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

Synthesis of pyrrothiamine, a novel thiamine analogue, and evaluation of its derivatives as potent and selective inhibitors of pyruvate dehydrogenase

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Supplementary Information (SI)

Enzyme assays

Evaluation of the inhibitory activity of compounds against Porcine PDH E1 *in vitro*

Porcine PDH E1 was purchased from Sigma. Porcine PDH E1 activity was determined by monitoring 2,6-dichlorophenolindophenol (DCPIP) reduction at 600 nm using a microplate reader (CLARIOstar) and conducted as described by Feng *et al*.[1] with some modifications. The percentage inhibition of compounds against porcine PDHc-E1 was assayed in triplicate at final concentrations of 0 µM (positive control) and 50 µM. The reaction buffer (50 mM KH_2PO_4 and 1 mM $MgCl_2$, pH 7) contained 50 µM thiamine pyrophosphate (TPP), 0.25 mM DCPIP, and 2 mg/ml PDH E1. The reaction mixture was preincubated at 37 ^oC for 30 min, then reaction was initiated by adding pyruvate to a final concentration of 50 mM. The same reaction mixture with TPP at 500 µM was used to determine whether the compound is competitive with respect to TPP. To determine the half-maximal inhibitory concentration (IC_{50}) TPP concentration was lowered to 10 µM, and inhibitor concentration was varied from 0 to 100 µM. Specific activity was calculated using the molar extinction coefficient of DCPIP, 21 mM^{-1} cm^{-1}.[2] The enzyme IC_{50} values were calculated from non-linear regression curve fitting using GraphPad Prism. The compound affinity (K_I) values were calculated and compared to the affinity of TPP (K_D); K_D was found to be 0.05 µM which is consistent with the value previously reported.[3]

Evaluation of the inhibitory activity of compounds against *S. cerevisiae* PDC *in vitro*

S. cerevisiae PDC was purchased from Sigma. *S. cerevisiae* PDC activity was determined by monitoring 2,6-dichlorophenolindophenol (DCPIP) reduction at 600 nm using a microplate reader (CLARIOstar) and conducted as described above with some modifications. The percentage inhibition of compounds against *S. cerevisiae* PDC was assayed in triplicate at final concentrations of 0 µM (positive control) and 200 µM. The reaction buffer (50 mM KH_2PO_4 and 1 mM $MgCl_2$, pH 7) contained 200 µM TPP, 0.27 mM DCPIP, and 0.15 mg/ml *S. cerevisiae* PDC. The reaction mixture was preincubated at 37 ^oC for 1 h, then reaction was initiated by adding pyruvate to a final concentration of 70 mM. Specific activity was calculated using the molar extinction coefficient of DCPIP, 21 mM^{-1} cm^{-1}.[2] *S. cerevisiae* PDC enzyme was chosen as a selectivity test since both PDC and PDH E1 enzymes use pyruvate as the donor substrate. Though humans do not express PDC, yeast PDC represents a member of eukaryotic TPP-dependent enzymes.

Evaluation of the inhibitory activity of compounds against *E. coli* OGDH *in vitro*

E. coli OGDH was from our previously reported article donated by R. Frank. *E. coli* OGDH activity was determined by monitoring DCPIP reduction at 600 nm using a microplate reader (CLARIOstar) and conducted as described by Mann *et al*. with some modifications.[4] The percentage inhibition of compounds against *E. coli* OGDH was assayed in triplicate at final concentrations of 0 µM (positive control) and 40 µM. The reaction buffer (50 mM KH_2PO_4 and 2 mM $MgCl_2$, pH 7) contained 40 µM TPP, 0.5 mM DCPIP, and 6.7 mg/ml *E. coli* OGDH. The reaction mixture was preincubated at 37 ^oC for 1 h, then reaction was initiated by adding α-ketoglutarate to a final concentration of 10 mM. Specific activity was calculated using the molar extinction coefficient of DCPIP, 21 mM^{-1} cm^{-1}.[3] *E. coli* OGDH E1 enzyme was also selected as another selectivity test because both OGDH E1 and PDH E1 enzymes use lipoamide as the acceptor substrate. The use of a bacterial enzyme was acceptable given that humans also possess OGDH E1 and that the amino acid sequence and the structure of the TPP pocket are highly conserved across species.[5]

Table S1. Summary of inhibitory activity of compounds 15, 16 and 19 on PDH E1.

Compounds	Inhibition (%)[a,b] [compound] = [TPP]	Inhibition (%)[a,c] [compound] = 10 x [TPP]
16	32 ± 3	< 10
15	41 ±5	< 10
19	78 ± 3	30 ± 5

[a] Data are the means of measurements in three technical replicates. [b] Inhibition (%) determined for compounds at 50 µM with [TPP] = 50 µM. [c] Inhibition (%) determined for compounds at 50 µM with [TPP] = 500 µM.

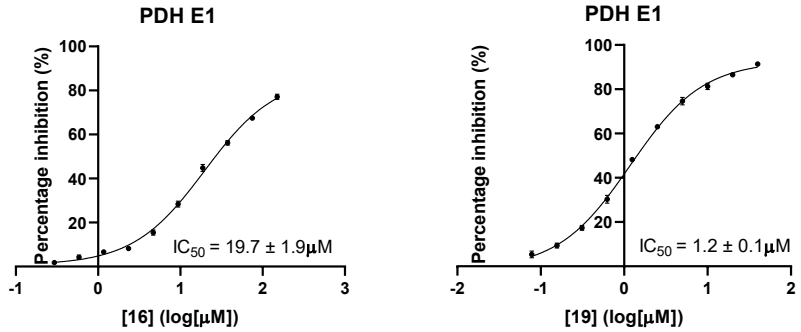


Figure S1. Determination of IC_{50} values for **16** and **19** under assay conditions as described above. Measurements were made in triplicate. Where the error bars are not visible, they are smaller than the symbols. Best-fit nonlinear regression curves are shown. Based on the K_D of TPP for PDH E1 to be 0.05 µM,[3] the K_I values of **16** and **19** were calculated to be 99 nM and 6 nM respectively.

Molecular Docking:

Docking of TPP and compound **19** was executed using GOLD docking program with human PDH (PDB: 6CFO) as the target. The ligand A5X401 was selected as the binding site. Our molecules were generated using Mercury. GA runs were set at 20 and was user defined with population size of 200 and 200000 number of operations. No early termination was permitted. Similarity and scaffold constraint to the original ligand were implemented on our compounds to mimic their binding positions. CHEMPLP and GoldScore were the docking scoring and rescoring respectively.[6] The best scoring conformations were chosen.

Synthetic Procedures

General synthesis methods

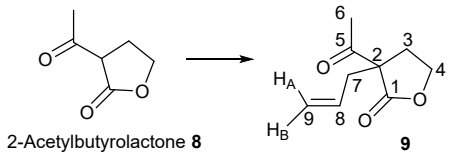
Oxygen- and moisture-sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Unless otherwise stated, all chemicals and reagents were purchased from commercial suppliers and used without further purification. Reaction progress was monitored by analytical thin-layer chromatography (TLC). TLC was conducted using Merck glass plates with silica Kieselgel 60 F254 of thickness 0.25 mm and visualised under 254 nm UV lamp or potassium permanganate staining solution (with gentle heating). Flash column chromatography was carried out in the indicated solvent system using prepacked silica gel cartridges for use on the Biotage Purification System. All solvents were removed under reduced pressure using a Büchi rotary evaporator with dry ice traps.

All yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Known compounds were characterised by, at minimum, ^{1}H NMR spectroscopy. New synthetic intermediates were characterised by, at minimum, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy and ESI-MS unless otherwise stated. Compounds subjected to biological assays were characterised by, at minimum, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy and HRMS. Further NMR experiments were performed as needed to confirm the structural assignment.

^{1}H NMR spectra were recorded at 400 MHz or 700 MHz in $CDCl_3$, CD_3OD or CD_3SOCD_3 solution on a Bruker 400 MHz or 700 MHz spectrometer and chemical shifts were recorded in parts per million (ppm). ^{13}C NMR spectra were recorded on either a Bruker 400 MHz or 700 MHz spectrometer. Resonances are described using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), qn (quintet), m (multiplet), br (broad), dd (doublet of doublets), etc. Coupling constants (*J*) are given in Hz and are rounded to the nearest 0.1 Hz. All NMR data were collected at 25 °C. Mass spectra used electrospray ionisation (ESI). Melting points of compounds were measured using a Reichert machine and are uncorrected.

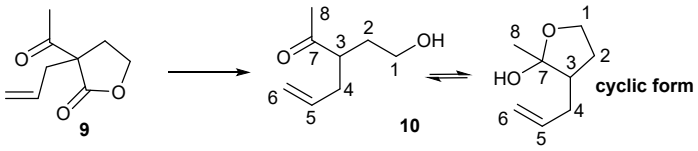
Experimental procedures – Synthesis

2-Acetyl-2-allylbutyrolactone **9**



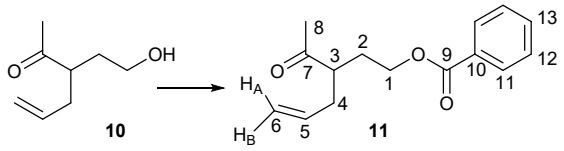
To a stirred solution of 2-acetylbutyrolactone **8** (2.15 mL, 20 mmol) in dry THF (100 mL, 0.2 M) under nitrogen at 0 °C was added sodium hydride (588 mg, 22 mmol) in three portions. The resultant mixture was stirred at r.t. for 30 min, treated with allyl bromide (2.6 mL, 30 mmol) dropwise, and stirred at r.t. overnight, concentrated under reduced pressure (to remove THF), diluted with EtOAc (150 mL), washed with aqueous phosphate buffer (pH 7) (50 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography to yield racemic **9** as a colourless oil (2.85 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.62 (dddd, 1H, *J* = 6.5, 8.0, 11.2 and 16.1 Hz, H-8), 5.20 (m, 1H, H-9_A), 5.19 (m, 1H, H-9_B), 4.30 (td, 1H, *J* = 3.5, 8.9 and 8.9 Hz, H-4_A), 4.20 (td, 1H, *J* = 7.5, 8.9 and 8.9 Hz, H-4_B), 2.89 (ddd, 1H, *J* = 3.5, 7.5 and 13.1 Hz, H-3_A), 2.79 (ddt, 1H, *J* = 0.9, 0.9, 8.0 and 14.5 Hz, H-7_A), 2.65 (ddt, 1H, *J* = 1.4, 1.4, 6.5 and 14.5 Hz, H-7_B), 2.36 (s, 3H, H-6), 2.12 (dt, 1H, *J* = 8.9, 8.9 and 13.1 Hz, H-3_B). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.0 (C-5), 175.2 (C-1), 131.3 (C-8), 120.4 (C-9), 66.4 (C-4), 61.0 (C-2), 39.2 (C-7), 28.8 (C-3), 25.8 (C-6). HRMS (ESI) *m/z*: [M+H$^+$] calculated for $C_9H_{12}O_3$: 169.0864; found: 169.0872. These data are consistent with those previously reported for this compound.[8]

3-Acetylhex-5-enol **10**



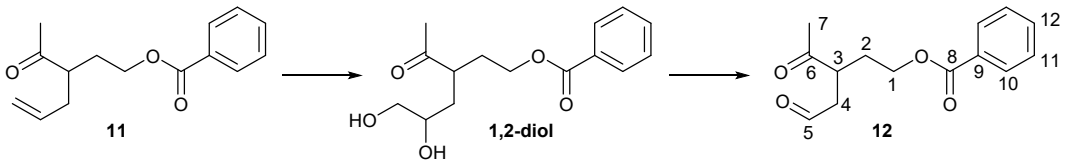
To a stirred solution of **9** (1.68 g, 10 mmol) in THF (65 mL) and water (35 mL) was added lithium hydroxide monohydrate (1.25 g, 30 mmol). The resultant mixture was stirred at 40 °C for 3 h, concentrated under reduced pressure, diluted with water (20 mL), acidified with conc. HCl to pH = 2-3, and extracted with EtOAc (200 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography to yield racemic **10** as a pale-yellow oil (566 mg, 40%). In $CDCl_3$, both the cyclic and acyclic forms coexist, complicating the analysis of the NMR spectra. In CD_3OD, **10** is mainly in its cyclic form with one diastereoisomer predominating: ^{1}H NMR (400 MHz, CD_3OD) δ 5.83 (m, 1H, H-5), 4.9-5.1 (m, 2H, H-6), 3.80 (m, 2H, H-1), 2.1-2.3 (m, 2H), 2.05 (m, 1H), 1.91 (m, 1H), 1.74 (m, 1H), 1.36 (s, 3H, H-8). ^{13}C NMR (100 MHz, CD_3OD) δ 137.1 (C-5), 114.3 (C-6), 106.2 (C-7), 65.1 (C-1), 48.9 (C-3), 33.3 (C-4), 30.0 (C-2), 18.4 (C-8). HRMS (ESI) *m/z*: [M+H$^+$] calculated for $C_8H_{14}O_2$: 143.1071; found: 143.1062.

3-Acetylhex-5-enyl benzoate **11**



To a stirred solution of **10** (1.42 g, 10 mmol) in dry pyridine (25 mL, 0.4 M) under nitrogen at 0 °C was added benzoyl chloride (1.22 mL, 10.5 mmol) dropwise. The resultant mixture was stirred at 40 °C for 5 h, concentrated under reduced pressure, diluted with EtOAc (100 mL), washed with 1 M HCl (100 mL) and then with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography to yield racemic **11** as a colourless oil (1.89 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, 2H, *J* = 7.7 Hz, H-11), 7.59 (t, 1H, *J* = 7.7 Hz, H-13), 7.47 (t, 2H, *J* = 7.7 Hz, H-12), 5.74 (m, 1H, H-5), 5.11 (m, 1H, H-6$_A$), 5.08 (m, 1H, H-6$_B$), 4.34 (t, 2H, *J* = 6.4 Hz, H-1), 2.79 (m, 1H, H-3), 2.43 (m, 1H, H-4$_A$), 2.30 (m, 1H, H-4$_B$), 2.20 (s, 3H, H-8), 2.17 (m, 1H, H-2$_A$), 1.90 (m, 1H, H-2$_B$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.0 (C-7), 166.6 (C-9), 134.7 (C-5), 133.0 (C-13), 130.1 (C-10), 129.6 (C-11), 128.4 (C-12), 117.6 (C-6), 63.1 (C-1), 49.3 (C-3), 35.9 (C-4), 29.7 (C-8), 29.6 (C-2). HRMS (ESI) *m/z*: [M+H$^+$] calculated for $C_8H_{14}O_2$: 247.1334; found: 247.1348.

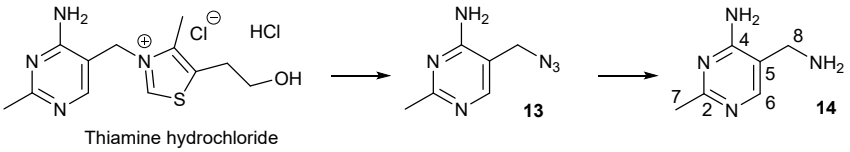
3-Acetyl-5-oxopentyl benzoate **12**



To a stirred solution of **11** (1230 mg, 5 mmol) in *t*-BuOH (25 mL) and water (25 mL, 0.1 M) was added AD-mix-α (10 g) and methanesulfonamide (2.4 g, 25 mmol). The resultant mixture was stirred at r.t. overnight, quenched with sodium sulfite (3.1 g), concentrated under reduced pressure, diluted with water (50 mL), and extracted with DCM (3 x 100 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated under reduced pressure to yield the **1,2-diol** (mixture of diatereomers) as a viscous colourless oil, which was used in the next step without further purification. To a stirred solution of the resultant oil in acetone (13 mL) and water (12 mL, 0.2 M) at 0 °C was added

$NaIO_4$ (1070 mg, 5 mmol). The resultant mixture was stirred at r.t. overnight, concentrated under reduced pressure, diluted with water (150 mL), and extracted with DCM (3 x 100mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography to yield racemic **12** as a colourless oil (990 mg, 80% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (s, 1H, H-5), 8.03 (dd, 2H, *J* = 1.4 and 8.5 Hz, H-10), 7.59 (tt, 1H, *J* = 1.4, 7.4 and 7.4 Hz, H-12), 7.47 (m, 2H, H-11), 4.36 (m, 2H, H-1), 3.26 (dtd, 1H, *J* = 4.1, 6.7, 6.7 and 9.2 Hz, H-3), 3.07 (dd, 1H, *J* = 9.2 and 18.5 Hz, H-4), 2.62 (dd, 1H, *J* = 4.1 and 18.5 Hz, H-4), 2.32 (s, 3H, H-7), 2.17 (dq, 1H, *J* = 6.7, 6.7, 6.7 and 13.0, H-2_A), 1.93 (dq, 1H, *J* = 6.7, 6.7, 6.7 and 13.0, H-2_B). ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.5 (C-6), 200.0 (C-5), 166.6 (C-8), 133.3 (C-12), 129.8 (C-9), 129.6 (C-10), 128.6 (C-11), 62.2 (C-1), 44.9 (C-4), 43.0 (C-3), 30.2 (C-2), 29.6 (C-7). HRMS (ESI) *m/z*: [M+H$^+$] calculated for $C_8H_{14}O_2$: 249.1127; found: 249.1139.

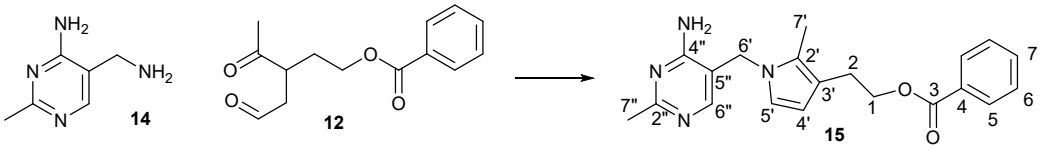
5-(Aminomethyl)-2-methylpyrimidin-4-amine **14**



Thiamine hydrochloride

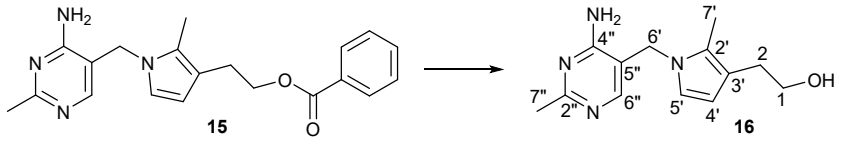
The synthesis and characterisation data for **13** have been previously described.[7] A stirred solution of **13** (1.02 g, 6.2 mmol) in MeOH (50 mL, 0.2 M) at r.t. was treated with 10% Pd/C (100 mg) under nitrogen. The flask was evacuated and flushed with hydrogen gas (three times). The resultant mixture was stirred vigorously at r.t. under an atmosphere of hydrogen (1 atm, H_2 balloon) for 4 h, filtered through Celite, and concentrated under reduced pressure to yield **14** as a white solid (847 mg, 98%), which was used in the next step without further purification. m.p. 143 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (s, 1H, H-6), 3.68 (s, 2H, H-8), 2.40 (s, 3H, H-7). ^{13}C NMR (100 MHz, CD_3OD) δ 165.6 (C-2), 162.4 (C-4), 152.2 (C-6), 114.7 (C-5), 39.0 (C-8), 23.2 (C-7). HRMS (ESI) *m/z*: [M+H$^+$] calculated for $C_6H_{10}N_4$: 139.0983; found: 139.0968.

2-(1-((4-Amino-2-methylpyrimidin-5-yl)methyl)-2-methyl-1H-pyrrol-3-yl)ethyl benzoate (pyrrothiamine benzoate) **15**



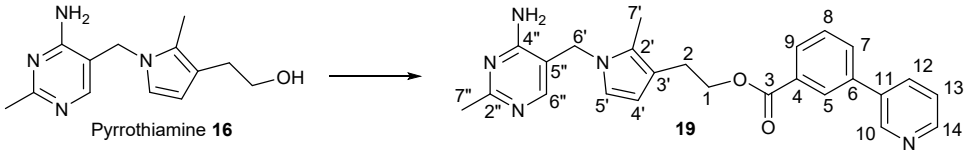
To a stirred solution of **12** (172 mg, 0.69 mmol) in dry DMF (3.5 mL, 0.2 M) under nitrogen was added **14** (106 mg, 0.76 mmol) and some molecular sieves (4 Å). The resultant mixture was stirred at 45 °C for 3 days, filtered, diluted with DCM (150 mL), washed with sat. aq. $NaHCO_3$ (80 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography to yield product **15** as a white solid (101 mg, 42%). m.p. 178 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (m, 2H, H-5), 7.85 (s, 1H, H-6”), 7.55 (m, 1H, H-7), 7.42 (m, 2H, H-6), 6.51 (d, 1H, *J* = 2.8 Hz, H-5’), 6.10 (d, 1H, *J* = 2.8 Hz, H-4’), 4.86 (br, 2H, NH_2), 4.81 (s, 2H, H-6’), 4.41 (t, 2H, *J* = 7.2 Hz, H-1), 2.86 (t, 2H, *J* = 7.2 Hz, H-2), 2.50 (s, 3H, H-7”), 2.13 (s, 3H, H-7’). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.5 (C-2”), 166.6 (C-3), 161.2 (C-4”), 155.1 (C-6”), 132.8 (C-7), 130.4 (C-4), 129.5 (C-5), 128.3 (C-6), 125.9 (C-2’), 119.0 (C-5’), 116.9 (C-3’), 109.9 (C-5”), 109.3 (C-4’), 65.8 (C-1), 46.0 (C-6’), 26.2 (C-2), 25.6 (C-7”), 10.0 (C-7’). HRMS (ESI) *m/z*: [M+H$^+$] calculated for $C_{20}H_{22}N_4O_2$: 351.1821; found: 351.1835.

Pyrrothiamine **16**



To a stirred solution of **15** (122 mg, 0.35 mmol) in dry MeOH (1.8 mL, 0.2 M) under nitrogen was added potassium carbonate (97 mg, 0.7 mmol). The resultant mixture was stirred at r.t. for 3 h, concentrated under reduced pressure, diluted with *n*-BuOH (50 mL), washed with sat. aq. $NaHCO_3$ (10 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography to yield product **16** as a white solid (61 mg, 71%). m.p. 150 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.05 (s, 1H, H-6"), 6.61 (d, 1H, *J* = 2.8 Hz, H-5'), 6.00 (d, 1H, *J* = 2.8 Hz, H-4'), 4.84 (s, 2H, H-6'), 3.63 (t, 2H, *J* = 7.8 Hz, H-1), 2.65 (t, 2H, *J* = 7.8 Hz, H-2), 2.40 (s, 3H, H-7"), 2.07 (s, 3H, H-7'). ^{13}C NMR (100 MHz, CD_3OD) δ 165.7 (C-2"), 160.8 (C-4"), 151.4 (C-6"), 124.8 (C-2'), 119.2 (C-5'), 116.9 (C-3'), 112.0 (C-5"), 108.1 (C-4'), 63.0 (C-1), 43.6 (C-6'), 29.8 (C-2), 23.4 (C-7"), 8.0 (C-7'). HRMS (ESI) *m/z*: [M+H$^+$] calculated for $C_{13}H_{18}N_4O$: 247.1558; found: 247.1566.

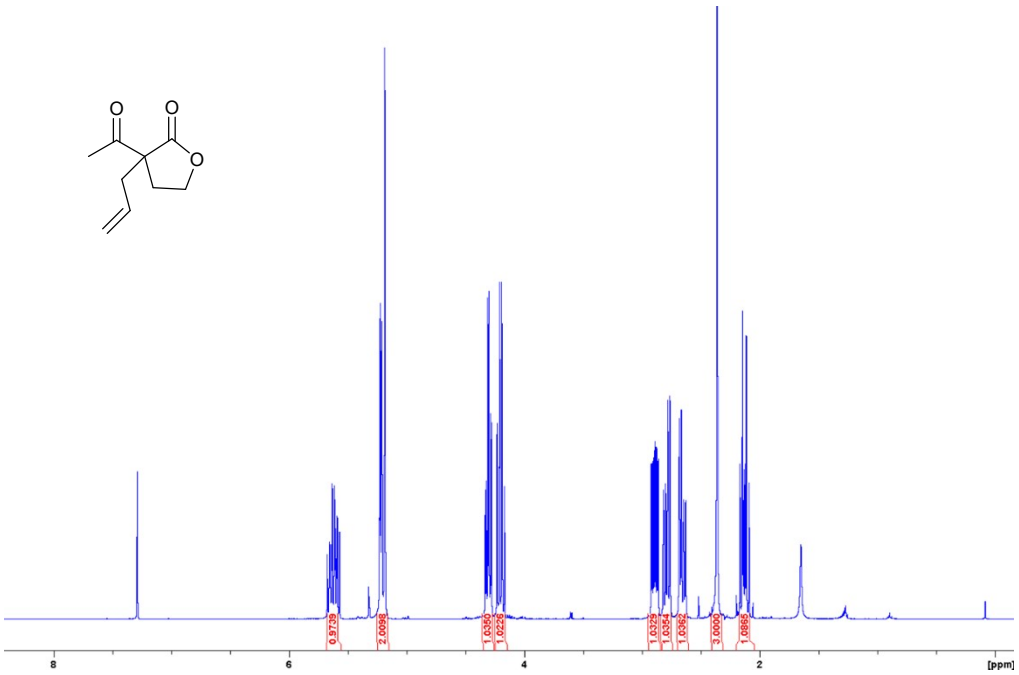
Pyrrothiamine 3-(pyridin-3-yl)benzoate **19**



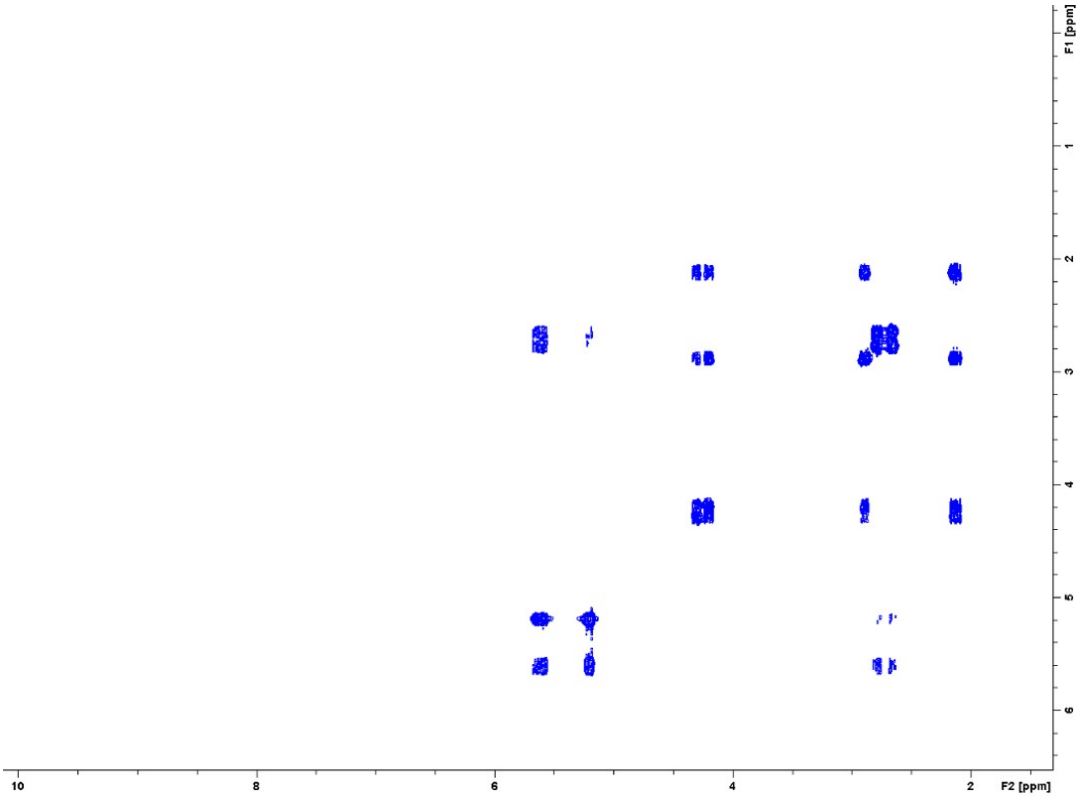
To a stirred solution of *m*-pyridin-3-ylbenzoic acid (30 mg, 0.148 mmol) and DCC (70 mg, 0.34 mmol) in dry DMF (1.2 mL, 0.1 M) under nitrogen at 0 °C was added DMAP (18 mg, 0.148 mmol) and **16** (28 mg, 0.11 mmol). The resultant mixture was stirred at r.t. for 2 days, diluted with DCM, filtered through cotton wool (to remove DCC/DCU), washed with aqueous phosphate buffer (pH 7) (50 mL), and extracted with DCM (100 mL). The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica flash chromatography to yield product **19** as a white solid (13 mg, 27%). m.p. 212 °C. ^{1}H NMR (400 MHz, CD_3SOCD_3) δ 8.92 (m, 1H, H-10), 8.62 (m, 1H, H-14), 8.22 (m, 1H, H-5), 8.11 (m, 1H, H-12), 8.02 (m, 2H, H-7 and H-9), 7.66 (t, 1H, *J* = 7.6 Hz, H-8), 7.52 (m, 1H, H-13), 7.05 (s, 1H, H-6"), 6.73 (br, 2H, NH_2), 6.64 (d, 1H, *J* = 2.8 Hz, H-5'), 5.97 (d, 1H, *J* = 2.8 Hz, H-4'), 4.78 (s, 2H, H-6'), 4.37 (t, 2H, *J* = 7.0 Hz, H-1), 2.82 (t, 2H, *J* = 7.0 Hz, H-2), 2.26 (s, 3H, H-7"), 2.04 (s, 3H, H-7'). ^{13}C NMR (100 MHz, CD_3SOCD_3) δ 166.0 (C-2"), 165.9 (C-3), 160.9 (C-4"), 153.1 (C-6"), 149.5 (C-14), 148.2 (C-10), 138.1 (C-6), 135.1 (C-11), 134.8 (C-12), 132.1 (C-7), 131.3 (C-4), 130.2 (C-8), 129.2 (C-9), 128.0 (C-5), 125.4 (C-2'), 124.5 (C-13), 120.4 (C-5'), 115.8 (C-3'), 111.4 (C-5"), 108.2 (C-4'), 66.2 (C-1), 44.1 (C-6'), 26.1 (C-2), 25.5 (C-7"), 9.6 (C-7'). HRMS (ESI) *m/z*: [M+H$^+$] calculated for $C_{25}H_{25}N_5O_2$: 428.2086; found: 428.2081.

NMR spectra

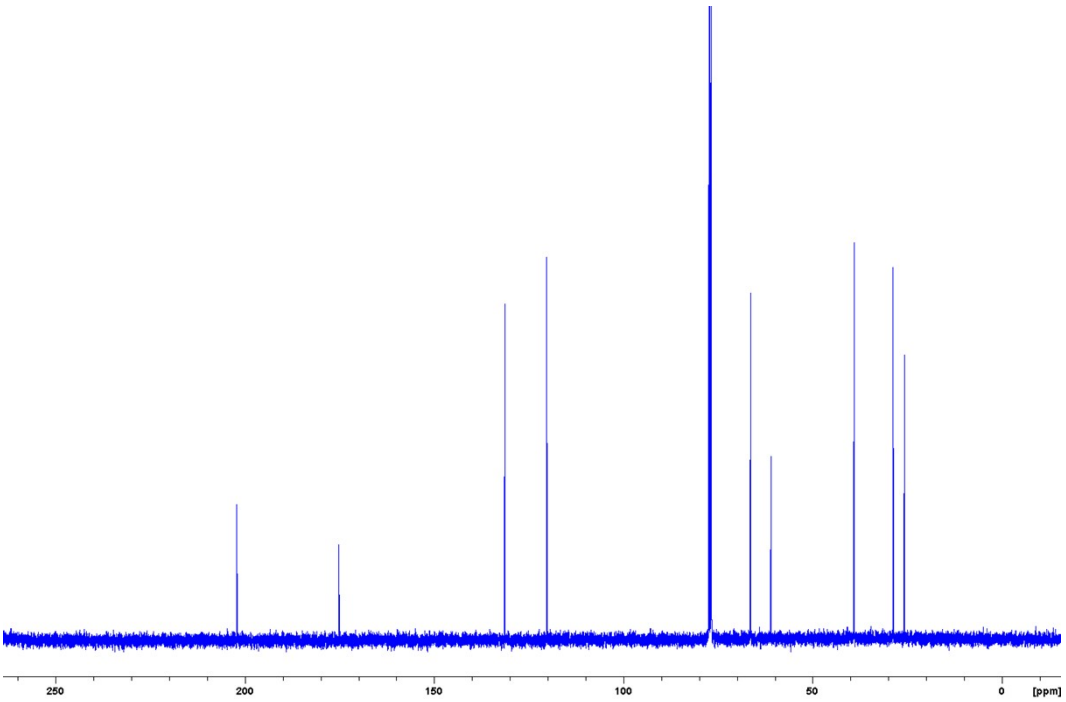
^{1}H NMR of **9** in $CDCl_3$:



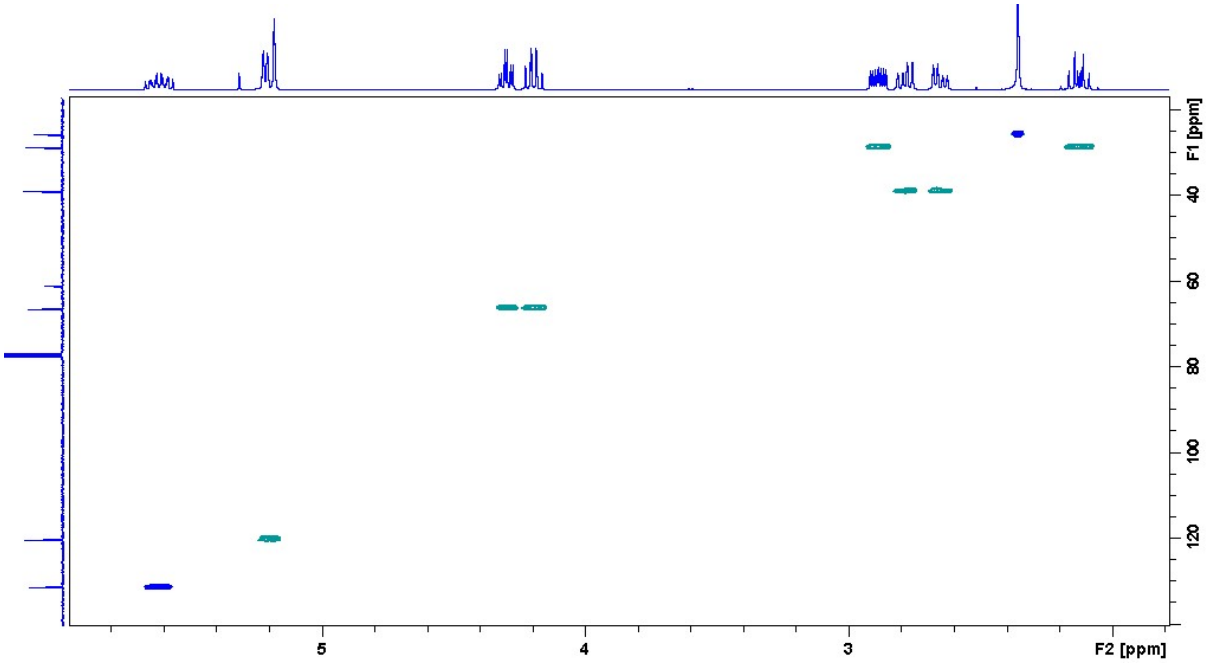
^{1}H COSY NMR of **9** in $CDCl_3$:



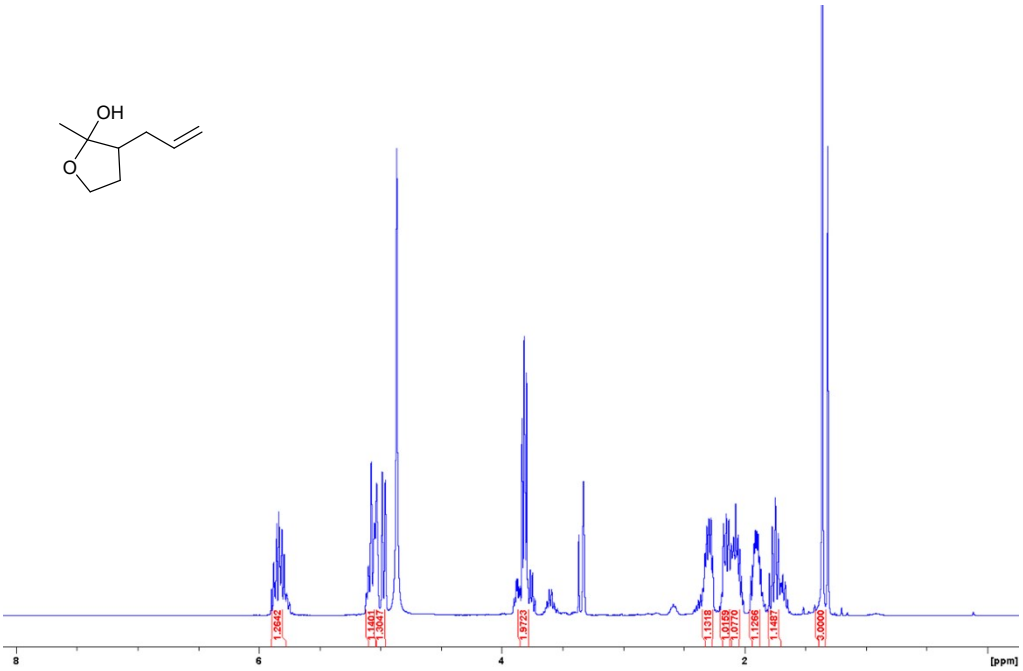
^{13}C NMR of **9** in $CDCl_3$:



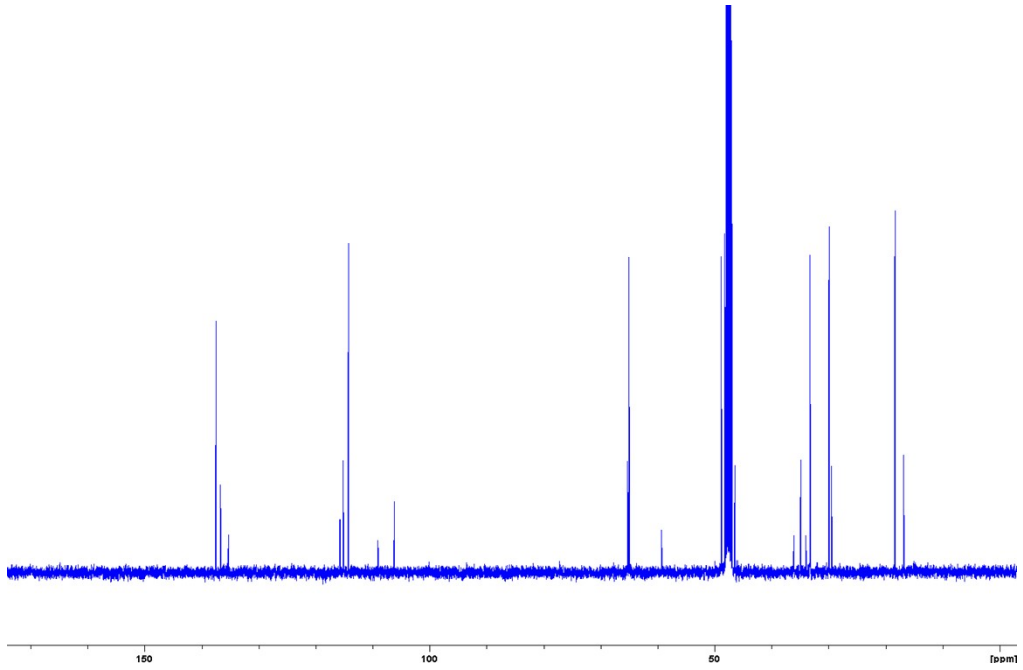
^{1}H-^{13}C HSQC NMR of **9** in $CDCl_3$:



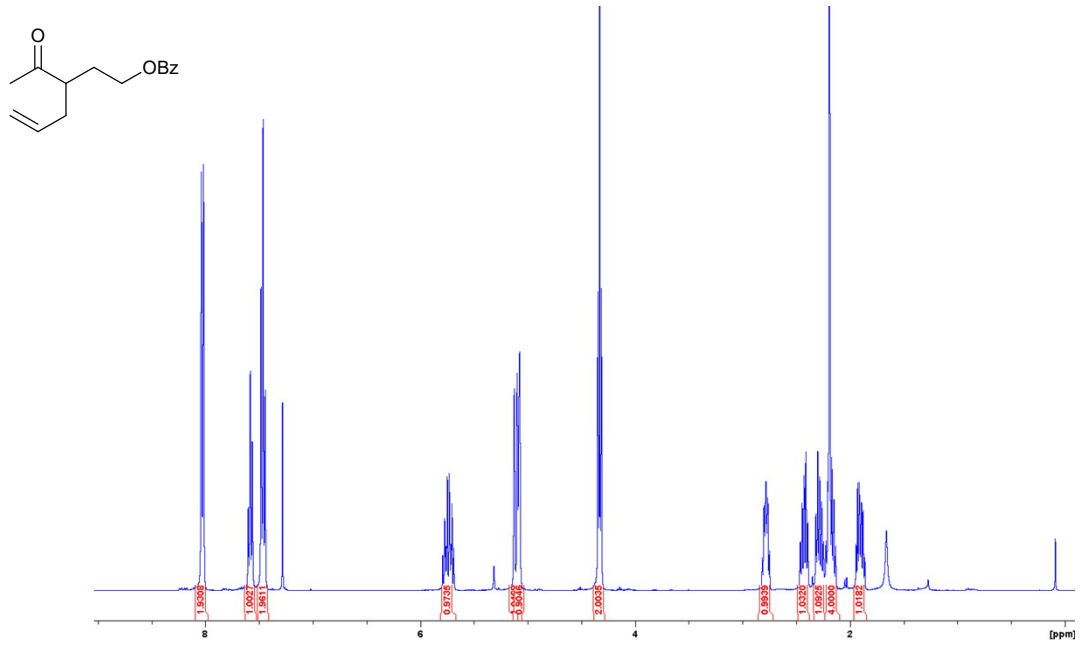
^{1}H NMR of **10** in CD_3OD: (the small peaks belong to the protons of the minor diastereomer and of the acyclic form)



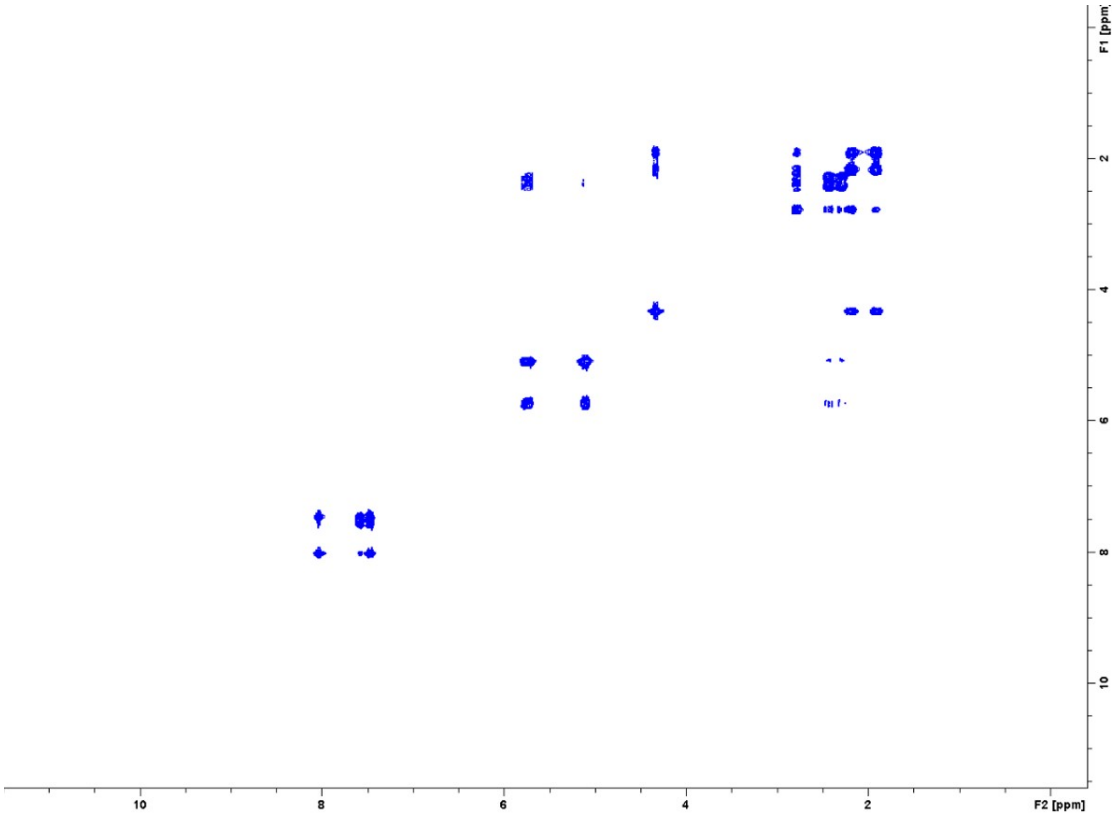
^{13}C NMR of **10** in CD_3OD: (the small peaks belong to the carbons of the minor diastereomer and of the acyclic form)



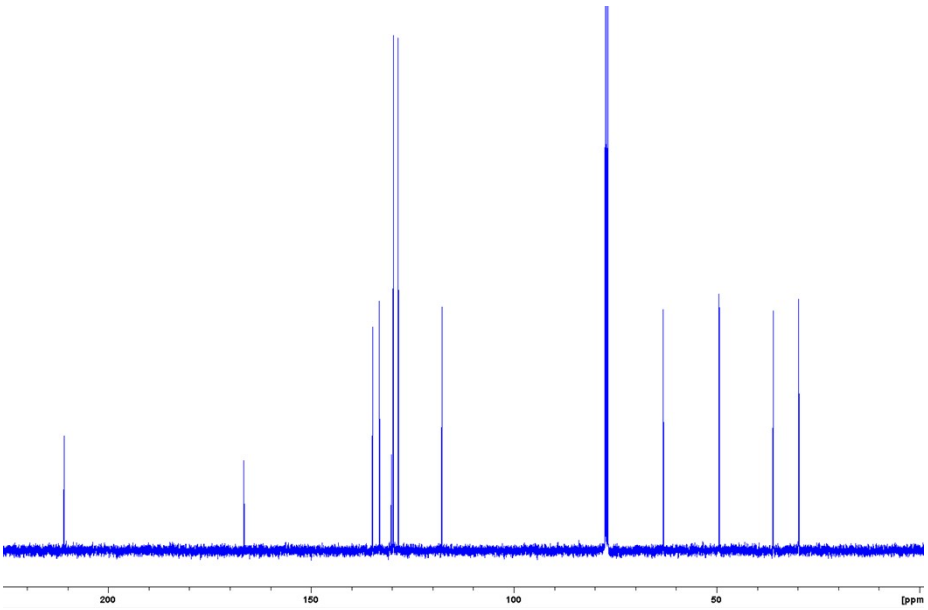
^{1}H NMR of **11** in $CDCl_3$:



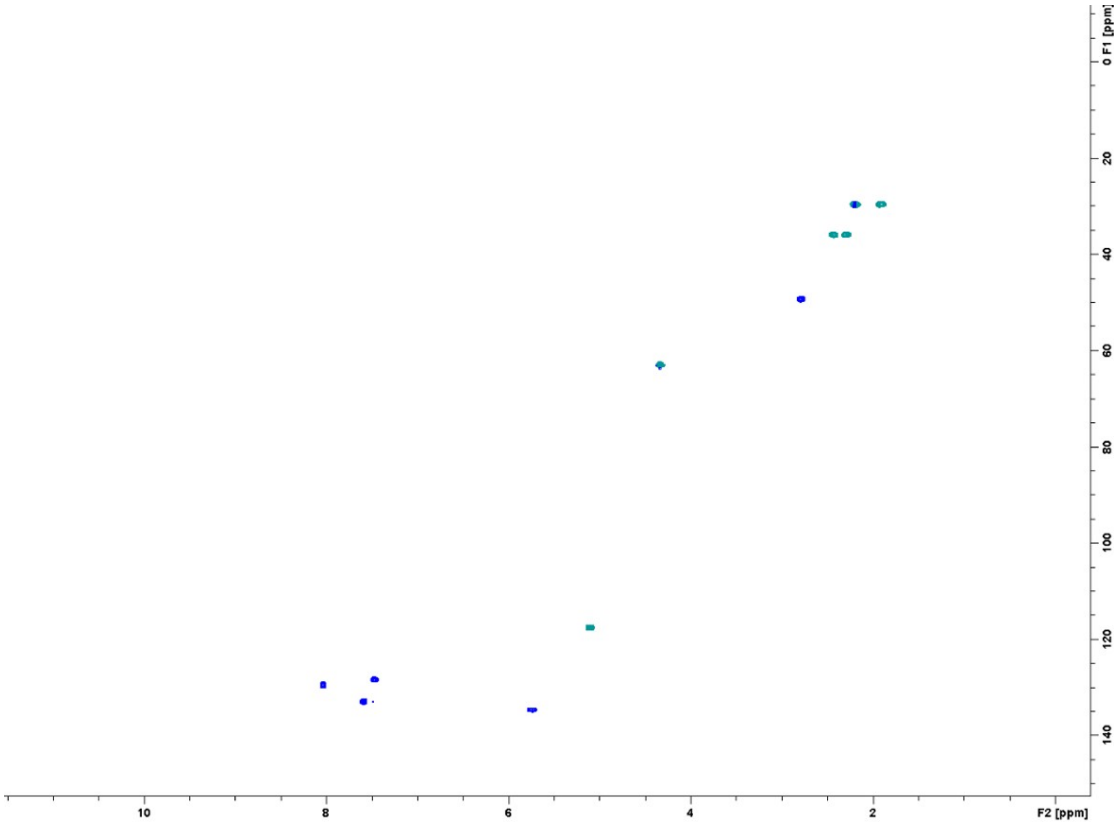
^{1}H COSY NMR of **11** in $CDCl_3$:



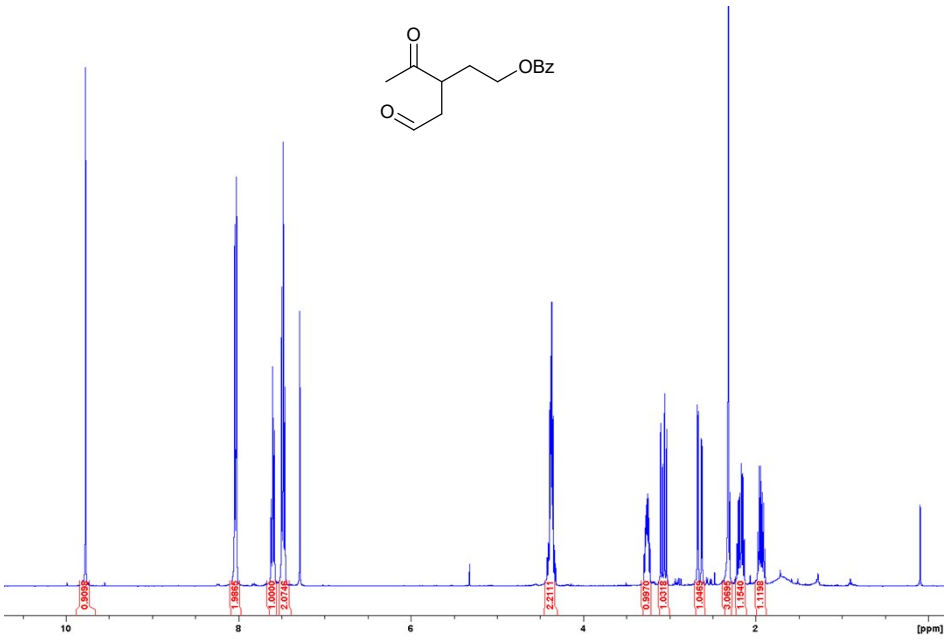
^{13}C NMR of **11** in $CDCl_3$:



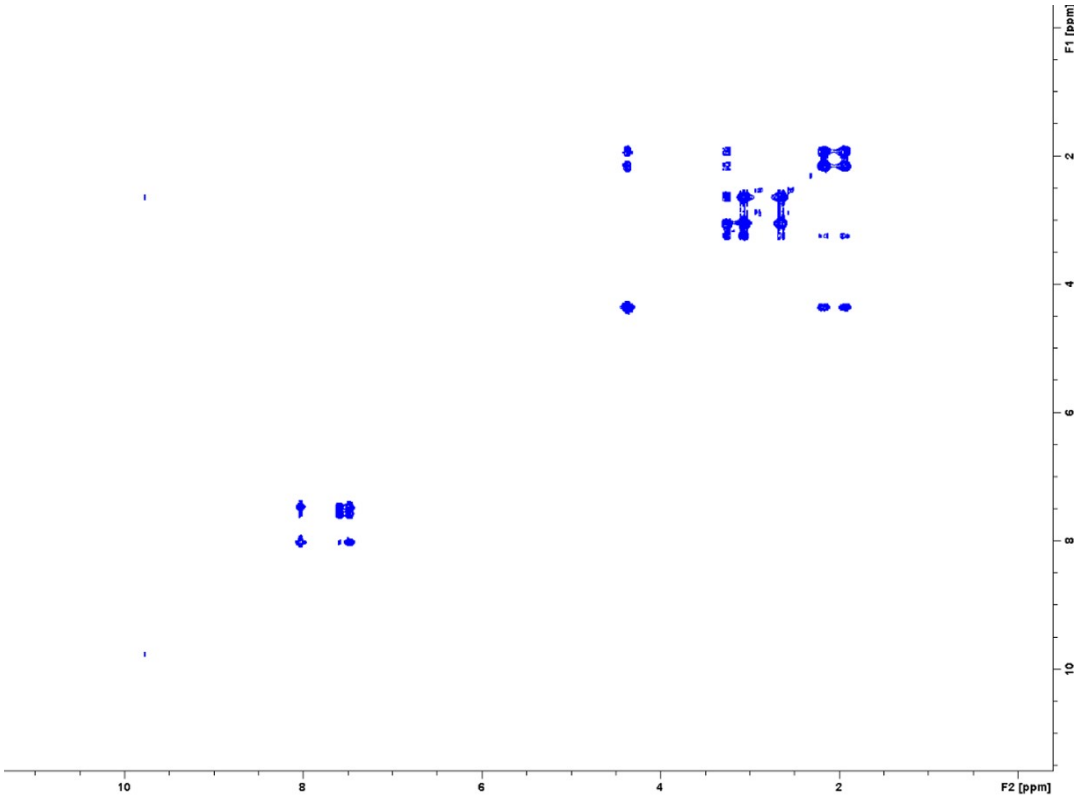
^{1}H-^{13}C HSQC NMR of **11** in $CDCl_3$:



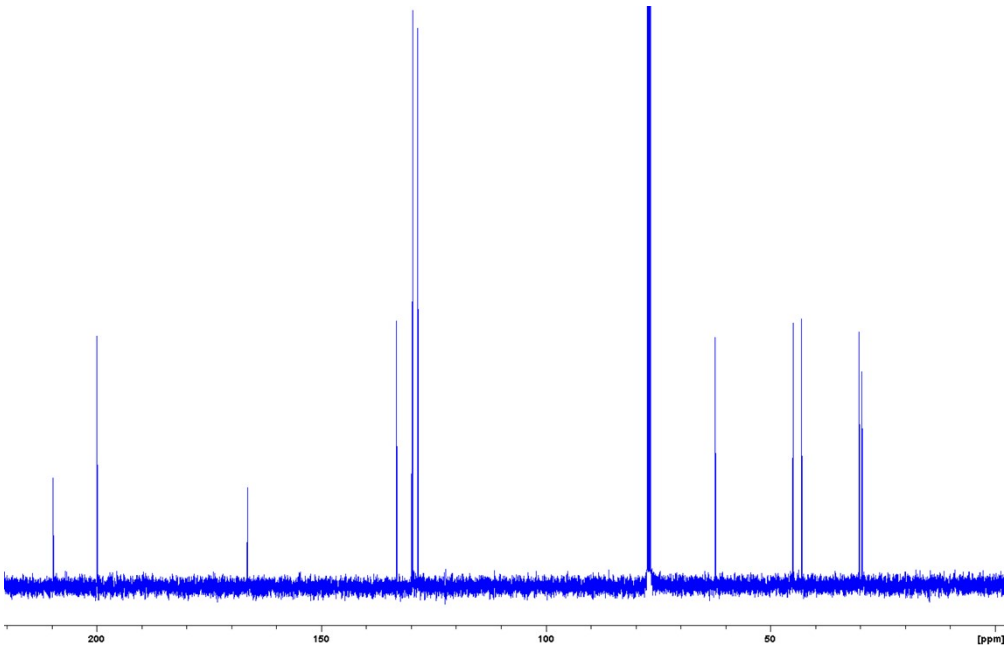
^{1}H NMR of **12** in $CDCl_3$:



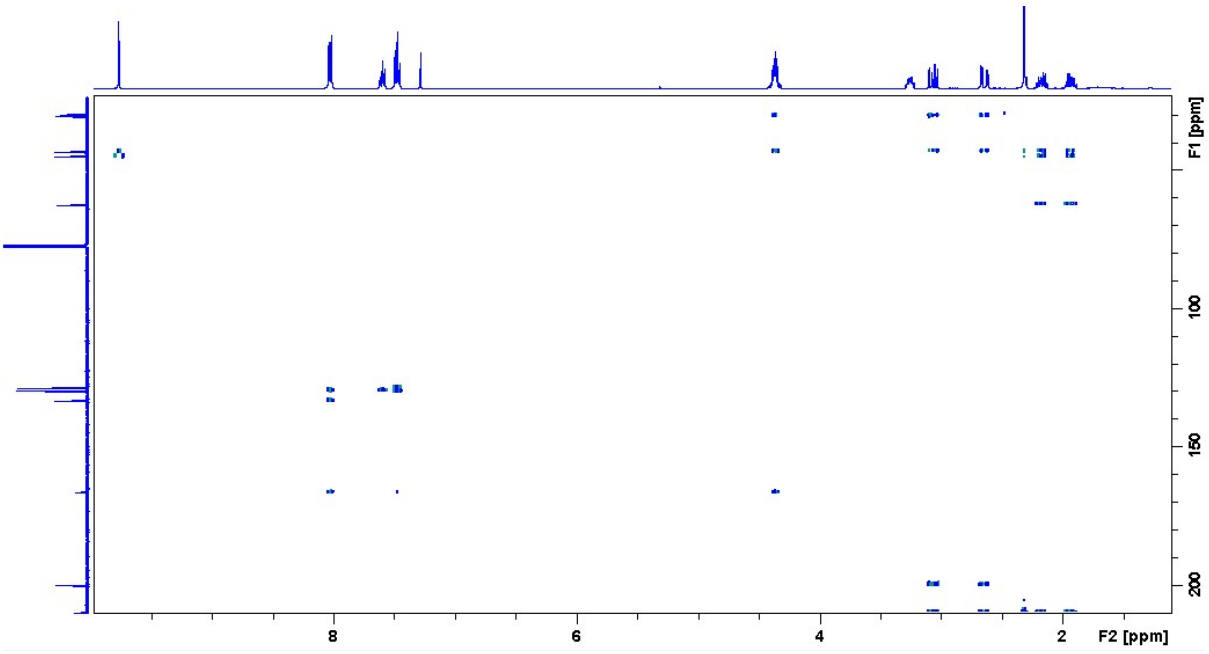
^{1}H COSY NMR of **12** in $CDCl_3$:



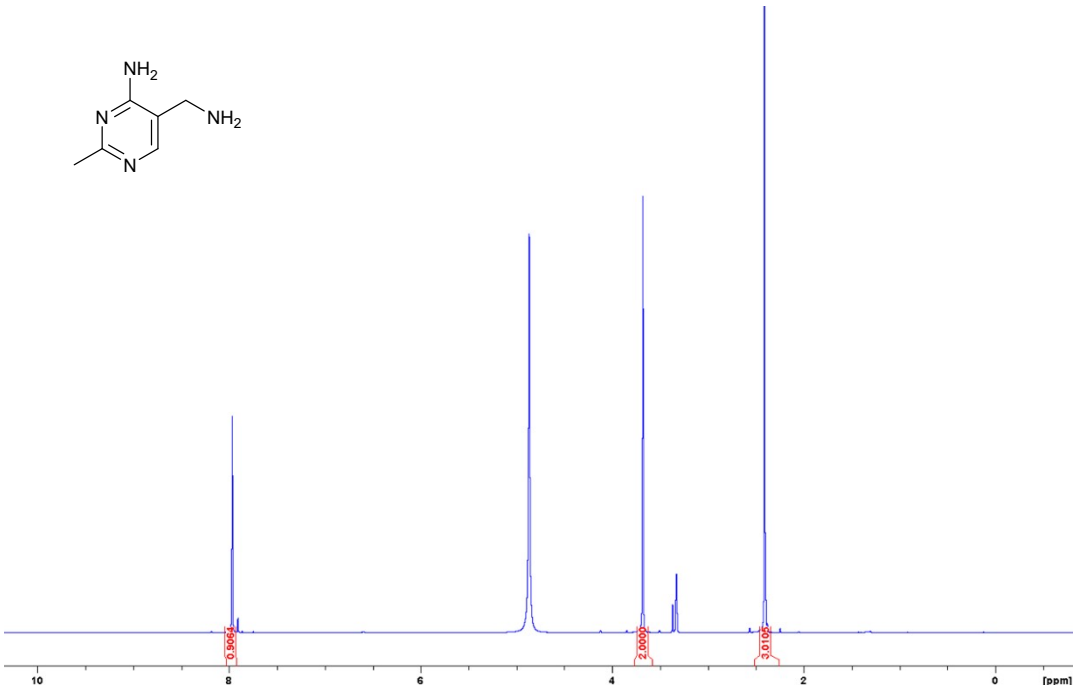
^{13}C NMR of **12** in $CDCl_3$:



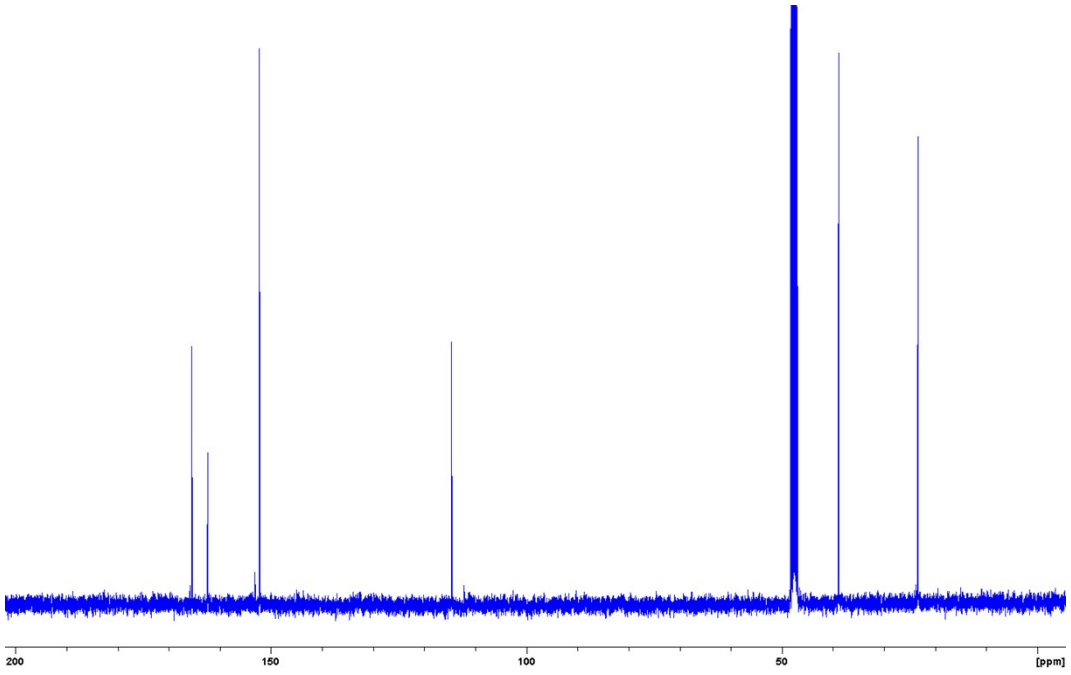
^{1}H-^{13}C HMBC NMR of **12** in $CDCl_3$:



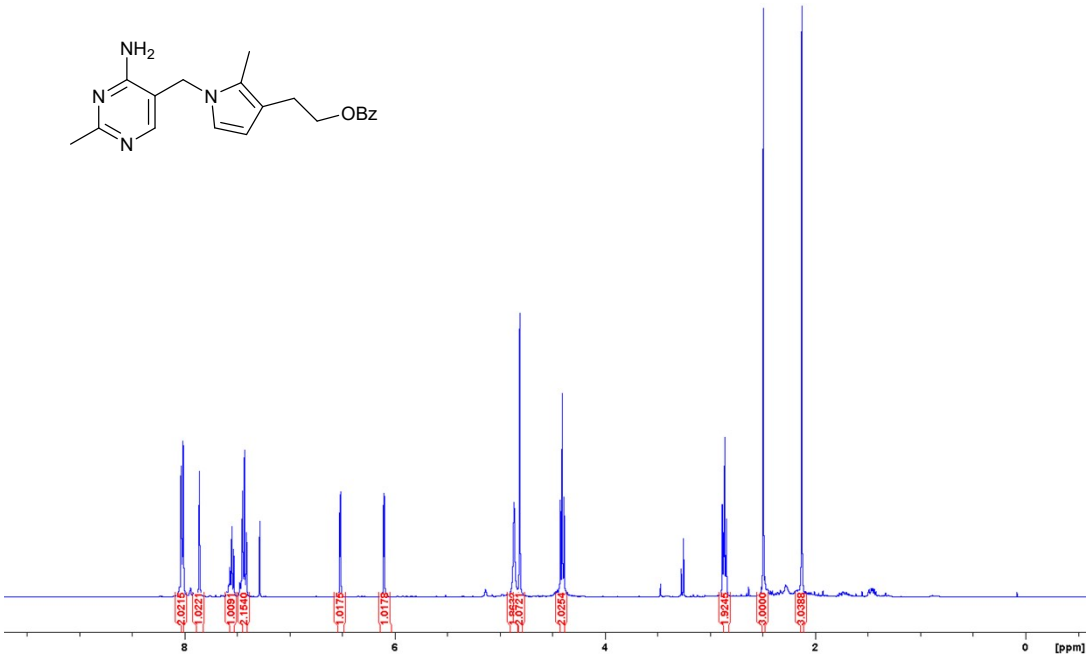
^{1}H NMR of **14** in CD_3OD:



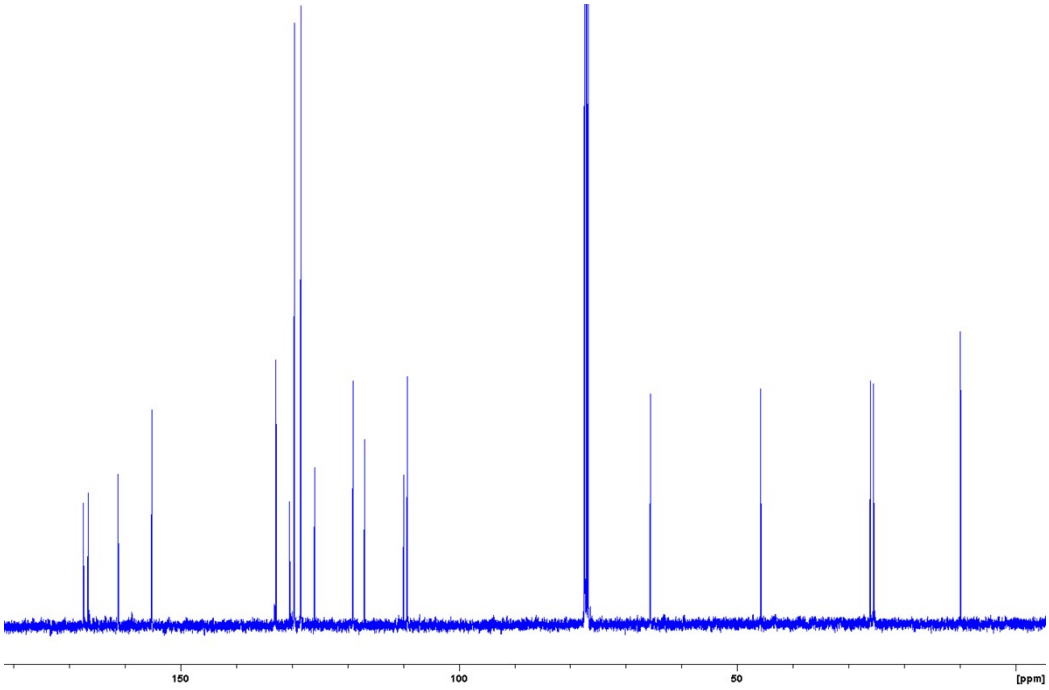
^{13}C NMR of **14** in CD_3OD:



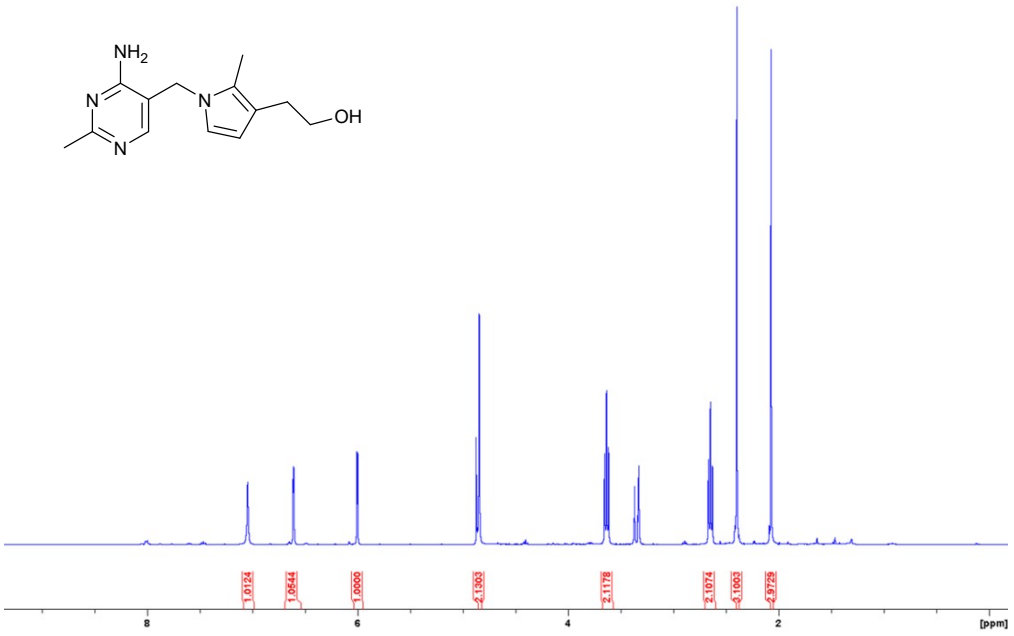
^{1}H NMR of **15** in $CDCl_3$:



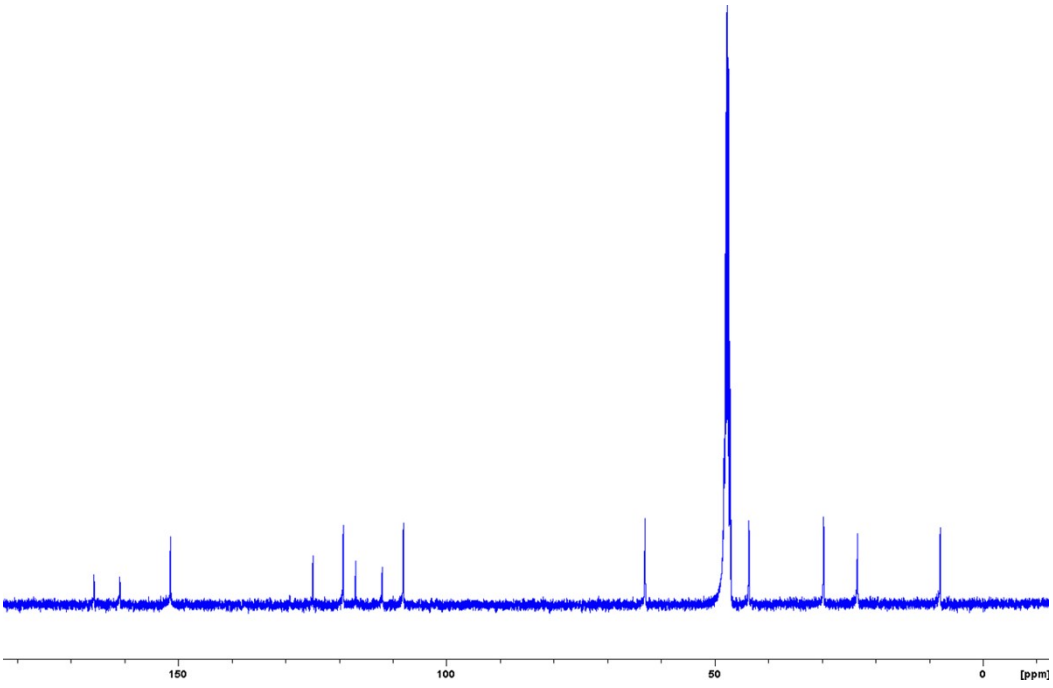
^{13}C NMR of **15** in $CDCl_3$:



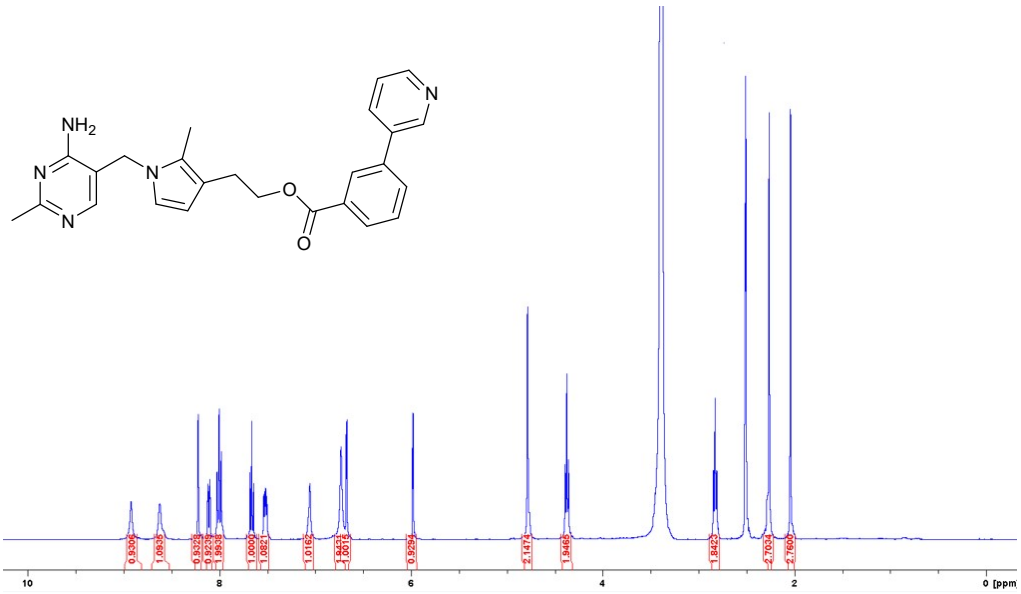
^{1}H NMR of **16** in CD_3OD:



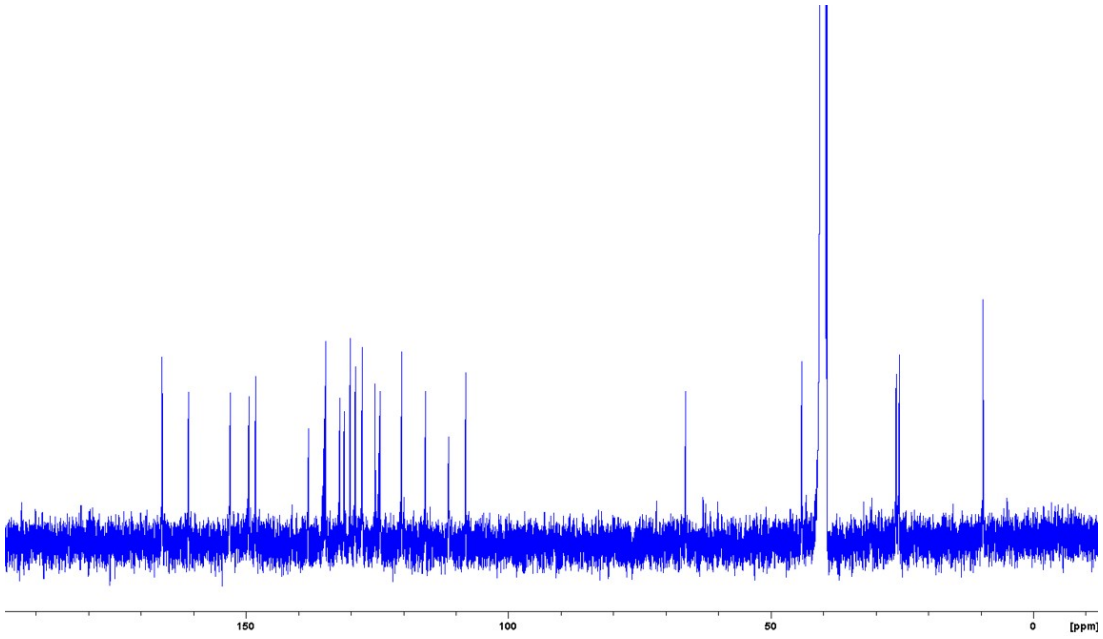
^{13}C NMR of **16** in CD_3OD:



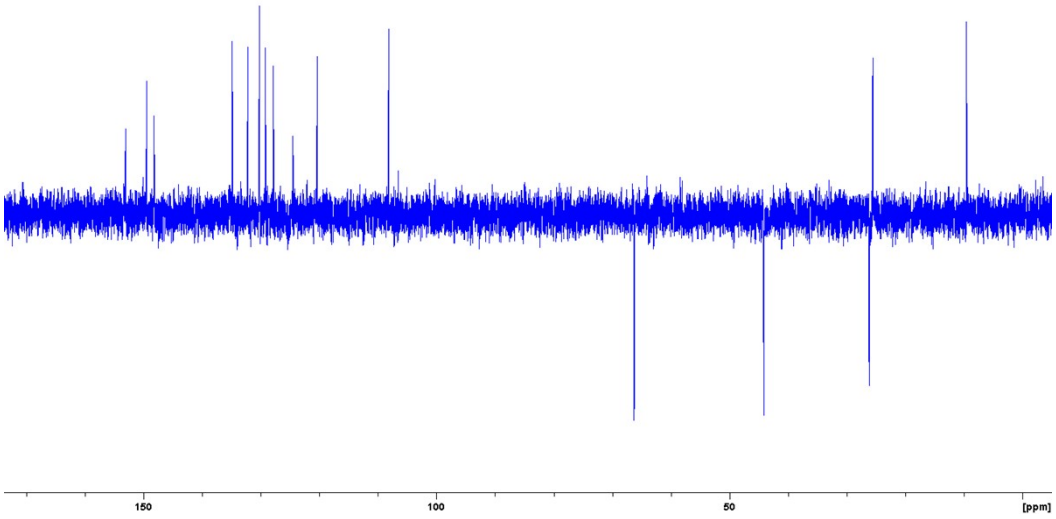
^{1}H NMR of **19** in CD_3SOCD_3:



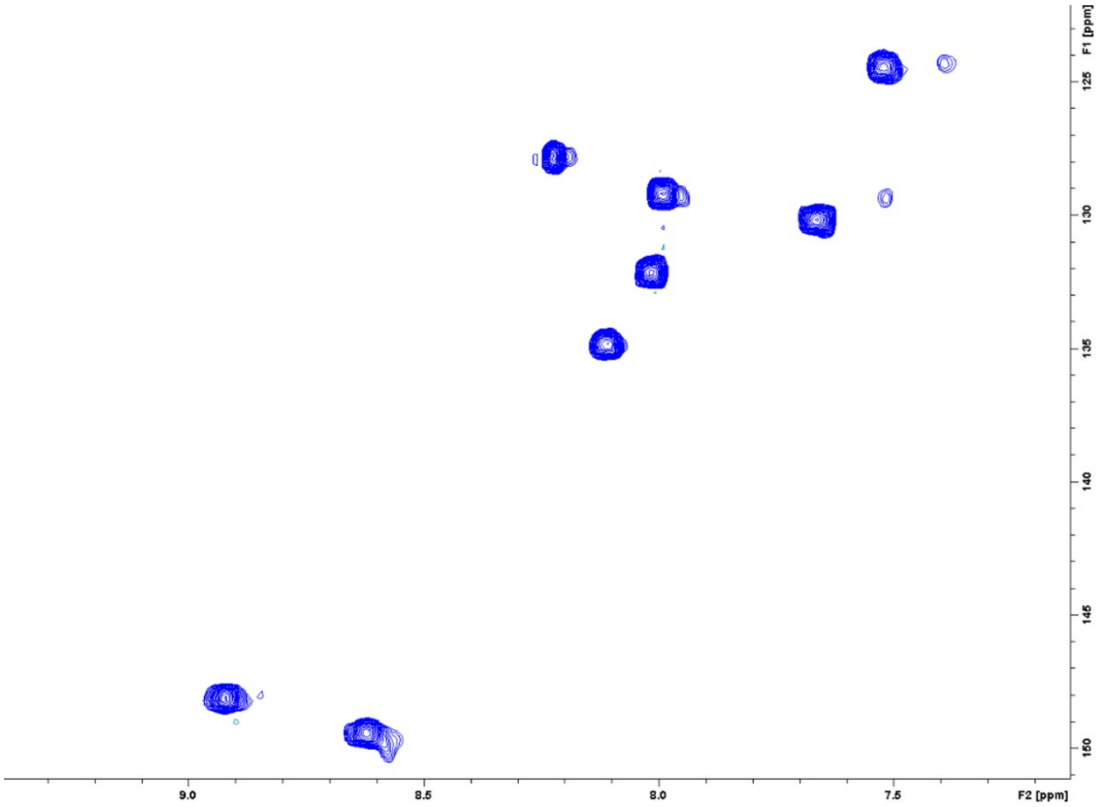
^{13}C NMR of **19** in CD_3SOCD_3:



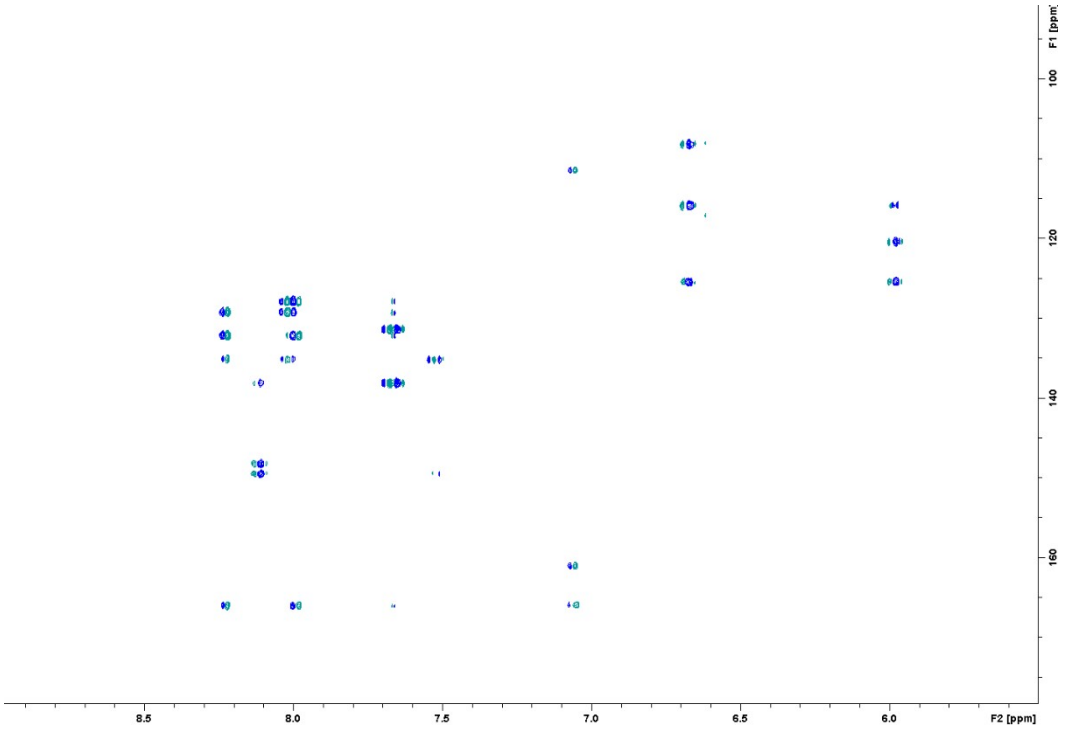
^{13}C DEPT-135 NMR of **19** in CD_3SOCD_3:



^{1}H-^{13}C HSQC NMR of **19** in CD_3SOCD_3 (aromatic region):



^{1}H-^{13}C HMBC NMR of **19** in CD_3SOCD_3 (aromatic region):



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