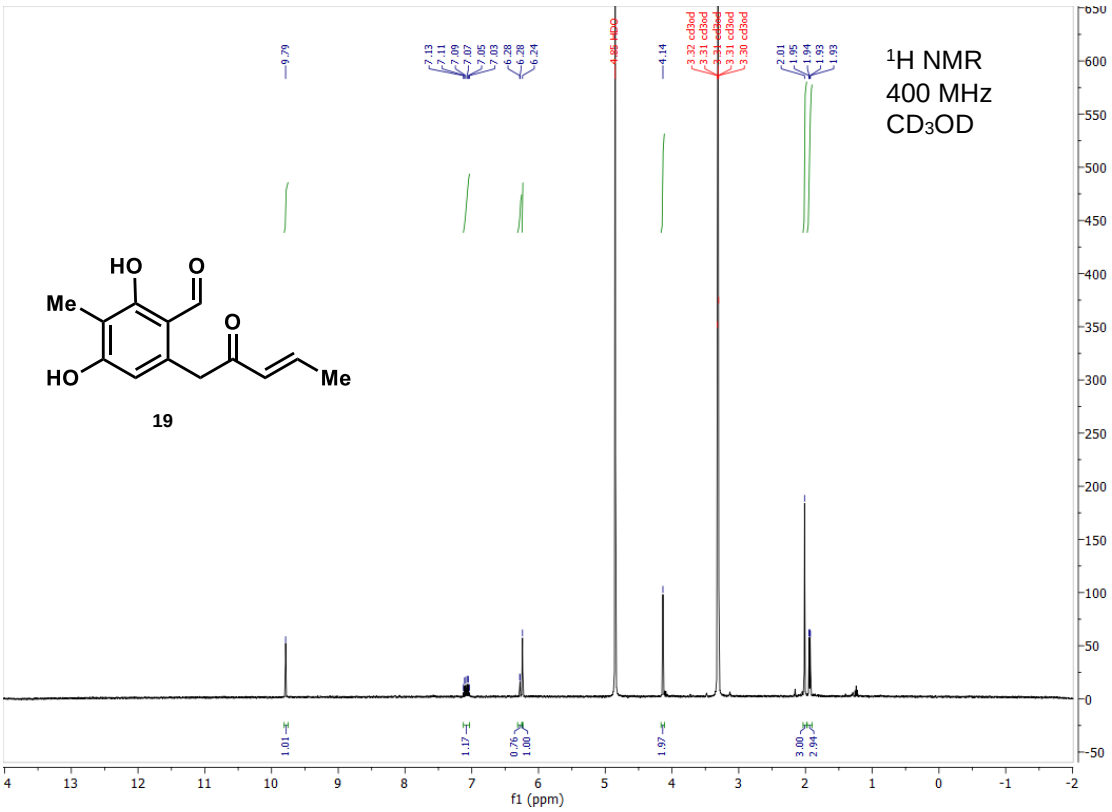
$^{1}$H NMR
400 MHz
$CDCl_3$

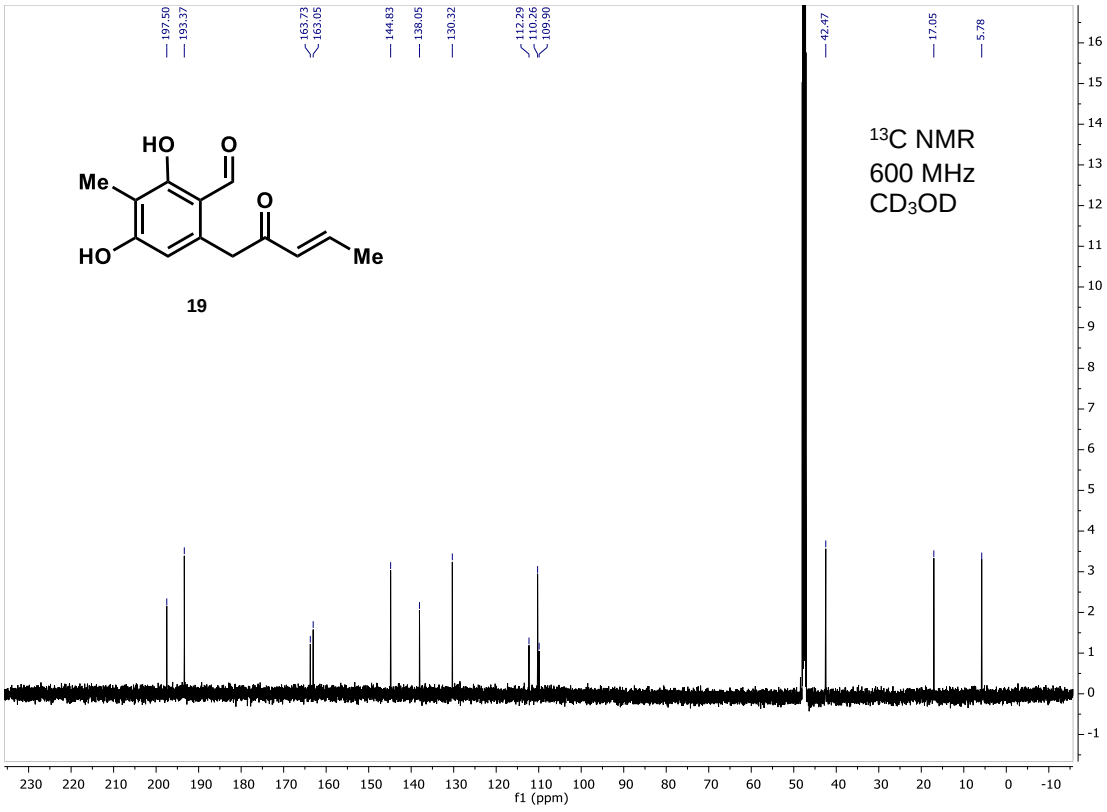


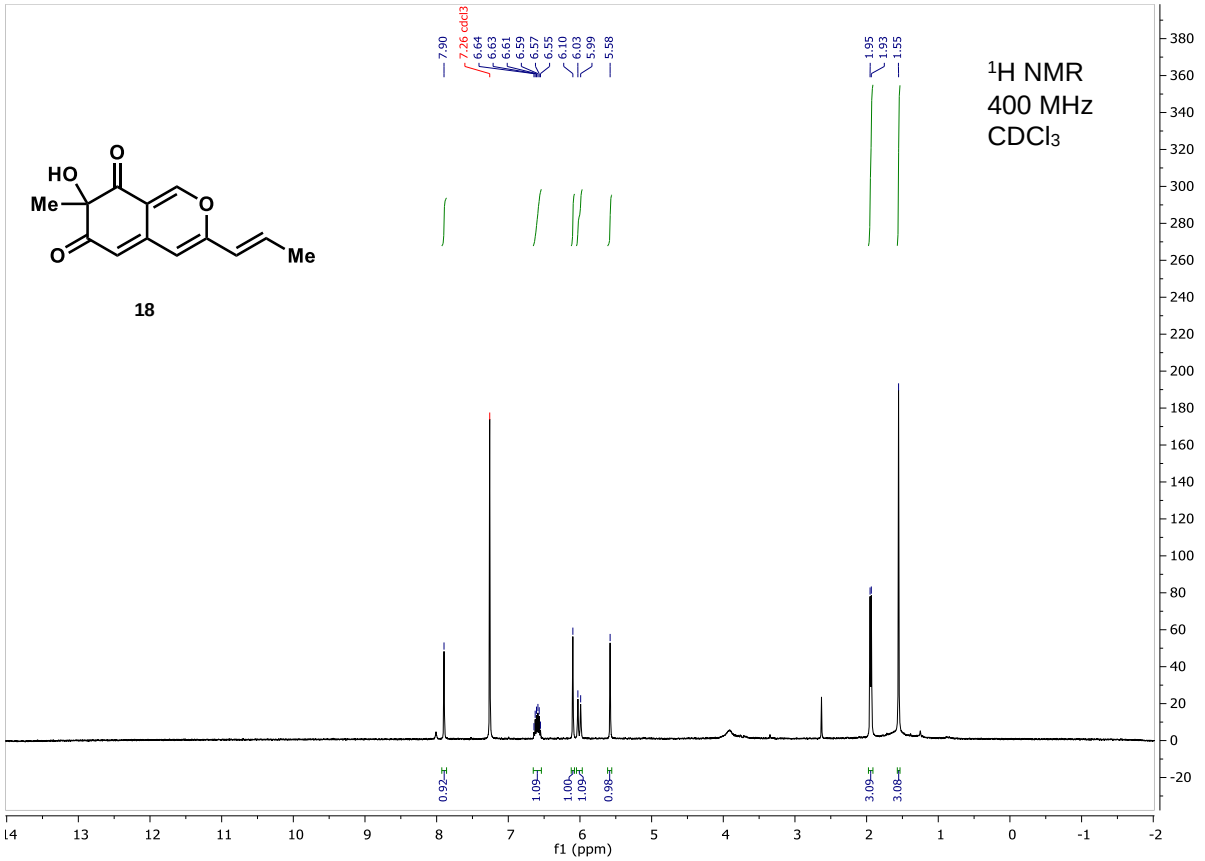



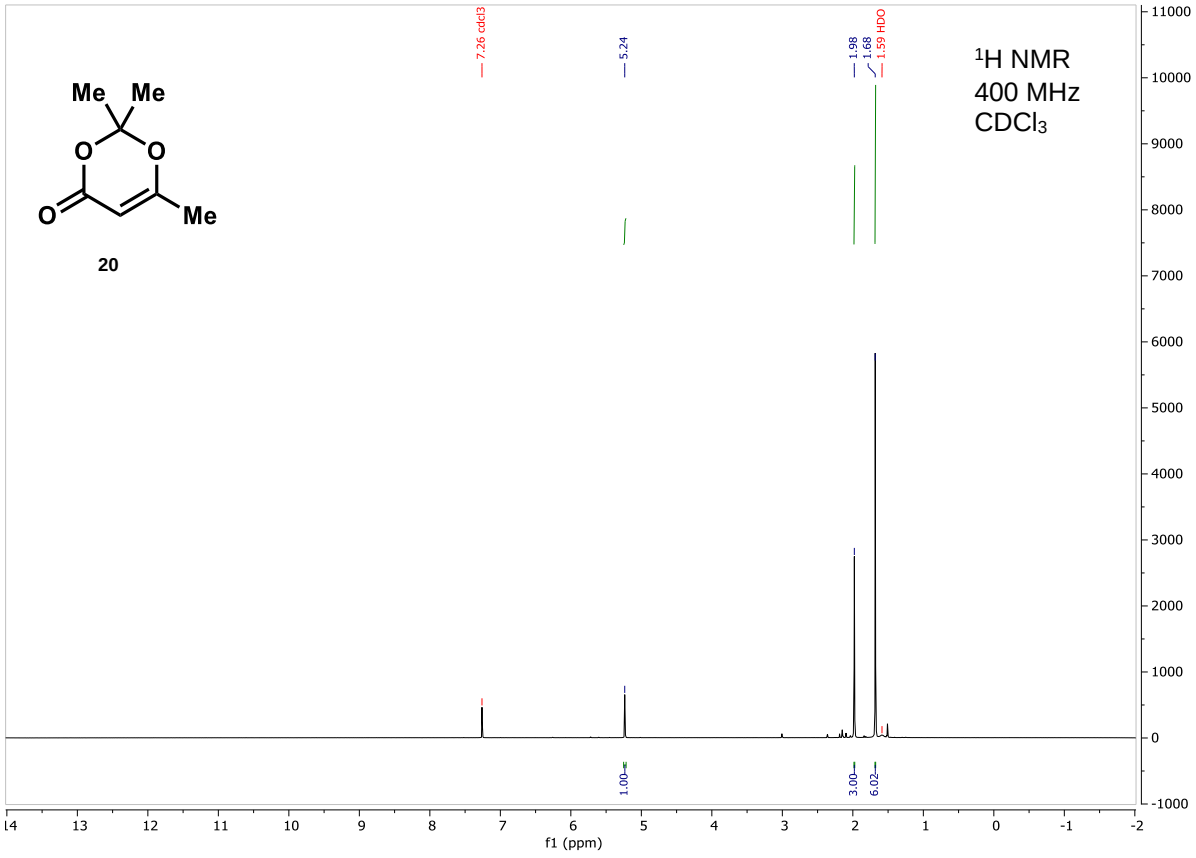

S8

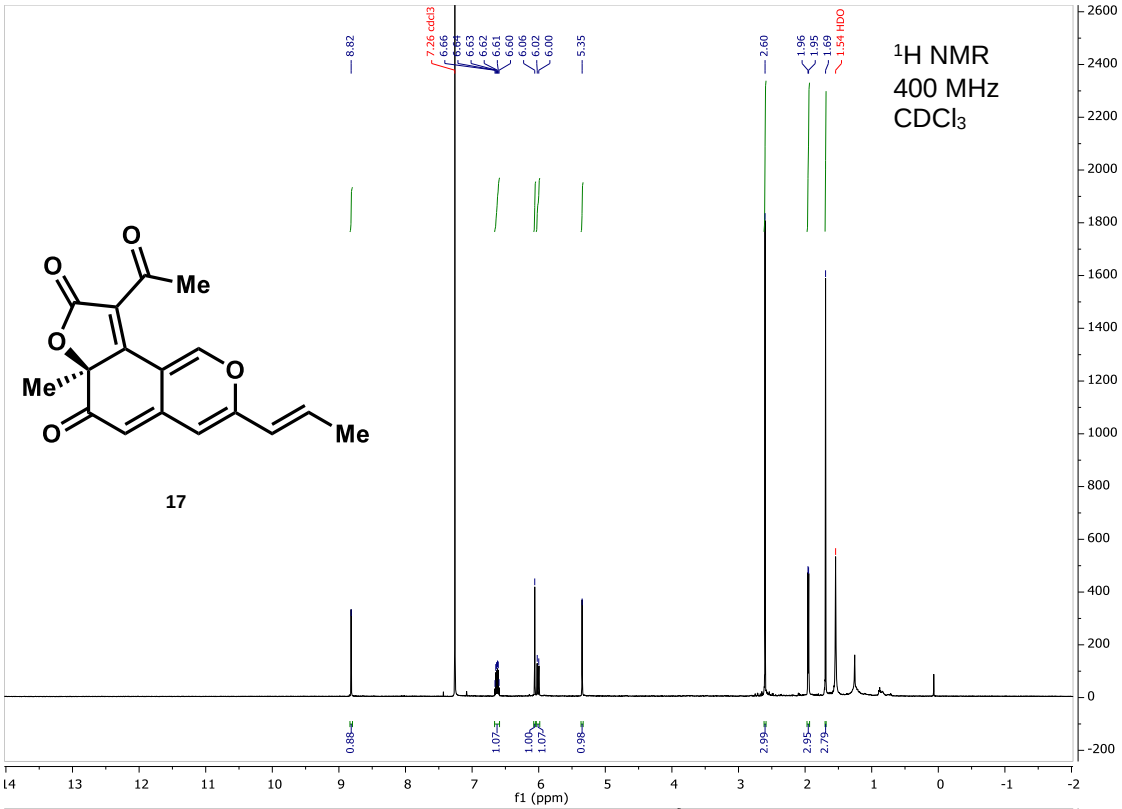
$^{13}$C NMR
600 MHz
$CDCl_3$

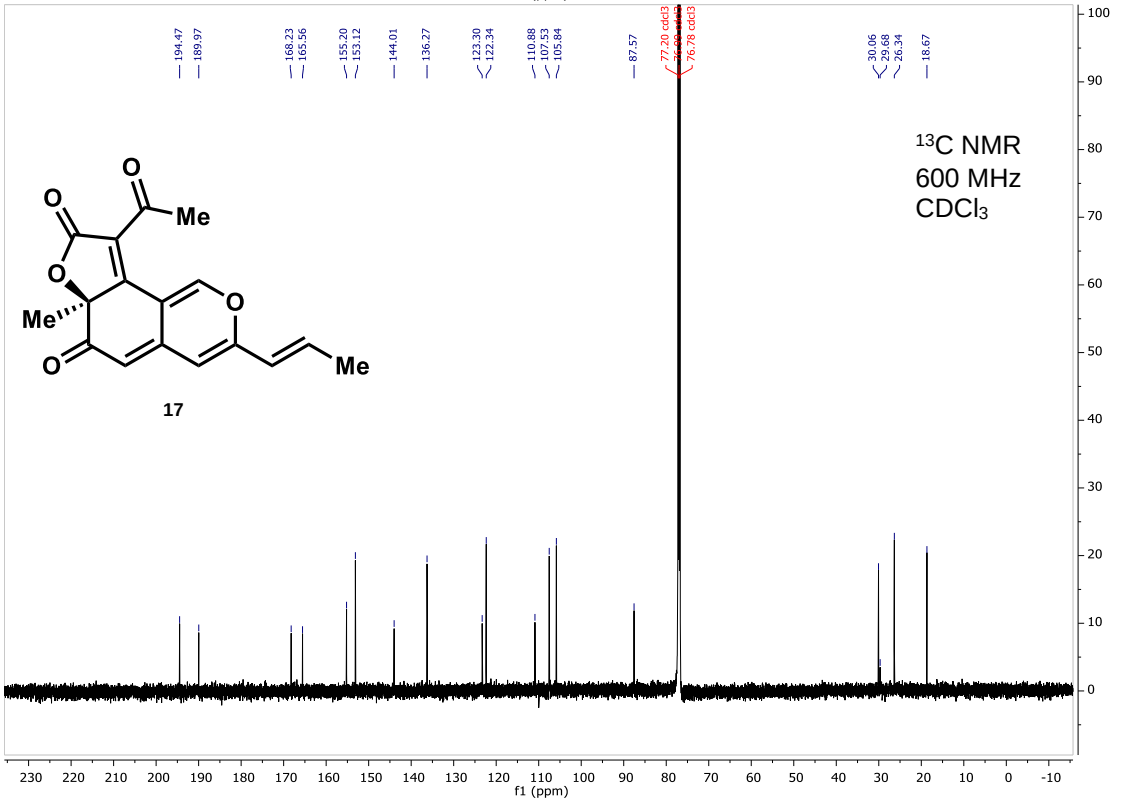

$^{1}$H NMR
400 MHz
$CD_3OD$
HO
O
Me
O
HO
Me
19
f1 (ppm)

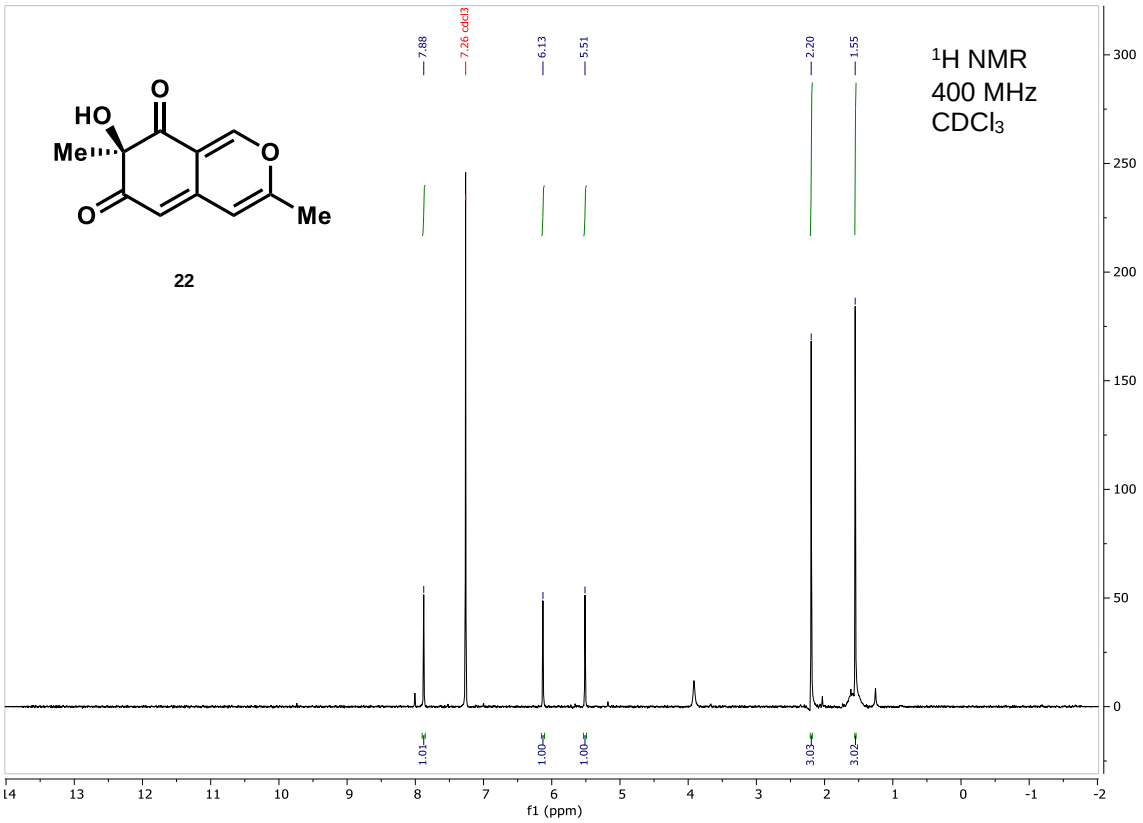

$^{13}$C NMR
600 MHz
$CD_3OD$
HO
O
Me
O
HO
Me
19
f1 (ppm)

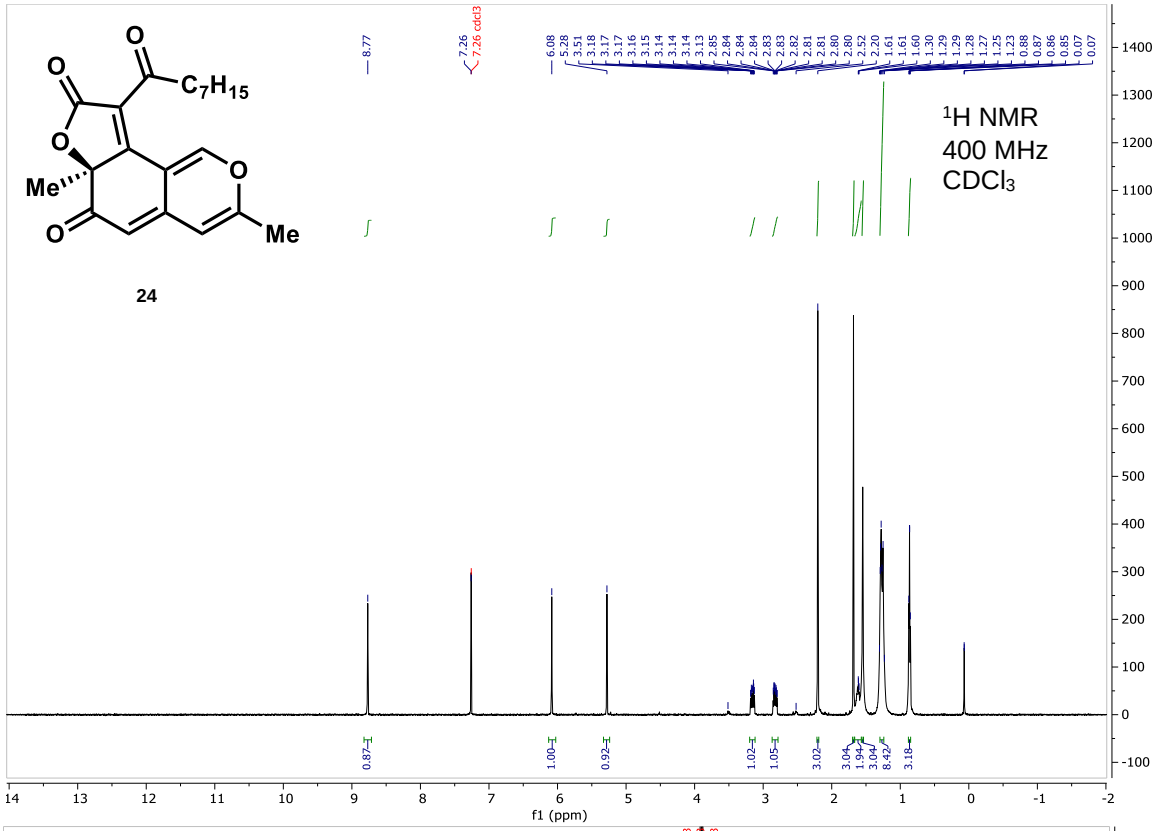

HO
Me
O
O
O
Me
18
7.90
7.26 cdcl3
6.64
6.63
6.61
6.59
6.57
6.55
6.10
6.03
5.99
5.58
1.95
1.93
1.55
0.92
1.09
1.00
1.09
0.98
3.09
3.08
f1 (ppm)
1H NMR
400 MHz
CDCl3

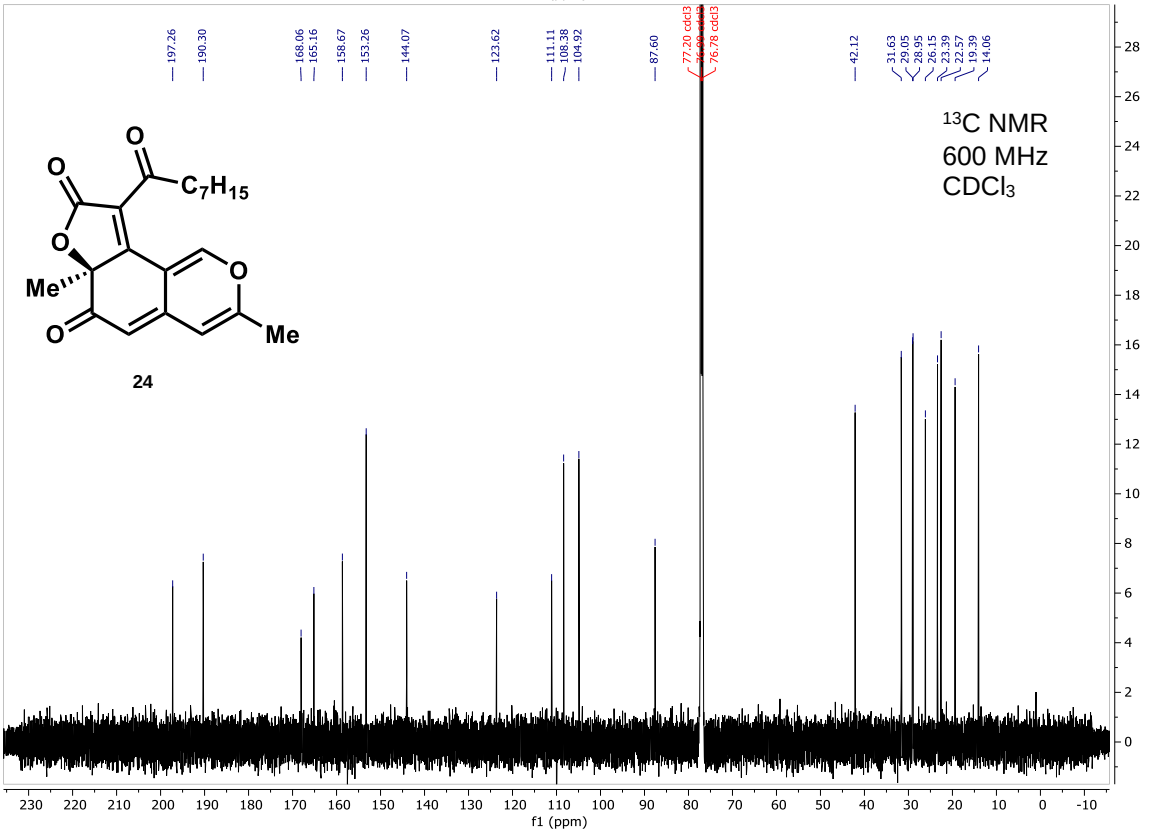

Me
Me
O
O
O
Me
20
7.26 cdcl3
5.24
1.98
1.68
1.59 HDO
1.00
3.00
6.02
f1 (ppm)
1H NMR
400 MHz
CDCl3

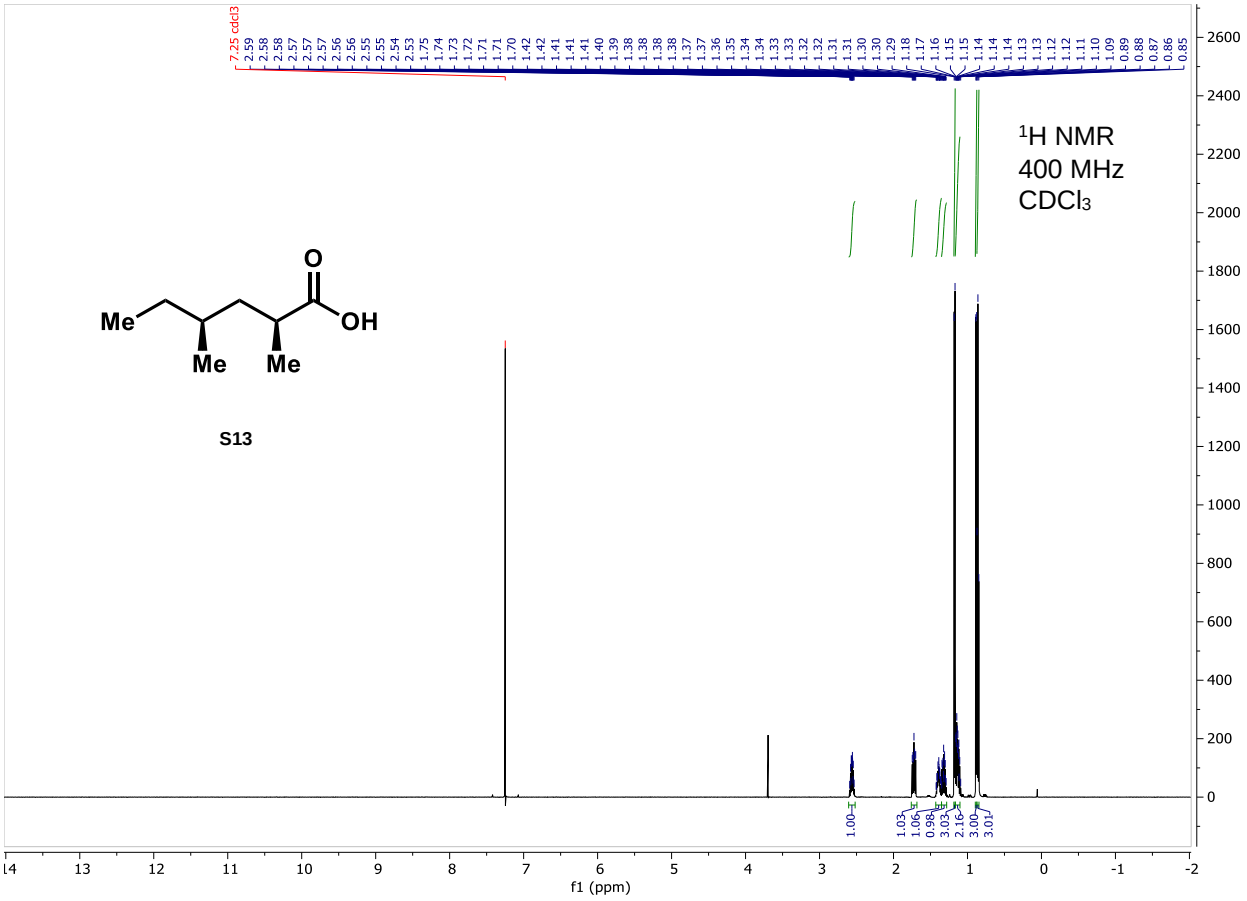

$^{1}$H NMR
400 MHz
$CDCl_3$
17
f1 (ppm)

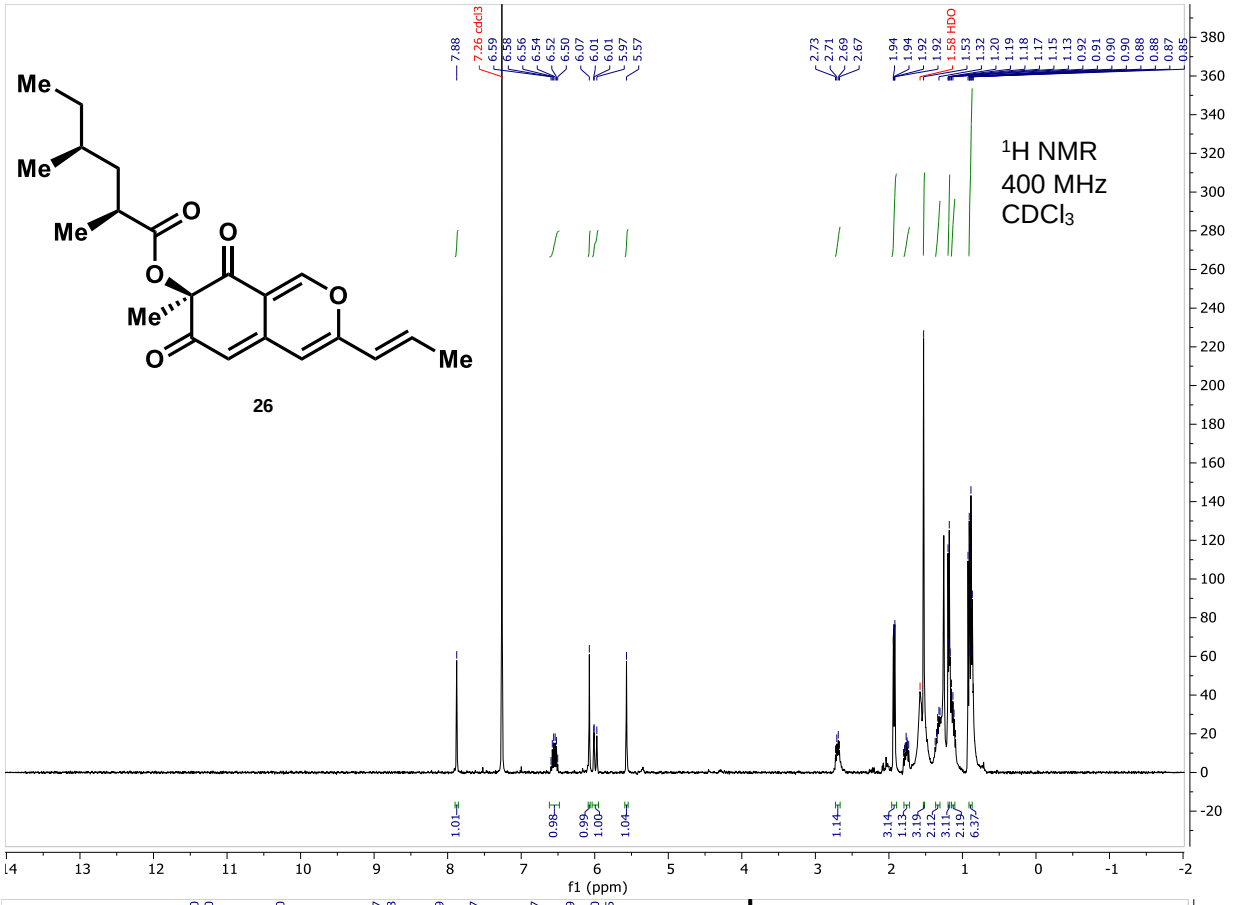

$^{13}$C NMR
600 MHz
$CDCl_3$
17
f1 (ppm)

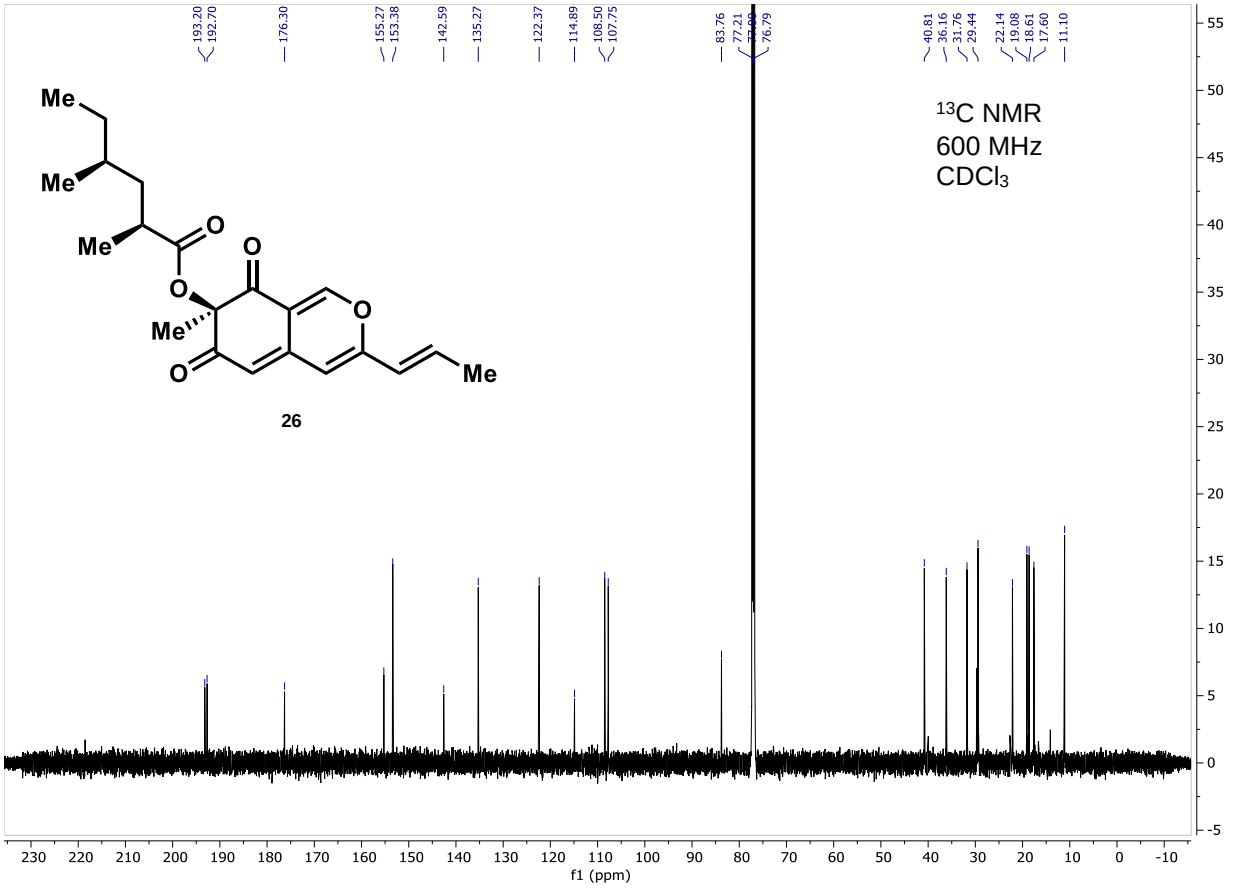

HO
Me
O
O
O
Me
22
7.88
7.26 cdcl3
6.13
5.51
2.20
1.55
1H NMR
400 MHz
CDCl3
1.01
1.00
1.00
3.03
3.02
f1 (ppm)

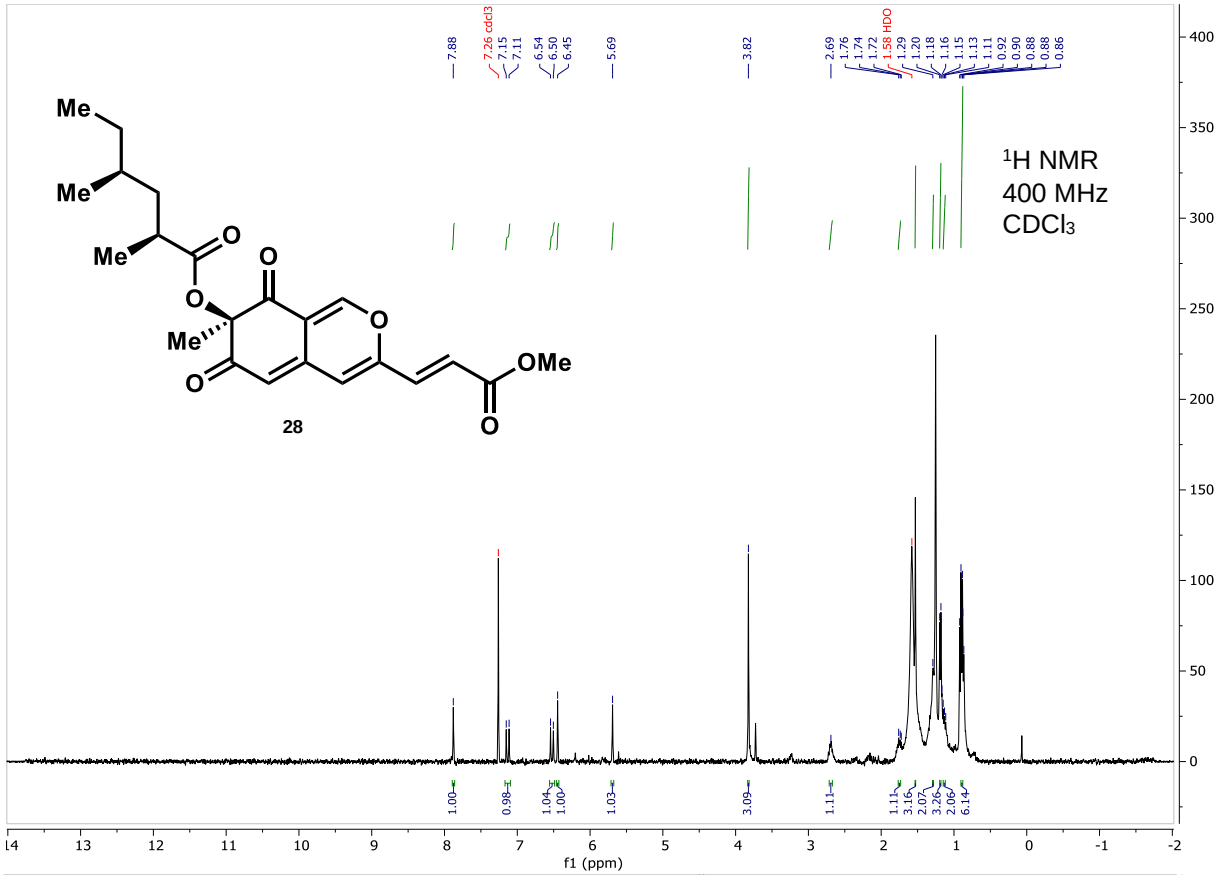

24
$^{1}$H NMR
400 MHz
$CDCl_3$
f1 (ppm)

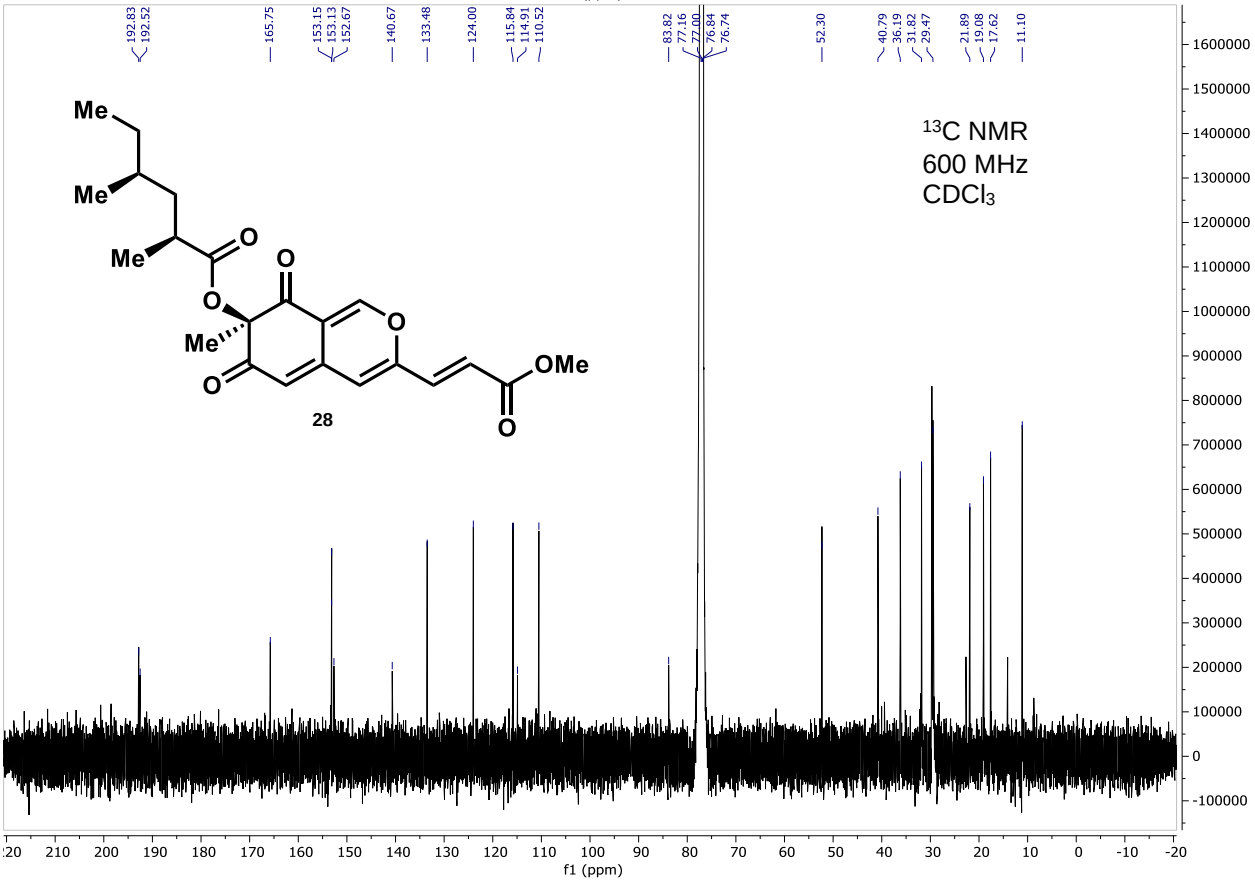

24
$^{13}$C NMR
600 MHz
$CDCl_3$
f1 (ppm)


$^1$H NMR
400 MHz
$CDCl_3$
Me
Me
Me
OH
O
S13
f1 (ppm)


Me
Me
Me
O
O
O
Me
O
O
Me
26
$^{1}$H NMR
400 MHz
$CDCl_3$
f1 (ppm)


Me
Me
Me
O
O
O
Me
O
O
Me
26
$^{13}$C NMR
600 MHz
$CDCl_3$
f1 (ppm)


7.88
7.26 cdcl3
7.15
7.11
6.54
6.50
6.45
5.69
3.82
2.69
1.76
1.74
1.72
1.58 HDO
1.29
1.20
1.18
1.16
1.15
1.13
1.11
0.92
0.90
0.88
0.88
0.86
1.00
0.98
1.04
1.00
1.03
3.09
1.11
1.11
3.15
2.07
3.26
2.06
6.14
Me
Me
Me
O
O
O
Me
O
O
O
OMe
O
28
1H NMR
400 MHz
CDCl3
f1 (ppm)

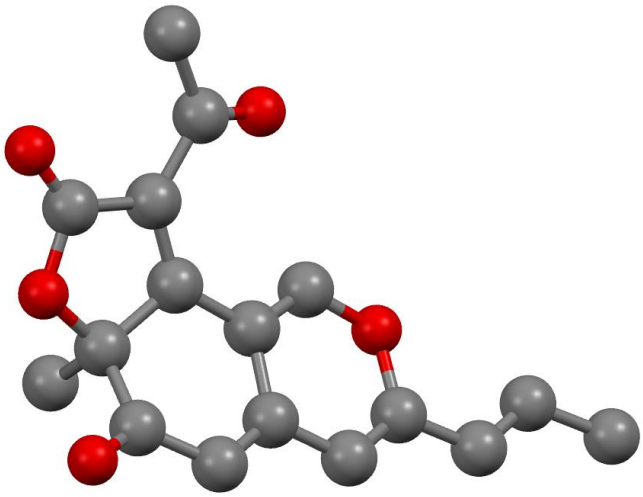

192.83
192.52
165.75
153.15
153.13
152.67
140.67
133.48
124.00
115.84
114.91
110.52
83.82
77.16
77.00
76.84
76.74
52.30
40.79
36.19
31.82
29.47
21.89
19.08
17.62
11.10
Me
Me
Me
O
O
O
Me
O
O
O
OMe
O
28
13C NMR
600 MHz
CDCl3
f1 (ppm)

## Part XI. Single-Crystal Structure Determination

Single-crystal X-ray diffraction data were collected using a Rigaku XtaLAB Synergy-S X-ray diffractometer configured in a kappa goniometer geometry. The diffractometer is equipped with a PhotonJet-S microfocus Cu source (λ = 1.54187 Å) set at a rough divergence of 9.5 and operated at 50 kV and 1 mA. X-ray intensities were measured at 298(1) K with the HyPix-6000HE detector placed 34.00 mm from the sample. The data were processed with CrysAlisPro v38.46 (Rigaku Oxford Diffraction) and corrected for absorption. The structures were solved in OLEX2[17] using SHELXTL[18] and refined using SHELXL.[19] All non-hydrogen atoms were refined anisotropically with hydrogen atoms placed at idealized positions.

### Table of Crystallographic Parameters

| Material | exp_589 |
|---|---|
| Space Group | $P2_12_12_1$ |
| *a* Å | 5.39440(6) |
| *b* Å | 18.0328(2) |
| *c* Å | 29.9372(4) |
| Volume (Å$^3$) | 2912.18 |
| Flack | 0.00(6) |
| Temperature | 298(1) |
| $\rho_{calc}$ (g cm$^{-3}$) | 1.361 |
| $R_1/wR_2$ | 3.00/8.46 |
| GOF | 1.052 |



**ORTEP Diagram**

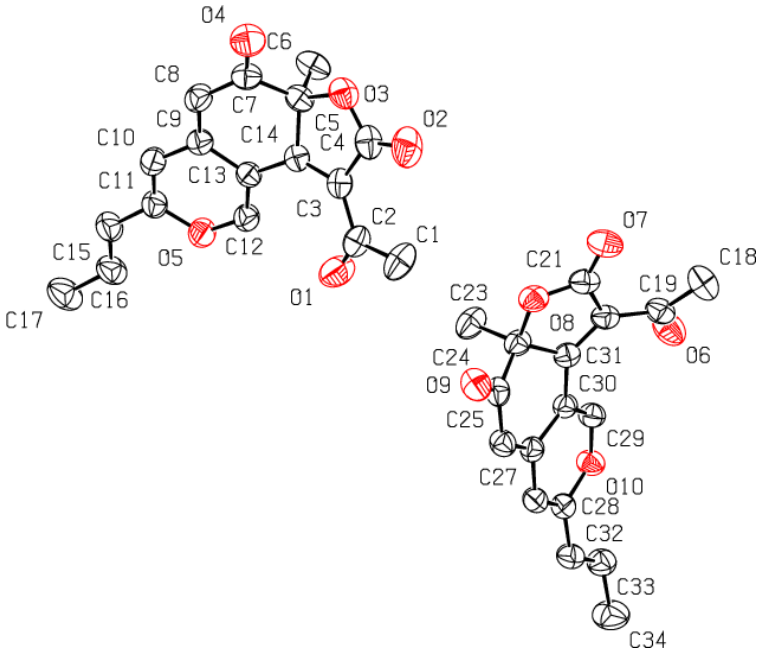



**Figure S21.** A view of exp_589 showing the atom labelling scheme. Thermal ellipsoids are drawn at the 50% probability level and H atoms are omitted for clarity.

## Part XII. Circular Dichroism Spectroscopy of Azaphilones **18**, **25**, and **30**

### Instruments and methods

Circular dichroism (CD) spectra were collected on a J-1500 Circular Dichroism Spectrophotometer (Jasco). Samples were prepared in UPLC grade MeCN at a final concentration of 750 μM. Data points were collected at room temperature from 190 to 500 nm with a scan rate of 100 nm/min in a quartz cell with an optical path of 1 mm.

### ECD Methodology

The general approach for absolute configuration assignment using ECD, including the detailed computational workflow, has been published elsewhere.[20-22] A subset of the details of the computational methodology is provided here. Conformers of each test structure were geometry optimized at the B3LYP/6-31G** level and stationary points were confirmed by performing frequency calculations.[23-31] All calculations were performed using Gaussian 09.[32] Output conformers were ranked according to DFT energy and a clustering was performed in order to remove duplicates. Initial duplicate identification was performed solely on an electronic energy basis where two compounds were considered identical if the difference in Hartrees was less than 0.01. Rounding the differences led to inconsistencies in identification of duplicates. It became better to cluster the DFT minima by energy and then re-cluster each energy bucket by structure using an all atom RMS of 0.6 Å. This process removed just identical compounds. Two Boltzmann distributions were calculated based on the free energy (G) and the electronic energy (E).

To calculate UV and ECD spectra, B3LYP geometries were used as input. The spectra were then calculated using either the B3LYP or CAM-B3LYP[33] functionals, along with the 6-31++G** basis set[34, 35] in vacuo. Only conformers which contributed more than 5.0% to the total in vacuo conformer distribution were selected for UV and ECD calculation. Time-dependent Density Functional Theory (TDDFT)[36] methodology was employed using the following keywords: TD=full,singlet, Nstates=100, and integral=ultrafinegrid. Spectral display, Boltzmann weighting, and curve fitting were carried out using SpecDis,[37, 38] and were displayed with a wavelength shift and band broadening sigma values in order to best match the calculated and experimental UV spectra. This shift and band broadening were then applied to the ECD spectra, and the area under the curve fit was determined by SpecDis.

*Trichoflectin* (**17**)

Calculations of the ECD and UV spectra (CAM-B3LYP/6-31++G**) involved modeling the (*R*)- enantiomer of the natural product. Since no other stereoisomers were possible, it should be noted that the (*S*)-enantiomer is assumed to have a spectrum that will be equal and opposite at all wavelengths.

Figure S22 provides an overlay of the calculated and measured UV and ECD spectra using the theoretical spectrum of the (*R*)-enantiomer. The calculated spectrum has been shifted 30 nm and a band broadening of σ=0.39 eV applied in order to optimize the UV spectral match. A high degree of confidence is derived from the statistical and visual matching of the spectra, with the experimental spectrum matching the mirror image of the calculated (*R*)-enantiomer. Hence, the absolute configuration of trichoflectin can confidently be assigned as (*S*). Figure S23 shows the one conformer of the *(R*) enantiomer that contributed >5% to the Boltzmann weighted spectrum, and the coordinates of this conformer are shown with the electronic energy below. Assigned absolute configuration of the desired compounds is provided in Scheme S1.

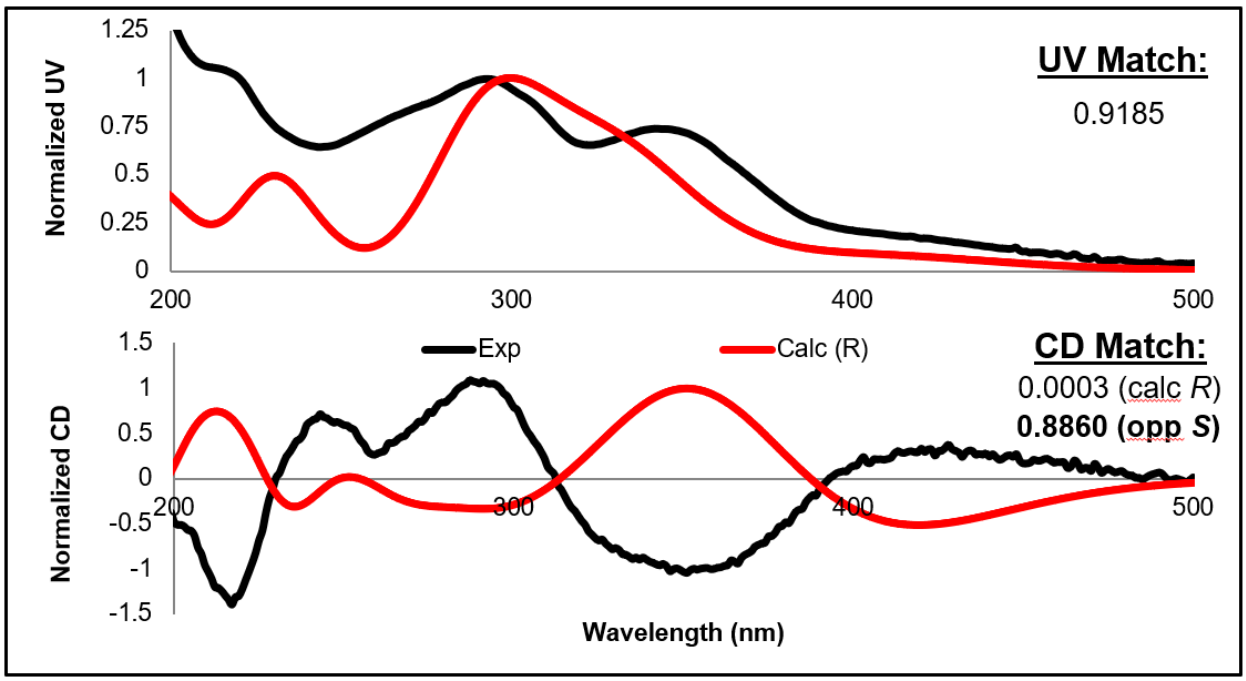
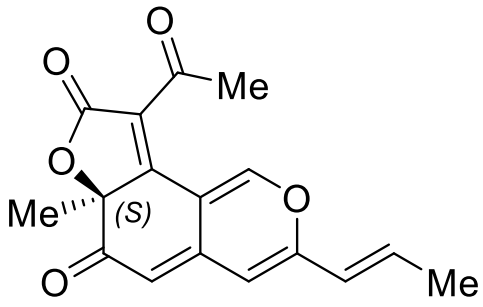
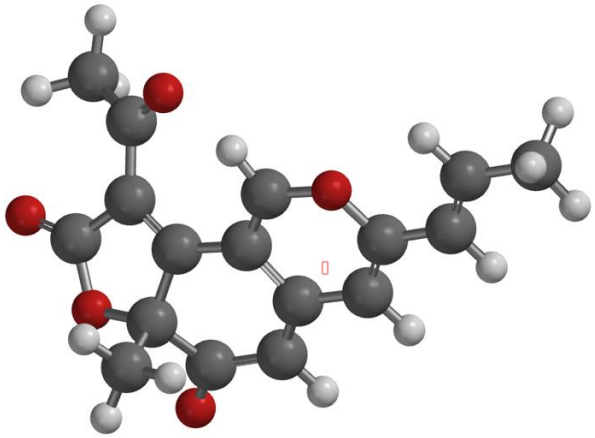



**Figure S22.** Comparison between experimental (black) and calculated (red) UV (top) and ECD (bottom) spectra. The calculated (R)-enantiomer is opposite of the experimental spectrum, with a large difference in fits (Δ=0.8857) suggesting a confident assignment. The calculated spectrum has been shifted 30 nm, and a band broadening of 0.39 eV has been applied.




**Scheme S1.** Assigned absolute configuration of trichoflectin based on ECD analysis



**Conformer 1:** 93.3%

**Figure S23.** One conformer of the (*R*)-enantiomer of trichoflectin that contributes >5% to the Boltzmann distribution. Note the percentage shown above based is on *in vacuo* electronic energies.

**Coordinates and electronic energies for B3LYP/6-31G** conformational minima contributing >5% to the *in vacuo* Boltzmann distribution.**

Conformer 1: -1032.346471 hartrees

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | -1.92652 | -1.24179 | 0.454749 | 8 | -4.4906 | 0.97176 | -0.14711 |
| 6 | -1.43886 | -2.36436 | -0.50656 | 6 | 6.76245 | 0.716682 | 0.01421 |
| 6 | -0.00398 | -2.37628 | -0.72861 | 6 | -2.84313 | 3.30205 | -0.90379 |
| 6 | 0.811717 | -1.3366 | -0.3621 | 6 | -1.81538 | -1.7794 | 1.89715 |
| 6 | 0.287916 | -0.07181 | 0.177876 | 1 | 7.0976 | 1.54991 | -0.61613 |
| 6 | -1.16113 | 0.044932 | 0.262781 | 1 | 7.1483 | -0.21358 | -0.41144 |
| 6 | 1.15752 | 0.916672 | 0.51399 | 1 | 7.22791 | 0.866265 | 0.996514 |
| 6 | 2.24341 | -1.37366 | -0.51756 | 1 | -2.48918 | 4.32822 | -1.00616 |
| 8 | -2.21691 | -3.20925 | -0.91931 | 1 | -3.79449 | 3.26342 | -0.3666 |
| 6 | 3.04043 | -0.3412 | -0.14027 | 1 | -3.0406 | 2.8698 | -1.89086 |
| 6 | 4.48685 | -0.31902 | -0.24494 | 1 | -2.17304 | -1.0227 | 2.60047 |
| 8 | 2.49748 | 0.804537 | 0.396205 | 1 | -0.78389 | -2.04131 | 2.14313 |
| 6 | 5.27154 | 0.705286 | 0.129229 | 1 | -2.44014 | -2.67163 | 1.98075 |
| 6 | -2.0555 | 1.05447 | 0.067487 | 1 | 0.408491 | -3.25877 | -1.20725 |
| 6 | -3.41789 | 0.444905 | 0.026975 | 1 | 0.849272 | 1.88074 | 0.892321 |
| 8 | -3.29409 | -0.90399 | 0.221383 | 1 | 2.70889 | -2.25918 | -0.93574 |
| 6 | -1.79661 | 2.49736 | -0.17149 | 1 | 4.92704 | -1.22041 | -0.66473 |
| 8 | -0.75437 | 3.01999 | 0.214502 | 1 | 4.80462 | 1.59524 | 0.545569 |

*Lunatoic Acid A Methyl Ester* (**28**)

Calculations of the ECD and UV spectra (CAM-B3LYP/6-31++G**) involved modeling the (*R*) enantiomer of the natural product methyl ester. While the compound contains an ester chain with two stereocenters, the majority of the UV absorption and thus the ECD signal is expected to come from the stereocenter on the ring. Calculations of a hypothetical diastereomer with inversion at the stereocenter alpha to the ester carbonyl showed an analogous signal to that for the expected structure, indicating that this prior assumption is correct.

Figure S26 provides overlays of the calculated and measured UV and ECD spectra using the theoretical spectrum of the (*R*)-enantiomer. The calculated spectrum has been shifted -46 nm and a band broadening of σ=0.28 eV applied in order to optimize the UV spectral match. A high degree of confidence is derived from the statistical and visual matching of the spectra, with the experimental spectrum matching the calculated (*R*)-enantiomer. Hence, the absolute configuration of the methyl ester of Lunatoic Acid A can confidently be assigned as (*R*). Figure S27 shows the six conformers of the *(R*) enantiomer that contributed >5% to the Boltzmann weighted spectrum, and the coordinates of these conformers are shown with the electronic energies listed below. Assigned absolute configuration of the desired compounds is provided in Scheme S2.

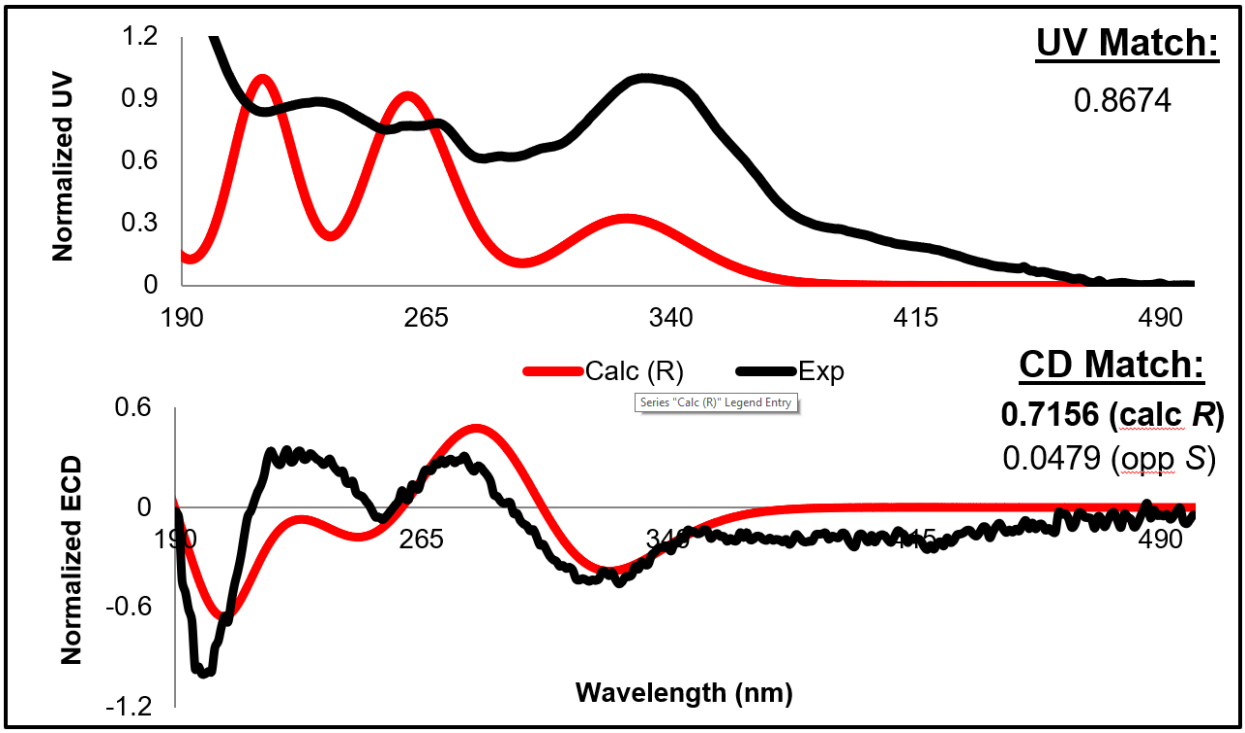
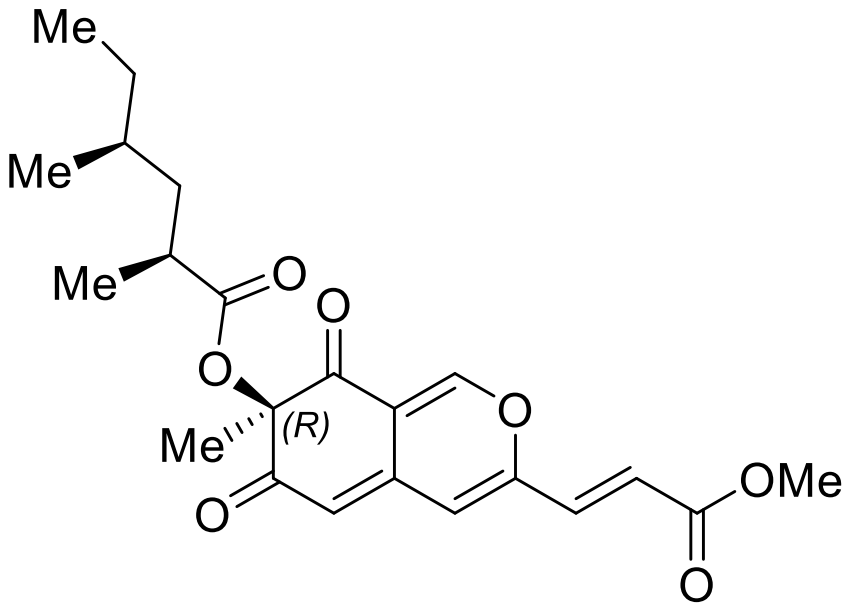



**Figure S24.** Comparison between experimental (black) and calculated (red) UV (top) and ECD (bottom) spectra. The calculated (*R*)-enantiomer is a good match to the experimental, with a large difference in fits (Δ=0.6677) suggesting a confident assignment. The calculated spectrum has been shifted -46 nm, and a band broadening of 0.28 eV has been applied.




**Scheme S2.** Assigned absolute configuration of lunatoic acid A methyl ester based on ECD analysis.

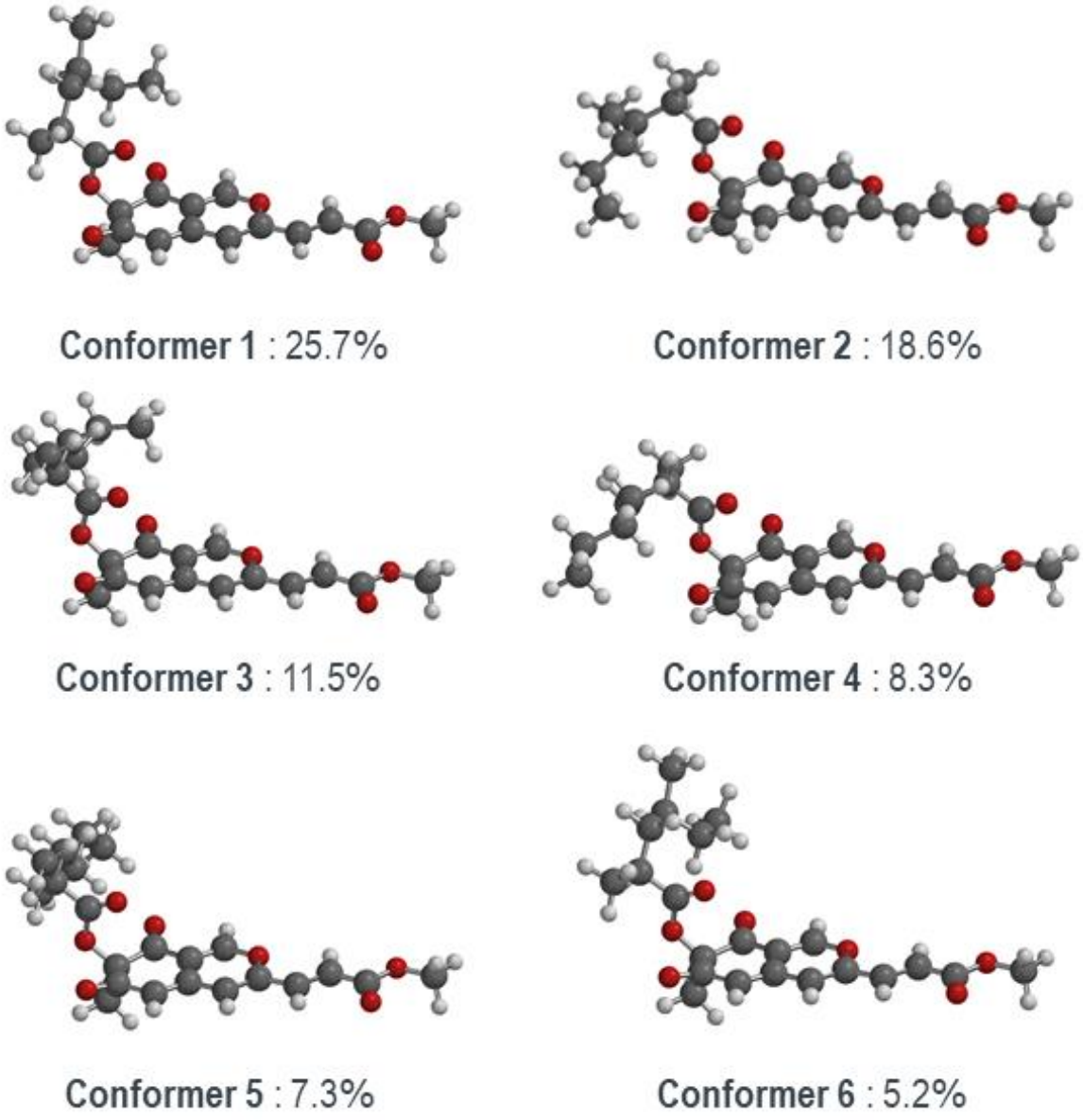



**Figure S25.** Six conformers of the (*R*)-enantiomer of lunatoic acid A methyl ester that contribute >5% to the Boltzmann distribution. Note the percentage shown above based is on *in vacuo* electronic energies.

**Coordinates and electronic energies for B3LYP/6-31G** conformational minima contributing >5% to the *in vacuo* Boltzmann distribution.**

Conformer 1: -1380.662338 hartrees

| | | | |
|---|---|---|---|
| 6 | 1.78282 | -1.68543 | 0.941074 |
| 6 | 1.32039 | -2.58496 | -0.23991 |
| 6 | -0.08437 | -2.56709 | -0.60377 |
| 6 | -0.9426 | -1.60841 | -0.14681 |

| | | | |
|---|---|---|---|
| 6 | -0.5196 | -0.5554 | 0.767273 |
| 6 | 0.874425 | -0.48446 | 1.26383 |
| 6 | -2.32929 | -1.52556 | -0.54115 |
| 6 | -3.1488 | -0.54276 | -0.08739 |
| 8 | -2.69057 | 0.431987 | 0.778282 |
| 6 | -1.39911 | 0.390925 | 1.16506 |
| 6 | -4.54266 | -0.40614 | -0.4433 |
| 6 | -5.36122 | 0.565606 | -0.00381 |
| 6 | -6.77309 | 0.588333 | -0.44821 |
| 8 | -7.42645 | 1.63913 | 0.094655 |
| 8 | -7.29006 | -0.21849 | -1.19745 |
| 6 | -8.81115 | 1.75329 | -0.27781 |
| 8 | 1.27673 | 0.428157 | 1.96608 |
| 8 | 2.14123 | -3.32308 | -0.7703 |
| 6 | 1.88912 | -2.55832 | 2.19714 |
| 8 | 3.11592 | -1.21925 | 0.681905 |
| 6 | 3.21769 | -0.42005 | -0.4067 |
| 6 | 4.65684 | -0.13761 | -0.77387 |
| 8 | 2.24322 | -0.05119 | -1.03495 |
| 6 | 4.86859 | 1.32854 | -1.19008 |
| 6 | 5.00568 | 2.33153 | -0.02596 |
| 6 | 3.77038 | 2.4135 | 0.898726 |
| 6 | 5.40257 | 3.71242 | -0.57045 |
| 6 | 5.00231 | -1.11961 | -1.91571 |
| 6 | 2.48952 | 2.96521 | 0.26043 |
| 1 | -9.18284 | 2.6388 | 0.236805 |
| 1 | -9.36624 | 0.864184 | 0.032778 |
| 1 | -8.90866 | 1.86616 | -1.36077 |
| 1 | 2.26945 | -1.95226 | 3.02238 |
| 1 | 2.57353 | -3.38374 | 1.99027 |
| 1 | 0.910116 | -2.96483 | 2.46741 |
| 1 | 5.50425 | 4.44226 | 0.240151 |
| 1 | 4.65198 | 4.09497 | -1.27036 |
| 1 | 6.35778 | 3.66779 | -1.10507 |
| 1 | 6.06683 | -1.05241 | -2.15839 |
| 1 | 4.42381 | -0.8727 | -2.81099 |
| 1 | 4.76711 | -2.14983 | -1.63287 |
| 1 | 1.67012 | 2.92449 | 0.983973 |
| 1 | 2.60967 | 4.00572 | -0.05882 |
| 1 | 2.18371 | 2.37216 | -0.60516 |
| 1 | 4.05069 | 1.6223 | -1.85843 |
| 1 | 5.78692 | 1.37485 | -1.78889 |
| 1 | 4.0386 | 3.04453 | 1.75683 |
| 1 | 3.55559 | 1.42696 | 1.32415 |
| 1 | 5.27726 | -0.37544 | 0.096936 |
| 1 | 5.83819 | 1.97483 | 0.600279 |
| 1 | -0.4089 | -3.31549 | -1.31925 |
| 1 | -2.73808 | -2.26244 | -1.22363 |
| 1 | -1.13259 | 1.20076 | 1.8328 |
| 1 | -4.9434 | -1.15583 | -1.12031 |
| 1 | -5.02585 | 1.34477 | 0.670543 |

Conformer 2: -1380.662034 hartrees

| | | | |
|---|---|---|---|
| 6 | 1.60301 | -0.03006 | 1.10298 |
| 6 | 1.3789 | -1.37343 | 0.355659 |
| 6 | 0.014003 | -1.7783 | 0.076571 |
| 6 | -1.04379 | -0.9249 | 0.210908 |
| 6 | -0.88031 | 0.452365 | 0.656243 |
| 6 | 0.458903 | 0.995158 | 0.987229 |
| 6 | -2.40696 | -1.28817 | -0.0968 |
| 6 | -3.43234 | -0.40746 | 0.032594 |
| 8 | -3.21705 | 0.887649 | 0.463061 |
| 6 | -1.95435 | 1.26785 | 0.747312 |
| 6 | -4.81605 | -0.71065 | -0.25362 |
| 6 | -5.83805 | 0.154289 | -0.13215 |
| 6 | -7.21264 | -0.28798 | -0.45871 |
| 8 | -8.09295 | 0.721015 | -0.27846 |
| 8 | -7.52427 | -1.40097 | -0.83766 |
| 6 | -9.4627 | 0.393566 | -0.57157 |
| 8 | 0.650022 | 2.17255 | 1.23702 |
| 8 | 2.3459 | -2.0847 | 0.107215 |
| 6 | 1.81116 | -0.3468 | 2.59071 |
| 8 | 2.82306 | 0.578009 | 0.669392 |
| 6 | 2.82824 | 0.976139 | -0.62908 |
| 6 | 4.13858 | 1.63788 | -1.00285 |
| 8 | 1.86405 | 0.836693 | -1.35396 |
| 6 | 5.34521 | 0.822473 | -0.50325 |
| 6 | 5.39588 | -0.62932 | -1.02382 |
| 6 | 6.2899 | -1.51713 | -0.1358 |
| 6 | 5.85781 | -0.67645 | -2.48719 |
| 6 | 4.13868 | 3.07093 | -0.43476 |
| 6 | 5.70701 | -1.793 | 1.25639 |
| 1 | -10.0311 | 1.3026 | -0.37759 |
| 1 | -9.80742 | -0.42213 | 0.069435 |

| | | | |
|---|---|---|---|
| 1 | -9.56843 | 0.086968 | -1.61553 |
| 1 | 2.01622 | 0.581948 | 3.12786 |
| 1 | 2.65955 | -1.02784 | 2.68593 |
| 1 | 0.921112 | -0.82186 | 3.01336 |
| 1 | 5.82731 | -1.6996 | -2.87624 |
| 1 | 6.88894 | -0.31306 | -2.58176 |
| 1 | 5.22695 | -0.06024 | -3.13642 |
| 1 | 5.05623 | 3.58945 | -0.72908 |
| 1 | 4.0866 | 3.04841 | 0.657594 |
| 1 | 3.2833 | 3.64439 | -0.80353 |
| 1 | 6.33759 | -2.49318 | 1.81458 |
| 1 | 5.62542 | -0.87924 | 1.85454 |
| 1 | 4.70273 | -2.219 | 1.16926 |
| 1 | 5.31673 | 0.82973 | 0.590032 |
| 1 | 6.26441 | 1.34783 | -0.79631 |
| 1 | 7.28637 | -1.05919 | -0.04828 |
| 1 | 6.44131 | -2.47593 | -0.64853 |
| 1 | 4.13371 | 1.68799 | -2.09586 |
| 1 | 4.38433 | -1.05266 | -0.96904 |
| 1 | -0.12316 | -2.78673 | -0.29964 |
| 1 | -2.63015 | -2.2909 | -0.44405 |
| 1 | -1.88497 | 2.30284 | 1.05848 |
| 1 | -5.02839 | -1.72114 | -0.59244 |
| 1 | -5.69587 | 1.17646 | 0.198431 |

Conformer 3: -1380.661580 hartrees

| | | | |
|---|---|---|---|
| 6 | 1.80645 | -1.44169 | 0.96146 |
| 6 | 1.25025 | -2.64964 | 0.155399 |
| 6 | -0.17932 | -2.70503 | -0.09509 |
| 6 | -0.9995 | -1.63538 | 0.118238 |
| 6 | -0.5044 | -0.36532 | 0.63408 |
| 6 | 0.927277 | -0.17683 | 0.961251 |
| 6 | -2.41536 | -1.64472 | -0.16665 |
| 6 | -3.19794 | -0.55479 | 0.039268 |
| 8 | -2.67158 | 0.624928 | 0.532123 |
| 6 | -1.35133 | 0.67509 | 0.80152 |
| 6 | -4.61807 | -0.50162 | -0.22184 |
| 6 | -5.40103 | 0.573276 | -0.02404 |
| 6 | -6.84641 | 0.491423 | -0.33301 |
| 8 | -7.45656 | 1.66853 | -0.07229 |
| 8 | -7.42178 | -0.4906 | -0.76212 |
| 6 | -8.86935 | 1.69464 | -0.34157 |
| 8 | 1.38571 | 0.902317 | 1.30141 |
| 8 | 2.02181 | -3.54237 | -0.16765 |
| 6 | 2.00108 | -1.88773 | 2.41606 |
| 8 | 3.1193 | -1.11135 | 0.482608 |
| 6 | 3.14307 | -0.63909 | -0.78697 |
| 6 | 4.53741 | -0.2664 | -1.24181 |
| 8 | 2.13028 | -0.48912 | -1.44328 |
| 6 | 4.66904 | 1.27368 | -1.1446 |
| 6 | 4.59209 | 1.85383 | 0.282993 |
| 6 | 4.31946 | 3.37107 | 0.257395 |
| 6 | 5.87038 | 1.54384 | 1.07516 |
| 6 | 4.76159 | -0.76217 | -2.67659 |
| 6 | 2.91313 | 3.7473 | -0.22612 |
| 1 | -9.20045 | 2.69967 | -0.08196 |
| 1 | -9.389 | 0.947829 | 0.264516 |
| 1 | -9.06233 | 1.48472 | -1.39691 |
| 1 | 2.44538 | -1.06879 | 2.98614 |
| 1 | 2.66392 | -2.75546 | 2.42639 |
| 1 | 1.04297 | -2.16464 | 2.86562 |
| 1 | 5.79709 | 1.92248 | 2.1002 |
| 1 | 6.74457 | 2.01411 | 0.607319 |
| 1 | 6.06246 | 0.46803 | 1.13768 |
| 1 | 5.75721 | -0.4714 | -3.02445 |
| 1 | 4.01456 | -0.32844 | -3.34689 |
| 1 | 4.67943 | -1.85142 | -2.73795 |
| 1 | 2.74341 | 4.82575 | -0.13886 |
| 1 | 2.75264 | 3.47711 | -1.27494 |
| 1 | 2.15184 | 3.22853 | 0.365762 |
| 1 | 3.87972 | 1.70055 | -1.77273 |
| 1 | 5.62504 | 1.56475 | -1.60085 |
| 1 | 5.07915 | 3.86872 | -0.36329 |
| 1 | 4.4569 | 3.76179 | 1.27411 |
| 1 | 5.25224 | -0.74403 | -0.56586 |
| 1 | 3.74888 | 1.38755 | 0.806291 |
| 1 | -0.55972 | -3.62808 | -0.52008 |
| 1 | -2.87746 | -2.54332 | -0.56017 |
| 1 | -1.03229 | 1.64273 | 1.16855 |
| 1 | -5.07182 | -1.41047 | -0.60798 |
| 1 | -5.01289 | 1.51069 | 0.356631 |

Conformer 4: -1380.661272 hartrees

| | | | |
|---|---|---|---|
| 6 | -1.55596 | 0.525882 | -1.24688 |
| 6 | -1.39326 | -1.0005 | -1.00539 |
| 6 | -0.05116 | -1.52379 | -0.8291 |
| 6 | 1.02845 | -0.71386 | -0.622 |
| 6 | 0.913797 | 0.737612 | -0.57223 |
| 6 | -0.39731 | 1.40781 | -0.74319 |
| 6 | 2.36829 | -1.20795 | -0.4078 |
| 6 | 3.41619 | -0.37277 | -0.18895 |
| 8 | 3.24754 | 0.998342 | -0.15597 |
| 6 | 2.00729 | 1.49677 | -0.33804 |
| 6 | 4.77861 | -0.80378 | 0.026419 |
| 6 | 5.82249 | 0.014168 | 0.247073 |
| 6 | 7.1711 | -0.5609 | 0.452202 |
| 8 | 8.07907 | 0.417463 | 0.662086 |
| 8 | 7.44234 | -1.74653 | 0.437014 |
| 6 | 9.42675 | -0.03819 | 0.875153 |
| 8 | -0.55242 | 2.60508 | -0.57627 |
| 8 | -2.38406 | -1.71879 | -1.06503 |
| 6 | -1.70204 | 0.748363 | -2.75855 |
| 8 | -2.78252 | 0.991689 | -0.67293 |
| 6 | -2.83081 | 0.930493 | 0.682381 |
| 6 | -4.13767 | 1.4721 | 1.21872 |
| 8 | -1.908 | 0.500183 | 1.34539 |
| 6 | -5.34191 | 0.698497 | 0.642041 |
| 6 | -5.24923 | -0.83741 | 0.727787 |
| 6 | -6.51003 | -1.46636 | 0.103601 |
| 6 | -5.01215 | -1.3188 | 2.16701 |
| 6 | -4.23525 | 2.97921 | 0.918661 |
| 6 | -6.4124 | -2.97899 | -0.12288 |
| 1 | 10.0219 | 0.861883 | 1.02621 |
| 1 | 9.78444 | -0.5968 | 0.006194 |
| 1 | 9.47759 | -0.68702 | 1.75349 |
| 1 | -1.86965 | 1.81069 | -2.94948 |
| 1 | -2.55413 | 0.164679 | -3.11348 |
| 1 | -0.80108 | 0.422162 | -3.28621 |
| 1 | -4.9906 | -2.41061 | 2.22314 |
| 1 | -5.8082 | -0.96452 | 2.83488 |
| 1 | -4.05431 | -0.96166 | 2.55624 |
| 1 | -5.1559 | 3.38547 | 1.34882 |
| 1 | -4.2481 | 3.15507 | -0.1603 |
| 1 | -3.38633 | 3.52528 | 1.3407 |
| 1 | -7.29867 | -3.35722 | -0.64326 |
| 1 | -5.53364 | -3.22271 | -0.73012 |
| 1 | -6.32747 | -3.52973 | 0.819164 |
| 1 | -5.46021 | 0.98631 | -0.40951 |
| 1 | -6.24213 | 1.04551 | 1.16859 |
| 1 | -6.70048 | -0.97928 | -0.86224 |
| 1 | -7.38038 | -1.23849 | 0.736557 |
| 1 | -4.08389 | 1.32562 | 2.30077 |
| 1 | -4.39419 | -1.15298 | 0.115844 |
| 1 | 0.047924 | -2.60425 | -0.81956 |
| 1 | 2.55469 | -2.27614 | -0.41827 |
| 1 | 1.97342 | 2.57747 | -0.27647 |
| 1 | 4.9545 | -1.87597 | 0.005158 |
| 1 | 5.71658 | 1.09205 | 0.282637 |

Conformer 5: -1380.661154 hartrees

| | | | |
|---|---|---|---|
| 6 | -1.70828 | -1.20718 | -1.044 |
| 6 | -1.12066 | -2.5505 | -0.52456 |
| 6 | 0.316767 | -2.63888 | -0.33668 |
| 6 | 1.12181 | -1.53681 | -0.34376 |
| 6 | 0.599747 | -0.19309 | -0.55821 |
| 6 | -0.846 | 0.041816 | -0.78461 |
| 6 | 2.54702 | -1.58687 | -0.11521 |
| 6 | 3.3148 | -0.46736 | -0.11168 |
| 8 | 2.76371 | 0.782449 | -0.32434 |
| 6 | 1.43357 | 0.870572 | -0.52933 |
| 6 | 4.74316 | -0.45144 | 0.10636 |
| 6 | 5.51166 | 0.651664 | 0.116227 |
| 6 | 6.96763 | 0.525948 | 0.352105 |
| 8 | 7.56038 | 1.74003 | 0.329591 |
| 8 | 7.56432 | -0.51695 | 0.541498 |
| 6 | 8.98158 | 1.72773 | 0.551402 |
| 8 | -1.3222 | 1.16282 | -0.85634 |
| 8 | -1.87601 | -3.50042 | -0.37132 |
| 6 | -1.91367 | -1.33244 | -2.55985 |
| 8 | -3.0199 | -1.01853 | -0.49685 |
| 6 | -3.04852 | -0.83734 | 0.847156 |
| 6 | -4.45833 | -0.65166 | 1.36568 |
| 8 | -2.03566 | -0.79038 | 1.51714 |
| 6 | -4.77348 | 0.863851 | 1.43291 |
| 6 | -4.66903 | 1.65075 | 0.111329 |

| | | | |
|---|---|---|---|
| 6 | -4.92025 | 3.14662 | 0.387374 |
| 6 | -5.61908 | 1.09458 | -0.95946 |
| 6 | -4.59634 | -1.31114 | 2.74352 |
| 6 | -4.59509 | 4.06869 | -0.79281 |
| 1 | 9.29665 | 2.76957 | 0.502455 |
| 1 | 9.48449 | 1.13527 | -0.21748 |
| 1 | 9.21301 | 1.29956 | 1.53026 |
| 1 | -2.38161 | -0.4191 | -2.93419 |
| 1 | -2.56102 | -2.19071 | -2.75188 |
| 1 | -0.95718 | -1.48515 | -3.06813 |
| 1 | -5.57012 | 1.68249 | -1.88047 |
| 1 | -6.65876 | 1.11124 | -0.6076 |
| 1 | -5.3616 | 0.065677 | -1.22357 |
| 1 | -5.60462 | -1.15335 | 3.13773 |
| 1 | -3.87404 | -0.88278 | 3.44345 |
| 1 | -4.41277 | -2.38806 | 2.68718 |
| 1 | -4.71353 | 5.12105 | -0.51411 |
| 1 | -3.56092 | 3.92231 | -1.12436 |
| 1 | -5.2485 | 3.88323 | -1.65103 |
| 1 | -4.09515 | 1.31017 | 2.17161 |
| 1 | -5.79004 | 0.969011 | 1.83696 |
| 1 | -4.30819 | 3.45048 | 1.24707 |
| 1 | -5.9671 | 3.28843 | 0.693539 |
| 1 | -5.138 | -1.13152 | 0.656004 |
| 1 | -3.64387 | 1.55535 | -0.2667 |
| 1 | 0.7176 | -3.6258 | -0.13015 |
| 1 | 3.02866 | -2.54208 | 0.062222 |
| 1 | 1.09511 | 1.88971 | -0.66938 |
| 1 | 5.21619 | -1.41532 | 0.274219 |
| 1 | 5.10413 | 1.64318 | -0.04225 |

Conformer 6: -1380.660826 hartrees

| | | | |
|---|---|---|---|
| 6 | 1.72315 | -1.62726 | 1.01081 |
| 6 | 1.24166 | -2.62238 | -0.08242 |
| 6 | -0.16608 | -2.619 | -0.43605 |
| 6 | -1.00892 | -1.61352 | -0.05807 |
| 6 | -0.56392 | -0.48662 | 0.75176 |
| 6 | 0.838572 | -0.38436 | 1.22068 |
| 6 | -2.3998 | -1.55165 | -0.44166 |
| 6 | -3.2047 | -0.52476 | -0.06623 |
| 8 | -2.72664 | 0.518295 | 0.70386 |
| 6 | -1.42978 | 0.499758 | 1.0748 |
| 6 | -4.60218 | -0.40712 | -0.41468 |
| 6 | -5.40691 | 0.606813 | -0.05153 |
| 6 | -6.82459 | 0.603336 | -0.47743 |
| 8 | -7.46216 | 1.70327 | -0.01965 |
| 8 | -7.35838 | -0.26105 | -1.14638 |
| 6 | -8.85109 | 1.79673 | -0.38191 |
| 8 | 1.26131 | 0.588955 | 1.82173 |
| 8 | 2.04829 | -3.41738 | -0.54816 |
| 6 | 1.80693 | -2.38374 | 2.34257 |
| 8 | 3.06546 | -1.2171 | 0.716302 |
| 6 | 3.19106 | -0.52486 | -0.44267 |
| 6 | 4.6419 | -0.30463 | -0.81074 |
| 8 | 2.23111 | -0.19526 | -1.11171 |
| 6 | 4.86933 | 1.08758 | -1.42272 |
| 6 | 4.94597 | 2.25717 | -0.4196 |
| 6 | 3.68055 | 2.36787 | 0.452821 |
| 6 | 5.23967 | 3.55856 | -1.18128 |
| 6 | 5.00999 | -1.43674 | -1.79549 |
| 6 | 3.69924 | 3.51074 | 1.47278 |
| 1 | -9.20878 | 2.72677 | 0.058749 |
| 1 | -9.40842 | 0.942826 | 0.012222 |
| 1 | -8.96316 | 1.81602 | -1.46917 |
| 1 | 2.19988 | -1.71345 | 3.11027 |
| 1 | 2.47304 | -3.23965 | 2.2149 |
| 1 | 0.818603 | -2.74182 | 2.64538 |
| 1 | 5.42632 | 4.39555 | -0.50293 |
| 1 | 4.39167 | 3.82928 | -1.82237 |
| 1 | 6.1209 | 3.45106 | -1.82334 |
| 1 | 6.08112 | -1.40892 | -2.01595 |
| 1 | 4.45828 | -1.31408 | -2.73249 |
| 1 | 4.75794 | -2.4165 | -1.37973 |
| 1 | 2.8286 | 3.4425 | 2.13228 |
| 1 | 4.59742 | 3.46547 | 2.10064 |
| 1 | 3.67856 | 4.49506 | 0.994432 |
| 1 | 4.06901 | 1.27588 | -2.14954 |
| 1 | 5.80757 | 1.0558 | -1.99064 |
| 1 | 3.53263 | 1.43619 | 1.00763 |
| 1 | 2.80314 | 2.46653 | -0.19955 |
| 1 | 5.23904 | -0.42073 | 0.100448 |
| 1 | 5.79589 | 2.06093 | 0.254076 |
| 1 | -0.50645 | -3.42507 | -1.07773 |
| 1 | -2.82413 | -2.34211 | -1.05077 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | -1.14669 | 1.36374 | 1.6632 | 1 | -5.05606 | 1.43894 | 0.547458 |
| 1 | -5.01815 | -1.20969 | -1.01787 | | | | |

*Deflectin-1a* (**24**)

Calculations of the ECD and UV spectra (B3LYP/6-31++G**) involved modeling the truncated (*R*) enantiomer of the natural product, shortening the exocyclic alkyl chain to a propyl group. This way there was still some conformational flexibility, but at a reduced computational cost. Since no other stereoisomers were possible, it should be noted that the (*S*)-enantiomer is assumed to have a spectrum that will be equal and opposite at all wavelengths.

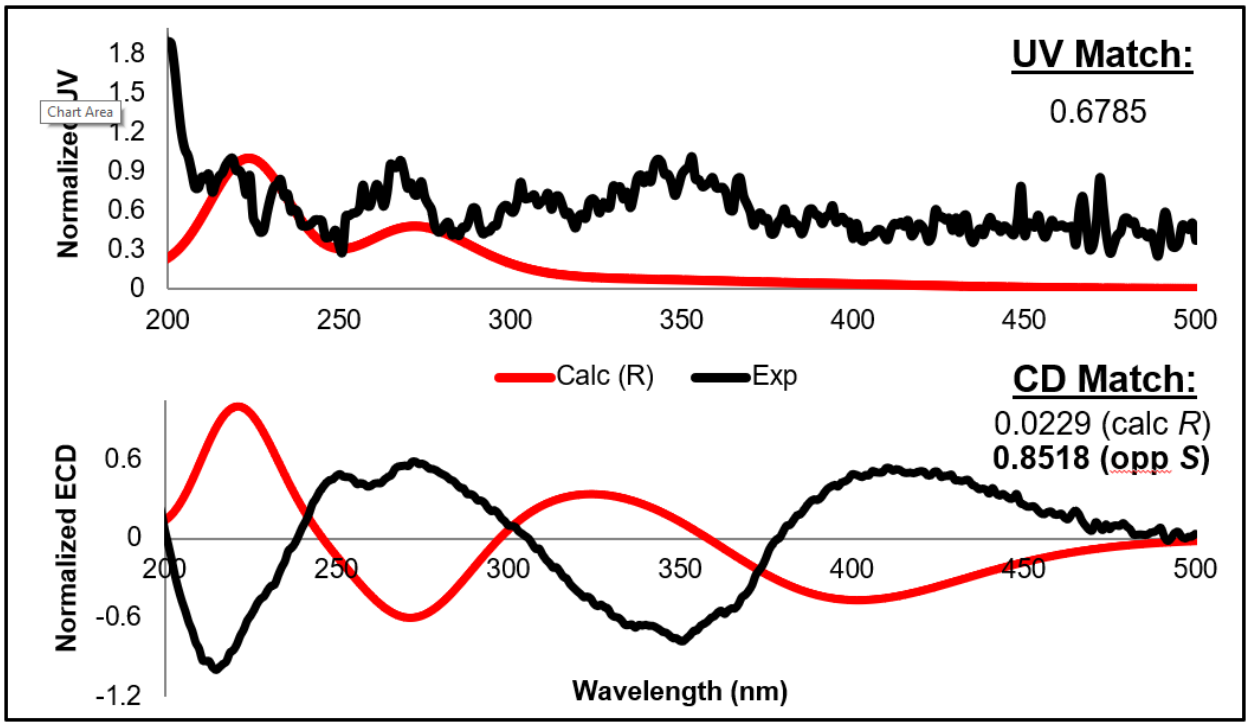
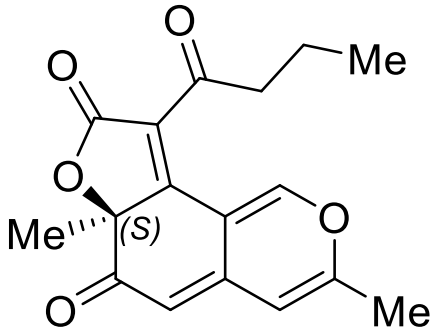



**Figure S26.** Comparison between experimental (black) and calculated (red) UV (top) and ECD (bottom) spectra. The calculated (*R*)-enantiomer is opposite of the experimental spectrum, with a large difference in fits (Δ=0.8289) suggesting a confident assignment. The calculated spectrum has been shifted -34 nm, and a band broadening of 0.3 eV has been applied.




**Scheme S3.** Assigned absolute configuration of truncated deflectin based on ECD analysis.

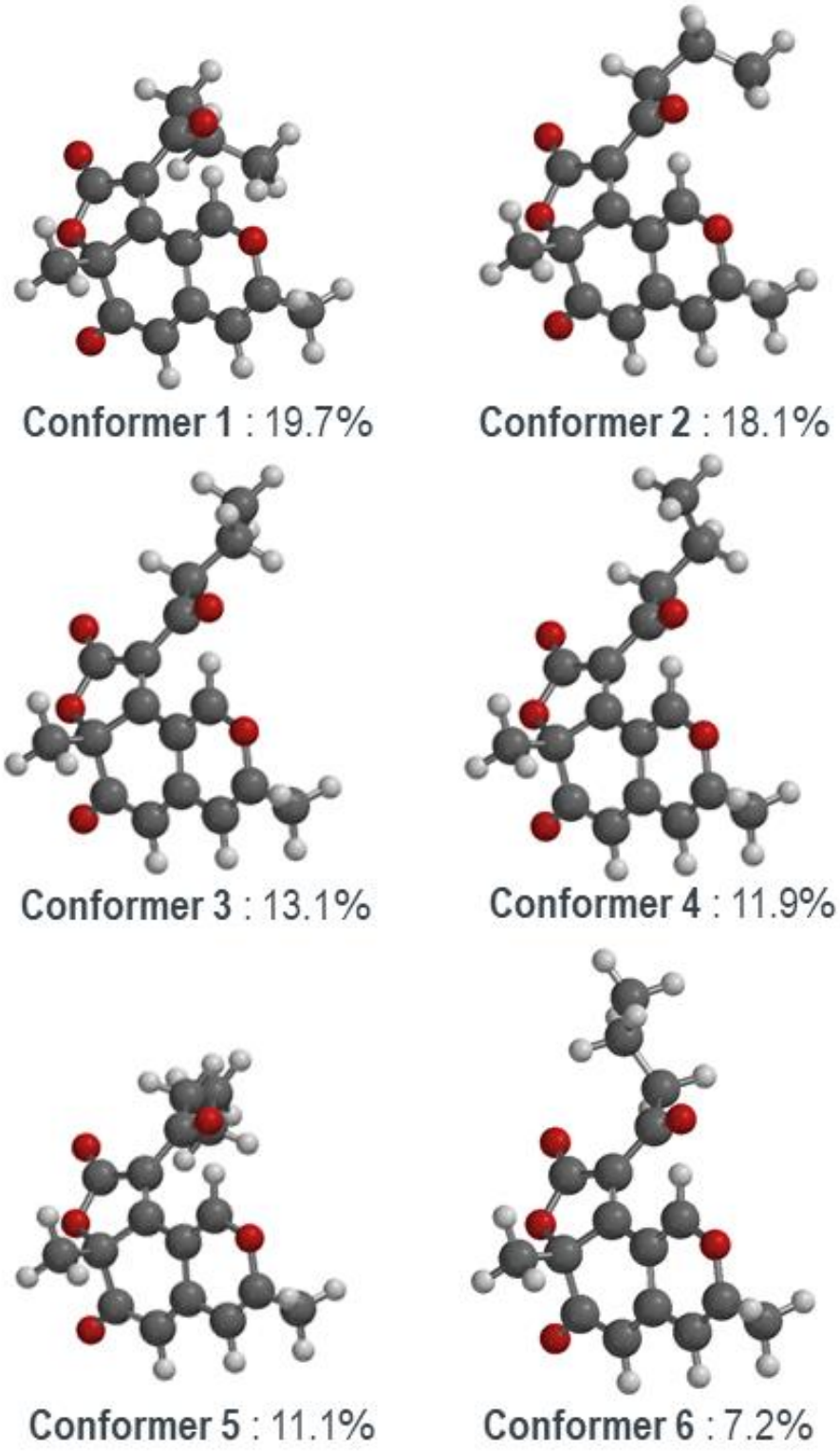



**Figure S27.** Six conformers of the (*R*)-enantiomer of truncated deflectin-1a that contribute >5% to the Boltzmann distribution. Note the percentage shown above based is on *in vacuo* electronic energies.

**Coordinates and electronic energies for B3LYP/6-31G** conformational minima contributing >5% to the *in vacuo* Boltzmann distribution.**

Conformer 1: -1033.613962 hartrees

| | | | |
|---|---|---|---|
| 6 | 1.12532 | -0.04345 | -0.39212 |
| 6 | 1.99275 | 0.732157 | 0.506105 |
| 6 | 3.30798 | 0.164545 | 0.702653 |
| 6 | 3.70807 | -0.95125 | 0.058724 |
| 8 | 2.8718 | -1.61446 | -0.80807 |
| 6 | 1.61067 | -1.16264 | -0.98929 |
| 6 | -0.24074 | 0.436799 | -0.53748 |
| 6 | -0.45225 | 1.9203 | -0.3767 |
| 6 | 0.276203 | 2.47925 | 0.879938 |
| 6 | 1.57228 | 1.87365 | 1.13487 |
| 6 | 5.0355 | -1.62273 | 0.154105 |
| 8 | -0.18568 | 3.42614 | 1.49476 |
| 6 | -1.45471 | -0.16625 | -0.63897 |
| 6 | -2.49589 | 0.888061 | -0.45779 |
| 8 | -1.86713 | 2.0917 | -0.28838 |
| 8 | -3.69893 | 0.782672 | -0.43922 |
| 6 | -1.75682 | -1.61382 | -0.78063 |
| 8 | -0.96298 | -2.36216 | -1.34426 |

Conformer 2: -1033.613885 hartrees

| | | | |
|---|---|---|---|
| 6 | 1.12455 | -0.26641 | 0.307283 |
| 6 | 2.26771 | 0.221135 | -0.47835 |
| 6 | 3.34533 | -0.7326 | -0.62018 |
| 6 | 3.31263 | -1.94174 | -0.02342 |
| 8 | 2.23687 | -2.33275 | 0.738887 |
| 6 | 1.17513 | -1.50542 | 0.861629 |
| 6 | 0.02309 | 0.624155 | 0.393856 |
| 6 | 0.26797 | 2.1012 | 0.310862 |
| 6 | 1.23803 | 2.44194 | -0.8567 |
| 6 | -3.0367 | -2.1407 | -0.16933 |
| 6 | -3.06518 | -1.968 | 1.36533 |
| 6 | -1.88548 | -2.64397 | 2.07088 |
| 6 | 0.047576 | 2.71521 | -1.59817 |
| 1 | 4.91526 | -2.65373 | 0.503621 |
| 1 | 5.69066 | -1.08748 | 0.843001 |
| 1 | 5.51202 | -1.66536 | -0.83119 |
| 1 | -1.96232 | -2.53676 | 3.1572 |
| 1 | -1.84539 | -3.71359 | 1.8375 |
| 1 | -0.92928 | -2.20641 | 1.76156 |
| 1 | -0.18359 | 3.77097 | -1.44072 |
| 1 | -0.46423 | 2.36183 | -2.49698 |
| 1 | 1.12617 | 2.59644 | -1.7238 |
| 1 | -3.10279 | -3.19773 | -0.44239 |
| 1 | -3.88139 | -1.59557 | -0.60229 |
| 1 | -4.00926 | -2.38602 | 1.73215 |
| 1 | -3.09299 | -0.90045 | 1.60707 |
| 1 | 4.00284 | 0.655146 | 1.37472 |
| 1 | 1.02743 | -1.78942 | -1.64834 |
| 1 | 2.20866 | 2.37403 | 1.8575 |
| 6 | 2.28943 | 1.45998 | -1.06108 |
| 6 | 4.35993 | -3.00144 | -0.06963 |
| 8 | 1.15626 | 3.51001 | -1.44011 |
| 6 | 1.37198 | 0.449204 | 0.391607 |
| 6 | 1.99964 | 1.79257 | 0.218404 |
| 8 | 1.00322 | 2.72868 | 0.145072 |
| 8 | 3.16773 | 2.08818 | 0.142914 |
| 6 | 2.14281 | -0.81834 | 0.425308 |
| 8 | 1.66299 | -1.82714 | 0.93665 |
| 6 | 3.53106 | -0.82161 | -0.18453 |
| 6 | 4.14405 | -2.21836 | -0.30714 |
| 6 | 3.46052 | -3.08584 | -1.36975 |
| 6 | 0.89721 | 2.64077 | 1.61047 |
| 1 | 3.94811 | -3.92427 | -0.492 |

| | | | |
|---|---|---|---|
| 1 | -5.20818 | -2.68012 | -0.67588 |
| 1 | -4.71146 | -3.23342 | 0.941464 |
| 1 | 3.94002 | -4.06679 | -1.44702 |
| 1 | 2.40785 | -3.24503 | -1.12034 |
| 1 | 3.51058 | -2.61139 | -2.35686 |
| 1 | -1.0324 | 3.71945 | 1.5056 |
| 1 | -0.22707 | 2.43922 | 2.4501 |
| 1 | -1.86575 | 2.17166 | 1.79764 |

Conformer 3: -1033.613578 hartrees

| | | | |
|---|---|---|---|
| 6 | -1.23669 | 0.367215 | -0.25506 |
| 6 | -2.39903 | -0.12519 | 0.498848 |
| 6 | -3.45192 | 0.849675 | 0.677926 |
| 6 | -3.38244 | 2.08379 | 0.13827 |
| 8 | -2.29045 | 2.48058 | -0.59741 |
| 6 | -1.2499 | 1.63178 | -0.7509 |
| 6 | -0.11296 | -0.54982 | -0.37714 |
| 6 | -0.44715 | -2.02001 | -0.36658 |
| 6 | -1.4339 | -2.38875 | 0.778195 |
| 6 | -2.45933 | -1.38886 | 1.02254 |
| 6 | -4.40172 | 3.16789 | 0.226283 |
| 8 | -1.38534 | -3.48506 | 1.31077 |
| 6 | 1.24071 | -0.41422 | -0.36279 |
| 6 | 1.82764 | -1.78285 | -0.25038 |
| 8 | 0.804028 | -2.69148 | -0.22508 |
| 8 | 2.98556 | -2.11847 | -0.18343 |
| 6 | 2.04843 | 0.831619 | -0.33746 |
| 8 | 1.59408 | 1.88107 | -0.78567 |

Conformer 4: -1033.613481 hartrees

| | | | |
|---|---|---|---|
| 6 | -1.16263 | -0.38101 | 0.220523 |
| 6 | -2.40001 | 0.133099 | -0.38523 |
| 6 | -3.44651 | -0.85331 | -0.53626 |
| 6 | -3.30464 | -2.1192 | -0.09307 |
| 8 | -2.1423 | -2.53751 | 0.51102 |
| 1 | -4.14471 | -0.15612 | 0.43636 |
| 1 | -3.49183 | -0.31005 | -1.15492 |
| 1 | -4.08734 | -2.71781 | 0.665863 |
| 1 | -5.20723 | -2.10655 | -0.54866 |
| 1 | -4.21514 | -0.46655 | -1.20995 |
| 1 | -0.36813 | -1.93743 | 1.43516 |
| 1 | -3.11123 | 1.75434 | -1.70571 |
| 6 | 3.43612 | 0.767733 | 0.264145 |
| 6 | 4.12596 | 2.12836 | 0.346454 |
| 6 | 5.53042 | 2.02448 | 0.946302 |
| 6 | -1.08342 | -2.47786 | -1.69396 |
| 1 | -3.96898 | 4.05911 | 0.693254 |
| 1 | -5.26285 | 2.84194 | 0.811555 |
| 1 | -4.73918 | 3.45517 | -0.77537 |
| 1 | 6.01292 | 3.00558 | 0.997751 |
| 1 | 5.49985 | 1.6137 | 1.96193 |
| 1 | 6.17009 | 1.36781 | 0.346005 |
| 1 | -1.25056 | -3.55592 | -1.64194 |
| 1 | -0.40215 | -2.25596 | -2.5194 |
| 1 | -2.03663 | -1.97206 | -1.86332 |
| 1 | 4.01855 | 0.049544 | -0.32738 |
| 1 | 3.36471 | 0.290609 | 1.25149 |
| 1 | 3.50852 | 2.81195 | 0.940619 |
| 1 | 4.17124 | 2.56697 | -0.65634 |
| 1 | -4.33325 | 0.579987 | 1.24865 |
| 1 | -0.42639 | 2.06877 | -1.29639 |
| 1 | -3.29392 | -1.69019 | 1.64721 |
| 6 | -1.10736 | -1.67595 | 0.626863 |
| 6 | -0.04957 | 0.55392 | 0.301997 |
| 6 | -0.41246 | 2.01128 | 0.432348 |
| 6 | -1.51029 | 2.43529 | -0.58532 |
| 6 | -2.53386 | 1.42861 | -0.80765 |
| 6 | -4.30737 | -3.21985 | -0.16391 |

| | | | |
|---|---|---|---|
| 8 | 1.53384 | 3.56825 | -1.03667 |
| 6 | 1.29868 | 0.455493 | 0.146455 |
| 6 | 1.84474 | 1.84502 | 0.081729 |
| 8 | 0.80578 | 2.72429 | 0.224366 |
| 8 | 2.98285 | 2.21887 | -0.06994 |
| 6 | 2.11868 | -0.76834 | -0.04675 |
| 8 | 1.72595 | -1.85224 | 0.377492 |
| 6 | 3.42359 | -0.63768 | -0.80651 |
| 6 | 4.20384 | -1.94904 | -0.92925 |
| 6 | 4.84226 | -2.39582 | 0.390592 |
| 6 | 0.92807 | 2.35529 | 1.84364 |
| 1 | 3.90327 | -4.06598 | -0.72992 |
| 1 | 5.22487 | -2.87623 | -0.64395 |
| 1 | 4.54526 | -3.58327 | 0.841596 |
| 1 | 5.41245 | -3.32099 | 0.258882 |
| 1 | 5.52894 | -1.63143 | 0.772205 |
| 1 | 4.07651 | -2.57643 | 1.14925 |
| 1 | 1.12083 | 3.42941 | 1.88756 |
| 1 | 0.16683 | 2.09296 | 2.58284 |
| 1 | -1.8505 | 1.81319 | 2.06358 |
| 1 | 4.01663 | 0.149602 | -0.32628 |
| 1 | 3.17871 | -0.22008 | -1.79379 |
| 1 | 4.98355 | -1.81297 | -1.68758 |
| 1 | 3.53375 | -2.73376 | -1.29737 |
| 1 | 4.38175 | -0.5671 | -1.00381 |
| 1 | 0.22974 | -2.12859 | 1.06416 |
| 1 | 3.42927 | 1.75158 | -1.32867 |

Conformer 5: -1033.613417 hartrees

| | | | |
|---|---|---|---|
| 6 | -1.28767 | 0.218168 | -0.29924 |
| 6 | -2.21665 | -0.5007 | 0.585253 |
| 6 | -3.45445 | 0.199085 | 0.84786 |
| 6 | -3.74166 | 1.38401 | 0.271162 |
| 8 | -2.8556 | 1.99399 | -0.58535 |
| 6 | -1.65796 | 1.41507 | -0.82441 |
| 6 | 0.010014 | -0.40554 | -0.5129 |
| 6 | 0.047735 | -1.9102 | -0.42909 |
| 6 | -0.70447 | -2.44705 | 0.822674 |
| 6 | -1.91543 | -1.71289 | 1.14633 |
| 6 | -4.98261 | 2.19305 | 0.436761 |
| 8 | -0.33731 | -3.47014 | 1.37615 |
| 6 | 1.28483 | 0.053474 | -0.64059 |
| 6 | 2.19597 | -1.1267 | -0.54633 |
| 8 | 1.43292 | -2.25264 | -0.40061 |
| 8 | 3.40297 | -1.17042 | -0.57187 |
| 6 | 1.75903 | 1.45745 | -0.75213 |
| 8 | 1.01665 | 2.33155 | -1.19275 |
| 6 | 3.16063 | 1.78466 | -0.28827 |
| 6 | 3.34581 | 1.5836 | 1.23018 |
| 6 | 4.76986 | 1.9244 | 1.67516 |
| 6 | -0.57977 | -2.57506 | -1.67001 |
| 1 | -4.7392 | 3.18535 | 0.831304 |
| 1 | -5.6774 | 1.69967 | 1.11806 |
| 1 | -5.47437 | 2.33802 | -0.53116 |
| 1 | 4.88825 | 1.7911 | 2.75506 |
| 1 | 5.49623 | 1.2759 | 1.17413 |
| 1 | 5.02313 | 2.96293 | 1.4345 |
| 1 | -0.4718 | -3.6575 | -1.57239 |
| 1 | -0.05535 | -2.23779 | -2.56775 |
| 1 | -1.63953 | -2.32352 | -1.75203 |
| 1 | 3.35205 | 2.82513 | -0.56546 |
| 1 | 3.86638 | 1.13206 | -0.81272 |
| 1 | 3.12583 | 0.542541 | 1.48743 |
| 1 | 2.62291 | 2.2093 | 1.76876 |
| 1 | -4.18405 | -0.24573 | 1.51496 |
| 1 | -1.02206 | 2.00861 | -1.46489 |
| 1 | -2.58722 | -2.17362 | 1.86324 |

Conformer 6: -1033.613007 hartrees

| | | | |
|---|---|---|---|
| 6 | -1.33004 | -0.33625 | 0.191808 |
| 6 | -2.46505 | 0.40933 | -0.37277 |
| 6 | -3.67628 | -0.36659 | -0.52076 |
| 6 | -3.75735 | -1.64969 | -0.11357 |
| 8 | -2.67727 | -2.28908 | 0.448706 |
| 6 | -1.50175 | -1.63261 | 0.561122 |
| 6 | -0.06485 | 0.378031 | 0.271432 |
| 6 | -0.15474 | 1.8741 | 0.43505 |

| | | | |
|---|---|---|---|
| 6 | -1.17503 | 2.515 | -0.54881 |
| 6 | -2.36977 | 1.71762 | -0.7651 |
| 6 | -4.94413 | -2.5486 | -0.1861 |
| 8 | -0.99925 | 3.64314 | -0.97797 |
| 6 | 1.24305 | 0.040367 | 0.096574 |
| 6 | 2.0288 | 1.30911 | 0.044704 |
| 8 | 1.16892 | 2.35948 | 0.216565 |
| 8 | 3.21456 | 1.47291 | -0.11853 |
| 6 | 1.83025 | -1.30289 | -0.13255 |
| 8 | 1.2409 | -2.31551 | 0.238784 |
| 6 | 3.18478 | -1.41488 | -0.79951 |
| 6 | 4.30321 | -1.49201 | 0.263832 |
| 6 | 5.68656 | -1.61045 | -0.37837 |
| 6 | -0.57534 | 2.27283 | 1.86396 |
| 1 | -4.71207 | -3.43639 | -0.78408 |
| 1 | -5.79421 | -2.0308 | -0.63283 |
| 1 | -5.22243 | -2.89313 | 0.815677 |
| 1 | 6.46885 | -1.68168 | 0.384072 |
| 1 | 5.75537 | -2.49987 | -1.01506 |
| 1 | 5.90155 | -0.73386 | -0.99846 |
| 1 | -0.5682 | 3.36281 | 1.93305 |
| 1 | 0.138091 | 1.85931 | 2.58141 |
| 1 | -1.57714 | 1.90262 | 2.09278 |
| 1 | 3.37509 | -0.5662 | -1.46007 |
| 1 | 3.1723 | -2.33988 | -1.38505 |
| 1 | 4.10728 | -2.35242 | 0.914763 |
| 1 | 4.26382 | -0.59047 | 0.882688 |
| 1 | -4.55345 | 0.097571 | -0.95702 |
| 1 | -0.7104 | -2.24947 | 0.96088 |
| 1 | -3.20125 | 2.20988 | -1.25887 |

## Part XIII. Bibliography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