Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Systematic methodology for the development of biocatalytic hydrogen-borrowing cascades: Application to the synthesis of chiral α-substituted carboxylic acids from α-substituted α-β unsaturated aldehydes.

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Source and cloning/expression conditions for recombinant proteins

Table S1. Source and cloning/expression conditions for ene-reductases and aldehyde dehydrogenases used in this study.

name	source	plasmid	restr. sites	*E. coli* strain	IPTG [mM]	OD_{600}	expression	His6-tag	ref.
PETNR	*Enterobacter cloacae*	pET21a	*Nde*I/*Not*I	BL21-DE3 Star	1	0.8-1	25 °C/o.n.	C-term	[1]
TOYE	*Thermoanaerobacter pseudethanolicus*	pET21b	*Nde*I/*Xho*I	Arctic Express RP	0.5	0.6	25 °C/o.n.	C-term	[2]
OYE2	*Saccharomyces cerevisiae*	pET21b	*Nde*I/*Xho*I	BL21-DE3	0.5	0.8-1	25 °C/o.n.	C-term	[3]
OYE3	*Saccharomyces cerevisiae*	pET21b	*Nde*I/*Xho*I	BL21-DE3	0.5	0.8-1	25 °C/o.n.	C-term	[3]
XenA	*Pseudomonas putida*	pET21a	*Nde*I/*Not*I	BL21-DE3	0.5	0.6	25 °C/o.n.	C-term	[4]
XenB	*Pseudomonas fluorescens*	pET21a	*Nde*I/*Not*I	BL21-DE3	0.5	0.6	25 °C/o.n.	C-term	[4]
LeOPR1	*Lycopersicon esculentum*	pET21a	*Nde*I/*Xho*I	BL21-DE3	1	0.6-0.8	25 °C/o.n.	C-term	[5]
NerA	*Agrobacterium radiobacter*	pET21a	*Nde*I/*Not*I	BL21-DE3	0.5	0.6	25 °C/o.n.	C-term	[6]
GluOx	*Gluconobacter oxydans*	pET28b	*Nde*I/*Xho*I	BL21-DE3	0.5	0.8-1	25 °C/o.n.	N-term	[7]
YqjM	*Bacillus subtilis*	pET21b	*Nde*I/*Xho*I	BL21-DE3	1	0.8-1	25 °C/o.n.	no tag	[8]
MR	*Pseudomonas putida*	pET21a	*Nde*I/*Not*I	BL21-DE3	1	0.8-1	25 °C/o.n.	no tag	[9]
Ald-DH-EC	*E. coli*	pET28b	*Nde*I/*Xho*I	BL21-DE3	0.5	0.8-1	25 °C/o.n.	N-term	[10]
Ald-DH BOV	*Bos taurus*	pET28b	*Nde*I/*Xho*I	BL21-DE3	0.5	0.5	25 °C/o.n	N-term	[11]
Ald-DH-HL	*Equus caballus*	pET-SUMO	-	BL21-DE3	0.6	0.5	25 °C/o.n	N-term	[12]

Expression and purification of recombinant proteins in *E. coli* host cells

For recombinant expression 800 mL of LB medium supplemented with the appropriate antibiotic (100 µg/mL ampicillin for pET21a or pET21b) and 50 µg/mL kanamycin for pET28b and pET-SUMO plasmids) were inoculated with 15 mL of an overnight culture harboring the desired vector with genes for the expression of the ERs and Ald-DHs. Cells were grown at 37 °C until the desired OD_{600} was reached and expression of protein was induced by the addition of IPTG. Protein expression was carried out overnight and after harvesting of the cells (4 °C, 8 x 10^3 rpm, 10 min), the remaining cell pellet was resuspended in buffer and cells were disrupted using a French Press. For flavin-containing proteins a small spatula of free FMN was added to the lysate before cell disruption. The cell debris was removed by centrifugation at 18 x 10^3 rpm for 30 min at 4 °C.

His_6-tagged proteins were resuspended in lysis buffer (50 mM KH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) prior to cell disruption and protein purification was performed by Ni-NTA affinity chromatography using pre-packed Ni-NTA HisTrap HP columns (GE Healthcare), previously equilibrated with lysis buffer. After loading of the filtered lysate, the column was washed with sufficient amounts of wash buffer (50 mM KH_2PO_4, 300 mM NaCl, 25 mM imidazole,pH 8.0), and bound protein was recovered with elution buffer (50 mM KH_2PO_4, 300 mM NaCl, 200 mM imidazole, pH 8.0).

Non-tagged proteins (YqjM and MR) were purified by anion exchange chromatography using a HiPrepQ HP 16/10 column (GE Healthcare). The lysate was dissolved in start buffer (20 mM Tris/HCl, pH 8.0 buffer) and after cell disruption and centrifugation loaded onto the column. The elution of the proteins was performed with a gradient between start buffer and elution buffer (20 mM Tris/HCl, 1M NaCl, pH 8.0 buffer).

After SDS-PAGE, fractions containing the desired proteins in a sufficient purity were pooled and dialyzed against 50 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.0) overnight, and concentrated using Centripreps (Millipore). The final concentration was determined at 280 nm or for flavin containing proteins using the wavelength/extinction coefficient for the protein bound flavin (approx. 450 nm/11,300 M^{-1} cm^{-1}).

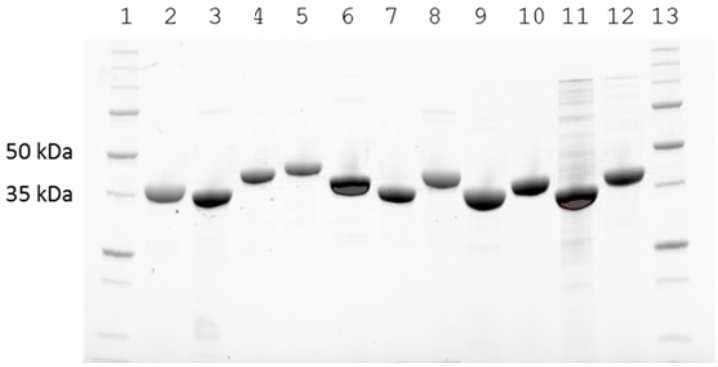


Figure S1. SDS-PAGE of purified ene-reductases. Lane 1: Biorad Precision Plus Protein™ Unstained Standard, lane 2: PETNR, lane 3: TOYE, lane 4: OYE2, lane 5: OYE3, lane 6: XenA, lane 7: XenB, lane 8: LeOPR1, lane 9: NerA, lane 10: GluOx, lane 11: YqjM, lane 12: MR, lane 13: Biorad Precision Plus Protein™ Unstained Standard.

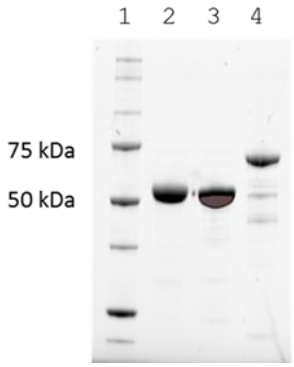
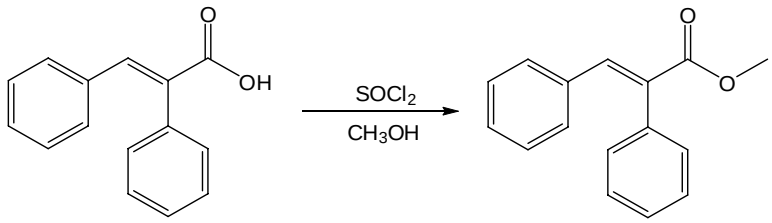


Figure S2. SDS-PAGE of purified aldehyde dehydrogenases. Lane 1: Biorad Precision Plus Protein™ Unstained Standard, lane 2: Ald-DH-BOV, lane 3: Ald-DH-EC, lane 4: Ald-DH-HL.

Chemical synthesis of substrates and reference compounds

1) Synthesis of (*E*)-2,3-diphenylacrylaldehyde (α-phenylcinnamaldehyde) 2a

Synthesis of (*E*)-methyl 2,3-diphenylacrylate (α-phenylcinnamic acid methyl ester)

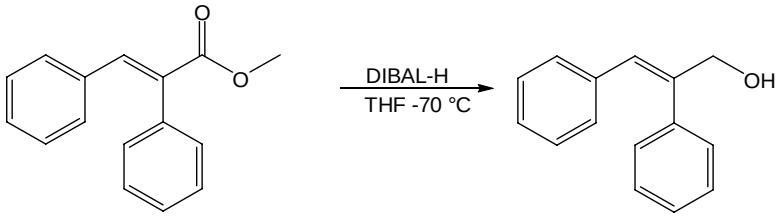


(*E*)-2,3-diphenylacrylic acid (α-phenylcinnamic acid) (1 g , 4.46 mmol) was dissolved in dried MeOH (30 mL). The reaction mixture was cooled down to 4 °C and a solution of thionylchloride (650 µL, 2 eq., 8.92 mmol) dissolved in dried MeOH (2 mL), prior cooled to 4 °C, was added dropwise into the reaction mixture. The solution was then refluxed overnight at 42 °C. The progress of the reaction was analyzed by TLC (Petroleum ether/EtOAc, 9:1, v v^{-1}, Rf ester product = 0.5; the carboxylic acid does not elute from the base). Methanol was evaporated, the residue was treated with sodium carbonate (20 mL, 5% w w^{-1}) and extracted with EtOAc (2 × 15 mL). The combined organic phase was dried with $MgSO_4$, filtered and evaporated. The product was recovered as pure solid in 89% yield (784 mg, 3.99 mmol).

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz): 3.72 (3H, CH_3, s), 6.95 – 6.97 (2H, CH, m), 7.04 – 7.18 (5H, CH, m), 7.26 – 7.33 (3H, CH, m), 7.78 (1H, CH, s).

GC-MS: (m/z, (rel. intensity)): 239 (100), 205 (4), 178 (5), 121 (10). GC-MS was measured with a Varian CP3800 gas chromatograph connected to a Varian Saturn 2000 GC/MS/MS (Agilent DB-Wax column).

Synthesis of (*E*)-2,3-diphenylprop-2-en-1-ol (α-phenylcinnamic alcohol)



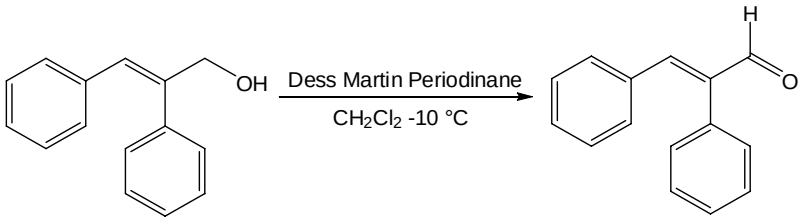
Initial attempts aimed at obtaining directly the saturated aldehyde. However, over-reduction to the alcohol was always observed. Therefore the ester was completely reduced to the alcohol with an excess of the reducing reagent.

Under anaerobic conditions, (*E*)-methyl 2,3-diphenylacrylate (α-phenylcinnamic acid methyl ester) (794 mg, 3.33 mmol) was dissolved in anhydrous THF (10 mL). The solution was cooled and kept at ca. -70 °C. A solution of DIBAL-H in anhydrous THF (1M, 10 mL, 9.99 mmol) was added dropwise and the reaction was stirred for additional 2 h at -10 °C/-20 °C. The progress of the reaction was analyzed by TLC (Petroleum ether/EtOAc, 7:3, v v^{-1}, Rf ester = 0.8; Rf alcohol = 0.5). The TLC showed that the ester was completely reduced to the alcohol. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl. This solution was added dropwise at -10 °C/-20 °C until bubbling ceased and then diluted with distilled water up to 120 – 130 mL. The precipitated colloidal aluminium was removed by filtration over celite, THF was partially evaporated and the alcohol was extracted with EtOAc (2 x 50 mL). The product was isolated as yellow oil that crystallized under reduced pressure in 80% yield (561.55 mg, 2.671 mmol).

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz): 1.21 (1H, OH, ^{3}J = 6.8 Hz), 4.38 (2H, CH_2, d, ^{3}J = 1.2 Hz), 6.61 (1H, CH, s), 6.91 – 6.93 (2H, CH aromatic, m), 7.01 – 7.05 (3H, CH aromatic, m), 7.14 -7.18 (2H, CH aromatic, m), 7.20 – 7.28 (3H, CH aromatic, m).

GC-MS: (m/z, (rel. intensity)): 210 (75), 193 (5), 178 (15), 152 (5), 133 (15), 115 (10), 105 (100), 91 (50), 77 (30), 59 (25), 45 (90), GC-MS was measured with a Varian CP3800 gas chromatograph connected to a Varian Saturn 2000 GC/MS/MS (Agilent DB-Wax column).

Synthesis of (*E*)-2,3-diphenylacrylaldehyde (α-phenylcinnamaldehyde)



(*E*)-2,3-diphenylprop-2-en-1-ol (α-phenylcinnamic alcohol) (561.5 mg, 2.67 mmol) was dissolved in CH_2Cl_2 (10 mL) at -10 °C. Dess Martin Periodinane (1,1,1-Tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one, 2.15 g, 1.9 equivalents, 5.073 mmol) was added in small portions and the heterogeneous reaction was stirred at -10 °C for 2.5 h. The progress of the reaction was analyzed by TLC (Petroleum ether/EtOAc, 7:3, v v^{-1}, Rf alcohol = 0.5; Rf aldehyde = 0.7). The reaction was quenched by the addition of an aqueous solution of NaOH (5%, w w^{-1}, 10 mL) and then diluted with CH_2Cl_2 (10 mL), the same volume of NaOH solution (10 mL) and distilled water (15 mL). The organic phase was removed and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phase was washed with brine and dried with $MgSO_4$. After filtration, the solvent was evaporated. The product was isolated as yellow crystals in 79% yield (439.26 mg, 2.109 mmol).

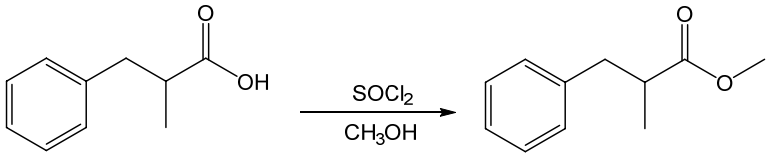
^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz): 7.11 -7.24 (7H, CH, m), 7.29 – 7.36 (4H, CH, m), 9.70 (1H, CH aldehyde, s).

^{13}C NMR ($CDCl_3$, 400 MHz, δ (ppm)): 127.3, 127.5, 127.8, 128.3, 129.2, 129.7, 132.3, 133.0, 140.8, 149.2, 192.9

GC-MS: (m/z, (rel. intensity)): 209 (100), 178 (25), 152 (5). GC-MS was measured with a Varian CP3800 gas chromatograph connected to a Varian Saturn 2000 GC/MS/MS (Agilent DB-Wax column).

2) Synthesis of (*E*)-2-methyl-3-phenylpropanal (α-methylhydrocinnamaldehyde) 1b

Synthesis of (*E*)-methyl 2-methyl-3-phenylproanoate



(*E*)-2-methyl hydrocinnamic acid (2.004 g, 12.18 mmol) was dissolved in dried methanol (50 mL) and cooled down to 4 °C in an ice bath. Thionylchloride (1.77 mL, 2 eq., 24.36 mmol) was diluted in dried methanol (5 mL), previously cooled to 4 °C in an ice bath. The thionylchloride solution was finally dropped into the reaction mixture, always keeping the reaction mixture on ice. Afterwards, the reaction was heated up to 42 °C and kept at this temperature overnight. The progress of the reaction was checked by TLC (Petroleum ether/EtOAc 9:1, v v^{-1}, Rf substrate: 0.23; Rf product: 0.57). The organic solvent was evaporated and the crude oil was treated with aqueous Na_2CO_3 (10% w w^{-1}, 4 mL) and extracted with EtOAc (2 x 30 mL). The combined organic phases were then dried with $MgSO_4$ anhydrous,

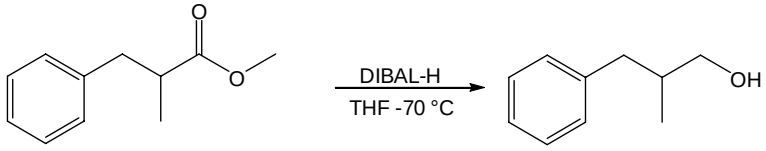
filtered and evaporated. Extraction was checked by TLC. The pure product was isolated as colorless oil (2.095 g, 11.76 mmol) in 97% yield.

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 1.08 (3H, CH_3, d, ^{3}J= 6.8 Hz), 2.57 (1H, CH_2, dd), 2.65 (1H, CH, m), 2.95 (1H, CH_2, dd, ^{2}J= 13.2 Hz, ^{3}J= 6.4 Hz), 3.56 (3H, CH_3, s), 7.07 – 7.27 (5H, C-H aromatic, m).

^{13}C-NMR ($CDCl_3$, 400 MHz, δ (ppm): 14.9, 37.9, 39.6, 49.7, 124.5, 126.7, 127.0, 137.5, 174.7.

GC-MS: (m/z, (rel. intensity)): 178 (22), 147 (4), 131 (5), 118 (55), 91 (100), 77 (4), 65 (8), 51 (3). GC-MS was measured with a Varian CP3800 gas chromatograph connected to a Varian Saturn 2000 GC/MS/MS (Agilent DB-Wax column).

Synthesis of (*E*)-2-methyl-3-phenylpropanol (α-methyl-hydrocinnamic alcohol) **1c**



The reduction of the ester intermediate with the mild reducing reagent DIBAL-H afforded the alcohol as the sole product. Although the reaction was carefully attempted few times, the aldehyde intermediate was never observed. DIBAL-H was added in small aliquots (initial 0.5 eq., further 0.3 eq. and final 0.2 eq.) during the time (15 – 20 min per addition). However, only the fully reduced alcohol was observed by TLC (0.5 eq, 0.8 eq, 1 eq.). The addition of 1 eq. produced the reduction of half of the ester starting material, according to the stoichiometry of the reaction. Therefore the reaction was repeated on a larger scale adding an excess of DIBAL-H (more than 2 equivalents) in order to obtain the quantitative reduction of the ester into the alcohol product. (*E*)-methyl 2-methyl-3-phenylproanoate (2.045 g, 11.47 mmol) was dissolved in THF anhydrous (25 mL) under inert atmosphere and cooled to -70 °C. DIBAL-H (1 M solution in THF, 25.25 mL, 2.2 eq, 25.25 mmol,) was slowly dropped into the solution and the temperature was carefully kept at -70 °C. The reaction mixture was stirred at this temperature and the progress of the reaction was checked by TLC (Petroleum ether/EtOAc 7:3, v v^{-1}, Rf substrate: 0.73; Rf product: 0.42). The reaction was complete after approximately 1 h and was quenched at -70 °C with NH_4Cl (50 mL) followed by the addition of water (50 mL). Precipitation of colloidal aluminium salts was observed. Therefore the solution was filtered over celite. THF was partially evaporated from the solution and the aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic phases were dried with $MgSO_4$ anhydrous, filtered and evaporated. The pure product was isolated as colorless oil (1.705 g, 11.36 mmol) in 99% yield.

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 0.83 (3H, CH_3, d, ^{3}J= 6.8 Hz), 1.61 (1H, OH), 1.86 (1H, CH, m), 2.33 (1H, CH_2, dd, ^{2}J= 13.2 Hz, ^{3}J= 8 Hz), 2.68 (1H, CH_2, dd), 3.41(2H, CH_2, m), 7.08 – 7.22 (5H, C-H aromatic, m).

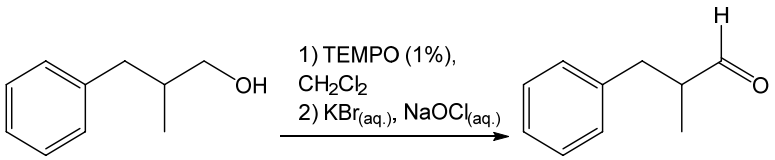
^{13}C-NMR ($CDCl_3$, 400 MHz, δ (ppm): 14.2, 35.5, 37.5, 65.4, 123.6, 126.0, 126.9, 138.4.

GC-MS: (m/z, (rel. intensity)): 150 (21), 132 (21), 117 (71), 91 (100), 77 (7), 65 (11), 51 (4). GC-MS was measured with a Varian CP3800 gas chromatograph connected to a Varian Saturn 2000 GC/MS/MS (Agilent DB-Wax column).

Synthesis of (*E*)-ethyl 2-methyl-3-phenylpropanal

First, it was tried to oxidize the alcohol using Dess-Martin periodinane. However, oxidation of the alcohol into the aldehyde was not observed at all.

Therefore an alternative method was applied from literature.[13]



2,2,6,6-tetramethyl-piperidinyloxy free radical (TEMPO), (10.40 mg, 1 mol%, 0.06657 mmol) was dissolved in CH_2Cl_2 (5 mL) prior to the addition of (*E*)-2-methyl-3-phenylpropanol (1.000 g, 6.696 mmol). A solution of KBr (79.22 mg, 0.1 eq., 0.6657 mmol) in water (0.5 mL) was added into the CH_2Cl_2 solution. The heterogeneous system was stirred vigorously and cooled at -10 °C. A solution of NaOCl (0.67 M) was buffered at pH ca. 9.5 with $NaHCO_3$ (added in small portions as solid). The buffered NaOCl solution (10.93 mL, 1.1 eq., 7.328 mmol) was slowly dropped (ca. 10 min) into the reaction mixture using a dropping funnel and the temperature was carefully maintained between -5/-10 °C. The oxidation was run for additional 15 min. The progress of the reaction was checked by TLC (Petroleum ether/EtOAc 8:2, v v^{-1}, Rf substrate: 0.35; Rf product: 0.71), showing that all the reagent was converted into the product. The organic phase was separated from the aqueous phase. The aqueous phase was then extracted once with CH_2Cl_2 (10 mL). The combined organic phases were washed first with a solution of HCl (10% v v^{-1}, 4 mL) containing KI (64 mg), then with a solution of $Na_2S_2O_3$ (10% v v^{-1}, 20 mL) and finally with water (20 mL). The organic phase was dried with $MgSO_4$, filtered and the solvent was evaporated. The pure product was isolated as pale yellow oil without any further purification (0.836 g, 5.640 mmol) in 84% yield. ^{1}H-NMR and ^{13}C-NMR were measured. All the experimental data were according to literature.[13]

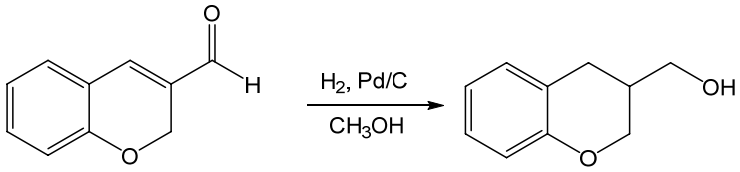
^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 1.00 (3H, CH_3, d, ^{3}J= 6.8 Hz), 2.52 (1H, CH_2, dd), 2.58 (1H, CH, m), 3.01 (1H, CH_2, dd, ^{2}J= 13.2 Hz, ^{3}J= 5.6 Hz), 7.08 – 7.23 (5H, C-H aromatic, m), 9.63 (1H, CH aldehyde, ^{3}J= 1.6 Hz).

^{13}C-NMR ($CDCl_3$, 400 MHz, δ (ppm): 13.2, 36.7, 48.1, 126.4, 128.6, 129.0, 138.9, 204.4.

GC-MS: (m/z, (rel. intensity)): 148 (38), 133 (11), 115 (8), 105 (16), 91 (100), 78 (16), 65 (11), 51 (5). GC-MS was measured with a Varian CP3800 gas chromatograph connected to a Varian Saturn 2000 GC/MS/MS (Agilent DB-Wax column).

3) Synthesis of chromane-3-carbaldehyde 5b

Synthesis of chroman-3-ylmethanol



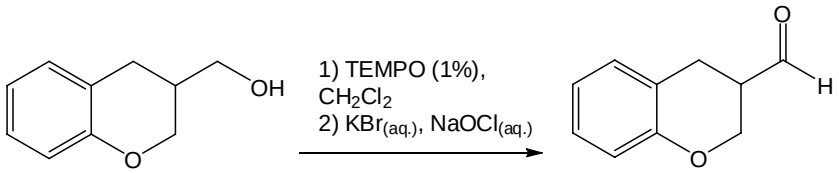
This reaction was aimed at directly obtaining the saturated aldehyde through the selective reduction of the C=C double bond by catalytic hydrogenation. However, the reduction of the C=C double bond occurred concomitantly with the reduction of the aldehyde moiety. Therefore, the saturated alcohol was isolated after the first step.

Nevertheless, a minor aliquot of the desired aldehyde was also obtained as judged by the ^{1}H-NMR spectra. Comparing the intensity of the aldehyde peak with the intensity of the characteristic peaks for the alcohol, it was possible to esteem the amount of aldehyde to be ca. 18%. The remaining 82% was the alcohol. No further products were detected as determined by GC-FID and GC-MS.

2*H*-Chromene-3-carbaldehyde (300 mg, 1.873 mmol) was dissolved in dried methanol (10 mL) under inert atmosphere in a two neck round-bottom flask. Then catalytic Pd/C (30 mg, 10% Pd content) was carefully added. The nitrogen was evacuated from the flask and pure hydrogen was introduced at atmospheric pressure. The hydrogenation was run until no further consumption of hydrogen was observed.

The reaction mixture was filtered through celite for removing the Pd/C. The solvent was evaporated and the product was isolated as colorless oil (305 mg). The mixture of chroman-3-ylmethanol and chroman-3-carbaldehyde was subjected to the final oxidation to get the chroman-3-carbaldehyde as the sole product.

Synthesis of chroman-3-carbaldehyde



The oxidation with TEMPO/NaOCl was repeated as previously described. Chroman-3-ylmethanol (305 mg, 1.875 mmol) was dissolved in a solution of TEMPO (2.58 mg, 0.020 mmol) in CH_2Cl_2 (2 mL). The oxidation was carried out as described before, using aqueous KBr (24.2 mg, 0.200 mmol) and a buffered solution of NaOCl (pH 9.5, 0.67 M, 3.35 mL). Work-up was performed as before. The product was isolated as colorless oil that becomes a waxy solid by standing at 4 °C (146 mg, 0.898 mmol). Isolated yield was 48%.

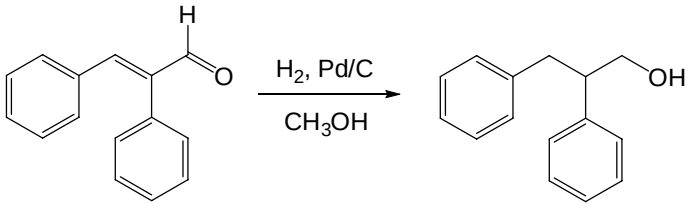
^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 2.88 (1H, CH, m), 2.94 (1H, CH_2, dd), 3.04 (1H, CH_2, dd, ^{2}J= 16.4 Hz, ^{3}J= 7.2 Hz), 4.24 – 4.35 (2H, CH_2, dddd, ^{2}J= 11.2 Hz, ^{3}J= 3.2 Hz, ^{4}J= 0.8 Hz), 6.72 – 6.83 (2H, C-H aromatic, m), 7.01 – 7.46 (2H, C-H aromatic, m).

^{13}C-NMR ($CDCl_3$, 400 MHz, δ (ppm): 23.7, 44.3, 64.0, 115.5, 120.2, 126.9, 128.8, 153.3, 200.3.

GC-MS: (m/z, (rel. intensity)): 162 (78), 144 (17), 131 (100), 115 (15), 105 (30), 105 (33), 91 (17), 77 (33), 63 (8), 51 (13). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

4) Synthesis of (*E*)-2,3-diphenylpropanal (α-phenylhydrocinnamaldehyde) 2b

Synthesis of (*E*)-2,3-diphenylpropan-1-ol

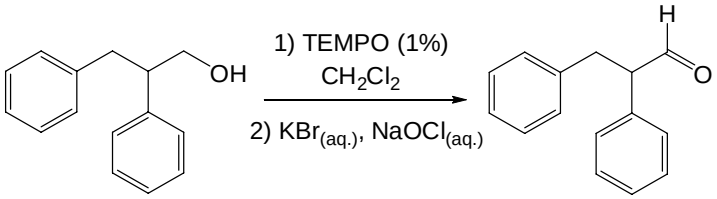


The catalytic hydrogenation was carried out as previously described. (*E*)-2,3-diphenylacrylaldehyde (23.9 mg, 0.115 mmol) was dissolved in dried methanol (5 mL) under inert atmosphere in a two neck round-bottom flask. Pd/C (10 mg, 10% Pd content) was added and the reagent was subjected to hydrogenation. The product was isolated as colorless oil (23.9 mg, 0.113 mmol) in 98% yield.

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 1.40 (1H, OH, broad), 2.82 (1H, CH_2, dd, ^{2}J= 13.2 Hz, ^{3}J= 7.2 Hz), 2.97 (1H, CH_2, dd), 3.01 (1H, CH, m), 6.96 – 7.25 (10H, C-H aromatic).

^{13}C-NMR ($CDCl_3$, 400 MHz, δ (ppm): 37.7, 49.1, 65.4, 125.0, 125.8, 127.1, 127.2, 127.6, 128.0, 138.9, 140.1.

Synthesis of (*E*)-2,3-diphenylpropanal



The oxidation with TEMPO/NaOCl was employed. (*E*)-2,3-diphenylpropan-1-ol (23.9 mg, 0.113 mmol) was reacted with catalytic TEMPO (0.117 mg, 0.001 mmol) in CH_2Cl_2 (500 µL) and aqueous KBr (1.34 mg, 0.011 mmol) with aqueous buffered NaOCl (pH 9.5, 0.67 M, 186 µL). Work-up was performed as before. The product was isolated as white solid (23.4 mg, 0.111 mmol) in 98% yield.

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 2.89 (1H, CH_2, dd, ^{2}J= 14.0 Hz, ^{3}J= 7.6 Hz) 3.40 (1H, CH_2, dd), 3.77 (1H, CH, m), 6.97 – 7.29 (10H, C-H aromatic), 9.67 (1H, CH aldehyde, d, ^{3}J= 1.6 Hz).

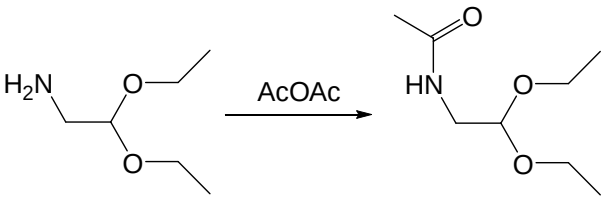
^{13}C-NMR ($CDCl_3$, 400 MHz, δ (ppm): 35.2, 60.0, 125.3, 126.7, 127.3, 128.0, 134.7, 137.8, 198.9.

GC-MS: (m/z, (rel. intensity)): 210 (50), 181 (25), 165 (17), 152 (3), 118 (3), 103 (17), 91 (100), 77 (12), 65 (10), 51 (4). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

5) Synthesis of (*Z*)-N-(1-(4-chlorophenyl)-3-oxoprop-1-en-2-yl)acetamide (α-acetamido *para*-chloro *trans*-cinnamaldehyde) 4a

The synthesis of the compound was performed according to literature.[14]

Synthesis of acetamido acetaldehyde diethyl acetal



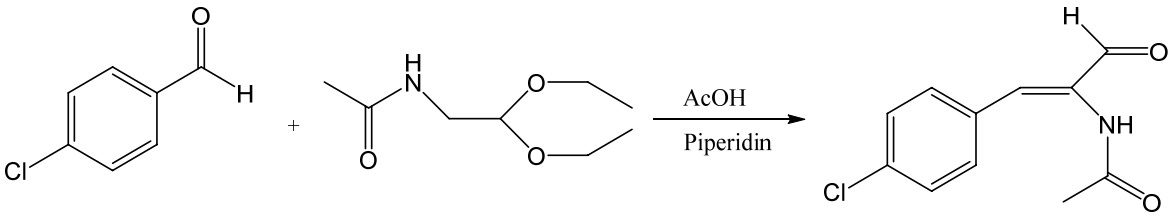
Aminoacetaldehyde diethyl acetal (5 mL, ρ 0.916 g mL^{-1}, 34 mmol) was cooled to 4 °C. Acetic anhydride was added (4.5 mL) and the reaction was stirred for 2 days at 4 °C. The dark brown reaction mixture was distilled under vacuum (0.1 torr) and the pure product was recovered as colorless viscous oil (5.72 g, 32.6 mmol) in 96% yield.

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 1.15 (6H, CH_3, t, ^{3}J= 7.2 Hz), 1.94 (3H, CH_3, s), 3.33 (2H, CH_2, t, ^{3}J= 5.6 Hz), 3.48 (1H, CH_2, m), 3.63 (1H, CH_2, m), 4.44 (1H, CH, t, ^{3}J = 5.2 Hz), 5.81 (1H, NH, broad).

^{13}C-NMR ($CDCl_3$, 400 MHz, δ (ppm): 14.3, 22.1, 40.8, 62.1, 99.6, 169.7.

GC-MS: (m/z, (rel. intensity)): 130 (27), 108 (100), 88 (29), 75 (55), 60 (25). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

Synthesis of α-acetamido *para*-chloro cinnamaldehyde



Para-chlorobenzaldehyde (1.214 g, 8.64 mmol), and acetamido acetaldehyde diethyl acetal (1.861 g, 10.62 mmol) were dissolved in glacial acetic acid (10 mL). Then, piperidine (5 drops) was added and the reaction mixture was heated up under reflux for ca. 15 min. The solution gradually became bright purple. Afterwards, the reaction mixture was cooled down to 20 °C and stirred at this temperature for two days. During this time, the reaction was carefully kept in darkness by wrapping alumina foil around the round bottom flask. After this time, the solution became dark brown. Formation of the product was detected by GC-MS and TLC (CH_2Cl_2 / CH_3OH, 98:2, v v^{-1}, Rf product= 0.4). However, lots of impurities were also detected by GC-MS and TLC.

Purification was quite cumbersome and required two column chromatographic steps. The first column chromatography was performed using a polar gradient as follows: CH_2Cl_2 pure; CH_2Cl_2/CH_3OH (98:2 v v^{-1}); CH_2Cl_2/CH_3OH (95:5 v v^{-1}) and CH_2Cl_2/CH_3OH (9:1 v v^{-1}). After TLC analysis of the collected fractions, selected pooled fractions were further analyzed by GC-MS. The pooled fraction containing the desired product was further purified with a second column chromatography using a polar gradient as follows: CH_2Cl_2 pure; CH_2Cl_2/CH_3OH (99:1 v v^{-1}); CH_2Cl_2/CH_3OH (98:2 v v^{-1}). After TLC analysis of the collected fractions, selected pooled fractions were further analyzed by GC-MS. GC-MS analysis as well as TLC analysis showed that the product was present in high purity (> 99%) The solvent was evaporated to afford a yellow solid (830 mg, 3.71 mmol) in 43% yield.

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 2.11 and 2.12 (3H, CH_3, s), 6.87 and 8.73 (1H, CH, s), 7.27 – 7.33 (4H, C-H aromatic, m), 7.32 and 7.78 (1H, NH, s), 9.32 and 9.46 (1H, CH aldehyde, s)

^{13}C-NMR ($CDCl_3$, 400 MHz, δ (ppm): 23.8, 127.7, 127.8, 129.8, 130.3, 130.3, 130.4, 133.1, 134.1, 168.1, 185.3, 188.7.

GC-MS: (m/z, (rel. intensity)): 223 (20), 207 (18), 181 (100), 164 (10), 152 (60), 138 (6), 127 (10), 117 (40), 89 (46), 75 (10), 63 (15), 51 (8). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

Biocatalytic synthesis of reference compounds

1 Biocatalytic synthesis of N-(1-(4-chlorophenyl)-3-oxopropan-2-yl)acetamide 4b

N-(1-(4-chlorophenyl)-3-oxopropan-2-yl)acetamide (**4b**) was obtained through enzymatic reduction of the related substrate. A 30 mL reaction was performed in phosphate buffer (50 mM KH_2PO_4/K_2HPO_4, pH 7.0). The reaction mixture consisted of **4a** (5 mM, 30 mg, dissolved as a stock solution in DMSO, 2% final co-solvent concentration), GluOx (6 µM) and NAD (6 mM) and was shaken at 30 °C, 190 rpm in an orbital shaker for 12 h. After extraction with EtOAc (2 x 15 mL) and drying over $MgSO_4$ the solvent was evaporated. A purple oil was obtained. The correct m/z ratio was identified by GC-MS (measured as the corresponding methylester).

GC-MS: (m/z, (rel. intensity)): 225 (6), 166(20), 154 (100), 138 (21), 125 (86), 117 (28), 94 (21), 58 (17). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

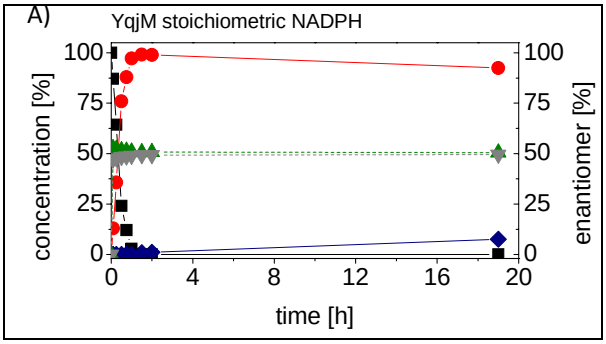
2 Biocatalytic synthesis of (*Z*)-2-acetamido-3-(4-chlorophenyl)acrylic acid 4e and 2*H*-chromene-3-carboxylic acid 5e

(*Z*)-2-acetamido-3-(4-chlorophenyl)acrylic acid (**4e**) and 2*H*-chromene-3-carboxylic acid (**5e**) were obtained through enzymatic oxidation of the related aldehyde substrates **4a** and **5a** using Ald-DH-BOV (for **4a**) and Ald-DH-EC (for **5a**). The reaction mixture (1 mL, performed in phosphate buffer 50 mM KH_2PO_4/K_2HPO_4, pH 7.0) consisted of substrates (5 mM, dissolved as a stock solution in DMSO, 2% final co-solvent concentration), Ald-DH (5 µM) and NAD^+ (7 mM) and was shaken at 30 °C, 190 rpm in an orbital shaker for 5 h. After extraction with MTBE (2 x 400 µL) and derivatization to the corresponding methylester, the correct m/z ratio was identified by GC-MS.

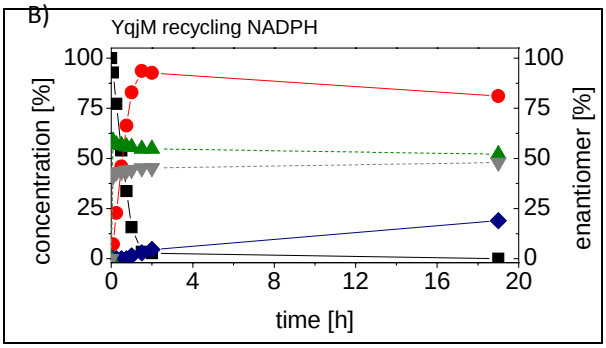
4e: GC-MS: (m/z, (rel. intensity)): 253 (12), 221 (7), 211 (100), 151 (81), 116 (12), 89 (27), 63 (6). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

5e: GC-MS: (m/z, (rel. intensity)): 190 (51), 175 (95), 159 (14), 131 (100), 115 (10), 102 (14), 95 (5), 77 (25), 63 (4), 51 (12). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

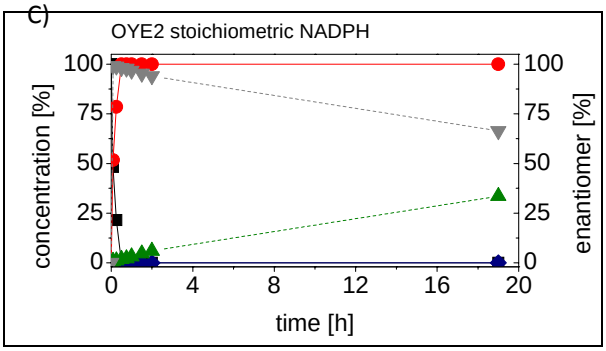
Experiments performed for the conversion of α-methyl-*trans*-cinnamaldehyde (1a)



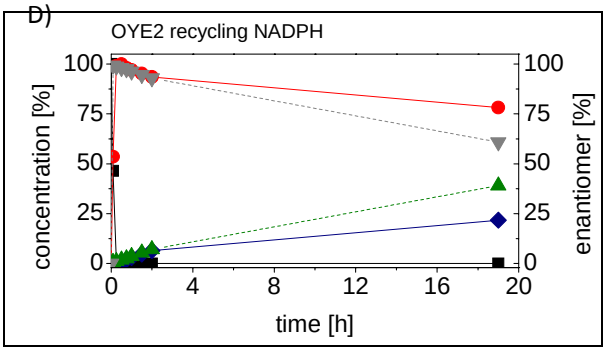
time [min]	conv. [%]	**1c** [%]	**1b** [%]	*ee* (*R*)-**1b** [%]	
5	13	n.m.	13	5	NADPH stoichiometric
15	36	n.m.	36	4	
30	76	n.m.	73	3	
45	88	n.m.	88	3	
60	97	n.m.	97	2	
90	>99	1	99	2	
120	>99	1	99	2	
19 h	>99	8	92	1	



time [min]	conv. [%]	**1c** [%]	**1b** [%]	*ee* (*R*)-**1b** [%]	
5	7	n.m.	7	17	NADPH recycling
15	23	n.m.	23	14	
30	46	n.m.	46	13	
45	66	n.m.	66	12	
60	84	n.m.	83	11	
90	96	2	94	10	
120	97	4	93	9	
19 h	>99	19	81	4	



time [min]	conv. [%]	**1c** [%]	**1b** [%]	*ee* (*S*)-**1b** [%]	
5	52	n.m.	52	98	NADPH stoichiometric
15	78	n.m.	78	98	
30	>99	n.m.	100	96	
45	>99	n.m.	100	96	
60	>99	n.m.	100	94	
90	>99	n.m.	100	90	
120	>99	n.m.	100	88	
19 h	>99	n.m.	100	33	



time [min]	conv. [%]	**1c** [%]	**1b** [%]	*ee* (*S*)-**1b** [%]	
5	53	n.m.	53	99	NADPH recycling
15	99	n.m.	99	98	
30	>99	n.m.	100	95	
45	>99	2	98	94	
60	>99	3	97	93	
90	>99	5	95	89	
120	>99	6	94	86	
19 h	>99	22	78	23	

Figure S3. Progress curve for the biotransformation of **1a** by A) YqjM using stoichiometric amounts of cofactor, B) YqjM using the GDH/glucose recycling system, C) OYE2 using stoichiometric amounts of cofactor and D) OYE2 using the GDH/glucose recycling system. Concentration of α-methylcinnamaldehyde **1a** (■), α-methylhydrocinnamaldehyde **1b** (●), α-methylhydrocinnamic alcohol **1c** (♦) and (*R*)-α-methylhydrocinnamaldehyde (▲) and (*S*)-α-methylhydrocinnamaldehyde (▼). Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [ER] = 2 µM, [**1a**] = 5 mM, [NADPH] = 10 µM, [GDH] = 10 U, [glucose] = 15 mM or [NADPH] = 11 mM; extraction with MTBE (2 x 500 µL). Conversion measured by achiral GC (DB-Wax); *ee* measured by chiral HPLC (Chiralsil OJ-H). n.m. not measurable.

Table S2. pH-Time-NAD(P)H study for the conversion of **1b** to **1d** using aldehyde dehydrogenases.

	time [h]	conv [%] pH 6 (NAD^+)		conv [%] pH 7 (NAD^+)		conv [%] pH 8 (NAD^+)		conv [%] pH 9 (NAD^+)	
Ald-DH-BOV	0.5	6		12		20		65	
	1	7		16		23		68	
	2	8		23		34		69	
	4	10		31		41		62	
	7	12		37		50		73	
	23	20		52		63		74	
Ald-DH-HL	0.5	5		6		7		48	
	1	5		7		8		51	
	2	5		8		10		50	
	4	6		11		14		48	
	7	6		14		18		52	
	23	7		32		32		51	
Ald-DH-EC		NAD^+	$NADP^+$	NAD^+	$NADP^+$	NAD^+	$NADP^+$	NAD^+	$NADP^+$
	0.5	5	2	71	18	92	28	>99	75
	1	7	2	81	21	>99	35	>99	76
	2	6	3	82	28	>99	36	>99	73
	4	6	3	>99	35	>99	45	>99	75
	7	7	-	>99	-	>99	-	>99	-
	23	7	3	>99	44	>99	66	>99	70

Experimental conditions: reaction volume = 1 mL, 50 mM KPi (pH 6.0, 7.0, 8.0) and 50 mM Tris/HCl pH 9.0, 30 °C, [Ald-DH] = 2 µM, [**1b**] = 5 mM, [$NAD(P)^+$] = 7 mM; extraction under acidic conditions with MTBE (2 x 500 µL) and derivatization with (trimethylsilyl)diazomethane to methylester. Conversion measured by achiral GC (DB-Wax).

Table S3. First experiment one-pot two enzyme cascade reaction for the conversion of **1a** to **1d** (The plot is shown in the main paper Figure 2).

time [min]	conversion [%]	**1a** [%]	**1b** [%]	**1d** [%]	**1e** [%]
15	15	85	1	10	4
30	19	81	1	13	4
60	27	73	2	20	5
90	32	68	2	24	6
120	32	68	2	24	6
180	39	61	2	30	7
240	42	58	2	33	7
360	45	55	1	36	8

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [OYE2] = 2 µM, [Ald-DH-EC] = 2 µM, [**1a**] = 5 mM, [NADH] = 10 µM; two-step selective extraction with MTBE: (I) under basic conditions (aldehydes) and (II) acidic conditions and derivatization with (trimethylsilyl)diazomethane (acids(ester)); IS = 2-phenylethanol. Conversion was measured by achiral GC (DB-Wax).

Table S4. Time study optimized one-pot two enzyme cascade reaction for the conversion of **1a** to **1d** (The plot is shown in the main paper Figure 3).

time [min]	conversion [%]	**1a** [%]	**1b** [%]	**1d** [%]	**1e** [%]	*ee* (*S*)-**1d** [%]
5	10	90	2	6	1	n.d.
10	17	83	3	12	2	n.d.
15	26	74	4	19	3	>98
20	32	68	4	25	3	>99
30	47	53	4	39	4	>99
45	68	32	4	60	5	99
60	91	9	3	83	5	99
80	>99	n.m.	n.m.	95	5	99
100	>99	n.m.	n.m.	95	5	>98

n.m. not measurable

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [OYE2] = 10 µM, [Ald-DH-EC] = 5 µM, [NADH] = 25 µM, [**1a**] = 5 mM; two-step selective extraction with MTBE: (I) under basic conditions (aldehydes) and (II) acidic conditions and derivatization with (trimethylsilyl)diazomethane (acids(ester)); IS = 2-phenylethanol. Conversion was measured by achiral GC (DB-Wax) and *ee* by chiral HPLC (Chiralsil OJ-3).

Table S5. One-pot two enzyme cascade reaction for the conversion of **1a** to **1d** using higher substrate concentrations.

substrate [mM]	time [h]	OYE2 [µM]	Ald-DH-EC [µM]	conv. [%]	**1d** [%]	**1e** [%]	*ee* (*S*)-**1d** [%]
10	6	10	5	97	93	4	99
25	6	10	5	38	33	5	n.d.
25	24	20	10	>99	96	4	96
50	6	10	5	16	14	2	n.d.
50	24	20	10	56	51	5	n.d.

n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [NADH] = 100 µM, [**1a**] = 5 mM; two-step selective extraction with MTBE: (I) under basic conditions (aldehydes) and (II) acidic conditions and derivatization with (trimethylsilyl)diazomethane (acids(ester)); IS = 2-phenylethanol. Conversion was measured by achiral GC (DB-Wax) and *ee* by chiral HPLC (Chiralsil OJ-3).

Experiments performed for the conversion of α-phenyl-*trans*-cinnamaldehyde (2a)

Table S6. Conversion of **2a** to **2d** using aldehyde dehydrogenases.

Ald-DH	time [h]	cofactor	conversion [%]	**2d**	side product 1 [%]	side product 2 [%]
EC	6	$NADP^+$	30	14	16	n.m.
EC	24	$NADP^+$	35	16	3	16
EC	6	NAD^+	20	16	4	n.m.
EC	24	NAD^+	51	18	12	21
BOV	6	NAD^+	>99	>99	n.m.	n.m.
BOV	24	NAD^+	>99	>99	n.m.	n.m.
HL	24	NAD^+	95	95	n.m.	n.m.
-	24	NAD^+	31	9	3	20

n.m. not measurable

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [Ald-DH] = 5 µM, [$NAD(P)^+$] = 7 mM, [**2a**] = 5 mM; extraction under acidic conditions with MTBE (2 x 500 µL), derivatization with (trimethylsilyl)diazomethane to methylester. Conversion was measured by achiral GC (DB-Wax).

Table S7. Cascade reaction combining all ERs with Ald-DH-BOV to determine the best ene reductase for the conversion of **2a**.

ER	conversion [%]	**2d** [%]	**2e [%]**	*ee* (*R*)-**2d** [%]
PETNR	96	89	7	94
TOYE	47	5	42	n.d.
OYE2	88	64	24	97
OYE3	86	47	39	95
XenA	70	4	66	n.d.
XenB	97	86	12	96
LeOPR1	98	83	15	93
NerA	92	77	16	95
GluOx	95	91	4	70
YqjM	97	48	49	46
MR	76	7	69	n.d.

n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [ER] = 20 µM, [Ald-DH-BOV] = 4 µM, [NADH] = 500 µM, [**2a**] = 5 mM; two-step selective extraction with MTBE: (I) under basic conditions (aldehydes) and (II) acidic conditions and derivatization with (trimethylsilyl)diazomethane (acids(ester)); IS = 2-phenylethanol. Conversion was measured by achiral GC (DB-Wax) and *ee* by chiral HPLC (Chiralsil OJ-3).

Table S8. Time study one-pot two enzyme cascade reaction for the conversion of **2a** to **2d** by PETNR and Ald-DH-BOV (The plot is shown in the main paper Figure 5A).

time [h]	conversion [%]	**2a** [%]	**2b** [%]	**2d** [%]	**2e** [%]	*ee* (*R*)-**2d** [%]
0.5	47	53	1	36	10	n.d.
1	53	47	2	44	7	n.d.
1.5	72	28	<1	65	7	95
2	84	16	n.m.	77	7	96
3	91	9	n.m.	82	8	97
4	92	8	n.m.	84	8	97

n.m. not measurable; n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [PETNR] = 25 µM, [Ald-DH-BOV] = 4 µM, [NADH] = 50 µM, [**2a**] = 5 mM; two-step selective extraction with MTBE: (I) under basdic conditions (aldehydes) and (II) acidic conditions and derivatization with (trimethylsilyl)diazomethane (acids(ester)); IS = 2-phenylethanol. Conversion was measured by achiral GC (DB-Wax) and *ee* by chiral HPLC (Chiralsil OJ-3).

Table S9. Time study one-pot two enzyme cascade reaction for the conversion of **2a** to **2d** by XenB and Ald-DH-BOV (The plot is shown in the main paper Figure 5B).

time [h]	conversion [%]	**2a** [%]	**2b** [%]	**2d**[%]	**2e** [%]	*ee* (*R*)-**2d** [%]
0.5	50	50	1	40	10	n.d.
1	62	38	<1	53	9	97
1.5	75	25	n.m.	66	9	98
2	86	14	n.m.	77	9	98
3	90	10	n.m.	82	8	98
5	91	9	n.m.	86	5	99

n.m. not measurable; n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [XenB] = 25 µM, [Ald-DH-BOV] = 4 µM, [NADH] = 50 µM, [**2a**] = 5 mM; two-step selective extraction with MTBE: (I) under basic conditions (aldehydes) and (II) acidic conditions and derivatization with (trimethylsilyl)diazomethane (acids(ester)); IS = 2-phenylethanol. Conversion was measured by achiral GC (DB-Wax) and *ee* by chiral HPLC (Chiralsil OJ-3).

Table S10. Additional experiments one-pot two enzyme cascade reaction for the conversion of **2a** to **2d** using increased concentration of cofactor and decreased concentration of Ald-DH-BOV.

ER	Ald-DH [µM]	conv. [%]	**2a** [%]	**2d** [%]	**2e** [%]	*ee* (*R*)-**2d** [%]
PETNR	4	75	25	67	7	>97
PETNR	2	69	31	64	6	96
XenB	4	91	9	76	14	>98
XenB	2	86	14	75	12	97
OYE2	4	78	22	64	13	98
OYE2	2	69	31	58	12	96

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [ER] = 25 µM, [Ald-DH-BOV] = 2 µM and 4 µM, [NADH] = 150 µM, [**2a**] = 5 mM; two-step selective extraction with MTBE: (I) under basic conditions (aldehydes) and (II) acidic conditions and derivatization with (trimethylsilyl)diazomethane (acids(ester)); IS = 2-phenylethanol. Conversion was measured by achiral GC (DB-Wax) and *ee* by chiral HPLC (Chiralsil OJ-3).

Experiments performed for the conversion of *trans*-2-methyl-2-pentenal (3a)

Table S11. Asymmetric bioreduction of **3a** using ene-reductases.

ER	conversion [%]	*ee* value (*S*)-**3b** [%]
PETNR	10	*rac*
TOYE	72	19 (*S*)
OYE2	96	97 (*S*)
OYE3	18	*rac*
XenA	22	8 (*S*)
XenB	68	4 (*S*)
LeOPR1	48	68 (*R*)
NerA	>99	78 (*S*)
GluOx	63	77 (*S*)
YqjM	49	17 (*R*)
MR	>99	5 (*S*)

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 1 h, [ER] = 2 µM, [**3a**] = 5 mM, [NADH] = 10 µM, [GDH] = 10 U, [glucose] = 15 mM; extraction with MTBE (2 x 500 µL). Conversion measured by achiral GC (DB1701); *ee* measured by chiral GC (Restek Rt-βDEXsa).

Table S12. Conversion of **3b** to **3d** using aldehyde dehydrogenases.

Ald-DH	conversion [%]
EC	>99
BOV	74
HL	22

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 1 h, [Ald-DH] = 5 µM, [**3b**] = 5 mM, [NAD^+] = 7 mM; extraction under acidic conditions with MTBE (2 x 500 µL), derivatization with (trimethylsilyl)diazomethane to methylester. Conversion was measured by achiral GC (DB1701).

Table S13. Time study one-pot two enzyme cascade reaction for the conversion of **3a** to **3d** by OYE2 and Ald-DH-EC (The plot is shown in the main paper Figure 6).

time [min]	conversion [%]	**3a** [%]	**3b** [%]	**3d** [%]	**3e** [%]	*ee* (*S*)-**3d** [%]
5	7	93	3	2	1	n.d.
10	14	86	5	4	4	n.d.
15	20	80	6	9	6	n.d.
20	28	72	7	14	8	n.d.
30	48	52	8	29	11	n.d.
45	96	4	6	77	13	98
60	>99	n.m.	n.m.	87	13	98

n.m. not measurable; n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [OYE2] = 10 µM, [Ald-DH-EC] = 3 µM, [NADH] = 25 µM, [**3a**] = 5 mM; extraction under acidic conditions with MTBE (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion was measured by achiral GC (DB1701) and *ee* by chiral GC (Restek Rt-ßDEXsm).

Table S14. Additional experiments one-pot two enzyme cascade reaction for the conversion of **3a** to **3d** using various concentrations of NADH, Ald-DH-EC and Ald-DH-BOV.

	Ald-DH [nM]	time [h]	NADH [µM]	conv [%]	**3a** [%]	**3b** [%]	**3d** [%]	**3e** [%]	*ee* (*S*)-**3d** [%]
Ald-DH-BOV	3000	5	25	>99	n.m.	n.m.	91	9	>98
	300	5	25	10	90	2	4	4	n.d.
	300	5	100	19	81	3	10	5	n.d.
	30	5	25	n.m.	100	n.m.	n.m.	n.m.	n.d.
	30	5	100	n.m.	100	n.m.	n.m.	n.m.	n.d.
Ald-DH-BOV	3000	12	25	>99	n.m.	n.m.	92	8	>98
	300	12	25	3	97	n.m.	n.m.	3	n.d.
	300	12	100	5	95	3	n.m.	2	n.d.
	30	12	25	n.m.	100	n.m.	n.m.	n.m.	n.d.
	30	12	100	n.m.	100	n.m.	n.m.	n.m.	n.d.
Ald-DH-EC	300	5	25	>99	n.m.	n.m.	85	15	96
	300	5	100	>99	n.m.	n.m.	85	15	96
	30	5	25	5	95	2	n.m.	3	n.d.
	30	5	100	8	92	3	n.m.	5	n.d.
Ald-DH-EC	300	12	25	>99	n.m.	n.m.	86	14	96
	300	12	100	>99	n.m.	n.m.	87	13	96
	30	12	25	20	80	5	7	8	n.d.
	30	12	100	16	84	5	5	7	n.d.

n.m. not measurable; n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [ER] = 10 µM, [NADH] = 25 µM, [**3a**] = 5 mM; extraction under acidic conditions with MTBE (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion was measured by achiral GC (DB1701) and *ee* by chiral GC (Restek Rt-ßDEXsm).

Experiments performed for the conversion of (*Z*)-N-(1-(4-chlorophenyl)-3-oxoprop-1-en-2-yl)acetamide (4a)

Table S15. Asymmetric bioreduction of **4a** using ene-reductases.

ER	conversion [%]	*ee* (*S*)-**4b** [%]
PETNR	>99	61
TOYE	>99	*rac*
OYE2	>99	70
OYE3	>99	73
XenA	65	*rac*
XenB	>99	51
LeOPR1	>99	33
NerA	>99	*rac*
GluOx	>99	*rac*
YqjM	>99	*rac*
MR	93	*rac*

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [ER] = 4 µM, [**4a**] = 5 mM, [NADH] = 6 mM; extraction with MTBE (2 x 500 µL). Conversion measured by achiral GC (HP5); *ee* measured by chiral HPLC (Chiralsil OJ-3).

Table S16. Conversion of **4a** to **4e** using aldehyde dehydrogenases.

Ald-DH	conversion [%]
EC	n.m.
BOV	21
HL	n.m.

n.m. not measurable

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [Ald-DH] = 5 µM, [**4a**] = 5 mM, [NAD^+] = 6 mM; extraction under acidic conditions with MTBE (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (HP5).

Table S17. Conversion of **4b** to **4d** using aldehyde dehydrogenases.

Ald-DH	conversion [%]
EC	34
BOV	77
HL	55

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [Ald-DH] = 5 µM, [**4b**] = 5 mM, [NAD^+] = 6 mM; extraction under acidic conditions with MTBE (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (HP5).

Table S18. One-pot two enzyme cascade reaction for the conversion of **4a** to **4d**.

ER	Ald-DH	conv [%]	**4a** [%]	**4b** [%]	**4d** [%]	**4e** [%]	*ee* (*S*)-**4d** [%]
PETNR	BOV	99	1	n.m.	81	18	85
OYE2	BOV	99	1	n.m.	81	18	94
OYE3	BOV	99	1	3	78	18	93
PETNR	EC	13	87	4	6	4	n.d.
OYE2	EC	32	68	11	17	4	n.d.
OYE3	EC	20	80	5	11	4	n.d.

n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [ER] = 10 µM, [Ald-DH] = 10 µM, [**4a**] = 5 mM, [NADH] = 100 µM; extraction under acidic conditions with EtOAc (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (HP5); *ee* measured by chiral GC (DEX-CB).

Table S19. One-pot two enzyme cascade reaction for the conversion of **4a** to **4d** using various concentrations of OYE2 and NADH in combination with Ald-DH-BOV.

OYE2 [µM]	NADH [µM]	conv [%]	**4a** [%]	**4b** [%]	**4d** [%]	**4e** [%]	*ee* (*S*)-**4d** [%]
10	100	99	1	n.m.	83	16	95
10	50	99	1	n.m.	82	16	95
10	25	95	2	1	80	18	95
5	100	98	2	n.m.	82	17	94
5	50	99	4	1	81	17	95
5	25	98	2	1	80	16	94

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [Ald-DH-BOV] = 10 µM, [**4a**] = 5 mM; extraction under acidic conditions with EtOAc (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (HP5); *ee* measured by chiral GC (DEX-CB).

Table S20. Time study one-pot two enzyme cascade reaction for the conversion of **4a** to **4d** by OYE2 and Ald-DH-BOV (The plot is shown in the main paper Figure 7).

time [min]	conversion [%]	**4a** [%]	**4b** [%]	**4d** [%]	**4e** [%]	*ee* (*S*)-**4d** [%]
5	n.m.	100	n.m.	n.m.	n.m.	n.d.
10	n.m.	100	n.m.	n.m.	n.m.	n.d.
15	1	99	1	n.m.	n.m.	n.d.
25	5	95	3	n.m.	2	n.d.
40	14	86	5	2	7	n.d.
60	24	76	8	6	11	n.d.
80	41	59	12	14	16	n.d.
100	55	45	14	22	19	n.d.
140	77	23	14	43	20	95
180	99	1	7	73	19	95
240	99	1	3	78	18	94
300	99	1	n.m.	81	18	94

n.m. not measurable; n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [OYE2] = 10 µM, [Ald-DH-BOV] = 10 µM, [NADH] = 50 µM, [**4a**] = 5 mM; extraction under acidic conditions with EtOAc (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (HP5) and *ee* value by chiral GC (DEX-CB).

Table S21. Additional experiments one-pot two enzyme cascade reaction for the conversion of **4a** to **4d** using increased concentrations of Ald-DH-BOV.

Ald-DH-BOV [µM]	conversion [%]	**4d** [%]	**4e** [%]	*ee* (*S*)-**4d** [%]
20	>99	82	18	95
30	>99	82	18	95
40	>99	82	18	96
50	>99	83	17	96

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [OYE2] = 10 µM, [NADH] = 50 µM, [**4a**] = 5 mM; extraction under acidic conditions with EtOAc (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (HP5) and *ee* value by chiral GC (DEX-CB).

Experiments performed for the conversion of 2*H*-chromene-3-carbaldehyde (5a)

Table S22. Asymmetric bioreduction of **5a** using ene-reductases.

ER	conversion [%]	**5b** [%]	side product 1 [%]	side product 2 [%]	*ee* value **5b** [%]
PETNR	97	62	26	9	*rac*
TOYE	29	22	7	n.m.	*rac*
OYE2	79	54	25	n.m.	82 (*S*)
OYE3	94	65	29	n.m.	75 (*S*)
XenA	43	35	8	n.m.	*rac*
XenB	96	72	24	n.m.	*rac*
LeOPR1	95	82	13	n.m.	*rac*
NerA	70	48	23	n.m.	*rac*
GluOx	79	54	25	n.m.	84 (*S*)
YqjM	82	54	26	2	*rac*
MR	52	47	5	n.m.	*rac*

n.m. not measurable

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [ER] = 4 µM, [**5a**] = 5 mM, [NADH] = 6 mM; extraction with MTBE (2 x 500 µL). Conversion measured by achiral GC (DB-Wax); *ee* measured by chiral HPLC (Chiralsil OJ-3).

Table S23. Conversion of **5a** to **5e** using aldehyde dehydrogenases.

Ald-DH	conversion [%]
EC	>99
BOV	>99

n.m. not measurable

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [Ald-DH] = 5 µM, [**5a**] = 5 mM, [NAD^+] = 6 mM; extraction under acidic conditions with MTBE (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (DB-Wax).

Table S24. Conversion of **5b** to **5d** using aldehyde dehydrogenases.

Ald-DH	conversion [%]
EC	>99
BOV	>99
HL	18

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, reaction time = 5 h, [Ald-DH] = 5 µM, [**5b**] = 5 mM, [NAD^+] = 6 mM; extraction under acidic conditions with MTBE (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (DB-Wax).

Table S25. Time study one-pot two enzyme cascade reaction for the conversion of **5a** to **5d** by OYE2 and Ald-DH-EC. (The plot is shown in the main paper Figure 8A).

time [h]	conversion [%]	**5a** [%]	**5b** [%]	**5d** [%]	**5e** [%]	*ee* (*S*)-**5d**[%]
0.5	50	50	4	23	17	n.d.
1	99	1	3	76	20	96
1.5	99	1	1	80	19	95
2	99	1	n.m.	81	19	95
2.5	>99	n.m.	n.m.	81	19	93
3	>99	n.m.	n.m.	81	19	93

n.m. not measurable; n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [OYE2] = 10 µM, [Ald-DH-EC] = 3 µM, [**5a**] = 5 mM, [NADH] = 100 µM; extraction under acidic conditions with EtOAc (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (DB-Wax); *ee* measured by chiral GC (Restek Rt-βDEXsm).

Table S26. Time study one-pot two enzyme cascade reaction for the conversion of **5a** to **5d** by GluOx and Ald-DH-EC. (The plot is shown in the main paper Figure 8B).

time [h]	conversion [%]	**5a** [%]	**5b** [%]	**5d** [%]	**5e** [%]	*ee* (*S*)-**5d** [%]
0.5	73	27	3	31	17	n.d.
1	99	1	3	77	20	96
1.5	99	1	1	80	19	>96
2	99	<1	n.m.	82	17	95
2.5	>99	n.m.	n.m.	82	18	95
3	>99	n.m.	n.m.	82	18	95

n.m. not measurable; n.d. not determined

Experimental conditions: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [GluOx] = 10 µM, [Ald-DH-EC] = 3 µM, [**5a**] = 5 mM, [NADH] = 100 µM; extraction under acidic conditions with EtOAc (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (DB-Wax); *ee* measured by chiral GC (Restek Rt-βDEXsm).

Table S27. Additional experiments one-pot two enzyme cascade reaction for the conversion of **5a** to **5d** using OYE2 and various concentrations of NADH, Ald-DH-EC and Ald-DH-BOV.

	Ald-DH [nM]	time [h]	NADH [µM]	conv [%]	**5a** [%]	**5b** [%]	**5d** [%]	**5e** [%]	side pr. [%]	*ee* (*S*)-**5d** [%]
Ald-DH-EC	3000	5	20	>99	n.m.	n.m.	81	19	n.m.	>93
	300	5	20	13	87	2	2	9	n.m.	n.d.
	300	5	100	24	76	5	6	13	n.m.	n.d.
	30	5	20	3	97	n.m.	n.m.	3	n.m.	n.d.
	30	5	100	3	97	n.m.	n.m.	3	n.m.	n.d.
Ald-DH-EC	3000	12	20	>99	n.m.	n.m.	80	19	1	>92
	300	12	20	8	92	n.m.	n.m.	8	n.m.	n.d.
	300	12	100	17	83	n.m.	3	14	n.m.	n.d.
	30	12	20	4	96	n.m.	n.m.	4	n.m.	n.d.
	30	12	100	5	95	n.m.	n.m.	5	n.m.	n.d.
Ald-DH-BOV	3000	5	20	14	86	n.m.	n.m.	5	8	n.d.
	300	5	20	3	97	n.m.	n.m.	3	n.m.	n.d.
	300	5	100	3	97	n.m.	n.m.	3	n.m.	n.d.
	30	5	20	2	98	n.m.	n.m.	2	n.m.	n.d.
	30	5	100	2	98	n.m.	n.m.	2	n.m.	n.d.
Ald-DH-BOV	3000	5	100	7	93	n.m.	n.m.	7	5	n.d.
	300	12	20	3	97	n.m.	n.m.	3	n.m.	n.d.
	300	12	100	10	90	n.m.	n.m.	7	n.m.	n.d.
	30	12	20	3	97	n.m.	n.m.	3	n.m.	n.d.
	30	12	100	3	97	n.m.	n.m.	3	n.m.	n.d.

n.m. not measurable; n.d. not determined

Table S28. Additional experiments one-pot two enzyme cascade reaction for the conversion of **5a** to **5d** using GluOx and various concentrations of NADH, Ald-DH-EC and Ald-DH-BOV.

	Ald-DH [nM]	time [h]	NADH [µM]	conv [%]	**5a** [%]	**5b** [%]	**5d** [%]	**5e** [%]	side pr. [%]	*ee* (*S*)-**5d** [%]
Ald-DH-EC	3000	5	20	99	1	n.m.	78	19	2	>93
	300	5	20	14	86	2	3	9	n.m.	n.d.
	300	5	100	19	81	4	5	11	n.m.	n.d.
	30	5	20	3	97	1	n.m.	3	n.m.	n.d.
	30	5	100	4	96	1	n.m.	3	n.m.	n.d.
Ald-DH-EC	3000	12	20	98	2	n.m.	80	18	n.m.	>92
	300	12	20	11	89	n.m.	2	9	n.m.	n.d.
	300	12	100	13	87	n.m.	3	11	n.m.	n.d.
	30	12	20	12	88	n.m.	n.m.	4	8	n.d.
	30	12	100	13	87	n.m.	n.m.	9	4	n.d.
Ald-DH-BOV	3000	5	20	8	92	2	1	5	n.m.	n.d.
	300	5	20	3	97	n.m.	n.m.	3	n.m.	n.d.
	300	5	100	3	97	n.m.	n.m.	3	n.m.	n.d.
	30	5	20	9	91	n.m.	n.m.	2	7	n.d.
	30	5	100	3	97	n.m.	n.m.	3	n.m.	n.d.
Ald-DH-BOV	3000	5	100	7	93	n.m.	n.m.	7	n.m.	n.d.
	300	12	20	18	82	n.m.	n.m.	4	14	n.d.
	300	12	100	4	96	n.m.	n.m.	4	n.m.	n.d.
	30	12	20	18	82	n.m.	n.m.	3	14	n.d.
	30	12	100	3	97	n.m.	n.m.	3	n.m.	n.d.

n.m. not measurable; n.d. not determined

Experimental conditions Table S27 and S28: reaction volume = 1 mL, 50 mM KPi pH 7.0, 30 °C, [OYE2] = [GluOx] = 10 µM, [**5a**] = 5 mM; extraction under acidic conditions with EtOAc (2 x 400 µL) and derivatization with (trimethylsilyl)diazo-methane to methylester. Conversion measured by achiral GC (DB-Wax); *ee* measured by chiral GC (Restek Rt-βDEXsm).

Analytical methods and determination of absolute configuration

GC measurements were performed using a 7890A GC System (Agilent Technologies) equipped with a FID-Detector and a 7693 autosampler.

The conversions of **1a**, **2a** and **5a** were determined using a DB Wax column from Agilent (30m x 0.32mm, 0.25µm film); **1a**: split ratio 10:1; Temp. program 100 °C hold 0 min; 4 °C/min to 200 °C hold 0 min, 20 °C/min to 240 °C hold 1 min. Retention times: **1a** 16.36 min; **1b** 11.13 min; **1e**-methylester 17.62 min; **1d**-methylester 12.16 min; **1c** 17.86 min; IS 2-phenylethanol 14.42 min. **2a**: split ratio 10:1; Temp. program 100 °C hold 2 min; 10 °C/min to 240 °C hold 4 min. Retention times: **2a** 17.88 min; **2b** 16.05 min; **2d**-methylester 16.30 min; **2e**-methylester 17.63 min; IS 2-phenylethanol 10.04 min. **5a**: split ratio 10:1; Temp. program 100 °C hold 0 min; 4 °C/min to 200 °C hold 0 min, 20 °C/min to 240 °C hold 1 min. Retention times: **5a** 24.42 min; **5b** 23.25 min; **5d**-methylester 23.79 min; 12.16 min; **5e**-methylester 25.36 min. The conversion for **3a** was measured using a DB1701 column from Agilent (30 m x 250 µm x 0.25 µm); split ratio 40:1; Temp. program 40 °C hold 2 min; 2 °C/min to 75 °C hold 2 min; 10 °C/min to 180 °C hold 2 min; 20 °C/min to 280 °C hold 0 min. Retention times: **3a** 10.39 min; **3b** 6.58 min; **3d**-methylester 10.67 min; **3e**-methylester 16.65 min. The conversion for **4a** was measured with an Agilent HP5 column (30 m x 320 µm x 0.25 µm); split ratio 50:1; constant flow: 1.6 mL/min; Temp. program 80 °C hold 2 min; 2 °C/min to 130 °C hold 0 min; 20 °C/min to 300 °C hold 2 min. Retention times: **4a** 32.62 min; **4b** 32.03 min; **4d**-methylester 32.56 min; **4e**-methylester 33.37 min.

The enantiomeric excess for the aldehyde **3b** was measured on a Restek Rt-ßDEXsa column (30m, 0.25 mm, 0.25 µm); split ratio 10:1;, injector 180 °C, detector 200 °C, flow 1 mL/min; Temp. program: 80 °C hold 10 min; 4 °C/min to 120 °C hold 2 min; 20 °C/min to 180 °C hold 1 min[15]. Retention times: (*R*)-**3b** 13.42 min, (*S*)-**3b** 13.84 min. The absolute configuration was assigned by comparison with literature data.[16] The *ee* values for the methylester **3d** and **5d** were measured using a Restek Rt-ßDEXsm column (30m, 0.25 mm, 0.25 µm); **3d**: split ratio 20:1; flow 1.2 mL/min; Temp. program: 70 °C hold 13 min; 10 °C/min to 200 °C hold 1 min; Retention times: (*R*)-**3d**-methylester 9.21 min, (*S*)-**3d**-methylester 9.49 min. **5d**: split ratio 20:1; temperature program: 150 °C hold 30 min; 10 °C/min to 200 °C hold 10 min; Retention times: (*S*)-**5d**-methylester 26.30 min; (*R*)-**5d**-methylester 27.05 min. The *ee* value for the methylester of **4d** was determined using a Agilent Chirasil Dex-CB column (25m x 320 µm x 0.25 µm nominal); split ratio: 10:1; constant flow: 1.5 mL/min; Temp. program 100 °C hold 0 min; 10 °C/min to 150 °C hold 60 min; 10 °C/min to 180 °C hold 1 min. Retention times: (*R*)-**4d**-methylester 60.13 min, (*S*)-**4d**-methylester 62.78 min.

HPLC analyses were performed on an Agilent 1200 analytical system.

The enantiomeric excess of aldehyde **1b** was measured on a Chiralsil OJ-H column (Chiralcel, 3 µm, 250 x 4.6 mm); mobile phase n-hexane/isopropanol 99:1 (isocratic); temperature 18 °C; flow rate 0.8 mL/min; UV = 215 nm. Retention times: **1a** 12.0 min, (*R*)-**1b** 11.8 min, (*S*)-**1b** 13.5 min, *rac*-**1c** 15.9 min. The absolute configuration was assigned by comparison with literature data.[17]

The enantiomeric excess values for the methylester of **1d**, and **2d** and for the aldehydes **4b** and **5b** were determined using a Chiralsil OJ-3 column (Chiralcel, 3 µm, 250 x 4.6 mm); **1c**: mobile phase n-hexane/EtOH 98:2 (isocratic); temperature: column was kept in an ice box; flow rate 1 mL/min; UV = 215 nm. Retention times: **1e**-methylester 11.6 min, (*R*)-**1d**-methylester 12.6 min, (*S*)-**1d**-methylester 14.6 min. **2d** and **5b**: mobile phase n-hexane/EtOH 98:2 (isocratic); temperature 10 °C; flow rate 1 mL/min. Retention times: IS 2-phenylethanol 14.3 min, (*R*)-**2d**-methylester 16.7 min, (*S*)-**2d**-methylester 24.6 min, **2e**-methylester 26.1 min. The absolute configuration of **5b** was assigned by comparison with literature data.[18] **4b**: mobile phase n-hexane/EtOH 90:10 (isocratic); temperature 10 °C; flow rate 1 mL/min; UV = 215 nm. Retention times: (*S*)-**4b** 14.1 min, (*R*)-**4b** 15.5 min, **4a** 25.3 min.

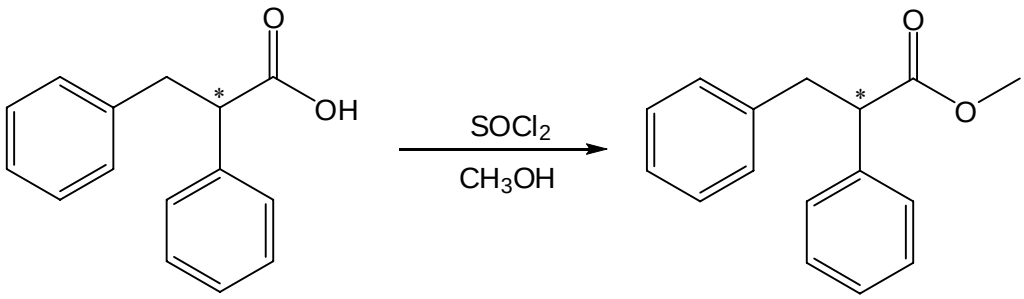
Absolute configuration of **4d**:

After separation of the enantiomers **4d** by preparative HPLC using a chiral preparative OJ-H column (Chiralcel 5 µm, 250 x 20 mm; mobile phase: n-hexane (0.1% TFA)/EtOH 95:5 (isocratic); flow rate 15 mL/min), the specific optical rotation of the acids (in MeOH) was measured on an ADP440+ Polarimeter (Bellington + Stanley). Peak 1: c = 0.258 g·dL^{-1}, *ee* = >99%, T = 20 °C, $[\alpha]_D$ = -45.4. Peak 2: c = 0.315 g·dL^{-1}, *ee* = >99%, T = 20 °C, $[\alpha]_D$ = +43.4. A comparison with literature data identified peak 1 as (*R*)-**4d** and peak 2 as (*S*)-**4d**.[19] The acids were derivatized to the corresponding methylester and according to their retention times (Chirasil Dex-CB column), the stereochemical outcome of the cascade reaction was determined to be (*S*).

Absolute configuration of enantiopure **2d**:

Separation of the enantiomers of **2d** was achieved by preparative HPLC using a chiral preparative OJ-H column (Chiralcel 5 µm, 250 x 20 mm; mobile phase: n-hexane (0.1% TFA)/EtOH 95:5 (isocratic); flow rate 15 mL/min). The enantiopure 2,3-diphenyl-propanoic acid (1st peak from preparative HPLC) was converted into 2,3 diphenyl 1-propanol, whose absolute configuration is known from literature. This is a two-step synthesis:

Reaction 1: Synthesis of the enantiopure methyl 2,3-diphenyl-propanoate



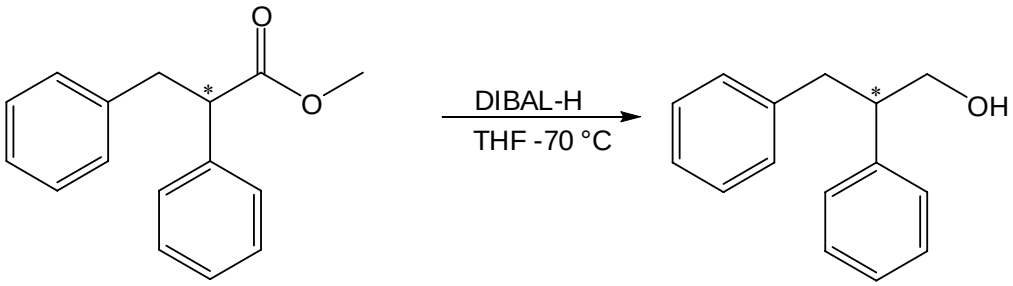
Enantiopure 2,3-diphenyl propanoic acid (143 mg , 0.632 mmol) was dissolved in dried MeOH (5 mL). The reaction mixture was cooled down to 4 °C and a solution of thionylchloride (140 µL, 3 eq., 1.918 mmol) dissolved in dried MeOH (1 mL), prior cooled to 4 °C, was added dropwise into the reaction mixture. The solution was then refluxed overnight at 42 °C. The progress of the reaction was analyzed by TLC (Petroleum ether/EtOAc, 9:1, v v^{-1}, Rf ester product = 0.65; the carboxylic acid does not elute from the base). Methanol was evaporated, the residue was treated with sodium carbonate (5 mL, 5% w w^{-1}) and extracted with EtOAc (2 × 5 mL). The combined organic phase was dried with $MgSO_4$, filtered and evaporated. The product was recovered as colourless oil in 90% yield (137 mg, 0.570 mmol).

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 2.94 (1H, CH_2, dd, ^{2}J= 13.6 Hz; ^{3}J= 6.4 Hz), 3.34 (1H, CH_2, dd, ^{3}J= 8.8 Hz), 3.52 (3H, CH_3, s), 3.77 (1H, CH, dd), 7.03 – 7.23 (10H, C-H aromatic)

^{13}C NMR ($CDCl_3$, 400 MHz, δ (ppm)): 38.8, 51.0, 52.6, 125.3, 126.4, 126.9, 127.3, 127.6, 127.9, 137.6, 138.0, 172.8

GC-MS (m/z, (rel. intensity)): 240 (36), 208 (5), 181 (33), 165 (13), 149 (7), 121 (15), 103 (9), 91 (100), 77 (13), 65 (7), 51(4). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

Reaction 2: Synthesis of the enantiopure 2,3-diphenyl 1-propanol



Under anaerobic conditions, enantiopure methyl 2,3-diphenyl-propanoate (137 mg, 0.570 mmol) was dissolved in anhydrous THF (3 mL). The solution was cooled and kept at ca. -70 °C. A solution of DIBAL-H in anhydrous THF (1M, 1.71 mL, 1.71 mmol) was added dropwise and the reaction was stirred for additional 2 h at -10 °C/-20 °C. The progress of the reaction was analyzed by TLC (Petroleum ether/EtOAc, 9:1, v v^{-1}, Rf ester = 0.65; Rf alcohol = 0.18). The TLC showed that the ester was completely reduced to the alcohol. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl. This solution was added dropwise at -10 °C/-20 °C until bubbling ceased and then diluted with distilled water up to 25 mL. The precipitated colloidal aluminium hydroxide was removed by filtration over celite. THF was partially evaporated and the alcohol was extracted with EtOAc (2 x 20 mL). The product was isolated as white oil in 75% yield (91.04 mg, 0.429 mmol).

^{1}H-NMR ($CDCl_3$, 400 MHz, δ (ppm), J (Hz)): 1.39 (1H, OH, s, broad), 2.82 (1H, CH_2, dd, ^{2}J= 13.2 Hz, ^{3}J= 7.6 Hz), 2.94 (1H, CH_2, dd), 3.01 (1H, CH, m), 3.69 (2H, CH_2, dd, ^{3}J= 5.6 Hz, ^{3}J= 0.8 Hz), 7.00 – 7.24 (10H, C-H aromatic).

^{13}C NMR ($CDCl_3$, 400 MHz, δ (ppm)): 37.7, 49.1, 65.3, 125.0, 125.8, 127.1, 127.2, 127.6, 128.0, 138.9, 140.9

GC-MS: (m/z, (rel. intensity)): 212 (9), 194 (92), 181 (50), 165 (31), 152 (), 121 (7), 103 (92), 91 (77), 77 (35), 65 (14), 51(8). GC-MS was measured with a Hewlett Packard HP6890 GC System connected to a HP5973 Mass selective detector (Agilent HP-1ms column).

The optical rotation of the enantiopure 2,3-diphenyl 1-propanol was measured on an ADP440+ Polarimeter (Bellington + Stanley).

c = 1.023 g·dL^{-1}, *ee* = >99%, T = 20 °C, $[\alpha]_D$ = -84.4.

Comparing to literature, the absolute configuration of 2,3-diphenyl 1-propanol was assigned to be (*R*). Therefore the enantiopure 2,3-diphenyl propanoic acid, achieved through biotransformation using ER and Ald-DH was also (*R*).[20]

Chiral HPLC chromatograms

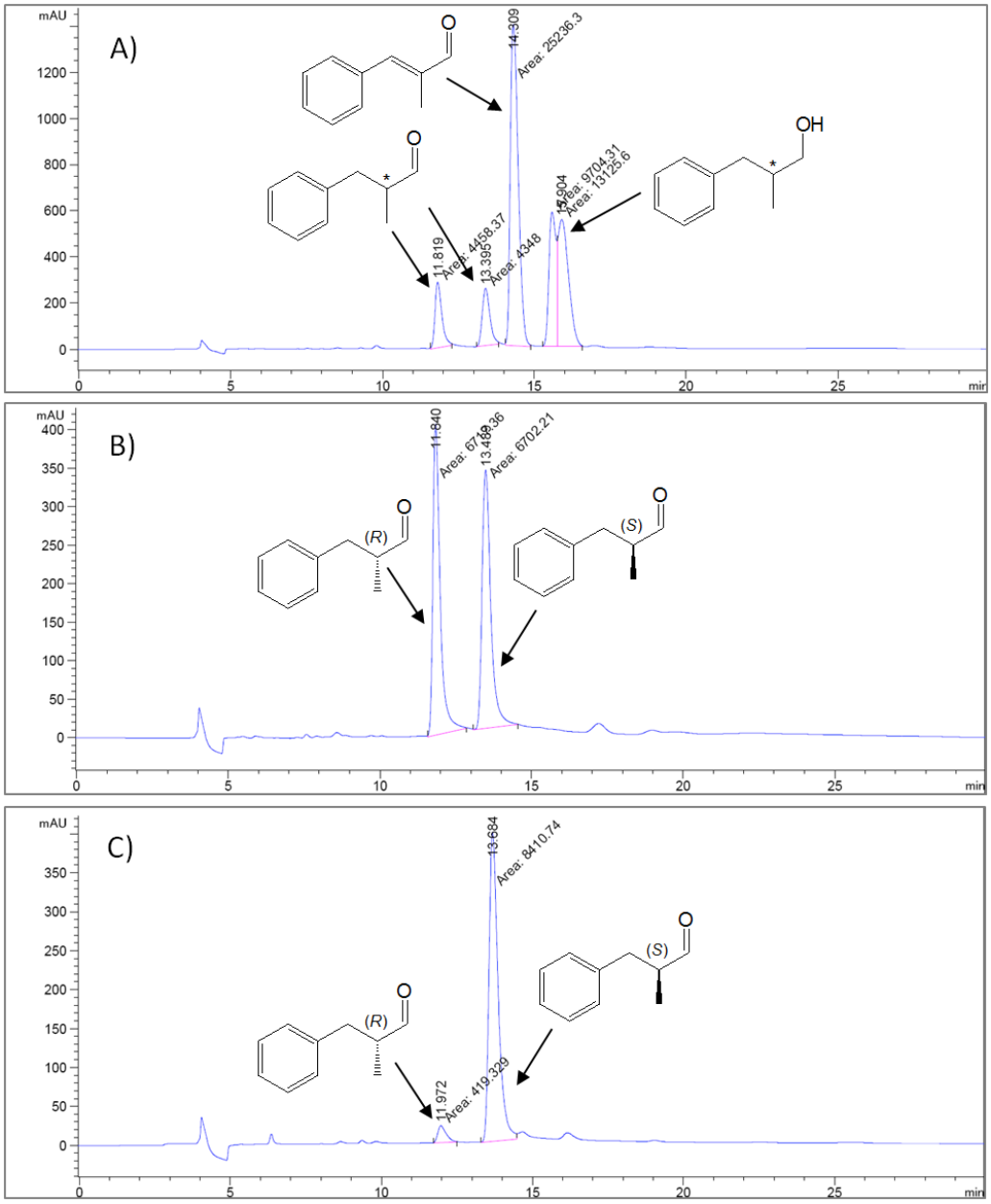


Figure S4. Chiral HPLC chromatograms (Chiralcel OJ-H) of A) **1a**, *rac*-**1b**, *rac*-**1c**, B) *rac*-**1b** and C) (*S*)-**1b** obtained from 1 h asymmetric bioreduction with OYE2.

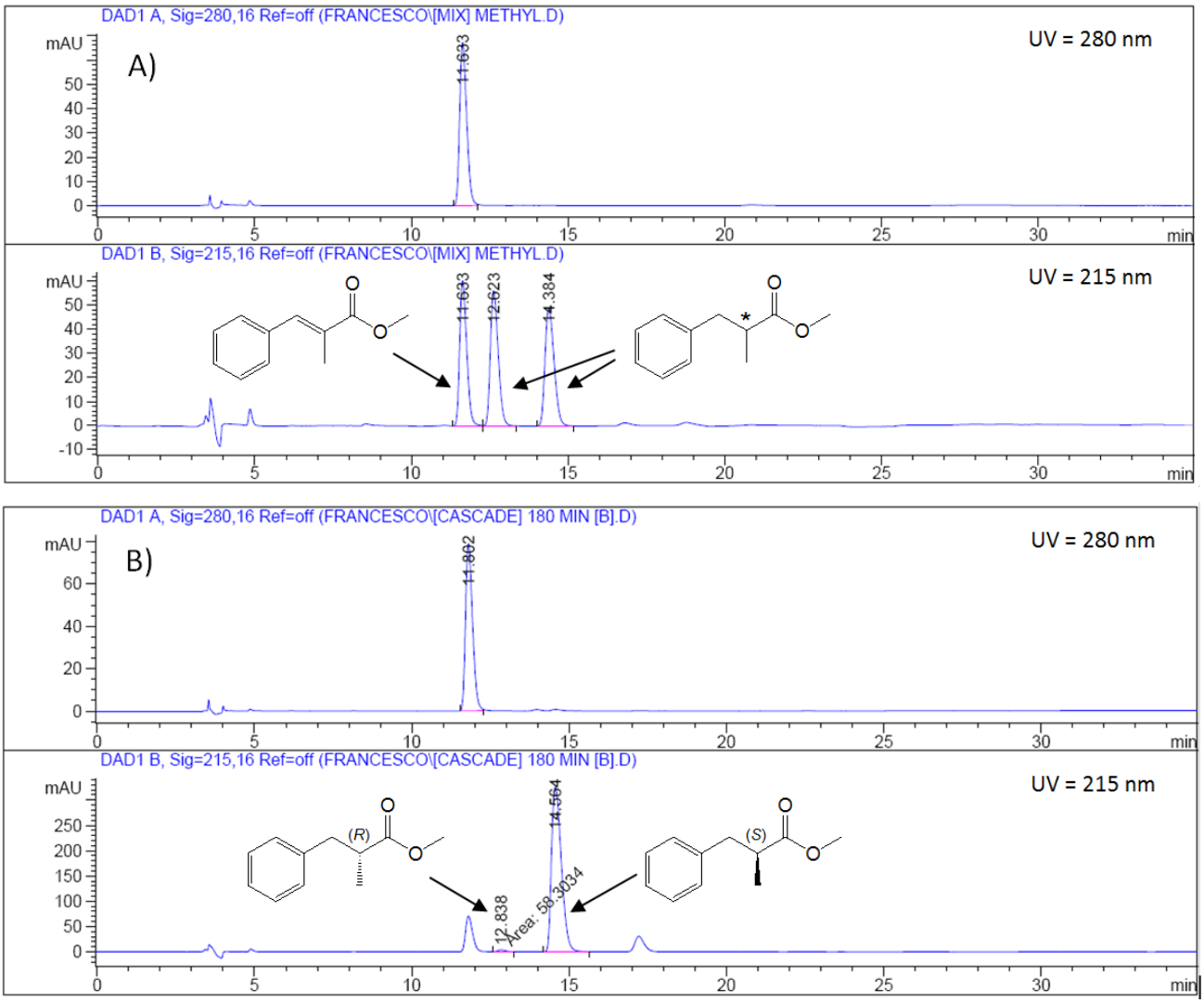


Figure S5. Chiral HPLC chromatograms (Chiralcel OJ-3) of A) **1e**-methylester and *rac*-**1d**-methylester, B) (*S*)-**1d**-methylester obtained from the cascade reaction using OYE2 and AId-DH-EC.

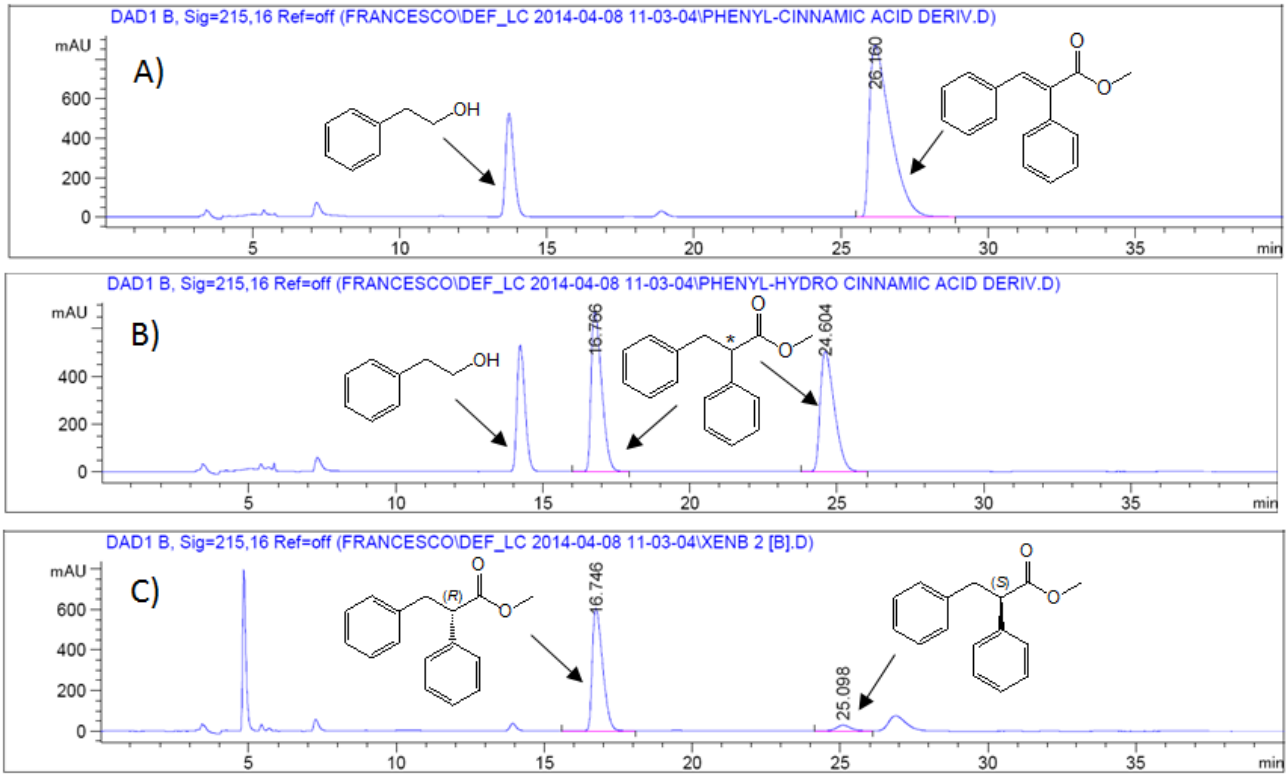


Figure S6. Chiral HPLC chromatograms (Chiralcel OJ-3) of A) **2e**-methylester and 2-phenylethanol (IS), B) *rac*-**2d**-methylester and 2-phenylethanol (IS) and C) (*R*)-**2d**-methylester obtained from the cascade reaction using XenB and Ald-DH-BOV.

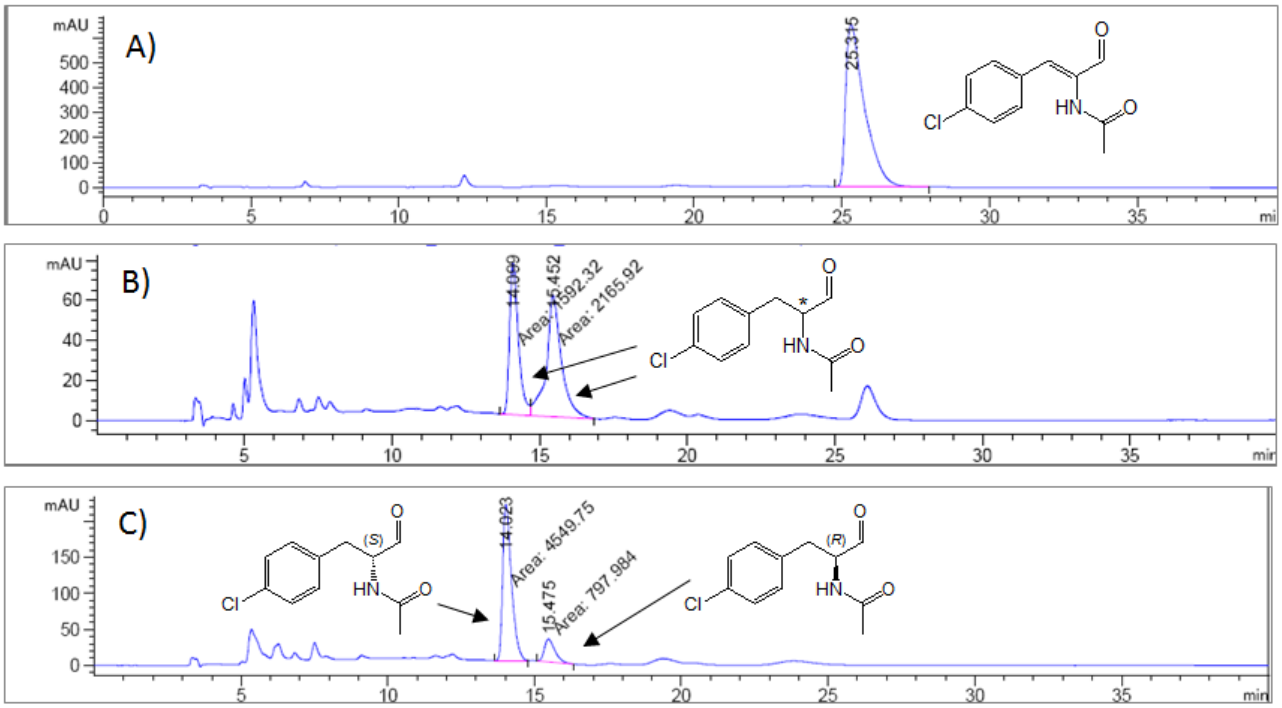


Figure S7. Chiral HPLC chromatograms (Chiralcel OJ-3) of A) **4a**, B) *rac*-**4b** obtained from a biotransformation using GluOx and C) (*S*)-**4b** obtained from the asymmetric biotransformation with OYE2.

Chiral GC chromatograms

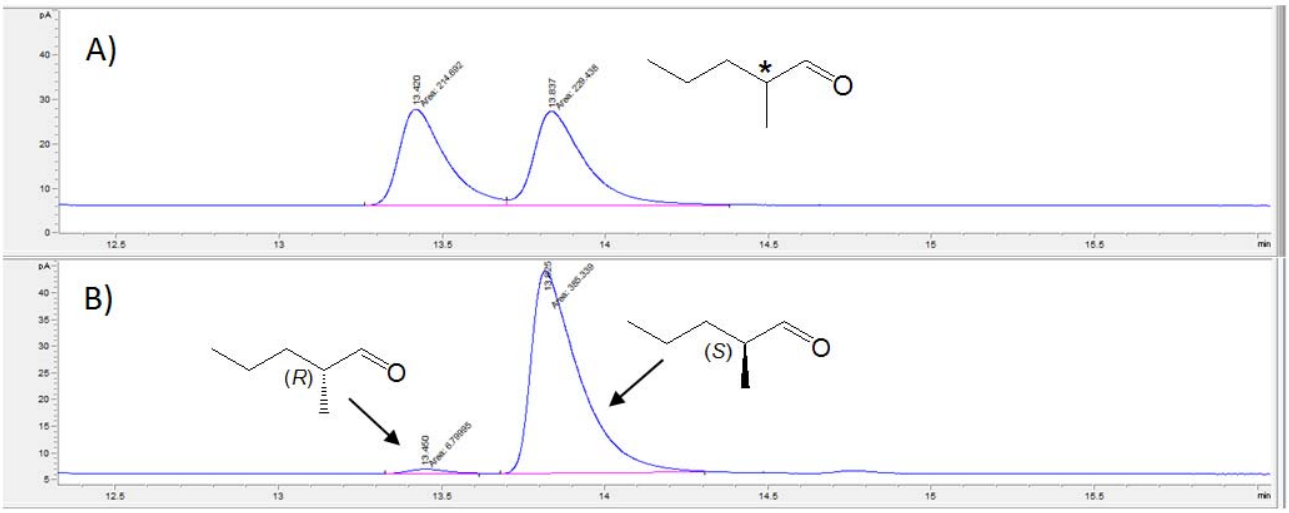


Figure S8. Chiral GC chromatograms (Restek Rt-βDEXsa) of A) *rac*-**3b** and B) (*S*)-**3b** obtained from the 1 h asymmetric bioreduction of **3a** using OYE2.

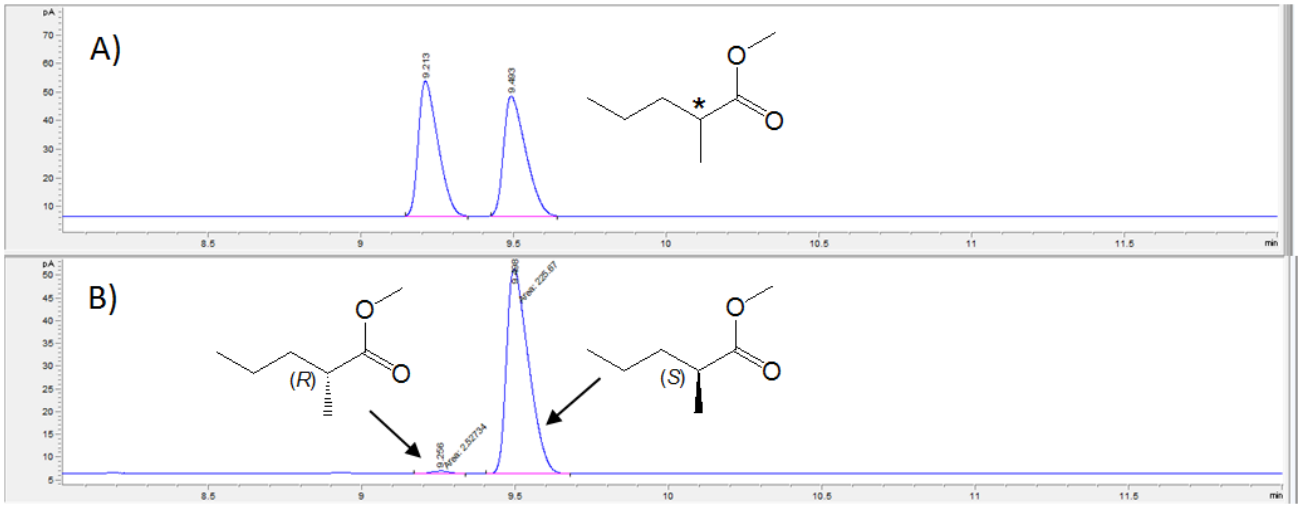


Figure S9. Chiral GC chromatograms (Restek Rt-βDEXsm) of A) *rac*-**3d**-methylester and B) (*S*)-**3d**-methylester obtained from the cascade reaction of **3a** using OYE2 and Ald-DH-BOV.

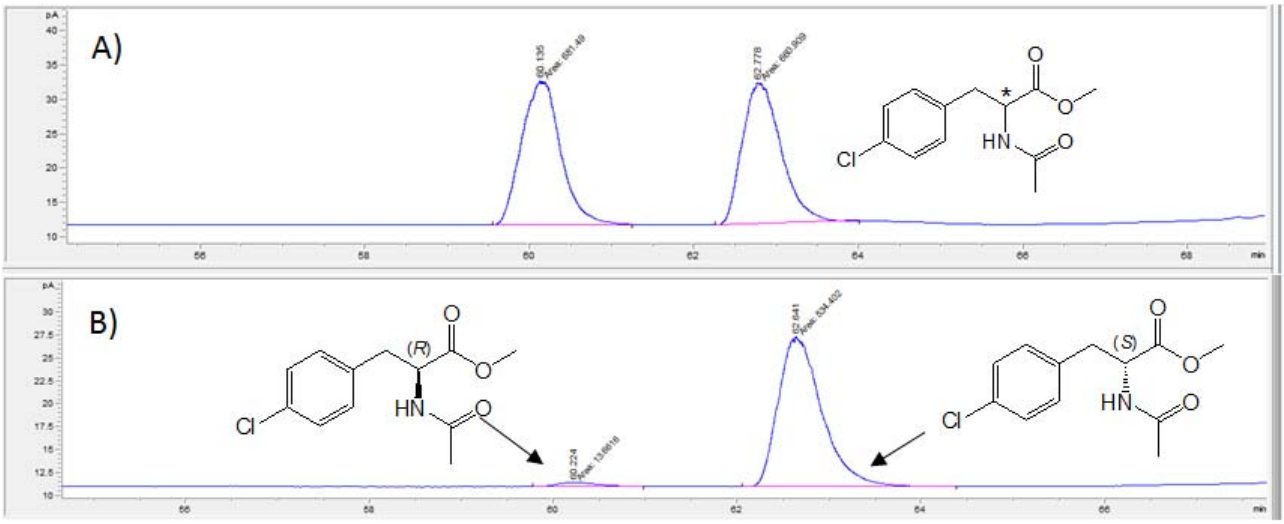


Figure S10. Chiral GC chromatograms (Chiralsil DEX-CB) of A) *rac*-**4d**-methylester and B) (*S*)-4**d**-methylester obtained from the cascade reaction of **4a** using OYE2 and Ald-DH-BOV.

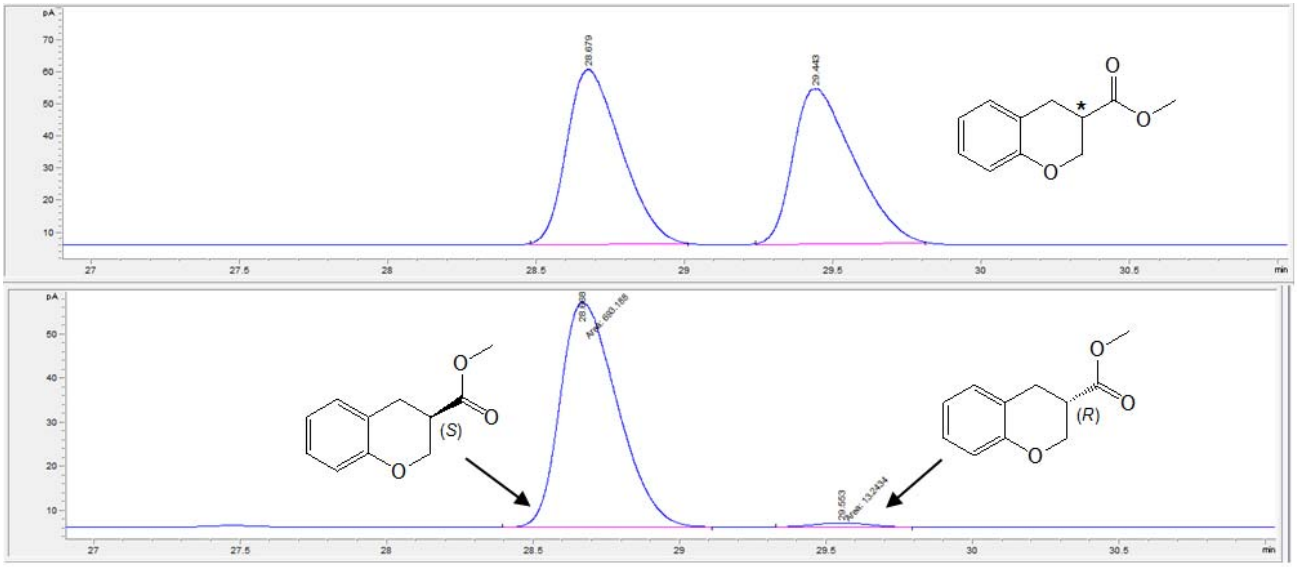
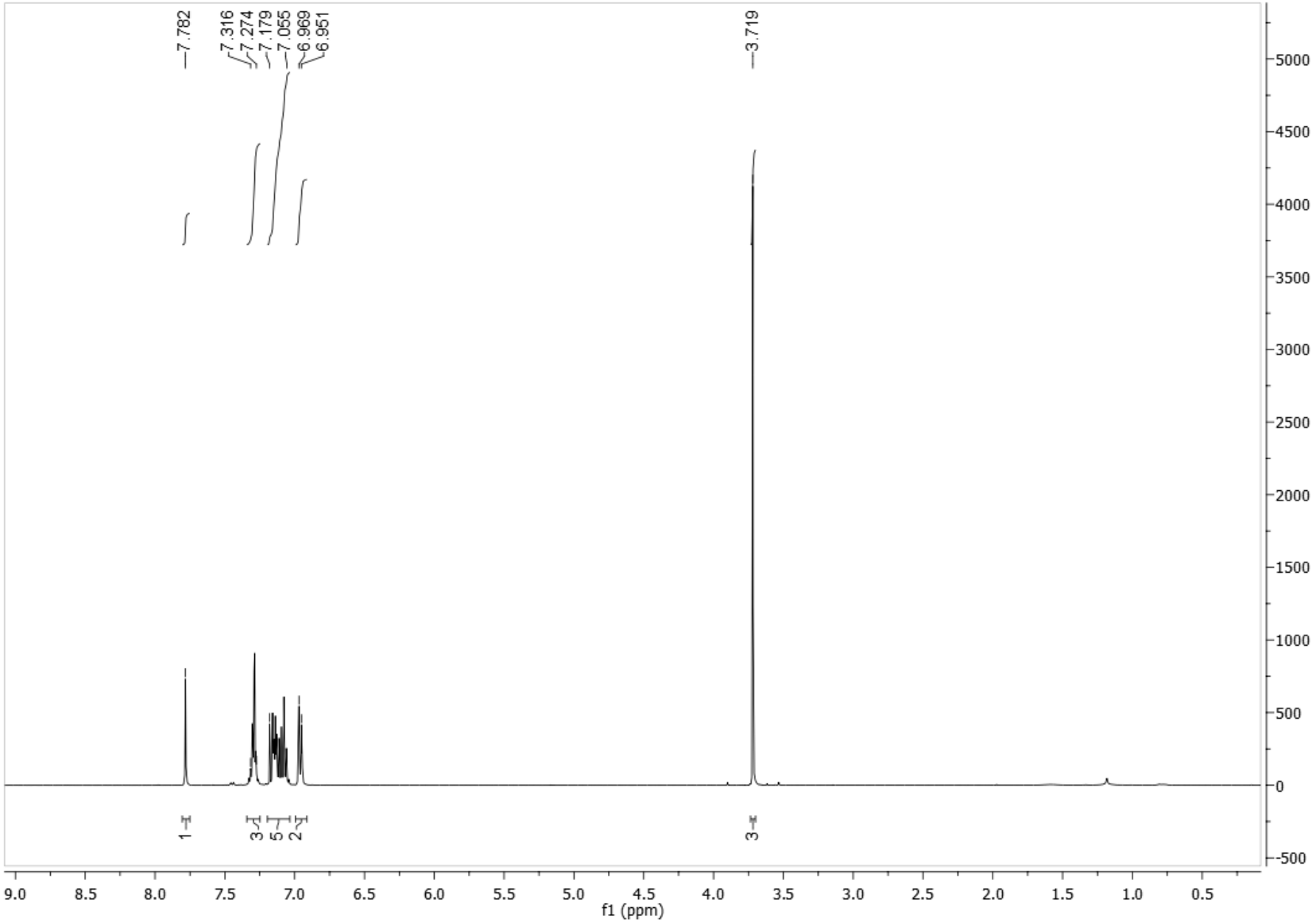


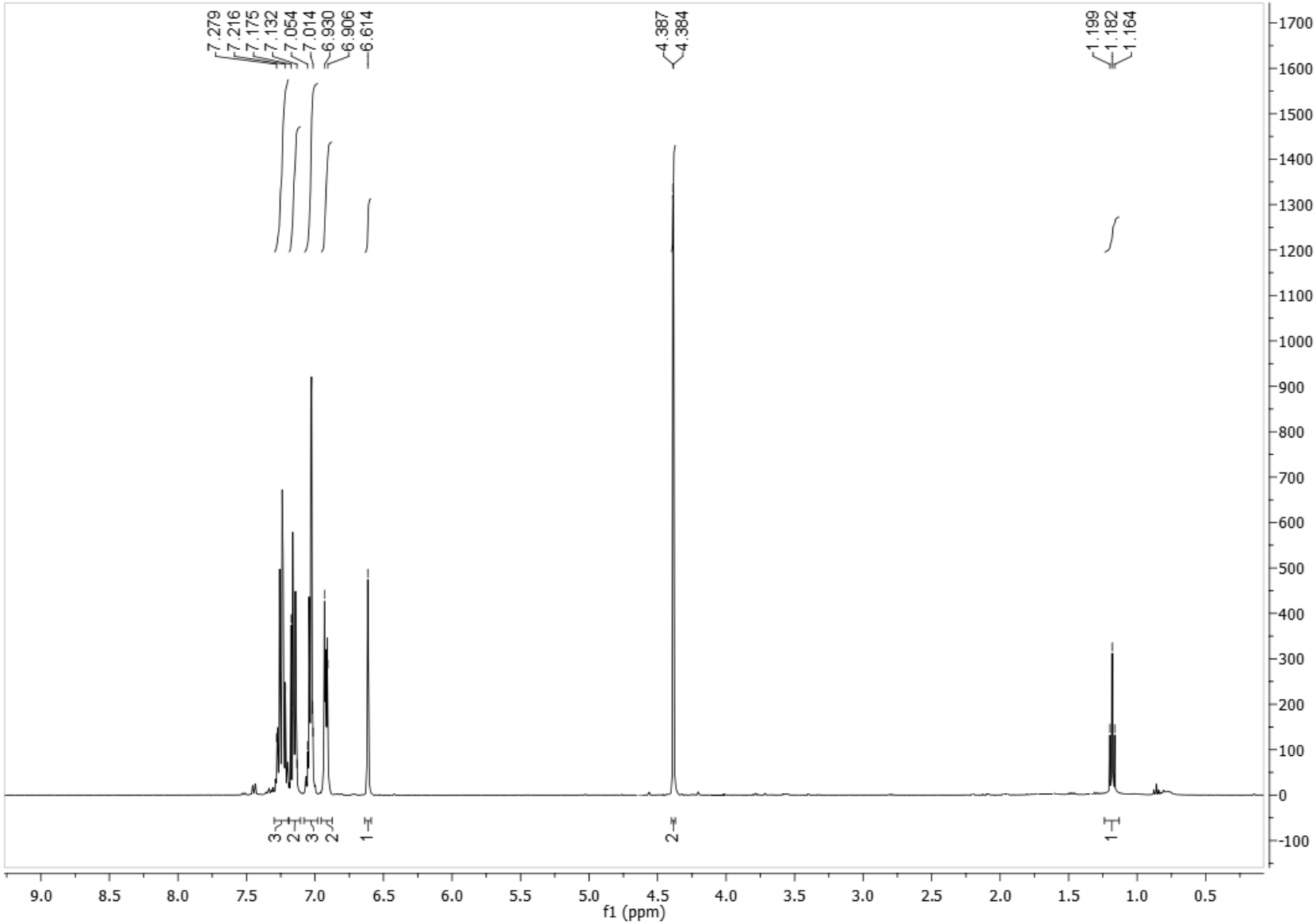
Figure S11. Chiral GC chromatograms (Restek Rt-βDEXsm) of A) *rac*-**5d**-methylester and B) (*S*)-**5d**-methylester obtained from the cascade reaction of **5a** using GluOx and Ald-DH-EC.

NMRs

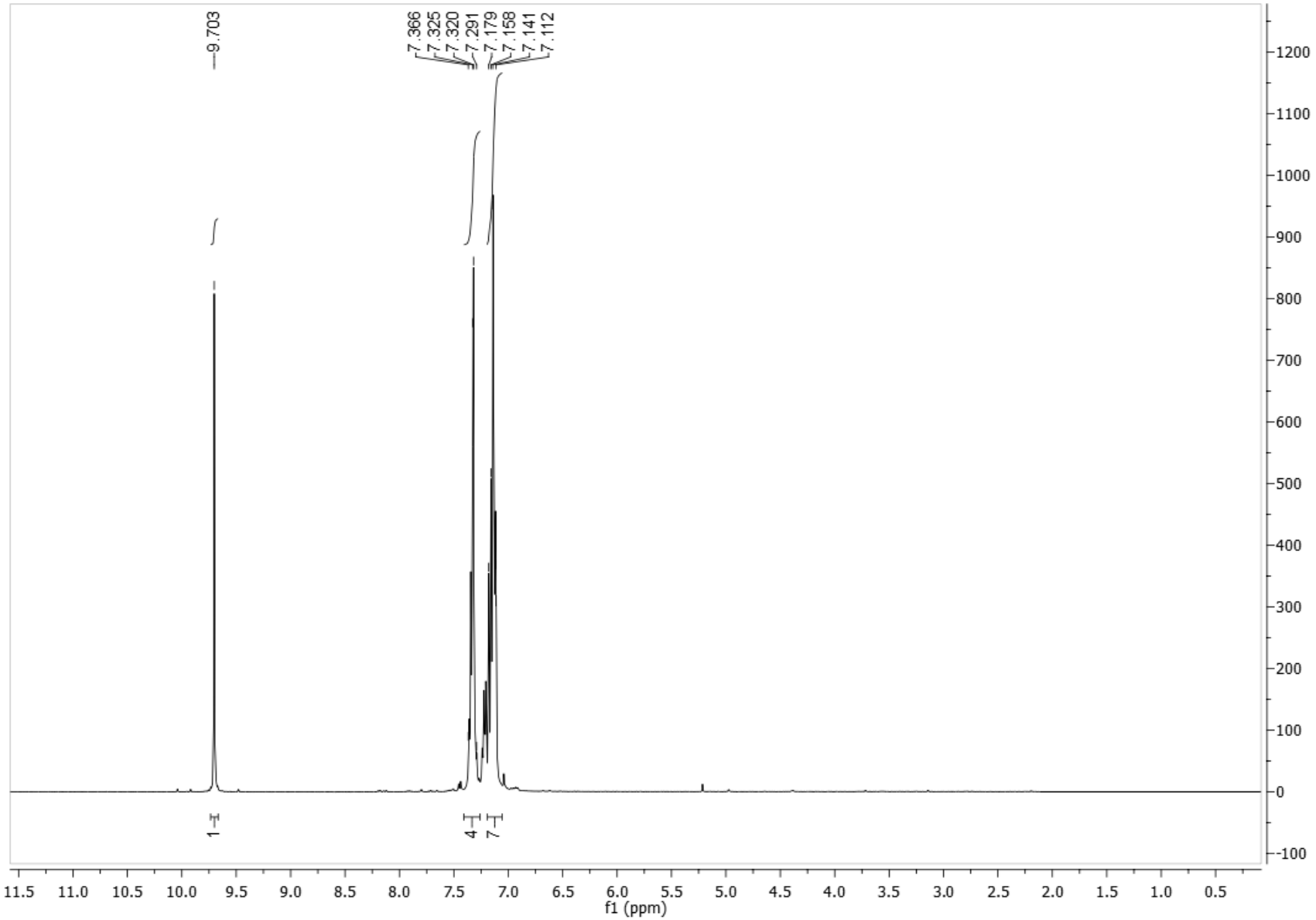
(*E*)-methyl 2,3-diphenylacrylate (α-phenylcinnamic acid methyl ester)



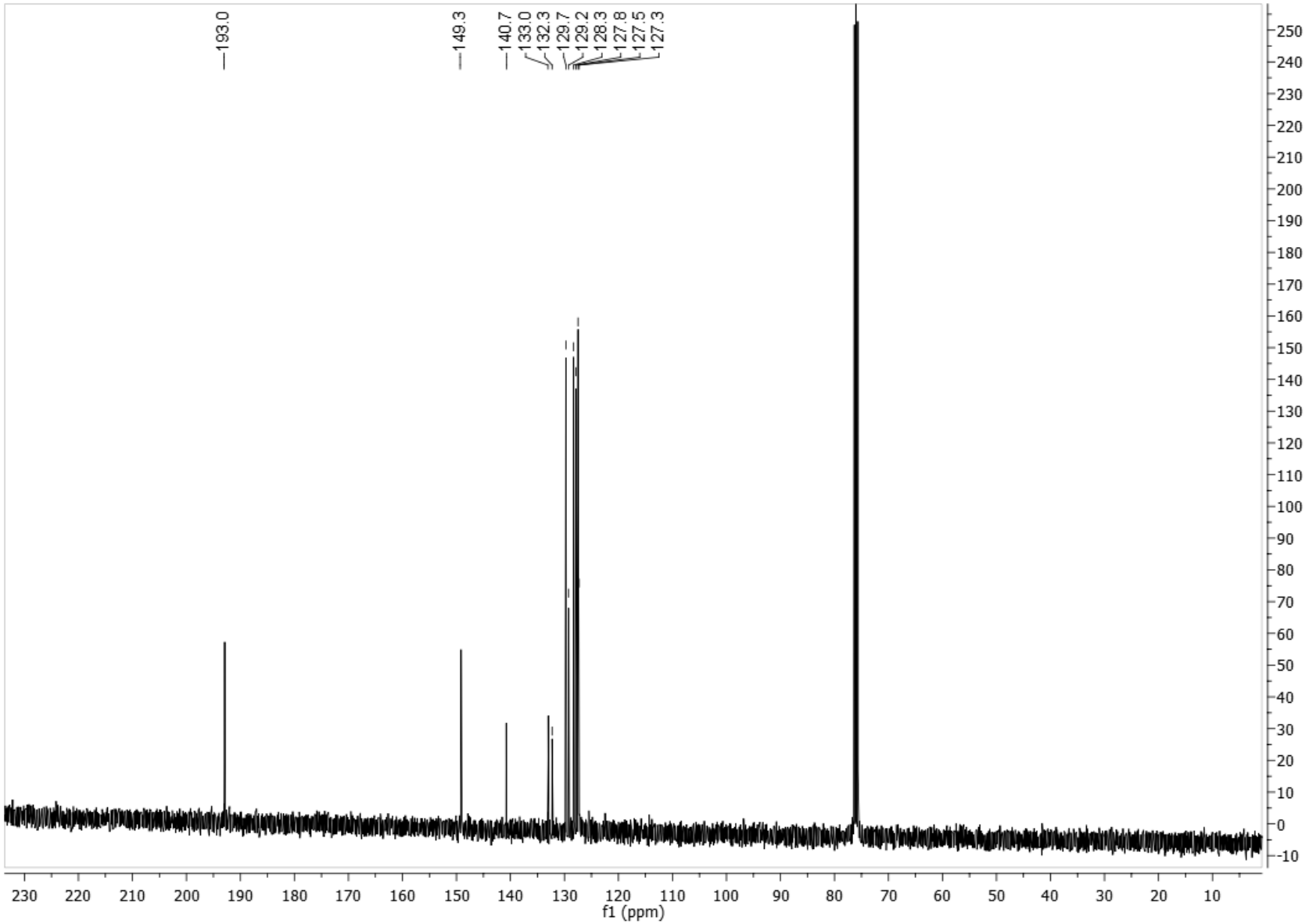
(*E*)-2,3-diphenylprop-2-en-1-ol (α-phenylcinnamic alcohol)



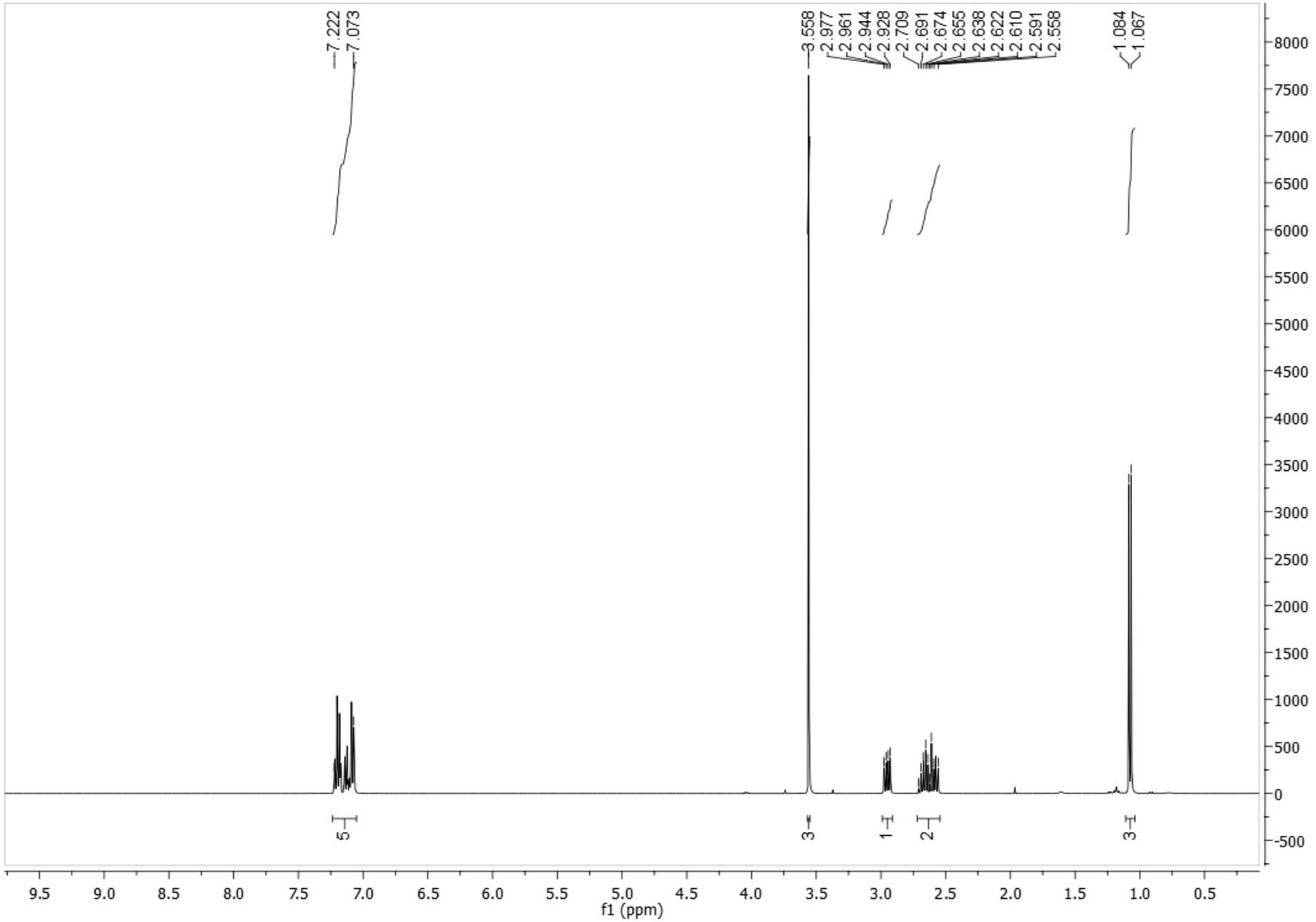
(*E*)-2,3-diphenylacrylaldehyde (α-phenylcinnamaldehyde) 2a (^{1}H-NMR)



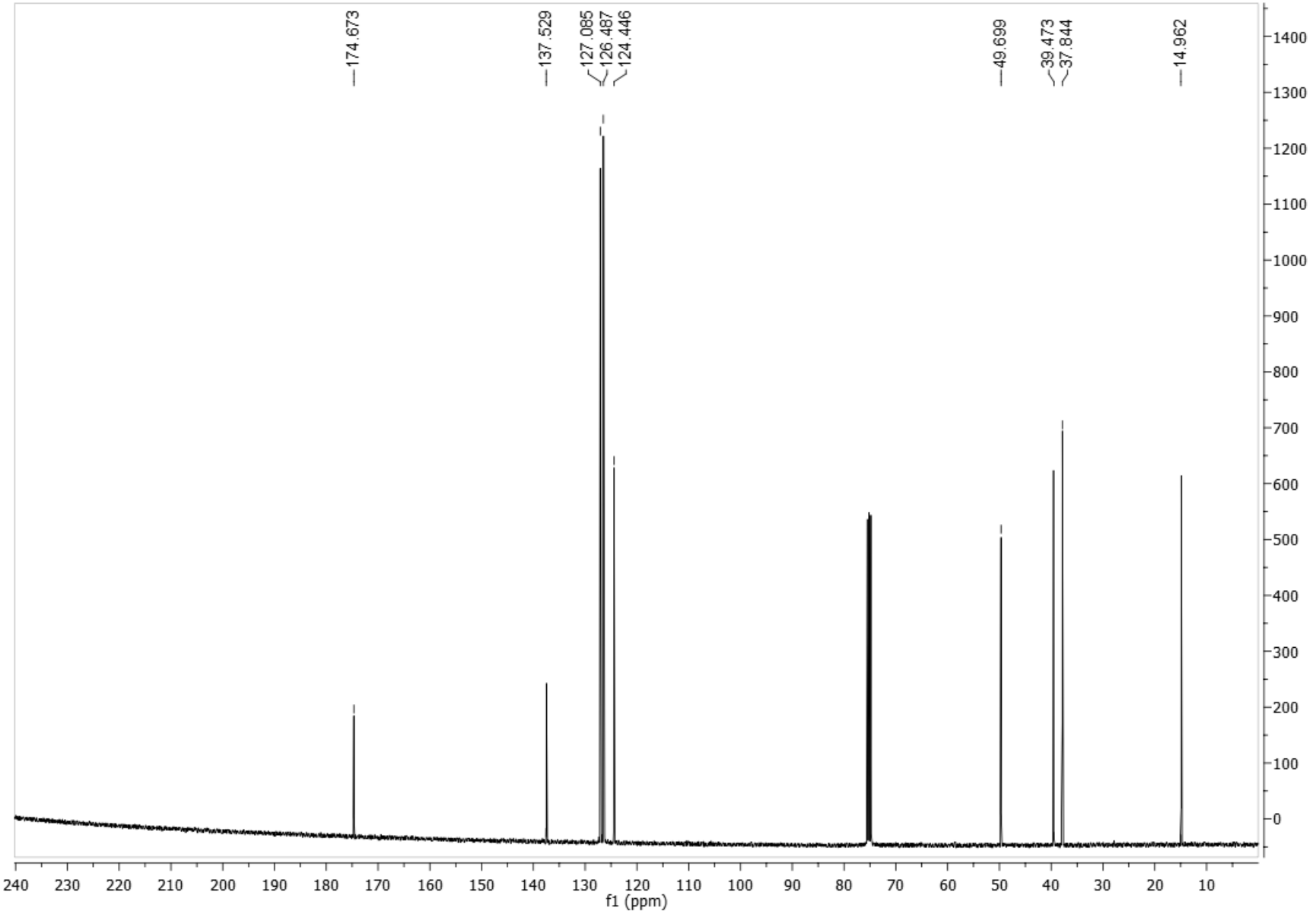
(*E*)-2,3-diphenylacrylaldehyde (α-phenylcinnamaldehyde) 2a (^{13}C-NMR)



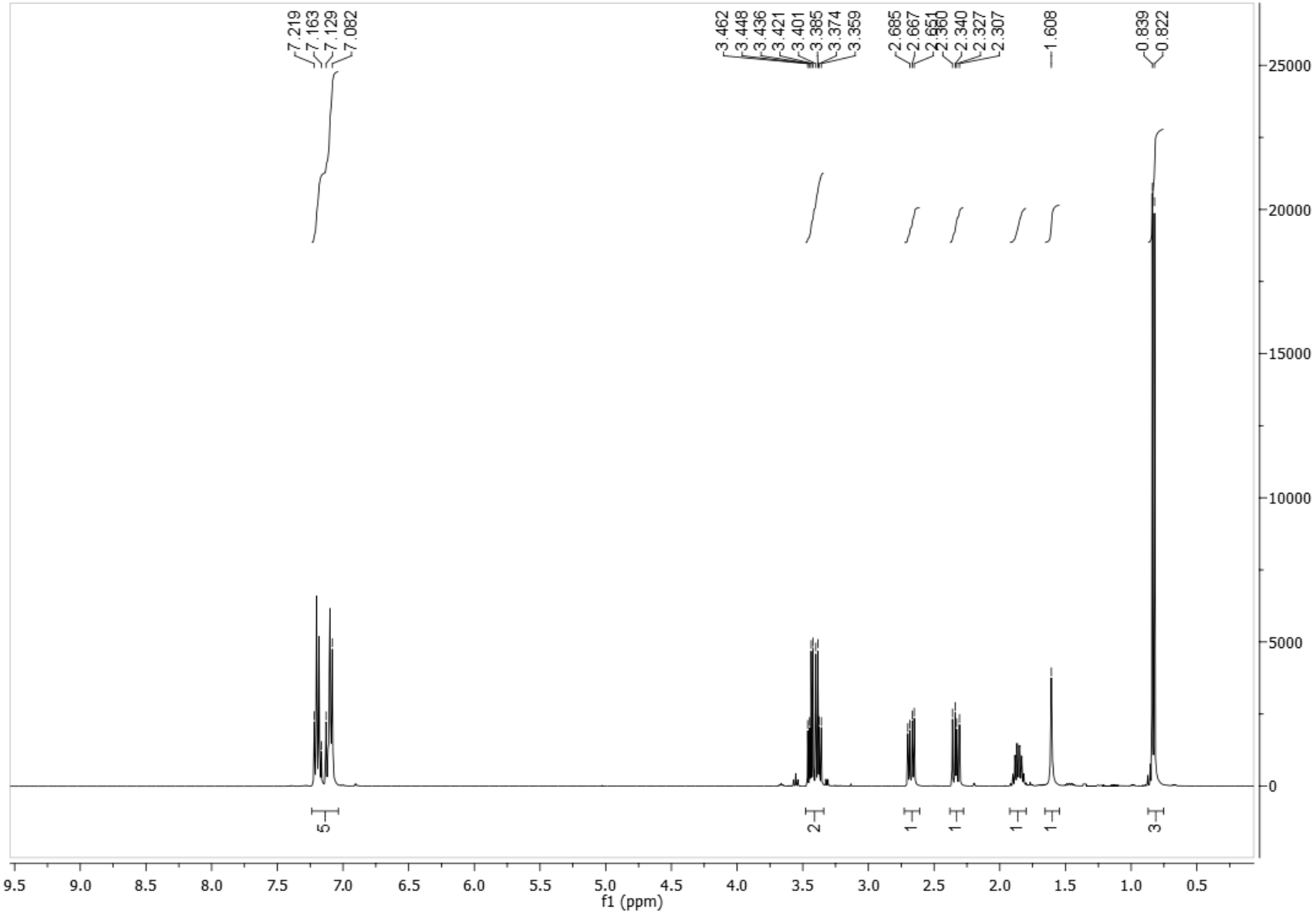
(*E*)-methyl 2-methyl-3-phenylproanoate (^{1}H-NMR)



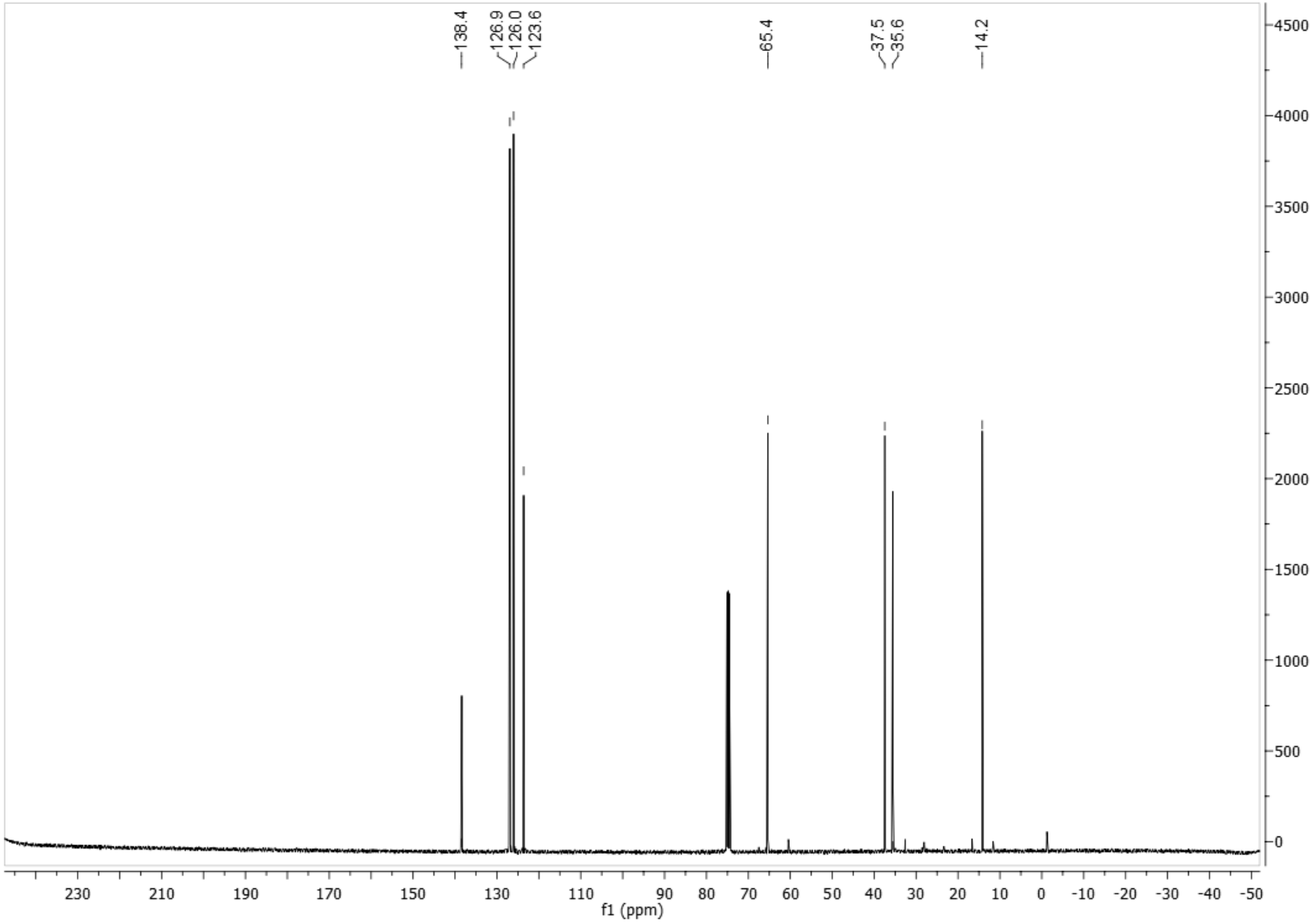
(*E*)-methyl 2-methyl-3-phenylproanoate (^{13}C-NMR)



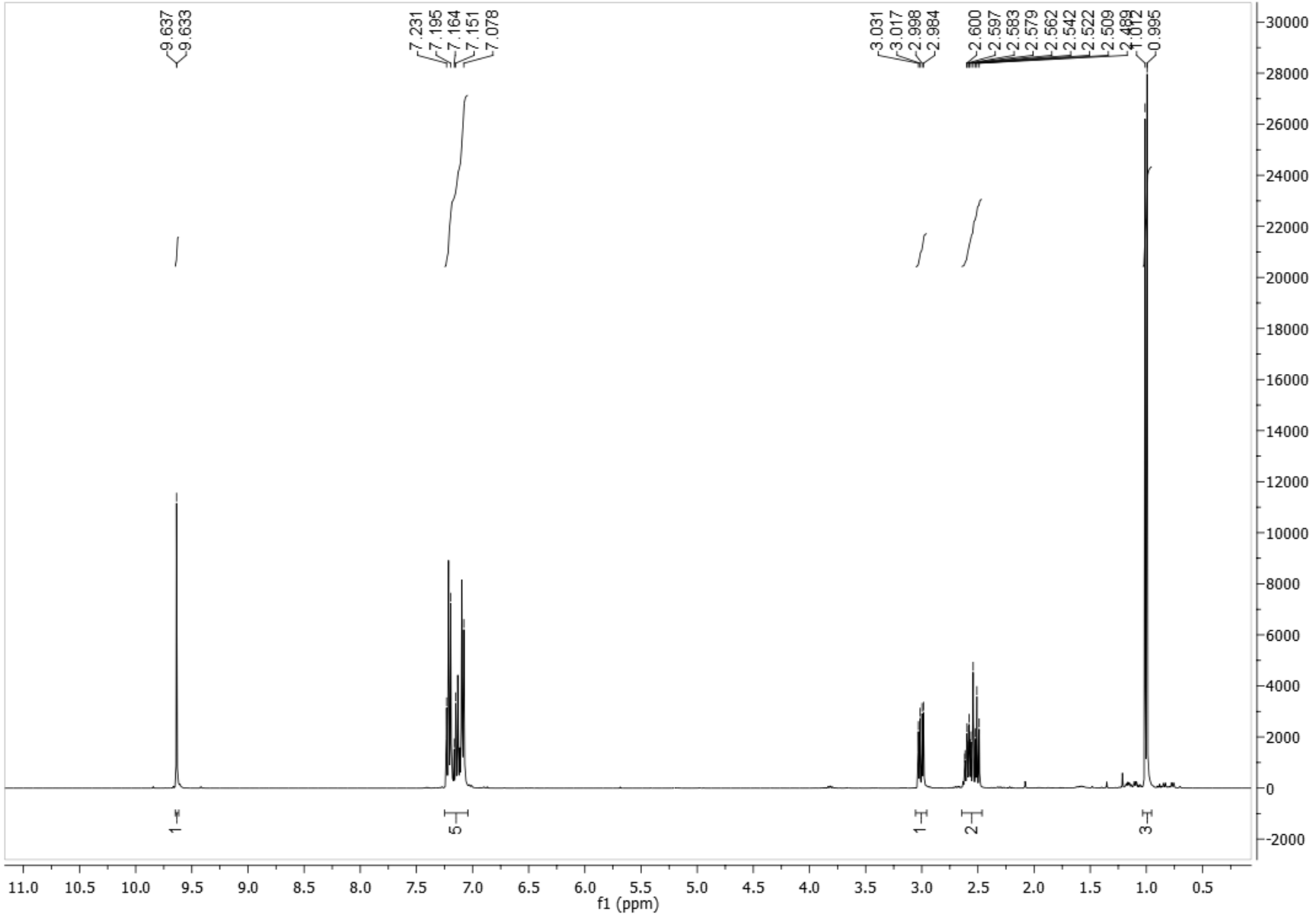
(*E*)-2-methyl-3-phenylpropanol (α-methyl-hydrocinnamic alcohol) **1c** (^{1}H-NMR)



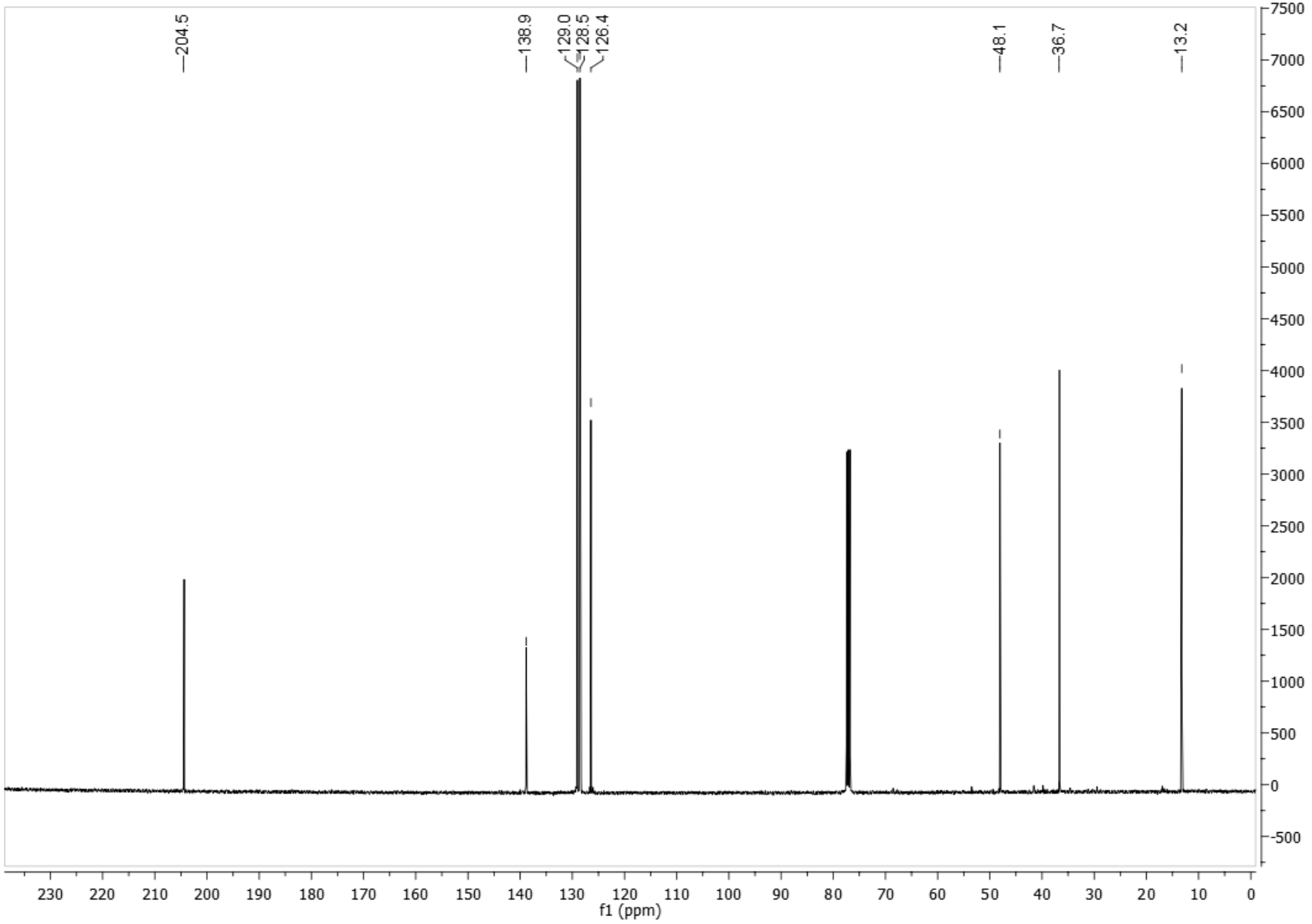
(*E*)-2-methyl-3-phenylpropanol (α-methyl-hydrocinnamic alcohol) **1c** (^{13}C-NMR)



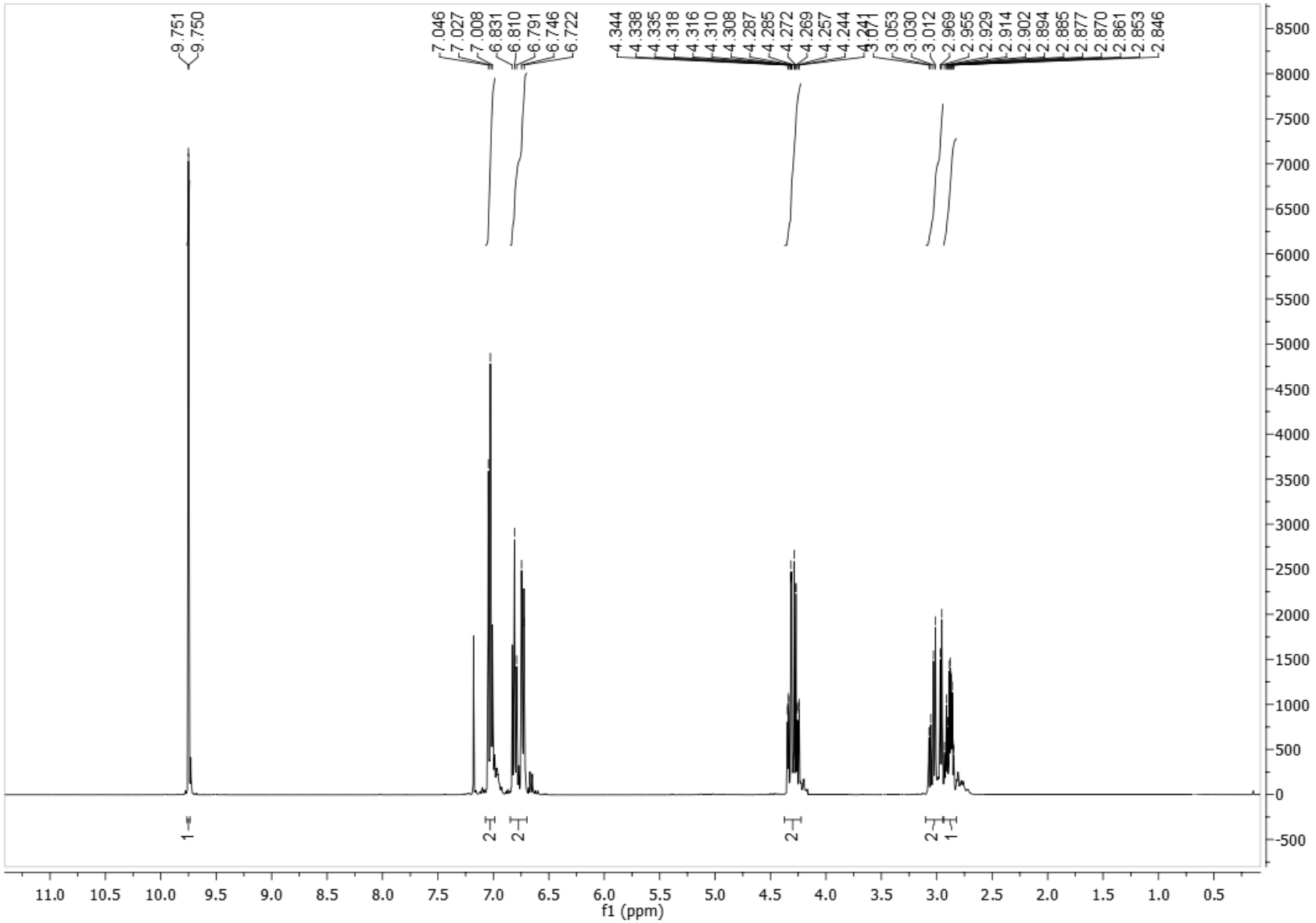
(*E*)-2-methyl-3-phenylpropanal (α-methylhydrocinnamaldehyde) 1b (^{1}H-NMR)



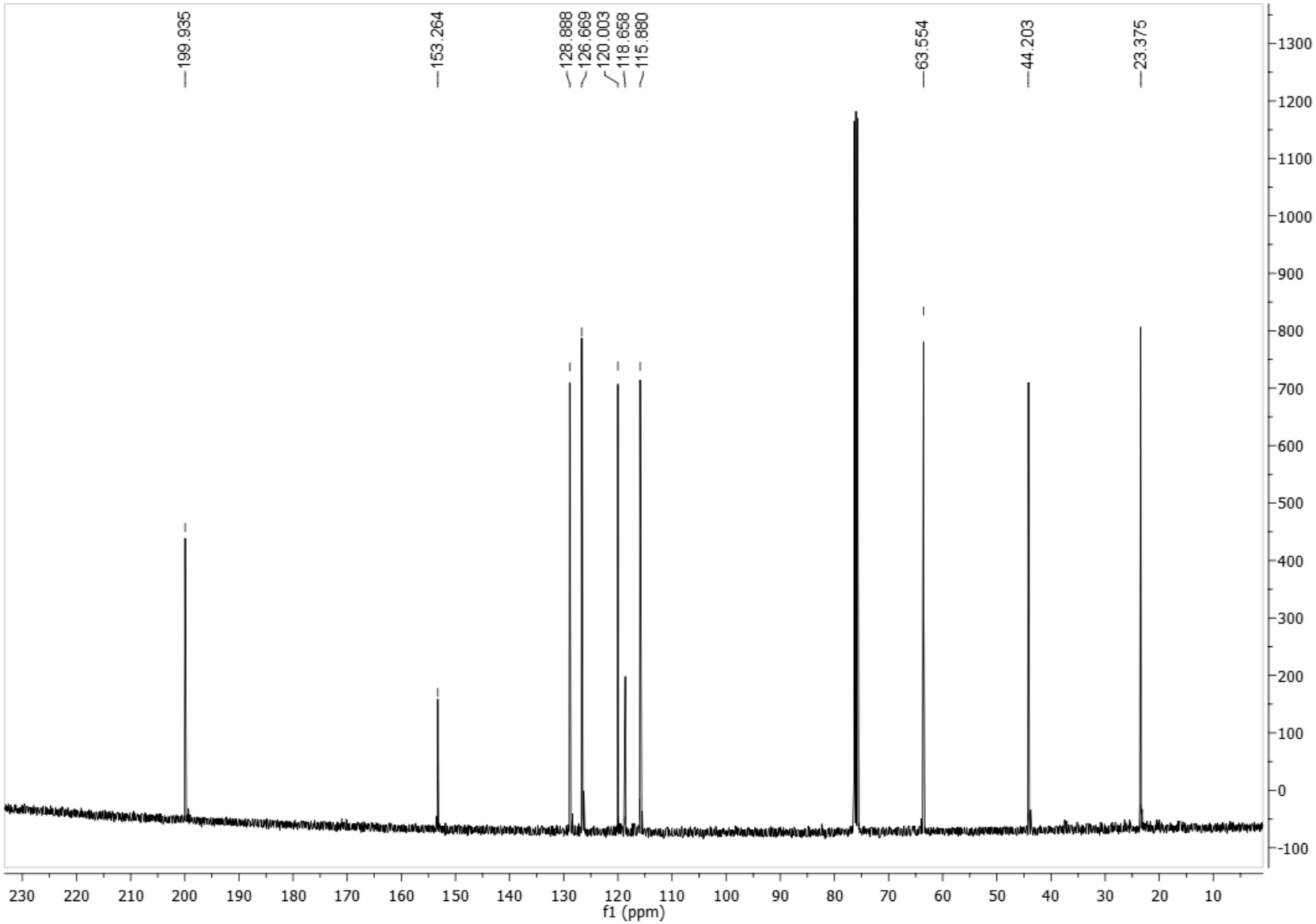
(*E*)-2-methyl-3-phenylpropanal (α-methylhydrocinnamaldehyde) 1b (^{13}C-NMR)



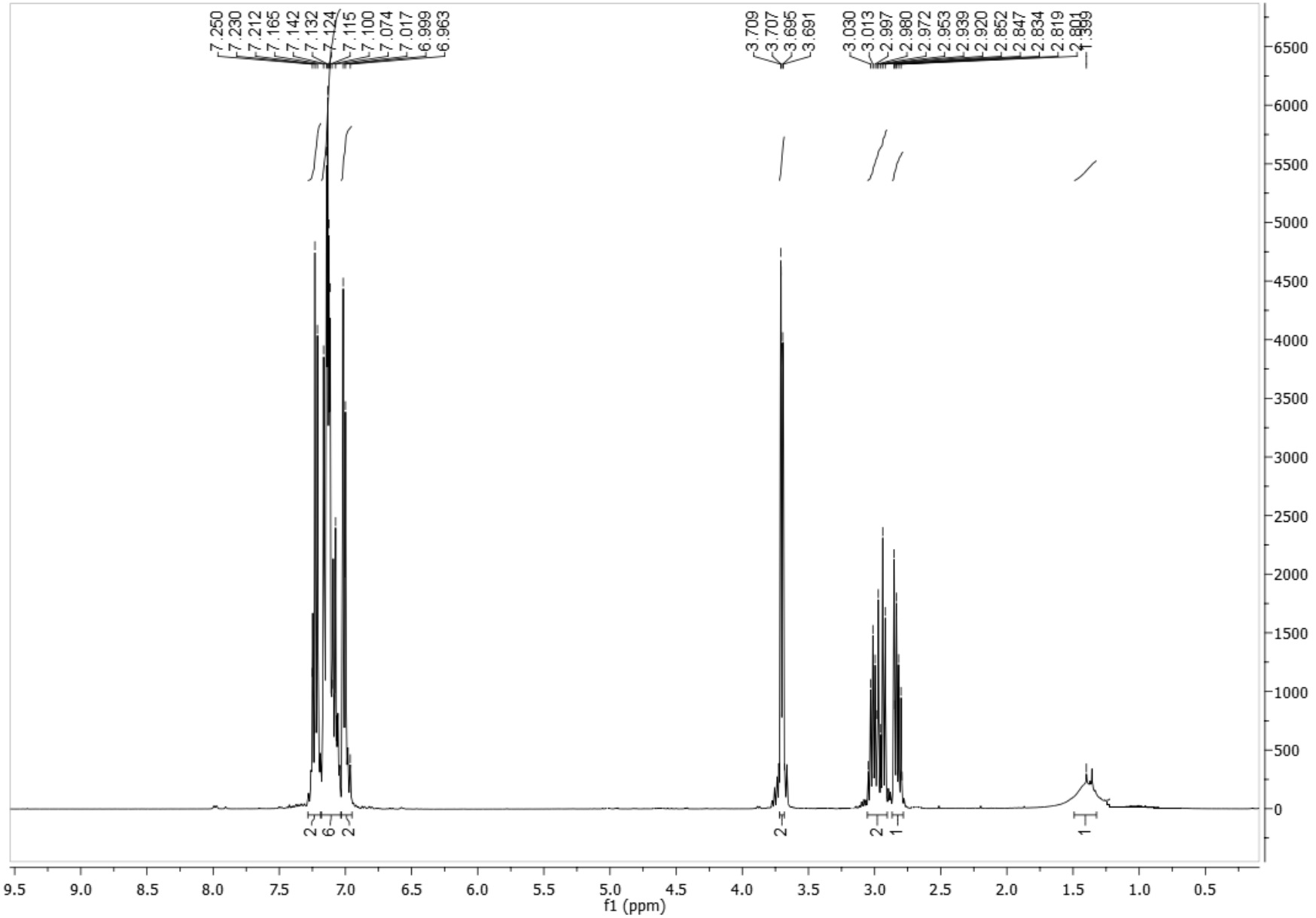
chromane-3-carbaldehyde 5b (^{1}H-NMR)



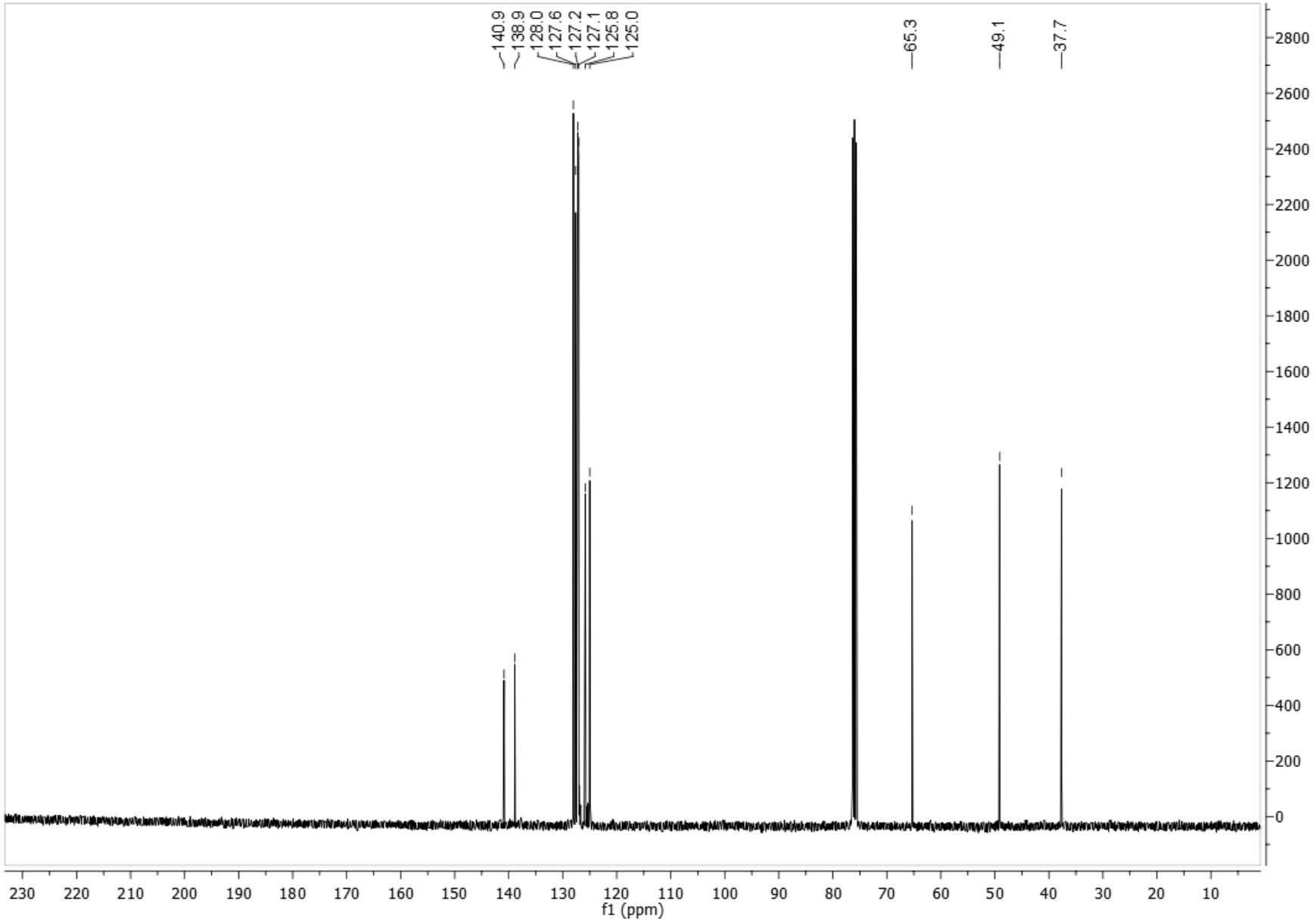
chromane-3-carbaldehyde 5b (^{13}C-NMR)



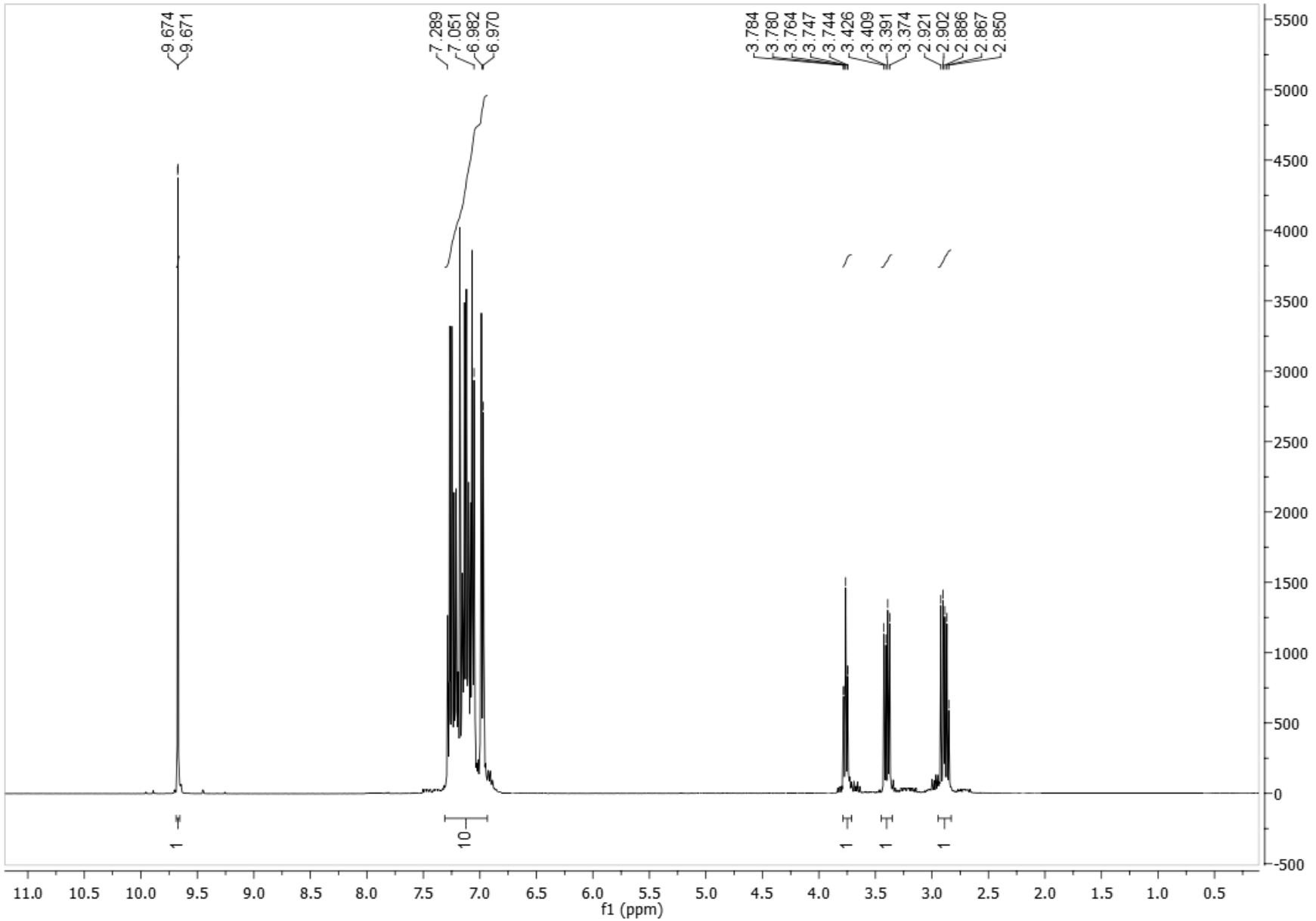
(*E*)-2,3-diphenylpropan-1-ol (^{1}H-NMR)



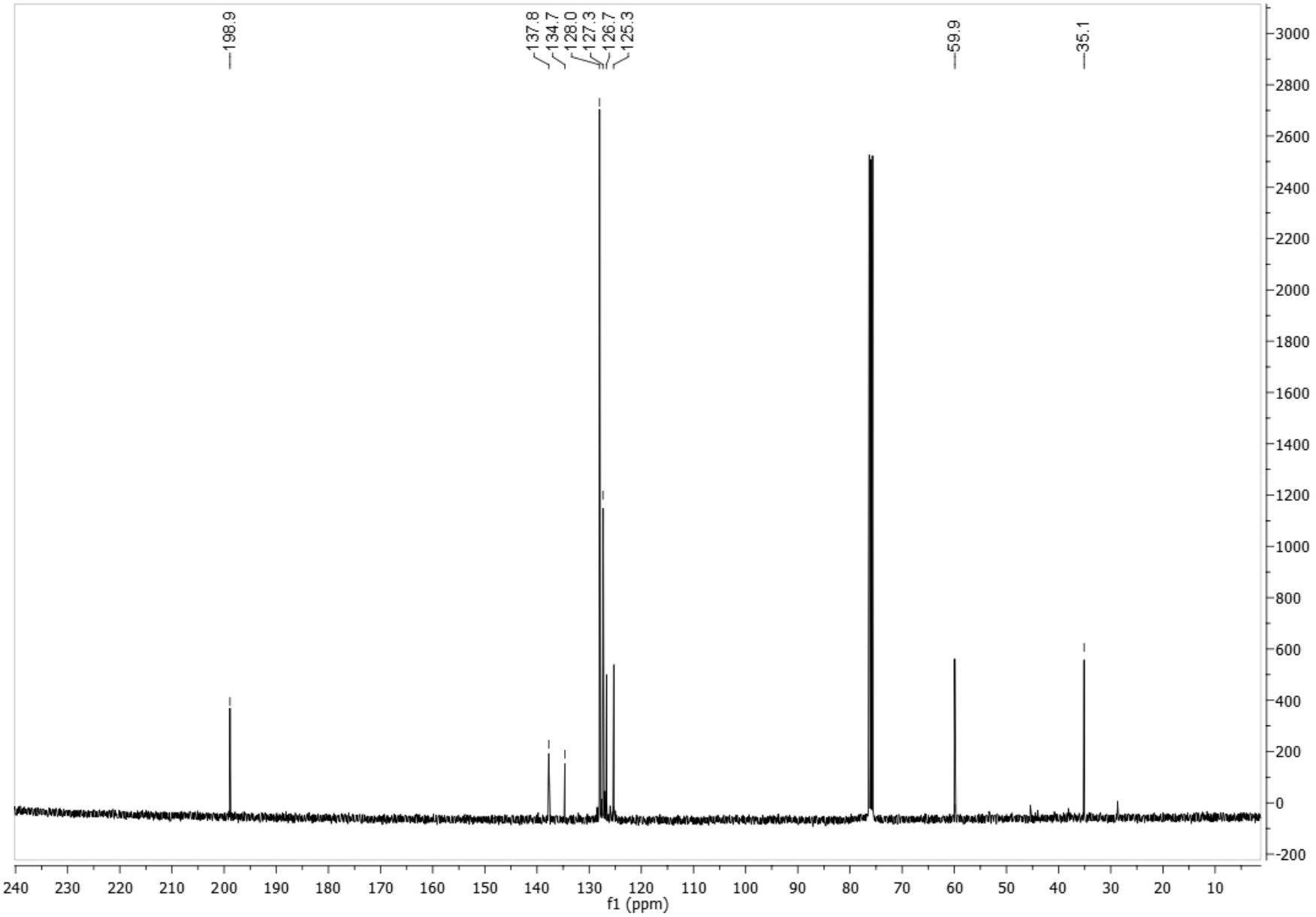
(*E*)-2,3-diphenylpropan-1-ol (^{13}C-NMR)



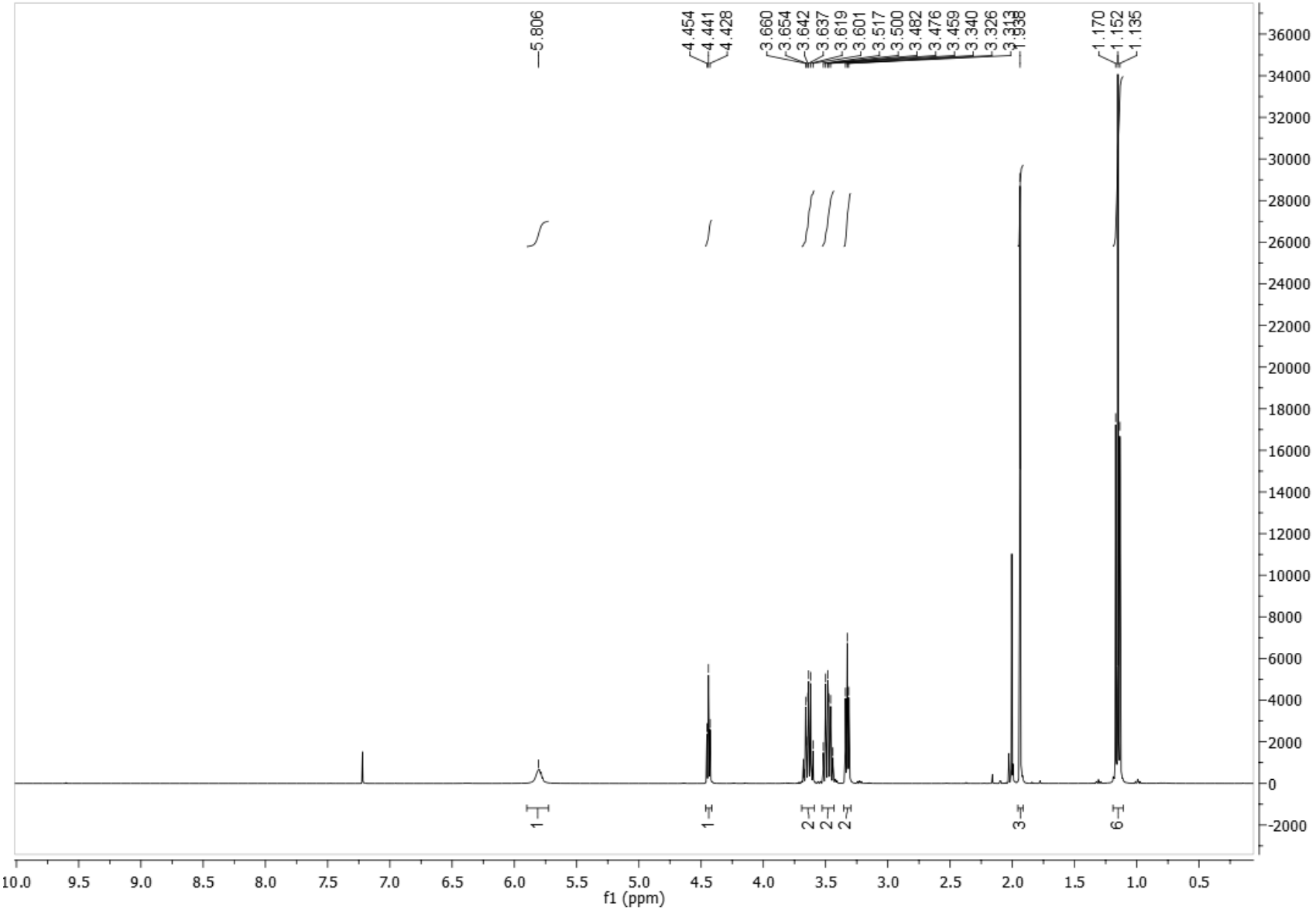
(*E*)-2,3-diphenylpropanal (α-phenylhydrocinnamaldehyde) 2b (^{1}H-NMR)



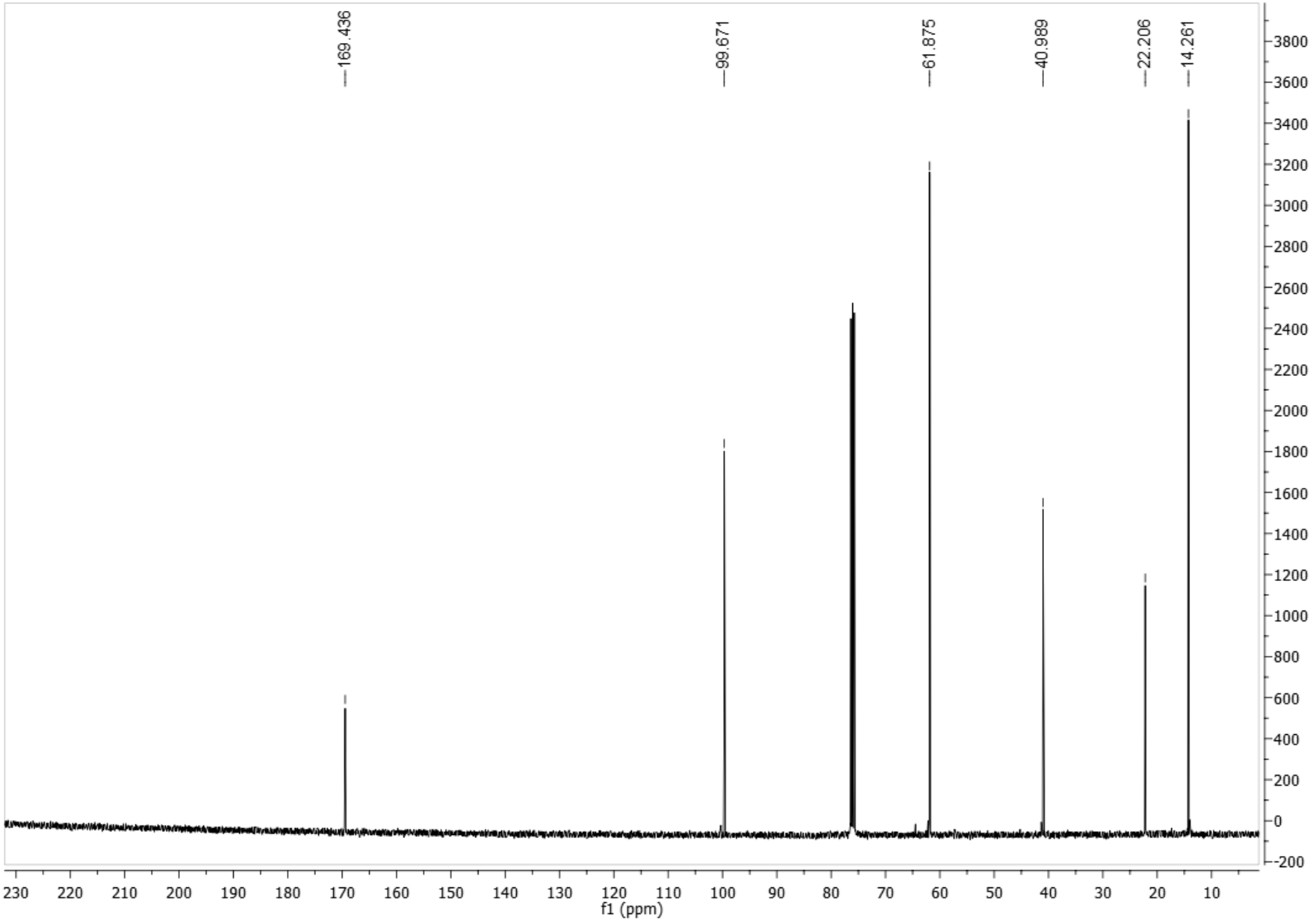
(*E*)-2,3-diphenylpropanal (α-phenylhydrocinnamaldehyde) 2b (^{13}C-NMR)



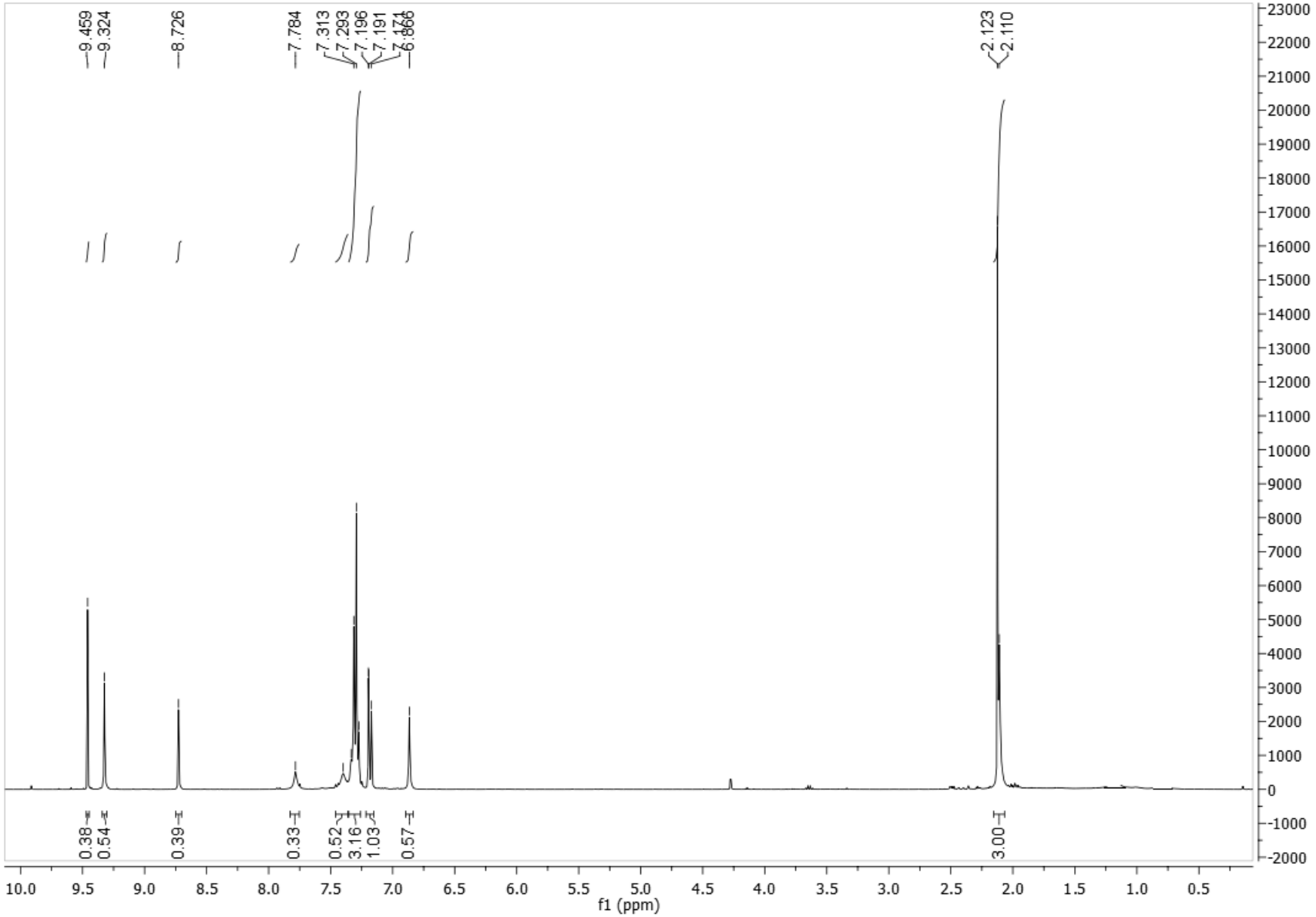
acetamido acetaldehyde diethyl acetal (^{1}H-NMR)



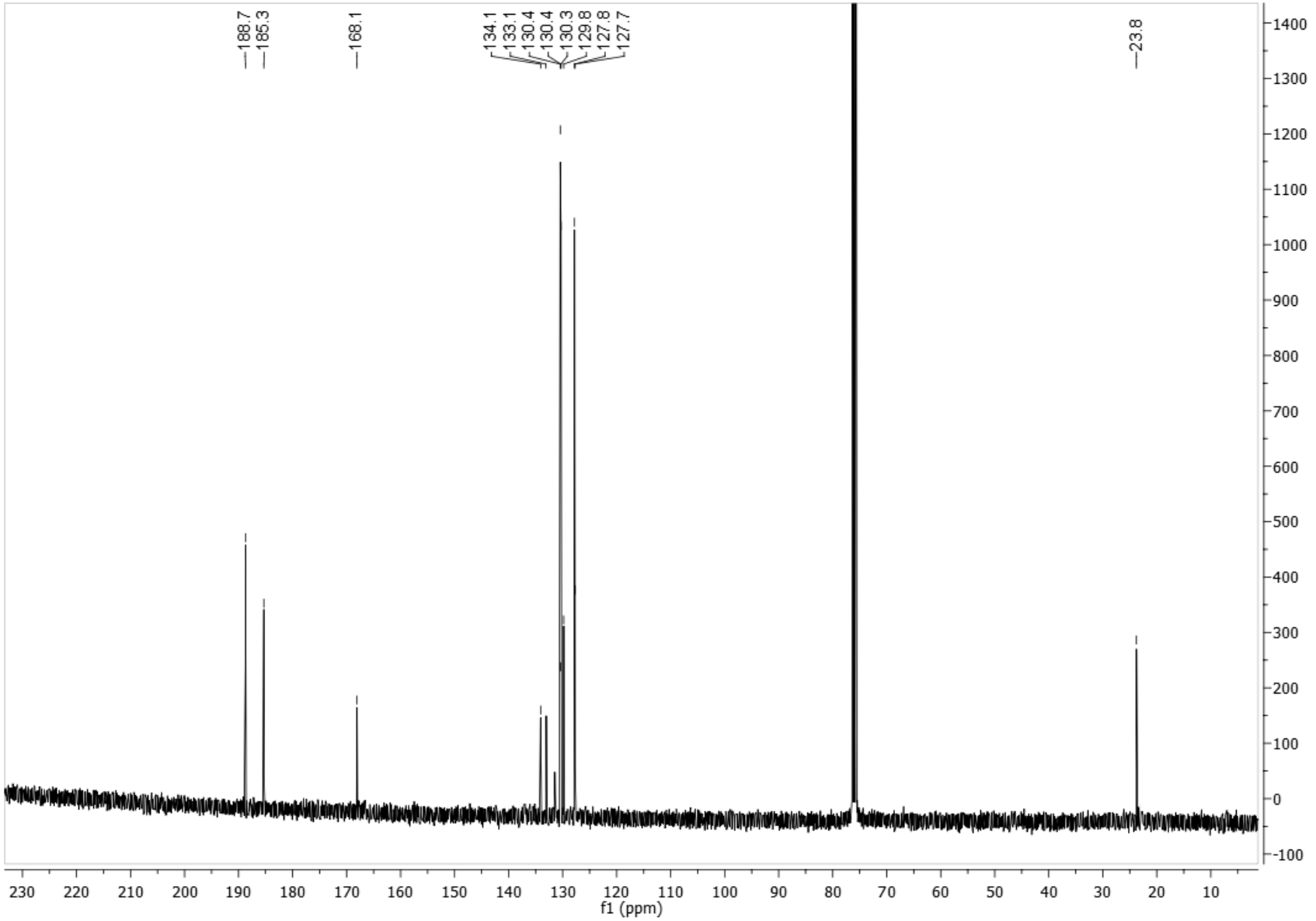
acetamido acetaldehyde diethyl acetal (^{13}C-NMR)



α-acetamido *para*-chloro cinnamaldehyde (^{1}H-NMR)



α-acetamido *para*-chloro cinnamaldehyde (^{13}C-NMR)



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