

Supporting Information for

A Transaminase Triggered Aza-Michael Approach for the Enantioselective Synthesis of Piperidine Scaffolds

James Ryan,[†] Mindaugas Šiaučiulis,[‡] Andrew Gomm,[‡] Beatriz Maciá,^{*,†} Elaine O'Reilly,^{*,‡}
Vittorio Caprio^{*,†}

[†]Faculty of Science & Engineering, Division of Chemistry & Environmental Science,
Manchester Metropolitan University, Chester Street, Manchester M1 5GD, United Kingdom

[‡]School of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD,
United Kingdom

Email:

v.caprio@mmu.ac.uk; elaine.oreilly@nottingham.ac.uk; b.macia-ruiz@mmu.ac.uk

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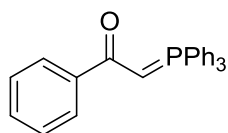
General Methods and Materials

General: NMR spectra were recorded on a JEOL ECS 400 NMR spectrometer (^1H 400 MHz, and ^{13}C 100 MHz). The chemical shifts were recorded in ppm with the residual CHCl_3 signal referenced to 7.26 ppm and 77.00 ppm for ^1H and ^{13}C respectively. Coupling constants (J) are reported in Hz, are corrected and refer to the apparent peak multiplicities. Thin layer chromatography was performed on Alfa Aesar silica gel 60 F254 plates. Flash column chromatography was performed on silica gel (60 Å, 230-400 mesh). GC-MS spectra were recorded on a HP 5973, HP-5MS (30 m \times 0.25 mm \times 0.25 μm), Helium carrier gas, flow 1 mL/min. Infrared spectra were recorded using a Thermo Nicolet 380 FT-IR. GC-FID analysis was performed on Agilent 6850 equipped with a CP CHIRASIL-DEX CB (25 m \times 0.25 mm) DF = 0.25 column. HPLC analysis was performed on an Agilent 1100 series equipped with a Lux Amylose-1 chiral column from Phenomenex. All racemic standards were prepared by mixing equimolar quantities of enantiomerically pure biotransformation products.

Materials: Commercially available reagents, purchased from Sigma Aldrich or Acros, were used throughout without further purification. Anhydrous THF, CH_2Cl_2 , diethyl ether and toluene were obtained from a Pure Solvent apparatus. Commercially available transaminases, ATA113 and ATA117 were purchased from Codexis in the form of lyophilised cell extract. All biotransformations were carried out in HEPES buffer (100 mM, pH 7.5) at 30 °C.

Procedure for the preparation of Wittig reagents

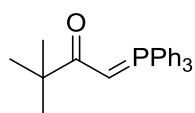
1-phenyl-2-(triphenylphosphanylidene) ethanone^[1]



To a solution of triphenylphosphine (7.9 g, 30.14 mmol) in CH_2Cl_2 (20 mL), 2-bromoacetophenone (5 g, 25.12 mmol) was added and the mixture was stirred for 24 h. Diethyl ether (100 mL) was then added and stirred for a further hour. The resulting precipitate was suspended in a mixture of water/methanol (100 mL, 1:1) and stirred for 1 h. Next, aqueous NaOH (2 M) was added until pH 7-8 was reached and the mixture was stirred for 5 h at r.t. Methanol was then removed under reduced pressure and the aqueous layer was extracted with CH_2Cl_2 (3 \times 20 mL). The combined organic extracts were then dried over Na_2SO_4 and concentrated under reduced pressure to provide the title compound as a white solid (9.03 g, 94% yield). ^1H NMR (400 MHz, CDCl_3):

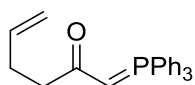
δ 8.02-7.91 (m, 2H), 7.78-7.65 (m, 6H), 7.60-7.42 (m, 9H), 7.40-7.28 (m, 3H), 4.43 (d, J = 24.7 Hz, 1H). In accordance with literature data.

3,3-dimethyl-1-(triphenylphosphoranylidene)-2-butanone^[2]



To a solution of triphenylphosphine (9.7 g, 37 mmol) in toluene (50 mL), 1-bromo-3,3-dimethylbutan-2-one (5 mL, 37 mmol) was added and the mixture was refluxed for 4 h. Solid precipitate was filtered and washed with diethyl ether (3 \times 100 mL). The phosphonium salt was then dissolved in the minimal amount of water/CH₂Cl₂ (1.5:1) and NaOH (2M, 100 mL) was added and the mixture stirred for 2 h. The reaction mixture was extracted with CH₂Cl₂ (3 \times 100 mL) and washed with brine (300 mL), dried over Na₂SO₄ and concentrated under reduced pressure to provide title compound as a beige solid (10.58 g, 79% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.68-7.57 (m, 6H), 7.56-7.47 (m, 3H), 7.47-7.38 (m, 6H), 3.77 (d, J = 27.0 Hz, 1H), 1.20 (s, 9H). In accordance with literature data.

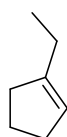
1-(triphenylphosphoranylidene)-5-hexen-2-one^[3]



To a solution of 1-(triphenylphosphoranylidene)-2-propanone (1.00 g, 3.14 mmol) in dry THF (50 mL), *n*-BuLi (1.9 mL, 2.5 M in hexanes) was added at -60°C and the mixture was stirred for 30 min. Excess of allylbromide (1.9 mL, 22.0 mmol) was then added and the reaction left to warm to room temperature slowly. The reaction mixture was quenched with water (100 mL) and extracted with CH₂Cl₂ (3 \times 100 mL), washed with brine (300 mL), dried over Na₂SO₄ and concentrated under reduced pressure to provide title compound as a yellow oil (990 mg, 88% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.68-7.59 (m, 6H), 7.59-7.51 (m, 3H), 7.49-7.41 (m, 6H), 5.99-5.83 (m, 1H), 5.11-4.92 (m, 2H), 3.71 (d, J = 26.6 Hz, 1H), 2.48-2.36 (m, 4H). In accordance with literature data.

Preparation of conjugated substrates

1-ethylcyclopentene^[4]



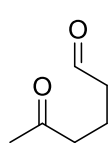
Ethyl magnesium bromide (1.2 equiv, 48 mmol, 16 mL, 3 M in Et₂O) was added to cyclopentanone (1 equiv, 40 mmol, 3.54 mL) in Et₂O (40 mL) under nitrogen at 0 °C. The mixture was then allowed to warm to room temperature and stirred for a further 2 h. The reaction mixture was then carefully quenched with cold water followed by sat.

NH₄Cl. The organic phase was separated and the aqueous layer extracted with Et₂O (2 × 40 mL). The combined organic extracts were dried over Na₂SO₄ and concentrated under reduced pressure to provide the crude 1-ethylcyclopentanol (3.90 g, 86% yield). To this crude, 85% wt. phosphoric acid (0.05 equiv, 1.71 mmol, 0.20 mL) was added and the mixture was heated to 115 °C. The product was distilled off as it formed and the biphasic solution was separated to provide crude 1-ethylcyclopentene as a colourless oil (1.84 g, 48% yield). ¹H NMR (400 MHz, CDCl₃): δ 5.35-5.29 (m, 1H), 2.35-2.26 (m, 2H), 2.26-2.19 (m, 2H), 2.11-2.02 (m, 2H), 1.91-1.80 (m, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). In accordance with literature data.

General Procedure for Oxidative Ring Opening of 1-methyl- and 1-ethylcyclopentene

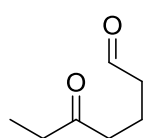
The corresponding 1-alkylcyclopentene (1 equiv, 16.6 mmol) and OsO₄ (4% w.t/v *t*BuOH, 0.01 equiv, 0.17 mmol, 1.06 mL) were added to a solution of *N*-methylmorpholine *N*-oxide (2 equiv, 33.2 mmol, 3.89 g) in acetone (20 mL). The mixture was stirred at room temperature for 4 h and then quenched with sat. Na₂SO₃ solution (80 mL) and extracted with CH₂Cl₂ (3 × 60 mL). The combined organic extracts were dried over Na₂SO₄ and concentrated under reduced pressure. The crude material was dissolved in water (20 mL) and sodium periodate (2 equiv, 33.2 mmol, 7.10 g) was added. The resulting mixture was stirred for 1 h at room temperature and then diluted with water (60 mL) and extracted with CH₂Cl₂ (3 × 30 mL). Next, the combined organic extracts were dried over Na₂SO₄ and concentrated under reduced pressure to provide the desired aldehyde.

5-oxohexanal^[5]



Colourless oil (460 mg, 85% yield). FTIR (neat) *V*_{max}: 2941, 2729, 1709, 1369, 1162 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 9.75 (s, 1H), 2.55-2.45 (m, 4H), 2.13 (s, 3H), 1.93-1.83 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 208.0, 201.9, 42.9, 42.2, 29.9, 15.9; Calculated C₆H₁₁O₂⁺ [M+H]⁺: 115.0754, found: 115.0758. In accordance with literature data.

5-oxoheptanal^[4]

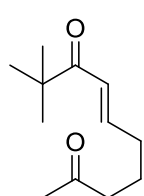


Pale yellow oil (850 mg, 40% yield). ¹H NMR (400 MHz, CDCl₃): δ 9.76-9.75 (m, 1H), 2.51-2.44 (m, 4H), 2.41 (q, *J* = 7.3 Hz, 2H), 1.90 (m, 2H), 1.04 (t, *J* = 7.3 Hz, 3H).

General Procedure for the Wittig Reaction (synthesis of 3a-f)

To 5-oxohexanal (228 mg, 2 mmol) or 5-oxoheptanal (256 mg, 2 mmol), in dry CH₂Cl₂ (20 mL) the corresponding Wittig reagent (1.25 equiv, 2.5 mmol) was added and the resulting mixture was stirred for 24 h at room temperature (unless stated otherwise). The reaction mixture was then concentrated under reduced pressure and the crude material was purified by column chromatography on silica gel (hexane/EtOAc, 7:3) to provide the isolated compounds **3a-f**.

(6E)-9,9-dimethyldec-6-ene-2,8-dione (**3a**)^[6]

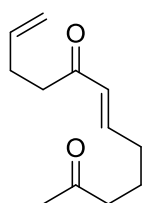


The synthesis of **3a** was carried out according the general procedure for Wittig reactions, but in this case, the reaction mixture was refluxed for 24 h in THF.

Colourless oil (537 mg, 58% yield). R.f. 0.48 (EtOAc/hexane 1:1); FTIR (neat)

V_{\max} : 2967, 1714, 1687, 1622, 1365, 1077, 983 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 6.94-6.82 (m, 1H), 6.50 (d, J = 15.1 Hz, 1H), 2.46 (t, J = 7.3 Hz, 2H), 2.25-2.20 (m, 2H), 2.14 (s, 3H), 1.80-1.70 (m, 2H), 1.14 (s, 9H); ¹³C NMR (100 MHz, CDCl₃): 208.3, 204.2, 146.2, 124.7, 42.8, 42.6, 31.5, 30.0, 26.1, 22.0; LC-MS (m/z): Calculated C₁₂H₂₁O₂⁺ [M+H]⁺: 197.1536, found: 197.1544. In accordance with literature data.

(6E)-dodeca-6,11-diene-2,8-dione (**3b**)



Pale yellow oil (308 mg, 57% yield). R.f. 0.45 (EtOAc/hexane 1:1); FTIR (neat)

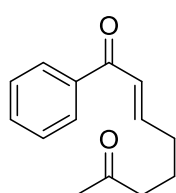
V_{\max} : 3078, 2935, 1713, 1668, 1628, 1360, 980, 912 cm⁻¹; ¹H NMR (400 MHz,

CDCl₃): δ 6.78 (dt, J = 16.0, 6.9 Hz, 1H), 6.20-5.99 (m, 1H), 5.94-5.62 (m, 1H),

5.06-4.94 (m, 2H), 2.63 (t, J = 7.3 Hz, 2H), 2.45 (t, J = 7.3 Hz, 2H), 2.38-2.31

(m, 2H), 2.25-2.18 (m, 2H), 2.13 (s, 3H), 1.79-1.68 (m, 2H); ¹³C NMR (100 MHz, CDCl₃): δ 208.1, 199.5, 146.2, 137.2, 130.7, 115.1, 42.5, 39.1, 31.6, 30.0, 28.0, 21.8; LC-MS (m/z): Calculated C₁₂H₁₉O₂⁺ [M+H]⁺: 195.1380, found: 195.1382.

(2E)-1-phenyloct-2-ene-1,7-dione (**3c**)^[6]

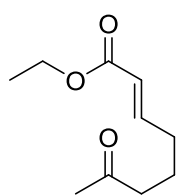


White solid (328 mg, 76% yield). R.f. 0.42 (EtOAc/hexane 1:1); M.p. 68 – 71

°C; FTIR (neat) V_{\max} : 3055, 3000, 2955, 2934, 1709, 1663, 1616, 1356, 1244,

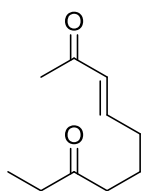
982, 699 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.96-7.90 (m, 2H), 7.60-7.53 (m, 1H), 7.51-7.44 (m, 2H), 7.03 (dt, $J = 15.5, 6.8$ Hz, 1H), 6.90 (d, $J = 15.5$ Hz, 1H), 2.51 (t, $J = 7.3$ Hz, 2H), 2.37-2.31 (m, 2H), 2.15 (s, 3H), 1.87-1.78 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3): δ 208.2, 190.7, 148.6, 137.8, 132.7, 128.5 (2C), 126.4, 42.6, 31.9, 30.0, 22.0; LC-MS (m/z): Calculated $\text{C}_{14}\text{H}_{17}\text{O}_2^+$ $[\text{M}+\text{H}]^+$: 217.1223, found: 217.1221. In accordance with literature data.

ethyl (2*E*)-7-oxooct-2-enoate (3d)^[5b]



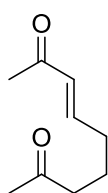
Colourless oil (171 mg, 66% yield). R.f. 0.41 (EtOAc/hexane 1:1); FTIR (neat) V_{max} : 2939, 1709, 1653, 1266, 1178, 1148 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 6.96-6.85 (dt, $J = 15.6, 7.0$ Hz, 1H), 5.82 (d, $J = 15.6$ Hz, 1H), 4.18 (q, $J = 7.2$ Hz, 2H), 2.45 (t, $J = 7.3$ Hz, 2H), 2.23-2.17 (m, 2H), 2.13 (s, 3H), 1.79-1.69 (m, 2H), 1.28 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 208.2, 166.5, 148.0, 122.0, 60.2, 42.6, 31.3, 30.0, 21.8, 14.2; LC-MS (m/z): Calculated $\text{C}_{10}\text{H}_{17}\text{O}_3^+$ $[\text{M}+\text{H}]^+$: 185.1172, found: 185.1147. In accordance with literature data.

(3*E*)-dec-3-ene-2,8-dione (3e)



The synthesis of **3e** was carried out according the general procedure for Wittig reactions, but in this case, the reaction mixture was refluxed for 24 h in THF. Pale yellow oil (770 mg, 69% yield). R.f. 0.37 (EtOAc/hexane 1:1); FTIR (neat) V_{max} : 2976, 2938, 1711, 1698, 1672, 1626, 1458, 1415, 1360, 1253, 976 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 6.75 (dt, $J = 16.0, 6.9$ Hz, 1H), 6.06 (d, $J = 16.0$ Hz, 1H), 2.48-2.36 (m, 4H), 2.28-2.18 (m, 2H), 2.23 (s, 3H), 1.82-1.71 (m, 2H), 1.04 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 210.8, 198.6, 147.3, 131.7, 41.2, 36.0, 31.7, 26.9, 21.9, 7.8; LC-MS (m/z): Calculated $\text{C}_{10}\text{H}_{17}\text{O}_2^+$ $[\text{M}+\text{H}]^+$: 169.1223, found: 169.1220.

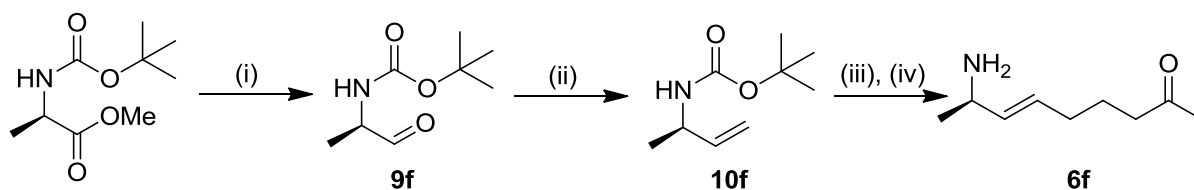
(3*E*)-non-3-ene-2,8-dione (3f)^[6]



Colourless oil (367 mg, 79% yield). R.f. 0.25 (EtOAc/hexane 1:1); FTIR (neat) V_{max} : 3004 2938, 1712, 1670, 1626, 1358 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 6.75 (dt, $J = 16.0, 6.9$ Hz, 1H), 6.06 (d, $J = 16.0$ Hz, 1H), 2.50-2.43 (m, 2H), 2.28-2.18 (m, 5H), 2.13 (s, 3H), 1.81-1.70 (m, 2H); ^{13}C NMR (100

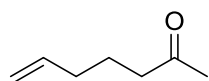
MHz, CDCl₃): δ 208.1, 198.5, 147.2, 131.7, 42.6, 31.6, 30.0, 26.9, 21.8; LC-MS (m/z): Calculated C₉H₁₄O₂Na⁺ [M+Na]⁺: 177.0886, found: 177.0870. In accordance with literature data.

Preparation of (*R*),(*E*)-8-aminonon-6-en-2-one (**6f**)



Scheme S1: Synthesis of (*R*),(*E*)-8-aminonon-6-en-2-one (**6f**): (i) DIBAL-H, DCM; (ii) Methylphenylphosphonium bromide, KHMDS, THF; (iii) Hoveyda-Grubbs II, hept-6-en-2-one, DCM; (iv) TFA, DCM.

hept-6-en-2-one^[7]

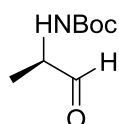


To a flame-dried flask under N₂, magnesium turnings (600 mg, 25.0 mmol), dry THF (20 mL) and a crystal of iodine were added. A portion (*ca.* 1/5) of 5-bromopentene (1.78 mL, 15.0 mmol) was added and the resulting solution was heated vigorously until the brown colour disappeared. The solution was then heated at 70 °C and the remaining 5-bromopentene was added dropwise at a rate to maintain gentle reflux (*ca.* 30 min). The reaction mixture was then refluxed for 3 h, cooled to r.t. and used immediately.

To a suspension of copper(I) iodide (143 mg, 0.750 mmol) in dry THF (20 mL) at –20 °C acetyl chloride (1.07 mL, 15.0 mmol) was added. To the resulting suspension, previously prepared pent-4-en-1-ylmagnesium bromide was added dropwise over 45 min, and the reaction mixture was subsequently stirred for 1 h at –20 °C and then allowed to reach room temperature. The majority of solvent was removed *in vacuo* and DCM (25 mL) and 1M HCl (25 mL) were added to the residue. The organic layer was separated, and the aqueous layer was washed with DCM (25 mL). Combined organic fractions were filtered to remove copper salts, washed with NaHCO₃ (2 × 25 mL), dried (over MgSO₄), filtered, and solvent was removed *in vacuo*. Crude product was purified by column chromatography (petrol/EtOAc 9:1, R.f. 0.21) to afford the title compound as a pale yellow oil (1.12 g, 10.0 mmol, 67%). δ_{H} (400 MHz, CDCl₃) 5.76 (m, 1H), 5.04–4.94 (m, 2H) 2.43 (t, *J* = 7.4 Hz, 2H), 2.13 (s, 3 H),

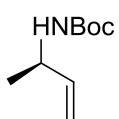
2.09-2.01 (m, 2H), 1.67 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) δ 209.0, 138.1, 115.4, 43.0, 33.2, 30.1, 23.0; GC-EIMS: calculated $\text{C}_7\text{H}_{12}\text{O}$ [M]: 112.0883, found 112.0887. In accordance with literature data.

***tert*-butyl (*R*)-(1-oxopropan-2-yl)carbamate (9f)^[8]**



A solution of commercially available (*R*)-Boc-Ala-OMe (1.02 g, 5 mmol) in dry dichloromethane (100 mL) was cooled to -78°C . To this, a solution of diisobutylaluminium hydride (DIBAL-H) in hexane (1 M, 12.5 mL) was added dropwise. The mixture was then left to stir and monitored by TLC until no starting material remained (*ca.* 45 minutes). MeOH (5 mL) was then added at -78°C , the solution was then warmed to r.t., and poured into sat. aq sodium potassium tartrate solution. The aqueous layer was separated and extracted with Et_2O (3×10 mL), and the combined organic layers were dried (MgSO_4), filtered, and evaporated. The residue was purified by silica gel column chromatography (petrol/EtOAc 5:3) to give the final product as a clear oil (613 mg, 3.54 mmol, 71% yield). ^1H NMR (300 MHz, CDCl_3) δ 9.55 (s, 1H), 5.18 (br s), 4.29-4.14 (m, 1H), 1.44 (s, 9H), 1.32 (d, $J = 7.4$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 199.7, 155.2, 80.1, 55.5, 28.3, 14.8; GC-EIMS: calculated $\text{C}_7\text{H}_{14}\text{NO}_2$ [M]: 144.1019, found 144.1021. In accordance with literature data.

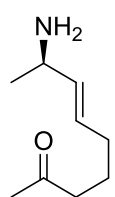
***tert*-butyl (*R*)-but-3-en-2-ylcarbamate (10f)^[9]**



To a solution of methyltriphenylphosphonium bromide (2.55 g, 7.14 mmol) in dry THF (21 mL), potassium hexamethyldisilazide (KHMDs) in toluene (0.7 M, 10.2 mL) was added dropwise at -78°C . The mixture was stirred for 1 h and then a solution of *tert*-butyl (*R*)-(1-oxopropan-2-yl)carbamate (585 mg, 3.38 mmol) in THF (12 mL) was added dropwise and stirred for 15 minutes at -78°C and then warmed to room temperature and left to stir overnight. The yellow solution was then quenched with saturated potassium tartrate solution and extracted with Et_2O (3×10 mL). The organic extracts were then reduced *in vacuo* and purified *via* flash silica gel column chromatography (petrol/EtOAc 9:1) to give the final product as a colourless oil (256 mg, 44%). ^1H NMR (400 MHz, CDCl_3) δ 5.84 (ddd, $J = 17.3, 10.4, 5.0$ Hz, 1H), 5.17 (d, $J = 17.3$ Hz, 1H), 5.08 (d, $J = 10.4$ Hz, 1H), 4.48 (br s, 1H), 4.24 (br s, 1H), 1.47 (s, 9H), 1.24 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR

(101 MHz, CDCl₃) δ 155.2, 140.2, 113.5, 79.2, 48.1, 28.4, 20.6; HRMS-ESI: calculated C₉H₁₇NO₂ [M]: 171.1259, found 171.1254. In accordance with literature data.

(R),(E)-8-aminonon-6-en-2-one (6f)



tert-butyl (*R*)-but-3-en-2-ylcarbamate (**10f**) (50 mg, 0.29 mmol) was added to hept-6-en-2-one (98 mg, 0.87 mmol) and dissolved in dichloromethane (2.5 mL). To this mixture Hoveyda-Grubbs II catalyst (18 mg, 10% mol) was added and the solution left to reflux for 24 h. The mixture was then reduced *in vacuo* and purified *via* silica gel column chromatography (petrol/Et₂O 1:1). This afforded a pale yellow oil (40 mg) that was identified by NMR as a 1:1 mixture of the desired product and homocoupled ketone. The pale yellow oil was dissolved in DCM (0.8 mL) and to this, trifluoroacetic acid (TFA, 0.4 mL) was added, with the mixture being allowed to stir for 30 minutes. The mixture was then reduced *in vacuo* and the residue dissolved in DCM and washed with 1M HCl. The acidic aqueous layer was basified with 12 M NaOH until pH 12 was reached and then extracted with DCM (3 \times 10 mL). The organic extracts were then combined, dried with MgSO₄ and the solvent removed *in vacuo* to give a clear foam (10 mg, 0.06 mmol, 21% over two steps). FTIR (ATR) V_{\max} : 2933, 1673, 1449, 1201, 1135, 799, 721 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 5.51-5.45 (m, 2H), 3.52-3.39 (m, 1H), 2.44 (t, *J* = 7.4 Hz, 2H), 2.15 (s, 3H), 2.08-1.94 (m, 2H), 1.73-1.59 (m, 2H), 1.15 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 209.1, 137.4, 127.5, 48.9, 43.0, 31.5, 30.0, 24.1, 23.3; HRMS-ESI calculated C₉H₁₇NO⁺ [M+H]⁺: 156.1343, found: 156.1380.

Biotransformations

General procedure for the preparation of compounds 4a-d,f

Commercially available (*S*)-selective ATA113 or (*R*)-selective ATA117 (25 mg) was rehydrated in HEPES buffer (4.5 mL, 100 mM, pH 7.5) containing PLP (2.00 mM) and isopropylamine (0.5 mmol, 2 equiv). The pH of the mixture was adjusted to 7.5, using aq HCl solution (1 M), and the total volume of the reaction was adjusted to 5 mL by addition of HEPES buffer. The ketoenone substrate **3a-e** was added (50 mM, 0.5 mL from a 500 mM stock solution in DMSO) and the reaction mixture incubated at 30 °C, 150 rpm for 24-48 h. The reaction was monitored by TLC. After completion, the pH of the supernatant was adjusted to 12 using aq NaOH solution (4 M) and the resulting mixture was extracted with Et₂O (3 \times 10 mL). The combined organic extracts were dried over Na₂SO₄ and concentrated under reduced pressure and analysed by GC.

Preparative scale biotransformation of (*R*),(*E*)-8-aminonon-6-en-2-one (**6f**) with ATA117

Commercially available (*R*)-selective ATA 117 (2.0 mg/mL) was rehydrated in HEPES buffer (1 mL, 100 mM, pH 7.5) containing PLP (1 mM). The pH of the mixture was adjusted to 7.5 and then (*R*),(*E*)-8-aminonon-6-en-2-one (**6e**) was added (5 mM, 50 μ L from a 100 mM stock in DMSO) and the reaction mixture incubated at 30 °C, 200 rpm for 24 h. The reaction was then analysed by GC-FID following extraction of the basified reaction mixture (adjusted to pH 12 with conc. NaOH) with EtOAc (750 μ L) and derivatisation (10 μ L acetic anhydride, 10 μ L triethylamine).

General procedure for the preparation of compounds **4e**

Commercially available (*S*)-selective ATA256 or (*R*)-selective ATA025 (25 mg) was rehydrated in HEPES buffer (4.5 mL, 100 mM, pH 7.5) containing PLP (2.00 mM) and isopropylamine (4 equiv, 86 μ L, 200mM). The pH of the mixture was adjusted to 7.5, using aq HCl solution (1 M), and the total volume of the reaction was adjusted to 4.5 mL by addition of HEPES buffer. The ketoenone substrate **3e** was added (50 mM, 0.5 mL from a 500 mM stock solution in DMSO) and the reaction mixture incubated at 50 °C, 150 rpm for 48 h. The reaction was monitored by GC-MS. After completion, the pH of the supernatant was adjusted to 12 using aq NaOH solution (4 M) and the resulting mixture was extracted with EtOAc (4 \times 20 mL). The combined organic extracts were dried over Na₂SO₄ and concentrated under reduced pressure and analysed by GC.

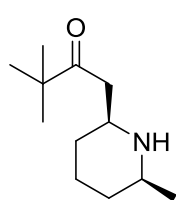
Epimerization procedure

The crude mixture was dissolved in an alcoholic solvent (5 mL) and stirred at the stated temperature for 24-48 h (see specific epimerization conditions in the table below), to allow epimerisation to take place. If required, the crude material was purified by column chromatography on silica gel (gradient from CH₂Cl₂ to 15% MeOH:CH₂Cl₂) to provide pure compounds **4a-f**.

| <i>Epimerization conditions</i> | | | | | |
|---------------------------------|-------------|----|------------------|---------|----------|
| Product | R | R' | Temperature (°C) | Solvent | Time (h) |
| 4a | <i>t</i> Bu | Me | 65 | MeOH | 24 |

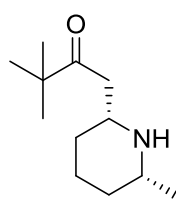
| | | | | | |
|-----------|--|----|------|------|----|
| 4b | CH ₂ =CHCH ₂ CH ₂ | Me | r.t. | MeOH | 48 |
| 4c | Ph | Me | r.t. | MeOH | 48 |
| 4d | EtO | Me | 80 | EtOH | 24 |
| 4e | Me | Et | r.t. | MeOH | 24 |
| 4f | Me | Me | r.t. | MeOH | 48 |

3,3-dimethyl-1-[(2*S*,6*S*)-6-methylpiperidin-2-yl]butan-2-one (**4a**)



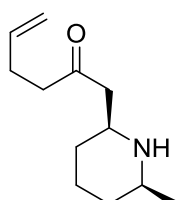
Yellow oil (38 mg, 78% yield, 99% *ee*). FTIR (neat) V_{\max} : 2962, 2972, 1700, 1466, 1366, 1062, 732, 570 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.01-2.90 (m, 1H), 2.73-2.62 (m, 1H), 2.61-2.48 (m, 2H), 1.77-1.66 (m, 1H), 1.60-1.44 (m, 2H), 1.43-1.28 (m, 1H), 1.18-0.98 (m, 2H), 1.09 (s, 9H), 1.03 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 215.9, 52.6, 52.2, 44.1, 43.4, 33.6, 31.6, 26.3, 24.5, 22.8; LC-MS (m/z): Calculated C₁₂H₂₄NO⁺ [M+H]⁺: 198.1852, found: 198.1850. $[\alpha]_D^{24}$ = +15 (c 2.6, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 110 °C, P = 6 psi, retention times: $t_r(S,S)$ = 47.4 min (only enantiomer), $t_r(R,R)$ = 44.8 min (not observed).

3,3-dimethyl-1-[(2*R*,6*R*)-6-methylpiperidin-2-yl]butan-2-one (**4a**)



Yellow oil (43 mg, 88% yield, 99% *ee*). $[\alpha]_D^{24}$ = -16 (c 3.8, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 110 °C, P = 6 psi, retention times: $t_r(S,S)$ = 47.4 min (not observed), $t_r(R,R)$ = 44.8 min (only enantiomer).

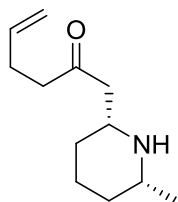
1-[(2*S*,6*S*)-6-methylpiperidin-2-yl]hex-5-en-2-one (**4b**)



Yellow oil (45 mg, 92% yield, 99% *ee*). FTIR (neat) V_{\max} : 2925, 1709, 1440, 1373, 1318, 1128, 995, 911, 747, 535 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 5.83-5.67 (m, 1H), 5.05-4.87 (m, 2H), 3.04-2.89 (m, 1H), 2.69-2.59 (m, 1H), 2.51-2.40 (m, 4H), 2.31-2.19 (m, 3H), 1.76-1.66 (m, 1H), 1.60-1.42 (m, 2H), 1.41-1.25 (m, 1H), 1.12-0.92 (m, 2H), 1.00 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃): δ 209.8, 136.9, 115.2, 52.3, 52.0, 49.6, 42.3, 33.7, 31.8, 27.5, 24.5, 22.9; LC-MS (m/z): Calculated C₁₂H₂₂NO⁺ [M+H]⁺: 196.1696, found: 196.1689. $[\alpha]_D^{24}$ = +28 (c 2.1, MeOH). *Ee*

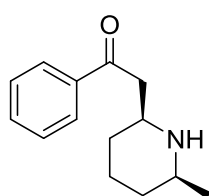
determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 125 °C, P = 6 psi, retention times: $t_r(S,S)$ = 43.2 min (only enantiomer), $t_r(R,R)$ = 40.6 min (not observed).

1-[(2*R*,6*R*)-6-methylpiperidin-2-yl]hex-5-en-2-one (4b)



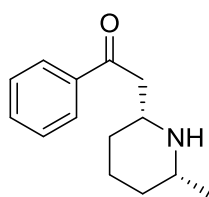
The synthesis of (*R,R*)-**4b** was carried out according the general procedure for biotransformations using ATA117 and 4 equiv of isopropylamine. Yellow oil (44 mg, 90% yield, 99% *ee*). $[\alpha]_D^{24} = -27$ (*c* 4.4, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, T = 125 °C, P = 6 psi, retention times: $t_r(S,S)$ = 43.2 min (not observed), $t_r(R,R)$ = 40.6 min (only enantiomer).

2-[(2*S*,6*S*)-6-methylpiperidin-2-yl]-1-phenylethanone (4c)



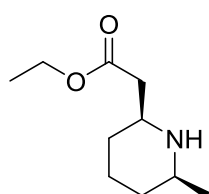
Yellow oil (41 mg, 76% yield, 99% *ee*). FTIR (neat) V_{\max} : 2926, 1680, 1448, 1223, 751, 731, 689, 572 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.97-7.89 (m, 2H), 7.59-7.51 (m, 1H), 7.48-7.40 (m, 2H), 3.26-3.15 (m, 1H), 3.11-3.03 (m, 2H), 2.82-2.69 (m, 1H), 2.55 (br s, 1H), 1.83-1.72 (m, 1H), 1.68-1.55 (m, 2H), 1.49-1.34 (m, 1H), 1.30-1.17 (m, 1H), 1.17-1.02 (m, 1H), 1.07 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 199.5, 136.9, 133.2, 128.6, 128.0, 52.8, 52.2, 45.4, 33.8, 32.0, 24.6, 22.9; LC-MS (m/z): Calculated $\text{C}_{14}\text{H}_{20}\text{NO}^+$ $[\text{M}+\text{H}]^+$: 218.1539, found: 218.1543. $[\alpha]_D^{24} = +20$ (*c* 3.0, MeOH). *Ee* determination by chiral HPLC analysis, Lux Amylose-1 column, flow = 0.5 mL/min, hexane/2-propanol 9:1, retention times: $t_r(S,S)$ = 11.8 min (only enantiomer), $t_r(R,R)$ = 27.0 min (not observed).

2-[(2*R*,6*R*)-6-methylpiperidin-2-yl]-1-phenylethanone (4c)



The synthesis of (*R,R*)-**4c** was carried out according the general procedure for biotransformations using ATA117 enzyme and 4 equiv of isopropylamine. Yellow oil (24 mg, 44% yield, 99% *ee*). $[\alpha]_D^{24} = -21$ (*c* 1.0, MeOH). *Ee* determination by chiral HPLC analysis, lux amylose-1 column, flow = 0.5 mL/min, 90% hexane: 10% 2-propanol, retention times: $t_r(S,S)$ = 11.8 min (not observed), $t_r(R,R)$ = 27.0 min (only enantiomer).

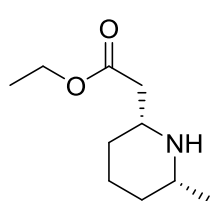
ethyl [(2*S*,6*S*)-6-methylpiperidin-2-yl]acetate (4d)



Yellow oil (33 mg, 72% yield, 99% *ee*). FTIR (neat) V_{\max} : 2927, 1729, 1288, 1188, 115, 1096, 1027, 773, 557 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3):

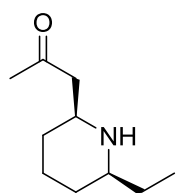
δ 4.12 (q, $J = 7.1$ Hz, 2H), 3.01-2.90 (m, 1H), 2.74-2.61 (m, 1H), 2.44-2.32 (m, 2H), 2.06 (br s, 1H), 1.80-1.70 (m, 1H), 1.63-1.53 (m, 2H), 1.44-1.29 (m, 1H), 1.24 (t, $J = 7.1$ Hz, 3H), 1.16-0.96 (m, 2H), 1.05 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 172.5, 60.3, 53.4, 52.2, 41.5, 33.8, 31.9, 24.5, 22.9, 14.2; LC-MS (m/z): Calculated $\text{C}_{10}\text{H}_{20}\text{NO}_2^+$ $[\text{M}+\text{H}]^+$: 186.1489, found: 186.1502. $[\alpha]_{\text{D}}^{24} = +21$ (c 3.8, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, $T = 110$ °C, $P = 6$ psi, retention times: $t_{\text{r}}(S,S) = 34.0$ min (only enantiomer), $t_{\text{r}}(R,R) = 32.0$ min (not observed).

ethyl [(2*R*,6*R*)-6-methylpiperidin-2-yl]acetate (4d)



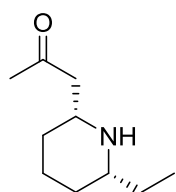
Yellow oil (32 mg, 70% yield, 99% *ee*). $[\alpha]_{\text{D}}^{24} = -20$ (c 3.0, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, $T = 110$ °C, $P = 6$ psi, retention times: $t_{\text{r}}(S,S) = 34.0$ min (not observed), $t_{\text{r}}(R,R) = 32.0$ min (only enantiomer).

(*S,S*)-(1-[6-ethylpiperidin-2-yl]propan-2-one) (4e)



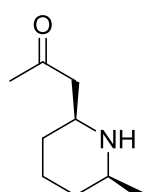
Yellow oil (21 mg, 50% yield, 70% *ee*). FTIR (neat) V_{max} : 3326, 2927, 2855, 1709, 1458, 1359, 1155, 1111, 963 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 3.03-2.88 (m, 1H), 2.57-2.49 (m, 2H), 2.47-2.35 (m, 1H), 2.11 (s, 3H), 1.79-1.69 (m, 1H), 1.69-1.60 (m, 1H), 1.54-1.45 (m, 1H), 1.41-1.24 (m, 3H), 1.16-0.90 (m, 2H), 0.87 (t, $J = 7.6$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 208.5, 58.3, 52.4, 50.3, 32.1, 31.5, 30.6, 29.9, 24.4, 10.3; LC-MS (m/z): Calculated $\text{C}_{10}\text{H}_{20}\text{NO}^+$ $[\text{M}+\text{H}]^+$: 170.1538, found: 170.1539. $[\alpha]_{\text{D}}^{24} = +18$ (c 3.4, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, $T = 110$ °C, $P = 6$ psi, retention times: $t_{\text{r}}(S,S) = 29.4$ min (85%), $t_{\text{r}}(R,R) = 27.4$ min (15%).

(*R,R*)-(1-[6-ethylpiperidin-2-yl]propan-2-one) (4e)



Yellow oil (1 mg, 2% yield, 99% *ee*). $[\alpha]_{\text{D}}^{24} = -60$ (c 0.3, MeOH). *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, $T = 110$ °C, $P = 6$ psi, retention times: $t_{\text{r}}(S,S) = 29.4$ min (not observed), $t_{\text{r}}(R,R) = 27.4$ min (only enantiomer).

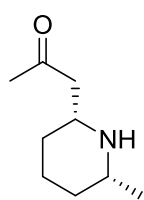
(*S,S*)-(1-[6-methylpiperidin-2-yl]propan-2-one); (*S,S*)-(+)-pinidinone (4f)^[10]



Yellow oil (36 mg, 93% yield, 99% *ee*). FTIR (neat) V_{max} : 2927, 2859, 1706, 1360, 1159, 734, 552 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 3.06-2.96 (m, 1H),

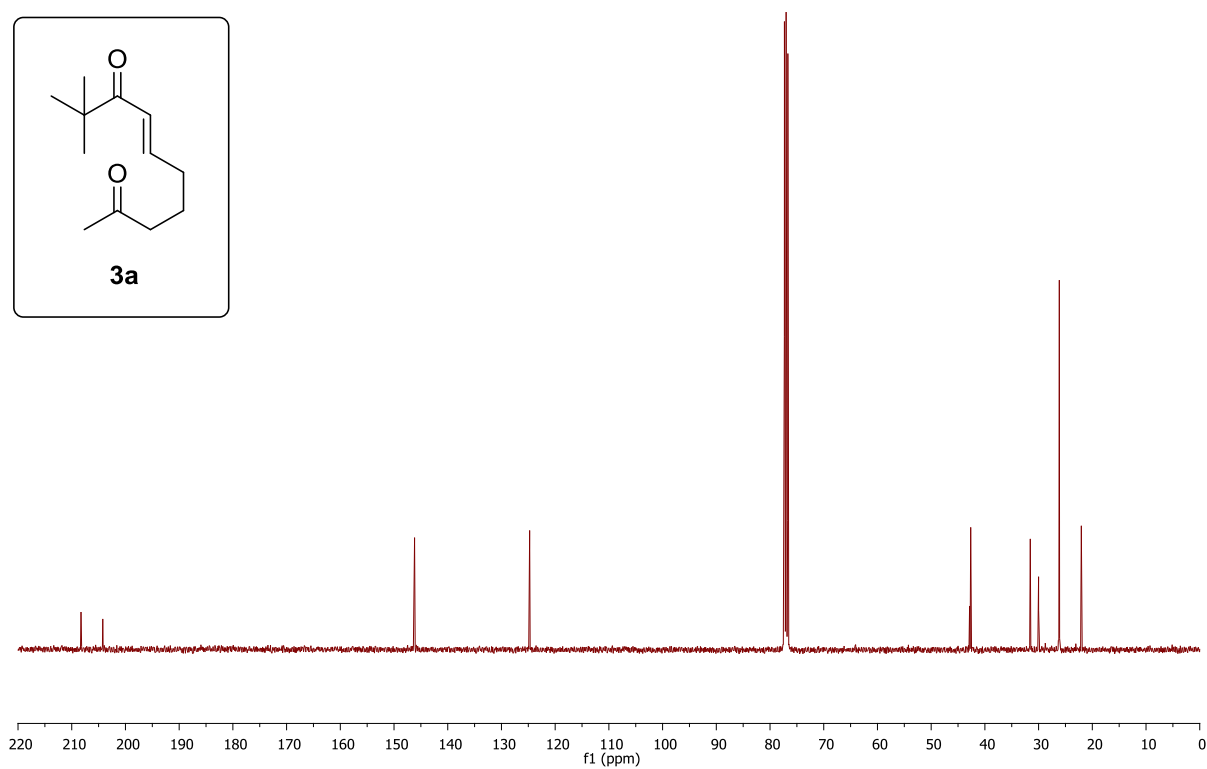
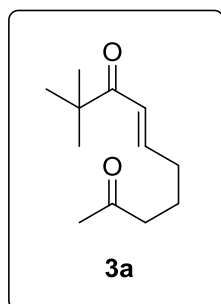
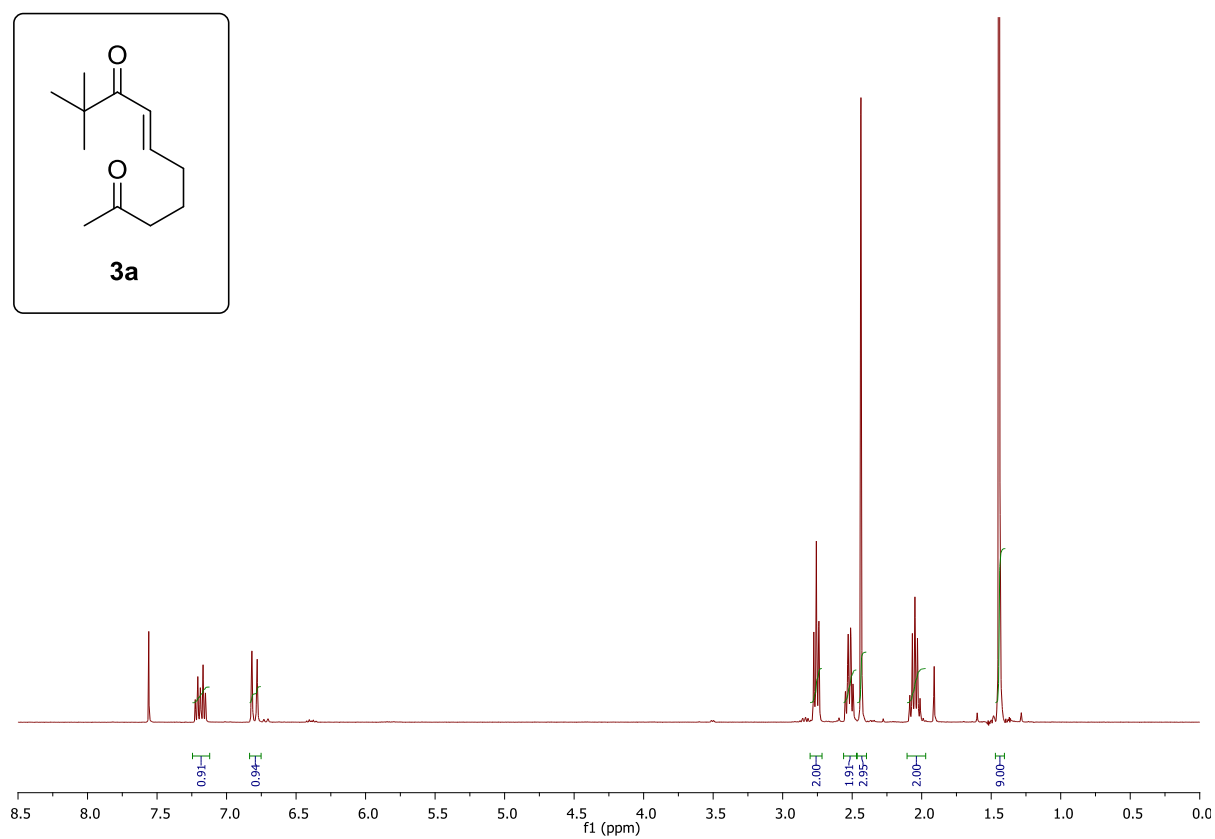
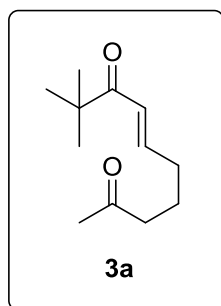
2.74-2.63 (m, 1H), 2.54 (d, $J = 6.4$ Hz, 2H), 2.13 (s, 3H), 2.00 (br, s, 1H), 1.75 (m, 1H), 1.64-1.55 (m, 1H), 1.55-1.47 (m, 1H), 1.43-1.21 (m, 2H), 1.15-0.97 (m, 2H), 1.04 (d, $J = 6.4$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3): δ 208.5, 52.3, 52.0, 50.6, 33.8, 31.8, 30.6, 24.6, 23.0; LC-MS (m/z): Calculated $\text{C}_9\text{H}_{18}\text{NO}^+$ $[\text{M}+\text{H}]^+$: 156.1383, found: 156.1385. $[\alpha]_{\text{D}}^{24} = +26$ (c 3.9, MeOH) $\{\text{Lit.}[\alpha]_{\text{D}}^{24} = +25$ (c 0.4, MeOH) $\}$. *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, $T = 105$ °C, $P = 6$ psi, retention times: $t_{\text{r}}(S,S) = 26.2$ min (only enantiomer), $t_{\text{r}}(R,R) = 24.1$ min (not observed). In accordance with literature data.

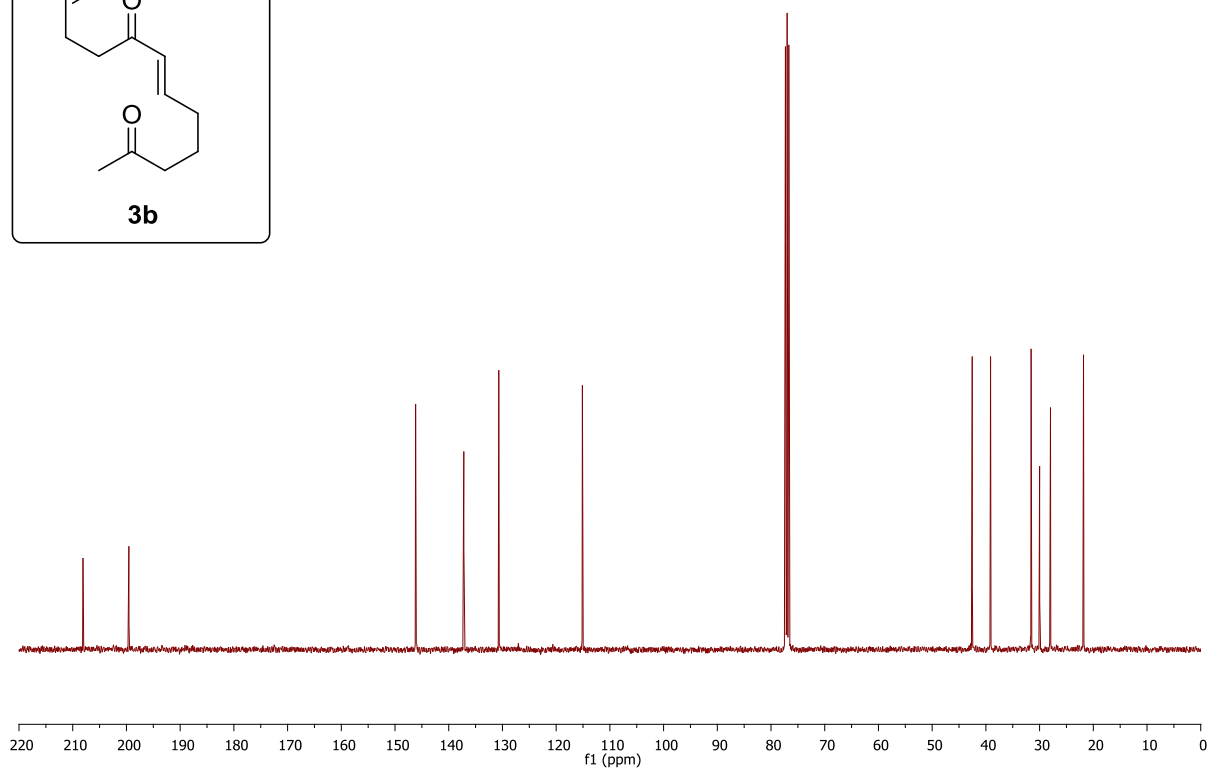
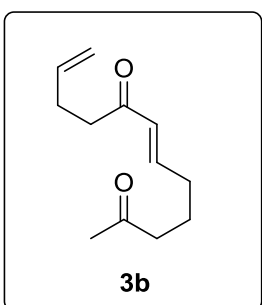
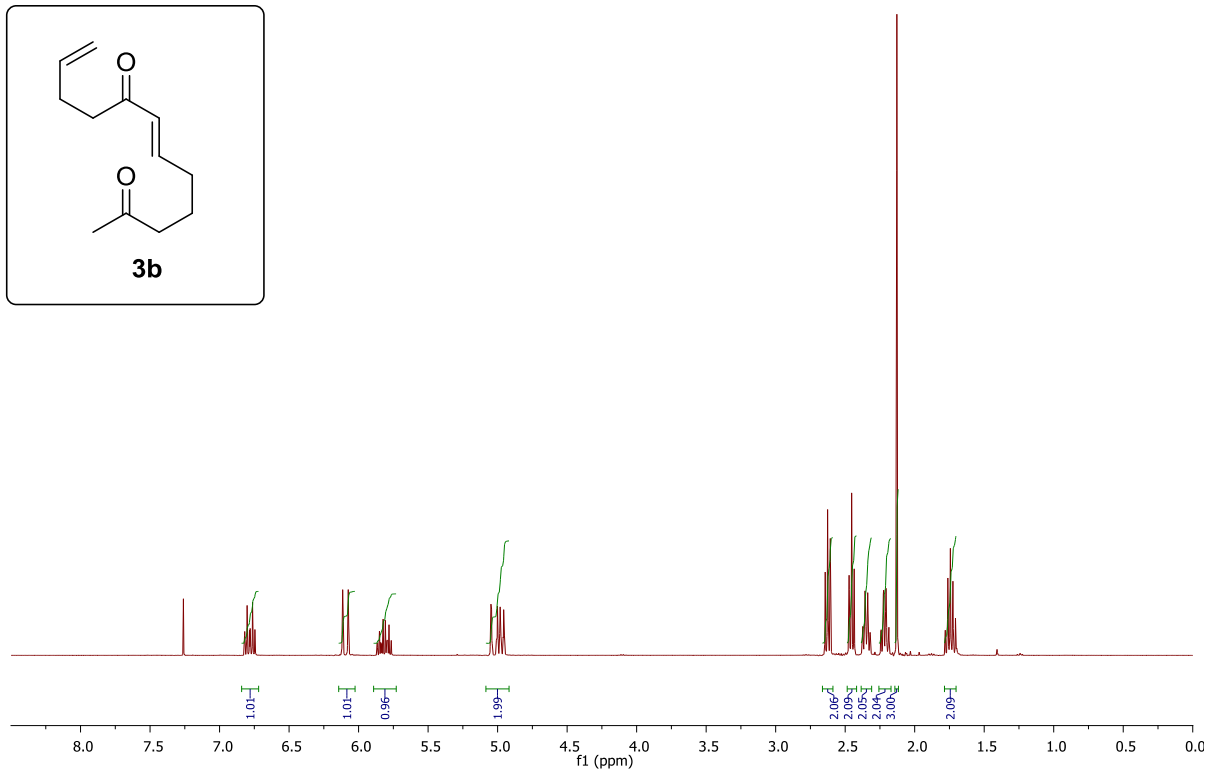
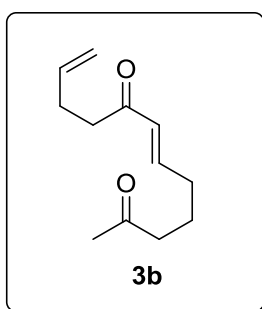
(*R,R*)- 1-[6-methylpiperidin-2-yl]propan-2-one); (*R,R*)-(-)-pinidinone (4f)^[11]

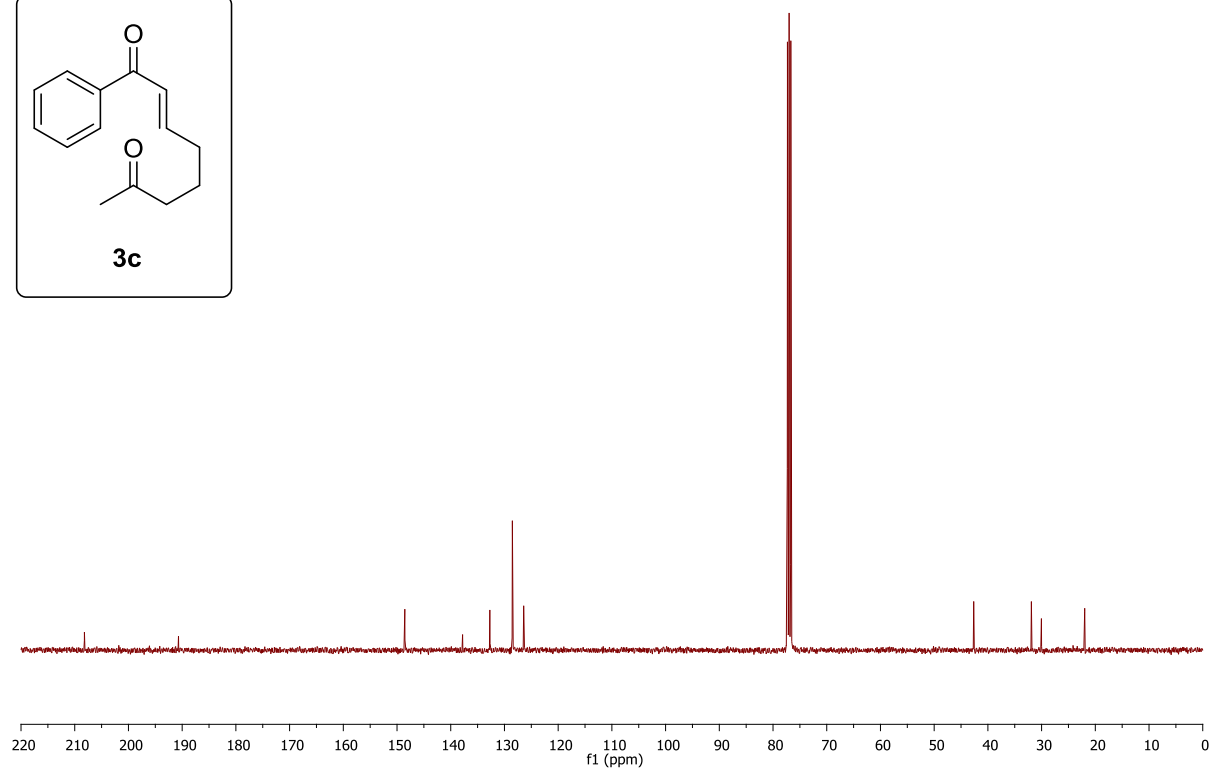
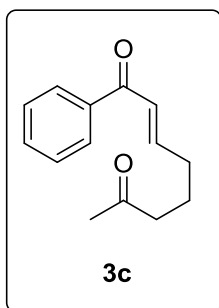
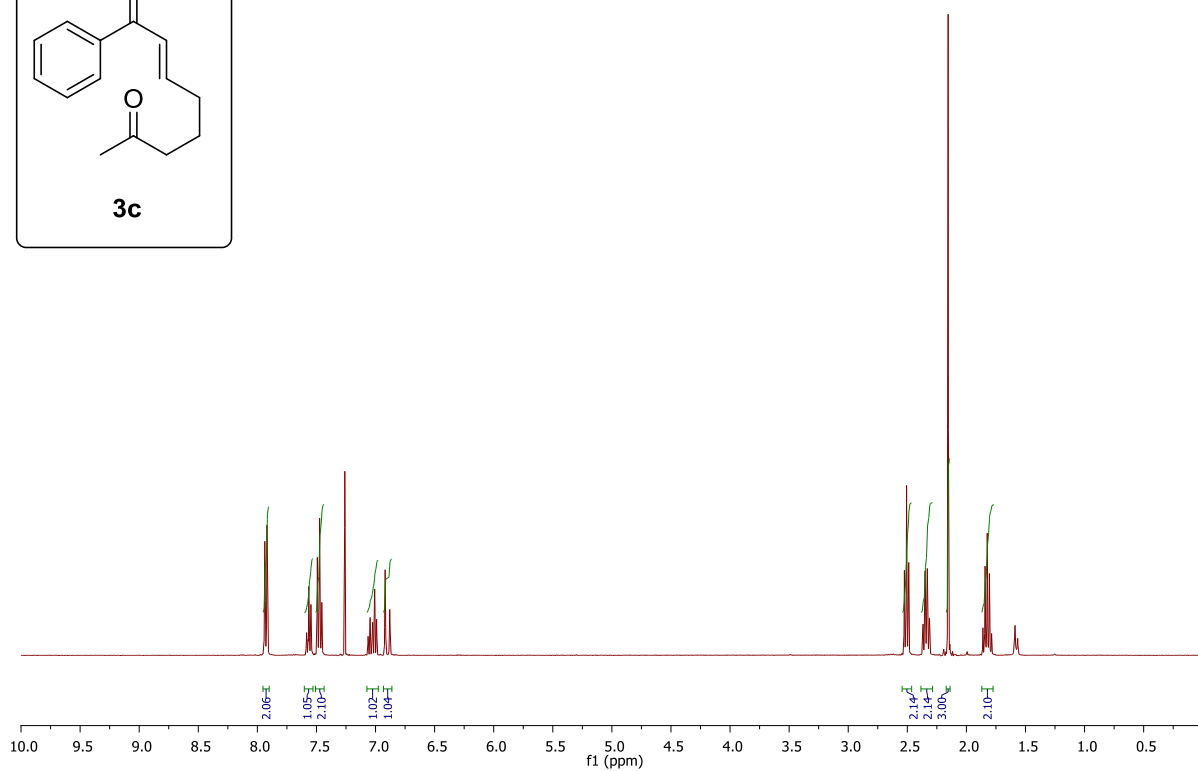
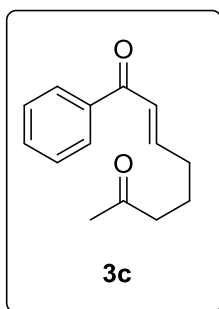


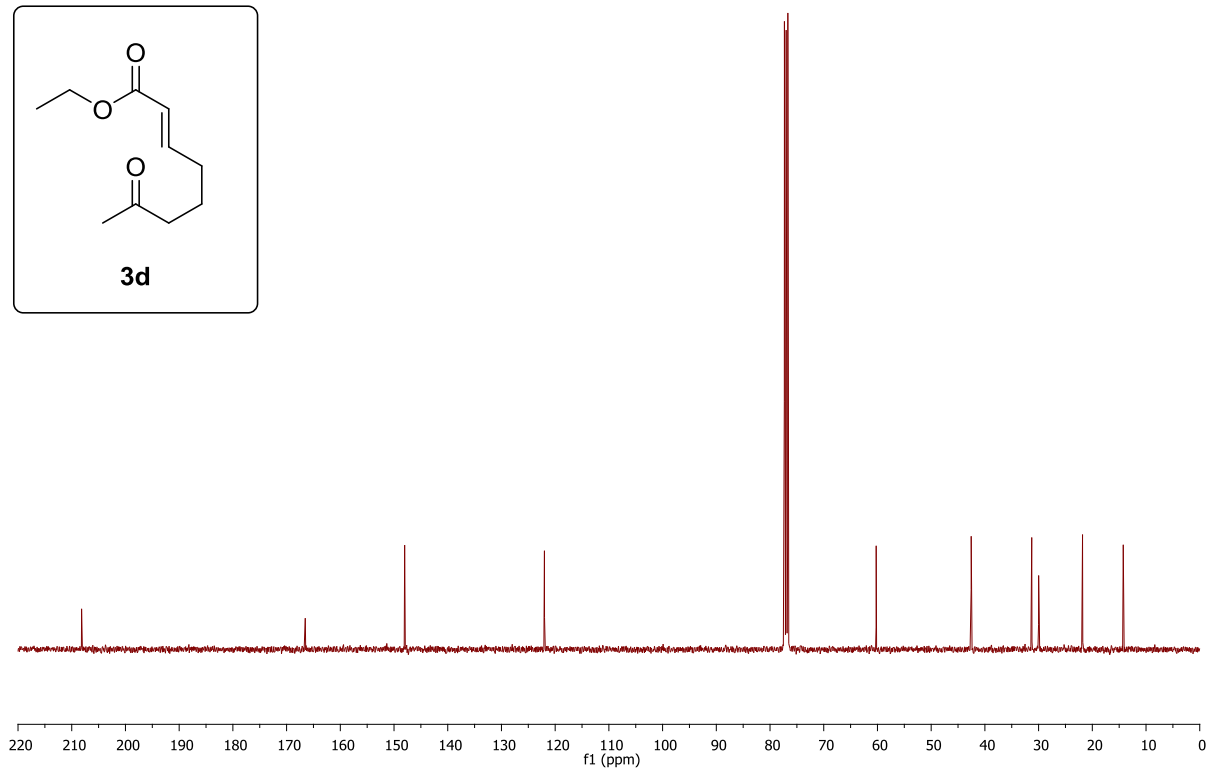
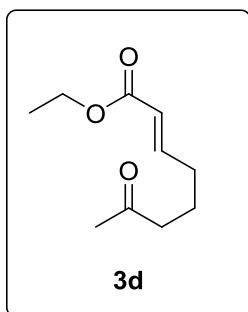
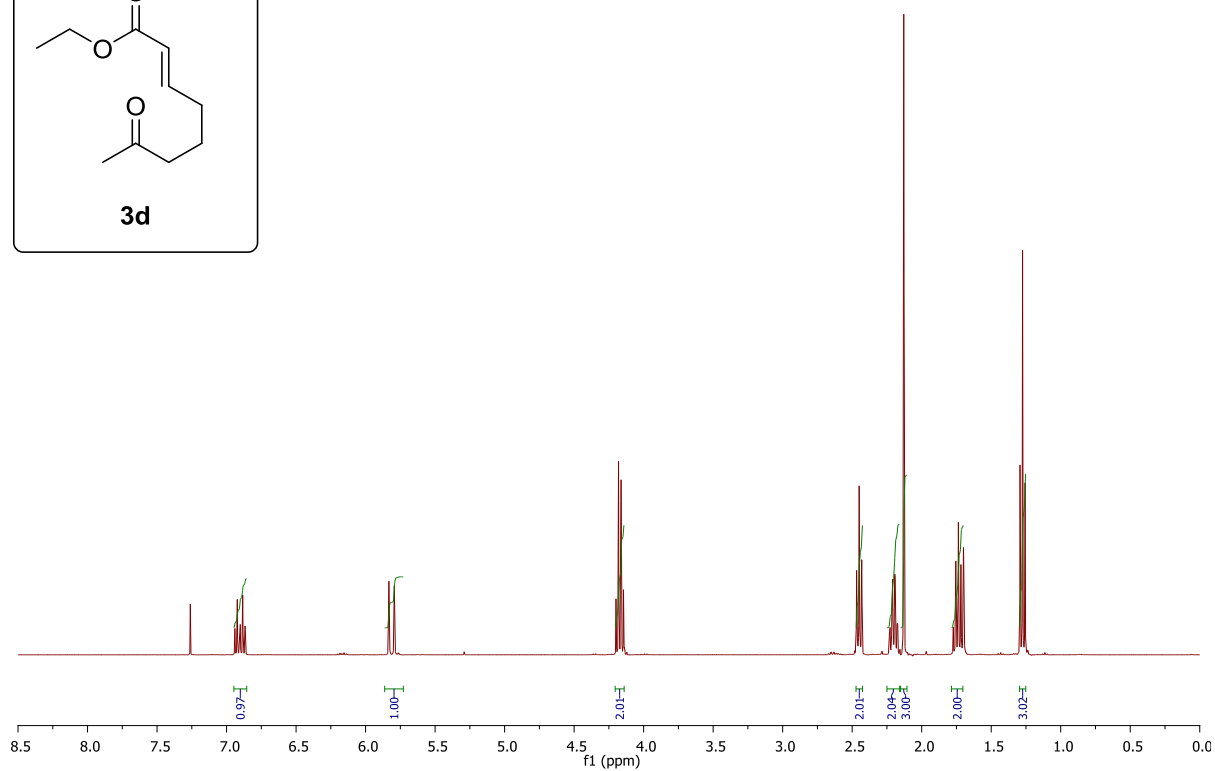
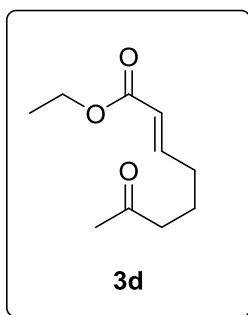
Yellow oil (35 mg, 91% yield, 99% *ee*). $[\alpha]_{\text{D}}^{24} = -25$ (c 2.4, MeOH) $\{\text{Lit.}[\alpha]_{\text{D}}^{24} = -4$ (c 3.5, MeOH) $\}$. *Ee* determination by chiral GC analysis, CP-Chirasil-DEX CB column, $T = 105$ °C, $P = 6$ psi, retention times: $t_{\text{r}}(S,S) = 26.2$ min (not observed), $t_{\text{r}}(R,R) = 24.1$ min (only enantiomer). In accordance with literature data.

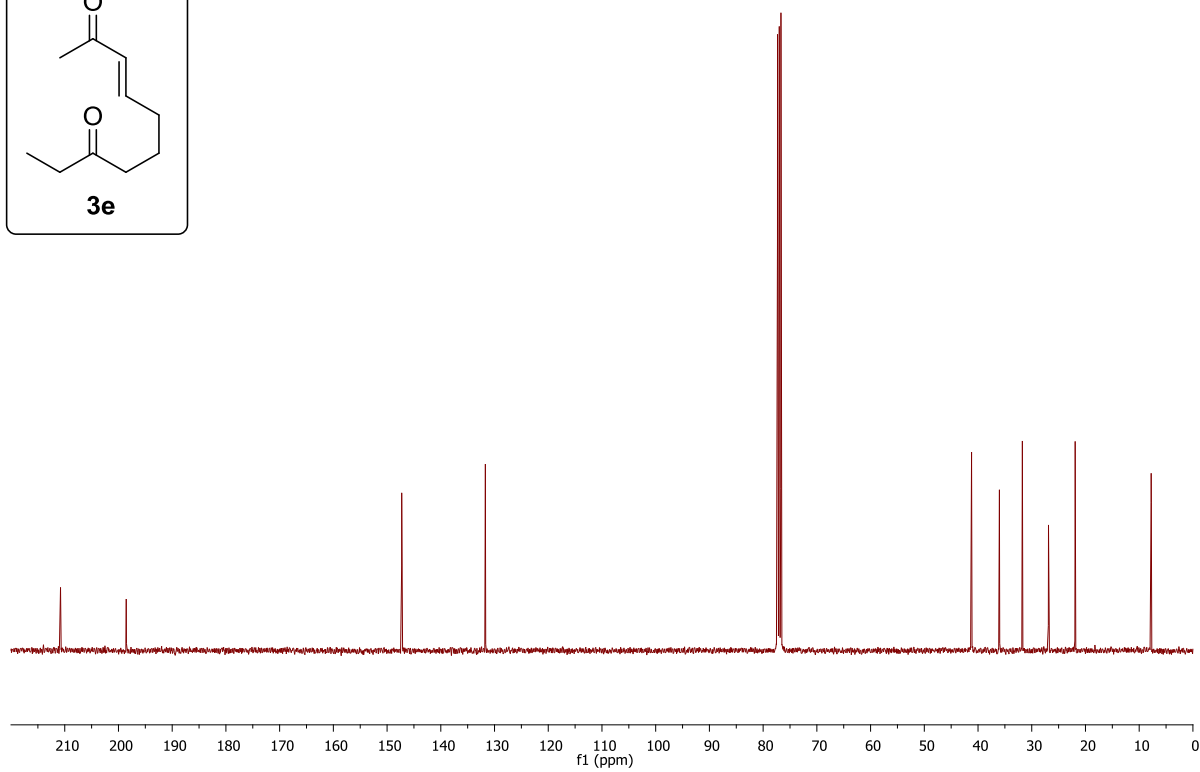
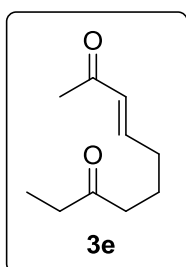
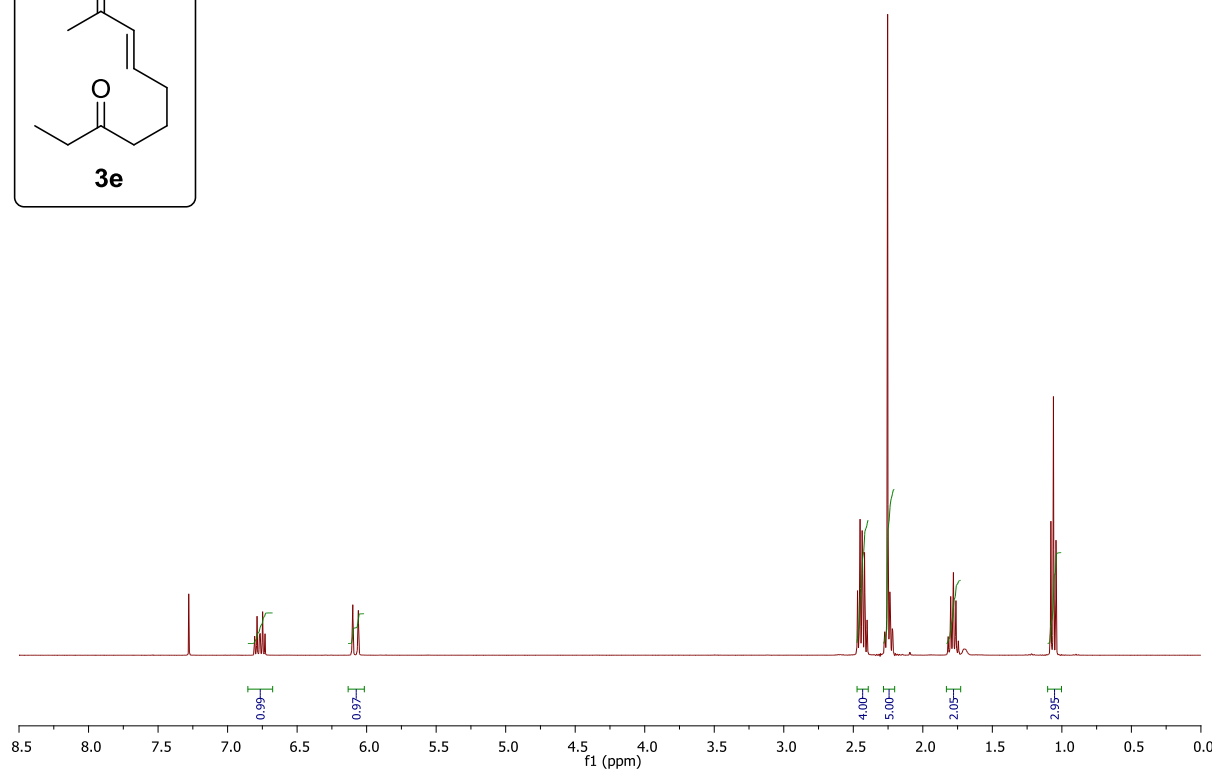
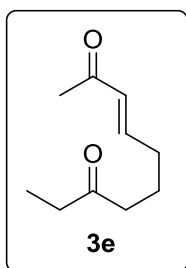
^1H , ^{13}C and NOE NMR Spectra

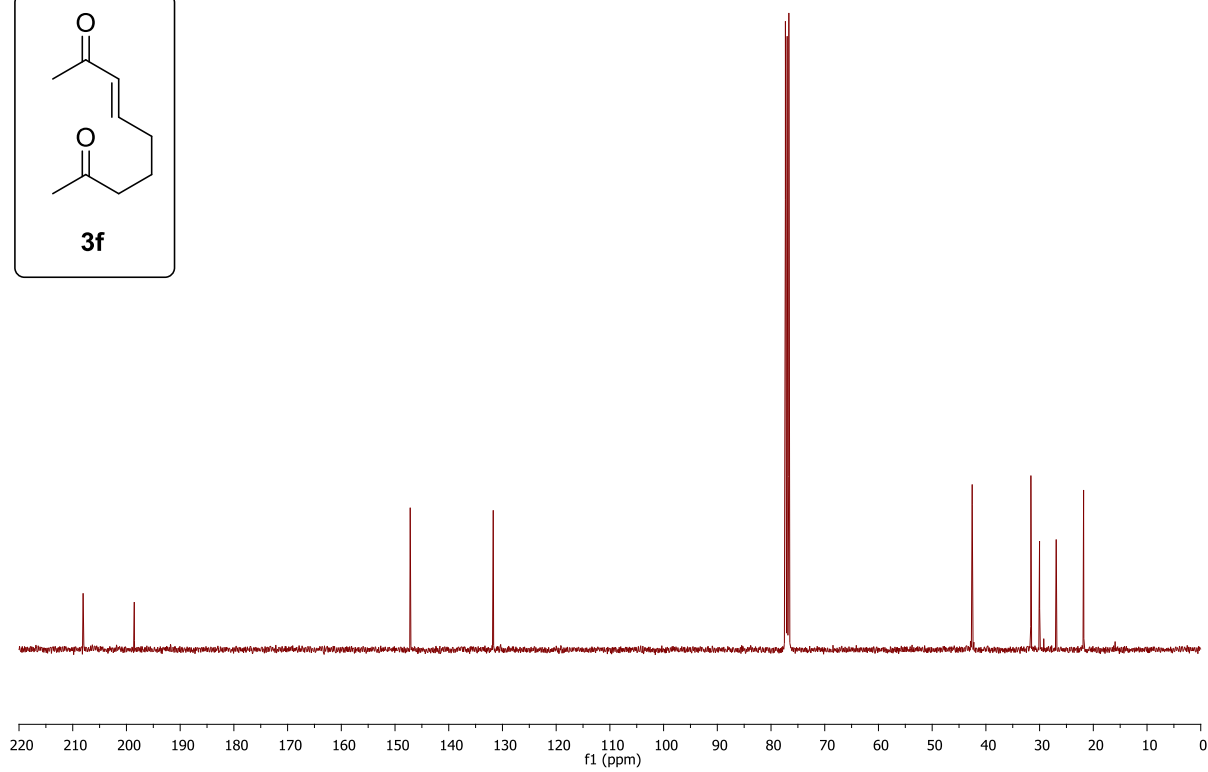
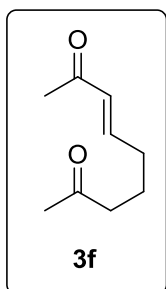
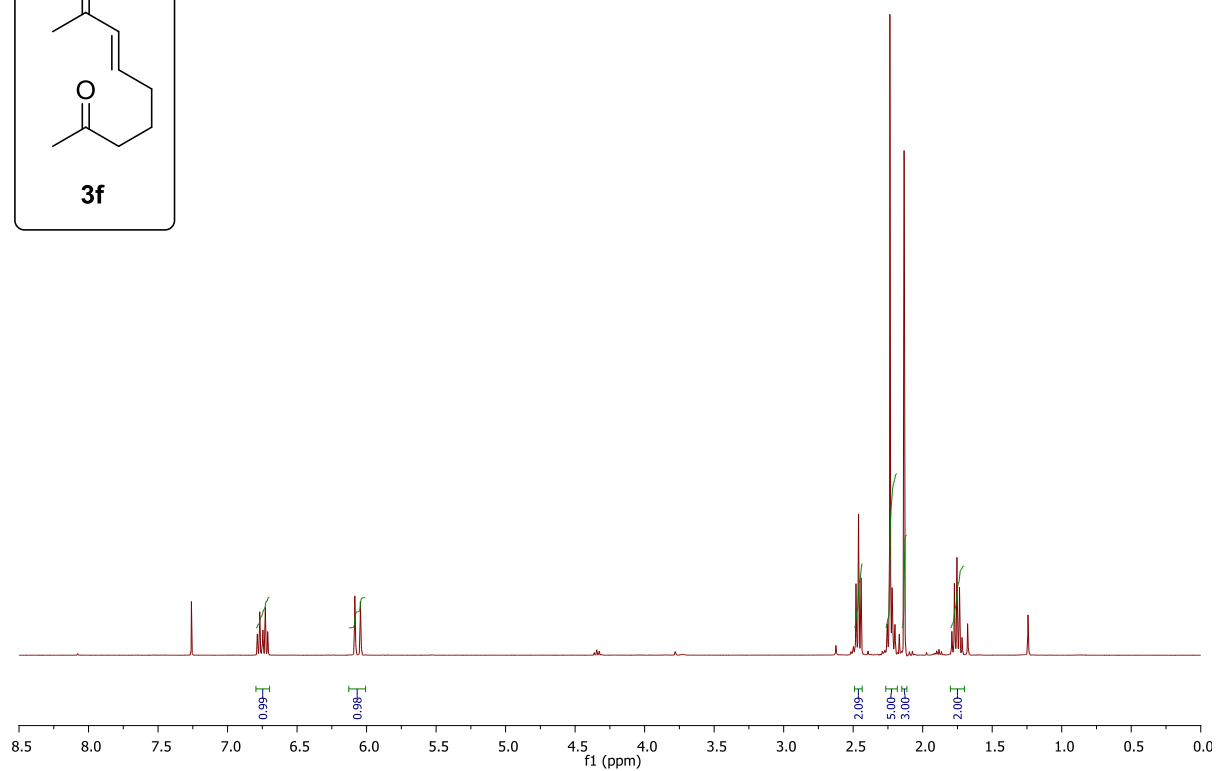
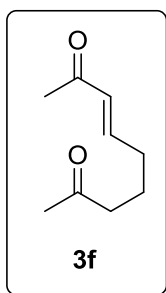


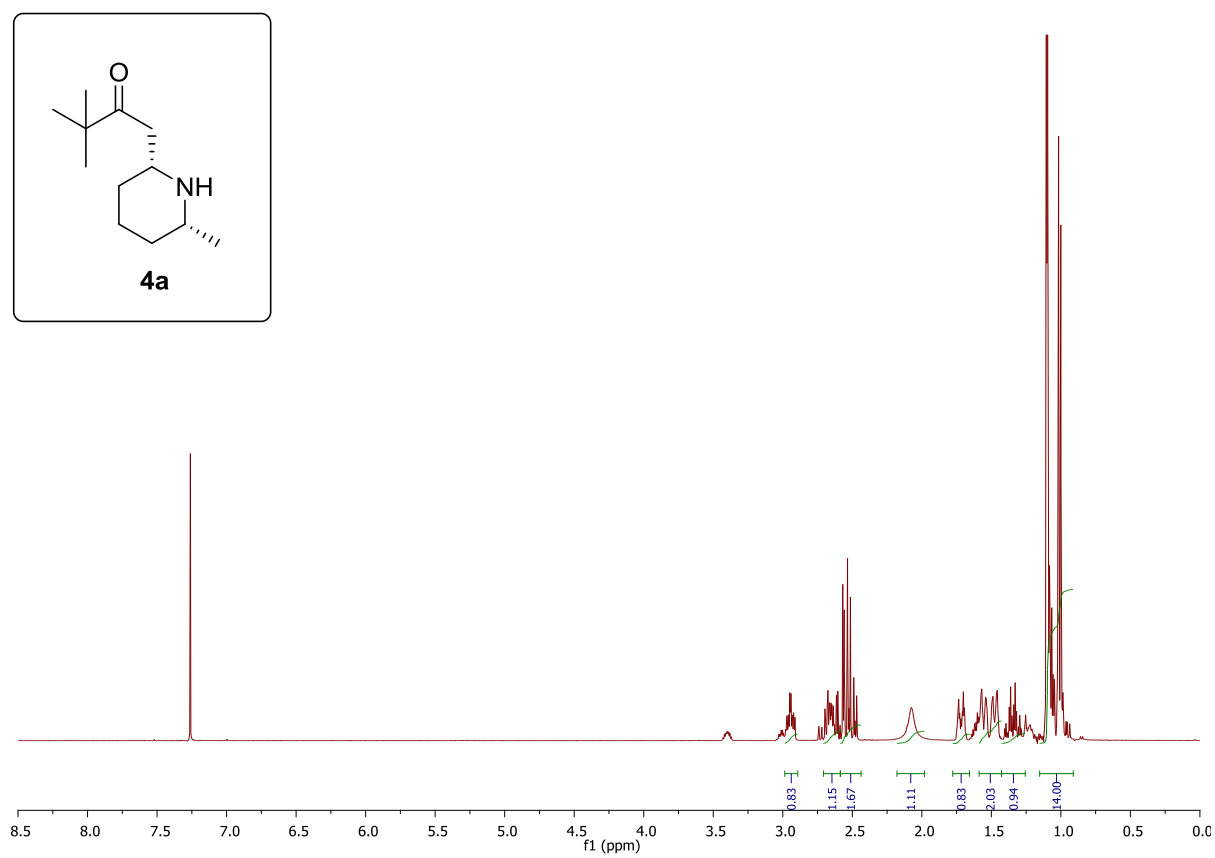
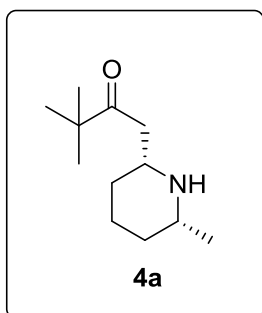
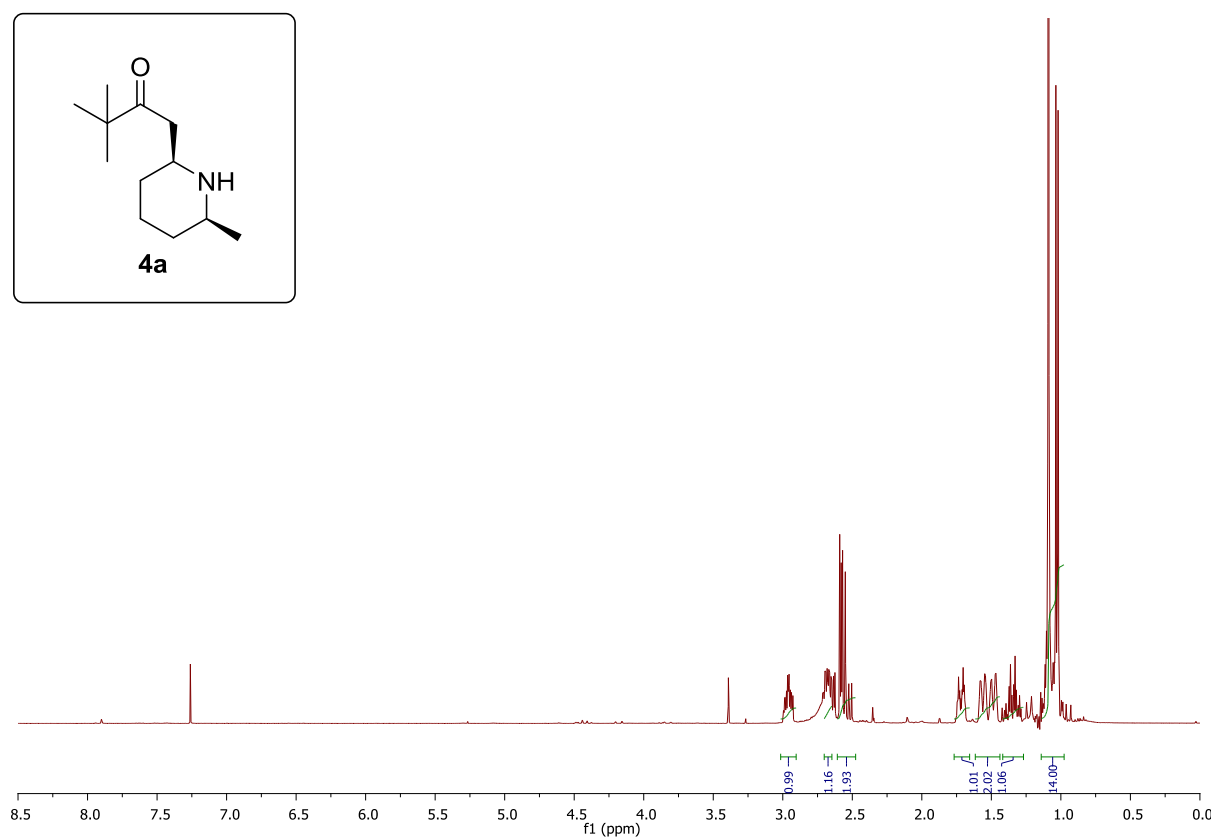
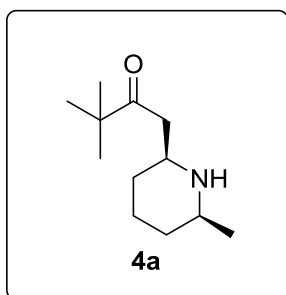


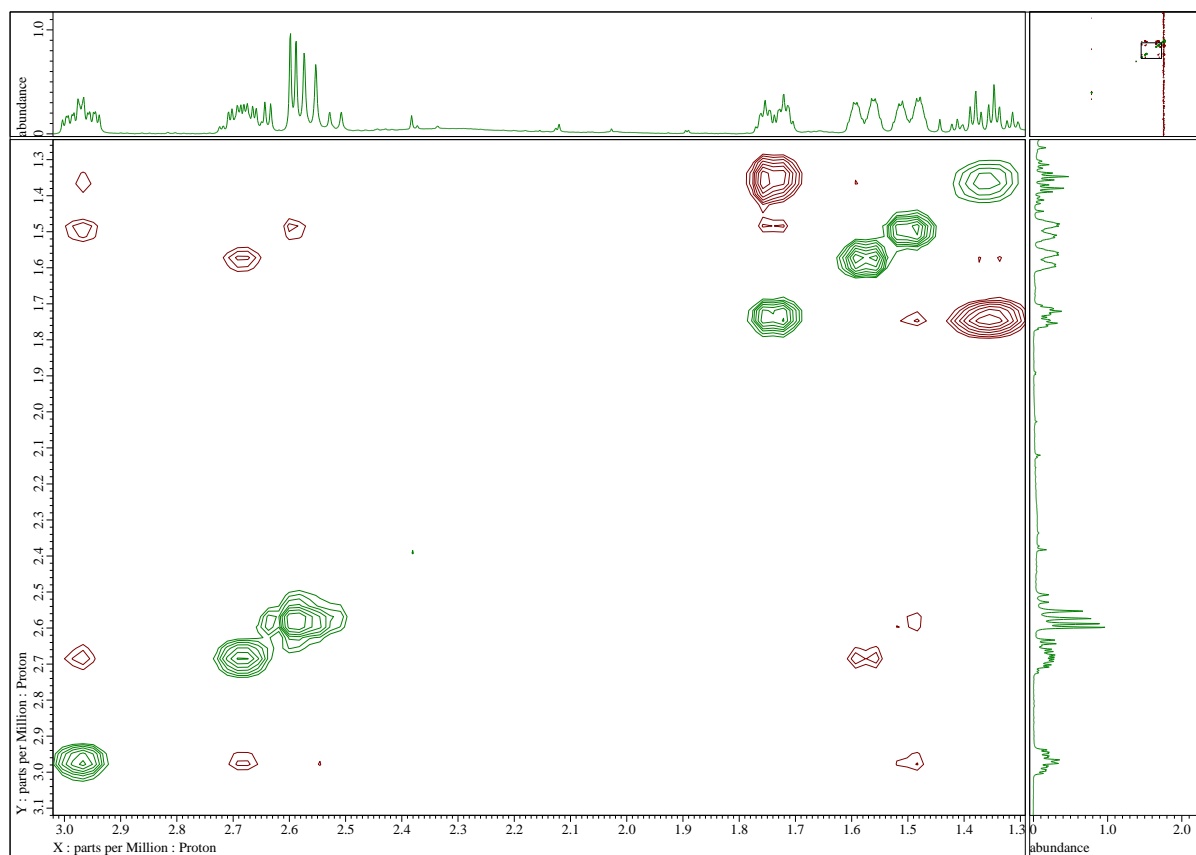
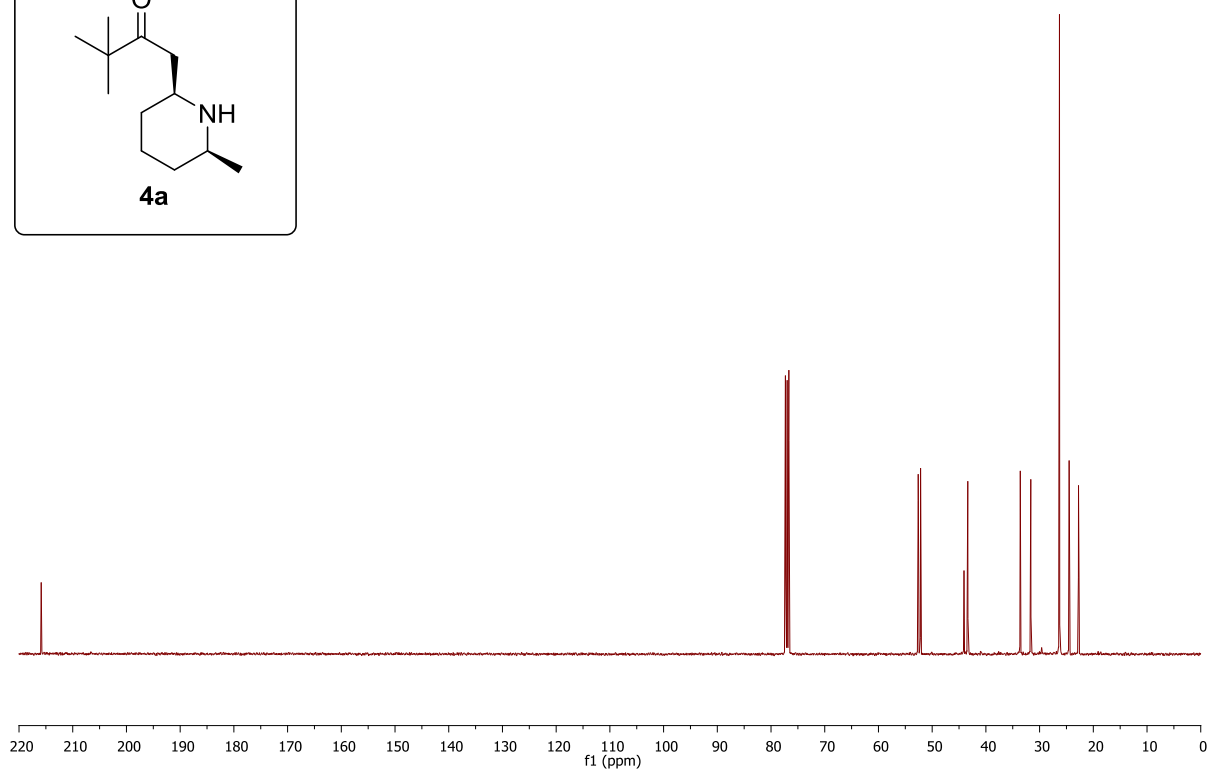
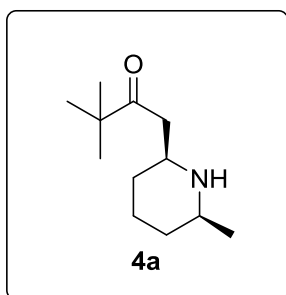


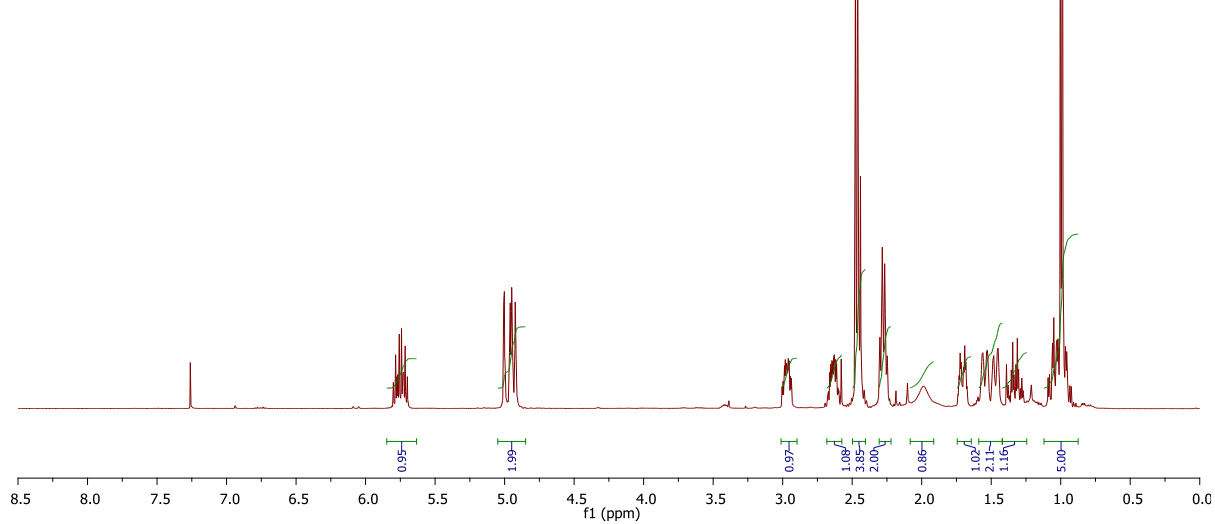
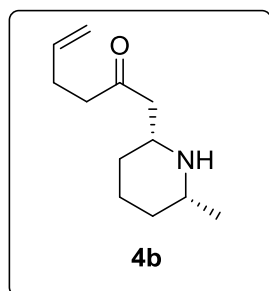
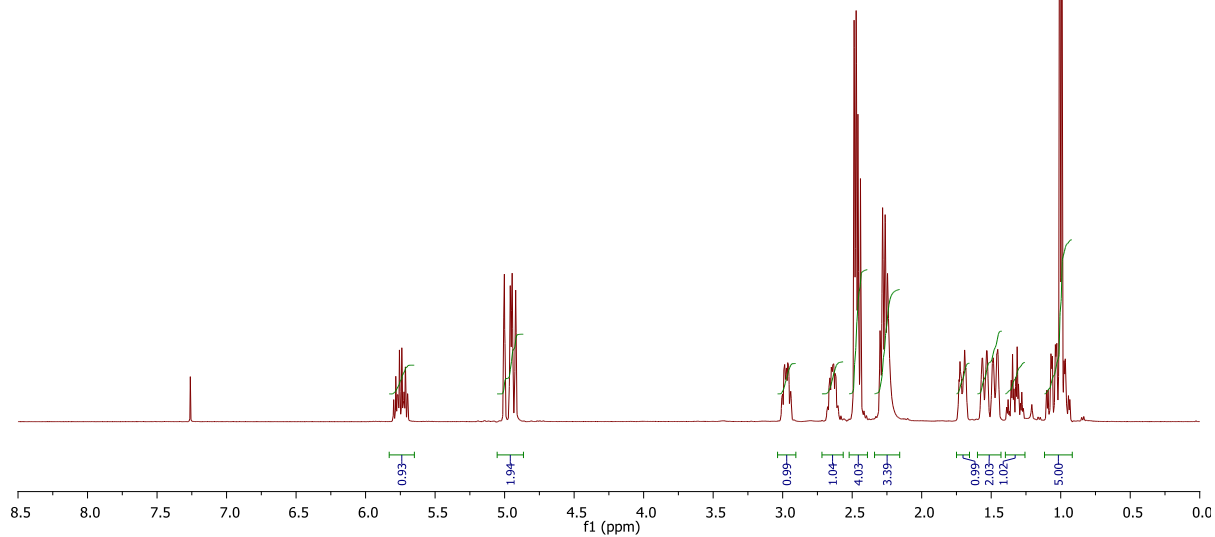
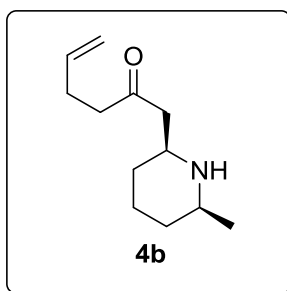


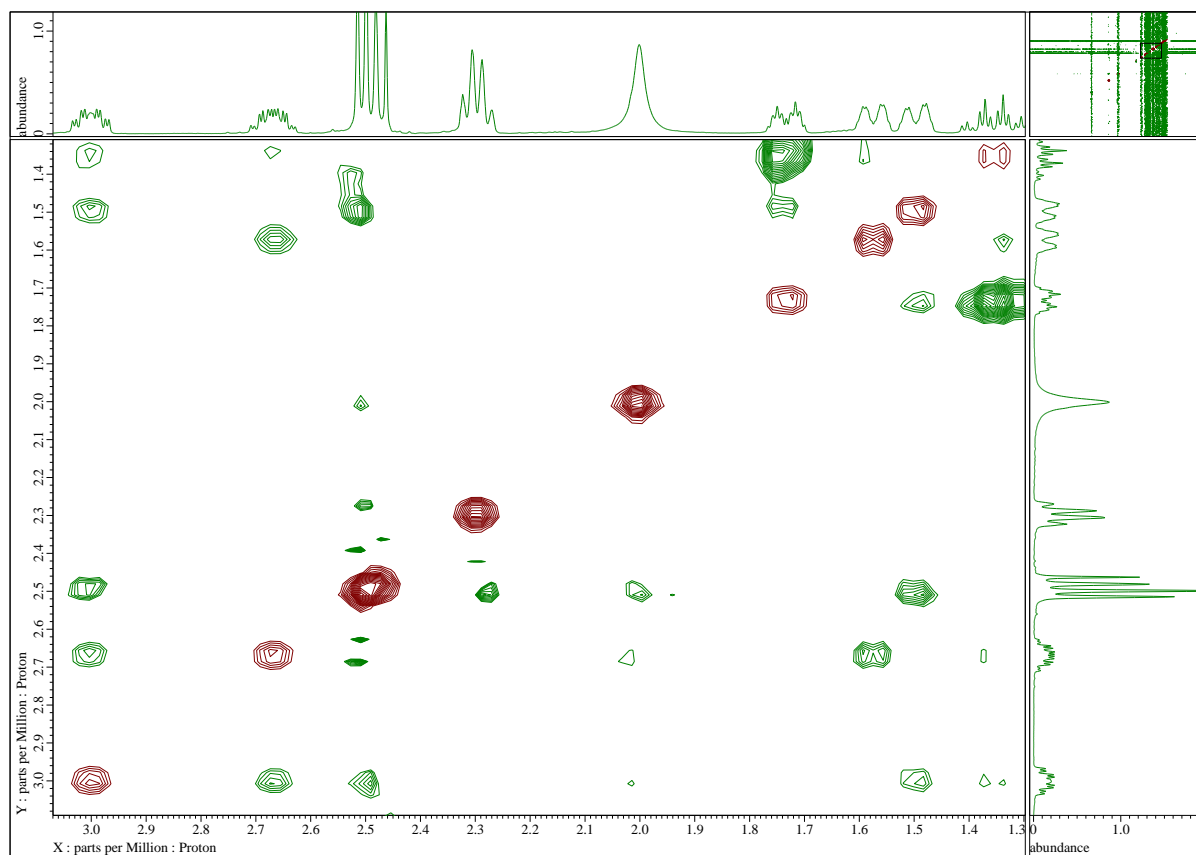
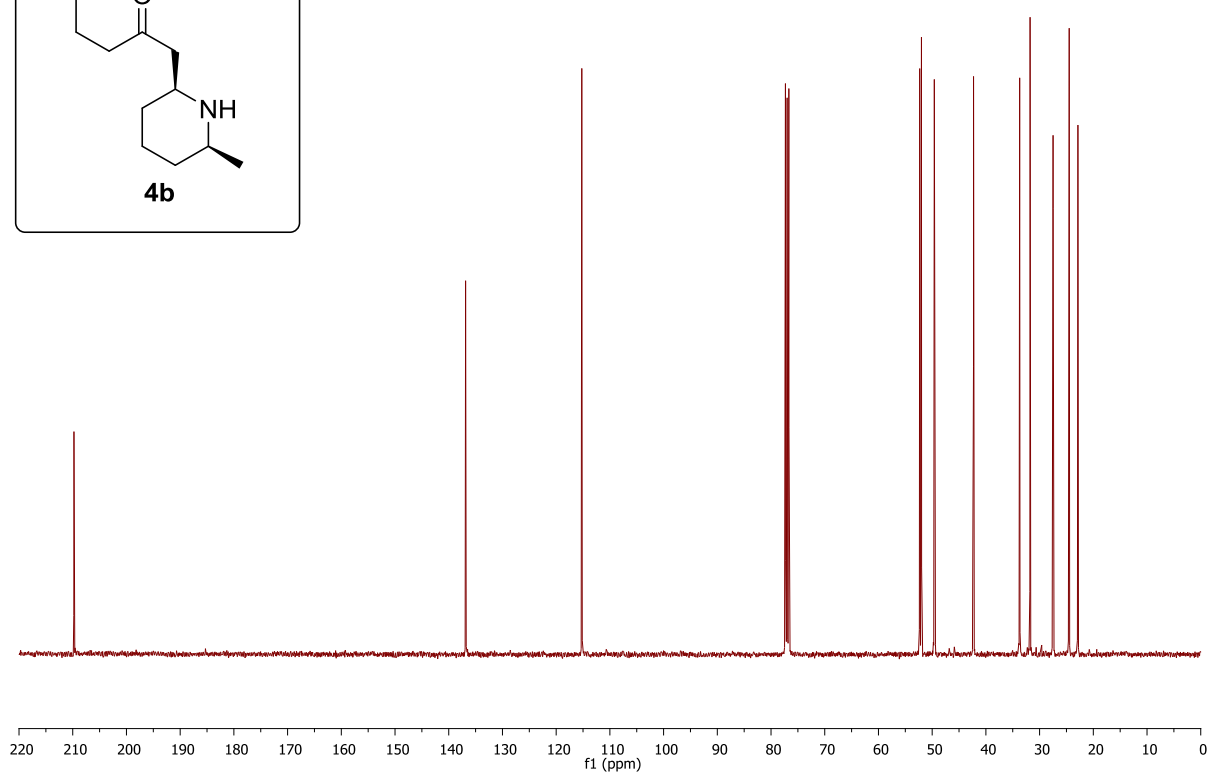
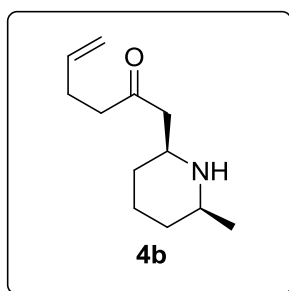


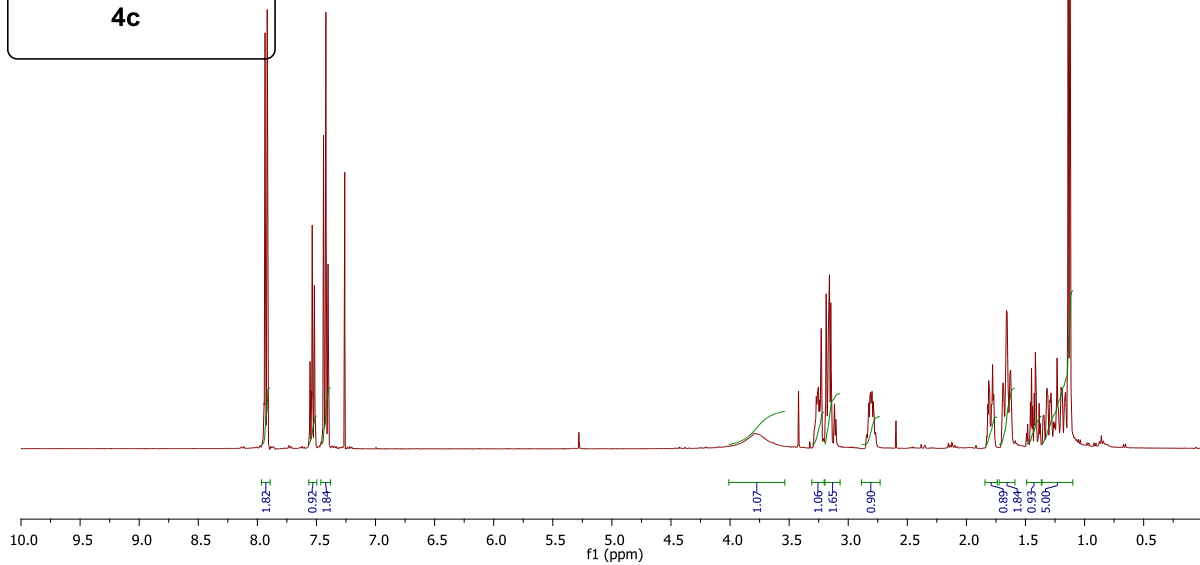
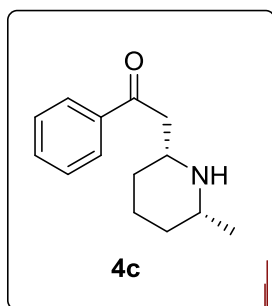
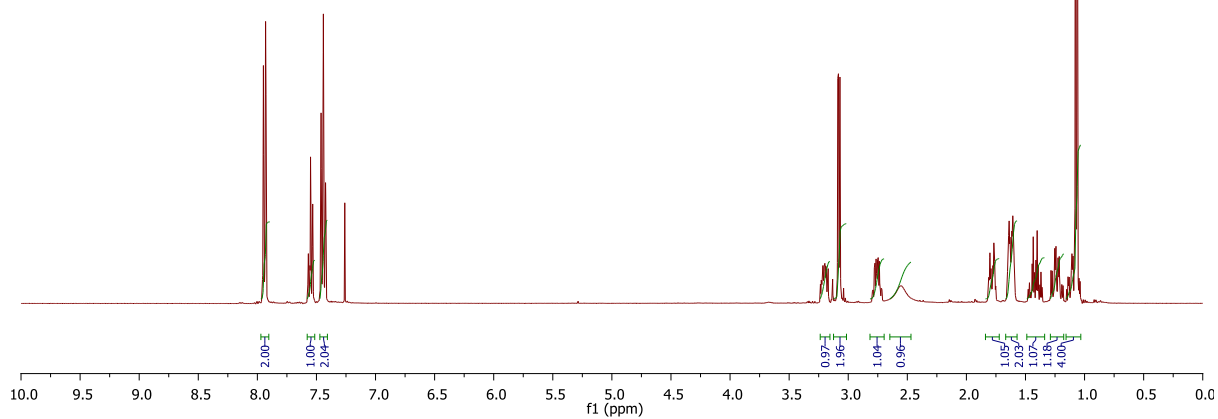
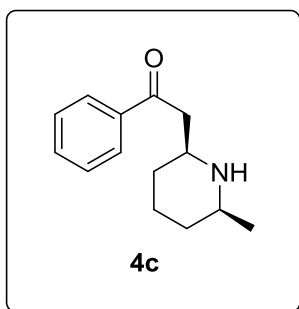


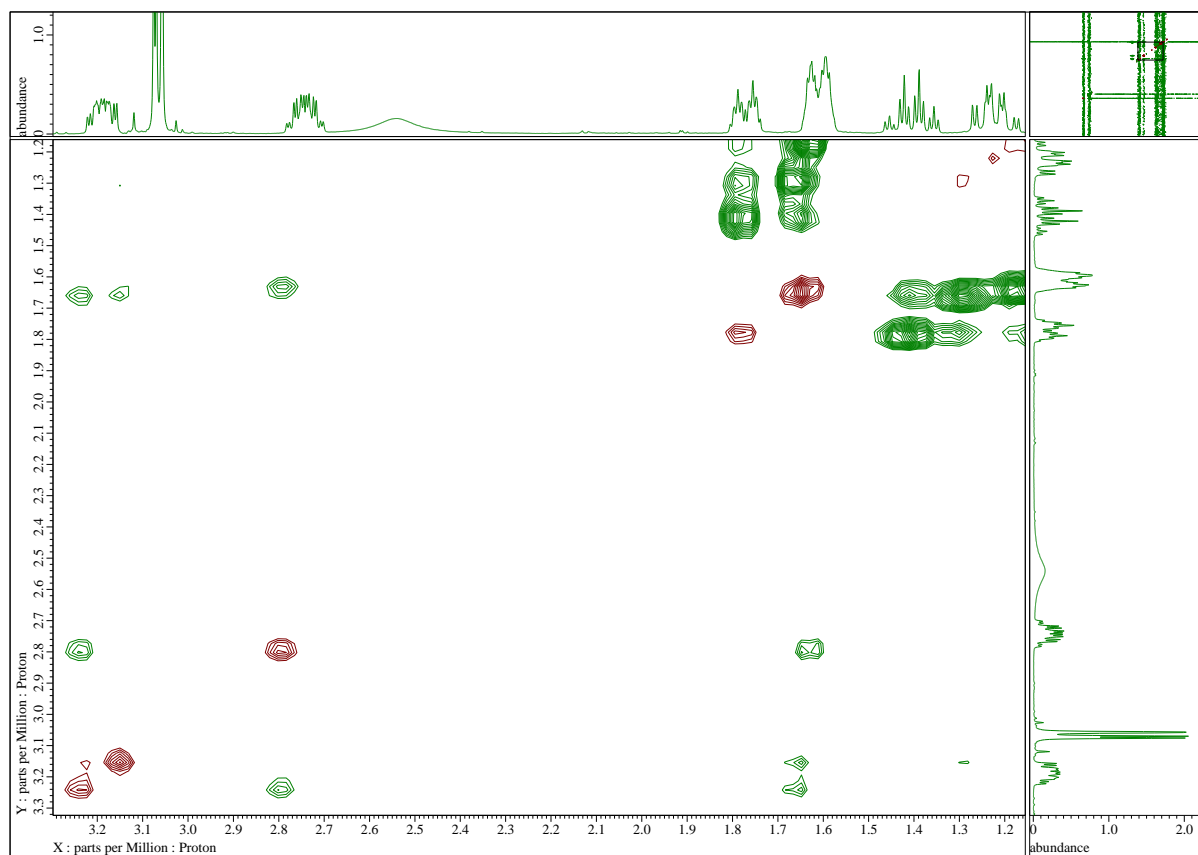
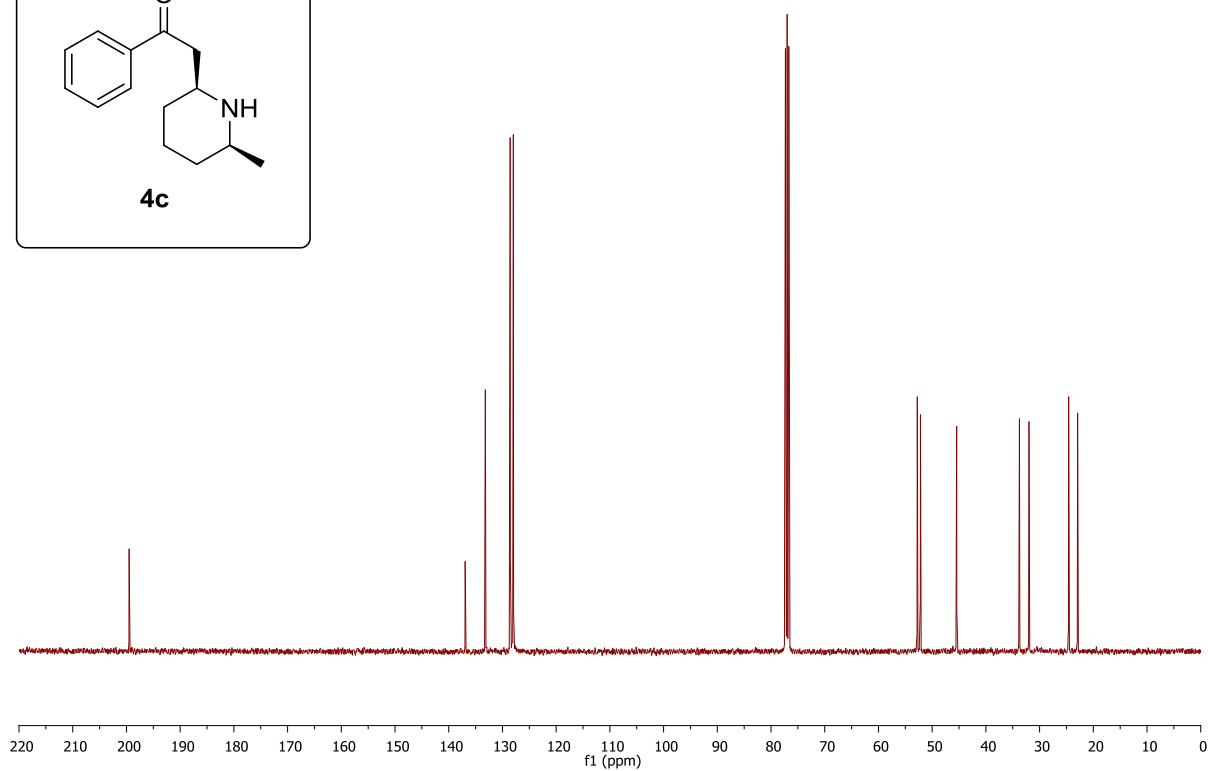
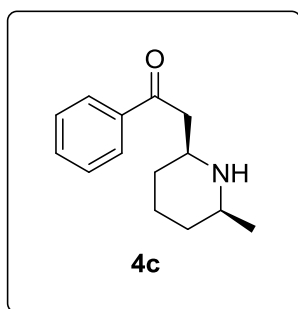


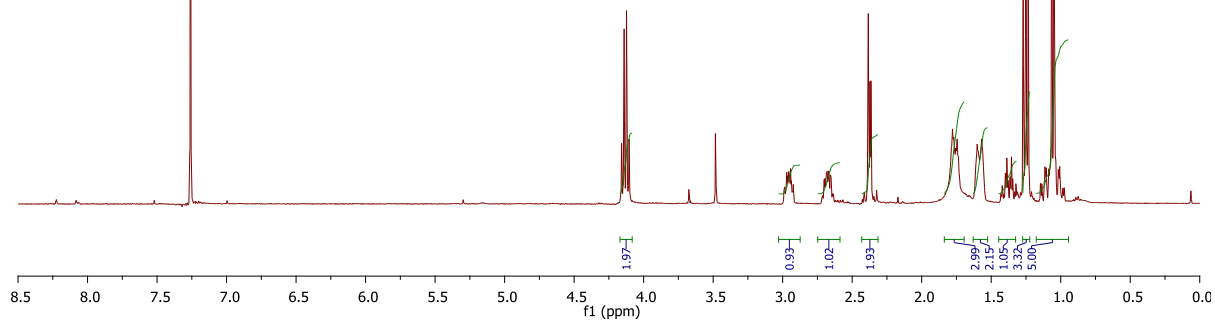
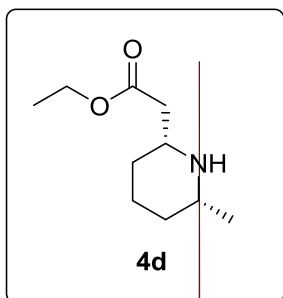
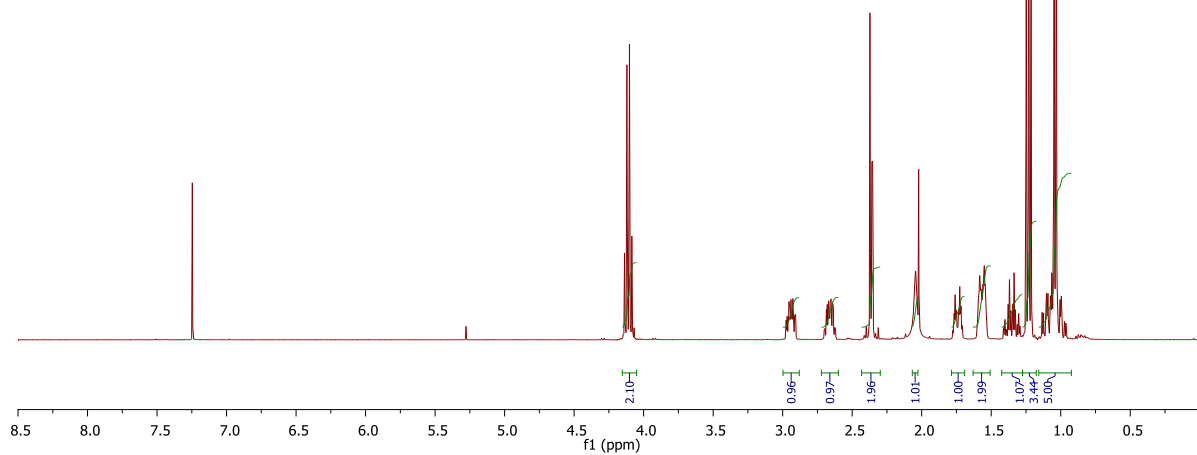
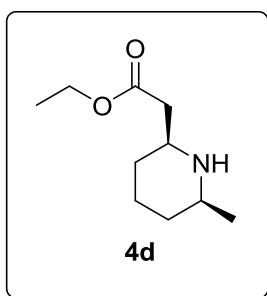


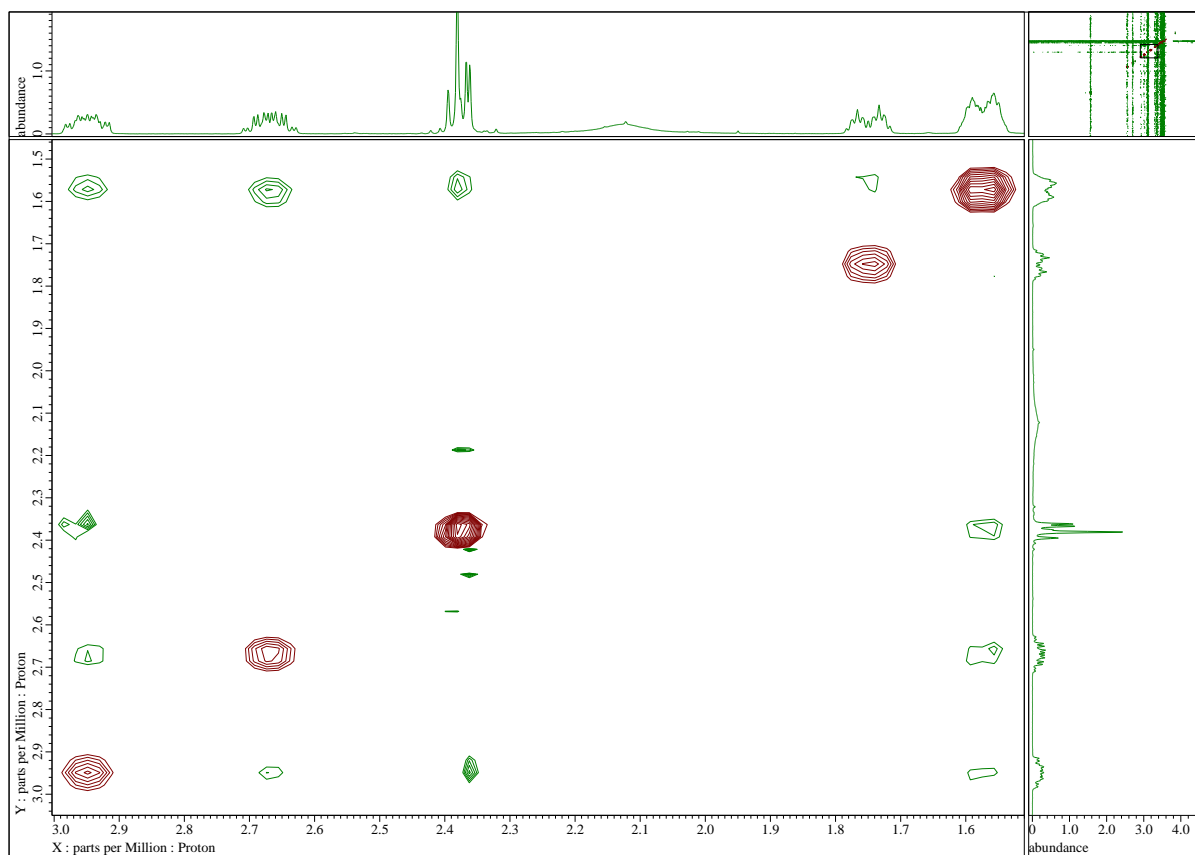
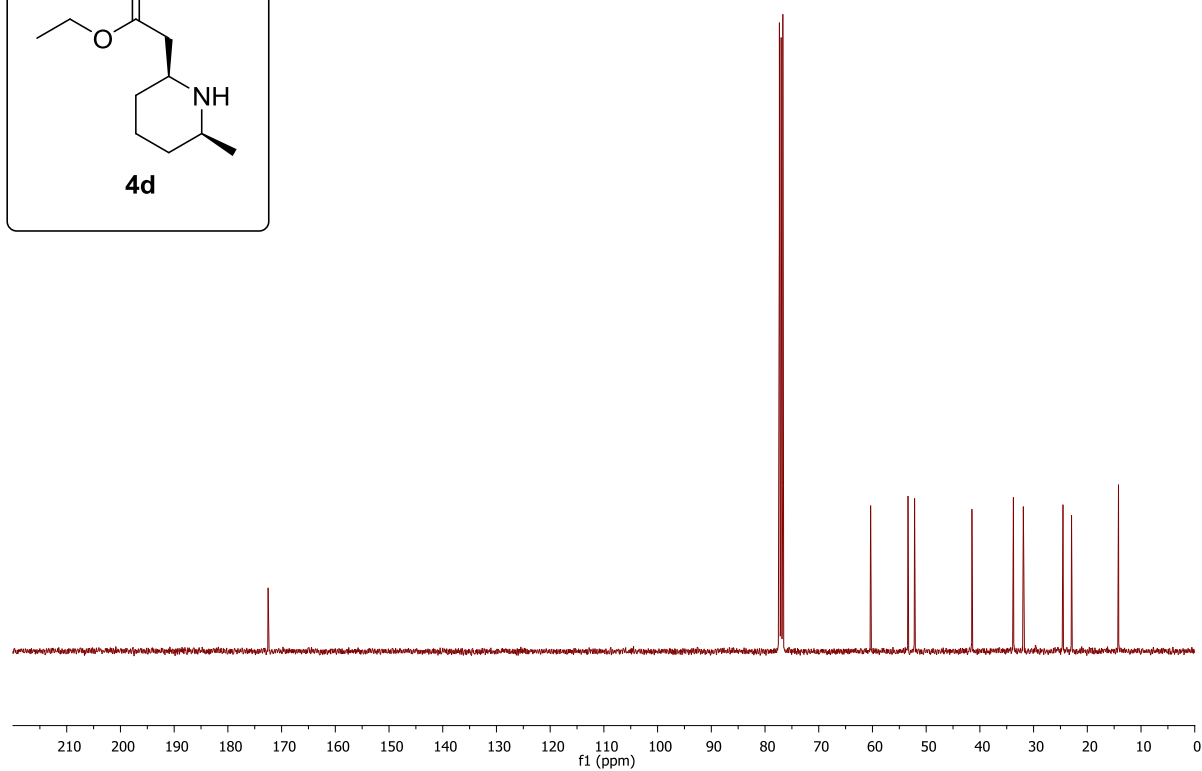
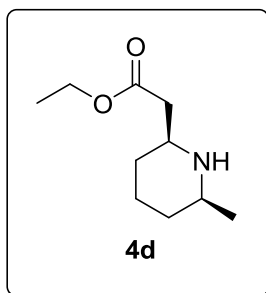


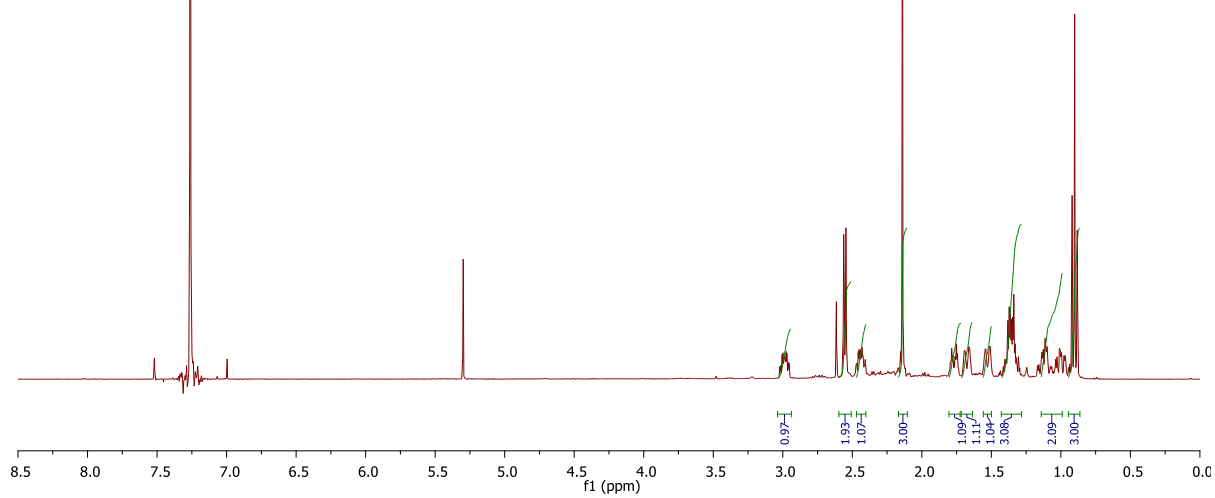
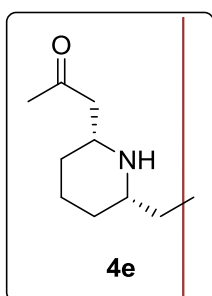
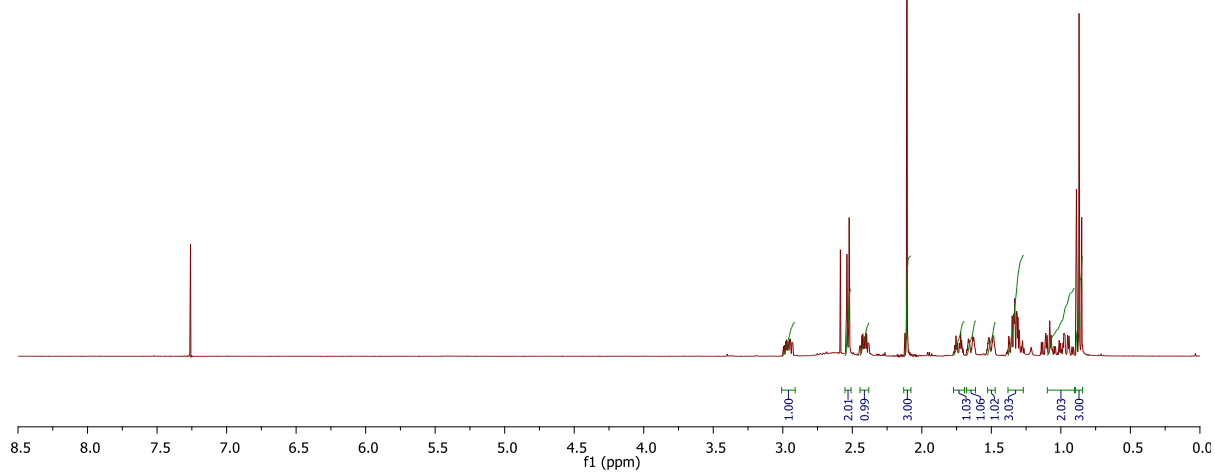
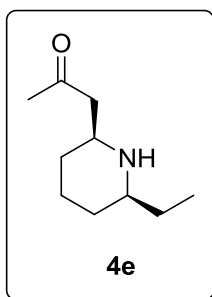


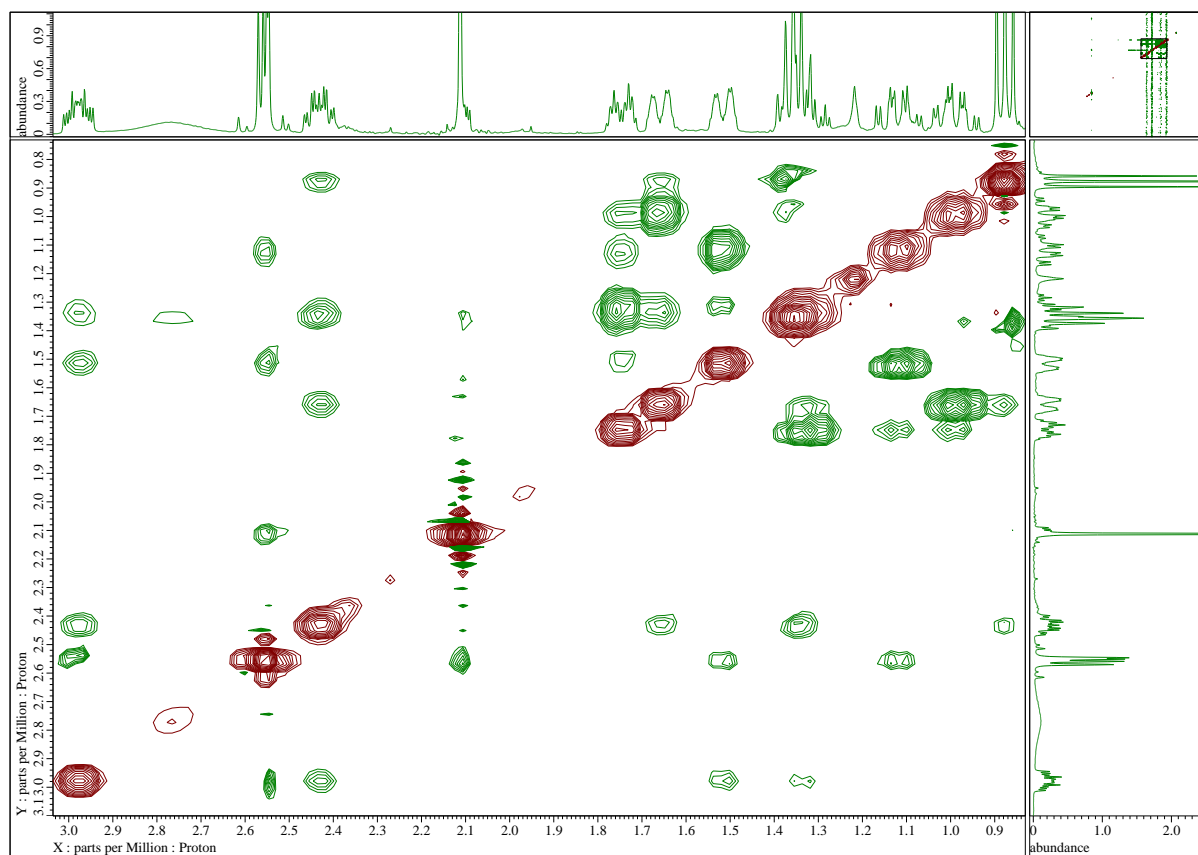
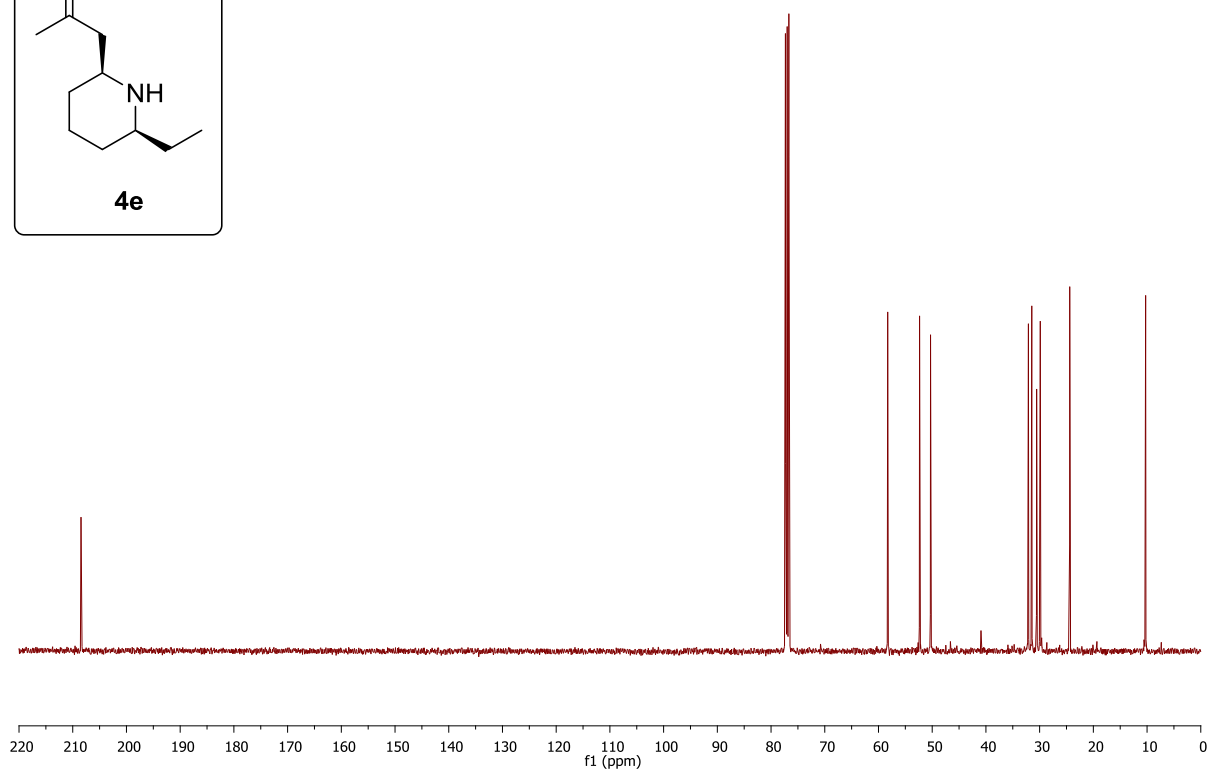
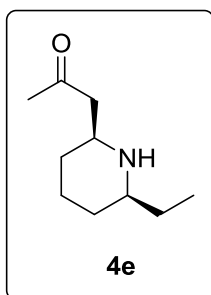


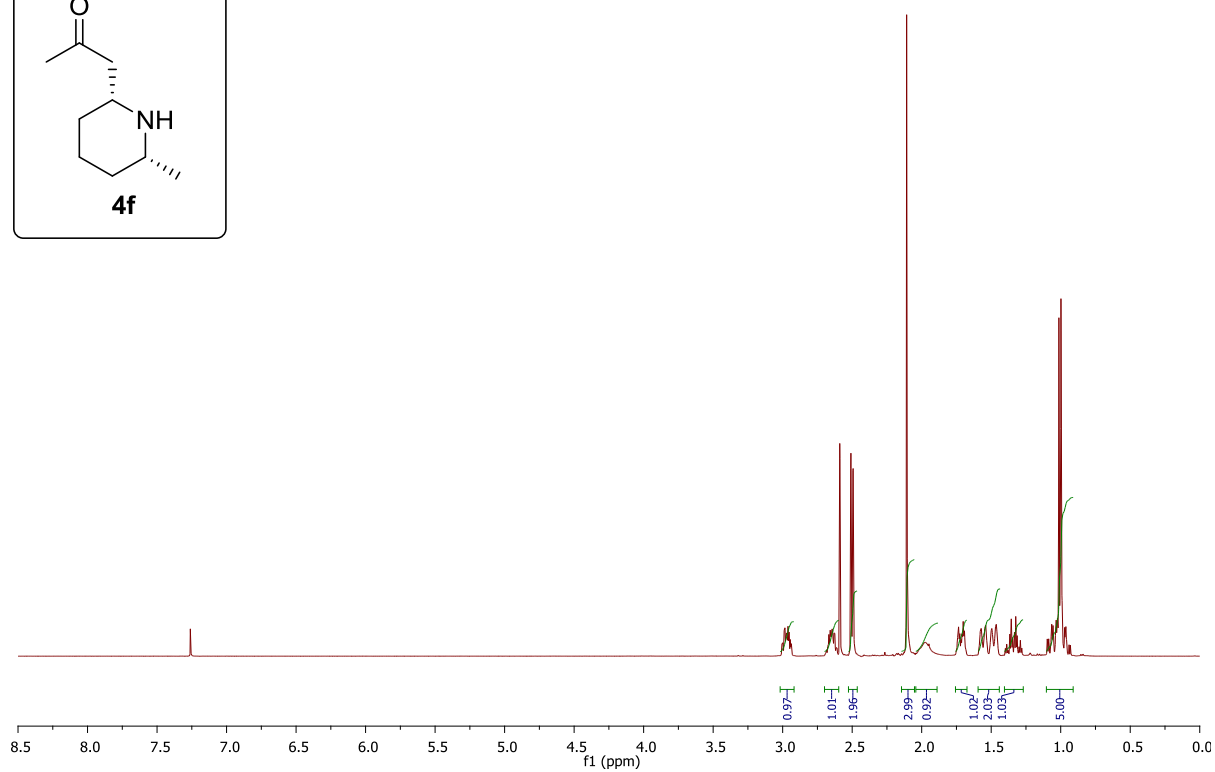
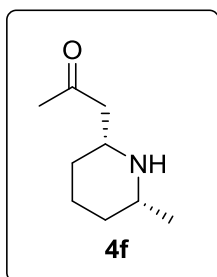
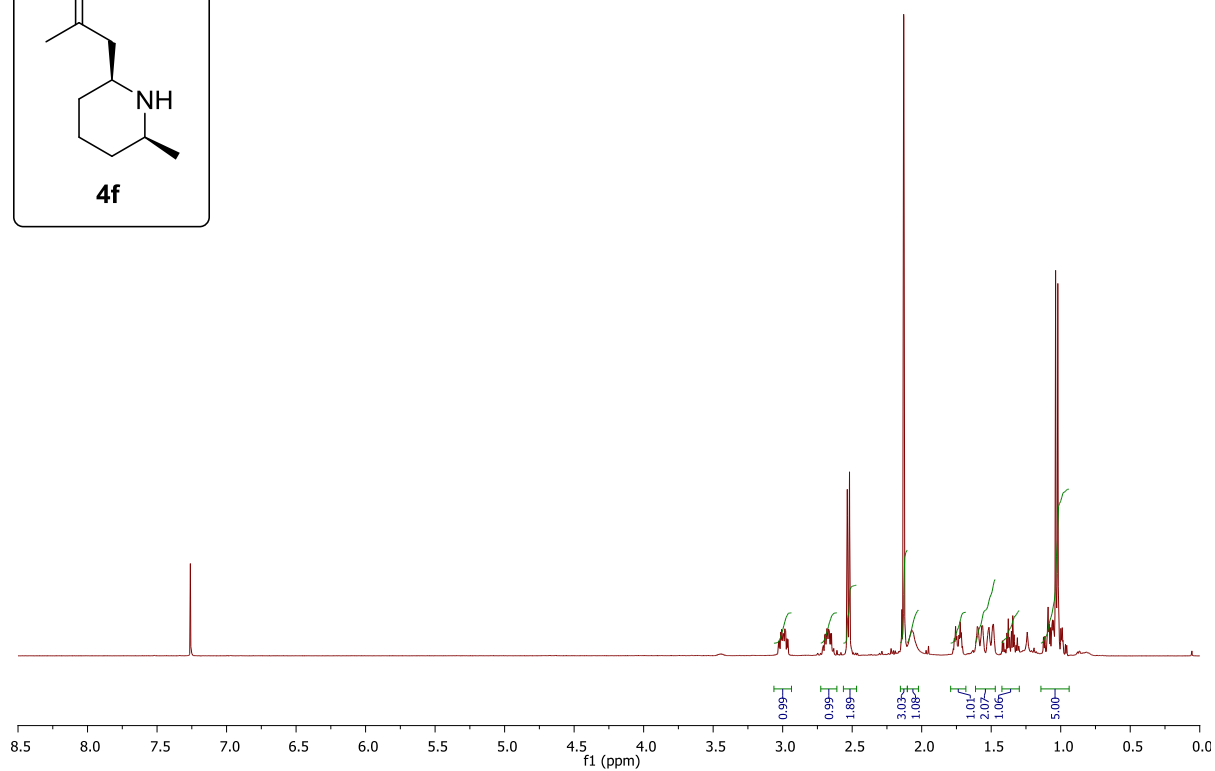
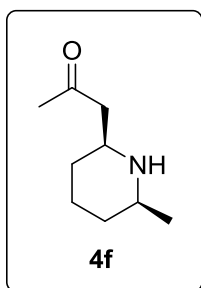


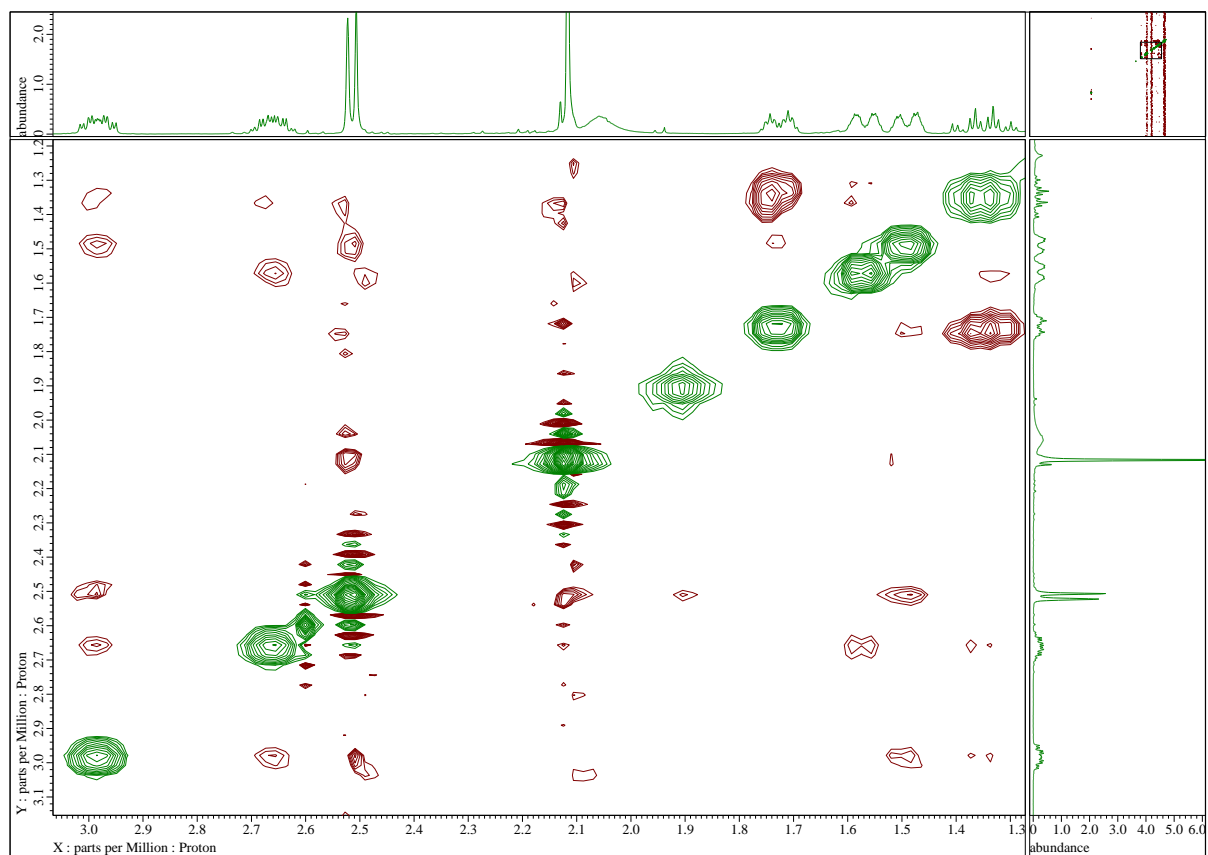
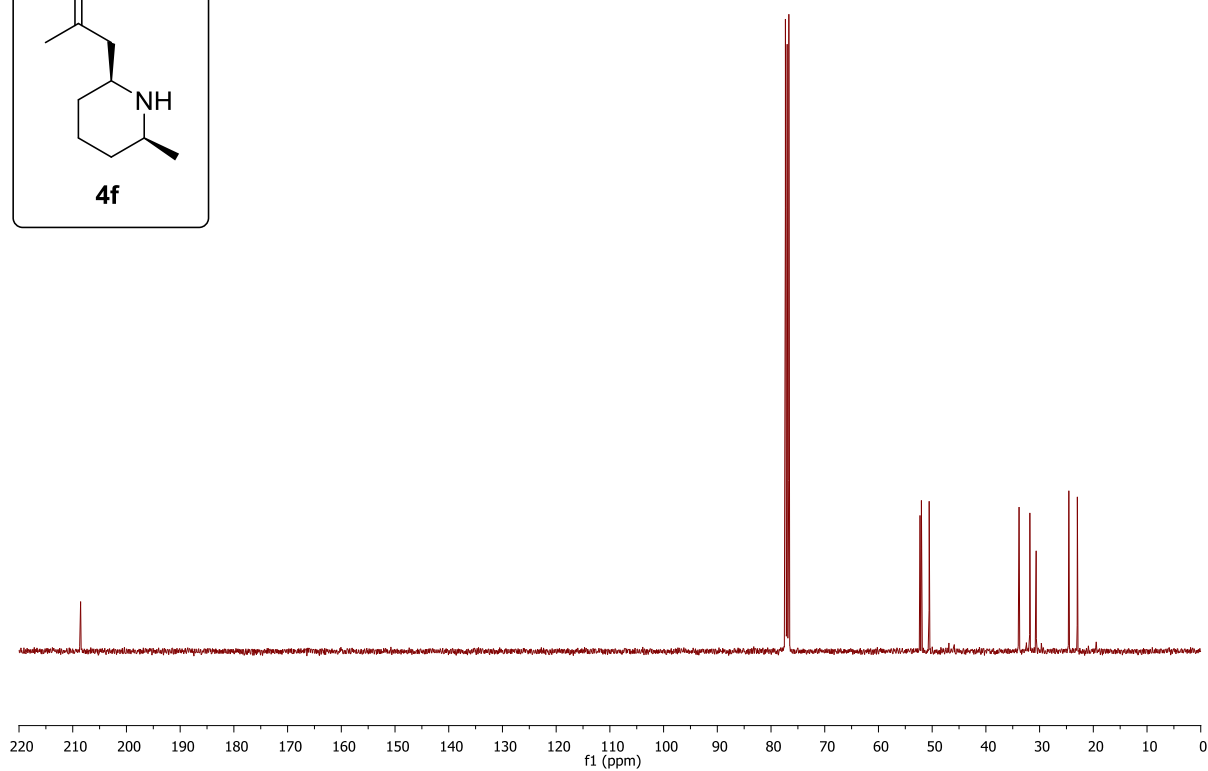
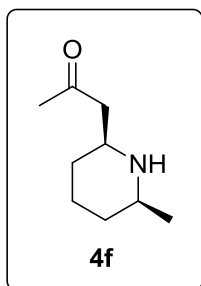


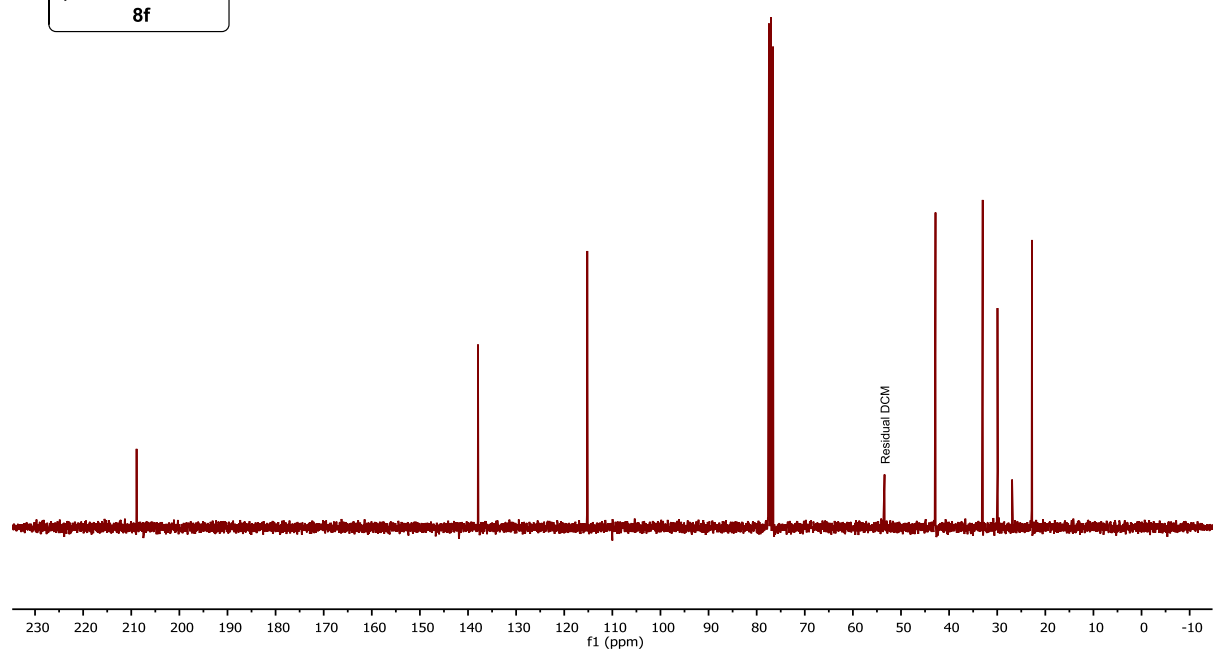
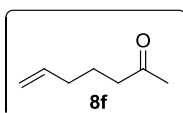
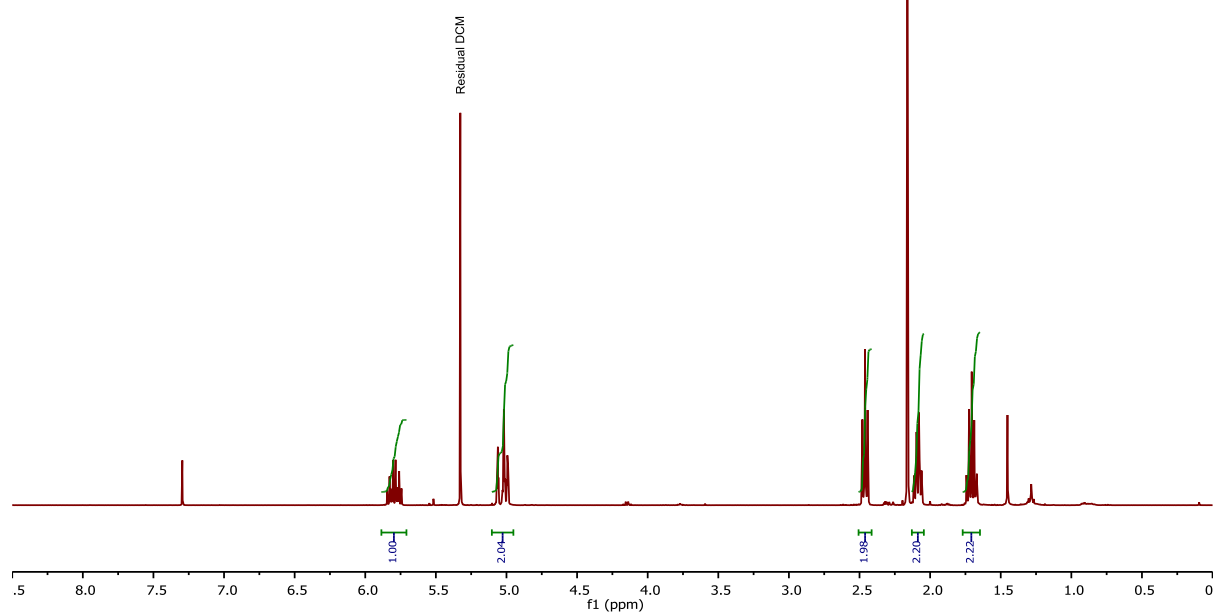
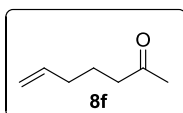


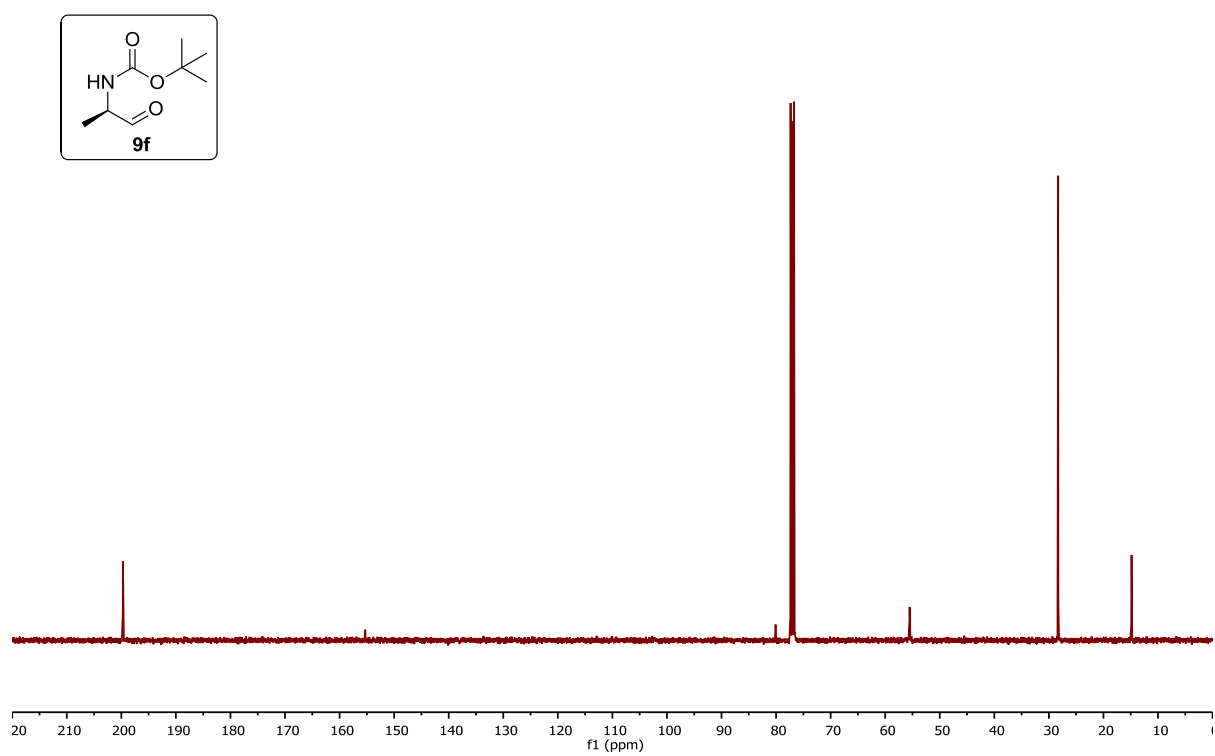
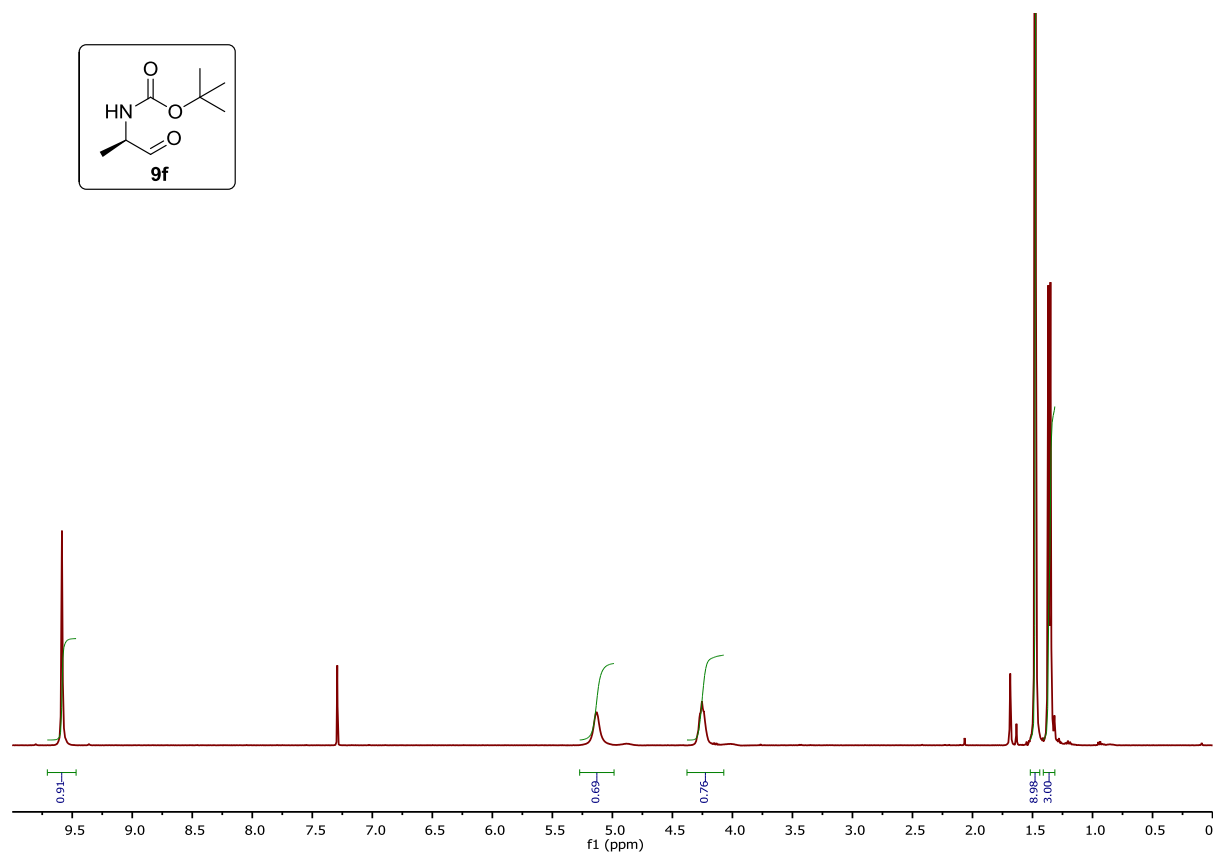


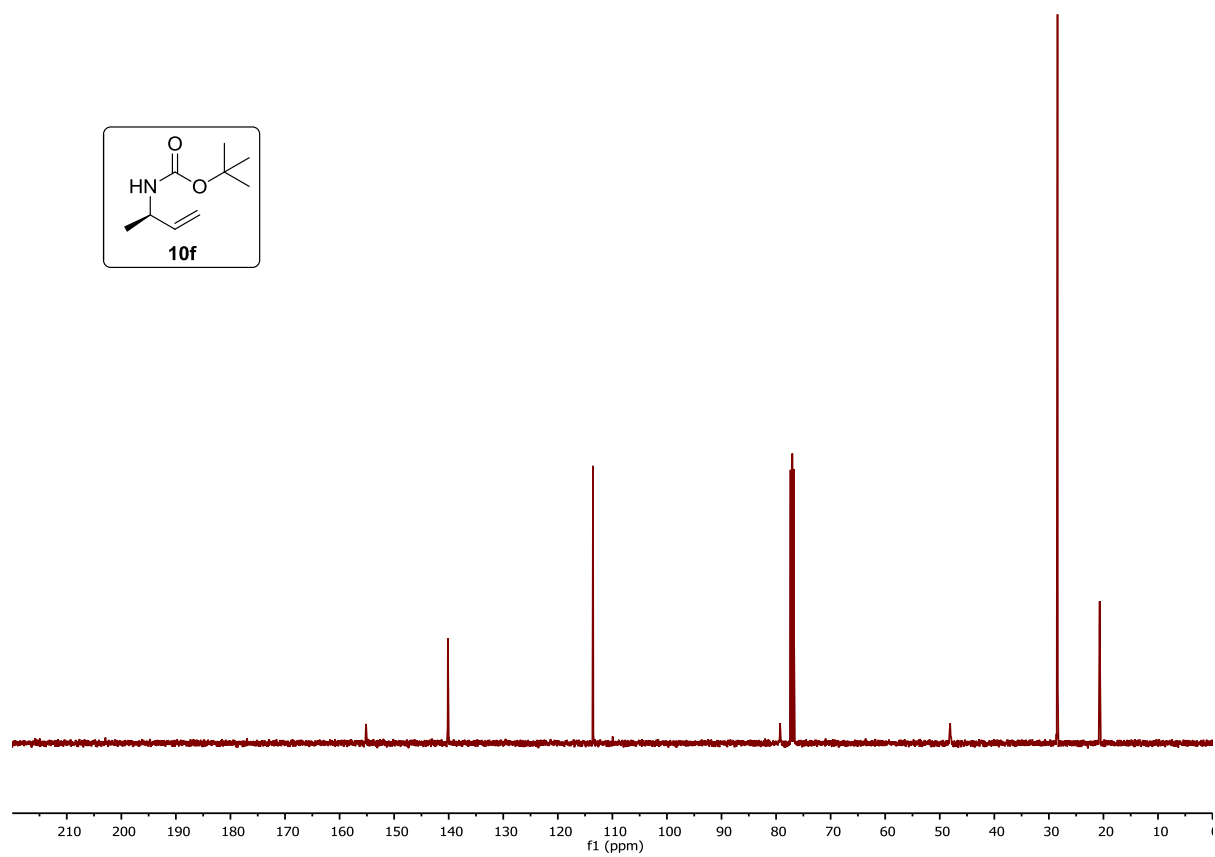
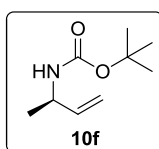
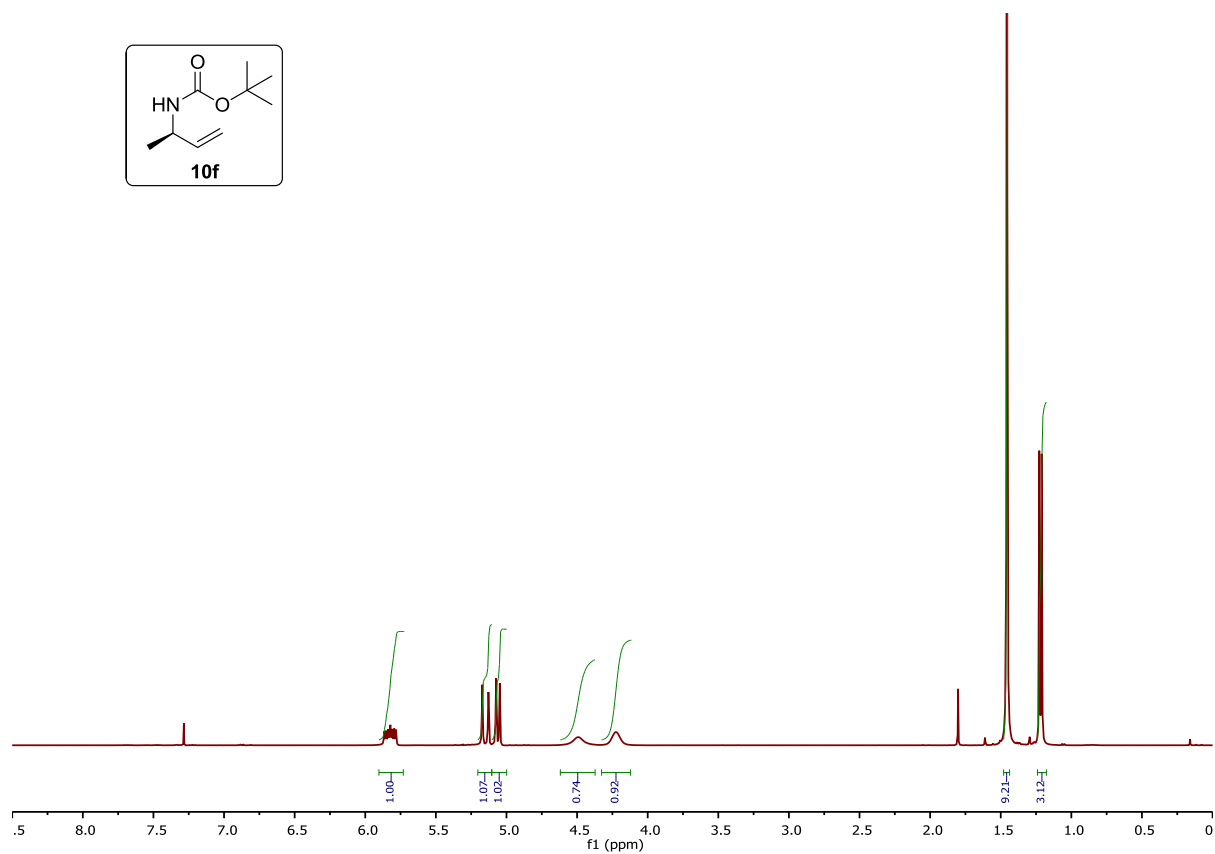
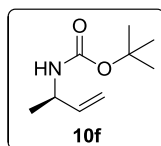


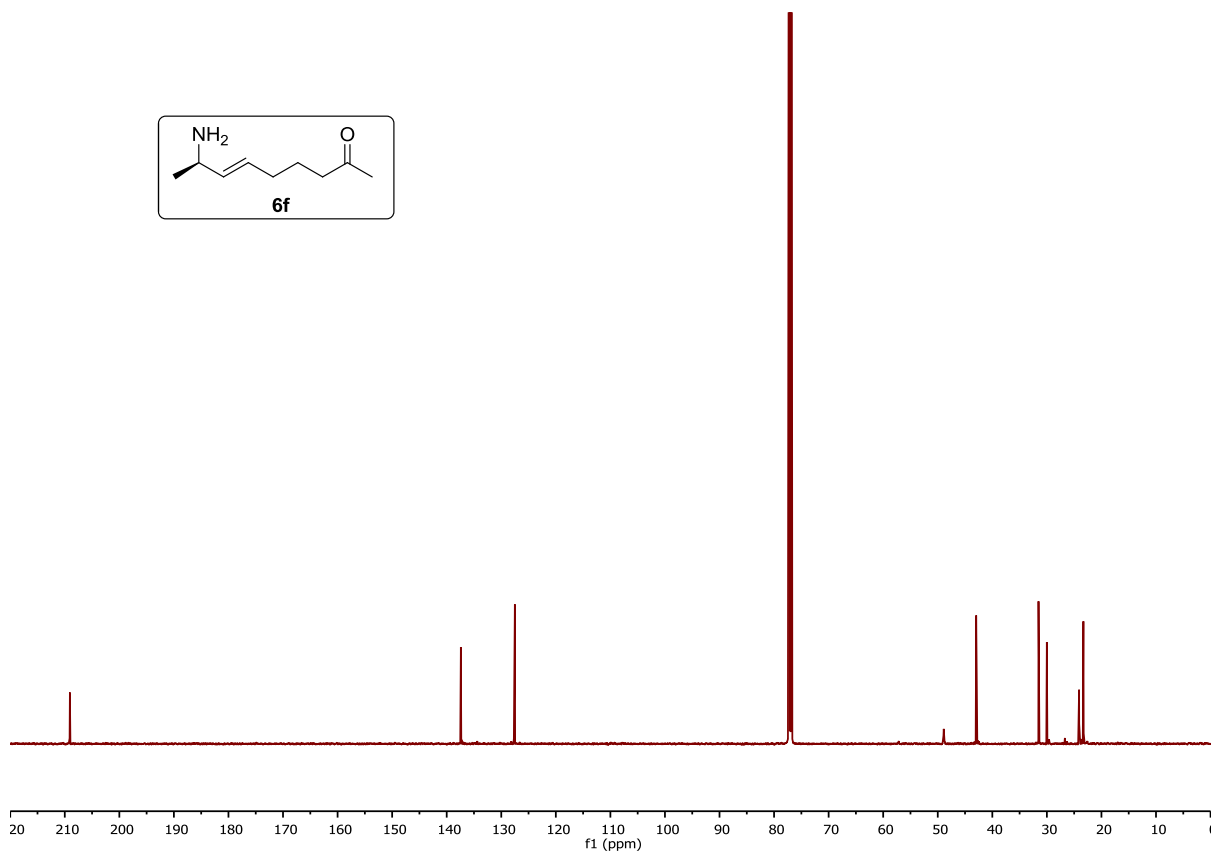
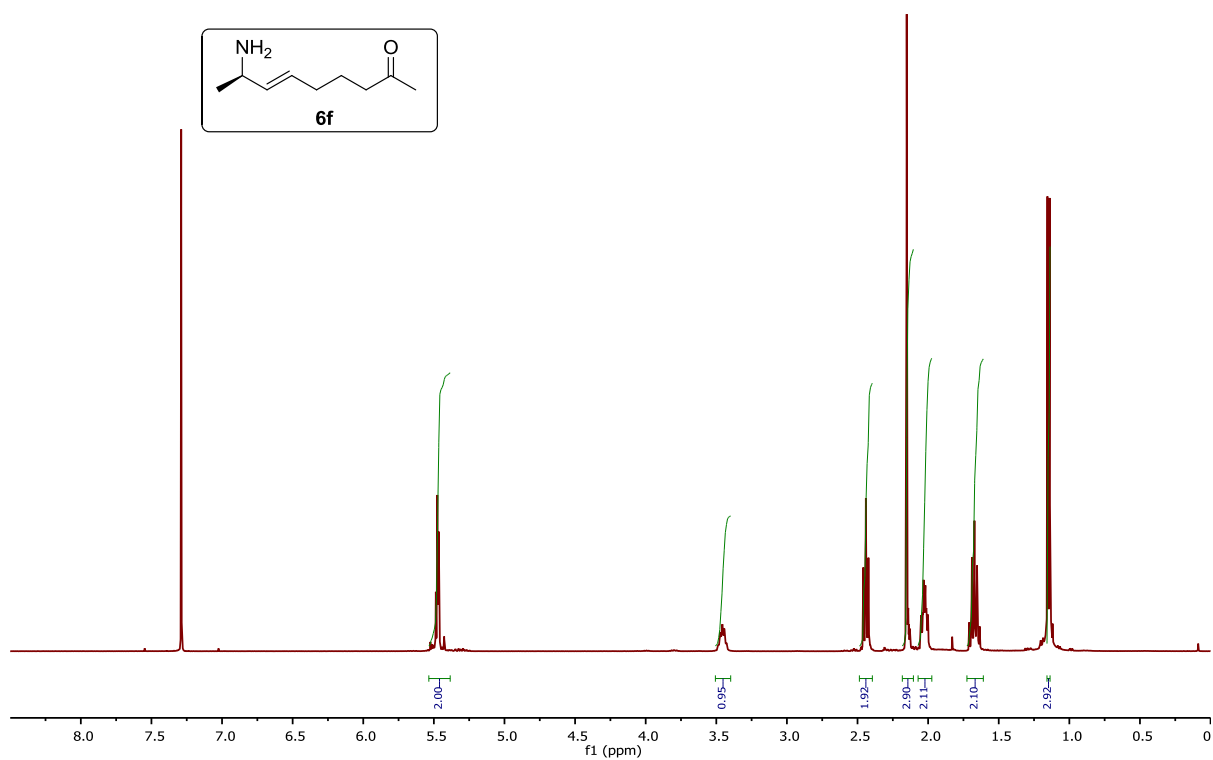




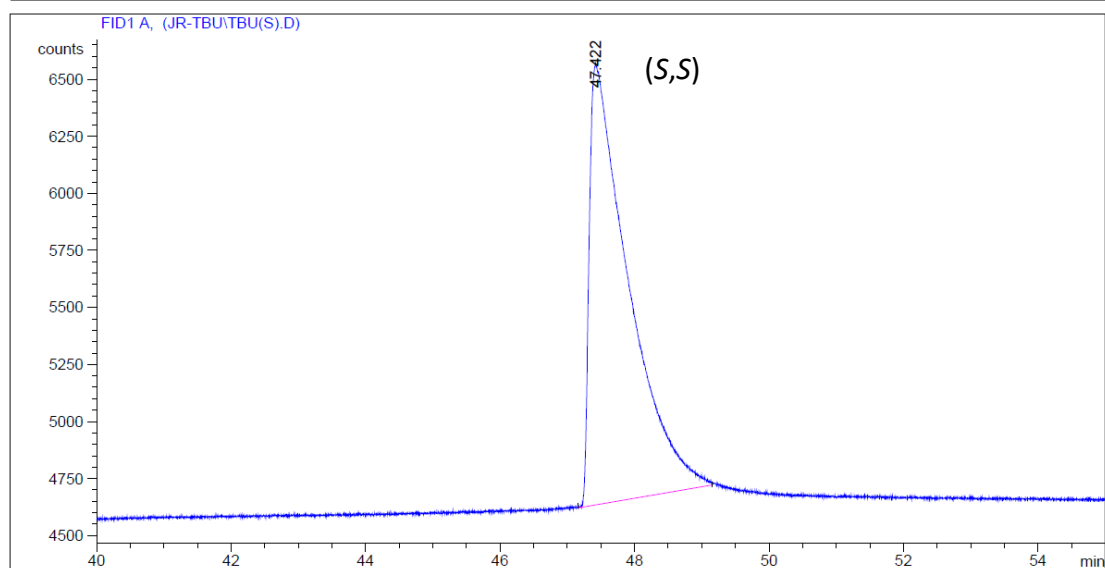
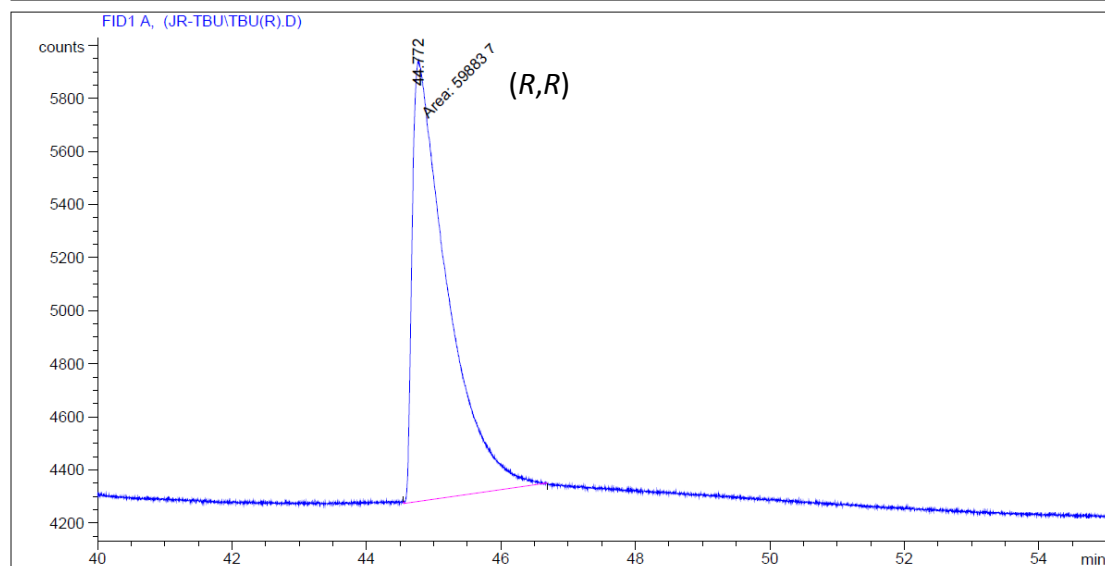
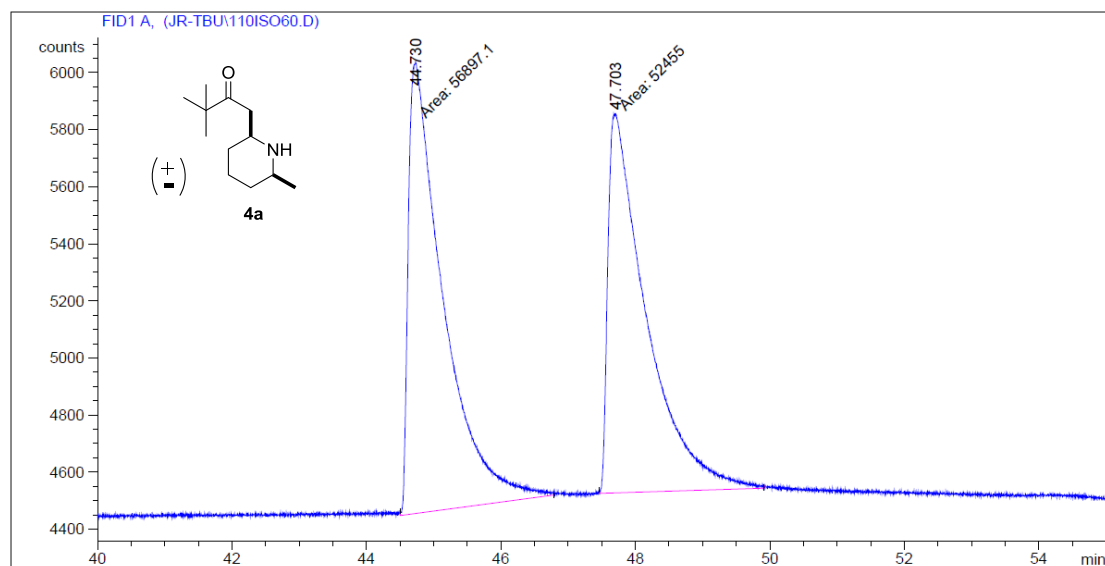


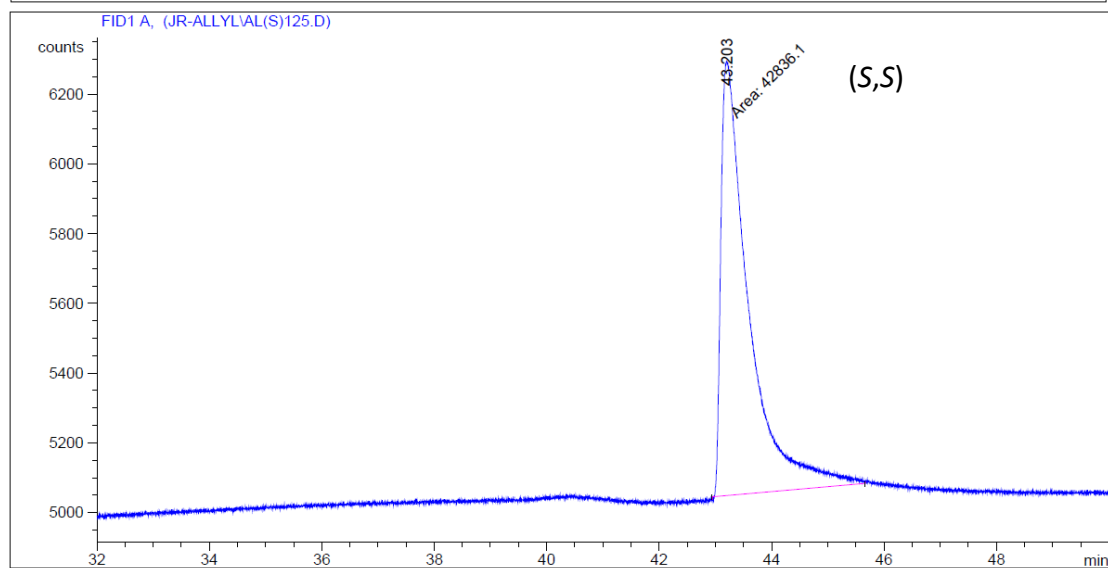
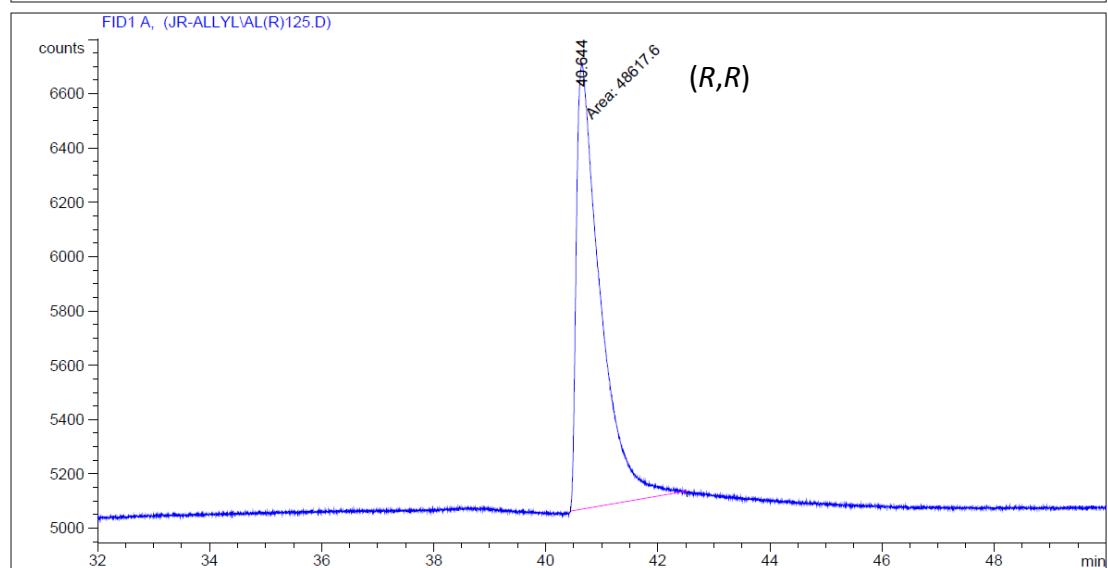
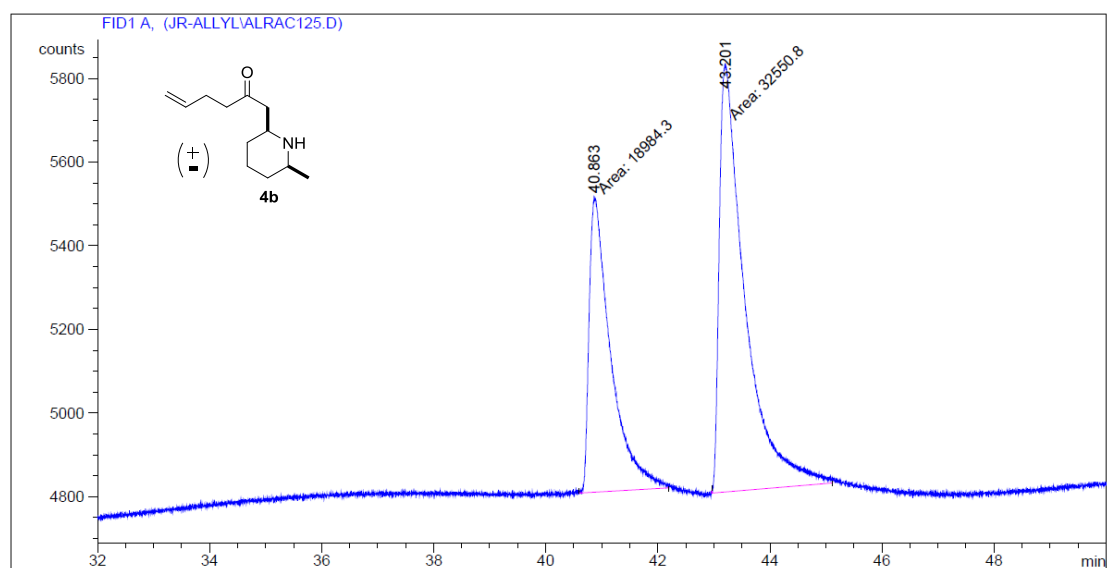


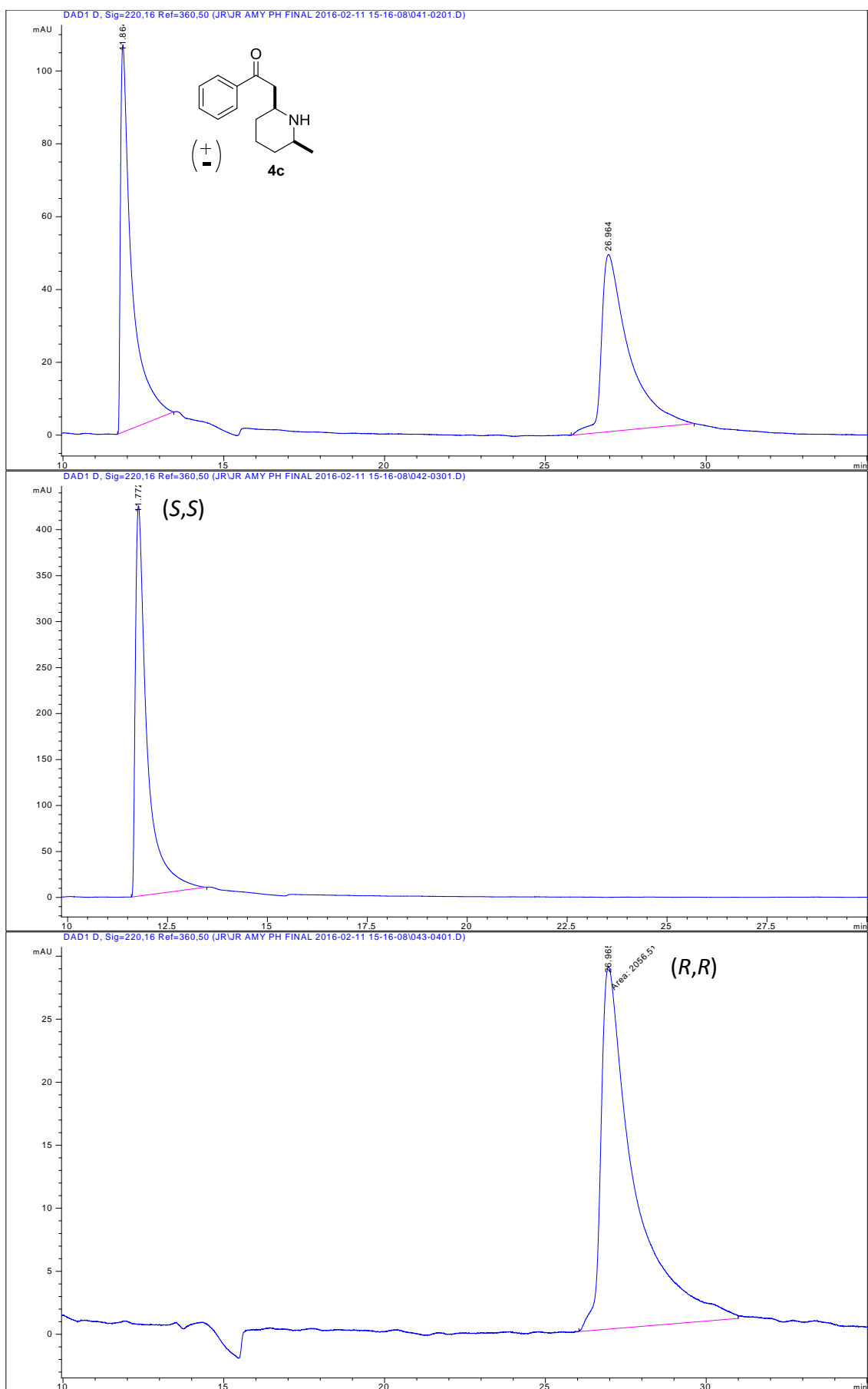


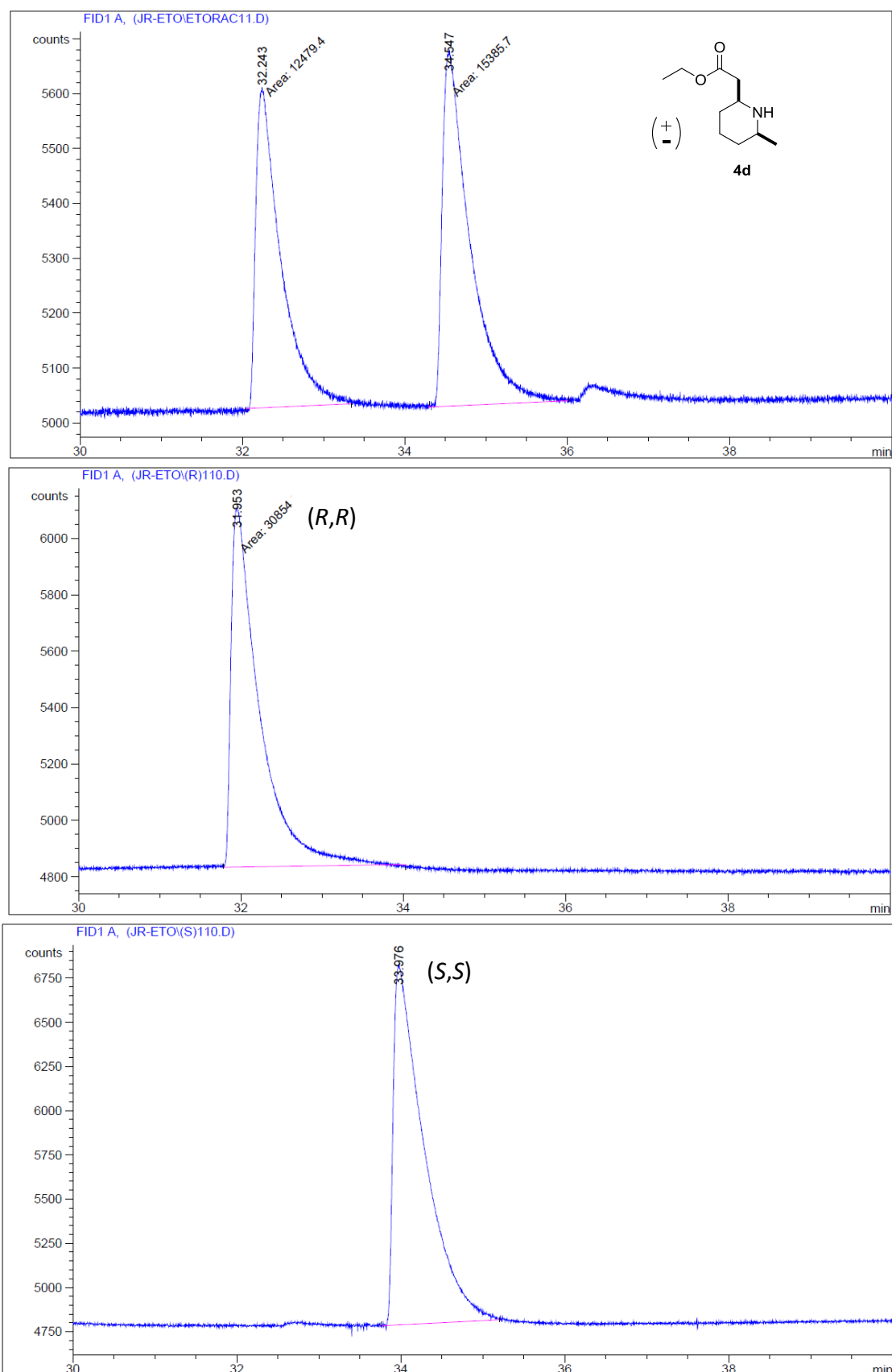


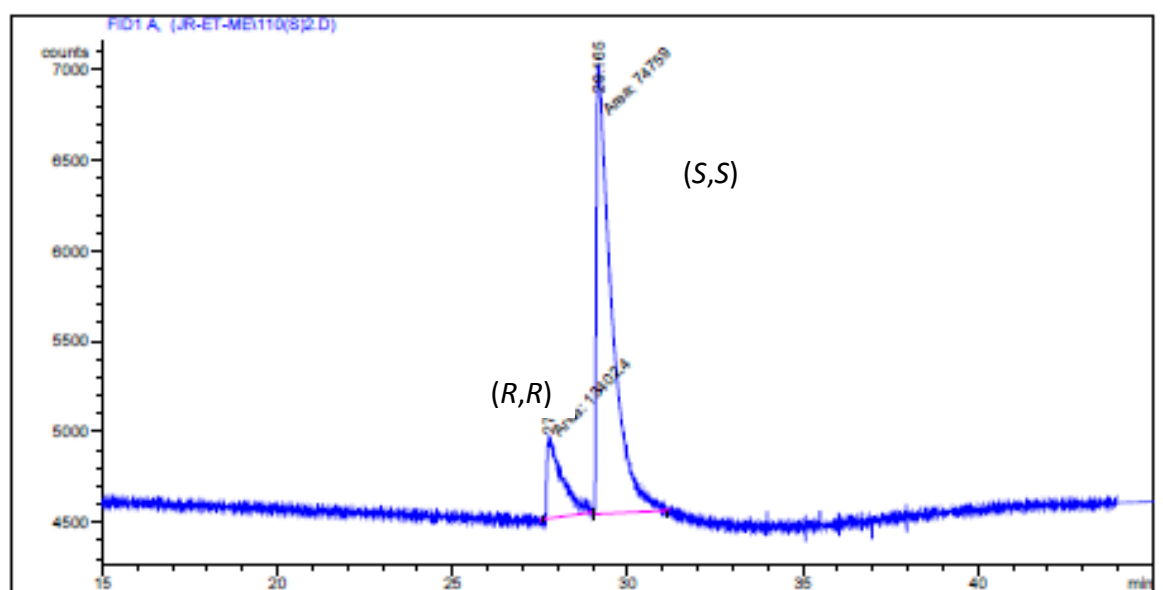
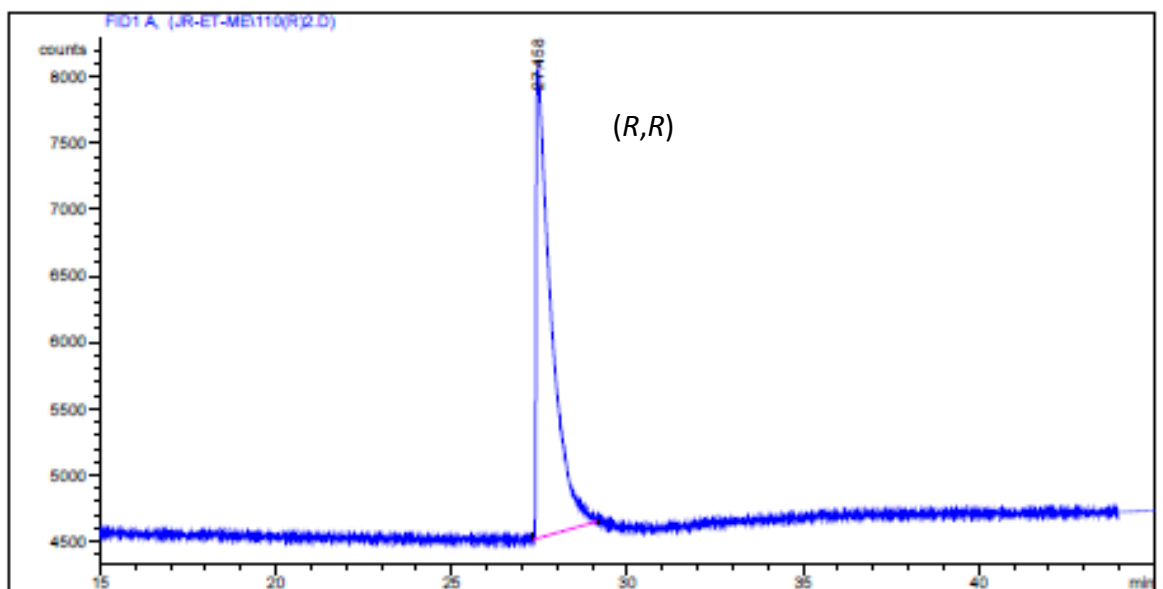
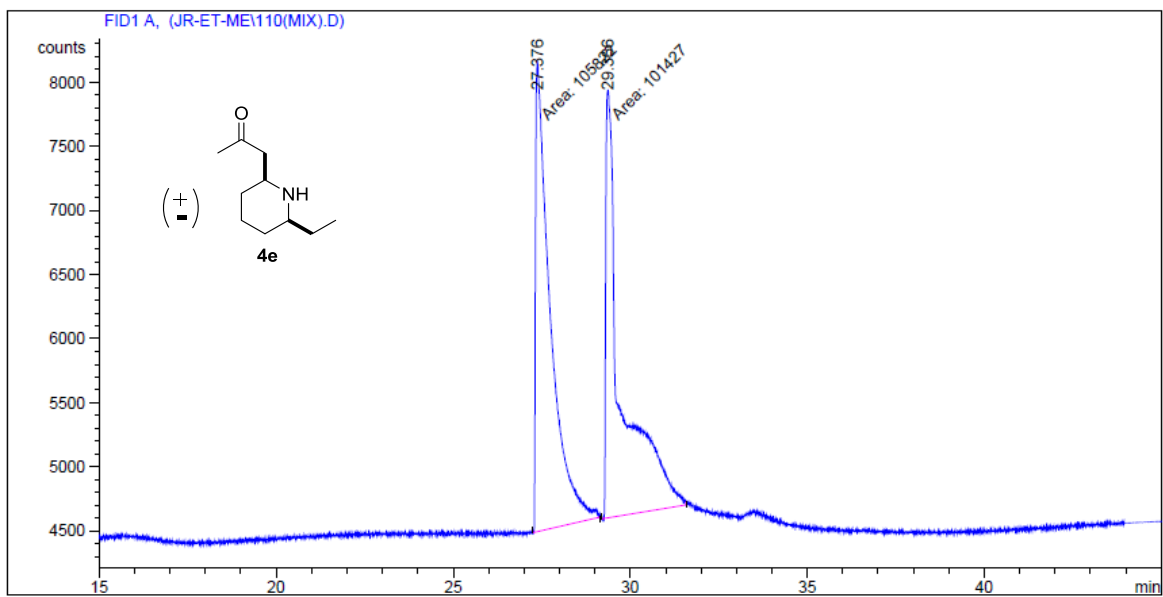
GC-MS and HPLC Chromatograms

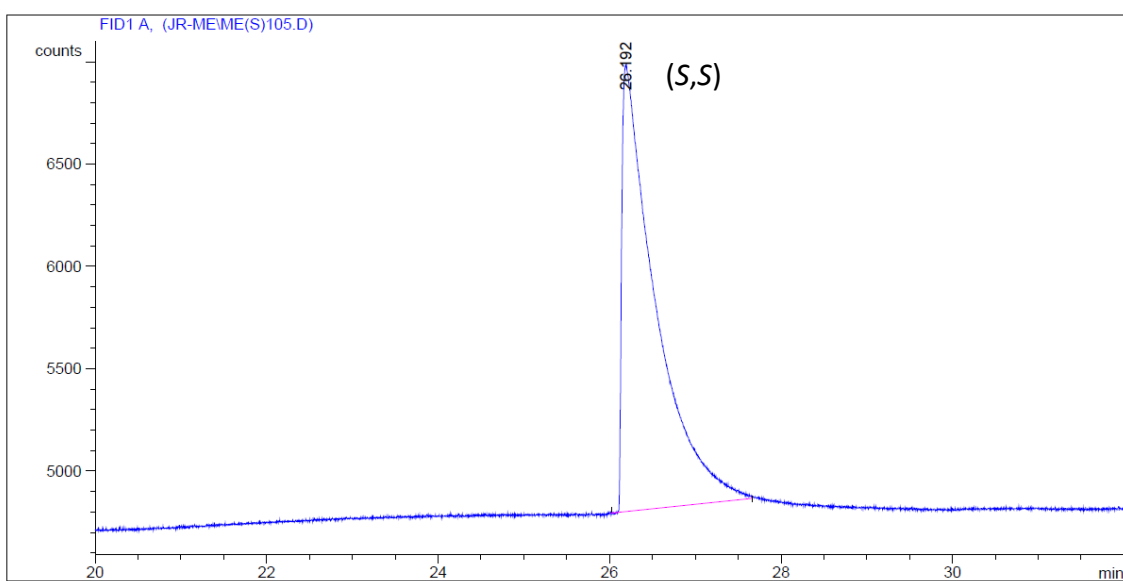
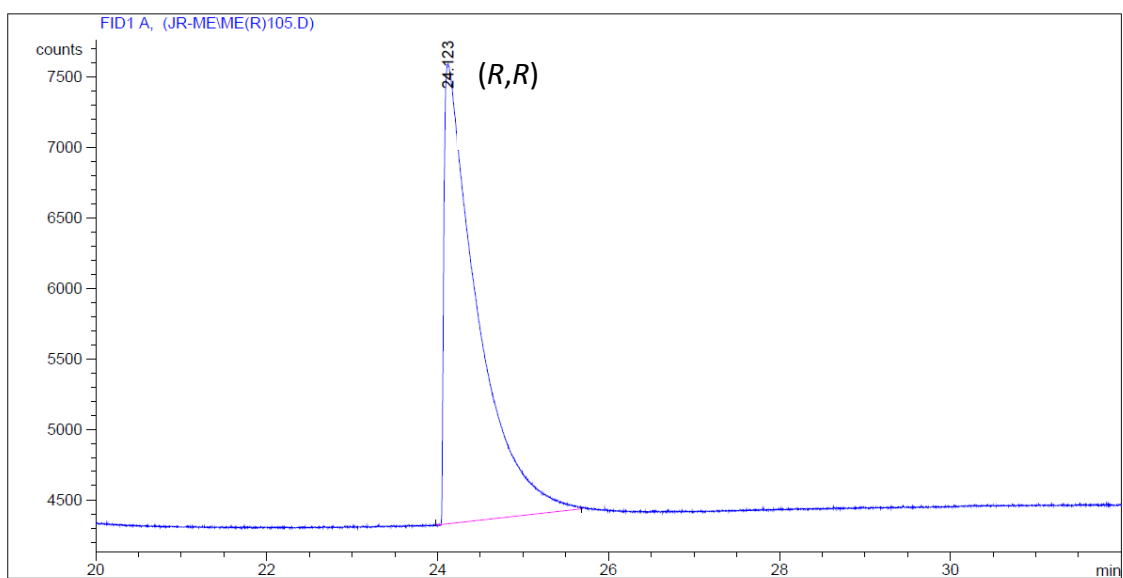
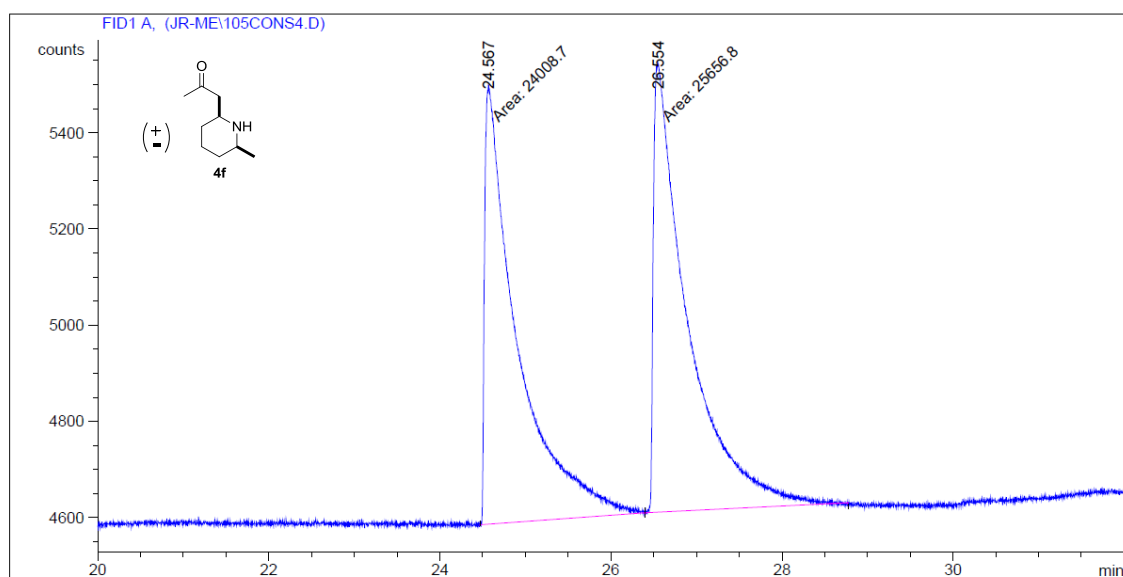












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