Supplementary Information (SI) for Organic & Biomolecular Chemistry.


# Transaminase-Triggered Synthesis of Disubstituted Pyrrolidines

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## 1. General Methods

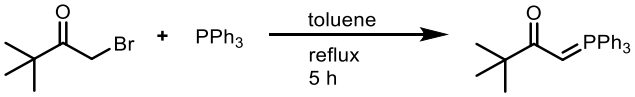
Nuclear magnetic resonance (NMR) spectra were recorded on Varian VnmrS 400, and 500 MHz spectrometers, and a Bruker Avance Neo 600MHz spectrometer, operating at 400.13 MHz for $^{1}H$ and 100.61 MHz for $^{13}C$, 500 MHz for $^{1}H$ and 126 MHz for $^{13}C$ and 600 MHz for $^{1}H$ and 151 MHz for $^{13}C$ respectively. Some experiments were also run on an Agilent DD2 500 spectrometer, operating at 500 MHz for $^{1}H$ and 126 MHz for $^{13}C$ and a JEOL 400 MHz spectrometer operating at 400.13 MHz for $^{1}H$ and 100.61 MHz for $^{13}C$ spectra. NMR were processed using MestReNova 16.0.0 software. The chemical shift values (δ), all reported in ppm, are referenced to the residual solvent peak, CDCl3 (δ 7.26 for $^{1}H$-NMR, δ 77.16 for $^{13}C$ NMR), and trimethyl silane (δ 0.00 for $^{1}H$-NMR, δ 77.0 for $^{13}C$ NMR). When $D_2O$ was used, the residual signal was referenced to 4.79 ppm for $^{1}H$ NMR. Coupling constants (*J*), reported in Hz, refer to the observed peak multiplicities. Data for $^{1}H$ NMR are reported as follows: chemical shift (δ/ppm) (multiplicity, coupling constant (Hz), integration, identity). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets. The assignment of signals in all NMR spectra was attained through $^{1}H$ NMR, $^{13}C$ NMR, $^{1}H^{1}H$ correlation spectroscopy (COSY), $^{1}H^{13}C$ heteronuclear single-quantum correlation spectroscopy (HSQC),$^{1}H^{13}C$ heteronuclear multiple bond correlation spectroscopy (HMBC) and Nuclear Overhauser Effect Spectroscopy (NOESY). Thin layer chromatography was performed on Merck silica gel 60 $F_{254}$ plates or Merck aluminium oxide 60 $F_{254}$ neutral plates. Flash column chromatography was performed on silica gel (60 Å, 230-400 mesh) or aluminium oxide 90 standardised. High resolution mass spectrometry (HRMS) analysis was carried out using the Agilent 1260 Infinity Prime II LC coupled to Agilent 6546 Quadrupole Time-Of-Flight (QTOF) MS system operated by the School of Chemistry Mass Spectrometry Facility at University College Dublin. Data acquisition and processing was performed using Agilent MassHunter software. Commercially available reagents, purchased from Sigma Aldrich, Thermo Scientific, Fluorochem, Merck, VWR Life Science, were used without further purification. Anhydrous THF and DCM were obtained from a Pure Solvent apparatus. Commercially available transaminases were purchased from Codexis ® in the form of lyophilised cell extract.

Chiral HPLC analysis was performed using an Agilent Technologies 1200 series equipped with a binary pump (G1312A) and automated liquid sampler (G1329A).

## 2. Procedures for the Preparation of Wittig Reagents

All the stabilised ylides used were commercially available apart from **S1,** and (benzoylmethylene)triphenylphosphorane (**S2**) was relatively expensive; therefore, it was synthesised from its bromine derivative.

### 3,3-Dimethyl-1-(triphenylphosphoranylidene)-2-butanone (S1)

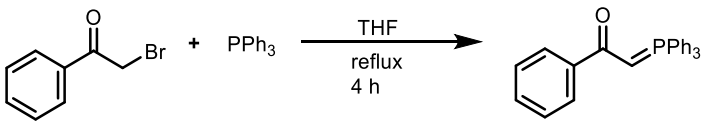



1-Bromopinacolone (2.7 mL, 20 mmol) was added to a solution of triphenylphosphine (5.270 g, 20.09 mmol, 1 equiv.) in toluene (30 mL) and refluxed for 5 h. The white solid filtrate was washed with diethyl ether (3 × 50 mL), before it was re-dissolved in the minimum amount of water/DCM (60:40, 60 mL). 2 M NaOH (100 mL) was added to the solution, and it was allowed to stir for 1.5 h. The reaction mixture was extracted with DCM (3 × 30 mL). The organic extracts were combined, washed with brine (100 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give an off-white solid (5.800 g, 81% yield).

**$^1$H-NMR** (400 MHz, $CDCl_3$) δ 7.70-7.40 (m, 15H), 3.78 (d, *J* = 25.4 Hz, 1H), 1.20 (s, 9H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 200.5, 133.2, 133.1, 131.8, 128.9, 128.8, 48.0, 46.9, 28.9. In accordance with literature data.[1]

### (Benzoylmethylene)triphenylphosphorane (S2)




2-Bromoacetophenone (4.959 g, 25 mmol) was added to a solution of triphenylphosphine (7.212 g, 27.5 mmol, 1.1 equiv.) in THF (52 mL) and refluxed for 4 h. The solid white precipitate was filtered and re-dissolved in DCM (100 mL). 5 M NaOH (80 mL) was added to the solution, and allowed to stir for 10 min. The organic layer was

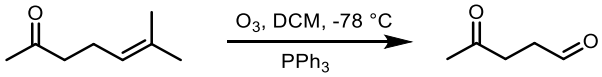
isolated, and the aqueous layer was extracted with DCM (3 × 40 mL). The organic extracts were combined, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a white solid (6.276 g, 66% yield).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 8.05-7.29 (m, 20H), 4.43 (d, *J* = 24.5 Hz, 1H).

**$^{13}$C-NMR** (126 MHz, $CDCl_3$, *J* P-C) δ 184.9, 141.3 (d, *J* = 14.7 Hz), 133.2 (d, *J* = 10.0 Hz), 132.1 (d, *J* = 2.3 Hz), 129.4, 128.9 (d, *J* = 12.4 Hz), 127.8, 127.1 (d, *J* = 88.2 Hz), 126.9, 50.8 (d, *J* = 112.1 Hz). In accordance with literature data. [2]

## 3. Preparation of Aldehyde Precursors

### 4-Oxopentanal (2) – ozonolysis of 6-methylhept-5-en-2-one (1)




6-Methylhept-5-en-2-one (**1**) (2 mL, 13.6 mmol) was dissolved in dry DCM (100 mL) in a dry 3-neck round bottom flask (250 mL). The solution was cooled to -78 °C while a stream of oxygen was passed through the solution. Once cooled, a stream of ozone was passed through the reaction mixture (~ 1 min/mmol) for 14 minutes. Once a pale blue colour was observed in the flask the reaction was stopped, and the solution was sparged with oxygen. Triphenylphosphine (4.040 g, 15.4 mmol, 1.1 equiv.) was added to the solution, and it was allowed to stir overnight. The reaction mixture was concentrated under reduced pressure.

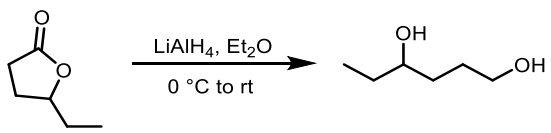
**Option A:** The crude product was dry loaded onto silica and purified by column chromatography (silica, cyclohexane/ethyl acetate, 9:1) to give a colourless oil (0.584 g, 43% yield).

**Option C:** The crude product was dissolved in the minimum amount of DCM and diethyl ether (100 mL) was added to the solution. The solution was cooled to 0 °C, and the resulting white precipitate was filtered under vacuum. The filtrate was concentrated under reduced pressure to give a yellow oil which turned to a solid at room temperature.

**$^{1}$H-NMR** (400 MHz, $CDCl_3$, purified) δ 9.7 (apparent s, 1H), 2.76-2.55 (m, 4H), 2.10 (s, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$, purified) δ 206.5, 200.5, 37.3, 35.4, 29.7. In accordance with literature data.[3]

### 4-Oxohexanal - $LiAlH_4$ reduction of γ-caprolactone to form hexane-1,4-diol (S3)



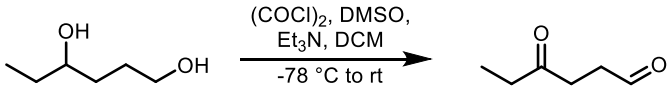

$LiAlH_4$ (1.090 g, 28.72 mmol, 2 equiv.) was slowly added to dry diethyl ether (100 mL) in a flame-dried 2-neck round bottom flask (500 mL) at 0 °C. Y-Caprolactone (1.6 mL, 14.3 mmol) in dry diethyl ether (20 mL) was added dropwise to the $LiAlH_4$ suspension at 0 °C. The solution was stirred overnight and allowed to warm to room temperature. The

reaction mixture was cooled to 0 °C and a saturated $Na_2SO_4$ solution (6 mL) was added dropwise slowly to quench the reaction. The solution was allowed to stir vigorously for 1 h. The white precipitate was filtered under vacuum. The resulting filtrate was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a colourless oil (1.738 g, >99% yield). The crude product was used directly in the next step without further purification.

**$^1$H-NMR** (500 MHz, $CDCl_3$) δ 3.69-3.63 (m, 1H), 3.62-3.56 (m, 1H), 3.55-3.48 (m, 1H), 3.34 (br. s, 2H), 1.69-1.58 (m, 3H), 1.51-1.40 (m, 3H), 0.91 (t, *J* = 6.4 Hz, 3H).

**$^{13}$C-NMR** (126 MHz, $CDCl_3$) δ 73.0, 62.6, 33.7, 30.1, 28.9, 9.9. In accordance with literature data.[4]

**Swern oxidation to form 4-oxohexanal (S4)**

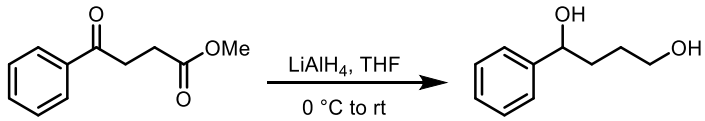



Oxalyl chloride (3.2 mL, 36.78 mmol) was dissolved in dry DCM (50 mL) in a flame-dried 2-neck round bottom flask (250 mL) with a stir bar, and the solution was cooled to -78 °C. Dry DMSO (4.1 mL, 57.72 mmol) in DCM (17 mL) was added dropwise and allowed to stir for 5 min. 4-Oxohexanal (1.690 g, 14.3 mmol) was dried in a minimum amount of toluene, before it was dissolved in dry DCM (17 mL), and added dropwise to the reaction mixture at -78 °C. The solution was allowed to stir for 1.5 h at -78 °C. Triethylamine (18 mL, 0.129 mol) was added dropwise to the reaction mixture at -78 °C and then allowed to stir overnight. Sodium bicarbonate solution (20 mL) was added to the reaction mixture after 18 h, and it was allowed to stir for 5 min. The organic layer was isolated and aqueous layer was extracted with ethyl acetate (2 × 40 mL). The organic extracts were combined, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give an orange/brown solid. The crude product was dry loaded onto celite and purified by column chromatography (silica, cyclohexane/ethyl acetate, 8:2 to 7:3). The title compound was isolated as an orange oil (0.770 g, 47% yield).

**$^1$H-NMR** (400 MHz, $CDCl_3$) δ 9.77 (t, *J* = 0.6 Hz, 1H), 2.76-2.67 (m, 4H), 2.47 (q, *J* = 7.4 Hz, 2H), 1.05 (t, *J* = 7.4 Hz, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 209.4, 200.7, 37.6, 36.0, 34.3, 7.9. In accordance with literature data.[5]

**4-Oxo-4-phenyl-butyraldehyde (S5)**

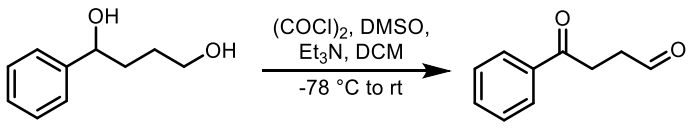



$LiAlH_4$ (1.180 g, 33.8 mmol) was slowly added to dry THF (60 mL) in a flame-dried 2-neck round bottom flask (250 mL) at 0 °C. Methyl 3-benzoylpropionate (1.610 g, 8.38 mmol) in dry THF (20 mL) was added dropwise to the $LiAlH_4$ suspension at 0 °C. The reaction mixture was allowed to stir for 5 h. The reaction mixture was cooled to 0 °C before $Na_2SO_4$ solution (6 mL) was added dropwise slowly. The reaction mixture was allowed to stir vigorously for 1 h. The white precipitate was filtered under vacuum and the filtrate was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give a colourless oil (1.120 g, 81% yield). The title compound was used directly in the next step without further purification.

**$^{1}$H-NMR** (500 MHz, $CDCl_3$) δ 7.29-7.25 (m, 4H), 7.23-7.18 (m, 1H), 4.59, (dd, *J* = 7.3, 5.4 Hz, 1H), 3.7-3.64 (br. m, 1H), 3.57-3.47 (m, 2H), 1.82-1.71 (m, 2H), 1.63-1.48 (m, 2H). One OH signal is broad along the baseline (3-4 ppm) and cannot be integrated.

**$^{13}$C-NMR** (126 MHz, $CDCl_3$) δ 144.8 128.5, 127.5, 125.9, 74.3, 62.7, 36.4, 29.2. In accordance with literature data.[6]

**Swern oxidation to form 4-oxo-4-phenyl-butyraldehyde (S6)**




Oxalyl chloride (1.6 mL, 17.34 mmol) was added to dry DCM (25 mL) in a flame-dried 2-neck round bottom flask (100 mL) with a stir bar, and the solution was cooled to -78 °C. Dry DMSO (2 mL, 26.96 mmol) in dry DCM (8 mL) was added dropwise to the reaction mixture and allowed to stir for 5 min. 1-Phenyl-butane-1,4-diol (1.120 g, 6.74 mmol) was dried in a minimum amount of toluene, before it was dissolved in dry DCM (16 mL) and

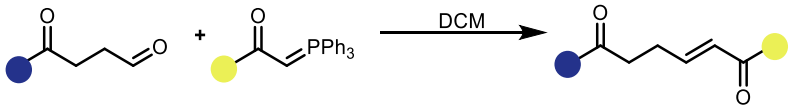
added dropwise to the reaction mixture at -78 °C. The reaction mixture was allowed to stir for 1 h at -78 °C, before triethylamine (8.5 mL, 61.32 mmol) was added dropwise. The solution was allowed to stir overnight. Sodium bicarbonate solution (10 mL) was added to the reaction mixture after 18 h, and it was allowed to stir for 30 min. The organic layer was isolated. The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The organic extracts were combined, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give an orange/brown solid. The crude product was dry loaded onto celite and purified by column chromatography (silica, cyclohexane/ethyl acetate, 8:2). The title compound was isolated as an orange oil (0.817 g, 75% yield).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 9.90 (t, *J* = 0.7 Hz, 1H), 8.01-7.95 (m, 2H), 7.60-7.54 (m, 1H), 7.50-7.43 (m, 2H), 3.32 (t, *J* = 6.2 Hz, 2H), 2.92 (td, *J* = 6.2, 0.7 Hz, 2H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 200.7, 197.9, 136.5, 133.4, 128.8, 128.2, 37.7, 31.1. In accordance with literature data.[7]

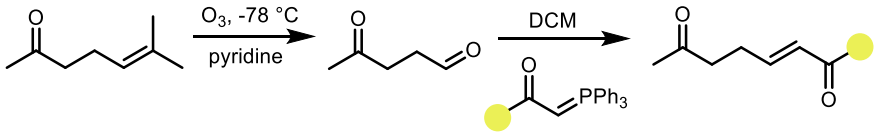
## 4. Ketoenone Synthesis

### General Procedure for the Wittig Reaction (GP1)



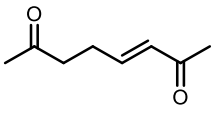

The appropriate aldehyde (6.5 mmol) was dissolved in dry DCM (65 mL), and the corresponding Wittig reagent (1.5 equiv., 9.75 mmol) was added to the solution. The reaction mixture was stirred for 24 h at room temperature (unless stated otherwise). The reaction mixture was then concentrated under reduced pressure. The crude product was dry loaded onto celite and purified by column chromatography (silica, cyclohexane/ethyl acetate) to provide the isolated ketoenone compounds.

### General Procedure for the Tandem Ozonolysis-Wittig Reaction (GP2)




**Option B:** 6-Methylhept-5-en-2-one (**1**) (1.5 mL, 10.16 mmol) was dissolved in dry DCM (100 mL) in a flame-dried 3-neck round bottom flask (250 mL). Dry pyridine (2.6 mL, 30.48 mmol, 3 equiv.) was added to the solution. A stream of oxygen was passed through the solution, and it was cooled to -78 °C. Once cooled, a stream of ozone was passed through the reaction mixture (~1 min/mmol) for 11 minutes. A dark yellow solution was observed. Upon completion, the solution was sparged with oxygen. The flask was allowed to warm up to room temperature, before the corresponding Wittig reagent (1.5 equiv., 15.24 mmol) was added to the solution. TLC was used to monitor the reaction, and after 24 h the reaction mixture was washed with 1 M HCl (3 × 50 mL). The reaction mixture was concentrated was under reduced pressure. The crude product was dry loaded onto celite and purified by column chromatography (silica, cyclohexane/ethyl acetate) to provide the isolated ketoenone compounds.

### (*E*)-oct-3-ene-2,7-dione (3a)



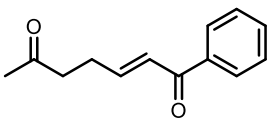

The synthesis of **3a** was carried out according to **GP1** with 4-oxopentanal and 1-triphenylphosphoranylidene-2-propanone. The title

compound was isolated as a yellow oil (0.565 g, 59% yield) after purification by column chromatography (cyclohexane/ethyl acetate, 99:1 to 7:3).

**$^{1}$H-NMR** (500 MHz, $CDCl_3$) δ 6.77 (dt, *J* = 16.0, 6.7 Hz, 1H), 6.06 (dt, *J* = 16.0, 1.6 Hz, 1H), 2.62 (t, *J* = 7.1 Hz, 2H), 2.51-2.44 (m, 2H), 2.22 (s, 3H), 2.16 (s, 3H).

**$^{13}$C-NMR** (126 MHz, $CDCl_3$) δ 206.9, 198.6, 146.3, 131.8, 41.6, 30.1, 27.1, 26.2. In accordance with literature data.[3]

**(*E*)-1-phenylhept-2-ene-1,6-dione (3b)**

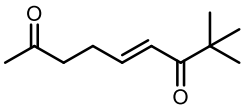


The synthesis of **3b** was carried out according to **GP1** with 4-oxopentanal and (benzoylmethylene) triphenylphosphorane (**S2**). The title compound was isolated as an orange oil (0.726 g, 53% yield) after purification by column chromatography (cyclohexane/ethyl acetate, 9:1).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 7.92-7.83 (m, 2H), 7.56-7.38 (m, 3H), 6.97 (dt, *J* = 15.4, 6.4 Hz, 1H), 6.87 (dt, *J* = 15.4, 1.2 Hz, 1H), 2.65-6.62 (m, 2H), 2.59-2.50 (m, 2H), 2.14 (s, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 207.3, 191.1, 147.9, 138.2, 133.2, 129.0, 129.0, 127.1, 42.1, 30.4, 27.0. In accordance with literature data.[3]

**(*E*)-8,8-dimethylnon-5-ene-2,7-dione (3c)**

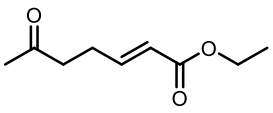


The synthesis of **3c** was carried out according to **GP2** with 6-methylhept-5-en-2-one (**1**) and 3,3-dimethyl-1-(triphenylphosphoranylidene)-2-butanone (**S1**). The title compound was isolated as an orange oil (0.300 g, 22% yield) after purification by column chromatography (cyclohexane/ethyl acetate, 9:1 to 8:2).

**$^{1}$H-NMR** (500 MHz, $CDCl_3$) δ 6.85 (dt, *J* = 15.3, 6.9 Hz, 1H), 6.51 (dt, *J* = 15.3, 1.6 Hz, 1H), 2.59 (t, *J* = 6.9 Hz, 2H), 2.50-2.44 (m, 2H), 2.15 (s, 3H), 1.13 (s, 9H).

**$^{13}$C-NMR** (126 MHz, $CDCl_3$) δ 207.0, 204.3, 145.3, 125.1, 43.0, 41.9, 30.1, 26.5, 26.3. In accordance with literature data.[3]

**Ethyl (*E*)-6-oxohept-2-enoate (3d)**

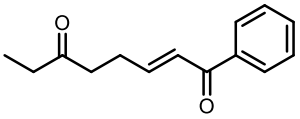


The synthesis of **3d** was carried out according to **GP2** with 6-methylhept-5-en-2-one and ethyl (triphenylphosphoranylidene) acetate. The title compound was isolated as a yellow oil (1.140 g, 49% yield) after purification by column chromatography (cyclohexane/ethyl acetate, 9:1).

**$^1$H-NMR** (500 MHz, $CDCl_3$) δ 6.91 (dt, *J* = 15.7, 6.7 Hz, 1H), 5.81 (dt, *J* = 15.7, 1.6 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.50-2.42 (m, 2H), 2.15 (s, 3H), 1.27 (t, *J* = 7.1 Hz, 3H).

**$^{13}$C-NMR** (126 MHz, $CDCl_3$) δ 206.7, 166.3, 146.9, 122.0, 60.1, 41.4, 29.8, 25.8, 14.1. In accordance with literature data.[3]

**(*E*)-1-phenyloct-2-ene-1,6-dione (3e)**

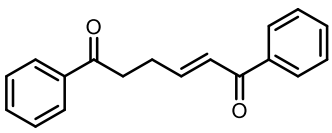


The synthesis of **3e** was carried out according to **GP1** with 4-oxohexanal and (benzoylmethylene) triphenylphosphorane (**S2**), but in this case, the reaction mixture was refluxed for 24 h in toluene. The title compound was isolated as an orange oil (1.002 g, 71% yield) after purification by column chromatography (cyclohexane/ethyl acetate, 9:1).

**$^1$H-NMR** (400 MHz, $CDCl_3$) δ 7.97-7.89 (m, 2H), 7.56-7.53 (m, 1H), 7.49-7.44 (m, 2H), 7.00 (dt, *J* = 15.5, 6.4 Hz, 1H), 6.9 (dt, *J* = 15.5, 1.2 Hz, 1H), 2.70-2.54 (m, 4H), 2.46 (q, *J* = 7.4 Hz, 2H), 1.07 (t, *J* = 7.4 Hz, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 209.7, 190.8, 147.8, 137.9, 132.9, 128.7, 126.7, 40.5, 36.2, 26.8, 7.9.

**(*E*)-1,6-diphenylhex-2-ene-1,6-dione (3f)**



The synthesis of **3f** was carried out according to **GP1** with 4-oxo-4-phenyl-butyraldehyde and (benzoylmethylene) triphenylphosphorane (**S2**), but in this case, the reaction mixture was refluxed for 24 h in toluene. The title compound was isolated as a yellow solid (0.877 g, 70% yield) after purification by column chromatography (cyclohexane/ethyl acetate, 9:1).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 8.03-7.89 (m, 4H), 7.64-7.53 (m, 2H), 7.52-7.42 (m, 4H), 7.10 (dt, *J* = 15.4, 6.7 Hz, 1H), 6.97 (dt, *J* = 15.4, 1.4 Hz, 1H), 3.22 (t, *J* = 7.1 Hz, 2H), 2.82-2.74 (m, 2H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 198.5, 190.9, 147.9, 138.0, 136.8, 133.4, 132.9, 128.9, 128.7, 128.7, 128.2, 126.9, 37.0, 27.2.

## 5. ATA Screening

Each well in a 96-well plate contained ketoenone substrate (5 mM) dissolved in DMSO (50 µL, 10% v/v). HEPES buffer pH 7.5 (450 µL), PLP (2 mM), *o*-xylylenediamine (5 mM), and ATA (1.5 mg/mL) was added to each well and incubated at 45 °C. The 96-well plate was observed every 30 mins for 3 h. A positive hit was identified when a black precipitate was observed in the well.

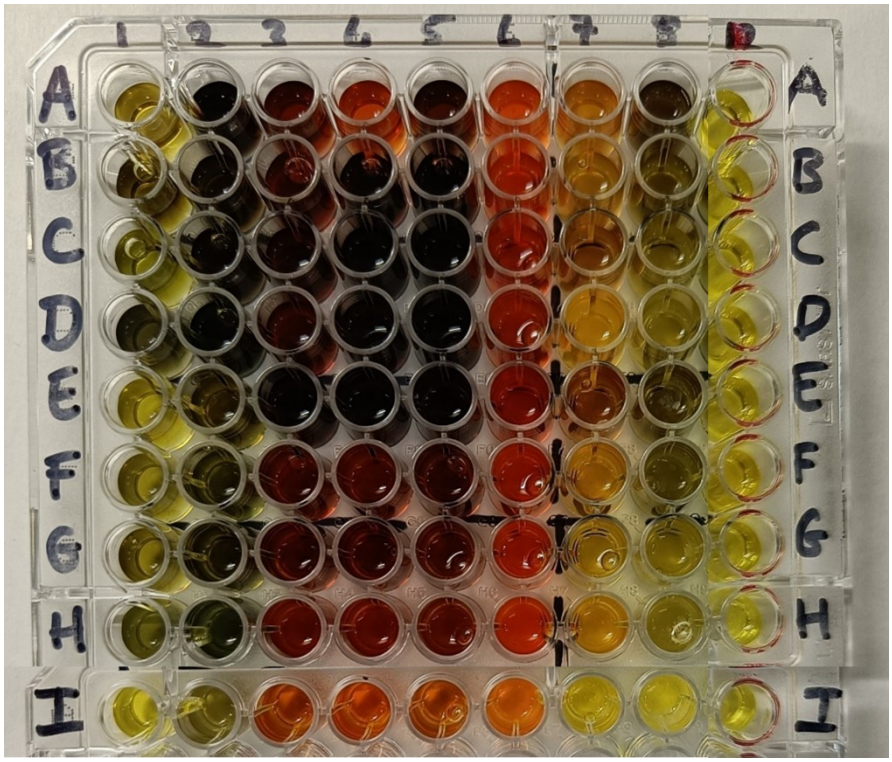



***Figure S1*. Colorimetric screen at 45 °C after 2 h.** This figure was spliced for clarity. Columns 1-12 contain different substrates; **(1)** No substrate, **(2)** Acetophenone, **(3) 3a**, **(4) 3c**, **(5) 3d**, **(6) 3b**, **(7) 3e**, **(8) 3f**, **12)** No substrate and no enzyme. Rows A-I contain different ATAs; **(A)** ATA217, **(B)** ATA234, **(C)** ATA237, **(D)** ATA238, **(E)** ATA251, **(F)** ATA254, **(G)** ATA256, **(H)** 260, **(I)** CDX043.

Each well in a 96-well plate contained ketoenone substrate (5 mM) dissolved in DMSO (50 µL, 10% v/v). HEPES buffer pH 7.5 (450 µL), PLP (2 mM), *o*-xylylenediamine (5 mM), and ATA (1.5 mg/mL) was added to each well and incubated at 30 °C. The 96-well plate was observed every 30 mins for 3 h. A positive hit was identified when a black precipitate was observed in the well.

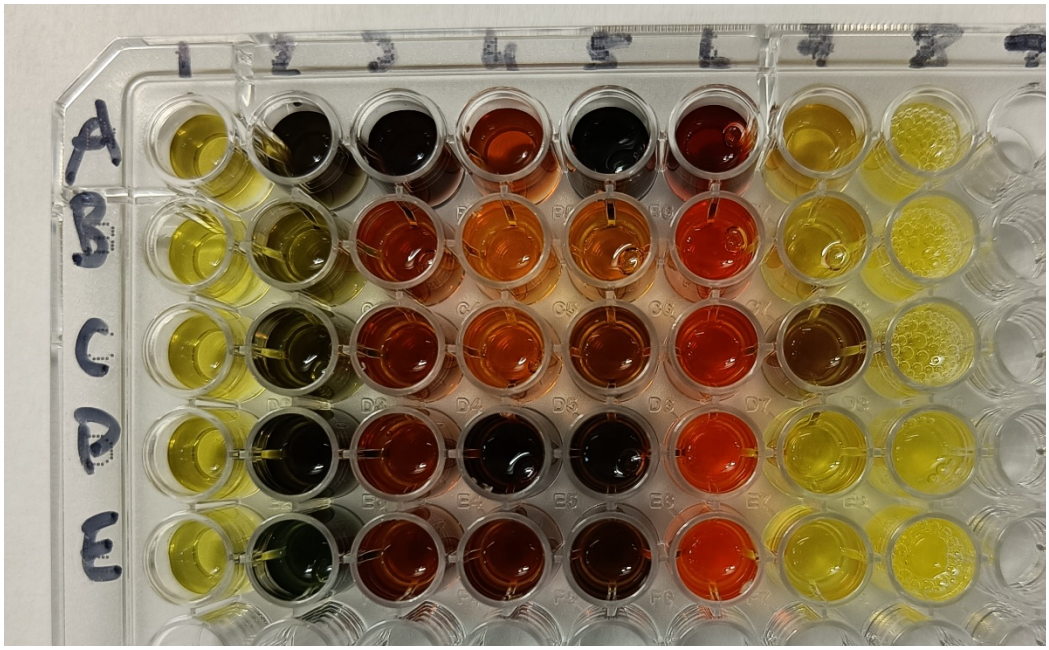
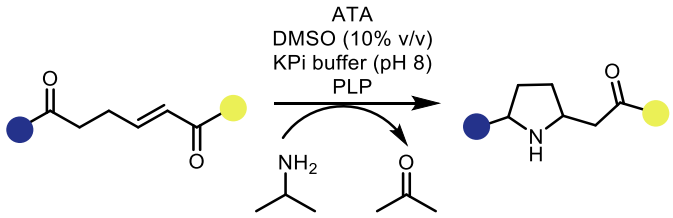


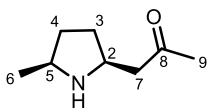

***Figure S2*. Colorimetric screen at 30 °C after 2h.** Columns 1-8 contain different substrates; **(1)** No substrate, **(2)** Acetophenone, **(3) 3a**, **(4) 3c**, **(5) 3d**, **(6) 3b**, **(7) 3e**, **(8) 3f**. Rows A-E contain different ATAs; **(A)** ATA113, **(B)** ATA200, **(C)** ATAP1B04, **(D)** ATAP1F03, **(E)** ATAP1G05.

## 6. Biotransformations - General Procedure for the Preparation of Disubstituted Pyrrolidines (GP3)

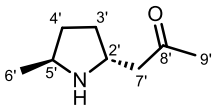



In a 50 mL falcon tube, the ketoenone substrate (0.5 mmol) was dissolved in DMSO (0.5 mL, 10% v/v). The total volume of the reaction mixture was adjusted to 10 mL by the addition of KPi buffer (100 mM, pH 8.0). PLP (4.9 mg, 0.02 mmol, 2 mM) and isopropylamine hydrochloride (0.096 g, 1 mmol, 2 equiv.) were added to the solution. The pH of the reaction mixture was checked with indicator paper, and it was adjusted with 1 M HCl or 1 M NaOH if required. The commercially available transaminase (50 mg, 5 mg/mL) was added to the reaction mixture and incubated at 50 °C, 200 rpm, for 24 h unless stated otherwise. Upon completion, the pH of the reaction mixture was adjusted to pH 12 using aqueous NaOH solution (10 M), and the resulting reaction mixture was extracted with diethyl ether (3 × 10 mL). The reaction mixture was centrifuged at 4,000 rpm for 5 min to break up the emulsion in between each extraction. The organic extracts were combined, dried with $Na_2SO_4$, filtered, and concentrated under reduced pressure to give isolated disubstituted pyrrolidine products. If required the product was purified *via* column chromatography (neutral alumina, methanol/dichloromethane). All isolated products were analysed by NMR spectroscopy.

### *(5S)*-1-(2,5-methylpyrrolidin-2-yl)propan-2-one (5a)




*cis* (**major**)




*trans* (**minor**)

The synthesis of **5a** was carried out according to **GP3** with **3a** and (*S*)-selective ATA256. The title compound was isolated as a brown oil (0.068 g, 34% yield, *cis/trans*, 62:38 *dr* – ratio was determined from the integration of H2 in each isomer) without further purification.

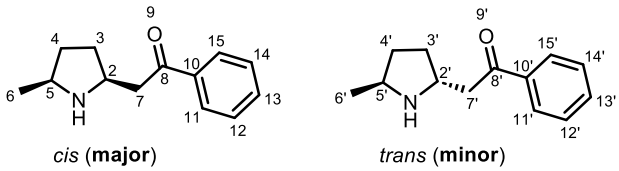
**$^1$H-NMR** (400 MHz, $CDCl_3$, *cis/trans*, 62:38 *dr*) δ 3.66 (m, 1H, H**5'**), 3.37 (m, 1H, H**5**), 3.25 (m, 1H, H**2'**), 3.14 (m, 1H, H**2**), 2.70-2.55 (m, 2H, H**7'**), 2.57 (m, 2H, H**7**), 2.14 (s, 6H, H**9,**

H**9'**), 2.05-1.91 (m, 4H, H**3** and H**3'**), 2.39-1.23 (m, 4H, H**4** and H**4'**), 1.13 (m, 6H, H**6** and H**6'**).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans*, 62:38 *dr*) δ 209.0, 208.9, 54.6, 54.5, 53.8, 53.7,51.3, 50.9, 34.6, 32.8, 32.7, 31.3, 31.0, 30.8, 22.3, 22.0.

**HRMS-ESI (m/z):** $C_8H_{15}NO^+$ $[M+H]^+$ theoretical 142.1232, found 142.1227.

**(5*S*)-2-(2,5-methylpyrrolidin-2-yl)-1-phenylethan-1-one (5b)**



*cis* (**major**) *trans* (**minor**)

The synthesis of **5b** was carried out according to **GP3** with **3b** and (*S*)-selective ATA256. The title compound was isolated as a brown oil (0.045 g, 63% yield, *cis/trans* 63:37 *dr* – ratio was determined from the integration of H2 in each isomer) after purification by column chromatography (DCM to 2% MeOH/DCM).

**Major diastereoisomer:**

**$^{1}$H-NMR** (600 MHz, $CDCl_3$) δ 7.98-7.92 (m, 2H, **Ar**H), 7.60-7.51 (m, 1H, **Ar**H), 7.48-7.42 (m, 2H, **Ar**H), 3.62-3.5 (m, 1H, H**2**), 3.24-3.19 (m, 3H, H**5**, H**7**), 2.03-1.95 (m, 1H, H**3**), 1.93-1.86 (m, 1H, H**4**), 1.54-1.46 (m, 1H, H**3**), 1.39-1.32 (m, 1H, H**4**), 1.17 (d, *J* = 6.5 Hz, 3H, H**6**).

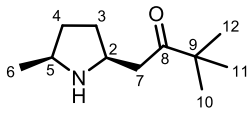
**Minor diastereoisomer:**

**$^{1}$H-NMR** (600 MHz, $CDCl_3$) δ 7.98-7.92 (m, 2H, **Ar**H'), 7.60-7.51 (m, 1H, **Ar**H'), 7.48-7.42 (m, 2H, **Ar**H'), 3.88-3.82 (m, 1H, H**2'**), 3.36-3.32 (m, 1H, H**5'**), 3.19 (dd, *J* = 16.8, 7.6 Hz, 1H, H**7'**), 3.12 (dd, *J* = 16.8, 5.3 Hz, 1H, H**7'**), 2.15-2.09 (m, 1H, H**3'**), 2.04-1.98 (m, 1H, H**4'**), 1.52-1.45 (m, 1H, H**3'**), 1.38-1.31 (m, 1H, H**4'**), 1.18 (d, *J* = 6.3 Hz, 3H, H**6'**).

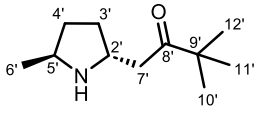
**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans* 63:37 *dr*) δ 199.8 (2C), 137.3, 137.2, 133.2, 133.1, 128.7 (2C), 128.2, 128.2, 54.6, 54.1, 53.7, 53.4, 46.1, 45.7, 34.3, 32.6, 32.4, 31.1, 22.1, 21.8.

**HRMS-ESI (m/z):** $C_{13}H_{17}NO^+$ $[M+H]^+$ theoretical 204.1388, found 204.1381.

**(5*S*)-3,3-dimethyl-1-(2,5-methylpyrrolidin-2-yl)butan-2-one (5c)**




*cis* (**major**)




*trans* (**minor**)

The synthesis of **5c** was carried out according to **GP3** with **3c** and (*S*)-selective ATA256. The title compound was isolated as an orange oil (0.168 g, 57% yield, *cis/trans* 67:33 *dr* – ratio was determined from the integration of H2 in the *trans*-isomer and H5 in the *cis*-isomer) without further purification.

**Major diastereoisomer:**

**$^{1}$H-NMR** (600 MHz, $CDCl_3$) δ 3.39-3.34 (m, 1H, H**2**), 3.20-3.12 (m, 1H, H**5**), 2.75 (dd, *J* = 17.9, 5.1 Hz, 1H, H**7**), 2.70 (dd, *J* = 17.9, 7.6 Hz, 1H, H**7**), 1.93-1.82 (m, 2H, H**4**), 1.40-1.27 (m, 2H, H**3**), 1.16 (d, *J* = 6.3 Hz, 3H, H**6**), 1.11 (s, 9H, H**10**, H**11**, H**12**).

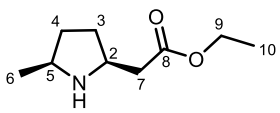
**Minor diastereoisomer:**

**$^{1}$H-NMR** (600 MHz, $CDCl_3$) δ 3.67-3.62 (m, 1H, H**2'**), 3.34-3.30 (m, 1H, H**5'**), 2.69 (apparent d, *J* = 6.3 Hz, 2H, H**7'**), 2.06-1.95 (m, 2H, H**4'**), 1.40-1.28 (m, 2H, H**3'**), 1.16 (d, *J* = 6.3 Hz 3H, H**6'**), 1.10 (s, 9H, H**10'**, H**11'**, H**12'**).

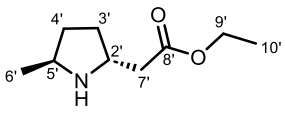
**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans* 67:33 *dr*) δ 216.3, 216.2, 54.9, 54.4, 53.7, 53.6, 44.46, 44.4, 44.0, 43.6, 34.3, 32.8, 32.6, 31.3, 26.9, 26.8, 22.4, 21.9.

**HRMS-ESI (m/z):** $C_{11}H_{21}NO^{+}$ $[M+H]^{+}$ theoretical 184.1701, found 184.1698.

**(5*S*)-Ethyl 2-(2,5-methylpyrrolidin-2-yl)acetate (5d)**




*cis* (**minor**)




*trans* (**major**)

The synthesis of **5d** was carried out according to **GP3** with **3d** and (*S*)-selective ATA256. The title compound was isolated as an orange oil (0.032 g, 10% yield, *cis/trans,* 33:67 *dr* - ratio was determined from the integration of H2 in each isomer) without further purification.

**Major diastereoisomer:**

**$^{1}$H-NMR** (600 MHz, $CDCl_3$) δ 4.13 (q, *J* = 7.2 Hz, 2H, H**9'**), 3.73-3.67 (m, 1H, H**2'**), 3.35-3.31 (m, 1H, H**5'**), 2.51-2.43 (m, 2H, H**7'**), 2.10-2.04 (m, 1H, H**4'**), 2.03-1.97 (m, 1H, H**3'**),

1.49-1.42 (m, 1H, H**4'**), 1.37-1.31 (m, 1H, H**3'**), 1.25 (t, *J* =7.2 Hz, 3H, H**10'**), 1.18 (d, *J* = 6.3 Hz, 3H, H**6'**).

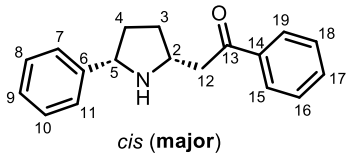
**Minor diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 4.13 (q, *J* = 7.2 Hz, 2H, H**9**), 3.47-3.41 (m, 1H, H**2**), 3.22-3.18 (m, 1H, H**5**), 2.55 (dd, *J* = 16.2, 5.2 Hz, 1H, H**7**), 2.50 (dd, *J* = 16.2, 7.7 Hz, 1H, **H7**), 1.97-1.86 (m, 2H, H**3**), 1.49-1.42 (m, 1H, H**4**), 1.38-32 (m, 1H, H**4**), 1.25 (t, *J* =7.2 Hz, 3H, H**10**), 1.19 (d, *J* = 6.2 Hz, 3H, H**6**).

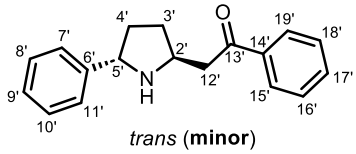
**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans,* 33:67 *dr*) δ 172.7, 172.6, 60.5, 55.2, 54.4, 53.5, 41.6, 41.1, 34.3, 32.7, 32.3, 31.0, 29.8, 21.8, 21.6, 14.4.

**HRMS-ESI (m/z):** $C_9H_{17}NO_2^+$ $[M+H]^+$ theoretical 172.1337, found 172.1334.

**(5*S*)-1-phenyl-2-(2,5-phenylpyrrolidin-2-yl)ethan-1-one (5f)**



*cis* (**major**)



*trans* (**minor**)

The synthesis of **5f** was carried out according to **GP3** with **3f** and (*S*)-selective ATA251, except 5 equiv. of IPA.HCl was used as the amino donor, and the reaction was allowed to react for 48 h. The title compound was isolated as a brown oil (0.029 g, 27% yield, *cis/trans*, 67:33 *dr* – ratio was determined from the integration of H2 in each isomer) after purification *via* column chromatography (alumina, DCM to 1% MeOH/DCM).

**Major diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 8.04-7.94 (m, 4H, **Ar**H), 7.58-7.19 (m, 6H, **Ar**H), 4.27 (t, *J* = 7.9 Hz, 1H, H**5**), 3.82-3.72 (m, 1H, H**2**), 3.34-3.26 (m, 2H, H**12**), 2.25-2.19 (m, 1H, H**4**), 2.14-2.08 (m, 1H, H**3**), 1.80-1.74 (m, 1H, H**4**), 1.69-1.63 (m, 1H, H**4**).

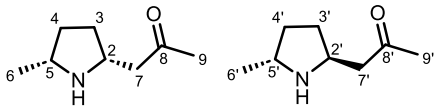
**Minor diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 8.04-7.94 (m, 4H, **Ar**H'), 7.58-7.19 (m, 6H, **Ar**H'), 4.40 (t, *J* = 7.6 Hz, 1H, H**2'**), 4.10-4.04 (m, 1H, H**5'**), 3.29-3.22 (m, 2H, H**7'**), 2.39-2.32 (m, 1H, H**3'**), 2.28-2.22 (m, 1H, H**4'**), 1.90-1.83 (m, 1H, H**3'**), 1.72-1.65 (m, 1H, H**4'**).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans*, 67:33 *dr*) δ 199.9, 199.7, 145.8, 145.2, 137.3, 133.2, 133.2, 128.7, 128.7, 128.7, 128.4, 128.2, 127.0, 126.8, 126.7, 126.4, 62.2, 61.7, 54.6, 54.5, 46.0, 45.9, 34.9, 34.8, 33.6, 32.6, 31.0.

**HRMS-ESI (m/z):** $C_{18}H_{19}NO^+$ $[M+H]^+$ theoretical 266.1545, found 266.1542.

**(5*R*)-1-(2,5-methylpyrrolidin-2-yl)propan-2-one (6a)**



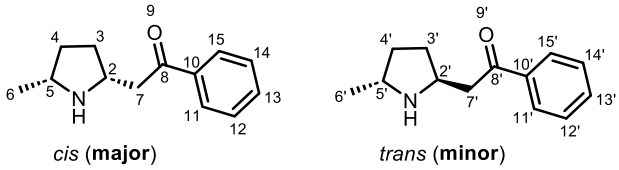

The synthesis of **6a** was carried out according to **GP3** with **3a** and (*R*)-selective ATA025. The title compound was isolated as a brown oil (62:38, 0.050 g, 57% yield, *cis/trans,* 62:38 *dr*) without further purification.

**$^1$H-NMR** (400 MHz, $CDCl_3$, *cis/trans*, 62:38 *dr*) δ 3.66 (m, 1H, H**5'**), 3.37 (m, 1H, H**5**), 3.25 (m, 1H, H**2'**), 3.14 (m, 1H, H**2**), 2.70-2.55 (m, 2H, H**7'**), 2.57 (m, 2H, H**7**), 2.14 (s, 6H, H**9,** **H**9'), 2.05-1.91 (m, 4H, H**3** and H**3'**), 2.39-1.23 (m, 4H, H**4** and H**4'**), 1.13 (m, 6H, H**6** and H**6'**).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans*, 62:38 *dr*) δ 209.0, 208.9, 54.6, 54.5, 53.8, 53.7, 51.3, 50.9, 34.6, 32.8, 32.7, 31.3, 31.0, 30.8, 22.3, 22.0.

**HRMS-ESI (m/z):** $C_8H_{15}NO^+$ $[M+H]^+$ theoretical 142.1232, found 142.1226.

**(5*R*)-2-(2,5-methylpyrrolidin-2-yl)-1-phenylethan-1-one (6b)**




The synthesis of **6b** was carried out according to **GP3** with **3b** and (*R*)-selective ATA025. The title compound was isolated as a brown oil (0.081 g, 54% yield, *cis/trans*, 64:36 *dr* – ratio was determined from the integration of H2 in each isomer) after purification *via* column chromatography (alumina, DCM to 2% MeOH/DCM).

**<u>Major diastereoisomer:</u>**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 7.98-7.92 (m, 2H, **Ar**H), 7.60-7.51 (m, 1H, **Ar**H), 7.48-7.39 (m, 2H, **Ar**H), 3.65-3.57 (m, 1H, H**2**), 3.27-3.19 (m, 3H, H**5**, H**7**), 2.06-1.97 (m, 1H, H**3**), 1.94-1.87 (m, 1H, H**4**), 1.56-1.48 (m, 1H, H**3**), 1.41-1.33 (m, 1H, H**4**), 1.19 (d, *J* = 6.5 Hz, 3H, H**6**).

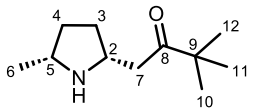
**Minor diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 7.98-7.92 (m, 2H, **Ar**H'), 7.60-7.51 (m, 1H, **Ar**H'), 7.48-7.39 (m, 2H, **Ar**H'), 3.91-3.85 (m, 1H, H**2'**), 3.42-3.36 (m, 1H, H**5'**), 3.24 (dd, *J* = 16.9, 7.5 Hz, 1H, H**7'**), 3.14 (dd, *J* = 16.9, 5.6 Hz, 1H, H**7'**), 2.17-2.11 (m, 1H, H**3'**), 2.06-1.99 (m, 1H, H**4'**), 1.55-1.47 (m, 1H, H**3'**), 1.41-1.33 (m, 1H, H**4'**), 1.20 (d, *J* = 6.5 Hz, 3H, H**6'**).

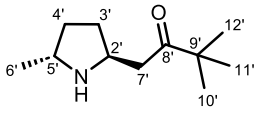
**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans*, 64:36 *dr*) δ 199.7, 199.6, 137.1, 137.0, 133.2, 128.7(2C), 128.2, 128.1, 54.6, 54.2, 53.7, 53.5, 45.7, 45.4, 41.1, 34.1, 32.5, 32.3, 31.0, 21.8, 22.0.

**HRMS-ESI (m/z):** $C_{13}H_{17}NO^+$ $[M+H]^+$ theoretical 204.1388, found 204.1384.

**(5*R*)-3,3-dimethyl-1-(2,5-methylpyrrolidin-2-yl)butan-2-one (6c)**



*cis* (**major**)



*trans* (**minor**)

The synthesis of **6c** was carried out according to **GP3** with **3c** and (*R*)-selective ATA025. The title compound was isolated as an orange oil (0.061 g, 58% yield, *cis/trans* 55:45 *dr*). No further purification was required.

**Major diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 3.39-3.33 (m, 1H, H**2**), 3.19-3.12 (m, 1H, H**5**), 2.75 (dd, *J* = 17.8, 4.9 Hz, 1H, H**7**), 2.69 (dd, *J* = 17.8, 7.6 Hz, 1H, H**7**), 1.94-1.81 (m, 2H, H**4**), 1.39-1.25 (m, 2H, H**3**), 1.11 (d, *J* = 6.3 Hz 3H, H**6**), 1.11 (s, 9H, H**10**, H**11**, H**12**).

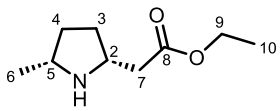
**Minor diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 3.67-3.60 (m, 1H, H**2'**), 3.32-3.28 (m, 1H, H**5'**), 2.71-2.63 (m, 2H, H**7'**), 2.05-1.94 (m, 2H, H**4'**), 1.38-1.26 (m, 2H, H**3'**), 1.15 (d, *J* = 6.4 Hz 3H, H**6'**), 1.11 (s, 9H, H**10'**, H**11'**, H**12'**).

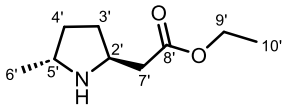
**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans* 55:45 *dr*) δ 215.9, 215.8, 54.5, 54.0, 53.2, 53.1, 44.1, 44.0, 43.8, 43.3, 34.0, 32.4, 32.2, 30.9, 26.5, 26.5, 22.1, 21.6.

**HRMS-ESI (m/z):** $C_8H_{15}NO^+$ $[M+H]^+$ theoretical 184.1701, found 184.1698.

**(5*R*)-Ethyl 2-(2,5-methylpyrrolidin-2-yl)acetate (6d)**




*cis* (**minor**)




*trans* (**major**)

The synthesis of **6d** was carried out according to **GP3** with **3d** and (*R*)-selective ATA025. The title compound was isolated as an orange oil (0.010 g, 10% yield, *cis/trans* 43:57 *dr*) without further purification.

**Major diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 4.13 (q, *J* = 7.2 Hz, 2H, H**9'**), 3.73-3.67 (m, 1H, H**2'**), 3.35-3.31 (m, 1H, H**5'**), 2.51-2.43 (m, 2H, H**7'**), 2.10-2.04 (m, 1H, H**4'**), 2.03-1.97 (m, 1H, H**3'**), 1.49-1.42 (m, 1H, H**4'**), 1.37-1.31 (m, 1H, H**3'**), 1.25 (t, *J* =7.2 Hz, 3H, H**10'**), 1.18 (d, *J* = 6.3 Hz, 3H, H**6'**).

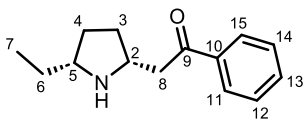
**Minor diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 4.13 (q, *J* = 7.2 Hz, 2H, H**9**), 3.47-3.41 (m, 1H, H**2**), 3.22-3.18 (m, 1H, H**5**), 2.55 (dd, *J* = 16.2, 5.2 Hz, 1H, H**7**), 2.50 (dd, *J* = 16.2, 7.7 Hz, 1H, **H7**), 1.97-1.86 (m, 2H, H**3**), 1.49-1.42 (m, 1H, H**4**), 1.38-32 (m, 1H, H**4**), 1.25 (t, *J* =7.2 Hz, 3H, H**10**), 1.19 (d, *J* = 6.2 Hz, 3H, H**6**).

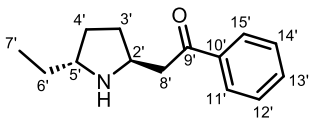
**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans* 43:57 *dr*) δ 172.7, 172.6, 60.5, 60.5, 55.2, 54.4, 53.4, 41.6, 41.1, 34.3, 32.7, 32.3, 31.0, 30.5, 21.8, 21.6, 14.4 (2C).

**HRMS-ESI (m/z):** $C_8H_{15}NO^+$ $[M+H]^+$ theoretical 172.1337, found 172.1333.

**(5*R*)-2-(2,5-ethylpyrrolidin-2-yl)-1-phenylethan-1-one (6e)**




*cis* (**major**)




*trans* (**minor**)

The synthesis of **6e** was carried out according to **GP3** with **3e** and (*R*)-selective ATA025 or ATA251 and was allowed to react for 48 h.

ATA025: The title compound was isolated as a brown oil (0.012 g, 25% yield, *cis/trans* 65:35 *dr*– ratio was determined from the integration of H2 in each isomer) after purification *via* column chromatography (alumina, DCM to 2% MeOH/DCM).

ATA251*: The title compound was isolated as a brown oil (0.064 g, 57% yield, *cis/trans*, 65:35 *dr*) after purification *via* column chromatography (alumina, DCM to 2% MeOH/DCM).

**Major diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 7.95-7.92 (m, 2H, **Ar**H), 7.55-7.51 (m, 1H, **Ar**H), 7.45-7.41 (m, 2H, **Ar**H), 3.59-3.52 (m, 1H, H**2**), 3.17 (apparent d, *J* = 6.5 Hz, 2H, H**8**), 3.02-2.95 (m, 1H, H**5**), 1.98-1.82 (m, 2H, H**3'**), 1.53-1.39 (m, 2H, H**4'**), 1.38-1.30 (m, 2H, H**6**), 0.91 (t, *J* = 7.5 Hz, 3H, H**7**).

**Minor diastereoisomer:**

**$^1$H-NMR** (600 MHz, $CDCl_3$) δ 7.95-7.92 (m, 2H, **Ar**H'), 7.55-7.51 (m, 1H, **Ar**H'), 7.45-7.41 (m, 2H, **Ar**H'), 3.80-3.72 (m, 1H, H**2'**), 3.21-3.07 (m, 3H, H**5'**, H**8'**), 2.10-2.02 (m, 1H, H**3'**), 2.02-1.94 (m, 1H, H**4'**), 1.51-1.40 (m, 2H, H**3'**), 1.40-1.29 (m, 2H, H**6'**), 0.91 (t, *J* = 7.5 Hz, 3H, H**7'**).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans* 65:35 *dr*) δ 199.8, 199.7, 137.2, 137.2, 133.2 (2C), 128.7, 128.7, 128.2, 128.2, 60.7, 59.8, 54.4, 53.8, 45.6, 45.4, 32.1, 31.7, 30.7, 30.2, 29.8, 29.6, 11.8, 11.5.

**HRMS-ESI (m/z):** $C_8H_{15}NO^+$ $[M+H]^+$ theoretical 218.1545, found 218.1541.

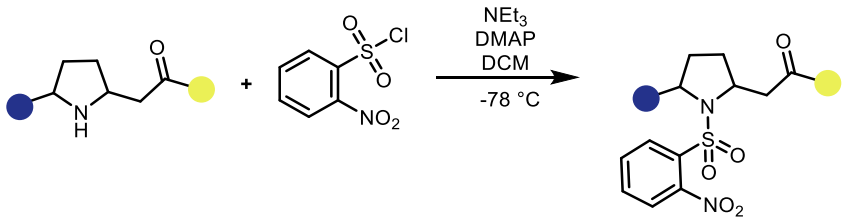
*Upon *ee* analysis of the nosylated compound **8e**, we identified that ATA251 displayed *R*-selectivity in the enzyme triggered reaction rather than *S*-selectivity as annotated by Codexis in their screening kit.

**General Procedure for Water Suppression NMR experiment**

The ketoenone substrate (0.25 mmol) was dissolved in DMSO (0.5 mL, 10% v/v). The total volume of the reaction mixture was adjusted to 5 mL by the addition of KPi buffer (100 mM, pH 8.0). PLP (2.5 mg, 0.01 mmol, 2 mM) and isopropylamine hydrochloride (0.048 g, 2 equiv.) were added to the solution. ATA256 (5 mg/mL) was added to the reaction mixture and incubated at 50 °C, 200 rpm, for 24 h. After 24 h, the reactions were centrifuged, an aliquot (140 µL) was removed from the supernatant, and added to 560 µL of maleic acid (12.2 mM in $D_2O$) in an NMR tube. Water suppression $^1$H-NMR experiments were carried out on a Varian VNMR 600 MHz spectrometer ($^1$H 600 MHz). These experiments were carried out at 298 K, run with 64 scans, a relaxation delay of 2.0 seconds and an acquisition time of 5.0 seconds.

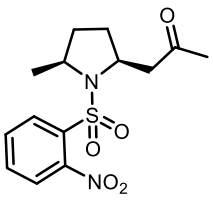
Retrospectively, in order to verify the quantitative-NMR conversions from the water suppression NMR experiment, a sample from the biocatalysed production of compound **5c** was analysed as described, but with an increased relaxation delay of 60 seconds. Based on this experiment, we found that 2 s and 60 s relaxation delay gave a negligible difference in q-NMR conversions.

## 7. General Procedure for Nosylation (GP4)




The disubstituted pyrrolidine (0.12 mmol) was dissolved in DCM (1.2 mL, 0.1 M) in a round bottom flask (5 mL) and stir bar. The solution was cooled to -78 °C, and triethylamine (25.4 µL, 0.18 mmol, 1.5 equiv.) and DMAP (1.5 mg, 0.012 mmol, 0.1 equiv.) were added to the solution. Once cooled, 2-nosyl chloride (0.040 g, 0.18 mmol,1.5 equiv.) was added to the solution and the reaction mixture was allowed to stir for 1 h. The reaction mixture was then concentrated under reduced pressure. The crude product was dry loaded onto celite and the purified by column chromatography (silica, pentane/ethyl acetate).

**1-((2*S*,5*S*)-5-methyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)propan-2-one (7a)**



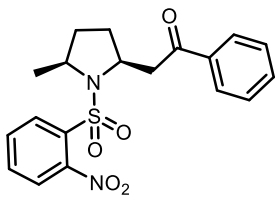
The synthesis of **7a** was carried out according to **GP4** with **5a**. The title compound was isolated as an orange oil (0.047 g, 34% yield, 67% *de*, 88% *ee*) after purification *via* column chromatography (pentane/ethyl acetate, 8:2 to 7:3).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 8.03-7.92 (m, 1H), 7.73-7.64 (m, 2H), 7.57-7.50 (m, 1H), 4.21-4.11 (m, 1H), 4.05-3.91 (m, 1H), 3.25 (dd, *J* = 17.6, 3.4 Hz, 1H), 2.65 (dd, *J* = 17.6, 9.7 Hz, 1H), 2.17 (s, 3H), 2.07-1.97 (m, 1H), 1.86-1.76 (m, 1H), 1.74-1.49 (m, 3H), 1.31 (d, *J* = 6.4 Hz, 3H).

**$^{13}$C-NMR** (126 MHz, $CDCl_3$) δ 206.8, 148.9, 133.8, 131.5, 130.8, 123.9, 57.9, 57.4, 51.1, 32.0, 30.8, 30.6, 23.4.

**HRMS-ESI (m/z):** $C_{14}H_{18}N_2O_5S^+$ $[M+H]^+$ theoretical 327.1009, found 327.1011.

**2-((2*S*,5*S*)-5-methyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)-1-phenylethan-1-one (7b)**



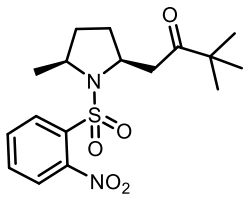
The synthesis of **7b** was carried out according to **GP4** with **5b**. The title compound was isolated as an orange oil (0.023 g, 51% yield, 92% *de*, 72% *ee*) after purification *via* column chromatography (pentane/ethyl acetate, 9:1 to 7:3).

**$^1$H-NMR** (400 MHz, $CDCl_3$) δ 8.04-7.97 (m, 3H), 7.74-7.66 (m, 2H), 7.62-7.54 (m, 2H), 7.52-7.45 (m, 2H), 4.43-4.35 (m, 1H), 4.08-3.99 (m, 1H), 3.87 (dd, *J* = 17.2, 3.1 Hz, 1H), 3.15 (dd, *J* = 17.2, 10.3 Hz, 1H), 2.17-2.06 (m, 1H), 1.94-1.84 (m, 1H), 1.79-1.69 (m, 1H), 1.66-1.56 (m, 1H), 1.37 (d, *J* = 6.4 Hz, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 198.2, 136.6, 133.8, 133.6, 131.5, 131.3, 131.0, 128.8, 128.3, 124.0, 58.3, 57.9, 46.7, 32.2, 30.8, 23.4.

**HRMS-ESI (m/z):** $C_{19}H_{20}N_20_5S^+$ $[M+Na]^+$ theoretical 411.0986, found 411.0987.

**3,3-dimethyl-1-((2*S*,5*S*)-5-methyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)butan-2-one (7c)**



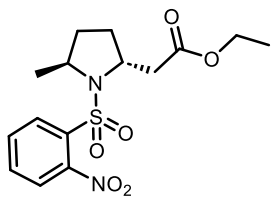
The synthesis of **7c** was carried out according to **GP4** with **5c**. The title compound was isolated as an orange oil (0.011 g, 25% yield, 86% *de*, 88% ee) after purification *via* column chromatography (pentane/ethyl acetate, 8:2).

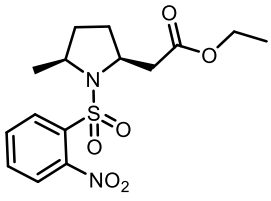
**$^1$H-NMR** (400 MHz, $CDCl_3$) δ 8.01-7.97 (m, 1H), 7.74-7.64 (m, 2H), 7.58-7.50 (m, 1H), 4.22-4.14 (m, 1H), 4.09-3.96 (m, 1H), 3.32 (dd, *J* = 17.9, 3.1 Hz, 1H), 2.72 (dd, *J* = 17.9, 10.2 Hz, 1H), 2.09-1.98 (m, 1H), 1.91-1.77 (m, 1H), 1.61-1.52 (m, 3H), 1.34 (d, *J* = 6.4 Hz, 3H), 1.16 (s, 9H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 214.2, 133.6, 131.3, 131.1, 130.8, 123.8, 58.0, 57.7, 44.6, 44.1, 31.9, 30.9, 26.2, 23.3.

**HRMS-ESI (m/z):** $C_{17}H_{24}N_2O_5S^+$ $[M+Na]^+$ theoretical 391.1299, found 391.1299.

**Ethyl 2-((2*R*,5*S*)-5-methyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)acetate (7d)**


*trans*
(**major**)


*cis*

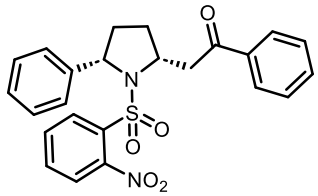
The synthesis of **7d** was carried out according to **GP4** with **5d**. The title compound was isolated as an orange oil (0.008 g, 39% yield, 10% *de*) after purification *via* column chromatography (pentane/ethyl acetate, 8:2).

**$^1$H-NMR** (400 MHz, $CDCl_3$, *cis/trans* 43:57 *dr* – ratio was determined on the integration of H2 in *trans*-**22** and H5 in *cis*-**22**) δ 8.09-8.0 (m, 2H), 7.74-7.54 (m, 6H), 4.47-4.39 (m, 1H), 4.27-4.06 (m, 6H), 4.06-3.96 (m, 1H), 3.07 (dd, *J* = 15.9, 4.0 Hz, 1H), 2.90 (dd, *J* = 16.2, 3.4 Hz, 1H), 2.50 (dd, *J* = 16.2, 10.0 Hz, 1H), 2.38 (dd, *J* = 15.9, 10.7 Hz, 1H), 2.32-2.13 (m, 2H), 2.06-1.95 (m, 1H), 1.94-1.84 (m, 1H), 1.68-1.55 (m, 2H), 1.34 (d, *J* = 6.5 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 2H), 1.24 (t, *J* = 5.9 Hz, 2H), 1.13 (d, *J* = 6.4 Hz, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 171.1, 170.9, 135.8, 133.8, 133.6, 131.9, 131.5, 131.4, 131.1, 130.2, 124.4, 124.0, 60.8, 60.7, 58.2, 58.1, 58.0, 57.4, 42.0, 39.2, 32.1, 31.1, 30.6, 29.2, 23.4, 20.9, 14.3.

**HRMS-ESI (m/z):** $C_{15}H_{20}N_2O_6S^+$ $[M+H]^+$ theoretical 357.1115, found 357.1118.

**2-((2*R*,5*S*)-1-((2-nitrophenyl)sulfonyl)-5-phenylpyrrolidin-2-yl)-1-phenylethan-1-one (7f)**



The synthesis of **7f** was carried out according to **GP4** with **5f**. The title compound was isolated as an orange oil (0.003 g, 38% yield, >99% *de*, 94% *ee*) after purification *via* column chromatography (pentane/ethyl acetate, 8:2).

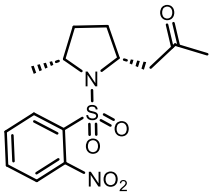
**$^1$H-NMR** (400 MHz, $CDCl_3$) δ 8.09-7.99 (m, 2H), 7.94-7.84 (m, 1H), 7.72-7.48 (m, 6H), 7.42-7.28 (m, 4H), 7.25-7.18 (m, 1H), 5.09-5.03 (m, 1H), 4.69-4.51 (m, 1H), 4.10 (dd, *J* = 17.1, 3.0 Hz, 1H), 3.27 (dd, *J* = 17.1, 10.5 Hz, 1H), 2.31-2.12 (m, 2H), 2.07-1.93 (m, 1H), 1.84-1.67 (m, 1H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 198.2, 141.8, 136.7,134.0, 133.6, 131.7, 131.4, 128.9, 128.6 (2C), 128.4, 127.5, 126.3, 124.1, 65.3, 58.6, 45.6, 34.6, 31.0.

**HRMS-ESI (m/z):** $C_{24}H_{22}N_2O_5S^+$ $[M+H]^+$ theoretical 451.1323, found 451.1324.

**7f** was recrystallised in isopropanol and submitted for single crystal X-ray diffraction.

**1-((2*R*,5*R*)-5-methyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)propan-2-one (8a)**



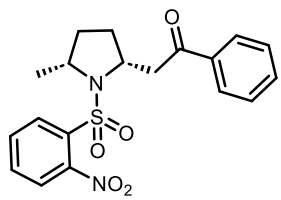
The synthesis of **8a** was carried out according to **GP4** with **6a**. The title compound was isolated as yellow oil (0.002 g, 2% yield, >99% *de*, 96% *ee*) after purification *via* column chromatography (pentane/ethyl acetate, 8:2).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 8.02-7.96 (m, 1H), 7.73-7.65 (m, 2H), 7.58-7.53 (m, 1H), 4.21-4.14 (m, 1H), 4.06-3.97 (m, 1H), 3.29 (dd, *J* = 17.6, 3.4 Hz, 1H), 2.65 (dd, *J* = 17.6, 9.7 Hz, 1H), 2.19 (s, 3H), 2.11-2.01 (m, 1H), 1.87-1.78 (m, 1H), 1.72-1.57 (m, 3H), 1.31 (d, *J* = 6.4 Hz, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 206.8, 148.9, 133.8, 131.5, 130.8, 123.9, 57.9, 57.4, 51.1, 32.0, 30.8, 30.6, 23.4.

**HRMS-ESI (m/z):** $C_{14}H_{18}N_2O_5S^+$ $[M+H]^+$ theoretical 327.1009, found 327.1010.

**2-((2*R*,5*R*)-5-methyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)-1-phenylethan-1-one (8b)**



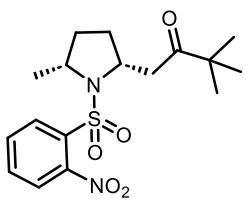
The synthesis of **8b** was carried out according to **GP4** with **6b**. The title compound was isolated as yellow oil (0.011 g, 18% yield, >99% *de*, 86% *ee*) after purification *via* column chromatography (pentane/ethyl acetate, 9:1 to 8:2).

**$^{1}$H-NMR** (500 MHz, $CDCl_3$) δ 8.03-7.98 (m, 3H), 7.73-7.66 (m, 2H), 7.61-7.54 (m, 2H), 7.52-7.46 (m, 2H), 4.43-4.36 (m, 1H), 4.08-3.99 (m, 1H), 3.88 (dd, *J* = 17.2, 3.1 Hz, 1H), 3.15 (dd, *J* = 17.2, 10.3 Hz, 1H), 2.16-2.07 (m, 1H), 1.93-1.85 (m, 1H), 1.79-1.71 (m, 1H), 1.66-1.60 (m, 1H), 1.37 (d, *J* = 6.4 Hz, 3H).

**$^{13}$C-NMR** (126 MHz, $CDCl_3$) δ 198.3, 136.6, 133.8, 133.6, 131.5, 131.3, 131.0 128.9, 128.3, 124.0, 58.4, 57.9, 46.7, 32.2, 30.8, 29.8, 23.4.

**HRMS-ESI (m/z):** $C_{19}H_{20}N_20_5S^+$ $[M+Na]^+$ theoretical 411.0986, found 411.0988.

**3,3-dimethyl-1-((2*R*,5*R*)-5-methyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)butan-2-one (8c)**



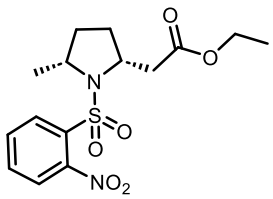
The synthesis of **8c** was carried out according to **GP4** with **6c**. The title compound was isolated as a colourless oil (0.018 g, 30% yield, 94% *de*, 94% *ee*) after purification *via* column chromatography (pentane/ethyl acetate, 95:5 to 8:2).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 8.05-7.95 (m, 1H), 7.74-7.63 (m, 2H), 7.58-7.49 (m, 1H), 4.23-4.13 (m, 1H), 4.08-3.96 (m, 1H), 3.32 (dd, *J* = 17.9, 3.1 Hz, 1H), 2.71 (dd, *J* = 17.9, 10.2 Hz, 1H), 2.10-1.98 (m, 1H), 1.89-1.78 (m, 1H), 1.62-1.50 (m, 3H), 1.34 (d, *J* = 6.4 Hz, 3H), 1.15 (s, 9H).

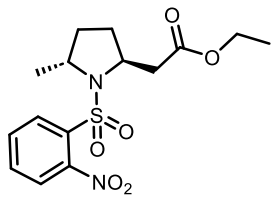
**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 214.4, 133.8, 131.5, 130.9, 123.9, 58.2, 57.9, 44.7, 32.1, 31.1, 26.4, 23.5.

**HRMS-ESI (m/z):** $C_{17}H_{24}N_2O_5S^+$ $[M+Na]^+$ theoretical 391.1299, found 391.1298.

**Ethyl 2-((2*R*,5*R*)-5-methyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)acetate (8d)**



*cis*-(**major**)



*trans*-(**minor**)

The synthesis of **8d** was carried out according to **GP4** with **6d**. The title compound was isolated as a colourless oil (0.003 g, 14% yield, 58% *de*) after purification *via* column chromatography (pentane/ethyl acetate, 8:2).

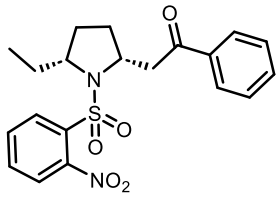
**$^{1}$H-NMR** (400 MHz, $CDCl_3$, *cis/trans* 79:21 *dr* – ratio was determined on the integration of H2 in *trans*-**23** and H5 in *cis*-**23**) δ 8.09-8.00 (m, 2H), 7.74-7.54 (m, 6H), 4.47-4.39 (m, 1H), 4.26-4.07 (m, 6H), 4.06-3.96 (m, 1H), 3.07 (dd, *J* = 15.9, 4.0 Hz, 1H), 2.91 (dd, *J* = 16.2, 3.4 Hz, 1H), 2.50 (dd, *J* = 16.2, 10.0 Hz, 1H), 2.38 (dd, *J* = 15.9, 10.7 Hz, 1H), 2.32-2.13 (m, 2H),

2.06-1.95 (m, 1H), 1.94-1.84 (m, 1H), 1.68-1.55 (m, 2H), 1.34 (d, *J* = 6.5 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 2H), 1.24 (t, *J* = 5.9 Hz, 2H), 1.13 (d, *J* = 6.4 Hz, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans* 79:21 *dr*) δ 171.1, 170.9, 135.8, 133.8, 133.6, 131.9, 131.5, 131.4, 131.1, 130.2, 124.4, 124.0, 60.8, 60.7, 58.2, 58.1, 58.0, 57.4, 42.0, 39.2, 32.1, 31.1, 30.6, 29.2, 23.4, 20.9, 14.3.

**HRMS-ESI (m/z):** $C_{15}H_{20}N_2O_6S^+$ $[M+H]^+$ theoretical 357.1115, found 357.1118.

**2-((2*R*,5*R*)-5-ethyl-1-((2-nitrophenyl)sulfonyl)pyrrolidin-2-yl)-1-phenylethan-1-one (8e)**



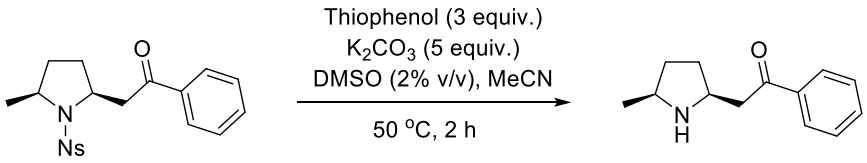
The synthesis of **7e** was carried out according to **GP4** with **5e**. The title compound was isolated as a yellow oil (0.032 g, 56% yield, >99% *de*, 96% *ee*) after purification *via* column chromatography (pentane/ethyl acetate, 9:1).

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 8.03-7.97 (m, 3H), 7.74-7.66 (m, 2H), 7.62-7.53 (m, 2H), 7.52-7.46 (m, 2H), 4.43-4.35 (m, 1H), 3.92-3.84 (m, 2H), 3.12 (dd, *J* = 17.3, 10.4 Hz, 1H), 2.18-2.08 (m, 1H), 1.95-1.83 (m, 1H), 1.82-1.65 (m, 3H), 1.59-1.47 (m, 1H), 0.97 (t, *J* = 7.5 Hz, 3H).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$) δ 198.3, 136.6, 133.8, 133.6, 131.5, 131.2, 131.0, 128.9, 128.3, 124.0, 63.6, 58.2, 46.6, 31.0, 29.9, 29.7, 10.7.

**HRMS-ESI** (m/z): $C_{20}H_{22}N_2O_5S^+$ $[M+H]^+$ theoretical 403.1323, found 403.1324.

## 8. Deprotection of 7b




*cis*/*trans* 96:4 *dr* *cis*/*trans* 64:36 *dr*

**7b** (0.017 g, 0.05 mmol) was dissolved in acetonitrile (1.5 mL) and DMSO (30 µL, 2% v/v) in a 2-neck round bottom flask (25 mL) with a stir bar. A sealed system was used where the round bottom flask was connected to 2 bubblers that contain a concentrated bleach solution. Potassium carbonate (0.026 g, 0.19 mmol) and thiophenol (16.6 µL, 0.162 mmol, 3 equiv.) were added to the solution and allowed to stir at 50 °C for 2 h. The reaction mixture was concentrated under reduced pressure to give a brown oil and was submitted for $^{1}$H-NMR spectroscopy.

**Major diastereoisomer (*cis*-5b):**

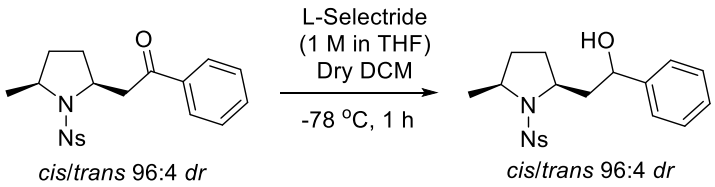
**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 7.98-7.92 (m, 2H, **Ar**H), 7.60-7.51 (m, 1H, **Ar**H), 7.45 (m, 2H, **Ar**H), 3.62-3.5 (m, 1H, H**2**), 3.24-3.19 (m, 3H, H**5**, H**7**), 2.03-1.95 (m, 1H, H**3**), 1.93-1.86 (m, 1H, H**4**), 1.54-1.46 (m, 1H, H**3**), 1.39-1.32 (m, 1H, H**4**), 1.17 (d, *J* = 6.5 Hz, 3H, H**6**).

**Minor diastereoisomer (*trans*-5b):**

**$^{1}$H-NMR** (400 MHz, $CDCl_3$) δ 7.98-7.92 (m, 2H, **Ar**H'), 7.60-7.51 (m, 1H, **Ar**H'), 7.45 (m, 2H, **Ar**H'), 3.88-3.82 (m, 1H, H**2'**), 3.36-3.32 (m, 1H, H**5'**), 3.19 (dd, *J* = 16.8, 7.6 Hz, 1H, H**7'**), 3.12 (dd, *J* = 16.8, 5.3 Hz, 1H, H**7'**), 2.15-2.09 (m, 1H, H**3'**), 2.04-1.98 (m, 1H, H**4'**), 1.52-1.45 (m, 1H, H**3'**), 1.38-1.31 (m, 1H, H**4'**), 1.18 (d, *J* = 6.3 Hz, 3H, H**6'**).

**$^{13}$C-NMR** (101 MHz, $CDCl_3$, *cis/trans* 63:37 *dr*) δ 199.8 (2C), 137.3, 137.2, 133.2, 133.1, 128.7 (2C), 128.2, 128.2, 54.6, 54.1, 53.7, 53.4, 46.1, 45.7, 34.3, 32.6, 32.4, 31.1, 22.1, 21.8.

## 9. Reduction of 7b to S7




*cis*/*trans* 96:4 *dr* *cis*/*trans* 96:4 *dr*

*cis*-**7b** (0.023 g, 0.06 mmol) was dissolved in dry DCM (1 mL) in a flame-dried 2-neck round bottom flask (25 mL) with a stir bar. The solution was cooled to -78 °C in a dry ice-acetone bath before L-Selectride® (0.1 mL, 1 M in THF) was added to it. The solution was allowed to stir at -78 °C for 1 h and then saturated ammonium chloride solution (0.5 mL) was added dropwise to the reaction mixture at room temperature. The aqueous solution was extracted with DCM (3 × 5 mL). The organic extracts were combined, dried with $Na_2SO_4$, filtered, and concentrated under reduced pressure to give a yellow oil (0.018 g, 75% yield). The crude product was dry loaded onto celite and purified by column chromatography (cyclohexane/ethyl acetate, 7:3 to 6:4) to give compound **S7** as a yellow oil (0.008 g, 33% yield, a mixture of stereoisomers).

**$^1$H-NMR** (400 MHz, $CDCl_3$, a mixture of stereoisomers 1:2 *dr* – ratio was determined on the integration of H8 in each isomer) δ 7.99-7.87 (m, 3H), 7.78-7.47 (m, 10H), 7.45-7.29 (m, 15H), 5.02 (d, *J* = 10.3 Hz, 1H), 4.79 (d, *J* = 10.0 Hz, 2H), 4.32-4.24 (m, 1H), 4.14-4.06 (m, 2H), 4.06-3.97 (m, 2H), 3.96-3.86 (m, 1H), 2.51-2.44 (m, 2H), 1.92-1.76 (m, 11H), 1.73-1.52 (m, 10H), 1.44 (d, *J* = 6.3, 3H), 1.35 (d, *J* = 6.4, 6H).

**$^{13}$C NMR** (101 MHz, $CDCl_3$, a mixture of stereoisomers 1:2 *dr*) δ 144.4, 144.3, 133.9, 133.6, 131.5, 131.5, 131.4, 131.3, 131.2, 131.2, 128.7, 128.6, 127.9, 127.5, 125.9, 125.9, 124.0, 123.9, 72.4, 70.5, 59.6, 59.5, 57.9, 57.6, 46.7, 46.4, 32.3, 32.2, 31.2, 30.4, 24.2, 23.5.

**HRMS-ESI (m/z):** $C_{19}H_{22}N_20_5S^+$ $[M+Na]^+$ theoretical 413.1142, found 413.1143.

**S7** (0.017 g, 0.05 mmol) was deprotected as described for the *N*-Ns deprotection of compound **7b** to give compound **S8**. Crude $^1$H-NMR analysis (A127) showed no change in ratio of diastereoisomers after *N*-Ns deprotection. Compound **S8** decomposed during attempted purification on Silica.

## 10. Epimerisation attempts of 5a and 5b

**5a** was dissolved in deuterated methanol and the ratio of isomers was monitored using $^{1}$H-NMR spectroscopy at different time intervals (***Figure S3***).

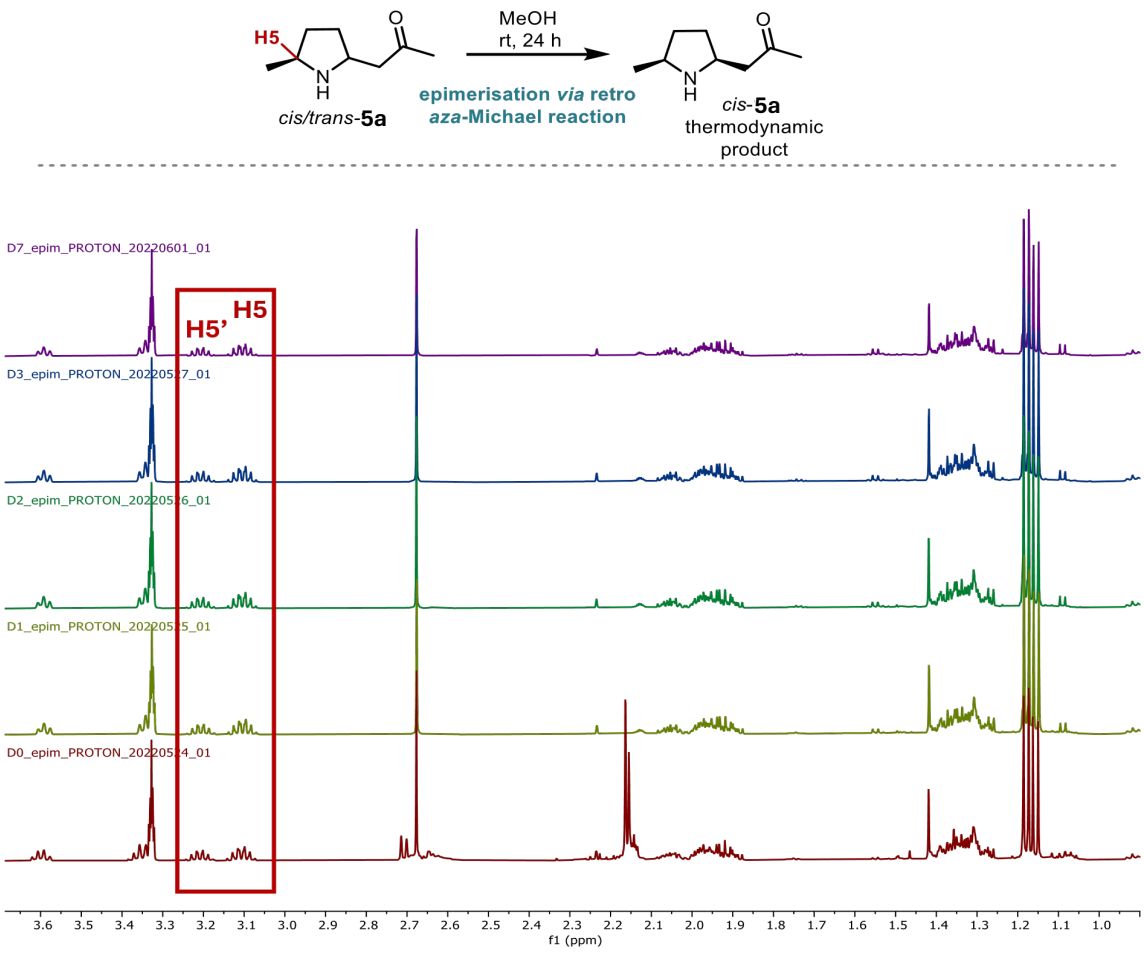



***Figure S3*** Stacked $^{1}$H-NMR spectra of the epimerisation attempt of **5a** over 7 days shows no change was observed in the ratio of H5 and H5'.

The multiplets between 3.23 and 3.07 ppm in **Figure S3**, which corresponded to H5' and H5 in *trans*-**5a** and *cis*-**5a** respectfully, were monitored over the time-course experiment. Across the time intervals, it was expected that the ratio of the multiplets would adjust until only one remained; however, no change in the ratio of the highlighted multiplets was observed. However we do observe complete deuterium exchange of the α-protons in **5a** after 24 hours in deuterated methanol (**Figure S4**). This observation could suggest that the forward and reverse *aza* Michael is taking place, and has reached its ratio equilibrium at the cis/trans ratio 62:38.

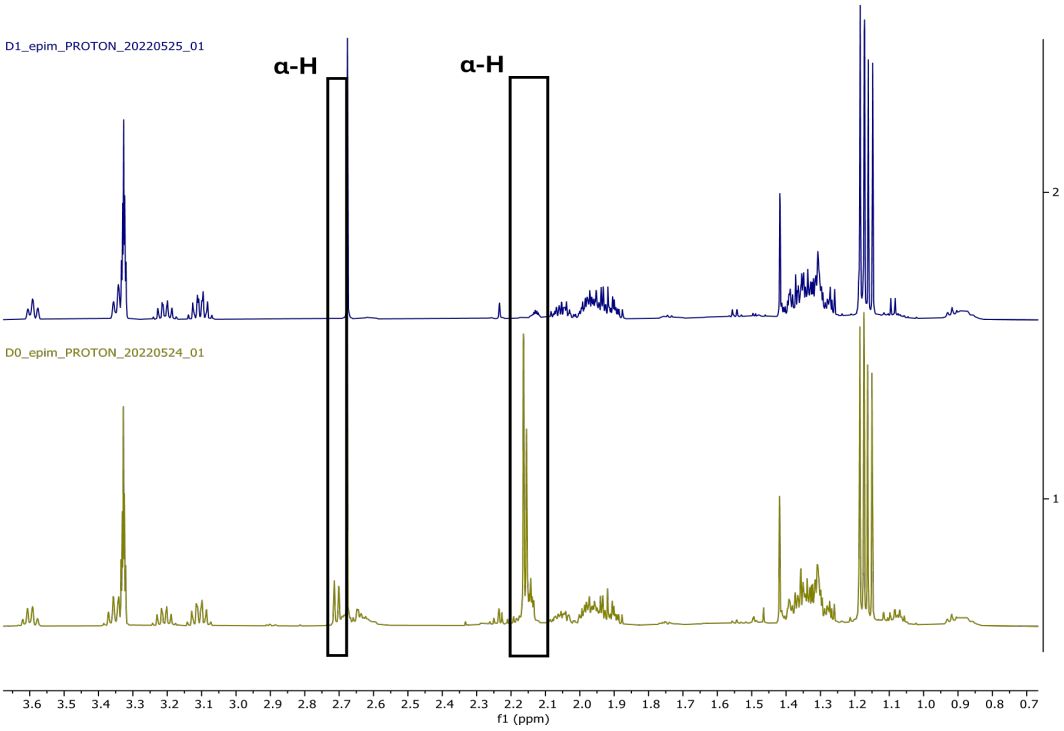
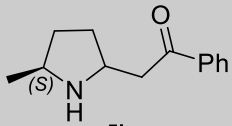
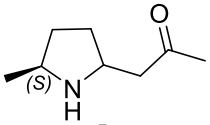
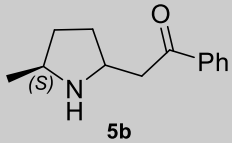
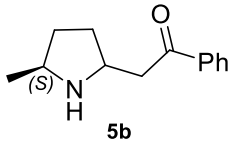
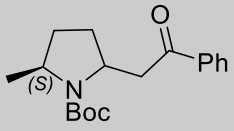



***Figure S4*** Stacked $^{1}$H-NMR spectra showed the disappearance of the α-protons in **5a** after stirring in methanol for 24 h. This observation was due to H/D exchange by keto-enol tautomerisation.

**Table S1. Epimerisation attempts 5a and 5b**

| Entry | Substrate | Solvent | Temp. (°C) | Time | Base | Change in *dr* |
|---|---|---|---|---|---|---|
| 1 |  **5b** *cis/trans*, 64:36 *dr* | methanol | room temp. | 24 h | - | No change |
| 2 |  **5a** *cis/trans*, 62:38 *dr* | Deuterated methanol | room temp. | 7 d | - | No change[a] |
| 3 |  **5b** *cis/trans*, 64:36 *dr* | methanol | reflux | 24 h | - | Decomp. |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 |  **5b** *cis/trans*, 64:36 *dr* | DMF/THF (2:1) | -78 °C | 1 h | KO*t*Bu | Decomp. |
| 5 |  *N*-Boc **5b** *cis/trans*, 64:36 *dr* | DMF/THF (2:1) | -78 °C | 1 h | KO*t*Bu | No change |

## 11. Epimerisation attempts of 7b

**Table S2. Epimerisation attempts 7b**

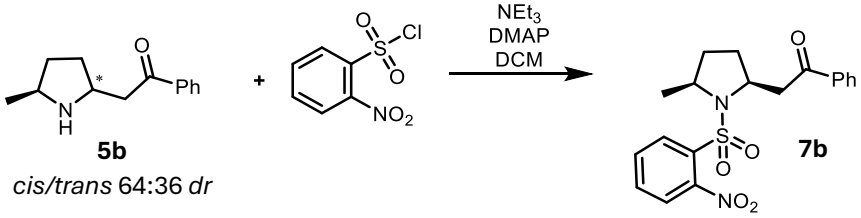
| Entry | 7b (starting *dr*) | Solvent | Temp. (°C) | Time | Nosyl chloride | Catalyst | *dr* |
|---|---|---|---|---|---|---|---|
| 1 | 80:20 | MeOH | room temp. | 6 h | - | - | 80:20 |
| 2 | 80:20 | MeOH | reflux | 6 h | - | - | 80:20 |
| 3 | 80:20 | MeOH | room temp. | 6 h | 10 equiv. | - | 93:7* |
| 4 | 80:20 | MeOH | reflux | 6 h | 10 equiv. | - | 92:8* |
| 5 | 83:17 | DCM | room temp. | 24 h | - | DMAP (1 equiv.) | 83:17 |
| 6 | 80:20 | DCM | -78 °C | 6 h | - | - | 80:20 |
| 7 | 80:20 | MeOH.HCl | room temp. | 12 h | - | - | 80:20 |

***** significant decomposition was observed.

[a] no nosyl chloride was used in entries 1-2 or 5-7.

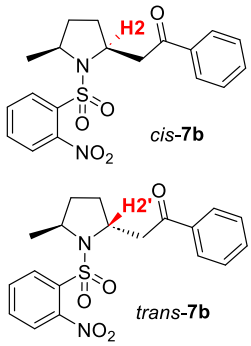
[b] no catalyst was used in entries 1-4 or 6-7.

## 12. Optimisation of the stereoselective nosylation (GP4)




The synthesis of **7b** was carried out according to **GP4** with **5b**, except the reaction was carried out at the stated temperature (**Figure S5**).

a.)




b.) **Table S3**

| Entry | Temp. (°C) | 7b *cis/trans dr* |
|---|---|---|
| 1 | room temp. | 80:20 |
| 2 | 0 | 80:20 |
| 3 | -20 | 80:20 |
| 4 | -78 | 96:4 |

c.)

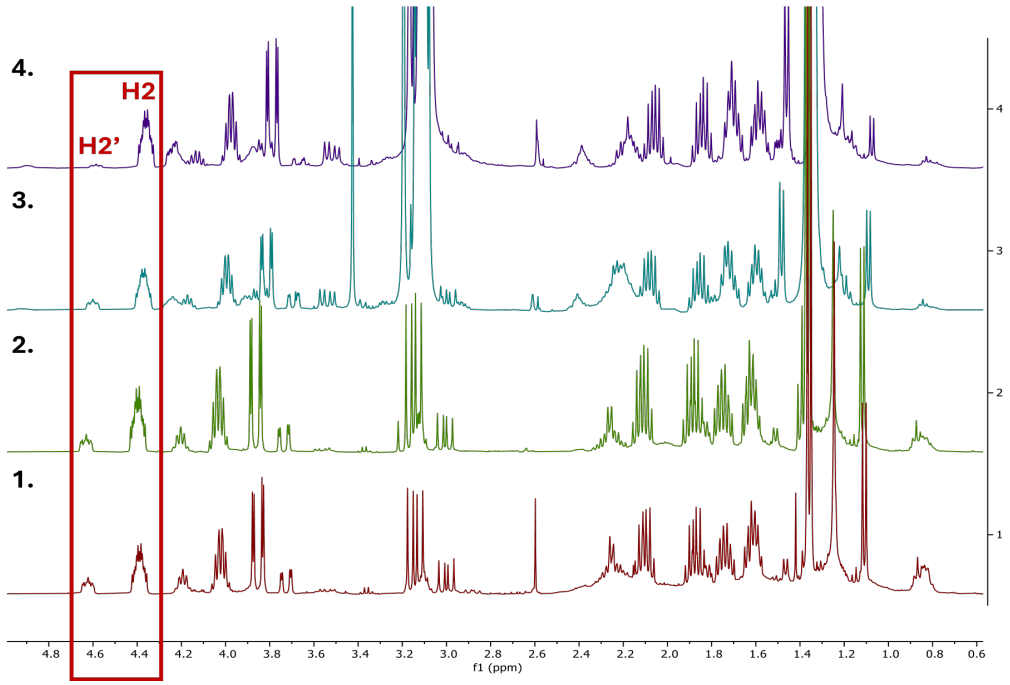



***Figure S5 a.)*** The structure of *cis*- and *trans*-**7b** compounds following the protection of **5b**. ***b.)*** **Table 3.3.** Comparison of *dr* values following the protection of **5b** at a range of temperatures. ***c.)*** The stacked crude $^{1}$H-NMR spectra of the reactions described in **Table 3.3.** Spectra **3.** and **4.** contain triethylamine peaks, which overlap with some diastereotopic protons (3.2-3.0 ppm), as no acidic work-up was carried out prior to $^{1}$H-NMR analysis in order to get a complete insight to the progress of the reaction.

A crude NMR study of the *N*-nosylation reaction in $CDCl_3$ (aliquots removed at time points 0, 2min, 10 min, 20 mins) showed that the protection step was extremely fast, and completed within two minutes at room temperature (**Figure S6**). At time zero (prior to the addition of 2-nosyl chloride) the NMR analysis showed that the *cis*/*trans* ratio of model compound **5b** was approx. 6:4 at room temperature.

## 13. Mass balance discussion

It was also noted that at time zero (prior to the addition of 2-nosyl chloride) the concentration of **5b** was less than initially measured by weight of purified **5b** from the biotransformation (**Figure S6**). This observation was determined on comparing the integration of **5b** protons with peaks that correspond to triethylamine. This observation was verified by the addition of 1,3,5 trimethoxybenzene (1,3,5-TMB) (a known internal standard (1 equiv.)) to the sample and by analysing it by quantitative $^{1}$H-NMR spectroscopy in $CDCl_3$ (**Figure S7**). This analysis showed that the quantity of **5b** at time zero was 50% less than initially measured by weight, despite $^{1}$H NMR and $^{13}$C-NMR spectroscopy, and HRMS indicating that the **5b** starting material had been successfully purified. As experienced previously, these disubstituted pyrrolidines are quite unstable and it was likely that the loss of starting material in the reaction mixture was due to some kind of non-stereoselective decomposition. Once the protection of **5b** was complete, a 48% NMR yield (1,3,5-trimethoxybenzene was used as the internal standard) was measured and compound **7b** was isolated with a 48% yield after purification by column chromatography. If the altered substrate concentration measured at time zero was taken into consideration compound **7b** was formed in a 96% conversion (**Scheme S1**), which would support the proposed mechanism of stereoselective nosylation during retro *aza* Michael epimerisation. These results also suggest that the mass balance issue is not connected to the nosylation reaction itself.

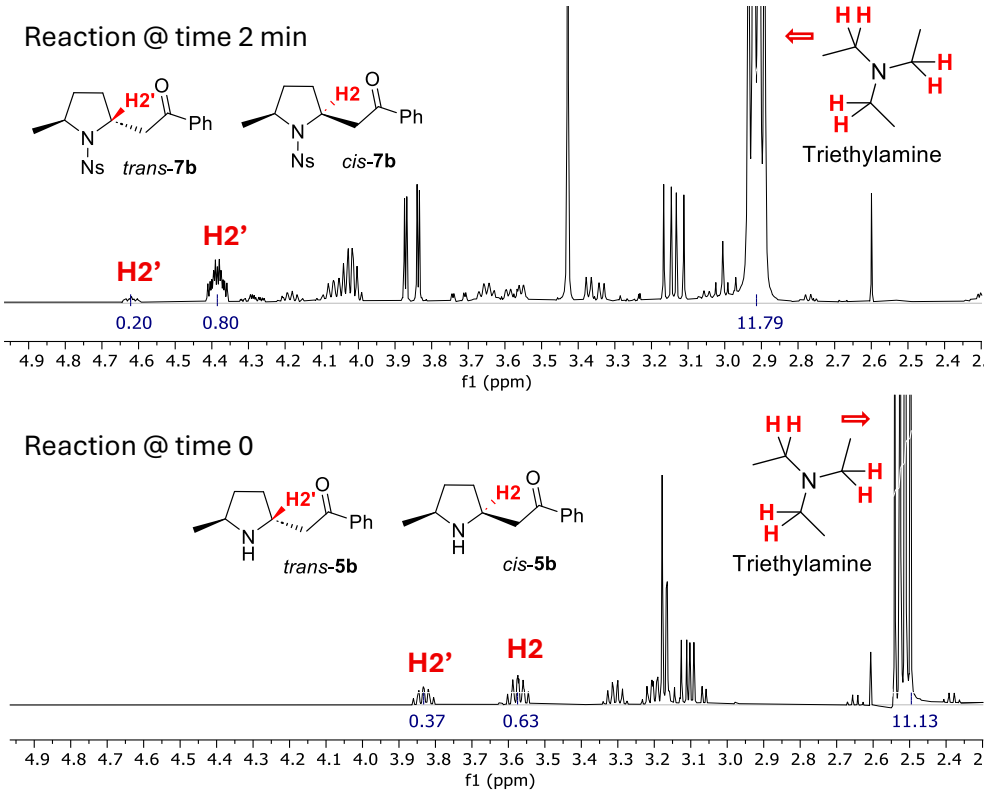



**Figure S6**. The nosylation reaction complete within 2 minutes and the *cis/trans* ratio converts from 63:37 (**5b**) to 80:20 (**7b**) at room temp. 1 equiv. of triethylamine should integrate 1:6 and not 1:~11, however, the ratio between pyrrolidines (**5b**/**7b**) and triethylamine indicates ~95% conversion.

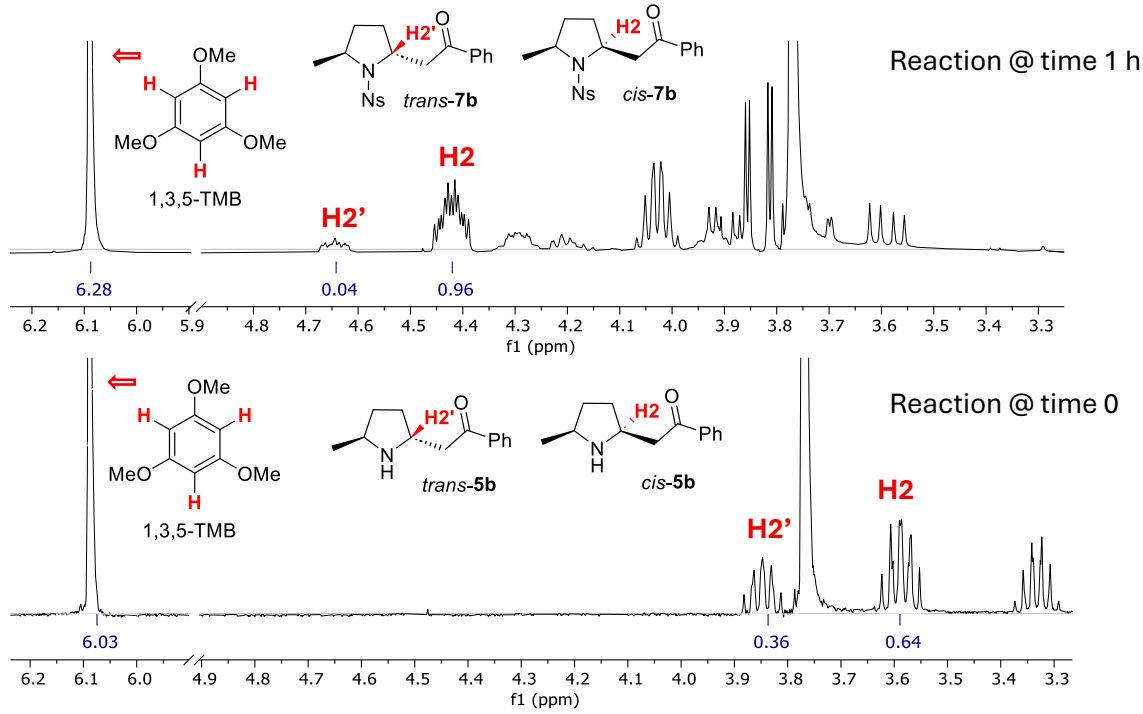
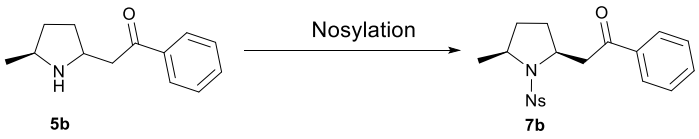



**Figure S7.** Nosylation at -78 °C with "1 equiv." of 1,3,5-TMB at time zero shows the presence of ~50% starting material **5b**. Crude NMR analysis indicates 96% conversion of **5b** to **7b**.




**5b** **7b**

-> [**5b**] = 93.3 mM
(based on the weight of **5b** added to solvent)
-> q-$^1$H-NMR [**5b**] = 46.7 mM

-> 48% NMR yield (44.8 mM)
-> 48% isolated yield
-> 96% adjusted NMR yield (conversion based on initial [**5b**])

**Scheme S1** An investigation into the nosylation of **5b** revealed that there was a discrepancy in the concentration of the model substrate prior to the addition of 2-nosyl chloride (NMR data in Figure S7). When this was taken into consideration the NMR yield/conversion was adjusted to 96%.

## 14. X-Ray Data

Single crystals of $C_{24}H_{22}N_2O_5S$ (**7f**) were recrystallised in isopropanol and appeared as yellow/brown blocks of approximately 0.19 × 0.7 × 0.05 mm. A suitable crystal was selected and mounted on a MiTeGen micromount with NVH immersion oil before measuring on a SuperNova, Dual source diffractometer, operating with Cu at UCD. The crystal was kept at 122(20) K during data collection. Using Olex2,[8] the structure was solved with the SHELXT structure solution program using Intrinsic Phasing and refined with the SHELXL refinement package using Least Squares minimisation.[9, 10]

**Structure Table**

| | |
|---|---|
| Empirical formula | $C_{24}H_{22}N_2O_5S$ |
| Formula weight | 450.49 |
| Temperature (K) | 122(20) |
| Crystal system | orthorhombic |
| Space group | P212121 |
| a (Å) | 7.63070(10) |
| b (Å) | 15.0624(2) |
| c (Å) | 18.5993(3) |
| α (°) | 90 |
| β (°) | 90 |
| γ (°) | 90 |
| Volume (Å3) | 2137.74(5) |

| | |
|---|---|
| Z | 4 |
| $\rho_{calc}$ (g/cm3) | 1.400 |
| μ (mm-1) | 1.686 |
| F (000) | 944.0 |
| Crystal size ($mm^3$) | 0.19 × 0.07 × 0.05 |
| Radiation | Cu Kα (λ = 1.54184) |
| 2Θ range for data collection (°) | 7.552 to 153.476 |
| Index ranges | -9 ≤ h ≤ 9, -18 ≤ k ≤ 18, -23 ≤ l ≤ 23 |
| Reflections collected | 31365 |
| Independent reflections | 4478<br>$R_{int}$ = 0.0620<br>$R_{sigma}$ = 0.0322] |
| Data/restraints/parameters | 4478/0/289 |
| Goodness-of-fit on F2 | 1.047 |
| Final R indexes<br>(I≥2σ (I))<br>Final R indexes (<br>all data)<br>Largest diff. peak/hole (e $Å^{-3}$) | $R_1$ = 0.0352<br>$wR_2$ = 0.0867<br>$R_1$ = 0.0414<br>$wR_2$ = 0.0910<br>0.18/-0.36 |
| Flack parameter | -0.008(8) |

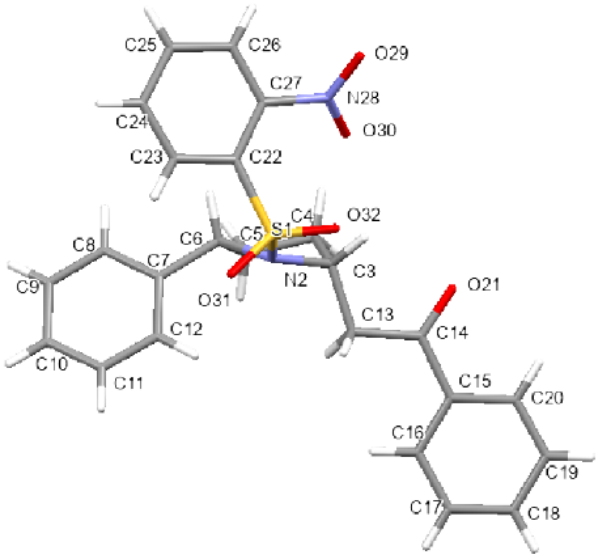
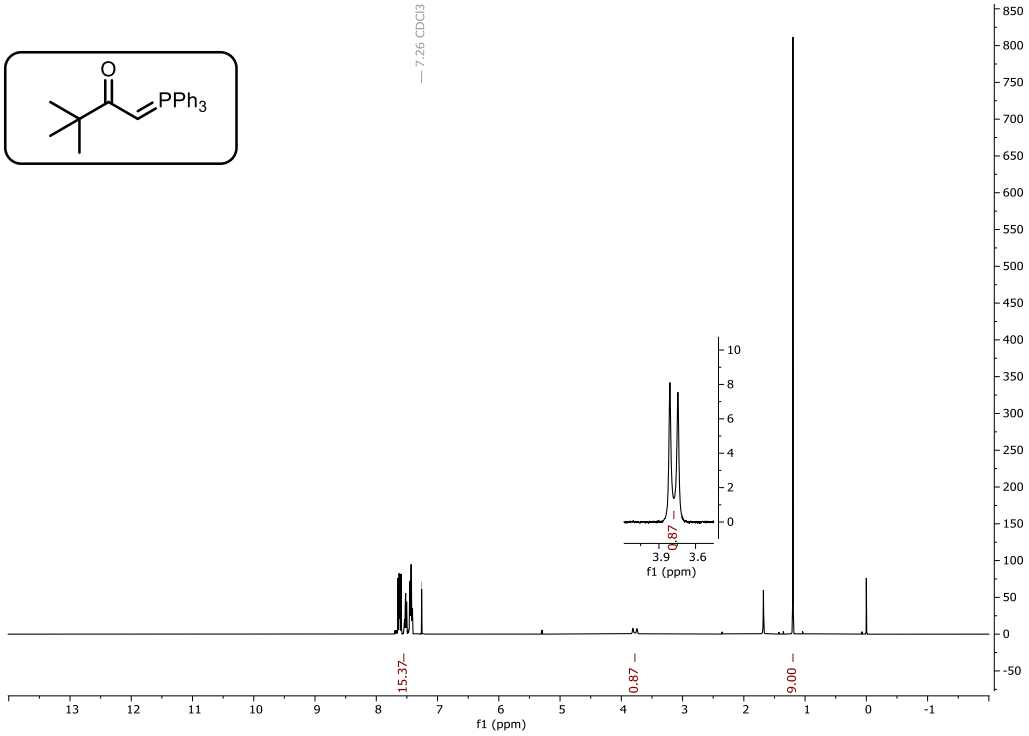


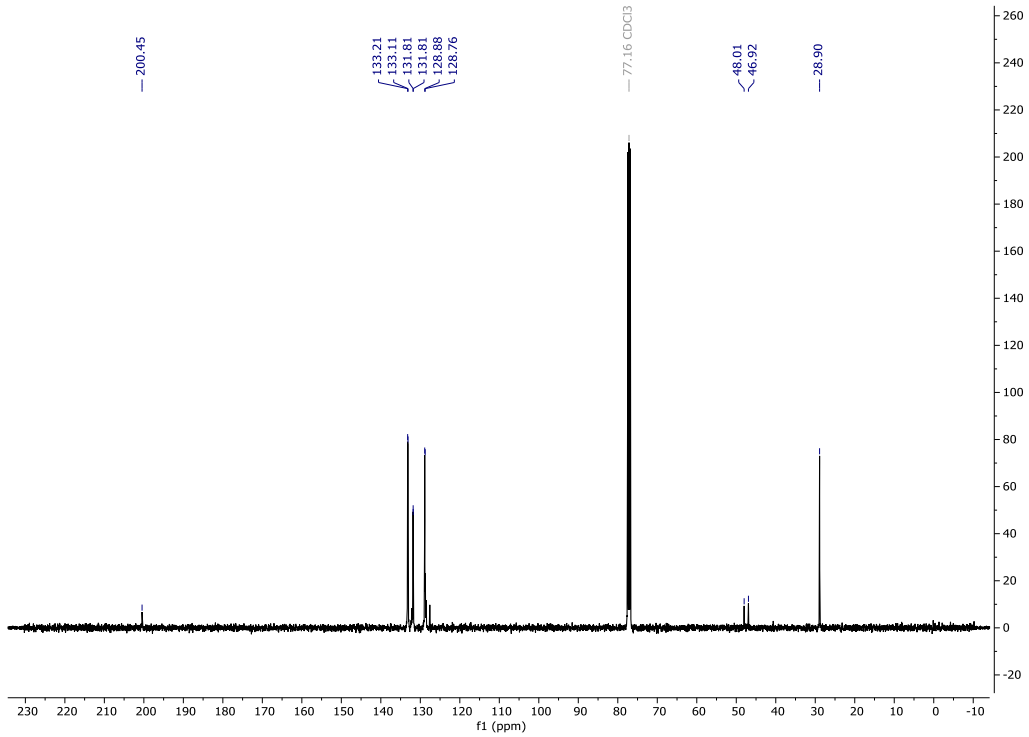

***Figure S8.*** Fully labelled molecular structure of **7f**.

The X-ray data was deposited by Dr Christopher Thomas with ref number CCDC 2506209.

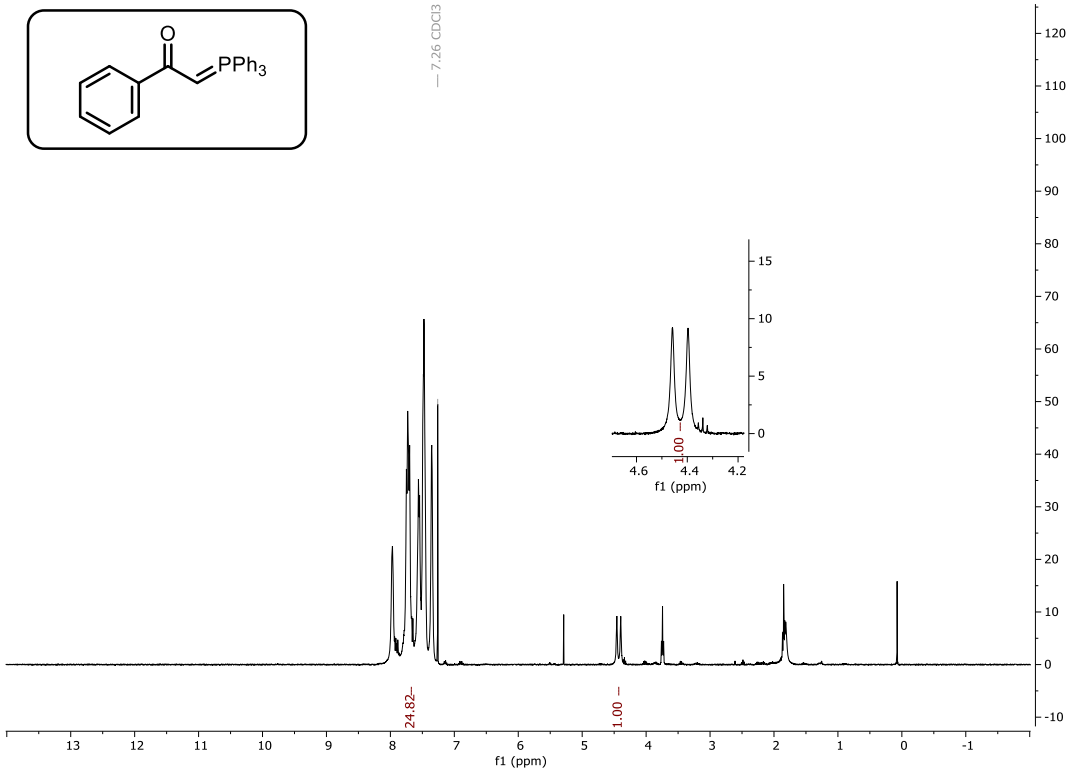
## 15. NMR Characterisation



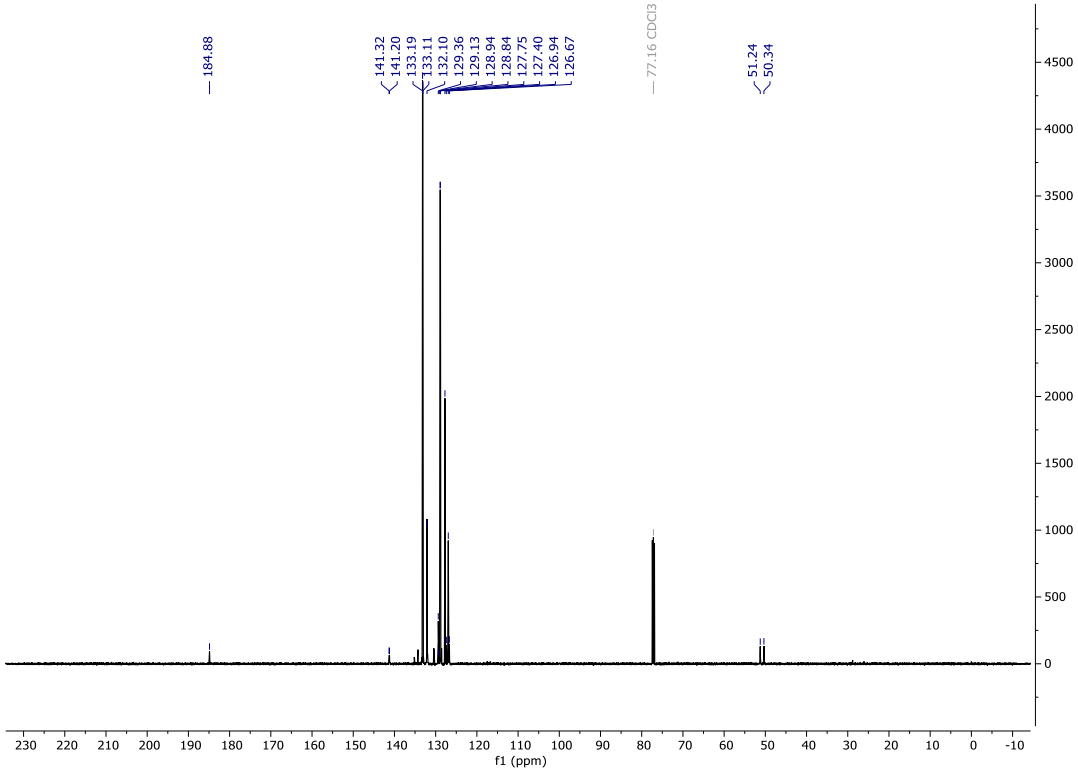

**A1:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **S1.**



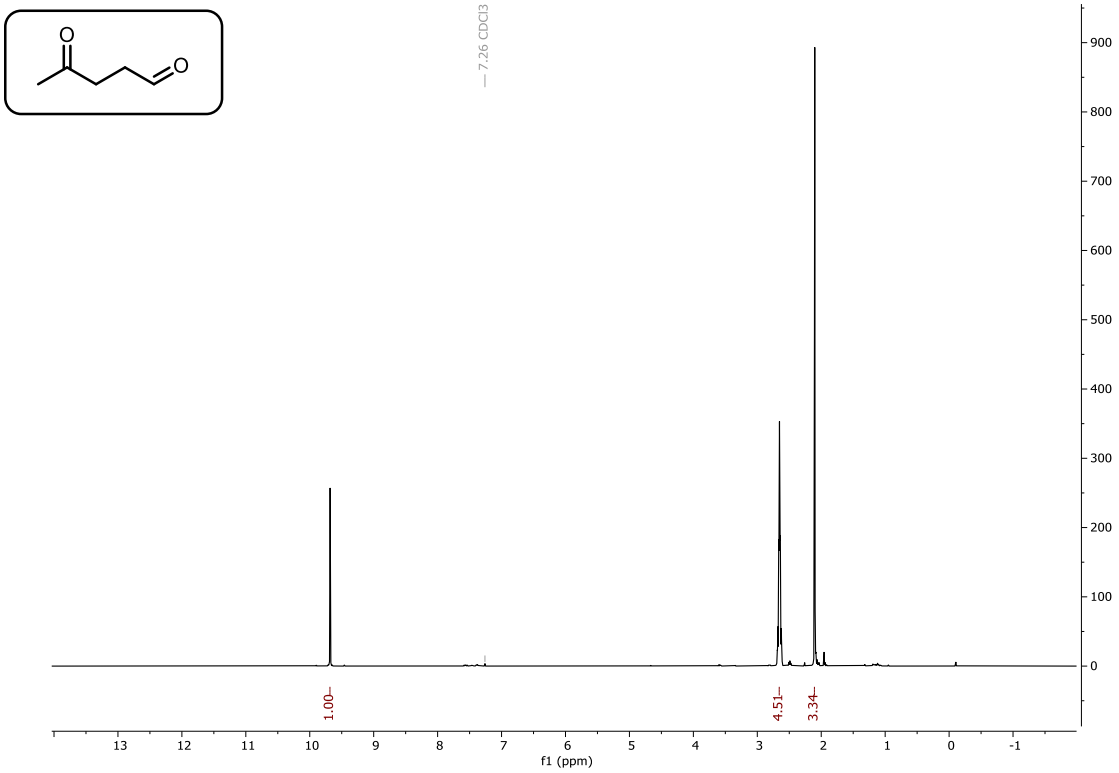

**A2:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **S1.**



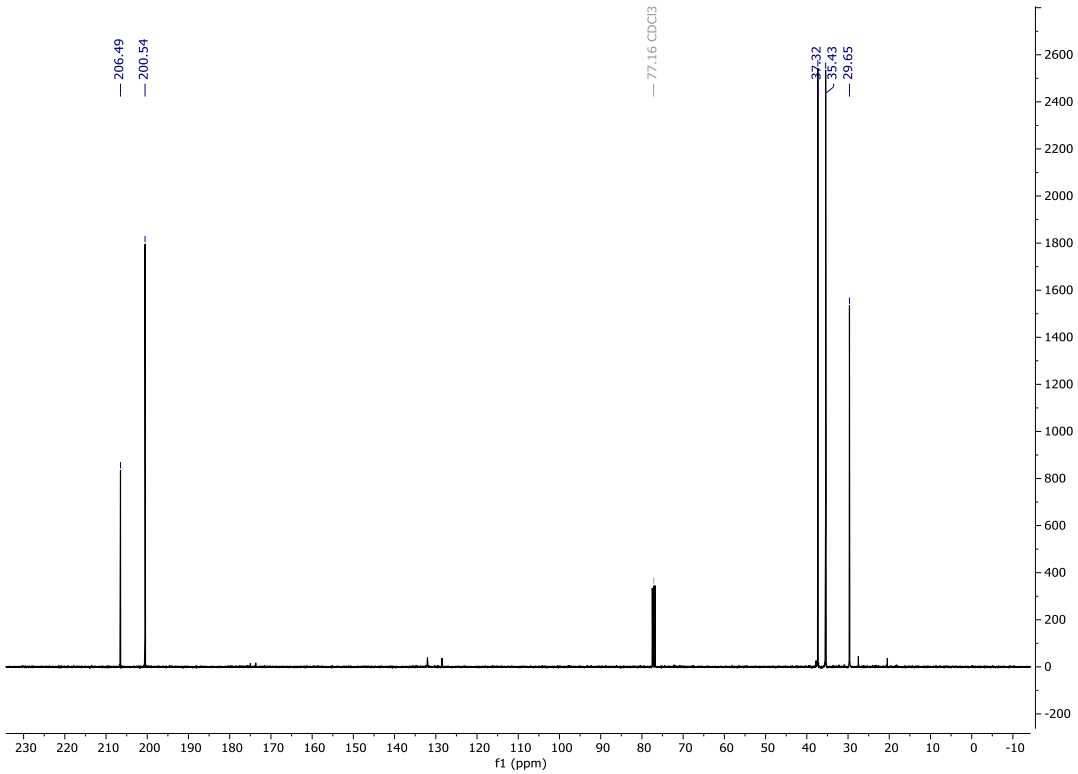

**A3:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **S2.**



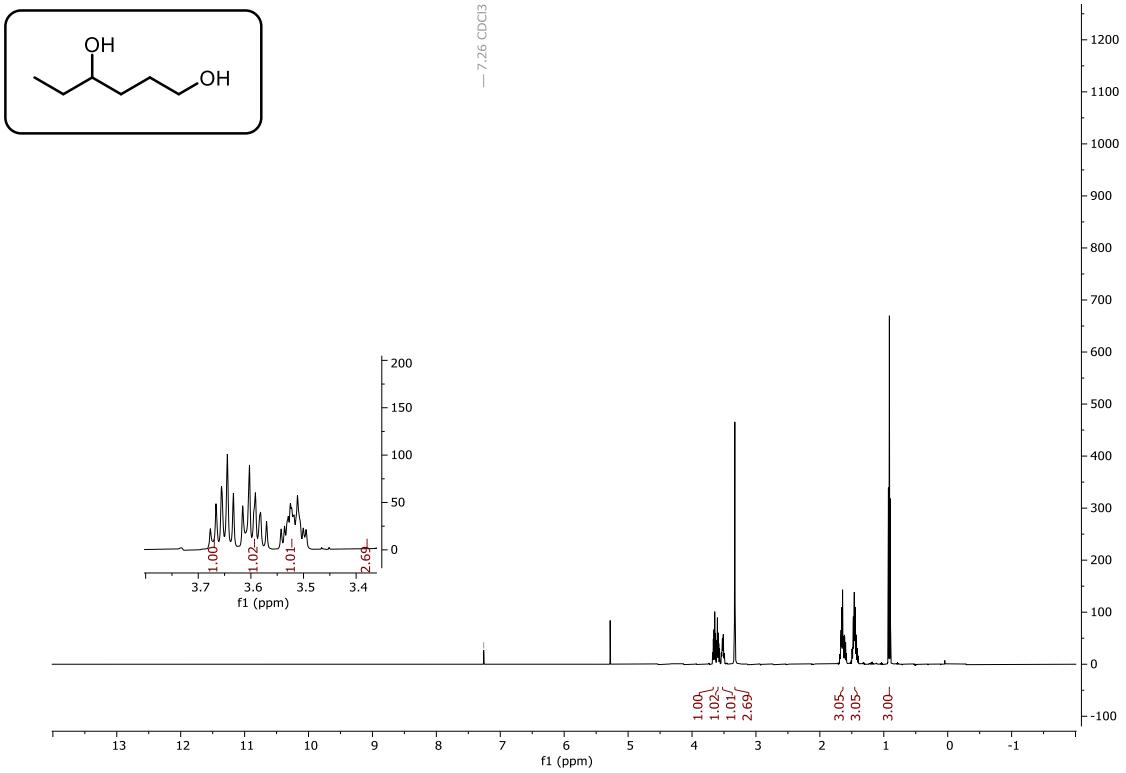

**A4:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **S2.**



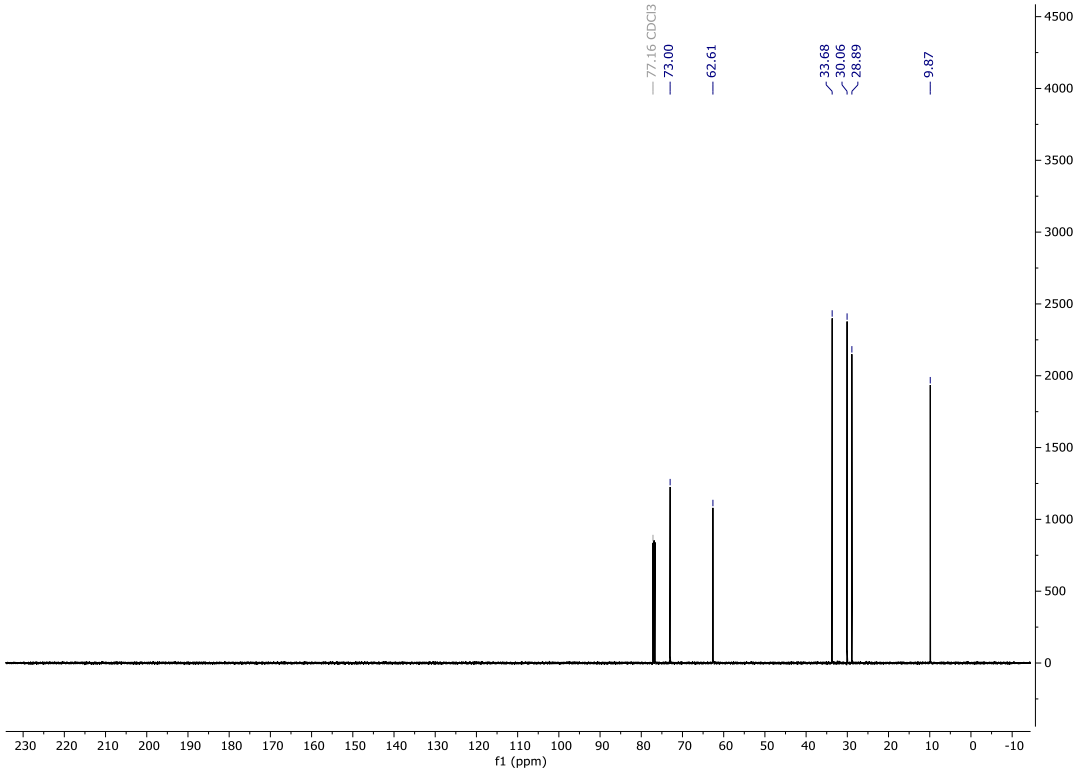

**A5:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **2.**



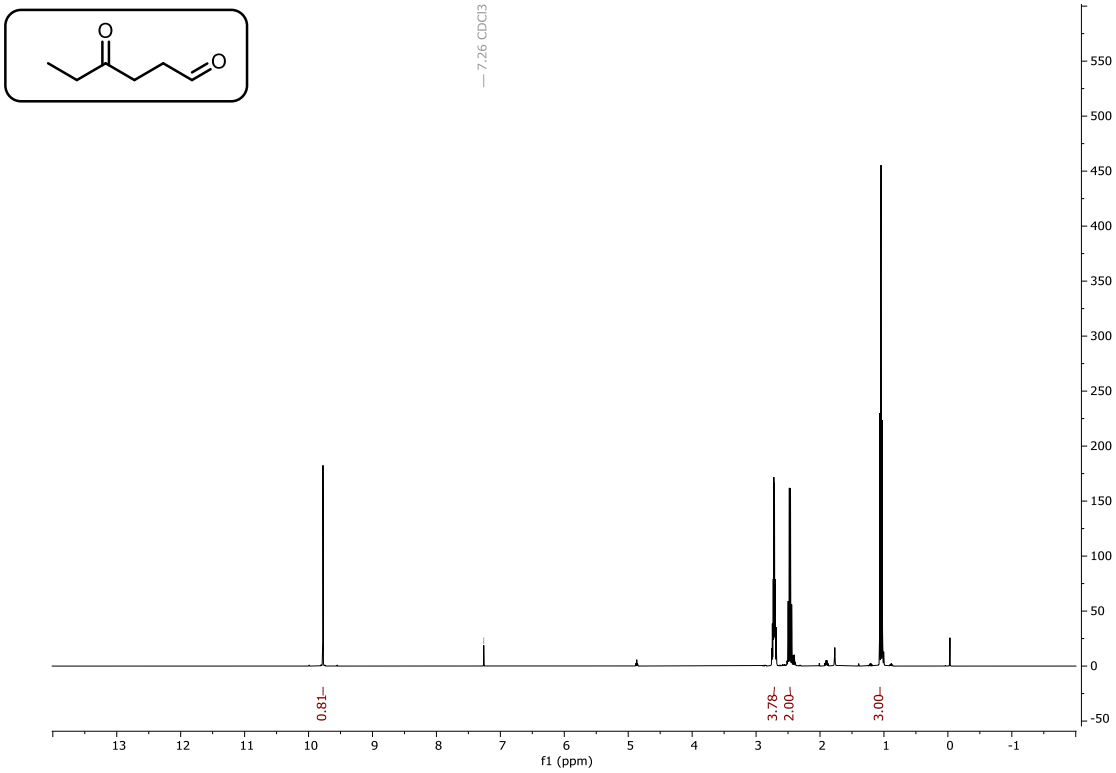

**A6:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **2.**



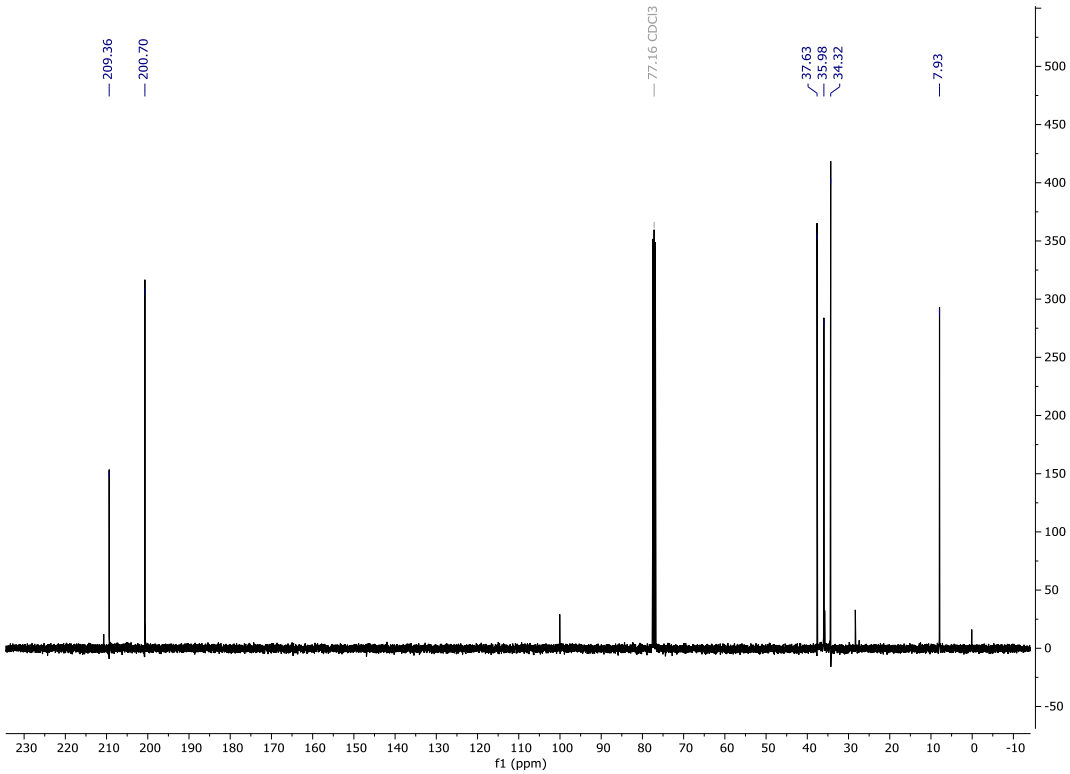

**A7:** $^{1}$H-NMR (500 MHz, $CDCl_3$) of compound **S3.**



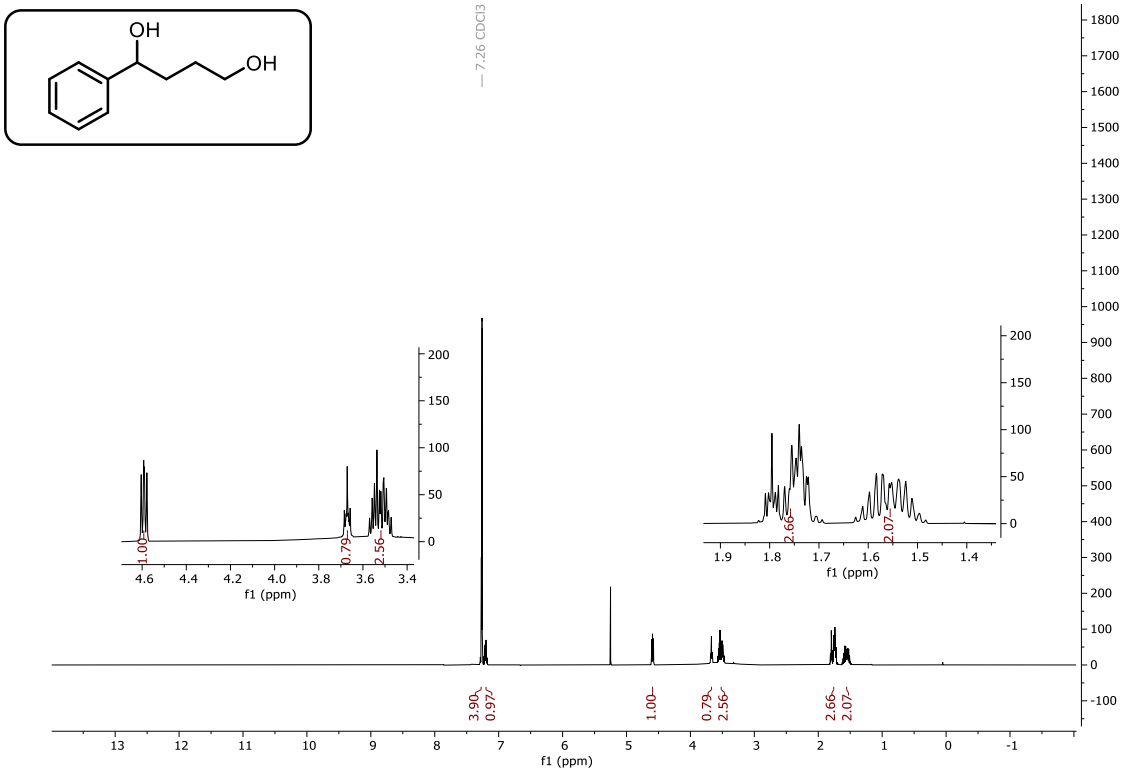

**A8:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **S3.**



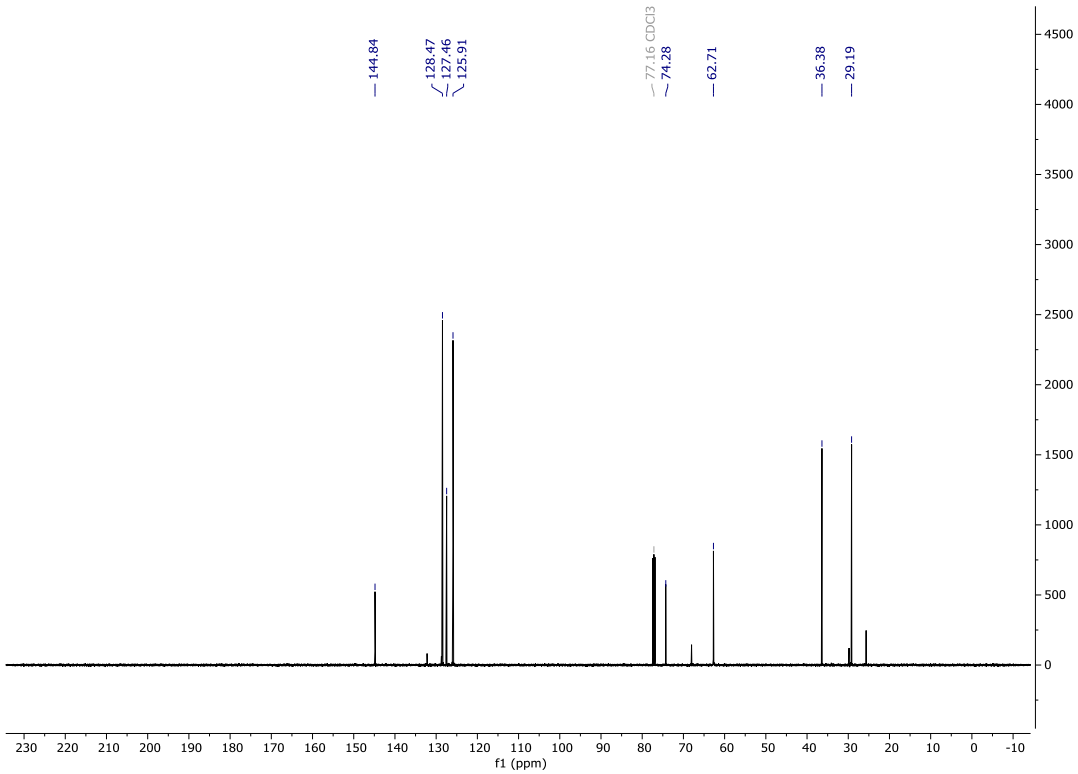

**A9:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **S4.**



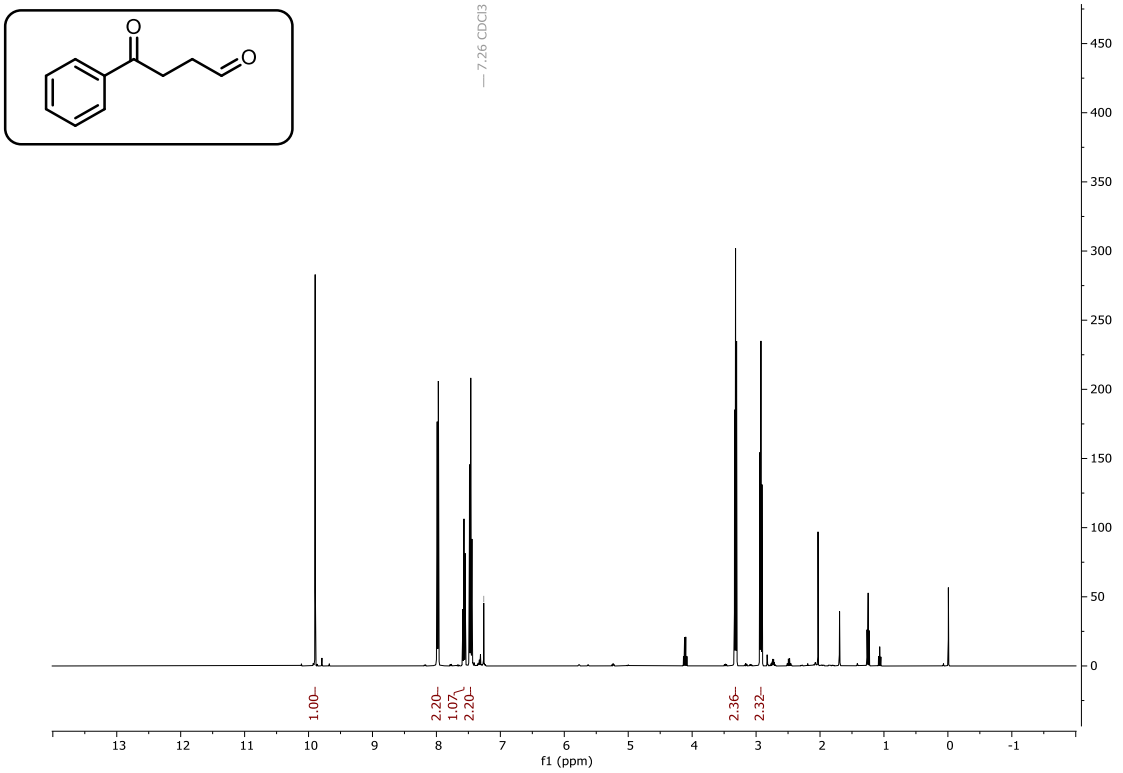

**A10:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **S4.**



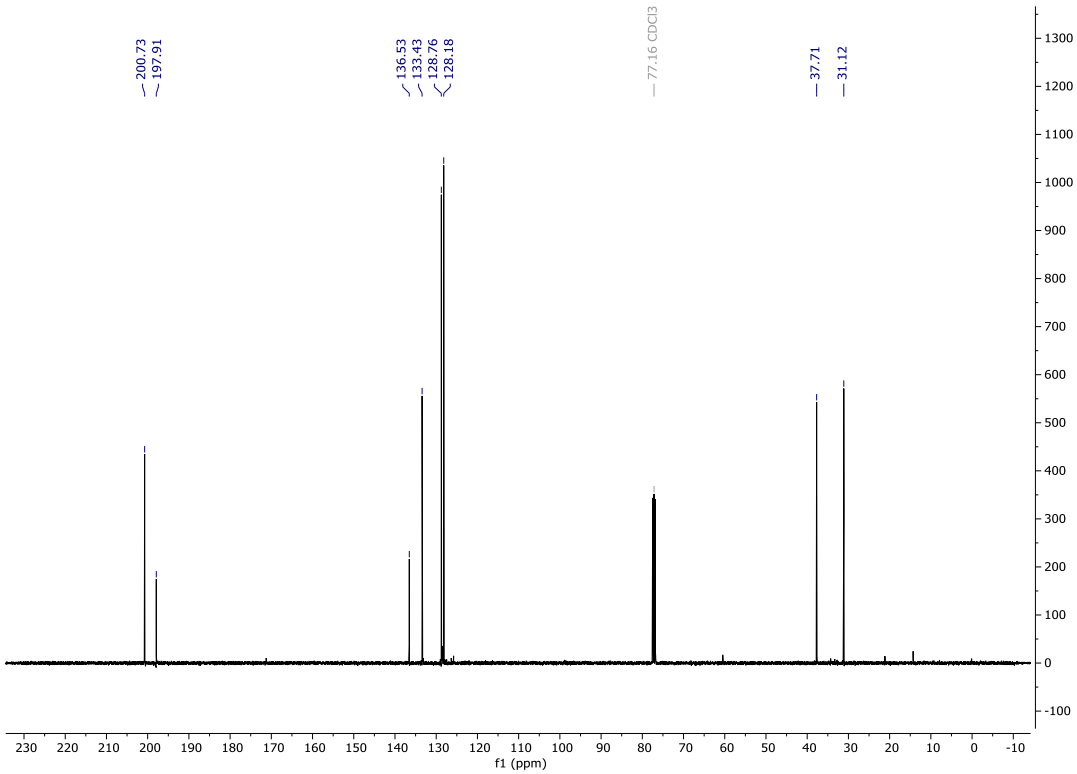

**A11:** $^{1}$H-NMR (500 MHz, $CDCl_3$) of compound **S5.**



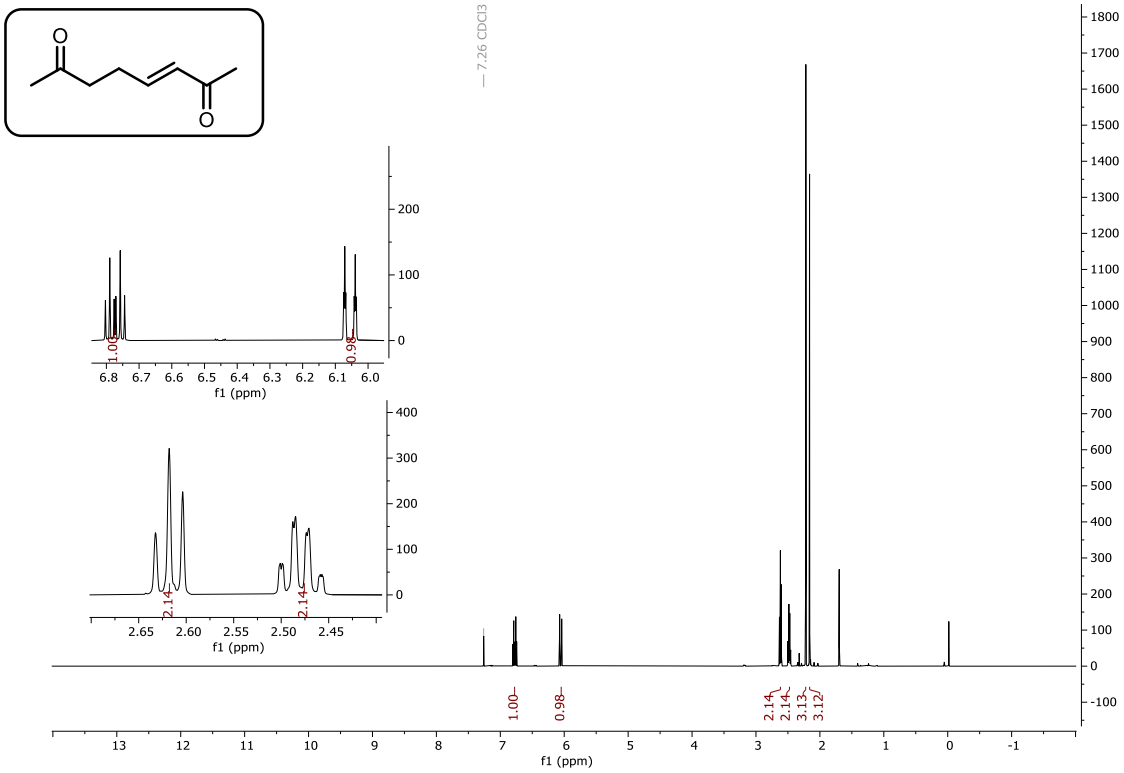

**A12:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **S5.**



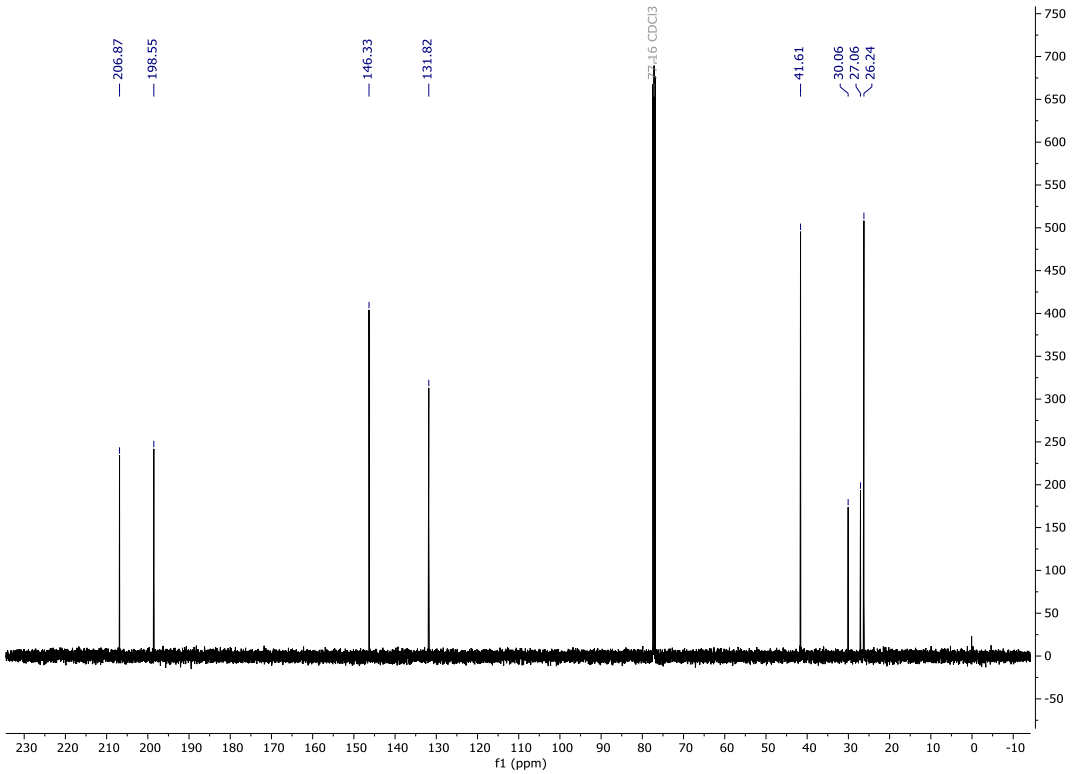

**A13:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **S6.**



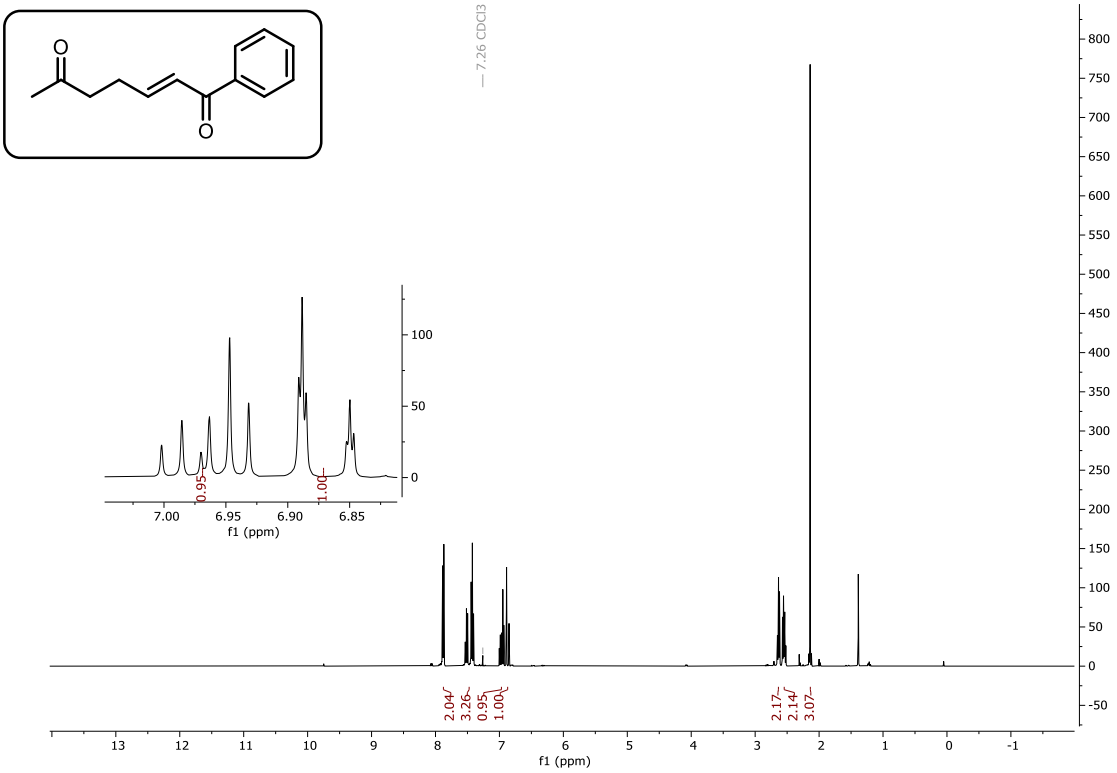

**A14:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **S6.**



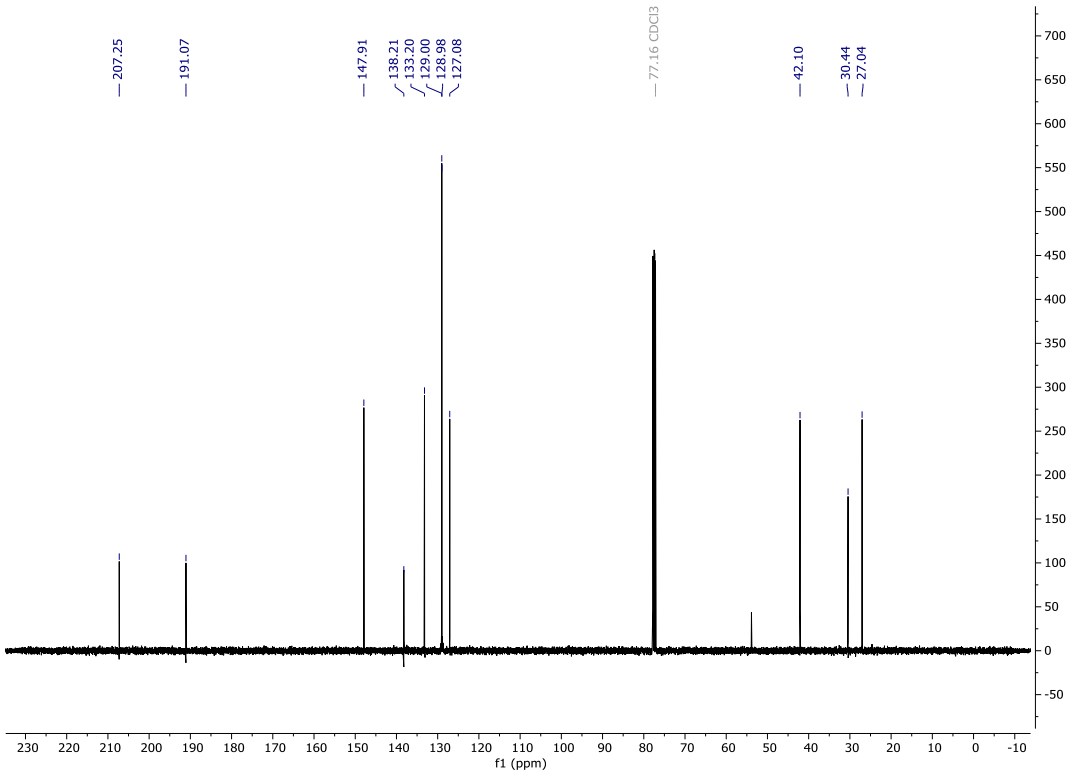

**A15:** $^{1}$H-NMR (500 MHz, $CDCl_3$) of compound **S7.**



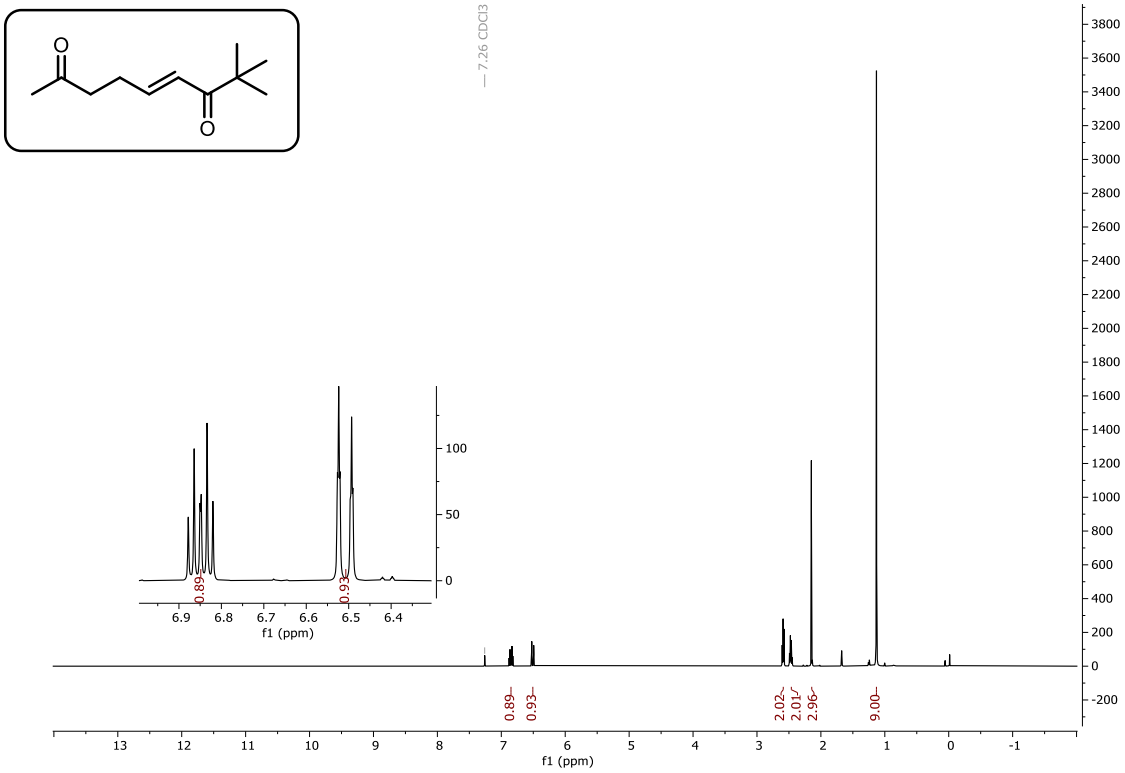

**A16:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **S7.**



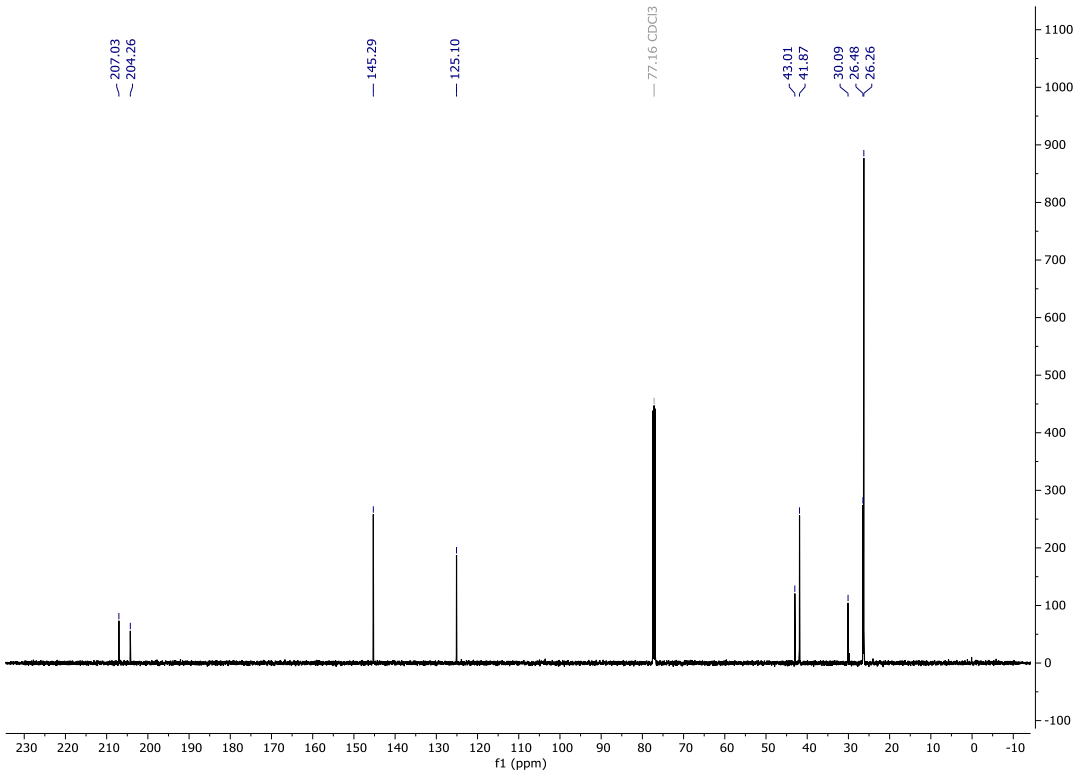

**A17:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **S8**.



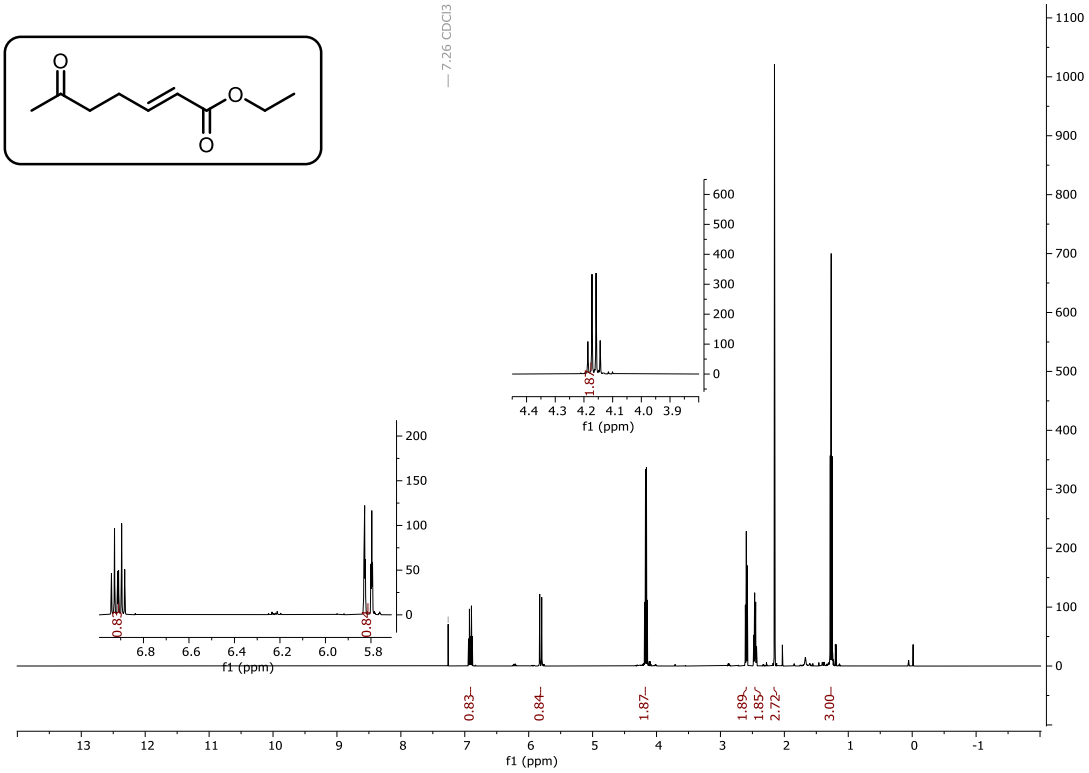

**A18:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **S8.**



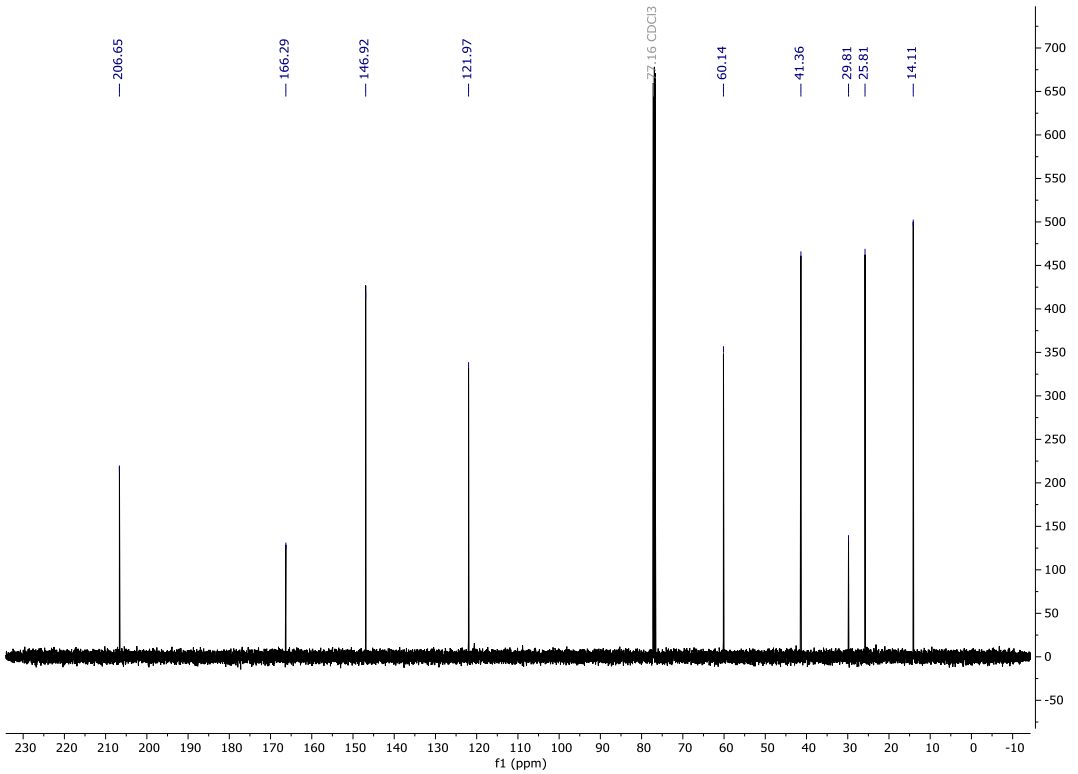

**A19:** $^{1}$H-NMR (500 MHz, $CDCl_3$) of compound **S9.**



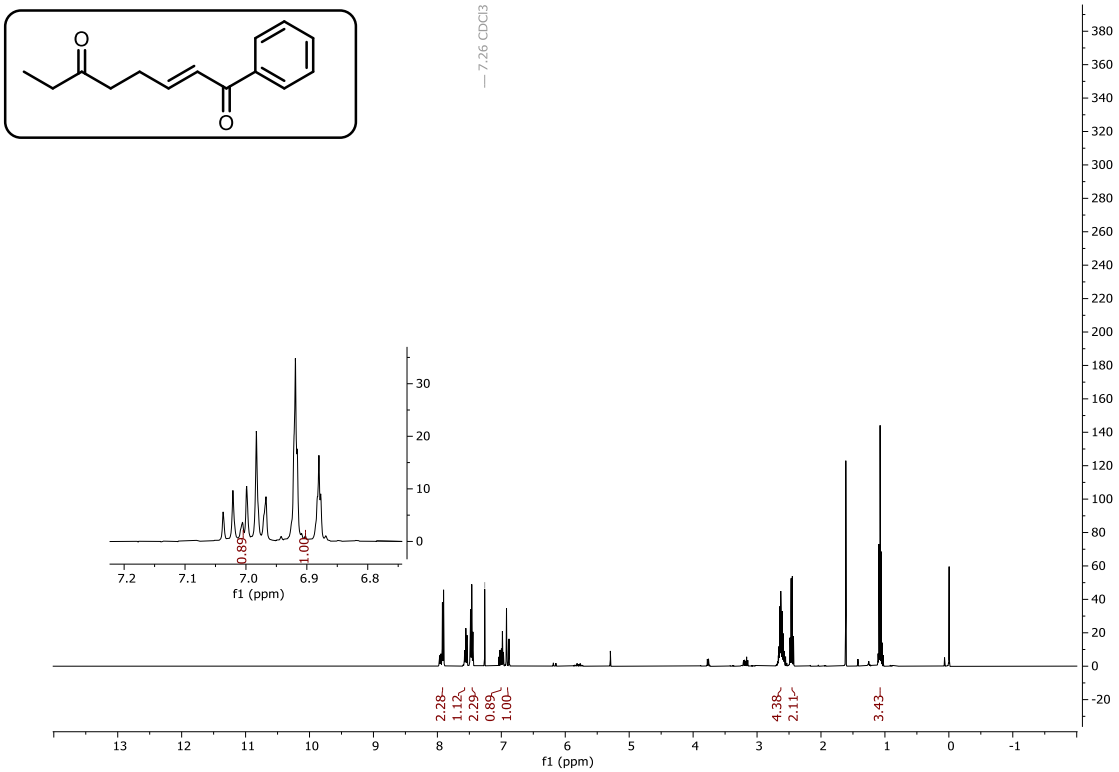

**A20:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **S9.**



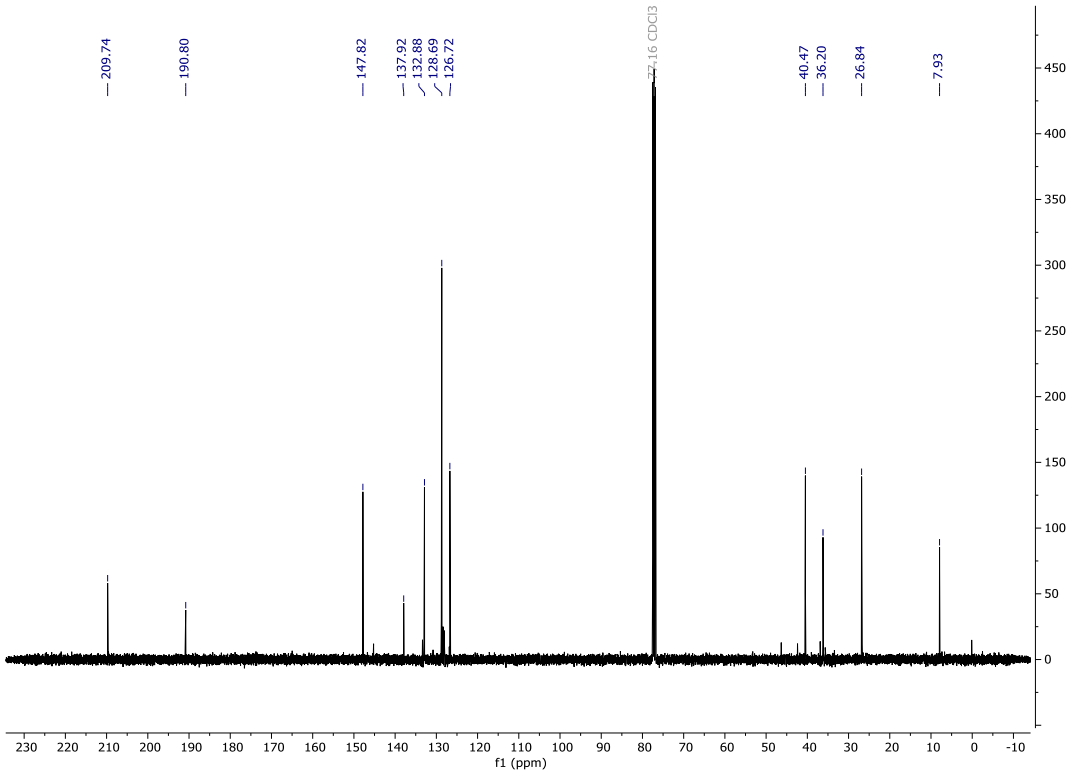

**A21:** $^{1}$H-NMR (500 MHz, $CDCl_3$) of compound **S10.**



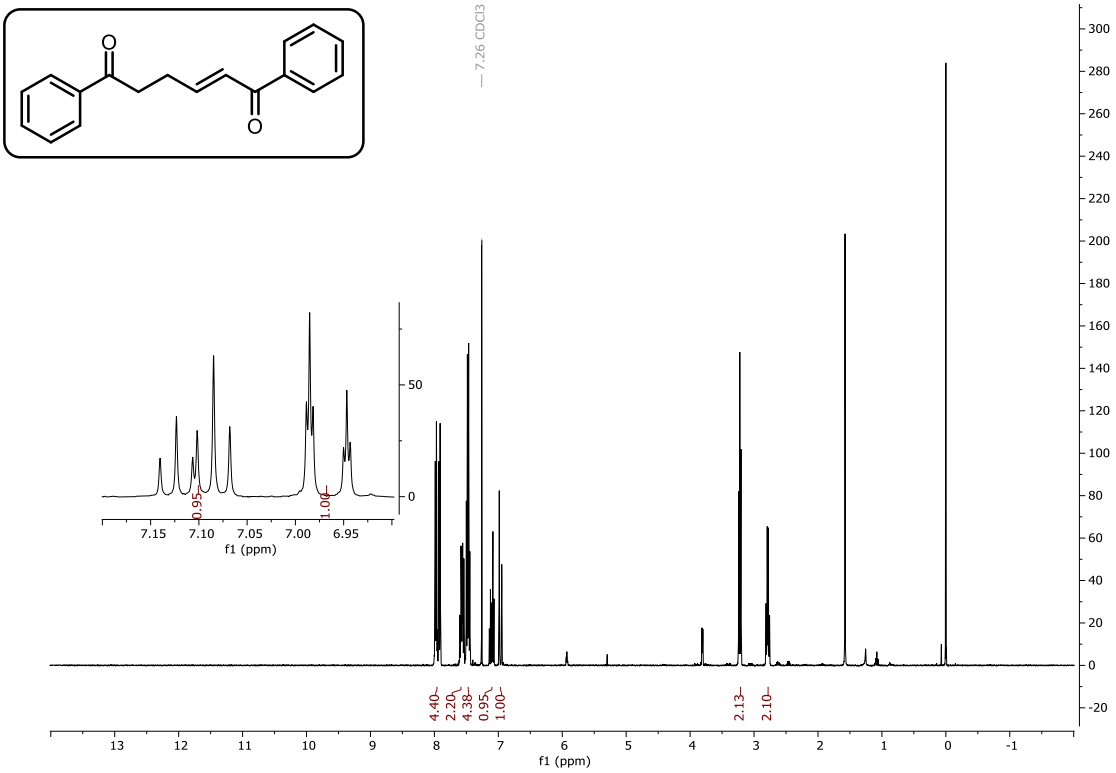

**A22:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **S10.**



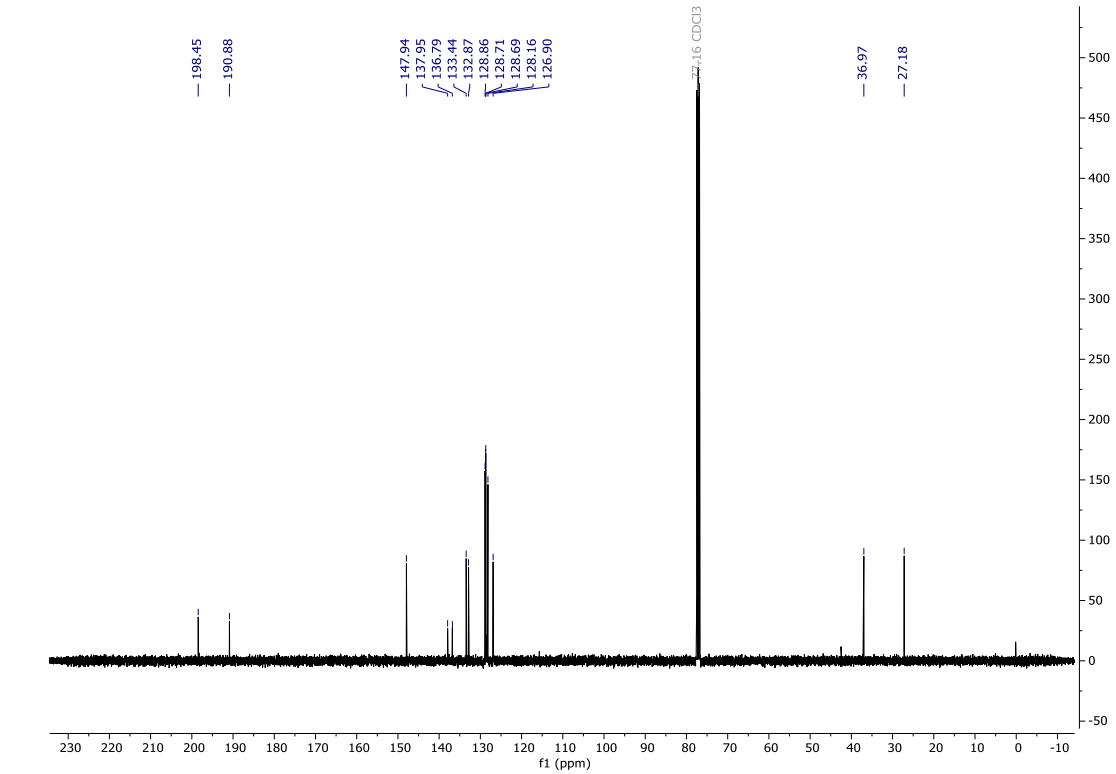

**A23:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **3e.**



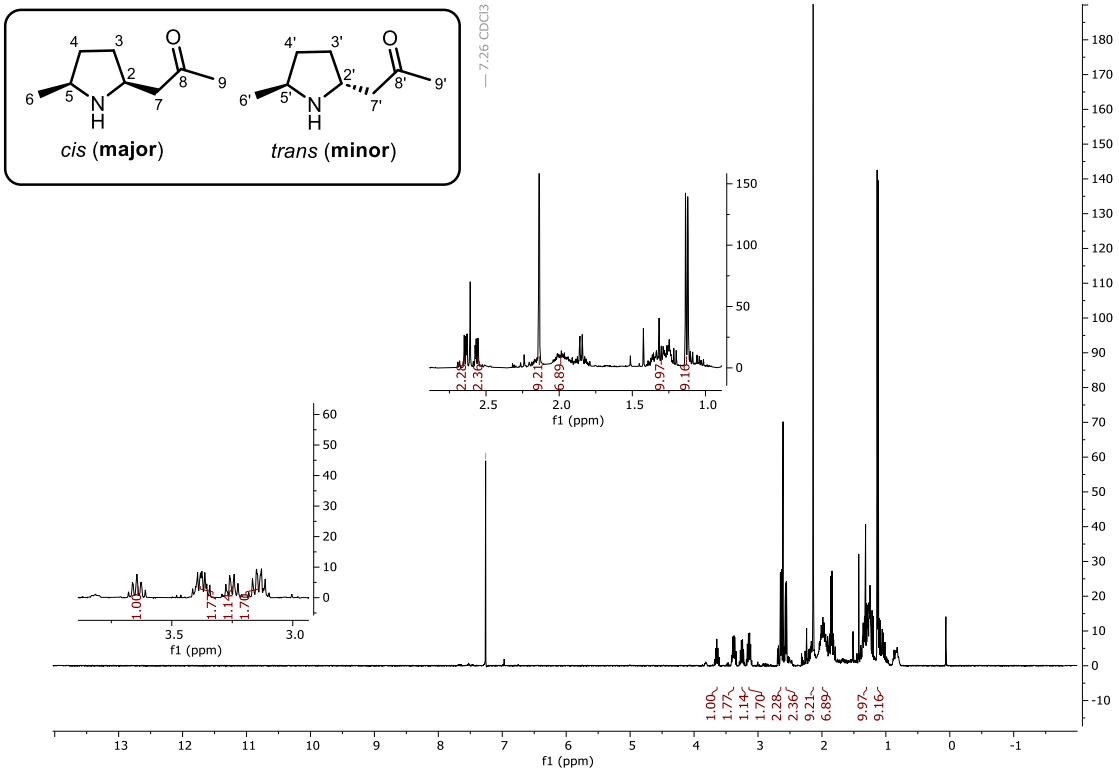

**A24:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **3e.**



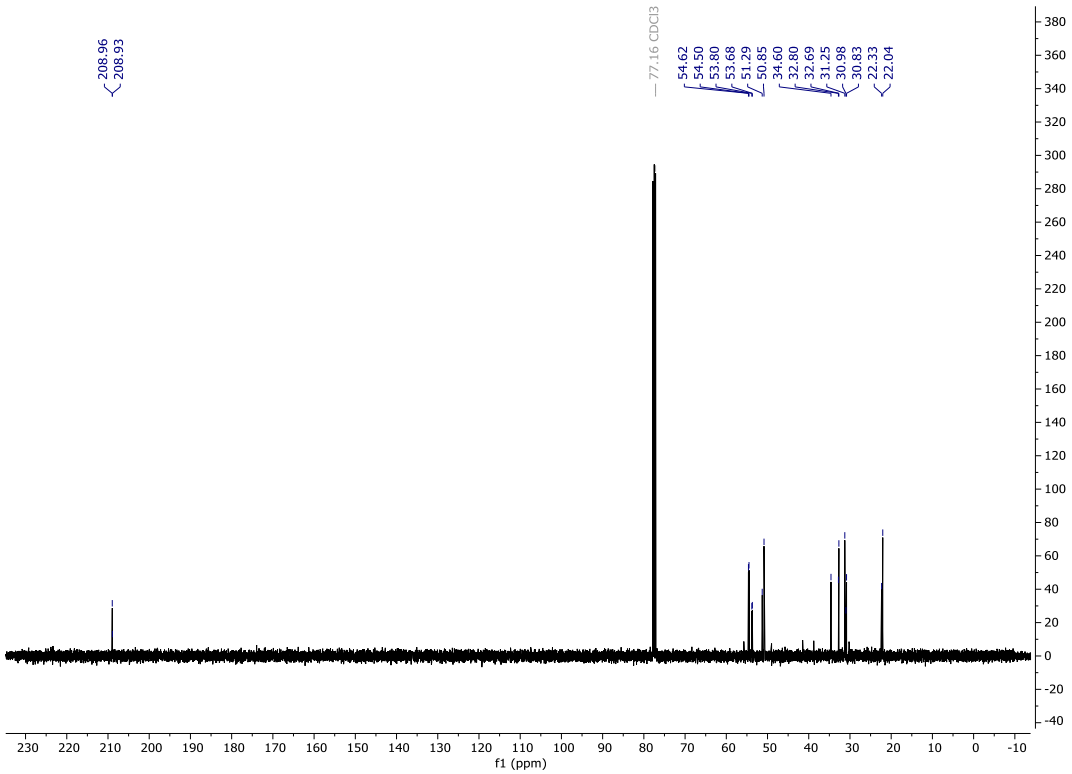

**A25:** $^{1}$H-NMR (500 MHz, $CDCl_3$) of compound **3f.**



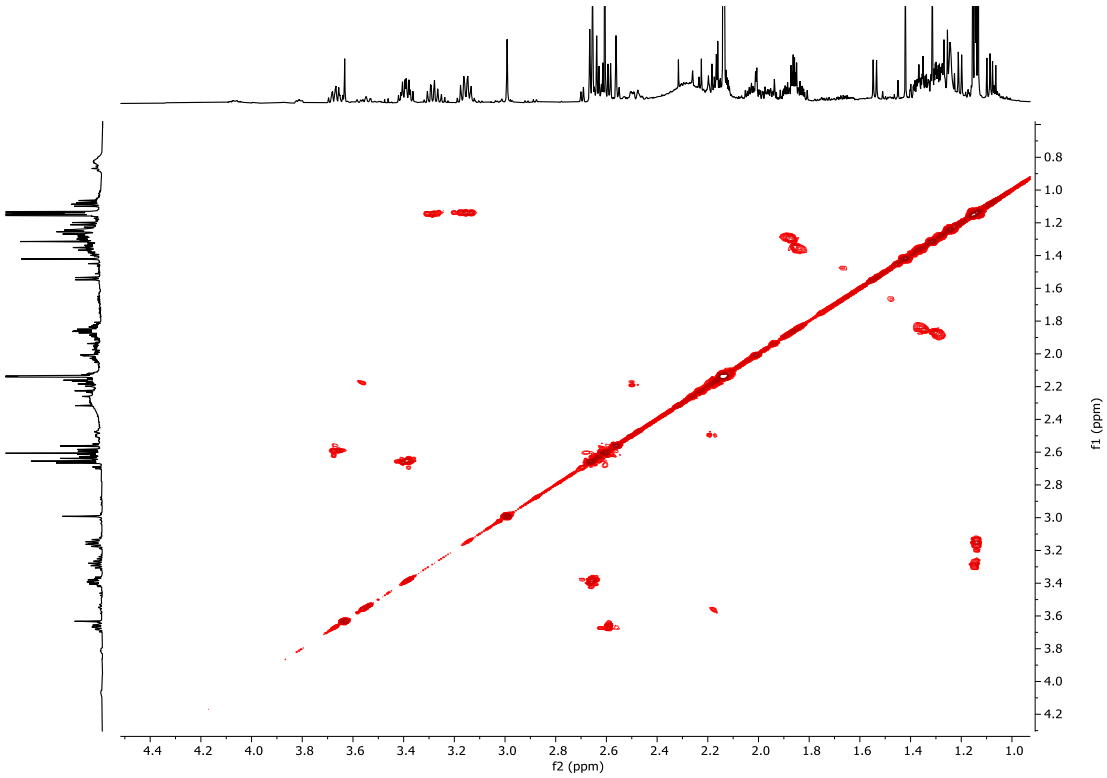

**A26:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **3f.**



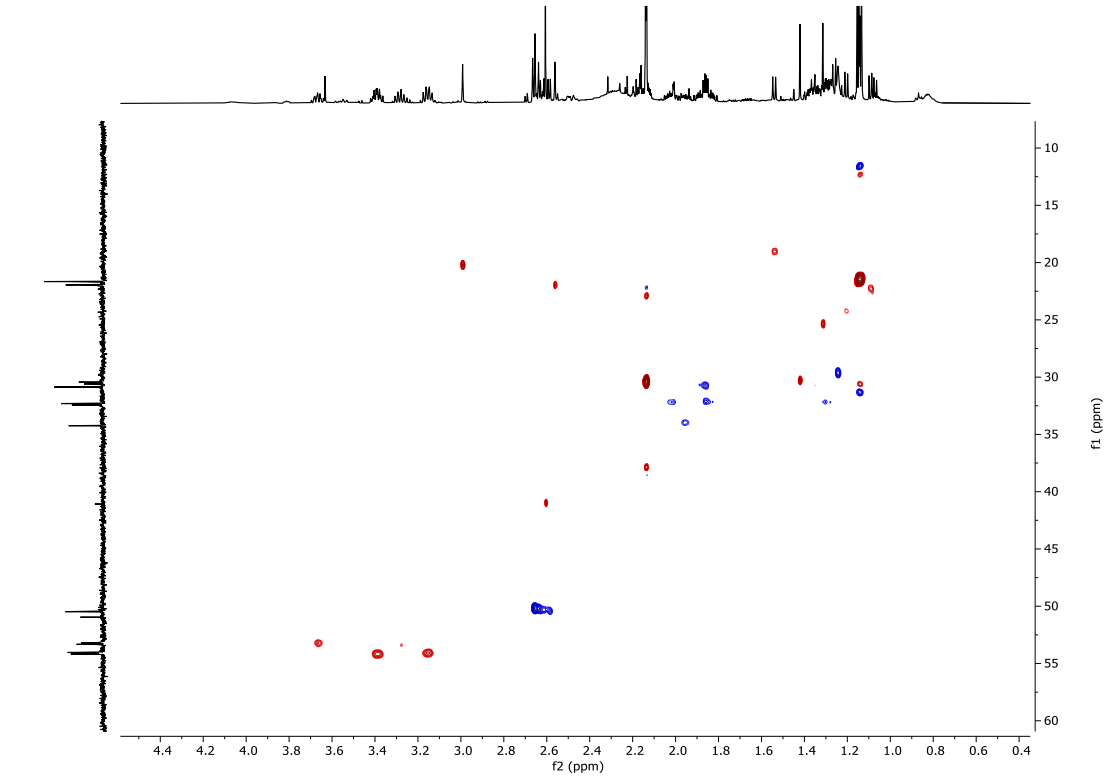

**A27:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **5a.**



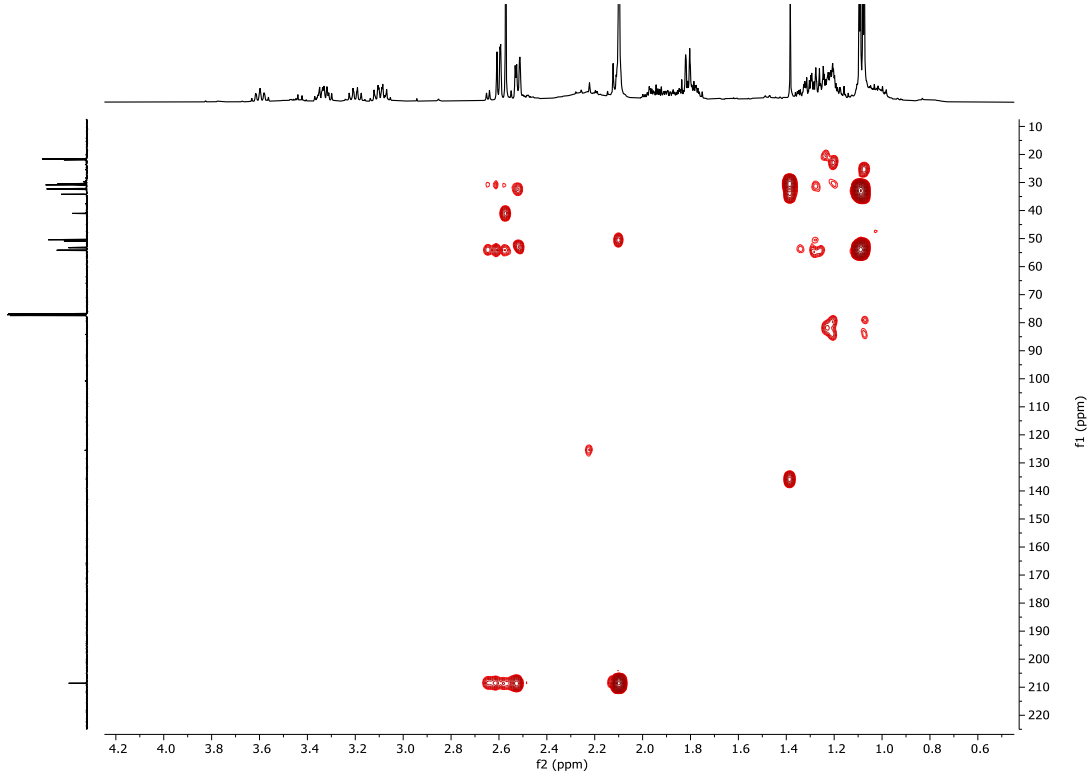

**A28:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **5a.**



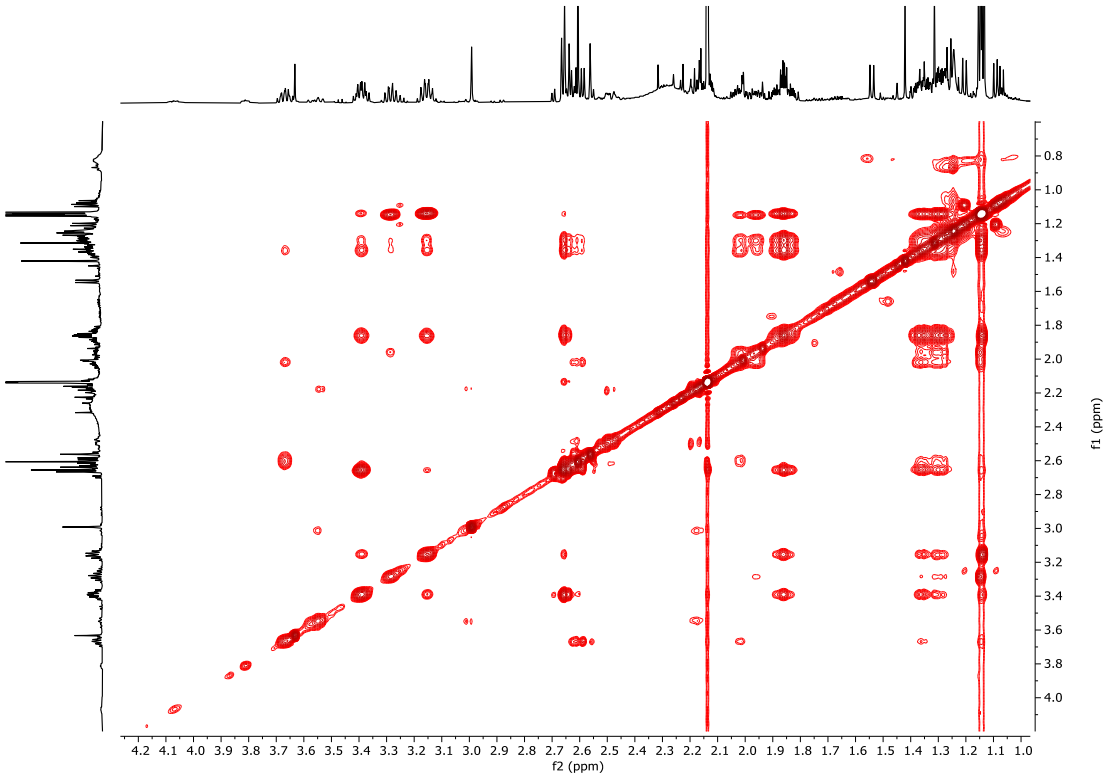

**A29:** COSY NMR (500 MHz, $CDCl_3$) of compound **5a**.



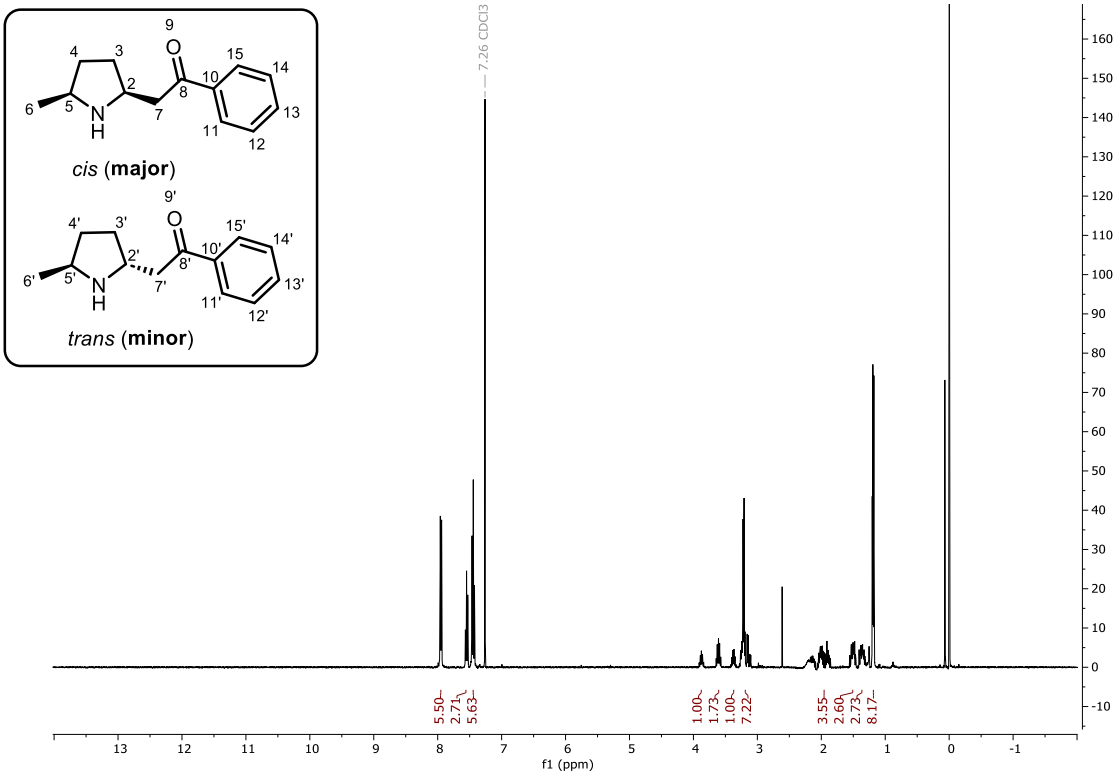

**A30:** HSQC NMR (500 MHz, $CDCl_3$) of compound **5a**.



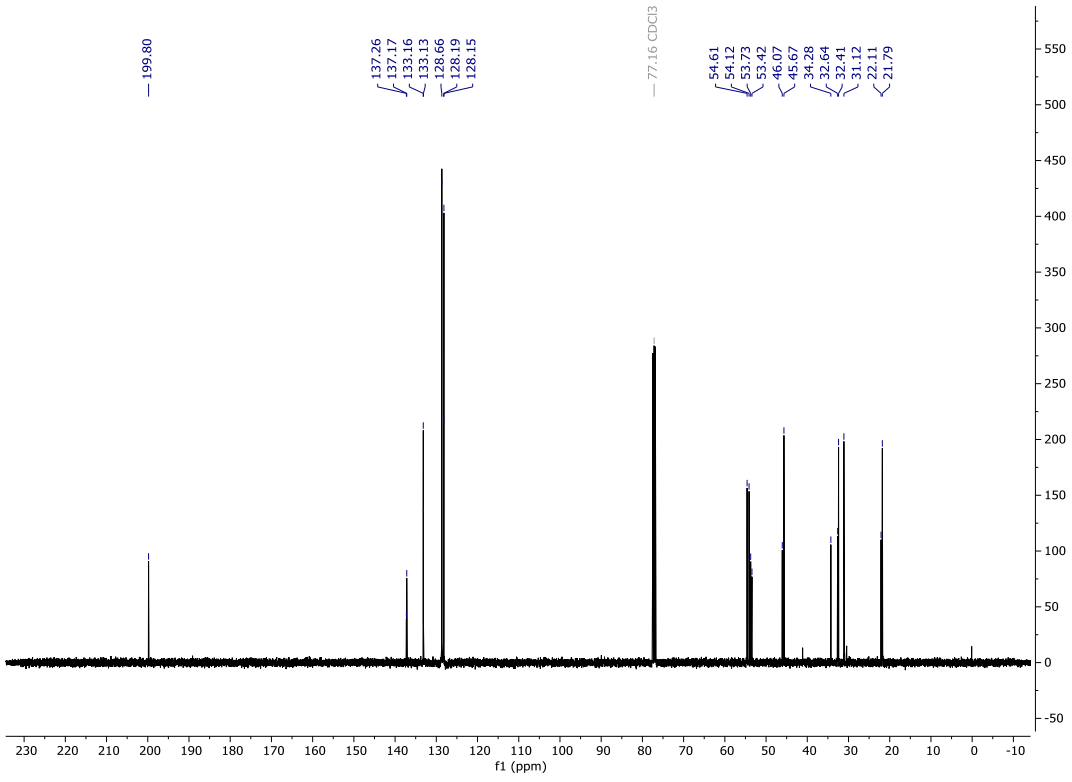

**A31:** HMBC NMR (400 MHz, $CDCl_3$) of compound **5a**.



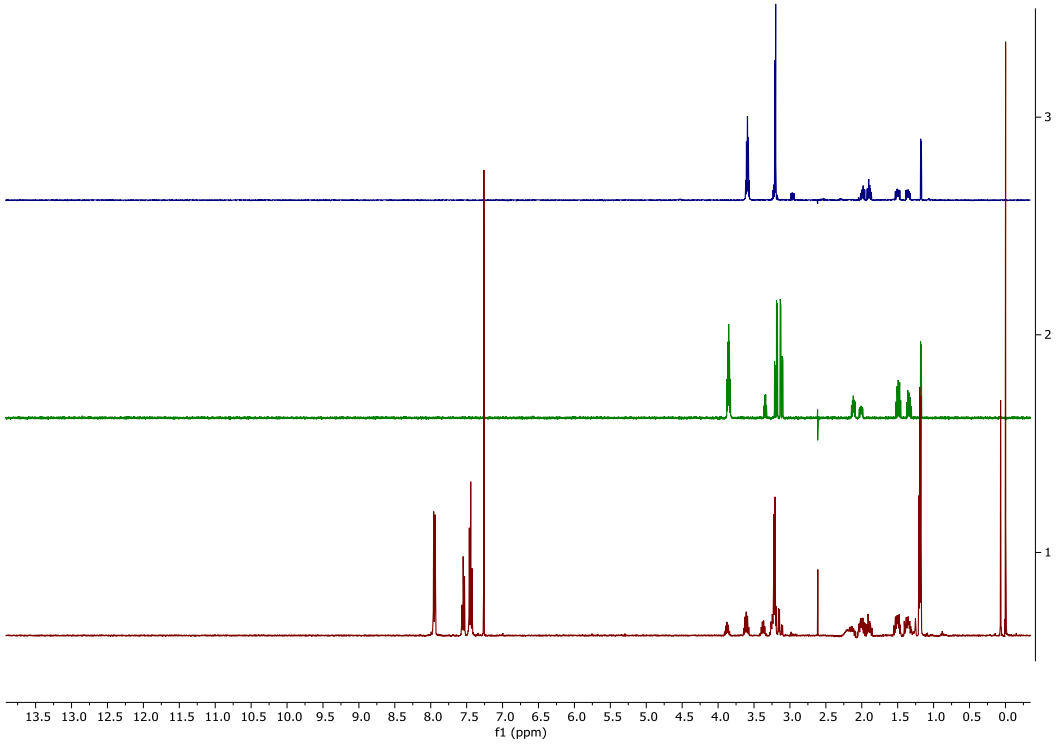

**A32:** TOCSY NMR (500 MHz, $CDCl_3$) of compound **5a**.



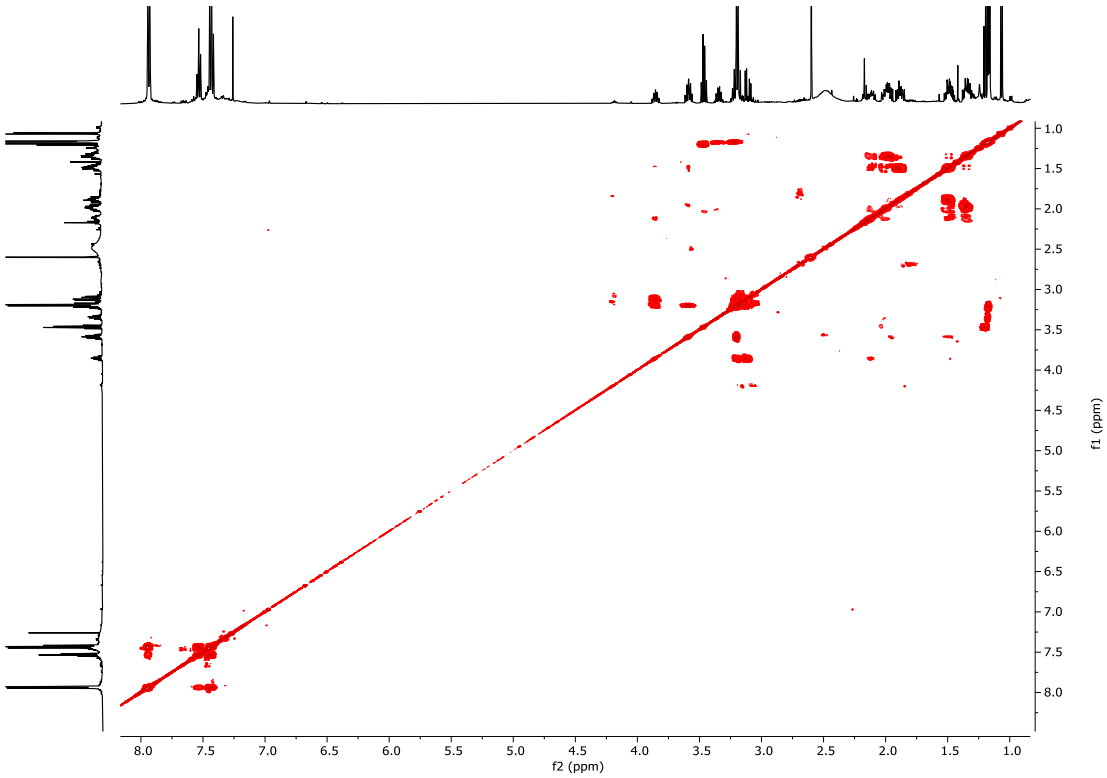

**A33:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **5b.**



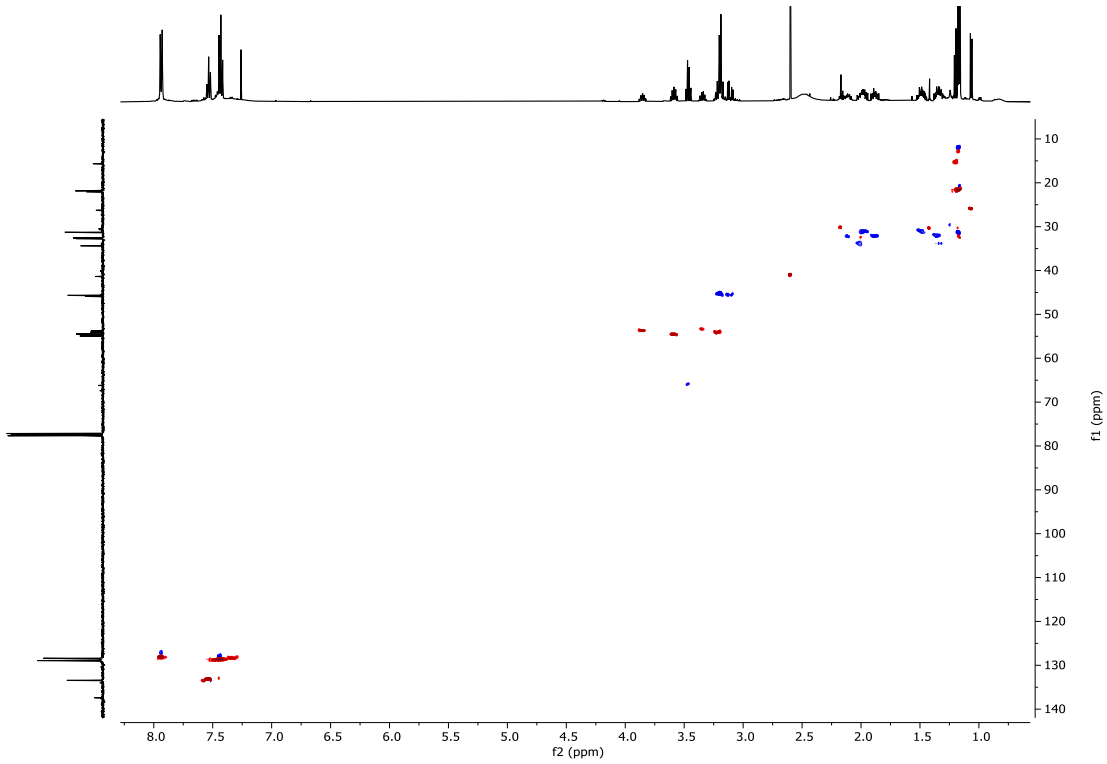

**A34:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **5b.**



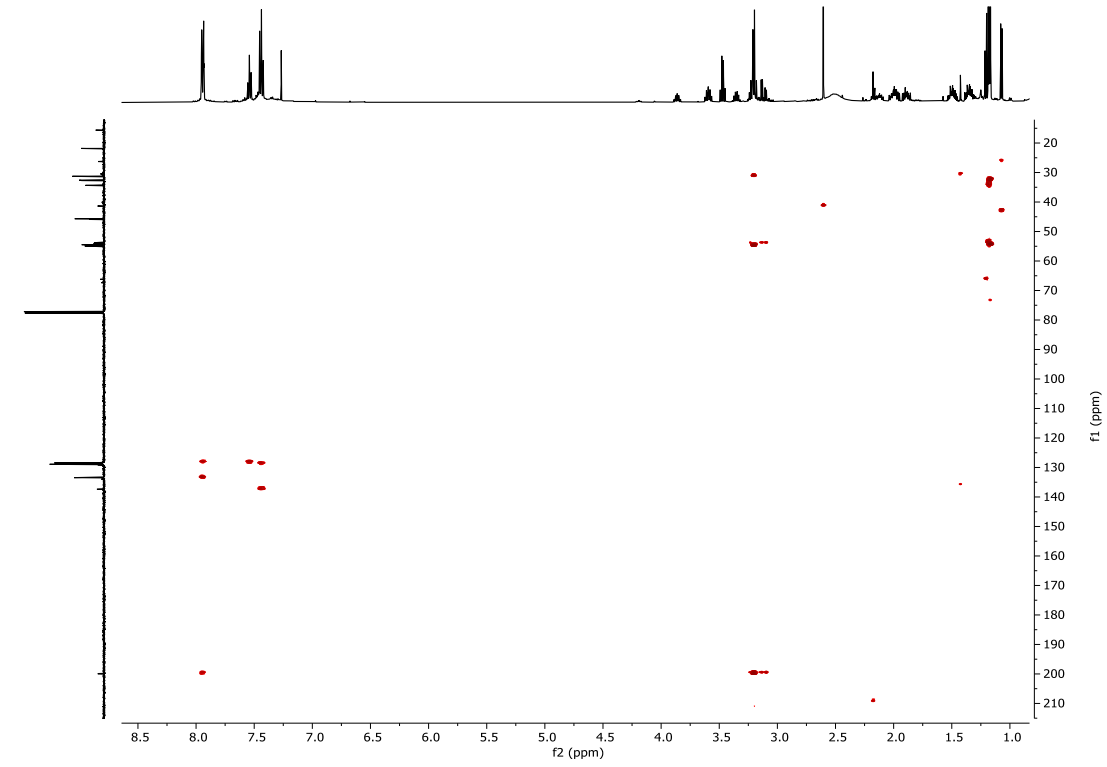

**A35:** Stacked selTOCSY NMR (600 MHz, $CDCl_3$) spectra of **5b**. Top spectrum = *cis*-**5b**. Middle spectrum = *trans*-**5b**. Bottom spectrum = mixture of *cis*-**5b** and *trans*-**5b**.



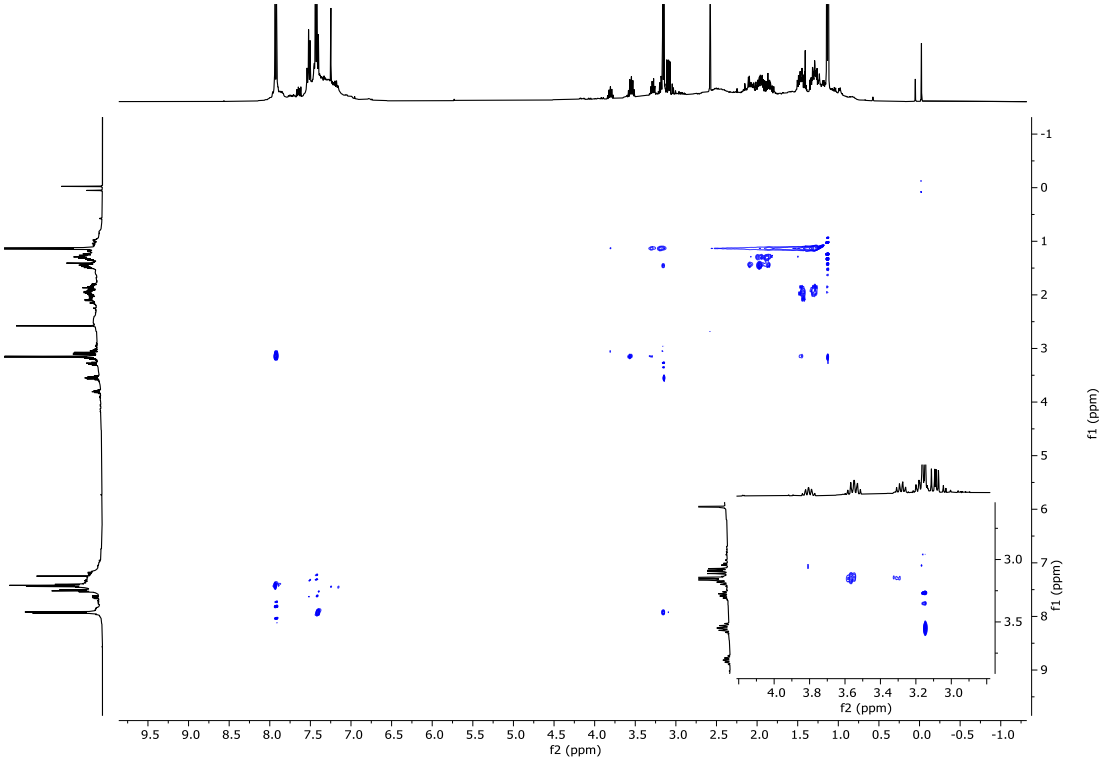

**A36:** COSY NMR (500 MHz, $CDCl_3$) of compound **5b**.



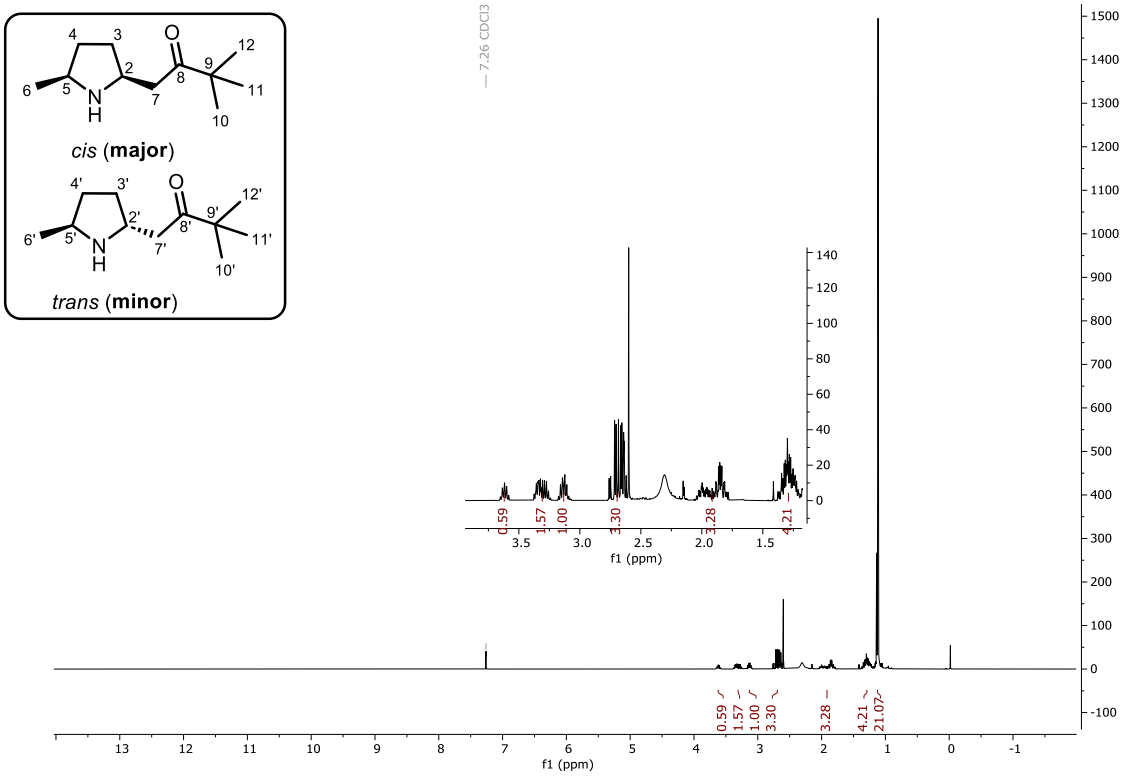

**A37:** HSQC NMR (500 MHz, $CDCl_3$) of compound **5b**.



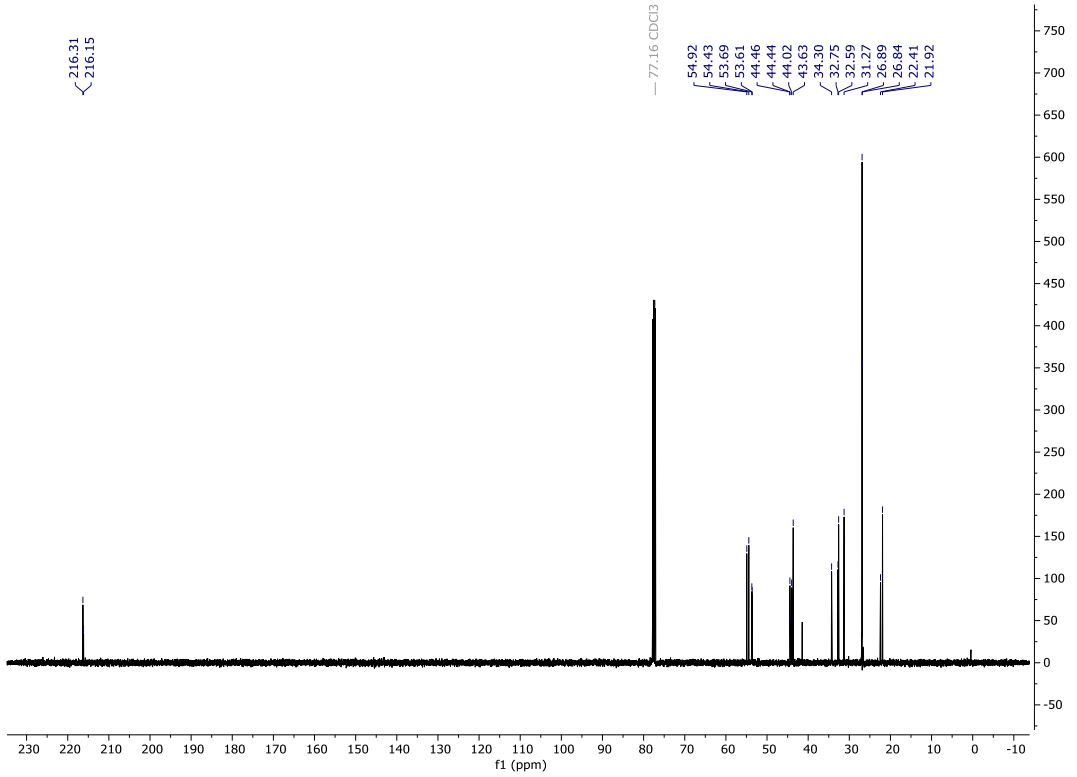

**A38:** HMBC NMR (500 MHz, $CDCl_3$) of compound **5b**.



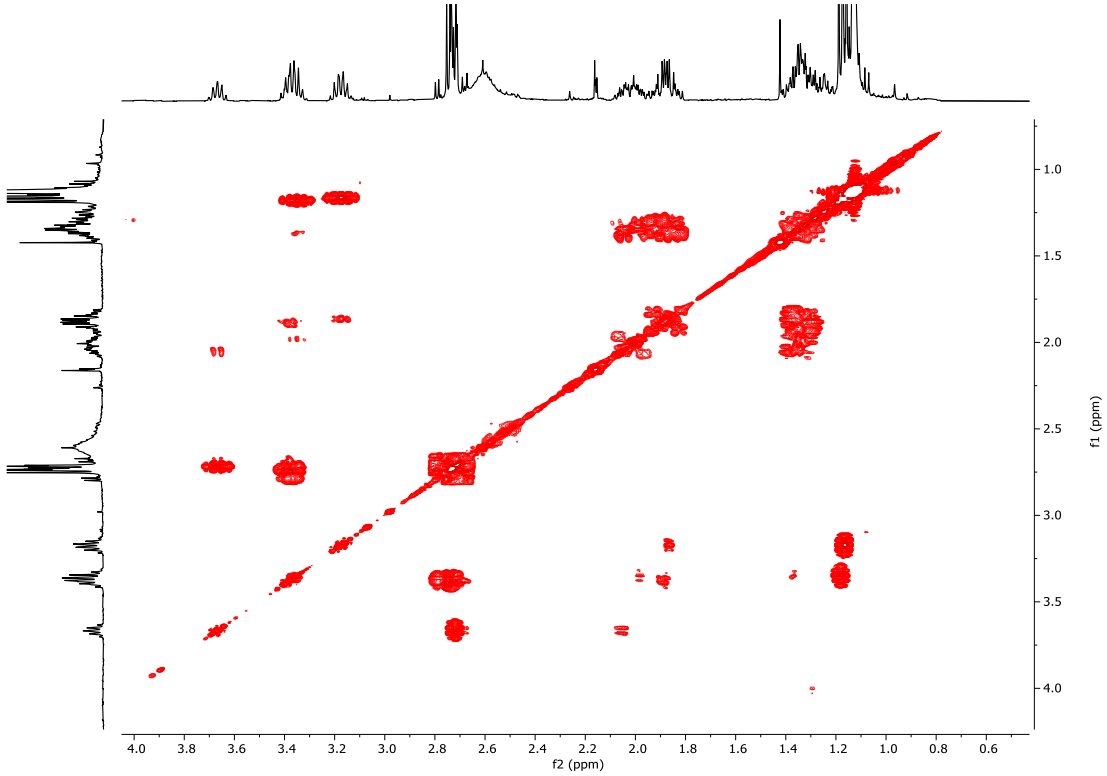

**A39:** NOESY NMR (400 MHz, $CDCl_3$) of compound **5b**.



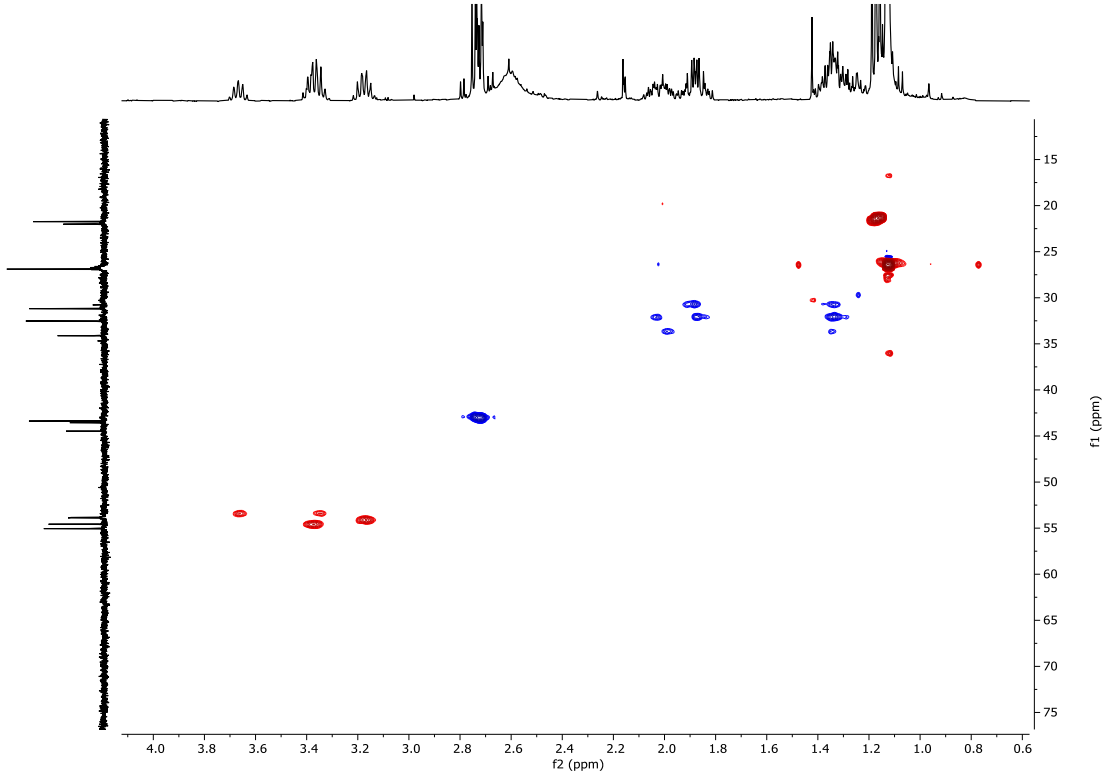

**A40:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **5c.**



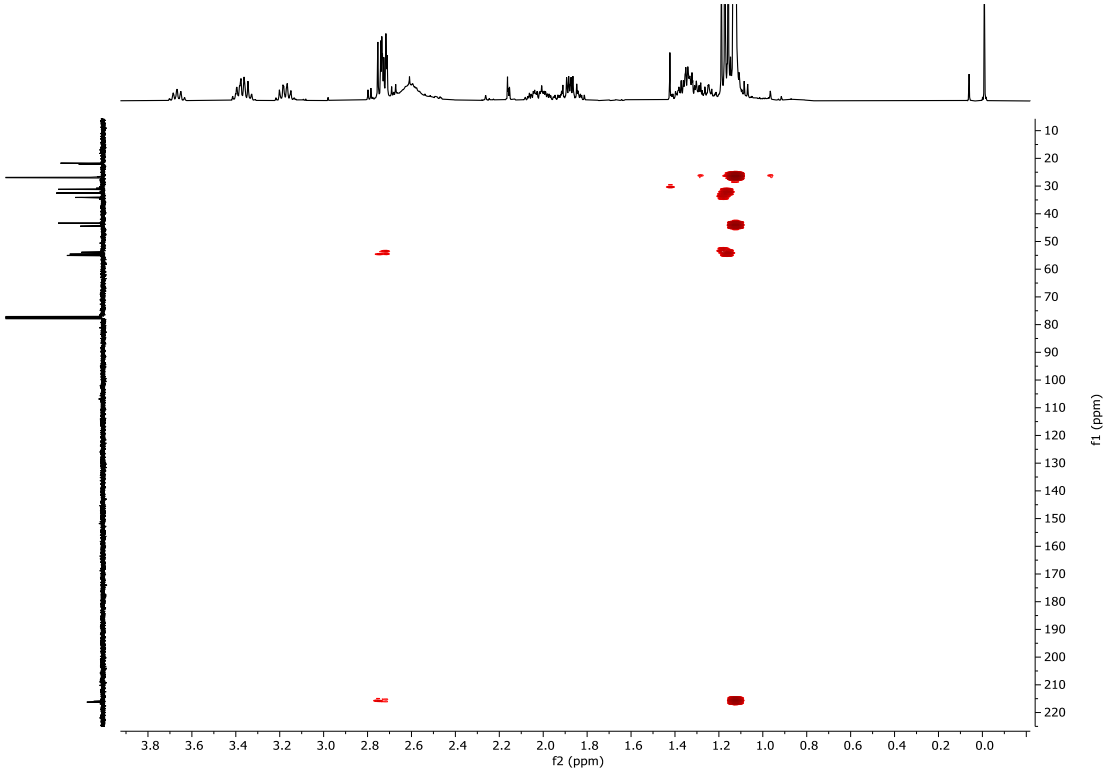

**A41:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **5c.**



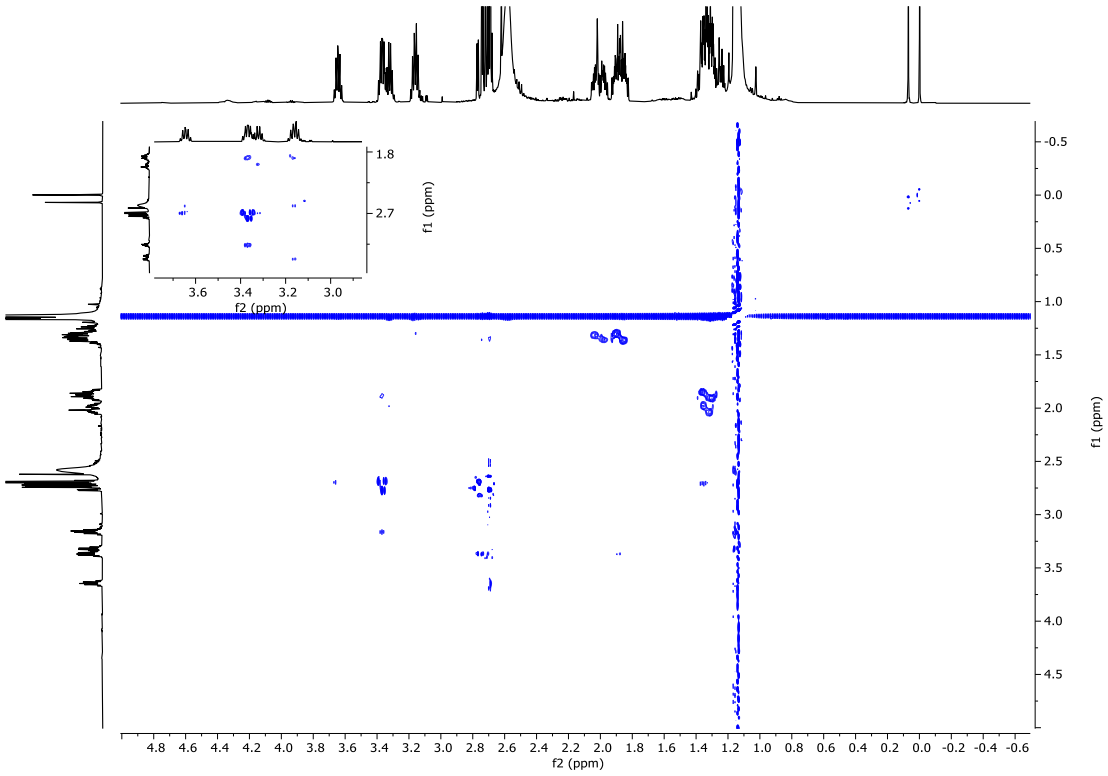

**A42:** COSY NMR (400 MHz, $CDCl_3$) of compound **5c.**



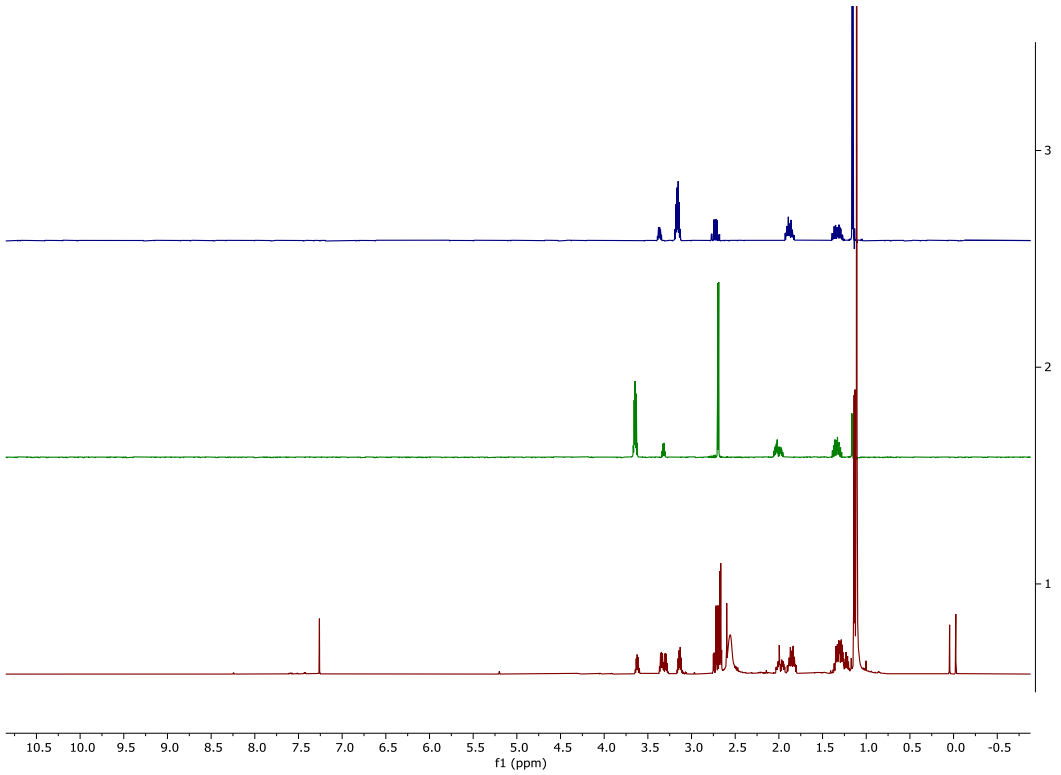

**A43:** HSQC NMR (400 MHz, $CDCl_3$) of compound **5c**.



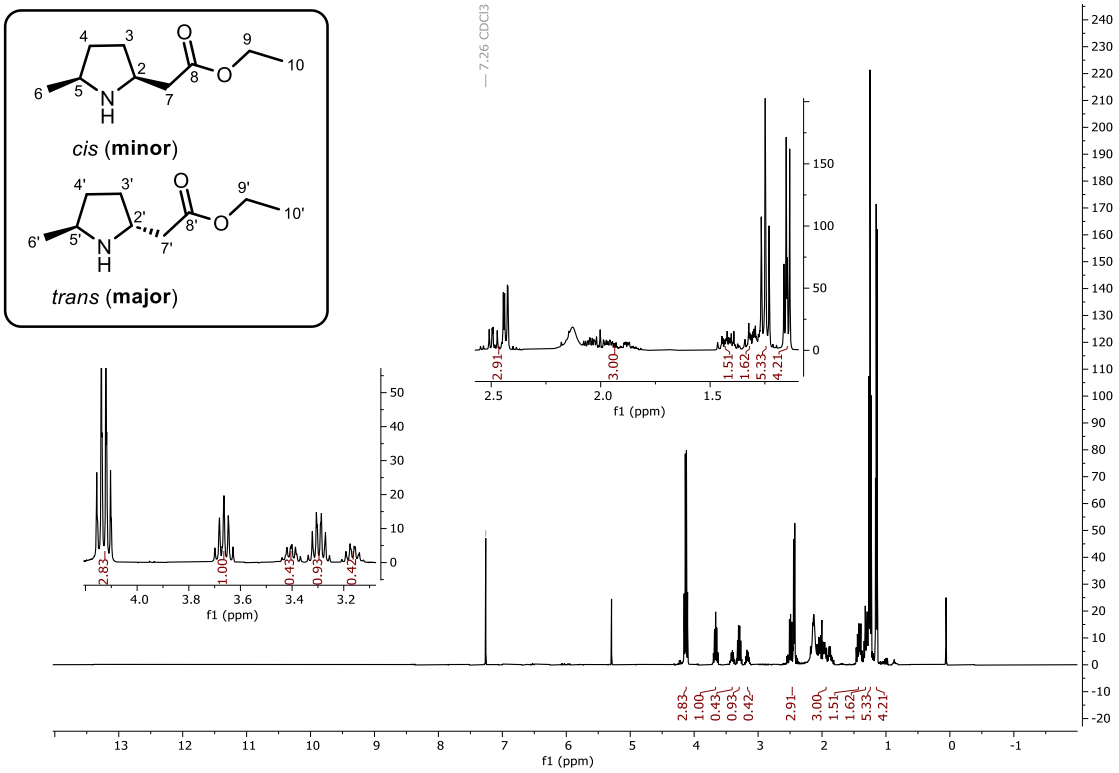

**A44:** HMBC NMR (400 MHz, $CDCl_3$) of compound **5c**.



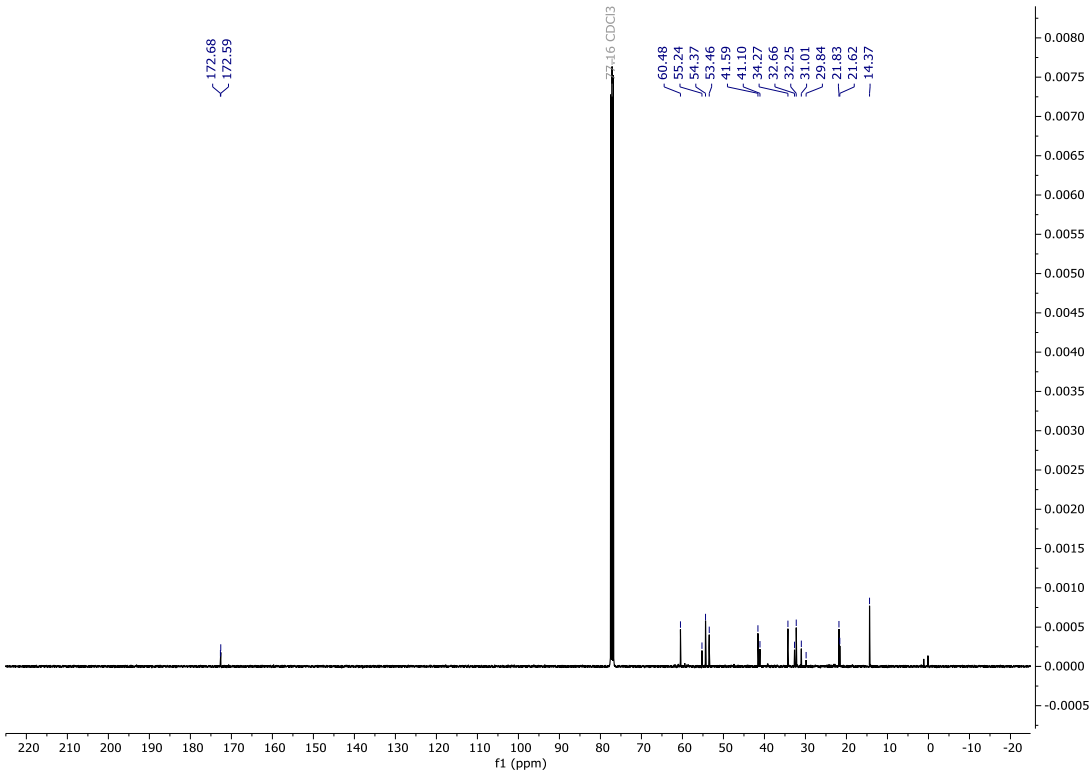

**A45:** NOESY NMR (600 MHz, $CDCl_3$) of compound **5c**.



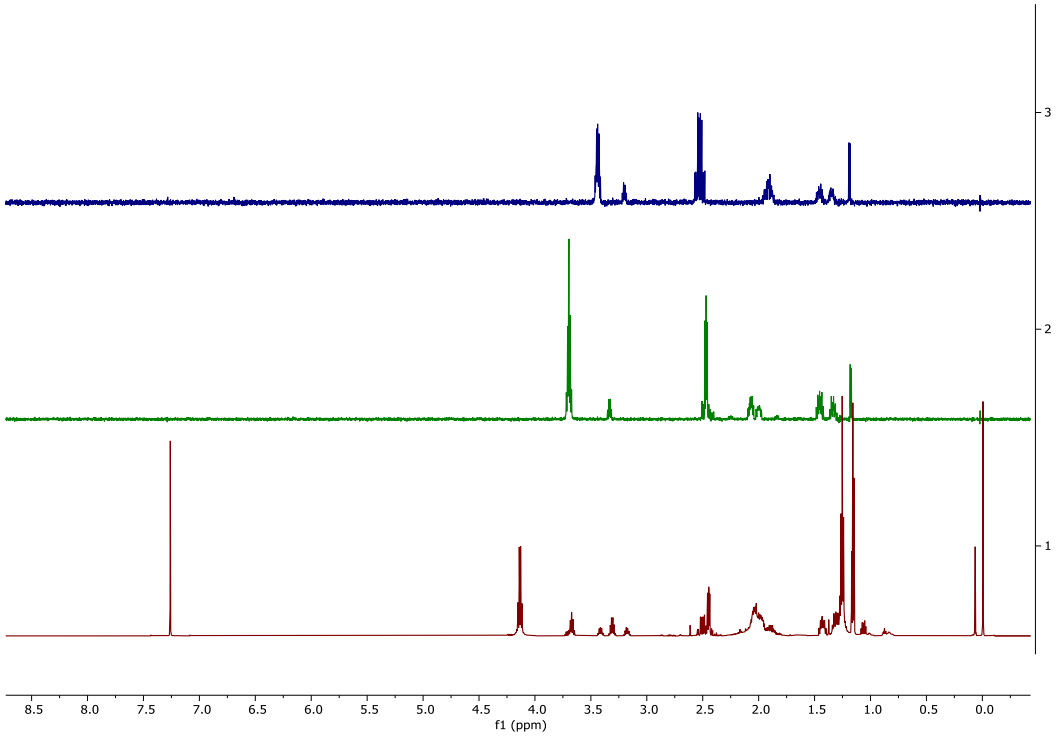

**A46:** Stacked selTOCSY NMR (600 MHz, $CDCl_3$) spectra of compound **5c**. Top spectrum = *cis*-**5c**. Middle spectrum = *trans*-**5c**. Bottom spectrum = mixture of *cis*-**5c** and *trans*-**5c**.



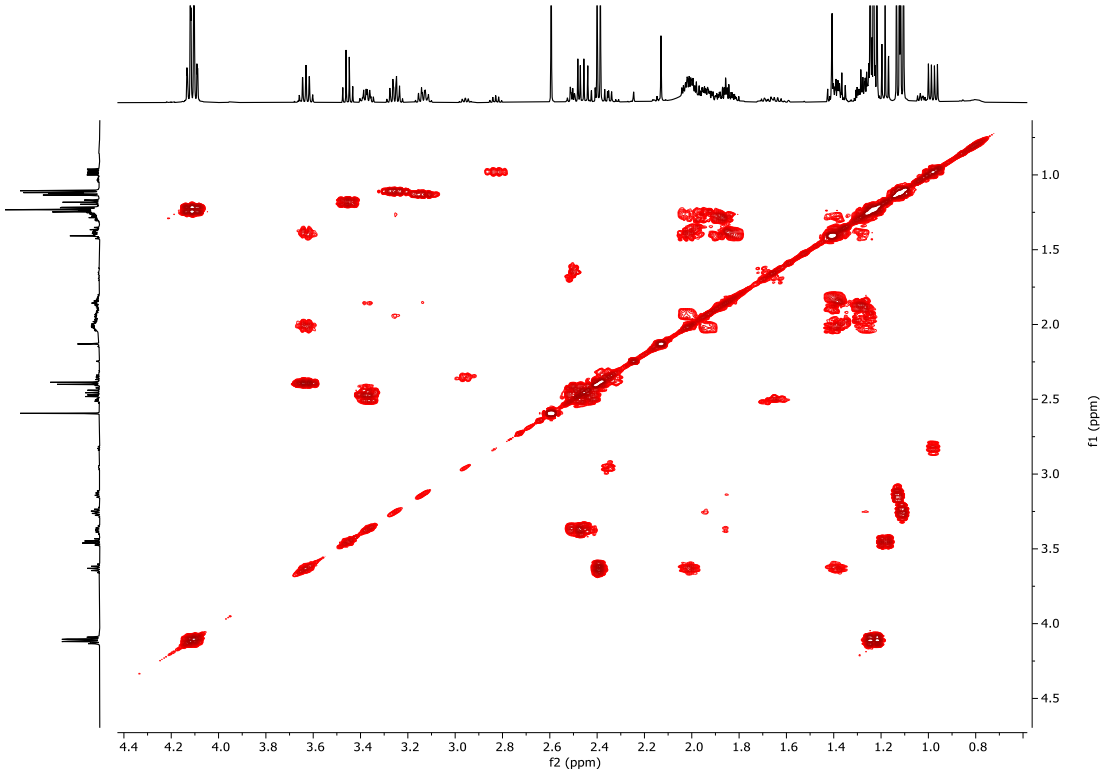

**A47:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **5d.**



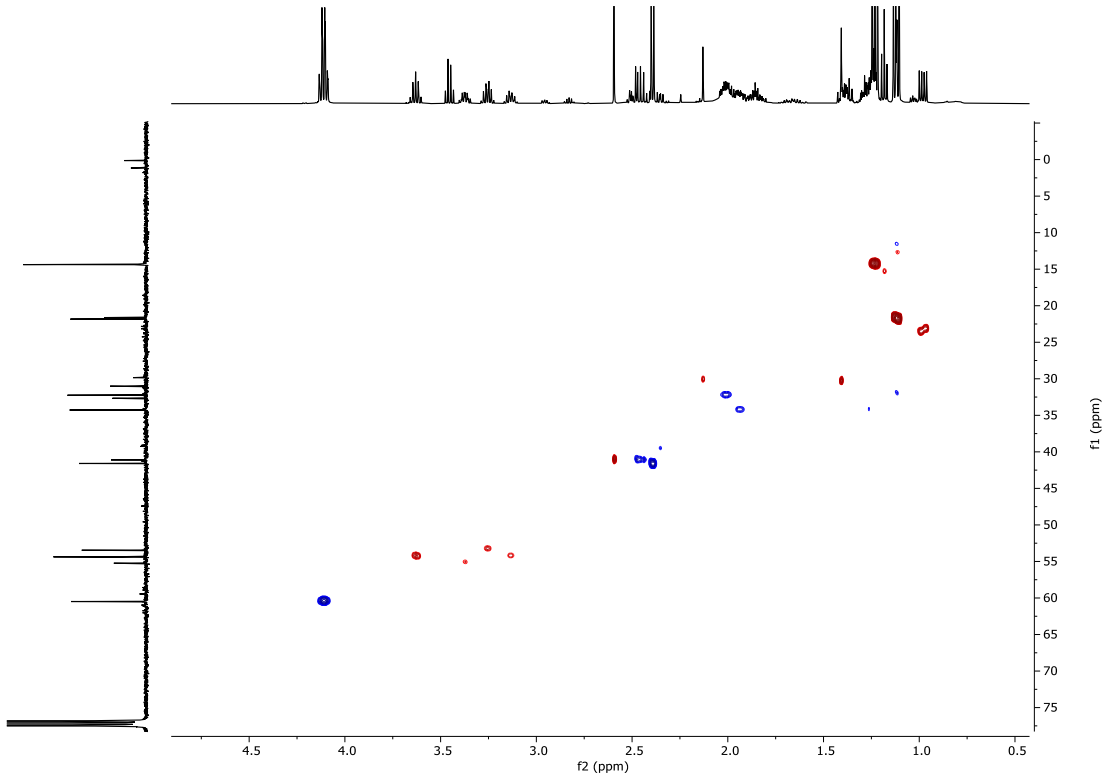

**A48:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **5d.**



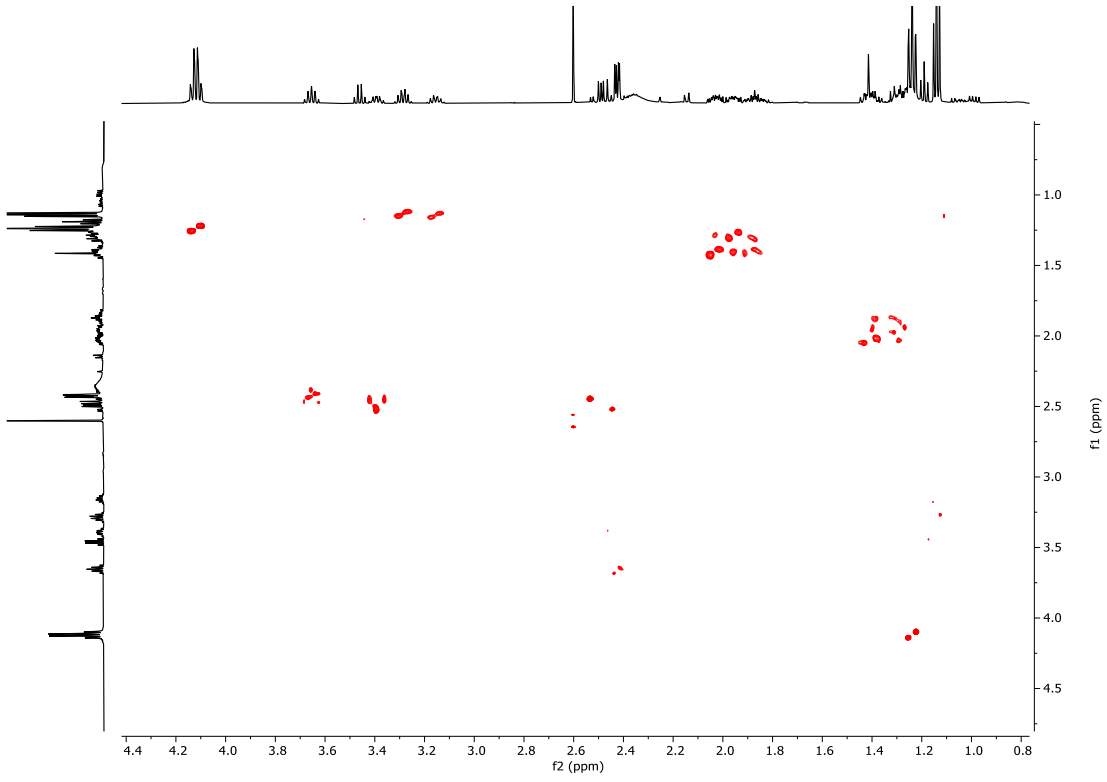

**A49:** Stacked selTOCSY NMR (600 MHz, $CDCl_3$) of compound **5d**. Top spectrum = *cis*-**5d**. Middle spectrum = *trans*-**5d**. Bottom spectrum = mixture of *cis*-**5d** and *trans*-**5d**.



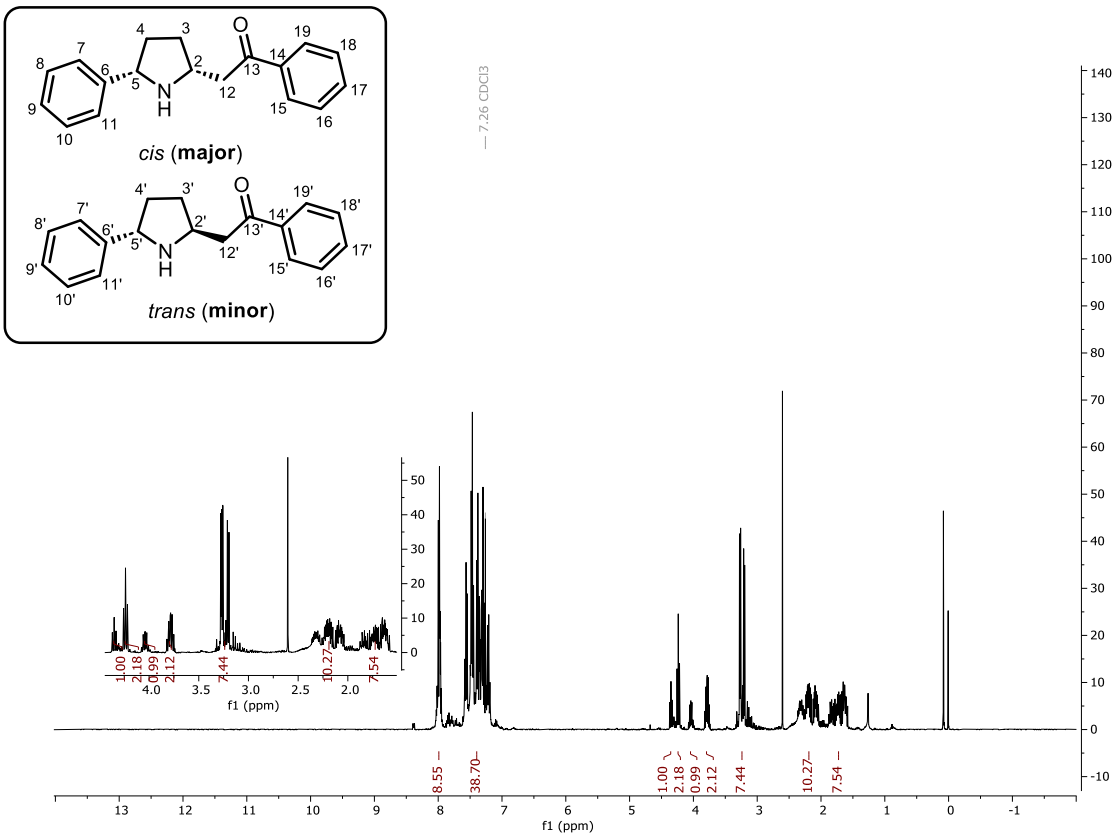

**A50:** COSY NMR (500 MHz, $CDCl_3$) of compound **5d**.



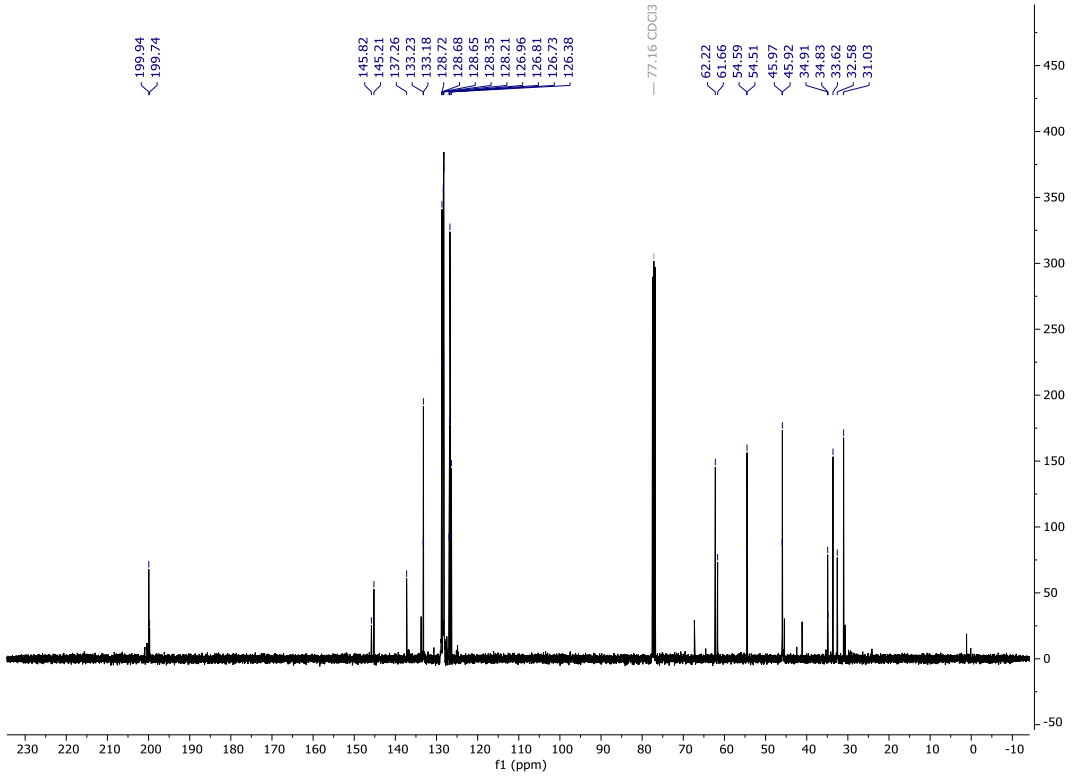

**A51:** HSQC NMR (500 MHz, $CDCl_3$) of compound **5d**.



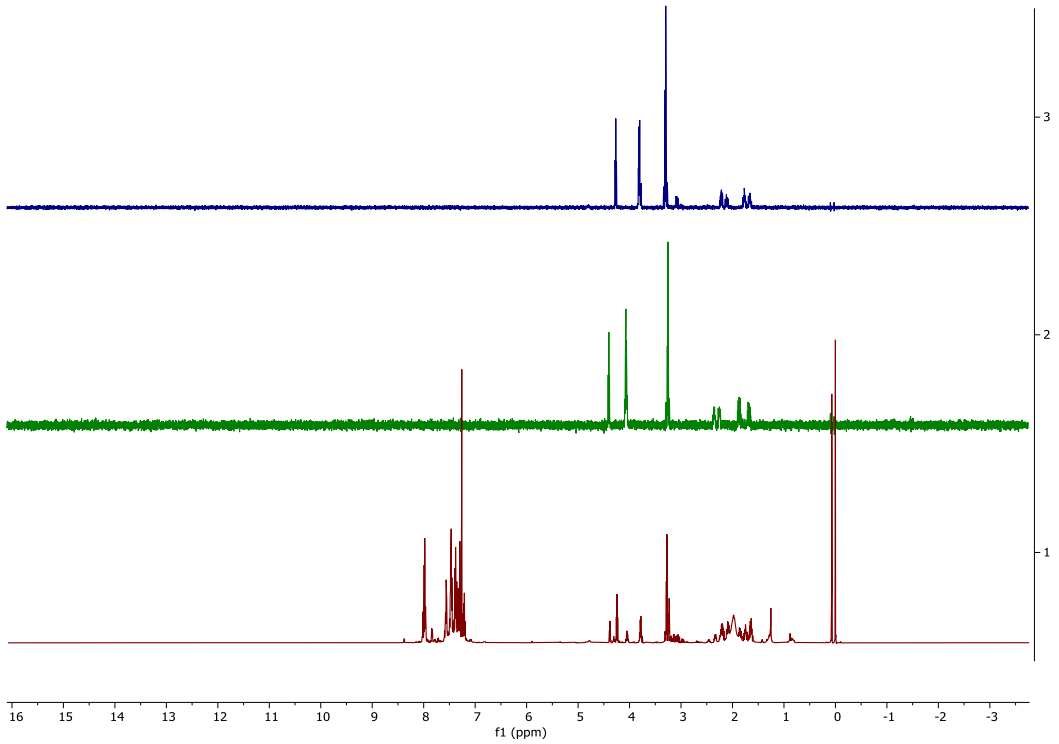

**A52:** NOESY NMR (500 MHz, $CDCl_3$) of compound **5d**.



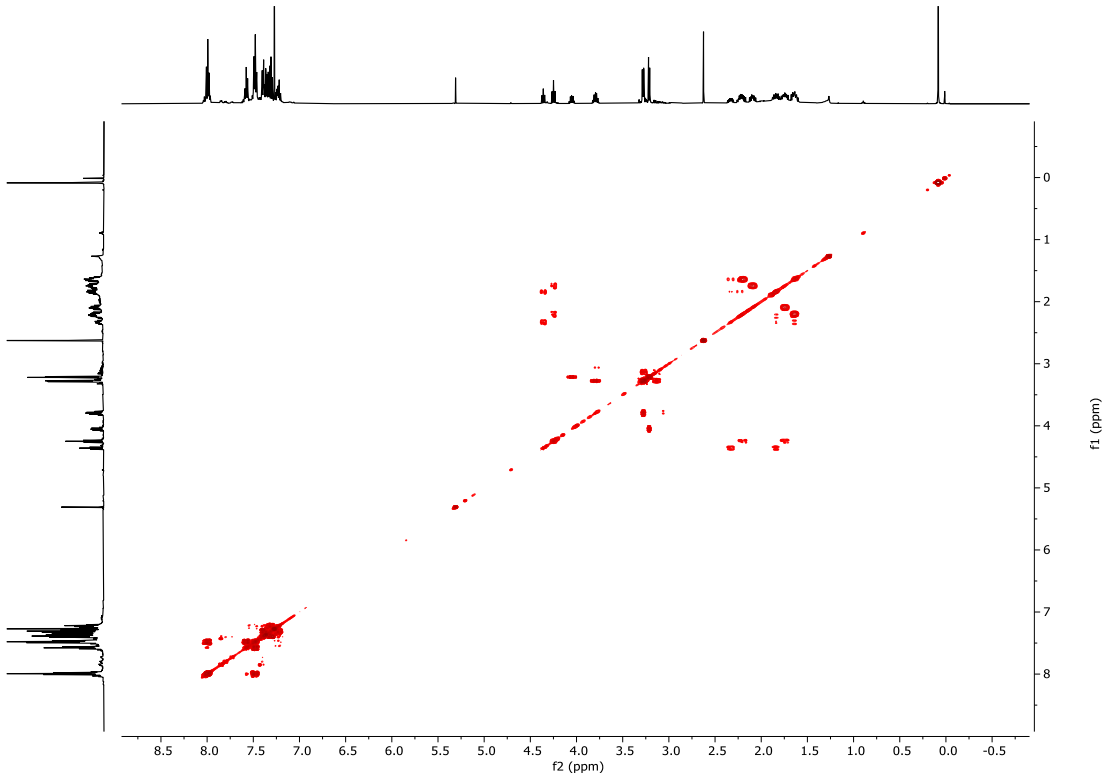

**A53:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **5f.**



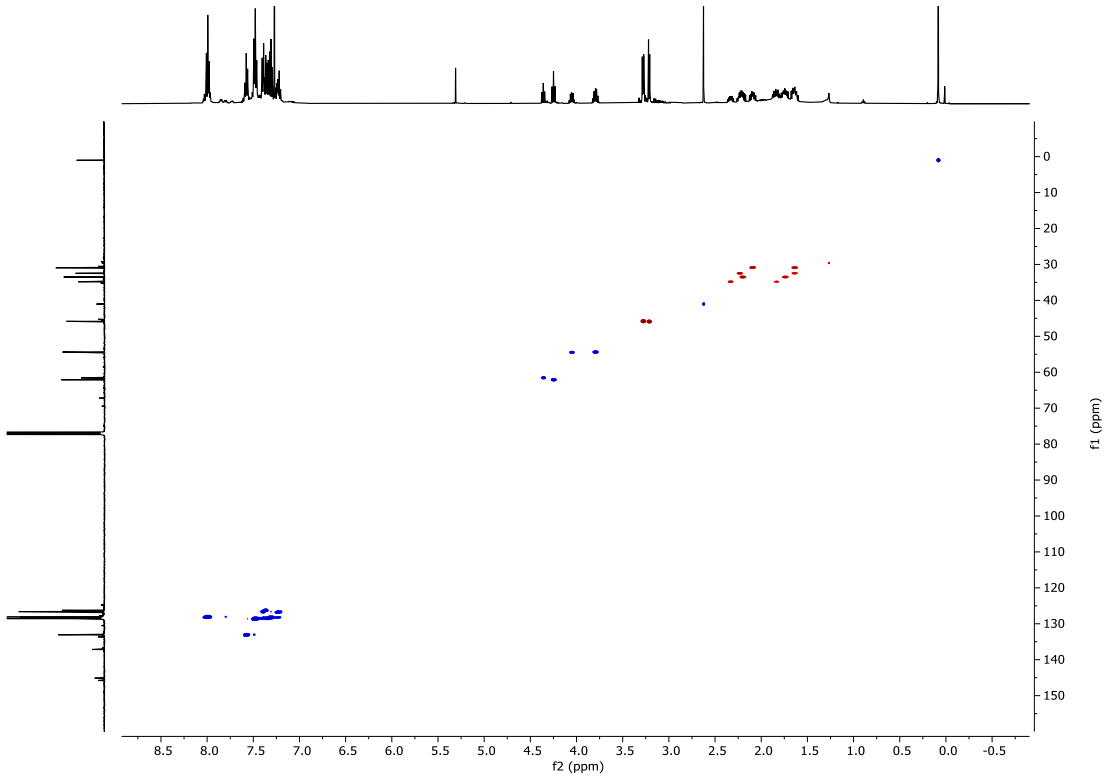

**A64:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **5f.**



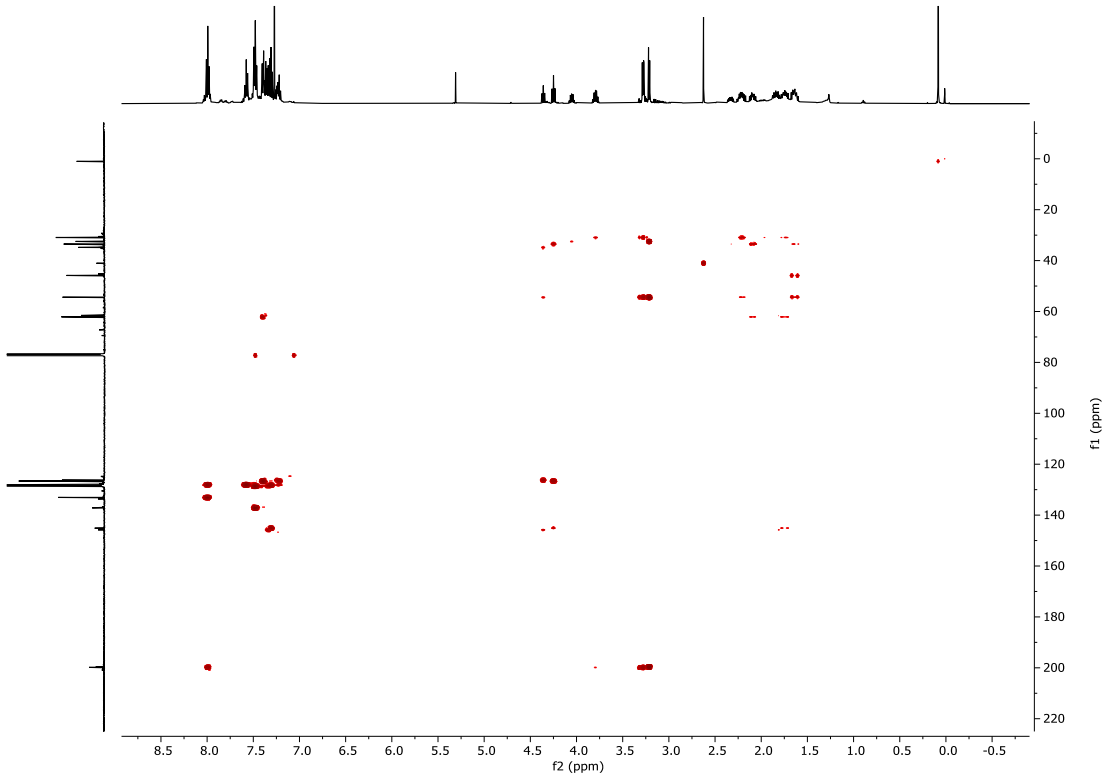

**A55:** Stacked selTOCSY NMR (600 MHz, $CDCl_3$) of compound **5f**. Top spectrum = *cis*-**5f**. Middle spectrum = *trans*-**5f**. Bottom spectrum = mixture of *cis*-**5f** and *trans*-**5f.**



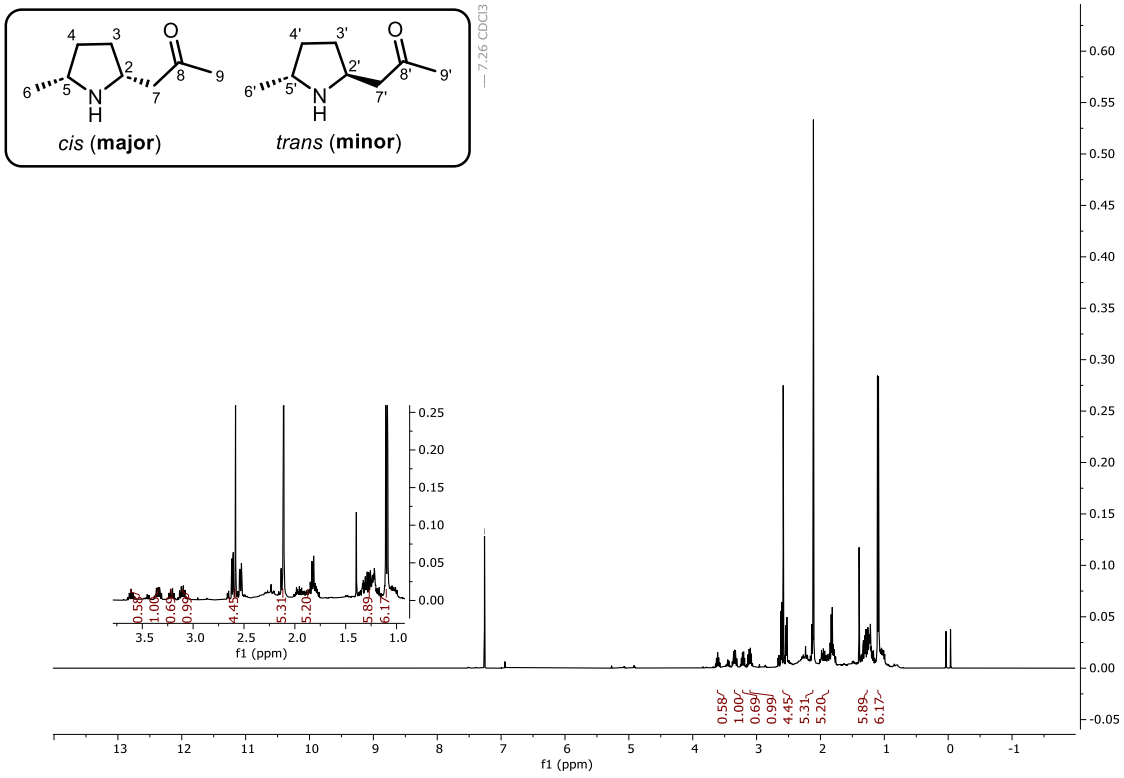

**A56:** COSY NMR (500 MHz, $CDCl_3$) of compound **5f**.



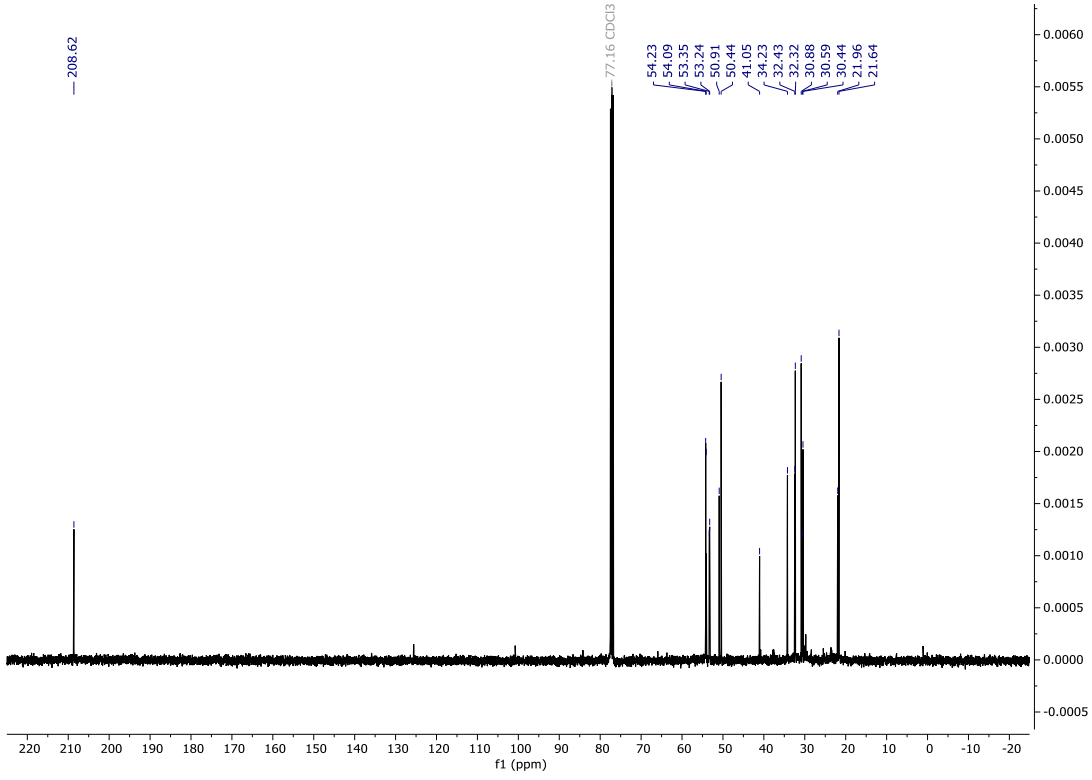

**A57:** HSQC NMR (500 MHz, $CDCl_3$) of compound **5f**.



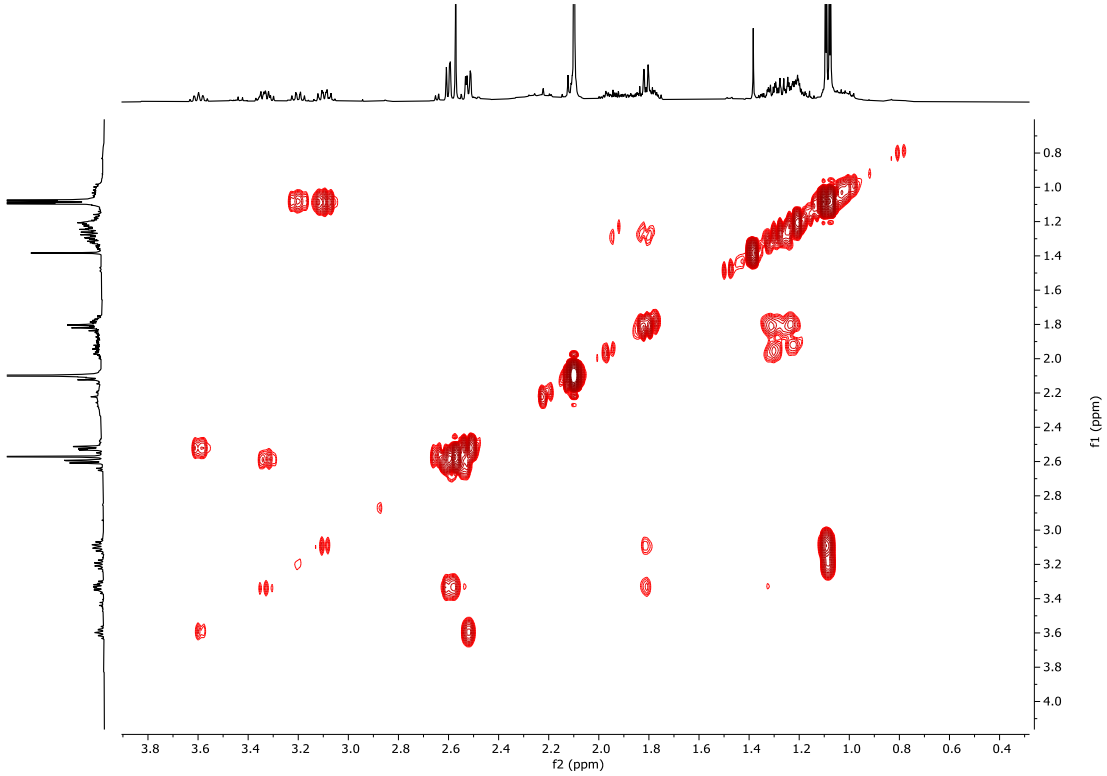

**A58:** HMBC NMR (500 MHz, $CDCl_3$) of compound **5f**.



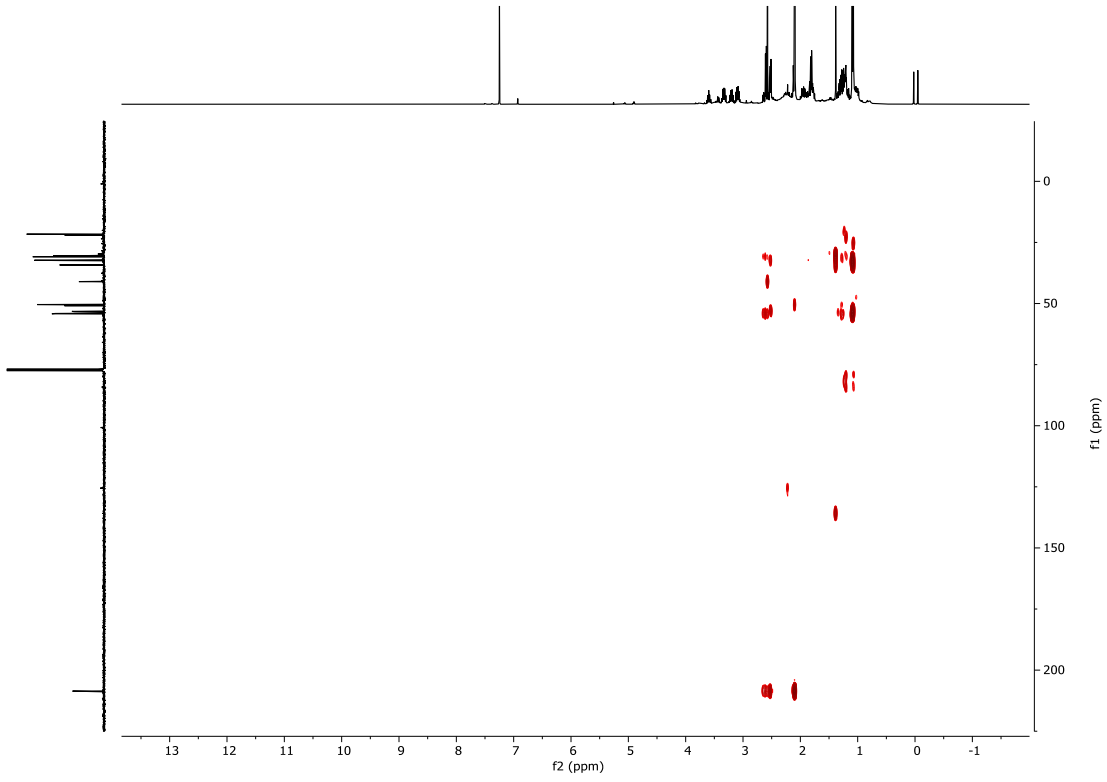

**A59:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **6a.**



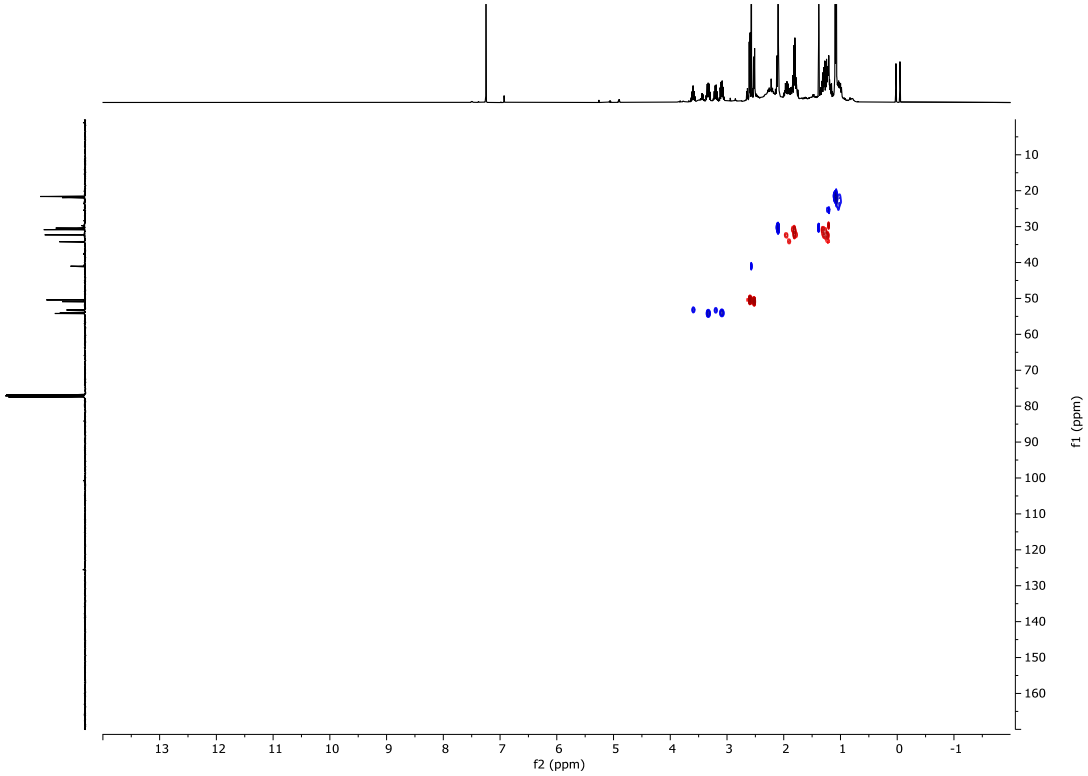

**A60:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **6a.**



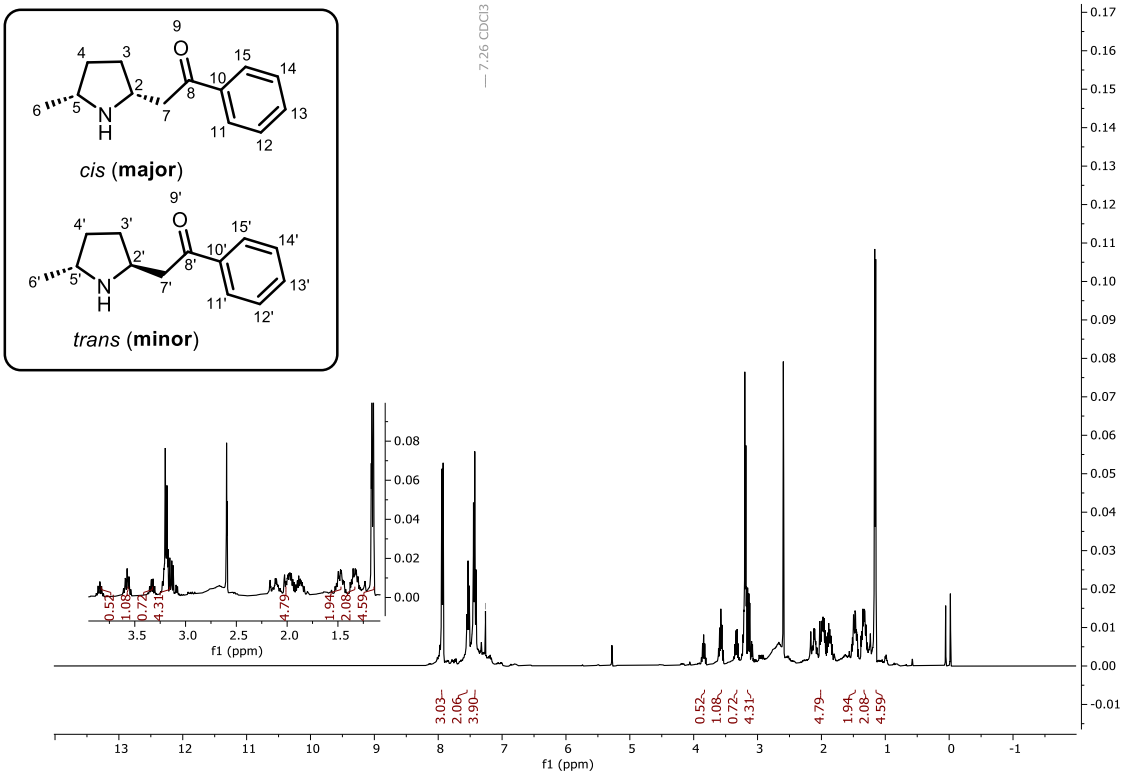

**A61:** COSY NMR (400 MHz, $CDCl_3$) of compound **6a.**



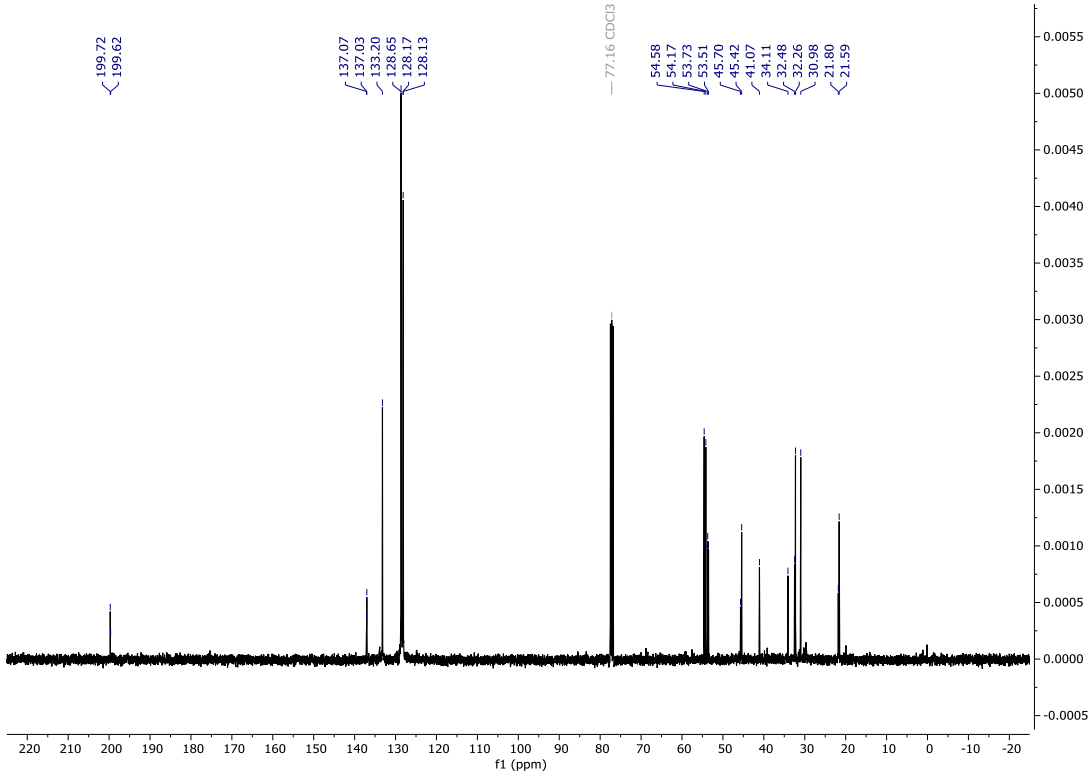

**A62:** HMBC NMR (400 MHz, $CDCl_3$) of compound **6a**.



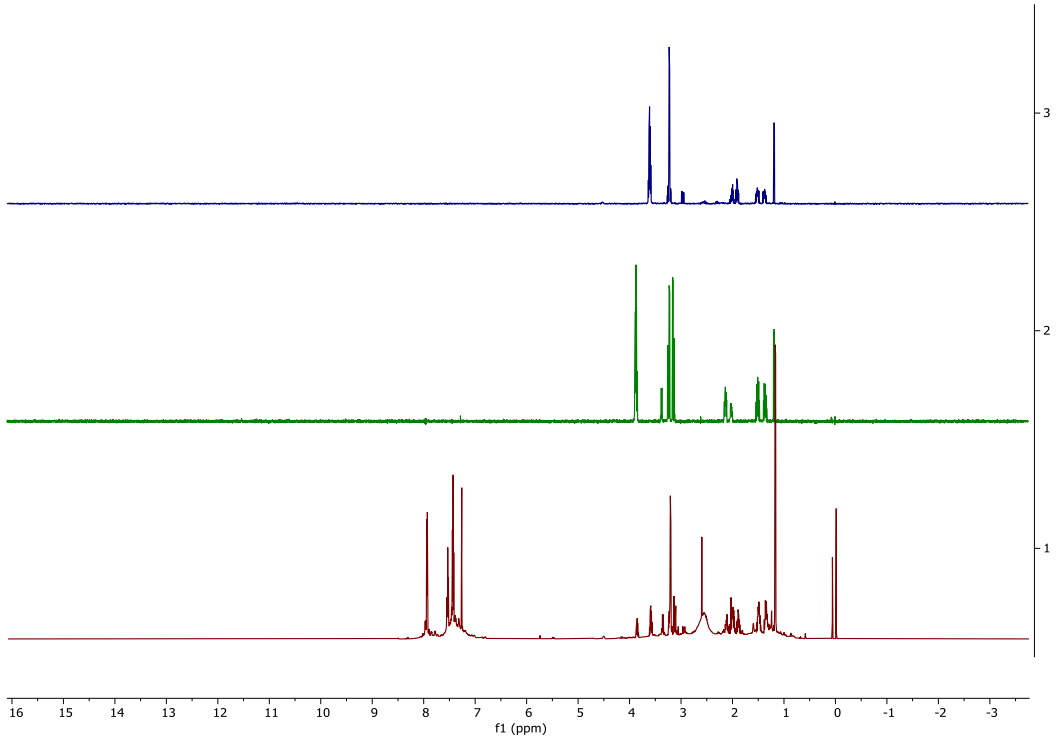

**A63:** HSQC NMR (400 MHz, $CDCl_3$) of compound **6a**.



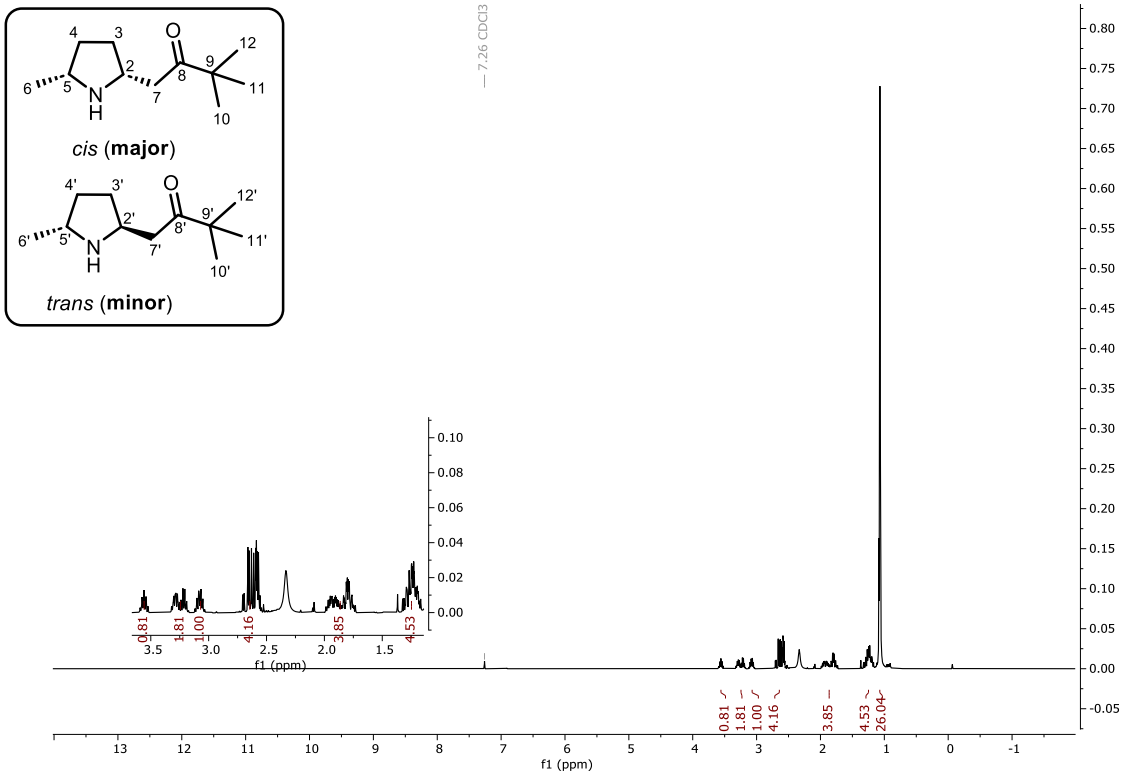

**A64:** $^1$H-NMR (400 MHz, $CDCl_3$) of compound **6b.**



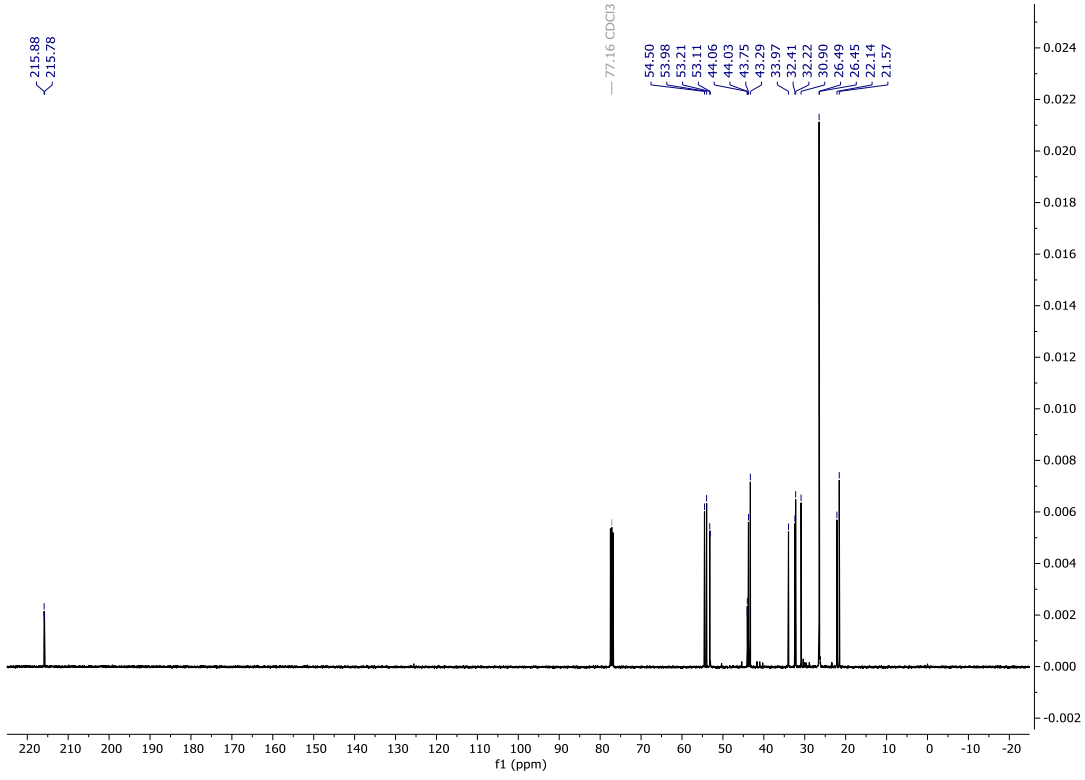

**A65:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **6b.**



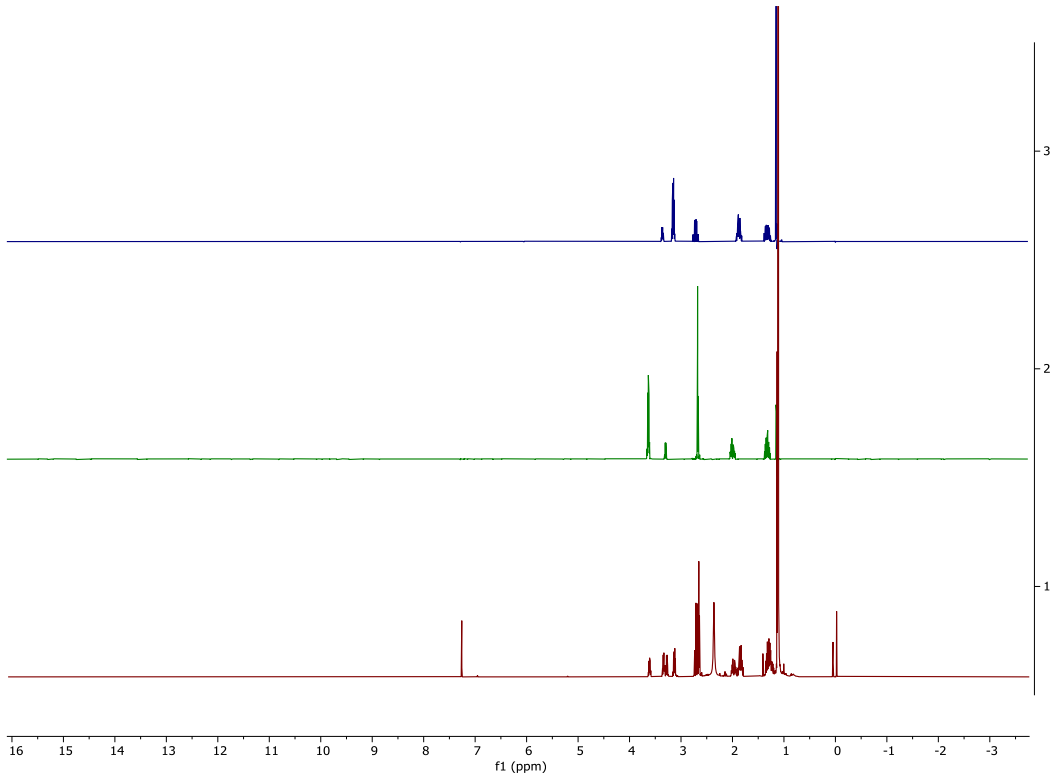

**A66:** Stacked selTOCSY NMR (600 MHz, $CDCl_3$) of compound **6b**. Top spectrum = *cis*-**6b**. Middle spectrum = *trans*-**6b**. Bottom spectrum = mixture of *cis*-**6b** and *trans*-**6b.**



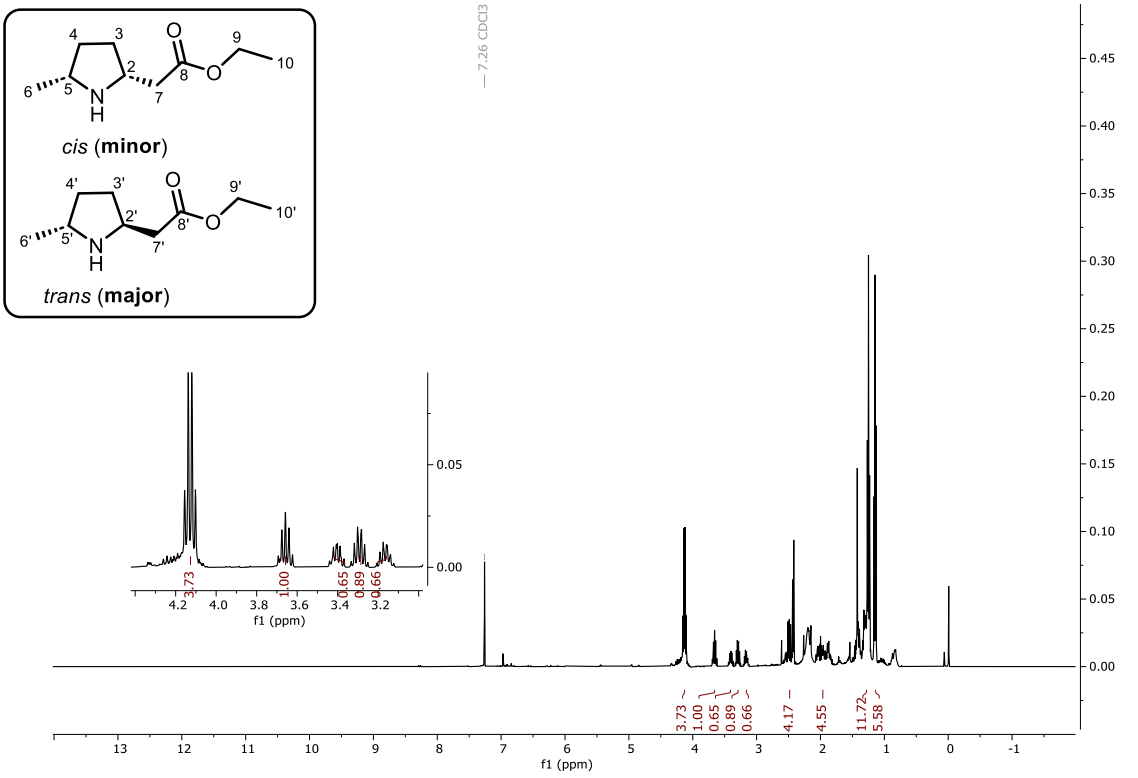

**A67:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **6c.**



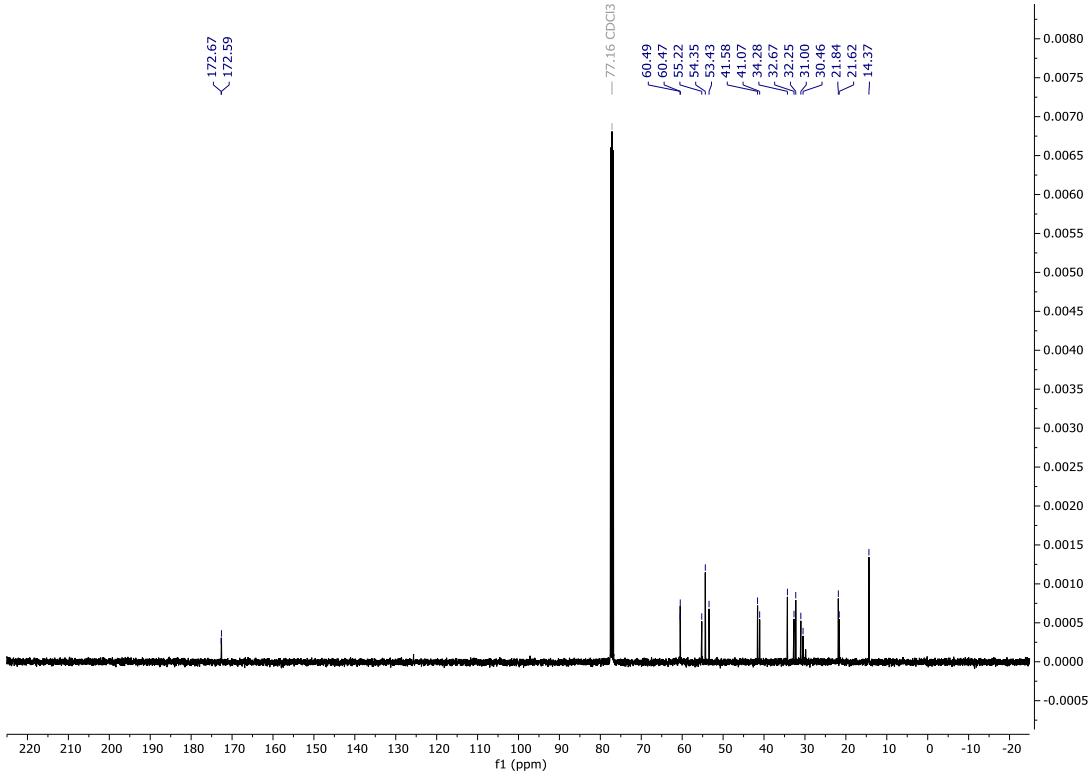

**A68:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **6c.**



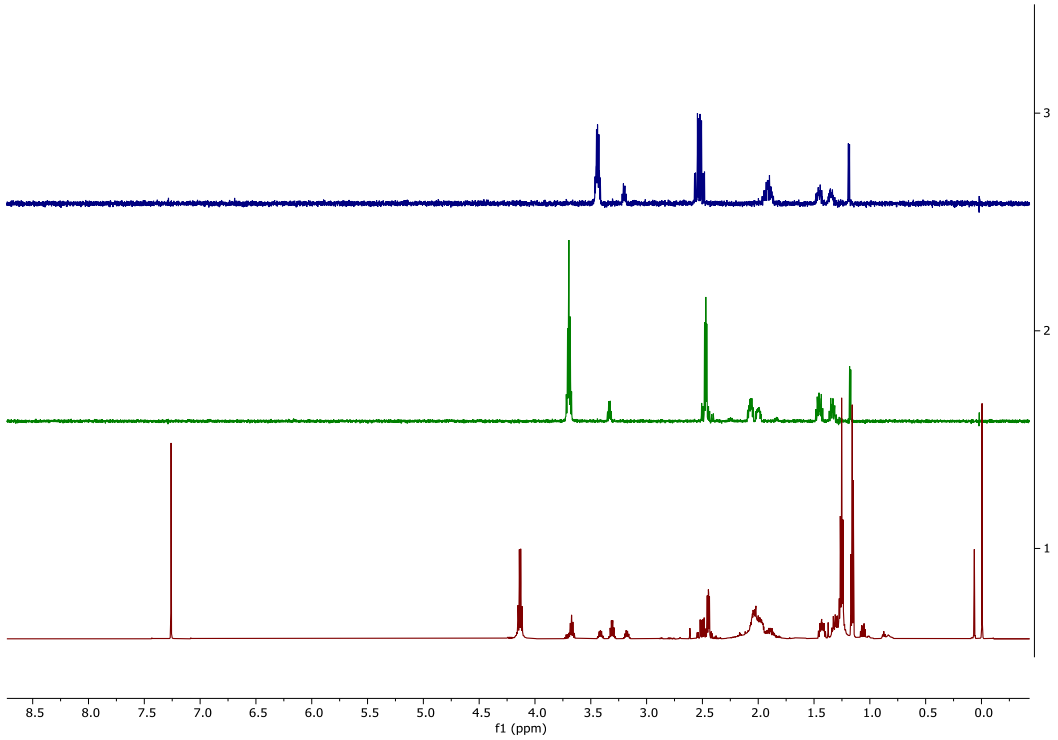

**A69:** Stacked selTOCSY NMR (600 MHz, $CDCl_3$) of compound **6c**. Top spectrum = *cis*-**6c**. Middle spectrum = *trans*-**6c**. Bottom spectrum = mixture of *cis*-**6c** and *trans*-**6c.**



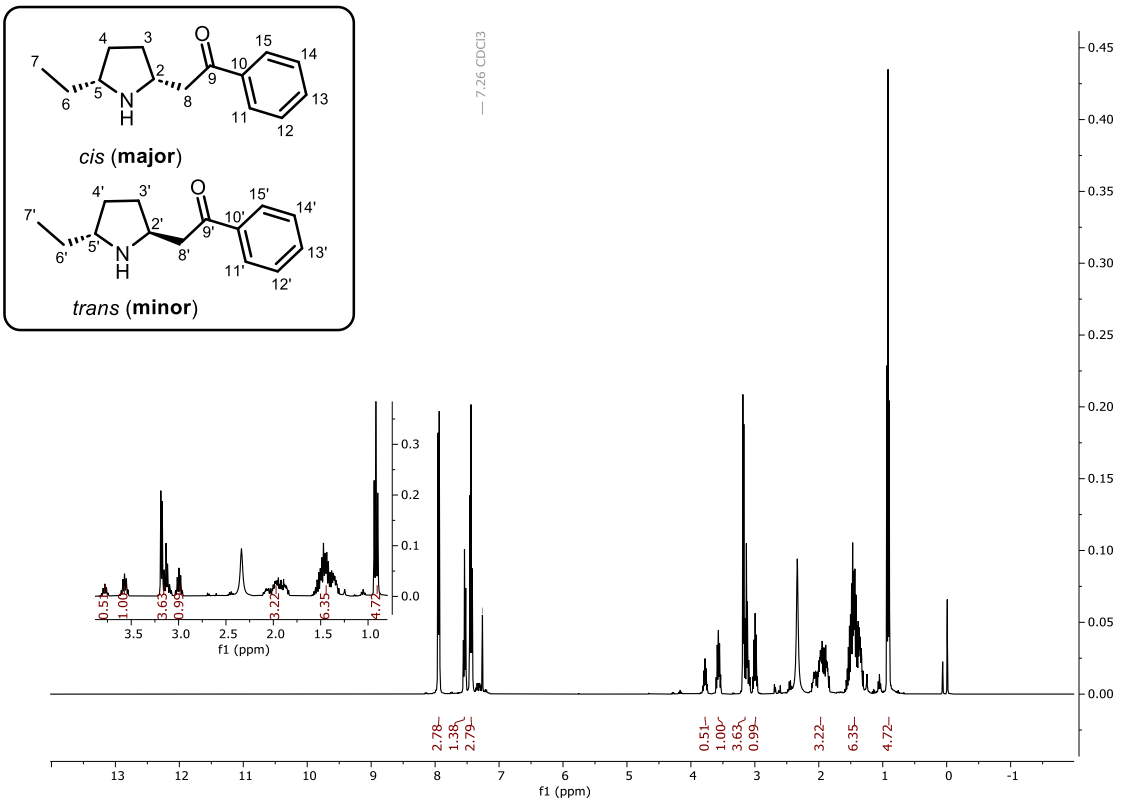

**A70:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **6d.**



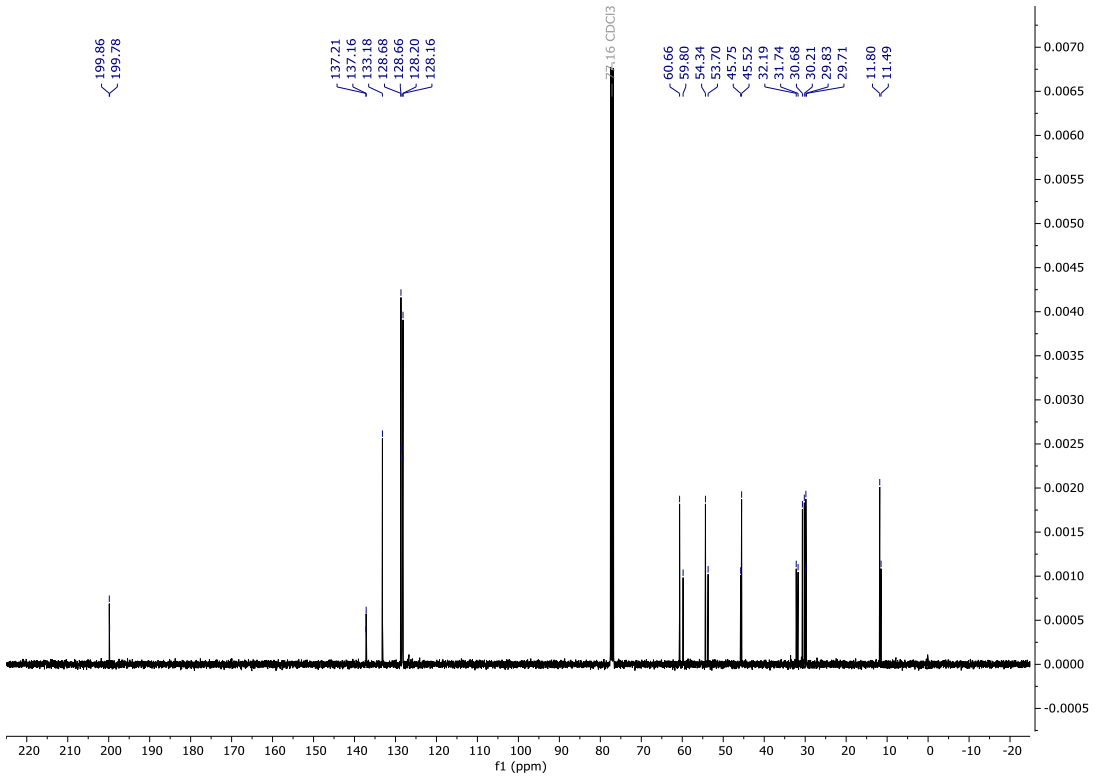

**A71:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **6d.**



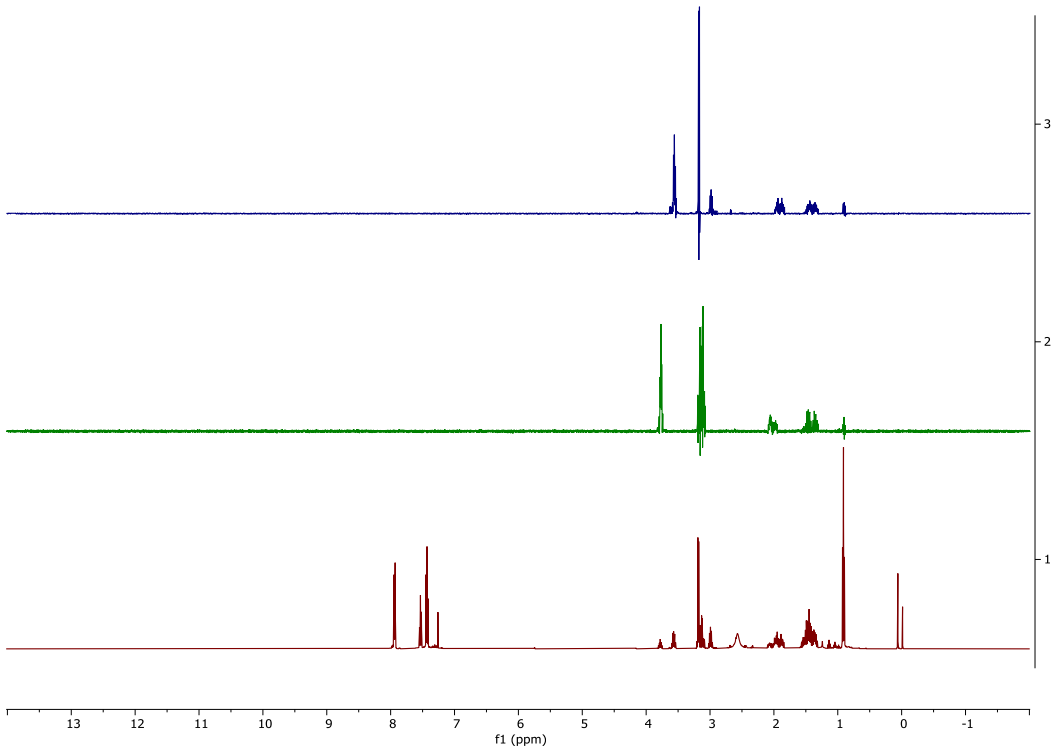

**A72:** Stacked selTOCSY NMR (600 MHz, $CDCl_3$) of compound **6d**. Top spectrum = *cis*-**6d**. Middle spectrum = *trans*-**6d**. Bottom spectrum = mixture of *cis*-**6d** and *trans*-**6d.**



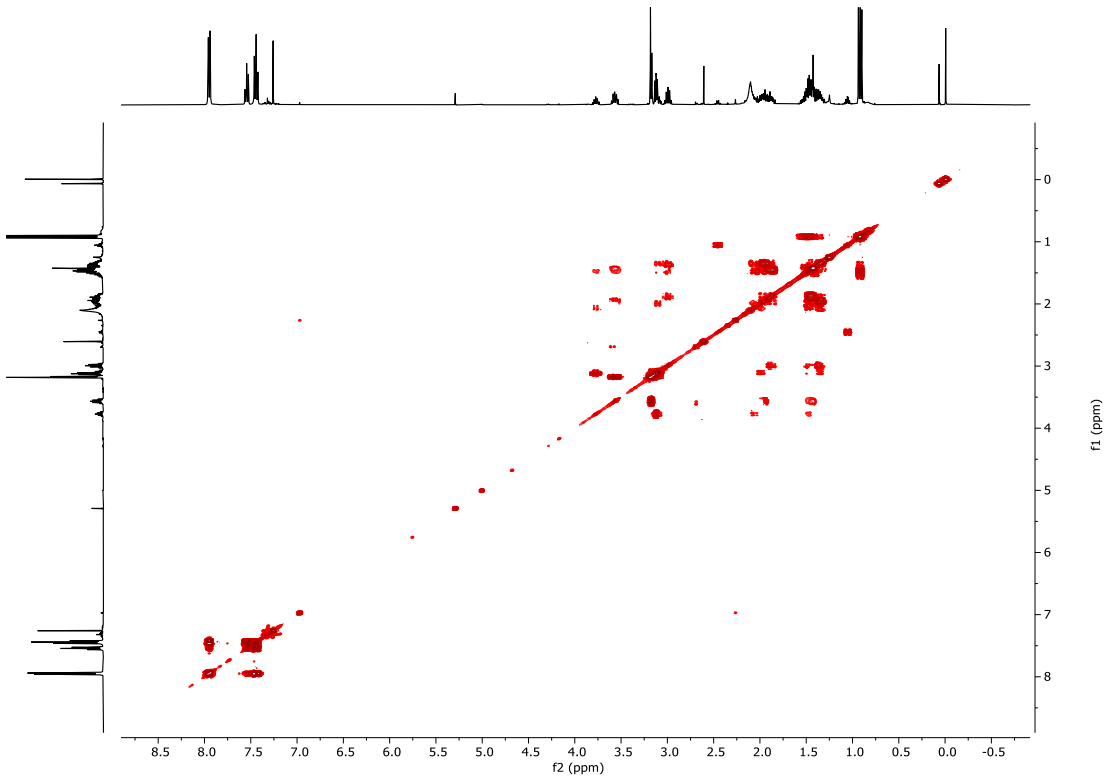

**A73:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **6e** from ATA251 reaction.



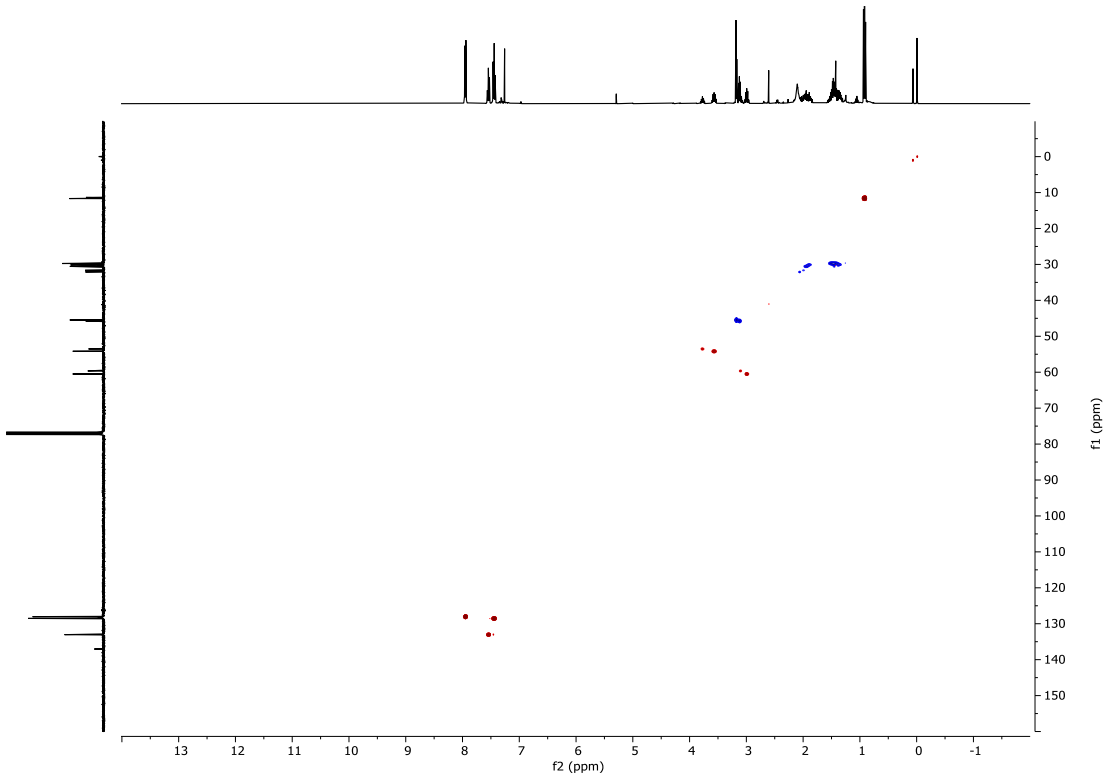

**A74:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **6e** from ATA251 reaction.



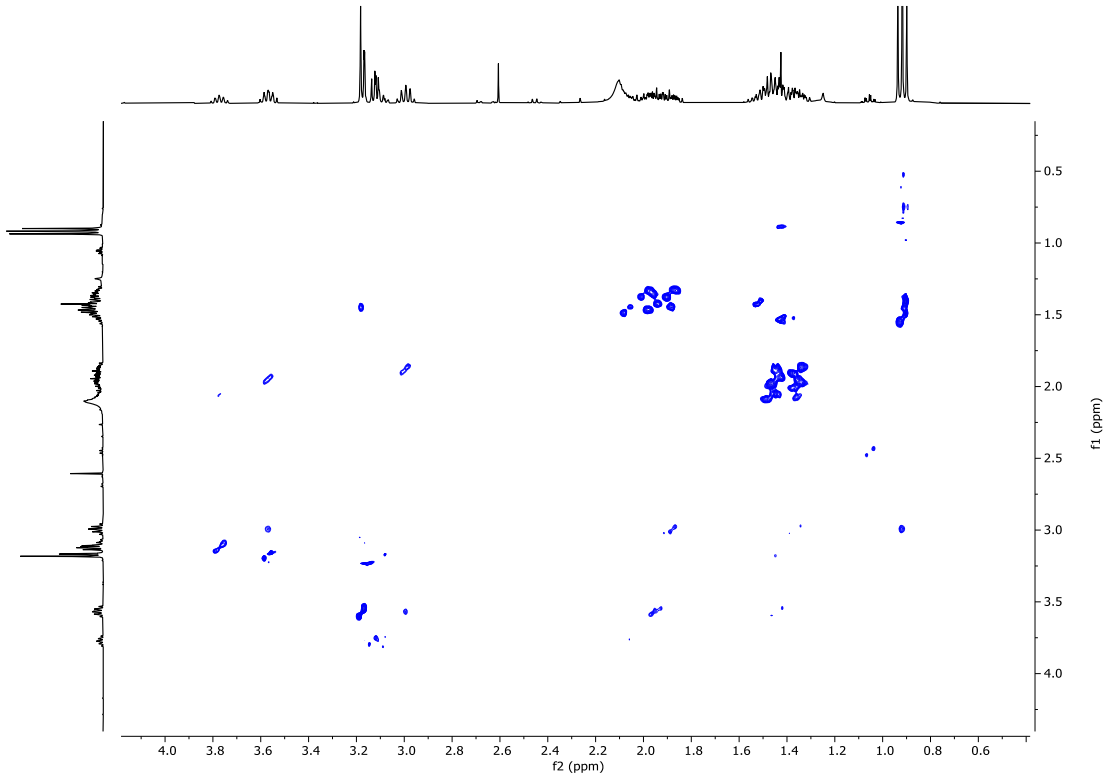

**A75:** Stacked selTOCSY NMR (500 MHz, $CDCl_3$) of compound **6e** from ATA251 reaction. Top spectrum = *cis*-**6e**. Middle spectrum = *trans*-**6e**. Bottom spectrum = mixture of *cis*-**6e** and *trans*-**6e**.



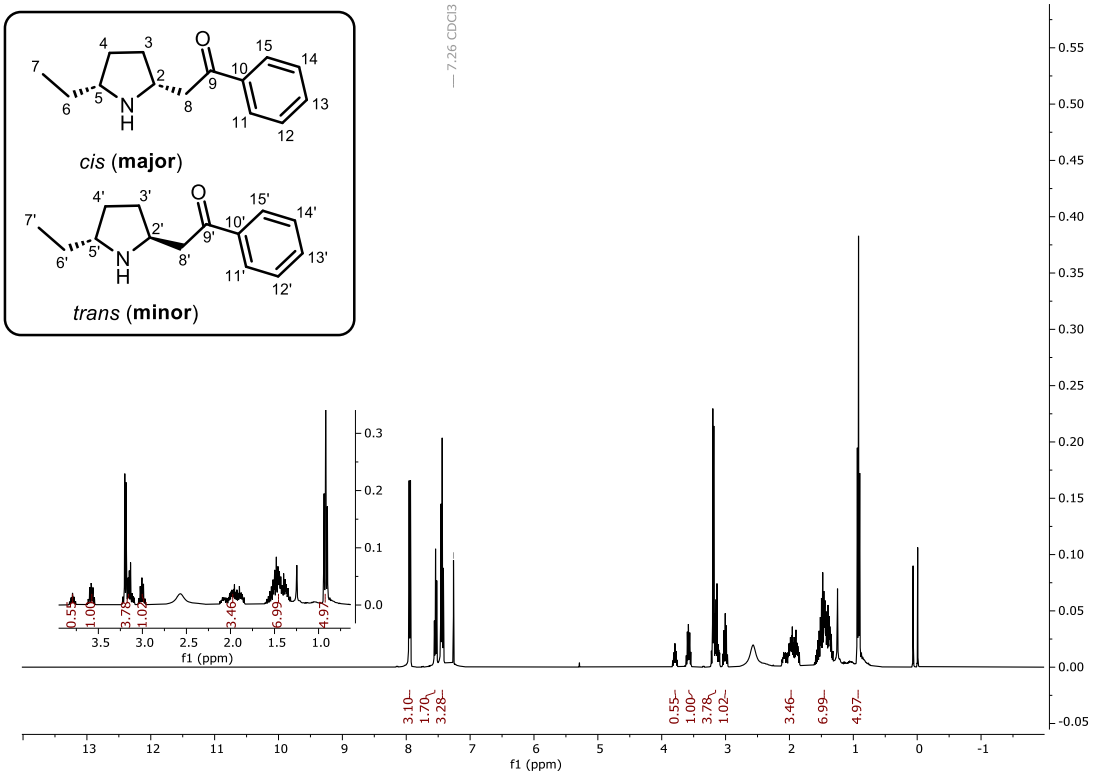

**A76:** COSY NMR (400 MHz, $CDCl_3$) of compound **6e** from ATA251 reaction.



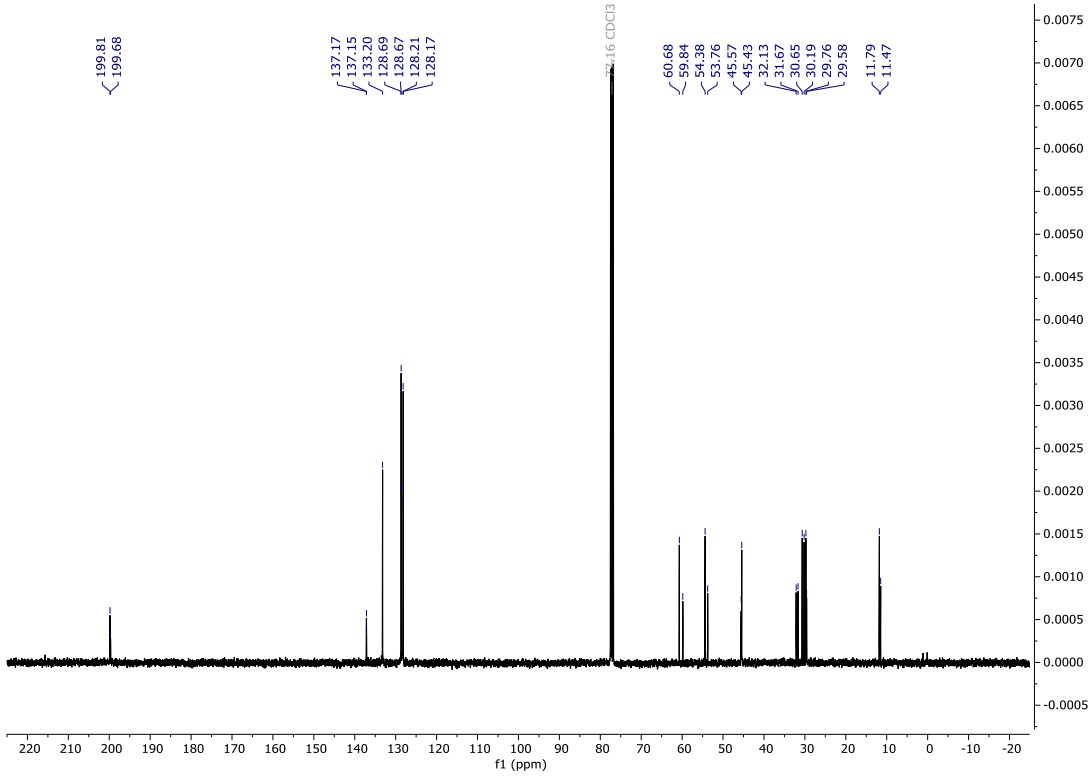

**A77:** HSQC NMR (400 MHz, $CDCl_3$) of compound **6e** from ATA251 reaction.



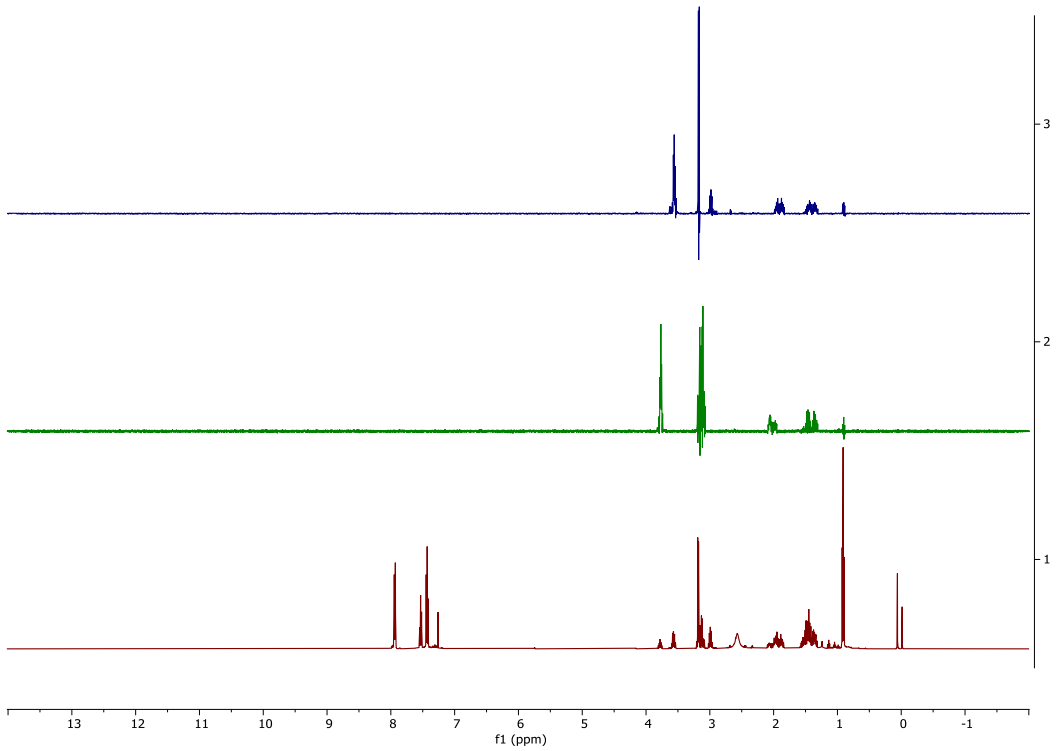

**A78:** NOESY (500 MHz, $CDCl_3$) of compound **6e** from ATA251 reaction.




**A79:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **6e** from ATA025 reaction.

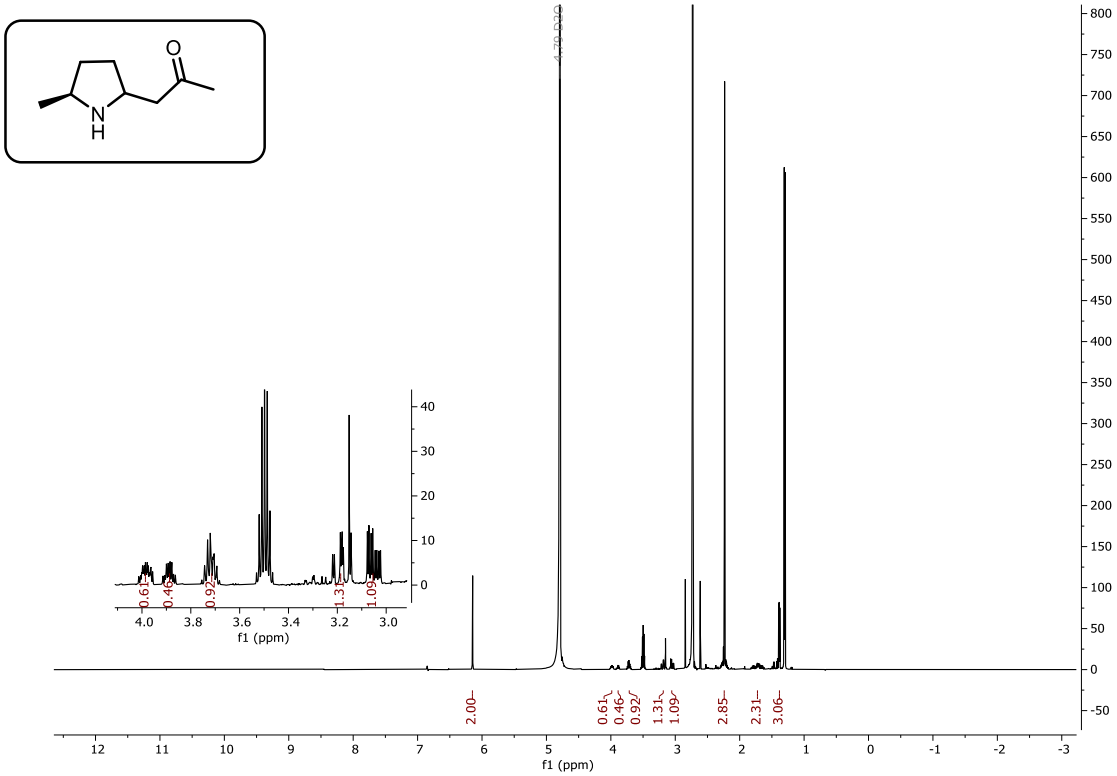



**A80:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **6e** from ATA025 reaction.

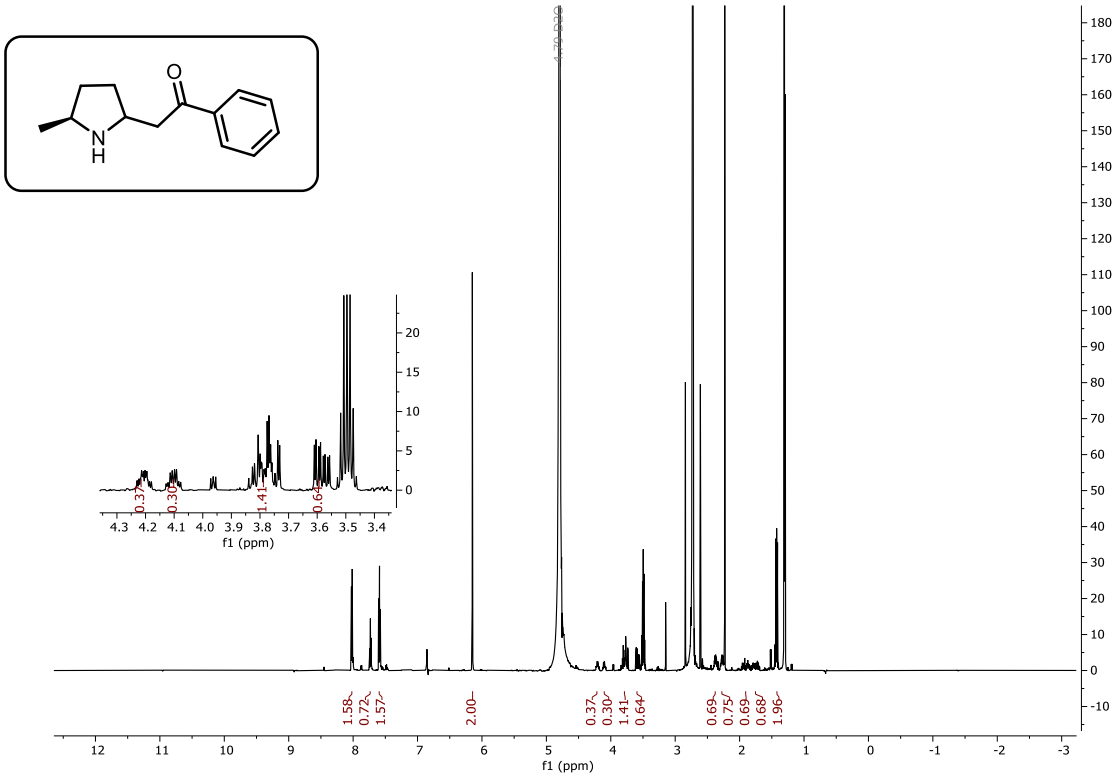



**A81:** Stacked selTOCSY NMR (500 MHz, $CDCl_3$) of compound **6e**. Top spectrum = *cis*-**6e**. Middle spectrum = *trans*-**6e**. Bottom spectrum = mixture of *cis*-**6e** and *trans*-**6e.**

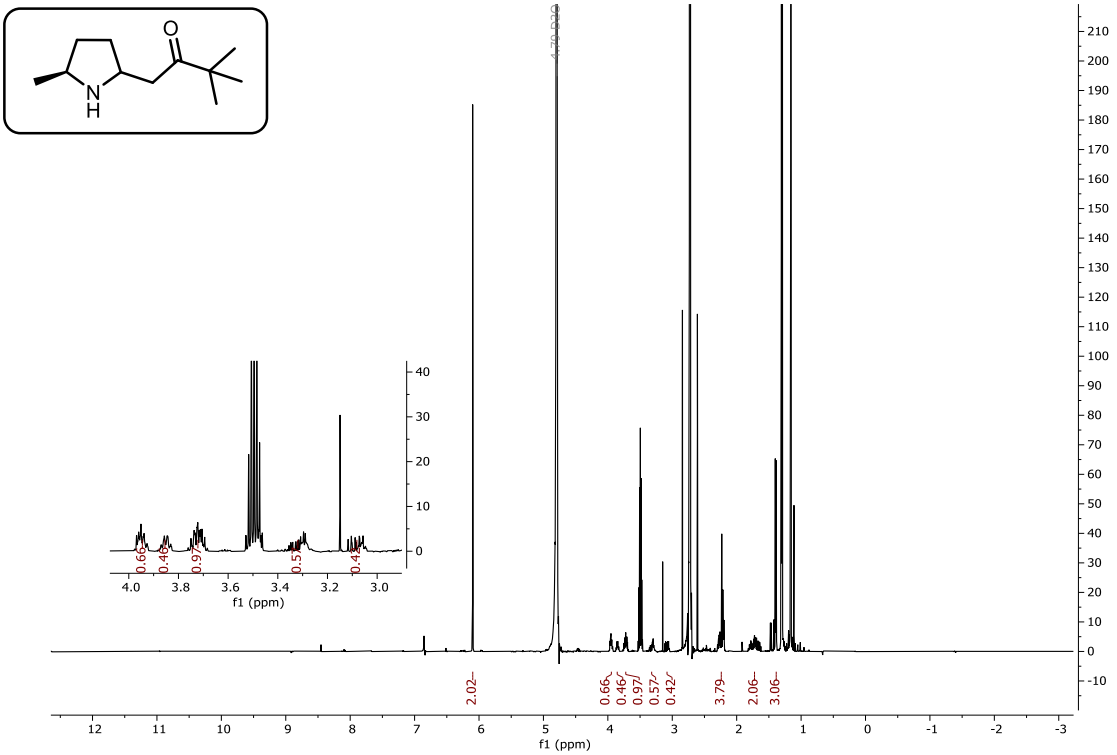
# Water Suppression $^{1}$H-NMR Spectra



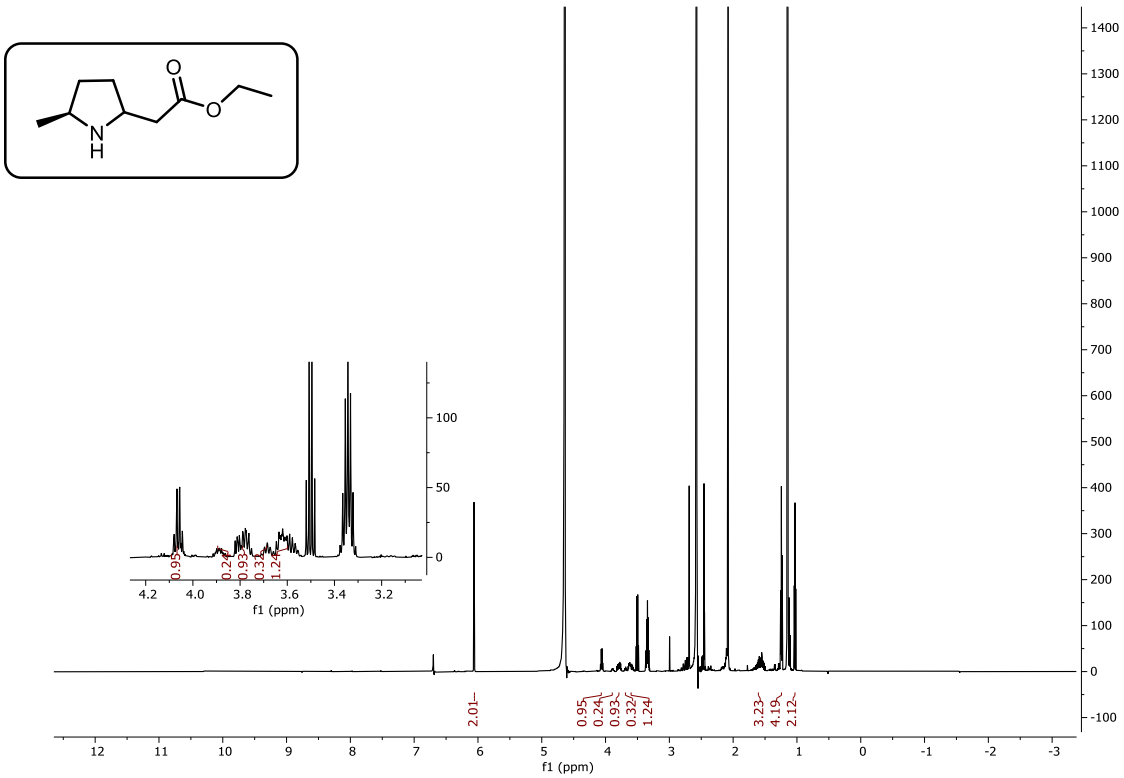

**A82:** Water suppression $^{1}$H-NMR (600 MHz, $D_2O$) of compound **5a.**



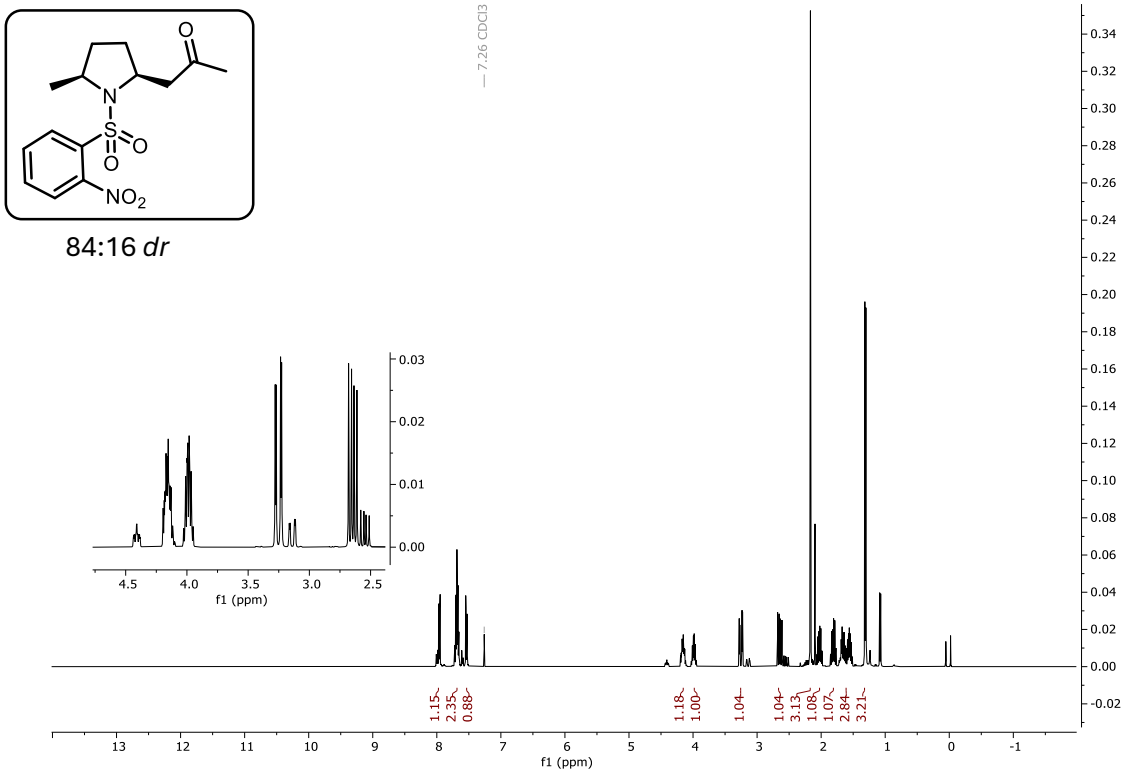

**A83:** Water suppression $^{1}$H-NMR (600 MHz, $D_2O$) of compound **5b.**



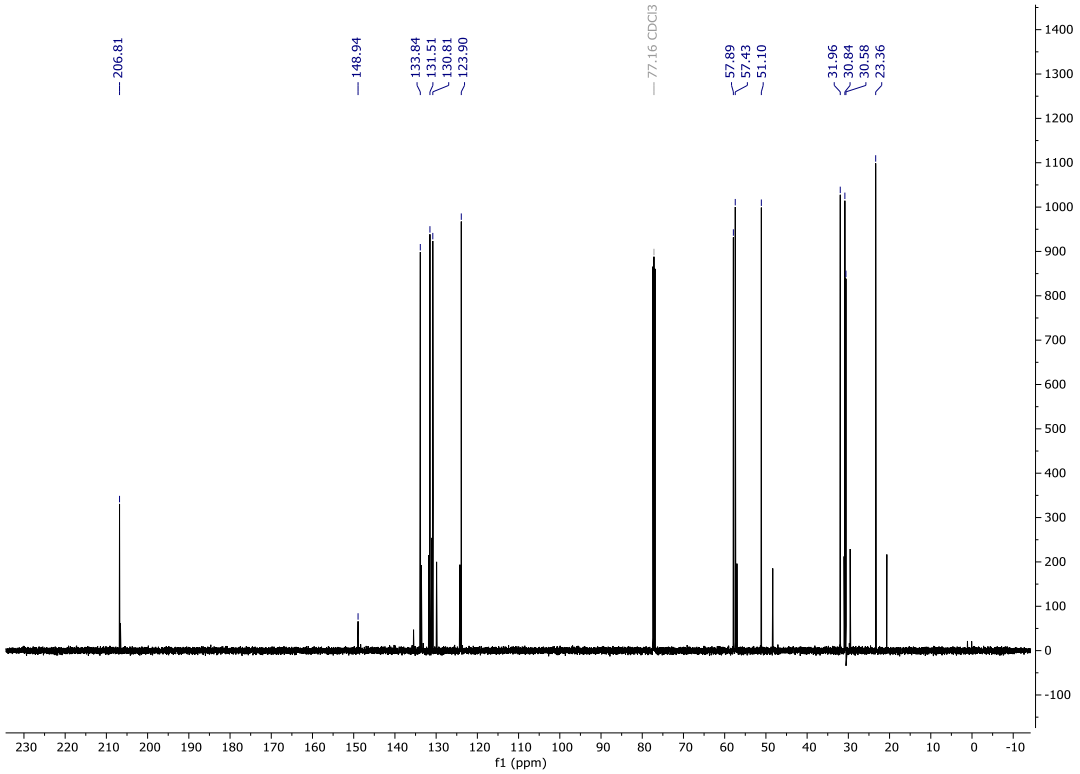

**A84:** Water suppression $^{1}$H-NMR (600 MHz, $D_2O$) of compound **5c.**



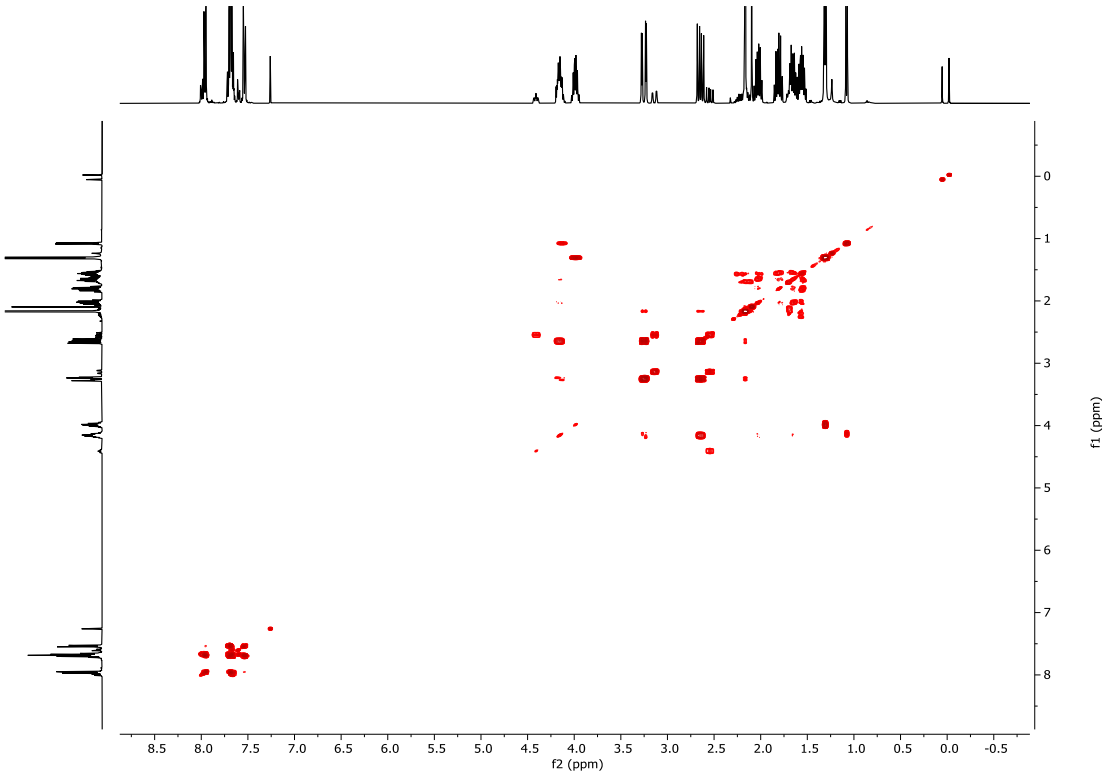

**A85:** Water suppression $^{1}$H-NMR (600 MHz, $D_2O$) of compound **5d.**



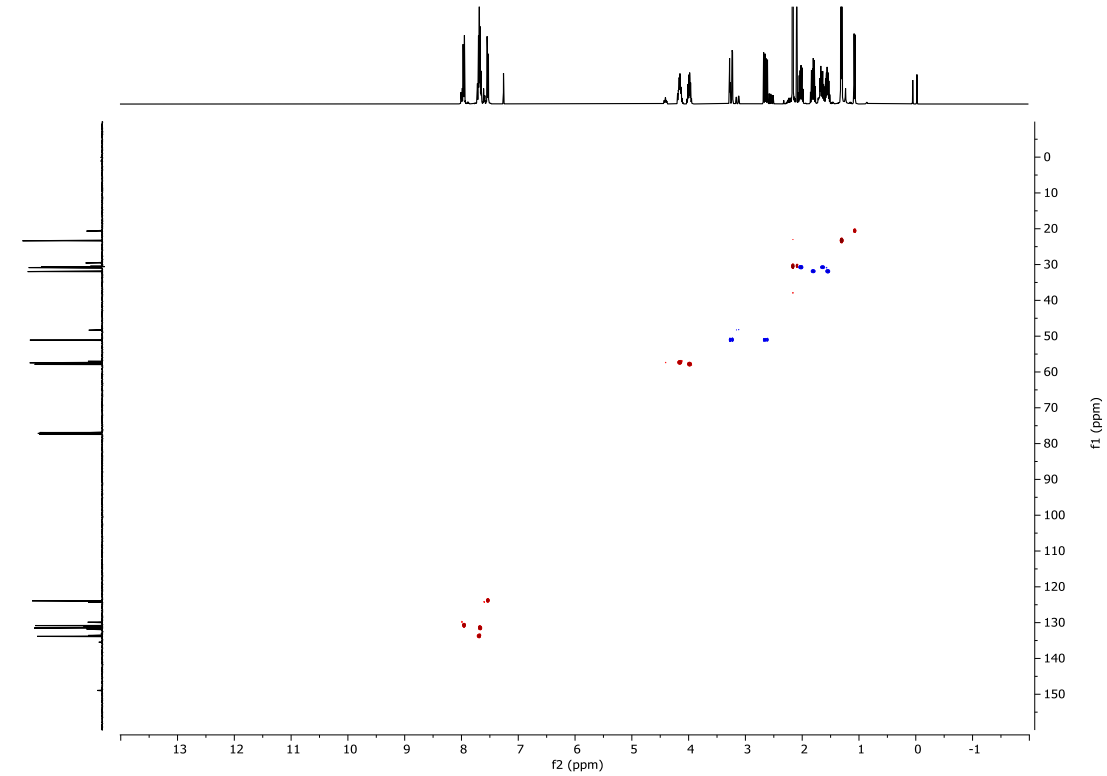

**A86:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **7a.**



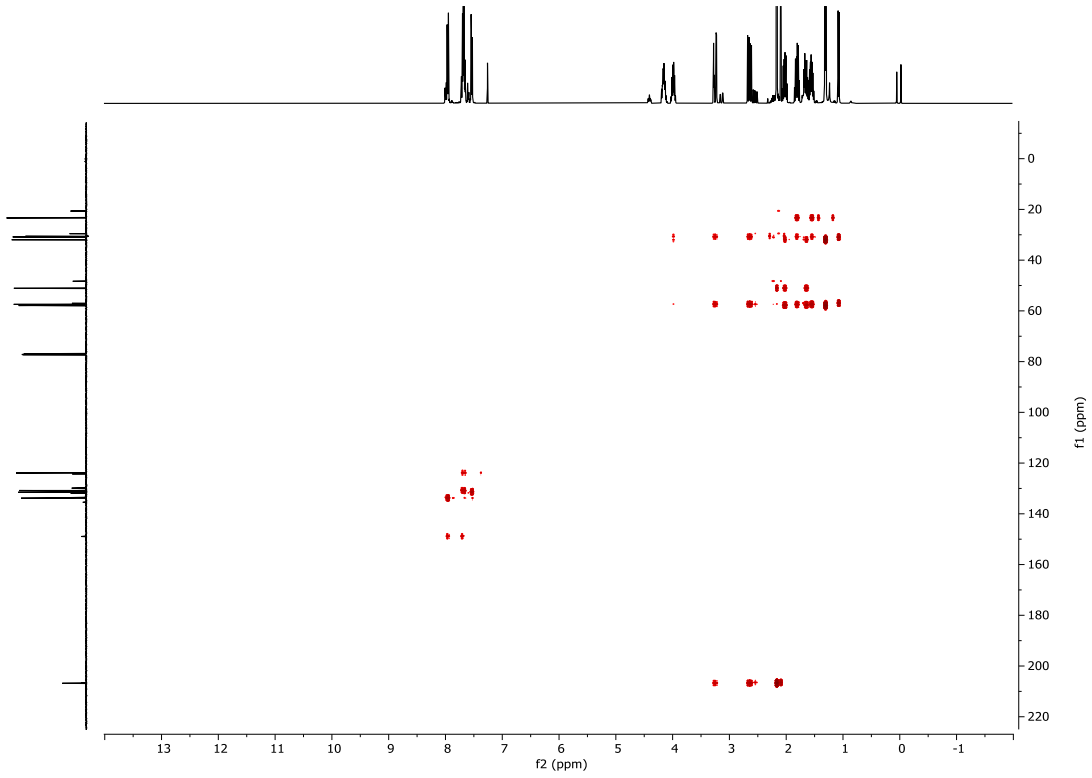

**A87:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **7a.**



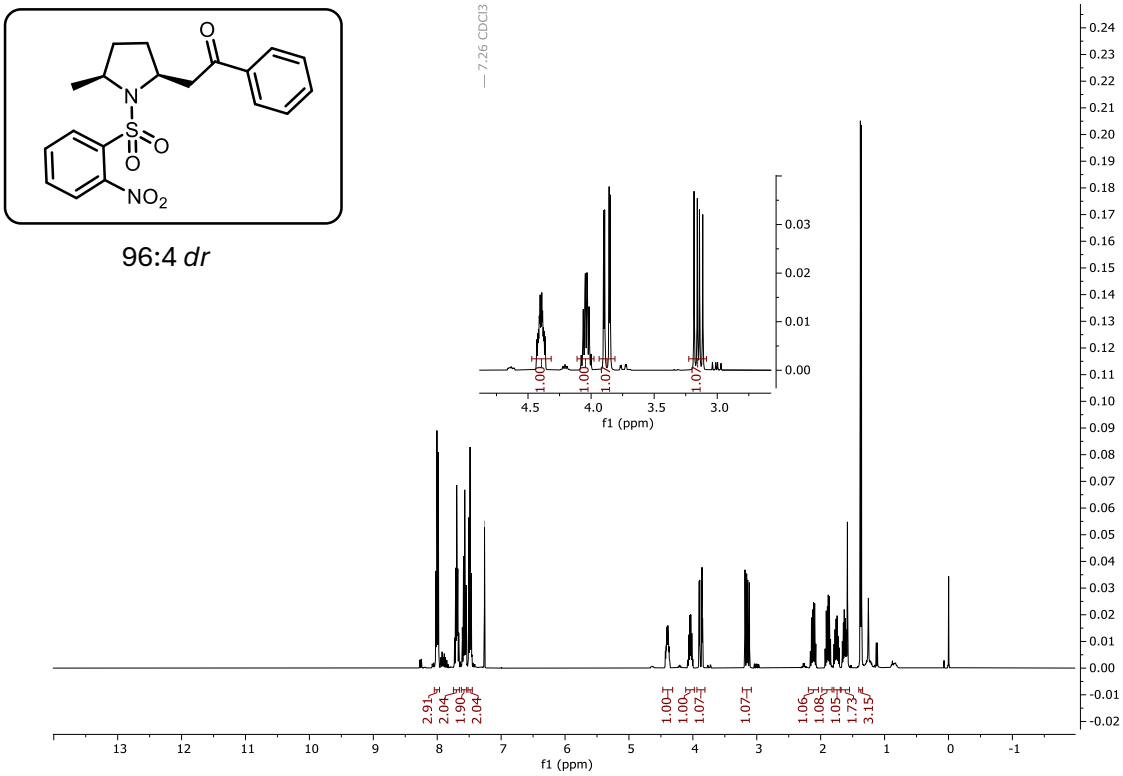

**A88:** COSY NMR (400 MHz, $CDCl_3$) of compound **7a.**



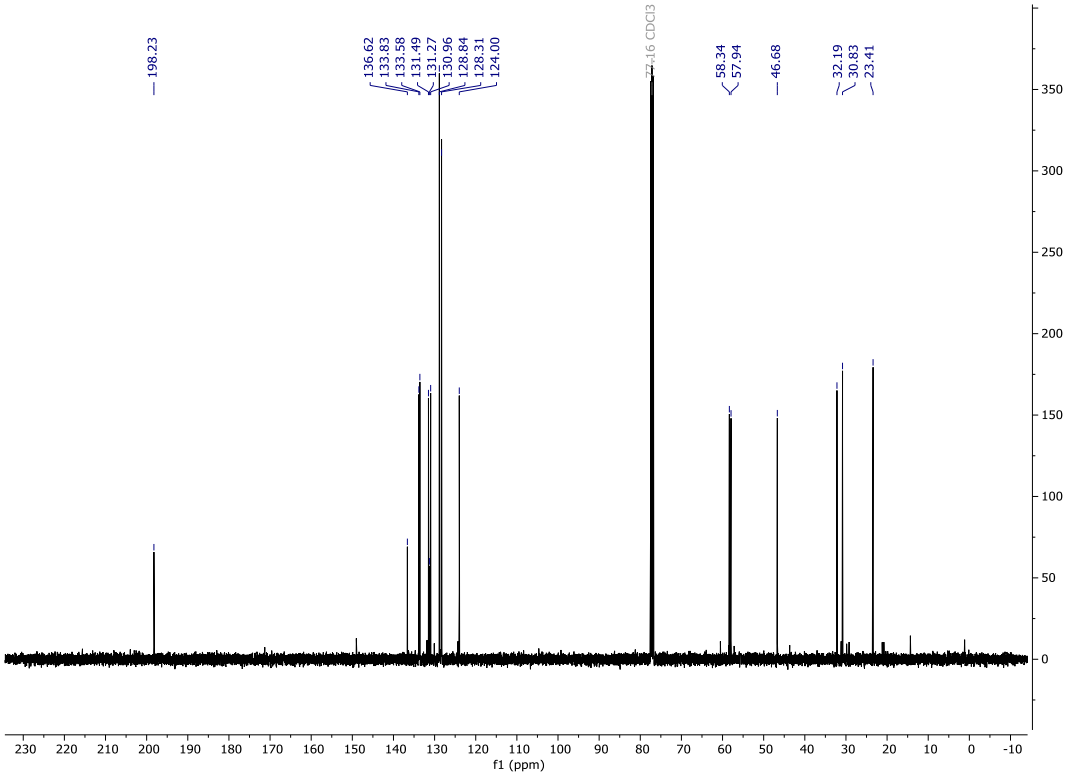

**A89:** HSQC NMR (400 MHz, $CDCl_3$) of compound **7a.**



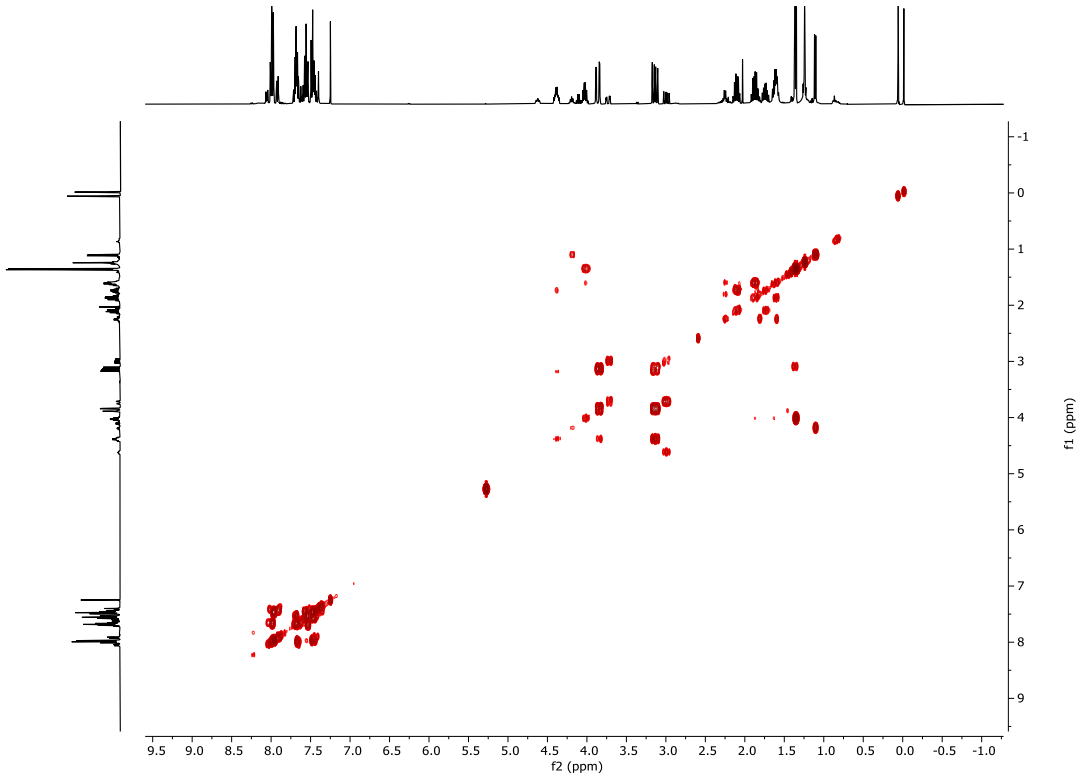

**A90:** HMBC NMR (400 MHz, $CDCl_3$) of compound **7a.**



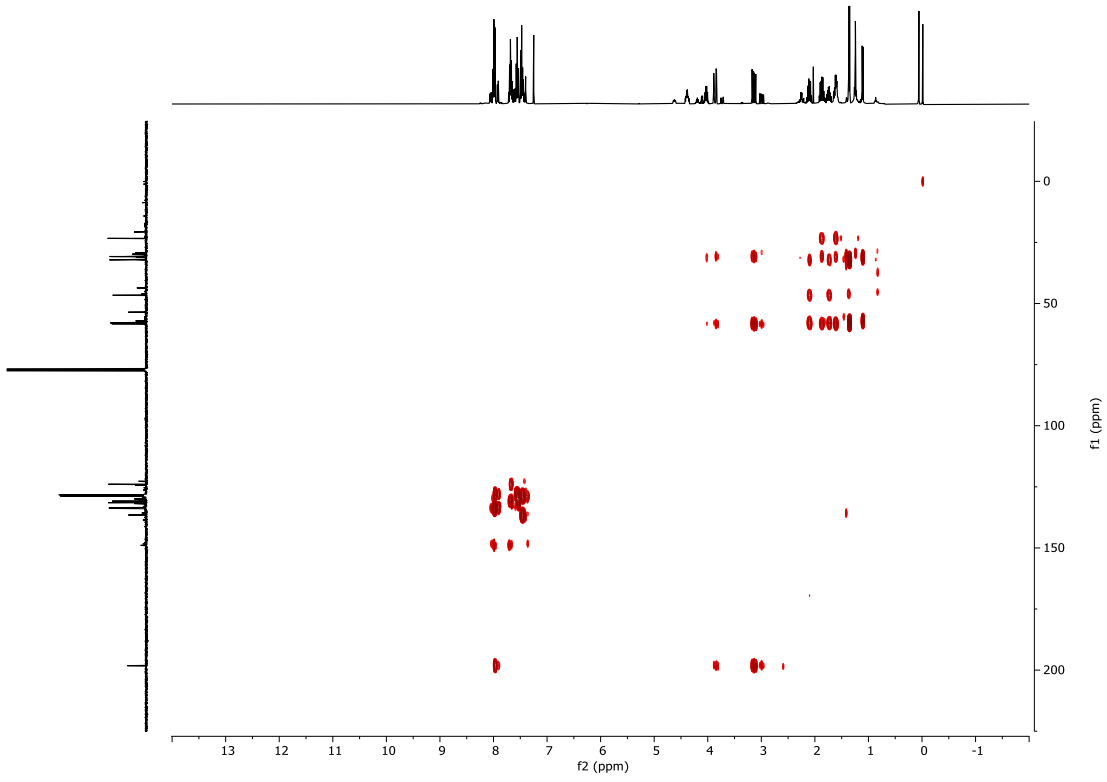

**A91:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **7b.**



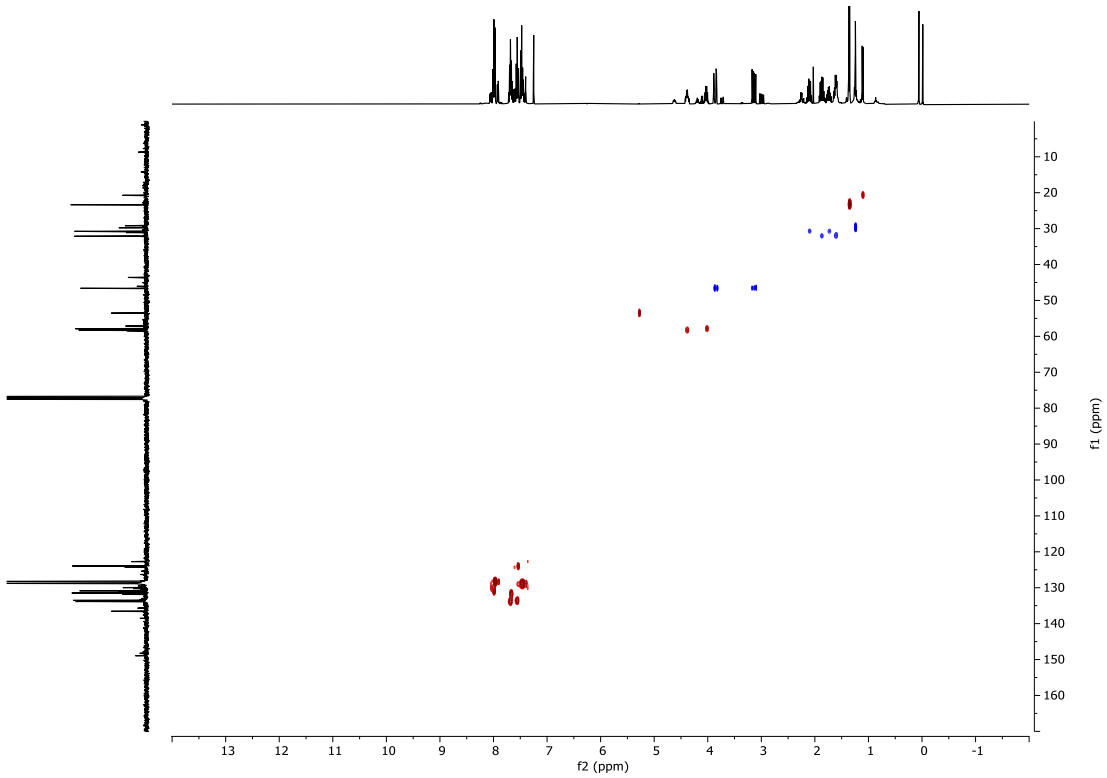

**A92:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **7b.**



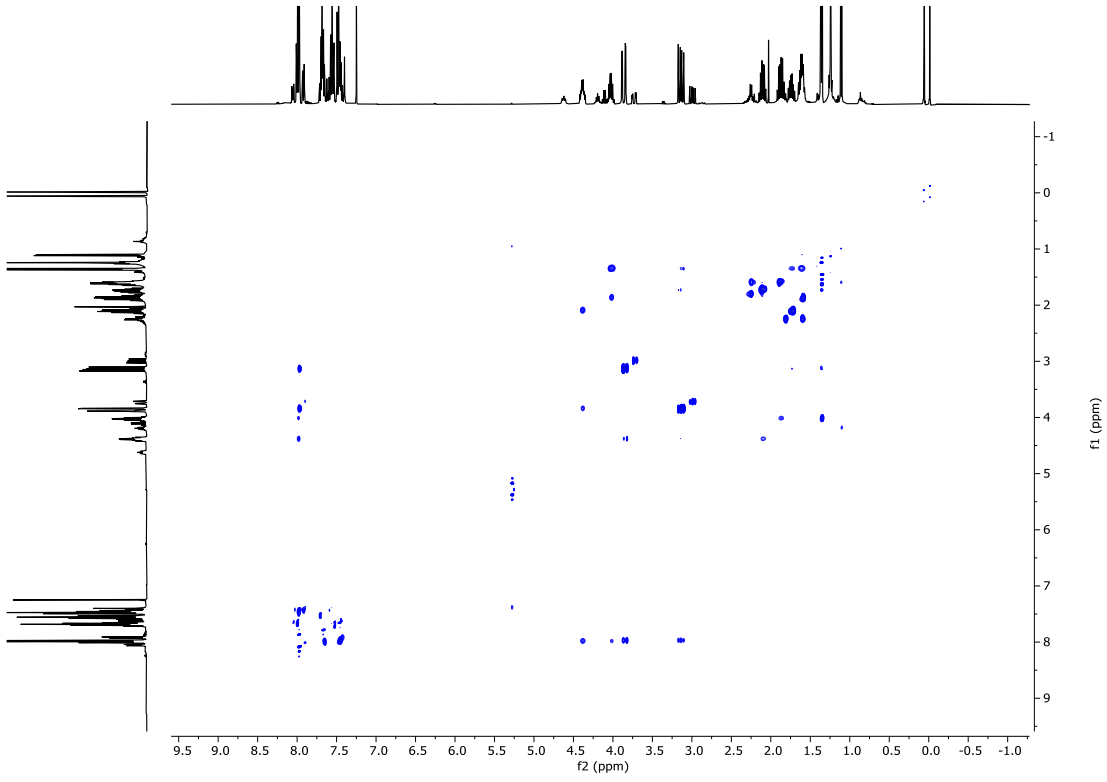

**A93:** COSY NMR (400 MHz, $CDCl_3$) of compound **7b**.



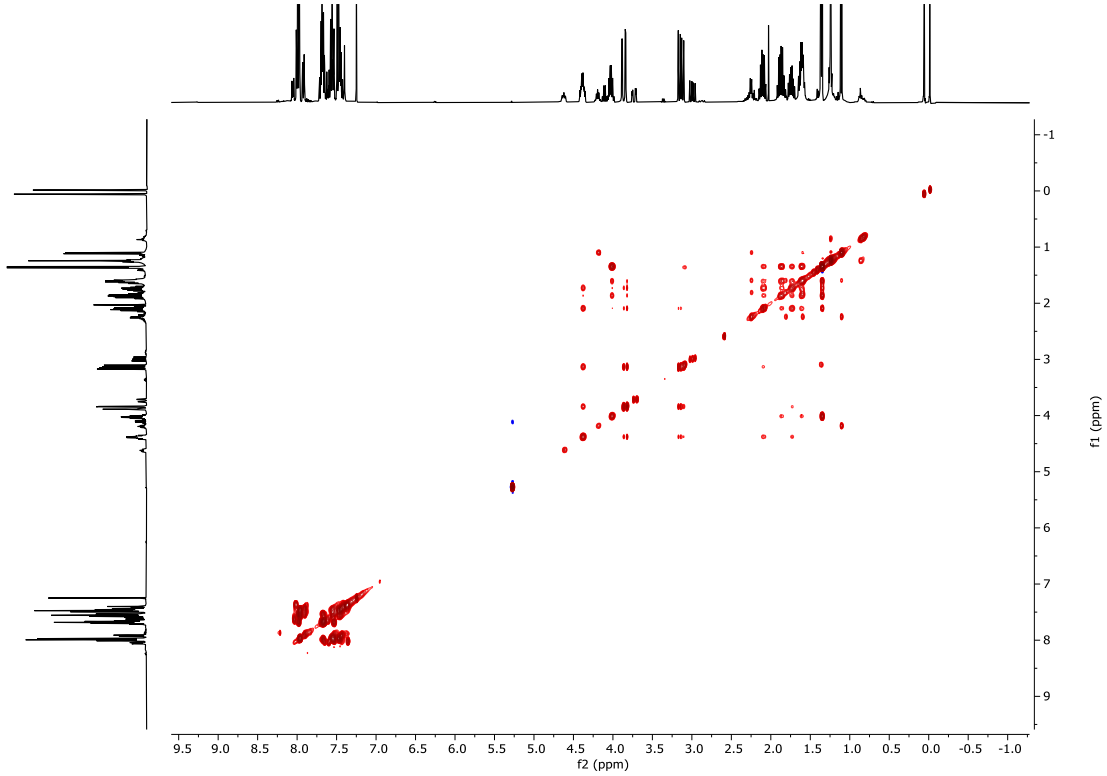

**A94:** HMBC NMR (400 MHz, $CDCl_3$) of compound **7b**.



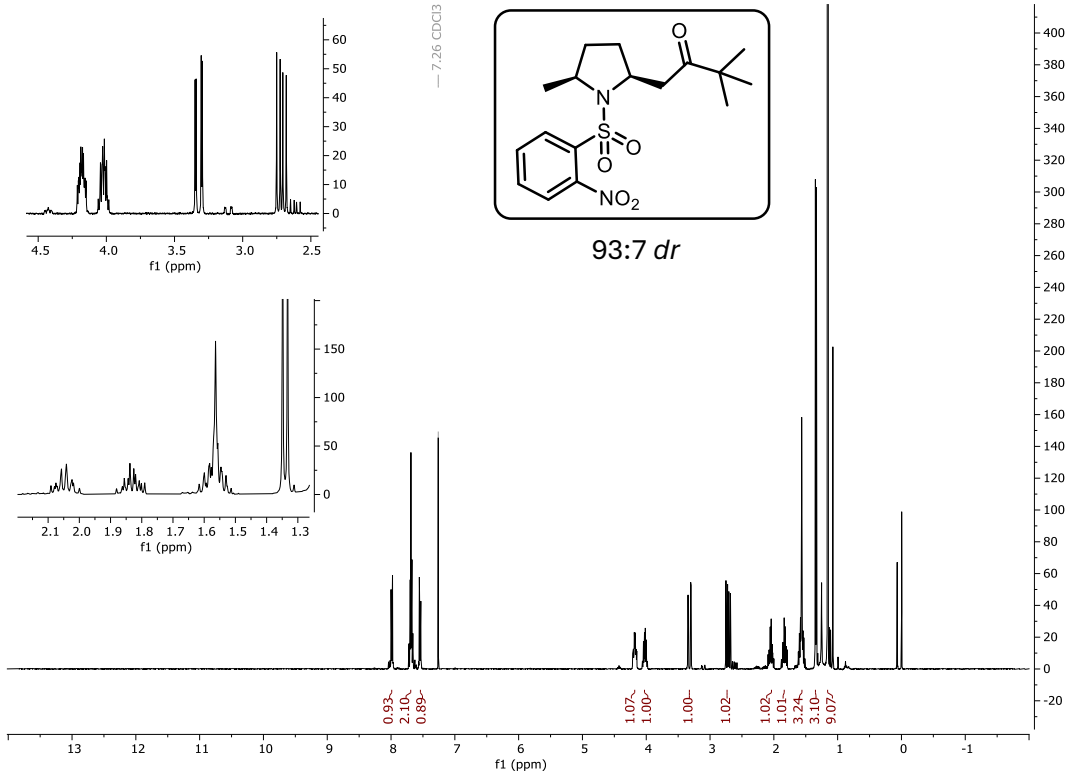

**A95:** HSQC NMR (400 MHz, $CDCl_3$) of compound **7b.**



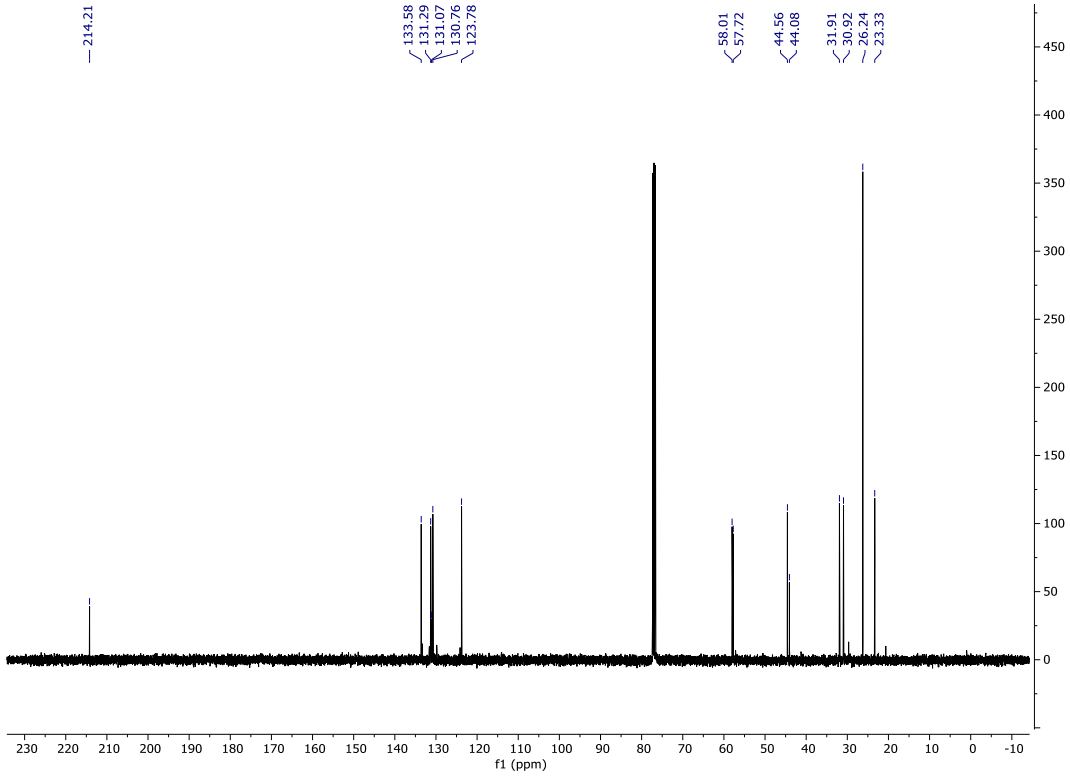

**A96:** NOESY NMR (400 MHz, $CDCl_3$) of compound **7b.**



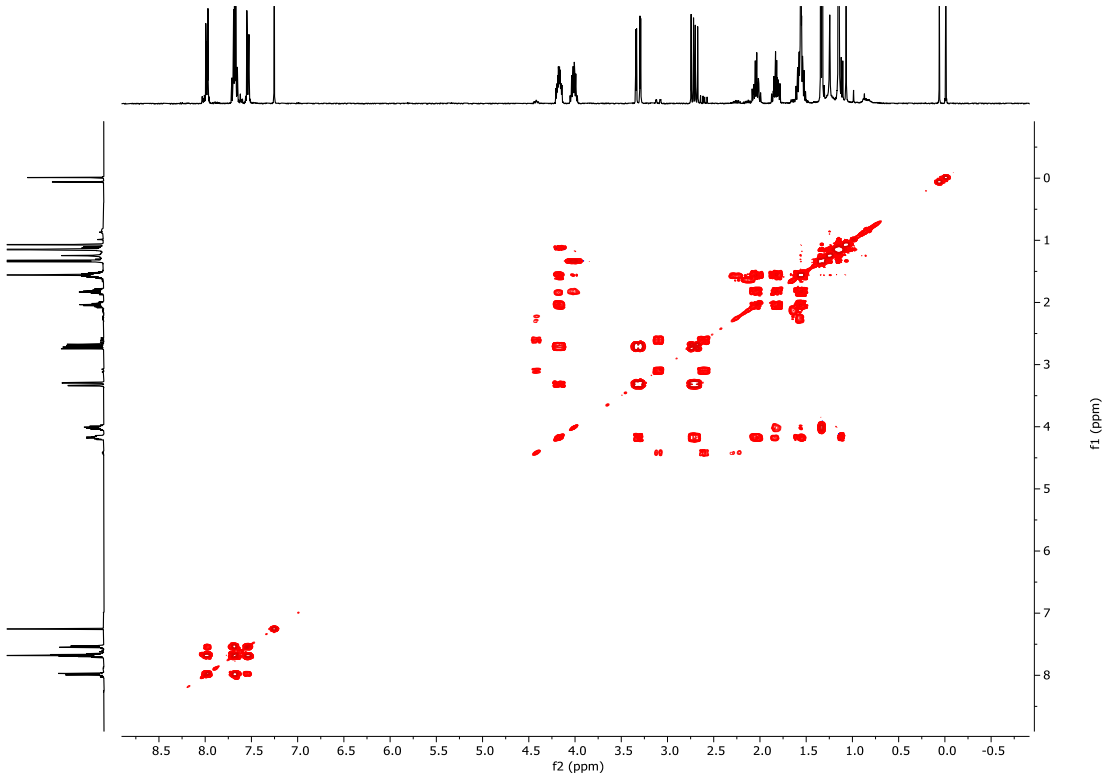

**A97:** TOCSY NMR (400 MHz, $CDCl_3$) of compound **7b.**



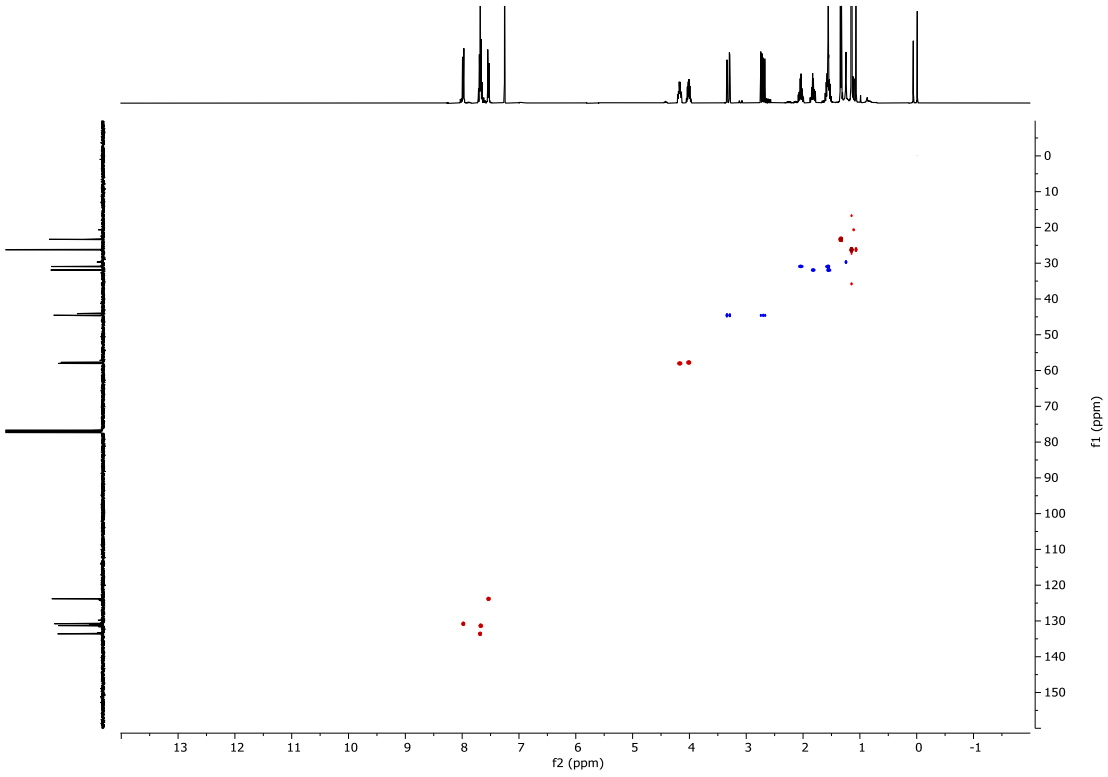

**A98:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **7c.**



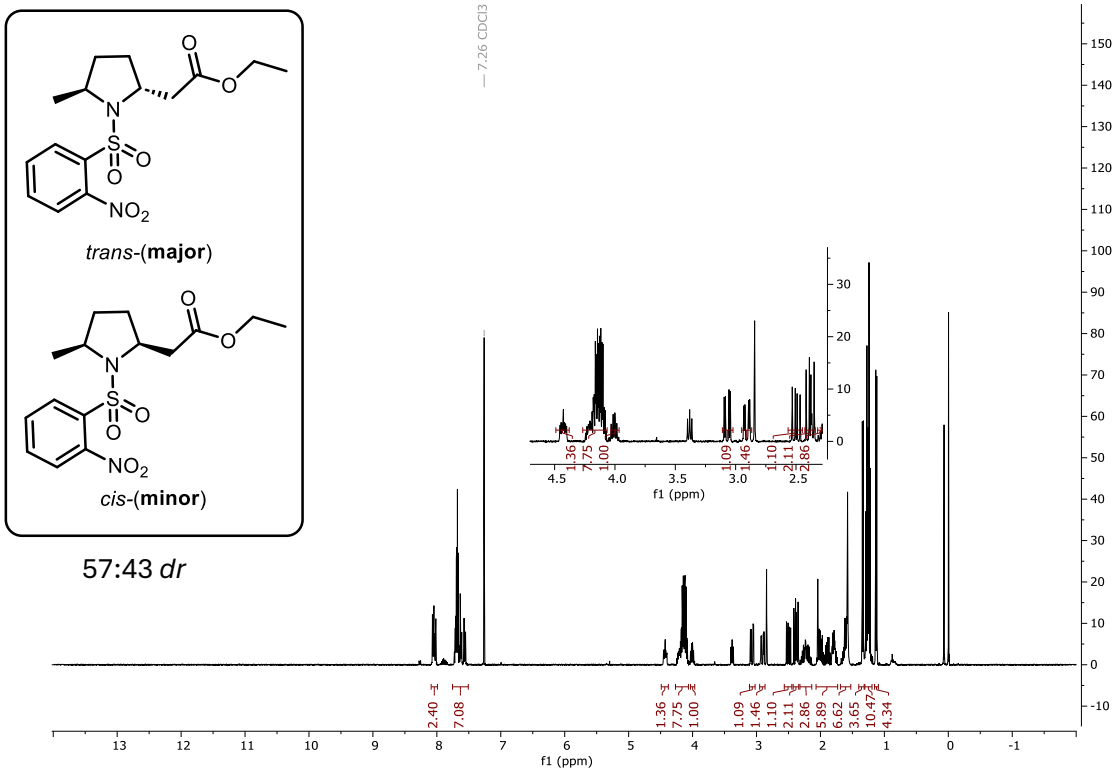

**A99:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **7c.**



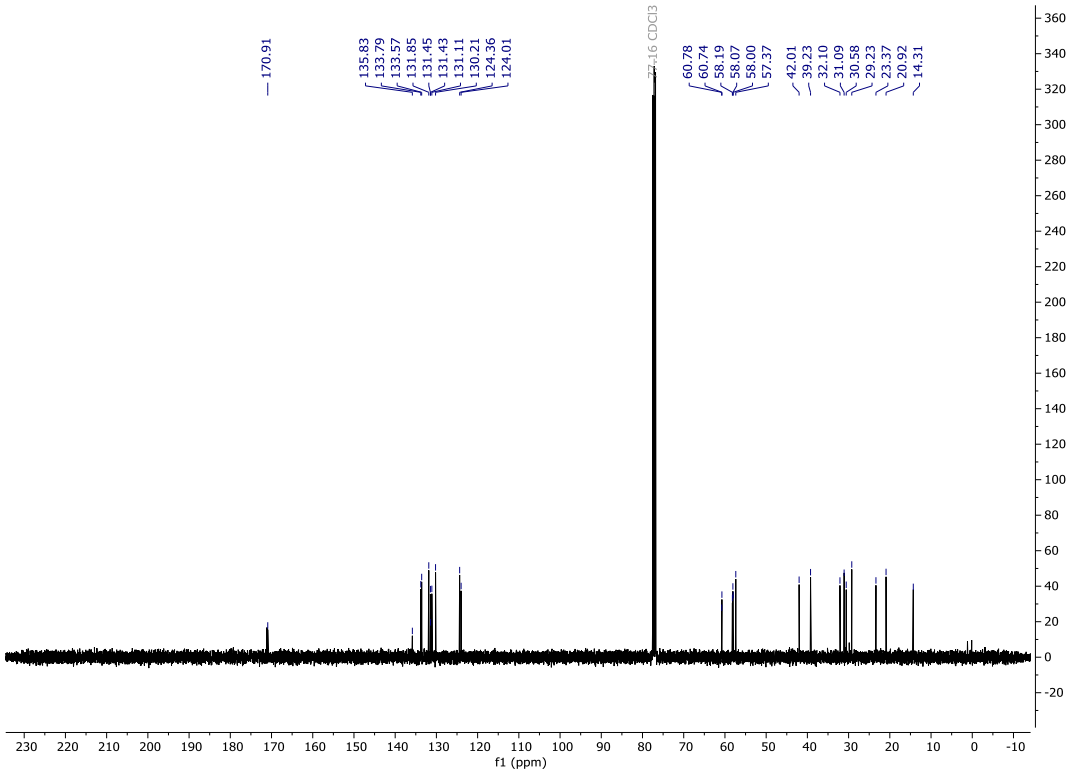

**A100:** COSY NMR (400 MHz, $CDCl_3$) of compound **7c.**



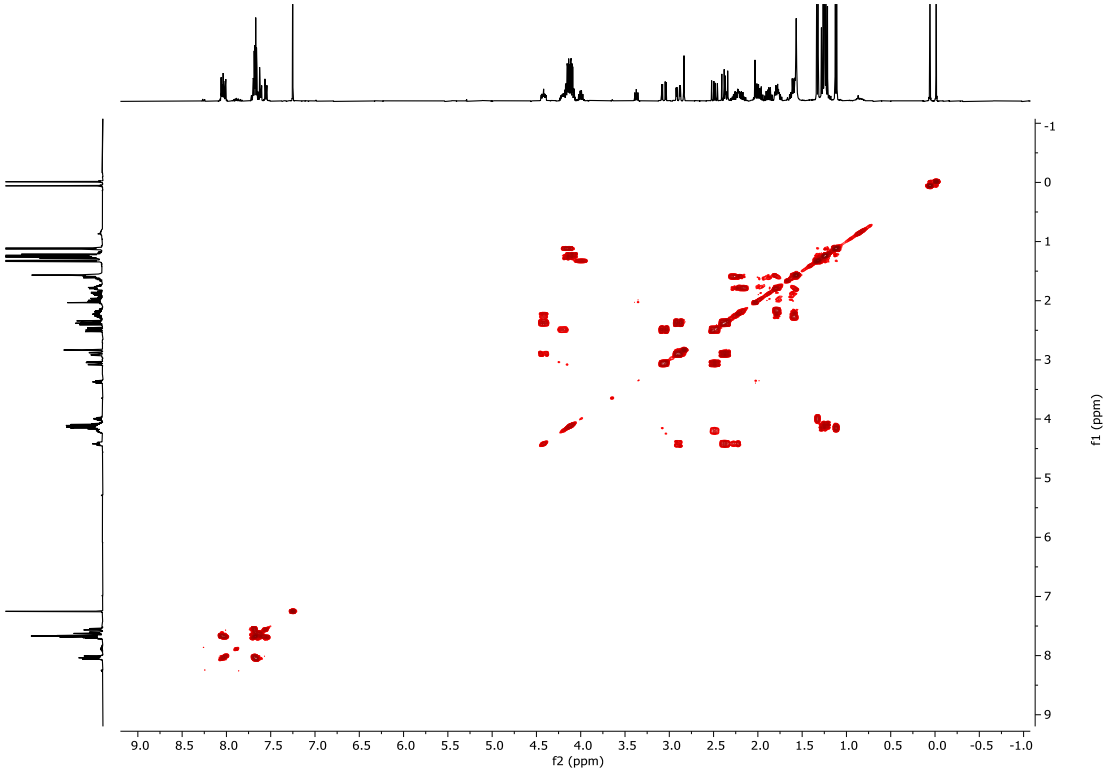

**A101:** HSQC NMR (400 MHz, $CDCl_3$) of compound **7c.**



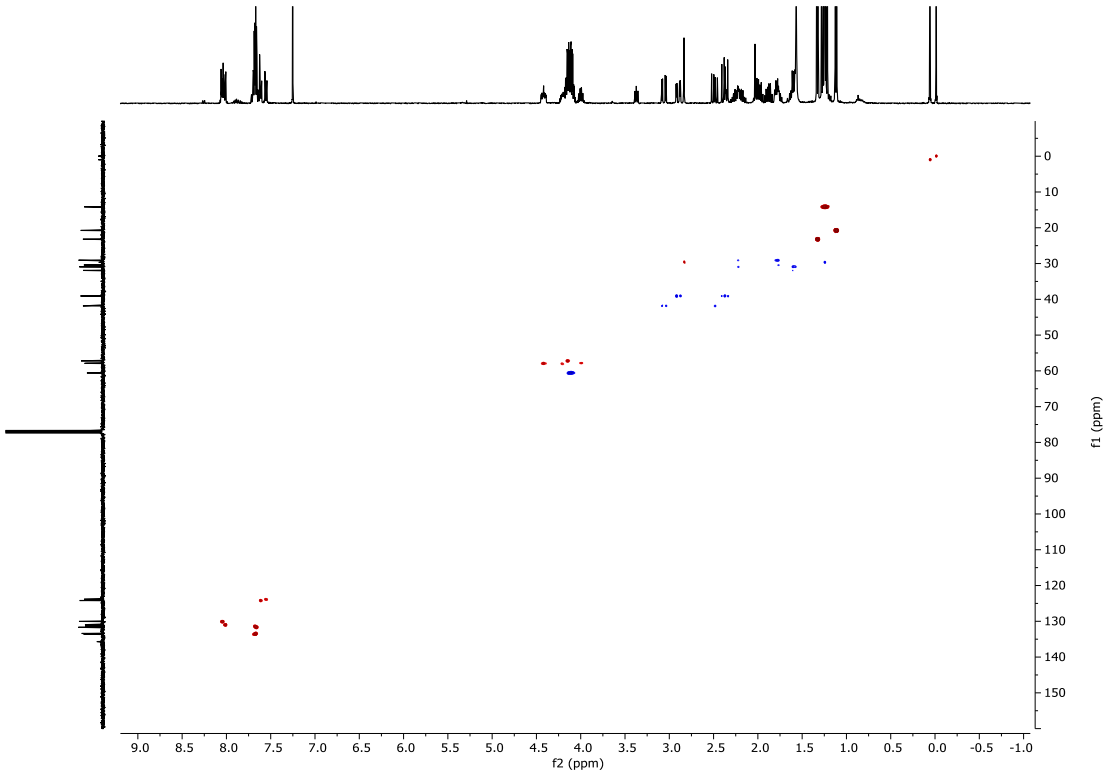

**A102:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **7d.**



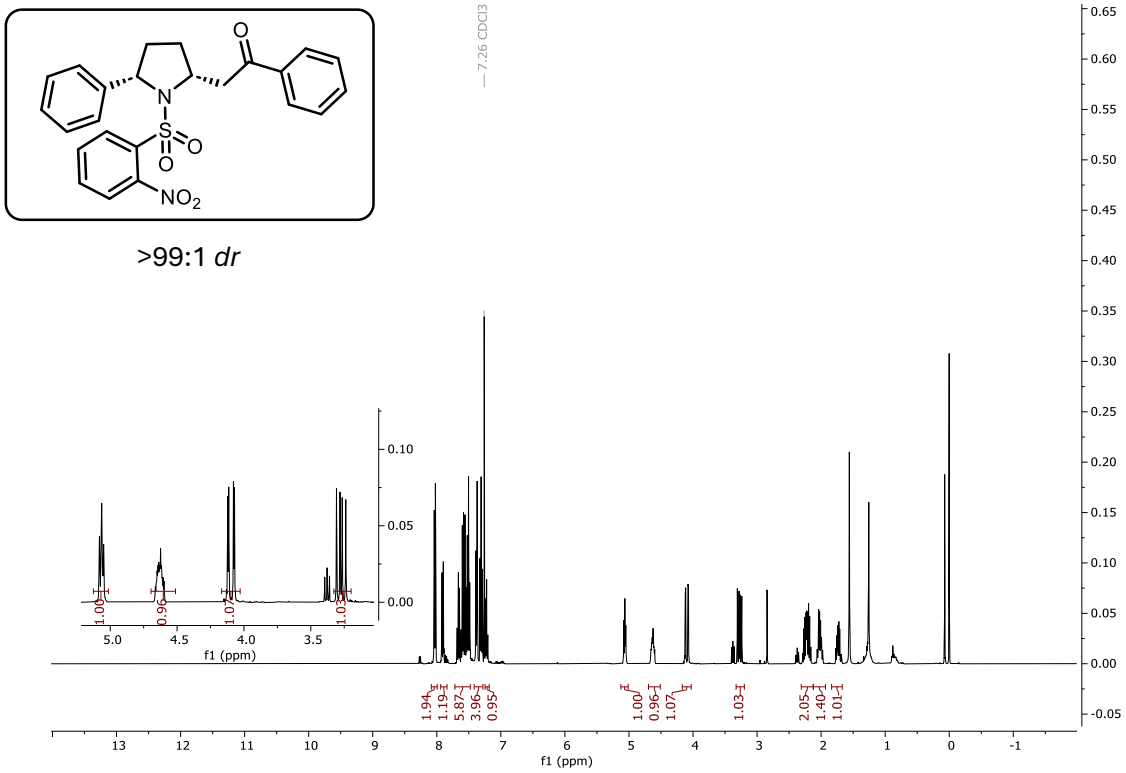

**A103:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **7d.**



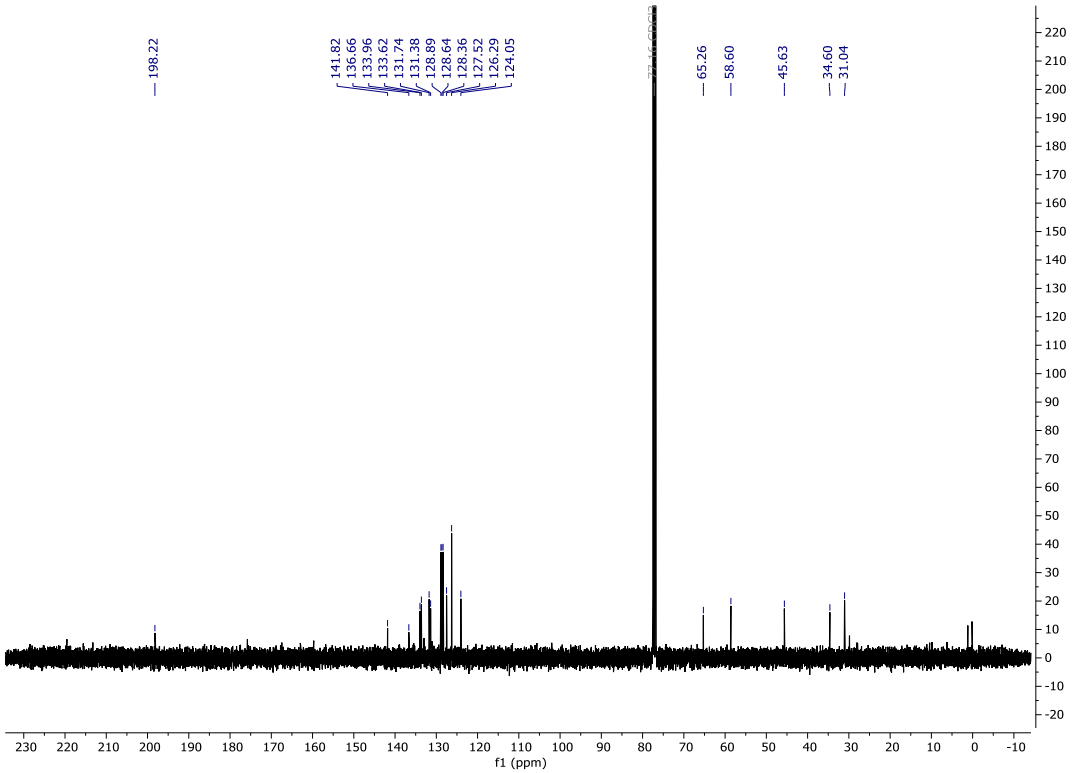

**A104:** COSY NMR (400 MHz, $CDCl_3$) of compound **7d.**



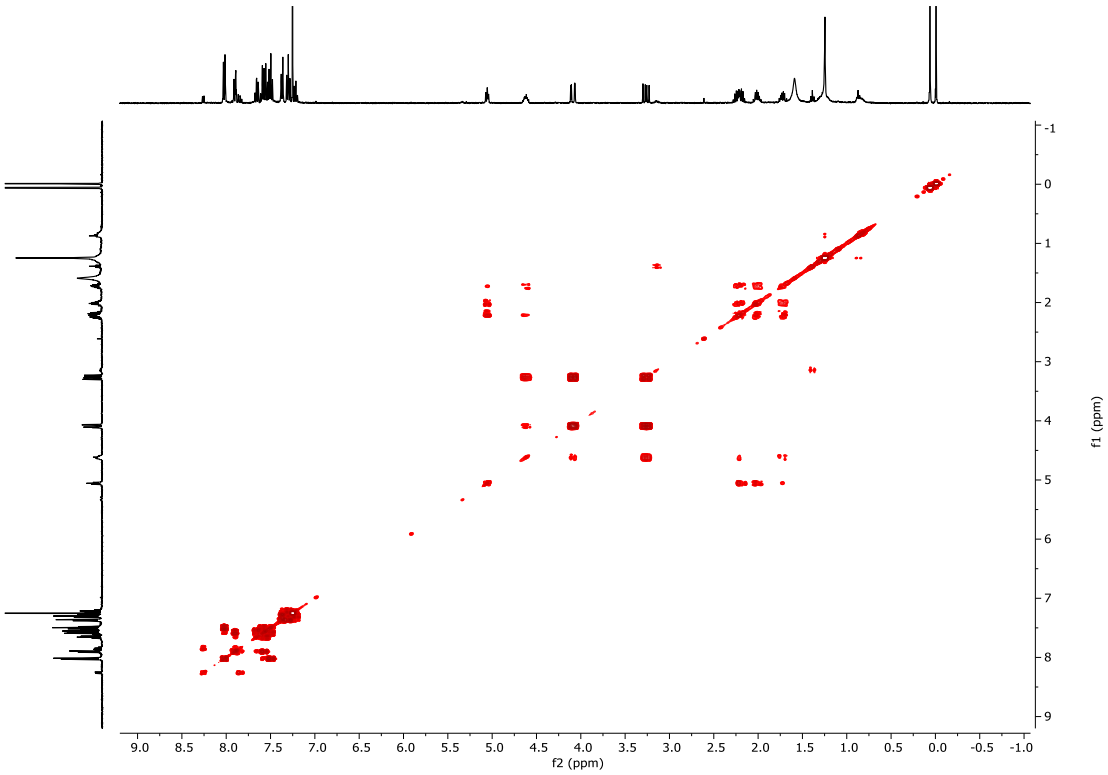

**A105:** HSQC NMR (400 MHz, $CDCl_3$) of compound **7d.**



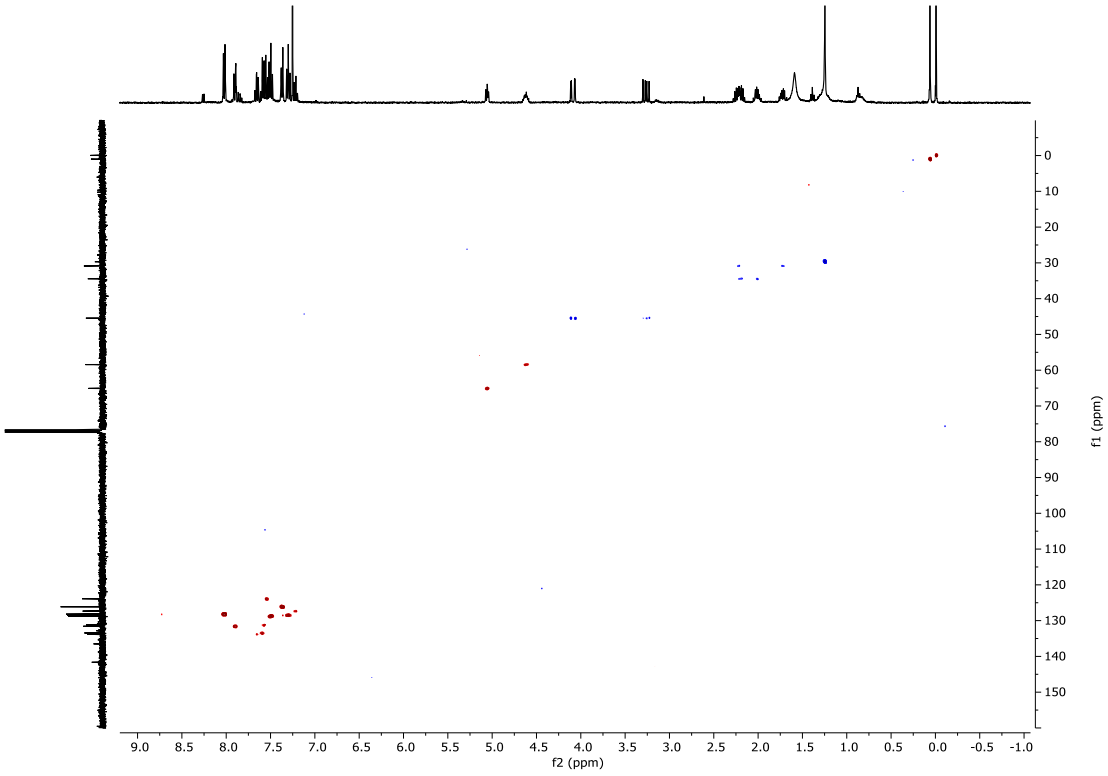

**A106:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **7f.**



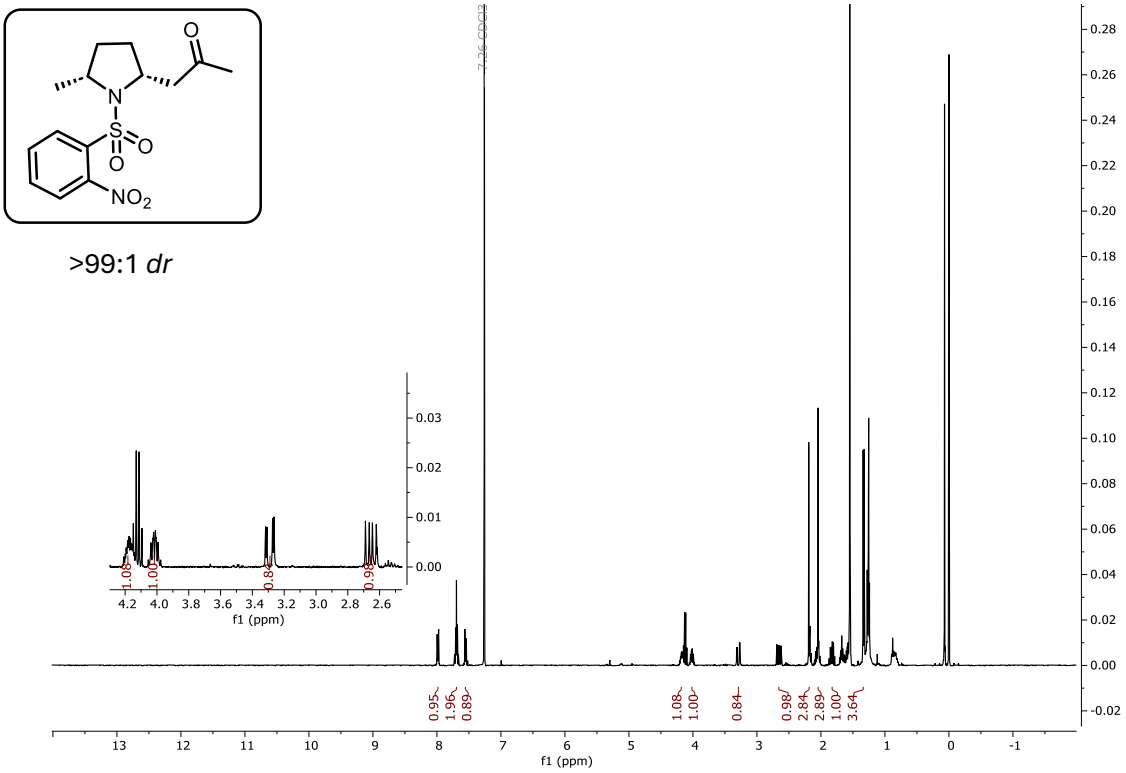

**A107:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **7f.**



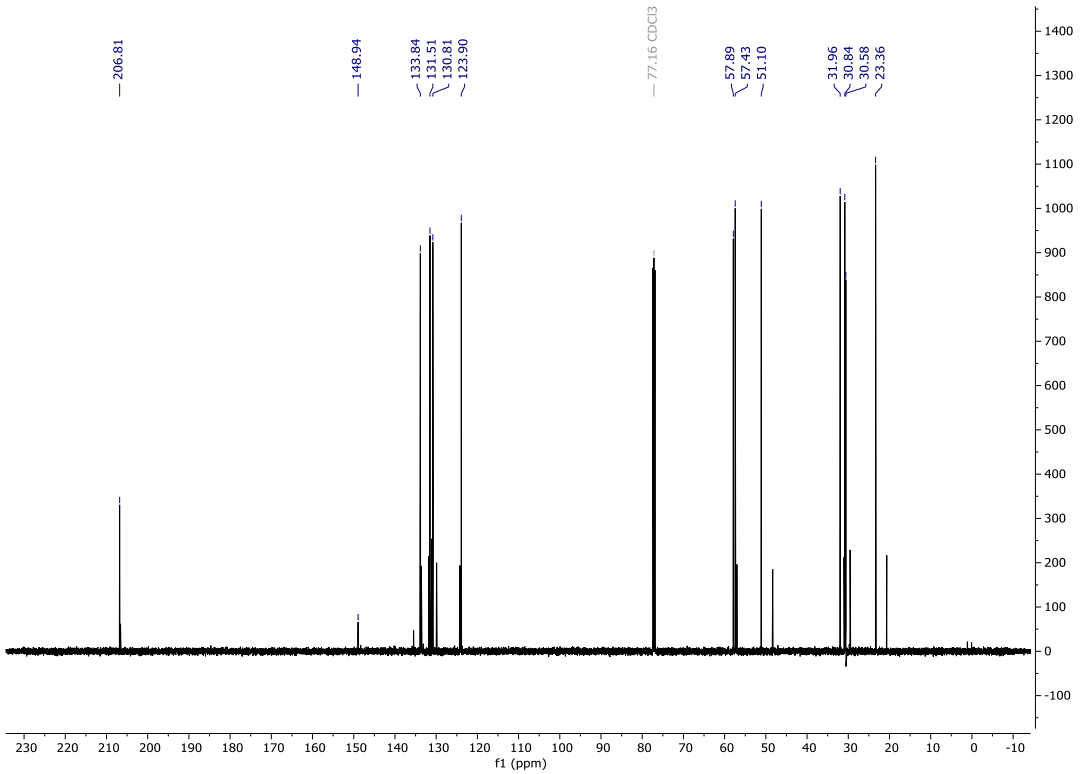

**A108:** COSY NMR (400 MHz, $CDCl_3$) of compound **7f.**



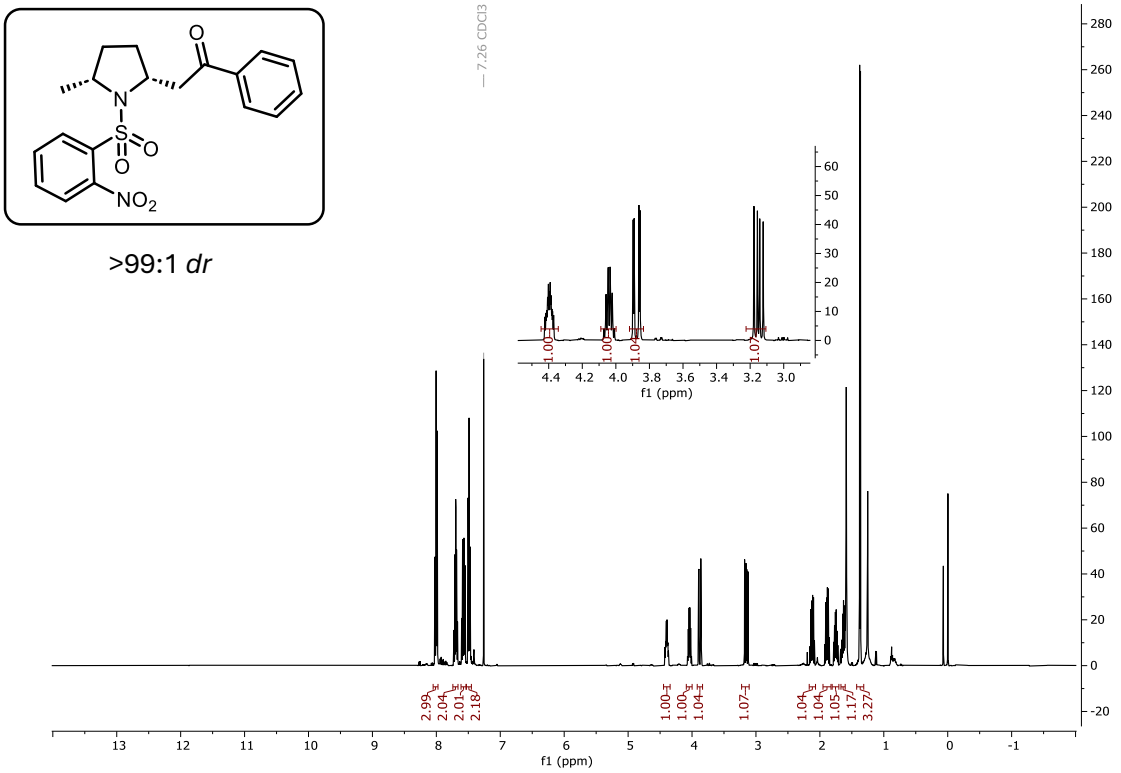

**A109:** HSQC NMR (400 MHz, $CDCl_3$) of compound **7f.**



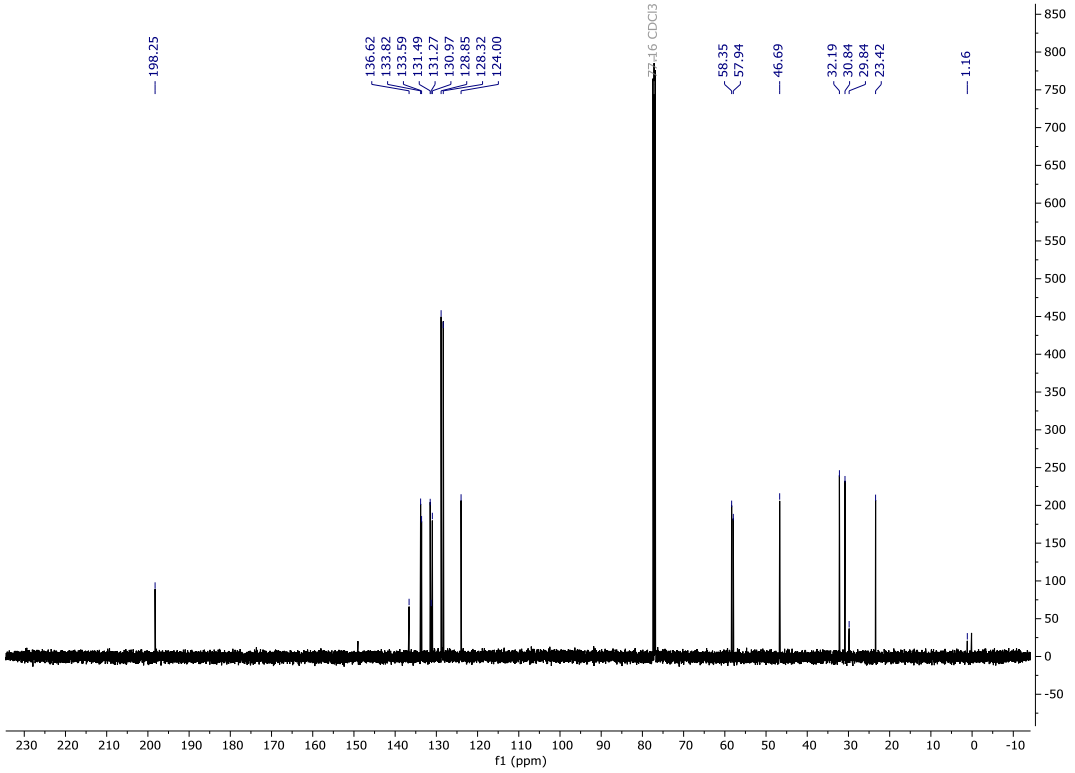

**A110:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **8a.**



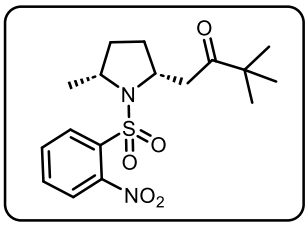

**A111:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **8a.**




**A112:** $^{1}$H-NMR (500 MHz, $CDCl_3$) of compound **8b.**



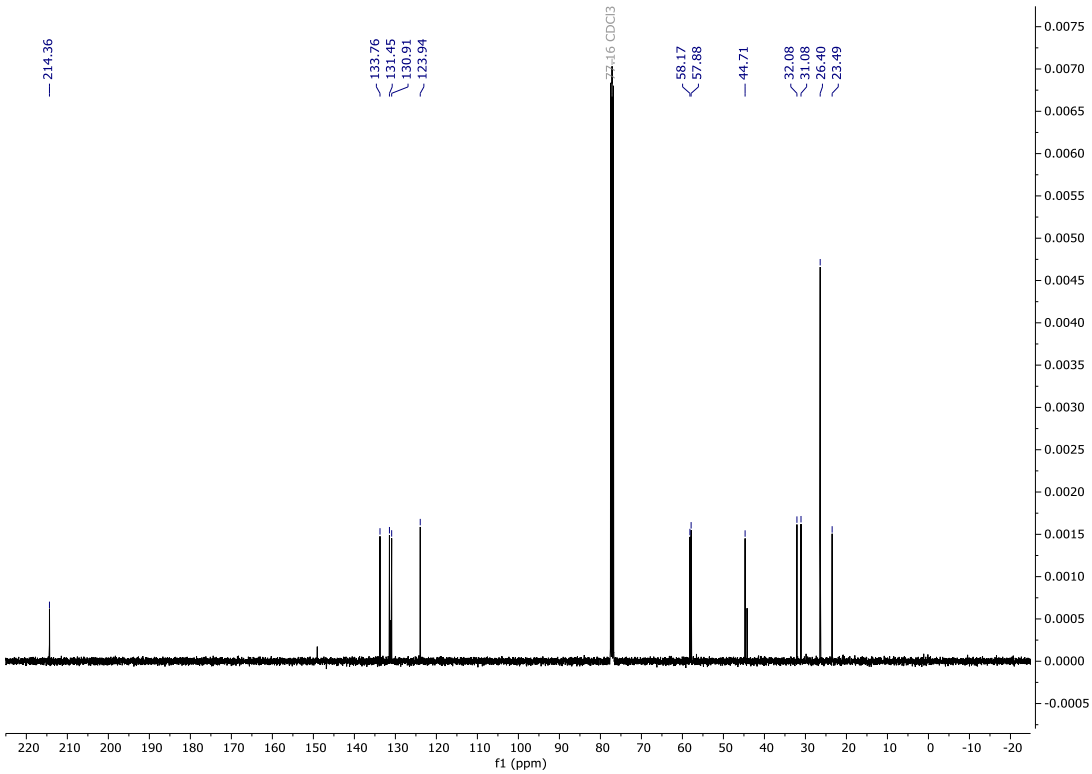

**A113:** $^{13}$C-NMR (126 MHz, $CDCl_3$) of compound **8b.**



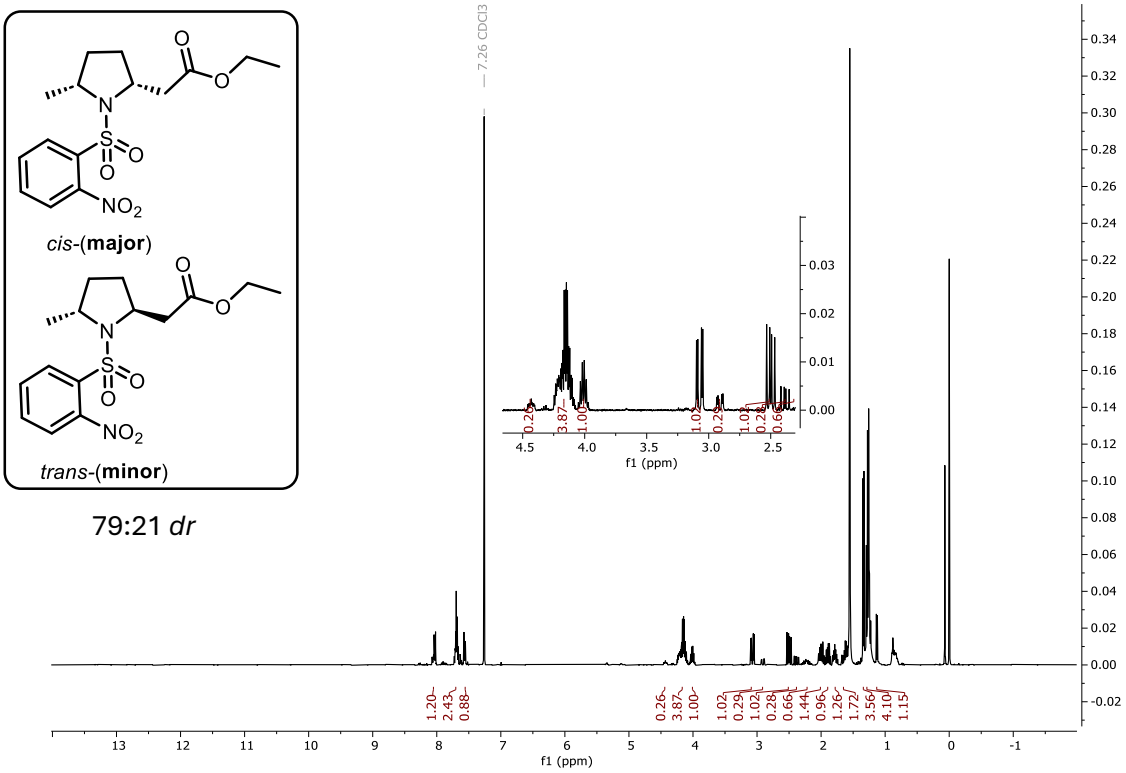
97:3 *dr*



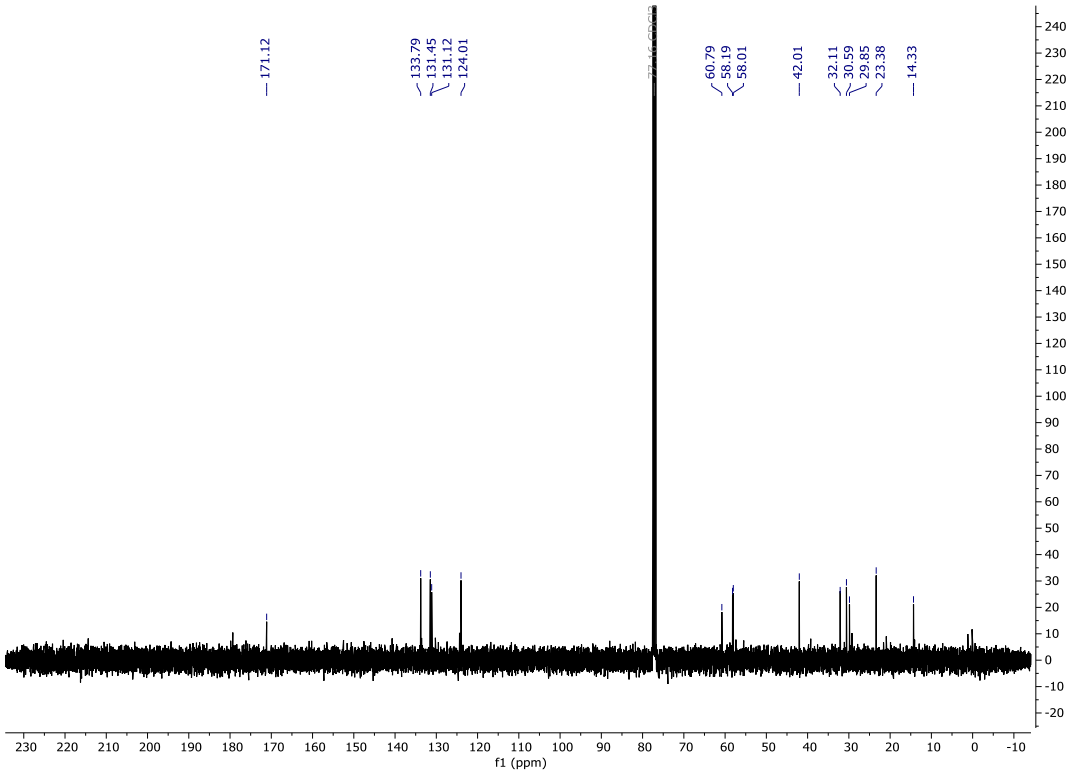

**A114:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **8c.**



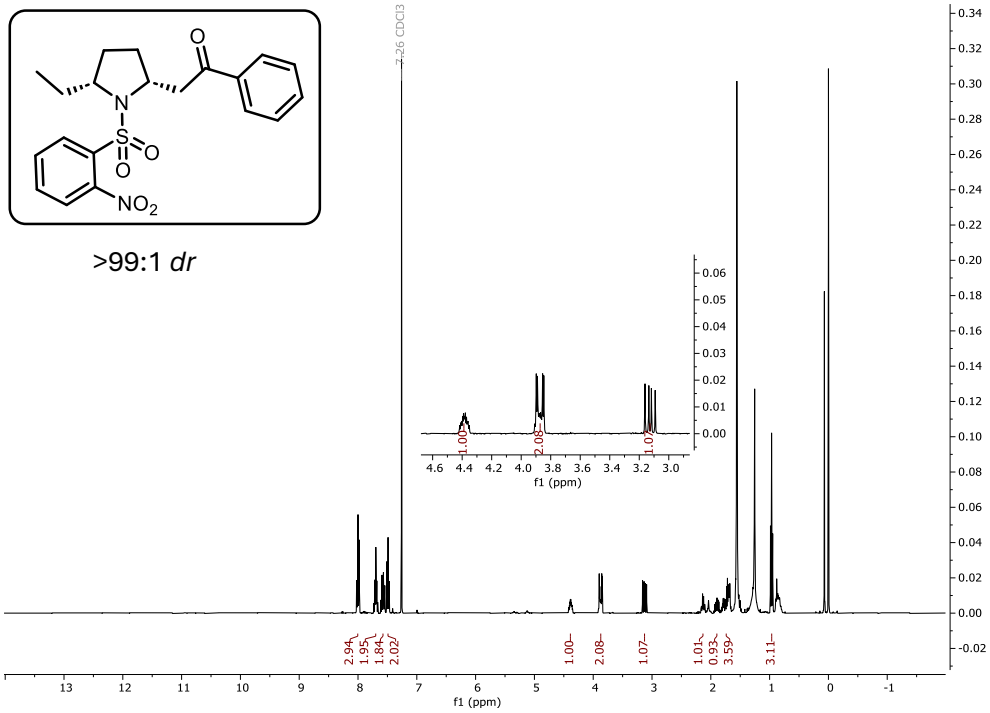

**A115:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **8c.**



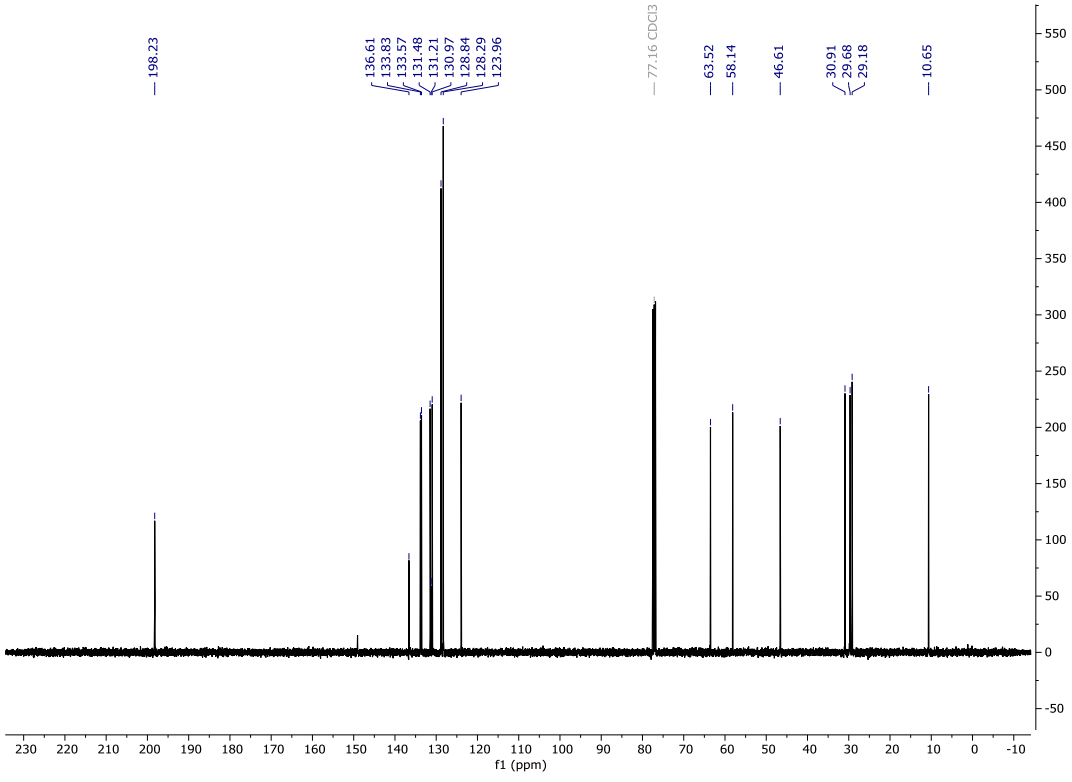

**A116:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **8d.**



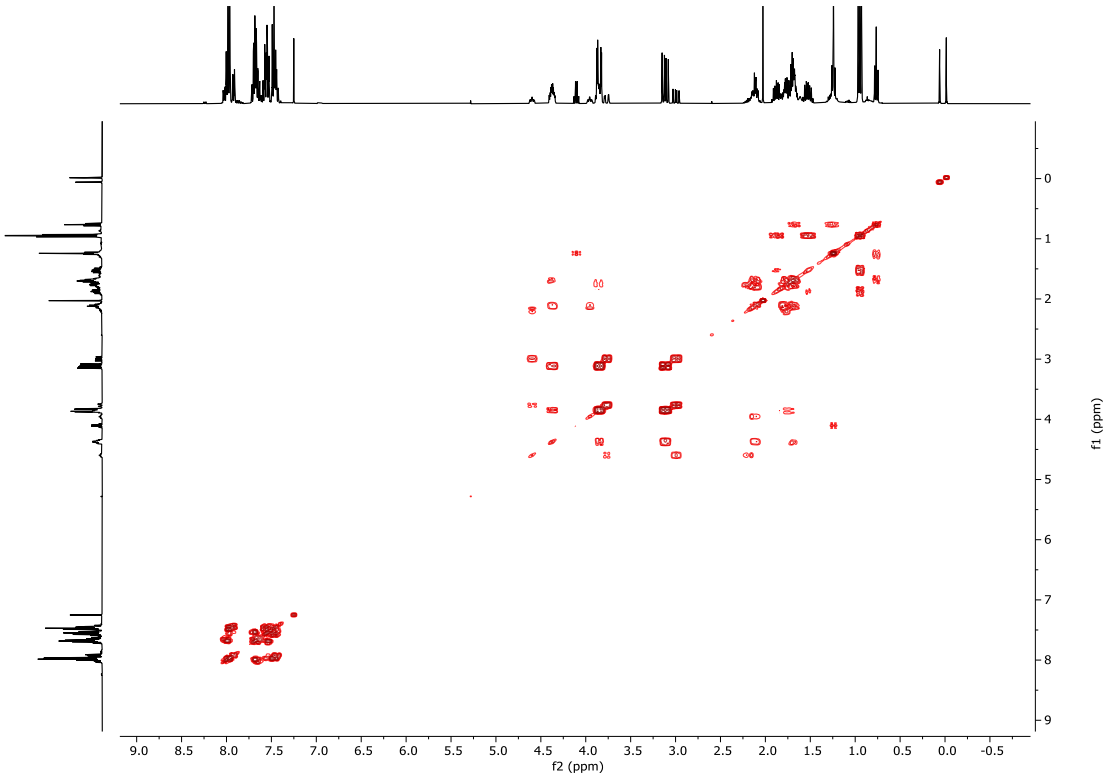

**A117:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **8d.**



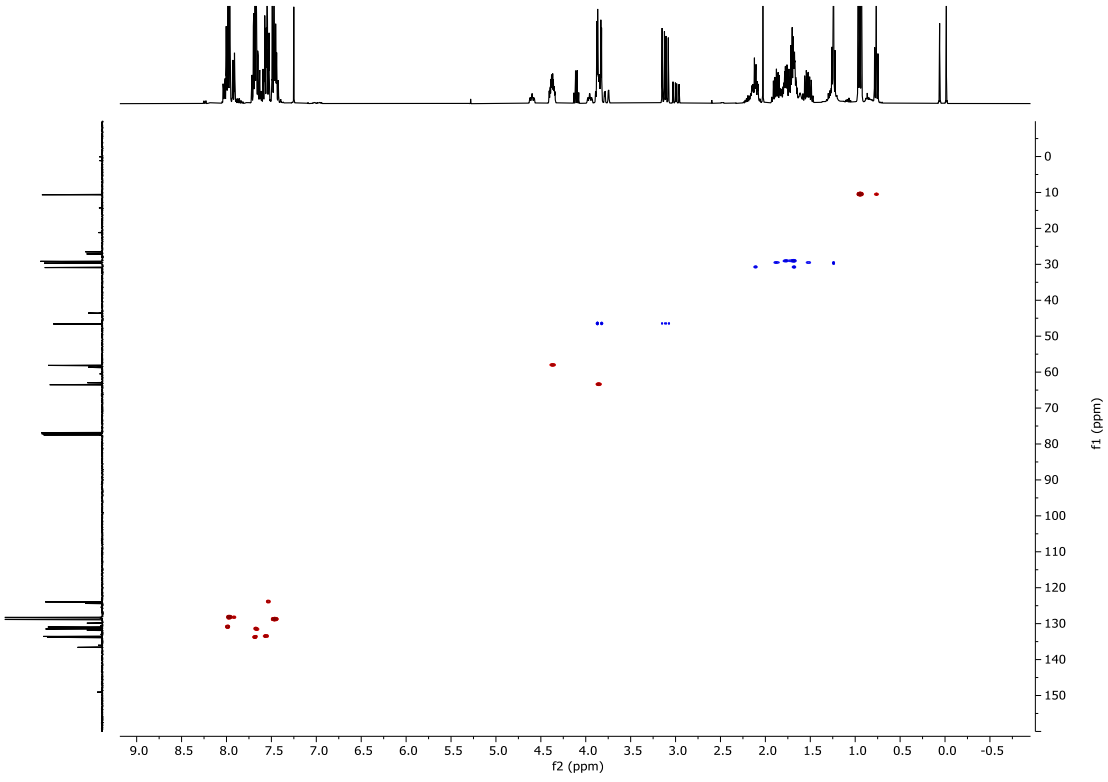

**A118:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **8e.**



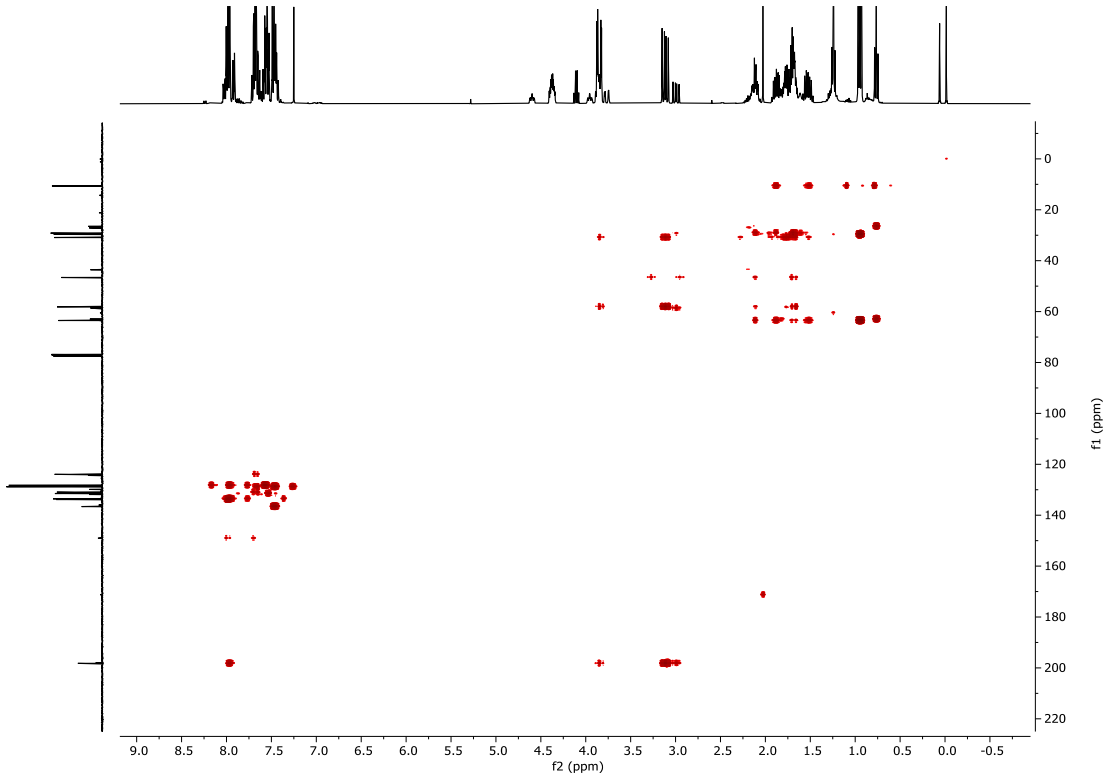

**A119:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **8e.**



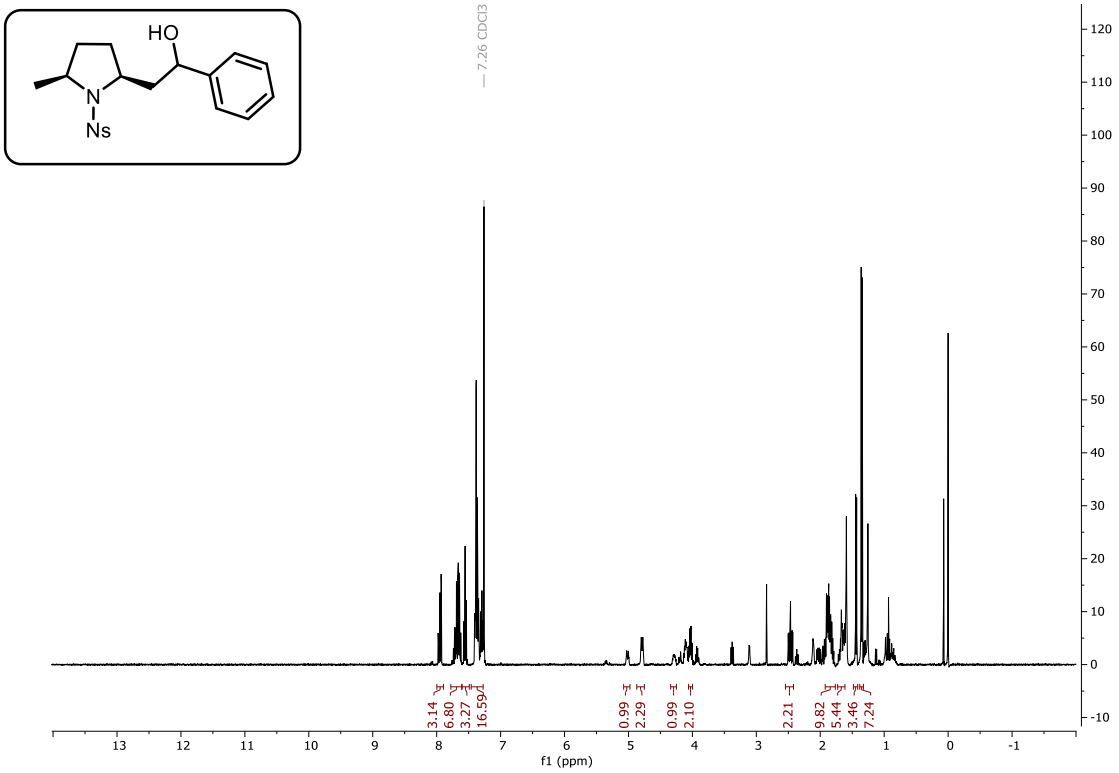

**A120:** COSY NMR (400 MHz, $CDCl_3$) of compound **8e.**



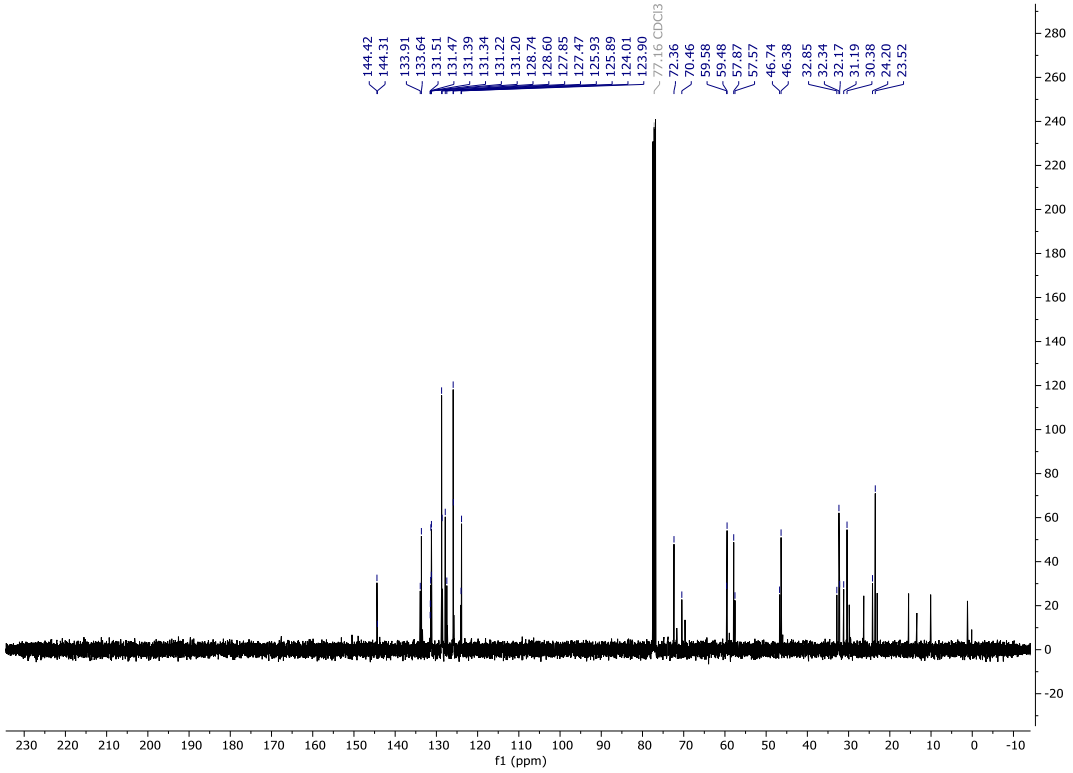

**A121:** HSQC NMR (400 MHz, $CDCl_3$) of compound **8e.**



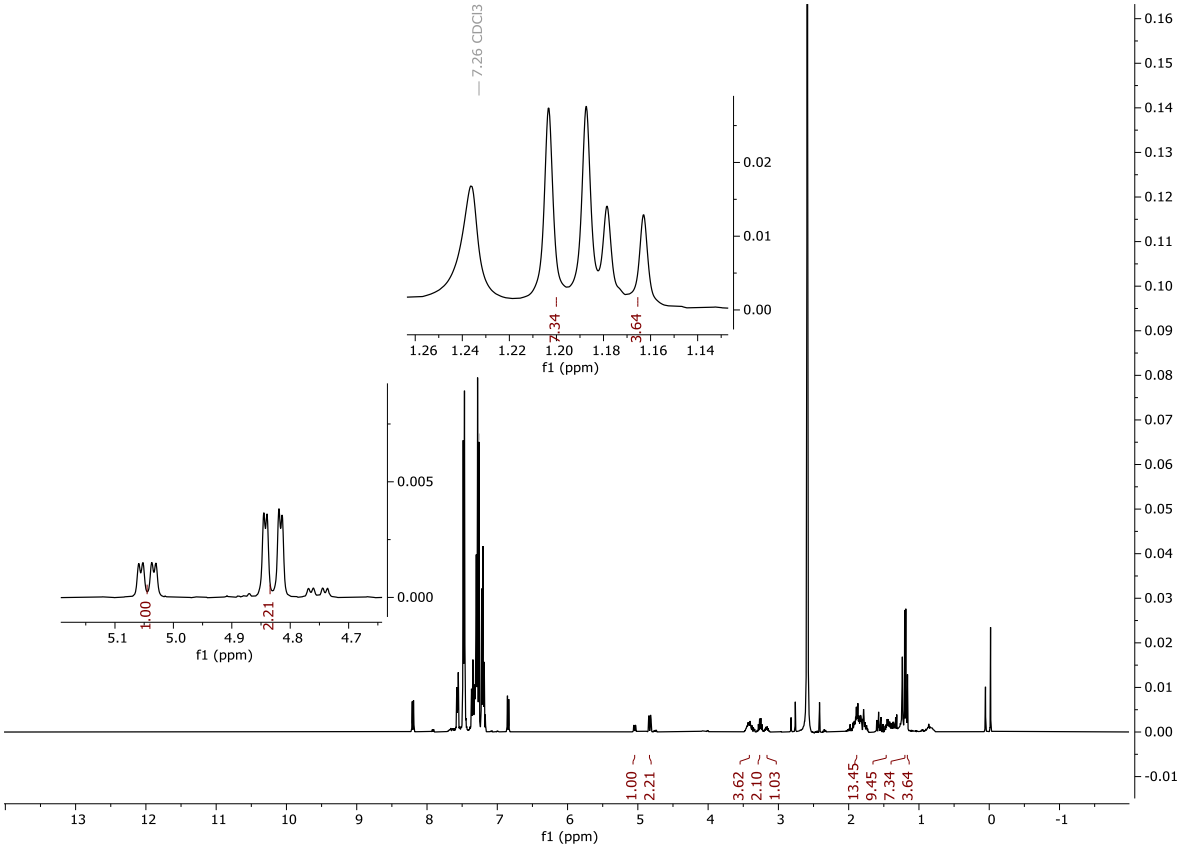

**A122:** HMBC NMR (400 MHz, $CDCl_3$) of compound **8e.**




**A123:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **S7.**




**A124:** $^{13}$C-NMR (101 MHz, $CDCl_3$) of compound **S7.**

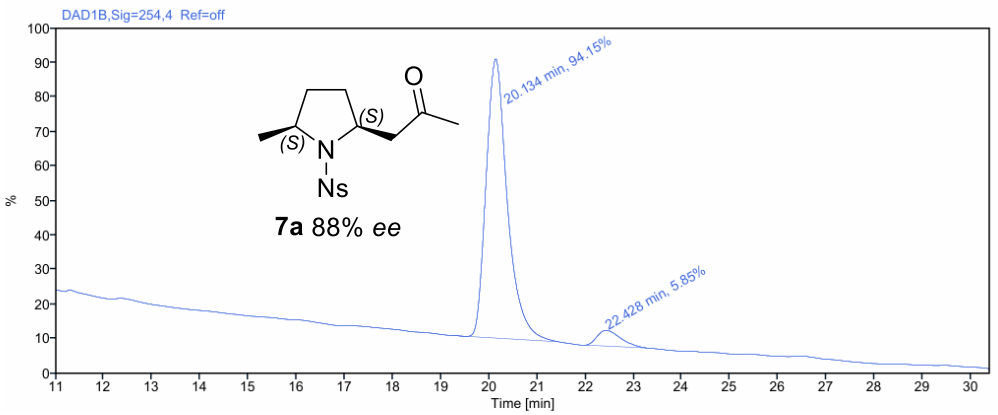



**A125:** $^{1}$H-NMR (400 MHz, $CDCl_3$) of compound **S8.**

## 16. HPLC data for enantiomeric excess

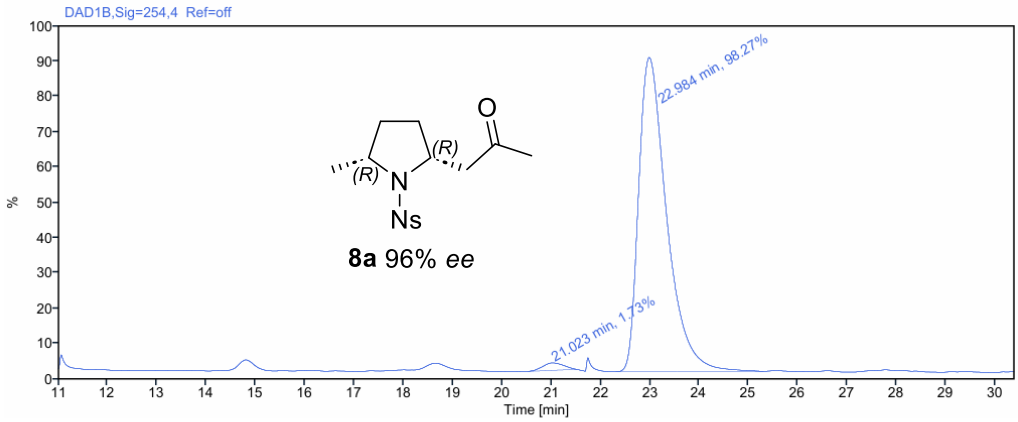
Method: Chiralpak IA, 9:1 Heptane/isopropanol, 0.8 mL/min, 210 nm or 254 nm

The integrated peaks display the same isoabsorbance plot.



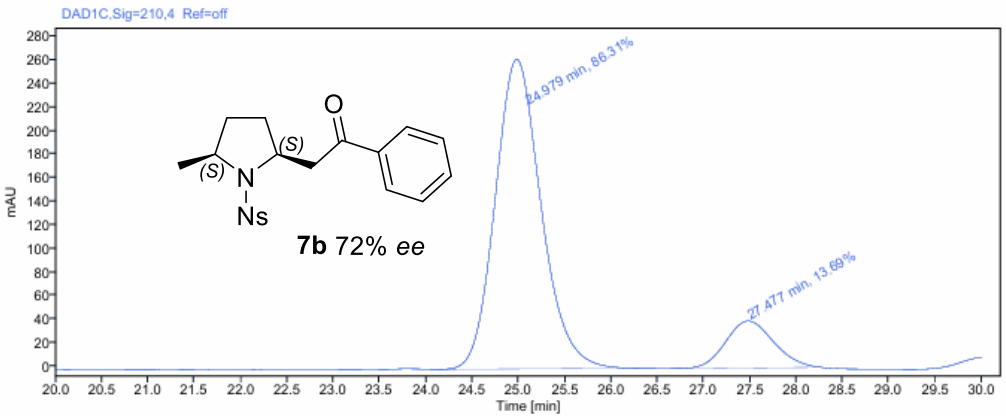

**Peak Results (Area Percent at least 1%)**

| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 20.134 | DAD1B,Sig=254,4 Ref=off | 2.196 | 17179.0 | 558.7 | 94.15 |
| 22.428 | DAD1B,Sig=254,4 Ref=off | 1.275 | 1068.0 | 31.6 | 5.85 |
| | | **Sum DAD1B** | 18247.0 | | |



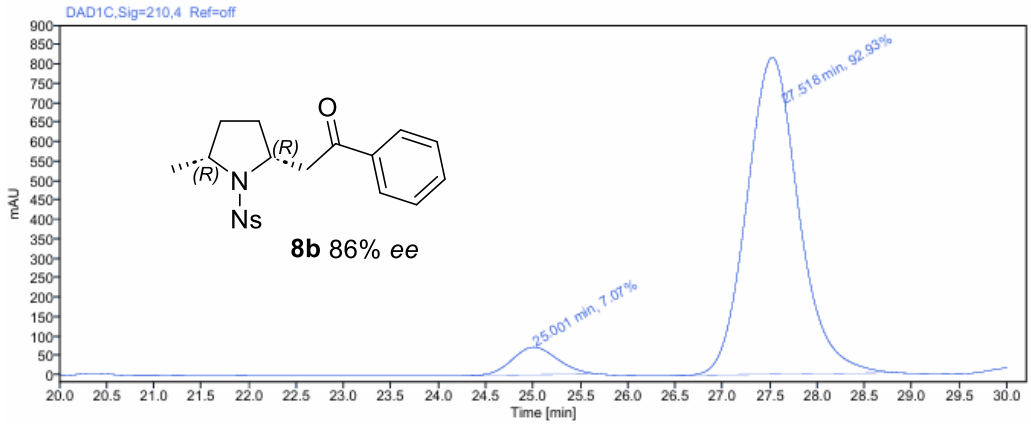

**Peak Results (Area Percent at least 1%)**

| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 21.023 | DAD1B,Sig=254,4 Ref=off | 0.973 | 114.9 | 3.9 | 1.73 |
| 22.984 | DAD1B,Sig=254,4 Ref=off | 2.999 | 6527.6 | 165.1 | 98.27 |
| | | **Sum DAD1B** | 6642.5 | | |



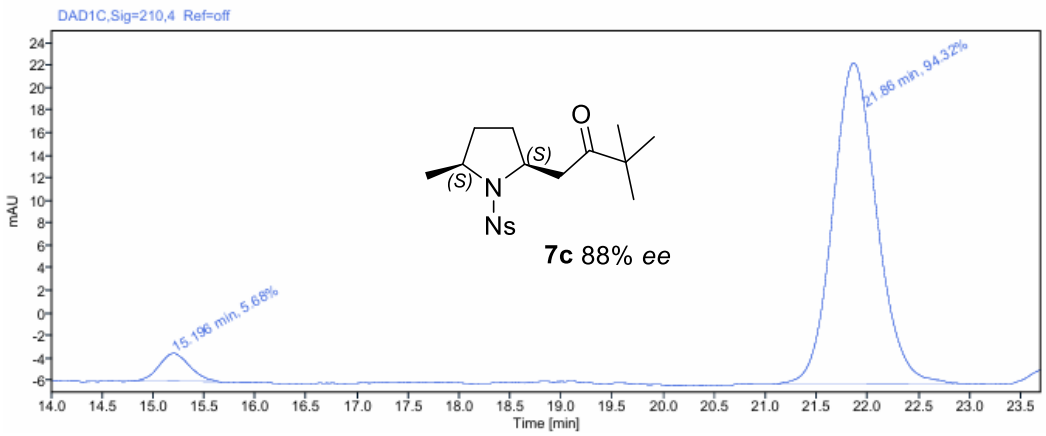

**Peak Results (Area Percent at least 1%)**

| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 24.979 | DAD1C,Sig=210,4 Ref=off | 2.166 | 9052.6 | 262.9 | 86.31 |
| 27.477 | DAD1C,Sig=210,4 Ref=off | 1.537 | 1435.8 | 39.8 | 13.69 |
| | | **Sum DAD1C** | 10488.4 | | |



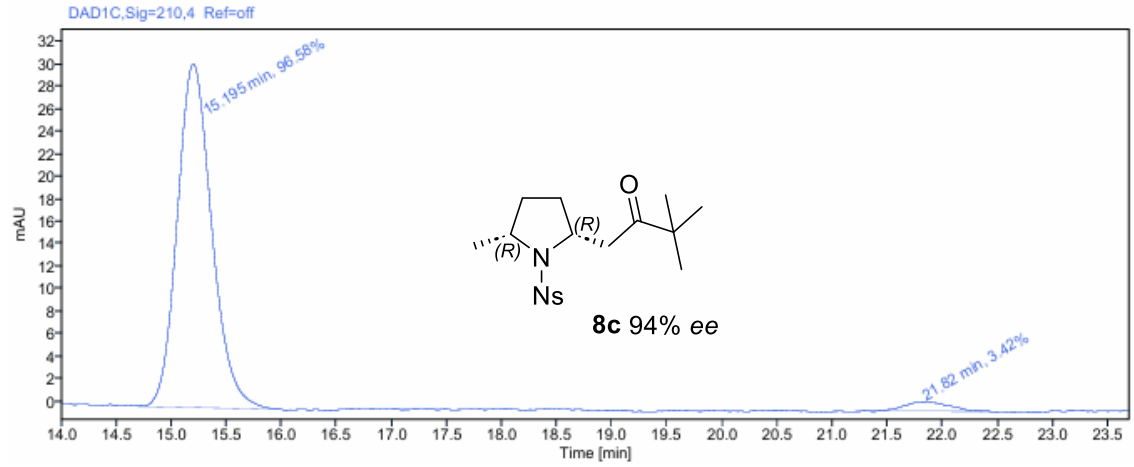

**Peak Results (Area Percent at least 1%)**

| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 25.001 | DAD1C,Sig=210,4 Ref=off | 1.441 | 2359.8 | 71.1 | 7.07 |
| 27.518 | DAD1C,Sig=210,4 Ref=off | 2.406 | 31041.4 | 815.5 | 92.93 |
| | | **Sum DAD1C** | 33401.2 | | |




**Peak Results (Area Percent at least 1%)**

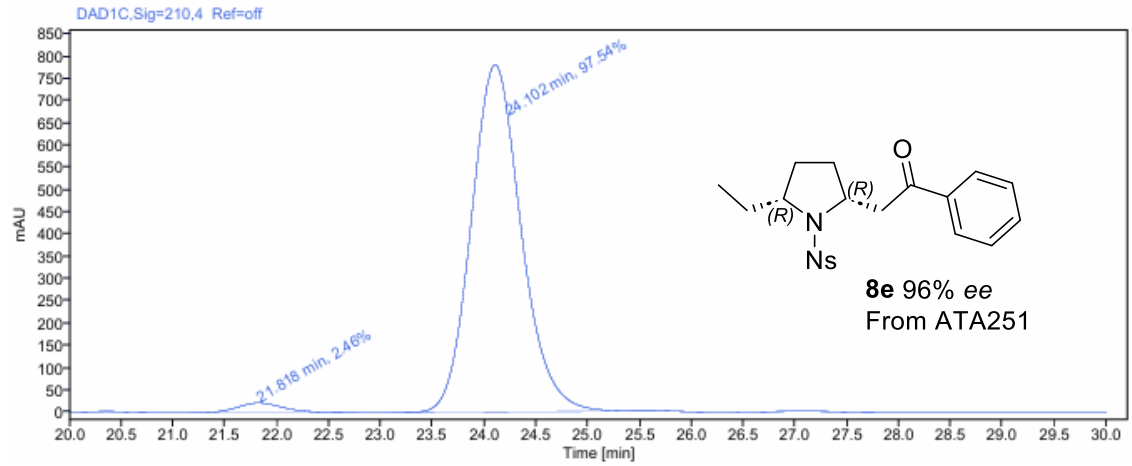
| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 15.196 | DAD1C,Sig=210,4 Ref=off | 0.919 | 51.4 | 2.5 | 5.68 |
| 21.860 | DAD1C,Sig=210,4 Ref=off | 1.891 | 854.0 | 28.5 | 94.32 |
| | | **Sum DAD1C** | 905.5 | | |




**Peak Results (Area Percent at least 1%)**

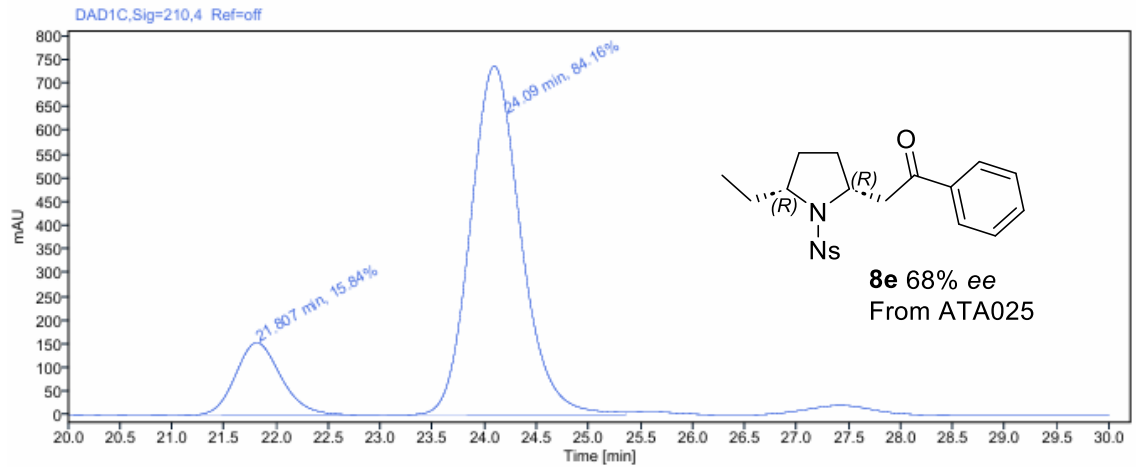
| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 15.195 | DAD1C,Sig=210,4 Ref=off | 1.324 | 667.9 | 30.6 | 96.58 |
| 21.820 | DAD1C,Sig=210,4 Ref=off | 1.155 | 23.7 | 0.9 | 3.42 |
| | | **Sum DAD1C** | 691.6 | | |

Note: From the HLPC spectra, compounds **7d** and **8d** are clearly not of the same R/S selectivity, however because compounds **7d** and **8d** displayed different *cis*/*trans* ratios depending on the chirality of the methyl group it was not feasible to record *ee* for these compounds.



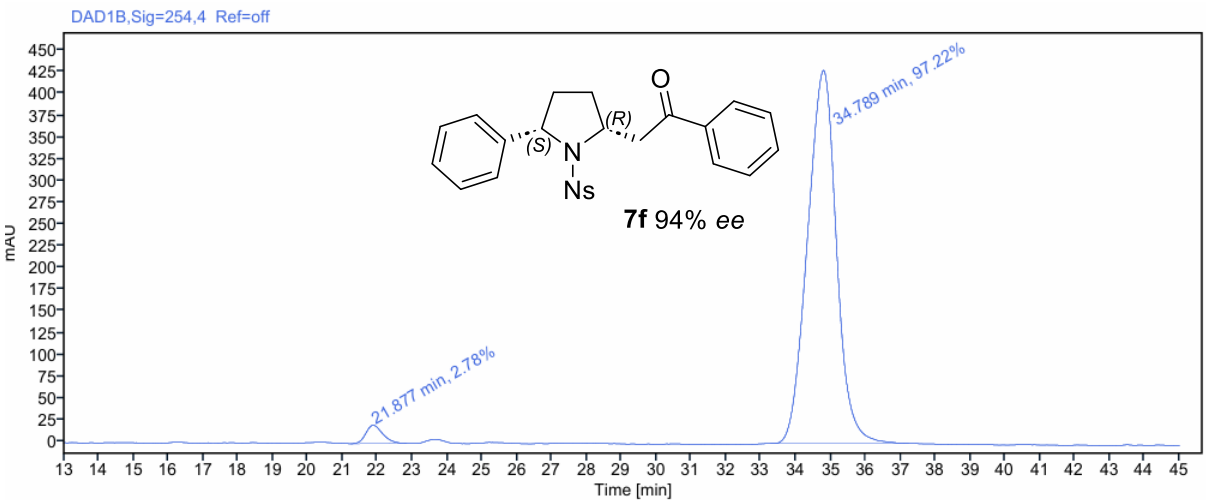

**Peak Results (Area Percent at least 1%)**

| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 21.818 | DAD1C,Sig=210,4 Ref=off | 1.603 | 659.6 | 21.6 | 2.46 |
| 24.102 | DAD1C,Sig=210,4 Ref=off | 2.598 | 26160.1 | 781.6 | 97.54 |
| | | **Sum DAD1C** | 26819.7 | | |




**Peak Results (Area Percent at least 1%)**

| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 21.807 | DAD1C,Sig=210,4 Ref=off | 1.937 | 4675.1 | 153.0 | 15.84 |
| 24.090 | DAD1C,Sig=210,4 Ref=off | 2.297 | 24831.6 | 736.7 | 84.16 |
| | | **Sum DAD1C** | 29506.7 | | |




**eak Results (Area Percent at least 1%)**

| RT (min) | Signal Description | Width (min) | Area | Height | Area% |
|---|---|---|---|---|---|
| 21.877 | DAD1B,Sig=254,4 Ref=off | 1.605 | 709.1 | 21.1 | 2.78 |
| 34.789 | DAD1B,Sig=254,4 Ref=off | 3.960 | 24839.0 | 429.3 | 97.22 |
| | | **Sum DAD1B** | 25548.1 | | |

## 17. References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