Synthesis of Hybrid Anticancer Agents Based on Kinase and Histone Deacetylase Inhibitors

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Experimental and analytical procedures for chemistry have been outlined elsewhere.[1]

All *Xenopus* experiments were performed in compliance with the relevant laws and institutional guidelines at the University of Portsmouth. Embryos were generated and screened according to Guille[2] and incubated at 18°c in the jelly coating until stage 9. The jelly coat was removed with 2% cysteine and embryos were arrayed by Pasteur pipette as 12 embryos in each well of 24 well plates containing 1000 µl of 0.1x MBS (Modified Barth's Saline, supplemented with penicillin 10000U/ml and streptomycin 10mg/ml). The compounds were added to the wells to the final concentration shown. Embryos were incubated at 18°C for 72 hrs and fixed in MEMFA before being analysed by WISH (wholemount in situ hybridisation5 using RNA probe for Egfl7 (fitch) obtained from the European Xenopus Resource Centre (WEB). Embryos for western blot were generated and screened according to (The jelly coat was removed at stage 2 with 2% cysteine (Guille) and embryos were arrayed by Pasteur pipette as 12 embryos in each well of 24 well plates containing 1000 µl of 0.1x MBS (Modified Barth's Saline, supplemented with penicillin 10000U/cm3 and streptomycin 10mg/cm3). The ISC-2-051-1 and SAHA were added to the wells to a final concentration of 100µM. Embryos were incubated at 18°C until stage 14. One embryo equivalent of freon extracted stage 14 whole embryo lysate was assayed for acetylated alpha tubulin and

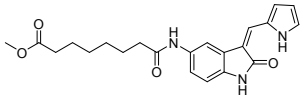
alpha tubulin by 12% SDS-PAGE and Western blotting (Robinson et al) using acetylated alpha tubulin (ab24610) and alpha tubulin (ab15246) antibodies.

HDAC inhibition assays were performed using a fluorimetric *in vitro* histone deacetylase assay (Merck Millipore #17-372) and according to instructions using HeLa cell enzyme extract (Enzo #BML-KI140-0100) in a 1:10 dilution. During assay incubation, the enzymatic activity from HeLa cell extract deacetylates a substrate unless inhibited by the compound of interest. Deacetylation of the substrate allows a fluorescent compound (the activator) to bind, leading to an amplified signal with increasing deacetylation. Inhibitor-containing reactions were compared to a vehicle control sample to ascertain compound HDAC-inhibitory activity.[5]

The synthesis and data for the hybrids are described in a patent.[6] Details for the key hybrid, **6**, are below:

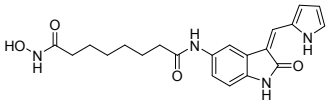
(*Z*)-*N*1-(3-((1*H*-Pyrrol-2-yl)methylene)-2-oxoindolin-5-yl)-*N*-8-hydroxyoctanediamide, **6**.

Part i) Synthesis of (*Z*)-methyl-8-(3-((*1H*-pyrrol-2-yl)methylene)-2-oxoindolin-5-ylamino)-8-oxooctanoate



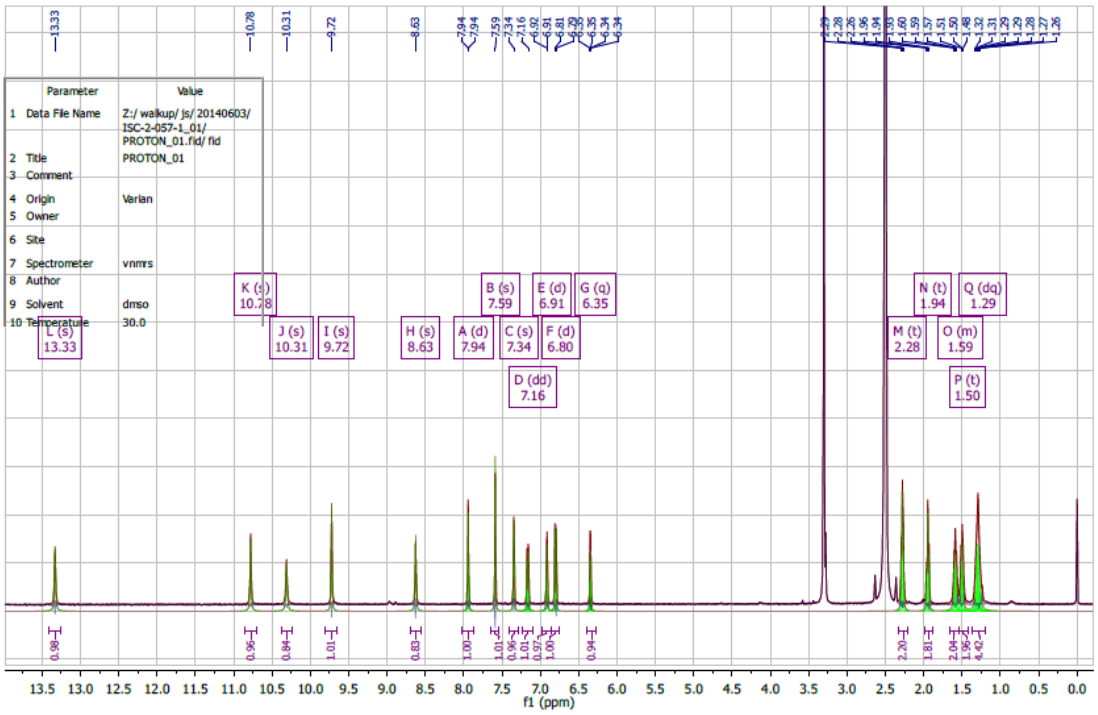
In a microwave vial (35 mL) were placed (3Z)-5-amino-3-(1H-pyrrol-2-ylmethylidene)-2,3-dihydro-1H-indol-2-one, methyl-8-chloro-8-oxooctanoate (1.2 equivalents), triethylamine (1 equivalent), THF (10 mL). The sealed vessel (with a septum) was stirred under initial microwave irradiation of 200 W with the temperature ramped from RT to 90 °C. Once the latter was reached, the reaction vessel was held at this temperature for 30 min by moderation of the initial microwave power. Thereafter, the mixture was cooled to RT, and then the reaction mixture was extracted with ethyl acetate (35 mL) and washed with water (2 x 50 mL). The organic layer was dried ($MgSO_4$). Filtration, then evaporation of the solvent, afforded a yellow solid. The product was recrystallized from ethyl acetate or chloroform with hexane. ^{1}H NMR (DMSO-d_6) δ = 13.35 (1H, brs), 10.81 (1H, brs), 9.76 (1H, brs), 7.95 (1H, d, J = 1.8 Hz), 7.59 (1H, s), 7.35 (1H, s), 7.14 (1H, dd, J_1, J_2 = 1.8 Hz), 6.91 (1H, m), 6.78 (1H, d, J = 8.4 Hz), 6.34 (1H, m), 3.57 (3H, s), 2.27 (4H, m), 1.56-1.31 (8H, m). ^{13}C NMR (DMSO-d_6) δ = 173.3, 170.7, 169.2, 134.8, 133.4, 129.4, 126.1, 125.7, 120.6, 118.7, 116.9, 110.4, 110.5, 109.4, 51.1, 36.1, 33.2, 30.6, 28.3 (2C), 25.0, 24.3. HRMS m/z calculated for $C_{22}H_{26}N_3O_4$ (MH+): 396.1918, Found: 396.1915.

Part ii)

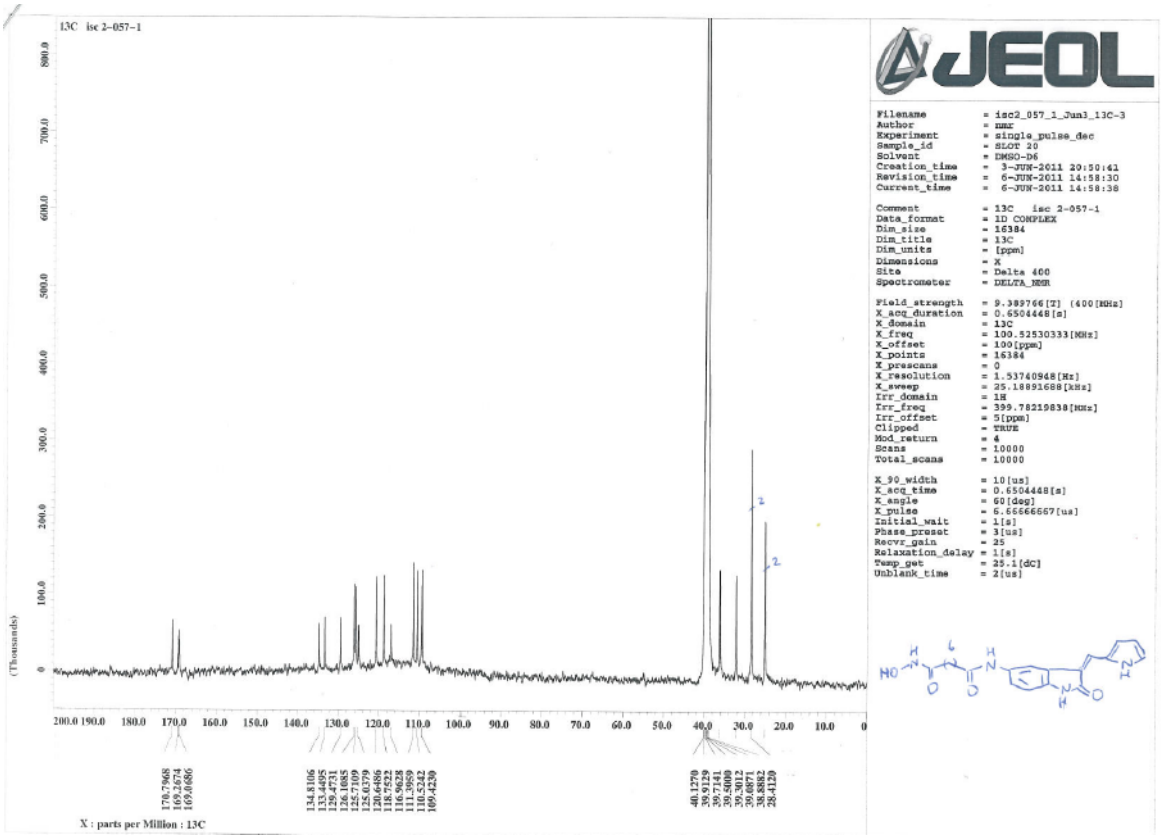


The previous methyl ester (250 mg, 0.63 mmol), was taken up in a mixture of MeOH: THF: 50% aqueous NH_2OH (5 mL:5 mL:2.5 mL). To this was added potassium cyanide[7] (1 equiv., 41 mg) and the reaction mixture was stirred at room temperature for 7 days. Volatiles were removed in *vacuo*, to the residue was added water, the resulting precipitate was filtered, washed with water, air-dried to give a bright yellow solid (210 mg, 84%).

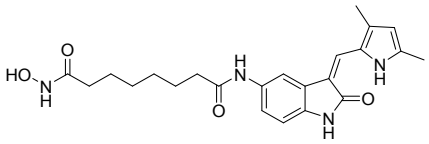
^{1}H NMR (dmso-$_{d6}$): 13.34 (1H, s), 10.81 (1H, s), 10.34 (1H, s), 9.76 (1H, s), 8.67 (1H, s), 7.96 (1H, s), 7.60 (1H, s), 7.36 (1H, s), 7.17 (1H, d, J = 8.6 Hz), 6.92 (1H, s), 6.80 (1H, d, J = 8.2 Hz), 6.36-6.34 (1H, m), 2.30-2.25 (2H, m), 1.97-1.92 (2H, m), 1.61-1.46 (4H, m), 1.34-1.24 (4H, m). ^{13}C NMR (dmso-d6): 170.8, 169.3, 169.1, 134.8, 133.4, 129.5, 126.1, 125.7, 125.0, 120.6, 118.8, 117.0, 111.4, 110.5, 109.4, 36.2, 32.2, 28.4 (2), 25.1 (2). HRMS: found 397.1871, theoretical 397.1870: LCMS purity >97%. Elemental Analysis CHN (%) calcd for $C_{21}H_{24}N_4O_4$ 0.5 H_2O: C, 62.4; H, 6.2; N, 13.6; found: C 62.2, H 6.2, N, 13.8.



^{1}H NMR (500 MHz, DMSO-d_6) δ 13.33 (s, 1H), 10.78 (s, 1H), 10.31 (s, 1H), 9.72 (s, 1H), 8.63 (s, 1H), 7.94 (d, J = 2.1 Hz, 1H), 7.59 (s, 1H), 7.34 (s, 1H), 7.16 (dd, J = 8.2, 2.1 Hz, 1H), 6.91 (d, J = 3.6 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 6.35 (q, J = 2.6 Hz, 1H), 2.28 (t, J = 7.5 Hz, 2H), 1.94 (t, J = 7.4 Hz, 2H), 1.66 – 1.55 (m, 2H), 1.50 (t, J = 7.3 Hz, 2H), 1.29 (dq, J = 12.7, 6.8, 6.3 Hz, 4H).

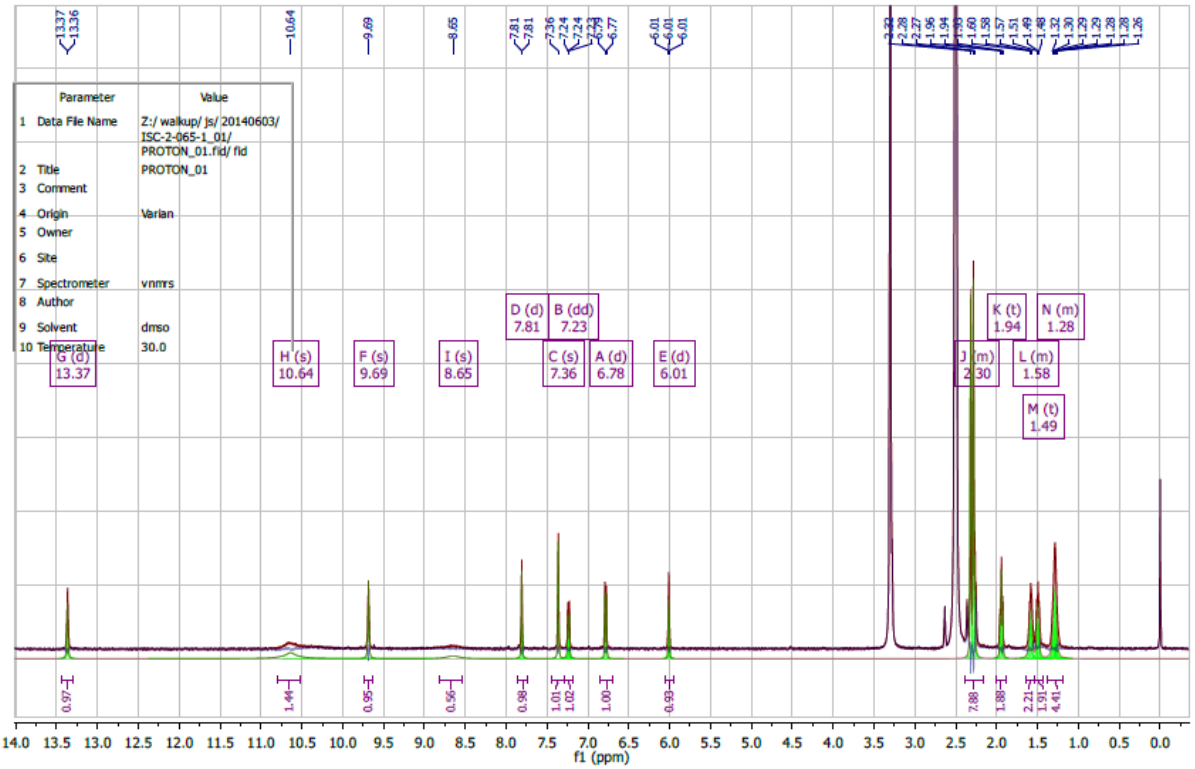


(Z)-N1-(3-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxoindolin-5-yl)-N8-hydroxyoctanediamide **7**.

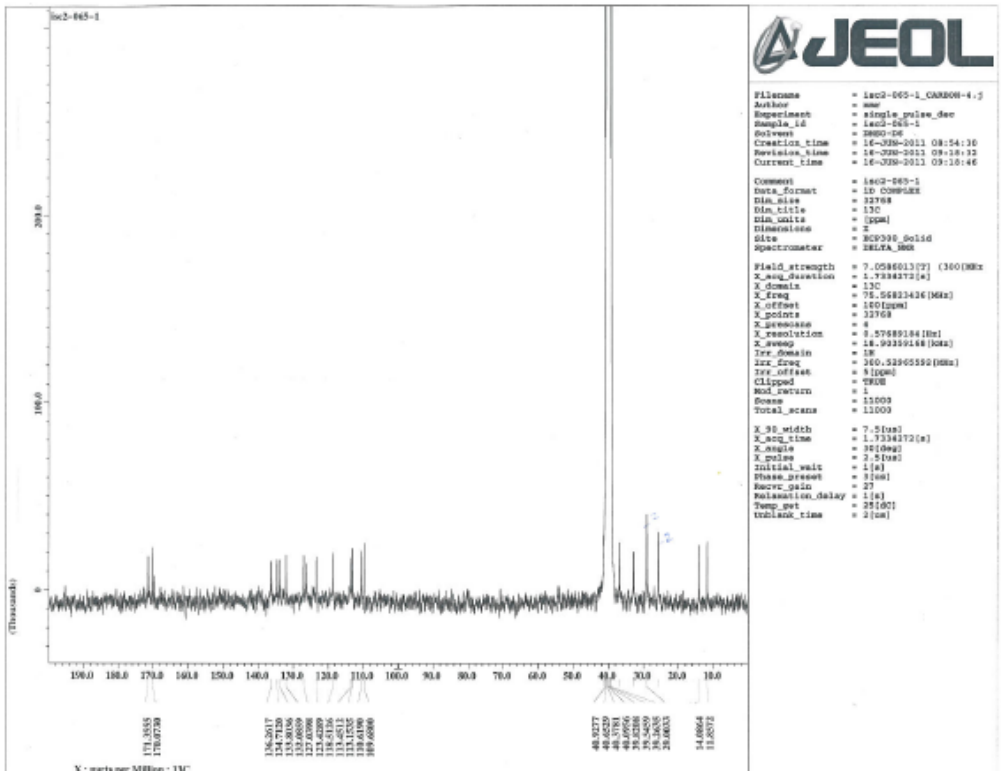


Methyl ester (*Z*)-methyl 8-(3-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-2-oxoindolin-5-ylamino)-8-oxooctanoate (125 mg, 0.30 mmol), synthesised as above, was taken up in a mixture of MeOH:THF:50% aqueous NH_2OH (3 mL:3 mL:1.5 mL). To this was added potassium cyanide (1 equiv., 19 mg) and the reaction mixture was stirred at room temperature for 3 days. Volatiles were removed in *vacuo*, to the residue added water, the resulting precipitate was filtered, washed with water, air-dried to give a bright yellow solid (110 mg).

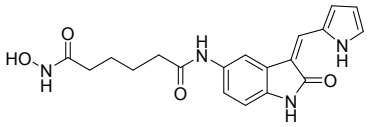
^{1}H NMR (dmso-d6): 13.38 (1H, s), 10.71 (1H, br s), 10.35 (1H, br s), 9.71 (1H, s), 8.69 (1H, br s), 7.82 (1H, s), 7.37 (1H, s), 7.24 (1H, d, J = 8.20 Hz), 6.78 (1H, d, J = 8.20 Hz), 6.02 (1H, s), 2.32 (3H, s), 2.29 (3H, s), 2.30-2.25 (2H, m), 1.98-1.94 (2H, m), 1.65-1.48 (4H, m), 1.35-1.28 (4H, m). ^{13}C NMR (dmso-d6): 171.4, 170.1, 169.3, 136.3, 134.7, 133.8, 132.1, 127.0, 126.2, 123.4, 118.5, 113.4, 113.1, 110.6, 109.7, 36.8, 32.7, 29.0 (2), 25.8 (2), 14.1, 11.8. MS: MH+ 425.3 (100%). HRMS: found 425.2184, theoretical 425.2183. LC purity > 96%. Elemental Analysis CHN (%) calcd for $C_{23}H_{28}N_4O_4.0.3\ CHCl_3$: C 60.8, H 6.2, N 12.2; found: C 60.9, H 6.6, N 12.2.



^{1}H NMR (500 MHz, DMSO-d_6) δ 13.37 (d, J = 2.6 Hz, 1H), 10.64 (s, 1H), 9.69 (s, 1H), 8.65 (s, 1H), 7.81 (d, J = 2.1 Hz, 1H), 7.36 (s, 1H), 7.23 (dd, J = 8.2, 2.0 Hz, 1H), 6.78 (d, J = 8.3 Hz, 1H), 6.01 (d, J = 2.7 Hz, 1H), 2.38 – 2.17 (m, 8H), 1.94 (t, J = 7.4 Hz, 2H), 1.65 – 1.53 (m, 2H), 1.49 (t, J = 7.2 Hz, 2H), 1.37 – 1.19 (m, 4H).

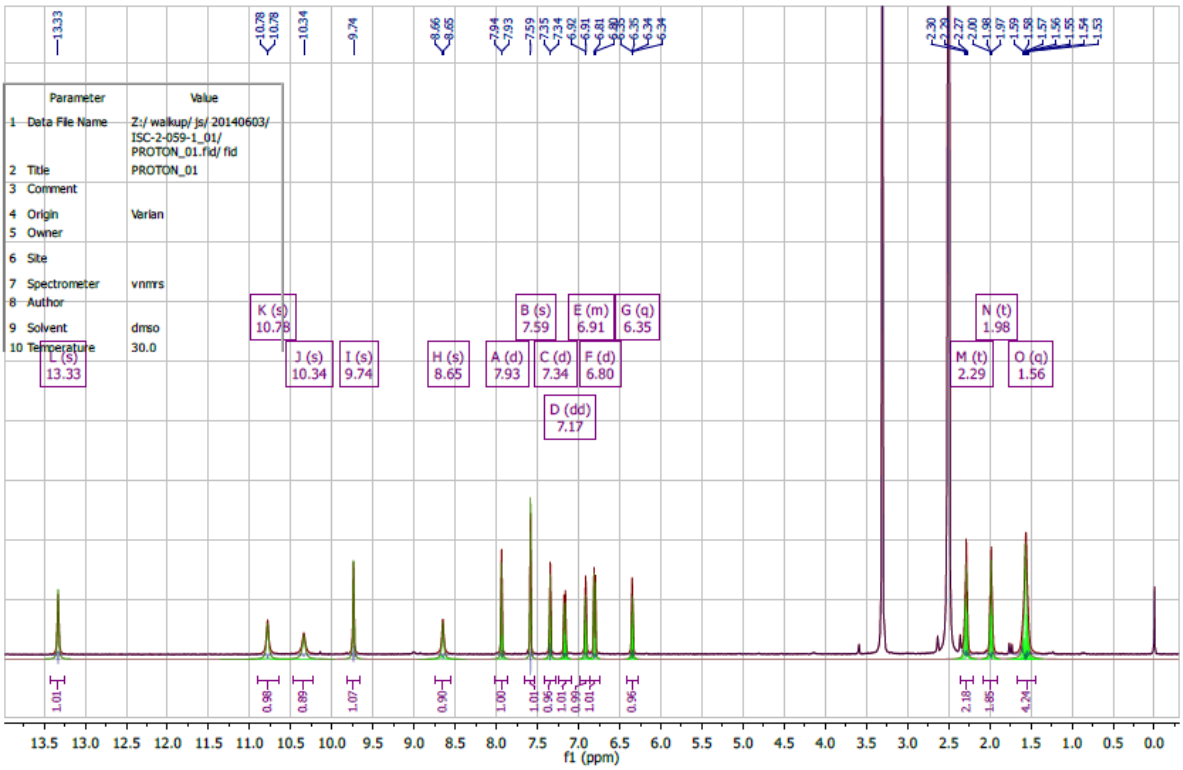


(Z)-N1-(3-((1H-pyrrol-2-yl)methylene)-2-oxoindolin-5-yl)-N6-hydroxyadipamide **8**

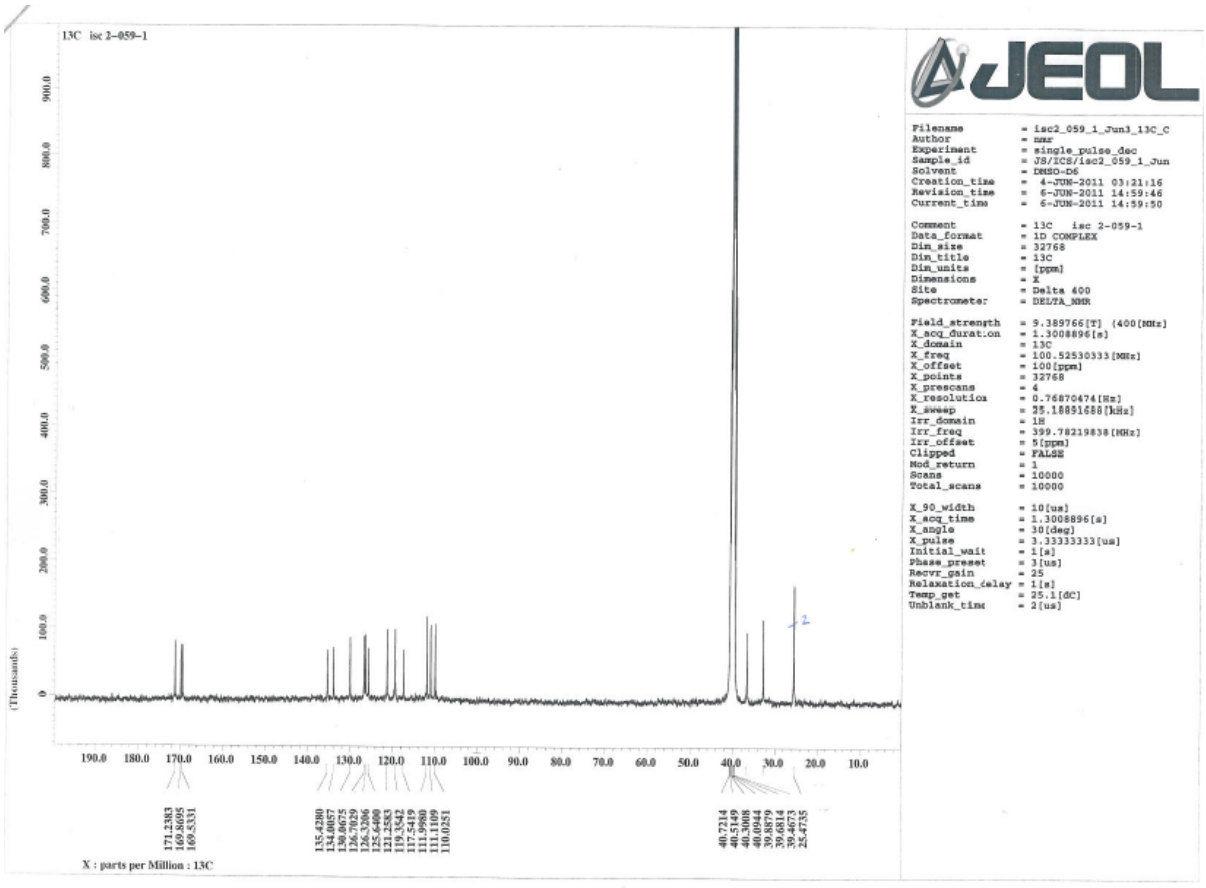


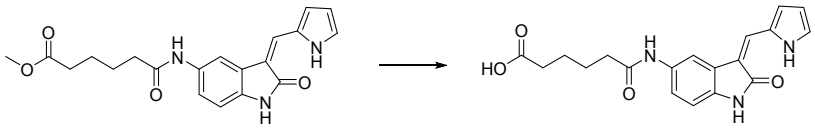
Methyl ester, (Z)-methyl 6-(3-((1H-pyrrol-2-yl)methylene)-2-oxoindolin-5-ylamino)-6-oxohexanoate (220 mg, 0.60 mmol), synthesised as above, was taken up in a mixture of MeOH :THF : 50% aqueous NH_2OH (5mL : 5mL : 2.5 mL). To this was added potassium cyanide (1 equiv., 39 mg) and the reaction mixture was stirred at room temperature for 5 days. Volatiles were removed in *vacuo*, to the residue added water, the resulting precipitate was filtered, washed with water, air-dried to give a bright yellow solid (190 mg).

^{1}H NMR (dmso-d6): 13.34 (1H, s), 10.81 (1H, s), 10.37 (1H, s), 9.77 (1H, s), 8.69 (1H, s), 7.95 (1H, s), 7.60 (1H, s), 7.36 (1H, s), 7.16 (1H, dd, J = 8.20, 1.95 Hz), 6.92 (1H, s), 6.80 (1H, d, J = 8.20 Hz), 6.36-6.34 (1H, m), 2.32-2.28 (2H, m), 2.00-1.97 (2H, m), 1.60-1.40 (4H, m). ^{13}C NMR (dmso-d6): 171.2, 169.9, 169.5, 135.4, 134.0, 130.1, 126.7, 126.3, 125.6, 121.3, 119.3, 117.5, 112.0, 111.1, 110.0, 36.2, 32.2, 25.5 (2). MS: MH+ 369.2 (100%). HRMS: found 369.1561, theoretical 369.1557. LC purity >97%. Elemental Analysis CHN (%) calcd for $C_{19}H_{20}N_4O_4$ 0.2 $CHCl_3$: C 58.8, H 5.2, N 14.3; found: C 59.0, H 5.5, N 14.4.



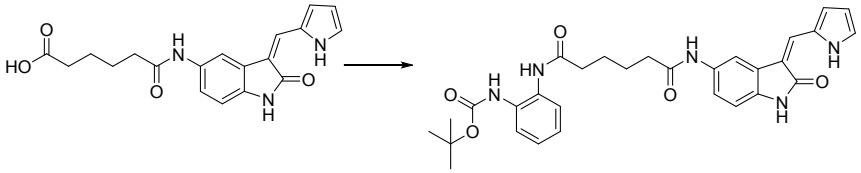
^{1}H NMR (500 MHz, DMSO-d_6) δ 13.33 (s, 1H), 10.78 (s, 1H), 10.34 (s, 1H), 9.74 (s, 1H), 8.65 (s, 1H), 7.93 (d, J = 2.1 Hz, 1H), 7.59 (s, 1H), 7.34 (d, J = 2.8 Hz, 1H), 7.17 (dd, J = 8.2, 2.1 Hz, 1H), 6.98 – 6.86 (m, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.35 (q, J = 2.5 Hz, 1H), 2.29 (t, J = 6.6 Hz, 2H), 1.98 (t, J = 6.8 Hz, 2H), 1.56 (q, J = 6.9, 5.7 Hz, 4H).





The methyl ester (305 mg, 0.83 mmol) was suspended in THF (5 mL) and MeOH (1 mL), to this was added a solution of sodium hydroxide (2 equiv., 1.66 mmol, 67 mg) in water (1 ml). The reaction mixture was stirred at room temperature overnight. After that, the organic volatiles were removed *in vacuo*, the reaction mixture was diluted with water and acidified with conc. HCl to pH ~ 2-3. A precipitate formed which was filtered under vacuum, washed with water and air-dried to give the expected product as a brown solid (275 mg).

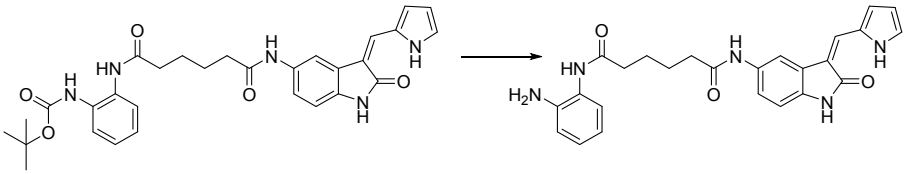
^{1}H NMR (dmso-d6): 13.34 (1H, s), 12.04 (1H, br s), 10.82 (1H, s), 9.79 (1H, s), 7.95 (1H, s), 7.60 (1H, s), 7.34 (1H, s), 7.14 (1H, d, J = 8.20 Hz), 6.92 (1H, s), 6.80 (1H, d, J = 8.2 Hz), 6.35 (1H, s), 2.32-2.22 (4H, m), 1.65-1.50 (4H, m). MS (ESI, MH^+) *m/z* 354.2 (25%)



The above carboxylic acid (275 mg, 0.75 mmol) and *N*-BOC-*o*-phenylenediamine (0.82 mmol, 171 mg) were dissolved in CH_2Cl_2 (6 ml), to this Et_3N (4.50 mmol, 0.63 ml) was added and the mixture was cooled in ice bath. Next, propane phosphonic acid anhydride T_3P (50% solution in DMF, 1.125 mmol, 0.73 ml) was added and the reaction mixture was allowed to warm up to room temperature overnight. Then the mixture was poured into saturated solution of K_2CO_3, stirred for 30 min and extracted into CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered and evaporated *in vacuo*. The residue was purified by ISCO Combiflash purification unit (10 – 30% MeOH in CH_2Cl_2) to give the expected product as an orange solid (175 mg).

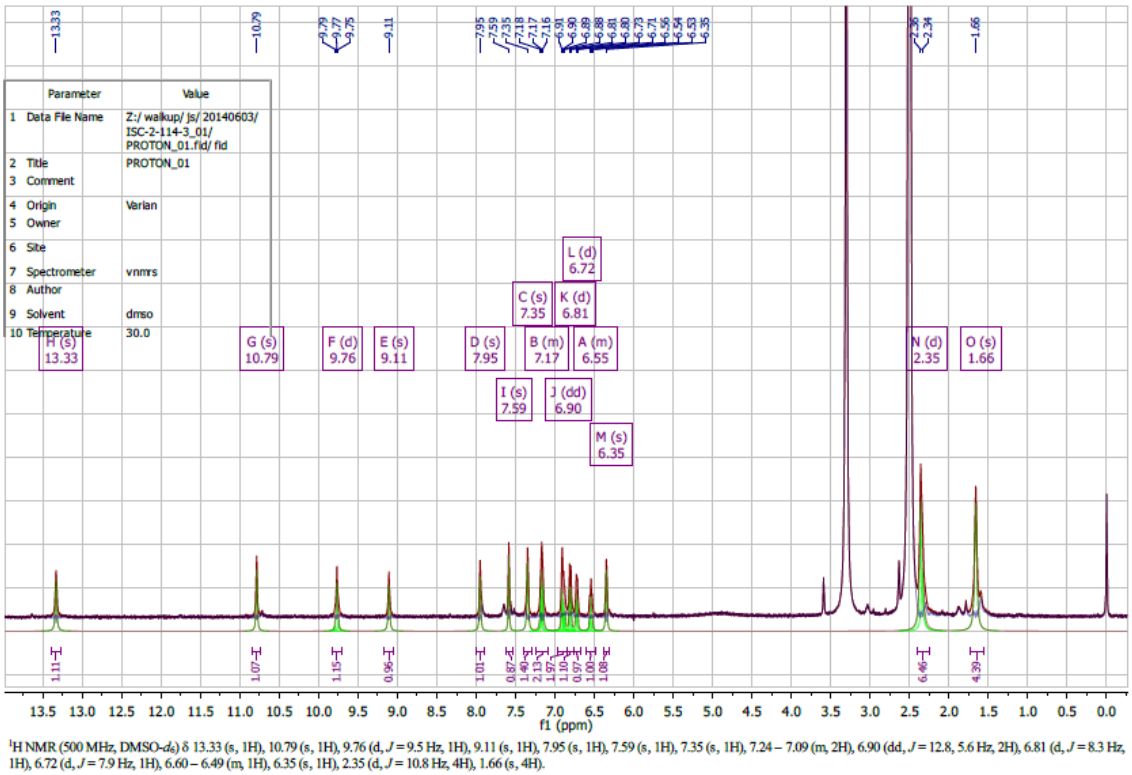
^{1}H NMR (dmso-d6): 13.48 (1H, s), 10.82 (1H, s), 9.81 (1H, s), 9.75 (1H, s), 8.35 (1H, s), 7.96 (1H, s), 7.58 (1H, s), 7.52 (1H, d, J = 8.6 Hz), 7.41 (1H, d, J = 7.8 Hz), 7.36 (1H, s), 7.22-7.03 (3H, m), 6.92 (1H, s), 6.80 (1H, d, J = 10.6 Hz), 6.35 (1H, s), 2.45-2.28 (4H, m), 1.70-1.55 (4H, m), 1.45 (9H, s). MS (ESI, MH^+) *m/z* 544.2 (30%)

Synthesis of (Z)-N1-(3-((1H-pyrrol-2-yl)methylene)-2-oxoindolin-5-yl)-N6-(2-aminophenyl)adipamide **9**

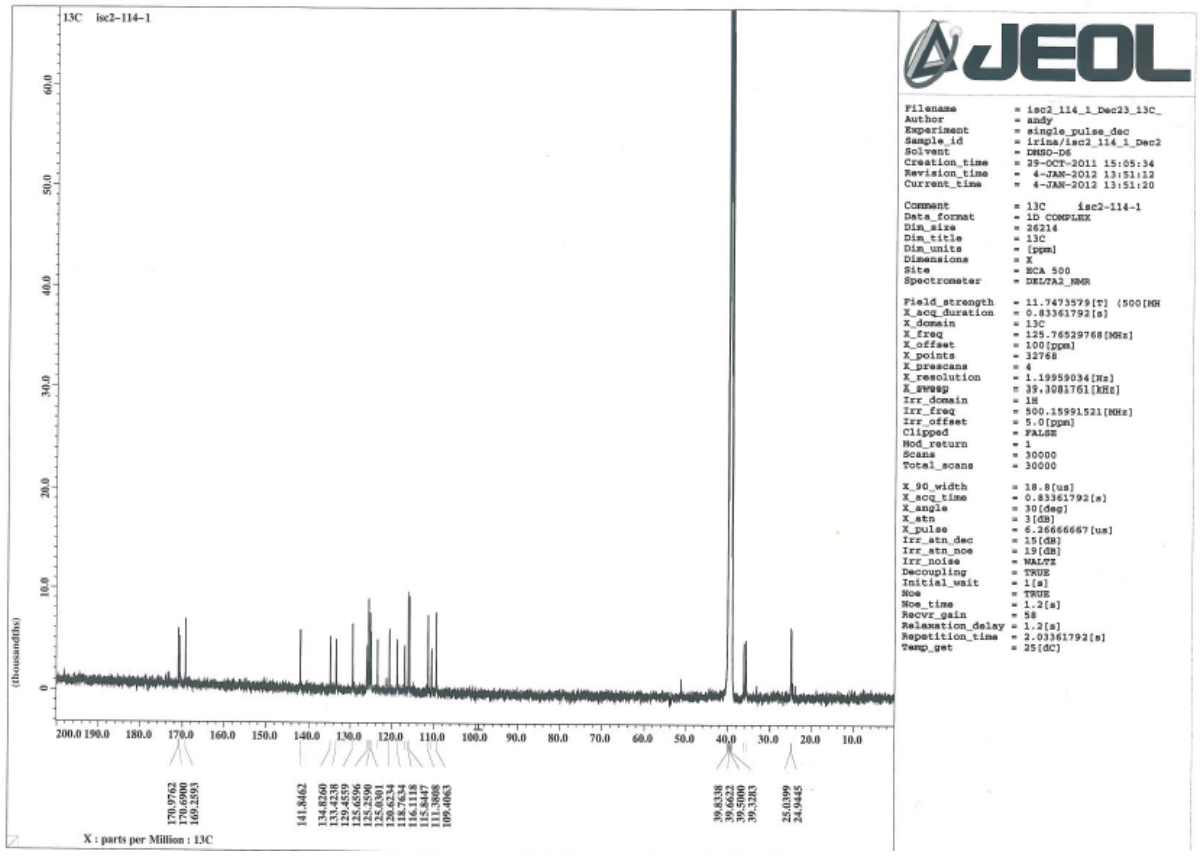


The Boc-protected compound (175 mg, 0.31 mmol) was suspended in CH_2Cl_2 (10 mL) and MeOH (1 mL). To this mixture 4N HCl/dioxane (2 ml) was added and stirred at room temperature overnight. The volatiles were removed *in vacuo*, then a saturated solution of sodium carbonate was added to the residue and sonicated. The precipitate was collected by suction and washed on the frit with water, dried, triturated with hot CH_2Cl_2 to give the title compound as an orange solid (94 mg, 87%).

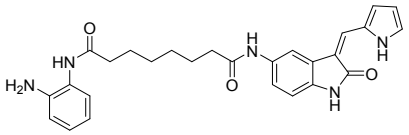
^{1}H NMR (dmso-d6): 13.34 (1H, s), 10.82 (1H, s), 9.80 (1H, s), 9.14 (1H, s), 7.96 (1H, s), 7.60 (1H, s), 7.36 (1H, s), 7.17-7.14 (2H, m), 6.92-6.86 (2H, m), 6.80 (1H, d, J = 8.2 Hz), 6.72 (1H, d, J = 7.4 Hz), 6.53 (1H, t, J = 7.4 Hz), 6.35 (1H, s), 5.00-4.80 (2H, br, NH_2), 2.40-2.25 (4H, m), 1.70-1.57 (4H, m). ^{13}C NMR (dmso-d6): 171.0, 170.7, 169.3, 141.8, 134.8, 133.4, 129.4, 126.1, 125.7, 125.3, 125.0, 123.5, 120.6, 118.8, 117.0, 116.1, 115.8, 111.4, 110.5, 109.4, 36.1, 35.6, 25.0, 24.9 (one quaternary C is invisible). MS (ESI, MH^+) *m/z* 444.3 (100%). HRMS (ESI, MH^+) *m/z* 444.2030, (calcd for $C_{25}H_{26}N_5O_3$ 444.2030). Elemental Analysis CHN (%) found: C64.0, H 5.6, N 14.3; calcd for $C_{25}H_{26}N_5O_3$ .0.4CH_2Cl_2: C 63.9, H 5.5, N 14.6.



^{1}H NMR (500 MHz, DMSO-d_6) δ 13.33 (s, 1H), 10.79 (s, 1H), 9.76 (d, J = 9.5 Hz, 1H), 9.11 (s, 1H), 7.95 (s, 1H), 7.59 (s, 1H), 7.35 (s, 1H), 7.24 – 7.09 (m, 2H), 6.90 (dd, J = 12.8, 5.6 Hz, 2H), 6.81 (d, J = 8.3 Hz, 1H), 6.72 (d, J = 7.9 Hz, 1H), 6.60 – 6.49 (m, 1H), 6.35 (s, 1H), 2.35 (d, J = 10.8 Hz, 4H), 1.66 (s, 4H).



(*Z*)-*N*1-(3-((*1H*-pyrrol-2-yl)methylene)-2-oxoindolin-5-yl)-*N8*-(2-aminophenyl)octanediamide **10**

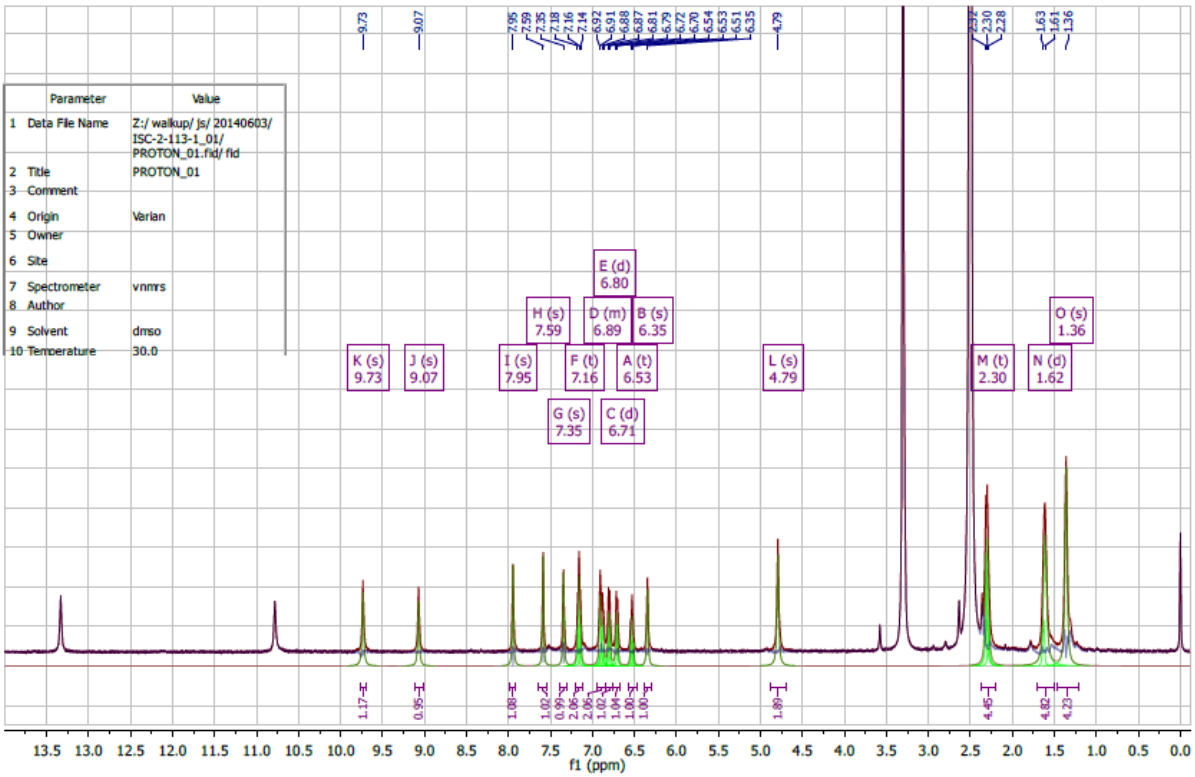


The Boc-protected compound, synthesised as above, (135 mg, 0.24 mmol) was suspended in CH_2Cl_2 (10 mL) and MeOH (1 mL). To this mixture 4N HCl/dioxane (2 mL) was added and stirred at room temperature overnight. The volatiles were removed *in vacuo*, then a saturated solution of sodium carbonate was added to the residue and sonicated. The precipitate was collected by suction and washed on the frit with water, dried, triturated with hot CH_2Cl_2 to give the title compound as an orange solid (108 mg, 95%).

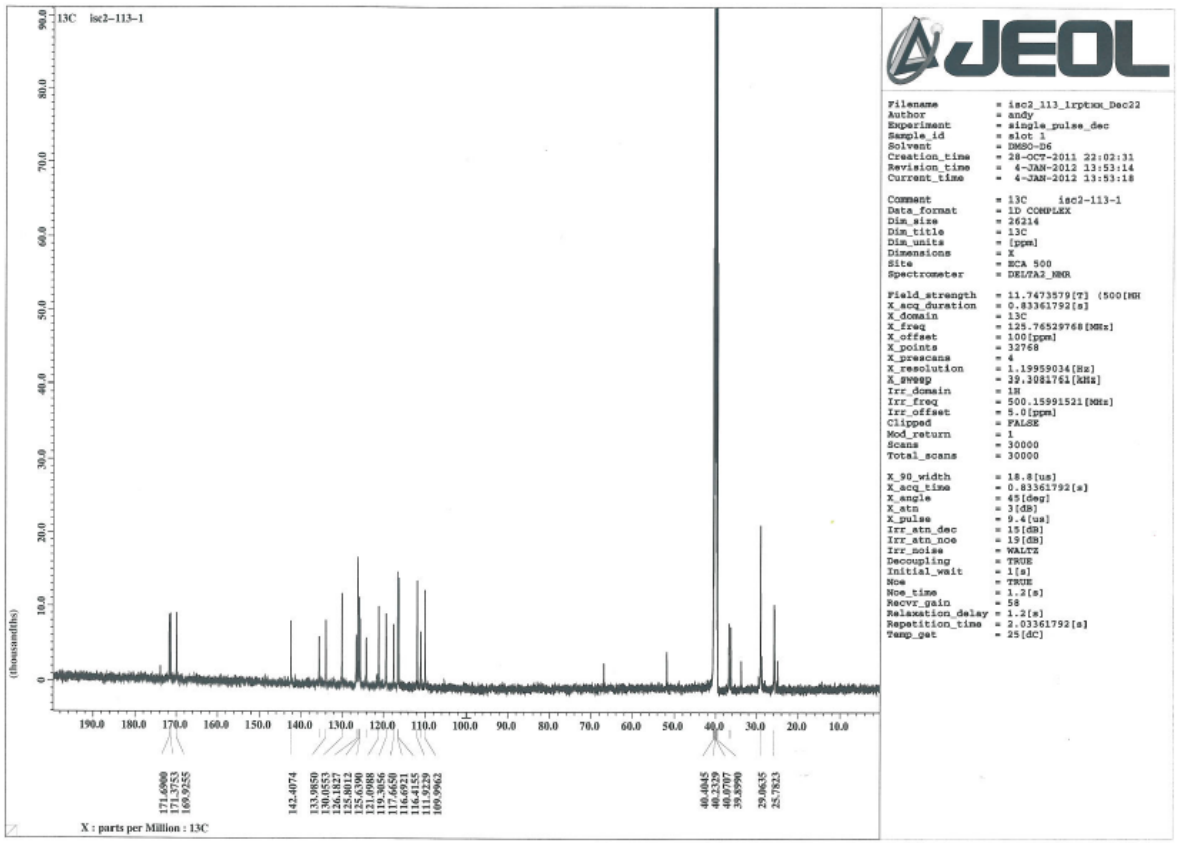
^{1}H NMR (dmso-d6): 13.34 (1H, s), 10.82 (1H, s), 9.77 (1H, s), 9.10 (1H, s), 7.96 (1H, s), 7.60 (1H, s), 7.36 (1H, s), 7.17-7.14 (2H, m), 6.92-6.86 (2H, m), 6.80 (1H, d, J = 8.2 Hz), 6.71 (1H, d, J = 7.4 Hz), 6.53 (1H, t, J = 7.4 Hz), 6.35 (1H, s), 4.82 (2H, s, NH_2), 2.32-2.28 (4H, m), 1.62-1.57 (4H, m), 1.36-1.30 (4H, m). ^{13}C NMR (dmso-d6): 171.7, 171.4, 169.9, 142.4, 135.6, 134.0, 130.1, 126.6, 126.2, 125.8, 125.6, 124.1, 121.1, 119.3, 117.7, 116.7, 116.4, 111.9, 111.0, 110.0, 36.8, 36.3, 29.0 (2C), 25.8 (2C) (one quaternary C is invisible).

MS (ESI, MH^+) *m/z* 472.3 (100%). HRMS (ESI, MH^+) *m/z* 472.2339, (calcd for $C_{27}H_{30}N_5O_3$ 472.2343).

Elemental Analysis CHN (%) found: C 68.8, H 6.3, N 14.7; calcd for $C_{27}H_{29}N_5O_3$: C 68.8, H 6.2, N 14.9.

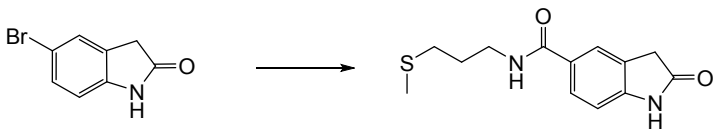


^{1}H NMR (500 MHz, DMSO-d_6) δ 9.73 (s, 1H), 9.07 (s, 1H), 7.95 (s, 1H), 7.59 (s, 1H), 7.35 (s, 1H), 7.16 (t, J = 8.8 Hz, 2H), 6.96 – 6.84 (m, 2H), 6.80 (d, J = 8.3 Hz, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.53 (t, J = 7.6 Hz, 1H), 6.35 (s, 1H), 4.79 (s, 2H), 2.30 (t, J = 8.6 Hz, 4H), 1.62 (d, J = 9.5 Hz, 4H), 1.36 (s, 4H).



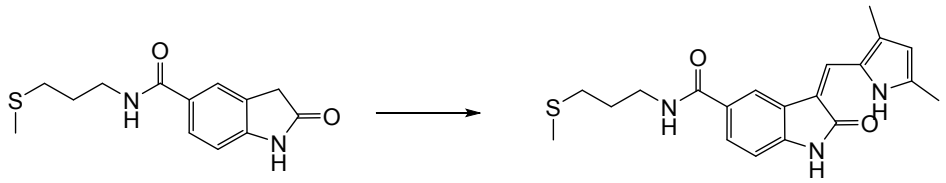
(Z)-3-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(3-(methylthio)propyl)-2-oxoindoline-5-carboxamide **13**

Part i)

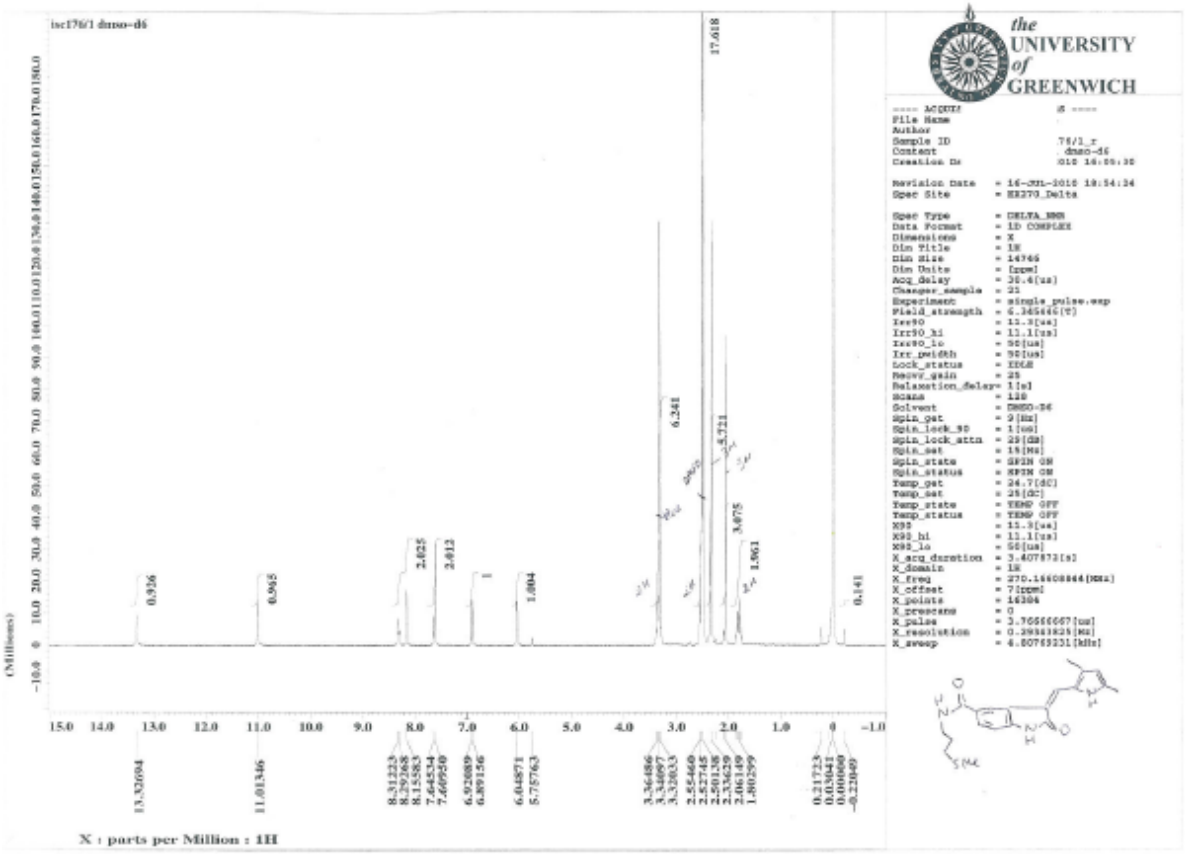


5-Bromo-1,3-dihydro-2*H*-indol-2-one (100 mg, 0.47 mmol), 3-(methylthio)propylamine (1.4 equiv., 69 mg), DBU (0.67 equiv., 48 mg), *trans*-di-µ-acetatobis[2-(di-o-tolyl-phosphino)-benzyl]di-palladium(II) (0.01 equiv., 4.7 mg), tri-*tert*-butylphosphonium tetrafluoroborate (0.03 equiv., 3.9 mg) and molybdenum hexacarbonyl (0.5 equiv., 59 mg) were heated in THF (2 ml) in microwave (150^{o}C, 45 min). DCM/brine extraction and purification by Combiflash afforded the title compound as a beige solid (33 mg). ^{1}H NMR ($CDCl_3$): 8.17 (1H, br s), 7.68-7.62 (2H, m), 6.88 (1H, d), 6.39 (1H, br s), 3.60-3.52 (4H, m), 2.59 (2H, t), 2.10 (3H, s), 1.94 (2H, t,). MS: MH+ 265.1

ii)



The above product (33 mg, 0.125 mmol), 3,5-dimethyl-1*H*-pyrrole-2-carboxaldehyde (1.2 equiv, 18.5 mg) and piperidine (cat.) were heated in EtOH (1 ml) in microwave (150°C, 30 min). The reaction mixture was cooled to room temperature and diluted with diethyl ether. The precipitate was filtered, washed with diethyl ether, air-dried to give the product as an orange crystalline solid (35 mg). ^{1}H NMR (dmso-d6): 13.32 (1H, s), 11.01 (1H, s), 8.31 (1H, br t), 8.16 (1H, s), 7.64-7.60 (2H, m), 6.90 (1H, d), 6.05 (1H, s), 3.36-3.30 (2H, m, under H_2O peak), 2.55-2.50 (2H, m, under DMSO peak), 3H under DMSO peak, 2.33 (3H, s), 2.06 (3H, s), 1.80 (2H, m). ^{1}H NMR ($CDCl_3$): 13.06 (1H, br s), 7.98 (1H, s), 7.80 (1H, s), 7.47-7.44 (2H, m), 6.89 (1H, d), 6.43 (1H, br s), 6.00 (1H, s), 3.62-3.55 (2H, m), 2.62 (2H, t), 2.37 (3H, s), 2.33 (3H, s), 2.12 (3H, s), 2.00-1.90 (2H, m). ^{13}C NMR (dmso-d6): 170.3, 167.1, 140.7, 136.9, 132.9, 128.1, 127.3, 126.1, 125.9, 124.4, 117.4, 113.4, 112.4, 109.2, 31.4 (2), 29.4, 15.2, 14.1, 11.9. MS: MH+ 370.2. HRMS: found 370.1586, theoretical 370.1584. Elemental Analysis CHN (%) calcd for $C_{20}H_{23}N_3O_2S$ + 0.355 moles DCM: C 61.2; H 6.0; N 10.5; found: C 61.2, H 6.0, N 10.3.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6.241
5.721
3.675
1.861
0.926
0.965
2.025
2.013
1
1.004
0.161
2H
2H
1H
3H
2H
13.32694
11.01346
8.31223
8.29268
8.15583
7.64534
7.60950
6.92689
6.89156
6.04871
5.75763
3.36486
3.34697
3.32033
2.55460
2.52745
2.50138
2.33629
2.06149
1.80299
0.21723
0.03041
0.00000
-0.22049
X : parts per Million : 1H

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File Name
Author
Sample ID T6/1_r
Content dmso-d6
Creation Dr :010 16:55:30
Revision Date = 16-JUL-2010 10:54:34
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Irr90_hi = 11.1[us]
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Irr_pwidth = 50[us]
Lock_status = IDLE
Recvr_gain = 25
Relaxation_delay= 1[s]
Scans = 128
Solvent = DMSO-D6
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Spin_set = 15[Hz]
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Temp_status = TEMP OFF
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X90_hi = 11.1[us]
X90_lo = 50[us]
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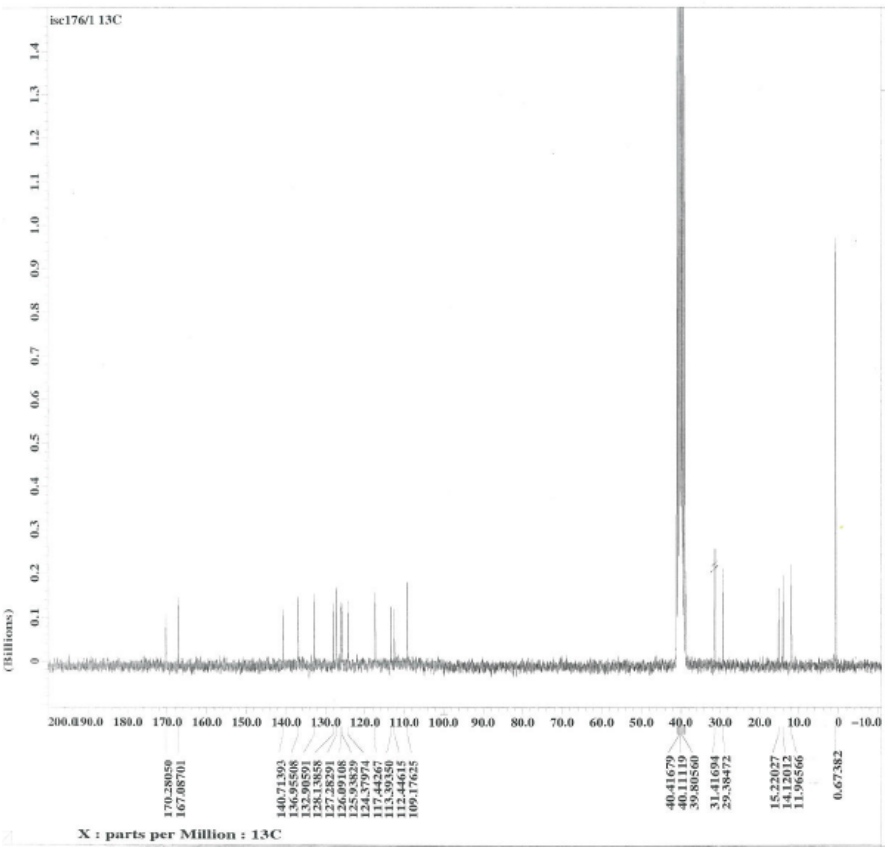
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128.13858
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126.60168
125.93826
124.37974
117.44267
113.39350
112.44615
109.17625
40.41679
40.11119
39.80560
31.41694
29.38472
15.22027
14.12012
11.96566
0.67382
X : parts per Million : 13C


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Relaxation_delay= 1[s]
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Spin_lock_90 = 1[us]
Spin_lock_attn = 29[dB]
Spin_set = 15[Hz]
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Spin_status = SPIN ON
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Temp_set = 25[dC]
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Temp_status = TEMP OFF
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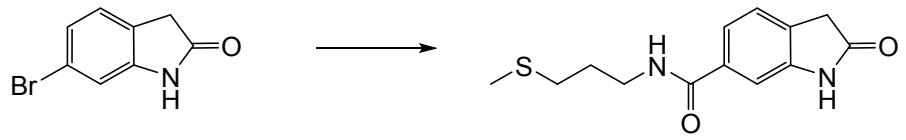
Synthesis of (Z)-3-((3,5-dimethyl-1H-pyrrol-2-yl)methylene)-N-(3-(methylthio)propyl)-2-oxoindoline-6-carboxamide **14**

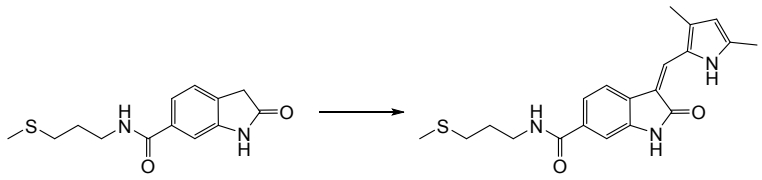
Part i)



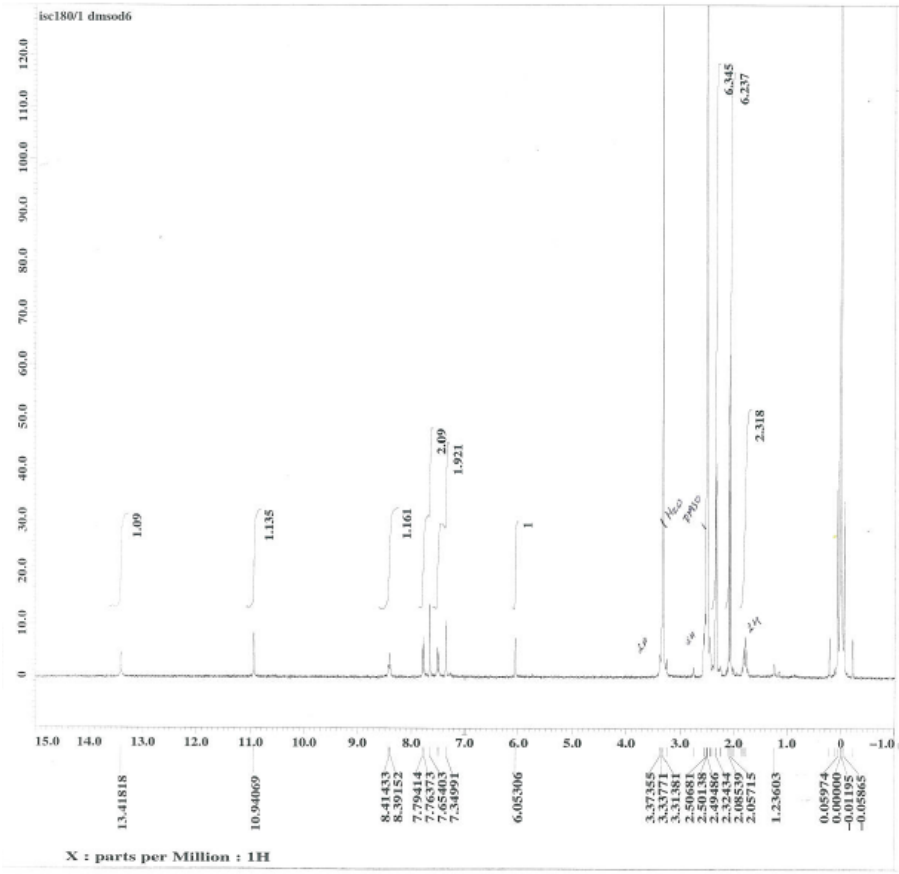
6-Bromo-1,3-dihydro-2*H*-indol-2-one (100 mg, 0.47 mmol), 3-(methylthio)propylamine (1.4 equiv., 69 mg), DBU (0.67 equiv., 48 mg), *trans*-di-μ-acetatobis[2-(di-o-tolyl-phosphino)-benzyl]di-palladium(II) (0.01 equiv., 4.7 mg), tri-tert-butylphosphonium tetrafluoroborate (0.03 equiv., 3.9 mg) and molybdenum hexacarbonyl (0.5 equiv., 59 mg) were heated in THF (2 ml) in a microwave (170°C, 30 min). DCM/brine extraction and purification by Combiflash afforded the title compound as an off-white solid (33 mg).

^{1}H NMR (dmso-d6): 10.60 (1H, s), 8.48 (1H, t), 7.57 (1H, d), 7.44 (1H, d), 7.27 (1H, s), 3.35-3.28 (4H, m), 2.53-2.47 (2H, under DMSO peak), 2.05 (3H, s), 1.78 (2H, m). MS: (MH+40) 305.1, MH+ 265.1 (5%).

Part ii)



N-(3-(Methylthio)propyl)-2-oxoindoline-6-carboxamide, made as above, (30 mg, 0.12 mmol), 3,5-dimethyl-1*H*-pyrrole-2-carboxaldehyde (1.2 equiv, 18 mg) and piperidine (cat.) were heated in EtOH (1 ml) in microwave (150°C, 30 min). Volatiles were removed *in vacuo*, and the residue was purified by Combiflash (30% EtOAc/DCM) to give a yellow solid (10 mg). ^{1}H NMR (dmso-d6): 13.42 (1H, br s), 10.94 (1H, s), 8.40 (1H, br t), 7.78 (1H, d), 7.65 (1H, s), 7.51 (1H, d), 7.35 (1H, s), 6.05 (1H, s), 3.37-3.31 (2H, under H_2O peak), 2.53-2.47 (2H, under DMSO peak), 2.32 (3H, s), 2.08 (3H, s), 2.05 (3H, s), 1.80 (2H, m). ^{13}C NMR ($CDCl_3$): 170.1, 166.8, 138.2, 137.5, 133.5, 132.2, 129.3, 127.5, 125.3, 120.5, 117.9, 113.6, 112.3, 108.8, 31.4 (2), 29.3, 15.2, 14.2, 11.9. MS: MH+ 370.2. HRMS: found 370.1583, theoretical 370.1584. Elemental Analysis CHN (%) calcd for $C_{20}H_{23}N_3O_2S$ + 0.095 moles of chloroform: C 63.4, H 6.1, N 11.0; found: C 63.4, H 6.1 (small amount).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1.135
1.161
2.09
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6.237
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13.41818
10.94069
8.41433
8.39152
7.79414
7.76373
7.65403
7.54991
6.05306
3.37355
3.33771
3.31381
2.50681
2.50138
2.49486
2.32434
2.08539
2.05715
1.23603
0.05974
0.00000
−0.01195
−0.05865
X : parts per Million : 1H


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Author
Sample ID
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Creation Da :010 14:52:52
Revision Date = 21-JUL-2010 17:44:32
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Temp_set = 25[dC]
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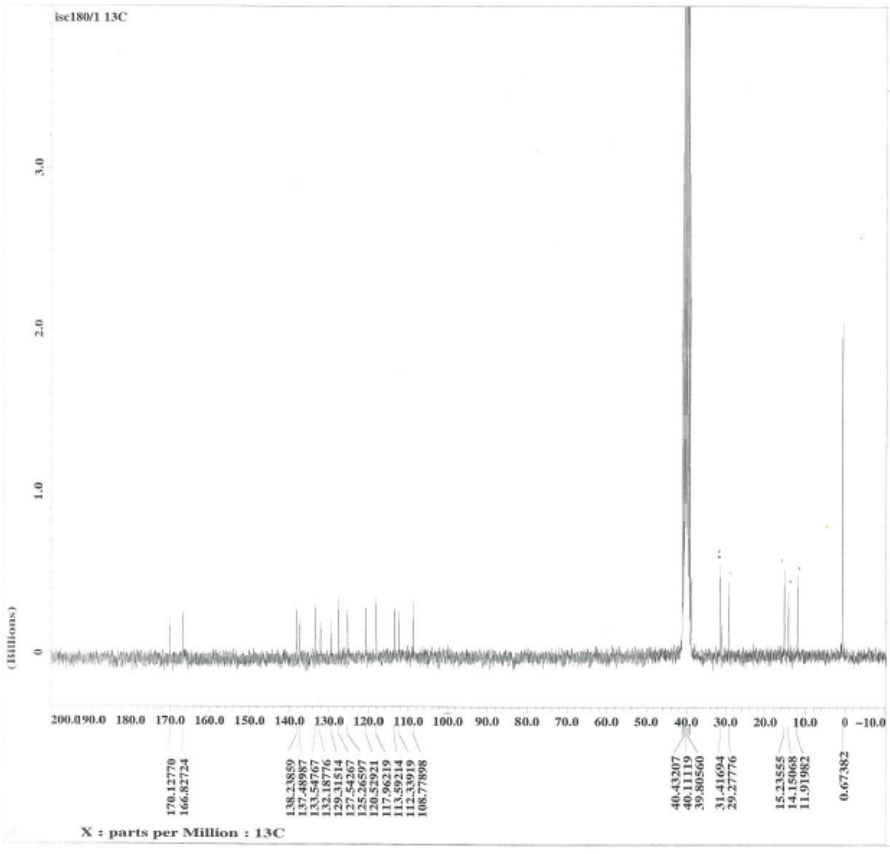
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39.80560
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X : parts per Million : 13C


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Author
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Experiment = single_pulse_dec
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Spin_set = 15[Hz]
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Spin_status = SPIN ON
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Temp_set = 25[dC]
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Temp_status = TEMP OFF
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X90_hi = 8.6[us]
X90_lo = 50[us]
X_acq_duration = 0.9633792[s]
X_domain = 13C
X_freq = 67.93330993[MHz]
X_offset = 100[ppm]
X_points = 16384
X_prescans = 0
X_pulse = 5.33333333[us]
X_resolution = 1.03801286[Hz]
X_sweep = 17.00680272[kHz]

Biochemical Kinase/HDACi Assays, performed by Reaction Biology

(http://www.reactionbiology.com/webapps/site/).

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