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

Stereoselective synthesis of (*R*)- and (*S*)-1,2-diazetidine-3-carboxylic acid derivatives for peptidomimetics

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Supporting Information

Table of Contents

1. General information

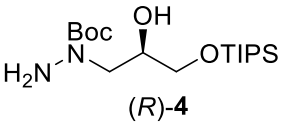
Anhydrous solvents were purchased from Sigma-Aldrich or Thermo Fisher Scientific in Sure-Seal™ bottles for use as reaction solvents. All other solvents were reagent grade and used as received. Petroleum ether refers to the fraction that boils in the range 40–60 °C. Commercially available starting materials were used without purification unless otherwise stated. Magnesium turnings were activated by washing with 1 M HCl, water and methanol (3×) followed by drying under high vacuum. For deprotections of tosyl groups, an excess of activated magnesium turnings was used (>10 equiv).

Thin layer chromatography was performed on pre-coated aluminium-backed plates (Merck Silicagel 60 F254), visualised by UV 254 nm, then stained with phosphomolybdic acid (PMA) or potassium permanganate ($KMnO_4$) dip and heated. Flash column chromatography was performed using 40–63 µm silica gel.

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance III HD (400 MHz) or AV (400 or 500 MHz) spectrometers. Chemicals shifts (δ) are reported in parts per million (ppm) relative to the solvent residual peaks ($CDCl_3$ δ_H: 7.26 ppm, δ_C: 77.16 ppm). Coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br.), or some combination of these.

HPLC analysis was conducted on an Agilent Technologies 1200 Series HPLC, using HPLC grade hexane and propan-2-ol as the eluent, and detection by UV at 254 nm. Low-resolution mass spectra were recorded on an Agilent Technologies 6130B Quadrupole LC-MS instrument. High-resolution mass spectra were recorded using a Bruker MaXis Impact Q-TOF. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer and are given in cm^{-1}. Melting points were recorded with a Gallenkamp MPD350 apparatus and are reported as observed. Optical rotations were measured using an AA-1000 polarimeter using a 2 dm cuvette and reported as observed.

2. Detailed procedures and analytical data



(*R*)-**4**

***tert*-Butyl (*R*)-1-(2-hydroxy-3-((triisopropylsilyl)oxy)propyl)hydrazine-1-carboxylate ((*R*)-4).** (*R*)-triisopropyl(oxiran-2-ylmethoxy)silane ((*R*)-**3**) (2.30 g, 10.0 mmol, 1.0 equiv), synthesised from (*S*)-glycidol ((*S*)-**2**),[1] was added dropwise to a solution of hydrazine monohydrate (4.85 mL, 100 mmol, 10.0 equiv) and water (180 µL, 10.0 mmol, 1.0 equiv) in methanol (40 mL) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C before stirring was continued overnight at room temperature. The mixture was concentrated *in vacuo* and co-evaporated with methanol (3 x 20 mL) to remove any excess hydrazine. A white solid of ring-opened epoxide (2.60 g, 9.91 mmol) was obtained, which was dissolved in anhydrous methanol (40 mL). At 0 °C, a solution of Boc_2O (2.16 g, 9.91 mmol, 0.99 equiv) in methanol (20 mL) was added over a 2 h period via syringe pump. The reaction mixture was allowed to warm to room temperature and stirred overnight. The mixture was concentrated *in vacuo* and purified by column chromatography on silica gel (20-50% EtOAc in petroleum ether) to yield (*R*)-**4** (2.34 g, 6.45 mmol, 65% yield) as a viscous colourless oil.

R_f = 0.21 (33% EtOAc in petroleum ether).

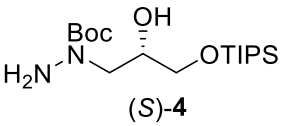
$[\alpha]^{28}_D$ +13.2 (*c* 0.62, $CHCl_3$).

IR (neat) 3336, 2941, 2866, 1692, 1366, 1170, 1122, 1063, 882, 682 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 3.99–3.92 (m, 1H), 3.70 (d, *J* = 5.5 Hz, 2H), 3.65 (dd, *J* = 14.2, 3.4 Hz, 1H), 3.52–3.44 (m, 1H), 1.49 (s, 9H), 1.10–1.07 (m, 21H). *N.B.* NH_2 and OH not observed.

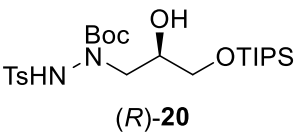
δ_C (126 MHz, $CDCl_3$) 81.3, 71.6, 65.3, 52.7, 28.5, 18.1, 12.0. *N.B.* Quaternary carbonyl peak not observed.

HRMS (ESI^+) calculated for $C_{17}H_{38}N_2NaO_4Si$ $[M+Na]^+$ 385.2499, measured 385.2482.



(*S*)-**4**

***tert*-Butyl (*S*)-1-(2-hydroxy-3-((triisopropylsilyl)oxy)propyl)hydrazine-1-carboxylate ((*S*)-4).** (*S*)-**4** was synthesised following the same procedure as described above starting from (*S*)-triisopropyl(oxiran-2-ylmethoxy)silane ((*S*)-**3**) (derived from (*R*)-glycidol ((*R*)-**2**)).[1] All analytical data were identical to compound (*R*)-**4** except: $[\alpha]^{28}_D$ –12.0 (*c* 1.00, $CHCl_3$).



(*R*)-**20**

***tert*-Butyl (*R*)-1-(2-hydroxy-3-((triisopropylsilyl)oxy)propyl)-2-tosylhydrazine-1-carboxylate ((*R*)-20).** To a solution of (*R*)-**4** (2.07 g, 5.71 mmol, 1.0 equiv) and TsCl (1.31 g, 6.85 mmol, 1.1 equiv) in anhydrous THF (57 mL) was slowly added pyridine (2.49 mL, 30.8 mmol, 5.0 equiv) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. 1 M HCl (30.8 mL, 30.8 mmol, 5.0 equiv) was added slowly, and the mixture was extracted with dichloromethane (3 x 20 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (10-20% EtOAc in petroleum ether) to yield (*R*)-**20** (2.80 g, 5.42 mmol, 95% yield) as a colourless viscous oil.

R_f = 0.21 (20% EtOAc in petroleum ether).

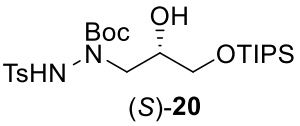
$[\alpha]^{28}_D$ +33.9 (*c* 0.29, $CHCl_3$).

IR (neat) 3202, 2942, 2866, 1709, 1599, 1342, 1160, 881, 670, 567 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.72 (d, *J* = 8.1 Hz, 2H), 7.23 (d, *J* = 7.7 Hz, 2H), 6.93 (s, 1H), 3.99 (s, 1H), 3.82–3.61 (m, 2H), 3.61–3.42 (m, 2H), 2.34 (s, 3H), 2.34 (br. s, 1H), 1.11 (s, 9H), 1.03–0.94 (m, 21H).

δ_C (101 MHz, $CDCl_3$) 144.5, 129.6, 128.9, 82.6, 65.1, 27.8, 21.7, 18.1, 12.0. *N.B.* Quaternary carbonyl peak not observed.

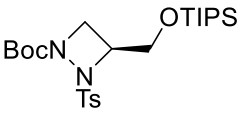
HRMS (ESI^+) calculated for $C_{24}H_{44}N_2NaO_6SSi$ $[M+Na]^+$ 539.2582, measured 539.2576.



(*S*)-**20**

***tert*-Butyl (*S*)-1-(2-hydroxy-3-((triisopropylsilyl)oxy)propyl)-2-tosylhydrazine-1-carboxylate ((*S*)-20).** (*S*)-**20** was synthesised following the same procedure as described above starting from (*S*)-**4**. All analytical data were identical to compound (*R*)-**20** except:

$[\alpha]^{28}_D$ –26.7 (*c* 1.00, $CHCl_3$).



(*S*)-**5**

***tert*-Butyl (*S*)-2-tosyl-3-(((triisopropylsilyl)oxy)methyl)-1,2-diazetidine-1-carboxylate ((*S*)-5).** To a solution of (*R*)-**20** (2.34 g, 4.52 mmol, 1.0 equiv) and triphenylphosphine (2.97 g, 11.3 mmol, 2.5 equiv) in THF (450 mL) at 0 °C was slowly added DIAD (2.22 mL, 11.3 mmol, 2.5 equiv). The reaction mixture was allowed to warm to room temperature and stirred for 24 h. Then, the solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel (10-20% EtOAc in petroleum ether) to give (*S*)-**5** (947 mg, 1.90 mmol, 42% yield) as a colourless oil, which crystallised to a white solid upon standing.

Enantiomeric excess (>98% *ee*) was determined by HPLC analysis (25 °C). [Chiralpak IA column 2-propanol/hexane = 2/98; flow rate = 1.0 mL/min; detection wavelength = 254 nm] t_R 7.5 min (major); t_R 11.5 min (minor).

R_f = 0.43 (20% EtOAc in petroleum ether).

M.p. 62–64 ºC.

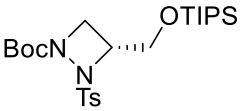
$[\alpha]^{28}_D$ +30.9 (*c* 0.19, $CHCl_3$).

IR (neat) 2943, 2866, 1714, 1599, 1365, 1326, 1156, 687, 539 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.76 (d, *J* = 7.9 Hz, 2H), 7.29 (d, *J* = 7.9 Hz, 2H), 4.02–3.94 (m, 2H), 3.89 (dd, *J* = 11.7, 3.2 Hz, 1H), 3.74 (d, *J* = 10.8 Hz, 1H), 3.70–3.62 (m, 1H), 2.39 (s, 3H), 1.33 (s, 9H), 1.03–0.96 (m, 21H).

δ_C (126 MHz, $CDCl_3$) 159.2, 145.1, 130.8, 130.1, 130.0, 82.6, 63.5, 60.3, 50.3, 28.0, 21.9, 18.1, 12.0.

HRMS (ESI^+) calculated for $C_{24}H_{42}N_2NaO_5SSi$ $[M+Na]^+$ 521.2476, measured 521.2474.

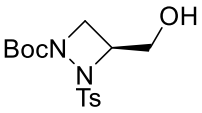


(*R*)-**5**

***tert*-Butyl (*R*)-2-tosyl-3-(((triisopropylsilyl)oxy)methyl)-1,2-diazetidine-1-carboxylate ((*R*)-5).** (*R*)-**5** was synthesised following the same procedure as described above starting from (*S*)-**20**. All analytical data were identical to compound (*S*)-**5** except:

$[\alpha]^{28}_{D}$ –26.2 (*c* 1.00, $CHCl_3$).

Enantiomeric excess (>98% *ee*) was determined by HPLC analysis (25 °C). [Chiralpak IA column 2-propanol/hexane = 2/98; flow rate = 1.0 mL/min; detection wavelength = 254 nm] t_R 7.5 min (minor); t_R 10.5 min (major).



(*S*)-**21**

***tert*-Butyl (*S*)-3-(hydroxymethyl)-2-tosyl-1,2-diazetidine-1-carboxylate ((*S*)-21).** To a solution of (*S*)-**5** (75 mg, 0.15 mmol, 1.0 equiv) in anhydrous THF (5.0 mL) was added tetrabutylammonium fluoride trihydrate (TBAF•$3H_2O$, 57 mg, 0.18 mmol, 1.2 equiv) at 0 °C and the mixture was stirred for 1 h. Brine was added (5.0 mL), and the mixture was extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to yield (*S*)-**21** (52 mg, 0.15 mmol, 99% yield) as a white solid. The analytical data match those reported in the literature.[2]

R_f = 0.25 (50% EtOAc in petroleum ether).

M.p. 92–93 ºC (Lit. 75–76 ºC).[2]

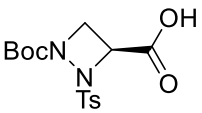
$[\alpha]^{28}_{D}$ +89.8 (*c* 0.26, $CHCl_3$). Lit. $[\alpha]^{27}_{D}$ +105.7 (*c* 0.48, $CHCl_3$).[2]

IR (neat) 3515, 2979, 1715, 1597, 1356, 1322, 1151, 1088, 695 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.86 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 4.10–4.03 (m, 1H), 3.96 (dd, *J* = 8.2, 5.6 Hz, 1H), 3.88 (dd, *J* = 12.7, 2.3 Hz, 1H), 3.74 (t, *J* = 8.3 Hz, 1H), 3.61 (dd, *J* = 12.7, 2.9 Hz, 1H), 2.47 (s, 3H), 2.31 (br. s, 1H), 1.43 (s, 9H).

δ_C (191 MHz, $CDCl_3$) 159.3, 145.4, 130.3, 130.1, 129.8, 83.1, 62.3, 61.4, 50.7, 28.1, 21.9.

HRMS (ESI^+) calculated for $C_{15}H_{22}N_2NaO_5S$ $[M+Na]^+$ 365.1142, measured 365.1145.



(*S*)-**22**

(*S*)-1-(*tert*-Butoxycarbonyl)-2-tosyl-1,2-diazetidine-3-carboxylic acid ((*S*)-22). To a solution of (*S*)-**5** (522 mg, 1.05 mmol, 1.0 equiv) in anhydrous THF (10 mL) was added tetrabutylammonium fluoride trihydrate (TBAF•$3H_2O$, 296 mg, 1.26 mmol, 1.2 equiv) at 0 °C and the mixture was stirred for 1 h. Brine was added (5.0 mL), and the mixture was extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. MS(ESI^+) confirmed complete conversion of the starting material to the intermediate primary alcohol. To a suspension of sodium periodate (2.70 g, 12.6 mmol, 12.0 equiv) and $RuCl_3$ (22 mg, 105 µmol, 0.1 equiv) in water (10 mL) was added a solution of the deprotected alcohol in acetone (10 mL). The reaction mixture was stirred overnight at room temperature and then filtered through a short plug of Celite eluting with EtOAc. To the filtrate was added NaOH solution (2.0 M, 10 mL), the phases were separated, and the aqueous layer was extracted

with EtOAc (2 x 10 mL). The aqueous layer was acidified with aqueous HCl (2.0 M, 20 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to yield carboxylic acid (*S*)-**22** (324 mg, 0.91 mmol, 87% yield) as a pale-yellow viscous oil.

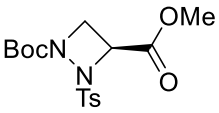
$[\alpha]^{28}_D$ +89.1 (*c* 0.06, $CHCl_3$).

IR (neat) 3271, 2926, 1724, 1631, 1313, 1155, 1008, 816, 713 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.88 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.19 (br. s, 1H), 4.52 (dd, *J* = 9.0, 6.0 Hz, 1H), 4.17 (dd, *J* = 9.0, 6.0 Hz, 1H), 4.00 (t, *J* = 9.0 Hz, 1H), 2.48 (s, 3H), 1.41 (s, 9H).

δ_C (101 MHz, $CDCl_3$) 170.5, 159.0, 146.0, 130.2, 130.0, 129.7, 83.9, 57.4, 51.6, 28.0, 21.9.

HRMS(ESI$^+$) calculated for $C_{15}H_{20}N_2NaO_5S$ $[M+Na]^+$ 379.0934, measured 379.0936.



(*S*)-**6**

1-(*tert*-Butyl) 3-methyl (*S*)-2-tosyl-1,2-diazetidine-1,3-dicarboxylate ((*S*)-6). Carboxylic acid (*S*)-**22** was synthesised from diazetidine (*S*)-**5** (377 mg, 0.75 mmol, 1.0 equiv) as describe above. To a solution of crude acid (*S*)-**22** in anhydrous DMF (5.0 mL) was added iodomethane (113 µL, 1.82 mmol, 2.4 equiv) and *N*,*N*-diisopropylethylamine (317 µL, 1.82 mmol, 2.4 equiv) and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with H_2O (3 x 10 mL) and brine (10 mL), dried over Na_2SO_4 and filtered. The mixture was concentrated *in vacuo* and purified by column chromatography on silica gel (20-33% EtOAc in petroleum ether) to yield (*S*)-**6** (227 mg, 0.61 mmol, 81% yield from (*S*)-**5**) as a white solid.

Alternatively, following a procedure by Presser and Hüfner,[3] the methyl ester (*S*)-**6** could be synthesised from carboxylic acid (*S*)-**22** using commercially available trimethylsilyldiazomethane ($TMSCH_2N_2$) solution in hexanes.

Enantiomeric excess (>98% *ee*) was determined by HPLC analysis (25 °C). [Chiralpak IA column 2-propanol/hexane = 1/9; flow rate = 1.0 mL/min; detection wavelength = 254 nm] t_R 11.6 min (minor); t_R 13.0 min (major).

R_f = 0.27 (33% EtOAc in petroleum ether).

M.p. 74–75 ºC.

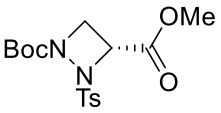
$[\alpha]^{28}_D$ +20.5 (*c* 0.88, $CHCl_3$).

IR (neat) 2985, 1752, 1737, 1363, 1300, 1166, 1146, 1084, 711 cm^{-1}.

δ_H (500 MHz, $CDCl_3$) 7.89 (d, *J* = 8.2 Hz, 2H), 7.38 (d, *J* = 8.0 Hz, 2H), 4.53 (dd, *J* = 9.0, 5.8 Hz, 1H), 4.16 (dd, *J* = 8.7, 5.8 Hz, 1H), 3.99 (t, *J* = 8.9 Hz, 1H), 3.82 (s, 3H), 2.47 (s, 3H), 1.41 (s, 9H).

δ_C (126 MHz, $CDCl_3$) 168.3, 158.9, 145.7, 130.4, 130.2, 129.8, 83.4, 57.6, 53.3, 51.5, 28.0, 21.9.

HRMS (ESI$^+$) calculated for $C_{32}H_{44}N_4NaO_{12}S_2$ $[2M+Na]^+$ 763.2289, measured 763.2280.

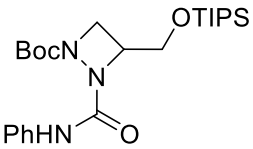


(*R*)-**6**

1-(*tert*-Butyl) 3-methyl (*R*)-2-tosyl-1,2-diazetidine-1,3-dicarboxylate ((*R*)-6). (*R*)-**6** was synthesised following the same procedure as described above starting from (*R*)-**5**. All analytical data were identical to compound (*S*)-**6** except:

$[\alpha]^{28}_{D}$ –26.6 (*c* 0.13, $CHCl_3$).

Enantiomeric excess (>98% *ee*) was determined by HPLC analysis (25 °C). [Chiralpak IA column 2-propanol/hexane = 1/9; flow rate = 1.0 mL/min; detection wavelength = 254 nm] t_R 11.6 min (major); t_R 12.9 min (minor).



8

***tert*-Butyl 2-(phenylcarbamoyl)-3-(((triisopropylsilyl)oxy)methyl)-1,2-diazetidine-1-carboxylate (8).** To a solution of diazetidine *rac*-**5** (65 mg, 0.13 mmol, 1.0 equiv) in anhydrous methanol (10 mL) was added an excess of activated magnesium turnings and the suspension was stirred (800 rpm) at room temperature for 2 h. The solution was quenched with saturated NH_4Cl solution (10 mL), extracted with dichloromethane (3 × 10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was re-dissolved in anhydrous dichloromethane (2.5 mL), phenyl isocyanate (22 µL, 0.20 mmol, 1.5 equiv) was added dropwise and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and purified by column chromatography on silica gel (10-20% EtOAc in petroleum ether) to give **8** as a colourless viscous oil (44 mg, 95 µmol, 73% yield).

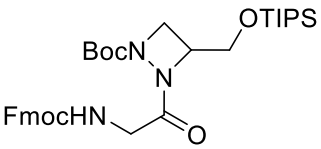
R_f = 0.44 (20% EtOAc in petroleum ether).

IR (neat) 2941, 2873, 1699, 1558, 1313, 1165, 994, 882, 763, 698 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 8.57 (br. s, 1H), 7.45 (d, *J* = 7.7 Hz, 2H), 7.29 (t, *J* = 7.9 Hz, 2H), 7.04 (t, *J* = 7.4 Hz, 1H), 4.63–4.53 (m, 1H), 4.32 (t, *J* = 7.3 Hz, 1H), 4.09 (t, *J* = 8.1 Hz, 1H), 3.98 (dd, *J* = 11.4, 3.9 Hz, 1H), 3.82 (dd, *J* = 11.4, 2.8 Hz, 1H), 1.54 (s, 9H), 1.11–1.05 (m, 21H).

δ_C (101 MHz, $CDCl_3$) 161.4, 138.4, 129.1, 123.4, 119.2, 83.5, 63.1, 59.6, 49.7, 28.3, 18.1, 12.1. *N.B.* One quaternary carbonyl peak not observed.

HRMS (ESI^+) calculated for $C_{24}H_{41}N_3NaO_4Si$ $[M+Na]^+$ 486.2759, measured 486.2751.



9

***tert*-Butyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)-3-(((triisopropylsilyl)oxy)methyl)-1,2-diazetidine-1-carboxylate (9).** To a solution of diazetidine *rac*-**5** (65 mg, 0.13 mmol, 1.0 equiv) in anhydrous methanol (10 mL) was added an excess of activated magnesium turnings and the suspension was stirred (800 rpm) at room temperature for 2 h. The solution was quenched with saturated ammonium chloride solution (10 mL), extracted with dichloromethane (3 × 10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was dissolved in anhydrous dichloromethane (5.0 mL), Fmoc-Gly-OH (58 mg, 0.20 mmol, 1.5 equiv), HATU (74 mg, 0.20 mmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (91 µL, 0.52 mmol, 4.0 equiv) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was

diluted with dichloromethane (10 mL), washed with 10% citric acid solution (10 mL) and saturated sodium hydrogen carbonate solution (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (10→33% EtOAc in petroleum ether) to give **9** as a colourless wax (44 mg, 71 μmol, 54% yield).

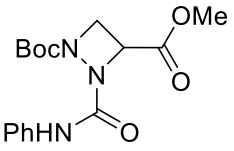
R_f = 0.52 (33% EtOAc in petroleum ether).

IR (neat) 3350, 2926, 2866, 1751, 1736, 1365, 1300, 1148, 739, 711 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (td, *J* = 7.5, 0.8 Hz, 2H), 5.46 (t, *J* = 4.9 Hz, 1H), 4.64–4.55 (m, 1H), 4.38 (d, *J* = 7.3 Hz, 2H), 4.31–4.13 (m, 5H), 4.08 (dd, *J* = 11.3, 3.6 Hz, 1H), 3.81 (dd, *J* = 11.4, 2.8 Hz, 1H), 1.52 (s, 9H), 1.11–1.03 (m, 21H).

δ_C (101 MHz, $CDCl_3$) 159.5, 156.4, 144.1, 144.0, 141.4, 127.8, 127.2, 125.3, 120.1, 83.5, 67.3, 63.2, 62.3, 51.3, 47.3, 42.2, 28.2, 18.1, 12.0.

HRMS (ESI^+) calculated for $C_{34}H_{49}N_3NaO_6Si$ $[M+Na]^+$ 646.3283, measured 646.3277.



10

1-(*tert*-Butyl) 3-methyl 2-(phenylcarbamoyl)-1,2-diazetidine-1,3-dicarboxylate (10). To a solution of diazetidine *rac*-**6** (64 mg, 0.18 mmol, 1.0 equiv) in anhydrous methanol (10 mL) was added an excess of activated magnesium turnings and the suspension was stirred (800 rpm) at room temperature for 2 h. The solution was quenched with saturated NH_4Cl solution (10 mL), extracted with dichloromethane (3 × 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was re-dissolved in anhydrous dichloromethane (2.5 mL), phenyl isocyanate (19 μL, 0.18 mmol, 1.0 equiv) was added dropwise and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and purified by column chromatography on silica gel (33% EtOAc in petroleum ether) to give **10** as a colourless wax (33 mg, 103 μmol, 60% yield).

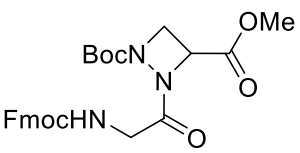
R_f = 0.51 (50% EtOAc in petroleum ether).

IR (neat) 2980, 1741, 1696, 1600, 1542, 1446, 1312, 1248, 1211, 1149, 752, 692 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 8.76 (br. s, 1H), 7.46 (d, *J* = 7.9 Hz, 2H), 7.30 (t, *J* = 7.9 Hz, 2H), 7.07 (t, *J* = 7.4 Hz, 1H), 5.02 (t, *J* = 8.1 Hz, 1H), 4.31 (d, *J* = 8.1 Hz, 2H), 3.82 (s, 3H), 1.56 (s, 9H).

δ_C (101 MHz, $CDCl_3$) 169.7, 161.1, 160.1, 138.0, 129.1, 123.8, 119.4, 84.6, 56.3, 53.0, 51.0, 28.3.

HRMS (ESI^+) calculated for $C_{16}H_{21}N_3NaO_5$ $[M+Na]^+$ 358.1373, measured 358.1369.



11

1-(*tert*-Butyl) 3-methyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)-1,2-diazetidine-1,3-dicarboxylate (11). To a solution of diazetidine *rac*-**6** (64 mg, 0.18 mmol, 1.0 equiv) in anhydrous methanol (10 mL) was added an excess of activated magnesium turnings and the suspension was stirred (800 rpm) at room temperature for 2 h. The solution was quenched with saturated

ammonium chloride solution (10 mL), extracted with dichloromethane (3 × 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in anhydrous dichloromethane (5.0 mL), Fmoc-Gly-OH (80 mg, 0.27 mmol, 1.5 equiv), HATU (103 mg, 0.27 mmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (125 µL, 0.72 mmol, 4.0 equiv) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (10 mL), washed with 10% citric acid solution (10 mL) and saturated sodium hydrogen carbonate solution (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (33→50% EtOAc in petroleum ether) to give **11** as a colourless viscous oil, which crystallised upon standing to form a white solid (50 mg, 101 µmol, 59% yield).

R_f = 0.33 (50% EtOAc in petroleum ether).

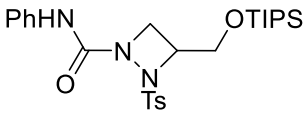
M.p. 55–57 ºC.

IR (neat) 3366, 2926, 1695, 1520, 1394, 1309, 1246, 1148, 759, 729 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.76 (d, *J* = 7.4 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 5.43 (br. s, 1H), 4.99 (dd, *J* = 7.7, 6.1 Hz, 1H), 4.44 (t, *J* = 8.6 Hz, 1H), 4.39 (d, *J* = 7.0 Hz, 2H), 4.35–4.23 (m, 3H), 4.23–4.15 (m, 1H), 3.82 (s, 3H), 1.54 (s, 9H).

δ_C (101 MHz, $CDCl_3$) 177.1, 168.7, 159.6, 156.5, 144.0, 141.4, 127.8, 127.2, 125.3, 120.1, 84.3, 67.4, 60.3, 53.2, 53.1, 47.2, 42.1, 28.2.

HRMS (ESI^+) calculated for $C_{26}H_{29}N_3NaO_7$ $[M+Na]^+$ 518.1898, measured 518.1898.



12

***N*-phenyl-2-tosyl-3-(((triisopropylsilyl)oxy)methyl)-1,2-diazetidine-1-carboxamide (12).** To a solution of diazetidine *rac*-**5** (65 mg, 0.13 mmol, 1.0 equiv) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL) and the mixture was stirred for 2 h at room temperature. The reaction mixture was then concentrated *in vacuo*, re-dissolved in dichloromethane (10 mL) and concentrated under reduced pressure (2×). The residue was dissolved in dichloromethane (10 mL), washed with saturated $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane (2.5 mL), phenyl isocyanate (22 µL, 0.20 mmol, 1.5 equiv) was added dropwise and the mixture was stirred overnight at room temperature. The mixture was concentrated *in vacuo* and the residue was purified by column chromatography (10→20% EtOAc in petroleum ether) to give **12** as a pale-yellow oil (44 mg, 85 µmol, 65% yield).

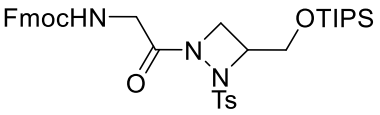
R_f = 0.32 (20% EtOAc in petroleum ether).

IR (neat) 3387, 2942, 2867, 1721, 1559, 1449, 1371, 1167, 998, 702 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 8.33 (s, 1H), 7.90 (d, *J* = 8.3 Hz, 2H), 7.52 (dd, *J* = 8.5, 0.9 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.08 (t, *J* = 7.4 Hz, 1H), 4.26 (dd, *J* = 8.4, 5.9 Hz, 1H), 3.98 (dd, *J* = 11.5, 3.6 Hz, 1H), 3.88 (ddd, *J* = 8.7, 6.1, 3.2 Hz, 1H), 3.77 (dd, *J* = 11.5, 2.8 Hz, 1H), 3.46 (t, *J* = 8.4 Hz, 1H), 2.50 (s, 3H), 1.06–0.99 (m, 21H).

δ_C (101 MHz, $CDCl_3$) 159.6, 146.1, 138.0, 130.4, 130.0, 129.0, 128.3, 123.7, 119.1, 63.1, 61.8, 48.5, 21.9, 18.0, 12.0.

HRMS (ESI$^+$) calculated for $C_{26}H_{39}N_3NaO_4SSi$ $[M+Na]^+$ 540.2323, measured 540.2321.



13

(9*H*-Fluoren-9-yl)methyl (2-oxo-2-(2-tosyl-3-(((triisopropylsilyl)oxy)methyl)-1,2-diazetidin-1-yl)ethyl)carbamate (13). To a solution of diazetidine *rac*-**5** (90 mg, 0.18 mmol, 1.0 equiv) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL) and the mixture was stirred for 2 h at room temperature. The mixture was then concentrated *in vacuo*, re-dissolved in dichloromethane (5.0 mL) and concentrated under reduced pressure (2×). The residue was dissolved in dichloromethane (10 mL), washed with saturated $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dissolved in anhydrous dichloromethane (5.0 mL), Fmoc-Gly-OH (80 mg, 0.27 mmol, 1.5 equiv), HATU (103 mg, 0.27 mmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (125 µL, 0.72 mmol, 4.0 equiv) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (10 mL), washed with 10% citric acid solution (10 mL) and saturated sodium hydrogen carbonate solution (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (20→33% EtOAc in petroleum ether) to give **13** as a colourless wax, which solidified upon standing (54 mg, 80 µmol, 44% yield).

R_f = 0.30 (33% EtOAc in petroleum ether).

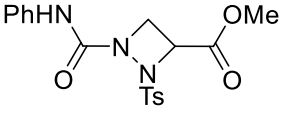
M.p. 59–61 ºC.

IR (neat) 3367, 2943, 2865, 1699, 1513, 1149, 1393, 1243, 1165, 1038, 994, 758, 740 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.90 (d, *J* = 7.9 Hz, 2H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.70 (br. s, 1H), 7.68–7.58 (m, 2H), 7.42 (d, *J* = 7.7 Hz, 2H), 7.40 (d, *J* = 7.7 Hz, 2H), 7.33 (t, *J* = 6.8 Hz, 2H), 5.37 (br. s, 1H), 4.45 (br. s, 1H), 4.38 (t, *J* = 8.4 Hz, 2H), 4.26 (t, *J* = 7.3 Hz, 1H), 4.23–4.17 (m, 1H), 4.01–3.92 (m, 2H), 3.76 (d, *J* = 9.4 Hz, 1H), 3.57 (t, *J* = 8.2 Hz, 1H), 2.50 (s, 3H), 1.09–0.99 (m, 21H).

δ_C (101 MHz, $CDCl_3$) 156.2, 146.1, 144.1, 141.4, 130.2, 130.2, 127.8, 127.2, 125.4, 120.1, 67.3, 63.1, 63.0, 51.0, 47.3, 42.4, 21.9, 18.0, 12.0. *N.B.* One quaternary carbonyl peak not observed.

HRMS (ESI$^+$) calculated for $C_{36}H_{47}N_3NaO_6SSi$ $[M+Na]^+$ 700.2847, measured 700.2849.



14

Methyl 1-(phenylcarbamoyl)-2-tosyl-1,2-diazetidine-3-carboxylate (14). To a solution of diazetidine *rac*-**6** (70 mg, 0.19 mmol, 1.0 equiv) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL) and the mixture was stirred for 2 h at room temperature. The mixture was then concentrated *in vacuo*, re-

dissolved in dichloromethane (10 mL) and concentrated under reduced pressure (2×). The residue was dissolved in dichloromethane (10 mL), washed with saturated $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane (2.5 mL), phenyl isocyanate (21 µL, 0.19 mmol, 1.0 equiv) was added dropwise and the mixture was stirred overnight at room temperature. The solution was concentrated *in vacuo* and the residue was purified by column chromatography (33% EtOAc in petroleum ether) to give **14** as a colourless viscous oil, which crystallised upon standing to form a white solid (48 mg, 0.12 mmol, 65% yield).

R_f = 0.14 (33% EtOAc in petroleum ether).

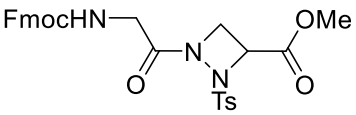
M.p. 53–54 ºC.

IR (neat) 3368, 2925, 1744, 1698, 1541, 1352, 1233, 1159, 1085, 751, 690 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 8.38 (br. s, 1H), 7.93 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.34 (t, *J* = 7.9 Hz, 2H), 7.11 (t, *J* = 7.4 Hz, 1H), 4.30 (dd, *J* = 6.3, 2.8 Hz, 1H), 4.27 (dd, *J* = 6.3, 3.0 Hz, 1H), 3.82 (s, 3H), 3.68–3.62 (m, 1H), 2.51 (s, 3H).

δ_C (101 MHz, $CDCl_3$) 167.5, 159.3, 146.8, 137.6, 130.6, 130.2, 129.1, 127.3, 124.1, 119.4, 58.4, 53.5, 49.8, 22.0.

HRMS (ESI$^+$) calculated for $C_{18}H_{19}N_3NaO_5S$ $[M+Na]^+$ 412.0938, measured 412.0932.



15

Methyl 1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)-2-tosyl-1,2-diazetidine-3-carboxylate (15). To a solution of diazetidine *rac*-**6** (70 mg, 0.19 mmol, 1.0 equiv) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL) and the mixture was stirred for 2 h at room temperature. The mixture was then concentrated *in vacuo*, re-dissolved in dichloromethane (5.0 mL) and concentrated under reduced pressure (2×). The residue was dissolved in dichloromethane (10 mL), washed with saturated $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dissolved in anhydrous dichloromethane (5.0 mL), Fmoc-Gly-OH (85 mg, 0.29 mmol, 1.5 equiv), HATU (108 mg, 0.29 mmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (132 µL, 0.72 mmol, 4.0 equiv) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (10 mL), washed with 10% citric acid solution (10 mL) and saturated sodium hydrogen carbonate solution (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (50% EtOAc in petroleum ether) to give **15** as a colourless wax, which solidified upon standing (54 mg, 98 µmol, 52% yield).

R_f = 0.22 (50% EtOAc in petroleum ether).

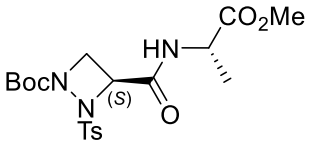
M.p. 64–66 ºC.

IR (neat) 3385, 2955, 1707, 1524, 1362, 1216, 1166, 1047, 739 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.92 (d, *J* = 7.9 Hz, 2H), 7.77 (d, *J* = 7.4 Hz, 2H), 7.63 (d, *J* = 7.1 Hz, 2H), 7.45 (d, *J* = 7.8 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.1 Hz, 2H), 5.35 (br. s, 1H), 4.61–4.49 (m, 1H), 4.47–4.30 (m, 4H), 4.30–4.19 (m, 2H), 3.85–3.72 (m, 4H), 2.50 (s, 3H).

δ_C (101 MHz, $CDCl_3$) 179.2, 167.5, 156.4, 146.8, 144.0, 141.4, 130.4, 130.3, 128.8, 127.8, 127.2, 125.3, 120.1, 67.3, 60.3, 53.6, 53.1, 47.3, 42.2, 22.0.

HRMS (ESI^+) calculated for $C_{28}H_{27}N_3NaO_7S$ $[M+Na]^+$ 527.1462, measured 527.1466.



(*S*,*S*)-**16**

***tert*-Butyl (*S*)-3-(((*S*)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-2-tosyl-1,2-diazetidine-1-carboxylate ((*S*,*S*)-16).** Carboxylic acid (*S*)-**22** was synthesised from diazetidine (*S*)-**5** (65 mg, 0.13 mmol, 1.0 equiv) as describe above. To a solution of crude acid (*S*)-**22** in anhydrous dichloromethane (3.0 mL), H_2N-Ala-OMe·HCl (28 mg, 0.20 mmol, 1.5 equiv), HATU (59 mg, 0.16 mmol, 1.2 equiv) and *N*,*N*-diisopropylethylamine (113 µL, 0.65 mmol, 5.0 equiv) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (10 mL), washed with 10% citric acid solution (10 mL) and saturated sodium hydrogen carbonate solution (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (33→50% EtOAc in petroleum ether) to give (*S*,*S*)-**16** as a white solid (38 mg, 86 µmol, 65% yield over 3 steps).

R_f = 0.34 (50% EtOAc in petroleum ether).

M.p. 128–129 ºC.

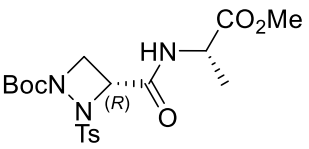
$[\alpha]^{25}_D$ –4.0 (*c* 0.25, $CHCl_3$).

IR (neat) 3368, 2925, 1743, 1728, 1697, 1540, 1288, 1150, 1086, 1042, 720, 680 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.89 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 2H), 4.57 (p, *J* = 7.2 Hz, 1H), 4.30 (dd, *J* = 9.3, 5.8 Hz, 1H), 4.00 (dd, *J* = 8.9, 5.8 Hz, 1H), 3.90 (t, *J* = 9.1 Hz, 1H), 3.78 (s, 3H), 2.49 (s, 3H), 1.48 (d, *J* = 7.2 Hz, 3H), 1.45 (s, 9H).

δ_C (101 MHz, $CDCl_3$) 172.4, 167.4, 158.8, 146.1, 130.3, 130.1, 129.2, 83.7, 58.7, 52.8, 52.7, 48.4, 28.0, 21.9, 18.2.

HRMS (ESI^+) calculated for $C_{19}H_{27}N_3NaO_7S$ $[M+Na]^+$ 464.1462, measured 464.1459.

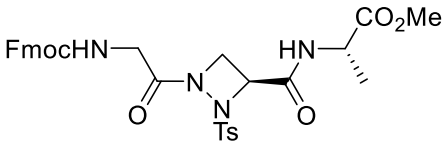


(*R*,*S*)-**16**

***tert*-Butyl (*R*)-3-(((*S*)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-2-tosyl-1,2-diazetidine-1-carboxylate ((*R*,*S*)-16).** (*R*)-**16** was synthesised following the same procedure as described above for (*S*,*S*)-**16** starting from (*R*)-**5**.

δ_H (400 MHz, $CDCl_3$) 7.88 (d, *J* = 8.1 Hz, 2H), 7.50 (d, *J* = 7.4 Hz, 1H), 7.42 (d, *J* = 8.9 Hz, 2H), 4.58 (p, *J* = 7.2 Hz, 1H), 4.28 (dd, *J* = 9.3, 5.7 Hz, 1H), 4.96 (dd, *J* = 8.8, 5.8 Hz, 1H), 3.89 (t, *J* = 9.1 Hz, 1H), 3.75 (s, 3H), 2.49 (s, 3H), 1.48 (d, *J* = 7.2 Hz, 3H), 1.47 (s, 9H).

δ_C (101 MHz, $CDCl_3$) 172.3, 167.4, 158.8, 146.1, 130.2, 130.1, 129.3, 83.8, 58.8, 53.1, 52.7, 48.3, 28.0, 21.9, 18.1.



17

Methyl ((*S*)-1-((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)-2-tosyl-1,2-diazetidine-3-carbonyl)-*L*-alaninate (17). To a solution of dipeptide (*S*,*S*)-**16** (31 mg, 70 μmol, 1.0 equiv) in CH_2Cl_2 (3.0 mL) was added trifluoroacetic acid (1.0 mL) and the mixture was stirred for 2 h at room temperature. The mixture was then concentrated *in vacuo*, re-dissolved in dichloromethane (5.0 mL) and concentrated under reduced pressure (2×). The residue was dissolved in CH_2Cl_2 (10 mL), washed with saturated $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dissolved in anhydrous CH_2Cl_2 (5.0 mL), Fmoc-Gly-OH (37 mg, 0.12 mmol, 1.5 equiv), HATU (47 mg, 0.12 mmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (57 μL, 0.33 mmol, 4.0 equiv) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 10% citric acid solution (10 mL) and saturated sodium hydrogen carbonate solution (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (50% EtOAc in petroleum ether→ EtOAc) to give **17** as a colourless wax, which solidified upon standing (22 mg, 35 μmol, 51% yield). The product was obtained as a mixture of confomers in a 58:42 ratio as determined by ^{1}H NMR in $CDCl_3$ at 298 K.

R_f = 0.12 (50% EtOAc in petroleum ether).

M.p. 61–62 ºC.

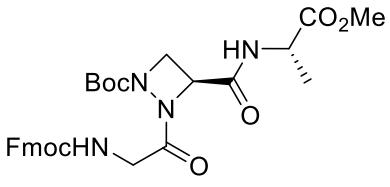
$[\alpha]^{28}_D$ +13.6 (*c* 0.04, $CHCl_3$).

IR (neat) 3346, 2926, 1736, 1677, 1527, 1358, 1213, 1162 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.96–7.87 (m, 4H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 6.7 Hz, 2H), 7.47–7.37 (m, 7H), 7.32 (t, *J* = 7.5 Hz, 2H), 5.42 (br. s, 1H), 4.68–4.51 (m, 3H), 4.42–4.07 (m, 8H), 3.82–3.71 (m, 8H), 3.35 (t, *J* = 8.9 Hz, 1H), 2.51 (s, 3H), 2.49 (s, 3H), 1.51 (d, *J* = 7.3 Hz, 3H), 1.49 (d, *J* = 7.1 Hz, 3H).

δ_C (101 MHz, $CDCl_3$) 172.4, 168.4, 166.1, 156.6, 147.1, 145.7, 143.9, 141.4, 130.6, 130.5, 130.4, 129.8, 127.9, 127.2, 125.3, 120.1, 67.5, 62.3, 60.3, 52.8, 48.7, 48.4, 47.2, 44.8, 41.6, 22.0, 18.3, 17.8.

HRMS (ESI$^+$) calculated for $C_{31}H_{32}N_4NaO_8S$ $[M+Na]^+$ 643.1833, measured 643.1835.



18

***tert*-Butyl (*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)-3-(((*S*)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-1,2-diazetidine-1-carboxylate (18).** To a solution of dipeptide (*S*,*S*)-**16** (95 mg, 0.22 mmol, 1.0 equiv) in anhydrous methanol (10 mL) was added an excess of activated magnesium turnings and the suspension was stirred (800 rpm) at room temperature for 2 h. The solution was quenched with saturated ammonium chloride solution (10 mL), extracted with dichloromethane (3 × 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in anhydrous dichloromethane (5.0 mL), Fmoc-Gly-OH (96 mg, 0.32 mmol, 1.5 equiv), HATU (122 mg, 0.32 mmol, 1.5 equiv)

and *N*,*N*-diisopropylethylamine (150 µL, 0.86 mmol, 4.0 equiv) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (10 mL), washed with 10% citric acid solution (10 mL) and saturated sodium hydrogen carbonate solution (10 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (50% EtOAc in petroleum ether→ EtOAc) to give **18** as a white foam (61 mg, 108 µmol, 50% yield).

R_f = 0.15 (50% EtOAc in petroleum ether).

M.p. 76–77 ºC.

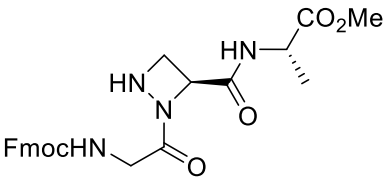
$[\alpha]^{29}_D$ –68.1 (*c* 0.11, $CHCl_3$).

IR (neat) 3298, 2979, 1693, 1600, 1543, 1446, 1313, 1248, 1211, 1148, 753, 692 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.76 (d, *J* = 7.4 Hz, 2H), 7.61 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 3H), 5.48 (br. s, 1H), 4.91 (dd, *J* = 7.9, 6.9 Hz, 1H), 4.55 (p, *J* = 7.1 Hz, 1H), 4.44–4.36 (m, 3H), 4.37–4.29 (m, 2H), 4.29–4.20 (m, 2H), 3.75 (s, 3H), 1.52 (s, 9H), 1.44 (d, *J* = 7.4 Hz, 3H).

δ_C (101 MHz, $CDCl_3$) 178.6, 172.7, 167.4, 159.6, 156.5, 143.9, 141.4, 127.8, 127.2, 125.2, 120.1, 84.3, 67.4, 61.3, 53.0, 52.7, 48.4, 47.2, 42.1, 28.1, 18.2.

HRMS (ESI$^+$) calculated for $C_{29}H_{34}N_4NaO_8$ $[M+Na]^+$ 589.2269, measured 589.2270.



19

Methyl ((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)-1,2-diazetidine-3-carbonyl)-L-alaninate (19). To a solution of pseudotripeptide **18** (55 mg, 97 µmol, 1.0 equiv) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL) and the mixture was stirred for 2 h at room temperature. The reaction mixture was then concentrated *in vacuo*, re-dissolved in dichloromethane (10 mL) and concentrated under reduced pressure (2×). The residue was dissolved in dichloromethane (10 mL), washed with saturated $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give **19** as a white solid (44 mg, 94 µmol, 97% yield).

M.p. 72–74 ºC.

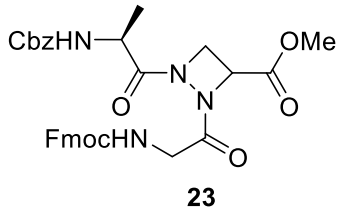
$[\alpha]^{25}_D$ –61.4 (*c* 0.04, $CHCl_3$).

IR (neat) 3307, 2920, 1715, 1667, 1529, 1449, 1215, 1158, 756, 729 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.3 Hz, 2H), 7.47 (br. s, 1H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.3 Hz, 2H), 5.56 (br. s, 1H), 5.26–4.96 (m, 1H), 4.61–4.50 (m, 1H), 4.39 (d, *J* = 6.7 Hz, 2H), 4.29–4.06 (m, 3H), 4.01–3.78 (m, 2H), 3.72 (s, 3H), 1.44 (d, *J* = 7.1 Hz, 3H).

δ_C (101 MHz, $CDCl_3$) 172.7, 168.4, 160.6, 156.6, 143.9, 141.4, 127.9, 127.2, 125.2, 120.1, 67.4, 64.5, 52.7, 48.4, 47.2, 45.3, 41.1, 18.1.

HRMS (ESI$^+$) calculated for $C_{24}H_{26}N_4NaO_6$ $[M+Na]^+$ 489.1745, measured 489.1748.



23

Methyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)glycyl)-1-(((benzyloxy)carbonyl)-L-alanyl)-1,2-diazetidine-3-carboxylate (23). To a solution of racemic pseudodipeptide **11** (25 mg, 50 µmol, 1.0 equiv) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL) and the mixture was stirred for 2 h at room temperature. The reaction mixture was then concentrated *in vacuo*, re-dissolved in dichloromethane (10 mL) and concentrated under reduced pressure (2×). The residue was dissolved in dichloromethane (10 mL), washed with saturated $NaHCO_3$ solution (10 mL), and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was dissolved in anhydrous dichloromethane (5.0 mL), Cbz-Ala-OH (17 mg, 76 µmol, 1.5 equiv), HATU (29 mg, 76 µmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (53 µL, 0.30 mmol, 4.0 equiv) were added, and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (10 mL), washed with 10% citric acid solution (10 mL) and saturated sodium hydrogen carbonate solution (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (50% EtOAc in petroleum ether→ EtOAc) to give **23** as a colourless wax (9 mg, 15 µmol, 30% yield). *NB* The pseudotripeptide **23** was obtained as a mixture of diastereomers as racemic **11** was used as starting material.

R_f = 0.61 (EtOAc).

IR (neat) 3335, 2925, 2854, 1709, 1522, 1451, 1245, 1059, 845, 760, 743 cm^{-1}.

δ_H (400 MHz, $CDCl_3$) 7.76 (d, *J* = 7.4 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.37–7.27 (m, 7H), 5.53–5.15 (m, 2H), 5.17–4.99 (m, 3H), 4.78–4.66 (m, 1H), 4.48–4.40 (m, 1H), 4.39 (d, *J* = 6.8 Hz, 2H), 4.23 (t, *J* = 6.8 Hz, 1H), 4.19–3.91 (m, 3H), 3.82 (br. s, 3H), 1.45–1.40 (m, 3H). *NB* Broad signals due to hindered rotation of the substituents and *N*-fluxionality around the four-membered diazetidine ring.

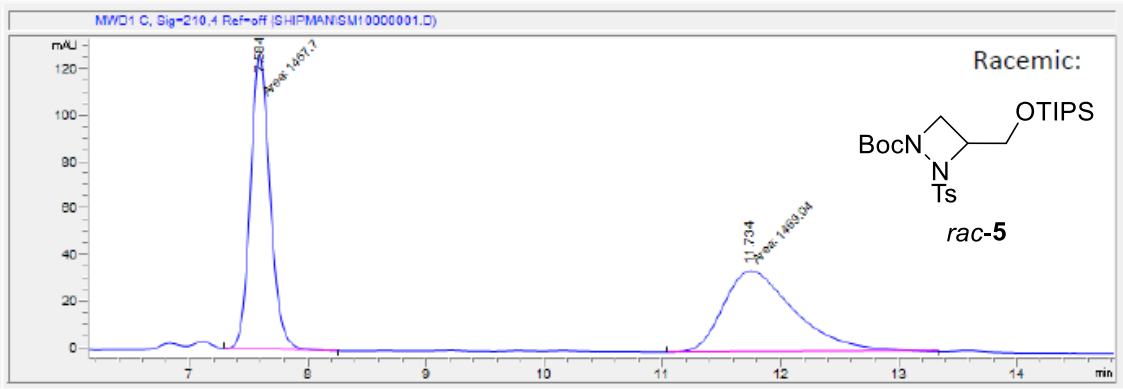
δ_C (126 MHz, $CDCl_3$) 176.3, 173.3, 156.6, 156.0, 143.9, 141.5, 128.7, 128.4, 128.3, 127.9, 127.3, 125.2, 124.9, 120.2, 67.5, 67.3, 64.8, 61.4, 60.6, 53.6, 49.5, 48.4, 47.2, 42.6, 18.5.

HRMS (ESI^+) calculated for $C_{32}H_{32}N_4NaO_8$ $[M+Na]^+$ 623.2112, measured 623.2121.

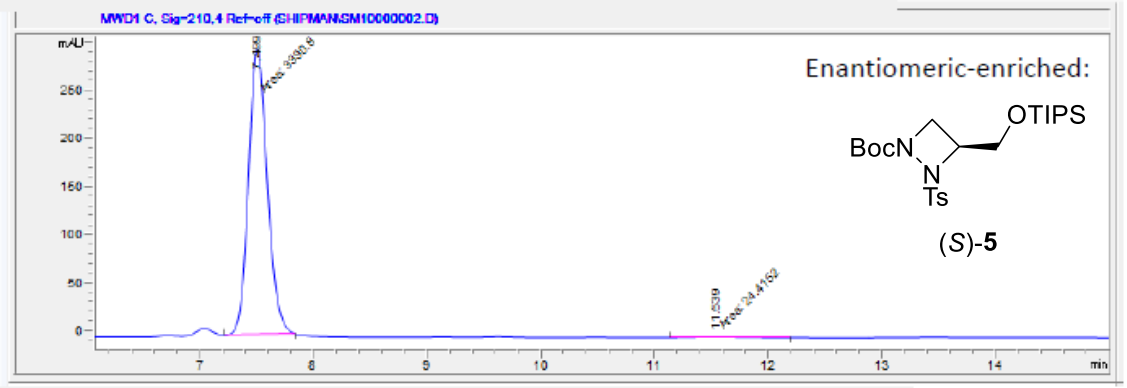
3. HPLC traces

HPLC profile of **5**

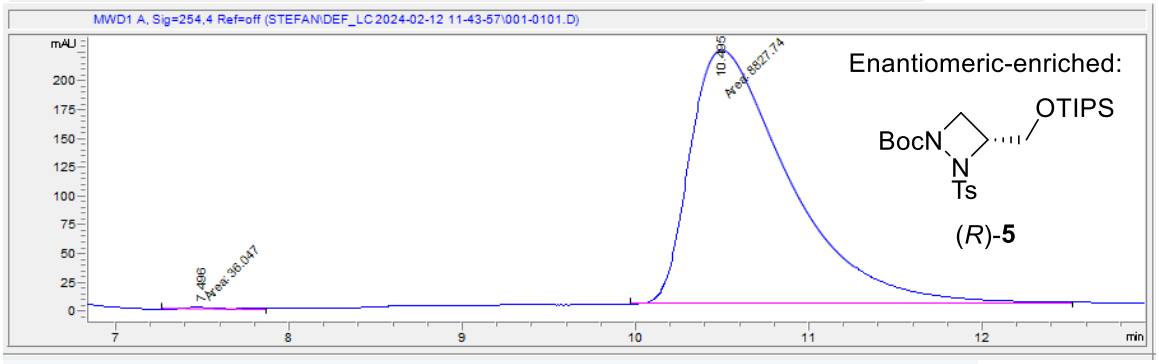
Chiralpak IA column (0.46 cm ø x 25 cm), 98:2 hexane:propan-2-ol, T = 25 °C, flow rate = 1.0 mL/min.



#	Time	Area	Height	Width	Area%	Symmetry
1	7.584	1457.7	126.7	0.1917	49.791	0.854
2	11.734	1469.9	34.8	0.7031	50.209	0.604



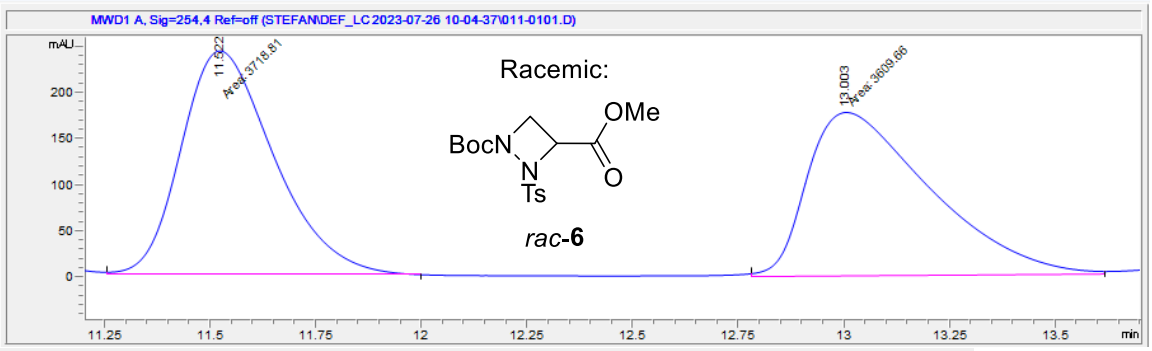
#	Time	Area	Height	Width	Area%	Symmetry
1	7.499	3390.8	294.7	0.1917	99.285	0.852
2	11.539	24.4	6.6E-1	0.6149	0.715	0.605



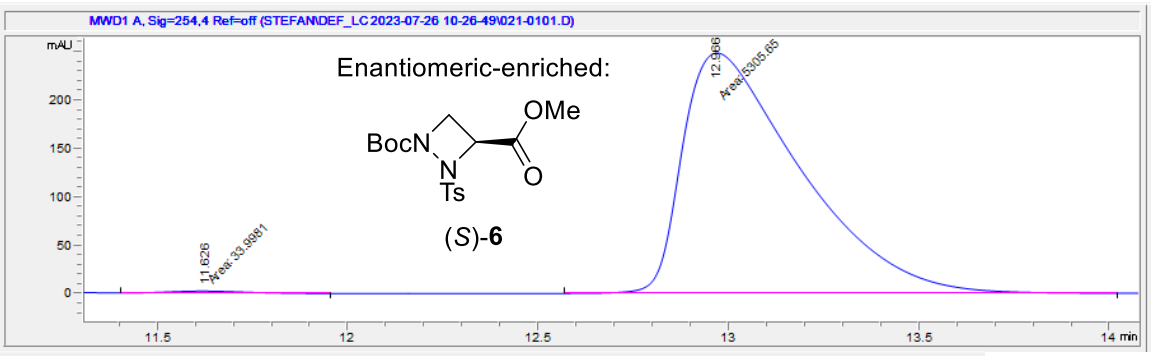
#	Time	Area	Height	Width	Area%	Symmetry
1	7.496	36	1.9	0.3122	0.407	0.647
2	10.495	8827.7	220.8	0.6664	99.593	0.455

HPLC profile of **6**

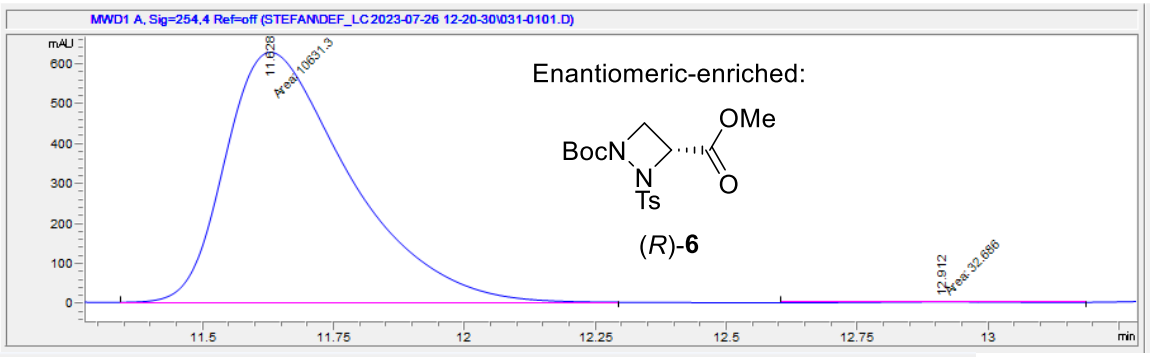
Chiralpak IA column (0.46 cm ø x 25 cm), 9:1 hexane:propan-2-ol, T = 25 °C, flow rate = 1.0 mL/min.



#	Time	Area	Height	Width	Area%	Symmetry
1	11.522	3718.8	242.3	0.2558	50.745	0.7
2	13.003	3609.7	176.9	0.34	49.255	0.449



#	Time	Area	Height	Width	Area%	Symmetry
1	11.626	34	2.3	0.2498	0.637	0.71
2	12.966	5305.6	247.5	0.3572	99.363	0.401



#	Time	Area	Height	Width	Area%	Symmetry
1	11.628	10631.3	635	0.279	99.693	0.551
2	12.912	32.7	1.9	0.2867	0.307	1.062

4. References

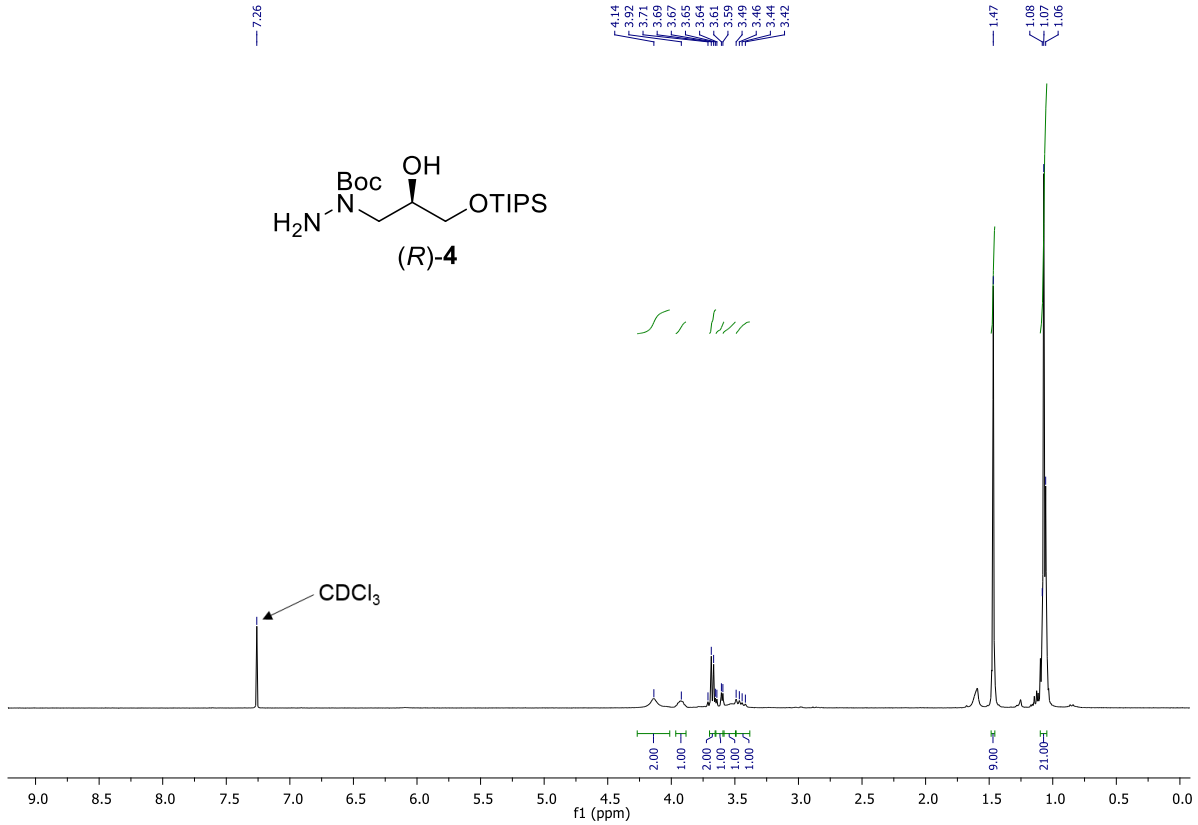
(1) D. Lainé, M. Fujita and S. V. Ley, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1639–1645.

(2) S. Rajkumar, G. J. Clarkson and M. Shipman, *Org. Lett.*, 2017, **19**, 2058–2061.

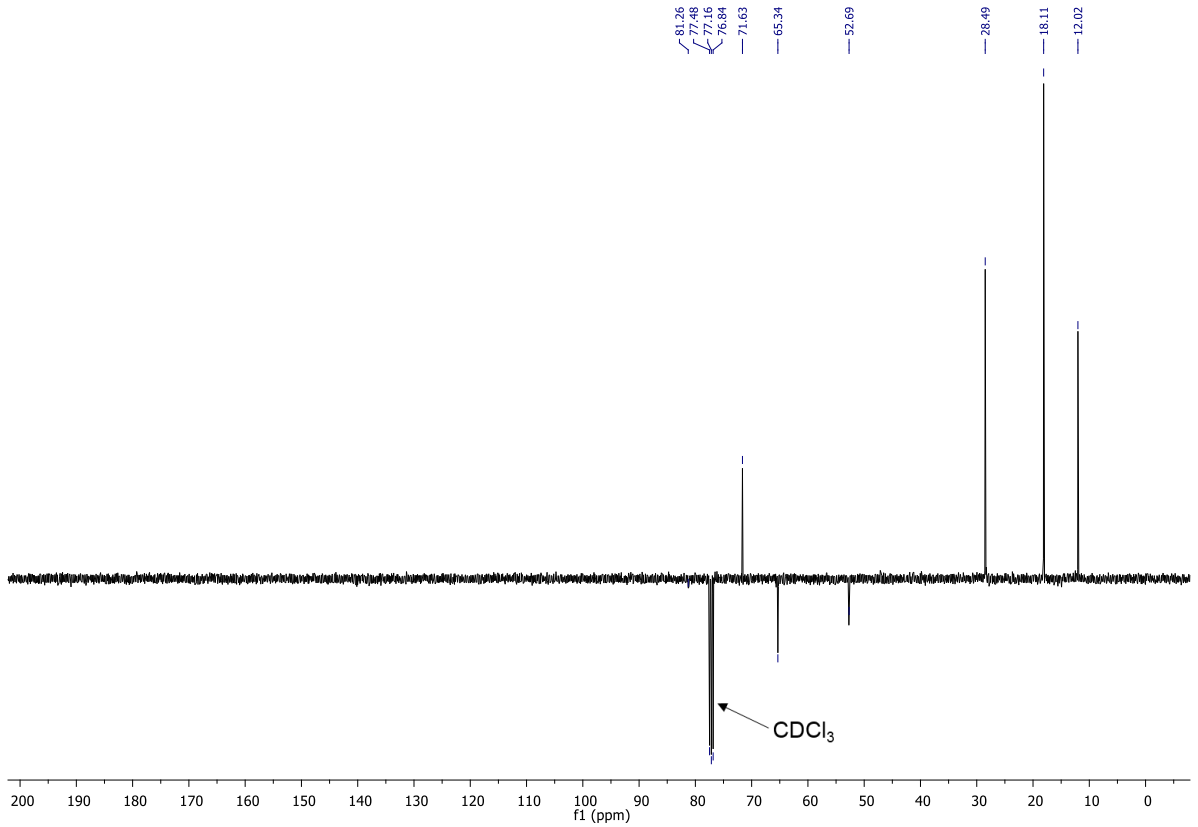
(3) A. Presser and A. Hüfner, *Monatsh. Chem.*, 2004, **135**, 1015–1022.

5. ^{1}H and ^{13}C NMR spectra

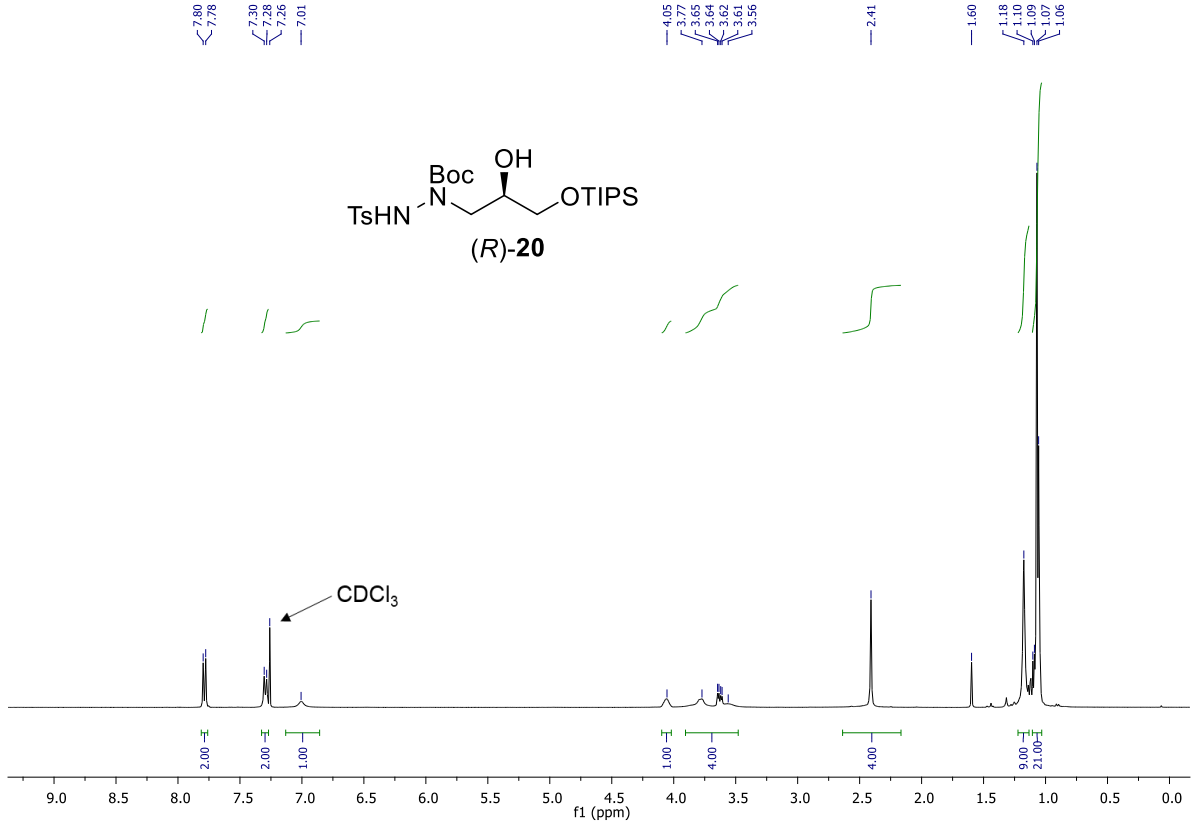
^{1}H NMR (400 MHz, $CDCl_3$)



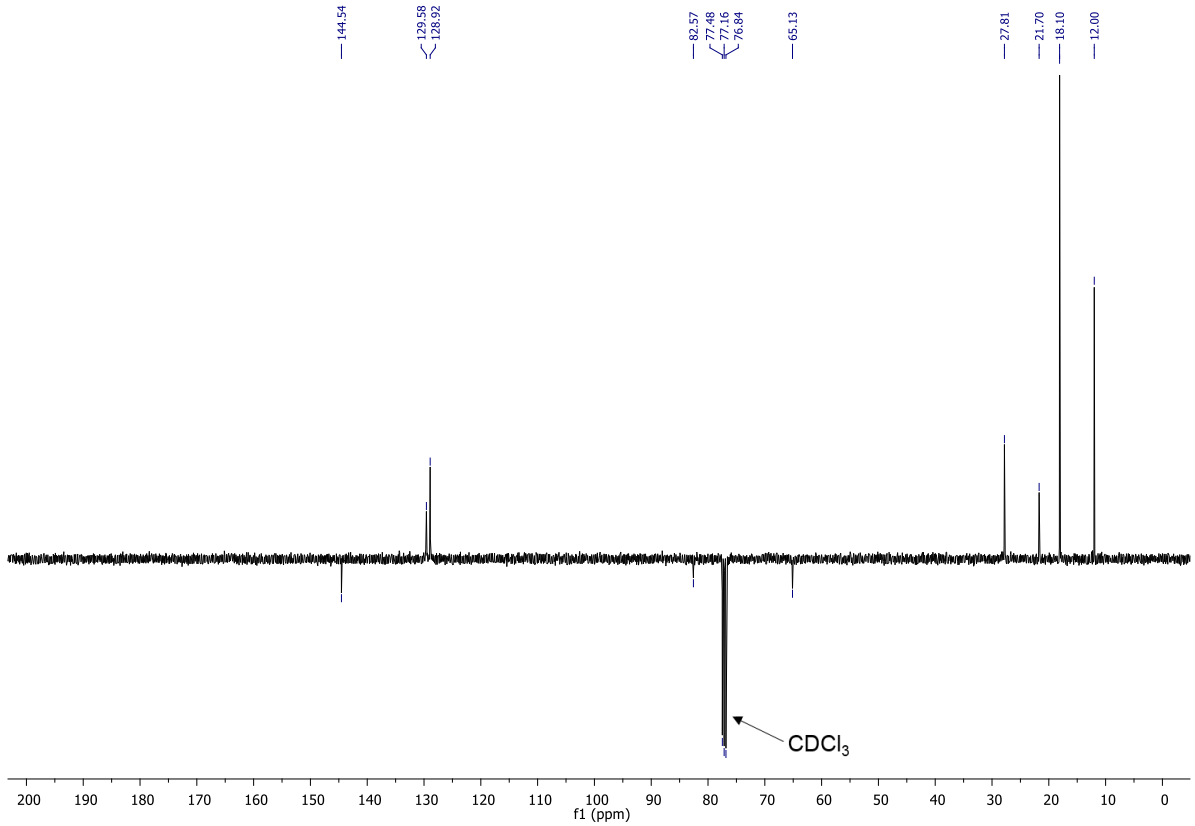
^{13}C NMR (126 MHz, $CDCl_3$)



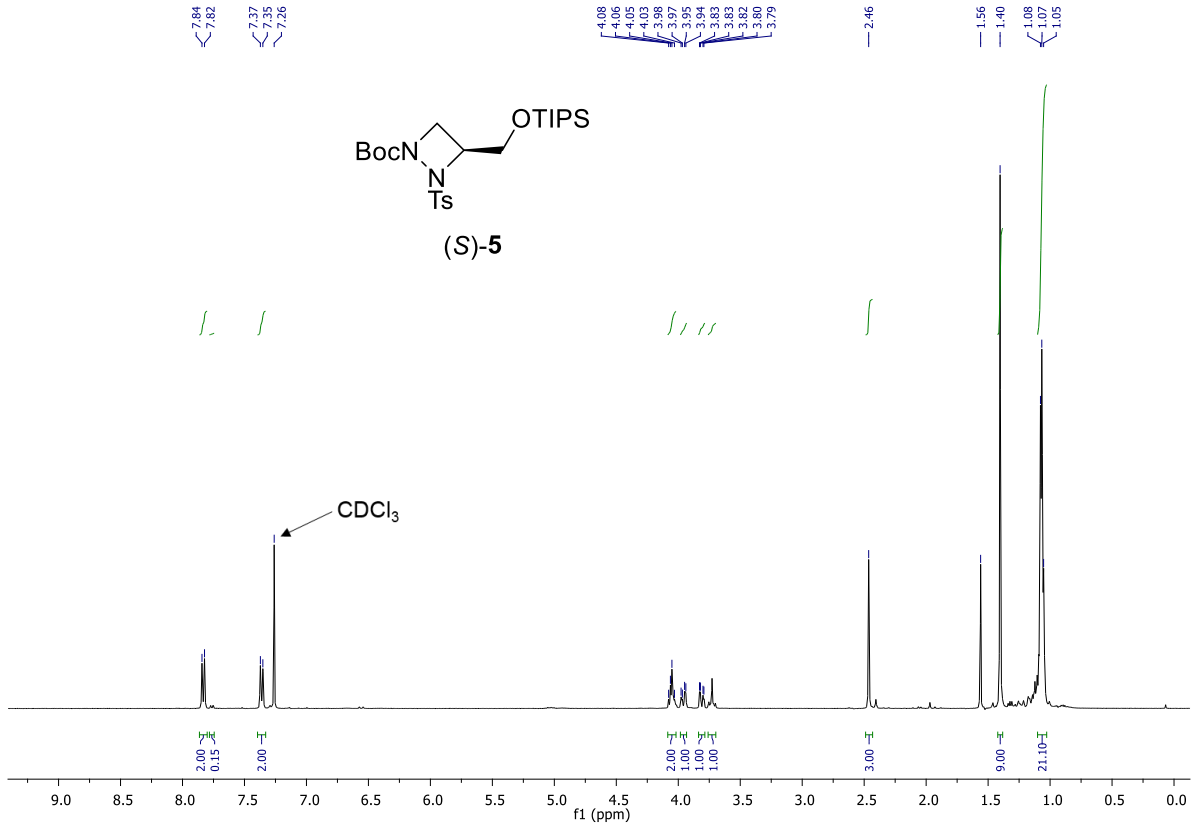
^{1}H NMR (400 MHz, $CDCl_3$)



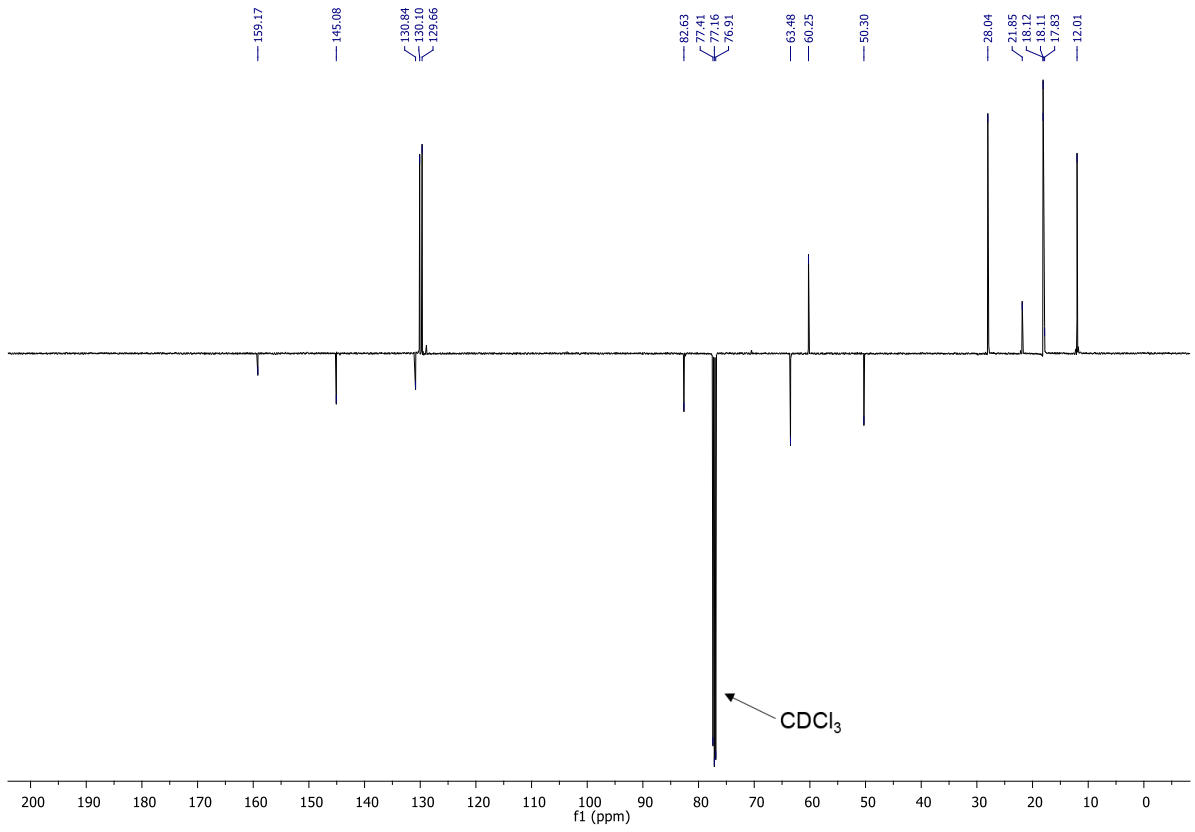
^{13}C NMR (101 MHz, $CDCl_3$)



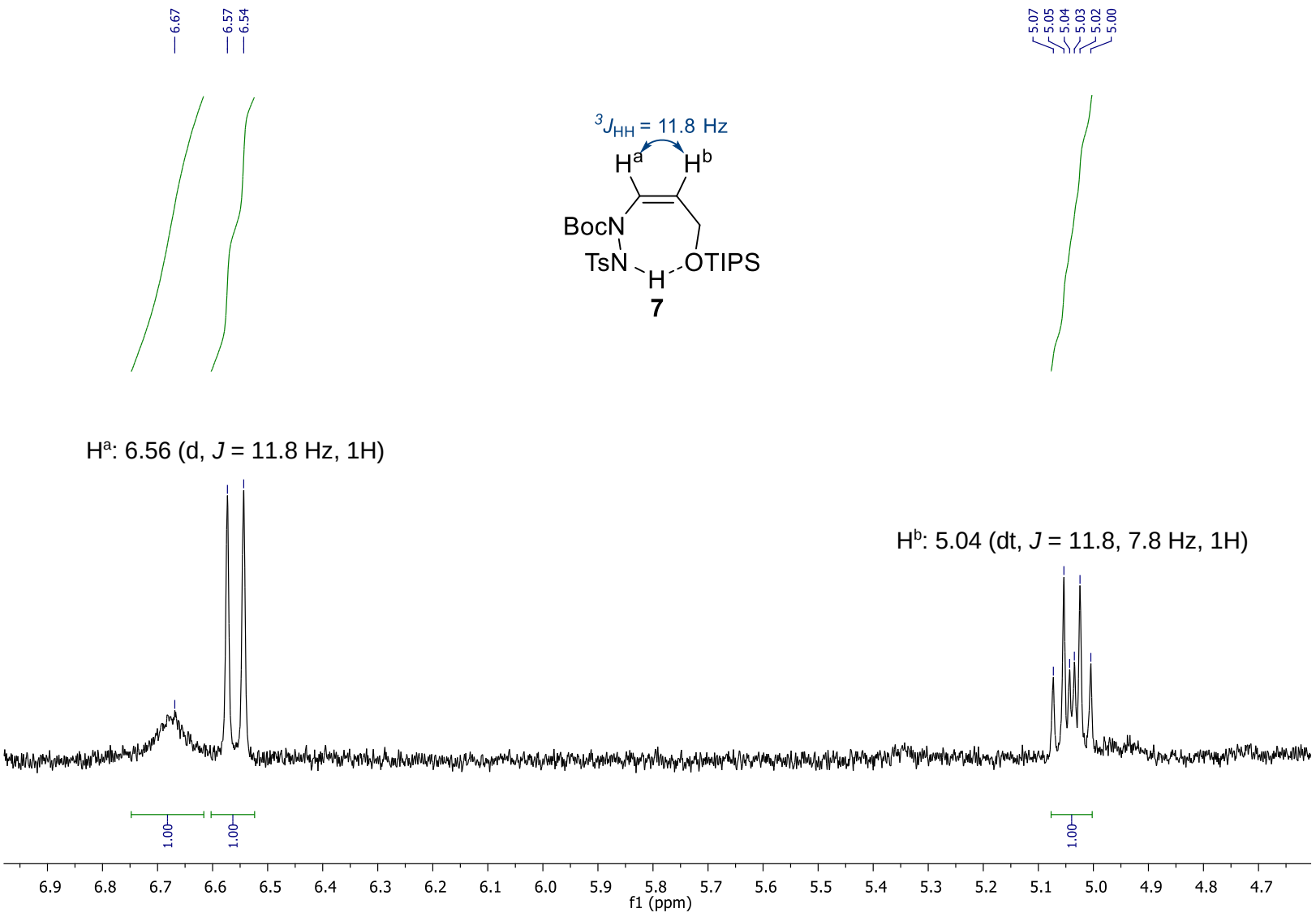
^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)



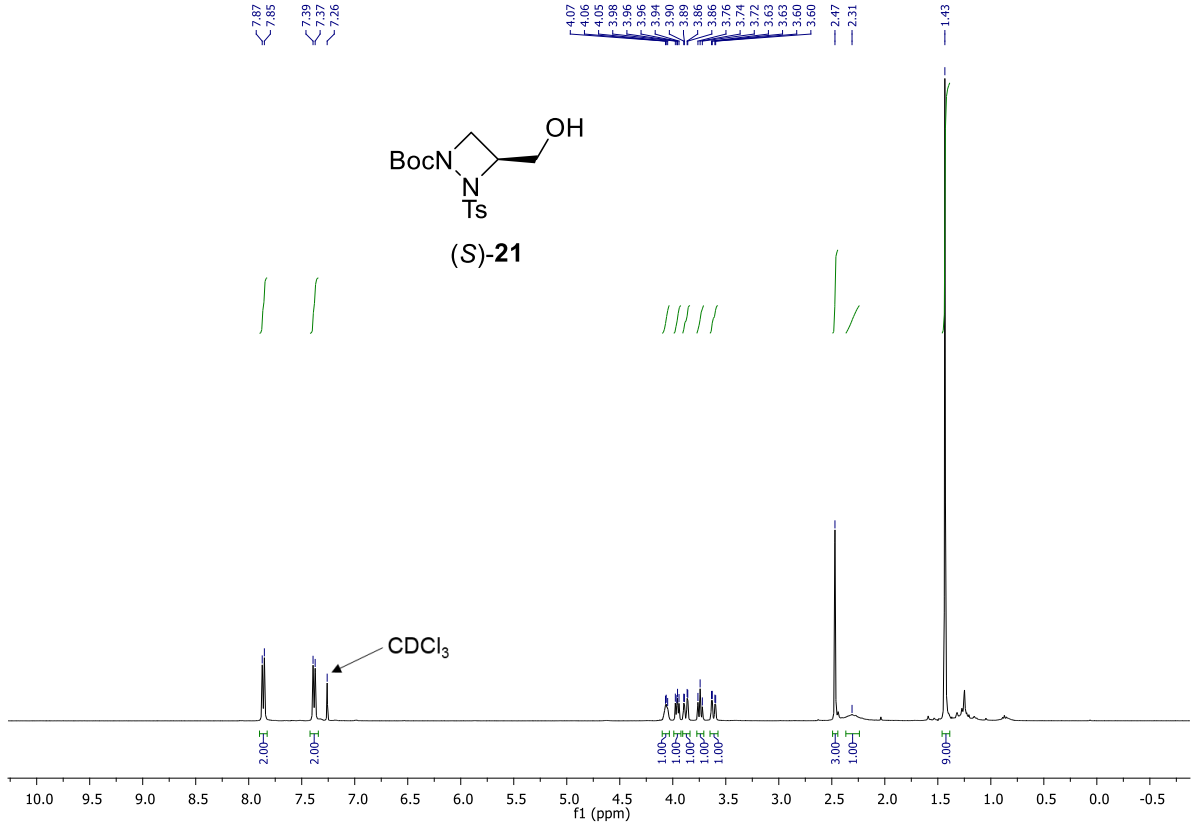
Section of ^{1}H NMR (400 MHz, $CDCl_3$) for alkene **7**



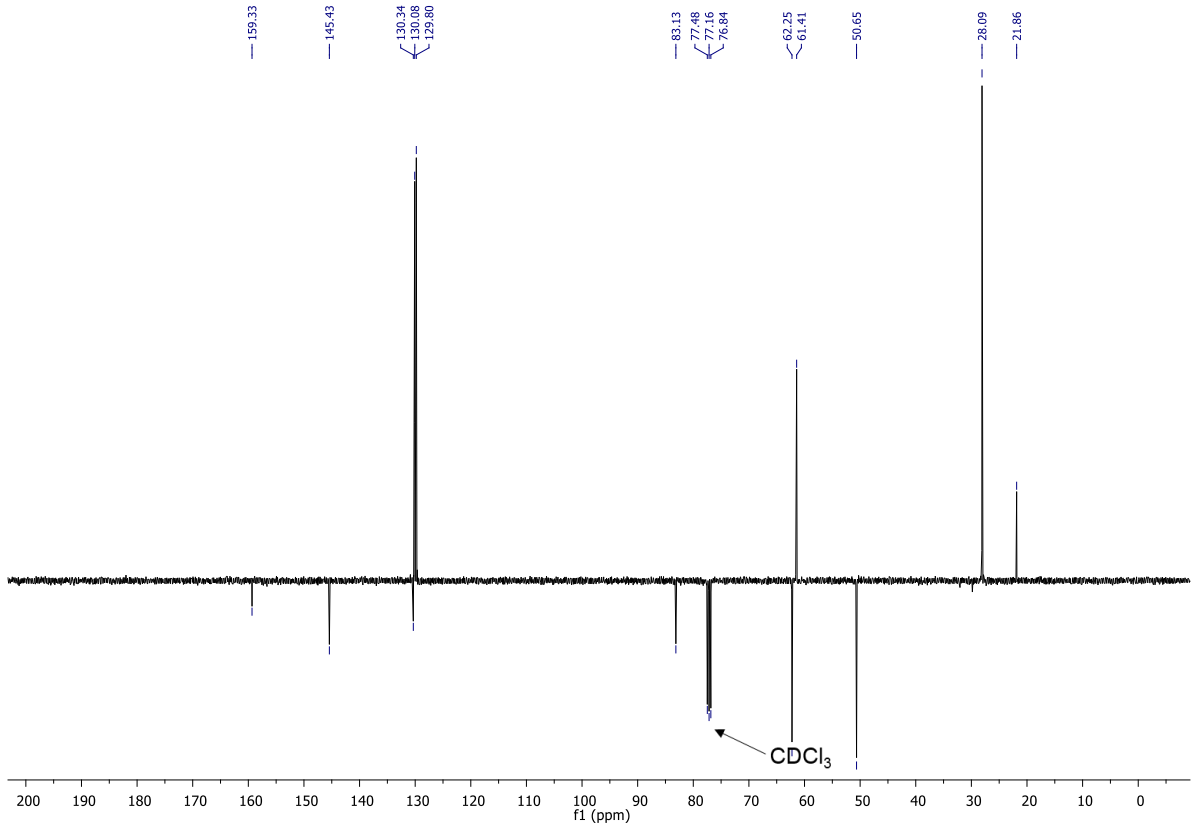
H^{a}: 6.56 (d, *J* = 11.8 Hz, 1H)

H^{b}: 5.04 (dt, *J* = 11.8, 7.8 Hz, 1H)

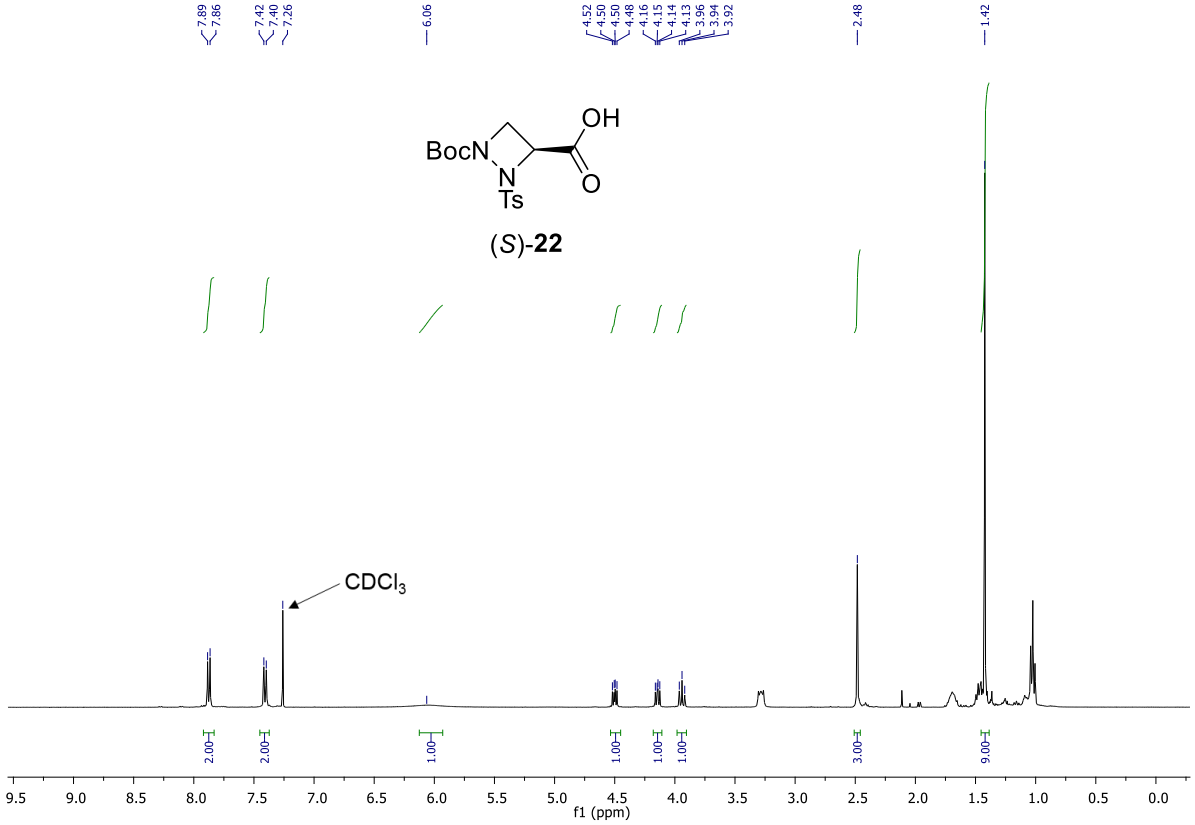
^{1}H NMR (400 MHz, $CDCl_3$)



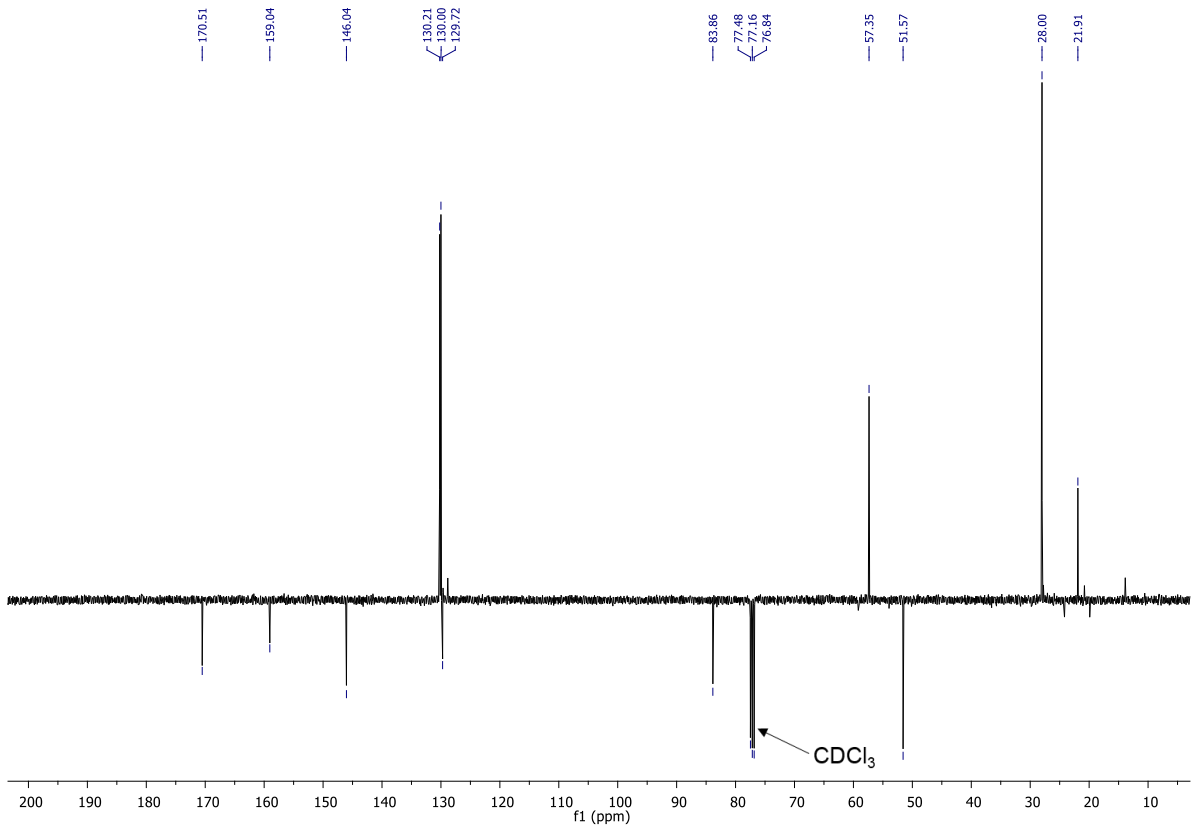
^{13}C NMR (101 MHz, $CDCl_3$)



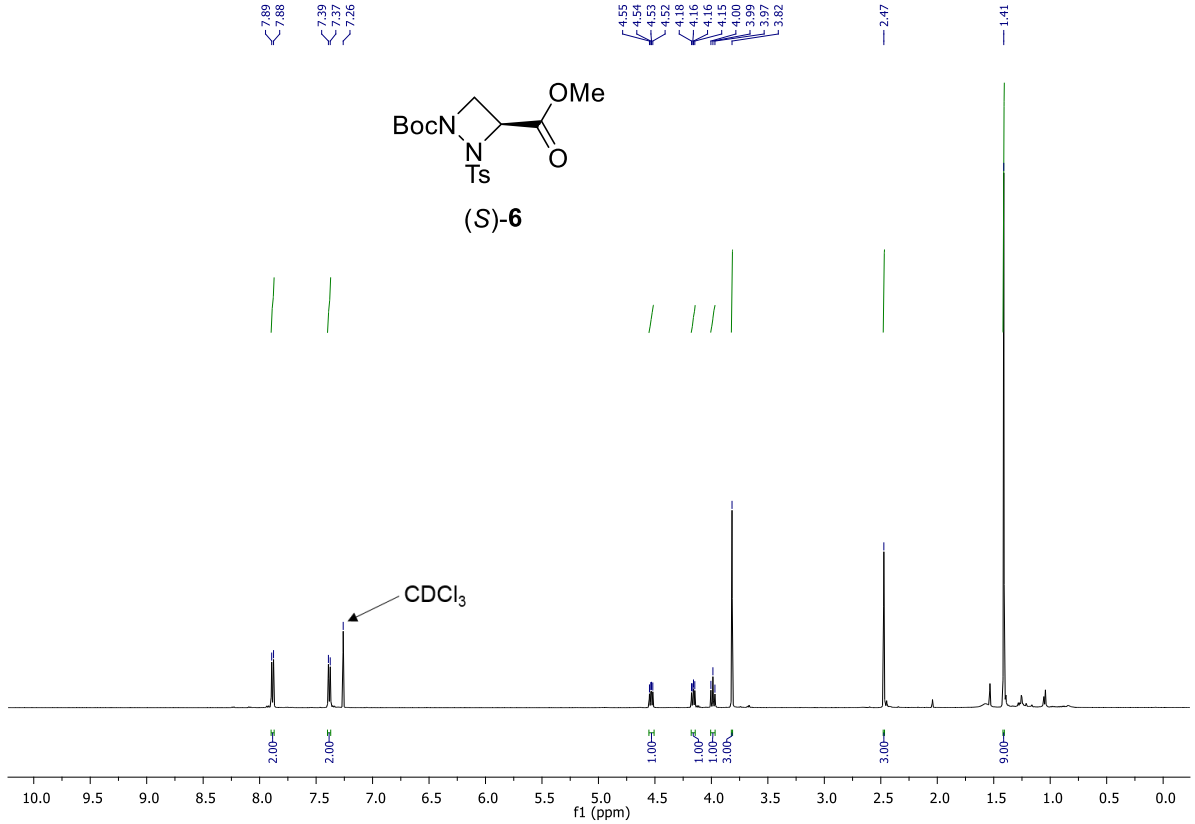
^{1}H NMR (400 MHz, $CDCl_3$)



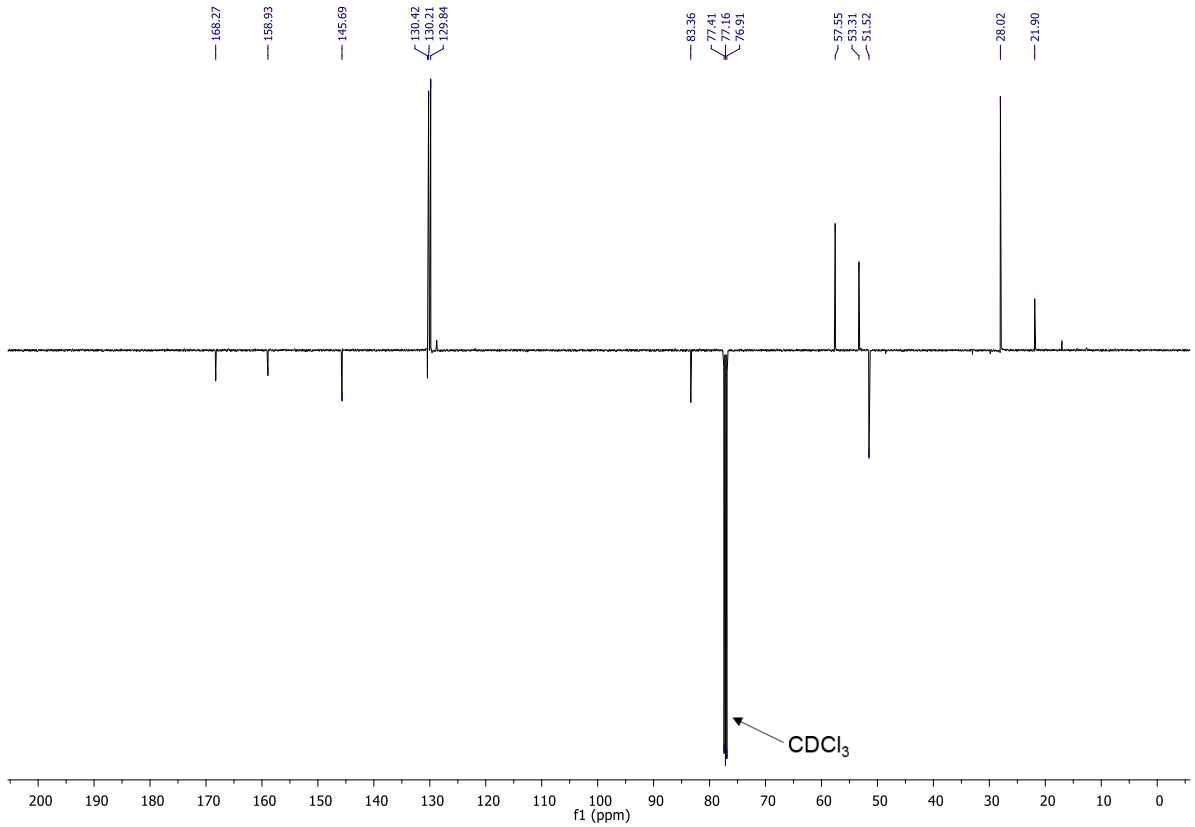
^{13}C NMR (101 MHz, $CDCl_3$)



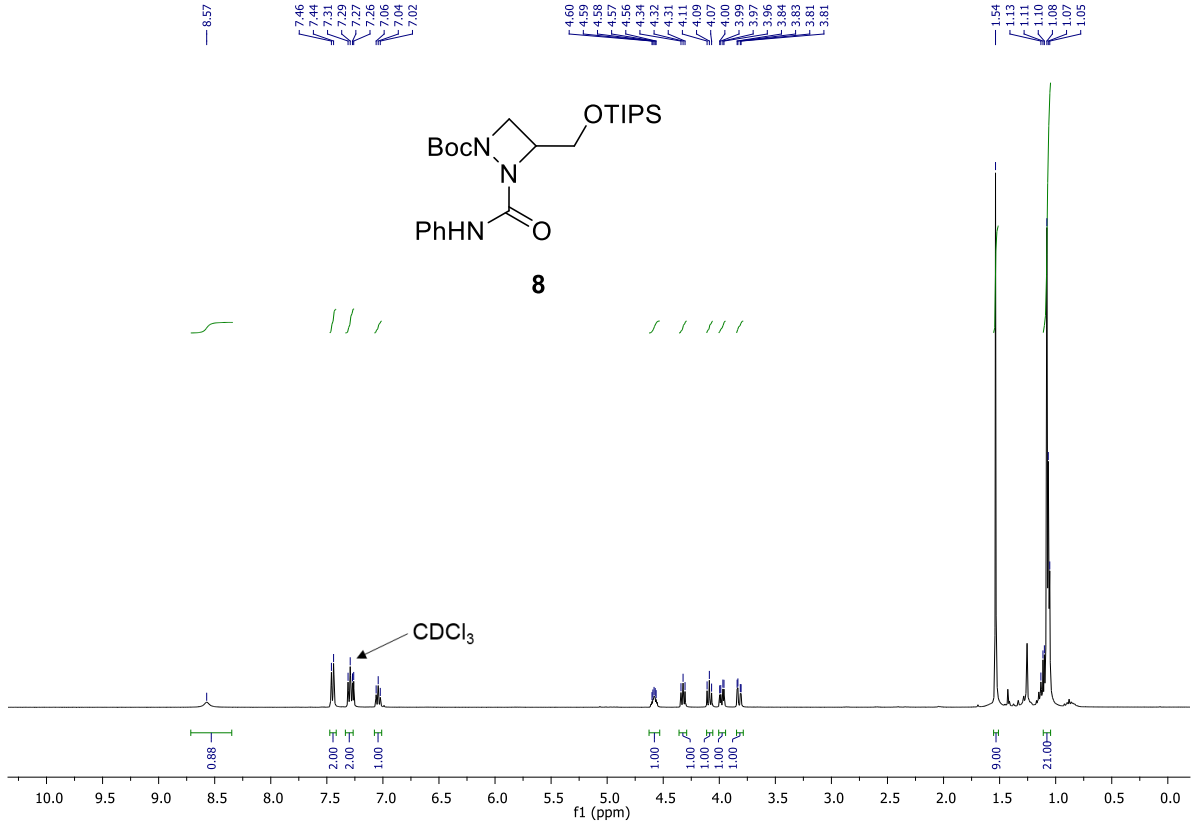
^{1}H NMR (500 MHz, $CDCl_3$)



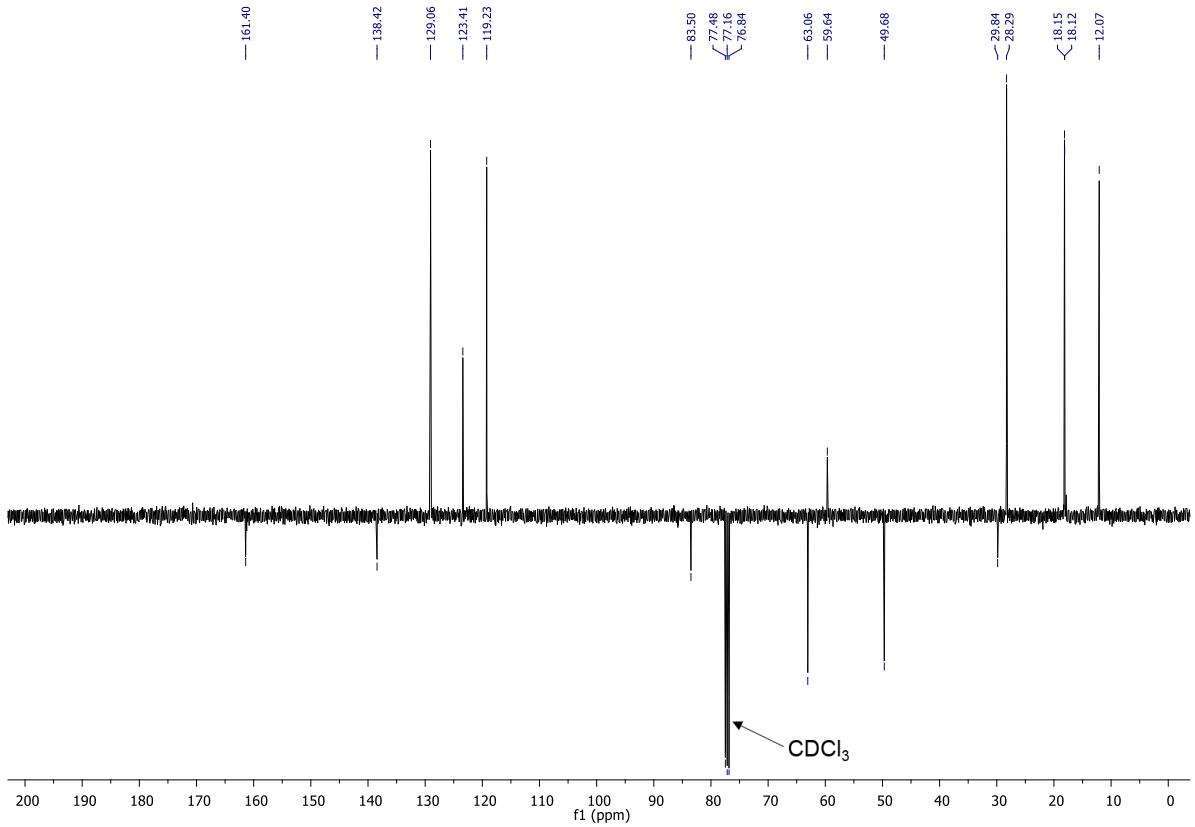
^{13}C NMR (126 MHz, $CDCl_3$)



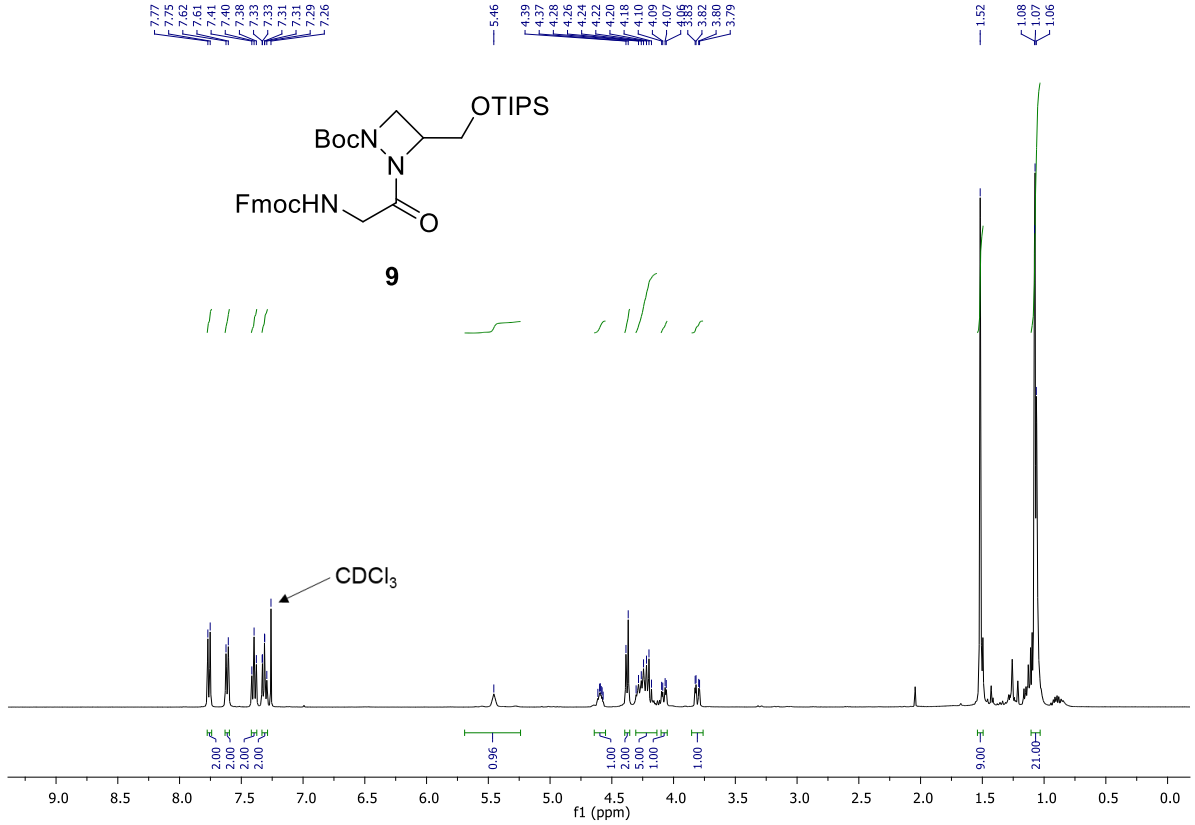
^{1}H NMR (400 MHz, $CDCl_3$)



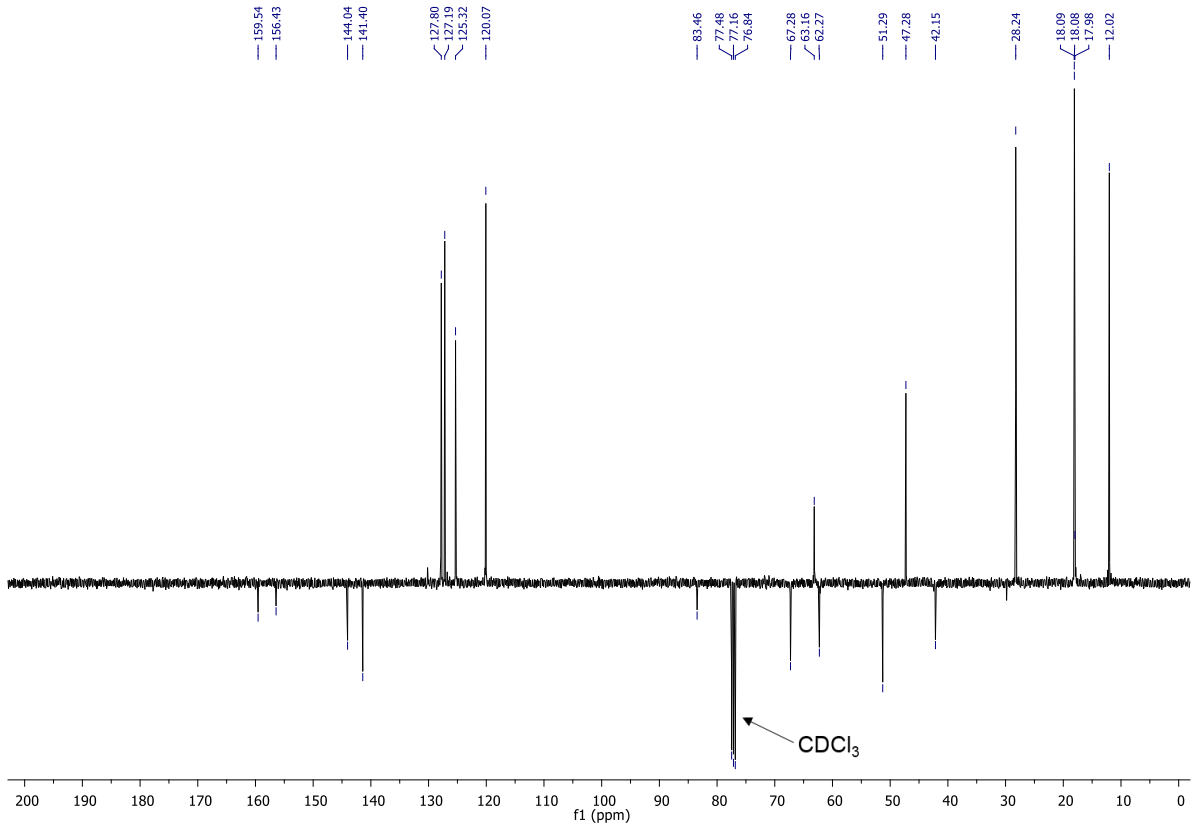
^{13}C NMR (101 MHz, $CDCl_3$)



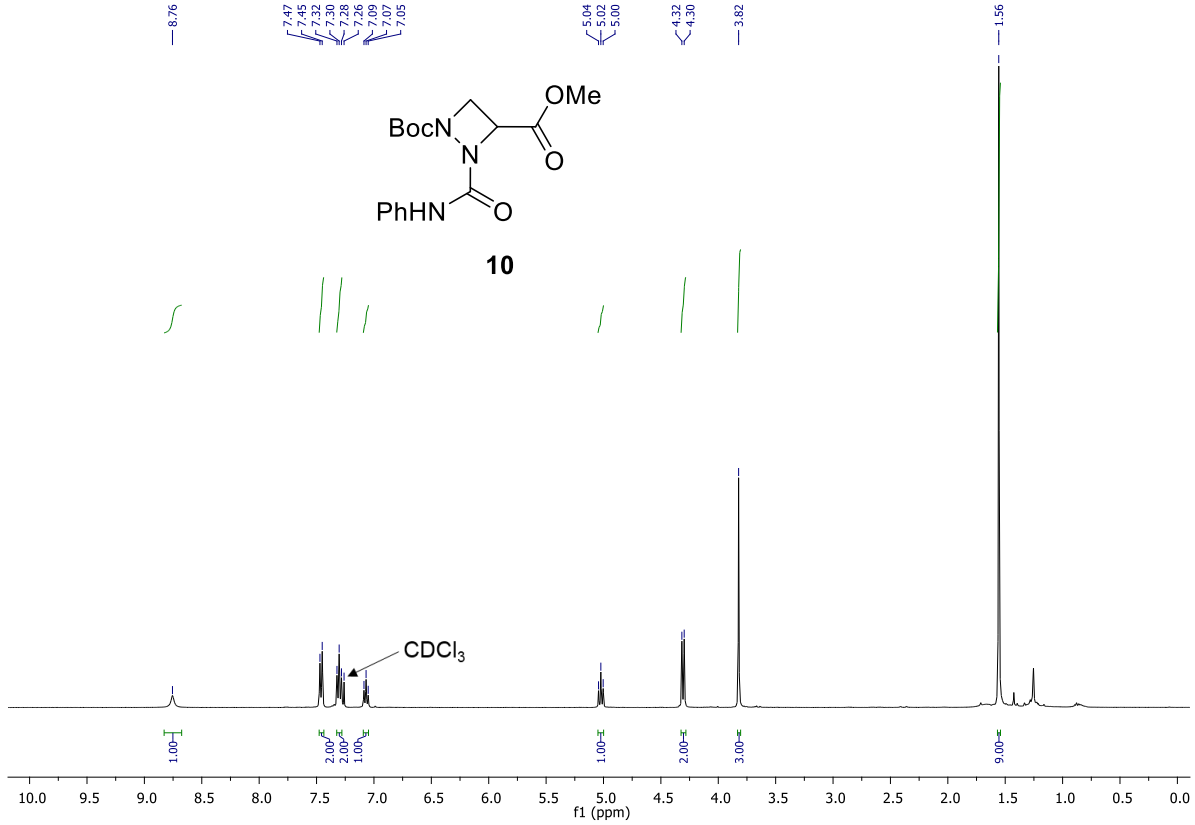
^{1}H NMR (400 MHz, $CDCl_3$)



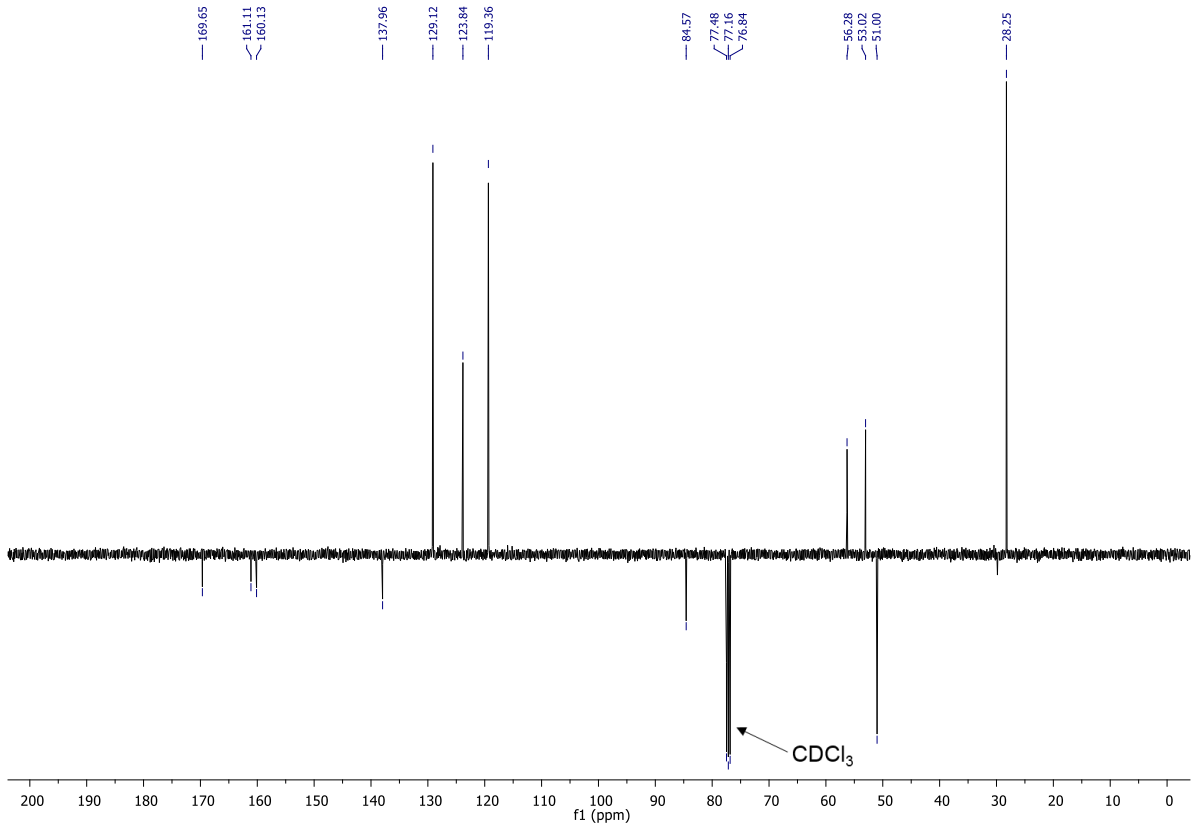
^{13}C NMR (101 MHz, $CDCl_3$)



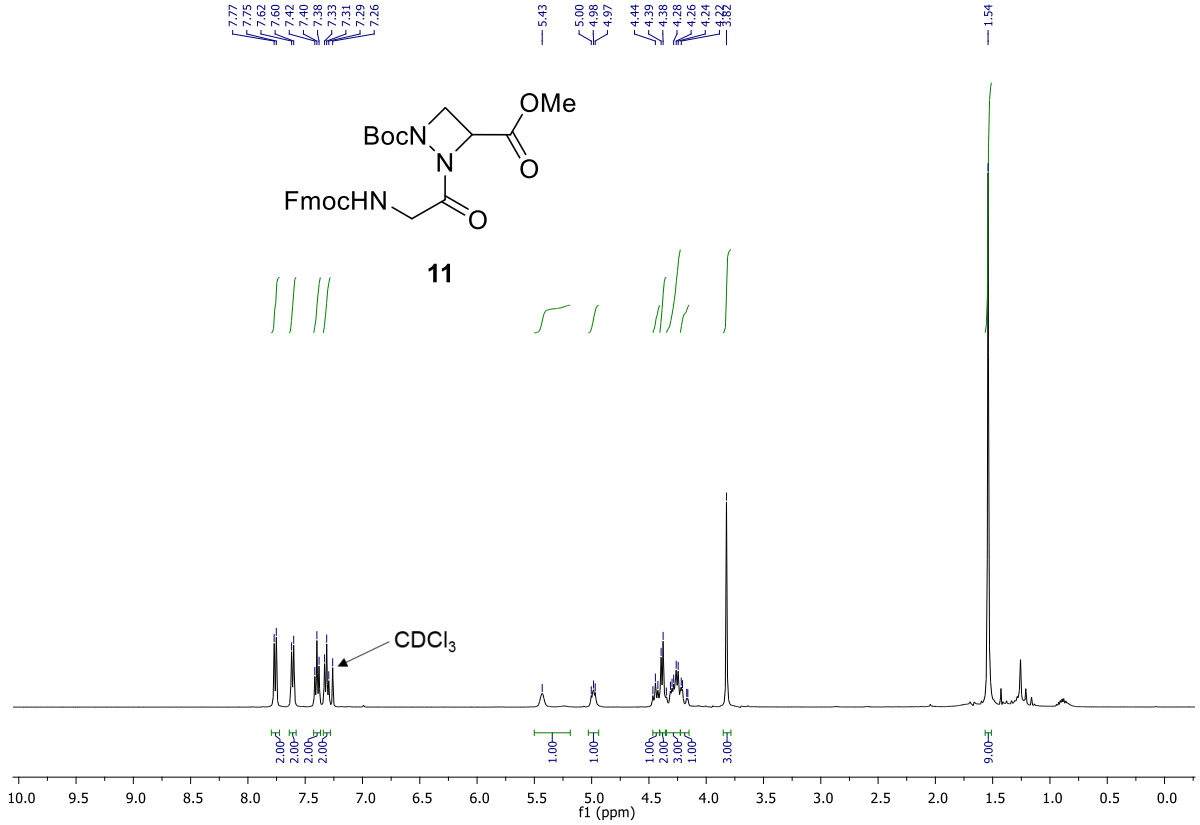
^{1}H NMR (400 MHz, $CDCl_3$)



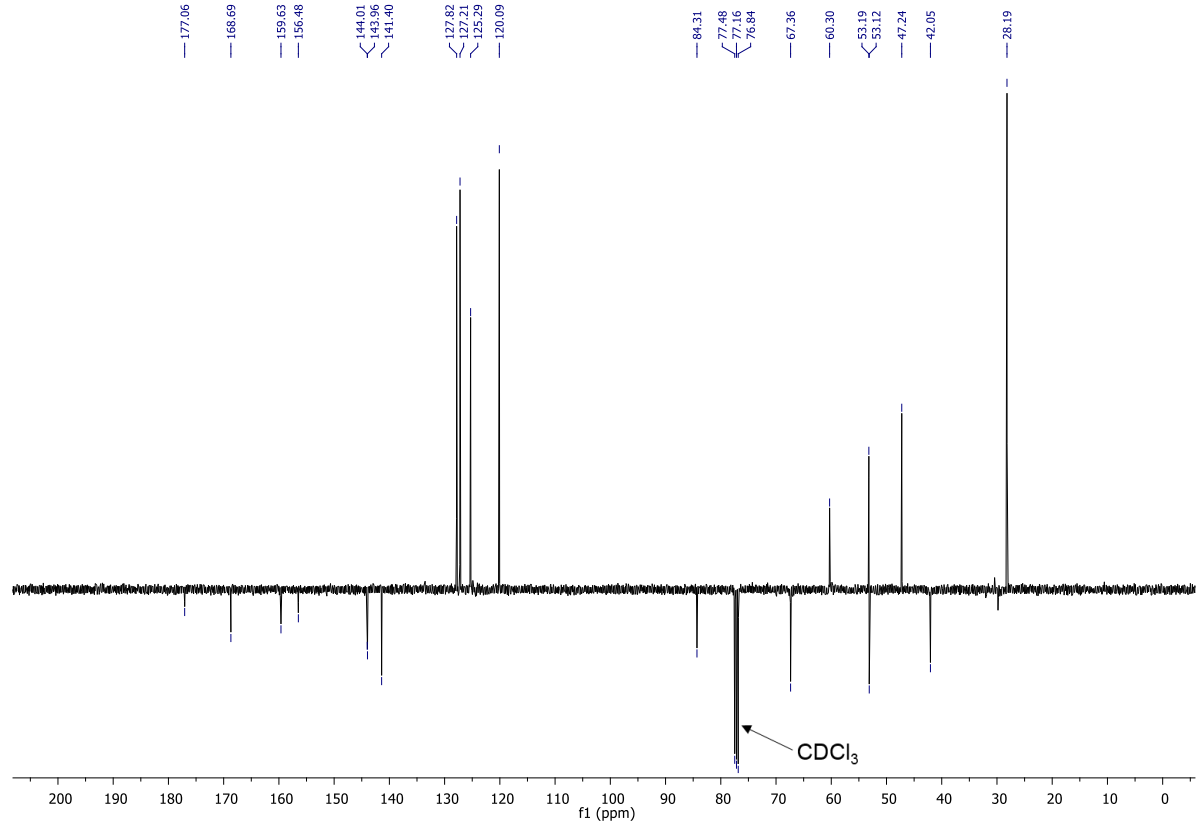
^{13}C NMR (101 MHz, $CDCl_3$)



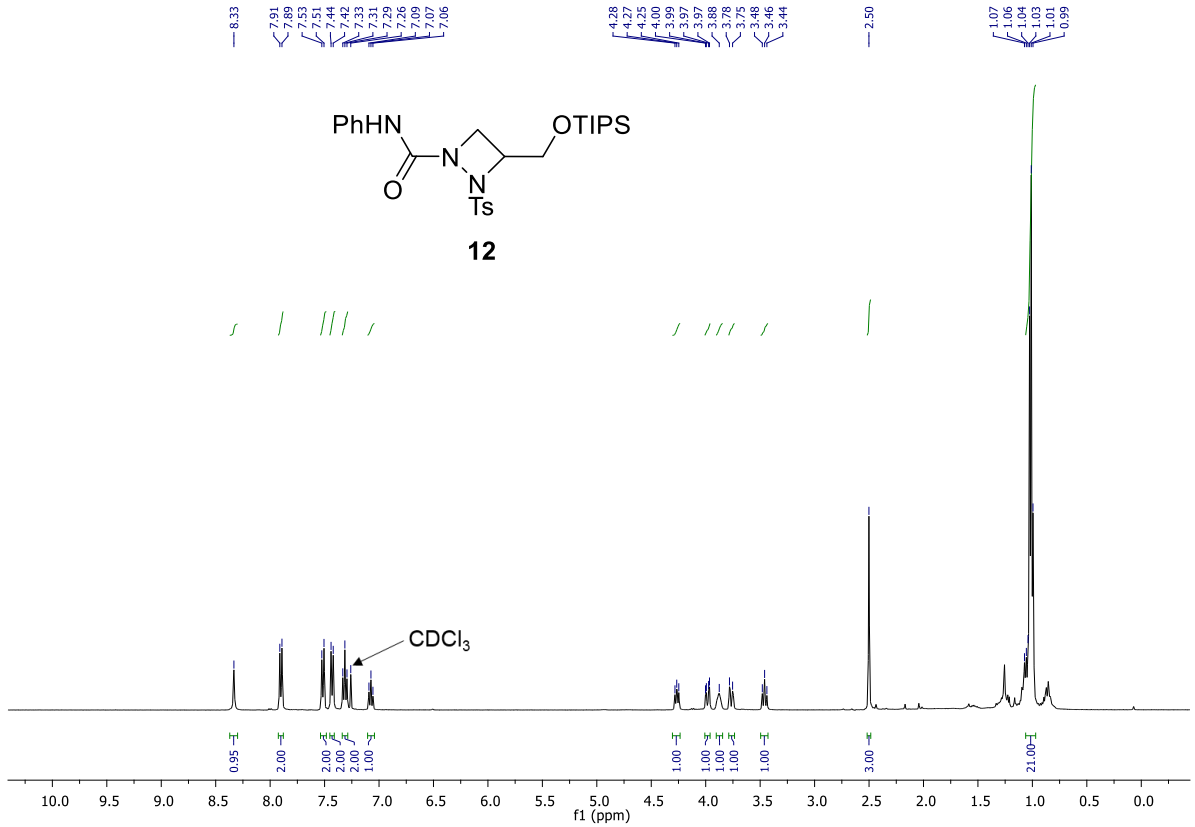
^{1}H NMR (400 MHz, $CDCl_3$)



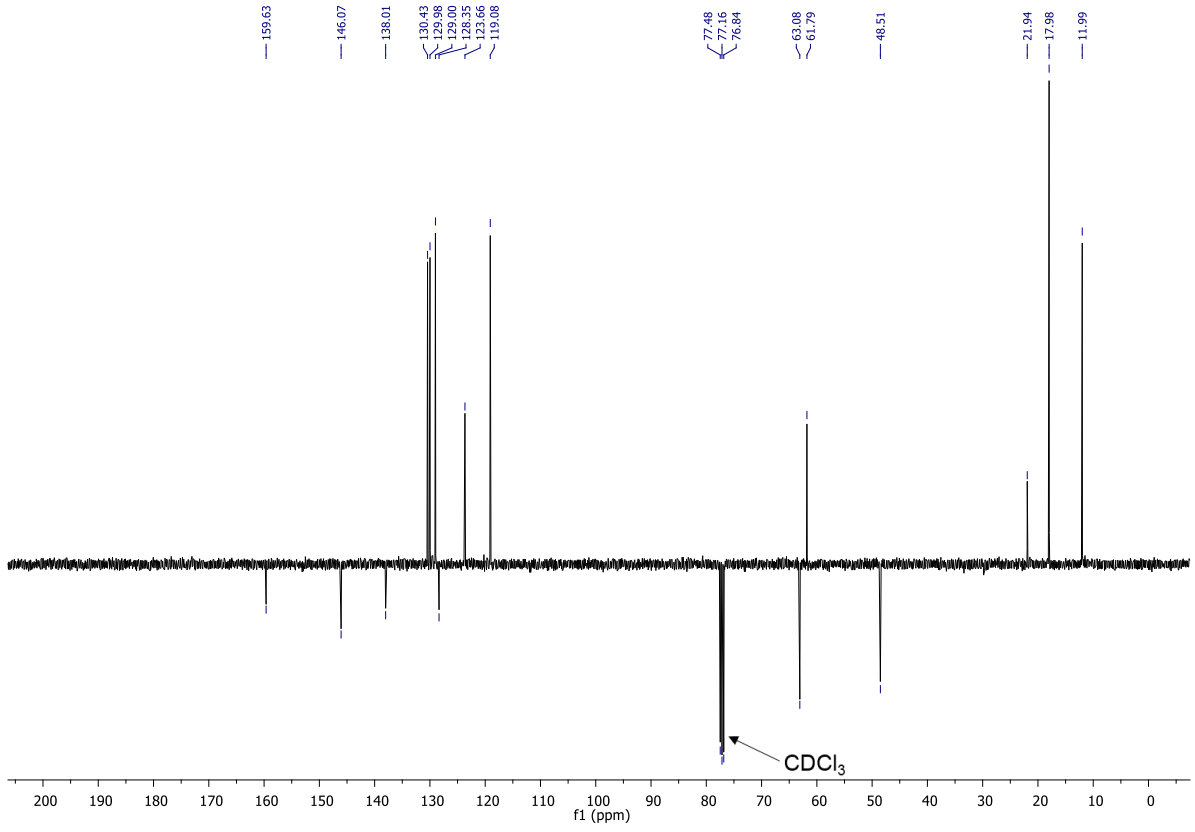
^{13}C NMR (101 MHz, $CDCl_3$)



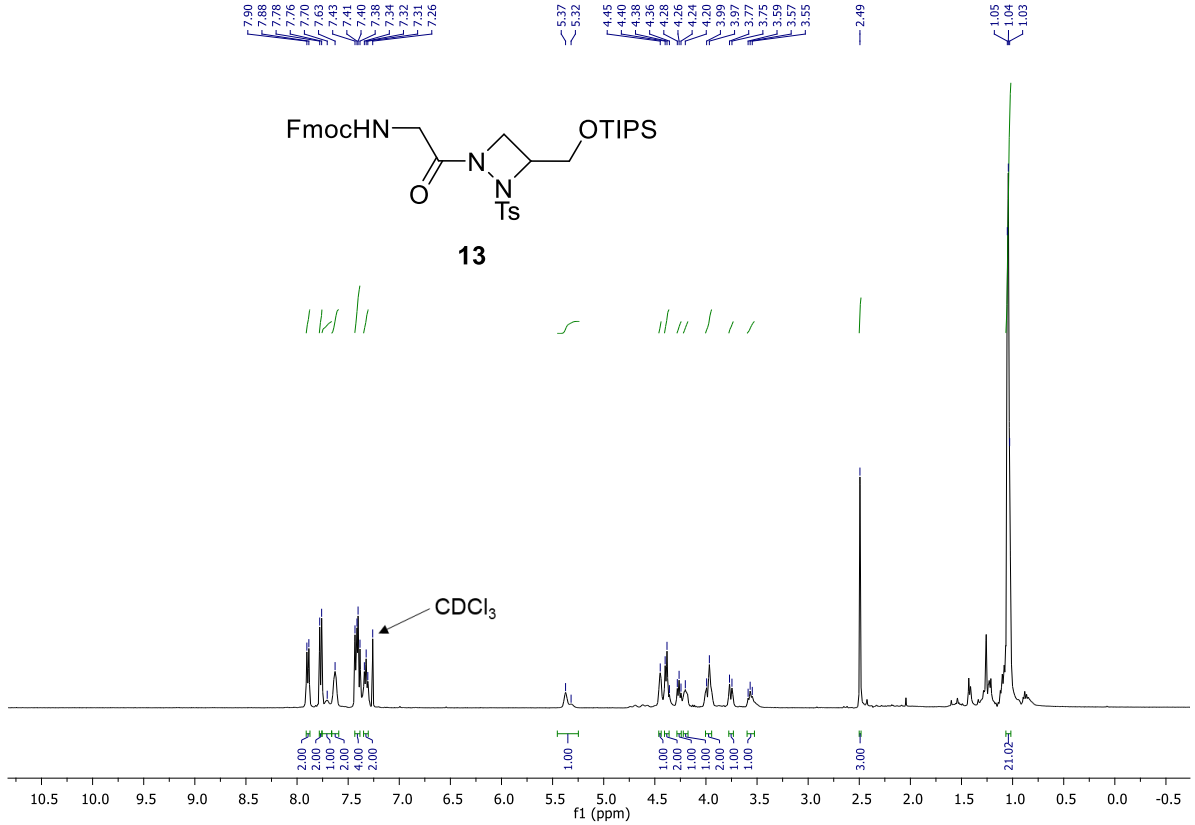
^{1}H NMR (400 MHz, $CDCl_3$)



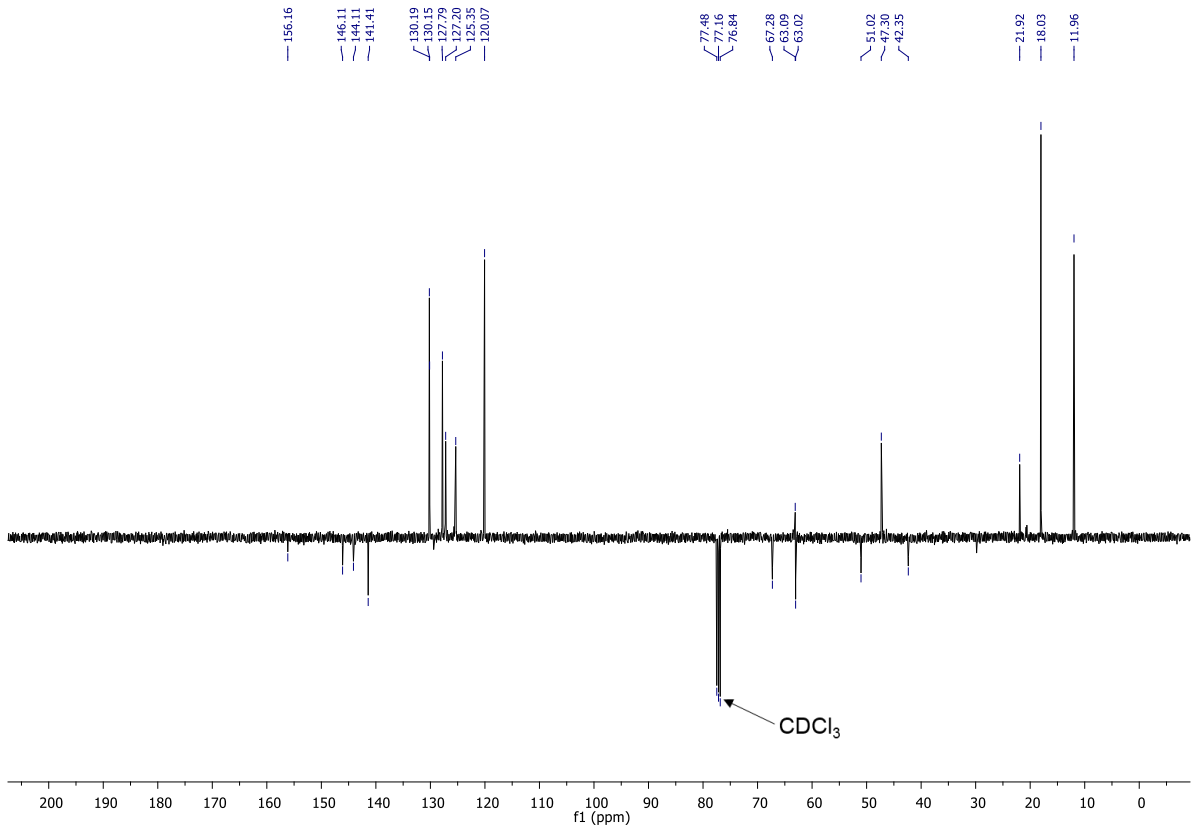
^{13}C NMR (101 MHz, $CDCl_3$)



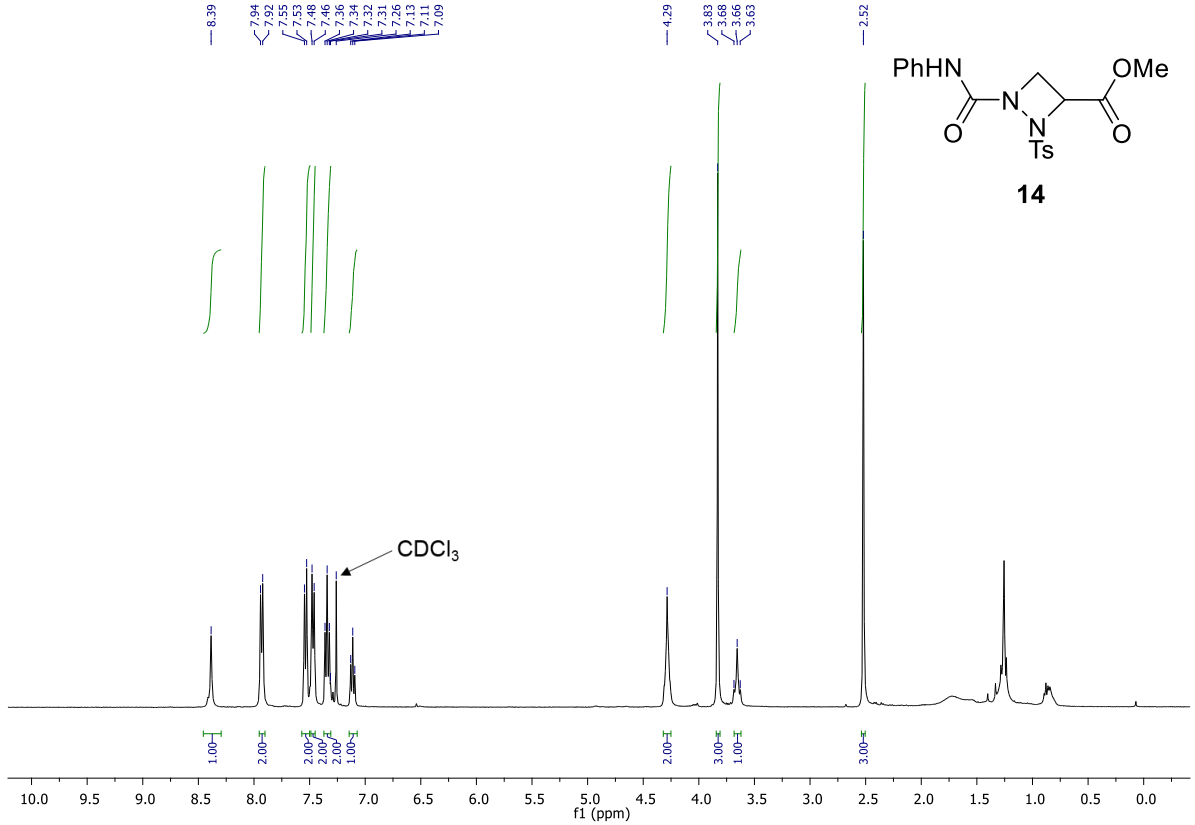
^{1}H NMR (400 MHz, $CDCl_3$)



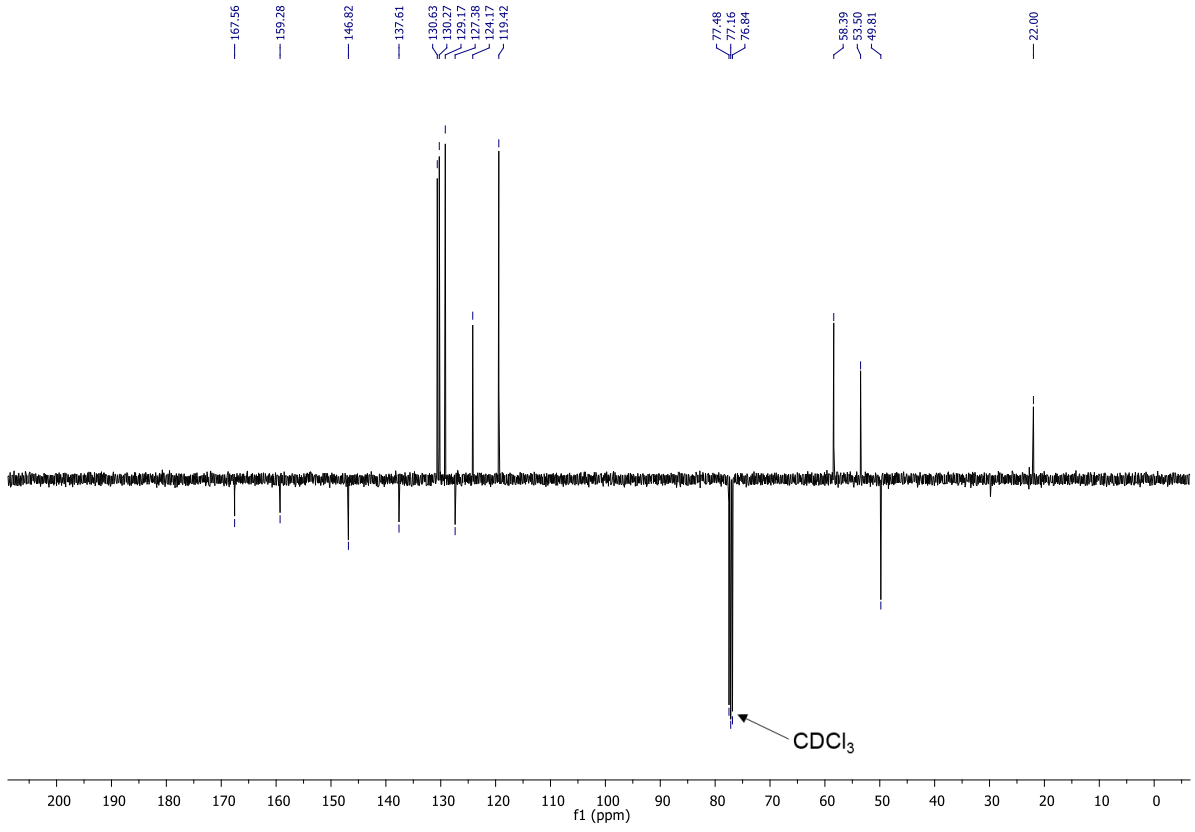
^{13}C NMR (101 MHz, $CDCl_3$)



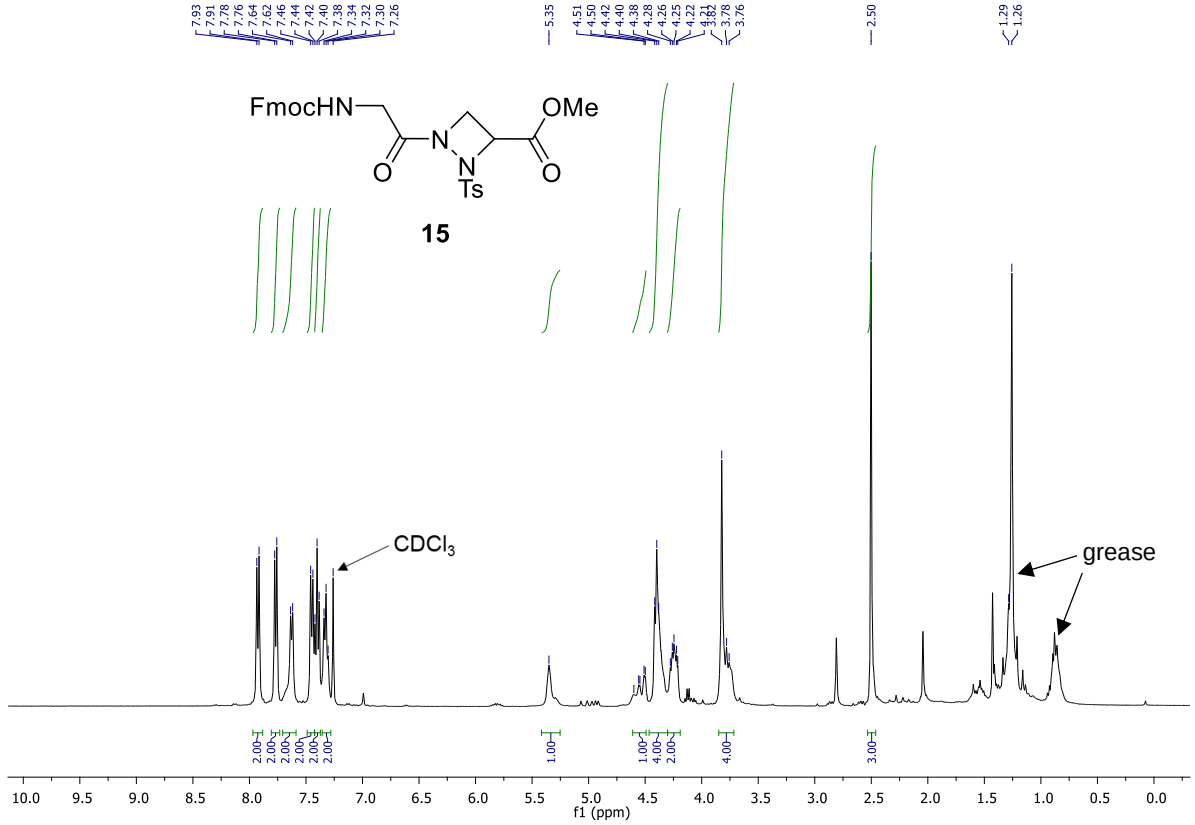
^{1}H NMR (400 MHz, $CDCl_3$)



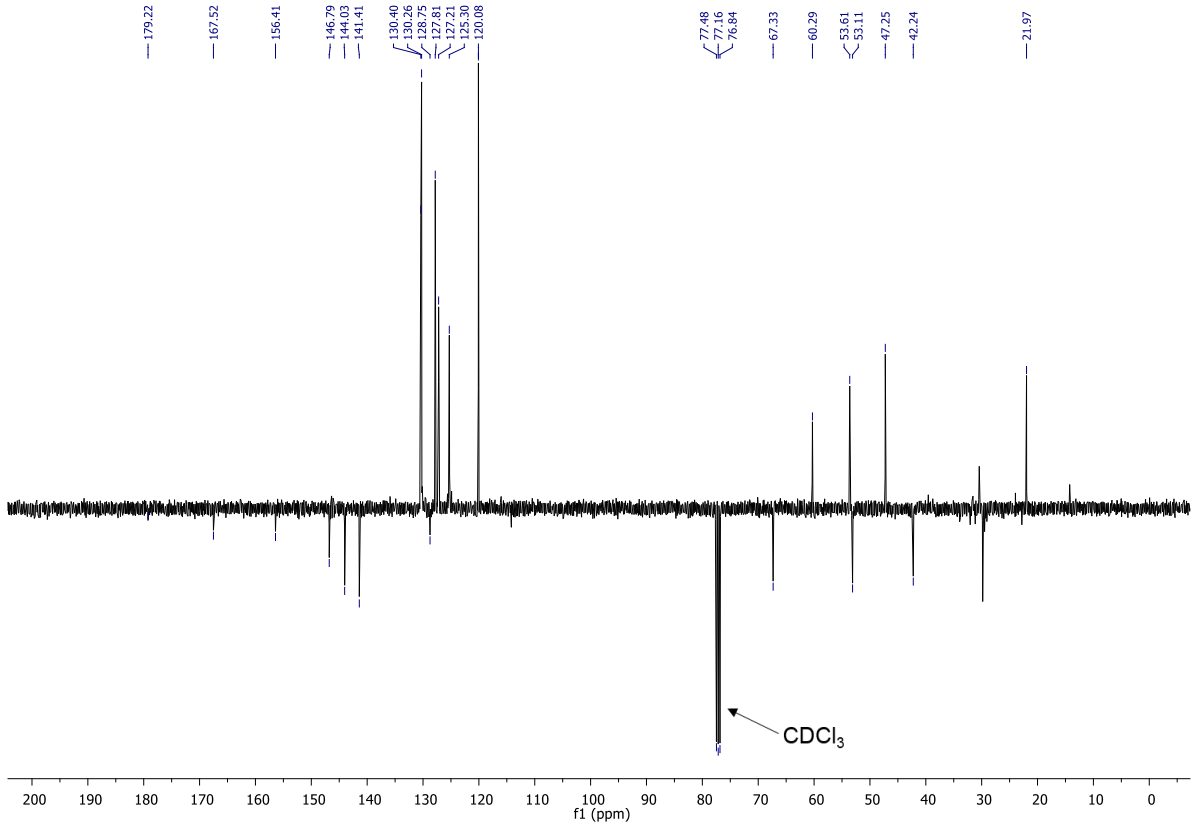
^{13}C NMR (101 MHz, $CDCl_3$)



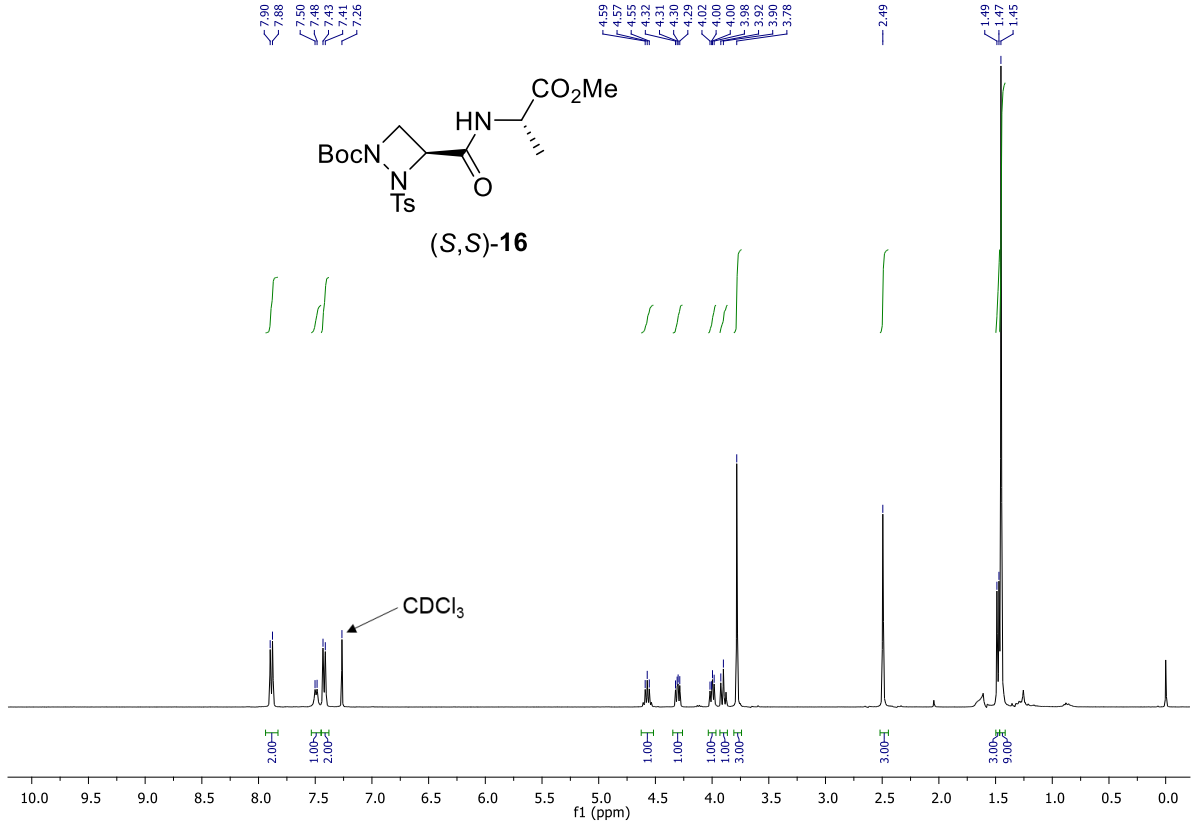
^{1}H NMR (400 MHz, $CDCl_3$)



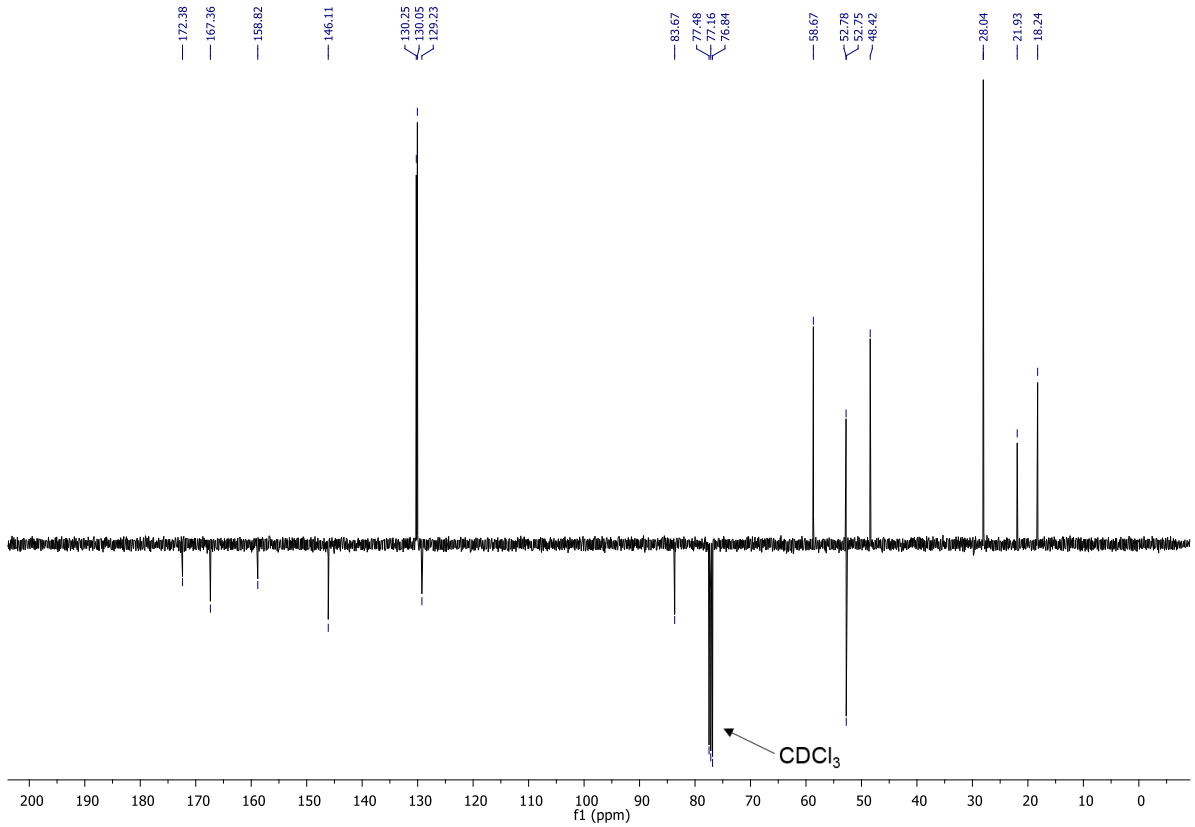
^{13}C NMR (101 MHz, $CDCl_3$)



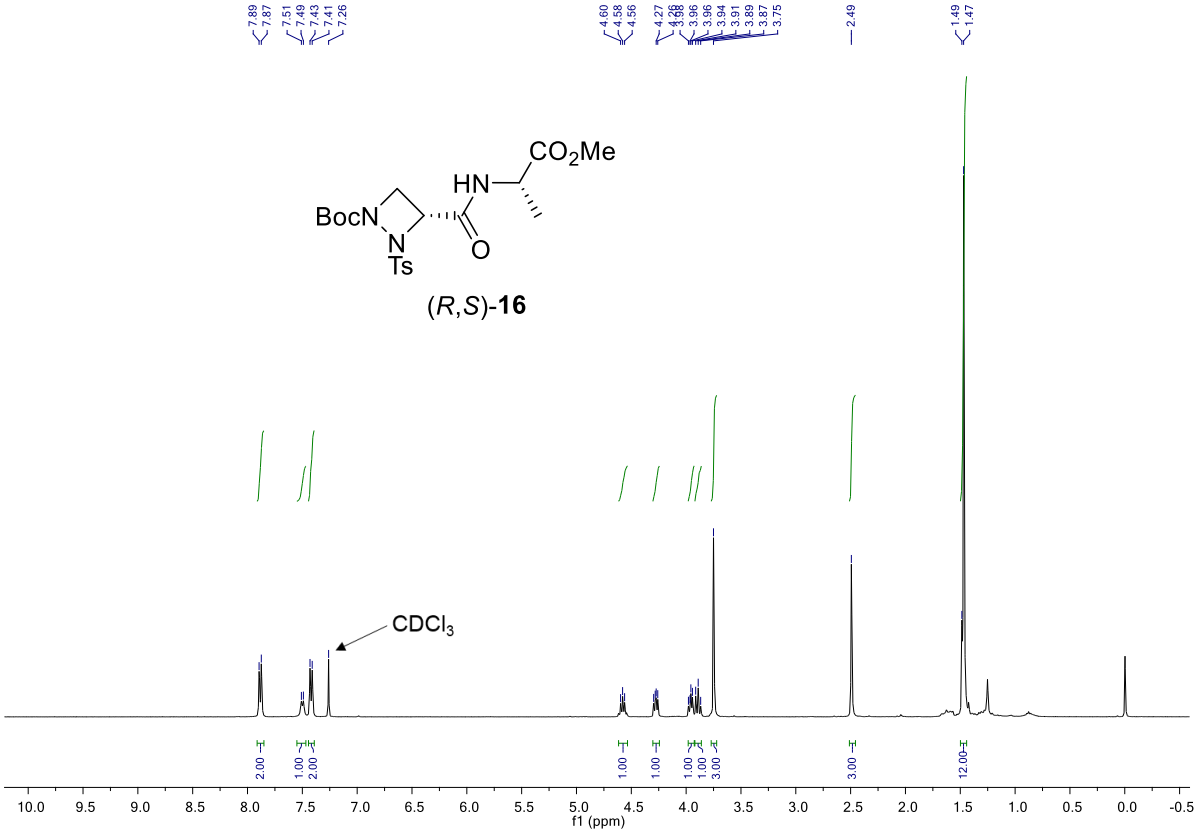
^{1}H NMR (400 MHz, $CDCl_3$)



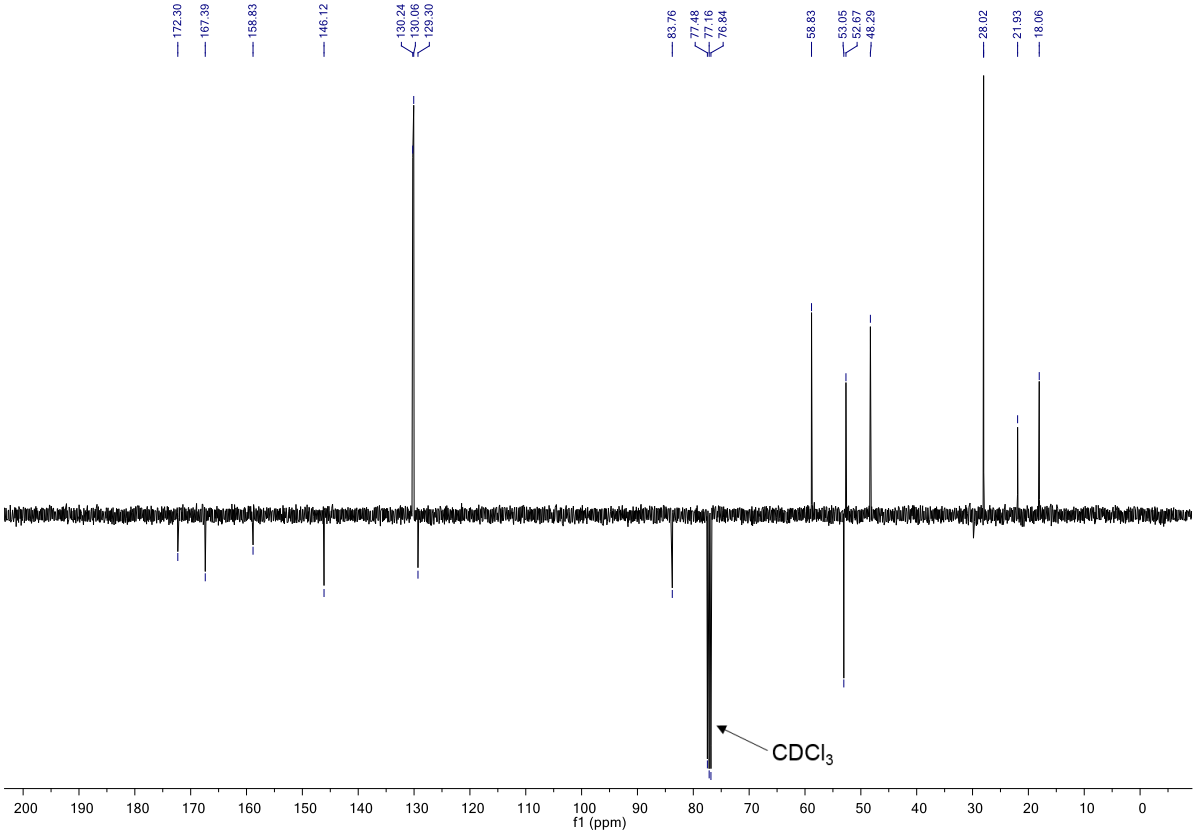
^{13}C NMR (101 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$)

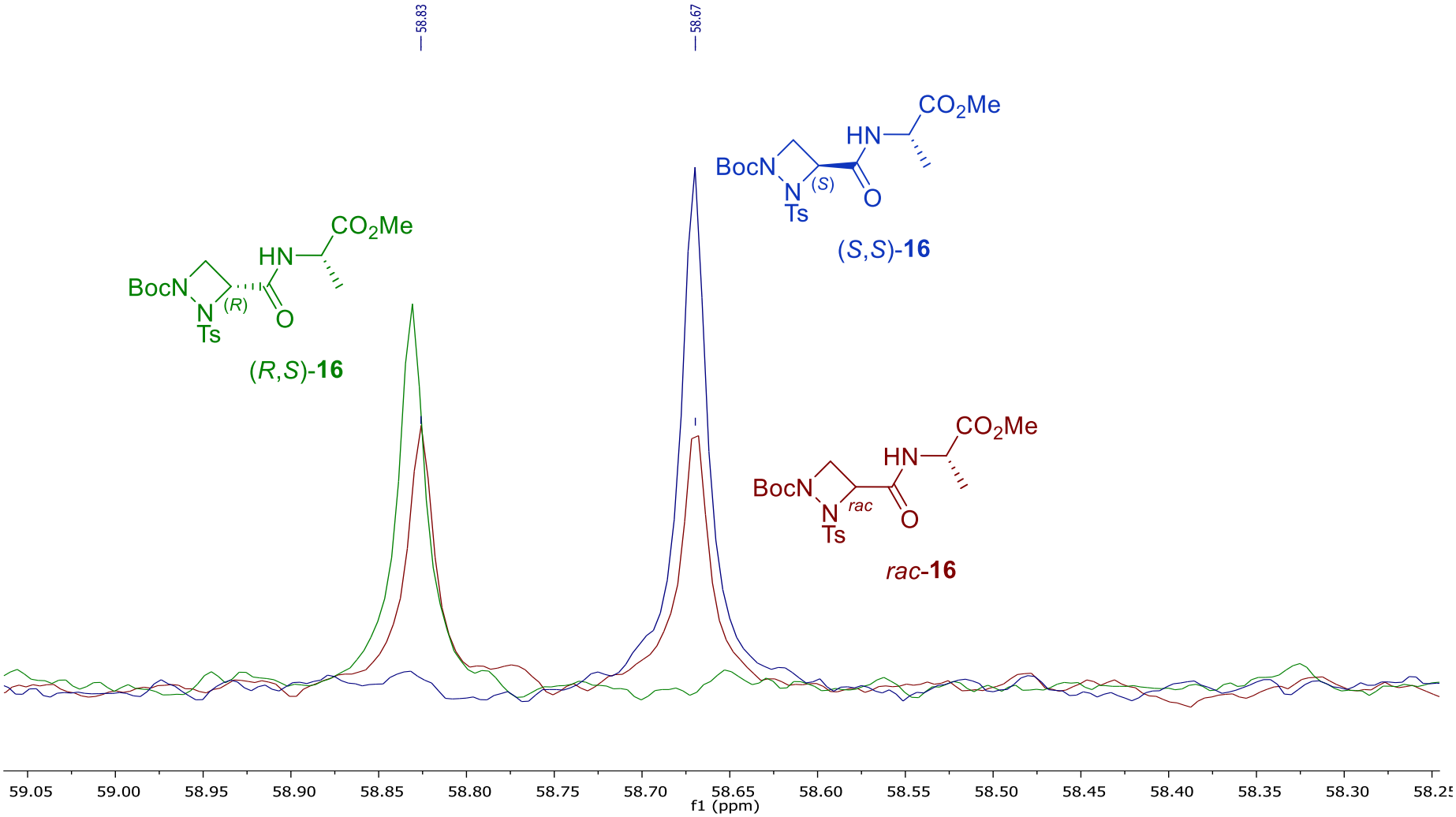


^{13}C NMR (101 MHz, $CDCl_3$)

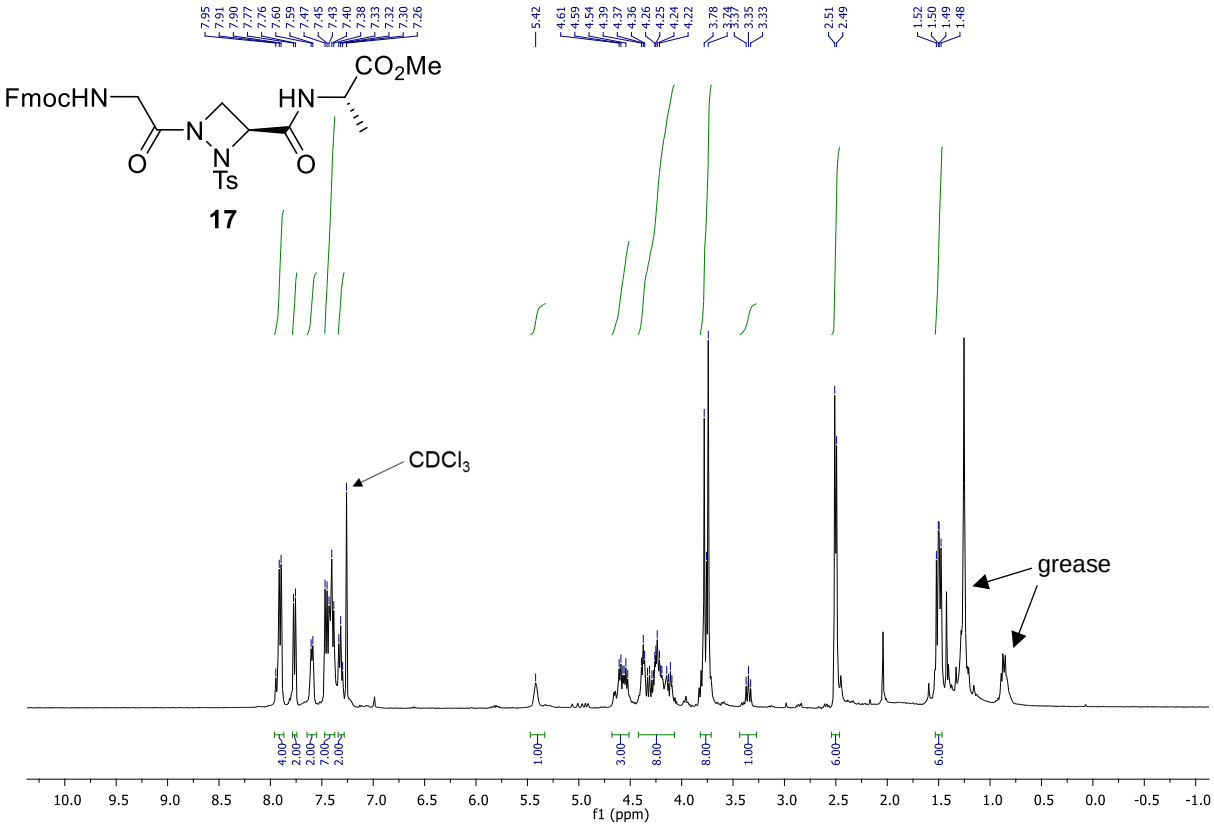


Comparison of *rac*-**16**, (*S*,*S*)-**16** and (*R*,*S*)-**16**

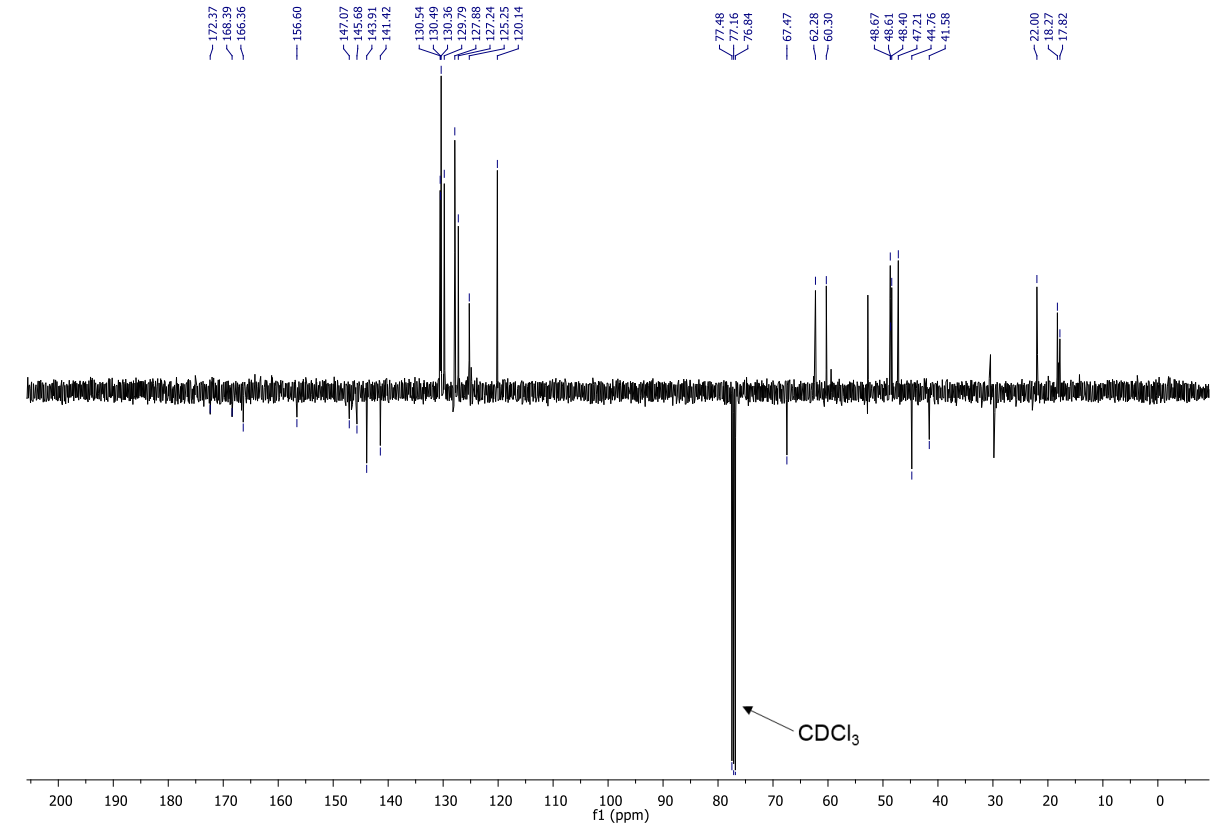
Section of ^{13}C NMR (101 MHz, $CDCl_3$)



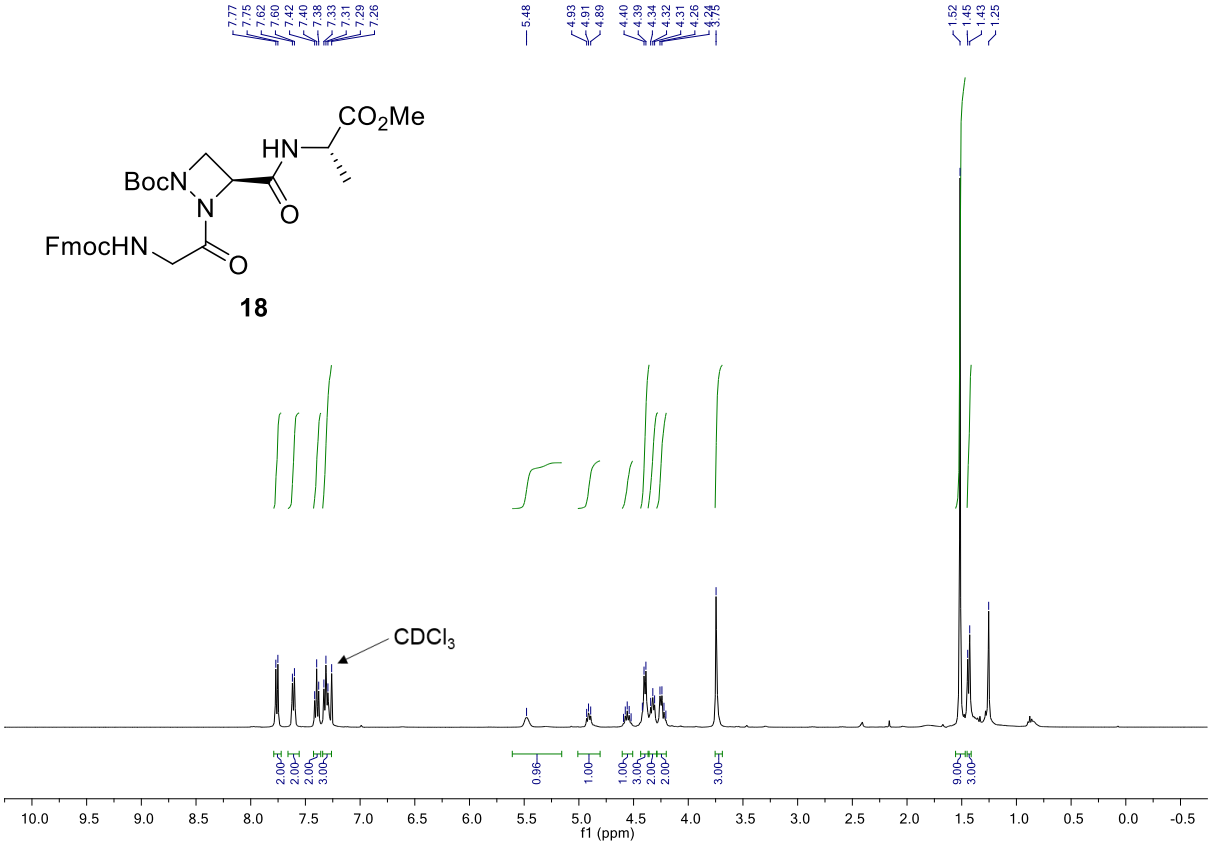
^{1}H NMR (400 MHz, $CDCl_3$)



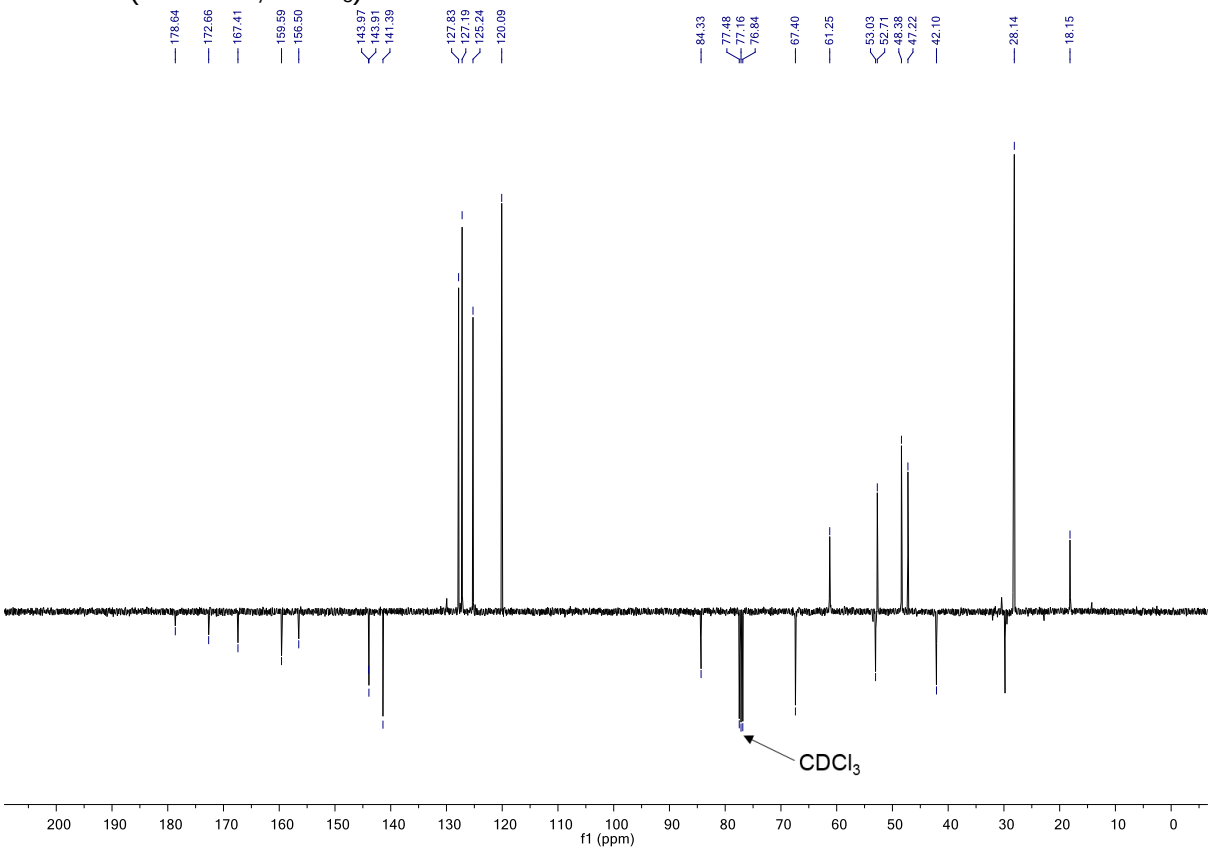
^{13}C NMR (101 MHz, $CDCl_3$)



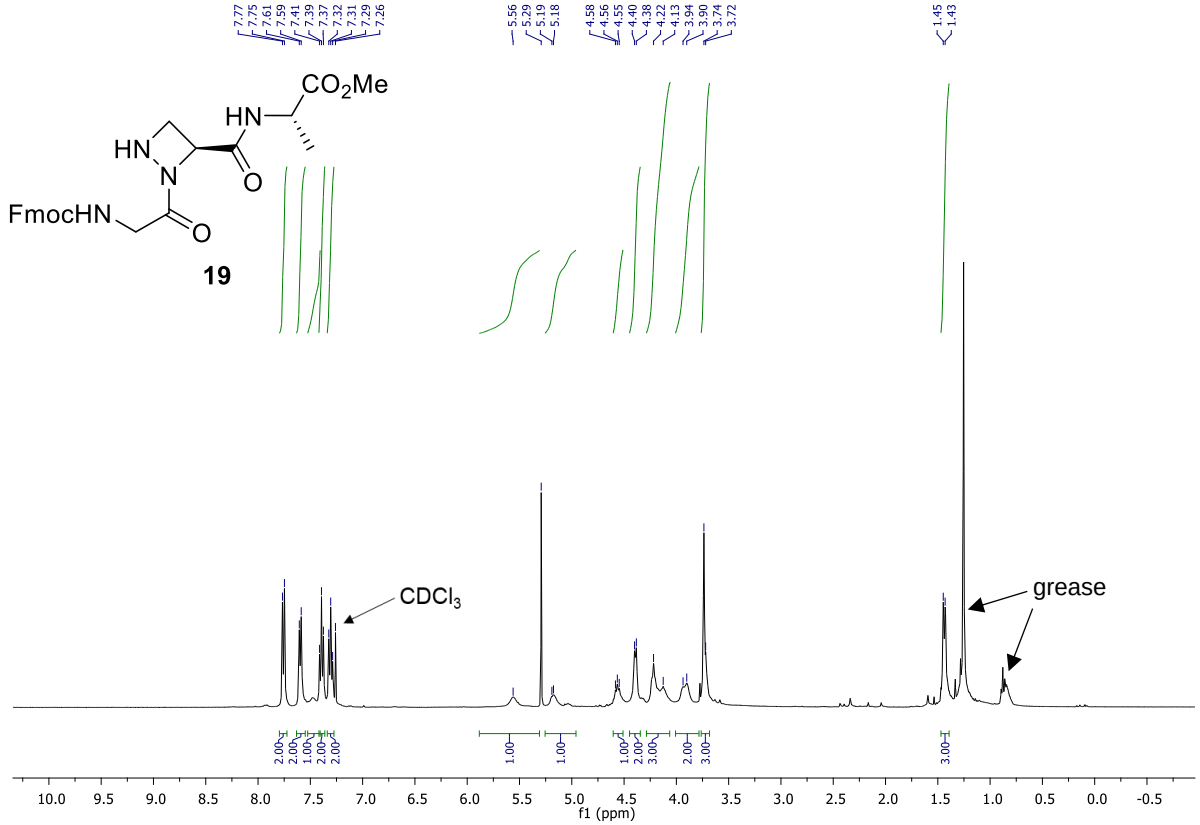
^{1}H NMR (400 MHz, $CDCl_3$)



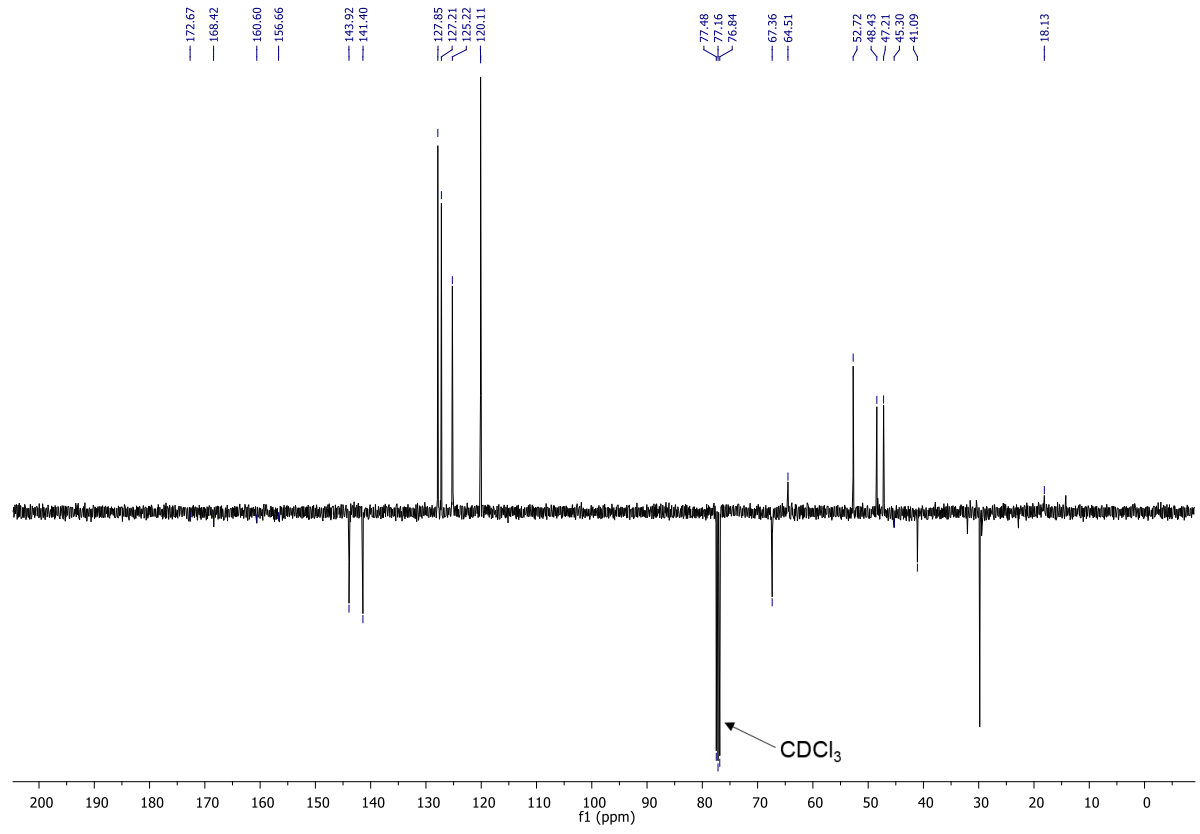
^{13}C NMR (101 MHz, $CDCl_3$)



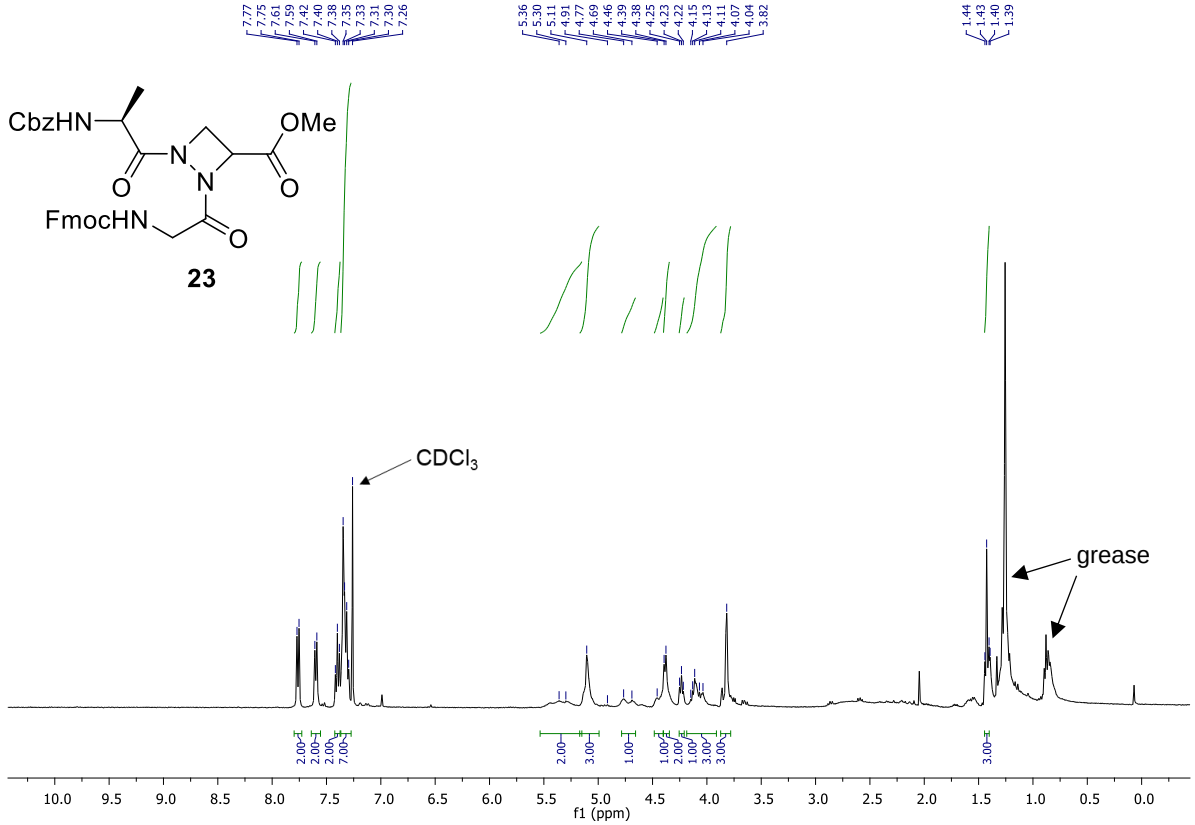
^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (101 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)