Supporting Information

The "gatekeeper" residue influences the binding mode of acetyl indoles to bromodomains

Andrea Unzue,[a] Hongtao Zhao,[b] Graziano Lolli,[b] Jing Dong,[b] Jian Zhu,[b]

Melanie Zechner,[a] Aymeric Dolbois,[a] Amedeo Caflisch,*[b] Cristina Nevado*[a]

Department of Chemistry[a] and Department of Biochemistry[b] University of Zürich

Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.

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*To whom correspondence should be addressed. Phone: (41) 446353945. Fax: (41) 446353948.
E-mail: cristina.nevado@chem.uzh.ch, caflisch@bioc.uzh.ch

1. Computational methods

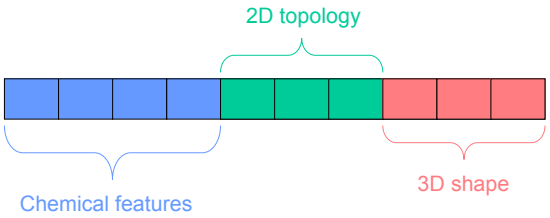


Figure S1. Scheme of the activity-oriented fingerprint (AoF), which consists of three sections: chemical features, 2D topology indices, and 3D shape descriptors. See below for detailed explanation.

Section A: Chemical features
1: number of heavy atoms divided by 2;
2: number of element phosphor
3: number of elements halogen (F + Cl + Br + I)
4: degrees of unsaturation
5: number of chemical rings
6: number of C.1
7: number of (N.ar + N.2)
8: number of (N.pl3 + N.am)
9: number of N.1
10: number of N.4
11: number of (O.3+S.3)
12: number of (O.2+S.2)
13: number of O.co2
14: number of S.o
15: number of S.o2
16: number of rotatable bonds.
*Atom types follow the definition of SYBYL atom types.

Section B: 2D topology
1: Randic first topology index divided by number of bonds scaled by a factor of 10
2: Randic second topology index divided by number of bonds scaled by a factor of 10

Section C: 3D shape
12 moments described in reference 1.

Similarity Index
In each section, a similarity index was computed between two molecules A and B by the following equation,[1]

$$simi = \frac{1}{1 + \frac{\sum_{i=1}^{n} | Ai - Bi |}{n}} \qquad (1)$$

The overall similarity index is the arithmetic mean of those from the three sections.

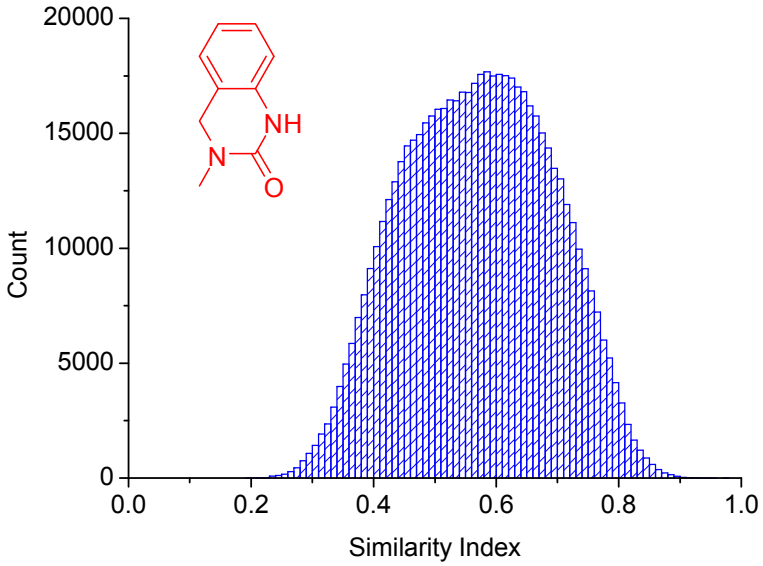


Figure S2. Distribution of similarity index between a query fragment and a 0.6 million fragments library. The 2D structure of the query molecule is shown in red.

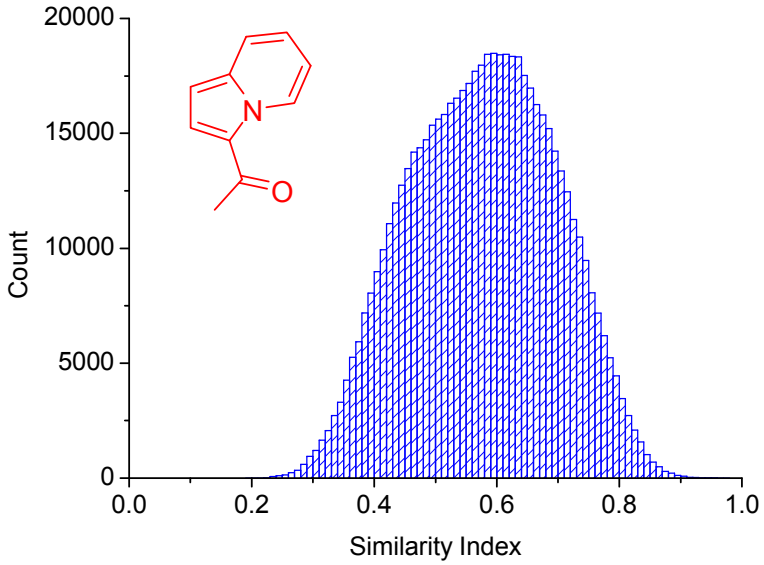


Figure S3. Distribution of similarity index between a query fragment and a 0.6 million fragments library. The 2D structure of the query molecule is shown in red.

2. Synthetic methods

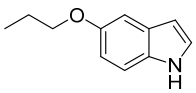
All reactions, unless otherwise stated, were carried out under a nitrogen atmosphere using standard Schlenk-techniques. All reagents were used as received unless otherwise noted. Solvents were purchased in the best quality available, degassed by purging thoroughly with nitrogen and dried over activated molecular sieves of appropriate size. Alternatively, they were purged with argon and passed through alumina columns in a solvent purification system (Innovative Technology). Reactions were monitored by thin layer chromatography (TLC) using Merck TLC silica gel 60 F_{254}. Flash column

chromatography was performed over silica gel (230-400 mesh). NMR spectra were recorded on AV 300, AV2 400 or AV2 500 MHz Bruker spectrometers. Chemical shifts are given in ppm. The spectra are calibrated to the residual ^{1}H and ^{13}C signals of the solvents. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), doublet-doublet (dd), quintet (quint), septet (sept), multiplet (m), and broad (br). Melting points were determined on a Mettler Toledo MP70 melting point instrument. Infrared spectra were recorded on a JASCO FT/IR-4100 spectrometer. High-resolution electrospray ionization mass spectrometry was performed on a Finnigan MAT 900 (Thermo Finnigan, San Jose, CA, USA) double-focusing magnetic sector mass spectrometer. Ten spectra were acquired. A mass accuracy ≤2 ppm was obtained in the peak matching acquisition mode by using a solution containing 2 µL PEG200, 2 µL PPG450, and 1.5 mg NaOAc (all obtained from Sigma-Aldrich, Buchs, Switzerland) dissolved in 100 mL MeOH (HPLC Supra grade, Scharlau, E-Barcelona) as internal standard. The purity of all tested compounds was determined by HPLC on a Waters Acquity UPLC (Waters, Milford, MA) Top spectrometer using an Acquity BEH C_{18} HPLC column (1.7 µm, 1× 50 mm, Waters) with a mixture of H_2O + 0.1 % HCOOH (A) and CH_3CN + 0.1 % HCOOH (B) solvent (0.1 mL flow rate, linear gradient from 5 % to 98 % B within 4 min followed by flushing with 98 % B for 1 min). Unless otherwise stated, all compounds showed ≥ 95 % purity.

The following compounds were prepared according to previously reported procedures: **30-32**,[2] **33**,[3] **51**,[4] and **53**.[5]

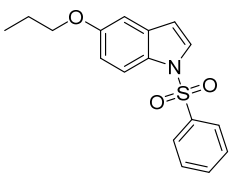
2.1 Synthesis of the indole derivative of GSK2801 (1a)

5-Propoxy-1H-indole (51)[4]



Yellow solid; Yield: 84 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ =8.02 (br, 1H), 7.30 – 7.22 (m, 1H), 7.16 (t, J = 2.9 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 6.88 (dd, J = 8.8, 2.4 Hz, 1H), 6.47 (ddd, J = 3.1, 2.0, 1.0 Hz, 1H), 3.97 (t, J = 6.6 Hz, 2H), 1.83 (h, J = 6.9 Hz, 2H), 1.06 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.6, 131.0, 128.3, 124.8, 112.9, 111.6, 103.6, 102.3, 70.4, 22.8, 10.6; IR (film): $\tilde{\upsilon}$ = 3411, 2962, 2933, 2875, 1623, 1580, 1282, 1221, 1156, 1122, 982, 799, 755, 719, 603, 427 cm^{-1}; MS (ESI): m/z: calcd for $C_{11}H_{14}NO^{+}$: 176.1, found: 176.0.

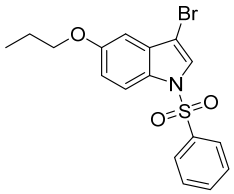
1-(Phenylsulfonyl)-5-propoxy-1*H*-indole (2)



To a solution of 5-propoxy-1*H*-indole (500 mg, 2.85 mmol) in H_2O (4.30 mL) and toluene (4.30 mL), 50 % aq. NaOH (2.90 mL) and tetra-*n*-butylammonium bromide (184 mg, 0.571 mmol) were sequentially added at

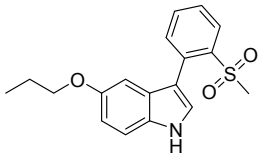
room temperature. Benzenesulfonyl chloride (509 µL, 3.99 mmol) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and it was stirred for 69 hours. The solvent was removed under reduced pressure, water (*ca.* 15.0 mL) was added and the remaining aqueous mixture was extracted with Et_2O three times. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure obtaining the desired product as a yellow solid (876 mg, 2.77 mmol, 97 % yield), which was used without further purification. Mp: 95-102 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.88 (d, *J* = 9.0 Hz, 1H), 7.85 (d, *J* = 1.1 Hz, 1H), 7.83 (d, *J* = 1.6 Hz, 1H), 7.55 – 7.46 (m, 2H), 7.41 (virt.t, *J* = 7.6 Hz, 2H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.93 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.58 (dd, *J* = 3.6, 0.8 Hz, 1H), 3.91 (t, *J* = 6.5 Hz, 2H), 1.80 (h, *J* = 7.4 Hz, 2H), 1.03 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.0, 138.2, 133.6, 131.8, 129.5, 129.2, 127.0, 126.6, 114.3, 114.3, 109.4, 104.5, 70.0, 22.6, 10.5; IR (neat): ῡ = 3133, 2966, 1608, 1445, 1363, 1337, 1260, 1224, 1174, 1154, 1142, 1120, 1088, 1022, 817, 802, 772, 724; HRMS (ESI): m/z: calcd for $C_{17}H_{17}NNaO_3S^+$: 338.0827, found: 338.0816.

3-Bromo-1-(phenylsulfonyl)-5-propoxy-1*H*-indole (3)



To a solution of 1-(phenylsulfonyl)-5-propoxy-1*H*-indole (400 mg, 1.27 mmol) in DMF (4.70 mL) a solution of bromine (65.0 µL, 1.27 mmol) in DMF (3.70 mL) was added dropwise. As the reaction did not show full conversion, a bromine solution (13.0 µL, 0.254 mmol) in DMF (0.740 mL) was added every hour a total of four times. After seven hours the reaction was poured into ice water (47.0 mL) containing 0.5 % NH_3 and 0.1 % $Na_2S_2O_3$. The precipitate was collected by filtration and washed with ice water (*ca.* 8.00 mL). Purification by flash column chromatography (silica gel; Hex/EtOAc = 15/1) afforded the desired product as a white solid (233 mg, 0.591 mmol, 47 % yield). Mp: 109-115 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ =7.91 – 7.82 (m, 3H), 7.57 (s, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.49 – 7.40 (m, 2H), 6.98 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.89 (d, *J* = 2.5 Hz, 1H), 3.95 (t, *J* = 6.5 Hz, 2H), 1.82 (h, *J* = 7.3 Hz, 2H), 1.04 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.0, 137.8, 134.0, 130.8, 129.3, 128.8, 126.8, 125.3, 115.8, 114.6, 102.7, 99.9, 70.1, 22.6, 10.5; IR (neat): ῡ = 3133, 2961, 2876, 1463, 1444, 1363, 1201, 1185, 1166, 1147, 1112, 1089, 1021, 837, 801, 724; HRMS (ESI): m/z: calcd for $C_{17}H_{16}BrNNaO_3S^+$: 415.9932, found: 415.9927.

3-(2-(Methylsulfonyl)phenyl)-5-propoxy-1*H*-indole (52)

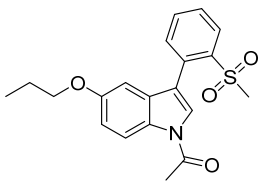


To a solution of 3-bromo-1-(phenylsulfonyl)-5-propoxy-1*H*-indole (1.0 eq) in degassed 1,2-dimethoxyethane (0.2 M), 5.00 mol % $Pd(PPh_3)_4$ was added and stirred at room temperature for 5 minutes. The reaction mixture was

heated to 85 °C and the corresponding boronic acid (1.2 eq) in 1,2-dimethoxyethane (0.6 M) and 1 M aq. $NaHCO_3$ (4.1 eq) were simultaneously added dropwise over 5 minutes and stirred at 85 °C. Although the conversion to the product was not complete, the reaction mixture was cooled to room temperature. The solvent was concentrated, water was added and the remaining aqueous mixture was extracted with DCM three times. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (silica gel; Hex/EtOAc = 3/1) obtaining the desired product, 3-(2-(methylsulfonyl)phenyl)-1-(phenylsulfonyl)-5-propoxy-1*H*-indole (**4**) as a yellow/brown solid (38.9 mg, 0.0828 mmol, 33 % yield).

To a solution of 3-(2-(methylsulfonyl)phenyl)-1-(phenylsulfonyl)-5-propoxy-1*H*-indole (43.0 mg, 0.0916 mmol) in MeOH (232 µL), 2 M aq. NaOH (300 µL) was added dropwise and the reaction was stirred at 85°C for 5 hours. The solvent was concentrated and after flash column chromatography (silica gel; Hex/EtOAc = 3/1) the desired product was obtained as a light purple oil (20.8 mg, 0.0631 mmol, 69 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.35 (br, 1H), 8.31 (dd, J = 8.1, 1.3 Hz, 1H), 7.96 (dd, J = 8.4, 1.4 Hz, 1H), 7.72 – 7.64 (m, 2H), 7.63 – 7.56 (m, 1H), 7.55 – 7.50 (m, 1H), 7.36 (dd, J = 8.5, 0.9 Hz, 1H), 6.93 (s, 1H), 3.90 (t, J = 6.6 Hz, 2H), 2.59 (s, 3H), 1.80 (h, J = 7.1 Hz, 2H), 1.03 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 154.5, 139.6, 134.4, 133.7, 133.4, 133.1, 129.4, 128.8, 127.6, 127.3, 126.9, 113.2, 112.1, 101.8, 70.3, 44.6, 22.7, 10.7; IR (neat): $\tilde{\upsilon}$ = 2962, 2928, 1978, 1485, 1466, 1301, 1206, 1149, 958, 908, 731; HRMS (ESI): m/z: calcd. for $C_{18}H_{19}NNaO_3S^+$: 352.0983, found: 352.0978.

1-(3-(2-(Methylsulfonyl)phenyl)-5-propoxy-1*H*-indol-1-yl)ethan-1-one (5)

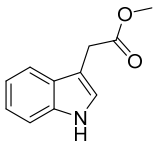


To a solution of 3-(2-(methylsulfonyl)phenyl)-5-propoxy-1*H*-indole (3.60 mg, 0.0109 mmol) in DCM (125 µL), freshly powdered NaOH (9.17 mg, 0.230 mmol) and TBAHS (1.90 mg, 0.000547 mmol) were sequentially added and stirred at room temperature for 5 minutes followed by the dropwise addition of acetyl chloride (7.80 µL, 0.109 mmol). The reaction was stirred for 13 hours. To obtain full conversion freshly powdered NaOH (4.59 mg, 0.115 mmol), TBAHS (0.950 mg, 0.000274 mmol) and acetyl chloride (3.90 µl, 0.055 mmol) were sequentially added twice within seven hours. The reaction was quenched with water (*ca.* 1.00 mL) followed by extraction with DCM three times. The combined organic layers were dried over $MgSO_4$ and the residue was purified by flash column chromatography (silica gel; Hex/EtOAc = 4/1) affording the final product as a light brown solid (2.50 mg, 0.00673 mmol, 63 % yield). Mp: 130-133 °C; Purity: 82 %; ^{1}H NMR (1:0.25 rotamer ratio, asteriks denote minor rotamer peaks, 500 MHz, DMSO-d_6): δ = 8.32* (d, J = 9.3

Hz, 1H), 8.27 (d, *J* = 8.8 Hz, 1H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.09* (d, *J* = 8.3 Hz, 1H), 8.04* (s, 1H), 7.99 (s, 1H), 7.84 (t, *J* = 7.7 Hz, 1H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 1H), 7.53* (d, *J* = 7.5 Hz, 1H), 7.21* (d, *J* = 9.2 Hz, 1H), 7.00 (dd, *J* = 9.0, 1.9 Hz, 1H), 6.69 (d, *J* = 1.6 Hz, 1H), 4.01* (t, *J* = 6.5 Hz, 2H), 3.86 (t, *J* = 6.4 Hz, 2H), 2.98* (s, 3H), 2.91 (s, 3H), 2.63 (s, 3H), 1.72-1.66 (m, 2H), 0.95 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.7, 156.6, 139.9, 133.3, 133.1, 132.1, 131.0, 129.9, 129.1, 128.3, 128.1, 117.8, 117.0, 114.1, 102.8, 70.0, 42.4, 23.8, 22.7, 10.6; IR (neat): $\tilde{\upsilon}$ = 2963, 2925, 2157, 2035, 2022, 1975, 1709, 1606, 1461, 1385, 1307, 1239, 1150, 967, 765; HRMS (ESI): m/z: calcd. for $C_{20}H_{21}NNaO_4S^+$: 394.1089, found: 394.1084.

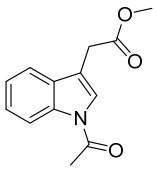
2.2 Synthesis of 1b and derivatives

Methyl 2-(1-acetyl-5-propoxy-1*H*-indol-3-yl)acetate (53)[5]



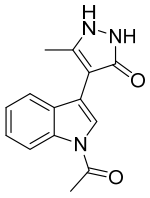
94 % yield. Mp: 43-47 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.12 (br, 1H), 7.68 – 7.54 (m, 1H), 7.31 – 7.24 (m, 1H), 7.21 – 7.16 (m, 1H), 7.16 – 7.11 (m, 1H), 7.03 (d, *J* = 2.4 Hz, 1H), 3.78 (d, *J* = 0.9 Hz, 2H), 3.70 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 136.1, 127.1, 123.1, 122.1, 119.6, 118.7, 111.2, 108.1, 51.9, 31.1; IR (neat): $\tilde{\upsilon}$ = 3358, 1717, 1457, 1432, 1336, 1242, 1201, 1157, 1094, 1009, 740; HRMS (ESI): m/z: calcd. for $C_{11}H_{11}NNaO_2^+$: 212.0687, found: 212.0682.

Methyl 2-(1-acetyl-1*H*-indol-3-yl)acetate (7)



To a solution of methyl 2-(1*H*-indol-3-yl)acetate (1.00 g, 5.29 mmol) in THF (2.10 mL), DMAP (103 mg, 0.846 mmol), acetic anhydride (8.50 mL, 89.8 mmol) and Et_3N (2.20 mL, 15.9 mmol) were sequentially added and the reaction was stirred at room temperature for 17 hours. It was quenched by the addition of a saturated aq. $NaHCO_3$ solution (*ca.* 10.00 mL) followed by extraction with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and the obtained residue was purified by flash column chromatography (silica gel; Hex/EtOAc = 5/1) affording the desired product as a yellow oil (1.10 g, 4.76 mmol, 90 % yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.43 (d, *J* = 8.1 Hz, 1H), 7.53 (ddd, *J* = 7.6, 1.4, 0.7 Hz, 1H), 7.45 (s, 1H), 7.41 – 7.34 (m, 1H), 7.30 (virt.td, 1H), 3.74 (s, 2H), 3.74 (s, 3H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.2, 168.3, 135.6, 130.0, 125.3, 123.8, 123.5, 118.7, 116.6, 114.8, 52.1, 30.6, 23.8; IR (neat): $\tilde{\upsilon}$ = 1736, 1699, 1449, 1437, 1384, 1371, 1350, 1330, 1245, 1222, 1194, 1163, 1144, 1016, 934; HRMS (ESI): m/z: calcd. for $C_{13}H_{13}NNaO_3^+$: 254.0793, found: 254.0788.

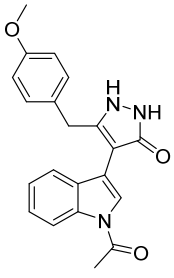
4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one (1b)



A solution of (^{i}Pr)$_2$NH (721 µL, 5.14 mmol), *n*-BuLi (2.5 M in hexane, 1.97 mL, 4.93 mmol) in THF (6.20 mL) was stirred at 0 °C for 15 minutes. After cooling to -78 °C a solution of methyl 2-(1-acetyl-1*H*-indol-3-yl)acetate (990 mg, 4.28 mmol) in THF (12.4 mL) was added dropwise and stirred at -78 °C for 1 hour. Then a solution of acetic anhydride (485 µL, 5.41 mmol) in THF (6.20 mL) was added dropwise and the reaction was stirred for 4 hours followed by quenching with a saturated aq. NH_4Cl solution (ca. 25.0 ml) and extraction with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and the residue was purified by flash column chromatography (silica gel; Hex/EtOAc 5/1). The desired product could not be obtained in pure form and it was used in the next step without further purification.

To a solution of unpure methyl (*Z*)-2-(1-acetyl-1*H*-indol-3-yl)-3-hydroxybut-2-enoate (148 mg, 0.542 mmol) in a 1:1 mixture of EtOH and toluene (14.0 mL of each solvent) camphoric acid (43.4 mg, 0.217 mmol) and hydrazine monohydrate (24.2 µL, 0.487 mmol) were sequentially added and the reaction was stirred at 93 °C for 40 minutes. The solvent was concentrated and the residue was purified by flash column chromatography (silica gel; DCM/MeOH 2 % to DCM/MeOH 9 %) affording the desired product as a white solid (77.7 mg, 0.305 mmol, 51 % yield over two steps). Mp: 88-93 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.52 (br, 1H), 9.76 (br, 1H), 8.35 (d, J = 8.3 Hz, 1H), 7.65 (s, 1H), 7.58 (d, J = 7.2 Hz, 1H), 7.33 (virt.td, 1H), 7.25 (virt.td, 1H), 2.66 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6): δ = 169.2, 159.3, 135.0, 130.1, 124.5, 123.7, 123.0, 121.0, 115.7, 113.7, 109.5, 94.7, 23.9, 10.9; IR (neat): $\tilde{\upsilon}$ = 3315, 3233, 2668, 1682, 1513, 1449, 1385, 1353, 1331, 1282, 1253, 1226, 1189, 1140, 1018, 965, 917, 818, 767, 747; HRMS (ESI): m/z: calcd. for $C_{14}H_{14}N_3O_3^+$: 256.1086, found: 256.1081.

4-(1-Acetyl-1*H*-indol-3-yl)-5-(4-methoxybenzyl)-1,2-dihydro-3*H*-pyrazol-3-one (10)



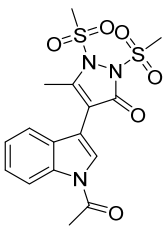
A solution of (^{i}Pr)$_2$NH (721 µl, 5.14 mmol), BuLi (1.6 M in hexane, 3.0 mL, 4.93 mmol) in THF (6.2 mL) was stirred at 0 °C for 15 minutes. After cooling to -78 °C, a solution of methyl 2-(1-acetyl-1*H*-indol-3-yl)acetate (990 mg, 4.28 mmol) in THF (12.4 mL) was added dropwise and stirred at -78 °C for 1 hour. Then a solution of 2-(4-methoxyphenyl)acetic anhydride (1.75 g, 5.56 mmol) in THF (6.2 mL) was added dropwise and the reaction was stirred for 2 hours followed by quenching with a saturated aq. NH_4Cl solution and extraction with EtOAc three times. The obtained organic layers were dried over $MgSO_4$ and the residue was purified by flash column chromatography (hexane:EtOAc, 4:1). The obtained product was then diluted in a 1:1

EtOH:Tol mixture (0.065 M) and hydrazine hydrate was added (1.0 eq). The reaction mixture was heated to reflux for 3 hours and concentrated under reduced pressure. Upon the addition of DCM, the desired product precipitated, it was filtered off and washed with DCM, obtaining the final product as a white solid in 29 % yield. mp 234-237 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.33 (d, J = 8.2 Hz, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.37 (s, 1H), 7.34 – 7.28 (m, 1H), 7.26 – 7.19 (m, 1H), 7.04 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 8.6 Hz, 2H), 3.87 (s, 2H), 3.68 (s, 3H), 2.56 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.1, 157.7, 135.0, 131.0, 130.2, 129.1, 124.5, 123.7, 124.0, 121.0, 115.7, 113.8, 113.5, 99.8, 99.0, 55.0, 23.8, two carbons are missing due to overlapping.; IR (film): ῡ = 3323, 3276, 1702, 1682, 1584, 1510, 1447, 1381, 1248, 1223, 1177, 1033, 963, 747, 633, 421, 414 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{21}H_{20}N_3O_3^+$: 362.1499, found: 362.1498.

General procedure for di-substituted amide and sulfonamide synthesis

4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one (**I**, 1.0 eq) was dissolved in DCM (0.2 M) and cooled to 0 °C. Et_3N (2.4 eq. in general, 4.2 eq. for **15** and **16**) and the corresponding acid or sulfonyl chloride (2.2 eq. in general, 4.0 eq. for compounds **15** and **16**) were added. The solution was allowed to warm to room temperature and stirred for 5-12 hours. The reaction mixture was quenched with water and extracted with EtOAc three times. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (1:1 hexane/EtOAc for compounds **11** and **13,** 2:1 hexane/EtOAc for compound **12**, 5:1 hexane/EtOAc for compounds **14** and **17** and 3:1 hexane/EtOAc for compounds **15**, **16** and **18**) affording the desired products in pure form. This procedure was followed to obtain the final compounds **11-18**.

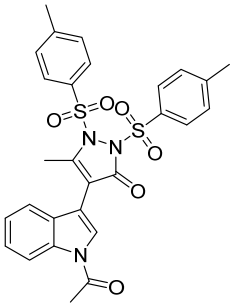
4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-1,2-bis(methylsulfonyl)-1,2-dihydro-3*H*-pyrazol-3-one (11)



Green oil; Yield: 89 %; ^{1}H NMR (1:0.5 rotamer ratio, asteriks denote minor rotamer peaks, 400 MHz, DMSO-d_6): δ = 8.40 (d, J = 8.6 Hz, 1H), 8.40* (d, J = 8.6 Hz, 1H), 8.08 (s, 1H), 7.97* (s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.50* (d, J = 7.8 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.41* (t, J = 7.6 Hz, 1H), 7.37 – 7.32 (m, 1H), 7.37 – 7.32* (m, 1H), 3.69* (s, 3H), 3.61 (s, 3H), 3.53* (s, 3H), 3.13 (s, 3H), 2.69 (s, 3H), 2.69* (s, 3H), 2.52* (s, 3H), 2.30 (s, 3H); ^{13}C NMR (presence of rotamers, 100 MHz, $CDCl_3$): δ = 168.7, 168.6, 168.6, 154.8, 153.0, 144.2, 140.8, 135.7, 135.6, 135.6, 129.5, 129.0, 128.9, 125.9, 125.8, 125.7, 125.6, 124.7, 124.1, 124.1, 123.9, 119.7, 119.6, 119.5, 116.9, 116.8, 109.3, 109.1, 108.0, 107.0, 53.4, 41.9, 41.8, 41.3, 39.7, 39.5, 39.4, 38.9, 31.5, 29.7, 24.0, 23.9, 14.1, 12.8, 11.2; IR (film): ῡ = 3015, 2932,

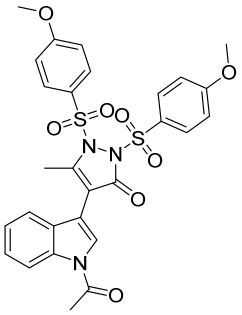
1708, 1450, 1372, 1330, 1225, 1177, 1036, 962, 748, 552, 517 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{16}H_{17}N_3NaO_6S_2^+$: 434.0451, found: 434.0446.

4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-1,2-ditosyl-1,2-dihydro-3*H*-pyrazol-3-one (12)



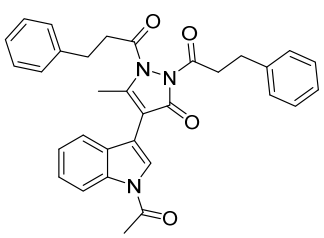
White solid; mp 189-193 °C; Yield: 69 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.35 (d, *J* = 8.3 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.34 – 7.27 (m, 3H), 7.20 – 7.14 (m, 2H), 7.09 (d, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 2H), 2.53 (s, 3H), 2.42 (s, 3H), 2.40 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.4, 154.6, 146.1, 145.5, 143.2, 135.5, 134.4, 132.7, 130.1, 129.3, 128.9, 128.4, 128.2, 125.6, 125.0, 123.9, 119.7, 116.7, 109.7, 107.8, 23.9, 21.8, 21.7, 12.9; IR (film): ῡ = 1701, 1451, 1442, 1377, 1334, 1256, 1194, 1174, 1156, 1090, 850, 809, 727, 712, 664, 647, 586, 541 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{28}H_{25}N_3NaO_6S_2^+$: 586.1077, found: 586.1074.

4-(1-Acetyl-1*H*-indol-3-yl)-1,2-bis((4-methoxyphenyl)sulfonyl)-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one (13)



White solid; mp 185-189 °C; Yield: 31 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.42 (d, *J* = 8.3 Hz, 1H), 7.95 (d, *J* = 9.0 Hz, 2H), 7.52 (d, *J* = 8.9 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.31 (s, 1H), 7.25 (t, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 9.0 Hz, 2H), 6.66 (d, *J* = 8.9 Hz, 2H), 3.91 (s, 3H), 3.80 (s, 3H), 2.61 (s, 3H), 2.50 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.4, 164.6, 164.0, 154.4, 142.9, 135.5, 130.7, 130.6, 128.9, 128.5, 126.8, 125.6, 124.9, 123.9, 119.7, 114.6, 113.8, 109.8, 107.5, 55.9, 55.6, 23.9, 12.8, one carbon is missing due to overlapping; IR (film): ῡ = 2922, 2848, 1713, 1592, 1578, 1498, 1445, 1379, 1372, 1259, 1223, 1199, 1169, 1158, 1091, 1019, 848, 833, 803, 753, 727, 669, 584, 569, 548, 503 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{28}H_{25}N_3NaO_8S_2^+$: 618.0975, found: 618.0972.

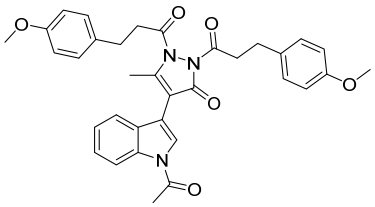
1,1'-(4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-3-oxo-1*H*-pyrazole-1,2(3*H*)-diyl)bis(3-phenylpropan-1-one) (14)



White solid; mp 164-170 °C; Yield: 85 %; Purity: 94 %; ^{1}H NMR (presence of rotamers, 300 MHz, $CDCl_3$): δ =8.49 (d, *J* = 8.1 Hz, 1H), 7.47 – 7.37 (m, 2H), 7.35 – 7.27 (m, 6H), 7.24 – 7.19 (m, 2H), 7.16 – 7.11 (m, 2H), 7.07 (dd, *J* = 6.7, 3.0 Hz, 1H), 7.02 – 6.96 (m, 1H), 3.53 – 3.40 (m, 2H), 3.10 (q, *J* = 7.4 Hz, 2H), 3.01 – 2.92 (m, 2H), 2.76 – 2.64

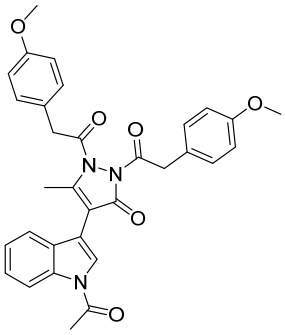
(m, 2H), 2.58 (s, 1.3H), 2.56 (s, 1.8 H), 2.56 (s, 1.1H), 2.26 (s, 1.8H); ^{13}C NMR (presence of rotamers, 126 MHz, $CDCl_3$): δ = 177.6, 172.9, 170.3, 168.5, 168.4, 161.8, 155.3, 143.7, 143.3, 140.3, 140.2, 139.4, 135.7, 129.3, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.0, 126.4, 126.4, 126.3, 125.8, 125.6, 124.6, 124.4, 123.9, 123.8, 120.2, 119.8, 110.8, 108.5, 105.1, 53.4, 36.8, 36.4, 35.4, 35.2, 30.6, 30.2, 30.1, 29.9, 24.0, 14.6, 14.0; IR (film): ῡ = 3026, 2927, 1706, 1561, 1448, 1372, 1329, 1221, 1191, 1033, 965, 746, 697, 643, 560 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{32}H_{29}N_3NaO_4^+$: 542.2050, found: 542.2045.

1,1'-(4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-3-oxo-1*H*-pyrazole-1,2(3*H*)-diyl)bis(3-(4-methoxyphenyl)propan-1-one) (15)



Colorless oil; Yield: 35 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.48 (d, *J* = 7.9 Hz, 1H), 7.48 – 7.37 (m, 2H), 7.33 – 7.27 (m, 2H), 7.22 – 7.15 (m, 3H), 6.98 (d, *J* = 8.6 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 6.86 (dd, *J* = 8.7, 2.9 Hz, 2H), 6.68 (dd, *J* = 8.6, 3.6 Hz, 2H), 3.80 (s, 3H), 3.75 (s, 3H), 3.43 (dt, *J* = 17.5, 7.9 Hz, 2H), 3.04 (dt, *J* = 12.3, 7.7 Hz, 2H), 2.85 (tt, *J* = 8.6, 4.5 Hz, 2H), 2.70 (tt, *J* = 8.9, 4.5 Hz, 2H), 2.58 (s, 1H), 2.57 (d, *J* = 1.5 Hz, 2H), 2.31 (d, *J* = 50.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 172.9, 171.6, 170.4, 170.1, 168.4, 158.1, 155.3, 151.0, 144.1, 143.3, 137.9, 135.7, 132.4, 131.6, 131.4, 129.5, 129.4, 129.3, 129.1, 129.0, 129.0, 128.2, 125.7, 125.7, 125.3, 124.4, 124.1, 123.9, 120.0, 119.8, 116.8, 113.9, 113.9, 113.8, 113.8, 110.9, 110.8, 108.4, 106.5, 55.3, 55.2, 54.4, 37.1, 35.4, 35.4, 33.5, 29.4, 29.3, 29.2, 29.2, 24.5, 23.9, 21.4, 20.8, 20.2, 14.1, 14.0, 13.9, presence of rotamers; IR (film): ῡ = 2927, 1769, 1733, 1710, 1611, 1512, 1448, 1373, 1328, 1245, 1220, 1177, 1103, 1033, 910, 824, 751, 729, 638, 520 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{34}H_{34}N_3O_6^+$: 580.2442, found: 580.2448.

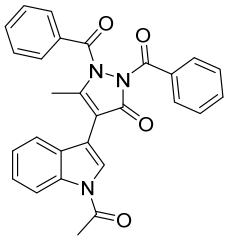
1,1'-(4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-3-oxo-1*H*-pyrazole-1,2(3*H*)-diyl)bis(2-(4-methoxyphenyl)ethan-1-one) (16)



Colorless oil; Yield: 45 %; Purity: 87 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.46 (d, *J* = 8.2 Hz, 1H), 7.39 (t, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.18 – 7.14 (m, 1H), 7.10 (s, 1H), 7.05 (t, *J* = 7.9 Hz, 1H), 6.98 (d, *J* = 7.7 Hz, 1H), 6.95 (s, 1H), 6.86 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.73 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.59 (d, *J* = 7.8 Hz, 1H), 6.57 (s, 1H), 4.44 (s, 2H), 3.82 (s, 3H), 3.69 (s, 2H), 3.62 (s, 3H), 2.53 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (presence of rotamers, 126 MHz, $CDCl_3$): δ = 171.5, 169.0, 168.4, 159.7, 159.6, 155.4, 145.0, 135.5, 134.7, 133.7, 129.6, 129.6, 129.3, 129.0, 128.2, 125.7, 125.3,

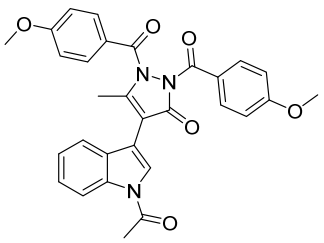
124.8, 123.9, 122.3, 121.2, 119.6, 116.8, 115.6, 114.6, 112.9, 112.9, 110.2, 108.8, 55.2, 55.0, 41.6, 40.8, 23.8, 14.0; IR (film): ῦ = 2927, 2838, 1769, 1734, 1710, 1599, 1585, 1490, 1448, 1377, 1330, 1258, 1221, 1148, 1105, 1037, 968, 906, 752, 729, 690, 641, 424 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{32}H_{30}N_3O_6^+$: 552.2129, found: 552.2131.

(4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-3-oxo-1*H*-pyrazole-1,2(3H)-diyl)bis(phenylmethanone) (17)



White solid; mp 169-175 °C; Yield: 45 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.44 (d, *J* = 8.1 Hz, 1H), 8.11 – 8.06 (m, 2H), 8.05 – 8.00 (m, 2H), 7.63 – 7.56 (m, 2H), 7.57 – 7.55 (m, 1H), 7.54 – 7.52 (m, 1H), 7.51 (d, *J* = 1.2 Hz, 1H), 7.45 (s, 1H), 7.44 – 7.38 (m, 2H), 7.36 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.33 – 7.27 (m, 1H), 2.74 (s, 3H), 2.56 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.4, 168.1, 164.2, 155.7, 144.4, 135.7, 134.2, 133.0, 132.4, 131.4, 130.4, 129.3, 128.6, 128.1, 127.9, 125.7, 124.6, 123.9, 119.9, 116.7, 111.0, 108.8, 23.9, 14.1; IR (film): ῦ = 3062, 2922, 2854, 1740, 1701, 1599, 1541, 1446, 1377, 1330, 1241, 1221, 1063, 1001, 937, 749, 712, 694, 668, 640, 423 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{28}H_{21}N_3NaO_4^+$: 486.1424, found: 486.1423.

(4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-3-oxo-1*H*-pyrazole-1,2(3*H*)-diyl)bis((4-methoxyphenyl)methanone) (18)



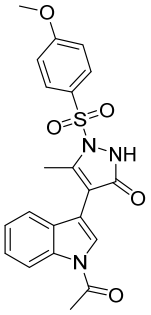
Colorless oil; Yield: 33 %; Purity: 93 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.44 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 2H), 8.00 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.44 (s, 1H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 8.8 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 2.71 (s, 3H), 2.56 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.4, 167.2, 164.3, 164.0, 163.6, 155.5, 144.2, 135.7, 134.1, 132.6, 129.5, 129.0, 128.2, 125.6, 125.3, 124.6, 124.4, 123.9, 120.2, 120.0, 116.7, 113.9, 113.5, 111.2, 108.4, 55.5, 23.9, 21.4, 14.0, presence of rotamers; IR (film): ῦ = 2927, 2833, 1740, 1699, 1602, 1510, 1448, 1329, 1243, 1223, 1165, 1062, 1026, 997, 932, 910, 843, 756, 729, 640, 621, 591 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{30}H_{25}N_3Na\ O_6^+$: 546.1636, found: 546.1632.

General procedure for mono-amide and sulfonamide synthesis

The corresponding acetyl indole (1.0 eq.) was dissolved in DCM (0.2 M) and cooled to 0 °C. Et_3N (1.6 eq.) and the corresponding acid or sulfonyl chloride (1.0 eq. in general, 1.5 eq. for compound **21**) was added. The solution was allowed to warm to room temperature and stirred for 12 hours. The reaction mixture was quenched with water and extracted with EtOAc three times. The combined organic phases

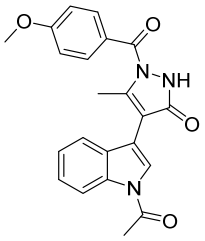
were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (1-3 % MeOH in DCM) affording the desired products in pure form. This procedure was followed to obtain the final compounds **19**-**22** and **28**.

4-(1-Acetyl-1*H*-indol-3-yl)-1-((4-methoxyphenyl)sulfonyl)-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one (19)



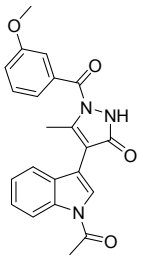
White solid; mp 219-222 °C; Yield: 51 %; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.76 (s, 1H), 8.28 (d, J = 8.1 Hz, 1H), 7.49 (s, 1H), 7.39 – 7.30 (m, 3H), 7.28 – 7.22 (m, 2H), 6.64 (d, J = 8.8 Hz, 2H), 3.71 (s, 3H), 2.62 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.1, 163.3, 151.5, 138.7, 134.8, 129.9, 129.0, 126.0, 124.7, 124.5, 123.2, 119.8, 115.8, 113.7, 110.8, 100.1, 55.5, 23.8, 10.5; IR (film): ῡ = 3266, 1677, 1451, 1390, 1368, 1336, 1267, 1189, 1163, 1093, 1019, 962, 843, 828, 804, 756, 721, 624, 565, 551 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{21}H_{19}N_3NaO_5S^+$: 448.0938, found: 448.0936.

4-(1-Acetyl-1*H*-indol-3-yl)-1-(4-methoxybenzoyl)-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one (20)



White solid; mp 173-179 °C; Purity: 88 %; Yield: 55 %; ^{1}H NMR (500 MHz, MeOD): δ = 8.35 (d, J = 8.3 Hz, 1H), 7.99 (d, J = 9.0 Hz, 2H), 7.59 (s, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.31 – 7.26 (m, 1H), 7.22 (td, J = 7.7, 1.1 Hz, 1H), 6.97 (d, J = 9.0 Hz, 2H), 3.85 (s, 3H), 2.58 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (126 MHz, MeOD): δ = 170.9, 165.9, 165.7, 137.0, 133.4, 132.8, 131.2, 126.2, 125.4, 124.6, 121.7, 121.1, 117.4, 115.1, 114.7, 113.9, 102.5, 56.1, 23.9, 10.8. IR (film): ῡ = 3349, 2922, 2495, 1712, 1604, 1512, 1447, 1375, 1253, 1225, 1170, 1144, 1069, 1017, 961, 844, 767, 749, 690, 636, 606, 482 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{22}H_{19}N_3NaO_4^+$: 412.1268, found: 412.1265.

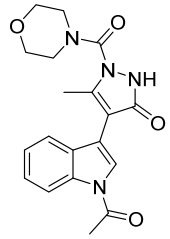
4-(1-Acetyl-1*H*-indol-3-yl)-1-(3-methoxybenzoyl)-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one (21)



White solid; mp 59-63 °C; Yield: 24 %; ^{1}H NMR (500 MHz, MeOD): δ = 8.36 (d, J = 8.2 Hz, 1H), 7.66 – 7.59 (m, 2H), 7.54 (d, J = 7.7 Hz, 1H), 7.51 (t, J = 1.7 Hz, 1H), 7.40 (s, 1H), 7.36 (t, J = 8.0 Hz, 1H), 7.29 (t, J = 7.3 Hz, 1H), 7.22 (t, J = 7.5 Hz, 1H), 7.17 (dd, J = 8.3, 2.0 Hz, 1H), 3.78 (s, 3H), 2.59 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (presence of rotamers, 126 MHz, MeOD): δ = 170.9, 165.7, 161.3, 155.2, 141.0, 137.0, 131.2, 130.9, 130.8, 126.2, 125.5, 124.7, 123.4, 121.4, 121.1, 120.7, 118.8, 117.5, 115.6, 114.0, 113.8, 102.4, 56.0, 55.9, 54.8, 25.1, 23.9, 10.8, 9.3; IR (film): ῡ = 3302, 2924, 1703, 1584, 1487, 1448, 1377, 1330, 1270,

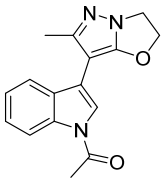
1258, 1224, 1214, 1142, 1030, 962, 745, 643, 425 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{22}H_{20}N_3O_4^+$: 390.1448, found: 390.1449.

4-(1-Acetyl-1*H*-indol-3-yl)-5-methyl-1-(morpholine-4-carbonyl)-1,2-dihydro-3*H*-pyrazol-3-one (22)



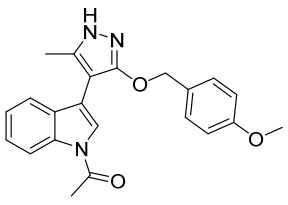
White solid; mp 217-221 °C; Yield: 48 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.47 (d, J = 7.7 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.43 – 7.36 (m, 2H), 7.30 – 7.27 (m, 1H), 3.57 – 3.41 (m, 8H), 2.64 (s, 3H), 2.31 (s, 3H); ^{13}C NMR (presence of rotamers, 126 MHz, $CDCl_3$): δ = 168.5, 154.2, 152.7, 139.1, 135.7, 129.9, 125.5, 123.5, 123.5, 120.0, 116.7, 112.8, 101.5, 66.4, 66.2, 53.4, 44.9, 44.2, 24.1, 11.0; IR (film): $\tilde{\upsilon}$ = 3297, 2927, 2865, 1725, 1691, 1450, 1409, 1387, 1279, 1268, 1234, 1116, 1067, 1037, 965, 856, 768, 745, 710, 629, 428 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{20}N_4NaO_4^+$: 391.1377, found: 391.1376.

1-(3-(6-Methyl-2,3-dihydropyrazolo[5,1-b]oxazol-7-yl)-1*H*-indol-1-yl)ethan-1-one (23)



To a solution of methyl-(*Z*)-2-(1-acetyl-1H-indol-3-yl)-3-hydroxybut-2-enoate (20.0 mg, 0.078 mmol) in DMF (0.3 mL) 1,2-dibromoethane (34 µL, 0.395 mmol) and K_2CO_3 (54.0 mg, 0.391 mmol) was added. The reaction mixture was heated to 80 °C for 7 hours. The reaction mixture was diluted with EtOAc and extracted with distilled water and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (EtOAc) followed by preparative HPLC, obtaining the final compound in pure form (9.0 mg, 41 %) as a light yellow oil. Purity: 94 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.46 (d, J = 7.4 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.39 (t, J = 7.7 Hz, 1H), 7.36 (s, 1H), 7.31 (t, J = 7.5 Hz, 1H), 5.14 (t, J = 7.9 Hz, 2H), 4.40 (t, J = 7.9 Hz, 2H), 2.67 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 168.3, 157.4, 152.8, 135.6, 129.7, 125.6, 124.0, 123.7, 121.9, 120.3, 116.6, 113.0, 75.5, 45.7, 24.1, 14.6; IR (film): $\tilde{\upsilon}$ = 2924, 2859, 1698, 1625, 1540, 1516, 1454, 1377, 1333, 1265, 1235, 1140, 1027, 1011, 950, 913, 874, 745, 699, 635, 608, 517, 407 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{16}H_{15}N_3NaO_2^+$: 304.1057, found: 304.1057.

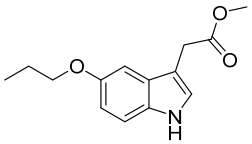
1-(3-(3-((4-Methoxybenzyl)oxy)-5-methyl-1*H*-pyrazol-4-yl)-1*H*-indol-1-yl)ethan-1-one (24)



To a solution of 4-(1-acetyl-1*H*-indol-3-yl)-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one (30.0 mg, 0.118 mmol) in DMF (502 µl), NaH (60 % in mineral oil, 4.70 mg, 0.118 mmol) was added portion-wise and the reaction was stirred at 0 °C for 15 minutes followed by the addition of PMBBr (23.6 mg, 0.118 mmol) and TBAI (1.04 mg, 0.00282 mmol).

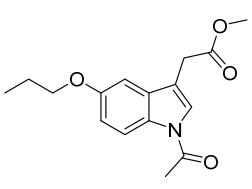
The reaction was stirred at room temperature for 1.5 hours, quenched with water and extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. After purification by flash column chromatography (silica gel; DCM/MeOH 0.5 % to DCM/MeOH 2 %) the desired product was obtained as a white solid (10.0 mg, 0.0266 mmol, 23 % yield). Mp: 197-102 °C; Purity: 92 %; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.81 (br, 1H), 8.35 (d, J = 8.1 Hz, 1H), 7.67 (s, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.32 (ddd, J = 8.3, 7.3, 1.3 Hz, 1H), 7.27 – 7.23 (m, 1H), 7.18 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H), 5.09 (s, 2H), 3.74 (s, 3H), 2.65 (s, 3H), 2.23 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ =168.5, 159.4, 159.2, 138.4, 135.8, 130.2, 128.7, 128.4, 128.3, 125.3, 123.5, 123.4, 120.7, 114.2, 113.8, 96.9, 55.3, 52.0, 24.1, 10.9; IR (neat): $\tilde{\upsilon}$ = 2957, 2926, 1701, 1513, 1448, 1378, 1330, 1304, 1248, 1226, 1175, 1144, 1031, 907, 817, 748, 728; HRMS (ESI): m/z: calcd. for $C_{22}H_{21}N_3NaO_3^+$: 398.1481, found: 398.1475.

Methyl 2-(1-acetyl-5-propoxy-1*H*-indol-3-yl)acetate (25)



To a solution of 5-propoxy-1*H*-indole (**51**, 950 mg, 5.32 mmol) in Et_2O (18.6 mL) at 0 °C, oxalyl chloride (512 µL, 5.96 mmol) was added. The reaction mixture was stirred for 30 min at 0 °C, and it was then cooled down to -78 °C. A solution of NaOMe in methanol (12.98 mmol of NaOMe in 3.0 mL of MeOH) was added dropwise at -78 °C. The reaction mixture was stirred at -78 °C for 3 hours and quenched by the addition of water. The formed precipitate was then dissolved in dioxane (23.0 mL) and water (3.8 mL), to which Pd/C (0.4 eq.) and NaH_2PO_2 (10 eq.) were added. The reaction mixture was stirred at 25 °C for 12 hours, filtered through a pad of celite, concentrated and redissolved in EtOAc. The EtOAc phase was then extracted three times with water, dried over $MgSO_4$ and concentrated under reduced pressure to obtain the desired methyl ester in 88 % over two steps as a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95 (br, 1H), 7.24 (d, J = 8.8 Hz, 1H), 7.14 (s, 1H), 7.06 (d, J = 2.3 Hz, 1H), 6.87 (dd, J = 8.8, 2.4 Hz, 1H), 3.98 (t, J = 6.6 Hz, 2H), 3.75 (s, 2H), 3.71 (s, 3H), 1.91 – 1.75 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.5, 153.6, 131.2, 127.6, 123.7, 113.0, 111.8, 108.1, 101.8, 70.4, 51.9, 31.2, 22.8, 10.6; IR (film): $\tilde{\upsilon}$ = 3405, 2962, 2876, 1727, 1585, 1485, 1455, 1201, 1145, 1061, 1012, 984, 797, 695, 631, 424 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{14}H_{18}NO_3^+$: 248.1281, found: 248.1278.

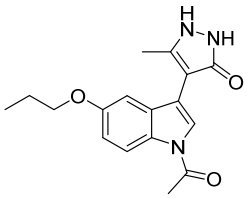
Methyl 2-(1-acetyl-5-propoxy-1*H*-indol-3-yl)acetate (26)



To a solution of methyl 2-(1-acetyl-5-propoxy-1*H*-indol-3-yl)acetate (**25**, 3.43 g, 22.14 mmol) in THF (8.7 mL), DMAP (0.43 g, 3.54 mmol), Et_3N (9.3 mL, 66.67 mmol) and acetic anhydride (35.5 mL, 372.34 mmol) was added. The

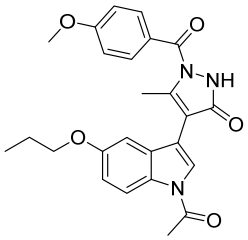
reaction mixture was stirred for 12 hours at 25 °C. It was quenched by the addition of a saturated $NaHCO_3$ solution and extracted with EtOAc three times. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained precipitate was purified by flash column chromatography (hexane: EtOAc, 5:1) to obtain the desired product in 42 % as a white solid; mp 75-78 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.31 (d, J = 8.9 Hz, 1H), 7.41 (s, 1H), 6.98 – 6.95 (m, 2H), 3.98 (t, J = 6.6 Hz, 2H), 3.74 (s, 3H), 3.69 (d, J = 1.1 Hz, 2H), 2.60 (s, 3H), 1.89 – 1.77 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.2, 168.0, 156.1, 131.0, 130.4, 124.4, 117.5, 114.7, 114.1, 102.7, 70.1, 52.2, 30.8, 23.7, 22.7, 10.6; IR (film): $\tilde{\upsilon}$ = 3105, 2970, 1739, 1789, 1602, 1454, 1402, 1391, 1330, 1268, 1217, 1188, 1163, 1077, 1014, 981, 943, 839, 805, 727, 653 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{16}H_{19}NNaO_4^+$: 312.1206, found: 312.1205.

4-(1-acetyl-5-propoxy-1H-indol-3-yl)-5-methyl-1,2-dihydro-3H-pyrazol-3-one (27)



A solution of (^{i}Pr)$_2$NH (581 µL, 4.15 mmol), BuLi (1.6 M in hexane, 2.5 mL, 3.98 mmol) in THF (5.0 mL) was stirred at 0 °C for 15 minutes. After cooling to -78 °C, a solution of indole **26** (1.00 g, 3.46 mmol) in THF (10.0 mL) was added dropwise and stirred at -78 °C for 1 hour. Then a solution of acetic anhydride (391 µL, 4.15 mmol) in THF (5.0 mL) was added dropwise and the reaction was stirred for 4 hours followed by quenching with a saturated aq. NH_4Cl solution and extraction with EtOAc three times. The obtained organic layers were dried over $MgSO_4$ and the residue was purified by flash column chromatography (hexane:EtOAc, 4:1). The obtained product was then diluted in 5.0 mL of EtOH and 5.0 mL of toluene and hydrazine hydrate was added (31 µL, 0.65 mmol). The reaction mixture was heated to reflux for 4 hours and concentrated under reduced pressure. Upon the addition of DCM, the desired product precipitated, it was filtered off and washed with DCM, obtaining the final product as a white solid in 18 % yield. mp 275-280 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.22 (d, J = 8.9 Hz, 1H), 7.61 (s, 1H), 7.03 (d, J = 2.4 Hz, 1H), 6.92 (dd, J = 8.9, 2.5 Hz, 1H), 3.91 (t, J = 6.5 Hz, 2H), 2.62 (s, 3H), 2.20 (s, 3H), 1.80 – 1.64 (m, 2H), 0.98 (t, J = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 168.6, 155.0, 131.2, 129.6, 124.2, 116.4, 113.6, 113.3, 104.5, 94.7, 69.2, 23.5, 22.0, 10.8, 10.3, two carbons are missing due to overlapping; IR (film): $\tilde{\upsilon}$ = 3315, 2967, 2932, 2902, 2869, 1684, 1589, 1563, 1514, 1455, 1389, 1335, 1256, 1210, 1174, 1141, 1106, 947, 858, 838, 813, 787, 678, 651, 583, 424 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{17}H_{20}N_3O_3^+$: 314.1499, found: 314.1498.

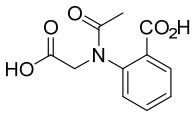
4-(1-Acetyl-5-propoxy-1*H*-indol-3-yl)-1-(4-methoxybenzoyl)-5-methyl-1,2-dihydro-3*H*-pyrazol-3-one (28)



Following the general procedure for mono-amide synthesis, compound **28** was obtained as a white solid in 19 % yield; mp 205-207 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.68 (s, 1H), 8.16 (d, J = 8.9 Hz, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.74 (s, 1H), 7.03 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 2.0 Hz, 1H), 6.85 (dd, J = 8.9, 2.1 Hz, 1H), 3.89 – 3.68 (m, 5H), 2.58 (s, 3H), 2.34 (s, 3H), 1.77 – 1.60 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ = 168.9, 163.9, 163.5, 155.3, 153.0, 138.6, 132.0, 130.3, 129.5, 125.0, 120.1, 116.7, 114.3, 114.0, 111.7, 102.8, 100.4, 69.2, 55.6, 23.6, 22.1, 10.6, 10.5; IR (film): ῡ = 2927, 1748, 1732, 1676, 1605, 1510, 1459, 1403, 1245, 1211, 1165, 1134, 1079, 1030, 965, 848, 761, 653, 635, 596 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{25}H_{26}N_3O_5^+$: 448.1867, found: 448.1869.

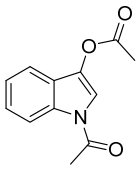
2.3 Synthesis of 1g derivatives

2-(*N*-(Carboxymethyl)acetamido)benzoic acid (30)[2]



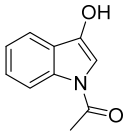
Light yellow solid; Yield: 33 % over two steps; mp 203-208 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.95 (br, 2H), 7.93 (dd, J = 7.7, 1.4 Hz, 1H), 7.67 (td, J = 7.7, 1.5 Hz, 1H), 7.60 – 7.55 (m, 1H), 7.52 (t, J = 7.6 Hz, 1H), 4.61 (d, J = 17.3 Hz, 1H), 3.63 (d, J = 17.3 Hz, 1H), 1.66 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.6, 169.0, 166.7, 142.4, 133.2, 131.3, 130.6, 129.4, 128.7, 50.8, 21.7; IR (film): ῡ = 2976, 2938, 1724, 1699, 1605, 1589, 1489, 1449, 1390, 1222, 1138, 1085, 1023, 893, 786, 747, 706, 652, 641, 585, 539, 466 cm^{-1}; MS (ESI): m/z: calcd for $C_{11}H_{11}NNaO_5^+$: 260.1, found: 259.9.

1-Acetyl-1*H*-indol-3-yl acetate (31)[2]



Light brown solid; Yield: 89 %; mp 66-72 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.36 (d, J = 8.3 Hz, 1H), 7.89 (s, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.42 – 7.36 (m, 1H), 7.33 – 7.28 (m, 1H), 2.62 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.4, 168.3, 133.6, 132.6, 125.7, 123.6, 123.5, 117.8, 116.0, 115.7, 23.7, 20.5; IR (film): ῡ = 1740, 1693, 1449, 1387, 1366, 1345, 1328, 1237, 1206, 1009, 932, 892, 733, 689, 633, 543, 419 cm^{-1}; MS (ESI): m/z: calcd for $C_{12}H_{11}NNaO_3^+$: 240.1, found: 240.0.

1-Acetylindolin-3-one (32)[2]



Light brown solid; Yield: 83 %; mp 139-142 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.44 (d, J = 8.3 Hz, 1H), 7.73 (ddd, J = 8.5, 7.3, 1.4 Hz, 1H), 7.69 (ddd, J = 7.7, 1.4, 0.7 Hz, 1H), 7.25 (td, J = 7.7, 0.9 Hz, 1H), 4.55 (s, 2H), 2.25 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 195.6, 168.6, 153.2, 136.9, 124.8, 123.7, 123.2, 117.6, 56.2, 24.0; IR (film): $\tilde{\upsilon}$ = 1716, 1674, 1605, 1586, 1459, 1380, 1354, 1282, 1234, 1193, 1139, 1010, 924, 761, 735, 648, 599, 508 cm^{-1}; MS (ESI): m/z: calcd for $C_{10}H_9NNaO_2^+$: 198.1, found: 197.9.

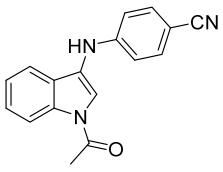
General procedure A for the condensation of 1-acetylindolin-3-one with anilines

A solution of 1-acetylindolin-3-one (1.0 eq.) and the desired aniline (1.0 eq.) in acetic acid (0.6 M) was refluxed for 8 hours. The reaction mixture was allowed to cool down, concentrated and the obtained precipitate was washed with Et_2O, obtaining the desired acetylated indoles in pure form (compound **33**). When no precipitate was observed, the intermediates were purified by flash column chromatography using DCM as eluent (compound **34**). This method was used to obtain intermediates **33, 34** and **37**.

General procedure B for the condensation of 1-acetylindolin-3-one with anilines

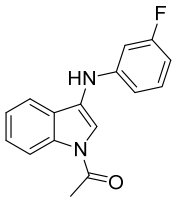
A solution of 1-acetylindolin-3-one (1.0 eq.), the desired aniline (1.0 eq.) and a catalytic amount of pTSA (0.1 eq.) in toluene (1.0 M) was refluxed in a Dean Stark for 2.5-4 hours. The reaction mixture was allowed to cool down and concentrated. The obtained residue was purified by flash column chromatography using DCM as eluent (compound **36**). For compounds **35** and **38**, the reaction mixture was concentrated, Et_2O was added and the obtained precipitate was filtered off. This method was used to obtain intermediates **35**, **36** and **38**.

4-((1-Acetyl-1*H*-indol-3-yl)amino)benzonitrile (33)[3]



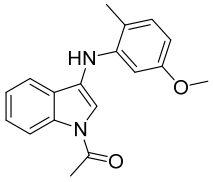
Light pink solid; Yield: 26 %; mp 208-212 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.48 (d, J = 8.0 Hz, 1H), 7.49 (d, J = 8.7 Hz, 2H), 7.44 – 7.37 (m, 3H), 7.30 (d, J = 7.3 Hz, 1H), 6.87 (d, J = 8.7 Hz, 2H), 2.64 (s, 3H), one proton is missing due to overlapping; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.2, 148.9, 134.8, 133.8, 126.4, 126.3, 123.8, 122.7, 119.8, 118.1, 117.0, 114.3, 101.4, 24.0, one carbon is missing due to overlapping; IR (film): $\tilde{\upsilon}$ = 3357, 2212, 1715, 1661, 1605, 1595, 1540, 1508, 1465, 1398, 1358, 1222, 1175, 1020, 939, 819, 741, 718, 654, 615, 535 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{17}H_{14}N_3O^+$: 276.1131, found: 276.1132.

1-(3-((3-Fluorophenyl)amino)-1*H*-indol-1-yl)ethan-1-one (35)



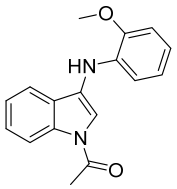
Brown solid; Yield: 52 %; mp 148-152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.47 (br, 1H), 7.45 – 7.39 (m, 2H), 7.39 (s, 1H), 7.35 (s, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 7.23 – 7.07 (m, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 6.66 (d, *J* = 10.6 Hz, 1H), 6.57 (t, *J* = 7.6 Hz, 1H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.2, 163.9 (d, *J* = 244.1 Hz), 146.5 (d, *J* = 10.3 Hz), 134.8, 130.9 (d, *J* = 9.0 Hz), 130.6 (d, *J* = 10.0 Hz), 129.1, 126.1, 125.7, 124.6, 123.5, 117.9, 113.8, 111.0 (d, *J* = 2.5 Hz), 106.4 (d, *J* = 21.6 Hz), 102.2 (d, *J* = 25.6 Hz), 24.2; IR (film): $\tilde{\upsilon}$ = 3333, 2872, 1614, 1580, 1556, 1490, 1403, 1177, 1147, 1120, 1031, 1008, 940, 809, 784, 745, 734, 686, 675, 569, 527, 517, 472, 449 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{16}H_{14}FN_2O^+$: 269.1085, found: 269.1085.

4-((1-Acetyl-1*H*-indol-3-yl)amino)benzonitrile (36)



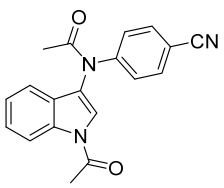
Light brown solid; Yield: 50 %; mp 108-110 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.49 (s, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.30 – 7.26 (m, 3H), 7.08 (d, *J* = 8.1 Hz, 1H), 6.59 (s, 1H), 6.40 (d, *J* = 7.3 Hz, 1H), 3.70 (s, 3H), 2.60 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.2, 159.1, 143.5, 134.9, 131.2, 126.6, 126.0, 125.5, 123.4, 117.9, 116.9, 116.5, 113.0, 104.6, 101.5, 55.3, 24.1, 16.7; IR (film): $\tilde{\upsilon}$ = 3406, 3119, 1684, 1583, 1518, 1445, 1379, 1331, 1268, 1216, 1198, 1162, 1116, 1039, 751 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{18}H_{19}N_2O_2^+$: 295.1441, found: 295.1442.

1-(3-((2-Methoxyphenyl)amino)-1*H*-indol-1-yl)ethan-1-one (37)



Brown solid; Yield: 56 %; mp 125-130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.48 (br, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.44 – 7.38 (m, 1H), 7.34 (s, 1H), 7.32 – 7.27 (m, 1H), 7.10 (dd, *J* = 8.1, 1.4 Hz, 1H), 6.99 – 6.89 (m, 3H), 6.88 – 6.83 (m, 1H), 3.96 (s, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.1, 147.4, 134.7, 133.8, 126.6, 126.0, 125.4, 123.3, 121.2, 119.3, 117.7, 116.9, 113.5, 110.8, 110.2, 55.7, 24.1; IR (film): $\tilde{\upsilon}$ = 3412, 1697, 1600, 1508, 1447, 1417, 1381, 1355, 1329, 1241, 1220, 1115, 1046, 1027, 1013, 935, 743, 647 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{17}H_{16}N_2NaO_2^+$: 303.1104, found: 303.1104.

***N*-(1-Acetyl-1*H*-indol-3-yl)-*N*-(4-cyanophenyl)acetamide (39)**



A solution of 4-((1-Acetyl-1H-indol-3-yl)amino)benzonitrile (50.0 mg, 0.182 mmol) and DMAP (2.2 mg, 0.018 mmol) in acetic anhydride (0.5 mL) was heated to reflux for 12 hours The reaction mixture was concentrated and the residue was

purified by column chromatography (1:1 DCM: Et_2O) obtaining the desired product in clean form (41.0 mg, 71 % yield) as a light yellow solid. mp 164-167 °C; Purity: 92 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.49 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.54 (s, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.47 – 7.44 (m, 1H), 7.38 – 7.32 (m, 2H), 2.67 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 168.2, 146.0, 134.8, 132.9, 126.8, 125.4, 125.0, 124.8, 122.9, 118.4, 118.0, 117.1, 24.0, 23.6, two carbons are missing due to overlapping; IR (film): ῡ = 2225, 1717, 1681, 1599, 1500, 1449, 1372, 1358, 1288, 1218, 1013, 955, 846, 745, 640, 556, 504 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{15}N_3NaO_2^+$: 340.1057, found: 340.1053.

General procedure for acylation

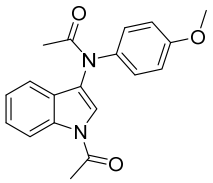
- **With acetyl chloride**

To a solution of the corresponding acetylated indole in toluene (1.0 M) acetyl chloride (4.0 eq.) was added. The reaction mixture was refluxed for 12 hours, cooled down and the formed precipitate was filtered off and washed with Et_2O, obtaining the desired acetylated final products in pure form. In the case of compounds **40** and **41**, the products were purified by flash column chromatography (1:1 DCM: Et_2O and 9:1, DCM:Et_2O respectively). This method was used to obtain the final products **40-44**.

- **With aryl-acylchlorides**

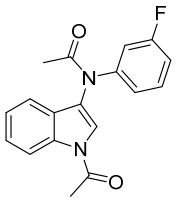
To a solution of the corresponding acetylated indole in toluene (0.1 M), the aryl acylchloride (3 eq.), Et_3N (4 eq.) and DMAP (0.1 eq.) were added. The reaction mixture was heated at 100 °C for 1 h, cooled down and concentrated under reduced pressure. The products were purified by flash column chromatography (toluene:EtOAc 9:1 for **45** and toluene:EtOAc 95:5 for **46**) and further purified by preparative HPLC (EtOAc:*n*-hexane 2:8). This method was used to obtain the final products **45-46**.

***N*-(1-Acetyl-1*H*-indol-3-yl)-*N*-(4-methoxyphenyl)acetamide (40)**



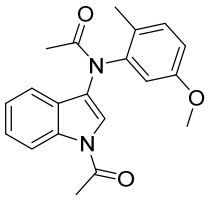
White solid; Yield: 36 % over two steps; mp 106-109 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.44 (d, J = 7.7 Hz, 1H), 7.63 – 7.52 (m, 1H), 7.42 – 7.30 (m, 1H), 7.29 (d, J = 8.9 Hz, 2H), 7.18 (br, 1H), 7.07 (br, 1H), 6.98 – 6.82 (m, 2H), 3.82 (s, 3H), 2.61 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (presence of rotamers, 101 MHz, $CDCl_3$): δ = 171.1, 168.4, 159.2, 158.1, 135.8, 134.6, 129.0, 127.1, 126.2, 125.5, 124.5, 123.7, 122.2, 120.1, 119.2, 118.3, 116.8, 114.8, 114.3, 55.5, 24.0, 23.3; IR (film): ῡ = 1716, 1663, 1506, 1452, 1375, 1363, 1295, 1247, 1222, 1029, 1018, 831, 752, 641, 548, 530, 427 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{18}N_2NaO_3^+$: 345.1210, found: 345.1207.

***N*-(1-Acetyl-1*H*-indol-3-yl)-*N*-(3-fluorophenyl)acetamide (41)**



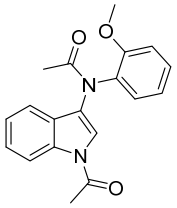
White solid; Yield: 70 %; mp 117-120 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.46 (d, *J* = 7.8 Hz, 1H), 7.54 (s, 1H), 7.50 – 7.36 (m, 1H), 7.38 – 7.28 (m, 2H), 7.21 – 7.05 (m, 3H), 6.96 (br, 1H), 2.65 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8, 168.3, 164.0, 161.5, 143.8, 134.7, 130.3, 126.7, 126.3, 125.6, 124.4, 122.1, 118.4, 117.0, 113.8, 24.0, 23.3, one carbon is missing due to overlapping; IR (film): ῡ = 3109, 3067, 1707, 1668, 1604, 1589, 1451, 1376, 1359, 1319, 1238, 1223, 1147, 1026, 813, 783, 762, 749, 693, 645, 553, 419 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{18}H_{15}N_2NaO_2^+$: 333.1010, found: 333.1006.

***N*-(1-acetyl-1*H*-indol-3-yl)-*N*-(5-methoxy-2-methylphenyl)acetamide (42)**



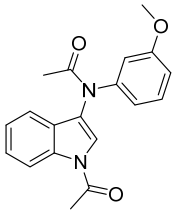
Light yellow oil; Yield: 63 %; Purity: 92 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.45 (d, *J* = 7.3 Hz, 1H), 7.77 (s, 1H), 7.31 (t, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 8.6 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 1H), 6.90 (s, 1H), 6.86 – 6.74 (m, 1H), 3.81 (s, 1H), 2.59 (s, 1H), 2.17 (s, 1H), 2.05 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.4, 168.5, 158.8, 142.0, 134.3, 132.3, 128.0, 125.5, 125.4, 124.5, 123.6, 119.7, 118.8, 116.6, 114.6, 114.5, 55.6, 24.1, 23.2, 17.0; IR (film): ῡ = 2937, 1709, 1673, 1504, 1449, 1374, 1352, 1328, 1292, 1245, 1225, 909, 730 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{20}H_{21}N_2O_3^+$: 337.1552, found: 337.1542.

***N*-(1-Acetyl-1H-indol-3-yl)-*N*-(2-methoxyphenyl)acetamide (43)**



White solid; Yield: 78 %; mp 192-195 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.41 (d, *J* = 8.0 Hz, 1H), 7.76 – 7.50 (m, 2H), 7.47 – 7.34 (m, 3H), 7.20 (s, 1H), 7.02 – 6.95 (m, 2H), 3.90 (s, 3H), 2.59 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.5, 168.4, 155.4, 134.6, 131.9, 130.0, 129.6, 126.7, 125.6, 125.4, 123.6, 121.4, 120.4, 119.3, 116.6, 112.3, 55.6, 23.9, 22.3; IR (film): ῡ = 3099, 1707, 1670, 1450, 1373, 1359, 1333, 1278, 1226, 1115, 1020, 750, 643, 546, 412 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{18}N_2NaO_3^+$: 345.1210, found: 345.1205.

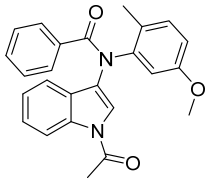
***N*-(1-Acetyl-1H-indol-3-yl)-*N*-(3-methoxyphenyl)acetamide (44)**



Brown solid; Yield: 63 % over two steps; mp 176-179 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.45 (d, *J* = 8.1 Hz, 1H), 7.56 (s, 1H), 7.41 – 7.33 (m, 1H), 7.32 – 7.21 (m, 3H), 6.96 (d, *J* = 7.8 Hz, 1H), 6.92 (s, 1H), 6.84 (br, 1H), 3.78 (s, 3H), 2.62 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 170.1, 160.0, 134.4,

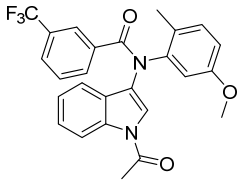
130.1, 128.5, 127.2, 126.0, 125.8, 125.8, 125.1, 124.2, 118.9, 116.6, 113.6, 55.8, 24.3, 23.3, one carbon is missing due to overlapping; IR (film): ῡ = 3109, 1718, 1673, 1601, 1585, 1489, 1452, 1376, 1361, 1321, 1303, 1286, 1222, 1158, 1130, 1042, 1024, 935, 860, 780, 747, 690, 644, 558 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{18}N_2NaO_3^+$: 345.1210, found: 345.12102.

***N*-(1-acetyl-1*H*-indol-3-yl)-*N*-(5-methoxy-2-methylphenyl)benzamide (45)**



Yellow sticky oil; Yield: 13 %; Purity: 94 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.43 (br. s., 1 H), 7.57 - 7.48 (m, 2 H), 7.40 - 7.30 (m, 3 H), 7.25 - 7.17 (m, 5 H), 6.77 (d, *J* = 7.9 Hz, 1 H), 6.70 (br. s., 1 H), 3.67 (s, 3 H), 2.49 (br. s., 3 H), 2.20 (br. s., 3 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.1, 168.2, 158.4, 135.4, 134.6, 132.0, 130.7, 128.5, 128.0, 126.9, 126.6, 126.0, 125.8, 123.9, 119.4, 116.7, 114.2, 113.4, 55.4, 24.0, 17.5, four carbons are missing due to overlapping; IR (film): ῡ = 2360, 2341, 1710, 1658, 1613, 1504, 1448, 1351, 1326, 1222, 1172, 1127, 1034, 747, 703 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{25}H_{23}N_2O_3^+$: 399.1709, found: 399.1706.

***N*-(1-acetyl-1*H*-indol-3-yl)-*N*-(5-methoxy-2-methylphenyl)-3-(trifluoromethyl)benzamide (46)**



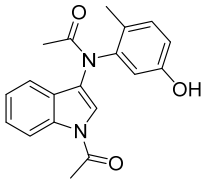
Yellow sticky oil; Yield: 21 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.45 (br. s., 1 H), 7.77 (br. s., 1 H), 7.67 (d, *J* = 7.9 Hz, 1 H), 7.59 (d, *J* = 7.5 Hz, 1 H), 7.43 - 7.31 (m, 3 H), 7.25 - 7.17 (m, 2 H), 7.12 (br. s., 1 H), 6.80 (d, *J* = 6.8 Hz, 1 H), 6.70 (br. s., 1 H), 3.68 (s, 3 H), 2.55 (br. s., 3 H), 2.11 (br. s., 3 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.5, 168.2, 158.6, 136.2, 134.6, 132.3, 131.6, 130.7, 130.4, 128.6, 127,6, 127.2, 126.8, 126.0, 125.6 (q, *J* = 4.0 Hz), 124.0, 123.5 (q, *J* = 272.9 Hz), 119.4, 116.7, 114.5, 55.5, 24.0, 17.4., 3 carbons are missing due to overlapping; IR (film): ῡ = 2360, 2341, 1714, 1661, 1613, 1505, 1449, 1351, 1325, 1223, 1167, 1123, 1070, 745 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{26}H_{22}F_3N_2O_3^+$: 467.1583 found: 467.1572.

General procedure for the demethylation reactions

The starting material (1.0 eq.) was dissolved in DCM (0.2 M) and 1 M BBr_3 solution in DCM was added (2.0 eq.) at 0 °C. The reaction mixture was stirred for 1-3 hours at 0 °C (in the case of compound **48**, 3 equivalents of BBr_3 were needed and the reaction was carried out for 12 hours at 25 °C), it was quenched with water and extracted with EtOAc three times. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The obtained residue was purified by flash column chromatography (DCM:Et_2O, 1:1) affording the desired products in pure form. This method was used to obtain final products **47-49**. In the case of **50**, the reaction mixture was quenched

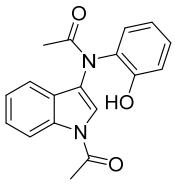
with sat. NaHCO3 and extracted with DCM, the residue was purified by preparative HPLC (EtOAc/*n*-hexane 3:7).

_N_-(1-Acetyl-1_H_-indol-3-yl)-_N_-(5-hydroxy-2-methylphenyl)acetamide (47)



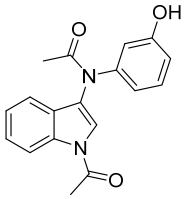
White solid; Yield: 78 %; mp 80-85 °C; ^{1}H NMR (1:0.4 rotamer ratio, asteriks denote minor rotamer peaks, 500 MHz, $CDCl_3$): δ = 8.44 (s, 1H), 8.44* (s, 1H), 7.68 (s, 1H), 7.55* (d, *J* = 7.6 Hz, 1H), 7.45 – 7.38* (m, 2H), 7.34* (t, *J* = 7.4 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.06* (d, *J* = 8.2 Hz, 1H), 6.89 (d, *J* = 7.7 Hz, 1H), 6.85 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.80 (d, *J* = 2.1 Hz, 1H), 6.67* (s, 1H), 6.62* (d, *J* = 8.2 Hz, 1H), 6.27 (s, 1H), 5.83* (s, 1H), 2.61* (s, 3H), 2.55 (s, 3H), 2.29* (s, 3H), 2.19* (s, 3H), 2.13 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (asteriks denote minor rotamer peaks, 126 MHz, $CDCl_3$): δ = 171.5*, 171.0, 168.7, 168.4*, 155.3, 154.8*, 141.8*, 141.8, 134.6*, 134.2, 132.5, 131.9*, 127.4, 126.9*, 126.5*, 126.4, 125.5, 124.6*, 124.5*, 123.7, 121.5*, 119.8, 119.0, 118.3*, 117.0*, 116.5, 115.6, 115.5*, 114.2*, 24.1*, 24.1, 23.1, 22.5*, 17.7*, carbons are missing due to overlapping 17.0; IR (film): ῡ = 3266, 2927, 1708, 1645, 1613, 1504, 1449, 1375, 1352, 1326, 1300, 1222, 907, 746, 728, 653, 548, 421 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{19}N_2O_3^+$: 323.1390, found: 323.1392.

_N_-(1-Acetyl-1_H_-indol-3-yl)-_N_-(2-hydroxyphenyl)acetamide (48)



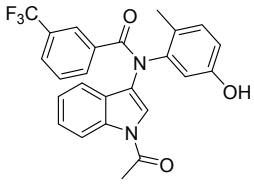
White solid; Yield: 47 %; mp 167-170 °C; ^{1}H NMR (1:0.6 rotamer ratio, asteriks denote minor rotamer peaks, 500 MHz, DMSO-d_6): δ = 8.33* (d, *J* = 8.6 Hz, 1H), 8.31 (d, *J* = 8.6 Hz, 1H), 8.20* (s, 1H), 7.92 (s, 1H), 7.74* (d, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.36* (t, *J* = 8.1 Hz, 1H), 7.32 – 7.28 (m, 2.2 H), 7.23 – 7.18 (m, 2H), 7.08* (t, *J* = 6.9 Hz, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.89* (d, *J* = 7.7 Hz, 1H), 6.85 (t, *J* = 7.5 Hz, 1H), 6.75 (t, *J* = 7.2 Hz, 1H), 2.66* (s, 3H), 2.61 (s, 3H), 2.01* (s, 3H), 1.97 (s, 3H); ^{13}C NMR (asteriks denote minor rotamer peaks, 126 MHz, DMSO-d_6): δ= 170.4, 169.7*, 169.6*, 169.3, 153.2, 133.8*, 133.7, 130.2, 129.6, 129.4, 129.3*, 129.2*, 128.3*, 126.9, 125.4*, 125.3*, 124.9, 124.6*, 123.8*, 123.1, 122.2, 119.8, 119.5, 119.1*, 118.6*, 116.8, 116.5, 116.1*, 115.8*, 23.8, 22.1*, 21.9, one carbon is missing due to overlapping; IR (film): ῡ = 3228, 1713, 1645, 1450, 1378, 1366, 1354, 1327, 1218, 749, 644, 428 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{18}H_{17}N_2O_3^+$: 309.1234, found: 309.1234.

***N*-(1-Acetyl-1*H*-indol-3-yl)-*N*-(3-hydroxyphenyl)acetamide (49)**



Light brown solid; Yield: 84 %; mp 210-214 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.55 (br, 1H), 8.36 (d, J = 8.2 Hz, 1H), 8.17 (br, 1H), 7.45 (d, J = 7.7 Hz, 1H), 7.37 (t, J = 7.7 Hz, 1H), 7.29 (t, J = 7.4 Hz, 1H), 7.16 (br, 1H), 6.91 (br, 1H), 6.86 (s, 1H), 6.66 (br, 1H), 2.65 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.0, 169.7, 157.8, 143.3, 133.9, 129.6, 126.8, 125.4, 124.9, 123.8, 118.41, 116.2, 113.7, 23.8, 22.8, three carbons are missing due to overlapping; IR (film): ῡ = 3203, 1714, 1634, 1601, 1449, 1378, 1327, 1219, 1133, 1032, 1013, 959, 852, 744, 717, 689, 645, 418 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{18}H_{17}N_2O_3^+$: 309.1234, found: 309.1234.

***N*-(1-acetyl-1*H*-indol-3-yl)-*N*-(5-hydroxy-2-methylphenyl)-3-(trifluoromethyl)benzamide (50)**



Yellow sticky oil; Yield: 62 %; Purity: 90 %; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.43 (br. s., 1 H), 7.77 (br. s., 1 H), 7.66 (d, J = 7.9 Hz, 1 H), 7.59 (d, J = 7.9 Hz, 1 H), 7.44 - 7.33 (m, 3 H), 7.26 - 7.18 (m, 2 H), 7.06 (br. s., 1 H), 6.68 (d, J = 8.7 Hz, 1 H), 6.65 (d, J = 2.6 Hz, 1 H), 5.33 (br. s., 1 H), 2.52 (br. s., 3 H), 2.09 (br. s, 3 H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.6, 168.4, 154.7, 136.0, 134.6, 132.4, 131.7, 130.8, 128.6, 127.4, 126.6, 126.1, 125.7, 124.1, 123.5 (q, J = 271.4 Hz), 119.8, 116.7, 115.7, 23.9, 17.4, four carbons are missing due to overlapping; IR (film): ῡ = 3295, 2360, 2342, 1715, 1645, 1615, 1508, 1449, 1361, 1322, 1211, 1167, 1128, 1095 cm^{-1}; HRMS (ESI): m/z: $C_{25}H_{19}F_3N_2NaO_3^+$: 475.1246, found: 475.1236.

3. Bromodomain expression and purification

Proteins were purified as described previously.[6] Briefly, His-tagged bromodomains were expressed in *Escherichia coli* BL21(DE3) cells upon induction with isopropyl thio-beta-D-galactoside (IPTG, final concentration 0.1 mM) for 16 h at 18 °C. Bacteria were lysated and (when required) the resulting extract was treated to remove DNA, adding 0.15% polyethylenimine (PEI). The His-tagged proteins were purified on HisTrap columns (GE Healthcare) and eluted using a step gradient of imidazole. The poly-Histidine tags were removed by overnight incubation with His-tagged tobacco etch virus (TEV) protease purified in-house (if required by the purification protocol, in the meantime the sample was exchanged via dialysis). A size-exclusion chromatography step (HiLoad 16/600 Superdex75 column) and a Ni-affinity chromatography step were subsequently performed to finally purify the cleaved bromodomains. Samples were then concentrated, flash frozen and stored at –80 °C.

4. X-ray crystallography

Crystallization, Data Collection, and Structure Determination

Crystals of the CREBBP bromodomain were grown at 4°C using the hanging drop vapor diffusion method. A 50 mM solution of compound **1b** (in 100 % DMSO) was added into the CREBBP bromodomain solution to reach a final DMSO concentration of 1 % (v/v) and the mixture was incubated on ice for 1 hour before crystallization. Then equal volumes of protein (with compound **1b**) and reservoir solutions (0.1 M MES pH 6.5, 0.10 $MgCl_2$, 20 % PEG 6000, 10 % ethylene glycol) were mixed and crystals appeared after 1 to 2 days. The crystals were flash-frozen in liquid nitrogen with extra 10 % ethylene glycol as cryoprotectant for measurements. Data sets were collected on a PILATUS 6MF detector at the Swiss Light Source beamline X06SA of the Paul Scherrer Institute (Villigen, Switzerland) and indexed, integrated and scaled with the XDS[7] and CCP4 programs.[8] The structures were solved by molecular replacement with PHASER[9] using the CREBBP structure (PDB entry 4NR5) as a search model and refined with PHENIX.[10] The atomic coordinates and structure factors of CREBBP in complex with inhibitor **1b** have been deposited with the Protein Data Bank as entry 4TQN.

The BAZ2B bromodomain was crystallized by vapour diffusion in sitting drops at 4°C. Crystallization buffer (pH 7.5) is composed by PEGs of different lengths PEG500MME (20%), PEG1000 (2%), PEG3350 (2%), PEG20000 (10%) and also contains MPD (2%). Overnight soaking of compounds **47** and **50** was performed by transferring the apo crystals in a soaking solution composed of 32% PEG500MME and 16% PEG20000 in which inhibitors were previously dissolved. Compounds were tested at saturating concentrations (< 5mM). Soaked crystals were frozen in liquid nitrogen. Diffraction data were collected at the Elettra synchrotron (Trieste, Italy), beamline XRD1 for compound **47** and at the Swiss Light Source beamline X06DA (Villigen, Switzerland) for compound **50**. Data were processed with XDS[7] and Aimless,[11] structures were solved by molecular replacement with Phaser[9] using PDB 4IR5 as search model. Initial models were refined alternating cycles of automatic refinement with Phenix[10] and manual model building with COOT.[12] Structures were deposited to the PDB with accession numbers 5E73 and 5E74.

The BRPF1 bromodomain was crystallized against reservoir buffer of 0.1 M Bis-Tris Propane (pH 6.5), 0.2 M Sodium Nitrate and 20% PEG3350, by a hanging drop vapor diffusion method at 4 °C. The apo crystals were transferred to the reservoir buffer containing 5 mM of compound **1b** for overnight soaking. Afterwards, crystals were cryo-protected using reservoir supplemented with additional glycerol and were flash frozen in liquid nitrogen. Data sets were collected at Swiss Light Source beam line X06SA using a PILATUS 6M detector and processed with XDS[7] and CCP4 program

suite.[8] Structure was solved by molecular replacement with Phaser,[9] using BRPF1 bromodomain structure (PDB entry 4LC2) as starting model. Refinement was done with Phenix[10] and iterative manual building in COOT.[12] Refined structure of BRPF1 in complex with XZ08 has been deposited to the PDB with accession code 5D7X.

Table S1

Compound	**1b**	**1b**	**47**	**50**
Bromodomain	CREBBP	BRPF1	BAZ2B	BAZ2B
Data collection				
Space group	P1 21 1	P 3_2 2 1	C 2 2 21	C 2 2 21
Unit cell				
a (Å)	24.73	60.37	83.18	80.65
b (Å)	46.16	60.37	96.48	96.58
c (Å)	56.96	63.61	57.73	57.91
alpha	90.00	90.00	90.00	90.00
beta	93.51	90.00	90.00	90.00
gamma	90.00	120.00	90.00	90.00
Resol. range (Å)	46.16 -2.00	40.39-1.35	42.56-1.71	48.29-1.78
Unique reflections	8757(1272)	29544(3961)	25278(1323)	21825(1105)
<I/σ(I)>	10.2(7.3)	21.6(5.7)	15.6(1.9)	28.5(3.6)
R merge	0.115(0.177)	0.060(0.311)	0.049(0.858)	0.042(0.548)
Completeness (%)	99.7(99.2)	98.8(92.5)	99.2(100)	99.3(90.4)
Multiplicity	6.2(6.2)	9.0(7.2)	4.4(4.4)	8.4(7.5)
Refinement				
Resol. range (Å)	35.84-2.00	30.18-1.35	33.74-1.71	48.29-1.78
R factor/R free	0.2087/0.2535	0.1682-0.1762	0.1904/0.2107	0.1847/0.2109
Mean B factors (A2)	19.20	16.57	33.63	36.52
RMS bonds (Å)	0.0069	0.008	0.007	0.007
RMS angles (°)	1.030	1.194	1.096	1.137
PDB ID code	4TS8	5D7X	5E73	5E74

4.1 $2mF_o - DF_c$ electron density maps of ligands 1b, 47 and 50

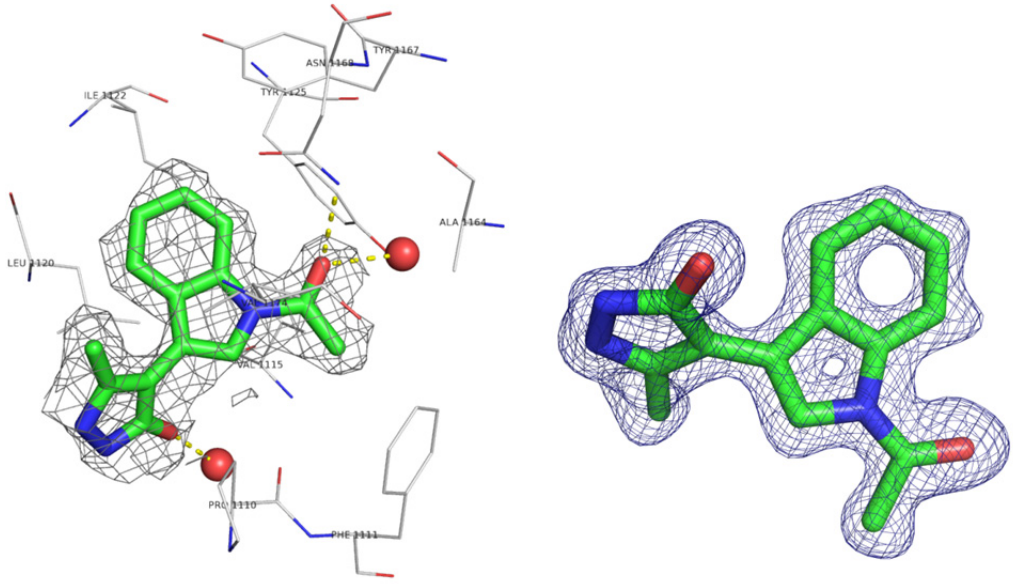


Figure S4. $2mF_o - DF_c$ electron density maps contoured at 1σ (grey mesh) were generated in a region within 1.6 Å for compound **1b** in CREBBP (left) and BRPF1 (right) using PHENIX and Pymol.

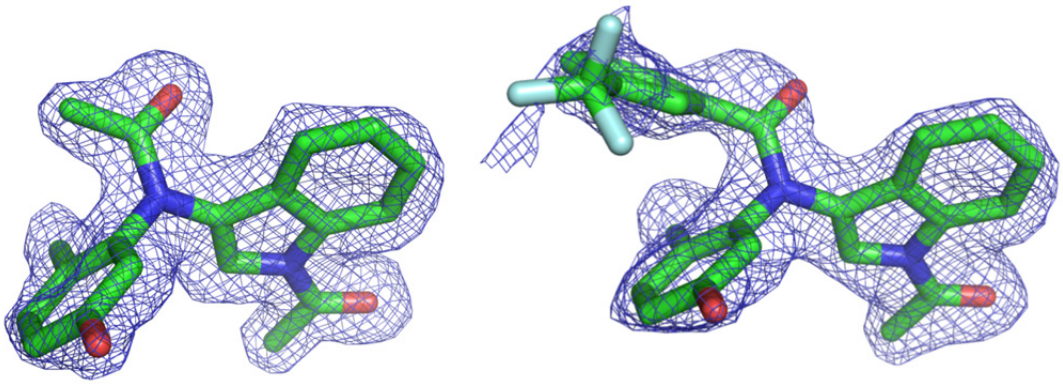


Figure S5. $2mF_o - DF_c$ electron density maps contoured at 1σ (grey mesh) were generated in a region within 1.6 Å for compounds **47** and **50** using PHENIX and Pymol.

5. Thermal shift measurements

Thermal shift measurements were carried out as previously described[13] with a final volume of 20 µl, ligand and protein concentrations 100 µM and 2 µM, respectively. The reported values (ΔT_m) are calculated as the difference between the transition midpoints of an individual sample and the average of the reference wells (containing the protein and the DMSO only) in the same plate. DMSO concentration was kept at 0.2% (v/v).

Table S2. DSF results of compounds **1b**, **1g** and **5** against a panel of different bromodomains. The median value of at least six measurements is given with the SEM values in parenthesis. Dashes indicate data not acquired.

	Compound **1b** ΔT_m (^oC)	Compound **1g** ΔT_m (^oC)	Compound **5** ΔT_m (^oC)
CREBBP	0.3 (0.1)	-0.4 (0.5)	0.0 (0.1)
BRD4(1)	-0.1 (0.1)	-0.9 (0.1)	0.0 (0.2)
BAZ2B	-0.1 (0.1)	-0.7 (0.2)	-0.4 (0.1)
SMARCA4	-0.2 (0.1)	-0.7 (0.1)	-0.1 (0.1)
TAF1(2)	0.0 (0.0)	-1.0 (0.1)	0.0 (0.1)
BRD4(2)	0.0 (0.1)	-0.4 (0.1)	-
EP300	0.4 (0.1)	-0.4 (0.3)	-
BRD9	1.3 (0.1)	-0.7 (0.1)	-
BRDT	-0.8 (0.5)	-2.1 (0.4)	-

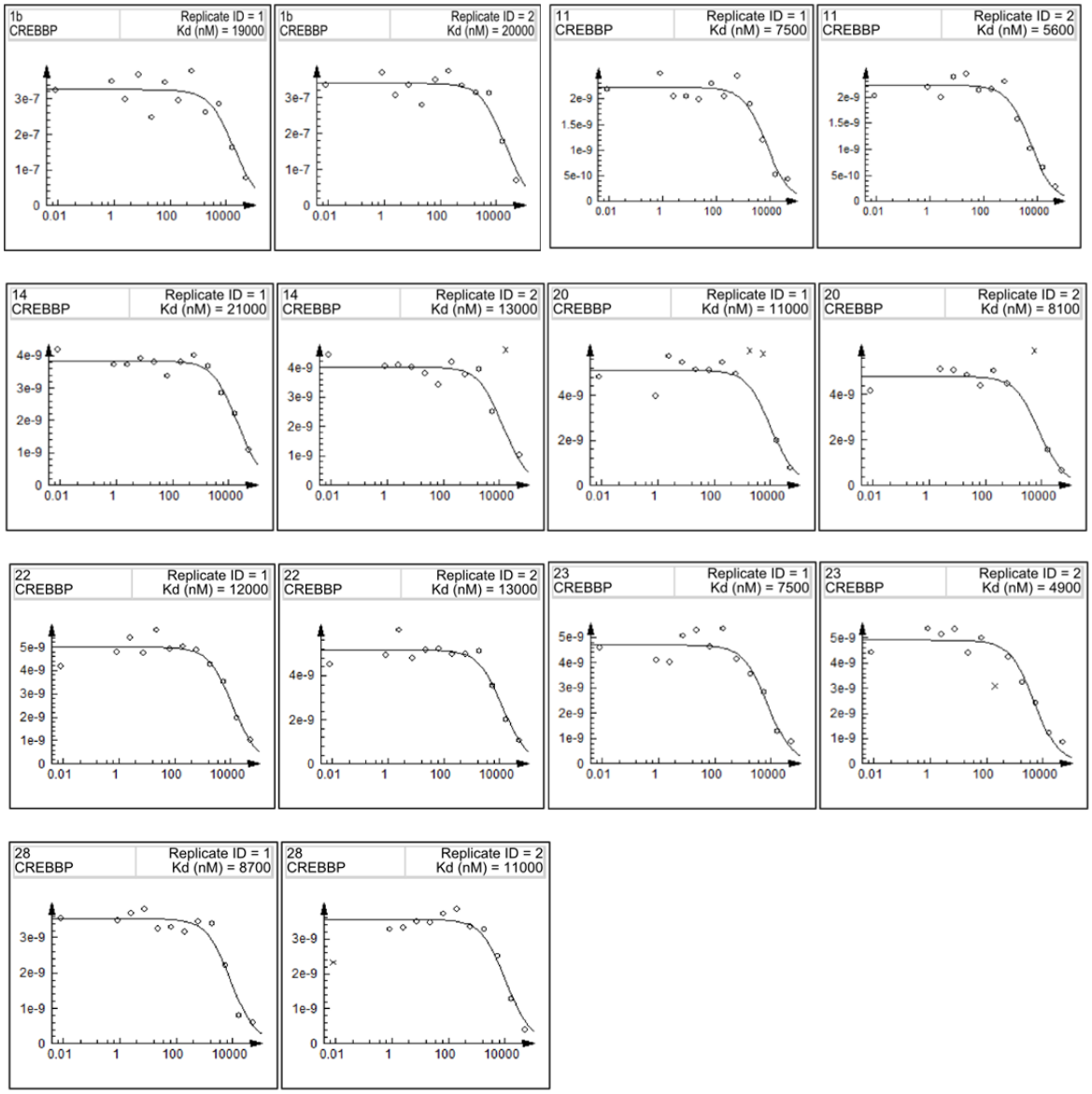
6. BROMOscan assays

K_D determinations by means of BROMOscan technology were carried out at DiscoveRx. E. *coli* derived from BL21 strain was used as host to grow T7 phage strains displaying the bromodomains. E. *coli*, grown to log-phase, were infected with T7 phage (from a frozen stock, being the multiplicity of infection 0.4) and incubated while shaking at 32 °C for 90-150 minutes, until lysis. In order to remove cell debris, lysates were centrifuged at 5,000 x g and filtered (0.2 μm). Affinity resins were obtained by treating streptavidin-coated magnetic beads with biotinylated acetylated peptide ligands for 30 minutes at 25°C. Those beads were then blocked with excess of biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % bovine serum albumin, BSA, 0.05 % Tween20, 1 mM dithiothreitol, (DTT) removing the unbound ligand and reducing non-specific phage binding.

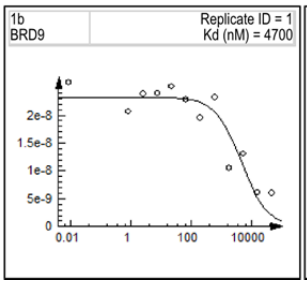
During the experiment, the bromodomain, ligand-bound affinity beads and test compounds were combined in a buffer composed of 17% SeaBlock, 0.33x phosphate-buffered solution, PBS, 0.04% Tween20, 0.02% BSA, 0.004% sodium azide and 7.4 mM DTT. Test compounds were prepared as 50 mM in pure DMSO and diluted to 5 mM with monoethylene gycol, MEG (100× concentrated in respect to the top screening concentration, 50 μM). During the assay a DMSO and MEG final concentration of 0.1% and 0.9% respectively was used. The assays were carried out in polystyrene 96-well plates in a final volume of 0.135 mL. The assay plates were incubated at 25 °C with shaking for 1 hour and the affinity beads were washed with a buffer composed of 0.05% Tween 20 in PBS. The beads were then re-suspended in the elution buffer (1x PBS, 0.05% Tween 20, 2 μM non-biotinylated affinity ligand) and incubated at 25°C with shaking for 30 minutes. The bromodomain concentration in the eluates was measured by qPCR. Binding constants (K_d) were calculated with a standard dose-

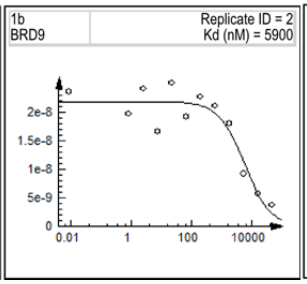
response curve using the Hill equation and curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm.

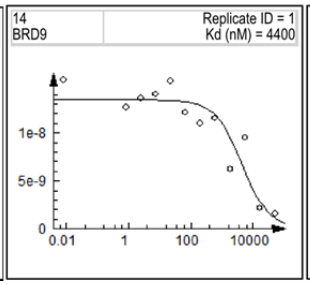
K_D determinations in CREBBP

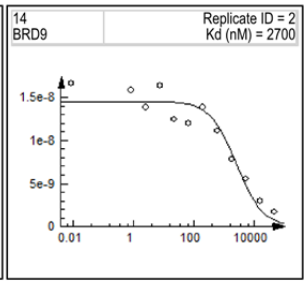


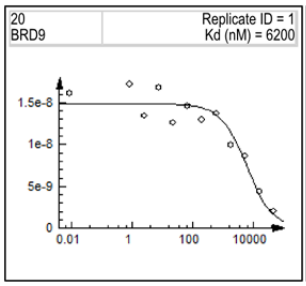
K_D determinations in BRD9

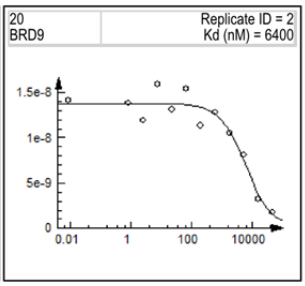


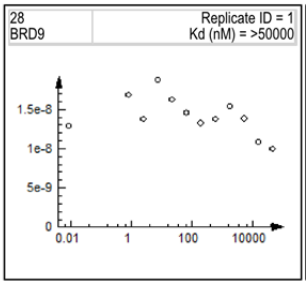


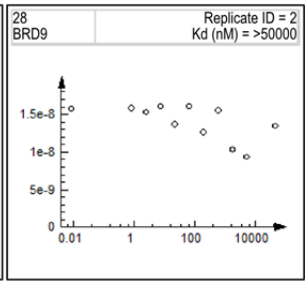




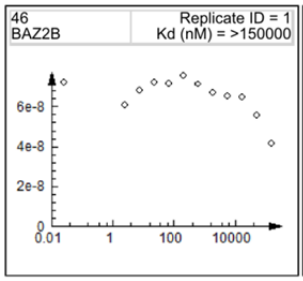


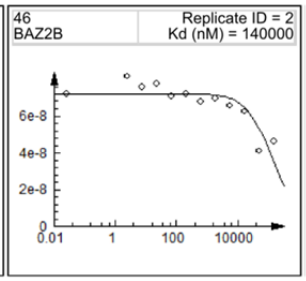


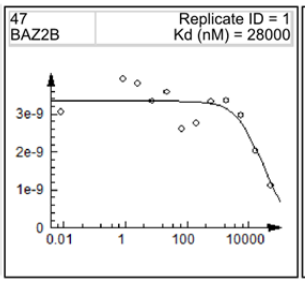


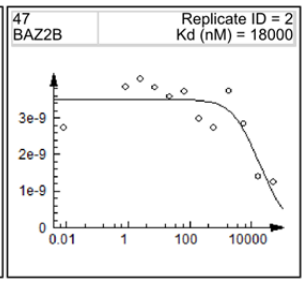


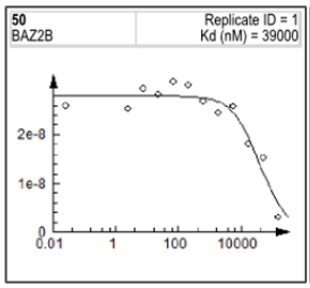
K_D determinations in BAZ2B

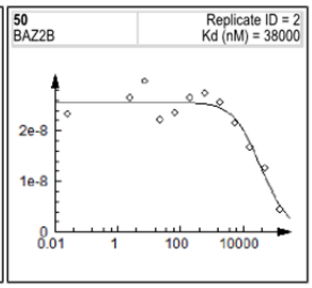




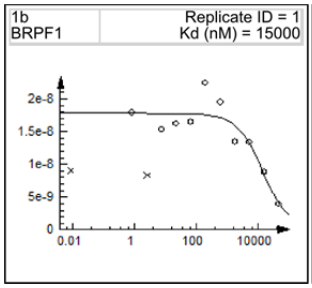


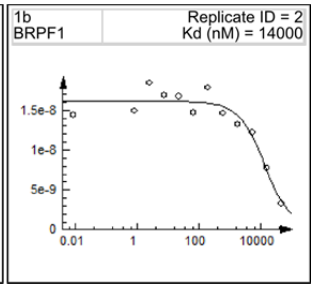






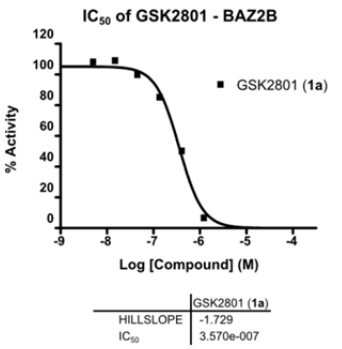
K_D determinations in BRPF1

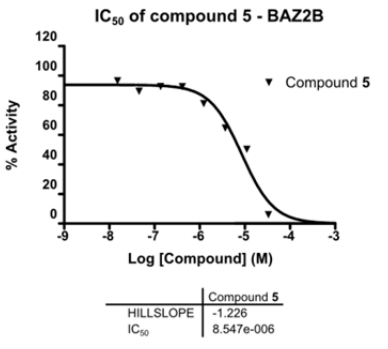


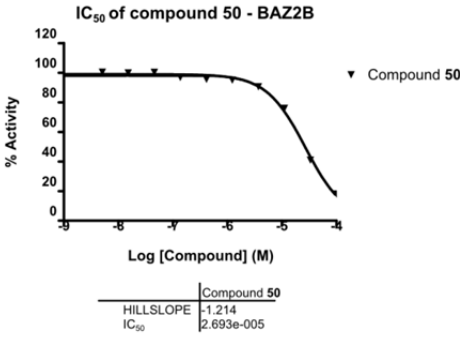


7. AlphaScreen assays

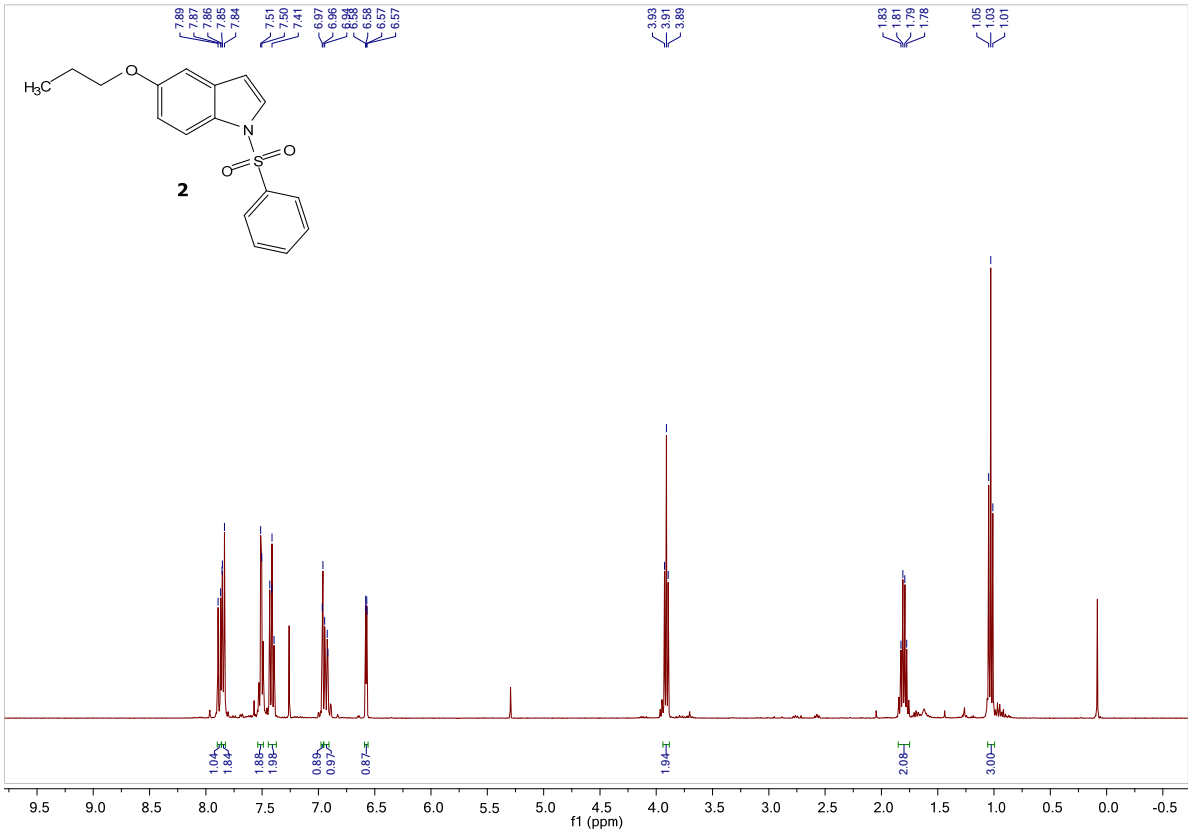
Single doses for binding of compounds **40-44**, **47**, **48** and **50** to BAZ2B were measured in duplicates by the AlphaScreen assay using a biotinylated histone H3 peptide (1-21) K9/14Ac as competitive ligand (measurements carried out at Reaction Biology Corp. Malvern, PA). Dose-response assays for compounds **5** and **50** were carried out by 10 dilution steps each, and the results are shown here:

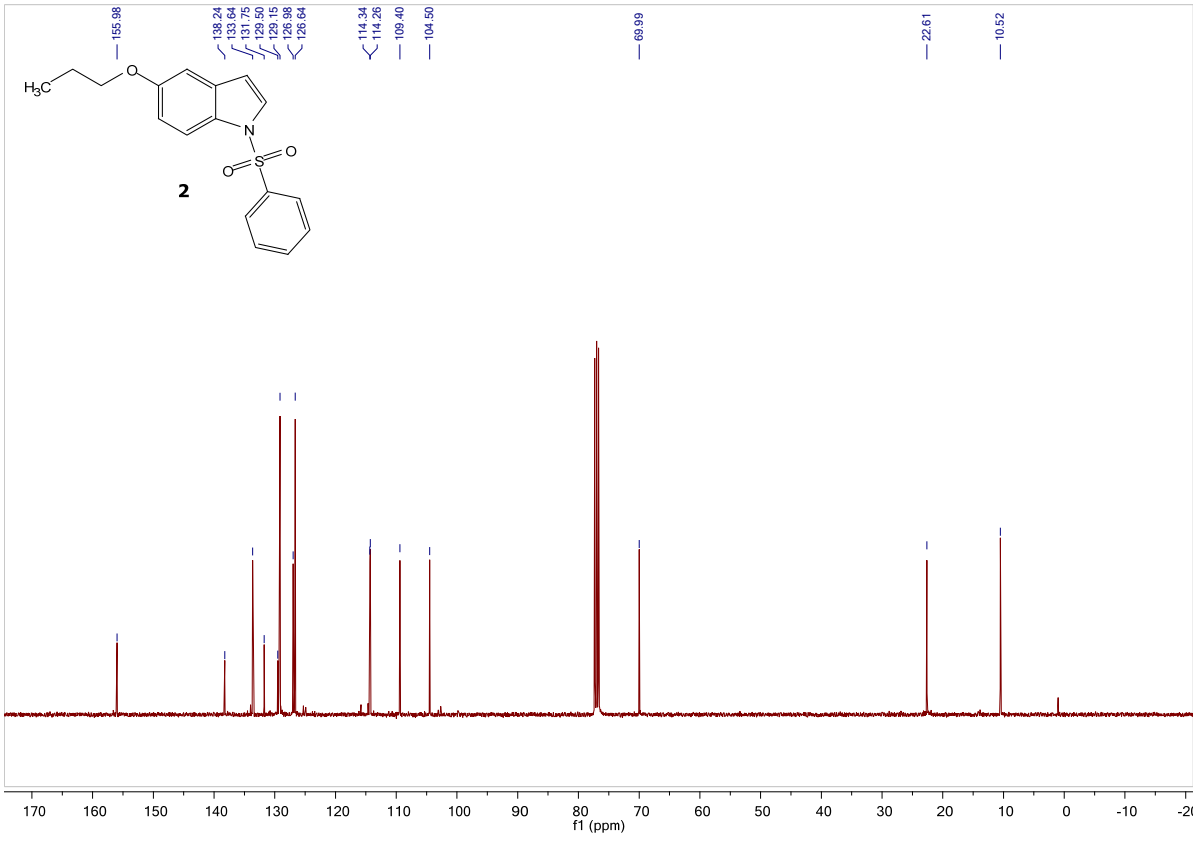


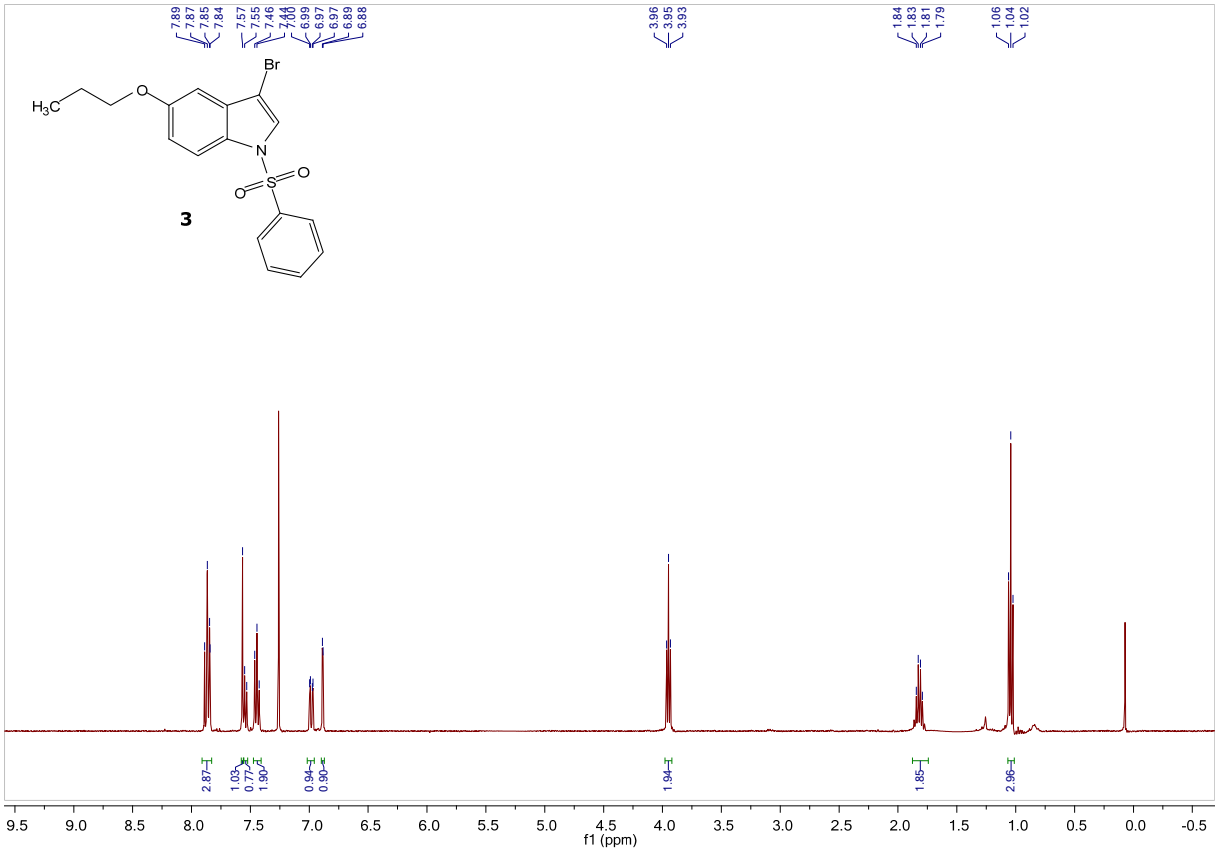




8. NMR traces of selected compounds






7.89
7.87
7.85
7.84
7.57
7.55
7.46
7.44
7.00
6.99
6.97
6.97
6.89
6.88
3.96
3.95
3.93
1.84
1.83
1.81
1.79
1.06
1.04
1.02
Br
H3C
O
N
S
O
O
3
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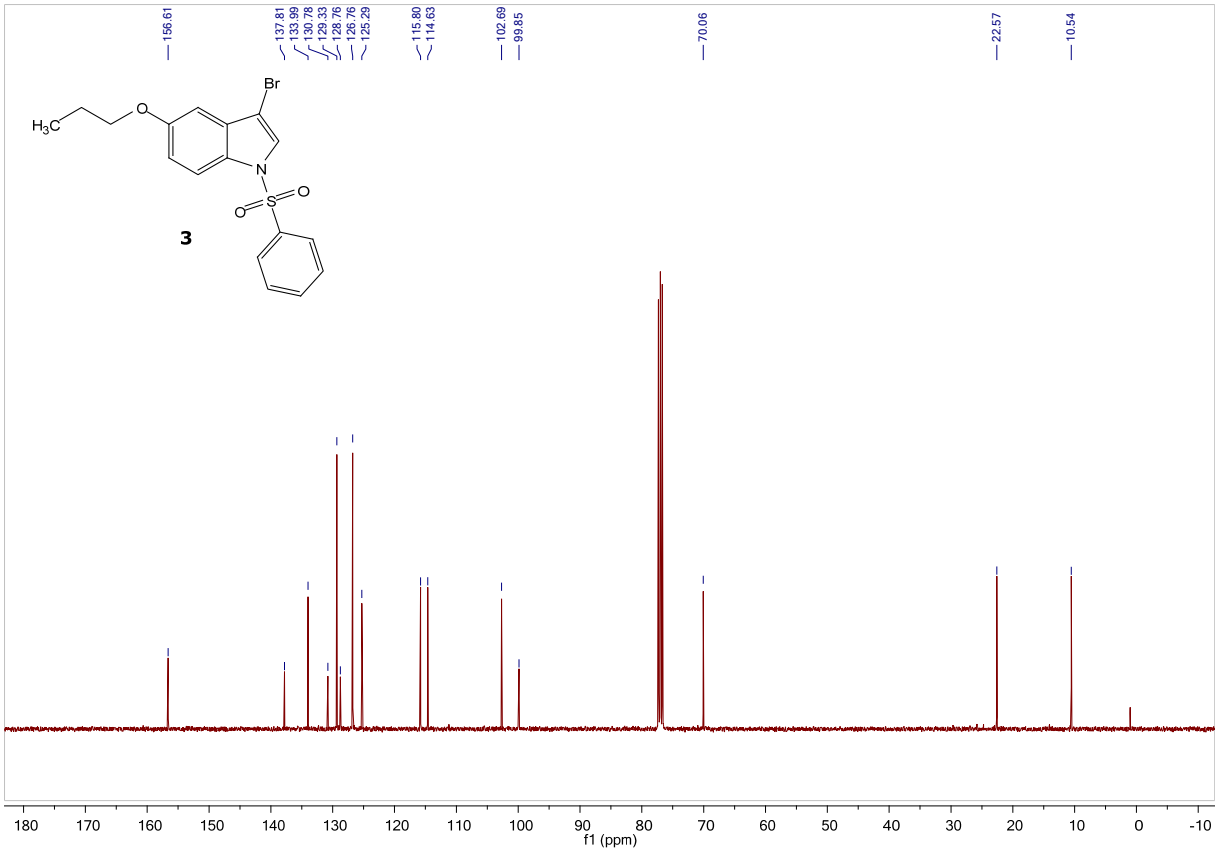
1.03
0.77
1.90
0.94
0.90
1.94
1.85
2.96
f1 (ppm)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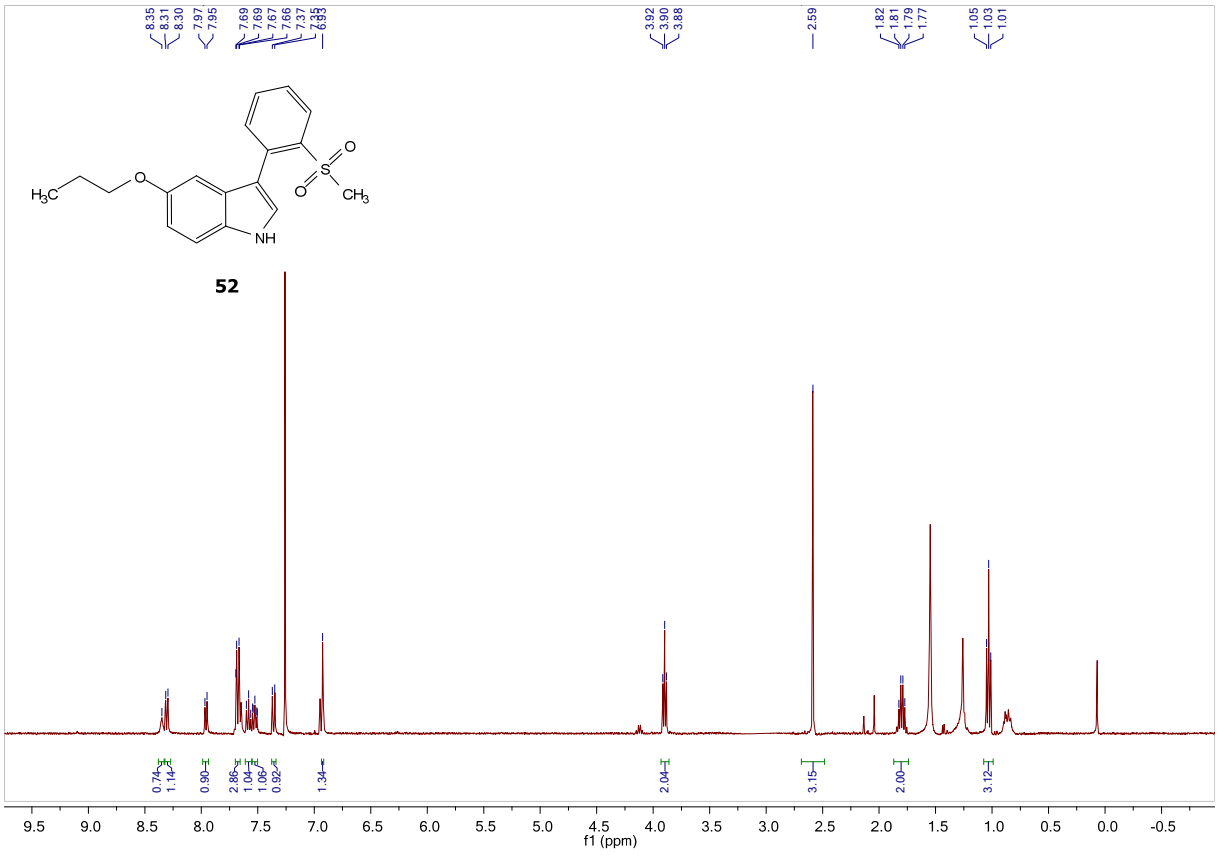
137.81
133.99
130.78
129.33
128.76
126.76
125.29
115.80
114.63
102.69
99.85
70.06
22.57
10.54
Br
H3C
O
N
S
O
O
3
f1 (ppm)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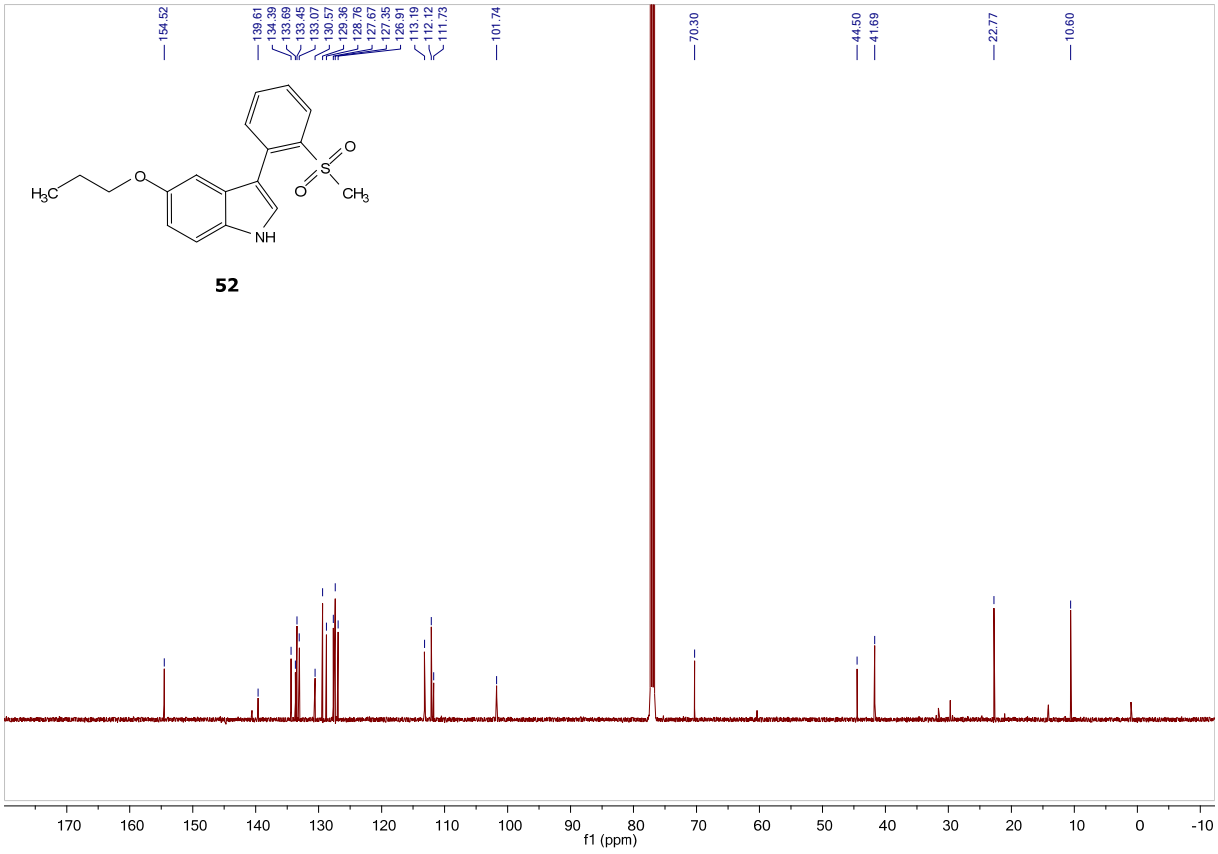
8.31
8.30
7.97
7.95
7.69
7.69
7.67
7.66
7.37
7.35
6.93
3.92
3.90
3.88
2.59
1.82
1.81
1.79
1.77
1.05
1.03
1.01
O
S
O
O
CH3
H3C
NH
52
0.74
1.14
0.90
2.86
1.04
1.06
0.92
1.34
2.04
3.15
2.00
3.12
f1 (ppm)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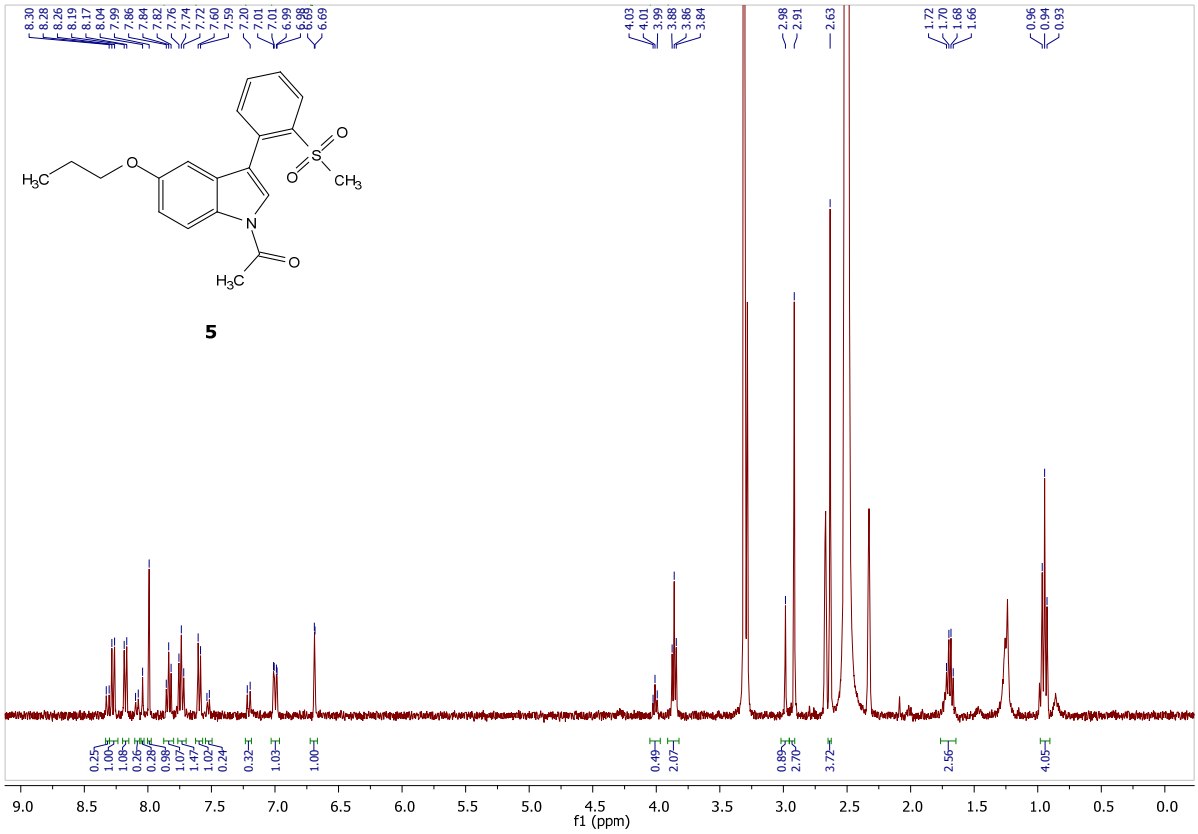
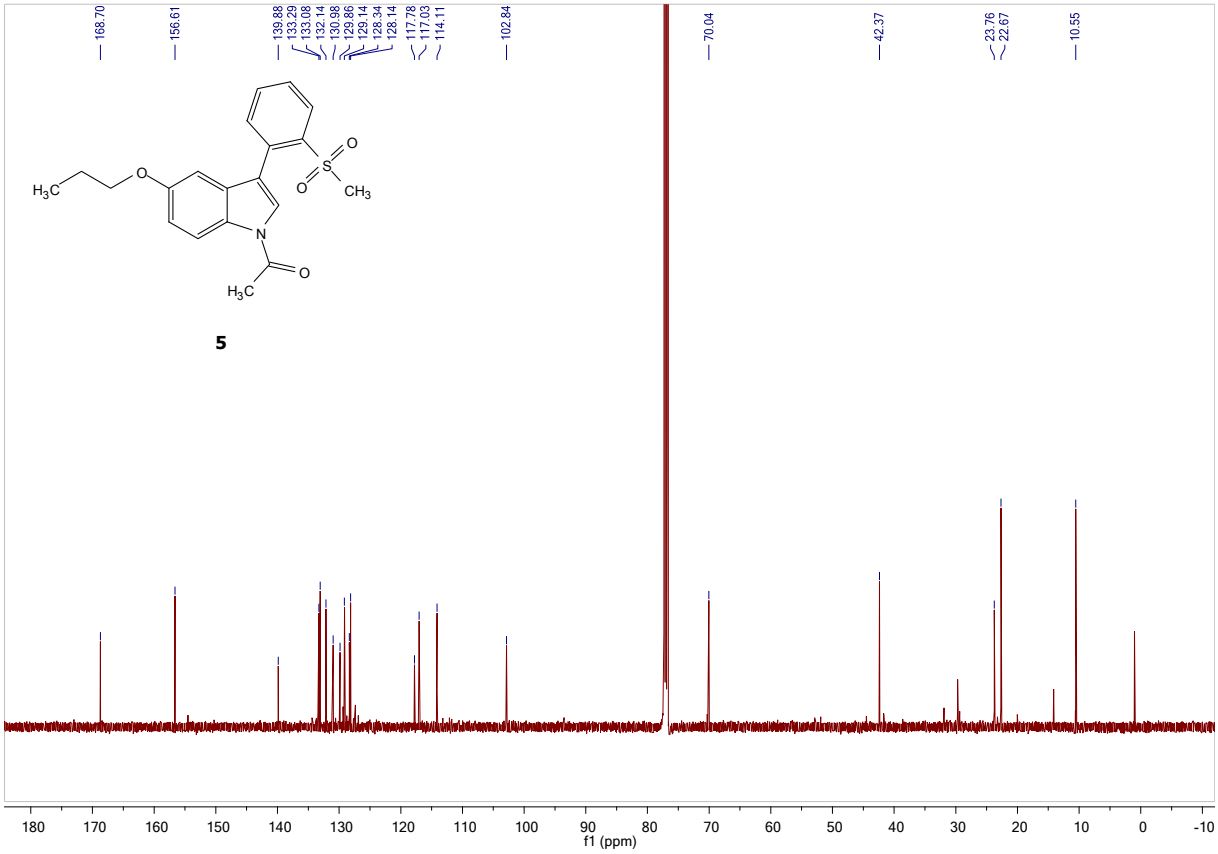
139.61
134.39
133.69
133.45
133.07
130.57
129.36
128.76
127.67
127.35
126.91
113.19
112.12
111.73
101.74
70.30
44.50
41.69
22.77
10.60
O
S
O
O
CH3
H3C
NH
52
f1 (ppm)


8.30
8.28
8.26
8.19
8.17
8.04
7.99
7.86
7.84
7.82
7.76
7.74
7.72
7.60
7.59
7.20
7.01
7.01
6.99
6.88
6.69
4.03
4.01
3.99
3.88
3.86
3.84
2.98
2.91
2.63
1.72
1.70
1.68
1.66
0.96
0.94
0.93
0.25
1.00
1.08
0.26
0.28
0.98
1.07
1.47
1.02
0.24
0.32
1.03
1.00
0.49
2.07
0.89
2.70
3.72
2.56
4.05
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
O
S
O
O
CH3
H3C
O
N
O
H3C

5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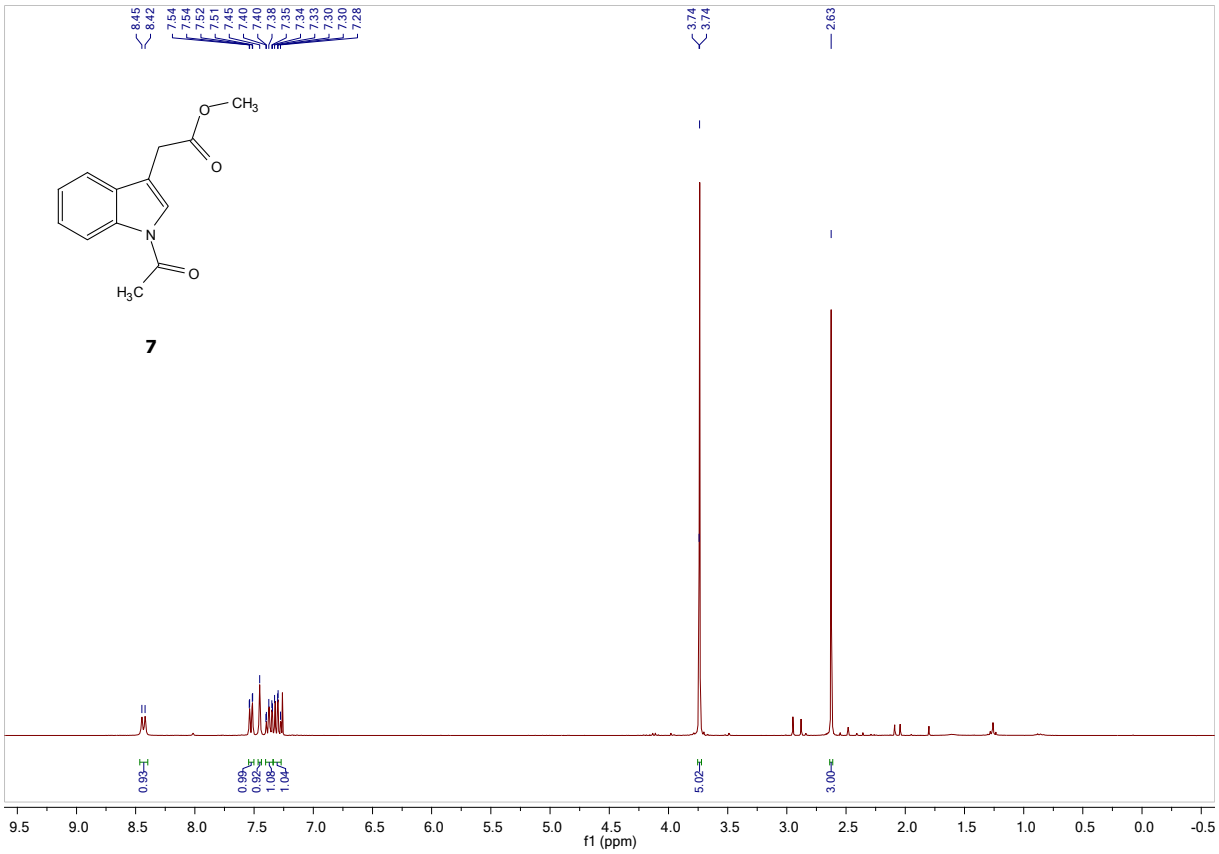
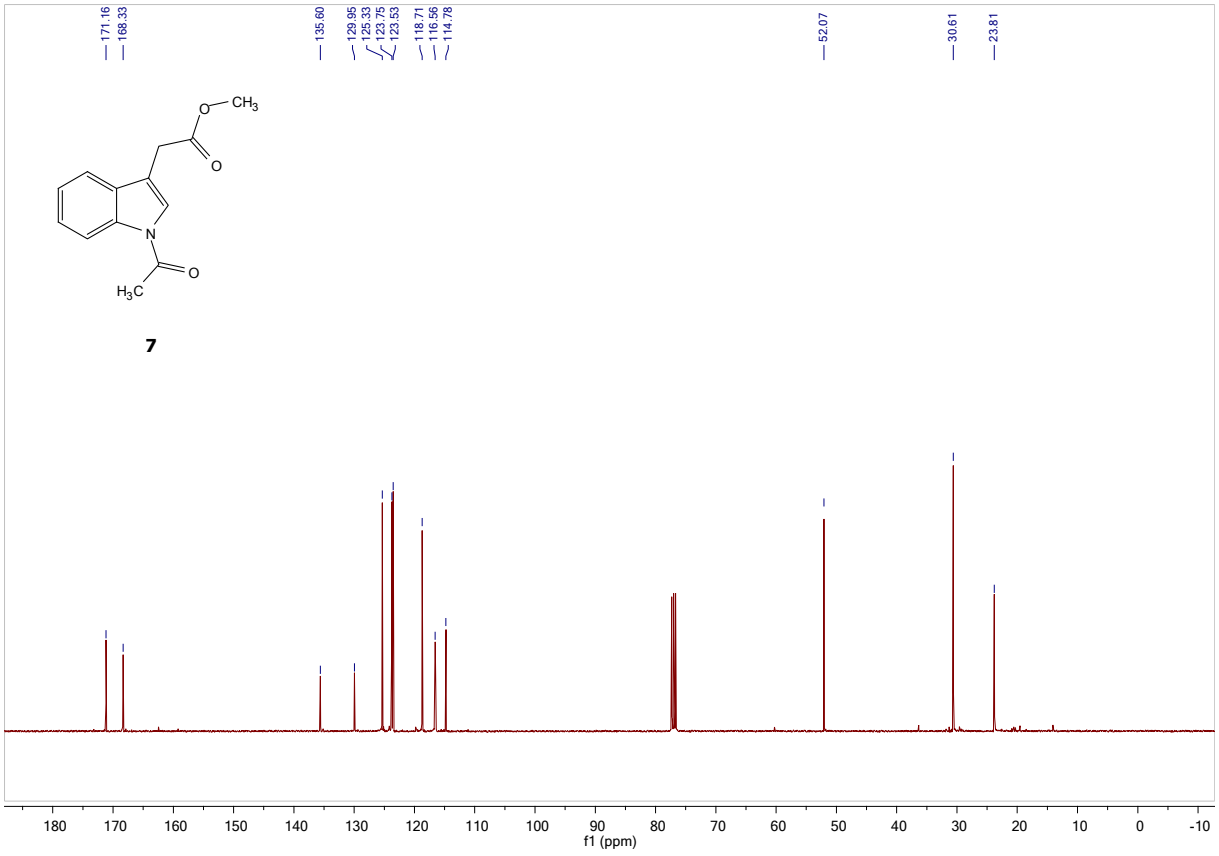
156.61
139.88
133.29
133.08
132.14
130.98
129.86
129.14
128.34
128.14
117.78
117.03
114.11
102.84
70.04
42.37
23.76
22.67
10.55
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)
O
S
O
O
CH3
H3C
O
N
O
H3C

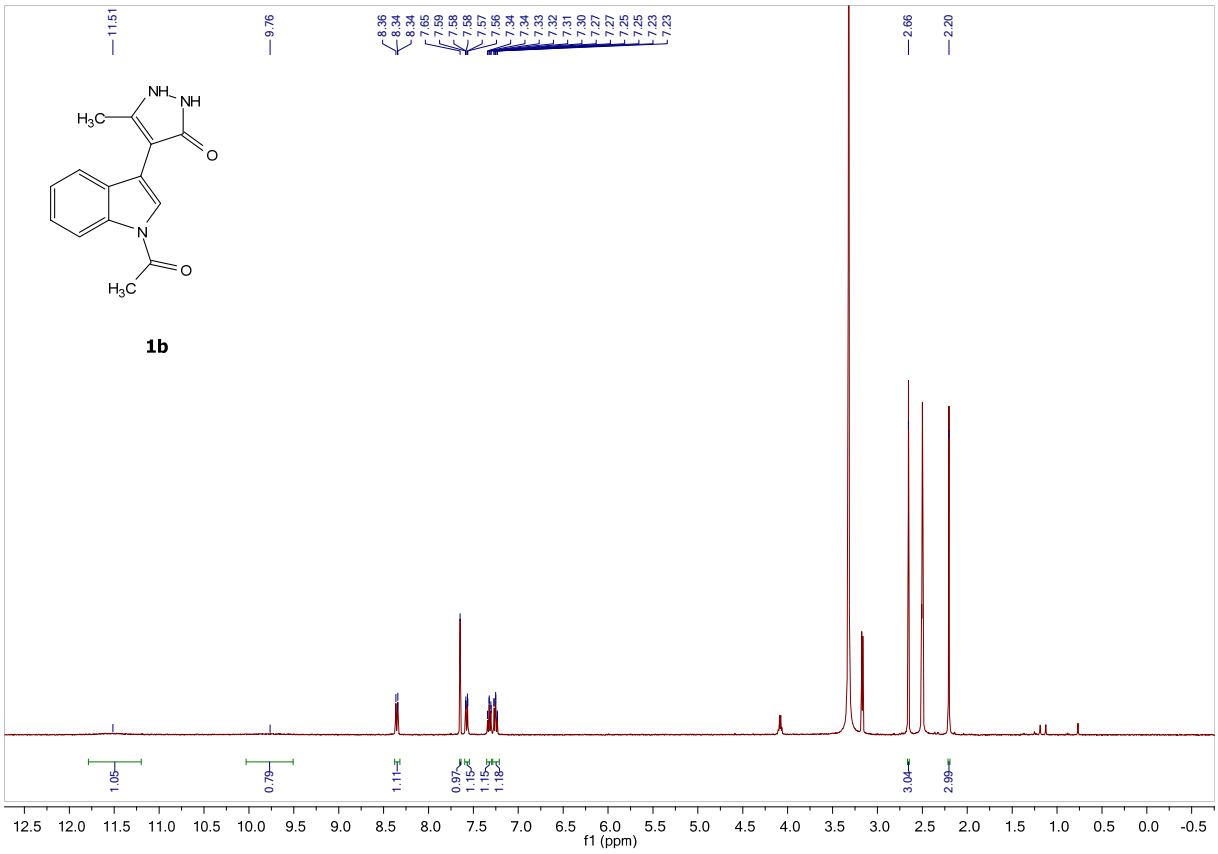
5

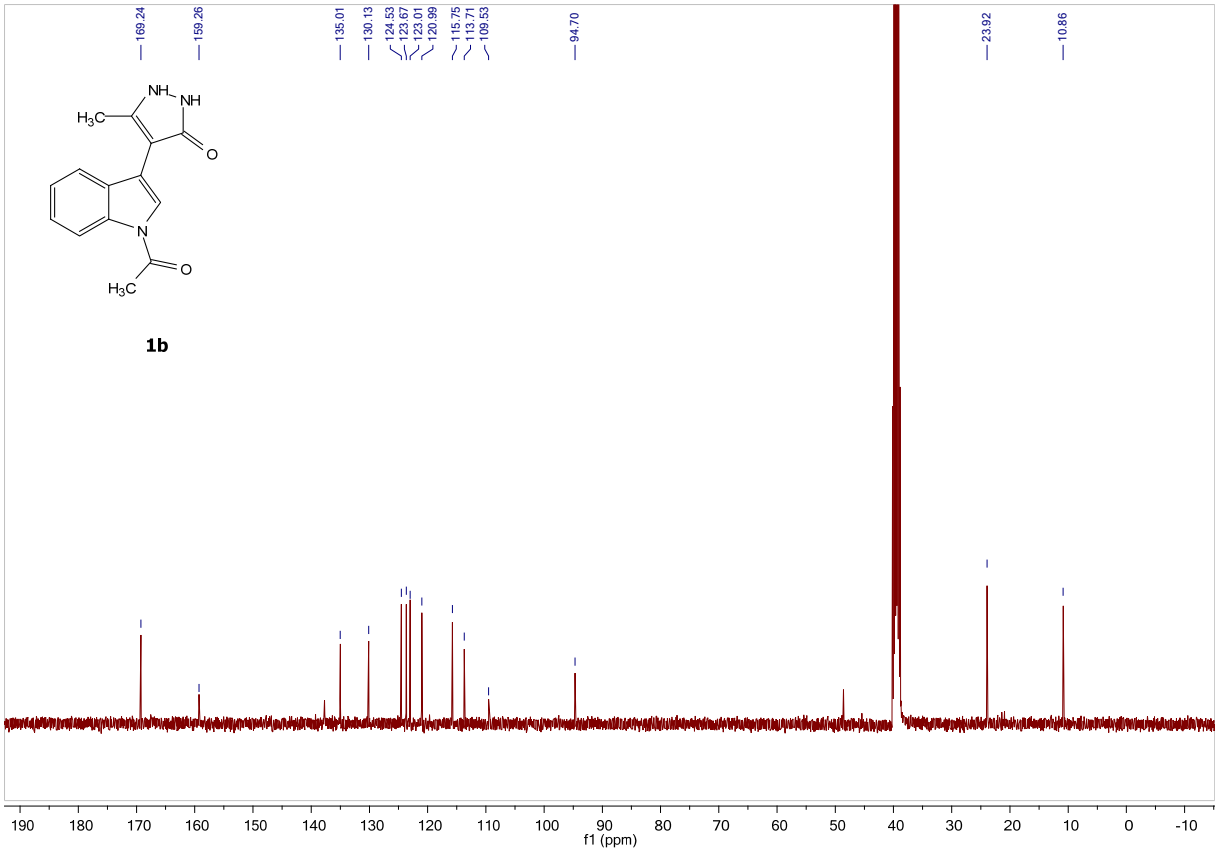

8.45
8.42
7.54
7.54
7.52
7.51
7.45
7.40
7.40
7.38
7.35
7.34
7.33
7.30
7.30
7.28
3.74
3.74
2.63
O
CH3
O
N
O
H3C
0.93
0.99
0.92
1.08
1.04
5.02
3.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

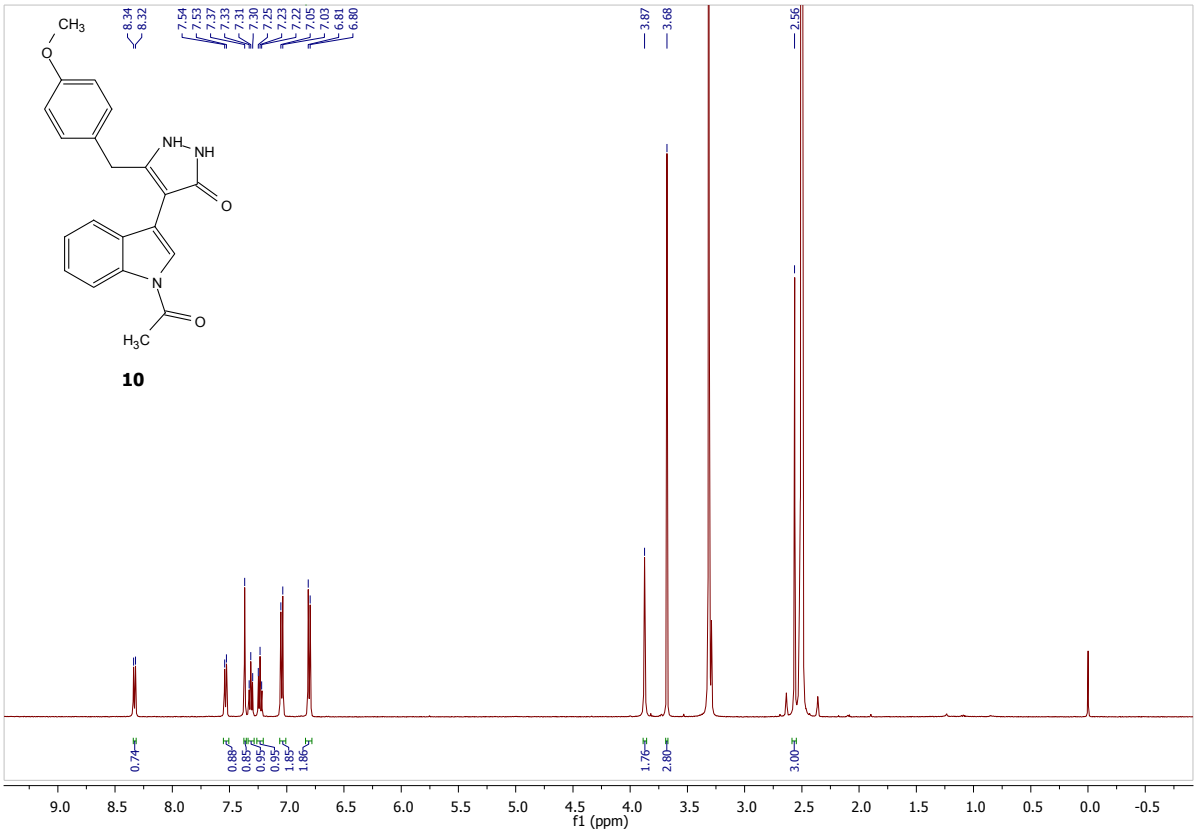
7

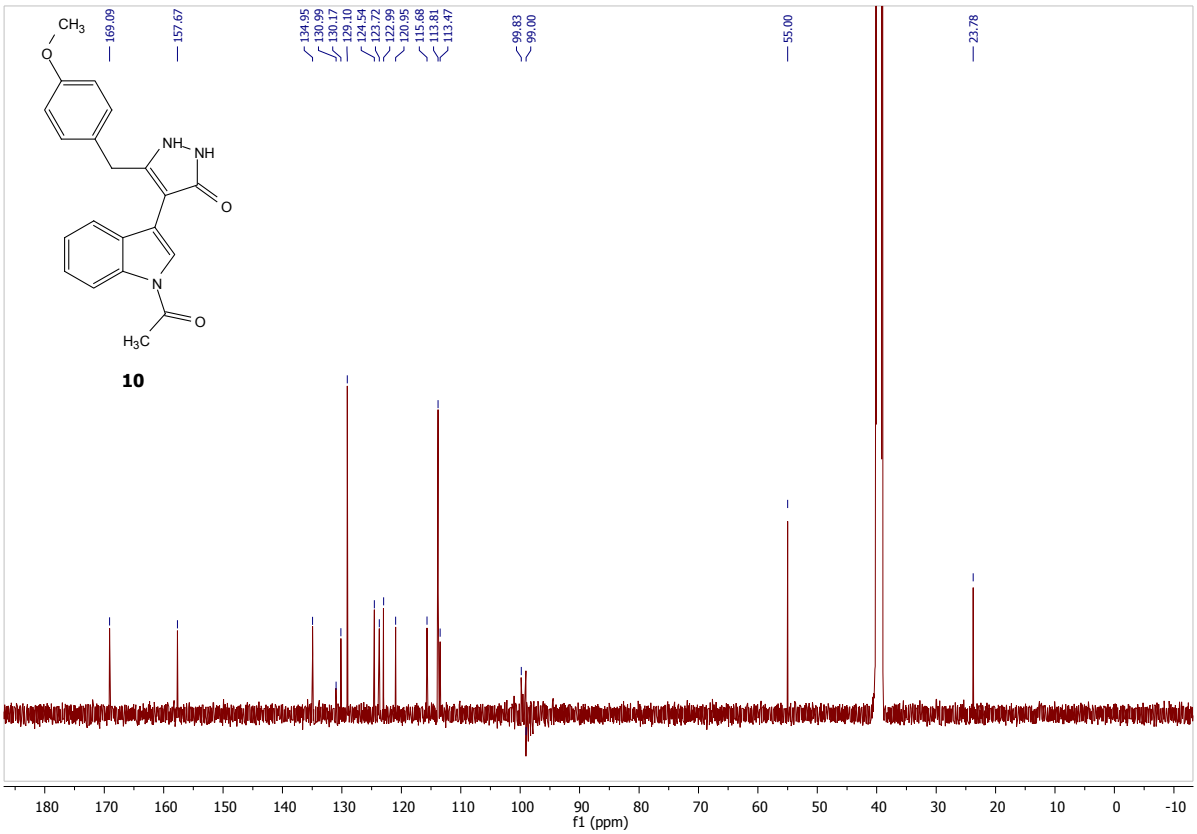

171.16
168.33
135.60
129.95
125.33
123.75
123.53
118.71
116.56
114.78
52.07
30.61
23.81
O
CH3
O
N
O
H3C
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

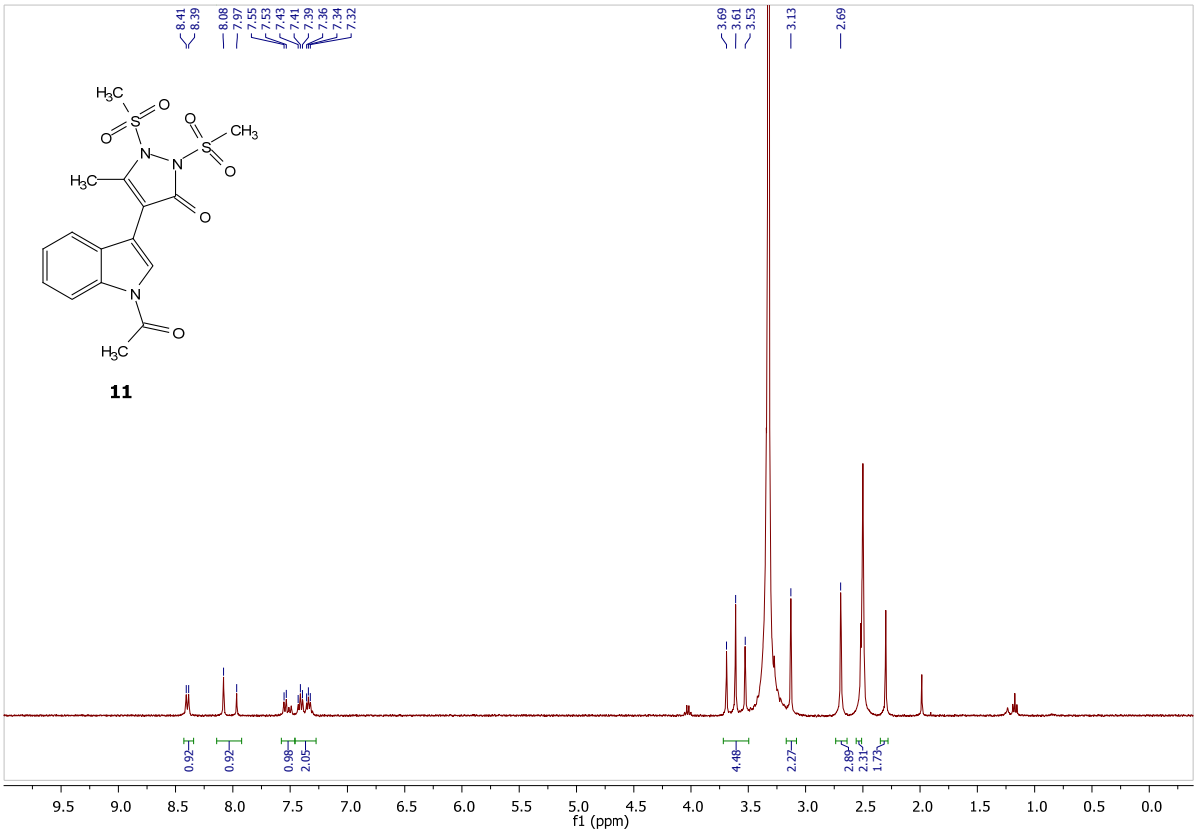
7


11.51
9.76
8.36
8.34
8.34
7.65
7.59
7.58
7.58
7.57
7.56
7.34
7.34
7.33
7.32
7.31
7.30
7.27
7.27
7.25
7.25
7.23
7.23
2.66
2.20
NH
NH
H3C
O
N
O
H3C
1b
1.05
0.79
1.11
0.97
1.15
1.15
1.18
3.04
2.99
f1 (ppm)

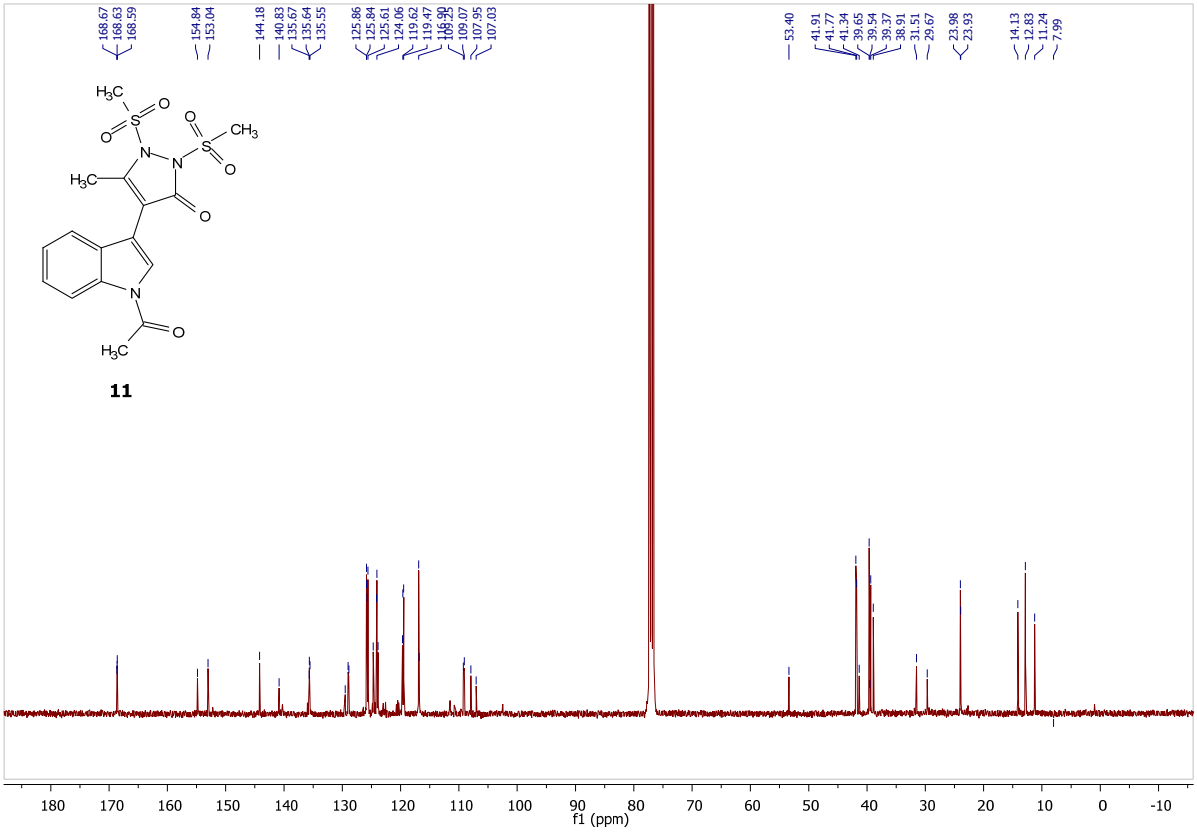

169.24
159.26
135.01
130.13
124.53
123.67
123.01
120.99
115.75
113.71
109.53
94.70
23.92
10.86
NH
NH
H3C
O
N
O
H3C
1b
f1 (ppm)


CH3
O
NH
NH
O
N
O
H3C
10
8.34
8.32
7.54
7.53
7.37
7.33
7.31
7.30
7.25
7.23
7.22
7.05
7.03
6.81
6.80
3.87
3.68
2.56
0.74
0.88
0.85
0.95
0.95
1.85
1.86
1.76
2.80
3.00
f1 (ppm)

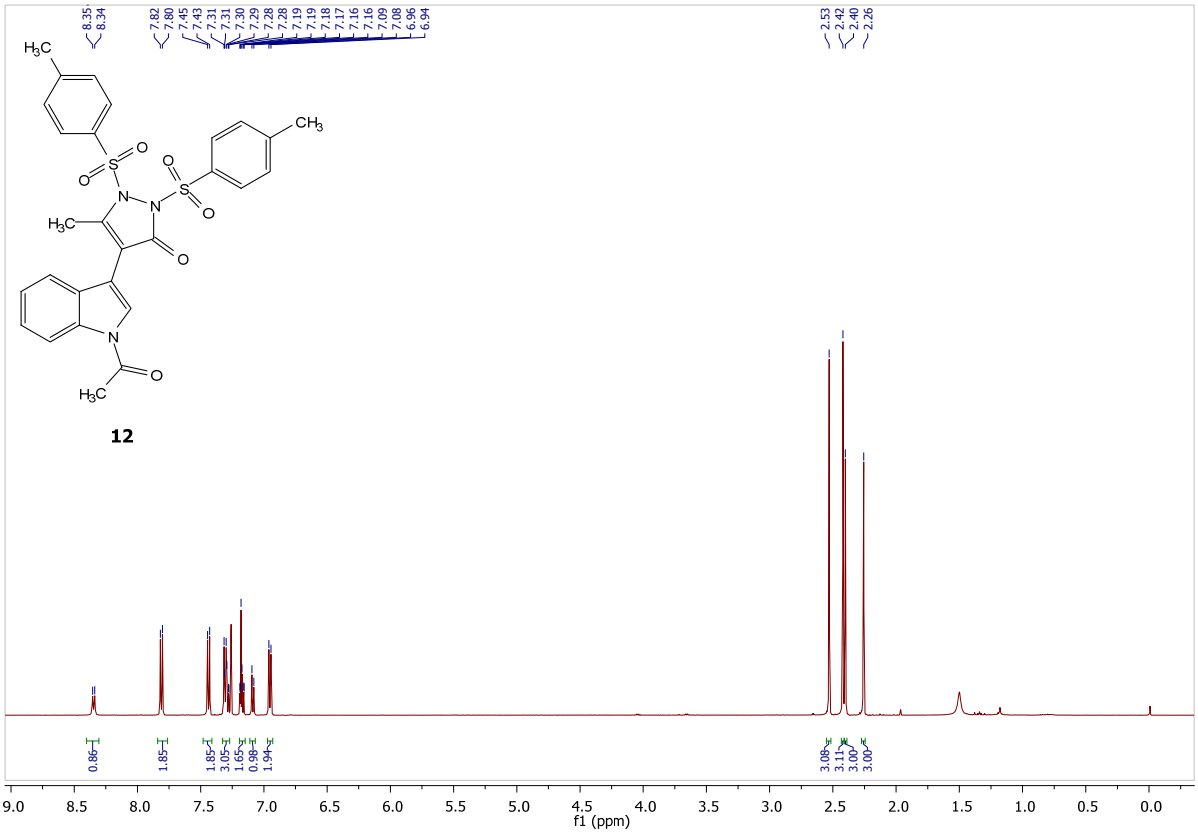

CH3
O
NH
NH
O
N
O
H3C
10
169.09
157.67
134.95
130.99
130.17
129.10
124.54
123.72
122.99
120.95
115.68
113.81
113.47
99.83
99.00
55.00
23.78
f1 (ppm)


8.41
8.39
8.08
7.97
7.55
7.53
7.43
7.41
7.39
7.36
7.34
7.32
3.69
3.61
3.53
3.13
2.69
0.92
0.92
0.98
2.05
4.48
2.27
2.89
2.31
1.73
f1 (ppm)

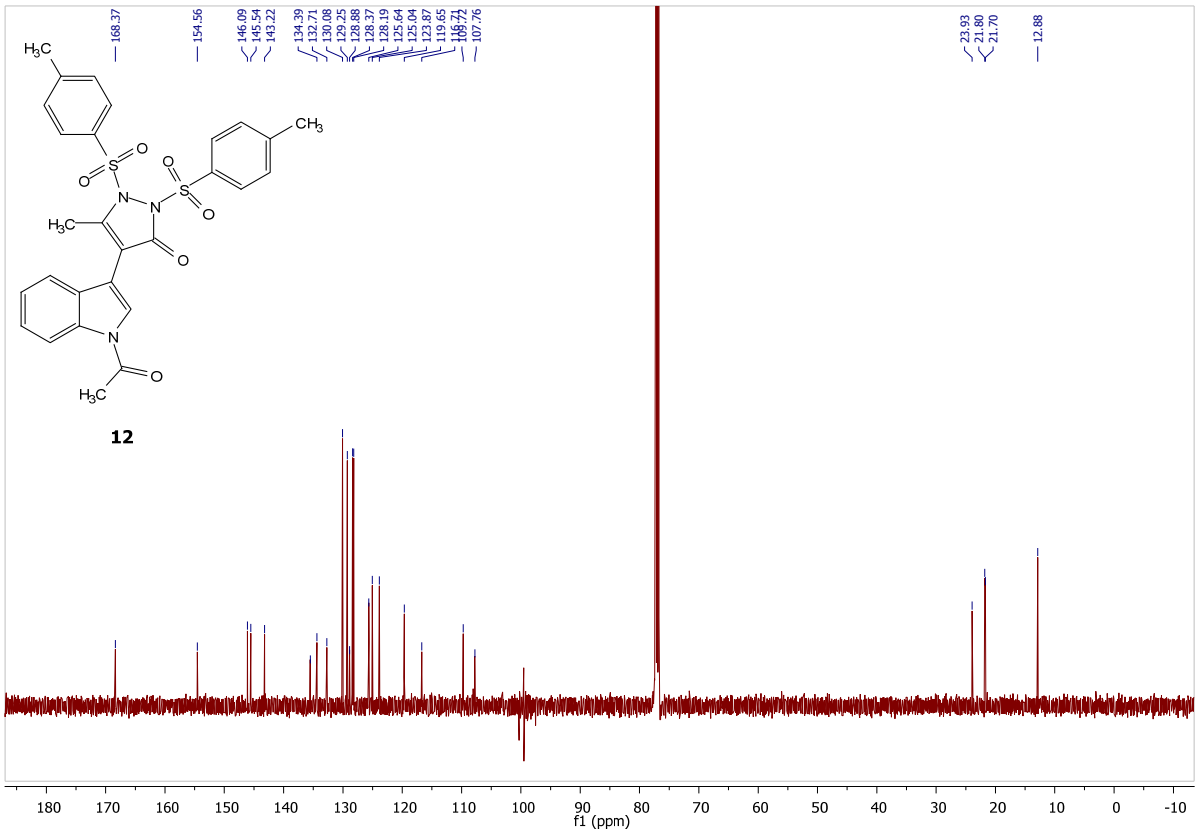
11


168.67
168.63
168.59
154.84
153.04
144.18
140.83
135.67
135.64
135.55
125.86
125.84
125.61
124.06
119.62
119.47
109.07
107.95
107.03
53.40
41.91
41.77
41.34
39.65
39.54
39.37
38.91
31.51
29.67
23.98
23.93
14.13
12.83
11.24
7.99
f1 (ppm)

11


8.35
8.34
7.82
7.80
7.45
7.43
7.31
7.31
7.30
7.29
7.28
7.28
7.19
7.19
7.18
7.17
7.16
7.16
7.09
7.08
6.96
6.94
2.53
2.42
2.40
2.26
0.86
1.85
1.85
3.05
1.65
0.98
1.94
3.08
3.11
3.00
3.00
f1 (ppm)

12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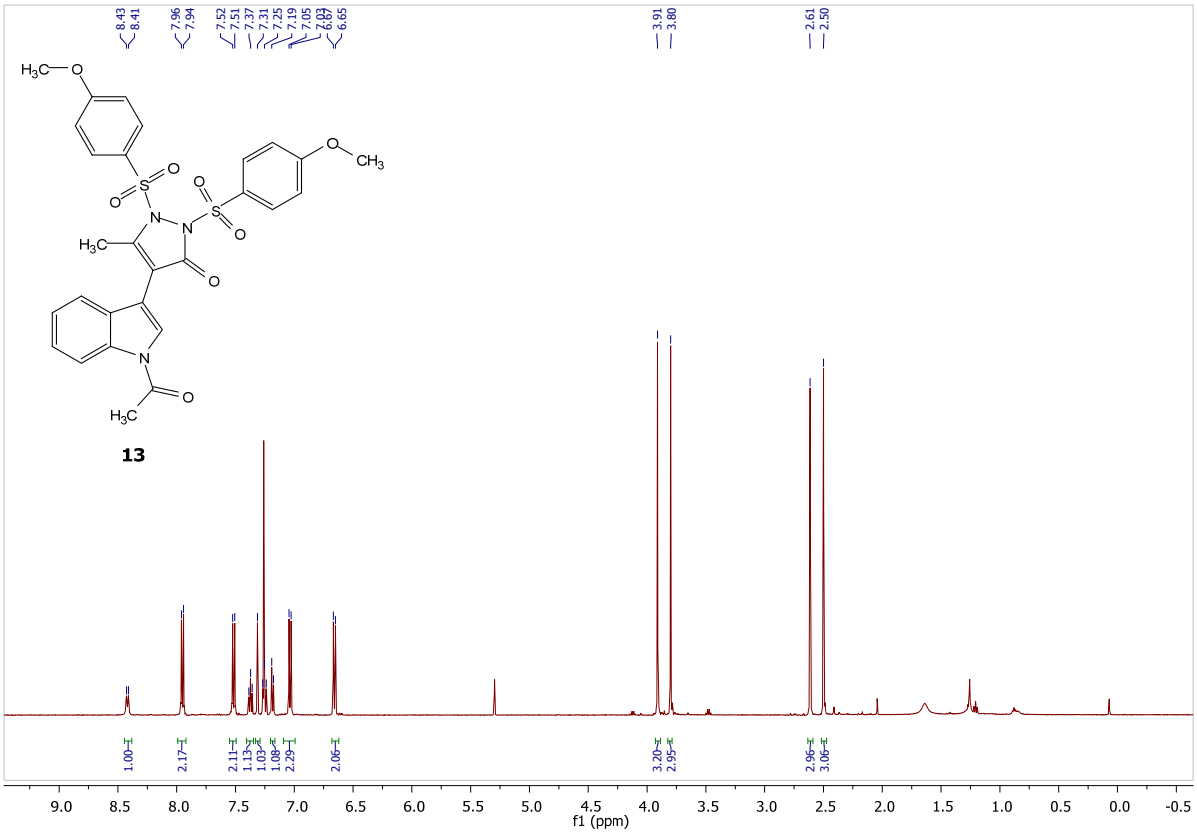
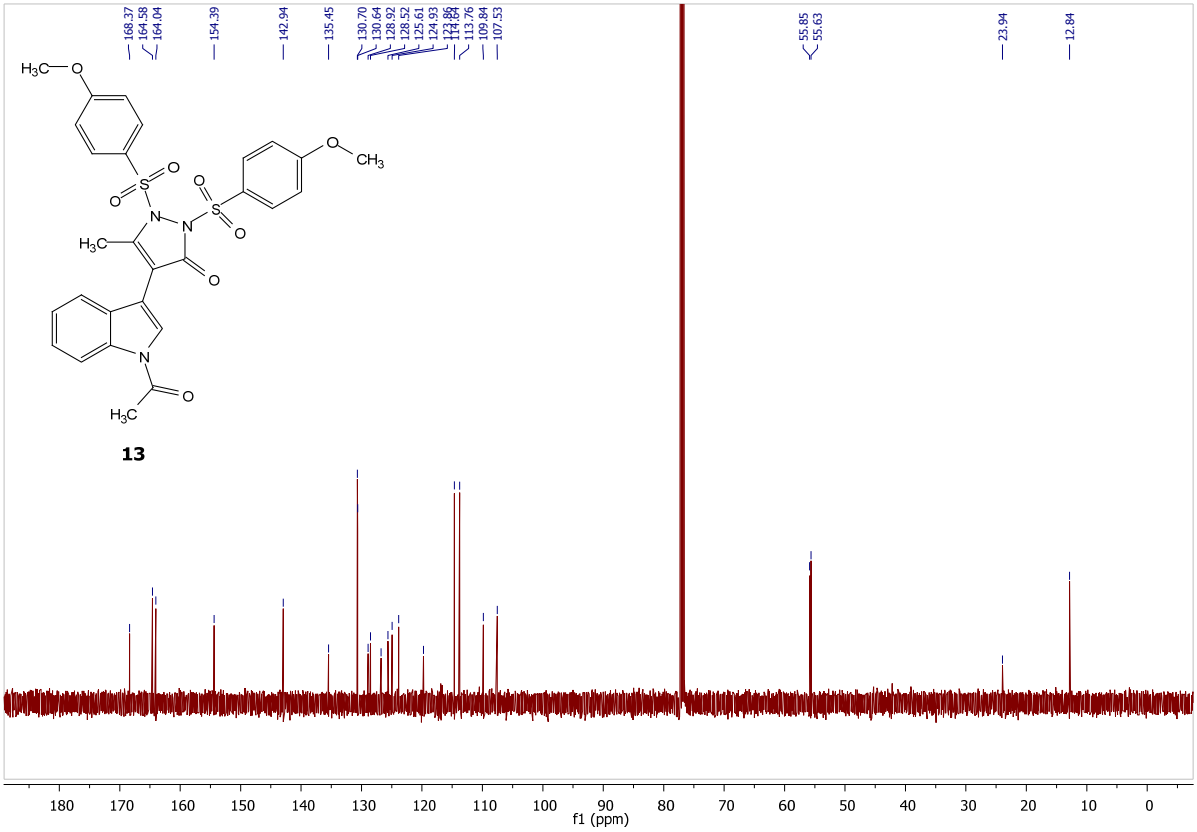
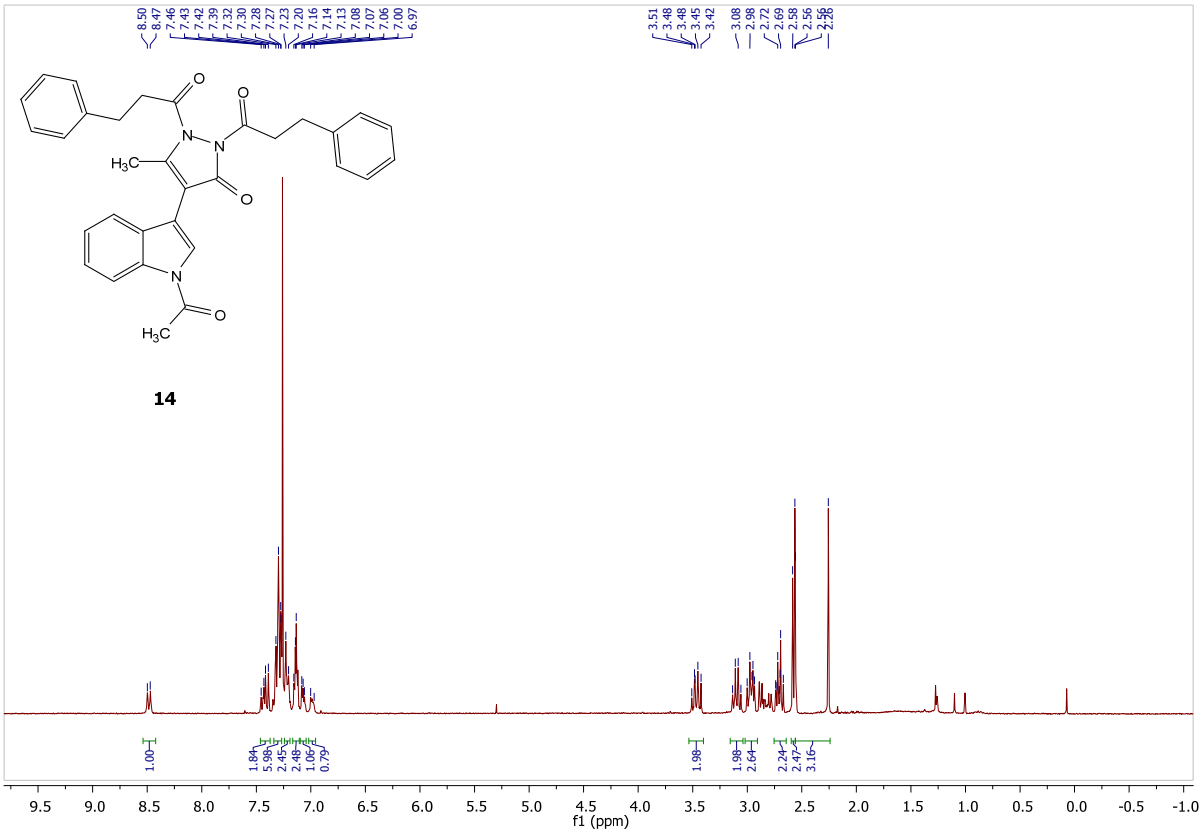
154.56
146.09
145.54
143.22
134.39
132.71
130.08
129.25
128.88
128.37
128.19
125.64
125.04
123.87
119.65
107.76
23.93
21.80
21.70
12.88
f1 (ppm)

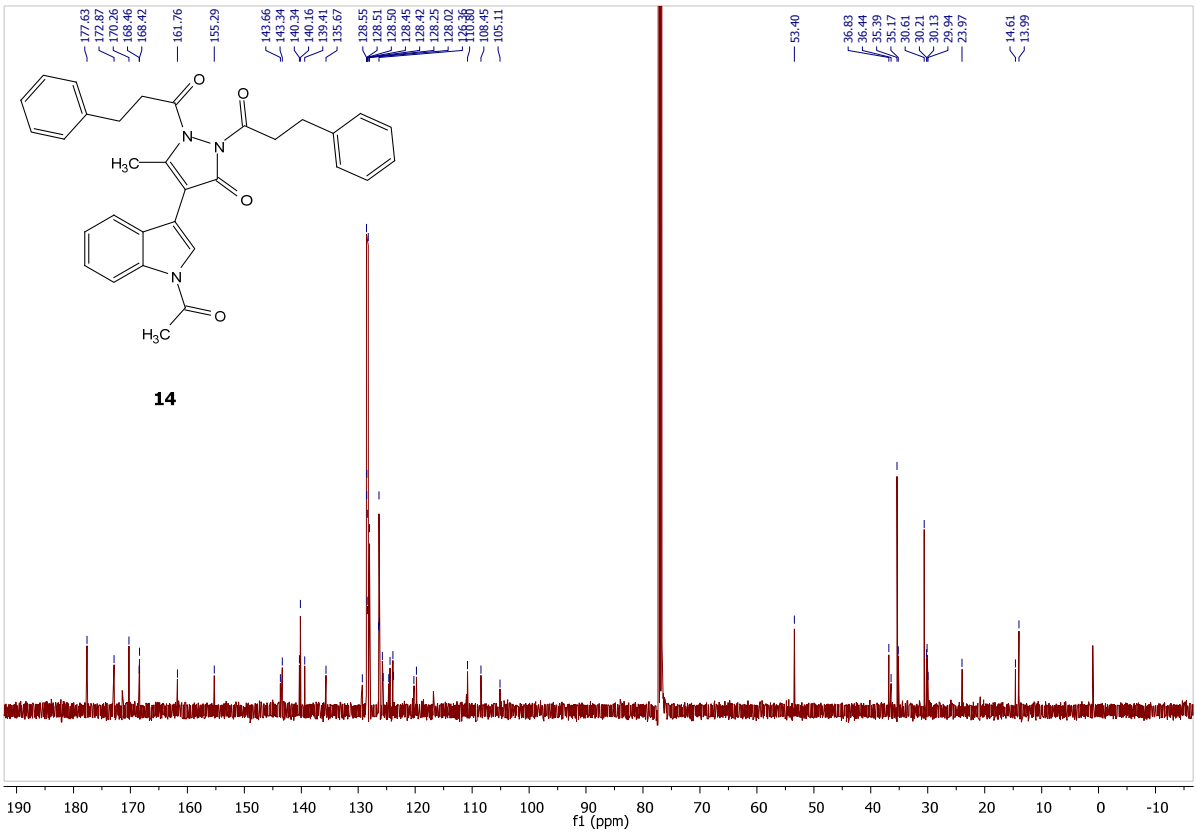
12


8.43
8.41
7.96
7.94
7.52
7.51
7.37
7.31
7.25
7.19
7.05
6.67
6.65
3.91
3.80
2.61
2.50
13
f1 (ppm)


168.37
164.58
164.04
154.39
142.94
135.45
130.70
130.64
128.92
128.52
125.61
124.93
113.76
109.84
107.53
55.85
55.63
23.94
12.84
13
f1 (ppm)


8.50
8.47
7.46
7.43
7.42
7.39
7.32
7.30
7.28
7.27
7.23
7.20
7.16
7.14
7.13
7.08
7.07
7.06
7.00
6.97
3.51
3.48
3.48
3.45
3.42
3.08
2.98
2.72
2.69
2.58
2.56
2.56
2.28
1.00
1.84
5.98
2.45
2.48
1.06
0.79
1.98
1.98
2.64
2.24
2.47
3.16
f1 (ppm)

14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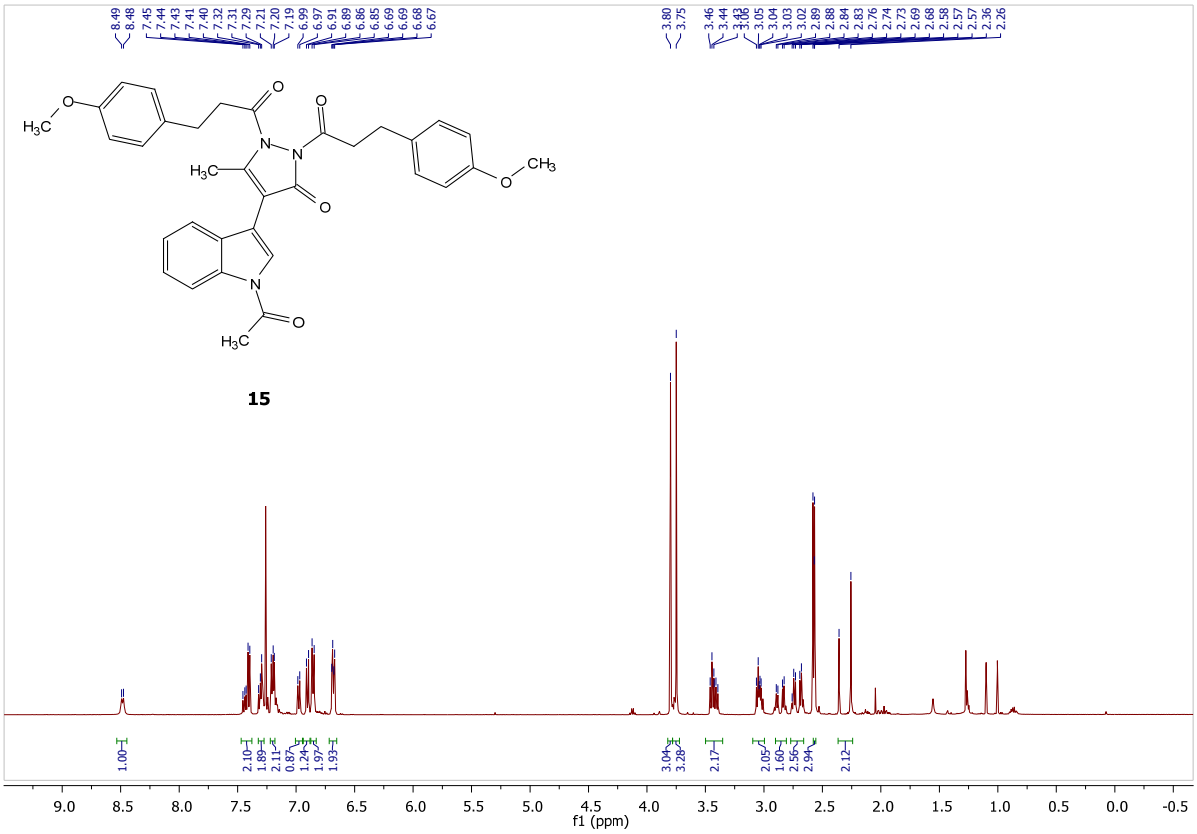
172.87
170.26
168.46
168.42
161.76
155.29
143.66
143.34
140.34
140.16
139.41
135.67
128.55
128.51
128.50
128.45
128.42
128.25
128.02
108.45
105.11
53.40
36.83
36.44
35.39
35.17
30.61
30.21
30.13
29.94
23.97
14.61
13.99
f1 (ppm)

14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8.48
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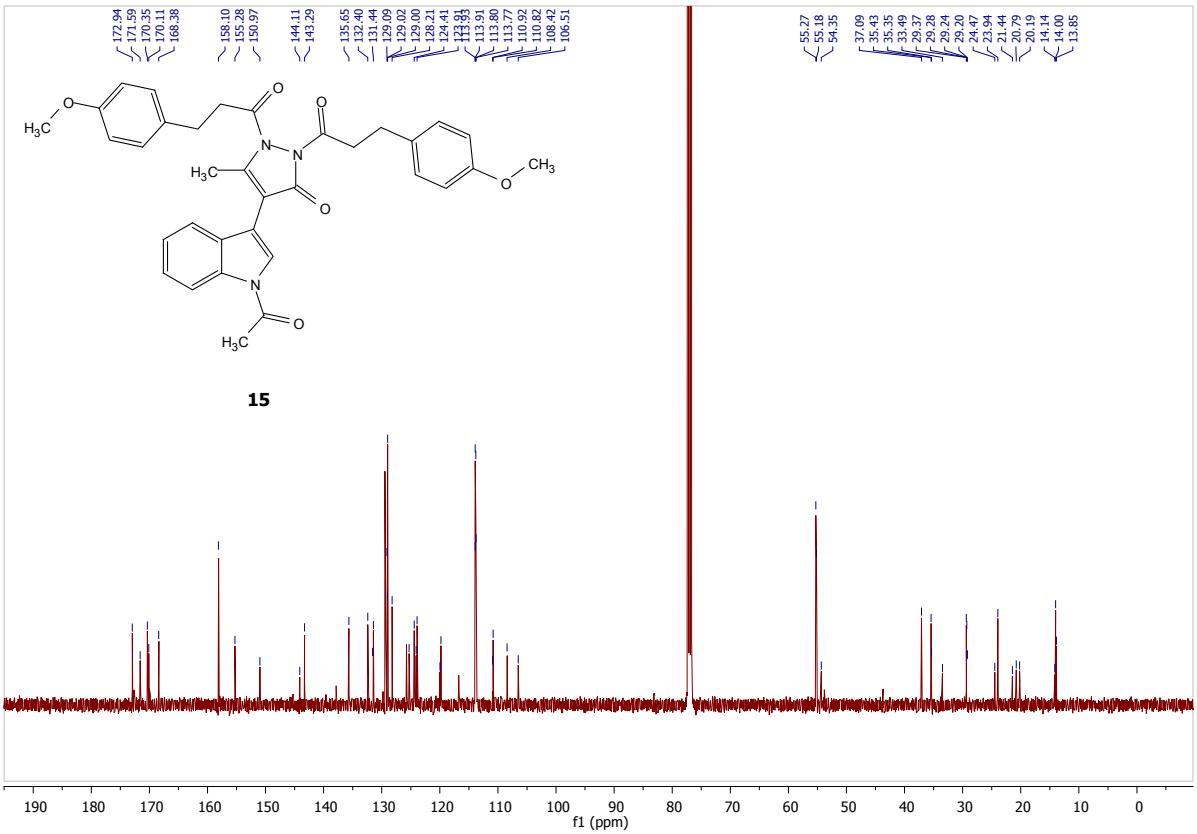
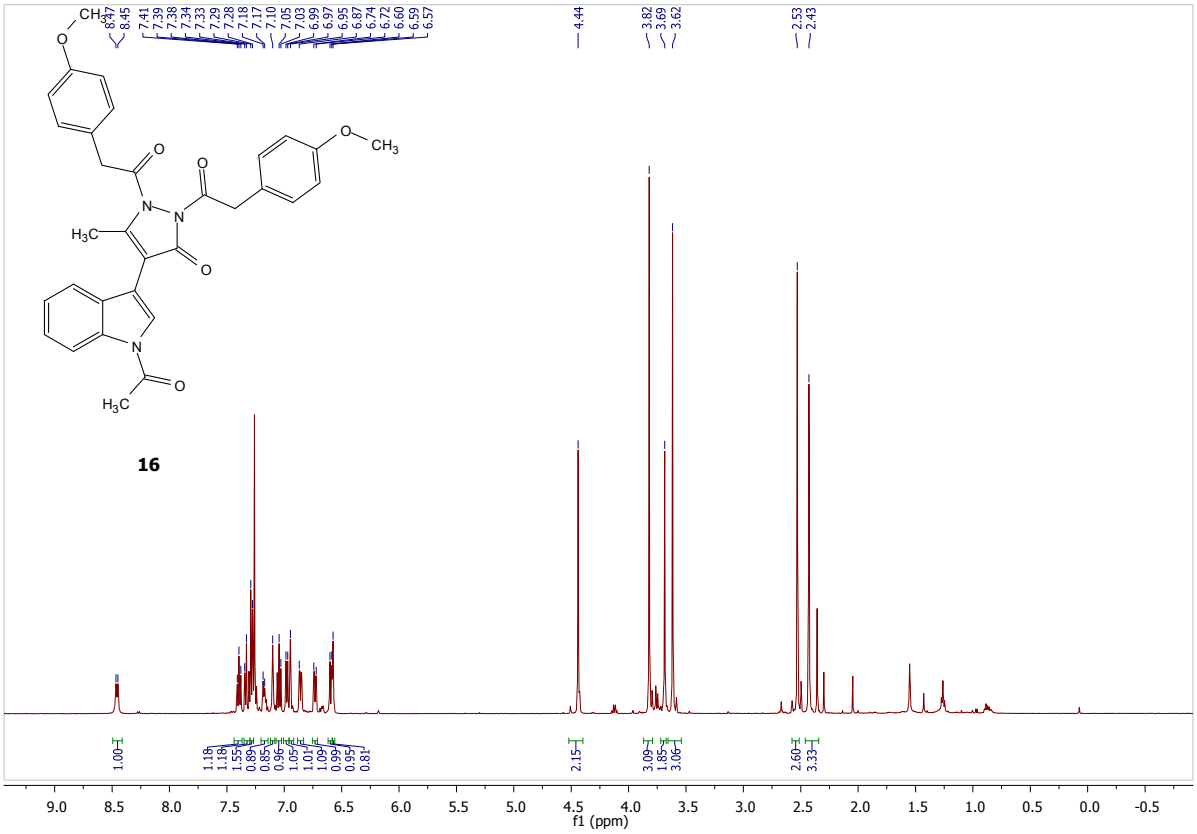
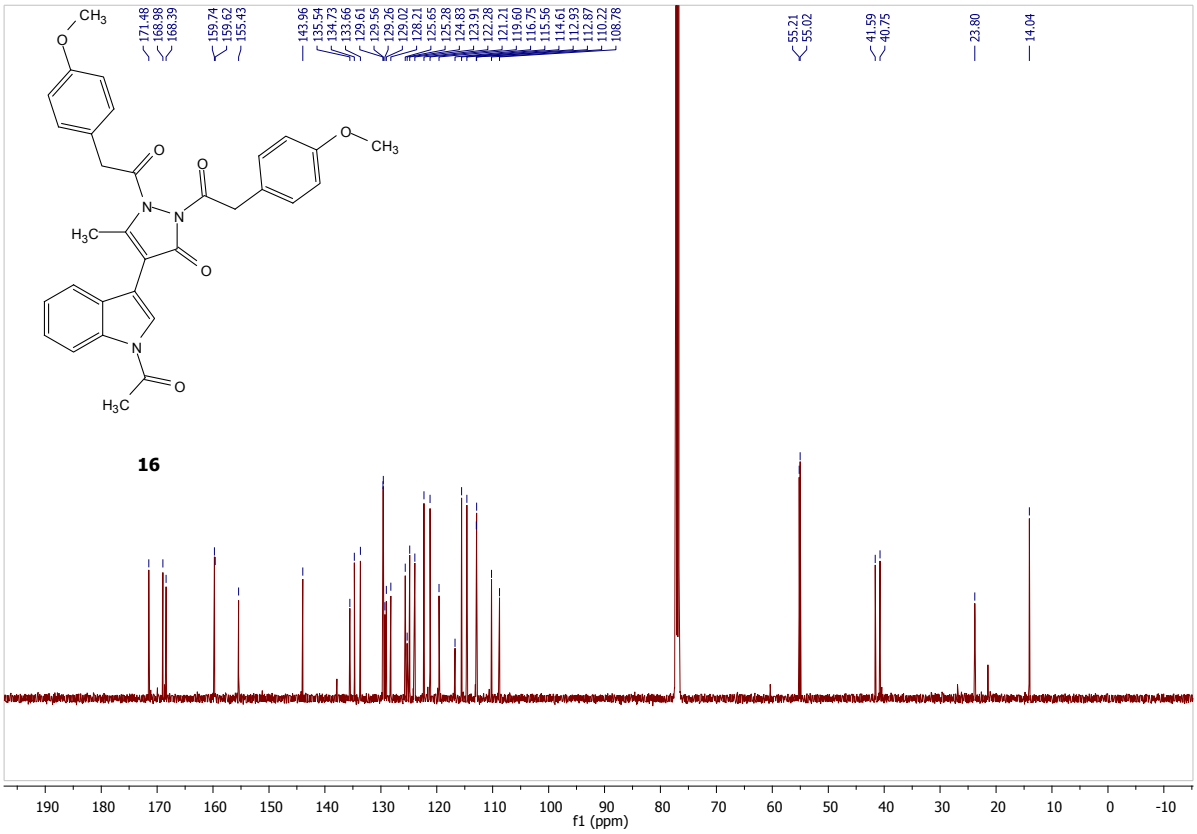
7.40
7.32
7.31
7.29
7.21
7.20
7.19
6.99
6.97
6.91
6.89
6.86
6.85
6.69
6.69
6.68
6.67
3.80
3.75
3.46
3.44
3.43
3.06
3.05
3.04
3.03
3.02
2.89
2.88
2.84
2.83
2.76
2.74
2.73
2.69
2.68
2.58
2.57
2.57
2.36
2.26
1.00
2.10
1.89
2.11
0.87
1.24
1.97
1.93
3.04
3.28
2.17
2.05
1.60
2.56
2.94
2.12
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

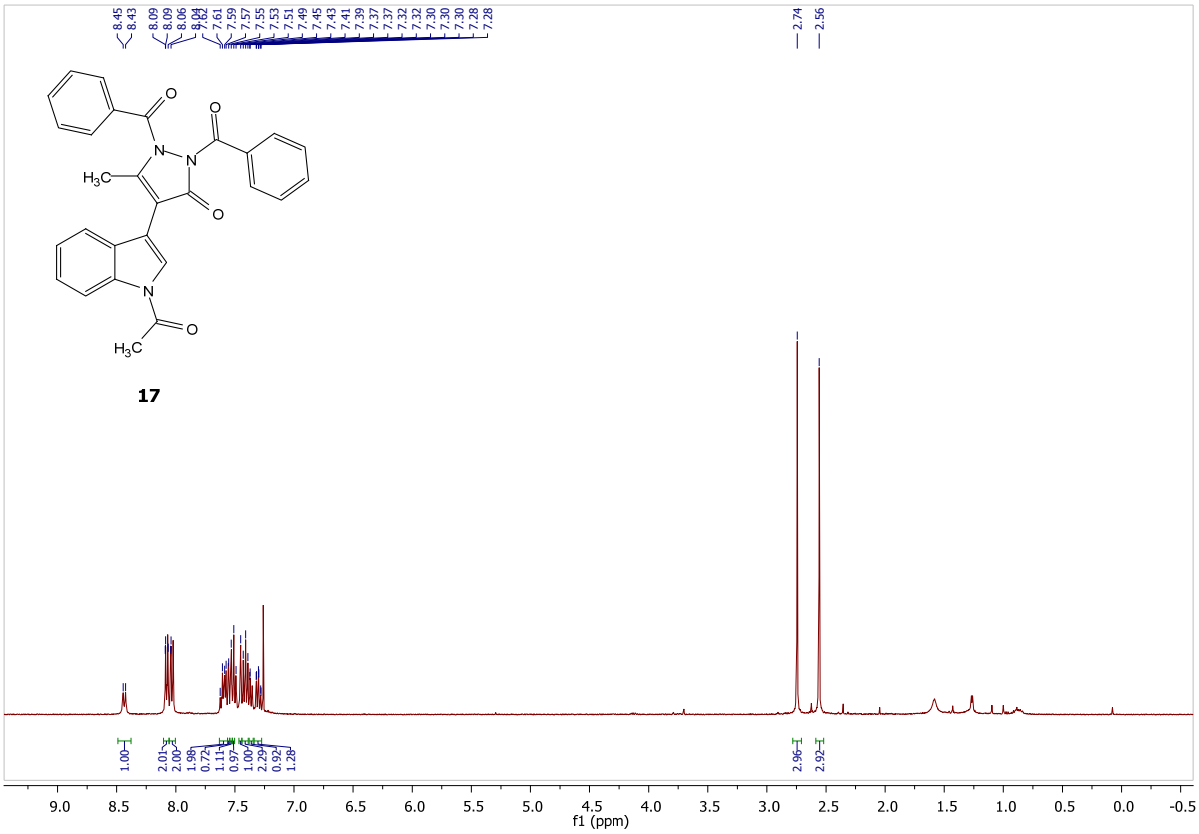
15


172.94
171.59
170.35
170.11
168.38
158.10
155.28
150.97
144.11
143.29
135.65
132.40
131.44
129.09
129.02
129.00
128.21
124.41
113.91
113.80
113.77
110.92
110.82
108.42
106.51
55.27
55.18
54.35
37.09
35.43
35.35
33.49
29.37
29.28
29.24
29.20
24.47
23.94
21.44
20.79
20.19
14.14
14.00
13.85
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

15


16
f1 (ppm)


16
f1 (ppm)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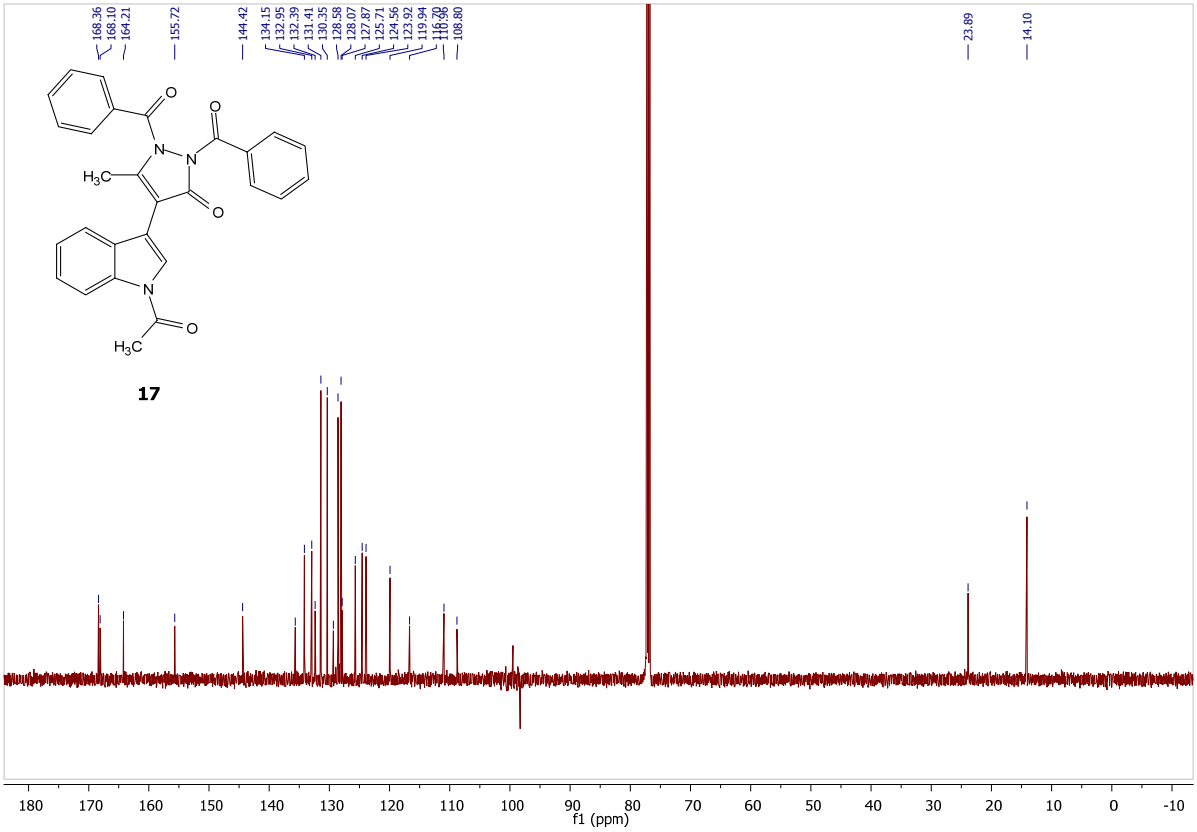
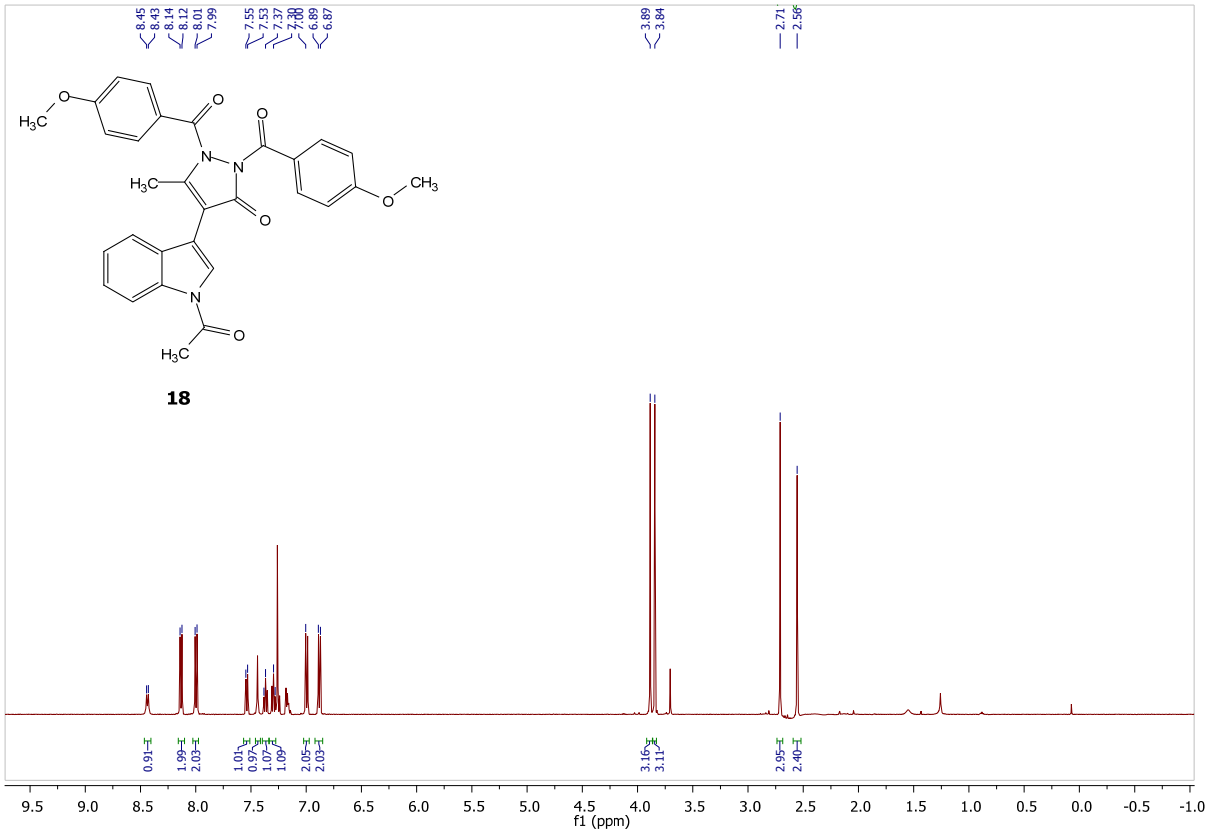
8.43
8.09
8.09
8.06
8.06
7.61
7.59
7.57
7.55
7.53
7.51
7.49
7.45
7.43
7.41
7.39
7.37
7.37
7.32
7.30
7.30
7.28
7.28
2.74
2.56
17
1.00
2.01
2.00
1.98
0.72
1.11
0.97
1.00
2.29
0.92
1.28
2.96
2.92
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


168.36
168.10
164.21
155.72
144.42
134.15
132.95
132.39
131.41
130.35
128.58
128.07
127.87
125.71
124.56
123.92
119.94
108.80
23.89
14.10
17
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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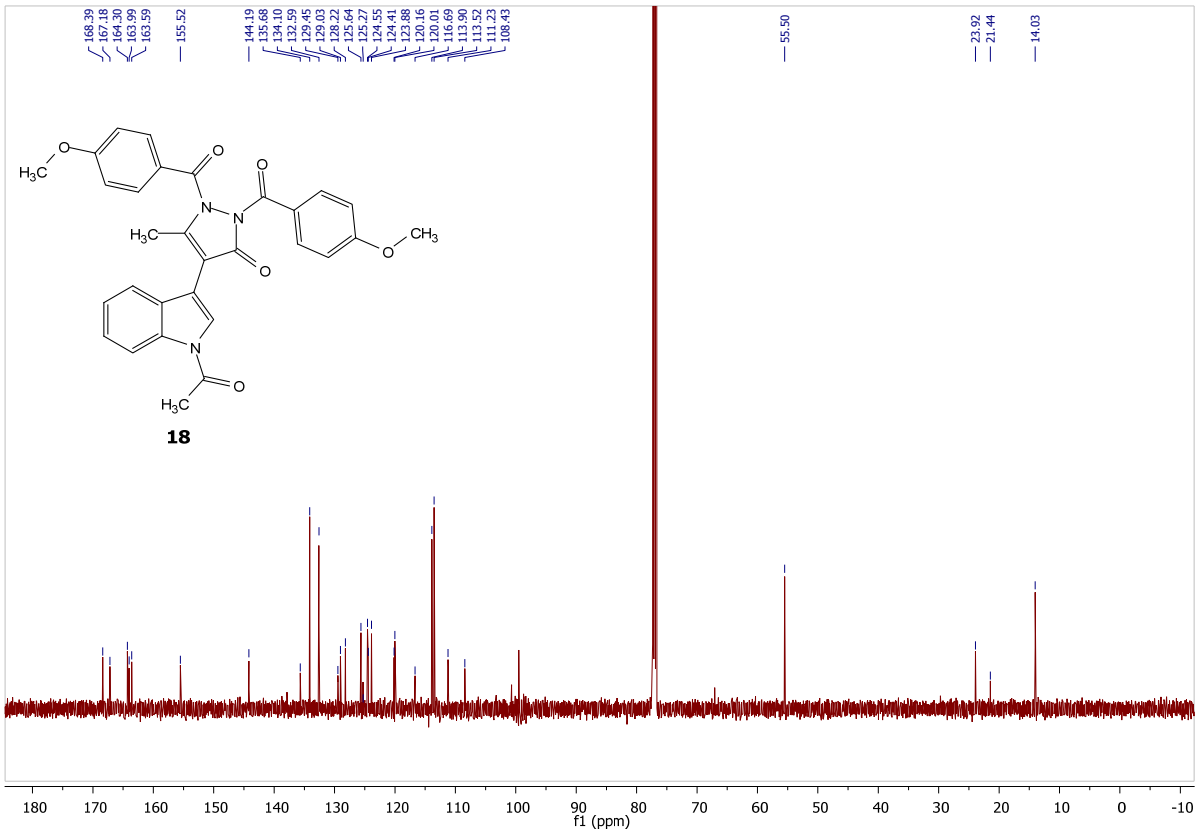
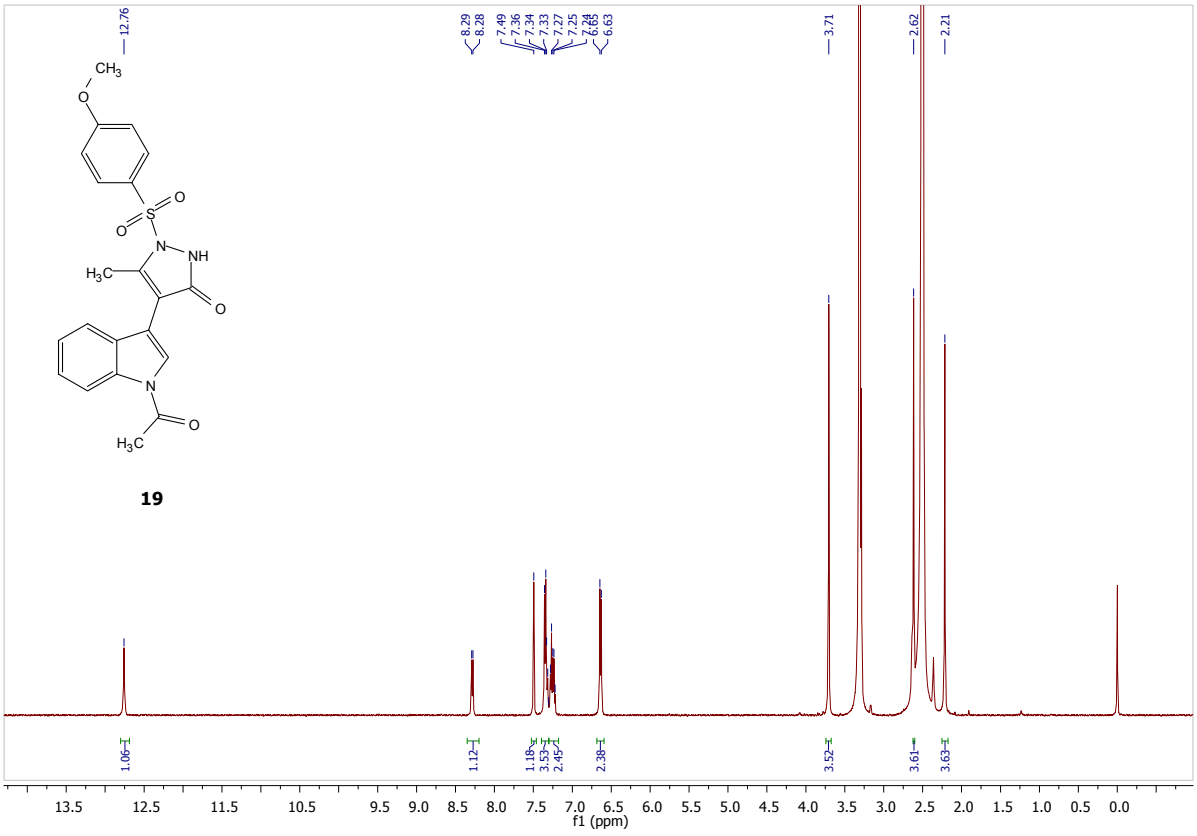
8.43
8.14
8.12
8.01
7.99
7.55
7.53
7.37
7.30
7.00
6.89
6.87
3.89
3.84
2.71
2.56
0.91
1.99
2.03
1.01
0.97
1.07
1.09
2.05
2.03
3.16
3.11
2.95
2.40
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

18


168.39
167.18
164.30
163.99
163.59
155.52
144.19
135.68
134.10
132.59
129.45
129.03
128.22
125.64
125.27
124.55
124.41
123.88
120.16
120.01
116.69
113.90
113.52
111.23
108.43
55.50
23.92
21.44
14.03
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

18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8.28
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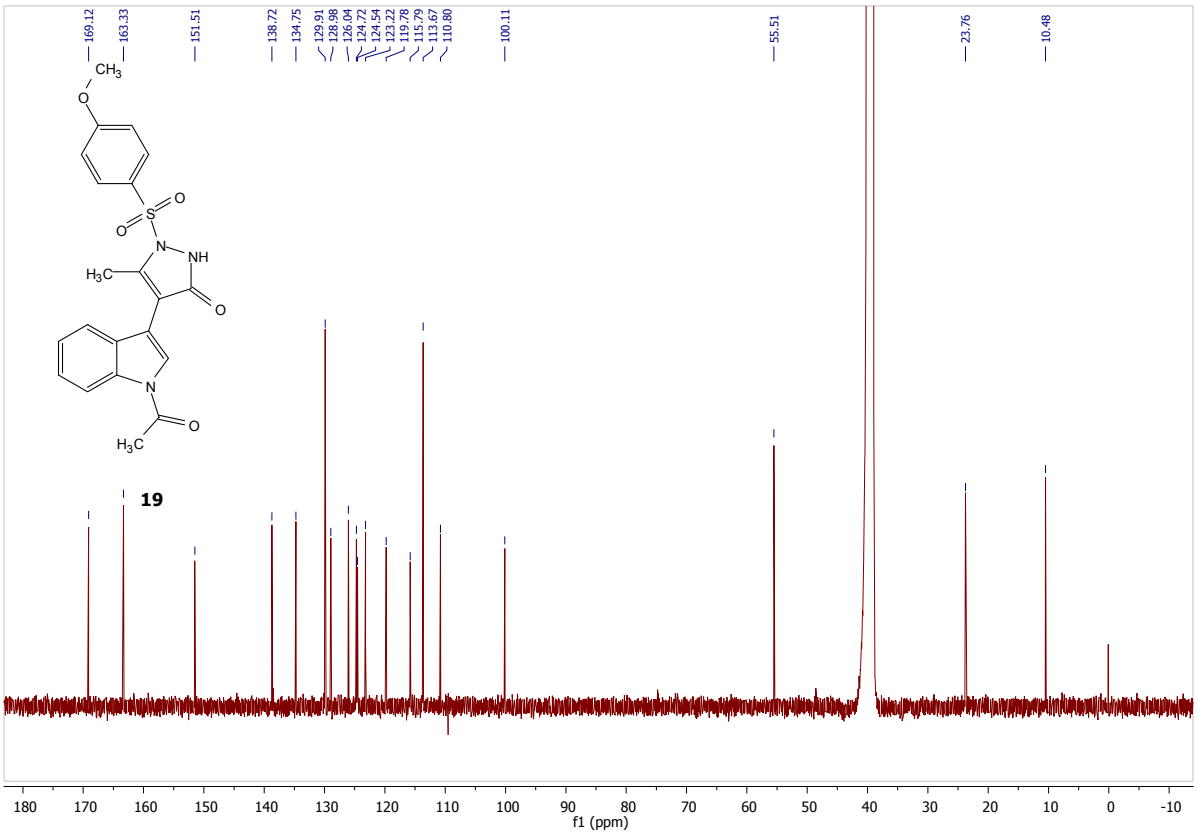
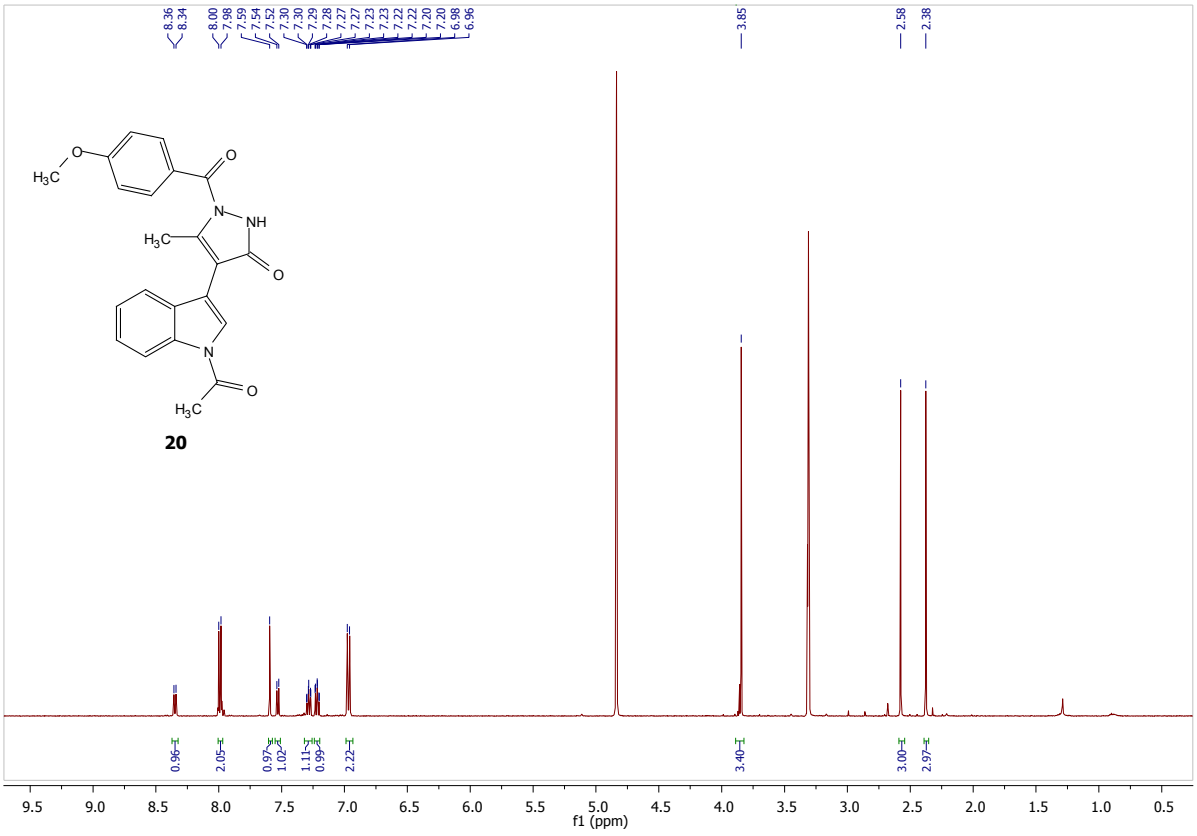
7.36
7.34
7.33
7.27
7.25
7.24
6.65
6.63
3.71
2.62
2.21
1.06
1.12
1.18
3.53
2.45
2.38
3.52
3.61
3.63
13.5
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

19


169.12
163.33
151.51
138.72
134.75
129.91
128.98
126.04
124.72
124.54
123.22
119.78
115.79
113.67
110.80
100.11
55.51
23.76
10.48
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

19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8.34
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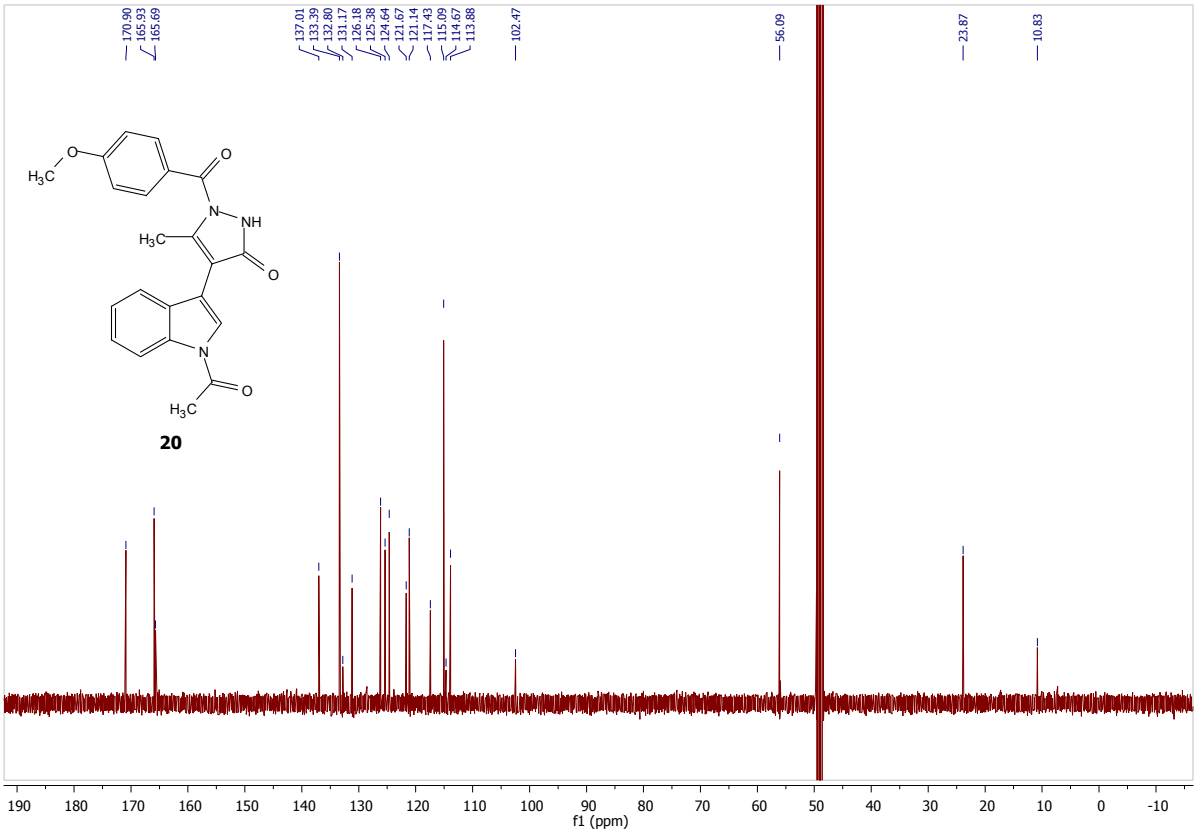
7.54
7.52
7.30
7.30
7.29
7.28
7.27
7.27
7.23
7.23
7.22
7.22
7.20
7.20
6.98
6.96
3.85
2.58
2.38
0.96
2.05
0.97
1.02
1.11
0.99
2.22
3.40
3.00
2.97
f1 (ppm)

20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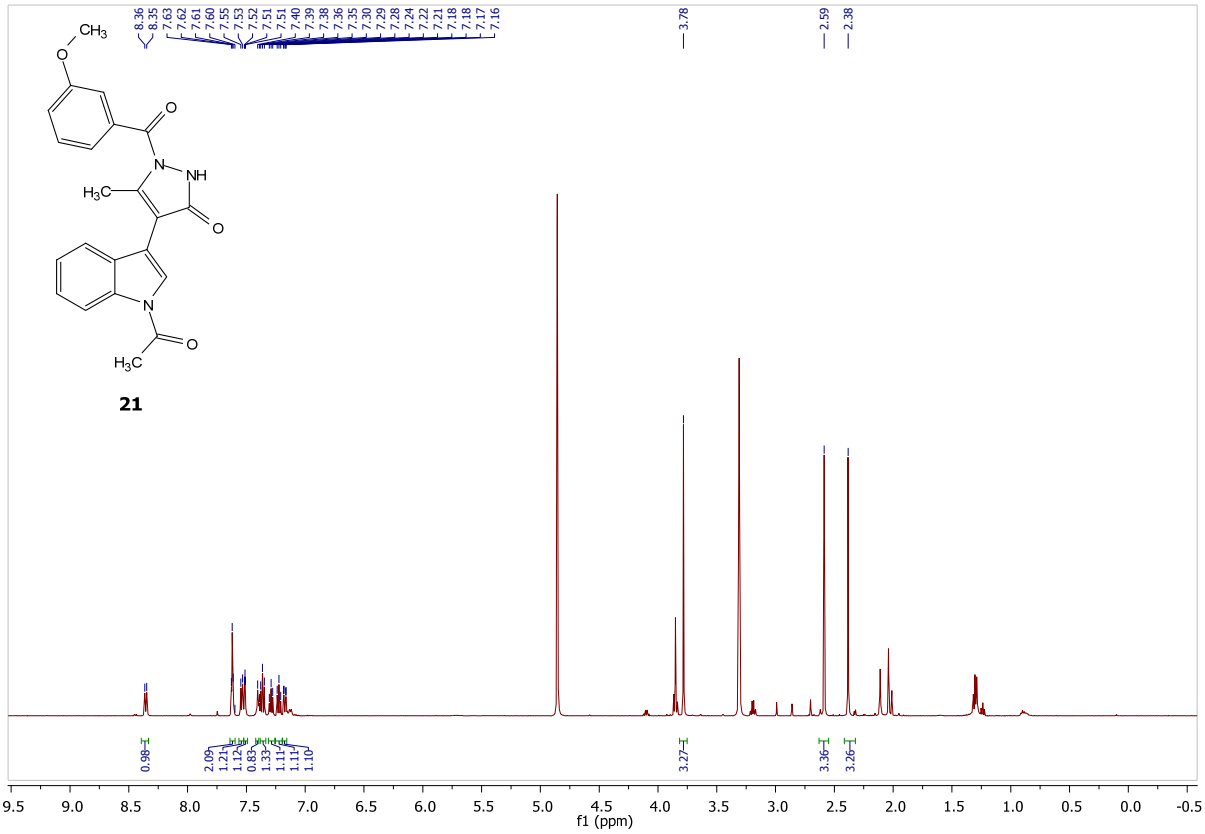
165.93
165.69
137.01
133.39
132.80
131.17
126.18
125.38
124.64
121.67
121.14
117.43
115.09
114.67
113.88
102.47
56.09
23.87
10.83
f1 (ppm)

20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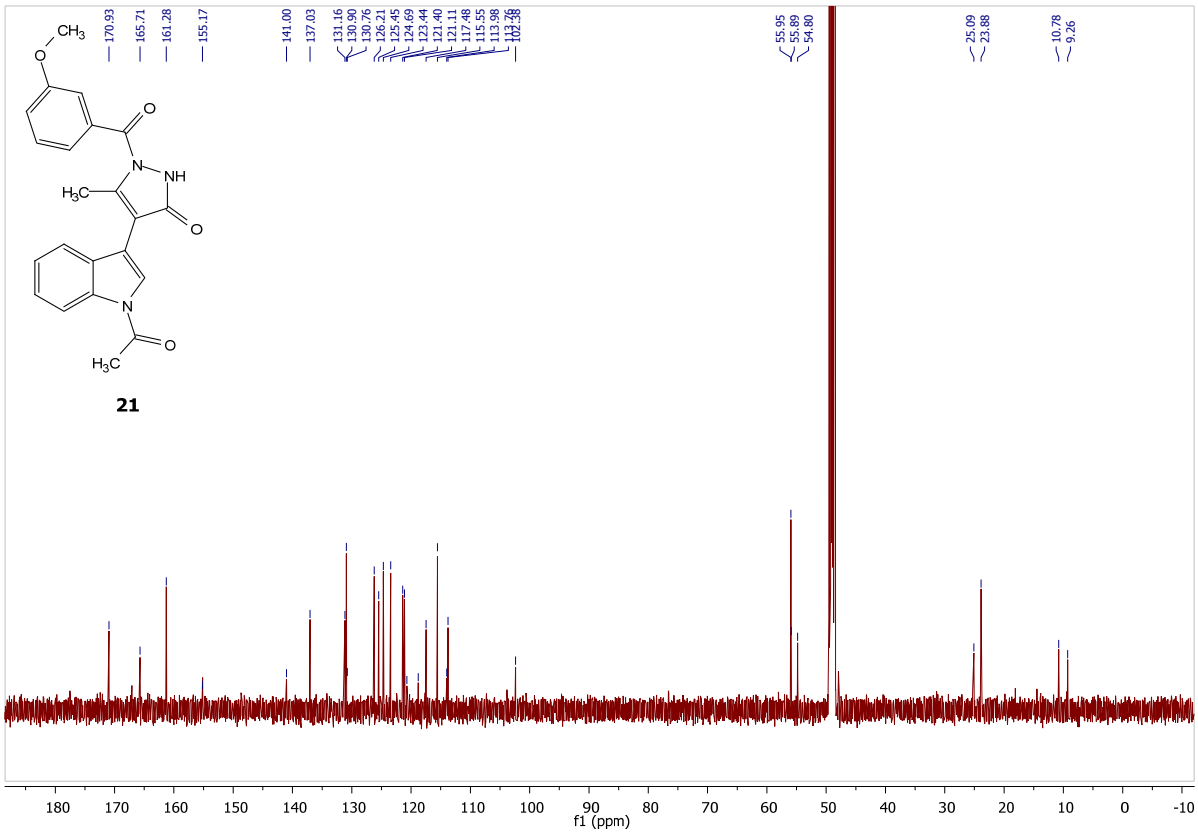
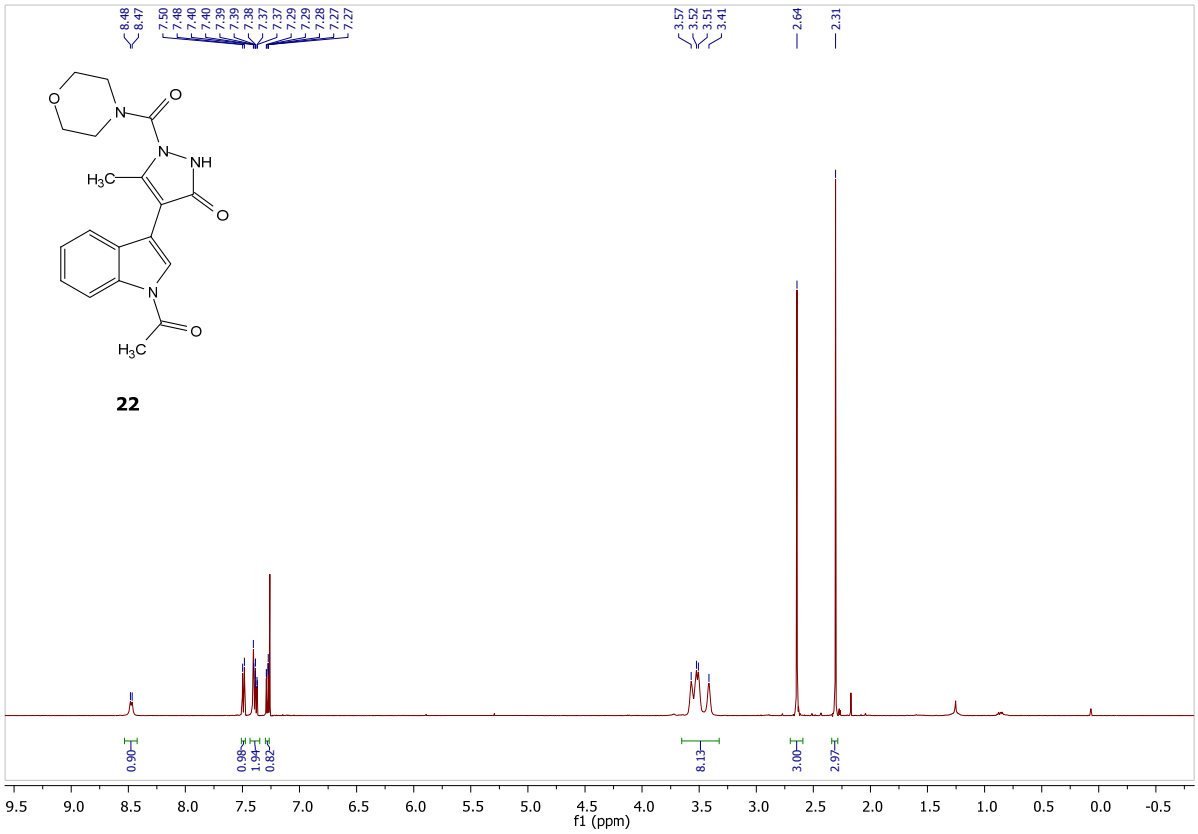
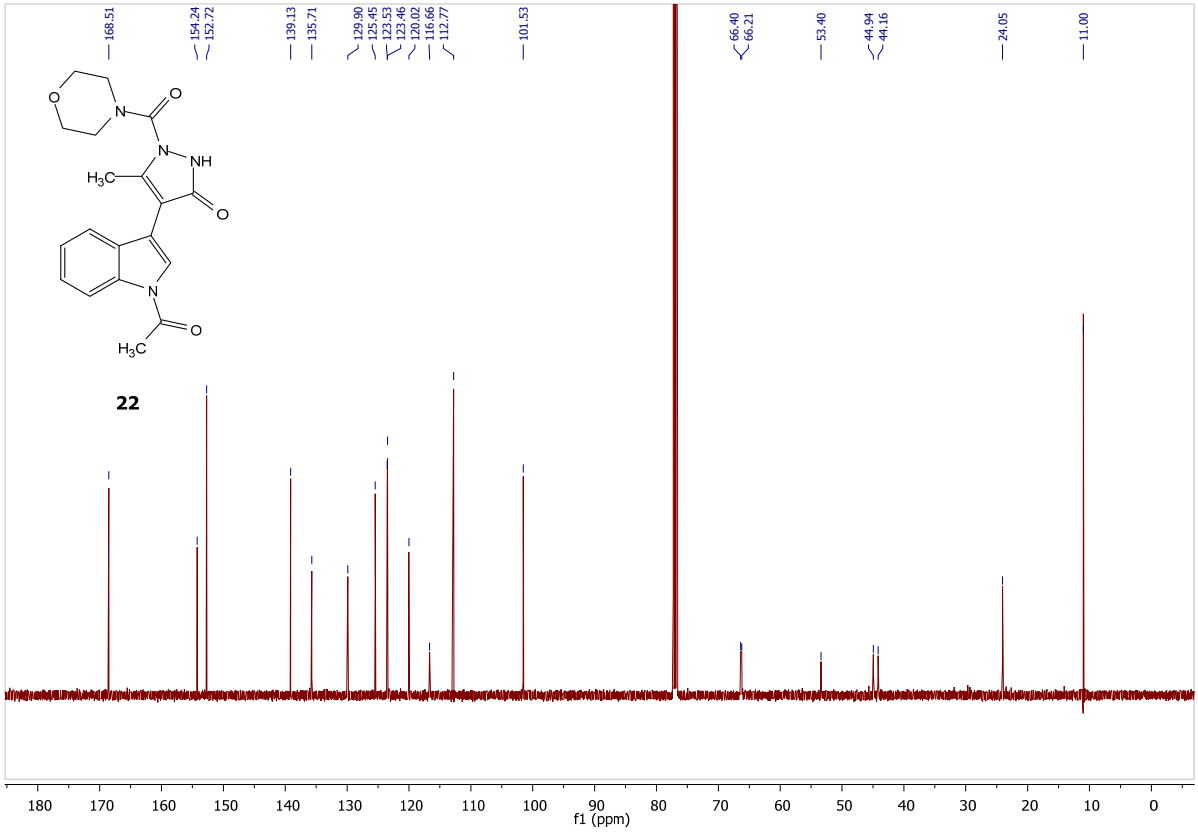
7.60
7.55
7.53
7.52
7.51
7.51
7.40
7.39
7.38
7.36
7.35
7.30
7.29
7.28
7.24
7.22
7.21
7.18
7.18
7.17
7.16
3.78
2.59
2.38
0.98
2.09
1.21
1.12
0.83
1.33
1.11
1.11
1.10
3.27
3.36
3.26
f1 (ppm)

21


170.93
165.71
161.28
155.17
141.00
137.03
131.16
130.90
130.76
126.21
125.45
124.69
123.44
121.40
121.11
117.48
115.55
113.98
55.95
55.89
54.80
25.09
23.88
10.78
9.26
f1 (ppm)

21


8.48
8.47
7.50
7.48
7.40
7.40
7.39
7.39
7.38
7.37
7.37
7.29
7.29
7.28
7.27
7.27
3.57
3.52
3.51
3.41
2.64
2.31
22
0.90
0.98
1.94
0.82
8.13
3.00
2.97
f1 (ppm)


168.51
154.24
152.72
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135.71
129.90
125.45
123.53
123.46
120.02
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112.77
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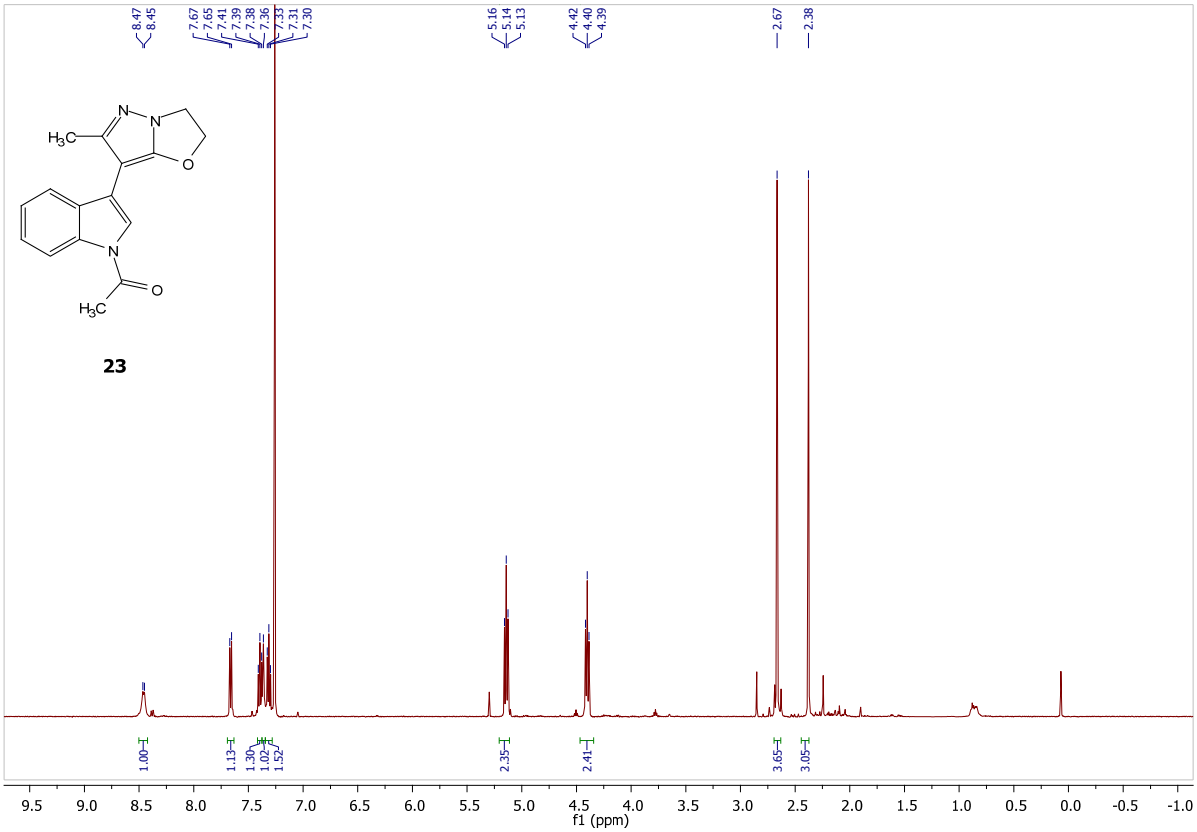
44.94
44.16
24.05
11.00
22
f1 (ppm)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7.65
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7.39
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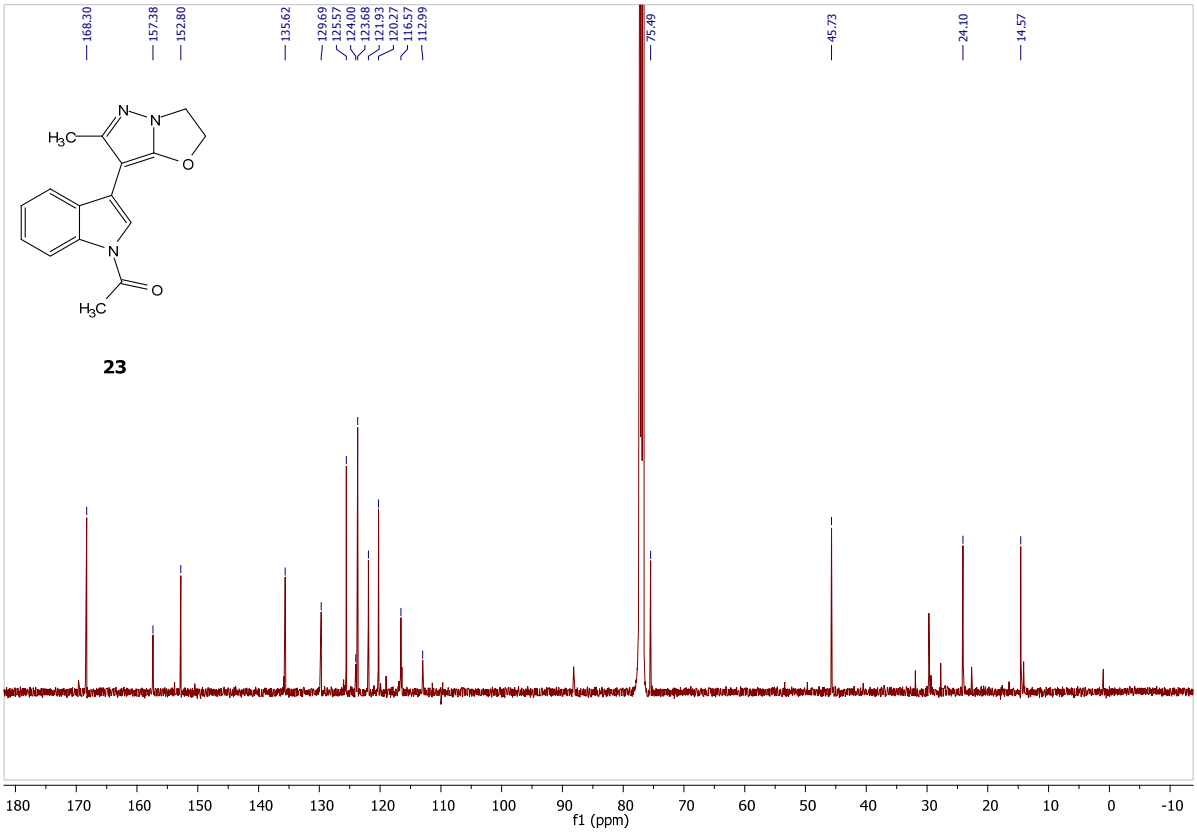
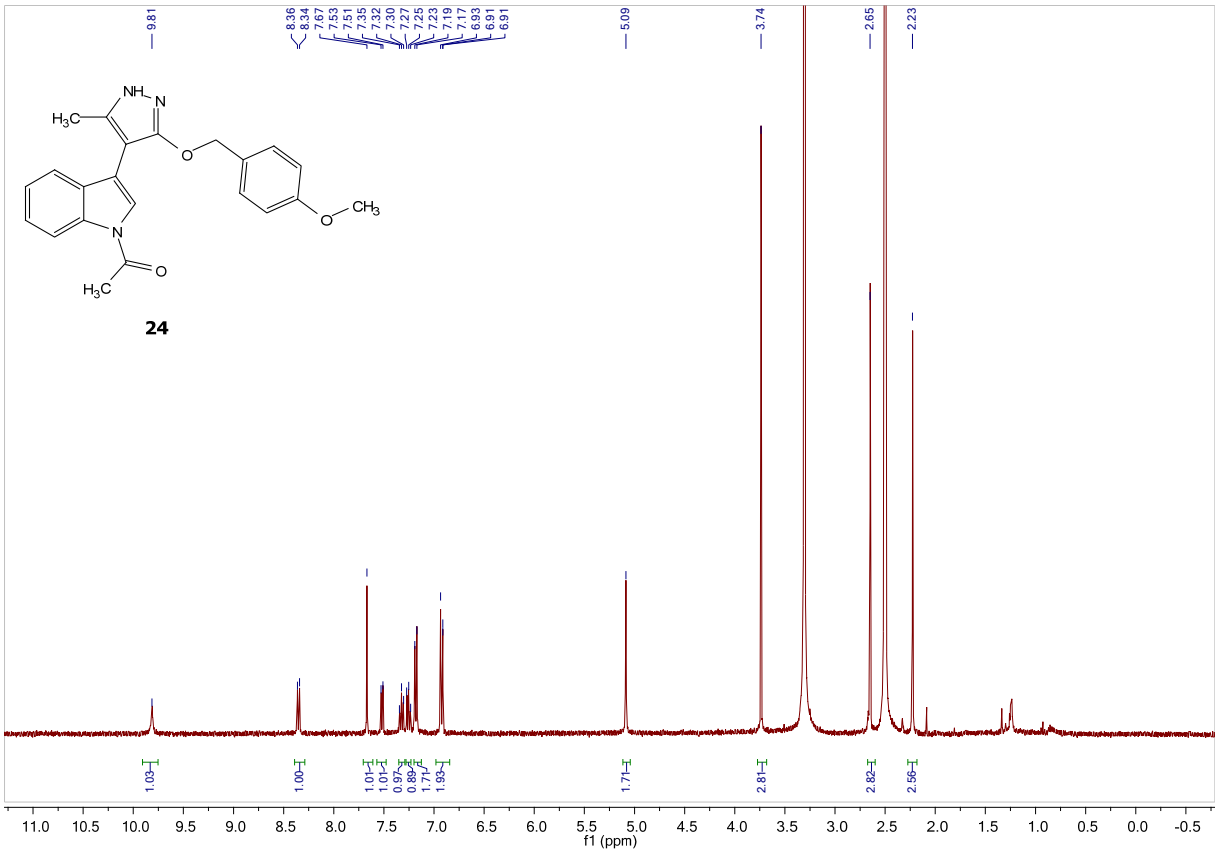
7.36
7.35
7.31
7.30
5.16
5.14
5.13
4.42
4.40
4.39
2.67
2.38
1.00
1.13
1.30
1.02
1.52
2.35
2.41
3.65
3.05
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)
23


168.30
157.38
152.80
135.62
129.69
125.57
124.00
123.68
121.93
120.27
116.57
112.99
75.49
45.73
24.10
14.57
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
23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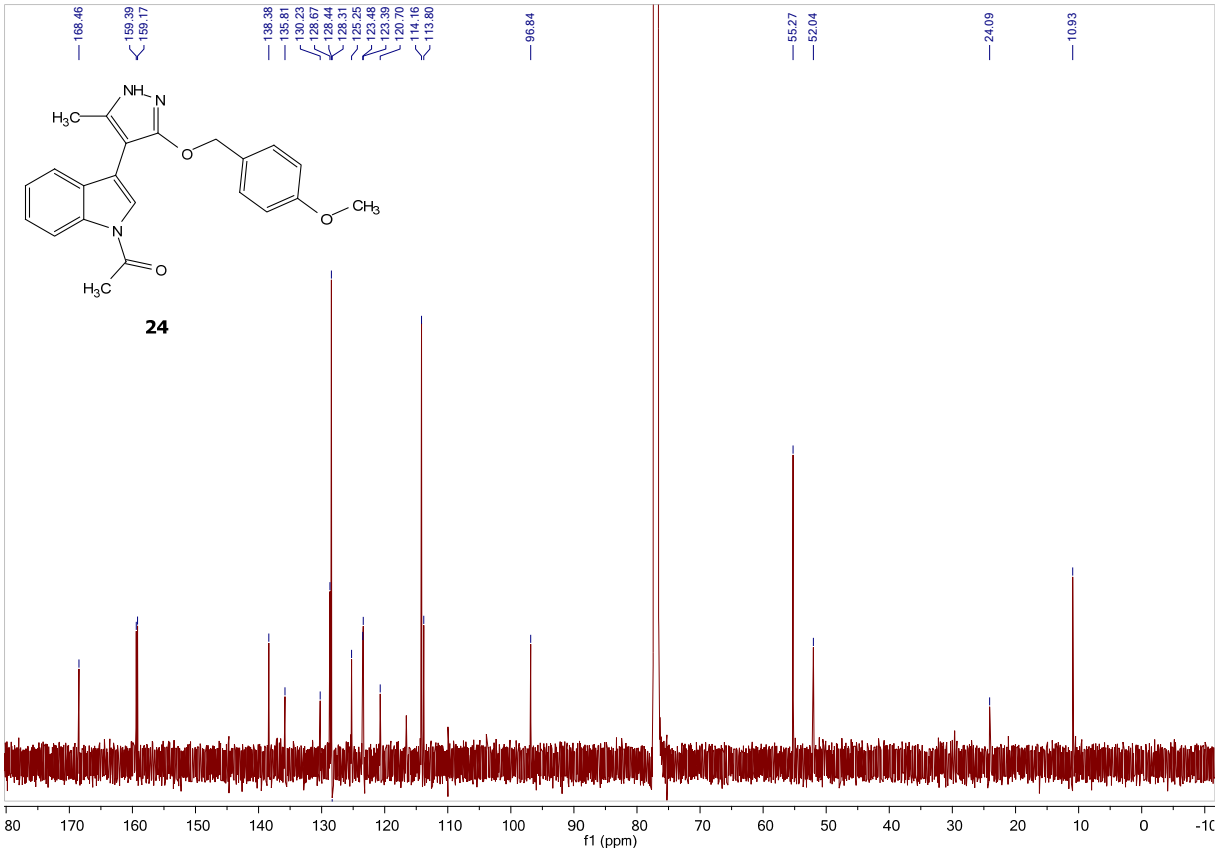
8.36
8.34
7.67
7.53
7.51
7.35
7.32
7.30
7.27
7.25
7.23
7.19
7.17
6.93
6.91
6.91
5.09
3.74
2.65
2.23
1.03
1.00
1.01
1.01
0.97
0.89
1.71
1.93
1.71
2.81
2.82
2.56
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
NH
N
H3C
O
O
CH3
N
O
H3C
24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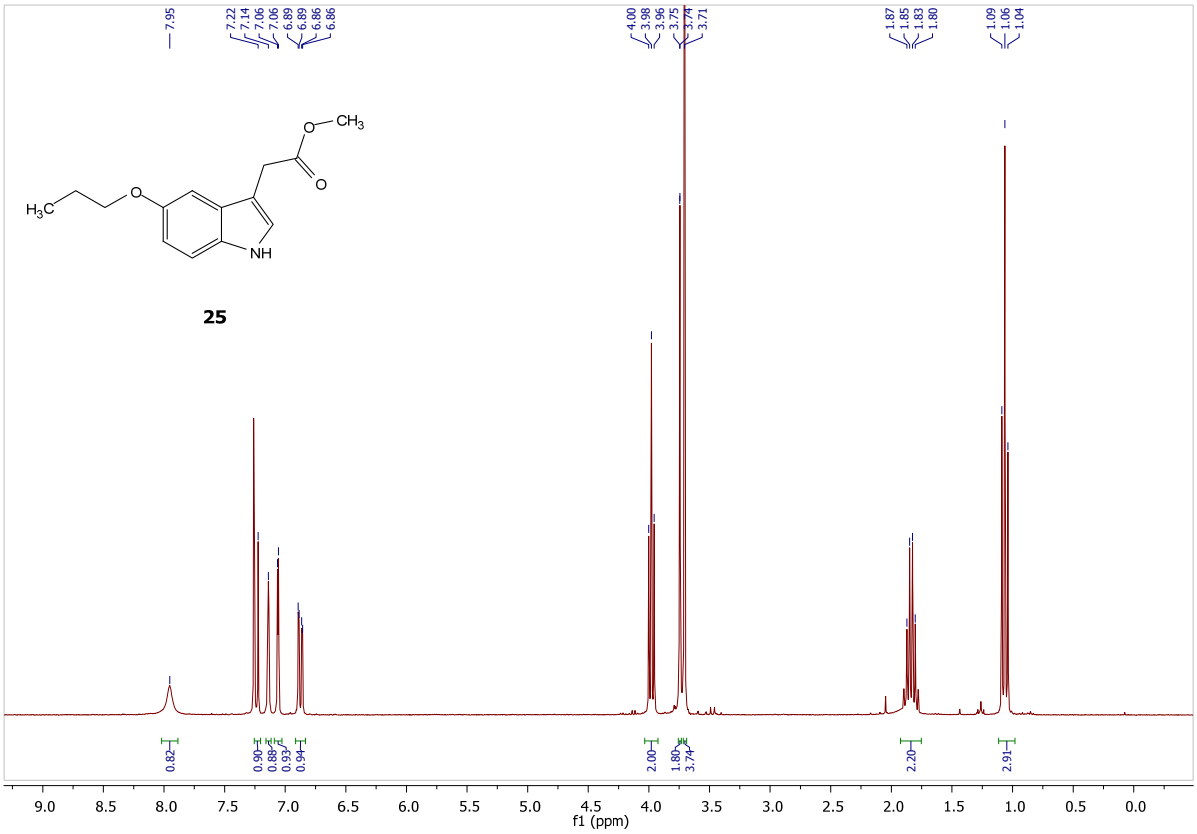
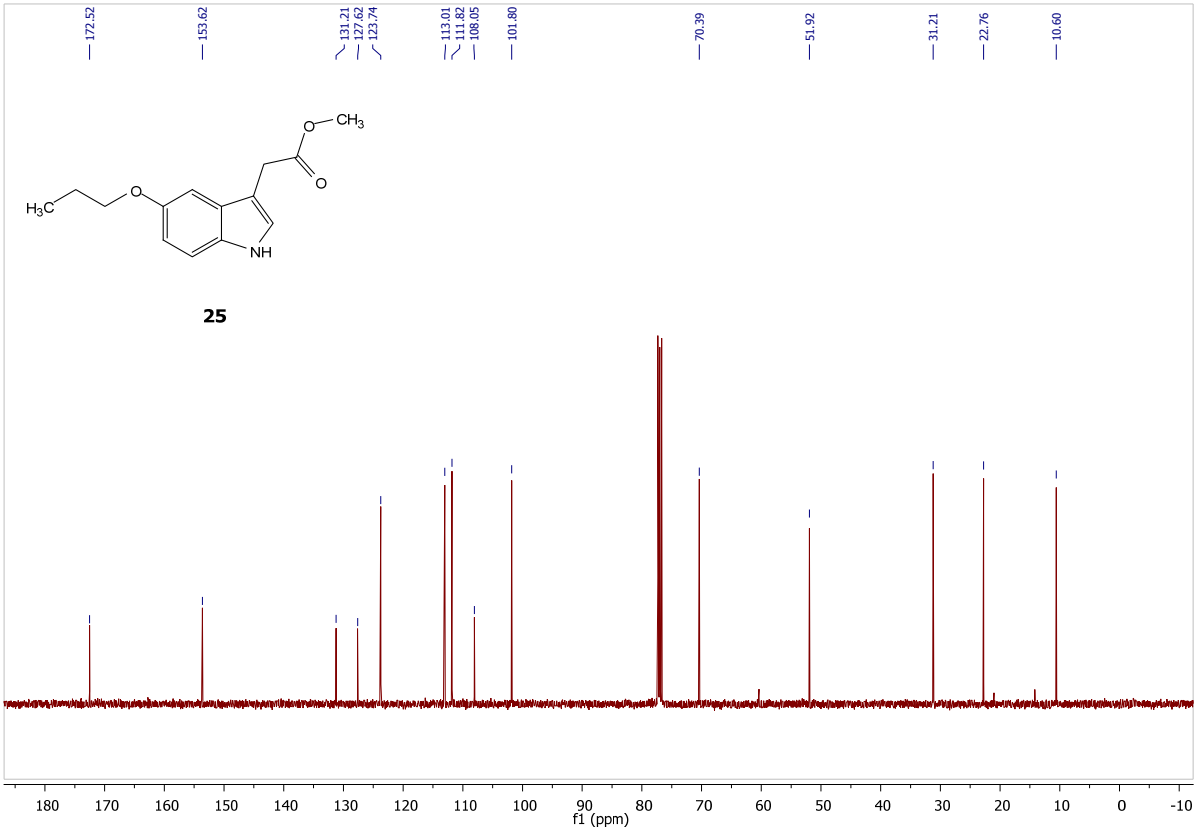
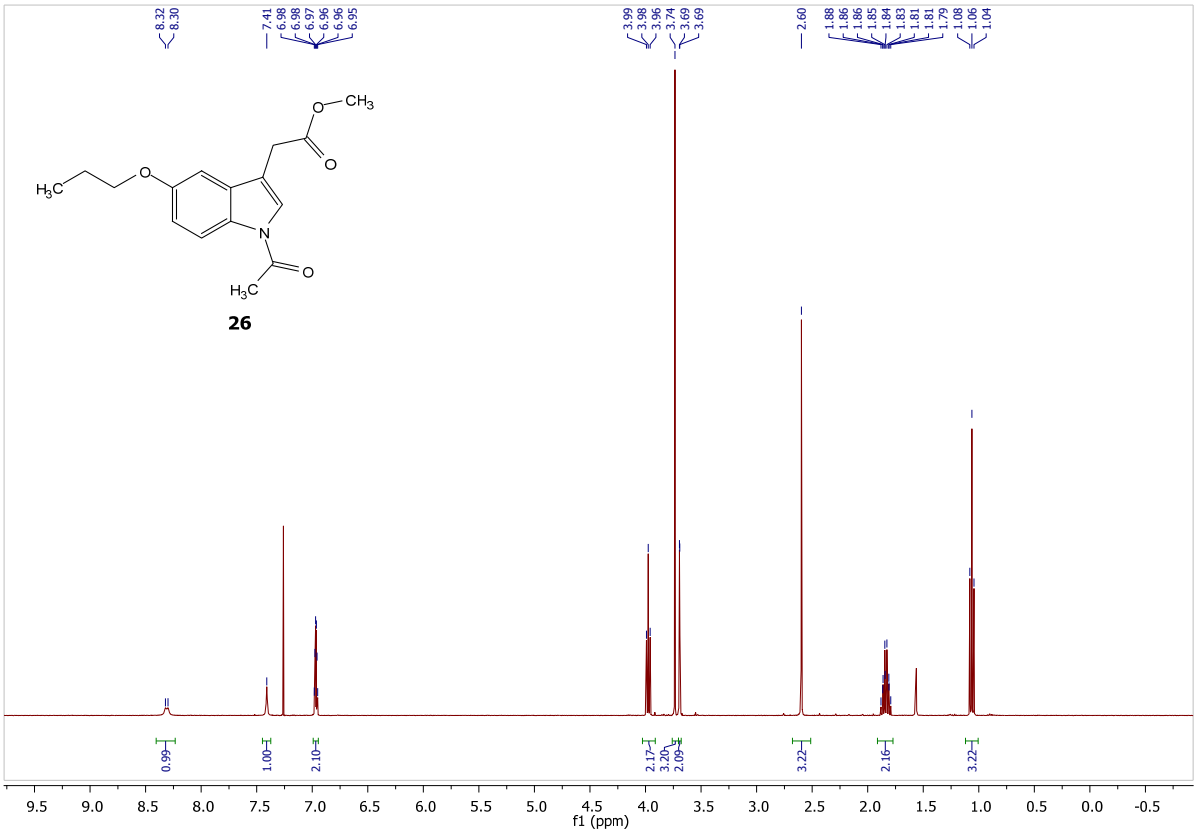
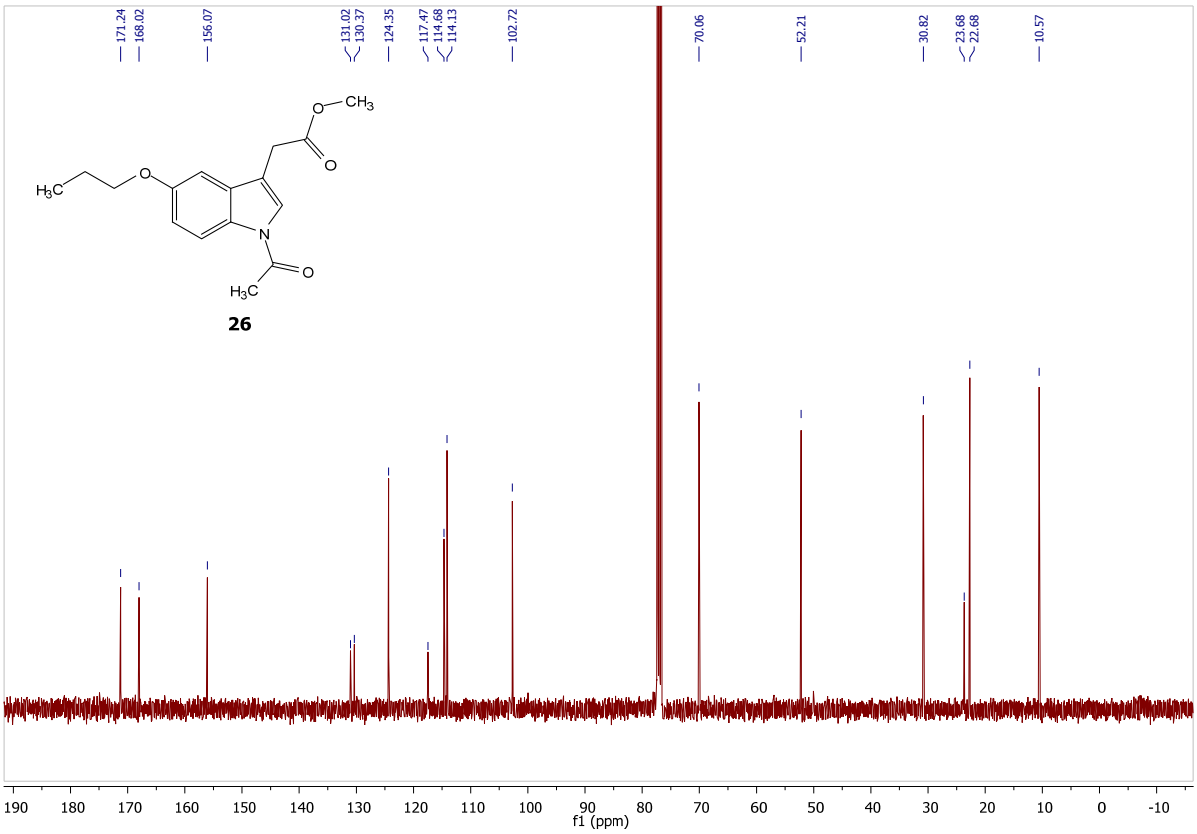
159.39
159.17
138.38
135.81
130.23
128.67
128.44
128.31
125.25
123.48
123.39
120.70
114.16
113.80
96.84
55.27
52.04
24.09
10.93
80 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
NH
N
H3C
O
O
CH3
N
O
H3C
24


7.95
7.22
7.14
7.06
7.06
6.89
6.89
6.86
6.86
4.00
3.98
3.96
3.75
3.74
3.71
1.87
1.85
1.83
1.80
1.09
1.06
1.04
O
CH3
O
H3C
O
NH
25
0.82
0.90
0.88
0.93
0.94
2.00
1.80
3.74
2.20
2.91
f1 (ppm)

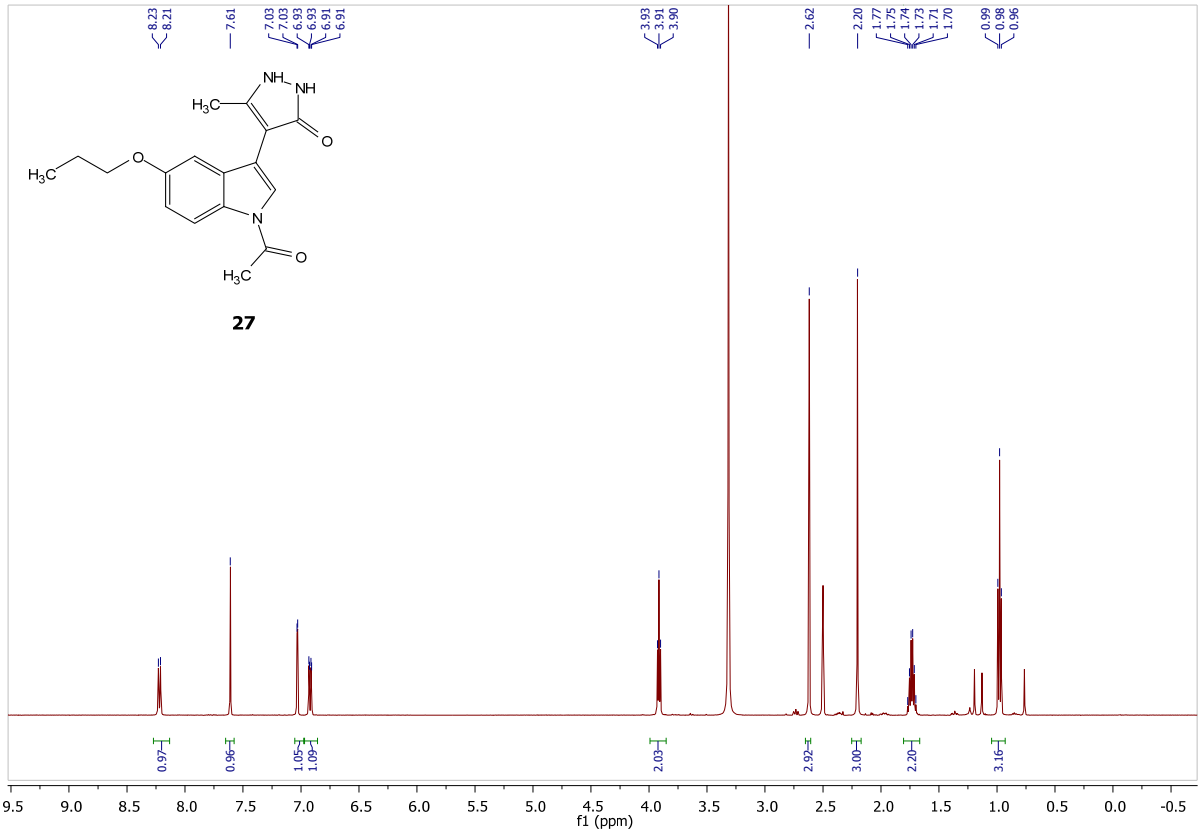

172.52
153.62
131.21
127.62
123.74
113.01
111.82
108.05
101.80
70.39
51.92
31.21
22.76
10.60
O
CH3
O
H3C
O
NH
25
f1 (ppm)


8.32
8.30
7.41
6.98
6.98
6.97
6.96
6.96
6.95
3.99
3.98
3.96
3.74
3.69
3.69
2.60
1.88
1.86
1.86
1.85
1.84
1.83
1.81
1.81
1.79
1.08
1.06
1.04
O
CH3
O
H3C
O
N
O
H3C
0.99
1.00
2.10
2.17
3.20
2.09
3.22
2.16
3.22
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

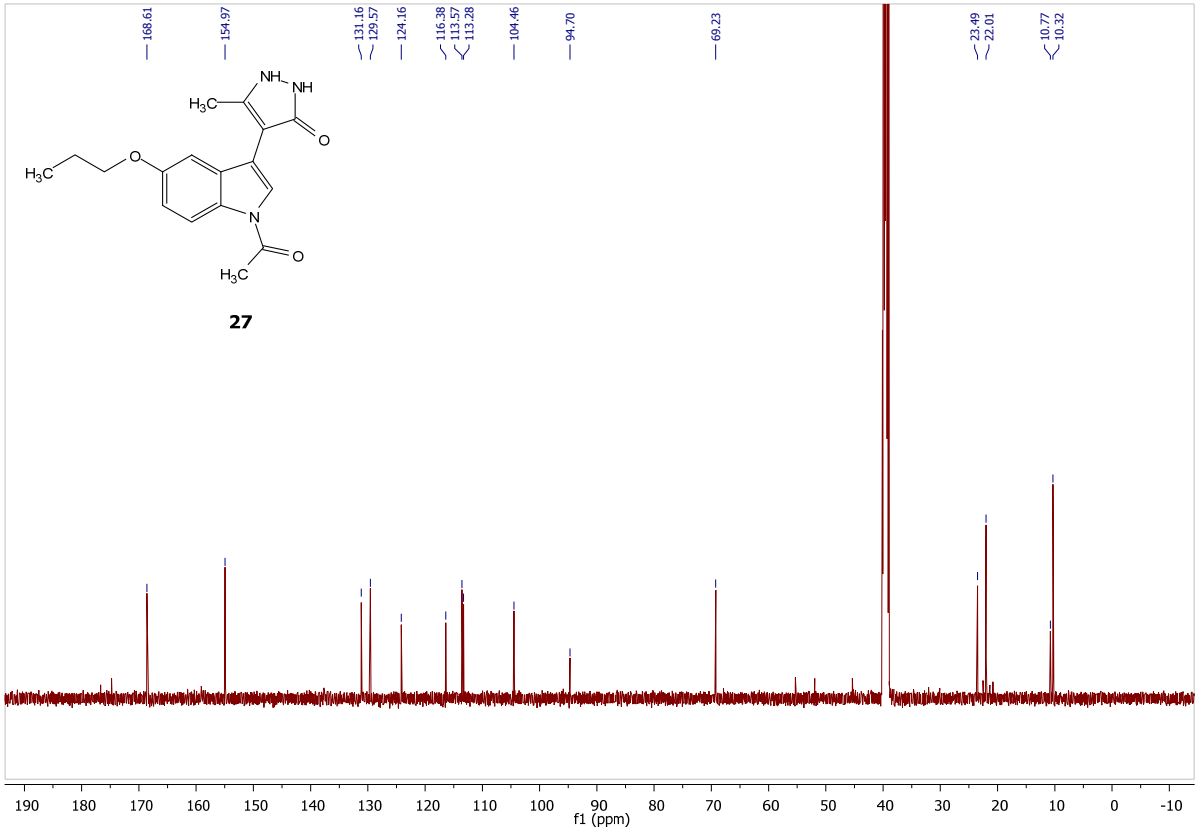
26


171.24
168.02
156.07
131.02
130.37
124.35
117.47
114.68
114.13
102.72
70.06
52.21
30.82
23.68
22.68
10.57
O
CH3
O
H3C
O
N
O
H3C
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

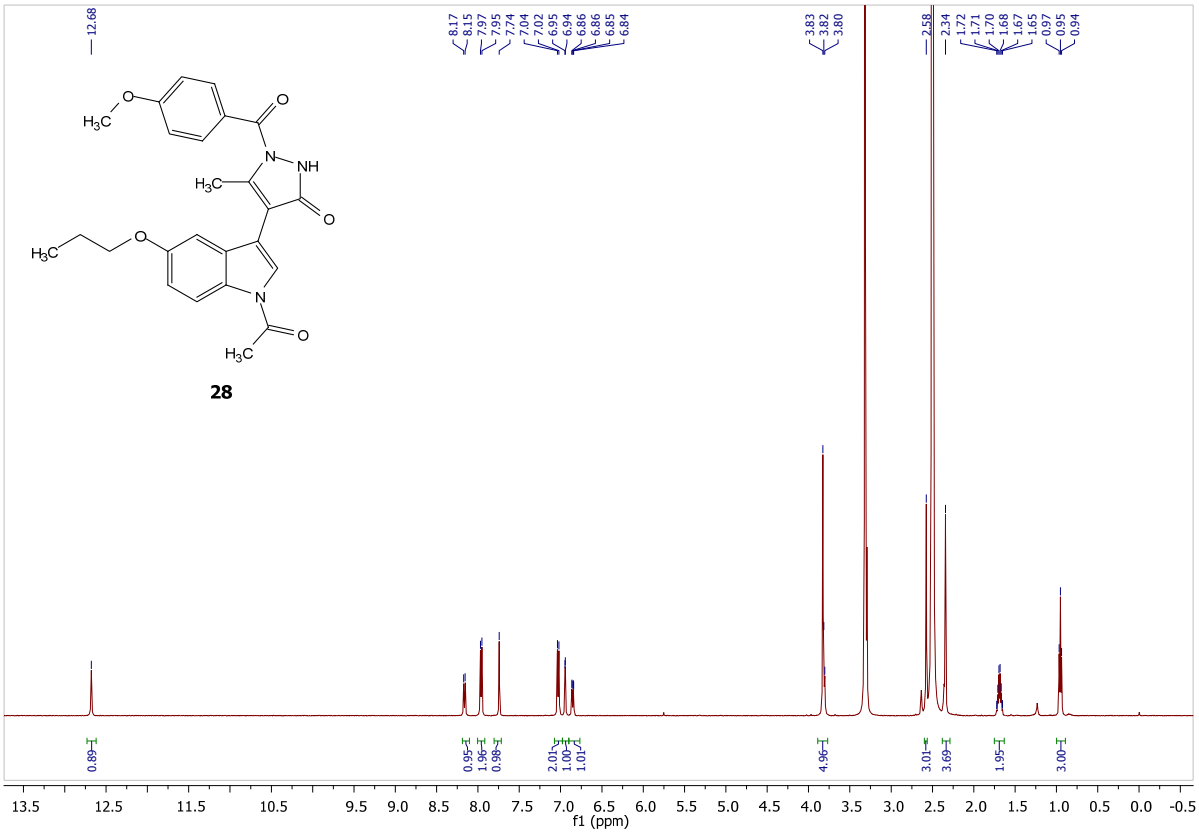
26


8.23
8.21
7.61
7.03
7.03
6.93
6.93
6.91
6.91
3.93
3.91
3.90
2.62
2.20
1.77
1.75
1.74
1.73
1.71
1.70
0.99
0.98
0.96
NH
NH
H3C
O
H3C
O
N
O
H3C
0.97
0.96
1.05
1.09
2.03
2.92
3.00
2.20
3.16
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

27


168.61
154.97
131.16
129.57
124.16
116.38
113.57
113.28
104.46
94.70
69.23
23.49
22.01
10.77
10.32
NH
NH
H3C
O
H3C
O
N
O
H3C
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

27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8.17
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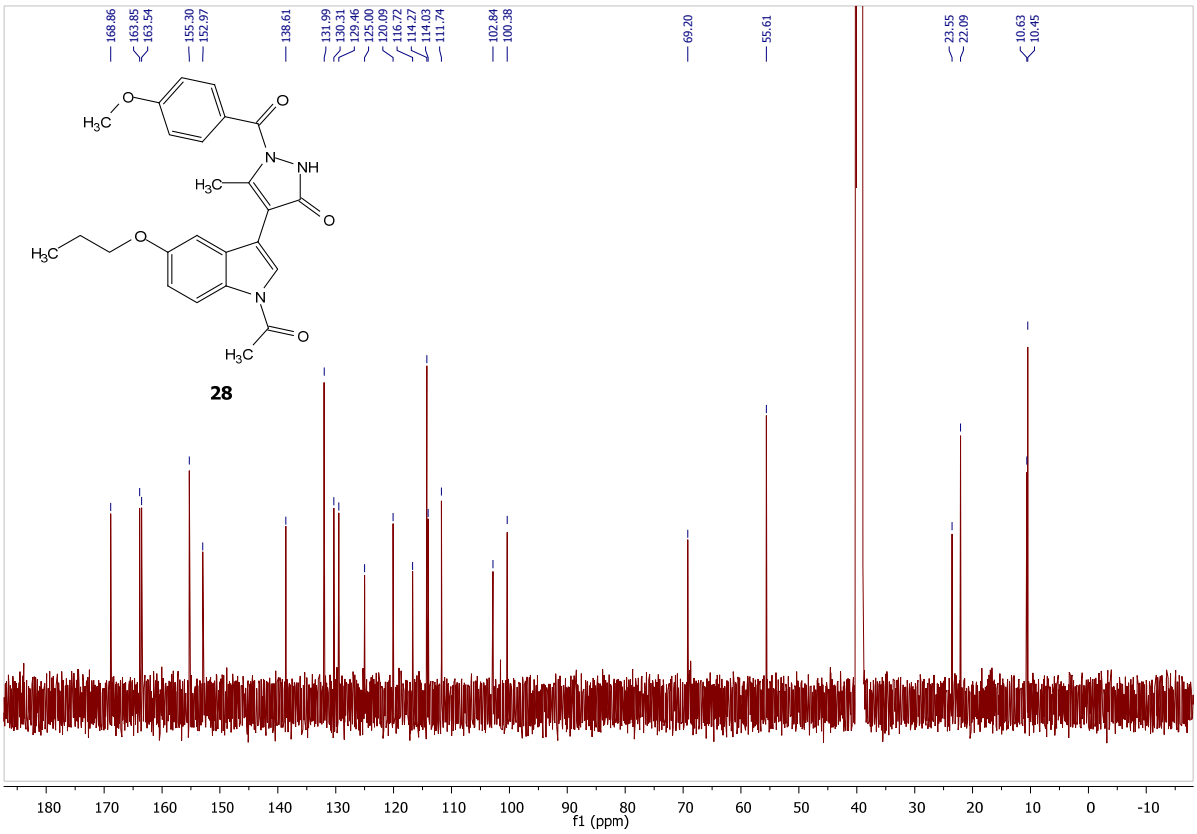
7.95
7.74
7.04
7.02
6.95
6.94
6.86
6.86
6.85
6.84
3.83
3.82
3.80
2.58
2.34
1.72
1.71
1.70
1.68
1.67
1.65
0.97
0.95
0.94
0.89
0.95
1.96
0.98
2.01
1.00
1.01
4.96
3.01
3.69
1.95
3.00
28
f1 (ppm)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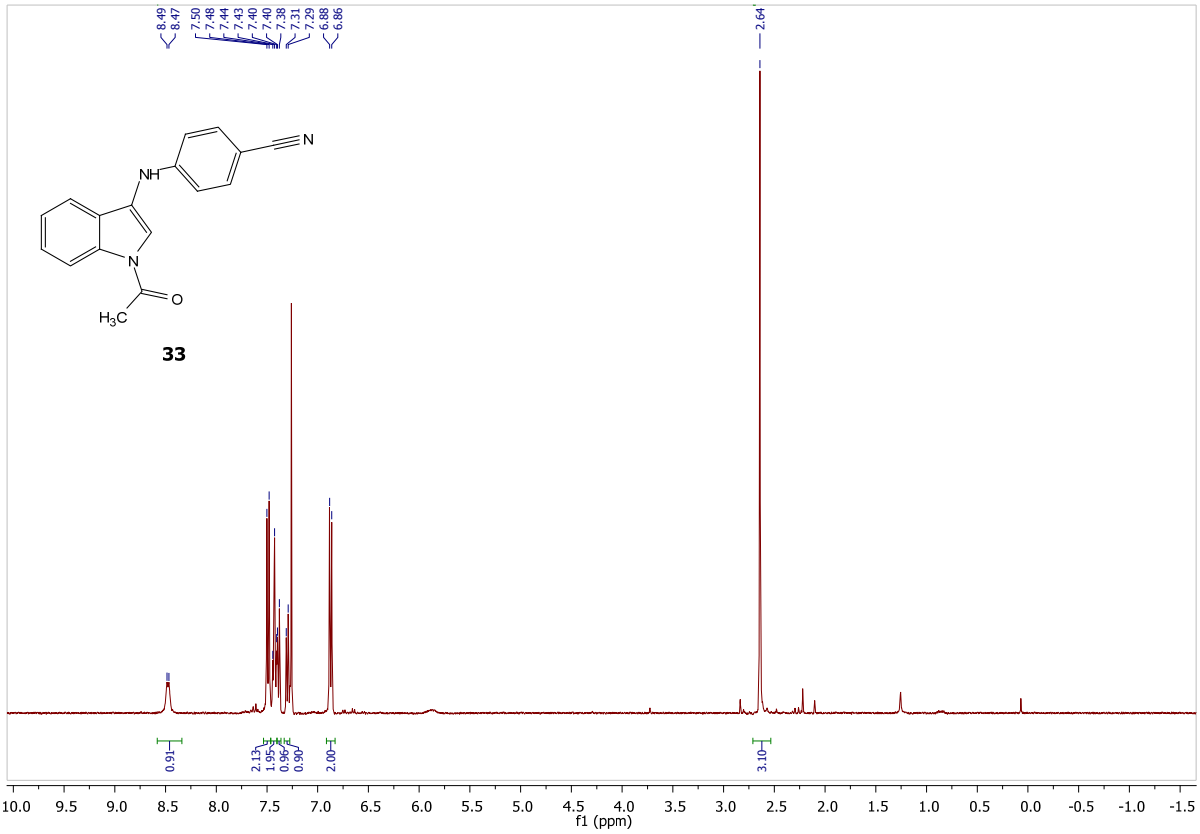
152.97
138.61
131.99
130.31
129.46
125.00
120.09
116.72
114.27
114.03
111.74
102.84
100.38
69.20
55.61
23.55
22.09
10.63
10.45
28
f1 (ppm)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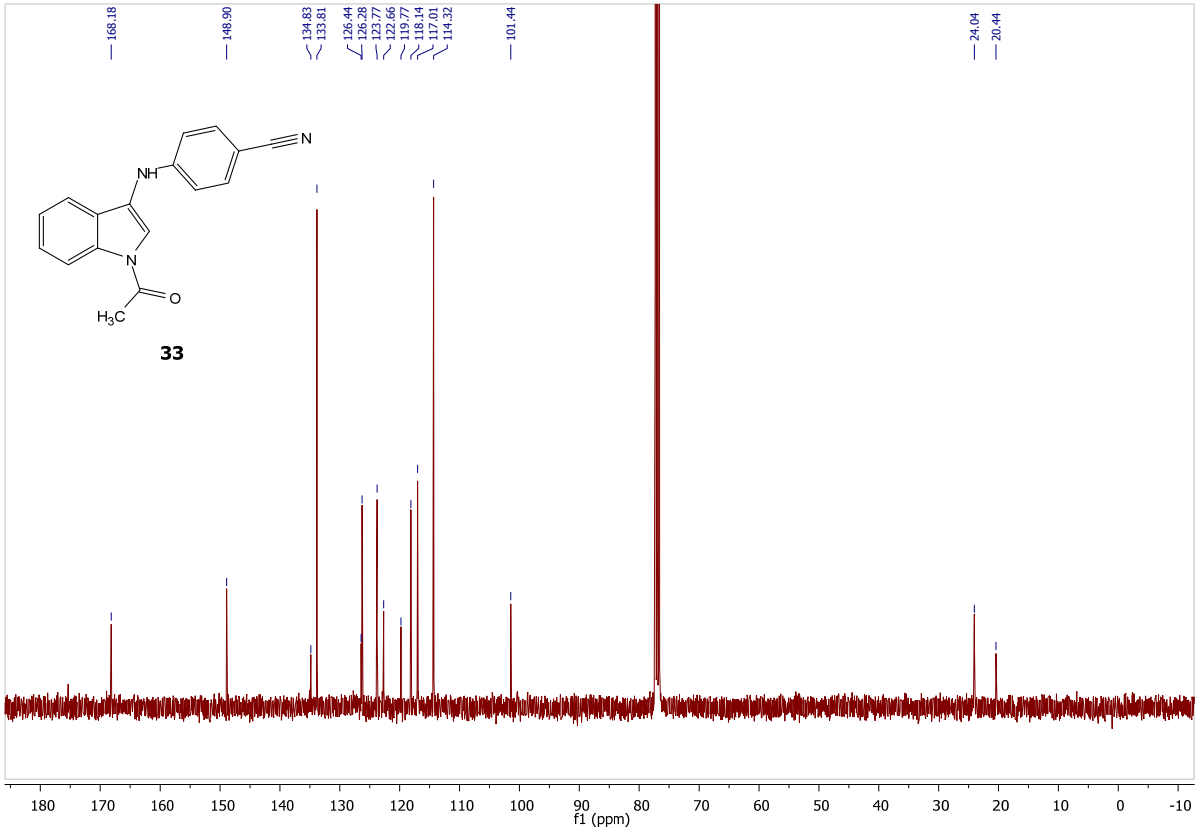
7.48
7.44
7.43
7.40
7.40
7.38
7.31
7.29
6.88
6.86
2.64
NH
N
O
H3C
33
0.91
2.13
1.95
0.96
0.90
2.00
3.10
f1 (ppm)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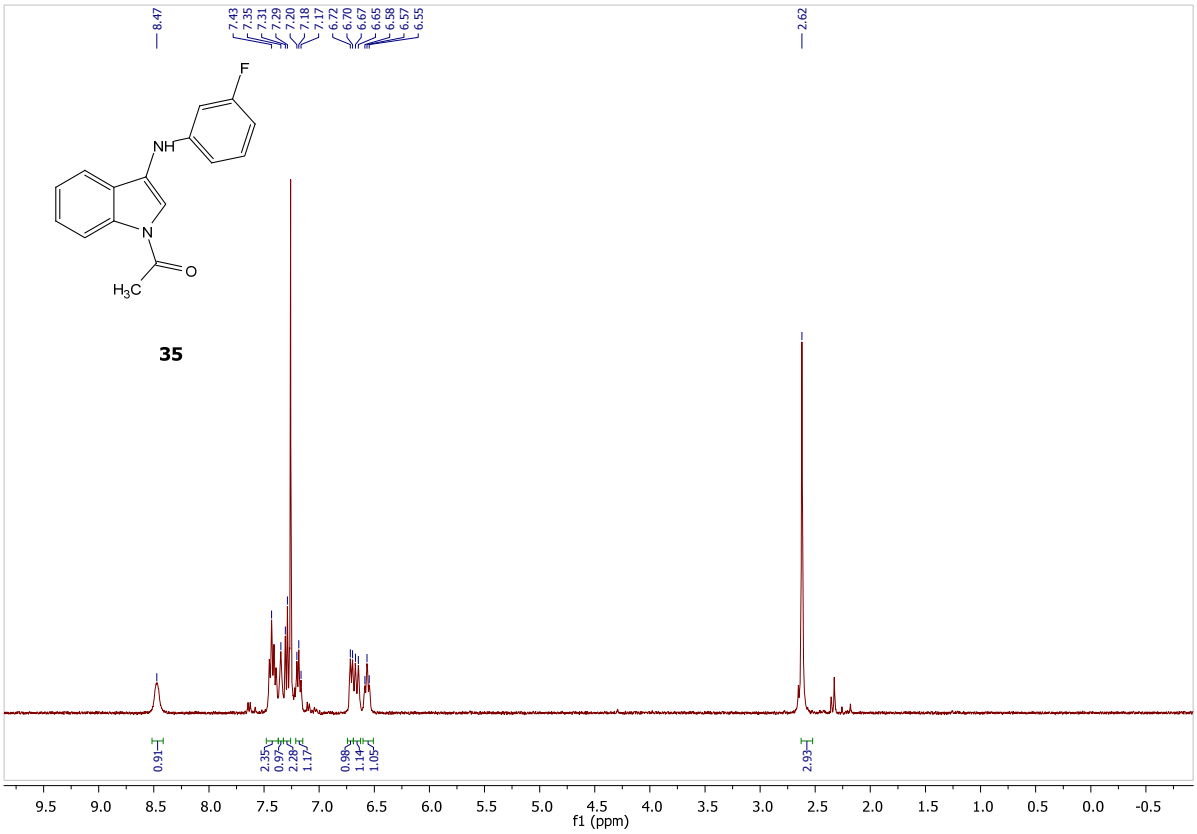
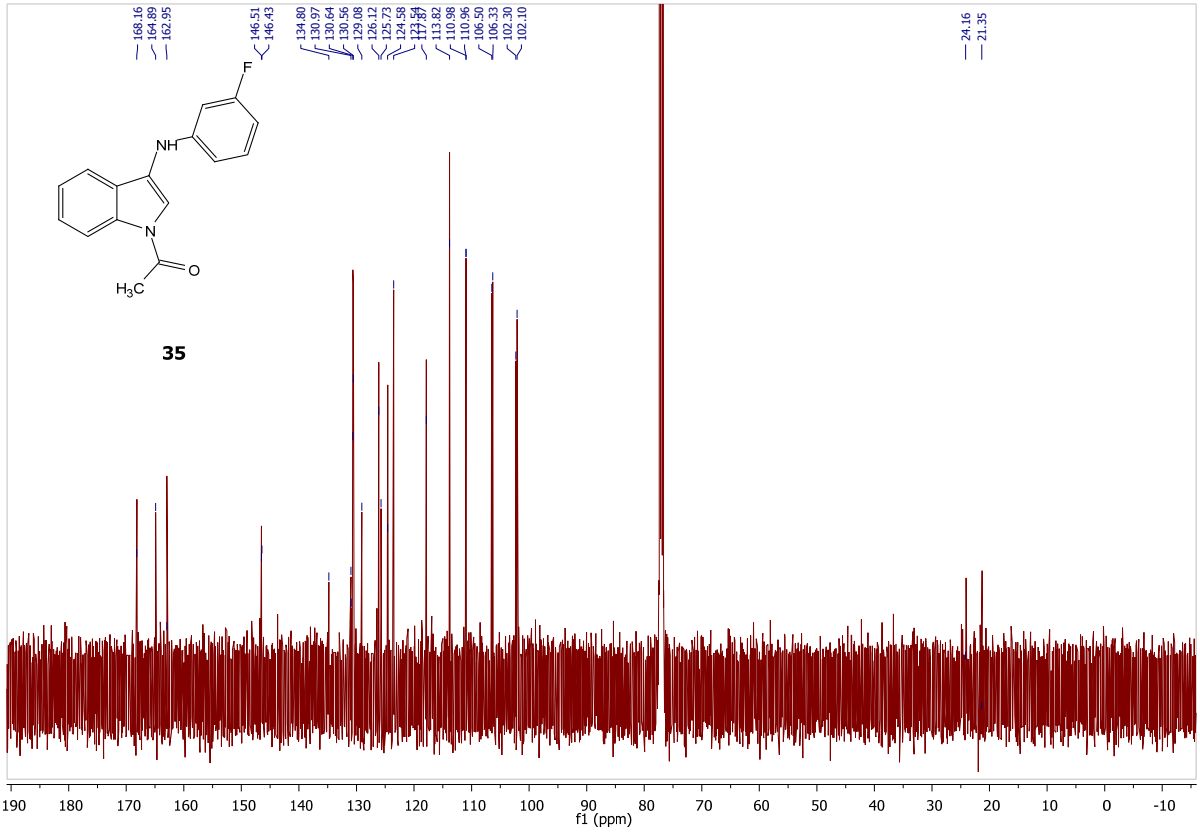
148.90
134.83
133.81
126.44
126.28
123.77
122.66
119.77
118.14
117.01
114.32
101.44
24.04
20.44
NH
N
O
H3C
33
f1 (ppm)


8.47
7.43
7.35
7.31
7.29
7.20
7.18
7.17
6.72
6.70
6.67
6.65
6.58
6.57
6.55
2.62
F
NH
N
O
H3C
35
0.91
2.35
0.97
2.28
1.17
0.98
1.14
1.05
2.93
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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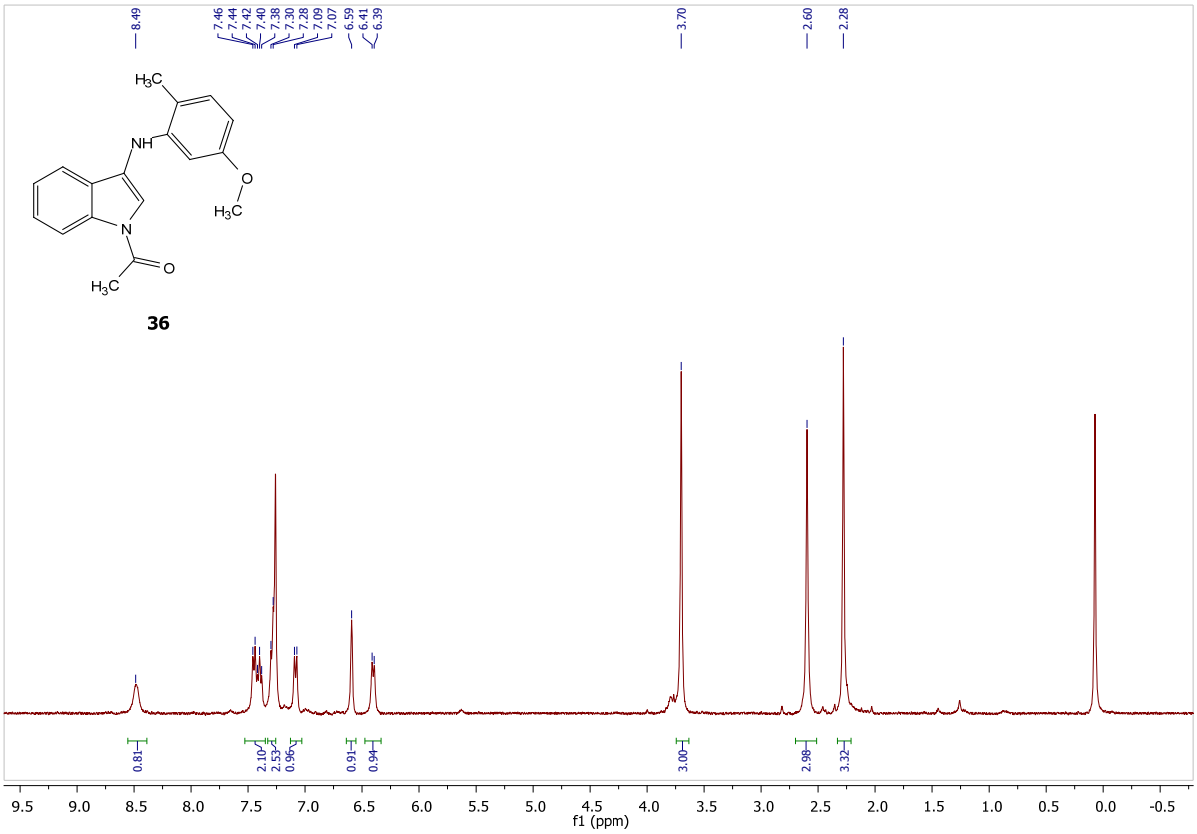
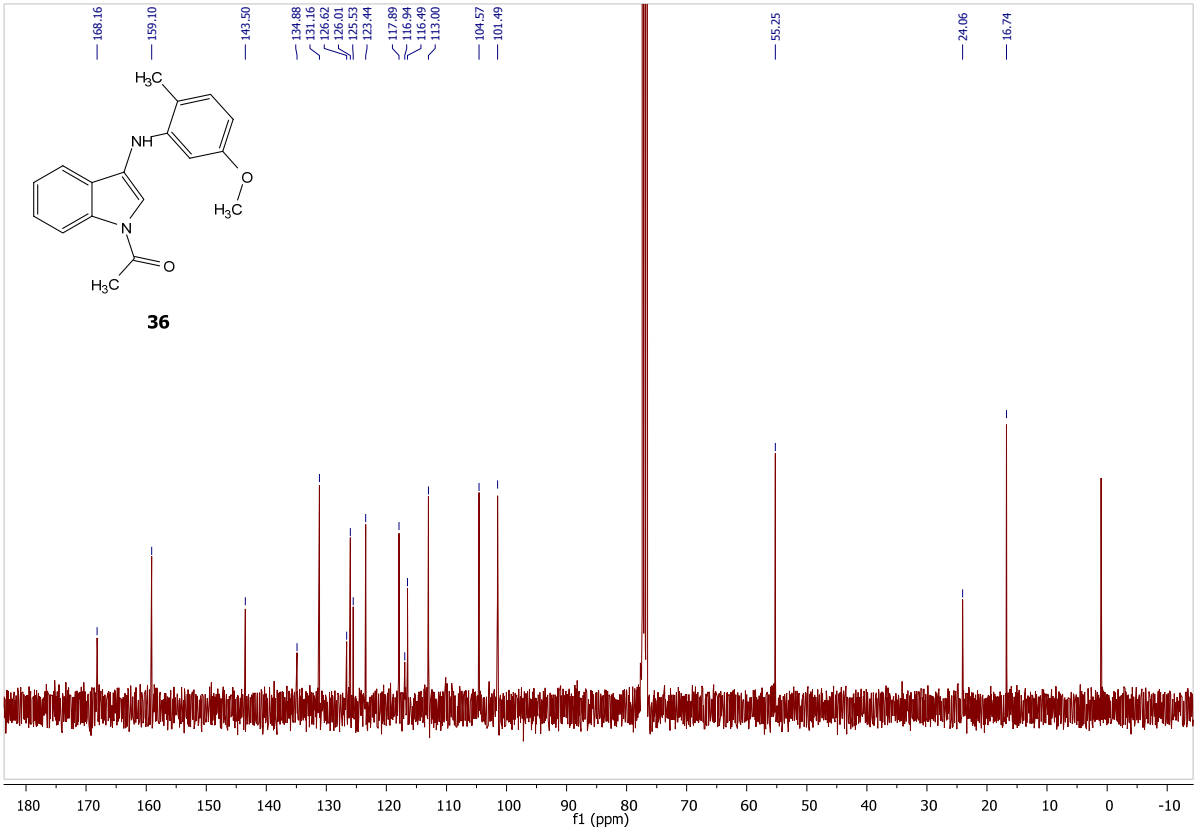
146.51
146.43
134.80
130.97
130.64
130.56
129.08
126.12
125.73
124.58
113.82
110.98
110.96
106.50
106.33
102.30
102.10
24.16
21.35
F
NH
N
O
H3C
35
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


8.49
7.46
7.44
7.42
7.40
7.38
7.30
7.28
7.09
7.07
6.59
6.41
6.39
3.70
2.60
2.28
H3C
NH
O
H3C
N
O
H3C
0.81
2.10
2.53
0.96
0.91
0.94
3.00
2.98
3.32
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

36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143.50
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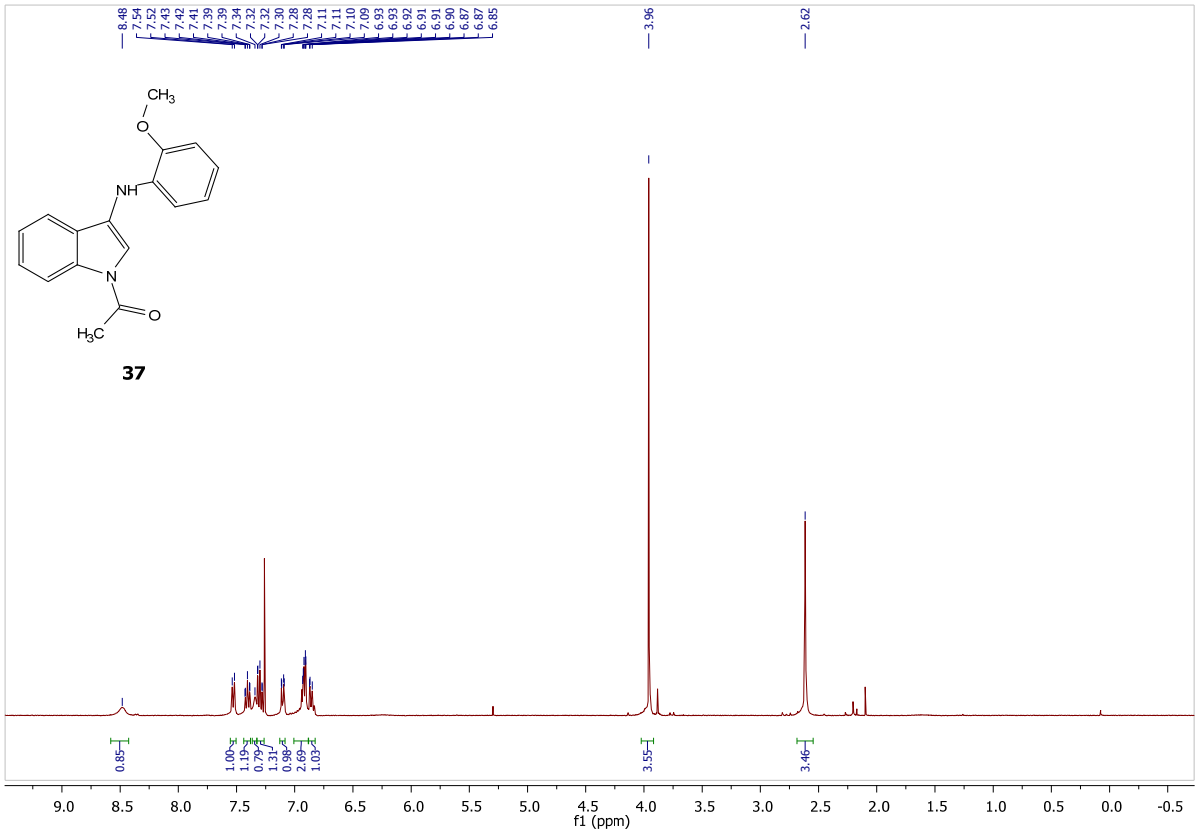
131.16
126.62
126.01
125.53
123.44
117.89
116.94
116.49
113.00
104.57
101.49
55.25
24.06
16.74
H3C
NH
O
H3C
N
O
H3C
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

36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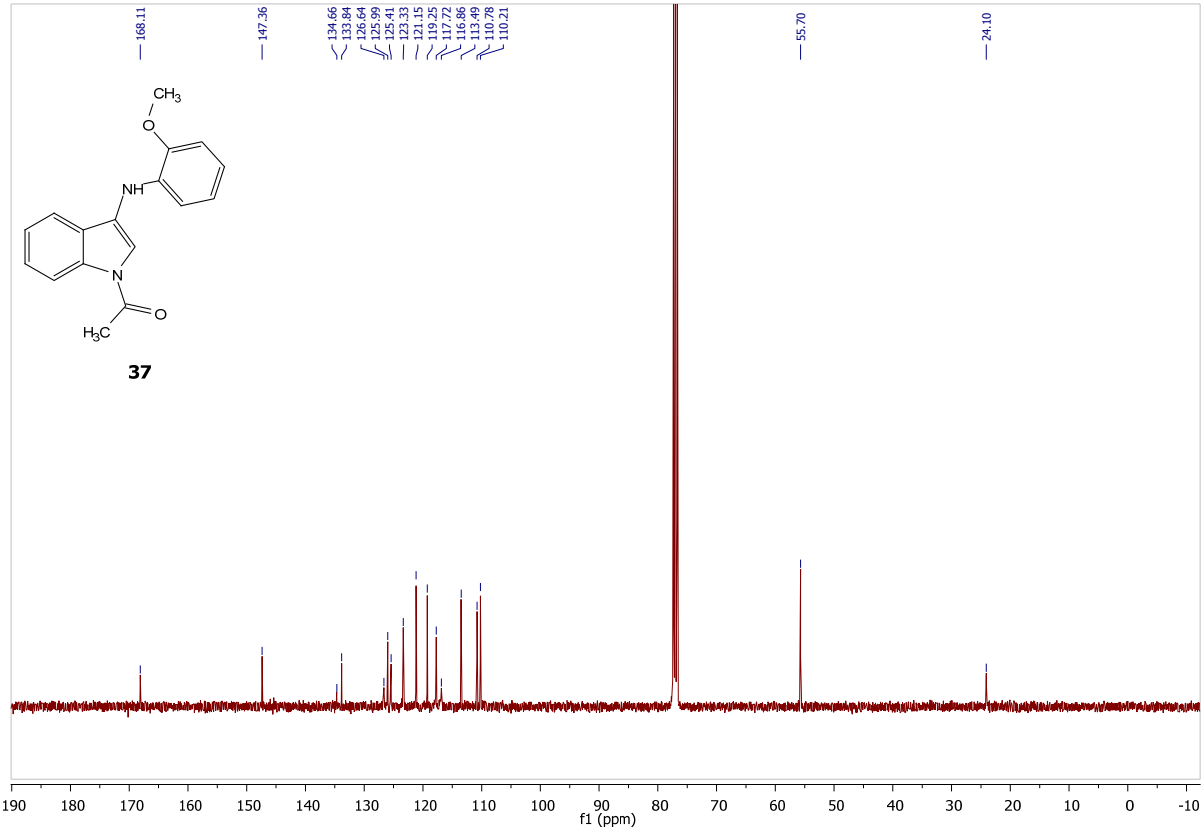
7.39
7.34
7.32
7.32
7.30
7.28
7.28
7.11
7.11
7.10
7.09
6.93
6.93
6.92
6.91
6.91
6.90
6.87
6.87
6.85
3.96
2.62
CH3
O
NH
N
O
H3C
37
0.85
1.00
1.19
0.79
1.31
0.98
2.69
1.03
3.55
3.46
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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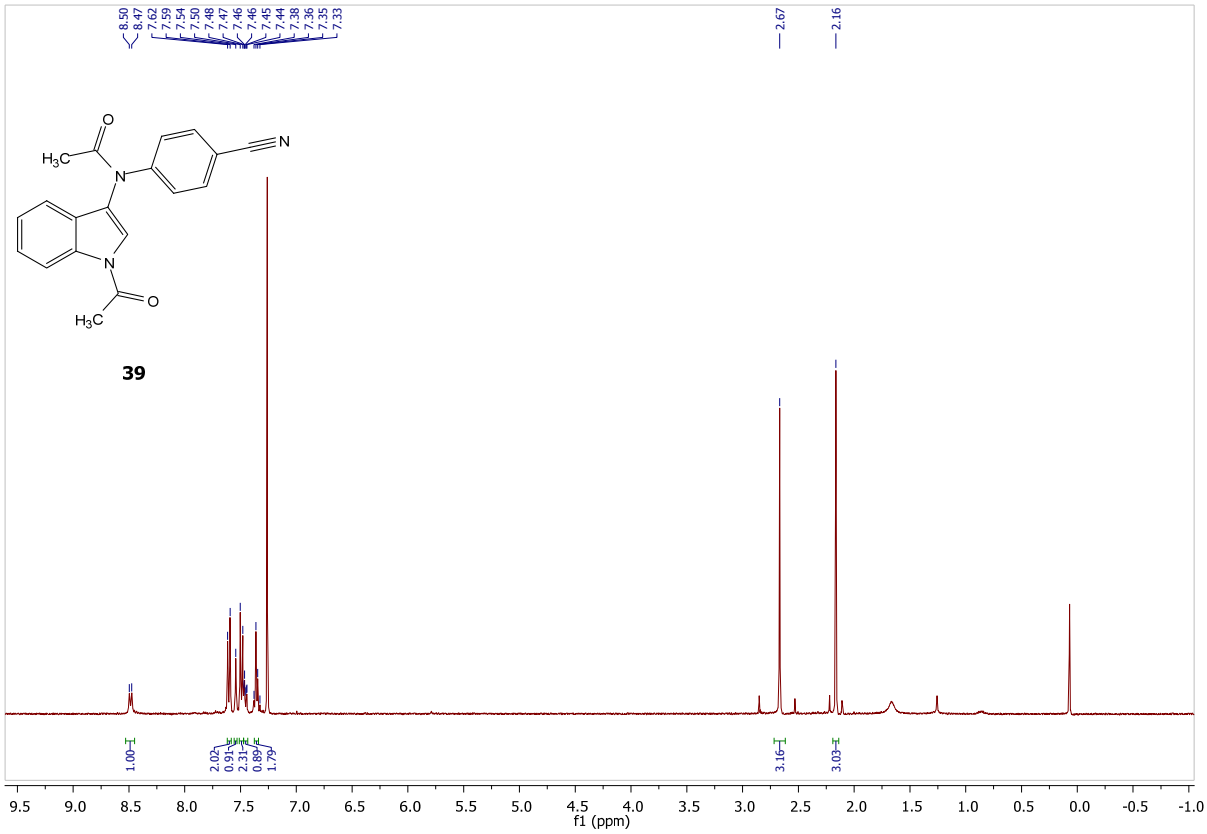
147.36
134.66
133.84
126.64
125.99
125.41
123.33
121.15
119.25
117.72
116.86
113.49
110.78
110.21
55.70
24.10
CH3
O
NH
N
O
H3C
37
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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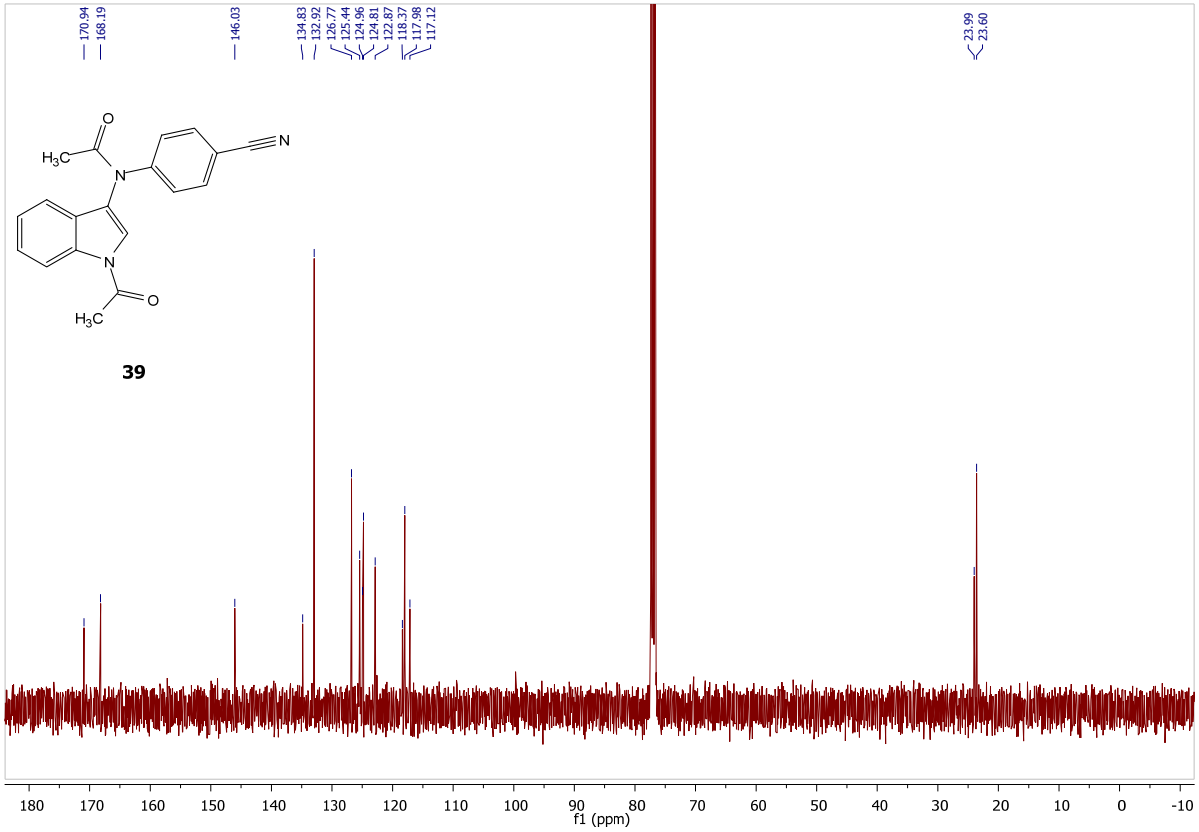
7.50
7.48
7.47
7.46
7.46
7.45
7.44
7.38
7.36
7.35
7.33
2.67
2.16
O
H3C
N
N
O
H3C
N
39
1.00
2.02
0.91
2.31
0.89
1.79
3.16
3.03
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)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146.03
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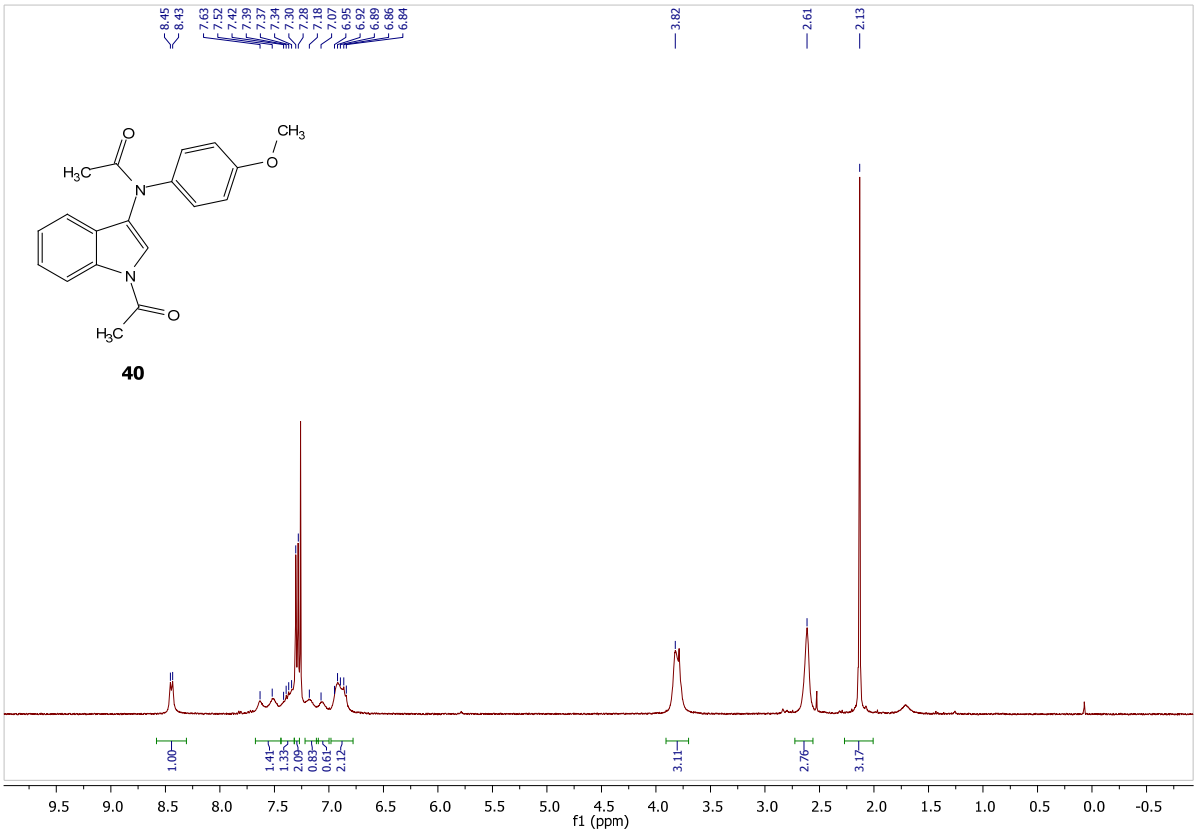
132.92
126.77
125.44
124.96
124.81
122.87
118.37
117.98
117.12
23.99
23.60
O
H3C
N
N
O
H3C
N
39
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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8.43
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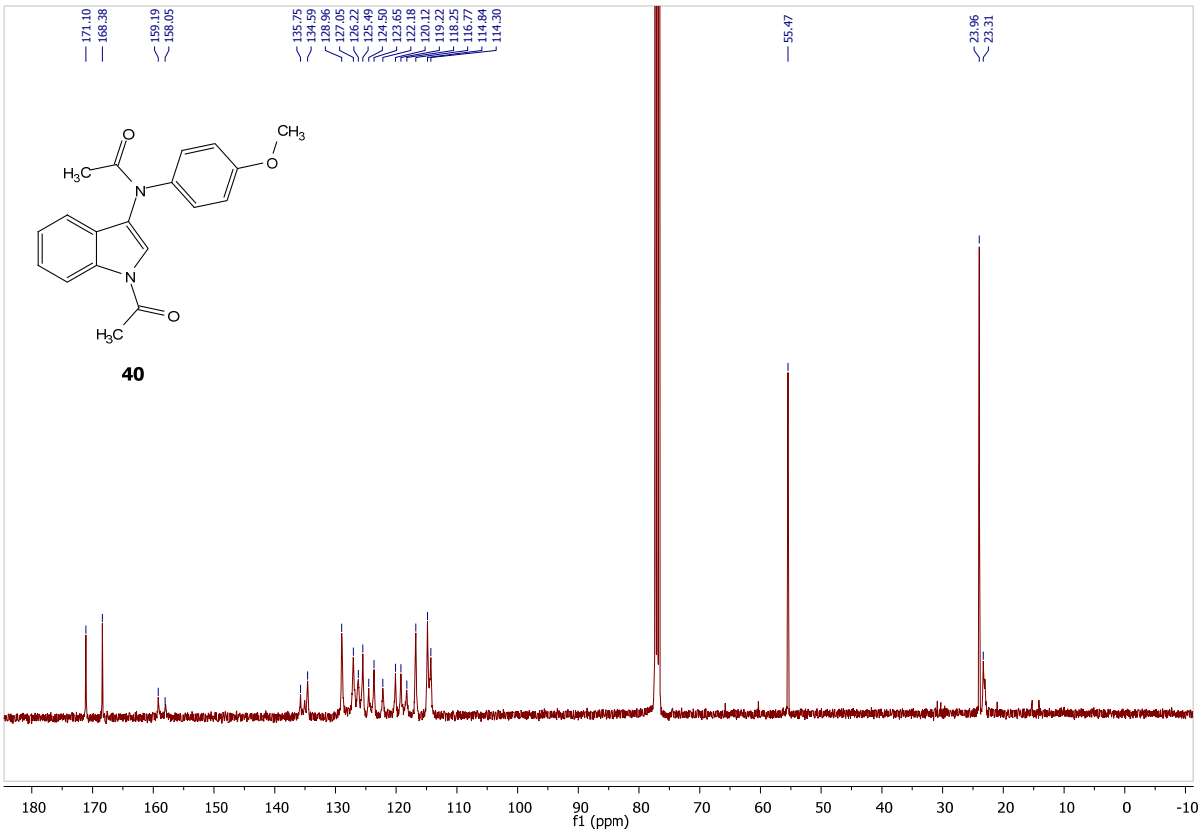
7.42
7.39
7.37
7.34
7.30
7.28
7.18
7.07
6.95
6.92
6.89
6.86
6.84
3.82
2.61
2.13
1.00
1.41
1.33
2.09
0.83
0.61
2.12
3.11
2.76
3.17
f1 (ppm)

40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168.38
159.19
158.05
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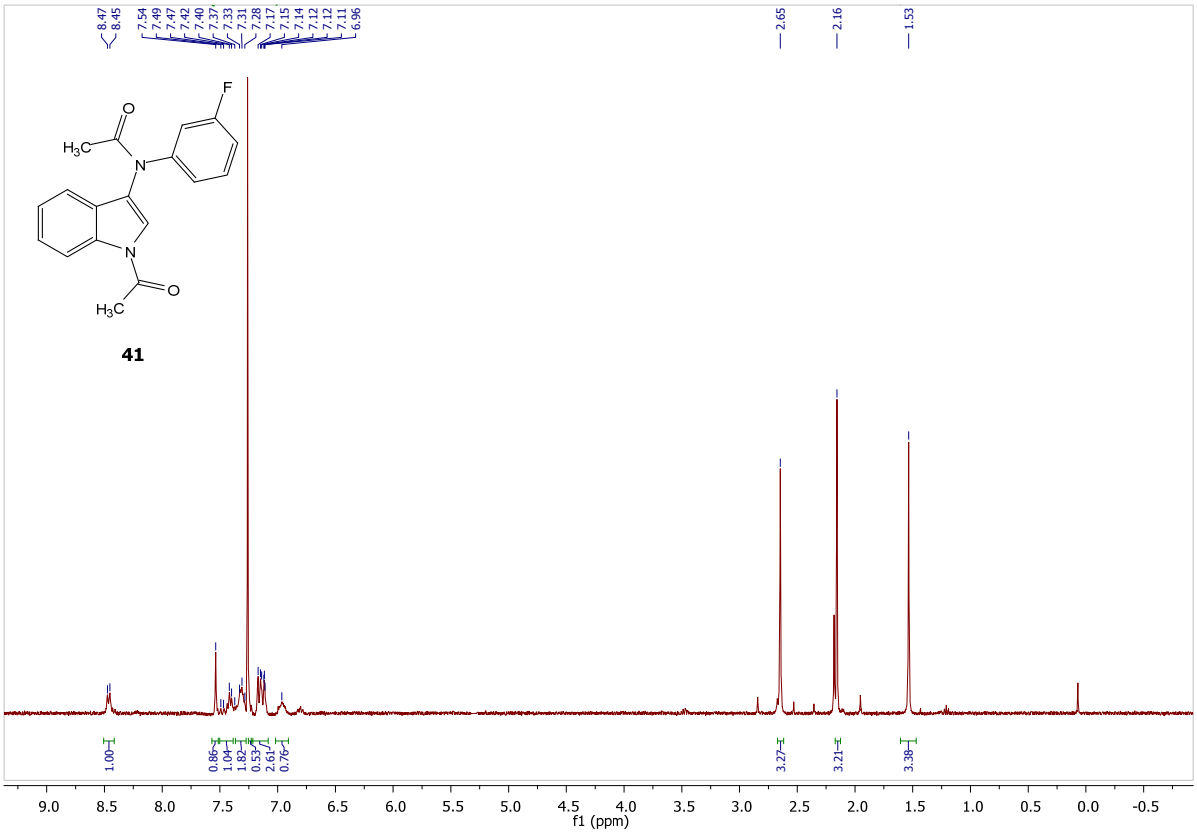
134.59
128.96
127.05
126.22
125.49
124.50
123.65
122.18
120.12
119.22
118.25
116.77
114.84
114.30
55.47
23.96
23.31
f1 (ppm)

40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7.49
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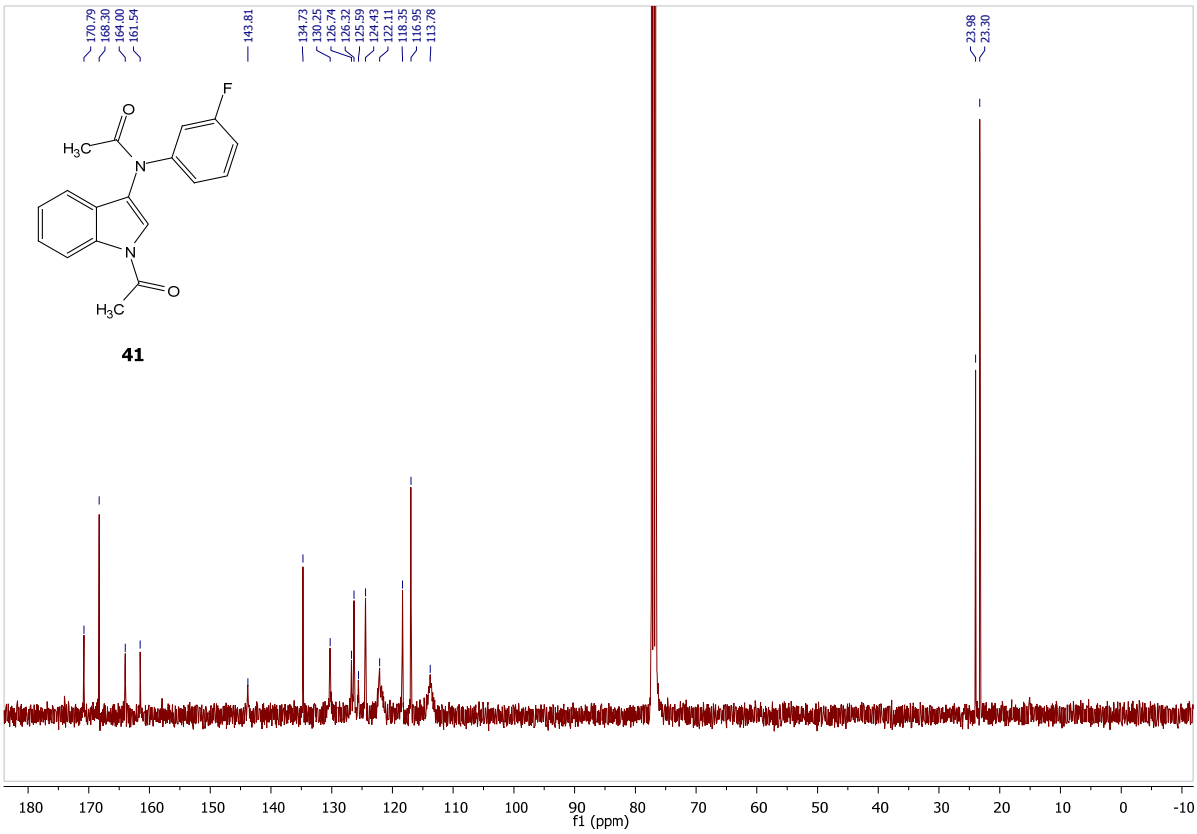
7.42
7.40
7.37
7.33
7.31
7.28
7.17
7.15
7.14
7.12
7.12
7.11
6.96
2.65
2.16
1.53
F
O
H3C
N
N
O
H3C
41
1.00
0.86
1.04
1.82
0.53
2.61
0.76
3.27
3.21
3.38
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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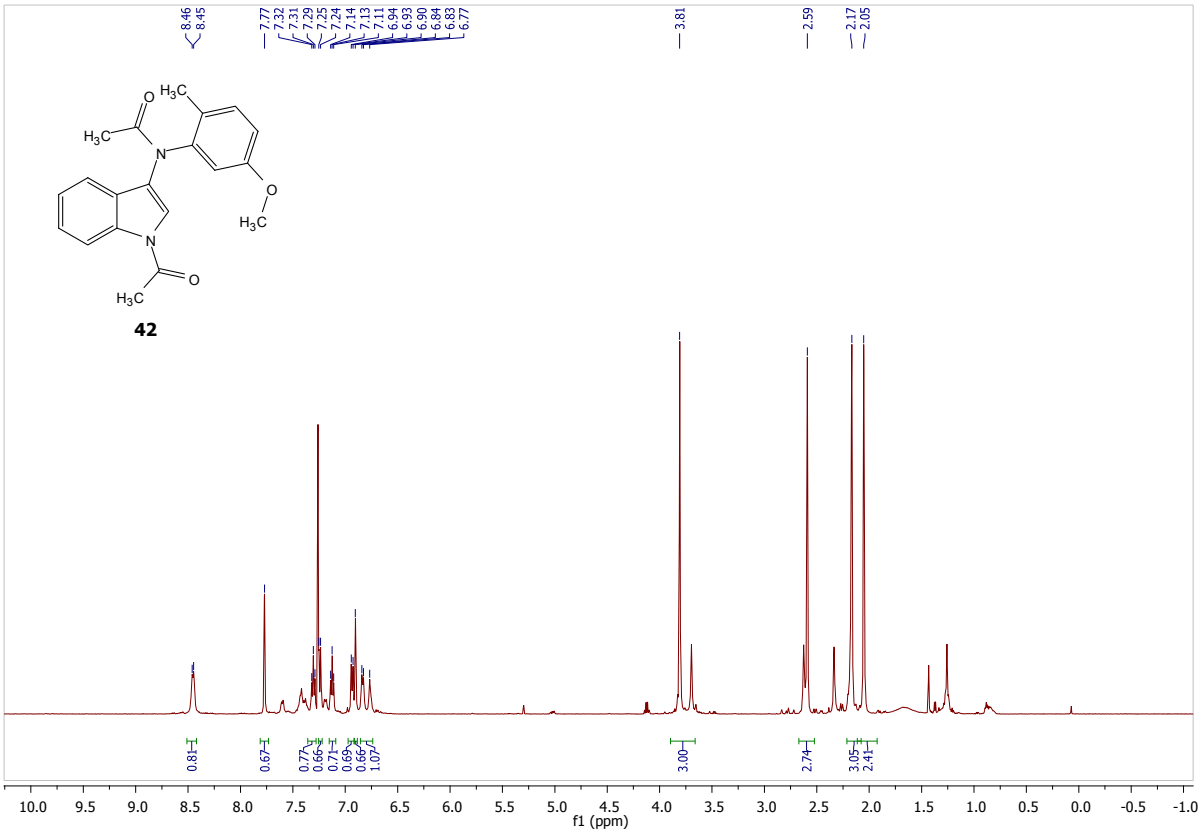
134.73
130.25
126.74
126.32
125.59
124.43
122.11
118.35
116.95
113.78
23.98
23.30
F
O
H3C
N
N
O
H3C
41
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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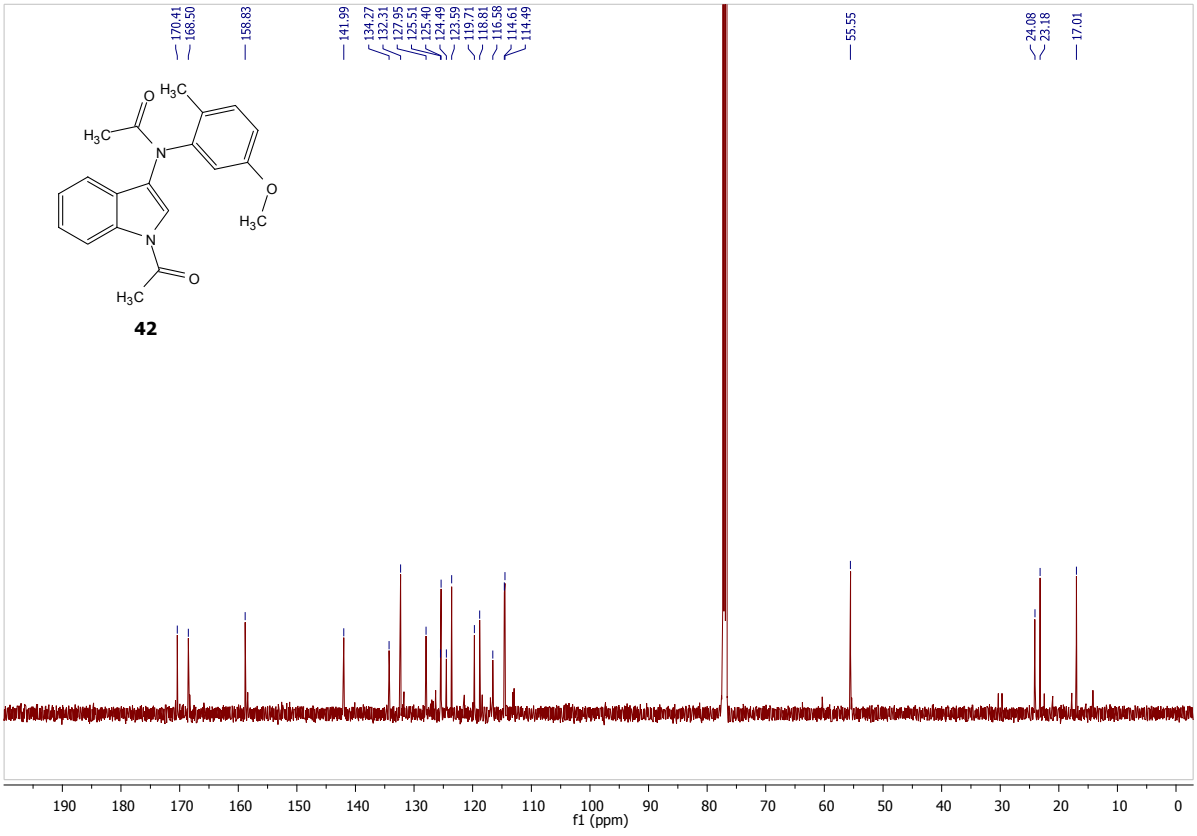
8.45
7.77
7.32
7.31
7.29
7.25
7.24
7.14
7.13
7.11
6.94
6.93
6.90
6.84
6.83
6.77
3.81
2.59
2.17
2.05
0.81
0.67
0.77
0.66
0.71
0.69
0.66
1.07
3.00
2.74
3.05
2.41
f1 (ppm)

42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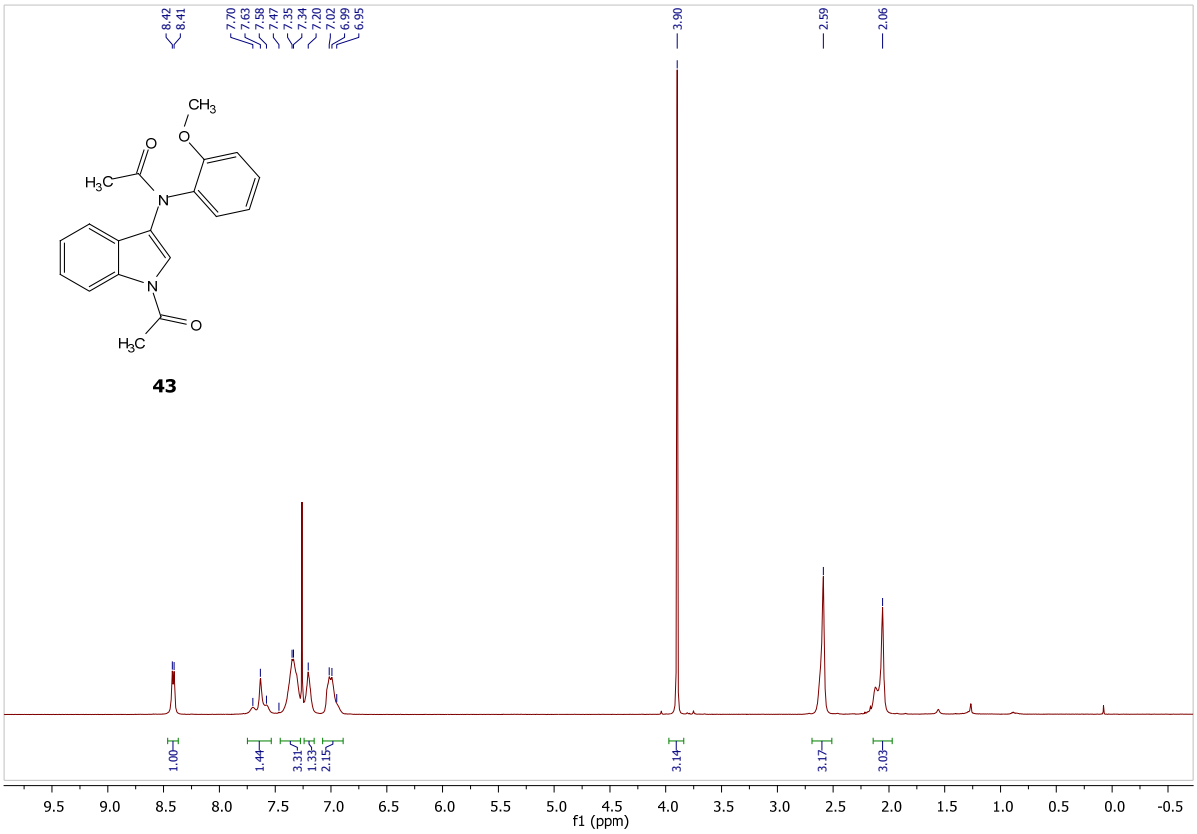
168.50
158.83
141.99
134.27
132.31
127.95
125.51
125.40
124.49
123.59
119.71
118.81
116.58
114.61
114.49
55.55
24.08
23.18
17.01
f1 (ppm)

42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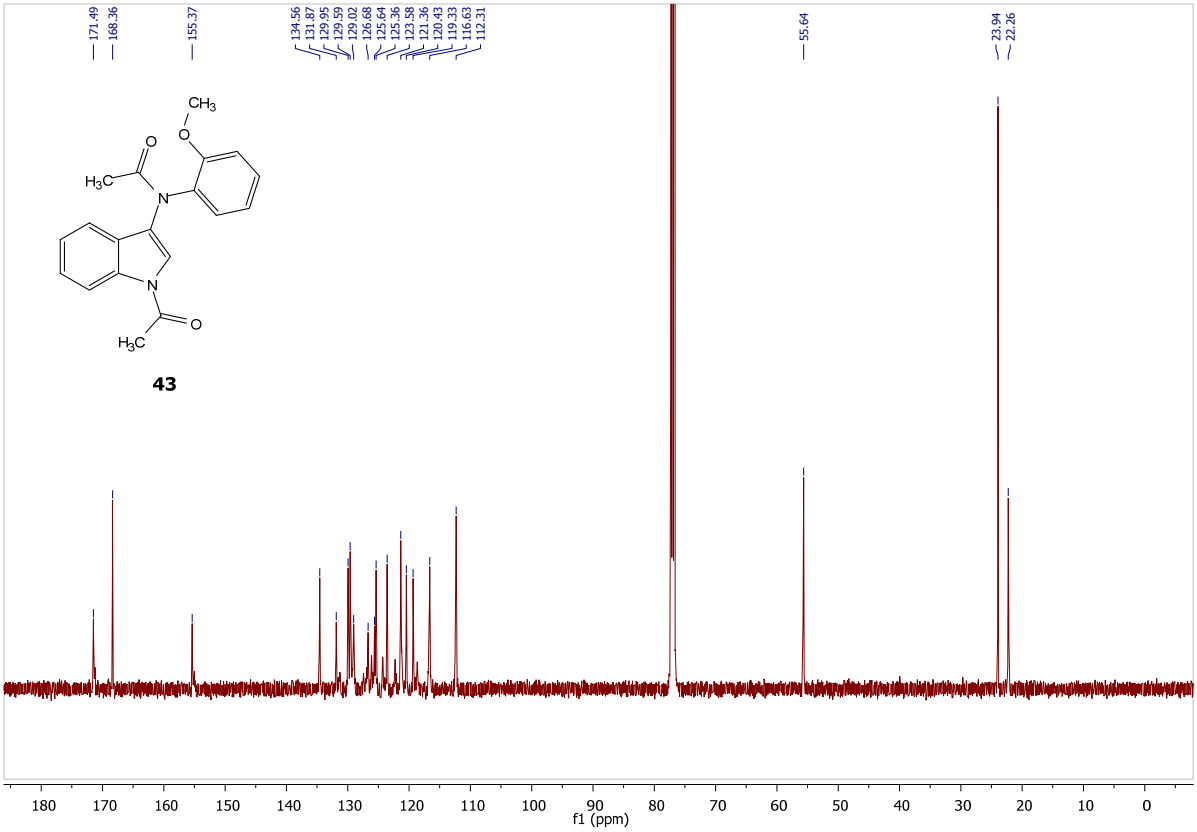
8.41
7.70
7.63
7.58
7.47
7.35
7.34
7.20
7.02
6.99
6.95
3.90
2.59
2.06
CH3
O
H3C
N
O
N
H3C
O
1.00
1.44
3.31
1.33
2.15
3.14
3.17
3.03
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

43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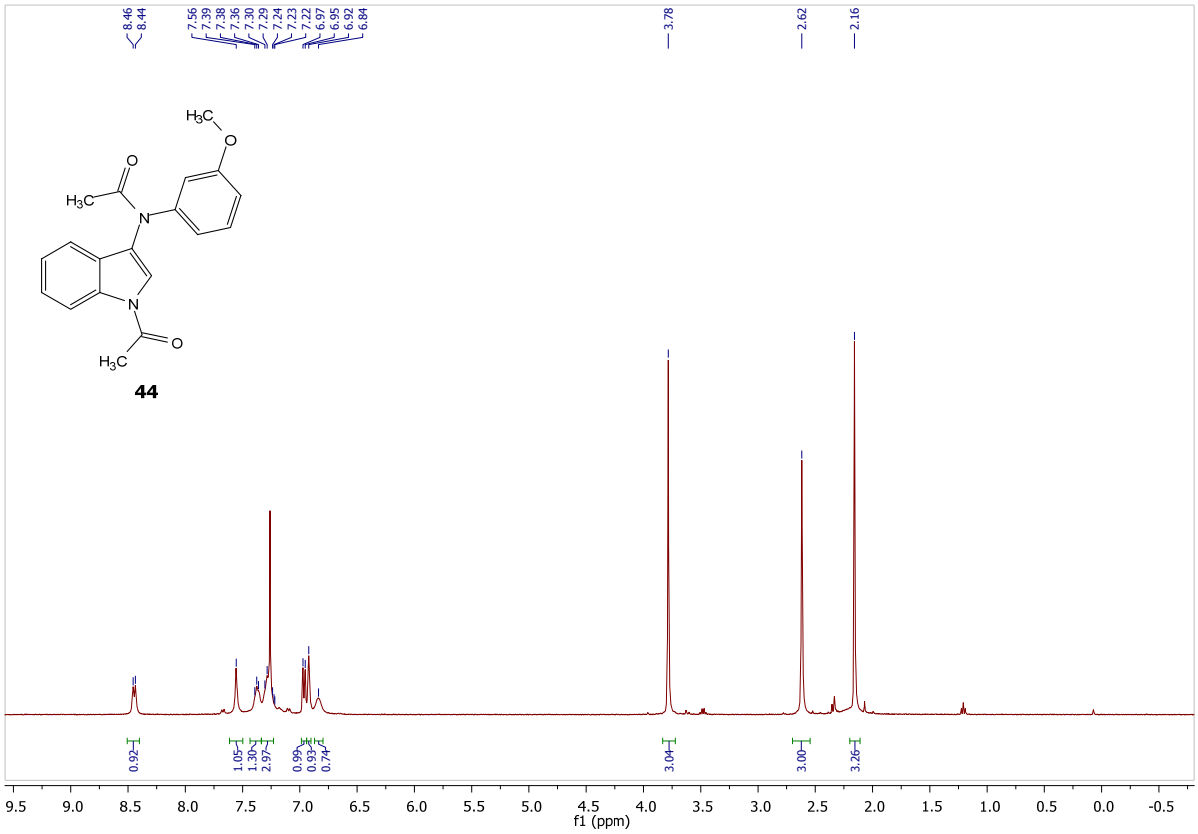
125.64
125.36
123.58
121.36
120.43
119.33
116.63
112.31
55.64
23.94
22.26
CH3
O
H3C
N
O
N
H3C
O
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

43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7.29
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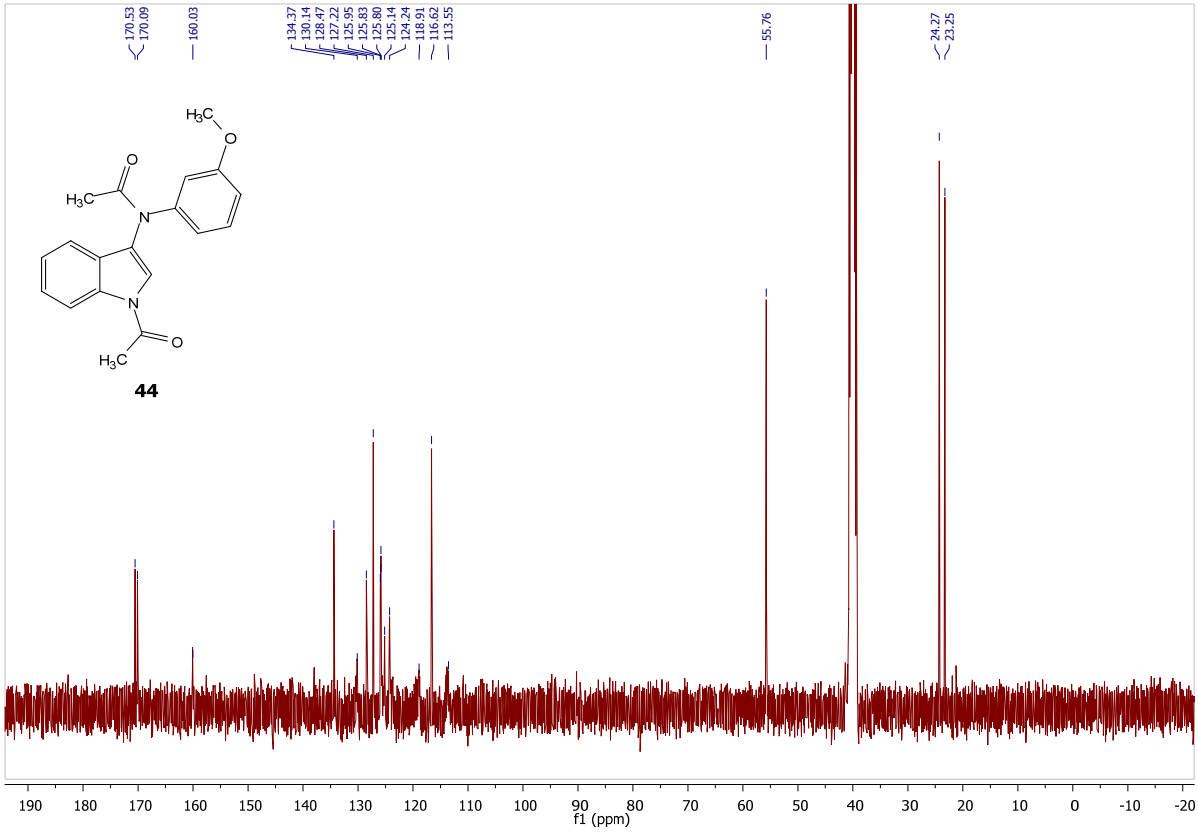
7.23
7.22
6.97
6.95
6.92
6.84
3.78
2.62
2.16
0.92
1.05
1.30
2.97
0.99
0.93
0.74
3.04
3.00
3.26
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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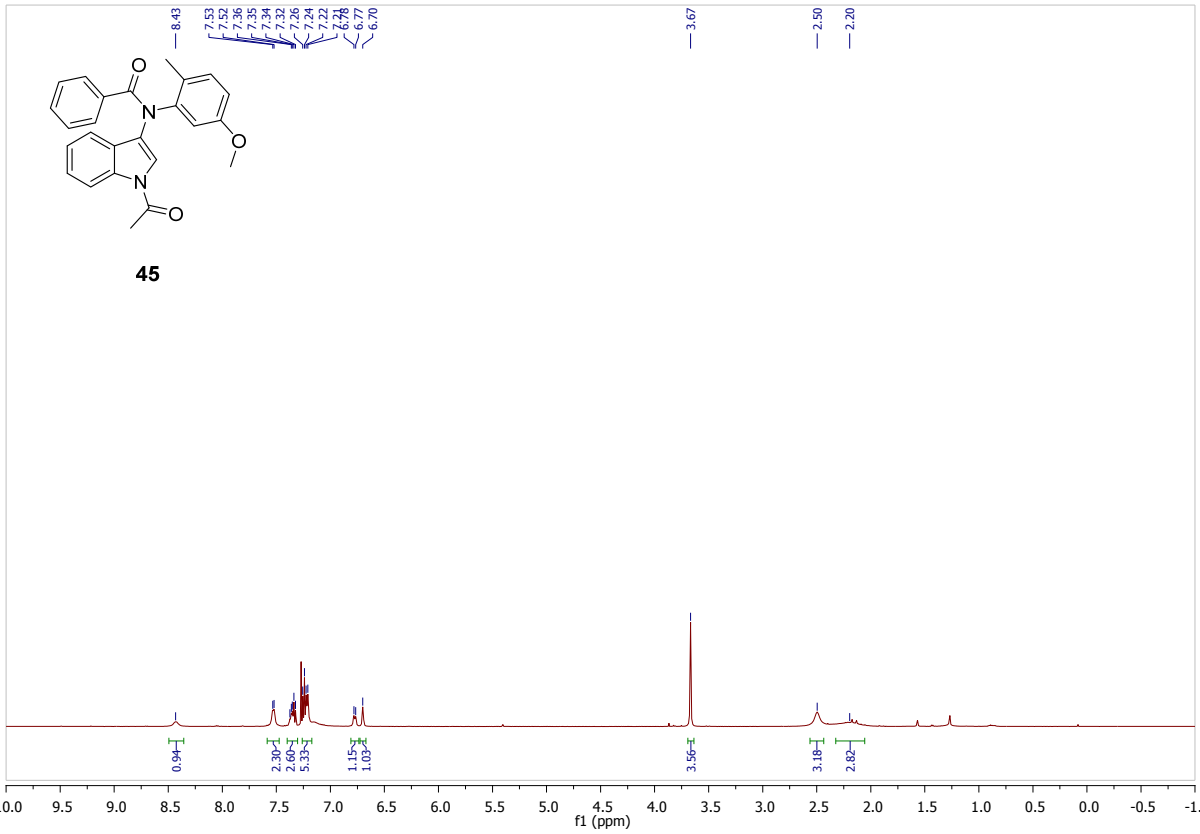
170.09
160.03
134.37
130.14
128.47
127.22
125.95
125.83
125.80
125.14
124.24
118.91
116.62
113.55
55.76
24.27
23.25
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

44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7.53
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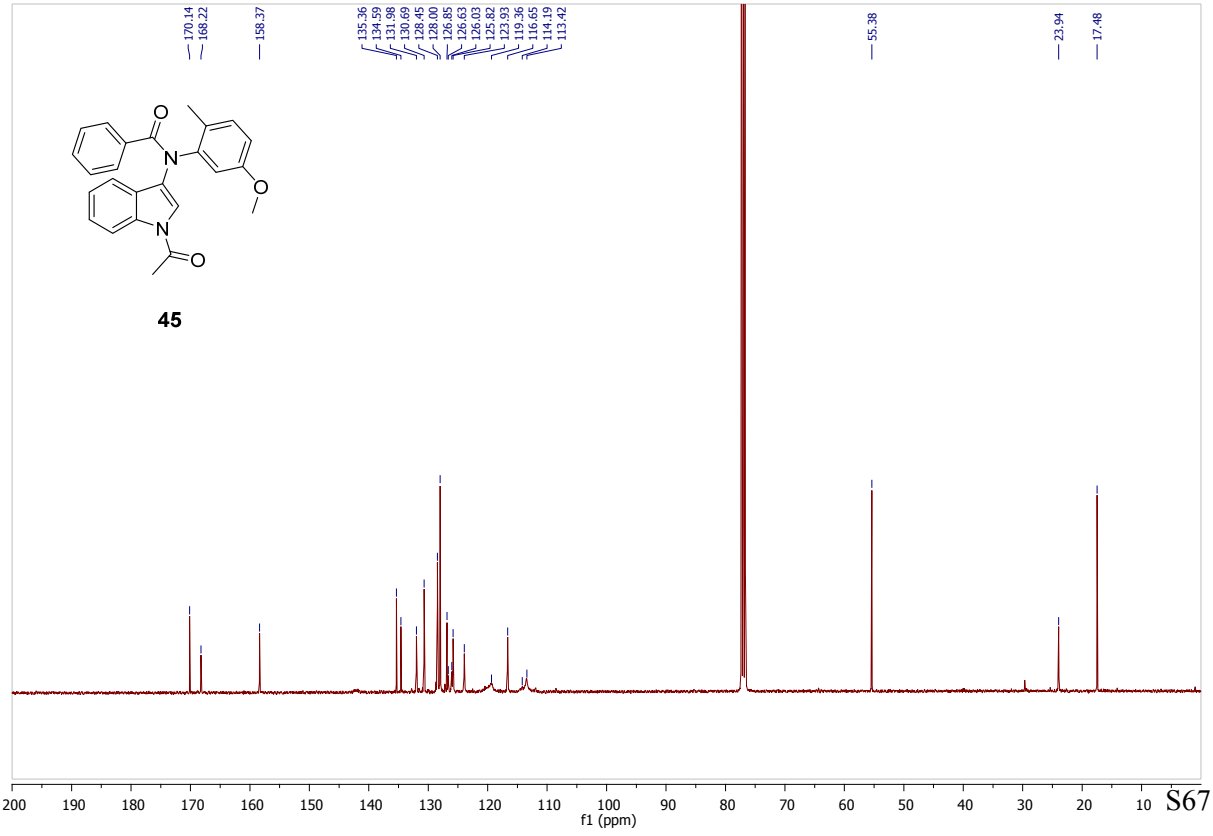
7.35
7.34
7.32
7.26
7.24
7.22
6.78
6.77
6.70
3.67
2.50
2.20
0.94
2.30
2.60
5.33
1.15
1.03
3.56
3.18
2.82
f1 (ppm)

45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168.22
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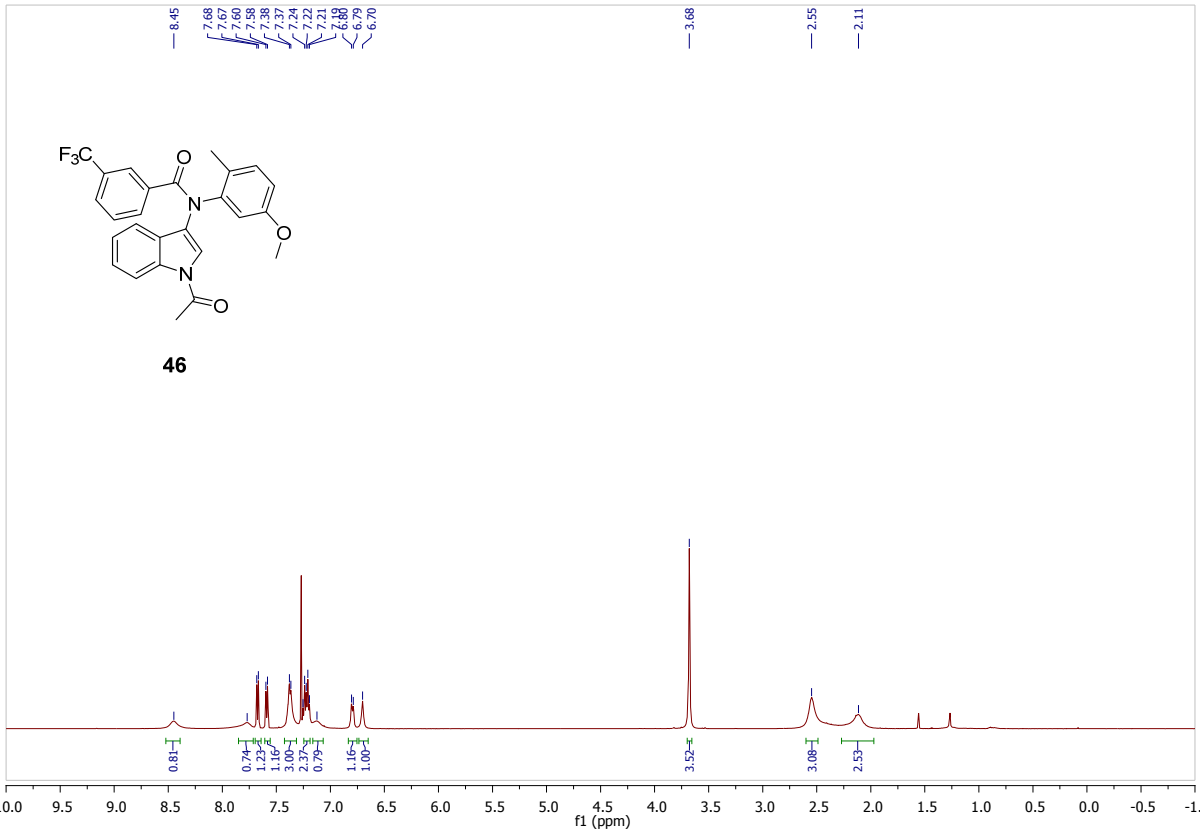
126.85
126.63
126.03
125.82
123.93
119.36
116.65
114.19
113.42
55.38
23.94
17.48
f1 (ppm)

45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7.24
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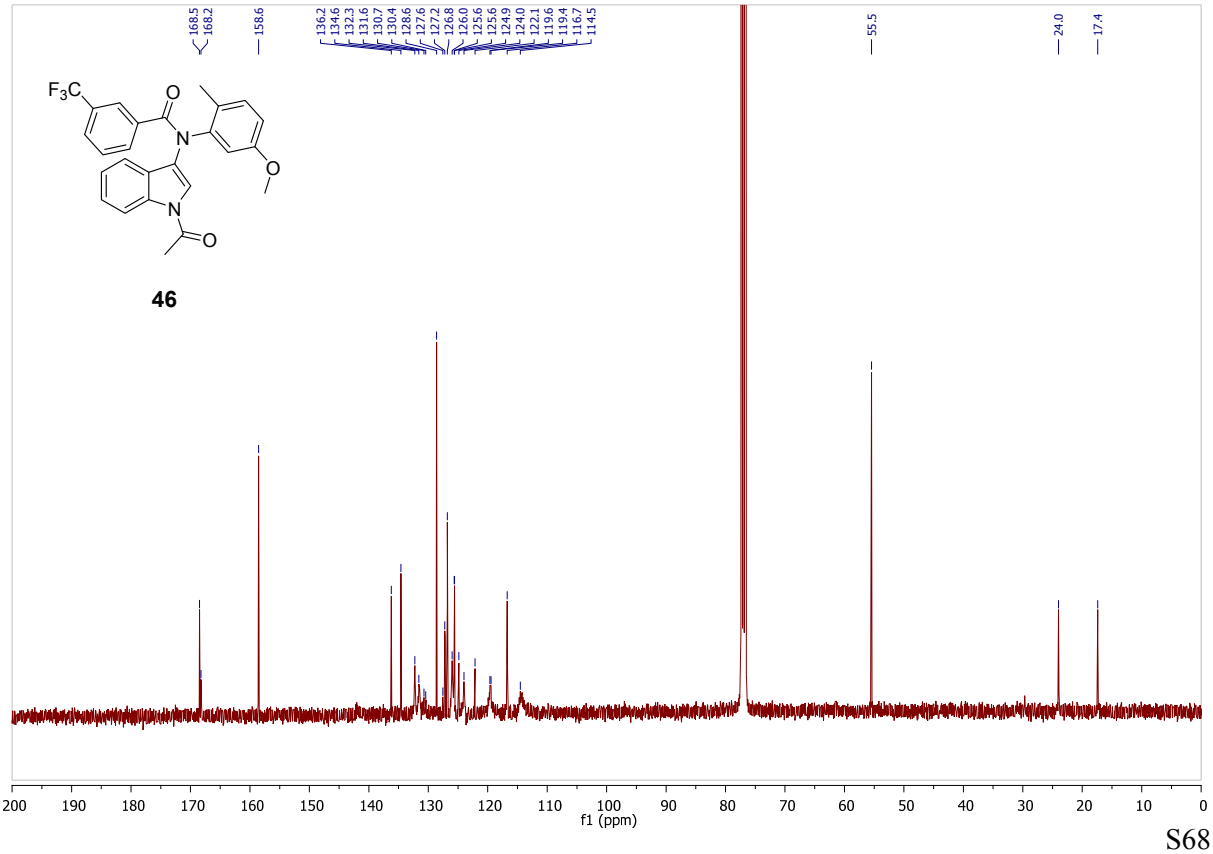
7.21
7.19
6.80
6.79
6.70
3.68
2.55
2.11
F3C
O
N
O
N
O
46
0.81
0.74
1.23
1.16
3.00
2.37
0.79
1.16
1.00
3.52
3.08
2.53
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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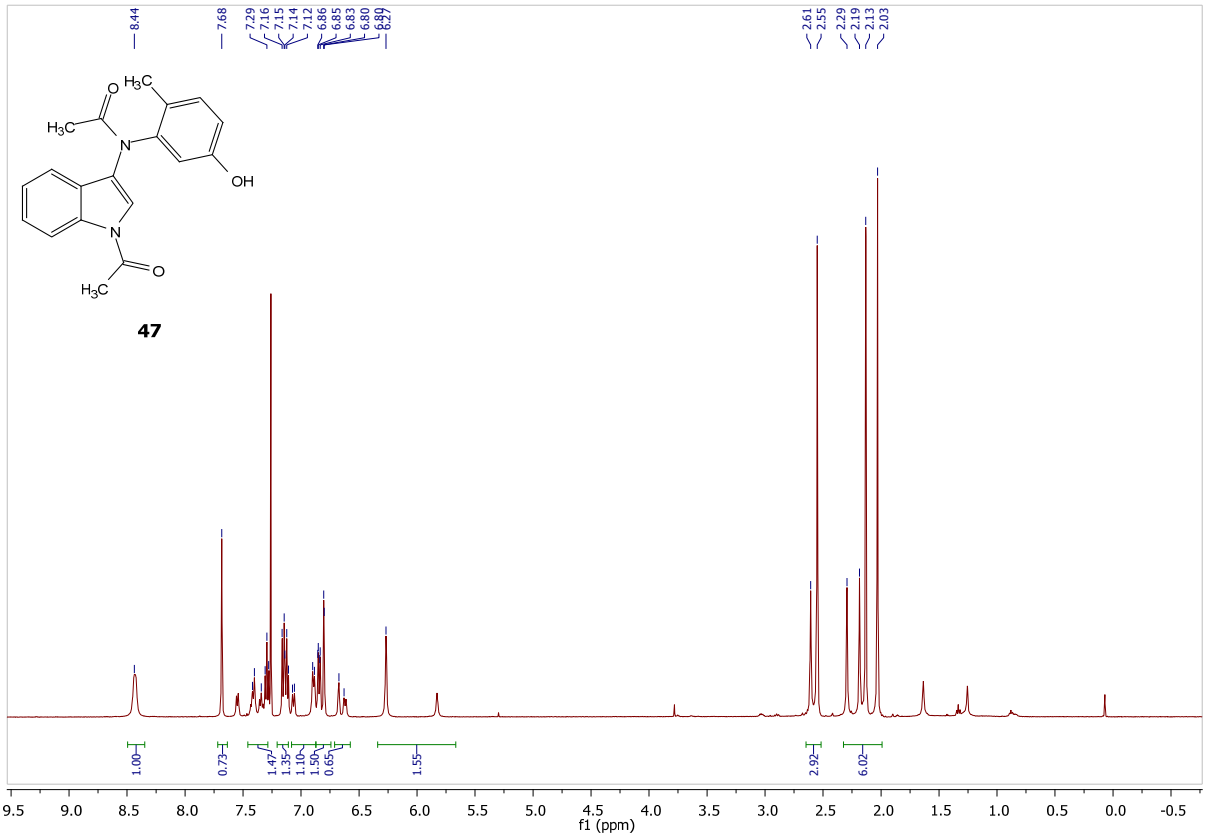
127.6
127.2
126.8
126.0
125.6
125.6
124.9
124.0
122.1
119.6
119.4
116.7
114.5
55.5
24.0
17.4
F3C
O
N
O
N
O
46
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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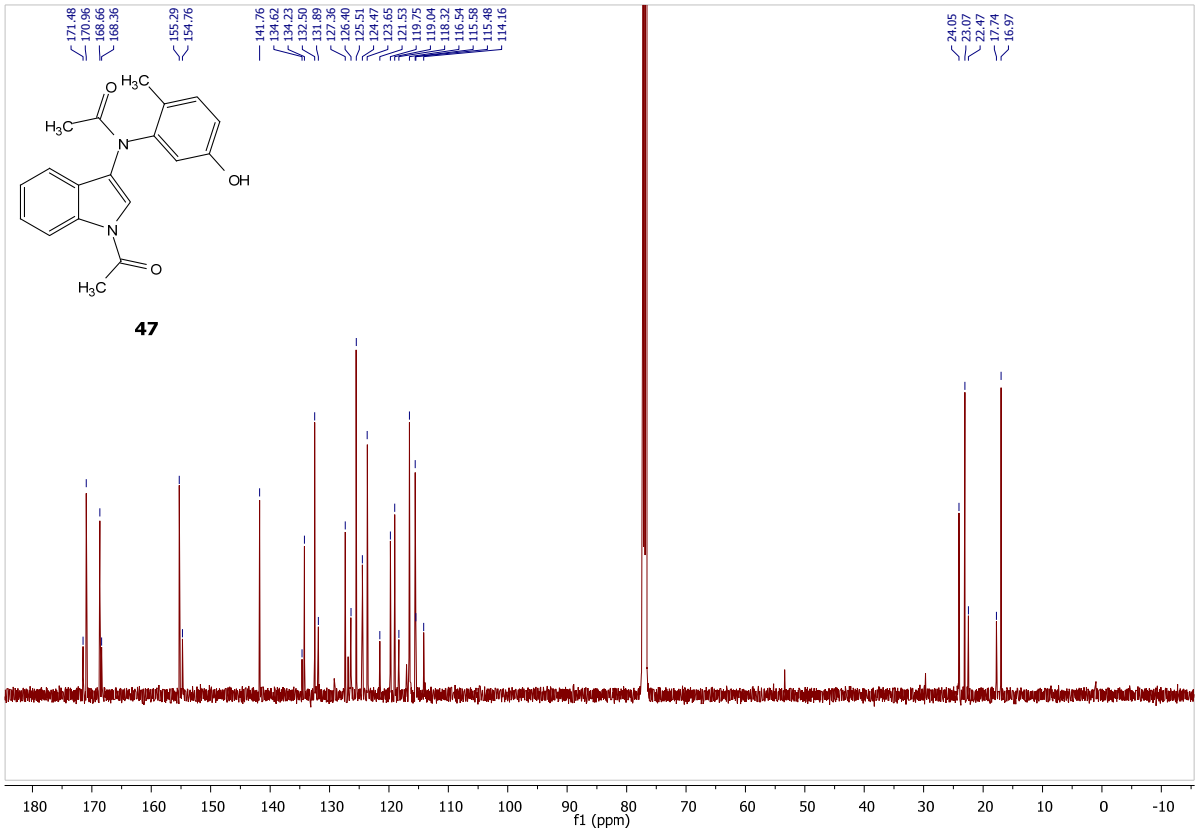
6.80
6.80
6.77
2.61
2.55
2.29
2.19
2.13
2.03
1.00
0.73
1.47
1.35
1.10
1.50
0.65
1.55
2.92
6.02
47
f1 (ppm)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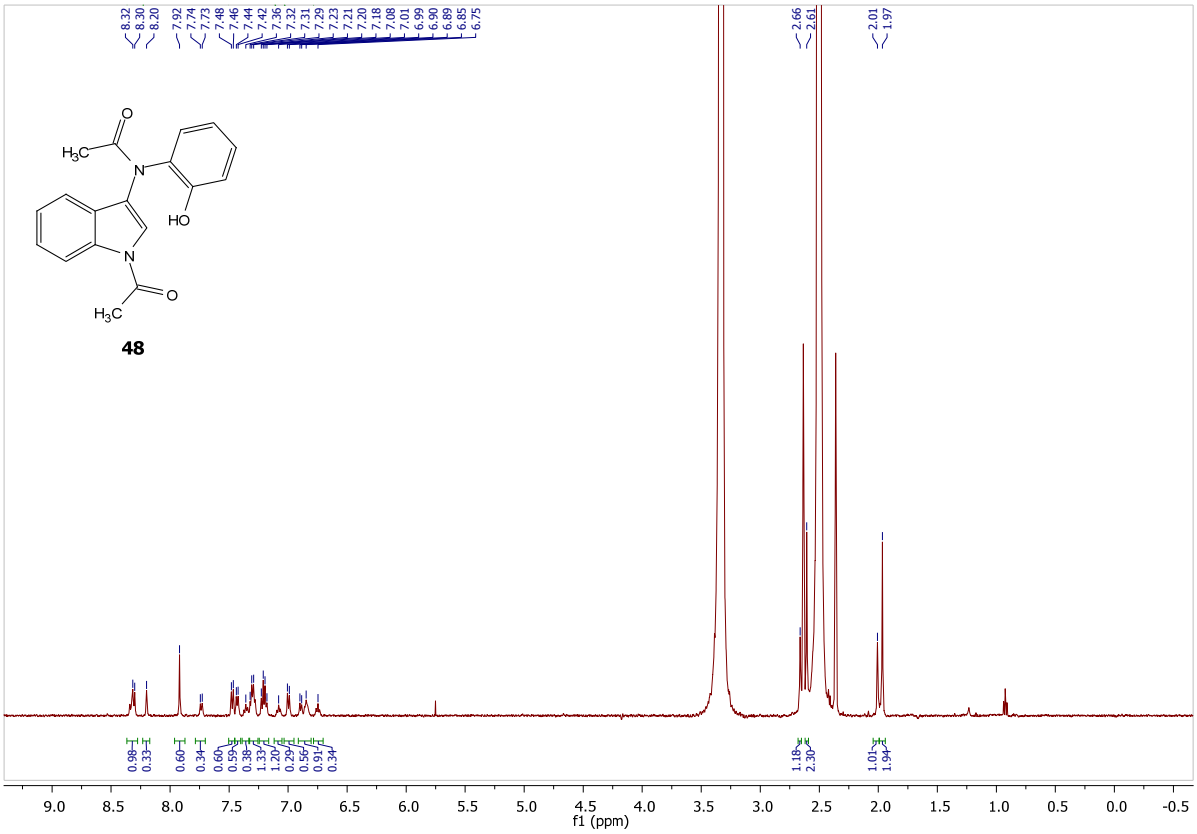
168.36
155.29
154.76
141.76
134.62
134.23
132.50
131.89
127.36
126.40
125.51
124.47
123.65
121.53
119.75
119.04
118.32
116.54
115.58
115.48
114.16
24.05
23.07
22.47
17.74
16.97
47
f1 (ppm)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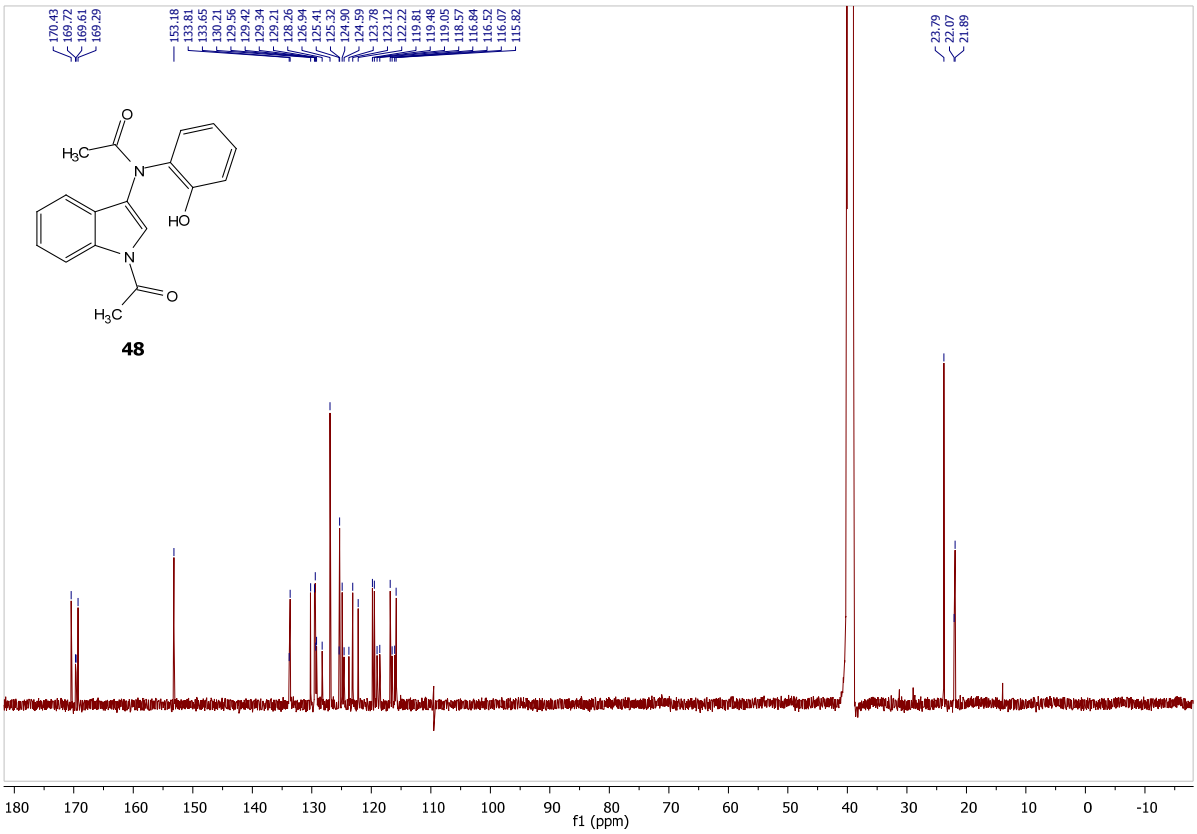
7.73
7.48
7.46
7.44
7.42
7.36
7.32
7.31
7.29
7.23
7.21
7.20
7.18
7.08
7.01
6.99
6.90
6.89
6.85
6.75
2.66
2.61
2.01
1.97
0.98
0.33
0.60
0.34
0.60
0.59
0.38
1.33
1.20
0.29
0.56
0.91
0.34
1.18
2.30
1.01
1.94
H3C
O
N
HO
O
H3C
48
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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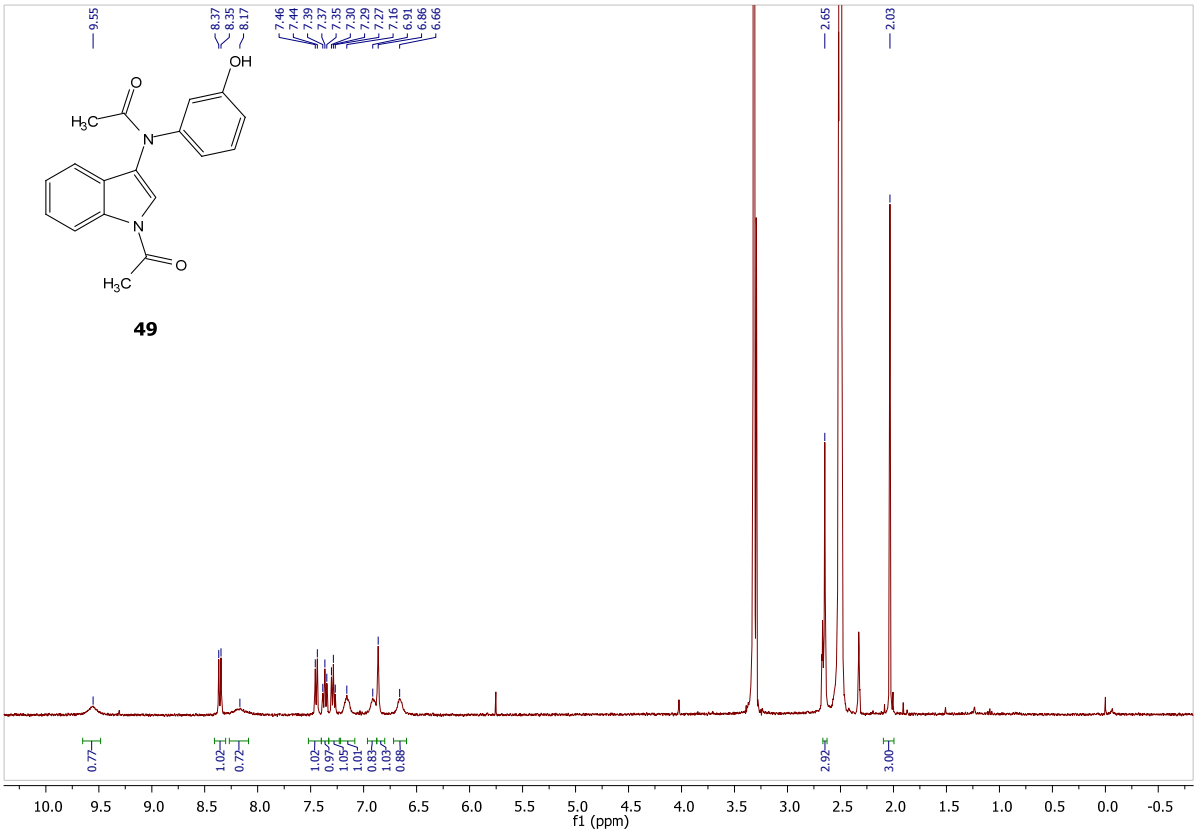
130.21
129.56
129.42
129.34
129.21
128.26
126.94
125.41
125.32
124.90
124.59
123.78
123.12
122.22
119.81
119.48
119.05
118.57
116.84
116.52
116.07
115.82
23.79
22.07
21.89
H3C
O
N
HO
O
H3C
48
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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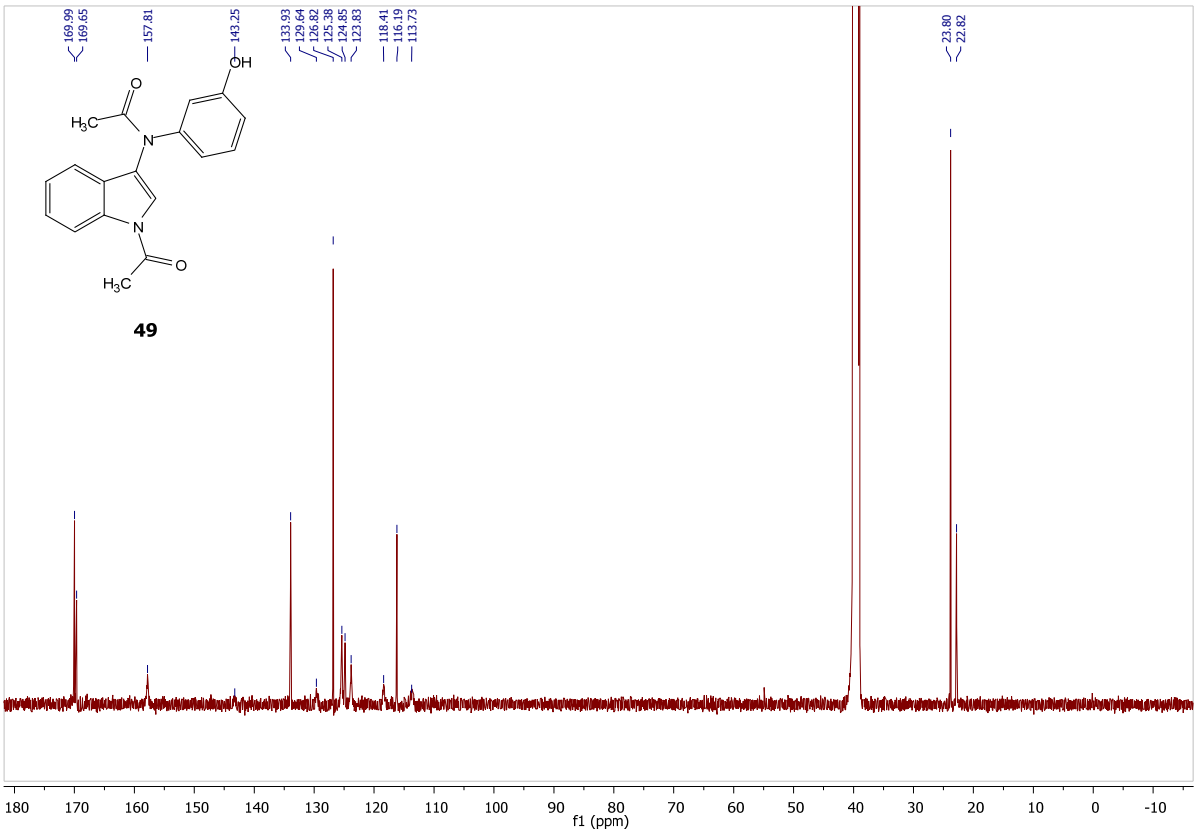
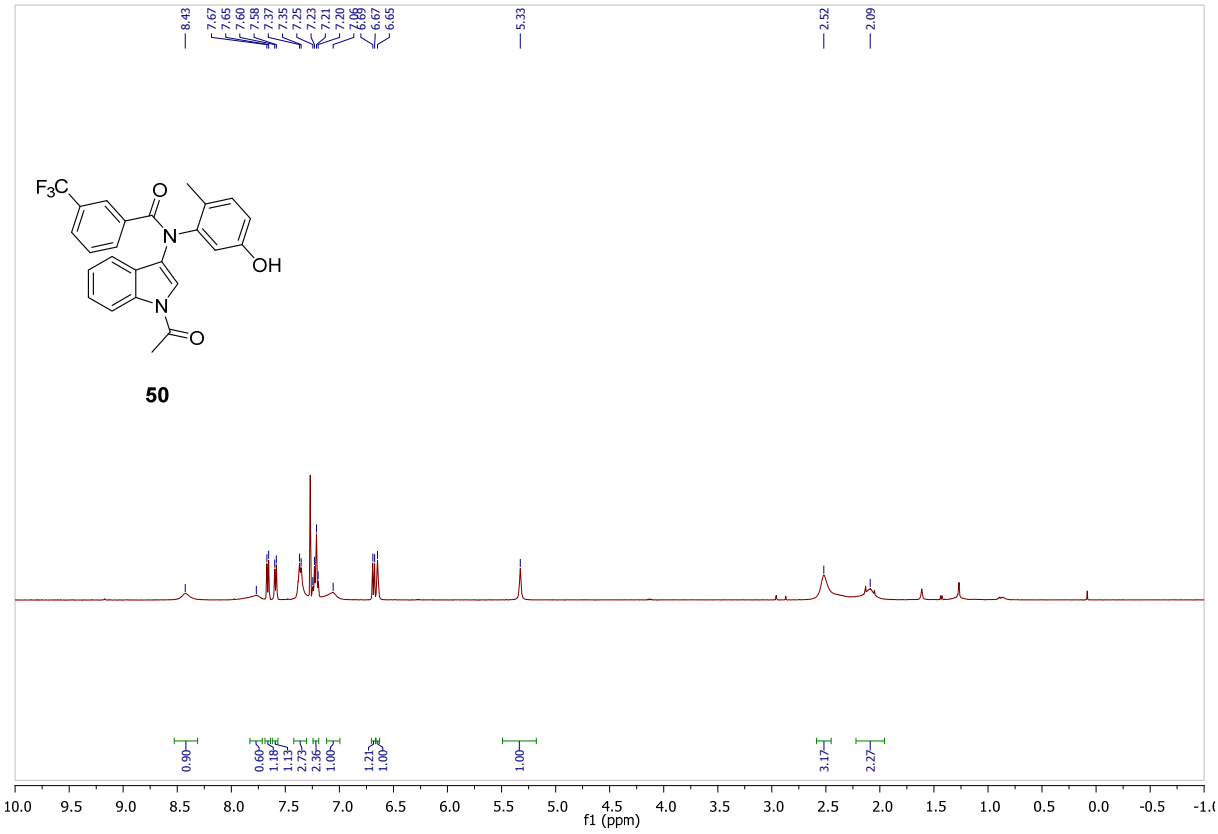
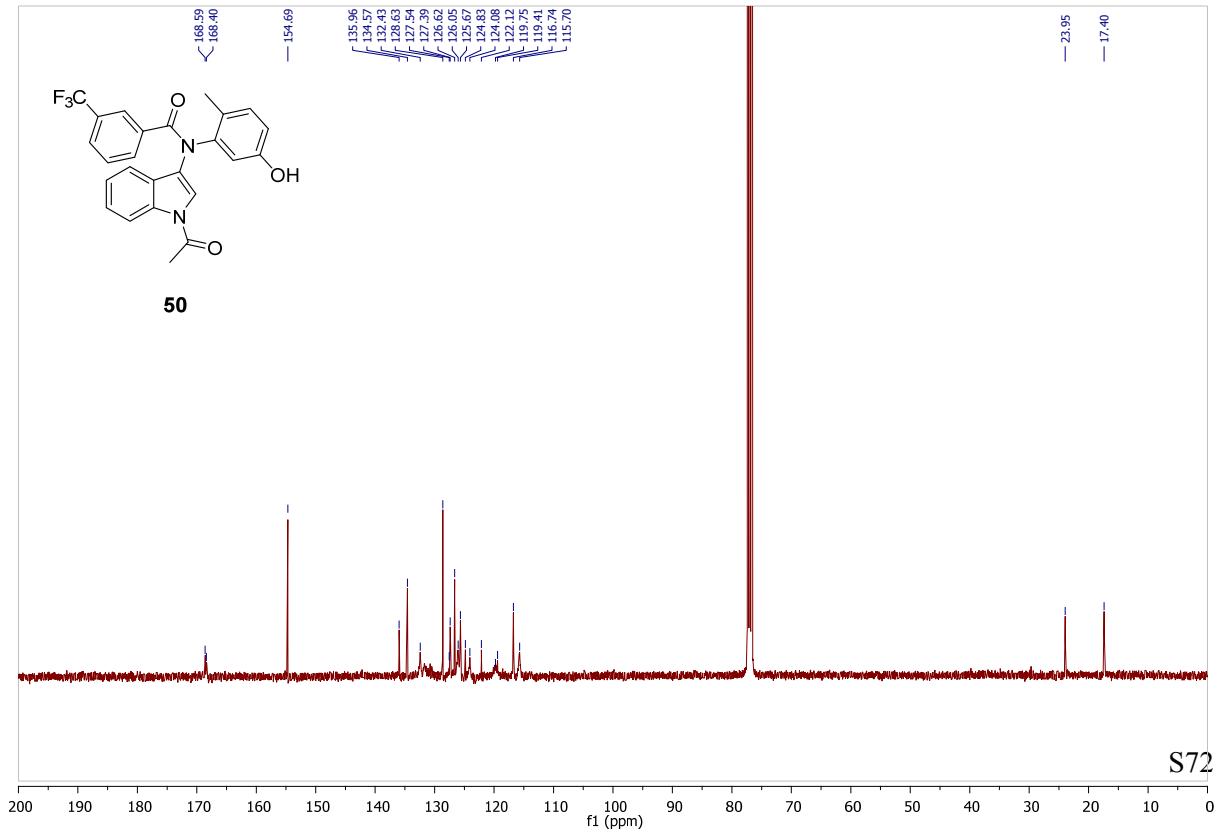
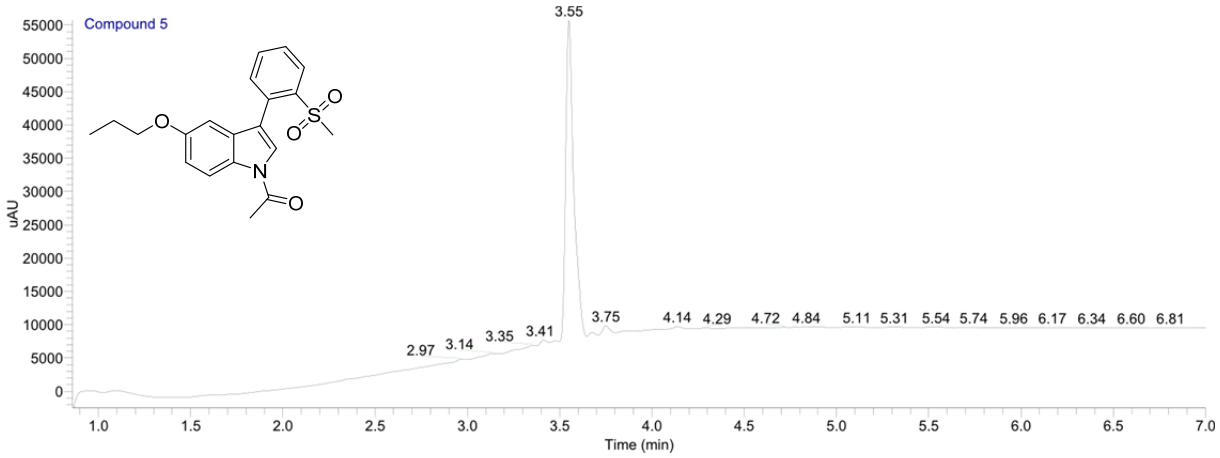
7.39
7.37
7.35
7.30
7.29
7.27
7.16
6.91
6.86
6.66
2.65
2.03
OH
O
H3C
N
N
O
H3C
49
0.77
1.02
0.72
1.02
0.97
1.05
1.01
0.83
1.03
0.88
2.92
3.00
f1 (ppm)


169.99
169.65
157.81
143.25
133.93
129.64
126.82
125.38
124.85
123.83
118.41
116.19
113.73
23.80
22.82
OH
O
H3C
N
N
O
H3C
49
f1 (ppm)

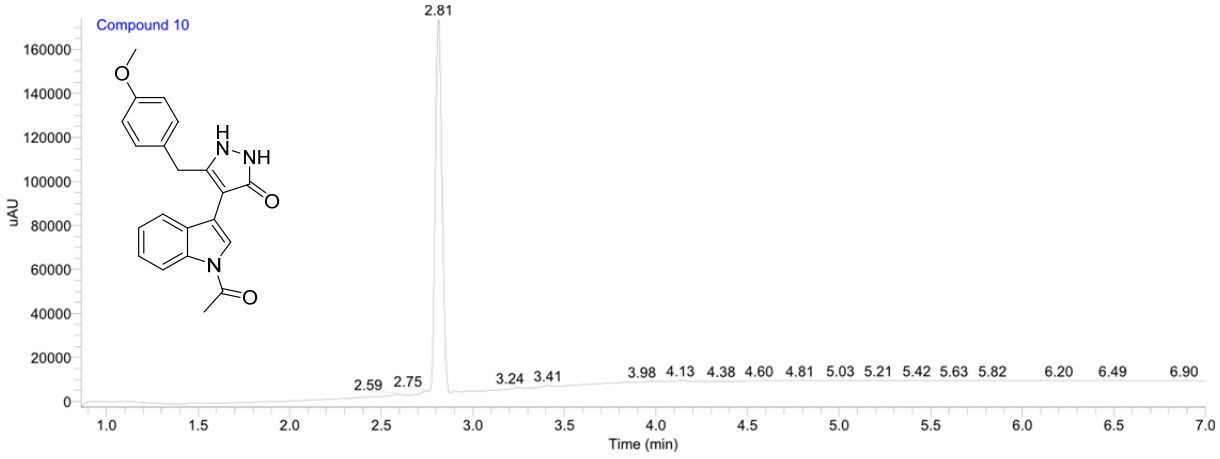

8.43
7.67
7.65
7.60
7.58
7.37
7.35
7.25
7.23
7.21
7.20
6.67
6.65
5.33
2.52
2.09
F3C
O
N
OH
N
O
50
0.90
0.60
1.18
1.13
2.73
2.36
1.00
1.21
1.00
1.00
3.17
2.27
f1 (ppm)


168.59
168.40
154.69
135.96
134.57
132.43
128.63
127.54
127.39
126.62
126.05
125.67
124.83
124.08
122.12
119.75
119.41
116.74
115.70
23.95
17.40
F3C
O
N
OH
N
O
50
f1 (ppm)

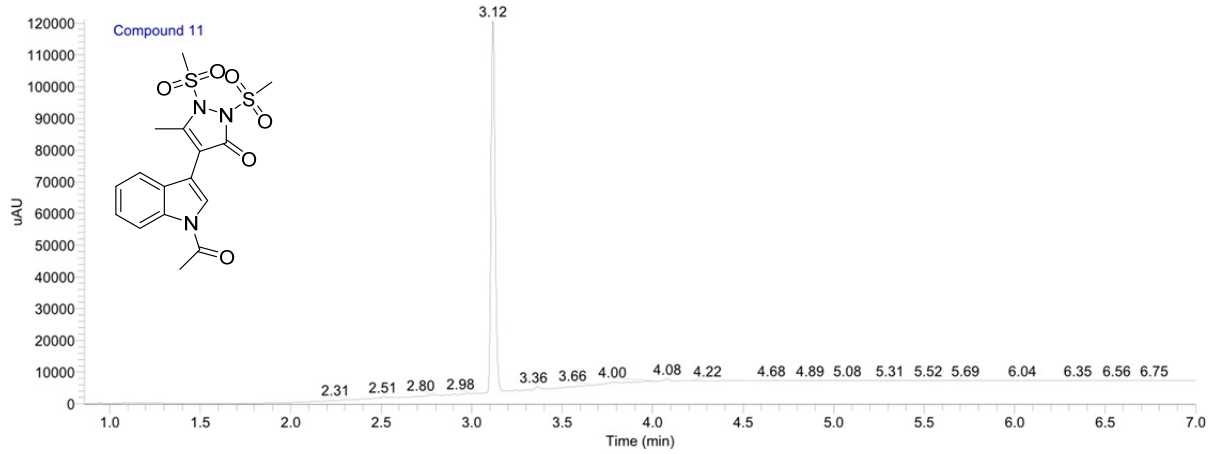
9. HPLC trace (for purity) of tested compounds



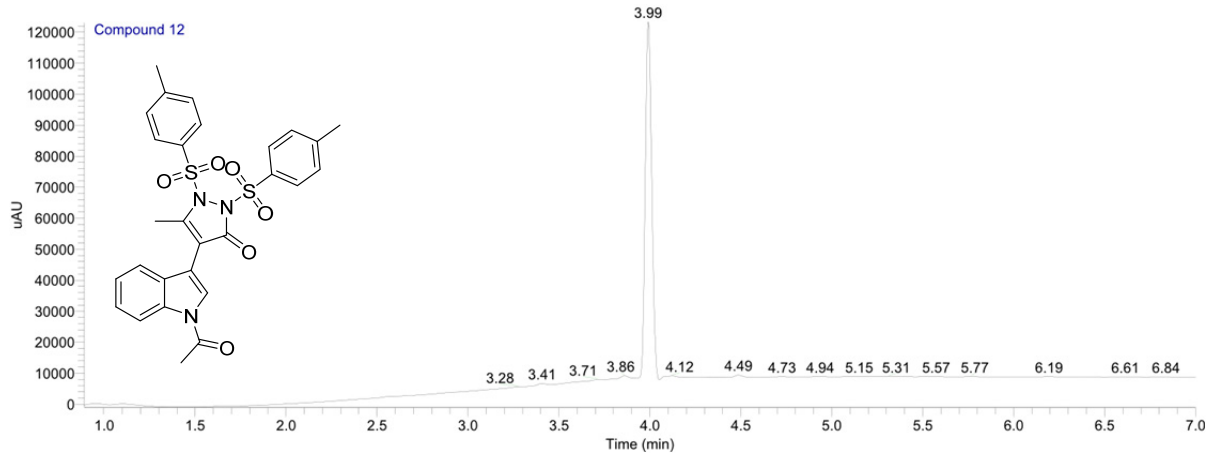
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.96	2.92	3	642.553	0.45	252.179	0.38
3.13	3.09	3.17	576.492	0.4	210.662	0.32
3.35	3.32	3.37	371.681	0.26	188.085	0.28
3.55	3.51	3.58	117094.052	81.97	47564.025	71.81
3.58	3.58	3.63	21374.281	14.96	16803.538	25.37
3.75	3.72	3.8	2502.816	1.75	1100.769	1.66
3.85	3.82	3.9	283.669	0.2	113.811	0.17



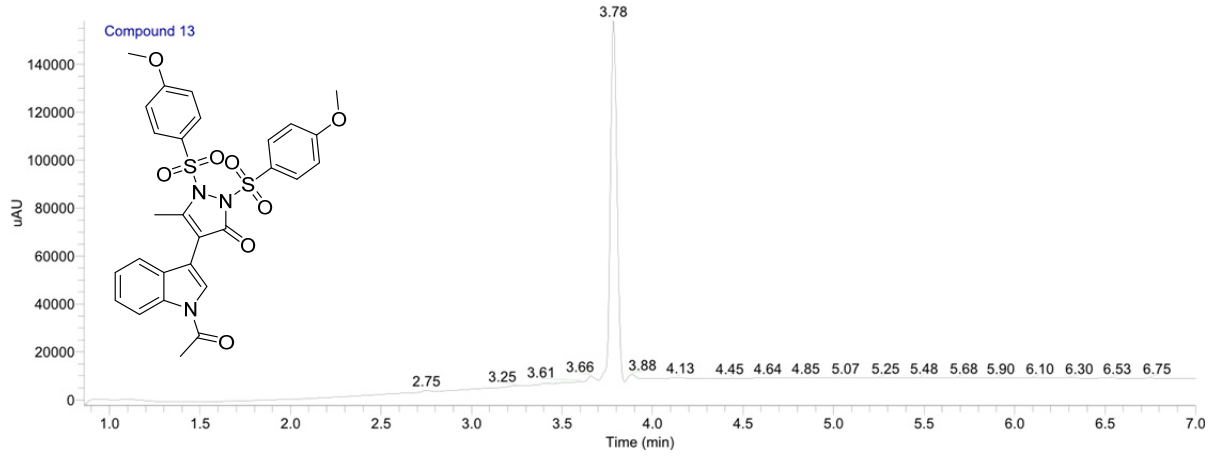
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.59	2.53	2.63	1750.043	0.4	567.526	0.33
2.74	2.7	2.76	2961.626	0.67	1271.616	0.74
2.81	2.77	2.88	435645.238	98.93	169690.148	98.93



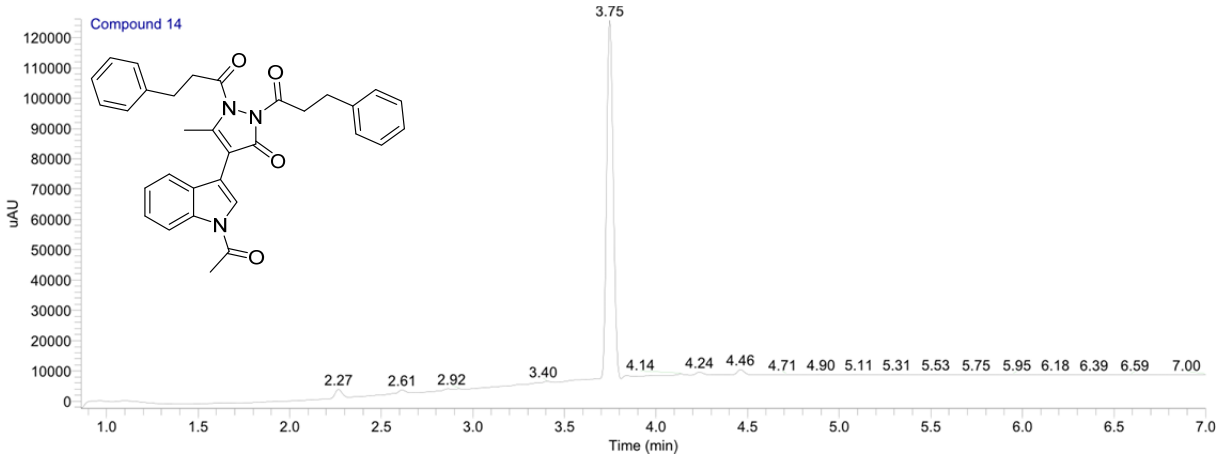
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.3	2.29	2.33	165.515	0.09	119.5	0.1
2.8	2.75	2.83	726.176	0.4	270.159	0.23
2.9	2.88	2.93	253.803	0.14	158.739	0.13
2.98	2.94	3	402.875	0.22	240.087	0.2
3.12	3.09	3.17	179878.747	98.27	117087.111	98.52
3.24	3.22	3.27	350.289	0.19	242.606	0.2
3.38	3.38	3.41	261.267	0.14	251.691	0.21
3.78	3.74	3.82	1016.065	0.56	474.359	0.4



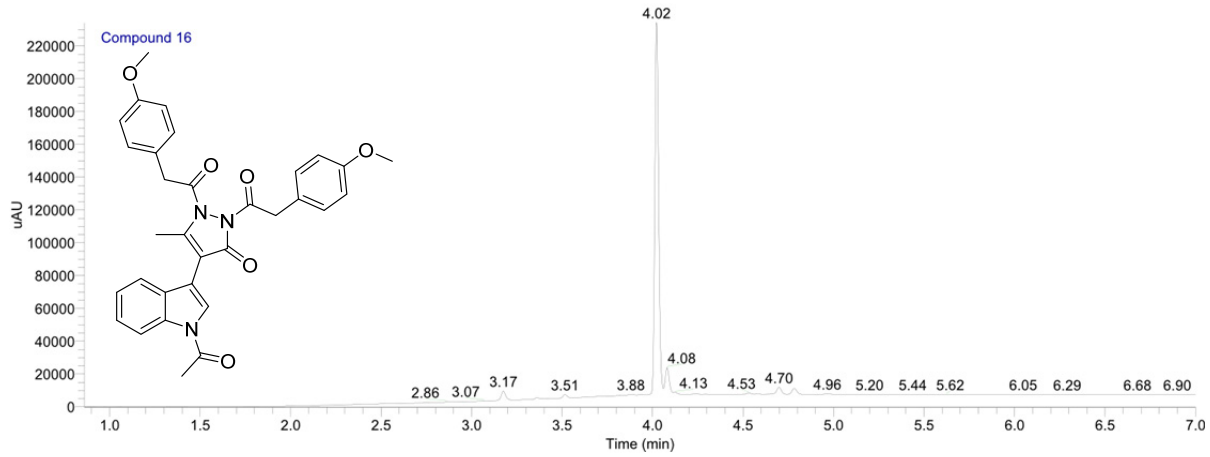
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
3.7	3.67	3.74	382.09	0.13	159.74	0.14
3.86	3.82	3.9	2017.611	0.7	835.573	0.72
3.99	3.94	4.05	285958.456	98.54	115114.707	98.57
4.49	4.44	4.55	1823.203	0.63	679.782	0.58



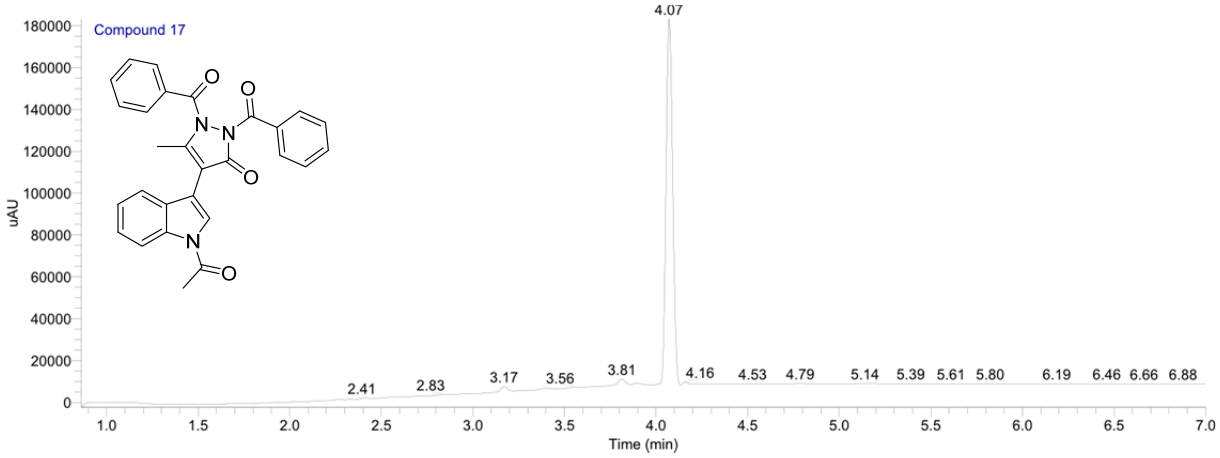
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.75	2.71	2.8	1741.503	0.45	660.636	0.41
3.52	3.48	3.56	539.026	0.14	223.987	0.14
3.66	3.63	3.7	6140.346	1.57	2349.567	1.47
3.74	3.71	3.74	5446.177	1.39	4765.362	2.98
3.78	3.75	3.84	370839.239	94.86	149806.336	93.55
3.88	3.85	3.94	6208.293	1.59	2322.263	1.45



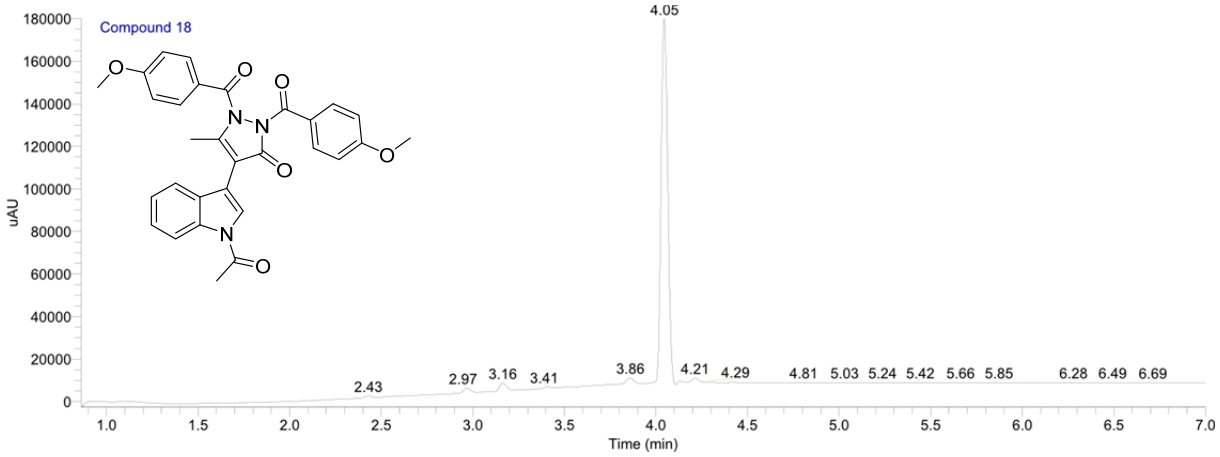
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.27	2.22	2.33	7516.469	2.38	2869.733	2.29
2.61	2.58	2.67	2632.958	0.83	1089.727	0.87
2.86	2.82	2.97	2305.929	0.73	556.803	0.44
3.34	3.31	3.37	379.372	0.12	173.413	0.14
3.75	3.69	3.8	295433.5	93.63	118100.5	94.1
4.24	4.19	4.3	2615.127	0.83	973.898	0.78
4.46	4.42	4.52	4639.038	1.47	1744.505	1.39



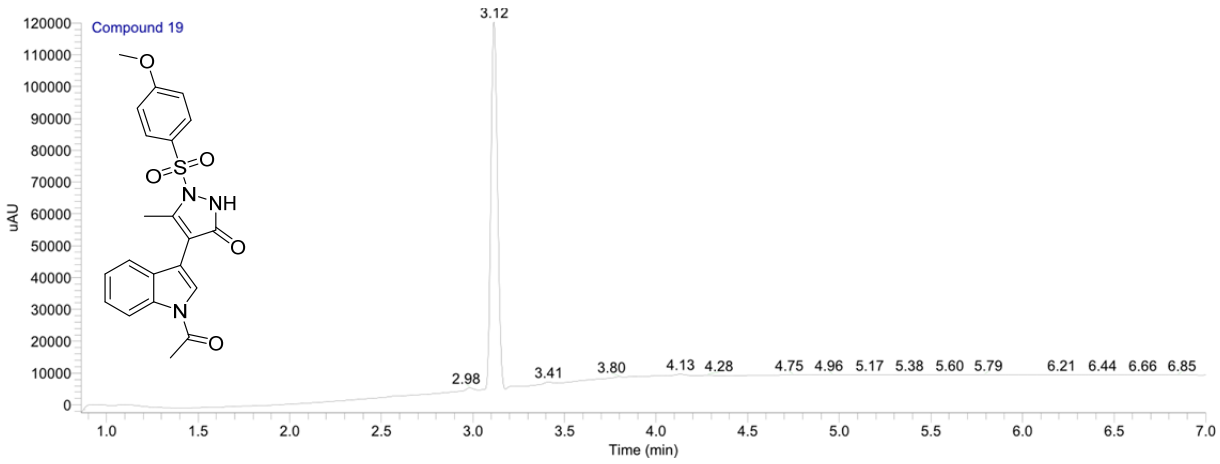
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.86	2.84	2.88	394.503	0.1	250.298	0.1
2.92	2.9	2.96	461.817	0.12	258.583	0.1
3.17	3.12	3.24	10480.059	2.69	5817.231	2.23
3.51	3.48	3.56	3473.517	0.89	2217.571	0.85
3.7	3.67	3.72	312.377	0.08	223.835	0.09
3.88	3.84	3.91	1077.044	0.28	564.895	0.22
4.02	3.99	4.06	330027.353	84.81	226422.87	86.64
4.08	4.06	4.11	23506.695	6.04	15798.679	6.05
4.24	4.2	4.32	1298.478	0.33	551.978	0.21
4.53	4.5	4.61	2017.606	0.52	854.579	0.33
4.7	4.64	4.74	8195.778	2.11	4311.426	1.65
4.78	4.75	4.84	6922.06	1.78	3612.794	1.38
4.96	4.93	5.01	975.332	0.25	458.87	0.18



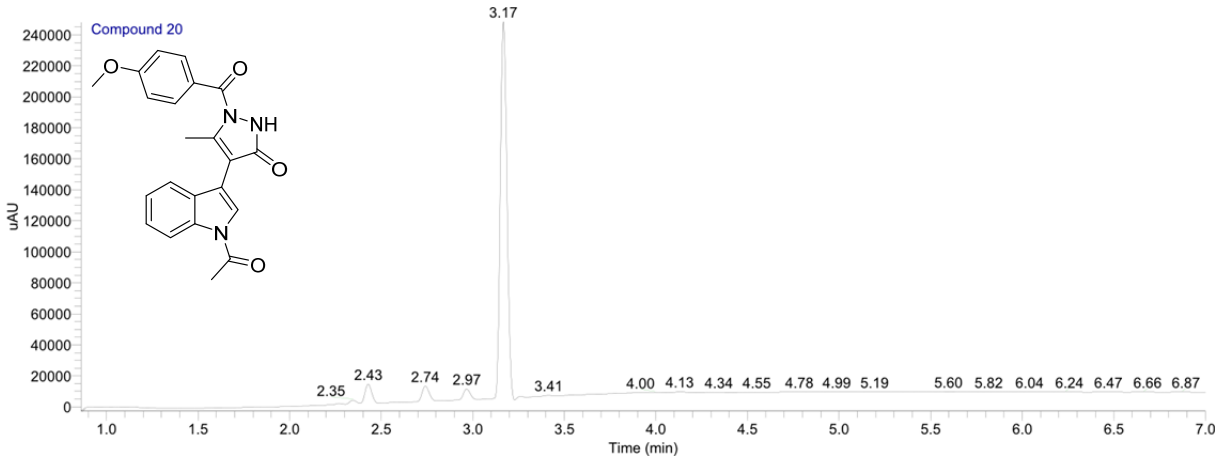
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.26	2.22	2.3	871.745	0.19	352.205	0.19
2.41	2.37	2.46	1129.794	0.25	391.083	0.21
2.82	2.78	2.86	601.083	0.13	239.01	0.13
3.17	3.12	3.22	6345.22	1.39	2437.072	1.34
3.81	3.75	3.86	7614.463	1.67	2895.641	1.59
3.89	3.86	3.94	1939.645	0.43	819.524	0.45
4.07	4.02	4.13	436857.776	95.86	174676.24	96
4.79	4.75	4.83	343.286	0.08	142.454	0.08



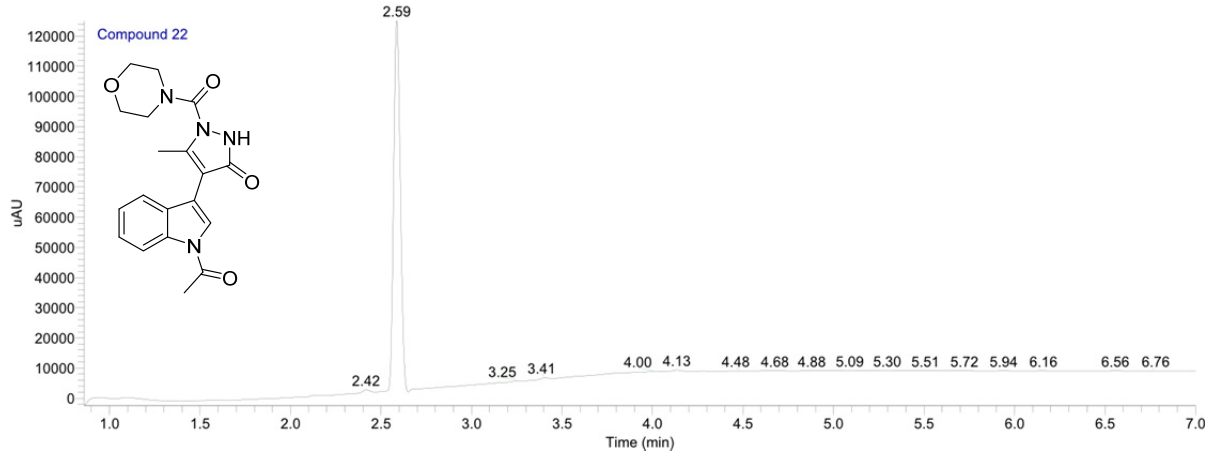
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.43	2.38	2.49	2181.413	0.48	839.009	0.46
2.97	2.92	3.03	5755.818	1.26	2219.446	1.21
3.16	3.11	3.22	9169.978	2	3682.757	2.01
3.86	3.8	3.92	7868.756	1.72	2829.776	1.55
4.05	3.99	4.1	426762.444	93.09	170896.539	93.32
4.21	4.17	4.26	5082.573	1.11	2021.285	1.1
4.3	4.27	4.34	949.038	0.21	393.821	0.22
4.42	4.37	4.47	651.379	0.14	242.953	0.13



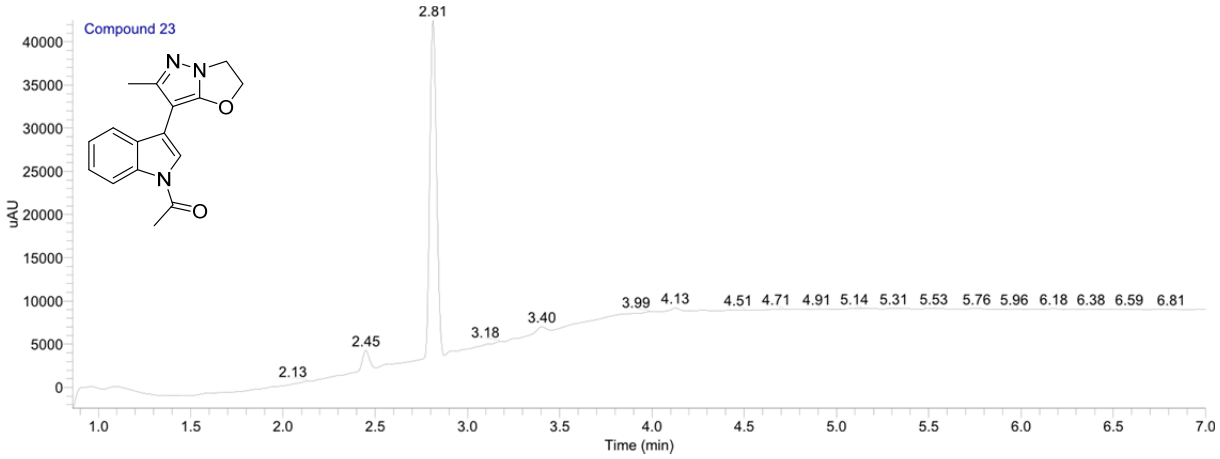
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.98	2.94	3.03	2249.85	0.76	876.844	0.75
3.12	3.07	3.17	291194.167	99	115408	99.02
3.79	3.76	3.84	701.369	0.24	267.686	0.23



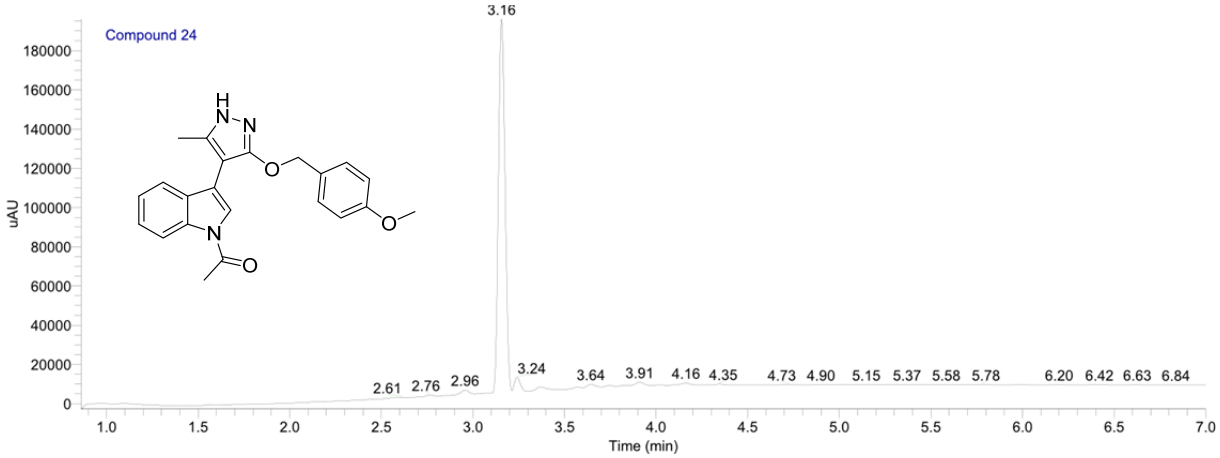
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.27	2.23	2.3	2295.876	0.33	919.853	0.33
2.34	2.31	2.38	5924.635	0.85	2445.805	0.89
2.43	2.39	2.49	31453.133	4.53	12412.399	4.5
2.74	2.69	2.81	26626.605	3.84	9939.185	3.6
2.97	2.91	3.03	18153.179	2.61	7042.946	2.55
3.17	3.12	3.22	609792.405	87.84	243076.767	88.12



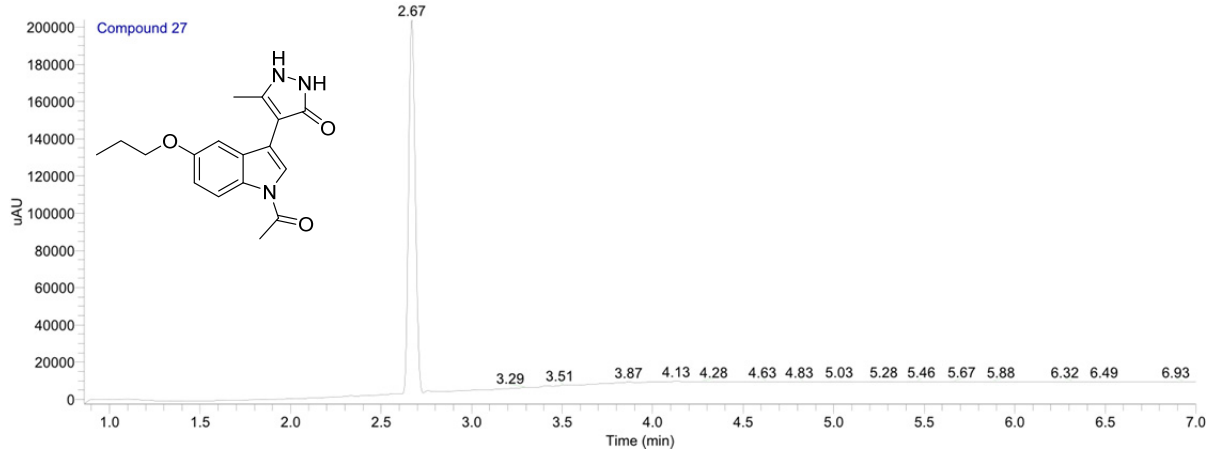
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.13	2.08	2.2	1174.811	0.37	341.176	0.27
2.42	2.37	2.47	2549.774	0.8	974.793	0.78
2.59	2.53	2.64	313101.658	98.71	122671.38	98.74
2.68	2.66	2.71	378.82	0.12	246.799	0.2



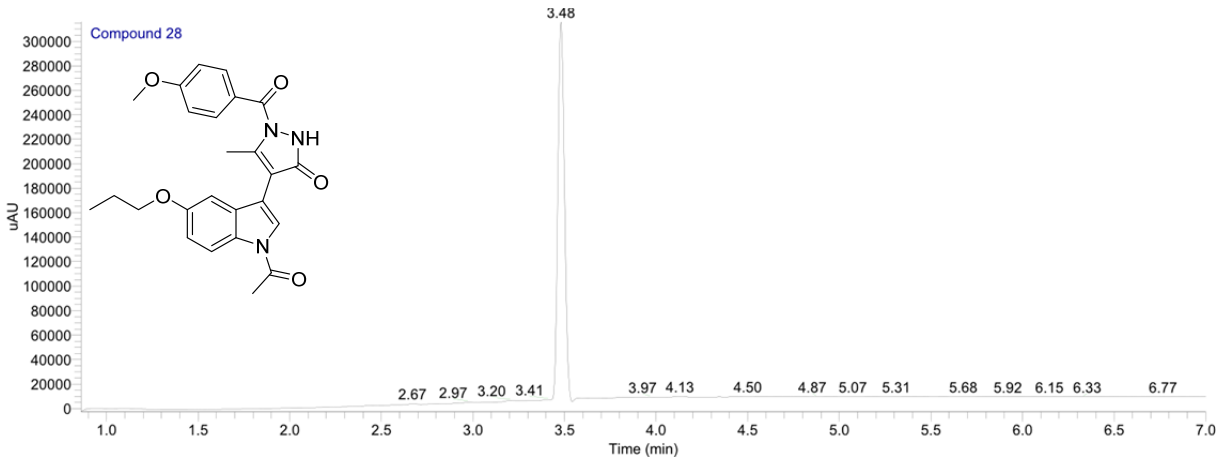
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.45	2.4	2.51	5917.481	5.64	2281.111	5.53
2.81	2.76	2.87	98968.781	94.36	38982.089	94.47



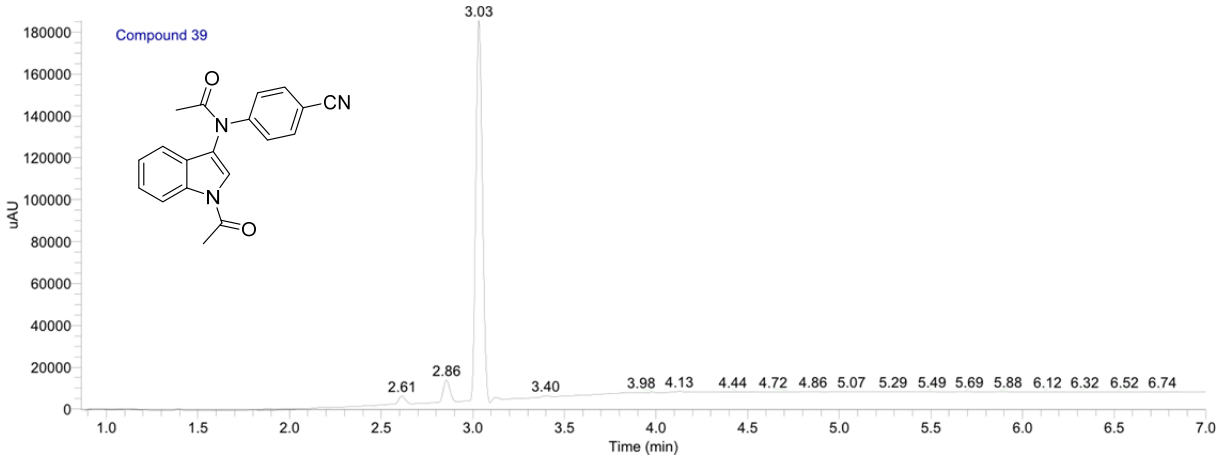
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.13	2.1	2.17	442.818	0.09	174.017	0.08
2.27	2.23	2.31	543.084	0.1	196.048	0.09
2.76	2.72	2.81	1856.827	0.36	755.568	0.36
2.96	2.89	3.01	7099.141	1.37	2361.127	1.14
3.16	3.11	3.21	475893.529	91.52	190025.433	91.52
3.24	3.22	3.28	14277.477	2.75	6737.97	3.25
3.37	3.33	3.4	3396.114	0.65	1455.253	0.7
3.57	3.53	3.6	1486.737	0.29	618.481	0.3
3.64	3.6	3.7	4526.911	0.87	1629.028	0.78
3.74	3.71	3.79	1303.747	0.25	526.741	0.25
3.91	3.85	3.96	5037.211	0.97	1712.702	0.82
4.16	4.11	4.22	2915.045	0.56	967.041	0.47
4.35	4.3	4.4	1196.633	0.23	472.508	0.23



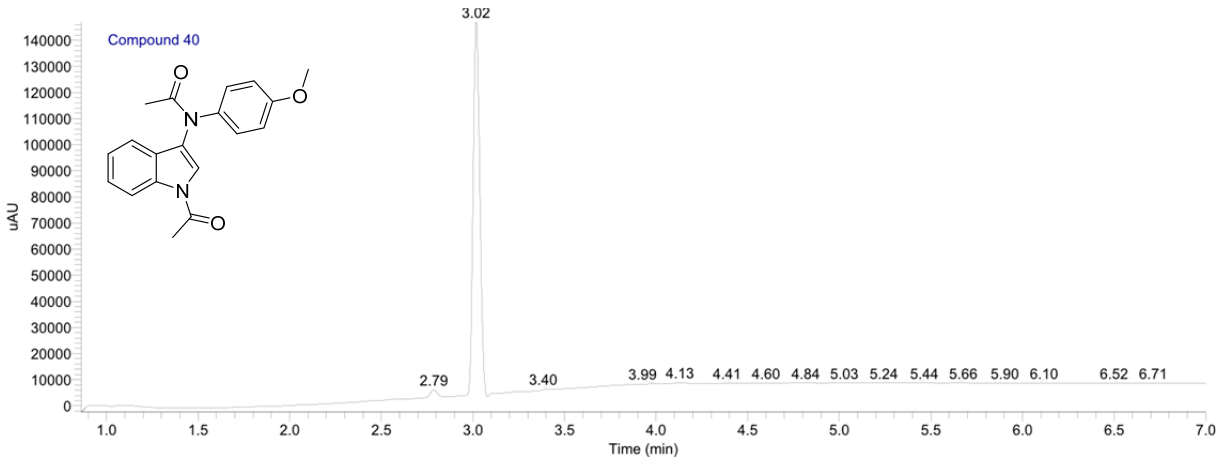
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.33	2.29	2.37	739.176	0.14	284.497	0.14
2.67	2.62	2.73	515666.487	99.26	200609.865	99.21
2.76	2.74	2.79	991.458	0.19	585.55	0.29
3.5	3.46	3.55	1031.187	0.2	357.317	0.18
3.86	3.82	3.92	1069.378	0.21	375.051	0.19



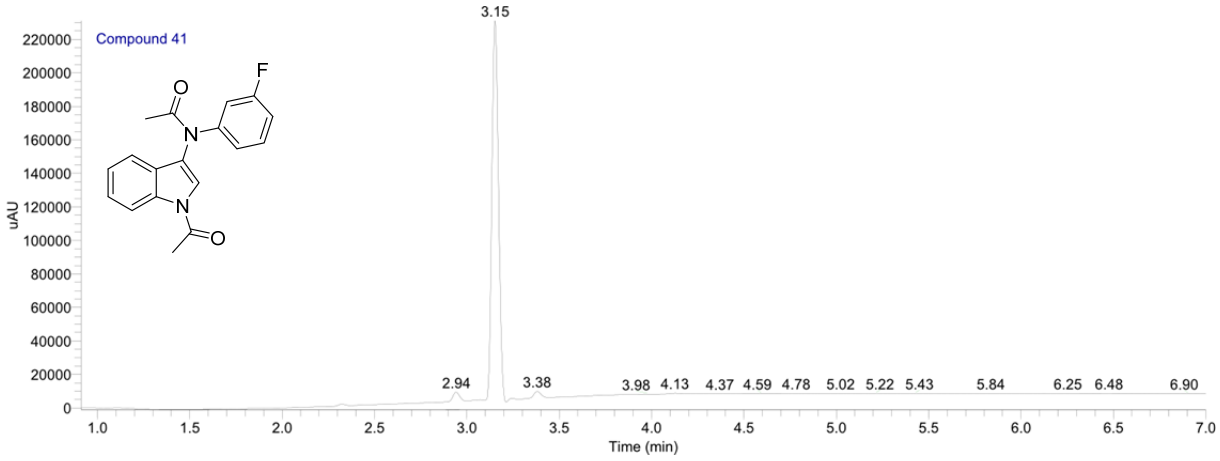
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.43	2.39	2.48	1319.905	0.17	527.585	0.17
2.67	2.63	2.72	2247.635	0.29	867.631	0.28
2.97	2.93	3.03	1883.183	0.24	731.893	0.23
3.2	3.16	3.27	2559.304	0.33	925.932	0.3
3.4	3.37	3.43	842.762	0.11	396.901	0.13
3.48	3.43	3.54	773136.231	98.45	308947.893	98.45
3.57	3.56	3.59	715.364	0.09	547.031	0.17
3.62	3.6	3.66	642.428	0.08	287.531	0.09
3.8	3.76	3.87	639.958	0.08	166.539	0.05
4.5	4.44	4.57	1290.322	0.16	399.024	0.13



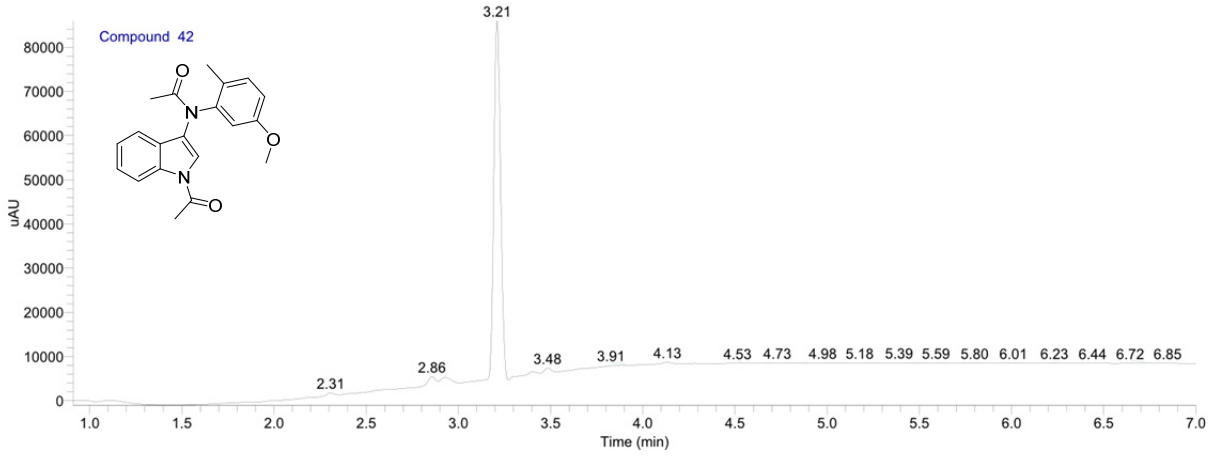
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.61	2.56	2.67	9967.94	2.02	3886.074	1.98
2.86	2.8	2.93	27547.37	5.59	10700.1	5.45
3.03	2.98	3.09	454960.2	92.38	181577.7	92.56



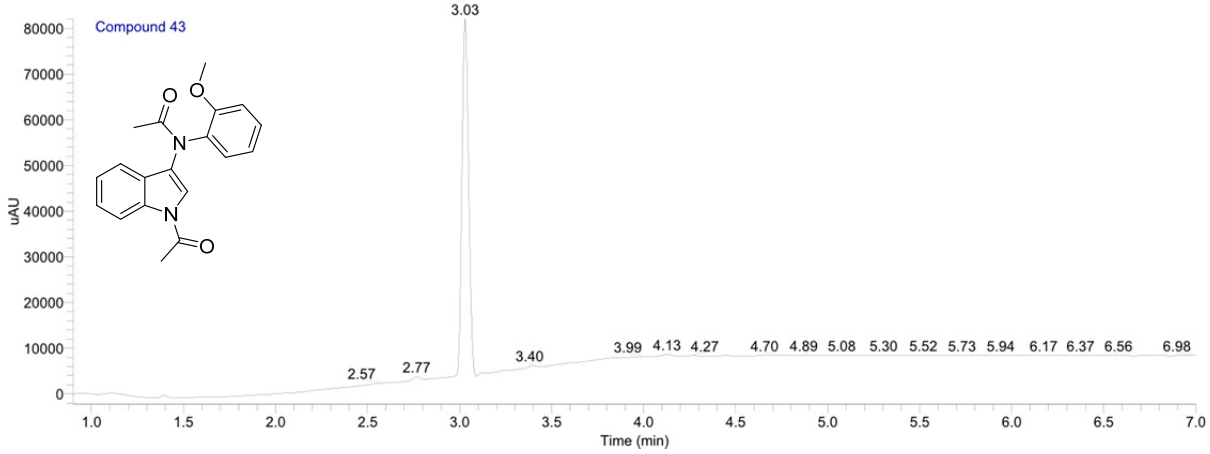
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.06	2.01	2.1	609.635	0.16	248.963	0.17
2.79	2.73	2.86	7703.717	2.08	2902.667	1.99
3.02	2.96	3.07	361464.5	97.75	142945.4	97.84



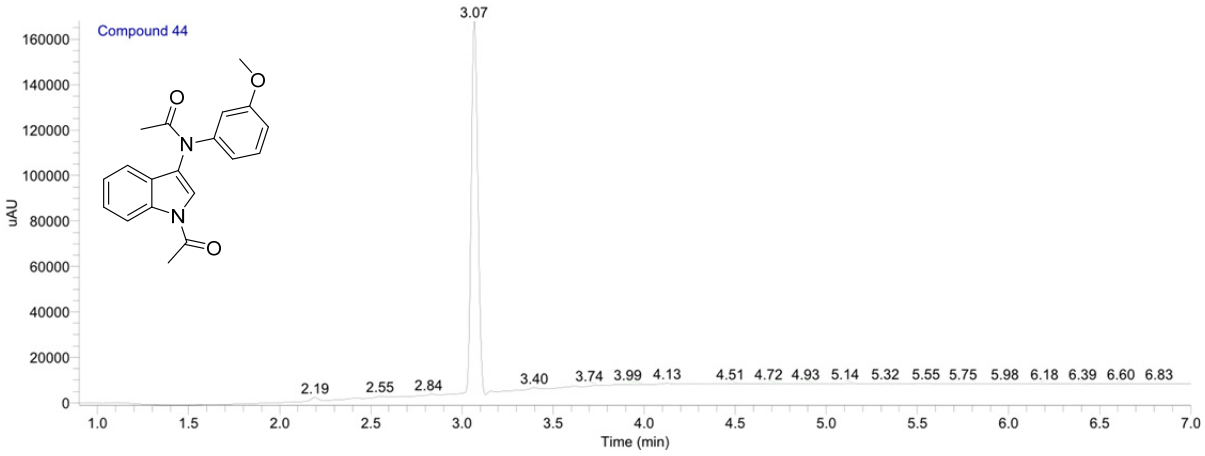
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.32	2.28	2.38	2787.125	0.47	1047.184	0.44
2.94	2.89	3	14463.35	2.45	5634.062	2.38
3.15	3.1	3.21	564786	95.67	226422.1	95.67
3.38	3.35	3.42	8329.927	1.41	3557.463	1.5



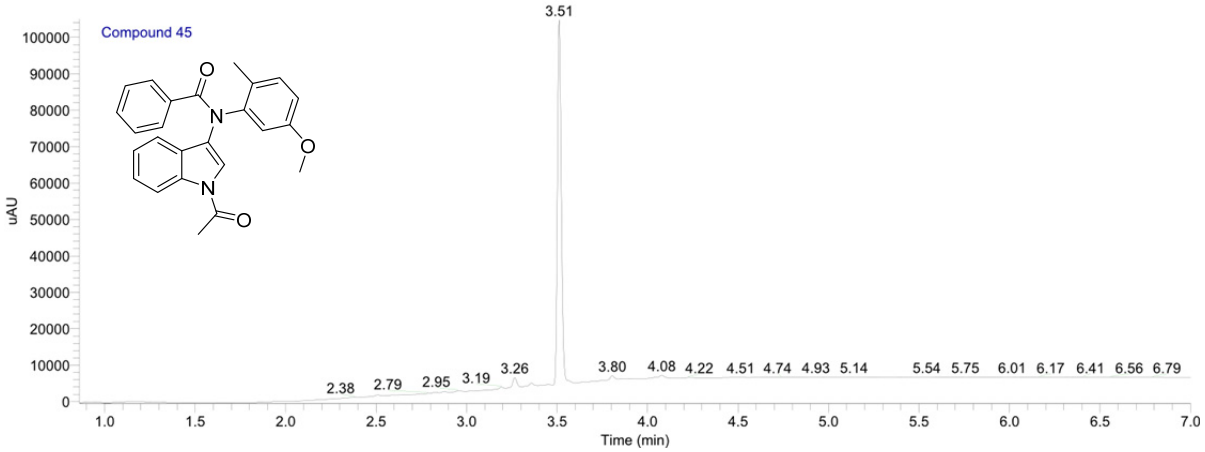
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.17	2.14	2.22	337.221	0.15	120.94	0.14
2.31	2.26	2.36	1713.898	0.78	636.303	0.75
2.86	2.79	3.01	12083.57	5.48	2055.576	2.42
3.21	3.16	3.26	203708.2	92.43	80918.66	95.43
3.48	3.45	3.54	2552.656	1.16	1066.647	1.26



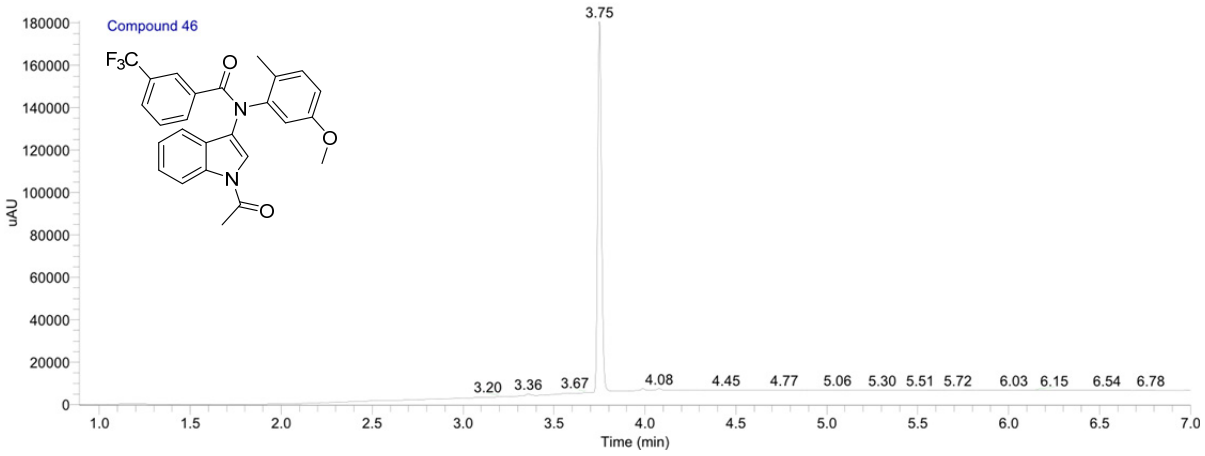
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
1.39	1.35	1.44	1451.394	0.71	568.176	0.71
2.76	2.72	2.81	1944.685	0.96	763.009	0.95
3.03	2.98	3.09	199637.3	98.33	78608.41	98.33



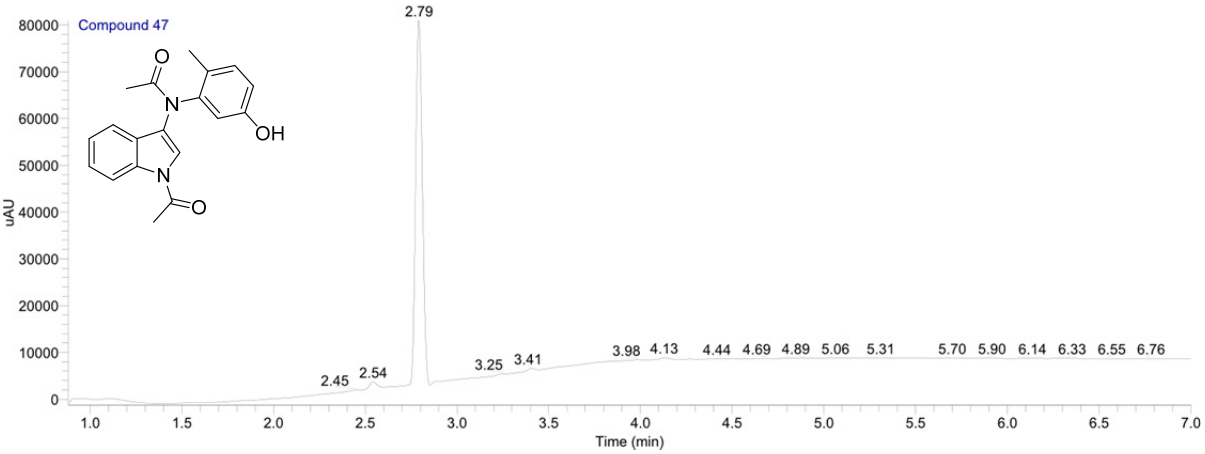
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.19	2.13	2.24	4709.689	1.12	1623.311	0.98
2.83	2.79	2.88	1046.48	0.25	371.877	0.22
3.07	3.01	3.12	412077	98.29	163525.8	98.46
3.61	3.58	3.65	615.347	0.15	242.017	0.15
3.73	3.7	3.77	816.102	0.19	326.066	0.2



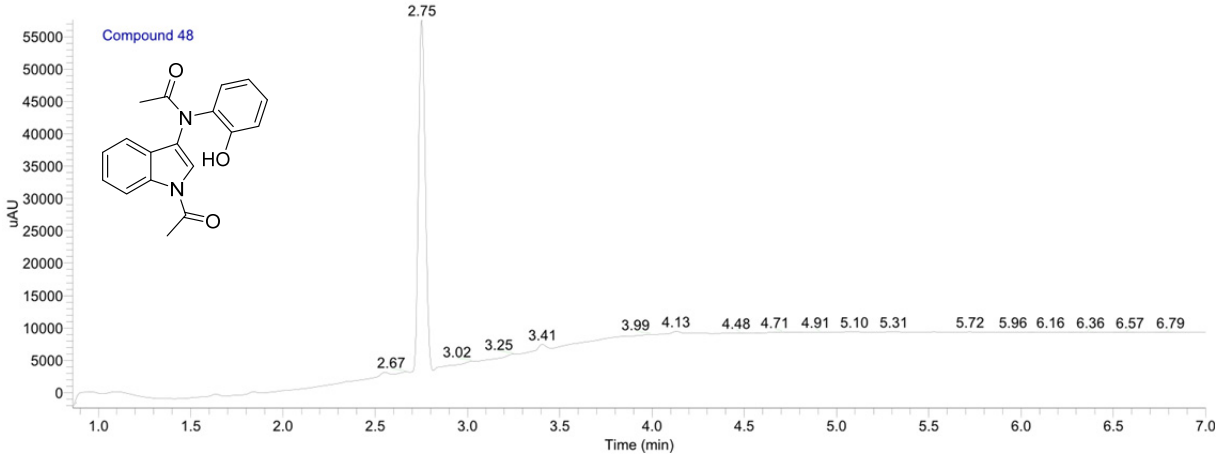
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
1.94	1.92	1.98	329.242	0.21	177.045	0.17
2.82	2.8	2.85	460.11	0.29	274.518	0.26
2.88	2.86	2.9	472.521	0.3	310.131	0.29
2.95	2.93	2.99	517.289	0.33	252.732	0.24
3.02	3	3.04	130.487	0.08	98.761	0.09
3.26	3.24	3.3	3986.27	2.53	2669.075	2.51
3.45	3.43	3.47	293.409	0.19	213.293	0.2
3.51	3.48	3.55	148263.38	94.12	99931.74	94.16
3.55	3.55	3.59	858.884	0.55	718.504	0.68
3.7	3.67	3.72	266.444	0.17	199.93	0.19
3.75	3.72	3.77	272.508	0.17	185.792	0.18
3.8	3.78	3.84	1679.586	1.07	1100.357	1.04



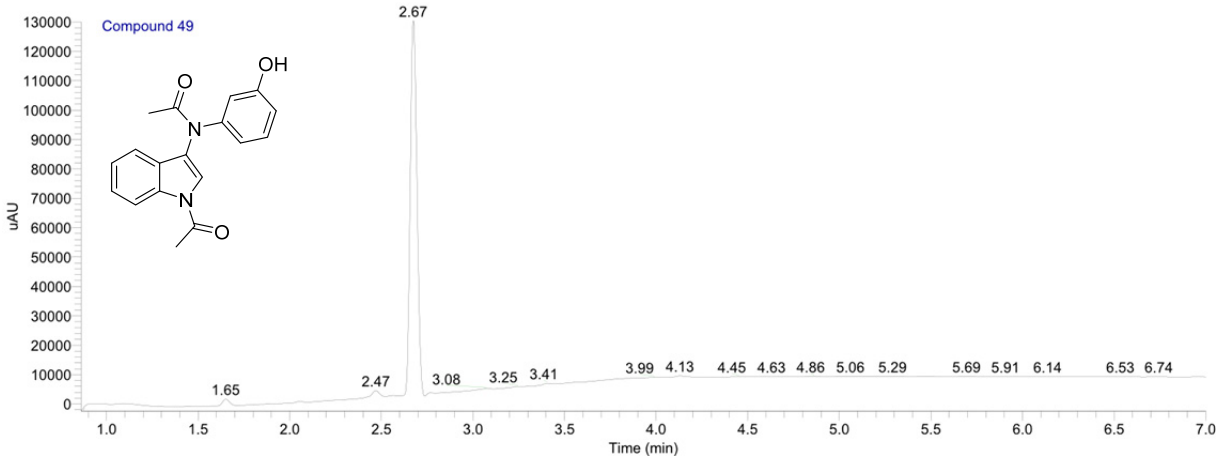
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.22	2.21	2.23	75.562	0.03	125.787	0.07
3.55	3.53	3.57	202.77	0.08	159.308	0.09
3.67	3.65	3.69	259.149	0.1	216.762	0.12
3.75	3.71	3.81	254472.851	99.28	174778.733	99.23
3.99	3.96	4.02	1305.743	0.51	861.574	0.49



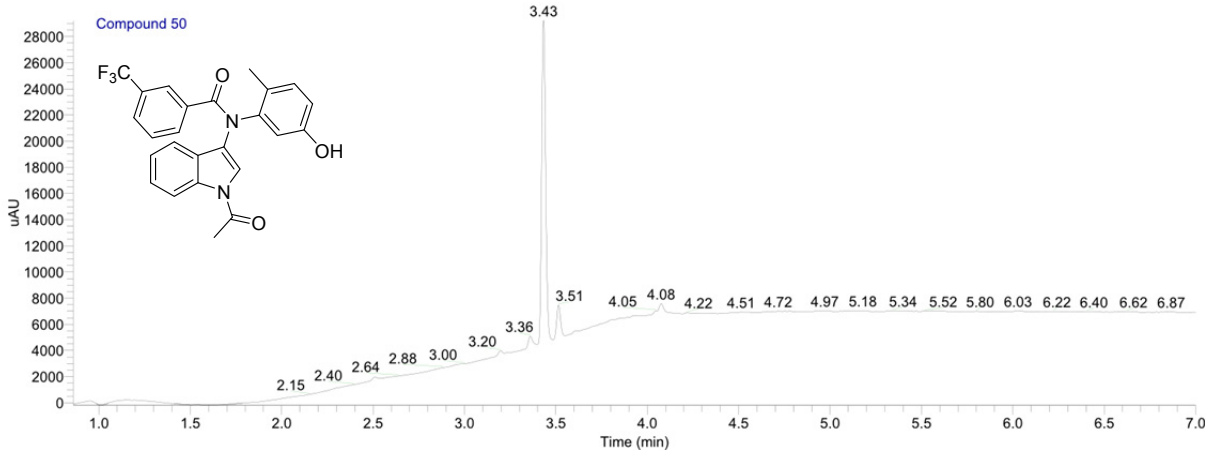
Apex RT	Start RT	End RT	Area	%Area	Height	%Height
2.54	2.5	2.6	4034.618	1.99	1467.067	1.85
2.79	2.74	2.85	198245.2	98.01	77695.04	98.15



Apex RT	Start RT	End RT	Area	%Area	Height	%Height
1.63	1.6	1.68	925.711	0.66	354.073	0.64
1.84	1.79	1.89	896.445	0.64	319.385	0.58
2.75	2.7	2.81	138527.539	98.3	54235.941	98.41
3.01	2.97	3.06	568.002	0.4	200.808	0.36



Apex RT	Start RT	End RT	Area	%Area	Height	%Height
1.65	1.6	1.71	5950.248	1.75	2258.58	1.7
2.05	2.02	2.1	928.978	0.27	390.907	0.29
2.47	2.39	2.52	6697.696	1.97	2291.604	1.73
2.67	2.62	2.73	325097.984	95.54	127282.075	95.99
3.07	2.99	3.12	1582.504	0.47	375.335	0.28



Apex RT	Start RT	End RT	Area	%Area	Height	%Height
3.43	3.4	3.48	36896.297	90.23	24638.591	90.13
3.51	3.49	3.55	3733.925	9.13	2540.89	9.3
3.61	3.59	3.63	260.667	0.64	155.749	0.57

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