Supporting Information

C3 and C6 Modification-Specific OYE Biotransformations of Synthetic Carvones and Sequential BVMO Chemoenzymatic Synthesis of Chiral Caprolactones**

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Supporting Information

C3 and C6 Modification-specific OYE biotransformations of synthetic carvones and sequential BVO chemoenzymatic synthesis of chiral caprolactones

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A hexane solution of $^n$BuLi (59.44 mL, 83.20 mmol, 1.4 M) was added dropwise to a cold solution (~10 °C under nitrogen gas) of DIPA (11.7 mL, 83.20 mmol) in anhydrous THF (60 mL) over a period of 10 min. The mixture was continuously stirred for 10 min to allow LDA formation. (-)-Carvone (9.6 g, 63.8 mmol) in anhydrous THF (90 mL) was slowly added and the mixture stirred for 45 min at the same temperature. The lithium enolate was then reacted with an excess of methyl iodide CH$_3$I (20 mL, 321.3 mmol), and the reaction mixture allowed stirring overnight at RT. It was extracted with diethyl ether (3 x 30 mL), acidified with 3M aq. HCl and then washed with NaHCO$_3$ and brine. The combined organic extracts were dried (MgSO$_4$), filtered, and solvent removed in vacuo. The residue was chromatographed (eluent: EtOAc:hexane 40:1–20:1) to give a 3:2 epimeric mixture of 6-methyl carvone (9.63 g, 85% yield) as a pale yellow oil.

To a diastereomeric mixture of 6-methylcarvone (1 g, 6.09 mmol) in CH$_2$Cl$_2$ (10 mL), was added DBU (0.9 mL, 6.09 mmol) and the reaction mixture was allowed to stir for 24 h at room temperature. The reaction mixture was washed with 1 M HCl (1 x 30 mL), water (1 x 30 mL) and brine (1 x 30 mL). The organic layer was dried (MgSO$_4$), solvents removed in vacuo and column chromatography eluting with hexane:EtOAc (40:1–50:1) afforded a 3:1 epimeric mixture of 6-methylcarvone. Partial separation of diastereomers (250 mg), providing major diastereomer 2 (80 mg, 32%, $R_f$ 0.54) as white crystals, and, minor diastereomer 3, (20 mg, 8%, $R_f$ 0.52) as a colourless oil, along with 150 mg of a diastereomeric mixture (60%).

The mixture of isomers (200 mg) was also recrystallized using hexane (~10 °C) to yield white crystals of 2 (76 mg, 38%).
Supporting Information

(6S)-Diastereomer (2): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.69–6.66 (m, 1H, CH=CCH$_3$, H-3), 4.81–4.79 (m, 2H, CH$_2$=C, H-9), 2.50–2.39 (m, 2H, CH$_2$CHCH and CH$_2$CHCH, H-6 and H-5), 2.35 (dt, $J = 13.0, 6.5$ Hz, 1H, CH$_2$CHCH, H-4), 2.29–2.21 (m, 1H, CH$_2$CHCH, H-4), 1.76 (dt, $J = 2.5, 1.3$ Hz, 3H, CH$_2$=CCH$_3$), 1.69 (dd, $J = 1.4, 0.8$ Hz, 3H, COCHCH$_3$), 1.03 (d, $J = 6.6$ Hz, 3H, CH$_2$CHC=O).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 201.9 (C=O), 145.8 (CH$_3$C=CH$_2$, C-8), 143.5 (CH$_3$C=CH, C-3), 134.9 (CH$_3$C=CH, C-2), 113.3 (CH$_3$C=CH$_2$, C-9), 50.7 (CH$_2$CHCH, C-5), 44.4 (CH$_2$CHCH, C-6), 31.3 (CH$_2$CHCH, C-4), 18.3 (CH$_2$=CCH$_3$, C-10), 16.3 (COCHCH$_3$, C-7), 12.7 (CH$_3$CHC=O, C-11).

MS (ES) $m/z$ 165 (MH$^+$, 100%); oil; Anal. Calc. for C$_{11}$H$_{16}$O, Expected C, 80.4; H, 9.8; Found C, 80.1; H, 10.0; IR $\nu_{\text{max}}$ 2986, 2971, 2924, 1664 (C=O), 1444, 1365, 1290, 1134, 1045. 951, 846 cm$^{-1}$; $[\alpha]_D$ +12.9° (c 1.7, CH$_2$Cl$_2$, 24 °C); m.p 37–38 °C.

(6R)-Diastereomer (3): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.72 (ddt, $J = 5.7, 2.7, 1.4$ Hz, 1H, CH=CCH$_3$, H-3), 4.91 (dq, $J = 2.7, 1.4$ Hz, 1H, CH$_2$=C, H-9), 4.72 (s, 1H, CH$_2$=C, H-9), 2.73–2.63 (m, 2H, CH$_2$CHCH and CH$_2$CHCH, H-6 and H-5), 2.50–2.40 (m, 1H, CH$_2$CHCH, H-4), 2.32–2.24 (m, 1H, CH$_2$CHCH, H-4), 1.77 (dt, $J = 2.5, 1.3$ Hz, 3H, CH$_2$=CCH$_3$), 1.69 (dt, $J = 1.3, 0.7$ Hz 3H, COCHCH$_3$), 0.90 (d, $J = 7.2$ Hz, 3H, CH$_2$CHC=O).

$^{13}$C NMR (100 MHz; CDCl$_3$) $\delta$ 203.7 (C=O), 145.0 (CH$_3$C=CH$_2$, C-8), 144.2 (CH$_3$C=CH, C-3), 133.8 (CH$_3$C=CH, C-2), 111.6 (CH$_3$C=CH$_2$, C-9), 44.9 (CH$_2$CHCH, C-5), 43.1 (CH$_2$CHCH, C-6), 26.4 (CH$_2$CHCH, C-4), 22.0 (CH$_2$=CCH$_3$, C-10), 16.1 (COCHCH$_3$, C-7), 10.6 (CH$_3$CHC=O, C-11).

MS (ES) $m/z$ 165 (MH$^+$, 80%); IR $\nu_{\text{max}}$ 2969, 2923, 1667 (C=O), 1450, 1365, 1249, 1130, 1061, 989, 888 cm$^{-1}$; oil; IR $\nu_{\text{max}}$ 2969, 2923, 1667 (C=O), 1450, 1365, 1249, 1130, 1061, 989, 888 cm$^{-1}$; $[\alpha]_D$ +28.5° (c 0.7, CH$_2$Cl$_2$, 24 °C).
Supporting Information

5S,6R)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-one, 4, and
(5S,6S)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-one, 5

DIPA (5.7 mL, 40.4 mmol) was purged to dry THF (50 mL) under N₂ gas, and a hexane solution of n-BuLi (19.4 mL, 40.6 mmol, 2.09 M) was slowly dropped at 0 °C. The reaction mixture was allowed to stir for further 2 h, and the vessel was cooled to −78 °C. Thence, R-(-)-carvone (5 g, 33.3 mmol) in fresh THF (50 mL) was dropwise added via cannula, and the reaction aged for 30 min. The reaction mixture was quenched with TMSCl (8.3 mL, 65.7 mmol in 50 mL THF), and the ice-acetone removed to allow the reaction to stir for an additional 60 min at ambient temperature. The solvent was removed in vacuo, and the concentrated organic layer was added to Celite®. The slurry was taken up with pentane (200 mL), and more pentane was added to wash the precipitate. The organic layer was washed with Na₂S₂O₃ (2 x 30 mL), saturated NaHCO₃ (2 x 30 mL) and water (2 x 50 mL), dried over (MgSO₄) and concentrated to give silylenol ether (6.80 g, 92%) as a yellow oil.

Dry DCM was added to a round bottomed flask (70 mL) containing silylenol ether (6.50 g, 27.3 mmol) under nitrogen atmosphere, and m-CPBA (7 g, 40.6 mmol) in dry DCM (150 mL) was dropwise transferred from second round flask at 0 °C. The reaction mixture was allowed to warm at RT, and stirred for further 60 min. Then, the mixture was washed with Na₂S₂O₃ (1 x 30 mL), and neutralised to pH = 7.0 via adding saturated NaHCO₃. The solid was filtered off by through a pad of Celite®, and the filtrate was concentrated under vacuum to yield a mixture of silylenol ether diastereomers (6.05 g, 76%) as a yellowish-green oil. HCl (50 mL, 1.5 M) was added to a mixture of silylenol ether (25.38 mmol), and allowed to stir overnight at RT. The mixture was neutralised with NaOH (0.1 M), and washed with water (3 x 20 mL), brine (3 x 20 mL), dried over (MgSO₄), and solvent removed in vacuo. The mixture was purified with flash column chromatography using
hex:EtOAc (4:1) to afford separable diastereomers of *anti* isomer of 6-hydroxycarvone 4 (2.49 g, 45%) and *syn* 5 (0.7 g, 13%) as a yellow oil.

**(6S)-Diastereomer (4):** ¹H NMR (400 MHz, CDCl₃) δ 6.75–6.71 (m, 1H, C=CH, H-3), 4.92–4.89 (m, 2H, CH₂=C, 2 x H-9), 4.13 (d, 1H, J = 12.7 Hz, CHOH, H-6), 3.78 (s, 1H, OH), 2.67 (ddd, J = 12.7, 11.0, 5.1 Hz, 1H, CH₂CHCH, H-5), 2.51–2.41 (m, 1H, CH₂CHCH, H-4) 2.39–2.31 (m, 1H, CH₂CHCH, H-4), 1.82–1.81 (m, 6H, 2 x CH₃).

¹³C NMR (100 MHz, CDCl₃) δ 200.7 (C=O), 145.8 (C=CH₂, C-8), 144.3 (C=CH, C-3), 133.1 (C=CH, C-2), 113.7 (C=CH₂, C-9), 74.5 (CHOH, C-9), 151.2 (CH₂CHCH, C-5), 30.8 (CH₂CHCH, C-4), 18.9 (CH₃), 15.5 (CH₃).

HRMS (ES) m/z for C₁₀H₁₄O₂Na [MNa]+, (Calc.: 189.0891, Found: 189.0882); oil; IR νmax 3476 OH), 2971, 2922, 2892, 1670 (C=O), 1646, 1434, 1371, 1286, 1136, 931 cm⁻¹, [α]D -33° (c 1.4, CH₂Cl₂, 24 °C).

**(6R)-Diastereomer (5):** ¹H NMR (400 MHz, CDCl₃) δ 6.65 (ddt, J = 4.1, 2.7, 1.4 Hz, 1H, C=CH, H-3), 4.83 (quint, J = 1.5 Hz, 1H, CH₂=C, H-9), 4.69–4.67 (m, 1H, CH₂=C, H-9), 4.40 (d, 1H, J = 5.9 Hz, CHOH, H-6), 3.60 (s, 1H, OH), 3.17–3.13 (m, 1H, CHCHOH, H-5), 2.74–2.65 (m, Hz, 1H, CH₂CHCH, H-4), 2.55–2.47 (m, 1H, CH₂CHCH, H-4) 1.80 (dt, J = 2.7, 1.4 Hz, 3H, COCH₃), 1.66–1.65 (m, 3H, CH₂=CH₂).

¹³C NMR (100 MHz, CDCl₃) δ 199.9 (C=O), 144.2 (C=CH₂, C-8), 143.1 (C=CH, C-3), 133.8 (C=CH, C-2), 113.9 (C=CH₂, C-9), 74.7 (CHOH, C-6), 47.1 (CH₂CHCH, C-5), 29.8 (CH₂CHCH, C-4), 23.2 (CH₃C=CH₂, C-10), 15.4 (COCH₃, C-7).

HRMS (ES) m/z for C₁₀H₁₄O₂Na [MNa]+, (Calc.: 189.0891, Found: 189.0885); oil; IR νmax 3463 OH), 2964, 2922, 1711(C=O), 1670, 1433, 1360, 1256, 1132, 870 cm⁻¹, [α]D -10.0° (c 0.88, CH₂Cl₂, 24 °C).

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**Supporting Information**
(5R,6R)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one, 7, and (5R,6S)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one, 8

The title compounds were prepared from S-(+)-carvone 6 according to the procedure used for 4 and 5, to afford anti 7 (1.88 g, 34%) and syn 8 (0.51 g, 9.2%) isomers as a colourless oil.

(6R)-Diastereomer (7): 1H NMR (400 MHz; CDCl₃) δ 6.76–6.74 (m, 1H, C=CH, H-3), 4.94–4.91 m, 2H, CH₂=C, 2 x H-9), 4.15 (dd, J = 12.7, 1.9 Hz, 1H, CHO, H-6), 3.79 (d, J = 1.8 Hz, 1H, OH), 2.69 (ddd, J = 12.7, 11.0, 5.1 Hz, 1H, CH₂CHCH, H-5), 2.52–2.43 (m, 1H, CH₂CHCH, H-4), 2.41–2.33 (m, 1H, CH₂CHCH, H-4), 1.84–1.83 (m, 6H, 2 x CH₃).

13C NMR (100 MHz, CDCl₃) δ 200.7 (C=O), 145.8 (C=CH₂, C-8), 144.3 (C=CH, C-3), 133.2 (C=CH₂, C-2), 113.7 (C=CH₂, C-9), 74.5 (CH₂OH, C-6), 51.2 (CH₂CHCH, C-5), 30.8 (CH₂CHCH, C-4), 18.9 (CH₃), 15.5 (CH₃).

MS (ES) m/z 189 (MNa⁺, 90%), 167 (MH⁺, 70%); oil; IR νmax 3477 (OH), 2971, 2892, 1724 (C=O), 1670, 1434, 1371, 1242, 1136, 891 cm⁻¹; [α]D +28 ° (c 0.50, CH₂Cl₂, 24 °C).

(6S)-Diastereomer (8): 1H NMR (400 MHz; CDCl₃) δ 6.67–6.66 (m, 1H, 1H, C=CH, H-3), 4.86 (quint, J = 1.5 Hz, 1H, CH₂=C, H-9), 4.71–4.70 (m, 1H, CH₂=C, H-9), 4.43 (dd, J = 6.0, 2.5 Hz, 1H, CH₂CHCH, H-6), 3.60 (d, J = 2.5 Hz, 1H, OH), 3.21–3.18 (m, 1H, CH₂CHCH, H-5), 2.78–2.69 (m, 1H, CH₂CHCH, H-4), 2.57–2.50 (m, 1H, CH₂CHCH, H-4), 1.83 (dt, J = 2.7, 1.4 Hz, 3H, COCH₃), 1.69 (dd, J = 1.3, 0.7 Hz, 3H, CH₃CH=CH₂).

13C NMR (100 MHz, CDCl₃) δ 200.0 (C=O), 144.2 (C=CH₂, C-8), 143.2 (C=CH, C-3), 133.9 (C=CH, C-2), 114.0 (C=CH₂, C-9), 74.8 (CH₂OH, C-6), 47.1 (CH₂CHCH, C-5), 29.9 (CH₂CHCH, C-4), 23.2 (CH₃C=CH₂, C-10), 15.4 (COCH₃, C-7).

MS (ES) m/z 189 (MNa⁺, 80%), 167 (MH⁺, 60%); oil; IR νmax 3477 (OH), 2971, 2892, 1724 (C=O), 1670, 1434, 1371, 1242, 1136, 891 cm⁻¹; [α]D −90 ° (c 0.2, CH₂Cl₂, 24 °C).
Supporting Information

(R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one, 10

Methylmagnesium iodide (2.9 mL, 3.0 M, 1.3 eq.) was added to diethyl ether (10 mL) in round bottomed flask (250 mL) under N<sub>2</sub> atmosphere. Then, S- (+)-carvone 6 (1 g, 6.65 mmol) in dry ether (7 mL) was slowly added over 30 min at -5 °C, and the colour of mixture changed from brown to grey. The reaction was allowed to warm gradually, and stirred for further 3 h at room temperature. The mixture was quenched with saturated NH<sub>4</sub>Cl (20 mL), extracted with diethyl ether (100 mL), and dried over (MgSO<sub>4</sub>). The solvent was removed under reduced pressure to afford alcohol intermediate product. A DCM solution of the alcohol was added to an orange suspension of PCC (2.5 g, 11.7 mmol, 1.8 eq.) and silica gel (2.5 g) in dry DCM (10 mL). The colour of the reaction mixture was altered to dark brown. The reaction mixture was allowed to stir for 3 h at ambient temperature, then, filtered off via silica gel using DCM as eluent, and solvents removed in vacuo. The product was purified via flash column chromatography using hex:EtOAc (40:1), to give 3-methylcarvone (1.01 g, 84%) as a yellow oil.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.79–4.77 (m, 1H, C=CH₂, H-9), 4.74–4.73 (m, 1H, C=CH₂, H-9), 2.66–2.58 (m, 1H, CH₂CHCH₂, H-6), 2.56–2.51 (m, 1H, CH₂CHCH₂, H-5), 2.37–2.32 (m, 2H, CH₂CHCH₂, 2 x H-4), 2.31–2.24 (m, 1H, CH₂CHCH₂, H-6), 1.95 (q, J = 0.8 Hz, 3H, 3 x H-7, CH₃C=CCH₃), 1.76–1.75 (m, 3H, 3 x H-11, CH₃C=CCH₃), 1.74–1.73 (m, 3H, CH₂=CHCCH₃).

<sup>13</sup>C NMR (100 MHz; CDCl<sub>3</sub>) δ 199.1 (C=O), 154.3 (CH₃C=CCH₃, C-3), 146.9 (CH₃C=CCH₃, C-2), 130.8 (C=CH₂, C-8), 110.3 (C=CH₂, C-9), 42.5 (CH₂CHCH₂, C-6), 41.4 (CH₂CHCH₂, C-5), 38.1 (CH₂CHCH₂, C-4), 21.7 (CH₃C=CCH₃, C-7), 20.6 (CH₃, CH₃C=CCH₃, C-11), 10.9 (CH₃C=CH₂, C-10).

MS (ES) m/z 165 (MH<sup>+</sup>, 100%); colourless oil; Anal. Calc. for C₁₁H₁₆O, Expected C, 80.4; H, 9.8; Found C, 79.3; H, 10.0; IR <i>ν</i><sub>max</sub> 2955, 2932, 2917, 1661 (C=O), 1433, 1376, 1257, 1142, 108, 889 cm<sup>-1</sup>; lit <i>[α]<sub>D</sub></i> -100.6 °, <i>[α]<sub>D</sub></i> -100.4 ° (c 0.56, CH₂Cl₂, 24 °C).
Supporting Information

\((2S,3R,6R)\)-2,6-Dimethyl-3- (prop-1-en-2-yl)cyclohexan-1-one, 11

\((2S,3R,6S)\)-2,6-Dimethyl-3- (prop-1-en-2-yl)cyclohexan-1-one, 12

\((2R,3R,6R)\)-2,6-Dimethyl-3- (prop-1-en-2-yl)cyclohexan-1-one, 13, and

\((2R,3R,6S)\)-2,6-Dimethyl-3- (prop-1-en-2-yl)cyclohexan-1-one, 14  

Sodium dithionite Na\(_2\)S\(_2\)O\(_4\) (5.05 g, 29 mmol, 9 eq.) was added in one-portion to a two-necked round-bottomed flask containing 6-methylcarvone (2 and 3) (530 mg, 3.22 mmol, 1 eq.), sodium bicarbonate (4.87 g, 59.96 mmol, 18 eq.) and phase transfer catalyst (adogen, 175 mg, 0.97 mmol, 0.3 eq.) in toluene-water solvent (40:40 mL). The mixture was gently heated and refluxed for 1.5 h under nitrogen atmosphere. Then, the reaction was cooled, the organic layer was separated, and the aqueous phase was washed with diethyl ether (2 x 25 mL). The combined organic layer was washed with water (1 x 25 mL), dried over (MgSO\(_4\)) and the solvent was removed in vacuo. The yield was purified with flash column chromatography (hexane:Et\(_2\)O 20:1), and further separation was with normal phase HPLC (hexane:Et\(_2\)O 97:3) to furnish four diastereomers of product with combined yield\% (76\%) and ratio (48\%, 12\%, 36\%, 4\% 11:12:13:14) as colorless oil.

\((2S,3R,6R)\)-Diastereomer (11): \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 4.77 (s, 1H, C=CH\(_2\), H-9), 4.73 (s, 1H, C=CH\(_2\), H-9), 2.42 (dq, \(J = 12.7, 6.3\) Hz, 1H, CH\(_2\)CH\(_2\)CH, H-6), 2.36 (dq, \(J = 12.2, 6.0\) Hz, 1H, CH\(_2\)CH\(_2\)CH and CHCH\(_2\)CH, H-5 and H-3), 1.85 (qd, \(J = 12.9, 3.6\) Hz, 1H, CH\(_2\)CH\(_2\)CH, H-5), 1.76–1.72 (m, 1H, CH\(_2\)CH\(_2\)CH, H-4), 1.70 (s, 3H, CH\(_3\)C=CH\(_2\)), 1.37 (qd, \(J = 13.2, 3.6\) Hz, 1H, CH\(_2\)CH\(_2\)CH, H-4), 1.03 (d, \(J = 6.4\) Hz, 3H, CH\(_3\)CH\(_2\)CH\(_2\)), 0.91 (d, \(J = 6.4\) Hz, 3H, CH\(_3\)CH\(_2\)CH=O).

\(^{13}\)C NMR (100 MHz; CDCl\(_3\)) \(\delta\) 214.1 (C=O), 146.3 (C=CH\(_2\), C-8), 112.3 (C=CH\(_2\), C-9), 55.8 (CH\(_2\)CH\(_2\)CH, C-3), 47.6 (CH\(_2\)CH\(_2\)CH, C-6), 45.2 (CH\(_2\)CH\(_2\)CH, C-2), 35.7 (CH\(_2\)CH\(_2\)CH, C-5), 31.5 (CH\(_2\)CH\(_2\)CH, C-4), 18.1 (CH\(_3\)C=CH\(_2\), C-10), 14.8 (CH\(_3\)CH\(_2\)CH=O, C-7), 12.1 (CH\(_3\)CH\(_2\)CH, C-11).
Supporting Information

HRMS (APCI) m/z for C₁₁H₁₈O, [M]⁺, (Calc.: 166.1358, Found: 166.1354); oil; Anal. Calc. for C₁₁H₁₈O, Expected C, 79.5; H, 10.9; Found C, 79.4; H, 11.1; IR νₘₚₙₙ 2969, 2930, 2859, 1709 (C=O), 1645, 1450, 1375, 1177, 979, 865 cm⁻¹; [α]D +27 ° (c 0.22, CH₂Cl₂, 24 °C).

(2S,3R,6S)-Diastereomer (12):
¹H NMR (600 MHz; CDCl₃) δ 4.78–4.76 (m, 1H, C=CH₂, H-9), 2.60–2.52 (m, 2H, CHCH₂CH₂ and CH₂CHCH₂, H-2 and H-6), 2.16–2.10 (m, 1H, CH₂CH₂CH₂, H-3), 1.92–1.84 (m, 2H, CHCH₂CH₂ and CHCH₂CH₂, H-4 and H-5), 1.71 (s, 3H, CH₃C=CH₂), 1.64 (td, J = 8.4, 4.0 Hz, 1H, CHCH₂CH₂, H-4), 1.17 (d, J = 7.2 Hz, 3H, CH₃CHCH), 0.96 (d, J = 6.7 Hz, 3H, CH₃CHC=O).

¹³C NMR (100 MHz; CDCl₃) δ 216.9 (C=O), 146.4 (C=CH₂, C-8), 112.3 (C=CH₂, C-9), 53.6 (CHCH₂CH₂, C-3), 43.9 (CH₂CH₂CH₂, C-6), 43.6 (CHCHCH₂, C-2), 31.6 (CH CH₂CH₂, C-5), 25.6 (CHCH₂CH₂, C-4), 18.9 (CH₃C=CH₂, C-10), 16.9 (CH₃CHC=O, C-7), 13.2 (CH₃CHCH, C-11).

MS (ES) m/z 167 (MH⁺, 70%), 189 (MNa⁺, 60%); oil; Anal. Calc. for C₁₁H₁₈O, Expected C, 79.5; H, 10.9; Found C, 79.5; H, 10.9; IR νₘₚₙₙ 2963, 2929, 2858, 1708 (C=O), 1644, 1456, 1376, 1133, 896 cm⁻¹; [α]D +18 ° (c 0.15, CH₂Cl₂, 24 °C).

(2R,3R,6R)-Diastereomer (13):
¹H NMR (600 MHz; CDCl₃) δ 4.89–4.88 (m, 1H, C=CH₂, H-9), 2.72-2.68 (m, 1H, CHCH₂CH₂, H-2), 2.59 (dq, J = 12.8, 6.4 Hz, 1H, CH₂CH₂CH₂, H-6), 2.36 (dt, J = 12.4, 4.0 Hz, 1H, CHCHCH₂, H-3), 2.06 (ddt, J = 13.2, 6.0, 3.4 Hz, 1H, CHCH₂CH₂, H-5), 1.90 (qd, J = 13.0, 3.7 Hz, 1H, CHCH₂CH₂, H-5), 1.70–1.68 (m, 1H, CHCH₂CH₂, H-4), 1.67 (s, 3H, CH₃C=CH₂), 1.29 (qd, J = 13.0, 3.7 Hz, 1H, CHCH₂CH₂, H-4), 1.01 (d, J = 6.4 Hz, 3H, CH₃CHCH), 0.94 (d, J = 7.4 Hz, 3H, CH₃CHC=O).

¹³C NMR (100 MHz; CDCl₃) δ 216.8 (C=O), 145.2 (C=CH₂, C-8), 111.3 (C=CH₂, C-9), 48.4 (CHCHCH₂, C-3), 46.8 (CHCHCH₂, C-2), 40.0 (CH₂CH₂CH₂, C-6), 34.4 (CHCH₂CH₂, C-4), 23.8 (CHCH₂CH₂, C-5), 22.1 (CH₃C=CH₂, C-10), 14.6 (CH₃CHC=O, C-7), 11.9 (CH₃, CH₃CHCH, C-11).

HRMS (APCI) m/z for C₁₁H₁₈O, [M]⁺, (Calc.: 166.1358, Found: 166.1353); Anal. Calc. for C₁₁H₁₈O, Expected C, 79.5; H, 10.9; Found C, 78.8; H, 11.2; IR νₘₚₙₙ 2969, 2931, 2869,1706 (C=O), 1645, 1451, 1375, 1129, 888 cm⁻¹; [α]D −46 ° (c 0.26, CH₂Cl₂, 24 °C).
Supporting Information

(2R,3R,6S)-Diastereomer (14): ¹H NMR (600 MHz; CDCl₃) δ 4.88 (quintet, J = 1.3 Hz 1H, C=CH₂, H-9), 4.38 (dq, J = 1.3, 0.9 Hz 1H, C=CH₂, H-9), 2.78–2.75 (m 1H, CH₂CH₂CH₂, H-6), 2.61 (qdd, J = 6.8, 5.6, 1.8 Hz 1H, CHCH₂CH₂, H-2), 2.36 (d quintet, J = 12.5, 6.3, 1.2 Hz 1H, CHCH₂CH₂, H-3), 2.01–1.93 (m, 1H, CHCH₂CH₂, H-5), 1.92–1.86 (m, 1H, CHCH₂CH₂, H-4), 1.83 (dq, J = 13.6, 3.4 Hz 1H, CHCH₂CH₂, H-7), 1.70–1.60 (m, 4H, CH₃C=CH₂ and CHCH₂CH₂, H-4), 1.06 (d, J = 7.0 Hz 3H, CH₃CHCH). 1.03 (d, J = 6.6 Hz 3H, CH₂CH₂=CH₃).

¹³C NMR (100 MHz; CDCl₃) δ 215.2 (C=O), 143.9 (C=CH₂, C-8), 113.1 (C=CH₂, C-9), 50.0 (CH₂CH₂CH₂, C-6), 47.4 (CHCH₂CH₂, C-2), 44.9 (CHCH₂CH₂, C-3), 31.1 (CHCH₂CH₂, C-5), 28.7 (CHCH₂CH₂, C-4), 24.2 (CH₃C=CH₂, C-10), 15.0 (CH₃CH₂CH₂=O, C-7), 12.4 (CH₃CHCH₂, C-11).

HRMS (APCI) m/z for C₁₁H₁₅O, [M]+, (Calc.: 166.1358, Found: 166.1355); oil; Anal. Calc. for C₁₁H₁₅O, Expected C, 79.5; H, 10.9; Found C, 79.0; H, 11.0; IR νmax 2970, 2934, 2873, 1708 (C=O), 1645, 1453, 1375, 1132, 892 cm⁻¹; [α]D +14 ° (c 0.10, CH₂Cl₂, 24 °C).

Scale up bioreduction of (5R,6R)-6-methylcarvone 3 via PETNR

The enzyme (1mL of 150 µM stock, diluted to 2 µM in reaction), substrate (63 mg dissolved in 1.5 mL EtOH), NADP⁺ (15 µM), G-6-PDH (750 U stock diluted to 10U in reaction), glucose (216 mg, final dilution reaction employed 15 mM) were added to 75 mL buffer solution of KP (pH 7.0, 50 mM), and the mixture incubated for 2.5 h at 30 °C at 135 rpm. TLC plate showed no starting material was detected, and the organic layer extracted and dried to afford pure isomer of (2R,3R,6R)-2-methylidihydrocarvone 13 (53 mg, 84%). The product was characterised with ¹H NMR spectroscopy, and showed identical spectrum to 13. It was run on GC using DB-Wax column to yield one peak at retention times 20.97 min.

(2S,3S,6R)-2-Hydroxy-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 15, and (2S,3S,6S)-2-Hydroxy-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 16 (mixture of isomers)
Supporting Information

To a two-necked round bottomed flask containing *anti* 6-hydroxycarvone 4 (300 mg, 1.8 mmol, 1 eq.), sodium bicarbonate (42.63 g, 32.4 mmol, 18 eq.) and phase transfer catalyst (adogen, 94.5 mg, 0.51 mmol, 0.3 eq.) in toluene-water solvent system (20:20 mL), sodium dithionite (2.73 g, 15.6 mmol, 9 eq.) were added. The mixture was gently heated and reflexed in oil-bath for 1 h under nitrogen atmosphere. Then, the reaction was cooled, and the organic layer was separated, and the aqueous phase was washed with diethyl ether (1 x 20 mL). The combined organic layer was washed with water (1 x 10 mL), dried over (MgSO₄) and solvent removed *in vacuo*. The product was purified by flash column chromatography (hexane:EtoAc 7:1) to furnish (96:4) diastereomers (212 mg, 70%, with Rᵣ 0.16 in hex: EtoAc 3:1) as a colourless oil.

**(6R)-Diastereomer (15):** ¹H NMR (400 MHz; CDCl₃) δ 4.89 (quint, J = 1.6, 1H, C=CH₂, H-9), 4.86–4.85 (m, 1H, C=CH₂, H-9), 4.09 (ddd, J = 11.4, 3.8, 1.6 Hz, 1H, HOH, H-2), 3.67 (d, J = 3.9 Hz, 1H, OH), 2.50 (dqd, J = 12.8, 6.4, 1.6 Hz, 1H, CH₂CH₂H, H-6), 2.25–2.18 (m, 1H, CHCH₂CH₂, H-3), 2.10 (ddt, J = 13.2, 6.3, 3.2 Hz, 1H, CHCH₂CH₂, H-5) 1.81 (dd, J = 1.5, 0.8 Hz, 3H, CH₃=C-CH₂), 1.79–1.78 (m, 1H, CHCH₂CH₂, H-5), 1.76 (d, J = 3.3 Hz, 1H, CHCH₂CH₂, H-4), 1.43–1.32 (m, 1H, CHCH₂CH₂, H-4), 1.11 (d, J = 6.5 Hz, 3H, CH₃CH₂CO₂H).

¹³C NMR (100 MHz; CDCl₃) δ 212.3 (C=O), 145.1 (C=CH₂, C-8), 112.6 (C=CH₂, C-9), 76.8 (CH₂OH, C-2), 56.1 (CH₂CH₂CH₂, C-6), 43.2 (CH₂CH₂CH₂, C-3), 34.9 (CH₂CH₂CH₂, C-4), 29.0 (CH₂CH₂CH₂, C-5), 19.3 (CH₂C=CH₂, C-10), 14.0 (CH₃C=CH₂, C-7).

**(248 and 249 mixture):** HRMS (APCI) m/z for C₁₀H₁₆O₂, [M⁺] (Calc.: 168.1145, Found: 168.1148); oil; IR νmax 3473 (OH), 2969, 2932, 2854, 1708 (C=O), 1666, 1448, 1375, 1233, 1126, 890 cm⁻¹.

**(2R,3S,6R)-2-Hydroxy-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 17, and (2R,3S,6S)-2-Hydroxy-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 18 (mixture of isomers)**
Supporting Information

The title compounds were prepared from syn 6-hydroxycarvone 5 according to the procedure used for 15 and 16. The product (170.0 mg, 56% with Rf 0.13 in hex; EtOAc 3:1, oil) was obtained as a colourless mixture of diastereomers (d.r. 95:5).

(6R)-Diastereomer (17): 1H NMR (400 MHz; CDCl3) δ 5.03–5.01 (m, 1H, CH=H2, H-9), 4.84–4.83 (m, 1H, CH=CH2, H-9), 4.11 (d, J = 3.1 Hz, 1H, CHO, H-2), 3.02–2.93 (m, 1H, CHCH2CH2, H-3), 2.46 (dt, J = 10.7, 3.3 Hz, 1H, CH2CH2CH2, H-6), 2.3 (bro s, 1H, OH), 2.01–1.98 (m, 2H, CH2CH2H, and CHCHH2, H-4 and H-5), 1.76 (dt, J = 1.3, 0.7 Hz, 3H, CH3=CH2), 1.68–1.58 (m, 1H, CHCH2CH2, H-5), 1.36–1.26 (m, 1H, CHCH2CH2, H-4) 1.06 (d, J = 6.7 Hz, 3H, CH3CH=O).

13C NMR (100 MHz; CDCl3) δ 213.7 (C=O), 143.9 (C=CH2, C-8), 113.3 (C=CH2, C-9), 75.1 (CHO, C-2), 51.2 (CH2CH2CH, C-6), 40.3 (CHCHCH2, C-3), 33.6 (CHCH2CH2, C-4), 23.1 (CHCH2CH2, C-5), 22.7 (CH3=C=CH2, C-10), 14.8 (CH3CH=O, C-7).

(17 and 18 mixture): HRMS (APCI) m/z for C10H16O2, [M]+ (Calc.: 168.1145, Found: 168.1147); oil, IR νmax 3452 (OH), 2968, 2932, 2855, 1709 (C=O), 1646, 1449, 1376, 1231, 1180, 855 cm−1.

Scale up bioreduction of (5S,6R)-6-hydroxycarvone 5

An absolute EtOH solution of substrate 5 (93 mg in 2.5 mL; dilution to 5 mM in reaction) was added to buffer solution of KP (PH 7.0, 50 mM), followed by addition of of OYE2 (2 mL of 112 μM stock to 115 mL total, 2 μM), NADP+ (15 μM), G-6-PDH (1200 U), glucose (320 mg total, 15 mM in final dilution) and the volume was completed to 115 mL with buffer solution. The reaction was incubated for 2.5 h at 30 °C, and extracted with ethyl acetate (3 x 50 mL). The organic layer was dried, concentrated, and purified via flash column chromatography using hex:EtOAc (5:1–3:1) as eluent to yield 10 mg (11%), d.e. >99% of one diastereomer of (2R,3S,6R)-2-hydroxydihydrocarvone 17. The product was confirmed using NMR, and IR spectroscopies, and run on GC using achiral DB-Wax column to give one peak at retention time 30.974 min.
(2R,3R,6S)-2-Hydroxy-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 20, and (2R,3R,6R)-2-Hydroxy-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 19 (mixture of isomers)

The title compounds were prepared from anti 6-hydroxycarvone 8 according to the procedure used for 15 and 16. The yielded was (72 mg, 24% with Rf 0.16 in hex; EtOAc 3:1) of diastereomers mixture (10:1) as colourless oil.

(6S)-Diastereomer (20): 1H NMR (400 MHz; CDCl₃) δ 4.91 (quint, J = 1.6 Hz, 1H, C=CH₂, H-9), 4.87–4.86 (m, 1H, C=CH₂, H-9), 4.10 (ddd, J = 11.3, 3.7, 1.5 Hz, 1H, CHO, H-2), 3.68 (d, J = 3.8 Hz, 1H, OH), 2.50 (d, quintet, J = 12.8, 6.4, 1.6 Hz, 1H, CH₂CH₂CH₂, H-6), 2.26–2.19 (m, 1H, CHCH₂CH₂, H-3), 2.12 (ddt, J = 13.3, 6.2, 3.2 Hz, 1H, CHCH₂CH₂, H-4), 1.81 (dd, J = 1.4, 0.7 Hz, 3H, CH₃C=CH₂), 1.80–1.77 (m, 2H, CHCH₂CH₂, H-4 and CHCH₂CH₂, H-5) 1.44–1.33 (m, 1H, CHCH₂CH₂, H-5), 1.11 (d, J = 6.5 Hz, 3H, CH₃CHC=O).

13C NMR (100 MHz; CDCl₃) δ 212.3 (C=O), 145.1 (C=CH₂, C-8), 112.6 (C=CH, C-3), 76.8 (CHOH, C-2), 56.1 (CH₂CH₂CH₂, C-6), 43.3 (CHCH₂CH₂, C-3), 35.0 (CHCH₂CH₂, C-4), 29.0 (CHCH₂CH₂, C-5), 19.4 (CH₃C=CH₂, C-10), 14.1 (CH₃CHC=O, C-7).

(20 and 19 mixture): HRMS (APCI) m/z for C₁₀H₁₆O₂, [M]+, (Calc.: 168.1145, Found: 168.1147); oil; IR νmax 3477 (OH), 2969, 2933, 2861, 1708 (C=O), 1647, 1448, 1375, 1272, 1180, 841 cm⁻¹.
Supporting Information

(2S,3R,5R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohexan-1-one, 22
(2R,3R,5R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohexan-1-one, 23
(2R,3S,5R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohexan-1-one, 24, and
(2S,3S,5R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohexan-1-one, 25 (mixture of isomers)\(^6\)

To magnetically stirred suspension of cupper bromide (CuBr, 5\% mole, 36 mg) in dry THF (4 mL), TMA (5 mmol, 2M in hexane) was added under N\(_2\) atmosphere, and the mixture allowed to stir for 5 min at RT. R-(−)-carvone 1 (5 mmol, 750 mg) was one-portion added, and the reaction mixture stirred for further 30 min. The mixture of reaction was carefully quenched with saturated NH\(_4\)Cl (1 mL), and left for 10 min to re-cooling. The solution was filtered with Duran-sintered disc filter, and the solid part washed with THF (3 x 10 mL). The combined organic layer was concentrated with reduced pressure evaporator, and the mixture was purified with flash column chromatography using hexane:EtOAc (10:1) to afford mixture of four diastereomers (1.5:1:1:0.1) as a colourless oil with yield (740 mg, 90\%), and further separation was run on normal phase HPLC.

(Mixture of isomers): \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.82–4.68 (m, 2H), 2.70–2.54 (m, 1H), 2.46–2.17 (m, 3H), 2.06–1.80 (m, 2H), 1.76–1.71 (m, 3H), 1.53–1.43 (m, 1H), major isomer (22): 1.11 (d, \(J = 6\) Hz, 3H), 1.06 (d, \(J = 6.4\) Hz, 3H); second isomer (23): 1.09 (d, \(J = 6.8\) Hz, 3H), 1.03 (d, \(J = 6.4\) Hz, 3H); third isomer (24): 0.98 (d, \(J = 6.8\) Hz, 3H), 0.82 (d, \(J = 6.8\) Hz, 3H).

\(^13\)C NMR (100 MHz; CDCl\(_3\)) \(\delta\) 213.5, 213.0, 212.6 (C=O), 147.8, 147.7, 147.0 (C=CH\(_2\), C-8), 111.8, 109.8, 109.7 (C=CH\(_2\), C-9), 51.7 (CH), 51.3 (CH), 48.5 (CH), 46.7 (CH\(_2\)), 46.6 (CH\(_2\)), 45.2 (CH), 44.1 (CH\(_2\)), 41.2 (CH\(_2\)), 40.9 (CH), 40.1 (CH), 40.0 (CH\(_2\)), 37.8 (CH\(_2\)), 36.4 (CH), 35.2 (CH\(_2\)), 35.0 (CH), 22.0, 20.9, 20.7, 20.6, 20.5, 14.1, 13.2, 12.1, 11.6 (CH\(_3\)).
Supporting Information

MS m/z 189 (MNa+, 80%), 167 (MH+, 60%); colourless oil; Anal. Calc. for C_{11}H_{18}O,
Expected C, 79.5; H, 10.9; Found C, 79.3; H, 10.7; IR ν_{max} 2969, 2929, 2873, 1708 (C=O),
1645, 1451, 1375, 1213, 889 cm\(^{-1}\).

General enzymatic procedure for Baeyer–Villiger oxidation using CHMOs

The standard enzymatic reaction (1 mL) was achieved in buffer solution of Tris.HCl (50
mM, pH 7.5), comprises [substrate] = 5 mM, [NADP+] = 15 M, GDH = 10 U, glucose or
glucose-6-phosphate 15 mM, [Enzyme] = 2 or 10 μM. The reaction mixture was shaken at
required temperature (25 or 30 or 37 °C) at 135 rpm for the desired time. The reaction
was then terminated by extraction into an organic layer with 800 μL of EtOAc:internal
standard (+)-limonene (99.5:0.5). The organic mixture was dried over (MgSO\(_4\)), and
analysed with Varianchiralsil-DEX CB column.

Scale up of BV oxidation of (2R,3R,6R)-2,6-dimethyl-3-(prop-1-en-2-yl)cyclohexan-1-one
21 by CHMOs

(3R,6S,7R)-3,7-Dimethyl-4-(prop-1-en-2-yl)oxepan-2-one, 21

In conical flask contains buffer solution of Tris.HCl pH 7.5, 60 mL, a solution of 13 (50 mg
dissolved in 1.2 mL absolute EtOH), NADP+ (15 μM), GDH (600 U), glucose (15 mM) and
CHMOs (120 μM stock diluted 1 mL to 60 mL, 2 μM employed) were rapidly mixed. The
enzymatic reaction mixture was incubated for 24 h at 25 °C, and monitored via TLC plate.
The organic layer was extracted with EtOAc (3 x 50 mL), dried over (MgSO\(_4\)), and
concentrated to afford a lactone 21 with yield (49 mg, 90%) as a colorless oil.

(3R,6S,7R)-Lactone (21): \(^1\)H NMR (400 MHz; CDCl\(_3\)) δ 4.88 (quint, \(J = 1.5\) Hz, 1H, C=CH\(_2\),
H-11), 4.77–4.75 (m, 1H, C=CH\(_2\), H-11), 4.66 (qd, \(J = 7.2, 3.3\) Hz, 1H, CHCHOCO, H-7), 2.81
(dqd, \(J = 10.6, 6.8, 5.0\) Hz, 1H, CHCH\(_2\)CH\(_2\), H-3), 2.40 (ddd, \(J = 8.7, 7.1, 3.3\) Hz, 1H, CHCHOCO,
H-6), 1.92–1.81 (m, 3H, CHCH\(_2\)CH\(_2\) and CHCH\(_2\)CH\(_2\), 2 x H-4 and H-5), 1.77 (dd, \(J = 1.4, 0.8\) Hz,
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3H, CH₃C=CH₂, 3 x H-12), 1.47–1.39 (m, 1H, CHCH₂CH₂, H-5), 1.34 (d, J = 7.2 Hz, 3H, CH₃CHOCO, 3 x H-9), 1.28 (d, J = 6.8 Hz, 3H, CH₃CHCH₂, 3 x H-8).

¹³C NMR (100 MHz; CDCl₃) δ 177.1 (C=O), 145.0 (C=CH₂, C-10), 113.6 (C=CH₂, C-11), 75.4 (CH₂CHCH, C-7), 50.7 (CH₂CHCH, C-6), 41.2 (CHCH₂CH₂, C-3), 30.2 (CHCH₂CH₂, C-4), 27.4 (CHCH₂CH₂, C-5), 22.2 (CH₃C=CH₂, C-12), 19.0 (CH₃CHCH₂, C-7), 16.1 (CH₃CH, C-9).

HRMS (ES) m/z for C₁₁H₁₉O₂, [MH]+, (Calc.: 183.1385, Found: 183.1383); oil; IR νₘₚₙ × 2972, 2934, 2874, 1717 (C=O ester), 1644, 1456, 1378, 1261, 1208, 1095 cm⁻¹; [α]₀ +50 ° (c 0.04, CH₂Cl₂, 24 °C), Retention time 13.868 min.

Scale up of BV oxidation of two isomers mixture of 3-methylidihydrocarvone 22 and 23 by CHMOs (3R,4R,6S)-3,4-Dimethyl-6-(prop-1-en-2-yl)oxepan-2-one, 26, and (3S,4R,6S)-3,4-Dimethyl-6-(prop-1-en-2-yl)oxepan-2-one, 27 (mixture of normal and abnormal lactones)

In conical flask contains buffer solution of Tris.HCl pH 7.5, 70 mL, a solution of 22 and 23 (63 mg dissolved in 1.5 mL absolute EtOH), NADP⁺ (15 μM), GDH (750 U diluted to 10 U in reaction), glucose (15 mM) and CHMOs (150 μM stock diluted 1 mL to 75 mL, 2 μM employed) were rapidly mixed. The enzymatic reaction mixture was incubated for 24 h at 25 °C, and monitored via TLC plate. The organic layer was extracted with EtOAc (3 x 50 mL), dried over (MgSO₄), and concentrated to afford a mixture of abnormal lactones 26 and 27 as a colorless volatile oil (62 mg, 90%).

(3R,4R,6S)-lactone (26): ¹H NMR (400 MHz; CDCl₃) δ 4.82–4.79 (m, 2H, C=CH₂, 2 x H-11), 4.15–4.12 (m, 2H, CH₂-O, 2 x H-7), 2.59 (dq, J = 10.1, 6.8 Hz, 1H, CH=O, H-3), 2.35 (ddt, J = 12.0, 8.8, 3.1 Hz, 1H, CHCH₂-O, H-6), 1.88–1.84 (m 1H, CH₂CHCH, H-5), 1.75–1.73 (m, 3H, CH₃C=CH₂, 3 x H-12), 1.69–1.59 (m, 1H, CHCHC=O, H-4), 1.48–1.46 (m, 1H, CHCHCH₂, H-
Supporting Information

$^{13}$C NMR (100 MHz; CDCl$_3$) $\delta$ 178.0 (C=O), 145.9 (C=CH$_2$, C-10), 111.2 (C=CH$_2$, C-11), 71.9 (CH$_2$-O, C-7), 46.4 (CHCH$_2$-O, C-6), 44.3 (CHCHCH$_2$, C-5), 41.5 (CH$_3$CH=O, C-3), 41.0 (CH$_3$CH=O, C-4), 22.0 (CH$_3$C=CH$_2$, C-12), 16.7 (CH$_3$CHCH, C-8), 13.7 (CH$_3$CH, C-9).

(3S,4R,6S)-lactone (27): $^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 4.71–4.69 (m, 2H, C=CH$_2$, H-11), 4.19–4.16 (m, 2H, CH$_2$-O, 2 x H-7), 3.02 (qd, $J$ = 6.8, 1.5 Hz, 1H, CHCH=O, H-3), 2.49 (dq, $J$ = 10.3, 5.4 Hz, 1H, CHCH$_2$-O, H-6), 1.98–1.92 (m, 2H, CH$_2$CHCH, 2 x H-5), 1.76–1.74 (m, 3H, CH$_3$C=CH$_2$, 3 x H-12), 1.19 (d, $J$ = 6.8 Hz, 3H, CH$_3$CH=O, 3 x H-8), 0.94 (d, $J$ = 7.2 Hz, 3H, CH$_3$CHCH=O, 3 x H-9).

Abnormal lactones mixture (26 and 27): HRMS (ES) m/z for C$_{11}$H$_{19}$O$_2$, [MH]$^+$, (Calc.: 183.1385, Found: 183.1384); oil; IR $\nu_{max}$ 2970, 2933, 2887, 1715 (C=O ester), 1640, 1458, 1391, 1278, 1234, 1111 cm$^{-1}$. Retention time 15.942 and 16.165 min of 26 and 27 respectively.
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2 Spectral data

(5R,6S)-(+)-5-Isopropenyl-2,6-dimethylcyclohexane-2-enone, 2
$^{13}$C (100 MHz): (5R,6S)-(+)-5-Isopropenyl-2,6-dimethylcyclohexane-2-enone, 2
COSY: (5R,6S)-(+)-5-Isopropenyl-2,6-dimethylcyclohexane-2-enone, 2
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(5R,6R)-(+)-5-Isopropenyl-2, 6-dimethylcyclohexane-2-enone, 3
Supporting Information

(5R,6R)-(+)-5-Isopropenyl-2, 6-dimethylcyclohexane-2-enone, 3
Supporting Information

(5R,6R)-(+)-5-isopropenyl-2,6-dimethylcyclohexane-2-enone, 3
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(5S,6R)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-one, 4
(5S,6S)-6-Hydroxy-2-methyl-5- (prop-1-en-2-yl) cyclohex-2-en-1-one, 5
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(2S,3R,6R)-2,6-Dimethyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 11
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(2S,3R,6S)-2,6-Dimethyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 12
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\((2R,3R,6R)-2,6\text{-Dimethyl-3-}(\text{prop-1-en-2-yl})\text{cyclohexan-1-one}, \text{13}\)
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(2R,3R,6S)-2,6-Dimethyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 14
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\((2S,3S,6R)-2\text{-Hydroxy-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one}, 15\)
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(2R,3S,6R)-2-Hydroxy-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one, 17
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(R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one, 10
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(2S,3R,5R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohexan-1-one, 22
(2R,3R,5R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohexan-1-one, 23
(2R,3S,5R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohexan-1-one, 24, and
(2S,3S,5R)-2,3-Dimethyl-5-(prop-1-en-2-yl)cyclohexan-1-one, 25 (mixture of isomers)
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(3R,6S,7R)-3,7-Dimethyl-4-(prop-1-en-2-yl)oxepan-2-one, 21
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(3R,4R,6S)-3,4-Dimethyl-6-(prop-1-en-2-yl)oxepan-2-one, 26, and (3S,4R,6S)-3,4-Dimethyl-6-(prop-1-en-2-yl)oxepan-2-one, 27 (mixture of lactones)
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(5R,6R)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one, 7
(5R,6S)-6-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one, 8
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3. Enzymology

3.1 General procedure: Biocatalysis

Biohydrogenation substrates were prepared as stock solutions (250 mM) in absolute ethanol. Glucose dehydrogenase from *Pseudomonas sp.* (GDH), glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (G-6-PDH), glucose-6-phosphate and potassium phosphate buffers KH$_2$PO$_4$ and K$_2$HPO$_4$ were obtained from Sigma-Aldrich. The oxidized and reduced forms of nicotinamide adenine dinucleotide phosphate (NADP*/NADPH), and nicotinamide adenine dinucleotide (NAD*/NADH) were obtained from Melford. Ketoreductase-to-go plates. The results were examined with achiral DB-Wax or GC and chiral Varianchiralsil-DEX CB column.

**Table S1** Temperature methods utilised to determine conversion and yield% of substrates

| Temperature Methods | Method 1 (DB-Wax column) | Method 3 Varianchiral sil-DEX CB column |
|---------------------|--------------------------|----------------------------------------|
| Split injector ratio (1:x) | 20: 1 | splitless |
| Flow rate (mL.min$^{-1}$) | 1.5 | 1.0 |
| Injector Temp (°C) | 220 | 200 |
| Start Temp (°C) | 40 | 70 |
| Start Temp Hold (min) | 2 | 2 |
| End Temp (°C) | 210 | 200 |
| End Temp Hold (min) | 3 | 3 |
| Temp ramping (°C/min) | 5 | 2 |
| Detector Temp (°C) | 250 | 200 |

Purified enzyme stocks of *pentaerythritol tetranitrate* reductase (PETNR) from *Enterobacter cloacae* and Old Yellow Enzyme 3 (OYE3) from *Saccharomyces cerevisiae* were employed. Additionally, the plasmid encoding the gene sequence of Old Yellow Enzyme 2 (OYE2) from *Saccharomyces cerevisiae* was used for re-expression of OYE2. Details of the plasmid-gene constructs and *Escherichia coli* expression strains of the three OYEs are found in Table S1. The
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protein sequences for OYE2 (UniProt: Q03558), OYE3 (UniProt: P41816) and PETNR (UniProt: P71278) were obtained from the UniProt online database (http://www.uniprot.org).

Table S2 Details of the three OYE gene constructs and expression strains

| Enzyme | Source     | Plasmid | Resr. Sites | E. coli Strain | His6-tag | Ref. |
|--------|------------|---------|-------------|----------------|----------|------|
| OYE2   | *S. cerevisiae* | pET21b  | Ndel/NotI   | BL21(DE3)      | C-term   | 171  |
| OYE3   | *S. cerevisiae* | pET21a  | NdeI/XhoI   | BL21(DE3)      | C-term   | 171  |
| PETNR  | *E. cloaca*   | pET21a  | NdeI/XhoI   | BL21(DE3)      | C-term   | 170  |

Resr. Sites = restriction sites through which the gene was cloned into the plasmid.

Cyclohexanone monooxygenase (CHMO_Phi1) from *Rhodococcus sp. Phi1*. UniProt amino acid sequence database ID = Q84H73.⁹

3.2 Expression and purification of recombinant OYE2

Expression and purification of OYE2 was performed using the method detailed below, based on the production and purification of OYE2 as described previously.⁸

3.2.1 Medium and sterile reagents preparation

Sterile stock solutions (1000x) of ampicillin (100 mgmL⁻¹) and isopropyl β-D-1-thiogalactopyranoside (IPTG; 0.5 M) in distilled water were filter sterilised (0.2 mm filtration) and stored at −20 °C prior to use. Super optimal broth (SOC) medium was prepared by combining tryptone (20 gL⁻¹), yeast extract (5 gL⁻¹), NaCl (10 mM), KCl (2.5 mM), MgCl₂ (10 mM) and MgSO₄ (10 mM) in distilled water and sterilising by autoclaving. A filter-sterilised stock of glucose in distilled water was prepared (1M; 0.2 mm filtration), and added aseptically to the sterile medium to a final concentration of 20 mM. Lysogeny broth-Miller (LB) was prepared by combining tryptone (10 gL⁻¹), yeast extract (5 gL⁻¹) and NaCl (10 gL⁻¹) followed by autoclaving. Lysogeny broth-Miller agar (LB agar) was prepared as for LB, except agarose (15 gL⁻¹) was added to the medium prior to autoclaving. After sterilisation, the medium was cooled to ~ 50 °C, and a sterile ampicillin solution was added (100 mgmL⁻¹) before pouring into sterile agar plates.
3.2.2 Transformation of competent cells
Purified OYE2 plasmid DNA (0.4 mL; Table S2) was mixed with pre-chilled competent *E. coli* BL21 (DE3) cells (50 mL) and incubated on ice for 30 minutes. The suspension was heat shocked by incubating at 42 °C for 30 seconds, followed by cooling on ice for 2 minutes. Cell recovery and growth was initiated by the addition of SOC medium (0.45 mL) and the culture was incubated at 37 °C for 1 hour at 200 rpm agitation. The cell suspension was spread aseptically on to LB agar, containing ampicillin (100 mgmL⁻¹), and incubated overnight at 37 °C.

3.2.4 Growth and expression of OYE2 clone
Starter cultures of OYE2 in *E. coli* BL21(DE3) were produced by inoculating LB media (5 mL) containing ampicillin (100 mgmL⁻¹) with a colony from the transformation plate (Section 3.2). Cultures were incubated for 1 hour at 37 °C with 200 rpm agitation. Aliquots of starter cultures (0.5 mL) were used to inoculate (6 x 1L LB) containing ampicillin (100 mgmL⁻¹) and incubated at 37 °C with 200 rpm agitation until the OD 600 nm reached ~ 0.5. Protein expression was initiated by the addition of sterile IPTG (0.5 mM) and the cultures were incubated overnight at 37 °C with 200 rpm agitation. Cultures were harvested by centrifugation (6,238 g, 5 °C, 10 minutes), and the cell pellet was frozen in liquid nitrogen and stored at −80 °C.

3.2.5 Purification of OYE2
The OYE2 cell pellet was resuspended in lysis buffer (50 mM KH₂PO₄/K₂HPO₄ pH 8.0 containing 10 mgmL⁻¹ DNAse and 1 x Complete EDTA-free protease inhibitor blend). Lysozyme (10 mgmL⁻¹) and flavin mononucleotide (FMN) were added, and the slurry was stirred until uniform. The cells were disrupted by sonication (BANDELIN SONOPULS) (20 minutes) and the cell debris was pelleted by centrifugation (4,3667 g, 1 hour, 4 °C). The supernatant was retained, and NaCl (3.9 mL, 0.3 M) and imidazole (0.65 mL, 10 M) were added.

The OYE2 supernatant was applied to a Ni-NTA affinity column (30 mL) pre-equilibrated in wash buffer 1 (50 mM KH₂PO₄/K₂HPO₄ pH 8.0 containing 10 mM imidazole). The column was washed with wash buffer 2 (50 mM KH₂PO₄/K₂HPO₄ pH 8.0 containing 300
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mM NaCl and 25 mM imidazole). Protein was recovered from the column in a step to elution buffer (50 mM KH$_2$PO$_4$/K$_2$HPO$_4$ pH 8.0 containing 300 mM NaCl and 200 mM imidazole).

Eluted protein fractions were checked for protein content and purity by SDS-PAGE using stain-free precast gels (Gel DOC EZ Imager BioRad) in standard running buffer (25 mM Tris containing 192 mM glycine and 0.1 % sodium dodecyl sulfate). Samples were prepared by combining protein fractions (5 mL) with water (5 mL) and 2 X SDS gel loading buffer (10 mL; 100 mM Tris pH 6.8 containing 4 % SDS, 0.2 % bromophenol blue, 200 mM 2-mercaptoethanol and 20 % glycerol). The samples were boiled at 95 °C for 5 min to denature the proteins, and centrifuged at 4 °C for 1 minute (13,000 g) to pellet any debris. Samples were loaded onto the precast gel, and run for 20 min at constant voltage of 180 V, and scanned using transilluminator.

The purest protein fractions (Figure S1) were dialysed against a storage buffer (10 mM Tris pH 7.0; 7 L) to remove the NaCl and imidazole prior to protein storage. The dialysis was performed overnight in the cold room (4 °C). The final protein concentration was determined by measuring the absorbance at 280 nm, and using the extinction coefficient of 10600 M$^{-1}$ cm$^{-1}$.

**Figure S1**: SDS-PAGE of the first OYE2 affinity chromatography purification with Ni$^{2+}$ NTA

Lanes 1-4 = elution fractions for first column and black box shows OYE2 bands
3.3 General enzymatic procedure for reduction by PETNR, OYE2 and OYE3

The standard enzymatic reaction (1 mL) was achieved in buffer solution of KH$_2$PO$_4$/K$_2$HPO$_4$ (50 mM, pH 7.0), comprises [substrate] = 5 mM, [NADP$^+$] = 15 μM, GDH or G-6-PDH = 10 U, glucose or glucose-6-phosphate 15 mM, [Enzyme] = 2 or 10 μM. The reaction mixture was shaken at required temperature 30 °C at 135 rpm for desired time. The reaction was then terminated by extraction into an organic layer of 800 μL of EtOAc: internal standard (+)-limonene (99.5:0.5). The organic mixture was dried over MgSO$_4$, and analysed with GC using DB-wax column to determine % of yield, conversion and diastereomeric excess.

3.3.1 Biocatalytic reduction of R-(−)-carvone 1

Table S3 The effect of temperature and co-factor on bioreduction of 1 by OYE2

| Co-factor            | T (22 °C) | T (30 °C) | T (37 °C) |
|----------------------|-----------|-----------|-----------|
|                      | Yield%    | d.e.%     | Yield%    | d.e.%     | Yield%    | d.e.%     |
| NADP$^+$/GDH         | 80        | 86        | 82        | 85        | 50        | 86        |
| NADP$^+$/G-6-DH      | 73        | 85        | 58        | 79        | 45        | 93        |
| NAD$^+$/GDH          | 82        | 85        | 85        | 85        | 46        | 95        |

1) Conditions: Enzyme (2 μM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, Co-factor (15 μM), GDH or G-6-DH (10 U), 130 rpm for 24 h. 2) No products were determined using co-factors NADPH or NADH.
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**Table S4:** The effect of time on bioconversion of 1 via OYE2 with respect to the yield and diastereomeric excess.

| Time (h) | Yield% | d.e% |
|----------|--------|------|
| 1        | 83     | 95   |
| 2        | 84     | 93   |
| 4        | 79     | 90   |
| 6        | 77     | 87   |
| 24       | 79     | 80   |

1) Conditions: Enzyme (2 µM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 µM), 30 °C at 130 rpm.

**Table S5** Biotransformation of 1 via OYE3 enzyme*

| Enzyme | 24 h Yield% | d.e.% | 2h Yield% | d.e.% |
|--------|--------------|------|-----------|------|
| OYE3   | 58           | 93   | 80        | 94   |

*Conditions: Enzyme (10 µM), pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 µM), 30 °C at 130 rpm

**Table S6** The influence of enzyme and NADP⁺ concentrations on bioreduction of 1 using OYE2

| Entry | [Enzyme] µM | [NADP⁺] µM | Yield% | d.e.% |
|-------|-------------|------------|--------|------|
| 1     | 2           | 15         | 79     | 80   |
| 2     | 2           | 25         | 57     | 93   |
| 3     | 2           | 50         | 57     | 93   |
| 4     | 2           | 100        | 54     | 93   |
| 5     | 10          | 15         | 82     | 85   |
| 6     | 10          | 25         | 65     | 66   |
| 7     | 10          | 50         | 65     | 69   |
| 8     | 10          | 100        | 55     | 64   |
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1) Conditions: Substrate (5 μM) pH 7.0 of 50 mM KP buffer solution, 30 °C at 130, 24 h.

**Figure S2**: GC chromatographs of A) Control solution of R-(-)-carvone B) Bio-catalytic reaction mixture using OYE2. Bio-reduction conditions: Enzyme (10 μM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 μM), GDH (10 U), Glucose (15 μM), 30 °C at 130 rpm for 24 h.

### 3.3.2 Biocatalytic reduction of 6-methylcarvone diastereomers mixture

![Diagram](attachment:diagram.png)
Supporting Information

**Figure S3**: GC chromatograms of A) Control solution of 6-methycarvone diastereomers mixture B) Bio-catalytic reaction mixture using PETNR. Bio-reduction conditions: Enzyme (10 µM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), 30 °C at 130 rpm for 24 h.

### 3.3.2.1 Biocatalytic reduction of 6-S-methylcarvone diastereomer

![Diagram of biocatalytic reduction](image-url)
Figure S4: The effect of temperature and co-factor on bioreduction of 2 by OYE2. Reaction conditions: Enzyme (2 µM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), at 130 rpm for 24 h.

Table S7: Bioconversion of 2 using OYE2 as a function of time (h). Bio-reduction conditions: Enzyme (2 µM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), 30 °C at 130 rpm for 24 h.

| Time | Yield% | d.e.% |
|------|--------|-------|
| 1    | 12     | ≥99   |
| 2    | 17     | ≥99   |
| 4    | 15     | ≥99   |
| 6    | 15     | ≥99   |
| 24   | 15     | ≥99   |

Table S8. Bioconversion optimisation of 2 using OYE2 enzyme and NADP⁺ concentration parameters Bio-reduction conditions: Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, 30 °C at 130 rpm for 24 h.

| [Enzyme] | [NADP⁺] | Yield% | d.e.% |
|----------|---------|--------|-------|
| 2        | 15      | 15     | ≥99   |
| 2        | 25      | 8      | ≥99   |
| 2        | 50      | 7      | ≥99   |
| 2        | 100     | 6      | ≥99   |
| 10       | 15      | 17     | ≥99   |
| 10       | 25      | 17     | ≥99   |
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|     | 10 | 50 | 13 | ≥99 |
|-----|----|----|----|-----|
|     | 10 | 100| 13 | ≥99 |

**Figure S5.** The effect of pH buffer solution and time parameter on biotransformation of 2 via OYE2 enzyme. Reaction conditions: Substrate (5 mM), NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), 30 °C at 130 rpm.
Figure S6. GC chromatographs of A) Control solution of 6-S-methylcarvone diastereomer 2 B) Bio-catalytic reaction mixture using OYE2 C) Standard reference of product. Bio-reduction conditions: Enzyme (2 µM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), 30 °C at 130 rpm for 24 h.

3.3.2.2 Biocatalytic reduction of 6-R-methylcarvone diastereomer
Figure S7: GC chromatographs of A) Control solution of 6-R-methycarvone diastereomer 3 B) Bio-catalytic reaction mixture using PETNR C) Standard reference of product. Bio-reduction conditions: Enzyme (10 μM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 μM), GDH (10 U), Glucose (15 mM), 30 °C at 130 rpm for 2 h. A small amount of 6-R-methyl carvone 3 has epimerised to 6-S-methyl carvone 2.
3.3.3 Biocatalytic reduction of 6-hydroxycarvone diastereomers 4 and 5

Figure S8. Optimisation pH buffer solution of KP of bioconversion of anti 4 and syn 5. Where, 1 mL scale, substrate (5 mM), OYE2 (2 µM), NADP⁺ (15 µM)/GDH (15 U), glucose (15 mM), pH 7.0 of KP buffer solution (50 mM), 24 h, 130 rpm. The figure showed the yield% of 15 and 17.
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**Table S9.** Optimisation of [NADP\(^+\)] and [OYE2] on outcomes of bioreduction of *anti* 4 and *syn* 5. Where, 1 mL scale, substrate (5 mM), pH 7.0 of KP buffer solution (50 mM), 24 h, 130 rpm

| Substrate | Product | [NADP\(^+\)] \(\mu\text{M}\) | [OYE2] \(\mu\text{M}\) | Yield % | d.e. % |
|-----------|---------|------------------|-----------------|--------|-------|
| 15        | 2       | 39               | ≥99             |
| 25        | 2       | 30               | ≥99             |
| 50        | 2       | 31               | ≥99             |
| 100       | 2       | 34               | ≥99             |
| 15        | 10      | 40               | ≥99             |
| 25        | 10      | 49               | ≥99             |
| 50        | 10      | 39               | ≥99             |
| 100       | 10      | 42               | ≥99             |

| Substrate | Product | [NADP\(^+\)] \(\mu\text{M}\) | [OYE2] \(\mu\text{M}\) | Yield % | d.e. % |
|-----------|---------|------------------|-----------------|--------|-------|
| 15        | 2       | 11               | ≥99             |
| 25        | 2       | 8                | ≥99             |
| 50        | 2       | 7                | ≥99             |
| 100       | 2       | 6                | ≥99             |
| 15        | 10      | 30               | 16              |
| 25        | 10      | 26               | 16              |
| 50        | 10      | 26               | 16              |
| 100       | 10      | 12               | 16              |
Table S10. Bioconversion of *anti* 4 and *syn* 5 by OYE3 within 2 and 24 h. Reaction conditions: substrate (5 mM), OYE3 (10 µM), NADP⁺ (15 µM)/GDH (15 U), glucose (15 mM), pH 7.0 of KP buffer solution (50 mM), 130 rpm.

| OYE3 | Time (h) | Yield % | d.e.% |
|------|----------|---------|-------|
|      |          |         |       |
|      | 2        | 14      | ≥99   |
|      | 24       | 17      | ≥99   |
|      | nd       | nd      | nd    |
|      | nd       | nd      | nd    |
Figure S9. GC chromatographs of A) Control solution of 6-S-hydroxycarvone diastereomer (anti) 4 B) Bio-catalytic reaction mixture using PETNR C) Standard reference of diastereomeric mixture of the product. Bio-reduction conditions: PETNR (10 µM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), 30 °C at 130 rpm for 24 h.
Figure S10. GC chromatographs of A) Control solution of 6-R-hydroxycarvone diastereomer (syn) 5  B) Bio-catalytic reaction mixture using PETNR Bio-reduction conditions: PETNR (10 µM), Substrate (5 mM) pH 7.0 0f 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), 30 °C at 130 rpm for 2 h.

3.3.4 Biocatalytic reduction of 6-R-hydroxycarvone diastereomer (anti) 8
Figure S11. GC chromatographs of A) Control solution of 6-R-hydroxycarvone diastereomer (anti) 8 B) Bio-catalytic reaction mixture using OYE2 C) Bio-catalytic reaction mixture using PETNR D) Standard reference of diastereomeric mixture of the product (Chemically synthesized). Bio-reduction conditions: Enzyme (10 µM), Substrate (5 mM) pH 7.0 of 50 mM KP buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), 30 °C at 130 rpm for 24 h.
3.4 Bio-oxidation of 6-methyldihydrocarvone using BVMOs

**Figure S12.** GC chromatograph of abnormal lactone 21 resulted from biooxidation of 6-Me-dihydrocarvone using CHMO_Phi1 from *Rhodococcus sp. Phi1*. Bio-oxidation conditions: Enzyme (2 µM), Substrate (5 mM) pH 7.0 of 50 mM Tris.HCl buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 mM), 25 °C at 130 rpm for 24 h.
Supporting Information

3.5 Structural analysis and Bio-oxidation of 3-methyldihydrocarvones using BVMOs

The structures of both isomers were incompletely confirmed through proton NMR but this was complicated due to overlapping of the peaks. Batey and co-workers separated two diastereomers (23:22 = 7:5) of from radical opening ring of carvone-derived cyclopropyl ketone precursor, and proved the major one to be 23, and minor to be 22.\(^{10}\) The proton NMR of 23 assigned therein is consistent with assignment of 23 (minor isomer of that mixture in the current study). This supports that the mixture of two isomers, separated in this work, consists of 22 and 23. Additionally, 22 and 23 have been reported (Siscovic and Roa\(^ {11}\)) in a 1:1 ratio through conjugate addition to carvone. Our comparative NMR data of a mixture of these two isomers, versus a total mixue of all 4 below allows a cross-analysis of the other minor components herein.
Supporting Information

Figure S13. GC chromatographs of abnormal lactones 26 and 27 resulted from biooxidation of mixture of four isomers of 3-Me-dihydrocarvone using CHMO_Phi1 from Rhodococcus sp. Phi1). Bio-oxidation conditions: Enzyme (2 µM), Substrate (5 mM) pH 7.0 of 50 mM Tris.HCl buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 µM), 25 °C at 130 rpm for 24 h.
Figure S14. GC chromatographs of abnormal lactones 26 and 27 resulted from biooxidation of mixture of two isomers of 3-Me-dihydrocarvone using CHMO Phi1 from *Rhodococcus* sp. Phi1). Bio-oxidation conditions: Enzyme (2 µM), Substrate (5 µM) pH 7.0 0f 50 mM Tris.HCl buffer solution, NADP⁺ (15 µM), GDH (10 U), Glucose (15 µM), 25 °C at 130 rpm for 24 h.

4 Modelling

**Figure S15.** Optimised DFT models of PETNR active site with FMNH₂ and (A,B) 6S-Me carvone and (C,D) 6R-Me carvone bound in two conformations.

5 X-ray structure

**Figure S16.** X-ray crystal structure and stereochemistry of 6S-Methylcarvone 2
Supporting Information

6. References

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