Supporting Information

A Multienzyme One-Pot Cascade for the Stereoselective Hydroxyethyl Functionalization of Substituted Phenols

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1. General information

Thin layer chromatography (tlc) was performed on Merck silica gel 60 F_{254} coated aluminium sheets and spots were visualized under UV light (λ = 245 nm) or with a potassium permanganate staining reagent [$KMnO_4$ (1.5 g), K_2CO_3 (10 g), NaOH (aq 10%, 1.25 mL), H_2O (200 mL)]. Flash column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm) using petrolether (VWR, bp 40–60 °C, distilled before use), cyclohexane, ethylacetate (both from Fisher Chemicals, analytical reagent grade) and methyl-*tert*-butylether (MtBE, Roth, ≥99.5%) as solvents. HPLC grade water and acetonitrile were acquired from Chem-Lab.

Optical rotation was measured in methanol (HPLC-grade, Chem-Lab) at 20 °C against the sodium D-line (λ = 589 nm) on a Perkin Elmer polarimeter 341 using a 10 cm pathlength cell. Circular dichrosim spectra were recorded on a JASCO J-715 spectropolarimeter in methanol (HPLC-grade, Chem-Lab) using a 1 cm quartz cuvette at analyte concentrations between 0.1 and 0.2 mg mL^{-1}.

GC-MS spectra were recorded with an Agilent 7890A GC-system, equipped with an Agilent 5975C quadrupole mass selective detector operated in ESI+ mode (70 eV) and a HP-5 MS column (30 m × 0.25 mm × 0.25 µm film) using He as carrier gas (flow = 0.55 mL min^{-1}) and the following standard temperature program: 100 °C (0.5 min hold) – [10 °C min^{-1}] – 300 °C.

NMR spectra were recorded on a Bruker Avance III 300 MHz unit at 20 °C. Chemical shifts are given in ppm and were referenced to residual solvent signals (^{1}H NMR: chloroform-d: s, 7.26 ppm; acetone-d6: p, 2.05 ppm, methanol-d4: s, 4.87 ppm) (^{13}C NMR: chloroform-d: t, 77.16 ppm; acetone-d6: h, 29.84 ppm; methanol-d4: h, 49.00 ppm). Signals are abbreviated as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quadruplet), p (pentet), h (heptet), m (undefined multiplet). High resolution mass spectra were measured on an Agilent 6230 TOF LC/MS. Analytes were ionized using Dual AJS electrospray ionization (ESI) with a capillary voltage of 3.5 and 5 kV operated either in the positive or negative mode, respectively.

Phosphate buffer salts [KH_2PO_4 (Honeywell/Fluka, ≥99.5%) and K_2HPO_4 (Roth, ≥99%)], ammonium chloride (Sigma Aldrich, ≥99%), pyridoxal 5-phosphate (PLP, Sigma Aldrich, 98%) and sodium pyruvate (Sigma Aldrich, ≥99%) were purchased from commercial sources. 1,2-Propanediol (propyleneglycol) was a gift from BASF (Ludwigshafen, Germany).

Phenols used as substrates in biotransformations were obtained from the following sources and were used without further purification unless otherwise stated: phenol (**1a**, Sigma Aldrich, 99%), 2-chlorophenol (**1b**, Acros Organics, >98%), *ortho*-cresol (**1e**, Sigma Aldrich, 99%), 2-fluorophenol (**1f**, Acros, 98%), 2-bromophenol (**1g**, Sigma Aldrich, 98%), 3-fluorophenol (**1i**, Sigma Aldrich, 98%), 3-chlorophenol [**1j**, gift from BASF (Ludwigshafen, Germany), purified by bulb-to-bulb distillation prior to use].

L-Tyrosine (**2a**, Fluka, 99%), 3-chloro-L-tyrosine (**2b**, Sigma Aldrich, 97%), 3-methyl-L-tyrosine (**2e**, Sigma Aldrich, 98%), 3-fluoro-DL-tyrosine (**2f**, Alfa Aesar, 98%) were available from commercial sources. Known tyrosines (**2g**, **2i**–**j**) were prepared according to literature procedures.[1]

trans-4-Hydroxycinnamic acid (**3a**, Alfa Aesar, 98%), ferulic acid (**3c**, Sigma Aldrich, 99%), 3-(3-ethoxy-4-hydroxyphenyl)acrylic acid (**3d**, ChemCollect GmbH, purity not given) were available from commercial sources. Known coumaric acids (**3b**, **3e**–**g**, **3i**–**j**) were prepared according to literature procedures.[1]

4-Vinylphenol (**4a**, Sigma Aldrich, 10% w/w solution in propylene glycol) and 2-naphthol (**4n**, Sigma Aldrich, >99%) were purchased from commercial sources. Known substituted 4-vinylphenols (**4b**, **4d**, **4e**) were prepared according to literature procedures.[2]

Racemic apocynol (**5c**, Sigma Aldrich, 97%) was available from commercial sources and other racemic benzylic alcohol reference compounds (**5a**–**b**, **5d**–**e**) were prepared according to literature procedures.[2] Enantiomerically pure (*S*)-**5a** was available from previous biotransformation studies.[3]

2. Preparation of biocatalysts and general biotransformation procedures

2.1. Activity measurements

All biocatalysts were heterologously expressed in *Escherichia coli* (*E. coli*) BL21 (DE3) according to literature[1-3] and used as follows for activity measurements:

Met379Val variant of a tyrosine phenol lyase from *Citrobacter freundii* as lyophilized cell-free extract (TPL_*Cf*, pEG 58, 1 L of bacterial culture yielded 760 mg lyophilisate, 44.8 mU mg^{-1} (1 unit refers to the amount of catalyst for the conversion of 1 µmol 2-chlorophenol to 3-chlorotyrosine per min). Tyrosine ammonia lyase from *Rhodobacter sphaeroides* as lyophilized *E. coli* whole cells (TAL_*Rs*), pEG 159, 1 L of bacterial culture yielded 820 mg lyophilisate, 0.88 mU mg^{-1}, (1 unit converts 1 µmol 3-chlorotyrosine to 3-chlorocoumaric acid per min). Ferulic acid decarboxylase from *Enterobacter* sp. (FDC_*E*s, pEG 156) as lyophilized *E. coli* whole cells (1.31 U mg^{-1}) or Ni-affinity purified enzyme (3.6 U μL^{-1}) (1 unit converts 1 µmol *p*-coumaric acid to 4-vinylphenol per min). Val46Glu or Val46Asp variant of FDC_*E*s (FDC_*E*s V46E and V46D, pEG 397 and 396, respectively) as Ni-affinity purified enzyme (storage concentration between 3–4 mmol L^{-1}).

2.2. General procedure for the hydration of hydroxystyrene derivatives (analytical scale)

Substrate 4-vinylphenol (**4a**–**n**, 10 mM, supplied as aliquot of a 10% w/w solution in propylene glycol) was added to potassium phosphate buffer (50 mM, pH 6.0, 1.0 mL) in a 2.0 mL Eppendorf reaction vessel. The reaction was started by the addition of a solution of Ni-affinity purified hydratase FDC_*E*s V46E (89 µM final

concentration) or FDC_*E*s V46D (100 µM final concentration) in reaction buffer and incubated in an Eppendorf Thermomixer at 25 °C, 700 rpm for 24 h.

2.3. General procedure for the decarboxylation/hydration cascade (analytical scale)

Substrate coumaric acid (**3a**–**c**, **3e**–**g**, **3i**–**j**, 10 mM, 100 µL of a 100 mM stock in ^{i}PrOH) was dissolved in potassium phosphate buffer (50 mM, pH 6.0) in a 2.0 mL Eppendorf reaction vessel. Ni-affinity purified decarboxylase FDC_*E*s (10 µM final concentration, 3.6 U) and purified hydratase FDC_*E*s V46E (100 µM final concentration) was added and the reaction was incubated in an Eppendorf Thermoshaker at 25 °C and 700 rpm. Samples (100 µL) were withdrawn between 17 min and 24 h (**3b**) or 3.5 h and 24 h (other substrates).

2.4. General procedures for the vinylation/hydration cascade (analytical scale)

Single-step approach:

Lyases TPL_*Cf* M379V (10 mg, 448 mU) and TAL_*Rs* (40 mg, 32 mU) as well as decarboxylase FDC_*E*s wt (2 mg, 3.2 U) and Ni-affinity purified FDC_*E*s V46E (100 µM) were suspended in reaction buffer (potassium phosphate buffer, 50 mM, pH 8.0) containing PLP (80 µM) and NH_4Cl (180 mM) (900 µL) in an Eppendorf Thermoshaker at 30 °C and 700 rpm for 15 min. Substrate phenol (**1a**–**b**, **1e**–**g**, **1i**–**j**, 10 mM, 50 µL of a 200 mM stock in ^{i}PrOH) and sodium pyruvate (92 mM) were added to start the reaction and incubation was continued for 24 h at 30 °C and 850 rpm.

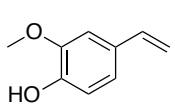
Two-step approach:

Lyases TPL_*Cf* M379V (10 mg, 448 mU) and TAL_*Rs* (40 mg, 35.2 mU) as well as decarboxylase FDC_*E*s wt (2 mg, 3.2 U) were suspended in reaction buffer (potassium phosphate buffer, 50 mM, pH 8.0) containing PLP (80 µM) and NH_4Cl (180 mM) (950 µL) in an Eppendorf Thermoshaker at 30 °C and 700 rpm for 15 min. Substrate phenol (**1a**–**b**, **1e**–**g**, **1i**–**j**, 10 mM, 50 µL of a 200 mM stock in ^{i}PrOH) and sodium pyruvate (92 mM) were added to start the vinylation reaction and incubation was continued for 24 h at 30 °C and 850 rpm. After 24 h the pH was adjusted to 6.0 by H_3PO_4 aq (1 M, 34 µL) followed by addition of Ni-affinity purified FDC_*E*s V46E (100 µM). The incubation was continued for 5.3 and 19 h at 25 °C and 700 rpm.

3. Preparation of substrates and reference material

Procedure A: The respective substituted coumaric acid (0.165 mmol) was suspended in potassium phosphate buffer (50 mM, pH 7.0, 10% v/v ^{i}PrOH, 3.3 mL, 50 mM substrate concentration) in an ultrasonic bath for 5 min. Ni-affinity purified FDC_*E*s wt was added (7.08 kU, 1 unit decarboxylates 1 mmol coumaric acid per minute) and the reaction was shaken in a 20 mL glass vial at 120 rpm and 23 °C. The reaction progress was monitored by tlc (cyclohexane/ethylacetate 5:1, $R_{f,\text{product}} \approx 0.4$) and after the denoted time below, the reaction was extracted with ethylacetate (2 × 3 mL). The combined extracts were dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The oily residue was filtered through a pad of silica (Ø 1 cm × 3 cm height) using a mixture of cyclohexane/ethylacetate 5:1 as solvent (5–8 mL). The filtrate was evaporated under reduced pressure to yield the pure product, which was dissolved immediately in in propylene glycol (10% w/w **4**) for storage.

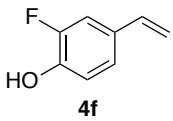
Procedure B: Methyltriphenylphosphonium bromide (5.2 g, 2 mmol, 2 equiv, Sigma Aldrich, 98%) was dispersed in anhydrous THF (2 mL) and cooled to 0 °C. Sodium bis(trimethylsilyl)amide (5.7 mL of a 1.0 M solution in THF, 2.2 mmol, 2.2 equiv, Sigma Aldrich) was added dropwise over 5 min. A yellow color developed and the reaction was stirred at 0 °C for 1.5 h. The respective benzaldehyde was added as a solid (1.0 mmol). Upon completion of addition, the mixture was stirred for 2.5 h at 21 °C after which tlc (cyclohexane/ethylacetate 3:1) showed full conversion of the aldehyde. The reaction was quenched by adding saturated aq NH_4Cl solution (40 mL) and extracted with ethylacetate (3 × 30 mL). Remaining triphenylphosphine was removed by washing the extract with H_2O_2 aq (10%, 50 mL). After drying the combined organic layers over Na_2SO_4 and removal of the solvent under reduced pressure, the product was purified on silica gel by flash-column chromatography if necessary.



4c

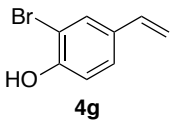
3-Methoxy-4-hydroxystyrene (4c). Modified procedure A: Ferulic acid (**3c**, 50 mg, 0.26 mmol) was suspended in potassium phosphate buffer (50 mM, pH 7.0, 12.5 mL, 20 mM **3c**) and lyophilized, Ni-affinity purified ferulic acid decarboxylase from *Enterobacter* sp. (FDC_*E*s) was added (7.08 kU, 1 U decarboxylates 1 mmol of *p*-coumaric acid per min). The reaction was shaken at 30 °C and 120 rpm for 19 h in a 50 mL Falcon tube before tlc (petrolether/ethylacetate 7:3) showed full conversion of **3c** to **4c**. The aqueous phase was saturated with NaCl, the pH adjusted to 5.0 with HCl (1 M) and extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give 37.9 mg of the title compound as colorless oil with a sweet scent (purity confirmed by NMR, 98% yield). The obtained oil was taken up in propylene glycol (340 mg, 10% w/w

4c) for storage. ^{1}H NMR (300 MHz, MeOH-d4) δ_H [ppm]: 7.00 (d, J = 1.8 Hz, 1H, 3–H), 6.84 (dd, J = 8.1 Hz, 1.8 Hz, 1H, 5–H), 6.72 (d, J = 8.1 Hz, 1H, 6–H), 6.61 (dd, J = 17.6, 10.9 Hz, 1H, 1'–H), 5.57 (dd, J = 17.6, 0.9 Hz, 1H, 2'–H_t), 5.03 (dd, J = 10.9 Hz, 0.9 Hz, 1H, 2'–H_c), 3.84 (s, 3H, 1–OCH_3). ^{13}C NMR (75 MHz, MeOH-d4) δ_C [ppm]: 149.0, 147.7, 138.1, 131.3, 120.7, 116.1, 111.1, 110.1, 56.3. GC-MS (EI+, 70 eV): t_R = 6.35 min; *m/z* (%) = 150 (100, M^+), 135 (84), 107 (37), 89 (7), 77 (41), 63 (9), 51 (13), 39 (10). The analytical data matches those reported in the literature.[4-5] [CAS 7786-61-0].



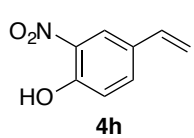
4f

2-Fluoro-4-vinylphenol (4f). Procedure A: Full conversion after 5 min. Colorless oil (18.6 mg, 83% yield). Tlc (cyclohexane/ethylacetate 5:1): R_f = 0.41. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H [ppm]: 7.15 (dd, J = 11.7, 2.0 Hz, 1H, 3–H), 7.07 (dd, J = 8.6, 1.7 Hz, 1H, 5–H), 6.94 (t, J = 8.6 Hz, 1H, 6–H), 6.60 (dd, J = 17.6, 10.8 Hz, 1H, 1'–H), 5.60 (d, J = 17.8 Hz, 1H, 2'–H_t), 5.18 (d, J = 10.9 Hz, 1H, 2'–H_c superimposed on bs, 1H, 1–OH). GC-MS (EI+, 70 eV): t_R = 3.94 min; *m/z* (%) = 139 (9), 138 (100, M^+), 137 (16), 112 (9), 109 (23), 90 (12), 89 (30), 83 (12), 63 (11), 57 (8). The analytical data matches those reported in the literature.[1] HR-MS: *m/z* = 138.048064 (calcd. 138.048093, diff. –0.21 ppm).



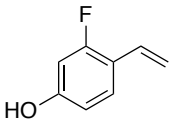
4g

2-Bromo-4-vinylphenol (4g). Procedure B: Colorless oil (379.1 mg, 75% yield). Tlc (cyclohexane/ethylacetate 5:1): R_f = 0.40. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H [ppm]: 7.52 (d, J = 2.1 Hz, 1H, 3–H), 7.27 (dd, J = 8.5, 2.0 Hz, 1H, 5–H), 6.98 (d, J = 8.4 Hz, 1H, 6–H), 6.58 (dd, J = 17.6, 10.9 Hz, 1H, 1'–H), 5.61 (dd, J = 17.5 Hz, 0.6 Hz, 1H, 2'–H_t), 5.53 (s, 1H, 1–OH), 5.18 (dd, J = 10.9, 0.4 Hz, 1H, 2'–H_c). GC-MS (EI+, 70 eV): t_R = 6.26 min; *m/z* (%) = 201 (9), 200 (97), 199 (15), 198 (100), 197 (7), 119 (25), 118 (15), 91 (41), 90 (22), 89 (52), 65 (23), 63 (30), 53 (10), 39 (14). The analytical data matches those reported in the literature.[1] HR-MS: *m/z* = 197.967944 (calcd. 197.968028, diff. –0.42 ppm).



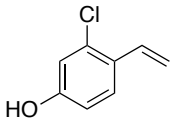
4h

2-Nitro-4-vinylphenol (4h). Procedure B: Yellow oil (260 mg, 62% yield). Tlc (cyclohexane/Ethylacetate 3:1): R_f = 0.63. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H [ppm]: 10.58 (s, 1H, C_{ar}–OH), 8.08 (d, J = 2.2 Hz, 1H, C_{ar}–H), 7.67 (dd, J = 8.7, 2.2 Hz, 1H, Cα–H), 7.13 (d, J = 8.7 Hz, 1H, C_{ar}–H), 6.64 (dd, J = 17.5, 10.9 Hz, 1H, Cα–H), 5.70 (d, J = 17.5 Hz, 1H, Cβ–H), 5.32 (d, J = 10.9 Hz, 1H, Cβ–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C [ppm]: 154.7, 135.0, 134.2 (2C), 130.6, 122.5, 120.3, 115.1. GC-MS (EI+, 70 eV): t_R = 6.86 min; *m/z* (%) = 165 (100), 148 (5), 119 (11), 91 (19), 89 (26), 65 (17). HR-MS: *m/z* = 165.042656 (calcd. 165.042593, diff. 0.38 ppm). No reference data was available for comparison.



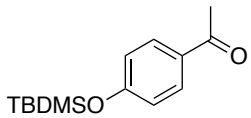
4i

3-Flouro-4-vinylphenol (4i). Procedure A: full conversion after 20 min, pale yellow oil (22.8 mg, 99% yield). Tlc (cyclohexane/ethylacetate 5:1): R_f = 0.40. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H [ppm]: 7.35 (t, J = 8.4 Hz, 1H, 5–H), 6.78 (dd, J = 17.8, 11.2 Hz, 1H, 1'–H), 6.63–6.50 (m, 2H, 2–H, 6–H), 5.68 (dd, J = 17.7, 1.1 Hz, 1H, 2'–H_t), 5.25 (dd, J = 11.2, 1.1 Hz, 1H, 2'–H_c), 5.13 (s, 1H, 1–OH). GC-MS (EI+, 70 eV): t_R = 5.39 min; *m/z* (%) = 139 (9), 138 (100), 137 (29), 109 (36), 89 (10), 83 (13), 63 (10), 57 (7), 39 (5). The analytical data matches those reported in the literature.[1] HR-MS: *m/z* = 138.048175 (calcd. 138.048093, diff. 0.59 ppm).



4j

3-Chloro-4-vinylphenol (4j). Procedure A: incomplete conversion after 24 h, colorless oil (11.3 mg, 48% yield). Tlc (cyclohexane/ethylacetate 3:1): R_f = 0.41. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H [ppm]: 7.46 (d, J = 8.6 Hz, 1H, 5–H), 7.02 (dd, J = 17.5, 11.0 Hz, 1H, 1'–H), 6.87 (d, J = 2.6 Hz, 1H, 2–H), 6.73 (dd, J = 8.6, 2.6 Hz, 1H, 6–H), 5.62 (dd, J = 17.5, 1.1 Hz, 1H, 2'–H_t), 5.27 (dd, J = 11.0, 1.1 Hz, 1H, 2'–H_c), 4.95 (s, 1H, 1–OH). GC-MS (EI+, 70 eV): t_R = 7.56 min; *m/z* (%) = 156 (32), 155 (11), 154 (100), 153 (8), 119 (56), 91 (34), 89 (23), 65 (14), 63 (23), 39 (9). The analytical data matches those reported in the literature.[1] HR-MS: *m/z* = 154.018577 (calcd. 154.018543, diff. 0.22 ppm).

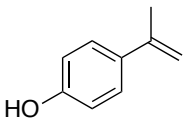


6

1-(4-((*tert*-Butyldimethylsilyl)oxy)phenyl)ethan-1-one (6). 4-Hydroxyacetophenone (4.23 g, 31.1 mmol, 1.1 equiv, 98%, Sigma Aldrich) and imidazole (4.57 g, 67.2 mmol, 2.4 equiv, >99.5%, Sigma Aldrich) were dissolved in dry *N*, *N*-dimethylformamide (10 mL, >99%, dried over 3 Å molecular sieve, Roth) under inert atmosphere and treated with *tert*-butyldimethylchlorosilane (4.22 g, 28.0 mmol, 1.0 equiv, 97%, Sigma Aldrich). The clear, yellow reaction mixture was stirred for 4 h at room temperature until tlc (petrol ether/ethylacetate 2:1) showed full conversion of the free phenol. The reaction was quenched by addition of saturated aq $NaHCO_3$ solution (100 mL) and extracted with petrolether (3 × 60 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure to yield the title compound as brownish crystalline solid (5.98 g, 83%, 95% purity), which was used without further purification. Tlc (petrolether/ethylacetate 2:1): R_f = 0.37 (4-hydroxyacetophenone), 0.84 (**6**). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H [ppm]: 7.88 (d, J = 8.8 Hz, 2H, 2–H, 6–H), 6.87 (d, J = 8.8 Hz, 2H, 3–H, 5–H), 2.55 (s, 3H, 2'–H_3), 0.99 (s, 9H, $SiC(CH_3)_3$), 0.23 (s, 6H, $Si(CH_3)_2$). *mp* = 36.2–38.1 °C (33–35 °C lit.).[6] GC-MS (EI+, 70 eV): t_R = 11.02 min; *m/z* (%) = 251 (4), 250 (20, M^+), 195 (6), 194 (21), 193 (100, M^+–57), 179 (11), 151 (32, M^+–99), 135 (7), 133 (7), 123 (6), 91 (7), 89 (7), 75 (6), 73 (7), 43 (22). The analytical data matches those reported in the literature.[7-8]

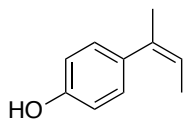
General procedure for the preparation of **4k** and **4m**: Sodium bis(trimethylsilyl)amide (1.05 g, 5.7 mmol, 2.1 equiv, 1 M in THF, Sigma Aldrich) was added to a stirred solution of the respective alkyl(triphenyl)phosphonium bromide (5.2 mmol, 1.9 equiv) in freshly distilled, anhydrous THF (8 mL), which caused colour change to yellow-orange. After 1.5 h at 21 °C, the protected ketone **6** (675 mg, 2.7 mmol, 95%) was added to the ylide solution. After stirring for 19 h at 21 °C the reaction mixture was acidified with H_2SO_4 aq. (10%) (pH 1–2) and extracted with CH_2Cl_2 (3 × 50 mL). After drying over anhydrous Na_2SO_4, the solvent was evaporated under reduced pressure and the crude product was redissolved in diethyl ether at 0 °C to precipitate excess triphenylphosphinoxide. The supernatant was separated and evaporated and the residue was purified by flash column chromatography (pure petrolether).

The protected products were subsequently dissolved in dry THF (10.6 mL per gram of product) and cooled to 0 °C. Tetrabutylammonium fluoride (2 equiv, 1 M solution in THF, Sigma Aldrich) was added and after 24 h the reaction mixtures were acidified to pH 3–4 with H_2SO_4 aq (0.1 M) and then extracted with CH_2Cl_2 (3 × 11 mL). The organic layers were washed with dd H_2O (3 × 10 mL), dried over Na_2SO_4 and evaporated under reduced pressure to yield the deprotected olefin products.



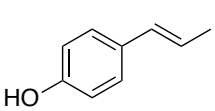
4k

4-(Prop-1-en-2-yl)phenol (4k). Colorless oil (137 mg, 38% over two steps). Tlc (petrolether/ethylacetate 9:1): R_f = 0.22. ^{1}H NMR (300 MHz, methanol-d4) δ_H [ppm]7.38–7.26 (m, 2H), 6.80–6.66 (m, 2H), 5.22 (dd, J = 1.5, 0.6 Hz, 1H), 4.91 (dd, J = 3.0 Hz, 1.5 Hz, 1H), 2.10–2.06 (m, 3H). ^{13}C NMR (75 MHz, methanol-d4) δ_C [ppm] 156.6, 142.8, 132.5, 126.2 (2C), 114.5 (2C), 108.7, 20.7. GC-MS (EI+, 70 eV): t_R = 6.14 min; *m/z* (%) = 134 (100, M^+), 119 (73), 91 (25), 77 (10), 65 (12). [CAS: 4286–23–1]. The analytical data matches those reported in the literature.[9]



4m

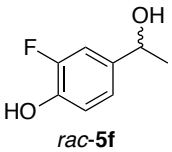
(*Z*)-4-(But-2-en-2-yl)phenol (4m). Colorless oil (69 mg, 37% over two steps). Tlc (petrolether/ethylacetate 9:1): R_f = 0.19. ^{1}H NMR (300 MHz, methanol-d4) δ_H [ppm] 7.04–6.97 (m, 2H), 6.78–6.71 (m, 2H), 5.48 (dq, J = 6.9, 1.5 Hz, 1H), 1.95 (p, J = 1.5 Hz, 3H), 1.56 (dq, J = 6.9, 15 Hz, 3H). ^{13}C NMR (75 MHz, methanol-d4) δ_C [ppm] 156.9, 137.9, 134.2, 130.1 (2C), 121.3, 115.7 (2C), 25.8, 15.1. GC-MS (EI+, 70 eV): t_R = 6.55 min; *m/z* (%) = 148 (100), 133 (82), 105 (26), 91 (16), 90 (12). *Z*-configuration was assigned by comparison with ^{1}H and ^{13}C NMR literature data of the *Z*-configured *O*-methylated analog.[10[



4l

(*E*)-4-(Prop-1-en-1-yl)phenol (4l). Methyl iodide (1.2 g, 99%, Lancaster) in anhydrous diethylether (3 mL) was added to stirred Mg-turnings (218 mg, purum for Grignard reactions, Aldrich Chemistry) under Ar atmosphere. The reaction mixture was stirred for 45 min until the Magnesium was completely dissolved. The ether was evaporated first under Ar-flow then under vacuum at 90 °C. After cooling to 21 °C, *trans*-anethole (1.016 g, 7.6 mmol, 99%, Sigma Aldrich) was added neat. The stirred red-brown reaction mixture was heated to 165 °C and after 3 h the reaction was cooled to 21 °C quenced by careful addition of ice. The quenched mixture was acidified with HCl aq (1 M, 5 mL) and extracted with diethyl ether (3 × 10 mL). The combined organic phases were washed with with brine (15 mL), dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (petrolether/ethylacetate 10:1) to yield the title compound as colorless oil (233 mg, 23%). Tlc (petrolether/ethylacetate 10:1): R_f = 0.14. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H [ppm] 7.25–7.17 (m, 2H, C_{ar}–H), 6.80–6.72 (m, 2H, C_{ar}–H), 6.33 (dd, *J* = 15.7, 1.5 Hz, 1H, Cα–H), 6.08 (dq, *J* = 15.7, 6.6 Hz, 1H, Cβ–H), 4.73 (s, 1H, Ar–OH), 1.85 (d, *J* = 6.6 Hz, 3H, $C_γ$–H_3). ^{13}C NMR (75 MHz, methanol-d4) δ_C [ppm] 157.5, 131.9, 131.1, 127.9 (2C), 123.1, 116.2 (2C), 18.5. Analytical data matches those reported in the literature.[11] GC-MS (EI+, 70 eV): t_R = 6.62 min; *m/z* (%) = 134 (100), 115 (12), 107 (28), 77 (18), 91 (12).

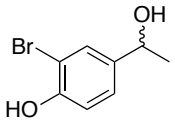
Procedure C: Substituted 4-hydroxyacetophenones (200 mg, 1.3 mmol) and cerium chloride heptahydrate (483 mg, 1.3 mmol, 1 equiv) was dissolved in methanol (5 mL). Sodium borohydride (52.8 mg, 1.4 mmol, 1.07 equiv) was added in small portions over 4 min upon which a colorless precipitate formed. After 40 min at 21 °C, tlc (petrolether/ethylacetate 2:1) confirmed full conversion of the substrate and the reaction was quenched by the addition of sat. aq NH_4Cl solution (8 mL). The mixture was extracted with ethyl acetate (3 × 6 mL) and the organic phases were washed with brine (10 mL), dried over Na_2SO_4 and evaporated under reduced pressure. Column chromatography on silica gel and petrolether/ethylacetate 2:1 as solvent and drying *in vacuo* over night afforded the solid product alcohols.



rac-**5f**

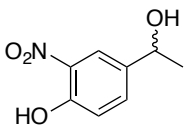
***rac*-2-Fluoro-4-(1-hydroxyethyl)phenol (*rac*-5f)**. Procedure C. Colorless solid (138 mg, 68%). Tlc (petrolether/ethylacetate 2:1): R_f = 0.30. ^{1}H NMR (300 MHz, acetone-d6) δ_H [ppm] 8.45 (s, 1H), 7.12 (dd, *J* = 12.4 Hz, 2.0 Hz, 1H), 7.00 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.92 (t, *J* = 8.5 Hz, 3H), 4.76 (q, *J* = 6.4 Hz, 1H), 4.18 (d, *J* = 4.1 Hz, 1H), 1.36 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d6) δ_C [ppm] 152.1 (d, *J* = 239.3 Hz), 144.1 (d, *J* = 13.1 Hz), 140.6 (d, *J* = 5.0 Hz), 122.2 (d, *J* = 3.2 Hz), 118.1 (d, *J* = 2.8 Hz), 113.7 (d, *J* = 18.7 Hz), 69.0 (dd, *J* = 8.9, 0.9 Hz), 26.1 (d, *J* = 3.5 Hz). *mp* = 76.0 –

80.1 °C (acetone). GC-MS (EI+, 70 eV): t_R = 5.77 min; *m/z* (%) = 157 (3), 156 (36), 141 (100), 139 (16), 138 (17), 113 (53), 93 (26), 83 (14), 65 (30), 57 (12), 43 (30). HR-MS: *m/z* = 156.058623 (calcd. 156.058658, diff. –0.22 ppm). No literature data was available for comparison.



rac-**5g**

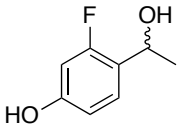
***rac*-2-Bromo-4-(1-hydroxyethyl)phenol (*rac*-5g)**. 3-Bromo-4-hydroxybenzaldehyde (100.0 mg, 0.497 mmol, Sigma Aldrich, 97%) was dissolved in anhydrous THF (2.5 mL) and cooled to –95 °C. Methylmagnesium bromide (3 M solution in diethylether, 414 µL, 1.24 mmol, 2.5 equiv, Sigma Aldrich) was added dropwise. A colorless precipitate formed and additional anhydrous THF (2 mL) was added to ease stirring. The reaction was allowed to warm slowly and the progress was followed by tlc (cyclohexane/ethylacetate 7:3). After 30 min (–45 °C) full conversion of the starting material to a single product with R_f = 0.29 was detected. The mixture was warmed to 0 °C in an ice bath and quenched by addition of sat. aq NH_4Cl solution (10 mL). The quenched mixture was extracted with MtBE (3 × 10 mL) and the combined organic phases were washed with brine (15 mL) before drying over anhydrous Na_2SO_4. The solvent was removed and the residue dried *in vacuo* to yield 103.6 mg (96% yield) of the title compound as yellowish solid without further purification. Tlc (cyclohexane/ethylacetate 7:3): R_f = 0.29. ^{1}H NMR (300 MHz, acetone-d6) δ_H [ppm] 8.66 (s, 1H), 7.51 (d, *J* = 2.0 Hz, 1H), 7.19 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.94 (d, *J* = 8.3 Hz, 1H), 4.84–4.68 (m, 1H), 4.18 (d, *J* = 4.0 Hz, 1H), 1.36 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d6) δ_C [ppm] 153.5, 141.3, 130.8, 126.6, 116.9, 109.9, 68.9, 26.2. *mp* = 80.4–82.9 °C (acetone). GC-MS (EI+, 70 eV): t_R = 8.45 min; *m/z* (%) = 218 (15), 216 (16), 203 (49), 201 (56), 200 (10), 198 (9), 173 (5), 137 (5), 119 (7), 94 (100), 65 (19), 63 (17), 53 (9), 43 (34), 39 (13). HR-MS: *m/z* = 215.978439 (calcd. 215.978592, diff. –0.71 ppm). No literature data was available for comparison.



rac-**5h**

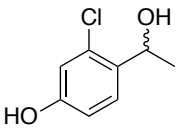
***rac*-2-Nitro-4-(1-hydroxyethyl)phenol (*rac*-5h)**. Modified procedure C: 4-hydroxy-3-nitroacetophenone (363 mg, 2 mmol, 97%, Acros) was dissolved in methanol (10 mL) and trifluoroacetic acid (TFA, 193 µL, 2.52 mmol, 1.26 equiv) was added. Sodium borohydride (Sigma Aldrich, 98%, 605 mg, 16 mmol, 8 equiv) was added in small portions in the course of 2 h. Upon complete addition, the reaction was heated to reflux for 15 h. The reaction was subsequently quenched by the initial addition of ice and then water (50 mL, pH 5 after quenching) and the aqueous phase was extracted with ethyl acetate (2 × 50 mL). The organic phase was again washed with water (50 mL) and brine (50 mL) before drying over anhydrous Na_2SO_4. Evaporation of the solvent yielded crude product and unreacted acetophenone as yellow oil (343.4 mg). Column chromatography with toluene/ethylacetate/acetic acid 90:9:1 as solvent ($R_{f,\text{product}}$

= 0.30) and drying *in vacuo* afforded 68.2 mg (19% yield) of the title alcohol as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H [ppm] 8.09 (s, 1H, C^{ar}–OH), 8.08 (d, J = 2.2 Hz, 1H, C^{ar}–H), 7.67 (dd, J = 2.2, 8.7 Hz, 1H, Cα–H), 7.13 (d, J = 8.7 Hz, 1H, C^{ar}–H), 6.64 (dd, J = 10.9, 17.5 Hz, 1H, Cα–H), 5.70 (d, J = 17.5 Hz, 1H, Cβ–H), 5.32 (d, J = 10.9 Hz, 1H, Cβ–H). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C [ppm] 154.7, 135.0, 134.2 (2C), 130.6, 122.5, 120.3, 115.1. *mp* = 78.5–80.1 °C ($CHCl_3$). GC-MS (EI+, 70 eV): t_R = 6.86 min; *m/z* (%) = 165 (100), 148 (5), 119 (11), 91 (19), 89 (26), 65 (17). HR-MS: *m/z* = 183.053003 (calcd. 183.053158, diff. –0.85). No literature data was available for comparison.



rac-**5i**

***rac*-3-Fluoro-4-(1-hydroxyethyl)phenol (*rac*-5i)**. Procedure C: Colorless solid (66.6 mg, 33%). Tlc (petrolether/ethylacetate 2:1): R_f = 0.27. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H [ppm] 8.62 (s, 1H), 7.37 (t, J = 8.8 Hz, 1H), 6.65 (dd, J = 8.5 Hz, 2.4 Hz, 1H), 6.51 (dd, J = 12.2, 2.4 Hz, 1H), 5.03 (q, J = 6.3 Hz, 1H), 4.14 (d, J = 4.3 Hz, 1H), 1.37 (d, J = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d6) δ_C [ppm] 160.8 (d, J = 242.6), 158.3 (d, J = 11.7 Hz), 128.3 (d, J = 6.9 Hz), 125.5 (d, J = 14.2 Hz), 112.0 (d, J = 2.9 Hz), 102.9 (d, J = 25.0 Hz), 63.4 (dd, J = 8.8, 2.4 Hz), 25.1 (d, J = 3.1 Hz). *mp* = 83.5–89.3 °C (acetone). GC-MS (EI+, 70 eV): t_R = 7.09 min; *m/z* (%) = 156 (19), 142 (8), 141 (100), 139 (12), 138 (17), 113 (21), 109 (11), 95 (14), 83 (11), 65 (17), 57 (8), 43 (16), 39 (6). HR-MS: *m/z* = 156.058636 (calcd. 156.058658, diff. –0.14 ppm). No literature data was available for comparison.



rac-**5j**

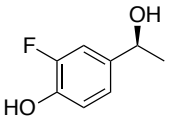
***rac*-3-Chloro-4-(1-hydroxyethyl)phenol (*rac*-5j)**. Modified procedure C: 1-(2-Chloro-4-hydroxyphenyl)ethan-1-one (100 mg, 586.2 µmol, Apollo Scientific) was dissolved in methanol (4 mL) and sodium borohydride (120 mg, 3.17 mmol, 5.4 equiv) was added in portions á 30 mg over 30 min whilst cooling the reaction on ice. Upon complete addition and ceased gas evolution, tlc (cyclohexane/ethylacetate 2:1) showed full conversion of the ketone and the reaction was quenched by addition of sat. aq NH_4SO_4 solution (10 mL). The suspension was diluted with water (5 mL), extracted with diethyl ether (3 × 10 mL) and the combined organic phases were washed with brine (15 mL) and dried over Na_2SO_4 before evaporation of the solvent under reduced pressure (30 °C, 900 mbar). The title compound was obtained as thick yellowish oil, which slowly crystallized upon storage (97 mg, 96% yield). The product was found to decompose with residual amounts of solvent (ethylacetate) upon storage at ambient atmosphere and 21 °C. Tlc (cyclohexane/ethylacetate 3:1): R_f = 0.34. ^{1}H NMR (300 MHz, acetone-d6) δ_H [ppm] 8.62 (bs, 1H), 7.50–7.46 (m, 1H), 6.84–6.79 (m, 2H), 5.16–5.08 (m, 1H), 4.23 (d, J = 3.6 Hz, 1H), 1.35 (d, J = 6.3 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d6) δ_C [ppm] 157.6, 136.2, 131.9, 128.4, 116.3, 115.3, 66.2, 24.9. *mp*

= 93.8–97.5 °C (acetone). GC-MS (EI+, 70 eV): t_R = 9.29 min; *m/z* (%) = 174 (6), 172 (17), 159 (31), 158 (8), 157 (100), 154 (12), 129 (13), 119 (7), 94 (15), 93 (28), 91 (10), 65 (25), 63 (13), 43 (17), 39 (11). HR-MS: *m/z* = 172.029238 (calcd. 172.029107, diff. 0.76 ppm). No literature data was available for comparison.

4. Isolation of hydration products from preparative scale biotransformations

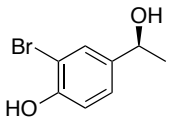
Enantiomerically pure products **5f**, **5g**, **5i**, and **5j** were isolated from 10-fold scaled biotransformations.

Lyases TPL_*Cf* M379V (120 mg, 5.37 U) and TAL_*Rs* (500 mg, 320 mU) as well as decarboxylase FDC_*Es* wt (50 mg, 80 U) and Ni-affinity purified FDC_*Es* V46E (100 µM) were suspended in reaction buffer (potassium phosphate buffer, 50 mM, pH 8.0) containing PLP (80 µM) and NH_4Cl (180 mM) (9.197 mL) in an INFORS cultivation shaker at 30 °C and 120 rpm for 15 min. Substrate phenol (**1f**–**g**, **1i**–**j**, 20 mM, 500 µL of a 400 mM stock in ^{i}PrOH) and sodium pyruvate (110 mg, 100 mM) were added to start the reaction and incubation was continued for 24 h at 30 °C and 120 rpm. Samples for HPLC were withdrawn (100 µL) and the reaction was extracted with MtBE (2 × 10 mL) after saturating the aqueous phase with sodium chloride. Phases were separated by centrifugation (4000 rpm, 20 min) and the combined organic layers were dried over anhydrous Na_2SO_4. After evaporation of the solvent and purification by column chromatography on silica gel using cyclohexane/MtBE 1:1 as eluent ($R_{f,\text{product}} \approx 0.30$) the product benzylic alcohols were obtained as colorless crystalline solids. The products could be recrystallized from cyclohexane/MtBE (75 °C) to enhance the *ee*



(*S*)-**5f**

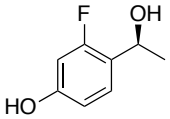
(*S*)-2-Fluoro-4-(1-hydroxyethyl)phenol [(*S*)-5f]. 26.7 mg (84% yield), 78% *ee* (*S*). ^{1}H NMR (300 MHz, acetone-*d6*) δ_H [ppm]: 8.44 (s, 1H, Ph–OH), 7.12 (dd, *J* = 12.4, 2.0 Hz, 1H, C_{ar}–H), 7.00 (dd, *J* = 8.3, 2.0 Hz, 1H, C_{ar}–H), 6.91 (t, *J* = 8.5 Hz, 1H, C_{ar}–H), 4.81–4.73 (m, 1H, Cα–H), 4.16 (d, *J* = 4.1 Hz, 1H, Cα–OH), 1.36 (d, *J* = 6.4 Hz, 3H, $C\beta H_3$). ^{13}C NMR (75 MHz, acetone-d6) δ_C [ppm]: 152.1 (d, *J* = 239.2 Hz), 144.2 (d, *J* = 13.1 Hz), 140.6 (d, *J* = 5.0 Hz), 122.2 (d, *J* = 3.2 Hz), 118.1 (d, *J* = 2.8 Hz), 113.7 (d, *J* = 18.7 Hz), 69.1 (d, *J* = 1.3 Hz), 26.17 (s). GC-MS (ESI+): matches racemic reference. *mp* = 88.0–89.0 °C (MeOH). $[\alpha]_D^{20}$ = –24.73° (c = 1.0, MeOH, *ee* = 87%). HR-MS *m/z* = 156.058689 (calcd. 156.058658, diff. 0.20 ppm).



(*S*)-**5g**

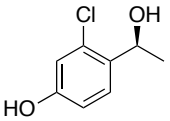
(*S*)-2-Bromo-4-(1-hydroxyethyl)phenol [(*S*)-5g]. 23.8 mg (58% yield), 88% *ee* (*S*). ^{1}H NMR (300 MHz, acetone-*d6*) δ_H [ppm]: 8.65 (s, 1H, Ph–OH), 7.51 (d, *J* = 2.0 Hz, 1H, C_{ar}–H), 7.19 (dd, *J* = 8.3, 2.1 Hz, 1H, C_{ar}–H), 6.94 (d, *J* = 8.3 Hz, 1H, C_{ar}–H), 4.81–4.73 (m, 1 H, Cα–H), 4.17 (d, *J* = 4.0 Hz, 1H, Cα–OH), 1.36 (d, *J* = 6.4 Hz, 3H, $C\beta H_3$). ^{13}C NMR (75 MHz, acetone-d6) δ_C [ppm]:

153.5, 141,3, 130.8, 126.7, 116.9, 109.9, 68.9, 26.2. GC-MS (ESI+): matches racemic reference. *mp* = 93.5–94.5 °C (MeOH). $[\alpha]_D^{20}$ = –25.93° (c = 1.1, MeOH, *ee* = 97%). HR-MS *m/z* = 215.978736 (calcd. 215.978592, diff. 0.66 ppm).



(*S*)-**5i**

(*S*)-3-Fluoro-4-(1-hydroxyethyl)phenol [(*S*)-5i]. 23.1 mg, (72% yield), 85% *ee* (*S*). ^{1}H NMR (300 MHz, acetone-*d6*) δ_H [ppm]: 8.61 (s, 1H, Ph–OH), 7.36 (t, *J* = 8.7 Hz, 1H, C_{ar}–H), 6.66 (d, *J* = 2.4 Hz, 1H, C_{ar}–H), 6.51 (dd, *J* = 12.2, 2.4 Hz, 1H, C_{ar}–H), 5.12–4.93 (m, 1H, Cα–H), 4.12 (d, *J* = 4.3 Hz, 1H, Cα–OH), 1.37 (d, *J* = 6.4 Hz, 3H, Cβ–H_3). ^{13}C NMR (75 MHz, acetone-d6) δ_C [ppm]: 160.8 (d, *J* = 242.7 Hz), 158.4 (d, *J* = 11.6 Hz), 128.3 (d, *J* = 6.9 Hz), 125.6 (d, *J* = 14.2 Hz), 112.0 (d, *J* = 2.9 Hz), 103.0 (d, *J* = 25.0 Hz), 63.4 (d, *J* = 2.6 Hz), 25.2 (d, *J* = 0.6 Hz). GC-MS (ESI+): matches racemic reference. *mp* = 104.5–107.5 °C (MeOH). $[\alpha]_D^{20}$ = –26.79° (c = 0.8, MeOH, *ee* = 98%). HR-MS *m/z* = 156.058715 (calcd. 156.058658, diff. 0.37 ppm).



(*S*)-**5j**

(*S*)-3-Chloro-4-(1-hydroxyethyl)phenol [(*S*)-5j]. 10.2 mg, (29% yield), 92% *ee* (*S*). ^{1}H NMR (300 MHz, acetone-*d6*) δ_H [ppm]: 8.60 (s, 1H, Ph–OH), 7.58–7.38 (m, 1H, C_{ar}–H), 6.91–6.73 (m, 2H, 2 × C_{ar}–H), 5.22–5.00 (m, 1H, Cα–H), 4.20 (d, *J* = 4.0 Hz, 1H, Cα–OH), 1.34 (d, *J* = 6.3 Hz, 3H, Cβ–H_3). ^{13}C NMR (75 MHz, acetone-d6) δ_C [ppm]: 157.6, 136.2, 131.9, 128.4, 116.3, 115.3, 66.2, 24.9. GC-MS (ESI+): matches racemic reference. *mp* = 122.4–125.7 °C (MeOH). $[\alpha]_D^{20}$ = –39.84° (c = 0.8, MeOH, *ee* >99%). HR-MS: *m/z* = 172.029090 (calcd. 172.029107, diff. –0.10 ppm).

5. Determination of conversion and enantiomeric excess (*ee*)

5.1. Compound quantification and determination of conversion

Conversion was determined after quenching an aliquot of the biotransformation (100 µL) with a mixture of water/acetonitrile 1:1 containing trifluoroacetic acid (TFA, 0.1% v/v) (900 µL). After incubation at 21 °C for 30 min and centrifugation at 14 000 rpm for 5 min, the clear supernatant was analyzed on an Agilent Infinity 1260 HPLC system equipped with a Phenomenex Luna C18(2) column (250 mm × 4.6 mm × 5 µm) and a diode array detector (DAD). The following method with water (A) and acetonitrile (B) modified with TFA (0.1% v/v) as solvents was used: 0–2 min (100% A), 2–15 min (100–0% A), 15–17 min (0% A), 17–19 min (0–100% A), 19–22 min (100% A); 1 mL min^{-1}; 5 µL sample injected. Peaks were integrated at λ = 280 nm and conversions were deduced from compound concentrations after calibration (R^2 ≥0.999) with independently synthesized or commercially obtained reference material (calibration factors and retention times see Table S1).

Table S1 HPLC retention times and calibration factors

	t_r [min] (calibration coefficient *k* [mAU/mM])[a]				
R (denotifier)	**1**	**2**	**3**	**4**	**5**
H (**a**)	12.0 (365.8)	8.6 (294.8)	10.9 (2318.0)	13.4 (614.6)	9.9 (324.5)
2-Cl (**b**)	13.3 (574.3)	9.2 (581.9)	12.0 (2593.3)	14.5 (547.3)	11.4 (589.1)
2-OMe (**c**)	–	–	12.5 (1046.4)	15.1 (839.7)	12.3 (719.2)
2-OEt (**d**)	–	–	12.0 (876.7)	14.6 (1201.3)	11.2 (721.0)
2-Me (**e**)	13.2 (381.9)	9.2 (255.0)	11.8 (1359.2)	14.4 (381.9)	10.9 (464.5)
2-F (**f**)	12.5 (161.6)	8.8 (309.3)	11.4 (2470.4)	13.8 (995.7)	10.6 (286.0)
2-Br (**g**)	13.6 (709.6)	9.4 (446.9)	12.2 (3073.4)	14.7 (709.6)	11.6 (393.9)
2-NO_2 (**h**)	–	–	12.5 (7291.7)	15.1 (1375.7)	12.3 (1000.4)
3-F (**i**)	12.7 (149.5)	8.8 (266.01)	11.6 (3036.1)	14.0 (653.6)	10.8 (304.2)
3-Cl (**j**)	13.7 (154.0)	9.3 (257.9)	12.0 (2297.3)	14.4 (684.1)	11.6 (451.7)

[a] Calibration function: *c*(analyte) = *area*(analyte) / *k*

5.2. Derivatization (acetylation) of biotransformation products and determination of the enantiomeric excess (ee)

After withdrawing aliquots for HPLC analytics, the biotransformations (900 µL, 10 mM substrate used) were extracted with ethyl acetate (2 × 500 µL) and the extract was dried over anhydrous $MgSO_4$. The dried extracts were transferred to a 1.5 mL Eppendorf vial with potassium carbonate (Sigma Aldrich, ≥99%, 10 mg) and acetic anhydride was added (>99%, Sigma Aldrich, 5 µL, 50 mM, 5 equiv) except for product **5h**, which had to be derivatized with catalytic amounts of *N*,*N*-dimethylaminopyridine (DMAP, 0.5 mg) instead of potassium

carbonate under otherwise identical conditions. The reaction was shaken for 15 min at 40 °C and 1000 rpm in an Eppendorf Thermomixer before quenching with water (200 µL) and continued incubation for 30 min.

The organic phase was withdrawn and dried again over $MgSO_4$ before analyzing the samples on an Agilent 7890 A GC system equipped with a flame ionization detector (FID). Separation of enantiomers was achieved using the chiral stationary phases and temperature programs listed in Table S2.

Table S2 Separation methods and enantiomer retention times on GC-FID.

compound	R	method	$t_{R,1}$	$t_{R,2}$
5a	H	A	11.4 (*R*)	11.7 (*S*)
5b	2-Cl	B	17.0 (*R*)	17.5 (*S*)
5c	2-OMe	B	17.3 (*R*)	17.6 (*S*)
5d	2-OEt	B	18.2	18.3
5e	2-Me	A	12.5 (*R*)	12.6 (*S*)
5f	2-F	A	11.1 (*R*)	11.5 (*S*)
5g	2-Br	C	21.9 (*R*)	22.1 (*S*)
5h	2-NO_2	D	24.5	24.8
5i	3-F	A	10.7 (*R*)	11.1 (*S*)
5j	3-Cl	C	17.0 (*R*)	19.1 (*S*)

Methods **A**–**C**: Agilent CP Chirasil DEX-CB (25 m × 0.32 mm × 0.25 µm film), H_2 carrier (1.3 mL min^{-1} const.), inlet temp 250 °C, split 50:1, 1 µL injection. 100 °C (1 min hold) – 10 °C min^{-1} – 160 °C (*X* min hold) – 20 °C min^{-1} – 180 °C (1 min hold); **A**: *X* = 6 min, **B**: *X* = 19 min, **C**: *X* =23 min. Method **D**: Restek Rt-bDEXse (30 m × 0.32 mm × 0.25 µm film), He carrier (1.8 mL min^{-1} const.), inlet temp 230 °C, split 50:1, 1 µL injection, 100 °C (1 min hold) – 10 °C min^{-1} – 170 °C (25 min hold) – 20 °C min^{-1} – 180 °C (1 min hold).

5.3. Determination of the absolute configuration

Absolute configuration of **5b**, **5c** and **5e** was determined to (*S*) *via* comparison of HPLC elution order to the literature.[2] (No reference data was available for **5d** and **5h**).

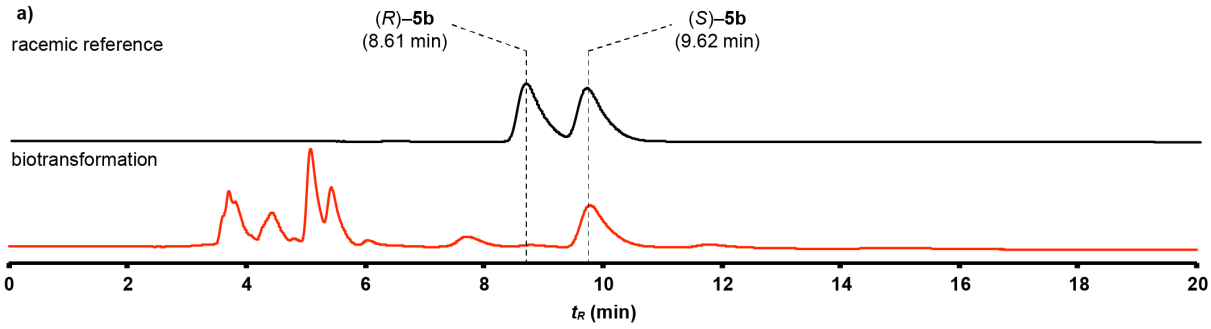


Figure S1 Example HPLC traces for the determination of the absolute configuration of chiral alcohols **5b**.

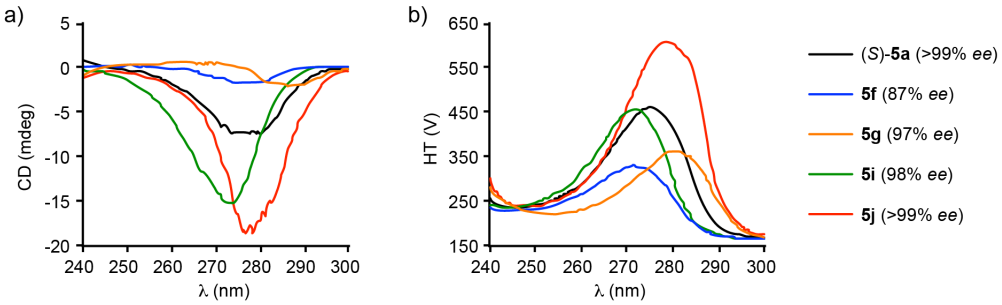


Figure S2 Circular dichroism (CD) spectra for determining the absolute configuration. The (*S*)-configured reference (*S*)-**5a** (black line) as well as all products from the biotransformation upscale show a negative phase in the CD-spectrum between 240 and 300 nm.

6. Additional results

Table S3 Comparison of a one-pot approach operating at pH 8.0 and a sequential approach with intermittent pH adjustment to 6.0 before addition of the hydratase.[a]

substrate	product	R	conv[b] (*ee*) [%] one-pot (24 h)	sequential (24 + 5 h)	sequential (24 +19 h)
1a	**5a**	H	73 (92)	n.d.	61 (83)
1b	**5b**	2-Cl	85 (83)	68 (90)	83 (85)
1e	**5e**	2-Me	12[c] (95)	n.d.	6[b] (87)
1f	**5f**	2-F	91 (81)	61 (76)	88 (75)
1g	**5g**	2-Br	84 (87)	49 (91)	68 (88)
1i	**5i**	3-F	80 (87)	n.d.	70 (81)
1j	**5j**	3-Cl	36 (95)	n.d.	28 (93)

[a]Reaction conditions: Lyophilized *E. coli* whole cells containing the heterologously expressed TPL_*Cf* M379V (10 mg mL^{-1}, 448 mU), TAL_*Rs* (40 mg mL^{-1}, 35 mU), FDC_*E*s wt (2 mg mL^{-1}, 3.2 U) in reaction buffer [potassium phosphate buffer (50 mM, pH 8.0) sodium pyruvate (92 mM), NH_4Cl (180 mM) and PLP (80 µM), pH adjusted to 8.0] with substrate phenol (10 mM, 50 µL of a 200 mM stock in ^{i}PrOH, 5% v/v). Incubate at 30 °C and 850 rpm for 24 h before adjusting the pH to 6.0 with H_3PO_4 aq (1 M, 34 µL mL^{-1}) and addition of purified FDC_*E*s V46E variant (100 µM, 1 mol %). Incubation continued for 19 h at 25 °C, 700 rpm. [b]Conversion to **5**, the corresponding vinylphenol **4** was detected as the major remaining intermediate. [c]Phenol **1** and tyrosine **2** detected as major constituents.

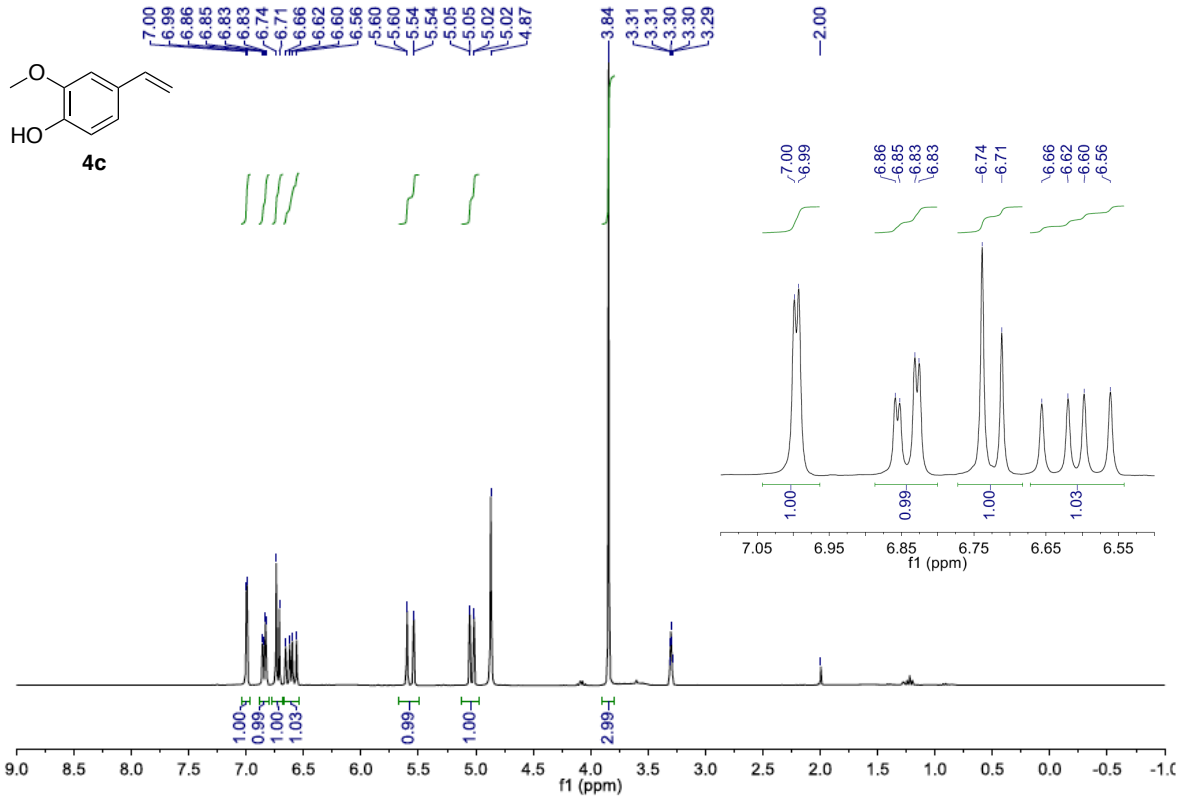
Table S4 Up-scaled hydroxyethylation cascade for isolation of products.

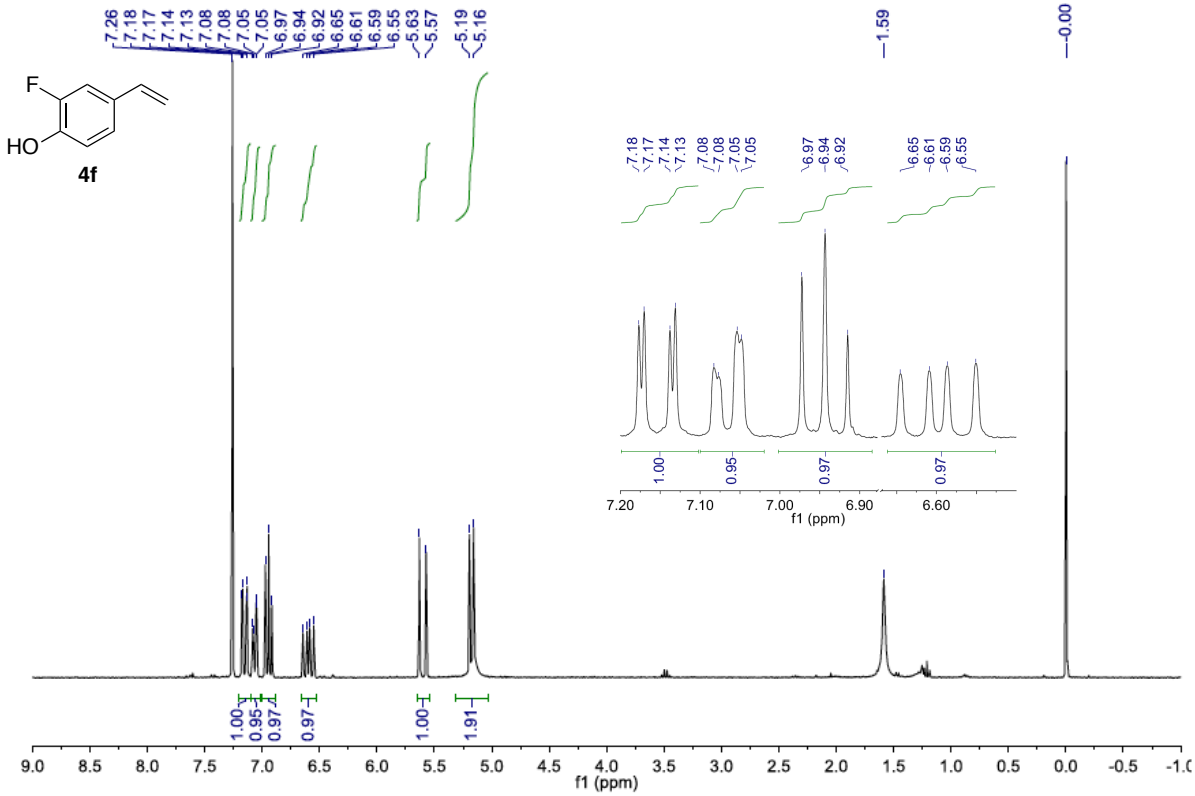
substrate	product	R	conv (isol. yield) [%]	*ee* [%]
1f	**5f**	2-F	91 (84)	78
1g	**5g**	2-Br	69 (58)	88
1i	**5i**	3-F	78 (72)	85
1j	**5j**	3-Cl	38 (29)[a]	92

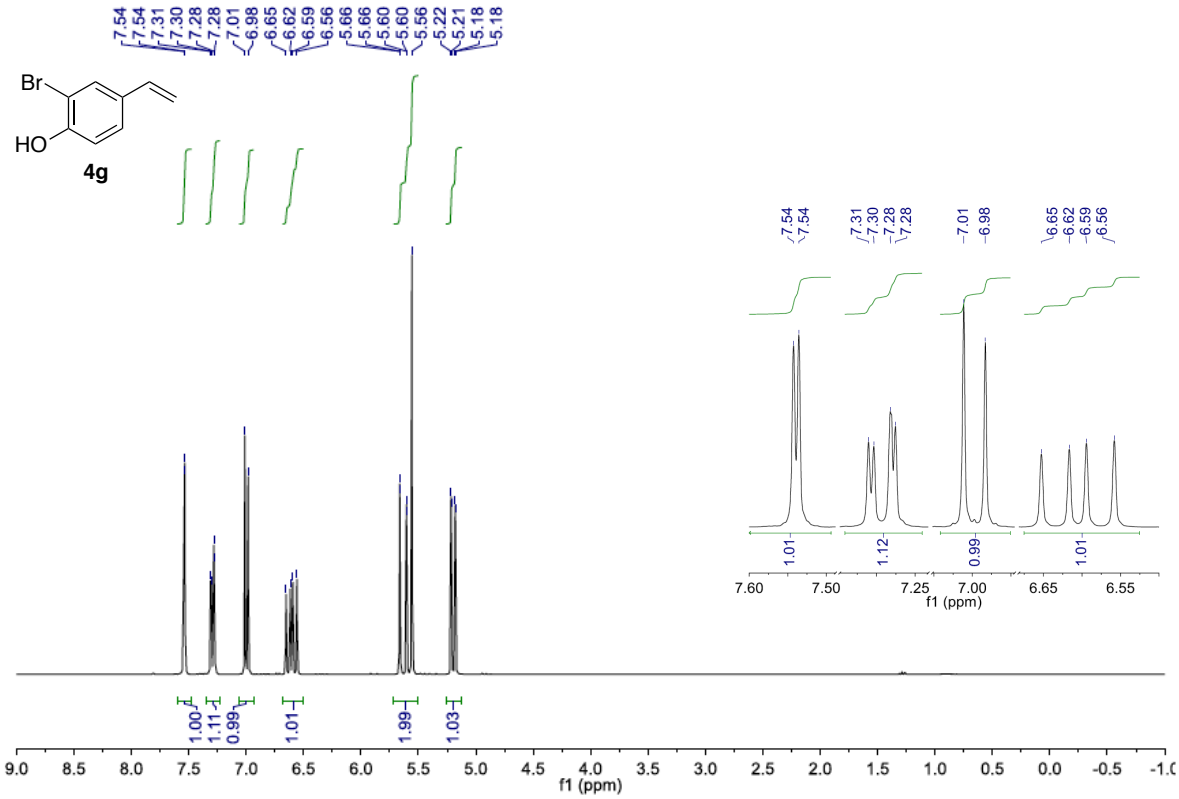
Reaction conditions: KP_i-buffer (pH 8.0; 50 mM, 9.197 mL) containing PLP (40 µM) and NH_4Cl (180 mM) (pH adjusted to 8.0 with KOH, 10 M); TPL_*Cf* M379V cfe (120 mg, 5.37 U); TAL_*Rs* whc (500 mg, 405 mU); FDC_*E*s wt whc (50 mg, 80 U); purified FDC_*E*s V46E (303 µL of a 3.3 mM stock, 100 µM), substrate **1** (20 mM, 500 µL of a 400 mM stock in ^{i}PrOH), sodium pyruvate (100 mM), 30 °C, 120 rpm. [a]Full conversion to vinylphenol **4j** was detected after 24 h and a second portion of FDC_*E*s V46E hydratase (100 µL) was added, incubation continued prolonged for another 14 h.

7. Spectroscopic data

7.1. *^{1}H NMR spectra*



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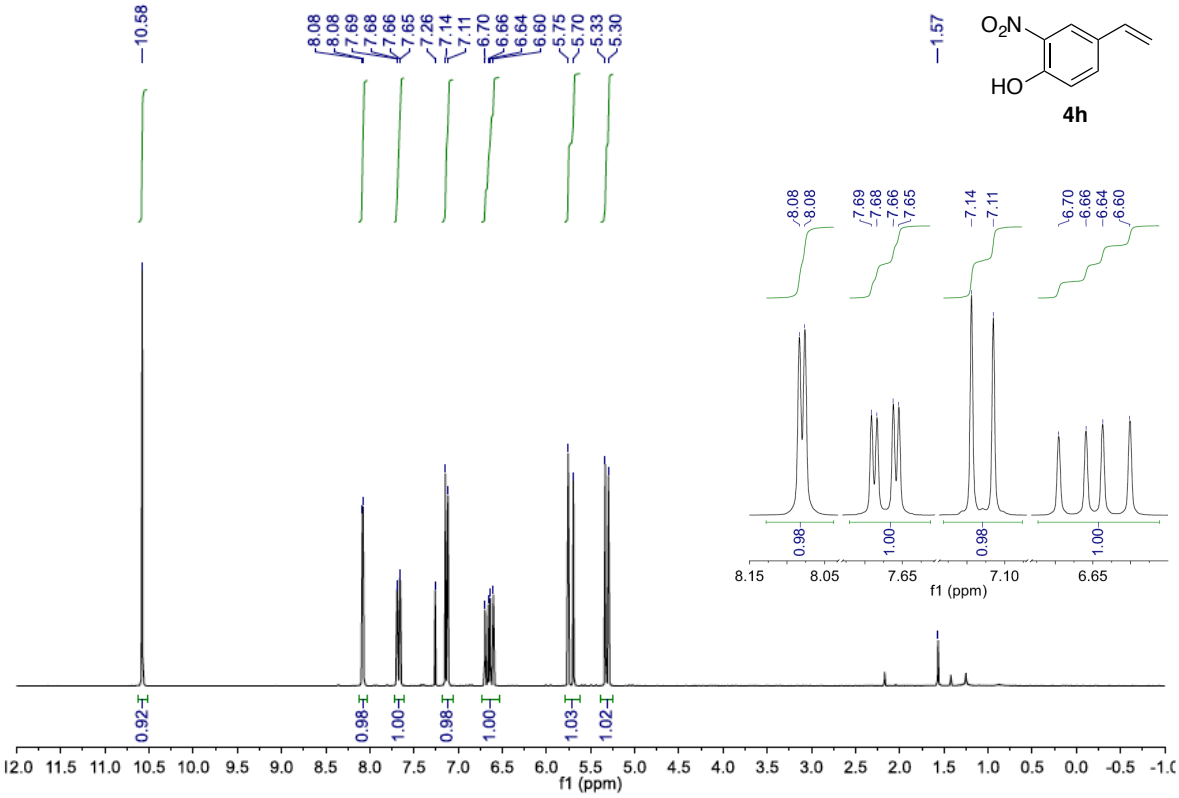
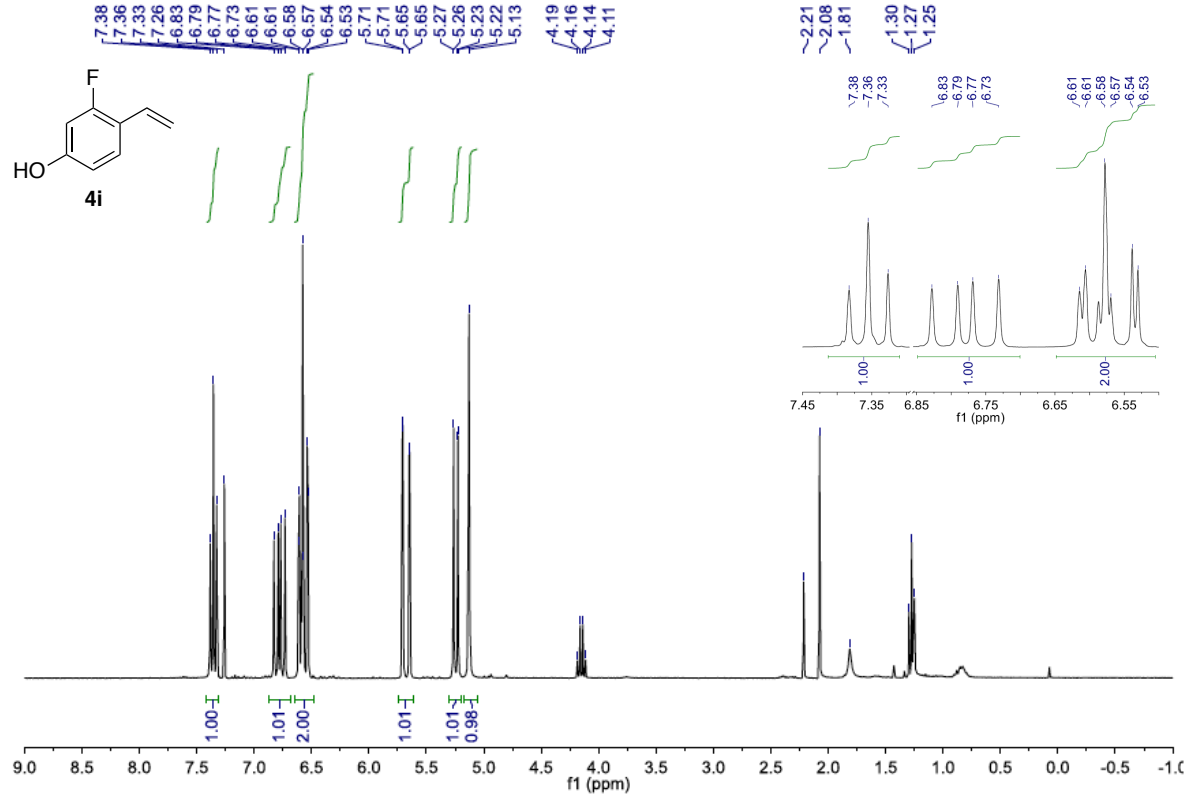
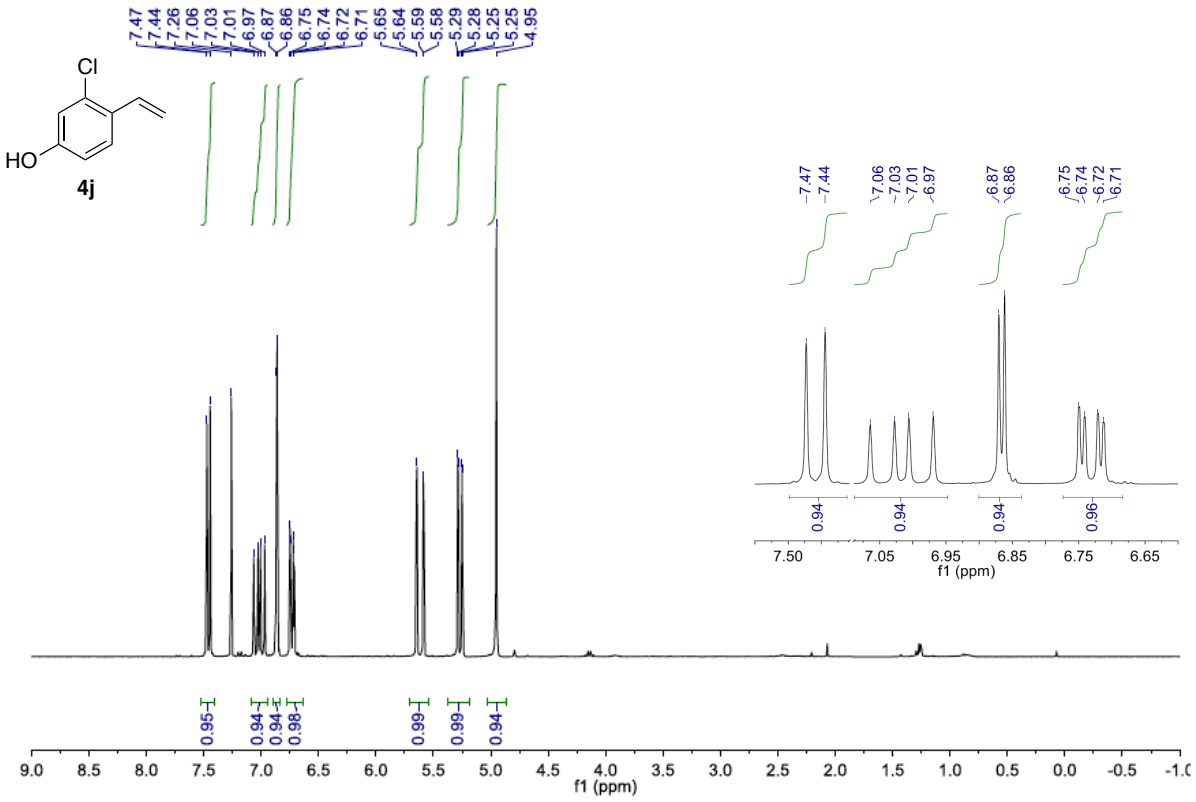
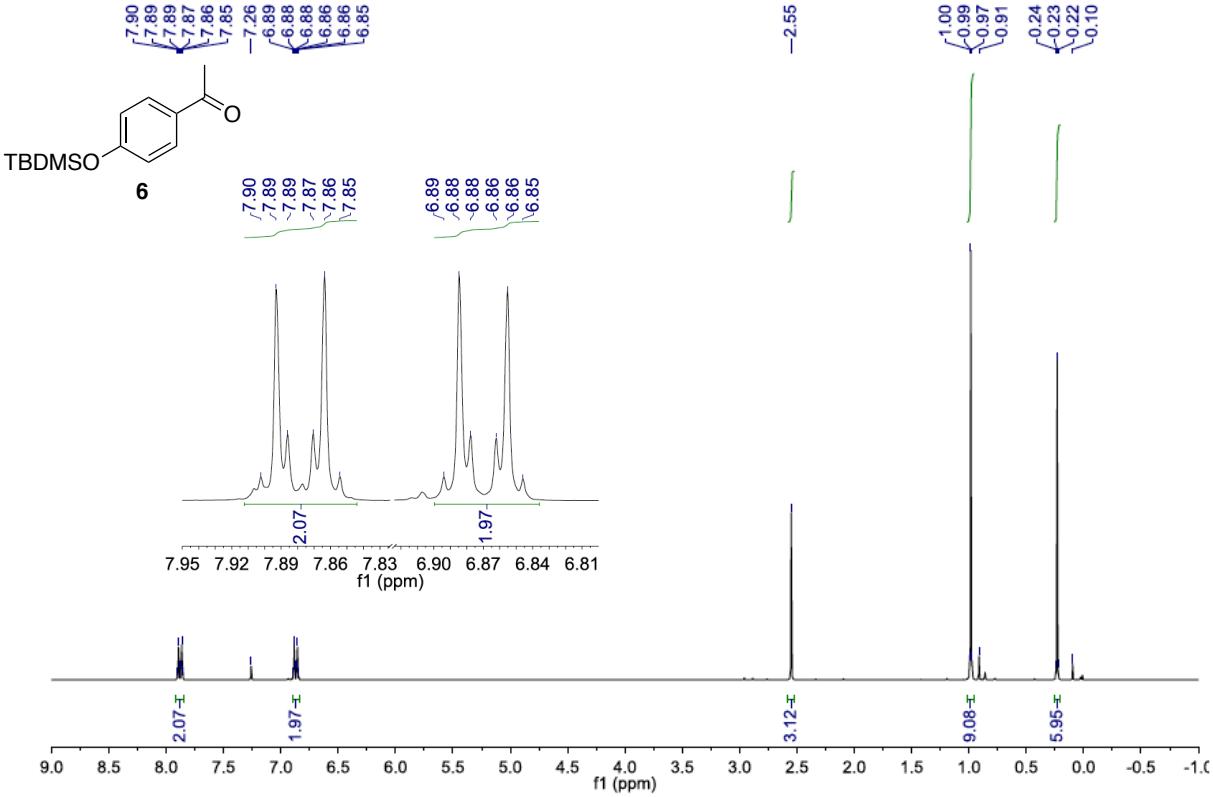
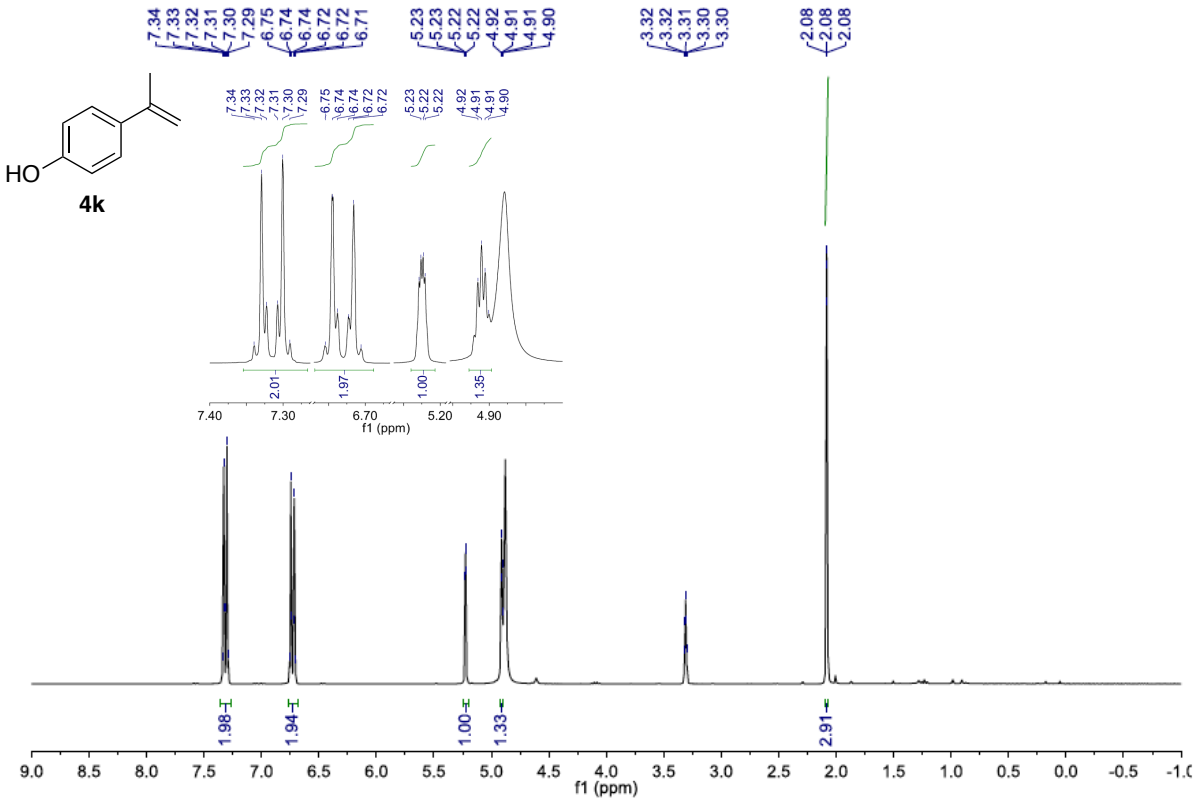
7.54
7.31
7.30
7.28
7.28
7.01
6.98
6.65
6.62
6.59
6.56
5.66
5.66
5.60
5.60
5.56
5.22
5.21
5.18
5.18
Br
HO
4g
1.00
1.11
0.99
1.01
1.99
1.03
f1 (ppm)
1.01
1.12
0.99
1.01


10.58
8.08
8.08
7.69
7.68
7.66
7.65
7.26
7.14
7.11
6.70
6.66
6.64
6.60
5.75
5.70
5.33
5.30
1.57
O2N
HO
4h
0.92
0.98
1.00
0.98
1.00
1.03
1.02
f1 (ppm)
0.98
1.00
0.98
1.00


F
HO
4i
f1 (ppm)


Cl
HO
4j
f1 (ppm)


TBDMSO
O
6
7.90
7.89
7.89
7.87
7.86
7.85
7.26
6.89
6.88
6.88
6.86
6.86
6.85
2.55
1.00
0.99
0.97
0.91
0.24
0.23
0.22
0.10
2.07
1.97
7.95 7.92 7.89 7.86 7.83 6.90 6.87 6.84 6.81
f1 (ppm)
2.07
1.97
3.12
9.08
5.95
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


HO
4k
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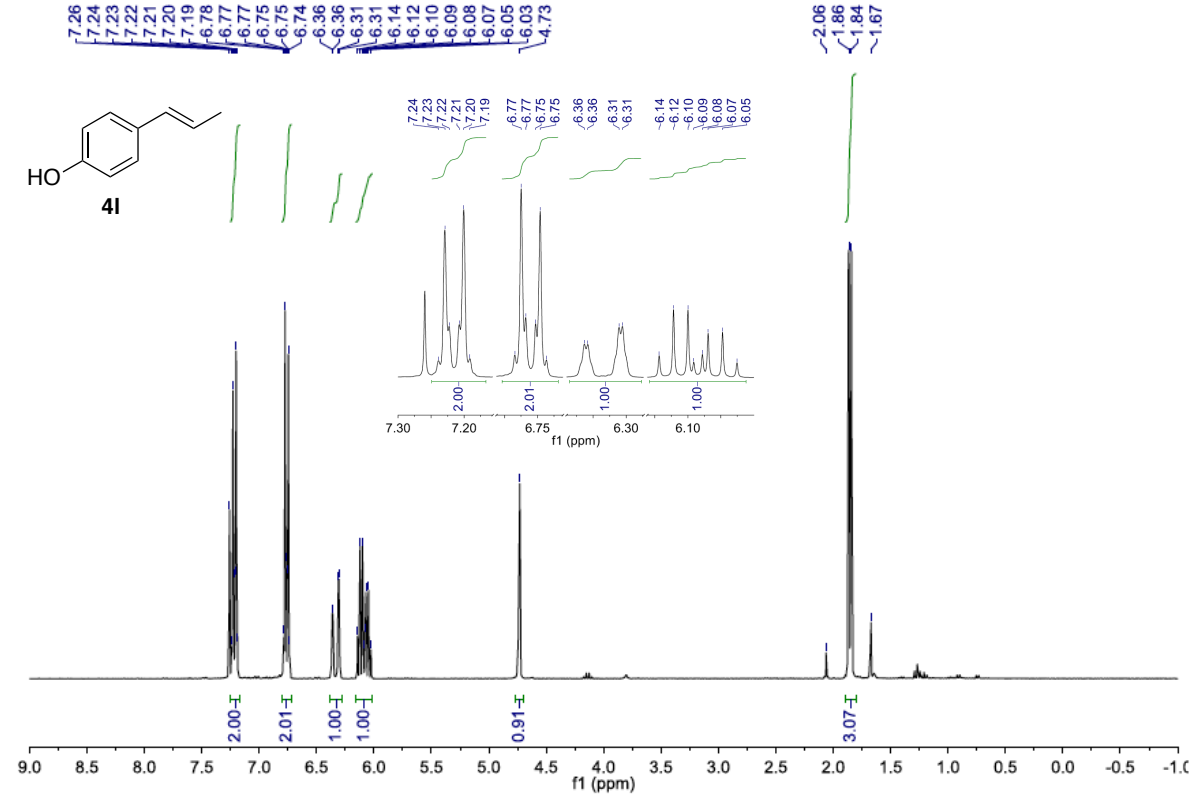
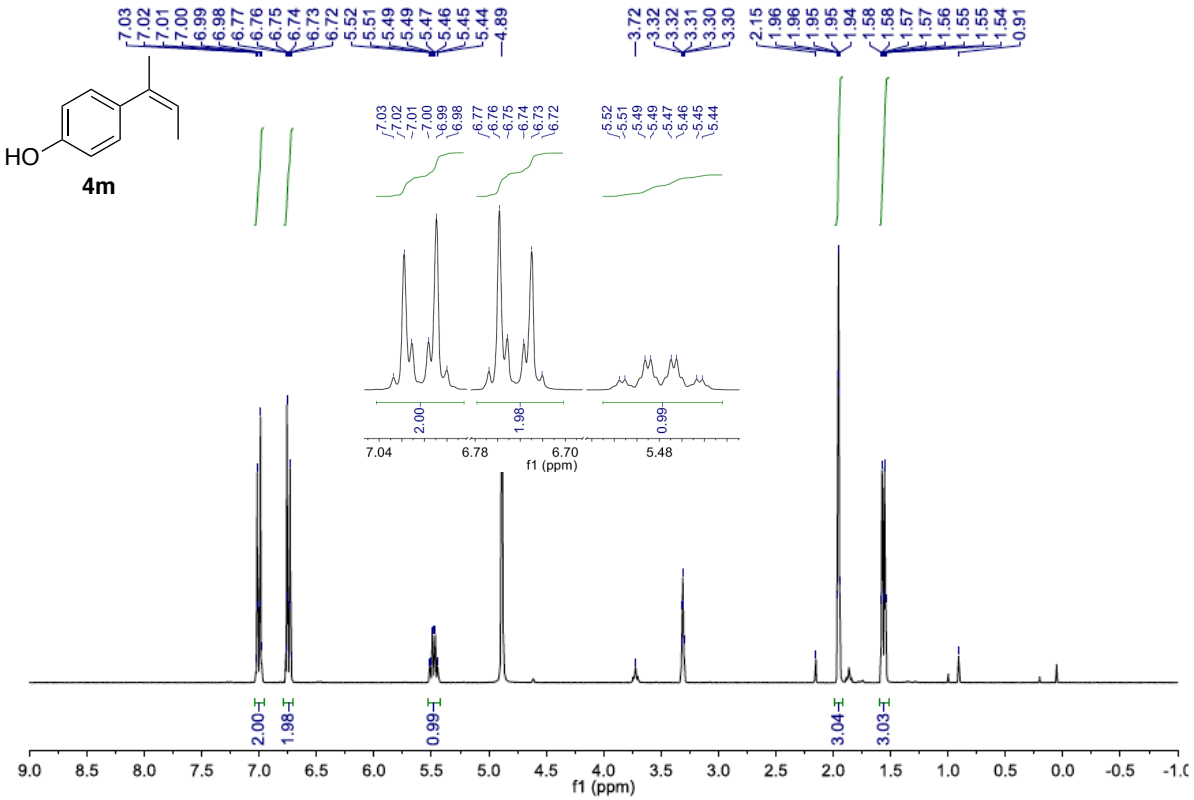
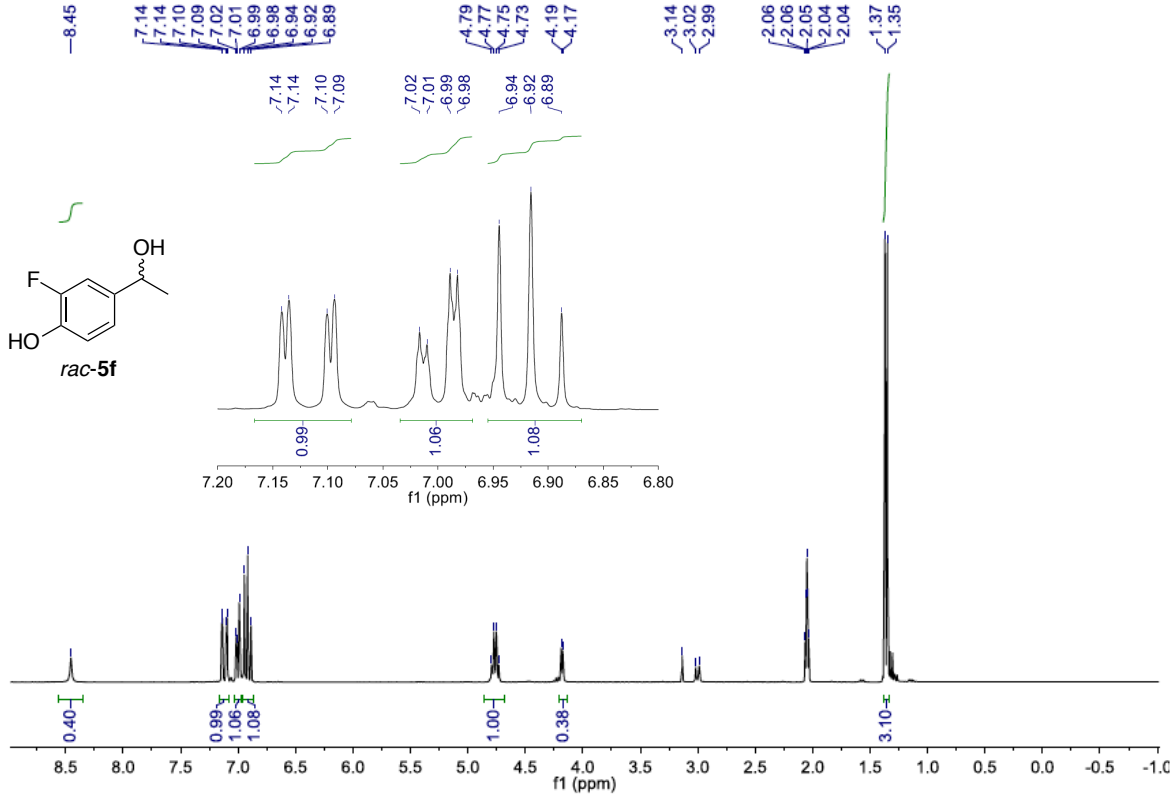
7.33
7.32
7.31
7.30
7.29
6.75
6.74
6.74
6.72
6.72
6.71
5.23
5.23
5.22
5.22
4.92
4.91
4.91
4.90
3.32
3.32
3.31
3.30
3.30
2.08
2.08
2.08
2.01
1.97
1.00
1.35
7.40 7.30 6.70 5.20 4.90
f1 (ppm)
1.98
1.94
1.00
1.33
2.91
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


HO
4l
f1 (ppm)


HO
4m
f1 (ppm)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7.14
7.14
7.10
7.09
7.02
7.01
6.99
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6.94
6.92
6.89
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4.77
4.75
4.73
4.19
4.17
3.14
3.02
2.99
2.06
2.06
2.05
2.04
2.04
1.37
1.35
OH
F
HO
rac-5f
0.99
1.06
1.08
f1 (ppm)
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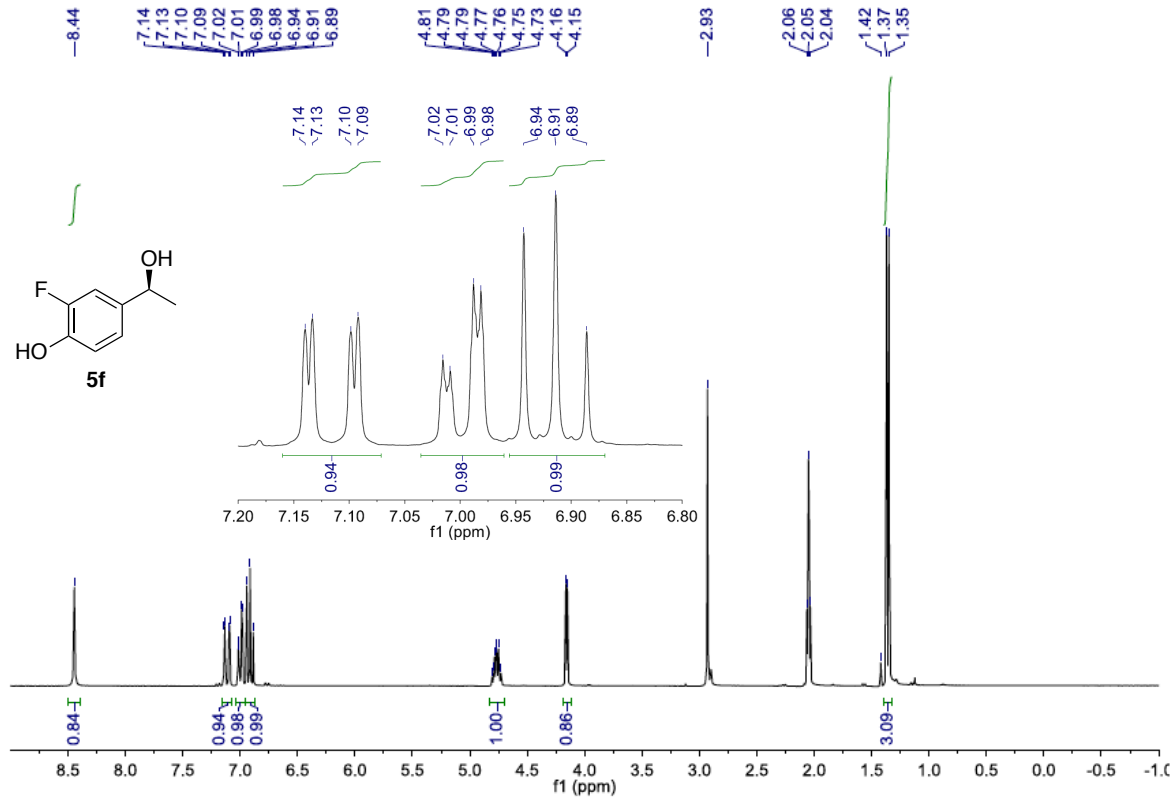
0.99
1.06
1.08
1.00
0.38
3.10
f1 (ppm)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7.01
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6.98
6.94
6.91
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4.81
4.79
4.79
4.77
4.76
4.75
4.73
4.16
4.15
2.93
2.06
2.05
2.04
1.42
1.37
1.35
OH
F
HO
5f
0.94
0.98
0.99
f1 (ppm)
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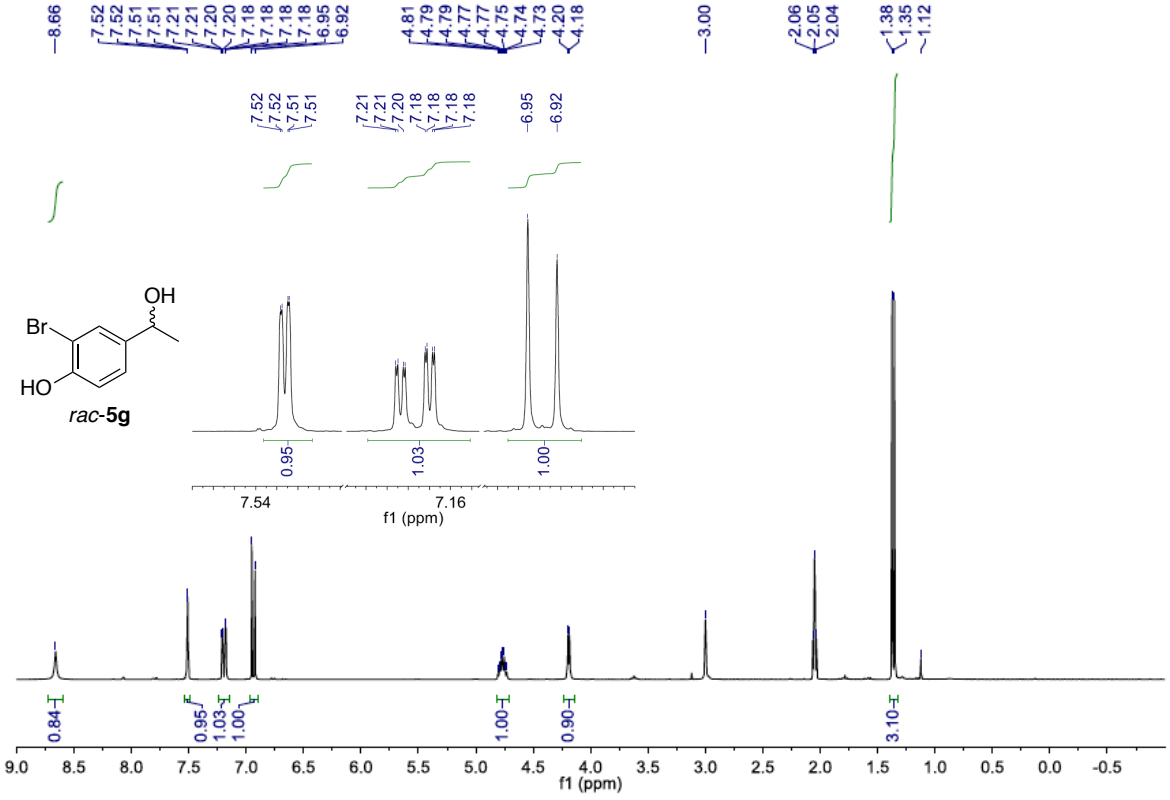
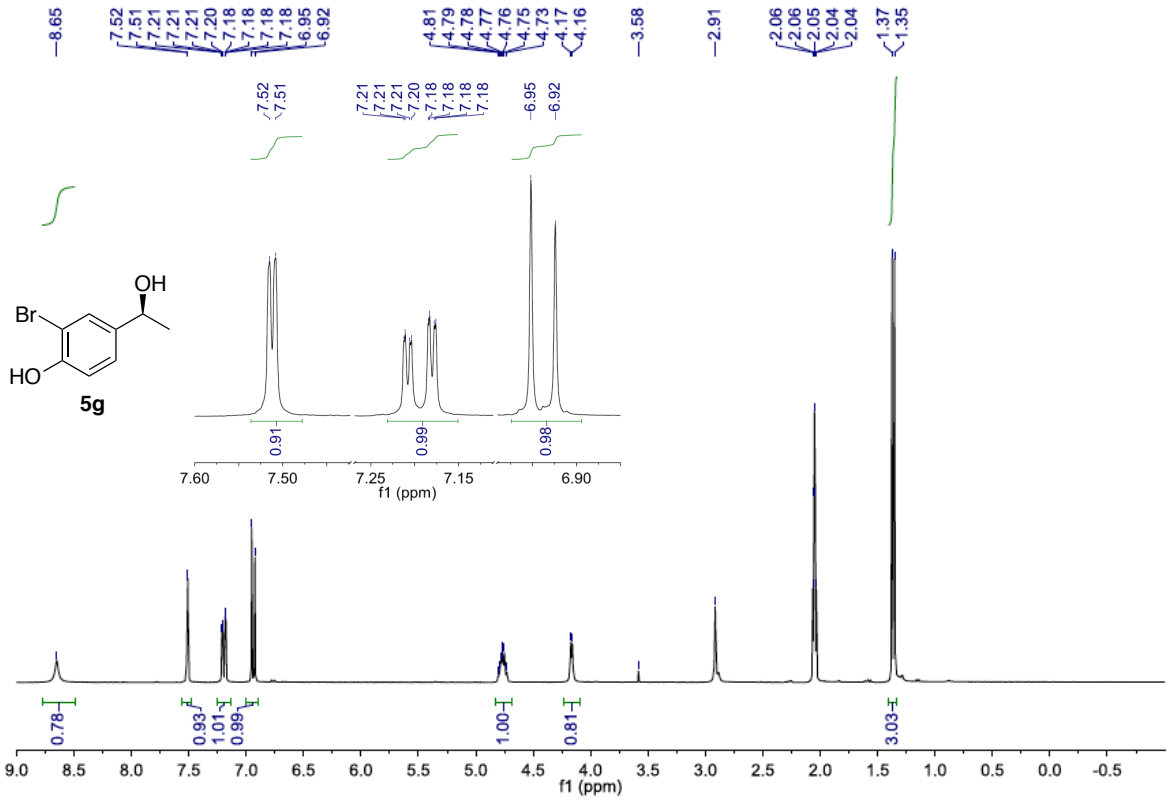
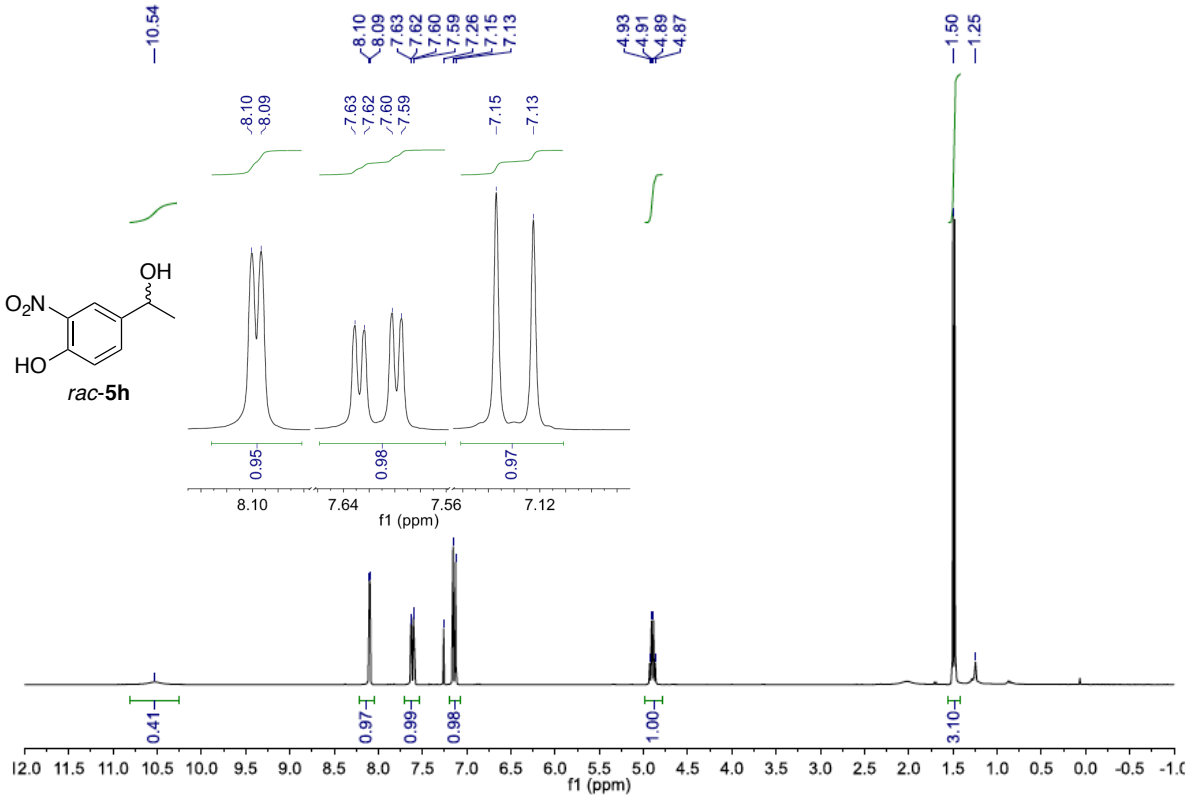
0.94
0.98
0.99
1.00
0.86
3.09
f1 (ppm)


OH
Br
HO
rac-5g
f1 (ppm)
f1 (ppm)


OH
Br
HO
5g
f1 (ppm)
f1 (ppm)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7.26
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7.13
4.93
4.91
4.89
4.87
1.50
1.25
OH
O2N
HO
rac-5h
0.95
0.98
0.97
8.10
7.64
7.56
7.12
f1 (ppm)
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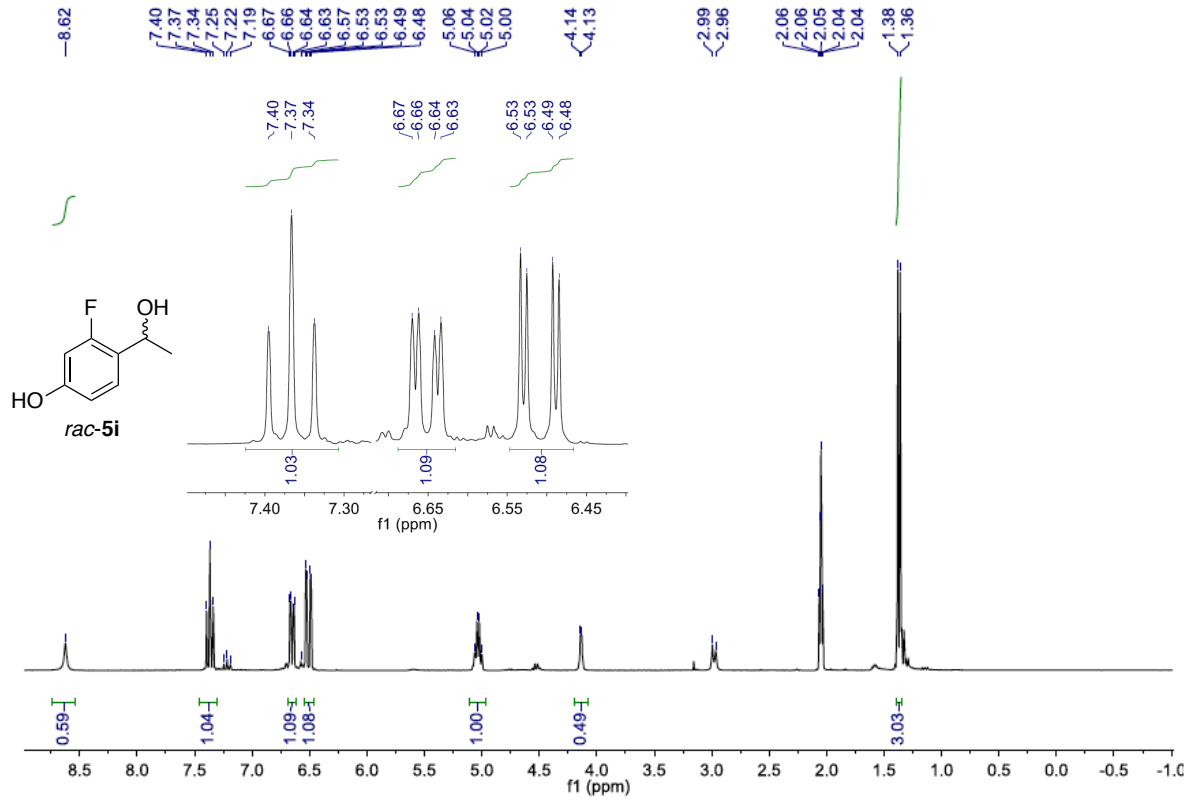
0.99
0.98
1.00
3.10
f1 (ppm)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7.19
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6.66
6.64
6.63
6.57
6.53
6.53
6.49
6.48
5.06
5.04
5.02
5.00
4.14
4.13
2.99
2.96
2.06
2.06
2.05
2.04
2.04
1.38
1.36
F
OH
HO
rac-5i
1.03
1.09
1.08
7.40
7.30
6.65
6.55
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f1 (ppm)
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1.08
1.00
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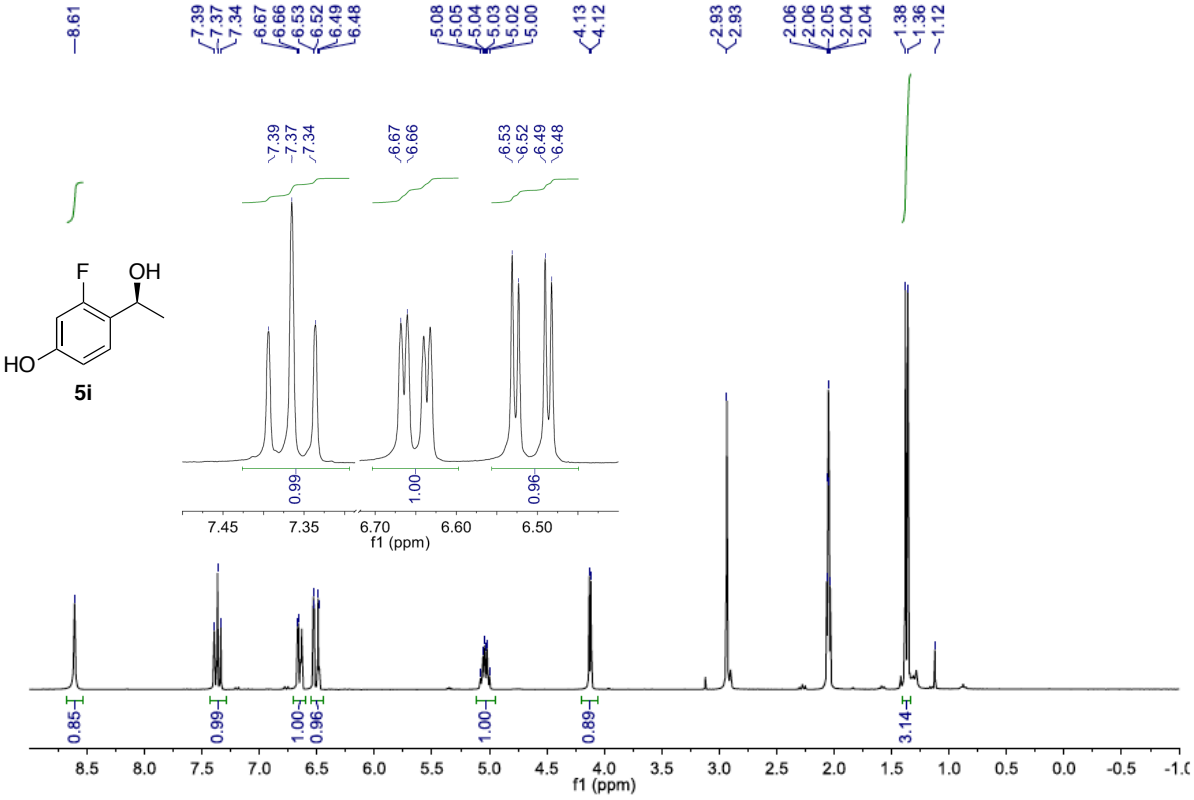
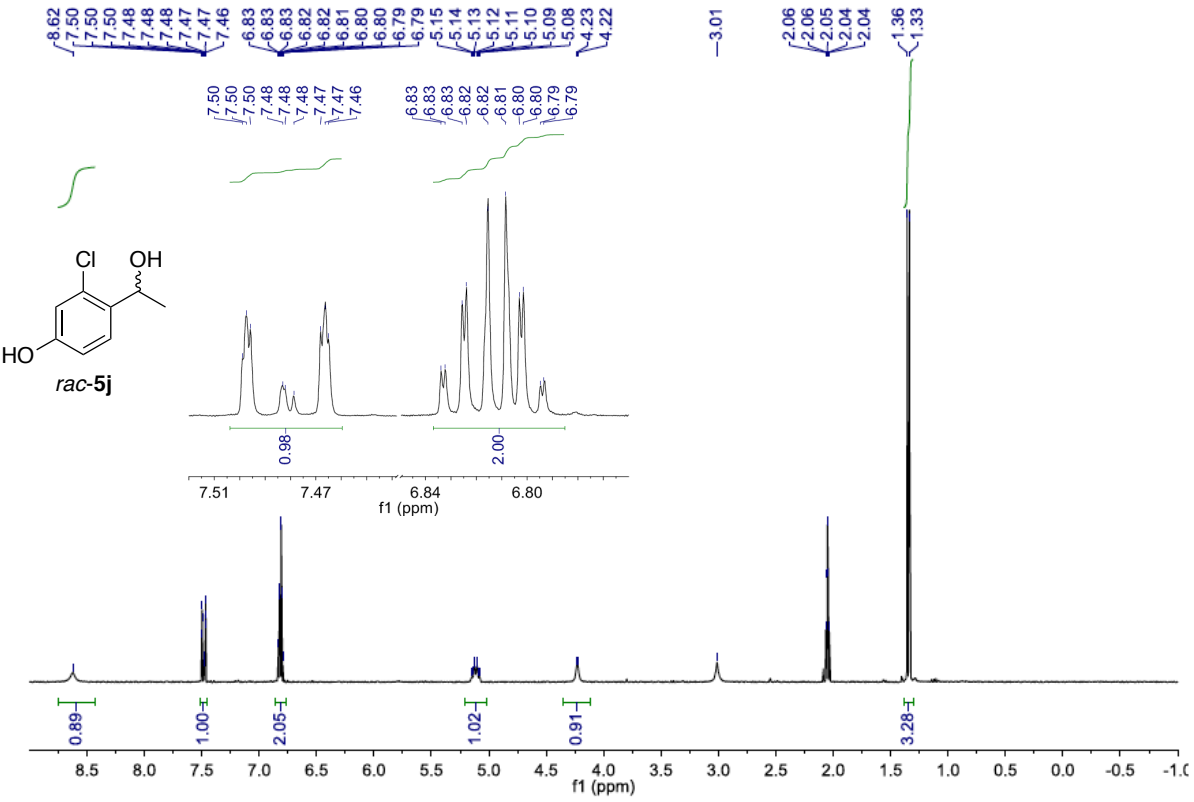
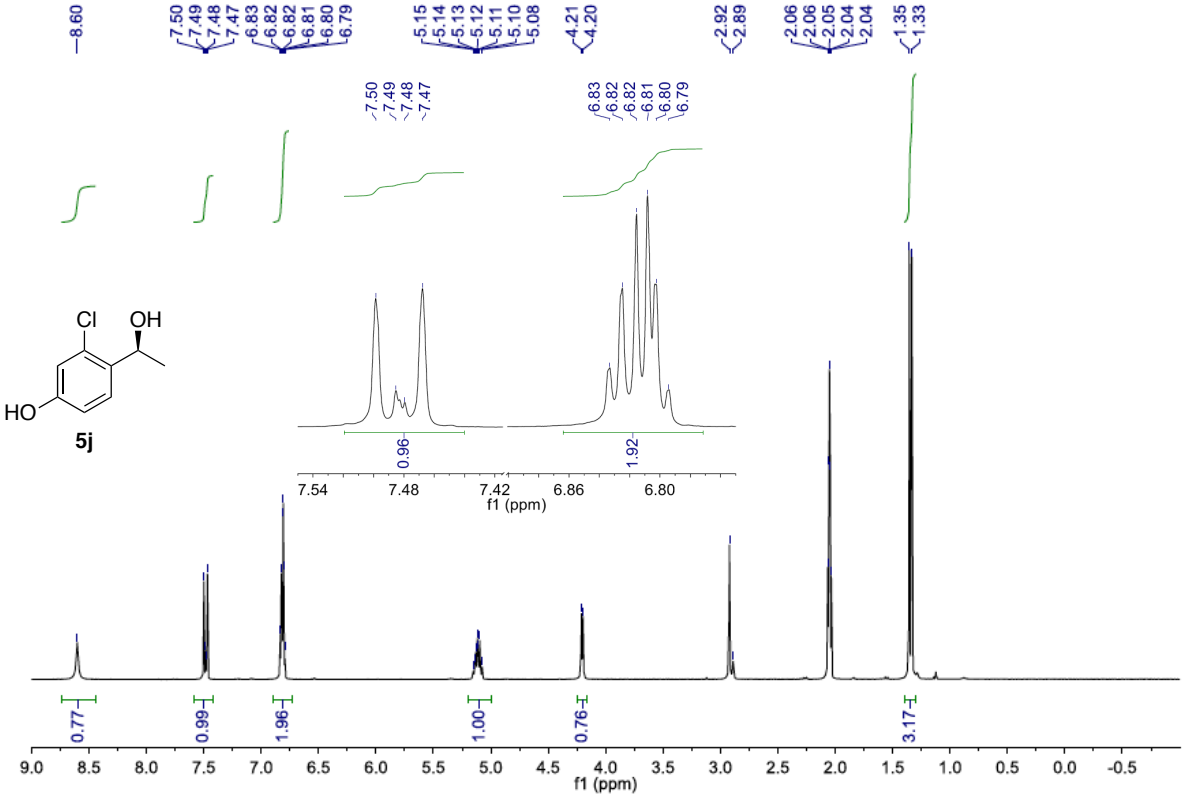
3.03
f1 (ppm)


F
OH
HO
5i
f1 (ppm)


Cl
OH
HO
rac-5j
f1 (ppm)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7.48
7.47
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6.82
6.82
6.81
6.80
6.79
5.15
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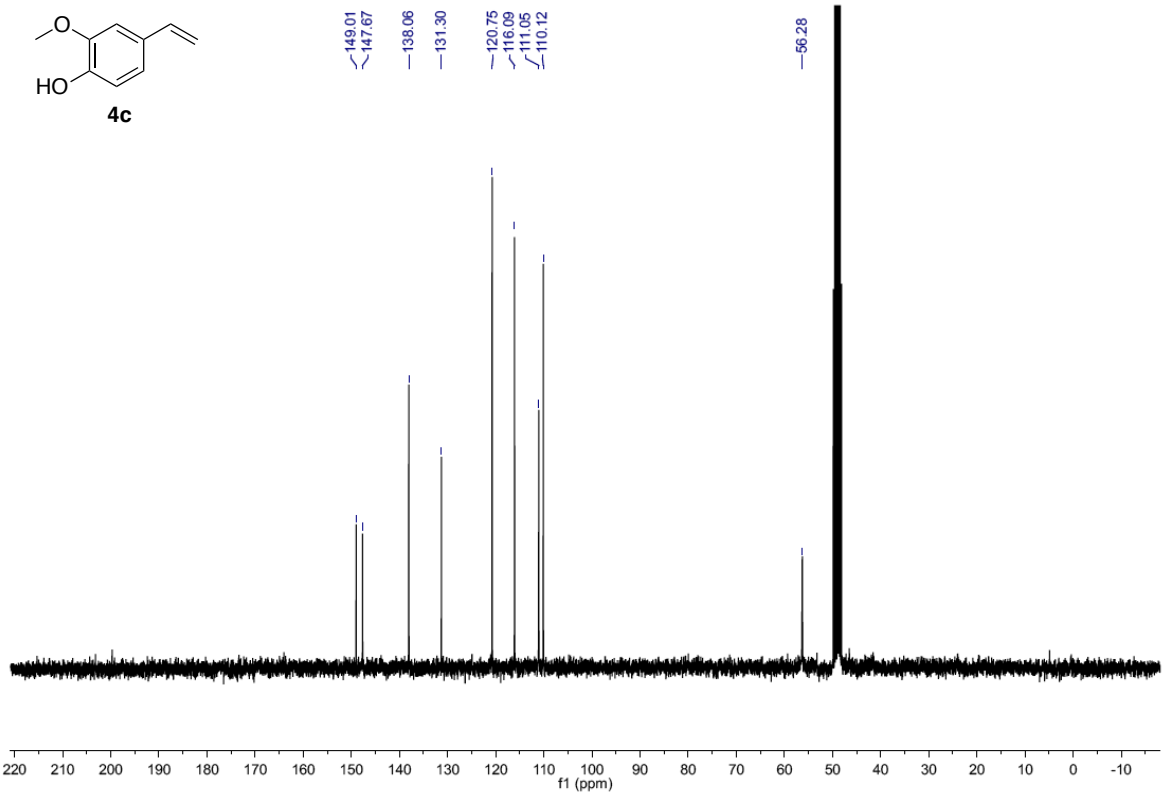
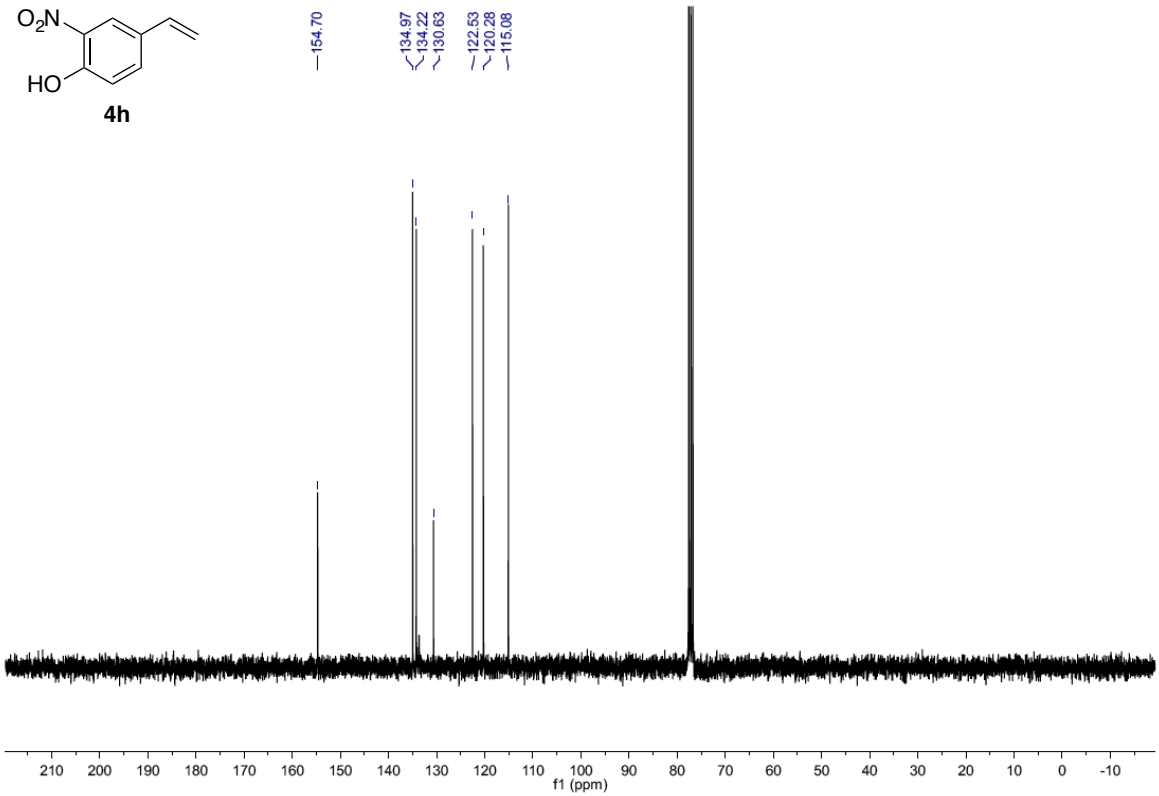
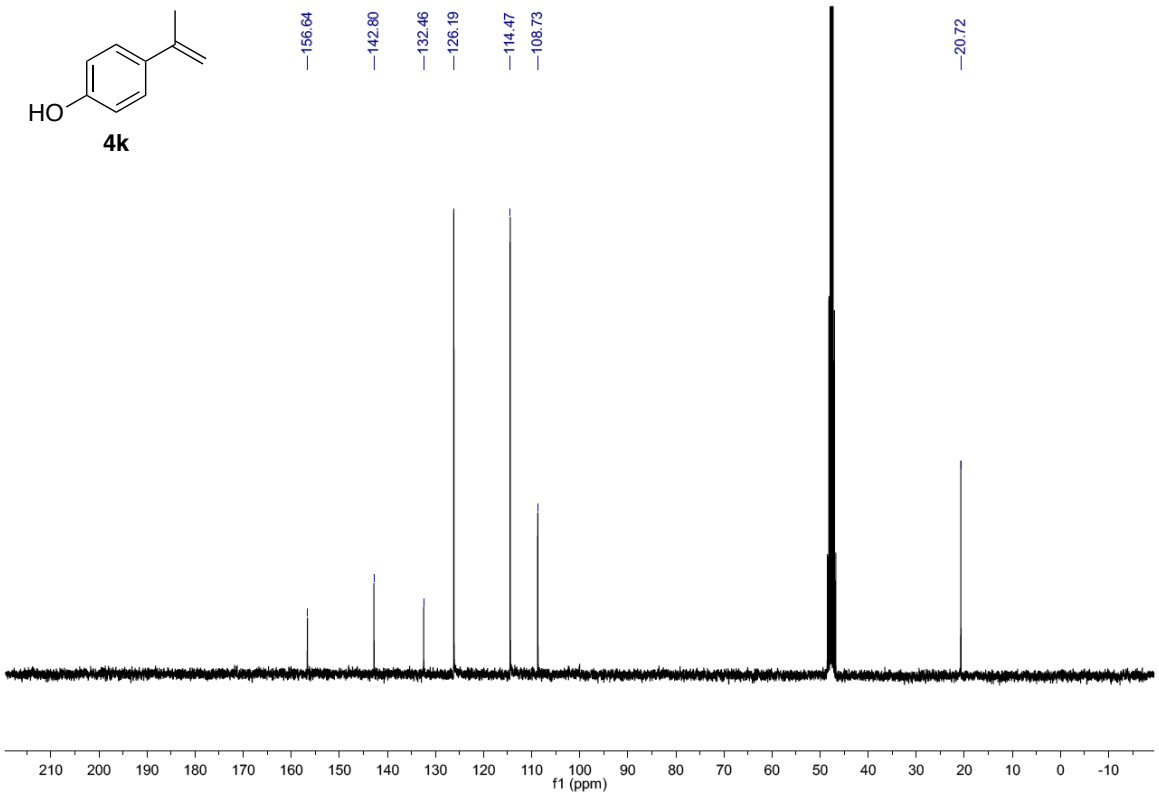
5.13
5.12
5.11
5.10
5.08
4.21
4.20
2.92
2.89
2.06
2.06
2.05
2.04
2.04
1.35
1.33
Cl
OH
HO
5j
0.96
1.92
7.54
7.48
7.42
6.86
6.80
f1 (ppm)
0.77
0.99
1.96
1.00
0.76
3.17
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

7.2. ^{13}C NMR spectra



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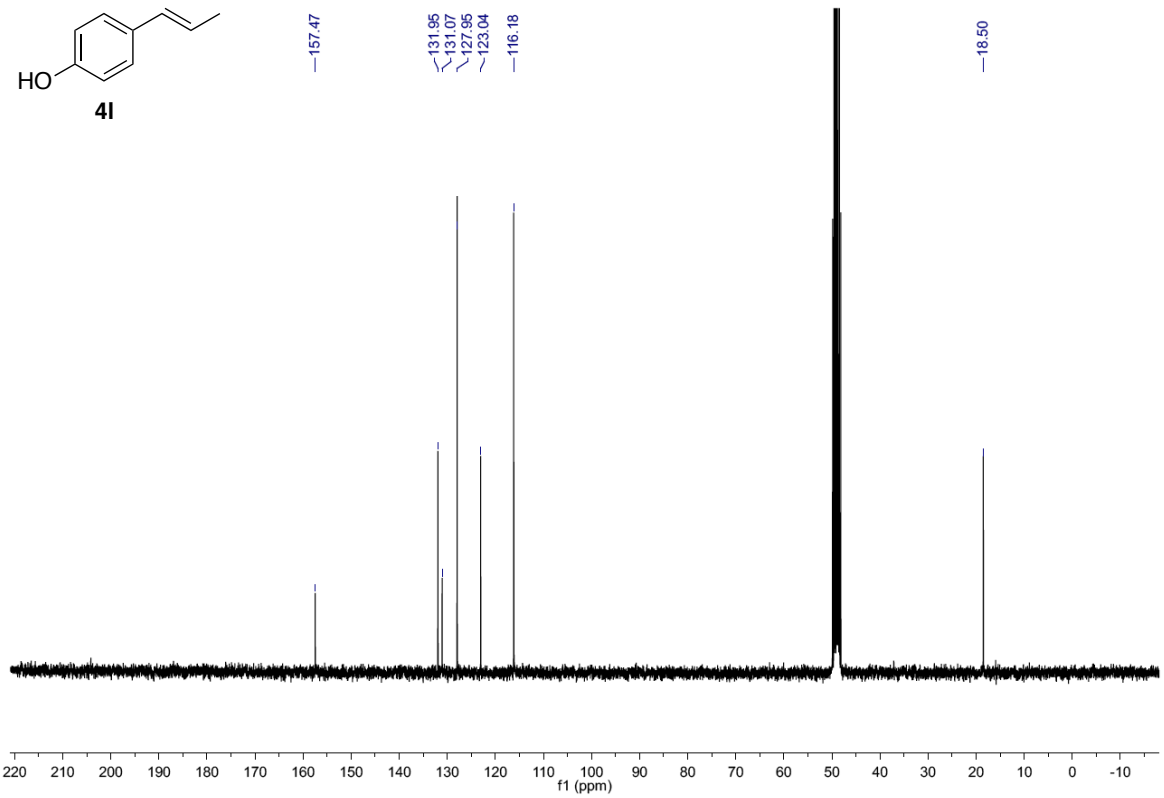
142.80
132.46
126.19
114.47
108.73
20.72
f1 (ppm)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4l
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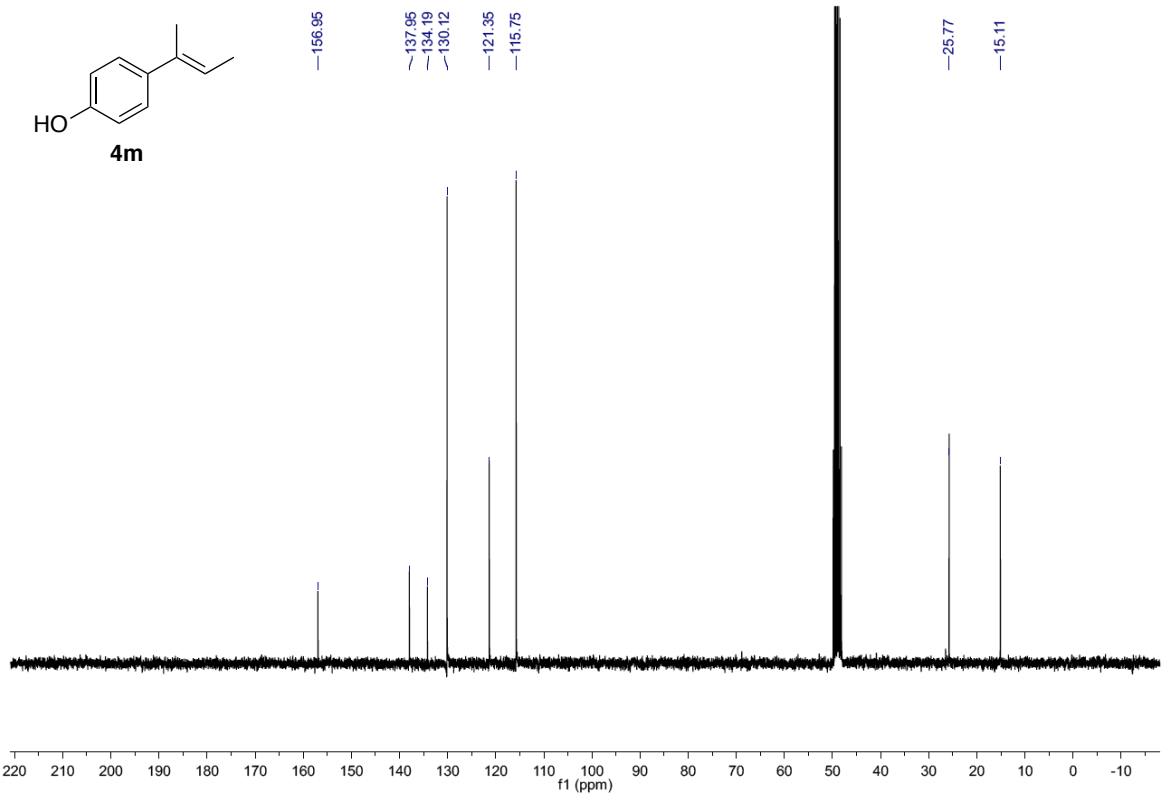
131.95
131.07
127.95
123.04
116.18
18.50
f1 (ppm)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4m
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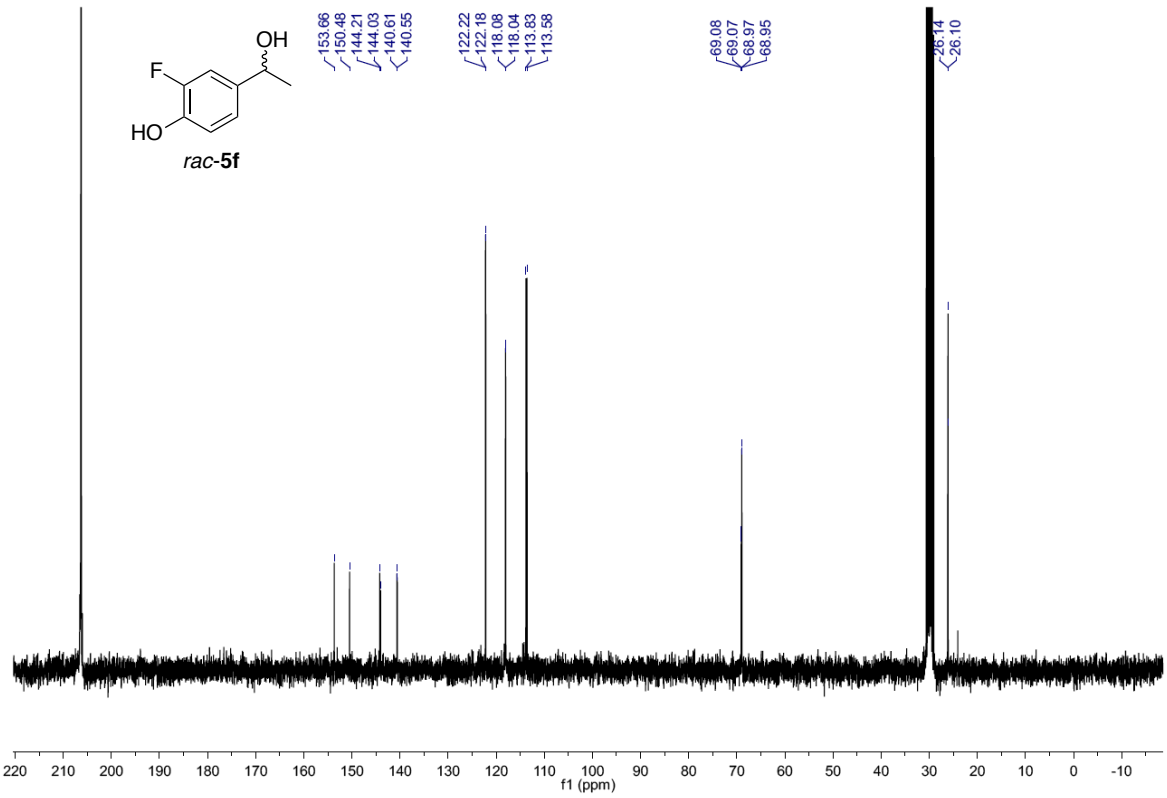
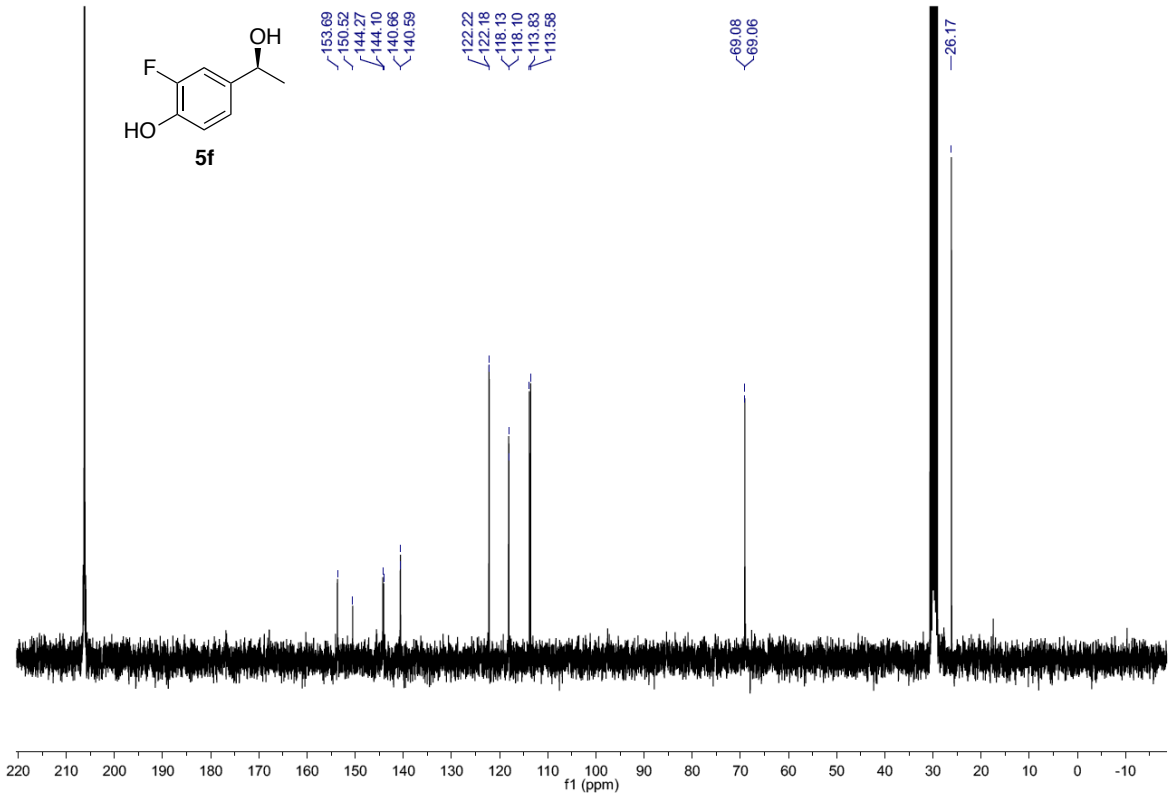
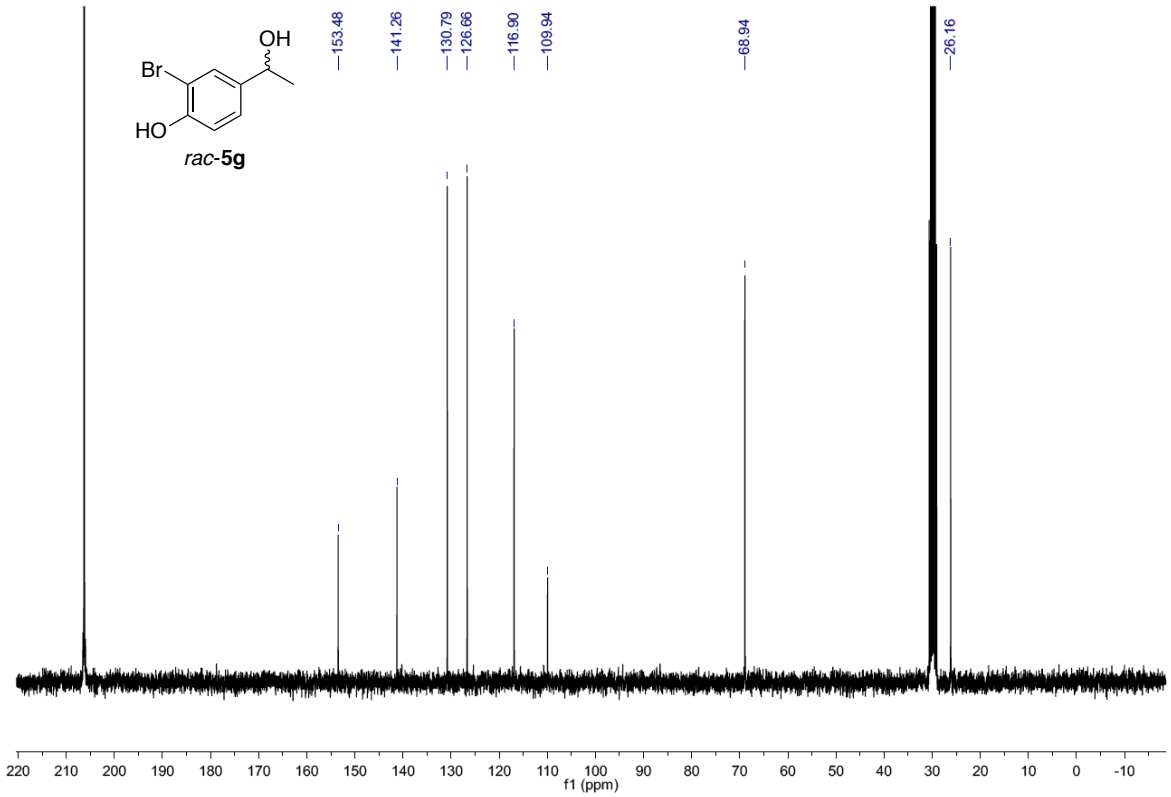
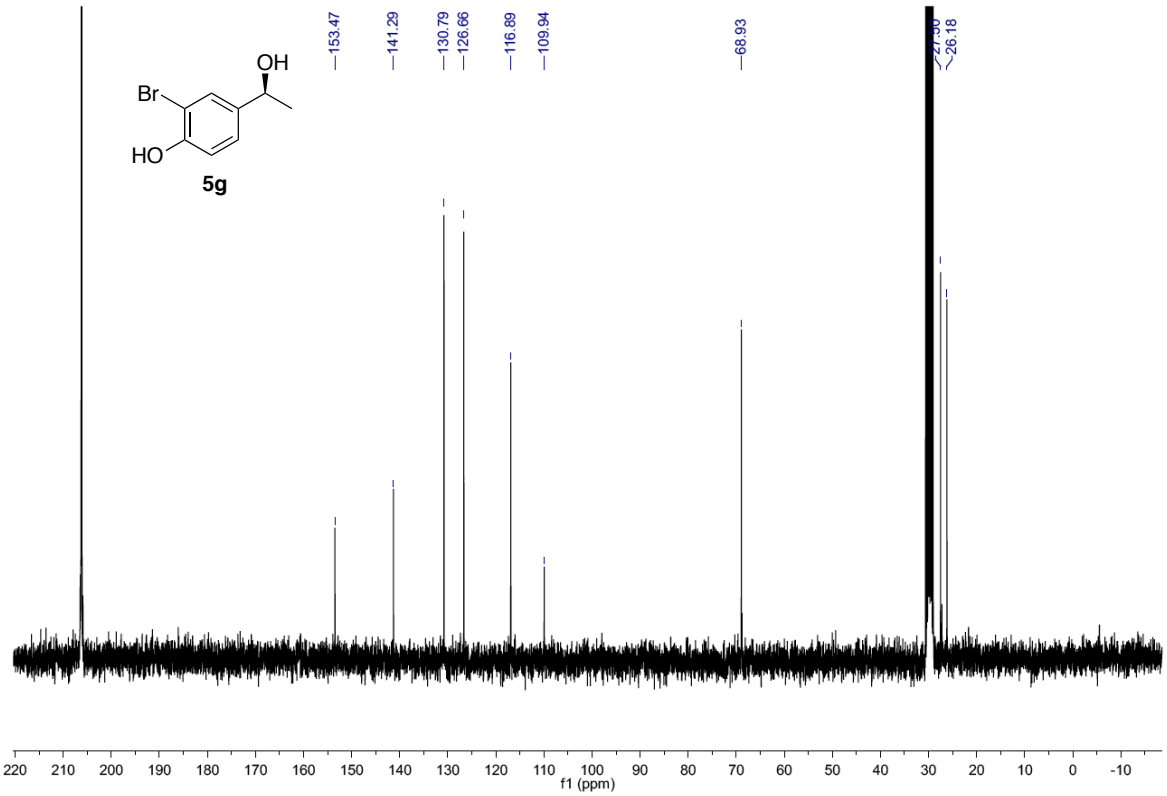
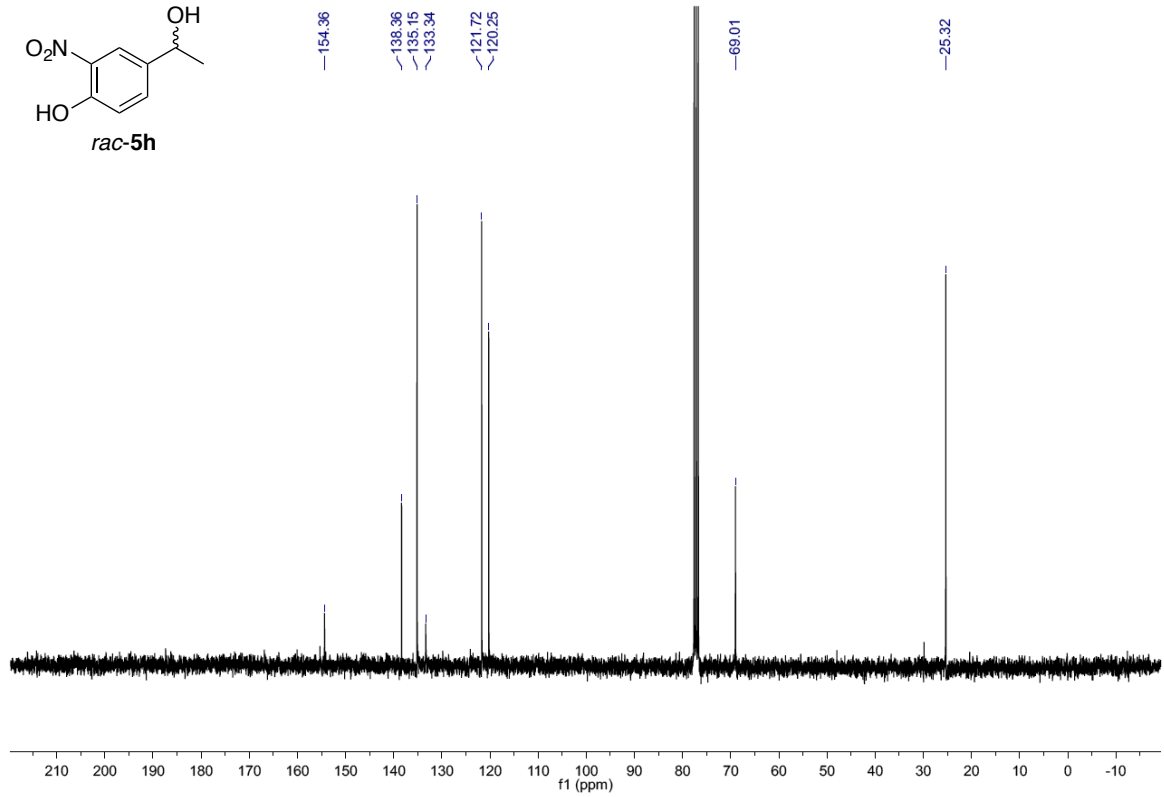
137.95
134.19
130.12
121.35
115.75
25.77
15.11
f1 (ppm)

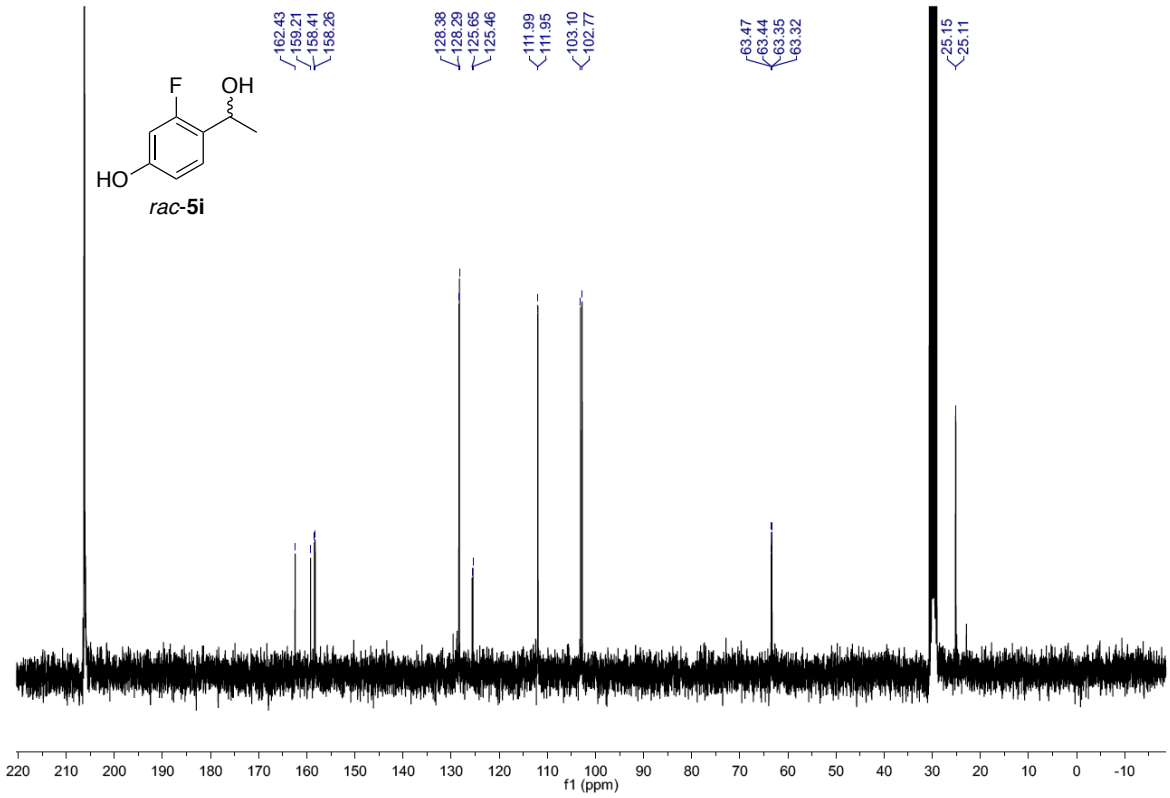

OH
F
HO
rac-5f
153.66
150.48
144.21
144.03
140.61
140.55
122.22
122.18
118.08
118.04
113.83
113.58
69.08
69.07
68.97
68.95
26.14
26.10
f1 (ppm)

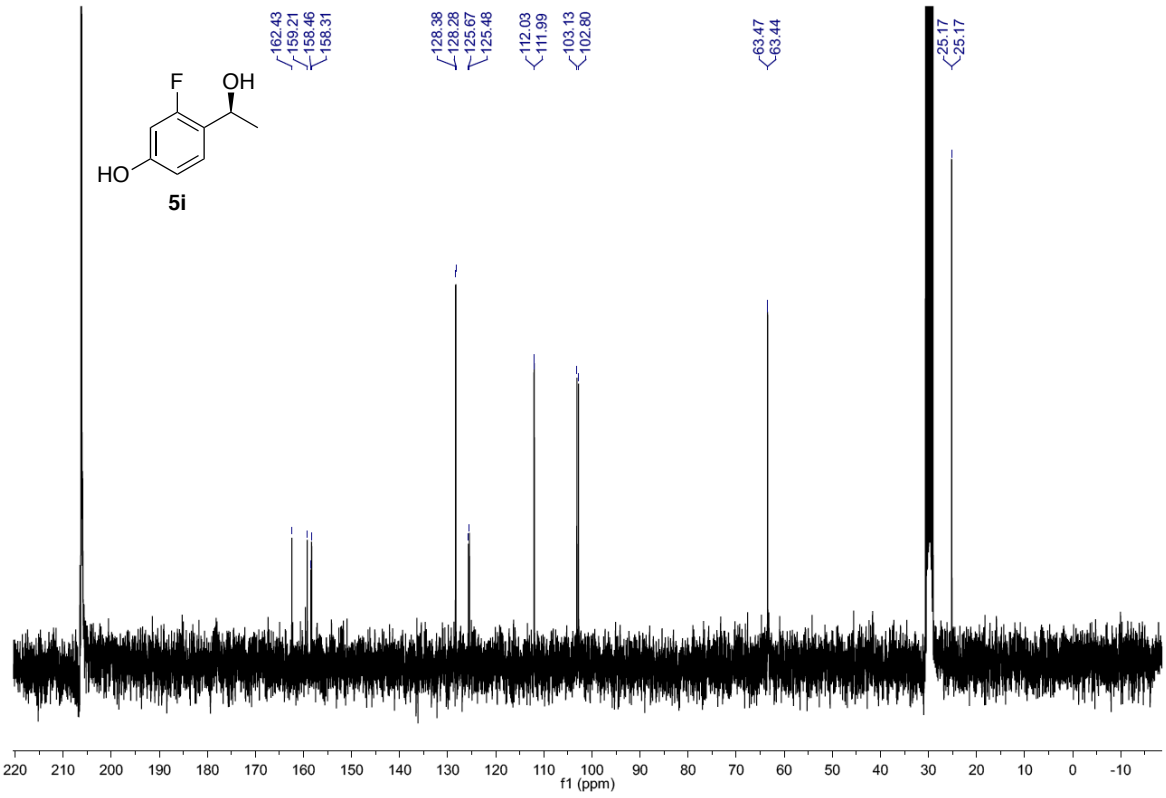

OH
F
HO
5f
153.69
150.52
144.27
144.10
140.66
140.59
122.22
122.18
118.13
118.10
113.83
113.58
69.08
69.06
26.17
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

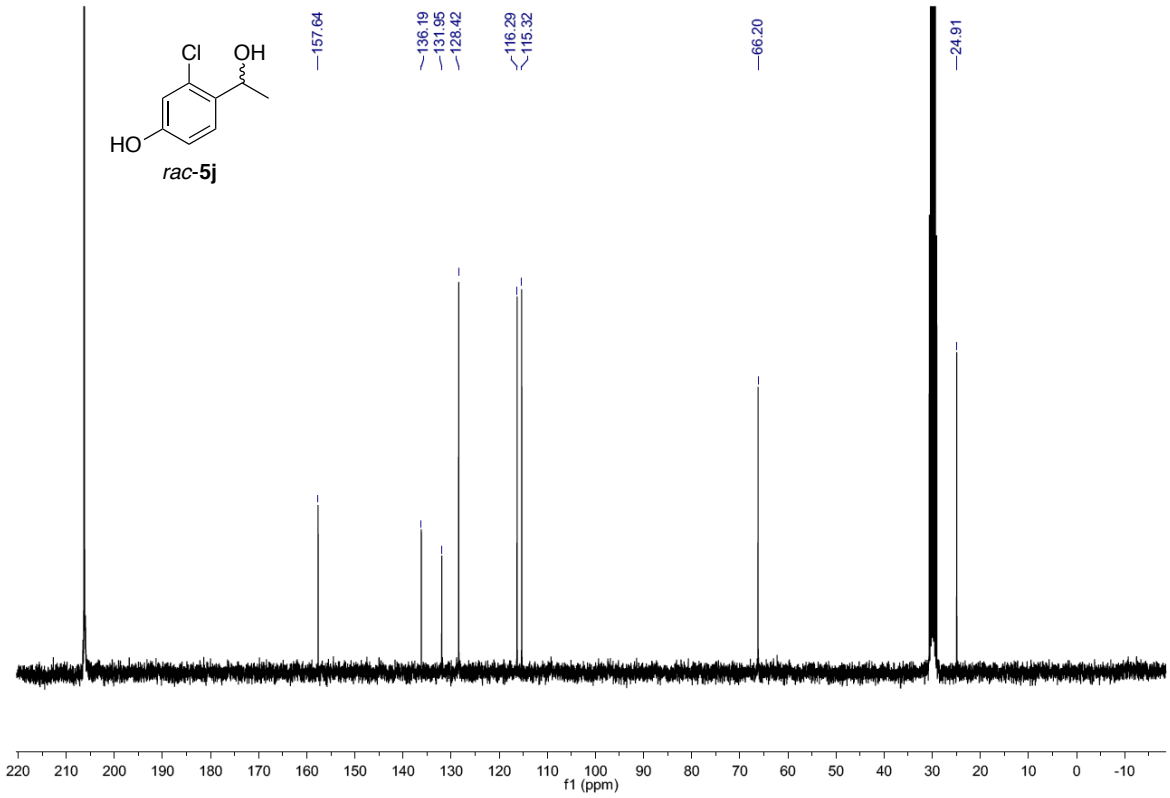

OH
Br
HO
rac-5g
153.48
141.26
130.79
126.66
116.90
109.94
68.94
26.16
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

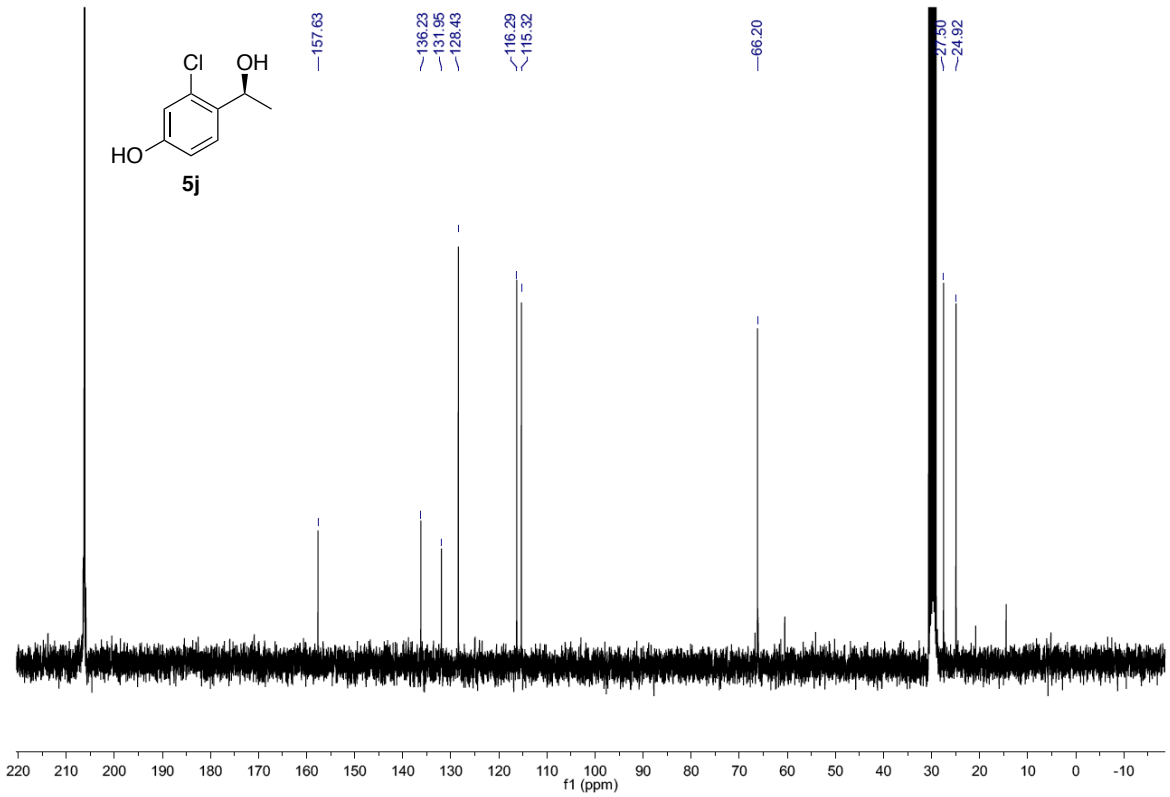

OH
Br
HO
5g
153.47
141.29
130.79
126.66
116.89
109.94
68.93
27.30
26.18
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


OH
O2N
HO
rac-5h
154.36
138.36
135.15
133.34
121.72
120.25
69.01
25.32
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

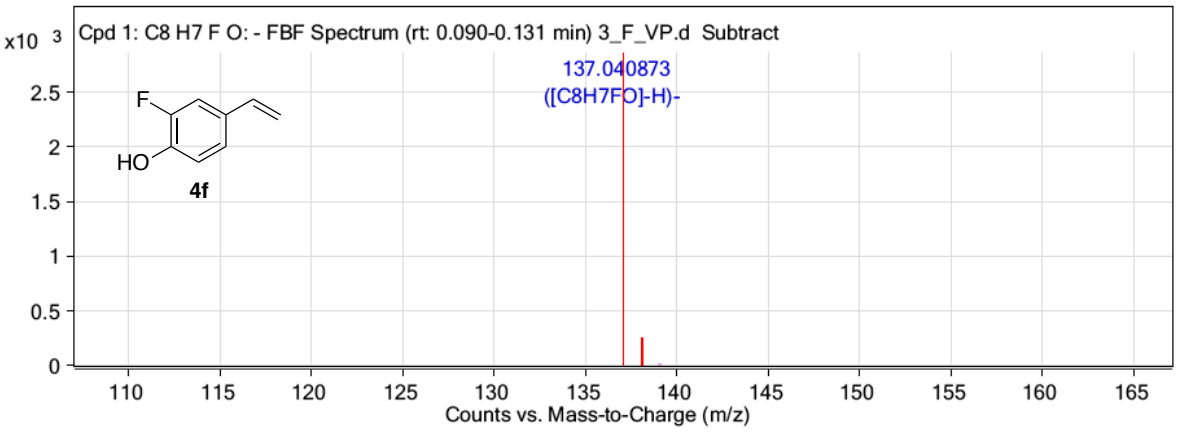

162.43
159.21
158.41
158.26
128.38
128.29
125.65
125.46
111.99
111.95
103.10
102.77
63.47
63.44
63.35
63.32
25.15
25.11
F
OH
HO
rac-5i
f1 (ppm)


162.43
159.21
158.46
158.31
128.38
128.28
125.67
125.48
112.03
111.99
103.13
102.80
63.47
63.44
25.17
25.17
F
OH
HO
5i
f1 (ppm)


Cl
OH
HO
rac-5j
157.64
136.19
131.95
128.42
116.29
115.32
66.20
24.91
f1 (ppm)

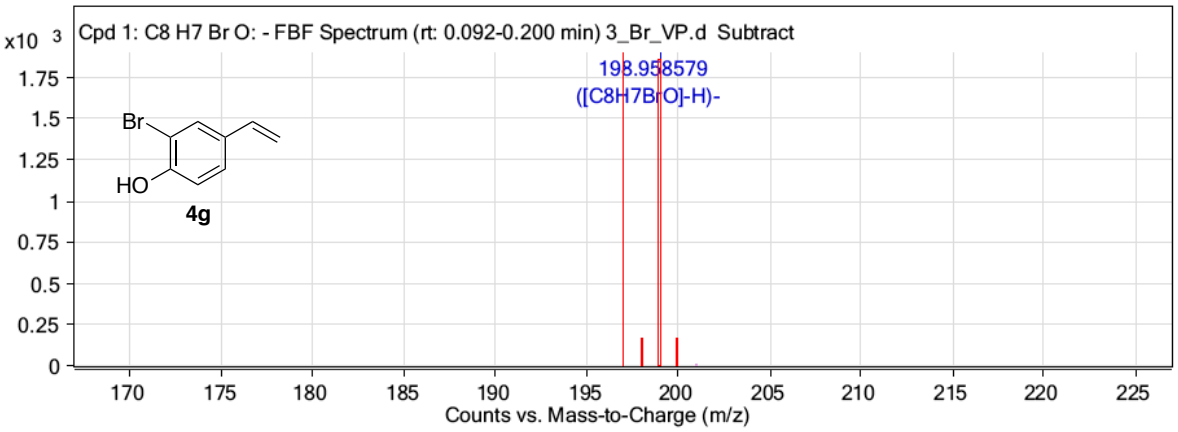

Cl
OH
HO
5j
157.63
136.23
131.95
128.43
116.29
115.32
66.20
27.50
24.92
f1 (ppm)

7.3. High-resolution mass spectra



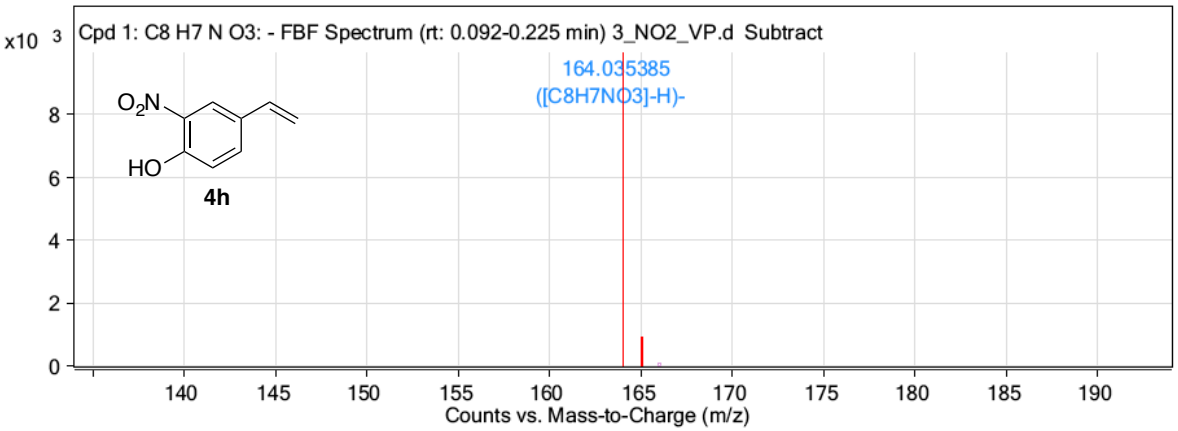
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
137.040873	1	2850.31	C8H7FO	(M-H)-
138.043116	1	231.51	C8H7FO	(M-H)-



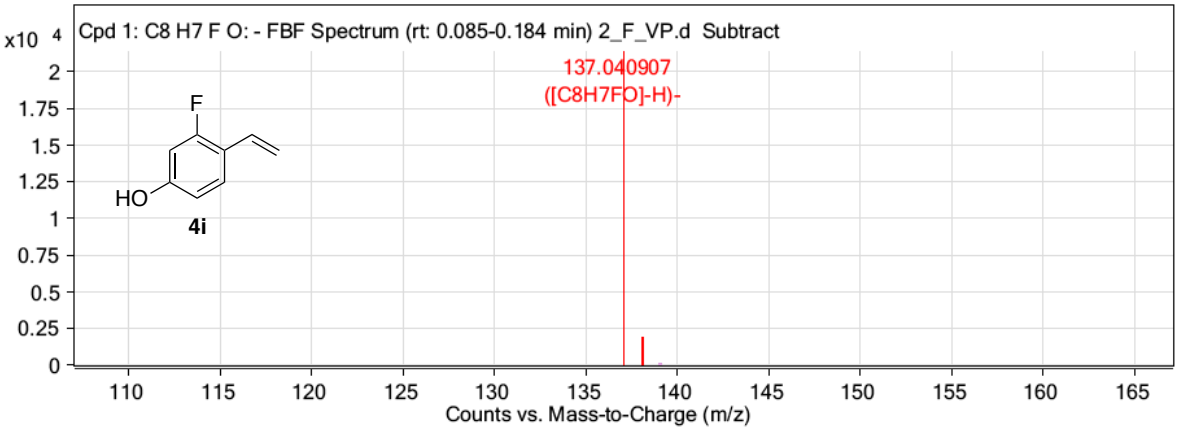
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
196.960742	1	1892.6	C8H7BrO	(M-H)-
197.963345	1	72.09	C8H7BrO	(M-H)-
198.958579	1	1893.49	C8H7BrO	(M-H)-
199.962863	1	85.84	C8H7BrO	(M-H)-



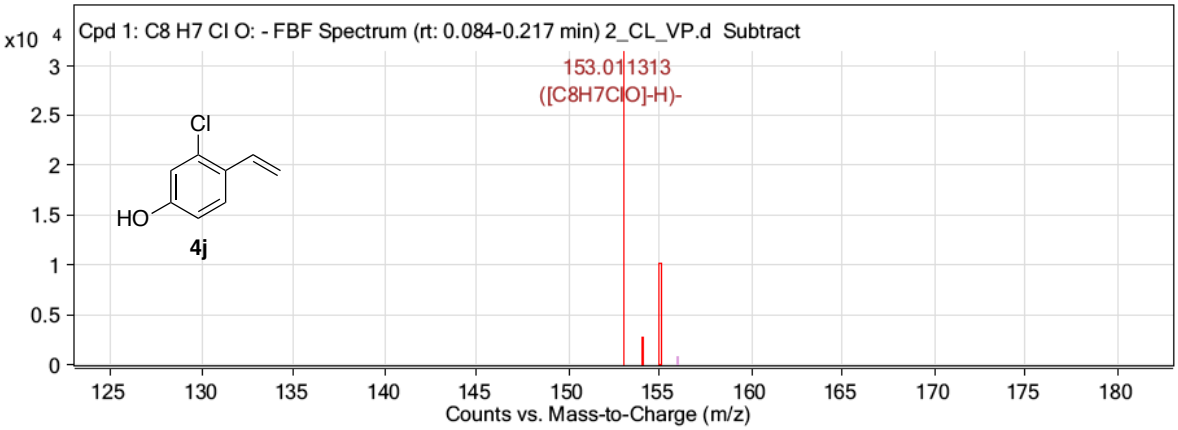
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
164.035385	1	9926.69	C8H7NO3	(M-H)-
165.038443	1	727.45	C8H7NO3	(M-H)-



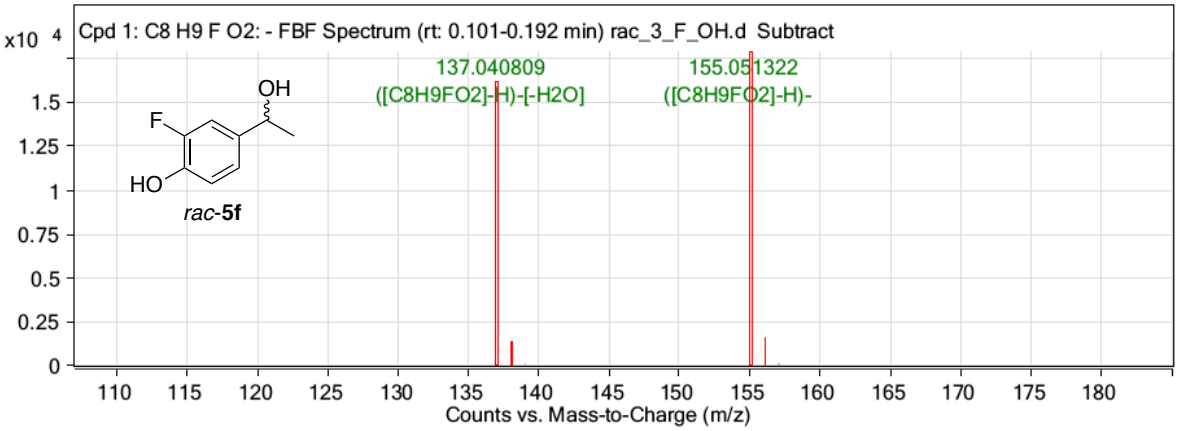
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
137.040907	1	21325.75	C8H7FO	(M-H)-
138.044148	1	1390.74	C8H7FO	(M-H)-



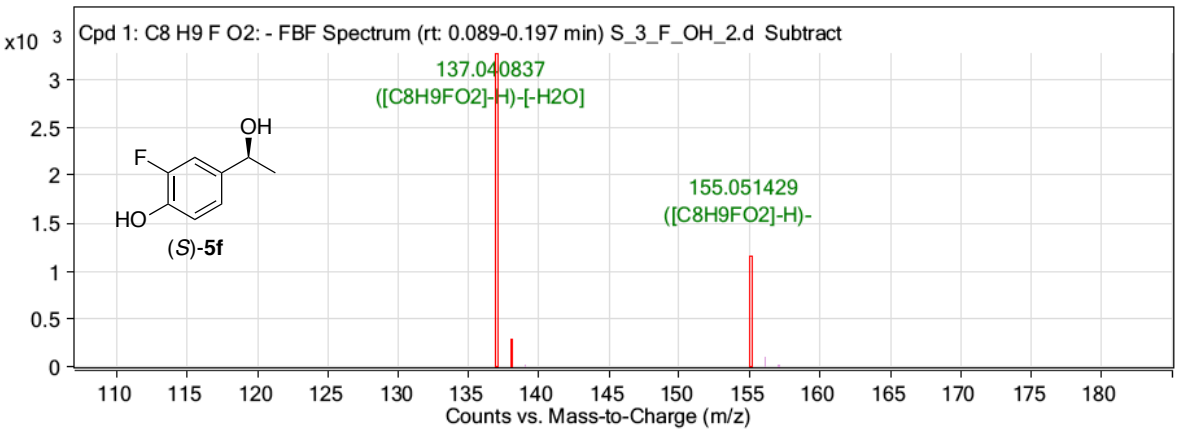
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
153.011313	1	31379.6	C8H7ClO	(M-H)-
154.01468	1	1930.47	C8H7ClO	(M-H)-
155.008448	1	8235.28	C8H7ClO	(M-H)-



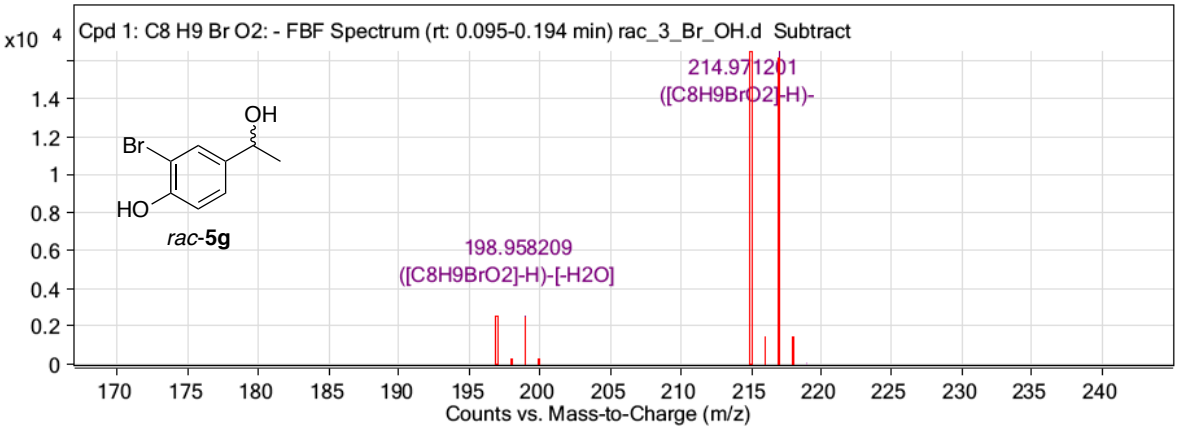
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
137.040809	1	16252.14	C8H9FO2	(M-H)-[-H2O]
138.044245	1	1107.78	C8H9FO2	(M-H)-[-H2O]
155.051322	1	17890.08	C8H9FO2	(M-H)-
156.054659	1	1240.88	C8H9FO2	(M-H)-



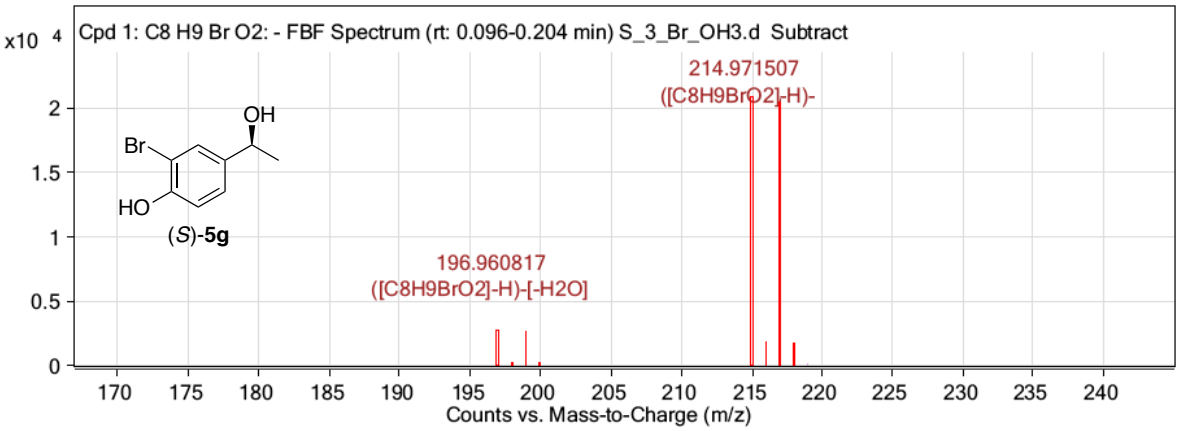
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
137.040837	1	3272.02	C8H9FO2	(M-H)-[-H2O]
138.044294	1	243.39	C8H9FO2	(M-H)-[-H2O]
155.051429	1	1170.56	C8H9FO2	(M-H)-



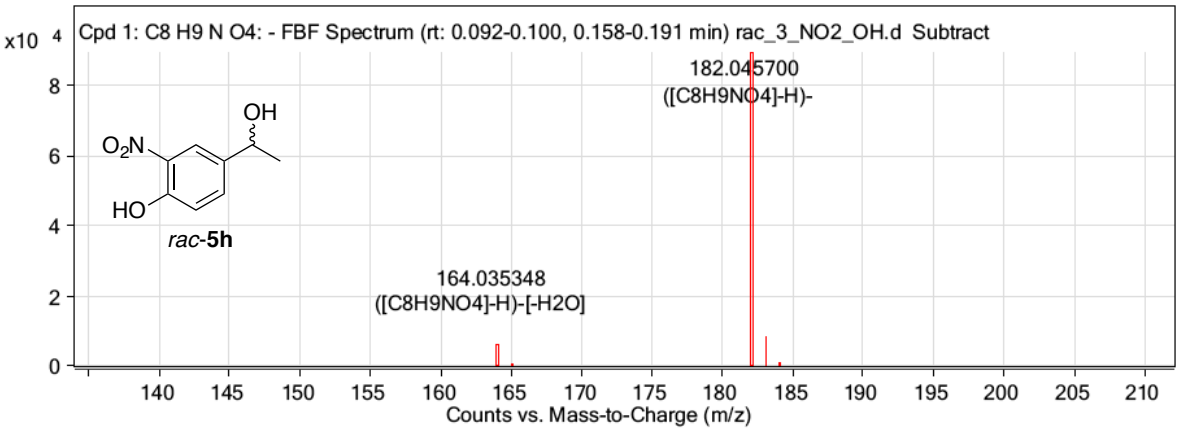
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
196.960559	1	2485.39	C8H9BrO2	(M-H)-[-H2O]
197.963906	1	151.24	C8H9BrO2	(M-H)-[-H2O]
198.958209	1	2555.99	C8H9BrO2	(M-H)-[-H2O]
199.96172	1	144.4	C8H9BrO2	(M-H)-[-H2O]
214.971201	1	16460.05	C8H9BrO2	(M-H)-
215.974783	1	1068.23	C8H9BrO2	(M-H)-
216.969202	1	16436.59	C8H9BrO2	(M-H)-
217.97232	1	1017.47	C8H9BrO2	(M-H)-



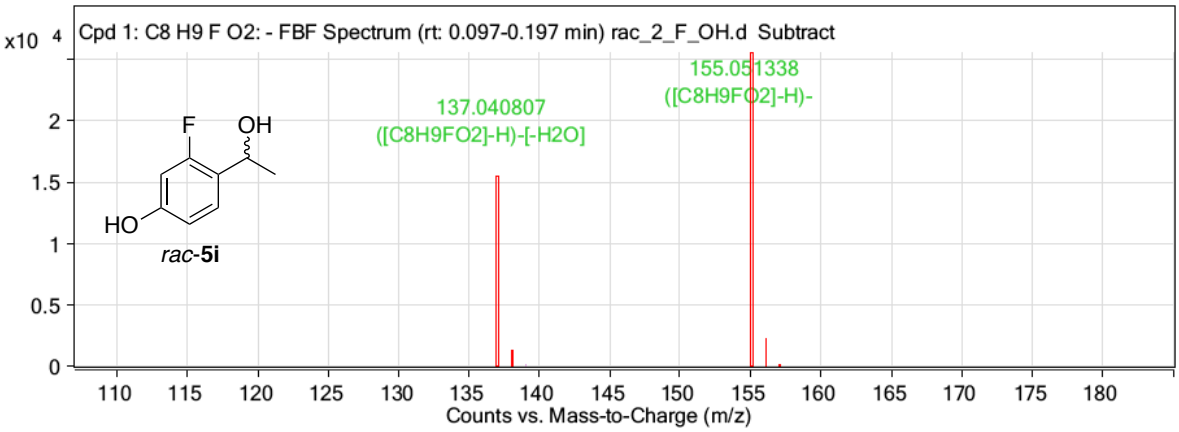
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
196.960817	1	2732.34	C8H9BrO2	(M-H)-[-H2O]
197.964276	1	164.21	C8H9BrO2	(M-H)-[-H2O]
198.959039	1	2642.37	C8H9BrO2	(M-H)-[-H2O]
199.961783	1	175.48	C8H9BrO2	(M-H)-[-H2O]
214.971507	1	20936.92	C8H9BrO2	(M-H)-
215.975069	1	1316.97	C8H9BrO2	(M-H)-
216.969426	1	20673.67	C8H9BrO2	(M-H)-
217.972487	1	1296.92	C8H9BrO2	(M-H)-



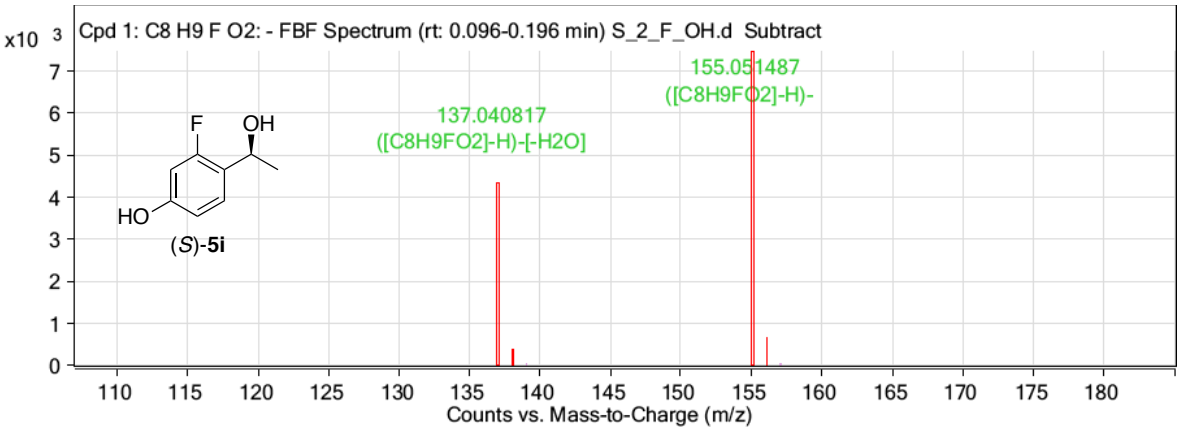
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
164.035348	1	5853.57	C8H9NO4	(M-H)-[-H2O]
165.038349	1	433.44	C8H9NO4	(M-H)-[-H2O]
182.0457	1	89215.25	C8H9NO4	(M-H)-
183.049107	1	4963.92	C8H9NO4	(M-H)-
184.050732	1	562.73	C8H9NO4	(M-H)-



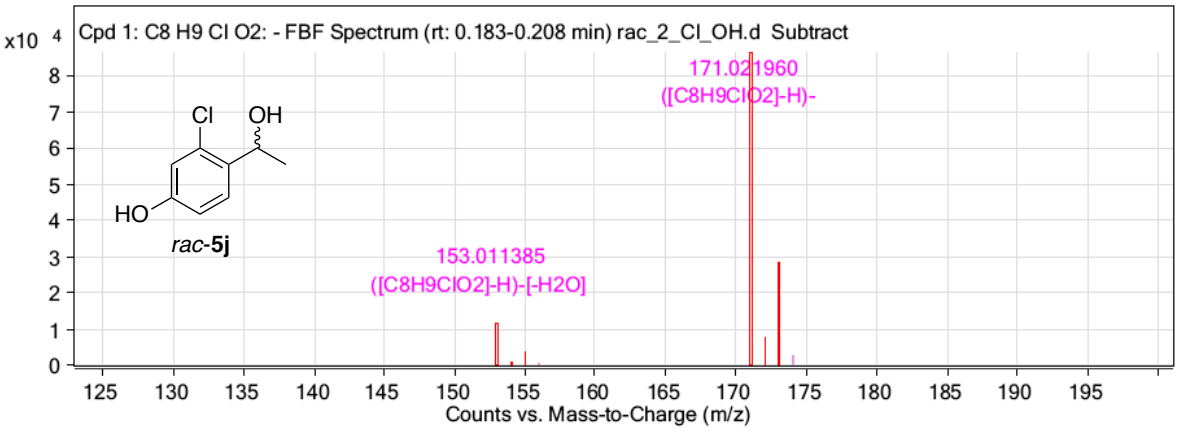
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
137.040807	1	15567.81	C8H9FO2	(M-H)-[-H2O]
138.04419	1	1028.89	C8H9FO2	(M-H)-[-H2O]
155.051338	1	25554.04	C8H9FO2	(M-H)-
156.054976	1	1613.59	C8H9FO2	(M-H)-
157.056495	1	33.8	C8H9FO2	(M-H)-



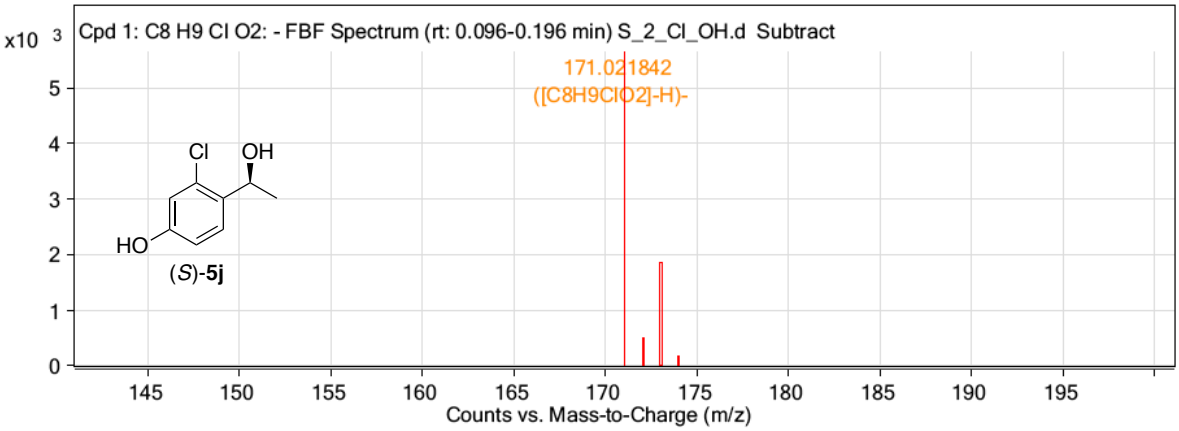
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
137.040817	1	4328.42	C8H9FO2	(M-H)-[-H2O]
138.043986	1	295.68	C8H9FO2	(M-H)-[-H2O]
155.051487	1	7443.24	C8H9FO2	(M-H)-
156.054778	1	516.98	C8H9FO2	(M-H)-



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
153.011385	1	11351.61	C8H9ClO2	(M-H)-[-H2O]
154.014918	1	781.01	C8H9ClO2	(M-H)-[-H2O]
155.008538	1	3200.45	C8H9ClO2	(M-H)-[-H2O]
171.02196	1	86238.39	C8H9ClO2	(M-H)-
172.025351	1	4886.95	C8H9ClO2	(M-H)-
173.019215	1	22504.63	C8H9ClO2	(M-H)-



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
171.021842	1	5639.76	C8H9ClO2	(M-H)-
172.024842	1	438.99	C8H9ClO2	(M-H)-
173.019028	1	1714.89	C8H9ClO2	(M-H)-
174.022911	1	87.37	C8H9ClO2	(M-H)-

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