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Supplementary Information

A small molecule that mimics the metabolic activity of copper-containing amine oxidases (CuAOs) toward physiological mono- and polyamines

Martine Largeron,*[a] Maurice-Bernard Fleury[a] and Margherita Strolin Benedetti[b]

[a] *UMR 8638 CNRS-Université Paris Descartes, Synthèse et Structure de Molécules d'Intérêt Pharmacologique, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire, 75270 Paris cedex 06, France; E-mail: martine.largeron@parisdescartes.fr*

[b] *UCB Pharma SA, 420 rue d'Estienne d'Orves, 92705 Colombes, France*

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I. Full experimental procedures

Instruments and reagents

Melting points were measured with a Köfler apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker 300 spectrometer operating at 300 and 75 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to internal tetramethylsilane (TMS) and *J* values are reported.

Chemicals were commercial products of the highest available purity and were used as supplied. Reduced catalyst $\mathbf{1_{red}}$ was synthesized in two steps from commercially available 2-nitroresorcinol, according to our published procedure.[1] Aminoacetone was prepared as its masked acetal as previously reported.[2]

Controlled-potential electrolysis was carried out in a cylindrical, three-electrode divided cell (9 cm diameter), using an electronic potentiostat. In the main compartment, a platinum grid (60 cm^2 area) served as the anode (working electrode). In the specific case of histamine, putrescine and spermidine (Table 1, entries 7-9), the platinum grid was replaced by a mercury pool because polyamines spontaneously attached to the electrode surface.[3] A platinum sheet was placed in the concentric cathodic compartment (counter electrode), which was separated from the main compartment with a glass frit. The reference electrode was an aqueous saturated calomel electrode (SCE), which was isolated from the bulk solution in a glass tube with a fine-porosity frit. The electrolyte solution (0.1 $mol.L^{-1}$ lithium perchlorate in methanol) was poured into the anodic and the cathodic compartments, as well as into the glass tube that contained the SCE electrode.

$\mathbf{1_{ox}}$-Mediated oxidation of benzylamine. *Method.*

Reduced catalyst $\mathbf{1_{red}}$ (0.1 mmol, 16.7 mg, 2 mol%) and an excess of benzylamine (5 mmol, 535 mg) were added to the electrolyte solution in the main anodic compartment (250 mL), and the resulting solution was oxidized, under nitrogen and magnetic stirring, at room temperature, at + 0.6 V *vs* SCE (initial current 40 mA). After exhaustive electrolysis, that is when a negligible current was recorded (0.5 mA), the solution was worked-up by the addition of 2,4-dinitrophenylhydrazine reagent (2.5 mmol (500 mg) in 5 mL of pure H_2SO_4, 15 mL of EtOH, and 5 mL of water),[4] the stoichiometry reflecting the fact that 5 mmol of benzylamine

gave only 2.5 mmol of N-benzylidenebenzylamine (see scheme 1). After 1h, the resulting solution was concentrated to a volume of 40 mL. The solid obtained was collected by filtration, washed with water and dried in a vacuum desiccator.

Benzaldehyde 2,4-dinitrophenylhydrazone.[4,5] Yield: 715 mg (50%), red crystals (recrystallized from toluene), m.p. 240-242 °C (lit.,[5] 237-238 °C). The NMR data are in accordance with those reported in the literature.[4]

Propionaldehyde 2,4-dinitrophenylhydrazone.[5] Yield: 380 mg (30%), orange solid, m.p. 152-154 °C (lit.,[5] 154-155 °C). ^{1}H NMR (300 MHz, DMSO D6/TMS): δ 1.08 (t, J = 5 Hz, 3H), 2.34-2.38 (m, 2H), 7.80 (d, J = 9.6 Hz, 1H), 8.02 (t, J = 5 Hz, 1H), 8.28 (dd, J = 9.6 Hz and J = 2.5 Hz, 1H), 8.78 (d, J = 2.5 Hz, 1H), 11.29 (s, 1H, D2O exchanged). ^{13}C NMR (75 MHz, DMSO D6/TMS): δ 10.6, 26.1, 116.7, 123.4, 128.9, 130.2, 136.8, 145.2, 156.3.

Methylglyoxal bis(2,4-dinitrophenylhydrazone).[6] Yield: 950 mg (44%, entry 4); 303 mg (14%, entry 5), red solid, m.p. 290-292°C (lit.,[6] 296 °C). ^{1}H NMR (300 MHz, DMSO D6/TMS): δ 2.39 (s, 3H), 7.95 (d, J = 9 Hz, 1H), 8.08 (d, J = 9.5 Hz, 1H), 8.42 (d, J = 9 Hz, 1H), 8.51 (m, 2H), 8.88 (m, 2H), 11.17 (s, 1H, D2O exchanged), 11.89 (s, 1H, D2O exchanged). The ^{13}C NMR spectrum was not recorded because of the poor solubility of methylglyoxal bis(2,4-dinitrophenylhydrazone) in DMSO D6.

3-Aminopropionaldehyde 2,4-dinitrophenylhydrazone.[7] Yield: 748 mg (41%), orange crystals (recrystallized from ethanol) m.p. 235-237 °C; (lit.,[7] 235 °C). ^{1}H NMR (300 MHz, DMSO D6/TMS): δ 1.82-1.87 (m, 2H), 2.41-2.49 (m, 2H), 2.89-2.91 (m, 2H), 7.69 (broad s, 3H, D2O exchanged), 7.84 (d, J = 9.6 Hz, 1H), 8.03 (t, J = 4.8 Hz, 1H), 8.33 (dd, J = 9.6 Hz and J = 2.6 Hz, 1H), 8.83 (d, J = 2.6 Hz, 1H), 11.37 (s, 1H, D2O exchanged); ^{13}C NMR (75 MHz, DMSO D6/TMS): δ 24.1, 29.5, 38.9, 116.6, 123.6, 129.2, 130.2, 137.0, 145.2, 154.1.

References

1 D. Xu, A. Chiaroni, M.-B. Fleury and M. Largeron, *J. Org. Chem.*, 2006, **71**, 6374 (see the supporting information).

2 (a) R. Calabretta, C. Giordano, C. Gallina, V. Morea, V. Consalvi and R. Scandurra, *Eur. J. Med. Chem.*, 1995, **30**, 931; (b) P. Cui, T. L. Macdonald, M. Chen and J. L. Nadler, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3401.

3 I. Gallardo, J. Pinson and N. Vilà, *J. Phys. Chem. B*, 2006, **110**, 19521.

4 P. Lue, W. Q. Fan and X. J. Zhou, *Synthesis*, 1989, 692.

5 M. Behforouz, J. L. Bolan and M. S. Flynt, *J. Org. Chem.*, 1985, **50**, 1186.

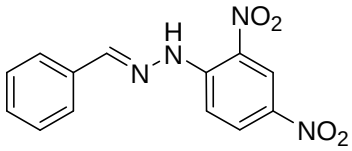
6 T. N. Mamashvili, N. A. Keiko, G. I. Sarapulova and M. G. Voronkov, *Russian Chem. Bull.* 1998, **47**, 2465.

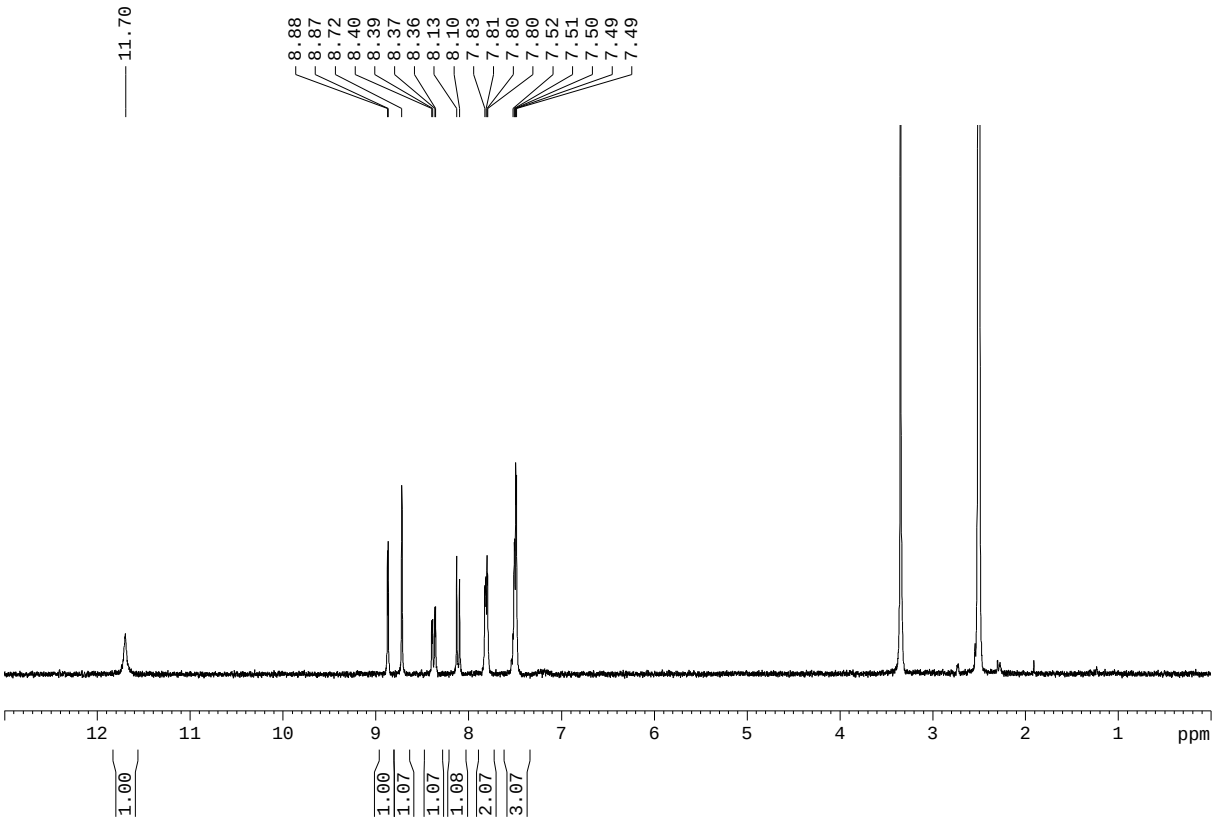
7 S. Ivanova, G. I. Botchkina, Y. Al-Abed, M. Meistrel III, F. Batliwalla, J. M. Dubinsky, C. Iadecola, H. Wang, P. K. Gregersen, J. W. Eaton and K. J. Tracey, *J. Exp. Med.* 1998, **188**, 327.

II. NMR spectra of isolated products

^{1}H NMR, 300 MHz, DMSO D6/TMS

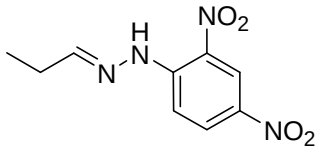
Benzaldehyde 2,4-dinitrophenylhydrazone (Table 1, Entry 1)

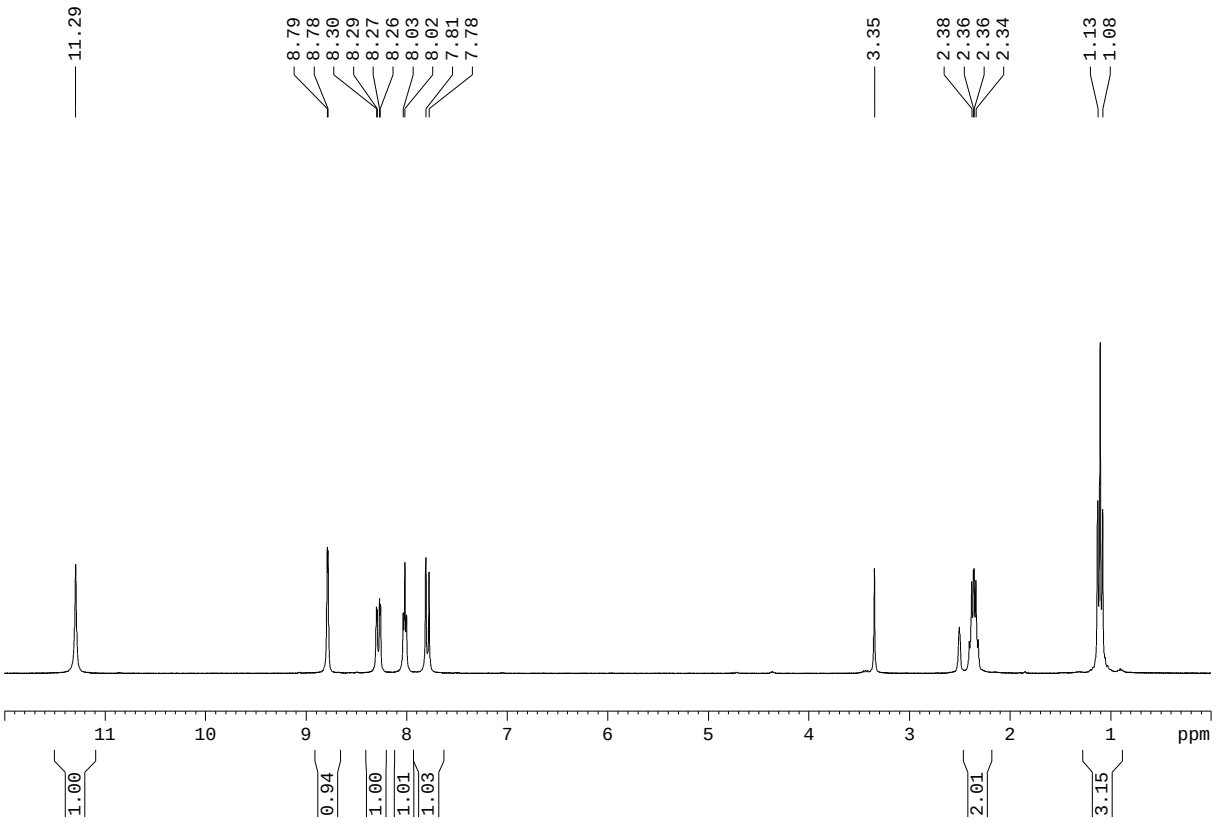




^{1}H NMR, 300 MHz, DMSO D6/TMS

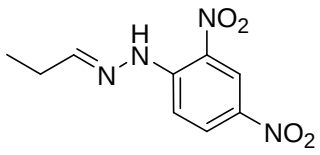
Propionaldehyde 2,4-dinitrophenylhydrazone (Table 1, Entry 3)

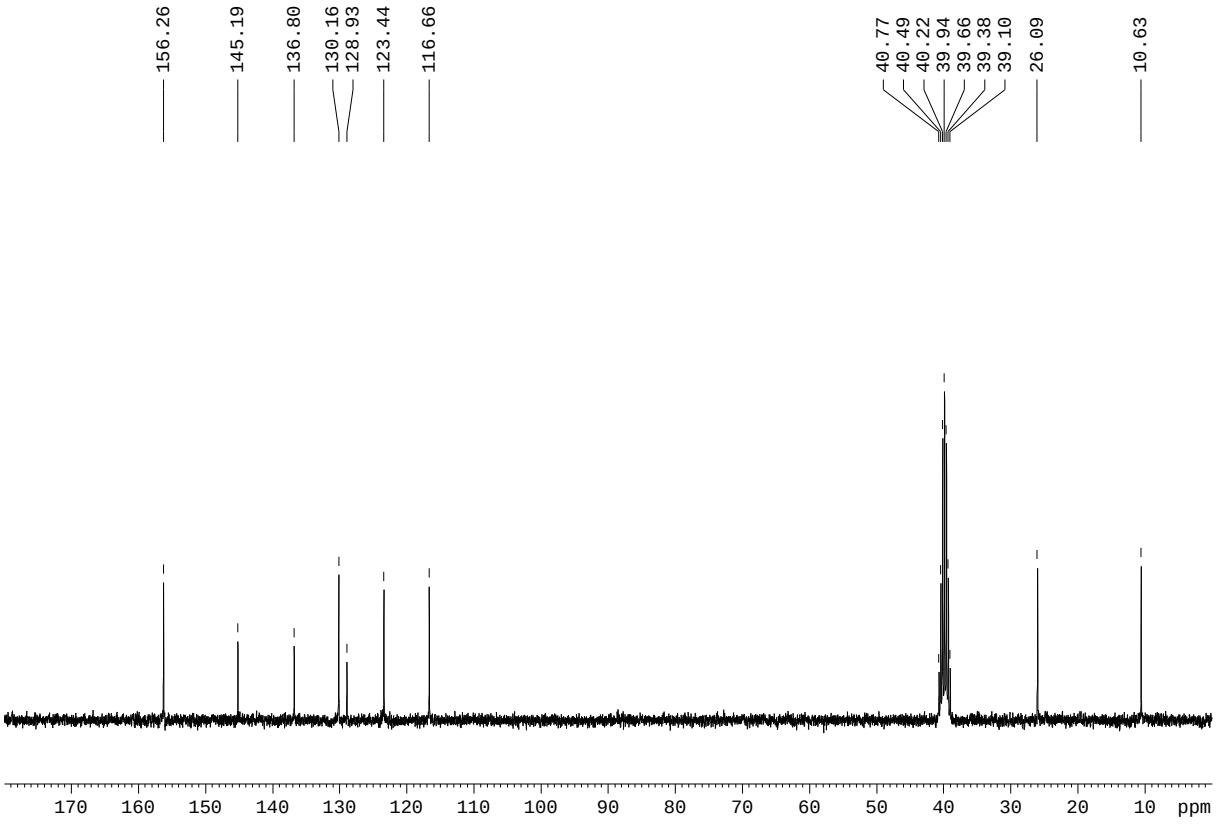




^{13}C NMR, 75 MHz, DMSO D6/TMS

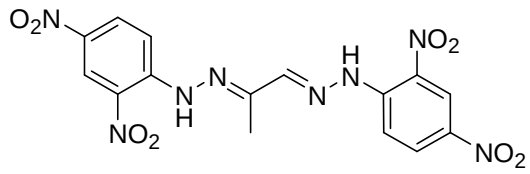
Propionaldehyde 2,4-dinitrophenylhydrazone (Table 1, Entry 3)

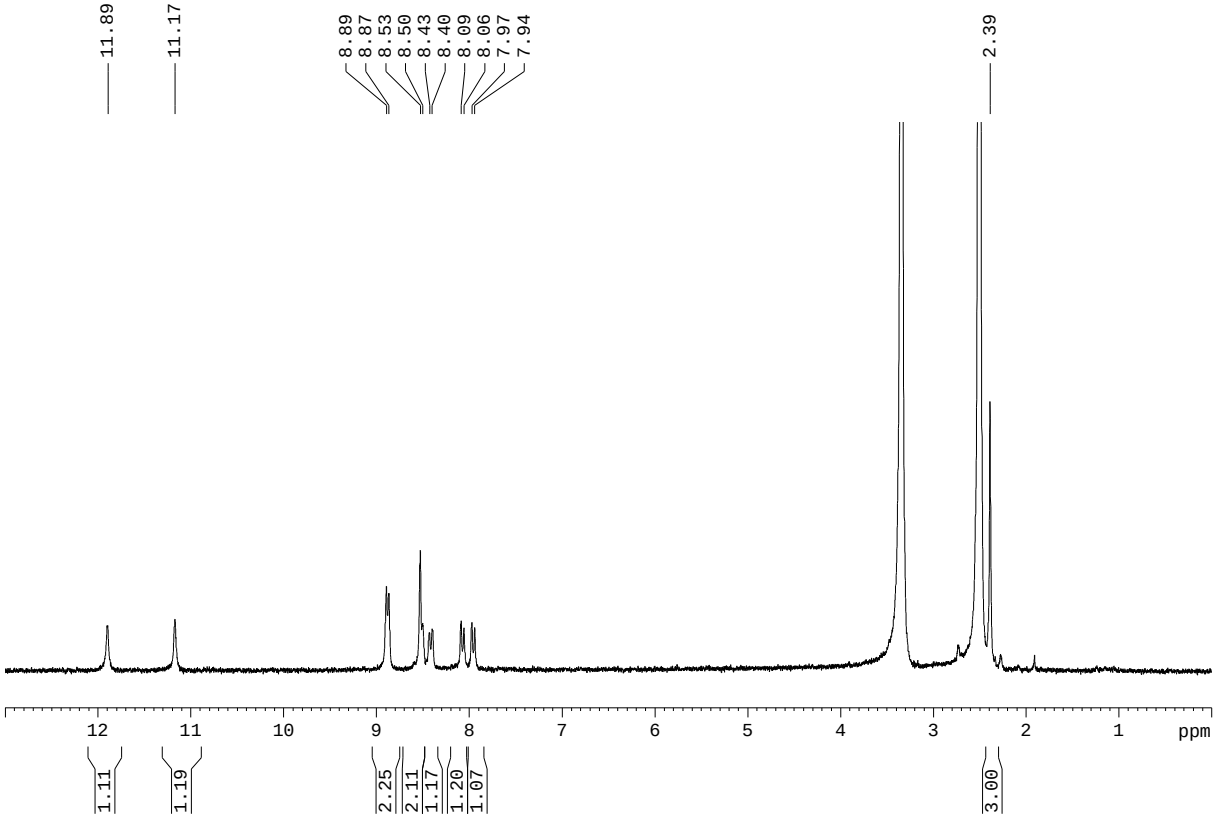




^{1}H NMR, 300 MHz, DMSO D6/TMS

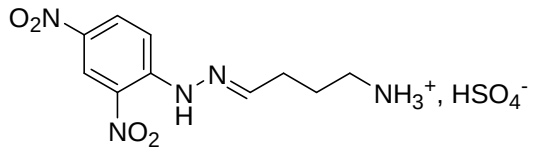
Methylglyoxal bis(2,4-dinitrophenylhydrazone) (Table 1, Entries 4 and 5)

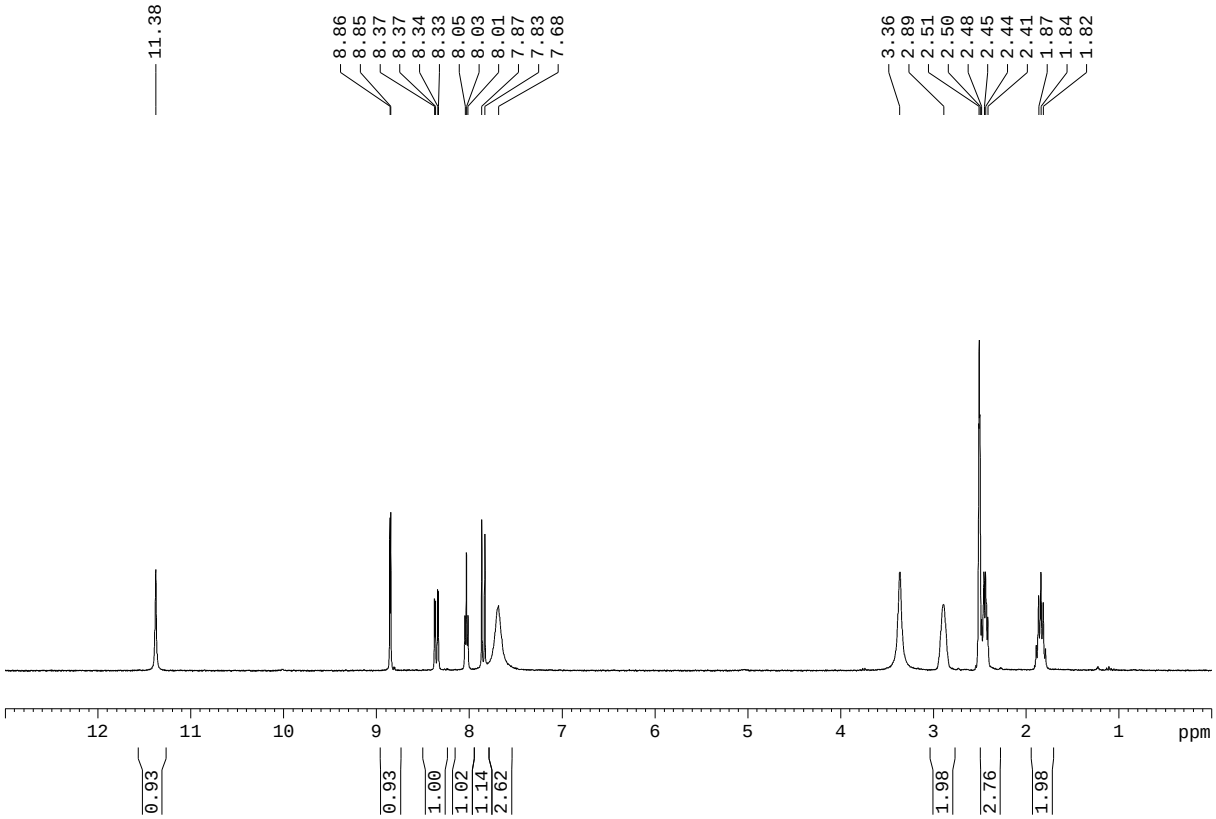




^{1}H NMR, 300 MHz, DMSO D6/TMS

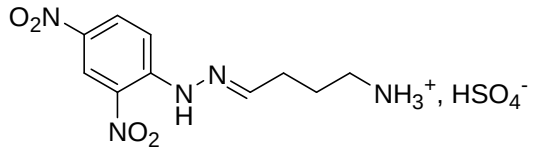
3-Aminopropionaldehyde 2,4-dinitrophenylhydrazone (Table 1, Entry 8)

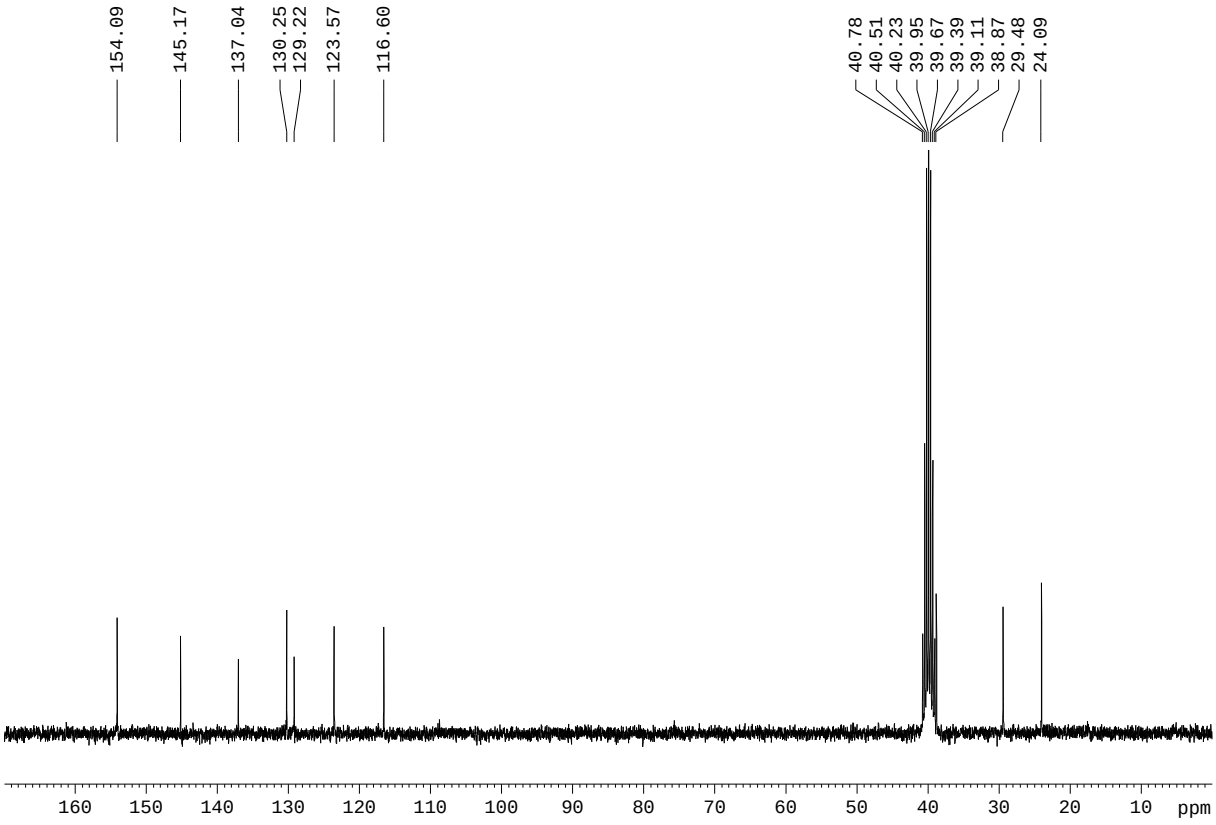




^{13}C NMR, 75 MHz, DMSO D6/TMS

3-Aminopropionaldehyde 2,4-dinitrophenylhydrazone (Table 1, Entry 8)





^{1}H NMR, 300 MHz, D_2O

Putrescine ammonium disulphate (Table 1, Entry 9)