Supporting Information

Mild Chemoenzymatic Oxidation of Allylic *sec*-Alcohols. Application to Biocatalytic Stereoselective Redox Isomerizations

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I. General considerations

Commercially available racemic secondary allylic alcohols **1a**, **2a**, **6a** and (5*R*)-**13a** were purchased from Sigma-Aldrich. α,β-unsaturated ketones **1b**-**4b**, **7b**, and **13b** were also obtained from Sigma-Aldrich, whereas the unsaturated ketone **5b** was obtained from Alfa-Aesar.

Non-commercially available racemic allylic alcohols **3a-5a**, **7a-12a**, and α,β-unsaturated ketones **8b**-**12b** were chemically synthesized, exhibiting physical and spectral data in agreement with those reported in the literature (see synthetic procedures and characterization data given below).

Saturated ketones obtained through this sequential approach exhibited physical and spectral data in agreement either with the ones obtained from commercial sources (**1c**-**2c**, **5c**-**7c**) or with those previously reported (**3c**, **4c**, **8c**-**13c**) (see characterization data given below).

Laccase from *Trametes versicolor* (LTv) was purchased from Sigma-Aldrich. Codex Ene Reductase Screening Kit and glucose dehydrogenase (GDH-105) were purchased from Codexis Inc. Glucose, $NADP^+$ and NADPH were purchased from Sigma-Aldrich. All other reagents were obtained from commercial sources and used as received.

Sequential reactions were performed in a test tube [(19 x 130 x 3) mm] and under magnetic stirring, otherwise indicated. The oxidation step mediated by the laccase-TEMPO catalytic system was performed open-to-air, while for the bioreduction step, the test tube was closed.

NMR spectra were recorded on a Bruker AV300 MHz spectrometer. All chemical shifts (δ) are given in parts per million (ppm) and referenced to the residual solvent signal as internal standard. Gas chromatography (GC) analyses were performed on an Agilent HP7820 GC or on an Agilent HP6890 GC chromatographs equipped with a FID detector. Thin-layer chromatography (TLC) was conducted with Merck Silica Gel 60 F254 precoated plates and visualized with UV and potassium permanganate stain. Column chromatographies were performed using Merck Silica Gel 60 (230-400 mesh).

In addition to those specified above, the following abbreviations and designations are used throughout the Supporting Information: Ar: aryl, Et_2O: diethyl ether; MeOH: methanol; MTBE: methyl *tert*-butyl ether, $NADP^+$: β-nicotinamide adenine dinucleotide phosphate hydrate, NADPH: β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate, TFA: trifluoroacetic acid, TEMPO: 2,2,6,6-tetramethyl-1-piperidinyloxyl radical.

II. Compounds described in this contribution

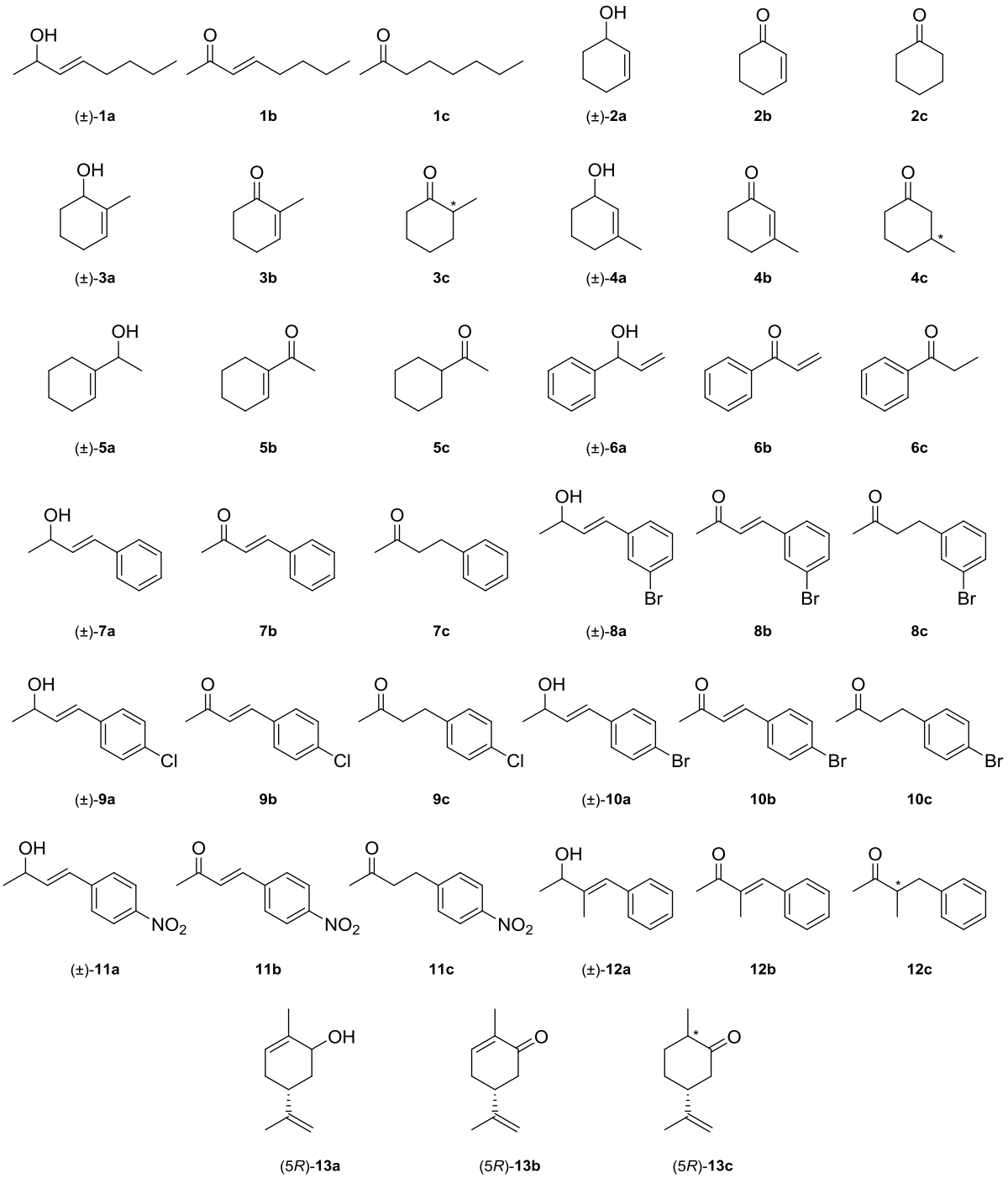


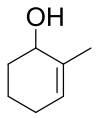
Figure S1. Structures of racemic allylic alcohols, α,β-unsaturated ketones and saturated ketones under study in this manuscript.

III. General protocol for the synthesis of racemic secondary allylic alcohols

The non-commercially available racemic secondary allylic alcohols were synthesized from the corresponding α,β-unsaturated ketones following the procedure reported by Gemal and Luche.[1]

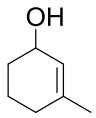
To a solution of the corresponding unsaturated ketone **3b-5b**, **7b-12b** (2 mmol) in MeOH (10 mL) at 0 ºC, $CeCl_3 \cdot 7\ H_2O$ (826 mg, 2.22 mmol), and sodium borohydride (86 mg, 2.28 mmol) were added. The mixture was stirred at 0 ºC for 1 h. After this time, the reaction was quenched by the addition of a saturated NH_4Cl aqueous solution (30 mL) and extracted with Et_2O (3 x 15 mL). The combined organics were washed with a saturated NaCl aqueous solution (2 x 15 mL), followed by H_2O (3 x 15 mL), dried over Na_2SO_4 and concentrated under vacuum. Further purification was not required.

Synthesized racemic secondary allylic alcohols **3a-5a**, **7a-12a** exhibited physical and spectral data in agreement with those previously reported in the literature:



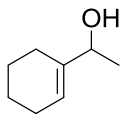
(±)-**3a**

(±)-2-Methylcyclohex-2-en-1-ol (3a). The title compound was obtained according to the general procedure as a reddish oil (141 mg, 1.26 mmol, yield: 63%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 5.53 (*s*, 1H), 3.98 (*s*, 1H), 2.00-1.56 (*m*, 10H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 135.7, 125.8, 68.8, 32.6, 25.8, 21.0, 18.6. Spectral properties are consistent with literature values.[2]



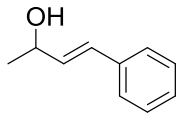
(±)-**4a**

(±)-3-Methylcyclohex-2-en-1-ol (4a). The title compound was obtained according to the general procedure as a colorless oil (145 mg, 1.3 mmol, yield: 65%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 5.49 (*s*, 1H), 4.16 (*s*, 1H), 1.92-1.54 (*m*, 10H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 138.9, 124.7, 66.2, 32.0, 30.4, 24.0, 19.4. Spectral properties are consistent with literature values.[3]



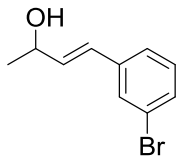
(±)-**5a**

(±)-1-(Cyclohex-1-en-1-yl)ethan-1-ol (5a). The title compound was obtained according to the general procedure as a colorless oil (131 mg, 1.04 mmol, yield: 52%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 5.68 (*s*, 1H), 4.18 (*q*, 1H, *J*= 6.4 Hz), 2.03 (*m*, 3H), 1.67-1.48 (*m* + *br s*, 6H), 1.27 (*d*, 3H, *J*= 6.5 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 141.6, 121.8, 72.5, 25.3, 24.0, 23.0 (2C), 21.9. Spectral properties are consistent with literature values.[4]



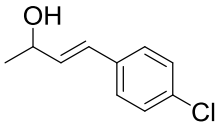
(±)-**7a**

(±)-*trans*-4-Phenylbut-3-en-2-ol (7a). The title compound was obtained according to the general procedure as a yellowish oil (273 mg, 1.84 mmol, yield: 92%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.43-7.24 (*m*, 5H), 6.60 (*d*, 1H, *J*= 15.9 Hz), 6.29 (*dd*, 1H, *J*= 15.9, 6.4 Hz), 4.52 (*m*, 1H), 1.73 (*br s*, 1H), 1.40 (*d*, 3H, *J*= 6.3 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 136.7, 133.5, 129.4, 128.6 (2C), 127.7, 126.5 (2C), 69.0, 23.4. Spectral properties are consistent with literature values.[5]



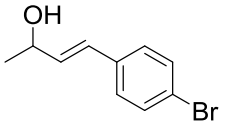
(±)-**8a**

(±)-*trans*-4-(3-Bromophenyl)but-3-en-2-ol (8a). The title compound was obtained according to the general procedure as a white solid (400 mg, 1.76 mmol, yield: 88%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.55 (*s*, 1H), 7.40-7.17 (*m*, 3H), 6.53 (*d*, 1H, *J*= 15.8 Hz), 6.29 (*dd*, 1H, *J*= 15.9, 6.0 Hz), 4.52 (*m*, 1H), 1.72 (*br s*, 1H), 1.40 (*d*, 3H, *J*= 6.4 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 138.9, 135.1, 130.5, 130.1, 129.3, 127.9, 125.1, 122.8, 68.6, 23.4. Spectral properties are consistent with literature values.[6]



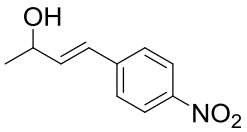
(±)-**9a**

(±)-*trans*-4-(4-Chlorophenyl)but-3-en-2-ol (9a). The title compound was obtained according to the general procedure as a white solid (347 mg, 1.90 mmol, yield: 95%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.30 (*m*, 4H), 6.53 (*d*, 1H, *J*= 17.3 Hz), 6.25 (*dd*, 1H, *J*= 15.9, 6.2 Hz), 4.50 (*m*, 1H), 1.81 (*br s*, 1H), 1.38 (*d*, 3H, *J*= 6.5 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 135.2, 134.2, 133.2, 128.7 (2C), 128.1, 127.7 (2C), 68.8, 23.4. Spectral properties are consistent with literature values.[7]



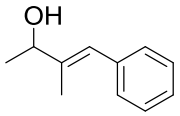
(±)-**10a**

(±)-*trans*-4-(4-Bromophenyl)but-3-en-2-ol (10a). The title compound was obtained according to the general procedure as a white solid (400 mg, 1.76 mmol, yield: 88%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.45 (*d*, 2H, *J*= 8.5 Hz), 7.25 (*d*, 2H, *J*= 8.4 Hz), 6.52 (*d*, 1H, *J*= 15.9 Hz), 6.27 (*dd*, 1H, *J*= 15.9, 6.2 Hz), 4.50 (*m*, 1H), 1.78 (*br s*, 1H), 1.39 (*d*, 3H, *J*= 6.4 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 135.7, 134.3, 131.7 (2C), 128.2, 128.0 (2C), 121.4, 68.8, 23.4. Spectral properties are consistent with literature values.[6]



(±)-**11a**

(±)-*trans*-4-(4-Nitrophenyl)but-3-en-2-ol (11a). The title compound was obtained according to the general procedure as a brown solid (367 mg, 1.9 mmol, yield: 95%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 8.16 (*d*, 2H, *J*= 8.8 Hz), 7.49 (*d*, 2H, *J*= 8.8 Hz), 6.66 (*d*, 1H, *J*= 15.9 Hz), 6.46 (*dd*, 1H, *J*= 16.0, 5.6 Hz), 4.56 (*m*, 1H), 2.08 (*br s*, 1H), 1.41 (*d*, 3H, *J*= 6.4 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 146.8, 143.4, 138.5, 126.9 (3C), 124.0 (2C), 68.4, 23.4. Spectral properties are consistent with literature values.[8]

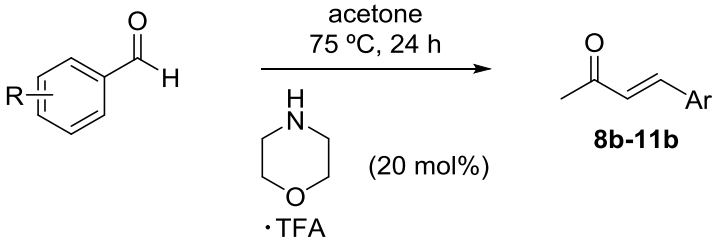


(±)-**12a**

(±)-*trans*-3-Methyl-4-phenylbut-3-en-2-ol (12a). The title compound was obtained according to the general procedure as a brown oil (292 mg, 1.8 mmol, yield: 90%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.39-7.24 (*m*, 5H), 6.55 (*s*, 1H), 4.41 (*q*, 1H, *J*= 6.5 Hz), 1.91 (*s*, 3H), 1.76 (*br s*, 1H), 1.40 (*d*, 3H, *J*= 6.4 Hz); **^{13}C NMR (75 MHz, CDCl3):** δ (ppm) 142.0, 138.0, 129.4 (2C), 128.5 (2C), 126.8, 124.8, 74.1, 22.2, 13.8. Spectral properties are consistent with literature values.[9]

IV. Synthesis of α,β-unsaturated ketones

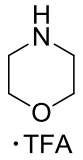
Following the procedure reported by List and co-workers,[10] ketones **8b**-**11b** were synthesized through an aldol condensation of acetone with the corresponding aldehydes, followed by dehydration (Scheme S1).



Scheme S1. Synthesis of ketones **8b**-**11b**.

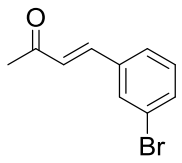
Synthesis of morpholinium trifluoroacetate

In order to carry out the aldol condensation, the catalyst morpholinium trifluoroacetate was synthesized as follows: To a solution of morpholine (1.7 mL, 20 mmol) in Et_2O (40 mL), another solution containing trifluoroacetic acid (22 mmol) in Et_2O (20 mL) was slowly added at 0 °C. After 1 h the reaction was allowed to warm up to room temperature and the white precipitate formed was filtered and washed with Et_2O and pentane to yield the desired catalyst as a white solid (3.9 g, 19.6 mmol, yield: 98%).



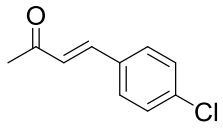
^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 9.00 (*s*, 2H), 3.77 (*t*, 4H, *J*= 4.8 Hz), 3.11 (*t*, 4H, *J*= 4.9 Hz). Spectral properties are consistent with literature values.[10]

To a solution of the corresponding aldehyde (3-bromobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde or 4-nitrobenzaldehyde for **8b**, **9b**, **10b** and **11b**, respectively, 5 mmol) in acetone (12.5 mL), the morpholinium trifluoroacetate salt was added (201.1 mg, 1 mmol). The reaction mixture was stirred at 75 °C in a test tube. After 24 h, the reaction was cooled to room temperature, and quenched with an aqueous $NaHCO_3$ saturated solution (15 mL). Acetone was removed under reduced pressure and the reaction was extracted with EtOAc (3 x 15 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum. The resulting crudes were purified by column chromatography (EtOAc/*n*-hexane), affording the desired α,β-unsaturated ketones.



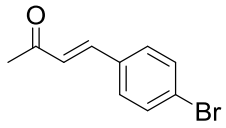
8b

***trans*-4-(3-Bromophenyl)but-3-en-2-one (8b).** The title compound was prepared using the previously described procedure and isolated after column chromatography (10% EtOAc/*n*-hexane) as a white solid (210 mg, 0.93 mmol, yield: 19%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.70 (*s*, 1H), 7.55-7.41 (*m*, 3H), 7.29 (*t*, 1H, *J*= 7.8 Hz), 6.71 (*d*, 1H, *J*= 7.8 Hz), 2.40 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 197.9, 141.5, 136.5, 133.2, 130.9, 130.5, 128.2, 126.8, 123.1, 27.8. Spectral properties are consistent with literature values.[6]



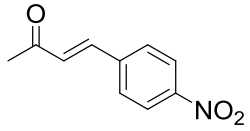
9b

***trans*-4-(4-Chlorophenyl)but-3-en-2-one (9b).** The title compound was prepared using the previously described procedure and isolated after column chromatography (30% EtOAc/*n*-hexane) as a white solid (624 mg, 3.45 mmol, yield: 69%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.48 (*m*, 3H), 7.39 (*d*, 2H, *J*= 8.3 Hz), 6.70 (*d*, 1H, *J*= 13.6 Hz), 2.39 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 198.1, 141.9, 136.4, 132.9, 129.4 (2C), 129.3 (2C), 127.5, 27.7. Spectral properties are consistent with literature values.[11]



10b

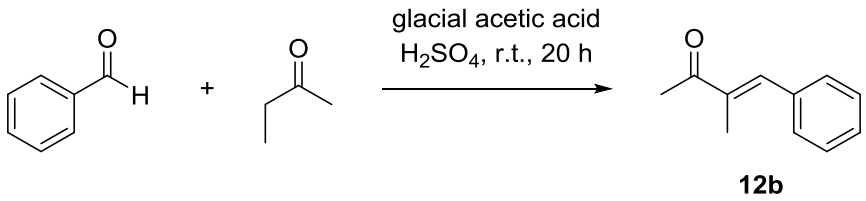
***trans*-4-(4-Bromophenyl)but-3-en-2-one (10b).** The title compound was prepared using the previously described procedure and isolated after column chromatography (30% EtOAc/*n*-hexane) as a brown solid (677 mg, 3.00 mmol, yield: 60%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.58-7.40 (*m*, 5H), 6.72 (*d*, 1H, *J*= 16.3 Hz), 2.40 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 198.1, 142.0, 133.3, 132.2 (2C), 129.6 (2C), 127.5, 124.8, 27.7. Spectral properties are consistent with literature values.[12]



11b

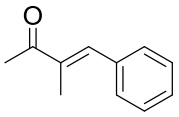
***trans*-4-(4-Nitrophenyl)but-3-en-2-one (11b).** The title compound was prepared using the previously described procedure and isolated after column chromatography (30% EtOAc/*n*-hexane) as a yellowish solid (204 mg, 1.06 mmol, yield: 21%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 8.23 (*d*, 2H, *J*= 8.8 Hz), 7.69 (*d*, 2H, *J*= 8.8 Hz), 7.53 (*d*, 1H, *J*= 16.3 Hz), 6.81 (*d*, 1H, *J*= 16.4 Hz), 2.41 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 197.6, 148.5, 140.7, 140.1, 130.4, 128.8 (2C), 124.2 (2C), 28.0. Spectral properties are consistent with literature values.[13]

Another aldol condensation with benzaldehyde and butan-2-one under different conditions led to the unsaturated ketone **12b**. This procedure was described by Buchwald and co-workers (Scheme S2).[14]



Scheme S2. Synthesis of ketone **12b**.

To a solution of butan-2-one (4.48 mL, 50 mmol) and benzaldehyde (2.55 mL, 25 mmol) in glacial acetic acid (20 mL), concentrated sulfuric acid was added (1.30 mL) and the reaction mixture was stirred for 20 h at room temperature. After this time the mixture was then poured into H_2O (100 mL) and then carefully neutralized with an aqueous NaOH 3 M solution. The resulting mixture was then extracted with EtOAc (3 x 30 mL), the combined organic layers were washed with an aqueous $NaHCO_3$ saturated solution (20 mL) and brine (20 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure, and the residue purified by column chromatography to afford the desired unsaturated ketone **12b**.



12b

***trans*-3-Methyl-4-phenylbut-3-en-2-one (12b).** The title compound was prepared using the previously described procedure and isolated after column chromatography (30% EtOAc/*n*-hexane) as a yellowish oil (2.13 g, 13.30 mmol, yield: 53%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.50 (*s*, 1H), 7.41-7.30 (*m*, 5H), 2.43 (*s*, 3H), 2.04 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 200.1, 139.7, 137.6, 135.9, 129.7 (2C), 128.6, 128.5 (2C), 25.8, 12.9. Spectral properties are consistent with literature values.[15]

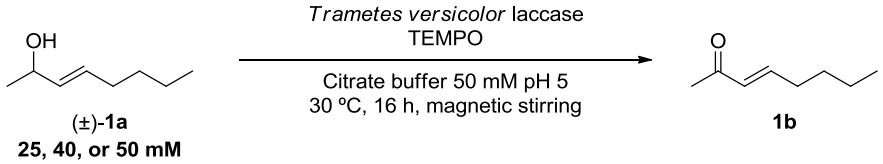
V. Oxidation of racemic allylic secondary alcohols with the laccase–TEMPO system

V.1. Optimization of the laccase–TEMPO reaction conditions

V.1.1. Effect of the substrate concentration

In an open-to-air test tube, TEMPO (4.1 mg, 33 mol%) was added to a solution of the racemic secondary allylic alcohol **1a** (10 mg, 0.08 mmol, at 25, 40, or 50 mM, respectively) in citrate buffer 50 mM pH 5 (3.2, 2 and 1.6 mL, respectively). The reaction mixture was stirred for a few minutes to dissolve all the reagents and then the laccase from *Trametes versicolor* (5 U) was added. The reaction was stirred for 16 h under magnetic stirring at 30 ºC. After this time, the mixture was extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4, and an aliquot was taken for the determination of the conversion value by GC analysis (Table S1).

Table S1. Oxidation of (±)-**1a** with the laccase-TEMPO system using different substrate concentrations.



Entry	Substrate concentration (mM)	**1b** (%)[a]
1	25	>99
2	40	>99
3	50	>99

[a] Conversion values measured by GC.

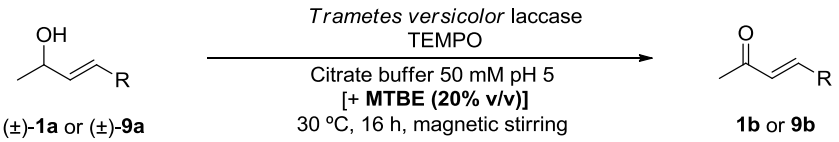
V.1.2. Effect of the organic solvent

A different effect in the presence of MTBE as organic co-solvent was observed depending on the substrate. Thus, when the racemic allylic alcohols were oils, total conversions were achieved in plain buffer media (except for **12a**), whereas in order to fully convert substrate **12a** and solid racemic alcohols, the addition of MTBE (20% v/v) was necessary due to their low solubility in the buffer.

In an-open-to-air test tube, TEMPO (4.1 mg, 33 mol%) was added to a solution of the racemic alcohol **1a** (oil partially soluble in the buffer) or **9a** (solid insoluble in the buffer)

(0.08 mmol, 50 mM) in 1.6 mL of citrate buffer 50 mM pH 5 or in a biphasic mixture containing citrate buffer 50 mM pH 5 and MTBE (20% v/v, for a total volume of 1.92 mL). The reaction mixture was stirred for a few minutes to dissolve all the reagents and then the laccase from *Trametes versicolor* (5 U) was added. The reaction was magnetically stirred for 16 h at 30 ºC. After this time, the mixture was extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4, and an aliquot was taken for the determination of the conversion values by GC analysis (Table S2).

Table S2. Effect of MTBE in the laccase-TEMPO-catalyzed oxidation of (±)-**1a** or (±)-**9a**.



Entry	Substrate	R	MTBE (% v/v)	Conv. **1b** or **9b** (%)[a]
1	**1a**	C_4H_9	no	>99
2	**1a**	C_4H_9	yes (20)	>99
3	**9a**	*p*-Cl-C_6H_4	no	85
4	**9a**	*p*-Cl-C_6H_4	yes (20)	>99

[a] Conversion values measured by GC.

*V.2. Oxidation of (5*R*)-carveol **13a** with the laccase–TEMPO system*

In an open-to-air test tube, TEMPO (4.1 mg, 33 mol%) was added to a solution of the mixture of isomers of (5*R*)-carveol **13a** (13 mg, 0.08 mmol, 40 mM) in citrate buffer 50 mM pH 5 (2 mL). The reaction mixture was stirred for a few minutes to dissolve all the reagents and then the laccase from *Trametes versicolor* (5 U) was added. The reaction was maintained under magnetic stirring at 30 ºC for 16 h, and then an aliquot was taken for the determination of the conversion value by GC analysis. To the remaining reaction crude, TEMPO (4.1 mg, 33 mol%) and laccase from *Trametes versicolor* (5 U) were added again. The reaction was stirred under the same conditions for other 16 h. After this time, the mixture was extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4, and an aliquot was taken for the determination of the new conversion value by GC analysis.

Assignment of isomers (1*R*,5*R*)-**13a** and (1*S*,5*R*)-**13a** was made by ^{1}H-NMR analysis in agreement with the spectra reported in literature for *cis*-**13a** and *trans*-**13a**, respectively.[2]

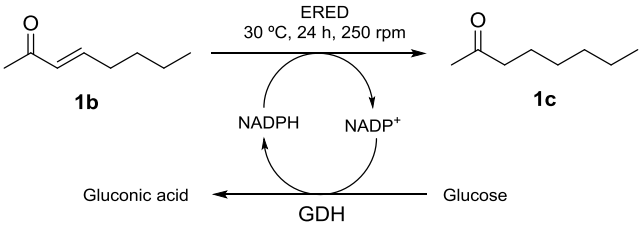
VI. Ene-reductase (ERED) screenings

All the bioreductions were carried out using two different buffer media: phosphate buffer 100 mM pH 7 (recommended for these reductases by Codexis Inc.), and citrate buffer 50 mM pH 5 (buffer used for the oxidation of racemic secondary alcohols with the laccase–TEMPO system, first step of this sequential process):

VI.1. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone ***1b***

The following conversion values were obtained according to the procedure that has already been reported in the Experimental section of the main manuscript (Table S3).

Table S3. Enzymatic screening for the reduction of **1b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



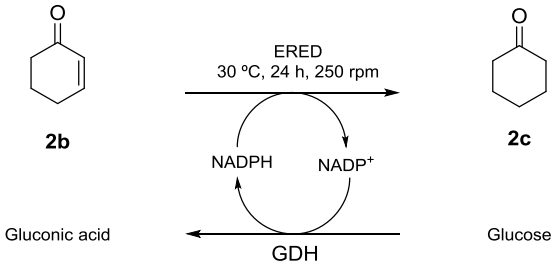
Entry	ERED	Phosphate buffer 100 mM pH 7	**Citrate buffer 50 mM pH 5**
		c (%)[a]	*c* (%)[a]
1	103	>99	>99
2	**110**	>99	**>99**
3	112	>99	>99
4	207	>99	>99
5	P1-A04	>99	>99
6	P1-E01	>99	>99
7	P1-H09	>99	>99

[a] Conversion values measured by GC analysis.

*VI.2. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **2b***

The following conversion values were obtained according to the procedure that has already been reported in the Experimental section of the main manuscript (Table S4).

Table S4. Enzymatic screening for the reduction of **2b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



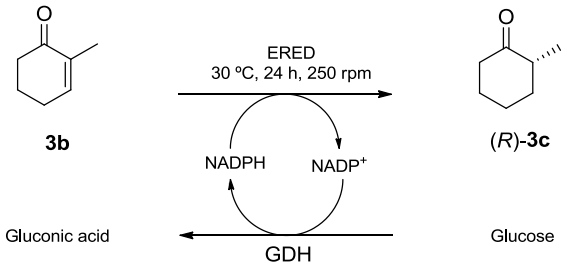
Entry	ERED	Phosphate buffer 100 mM pH 7	**Citrate buffer 50 mM pH 5**
		c (%)[a]	*c* (%)[a]
1	103	>99	>99
2	110	>99	>99
3	112	>99	>99
4	207	>99	75
5	**P1-A04**	>99	**>99**
6	P1-E01	>99	>99
7	P1-H09	>99	66

[a] Conversion values measured by GC analysis.

*VI.3. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **3b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S5).

Table S5. Enzymatic screening for the reduction of **3b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.

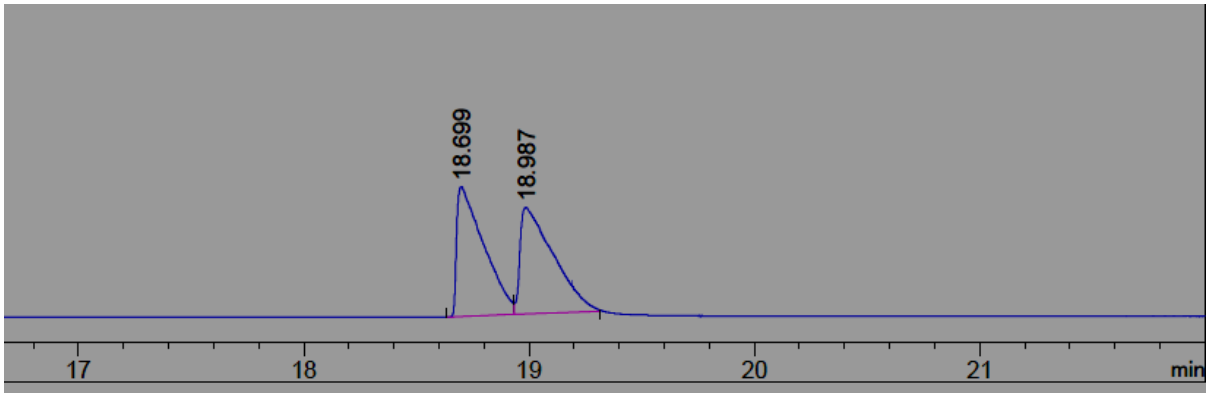


Entry	ERED	Phosphate buffer 100 mM pH 7		**Citrate buffer 50 mM pH 5**	
		c (%)[a]	*ee* (%)[b]	*c* (%)[a]	*ee* (%)[b]
1	103	>99	74 (*R*)	>99	86 (*R*)
2	110	>99	76 (*R*)	82	85 (*R*)
3	112	>99	78 (*R*)	11	81 (*R*)
4	207	>99	76 (*R*)	44	82 (*R*)
5	P1-A04	>99	74 (*R*)	>99	77 (*R*)
6	**P1-E01**	>99	82 (*R*)	>**99**	**87 (*R*)**
7	P1-H09	25	75 (*R*)	30	87 (*R*)

[a] Conversion values measured by GC analysis.

[b] Enantiomeric excess values were determined by chiral GC analysis (Figure S2).

(±)-**3c**



(*R*)-**3c**

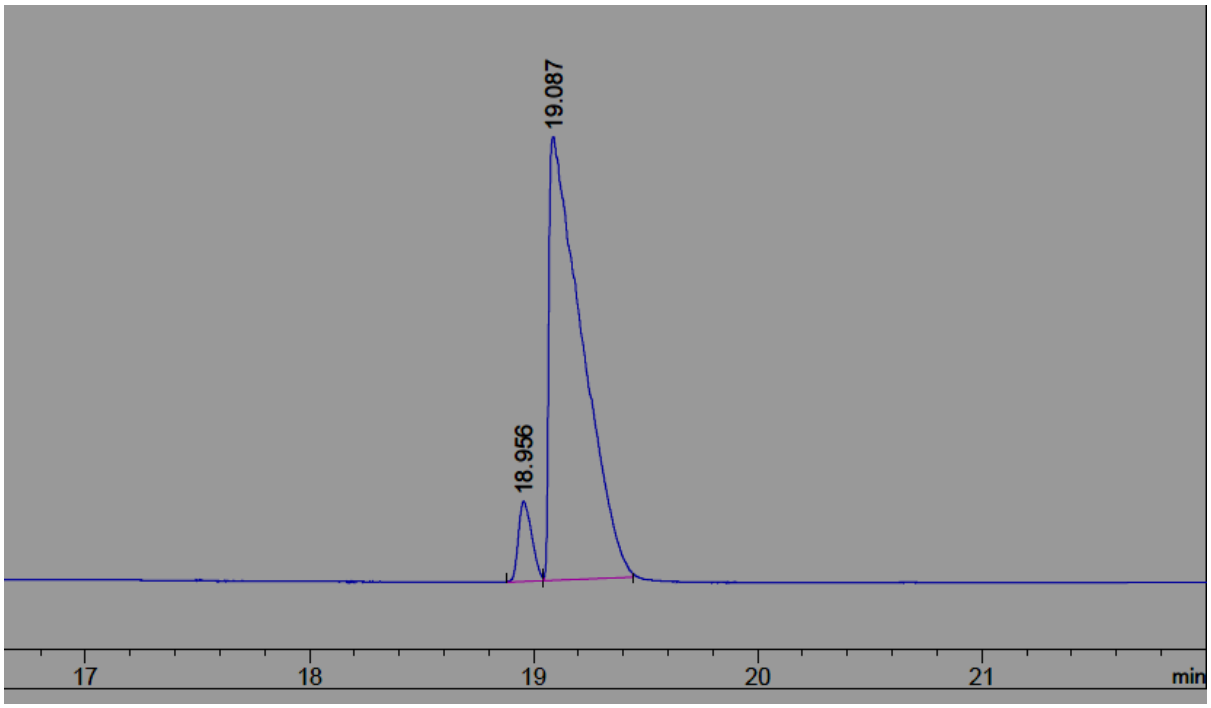
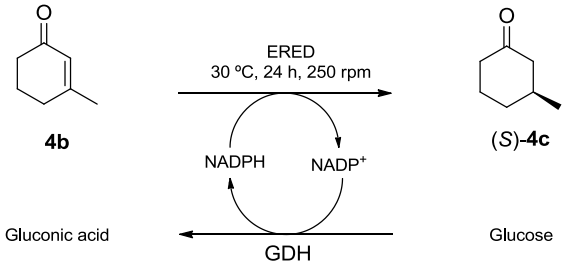


Figure S2. Analytical separation by chiral GC for racemic (above) and enantioenriched (below) **3c** obtained after carrying out the bioreduction of **3b** with ERED-P1-E01 in citrate buffer 50 mM pH 5.

*VI.4. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **4b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S6).

Table S6. Enzymatic screening for the reduction of **4b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.

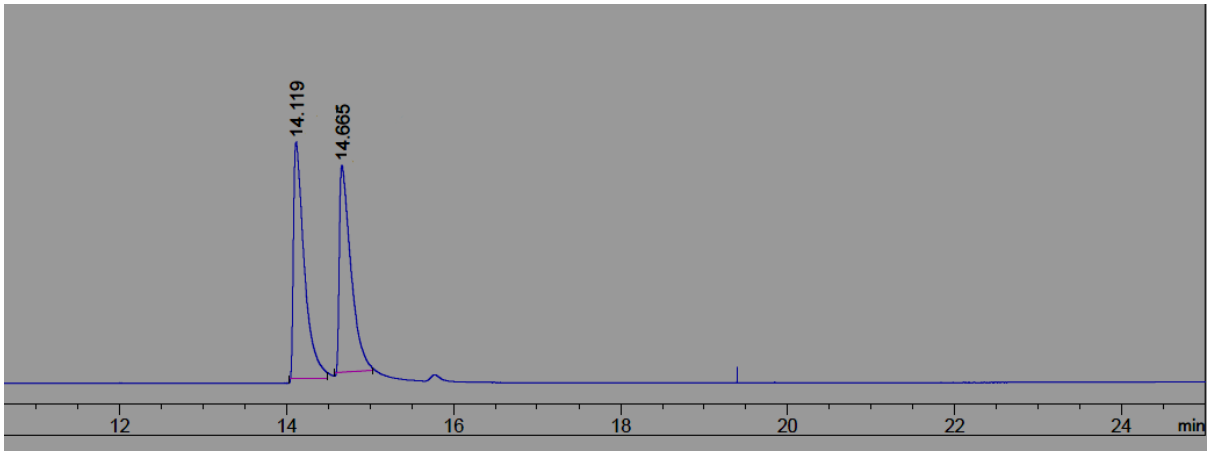


Entry	ERED	Phosphate buffer 100 mM pH 7		**Citrate buffer 50 mM pH 5**	
		c (%)[a]	*ee* (%)[b]	*c* (%)[a]	*ee* (%)[b]
1	103	84	>99 (*S*)	37	>99 (*S*)
2	110	85	>99 (*S*)	10	>99 (*S*)
3	112	25	>99 (*S*)	<1	n.d.
4	207	92	>99 (*S*)	8	>99 (*S*)
5	P1-A04	65	>99 (*S*)	25	>99 (*S*)
6	**P1-E01**	99	>99 (*S*)	**99**	**>99 (*S*)**
7	P1-H09	<1	n.d.	<1	n.d.

[a] Conversion values measured by GC analysis.

[b] Enantiomeric excess values were determined by chiral GC analysis (Figure S3). n.d.: not determined.

(±)-**4c**



(*S*)-**4c**

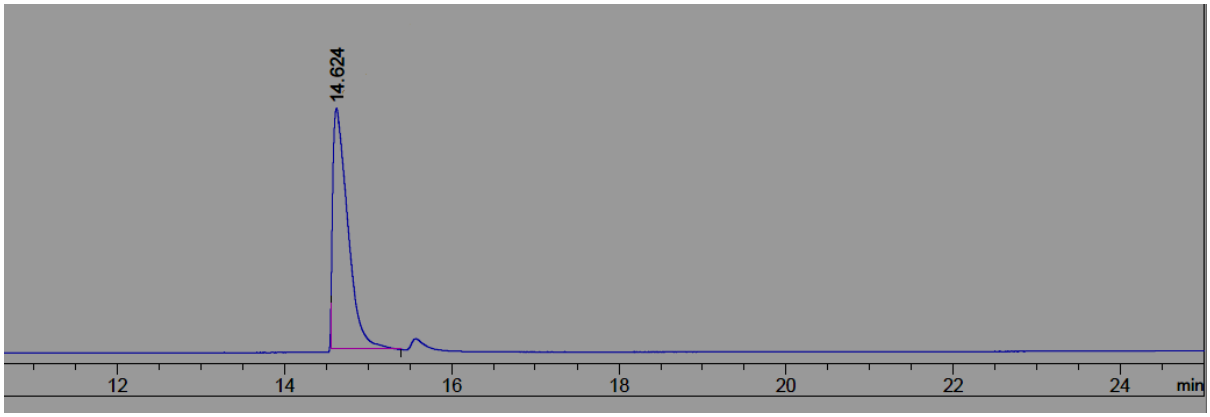
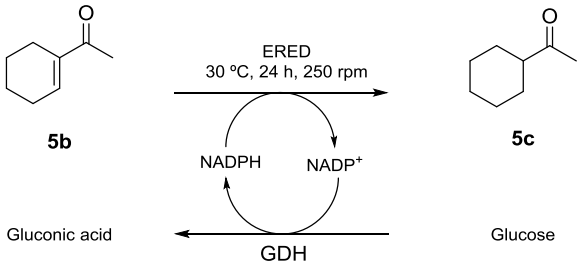


Figure S3. Analytical separation by chiral GC for racemic (above) and enantioenriched (below) **4c** obtained after carrying out the bioreduction of **4b** with ERED-P1-E01 in citrate buffer 50 mM pH 5.

*VI.5. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **5b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S7).

Table S7. Enzymatic screening for the reduction of **5b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



Entry	ERED	Phosphate buffer 100 mM pH 7	**Citrate buffer 50 mM pH 5**
		c (%)[a]	*c* (%)[a]
1	103	>99	>99
2	110	>99	23
3	112	16	<1
4	207	96	7
5	**P1-A04**	>99	**>99**
6	P1-E01	>99	>99
7	P1-H09	10	2

[a] Conversion values measured by GC analysis.

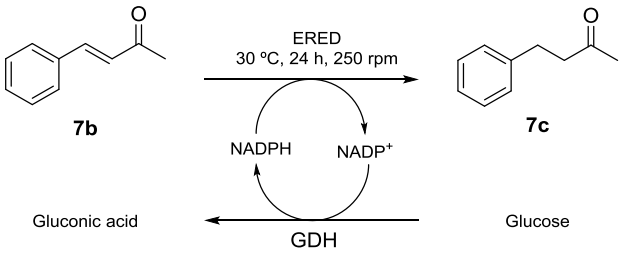
*VI.6. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **6b***

α,β-Unsaturated ketone **6b** could not be isolated starting from the commercially available vinyl alcohol (±)-**6a**, neither using the laccase from *Trametes versicolor*–TEMPO system, nor using other chemical oxidants such as Dess-Martin periodinane. For this reason, the full sequential process for the substrate (±)-**6a** was carried out selecting the ene-reductase that had reduced aromatic unsaturated ketones with the best results in terms of conversion, ERED-P1-E01. Furthermore, the sequential process starting from (±)-**6a** was scaled up to 50 mg, and the resulting propiophenone was characterized and isolated in good yield (*see scaled-up reactions Section*).

*VI.7. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **7b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S8).

Table S8. Enzymatic screening for the reduction of **7b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



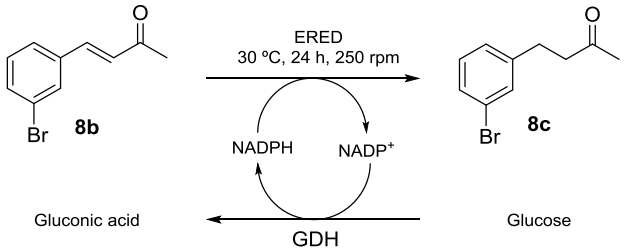
Entry	ERED	Phosphate buffer 100 mM pH 7	**Citrate buffer 50 mM pH 5**
		c (%)[a]	*c* (%)[a]
1	103	>99	80
2	110	>99	51
3	112	>99	12
4	207	>99	36
5	P1-A04	>99	99
6	**P1-E01**	>99	**>99**
7	P1-H09	89	33

[a] Conversion values measured by GC analysis.

*VI.8. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **8b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S9).

Table S9. Enzymatic screening for the reduction of **8b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



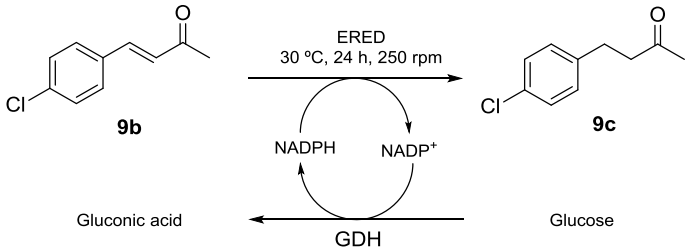
Entry	ERED	Phosphate buffer 100 mM pH 7	**Citrate buffer 50 mM pH 5**
		c (%)[a]	*c* (%)[a]
1	**103**	>99	**>99**
2	110	98	50
3	112	89	13
4	207	97	46
5	P1-A04	>99	99
6	P1-E01	98	99
7	P1-H09	87	43

[a] Conversion values measured by GC analysis.

VI.9. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone ***9b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S10).

Table S10. Enzymatic screening for the reduction of **9b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



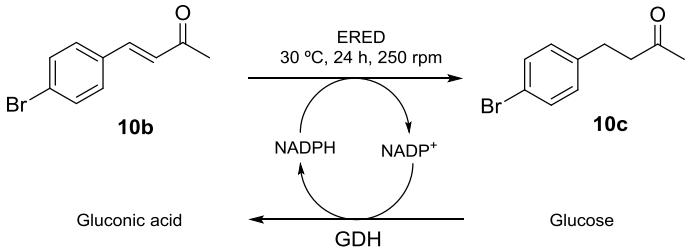
Entry	ERED	Phosphate buffer 100 mM pH 7	**Citrate buffer 50 mM pH 5**
		c (%)[a]	*c* (%)[a]
1	103	>99	99
2	110	>99	76
3	112	81	12
4	207	>99	28
5	**P1-A04**	>99	**>99**
6	P1-E01	>99	>99
7	P1-H09	36	8

[a] Conversion values measured by GC analysis.

*VI.10. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **10b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S11).

Table S11. Enzymatic screening for the reduction of **10b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



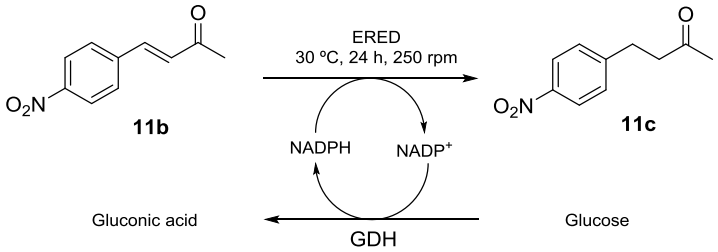
Entry	ERED	Phosphate buffer 100 mM pH 7	**Citrate buffer 50 mM pH 5**
		c (%)[a]	*c* (%)[a]
1	103	>99	>99
2	110	95	18
3	112	60	11
4	207	>99	>99
5	P1-A04	>99	>99
6	**P1-E01**	>99	**>99**
7	P1-H09	21	4

[a] Conversion values measured by GC analysis.

*VI.11. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **11b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S12).

Table S12. Enzymatic screening for the reduction of **11b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



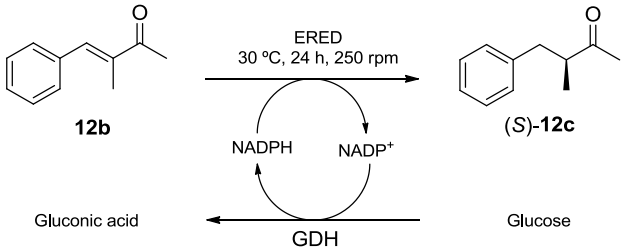
Entry	ERED	Phosphate buffer 100 mM pH 7	**Citrate buffer 50 mM pH 5**
		c (%)[a]	*c* (%)[a]
1	103	>99	>99
2	110	>99	>99
3	112	>99	57
4	207	>99	83
5	P1-A04	>99	>99
6	**P1-E01**	>99	**>99**
7	P1-H09	>99	67

[a] Conversions values measured by chiral HPLC.

*VI.12. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone **12b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S13).

Table S13. Enzymatic screening for the reduction of **12b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.

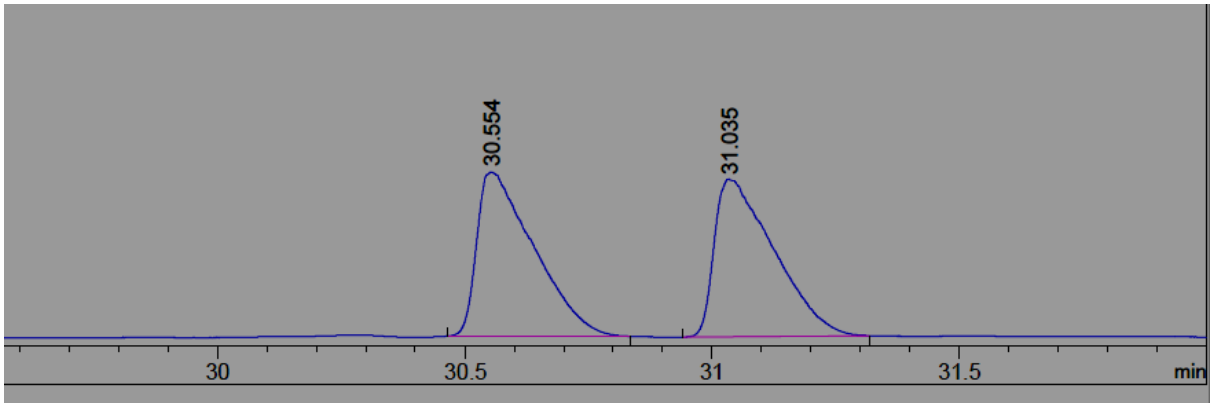


Entry	ERED	Phosphate buffer 100 mM pH 7		**Citrate buffer 50 mM pH 5**	
		c (%)[a]	*ee* (%)[b]	*c* (%)[a]	*ee* (%)[b]
1	103	>99	80 (*S*)	>99	82 (*S*)
2	110	96	58 (*S*)	29	<1
3	112	71	58 (*S*)	2	n.d.
4	207	90	25 (*S*)	14	39 (*S*)
5	P1-A04	>99	70 (*S*)	>99	74 (*S*)
6	**P1-E01**	>99	92 (*S*)	**>99**	**92 (*S*)**
7	P1-H09	29	n.d.	6	n.d.

[a] Conversion values measured by GC analysis.

[b] Enantiomeric excess values were determined by chiral GC analysis (Figure S4). n.d.: not determined.

(±)-**12c**



(*S*)-**12c**

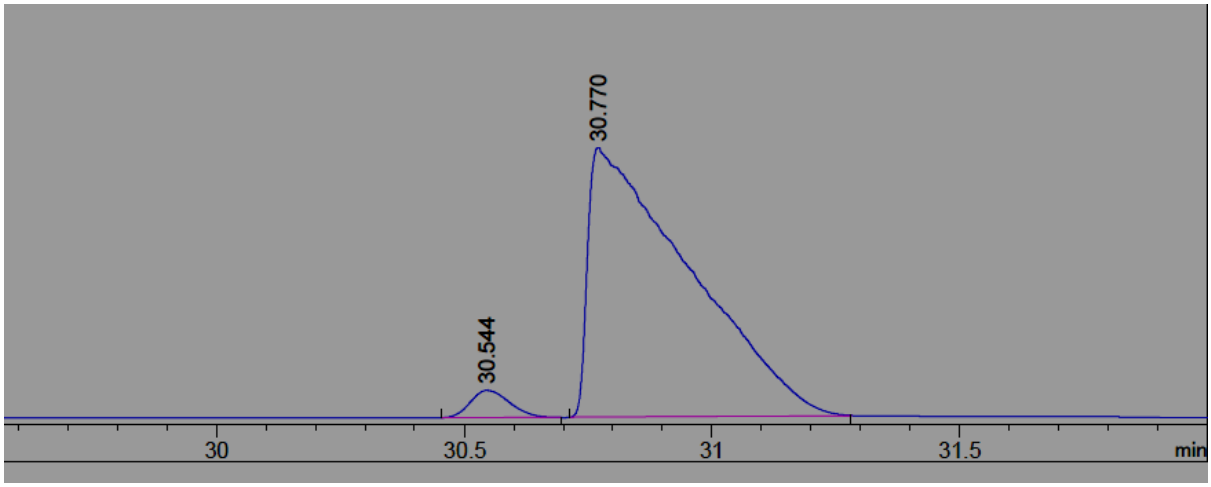
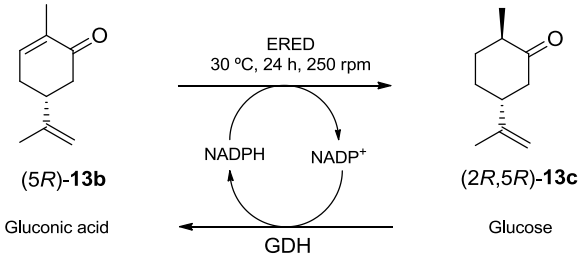


Figure S4. Analytical separation by chiral GC for racemic (above) and enantioenriched (below) **12c** obtained after carrying out the bioreduction of **12b** with ERED-P1-E01 in citrate buffer 50 mM pH 5.

*VI.13. Screening for ERED-catalyzed reactions with α,β-unsaturated ketone (*5R*)-**13b***

The following conversion values were obtained according to the procedure that has been already reported in the Experimental section of the main manuscript (Table S14).

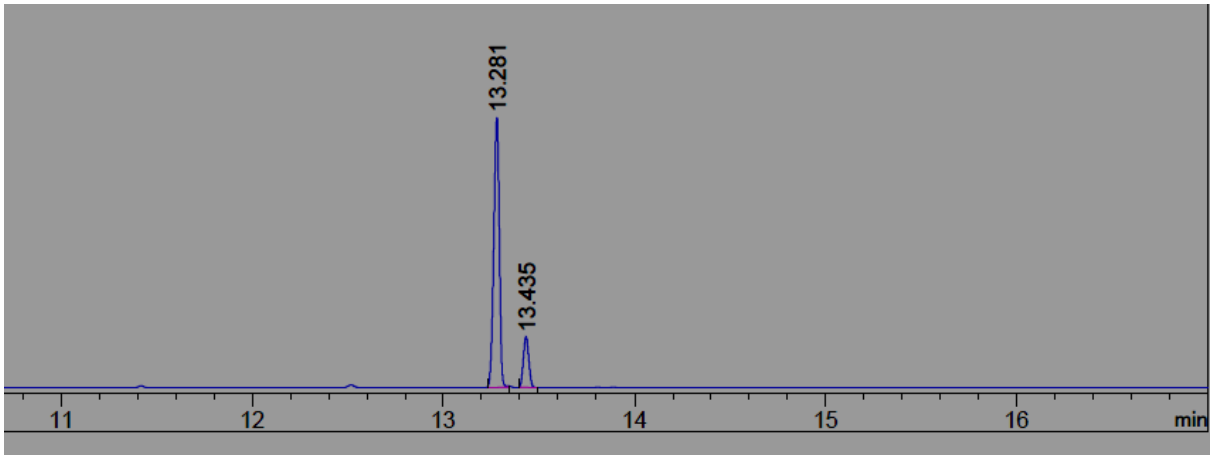
Table S14. Enzymatic screening for the reduction of (5*R*)-**13b** using commercially available ene-reductases in two different buffer media. The ERED and the buffer medium that were later selected for the bienzymatic sequential strategy appear in bold font.



Entry	ERED	Phosphate buffer 100 mM pH 7		**Citrate buffer 50 mM pH 5**	
		c (%)[a]	*de* (%)[a]	*c* (%)[a]	*de* (%)[a]
1	103	>99	86 (2*R*,5*R*)	98	90 (2*R*,5*R*)
2	110	>99	84 (2*R*,5*R*)	>99	90 (2*R*,5*R*)
3	112	>99	84 (2*R*,5*R*)	8	n.d.
4	207	>99	84 (2*R*,5*R*)	>99	86 (2*R*,5*R*)
5	P1-A04	>99	88 (2*R*,5*R*)	>99	90 (2*R*,5*R*)
6	**P1-E01**	>99	90 (2*R*,5*R*)	**>99**	**90 (2*R*,5*R*)**
7	P1-H09	>99	56 (2*S*,5*R*)	68	17 (2*R*,5*R*)

[a] Conversion and diastereomeric excess values were measured by GC analysis (Figure S5). n.d.: not determined.

(2*RS*,5*R*)-dihydrocarvone **13c**



(2*R*,5*R*)-**13c** obtained after the bioreduction with ERED-P1-E01

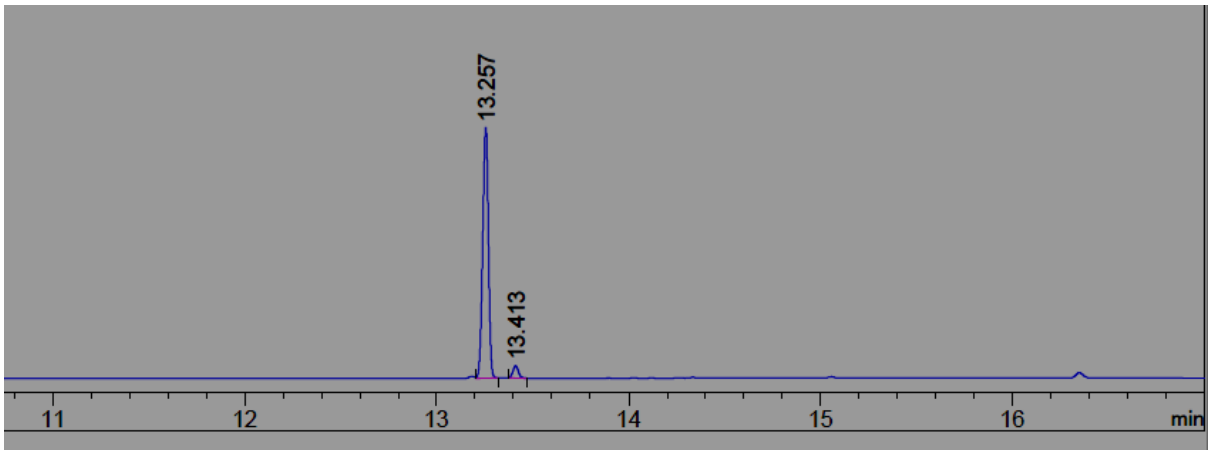


Figure S5. Analytical separation by achiral GC for the commercial mixture of isomers (above) and diastereoenriched (below) **13c** obtained after carrying out the bioreduction of (5*R*)-**13b** with ERED-P1-E01 in citrate buffer 50 mM pH 5.

VI.14. Characterization of the saturated ketones

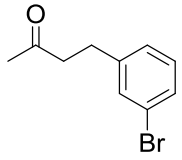
Commercial ketones

Saturated ketones **1c**-**7c** and **13c** obtained through these bioreduction processes exhibited physical and spectral data in agreement with the ones obtained from commercial sources.

Non-commercial ketones

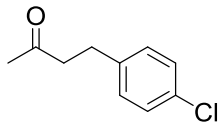
In order to characterize the saturated ketones **8c**-**12c**, bioreductions of the corresponding α,β-unsaturated ketones **8b**-**12b** with the selected EREDs (marked in bold, Tables S9-S13), were scaled-up to 25 mg of substrate:

In a test tube, D-(+)-glucose (2 equiv.), GDH-105 (4 mg per mmol substrate), and $NADP^+$ (0.6 mM) were added to a solution of the α,β-unsaturated ketone **8b**-**12b** (25 mg, 30 mM) in citrate buffer 50 mM pH 5. The reaction mixture was stirred for a few minutes to dissolve all the reagents and then the selected ERED (25 mg) was added. The test tube was closed and the reaction was stirred for 24 h under magnetic stirring at 30 ºC. After this time, the mixture was extracted with EtOAc (2 x 6 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure, isolating the corresponding saturated ketones **8c**-**12c**. Characterization data are given below:



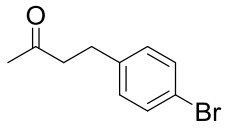
8c

4-(3-Bromophenyl)butan-2-one (8c). The title compound was synthesized using the previously described procedure and isolated after extraction as a yellowish oil (20 mg, 0.09 mmol, yield: 79%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.34 (*m*, 2H), 7.15 (*m*, 2H), 2.88 (*t*, 2H, *J*= 6.9 Hz), 2.77 (*t*, 2H, *J*= 7.2 Hz), 2.16 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 207.3, 143.4, 131.4, 130.1, 129.3, 127.1, 122.5, 44.8, 30.1, 29.2. Spectral properties are consistent with literature values.[16]



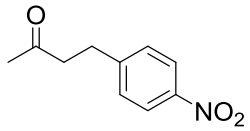
9c

4-(4-Chlorophenyl)butan-2-one (9c). The title compound was synthesized using the previously described procedure and isolated after extraction as a yellowish oil (18 mg, 0.10 mmol, yield: 71%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.26 (*d*, 2H, *J*= 8.5 Hz), 7.13 (*d*, 2H, *J*= 8.5 Hz), 2.88 (*t*, 2H, *J*= 7.5 Hz), 2.76 (*t*, 2H, *J*= 7.5 Hz), 2.16 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 207.7, 139.6, 132.0, 129.8 (2C), 128.7 (2C), 45.0, 30.2, 29.1. Spectral properties are consistent with literature values.[17]



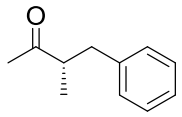
10c

4-(4-Bromophenyl)butan-2-one (10c). The title compound was synthesized using the previously described procedure and isolated after extraction as a yellowish oil (21 mg, 0.09 mmol, yield: 83%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.41 (*d*, 2H, *J*= 8.4 Hz), 7.08 (*d*, 2H, *J*= 8.5 Hz), 2.86 (*t*, 2H, *J*= 7.9 Hz), 2.75 (*t*, 2H, *J*= 7.9 Hz), 2.15 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 207.6, 140.1, 131.6 (2C), 130.2 (2C), 120.0, 44.9, 30.2, 29.2. Spectral properties are consistent with literature values.[17]



11c

4-(4-Nitrophenyl)butan-2-one (11c). The title compound was synthesized using the previously described procedure and isolated after extraction as a brown oil (18 mg, 0.09 mmol, yield: 71%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 8.16 (*d*, 2H, *J*= 8.7 Hz), 7.37 (*d*, 2H, *J*= 8.7 Hz), 3.02 (*t*, 2H, *J*= 7.2 Hz), 2.83 (*t*, 2H, *J*= 7.4 Hz), 2.18 (*s*, 3H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 206.7, 149.0, 146.5, 129.3 (2C), 123.7 (2C), 44.1, 30.0, 29.3. Spectral properties are consistent with literature values.[17]



(*S*)-12c

(*S*)-3-Methyl-4-phenylbutan-2-one [(*S*)-12c]. The title compound was synthesized using the previously described procedure and isolated after extraction as a yellowish oil (19 mg, 0.12 mmol, yield: 75%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.33-7.16 (*m*, 5H), 3.02 (*dd*, 1H, *J*= 13.4, 6.7 Hz), 2.85 (*ddq*, 1H, *J*= 7.6, 7.0, 6.7 Hz), 2.59 (*dd*, 1H, *J*= 13.4, 7.6 Hz), 2.11 (*s*, 3H), 1.11 (*d*, 3H, *J*= 6.9 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 212.3, 139.8, 129.0 (2C), 128.5 (2C), 126.3, 48.9, 39.0, 29.0, 16.3. Spectral properties are consistent with literature values.[18]

In order to measure the *ee* of **12c**, racemic ketone (±)-**12c** was prepared through hydrogenation using palladium on carbon (Pd/C) as catalyst:[19]

To a solution of the unsaturated ketone **12b** (40 mg, 0.25 mmol) in deoxygenated EtOAc (3 mL), Pd/C on charcoal (10% w w^{-1}, 3 mg) was carefully added and the reaction was stirred for 18 h at room temperature under H_2 pressure (balloon). Then the reaction was filtered over celite and washed with EtOAc (15 mL). The liquid residue was concentrated under vacuum to afford (±)-**12c** as a yellowish oil (28 mg, 0.17 mmol, yield: 69%).

VI.15. Absolute configuration assignment of the optically active saturated ketones

The absolute configuration of **3c** enantiomers was assigned according to the data reported in the literature by Okamoto *et al*.[20]

To assign the absolute configuration of **4c** enantiomers, the GC chromatograms obtained after the reduction of **4b** with ene-reductases were compared to the one obtained after the injection of commercially available (*R*)-**4c**.

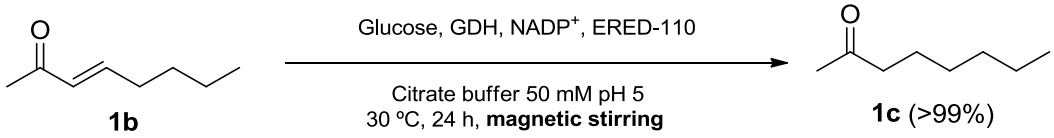
Absolute configuration assignment of **12c** enantiomers was done according to the data reported in the literature by Brenna *et al*.[18]

The absolute configuration of diastereoisomers (2*R*,5*R*)-**13c** and (2*S*,5*R*)-**13c** was assigned by NMR analysis, and the spectra are in agreement with those reported in the literature for *cis*-**13c** and *trans*-**13c**, respectively.[21]

VII. Optimization of the bienzymatic process

VII.1. Ene-reductase-catalyzed bioreductions under magnetic stirring

As it has been proved by us that the magnetic stirring leads to higher conversions for the oxidation of secondary alcohols than the orbital shaking;[22] in order to combine both steps of this sequential process, the reduction of the C=C double bond of the α,β-unsaturated ketone **1b** was studied under magnetic stirring (Scheme S3).

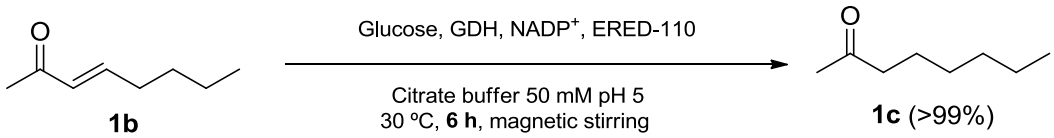


Scheme S3. ERED-catalyzed bioreduction of unsaturated ketone **1c** under magnetic stirring.

In a test tube, D-(+)-glucose (29 mg, 0.16 mmol), GDH-105 (4 mg), and $NADP^+$ (0.6 mM) were added to a solution of the α,β-unsaturated ketone **1b** (10 mg, 0.08 mmol, 40 mM) in citrate buffer 50 mM pH 5 (2 mL). The reaction mixture was stirred for a few minutes to dissolve all the reagents and then ERED-110 (20 mg) was added. The test tube was closed and the reaction was stirred for 24 h under magnetic stirring at 30 ºC. After this time, the mixture was extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4, and an aliquot was taken for the determination of the conversion value by GC analysis.

VII.2. Ene-reductase-catalyzed bioreductions at shorter reaction times

The ERED-110-catalyzed bioreduction of **1b** to **1c** was studied at shorter reaction times (6 h, Scheme S4).



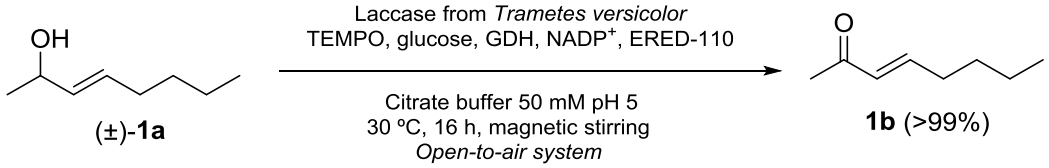
Scheme S4. ERED-catalyzed bioreduction of unsaturated ketones **1c** at short reaction times (6 h).

In a test tube, D-(+)-glucose (29 mg, 0.16 mmol), GDH-105 (4 mg), and $NADP^+$ (0.6 mM) were added to a solution of the α,β-unsaturated ketone **1b** (10 mg, 0.08 mmol, 40 mM) in

citrate buffer 50 mM pH 5 (2 mL). The reaction mixture was stirred for a few minutes to dissolve all the reagents and then ERED-110 (20 mg) was added. The test tube was closed and the reaction was stirred for 6 h under magnetic stirring at 30 ºC. After this time, the mixture was extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4, and an aliquot was taken for the determination of the conversion value by GC analysis.

VII.3. One-pot two-step cascade approach

Initially, we attempted to carry out this process as a bienzymatic cascade. For this reason, all enzymes and reagents required for both steps were mixed in the presence of the racemic allylic alcohol **1a** (Scheme S5).



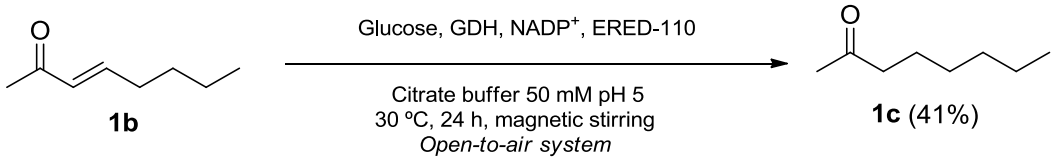
Scheme S5. Attempt of chemoenzymatic isomerization of **1a** through a one-pot two-step cascade.

In an open-to-air test tube, TEMPO (4.1 mg, 33 mol%), D-(+)-glucose (29 mg, 0.16 mmol), GDH-105 (4 mg), and $NADP^+$ (0.6 mM) were added to a solution of the racemic secondary alcohol **1a** (10 mg, 0.08 mmol, 40 mM) in citrate buffer 50 mM pH 5 (2 mL). The reaction mixture was stirred for a few minutes to dissolve all the reagents and then the laccase from *Trametes versicolor* (5 U) and ERED-110 (20 mg) were added. The reaction was stirred for 16 h under magnetic stirring at 30 ºC. After this time, the mixture was extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4, and an aliquot was taken for the determination of the conversion value by GC analysis.

Unfortunately, only the unsaturated ketone **1b** was obtained after 16 h. Regarding the results obtained, we speculate that the reaction catalyzed by the ene-reductase did not occur due to the inactivation of the NADPH recycling system. As the laccase-TEMPO system might catalyze the oxidation of the primary hydroxyl group present in the glucose,[23] GDH-105 was then unable to regenerate NADPH.

Moreover, molecular oxygen is necessary to complete the laccase catalytic cycle,[24] (therefore, it was necessary to work on oxygen-saturated or open-to-air media), whereas many ene-

reductases are unstable to the presence of oxygen (what often requires developing these bioreductions in deoxygenated media).[25] Thus, when the reduction of the unsaturated ketone **1b** took place in an open-to-air system (Scheme S6), the saturated ketone **1c** was only obtained in 41% conversion.



Scheme S6. ERED-catalyzed bioreduction of unsaturated ketone **1c** in the presence of air.

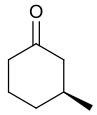
In an open-to-air test tube, D-(+)-glucose (29 mg, 0.16 mmol), GDH-105 (4 mg), and $NADP^+$ (0.6 mM) were added to a solution of the α,β-unsaturated ketone **1b** (10 mg, 0.08 mmol, 40 mM) in citrate buffer 50 mM pH 5 (2 mL). The reaction mixture was stirred for a few minutes to dissolve all the reagents and then ERED-110 (20 mg) was added. The reaction was stirred for 24 h under magnetic stirring at 30 ºC. After this time, the mixture was extracted with EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4, and an aliquot was taken for the determination of the conversion value by GC analysis.

VIII. Scaling-up reactions

In an open-to-air test tube (30 x 140 x 5 mm), TEMPO (33 mol%) was added to a solution of the racemic allylic alcohols **4a**, **6a** or **12a** (50 mg; 40 mM in the buffer medium for **4a** and **6a**; 30 mM in the reaction medium for **12a**) in citrate buffer 50 mM pH 5 (for substrates **4a** and **6a**, total volume: 11,1 and 9.3, respectively), or in a biphasic mixture composed by citrate buffer 50 mM pH 5 (10.25 mL) and MTBE (20% v/v, 2.05 mL) for **12a**. The reaction mixture was stirred for a few minutes to dissolve all the reagents, and then the laccase from *Trametes versicolor* (24 U for **4a**; 23 U for **6a**; 19 U for **12a**) was added. The reaction was maintained under magnetic stirring for 16 h at 30 ºC. Then, D-(+)-glucose (2 equiv.), GDH-105 (50.4 mg per mmol substrate), $NADP^+$ (0.6 mM), and ERED-P1-E01 (50 mg) were added. The test tube was closed and the reaction was maintained under magnetic stirring at 30 ºC for 24 h in order to reach total conversions. During this time, samples (200 µL) were taken from the reaction mixture and extracted with EtOAc (2 x 200 µL) for determination of the conversion (and enantiomeric excess) values by GC. After this time, the solution was centrifuged at 4,900 rpm for 7 min. The supernatant was decanted and extracted with EtOAc

(15 x 2 mL) for **6c** and **12c**, and Et_2O (15 x 2 mL) for **4c**. The organic layers were combined, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure providing (*S*)-**4c** (yield 69%, >99% *ee*), **6c** (yield 85%) and (*S*)-**12c** (yield 87%, 91% *ee*).

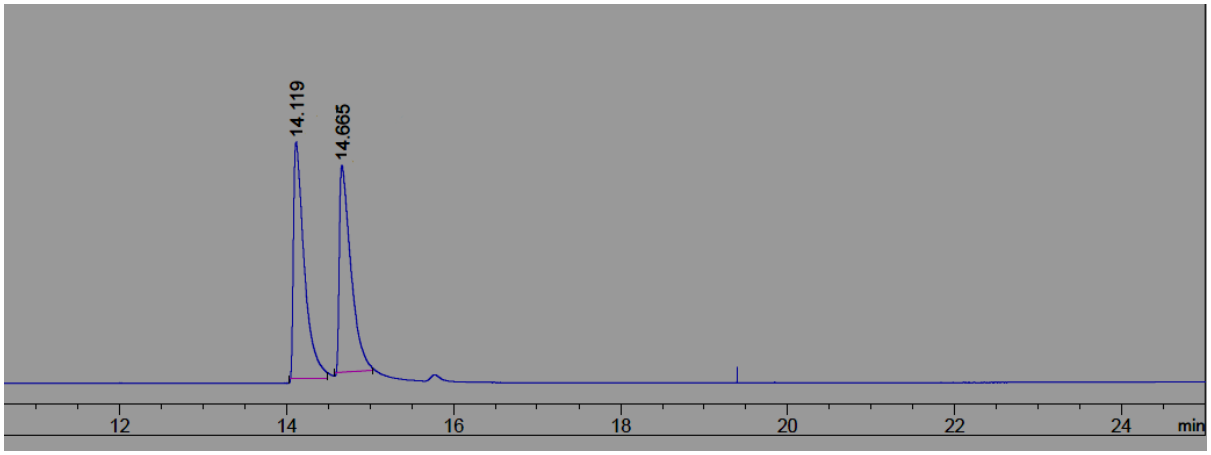
Characterization data are given below:



(*S*)-4c

(*S*)-3-Methylcyclohexanone [(*S*)-4c]. The title compound was obtained through the bienzymatic strategy as colorless oil (34.5 mg, 0.31 mmol, yield: 69%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 2.39-2.16 (*m*, 3H), 2.06-1.81 (*m*, 4H), 1.72-1.57 (*m*, 1H), 1.38-1.18 (*m*, 1H), 1.00 (*d*, 3H, *J*= 6.2 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 212.0, 50.0, 41.1, 34.2, 33.3, 25.3, 22.1. Spectral data and physical properties are in agreement with the ones obtained from a sample obtained from commercial sources.

(±)-**4c**



(*S*)-**4c**

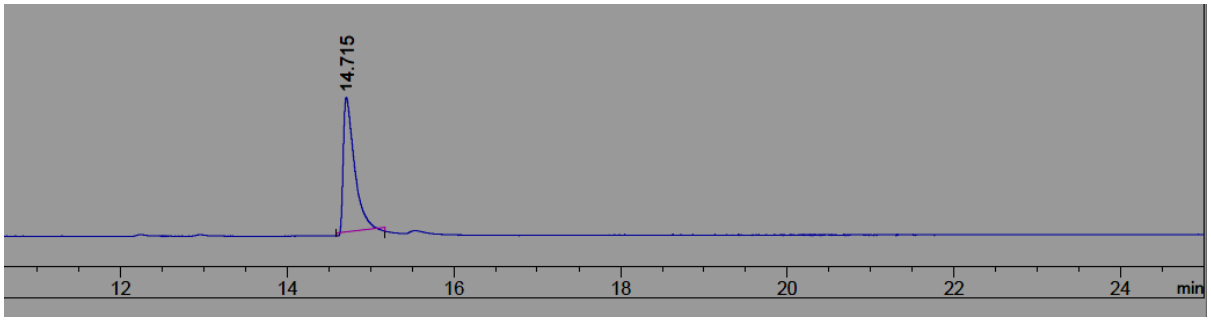
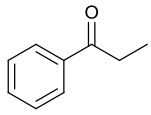
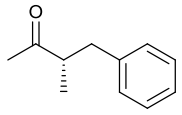


Figure S6. Analytical separation by chiral GC for racemic (above) and enantioenriched (below) **4c** obtained at 50-mg scale through the two-step sequential process starting from the racemic allylic alcohol **4a** using the laccase–TEMPO system and ERED-P1-E01.



6c

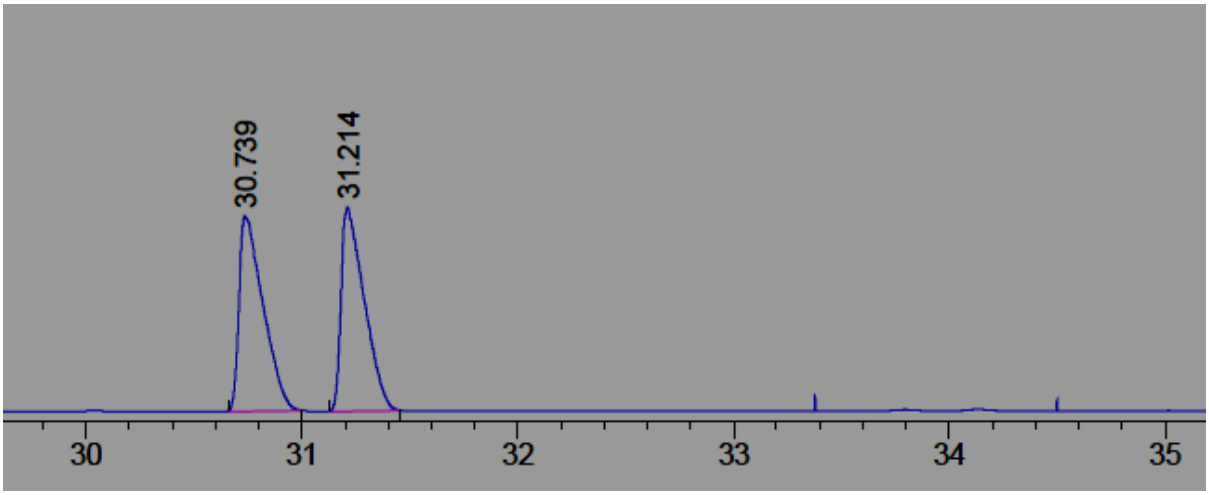
Propiophenone (6c). The title compound was obtained through the bienzymatic strategy as colorless oil (42.5 mg, 0.32 mmol, yield: 85%). **^{1}H NMR (300 MHz, $CDCl_3$):** δ (ppm) 7.99 (*d*, 2H, J= 8.1 Hz), 7.60-7.45 (*m*, 3H), 3.03 (*q*, 2H, *J*= 7.5 Hz), 1.25 (*t*, 3H, *J*= 7.2 Hz); **^{13}C NMR (75 MHz, $CDCl_3$):** δ (ppm) 201.0, 137.0, 133.0, 128.7 (2C), 128.1 (2C), 31.9, 8.3. Spectral data and physical properties are in agreement with the commercial sources.



12c

(*S*)-3-Methyl-4-phenylbutan-2-one [(*S*)-12c]. The title compound was obtained through the bienzymatic strategy as yellowish oil (44 mg, 0.27 mmol, yield: 87%). Spectral data and physical properties were in agreement with literature and with those previously reported in this Supporting Information (see Section VI.14).

(±)-**12c**



(*S*)-**12c**

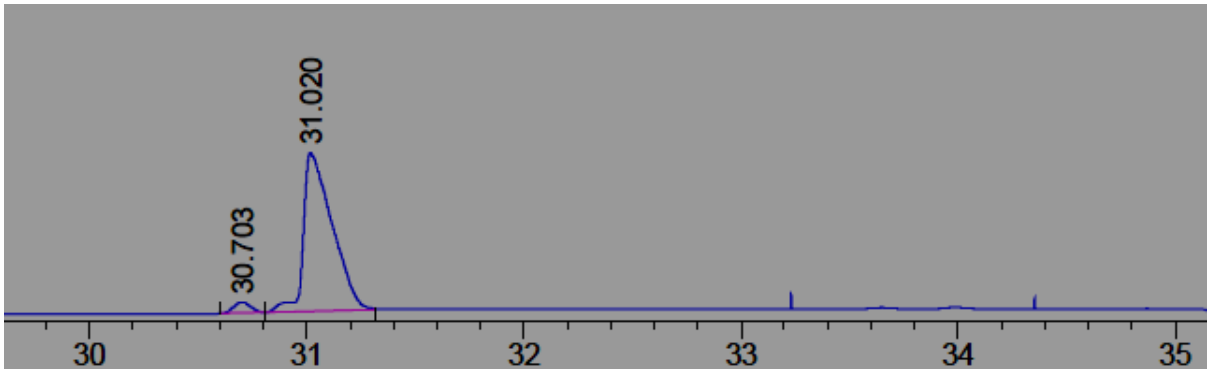


Figure S7. Analytical separation by chiral GC for racemic (above) and enantioenriched (below) **12c** obtained at 50-mg scale through the one-pot two-step sequential process starting from the racemic allylic alcohol **12a** using the laccase–TEMPO system and ERED-P1-E01.

IX. Analytical data

IX.1. GC analyses for the determination of ratio of products (and ee/de for chiral saturated ketones)

The following GC columns were used:

A: Hewlett Packard HP-1 (30 m x 0.32 cm x 0.25 µm, 12.2 psi N_2).

B: CP-ChiraSil-DEX CB (25 m x 0.32 cm x 0.25 µm, 12.2 psi N_2).

C: RT-BetaDEXe (30 m x 0.25 cm x 0.25 µm, 12.2 psi N_2).

D: DB-1701 (30 m x 0.25 cm x 0.25 µm, 12.2 psi N_2).

Table S15. Retention times for compounds **1-10**, **12** and **13** obtained by GC analysis.

Compound	Column	Program[a]	Retention time (min)			
			Alcohol	Unsaturated ketone	Saturated Ketone	
					(*R*)[b]	(*S*)[b]
1	**A**	40/0/2.5/80/0/20/200/0	5.5	6.6	5.1	
2	**A**	40/10/7/0/20/200/0	3.2	3.9	3.0	
3	**B**	50/3/2.5/120/0/20/180/4	26.8	20.5	19.5	19.2
4	**C**	70/0/2/140/0/20/180/5	18.6; 20.5[c]	23.3	14.4	15.0
5	**B**	50/3/2.5/120/0/20/180/4	28.2; 28.4[c]	23.8	20.9	
6	**B**	50/3/2.5/120/0/20/180/4	32.0; 32.3	24.1	25.2	
7	**A**	70/3/2.5/180/2	10.7	11.5	7.6	
8	**B**	110/0/2.5/120/0/10/160/0/20/180/7	12.8	11.8	10.0	
9	**B**	110/0/2.5/120/0/10/160/0/20/180/7	11.6	10.8	9.4	
10	**B**	110/0/2.5/120/0/10/160/0/20/180/7	13.4	12.5	10.4	
12	**B**	50/3/2.5/120/0/10/160/0/20/180/4	35.6	34.7	30.5	31.0
13	**D**	80/6.5/10/160/5/20/200/2/20/280/1	13.6; 13.9[d]	14.1	13.3[e]	13.4[f]

[a] GC programme: initial temp. (ºC) / time (min) / ramp (ºC/min) / temp. (ºC) / time (min) / ramp (ºC/min) / final temp. (ºC) / time (min).
[b] Only for chiral saturated ketones.
[c] The enantiomers of these racemic allylic alcohols appeared separated in these programs.
[d] The two isomers of (5*R*)-carveol appeared separated in this program: **first isomer** (1*S*,5*R*) and **second isomer** (1*R*,5*R*).
[e] *R* corresponds to the (2*R*,5*R*)-**13c** isomer. [f] *S* corresponds to the (2*S*,5*R*)-**13c** isomer.

*IX.2. HPLC analyses for the determination of ratio of products for compound **11***

The ratio of products obtained through the bienzymatic process for the saturated ketone 4-(4-nitrophenyl)butan-2-one **11c** was determined by HPLC analysis using calibration curves.

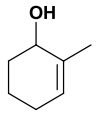
The following HPLC conditions were used:

Column Daicel Chiralpak OD (25 cm x 4.6 mm, 5 µm particle size); eluent: *n*-hexane / 2-PrOH (90:10), 30 ºC, flow 0.8 mL/min.

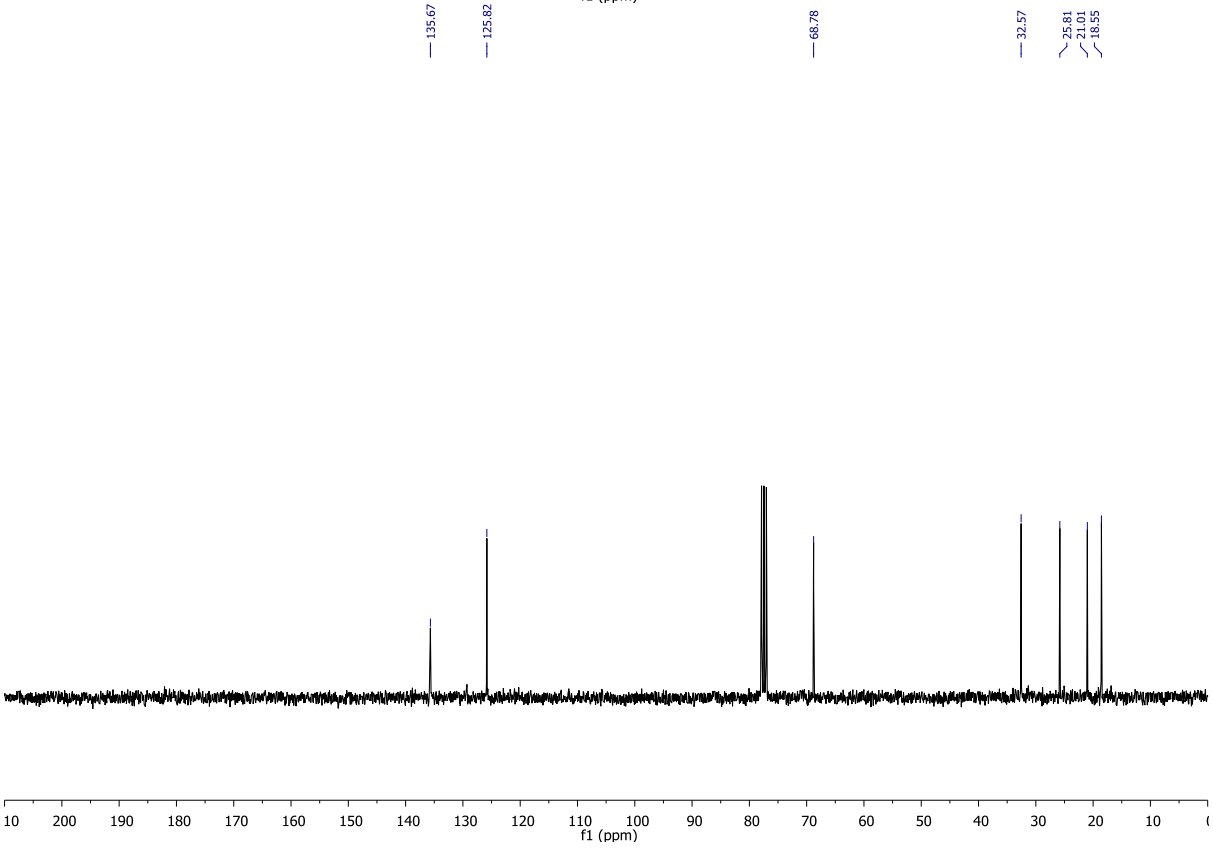
Table S16. Retention times for the compounds **11a**, **11b** and **11c** obtained by chiral HPLC under the previously described conditions.

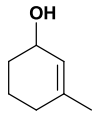
Retention time (min)		
11a	**11b**	**11c**
12.4	17.4	11.2

X. NMR spectra



(±)-**3a**

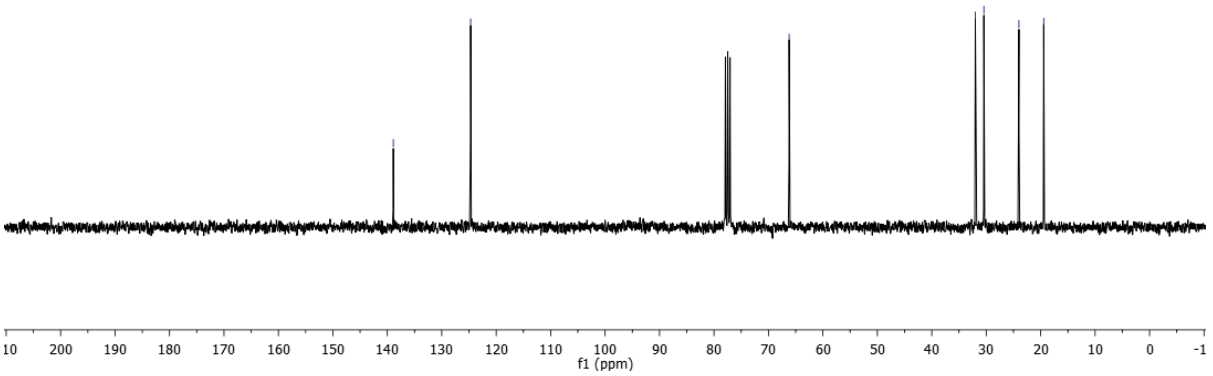


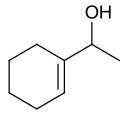

OH

(±)-**4a**

5.4881
4.1644
1.9171
1.8889
1.8734
1.8415
1.8365
1.8290
1.7986
1.7847
1.7686
1.7521
1.7346
1.6830
1.5989
1.5822
1.5651
1.5530
1.5419
1.00
1.00
11.36
f1 (ppm)

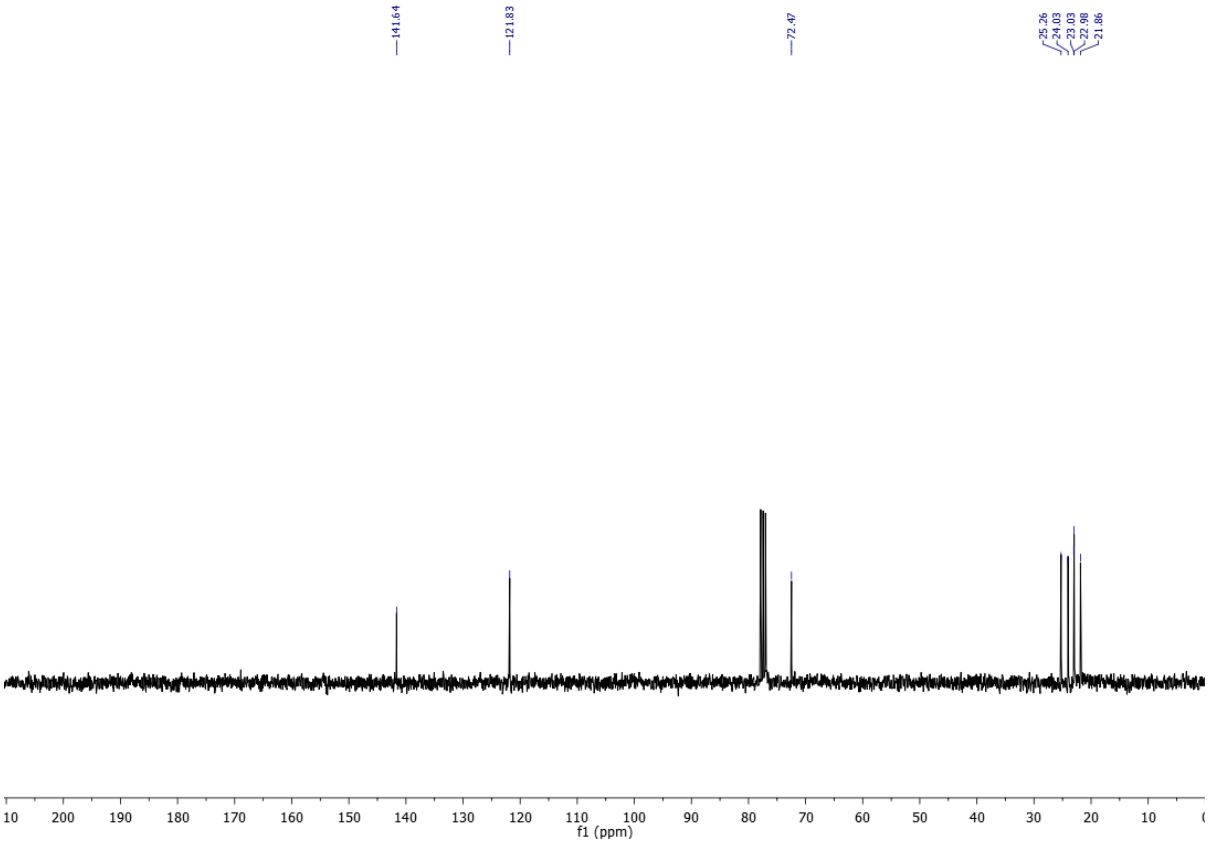
138.89
124.69
66.20
32.00
30.43
23.99
19.44

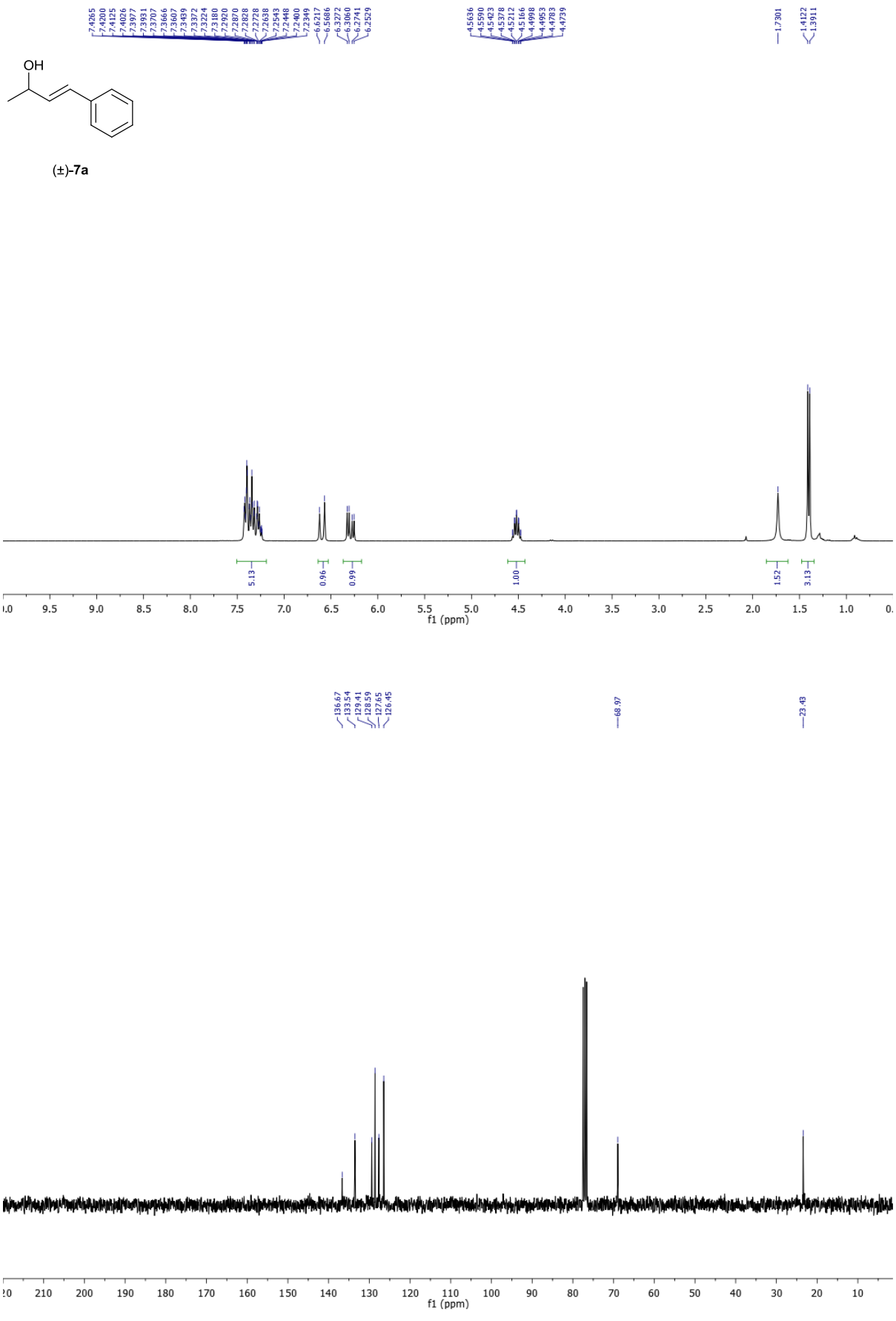

f1 (ppm)


OH

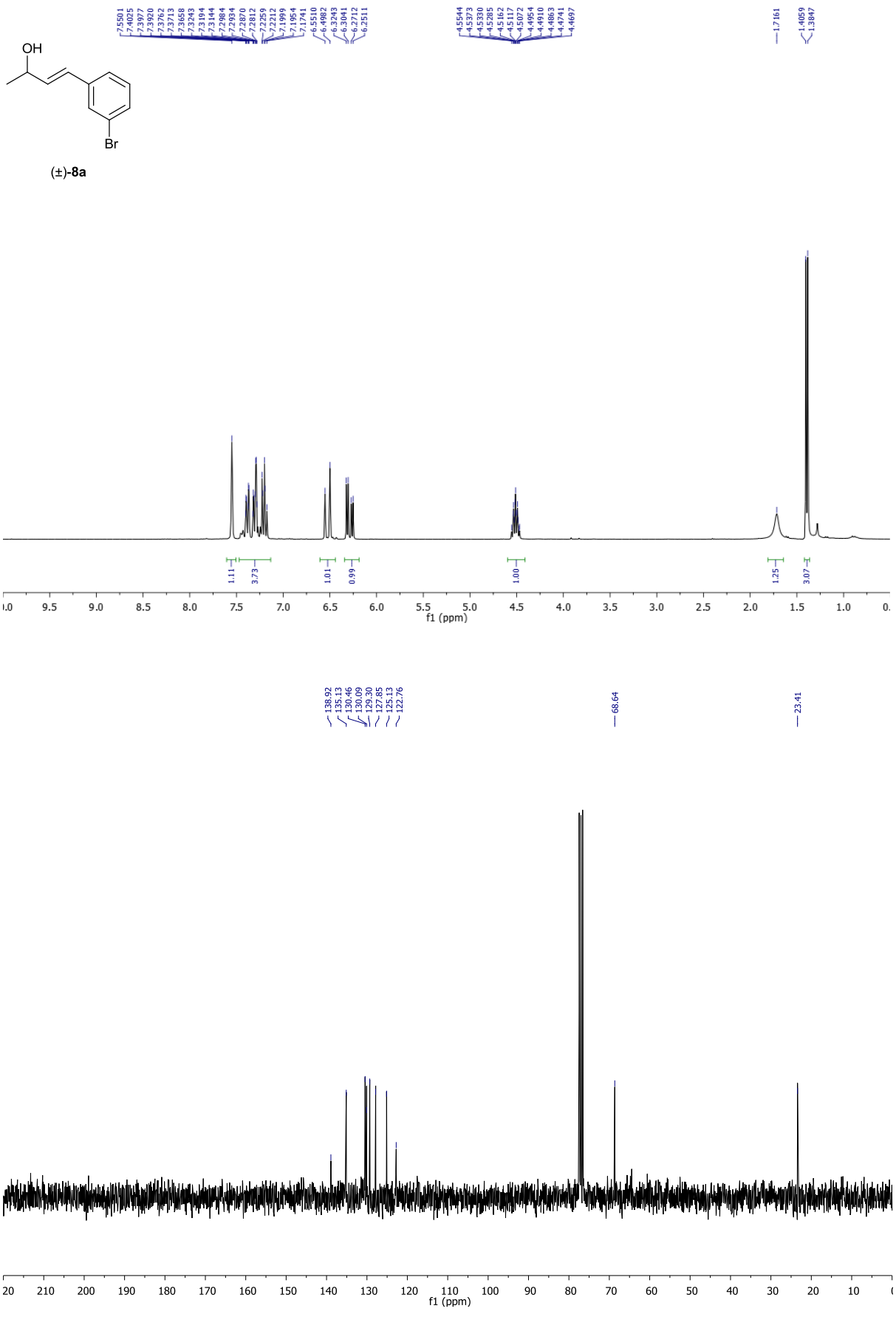
(±)-**5a**

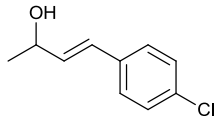
5.6838
4.2160
4.1947
4.1730
4.1515
2.0497
2.0437
2.0354
2.0292
2.0216
1.6739
1.6630
1.6550
1.6504
1.6388
1.6335
1.6268
1.6179
1.6141
1.4966
1.2862
0.99
1.00
3.54
5.72
3.16
f1 (ppm)


141.64
121.83
72.47
25.26
24.03
23.03
22.98
21.86
f1 (ppm)

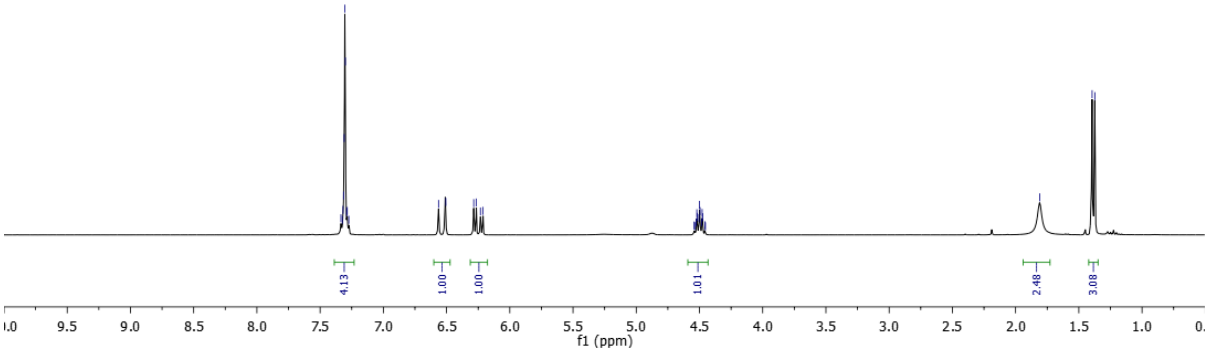

7.4265
7.4200
7.4125
7.4026
7.3977
7.3931
7.3707
7.3666
7.3607
7.3459
7.3372
7.3224
7.3180
7.2920
7.2870
7.2828
7.2728
7.2638
7.2543
7.2446
7.2400
7.2349
6.6417
6.6486
6.3272
6.3060
6.2741
6.2529
4.5636
4.5590
4.5543
4.5378
4.5212
4.5166
4.4998
4.4953
4.4783
4.4739
1.7301
1.4122
1.3911
OH
5.13
0.96
0.99
1.00
1.52
3.13
f1 (ppm)
136.67
133.54
129.41
128.59
127.65
126.45
68.97
23.43
f1 (ppm)

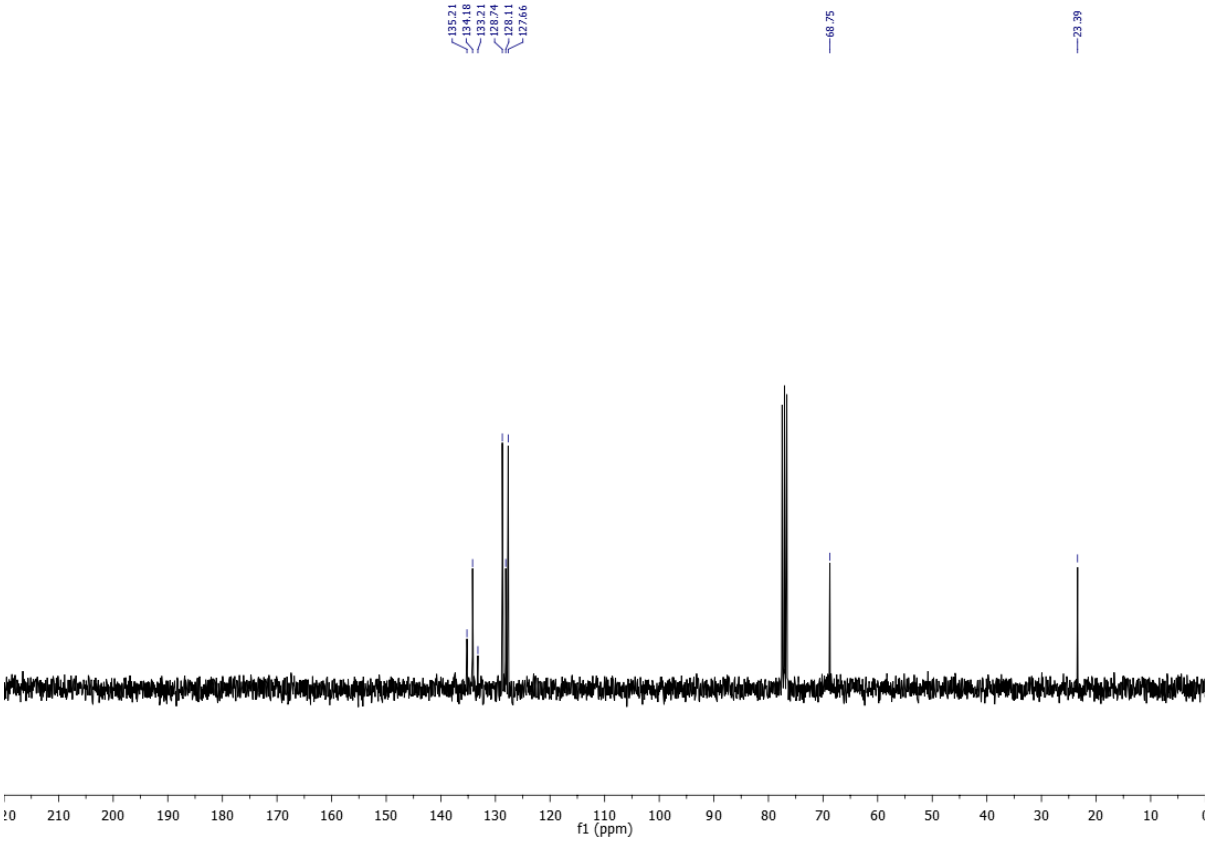
(±)-**7a**

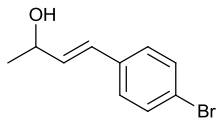

7.5501
7.4025
7.3977
7.3970
7.3762
7.3713
7.3658
7.3249
7.3194
7.3144
7.2984
7.2934
7.2870
7.2812
7.2259
7.2212
7.1999
7.1954
7.1741
6.5510
6.4982
6.3243
6.3041
6.2712
6.2511
4.5544
4.5373
4.5330
4.5285
4.5162
4.5117
4.5072
4.4954
4.4910
4.4863
4.4741
4.4697
1.7161
1.4059
1.3847
OH
Br
(±)-8a
1.11
3.73
1.01
0.99
1.00
1.25
3.07
f1 (ppm)
138.92
135.13
130.46
130.09
129.30
127.85
125.13
122.76
68.64
23.41
f1 (ppm)


OH
Cl

(±)-**9a**

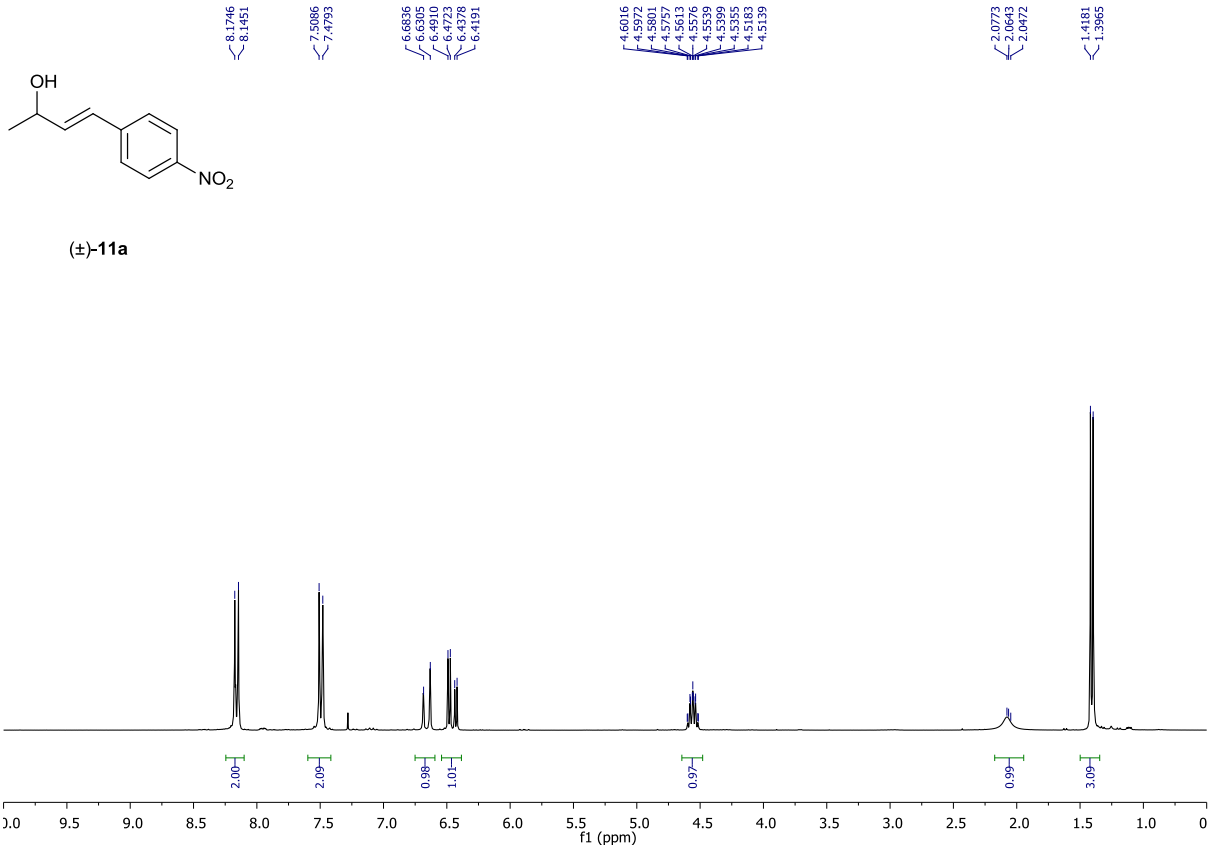

4.13
1.00
1.00
1.01
2.48
3.08
f1 (ppm)


135.21
134.18
133.21
128.74
128.11
127.66
68.75
23.39
f1 (ppm)


OH
Br

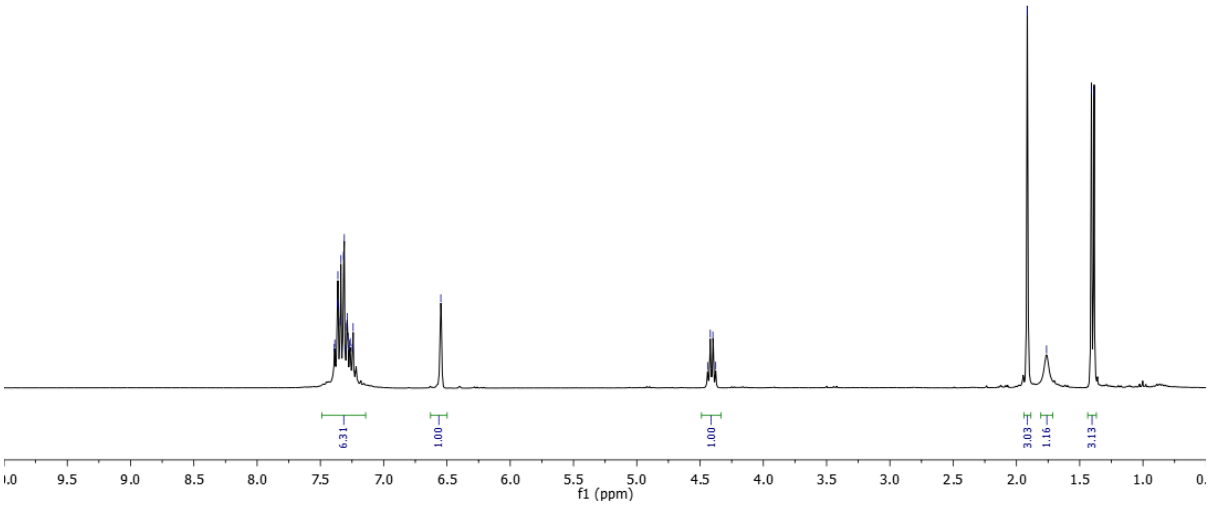
(±)-**10a**

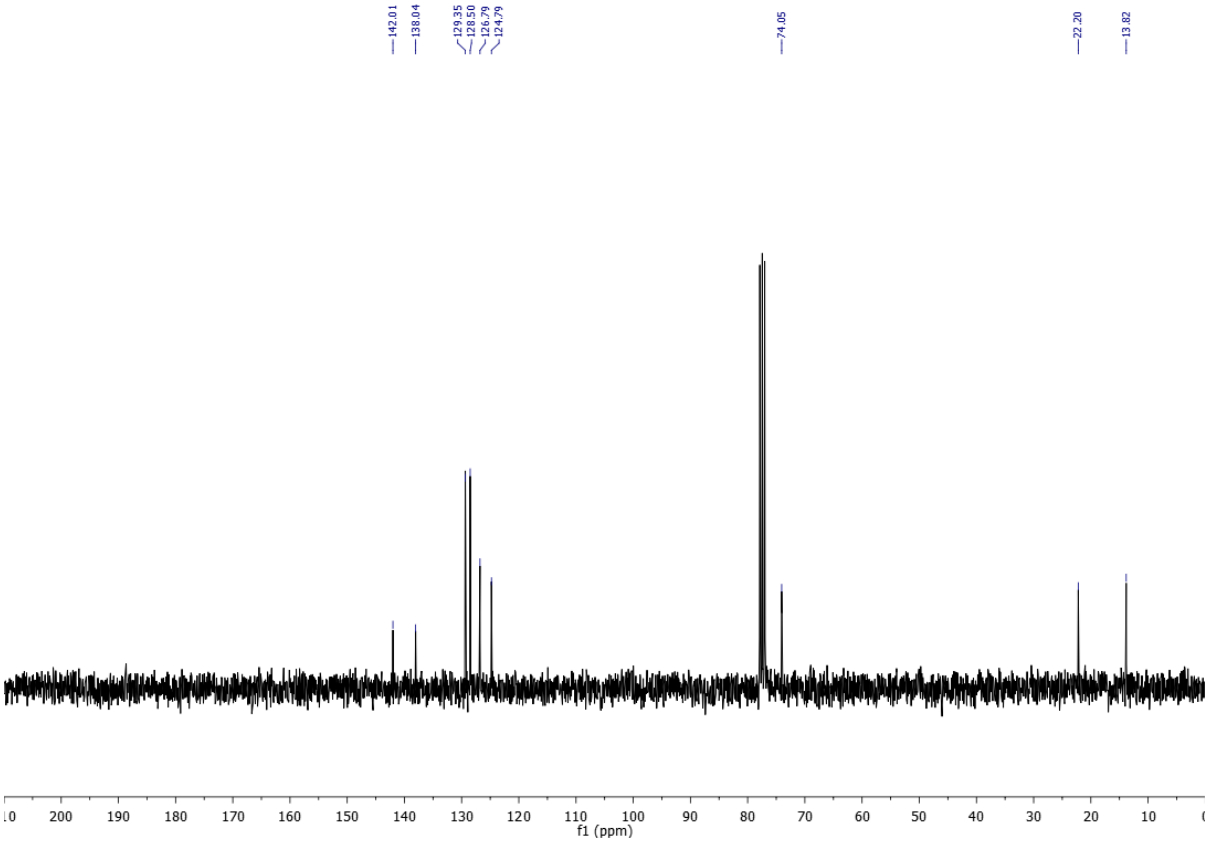
7.4667
7.4385
7.2687
7.2406
6.5512
6.4981
6.3115
6.3061
6.2994
6.2852
6.2585
6.2531
6.2374
6.2325
4.5458
4.5417
4.5300
4.5245
4.5203
4.5094
4.5032
4.4990
4.4880
4.4824
4.4782
4.4610
4.4569
1.7791
1.4005
1.3792
2.00
1.99
1.00
1.01
1.00
1.57
3.07
f1 (ppm)
135.65
134.31
131.68
128.16
127.99
121.37
68.77
23.39
f1 (ppm)

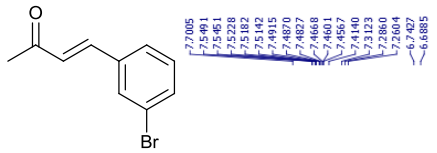

8.1746
8.1451
7.5086
7.4793
6.6836
6.6305
6.4910
6.4723
6.4378
6.4191
4.6016
4.5972
4.5901
4.5757
4.5613
4.5576
4.5539
4.5399
4.5355
4.5183
4.5139
2.0773
2.0643
2.0472
1.4181
1.3965
OH
NO2
(±)-11a
2.00
2.09
0.98
1.01
0.97
0.99
3.09
f1 (ppm)
146.83
143.37
138.47
126.94
123.97
68.35
23.35
f1 (ppm)


OH
7.3895
7.3861
7.3660
7.3616
7.3580
7.3493
7.3375
7.3165
7.3108
7.3015
7.2921
7.2871
7.2809
7.2693
7.2637
7.2473
7.2411
6.5483
4.4402
4.4191
4.3987
4.3763
1.9147
1.7621
1.4078
1.3865

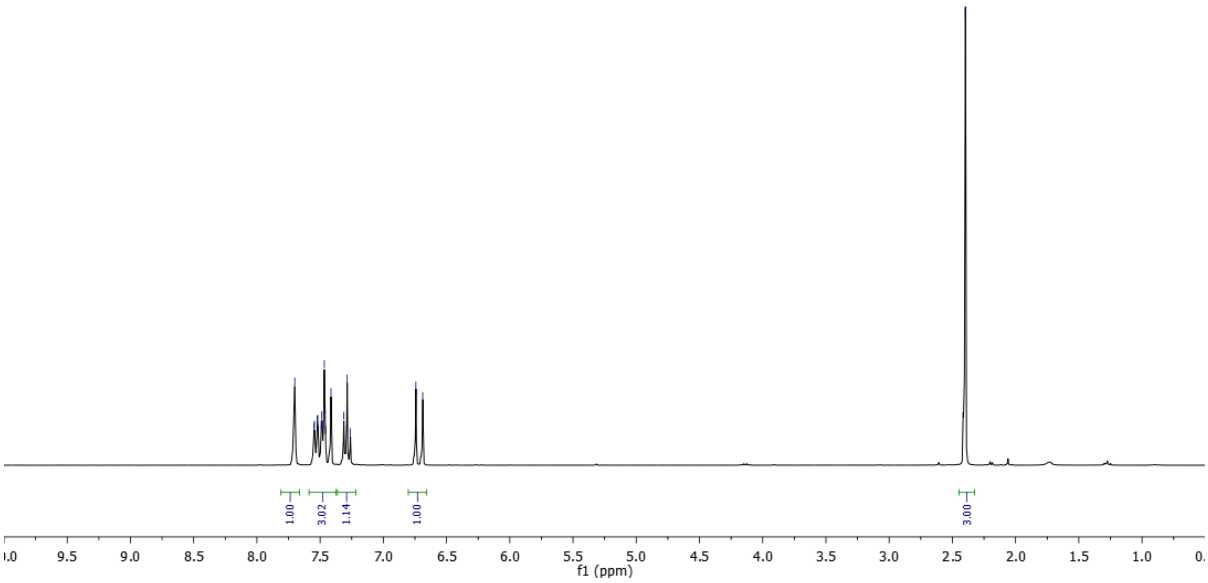
(±)-**12a**

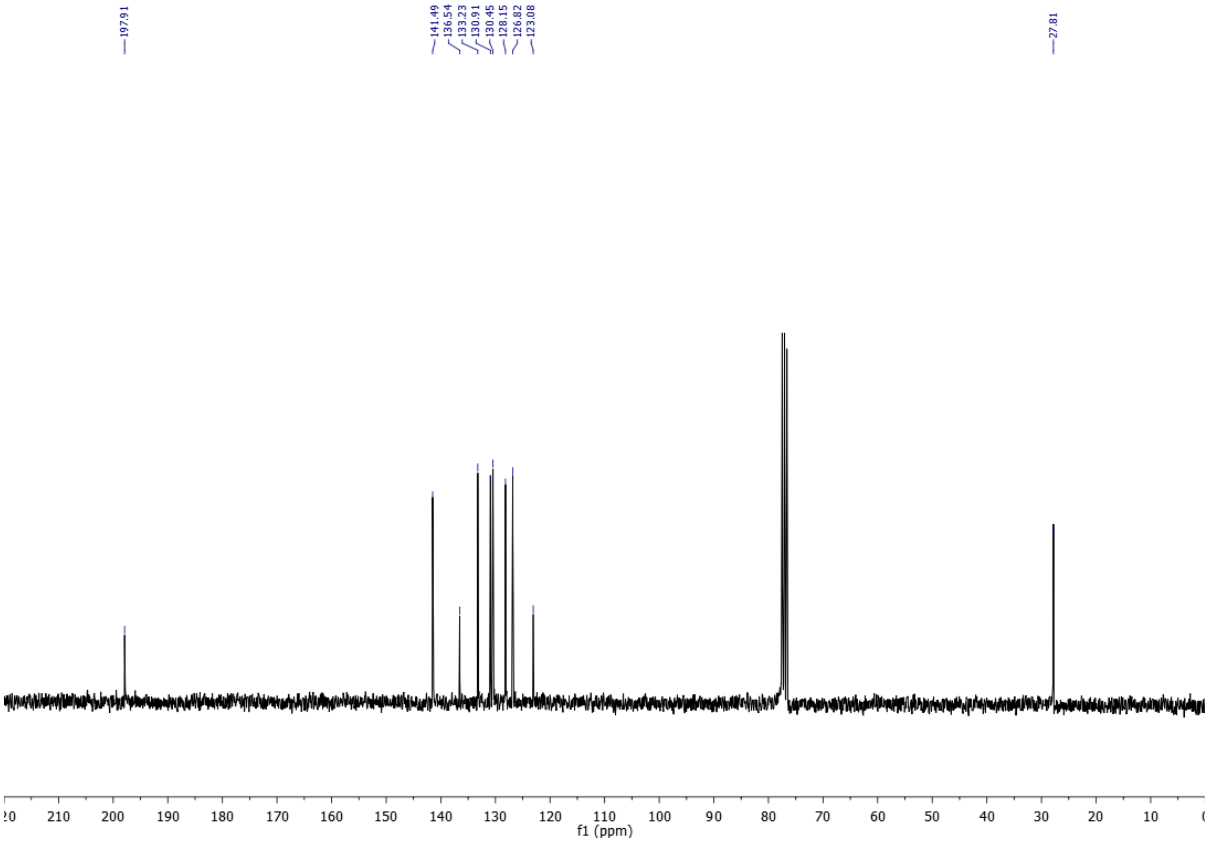

6.31
1.00
1.00
3.03
1.16
3.13
f1 (ppm)

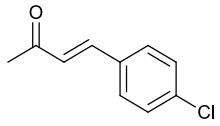

142.01
138.04
129.35
128.50
126.79
124.79
74.05
22.20
13.82
f1 (ppm)


O
Br
7.7005
7.5491
7.5461
7.5228
7.5182
7.5142
7.4915
7.4870
7.4627
7.4468
7.4461
7.4567
7.4140
7.3123
7.2860
7.2604
6.7427
6.6885
2.3972

8b

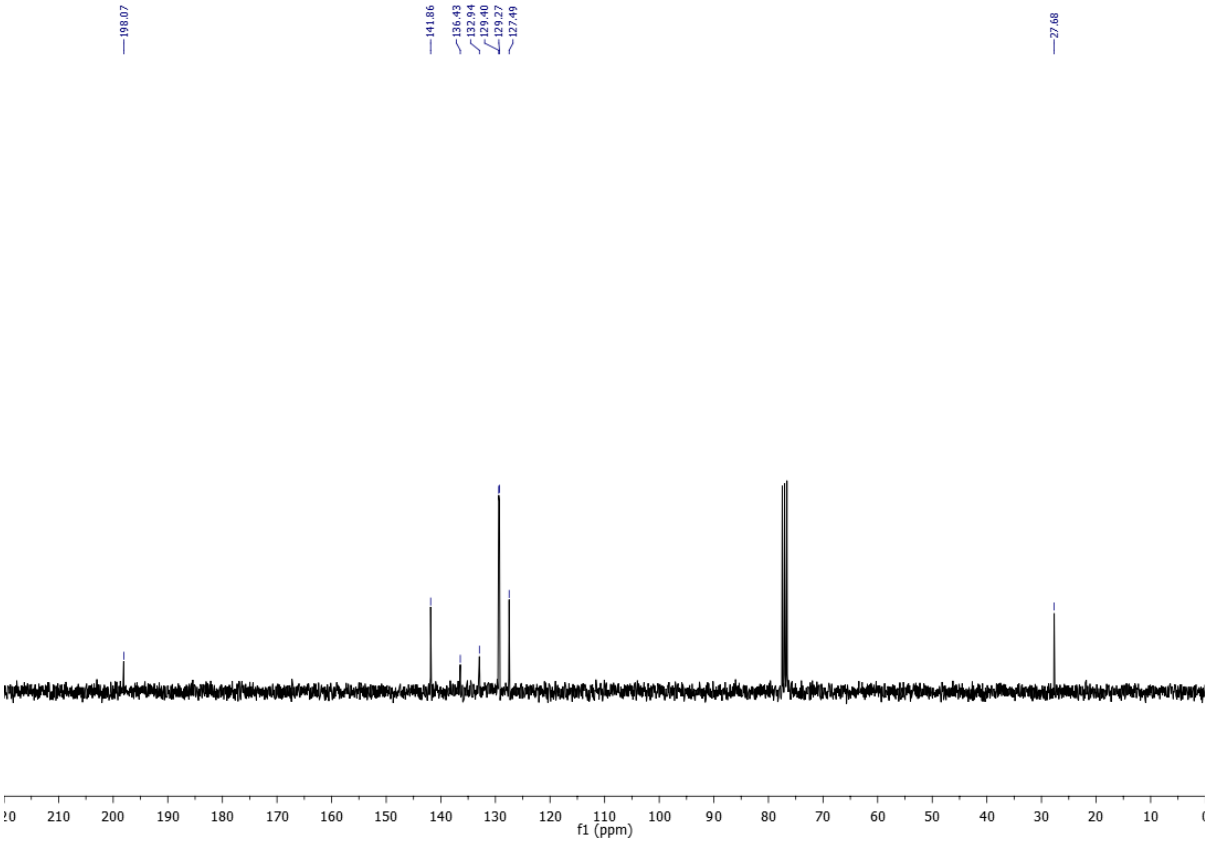

1.00
3.02
1.14
1.00
3.00
f1 (ppm)

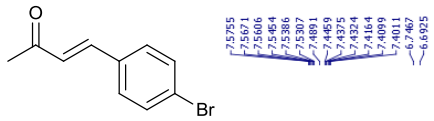

197.91
141.49
136.54
133.23
130.91
130.45
128.15
126.82
123.08
27.81
f1 (ppm)


O
Cl

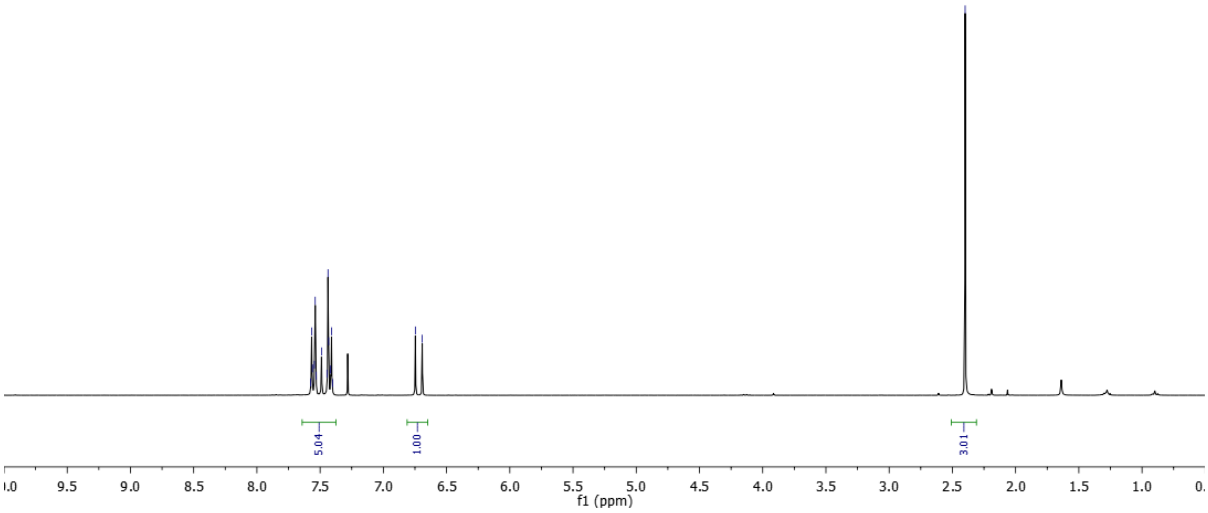
9b

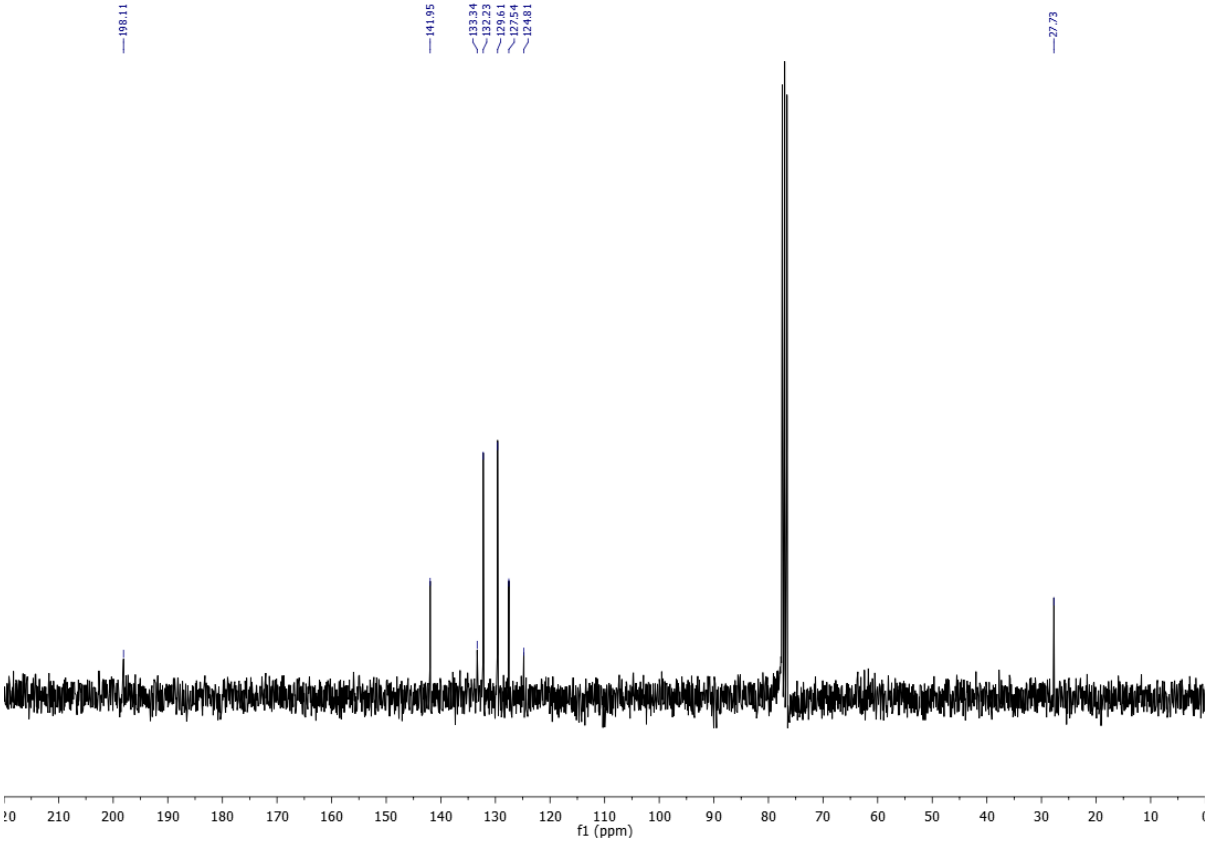
7.5043
7.4769
7.4485
7.3996
7.3720
6.7277
6.6735
2.3939
3.06
1.95
1.02
3.00
f1 (ppm)

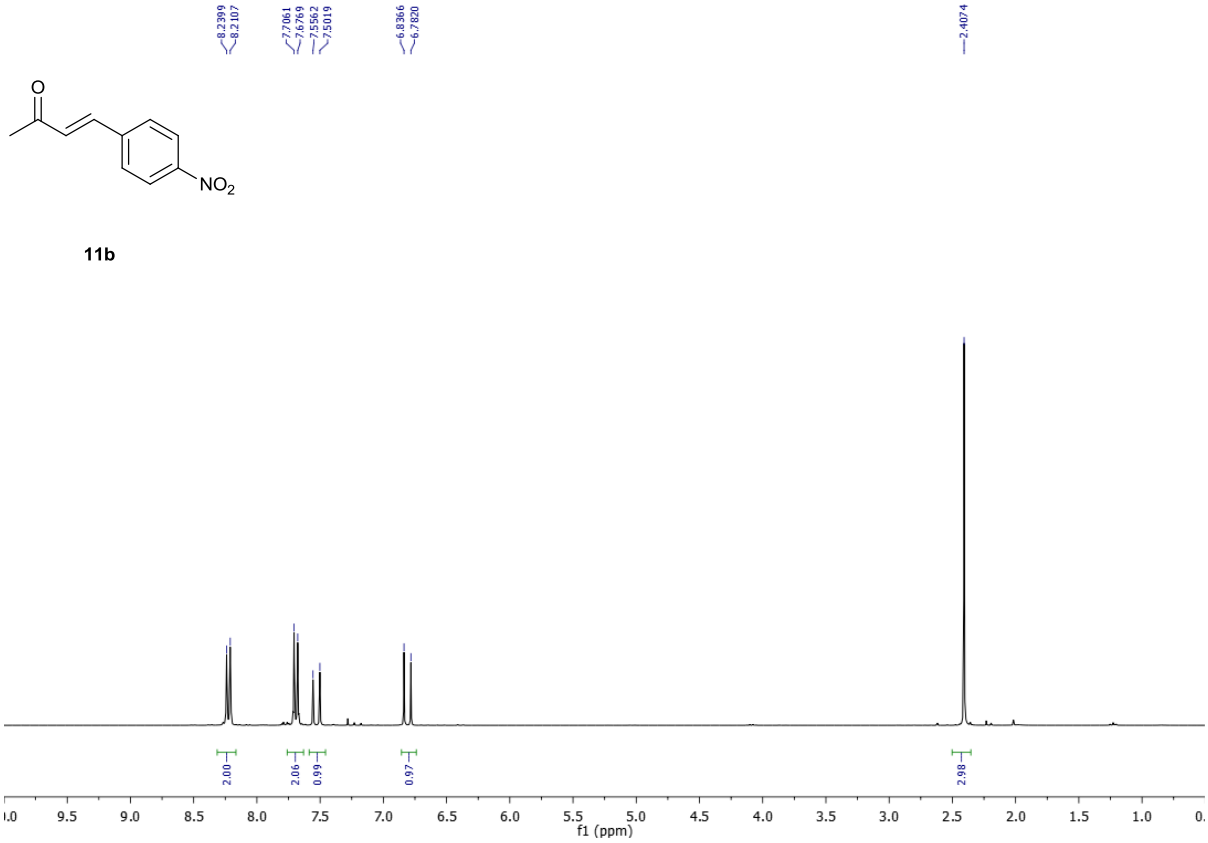

198.07
141.86
136.43
132.94
129.40
129.27
127.49
27.68
f1 (ppm)


O
Br
7.5755
7.5671
7.5606
7.5464
7.5386
7.5307
7.5091
7.4469
7.4375
7.4324
7.4164
7.4099
7.4011
6.7467
6.6925
2.3990

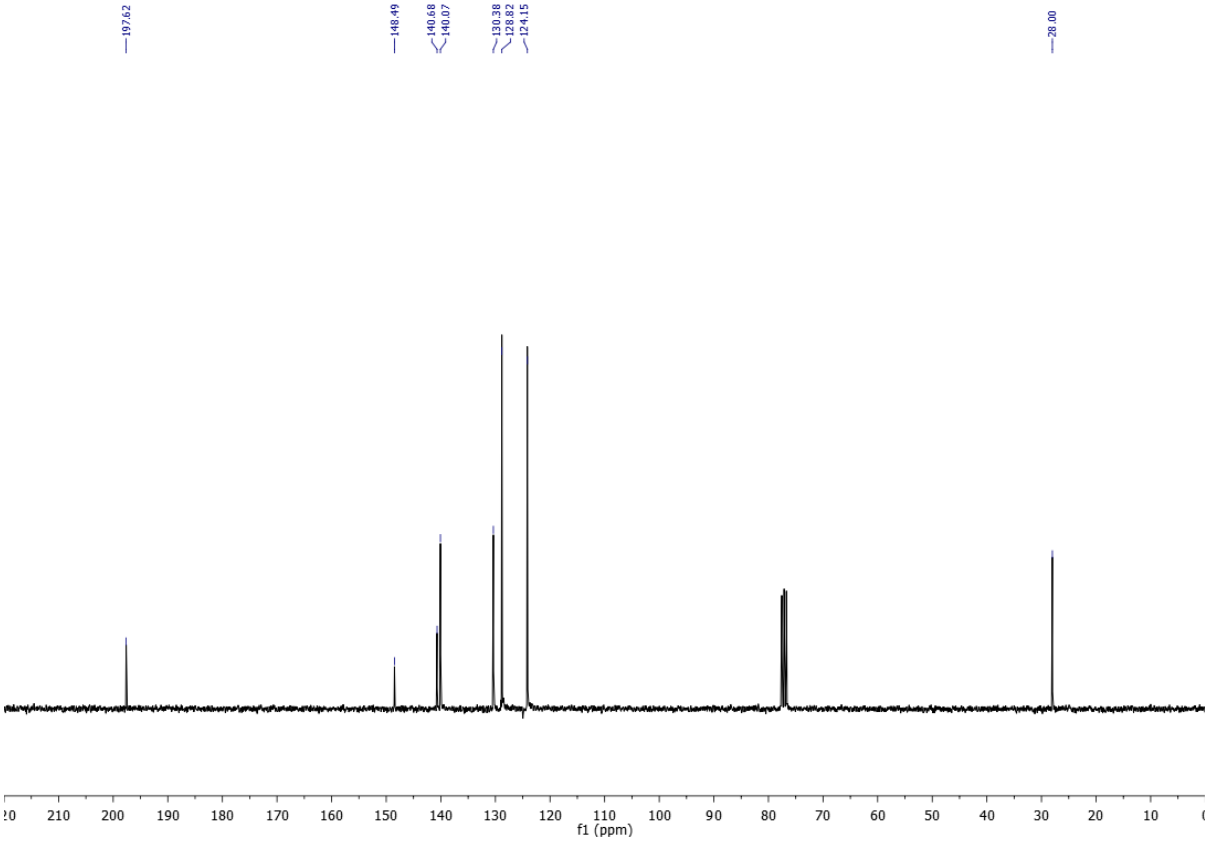
10b

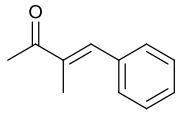

5.04
1.00
3.01
f1 (ppm)


198.11
141.95
133.34
132.23
129.61
127.54
124.81
27.73
f1 (ppm)


8.2399
8.2107
7.7061
7.6769
7.5562
7.5019
6.8366
6.7820
2.4074
O
NO2
2.00
2.06
0.99
0.97
2.98
f1 (ppm)

11b

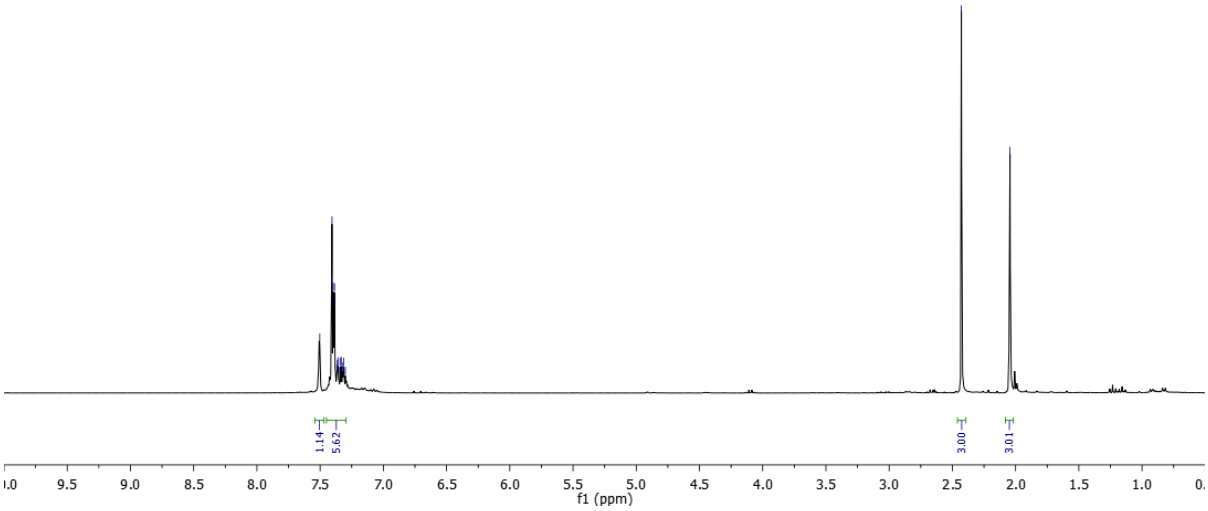

197.62
148.49
140.68
140.07
130.38
128.82
124.15
28.00
f1 (ppm)



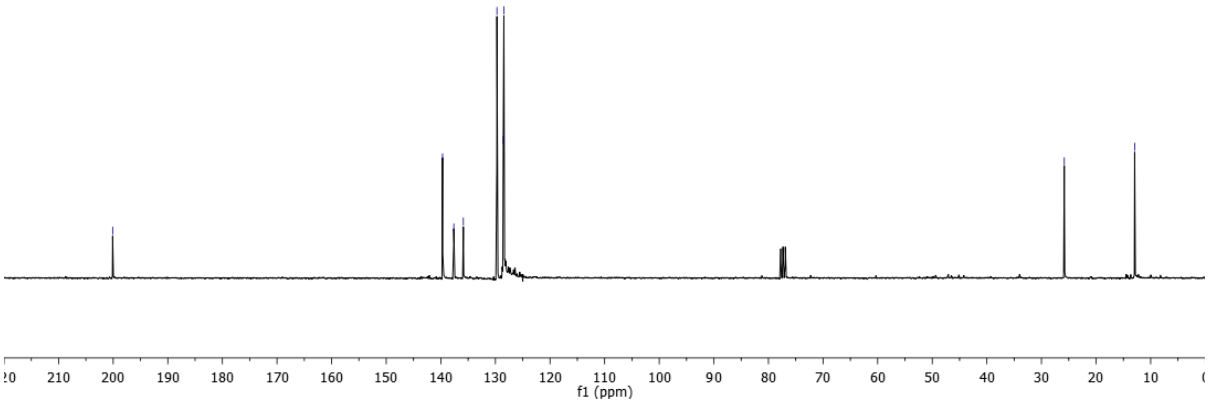
12b

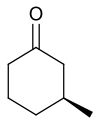

7.5025
7.4086
7.4059
7.3971
7.3890
7.3861
7.3681
7.3629
7.3593
7.3556
7.3419
7.3330
7.3248
7.3185
7.3129
7.3008


2.4286
2.0441


1.14
5.62
3.00
3.01
f1 (ppm)

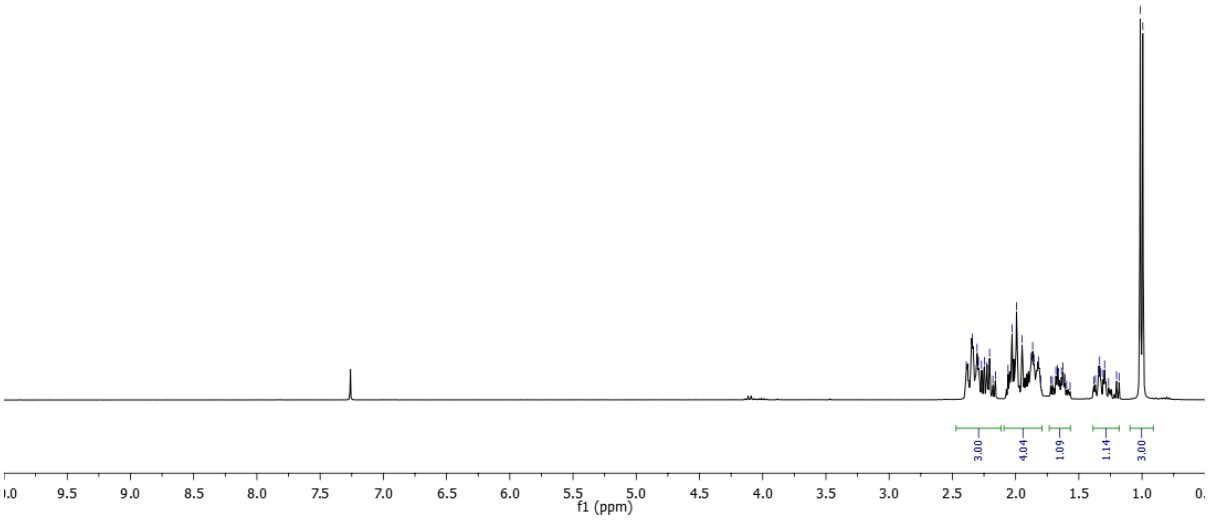

200.10
139.68
137.62
135.87
129.73
128.57
128.45
25.82
12.93

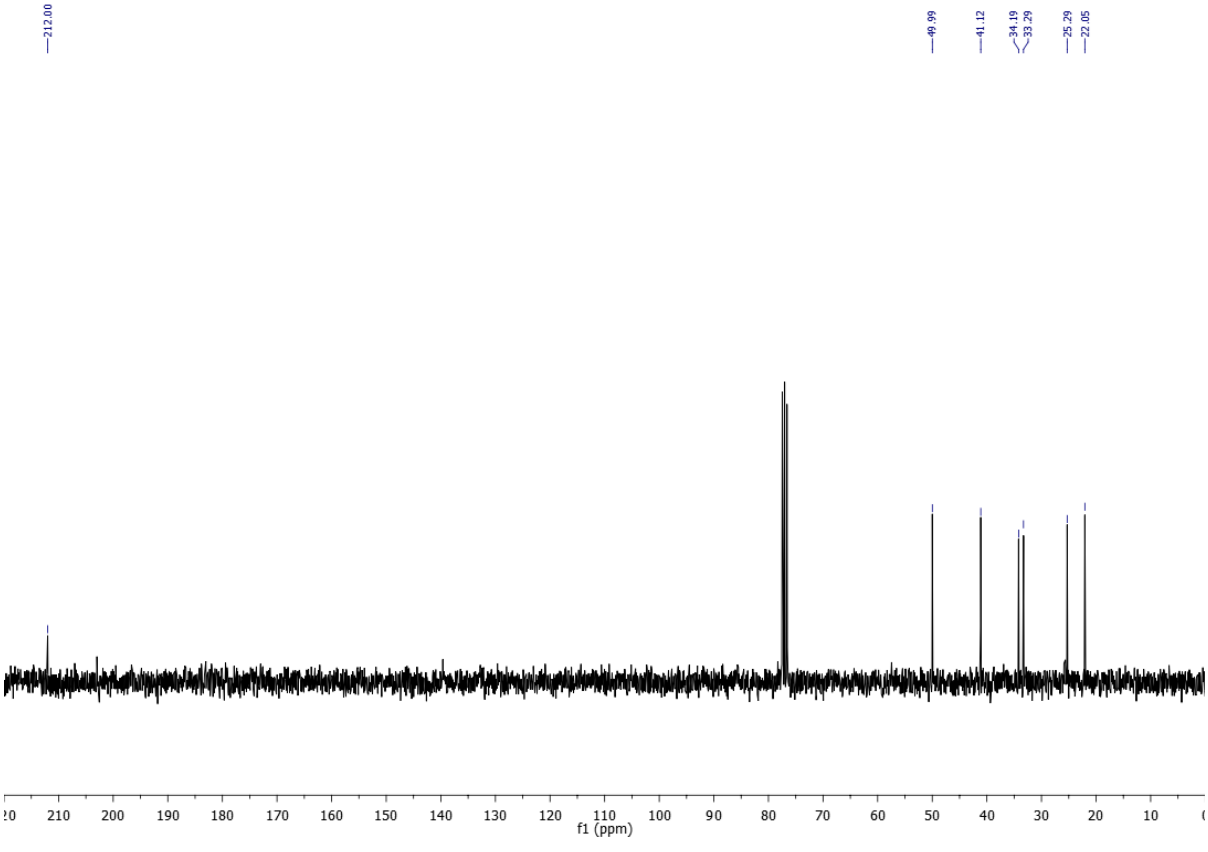

f1 (ppm)

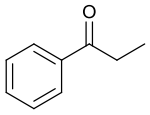


***(S)*-4c**


2.3903
2.3409
2.3048
2.2936
2.2699
2.2462
2.2293
2.2058
2.1792
2.1590
2.0597
2.0272
1.9929
1.9485
1.8773
1.8652
1.8548
1.8191
1.8009
1.7218
1.7095
1.6816
1.6696
1.6370
1.6251
1.6091
1.5969
1.5692
1.3814
1.3694
1.3463
1.3356
1.3256
1.3069
1.2961
1.2650
1.2022
1.1018
1.0444
0.9938


3.00
4.04
1.09
1.14
3.00
f1 (ppm)


212.00
49.99
41.12
34.19
33.29
25.29
22.05
f1 (ppm)

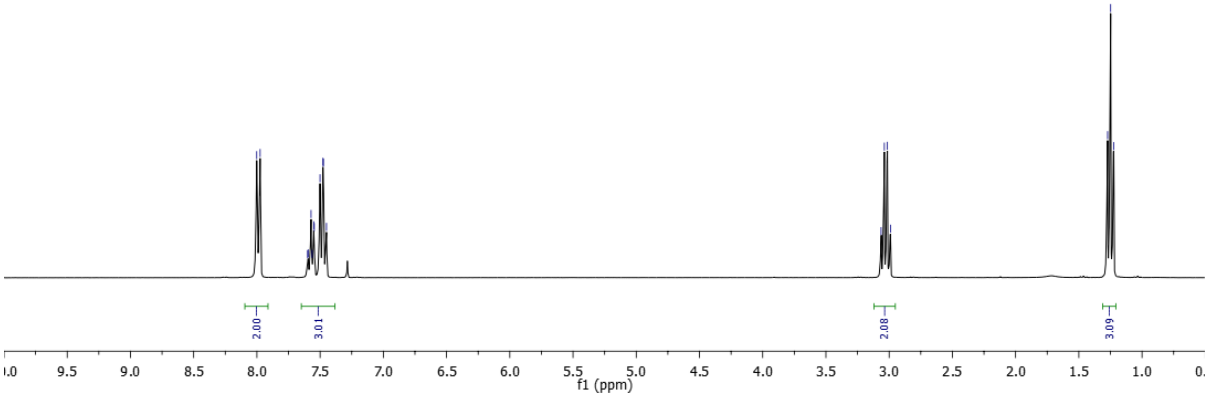


6c

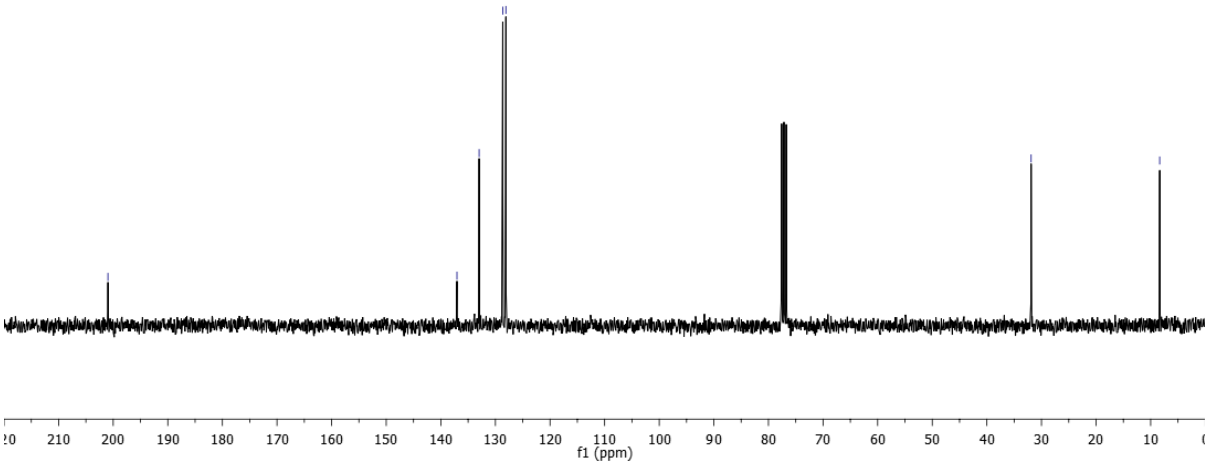

8.0013
7.9743
7.5987
7.5949
7.5717
7.5631
7.5503
7.5463
7.5007
7.4773
7.4741
7.4495

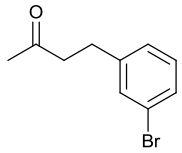

3.0638
3.0379
3.0137
2.9897


1.2723
1.2483
1.2241


2.00
3.01
2.08
3.09
f1 (ppm)

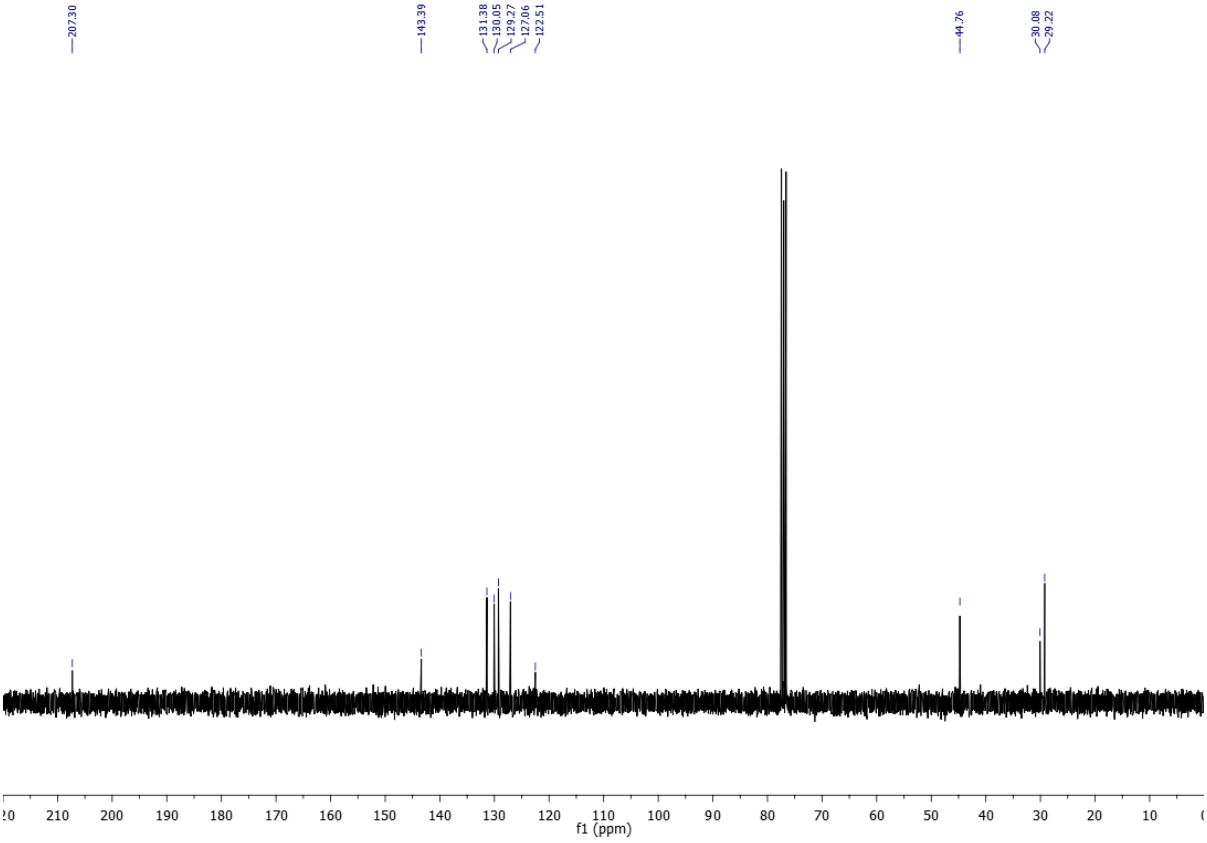

200.96
137.04
132.99
128.66
128.09
31.89
8.35

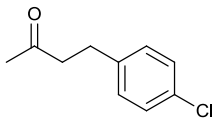

f1 (ppm)


O
Br

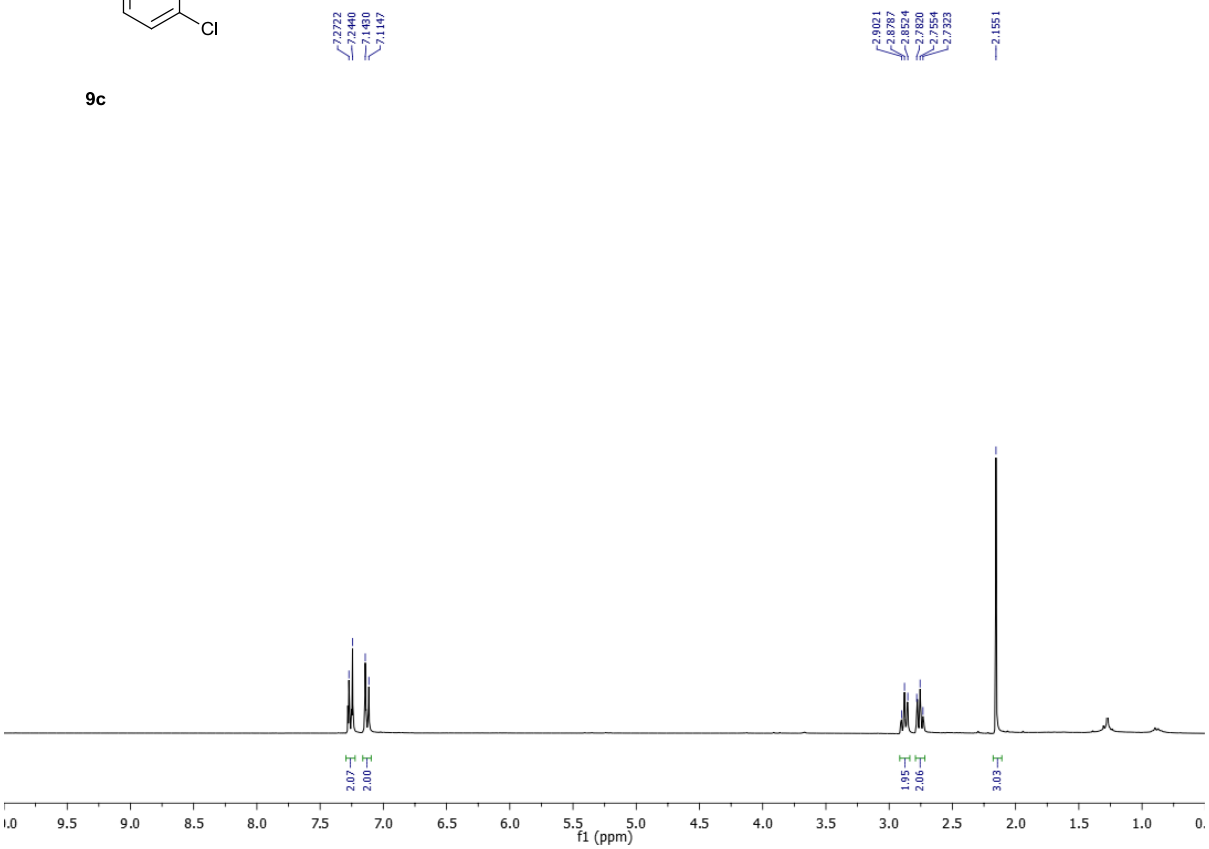
8c

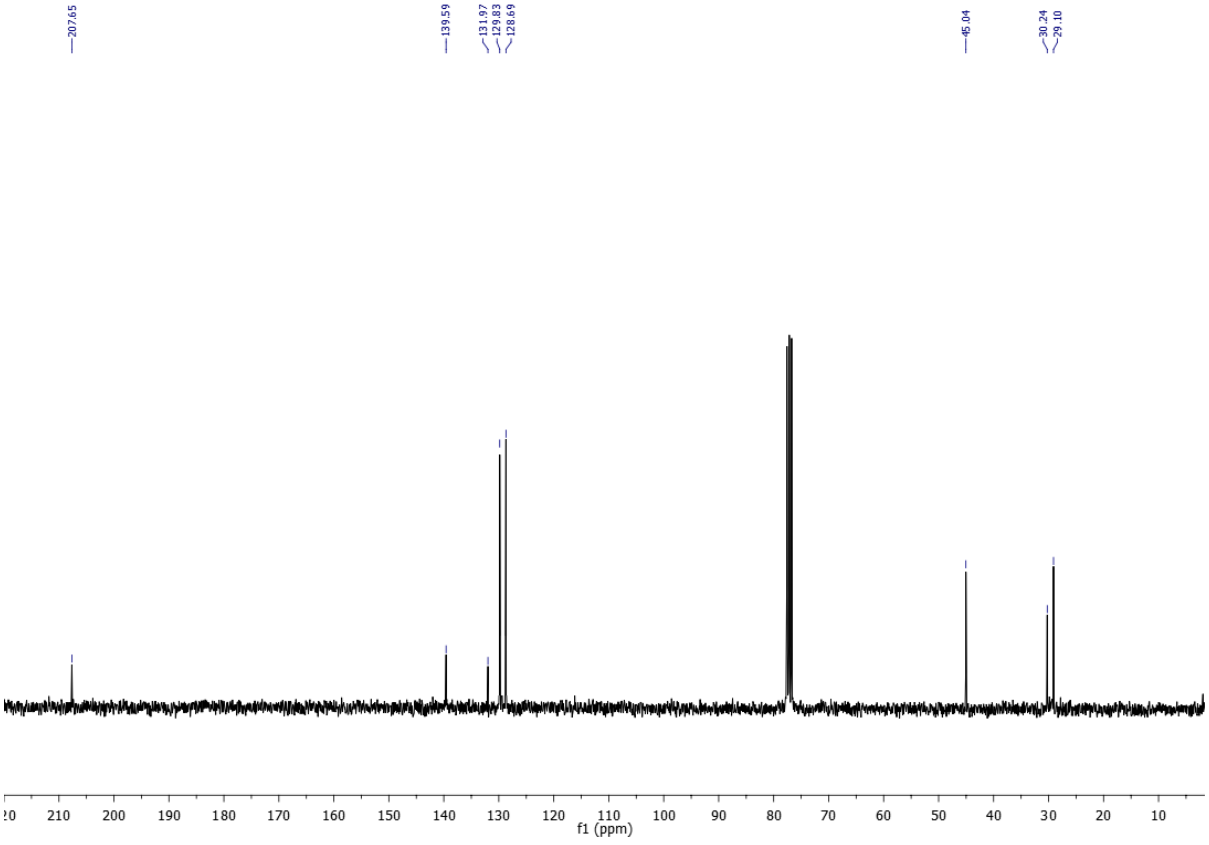
7.3549
7.3512
7.3344
7.3284
7.1894
7.1630
7.1368
7.1152
2.9038
2.8898
2.8578
2.7917
2.7672
2.7485
2.1631
1.96
1.95
2.02
2.00
2.97
f1 (ppm)

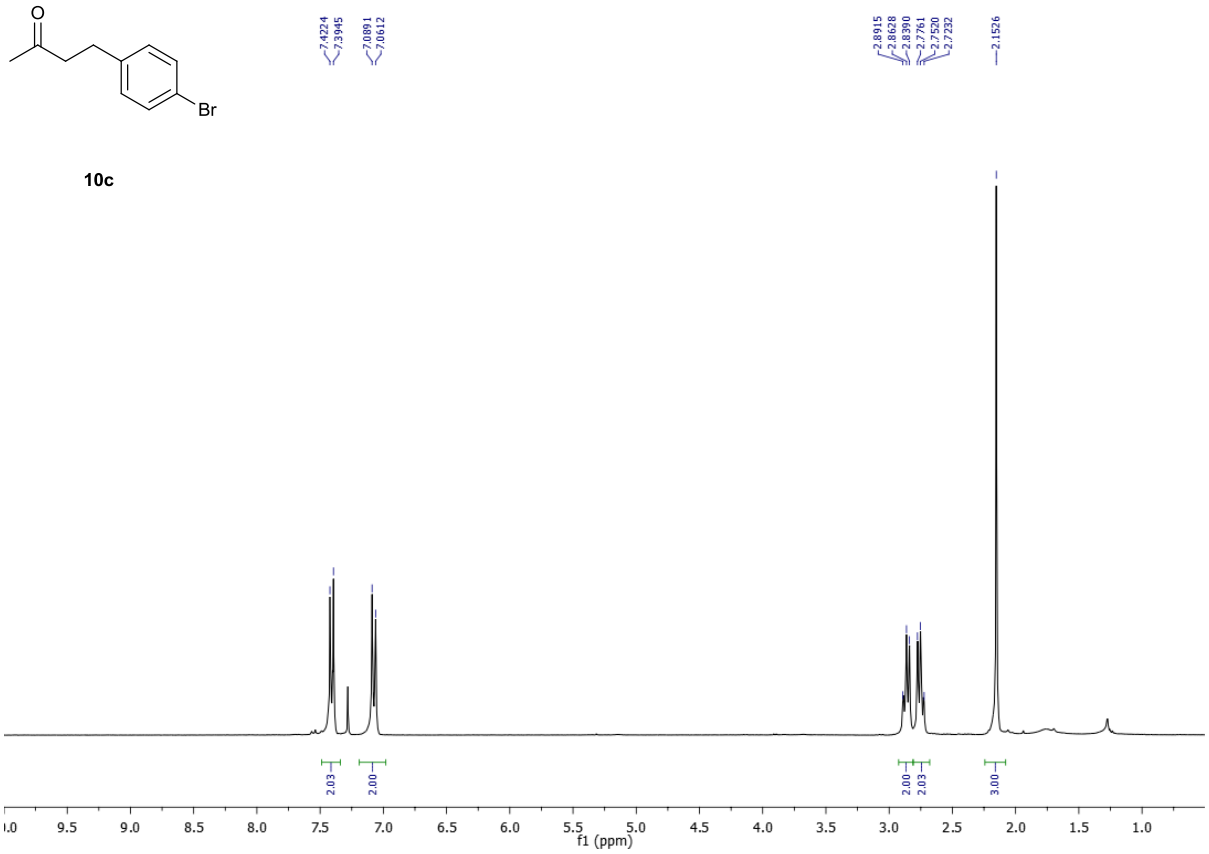

207.30
143.39
131.38
130.05
129.27
127.06
122.51
44.76
30.08
29.22
f1 (ppm)

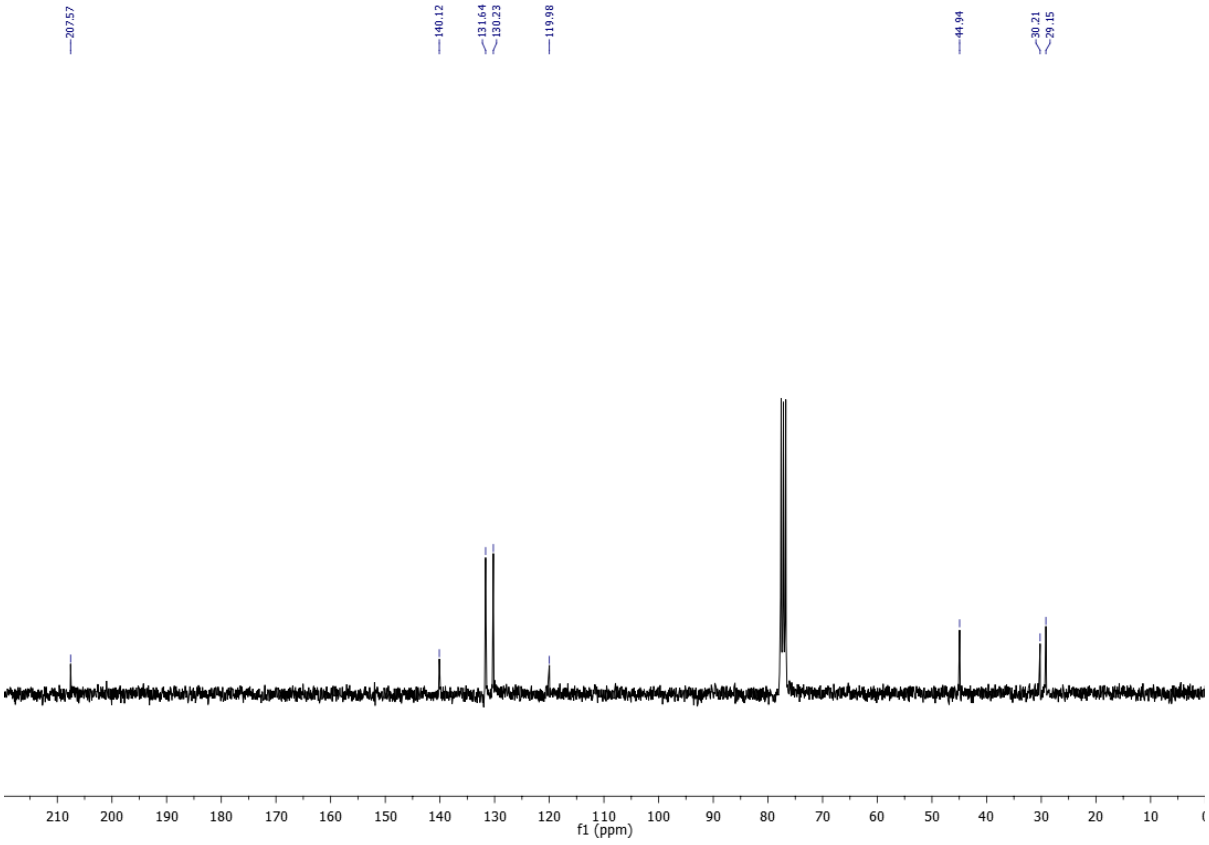

O
Cl

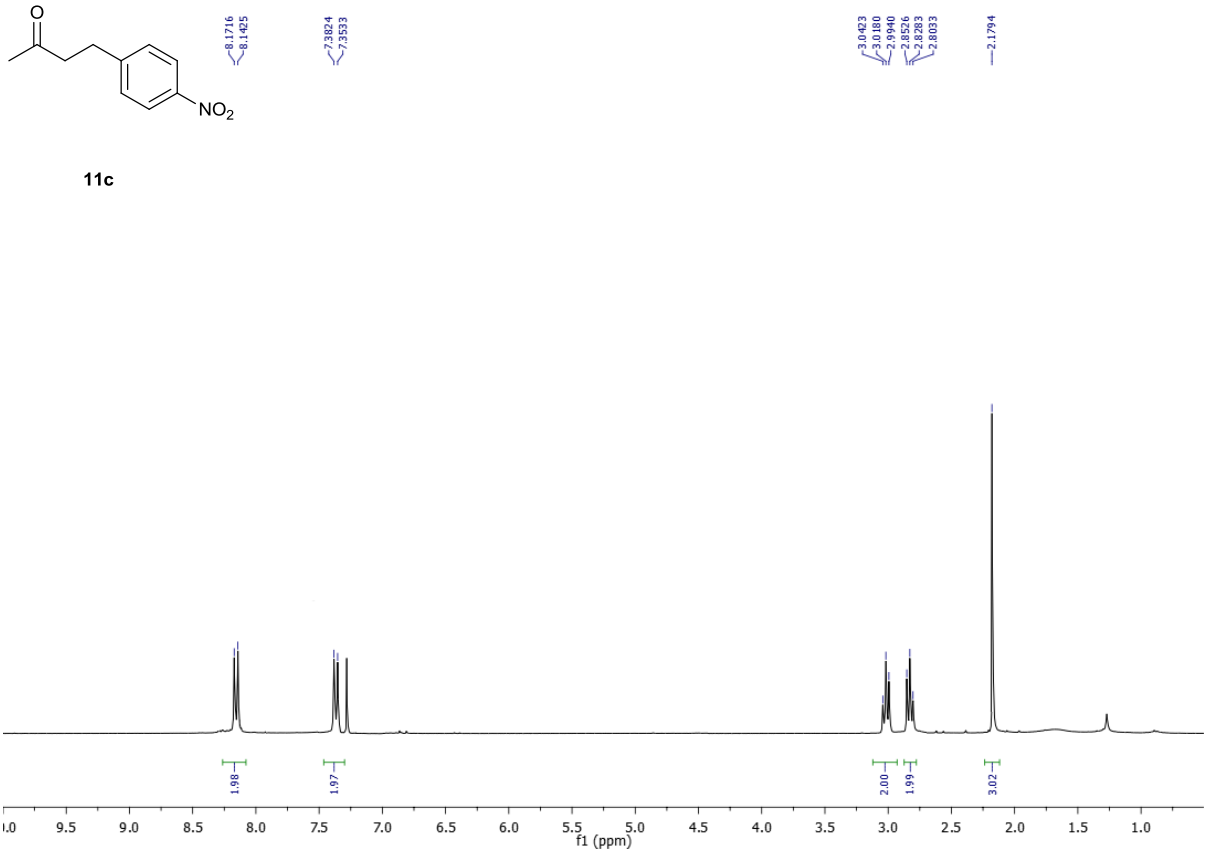
9c


7.2722
7.2440
7.1490
7.1147
2.9021
2.8787
2.8524
2.7820
2.7554
2.7323
2.1551
2.07
2.00
1.95
2.06
3.03
f1 (ppm)

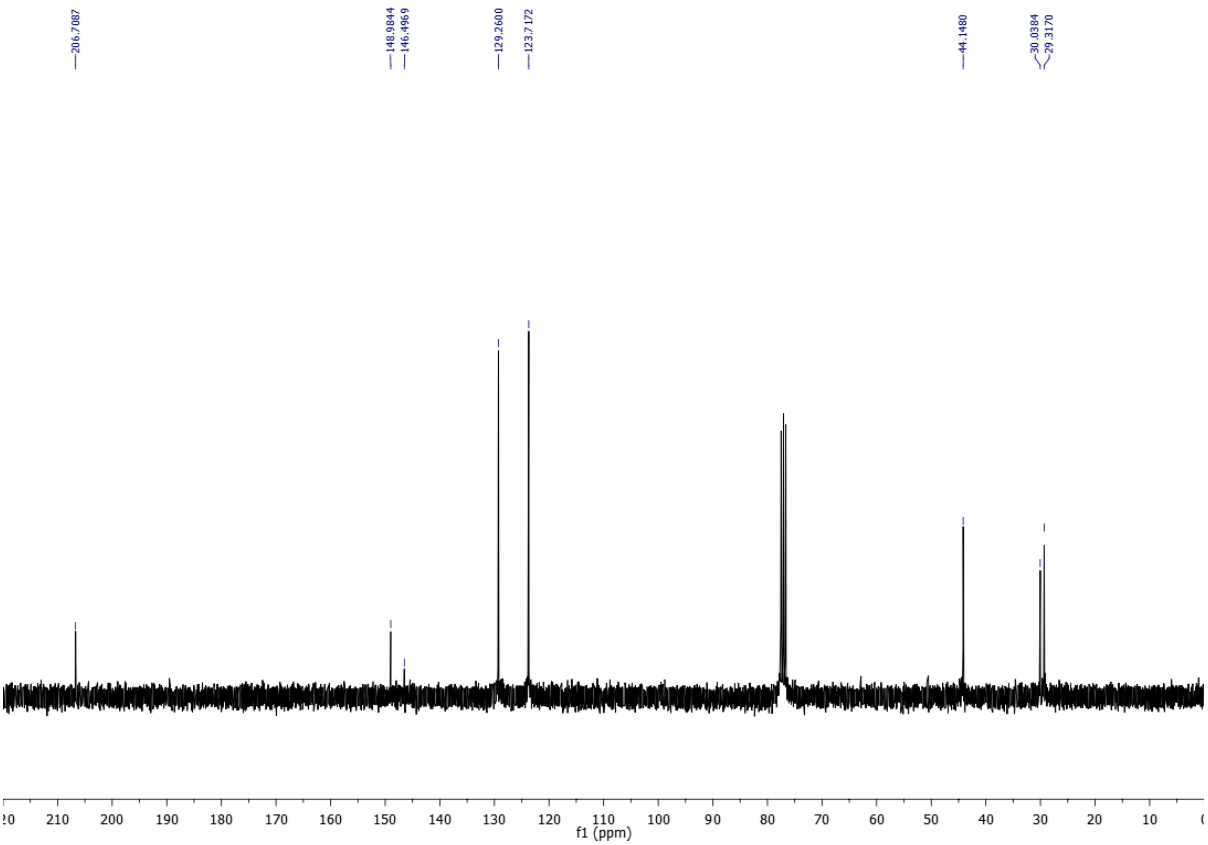

207.65
139.59
131.97
129.83
128.69
45.04
30.24
29.10
f1 (ppm)

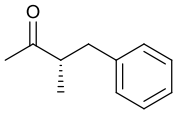

O
Br
10c
7.4224
7.3945
7.0891
7.0612
2.8915
2.8628
2.8390
2.7761
2.7520
2.7232
2.1526
2.03
2.00
2.00
2.03
3.00
f1 (ppm)


207.57
140.12
131.64
130.23
119.98
44.94
30.21
29.15
f1 (ppm)


O
NO2
8.1716
8.1425
7.3824
7.3553
3.0423
3.0180
2.9940
2.8526
2.8283
2.8033
2.1794
1.98
1.97
2.00
1.99
3.02
f1 (ppm)

11c


206.7087
148.9844
146.4969
129.2600
123.7172
44.1480
30.0384
29.3170
f1 (ppm)

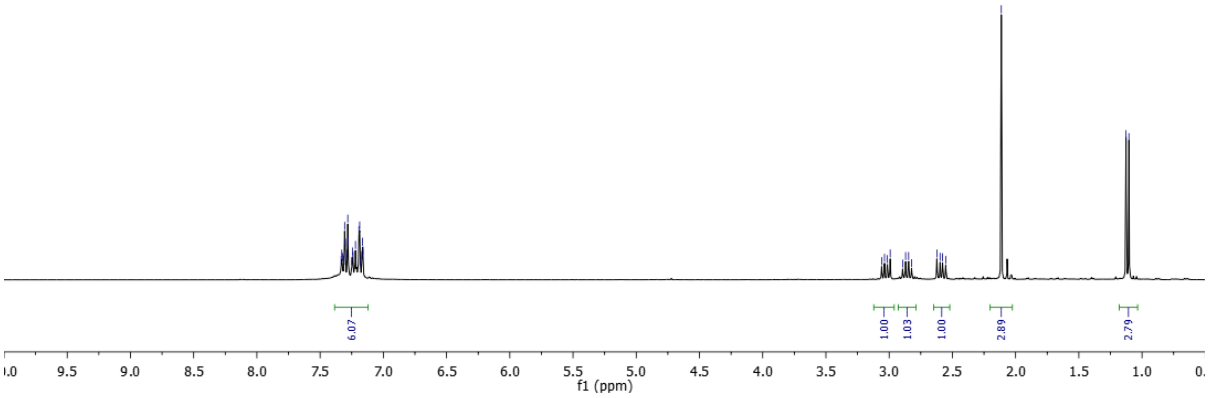


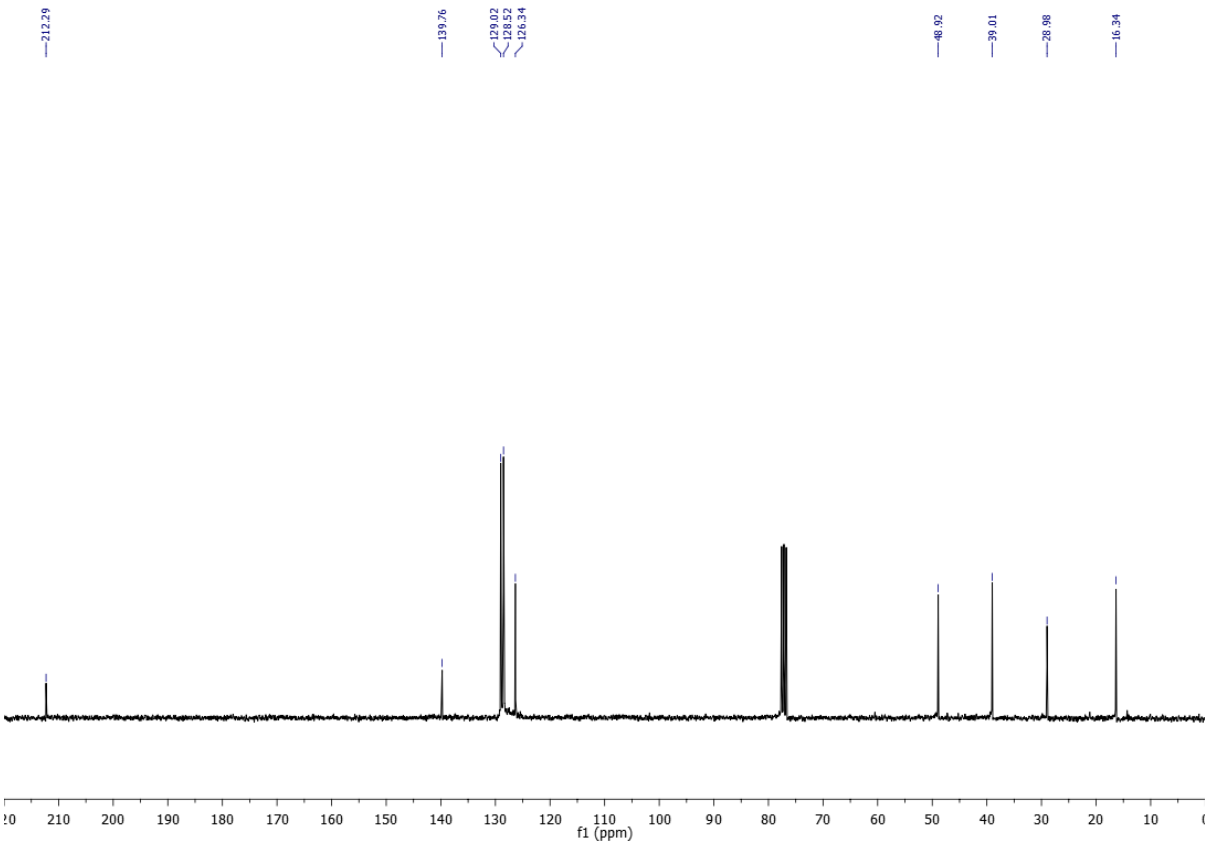
(*S*)-12c


7.3337
7.3288
7.3230
7.3129
7.3061
7.3007
7.2860
7.2812
7.2781
7.2495
7.2446
7.2397
7.2205
7.1930
7.1873
7.1799
7.1697
7.1650
7.1608


3.0591
3.0366
3.0141
2.9917
2.8934
2.8698
2.8461
2.8228
2.6226
2.6071
2.5778
2.5523
2.1131


1.1270
1.1039


6.07
1.00
1.03
1.00
2.89
2.79
f1 (ppm)


212.29
139.76
129.02
128.52
126.34
48.92
39.01
28.98
16.34
f1 (ppm)

XI. References

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