Supporting Information: Design, Synthesis and Evaluation of tryptophan analogues as tool compounds to study IDO1 activity

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Contents

1 – Chemical Synthesis Methods

1.1 – General Experimental

Heated reactions were performed on Heidolph Hei-Tec stirrer hotplates using fitted heating-mantels with the temperature being controlled *via* an external probe. Reactions that required cooling were performed with one of the following: ice/water (0 °C), ice/sodium chloride (−20 °C), dry ice/acetonitrile (−40 °C) or dry ice/acetone (−78 °C) bath. The term 'warmed passively to X °C' refers to the act of allowing a cooled reaction mixture in a non-maintained cooling bath to warm to the stated temperature.

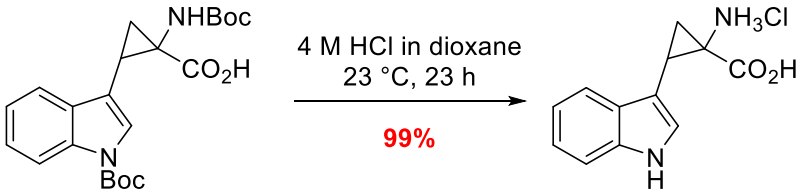
Unless stated otherwise, all solvents and reagents were used without further purification, from one of the following outlets: Alfa Aesar, Acros Organics, Fisher, Fluorochem, Sigma Aldrich, TCI, Merck or VWR chemicals. Petroleum ether refers to 'Petroleum ether 60:40'. Anhydrous solvents were either collected from a solvent purification system using alumina columns or dried from the bottle *via* activated (300 °C, >6 h, <1 mbar) 3 or 4 Å molecular sieves. Any reference to 'water' use refers to purified water collected from a PURELAB option-S 7 reverse osmosis water purifier.

Thin-layer chromatography (TLC) was performed using Merck silica gel F254, aluminium-backed TLC plates. Flash column chromatography was carried out using Sigma-Aldrich silica gel (Technical grade, 60 Å pore size, 230–400 mesh, 40–63 μm particle size) with the column diameter and collected fraction size determined using Still's reference guide.[1]

Melting points were determined *via* a Stuart SMP10 melting point apparatus with a digital temperature reading (1 °C graduations). Melting points are quoted as ranges where applicable. Infrared Spectra (IR) were recorded using either a Varian 660 FT-IR spectrometer (with UATR attachment for solid samples) or a Perkin Elmer Spectrum 100 FT-IR spectrometer (with UATR attachment for solid samples) at 23 °C. For ^{1}H-NMR spectra recorded at 400 MHz either a Bruker AVIII400, AV NEO 400 or NEO400 NMR spectrometer was used; for ^{1}H-NMR spectra recorded at 500 MHz a Bruker AV NEO 500 was used. ^{13}C-NMR spectra were recorded at 101 MHz using a Bruker AVIII400, AV NEO 400 or NEO400 NMR spectrometer and are proton decoupled, unless otherwise stated. All ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 25 °C, unless stated otherwise, and the data processed using MestReNova 12.0.2. Chemical shifts (δ) are reported in ppm relative to the standard solvent shift of the deuterated solvent used with coupling constants (*J*) expressed in units of Hertz (Hz). Proton assignments are presented in condensed formula form with the assigned proton being highlighted in italics (i.e. $CH_3C\mathit{H}_2CH_3$) and will proceed from left to right on the structure of the compound being assigned. COSY, HSQC, HMBC and pendant techniques were used to unambiguously assign

both ^{1}H-NMR and ^{13}C-NMR spectra, JMOD, DEPT45 and/or 135 experiments were used to identify quaternary carbons. The following abbreviations were used to assign resonance multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, dd = double doublet, td = triple doublet, dt = double triplets, tt = triple triplets, ddd = double double doublets, tdd = triple double doublets, dddd = double double double doublets, quin. = quintet, *app.* = apparent. 'Stack' is used to describe a resonance structure in which two or more non-equivalent nuclei are coincident. 'Multiplet', m, is used to describe a resonance that arises from a single, or multiple equivalent, nuclei where the coupling patterns cannot be identified and assigned. Low and high resolution mass spectra were recorded *via* electrospray (ES), affinity-purification (AP) or Atmospheric Solids Analysis Probe (ASAP) techniques using a Waters Xevo G2-XS or a Synapt G2S TOF Mass Spectrometer in positive or negative mode (technique and charge detection method stated in each MS report). LC-MS analysis was performed using either a Waters e2695 separation module coupled with a Waters SQD MS-detector (raw data was processed and extracted using MassLynx V4.1 SCN855), or a ThermoFisher LCQ-fleet LC-MS (raw data was processed and extracted using Xcaliber).

16) 1-Amino-2-(1H-indol-3-yl)cyclopropane-1-carboxylic acid hydrochloride



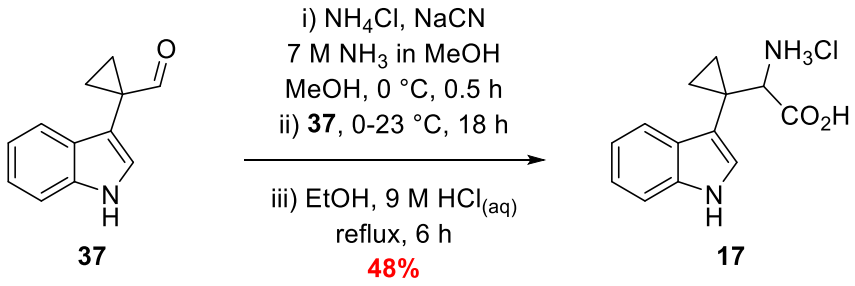
31 **16**

Acid **31** (80 mg, 0.19 mmol) was dissolved in a solution of hydrogen chloride in 1,4-dioxane (4 M, 2 mL) and stirred at 23 °C under an atmosphere of argon for 23 h. The resultant suspension was diluted with diethyl ether (5 mL) and the solid collected *via* suction filtration, the solid was washed further with diethyl ether (2 × 10 mL), transferred to a flask and dried under high-vacuum to afford a diastereomeric mixture (87:13, *E*/*Z*) of the indole **16** as a red solid (48 mg, 99%); **mp** = 260–261 °C; $\boldsymbol{\nu_{max}/cm^{-1}}$ (neat) 3372 (NH), 2863 br (OH), 1703 (CO); $\boldsymbol{\delta_H}$ **(400 MHz; CD_3OD) Resonances assignable to the major *E*-diastereomer**: 1.97 (1 H, dd, *J* = 10.2, 6.4, CHC*H*H'C), 2.19 (1 H, dd, *J* = 8.4, 6.4, CHCH*H*'C), 2.93–2.98 (1 H, m, CC*H*CHH', overlap with minor diastereomer), 7.22–7.27 (1 H, m, C*H*CHCC or C*H*CHCNH), 7.31 (1 H, *app.* dd, *J* = 7.3, 1.3, C*H*CHCC or C*H*CHCNH), 7.56 (1 H, *app.* d, *J* = 1.3, CC*H*NH), 7.71–7.75 (1 H, *app.* d, *J* = 7.0, CHC*H*CC or CHC*H*CNH), 8.13 (1 H, *app.* d, *J* = 8.1, CHC*H*CC or CHC*H*CNH). **Resonances assignable to the minor *Z*-diastereomer**: 1.91 (1 H, dd, *J* = 10.2, 6.2, CHC*H*H'C), 2.96–3.05 (1 H, m, CHC*H*H'C), 7.03 (1 H, app. t, *J* = 7.3, C*H*CHCC or C*H*CHCNH), 7.07–7.12 (1 H, m, C*H*CHCC or C*H*CHCNH), 7.13 (1 H, s, CC*H*NH), 7.34 (1 H, d, *J* = 6.9, CHC*H*CC or CHC*H*CNH), 7.69 (1 H, d, *J* = 7.8, CHC*H*CC or CHC*H*CNH), CHCH*H*'C resonance missing, assumed to be overlapping with the resonance at 2.19 ppm of the major diastereomer; $\boldsymbol{\delta_C}$ **(101 MHz; CD_3OD) Resonances assignable to the major *E*-diastereomer**: 17.6 (CH_2), 23.2 (CH), 40.5 (C), 116.2 (CH), 119.8 (CH), 123.9 (CH), 125.7 (CH), 126.6 (CH), 131.7 (C), 136.8 (C), 153.1 (C), 169.5 (C). **Resonances assignable to the minor *Z*-diastereomer**: 17.7 (CH_2), 24.4 (CH), 40.9 (C), 108.9 (CH), 112.4 (CH), 119.1 (CH), 120.1 (CH), 122.6 (CH), 125.5 (CH), 129.1 (C), 137.9 (C), 169.9 (C), missing C(8) of the indole

ring; **LRMS *m/z* (ASAP+)** 200.1 ([M – NH_2]$^+$, 100%); **HRMS *m/z* (ASAP+)** calcd. for $C_{12}H_{10}NO_2$ 200.0712, found 200.0720.

A known compound prepared according to a standard procedure.

17) 2-(1-(1H-Indol-3-yl)cyclopropyl)-2-aminoacetic acid hydrochloride



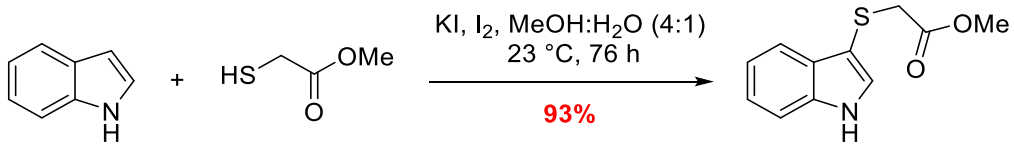
37 **17**

A solution of NH_3 in methanol (7.0 M, 47 µL) was added to a suspension of NH_4Cl (29 mg, 0.54 mmol) and NaCN (26 mg 0.54 mmol) in methanol (1 mL) and stirred vigorously at 0 °C. After 30 min, a solution of **37** (100 mg, 0.539 mmol) in methanol (1 mL) was added dropwise over 2 min and then the solution was allowed to warm to 23 °C. After 72 h, the aldehyde was no longer visible *via* TLC so the reaction mixture was diluted with water (5 mL), $NaHCO_{3(aq)}$ (sat. soln., 10 drops) was added and then the organics were extracted with ethyl acetate (2 × 10 mL). The organic was extracted with $HCl_{(aq)}$ (1 M, 3 × 10 mL) and the acidic aqueous layers combined, taken to ~pH 12 *via* the addition of $NaOH_{(aq)}$(1 M, 35 mL) and the remaining organic residues extracted with ethyl acetate (3 × 10 mL). The organics were combined, dried over $MgSO_4$, the solids filtered off and concentrated to afford the intermediate cyanoamine. The intermediate was immediately re-solublised in EtOH (0.5 mL) and $HCl_{(aq)}$ (9 M, 4 mL) was added and heated to reflux. After 24 h, the solution was allowed to cool to 23 °C, concentrated to dryness and dried under high vacuum to afford tryptophan analogue **17** as an off-white solid (69 mg, 48%); **mp** = 137–139 °C; **ν_{max}/cm^{-1}** (neat) 3243 br (COOH), 1614 br (CO); **δ_H (400 MHz; $CDCl_3$)** 0.89 (1 H, ddd, *J* = 9.2, 6.2, 4.2, C*H*^{1}H^{2}CH^{3}H^4), 1.02 (1 H, ddd, *J* = 9.2, 6.0, 4.5, CH^{1}H^{2}C*H*^{3}H^4), 1.16 (1 H, ddd, *J* = 9.7, 6.0, 4.2, CH^1*H*^{2}CH^{3}H^4), 1.35 (1 H, ddd, *J* = 9.5, 6.2, 4.5, CH^{1}H^{2}CH^3*H*^{4}), 3.47 (1 H, s, C*H*NH_3ClCO_2H), 7.03 (1 H, ddd, *J* = 7.9, 7.0, 1.0, C*H*CHCC or C*H*CHCNH), 7.09 (1 H, ddd, *J* = 8.1, 7.0, 1.2, C*H*CHCC or C*H*CHCNH), 7.24 (1

H, s, CC*H*NH), 7.33 (1 H, dt, *J* = 8.1, 1.0, CHC*H*CC or CHC*H*CNH), 7.85 (1 H, dt, *J* = 7.9, 1.2, CHC*H*CC or CHC*H*CNH); **δ_C (101 MHz; $CDCl_3$)** 12.1 (CH_2), 13.5 (CH_2), 19.5 (C), 63.7 (C), 112.4 (CH), 114.8 (C), 120.2 (Stack, 2 × CH), 122.7 (CH), 126.9 (CH), 128.7 (C), 138.1 (C); **LRMS *m/z* (ES+)** 253.1 ([M + Na]$^+$, 20%); 231.1 ([M + H]$^+$, 14); **HRMS *m/z* (ES+)** calcd for $C_{13}H_{14}N_2O_2Na$ 253.0953, found 253.0952.

A known compound prepared according to a modified literature procedure. The recorded data are in agreement with that reported in the literature with the exception of the missing COOH resonance in the ^{13}C-NMR spectra.[2]

18) Methyl 2-((1H-indol-3-yl)thio)acetate



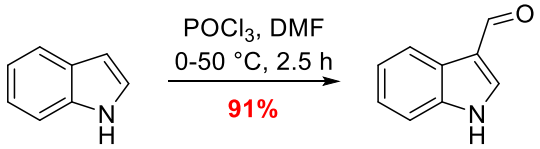
26 **18**

A solution of I_2 (3.60 g, 14.2 mmol) and KI (2.35 g, 14.2 mmol) in MeOH:H_2O (4:1, 14 mL) was added dropwise over 10 h to a solution of indole **26** (2.00 g, 17.0 mmol) and thioglycolic acid (1.27 mL, 14.2 mmol) in MeOH:H_2O (4:1, 30 mL) at 23 °C. The reaction mixture was stirred for 76 h before being quenched by the addition of $NaHCO_{3(aq)}$ (Sat. Soln., 40 mL) and then diluted further with ethyl acetate (35 mL). The resultant phases were separated, and the organic phase was washed sequentially with $Na_2S_2O_{3(aq)}$ (Sat. Soln., 20 mL), $NaOH_{(aq)}$ (1 M, 20 mL), water (20 mL) and brine (30 mL). The organic was then dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude material was subjected to flash column chromatography (R_f = 0.3, 6:4 *n*-hexane:ethyl acetate) to give sulfenylindole **18** as a colourless oil* (2.91 g, 93%); **ν_{max}/cm^{-1}** (neat) 3364 (NH), 2950 (CH), 1720 (CO); **δ_H (400 MHz; $CDCl_3$)** 3.46 (2 H, s, SC*H*$_2$CO$_2$CH$_3$), 3.67 (3 H, s, CO$_2$C*H*$_3$), 7.22–7.30 (2 H, Stack, C*H*CHCC and C*H*CHCNBoc), 7.38–7.42 (2 H, Stack, CHC*H*CC or CHC*H*CNBoc, and CC*H*NH), 7.76–7.81 (1 H, m, CHC*H*CC or CHC*H*CNBoc), 8.45 (1 H, s, N*H*); **δ_C (101 MHz; $CDCl_3$)** 38.8 (CH_2), 52.4 (CH_3), 104.5 (C), 111.8 (CH), 119.2 (CH), 120.8 (CH), 123.0 (CH), 129.1 (C), 130.5 (CH), 136.3 (C), 171.1 (C); **LRMS *m/z* (ES+)** 244.0 ([M +

Na]$^+$, 94%), 222.0 ([M + H]$^+$, 100); **HRMS *m/z* (ES+)** calcd for $C_{11}H_{12}NO_2S$ 222.0589, found 222.0596.

A known compound prepared according to a literature procedure.[3] *No spectral data is available within the literature. *The clear oil darkens on standing with no detectable degradation products via* ^{1}*H-NMR.*

27) Indole-3-carbaldehyde

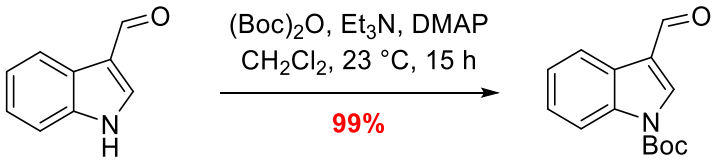


26 **27**

Freshly distilled phosphorus(V) oxychloride (1.91 mL, 20.4 mmol) was added to anhydrous *N,N'*-dimethylformamide (4 mL) at 0 °C in a flame-dried round-bottom flask and stirred under an atmosphere of argon for 30 min. Indole **26** (2.00 g, 17.0 mmol) in anhydrous *N,N'*-dimethylformamide (16 mL) was added to the cooled $POCl_3$/DMF mixture dropwise over 5 min. The resultant solution was heated to 50 °C and stirred for a further 2 h, after which the reaction was judged complete *via* TLC (R_f = 0.3, 1:1 ethyl acetate:*n*-hexane). The reaction mixture was allowed to cool to 23 °C before ice (~10 g) was added and stirred vigorously. The resultant solution was brought to pH 11, *via* dropwise addition of aqueous sodium hydroxide (1 M), forming a colourless precipitate. The suspension was brought to reflux for 15 min and then allowed to cool to 23 °C before the solid was collected *via* suction filtration. The solid was dried under reduced pressure to afford aldehyde **27**, with no need for further purification, as a white crystalline solid (2.24 g, 91%); **ν_{max}/cm^{-1}** (neat) 3166 (conj. NH), 2979 (CH), 2931 (CH), 2892 (CH), 2820.5 (CH), 1629.9 (conj. CO); **δ_H (400 MHz; CD_3OD)** 7.23 (1H, td, *J* = 7.2, 1.2, C*H*CHCC or C*H*CHCNH), 7.27 (1H, td, *J* = 7.2, 1.2, C*H*CHCC or C*H*CHCNH), 7.45–7.49 (1H, m, CHC*H*CC or CHC*H*CNH), 8.08 (1H, s, CC*H*NH), 8.14–8.17 (1H, m, CHC*H*CC or CHC*H*CNH), 9.88 (1H, s, C*H*O); **δ_C (101 MHz; CD_3OD)** 113.1 (CH), 120.1, (CH), 122.4 (CH), 123.6 (CH), 125.0 (CH), 125.7 (C), 138.9 (C), 139.6 (CH), 187.3 (CH); **LRMS *m/z* (ES+)** 146.1 ([M + H]$^+$, 100%), 118.1 (18).

A known compound prepared according to a literature procedure.[4] *The recorded data are in agreement with that reported in the literature.*[4]

28) 3-Formyl-indole-1-*tert*-butyl carboxylate

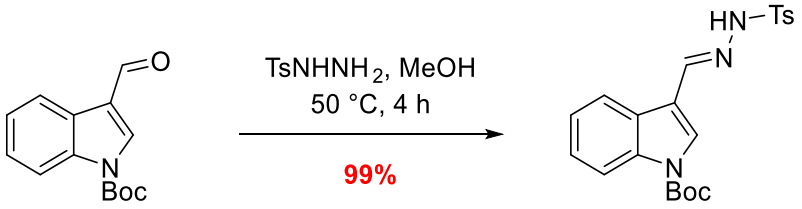


27 **28**

Di-*tert*-butyl dicarbonate (3.38 g, 15.5 mmol) was dissolved in dichloromethane (35 mL) and stirred. To this solution, indole **27** (1.50 g, 10.3 mmol), triethylamine (2.16 mL, 15.5 mmol) and DMAP (126 mg, 1.03 mmol) were added sequentially and the resulting solution was stirred at 23 °C for 15 h until determined complete *via* TLC (R_f = 0.7, 100% ethyl acetate). The reaction mixture was quenched with water (20 mL) and stirred for a further 5 minutes. The layers were separated and the organic washed with water (2 × 30 mL) and brine (1 × 40 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure to give *N*-Boc indole **28** as a pale yellow solid in sufficient purity (2.52 g, 99%); **ν_{max}/cm^{-1}** (neat) 1741.8 (CO), 1677.5 (CO); **δ_H (400 MHz; $CDCl_3$)** 1.71 (9 H, s, C(C*H*$_3$)$_3$), 7.34–7.44 (2 H, Stack, C*H*CHC and C*H*CHCNBoc), 8.15 (1 H, m, CHC*H*C or CHC*H*CNBoc), 8.23 (1 H, s, CC*H*NBoc), 8.26–8.30 (1 H, m, CHC*H*C or CHC*H*CNBoc), 10.09 (1 H, s, C*H*O); **δ_C (101 MHz; $CDCl_3$)** 28.1 (CH_3), 45.9 (C), 85.7 (C), 115.2 (CH), 121.6 (C), 122.1 (CH), 124.6 (CH), 126.1 (CH), 136.0 (C), 136.5 (CH), 148.8 (C), 185.8 (CO); **LRMS *m/z* (AP+)** 246.2 ([M + 1]$^+$, 2%), 190.1 ([M – ^{t}Bu + H]$^+$, 100), 146.1 ([M − Boc]$^+$, 21).

A known compound prepared according to a literature procedure.[5] *The recorded data are in agreement with that reported in the literature.*[5]

29) *E-tert*-Butyl-3-((2-tosylhydrazono)methyl)-indole-1-carboxylate

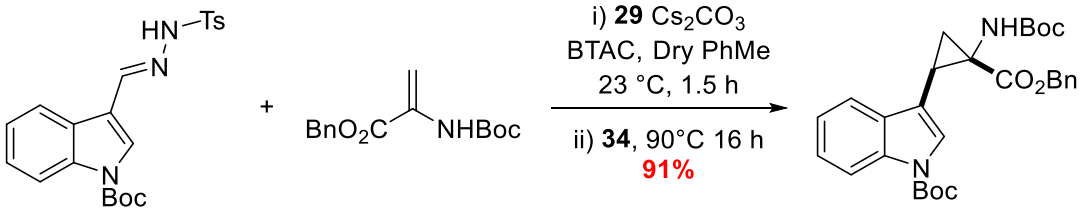


28 **29**

p-Toluenesulfonyl hydrazide (1.50 g, 6.10 mmol) was added to a solution of aldehyde **28** (1.14 g, 6.10 mmol) in methanol (30 mL) and stirred at 50 °C for 4 h until determined complete *via* TLC (R_f = 0.3, 1:1 ethyl acetate: *n*-hexanes). The reaction was taken off heat and allowed to cool to 23 °C before the methanol was removed under reduced pressure to afford *E*-hydrazide **29**, with no need for further purification, as a crystalline yellow solid (2.51 g, 99%); **mp** = 86–88 °C; **ν_{max}/cm^{-1}** (neat) 3194 (NH), 2979 (CH), 1736 (CO), 1551 (SO), 1151 (SO); **δ_H (400 MHz; $CDCl_3$)** 1.66 (9 H, s, C(CH_3)$_3$), 2.38 (3 H, s, Ts-CH_3), 7.30 (2 H, AA'BB', Ts-CH), 7.33 (1 H, td, *J* = 7.8, 1.2, C*H*CHCC or C*H*CHCNBoc), 7.38 (1 H, td, *J* = 7.8, 1.2, C*H*CHCC or C*H*CHCNBoc), 7.73 (1 H, s, CC*H*N), 7.83 (1 H, s, NH), 7.91 (2 H, AA'BB', *Ar-H*), 7.95 (1 H, s, CC*H*NBoc), 8.11 (1 H, d, *J* = 7.8, CHC*H*CC or CHC*H*CNBoc), 8.19 (1 H, dt, *J* = 7.8, 1.5, CHC*H*CC or CHC*H*CNBoc); **δ_C (101 MHz; $CDCl_3$)** 21.7 (CH_3), 28.3 (CH_3), 84.9 (C), 115.2 (CH), 116.0 (C), 122.9 (CH), 123.9 (CH), 125.7 (CH), 126.9 (C), 128.2 (CH), 129.3 (CH), 129.8 (CH), 135.4 (C), 136.0 (C), 143.6 (CH), 144.4 (C), 149.2 (C), quaternary carbamate CO not observed; **LRMS *m/z* (ES+)** 436.1 ({M + Na]$^+$, 100%), 414.2 ([M + H]$^+$, 33); **HRMS *m/z* (ES+)** calcd for $C_{21}H_{23}N_3O_4SNa$ 436.1307, found 436.1306.

A novel compound prepared according to a modified literature procedure.[6]

30) *tert*-Butyl 3-(2-((benzyloxy)carbonyl)-2-((*tert*-butoxycarbonyl)amino)cyclopropyl)-1*H*-indole-1-carboxylate



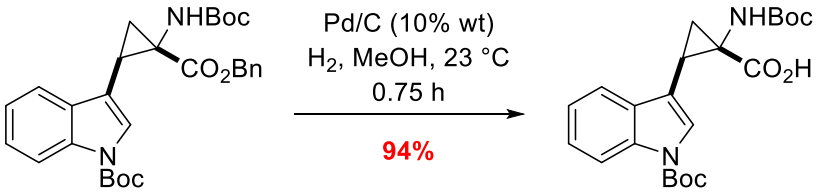
29 **34** **30**

An oven-dried two-neck flask, fitted with an oven-dried condenser, was charged with **29** (2.02 g, 4.88 mmol), caesium carbonate (1.59 g, 4.88 mmol), benzyltriethylammonium chloride (111 mg, 0.487 mmol) and anhydrous toluene (40 mL). The resulting suspension was stirred vigorously under an atmosphere of argon for 1.5 h to yield a bright white suspension. A solution of **34** (1.02 g, 3.67 mmol) in anhydrous toluene (40 mL) was then added and the overall solution was heated at 90 °C for 16 h until complete loss of the acrylate was seen *via* TLC. The reaction mixture was allowed to cool and the remaining solids were removed *via* suction filtration, the collected solids were washed with ethyl acetate (2 × 20 mL). The combined organics were diluted with water (20 mL), separated and the resultant organic phase was washed further with water (2 × 20 mL) and brine (30 mL). Drying over $MgSO_4$ and concentration under reduced pressure afforded the crude mixture. Analysis of the crude by ^{1}H-NMR revealed an 92:8 d.r.. (*E/Z*). The crude was subjected to flash column chromatography (R_f = 0.2, 8.5:1.5 *n*-hexane:ethyl acetate) to yield diastereomeric mixture (86:14, *E/Z*) of cyclopropane **30** as a pale yellow solid (1.69 g, 91%); **mp** = 126–128 °C; **ν_{max}/cm^{-1}** (neat) 3254 (NH), 2976 (CH), 1731 (CO), 1726 (CO), 1678 (CO); **δ_H (400 MHz; $CDCl_3$) Resonances assignable to the major *E*-diastereomer**: 1.46 (9 H, s), 1.65 (9 H, s), 2.16–2.22 (1 H, m), 2.69 (1 H, app. t, *J* = 8.8, CC*H*CHH'), 4.74 (2 H, Stack, CO_2CH_2Ph), 5.43 (1 H, s, N*H*), 6.88 (2 H, m, Ph-CH), 7.12–7.23 (4 H, Stack, 3 × Ph-CH & C*H*CHCC or C*H*CHCNBoc)), 7.30 (1 H, ddd, *J* = 8.4, 7.2, 1.4, C*H*CHCC or C*H*CHCNBoc), 7.34–7.37 (1 H, m, CC*H*NBoc), 7.73 (1 H, s, CHC*H*CC or CHC*H*CNBoc), 8.08 (1 H, d, *J* = 8.1, CHC*H*CC or CHC*H*CNBoc). **Resonances assignable to the minor *Z*-diastereomer**: 2.27 (1 H, m), 2.88

(1 H, app. t, *J* = 8.7, CC*H*CHH'). Used for purposes of identifying the diastereomer ratio; **δ_C (101 MHz; $CDCl_3$) Resonances assignable to the major *E*-diastereomer**: 21.4 (CH_2), 26.2 (CH), 28.4 (CH_3), 28.5 (CH_3), 67.1 (CH_2), 83.7 (C), 115.4 (CH), 119.4 (CH), 122.8 (CH), 124.5 (CH), 125.0 (C), 125.2 (CH), 128.0 (CH), 128.1 (CH), 128.3 (CH), 130.8 (C), 135.4 (C), 144.9 (C), 149.6 (C), 155.9 (C), 170.1 (C). Both quaternary $C(CH_3)_3$ carbons not observed. **Resonances assignable to the minor *Z*-diastereomer**: No resonances assignable; **LRMS *m/z* (ES+)** 529.2 ([M + Na]$^+$, 100%); **HRMS *m/z* (ES+)** calcd for $C_{29}H_{34}N_2O_6Na$ 529.2315, found 529.2317.

A novel compound prepared according to a modified literature procedure.[7]

31) 2-(1-(*tert*-Butoxycarbonyl)-1*H*-indol-3-yl)-1-((*tert*-butoxycarbonyl)amino)cyclopropane-1-carboxylic acid



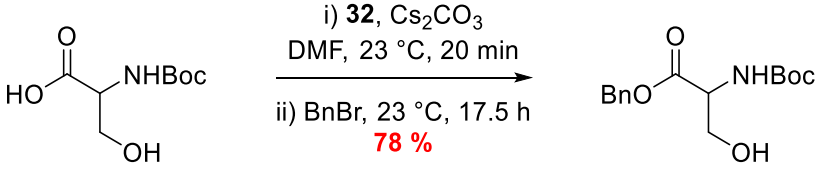
30 **31**

10% Pd/C (100 mg) was suspended in a solution of **30** (200 mg, 0.395 mmol) in methanol (8 mL) and stirred in a round-bottom flask fitted with a septa. $H_{2(g)}$ was then bubbled through the solution for 10 min *via* balloon and then for the remainder of the reaction the balloon needle was suspended above the surface of the solution. After 45 min, the reaction was determined complete *via* TLC. The reaction mixture was passed through a celite bung and was eluted with methanol (2 × 5 mL). The resulting liquor was collected and concentrated under reduced pressure to give a diastereomeric mixture of acid **31** (91:9, *E*/*Z*) as a white solid (154 mg, 94%); **mp** = 153–154 °C; **ν_{max}/cm^{-1}** (neat) 2978 (CH), 1726 (CO), 1702 (CO), Br. COOH absorbance seen but with no definable centre; **δ_H (400 MHz; CD_3OD) Resonances assignable to the major *E*-diastereomer**: 1.49 (9 H, s C(CH_3)$_3$), 1.56–1.60 (1 H, m, CHC*H*H'C), 1.66 (9 H, s, C(CH_3)$_3$), 2.10 (1 H, dd, *J* = 8.2, 5.1, CHCH*H*'C), 2.67 (1 H, td, *J* = 8.8, 8.1, 1.5, CC*H*CHH'), 7.21 (1 H, td, *J* = 7.4, 1.3, C*H*CHCC or C*H*CHCNBoc), 7.24–7.29 (1

H, m, C*H*CHCC or C*H*CHCNBoc), 7.39–7.41 (1 H, m, CC*H*NBoc), 7.89 (1 H, d, *J* = 7.6, CHC*H*CC or CHC*H*CNBoc), 8.05 (1 H, d, *J* = 8.1, CHC*H*CC or CHC*H*CNBoc). **Resonances assignable to the minor *Z*-diastereomer**: 2.80 (1 H, m, CC*H*CHH'). Used for purposes of identifying the diastereomer ratio; **δ_C (101 MHz; CDCl3) Resonances assignable to the major *E*-diastereomer**: 21.7 (CH_2), 26.3 (CH), 28.4 (CH_3), 28.8 (CH_3), 41.2 (C), 80.5 (C), 84.8 (C), 115.8 (CH), 117.8 (C), 120.9 (CH), 123.6 (CH), 125.3 (CH), 125.8 (CH), 132.3 (C), 136.7 (C), 151.0 (C), 158.6 (C), 173.9 (C). **Resonances assignable to the minor *Z*-diastereomer**: No resonances assignable; **LRMS *m/z* (ES+)** 439.2 ($[M + Na]^+$, 100%); **HRMS *m/z* (ES+)** calcd for $C_{22}H_{28}N_2O_6Na$ 439.1845, found 439.1851.

A novel compound prepared according to a standard procedure. ***Note: If left for >1 h under hydrogenolysis conditions the product rapidly degrades – presumably via reduction of the 2,3-double bond however this was never verified in our experiments.***[8]

33) Benzyl (*tert*-butoxycarbonyl)serinate



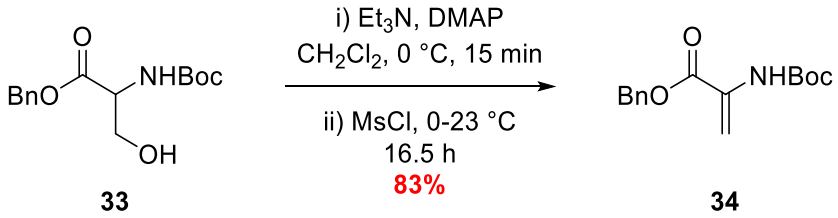
32 **33**

N-Boc Serine **32** (2.00 g, 9.27 mmol) was dissolved in a suspension of caesium carbonate (3.81 g, 11.7 mmol) in *N,N'*-dimethylformamide (40 mL) and stirred vigorously at 23 °C for 20 min. Benzyl bromide (1.39 mL, 11.7 mmol) was then added dropwise over 3 min and the reaction allowed to stir. After 17.5 h, the reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (30 mL). The organic and aqueous layers were separated and the organic was washed with water (2 × 15 mL) and brine (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was subjected to flash column chromatography (R_f = 0.2, 2:1 *n*-hexanes:ethyl acetate) to yield benzyl ester **33** compound as a colourless oil (2.23 g, 78%); **ν_{max}/cm^{-1}** (neat) 3391 (OH), 2976 (CH), 1688 (CO); **δ_H (400 MHz; $CDCl_3$)** 1.44 (9 H, s, C(C*H*$_3$)$_3$), 2.18 (1 H, t, *J* = 6.2, C*H*CH$_2$), 3.92 (1 H, dd, *J* = 6.2, 3.7, CHC*H*$_2$), 3.99 (1 H, dd, *J* = 6.2, 3.7, CHC*H*$_2$), 4.43 (1 H, s, O*H*), 5.20 (1 H, d, *J* = 12.4,

PhC*H*H'CO_2), 5.22 (1 H, d, *J* = 12.4, PhCH*H*'CO_2), 5.44 (1 H, s, N*H*), 7.31–7.40 (5 H, m, *Ar-H*); **δ_C (101 MHz; $CDCl_3$)** 28.4 (CH_3), 56.0 (CH), 63.7 (CH_2), 67.5 (CH_2), 128.3 (CH), 128.6 (CH), 128.8 (CH), 135.3 (C), 155.9 (CO) 170.8 (CO); **LRMS *m/z* (ES+)** 318.1 ([M + Na]$^+$ 100%).

A known compound prepared according to a literature procedure.[9] *The recorded data are in agreement with that reported in the literature with the exception of the missing quaternary carbon of the* ^{t}*Bu-group in the* ^{13}*C-NMR spectrum.*[10]

34) Benzyl 2-((*tert*-butoxycarbonyl)amino)acrylate

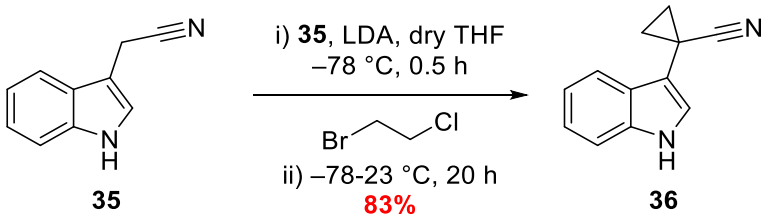


33 **34**

A solution of alcohol **33** (8.52 g, 28.9 mmol), triethylamine (10.1 mL, 72.1 mmol) and 4-dimethylaminopyridine (3.52 g, 28.9 mmol) in anhydrous dichloromethane (150 mL) was cool to 0 °C and stirred under an atmosphere of argon for 15 min. Methanesulfonyl chloride (3.70 mL, 37.5 mmol) was then added dropwise over the course of 5 min, the resultant mixture was allowed to warm to 23 °C over-night. After 16.5 h, the reaction mixture was diluted with $HCl_{(aq)}$ (1 M, 10 mL). The organic and aqueous layers were separated and the organic was washed with $HCl_{(aq)}$ (1M, 10 mL), water (2 × 10 mL) and brine (20 mL). This organic solution was dried with MgSO4 and concentrated under reduced pressure to give a light brown oil. The crude was subjected to flash column chromatography (R_f = 0.3, 9:1 *n*-hexane:ethyl acetate) to afford amino acrylate **34** as a white solid (6.64 g, 83%); **ν_{max}/cm^{-1}** (neat) 3397 (NH), 2980 (CH), 1705 (CO), 1630 (CC); **δ_H (400 MHz; $CDCl_3$)** 1.48 (9 H, s, C(CH_3)$_3$), 5.26 (2 H, s, PhCH_2CO_2), 5.79 (1 H, d, *J* = 1.5, CCH_aH$_b$ or CCH$_a$$H_b$), 6.18 (1 H, *app.* s, CCH_aH$_b$ or CCH$_a$$H_b$), 7.03 (1 H, s, N*H*), 7.32–7.40 (5 H, m, *Ar-H*); **δ_C (101 MHz; $CDCl_3$)** 28.4 (CH_3), 67.8 (CH_2), 80.9 (C), 105.6 (CH_2), 128.3 (CH), 128.7 (CH), 128.8 (CH), 131.5 (C), 135.3 (C), 152.7 (CO), 164.0 (CO); **LRMS *m/z* (ES+)** 300.1 ([M + Na]$^+$, 100%).

A known compound prepared according to a literature procedure.[11] *The recorded data are in agreement with that reported in the literature.*[11]

36) 1-(Indol-3-yl)cyclopropane-1-carbonitrile

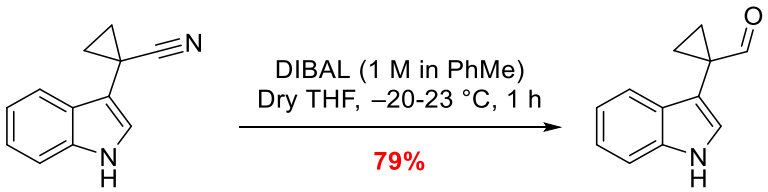


A solution of *n*-BuLi in hexanes (2.25 M, 22.8 mL, 51.2 mmol) was added to a flame-dried, two-neck round-bottom flask that had been charged with anhydrous tetrahydrofuran (50 mL) and cooled to –40 °C under an atmosphere of argon. Freshly distilled $(^{i}Pr)_2NH$ (7.20 mL, 51.2 mmol) was then added to the cooled *n*-BuLi/tetrahydrofuran solution dropwise over 5 min while maintaining the temperature at –40 °C. After 0.5 h, the resulting LDA solution was transferred *via* cannula (by positive pressure of argon) to a solution of nitrile **35** (2.00 g, 12.8 mmol) in anhydrous tetrahydrofuran (20 mL) which had been cooled to –78 °C. The resulting solution was allowed to mature for 0.5 h, in which time a yellow suspension had formed signifying the formation of the nitrile anion. 1-bromo-2-chloroethane (1.60 mL, 19.2 mmol) was then added in one portion and the solution immediately turned to a brown translucent solution. The resulting brown solution was allowed to warm passively to 23 °C and after 20 h the reaction was determined complete *via* TLC. The reaction mixture was quenched by slow addition of water (20 mL) and then diluted further with ethyl acetate (20 mL). The two layers were separated and the organic was washed with $HCl_{(aq)}$ (2 M, 2 × 20 mL), water (20 mL) and brine (40 mL). After drying over $MgSO_4$, the solids were filtered off and the organic was concentrated to give the crude as a brown oil. The crude was subjected to flash column chromatography (R_f = 0.5, 1:1 *n*-hexane:ethyl acetate) and the product-containing fractions were combined, concentrated and recrystallised from hot *n*-hexane and ethyl acetate to afford 1,1'-cyclopropane **36** as pale-yellow, needle-like crystals (1.94 g, 83%); **mp** = 168–170 °C; **ν_{max}/cm^{-1}** (neat) 3392 (NH), 3126 (CH), 2093 (CH), 3057 (CH), 2227 (CN); **δ_H (400 MHz; $CDCl_3$)** 1.34–1.39 (2 H, AA'BB', Cyclopropane CH_2), 1.64–1.69 (2 H, AA'BB', Cyclopropane

CH_2), 7.11 (1 H, d, *J* = 2.6, CC*H*NH), 7.21 (1 H, td, *J* = 8.1, 7.2, 1.4, C*H*CHCC or C*H*CHCNH), 7.26 (1 H, td, *J* = 7.7, 7.2, 1.4, C*H*CHCC or C*H*CHCNH), 7.38 (1 H, dd, *J* = 8.1, 1.4, CHC*H*CC or CHC*H*CNH), 7.83 (1 H, dd, *J* = 7.7, 1.4, CHC*H*CC or CHC*H*CNH), 8.13 (1 H, s, N*H*); **δ_C (101 MHz; $CDCl_3$)** 5.9 (C), 15.5 (2 × CH_2), 111.7 (CH), 112.0 (C), 119.0 (CH), 120.5 (CH), 123.1 (CH), 123.3 (CH), 126.5 (C), 136.3 (C), one quaternary-C (CN) resonance was not observed; **LRMS *m/z* (ES+)** 183.1 ([M + H]$^+$, 100%), 156.1 ([M − CN]$^+$, 95); **HRMS *m/z* (ES+)** calcd for $C_{12}H_{11}N_2$183.0922, found 183.0921.

A reported compound prepared according to a modified literature procedure.[2] *No reference data is available to compare the recorded spectra to.*

37) 1-(Indol-3-yl)cyclopropane-1-carbaldehyde



36 **37**

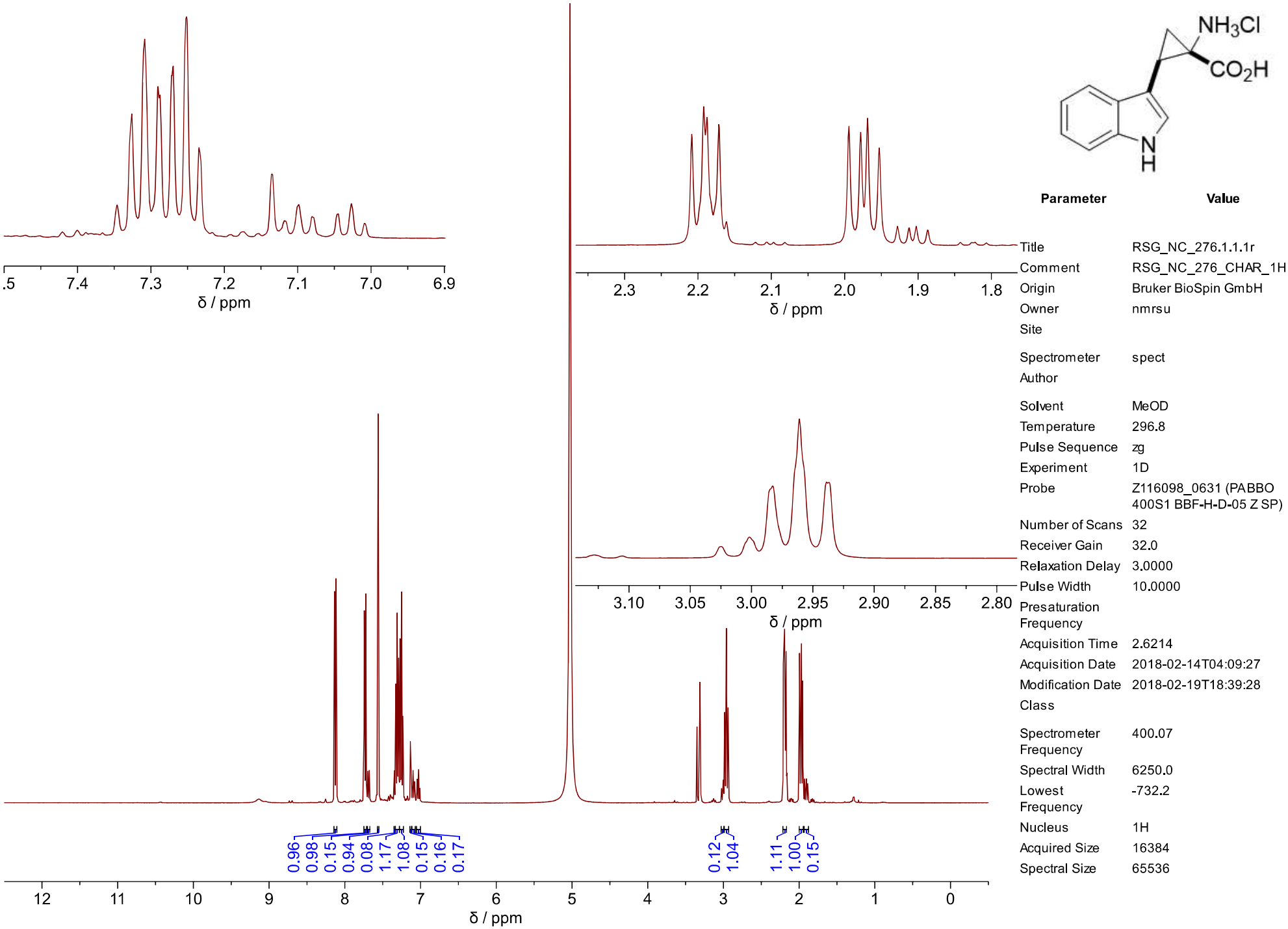
A solution of DIBAL (4.30 mL, 4.28 mmol, 1.0 M) in toluene was added dropwise over 20 min to a stirred solution of nitrile **36** (600 mg, 3.29 mmol) in anhydrous tetrahydrofuran (10 mL) which had been cooled to −20 °C under argon. After a further 40 min, the reaction was determined complete *via* TLC. Upon completion, the remaining DIBAL was quenched *via* dropwise addition of MeOH (2.5 mL) over 5 min. The reaction mixture was then diluted further with a solution of Rochelle's salt (Sat. soln, 20 mL) and allowed to stir. After 1 h, the mixture was extracted with ethyl acetate (15 mL). The two-phases were separated and the aqueous was back-extracted with ethyl acetate (2 × 10 mL) and the combined organics were washed with water (2 × 10 mL) and brine (20 mL), dried over $MgSO_4$, filtered and concentrated to afford the crude as a yellow oil. The crude oil was subjected to flash column chromatography (R_f = 0.4, 6:4 *n*-hexane:ethyl acetate) to afford aldehyde **37** as a colourless oil (481 mg, 79%); **ν_{max}/cm^{-1}** (neat) 3376 s (NH), 3092 (CH), 3055 (CH), 3009 (CH), 2829 (CH – aldehyde), 2721 (CH – aldehyde), 1699 (CO); **δ_H (400 MHz; $CDCl_3$)** 1.38–1.42 (2 H, AA'BB', CH_2), 1.63–1.67

(2 H, AA'BB', C*H'*$_2$), 7.12 (1 H, d, *J* = 2.5, CC*H*NH), 7.15 (1 H, ddd, *J* = 8.2, 7.2, 1.3, C*H*CHCC or C*H*CHCNH), 7.24 (1 H, ddd, *J* = 8.2, 7.2, 1.3, C*H*CHCC or C*H*CHCNH), 7.39 (1 H, dt, *J* = 8.1, 0.9, CHC*H*CC or CHC*H*CNH), 7.59 (1 H, dt, *J* = 8.1, 0.9, CHC*H*CC or CHC*H*CNH), 8.13 (1 H, s, NH), 9.45 (1 H, s, C*H*O); **δ_C (101 MHz; $CDCl_3$)** 16.5 (2 × CH_2), 29.0 (C), 111.6 (CH), 113.7 (C), 119.2 (CH), 120.1 (CH), 122.7 (CH), 124.3 (CH), 128.0 (C), 136.4 (C), 202.3 (CHO); **LRMS *m/z* (ES+)** 208.1 ($[M + Na]^+$, 100%); 186.1 ($[M + H]^+$, 5); **HRMS *m/z* (TOF ES+)** calcd for $C_{12}H_{11}NONa$ 208.0739, found 208.0738.

A literature compound prepared according to a modified literature procedure.[2] *The recorded data are in agreement with that reported in the literature.*

1.3 – ^{1}H- and ^{13}C-NMR Spectra

Page intentionally left blank, NMR spectra to follow.



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Author	
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Relaxation Delay	3.0000
Pulse Width	10.0000
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Modification Date	2018-02-19T18:39:28
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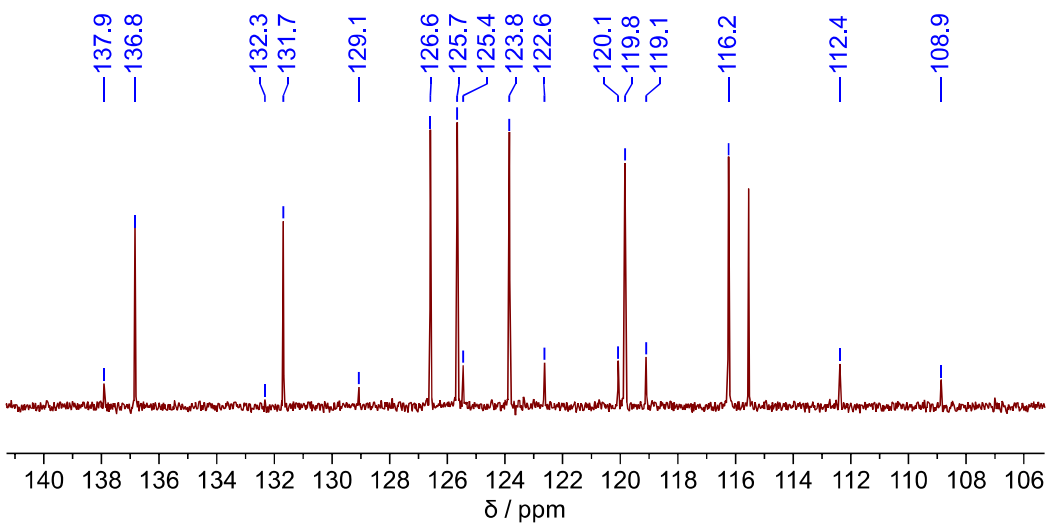

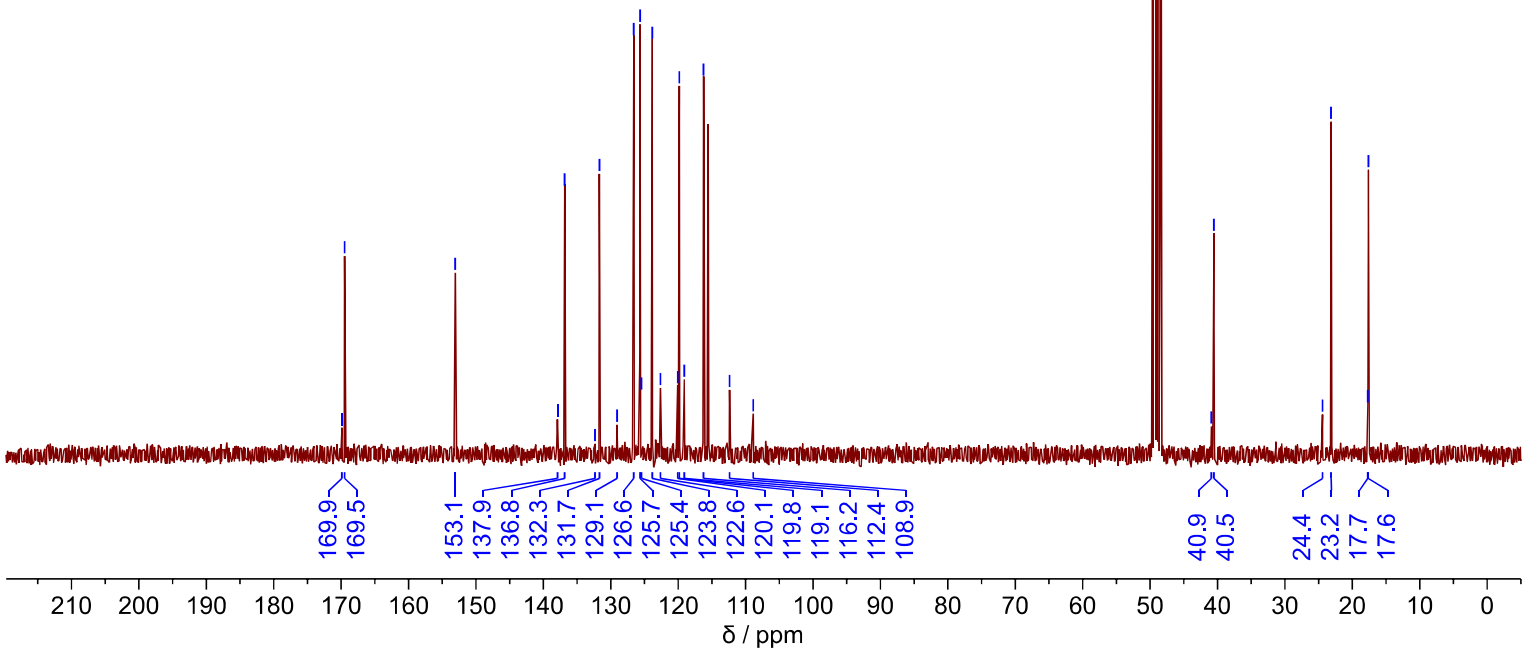

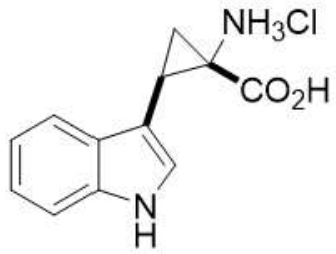

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Author	
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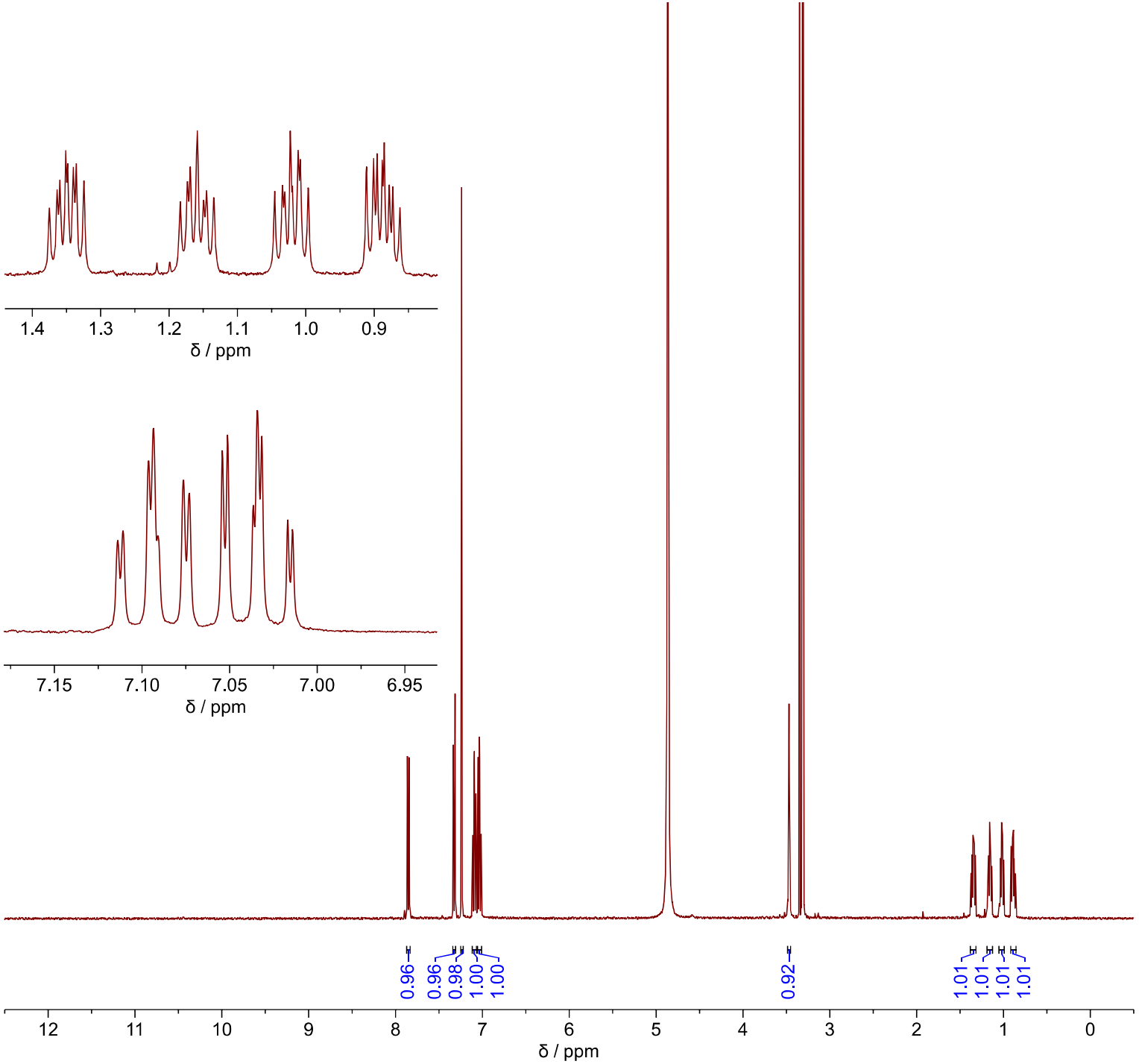
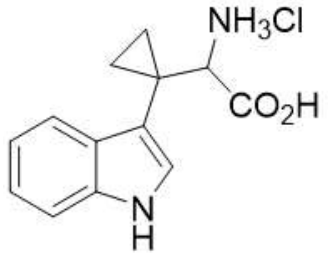
Spectral Size	65536





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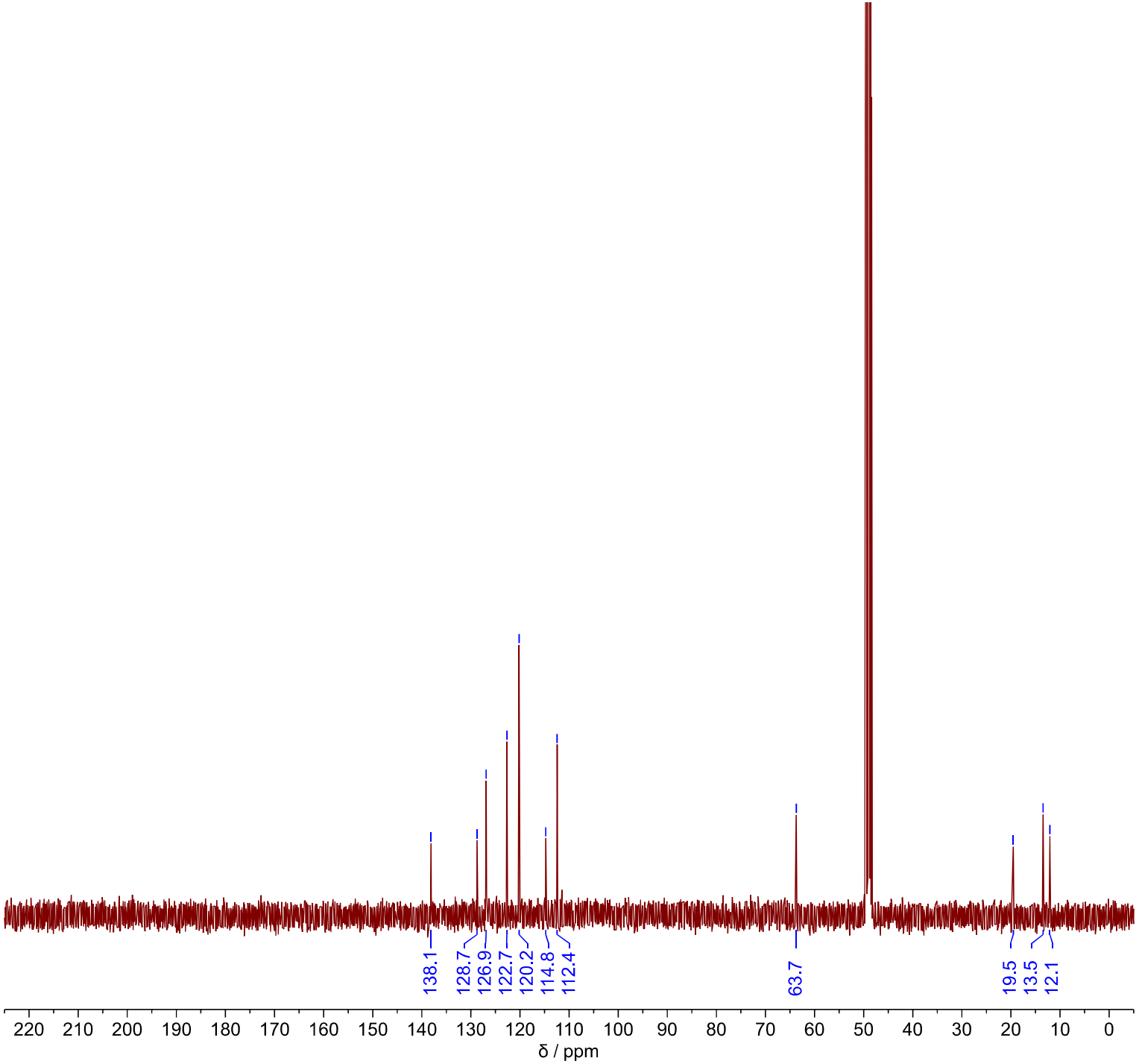

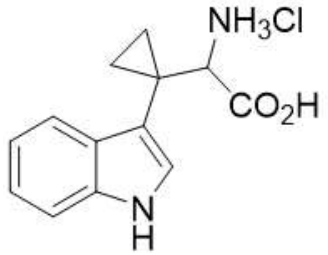

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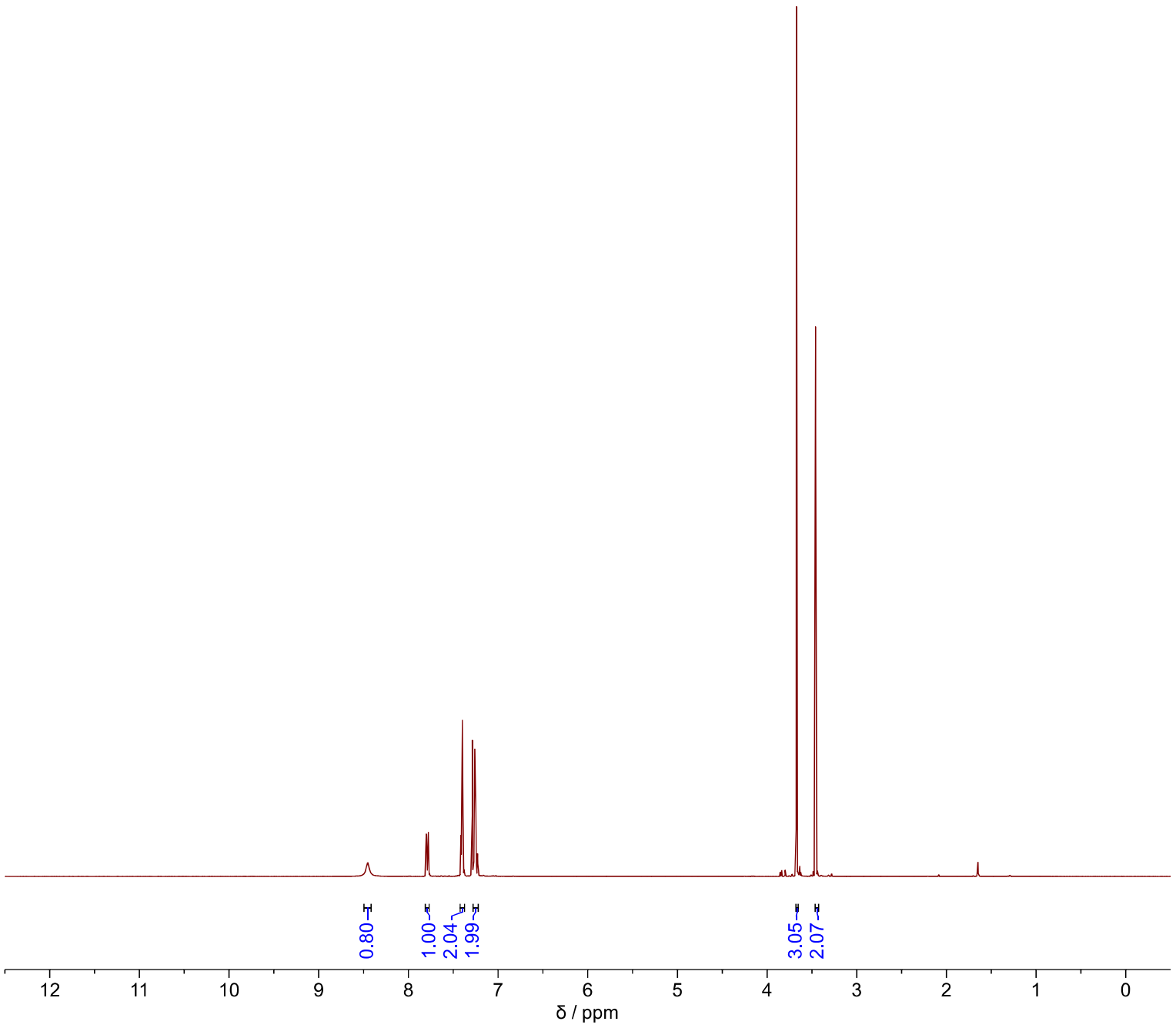

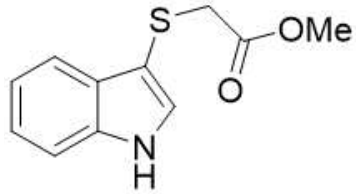

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Author	
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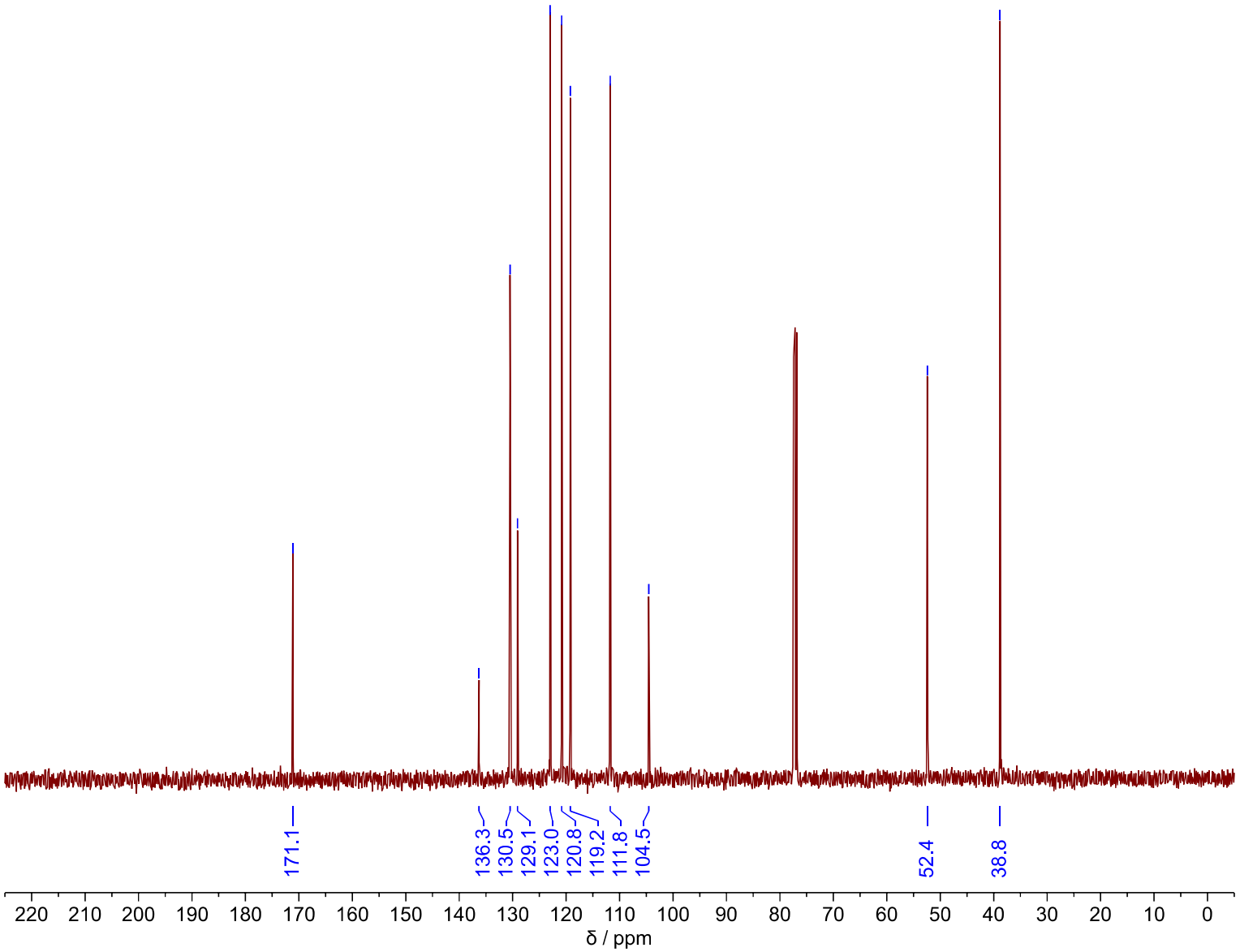

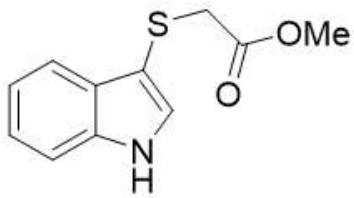

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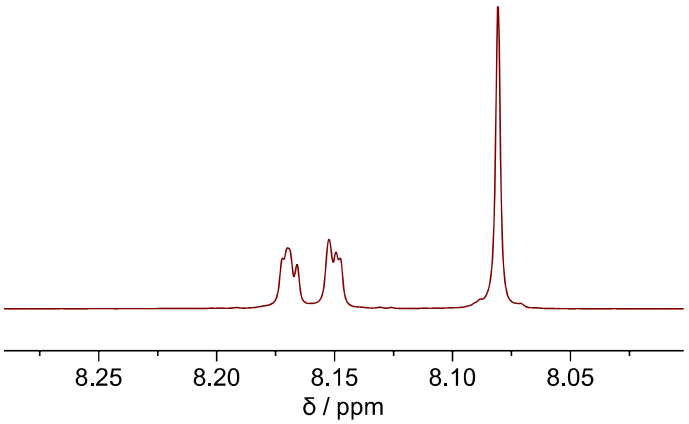
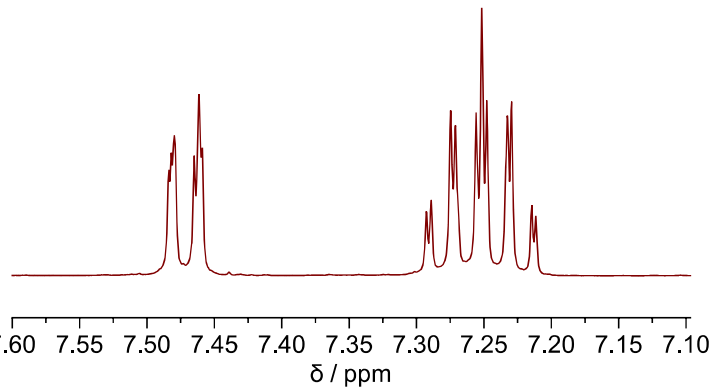
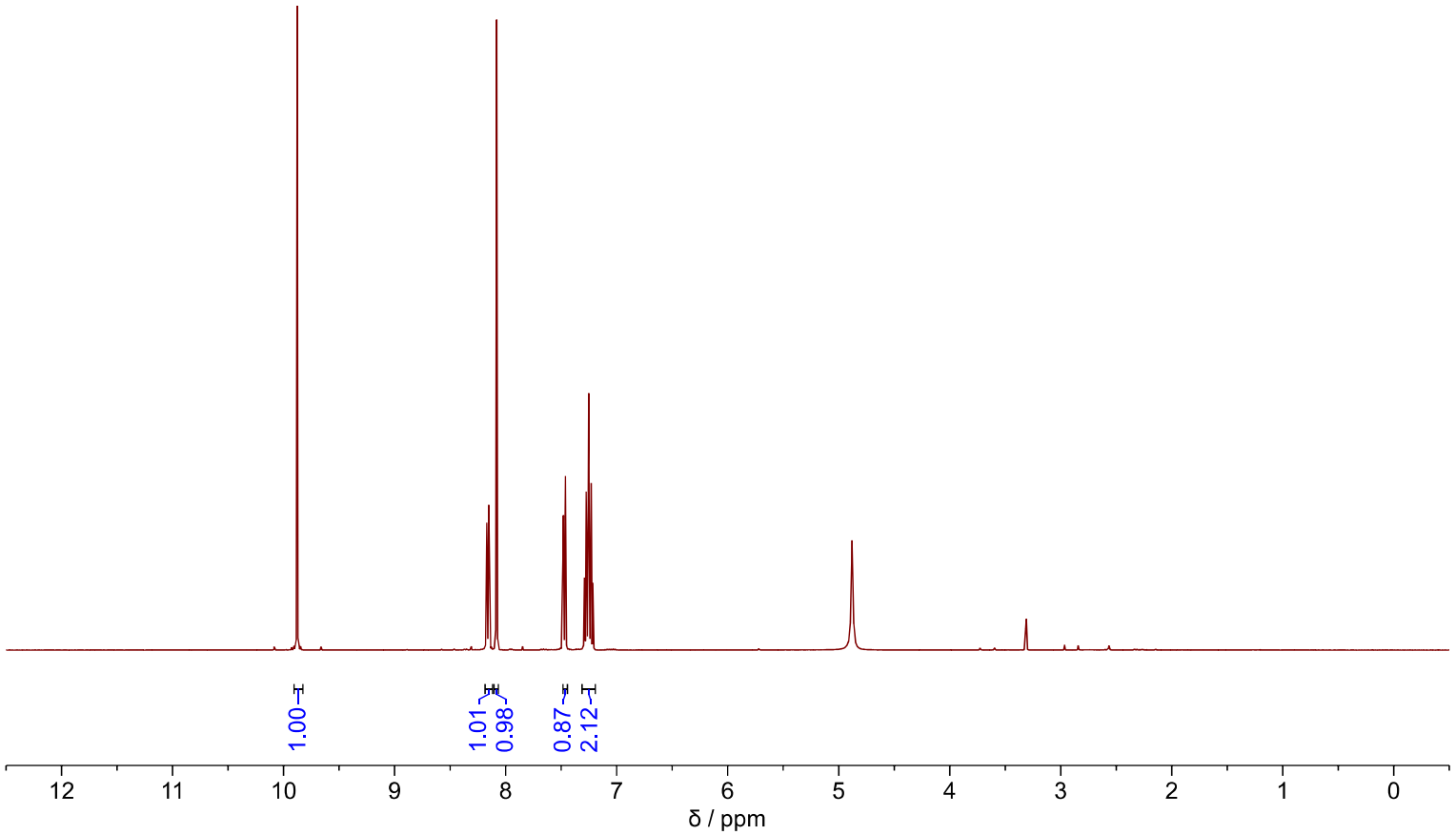
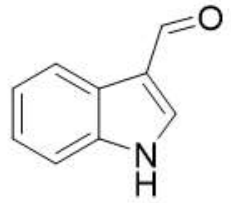
Modification Date	2019-01-03T14:33:04
Class	
Spectrometer Frequency	100.62
Spectral Width	25252.5
Lowest Frequency	-1114.7
Nucleus	13C
Acquired Size	9089
Spectral Size	65536

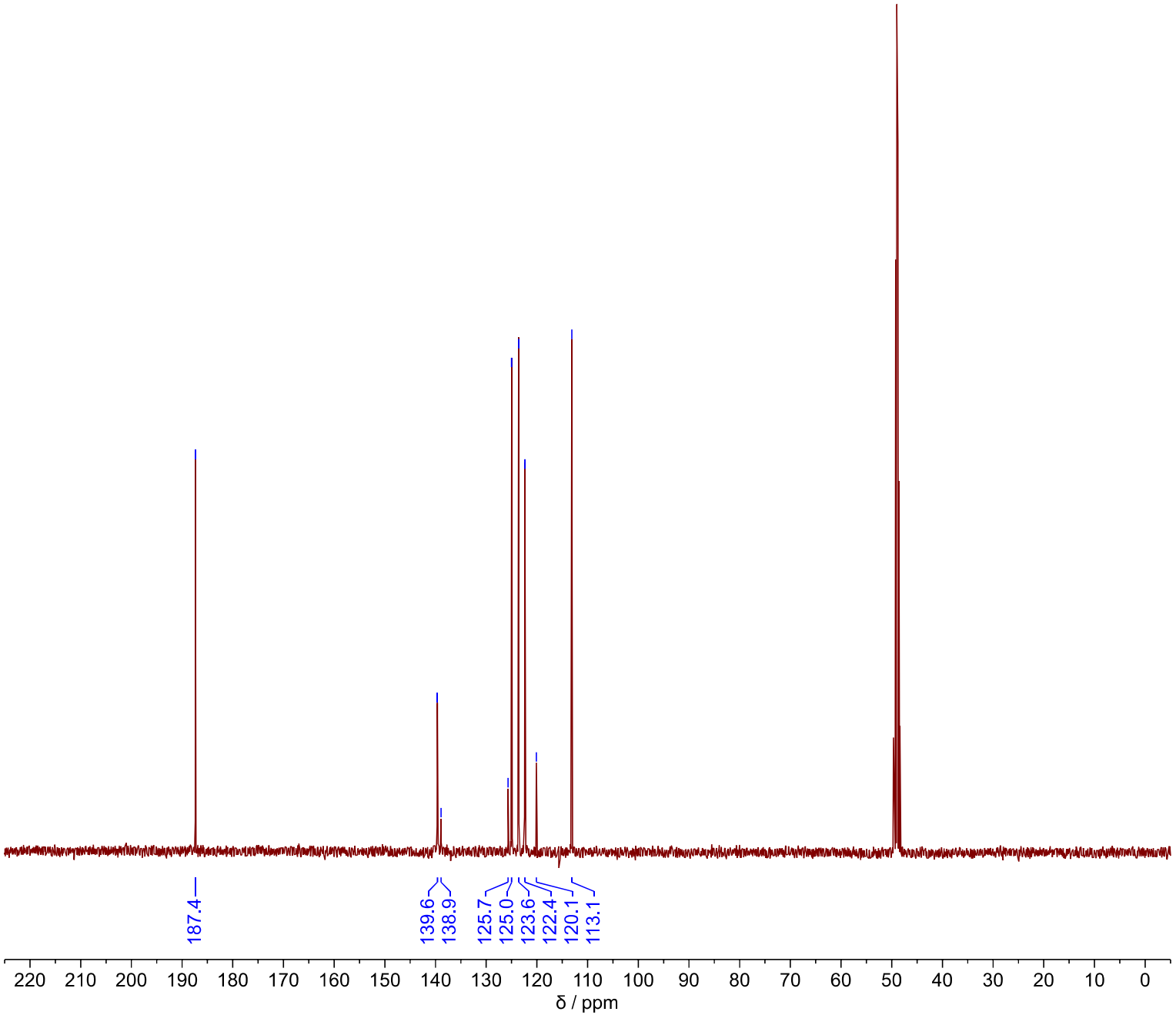


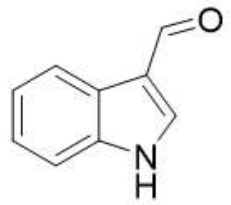




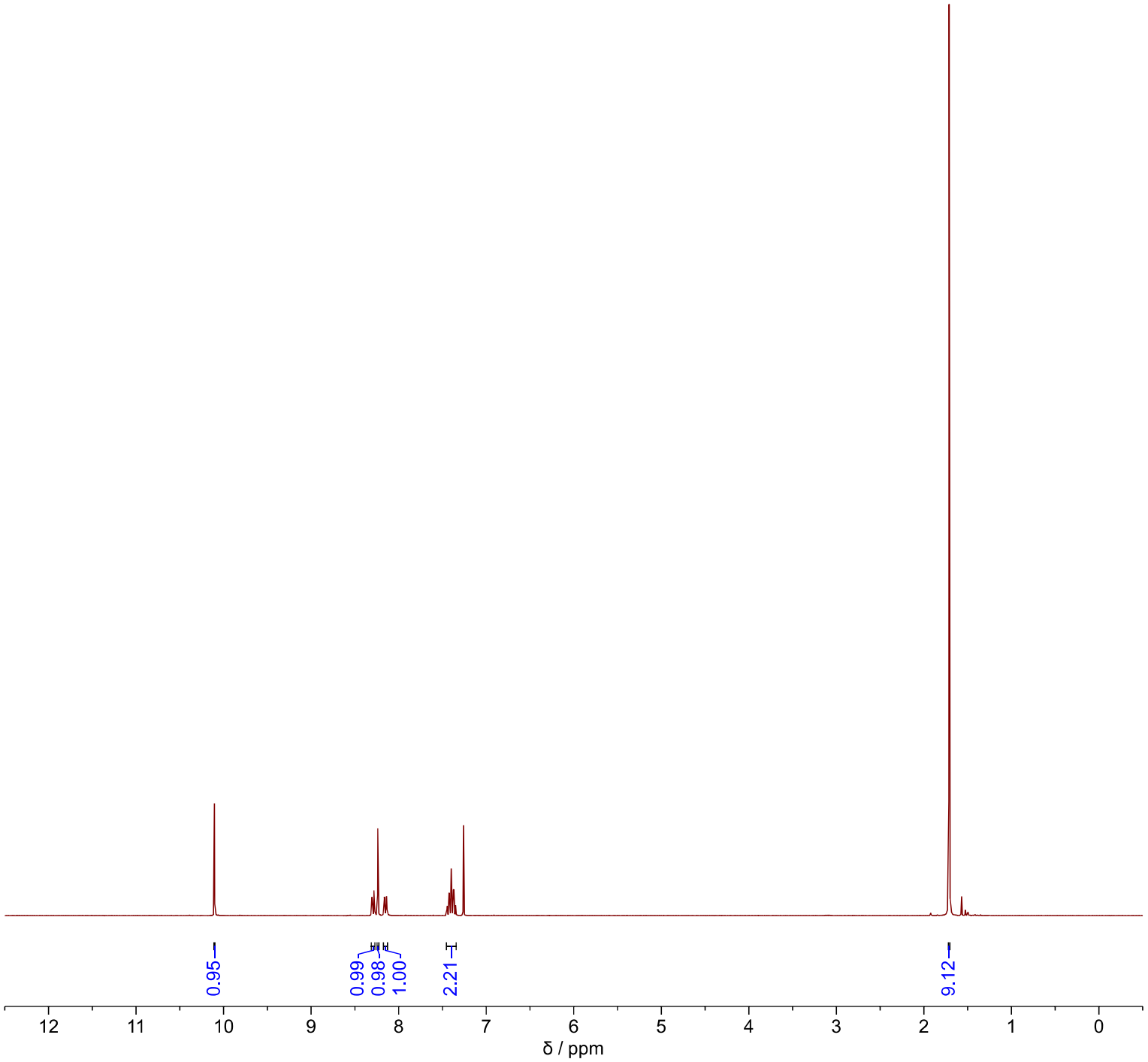


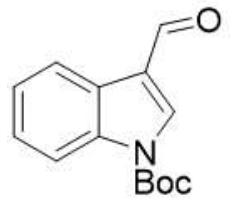
Parameter	Value
Title	RSG_NC_021_CHAR_1H.10.fid
Comment	RSG_NC_021_CHAR_1H
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	MeOD
Temperature	296.0
Pulse Sequence	zg30
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	32
Receiver Gain	322.0
Relaxation Delay	1.5000
Pulse Width	11.0600
Presaturation Frequency	
Acquisition Time	1.9923
Acquisition Date	2016-11-30T09:01:00
Modification Date	2016-11-30T18:33:54
Class	
Spectrometer Frequency	400.13
Spectral Width	8223.7
Lowest Frequency	-1718.7
Nucleus	1H
Acquired Size	16384
Spectral Size	65536



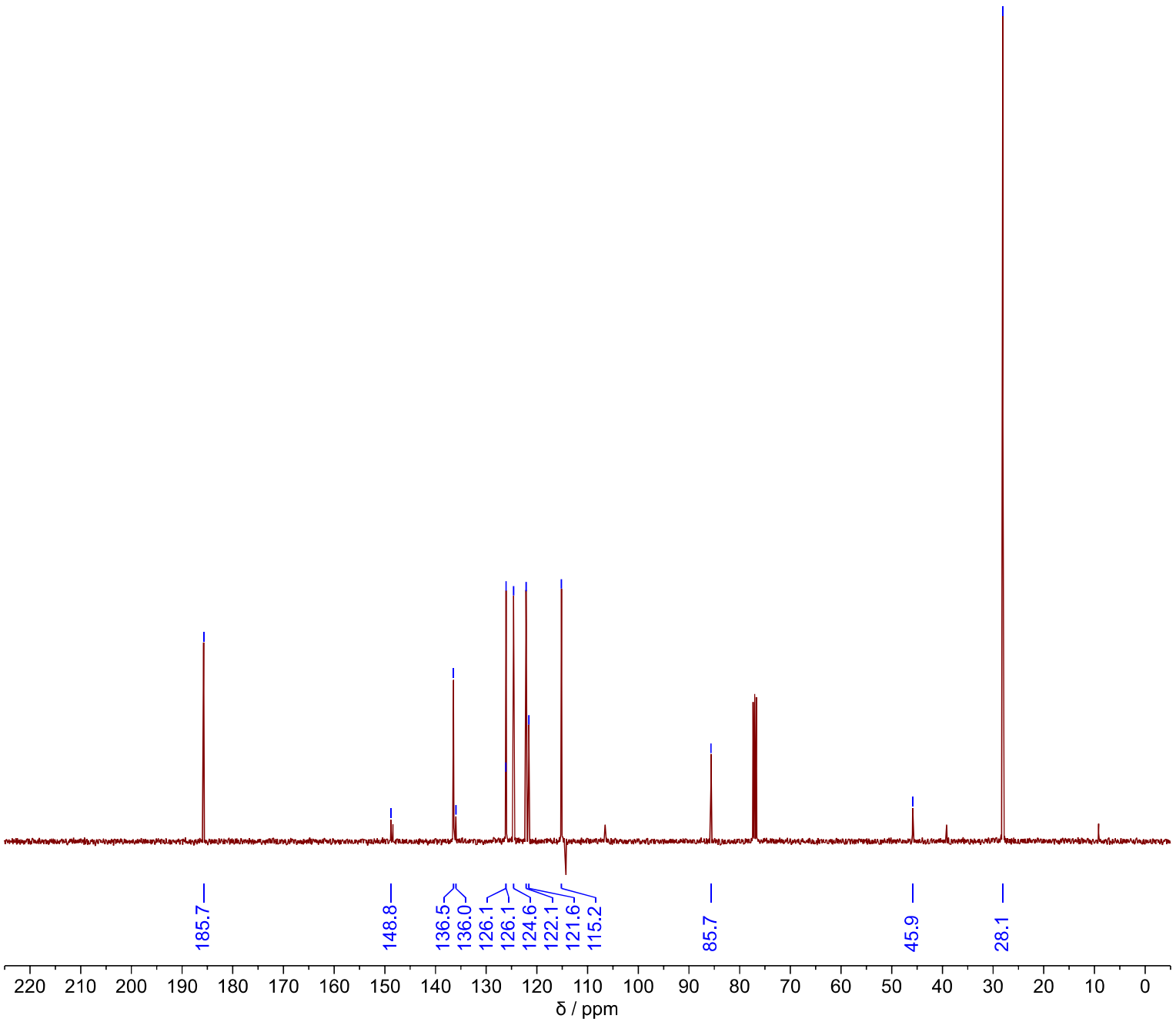


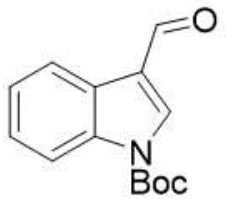
Parameter	Value
Title	RSG_NC_021_CHAR_13C.10.fid
Comment	RSG_NC_021_CHAR_13C
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	MeOD
Temperature	296.2
Pulse Sequence	udeft
Experiment	1D
Probe	5 mm PADUL 13C-1H/D Z-GRD Z108621/0010
Number of Scans	380
Receiver Gain	2050.0
Relaxation Delay	3.0000
Pulse Width	8.8000
Presaturation Frequency	
Acquisition Time	0.3599
Acquisition Date	2016-11-30T09:11:00
Modification Date	2016-11-30T18:33:52
Class	
Spectrometer Frequency	100.62
Spectral Width	25252.5
Lowest Frequency	-985.9
Nucleus	13C
Acquired Size	9089



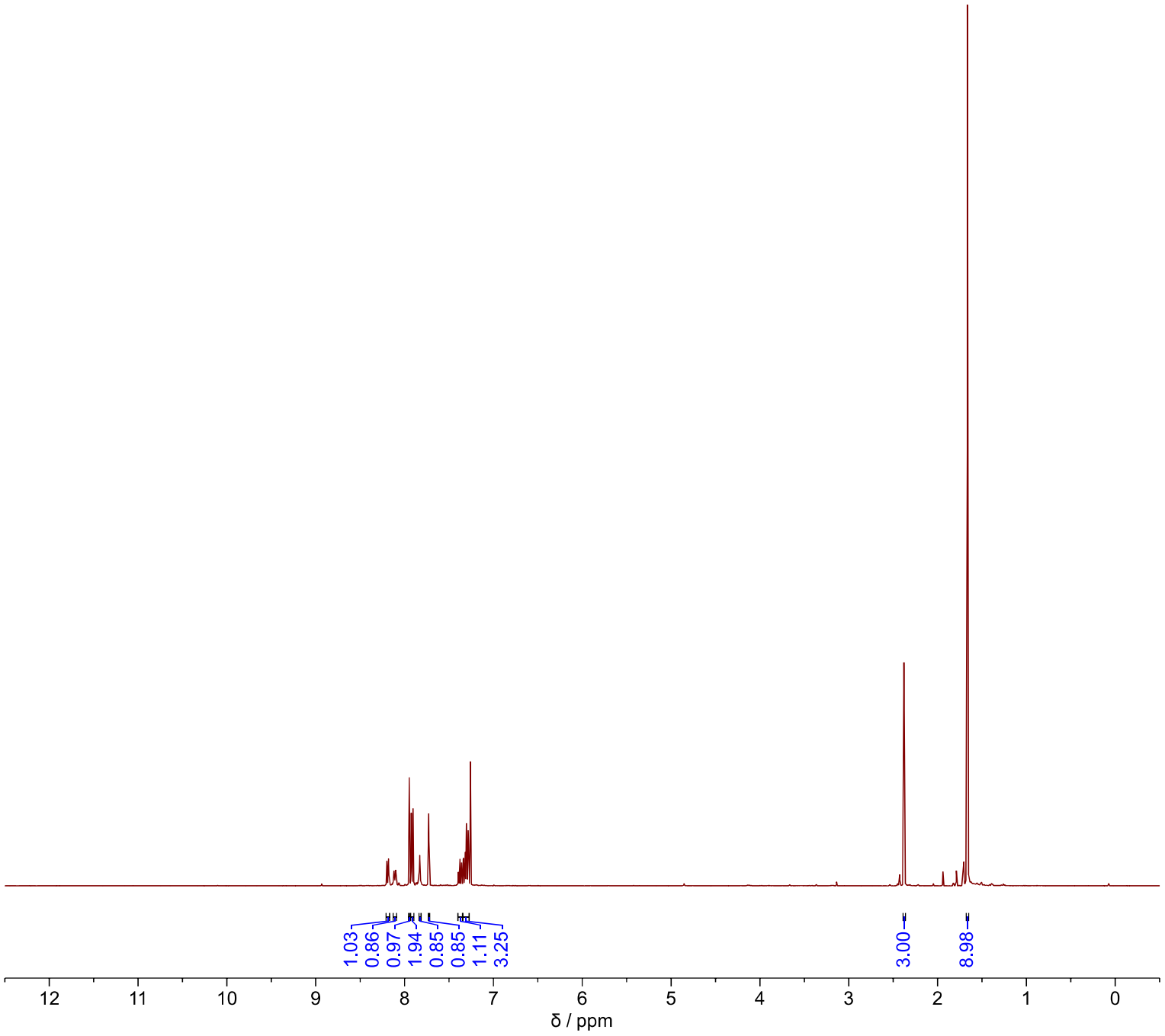


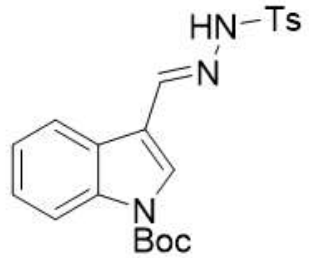
Parameter	Value
Title	RSG_NC_111.10.1.1r
Comment	RSG_NC_111_C_1H
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	292.9
Pulse Sequence	zg30
Experiment	1D
Probe	5 mm PABBO BB-1H/ D Z-GRD Z104275/ 0131
Number of Scans	32
Receiver Gain	512.0
Relaxation Delay	1.0000
Pulse Width	12.8000
Presaturation Frequency	
Acquisition Time	2.7263
Acquisition Date	2017-05-08T18:12:00
Modification Date	2017-05-19T12:02:18
Class	
Spectrometer Frequency	300.13
Spectral Width	6009.6
Lowest Frequency	-1151.4
Nucleus	1H
Acquired Size	16384
Spectral Size	32768



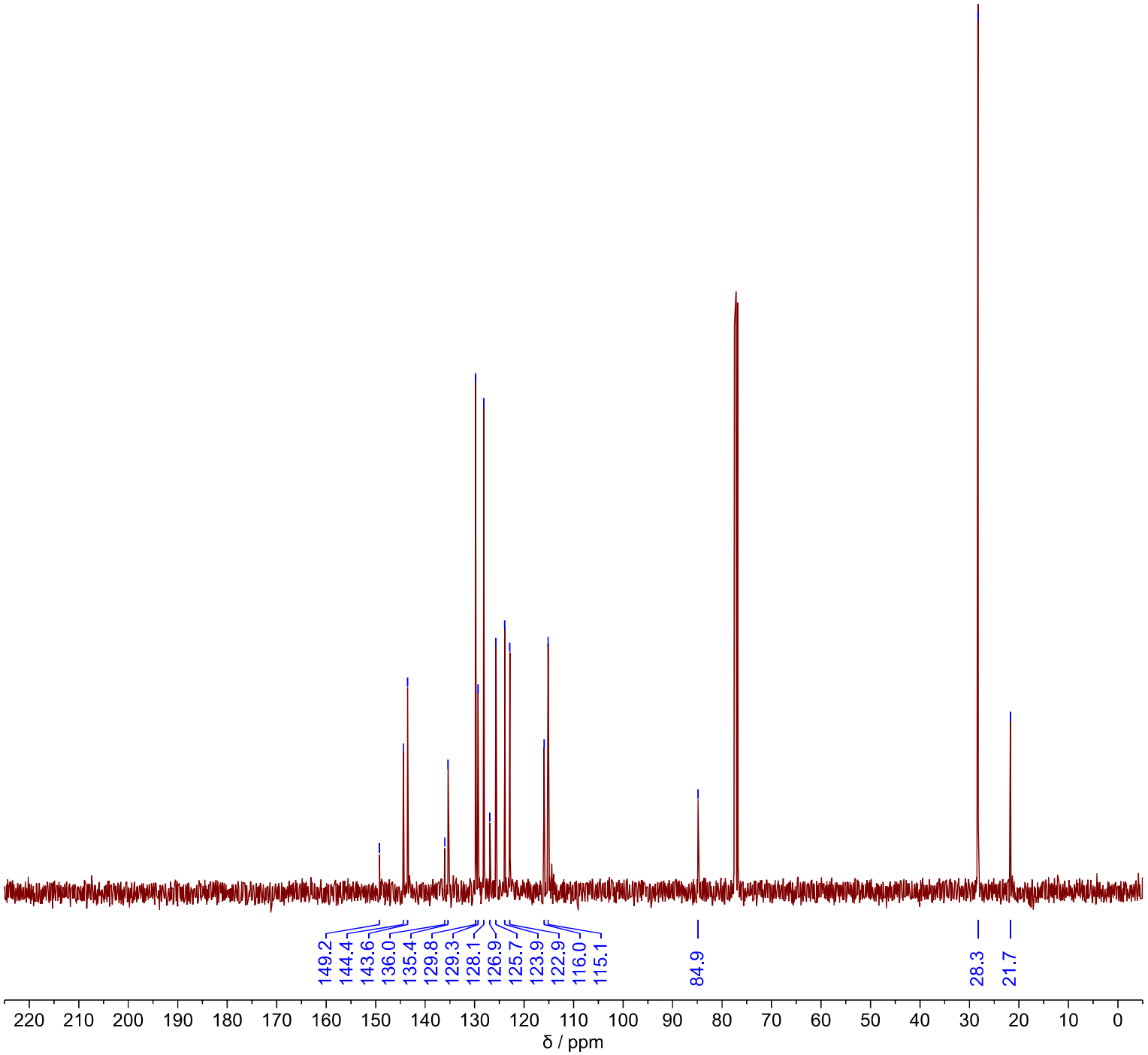


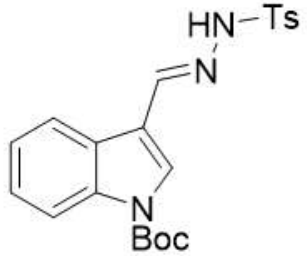
Parameter	Value
Title	RSG_NC_056_CHAR_13C.10.fid
Comment	RSG_NC_056_CHAR_13C
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	295.0
Pulse Sequence	udeft
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	380
Receiver Gain	2050.0
Relaxation Delay	3.0000
Pulse Width	8.8000
Presaturation Frequency	
Acquisition Time	0.3599
Acquisition Date	2017-02-17T02:17:00
Modification Date	2017-02-17T13:27:56
Class	
Spectrometer Frequency	100.62
Spectral Width	25252.5
Lowest Frequency	-1126.3
Nucleus	13C
Acquired Size	9089
Spectral Size	32768



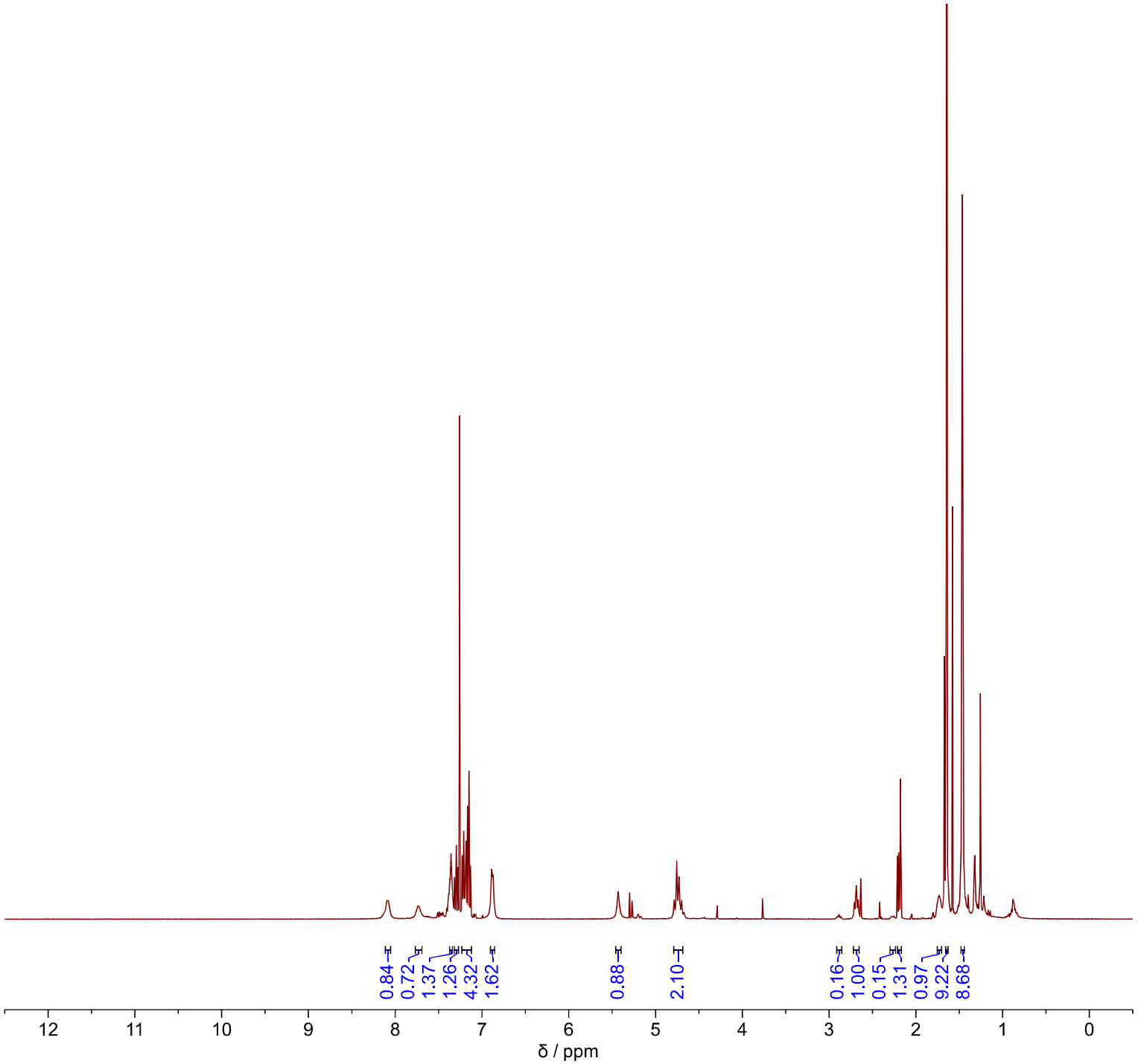


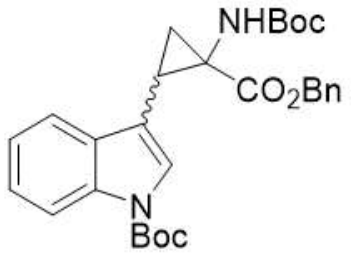
Parameter	Value
Title	RSG_NC_089.11.fid
Comment	RSG_NC_089_CHAR_1H
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	295.3
Pulse Sequence	zg30
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	32
Receiver Gain	362.0
Relaxation Delay	1.5000
Pulse Width	11.0600
Presaturation Frequency	
Acquisition Time	1.9923
Acquisition Date	2017-04-02T18:42:00
Modification Date	2017-05-16T17:21:40
Class	
Spectrometer Frequency	400.13
Spectral Width	8223.7
Lowest Frequency	-1720.7
Nucleus	1H
Acquired Size	16384
Spectral Size	65536



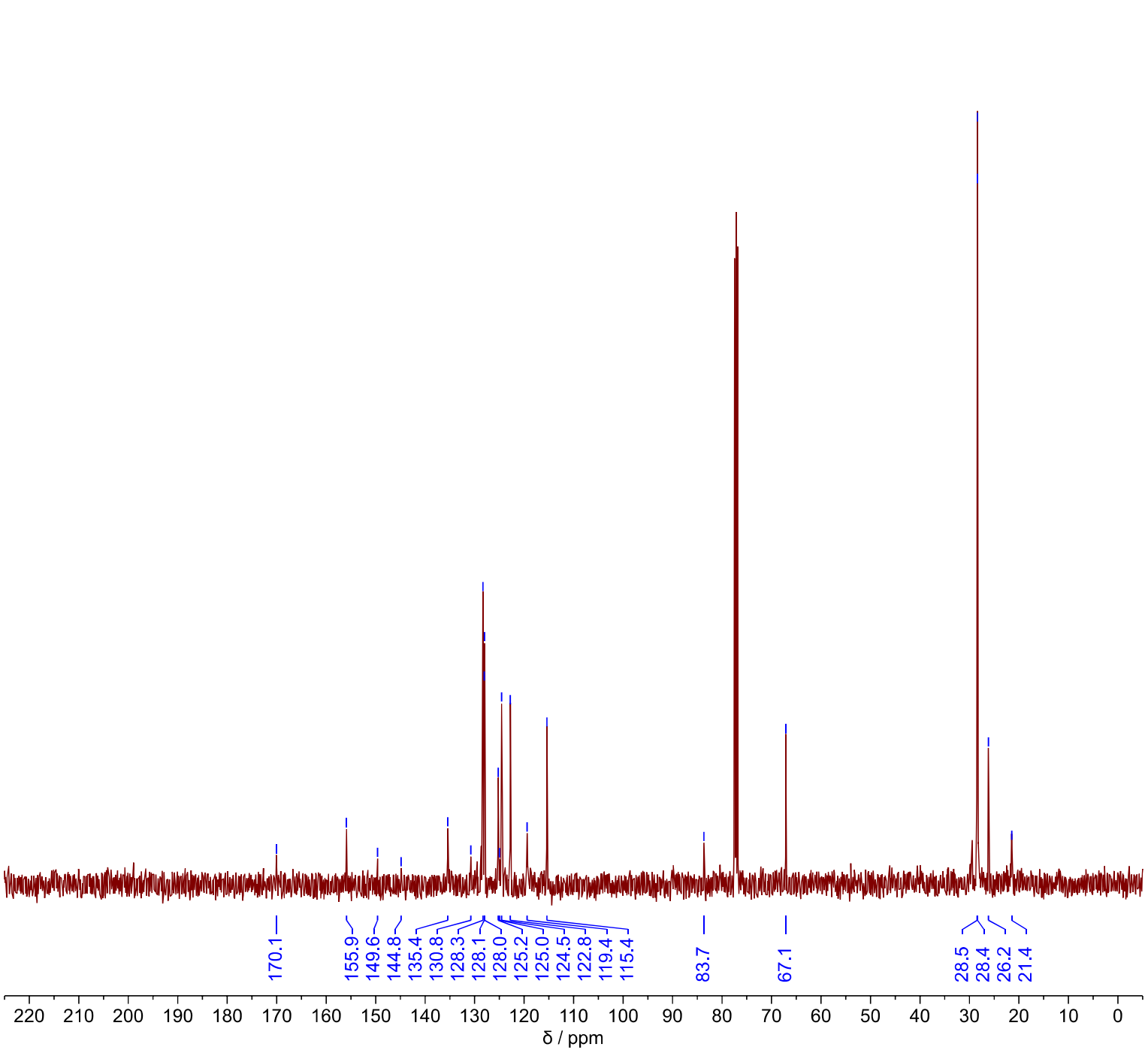


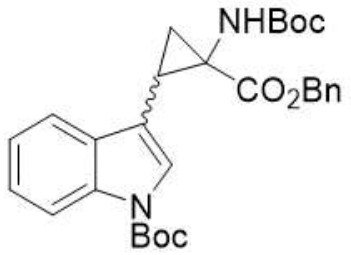
Parameter	Value
Title	RSG_NC_089.51.fid
Comment	RSG_NC_089_CHAR_13C
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	295.5
Pulse Sequence	udeft
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	380
Receiver Gain	2050.0
Relaxation Delay	3.0000
Pulse Width	8.8000
Presaturation Frequency	
Acquisition Time	0.3599
Acquisition Date	2018-02-28T11:52:00
Modification Date	2018-02-28T12:57:52
Class	
Spectrometer Frequency	100.62
Spectral Width	25252.5
Lowest Frequency	-1111.5
Nucleus	13C
Acquired Size	9089
Spectral Size	32768



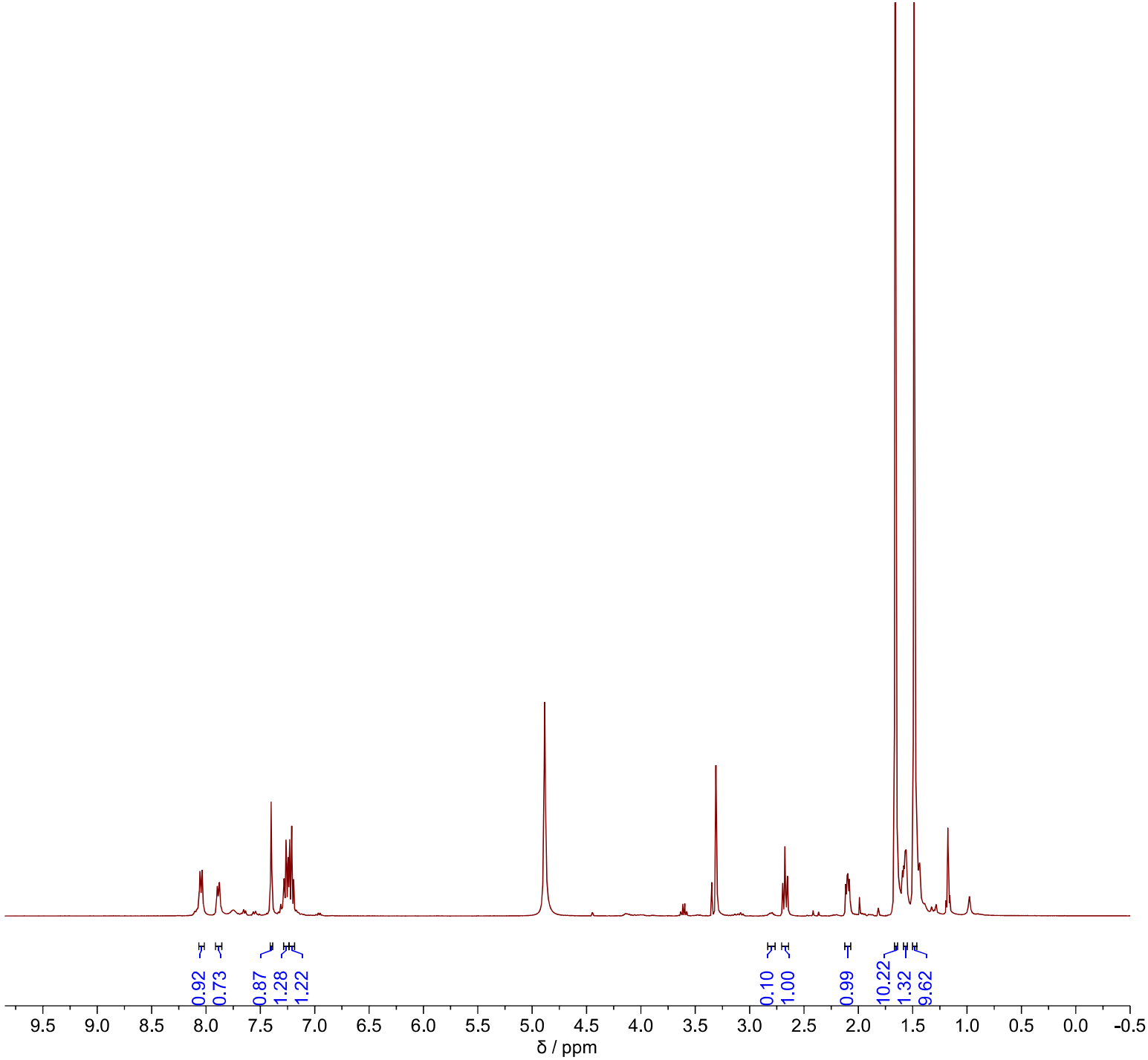


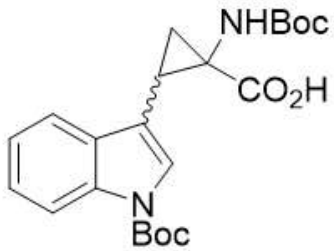
Parameter	Value
Title	RSG_NC_203.10.fid
Comment	RSG_NC_203_CHAR_1H
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	295.6
Pulse Sequence	zg30
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	32
Receiver Gain	362.0
Relaxation Delay	1.5000
Pulse Width	11.0600
Presaturation Frequency	
Acquisition Time	1.9923
Acquisition Date	2017-10-26T15:48:00
Modification Date	2018-02-28T11:21:56
Class	
Spectrometer Frequency	400.13
Spectral Width	8223.7
Lowest Frequency	-1721.1
Nucleus	1H
Acquired Size	16384
Spectral Size	65536



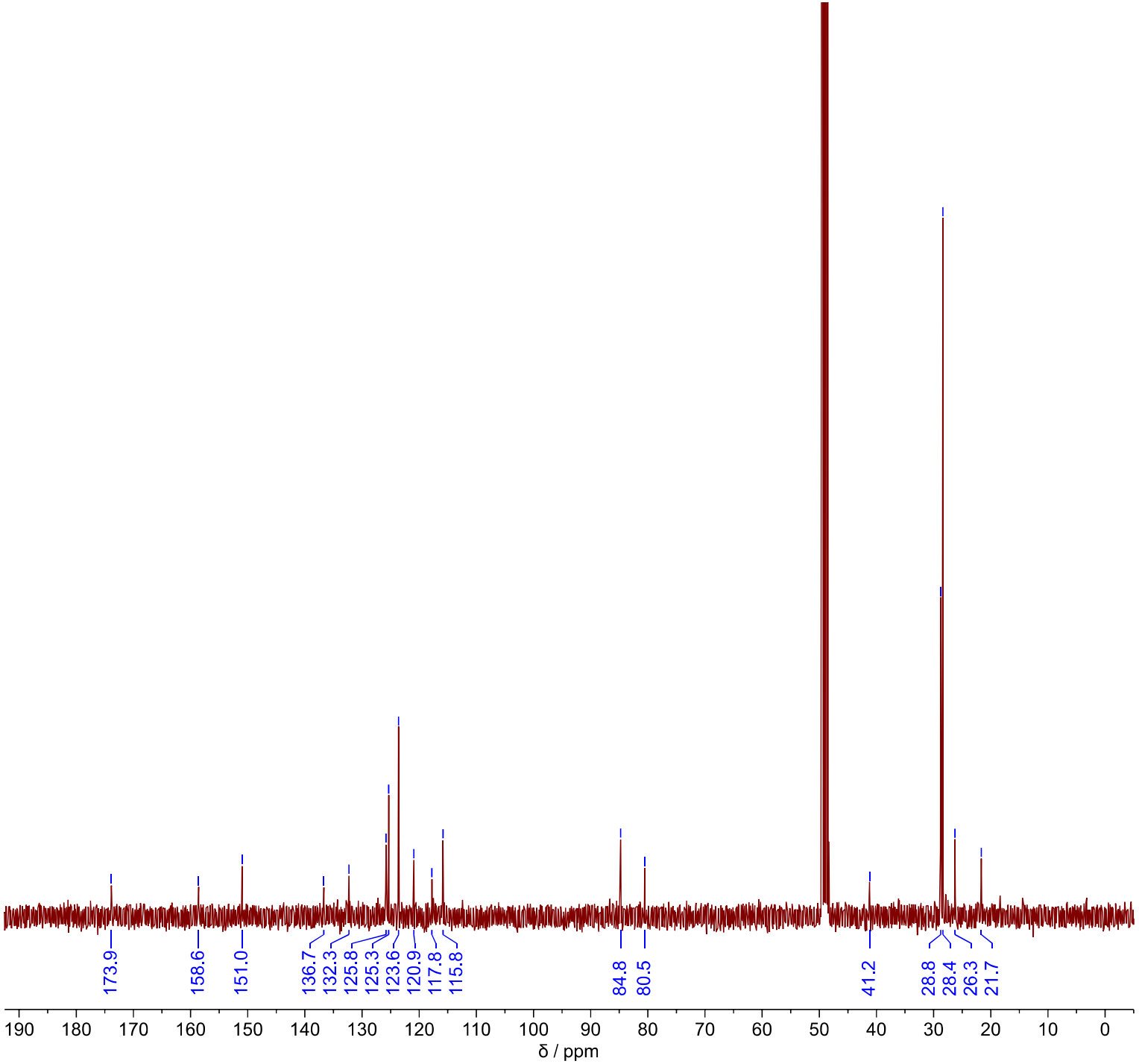


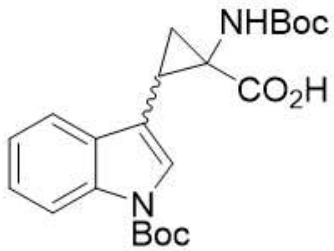
Parameter	Value
Title	RSG_NC_203.12.fid
Comment	RSG_NC_203_CHAR_13C
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	295.6
Pulse Sequence	udeft
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	380
Receiver Gain	2050.0
Relaxation Delay	3.0000
Pulse Width	8.8000
Presaturation Frequency	
Acquisition Time	0.3599
Acquisition Date	2017-10-26T16:24:00
Modification Date	2018-02-28T11:22:00
Class	
Spectrometer Frequency	100.62
Spectral Width	25252.5
Lowest Frequency	-1111.4
Nucleus	13C
Acquired Size	9089
Spectral Size	32768



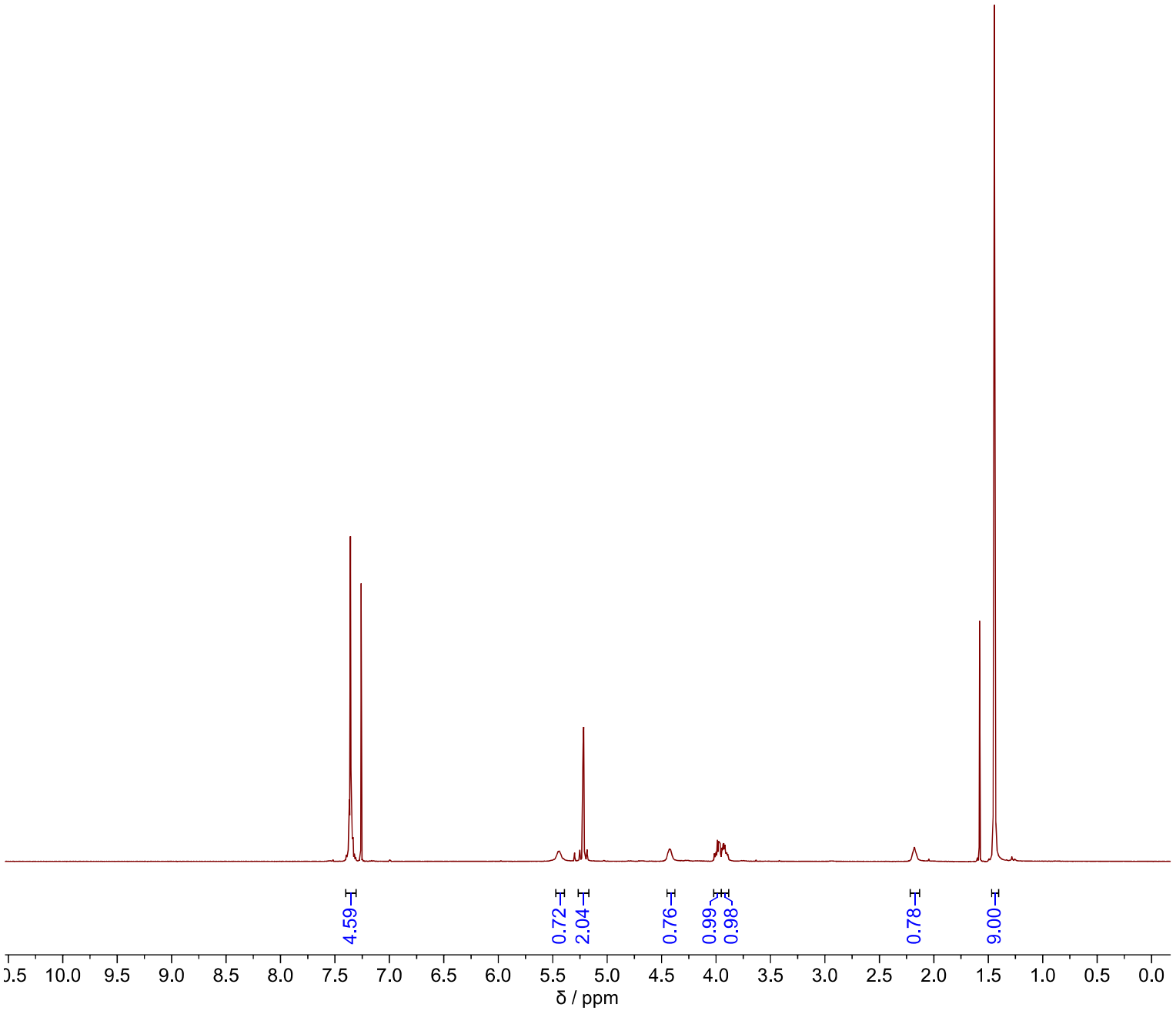


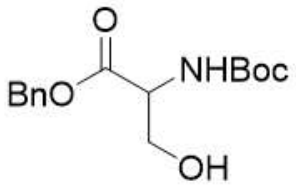
Parameter	Value
Title	RSG_NC_290.1.1.1r
Comment	RSG_NC_290_CHAR_1H
Origin	Bruker BioSpin GmbH
Owner	nmrsu
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	296.1
Pulse Sequence	zg
Experiment	1D
Probe	Z116098_0631 (PABBO 400S1 BBF-H-D-05 Z SP)
Number of Scans	32
Receiver Gain	32.0
Relaxation Delay	3.0000
Pulse Width	10.0000
Presaturation Frequency	
Acquisition Time	2.6214
Acquisition Date	2018-02-15T13:26:38
Modification Date	2018-02-19T18:38:32
Class	
Spectrometer Frequency	400.07
Spectral Width	6250.0
Lowest Frequency	-2308.9
Nucleus	1H
Acquired Size	16384
Spectral Size	65536



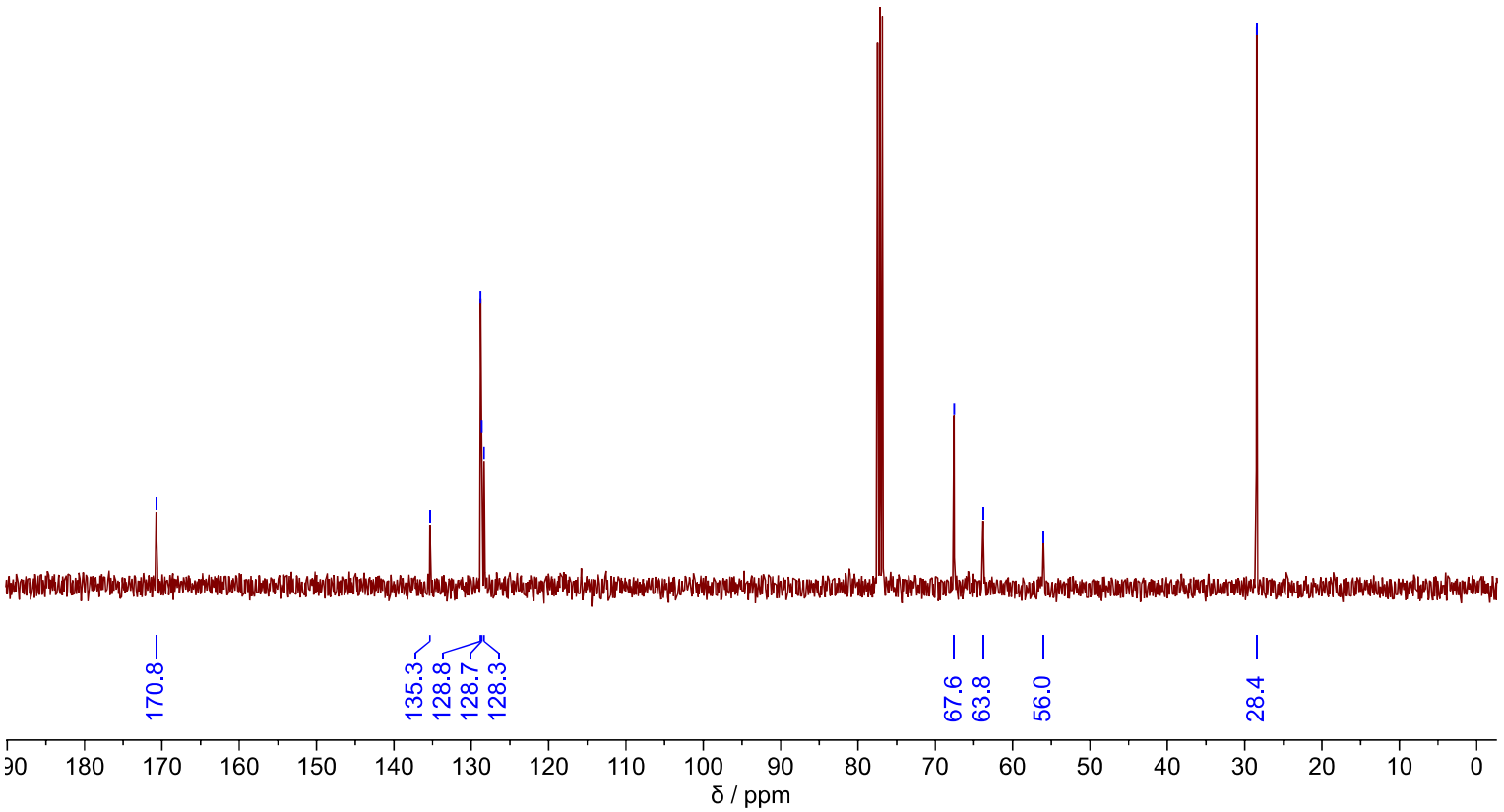


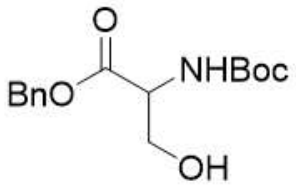
Parameter	Value
Title	RSG_NC_290.2.1.1r
Comment	RSG_NC_290_CHAR_13C
Origin	Bruker BioSpin GmbH
Owner	nmrsu
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	296.4
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z116098_0631 (PABBO 400S1 BBF-H-D-05 Z SP)
Number of Scans	480
Receiver Gain	39.1
Relaxation Delay	3.0000
Pulse Width	10.0000
Presaturation Frequency	
Acquisition Time	0.6881
Acquisition Date	2018-02-15T13:57:17
Modification Date	2018-02-19T18:38:34
Class	
Spectrometer Frequency	100.60
Spectral Width	23809.5
Lowest Frequency	-2101.3
Nucleus	13C
Acquired Size	16384
Spectral Size	65536



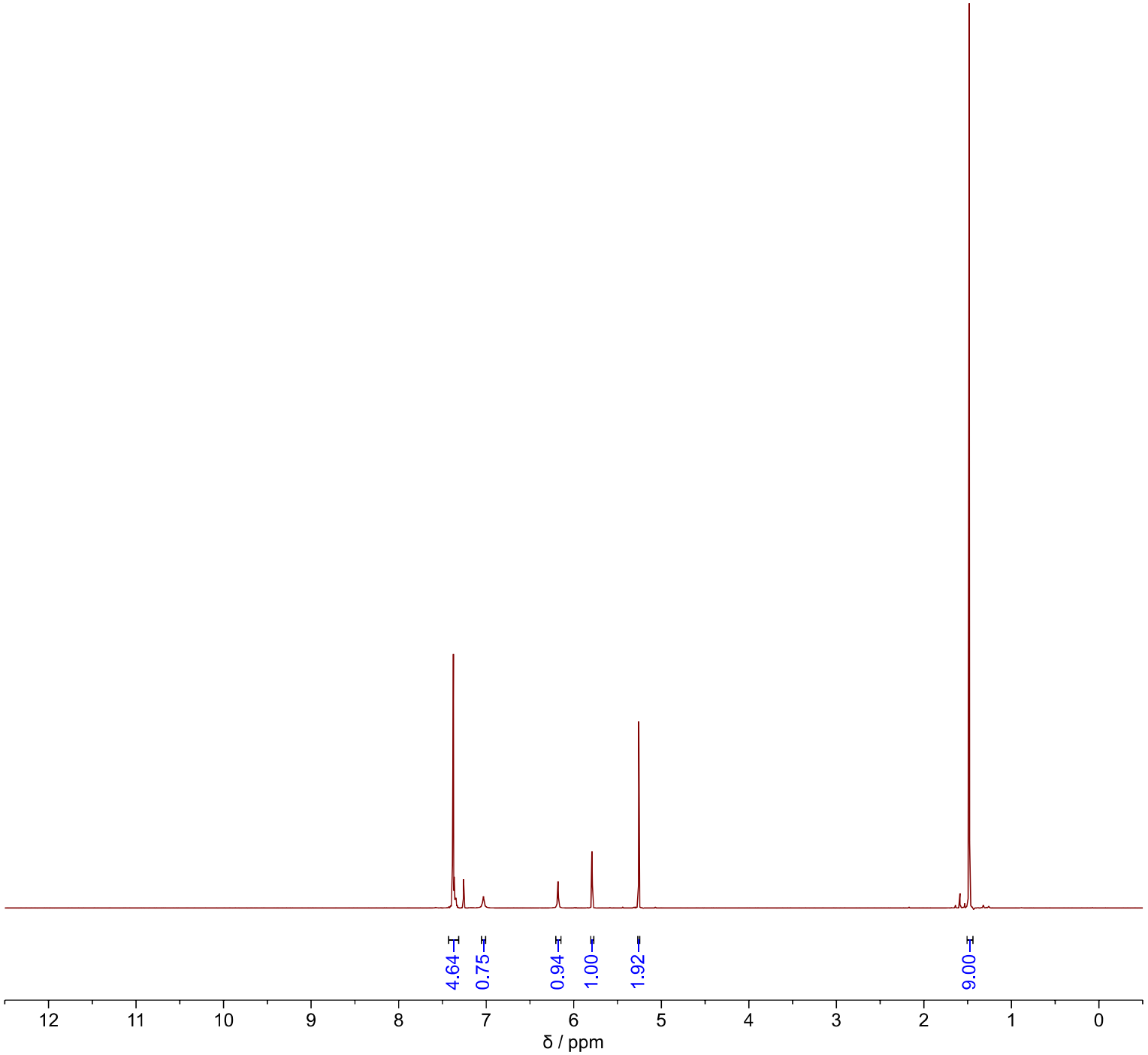


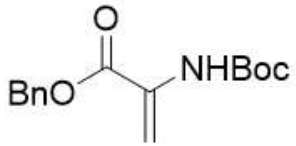
Parameter	Value
Data File Name	/ Volumes/ NICK CUNDY/ RSG_NC_190/ 10/ fid
Title	RSG_NC_190.10.fid
Comment	RSG_NC_190_CHAR_1H
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	296.0
Pulse Sequence	zg30
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	32
Receiver Gain	512.0
Relaxation Delay	1.5000
Pulse Width	11.0600
Presaturation Frequency	
Acquisition Time	1.9923
Acquisition Date	2017-10-06T07:10:00
Modification Date	2017-11-28T21:38:24
Class	
Spectrometer Frequency	400.13
Spectral Width	8223.7
Lowest Frequency	-1721.1
Nucleus	1H
Acquired Size	16384
Spectral Size	65536



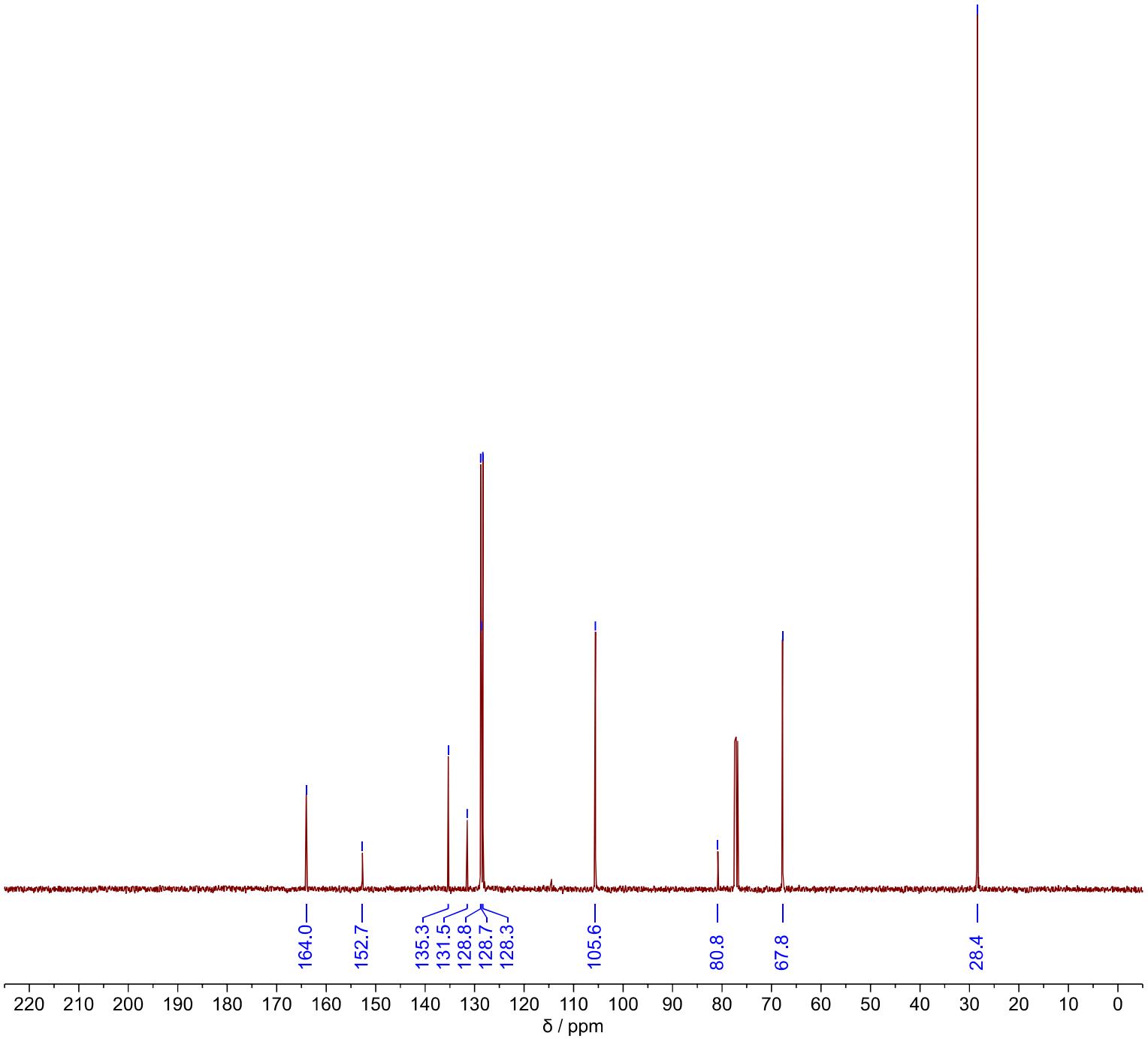


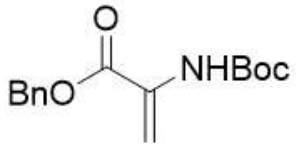
Parameter	Value
Data File Name	/ Volumes/ NICK CUNDY/ RSG_NC_190/ 12/ fid
Title	RSG_NC_190.12.fid
Comment	RSG_NC_190_CHAR_13C
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	296.4
Pulse Sequence	udeft
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	380
Receiver Gain	2050.0
Relaxation Delay	3.0000
Pulse Width	8.8000
Presaturation Frequency	
Acquisition Time	0.3599
Acquisition Date	2017-10-06T07:47:00
Modification Date	2017-11-28T21:38:28
Class	
Spectrometer Frequency	100.62
Spectral Width	25252.5
Lowest Frequency	-1110.6
Nucleus	13C
Acquired Size	9089
Spectral Size	32768



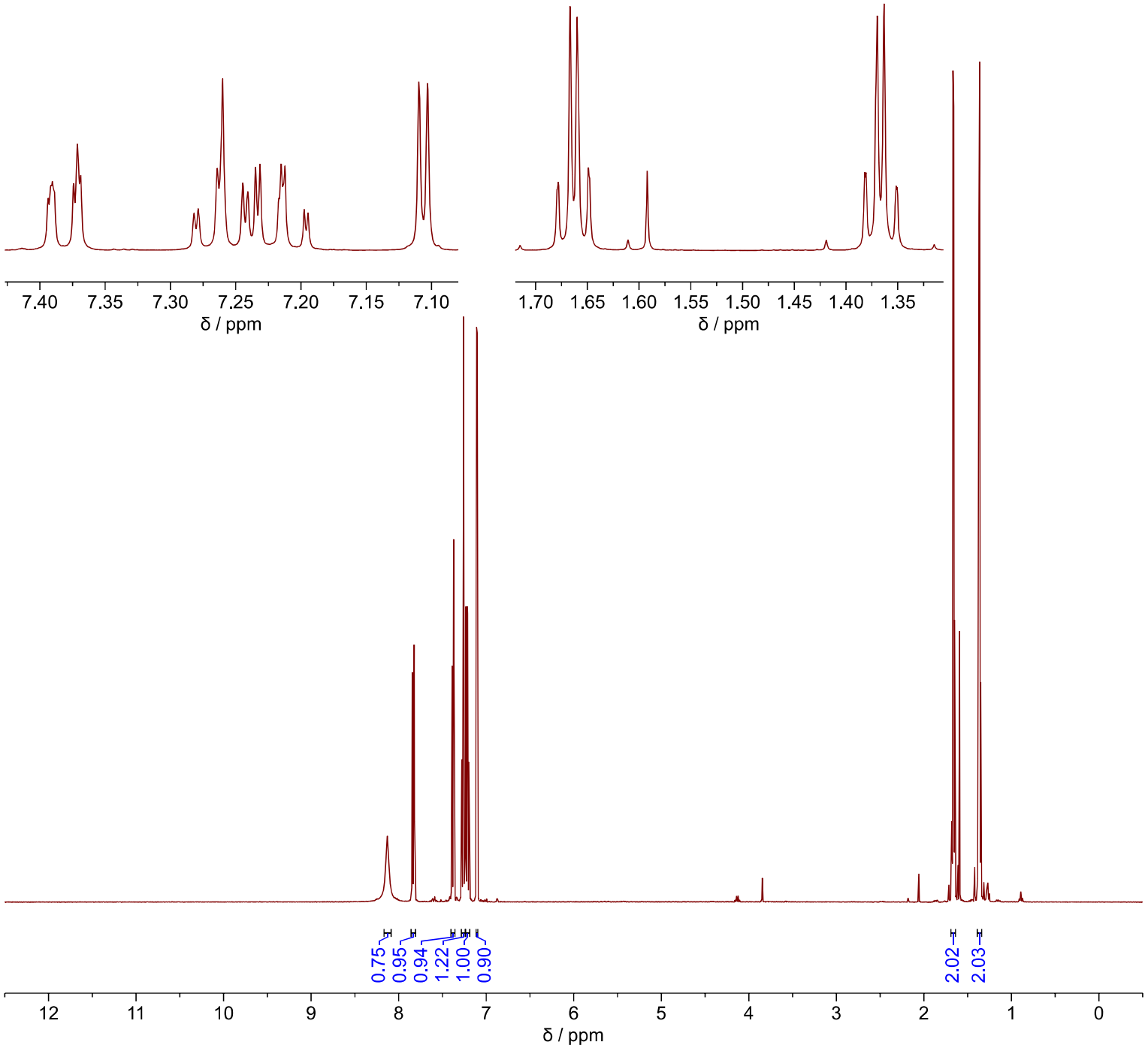


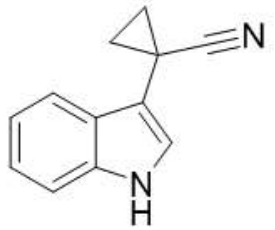
Parameter	Value
Title	RSG_NC_192.10.fid
Comment	RSG_NC_192_CHAR_1H
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	296.3
Pulse Sequence	zg30
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	32
Receiver Gain	203.0
Relaxation Delay	1.5000
Pulse Width	11.0600
Presaturation Frequency	
Acquisition Time	1.9923
Acquisition Date	2017-10-10T02:40:00
Modification Date	2018-02-27T16:23:36
Class	
Spectrometer Frequency	400.13
Spectral Width	8223.7
Lowest Frequency	-1721.1
Nucleus	1H
Acquired Size	16384
Spectral Size	65536





Parameter	Value
Title	RSG_NC_192.12.fid
Comment	RSG_NC_192_CHAR_13C
Origin	Bruker BioSpin GmbH
Owner	Facility
Site	
Spectrometer	spect
Author	
Solvent	CDCl3
Temperature	296.4
Pulse Sequence	udeft
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
Number of Scans	380
Receiver Gain	2050.0
Relaxation Delay	3.0000
Pulse Width	8.8000
Presaturation Frequency	
Acquisition Time	0.3599
Acquisition Date	2017-10-10T03:17:00
Modification Date	2018-02-27T16:23:42
Class	
Spectrometer Frequency	100.62
Spectral Width	25252.5
Lowest Frequency	-1112.0
Nucleus	13C
Acquired Size	9089
Spectral Size	32768





Parameter	Value
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Author	
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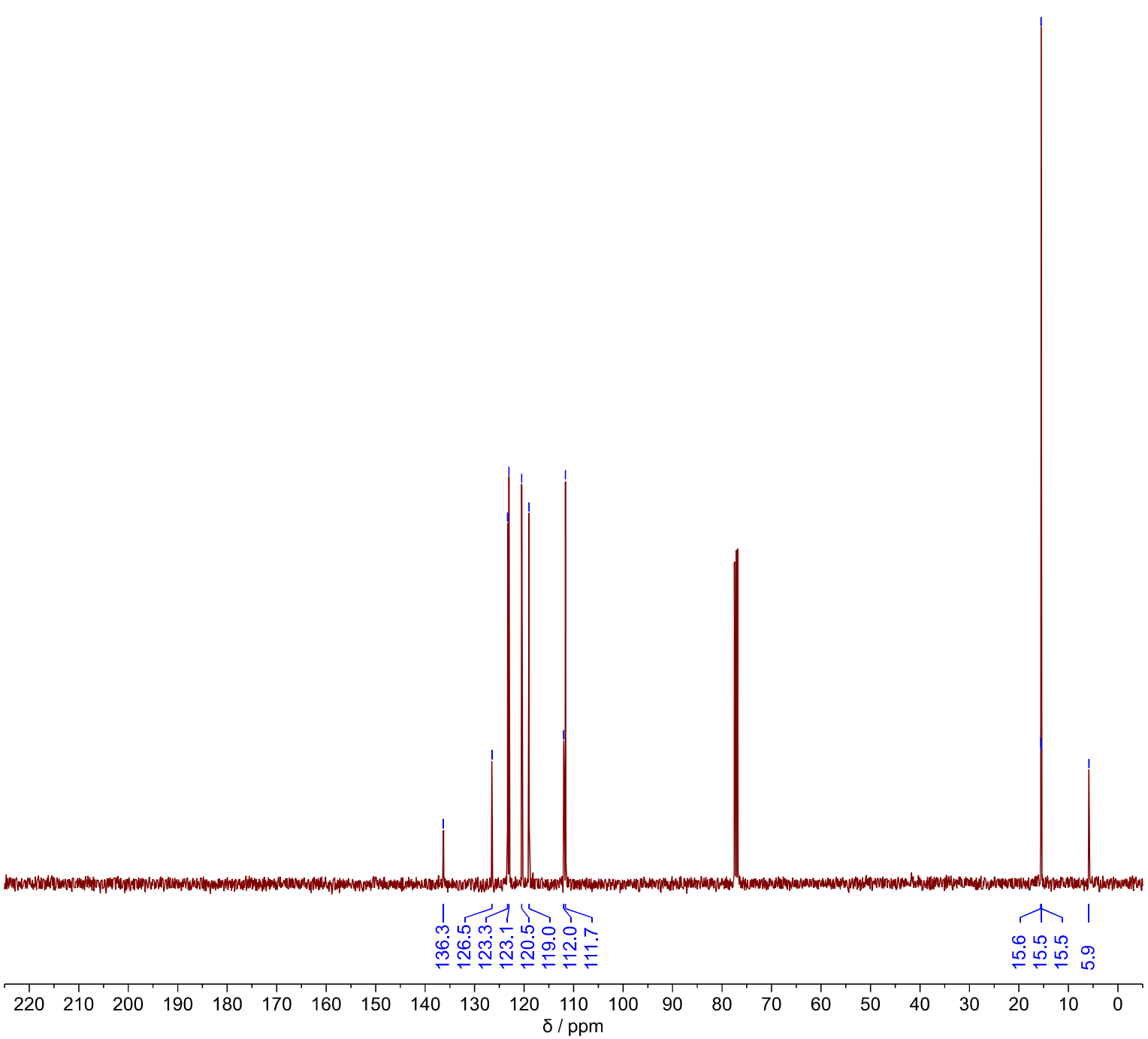

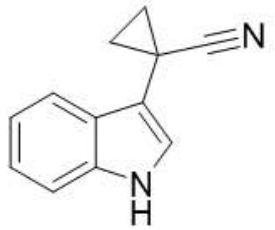

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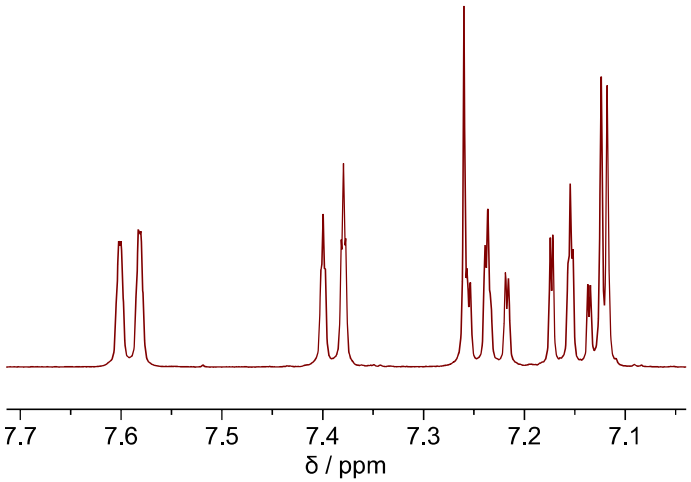

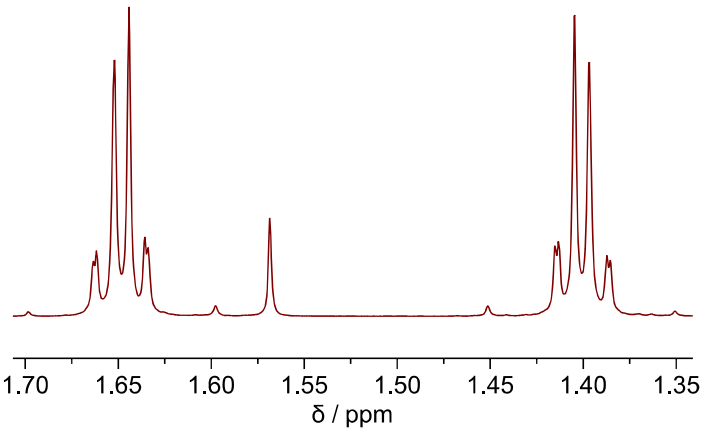

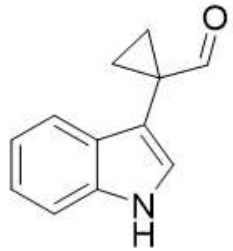

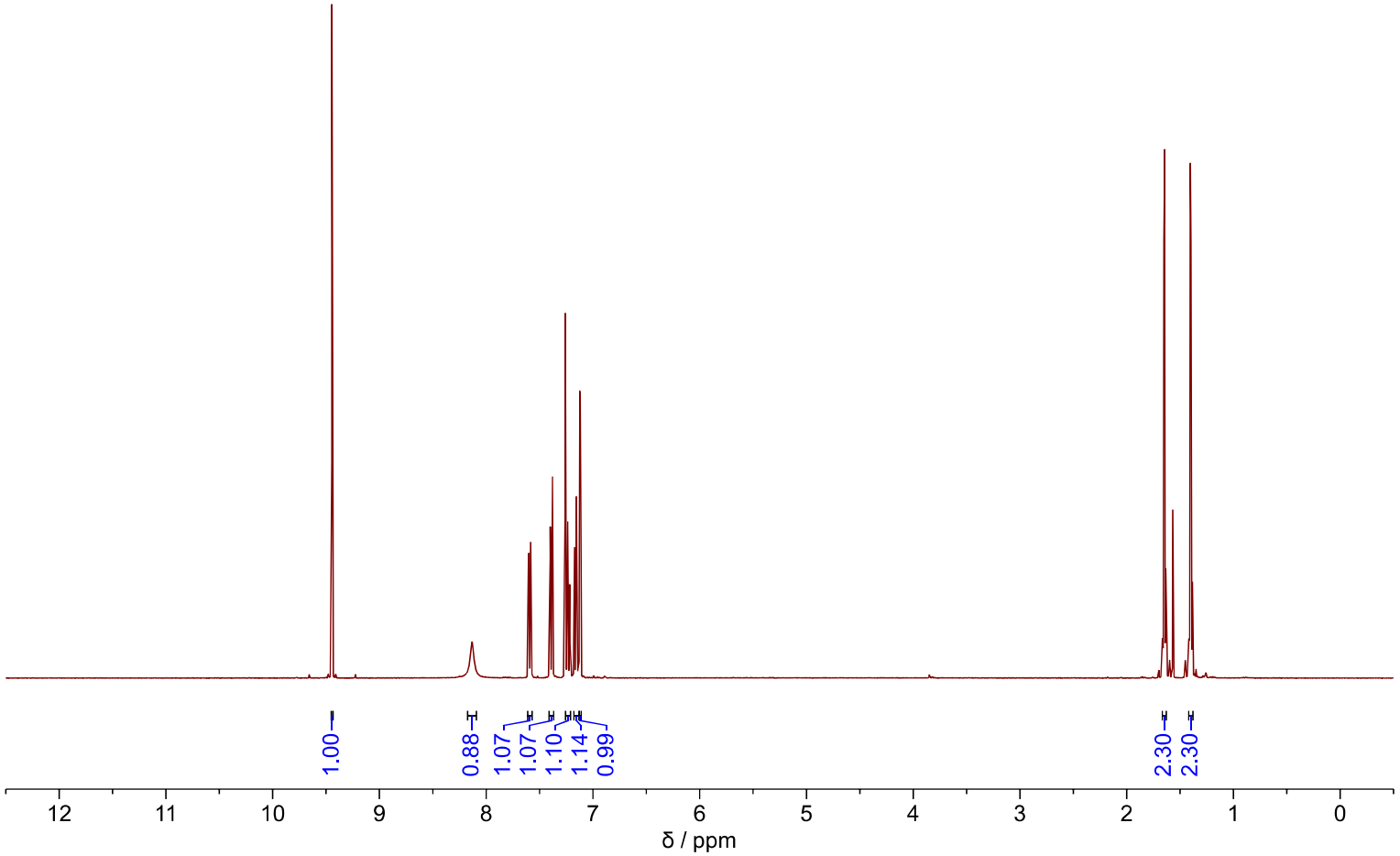

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Author	
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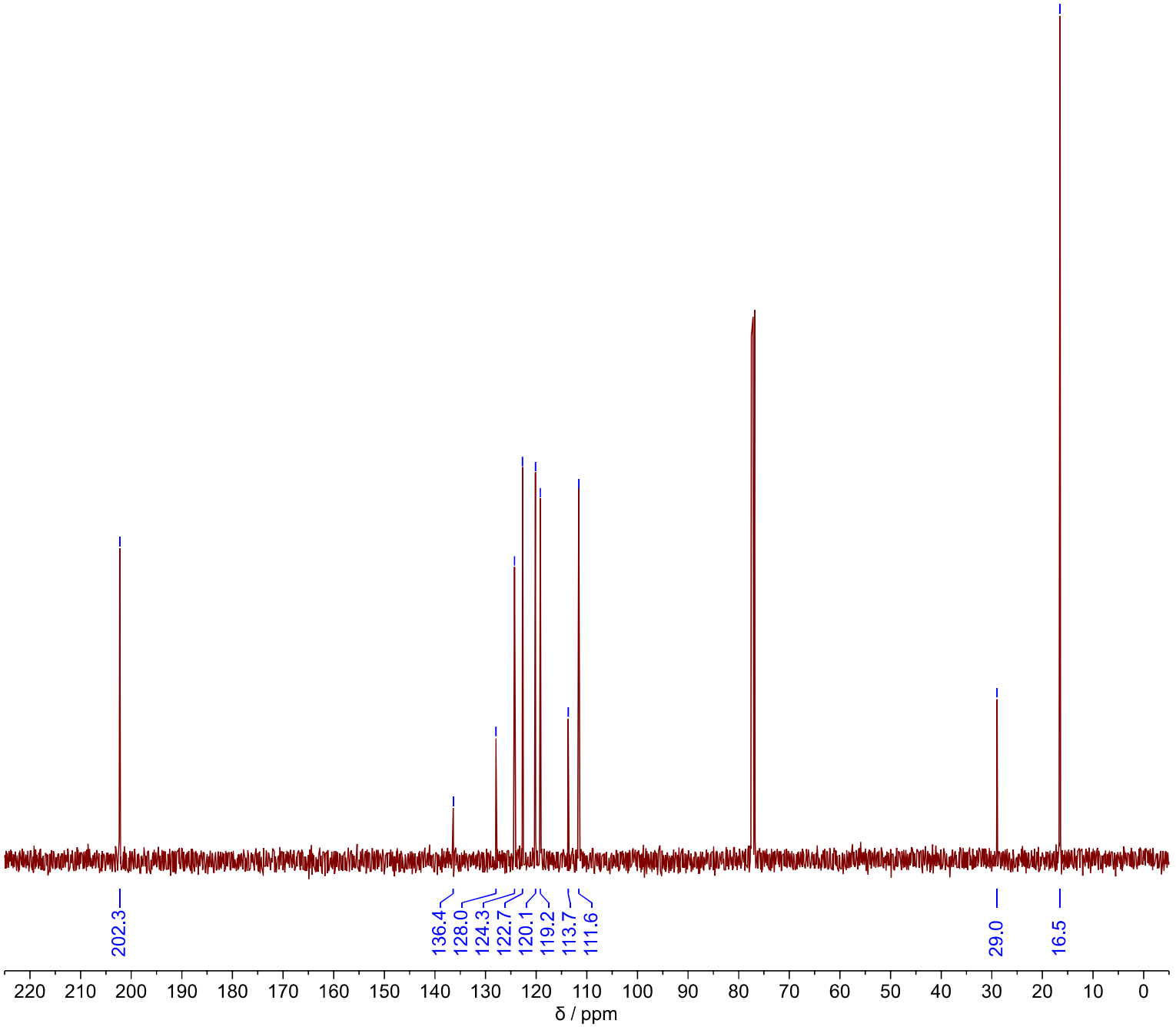

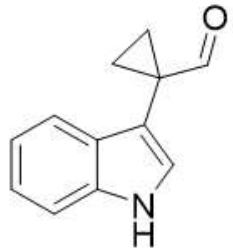

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Owner	Facility
Site	
Instrument	spect
Author	
Solvent	CDCl3
Temperature	294.6
Pulse Sequence	udeft
Experiment	1D
Probe	5 mm PADUL 13C-1H/ D Z-GRD Z108621/ 0010
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Presaturation Frequency	
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Spectrometer Frequency	100.62
Spectral Width	25252.5
Lowest Frequency	-1113.7
Nucleus	13C
Acquired Size	9089
Spectral Size	65536

2 – Biological Evaluation Methods

2.1 – General Methods

All reagents and solutions were purchased from Sigma-Aldrich or ThermoFisher at 99.5% purity or higher. Molecular biology grade water was used to make up all stock solutions, stock solutions of sodium ascorbate and catalase were stored for a maximum period of 6 weeks before a fresh solution was prepared.

The assays (with the exception of the thermal shift assay) were performed using the same component solutions, at the same concentrations but with differing volumes – volumes will be stated in the assay descriptions. Stock solution refers to a mixture of tris·HCl, pH 8 buffer (50 mM), sodium ascorbate (20 mM) and methylene blue (10 μM) – fresh stock solution was prepared for each experiment. Catalase (10 μg mL^{-1}) was prepared from a suspension of catalase solution (24 mg mL^{-1}) with an assayed activity of 11895 units mL^{-1}. Samples of recombinant IDO1 F164A were kindly provided by Prof. Emma L. Raven (University of Bristol, School of Chemistry) for use in the biochemical assays (5 μM). Trichloroacetic acid (TCA, 30% in water) was used to quench the assays prior to LC-MS analysis.

'UV-Vis' refers to UV-visible spectra recorded using a Varian Cary50 UV-Visible spectrophotometer measuring continuous (10 readings s^{-1}) absorbance at 321 nm.[12] LC-MS analysis was performed using either a Waters e2695 separation module coupled with a Waters SQD MS-detector (raw data was processed and extracted using MassLynx V4.1 SCN855), or a ThermoFisher LCQ-fleet LC-MS (raw data was processed and extracted using Xcaliber). All graphical data was plotted and analysed using Prism 5 or 6. All reported plots were generated from the data analysis performed in Prism.

2.2 – Experimental Detail

Steady State Assay

Steady state assay experiments were carried out at The University of Birmingham, School of Chemistry, by Nicholas Cundy. Total assay volume = 1 mL, performed in a sealed quartz cuvette of 1.2 mL maximum volume. Oxygenated stock solution (O_2 gas passed through the stock solution for 10 min) was transferred to the UV cuvette. Catalase and IDO1 was added to the oxygenated stock solution and the cuvette was sealed under an atmosphere O_2. After 5 min, Trp/1-MT/inhibitor (1 mM) was added, the cuvette was re-sealed under an atmosphere of O_2, the cuvette was transferred to the UV-vis spectrometer and absorbance readings were initiated to detect *N*-formyl-*L*-kynurenine (Abs = 321 nm). After 2 h, the absorbance readings were ceased, and the assay mixture was transferred to an Eppendorf vial containing TCA (100 μL). The assay mixture was subjected to centrifugation (13,000 rpm, 5 min), transferred to a HPLC vial and submitted for LC-MS analysis.

Inhibition Assay

Inhibition assay experiments were carried out at the University of Birmingham, School of Chemistry, by Nicholas Cundy. Total assay volume = 1 mL, performed in a sealed quartz cuvette of 1.2 mL maximum volume. Oxygenated stock solution (O_2 gas passed through the stock solution for 10 min) was transferred to the UV cuvette. Catalase and IDO1 was added to the oxygenated stock solution and the cuvette was sealed under an atmosphere O_2. After 5 min, inhibitor (Varied concentrations) was added, the cuvette was re-sealed under an atmosphere of O_2, the cuvette was transferred to the UV-vis spectrometer and absorbance readings were initiated (Abs = 321 nm). After 15 min, the UV recording was paused, Trp (1 mM) was added and then UV recording was resumed. After a further 1.75 h, the absorbance readings were ceased, and the assay mixture was transferred to an Eppendorf vial containing TCA (100 μL). The assay mixture was subjected to centrifugation (13,000 rpm, 5 min), transferred to a HPLC vial and submitted for LC-MS analysis.

Differential Scanning Fluorimetry/Thermal Shift Assay

Thermal melting experiments were carried out at the University of Manchester by Roseanna Hare using a Stratagene Mx3005p Real Time PCR machine (Agilent Technologies). All experiments were conducted at 200 uL volume in 96-well PCR plates, using 20 mM HEPES, pH 7.5, 140 mM NaCl, containing either 2% or 5% DMSO. IDO1 protein was used at a final concentration of 1 or 10 μM as required. Compounds were diluted into buffer from a 10 mM DMSO stock solution and added at a final concentration of 10 μM, 100 μM or 500 μM. Where required SYPRO Orange (Molecular Probes) was added as a fluorescence probe at a final dilution of 1:500, 1:1000 or 1:2000 (v/v). Excitation and emission filters for the SYPRO-Orange

dye were set to 465 nm and 590 nm, respectively. The temperature was raised with a step of 1 °C per minute from 25 °C to 96 °C, and fluorescence readings were taken at each interval. Experiments were performed in triplicate and the observed temperature shifts, ΔT_m^{obs}, were recorded as the difference between the transition midpoints of sample and reference wells containing protein without ligand in the same conditions and determined by non-linear least squares fit of the initial fluorescence event, reported in °C.

Microscale Thermophoresis Assay

Recombinant IDO was labelled with fluorescent dye using NHS-esterification of surface lysine residues. NT-650 dye (Nanotemper) which is a strong red-fluorescent dye with an excitation wavelength of 650 nm was used at a ratio of 2:1 dye to protein. IDO was diluted to 20 µM in phosphate buffered saline pH 7.4 (PBS) and mixed 1:1 with 40 µM NT-650 in PBS/DMSO. The sample was left for 30 minutes at room temperature before being buffer exchanged to in 20 mM Tris, 150 mM NaCl, 0.005% Tween-20, 1mM EDTA, 2 mM MgCL2, 4% DMSO (running buffer) using a 5 ml Hi-Trap desalting column (Cytiva). Labelled IDO was then centrifuged at 14,000 rpm for 10 minutes and checked for concentration using UV absorbance at 280 nm with an absorbance of 1.1 (1 cm pathlength) equivalent to 1 mg/ml. Final labelled IDO concentration was 6 µM. A binding check to determine if there is a change in thermophoresis caused by binding to compounds L-tryptophan (TRP), 1-MT and compound 17 was performed using 10 nM 650-IDO in running buffer, with and without 500 µM of each compound. The instrument (Nanotemper Pico) was set-up in expert mode with a medium MST power and LED-laser set to 10 %. MST-induces changes in fluorescence were plotted using the change in fluorescence at 2.5 s response time. A concentration dependant change in MST was conducted for compound-17 using the same conditions and a 16-sample serial dilution from 500 µM.

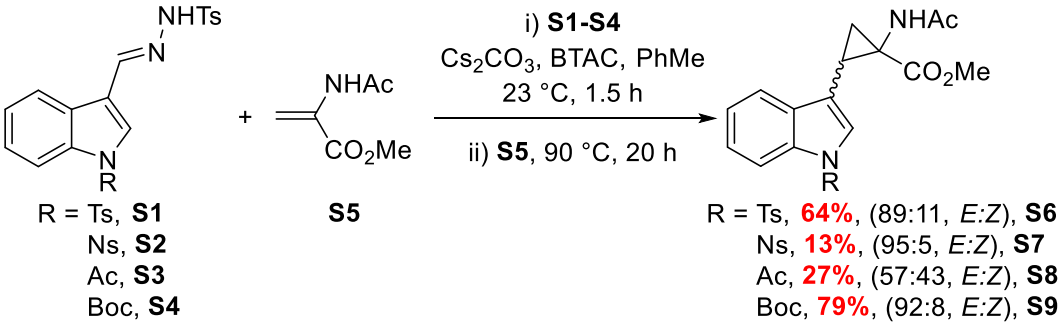
UV-vis analysis

UV-vis spectra were recorded on an Agilent Cary 60 UV-Vis Spectrophotometer at a final concentration of 10 µM IDO and/or 10 µM compounds/hemin in phosphate buffered saline pH 7.4 (PBS) containing 0.1% DMSO. All samples were incubated for 1-1.5 hours at ambient temperature to allow equilibration before measuring.

3 – Appendix

3.1 – Indole protecting group screen

Tosyl was primarily utilised as the protecting group for the 1,3-dipolar cycloaddition chemistry (Scheme SI1); tosyl hydrazone **S1** gave a moderate yield of tryptophan analogue **S5** with preference for the *E*-diasteroemer. Difficulty was faced when attempting to remove the tosyl group (Table SI1), as such alternative protecting groups were trialled. Nosyl- and acetyl hydrazones **S2** and **S3** gave poor conversion to the respective tryptophan analogues and thus were not viable protecting groups. Boc hydrazone **S4** proved superior in the cycloaddition chemistry giving a good yield with increased selectivity for the *E*-diastereomer.



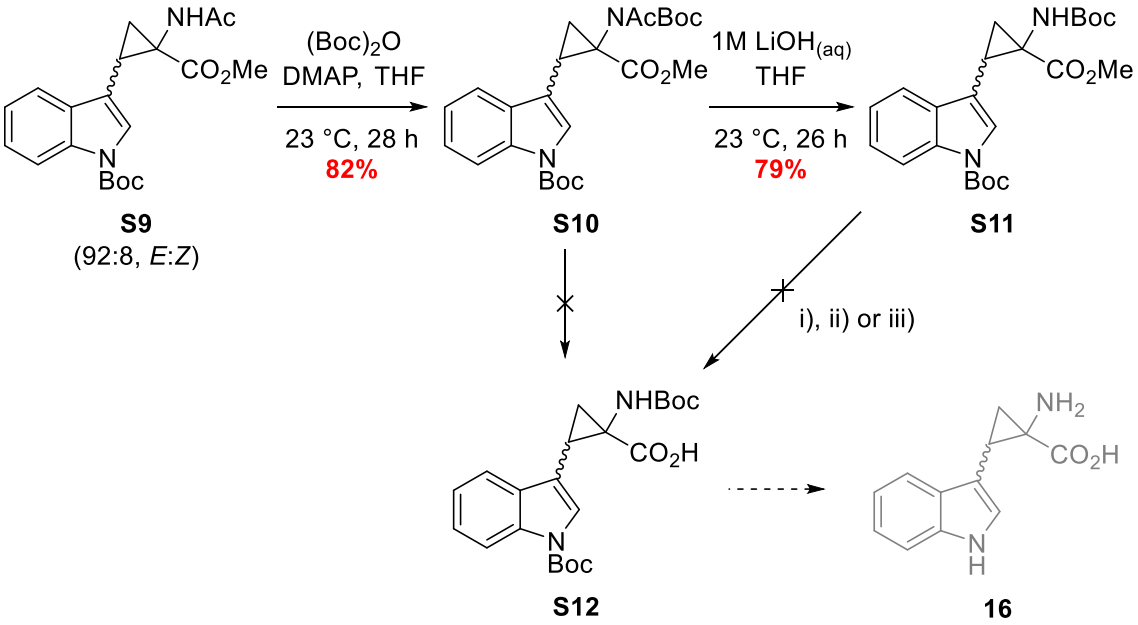
Scheme SI1 - Indole protecting group screen

Table SI1 – Attempted Tosyl Deprotection conditions

Entry	Conditions	Result
1	Mg(s), MeOH,))), 23 °C	**S6**
2	Mg(s), THF:MeOH (1:1),))), 23 °C	**S6**
3	Mg(s), NH_4Cl, MeOH,))) 23 °C	**S6**
4	Li(s), naphthalene, THF, −78 °C	**S6**
5	Na(s), MeOH, 23 °C	**S6**
6	Thioglycolic acid, LiOH, DMF, 23 °C	**S6**
7	Thioglycolic acid, LiOH, DMF, 70 °C	**S6**
8	KOH, MeOH, 80 °C	**S6**

With Boc selected as the optimised protecting group, a literature deprotection strategy was employed to ultimately access desired tryptophan analogue **16**.[13] Surprisingly, further difficulty was faced when attempts to deprotect the acetyl amide and methyl ester were made (Scheme

SI2). Boc-protection of acetamide **S9** was required to provide an activated imide-like functionality for the subsequent hydrolysis of the acetyl-group. Later attempts to saponify methyl ester **S11**, under a variety of conditions, ultimately lead to recovery of **S11** or degradation.



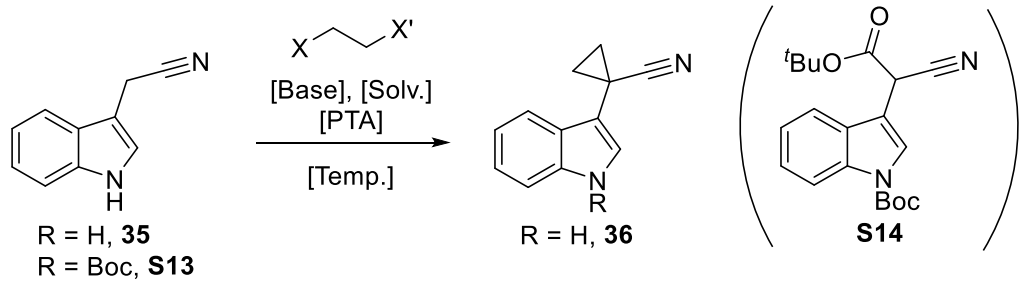
Scheme SI2 - Attempted deprotection sequence

i) 1M LiOH, H_2O:THF (1:2), reflux, 24 h, decomposed; ii) 1M LiOH, H_2O_2, H_2O:THF (1:2), 23–70 °C, 18 h, decomposed; iii) LiI, EtOAc, reflux, 20 h, ***S10*** *recovered.*

Amino acrylate **34** was synthesised to install more readily cleavable protecting groups to the protected tryptophan analogue. This strategy proved successful and allowed highly efficient access to tryptophan analogue **16** (See full text for discussion).

*3.2 – Conditions screen for the dialkylation of **35***

Due to sparing experimental detail within published report of cyclopropane **36**, an optimisation screen was required (Scheme SI3).[2] Initial trials utilising 1,2-dichloroethene and LDA lead to the recovery of Claisen-adduct **S13** only (Entry 1, Table SI2). Subsequent trials with aqueous base systems, with 1,2-dibromoethane or 1-bromo-2-chloroethane, failed to give any conversion and starting material was isolated in all instances (Entries 2–4, Table SI2). *N*-Boc nitrile **S12**, 1-bromo-2-chloroethane and LDA (4 eq.) lead to recovery of Claisen-adduct **S13** only – one equivalent of base was added to a solution of **S12** and 1-bromo-2-chloroethane with the remaining three equivalents added 30 mins later (Entry 5, Table SI2). Forgoing the Boc-group, addition of all equivalents of LDA followed by rapid addition of 1-bromo-2-chloroethane lead to the successful cyclodialkylation of nitrile **35** to give cyclopropyl **36** (Entry 6, Table SI2)



*Scheme SI3 - Optimisation of 1,1'-spirocyclic cyclopropane **36** synthesis*

*Table SI2 - Optimisation conditions for the synthesis of 1,1'-spirocyclic cyclopropane **36***

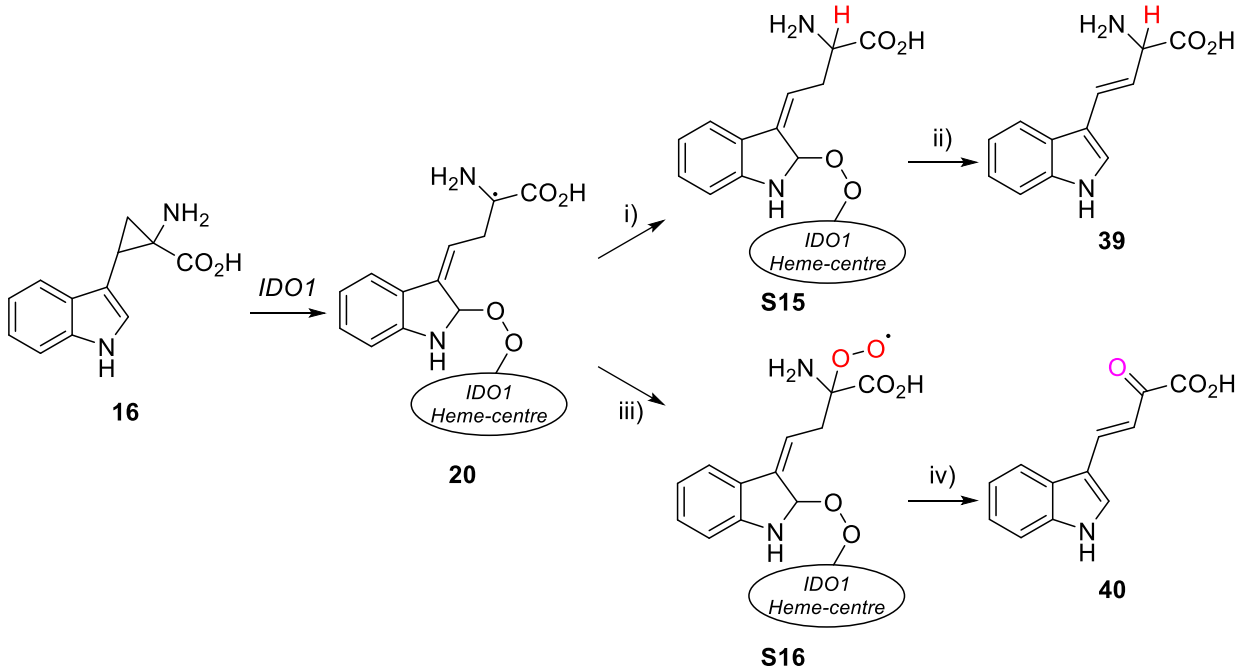
Entry	R	X	X'	Base	Solv.	PTC	Temp./°C	Result
1	Boc	Cl	Cl	LDA[a]	THF	N/A	−78–23	**S14**, **27%**
2	Boc	Br	Br	NaOH[b]	PhMe	TBAB	23	**S13**
3	Boc	Br	Cl	NaOH[b]	PhMe	BTAC	70	**S13**
4	Boc	Br	Cl	NaOH[b]	Neat.	BTAC	50	**S13**
5	Boc	Br	Cl	LDA	THF	N/A	−30–23	**S14**, **21%**
6	H	Br	Cl	LDA	THF	N/A	−40 to −78–23	**36**, **83%**

[a] *LDA solutions were prepared from freshly titrated nBuLi and distilled ($^{i}Pr)_2NH$.*

[b] *50% aqueous solutions.*

*3.3 – Metabolic by-products of **16** and **17***

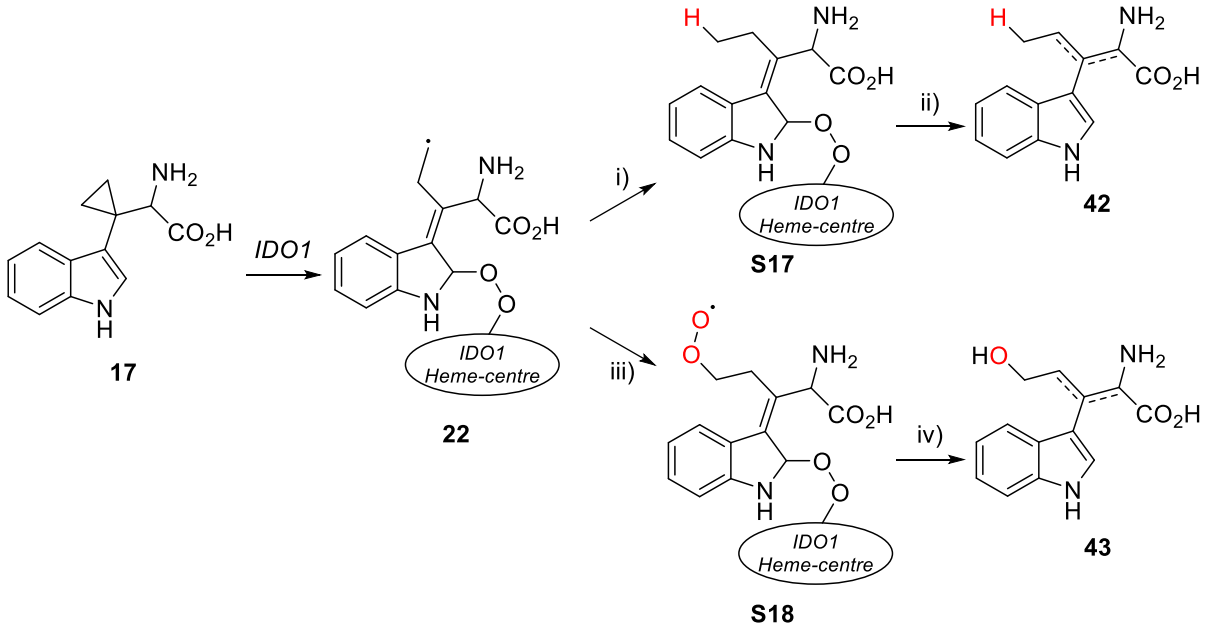
As part of this work, we hypothesised various metabolic by-products of 1,2-cyclopropyl tryptophan analogue **16** in order to survey the assay mixture post-incubation of IDO1 and **16**. Cyclopropyl *N*-formyl kynurenine analogue **38** (See full text) is a hypothesised product of an electrophilic-base mechanism of IDO1-mediate dioxygenation. Amino acid **39** is predicted to arise from radical ring-opening of **16** giving captodative radical **20** which could undergo a proton-abstraction (i, Scheme SI4) from an active site residue or the assay media give amino acid **S15**. Ultimately, metabolic product **39** is liberated from the heme-centre as a result of re-aromatisation on the indole ring system (ii, Scheme SI4). Alternatively, amino acid **20** could undergo a radical addition to molecular oxygen to give hemi-peroxy aminal **S16** (iii, Scheme SI4). This presumably unstable intermediate is predicted to collapse to give an imine and undergo rapid hydrolysis to give an α-keto acid. Upon liberation from the heme-centre and re-aromatisation of the indole ring, metabolite **40** could be present in the post-assay media (iv, Scheme SI4). If metabolite **S16** is liberated from the heme-centre prior to the degradation of the hemi-peroxy aminal it is also possible to envisage a mechanism by which the alkyl peroxy species is reduced to the alcohol by catalase present in the assay media. In this scenario, the resulting hemi aminal would likely degrade rapidly and giving α-keto acid **40**.



*Scheme SI4 - Plausible metabolic outcomes for **16***

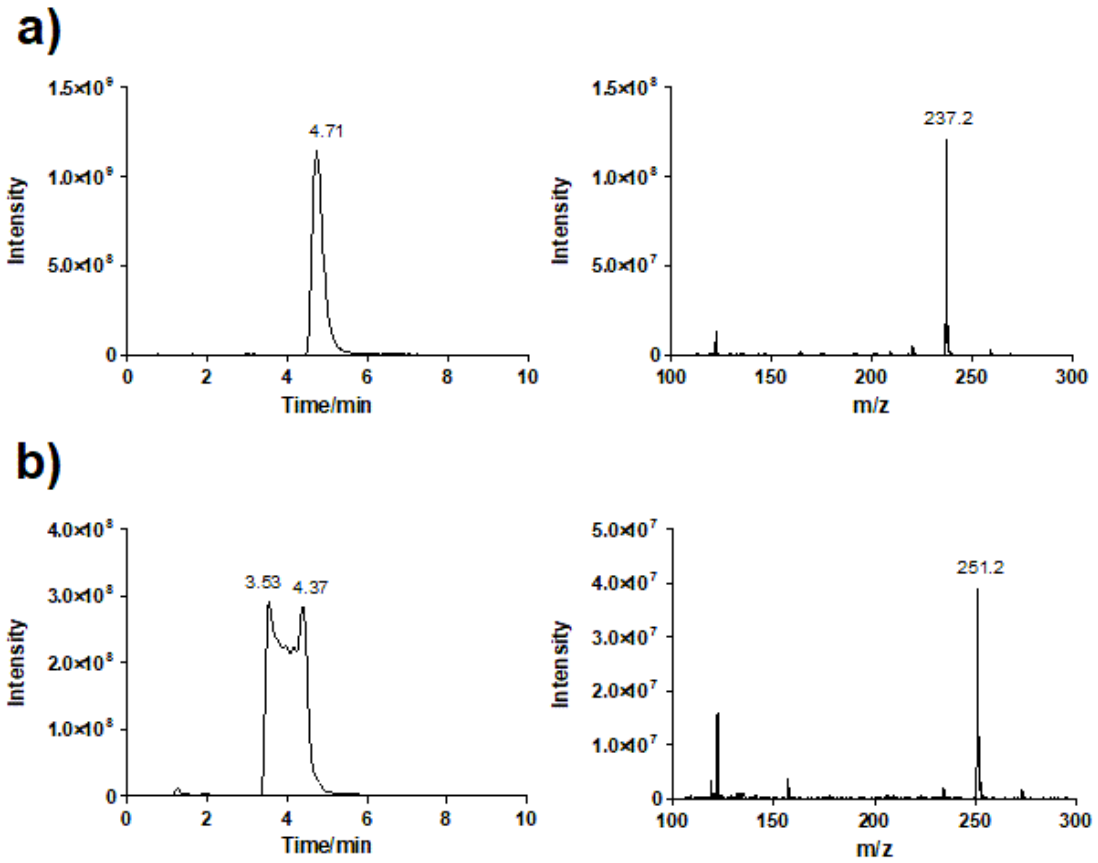
The same analysis was performed for inhibitor **17**. Spirocyclic cyclopropyl *N*-formyl kynurenine analogue **41** (see full text) is a hypothesised product of an electrophilic-base mechanism of IDO1-mediate dioxygenation. Radical ring-opening of **17** could lead to primary radical **22**. Hydrogen abstraction (i, Scheme SI5) from an active site residue or from media present in the active site would lead to amino acid **S17** – collapse of the heme-peroxy intermediate under

the quenching conditions is predicted to lead to metabolite **42** (ii, Scheme SI5). If the primary radical undergoes a radical recombination with molecular oxygen in solution, alkyl peroxy **S18** is predicted to form (iii, Scheme SI5). Upon release from the heme-centre, alcohol **43** is predicted to arise from degradation of the alkyl peroxy by catalase present within the assay media (vi, Scheme SI5).

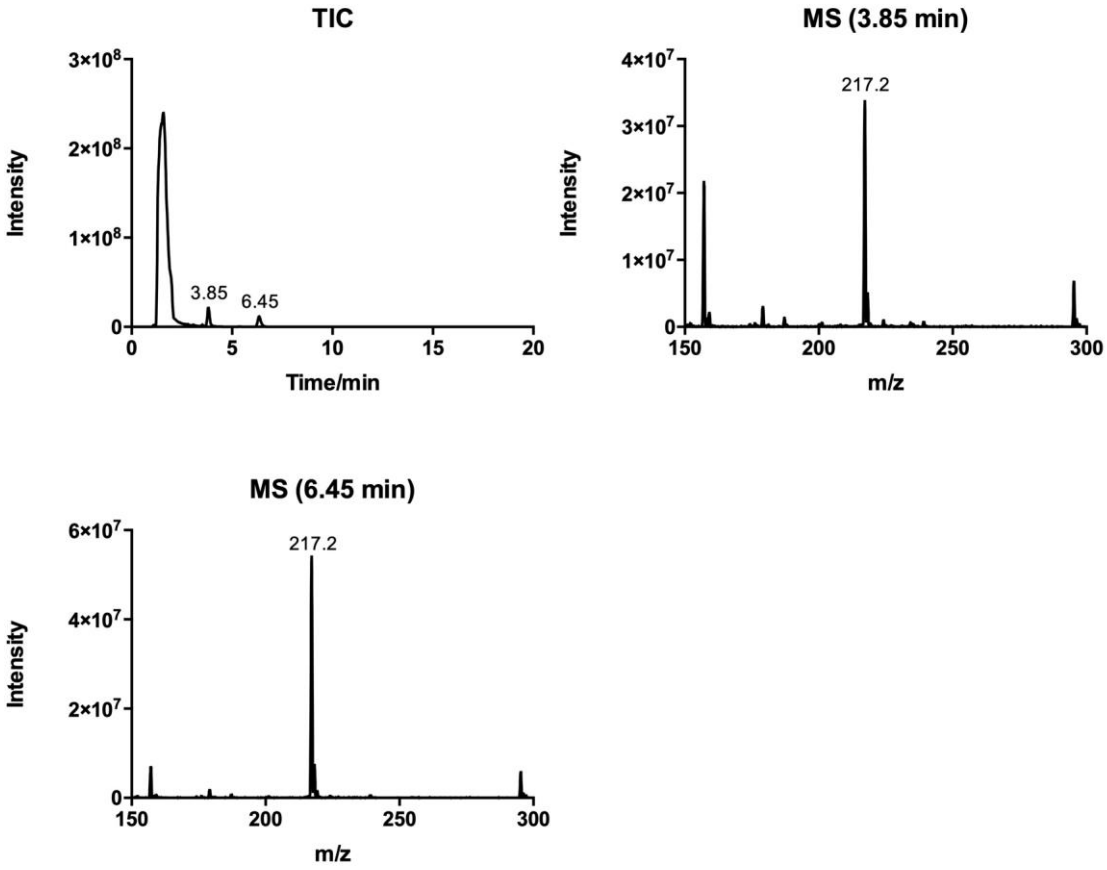


*Scheme SI5 - Plausible metabolic outcomes for **17***

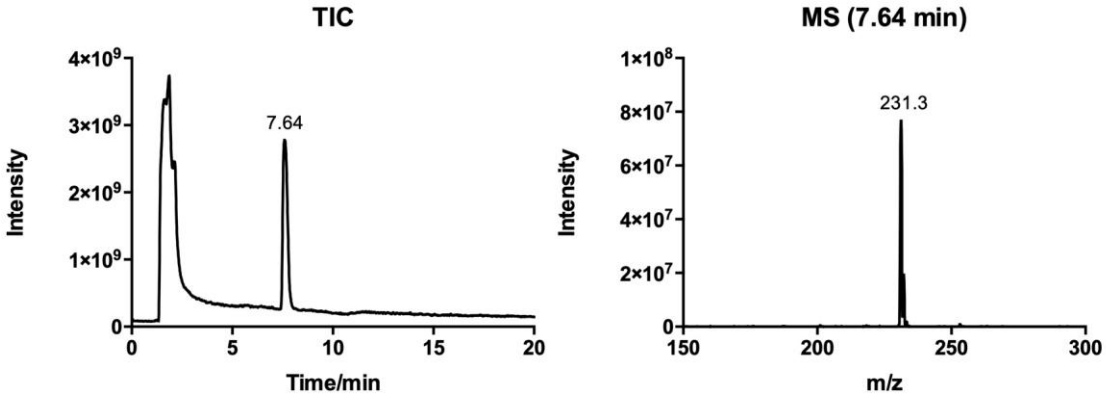
*3.4 – F164A IDO1 mediated turnover of **1** and **3***



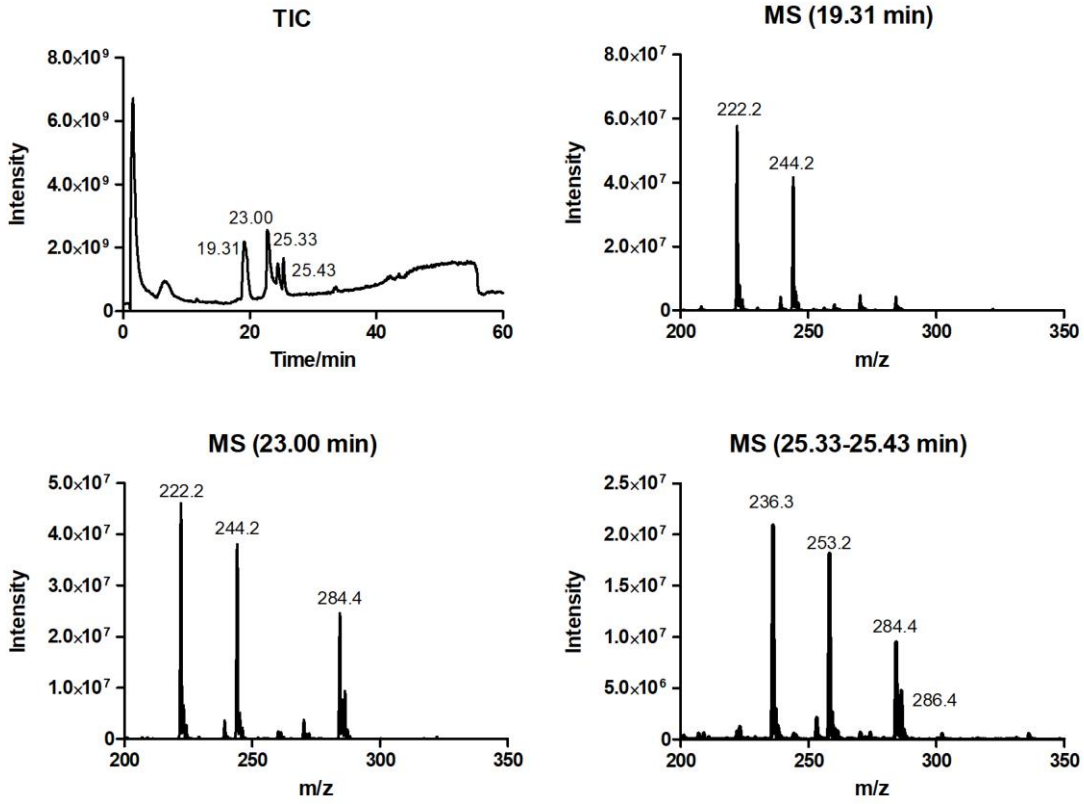
*3.5 – LC-MS data for **16**/IDO1 steady state assay*



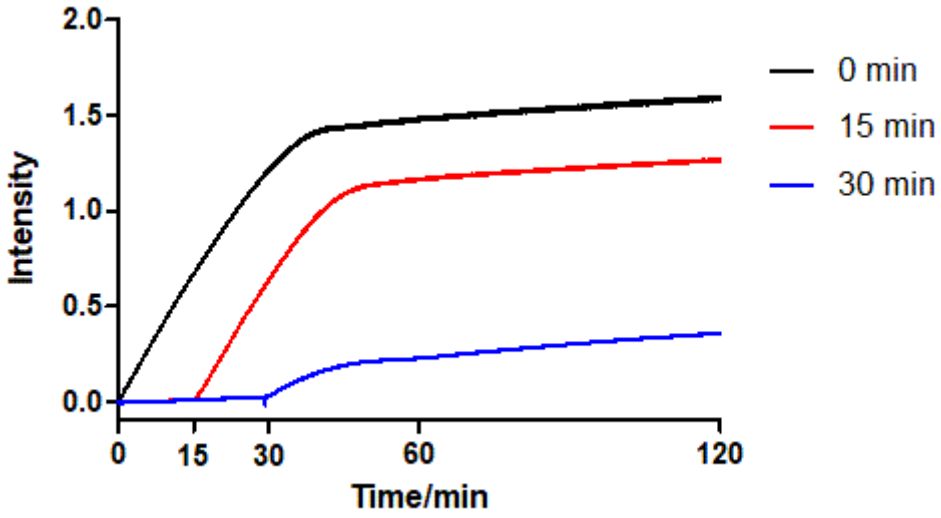
*3.6 – LC-MS data for **17**/IDO1 steady state assay*



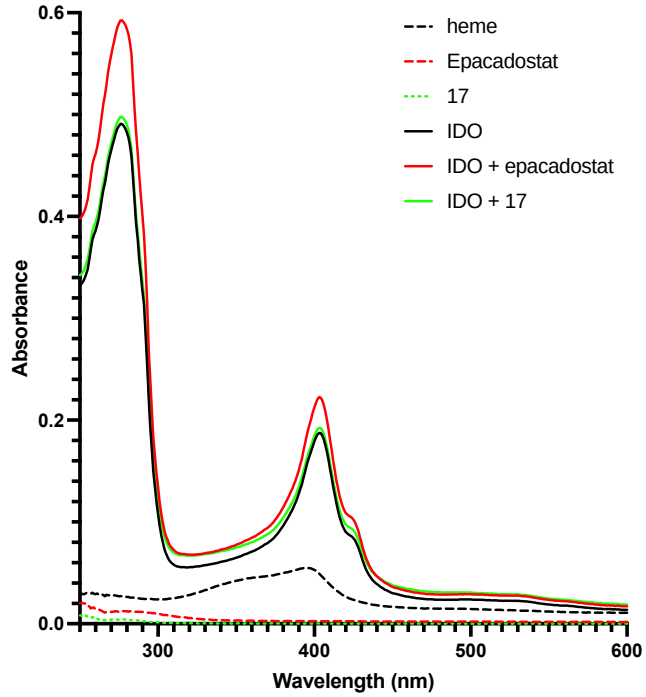
*3.7 – LC-MS data for **18**/IDO1 steady state assay*



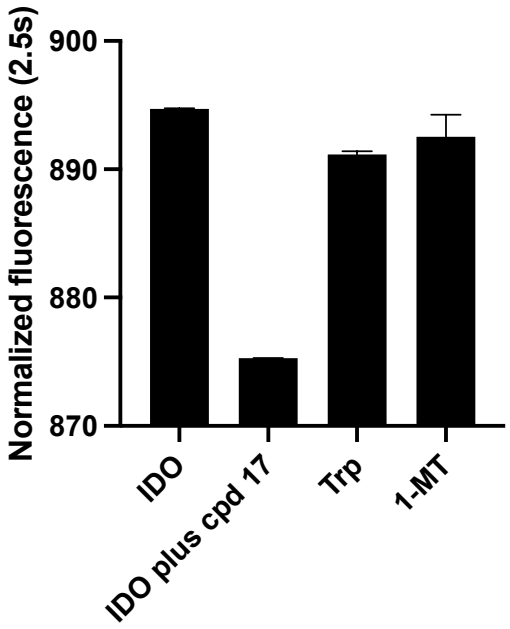
3.8 – Pre-incubation control experiments



3.9 – UV-vis spectra of IDO-1, heme, epacadostat, ***17****, IDO-1 + epacodostat and IDO-1 +* ***17****.*



*3.10 – MST changes induced by **17**, TRP and 1-MT*



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