Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Enantio- and regioselective *ene* reductions using $F_{420}H_2$ dependent enzymes

Sam Mathew[+], Milos Trajkovic[+], Hemant Kumar, Quoc-Thai Nguyen, Marco W. Fraaije*

Sam Mathew[+], Milos Trajkovic[+], Hemant Kumar, Quoc-Thai Nguyen, Marco W. Fraaije. Molecular Enzymology Group, University of Groningen, Nijenborgh 4, 9747 AG Groningen (The Netherlands)

Quoc-Thai Nguyen. Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City, 41 Dinh Tien Hoang Street, Ben Nghe Ward, District 1, Ho Chi Minh City (Vietnam)

Contents

Codon optimized nucleotide sequence of FDR from *Mycobacterium hassiacum* (FDR-Mha)

5′-
ATGGATCCGAAAAATAAACCGGCTCAACTGAACTCCCCGTGGGTCTCCAAAATCATGAAATATGGTGG
CAAAGCACACGTCGCAGTCTATCGTCTGACCGGCGGTCGCATTGGCAGTAAATGGCGTATCGGCGCTG
GTTTTAAAAAACCGGTTCCGACGCTGCTGCTGGAACATGTGGGCCGTAAAAGCGGTAAACGCTTCGTC
ACCCCGCTGGTGTATATTACGGATGGCCCGGACATCGTGGTTGTCGCATCTCAGGGCGGTCGTGATGAC
CACCCGCAATGGTATCGCAACCTGGTTGCCAATCCGGAAGCATACGTCGAAATTGGTCGTGAACGTCG
CGCAGTGCGTGCTGTTACCGCAGATCCGGAAGAACGTGCCCGCCTGTGGCCGAAACTGGTTGATGCGT
ACGCCGACTTTGACACCTACCAATCGTGGGCGAATCGTGAAATCCCGGTCGTTATCCTGCAGCCGCGTA
A-3′

Primers used for amplifying FDR-Rh1 (ro04677) and FDR-Rh2 (ro05392) genes from *Rhodococcus* RHA1 genomic DNA

	Sequence (5′→3′)	Template strand
FDR-Rh1 (RHA1_ro04677)	Reverse primer	**CTATCGGGGAGTGCAGACCA**
	Forward primer	**ATGAATGCACCCGCACCC**
FDR-Rh2 (RHA1_ro05392)	Reverse primer	**TCAGGTCGTGGGGTCGAG**
	Forward primer	**ATGCCGACGGACCGC**

Rh1: RHA1_ro04677
>NC_008268.1:c4929566-4929081 Rhodococcus jostii RHA1
5′-
ATGAATGCACCCGCACCCGCCCGACCGCCCGGCCTCGACTCGAAGTGGACGGTCTCCTTCATCAAGTG
GATGTCGAAGATCAACGTCGTGCTCTACCGGCGGACGGGCGGGCGCCTGGGCAGCAAGTGGCGGGTG
GGCAGCGCCTTCCCCCGCGGGTTGCCCGTCTGCCTGCTCACCACCACGGGACGGAAGAGCGGCGAGCC
GCGGATCAGCCCGCTGCTGTTCCTCGAGGACGGCGACCGCATCATCCTCGTCGCCTCGCAGGGCGGCCT
CCCGAAGCACCCGATGTGGTACCTCAACCTGCGCGCGAACCCCGACGTGACCGTCCAGGTGAAGTCGC
GGGTCCGGCCGATGACCGCCCACGTGGCGGACCCCGAGGAACGCGCGCGCCTGTGGCCGCGGCTCGTC
GCCATGTATCCGGATTTCGACAACTACCAGGCCTGGACCGACCGCACGATCCCCGTCGTGGTCTGCACT
CCCCGATAG-3′

Rh2: RHA1_ro05392
>NC_008268.1:c5741722-5741291 Rhodococcus jostii RHA1
5′-
ATGCCGACGGACCGCGGACTCAAGTTCATGAACGCCGCCCACCGCGCCCTCCTGCGCGTGACCGGCGG
GCGGGTGGGGCGGAGTTTCGGCAAGATGCCGGTGGTGGAGTTGACCACCGTCGGCCGCAGGACCGGG
AAGGTGCACAGCGTCATGCTGACCGTCCCGGTGAGGGAAGGCGACACGCTCGTCGTGGTGGCCTCACG
CGGCGGCGACGACCGCCACCCCGCGTGGTTCCTGAACCTGCAGGCCAACCCGGTGGTCCAACTGTCGC
TGCAGGGAAACCCCGCGCAGTCCATGCGCGCCCACGTGGCAACCCCGGAGGAGCGGGCCCGTCTGTGG
CCGAAAGTGACCGCCGCCTACAAGGGGTATGCCGGCTACCAGAAGAAAACGGACCGCGAGATCCCTCT
GGTCCTGCTCGACCCCACGACCTGA-3′

Cloning, expression and purification of FDRs

Rhodococcus jostii RHA1 was grown in lysogeny broth (LB) at 30 °C; subsequently its genomic DNA was extracted using the GenElute Bacterial Genomic DNA kit from Sigma. Two putative FDR genes (RHA1_ro04677 and RHA1_ro05392) were amplified from extracted the genomic DNA using Phusion High-Fidelity DNA polymerase (Thermo Scientific) along with the primers listed in table (page 3). The purified PCR products (100–200 ng) were treated with 0.5 U Taq polymerase (Roche) and 0.75 mM dATP by incubation at 72 °C for 15 min. The resulting insert DNA fragments were ligated into the pET-SUMO vector.

The plasmid was then introduced into the chemically competent *Escherichia coli* (BL21) cells using Hanahan method and the transformants were grown at 37 °C in 1 L of Terrific broth (TB) containing 50 μg/ml of kanamycin. When the OD_{600} reached 0.5–0.6, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM. After 48 h of induction at 24 °C, the over-expressed cells were centrifuged at 4000 rpm for 20 min at 4 °C. Cells were resuspended in lysis buffer [50 mM KPi pH 8.0, 400 mM NaCl, 100 mM KCl, 20% (v/v) glycerol, 1.0 mM β-mercaptoethanol, 0.5 mM phenylmethylsylfonyl fluoride (PMSF)] and disrupted by sonication using a VCX130 Vibra-Cell at 4 °C (5 s on, 5 s off, 70% amplitude, total of 15 min). The sonicated cells were then centrifuged at 39742 × g for 30 min. The N-terminal His6-tagged fusion protein was purified at 4 °C on a Ni-NTA agarose resin obtained from Qiagen (Hilden, Germany). Briefly, the crude extract was passed directly over a column containing 5 ml of Ni-NTA agarose resin. The column was then washed with KPi buffer (pH 8.0) containing 50 mM imidazole and the N-terminal His6-tagged protein was eluted with phosphate buffer (pH 8.0) containing 500 mM imidazole. The eluted solution containing purified protein was concentrated using an Amicon PM-10 ultrafiltration unit and then desalted against 50 mM Tris/HCl buffer (pH 8.0) containing 20% (v/v) glycerol and 1.0 mM β-mercaptoethanol. The desalted protein was stored at -20 °C for further studies. The concentration of purified enzyme was determined by using the Bradford assay.

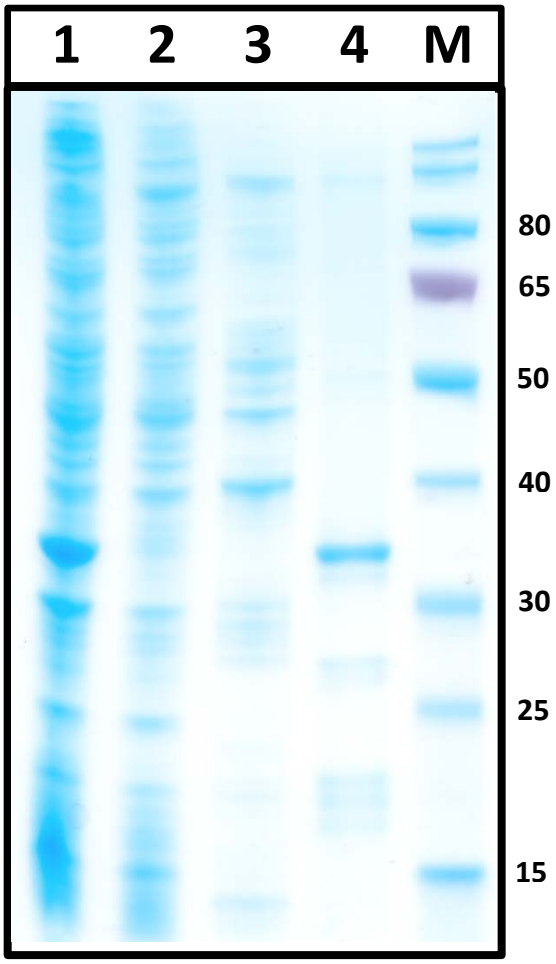


Fig S1 SDS-PAGE gel of SUMO-Rh1 FDR

1. Crude free extract
2. Flow through
3. Wash fraction (50 mM imidazole)
4. Elution fraction (500 mM imidazole)

M Marker

Yield: 25 mg/L

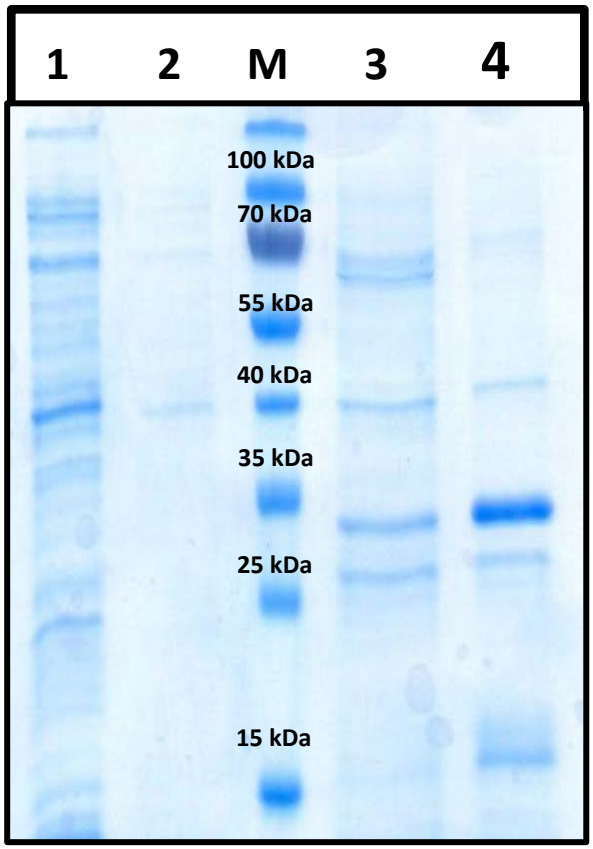


Fig S2 SDS-PAGE gel of SUMO-Rh2 FDR

1. Flow through
2. Wash fraction (buffer)
3. Wash fraction (50 mM imidazole)
4. Elution fraction (500 mM imidazole)

M Marker

Yield: 18 mg/L

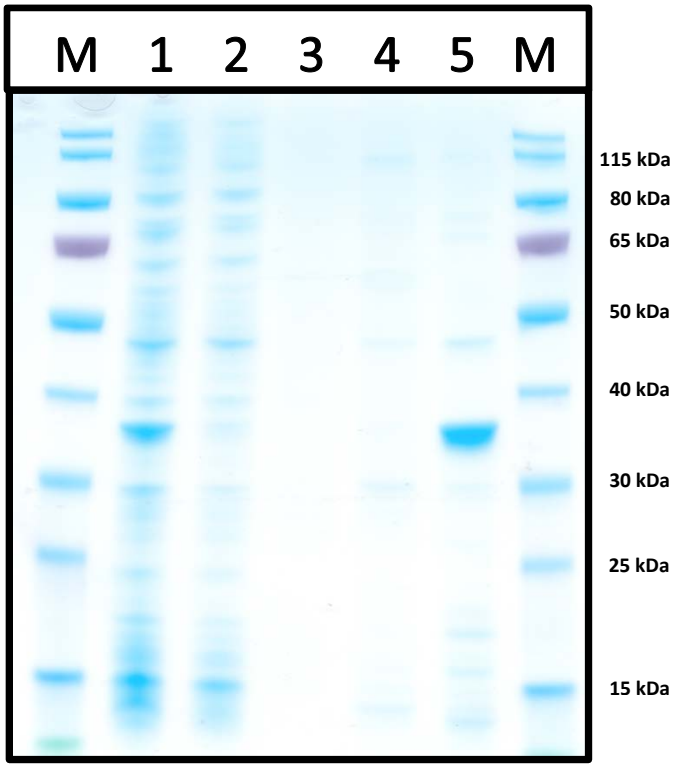


Fig S3 SDS-PAGE gel of SUMO-Mha FDR

1. Crude free extract
2. Flow through
3. Wash fraction (5 mM imidazole)
4. Wash fraction (50 mM imidazole)
5. Elution fraction (500 mM imidazole)

M Marker

Yield: 45 mg/L

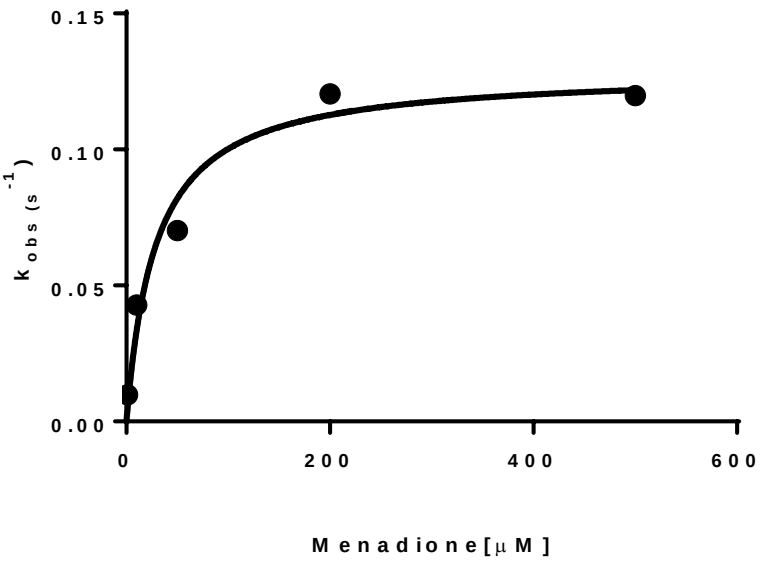


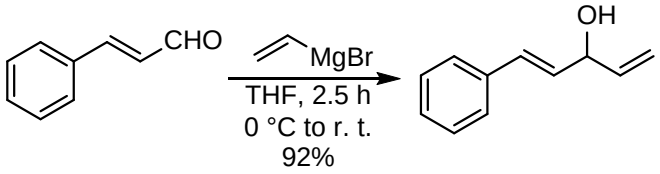
Fig. S4. Kinetic profile for FDR-Rh1-SUMO. F_{420} oxidation rates were measured in degassed 50 mM Tris/HCl buffer at pH 8.0 and 25 °C (0.1 μM enzyme, 20 μM $F_{420}H_2$, 5-100 μM menadione). Reduced F_{420} ($F_{420}H_2$) was prepared using a recombinant FGD1-RhA1[1] with SUMO-tag as described earlier, followed by heat inactivation of FGD1 at 55° C water bath for 15 minutes and ultra-filtration (Centricon 10 kDa). Steady-state kinetic parameters for FDR-Rh1 was determined by monitoring the oxidation of $F_{420}H_2$ at 400 nm using an absorption coefficient ε400 nm of 25.7 mM^{-1} cm^{-1} in a V-650 spectrophotometer from Jasco (IJsselstein, The Netherlands).[2] Kinetic data were analyzed using nonlinear regression to the Michealis–Menten equation using GraphPad Prism v. 6.0 (GraphPad Software Inc., La Jolla, CA, USA).

Synthesis of substrates and products

General. The F_{420} cofactor and FGD-Rha1 were supplied by GECCO-biotech. Commercially available compounds were purchased from Sigma–Aldrich, Acros Organics and Fluorochem, and used without further purification. All other solvents were obtained as analytical grade and used without further purification. Flash chromatography was performed with Silicycle silica gel SiliaFlash P60. NMR spectra were recorded on an Agilent 400-MR spectrometer (^{1}H and ^{13}C resonances at 400 MHz and 100 MHz, respectively). Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are reported in hertz (Hz). The residual ^{1}H signals from solvent were used as references.

Synthesis of (2*E*,4*E*)-5-phenylpenta-2,4-dienal (3S)[3]

A. (*E*)-1-phenylpenta-1,4-dien-3-ol

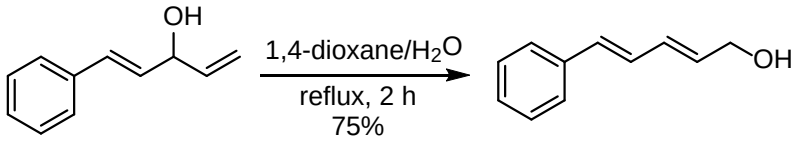


To a solution of cinnamaldehyde (2.35 g, 17.8 mmol) in anhydrous THF (55 mL) was added vinylmagnesium bromide (1.0 M in THF, 20.9 mL, 20.9 mmol) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and 2 h at room temperature, after which it was cooled down to 0 °C and then quenched with 10% aqueous NH_4Cl solution. The reaction mixture was extracted with EtOAc (3 × 120 mL). The combined organic layers were dried over $MgSO_4$, filtered and solvents evaporated under reduced pressure. The residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 8:2) to afford the desired product (2.62 g, 92%) as pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.39 (m, 2H), 7.38–7.32 (m, 2H), 7.32–7.25 (m, 1H), 6.65 (dd, *J* = 16.1, 1.3 Hz, 1H), 6.27 (dd, *J* = 15.9, 6.5 Hz, 1H), 6.01 (ddd, *J* = 17.2, 10.4, 5.9 Hz, 1H), 5.38 (dt, *J* = 17.2, 1.4 Hz, 1H), 5.23 (dt, *J* = 10.3, 1.3 Hz, 1H), 4.85 (t, *J* = 6.3 Hz, 1H), 1.84 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 139.4 (CH), 136.7 (C), 131.0 (CH), 130.5 (CH), 128.7 (2xCH), 127.9 (CH), 126.7 (2xCH), 115.6 (CH_2), 74.0 (CH). The characterization data for this compound matched that of a previous report.

B. (2*E*,4*E*)-5-phenylpenta-2,4-dien-1-ol

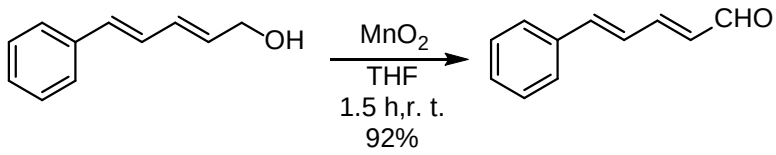


To a solution of (*E*)-1-phenylpenta-1,4-dien-3-ol (2.62 g, 16.4 mmol) in 1,4-dioxane (40 mL) was added H_2O (370 mL). Reaction mixture was stirred vigorously to reflux under N_2 atmosphere for 2 h. After that reaction mixture was cooled to room temperature and brine was added (100 mL). The mixture was extracted with EtOAc (3 × 300 mL), washed with brine, dried over $MgSO_4$, filtered and solvents evaporated under reduced pressure. The residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 2:1) to afford the desired product (1.98 g, 75%) as white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.28–7.20 (m, 1H), 6.80 (dd, *J* = 15.6, 10.5 Hz, 1H), 6.56 (d, *J* = 15.7 Hz, 1H), 6.43 (dd, *J* = 15.2, 10.4 Hz, 1H), 5.97 (dt, *J* = 15.2, 5.8 Hz, 1H), 4.25 (d, *J* = 5.9 Hz, 2H), 1.81 (br s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2 (C), 132.8 (CH), 132.6 (CH), 131.7 (CH), 128.7 (2xCH), 128.3 (CH), 127.7 (CH), 126.5 (2xCH), 63.5 (CH_2). The characterization data for this compound matched that of a previous report.

C. (2*E*,4*E*)-5-phenylpenta-2,4-dienal (3S)



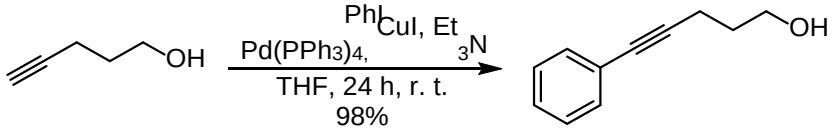
To a solution of the (2*E*,4*E*)-5-phenylpenta-2,4-dien-1-ol (601 mg, 3.8 mmol) in THF (36 mL) was added MnO_2 (3.24 g, 37.3 mmol). The reaction mixture was stirred for 1.5 h and then filtered through a pad of celite to remove inorganic compounds. The filtrate was concentrated and the residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 4:1) to afford the aldehyde **3S** (546.0 mg, 92%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (d, *J* = 7.9 Hz, 1H), 7.54–7.47 (m, 2H), 7.42–7.33 (m, 3H), 7.29–7.21 (m, 1H), 7.04–6.97 (m, 2H), 6.26 (dd, *J* = 15.2, 7.9 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 193.6 (CH), 152.1 (CH), 142.5 (CH), 135.7 (C), 131.7 (CH), 129.8 (CH), 129.0 (2xCH), 127.6 (2xCH), 126.3 (CH). The characterization data for this compound matched that of a previous report.

Synthesis of (*E*)-5-phenylpenta-4-enal (3P)

A. 5-phenylpent-4-yn-1-ol[4]

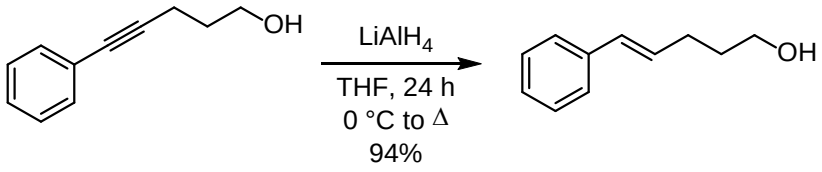


$Pd(PPh_3)_4$ (123 mg, 0.11 mmol, 1 mol %) was dissolved in anhydrous THF (5 mL) under N_2 atmosphere. 4-Pentyn-1-ol (1.0 mL, 10.8 mmol), iodobenzene (2.38 mL, 21.3 mmol), triethylamine (28.6 mL, 205.2 mmol) and CuI (40.5 mg, 0.21 mmol, 2 mol %) were added sequentially and the reaction mixture was stirred at room temperature for 24 h. After that reaction mixture was filtered through a pad of celite and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 2:1) to afford the desired product (1.68 g, 98%) as orange oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.36 (m, 2H), 7.31–7.24 (m, 3H), 3.81 (t, *J* = 6.2 Hz, 2H), 2.54 (t, *J* = 6.9 Hz, 2H), 1.85 (quint., *J* = 6.6 Hz, 2H), 1.75 (br s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 131.6 (2xCH), 128.3 (2xCH), 127.8 (CH), 123.8 (C), 89.5 (C), 81.2 (C), 61.9 (CH_2), 31.5 (CH_2), 16.1 (CH_2). The characterization data for this compound matched that of a previous report.

B. (*E*)-5-phenylpent-4-en-1-ol[4]



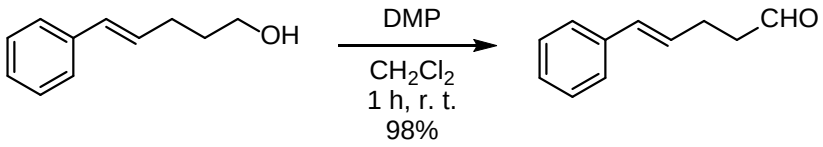
To a suspension of $LiAlH_4$ (2.00 g, 52.7 mmol) in anhydrous THF (9 mL) at 0 °C was added dropwise a solution of 5-phenylpent-4yn-1-ol (1.60, 10.0 mmol) in anhydrous THF (9 mL). The reaction mixture was stirred at 0 °C for 20 min, and then refluxed for 24 h. After that reaction mixture was cooled to 0 °C, and quenched by the sequential addition of water (2.0 mL), a 15% aqueous solution of NaOH (2.0 mL) and additional water (6.0 mL). The resulting white

precipitate was filtered through a pad of celite and washed with Et_2O. The filtrate was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 2:1) to afford the desired product (1.53 g, 94%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.28 (m, 4H), 7.25–7.18 (m, 1H), 6.44 (d, *J* = 15.7 Hz, 1H), 6.24 (dt, *J* = 15.8, 6.9 Hz, 1H), 3.71 (t, *J* = 6.5 Hz, 2H), 2.32 (qd, *J* = 7.1, 1.4 Hz, 2H), 1.77 (quint., *J* = 6.9 Hz, 2H), 1.71 (br s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7 (C), 130.5 (CH), 130.2 (CH), 128.6 (2xCH), 127.0 (CH), 126.0 (2xCH), 62.4 (CH_2), 32.3 (CH_2), 29.4 (CH_2). The characterization data for this compound matched that of a previous report.

C. (*E*)-5-phenylpenta-4-enal (3P)

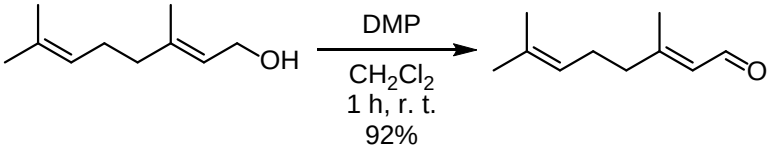


Dess-Martin periodinane (340.0 mg, 0.80 mmol) was added to solution of (*E*)-5-phenylpent-4-en-1-ol (100.0 mg, 0.62 mmol) in dichloromethane (12.3 mL) and the reaction mixture was stirred at room temperature for 1 h. After that reaction mixture was quenched with 10% aqueous solution of sodium thiosulfate (10.0 mL) and the organic layer washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 9:1) to afford the aldehyde **3P** (97.0 mg, 98%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (t, *J* = 1.5 Hz, 1H), 7.37 – 7.28 (m, 4H), 7.25 – 7.19 (m, 1H), 6.44 (d, *J* = 15.8 Hz, 1H), 6.21 (dt, *J* = 15.8, 6.6 Hz, 1H), 2.66 – 2.60 (m, 2H), 2.59 – 2.52 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 201.8 (CH), 137.3 (C), 131.2 (CH), 128.6 (2xCH), 128.2 (CH), 127.3 (CH), 126.1 (2xCH), 43.4 (CH_2), 25.6 (CH_2). The characterization data for this compound matched that of a previous report.[5]

Synthesis of geranial (4P)

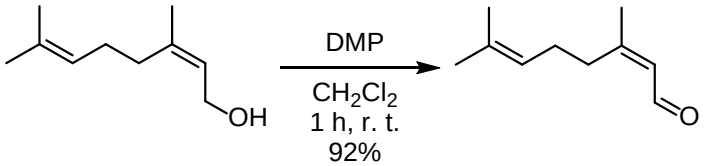


Dess-Martin periodinane (195.0 mg, 0.46 mmol) was added to solution of geraniol (50 μL, 0.28 mmol) in dichloromethane (2.0 mL) and the reaction mixture was stirred at room temperature for 1 h. After that reaction mixture was quenched with 10% aqueous solution of sodium thiosulfate (2.0 mL) and the organic layer washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 95:5) to afford the geranial **4P** (39.8 mg, 92%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (d, *J* = 8.0 Hz, 1H), 5.88 (dd, *J* = 8.2, 1.3 Hz, 1H), 5.11–5.03 (m, 1H), 2.27–2.17 (m, 4H), 2.17 (d, *J* = 1.3 Hz, 3H), 1.69 (s, 3H), 1.61 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 191.5 (CH), 164.0 (C), 133.1 (C), 127.6 (CH), 122.7 (CH), 40.8 (CH_2), 25.9 (CH_2), 25.8 (CH_3), 17.9 (CH_3), 17.8 (CH_3). The characterization data for this compound matched that of a previous report.[6]

Synthesis of neral (5P)



Dess-Martin periodinane (193.0 mg, 0.45 mmol) was added to solution of geraniol (50 μL, 0.28 mmol) in dichloromethane (2.0 mL) and the reaction mixture was stirred at room temperature for 1 h. After that reaction mixture was quenched with 10% aqueous solution of sodium thiosulfate (2.0 mL) and the organic layer washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash

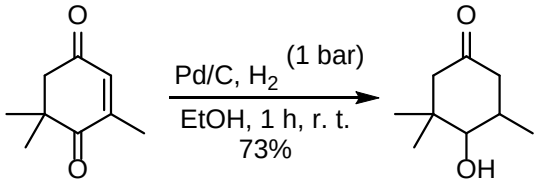
chromatography (SiO_2, pentane/ethyl acetate = 95:5) to afford the neral **5P** (40.0 mg, 92%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (d, *J* = 8.2 Hz, 1H), 5.87 (d, *J* = 8.2 Hz, 1H), 5.14–5.07 (m, 1H), 2.58 (t, *J* = 7.5 Hz, 2H), 2.23 (q, *J* = 7.4 Hz, 2H), 1.98 (d, *J* = 1.3 Hz, 3H), 1.68 (s, 3H), 1.59 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 190.9 (CH), 163.9 (C), 133.9 (C), 128.8 (CH), 122.4 (CH), 32.8 (CH_2), 27.2 (CH_2), 25.8 (CH_3), 25.2 (CH_3), 17.9 (CH_3). The characterization data for this compound matched that of a previous report.[7]

Synthesis of 2,2,6-trimethylcyclohexane-1,4-dione (15P)

A. 4-hydroxy-3,3,5-trimethylcyclohexan-1-one

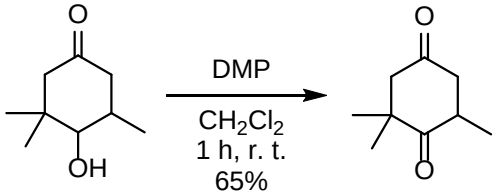


To suspension of ketoisophorone (21.9 mg, 0.14 mmol) and 10% palladium on carbon (8.8 mg, 0.01 mmol, 6 mol%) in ethanol (1.3 mL) was bubbled hydrogen at room temperature during 1 h. After that reaction mixture was filtered through a pad of celite and washed with ethanol. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 2:1) to afford the desired product (16.4 mg, 73%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.33 (s, 1H), 2.59 (d, *J* = 13.6 Hz, 1H), 2.35 (t, *J* = 13.3 Hz, 1H), 2.27–2.16 (m, 1H), 2.11–2.02 (m, 1H), 1.97 (br s, 1H), 1.88 (d, *J* = 13.6 Hz, 1H), 1.09 (s, 3H), 1.06 (d, *J* = 6.6 Hz, 3H), 0.87 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 214.8 (C), 79.8 (CH), 51.1 (CH_2), 45.7 (CH_2), 42.3 (C), 35.7 (CH), 29.8 (CH_3), 28.3 (CH_3), 20.9 (CH_3).

B. 2,2,6-trimethylcyclohexane-1,4-dione (11P)



Dess-Martin periodinane (92.0 mg, 0.22 mmol) was added to solution of 4-hydroxy-3,3,5-trimethylcyclohexan-1-one (27.7. mg, 0.18 mmol) in dichloromethane (2.1 mL) and the reaction mixture was stirred at room temperature for 1 h. After that reaction mixture was quenched with 10% aqueous solution of sodium thiosulfate (2.0 mL) and the organic layer washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, pentane/ethyl acetate = 2:1) to afford the desired product **15P** (17.9 mg, 65%) as colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06–2.91 (m, 1H), 2.79–2.74 (m, 1H), 2.74–2.71 (m, 1H), 2.51 (d, *J* = 15.4 Hz, 1H), 2.33 (dd, *J* = 17.7, 13.3 Hz, 1H), 1.20 (s, 3H), 1.14 (d, *J* = 6.6 Hz, 3H), 1.11 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 214.2 (C), 208.0 (C), 52.9 (CH_2), 45.0 (CH_2), 44.4 (C), 40.0 (CH), 26.7 (CH_3), 25.7 (CH_3), 14.7 (CH_3). The characterization data for this compound matched that of a previous report.[8]

Enzymatic reaction of substrates

A typical reaction mixture contained 400 µL of 50 mM Tris/HCl supplemented with 1 mM of substrate, 20 µM of F_{420}, 0.1 µM of FGD-Rha1[1], 10 mM glucose 6-phosphate, 25 µM SUMO-FDRs and DMSO (3% v/v). The reaction was performed in a closed 2 mL glass vial in the dark at 24 °C and 135 rpm.

Analysis of products

Substrates (**1**–**15**) were initially analyzed in HPLC to see the depletion of substrate at 240 nm. On the confirmation of complete depletion of substrates, the reaction mixture was extracted with equal volume of ethyl acetate containing 2 mM of mesitylene as an external standard. This mixture was then vortexed, centrifuged (13,000 rpm, 10 minutes), passed over anhydrous magnesium sulfate and finally analyzed using GC-MS QP_{2010} ultra (Shimadzu) with electron ionization and quadrupole separation. The column employed was a HP-1 (Agilent, 30 mx 0.25 mm x 0.25 µm) and the method used for the GC-MS is mentioned below.

Injection temp: 300 °C; Oven program: 40 °C for 2 min; 5 °C/min until 100 °C for 0 min; 10 °C/min until 250 °C for 10 mins.

3 µL was injected in to the GC and the split ratio was 5. The software to analyze chromatograms, MS spectra and to generate the figures was GCMSsolution Postrun Analysis 4.11 (Shimadzu). The library for the MS spectra was NIST11. The products were confirmed with commercial standards/synthesized products.

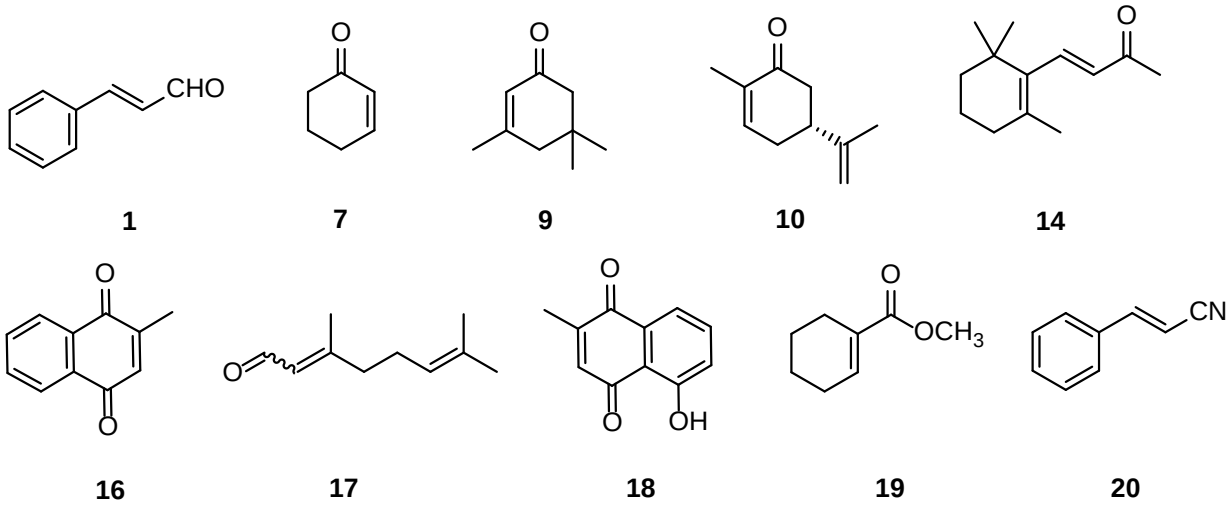


Fig. S5. Substrates used for the initial screening with crude extracts containing FDR-Mha, FDR-Rh1, and FDR-Rh2 respectively.

Table S1 Initial screening of substrates using FDR-Rh1, FDR-Rh2 and FDR-Mha whole cell reactions. Reaction conditions: 1.0 mM substrate, F_{420} (100 μM), G6P (10 mM), FGD-Rha1 (10 mg/mL), SUMO-FDRs (10 mg/mL), DMSO (10% v/v), Tris/HCl (50 mM, pH 8.0) at 25 °C for 18h. The degree of conversion was determined semi-quantitatively by analyzing GC peaks of substrates and products. The conversions are categorized as 100%, +++; >50%, ++; 1-50%, +; and 0%, -.

		Rh1	Rh2	Mha
Ketones	7	+++	++	++
	9	+	-	-
	10	++	+	+
	14	++	+	+
Quinones	16	+++	+++	+++
	18	+++	+++	+++
Ester	19	-	-	-
Nitrile	20	-	-	-
Aldehydes	1	+++	+++	+++
	17	+++	+++	+++

Table S2 Retention time of substrates and products.

Substrates	Retention time (min)	Products	Retention time (min)
1S	17.1	**1P**	14.6
2S	18.1	**2P**	15.7
3S	20.4	**3P**	20.0
4S	17.3	**4P**	14.8
5S	16.7	**5P**	14.8
6S	8.4	**6P**	7.2
7S	7.7	**7P**	6.8
8S	11.4	**8P**	8.6
9S	13.6	**9P**	11.3
10S	16.8	**10P**	15.8 (*trans*) 15.9 (*cis*)
11S	8.9	**11P**	5.9
12S	9.8	**12P**	8.5
13S	7.2	**13P**	5.8
14S	20.9	**14P**	20.2
15S	14.0	**15P**	14.5

Chiral GC analyses

The chiral analysis for **4**, **5**, **8, 9, 11, 12, 13** and **15** was determined by chiral GC-FID. GC–FID analyses were carried out with a Agilent Technologies 7890A GC system using a Agilent CP Chirasil-Dex CB capillary column (25 m x 0.25 mm, 0.25 μm film), injector and detector temperatures of 250 °C and a 50:1 split ratio were used for substrates **8, 9, 11, 12, 13** and **15**. For substrate **4** and **5** was used the same Agilent GC system with Aurora Borealis FS-Hydrodex-B-TBDAc capillary column (25 m × 0.25 mm, 0.25 μm film), injector and detector temperatures of 250 °C and a 50:1 split ratio.

The temperature program for **4** and **5** was: 40 °C hold 0 min, 10 °C min^{-1} to 80 °C, hold 2 min, 1 °C min^{-1} to 95 °C, hold 0 min, 0.5 °C min^{-1} to 100 °C, hold 5 min, 10 °C min^{-1} to 180 °C, hold 5 min. Retention times were: geranial **4**: 42.4 min, neral **5**: 41.1 min, (*R*)-citronellal: 32.9 min, (*S*)-citronellal: 32.3 min. The absolute configuration of (*R*)-citronellal was determined by commercially available (*R*)-citronellal (Sigma Aldrich).

The temperature program for **8** was: 40 °C hold 0 min, 5 °C min^{-1} to 95 °C, hold 10 min, 10 °C min^{-1} to 150 °C, hold 10 min. Retention times were: **8S**: 23.7 min, (*R*)-**8P**: 17.3 min, (*S*)-**8P**: 17.6 min. The absolute configuration of (*R*)-**8P** was determined by commercially available (*R*)-3-methylcyclohexan-1-one (Sigma Aldrich).

The temperature program for **9** was: 40 °C hold 0 min, 5 °C min^{-1} to 180 °C, hold 10 min. Retention times were: **9S**: 19.4 min, (*R*)-**9P**: 17.3 min, (*S*)-**9P**: 17.0 min. The absolute configuration of (*R*)-**9P** was determined by commercially available (5*R*)-3,3,5-trimethylcyclohexan-1-one (Enamine).

The temperature program for **11** was: 40 °C hold 0 min, 2 °C min^{-1} to 55 °C, hold 40 min, 10 °C min^{-1} to 150 °C, hold 5 min. Retention times were: **11S**: 53.0 min, (*R*)-**11P**: 33.6 min, (*S*)-**11P**: 34.0 min. The absolute configuration of (*R*)-**11P** was determined by commercially available (*R*)-3-methylcyclopentan-1-one (Sigma Aldrich).

The temperature program for **12** was: 80 °C hold 6.5 min, 10 °C min^{-1} to 130 °C, hold 3 min. Retention times were: **12S**: 11.4 min, (*S*)-**12P**: 10.9 min, (*R*)-**12P**: 11.0 min. The absolute configuration of (*R*)-**12P** was determined by described method for the same compound on the same column.[9]

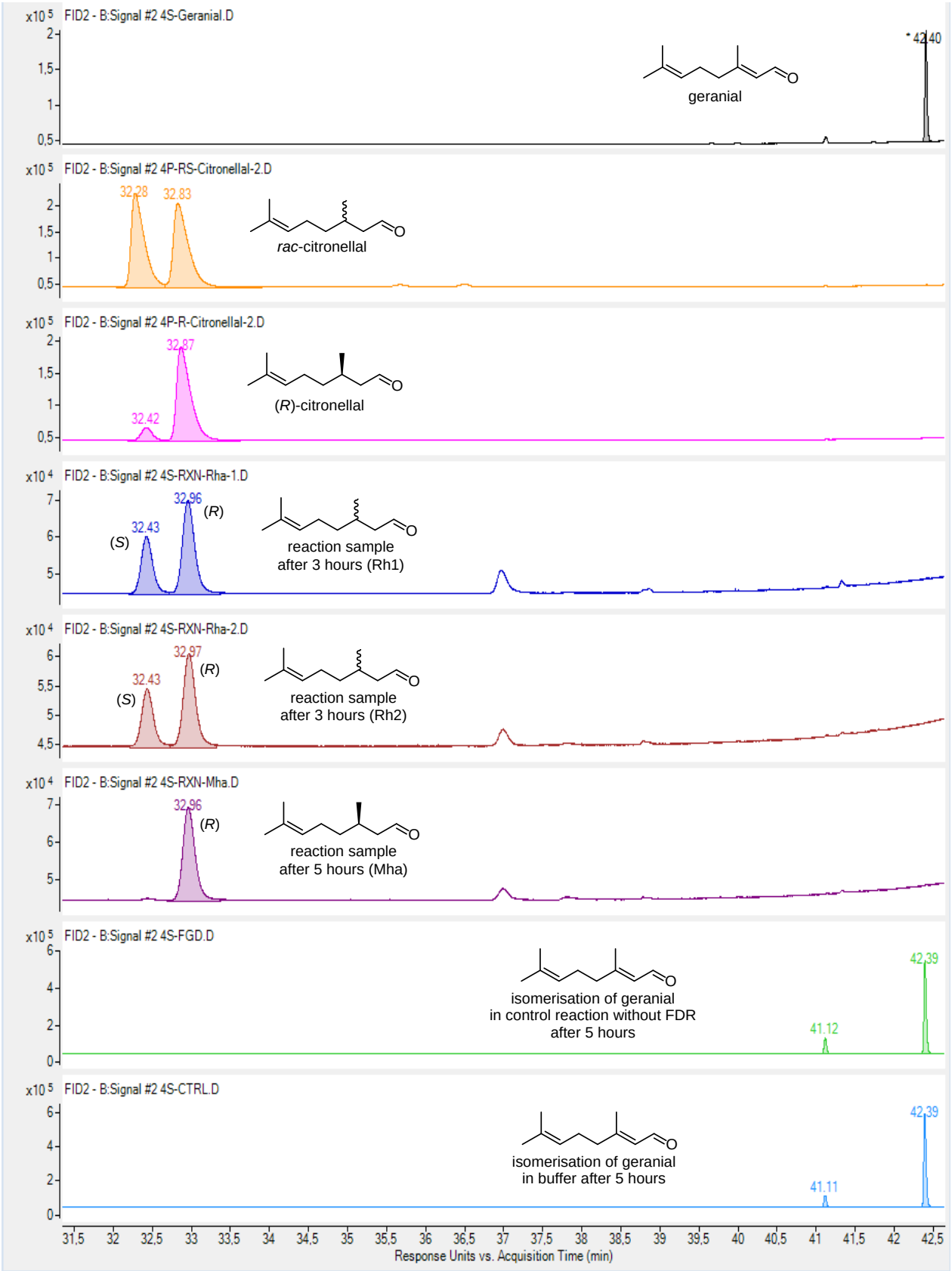
The temperature program for **13** was: 40 °C hold 0 min, 5 °C min^{-1} to 68 °C, hold 15 min, 10 °C min^{-1} to 160 °C, hold 2 min. Retention times were: **13S**: 20.8 min, (*R*)-**13P**: 16.7 min, (*S*)-**13P**: 17.0 min. The absolute configuration of (*R*)-**13P** was determined by described method for the same compound on the same column.[10]

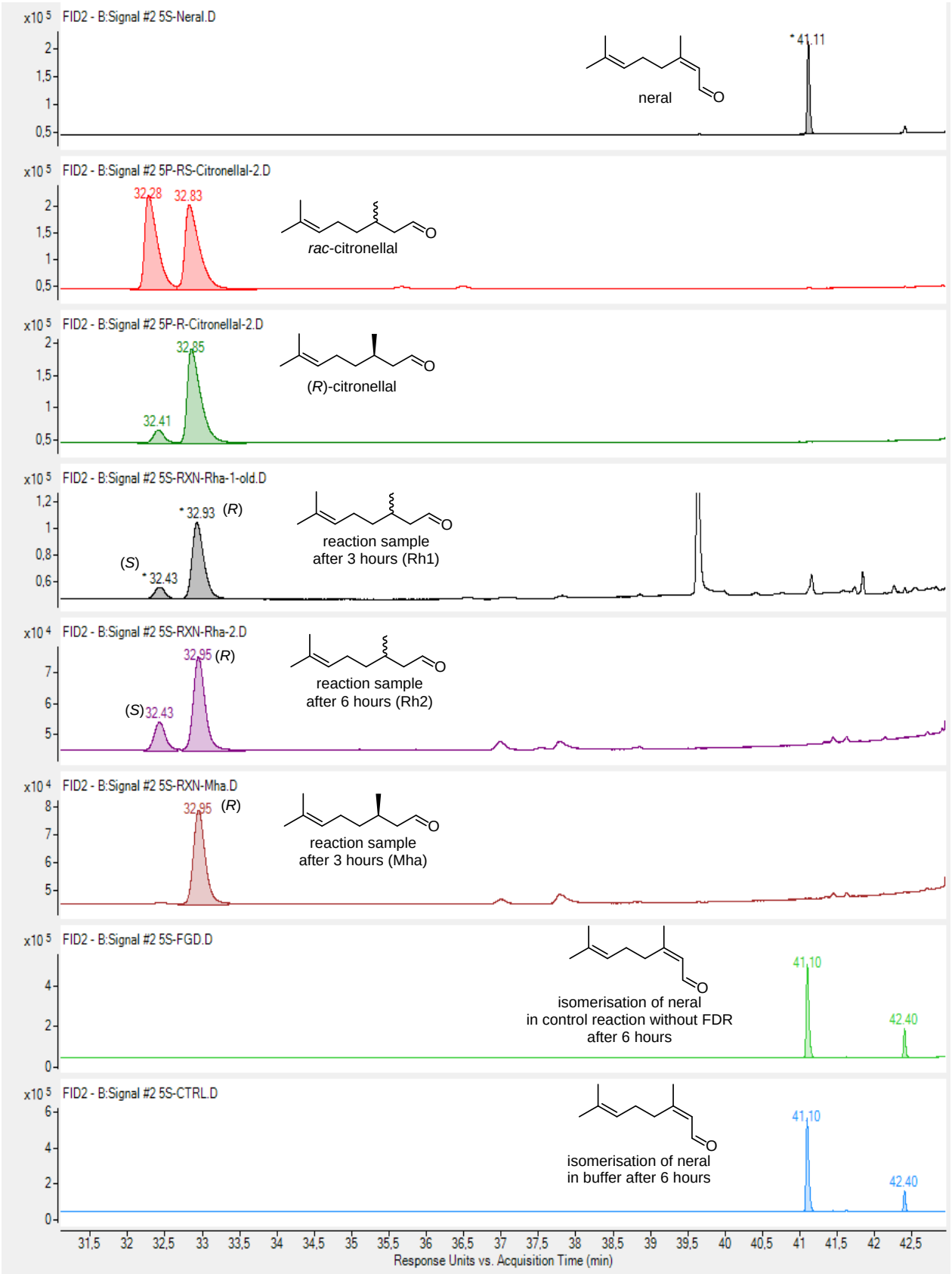
The temperature program for **15** was: 90 °C hold 2 min, 4 °C min^{-1} to 115 °C, hold 0 min, 10 °C min^{-1} to 180 °C, hold 2 min. Retention times were: **15S**: 12.2 min, (*R*)-**15P**: 12.7 min, (*S*)-**15P**:

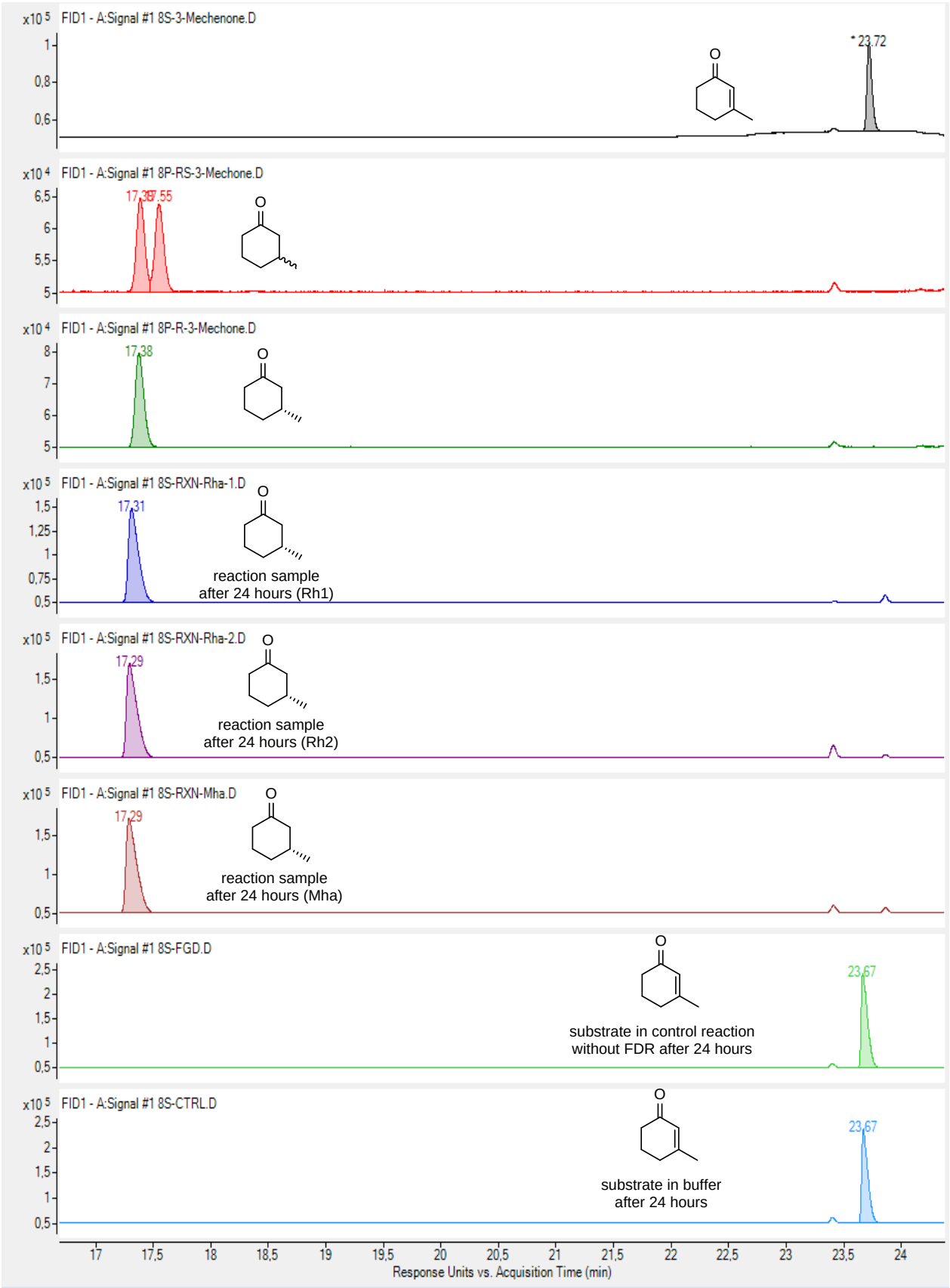
12.8 min. The absolute configuration of (*S*)-**15P** was determined by described method for the same compound on the same column.[11]

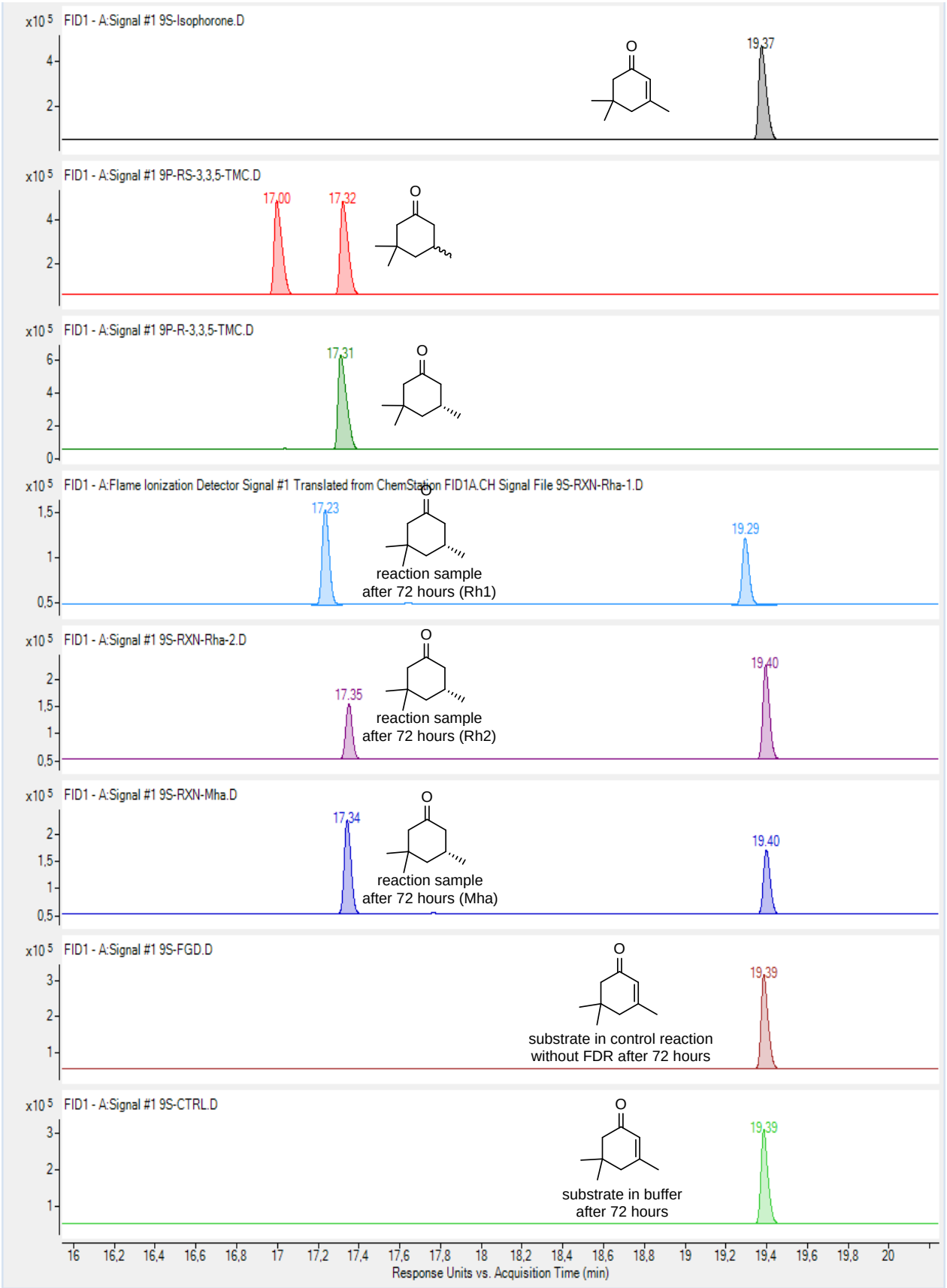
Table S3 Retention time of substrates and products for chiral analysis.

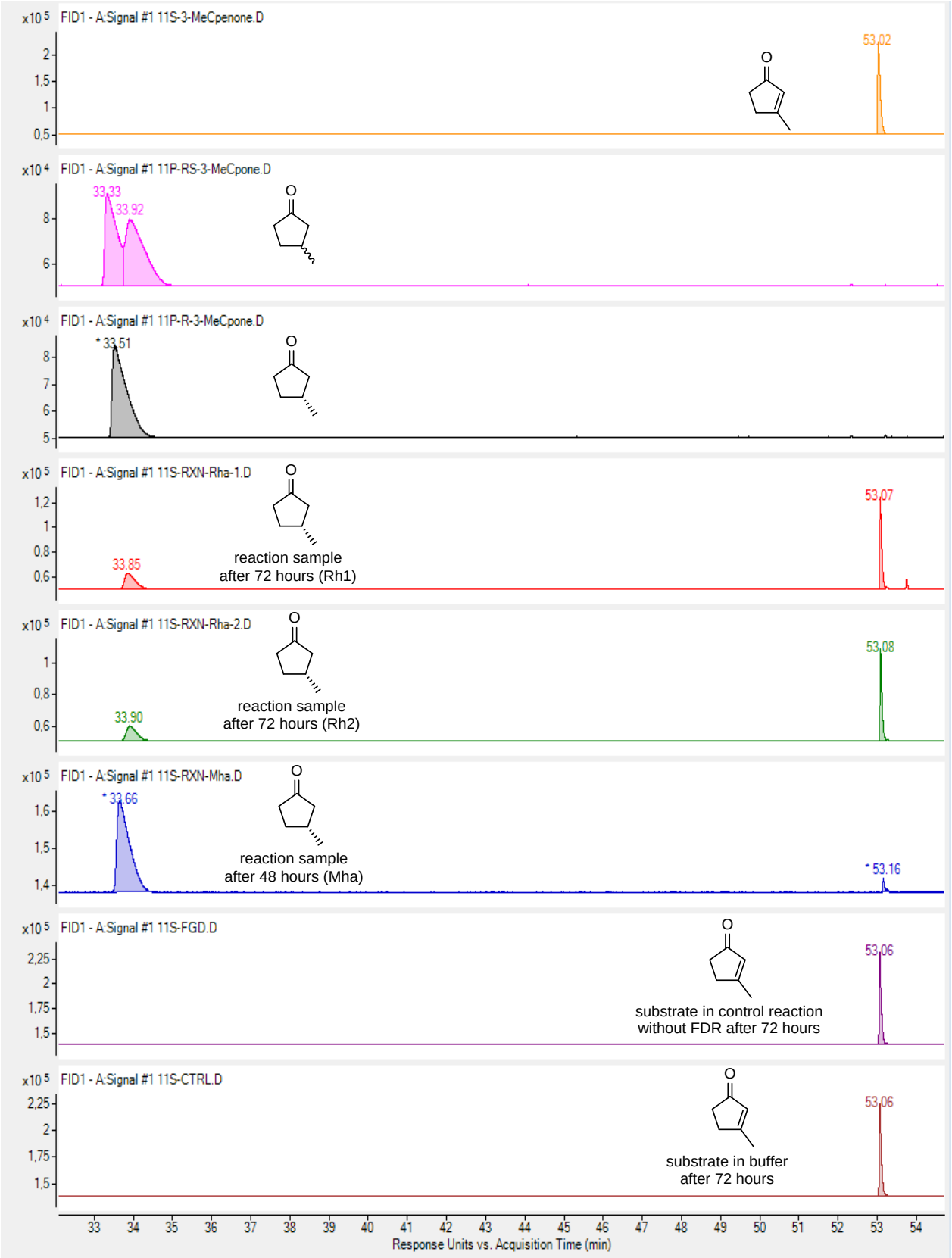
Substrates	Retention time (min)	Products	Retention time (min)
4S	42.4	**4P**	32.3 (*S*) 32.9 (*R*)
5S	41.1	**5P**	32.3 (*S*) 32.9 (*R*)
8S	23.7	**8P**	17.3 (*R*) 17.6 (*S*)
9S	19.4	**9P**	17.0 (*S*) 17.3 (*R*)
11S	53.0	**11P**	33.6 (*R*) 34.0 (*S*)
12S	11.4	**12P**	10.9 (*S*) 11.0 (*R*)
13S	20.8	**13P**	16.7 (*R*) 17.0 (*S*)
15S	12.2	**15P**	12.7 (*R*) 12.8 (*S*)

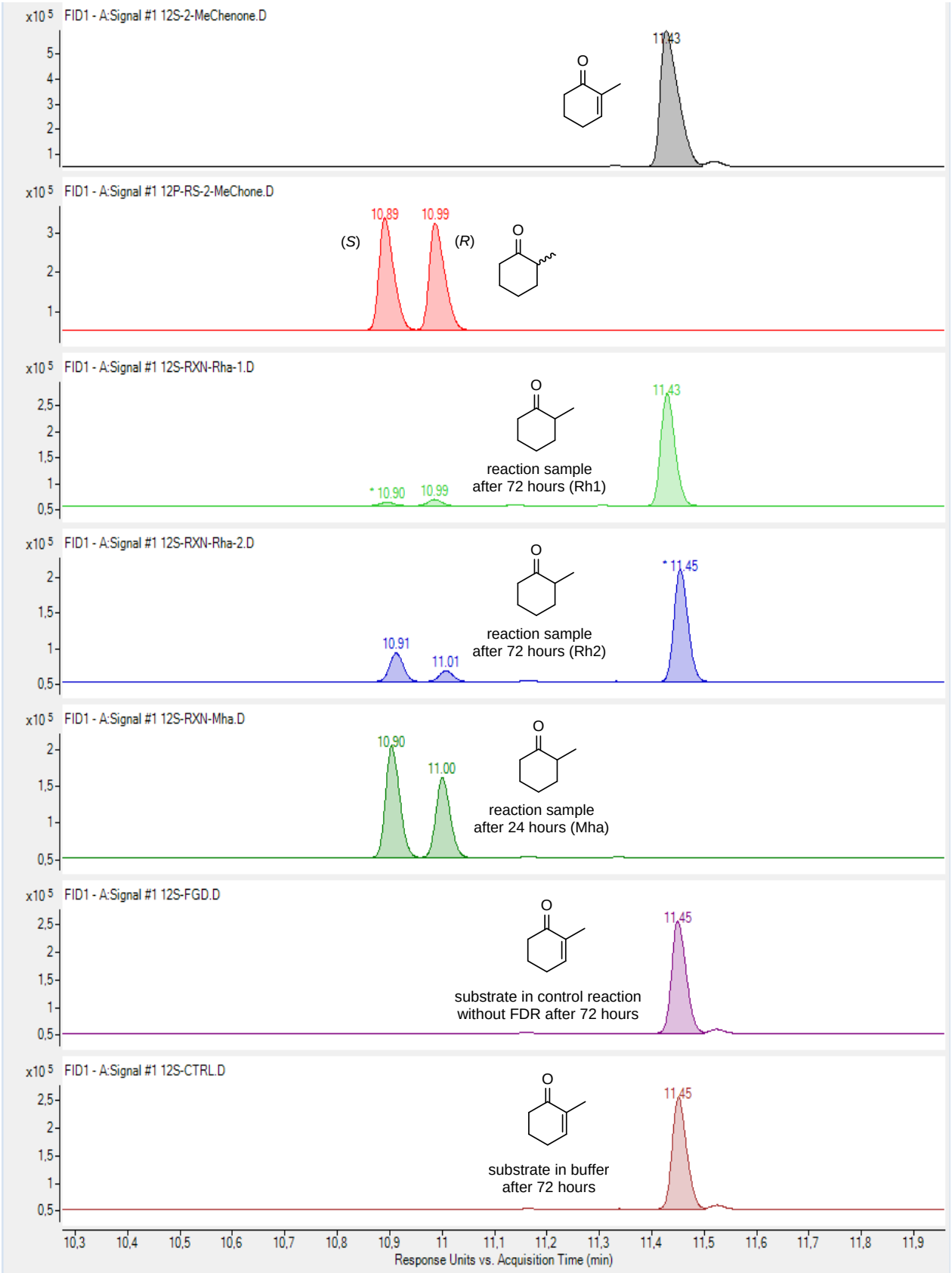

x10 5 FID2 - B:Signal #2 4S-Geranial.D
42.40
geranial
x10 5 FID2 - B:Signal #2 4P-RS-Citronellal-2.D
32.28
32.83
rac-citronellal
x10 5 FID2 - B:Signal #2 4P-R-Citronellal-2.D
32.87
32.42
(R)-citronellal
x10 4 FID2 - B:Signal #2 4S-RXN-Rha-1.D
32.96
(R)
32.43
(S)
reaction sample
after 3 hours (Rh1)
x10 4 FID2 - B:Signal #2 4S-RXN-Rha-2.D
32.97
(R)
32.43
(S)
reaction sample
after 3 hours (Rh2)
x10 4 FID2 - B:Signal #2 4S-RXN-Mha.D
32.96
(R)
reaction sample
after 5 hours (Mha)
x10 5 FID2 - B:Signal #2 4S-FGD.D
42.39
41.12
isomerisation of geranial
in control reaction without FDR
after 5 hours
x10 5 FID2 - B:Signal #2 4S-CTRL.D
42.39
41.11
isomerisation of geranial
in buffer after 5 hours
Response Units vs. Acquisition Time (min)

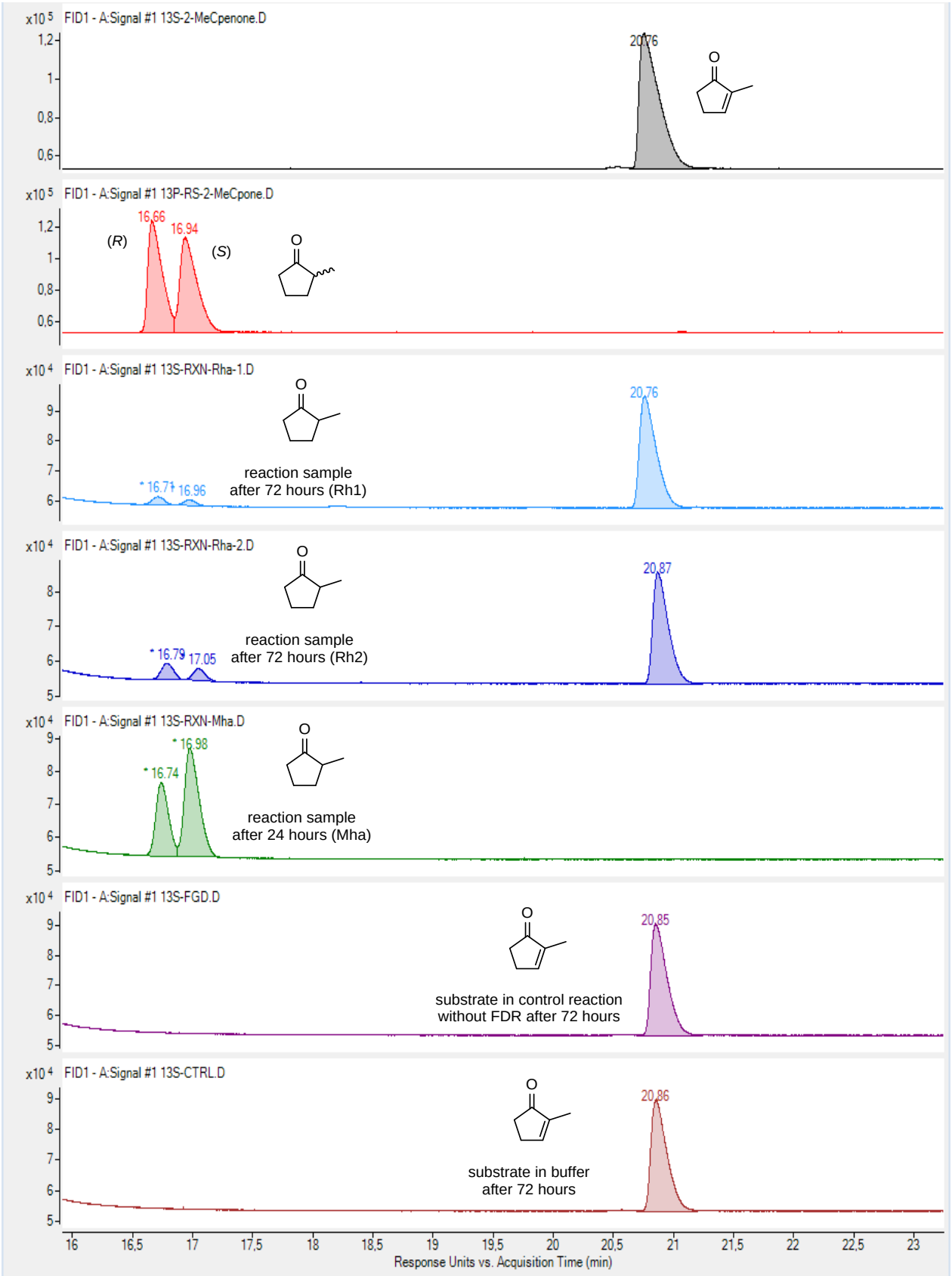

x10 5 FID2 - B:Signal #2 5S-Neral.D
neral
* 41.11
x10 5 FID2 - B:Signal #2 5P-RS-Citronellal-2.D
32.28
32.83
rac-citronellal
x10 5 FID2 - B:Signal #2 5P-R-Citronellal-2.D
32.95
32.41
(R)-citronellal
x10 5 FID2 - B:Signal #2 5S-RXN-Rha-1-old.D
* 32.93 (R)
(S)
* 32.43
reaction sample
after 3 hours (Rh1)
x10 4 FID2 - B:Signal #2 5S-RXN-Rha-2.D
32.95 (R)
(S) 32.43
reaction sample
after 6 hours (Rh2)
x10 4 FID2 - B:Signal #2 5S-RXN-Mha.D
32.95 (R)
reaction sample
after 3 hours (Mha)
x10 5 FID2 - B:Signal #2 5S-FGD.D
41.10
42.40
isomerisation of neral
in control reaction without FDR
after 6 hours
x10 5 FID2 - B:Signal #2 5S-CTRL.D
41.10
42.40
isomerisation of neral
in buffer after 6 hours
Response Units vs. Acquisition Time (min)

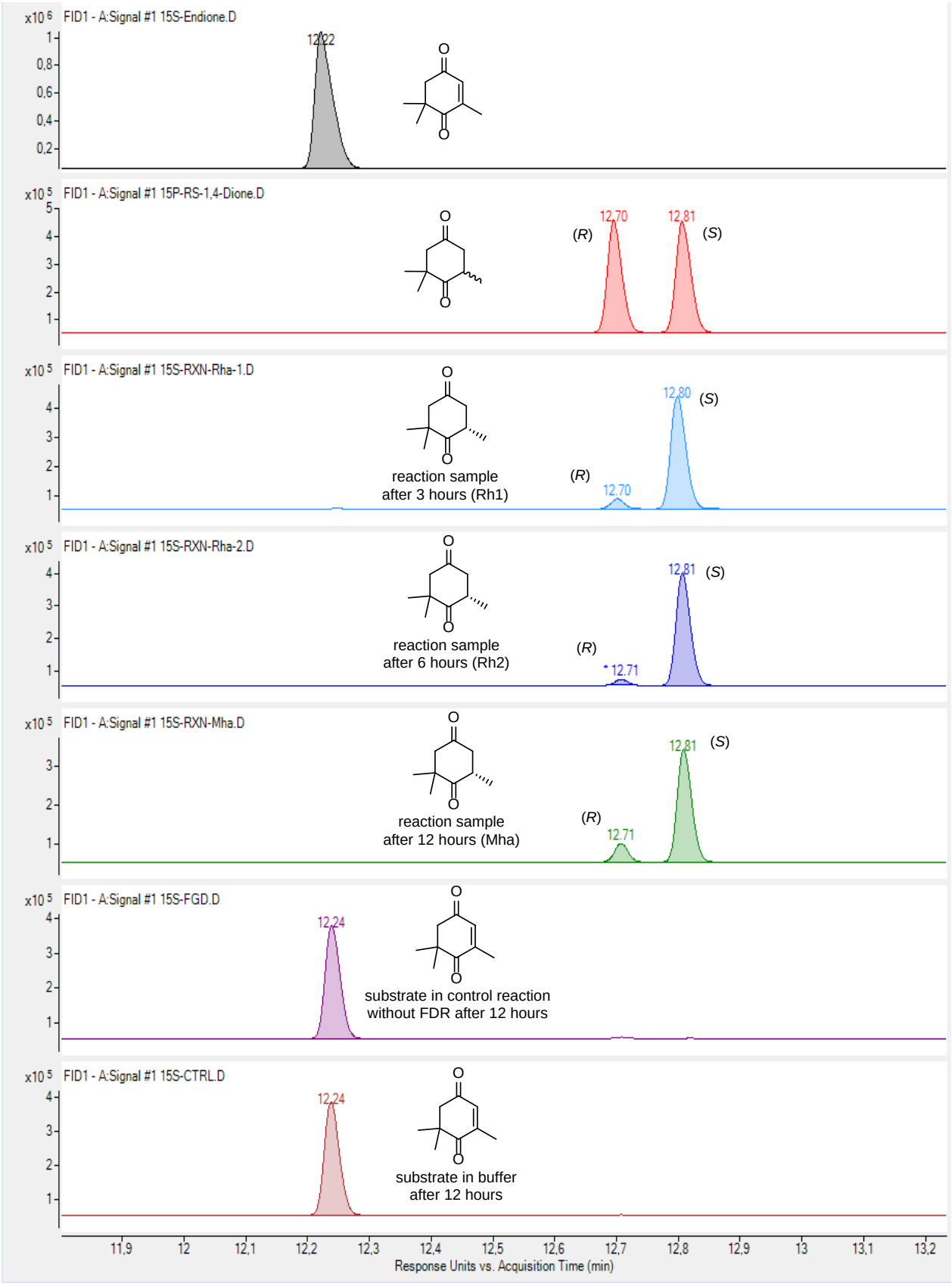

FID1 - A:Signal #1 8S-3-Mechenone.D
23.72
FID1 - A:Signal #1 8P-RS-3-Mechone.D
17.38
17.55
FID1 - A:Signal #1 8P-R-3-Mechone.D
17.38
FID1 - A:Signal #1 8S-RXN-Rha-1.D
17.31
reaction sample
after 24 hours (Rh1)
FID1 - A:Signal #1 8S-RXN-Rha-2.D
17.29
reaction sample
after 24 hours (Rh2)
FID1 - A:Signal #1 8S-RXN-Mha.D
17.29
reaction sample
after 24 hours (Mha)
FID1 - A:Signal #1 8S-FGD.D
23.67
substrate in control reaction
without FDR after 24 hours
FID1 - A:Signal #1 8S-CTRL.D
23.67
substrate in buffer
after 24 hours
Response Units vs. Acquisition Time (min)


x10 5 FID1 - A:Signal #1 9S-Isophorone.D
19.37
x10 5 FID1 - A:Signal #1 9P-RS-3,3,5-TMC.D
17.00
17.32
x10 5 FID1 - A:Signal #1 9P-R-3,3,5-TMC.D
17.31
x10 5 FID1 - A:Flame Ionization Detector Signal #1 Translated from ChemStation FID1A.CH Signal File 9S-RXN-Rha-1.D
17.23
19.29
reaction sample
after 72 hours (Rh1)
x10 5 FID1 - A:Signal #1 9S-RXN-Rha-2.D
17.35
19.40
reaction sample
after 72 hours (Rh2)
x10 5 FID1 - A:Signal #1 9S-RXN-Mha.D
17.34
19.40
reaction sample
after 72 hours (Mha)
x10 5 FID1 - A:Signal #1 9S-FGD.D
19.39
substrate in control reaction
without FDR after 72 hours
x10 5 FID1 - A:Signal #1 9S-CTRL.D
19.39
substrate in buffer
after 72 hours
16 16,2 16,4 16,6 16,8 17 17,2 17,4 17,6 17,8 18 18,2 18,4 18,6 18,8 19 19,2 19,4 19,6 19,8 20
Response Units vs. Acquisition Time (min)

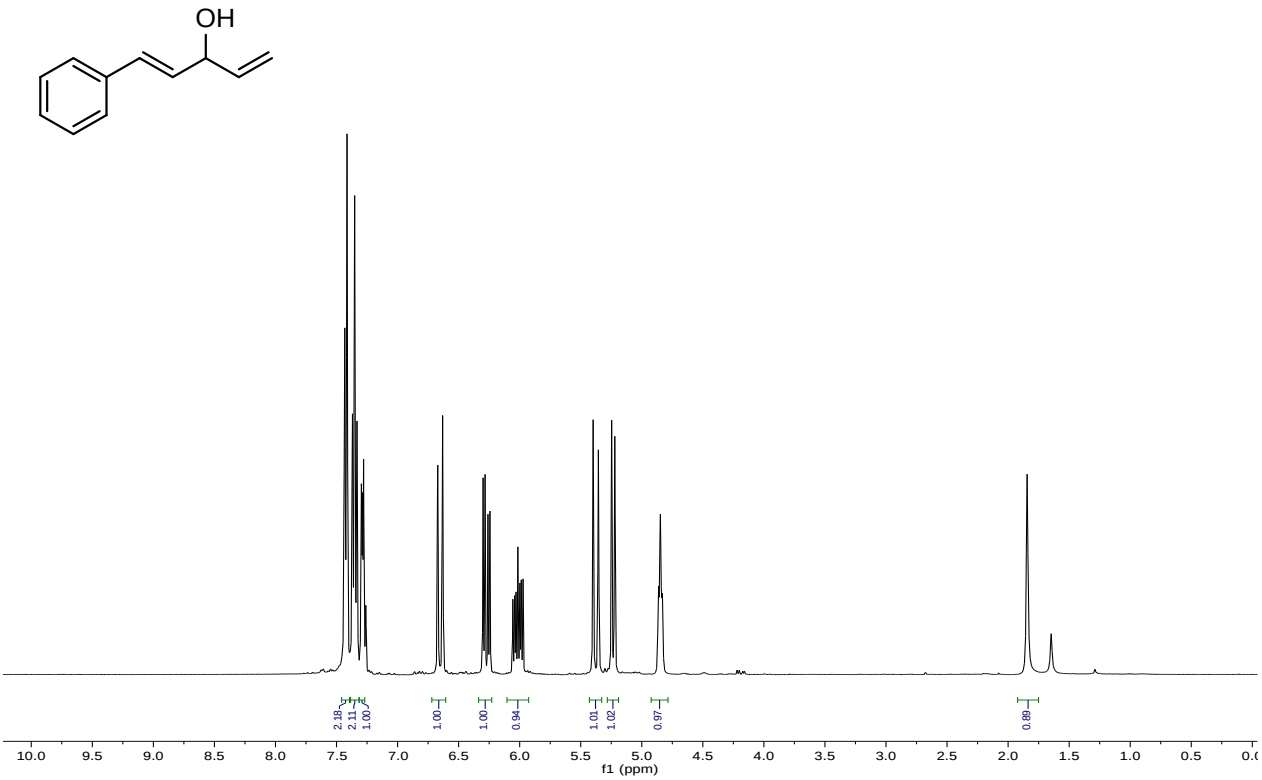

FID1 - A:Signal #1 11S-3-MeCpenone.D
53.02
FID1 - A:Signal #1 11P-RS-3-MeCpone.D
33.33
33.92
FID1 - A:Signal #1 11P-R-3-MeCpone.D
* 33.51
FID1 - A:Signal #1 11S-RXN-Rha-1.D
53.07
33.85
reaction sample
after 72 hours (Rh1)
FID1 - A:Signal #1 11S-RXN-Rha-2.D
53.08
33.90
reaction sample
after 72 hours (Rh2)
FID1 - A:Signal #1 11S-RXN-Mha.D
* 33.66
* 53.16
reaction sample
after 48 hours (Mha)
FID1 - A:Signal #1 11S-FGD.D
53.06
substrate in control reaction
without FDR after 72 hours
FID1 - A:Signal #1 11S-CTRL.D
53.06
substrate in buffer
after 72 hours
Response Units vs. Acquisition Time (min)

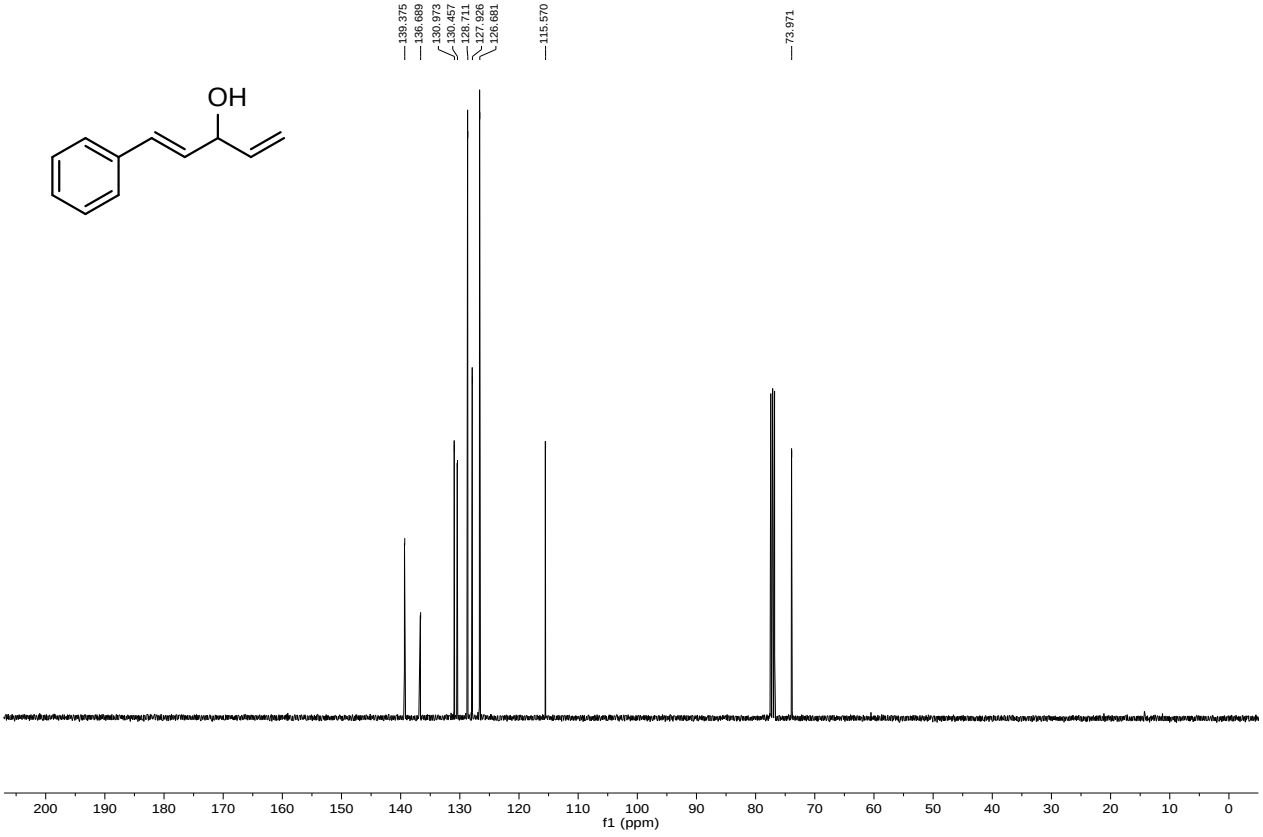

x10 5 FID1 - A:Signal #1 12S-2-MeChenone.D
11.43
x10 5 FID1 - A:Signal #1 12P-RS-2-MeChone.D
10.89
10.99
(S)
(R)
x10 5 FID1 - A:Signal #1 12S-RXN-Rha-1.D
11.43
reaction sample
after 72 hours (Rh1)
* 10.90
10.99
x10 5 FID1 - A:Signal #1 12S-RXN-Rha-2.D
* 11.45
reaction sample
after 72 hours (Rh2)
10.91
11.01
x10 5 FID1 - A:Signal #1 12S-RXN-Mha.D
10.90
11.00
reaction sample
after 24 hours (Mha)
x10 5 FID1 - A:Signal #1 12S-FGD.D
11.45
substrate in control reaction
without FDR after 72 hours
x10 5 FID1 - A:Signal #1 12S-CTRL.D
11.45
substrate in buffer
after 72 hours
10,3 10,4 10,5 10,6 10,7 10,8 10,9 11 11,1 11,2 11,3 11,4 11,5 11,6 11,7 11,8 11,9
Response Units vs. Acquisition Time (min)

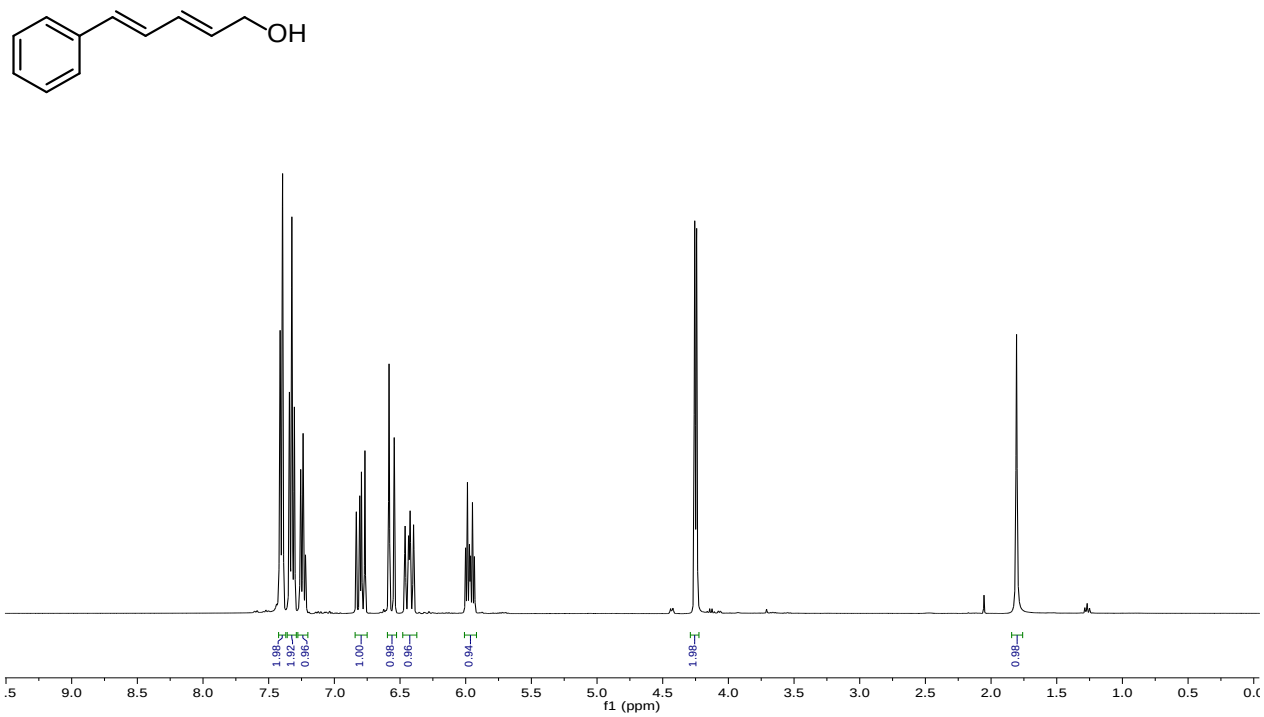

FID1 - A:Signal #1 13S-2-MeCpenone.D
20,76
FID1 - A:Signal #1 13P-RS-2-MeCpone.D
16,66
16,94
(R)
(S)
FID1 - A:Signal #1 13S-RXN-Rha-1.D
20,76
16,71
16,96
reaction sample
after 72 hours (Rh1)
FID1 - A:Signal #1 13S-RXN-Rha-2.D
20,87
16,79
17,05
reaction sample
after 72 hours (Rh2)
FID1 - A:Signal #1 13S-RXN-Mha.D
16,98
16,74
reaction sample
after 24 hours (Mha)
FID1 - A:Signal #1 13S-FGD.D
20,85
substrate in control reaction
without FDR after 72 hours
FID1 - A:Signal #1 13S-CTRL.D
20,86
substrate in buffer
after 72 hours
Response Units vs. Acquisition Time (min)

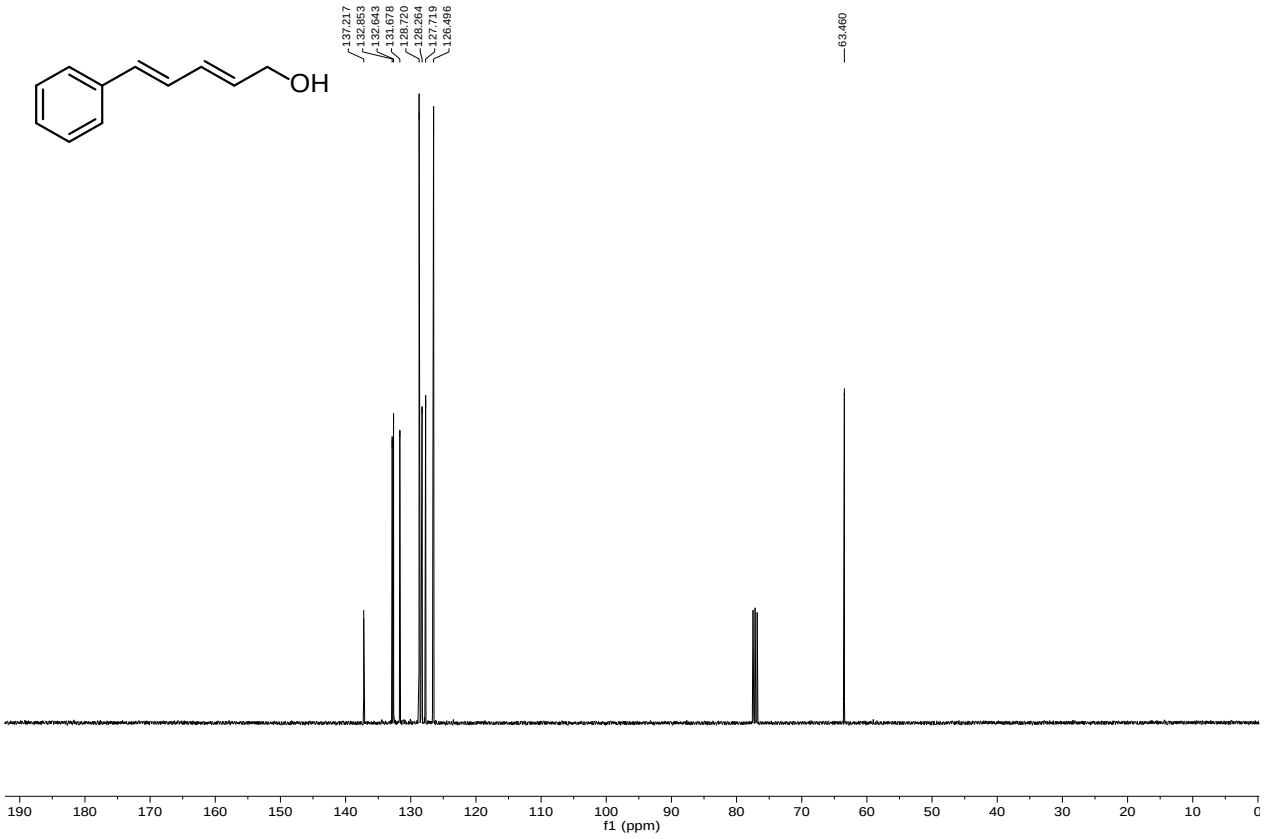

x10 6 FID1 - A:Signal #1 15S-Endione.D
12.22
x10 5 FID1 - A:Signal #1 15P-RS-1,4-Dione.D
12.70
12.81
(R)
(S)
x10 5 FID1 - A:Signal #1 15S-RXN-Rha-1.D
12.80
(S)
reaction sample
after 3 hours (Rh1)
(R)
12.70
x10 5 FID1 - A:Signal #1 15S-RXN-Rha-2.D
12.81
(S)
reaction sample
after 6 hours (Rh2)
(R)
12.71
x10 5 FID1 - A:Signal #1 15S-RXN-Mha.D
12.81
(S)
reaction sample
after 12 hours (Mha)
(R)
12.71
x10 5 FID1 - A:Signal #1 15S-FGD.D
12.24
substrate in control reaction
without FDR after 12 hours
x10 5 FID1 - A:Signal #1 15S-CTRL.D
12.24
substrate in buffer
after 12 hours
11,9
12
12,1
12,2
12,3
12,4
12,5
12,6
12,7
12,8
12,9
13
13,1
13,2
Response Units vs. Acquisition Time (min)

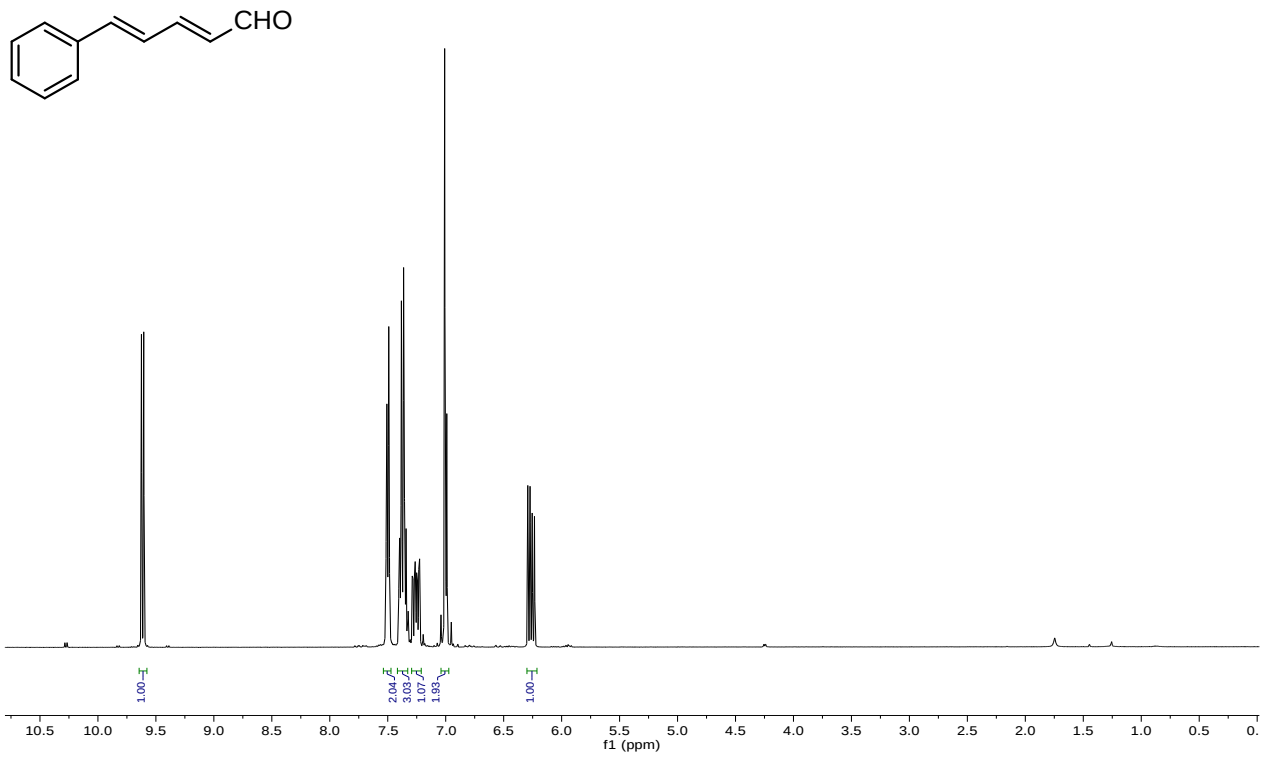
NMR spectra

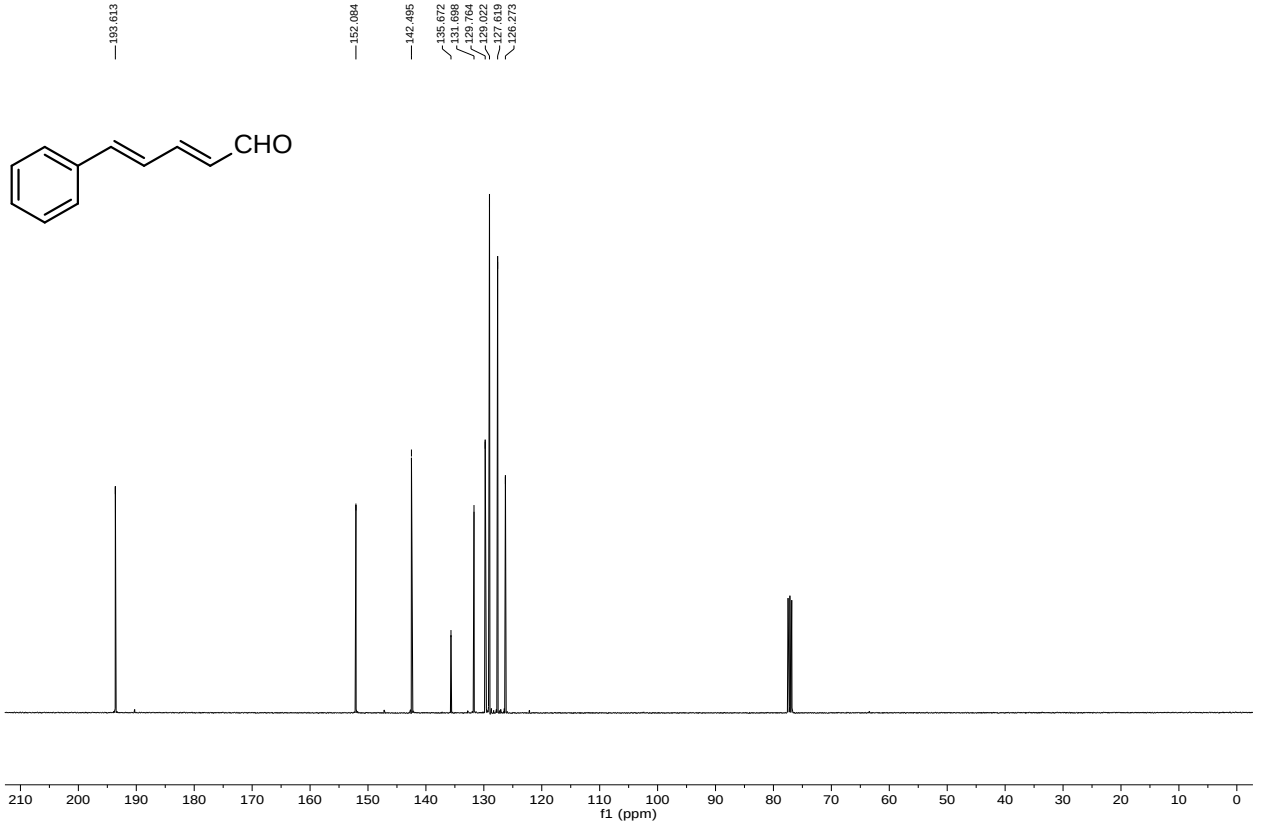

OH
2.18
2.11
1.00
1.00
1.00
0.94
1.01
1.02
0.97
0.89
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

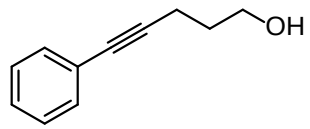

139.375
136.689
130.973
130.457
128.711
127.926
126.681
115.570
73.971
OH
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

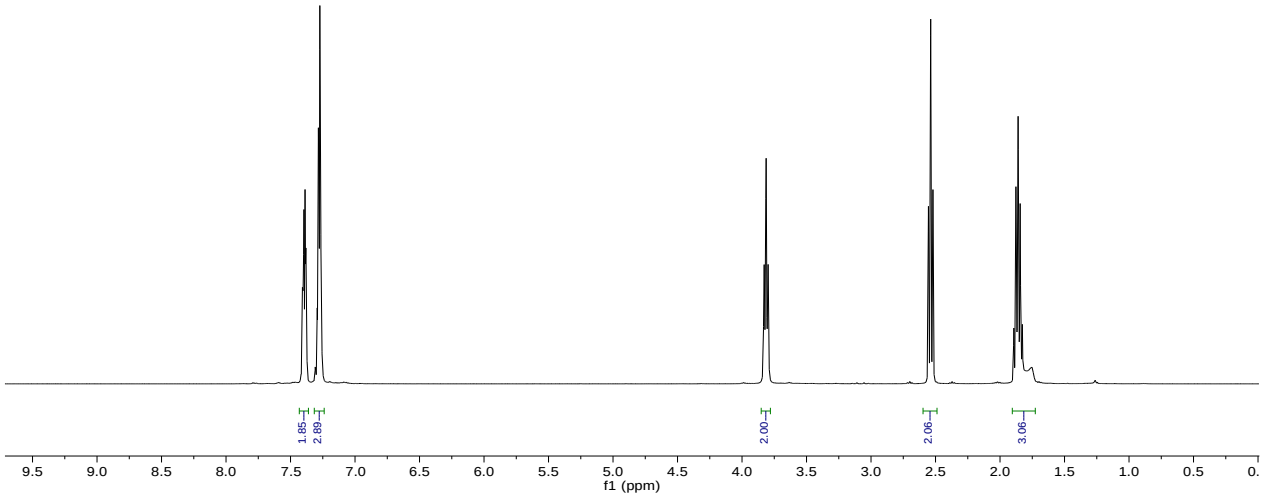

OH
1.98
1.92
0.96
1.00
0.98
0.96
0.94
1.98
0.98
f1 (ppm)

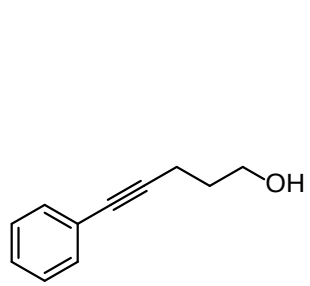

OH
137.217
132.853
132.643
131.678
128.720
128.264
127.719
126.496
63.460
f1 (ppm)

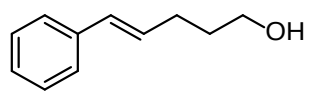

CHO
1.00
2.04
3.03
1.07
1.93
1.00
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

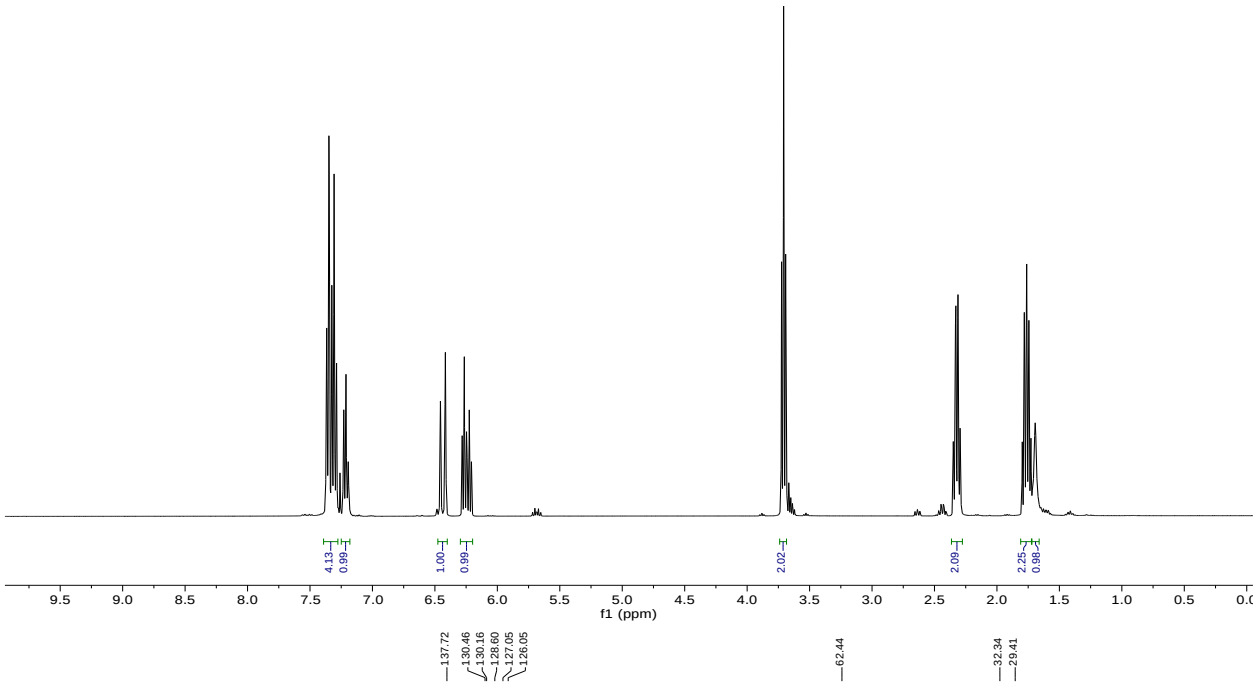

193.613
152.084
142.495
135.672
131.698
129.764
129.022
127.619
126.273
CHO
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

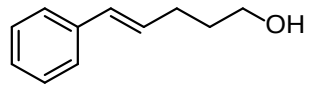

OH

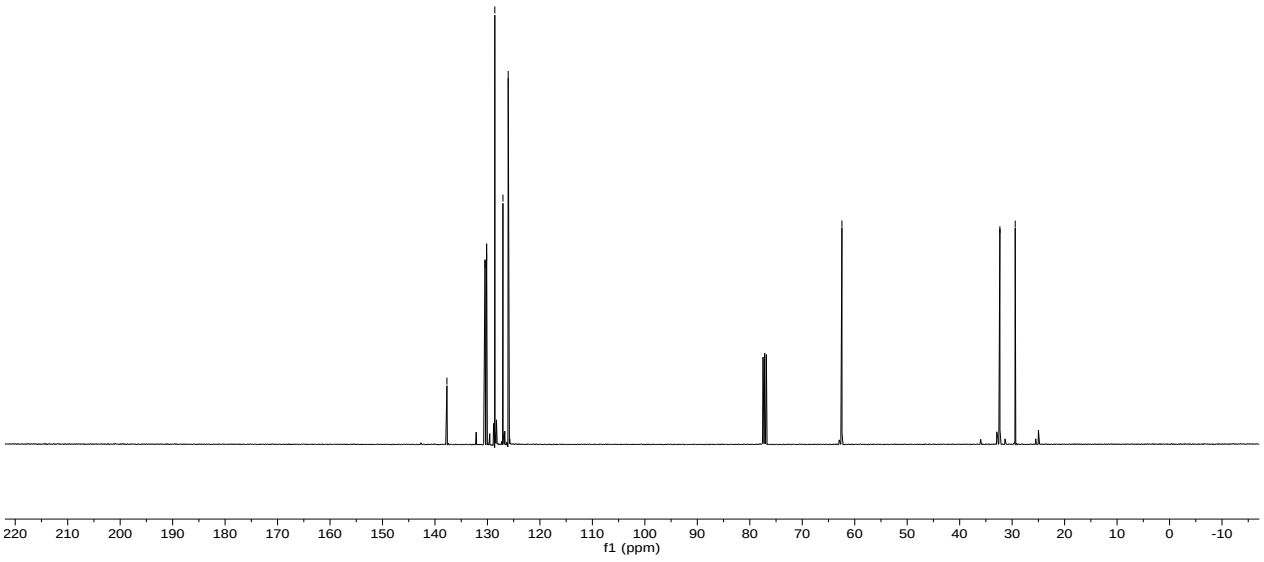

1.85
2.89
2.00
2.06
3.06
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

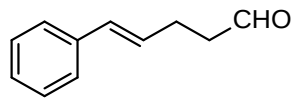

131.64
128.33
127.78
123.83
89.46
81.24
61.86
31.50
16.10
OH
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

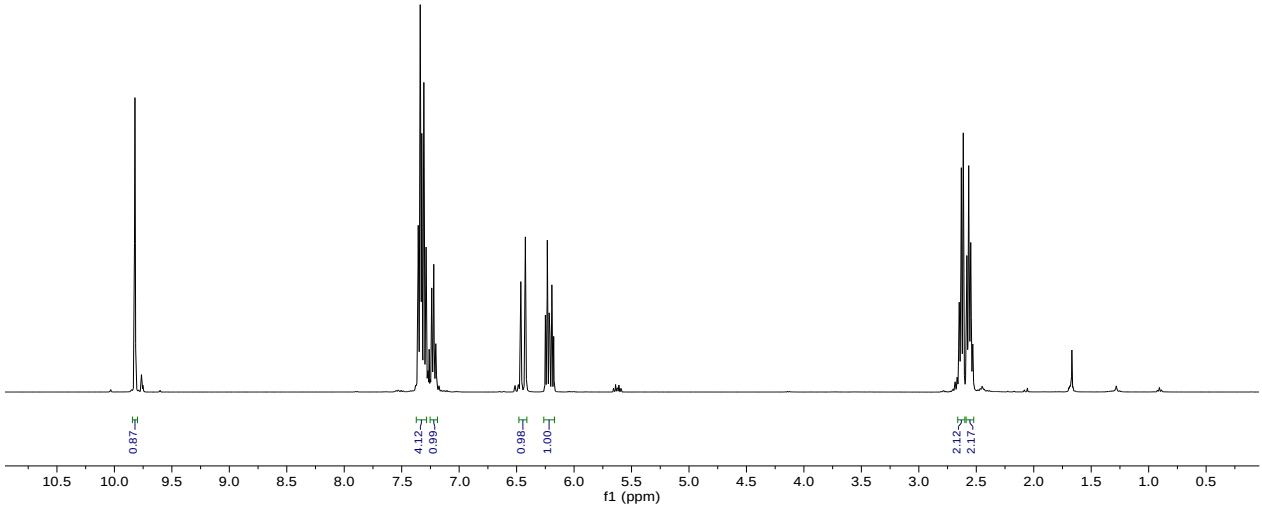

OH

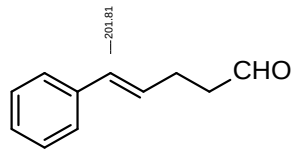

4.13
0.99
1.00
0.99
2.02
2.09
2.25
0.98
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)
137.72
130.46
130.16
128.60
127.05
126.05
62.44
32.34
29.41


OH

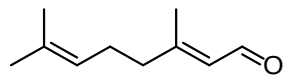

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

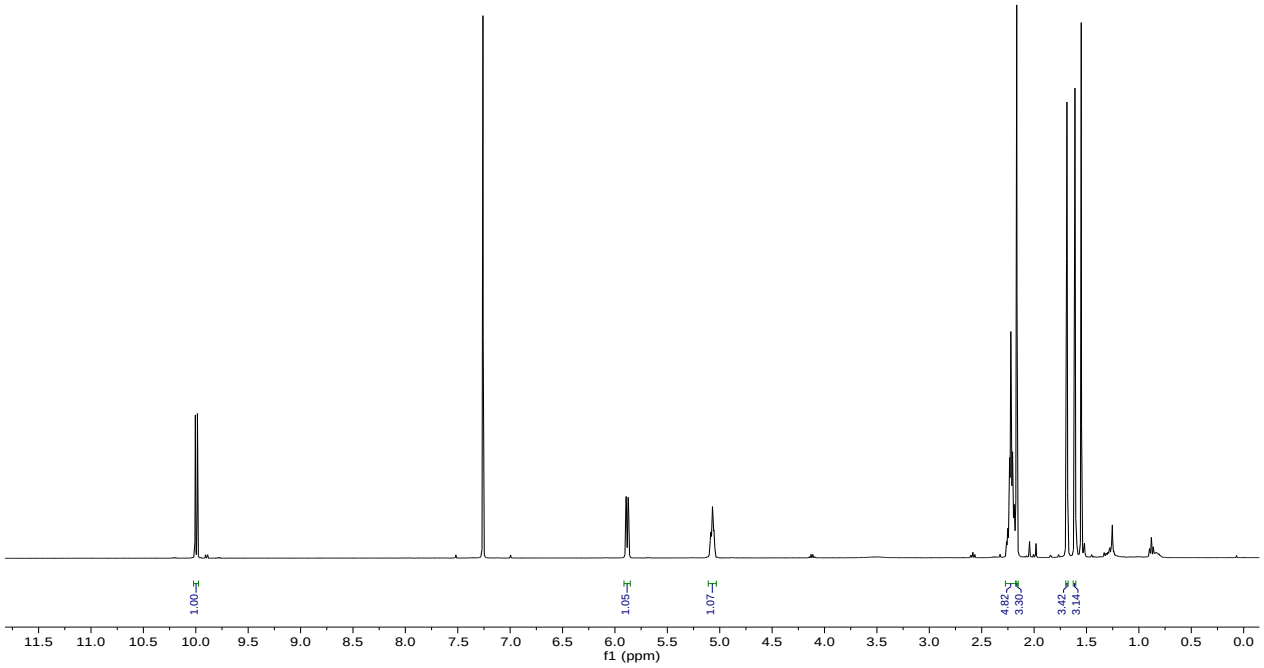

CHO

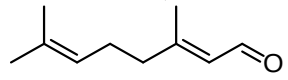

0.87
4.12
0.99
0.98
1.00
2.12
2.17
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


CHO

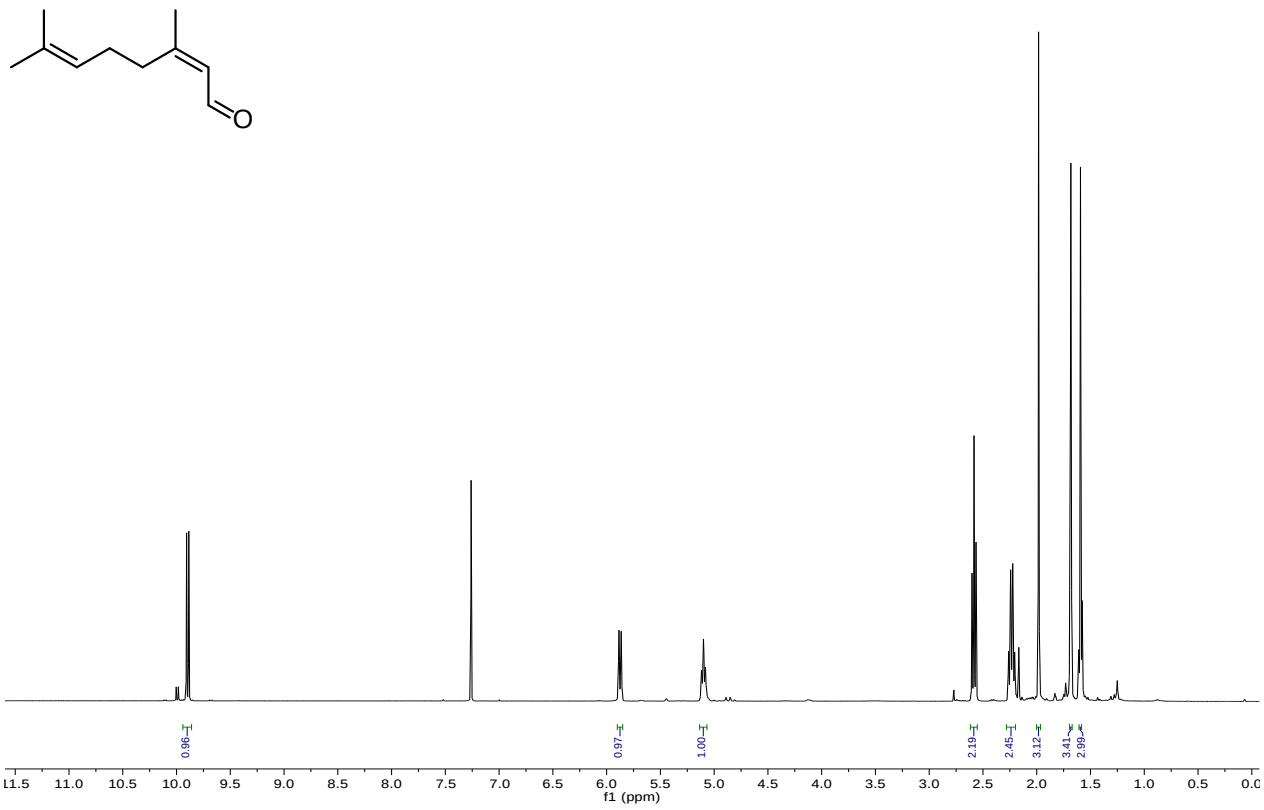
201.81
137.29
131.19
128.62
128.25
127.32
126.13
43.39
25.59
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

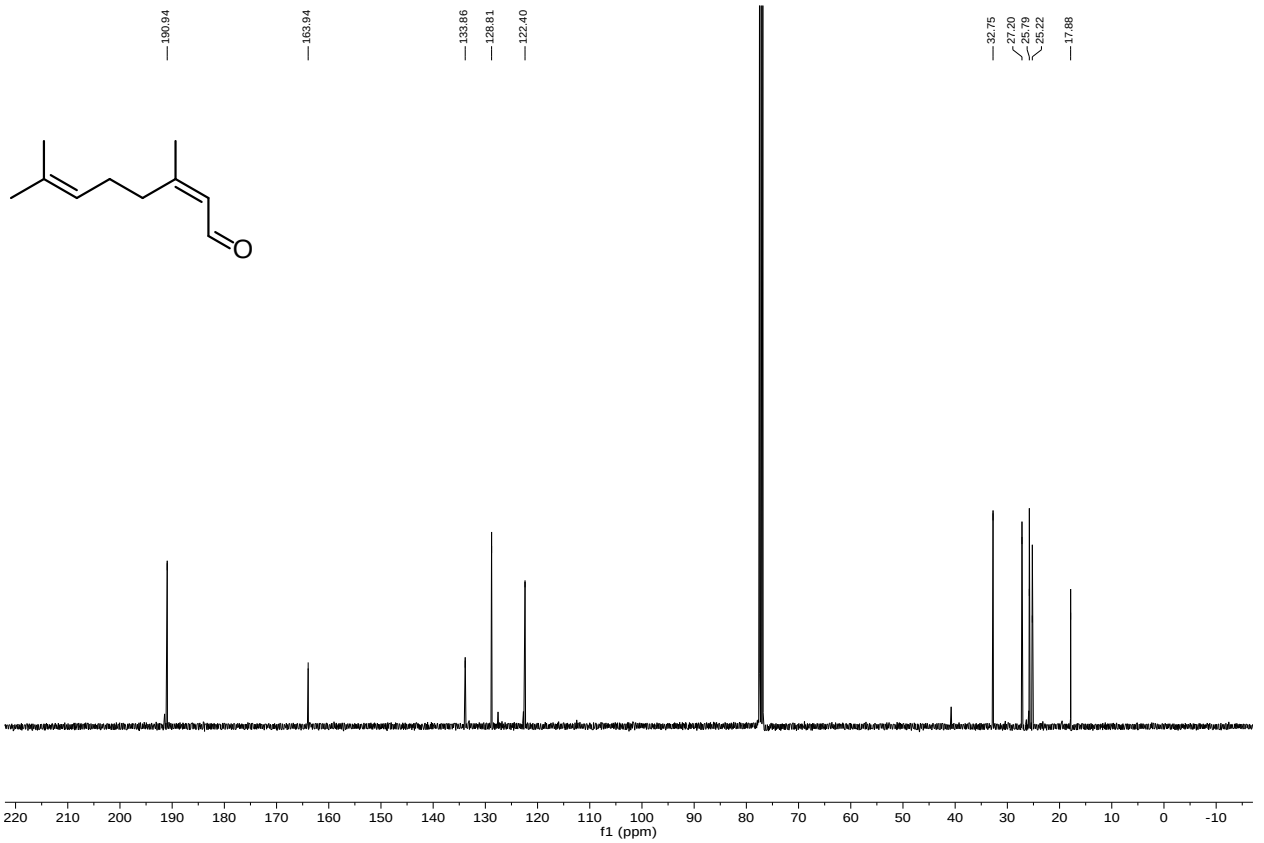


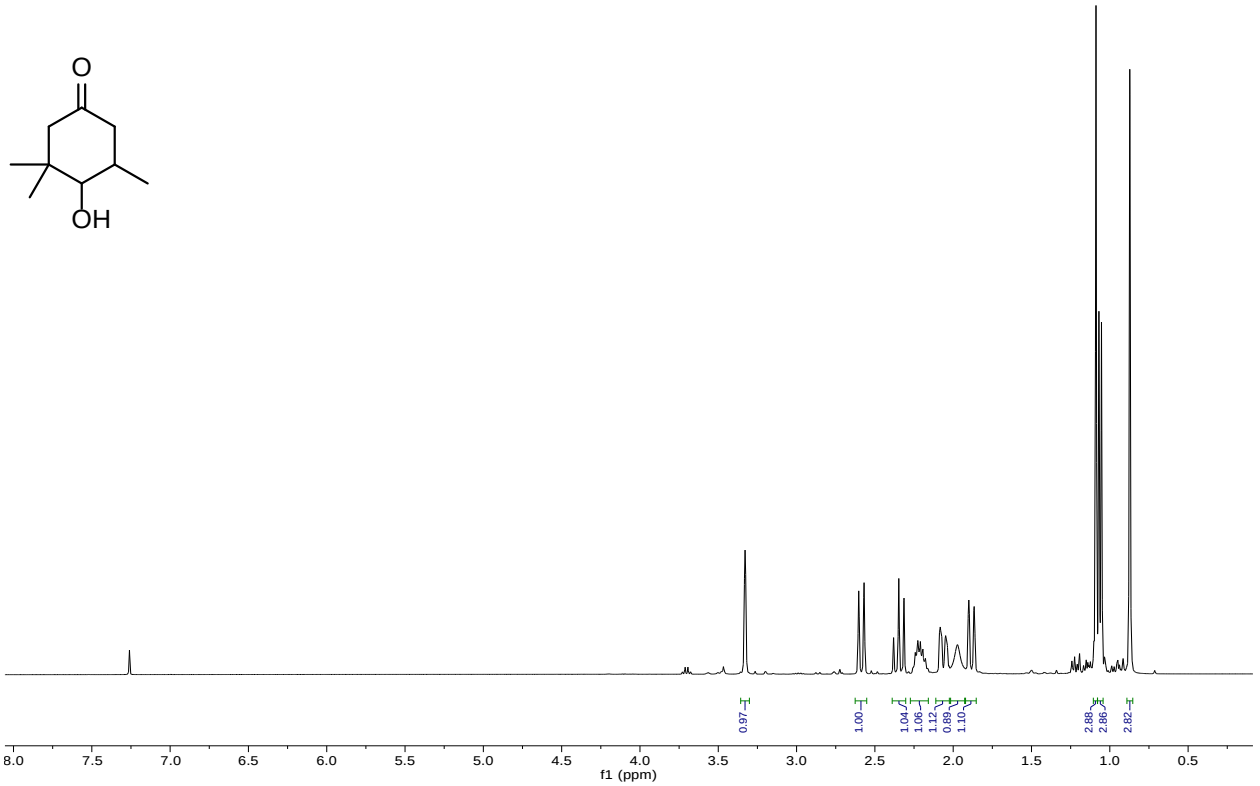

1.00
1.05
1.07
4.82
3.30
3.42
3.14
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

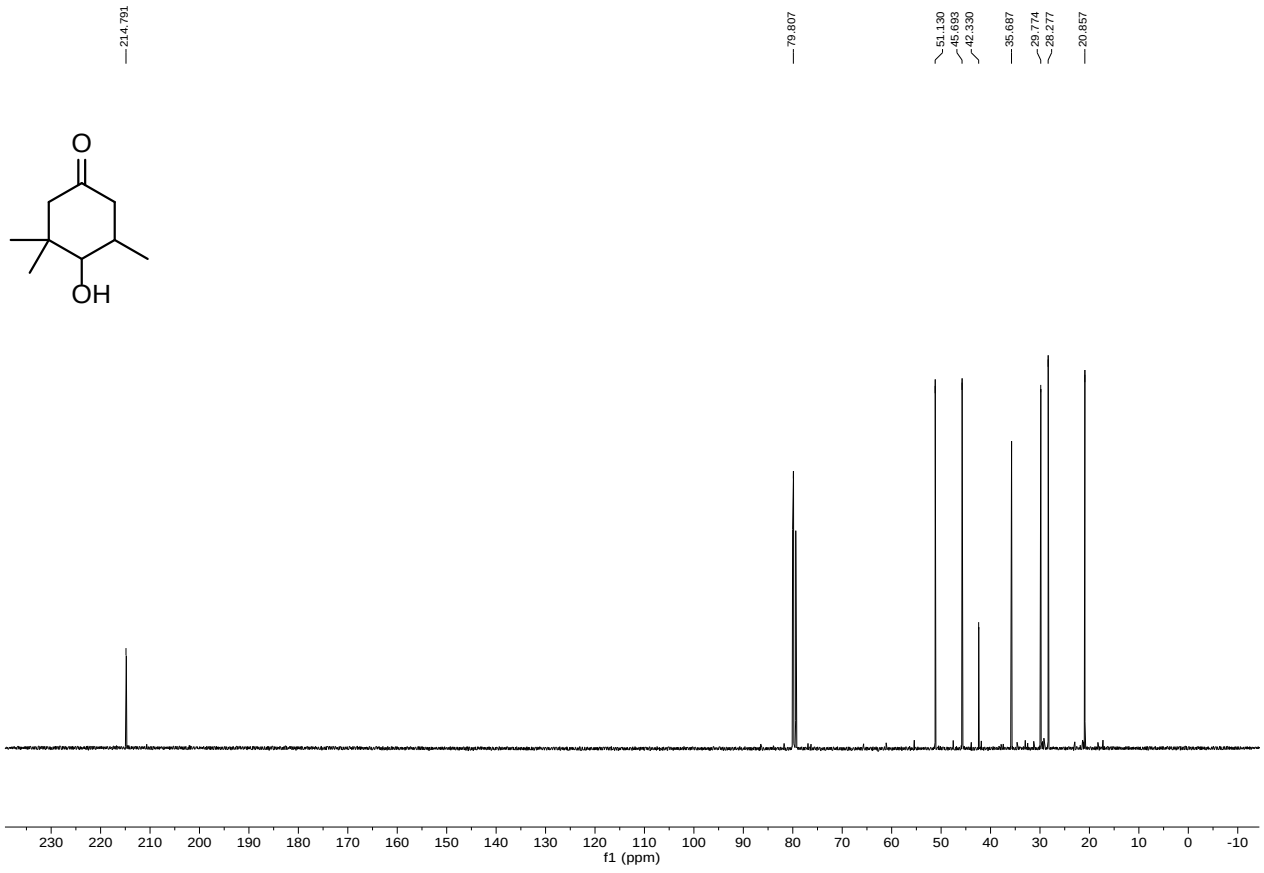


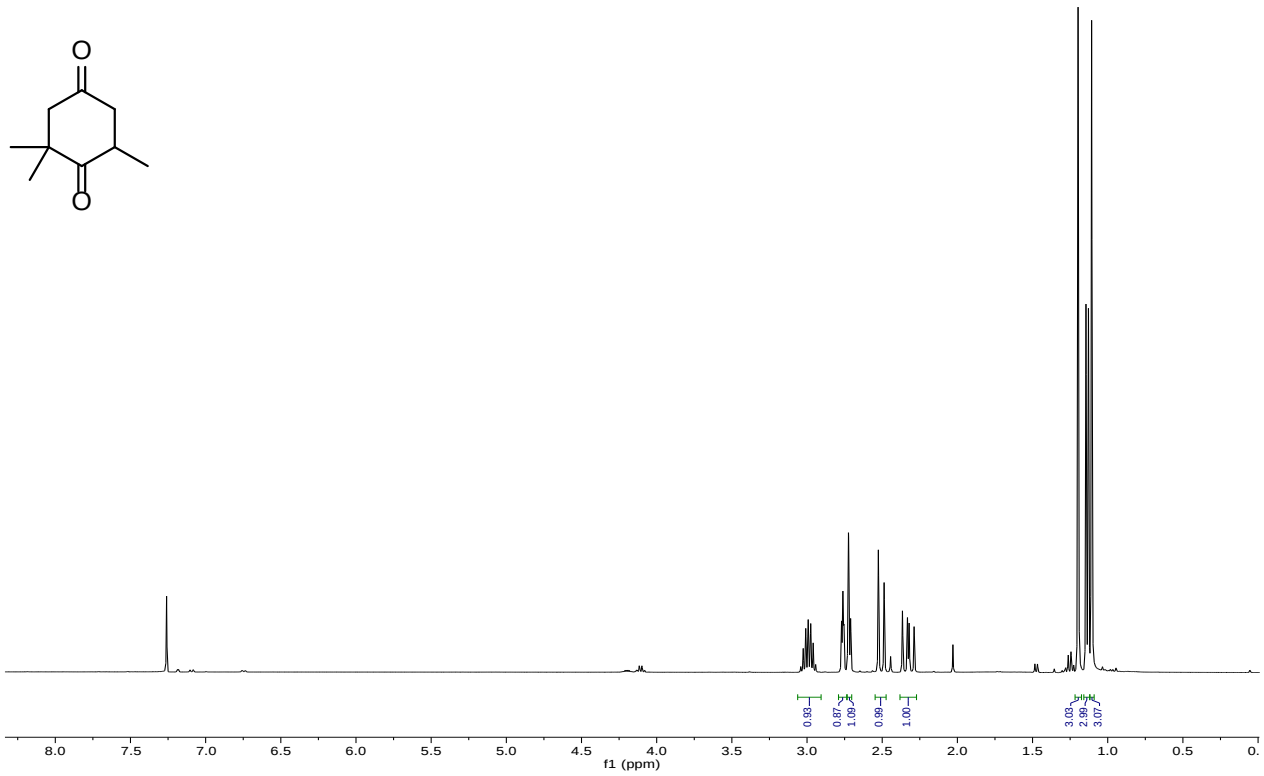
191.46
163.97
133.10
127.58
122.71
40.77
25.90
25.81
17.88
17.75
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

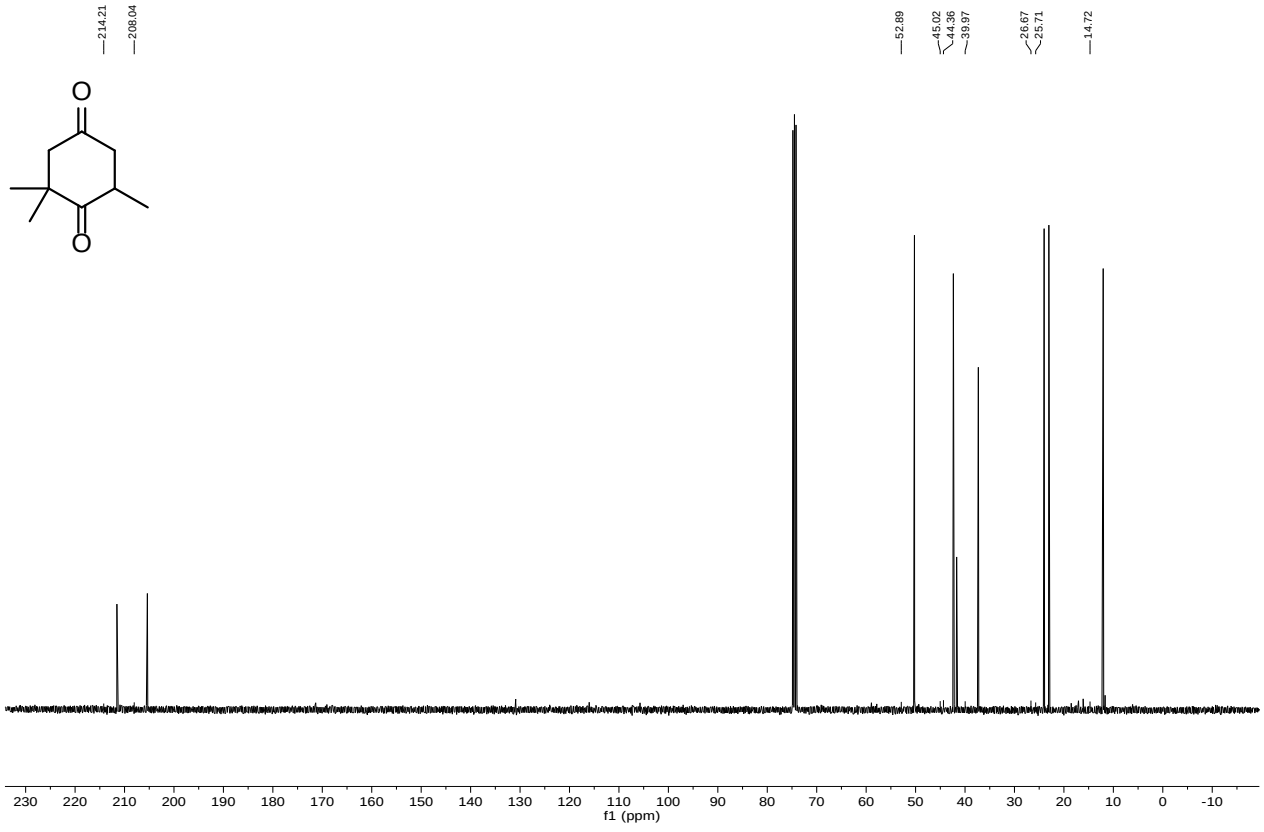

O
0.96
0.97
1.00
2.19
2.45
3.12
3.41
2.99
f1 (ppm)


190.94
163.94
133.86
128.81
122.40
32.75
27.20
25.79
25.22
17.88
O
f1 (ppm)


O
OH
0.97
1.00
1.04
1.06
1.12
0.89
1.10
2.88
2.86
2.82
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


214.791
79.807
51.130
45.693
42.330
35.687
29.774
28.277
20.857
O
OH
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


O
O
0.93
0.87
1.09
0.99
1.00
3.03
2.99
3.07
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


214.21
208.04
52.89
45.02
44.36
39.97
26.67
25.71
14.72
O
O
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

Homology model of FDR-Rh1

The structural homology model of FDR-Rh1 was built using the crystal structure of the F_{420}-complexed Ddn deazaflavin-dependent nitroreductase from *Nocardia farcinica* (PDB:3R5Z, 40% sequence identity with FDR-Rh1). For building the homology model, the Phyre[2] server (Protein Homology/analogY Recognition Engine V 2.0) was used in the default mode using the protein sequence of FDR-Rh1 (http:// http://www.sbg.bio.ic.ac.uk/phyre2).[12] For visualization, PyMol was used. The F_{420} cofactor, as bound to the Ddn, was superimposed in the FDR-Rh1 structural model.

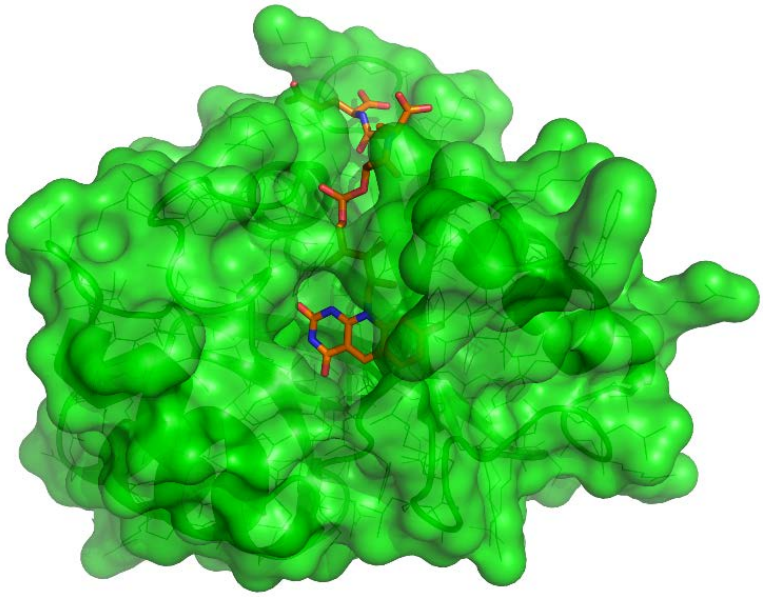


Fig. S6. Structural model of F_{420}-complexed FDR-Rh1 with the F_{420} cofactor in orange (C atoms).

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