Electronic Supplementary Material (ESI) for RSC Advances.
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General Methods. Amino Acids Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Phe-OH, and Fmoc-D-Phe-OH were obtained from Sigma-Aldrich. *L*-Threonine tert-butyl ester hydrochloride, Fmoc-*D*-*allo*-Thr(^{t}Bu)-OH and Fmoc-Gly-OH were from Chem-impex International, Inc and Fmoc-*D*-Leu-OH was from Advanced ChemTech.

Solution-phase reactions were done in a round-bottom flask. Organic solvent removal was performed using Eyela rotary evaporator with temperature not exceeding 40°C. Freeze-drying was done using a Labconco Lyophilizer or Labconco CentriVac. Solid-phase reactions were all performed at 25 °C in a glass column (15 mL) with a polyethylene porous disc and a stopcock using a protocol described by Amblard et al. (2005) with some modifications. Solvents, excess soluble reagents and washings were removed by suction filtration. Deprotection of Fmoc group was done using 20% Piperidine in DMF (1 x 2mL x 2min; 1 x 2mL x 20min). Washings after each step (coupling and deprotection) were done with DCM (3 x 2mL x 2min), MeOH (3 x 2mL x 1min), and DMF (3 x 2mL x 1min).

Purification of compounds by gravity column chromatography was performed using silica gel 60, 0.063-0.200 mm (70-230 mesh, Merck). Monitoring progress of solution-phase reactions was done by thin layer chromatography (TLC) using Silica gel 60 (Merck). The TLC plates were visualized first under a UV lamp set at long wavelength and stained using permanganate staining solution. All solvents used in reactions and column chromatography were A.R. grade.

Analytical HPLC was carried out on Phenomenex Luna C18 reversed-phase column (5µ 100A 250 x 4.6 mm) using a Shimadzu instrument with photodiode array detector (PDA) set at 220 nm using linear gradients of CH_3CN (0.1% TFA) in H_2O (0.1% TFA) run at 1.0 mL/min flow rate. Purification of Nobilamide B and analogues were carried out using semi-preparavive Varian C18 reversed-phase column (5µ 250 x 10.0 mm) or Phenomenex C18 reversed-phase column (5µ 100A 250 x 10.0 mm). All peptides were found to be >98% pure based on HPLC purity check.

NMR spectra were recorded on a 500 (^{1}H 500 MHz, ^{13}C 125 MHz) Agilent (Varian) NMR spectrometer with a 5 mm one-probe. Chemical shifts are reported in parts per million (ppm) and *J* (coupling constant) values are expressed in hertz, Hz. Multiplicity is indicated using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet), and tt (triple triplet). QSTAR Elite Q-TOF (Applied Biosystems/AB Sciex) Electron Spray Ionization Mass spectrometer (ESI-MS) was used to generate the high-resolution mass spectra.

Fmoc-Ala-Thr-O^{t}Bu (3): H-Thr-O^{t}Bu·HCl (1.78 g, 8.39 mmol, 1 eq.) was dissolved in dichloromethane (20 mL) and into this triethylamine (1.05 mL, 7.55 mmol, 0.90 eq.) was added. The mixture was stirred for 30 min. Fmoc-Ala-OH (3.40 g, 10.9 mmol, 1.30 eq.) was added till completely dissolved followed by the addition of EDC (1.78 g, 9.28 mmol, 1.31 eq.) and dichloromethane (20 mL). The mixture was stirred for 24 h at 25 °C under N_2. The mixture was concentrated in *vacuo* and purified by gravity column chromatography [DCM-MeOH (9:1)] to give Fmoc-Ala-Thr-O^{t}Bu (3.50 g, 7.49 mmol, 89.3 % yield). Appearance: White powder; TLC (SiO_2, DCM-MeOH 9:1) R_f = 0.73; Analytical HPLC (t_R 26.4 min; 3(A):7(B) to 1(A):0(B) over 30 min (A: 0.1% TFA in ACN, B: 0.1% TFA in H_2O), ESI-MS [M+Na]$^+$: 491.2393, calculated for $C_{26}H_{32}N_2O_6Na$: 491.5313; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.76 (2 H, d, $^3J_{HH}$ = 7.5, Fmoc Ph-**H**); 7.58 (2 H, d, $^3J_{HH}$ = 7.0, Fmoc Ph-**H**); 7.40 (2 H, t, $^3J_{HH}$ = 7.4, Fmoc Ph-**H**); 7.32 (2 H, t, $^3J_{HH}$ = 7.4, Fmoc Ph-**H**); 6.87 (1 H, d, $^3J_{HH}$ = 8.6, N**H** Thr); 5.65 (1 H, s, N**H** Ala); 4.49 (1 H, dd, $^3J_{HH}$ = 8.9; 2.4, C**H**^{α} Thr); 4.38 (2 H, m, C**H**$_2$ Fmoc); 4.30 (2 H, m, C**H**^{β} Thr and C**H**^{α} Ala); 4.21 (1 H, t, $^3J_{HH}$ = 6.9, C**H** Fmoc); 1.48 (9 H, s, ^{t}Bu C**H**$_3$); 1.44 (3 H, d, $^3J_{HH}$ = 6.7, C**H**$_3$ Ala); 1.20 (3 H, d, $^3J_{HH}$ = 6.4, C**H**$_3$ Thr); ^{13}C-NMR (125 MHz, $CDCl_3$): 172.80 (**C**=O Ala); 169.76 (**C**=O Thr); 156.06 (**C**=O Fmoc); 143.81 (**C** Ph Fmoc); 143.70 (**C** Ph Fmoc); 141.26 (**C** Ph Fmoc); 127.71 (**C** Ph Fmoc); 127. 07 (**C** Ph Fmoc); 125.09 (**C** Ph Fmoc); 119.96 (**C** Ph Fmoc); 82.63 (**C**q ^{t}Bu); 68.40 (**C**H^β Thr); 67.17 (**C**H$_2$ Fmoc); 57.94 (**C**H^α Thr); 50.63 (**C**H^α Ala); 47.06 (**C**H Fmoc); 28.00 (**C**H$_3$ ^{t}Bu); 20.05 (**C**H$_3$ Thr); 18.79 (**C**H$_3$ Ala)

Fmoc-Ala-(*Z*)-Dhb-O^{t}Bu (4): Fmoc-Ala-Thr-O^{t}Bu (2.03 g, 4.34 mmol, 1 eq.), EDC·HCl (1.60 g, 8.32 mmol, 1.92 equiv), and CuCl (497 mg, 5.02 mmol, 1.16 equiv) were dissolved together in 20 mL DCM/DMF (12:1) and the mixture was stirred for 48 h at 25 °C under N_2. The organic solvent was evaporated in *vacuo* and the residue was purified by gravity chromatography [DCM-MeOH (9:1)] to give Fmoc-Ala-(*Z*)-Dhb-O^{t}Bu (1.41 g, 3.13 mmol, 72.1 % yield). Appearance: White powder; TLC (SiO_2, DCM-MeOH 9:1) R_f = 0.88; Analytical HPLC (t_R 29.8 min; 3(A):7(B) to 1(A):0(B) over 30 min (A: 0.1% TFA in ACN, B: 0.1% TFA in H_2O), ESI-MS [M+Na]$^+$: 473.2266, calculated for $C_{26}H_{30}N_2O_5Na$: 473.5160; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.77 (2 H, d, $^3J_{HH}$ = 7.5, Fmoc Ph-**H**); 7.59 (2

H, d, $^3J_{HH}$ = 7.3, Fmoc Ph-**H**); 7.40 (2 H, t, $^3J_{HH}$ = 7.3, Fmoc Ph-**H**); 7.32 (2 H, t, $^3J_{HH}$ = 7.7, Fmoc Ph-**H**); 6.73 (1 H, q, $^3J_{HH}$ = 7.1 Hz, C**H**^{β} Dhb); 5.51 (1 H, bs, N**H** Ala); 4.43 (3 H, m, C**H**$_2$ Fmoc and C**H**^{α} Ala); 4.23 (1 H, t, $^3J_{HH}$ = 6.8, C**H** Fmoc); 1.73 (3 H, d, $^3J_{HH}$ = 6.74, C**H**$_3$ Dhb); 1.48 (12 H, s, ^{t}Bu C**H**$_3$; C**H**$_3$ Ala); ^{13}C-NMR (125 MHz, $CDCl_3$): 170.35 (**C**=O Ala); 163.38 (**C**=O Dhb); 155.97 (**C**=O Fmoc); 143.74 (**C** Ph Fmoc); 141.30 (**C** Ph Fmoc); 132.81 (**C**H Dhb); 127.72 (**C** Ph Fmoc); 127. 08 (**C** Ph Fmoc); 126.72 (**C** Dhb); 125.03 (**C** Ph Fmoc); 119.98 (**C** Ph Fmoc); 81.80 (**C**q ^{t}Bu); 67.08 (**C**H$_2$ Fmoc); 50.77 (**C**H^α Ala); 47.14 (**C**H Fmoc); 28.02 (**C**H$_3$ ^{t}Bu); 18.78 (**C**H$_3$ Ala); 14.66 (**C**H$_3$ Dhb).

Fmoc-Ala-(Z)-Dhb-OH (5): Fmoc-Ala-(*Z*)-Dhb-O^{t}Bu (303 mg, 0.675 mmol) was dissolved in 2 mL TFA-DCM (95:5) and was stirred for 3 hrs at 25 °C and was concentrated in *vacuo.* The TFA was removed by co-evaporations with DCM and diethylether to give Fmoc-Ala-(*Z*)-Dhb-OH (242 mg, 0.615 mmol, 91.1 % yield). Appearance: White powder; TLC (SiO_2, DCM-MeOH 9:1) R_f = 0.19; Analytical HPLC (t_R 21.4 min; 3(A):7(B) to 1(A):0(B) over 30 min (A: 0.1% TFA in ACN, B: 0.1% TFA in H_2O), ESI-MS [M+Na]$^+$: 417.1567, Calculated for $C_{22}H_{22}N_2O_5Na$: 417.4097; ^{1}H-NMR (500 MHz, $CDCl_3$): 7.78 (2 H, d, $^3J_{HH}$ = 7.5, Fmoc Ph-**H**); 7.67 (2 H, d, $^3J_{HH}$ = 8.4, Fmoc Ph-**H**); 7.38 (2 H, t, $^3J_{HH}$ = 7.4, Fmoc Ph-**H**); 7.30 (2 H, t, $^3J_{HH}$ = 7.4, Fmoc Ph-**H**); 6.86 (1 H, q, $^3J_{HH}$ = 6.8 Hz, C**H**^{β} Dhb); 4.37 (2 H, m, C**H**$_2$ Fmoc); and 4.28-4.22 (2 H, m, C**H**^{α} Ala, C**H** Fmoc); 1.74 (3 H, d, $^3J_{HH}$ = 7.5, C**H**$_3$ Dhb); 1.42 (3 H, t, $^3J_{HH}$ = 7, C**H**$_3$ Ala); ^{13}C-NMR (125 MHz, $CDCl_3$): 173.17 (**C**=O Ala); 165.84 (**C**=O Dhb); 156.83 (**C**=O Fmoc); 143.92 (**C** Ph Fmoc); 143.78 (**C** Ph Fmoc); 141.16 (**C** Ph Fmoc); 135.35 (**C**H Dhb); 127.36 (**C** Ph Fmoc); 126.99 (**C** Dhb); 126.76 (**C** Ph Fmoc); 126.73 (**C** Ph Fmoc); 124.84 (**C** Ph Fmoc); 124.77 (**C** Ph Fmoc); 119.49 (**C** Ph Fmoc); 66.53 (**C**H$_2$ Fmoc); 50.68 (**C**H^α Ala); 46.98 (**C**H Fmoc); 16.87 (**C**H$_3$ Ala); 12.79 (**C**H$_3$ Dhb).

Nobilamide B, Propanoyl–D-Phe–D-Leu–Phe–D-*a*-Thr–Val–Ala–(Z)-Dhb-OH (1): Barlos (chlorotrityl chloride) resin (86.9 mg, 0.148 mmol, 1 eq.; 1.7 mmol/g loading) was placed in a small column (described in 3.2). The resin was swelled by washing with DCM (3 x 2 mL x 1 min). A solution of Fmoc-Ala-(Z)-Dhb-OH (65.5 mg, 0.167 mmol, 1.13 eq.) and DIEA (51 µL, 0.294 mmol, 2.06 eq.) in DMF (2 mL) was added. The mixture was allowed to stir for 1.5 hrs. The resin was then washed with DCM (3 x 2mL x 2min), MeOH (3 x 2mL x 2min), DCM (3 x 2mL x 2min) and DMF (3 x 2mL x 2min). Fmoc-Ala-(*Z*)-Dhb-*O*-Barlos resin was subjected to Fmoc deprotection treatment: DMF (3 x 1mL x 1min), 20% piperidine in DMF (1 x 2mL x 2min), 20% Piperidine in DMF (1 x 2mL x 20min), DMF (4 x 2mL x 1min). All deprotection solutions were kept for Fmoc monitoring. The Kaiser test was positive after deprotection. The loading, as calculated by Fmoc assay was 1.67 mmol/g, 98.2%.[11,27]

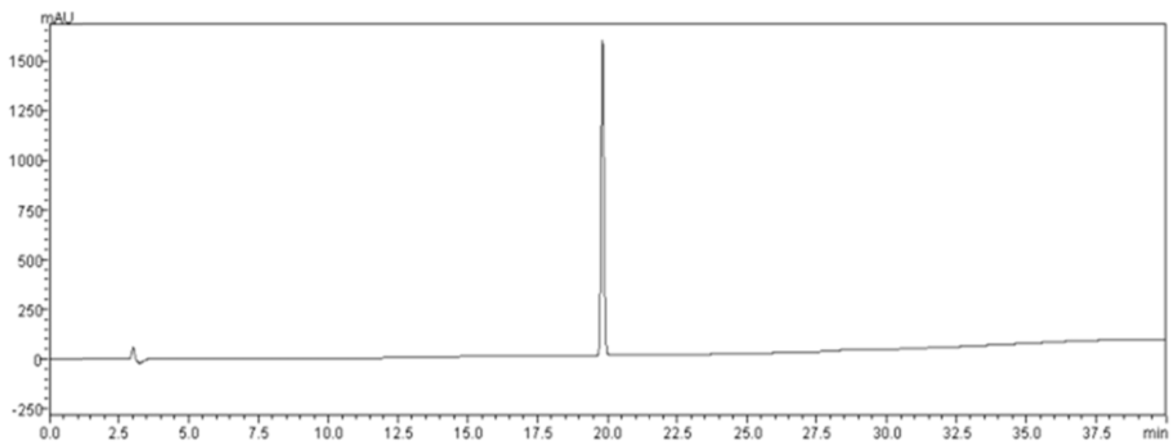
Fmoc-Val-OH (81.3 mg, 0.240 mmol, 1.62 eq.) was coupled to H-Ala-(*Z*)-Dhb-*O*-Barlos resin using 0.50 M HBTU/Oxyma Pure (444 µL, 0.222 mmol, 1.50 eq.) and DIEA (77 µL, 0.442 mmol, 3 eq.) in DMF for 1.5 hrs. Fmoc-Val-Ala-(*Z*)-Dhb-*O*-Barlos resin was washed and the coupling was successful as indicated by the negative result in Kaiser Test. Fmoc was removed as described previously and Fmoc-D-*a*-Thr(O^{t}Bu)-OH (88.0 mg, 0.221 mmol, 1.49 eq.), Fmoc-Phe-OH (89.8 mg, 0.232 mmol, 1.57 eq.), Fmoc-D-Leu-OH (77.3 mg, 0.219 mmol, 1.48 eq.), Fmoc-D-Phe-OH (85.4 mg, 0.220 mmol, 1.49 eq.), Propionic acid (17 µL, 0.228 mmol, 1.54 eq.) were coupled consecutively to H-Val-Ala-(*Z*)-Dhb-*O*-Barlos resin using 0.50 M HBTU/Oxyma Pure (444 µL, 0.222 mmol, 1.50 eq.) and DIEA (77 µL, 0.442 mmol, 2.99 eq.) with the same cycle of washing, deprotection and coupling. In each coupling, the Kaiser test was negative after 1.5 hrs of stirring. The peptide was cleaved from the resin using (1 x 2 mL x 90 min) TFA/DCM (95:5) cleavage cocktail and the mixture was filtered. Another fresh cleavage cocktail (1 x 2 mL x 30min) and (1 x 2 mL x 1 min) was added to the resin collecting all the filtrates in a round-bottom flask. TFA was removed in *vacuo* and by co-evaporations with DCM. The peptide was dissolved in methanol and dried under reduced pressure to give 98.8 mg of crude peptide. Nobilamide B (Propanoyl–*D*-Phe–*D*-Leu–Phe–*D*-*a*-Thr–Val–Ala–(*Z*)-Dhb-OH), was found to be a major product as seen in the HPLC profile Analytical HPLC (t_R 28.8 min; Condition 5:95 to 1:0 A in B for 40 min, A: 0.1% TFA ACN, B: 0.1% TFA H_2O). An aliquot of 67.5 mg of the crude peptide was purified using semi-preparavive Varian C18 reversed-phase column (5µ 250 x 10.0 mm) or Phenomenex C18 reversed-phase column (5µ 100A 250 x 10.0 mm). After analytical HPLC profiling, semi-preparative HPLC purification and mass spectroscopic analysis, 17.1 mg (20.5 µmoles, 25.3% yield) of pure peptide was obtained with >98% purity. Appearance: White powder; Analytical HPLC (t_R 19.8 min; Condition 3(A):7(B) to 1(A):0(B) over 30 min (A: 0.1% TFA in ACN, B: 0.1% TFA in H_2O), ES-MS [M+Na]$^+$: 858.5299, calculated for $C_{43}H_{61}N_7O_{10}Na$: 858.9746.

Dorsal Root Ganglion Primary Cell Culture Assay:

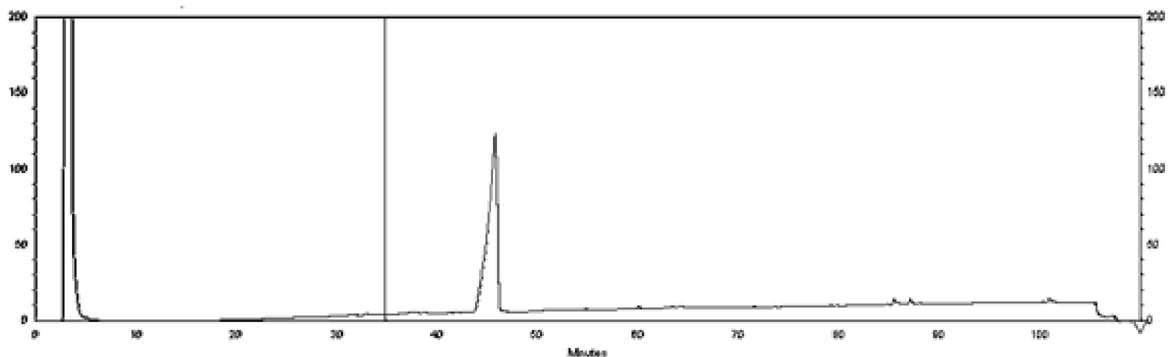
The neuroactivity of Nobilamide B was assessed using the dorsal root ganglion primary cell culture assay. DRG cells were harvested from the spine of a 21 day-old mouse using a dissecting microscope and the cells were plated and cultured in a 24-well plate. Individual cells were selected and monitored using the Olympus IX50 fluorescence microscope and Nikon camera attached to a computer running the NIS Elements Basic Research software. Intracellular calcium ions were viewed using Fluo-4 fluorescent probe.

Analytical HPLC profile
Nobilamide B

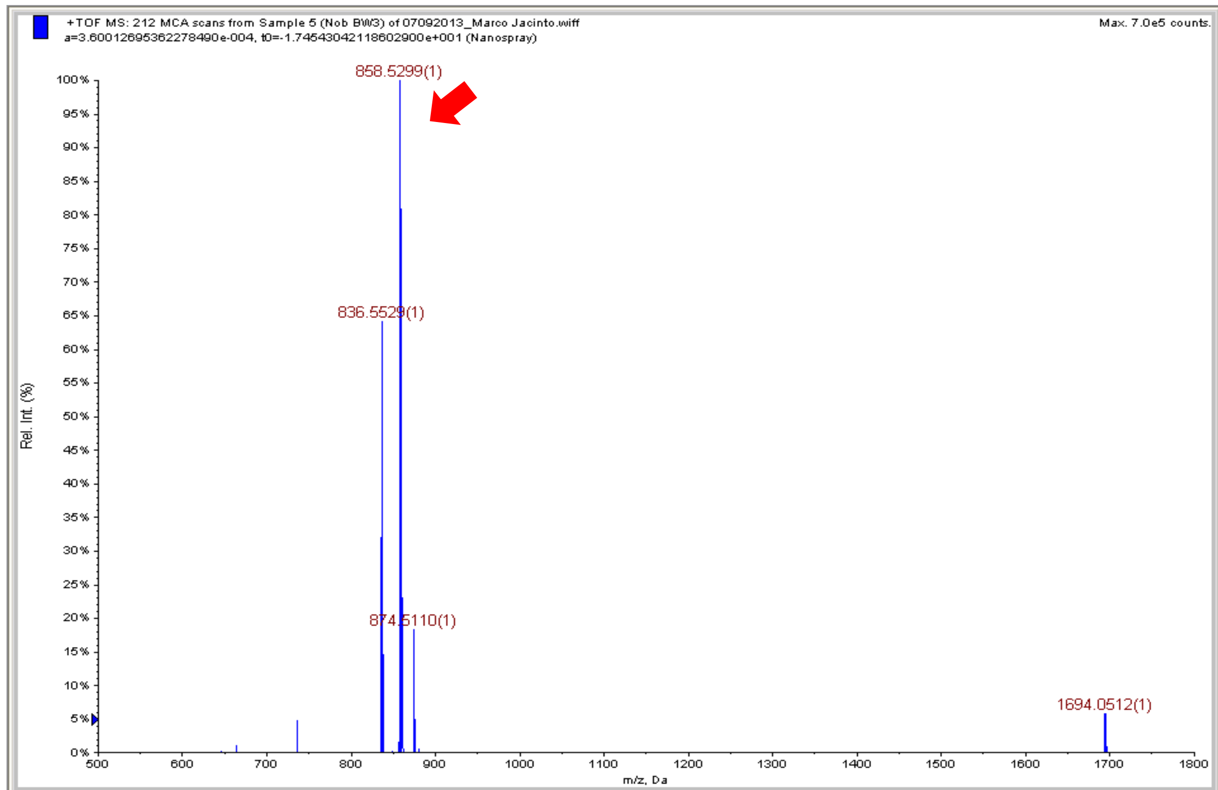
Conditions: C18 reversed-phase column; 5(A):95(B) to 1(A):0(B) over 40 min (A: 0.1% TFA in ACN, B: 0.1% TFA in H_2O at 1.0 mL/min flow rate; wavelength detector set at 220 nm)



Co-elution of synthetic and isolated nobilamide B
Conditions: C18 reversed-phase column; 5(A):95(B) to 1(A):0(B) over 120 min (A: ACN, B: 0.5% TFA in H_2O at 1.0 mL/min flow rate; wavelength detector set at 220 nm)



Mass Spectrum of nobilamide B



NMR data of Nobilamide B:

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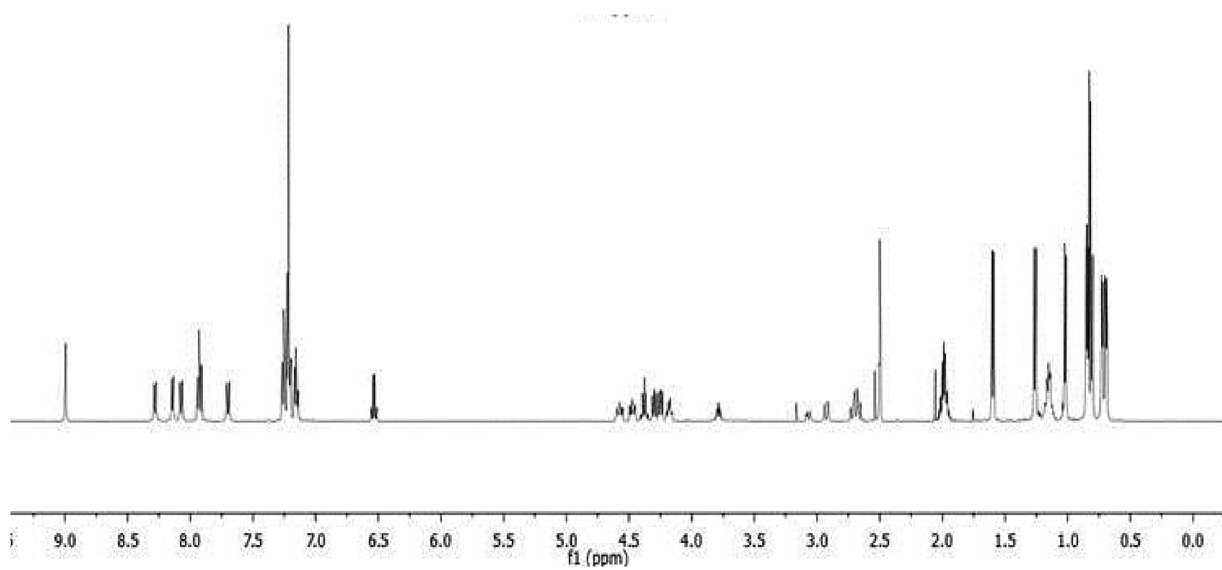
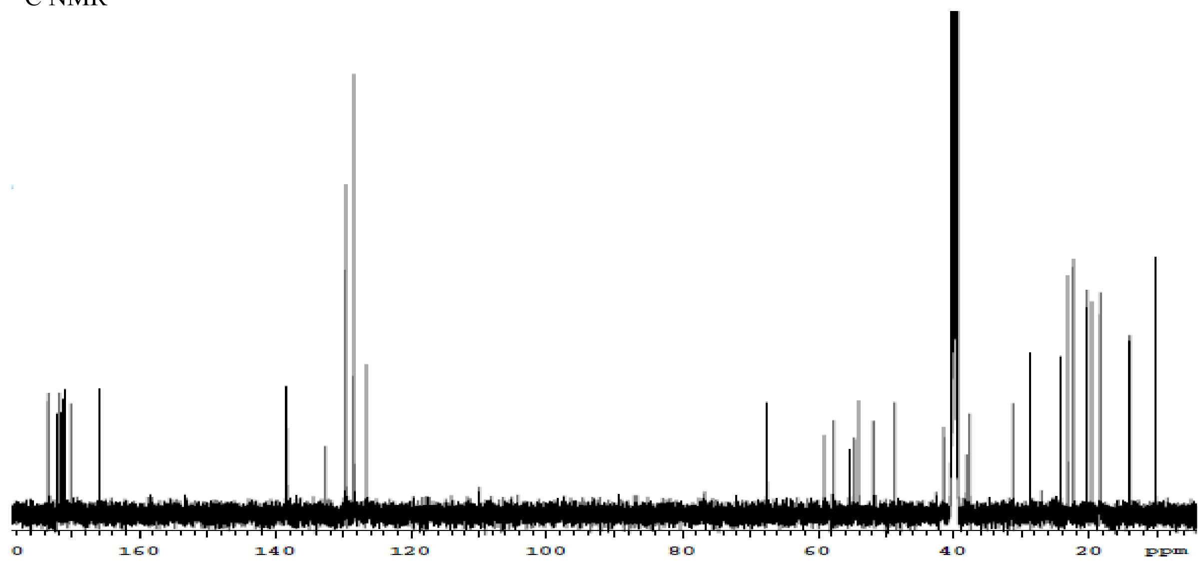
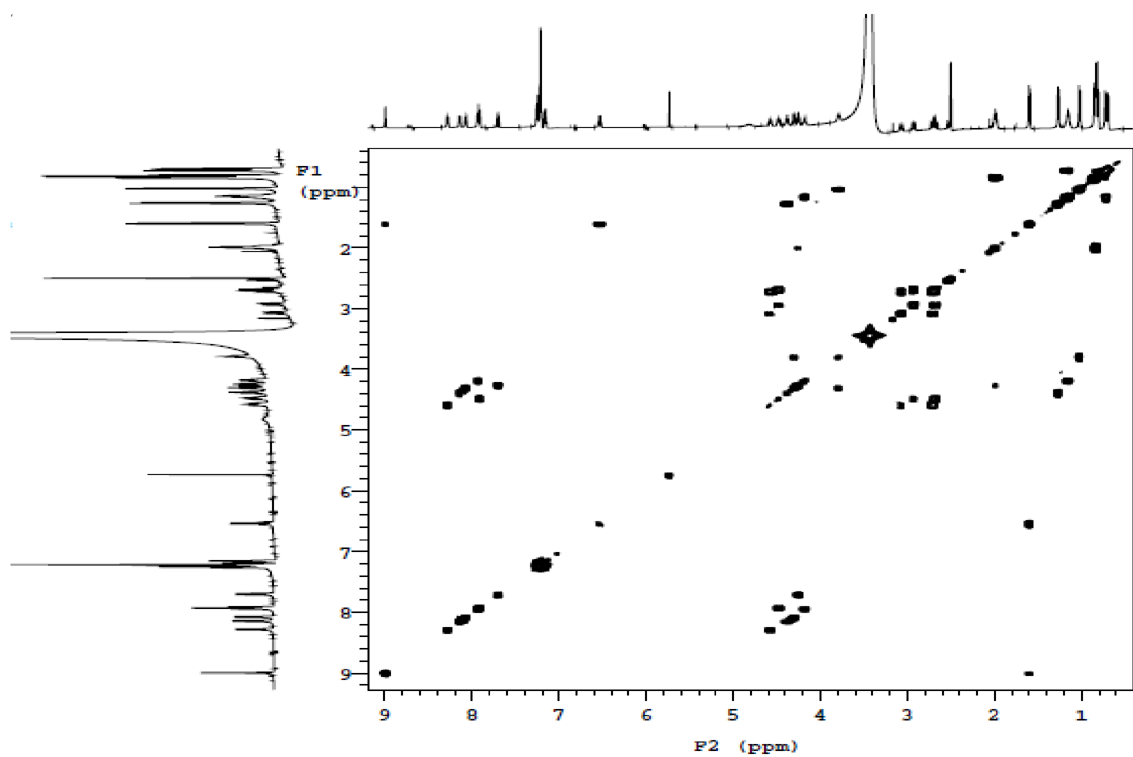


^{13}C NMR



^{1}H-^{1}H COSY



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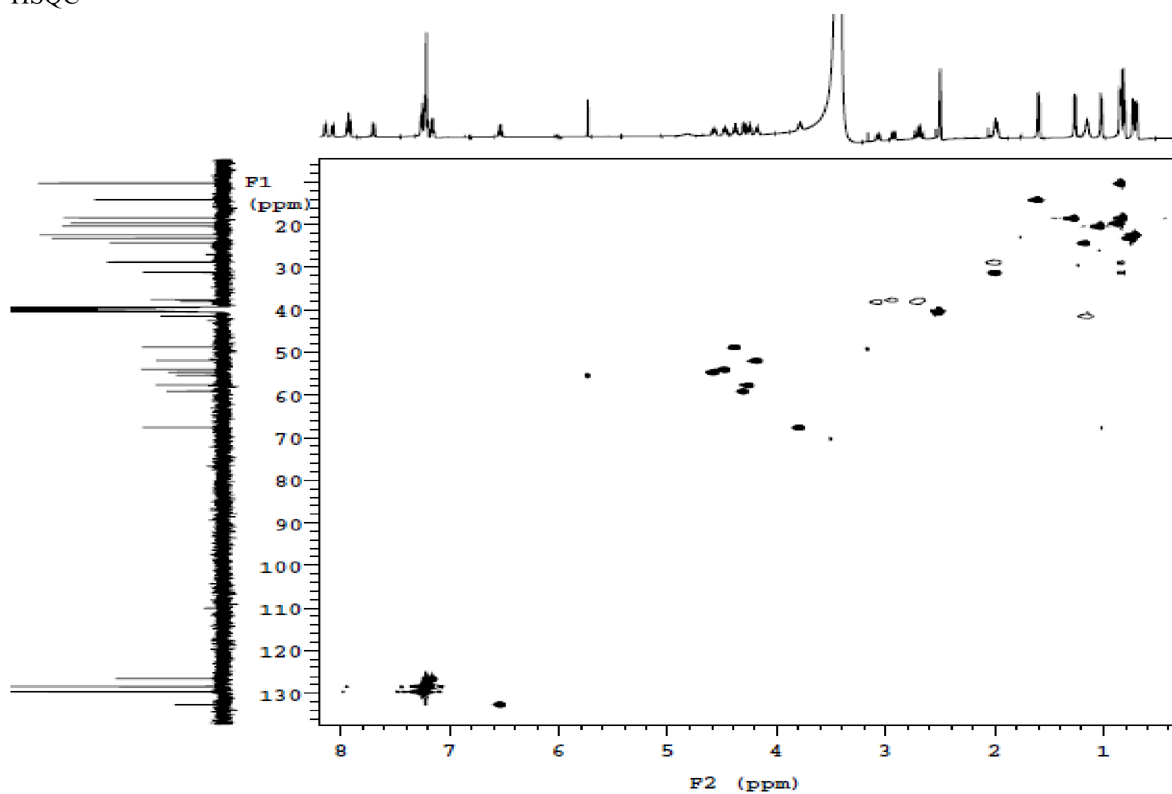

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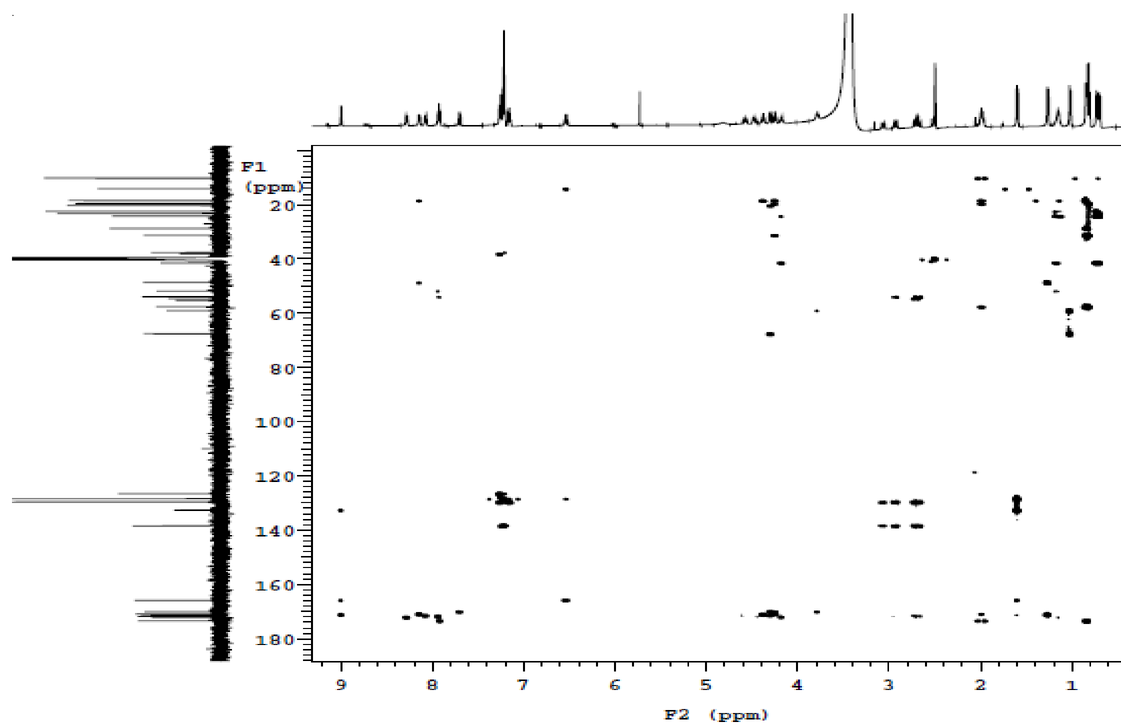

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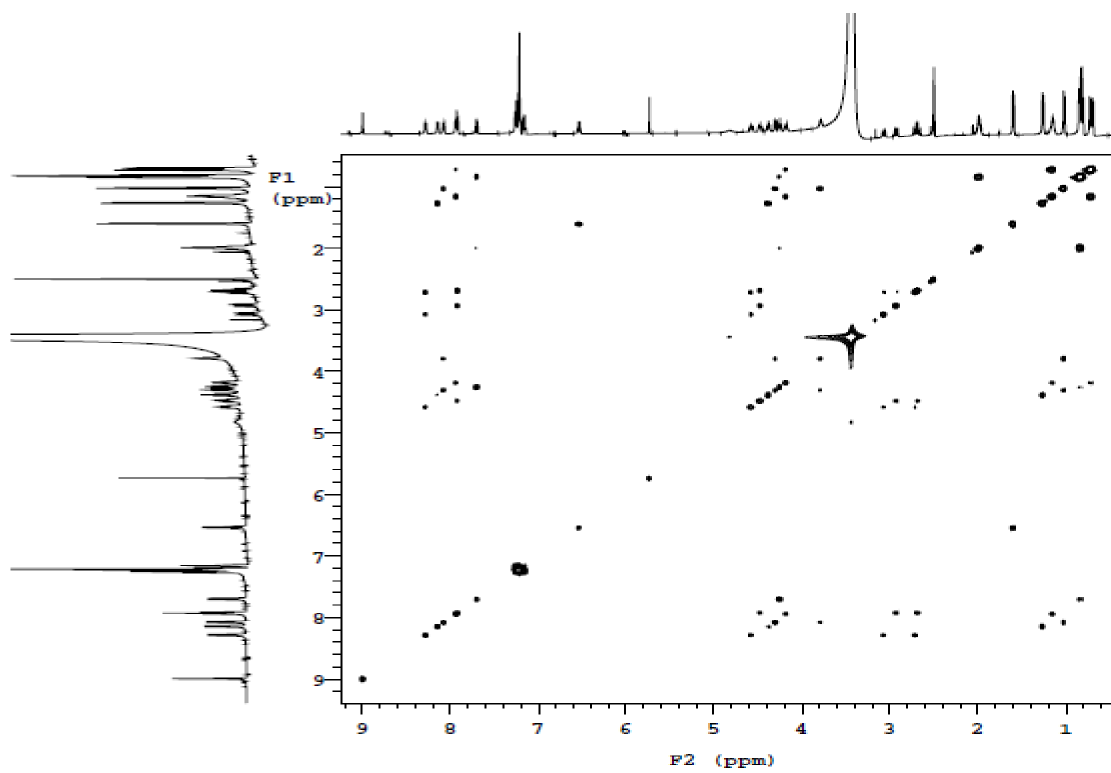

NOESY