Supporting Information

Differential Induction of Cytoplasmic Vacuolization and Methuosis by Novel 2-Indolyl-Substituted Pyridinylpropenones

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Chemistry

General methods

Reactions were performed in washed and oven-dried glassware (≥ 110 ^{o}C) under an Ar (g) atmosphere. Reactions were stirred using a magnetic stirring apparatus with Teflon-coated stir bars. Reagents were obtained from either Acros Organics, Sigma-Aldrich, or VWR and purified when necessary according to instructions in Perrin's Purification of Laboratory Chemicals.[1] Anhydrous solvents were purchased from Sigma-Aldrich, and dried over 3 Å molecular sieves if necessary. All reagent grade solvents (acetone, DCM, MeOH, ethyl acetate, and hexane) were purchased from VWR. Proton (^{1}H) NMR and carbon (^{13}C) NMR were determined using either a Unity-400 spectrophotometer (400 mHz), Varian Inova-600 spectrophotometer (600 mHz) or a Bruker Avance spectrophotometer (600 mHz). Chemical shifts were reported in ppm (δ) and were referenced to TMS. The chemical shifts for ^{1}H NMR were reported to the second decimal place while ^{13}C chemical shifts were reported to the first decimal place. When appropriate, 2 decimal places were reported. The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q= quartet, m = multiplet, dd = doublet of doublets. Coupling constants (*J*) were reported in Hertz (Hz). Melting points were determined on an Electrothermal digital melting point apparatus and were uncorrected. Melting point determinations were performed in triplicate to ensure accurate measurement. Analytical TLC was performed on Baker-flex TLC plates (2.5 x 7.5 cm) with a 254 nm fluorescent indicator (IB-F). Plates were developed in a covered chamber, usually with 5-10 mL of mobile phase, and visualized by UV-light. Flash chromatography was performed using Fisher Silica Gel 60, 200-425 mesh (40-60 mm) as a stationary phase. Flash columns were packed as described in the literature[2] and generally require about 50 times the amount of compound to be purified. All synthesized compounds were at least 95% pure as determined by elemental analysis (Atlantic Microlabs, Norcross, GA). Results were within ±0.4 % of the theoretical values.

trans-3-(5-Methoxy-2-methylindol-3-yl)-1-(4-pyridinyl)-2-propen-1-one, 'MOMIPP' (**1**)

To a dried two-neck flask purged with N_2 (g), 5-methoxy-2-methylindole-3-carboxyaldehyde (232 mg, 1.22 mmol) was dissolved in anhydrous methanol (6 mL). 4-Acetylpyridine (222 mg, 1.83 mmol) and piperidine (103 mg, 1.22 mmol) were added and the mixture was allowed to reflux for 18 hrs. An orange precipitate slowly began to form. Upon completion, the orange precipitate was collected by vacuum filtration, washed with chilled methanol (50 mL), and dried for 36 hours *in vacuo* at 40 $^{\circ}$C yielding an orange solid (314 mg, 88%): TLC R_f 0.25 in ethyl acetate: hexane (4:1). Melting point = 256-259 $^{\circ}$C (lit.[3] 252-256 $^{\circ}$C). ^{1}H NMR (400 mHz, d_6-DMSO) δ 11.91 (s, 1H), 8.82-8.81 (d, 2H, *J* = 6 Hz), 8.11-8.08 (d, 1H, *J* = 15.6 Hz), 7.96-7.94 (d, 2H, *J* = 6 Hz), 7.44 (d, 1H, *J* = 2 Hz), 7.39-7.35 (d, 1H, *J* = 15.2 Hz), 7.33-7.30 (d, 1H, *J* = 8.4 Hz), 6.86-6.84 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 3.87 (s, 3H), 2.58 (s, 3H); ^{13}C NMR (150 MHz , d_6-DMSO) δ 187.9, 155.0, 150.5, 145.7, 144.9, 139.5, 130.8, 126.4, 121.3, 112.6, 112.1,

110.8, 109.2, 103.4, 55.4, 12.0; Elemental analysis calculated for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58 Found: C, 73.73; H, 5.54; N, 9.52.

trans-3-(5-Methoxy-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (**2**)

To a dried two-neck flask purged with Ar (g), 5-methoxy-indole-3-carboxyaldehyde (300 mg, 1.71 mmol) was dissolved in anhydrous methanol (15 mL). 4-Acetylpyridine (414 mg, 3.42 mmol) and piperidine (0.34 mL, 3.42 mmol) were added and the mixture was allowed to reflux for 18 hrs. A yellow precipitate slowly began to form. Upon completion, the precipitate was collected by vacuum filtration, washed with chilled methanol (40 mL), and dried for 24 hours *in vacuo* at 40 $^{\circ}$C under an oil-driven vacuum pump yielding a yellow powder (418 mg, 88%): TLC R_f 0.32 in ethyl acetate: hexane (4:1). Melting point = 236.5-239 $^{\circ}$C (lit.[3] 235-237 $^{\circ}$C). ^{1}H NMR (600 mHz, d_6-DMSO) δ 11.93 (s, 1H), 8.82 (m, 2H), 8.16 (s, 1H), 8.12-8.09 (d, 1H, J = 15.48 Hz), 7.95 (m, 2H), 7.53-7.51 (d,1H, J = 15.42 Hz), 7.49 (d, 1H, J = 2.34 Hz), 7.41-7.40 (d, 1H, J = 8.76 Hz), 6.91-6.89 (dd, 1H, J_1 = 8.76 Hz, J_2 = 2.4 Hz), 3.87 (s, 3H); ^{13}C NMR (150 MHz , d_6-DMSO) δ 188.3, 155.0, 150.5, 144.8, 140.8, 134.0, 132.2, 125.9, 121.4, 114.2, 113.1, 112.6, 112.2, 102.6, 55.5.

trans-3-(5-Methoxy-2-methylindol-3-yl)-1-(3-pyridinyl)-2-propen-1-one (**3**)

To a dried two-neck flask purged with N_2 (g), 5-methoxy-2-methylindole-3-carboxyaldehyde (150 mg, 0.793 mmol) was dissolved in anhydrous methanol (10 mL). 3-Acetylpyridine (144 mg, 1.19 mmol) and piperidine (101 mg, 1.19 mmol) were added and the mixture was allowed to reflux for 18 hrs. A yellowish-orange precipitate slowly began to form. Upon completion, the precipitate was collected by vacuum filtration, washed with chilled methanol (50 mL), and dried for 24 hours *in vacuo* at 40 $^{\circ}$C under an oil-driven vacuum pump yielding yellowish-orange solid (134 mg, 58%): TLC R_f 0.42 in ethyl acetate: hexane (4:1). Melting point = 193-195 $^{\circ}$C. ^{1}H NMR (600 mHz, d_6-DMSO) δ 11.84 (s, 1H), 9.25 (d, 1H, J = 1.62 Hz), 8.79-8.78 (dd, 1H, J_1 = 4.74 Hz, J_2 = 1.62 Hz), 8.44-8.42 (td, 1H, J_1 = 7.98 Hz, J_2 = 1.86 Hz), 8.10-8.07 (d, 1H, J = 15.24 Hz), 7.60-7.58 (m, 1H), 7.46-7.45 (d, 1H, J = 2.34 Hz), 7.44-7.41 (d, 1H, J = 15.24 Hz), 7.31-7.30 (d, 1H, J = 8.64 Hz), 6.85-6.83 (dd, 1H, J_1 = 8.64 Hz, J_2 = 2.34 Hz), 3.87 (s, 3H), 2.58 (s, 3H); ^{13}C NMR (150 MHz , d_6-DMSO) δ 187.6, 155.0, 152.4, 149.0, 145.1, 138.6, 135.5, 133.9, 130.8, 126.5, 123.7, 113.2, 112.1, 110.8, 109.1, 103.3, 55.45, 12.1; Elemental analysis calculated for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58 Found: C, 73.56; H, 5.59; N, 9.43.

N-(*t*-Boc)-4-Methoxy-2-methylaniline (**4**)

4-Methoxy-2-methylaniline (2.2 g, 16.05 mmol) was dissolved in THF (50 mL) under an Argon (g) atmosphere. Di-*tert*-butyl-dicarbonate (5.01 g, 23 mmol) was added to the stirred solution and refluxed overnight. The solvents were distilled *in vacuo* and the residue was redissolved in DCM (50 mL). The organic layer was then washed with saturated $NaHCO_3$ (75 mL) and brine (75 mL). The organic layer was dried with sodium sulfate and concentrated *in vacuo* to yield a dark red oil which was purified by column chromatography using a gradient of 0-20%

EtOAc/hexanes to yield an orange solid (0.77 g). The orange solid was recrystallized in hexanes to yield white crystals (2.86 g, 74%): TLC R_f 0.61 in 20% ethyl acetate/hexane. Melting point = 90.5 - 92.5 °C (lit.[4] 80 – 82 °C). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 (s, 1H), 6.73-6.72 (m, 2H), 6.11 (s, 1H), 3.77 (s, 3H), 2.23 (s, 3H), 1.52 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.5, 153.8, 129.2, 124.3, 115.9, 111.5, 80.1, 55.4, 25.4, 18.0. Elemental analysis calculated for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90 Found: C, 65.90; H, 8.09; N, 5.91.

N-Methoxy-*N*-methylpropionamide (**5**)

To a two-neck flask purged with Ar (g) was added *O*,*N*-dimethylhydroxylamine hydrochloride (1.02 g,10.5 mmol) and propionyl chloride (0.93 g, 10 mmol) in anhydrous DCM (50 mL). The suspension was stirred for 15 minutes at 0 °C. Pyridine (1.69 mL, 21 mmol) was added dropwise over the course of two hours at 0 °C. After addition of pyridine was complete, the reaction was removed from the ice bath and stirred for 3.5 hours at ambient temperatures. The reaction mixture was washed twice with 0.5 N HCl (50 mL), then washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over sodium sulfate, which was filtered, and the DCM was distilled *in vacuo* leaving yellow tinted oil. Due to the product's relatively low boiling point, a bulb-to-bulb distillation was performed under water aspirator vacuum leaving colorless oil (905 mg, 77%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.69 (s, 3H), 3.17 (s, 3H), 2.45-2.42 (q, 2H, J = 7.2 Hz), 1.13-1.11 (t, 3H, J = 7.5 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.6, 61.3, 32.4, 25.4, 8.9. Elemental analysis calculated for $C_5H_{11}NO_2 \cdot 0.075\ H_2O$: C, 50.68; H, 9.48; N, 11.82 Found: C, 50.57; H, 9.56; N, 11.43.

N-Methoxy-*N*-methylbutanamide (**6**)

O,*N*-Dimethylhydroxylamine (1.02 g, 10.5 mmol) was dissolved in dichloromethane (50 mL) under an atmosphere of Argon (g). Butyryl chloride (1.06 g, 10 mmol) was added to the reaction mixture and the reaction was cooled to 0 °C. Pyridine (1.69 mL, 21 mmol) was added dropwise over 25 minutes. After the pyridine was added, the reaction mixture was allowed to warm to ambient temperatures and was stirred for an additional 3 hours. Upon completion, the DCM layer was washed twice with 0.5 N HCl (50 mL x 2) followed by washing with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield a colorless oil (1.28 g, 97%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.67 (s, 3H), 2.91 (s, 3H), 2.40-2.38 (t, 2H, J = 7.6 Hz), 1.68-1.62 (m, 2H) 0.96-0.94 (t, 3H, J = 7.44 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.9, 61.4, 33.9, 32.3, 18.2, 14.1. Elemental analysis calculated for $C_6H_{13}NO_2 \cdot 0.05\ CH_2Cl_2$: C, 53.66; H, 9.75; N, 10.34 Found: C, 53.72; H, 9.97; N, 10.22.

N-Methoxy-*N*-methylisopropanamide (**7**)

O,*N*-Dimethylhydroxylamine (2.04 g, 21 mmol) was dissolved in dichloromethane (60 mL) under an atmosphere of Ar (g). Isobutyryl chloride (2.13 g, 20 mmol) was added to the reaction mixture and the reaction was cooled to 0 °C. Pyridine (3.4 mL, 42 mmol) was added dropwise over 10 minutes. After the pyridine was added, the reaction mixture was allowed to warm to

room temperature and was stirred for an additional 3 hours. Upon completion, the reaction mixture was washed twice with 0.5 N HCl (50 mL x 2) followed by washing with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield a colorless oil (2.01g, 76%): ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.70 (s, 3H), 3.19 (s, 3H), 2.95 (m, 1H), 1.14-1.12 (d, 6H, J = 6.84 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.4, 172.8, 61.4, 35.1, 29.8, 19.1.

N-Methoxy-*N*-methylisobutanamide (**8**)

To a two-neck flask purged with Ar (g) was added *O*,*N*-dimethylhydroxylamine hydrochloride (1.70 g, 17.4 mmol) and isovaleryl chloride (2.0 g, 16.6 mmol) in anhydrous DCM (50 mL). The suspension was stirred for 15 minutes at 0 °C. Pyridine (2.8 mL, 34.9 mmol) was added dropwise over the course of 20 minutes at 0 °C. After addition of pyridine was complete, the reaction was removed from the ice bath and stirred for 3 hours at ambient temperatures. The reaction mixture was washed twice with 1 N HCl (75 mL), then washed with saturated $NaHCO_3$ (75 mL) and brine (75 mL). The organic layer was dried over sodium sulfate, which was filtered off, and the DCM was distilled *in vacuo* leaving yellow tinted oil. Due to the product's low boiling point, a bulb-to-bulb distillation was performed under water aspirator vacuum leaving colorless oil (1.44 g, 60 %): ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.68 (s, 3H), 3.18 (s, 3H), 2.30 (d, 2H, J = 6.7 Hz), 2.17 (m, 1H), 0.97 (d, 6H, J = 6.7 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2, 61.2, 40.7, 32.0, 25.2, 22.7. Elemental analysis calculated for $C_7H_{15}NO_2 \cdot 0.05\ H_2O \cdot 0.05\ C_4H_{10}O$: C, 57.73; H, 10.50; N, 9.35 Found: C, 57.57; H, 10.35; N, 8.95.

2-Ethyl-5-methoxyindole (**10**)

N-(*t*-Boc)-4-Methoxy-2-methylaniline (1.00 g, 4.2 mmol) was dissolved in anhydrous THF (15 mL) under an atmosphere of Ar (g). The solution was cooled to -40 °C and *sec*-butyllithium (1.4 M in cyclohexane, 6.65 mL, 0.60 g) was added slowly to maintain an internal temperature of <-25 °C. After reaching 1 equivalent of *sec*-butyllithium (~3.3 mL) the reaction mixture turned bright yellow signifying the deprotonation of the amide nitrogen. The reaction mixture was then cooled to -50 °C over 10 minutes and a solution of *N*-methoxy-*N*-methylpropanamide (520 mg, 4.4 mmol) in THF (3 mL) was added in a dropwise fashion. The reaction mixture was allowed to warm to -10 °C over 30 minutes while stirring. Upon completion, the mixture was partitioned between Et_2O (50 mL) and 0.5 N HCl (50 mL). The aqueous layer was extracted once more with Et_2O (25 mL). The combined ether layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* providing a yellowish-brown oil. The crude reaction mixture was dissolved in dichloromethane (20 mL) and trifluoroacetic acid (3 mL) was added to the reaction mixture and stirred at room temperature for 48 hours. When the reaction reached completion, DCM (50 mL) was added and the reaction mixture was transferred to a separatory funnel and washed with saturated $NaHCO_3$ (50 mL) followed by brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* giving a deep yellow oil which was purified by column chromatography (0-20 % ethyl acetate/ hexanes) to yield a yellow/brown oil

(0.261 g, 35%): TLC R_f 0.43 in 20% EtOAc/hexanes. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.20-7.18 (d, 1H, *J* = 8.64 Hz), 7.04 (d, 1H, *J* = 2.4 Hz), 6.80-6.78 (dd, 1H J_1 = 8.64 Hz, J_2 = 2.4 Hz), 6.20 (s, 1H), 3.86 (s, 3H), 2.82-2.76 (q, 2H, *J* = 7.62 Hz), 1.36-1.34 (t, 3H, *J* = 7.62 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.3, 142.4, 131.2, 129.5, 111.09, 110.96, 102.3, 98.8, 56.1, 21.7, 13.5. Elemental analysis calculated for $C_{11}H_{13}NO$: C, 76.16; H, 7.99; N, 7.40. Found: C, 76.28; H, 7.97; N, 7.39.

5-Methoxy-2-propylindole (**11**)

N-(*t*-Boc)-4-Methoxy-2-methylaniline (1.00 g, 4.2 mmol) was dissolved in THF (15 mL) under an atmosphere of Ar (g). The solution was cooled to -40 °C over 10 minutes and *sec*-butyllithium (1.4 M in cyclohexane, 6.66 mL) was added slowly to maintain an internal temperature of <-25 °C. After reaching 1 equivalent of *sec*-butyllithium (3.33 mL) the reaction mixture turned a bright yellow signifying the total deprotonation of the amide nitrogen. The reaction mixture was then cooled to -50 °C and a solution of *N*-methoxy-*N*-methylbutanamide (580 mg, 4.4 mmol) in THF (3 mL) was added over 10 minutes in a dropwise fashion. The reaction mixture was warmed to -10 °C over 30 minutes. The mixture was partitioned between Et_2O (50 mL) and 1 N HCl (50 mL). The aqueous layer was extracted an additional two times with Et_2O (25 mL). The organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield a brown solid. This above reaction was performed twice yielding crude brown solid (3 g), which was taken directly to the next step. Crude product (3 g) was dissolved in dichloromethane (20 mL) and trifluoroacetic acid (3 mL) was added to the solution and the reaction mixture was stirred at room temperature for 48 hours. When the reaction reached completion, the reaction mixture was added to a separatory funnel and washed with $NaHCO_3$ (30 mL) followed by brine (30 mL). The organic layer was dried over $NaSO_4$ and concentrated *in vacuo* giving a crude black oil (3.03 g) which was purified with column chromatography to yield a yellow/brown solid (1.1 g, 68%): TLC R_f 0.32 in 10% EtOAc/hexanes. Melting point = 64 – 65 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74 (s, 1H), 7.19-7.17 (d, 1H, *J* = 8.7 Hz), 7.01 (d, 1H, *J* = 2.46 Hz), 6.78-6.76 (dd, 1H J_1 = 8.7 Hz, J_2 = 2.46 Hz) 6.18-6.17 (m, 1H), 3.84 (s, 3H), 2.72-2.70 (t, 2H, *J* = 7.34 Hz), 1.77-1.71 (m, 2H), 1.01-0.99 (t, 3H, *J* = 7.32 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.3, 140.9, 131.1, 129.5, 111.08, 110.96, 102.16, 99.7, 56.1, 30.6, 22.7, 14.1. Elemental analysis calculated for $C_{12}H_{15}NO$: C, 76.16; H, 7.99; N, 7.40. Found: C, 76.28; H, 7.97; N, 7.39.

2-Isopropyl-5-methoxyindole (**12**)

N-(*t*-Boc)-4-Methoxy-2-methylaniline (500 mg, 2.1 mmol) was dissolved in THF (10 mL) under an atmosphere of Ar (g). The solution was cooled to -40 °C over 10 minutes and *sec*-butyllithium (1.4 M in cyclohexane, 3.33 mL) was added slowly to maintain an internal temperature of <-25 °C. After reaching 1 equivalent of *sec*-butyllithium (3.33 mL) the reaction mixture turned a bright yellow signifying the total deprotonation of the amide nitrogen. The reaction mixture was then cooled to -50 °C and a solution of *N*-Methoxy-*N*-methylisopropanamide (288 mg, 2.2

mmol) in THF (3 mL) was added over 5 minutes. The reaction mixture was warmed to -10 °C over 30 minutes. The mixture was partitioned between Et_2O (50 mL) and 1N HCl (50 mL). The aqueous layer was extracted an additional two times with Et_2O (25 mL). The Et_2O was then washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to yield dark brown oil. The described reaction was performed 2 more times (3 total) times yielding crude ketone (2.2 g), which was taken directly to the next step. Crude intermediate (2.2 g) was dissolved in dichloromethane (20 mL) and trifluoroacetic acid (3 mL) was added to the reaction mixture and stirred at room temperature for 48 hours. Upon completion, the reaction mixture was added to a separatory funnel and washed with $NaHCO_3$ (50mL) followed by brine (50 mL). The organic layer was dried over $NaSO_4$ and concentrated *in vacuo* giving a crude black oil (1.5 g) which was purified with column chromatography to yield a yellow solid (340 mg, 28%): TLC R_f 0.48 in 20% EtOAc/hexanes. Melting point = 68-71 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.23-7.21 (d, 1H, J = 8.7 Hz), 7.05 (d, 1H, J = 2.46 Hz), 6.81-6.79 (dd, 1H, J_1 = 2.46 Hz, J_2 = 8.64 Hz), 6.21-6.20 (m, 1H), 3.87 (s, 3H), 3.10-3.05 (m, 1H), 1.35-1.34 (d, 6H, J = 6.9 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.0, 146.8, 130.7, 129.0, 110.95, 110.83, 102.0, 97.3, 55.9, 36.6, 27.7, 24.7, 23.3. Elemental analysis calculated for $C_{12}H_{15}NO$: C, 76.16; H, 7.99; N, 7.40. Found: C, 75.95; H, 7.85; N, 7.17.

2-Isobutyl-5-methoxyindole (**13**)

N-(*t*-Boc)-4-Methoxy-2-methylaniline (1.00 g, 4.2 mmol) was dissolved in anhydrous THF (15 mL) under an atmosphere of Argon (g). The solution was cooled to -40 °C and *sec*-butyllithium (1.4 M in cyclohexane, 6.61 mL, 0.59 g) was added slowly as to maintain an internal temperature of < -25°C. After reaching 1 equivalent of *sec*-butyllithium (~3.3 mL) the reaction mixture turned bright yellow signifying the deprotonation of the amide nitrogen. Upon completion of the addition of *sec*-butyllithium, the reaction mixture was then cooled to -50 °C over 10 minutes and a solution of *N*-Methoxy-*N*-methylisobutanamide (0.67 g, 4.63 mmol) in THF (3 mL) was added. The reaction mixture was allowed to warm to -10 °C over 30 minutes while stirring. Upon completion, the mixture was partitioned between Et_2O (50 mL) and 0.5 N HCl (50 mL). The aqueous layer was extracted once more with Et_2O (25 mL). The combined ether layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* providing a yellowish-brown oil. The above reaction was performed a second time immediately after the first reaction was complete. Therefore, the total amount of starting material (N-(*t*-Boc)-4-methoxy-2-methylaniline, 2.0 g, 8.4 mmol) was doubled. The crude reaction mixture (from 2.0 g) was dissolved in dichloromethane (20 mL) and trifluoroacetic acid (3 mL) was added to the solution and stirred at room temperature for 48 hours. When the reaction reached completion, DCM (50 mL) was added and the reaction mixture was transferred to a separatory funnel and washed with saturated $NaHCO_3$ (50 mL) followed by brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* giving a deep yellow oil which was purified by column chromatography (0-20 % ethyl acetate/ hexanes) to yield an amber oil (1.21 g, 71%): TLC R_f 0.53 in 20% EtOAc/hexanes. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.18-7.17 (d, 1H, J =

8.7 Hz), 7.01 (d, 1H, J = 2.4 Hz), 6.78-6.76 (dd, 1H J_1 = 8.7 Hz, J_2 = 2.4 Hz), 6.16 (s, 1H), 3.84 (s, 3H), 2.60-2.58 (d, 2H, J = 7.14 Hz), 1.97-1.95 (m, 1H), 0.97-0.96 (d, 6H, J = 6.6 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.1, 139.8, 130.9, 129.3, 110.88, 110.72, 101.9, 100.4, 55.9, 37.8, 29.0, 22.5. Elemental analysis calculated for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 77.07; H, 8.50; N, 6.81.

2-Ethyl-5-methoxyindole-3-carboxaldehyde (**14**)

Dimethylformamide (1 mL) was cooled to 0 °C. Phosphorus oxychloride (0.25 mL) was added and the reaction mixture was stirred for 10 minutes. A solution of 2-ethyl-5-methoxyindole (226 mg, 1.7 mmol) in dimethylformamide (2 mL) was added to the reaction mixture dropwise over 10 minutes. The solution was stirred for an additional 40 minutes. The reaction mixture was added to ice cold 1 N NaOH (25 mL) and stirred for 10 minutes. The precipitate was collected, washed with cold water and dried overnight in a vacuum desiccator set at 40 °C equipped to an oil driven vacuum pump yielding a white solid (213 mg, 62%): TLC R_f 0.55 in 4:1 ethyl acetate/hexanes. Melting point = 211-212 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.84 (s,1H), 10.04 (s, 1H), 7.58 (d, 1H, J = 2.4 Hz), 7.30-7.28 (d, 1H, J = 9 Hz), 6.81-6.79 (dd, 1H, J_1 = 9 Hz, J_2 = 2.4 Hz), 3.77 (s, 3H), 3.08-3.04 (q, 2H, J = 7.8 Hz), 1.33-1.28 (t, 3H, J = 7.8 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 183.86, 155.48, 153.86, 130.09, 126.33, 112.68, 112.20, 111.95, 102.41, 55.25, 18.92, 14.54. Elemental analysis calculated for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.92; H, 6.97; N, 6.34.

5-Methoxy-2-propylindole-3-carboxaldehyde (**15**)

Dimethylformamide (2 mL) was cooled to 0 °C. Phosphorus oxychloride (0.5 mL) was added and the reaction mixture was stirred for 10 minutes. A solution of 5-methoxy-2-propylindole (325 mg, 1.7 mmol) in dimethylformamide (3 mL) was added to the reaction mixture dropwise over 10 minutes. The solution was stirred for an additional 40 minutes. The reaction mixture was added to ice cold 1 N NaOH (50 mL) and stirred for 10 minutes. The precipitate was collected and dried overnight in an oil driven vacuum pump equipped to a vacuum desiccator set at 40 °C yielding a white solid (318 mg, 86%): TLC R_f 0.67 in 4:1 ethyl acetate/hexanes. Melting point = 166-168 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.16 (s, 1H), 8.31 (s, 1H), 7.79 (d, 1H, J = 2.46 Hz), 7.23-7.22 (d, 1H, J = 8.82 Hz), 6.89-6.87 (dd, 1H, J_1 = 2.54, J_2 = 8.76 Hz), 3.89 (s, 3H), 3.06-3.04 (t, 2H, J = 7.5 Hz), 1.84-1.80 (m, 2H) 1.05-1.03 (t, 3H, J = 7.32 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.4, 156.5, 151.4401, 129.6, 126.7, 114.7, 113.5, 111.5, 103.0, 55.8, 28.3, 23.5, 13.8. Elemental analysis calculated for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.92; H, 6.97; N, 6.34.

2-Isopropyl-5-methoxyindole-3-carboxaldehyde (**16**)

Dimethylformamide (2 mL) was cooled to 0 °C. Phosphorus oxychloride (0.5 mL) was added and the reaction mixture was stirred for 10 minutes. A solution of 2-isopropyl-5-methoxyindole (200 mg, 1.05 mmol) in dimethylformamide (3 mL) was added to the reaction mixture dropwise

over 10 minutes. The solution was stirred for an additional 40 minutes. The reaction mixture was added to ice cold 1 N NaOH (50 mL) and stirred for 10 minutes. The precipitate was collected and dried overnight in an oil driven vacuum pump equipped to a vacuum desiccator set at 40 °C yielding a light brown solid (318 mg, 86%): TLC R_f 0.71 in 4:1 ethyl acetate/hexanes. Melting point = 181-183 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.22 (s, 1H), 8.63 (s, 1H), 7.79 (d, 1H, *J* = 2.46 Hz), 7.26-7.24 (d, 1H, *J* = 8.94 Hz), 6.89-6.87 (dd, 1H, J_1 = 2.52 Hz, J_2 = 8.76 Hz), 3.87 (s, 3H), 3.79-3.74 (m, 1H), 1.46-1.45 (d, 6H, *J* = 7.02 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.1, 156.52, 156.51, 129.4, 126.9, 113.5, 113.2, 111.6, 103.0, 55.8, 25.8, 22.7. Elemental analysis calculated for $C_{13}H_{15}NO_2$: C, 71.87; H, 6.96; N, 6.45. Found: C, 71.59; H, 6.98; N, 6.39.

2-Isobutyl-5-methoxyindole-3-carboxaldehyde (**17**)

Dimethylformamide (1 mL) was cooled to 0 °C. Phosphorus oxychloride (0.5 mL) was added and the reaction mixture was stirred for 10 minutes. A solution of 2-isobutyl-5-methoxyindole (375 mg, 1.8 mmol) in dimethylformamide (2 mL) was added to the reaction mixture dropwise over 10 minutes. The solution was stirred for an additional 40 minutes. The reaction mixture was added to ice cold 1 N NaOH (35 mL) and stirred for 10 minutes. The precipitate was collected, washed with cold water (30 mL) and dried overnight in a vacuum desiccator set at 40 °C equipped to an oil driven vacuum pump yielding a tan solid (393 mg, 94%): TLC R_f 0.45 in 1:1 ethyl acetate/hexanes. Melting point = 146-147 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.13 (s,1H), 8.74 (s, 1H), 7.81 (d, 1H, *J* = 2.5 Hz), 7.24-7.23 (d, 1H, *J* = 8.7 Hz), 6.89-6.87 (dd, 1H, J_1 = 8.7 Hz, J_2 = 2.5 Hz), 3.87 (s, 3H), 2.93-2.92 (d, 2H, *J* = 7.3 Hz), 2.11-2.05 (m, 1H), 1.02-1.01 (d, 6H, *J* = 6.7 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.62, 156.48, 150.72, 129.69, 126.68, 115.22, 113.59, 111.55, 102.99, 55.80, 35.46, 30.08, 22.51. Elemental analysis calculated for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.62; H, 7.52; N, 5.98.

trans-3-(2-Ethyl-5-methoxyindol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (**18**)

To a dried two-neck flask purged with N_2 (g), 2-ethyl-5-methoxyindole-3-carboxyaldehyde (191 mg, 0.940 mmol) was dissolved in anhydrous methanol (10 mL). 4-Acetylpyridine (200 mg, 1.65 mmol) and piperdine (135 mg, 1.58 mmol) were added and the mixture was allowed to reflux for 20 hrs. A yellow precipitate slowly began to form. Upon completion, the yellow precipitate was collected by vacuum filtration, washed with chilled methanol (40 mL), and dried for 36 hours *in vacuo* at 40 °C yielding a bright yellow solid (208 mg, 72%): TLC R_f 0.38 in ethyl acetate: hexane (4:1). Melting point = 214-215 °C. ^{1}H NMR (600 mHz, d_6-DMSO) δ 11.86 (s, 1H), 8.81 (m 2H), 8.11-8.08 (d, 1H, *J* = 15.24 Hz), 7.94-7.93 (m, 2H), 7.45 (d, 1H, *J* = 2.28 Hz), 7.39-7.37 (d, 1H, *J* = 15.24 Hz), 7.34-7.33 (d, 1H, *J* = 8.64 Hz), 6.87-6.86 (dd,1H, J_1 = 8.7 Hz, J_2 = 2.34 Hz), 3.87 (s, 3H), 2.98-2.94 (q, 2H, *J* = 7.62 Hz), 1.32-1.29 (t, 3H, *J* = 7.62 Hz); ^{13}C NMR (150 MHz , d_6-DMSO) δ 188.1, 155.2, 151.2, 150.6, 145.1, 139.3, 131.2, 126.4, 121.4, 112.9, 112.4, 111.0, 108.3, 103.8, 55.6, 19.3, 14.2; Elemental analysis calculated for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14. Found: C, 74.26; H, 5.92; N, 9.06.

trans-3-(5-Methoxy-2-propylindol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (**19**)

5-Methoxy-2-propylindole-3-carboxaldehyde (200 mg, 0.92 mmol) was dissolved in anhydrous methanol (7.5 mL) under an atmosphere of Argon (g). 4-Acetylpyridine (167.17 mg, 1.38 mmol) and piperidine (117.5 mg, 1.38 mmol) were added to the reaction mixture and the solution was refluxed for 24 hours. A precipitate slowly formed which was collected, washed with cold MeOH (30 mL) and dried overnight in an oil driven vacuum pump equipped to a vacuum desiccator heated to 40 °C to yield a bright yellow powder (253 mg, 85%): TLC R_f 0.43 in 4:1 ethyl acetate/hexanes. Melting point = 221-224 °C. ^{1}H NMR (600 MHz, d_6-DMSO) δ 11.87 (s, 1H), 8.82-8.82 (m, 2H), 8.10-8.07 (d, 1H, J = 15.24 Hz), 7.94-7.93 (m, 2H), 7.46-7.45 (d, 1H, J = 2.34), 7.40-7.37 (d, 1H, J = 15.24 Hz), 7.34-7.32 (d, 1H, J = 8.7 Hz), 6.87-6.86 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.7 Hz), 3.87 (s, 3H), 2.92-2.89 (t, 2H, J = 7.44 Hz) 1.74-1.70 (m, 2H) 0.95-0.92 (t, 3H, J = 14.7 Hz); ^{13}C NMR (150 MHz, d_6-DMSO) δ 188.0, 155.1, 150.5, 149.7, 145.0, 139.4, 131.1, 126.2, 121.3, 112.8, 112.3, 111.0, 109.0, 103.7, 55.5, 27.6, 22.8, 13.5. Elemental analysis calculated for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74. Found: C, 75.05; H, 6.32; N, 8.70.

trans-3-(2-Isopropyl-5-methoxyindol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (**20**)

2-Isopropyl-5-methoxyindole-3-carboxaldehyde (105 mg, 0.48 mmol) was dissolved in anhydrous methanol (5 mL) under an atmosphere of Argon (g). 4-Acetylpyridine (87.2 mg, 0.72 mmol) and piperidine (61.3 mg, 0.72 mmol) were added to the reaction mixture and the solution was refluxed for 24 hours. A precipitate slowly formed which was collected, washed with cold MeOH (30 mL) and dried overnight in an oil driven vacuum pump equipped to a vacuum desiccator heated to 40 °C to yield a bright yellow powder (102 mg, 66%): TLC R_f 0.38 in 4:1 ethyl acetate/hexanes. Melting point = 252-253 °C. ^{1}H NMR (600 MHz, d_6-DMSO) δ 11.78 (s, 1H), 8.82-8.81 (m, 2H), 8.15-8.13 (d, 1H, J = 15.24 Hz), 7.94 (m, 2H), 7.45 (d, 1H, J = 2.28 Hz), 7.41-7.38 (d, 1H, J = 15.18 Hz), 7.36-7.34 (d, 1H, J = 8.7 Hz), 6.88-6.87 (dd, 1H, J_1 = 2.34 Hz, J_2 = 8.64 Hz), 3.87 (s, 3H), 3.54-3.49 (m, 1H), 1.35-1.34 (d, 6H, J = 6.96 Hz); ^{13}C NMR (150 MHz, d_6-DMSO) δ 188.6, 155.7, 155.4, 151.1, 145.6, 139.6, 131.8, 126.6, 121.9, 113.5, 113.0, 111.6, 105.0, 104.4, 56.1, 26.0, 22.8. Elemental analysis calculated for $C_{20}H_{20}N_2O_2$: C, 74.98; H, 6.29; N, 8.74. Found: C, 74.78; H, 6.42; N, 8.68.

trans-3-(2-Isobutyl-5-methoxyindol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (**21**)

To a dried two-neck flask purged with N_2 (g), 2-isobutyl-5-methoxylindole-3-carboxyaldehyde (253 mg, 1.09 mmol) was dissolved in anhydrous methanol (10 mL). 4-Acetylpyridine (196 mg, 1.62 mmol) and piperdine (173 mg, 2.03 mmol) were added and the mixture was allowed to reflux for 20 hrs. A yellow precipitate slowly began to form. Upon completion, the yellow precipitate was collected by vacuum filtration, washed with chilled methanol (30 mL), and dried for 36 hours *in vacuo* at 40 °C yielding a bright yellow solid (302 mg, 83 %): TLC R_f 0.38 in ethyl acetate: hexane (4:1). Melting point = 217-218 °C. ^{1}H NMR (600 mHz, d_6-DMSO) δ 11.87 (s, 1H), 8.82-8.81 (m 2H), 8.08-8.06 (d, 1H, J = 15.24 Hz), 7.94-7.93 (m, 2H), 7.47-7.46 (d, 1H,

J = 2.34 Hz), 7.41-7.38 (d, 1H, *J* = 15.24 Hz), 7.34-7.33 (d, 1H, *J* = 8.7 Hz), 6.88-6.86 (dd,1H, J_1 = 8.7 Hz, J_2 = 2.34 Hz), 3.87 (s, 3H), 2.80-2.79 (d, 2H, *J* = 7.32 Hz), 2.03-2.01 (m, 1H), 0.93-0.92 (d, 6H); ^{13}C NMR (150 MHz , d_6-DMSO) δ 188.1, 155.1, 150.5, 148.9, 145.0, 139.6, 131.1, 126.1, 121.3, 112.9, 112.3, 111.0, 109.4, 103.8, 55.5, 34.7, 29.2, 22.2. Elemental analysis calculated for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found: C, 75.35; H, 6.65; N, 8.37.

Biology

Cell Culture

U251 human glioblastoma cells were purchased from the DCT Tumor Repository (National Cancer Institute, Frederick, MD). Cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) (Fisher Scientific, Wayne, MI) at 37^{o}C in an atmosphere of 5% CO_2/95% air. Prior to addition to cell cultures, all compounds were dissolved in dimethyl sulfoxide (DMSO) and serial dilutions were prepared in DMSO. All of the stated drug concentrations were achieved by diluting the appropriate DMSO stock solution into the cell culture medium at 1/1000. Control cultures received medium containing 0.1% DMSO.

Cell Morphology

To determine the effects of the compounds on cell morphology, U251 cells were plated in 35 mm plastic tissue culture dishes (100,000 cells/dish) and allowed to attach for 24 h. Test compounds were then added and phase-contrast images of live cells were obtained at 24 h and 48h, using an Olympus IX70 inverted microscope equipped with a digital camera and SPOT imaging software (Diagnostic Instruments, Inc., Sterling Heights, MI). Medium and test compounds were replenished after the first 24 h period.

Sulforhodamine B Assay

Total protein in adherent cells was measured by the sulforhodamine B (SRB) colorimetric assay, following standard procedures for anticancer drug screening used by the U.S. National Cancer Institute. Cells were seeded at 2,000 cells per well in 96-well plates, with four replicate wells for each drug concentration. On the day after plating, four wells were assayed to establish a pre-drug (time-0) baseline. Test compounds were diluted as described above and added to the remaining wells. The medium and compounds were replenished after 24 hr. SRB assays were performed at a 48 h endpoint as described,[5] using a SpectraMax Plus 384 plate reader (Molecular Devices, Sunnyvale, CA) to determine absorbance at 515 nm. The concentration of each compound producing 50% growth inhibition (GI_{50}) relative to the no-drug control was calculated according to the NCI recommendation (http://dtp.nci.nih.gov/branches/btb/ivclsp.html). To determine if the GI_{50}'s for compounds **1** and **18** were significantly different, the dose-response curves for these compounds were repeated seven times, and the calculated GI_{50} values were compared by Student's *t*-Test (Figure S1).

Trypan Blue Viability Assay

Our previous studies have established that in adherent cell cultures treated with MOMIPP detachment of cells accompanies the loss of cell viability. To directly compare the effects of different compounds on cell viability, we utilized the trypan blue dye-exclusion assay to distinguish live (unstained) from dead (blue) cells.[6] U251 cells were seeded in 35 mm diameter dishes at a density of 100,000 cells/dish. Twenty four hours later, test compounds were added in fresh medium at a final concentration of 10 μM. Controls received an equivalent volume of vehicle (DMSO). All cultures were treated for 2 days, with no change of medium. At the end of the incubation, floating and adherent cells were combined and collected by centrifugation. Cells were washed with Hank's balanced salt solution (HBSS) and incubated with 0.2% trypan blue solution (Sigma, St. Louis, MO) for 5 min at room temperature. The percentage of dead cells was determined by light microscopy using a hemocytometer. Three separate cultures were assayed for each compound (Figure S2).

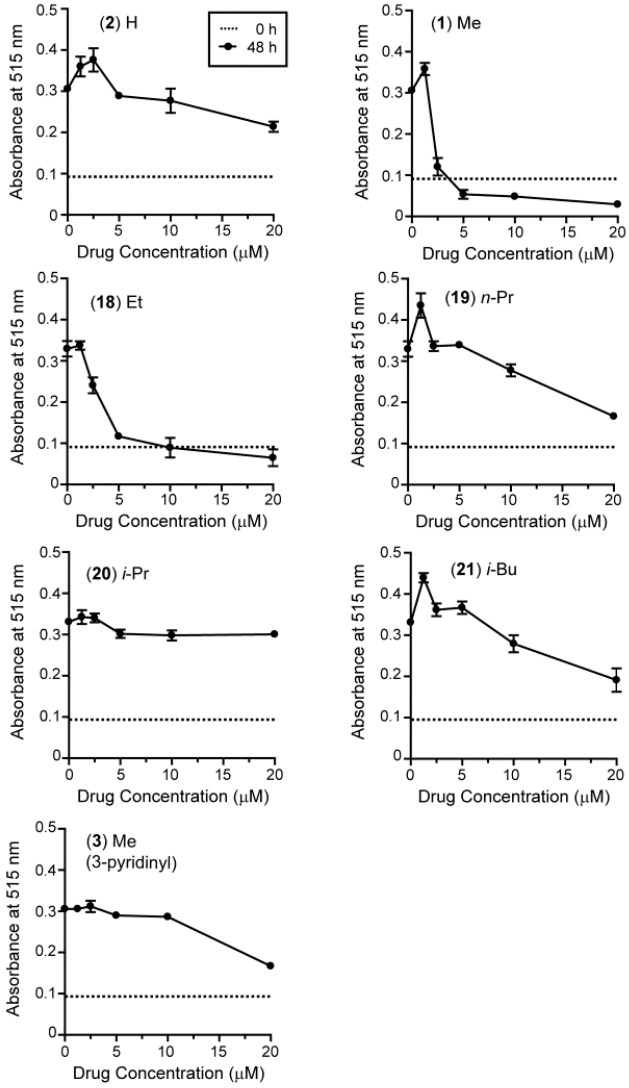


Figure S1. Effects of MOMIPP (**1**) and related 2-indolyl-substituted pyridinylpropenones on growth of U251 glioblastoma cells. Cells were seeded in 96-well plates as described in the methods. After 24 h, each compound was added at the indicated concentrations and SRB assays were performed after an additional 48 h. Each point represents the mean ± SD of values from four wells. The dotted line on each graph indicates the average absorbance of four wells sampled at time-0 (i.e., the time that the compounds were added). For each compound the concentration resulting in a 50% reduction in the net protein increase observed for the vehicle control (GI_{50}) was determined by the formula [(Ti-Tz)/C-Tz)] x 100 =50, where Tz is the absorbance measurement at time-0, C is the absorbance in the vehicle-treated controls after 48 h, and Ti is the absorbance in the drug-treated cultures at 48 h.

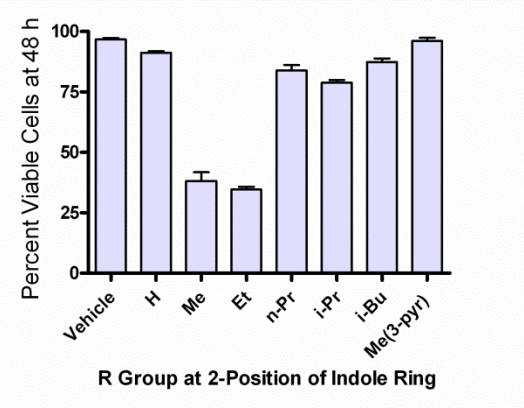


Figure S2. Effects of MOMIPP (1) and related 2-indolyl-substituted pyridinylpropenones on viability of U251 glioblastoma cells. Cells were seeded in 35 mm dishes and treated for 48 h with compounds at a final concentration of 10 μM. Trypan blue dye-exclusion assays were performed as described in the methods. Values are the mean ± SD of separate assays performed on three parallel cultures.

Computational Studies: Methods and Validation Data

Molecular modeling studies were performed using SYBYL 8.0 from Tripos Inc (St. Louis, MO). Energy minimizations were carried-out by applying general-purpose force-field parameters. Atomic charges were accepted on the basis of the MMFF94 parameterization. For solution or protein environments, an implicit solvent model was adopted by considering a 10 Å non-bonded cutoff and a distance-dependent dielectric function with a constant default value of ε = 4. NVT molecular dynamic simulations were conducted for durations of 1 ns with time-steps of 0.5 fs at a targeted temperature of T = 310 °K while applying a strong temperature coupling constant of 100 ps.

To validate our approach, both gas-phase and in-solution studies were first conducted on two simplified model compounds that display rotational properties relevant to our series of more structurally complex test agents. The two rotameric possibilities of interest to us are shown (Figure S3) for each of the model compounds. Importantly, acrolein has been well-studied in the literature and it has gas-phase experimental data[7] that can be used to strictly assess the results from our computational analyses. By convention, the nomenclature 's-*cis*' and 's-*trans*' refers to the relative positions of the two double bonds in a molecule when they are separated by a single bond. Conformational equilibration is possible by rotation about this single bond, as is well known for the classical prototype, 1,3-butadiene.

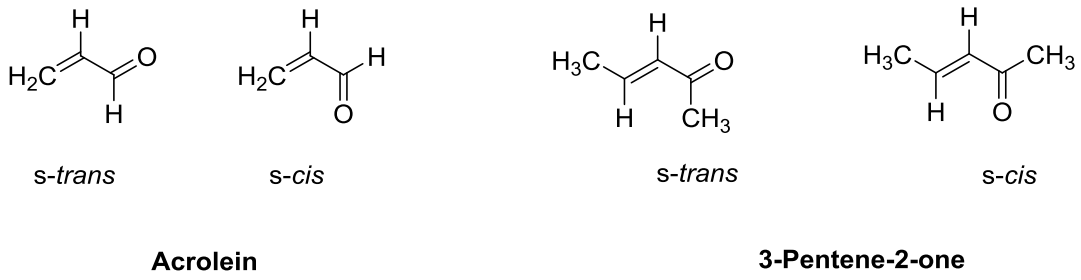


Figure S3. Rotameric possibilities (s-*trans* and s-*cis* conformations) for acrolein and 3-pentene-2-one.

Our gas-phase studies of acrolein indicate that this molecule prefers the C=C-C=O s-*trans* conformation by 2.2 kcal/mol compared to its s-*cis* conformation. Furthermore, while the calculated energy barrier for rotation (approximated by the structure having a CCCO torsion angle of 90°) is considerably higher in energy, it is surmountable under practical conditions (e.g. exposed to reasonably elevated temperatures or when placed within the environment of a specific receptor interaction where the latter might prefer a given rotomer). Most importantly, our computed energies agree closely with the experimental values previously recorded in the literature (Table S1). Interestingly, the solvent effect in the applied implicit approximation reduces the s-*trans* to s-*cis* energy separation by 1.8 kcal/mol and its barrier by 1.3 kcal/mol, thus maintaining only a modest preference of 0.4/mol kcal for the s-*trans* arrangement.

Within our 3-pentene-2-one model, classical *cis/trans* isomerization about the CC double bond is additionally possible due to the 1,2-disubstitution of the ethylene substructure. However, because NMR analysis of each of the compounds in our target series clearly showed that they all possess the more favorable *trans*-conformation as anticipated after the Claisen-Schmidt condensation, the computational problem reduces to just the s-*cis*/s-*trans* possibilities as described and depicted above. This strategy was likewise adopted during our subsequent analyses of the target compound series. In the case of 3-pentene-2-one, the s-*trans* conformer is preferred over the s-*cis* by 1.3 kcal/mol in the gas phase. This reduction in relative energy compared to the parent acrolein system is in accord with the observation that the H and a methyl-group adopt a nearly *'cis'* relationship that can lead to their repulsion. Indeed, the calculated

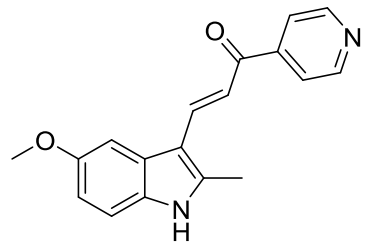
CCCO torsion angle for the s-*trans* conformation is 162-174° which is likely the consequence of relieving this interaction. In contrast, the heavy atom remains perfectly coplanar in the s-*cis* form. The relative height of the rotational barrier is likewise impacted by the higher energy of the s-*trans* conformer such that it becomes 6.4 kcal/mol. Upon performing a gas-phase molecular dynamics study utilizing the optimized s-*cis* conformation as a starting point, it was noted that the C=C-C=O torsion angle changes at about 0.4 ns and oscillates around 180°. This suggests that the structure is inclined toward adopting the s-*trans* arrangement. Alternatively, molecular simulations for the same molecule within the implicit solvent model and starting from the s-*trans* conformation turns into the s-*cis* arrangement, in accord with a very modest preference prompted by an optimized geometry that reflects only 0.9 kcal/mol.

Overall, these preliminary results are in accord with what can be expected for the unambiguous molecular models and, importantly, are in close agreement with the available experimentally-derived values recorded in the prior literature. Beyond this method validation, these results further suggest that while the presence of simple alkyl substituents across the 'double-double-bond' arrangements can have an impact upon the s-*cis*/s-*trans* equilibria, these effects are minimal when dealing with small substituents such as a methyl group. As their size increases, however, then became the focus of the studies that are reported across the target series delineated within the main text of this publication.

Table S1. Relative molecular-mechanics derived energies[a] for acrolein and 3-pentene-2-one and experimentally determined energies reported for acrolein.[7]

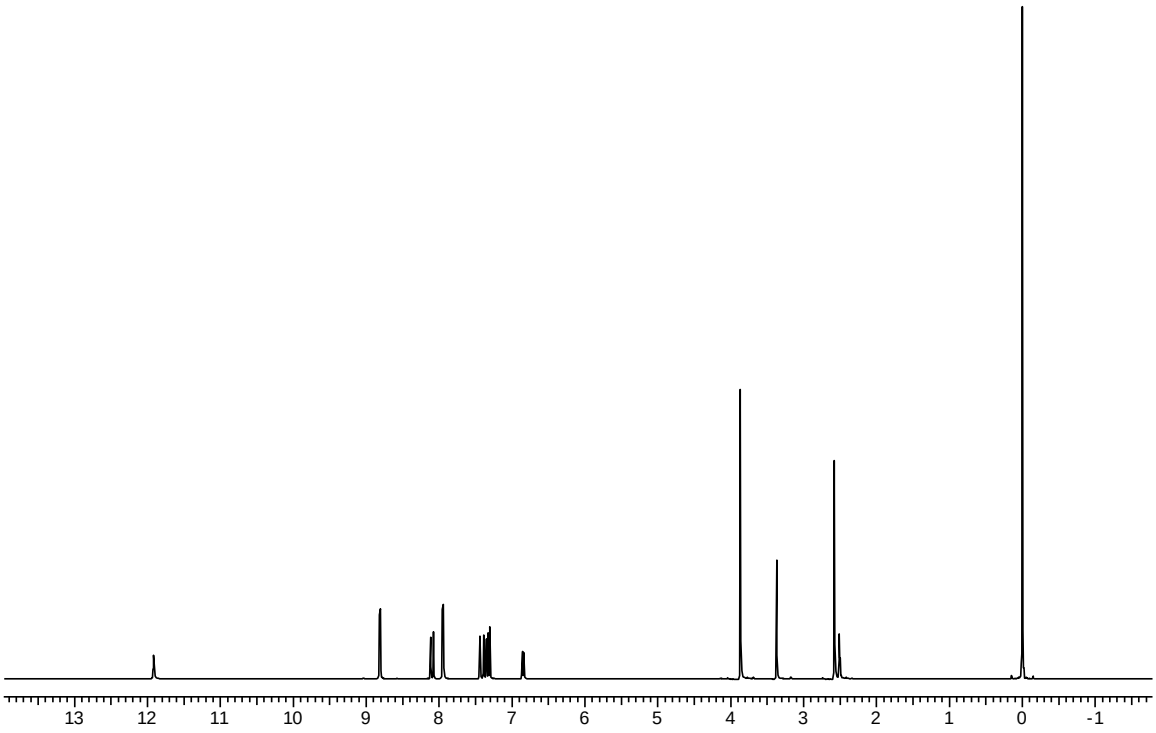
	Gas Phase	Implicit solvent[b]	Exp (gas phase)
Acrolein			
s-*cis*	2.2	0.4	1.6 - 2.2
CCCO=90^o	6.9	5.6	6.4
s-*trans*	0.0	0.0	0.0
3-Pentene-2-one			
s-*cis*	1.3	0.0	
CCCO = 90^o	6.4	4.7	
s-*trans*	0.0	0.9	

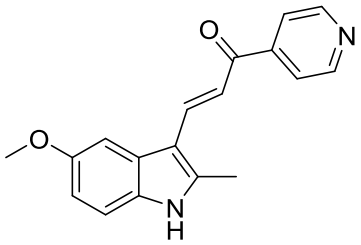
[a]Energies are recorded in kcal/mol. [b]Distance-dependent dielectric function with ε (dielectric constant) of 4.



1

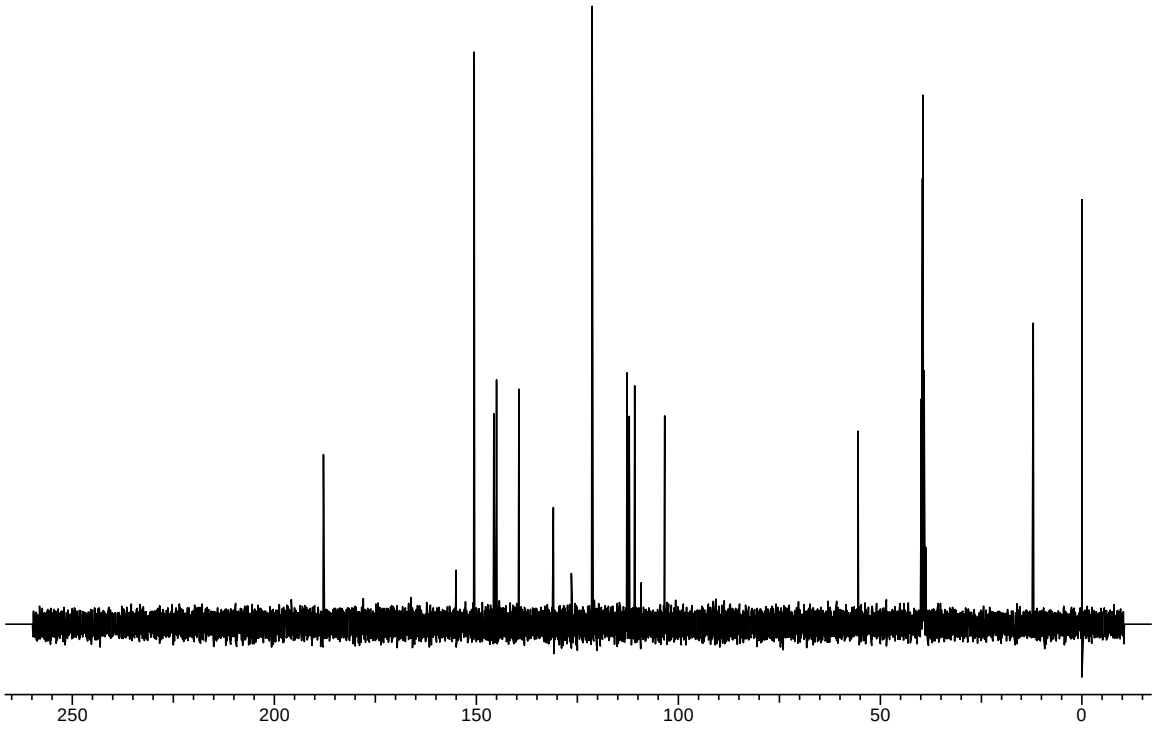
^{1}H NMR (600 mHz, d_6-DMSO)





1

^{13}C NMR (150 mHz, d_6-DMSO)



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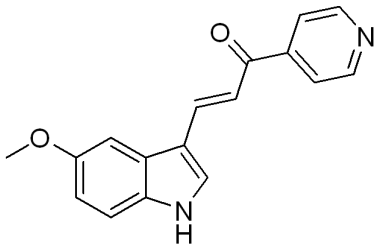

2

^{1}H-NMR (600 mHz, d_6-DMSO)

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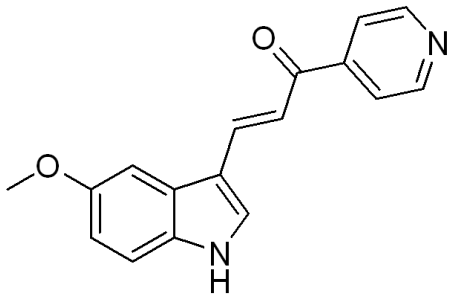
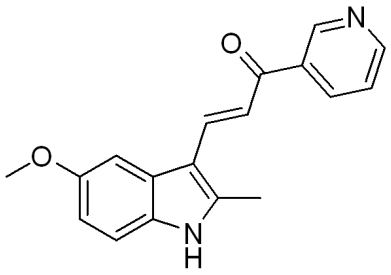


2

^{13}C-NMR (150 mHz, d_6-DMSO)

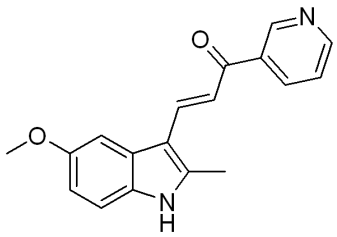
CT-1-111 1 1 /home/ctrabbi/avance600



3

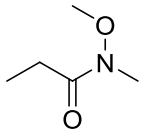
^{1}H-NMR (600 mHz, d_6-DMSO)

CT-1-111 3 1 /home/ctrabbi/avance600



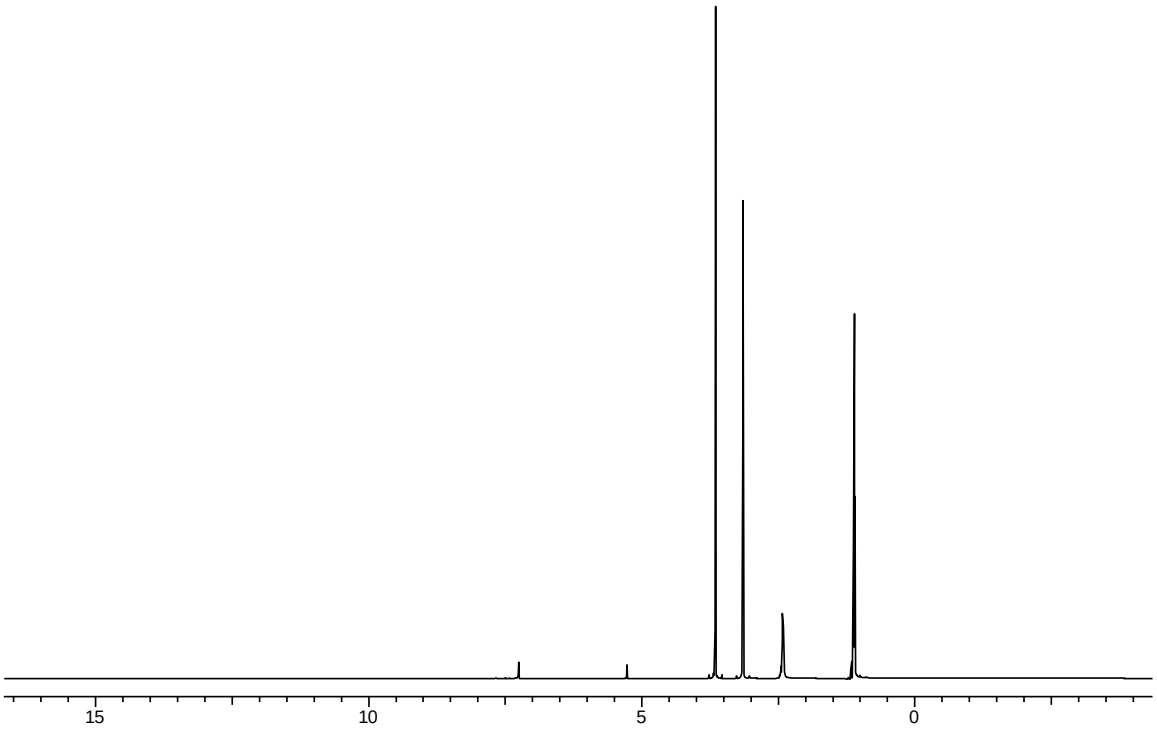
3

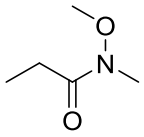
^{13}C-NMR (150 mHz, d_6-DMSO)



5

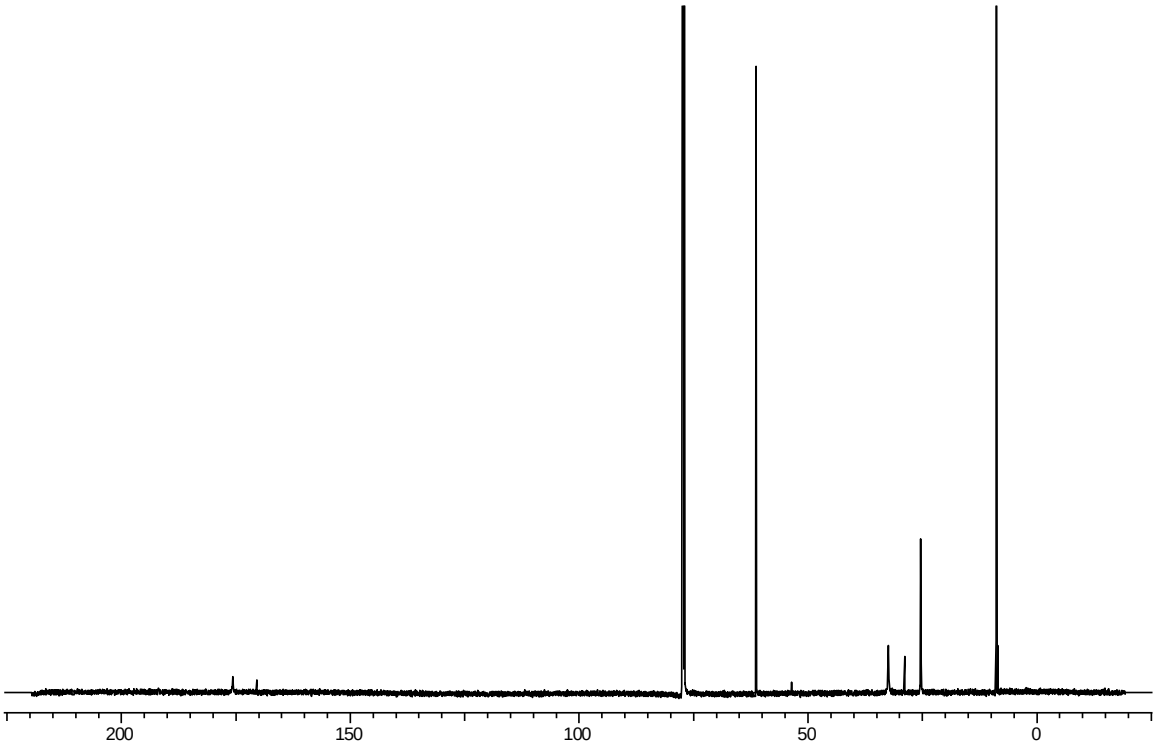
^{1}H NMR (600 MHz, $CDCl_3$)

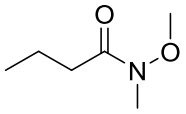




5

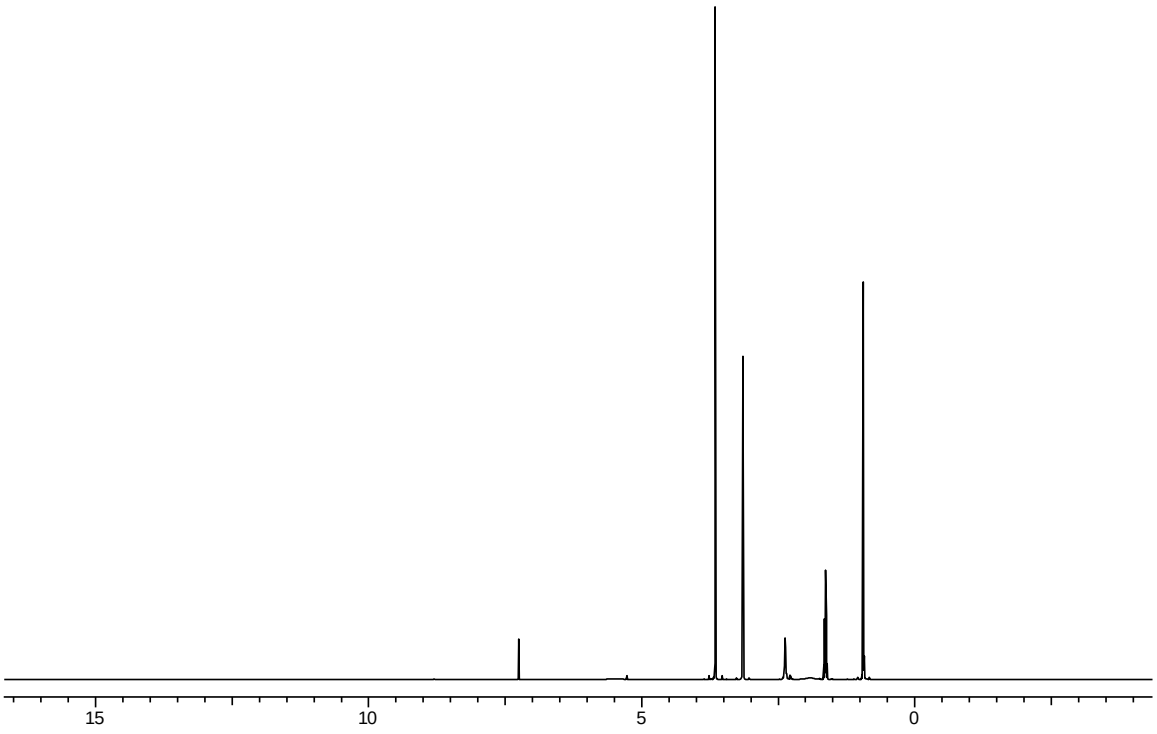
^{13}C NMR (150 MHz, $CDCl_3$)

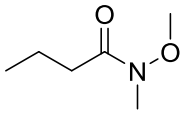




6

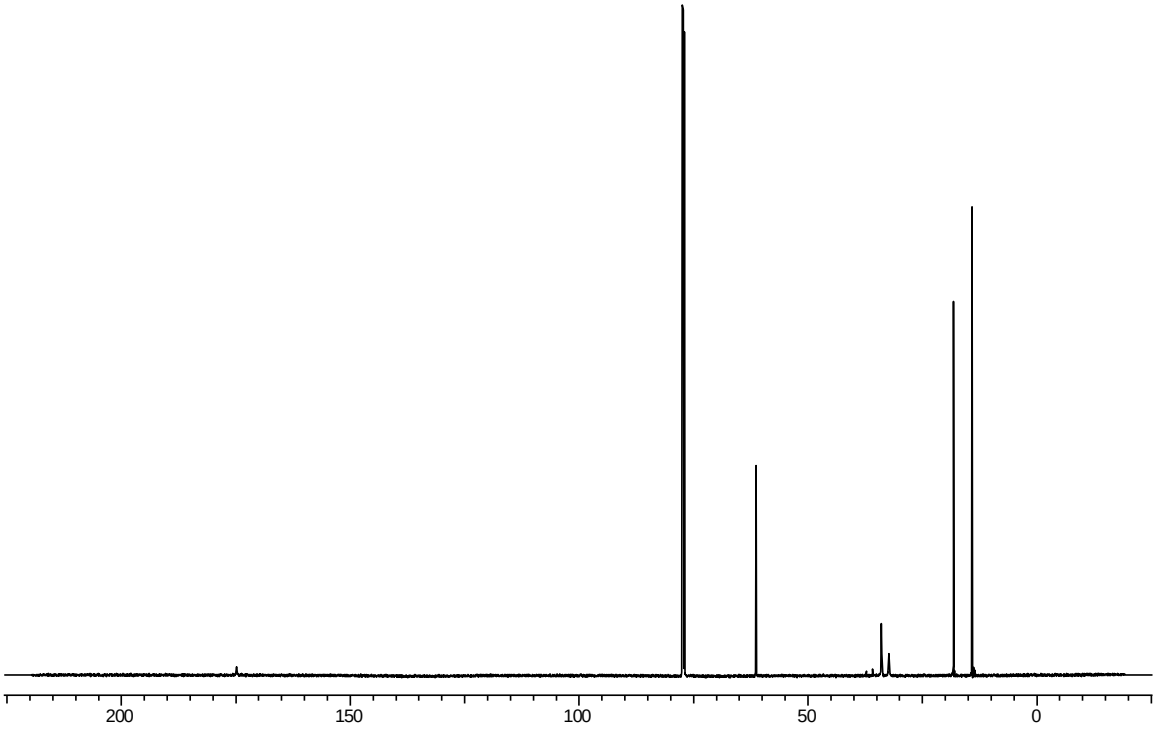
^{1}H NMR (600 MHz, $CDCl_3$)



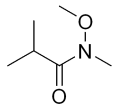


6

^{13}C NMR (150 MHz, $CDCl_3$)



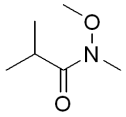
EA-1-11 1 1 /home/ctrabbi/avance600



7

^{1}H NMR (600 MHz, $CDCl_3$)

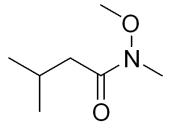
EA-1-11 3 1 /home/ctrabbi/avance600



7

^{13}C NMR (150 MHz, $CDCl_3$)

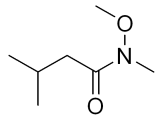
CT-1-95 1 1 /home/ctrabbi/avance600



8

^{1}H NMR (600 MHz, $CDCl_3$)

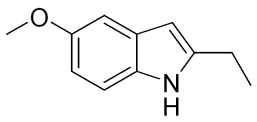
CT-1-95 3 1 /home/ctrabbi/avance600



8

^{13}C NMR (150 MHz, $CDCl_3$)

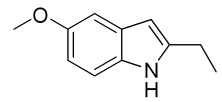
CT-1-94 1 1 /home/ctrabbi/avance600



10

^{1}H NMR (600 MHz, $CDCl_3$)

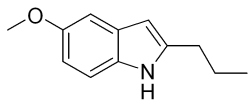
CT-1-94 3 1 /home/ctrabbi/avance600



10

^{13}C NMR (150 MHz, $CDCl_3$)

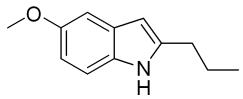
EA-1-9 1 1 /home/ctrabbi/avance600



11

^{1}H NMR (600 MHz, $CDCl_3$)

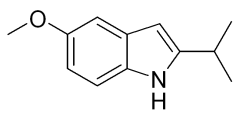
EA-1-9 3 1 /home/ctrabbi/avance600



11

^{13}C NMR (150 MHz, $CDCl_3$)

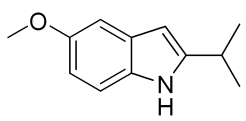
EA-1-13 1 1 /home/ctrabbi/avance600



12

^{1}H NMR (600 MHz, $CDCl_3$)

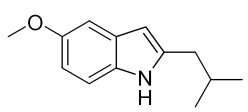
EA-1-13 3 1 /home/ctrabbi/avance600



12

^{13}C NMR (150 MHz, $CDCl_3$)

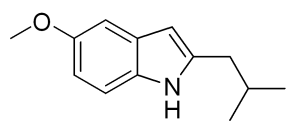
CT-1-99 1 1 /home/ctrabbi/avance600



13

^{1}H NMR (600 MHz, $CDCl_3$)

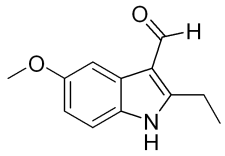
CT-1-99 3 1 /home/ctrabbi/avance600



13

^{13}C NMR (150 MHz, $CDCl_3$)

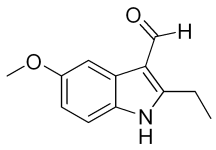
CT-1-96 1 1 /home/ctrabbi/avance600



14

^{1}H NMR (600 MHz, $CDCl_3$)

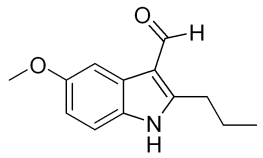
CT-1-96 3 1 /home/ctrabbi/avance600



14

^{13}C NMR (150 MHz, $CDCl_3$)

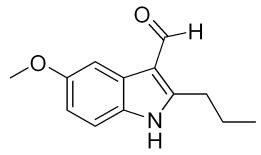
EA-1-10 1 1 /home/ctrabbi/avance600



15

^{1}H NMR (600 MHz, $CDCl_3$)

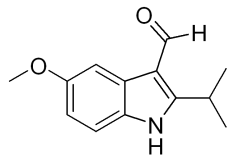
EA-1-10 4 1 /home/ctrabbi/avance600



15

^{13}C NMR (150 MHz, $CDCl_3$)

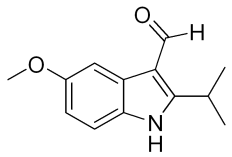
EA-1-14-1 1 1 /home/ctrabbi/avance600



16

^{1}H NMR (600 MHz, $CDCl_3$)

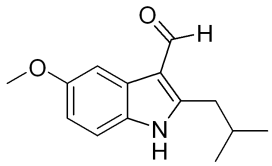
EA-1-14-1 3 1 /home/ctrabbi/avance600



16

^{13}C NMR (150 MHz, $CDCl_3$)

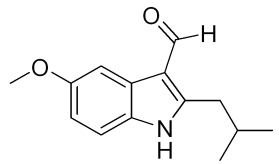
CT-1-100 1 1 /home/ctrabbi/avance600



17

^{1}H NMR (600 MHz, $CDCl_3$)

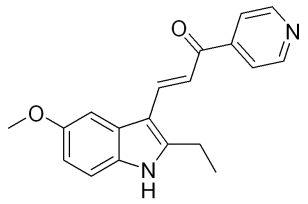
CT-1-100 3 1 /home/ctrabbi/avance600



17

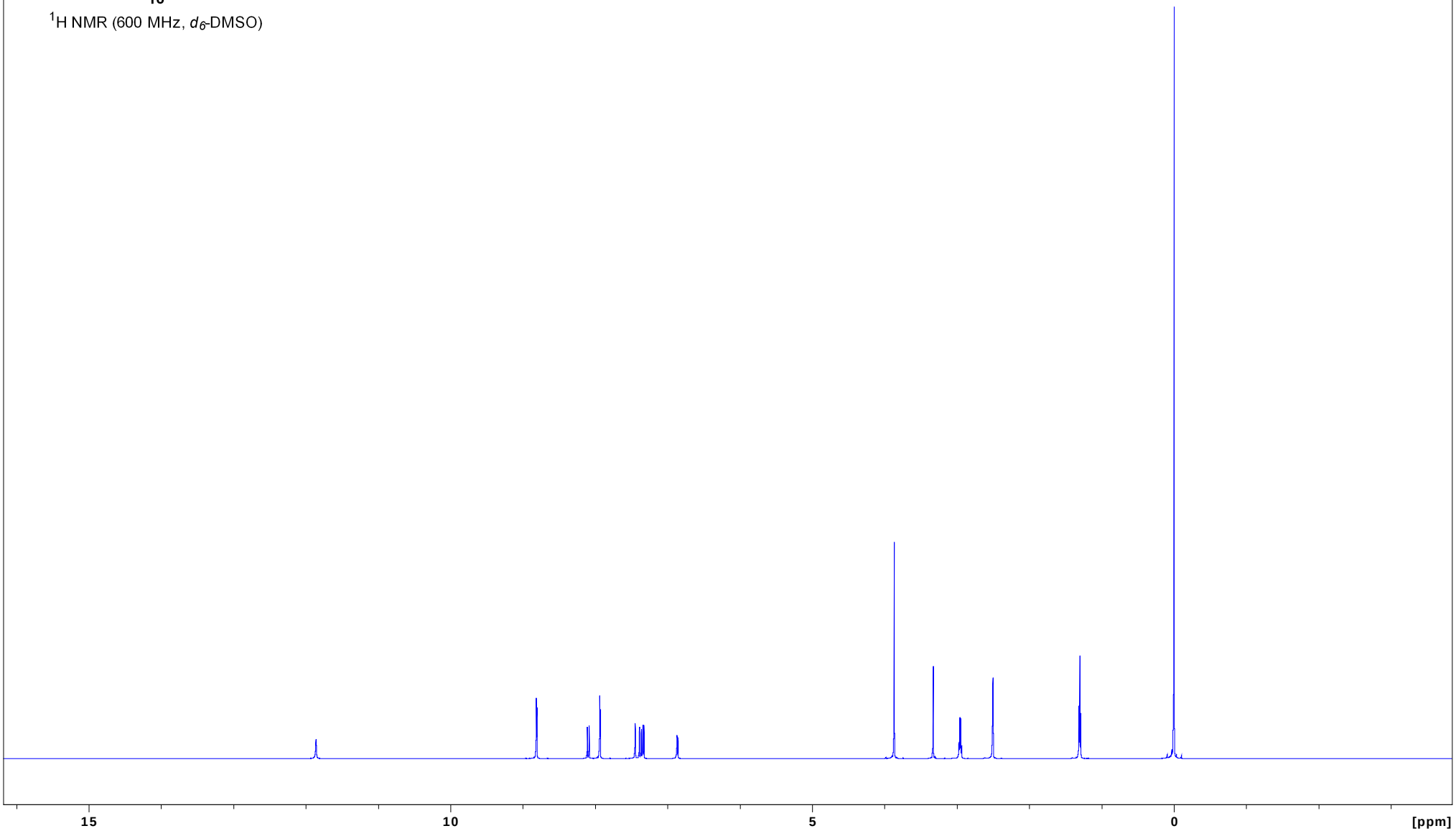
^{13}C NMR (150 MHz, $CDCl_3$)

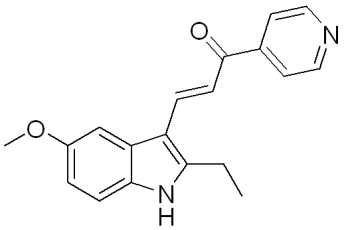
CT-1-97 1 1 /home/ctrabbi/avance600



18

^{1}H NMR (600 MHz, d_6-DMSO)





CT-1-97 2 1 /home/ctrabbi/avance600

18

COSY (d_6-DMSO)

F1 [ppm]

0

5

10

15

15

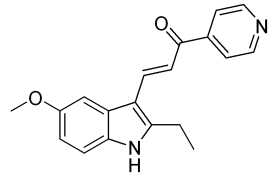
10

5

0

F2 [ppm]

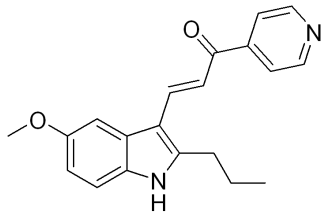
CT-1-97 3 1 /home/ctrabbi/avance600



18

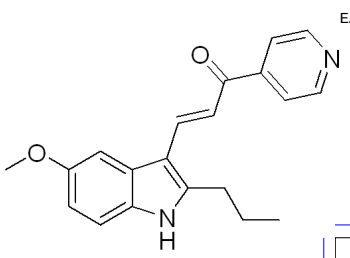
^{13}C NMR (150 MHz, d_6-DMSO)

EA-1-12 1 1 /home/ctrabbi/avance600



19

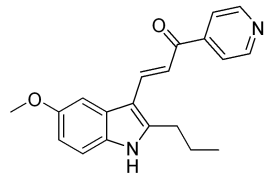
^{1}H NMR (600 MHz, d_6-DMSO)



19

COSY (d_6-DMSO)

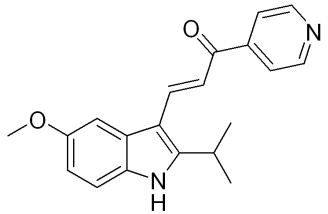
EA-1-12 3 1 /home/ctrabbi/avance600



19

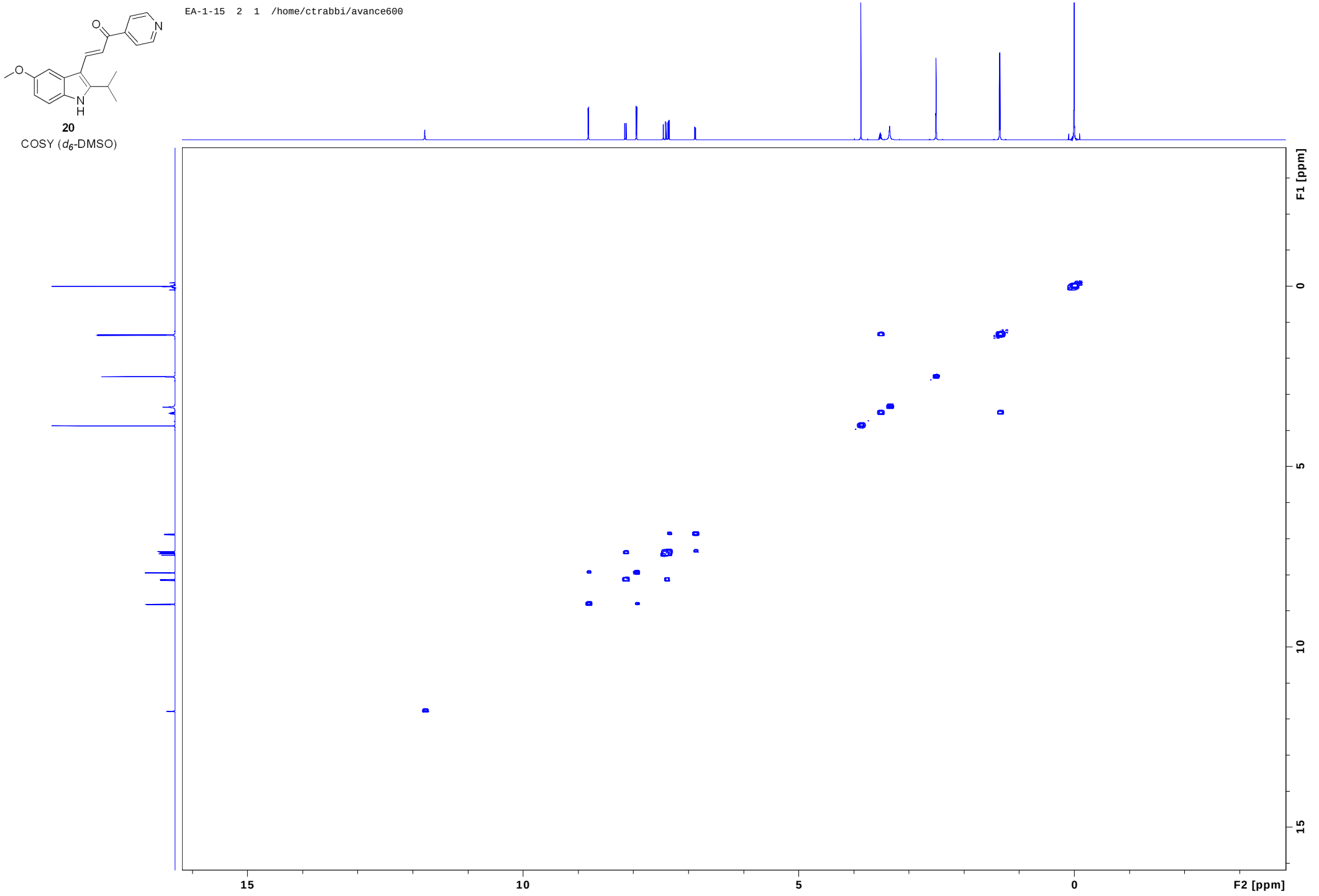
^{13}C NMR (150 MHz, d_6-DMSO)

EA-1-15 1 1 /home/ctrabbi/avance600



20

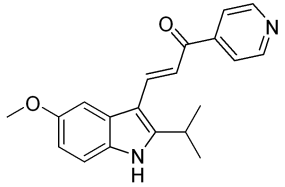
^{1}H NMR (600 MHz, d_6-DMSO)



20

COSY (d_6-DMSO)

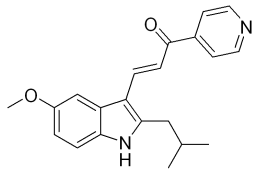
EA-1-15 3 1 /home/ctrabbi/avance600



20

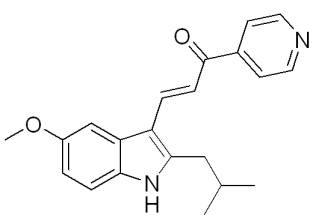
^{13}C NMR (150 MHz, d_6-DMSO)

CT-1-102 1 1 /home/ctrabbi/avance600



21

^{1}H NMR (600 MHz, d_6-DMSO)



21

COSY (d_6-DMSO)

CT-1-102 2 1 /home/ctrabbi/avance600

F1 [ppm]

0

5

10

15

F2 [ppm]

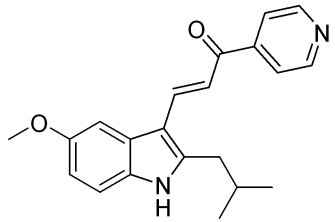
15

10

5

0

CT-1-102 3 1 /home/ctrabbi/avance600



21

^{13}C NMR (150 MHz, d_6-DMSO)

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