Quorum-quenching inhibitor discovery

Prashant B. Kapadnis, Evan Hall, Madeleine Ramstedt, Warren R. J. D. Galloway, Martin Welch and David R. Spring*

Supplementary Experimental Section

1.1 General experimental details

Reactions were performed using oven-dried glassware apparatus under an atmosphere of nitrogen with anhydrous, freshly distilled solvents unless otherwise stated. Dichloromethane, ethyl acetate, methanol and *n*-hexane were distilled from calcium hydride. Anhydrous dimethylformamide (DMF) and dimethylacetamide (DMA), were used as obtained from commercial sources. All other reagents were used as obtained from commercial sources.

Room temperature refers to ambient temperature. Temperatures of 0°C were maintained using an ice-water bath and temperatures below 0°C were maintained using an acetone-cardice bath

Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. All flash chromatography was carried out using slurry-packed Merck 9325 Keiselgel 60 silica gel. Were possible, reactions were monitored by thin layer chromatography (TLC) performed on commercially prepared glass plates precoated with Merck silica gel 60 F254 or aluminium oxide 60 F254. Visualisation was by the quenching of UV fluorescence (ν_{max} = 254 nm) or by staining with ceric ammonium molybdate, potassium permanganate or Dragendorff 's reagent (0.08% w/v bismuth subnitrate and 2% w/v KI in 3M aq. AcOH).

Infrared spectra were recorded neat on a Perkin-Elmer Spectrum One spectrometer with internal referencing. Selected absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}).

Melting points were obtained using a Büchi® melting point apparatus (model B-545) and are uncorrected.

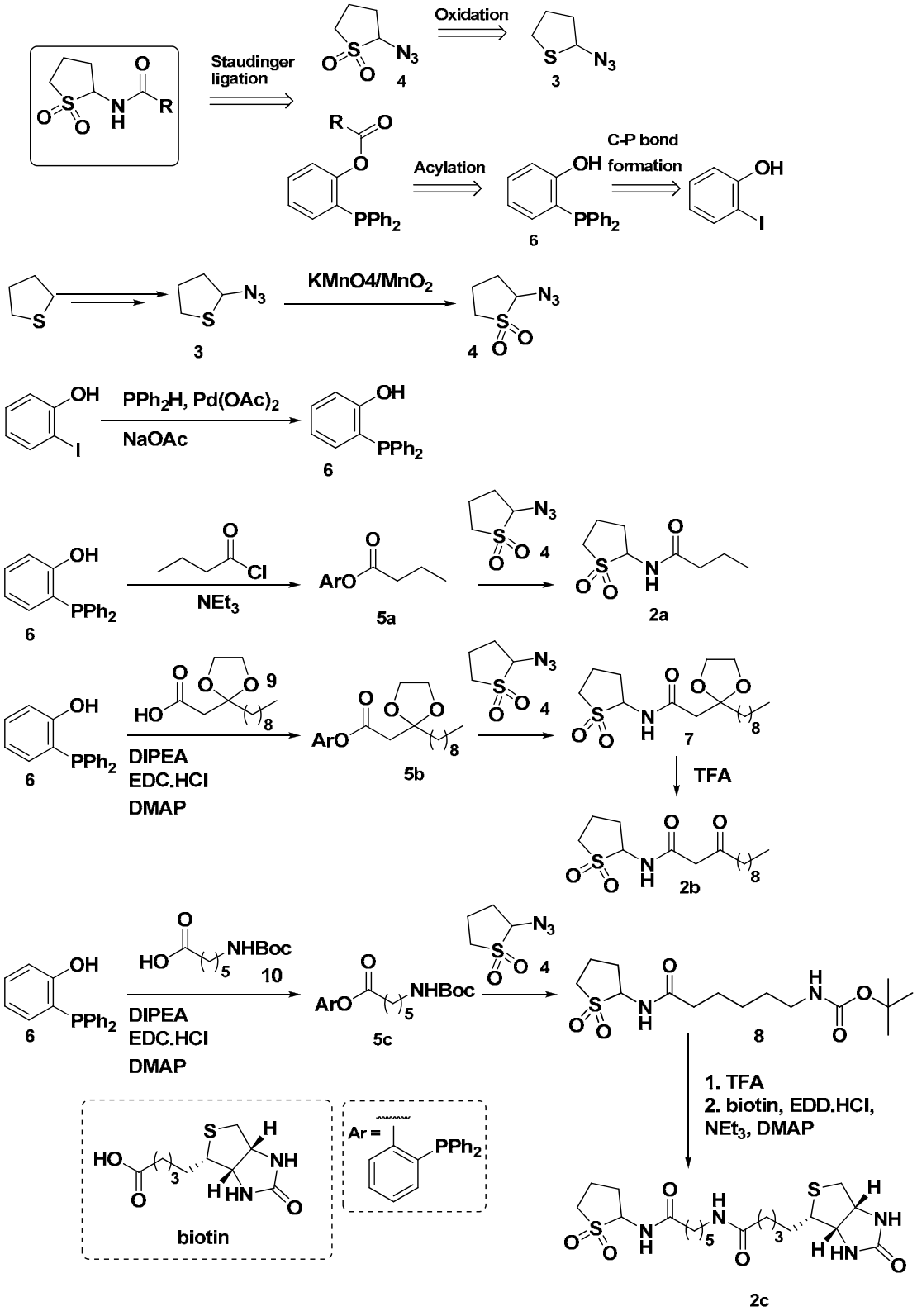
Proton magnetic resonance spectra were recorded using an internal deuterium lock at ambient probe temperatures (unless otherwise stated) on the following instruments: Bruker DPX-400 (400 MHz), Bruker Avance 400 QNP (400 MHz), Bruker Avance 500 BB ATM (500 MHz) and Bruker Avance 500 Cryo Ultrashield (500 MHz). Chemical shifts (δ_H) are quoted in ppm, to the nearest 0.01 ppm, and are referenced to the residual non-deuterated solvent peak. Coupling constants (*J*) are reported in Hertz to the nearest 0.5 Hz. Data are reported as follows: chemical shift, integration, multiplicity [br, broad; s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sept, septet; m, multiplet; or as a combination of these (*e.g.* dd, dt, *etc.*)], coupling constant(s) and assignment. Proton assignments were determined either on the basis of unambiguous chemical shift or coupling pattern, by patterns observed in 2D experiments (^{1}H-^{1}H COSY, HMBC and HMQC) or by analogy to fully interpreted spectra for related compounds. Diastereotopic protons are assigned as H and H'.

Carbon magnetic resonance spectra were recorded by broadband proton spin decoupling at ambient probe temperatures (unless otherwise stated) using an internal deuterium lock on the following instruments: Bruker DPX-400 (100 MHz), Bruker Avance 400 QNP (100 MHz), Bruker Avance 500 BB ATM (125 MHz) and Bruker Avance 500 Cryo Ultrashield (125 MHz). Chemical shifts (δ_C) are quoted in ppm, to the nearest 0.1 ppm, and are referenced to the residual non-deuterated solvent peak. Where appropriate, coupling constants are reported in Hertz to the nearest 0.5 Hz and data are reported as for proton magnetic resonance spectra without integration. Assignments were supported by DEPT editing and determined either on the basis of unambiguous chemical shift or coupling pattern, by patterns observed in 2D experiments (HMBC and HMQC) or by analogy to fully interpreted spectra for related compounds.

High resolution mass spectroscopy measurements were made by the EPSRC mass spectrometry service (Swansea) or recorded in-house using a Waters LCT Premier Mass Spectrometer or a Micromass Quadrapole-Time of Flight (Q-ToF) spectrometer. Mass values are reported within the error limits of ±5 ppm mass units. ESI = electrospray ionisation.

app = apparent
Boc = *tert*-butoxycarbonyl
DIPEA = di*iso*propylethylamine
DMAP = 4-dimethylaminopyridine
EDC= 1-ethyl-3-(3-dimethylaminopropy)carbodiimide
equiv = equivalents
TFA = trifluoroacetic acid
min = minute(s); hr = hour(s)

1.2 Outline of synthetic routes used



Scheme 1: Outline of synthetic routes used for analogue synthesis

1.3 General experimental procedures

1.3.1 General procedure 1: Synthesis of aryl diphosphine Staudinger ligation coupling partners

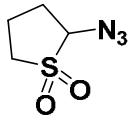
To a well stirred solution of 2-(diphenylphosphino) phenol (0.2 g, 1.0 equiv) in anhydrous CH_2Cl_2 (10 cm^3) at room temperature under nitrogen was added the appropriate acid derivative (1.2 equiv), diisopropylethylamine (0.175 cm^3, 1.4 equiv), EDC.HCl (0.193 g, 1.4 equiv) and DMAP (10 mg, 0.1 equiv). The resulting mixture was stirred at room temperature until TLC analysis showed completion of the reaction. The reaction mixture was diluted with CH_2Cl_2 (30 cm^3) and washed with 10% HCl solution. The organic layer was separated, dried ($MgSO_4$) and concentrated *in vacuo* to leave the crude product which was purified by column chromatography.

1.3.2 General Procedure 2: Staudinger ligation

The phosphine derivative (**5a-c**, 1.2 equiv) was added to a solution of the azide **4** (1 equiv) in anhydrous DMF (0.1 M) under nitrogen at room temperature. The resulting mixture was stirred at 70 °C until TLC analysis showed complete consumption of the azide. Water was added and the mixture was stirred for 16 hr at the same temperature. The solvent was removed *in vacuo* and the crude product was purified by column chromatography.

1.4 Experimental procedures

1.4.1 2-azido-tetrahydro-thiophen 1,1-dioxide (4)



4

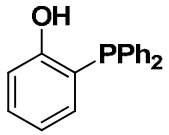
To a well stirred solution of 2-azidotetrahydrothiophene **(3)**[a] (0.1 g, 0.775 mmol) in CH_2Cl_2 (10 cm^3) at room temperature was added a well-ground mixture of $KMnO_4$ and MnO_2 (1:3 ratio, 1.55 g) slowly over 15 min. The resulting mixture was stirred at room temperature until TLC analysis showed that the reaction was complete (4 hr). The reaction mixture was then filtered through a sintered glass funnel to remove the oxidant. The filtrate was concentrated *in vacuo*. The crude material thus obtained was purified by column chromatography (SiO_2; CH_2Cl_2) to yield **4** as colourless oil (0.77 mg, 62%).

$\boldsymbol{\nu}_{max}$ (neat)/cm^{-1} 2107 (N_3), 1320 (SO_2), 1132 (SO_2); $\boldsymbol{\delta}_H$ (400 MHz; $CDCl_3$) 4.42 (1H, dd, *J* 6.0 Hz, 4.0 Hz, C<u>H</u>N_3), 3.11-2.98 (2H, m, CH_2), 2.38-2.30 (1H, m, CH_2), 2.25-2.05 (2H, m, CH_2), 2.02-1.94 (1H, m, CH); $\boldsymbol{\delta}_C$ (125 MHz; $CDCl_3$) 74.5 (CH), 48.9 (CH_2), 28.1 (CH_2), 18.9 (CH_2).

This data is in agreement with that previously reported.[a]

[a] Prepared by the method of I. W. J. Still, W. L. Brown, R. J. Colville and G. W. Kutney, *Can. J. Chem.*, 1984, **62**, 586-590.

1.4.2 2-(diphenylphosphino) phenol (6)

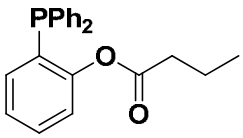


6

A mixture of *ortho*-iodophenol (1 g, 4.54 mmol), diphenylphosphine (0.8 cm^3, 4.54 mmol), palladium (II) acetate (10 mg, 0.045 mmol) and sodium acetate (0.4 g, 4.99 mmol) in anhydrous DMA (14 cm^3) under nitrogen was stirred at 110 °C for 16 hr. The reaction mixture was then filtered through a pad of celite. The filtrate was concentrated *in vacuo* and the crude material thus obtained was purified by column chromatography (SiO_2; CH_2Cl_2) to give **6** as white solid (0.962 g, 77%).

ν_{max} (neat)/cm^{-1} 3249 (OH), 1434 (P-Ph); δ_H (400 MHz; $CDCl_3$) 7.43-7.26 (11H, m, aryl CH), 7.05-6.86 (3H, m, aryl CH), 6.42 (1H, br s, OH); δ_C (125 MHz; $CDCl_3$) 159.1, 159.2, 135.0, 134.9, 134.7, 133.4, 133.2, 131.5, 128.9, 128.7, 128.6, 121.1, 121.0, 115.5; **HRMS** (ESI^+) *m/z* found $[M+H]^+$ 279.0927, $C_{18}H_{16}OP^+$ required 279.0939; **m.p.** 149-150 °C (CH_2Cl_2).

1.4.3 2-(diphenylphosphino)phenyl butyrate (5a)

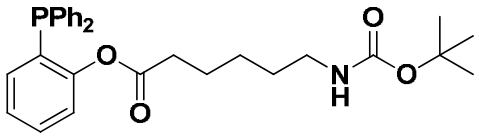


5a

To a well stirred solution of 2-(diphenylphosphino) phenol (**6**) (0.2 g, 0.718 mmol) in anhydrous CH_2Cl_2 (7 cm^3) at room temperature under nitrogen was added triethylamine (0.12 cm^3, 0.79 mmol) followed by drop-wise addition of butyryl chloride (0.084 cm^3, 0.79 mmol). The resulting mixture was stirred at room temperature until TLC analysis showed the reaction had gone to completion (2 hr). The reaction mixture was diluted with CH_2Cl_2 (30 cm^3) and washed with 5% $NaHCO_3$ solution. The organic layer was separated, dried ($MgSO_4$) and concentrated *in vacuo* to yield **5a** as off-white solid (0.17 g, 68%) which was analytically pure.

ν_{max} (neat)/cm^{-1} 1757 (C=O), 1432 (P-Ph); δ_H (400 MHz, $CDCl_3$) 7.41-7.27 (11H, m, aryl CH), 7.18-7.08 (2H, m, aryl CH), 6.82 (1H, app. ddd, *J* 7.5 Hz, 4.5 Hz, 1.5 Hz, aryl CH), 2.23 (2H, t, *J* 7.5 Hz, C(=O)CH_2), 1.54 (2H, app. sextet, *J* 7.5 Hz, C(=O)$CH_2C\underline{H_2}$), 0.88 (3H, t, *J* 7.5 Hz, CH_3); δ_C (125 MHz; $CDCl_3$) 171.4, 135.7, 135.6, 134.0, 133.8, 133.6, 129.8, 128.9, 128.5, 128.4, 122.5, 35.8, 17.9, 13.5; **HRMS** (ESI$^+$) *m/z* found $[M+H]^+$ 349.1364, $C_{22}H_{22}O_2P^+$ required 349.1357; **m.p.** 77-80 °C (CH_2Cl_2).

1.4.4 2-(diphenylphosphino)phenyl 6-(tert-butoxycarbonylamino)hexanoate (5c)

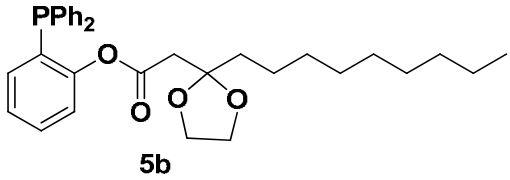


5c

Prepared by general procedure 1 using BOC-6-aminocaproic acid (**10**, obtained from commercial source). The desired product was obtained as colourless oil (99%).

ν_{max} (neat)/cm^{-1} 1765 (C(=O) ester) 1698 (C(=O) carbamate), 1438 (P-Ph),; δ_H (400 MHz, $CDCl_3$) 7.41-7.27 (11 H, m, aryl CH), 7.13 (2H, app. dd, *J* 12.5 Hz, 5.0 Hz, aryl CH), 6.84-6.77 (1H, m, aryl CH), 4.56 (1H, br s, NH), 3.08 (2H, app. br s, $C\underline{H_2}NH$), 2.25 (2H, t, *J* 7.5 Hz, C(=O)CH_2), 1.58-1.34 (13 H, m, 2 x CH_2 and 3 x CH_3), 1.26-1.15 (2H, m, CH_2); δ_C (125 MHz; $CDCl_3$) 171.7, 156.3, 153.2, 153.0, 143.2, 136.0, 135.9, 134.4, 134.1, 130.6, 130.3, 129.4, 128.9, 126.5, 122.9, 110.5, 34.2, 30.1, 28.8, 26.6, 24.5; **HRMS** (ESI$^+$) *m/z* found $[M+H]^+$ 492.2321, $C_{29}H_{35}NO_4P^+$ required 492.2304.

1.4.5 2-(diphenylphosphino)phenyl 2-(2-nonyl-1,3-dioxolan-2-yl)acetate (5b)



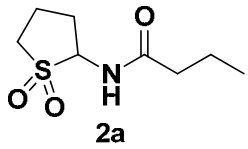
5b

Prepared by general procedure 1 using 2-(2-nonyl-1,3-dioxolan-2-yl)acetic acid **(9)**.[b] The crude material was purified by column chromatography (SiO_2; 3:10 EtOAc: hexane) to yield **5b** as colourless oil (84%).

ν_{max} (neat)/cm^{-1} 1765 (C=O), 1437 (P-Ph); δ_H (500 MHz, $CDCl_3$) 7.41-7.28 (11H, m, aryl CH), 7.22-7.09 (2H, m, aryl CH), 6.82 (1H, app. ddd, *J* 7.5 Hz, 4.5 Hz, 1.5 Hz, aryl CH), 3.93 (4H, app. br s, $OC\underline{H_2}CH_2O$ and $OCH_2C\underline{H_2}O$), 2.54 (2H, s, $C(=O)CH_2$), 1.84-1.70 (2H, m, CH_2), 1.43-1.11 (14 H, m, 7 x CH_2), 0.88 (3H, t, *J* 7.0 Hz, CH_3); δ_C (125 MHz; $CDCl_3$) 167.3, 152.7, 152.6, 135.7, 135.6, 134.0, 133.8, 133.6, 133.5, 130.1, 130.0, 129.8, 128.9, 128.6, 128.5, 126.0, 122.4, 109.2, 65.0, 41.8, 37.6, 31.8, 29.6, 29.5, 29.3, 23.3, 22.6, 14.1; **HRMS** (ESI^+) *m/z* found $[M+H]^+$ 519.2662, $C_{32}H_{40}O_4P^+$ required 519.2664.

[b] **9** was prepared according to the method of G. L. Thomas, C. M. Bohner, H. E. Williams, C. M. Walsh, M. Ladlow, M. Welch, C. E. Bryant and D. R. Spring, *Mol. Biosyst.* 2006, **2**, 132-137.

1.4.6 *N*-(tetrahydrothiophen 1,1-dioxide -2-yl)butyramide (2a)

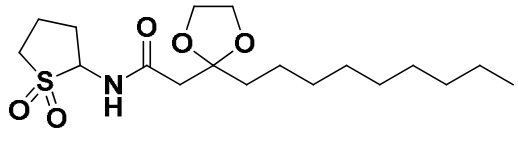


2a

Prepared by general procedure 2. The crude material was purified by column chromatography on (SiO_2; 0.4:10 MeOH: $CHCl_3$) to yield **2a** as off white solid (40%).

ν_{max} (neat)/cm^{-1} 3280 (NH), 1658 (C=O), 1299 (SO_2), 1143 (SO_2); δ_H (400 MHz, $CDCl_3$) 6.10 (1H, br s, NH), 5.14 (1H, dd, *J* 17.0 Hz, 8.5 Hz, C<u>H</u>NH), 3.23-3.14 (1H, m, SO_2C<u>H</u>H'), 3.04-2.95 (1H, m, SO_2CH<u>H</u>'), 2.60-2.49 (1H, m, CH_2), 2.24 (4H, m, 2 x CH_2), 1.95-1.84 (1H, m, CH_2), 1.75-1.63 (2H, m, CH_2), 0.96 (3H, t, *J* 7.5 Hz, CH_3); δ_C (125 MHz; $CDCl_3$) 173.2 (C=O), 66.5 (CH), 50.1 (CH_2), 38.2 (CH_2), 29.5 (CH_2), 18.8 (CH_2), 18.3 (CH_2), 13.6 (CH_3); **HRMS** (ESI^+) *m/z* found $[M+Na]^+$ 228.0673, $C_8H_{15}NNaO_3S^+$ required 228.0670; **m.p.** 120-121 °C ($CHCl_3$: MeOH).

1.4.7 *N*-(1,1-dioxidotetrahydrothiophen-2-yl)-2-(2-nonyl-1,3-dioxolan-2-yl)acetamide (7)

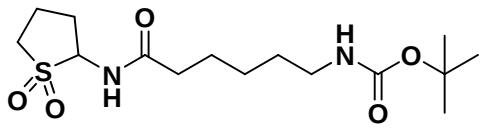


7

Prepared by general procedure 2. The crude material was purified by column chromatography (SiO_2; 0.4:10 MeOH: $CHCl_3$) to give **7** as off-white solid (50%).

ν_{max} (neat)/cm^{-1} 3280 (NH), 1668 (C=O); δ_H (400 MHz; $CDCl_3$) 7.05 (1H, d, *J* 9.0 Hz, NH), 5.12 (1H, app. q, *J* 9.0 Hz, C<u>H</u>NH), 4.15-3.94 (4H, m, OC<u>H</u>$_2$$CH_2$O and OCH_2C<u>H</u>$_2$O), 3.22-3.18 (1H, m, SO_2C<u>H</u>H'), 3.05-2.95 (1H, m, SO_2CH<u>H</u>'), 2.68-2.62 (2H, m, C(=O)CH_2), 2.59-2.50 (1H, m, CH_2), 2.25-2.10 (2H, m, CH_2), 1.95-1.85 (1H, m, CH_2), 1.72-1.68 (2H, m, CH_2), 1.32-1.20 (14H, m, CH_2), 0.87 (3H, t, *J* 7.0 Hz, CH_3); δ_C (125 MHz; $CDCl_3$) 169.4 (C=O), 109.5 (C), 66.4 (CH), 65.0 (CH_2), 64.9 (CH_2), 50.0 (CH_2), 44.2 (CH_2), 37.4 (CH_2), 31.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.408 (CH_2), 29.401 (CH_2) 29.2 (CH_2), 23.7 (CH_2), 22.6 (CH_2), 18.4 (CH_2), 14.1 (CH_3); **HRMS** (ESI^+) *m/z* found $[M+H]^+$ 376.2163, $C_{18}H_{34}NO_5S^+$ required 376.2158; **m.p.** 61-62 °C ($CHCl_3$: MeOH).

1.4.8 tert-butyl {6-[(1,1-dioxidotetrahydrothiophen-2-yl)amino]-6-oxohexyl}carbamate (8)

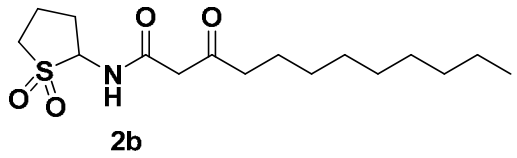


8

Prepared by general procedure 2. The crude material was purified by column chromatography (SiO_2; 0.4:10 MeOH: $CHCl_3$) to give **8** as off-white solid (71%).

ν_{max} (neat)/cm^{-1} 3355 (NH), 1688 (C=O amide), 1659 (C=O carbamate), 1140 (SO_2); δ_H (400 MHz; $CDCl_3$) 6.52 (1H, br s, NH), 5.13 (1H, app. q, *J* 8.5 Hz, C<u>H</u>NH), 4.62 (1H, br s, NH), 3.19-3.13 (1H, m, $SO_2C\underline{H}H'$), 3.09 (2H, app. t, *J* 7.0 Hz, $C\underline{H}_2NHC(=O)O^tBu$), 2.99 (1H, ddd, *J* 13.0 Hz, 10.0 Hz, 8.0 Hz, $SO_2CH\underline{H}'$), 2.58-2.51 (1H, m, $C\underline{H}H'CHNH$), 2.28 (2H, t, *J* 7.5 Hz, $C(=O)CH_2$), 2.22-2.18 (1H, m, $SO_2CH_2C\underline{H}H'$), 2.17-2.08 (1H, m, $SO_2CH_2CH\underline{H}'$), 2.00-1.90 (1H, m, $CH\underline{H}'CHNH$), 1.75-1.64 (2H, m, $C(=O)CH_2C\underline{H}_2$), 1.53-1.37 (13H, m, 2 x CH_2 and 3 x CH_3); δ_C (125 MHz; $CDCl_3$); 173.4 (C=O amide), 156.1 (C=O carbamate), 79.9 (C), 66.6 (CH), 50.1 (CH_2), 40.1 (CH_2), 35.9 (CH_2), 29.5 (CH_2) , 29.1 (CH_2), 28.4 (CH_3), 26.0 (CH_2), 24.8 (CH_2), 18.4 (CH_2); **HRMS** (ESI^+) *m/z* found $[M+H]^+$ 349.1812, $C_{15}H_{29}N_2O_5S^+$ required 349.1797, **m.p.** 119-120 °C ($CHCl_3$: MeOH).

1.4.9 *N*-(1,1-dioxidotetrahydrothiophen-2-yl)-3-oxododecanamide (2b)

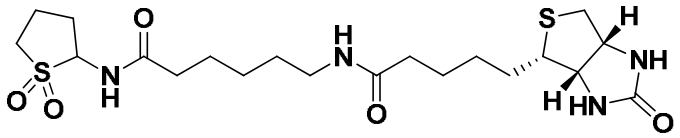


2b

To a well stirred solution of **7** (32 mg, 0.085 mmol) in anhydrous CH_2Cl_2 (0.5 cm^3) at room temperature under nitrogen was added trifluoroacetic acid (64 μL, 0.852 mmol) drop-wise. The resulting mixture was stirred at room temperature until TLC analysis showed that the reaction had gone to completion (1 hr). The reaction mixture was then diluted with CH_2Cl_2 (30 cm^3) and washed with 5% $NaHCO_3$ solution. The organic layer was separated, dried ($MgSO_4$) and concentrated *in vacuo* to give **2b** as off-white solid (23.5 mg, 83%).

ν_{max} (neat)/cm^{-1} 3272 (NH), 1714 (C=O ketone), 1625 (C=O amide), 1301 (SO_2), 1138 (SO_2); δ_H (400 MHz; $CDCl_3$) 7.76 (1H, d, *J* 8.0 Hz, NH), 5.10 (1H, app. q, *J* 8.0 Hz, C<u>H</u>NH), 3.51 (2H, app. d, *J* 3.5 Hz, C(=O)CH_2C(=O)), 3.24-3.08 (1H, m, SO_2C<u>H</u>H'), 3.02-2.92 (1 H, m, SO_2CH<u>H</u>'), 2.63-2.45 (3H, m, CH_2), 2.29-2.03 (2H, m, CH_2), 2.00-1.92 (1H, m, CH_2), 1.67-1.50 (2H, m, CH_2), 1.35-1.15 (12 H, m, 6 x CH_2), 0.88 (3H, t, *J* 7.0 Hz, CH_3); δ_C (125 MHz; $CDCl_3$) 207.1 (C=O ketone), 166.3 (C=O amide), 66.6 (CH), 50.1 (CH_2), 48.0 (CH_2), 43.9 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 23.3 (CH_2), 22.6 (CH_2), 18.6 (CH_2), 14.0 (CH_3); **HRMS** (ESI^+) *m/z* found $[M+H]^+$ 332.1891, $C_{16}H_{30}NO_4S^+$ required 332.1896, **m.p.** 119-120 °C (CH_2Cl_2).

1.4.10 *N*-(1,1-dioxidotetrahydrothiophen-2-yl)-6-({5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoyl}amino)hexanamide (2c)



2c

To a well stirred solution of **8** (82 mg, 0.235 mmol) in anhydrous CH_2Cl_2 (0.5 cm^3) at room temperature under nitrogen was added trifluoroacetic acid (175 cm^3, 2.35 mmol) drop-wise. The resulting mixture was stirred at room temperature until TLC analysis showed completion of the reaction (1 hr). The solvent was removed *in vacuo*. The residue was re-dissolved in anhydrous CH_2Cl_2 (2.2 cm^3) under nitrogen and biotin (64 mg, 0.258 mmol), EDC.HCl (63 mg, 0.328 mmol), DMAP (4 mg, 0.024 mmol) and triethylamine (83 μL, 0.586 mmol) were added at room temperature. The reaction mixture was stirred at room temperature for 16h. The solvent was then removed *in vacuo* and the crude product thus obtained was purified by chromatography (SiO_2; 1:4 MeOH: $CHCl_3$) to yield **2c** as white solid (0.50 mg, 50%).

ν_{max} (neat)/cm^{-1} 3284.5 (NH), 1669.1 (C=O), 1630.2 (C=O), 1307.0 (SO_2), 1150.2 (SO_2); δ_H (400 MHz; d_6 DMSO) 8.51 (1H, d, *J* 8.0 Hz, $SO_2CHN\underline{H}$), 7.72 (1H, t, *J* 5.5 Hz, $CH_2N\underline{H}$), 6.40 (1H, s, urea NH), 6.34 (1H, s, urea NH), 4.85 (1H, dd, *J* 16.0 Hz, 8.0 Hz, $SO_2C\underline{H}NH$), 4.33-4.27 (1H, m, $CH_2CHSCH_2C\underline{H}$), 4.18-4.06 (1H, m, $CH_2CH(S)C\underline{H}NH$), 3.17-3.03 (3H, m, $CH_2C\underline{H}S$ and SO_2CH_2), 3.00 (2H, app. dd, *J* 12.5 Hz, 6.5 Hz, $C\underline{H}_2NH$), 2.90-2.79 (2H, m, $CH_2CHSC\underline{H}_2$), 2.42-2.33 (1H, m, $SO_2CHNHC\underline{H}H'$), 2.16 (2H, t, *J* 7.5 Hz, $SO_2CHNHC(=O)C\underline{H}_2$), 2.13-2.07 (1H, m, $SO_2CH_2C\underline{H}H'$), 2.04 (2H, t, *J* 7.5 Hz, $CH_2NHC(=O)C\underline{H}_2$), 1.99-1.90 (1H, m, $SO_2CH_2CH\underline{H}'$), 1.90-1.80 (1H, m, $SO_2CHNHC\underline{H}H'$), 1.65-1.41 (6H, m, $C(=O)CH_2C\underline{H}_2CH_2CH_2CHS$, $C\underline{H}_2CHS$ and $SO_2CHNHC(=O)CH_2C\underline{H}_2$), 1.40-1.33 (2H, m, $CH_2C\underline{H}_2CH_2NHC(=O)$), 1.31-1.22 (4H, m,. $C\underline{H}_2CH_2CH_2NH$ and $C\underline{H}_2CH_2CHS$); δ_C (125 MHz; d_6 DMSO) 172.9 (C), 171.8 (C), 162.8 (C), 66.9 (CH), 61.1 (CH), 59.2 (CH), 55.5 (CH), 50.0 (CH_2), 45.7 (CH_2), 38.3 (CH_2), 35.3 (CH_2),

35.0 (CH_2), 29.0 (CH_2), 28.3 (CH_2), 28.1 (CH_2), 27.8 (CH_2), 26.1 (CH_2), 25.4 (CH_2), 24.9 (CH_2), 18.5 (CH_2); **HRMS** (ESI^+) *m/z* found $[M+H]^+$ 475.2067, $C_{20}H_{35}N_4O_5S_2^+$ required 475.2049, **m.p.** 90-95 °C (MeOH: $CHCl_3$).

1.5 AiiA inhibition

1.5.1 Protein expression and purification

E-coli expressing the recombinant protein were received from J.K. Lee (Korea Research Institute of Bioscience and Biotechnology (Kim *et al*. 2005)). Overnight cultures (10 ml) of transformed cells were added to flasks containing 500 ml Luria Broth with $ZnSO_4$ (final conc Zn^{2+} 10μM) and 5ml 20% glucose. All bacterial cultures were maintained in the presence of 50μg/ml carbenicillin. The fresh culture was grown at 37 ºC until mid-exponential phase (OD ~0.6) and isopropyl-β-D-thiogalactopyranoside (IPTG) was added to each flask to give a final concentration of 0.5 mM. The cells were left to express the protein overnight at 25 ºC. The cells were then harvested by centrifugation (20 min, 10,000 x *g*, 4 ºC).

The cell pellet was lysed in 25 ml ice cold lysis buffer (50 mM Tris-HCl, 300 mM NaCl, 10 mM imidazole, pH 8) using three 2-min pulses of ultrasound. The solutions were left to cool one ice between each pulse. The sample was clarified by centrifugation (20 min, 4000 x *g*, 4ºC). The solution was loaded onto a column of amylose resin (New England BioLabs) equilibrated with wash buffer 1 (20 mM Tris-HCl, 200 mM NaCl, pH 7.4). The column was washed overnight with approximately 75 column volumes of wash buffer 1. The protein was eluted with approximately 10 ml of elution buffer 1 (20mM Tris-HCl, 200 mM NaCl, 10 mM maltose, pH 8). Thereafter the protein was loaded onto a Ni-NTA agarose column (Qiagen) equilibrated with wash buffer 2 (50 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole, pH 8). The column was washed with approximately 35 column volumes with wash buffer 2. The protein was eluted in approximately 10 ml of elution buffer 2 (50 mM Tris-HCl, 300 mM NaCl and 250 mM imidazole, pH 8). The eluted protein was dialyzed four times against 1l 50 mM Tris-HCl pH 8. In the last dialysis buffer, 10 % (v/v) glycerol was added. The concentration of protein was determined using the absorbance at 280 nm and a calculated extinction coefficient of 85 890 M^{-1} cm^{-1}. The

protein was snap frozen in small aliquots using N_2(l) and stored at -80 ºC until use. SDS-PAGE of the purified protein gave a distinct band around 70 kDa.

Reference

Kim MH, Kang HO, Kang BS, Kin KJ, Choi WC, Oh TK, Lee CH, Lee JK, Crystallization and preliminary crystallographic analysis of *Bacillus thuringiensis* AHL-lactonase, Biochimical et Biophysica Acta, 2005, 1750, 5-8

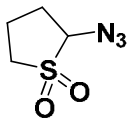
1.5.2 Isothermal titration calorimetry (ITC)

ITC measurements were carried out on a MicroCal VP-ITC microcalorimeter and data analysis was carried out on the accompanying Origin 7 software. The general specifications of the runs are as follows: cell volume = 1.4227 ml, cell temp = 25 °C, number of injections = 15. Injection volumes ranged from 2µl to 15µl with 320 seconds between each, an initial delay of 120 sec was used before starting the titration. The composition of the reaction cell varied with the amount of inhibitor present, but a typical run is shown below (Table 1).

Table 1. Composition of reaction cell and syringe for ITC kinetic assays.

Experiment	**Reaction Cell Composition**	**Syringe Composition**
AHL control	1 ml 2x buffer (20 mM TRIS, pH 8.0), 1 ml H_2O	5.15 mM HHL (in H_2O)
Enzyme + AHL	120 µl MBP-His_6-AiiA, 940 µl 2x buffer, 940 µl H_2O	5.15 mM HHL (in H_2O)
Enzyme + AHL + TS analogue (sample values)	120 µl MBP-His_6-AiiA, 940 µl 2x buffer, 929 µl H_2O 11 µl TS analogue (from 105 µM stock in H_2O)	5.15 mM HHL (in H_2O)

1.6 ^{1}H and ^{13}C NMR spectra



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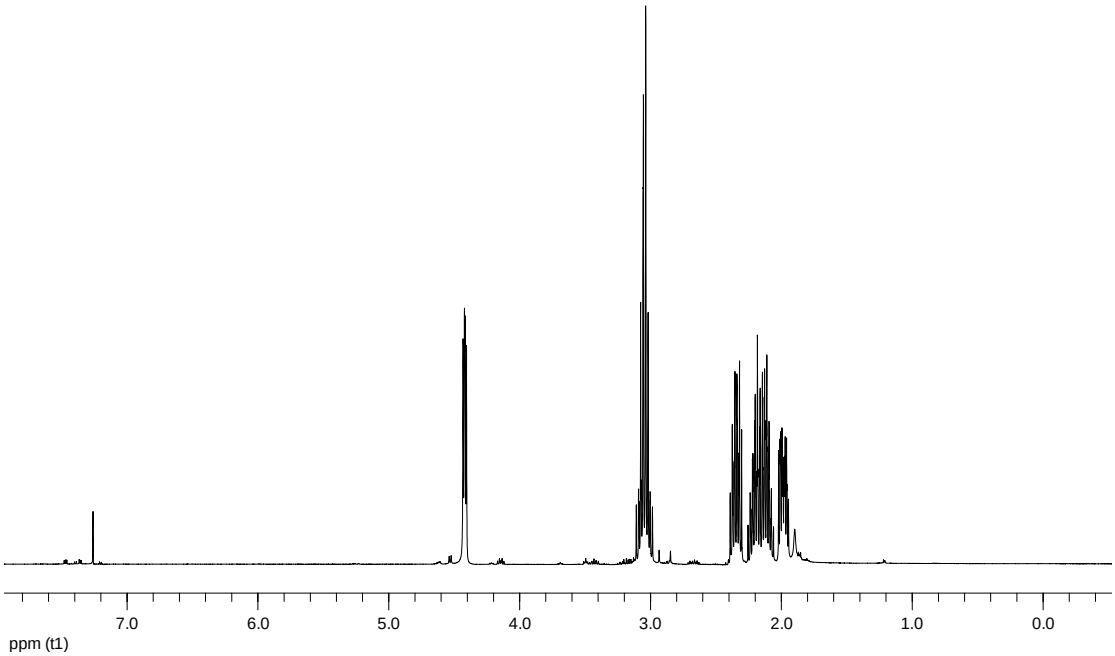

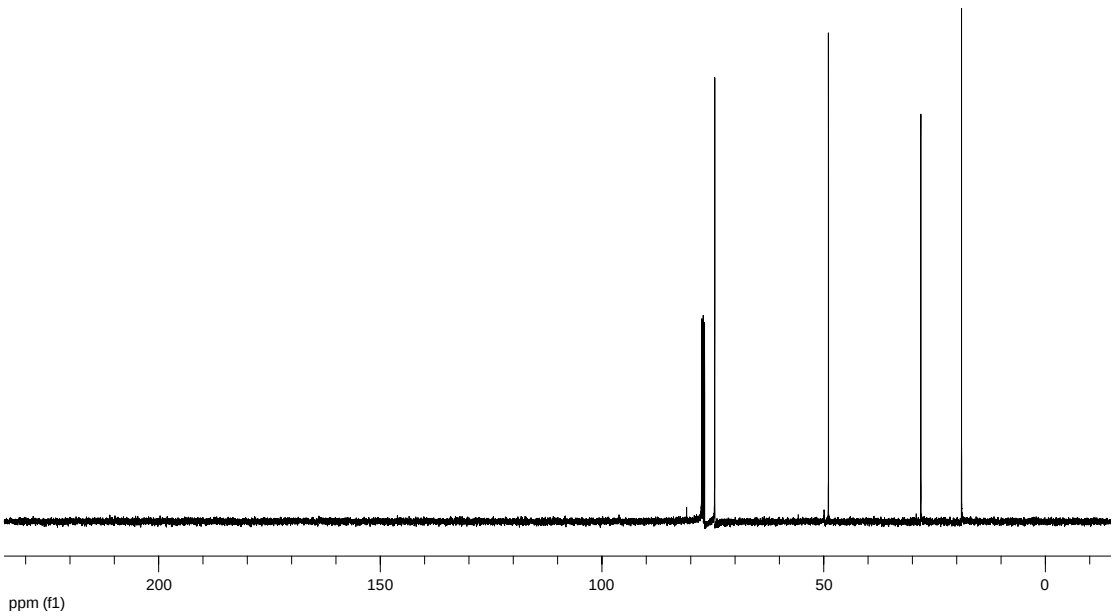

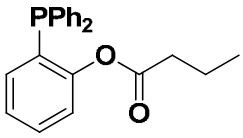

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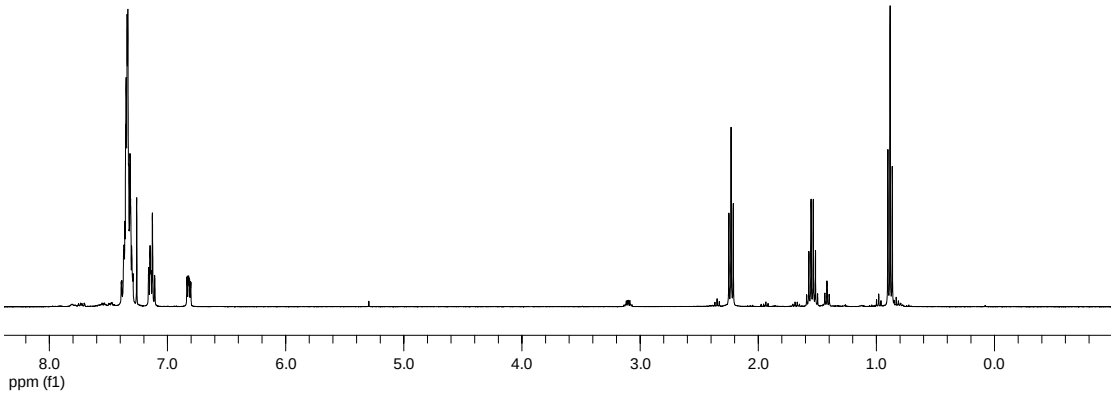

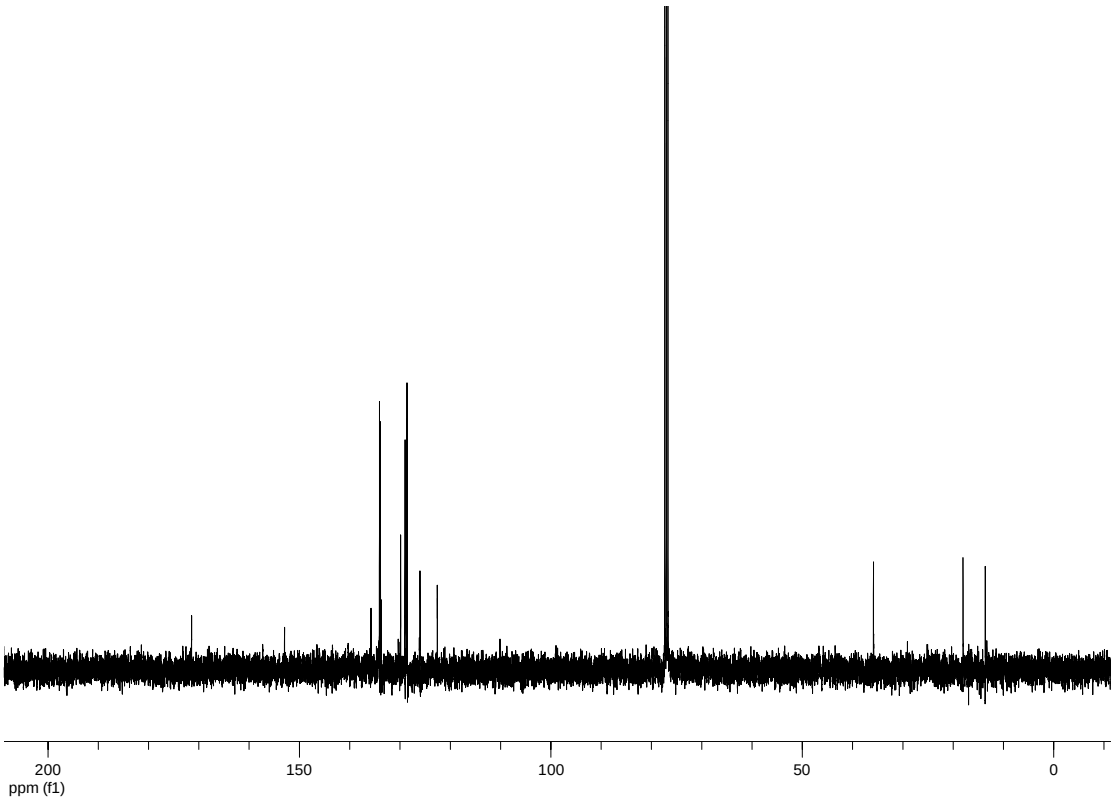

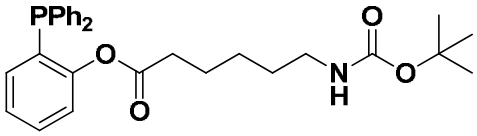

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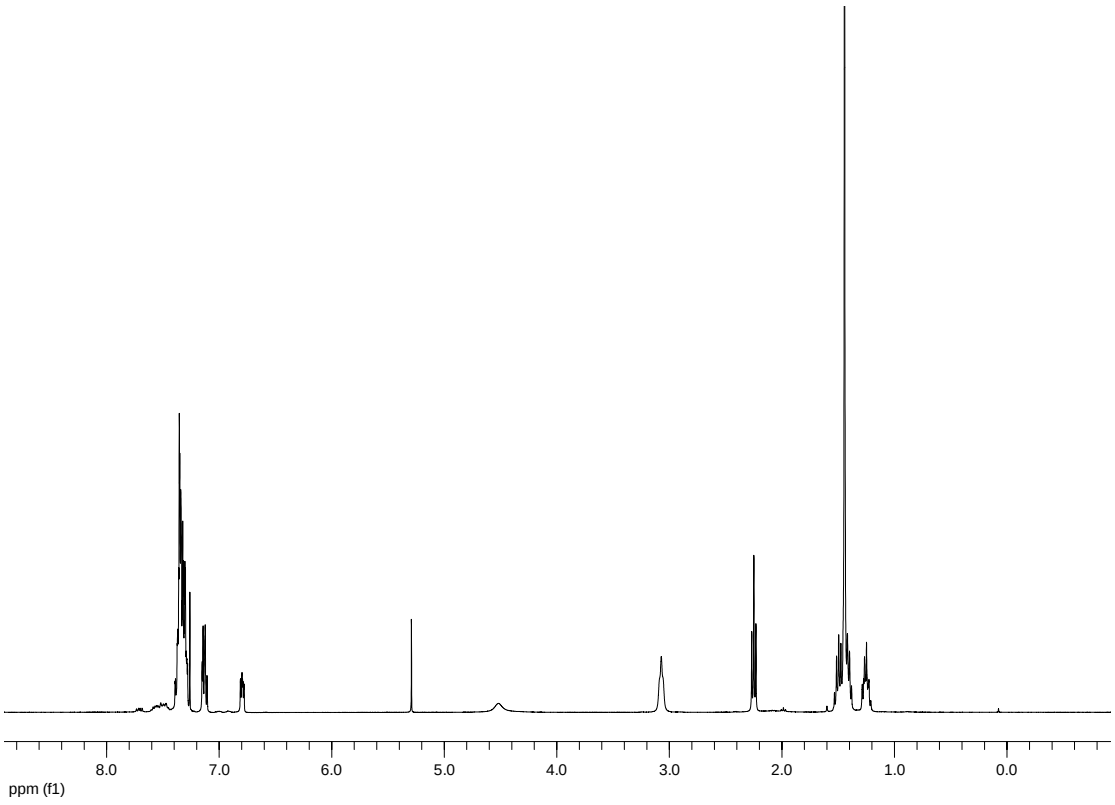

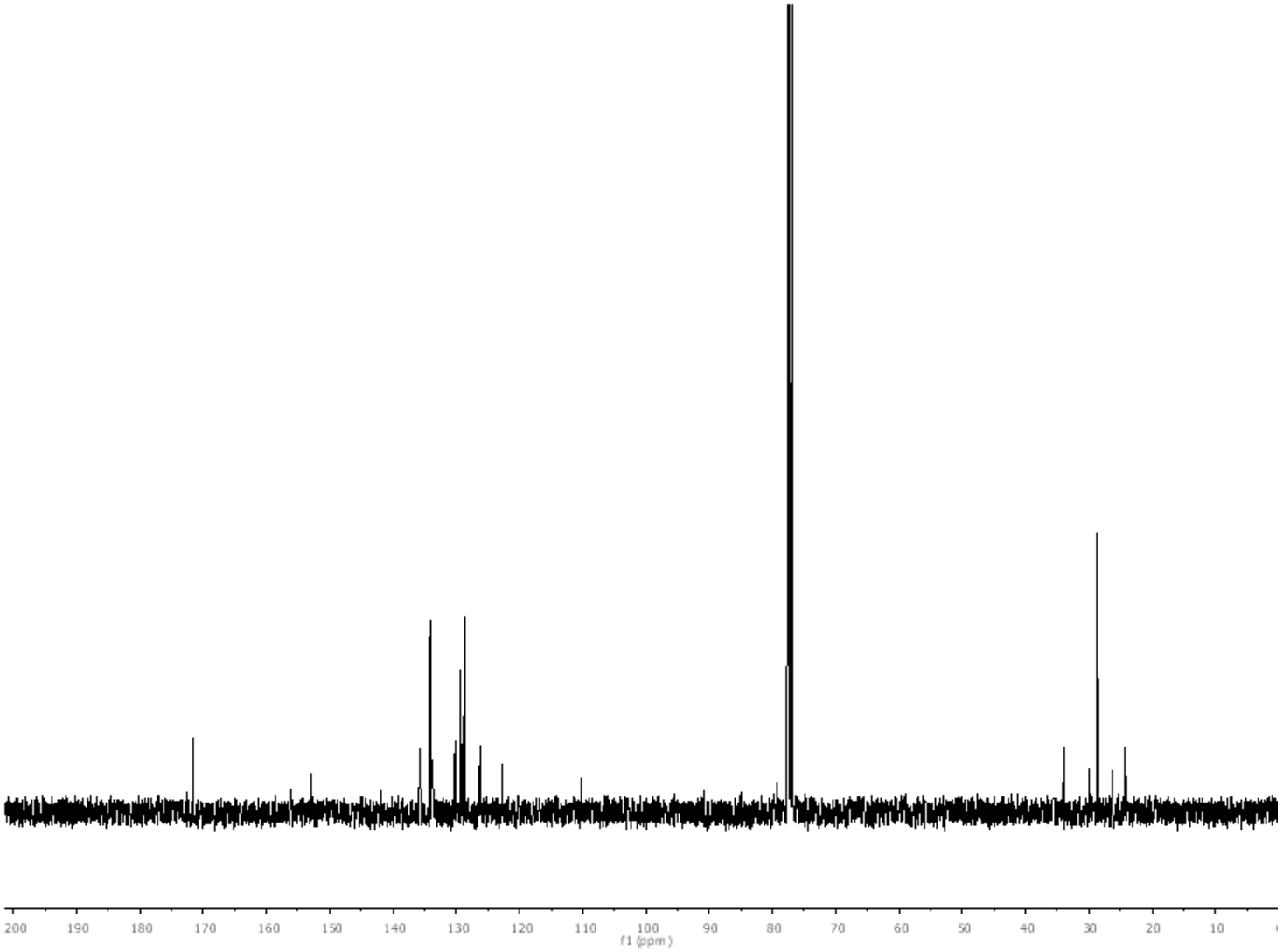
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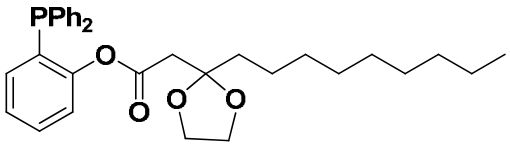

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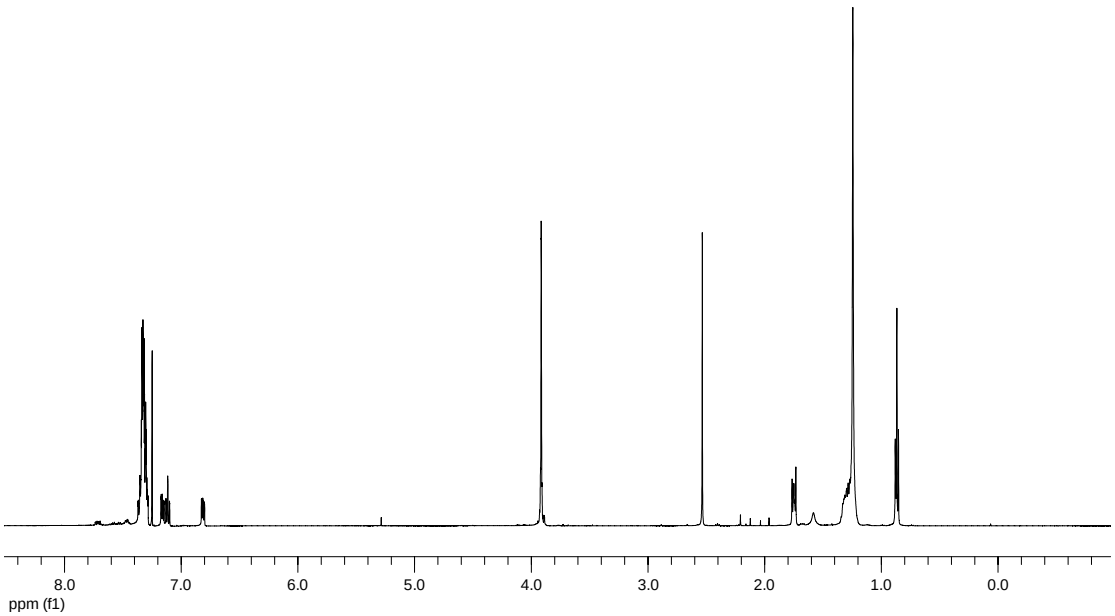

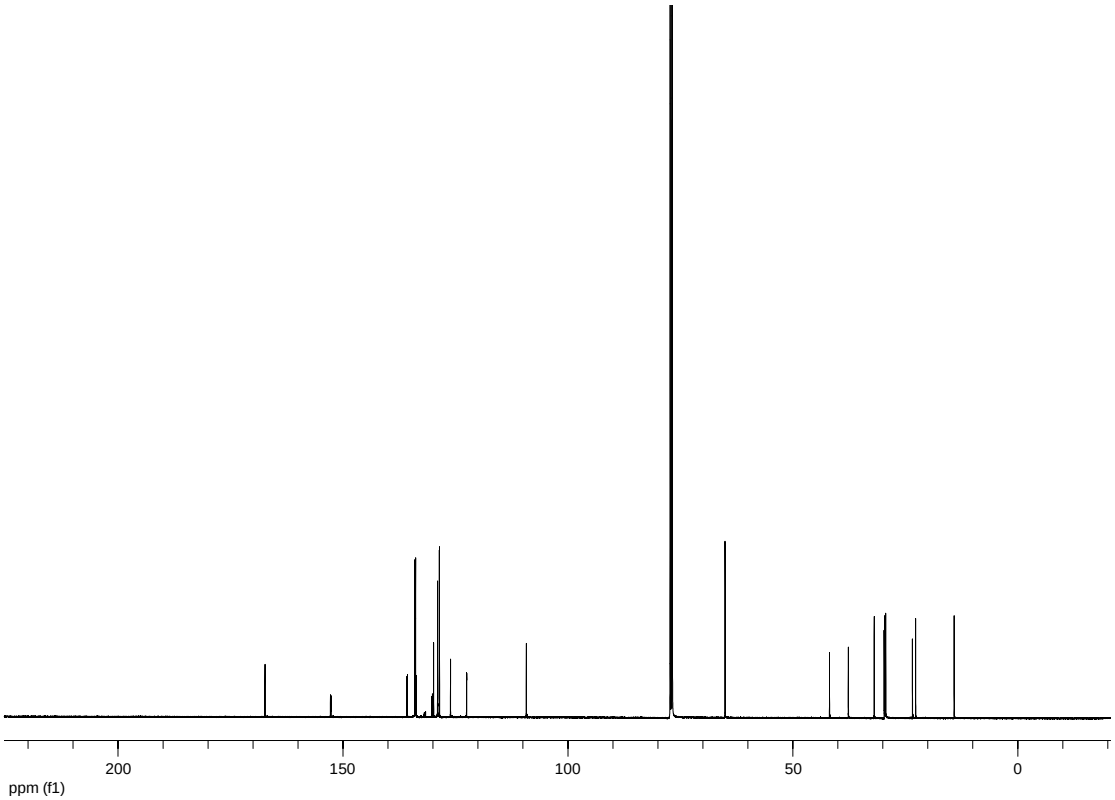

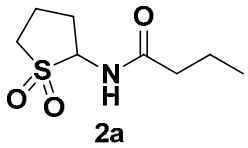

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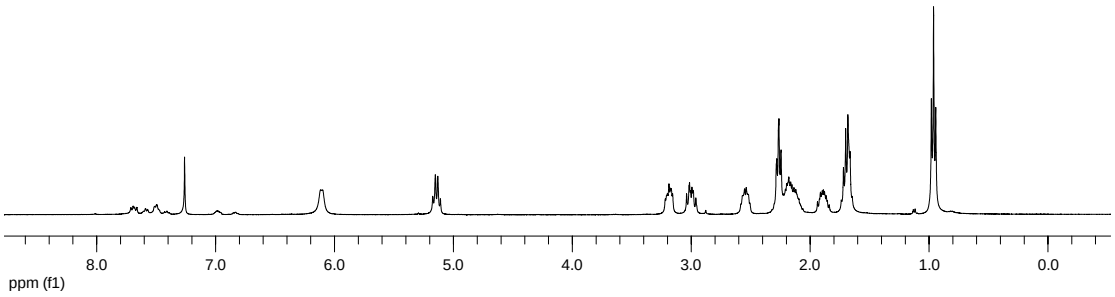

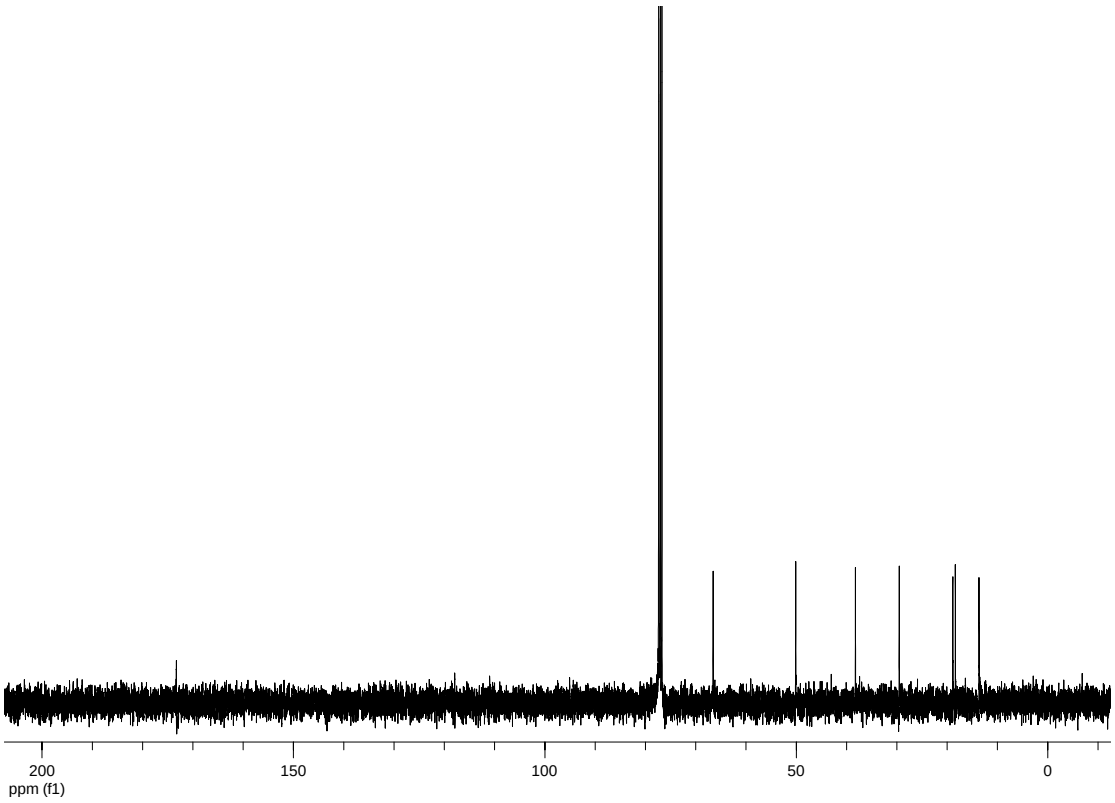

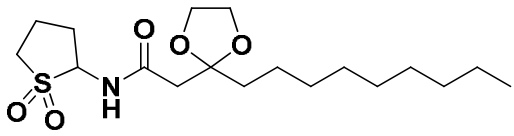

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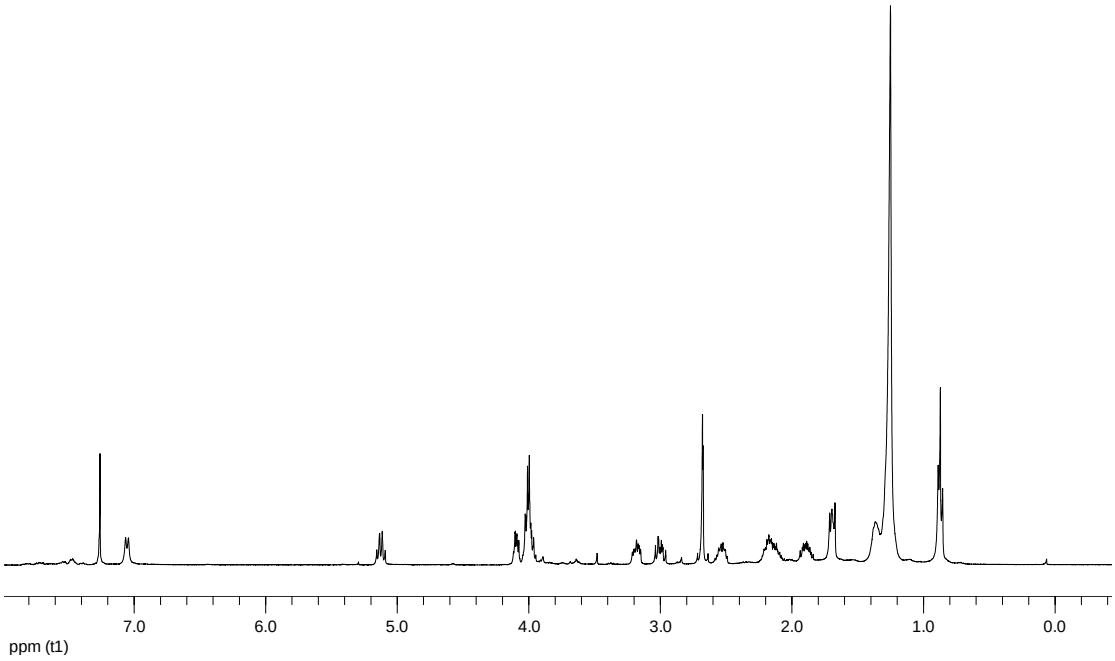

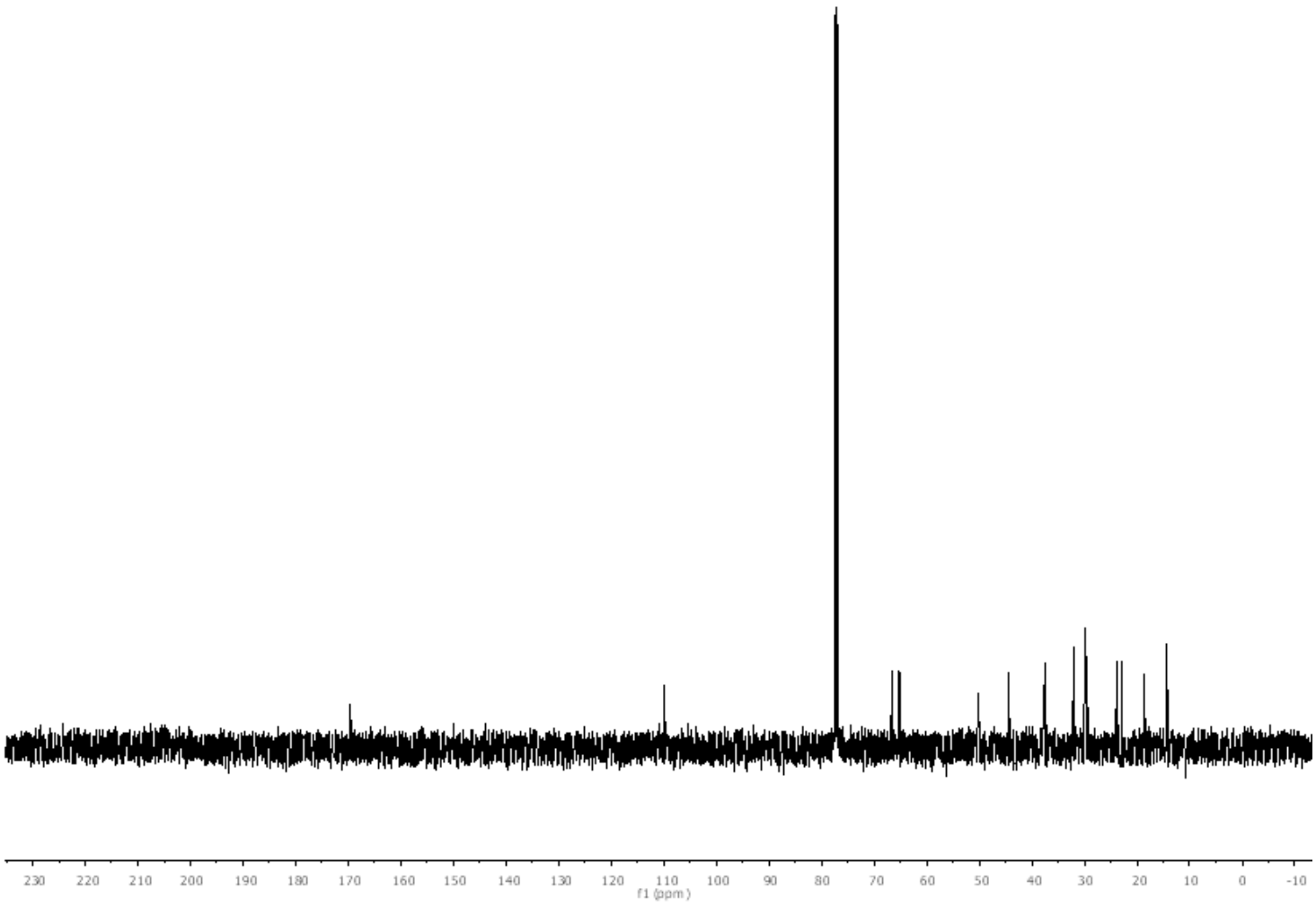
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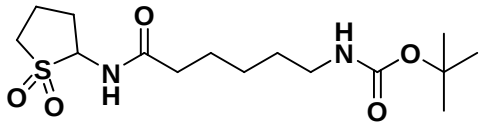

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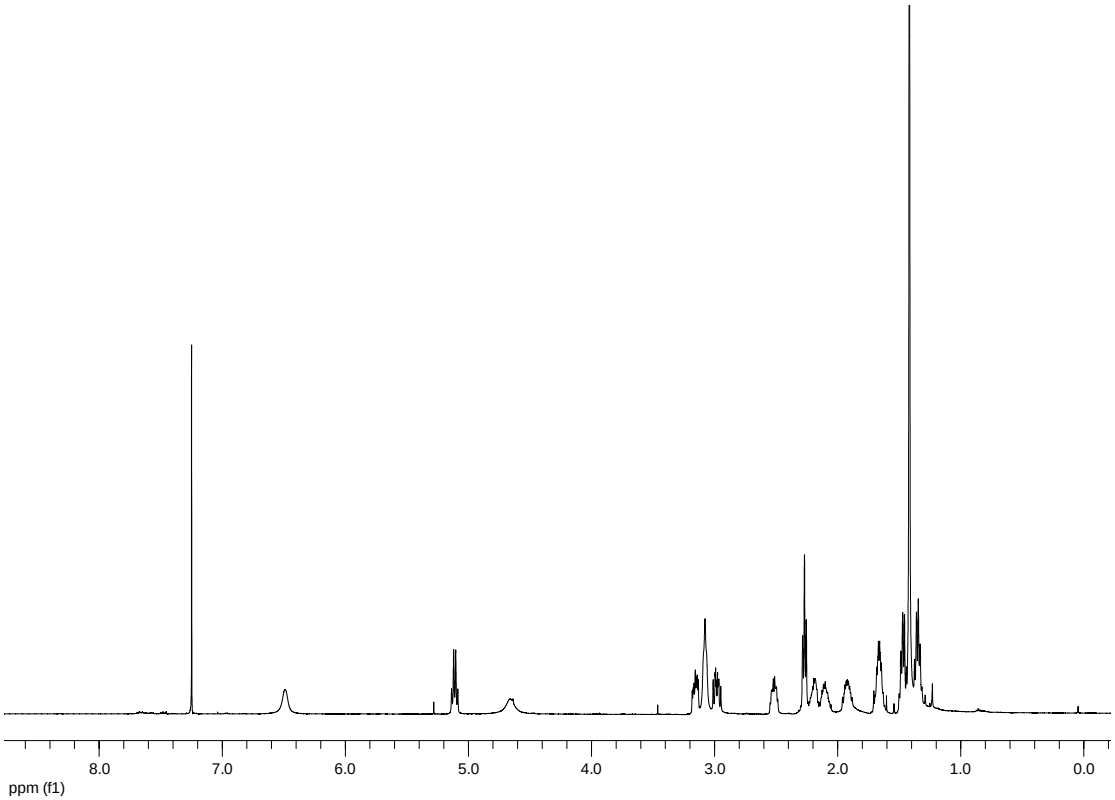

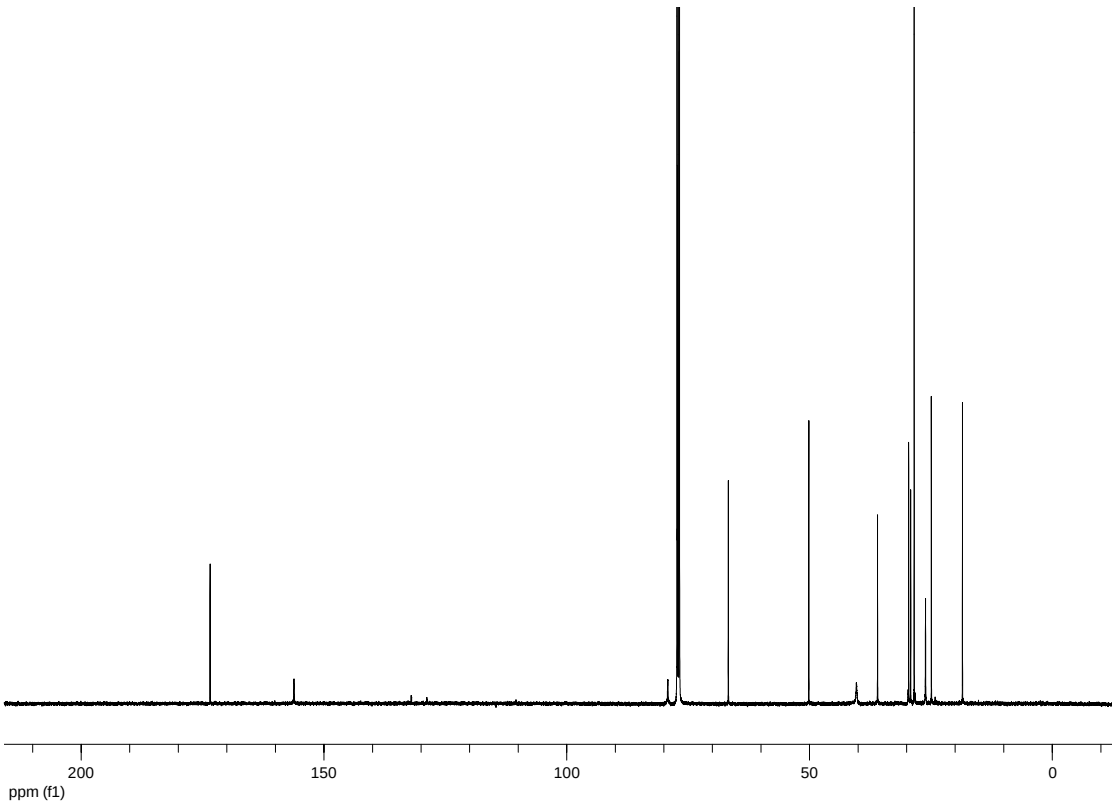

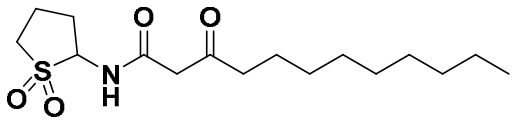

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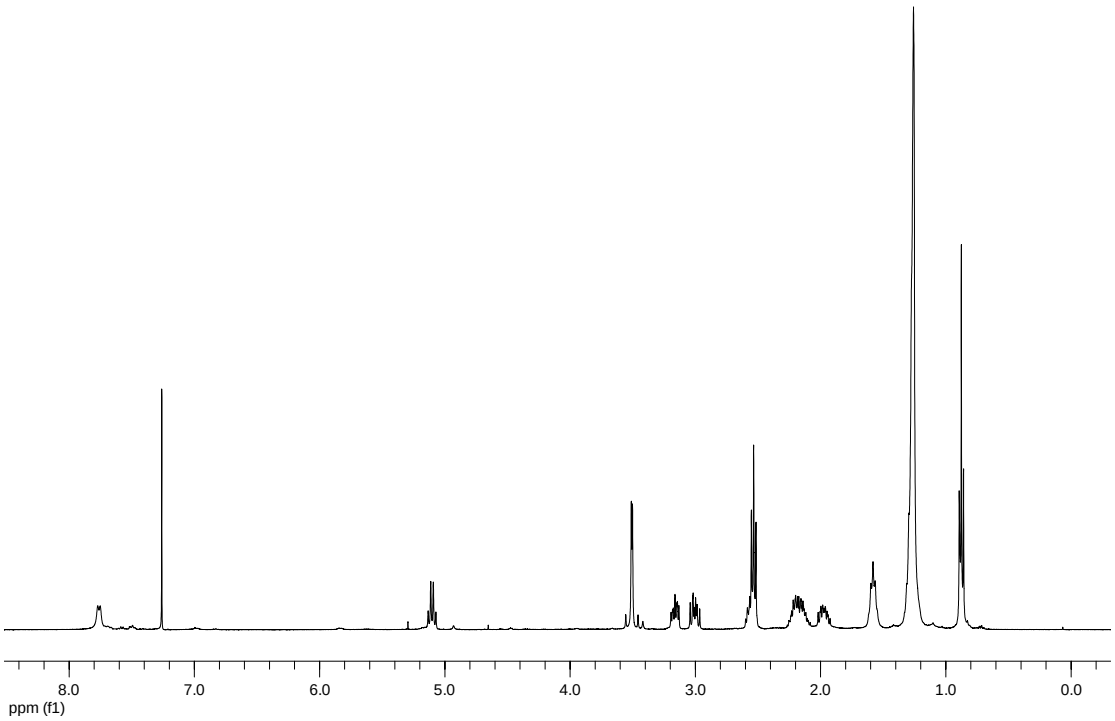

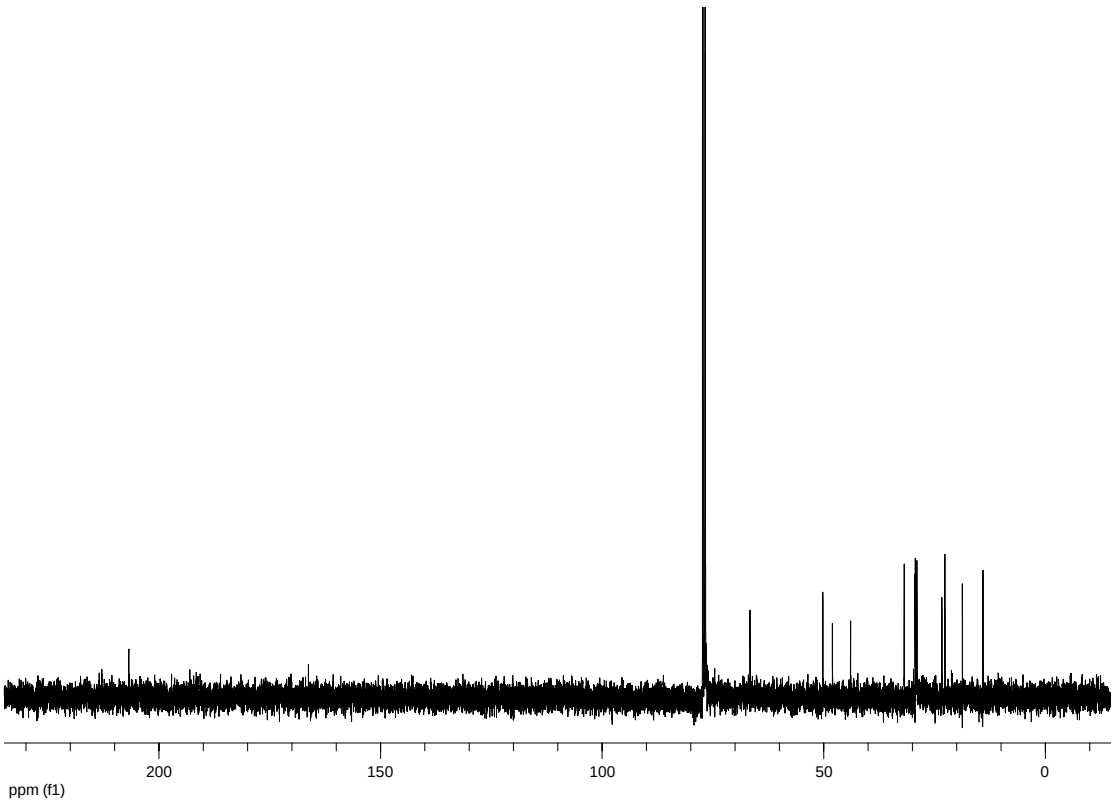

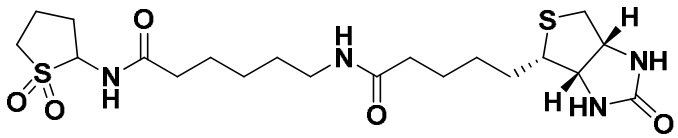

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