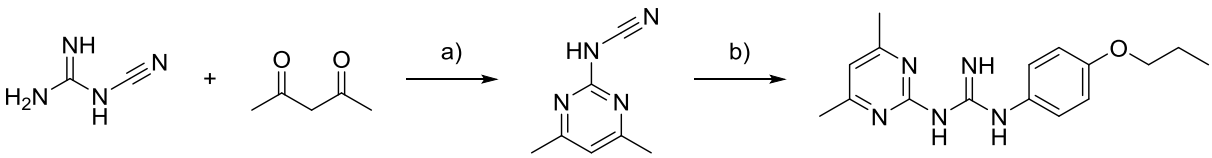
Synthesis of S9 and S9ox

General

Chemicals and solvents were either purchased from commercial suppliers. For thin-layer chromatography (TLC), silica gel plates Merck 60 F254 were used and compounds were visualized by irradiation with UV light and/or by treatment with a solution ninhydrine followed by heating. Column chromatography was performed using silica gel Merck 60 (particle size 0.063-0.200 mm). ^{1}H-NMR, ^{13}C-NMR spectra were recorded on Bruker AVANCE III 400. Chemical shifts for protons are given in δ relative to tetramethylsilane (TMS) and are referenced to residual protium in the NMR solvent (DMSO-d_6: δ = 2.50 ppm, Methanol-d_4: δ = 4.87 ppm). Chemical shifts for carbon are referenced to the carbon in NMR solvent (DMSO-d_6: δ = 39.52 ppm,, Methanol-d_4: δ = 49.00 ppm. The coupling constants J are given in Hz. IR DRIFT spectras were recorded with Nicolet AVATAR 370 FT-IR in cm^{-1}. High-resolution mass spectras were recorded with LCQ Fleet spectrometer.

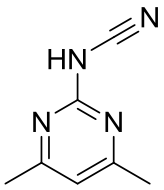
Reaction scheme



Scheme 1: Synthetic route to S9. Reagents and conditions: a) NaOH, H_2O, reflux, 22% yield, b) 4-propoxyaniline, EtOH, reflux, 47% yield.

Preparation of substrates

N-(4,6-Dimethylpyrimidin-2-yl)cyanamide

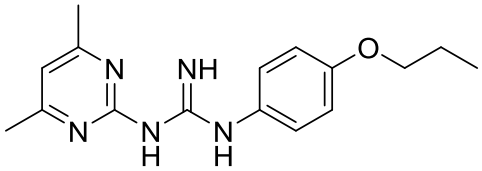


N-(4,6-Dimethylpyrimidin-2-yl)cyanamide was prepared according to previously reported procedure.[1]

Cyanoguanidine (5.0 g, 60 mmol), acetylacetone (9.0 g, 90 mmol) were added to a solution of NaOH (0.3M, 40 ml) and the reaction mixture was stirred under reflux 48 h. Then the mixture was cooled to 4 °C, solids were filtered and washed with minimal amount of water. Filtrate cake was recrystallized from boiling ethanol (approx. 120 ml).

22% **yield**, white solid, **m.p.** = 228.7 °C, **^{1}H-NMR:** (400 MHz, DMSO-d_6) δ = 12.58 (s, 1H), 6.63 (s, 1H), 2.31 (s, 6H). ppm, **^{13}C-NMR:** (101 MHz, DMSO-d_6) δ = 166.8, 160.2 (2C), 115.8, 109.7, 21.9 (2C) ppm, **IR** (KBr) ν = 3503, 3282, 3249, 3064, 3010, 2980, 2857, 2842, 2815, 2621, 2319, 2244, 2202, 2175, 2089, 1838, 1727, 1649, 1610, 1422, 1362, 1323, 1231, 1195, 1165, 1036, 1018, 985 cm^{-1}, **HRMS** (ESI+) *m*/*z*: calcd. for $C_7H_9N_4$ $[M+H]^+$: 149.0827, found: 149.0792.

1-(4,6-Dimethylpyrimidin-2-yl)-3-(4-propoxyphenyl)guanidine



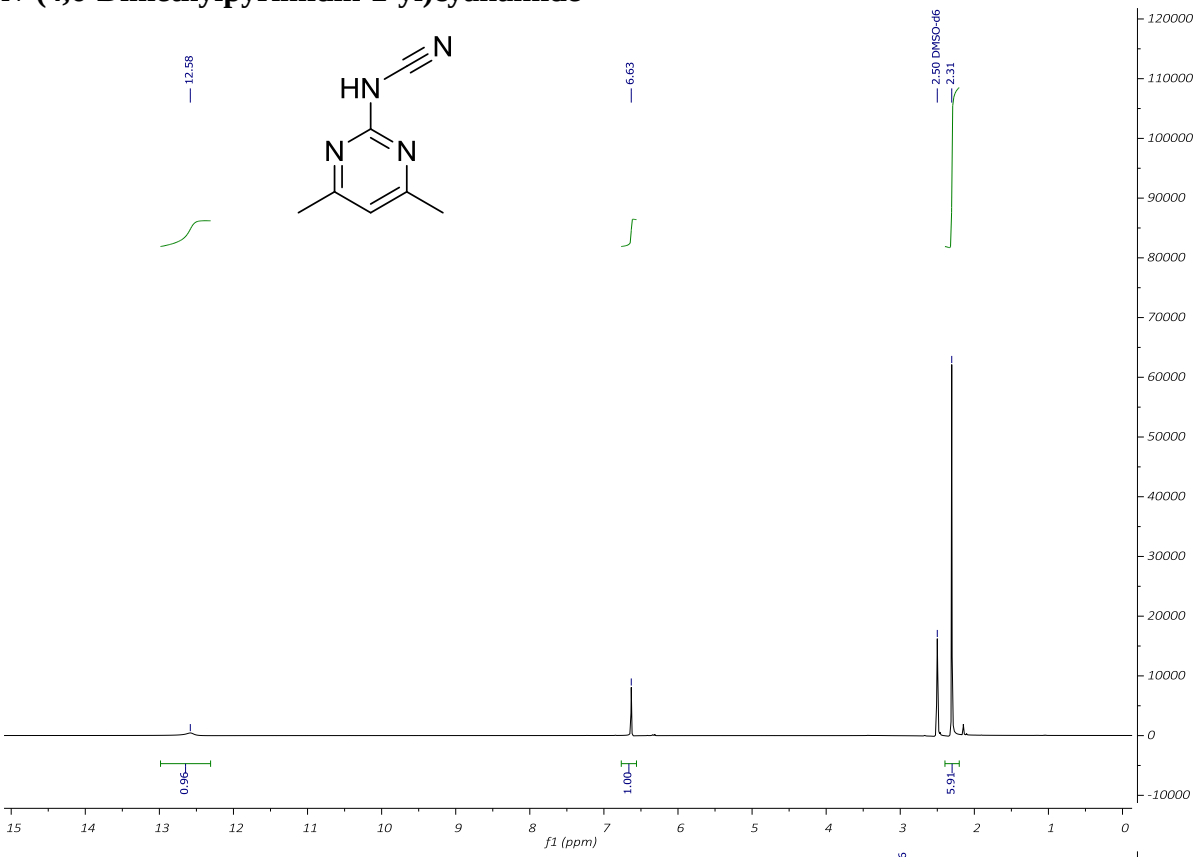
4-Propoxyaniline (307 mg, 2.03 mmol, 1.5 eq.) was added dropwise to a suspension of *N*-(4,6-dimethylpyrimidin-2-yl)cyanamide (200 mg, 1.35 mmol; 1.0 eq.) in anhydrous EtOH (4 ml). Solids were dissolved during addition of aniline. Reaction mixture was heated to reflux. At this temperature reaction mixture was stirred for 72 h and monitored by TLC (eluent: MeOH). Then reaction mixture was cooled to -35 °C and solution of NaOH (10 ml, 10% w/w) was added dropwise. Solids were filtered and washed with Et_2O (4 × 20 ml). Filtrate cake was dissolved in MeOH and purified by column chromatography on silica with MeOH as an eluent.

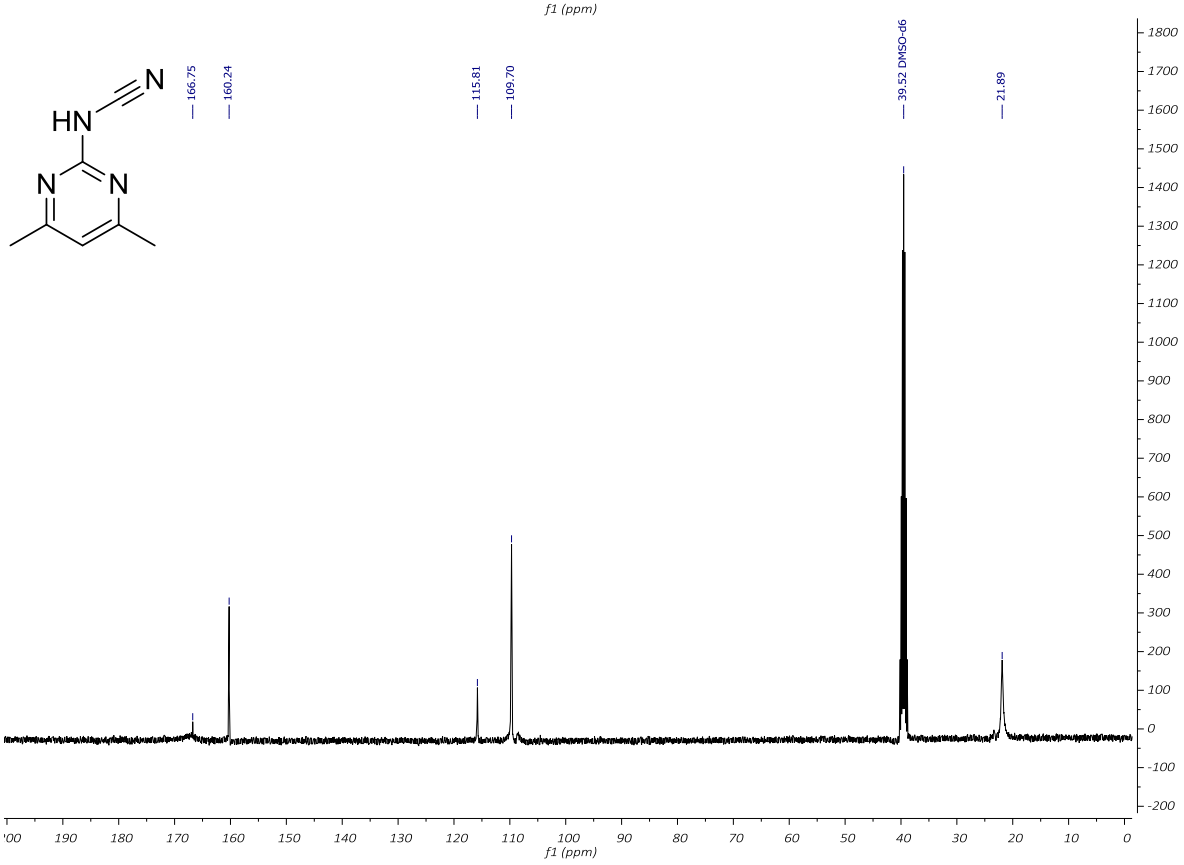
47% **yield**, white solid, **m.p.** = 191.5 °C, $\boldsymbol{R_f}$ = 0.36 (MeOH, ninhydrine), **^{1}H-NMR:** (400 MHz, Methanol-d_4) δ = 7.21 – 7.08 (m, 2H), 6.92 – 6.83 (m, 2H), 6.61 (s, 1H), 3.89 (t, J = 6.5 Hz, 2H), 3.27 (p, J = 1.6 Hz, 1H), 2.31 (s, 6H), 1.83 – 1.65 (m, 2H), 1.01 (t, J = 7.4 Hz, 3H) ppm, **^{13}C-NMR:** (101 MHz, Methanol-d_4) δ = 168.6 (2C), 165.2, 158.1, 157.3, 133.7, 126.6 (2C), 116.4 (2C), 112.9, 70.9, 23.74 (2C), 23.69, 10.9 ppm, **IR** (KBr) ν = 3312, 3106, 3088, 2959, 2893, 2869, 1631, 1577, 1527, 1509, 1419, 1383, 1344, 1237, 1171, 1117, 1075, 1048, 1024 cm^{-1}, **HRMS** (ESI+) *m*/*z*: calcd. for $C_{16}H_{22}N_5O$ $[M+?]^+$: 300.1824, found: 300.1773.

[1] WO2013/53726, **2013**, *A1*.

NMR spectra

N-(4,6-Dimethylpyrimidin-2-yl)cyanamide





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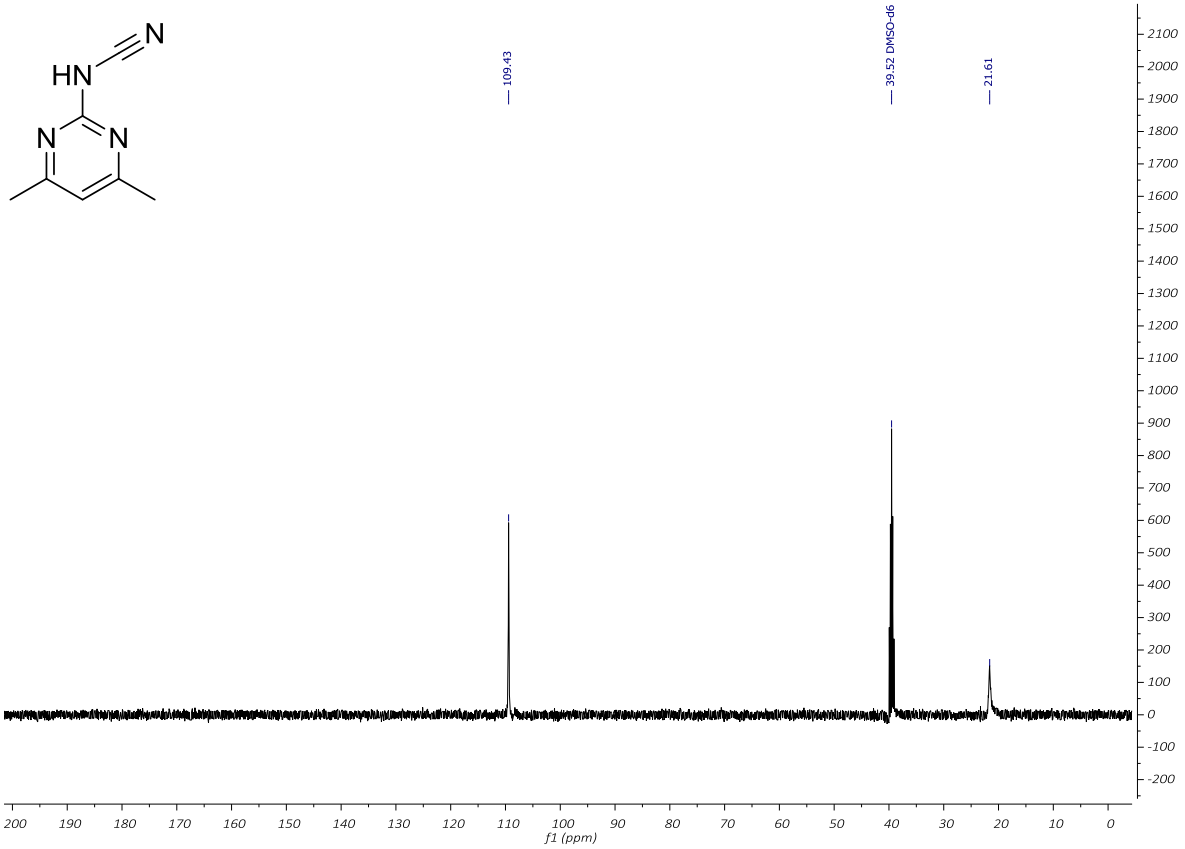
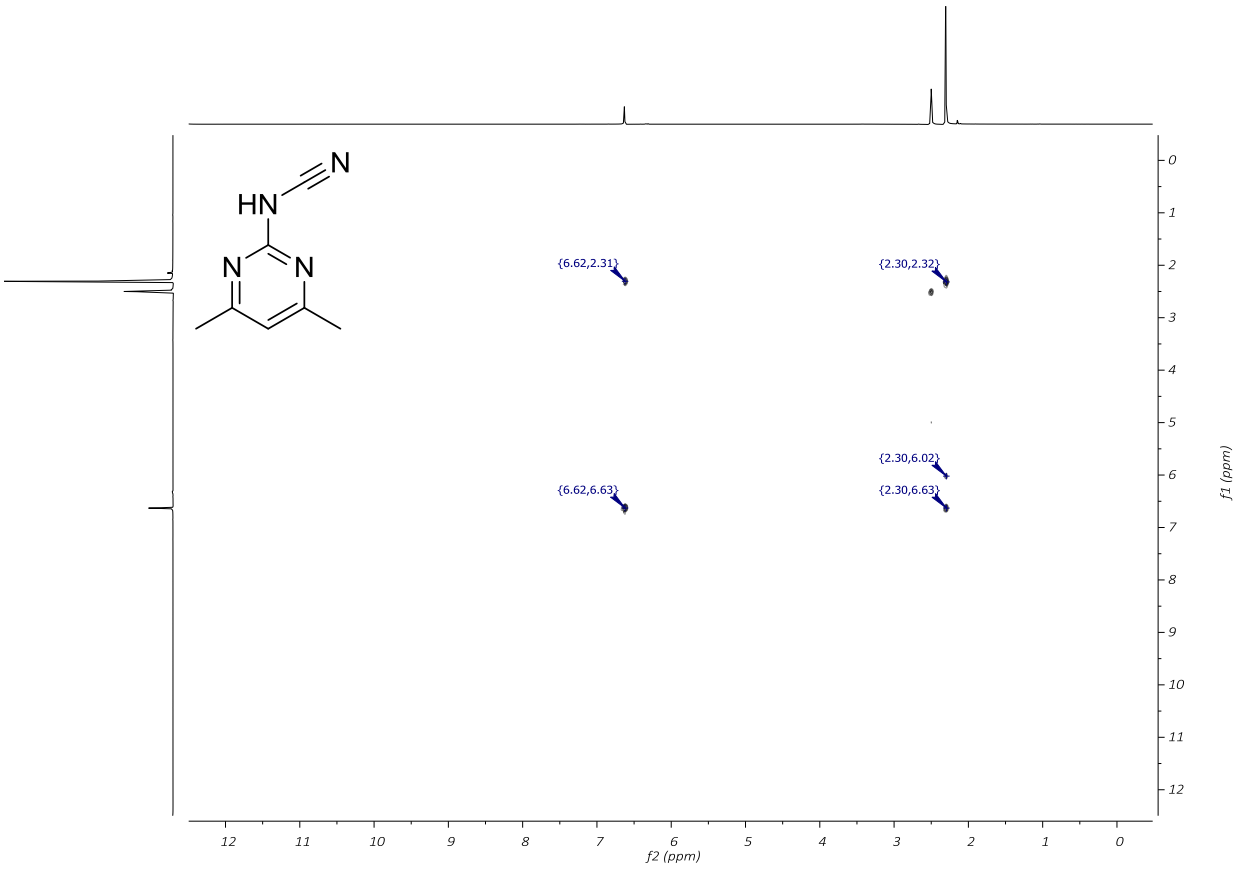


COSY



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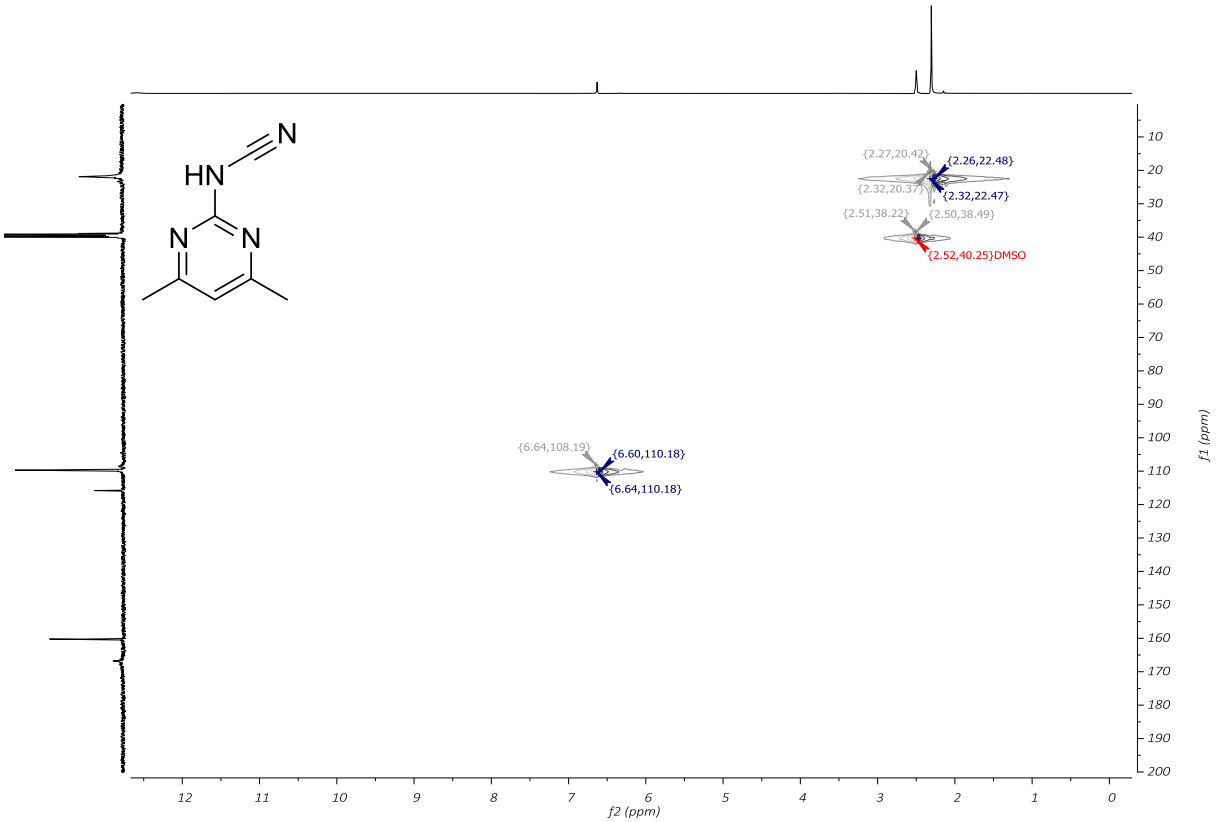
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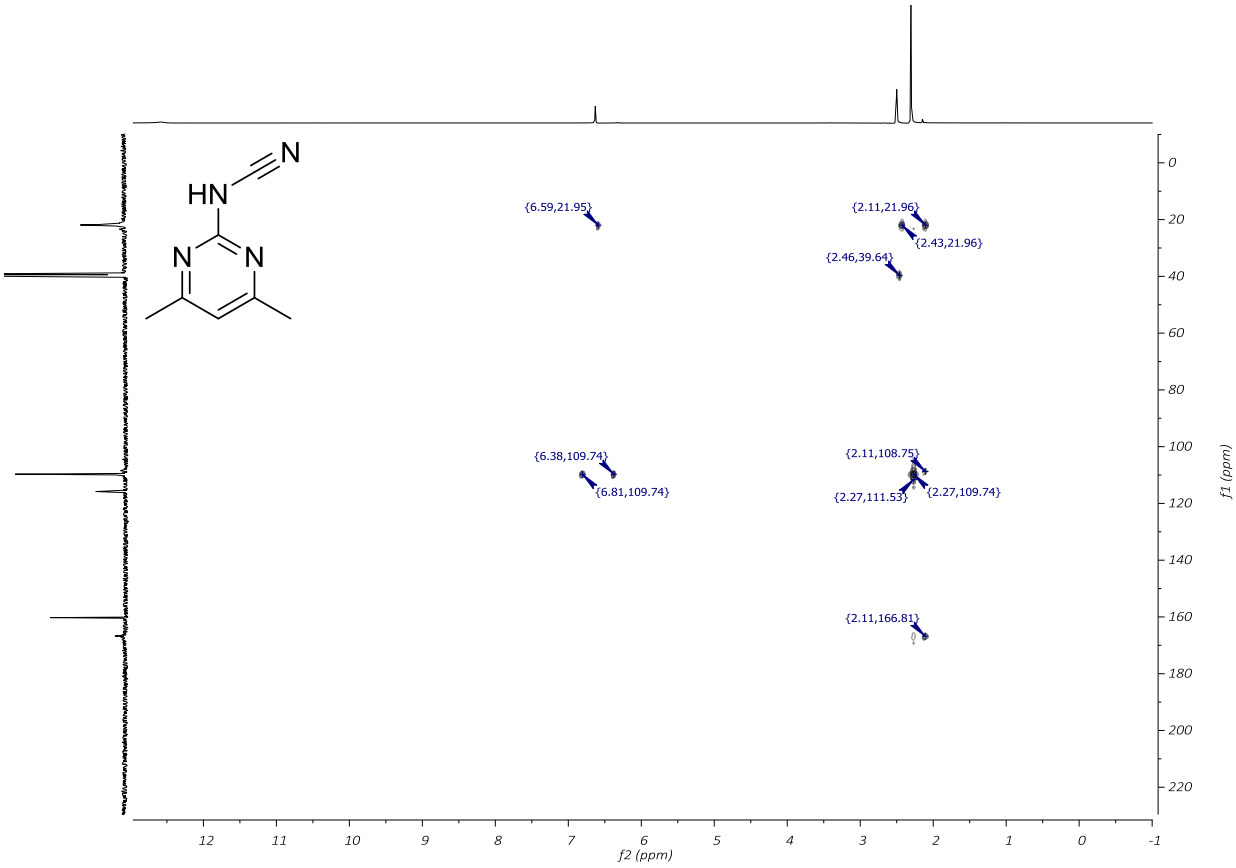
f2 (ppm)
f1 (ppm)

HMBC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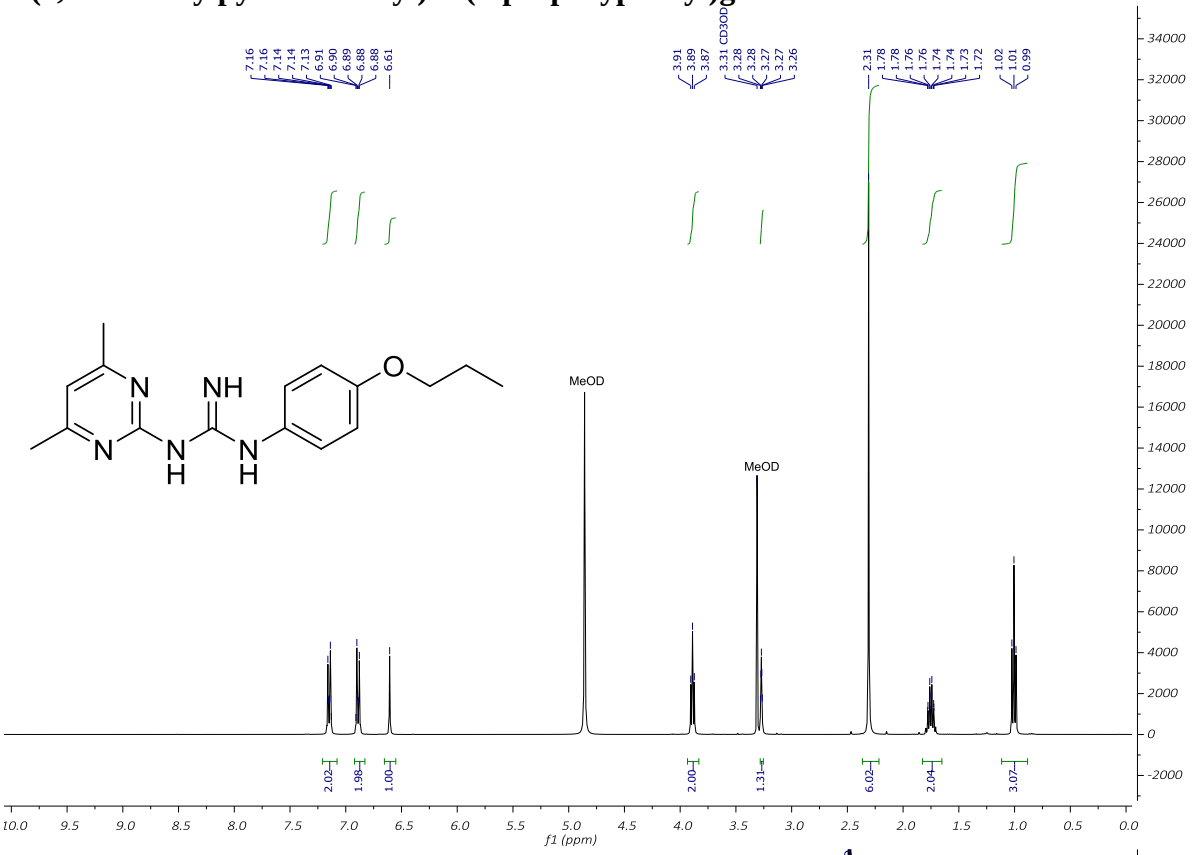
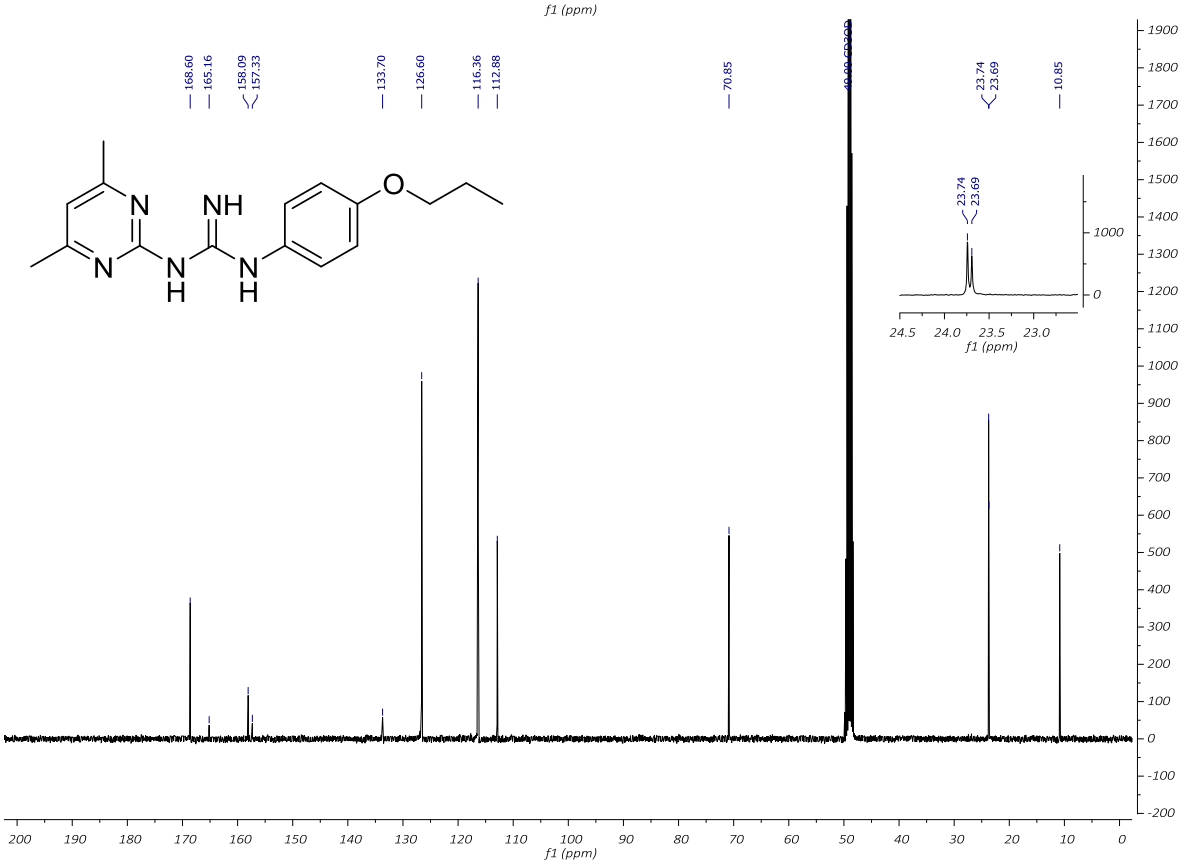
f2 (ppm)
f1 (ppm)

1-(4,6-Dimethylpyrimidin-2-yl)-3-(4-propoxyphenyl)guanidine





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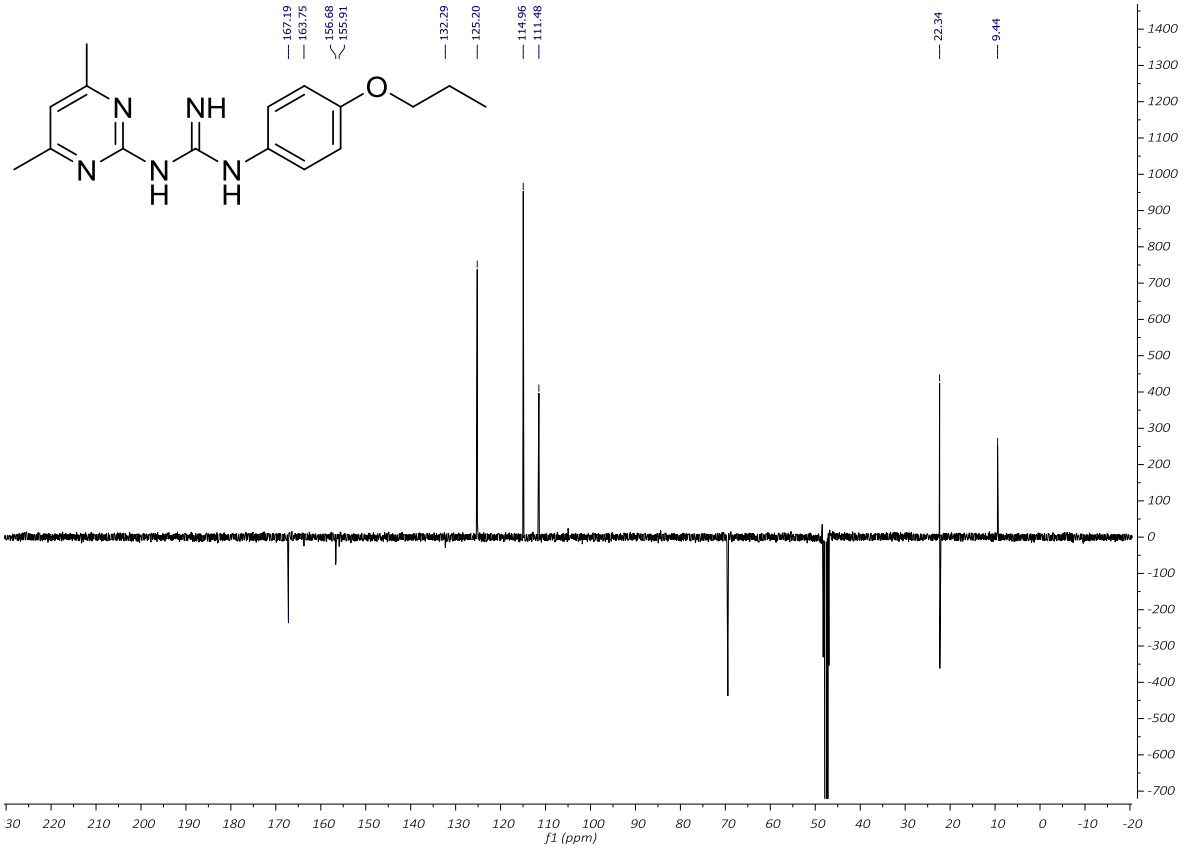

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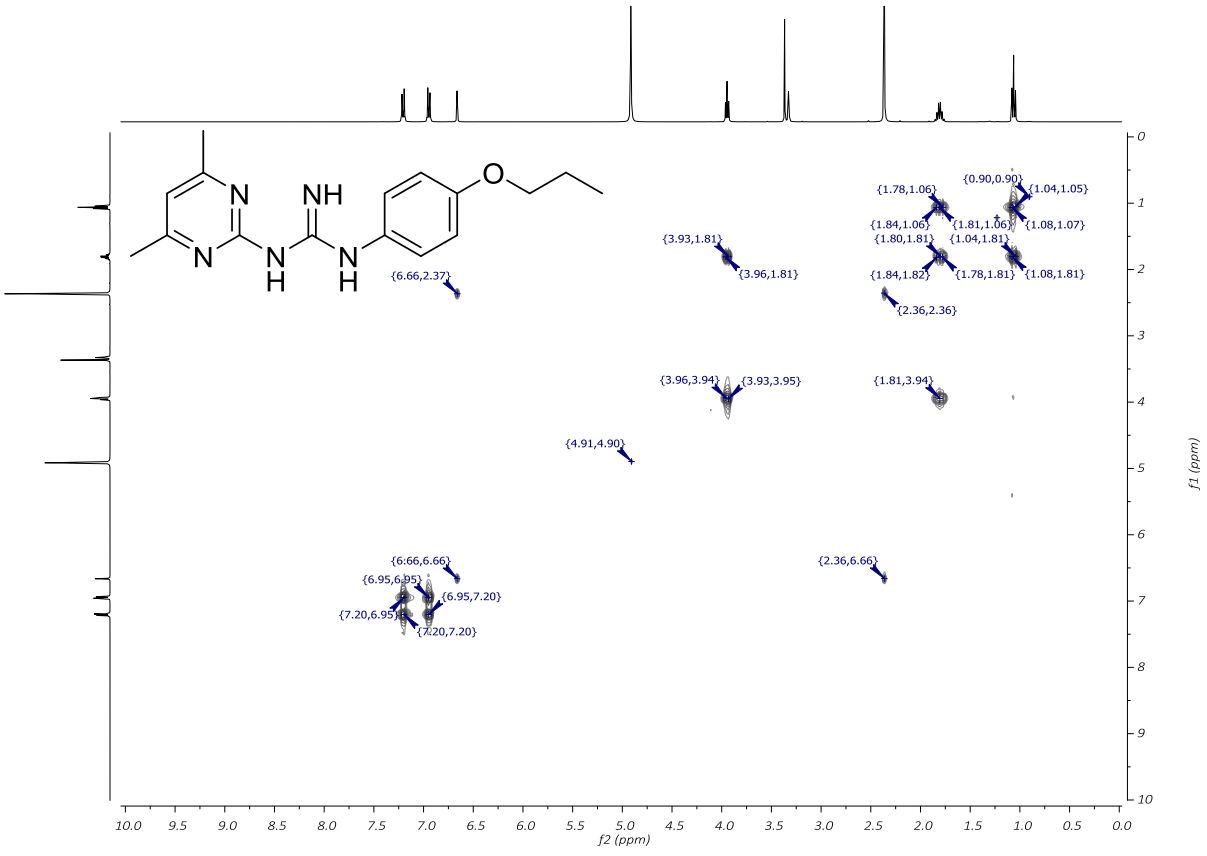

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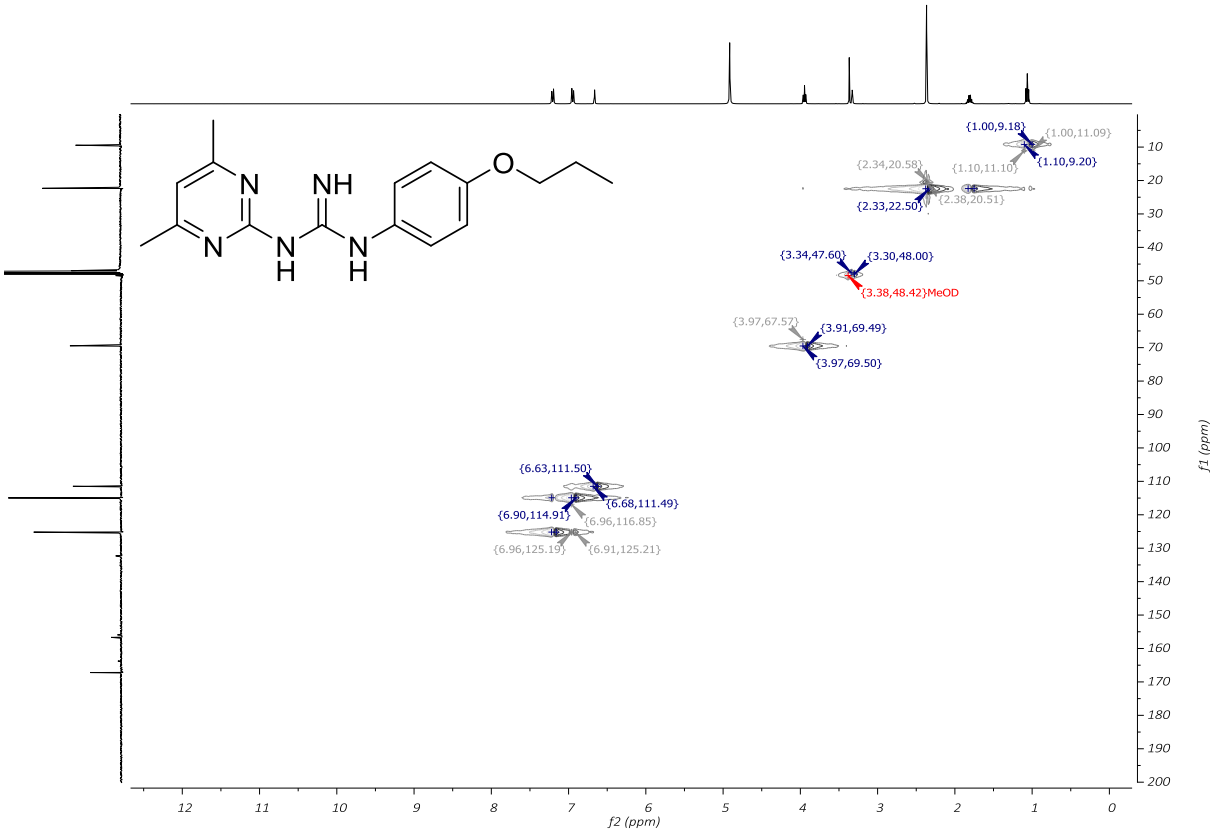
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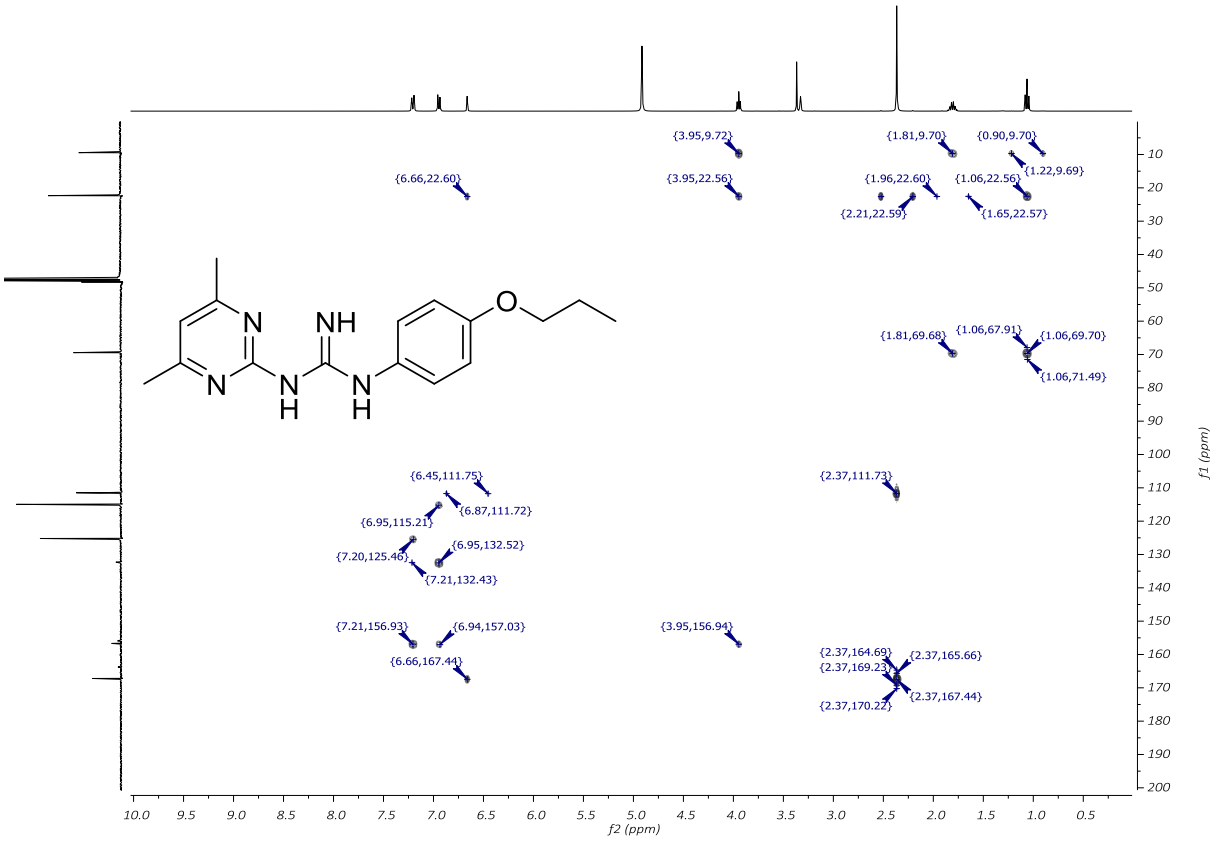
f2 (ppm)
f1 (ppm)

HMBC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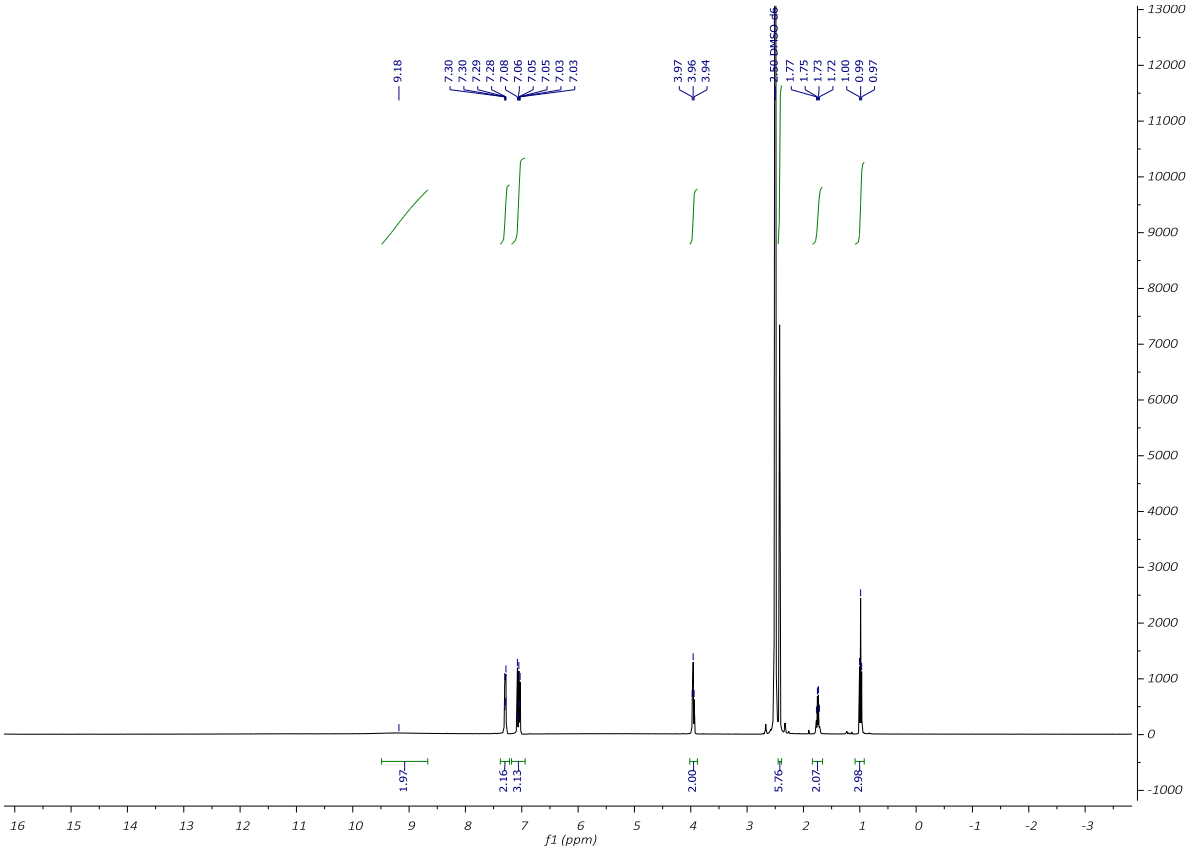
f2 (ppm)
f1 (ppm)

Preparation of oxalate salt derived from S9

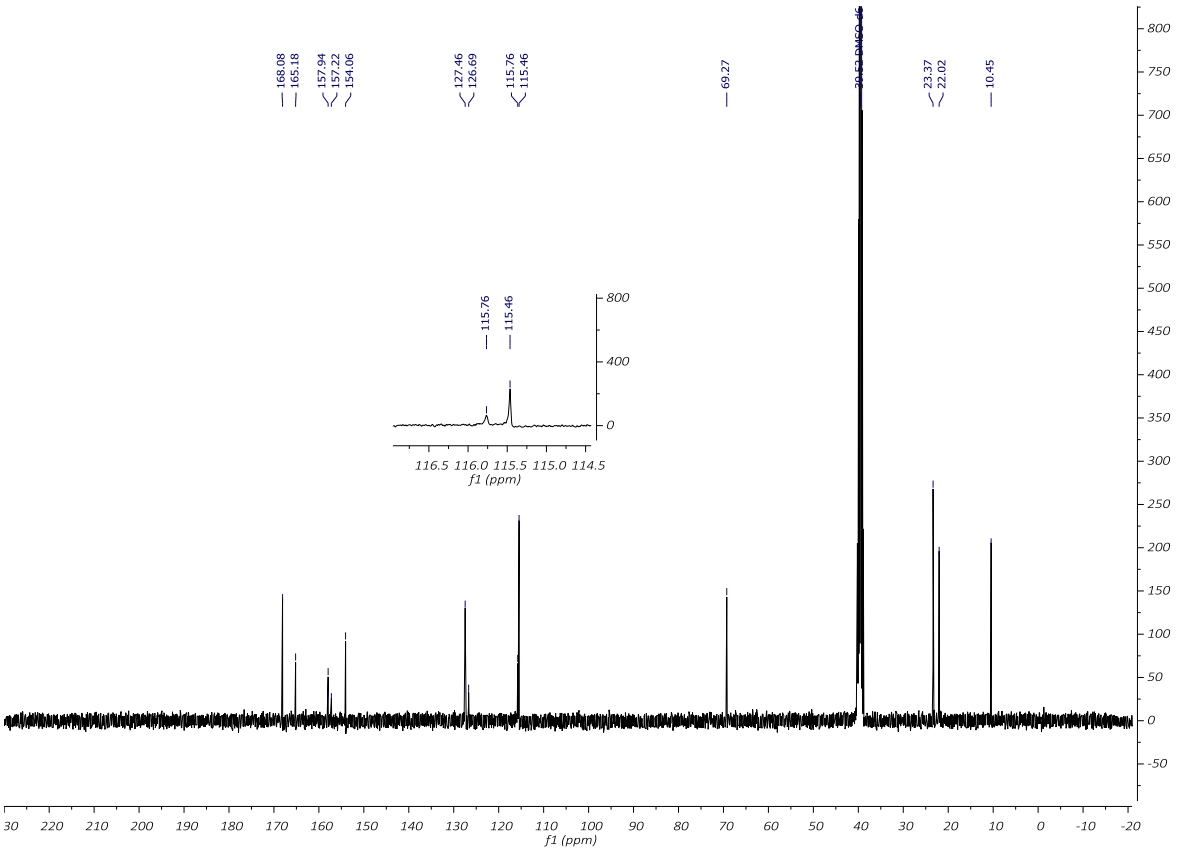
Corresponding oxalate salt derived from 1-(4,6-dimethylpyrimidin-2-yl)-3-(4-propoxyphenyl)guanidine was prepared according to previously reported procedure.[2]
Oxalate acid dihydrate (27.1 mg; 0.167 mmol; 1.0 eq.) was dissolved in distilled water (1.0 ml). To this solution was added 1-(4,6-dimethylpyrimidin-2-yl)-3-(4-propoxyphenyl) (20.0 mg, 0.167 mmol, 1.0 eq.). Reaction mixture was stirred for 24 h at room temperature. Water was evaporated. Resulting solid was used directly to further studies.
quantitative **yield**, white solid, **^{1}H-NMR:** (400 MHz, DMSO-d_6) δ = 9.18 (s, 2H), 7.38 – 7.22 (m, 2H), 7.18 – 6.94 (m, 3H), 3.96 (t, J = 6.5 Hz, 2H), 2.42 (s, 6H), 1.74 (q, J = 7.0 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H) ppm, **^{13}C-NMR:** (101 MHz, DMSO-d_6) δ = 168.1 (2C), 165.2, 157.9, 157.2, 154.1 (2C), 127.5 (2C), 126.7, 115.7 (2C), 115.5, 69.3, 23.4 (2C), 22.0, 10.5 ppm.
168.6 (2C), 165.2, 158.1, 157.3, 133.7, 126.6 (2C), 116.4 (2C), 112.9, 70.9, 23.74 (2C), 23.69, 10.9 ppm, **IR** (KBr) v = 3375, 3294, 3111, 2959, 2881, 1736, 1652, 1613, 1545, 1512, 1428, 1356, 1341, 1299, 1240, 1207, 1180, 1099, 1078, 1054, 1015 cm^{-1}.

[2] Israel, M.; Zoll, E. C.; Muhammad, N.; Modest, E. *J. Med. Chem.* **1973**, *16*, 1-5.

^{1}H-NMR



^{13}C-NMR



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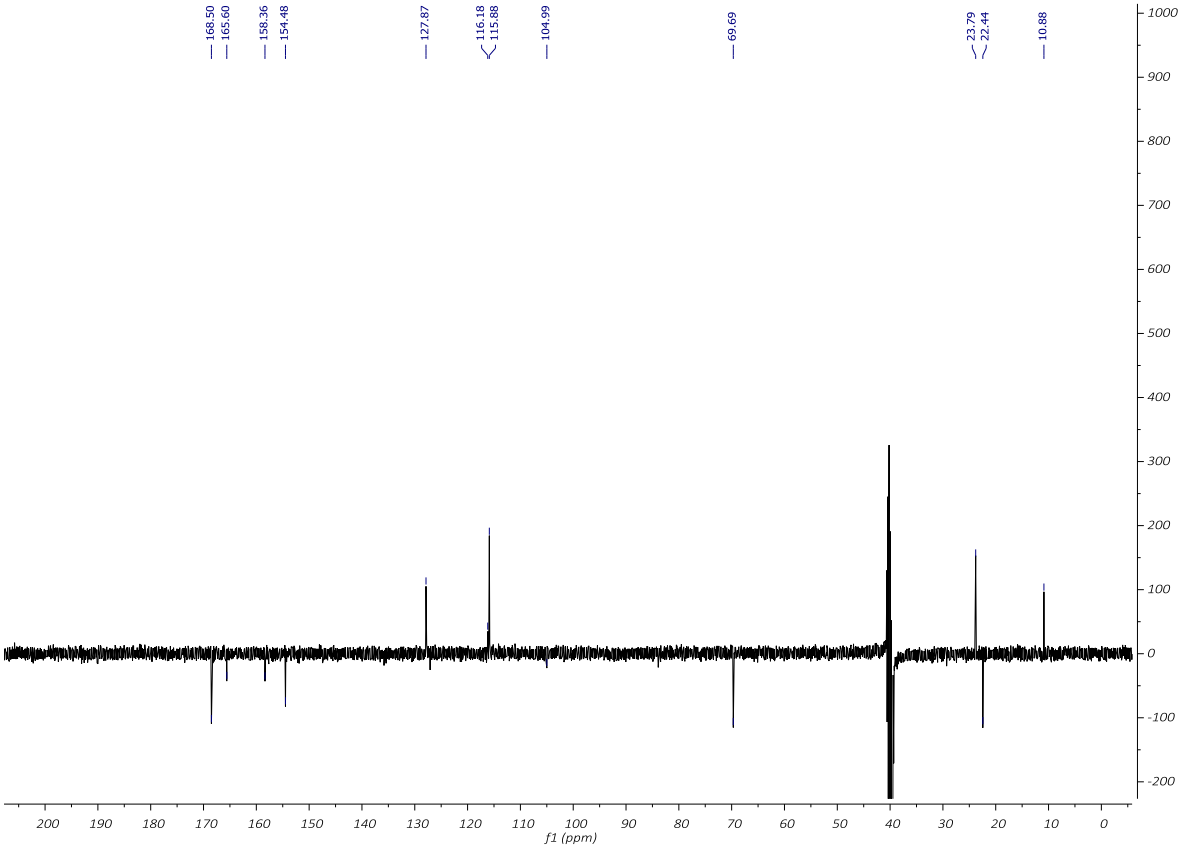

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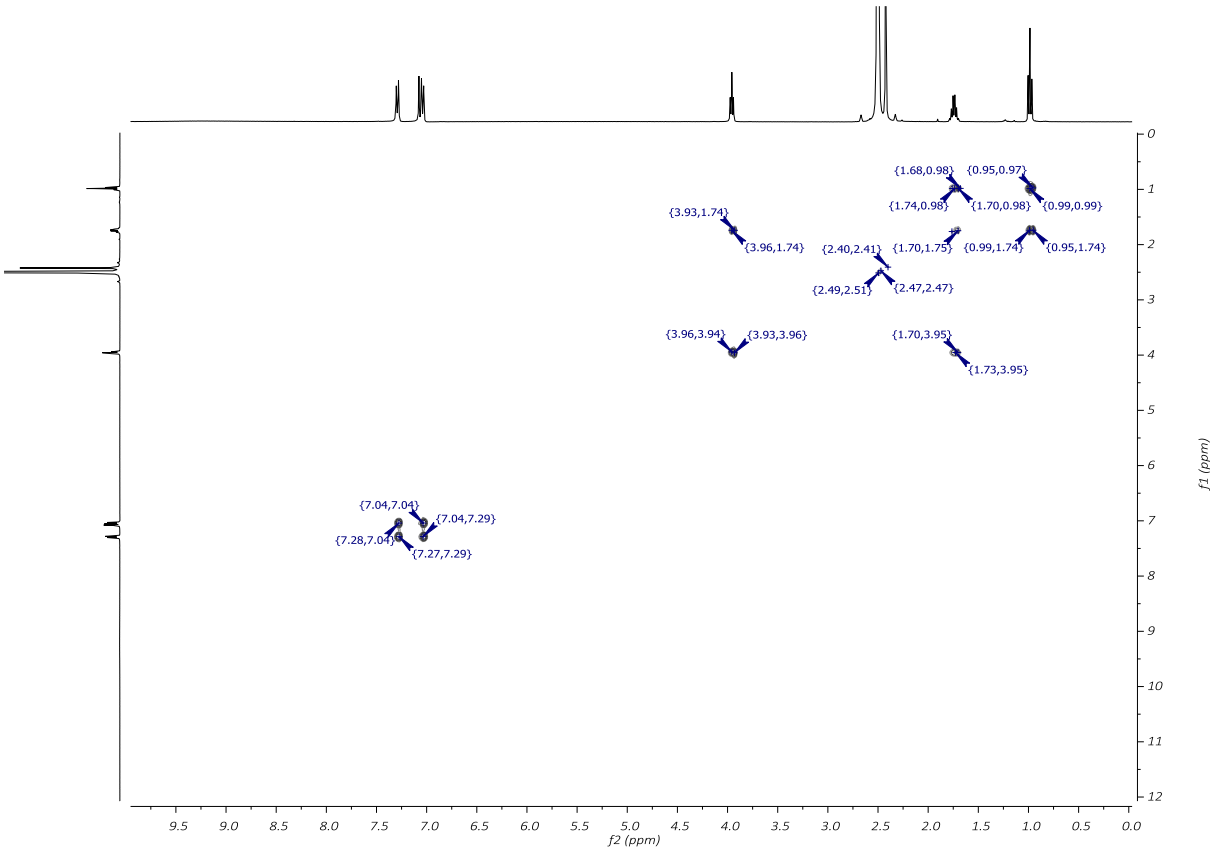

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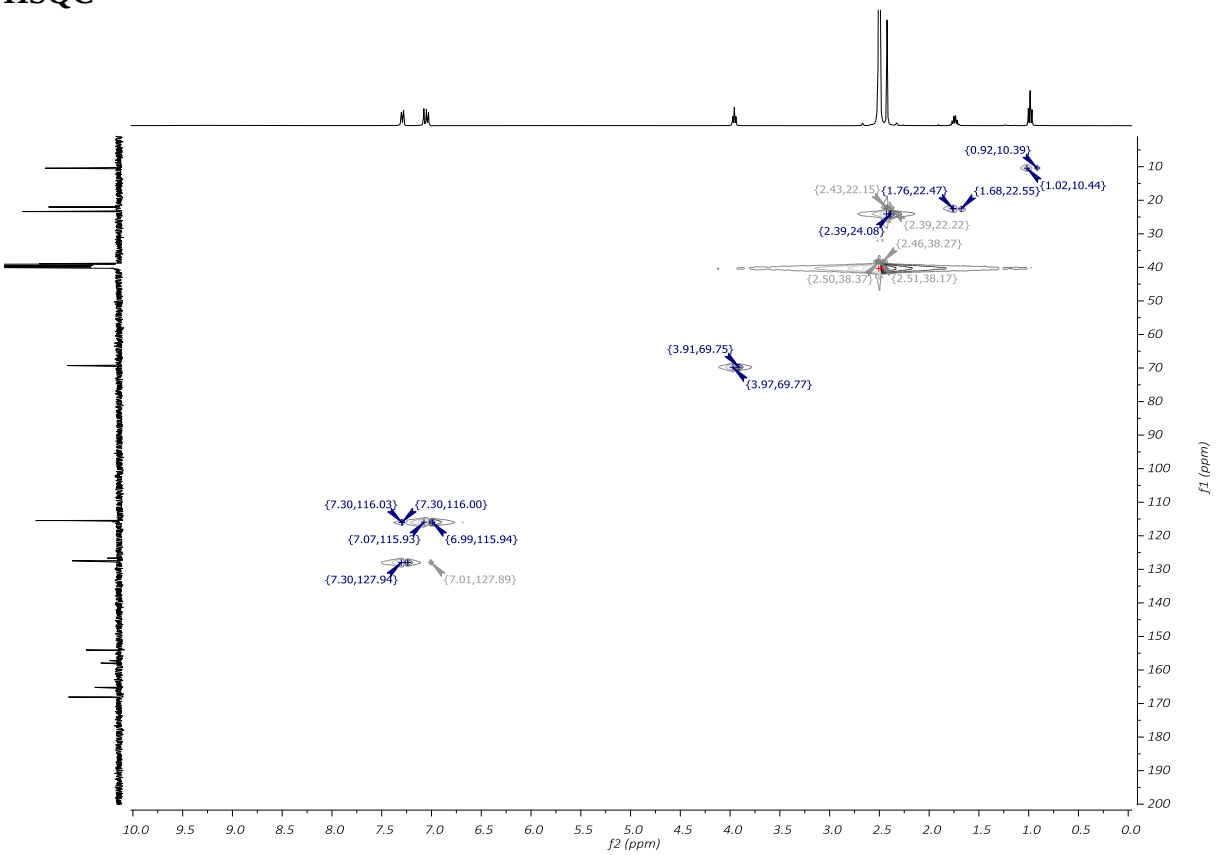
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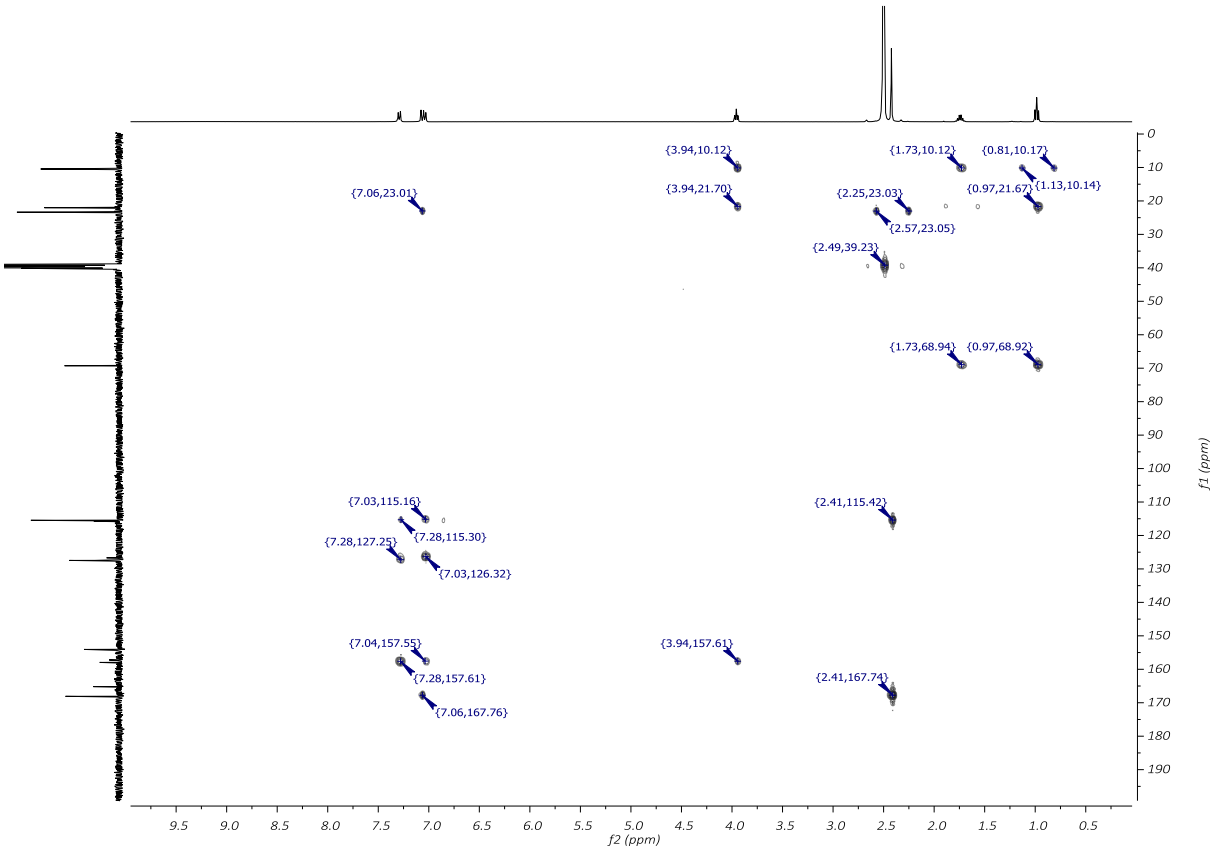
f2 (ppm)
f1 (ppm)

HMBC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f2 (ppm)
f1 (ppm)