Supplementary Information

Inhibitors of an Essential Mycobacterial Cell Wall Lipase (Rv3802c) as Tuberculosis Drug Leads

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General Synthetic Experimental

^{1}H and ^{13}C NMR spectra were recorded at 300K using a Bruker Avance DRX300, or DPX 400 spectrometer. Chemical shifts are reported in parts per million (ppm) and are referenced to solvent residual signals: $CDCl_3$ (δ 7.26 [^{1}H]) and (δ 77.16 [^{13}C]). ^{1}H NMR data is reported as chemical shift (δ_H), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets), coupling constant (*J* Hz) and assignment where possible. ^{13}C NMR data is listed, with peaks belonging to rotamers *italisized*. The presence of rotamers was detected with the assistance of 2D NOESY experiments.

Low resolution mass spectra were recorded on a Finnigan LCQ Deca ion trap mass spectrometer (ESI). High resolution mass spectra were recorded on a Bruker 7T Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR). Infrared (IR) absorption spectra were recorded on a Bruker ALPHA Spectrometer with Attenuated Total Reflection (ATR) capability, using OPUS 6.5 software. Optical rotations of enantioenriched compounds were recorded on a Perkin–Elmer 341 polarimeter at 589 nm (sodium D line) with a cell path length of 1 dm, and the concentrations are reported in g/100 mL.

Materials

Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Kieselgel 60 0.25 mm F254). Flash column chromatography was performed using 230-400 mesh Kieselgel 60 silica eluting with distilled solvents as described. Ratios of solvents used for TLC and column chromatography are expressed in v/v as specified. Compounds were visualised by UV light at 254 nm or using vanillin or cerium molybdate stain.

Commercial materials were used as received unless otherwise noted. Reagents that were not commercially available were synthesized following literature procedures. Dichloromethane and methanol were distilled from calcium hydride, and THF was distilled over sodium/benzophenone. Anhydrous DMF was purchased from Sigma Aldrich.

General Biological Experimental

Bacterial growth conditions

Virulent *M. tuberculosis* H37Rv (ATCC 27294) was grown in Middlebrook 7H9 broth medium supplemented with OADC (Difco Laboratories, Detroit, MI), 0.5% glycerol and 0.05% Tween-80. Freshly seeded cultures were grown at 37 °C, for approximately 14 days, to mid-exponential phase (OD_{600} 0.4-0.8) for use in Rv3802c inhibition assays.

Inhibition of *M. tuberculosis*

The inhibitors were screened using a modified resazurin reduction microplate assay, as previously described.[1] *M. tuberculosis,* grown to mid-exponential phase (OD_{600} 0.4-0.8), was diluted to OD_{600} 0.001 in 7H9S media (Middlebrook 7H9 with OADC, 0.5% glycerol, 0.02% tyloxapol, 1% tryptone) containing 0.5% DMSO. Microtitre plates (96-well) were setup with 100 μL of inhibitors, serially diluted into 7H9S. Diluted *M. tuberculosis* (100 μL), representing approx 2×10^4 CFU/mL was added to each well. Plates were incubated for 5 days at 37 °C in a humidified incubator prior to the addition of 30 μL of a 0.02% resazurin solution and 12.5 μL of 20% Tween 80 to each well. Sample fluorescence was measured after 48 h on a BMG Labtech POLARstar Omega with an excitation wavelength of 530 nm and emission at 590 nm. Changes in fluorescence relative to positive control wells (H37Rv with no inhibitor) minus negative control wells (no H37Rv) were plotted for determination of IC_{50} values.

Rv3802c Enzyme activity and inhibition assay

The lipolytic activity and inhibition of lipolytic activity of Rv3802c was determined by Tween cleavage, as previously reported,[2] with the following modifications. Briefly, a reaction mix was prepared with 66 mM $CaCl_2$ and 0.66% Tween 20 in buffer (50 mM Tris pH 8.0). An aliquot of the reaction mix (75 μL) was transferred to a 96-well microtitre plate where compounds **4–20** were 2-fold serially diluted across the plate. A solution of 50 mM Tris pH 8.0 (75 μL) containing 750 ng purified Rv3802c was added to each well to give a final volume of 150 μL. The assay was performed at 37 °C and

turbidity assessed at 405 nm after 60 min. Inhibition of enzyme activity is reported relative to a control containing no inhibitor.

Inhibitor Reversibility Studies

The enzyme assay was conducted as described above, except that inhibitors were pre-incubated with the enzyme for 30 min prior to addition of the substrate (1 mM, 5 mM, or 10 mM).

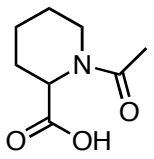
Cytotoxicity Studies

The human embryonic kidney cell line, HEK293, was plated in 96-well microtitre plates at 5×10^3 cells/well in DMEM supplemented with 10% FCS and cultured for 48 h at 37 °C in a humidified incubator with 5% CO_2. Inhibitors were added at reported concentrations and cells were incubated for a further 24 h. Following incubation, media was removed and replaced with 200 μL fresh DMEM/10% FCS, to which 30 μL 0.02% resazurin was added. Resazurin reduction was measured fluorescently in a BMG Labtech POLARstar Omega plate reader at 530 nm/590 nm (Ex/Em). Values are represented relative to controls containing no inhibitor.

Synthetic Experimental Procedures

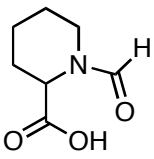
Preparation of Reagents:

1-acetylpiperidine-2-carboxylic acid



To a suspension of D/L-pipecolic acid (0.5 g, 3.9 mmol) in acetic anhydride (5 mL) at room temperature was added 5 drops of concentrated sulfuric acid. The resulting solution was allowed to stir for 24 hours, before diluting with H_2O (10 mL) and extracting with DCM (2 × 10 mL). The combined organic layer was dried (Na_2SO_4), and concentrated *in vacuo*. The resulting residue was re-dissolved in toluene (50 mL) and the solvent was removed *in vacuo* to afford the desired *N*-acetyl-D/L-pipecolic acid as a colourless oil (330 mg, 50%); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.19 (1H, br s, COOH), 5.57–4.55 (2H, m), 3.86–3.70 (1H, m), 2.16 (3H, s), 2.55–1.37 (6H, m). ^{1}H NMR data is in agreement with that previously reported by Pizzorno *et al.*[3]

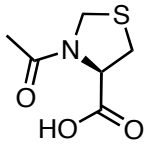
1-formylpiperidine-2-carboxylic acid



To a solution of D/L-pipecolic acid (0.5 g, 3.9 mmol) in formic acid (6.5 mL) at 0 ºC was added acetic anhydride (3.2 mL). The resulting mixture was allowed to warm to room temperature over 16 hours. The solvent was removed *in vacuo*, and the residue was diluted with H_2O (10 mL) and extracted with DCM (2 × 10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The resulting residue was re-dissolved in toluene (50 mL) and the

solvent was removed *in vacuo* to afford the desired *N*-formyl-D/L-pipecolic acid as a colourless oil (378 mg, 62%); **^{1}H NMR** (200 MHz, $CDCl_3$) δ 8.12 (0.7H, s), 8.07 (0.3H, s), 6.74 (1H, br s, COOH), 5.22–5.20 (1H, m), 3.61–3.29 (2H, m), 1.85–1.41 (6H, m). ^{1}H NMR data is in agreement with that previously reported by Pizzorno *et al.*[3]

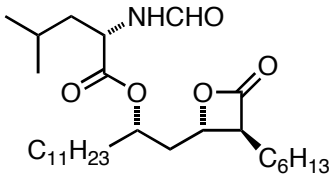
(*R*)-3-acetylthiazolidine-4-carboxylic acid



Fmoc-L-thiazolidine-4-carboxylic acid (100 mg, 0.28 mmol) was dissolved in 1:4 v/v piperidine/MeCN (10 mL) and allowed to stir for 1 hour before the solvent was removed *in vacuo* to afford L-thiazolidine carboxylic acid. This product was subsequently dissolved in 1:9 v/v acetic anhydride in pyridine (10 mL) and the reaction was stirred for 1 hour at room temperature. The solvent was removed *in vacuo*, and the resulting residue was re-dissolved in toluene (3 × 10 mL) and the solvent removed *in vacuo* to afford the desired carboxylic acid as a white solid (30 mg, 61%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.75 (1H, br s, COOH), 5.06 (1H, t, *J* = 5.2, 7.0 Hz), 4.62–4.56 (2H, m), 3.39–3.28 (2H, m), 2.21–2.12 (3H, m). ^{1}H NMR data is in agreement with that previously reported by Croce *et al.*[4]

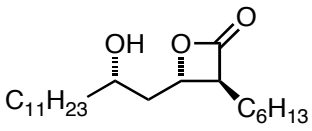
Preparation of Alcohol 3

Tetrahydrolipstatin (THL) (1)



Tetrahydrolipstatin (THL) (**1**) was extracted from the contents of 84 Xenical® capsules (10.1 g by weight of THL) by Soxhlet extraction using chloroform (300 mL). The residue was purified by column chromatography (eluent: 70:30 v/v hexane/EtOAc) to afford tetrahydrolipstatin (**1**) as a white solid (9.88 g, 98%). **m.p.** 44–46 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.22 (1H, s, CH), 6.02 (1H, d, *J* = 8.5 Hz, NH), 5.02 (1H, m, CH), 4.68 (1H, m, CH), 4.28 (1H, m, CH), 3.22 (1H, m, CH), 1.15–2.10 (35H, m, CH_2 + CH), 0.95 (6H, m, CH_3) 0.87 (6H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ 172.1, 170.9, 160.1, 72.8, 69.3, 57.1, 49.7, 41.6, 38.8, 34.1, 31.9, 31.8, 31.6, 29.7, 29.6, 29.5, 29.4, 29.1, 26.8, 25.2, 25.0, 22.9, 22.9, 22.7, 22.4, 14.1, 14.0 (1 signal obscured); **MS** (ESI) *m/z* 496.47 $(M+H)^+$. These data are in agreement with those previously reported by Ortar *et al*.[5]

(3*S*,4*S*)-3-hexyl-4-((*S*)-2-hydroxytridecyl)oxetan-2-one (3)



To a stirred solution of carboxylic acid **2** (0.54 g, 1.16 mmol) in pyridine (20 mL) at 0 °C was added benzenesulfonyl chloride (0.31 mL, 2.40 mmol). The reaction was stirred at 0 °C for 15 min before the reaction was incubated at –18 °C for 24 h. After this time, a further aliquot of benzenesulfonyl chloride (0.23 mL, 1.79 mmol) was added at 0 °C and the reaction mixture stirred at 0 °C for 10 min before incubating at –18 °C for a further 24 hours. The reaction mixture was subsequently diluted with water (75 mL) and extracted into ether (3 × 75 mL). The organic phase was washed with water (3 × 20 ml)

and brine (20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford a yellow oil. The residue was purified by column chromatography (eluent: CH_2Cl_2) to afford the protected β-lactone as a colourless oil (0.44 g, 74%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.45 (1H, dt, *J* = 4.2, 8.1 Hz, CH), 3.94 (1H, m, CH), 3.24 (1H, m, CH), 1.18–2.06 (35H, m, CH_2 + CH), 0.86 (6H, m, CH_3), 1.10 (18H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.0, 74.7, 69.7, 56.8, 40.7, 36.4, 32.0, 31.6, 29.8, 29.7, 29.7, 29.6, 29.4, 29.1, 27.8, 26.8, 25.2, 22.8, 22.6, 14.3, 14.2 (3 signals obscured). These data are in agreement with those previously reported by Ortar *et al*.[5] Tetrabutylammonium fluoride (1M solution in THF, 3.5 mL, 3.5 mmol) and acetic acid (69 μL, 1.18 mmol) were added dropwise to a stirred solution of the protected β-lactone (617 mg, 1.18 mmol) in THF (7 mL) at –20 °C. The reaction mixture was stirred at –20 °C for 15 min before incubating at –18 °C for 15 h. The mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 10 mL). The organic phase was washed with brine (20 mL), dried ($MgSO_4$), and concentrated *in vacuo* to afford a colourless oil. The residue was purified by column chromatography (eluent: 80:20 v/v hexane/EtOAc) to afford alcohol **3** as a white solid (264 mg, 63%). **m.p.** 49–51 ^{o}C; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.46 (1H, dt, *J* = 3.9, 6.3 Hz, CH), 3.79 (1H, m, CH), 3.31 (2H, m, CH + OH), 1.26–2.06 (32H, m, CH_2), 0.88 (6H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.0, 76.4, 69.7, 56.7, 41.3, 36.6, 32.9, 32.1, 29.9, 29.9, 29.7, 29.4, 28.2, 28.1, 27.1, 26.8, 25.4, 23.0, 22.6, 14.3, 14.2 (1 signal obscured); **MS** (ESI) *m/z* 355.2 $(M+H)^+$. These data are in agreement with those previously reported by Ortar *et al*.[5]

Preparation of Inhibitors:

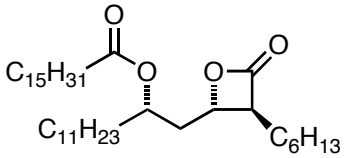
General Procedure A: Esterification of alcohol 3

To a stirred solution of carboxylic acid (2.0 equiv.) in CH_2Cl_2 (0.14 mL) at 0 °C under Argon was added DIC (2.0 equiv.). The reaction was stirred at 0 °C for 15 minutes before the solvent was concentrated *in vacuo*, and the residue dissolved in DMF (141 μL) and added to a solution of alcohol **3** (25 mg, 1.0 equiv.) and DMAP (0.1 equiv.) in DMF (70 μL). The reaction mixture was stirred at room temperature for 15 h before diluting with water and extracting with EtOAc (3 x 10 mL). The organic phase was washed with brine (3 x 10 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The resulting esters were purified by silica gel chromatography using eluting solvents as indicated.

General Procedure B: Esterification of alcohol 3

To a stirred solution of carboxylic acid (2.0 equiv.), DMAP (0.1 equiv.), and alcohol **3** (25 mg, 1.0 equiv.) in DMF (0.5 mL) at 0 °C under Argon was added DIC (2.0 equiv.). The reaction was warmed to room temperature for 15–72 h before diluting with water and extracting with Et_2O (3 × 10 mL). The organic phase was washed with brine (3 × 10 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The resulting esters were purified by silica gel chromatography using eluting solvents as indicated.

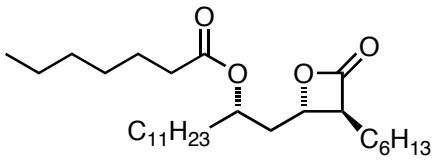
(*R*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl palmitate (4)



Palmitic acid (36 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 95:5 v/v hexane/Et_2O) to afford ester **4** as a colourless amorphous solid (29 mg, 69 %).

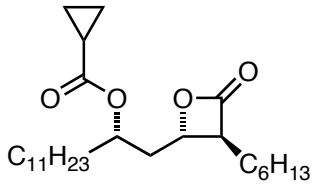
$[\alpha]_D^{25}$ –1.7 (*c* 0.75, $CHCl_3$); **IR** ν_{max}: 2920, 2852, 1827, 1735, 1704, 1465, 1378 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.03–4.96 (1H, m, CH), 4.33–4.29 (1H, m, CH), 3.20 (1H, ddd, *J* = 4.0, 7.6, 7.6 Hz, CH), 2.36–2.27 (4H, m, CH_2), 2.20–2.12 (1H, m, CH_2), 1.95 (1H, app. ddd, *J* = 3.9, 5.7, 14.7 Hz, CH_2), 1.81–1.25 (54H, m, CH_2), 0.89–0.85 (9H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ 173.6 (C=O), 171.3 (C=O), 75.0, 70.8, 57.0, 38.9, 34.7, 34.3, 32.1, 32.1, 31.6, 29.8, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.5, 29.5, 29.5, 29.3, 29.1, 27.8, 26.8, 25.4, 25.1, 22.8, 22.7, 14.3, 14.2 (7 signals obscured); **HRMS** (ESI) *m/z* calcd. for $C_{38}H_{72}O_4Na$ $(M+Na)^+$ 615.5329, found 615.5323.

(*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl hexanoate (5)



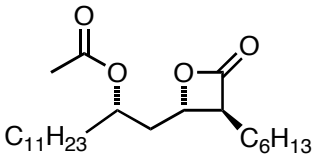
Hexanoic acid (18 μL, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 90:10 v/v hexane/Et_2O) to afford ester **5** as a colourless oil (33 mg, quant.). $[\alpha]_D^{25}$ –2.9 (*c* 1.4, $CHCl_3$); **IR** ν_{max}: 2926, 2856, 1826, 1734, 1465, 1378, 1244 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.04–4.96 (1H, m, CH), 4.31 (1H, ddd, *J* = 4.1, 6.0, 7.2 Hz, CH), 3.19 (1H, ddd, *J* = 4.1, 7.5, 7.5 Hz, CH), 2.30 (2H, t, *J* = 7.2 Hz, CH_2), 2.20–2.11 (1H, m, CH_2), 1.97 (1H, app. ddd, *J* = 3.9, 6.0, 14.8 Hz, CH_2), 1.84–1.25 (36H, m, CH_2), 0.92–0.86 (9H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 173.7 (C=O), 171.3 (C=O), 74.9, 70.8, 57.0, 38.9, 34.5, 34.3, 32.0, 31.6, 31.4, 29.7, 29.6, 29.5, 29.4, 29.4, 29.0, 27.7, 26.8, 25.3, 24.7, 22.7, 22.6, 22.4, 14.2, 14.1, 14.0 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{28}H_{52}O_4Na$ $(M+Na)^+$ 475.3764, found 475.3740.

(*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl cyclopropane carboxylate (6)



Cyclopropane carboxylic acid (11 μL, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 80:20 v/v hexane/EtOAc) to afford ester **6** as a colourless oil (16 mg, 54%). $[\alpha]_D^{25}$ –2.7 (*c* 1.1, $CHCl_3$); **IR** ν_{max}: 2931, 2855, 1827, 1728, 1262 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.02–4.94 (1H, m, CH), 4.34 (1H, ddd, *J* = 4.1, 6.5, 6.5 Hz, CH), 3.20 (1H, ddd, *J* = 4.0, 7.5, 7.5 Hz, CH), 2.23–2.13 (1H, m, CH_2), 2.01 (1H, app. ddd, *J* = 4.0, 6.3, 14.7 Hz, CH_2), 1.87–1.26 (30H, m, CH_2), 1.02–0.86 (11H, m, CH + CH_2 + CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.5 (C=O), 171.3 (C=O), 74.9, 71.1, 57.0, 38.8, 34.3, 32.1, 31.6, 29.9, 29.8, 29.7, 29.6, 29.5, 29.1, 27.8, 26.8, 25.4, 22.8, 22.7, 14.3, 14.2, 13.1, 8.6, 8.5 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{26}H_{46}O_4Na$ $(M+Na)^+$ 445.3294, found 445.3288.

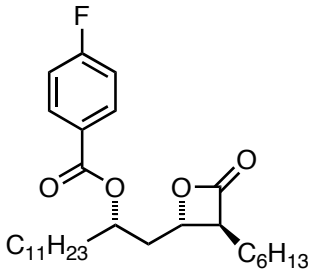
(*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl acetate (7)



To a solution of the alcohol **3** (25 mg, 0.07 mmol, 1.0 equiv.) in pyridine (0.5 mL) at room temperature was added acetic anhydride (0.5 mL, excess), and the reaction allowed to stir for 4 hours. The reaction mixture was diluted with H_2O (10 mL) and Et_2O (10 mL) and extracted with Et_2O (2 × 15 mL). The combined organic extract was dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was purified by column chromatography (eluent: 80:20 v/v hexane/EtOAc) to afford ester **7** as a colourless oil (22 mg, 78%).

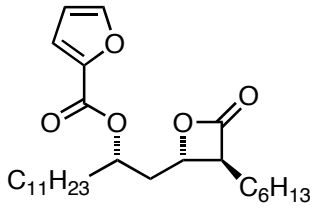
$[\alpha]_D^{25}$ –3.2 (*c* 0.12, CH_2Cl_2); **IR** ν_{max}: 2924, 2854, 1823, 1737, 1465, 1372, 1235, 1118, 1022 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.02–4.96 (1H, m, CH), 4.34–4.30 (1H, m, CH), 3.22–3.17 (1H, m, CH), 2.18–2.09 (1H, m, CH_2), 2.05 (3H, s, CH_3), 1.99–1.93 (1H, m, CH_2), 1.84–1.24 (30H, m, CH_2), 0.88–0.85 (6H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ 171.3 (C=O), 170.9 (C=O), 75.1, 71.2, 57.0, 38.9, 34.3, 32.0, 31.6, 29.7, 29.7, 29.6, 29.5, 29.5, 29.1, 27.8, 26.8, 25.4, 22.8, 22.6, 21.3, 14.3, 14.2 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{24}H_{44}O_4Na$ $(M+Na)^+$ 419.3132, found 419.3131.

(*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl 4-fluorobenzoate (8)



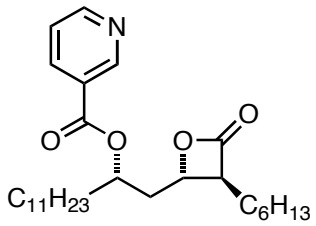
4-Fluorobenzoic acid (20 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 90:10 v/v hexane/EtOAc) to afford ester **8** as a colourless oil (12 mg, 36%). $[\alpha]_D^{25}$ –0.40 (*c* 0.49, $CHCl_3$); **IR** ν_{max}: 2925, 2855, 1825, 1717, 1605, 1508, 1465, 1270, 1239 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.07–8.02 (2H, m, Ar-H), 7.15–7.09 (2H, m, Ar-H), 5.26–5.18 (1H, m, CH), 4.40–4.35 (1H, m, CH), 3.24 (1H, ddd, *J* = 4.2, 7.5, 7.5 Hz, CH), 2.36–2.26 (1H, m, CH_2), 2.13 (1H, ddd, *J* = 4.0, 5.9, 14.8 Hz, CH_2), 1.79–1.24 (30H, m, CH_2), 0.89–0.83 (6H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.0 (C=O), 165.8 (d, $J_{C\text{-}F}$ = 253 Hz, *i*-ArC), 165.1 (C=O), 132.1 (d, $J_{C\text{-}F}$ = 9.3 Hz, *m*-ArC), 126.3, 115.6 (d, $J_{C\text{-}F}$ = 21.8 Hz, *o*-ArC), 74.7, 71.2, 56.9, 39.0, 34.4, 32.0, 31.6, 29.8, 29.7, 29.6, 29.5, 29.5, 29.1, 27.8, 26.8, 25.4, 22.8, 22.6, 14.3, 14.1 (4 signals obscured); **HRMS** (ESI) *m/z* calcd. for $C_{29}H_{45}FO_4Na$ $(M+Na)^+$ 499.3200, found 499.3194.

(*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl furan-2-carboxylate (9)



2-Furan-carboxylic acid (18 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 90:10 v/v hexane/EtOAc) to afford ester **9** as a colourless oil (9 mg, 28%). $[\alpha]_D^{25}$ –0.4 (*c* 1.23, $CHCl_3$); **IR** ν_{max}: 2924, 2854, 1826, 1725, 1580, 1471, 1396, 1294, 1230 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.59–7.58 (1H, m, Ar-H), 7.19 (1H, d, *J* = 3.6 Hz, Ar-H), 6.53–6.51 (1H, m, Ar-H), 5.24–5.16 (1H, m, CH), 4.40 (1H, ddd, *J* = 4.2, 6.6, 6.6 Hz, CH), 3.25 (1H, ddd, *J* = 4.0, 7.5, 7.5 Hz, CH), 2.36–2.26 (1H, m, CH_2), 2.08 (1H, app. ddd, *J* = 4.0, 6.4, 14.8 Hz, CH_2), 1.79–1.24 (30H, m, CH_2), 0.87–0.84 (6H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.4 (C=O), 158.6 (C=O), 146.9, 144.9, 118.6, 112.3, 75.0, 72.2, 57.4, 39.1, 34.5, 32.3, 31.8, 30.1, 30.0, 29.9, 29.8, 29.7, 29.3, 28.1, 27.1, 25.7, 23.1, 22.9, 14.5, 14.4 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{27}H_{44}O_5H$ $(M+H)^+$ 449.3267, found 449.3262.

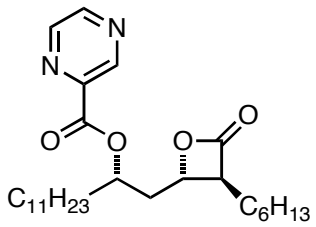
(*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl picolinate (10)



3-Pyridine carboxylic acid (17 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 80:20 v/v hexane/EtOAc) to afford ester **10** as a colourless oil (23 mg, 71%). $[\alpha]_D^{25}$ –1.7 (*c* 0.17, $CHCl_3$); **IR** ν_{max}: 2925, 2855, 1825, 1723, 1591, 1466,

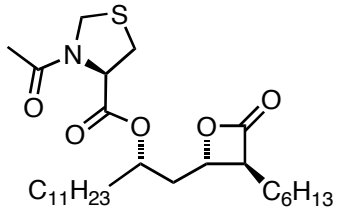
1419, 1280 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.22 (1H, br s, Ar-H), 8.80 (1H, dd, *J* = 1.5, 4.8 Hz, Ar-H), 8.28 (1H, app. dt, *J* = 1.9, 8.0 Hz, Ar-H), 7.40 (1H, dd, *J* = 4.8, 8.0 Hz, Ar-H), 5.32–5.24 (1H, m, CH), 4.41–4.36 (1H, m, CH), 3.24 (1H, ddd, *J* = 4.1, 7.4, 7.4 Hz, CH), 2.40–2.27 (1H, m, CH_2), 2.15 (1H, app. ddd, *J* = 4.2, 5.1, 14.8 Hz, CH_2), 1.84–1.11 (30H, m, CH_2), 0.89–0.83 (6H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 171.0 (C=O), 165.0 (C=O), 153.7, 151.0, 137.2, 126.1, 123.5, 74.9, 72.6, 57.1, 39.0, 34.3, 32.0, 31.5, 29.7, 29.6, 29.5, 29.4, 29.4, 29.0, 27.8, 26.8, 25.4, 22.8, 22.6, 14.2, 14.1 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{28}H_{45}NO_4H$ $(M+H)^+$ 460.3427, found 460.3421.

(*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl pyrazine-2-carboxylate (11)



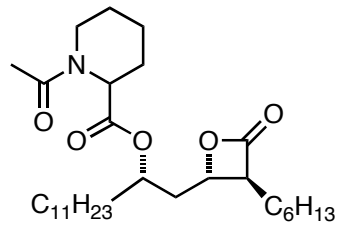
2-Pyrazine carboxylic acid (18 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 80:20 v/v hexane/EtOAc) to afford ester **11** as a colourless oil (27 mg, 84%). $[\alpha]_D^{25}$ –2.9 (*c* 2.2, $CHCl_3$); **IR** ν_{max}: 2923, 2854, 1822, 1720, 1466, 1301 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 9.29 (1H, br s, Ar-H), 8.76 (2H, m, Ar-H), 5.42–5.33 (1H, m, CH), 4.45–4.39 (1H, m, CH), 3.22 (1H, ddd, *J* = 4.1, 7.4, 7.4 Hz, CH), 2.42-–2.31 (1H, m, CH_2), 2.11 (1H, app. ddd, *J* = 4.0, 5.1, 14.9 Hz, CH_2), 1.91–1.26 (30H, m, CH_2), 0.88–0.83 (6H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 170.9 (C=O), 163.7 (C=O), 147.8, 146.4, 144.6, 143.5, 76.7, 74.2, 57.2, 39.0, 34.3, 32.0, 31.5, 29.6, 29.6, 29.5, 29.4, 29.4, 29.0, 27.8, 26.8, 25.4, 22.8, 22.6, 14.2, 14.1 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{27}H_{44}N_2O_4Na$ $(M+Na)^+$ 483.3193, found 483.3193.

(*R*)-((*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 3-acetylthiazolidine-2-carboxylate (12)



N-acetyl-L-thiazolidine carboxylic acid (24 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 80:20 v/v hexane/EtOAc) to afford ester **12** as a colourless oil (28 mg, 78%). $[\alpha]_D^{25}$ –5.0 (*c* 0.08, CH_2Cl_2); **IR** ν_{max}: 2924, 2855, 1824, 1740, 1665, 1465, 1402, 1195, 1130 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) 5.18–4.99 (2H, m, CH + CH_2), 4.81–4.29 (3H, m, CH + CH_2), 3.48–3.15 (3H, m, CH + CH_2), 2.17–1.25 (35H, m, CH_2 + CH_3), 0.89–0.86 (6H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) 171.2 (C=O), 169.7 (C=O), *169.6*, 168.9 (C=O), *75.4*, 75.1, *74.0*, 73.1, *62.9*, 61.5, *57.3*, 57.1, 49.3, *48.5*, *39.4*, 38.8, 34.4, 33.0, 32.1, 31.6, 29.8, 29.7, *29.6*, *29.6*, 29.6, 29.5, 29.5, *29.3*, 29.1, 29.1, 27.7, 26.8, 25.2, *23.2*, 22.8, 22.7, 22.7, *22.6,* 14.3, 14.2; **HRMS** (ESI) *m/z* calcd. for $C_{28}H_{49}NO_5SNa$ $(M+Na)^+$ 534.3224, found 534.3222.

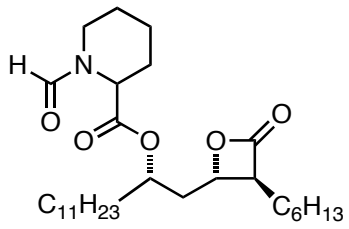
(*R*/*S*)-((S)-1-((2S,3S)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 1-acetylpiperidine-2-carboxylate (13)



N-acetyl-L/D-pipecolic acid (24 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure B, over 72 hours. The crude product was purified by column chromatography (eluent: 60:40 v/v hexane/EtOAc) to afford ester **13**

as a colourless oil (20 mg, 57%, *d.r.* 50:50). $[\alpha]_D^{25}$ –22.4 (*c* 0.17, CH_2Cl_2); **IR** ν_{max}: 2925, 2855, 1822, 1734, 1651, 1416, 1194, 1163, 1116 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.31–4.97 (3H, m, CH + CH_2), 4.57–4.28 (3H, m, CH + CH_2), 3.72–3.69 (2H, m, CH), 3.37–3.18 (4H, m, CH + CH_2), 2.32–1.24 (82H, m, CH_2 + CH_3), 0.88–0.85 (12H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ 171.4 (C=O), 171.3 (C=O), 171.2 (C=O), 170.9 (C=O), 170.9 (C=O), 170.8 (C=O), *170.7*, *75.6*, 74.9, 74.6, *73.0*, *72.9*, 72.3, 72.0, 57.2, *57.2*, 57.1, 57.0, 52.0, 51.9, 44.3, 44.2, *39.6*, *39.5*, *39.4*, *39.2*, 38.8, *34.5*, *34.5*, 34.3, 34.1, 32.0, 31.6, 31.6, 29.7, 29.7, 29.6, 29.6, 29.6, 29.5, 29.4, 29.4, 29.3, 29.1, 29.1, 27.7, 27.7, 27.7, *27.5*, *27.1*, 26.9, 26.8, 26.8, 26.7, 26.5, *25.4*, 25.4, 25.3, *24.6*, *24.6*, 22.8, 22.7, 22.6, 21.8, 21.8, 21.6, *21.1*, 21.0, 20.8, 14.3, 14.2 (6 signals obscured); **HRMS** (ESI) *m/z* calcd. for $C_{30}H_{53}NO_5Na$ $(M+Na)^+$ 530.3822, found 530.3816.

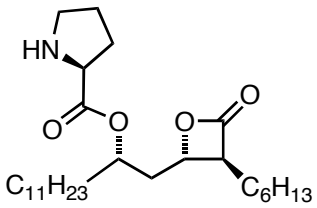
(*R*/*S*)-((*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 1-formylpiperidine-2-carboxylate (14)



N-formyl-L/D-pipecolic acid (22 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure B, over 15 hours. The crude product was purified by column chromatography (eluent: 60:40 v/v hexane/EtOAc) to afford ester **14** as a colourless oil (36 mg, quant., *d.r.* 50:50). $[\alpha]_D^{25}$ –18.2 (*c* 0.14, CH_2Cl_2); **IR** ν_{max}: 2925, 2856, 1823, 1737, 1680, 1427 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.08 (0.7H, s, CH), 8.07 (0.7H, s, CH), 8.02 (0.3H, s, CH), 8.01 (0.3H, s, CH), 5.15–4.99 (3H, m, CH + CH_2), 4.34–4.26 (3H, m, CH + CH_2), 3.54–3.17 (6H, m, CH + CH_2), 2.33–1.33 (76H, m, CH_2), 0.87–0.84 (12H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ 171.0 (C=O), 170.8 (C=O), *170.7*, 170.4 (C=O), 170.3 (C=O), *162.6*, *162.5*, 162.2 (C=O), 162.1 (C=O), *75.5*, *75.4*, 74.8, 74.7, *73.2*, *73.0*, 72.7, 72.5, *57.1*, 57.1, 57.0, 50.8, 50.7, 44.2, 44.2, *39.4*, *39.2*,

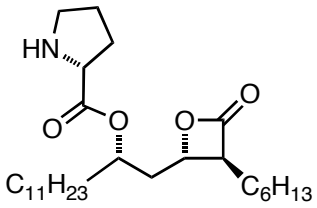
38.8, 38.8, *38.4*, *38.2*, 34.3, 34.2, 34.1, 32.0, 31.6, 31.5, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.1, 29.0, 27.7, 27.7, *27.6*, *27.4*, *27.1*, 26.8, 26.8, 26.8, 26.5, 26.3, 25.5, *25.4*, 25.3, *25.3*, 25.3, 24.2, 24.2, 22.8, 22.6, 21.7, *21.6*, *21.6*, 21.6, 14.2, 14.1; **HRMS** (ESI) *m/z* calcd. for $C_{29}H_{51}NO_5Na$ $(M+Na)^+$ 516.3665, found 516.3660.

(*S*)-((*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) pyrrolidine-2-carboxylate (15)



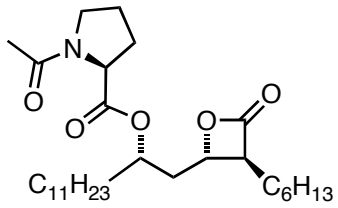
N-Cbz-L-proline (35 mg, 0.14 mmol), was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure B, over 72 hours. The crude product was purified by column chromatography (eluent: 60:40 v/v hexane/EtOAc) to afford ester **21** as a colourless oil (43 mg, quant.). A stirred suspension of ester **21** (30 mg, 0.05 mmol) and 10% Pd/C (10 mg) in MeOH (1.5 mL) at room temperature was allowed to react under an atmosphere of H_2 for 4 h. The suspension was filtered through a pad of celite® and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography (eluent: 30:70 v/v hexane/EtOAc + 0.05% Et_3N) to afford ester **15** as a colourless oil (14 mg, 62%). $[\alpha]_D^{25}$ –30.6 (*c* 0.085, CH_2Cl_2); **IR** ν_{max}: 2924, 2855, 1824, 1732, 1464, 1183, 1120 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.08–5.02 (1H, m, CH), 4.33–4.28 (1H, m, CH), 3.80 (1H, app. dd, *J* = 5.6, 7.6 Hz, CH), 3.22–2.93 (4H, m, CH + CH_2 + NH), 2.19–1.24 (36H, m, CH_2), 0.88–0.85 (6H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ 174.7 (C=O), 171.1 (C=O), 75.2, 72.3, 59.9, 57.1, 47.1, 39.1, 34.4, 32.0, 31.6, 30.2, 29.7, 29.7, 29.6, 29.5, 29.4, 29.1, 27.7, 26.8, 25.4, 25.3, 22.8, 22.6, 14.3, 14.2 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{27}H_{49}NO_5H$ $(M+H)^+$ 452.3734, found 452.3728.

(*R*)-((*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) pyrrolidine-2-carboxylate (16)



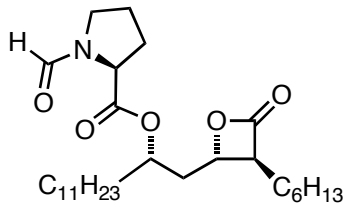
N-Cbz-D-proline (35 mg, 0.14 mmol), was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure B, over 72 hours. The crude product was purified by column chromatography (eluent: 60:40 v/v hexane/EtOAc) to afford ester **22** as a colourless oil (43 mg, quant.). A stirred suspension of ester **22** (30 mg, 0.05 mmol) and 10% Pd/C (10 mg) in MeOH (1.5 mL) at room temperature was allowed to react under an atmosphere of H_2 for 4 h. The suspension was filtered through a pad of celite® and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography (eluent: 30:70 v/v hexane/EtOAc + 0.05% Et_3N) to afford ester **16** as a colourless oil (16 mg, 68%). $[\alpha]_D^{25}$ +29.5 (*c* 0.095, CH_2Cl_2); **IR** ν_{max}: 2926, 2855, 1825, 1732, 1465, 1377, 1327, 1026, 1179, 1120 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.07–5.01 (1H, m, CH), 4.34–4.30 (1H, m, CH), 3.76–3.72 (1H, m, CH), 3.23–3.18 (1H, m, CH_2), 3.11–3.05 (1H, m, CH_2), 2.94–2.88 (1H, m, CH), 2.20–2.09 (1H, m, CH_2), 2.01–1.95 (1H, m, CH_2), 1.87–1.24 (35H, m, CH_2 + NH), 0.89–0.85 (6H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ 175.3 (C=O), 171.2 (C=O), 75.0, 71.7, 60.0, 57.1, 47.1, 39.0, 34.3, 32.1, 31.6, 30.5, 29.8, 29.7, 29.6, 29.5, 29.4, 29.1, 27.8, 26.9, 25.6, 25.4, 22.8, 22.7, 14.3, 14.2 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{27}H_{49}NO_4H$ $(M+H)^+$ 452.3734, found 452.3740.

(*S*)-((*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 1-acetylpyrrolidine-2-carboxylate (17)



N-acetyl-L-proline (22 mg, 0.14 mmol) was reacted with alcohol **3** (25 mg, 0.07 mmol) *via* general procedure A. The crude product was purified by column chromatography (eluent: 60:40 v/v hexane/EtOAc) to afford ester **17** as a colourless oil (18 mg, 51%). $[\alpha]_D^{25}$ –12.0 (*c* 1.0, $CHCl_3$); **IR** ν_{max}: 2926, 2855, 1823, 1740, 1655, 1416, 1354, 1278 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.15–4.97 (1H, m, CH), 4.43 (1H, dd, *J* = 3.7, 8.2 Hz, CH), 4.37–4.26 (1H, m, CH), 3.67–3.46 (2H, m, CH_2), 3.18 (1H, ddd, *J* = 4.0, 7.4, 7.4 Hz, CH), 2.29–1.25 (39H, m, CH_2 + CH_3), 0.89–0.85 (6H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.1 (C=O), 171.3 (C=O), *169.6*, 169.4 (C=O), *75.3*, 75.2, *73.0*, 72.2, *60.4*, 59.0, *57.3*, 57.1, 47.9, *46.4*, *39.4*, 39.0, *34.5*, 34.5, 32.0, 31.6, *31.4*, 29.8, 29.7, *29.6*, 29.6, 29.5, 29.4, *29.3*, 29.1, *29.1*, 27.7, *26.9*, 26.8, *25.4*, 25.3, 25.0, *22.9*, 22.8, 22.7, 22.4, 14.2, 14.1 (2 signals obscured); **HRMS** (ESI) *m/z* calcd. for $C_{29}H_{51}NO_5H$ $(M+H)^+$ 494.3845, found 494.3840.

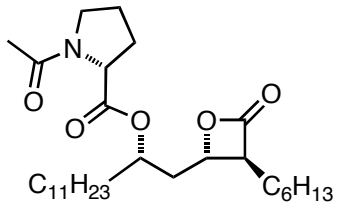
(*S*)-((*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 1-formylpyrrolidine-2-carboxylate (18)



To a solution of amine **15** (14 mg, 0.03 mmol) in DCM (0.7 mL) at room temperature was added formic acetic anhydride[6] (0.15 mL, excess), and the resulting mixture was

allowed to stir for 20 minutes. The reaction mixture was then diluted with Et_2O (10 mL) and H_2O (10 mL), extracted with Et_2O (2 × 15 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was purified by column chromatography (eluent: 60:40 v/v hexane/EtOAc) to afford ester **18** as a colourless oil (10.3 mg, 72%). $[\alpha]_D^{25}$ –61.3 (*c* 0.14, CH_2Cl_2); **IR** ν_{max}: 2926, 2855, 1823, 1741, 1676, 1379 cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.28 (0.7H, s, CH), 8.25 (0.3H, s, CH), 5.15–4.99 (1H, m, CH), 4.42–4.26 (2H, m, CH), 3.70–3.51 (2H, m, CH_2), 3.22–3.15 (1H, m, CH), 2.33–1.25 (36H, m, CH_2), 0.88 (6H, m, CH_3); **^{13}C NMR** (75 MHz, $CDCl_3$) δ *171.7*, 171.4 (C=O), 171.3 (C=O), *170.7*, *161.7*, 160.8, *75.5*, 75.1, *73.3*, 72.5, *59.1*, *57.3*, 57.1, 57.0, 46.4, *44.0*, *39.4*, 39.0, 34.5, 32.0, 31.6, 29.7, 29.7, *29.6*, *29.6*, 29.6, 29.5, 29.4, *29.4*, 29.1, *29.0*, 27.7, *26.9*, 26.8, *25.3*, 25.3, 24.2, *22.9*, 22.8, 22.6, 14.2, 14.1 (2 signals obscured); **HRMS** (ESI) *m/z* calcd. for $C_{28}H_{49}NO_5Na$ $(M+Na)^+$ 502.3509, found 502.3503.

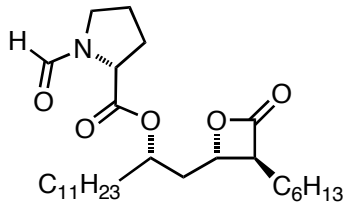
(*R*)-((*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 1-acetylpyrrolidine-2-carboxylate (19)



To a solution of amine **16** (7 mg, 0.015 mmol) in DCM (0.5 mL) at room temperature was added triethylamine (20 μL, 0.12 mmol), followed by acetyl chloride (10 μL, 0.10 mmol), and the resulting mixture was allowed to stir for 30 minutes. The reaction mixture was then diluted with Et_2O (10 mL) and H_2O (10 mL), extracted with Et_2O (2 × 15 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was purified by column chromatography (eluent: 60:40 v/v hexane/EtOAc) to afford ester **19** as a colourless oil (6 mg, 85%). $[\alpha]_D^{25}$ +48.6 (*c* 0.07, CH_2Cl_2); **IR** ν_{max}: 2926, 2855, 1824, 1743, 1655, 1466, 1419, 1191, 1123 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.15–4.94 (1H, m, CH), 4.50–4.45 (0.9H, m, CH), 4.39 (0.9H, dd, *J* = 3.8, 8.3 Hz, CH), 4.32–4.28 (0.2H, m, CH), 3.68–3.48 (2H, m, CH_2), 3.27–3.18 (1H, m, CH), 2.28–1.24 (39H, m, CH_2 +

CH_3), 0.89–0.86 (6H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ 172.4 (C=O), *171.9*, 171.7 (C=O), 169.3 (C=O), *75.5*, 74.5, *73.0*, 71.6, *60.6*, 58.9, *57.3*, 57.1, 47.9, *46.4*, *39.3*, 38.7, *34.5*, 34.0, 32.1, 31.6, *31.6*, *31.5*, *29.8*, 29.8, 29.7, 29.6, *29.6*, 29.5, 29.5, 29.4, *29.4*, 29.2, *29.1*, 27.7, *26.9*, 26.7, 25.4, *25.3*, 25.1, 22.8, 22.7, *22.7*, *22.5*, 22.3, 14.3, 14.2 (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{29}H_{51}NO_5Na$ $(M+Na)^+$ 516.3660, found 516.3656.

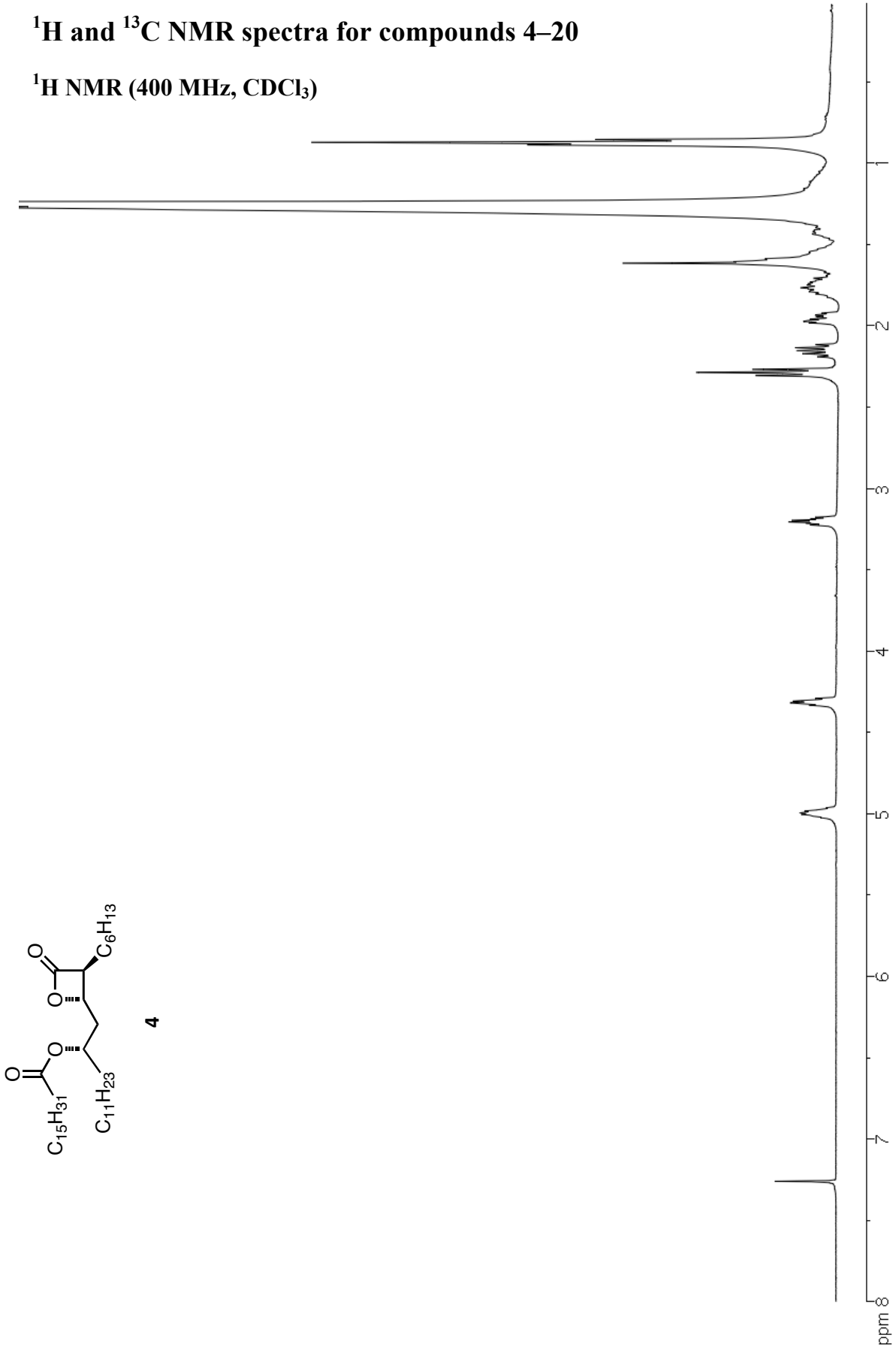
(*R*)-((*S*)-1-((2*S*,3*S*)-3-hexyl-4-oxooxetan-2-yl)tridecan-2-yl) 1-formylpyrrolidine-2-carboxylate (20)



To a solution of amine **16** (15 mg, 0.034 mmol) in DCM (0.8 mL) at room temperature was added formic acetic anhydride[6] (0.15 mL, excess), and the resulting mixture was allowed to stir for 30 minutes. The reaction mixture was then diluted with Et_2O (10 mL) and H_2O (10 mL), extracted with Et_2O (2 × 15 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude product was purified by column chromatography (eluent: 60:40 v/v hexane/EtOAc) to afford ester **20** as a colourless oil (9 mg, 55%). $[\alpha]_D^{25}$ +35.4 (*c* 0.12, CH_2Cl_2); **IR** ν_{max}: 3339, 2925, 2855, 1822, 1742, 1674, 1616, 1573, 1464, 1379 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.27 (0.7H, s, CH), 8.25 (0.3H, s, CH), 5.17–4.96 (1H, m, CH), 4.46–4.29 (2H, m, CH), 3.71–3.53 (2H, m, CH_2), 3.27–3.16 (1H, m, CH), 2.30–1.24 (36H, m, CH_2), 0.89–0.85 (6H, m, CH_3); **^{13}C NMR** (101.6 MHz, $CDCl_3$) δ *171.9*, 171.6 (C=O), 171.6 (C=O), *170.8*, *161.9*, 160.8, *75.6*, 74.6, *73.2*, 72.1, *59.1*, *57.2*, 57.1, 56.9, 46.4, *44.1*, *39.5*, 38.7, *34.5*, 34.1, 32.0, 31.6, *31.6*, 29.8, 29.7, *29.6*, 29.6, *29.6*, 29.5, 29.4, *29.3*, 29.1, *29.1*, 27.7, *27.7*, *26.9*, 26.7, 25.4, *25.3*, 24.3, *22.9*, 22.8, 22.7, 22.6, 14.3, 14.2, *14.2* (1 signal obscured); **HRMS** (ESI) *m/z* calcd. for $C_{28}H_{49}NO_5H$ $(M+H)^+$ 480.3691, found 480.3684.

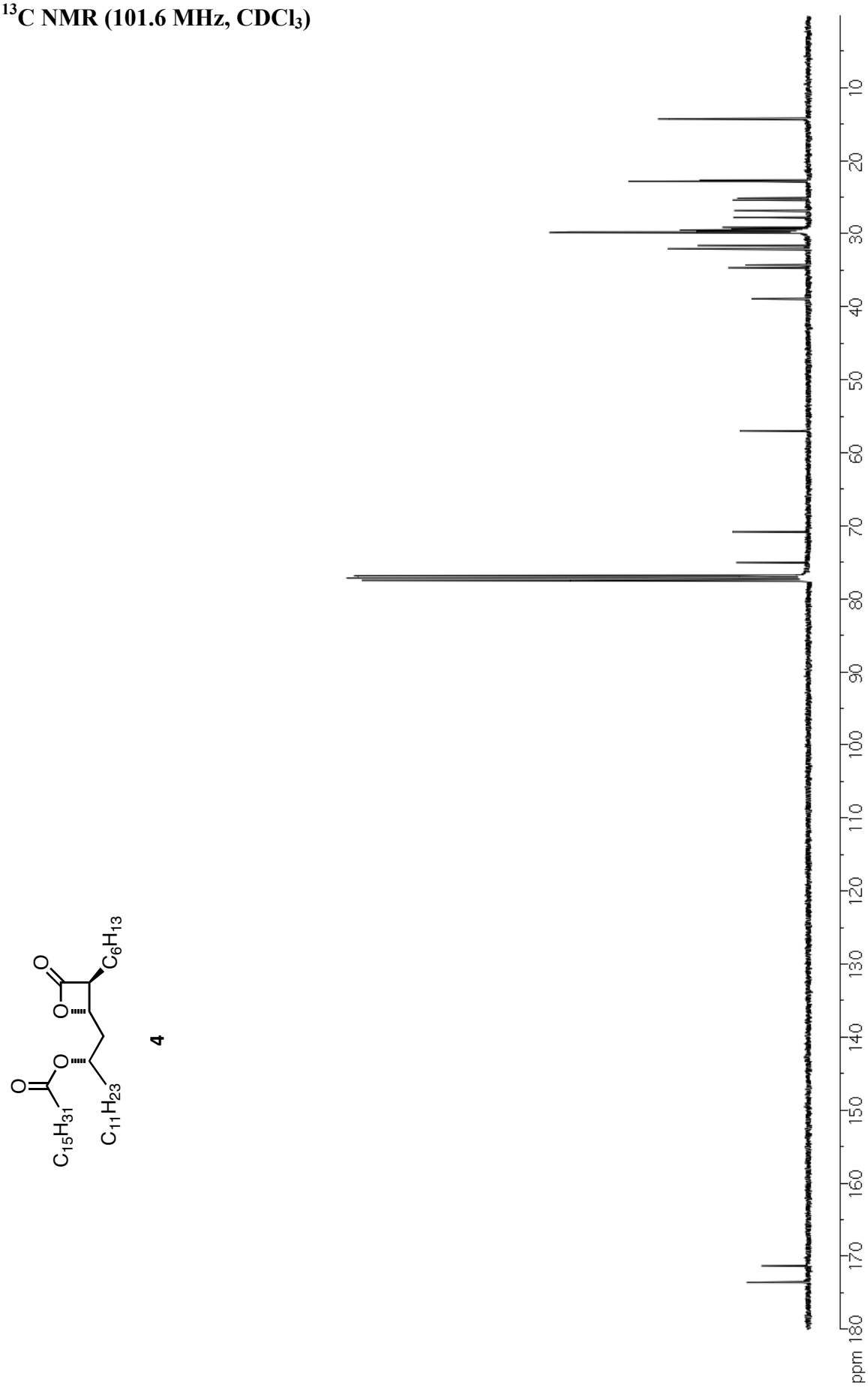
^{1}H and ^{13}C NMR spectra for compounds 4–20

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



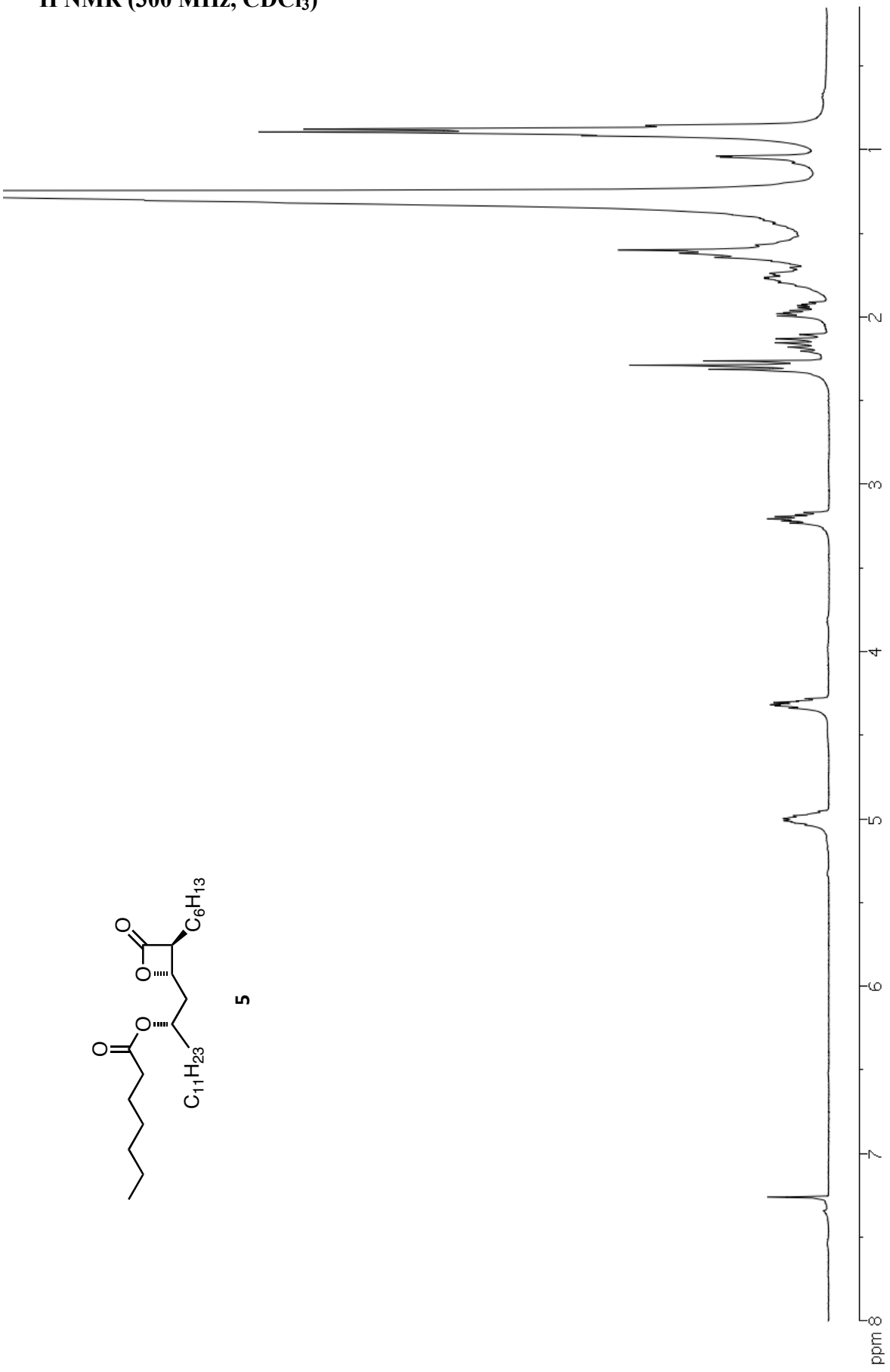
4

^{13}C NMR (101.6 MHz, $CDCl_3$)



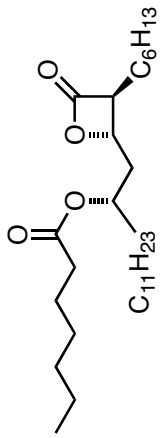
4

^{1}H NMR (300 MHz, $CDCl_3$)

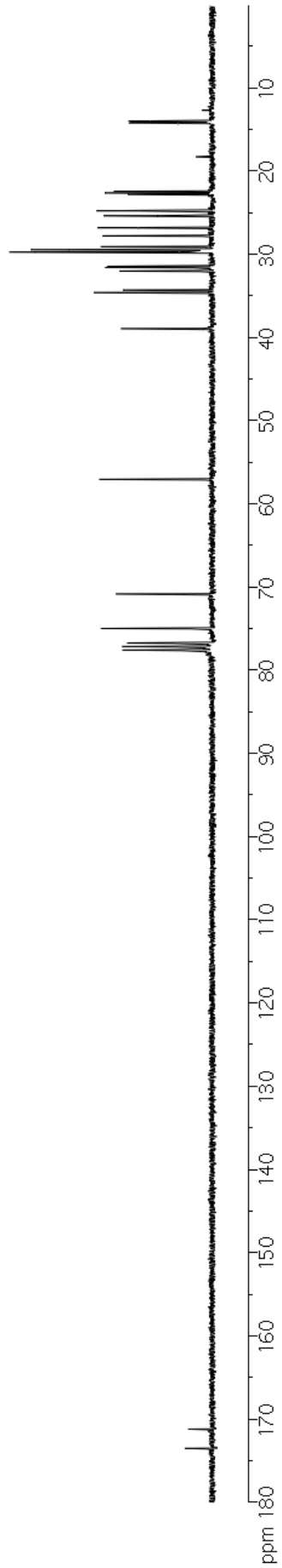


5

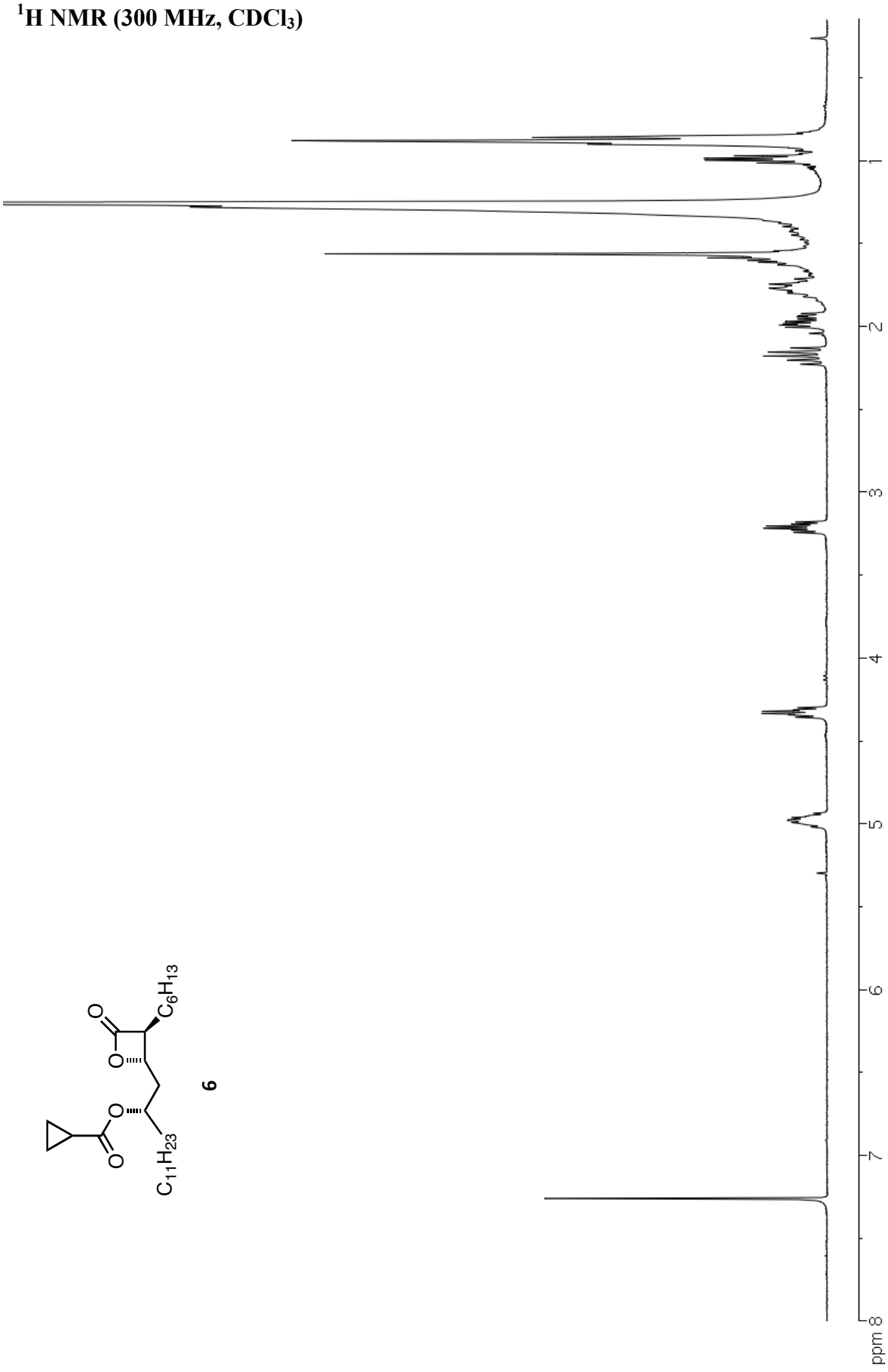
^{13}C NMR (75 MHz, $CDCl_3$)



5

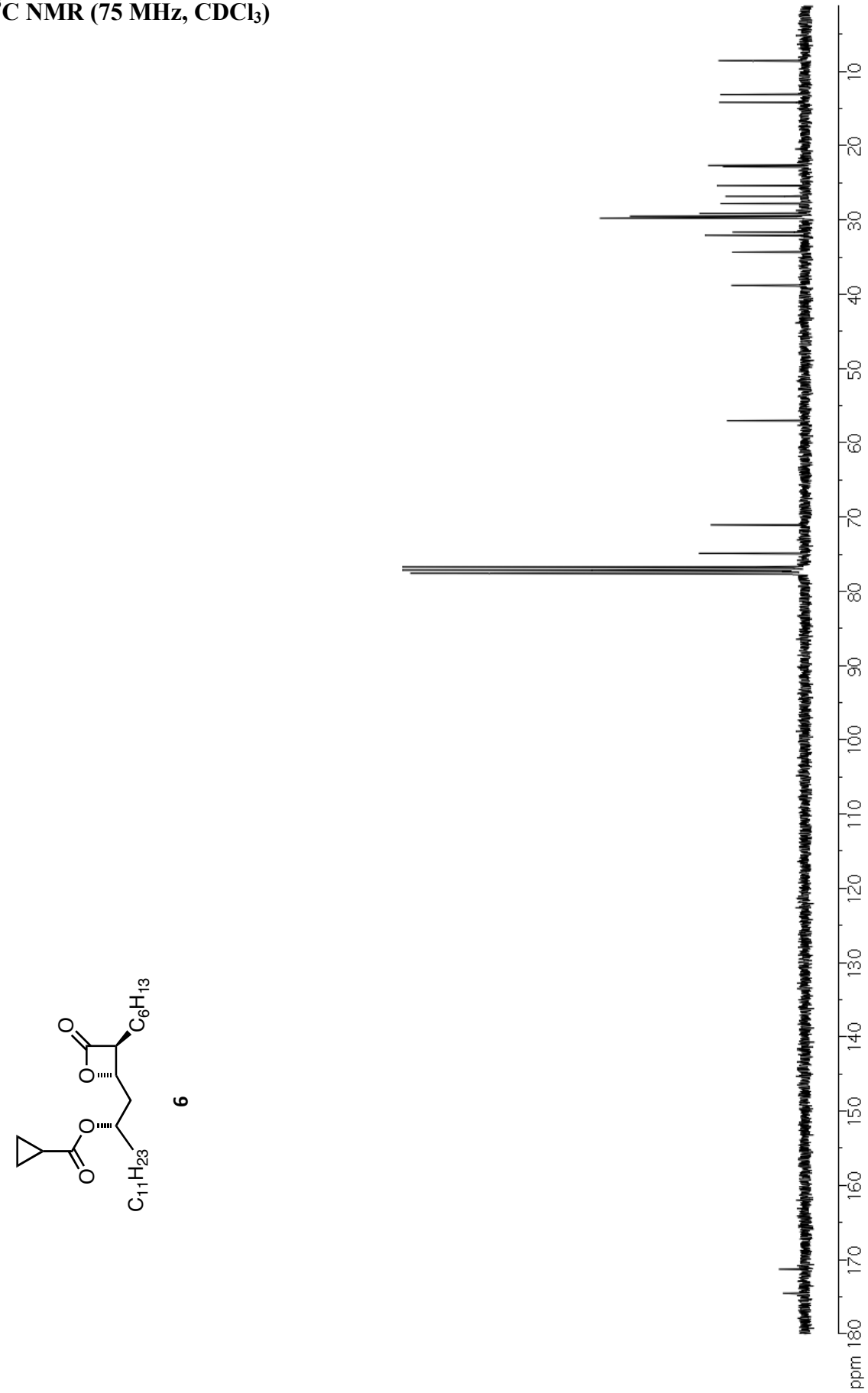


^{1}H NMR (300 MHz, $CDCl_3$)



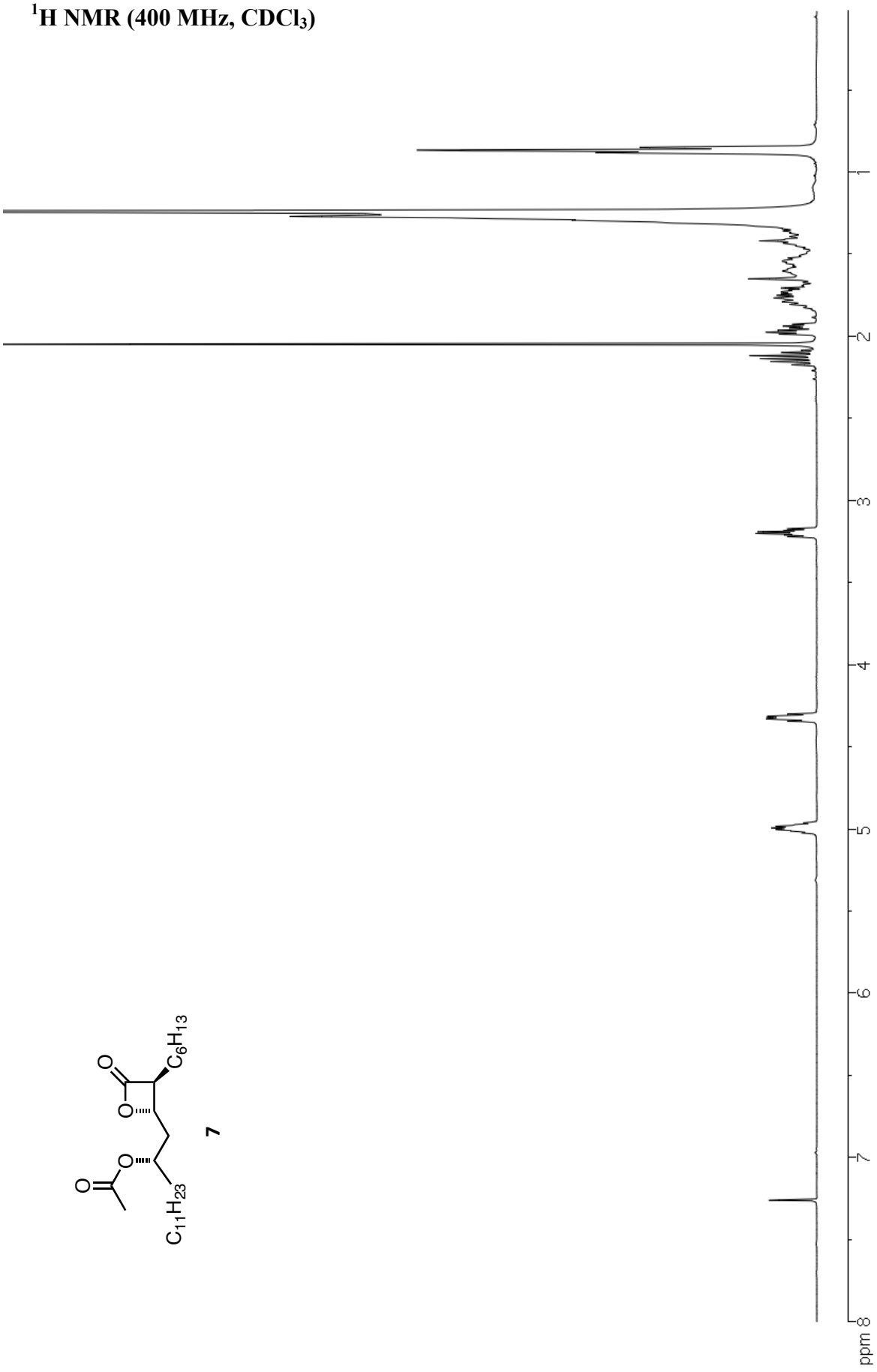
6

^{13}C NMR (75 MHz, $CDCl_3$)



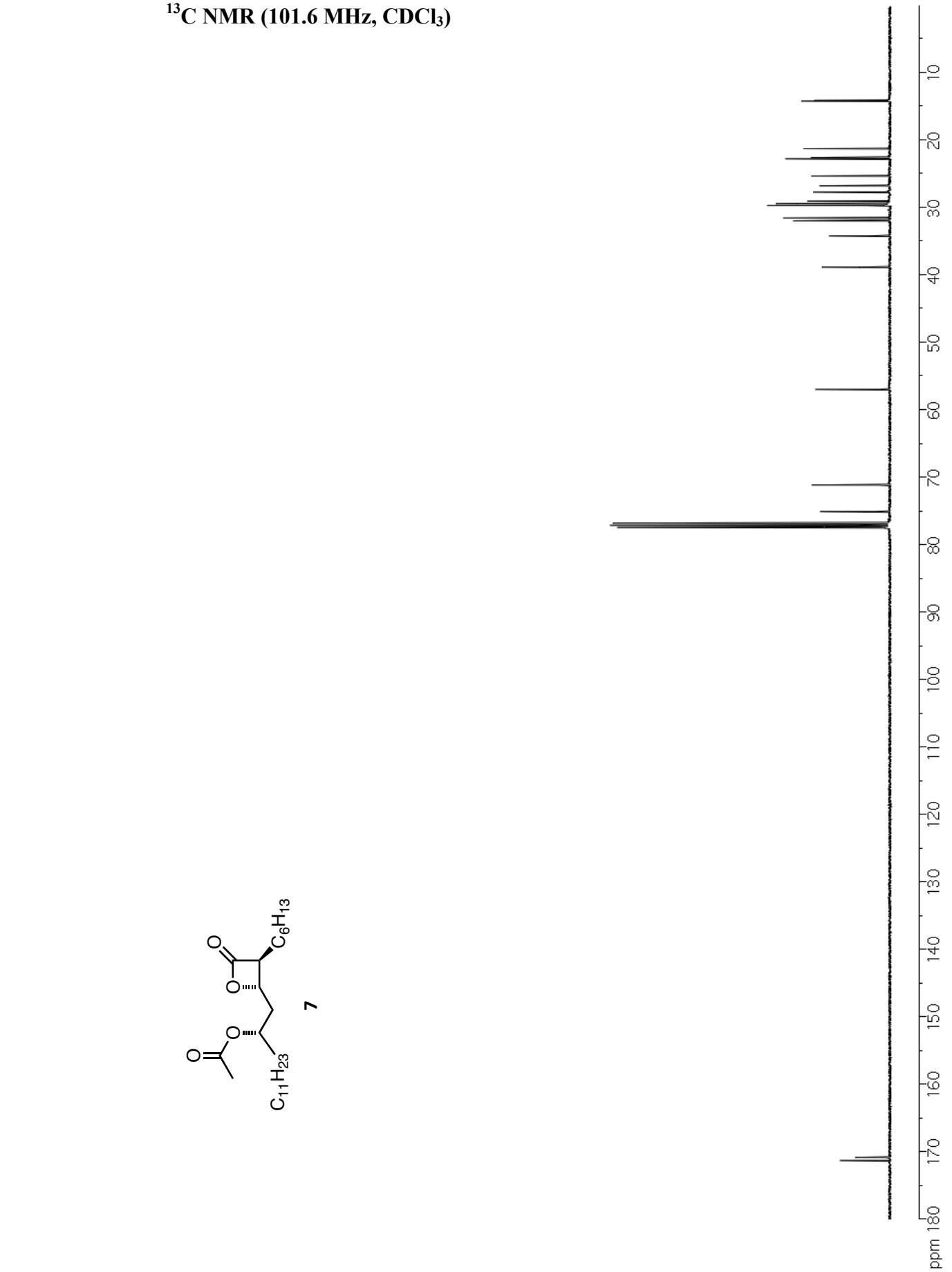
6

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



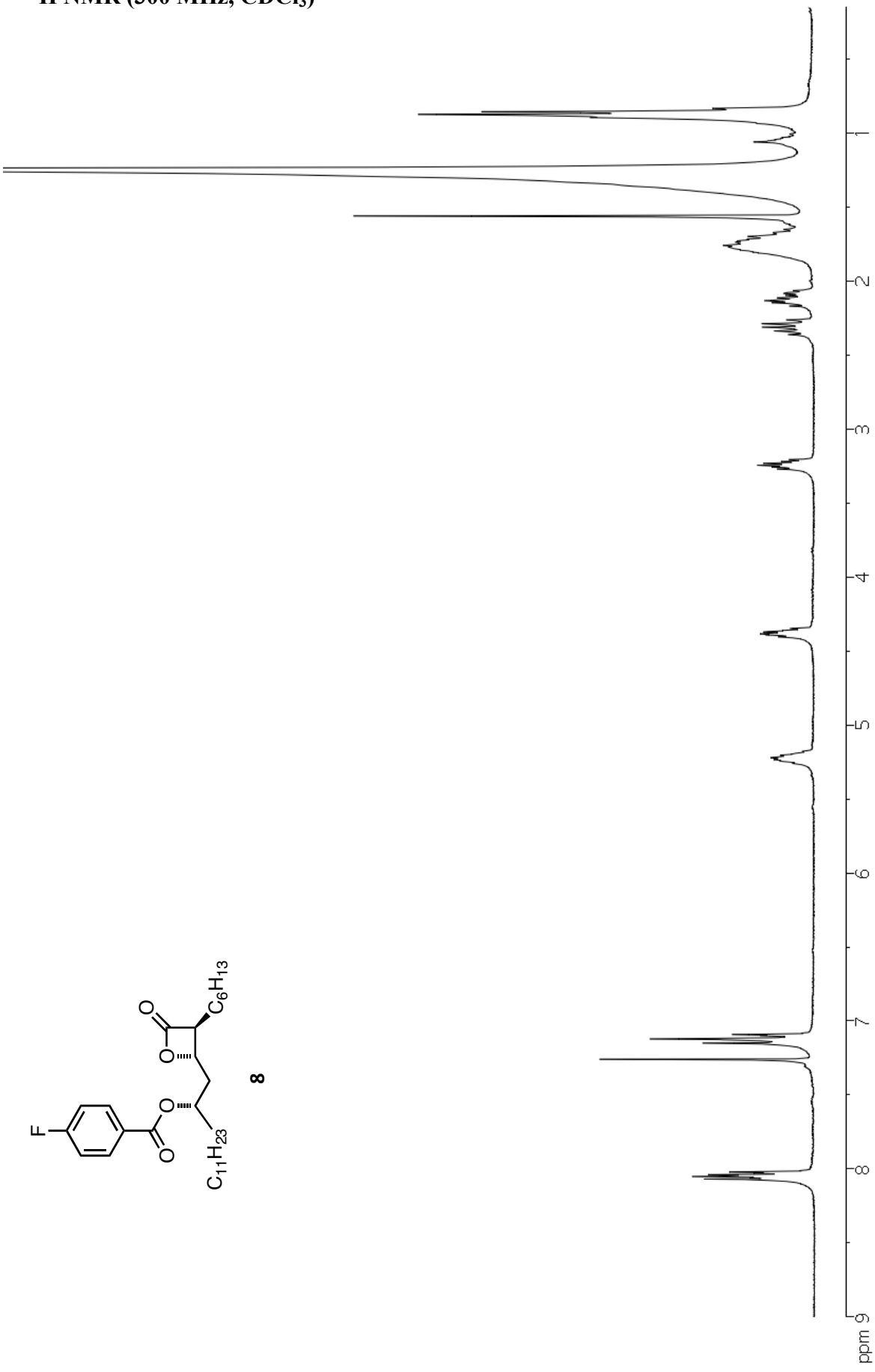
7

^{13}C NMR (101.6 MHz, $CDCl_3$)



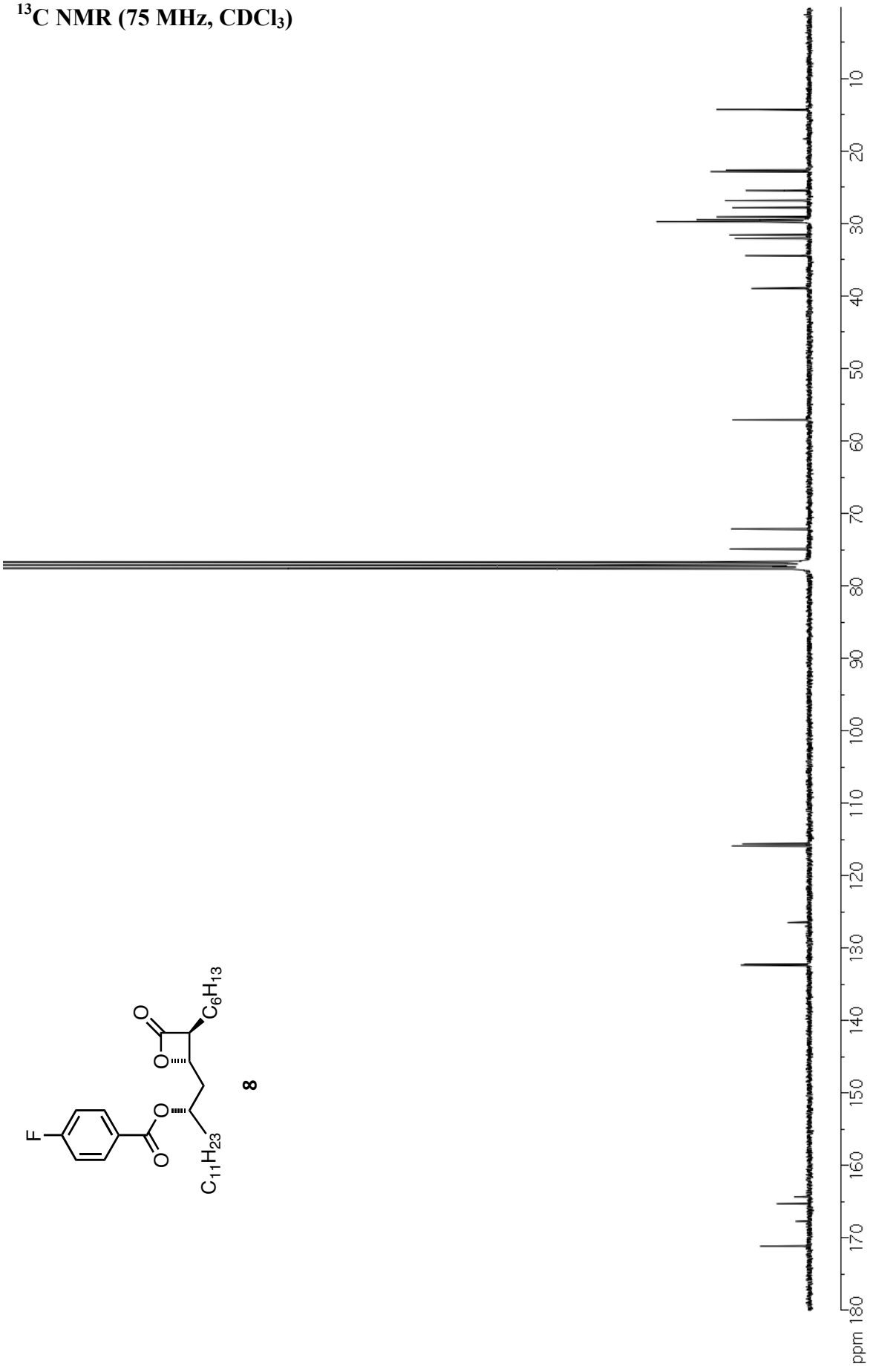
7

^{1}H NMR (300 MHz, $CDCl_3$)



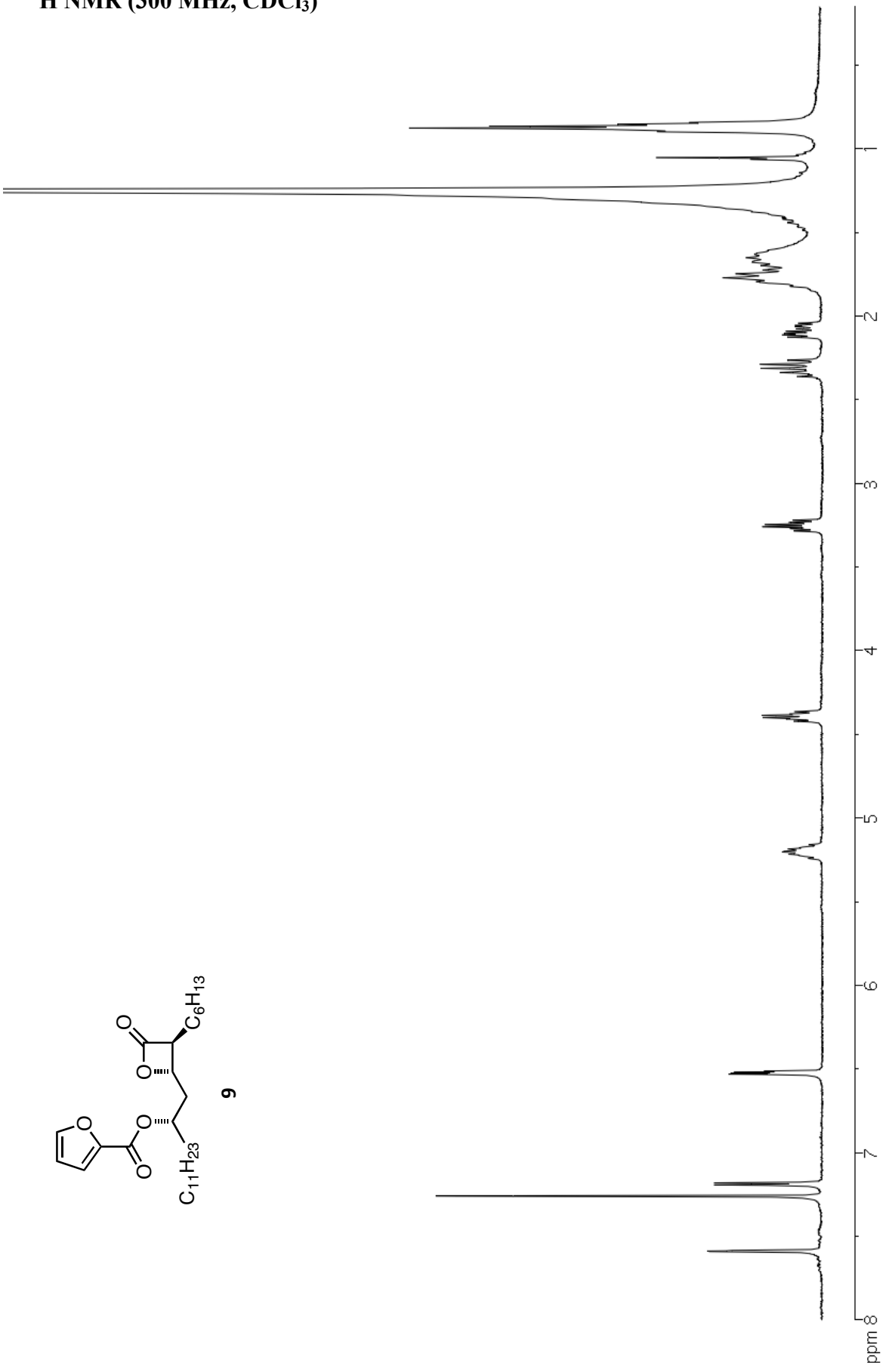
8

^{13}C NMR (75 MHz, $CDCl_3$)



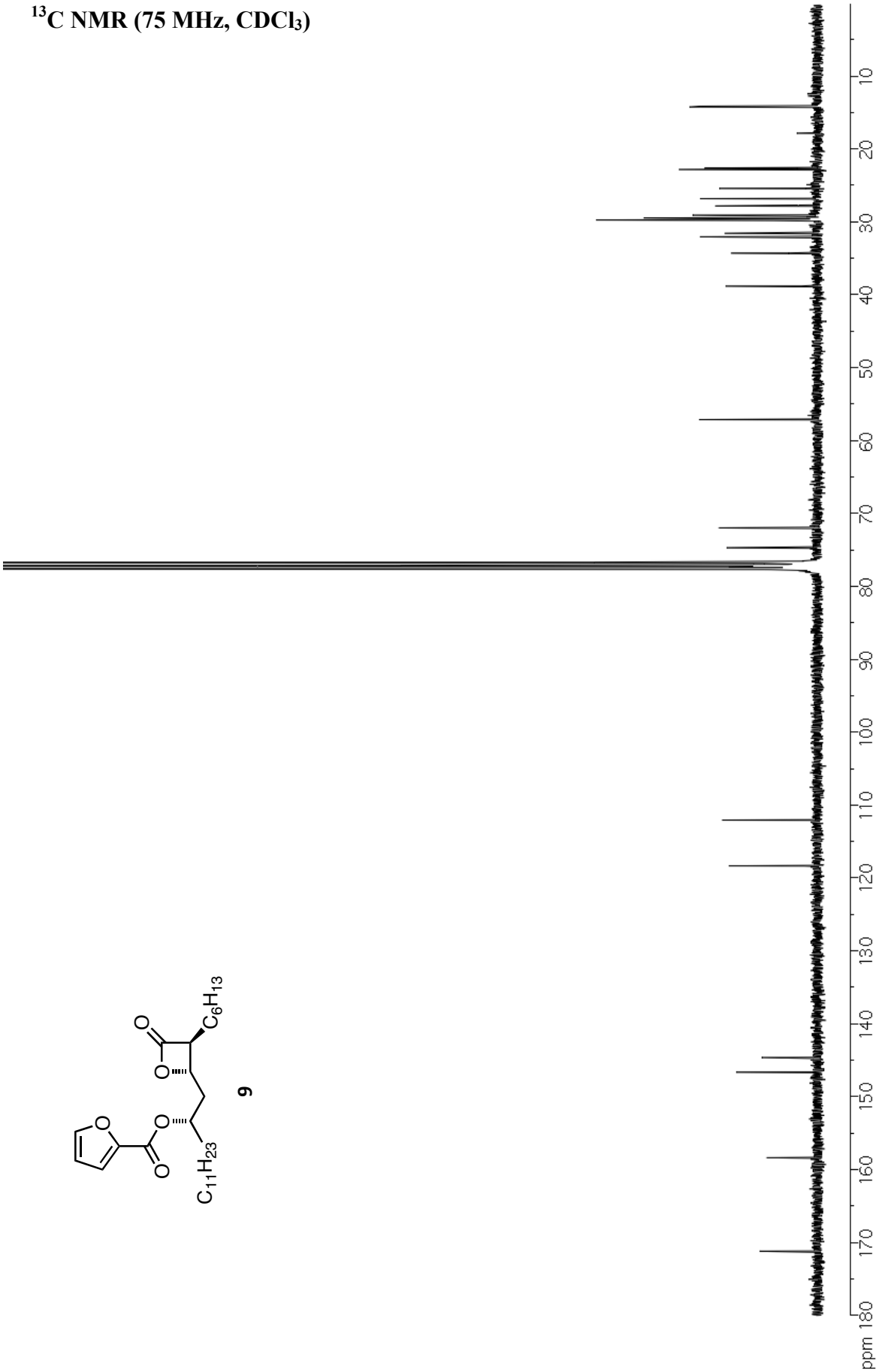
8

^{1}H NMR (300 MHz, $CDCl_3$)



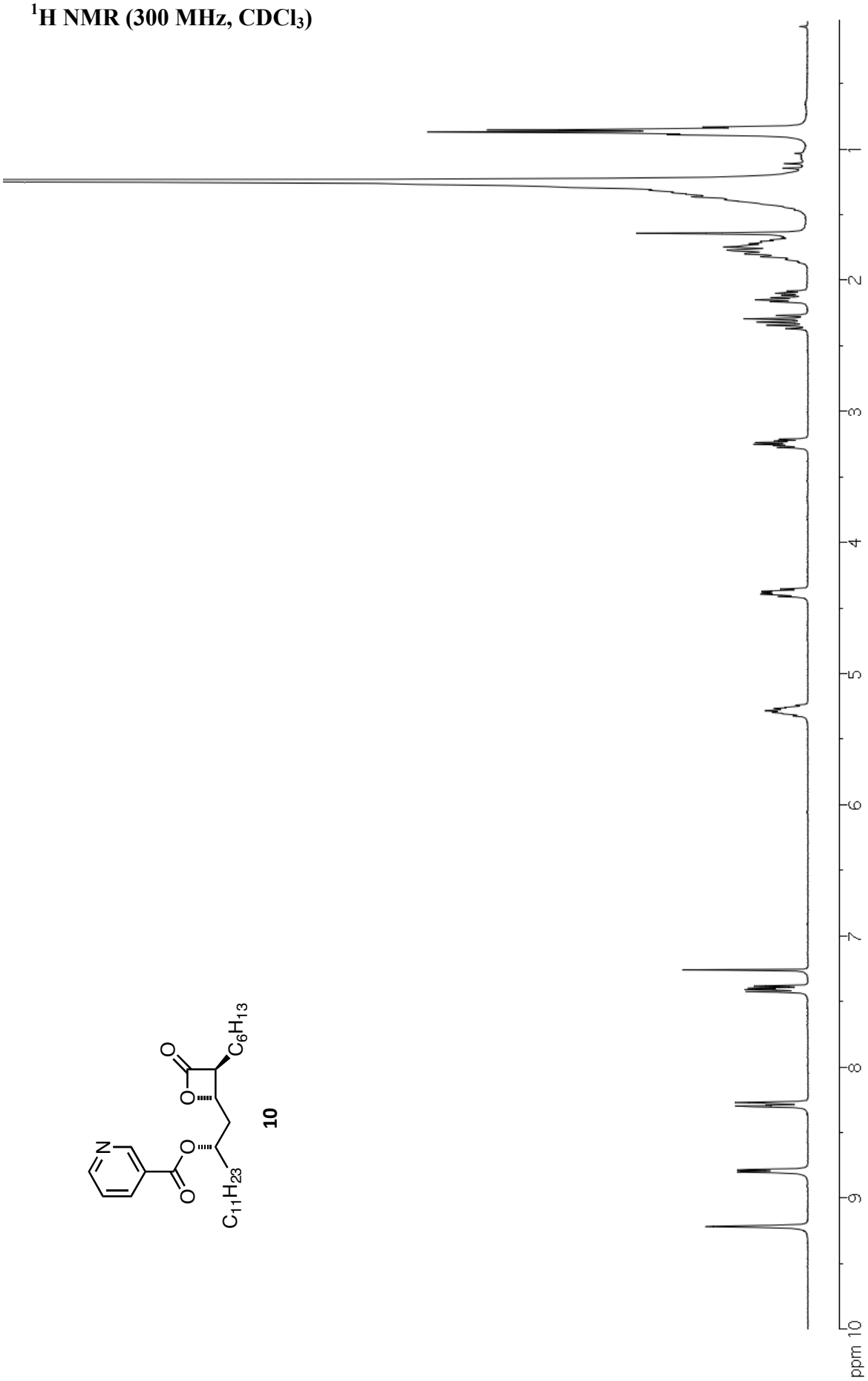
9

^{13}C NMR (75 MHz, $CDCl_3$)



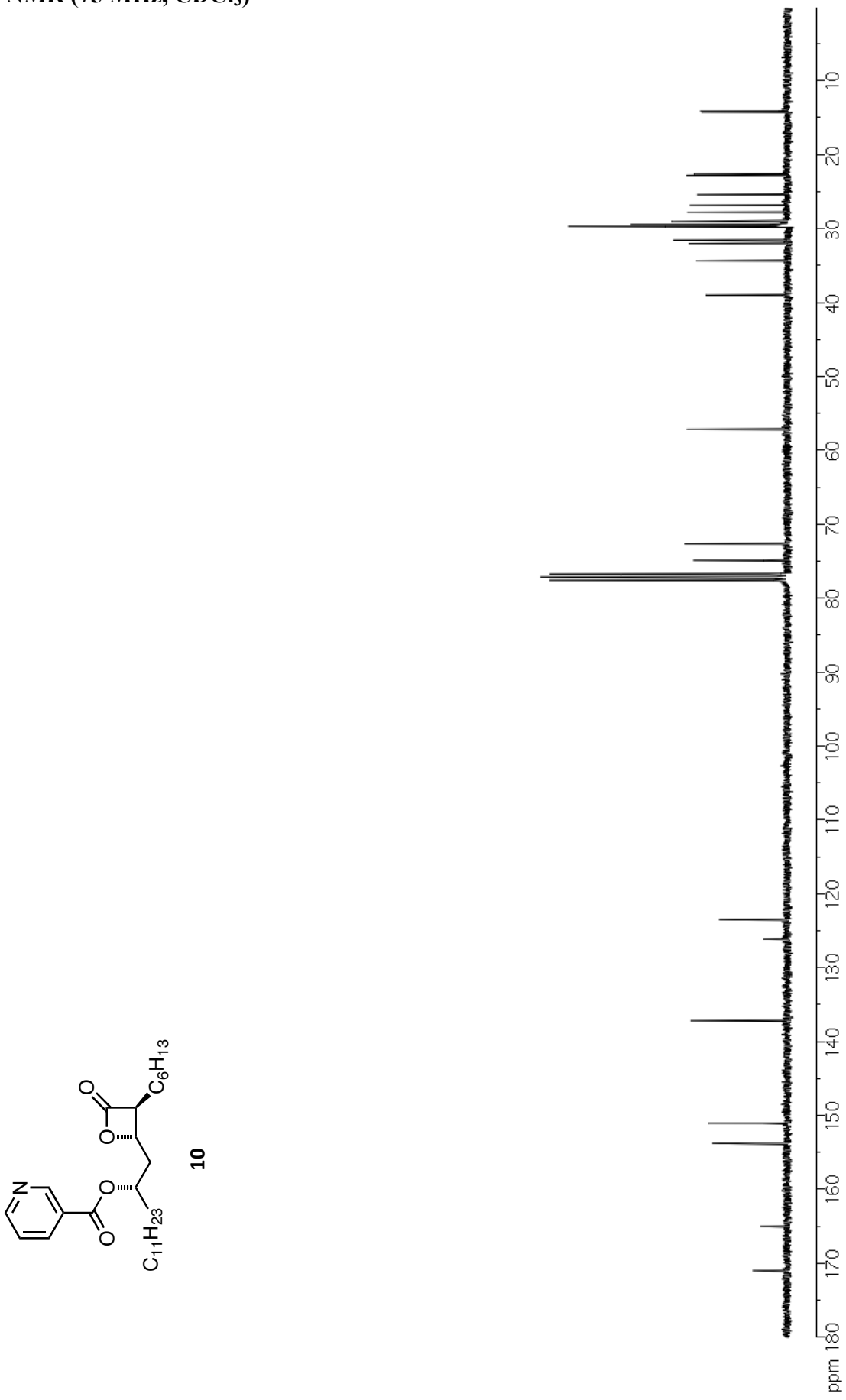
9

^{1}H NMR (300 MHz, $CDCl_3$)



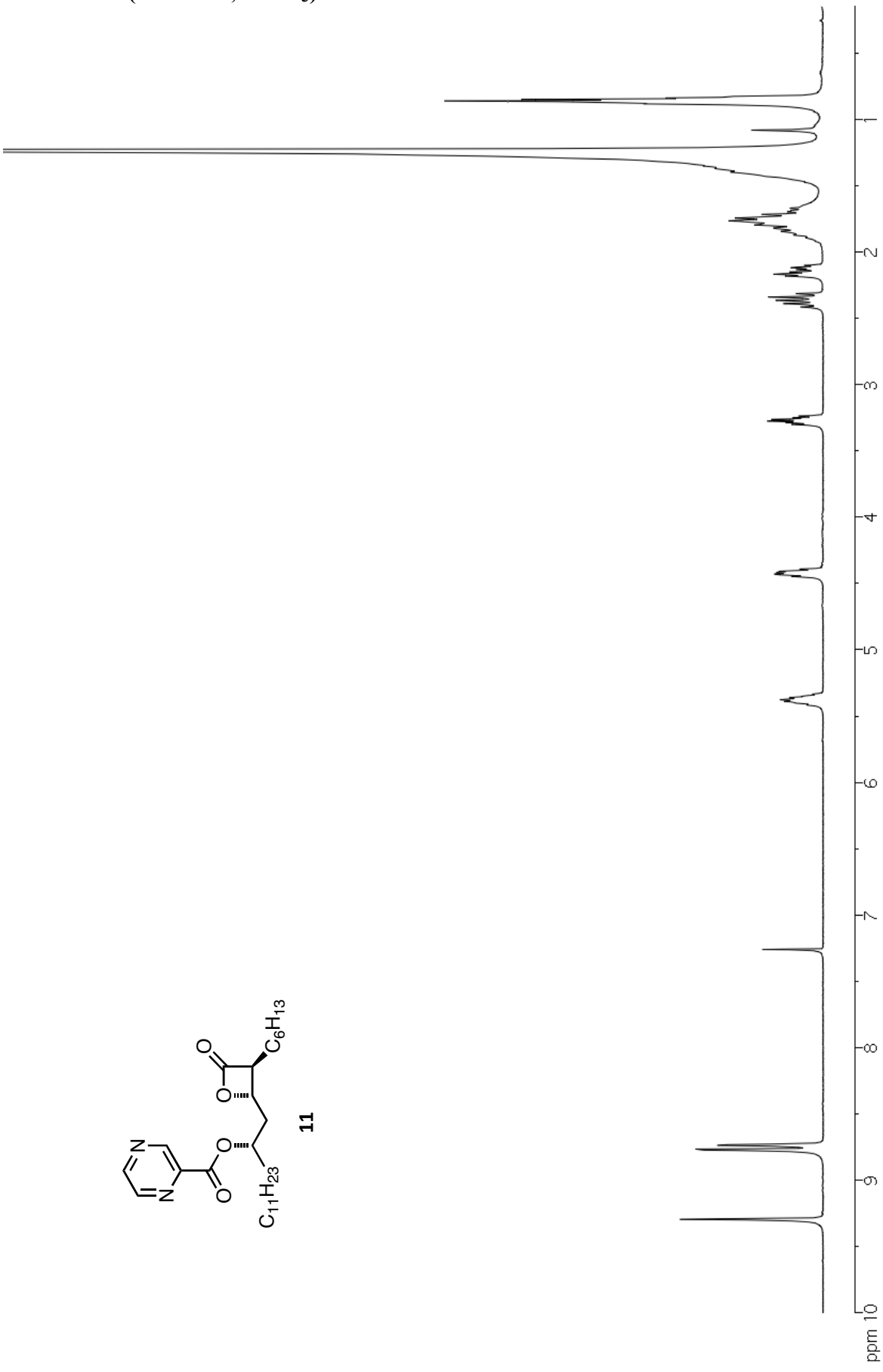
10

^{13}C NMR (75 MHz, $CDCl_3$)



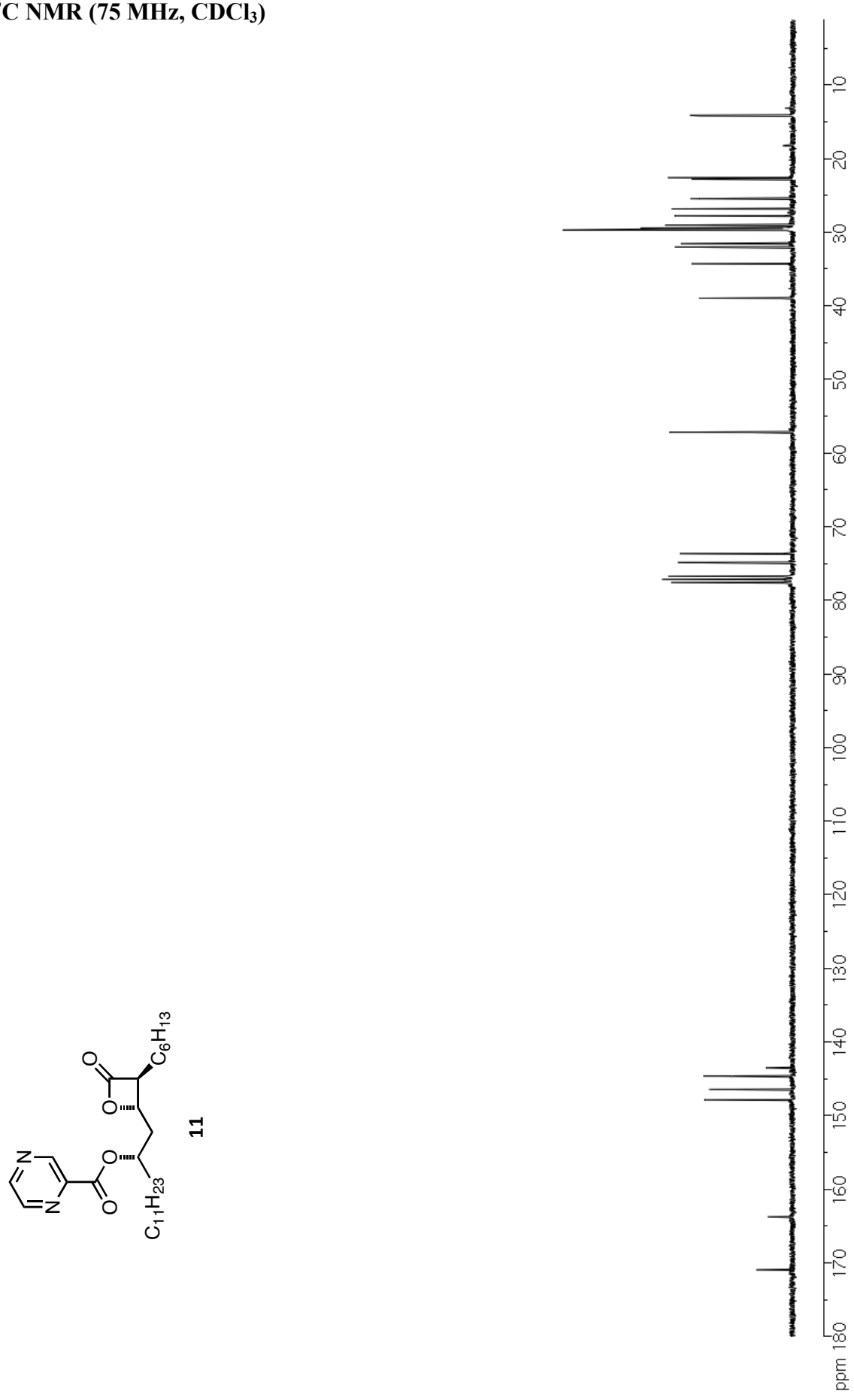
10

^{1}H NMR (300 MHz, $CDCl_3$)



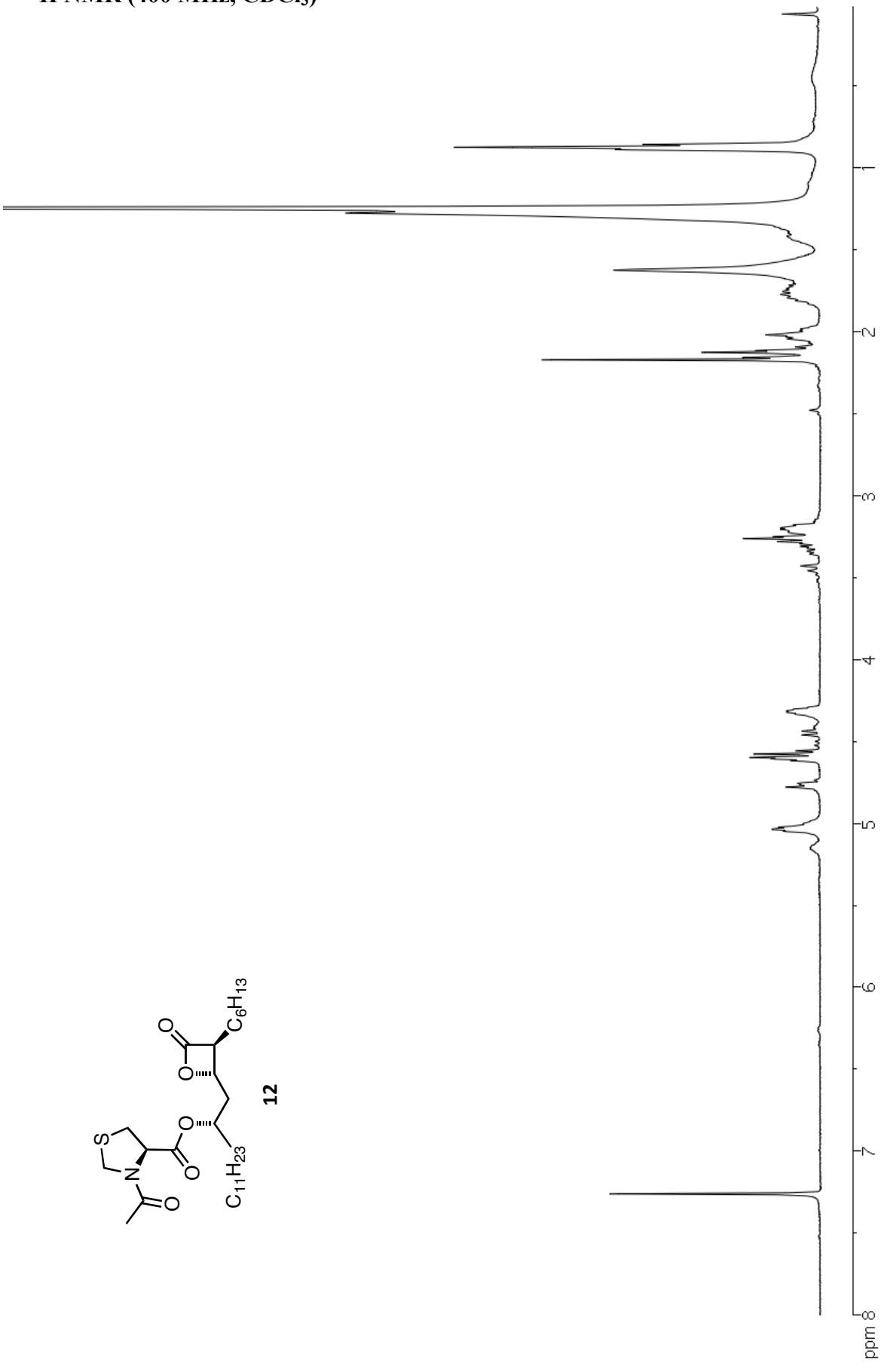
11

^{13}C NMR (75 MHz, $CDCl_3$)



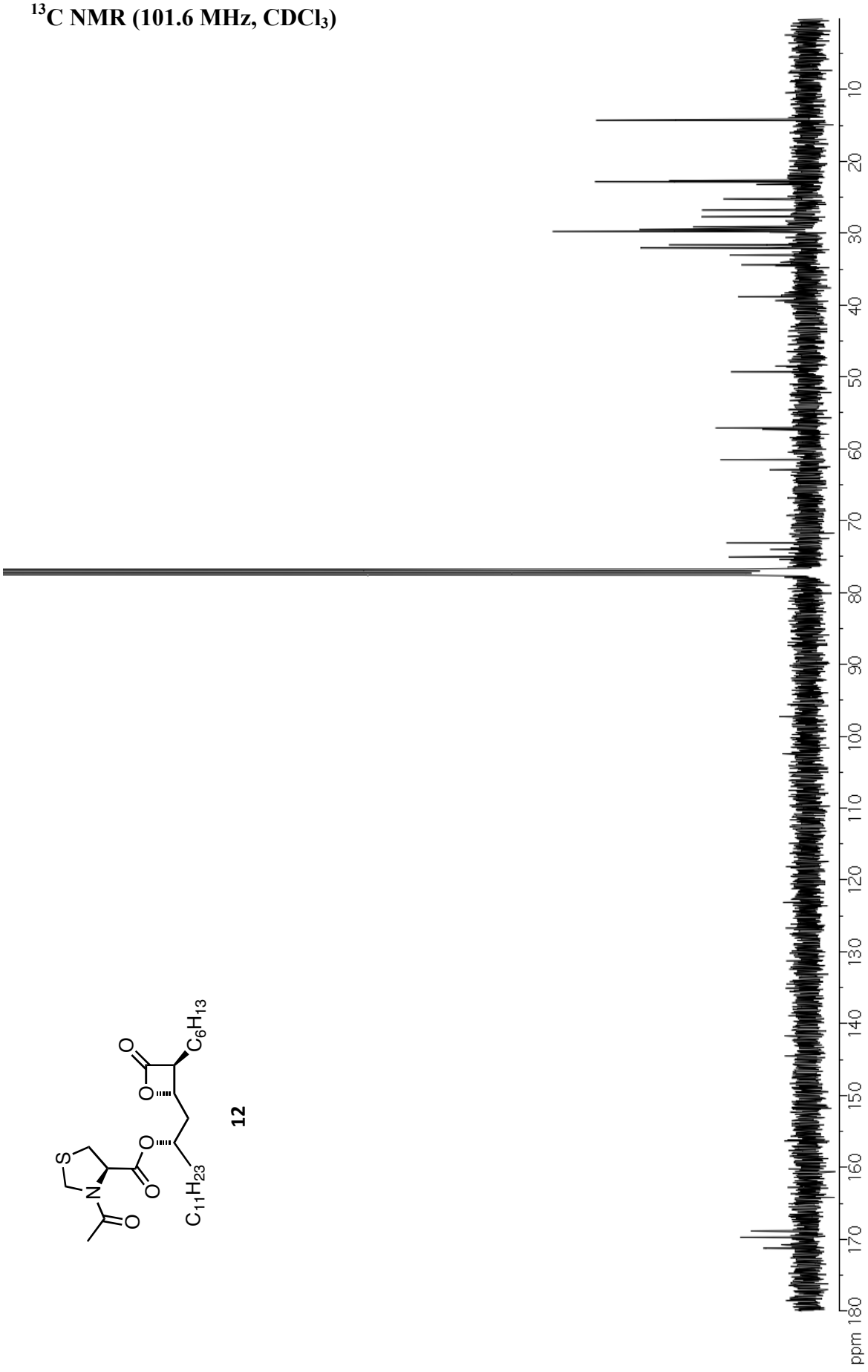
11

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



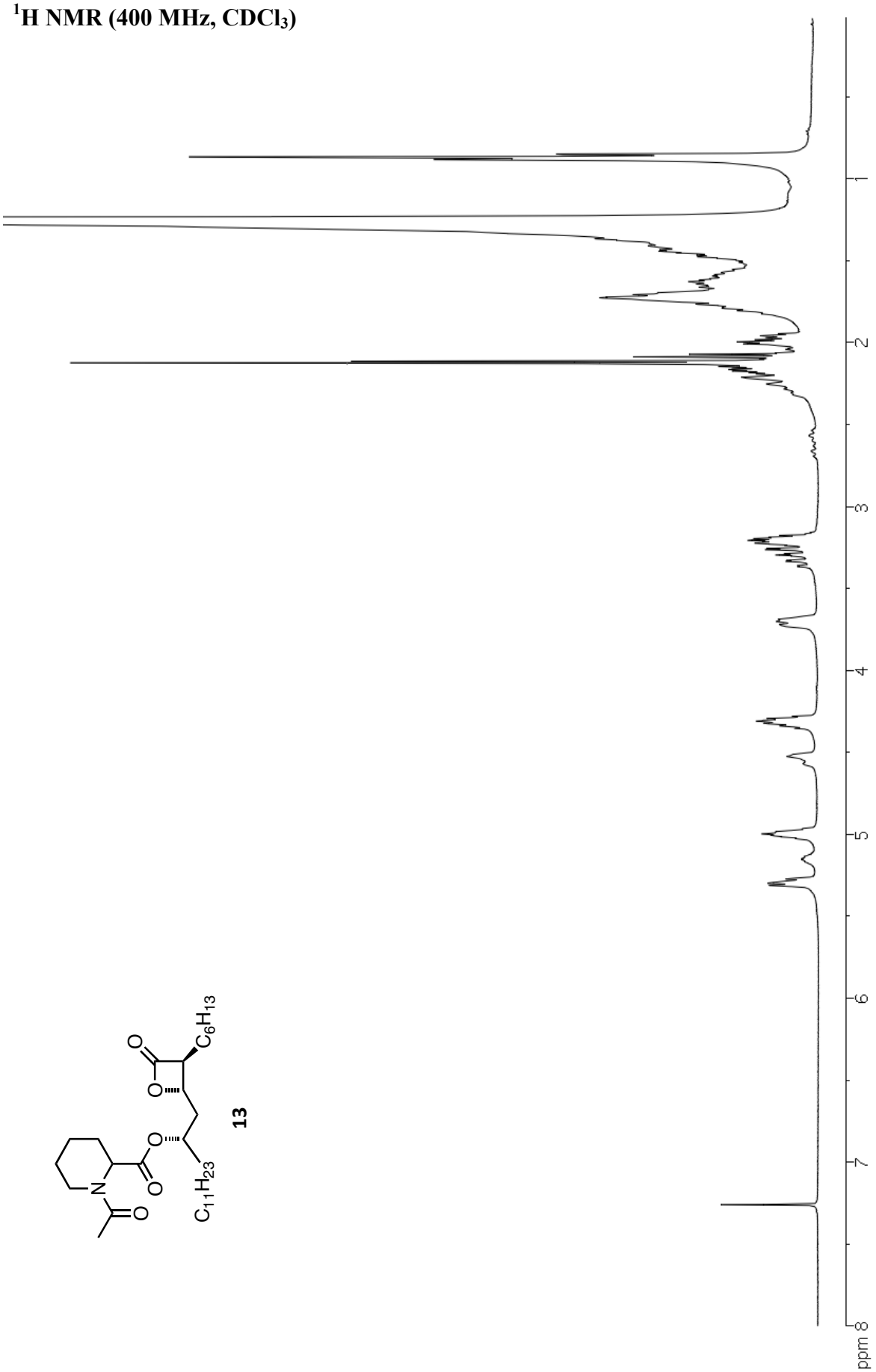
12

^{13}C NMR (101.6 MHz, $CDCl_3$)



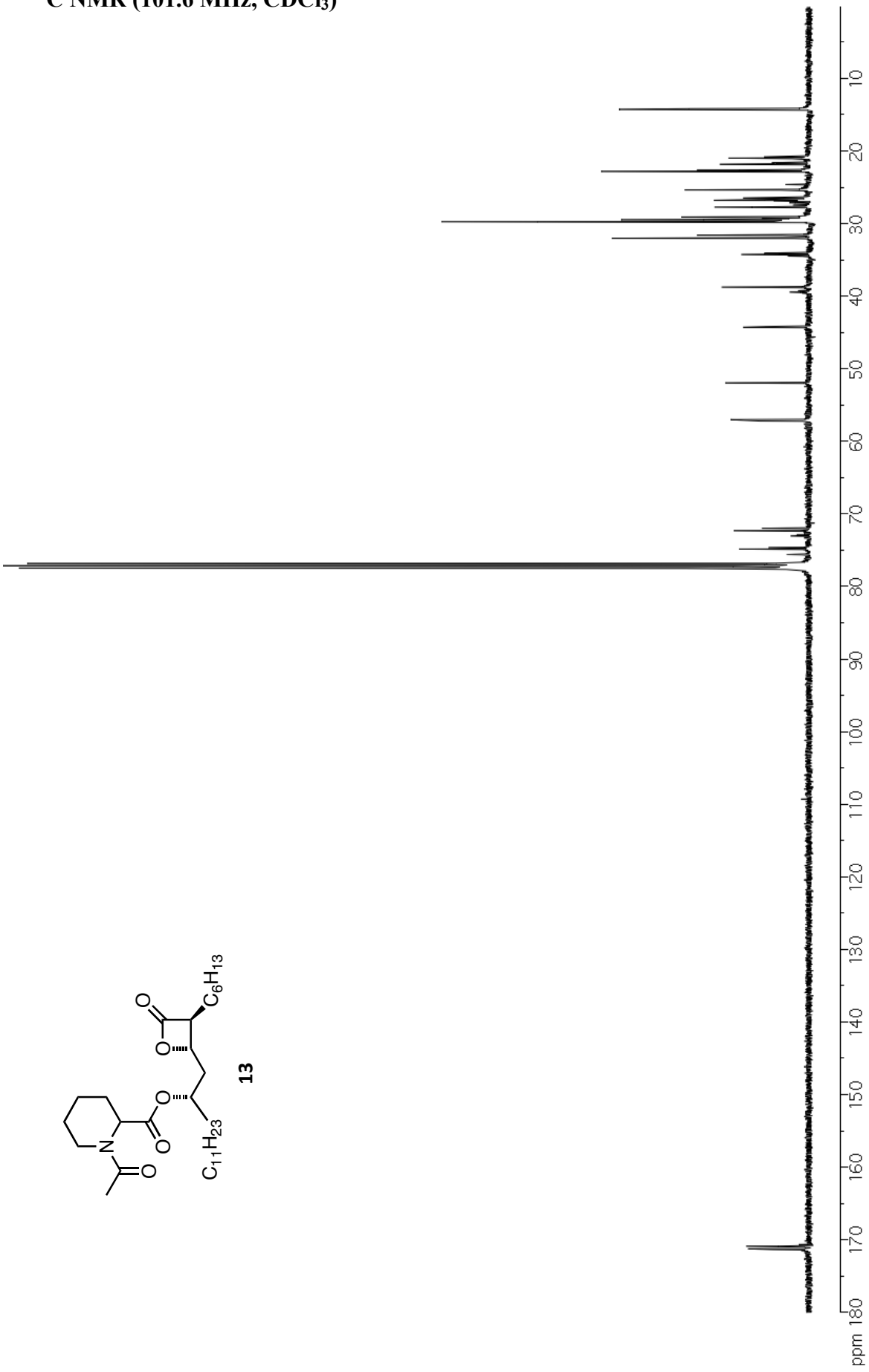
12

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



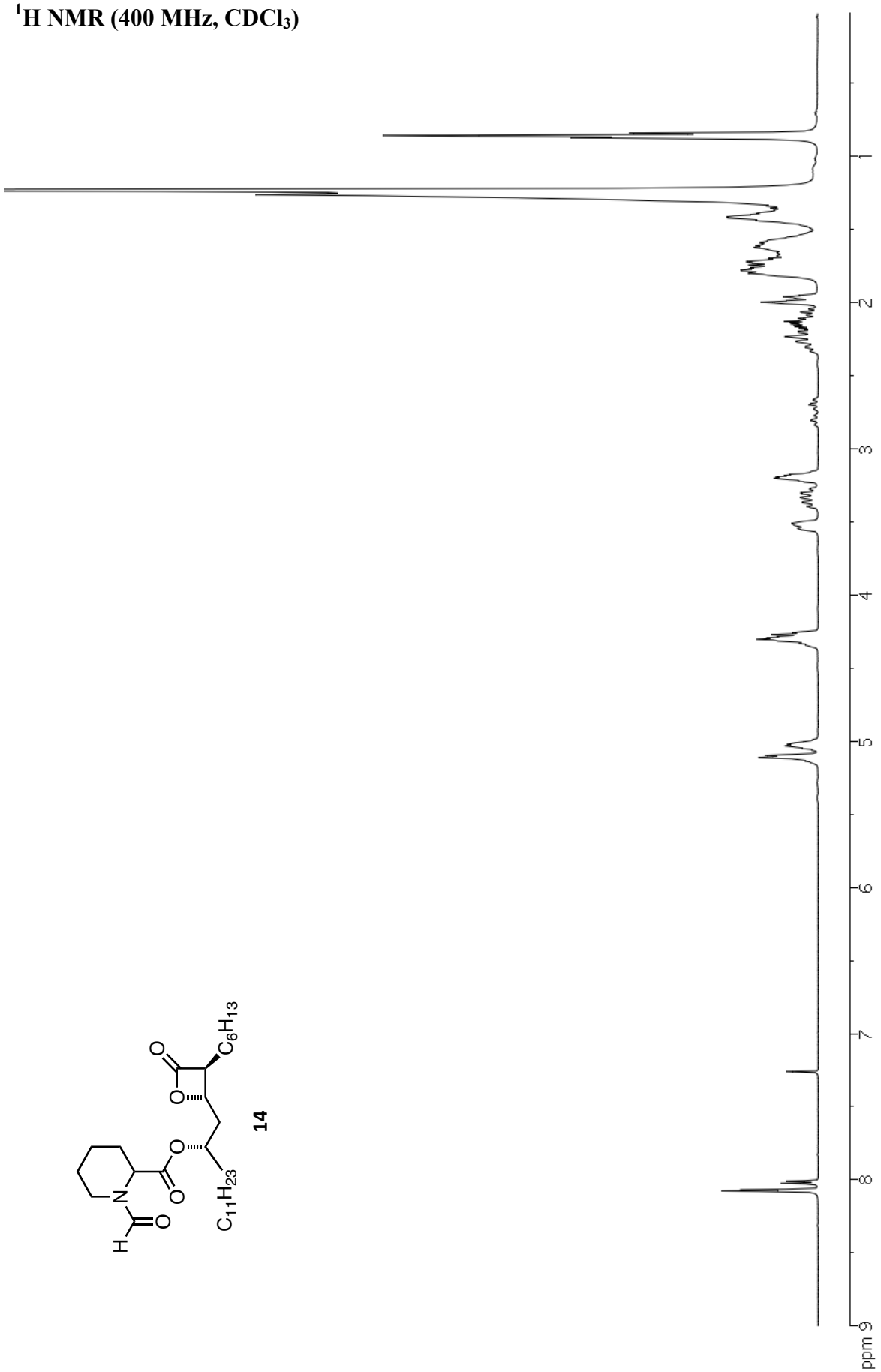
13

^{13}C NMR (101.6 MHz, $CDCl_3$)



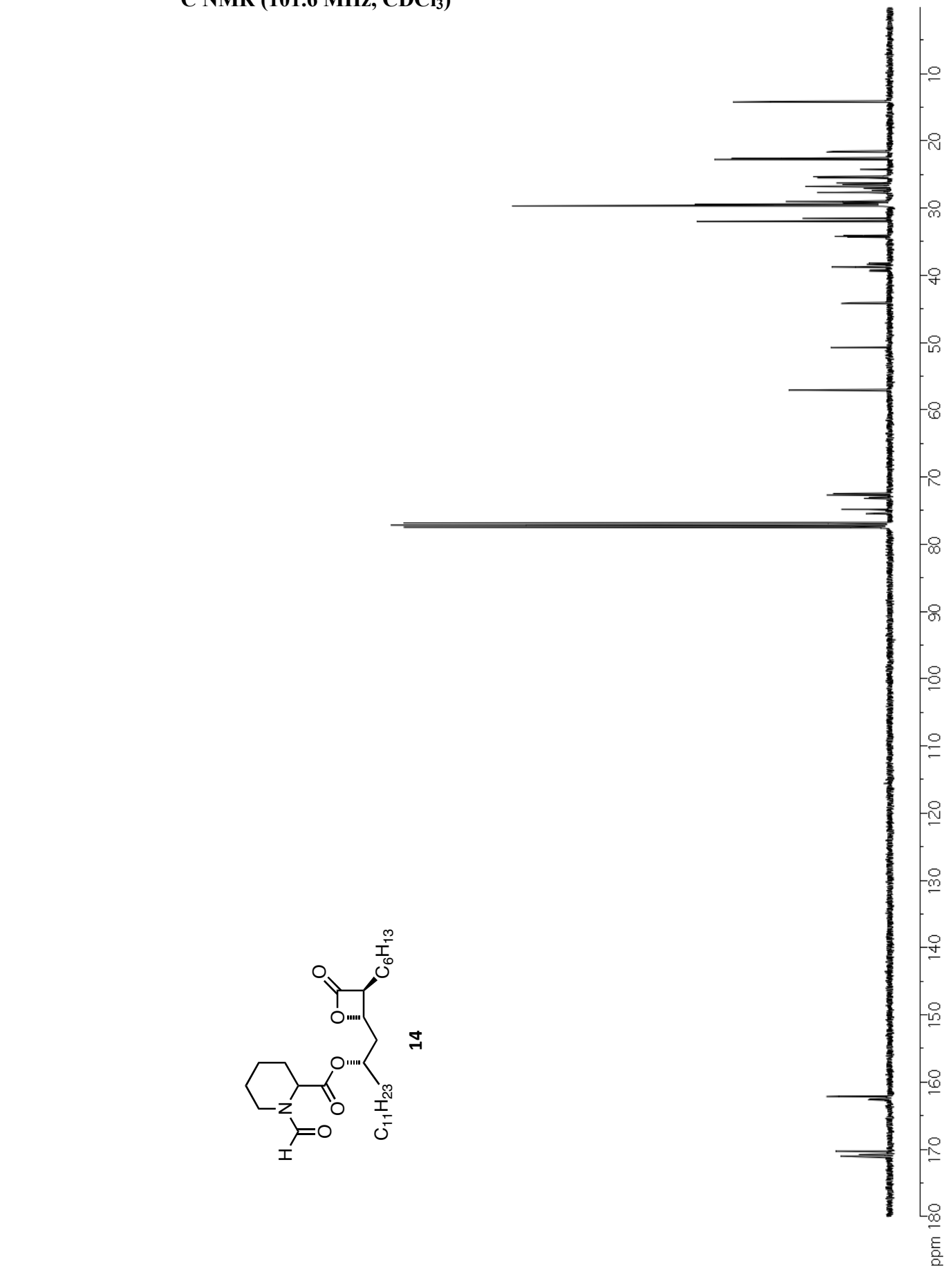
13

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



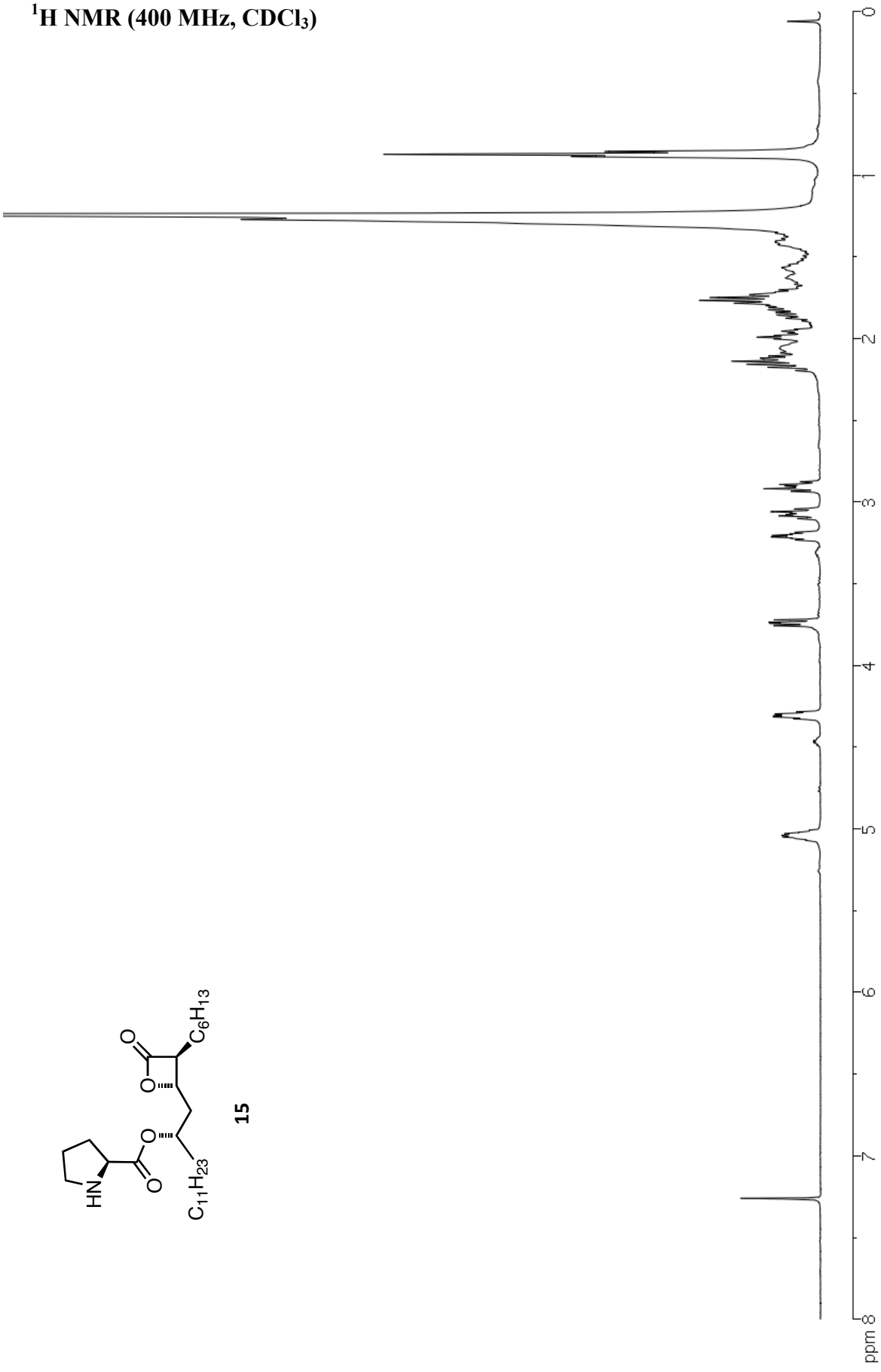
14

^{13}C NMR (101.6 MHz, $CDCl_3$)



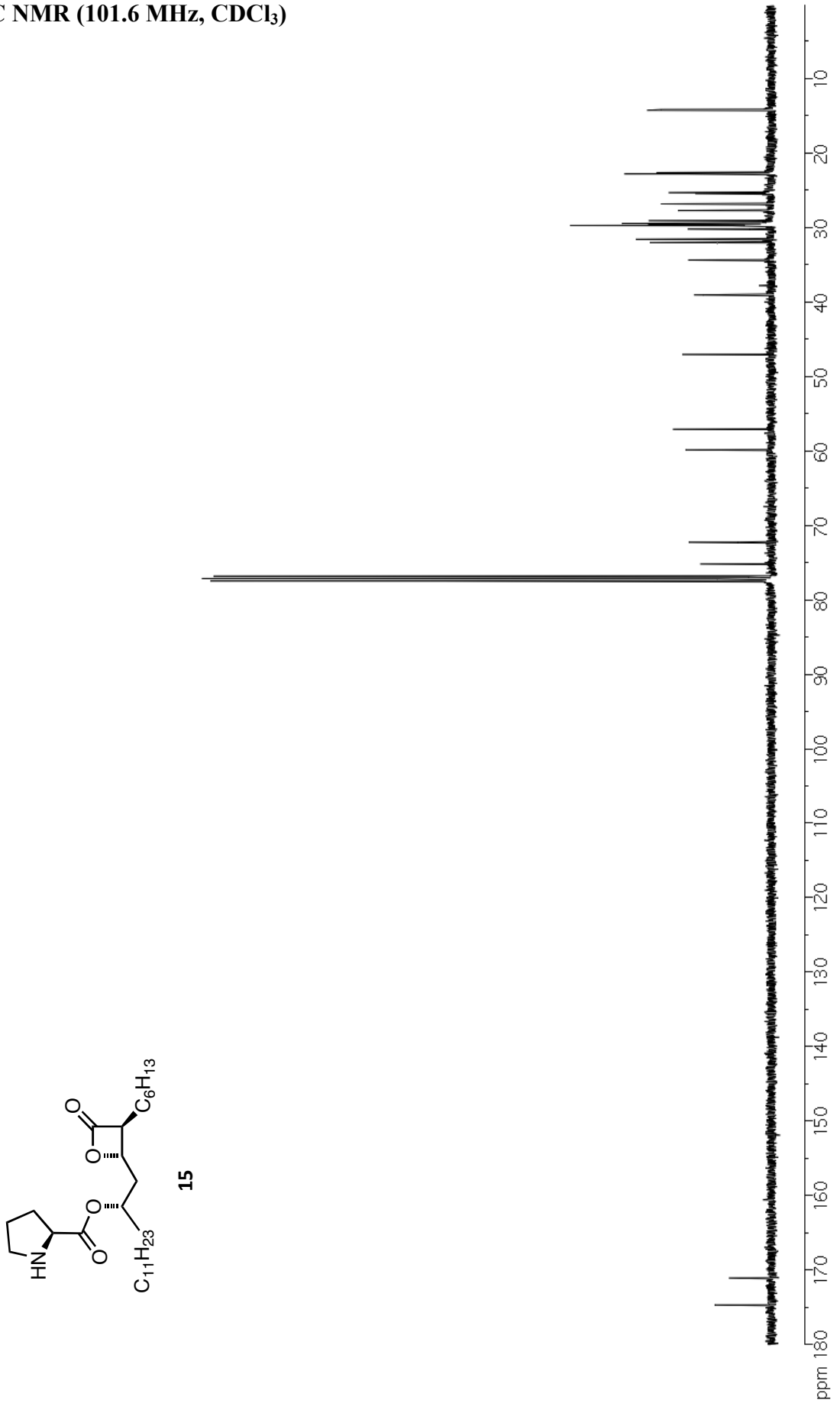
14

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



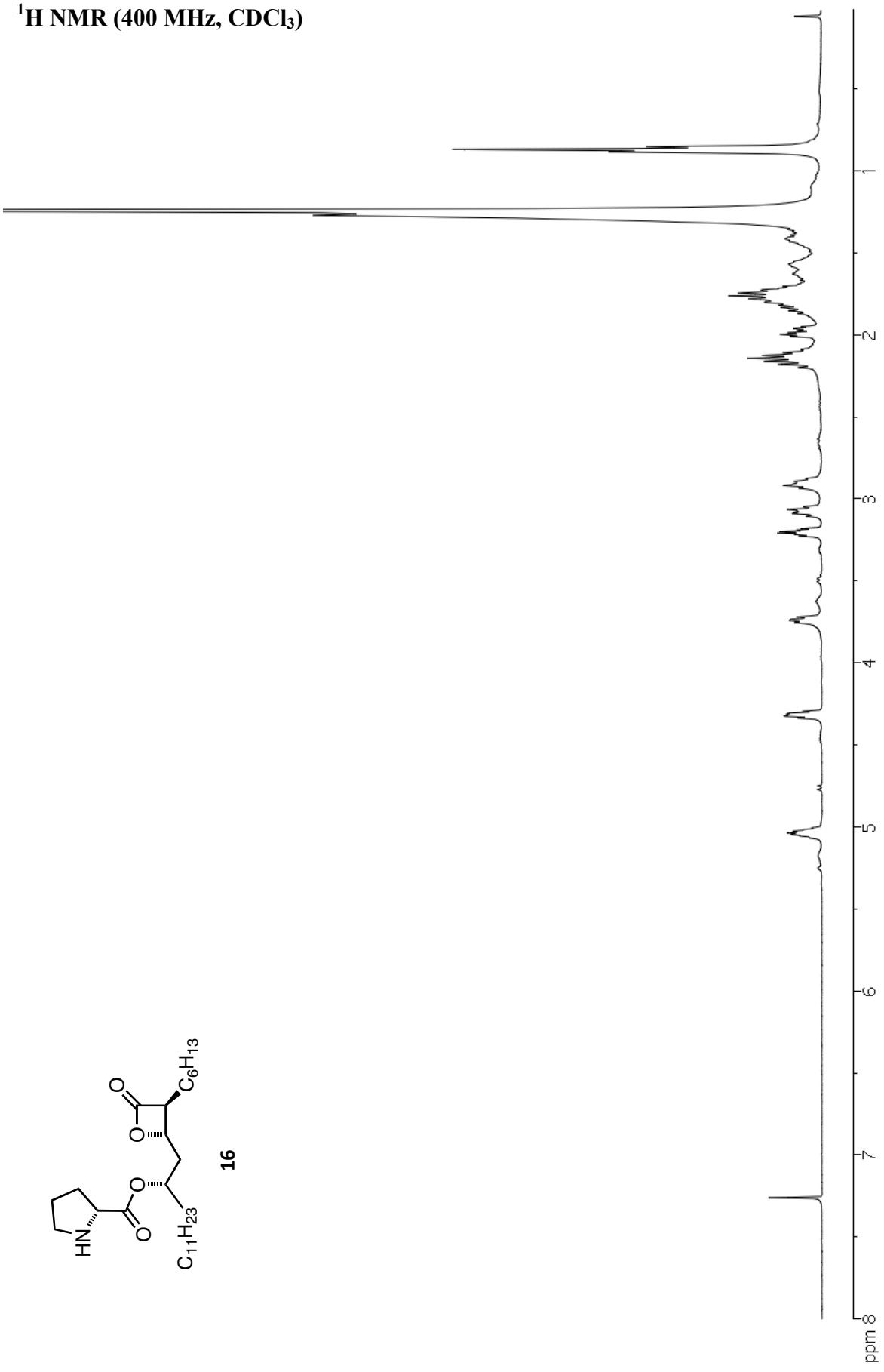
15

^{13}C NMR (101.6 MHz, $CDCl_3$)



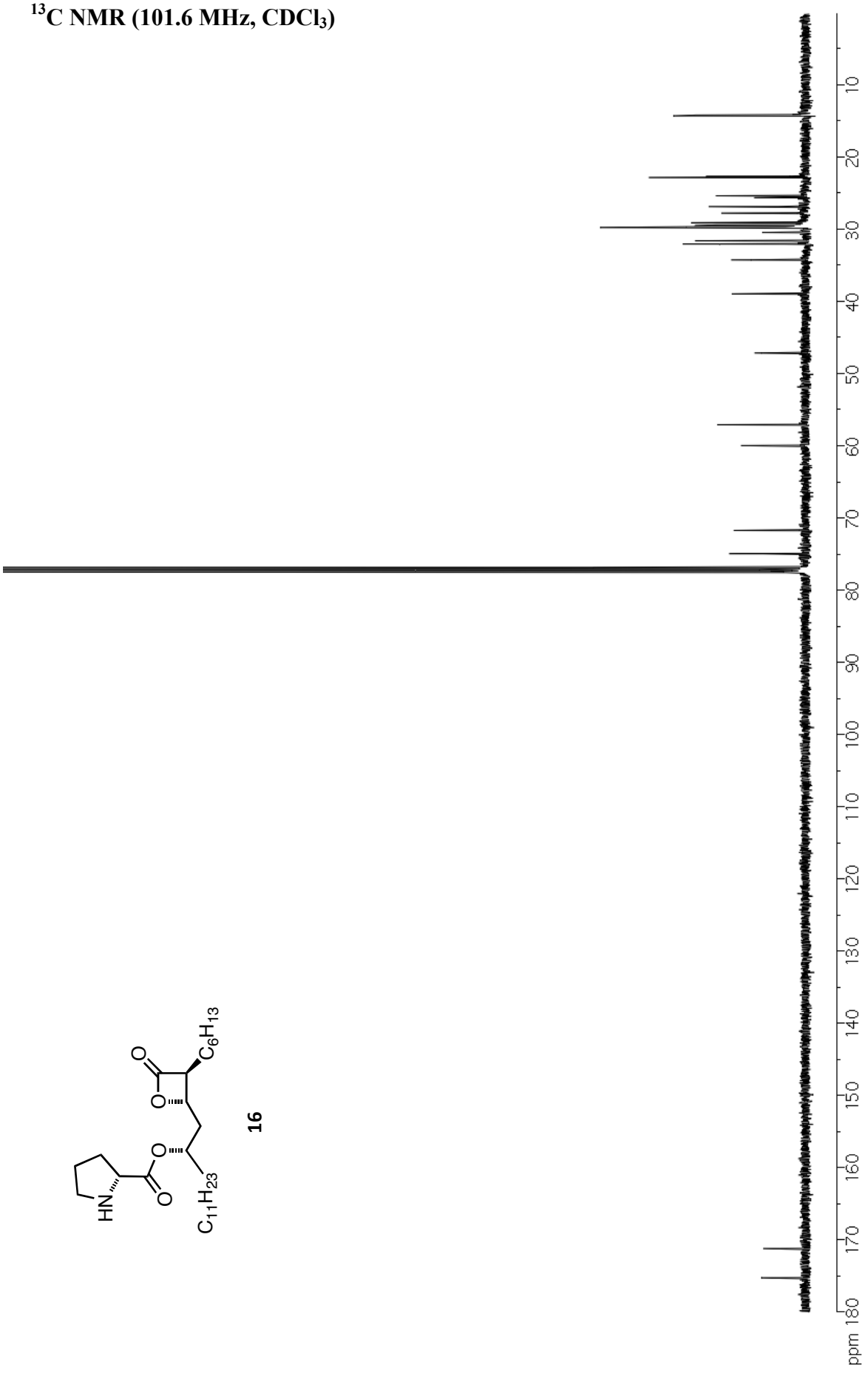
15

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



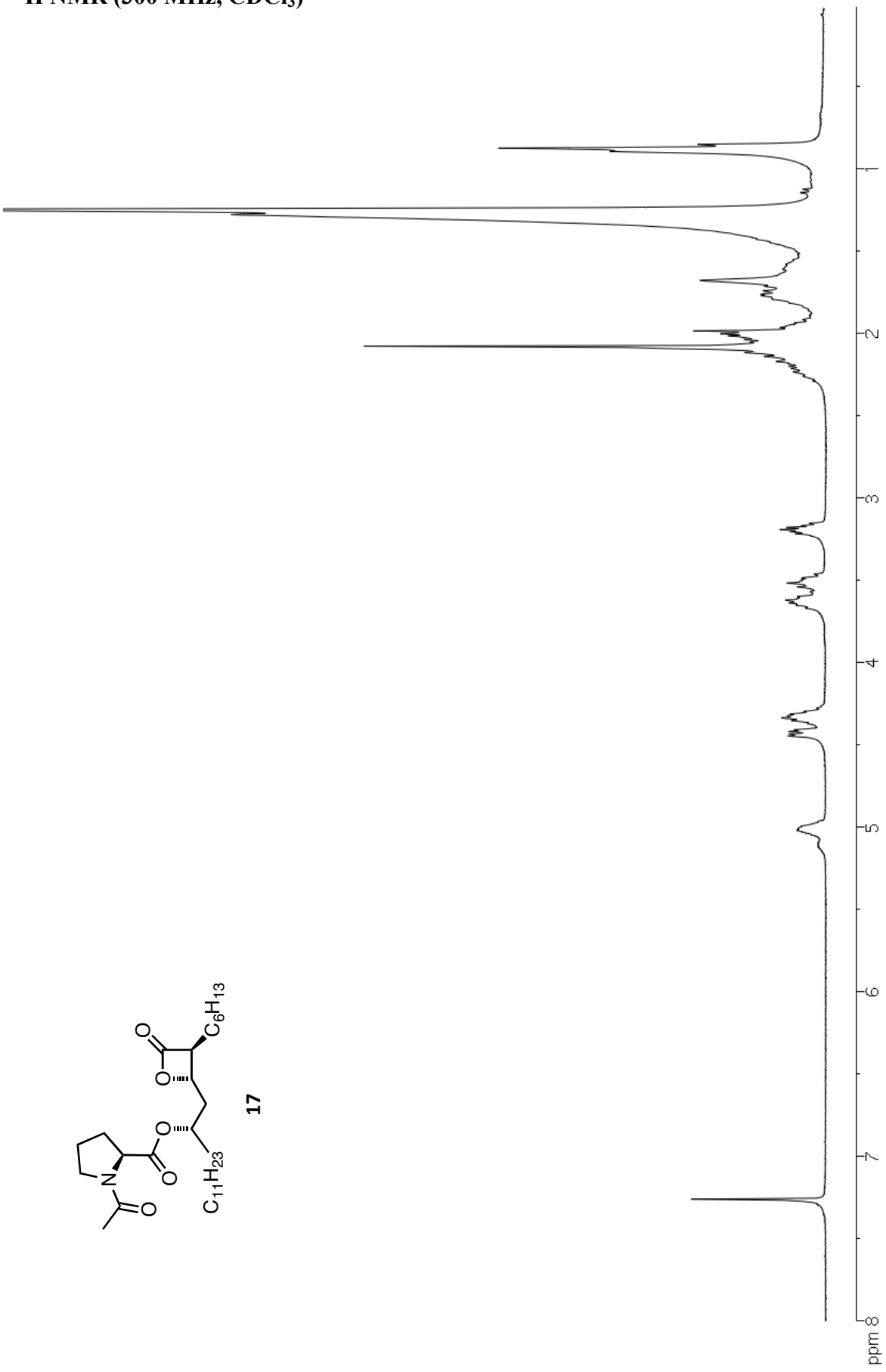
16

^{13}C NMR (101.6 MHz, $CDCl_3$)



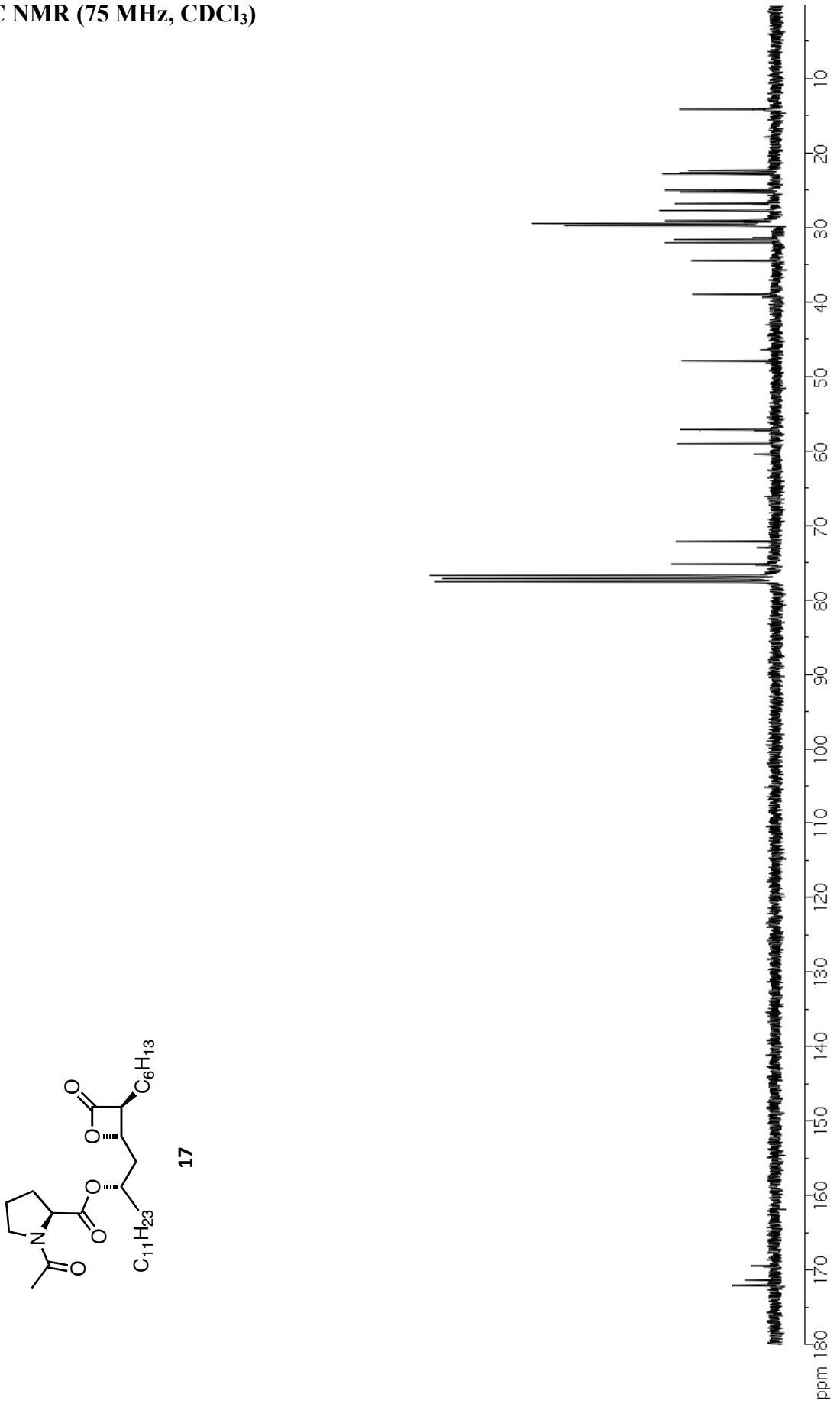
16

^{1}H NMR (300 MHz, $CDCl_3$)



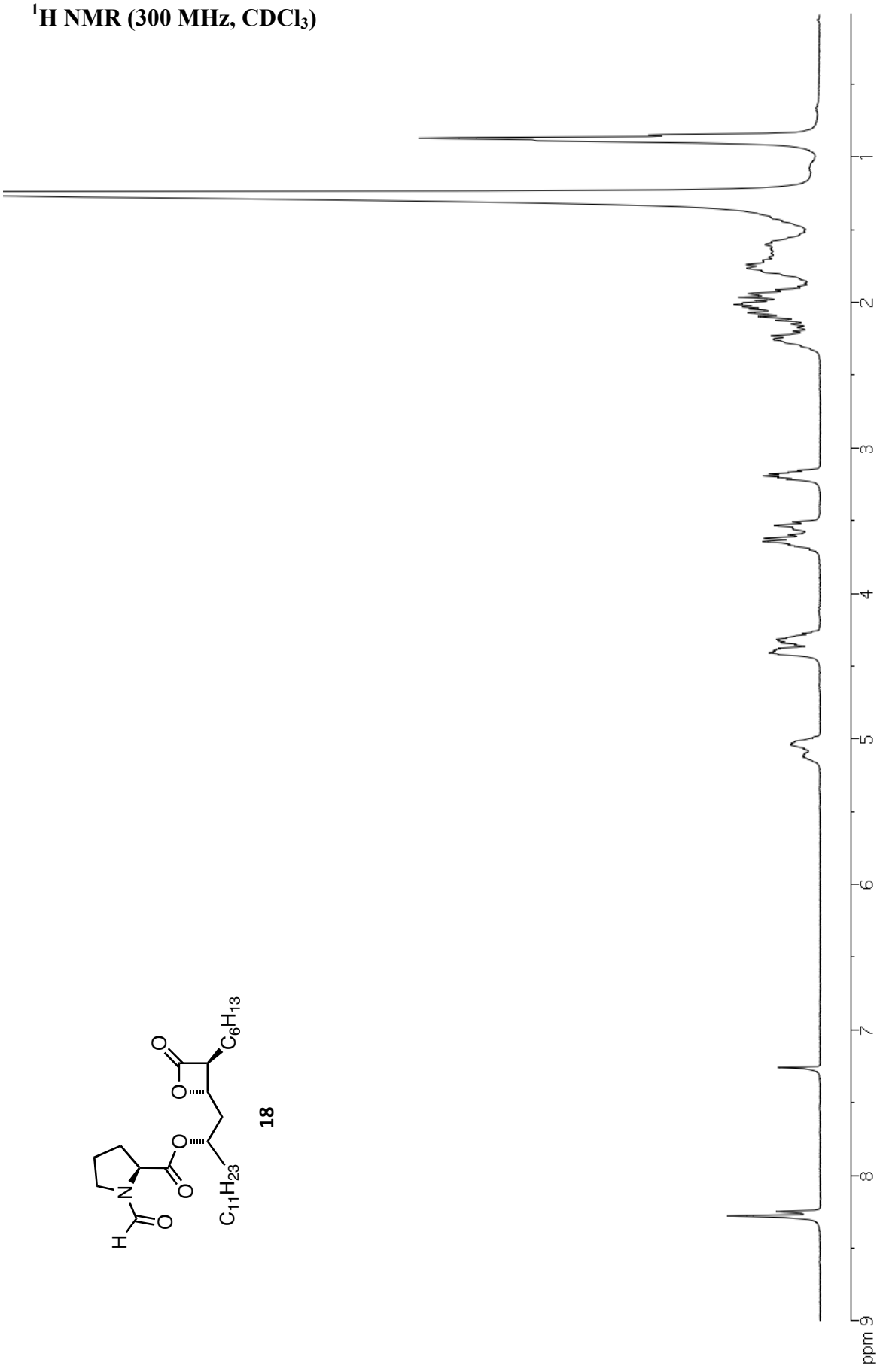
17

^{13}C NMR (75 MHz, $CDCl_3$)



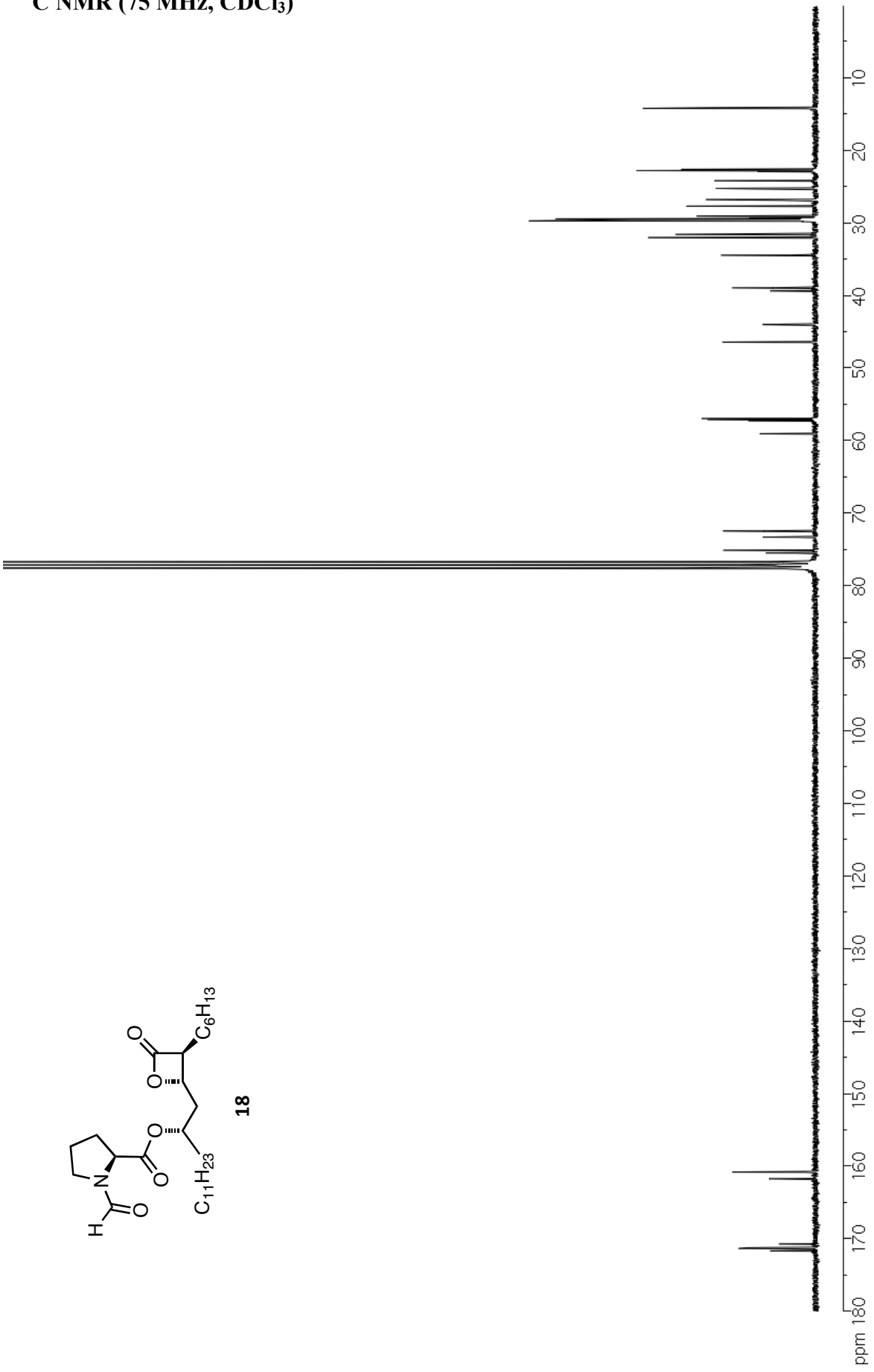
17

^{1}H NMR (300 MHz, $CDCl_3$)



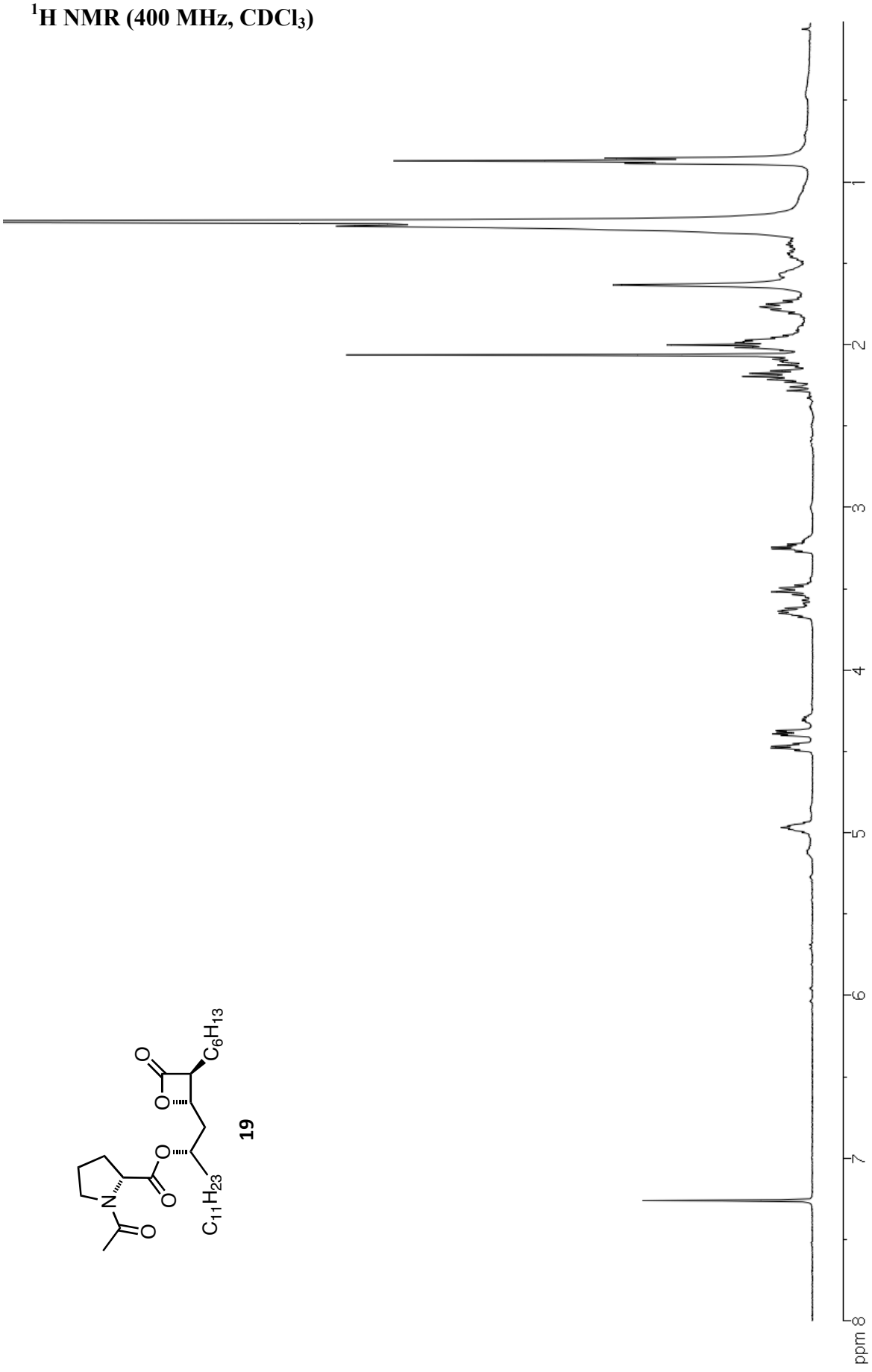
18

^{13}C NMR (75 MHz, $CDCl_3$)



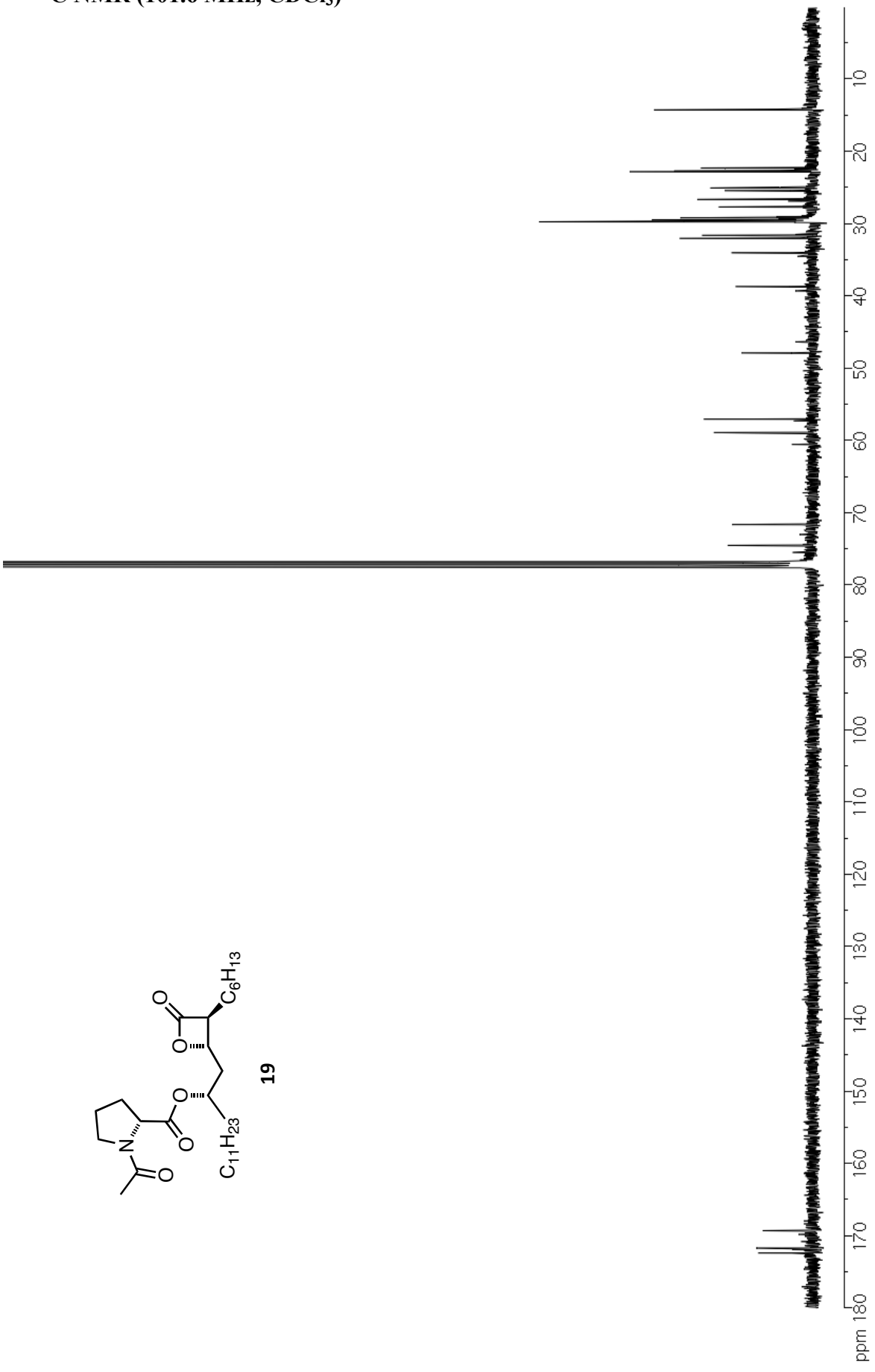
18

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



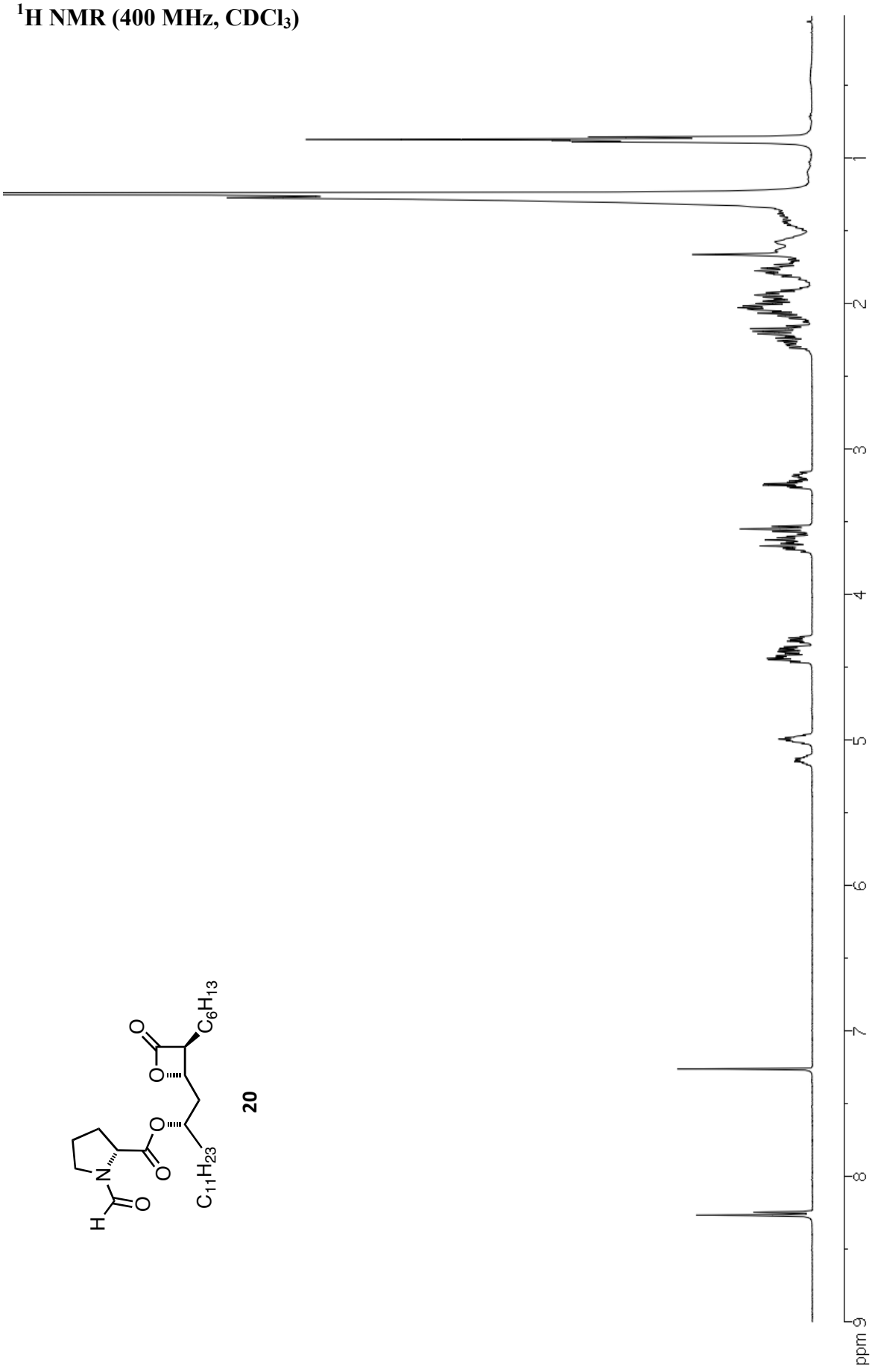
19

^{13}C NMR (101.6 MHz, $CDCl_3$)



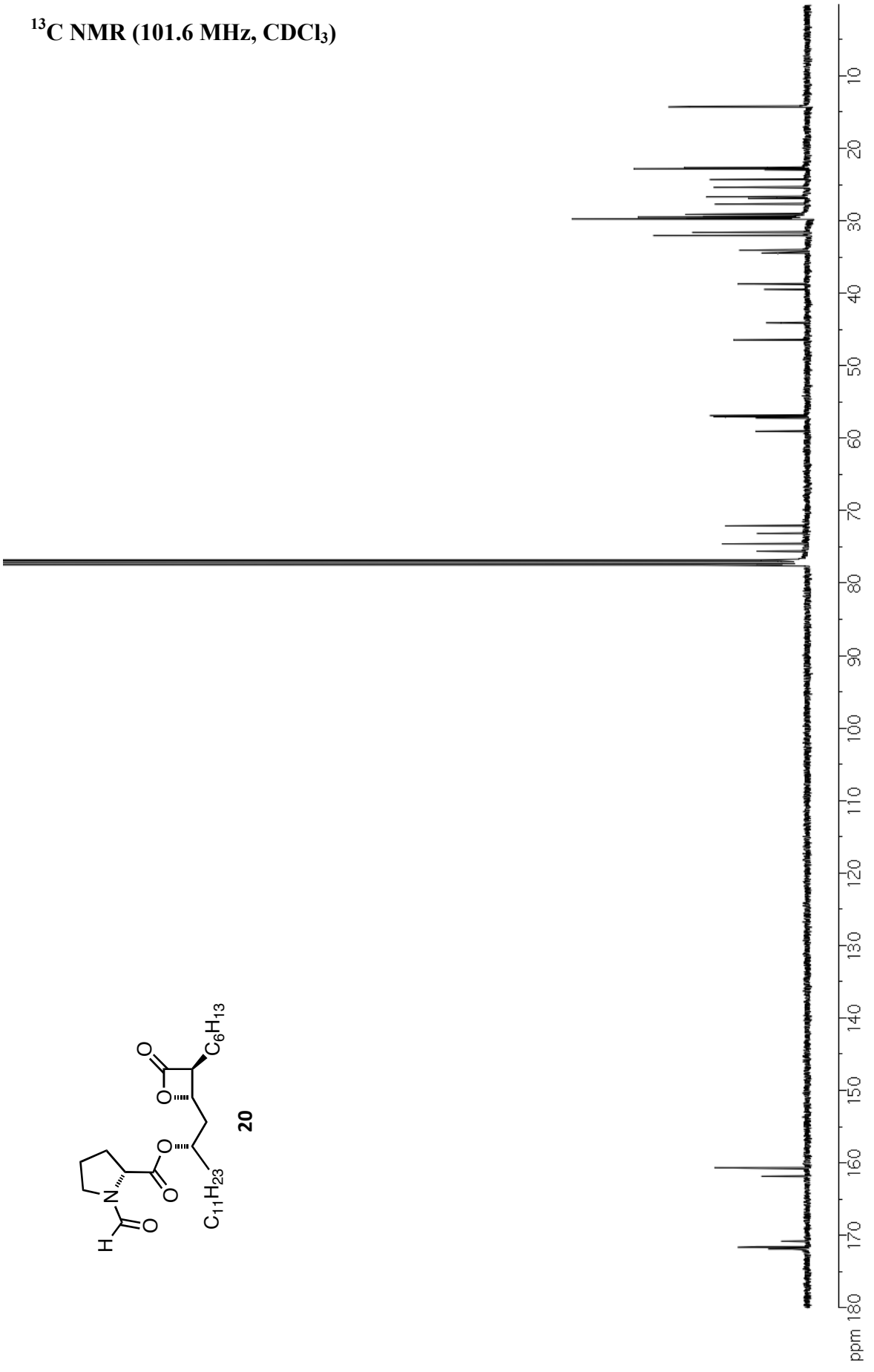
19

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



20

^{13}C NMR (101.6 MHz, $CDCl_3$)



20

Inhibitor Reversibility Studies against Rv3802c

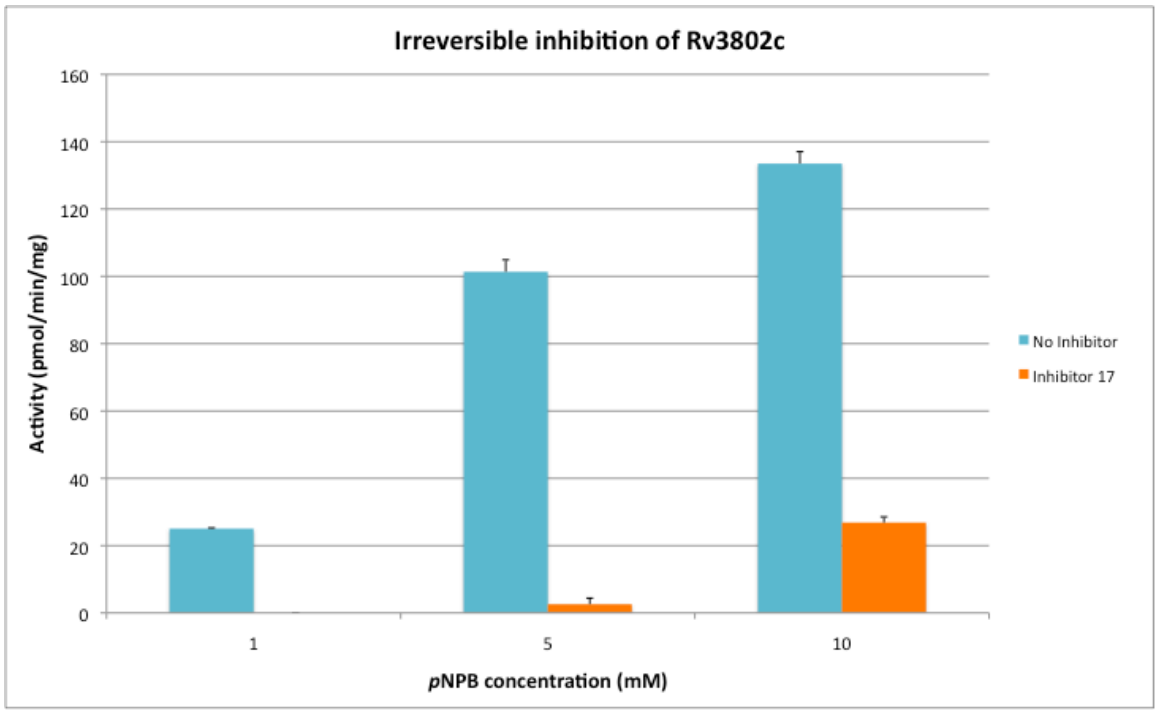


Figure S1. Covalent irreversible inhibition of Rv3802c by compound **17**.

Raw Data for Biological Assays

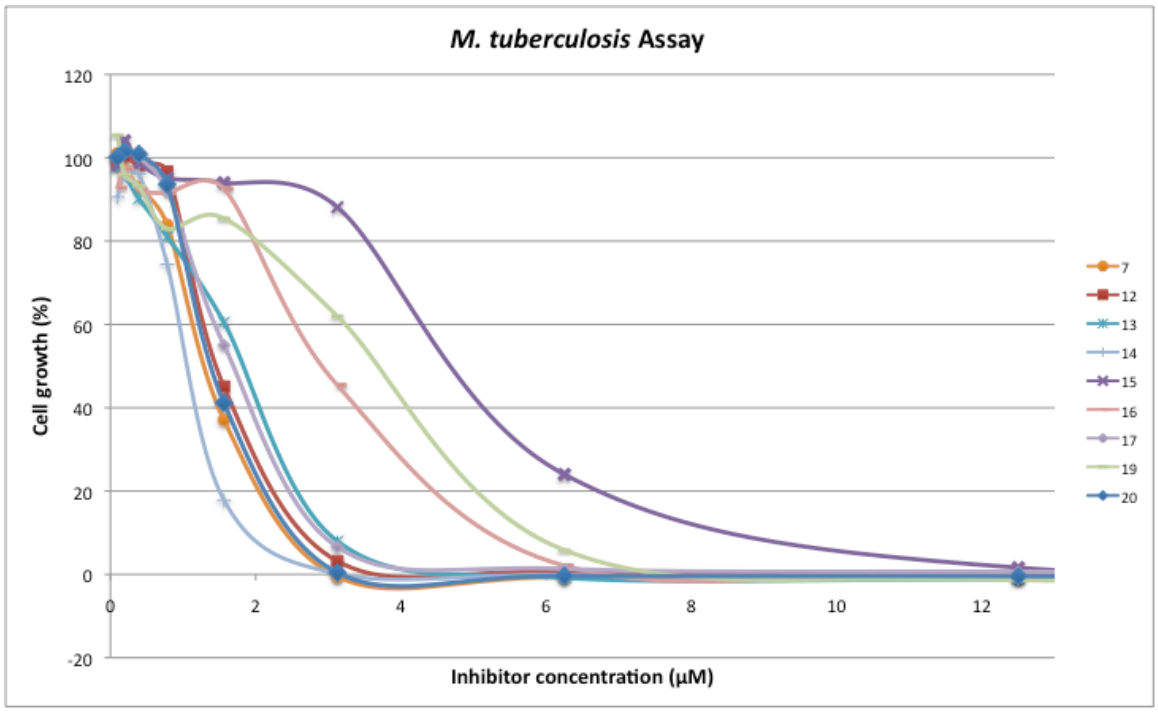


Figure S2: Growth of *M. tuberculosis* versus inhibitor concentration for compounds **7**, and **12**–**20**.

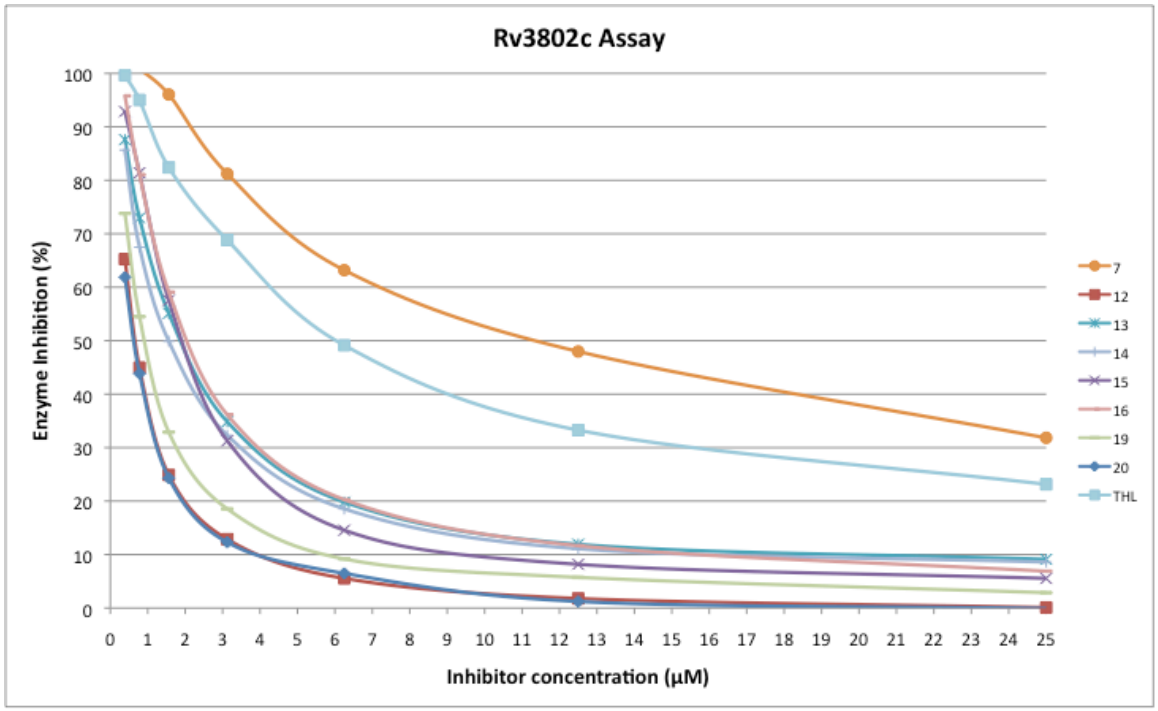


Figure S3: Percentage inhibition of Rv3802c versus inhibitor concentration for compounds **7**, and **12**–**20**.

Cytotoxicity Assay Results

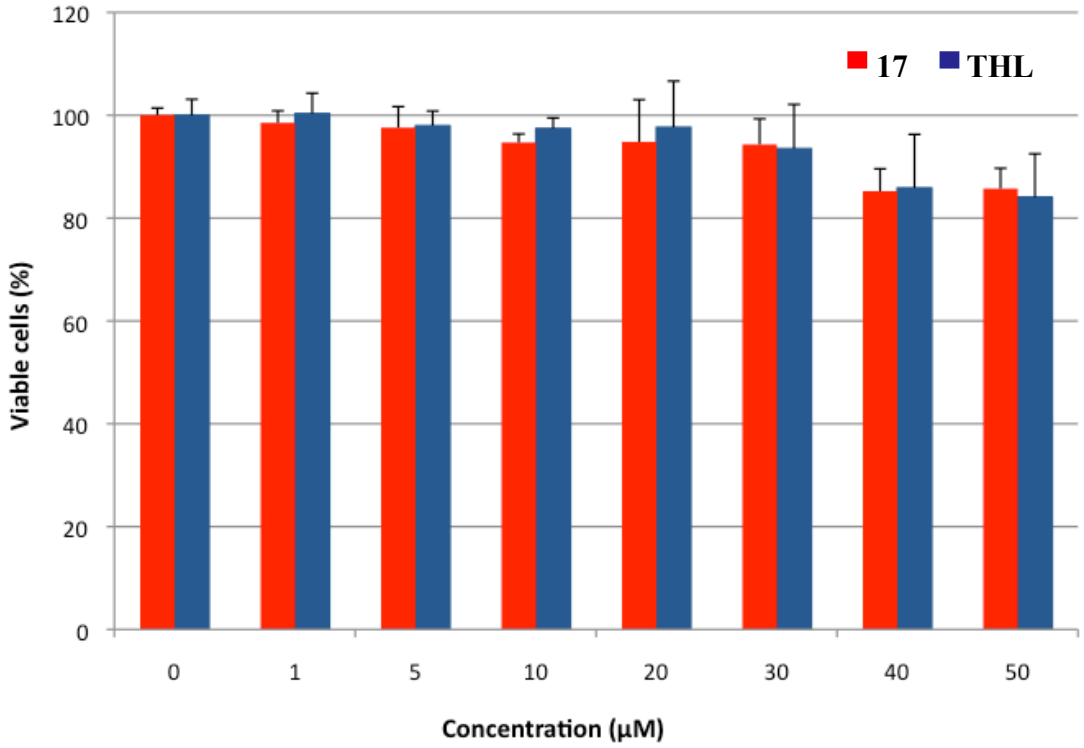


Figure S4: Cytotoxicity of inhibitor **17** and **THL** on HEK293 cells.

References

[1] N. K. Taneja, J. S. Tyagi, *J. Antimicrob. Chemother.* **2007**, *60*, 288-293.
[2] J. Pratt, J. D. Cooley, C. W. Purdy, D. C. Straus, *Curr. Microbiol.* **2000**, *40*, 306-309; N. P. West, F. M. Chow, E. J. Randall, J. Wu, J. Chen, J. M. Ribeiro, W. J. Britton, *FASEB J.* **2009**, *23*, 1694-1704.
[3] M. T. Pizzorno, S. M. Albonico, *J. Org. Chem.* **1977**, *42*, 909-910.
[4] P. D. Croce, R. Ferraccioli, C. La Rosa, *Tetrahedron* **1995**, *51*, 9385-9392.
[5] G. Ortar, T. Bisogno, A. Ligresti, E. Morera, M. Nalli, V. Di Marzo, *J. Med. Chem.* **2008**, *51*, 6970-6979.
[6] A. Crepin, N. Wattier, S. Petit, L. Bischoff, C. Fruit, F. Marsais, *Org. Biomol. Chem.* **2009**, *7*, 128-134.