< Supporting Information >

Pyrazolo[1,5-*a*]pyridine-Fused Pyrimidine-Based Fluorophore and Its Bioapplication to Probing Lipid Droplets

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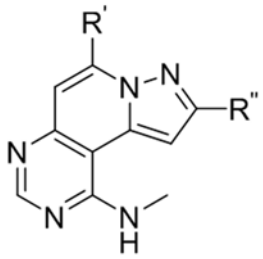
I. General Information

All commercially available reagents and solvents were used without further purification unless noted otherwise. All the solvents were purchased from commercial venders. ^{1}H and ^{13}C NMR spectra were obtained using Agilent 400–MR DD2 [Agilent, USA] or Varian Inova–500 [Varian Assoc., Palo Alto, USA] instruments. Chemical shifts were reported in ppm from tetramethylsilane (TMS) as internal standard or the residual solvent peak ($CDCl_3$; ^{1}H: δ = 7.26 ppm; ^{13}C: δ = 77.23 ppm). Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), dt (doublet of triplet), td (triplet of doublet), brs (broad singlet), and so on. Coupling constants are reported in hertz. Mass spectrometric analysis was performed using a Finnigan Surveyor MSQ Plus LC/MS [Thermo] or 6120 Quadrupole LC/MS [Agilent Technologies] with electrospray ionization (ESI). High resolution mass spectrometric analyses were conducted by Ultra High Resolution ESI Q-TOF mass spectrometer [Bruker]. The conversion of starting materials was monitored by thin–layer chromatography (TLC) using pre–coated glass–backed plates (silica gel 60; F_{254}=0.25 mm), and the reaction components were visualized by observation under UV light (254 and 365 nm) or by treatment of TLC plates with visualizing agents such as $KMnO_4$, phosphomolybdic acid, and ninhydrin followed by heating. Products were purified by flash column chromatography on silica gel (230–400 mesh) using a mixture of EtOAc/hexane or MeOH/CH_2Cl_2 as eluents. Absorption spectra and molar absorption coefficient at the absorption maxima of fluorescence compounds were measured by UV-VIS spectrophotometer UV-1650PC [Shimadzu, Japan]. Emission spectra was measured by Cary Eclipse Fluorescence spectrophotometer [Varian Associates] and absolute quantum yield was measured by QE-2000 [Otsuka Electronics]. All quantum mechanical calculations were performed in Gaussin09W. The ground state structures of fluoremidine compounds were optimized using density functional theory (DFT) at the B3LYP/6-31G* level. The energy of HOMO and LUMO values were calculated through time dependent density functional theory (TD-DFT) with the optimized structures of the ground state compare with experimental emission properties. Cell culture reagents including fetal bovine serum, calf serum, culture media, and antibiotic-antimycotic solution were purchased from GIBCO. Lysotracker Red DND-99 and MitoTracker Red CMXRos were purchased from Molecular Probes. The culture dish was purchased from CORNING. Insulin was purchased from Sigma Aldrich. Glass bottom chamber slide was purchased from Thermo scientific. All

antibodies for immunofluorescence imaging were purchased from Abcam. Fluorescence microscopy studies were carried with DeltaVision Elite imaging system [GE Healthcare] equipped with a sCMOS camera. Objective lenses are supported by Olympus IX-71 [Olympus] inverted microscope equipped with Plan APO 60×/Oil, 1.42 NA, WD 0.15 mm or Super-Plan APO 100×/Oil, 1.4 NA, WD 0.13 mm. DeltaVision Elite uses a solid state illumination system, InSightSSI fluorescence illumination module. Four-color standard filter set [GE Healthcare, 52-852113-003] and Seven-color combined filter set [GE Healthcare, 52-852113-024] were used to detect fluorescence signals.

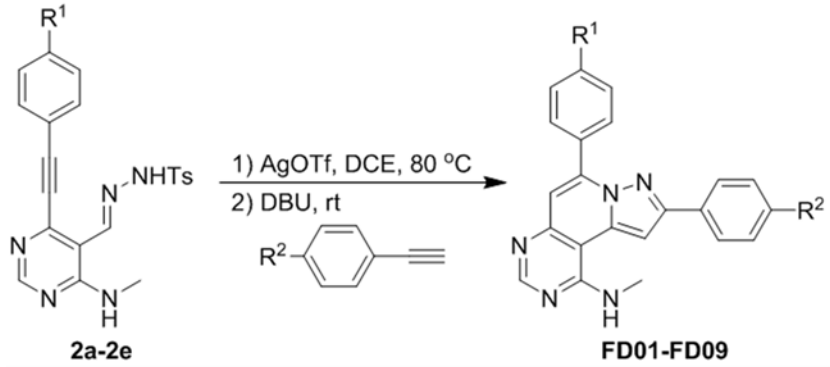
II. Supporting Figures and Tables



cpd	R`	R``	λ_{abs} (nm)[a]	λ_{em} (nm)[b]	ε
A01	*n*-propyl	cyclopropyl	325	386	4,000
A02	*n*-propyl	phenyl	324	388	8,000
A03	phenyl	phenyl	330	424	13,000

All experiment data obtained in dichloromethane (DCM). [a] Only the longest absorption maxima are shown. [b] Excited at the maximum excitation wavelength.

Table S1. Photophysical properties of A01–A03.



cpd	R^1	R^2	yield(%)[a]
FD01	H	H	70
FD02	CN	H	51
FD03	CF_3	H	77
FD04	OCH_3	H	72
FD05	$N(CH_3)_2$	H	77
FD06	H	CN	40
FD07	H	CF_3	55
FD08	H	OCH_3	53
FD09	H	$N(CH_3)_2$	64

[a] Isolated yield

Table S2. Synthetic yield results of key transformation for **FD01–FD09**.

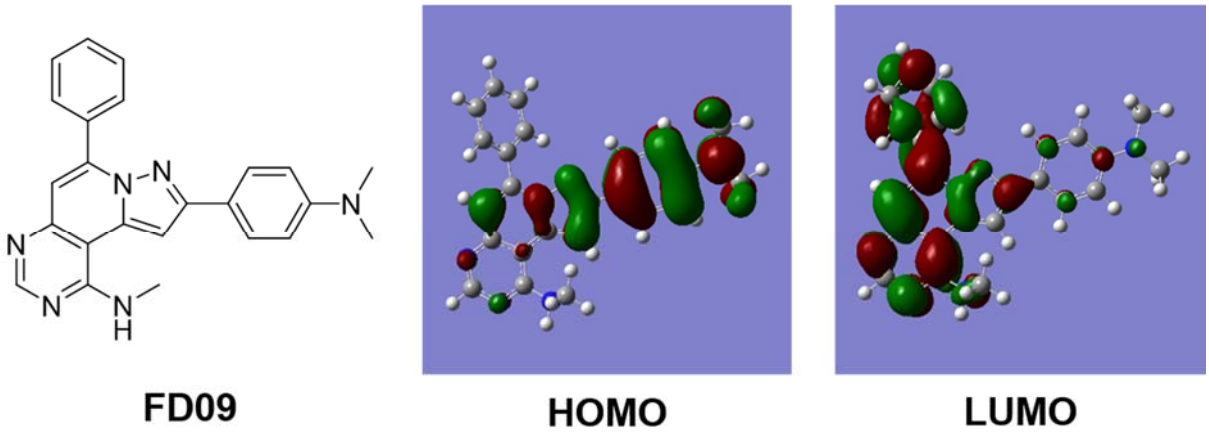


Figure S1. Chemical structure and electron density distribution of the HOMO and the LUMO for *N*,*N*-dimethylamino group at the R^2 position (**FD09**), calculated through DFT at the B3LYP/6-31G*level.

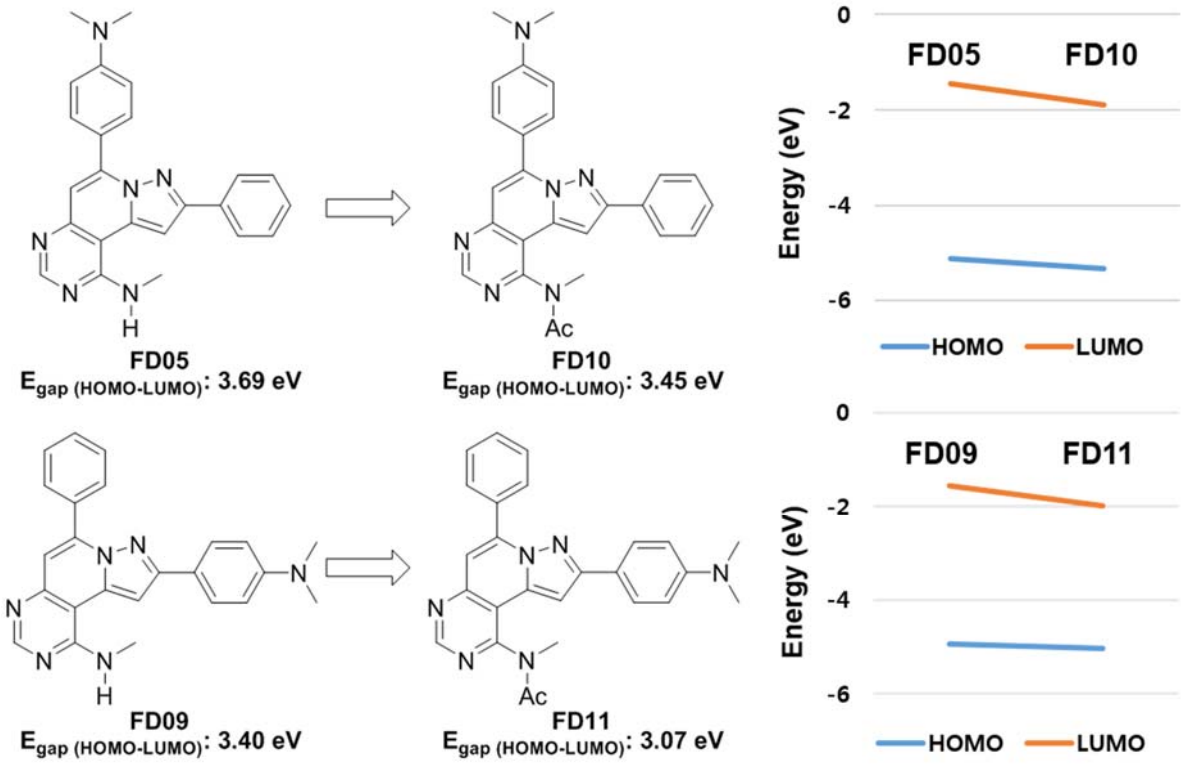


Figure S2. Chemical structures and energy levels of the HOMO and the LUMO of **FD05**, **FD09**, **FD10**, and **FD11**.

Cpd	R^1	R^2	R^3	Ether (nm)	DCM (nm)	DMF (nm)	MeOH (nm)
FD01	H	H	H	420	424	434	434
FD02	CN	H	H	452	456	476	472
FD03	CF_3	H	H	432	438	458	454
FD04	OCH_3	H	H	410	420	428	432
FD05	$N(CH_3)_2$	H	H	430	450	468	484
FD06	H	CN	H	416	422	432	430
FD07	H	CF_3	H	416	420	434	430
FD08	H	OCH_3	H	420	424	438	436
FD09	H	$N(CH_3)_2$	H	481	532	598	N.D.
FD10	$N(CH_3)_2$	H	Ac	474	504	542	N.D.
FD11	H	$N(CH_3)_2$	Ac	560	604	N.D.	N.D.
FD12	$N(CH_3)_2$	$N(CH_3)_2$	H	432	492	554	N.D.
FD13	$N(CH_3)_2$	$N(CH_3)_2$	Ac	528	582	N.D.	N.D.

Table S3. Emission wavelengths of **FD01–FD13** upon polarity changes at the different organic solvents.

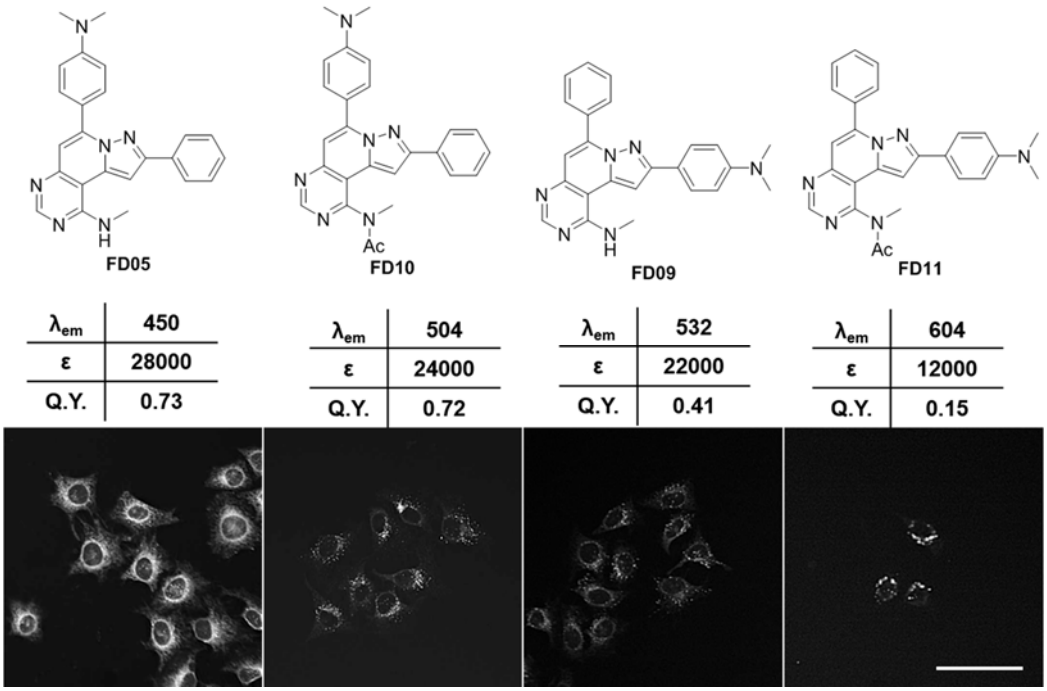


Figure S3. Live cell imaging after staining of A549 cells with probes. 20 μM of each probe was treated to A549 cells. After 1 h, cells were washed with PBS. Fluorescence images were taken by InCell Analyzer 2000 [GE Healthcare] equipped with 20× lens. Fluorescence signals of each probe were obtained using the following filter sets; **FD05** (DAPI/DAPI), **FD09** (DAPI/DAPI), **FD10** (DAPI/FITC), **FD11** (DAPI/FITC). Scale bars, 100 μm.

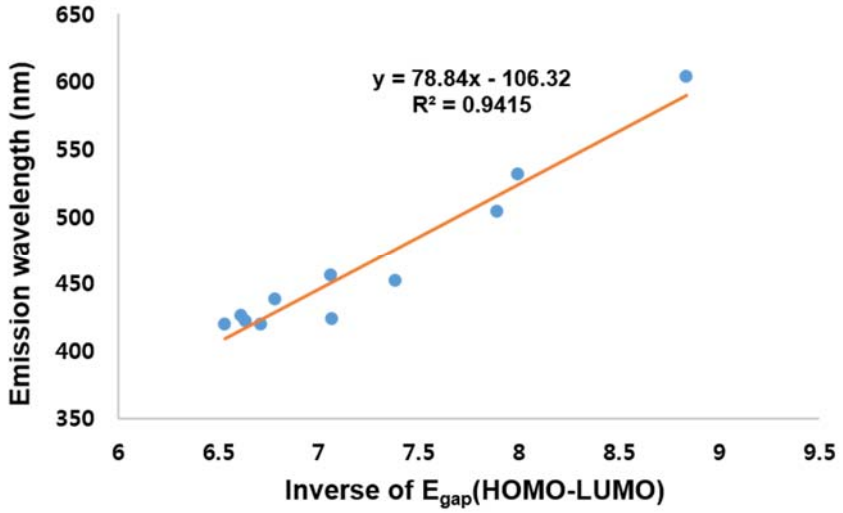
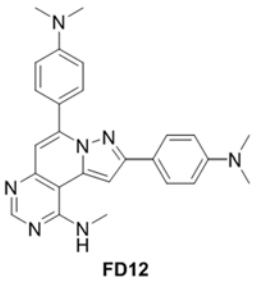


Figure S4. Correlation between the calculated HOMO-LUMO energy gap and the measured emission wavelength of **FD01–FD11**.

☞ Prediction of λ_{em} of for **FD12** and **FD13**

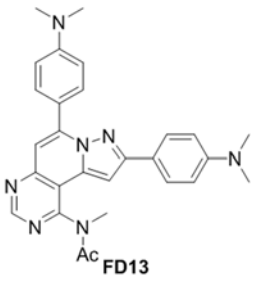
The estimation of emission wavelength (λ_{em}) = 78.84x – 106.32 (x: calculated 1/eV)

cpd	x (calculated 1/eV)	Estimated λ_{em}
FD12	7.6834422	499 nm
FD13	8.47745	562 nm



solvent	Abs (nm)[a]	Em (nm)[b]	ε	Q. Y.[c]
Ether	337	432	33000	0.45
DCM	352	492	40000	0.41
DMF	348	554	33000	0.23
MeOH	360	N.D.	31000	0.05

[a]Only the longest absorption maxima are shown. [b]Excited at the maximum excitation wavelength. [c]Absolute quantum yield.



solvent	Abs (nm)[a]	Em (nm)[b]	ε	Q. Y.[c]
Ether	392	528	35000	0.43
DCM	404	582	31000	0.38
DMF	400	N.D.	24000	0.06
MeOH	400	N.D.	24000	< 0.01

[a]Only the longest absorption maxima are shown. [b]Excited at the maximum excitation wavelength. [c]Absolute quantum yield.

Table S4. Photophysical properties of **FD12** and **FD13**

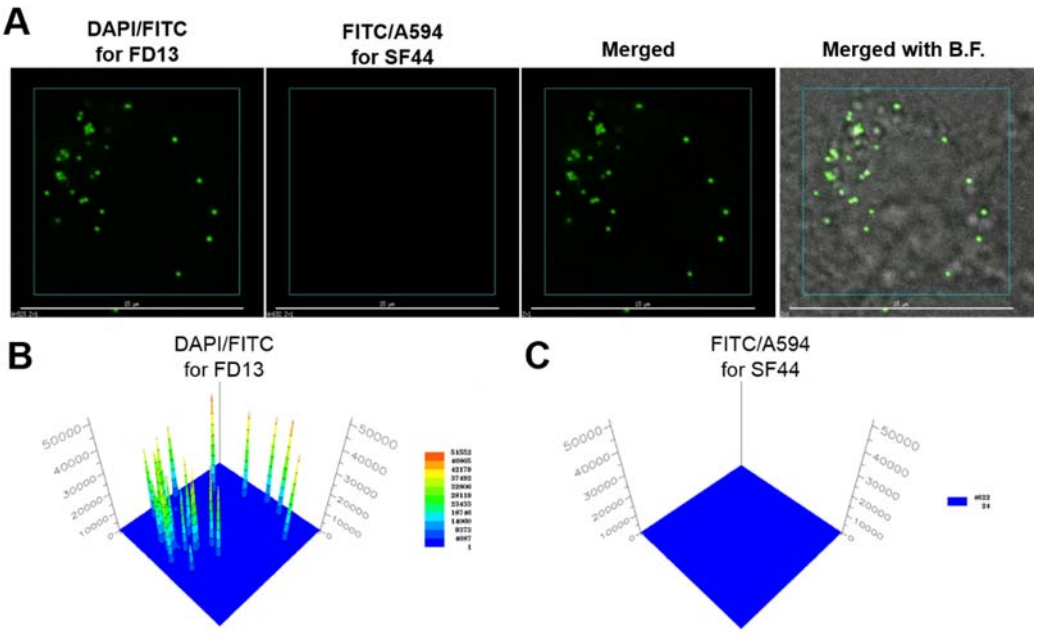


Figure S5. Single treatment of FD13 in Hela cells for fluorescence imaging. A) 20 μM of **FD13** is treated to Hela cells for 1 h in 5% CO_2 incubator. After 1 h, fluorescent images are taken without washing of probe within media. Fluorescence images are taken by DeltaVision imaging system equipped with 60× lens. Scale bars, 15 μm. B) Calculated DAPI/FITC fluorescence intensity (for **FD13**) within selected area (blue square) of cells. The color scale indicates the fluorescence intensity, from low (blue) to high (red). C) Calculated FITC/A594 fluorescence intensity (for **SF44**) within selected area (blue square) of cells. The color scale indicates the fluorescence intensity, from low (blue) to high (red).

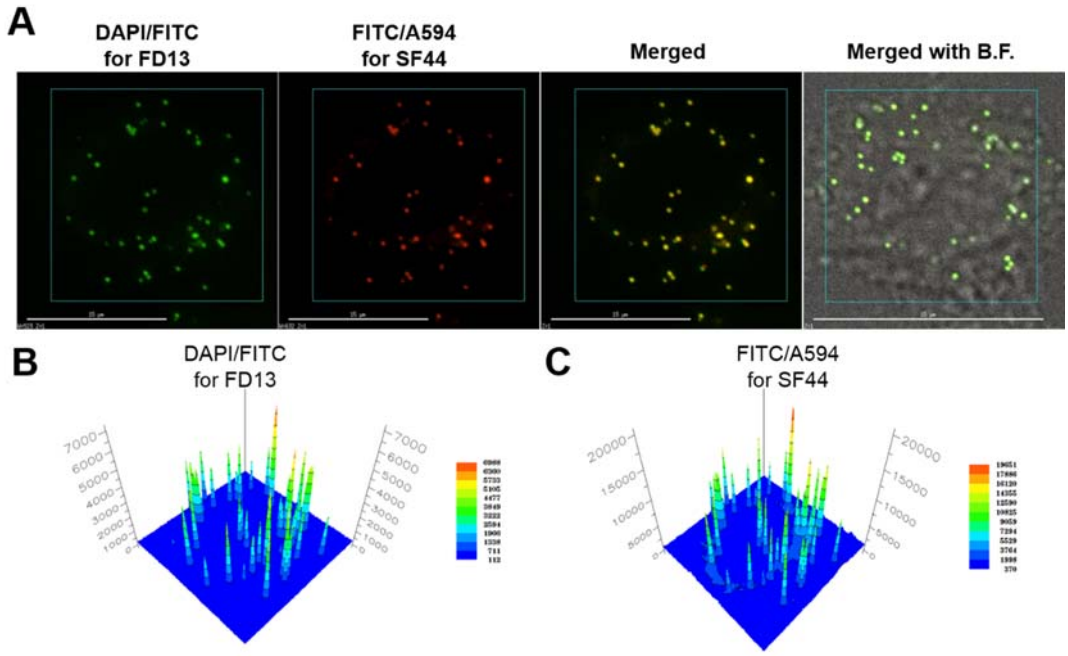


Figure S6. Dual treatment of FD13 and SF44 for fluorescence imaging. A) 20 μM of **FD13** is treated to Hela cells for 1 h in 5% CO_2 incubator. After 1 h, add 20 μM of SF44 and incubate for 30 min. Fluorescent images are taken without washing of probes within media. Fluorescence images are taken by DeltaVision imaging system equipped with 60× lens. Scale bars, 15 μm. B) Calculated DAPI/FITC fluorescence intensity (for **FD13**) within selected area (blue square) of cells. The color scale indicates the fluorescence intensity, from low (blue) to high (red). C) Calculated FITC/A594 fluorescence intensity (for **SF44**) within selected area (blue square) of cells. The color scale indicates the fluorescence intensity, from low (blue) to high (red).

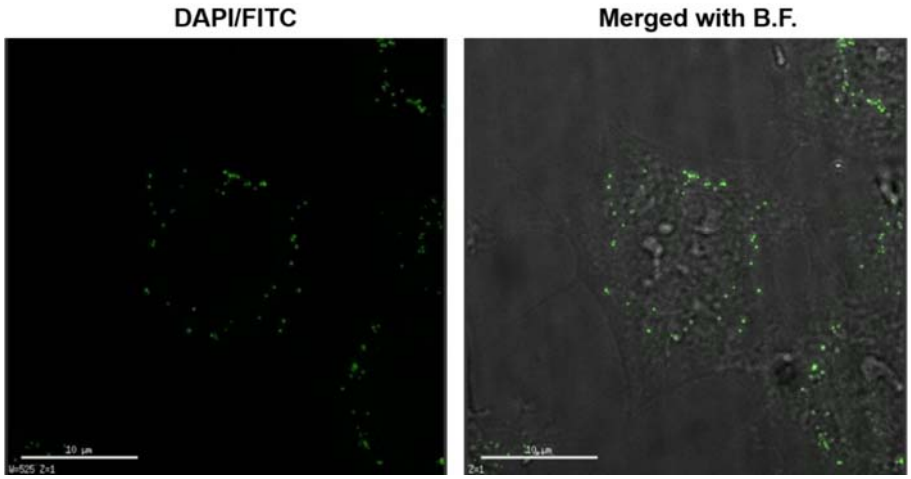


Figure S7. Hela cells are fixed with 3.7% para-formaldehyde for 15 min at r.t. After the fixation of cells, 20 μM of **FD13** is treated for 1 h at r.t. After washing with PBS buffer for three times, fluorescence images are taken by DeltaVision imaging system equipped with 60× lens. Scale bars, 10 μm.

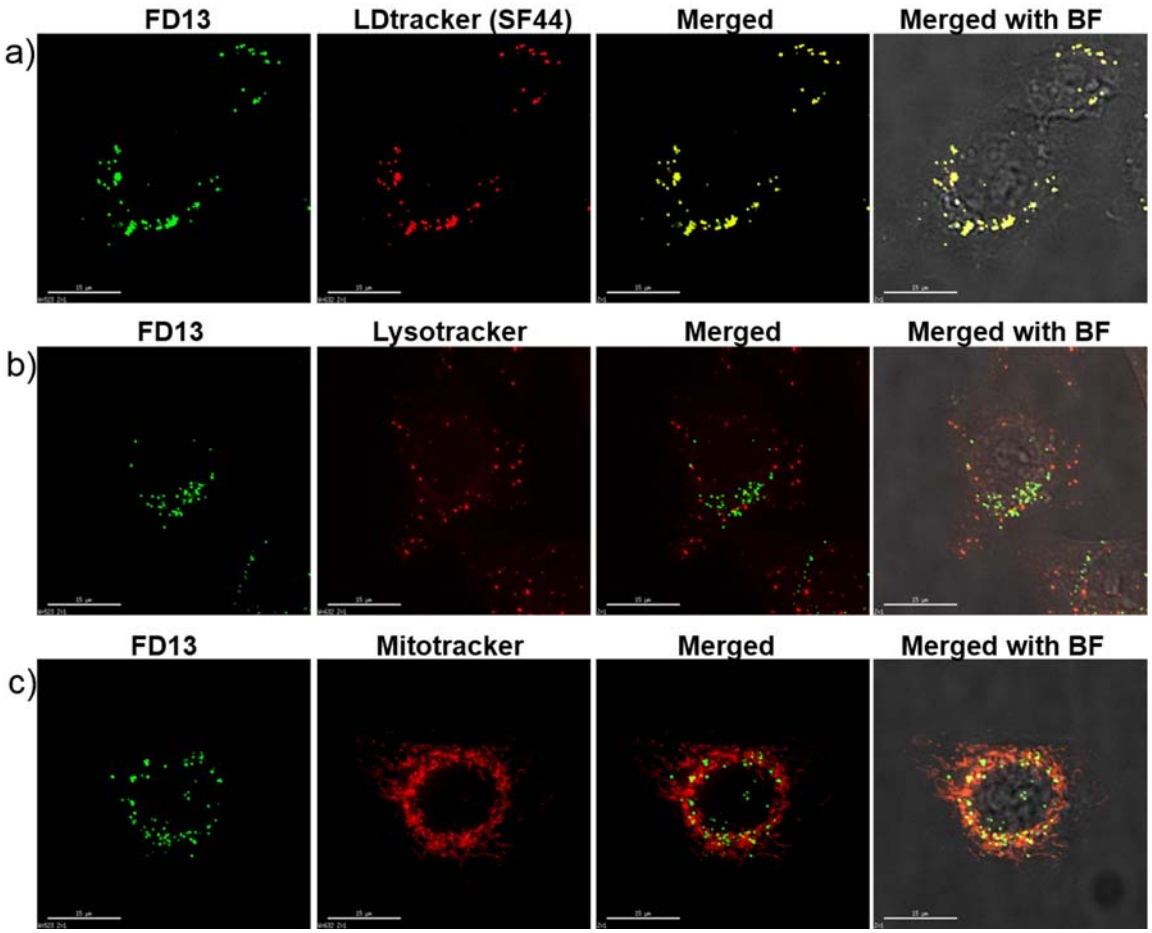
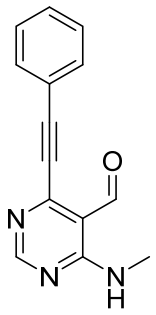


Figure S8. Colocalization of **FD13** and lipid droplet tracker (LD tracker, SF44). A549 cells were treated with 20 μM **FD13** for 1 h. Cellular lipid droplet, lysosome, and mitochondria were stained with SF44 (20 μM), Lysotracker Red (50 nM), and Mitotracker Red (20 nM), respectively, for 30 min. After washing, live cell images were obtained using DeltaVision imaging system equipped with a 60× lens. Merged images show specific colocalization of **FD13** and LD tracker (yellow). Scale bars, 15 μm. Images of a) **FD13** and LD tracker; b) FD13 and Lysotracker; and c) **FD13** and Mitotracker.

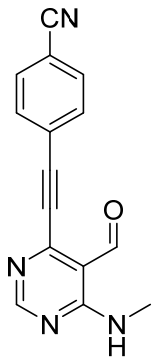
III. General Experimental Procedures and Spectroscopic Data

1. General synthetic procedure for compounds 1a–1e

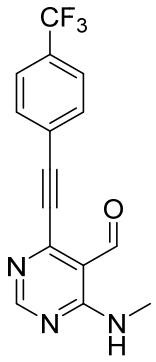
To the anhydrous DMF solution (60 mL) of 4-chloro-6-(methylamino)pyrimidine-5-carbaldehyde (1.0 g), $Pd(PPh_3)Cl_2$ (5 mol%), and CuI (20 mol%), terminal alkynes (2.0 equiv.), and triethylamine (1.6 mL, 2.0 equiv.) were added under argon atmosphere. After being stirred at room temperature for 4 h, the reaction mixture was quenched with deionized water (200 mL). The resultant was extracted with EtOAc (100 mL × 3) and combined organic layer was washed with brine (100 mL). After drying with anhydrous Na_2SO_4(s), the solvent was removed under the reduced pressure. The residue was purified by silica-gel flash column chromatography to obtain **1a–1e**.



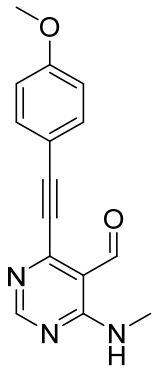
Compound 1a: Yield: 98%; pale-yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.55 (s, 1H), 8.91 (brs, 1H), 8.67 (s, 1H), 7.65–7.62 (m, 2H), 7.47–7.38 (m, 3H), 3.14 (d, J = 5.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.5, 161.5, 160.4, 155.0, 132.4, 130.3, 128.6, 120.6, 98.3, 83.8, 27.4; IR (neat) ν_{max}: 3337, 3076, 2934, 2859, 2214, 1764, 1652, 1582, 1538, 1400, 969, 878; LRMS (ESI) *m/z* calcd for $C_{14}H_{11}N_3O$ $[M+H]^+$: 238.09; Found: 237.99; Registration No.: 1477472-98-2.



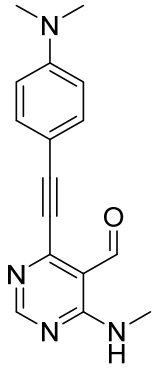
Compound 1b: Yield: 79%; brown solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.52 (s, 1H), 8.91 (brs, 1H), 8.70 (s, 1H), 7.71–7.73 (m, 4H), 3.17 (d, J = 5.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.0, 161.6, 160.4, 154.2, 132.8, 132.3, 125.4, 117.9, 113.7, 112.1, 95.1, 87.1, 27.5; IR (neat) ν_{max}: 3331, 2925, 2848, 2229, 1660, 1603, 1546, 1443, 1260, 1099, 966, 839; LRMS (ESI) *m/z* calcd for $C_{15}H_{10}N_4O$ $[M+H]^+$: 263.09; Found: 263.2.



Compound 1c: Yield: 91%; brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (s, 1H), 8.89 (brs, 1H), 8.67 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 3.14 (d, *J* = 4.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.2, 161.6, 160.4, 154.4, 132.6, 125.7, 125.61, 125.58, 124.4, 112.0, 95.9, 85.5, 27.5; IR (neat) ν_{max}: 3334, 3052, 2943, 2857, 2218, 1657, 1608, 1543, 1394, 1327, 1173, 1108, 967, 841; LRMS (ESI) *m/z* calcd for $C_{15}H_{10}F_3N_3O$ $[M+H]^+$: 306.08; Found: 306.1.



Compound 1d: Yield: 75%; brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.53 (s, 1H), 8.90 (d, *J* = 4.0 Hz, 1H), 8.64 (s, 1H), 7.57 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 3.85 (s, 3H), 3.13 (d, *J* = 5.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.7, 161.5, 161.3, 160.4, 155.3, 134.3, 114.3, 112.6, 111.5, 99.2, 83.3, 55.4, 27.4; IR (neat) ν_{max}: 3334, 2924, 2856, 2207, 1663, 1539, 1396, 1255, 1173, 1027, 967, 832; LRMS (ESI) *m/z* calcd for $C_{15}H_{13}N_3O_2$ $[M+H]^+$: 268.10; Found: 268.2.

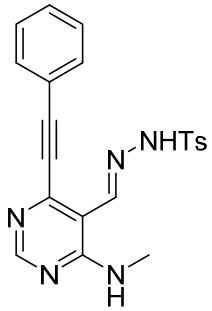


Compound 1e: Yield: 96%; reddish brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.55 (s, 1H), 8.90 (brs, 1H), 8.62 (s, 1H), 7.50 (d, *J* = 8.8 Hz, 2H), 6.66 (d, *J* = 8.8 Hz, 2H), 3.12 (d, *J* = 5.2 Hz, 3H), 3.04 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.9, 161.4, 160.5, 155.7, 151.3, 134.1, 111.5, 111.0, 106.5, 102.0, 83.5, 40.0, 27.4; IR (neat) ν_{max}: 3329, 2955, 2927, 2912, 2853, 2197, 1658, 1582, 1526, 1396, 1371, 1184, 967, 814; LRMS (ESI) *m/z* calcd for $C_{16}H_{16}N_4O$ $[M+H]^+$: 281.13; Found: 281.2.

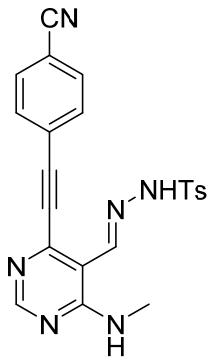
2. General synthetic procedure for compounds 2a–2e

To a methanol solution of **1a–1e**, tosylhydrazide (2.0 equiv.), and AcOH were added. After stirring at 40 °C until starting materials were consumed, the reaction mixture was quenched with deionized water. The resultant was extracted with dichloromethane (DCM) twice and

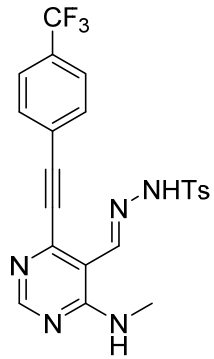
dried with anhydrous Na_2SO_4(s). After the solvent was removed under the reduced pressure, the residue was purified by silica-gel flash column chromatography to obtain **2a**–**2e**.



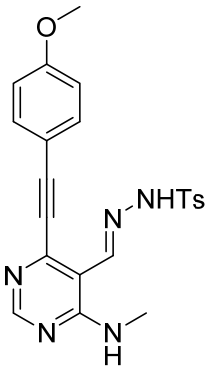
Compound 2a: Yield: 80%; yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.38 (s, 2H), 7.83 (d, J = 6.8 Hz, 2H), 7.30 (d, J = 7.6 Hz, 2H), 7.20 (t, J = 6.8 Hz, 1H), 7.12–7.06 (m, 4H), 3.04 (d, J = 5.2 Hz, 3H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.8, 157.5, 147.9, 145.5, 144.6, 135.1, 131.7, 129.8, 129.7, 128.1, 127.8, 120.5, 110.2, 97.7, 84.2, 27.7, 21.6; IR (neat) ν_{max}: 3295, 3181, 3057, 2864, 2752, 2214, 1608, 1544, 1267, 1074, 971; LRMS (ESI) *m/z* calcd for $C_{21}H_{19}N_5O_2S$ $[M+H]^+$: 406.13; Found: 405.93; Registration No.: 1477473-18-9.



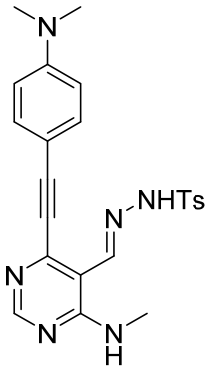
Compound 2b: Yield: 45%; yellowish brown solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.52 (s, 1H), 8.49 (brs, 1H), 8.46 (s, 1H), 7.99 (d, J = 6.0 Hz, 2H), 7.86 (d, J = 6.8 Hz, 2H), 7.78 (d, J = 6.8 Hz, 2H), 7.46 (d, J = 6.0 Hz, 2H), 3.03 (d, J = 4.0 Hz, 3H), 2.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.8, 158.1, 147.7, 145.4, 145.2, 134.6, 132.5, 132.1, 130.1, 127.8, 125.6, 118.0, 113.1, 110.5, 94.5, 88.0, 27.9, 21.7; IR (neat) ν_{max}: 3230, 3062, 2230, 1608. 1581, 1545, 1440, 1168, 1075, 970; LRMS (ESI) *m/z* calcd for $C_{22}H_{18}N_6O_2S$ $[M+H]^+$: 431.12; Found: 431.2.



Compound 2c: Yield: 39%; yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.35 (s, 1H), 8.27 (d, J = 4.8 Hz, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.30–7.21 (m, 6H), 2.97 (d, J = 5.2 Hz, 3H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 157.5, 147.2, 145.0, 144.9, 134.9, 132.0, 131.3, 131.0, 129.9, 127.8, 125.0, 124.1, 122.1, 95.6, 86.0, 27.6, 21.5; IR (neat) ν_{max}: 3219, 2923, 2218, 1609, 1545, 1324, 1165, 1130, 1086, 1066, 969; LRMS (ESI) *m/z* calcd for $C_{22}H_{18}F_3N_5O_2S$ $[M+H]^+$: 474.11; Found: 474.2.



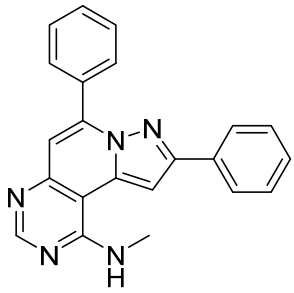
Compound 2d: Yield: 76%; pale yellow solid; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.44 (s, 1H), 8.36 (brs, 1H), 8.34 (s, 1H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 8.8 Hz, 2H), 6.61 (d, *J* = 8.8 Hz, 2H), 3.77 (s, 3H), 3.03 (d, *J* = 4.8 Hz, 3H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 161.2, 159.2, 157.7, 148.5, 146.3, 145.2, 135.6, 133.7, 130.2, 128.1, 114.2, 112.8, 110.2, 98.6, 83.9, 55.7, 27.9, 21.7; IR (neat) ν_{max}: 2966, 2207, 1604, 1581, 1544, 1511, 1304, 1254, 1168, 1075, 970; LRMS (ESI) *m/z* calcd for $C_{22}H_{21}N_5O_3S$ $[M+H]^+$: 436.14; Found: 436.2.



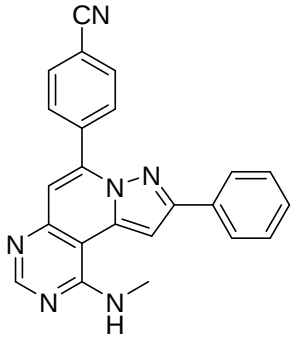
Compound 2e: Yield: 56%; yellow solid; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 11.91 (brs, 1H), 8.54 (s, 1H), 8.40–8.39 (m, 2H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.49–7.45 (m, 4H), 6.74 (d, *J* = 9.2 Hz, 2H), 3.01 (d, *J* = 4.8 Hz, 3H), 2.98 (s, 6H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 158.3, 157.6, 151.1, 148.5, 144.8, 144.1, 135.3, 133.5, 130.0, 127.2, 111.7, 108.9, 105.9, 99.3, 84.0, 48.6, 27.4, 21.1; IR (neat) ν_{max}: 3301, 3039, 2193, 1605, 1534, 1447, 1368, 1339, 1165, 1072, 969; LRMS (ESI) *m/z* calcd for $C_{23}H_{24}N_6O_2S$ $[M+H]^+$: 449.17; Found: 449.1.

3. General procedure for the preparation of FD01–FD09

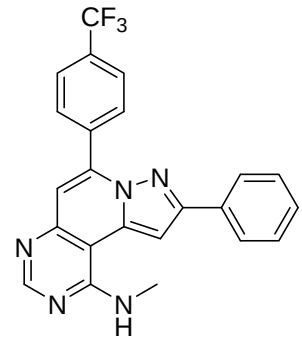
To the DCE solution of **2a–2e**, AgOTf (20 mol%) was added. After stirring at 80 ºC for 2 h, the reaction mixture was cooled to room temperature. After the addition of DBU (3.0 equiv.) and terminal alkyne (3.0 equiv.), the reaction mixture was stirred at room temperature for 16 h. The resultant was quenched with water and extracted with DCM in two times. After drying with anhydrous Na_2SO_4(s), the solvent was removed under the reduced pressure. The residue was purified by silica-gel flash column chromatography to obtain **FD01**–**FD09**.



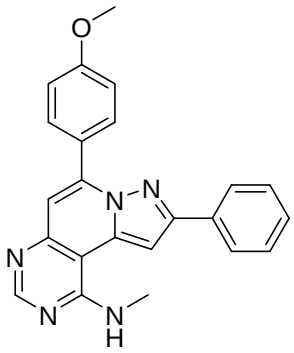
Compound FD01: Yield: 70%; off-white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.00–7.97 (m, 2H), 7.92 (d, *J* = 8.0, 2H), 7.55–7.53 (m, 3H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.13 (s, 1H), 7.07 (s, 1H), 5.77 (d, *J* = 5.6 Hz, 1H), 3.29 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3, 156.8, 153.6, 150.3, 144.8, 136.3, 132.7, 130.0, 129.7, 128.8, 128.7, 128.3, 126.4, 112.6, 103.7, 95.8, 28.7; IR (neat) ν_{max}: 3430, 3061, 2932, 1722, 1630, 1578, 1444, 1417, 1313, 1292, 740; HRMS (FAB+) *m/z* calcd for $C_{22}H_{17}N_5$ $[M+H]^+$: 352.1562; Found: 352.1556; Registration No.: 1477473-78-1.



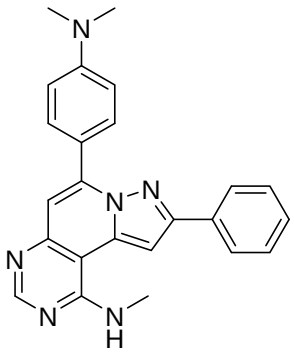
Compound FD02: Yield: 51%; yellowish brown solid; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.67 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 2H), 8.12 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 2H), 8.01 (d, *J* = 7.2 Hz, 2H), 7.53–7.52 (m, 2H), 7.45–7.41 (m, 1H), 7.27 (d, *J* = 4.4 Hz, 1H), 7.21 (s, 1H), 3.19 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 157.3, 156.9, 153.0, 149.6, 142.0, 136.9, 136.1, 132.2, 130.6, 129.0, 128.9, 126.2, 118.5, 113.3, 112.5, 103.6, 97.7, 28.7; IR (neat) ν_{max}: 3468, 3050, 2927, 2853, 2230, 1735, 1633, 1585, 1548, 1442, 1294, 743; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}N_6$ $[M+H]^+$: 377.1509; Found: 377.1511.



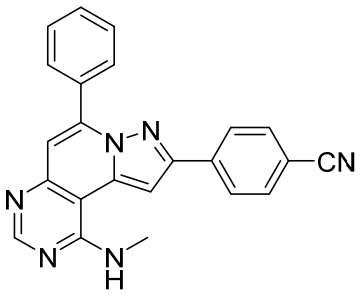
Compound FD03: Yield: 77%; pale brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.12 (d, *J* = 7.6 Hz, 2H), 7.95 (d, *J* = 7.6 Hz, 2H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.47–7.43 (m, 2H), 7.41–7.38 (m, 1H), 7.17 (s, 1H), 7.13 (s, 1H), 5.80 (brs, 1H), 3.34 (d, *J* = 4.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 157.0, 154.0, 150.1, 143.3, 136.3, 136.1, 132.1, 132.0, 131.7, 130.1 129.1, 128.8, 126.5, 125.3, 113.3, 104.1, 96.1, 28.8; IR (neat) ν_{max}: 3492, 3049, 2925, 2855, 1732, 1634, 1587, 1553, 1443, 1323, 1112, 1068, 843; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}F_3N_5$ $[M+H]^+$: 420.1431; Found: 420.1430.



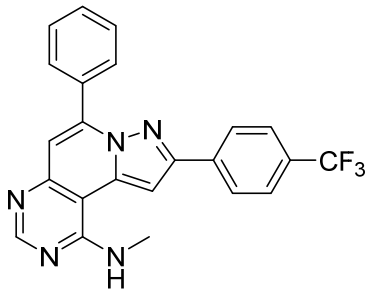
Compound FD04: Yield: 72%; orange solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.00 (d, *J* = 9.2 Hz, 2H), 7.97 (d, *J* = 7.2 Hz, 2H), 7.46–7.42 (m, 2H), 7.39–7.37 (m, 1H), 7.12 (s, 1H), 7.01 (s, 1H), 7.07 (d, *J* = 8.8 Hz, 2H), 5.79 (d, *J* = 4.4 Hz, 1H), 3.91 (s, 1H), 3.31 (d, *J* = 4.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 160.6, 157.2, 156.6, 152.7, 149.8, 143.6, 136.1, 132.5, 131.2, 128.9, 128.8, 126.1, 124.6, 113.7, 111.4, 102.7, 97.4, 55.4, 28.6; IR (neat) ν_{max}: 3346, 3117, 2923, 2852, 1734, 1628, 1561, 1510, 1443, 1258, 1180, 1034, 750; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}N_5O$ $[M+H]^+$: 382.1662; Found: 382.1664.



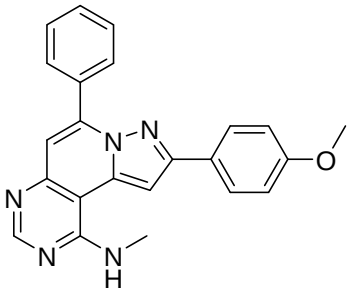
Compound FD05: Yield: 77%; orange solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.05–8.02 (m, 4H), 7.46–7.44 (m, 2H), 7.40–7.39 (m, 1H), 7.15 (s, 1H), 7.11 (s, 1H), 6.86 (d, *J* = 8.4 Hz, 2H), 5.76 (brs, 1H), 3.34 (d, *J* = 4.0 Hz, 3H), 3.01 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 156.7, 153.3, 151.5, 150.5, 145.4, 136.5, 132.7, 130.9, 128.7, 128.5, 128.4, 126.5, 119.7, 111.3, 110.7, 95.6, 40.2, 28.7; IR (neat) ν_{max}: 3409, 3049, 2930, 2813, 1732, 1574, 1522, 1460, 1365, 1265, 1167, 742; HRMS (ESI) *m/z* calcd for $C_{24}H_{22}N_6$ $[M+H]^+$: 395.1979; Found: 395.1978.



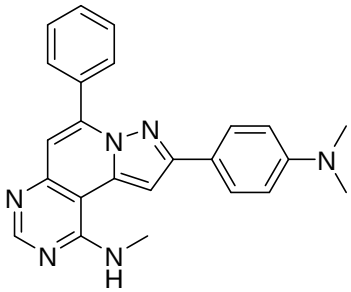
Compound FD06: Yield: 40%; pale brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 8.10 (d, *J* = 8.4 Hz, 2H), 8.01–7.98 (m, 2H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.60–7.58 (m, 3H), 7.24 (s, 1H), 7.20 (s, 1H), 5.75 (brs, 1H), 3.37 (d, *J* = 5.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 157.23, 157.22, 156.9, 151.0, 149.8, 143.8, 136.8, 136.3, 133.0, 132.3, 130.2, 129.6, 128.4, 126.6, 118.8, 113.1, 111.0, 103.1, 98.4, 28.7; IR (neat) ν_{max}: 3458, 3054, 2987, 2930, 2853, 2220, 1631, 1588, 1547, 1450, 1265, 749; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}N_6$ $[M+H]^+$: 377.1509; Found: 377.1510.



Compound FD07: Yield: 55%; pale orange solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.04 (d, *J* = 8.4 Hz, 2H), 7.98–7.96 (m, 2H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.56–7.54 (m, 3H), 7.18 (s, 1H), 7.15 (s, 1H), 5.79 (d, *J* = 4.4 Hz, 1H), 3.33 (d, *J* = 4.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 157.0, 152.1, 150.4, 144.8, 136.5, 135.8, 132.4, 130.7, 130.4, 130.2, 129.6, 128.3, 126.7, 125.72, 125.68, 125.64, 125.61, 113.3, 103.7, 96.2, 28.8 ; IR (neat) ν_{max}: 3385, 3115, 2926, 2848, 1738, 1622, 1563, 1539, 1409, 1330, 1173, 1117, 1068, 769; HRMS (ESI) *m/z* calcd for $C_{23}H_{16}F_3N_5$ $[M+H]^+$: 420.1431; Found: 420.1433.



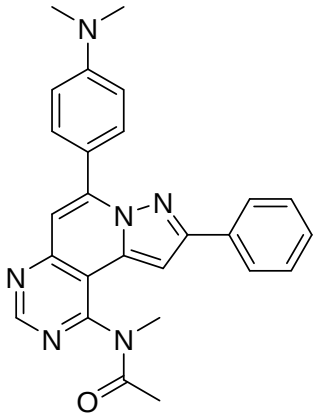
Compound FD08: Yield: 53%; white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.04–8.02 (m, 2H), 7.92 (d, *J* = 9.2 Hz, 2H), 7.57–7.56 (m, 3H), 7.15 (s, 1H), 7.06 (s, 1H), 6.98 (d, *J* = 8.8 Hz, 2H), 5.77 (brs, 1H), 3.87 (s, 1H), 3.35 (d, *J* = 4.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2, 158.4, 156.7, 153.6, 150.2, 144.8, 136.3, 132.8, 132.1, 130.0, 129.7, 128.2, 127.8, 125.1, 114.1, 112.2, 103.7, 95.4, 55.3, 28.8; IR (neat) ν_{max}: 2959, 2922, 2859, 1724, 1629, 1578, 1541, 1454, 1275, 1176, 749; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}N_5O$ $[M+H]^+$: 382.1662; Found: 382.1664.



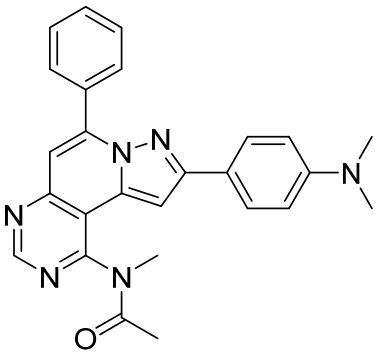
Compound FD09: Yield: 64%; yellow solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.04–8.03 (m, 2H), 7.87–7.85 (m, 2H), 7.56–7.55 (m, 3H), 7.12 (s, 1H), 7.02 (s, 1H), 6.78 (d, *J* = 8.8 Hz, 2H), 5.79 (brs, 1H), 3.35 (d, *J* = 4.8 Hz, 3H), 3.02 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 156.6, 154.3, 150.9, 150.2, 144.8, 136.2, 132.9, 129.9, 129.8, 128.2, 127.4, 120.3, 112.1, 111.7, 103.6, 95.0, 40.4, 28.7; IR (neat) ν_{max}: 3436, 3036, 2925, 2850, 2812, 1730, 1614, 1577, 1542, 1494, 1362, 1265, 1197, 736; HRMS (ESI) *m/z* calcd for $C_{24}H_{22}N_6$ $[M+H]^+$: 395.1979; Found: 395.1979.

4. General Procedure for the Preparation of FD10 and FD11

To freshly distilled DMF solution of sodium hydride (1.5 equiv.), **FD05** or **FD09** in anhydrous DMF was slowly added at 0 ºC. After the addition of acetic anhydride (3.0 equiv.), the reaction mixture was stirred at room temperature for 2 h. The resultant was extracted with EtOAc in three times and the combined organic layer was washed with brine. After drying with anhydrous Na_2SO_4(s), the solvent was removed under the reduced pressure. The residue was purified by silica-gel flash column chromatography to obtain **FD10** and **FD11**.

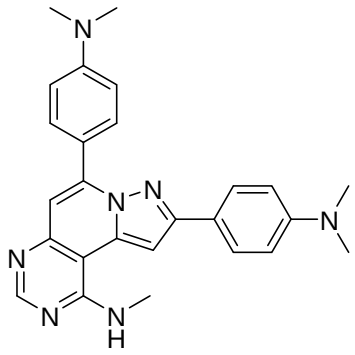


Compound FD10: Yield: 82%; orange solid; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 9.20 (s, 1H), 8.07 (d, *J* = 8.8 Hz, 2H), 8.00 (d, *J* = 7.2 Hz, 2H), 7.50–7.46 (m, 2H), 7.43–7.39 (m, 1H), 7.30 (s, 1H), 7.27 (s, 1H), 6.88 (d, *J* = 9.2 Hz, 2H), 3.37 (s, 3H), 3.11 (s, 6H), 1.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 158.9, 157.3, 154.7, 154.5, 152.0, 148.2, 135.6, 132.1, 131.2, 129.1, 128.8, 126.7, 118.8, 112.7, 111.6, 111.3, 109.2, 99.9, 40.2, 33.5, 22.4; IR (neat) ν_{max}: 3056, 2929, 2854, 1677, 1613, 1563, 1441, 1358, 1297, 1168, 736; HRMS (ESI) *m/z* calcd for $C_{26}H_{24}N_6O$ $[M+Na]^+$: 459.1904; Found: 459.1902.

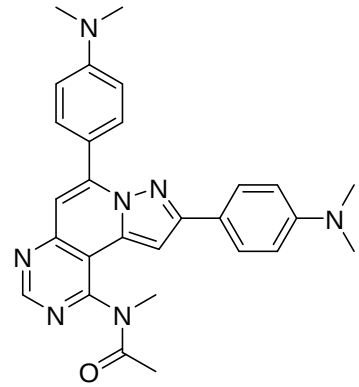


Compound FD11: Yield: 74%; yellowish orange solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.08–8.07 (m, 2H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.61–7.59 (m, 3H), 7.28 (s, 1H), 7.18 (s, 1H), 6.77 (d, *J* = 8.8 Hz, 2 H), 3.42 (s, 3H), 3.02 (s, 6H) 1.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 159.1, 157.2, 155.5, 154.5, 151.1, 147.6, 135.3, 132.3, 130.6, 129.8, 128.4, 127.7, 119.6, 113.4, 112.2, 110.5, 99.4, 40.3, 33.5, 22.4; IR (neat) ν_{max}: 2925, 2855, 1678, 1606, 1563, 1546, 1519, 1444, 1369, 1295, 1170, 766; LRMS (ESI) *m/z* calcd for $C_{26}H_{24}N_6O$ $[M+H]^+$: 459.1904; Found: 459.1906.

5. Designed probe FD12 and FD13



Compound FD12 was prepared using the general procedure of section 3 from compound **2e**.; Yield: 71%; yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.05 (d, *J* = 8.8 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.08 (s, 1H), 6.99 (s, 1H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 8.8 Hz, 2H), 5.81 (brs, 1H), 3.33 (d, *J* = 4.8 Hz, 3H), 3.08 (s, 6H), 3.02 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.7, 156.9, 153.8, 151.1, 150.4, 144.7, 136.6, 131.1, 127.5, 120.7, 119.9, 112.6, 111.6, 109.6, 102.4, 96.6, 40.4, 40.3, 29.1; IR (neat) ν_{max}: 3411, 3032, 2925, 2806, 1735, 1670, 1614, 1571, 1520, 1441, 1364, 1197, 1121, 819, 736; HRMS (ESI) *m/z* calcd for $C_{26}H_{27}N_7$ $[M+H]^+$: 438.2401; Found: 438.2402.



Compound FD13 was prepared using the general procedure of section 4 from **FD12**.; Yield: 82%; yellowish orange solid; ^{1}H NMR (500 MHz, CD_2Cl_2) δ 9.15 (s, 1H), 8.05 (d, *J* = 6.4 Hz, 2H), 7.84 (d, *J* = 7.2 Hz, 2H), 7.21 (s, 1H), 7.13 (s, 1H), 6.86 (d, *J* = 6.8 Hz, 2H), 6.77 (d, *J* = 7.2 Hz, 2H), 3.34 (s, 3H), 3.09 (s, 6H), 3.00 (s, 6H), 1.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 158.8, 157.1, 155.1, 154.6, 151.9, 151.0, 148.1, 135.5, 131.2, 127.7, 119.9, 119.1, 112.5, 112.2, 111.2, 108.3, 99.0, 40.4, 40.2, 33.4, 22.3; IR (neat) ν_{max}: 3053, 2928, 2854, 1724, 1677, 1610, 1562, 1522, 1440, 1265, 748; HRMS (ESI) *m/z* calcd for $C_{28}H_{29}N_7O$ $[M+H]^+$: 480.2506; Found: 480.2508

IV Experimental Procedures for Cell-based Fluorescence Image

1. Cell culture

A549 (human lung carcinoma) and Hela (human cervical cancer cell) cell lines were obtained from American Type Culture Collection [ATCC]. Cells were cultured in RPMI 1640 [GIBCO] supplemented with heat-inactivated 10% (v/v) fetal bovine serum [FBS, GIBCO] and 1% (v/v) antibiotic-antimycotic agent [GIBCO]. Cell lines were maintained in humidified atmosphere of 5% CO_2 and 95% air at 37 °C, and cultured in 100 mm cell culture dish [CORNING].

2. Live cell imaging of FD12 and FD13 in A549 cells

A549 cells were seeded on glass bottom chamber slide and incubated at 5 % CO_2, 37 °C for overnight. Cells were treated with **FD12** (20 μM) or **FD13** (20 μM) in media for 1 h. Then, probes were washed with PBS buffer for 3 times and fluorescence images were taken by fluorescence microscopy under PBS buffer with DeltaVision Elite imaging system [GE Healthcare] equipped with 60×/1.42 NA oil lens. Fluorescence signals of each probe were obtained using following filter sets; **FD12** (Ex; 390 nm with 18 nm bandwidth, Em; 435 nm with 48 bandwidth), **FD13** (Ex; 390 nm with 18 nm bandwidth, Em; 525 nm with 48 nm bandwidth).

3. Co-staining of FD13 with organelle staining markers

A549 cells were seeded on glass bottom chamber slide and incubated at 5 % CO_2, 37 °C for overnight. Cells were treated with 20 μM of **FD13** for 1 h. Then, 20 μM of LD tracker (SF44), 50 nM of Lysotracker Red or 20 nM of Mitotracker Red were treated to each well and incubated for 30 min. After treatment of each probe, cells were washed with PBS buffer for 3 times and then fluorescence images were taken by fluorescence microscopy under PBS buffer with DeltaVision Elite imaging system [GE Healthcare] equipped with 60×/1.42 NA oil lens. Fluorescence signals of each probe were obtained using following filter sets; **FD13** (Ex; 390 nm with 18 nm bandwidth, Em; 525 nm with 48 nm bandwidth), SF44 (Ex; 475 nm with 28 nm bandwidth, Em; 625 nm with 45 nm bandwidth), Lysotracker Red and Mitotracker Red (Ex; 575 nm with 25 nm bandwidth, Em; 625 nm with 45 nm bandwidth).

4. Differentiation of 3T3-L1 cell lines

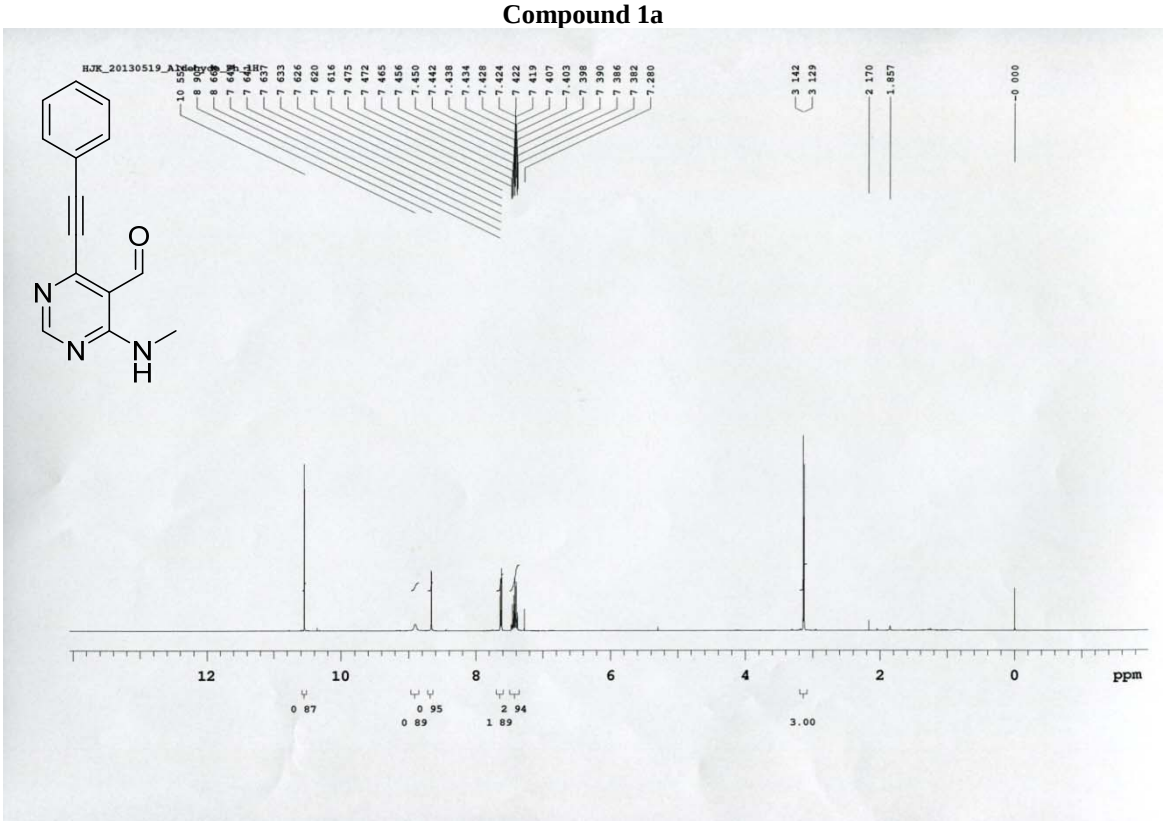
3T3-L1 cells were cultured to seeded on glass bottom chamber slide and incubated with DMEM, [Dulbecco's modified Eagle's medium] supplemented with heat inactivated 10% (v/v) calf serum and 1% (v/v) FBS (fetal bovine serum), 1 μM dexamethasone, 10 μM rosiglitazone, 5 μg/ml insulin. 2 days later, replaced the media to the DMEM supplemented with 10% (v/v) FBS, and 5 μg/ml insulin and refreshed the media with same condition every 2 day.

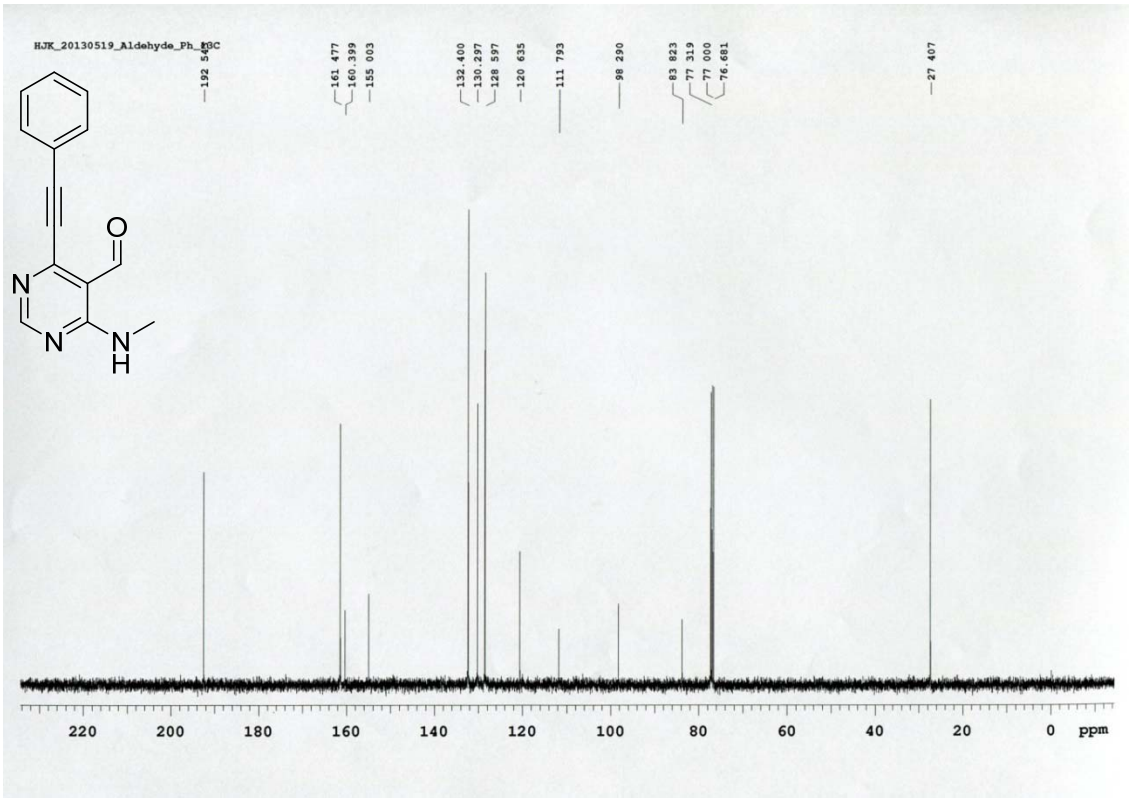
5. Immunohistochemistry with anti-ADRP and anti-perilipin antibodies

3T3-L1 cells were seeded on glass bottom chamber slide and differentiated by protocol as stated above. When the differentiation was completed, cells were fixed with 3.7% paraformaldehyde in PBS for 15 min at r.t., and washed with ice cold PBS for twice, decanted from glass bottom dish. Fixed cells on dish were introduced with diluted primary antibody solution (1:200) in PBST with 1% BSA, and incubated at 4 ^{o}C for overnight. Primary antibody was decanted and washed with PBS for 3 times. Diluted secondary antibody, conjugated with TRICT fluorescent dye, solution (1:100) was added, followed **FD13** (20 μM) was treated to the cell in PBS for 1 h at r.t., followed by PBS washing for 3 min twice. Fluorescence images were taken by Deltavision imaging system [GE Healthcare] equipped with 100×/1.4 NA oil lens. Fluorescence signal of **FD13** and TRITC-conjugated secondary antibody were obtained using following filter sets; **FD13** (Ex; 390 nm with 18 nm bandwidth, Em; 525 nm with 48 nm bandwidth), TRITC-conjugated secondary antibody (Ex; 542 nm with 27 nm bandwidth, Em; 597 nm with 45 nm bandwidth).

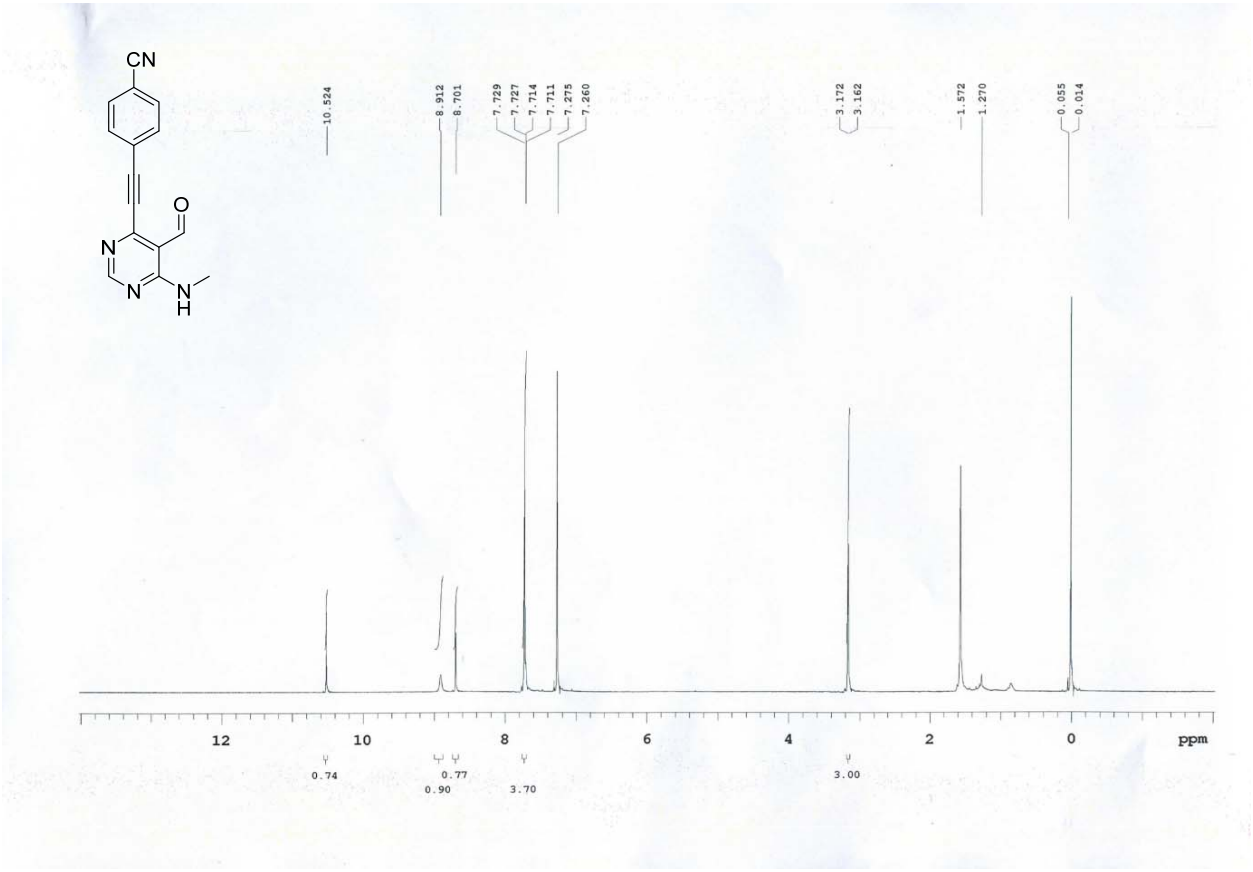
V. ^{1}H and ^{13}C NMR spectra

Compound 1a





Compound 1b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N
N
H
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7.727
7.714
7.711
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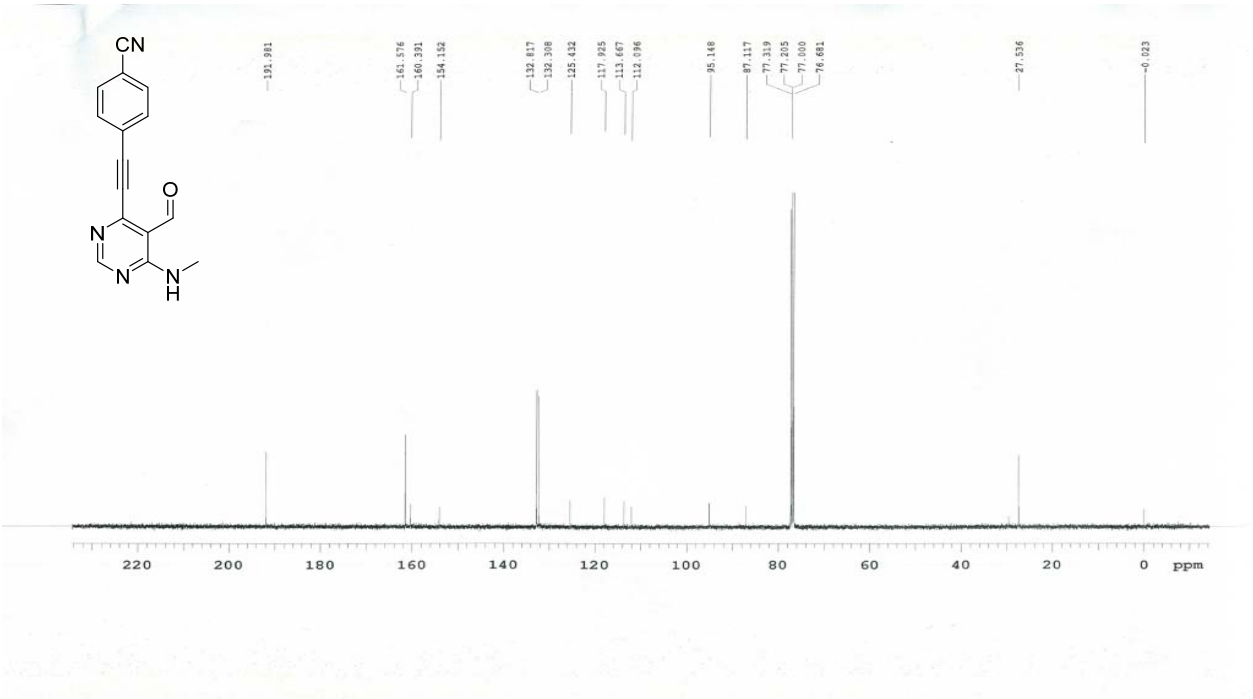
7.260
3.172
3.162
1.572
1.270
0.055
0.014
12
10
8
6
4
2
0
ppm
0.74
0.77
0.90
3.70
3.00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N
N
N
H
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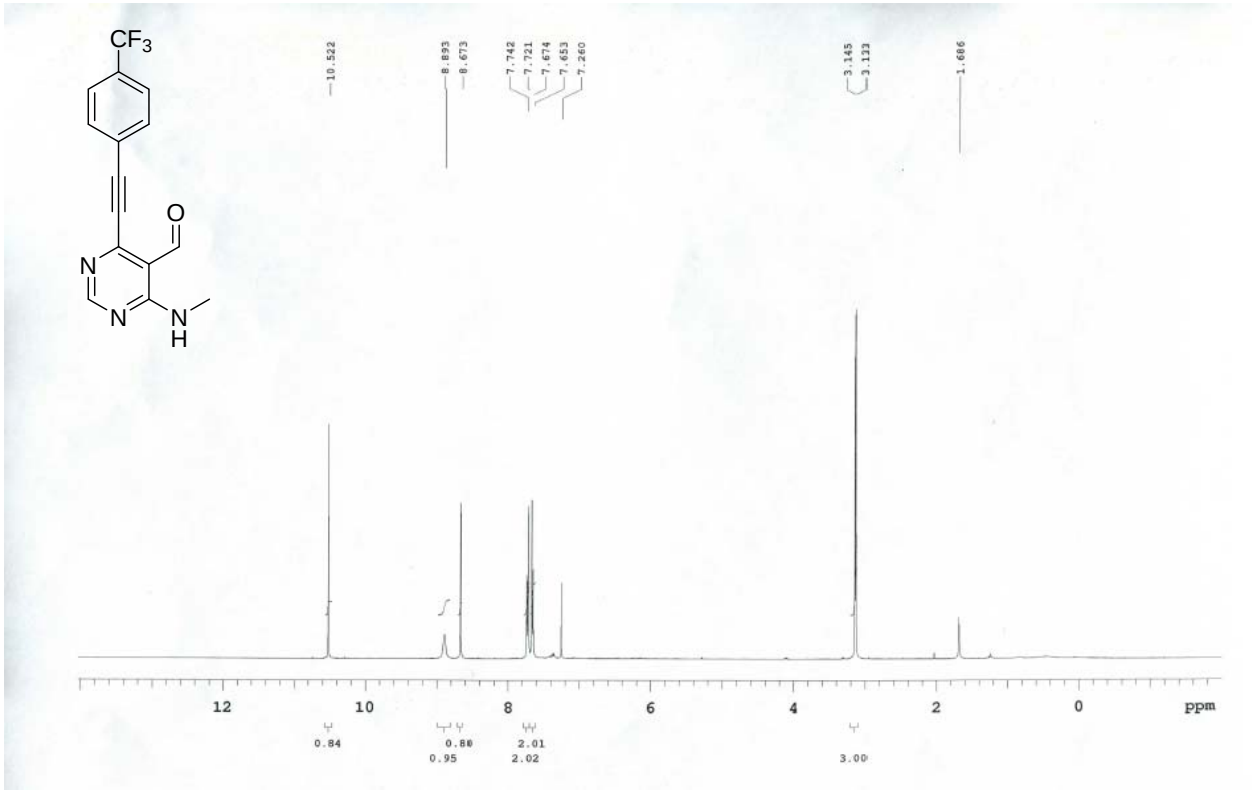
161.576
160.391
154.152
132.817
132.308
125.432
117.925
113.667
112.096
95.148
87.117
77.319
77.205
77.000
76.681
27.536
-0.023
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 1c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N
N
H
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7.721
7.674
7.653
7.260
3.145
3.133
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10
8
6
4
2
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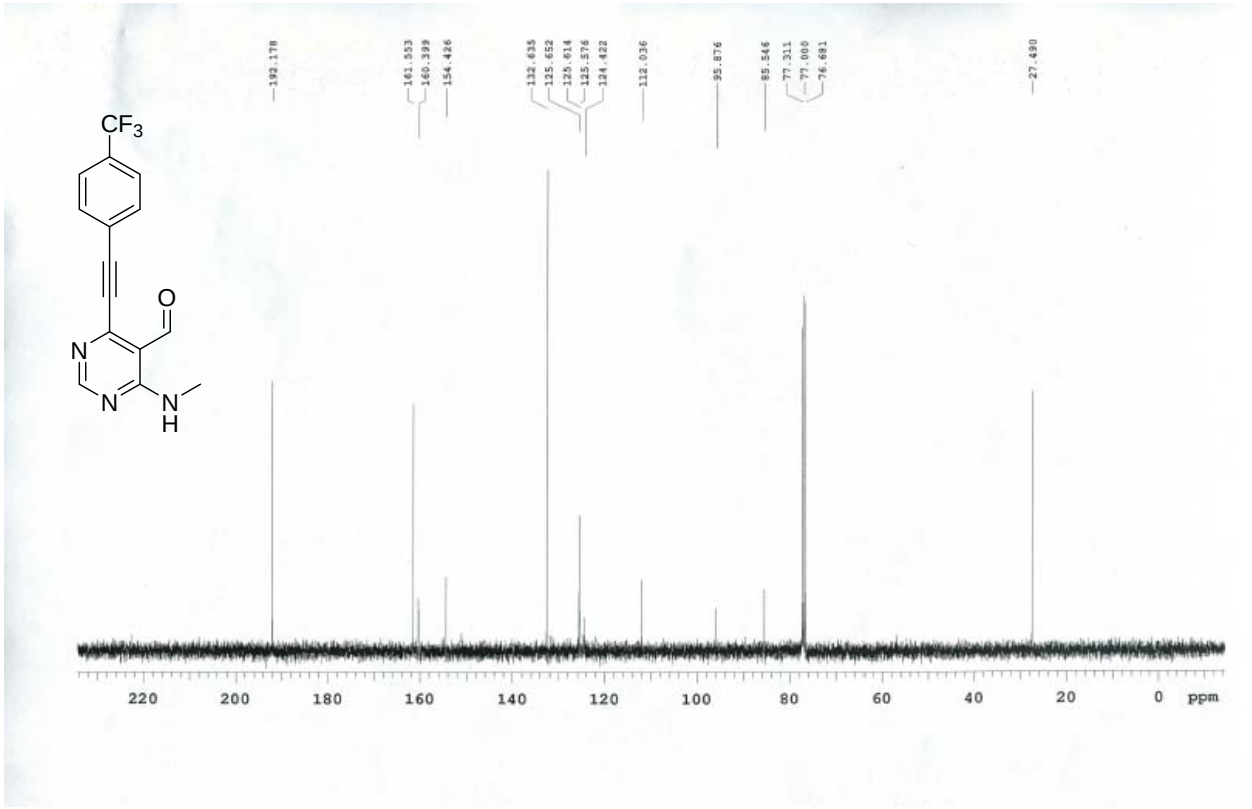
ppm
0.84
0.88
0.95
2.01
2.02
3.00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N
N
N
H
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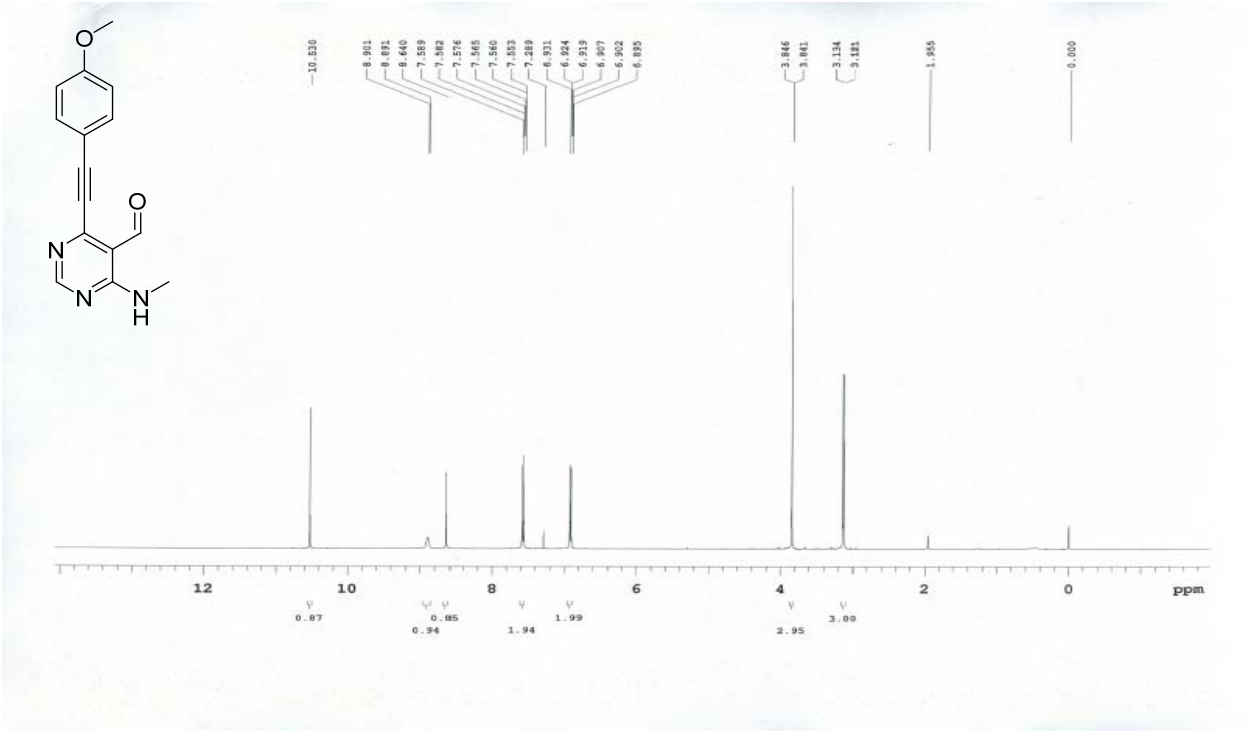
161.553
160.399
154.426
132.635
125.652
125.614
125.578
124.422
112.036
95.876
85.546
77.311
77.000
76.681
27.490
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 1d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7.582
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7.565
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7.289
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6.919
6.907
6.902
6.895
3.846
3.841
3.134
3.131
1.955
0.000
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10
8
6
4
2
0
ppm
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0.85
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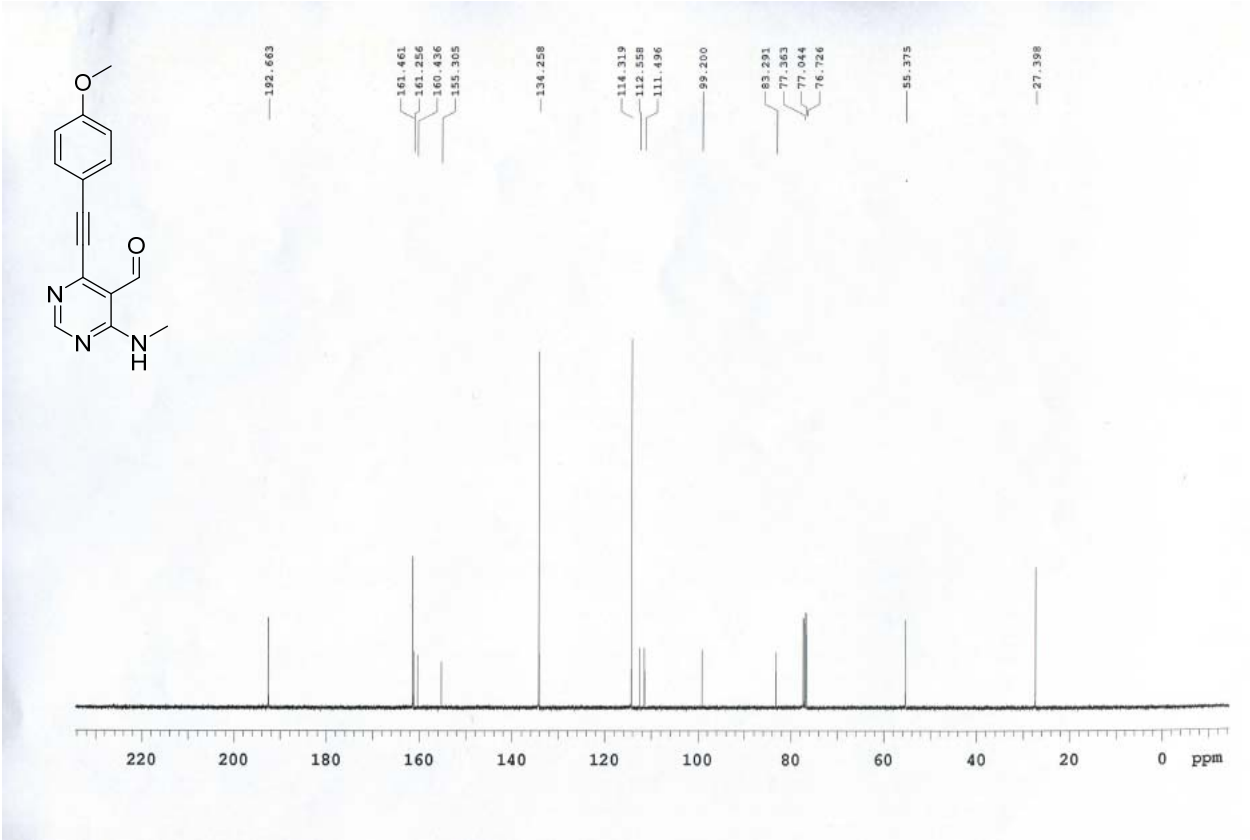
1.99
2.95
3.00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160.436
155.305
134.258
114.319
112.558
111.496
99.200
83.291
77.363
77.044
76.726
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27.398
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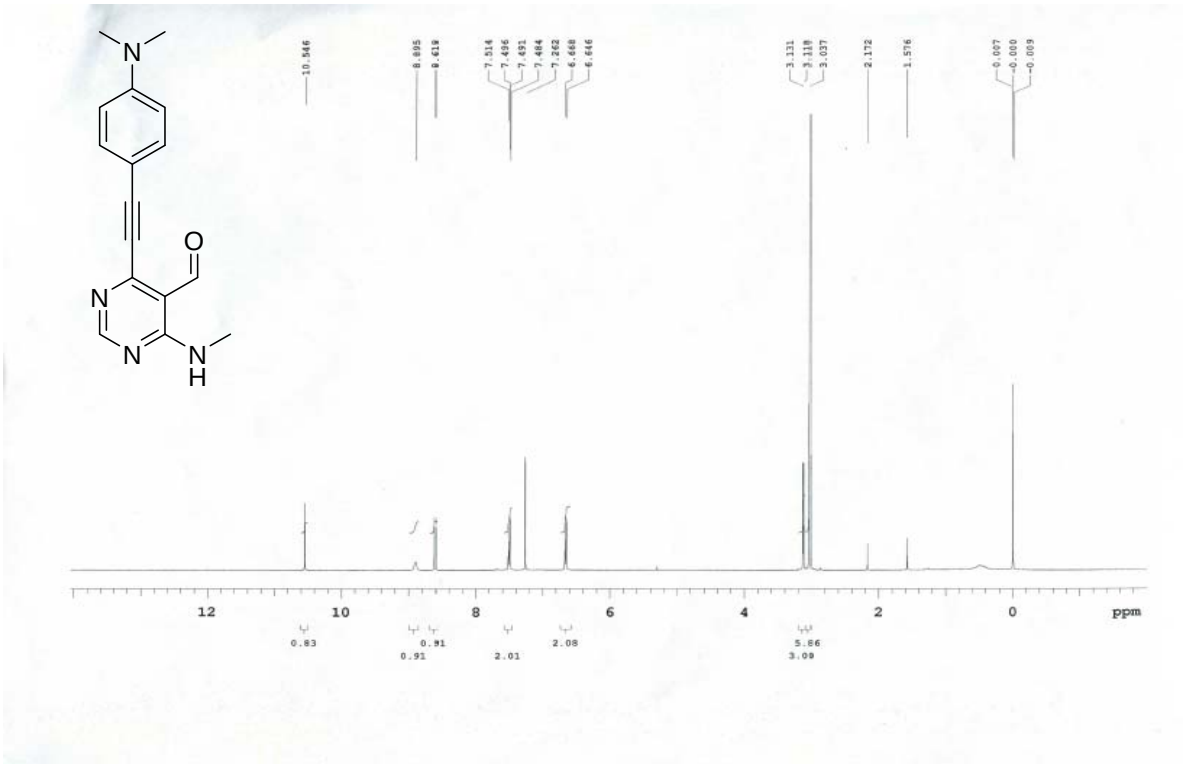
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 1e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7.496
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7.262
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6.646
3.131
3.118
3.037
2.172
1.576
0.007
0.000
-0.009
12
10
8
6
4
2
0
ppm
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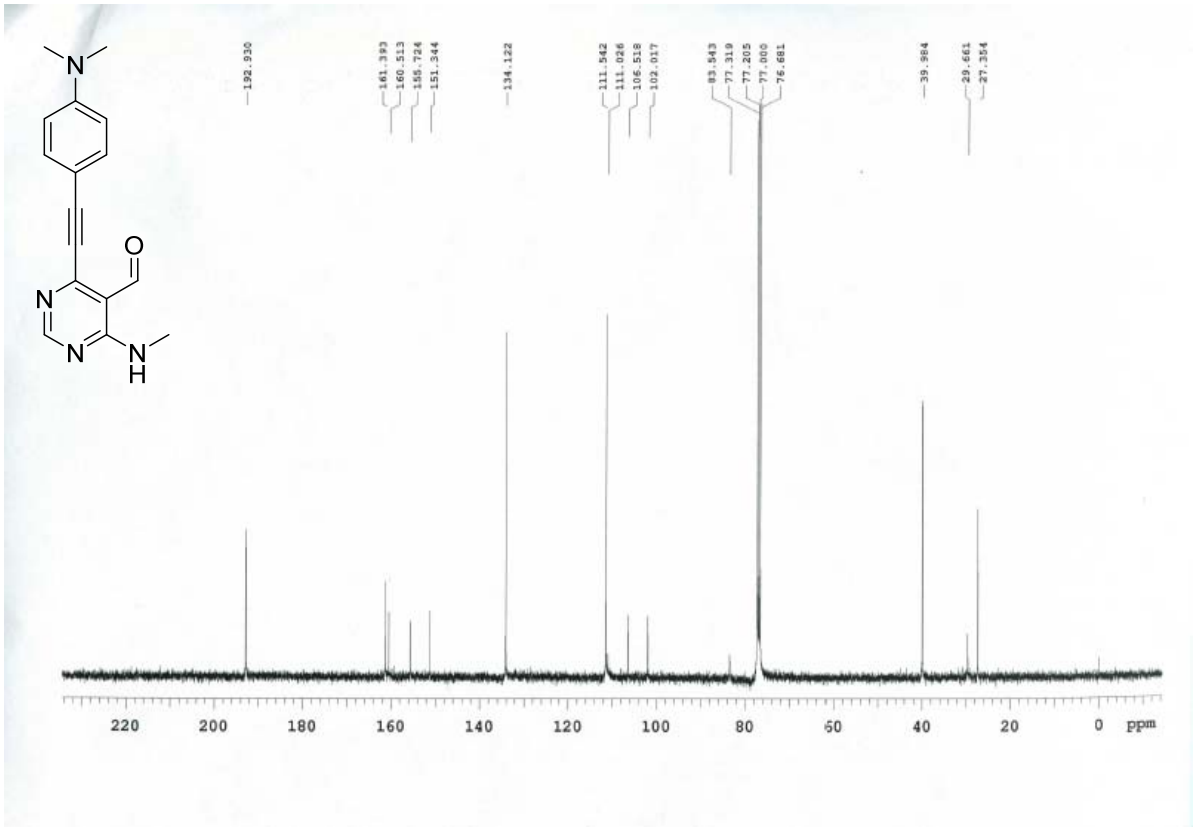
0.91
2.01
2.08
5.86
3.09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151.344
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111.542
111.026
106.518
102.017
83.543
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77.000
76.681
39.984
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220
200
180
160
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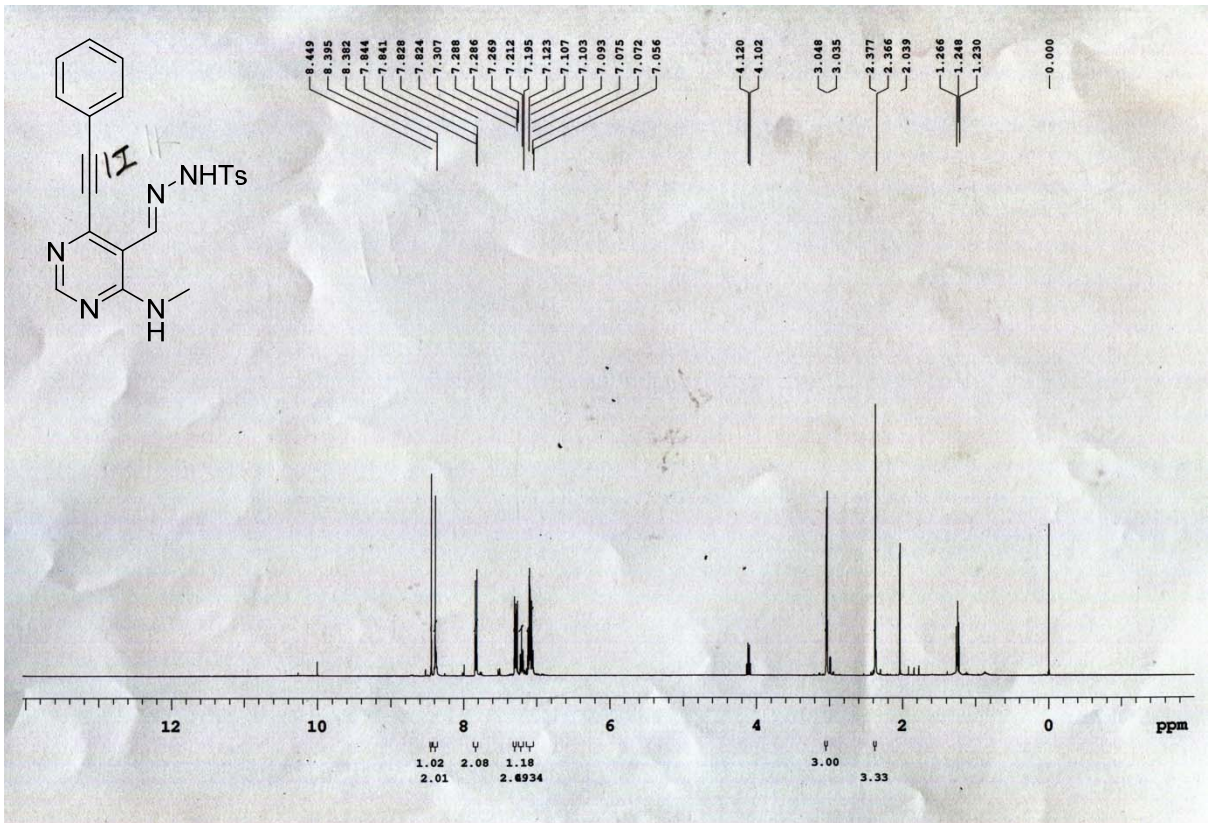
80
60
40
20
0
ppm

Compound 2a


NHTs
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N
N
H
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7.824
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7.288
7.286
7.269
7.212
7.195
7.123
7.107
7.103
7.093
7.075
7.072
7.056
4.120
4.102
3.048
3.035
2.377
2.366
2.039
1.266
1.248
1.230
0.000
12
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4
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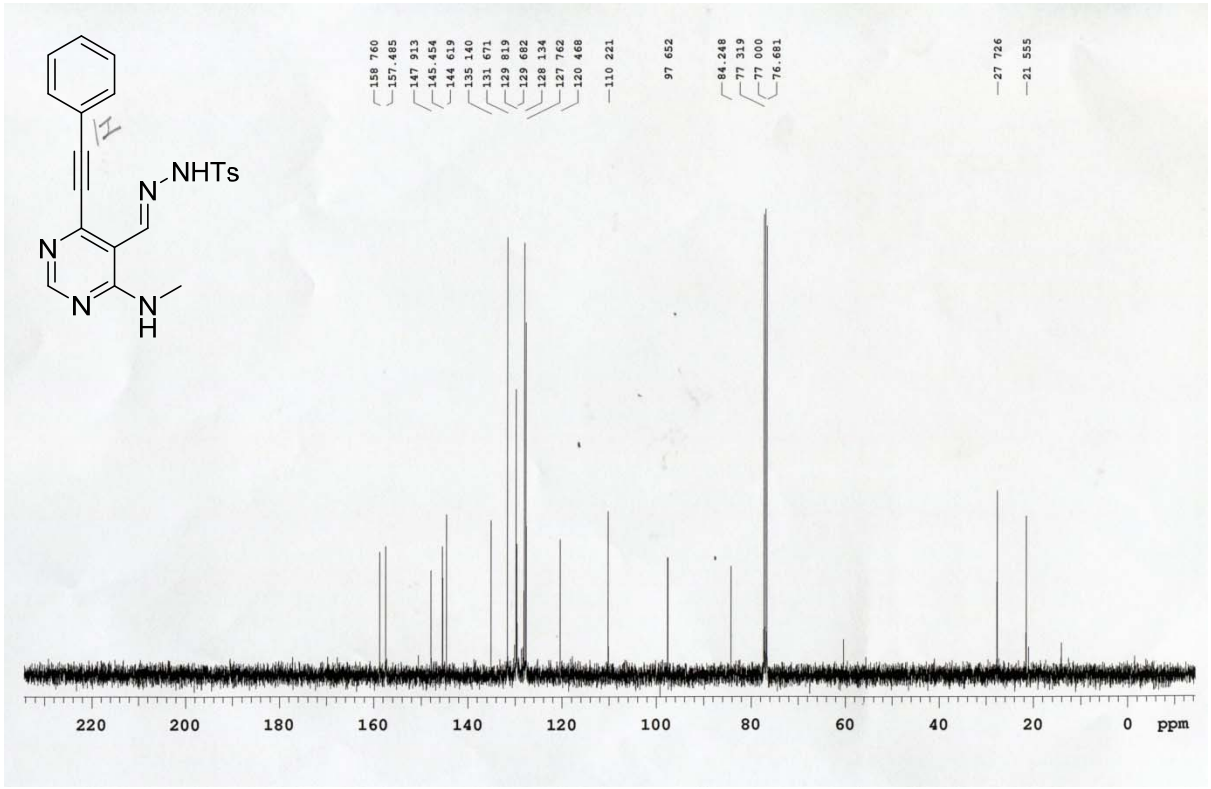
3.33


NHTs
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N
N
H
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144.619
135.140
131.671
129.819
129.682
128.134
127.762
120.468
110.221
97.652
84.248
77.319
77.000
76.681
27.726
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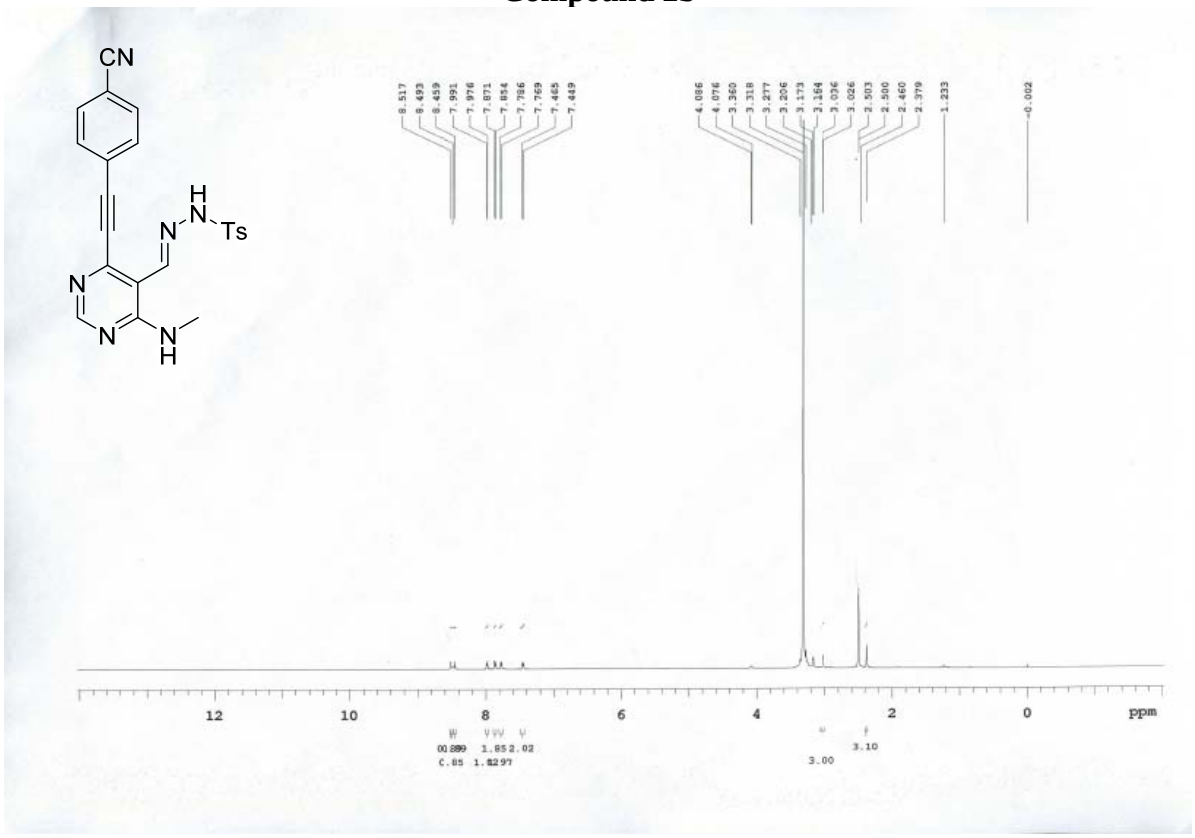
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 2b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H
N
N
Ts
N
N
N
H
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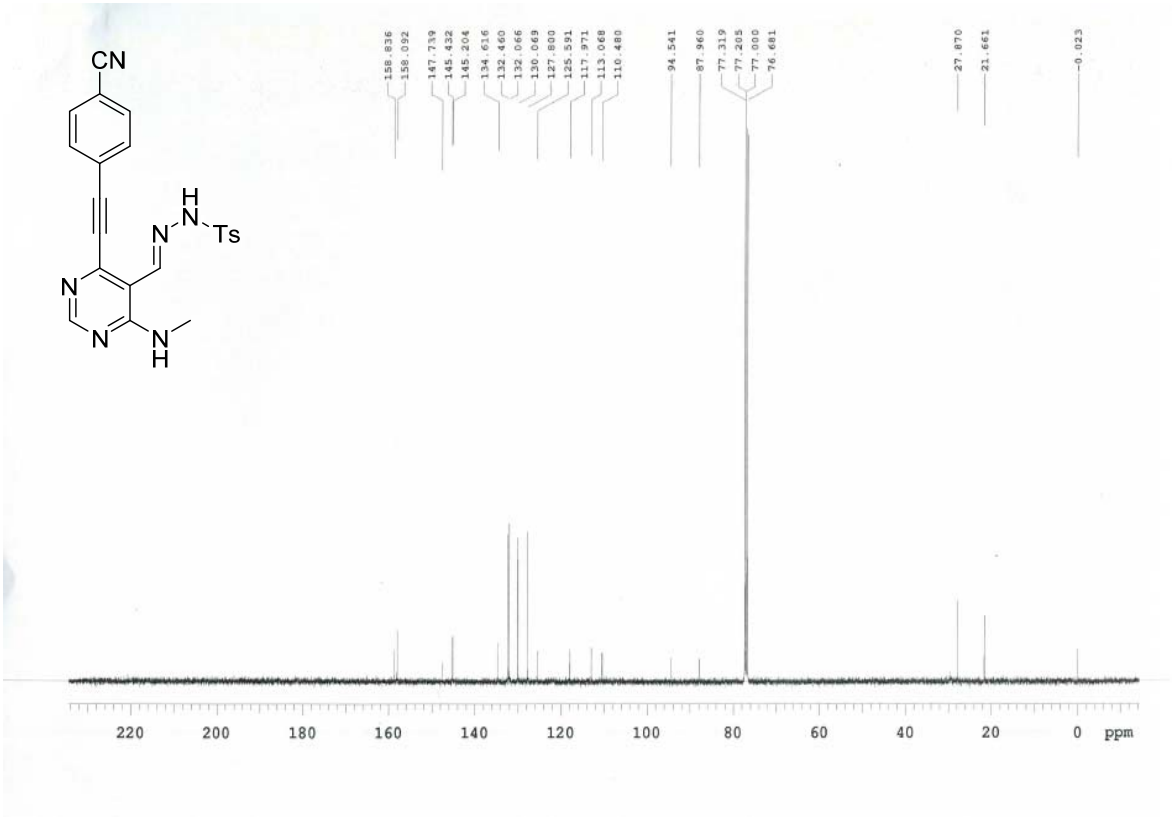
7.991
7.976
7.871
7.854
7.786
7.769
7.465
7.449
4.086
4.076
3.360
3.318
3.277
3.206
3.173
3.164
3.036
3.026
2.503
2.500
2.460
2.379
1.233
0.002
12
10
8
6
4
2
0
ppm
1.85
2.02
1.82
97
3.00
3.10


CN
H
N
N
Ts
N
N
N
H
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145.432
145.204
134.616
132.460
132.066
130.069
127.800
125.591
117.971
113.068
110.480
94.541
87.960
77.319
77.205
77.000
76.681
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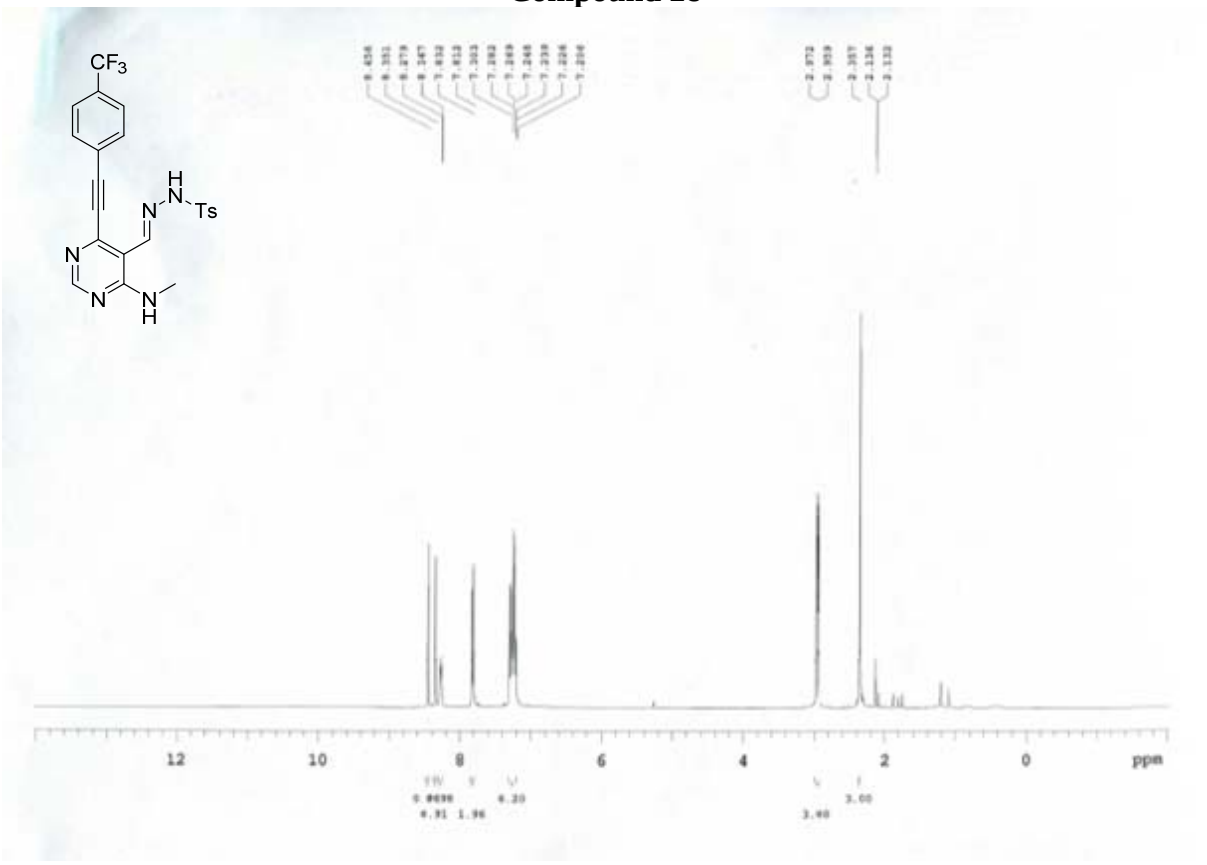
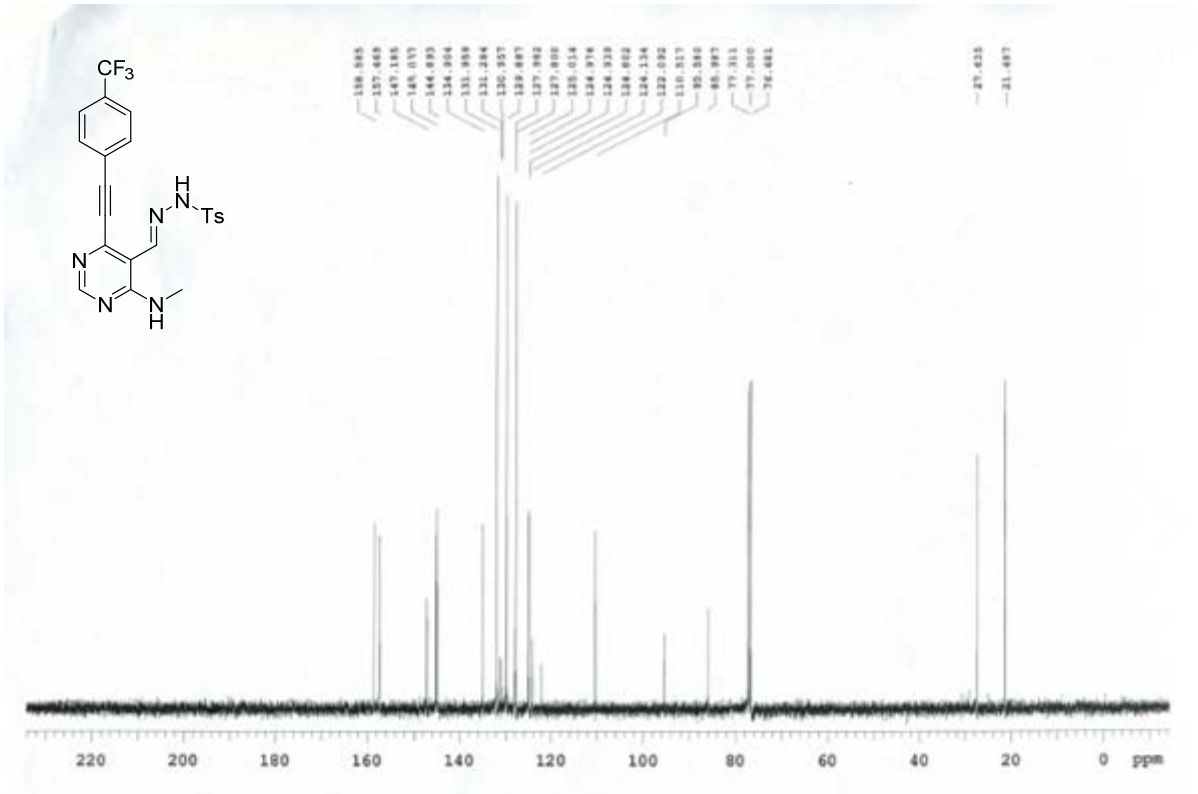
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 2c


CF3
H
N
Ts
N
N
N
H
12
10
8
6
4
2
0
ppm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H
N
Ts
N
N
N
H
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80
60
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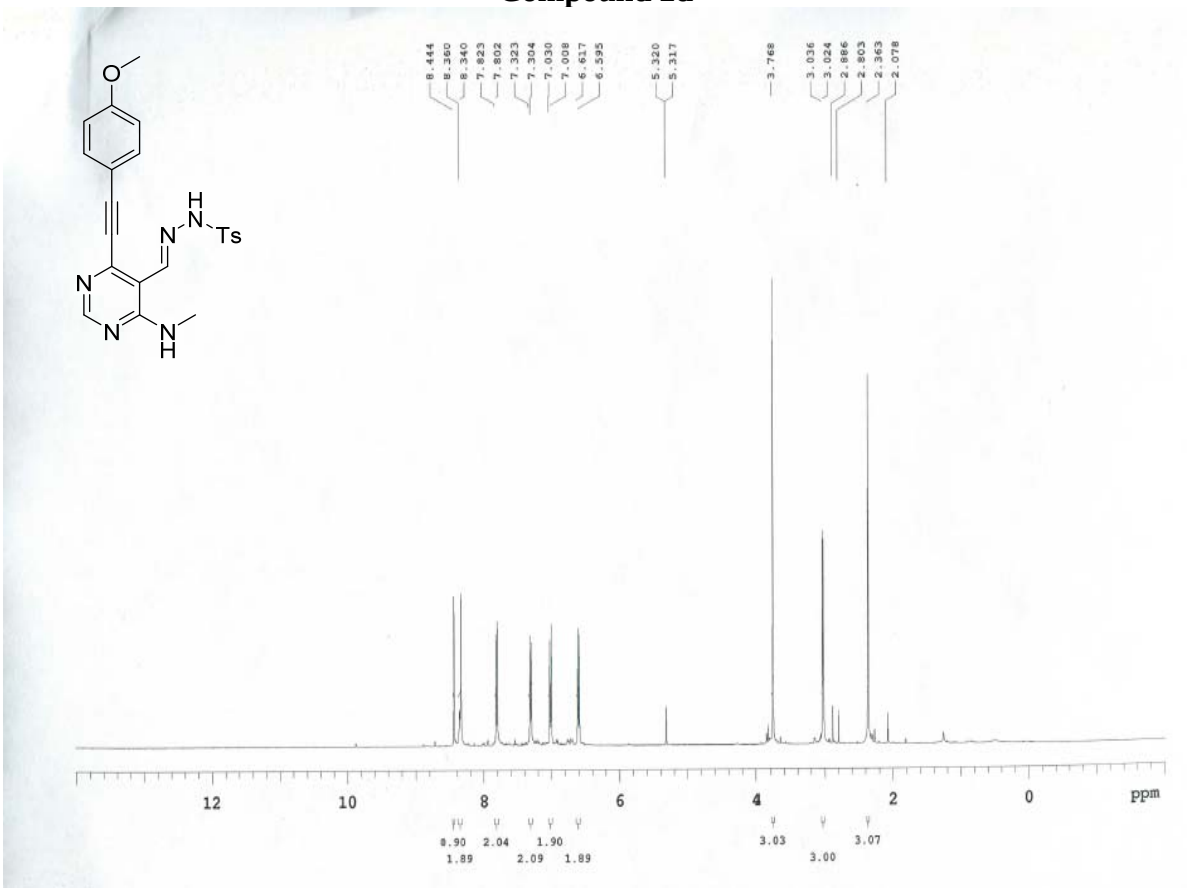
20
0
ppm

Compound 2d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7.030
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5.317
3.768
3.036
3.024
2.886
2.803
2.363
2.078
12
10
8
6
4
2
0
ppm
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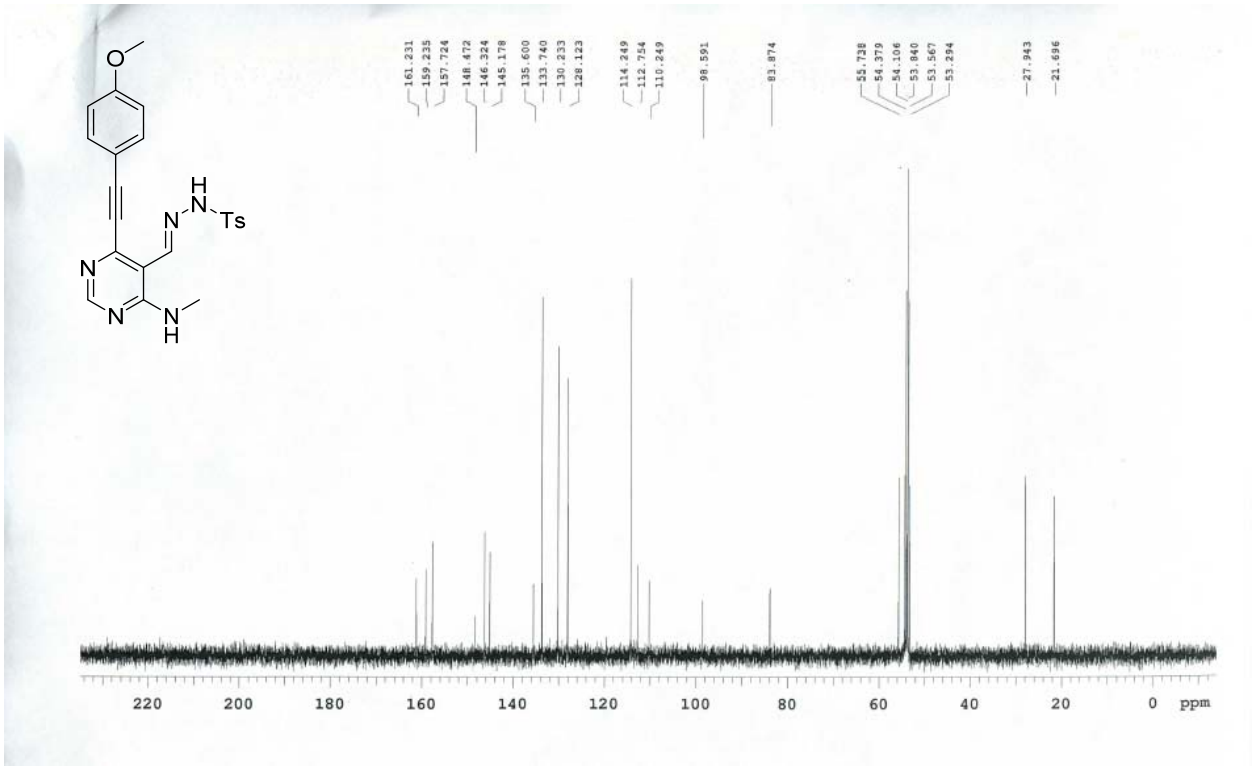
1.90
1.89
2.09
1.89
3.03
3.00
3.07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146.324
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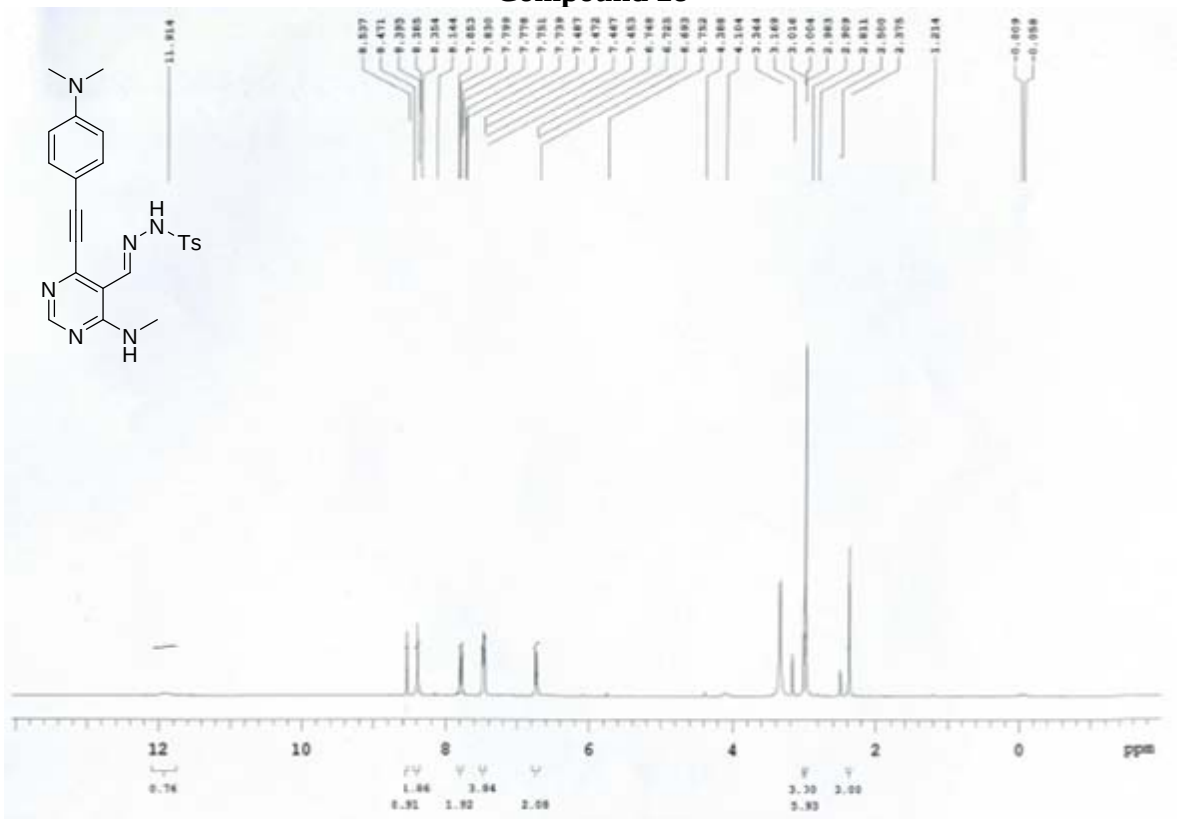
128.123
114.249
112.754
110.249
98.591
83.874
55.738
54.379
54.106
53.840
53.567
53.294
27.943
21.696
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound 2e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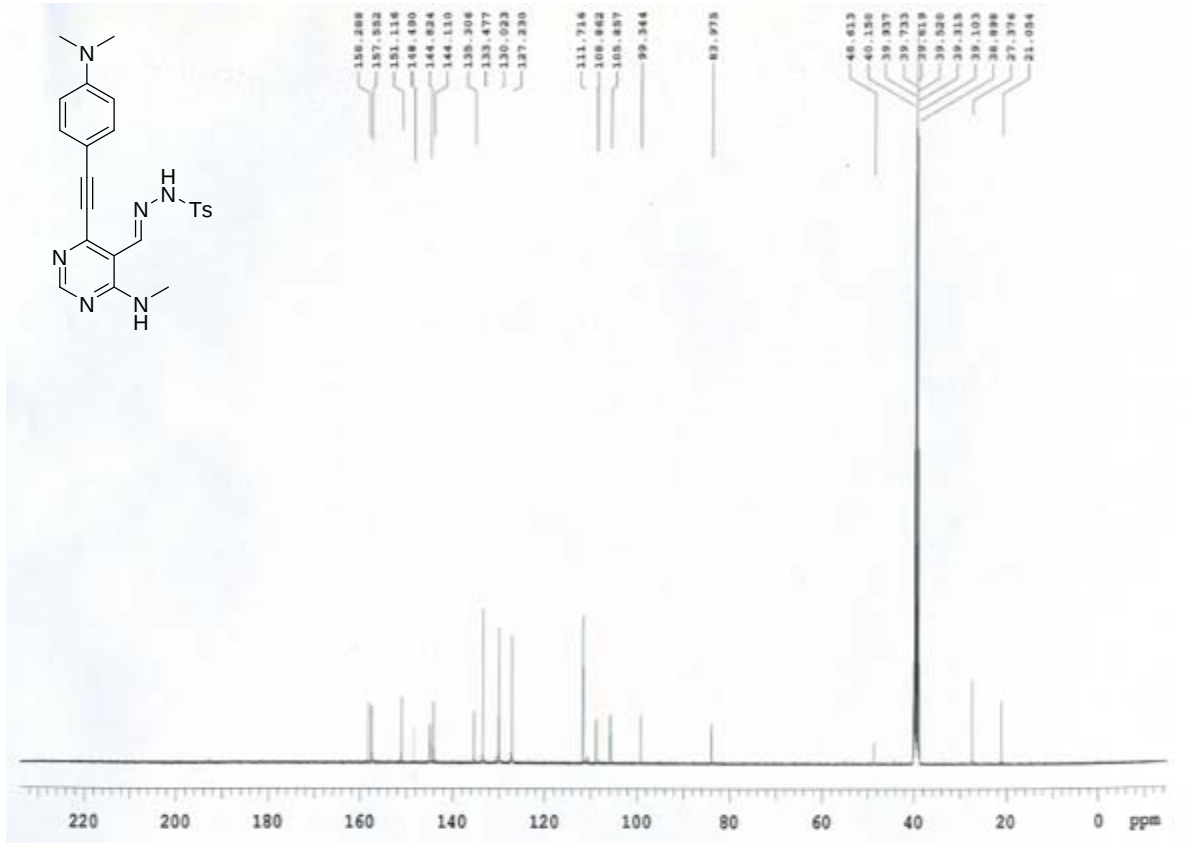
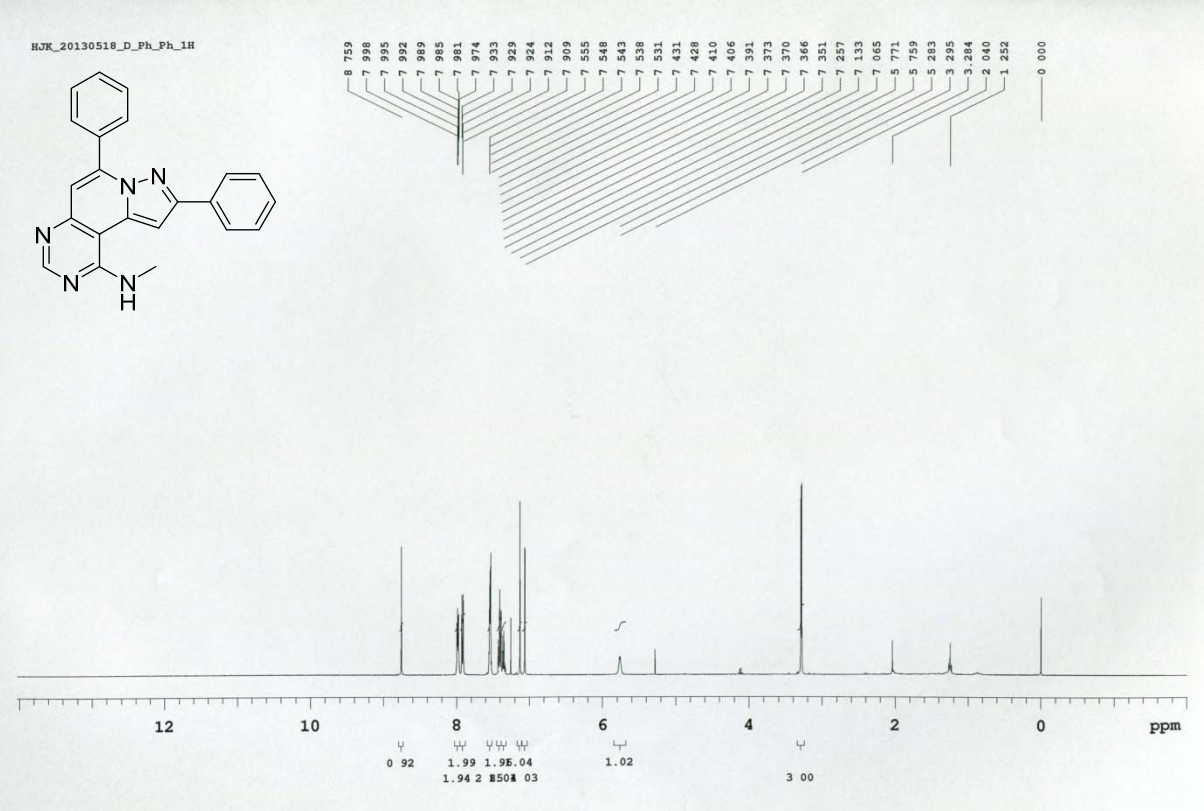
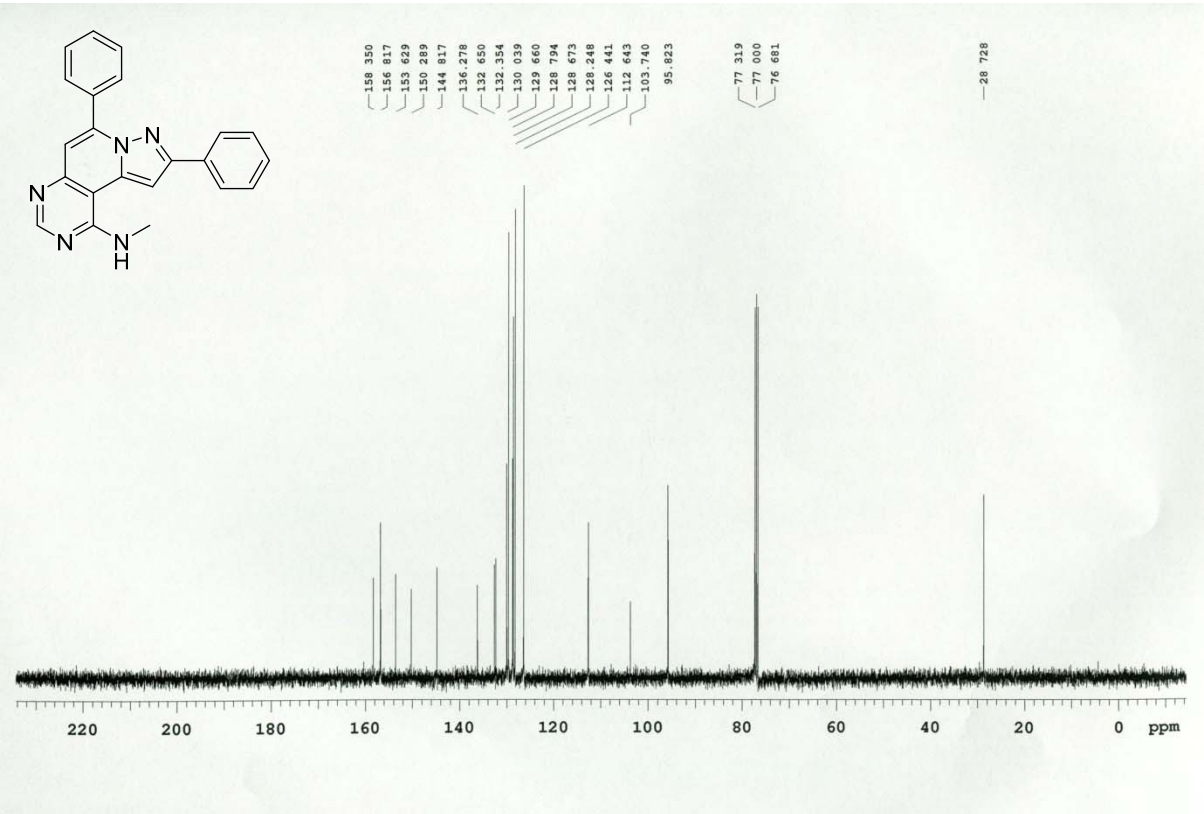
H
N
Ts
N
N
N
H
12
10
8
6
4
2
0
ppm


N
H
N
Ts
N
N
N
H
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Compound FD01





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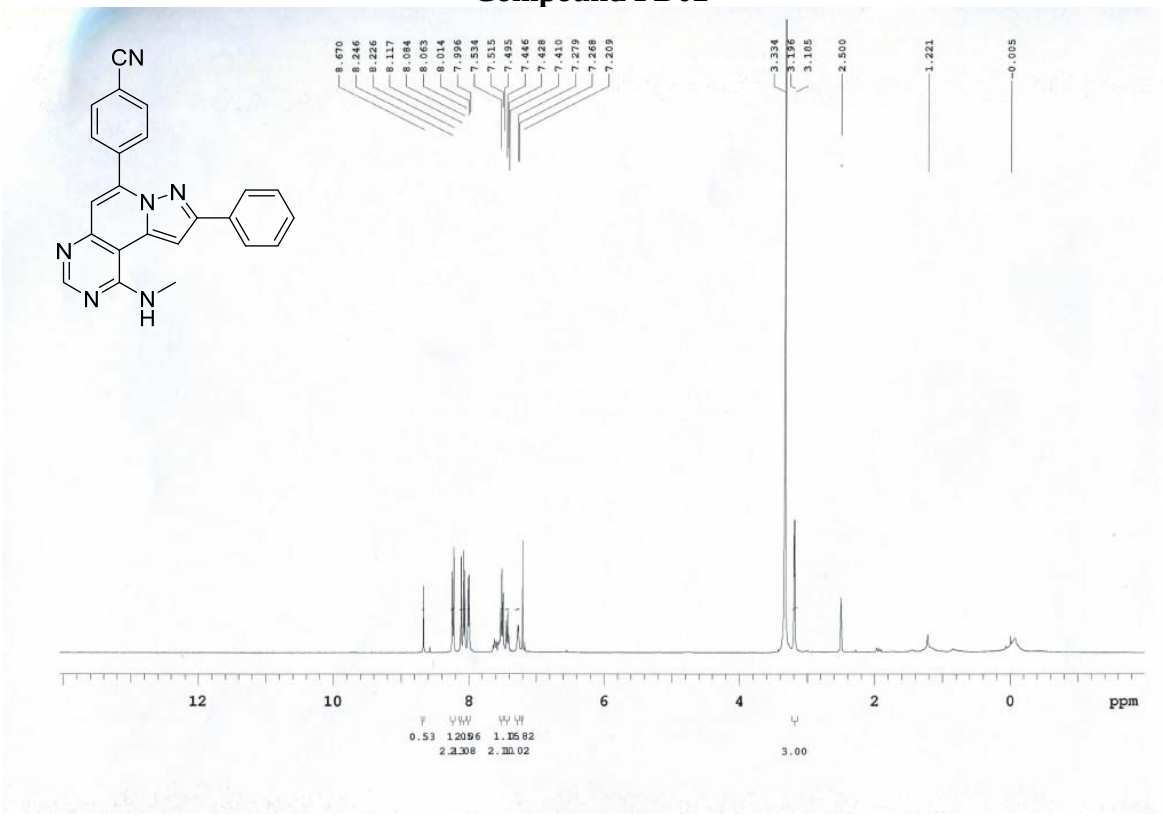

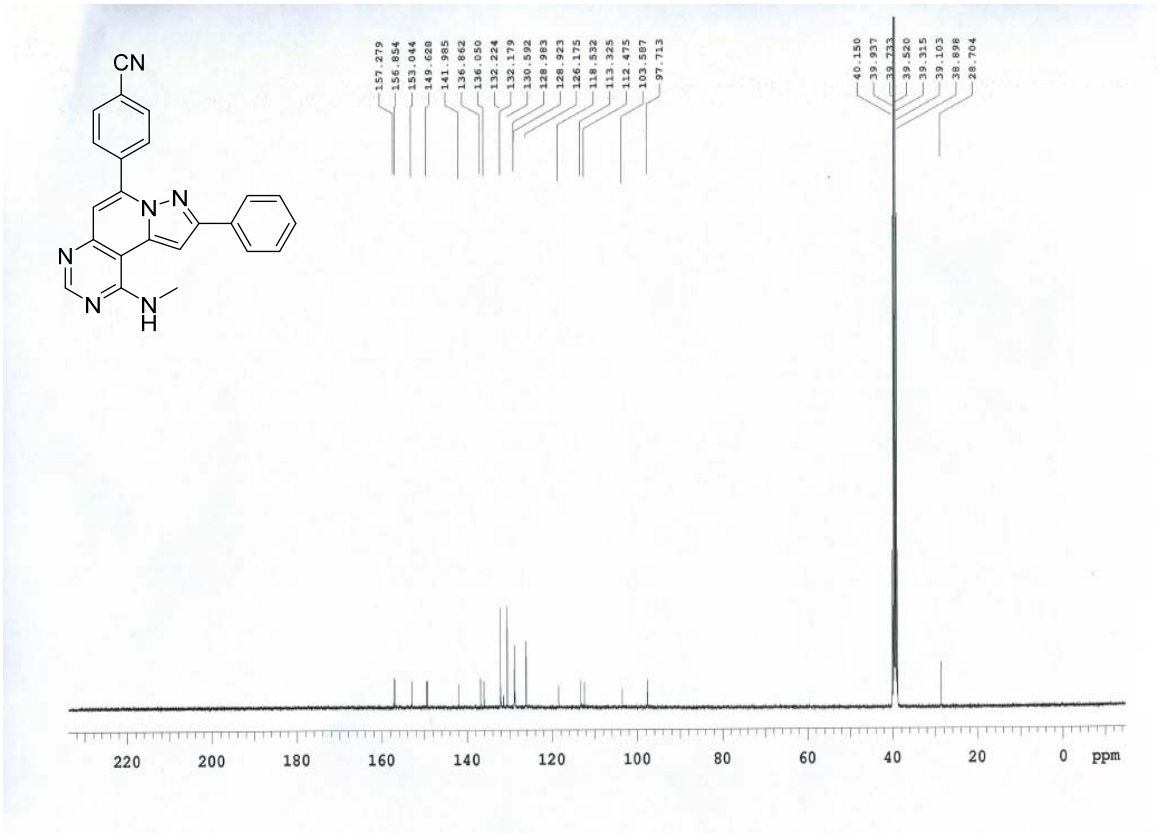

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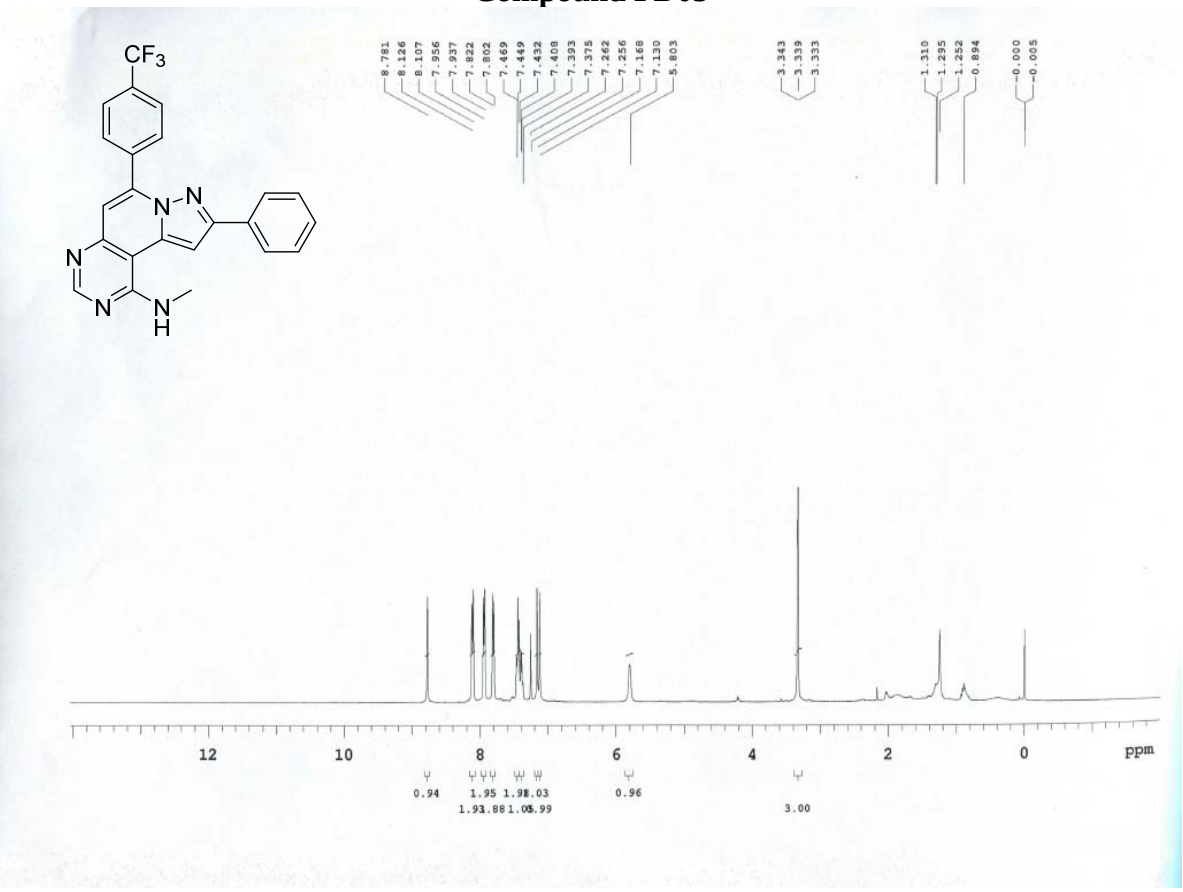
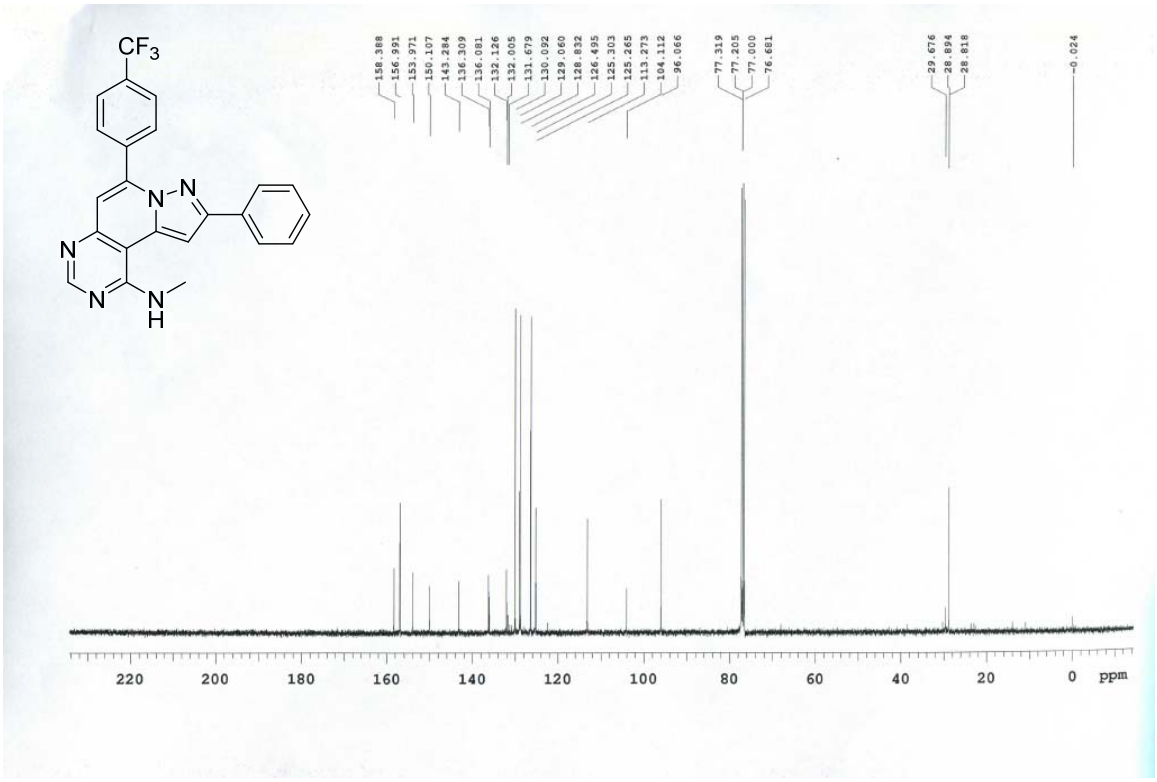
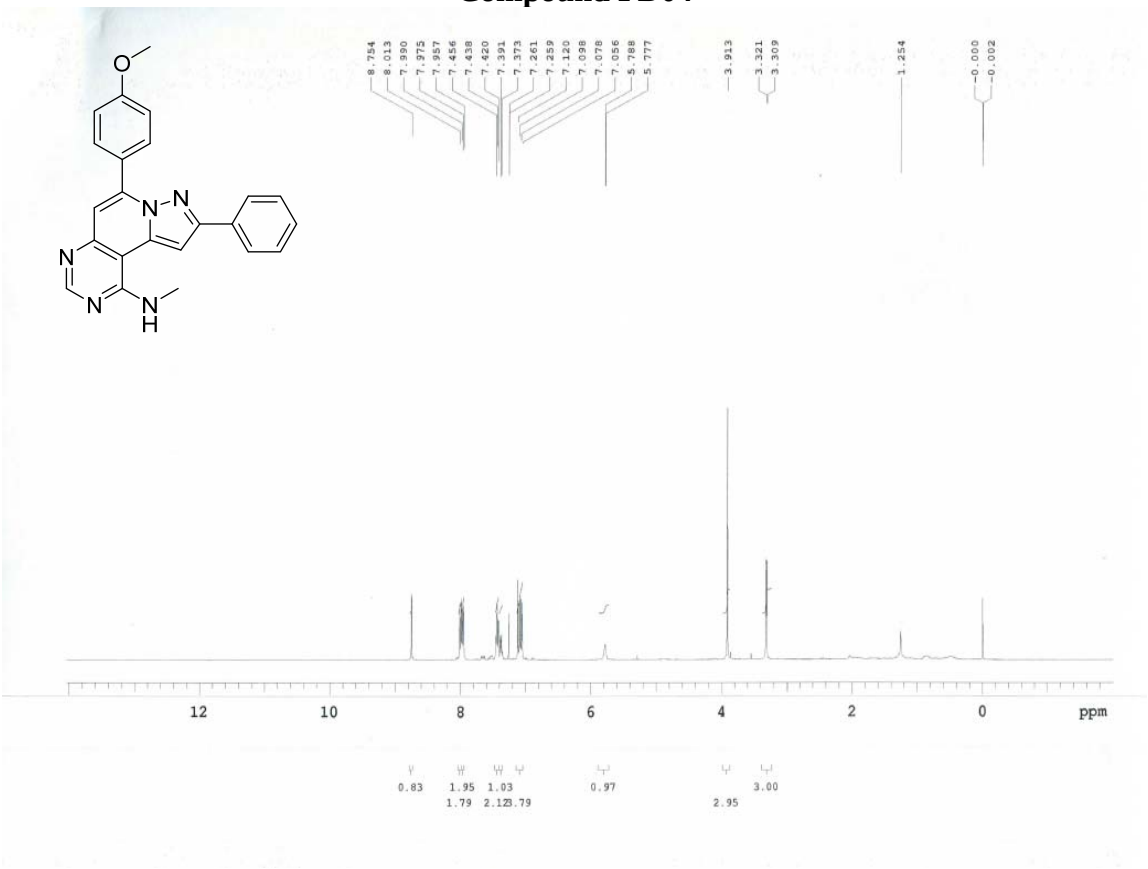
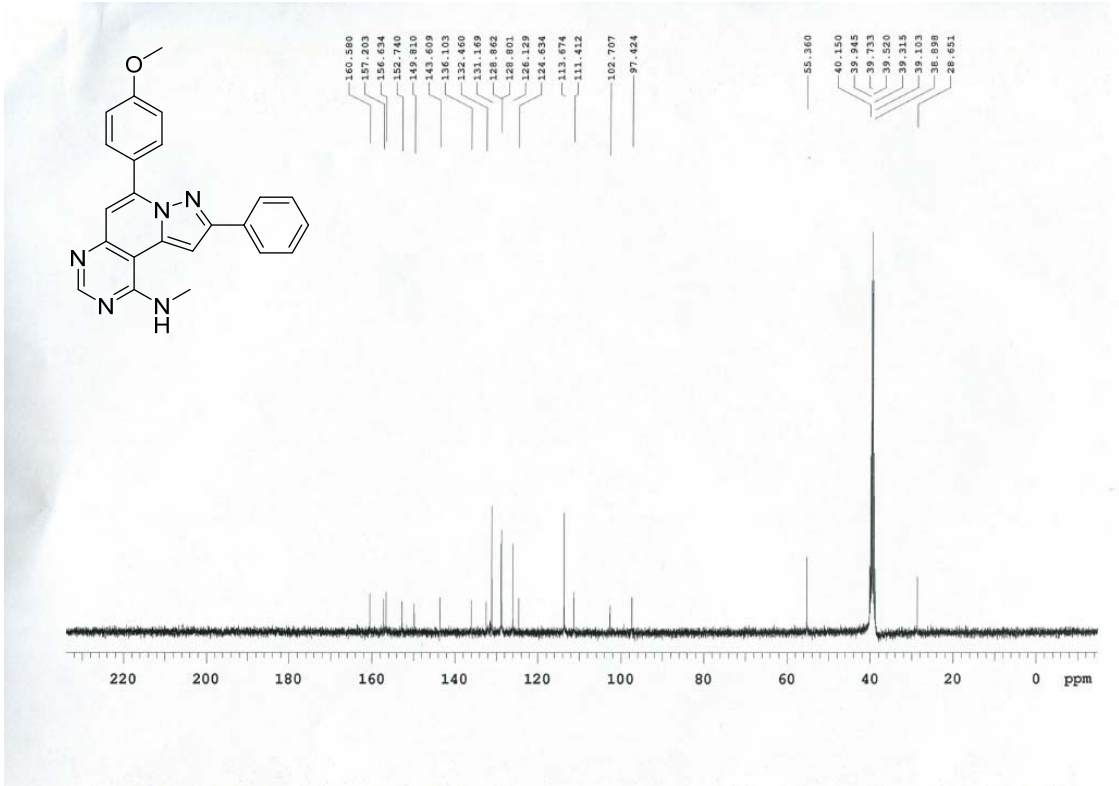




Compound FD04





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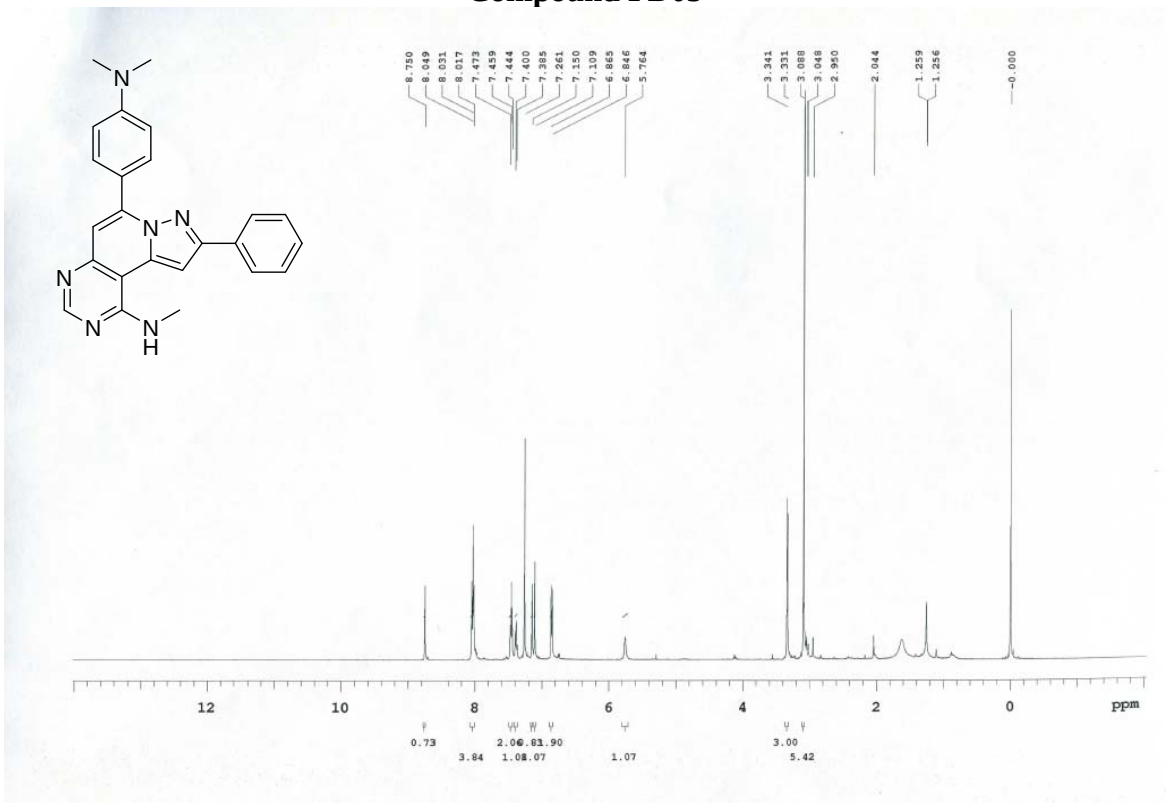

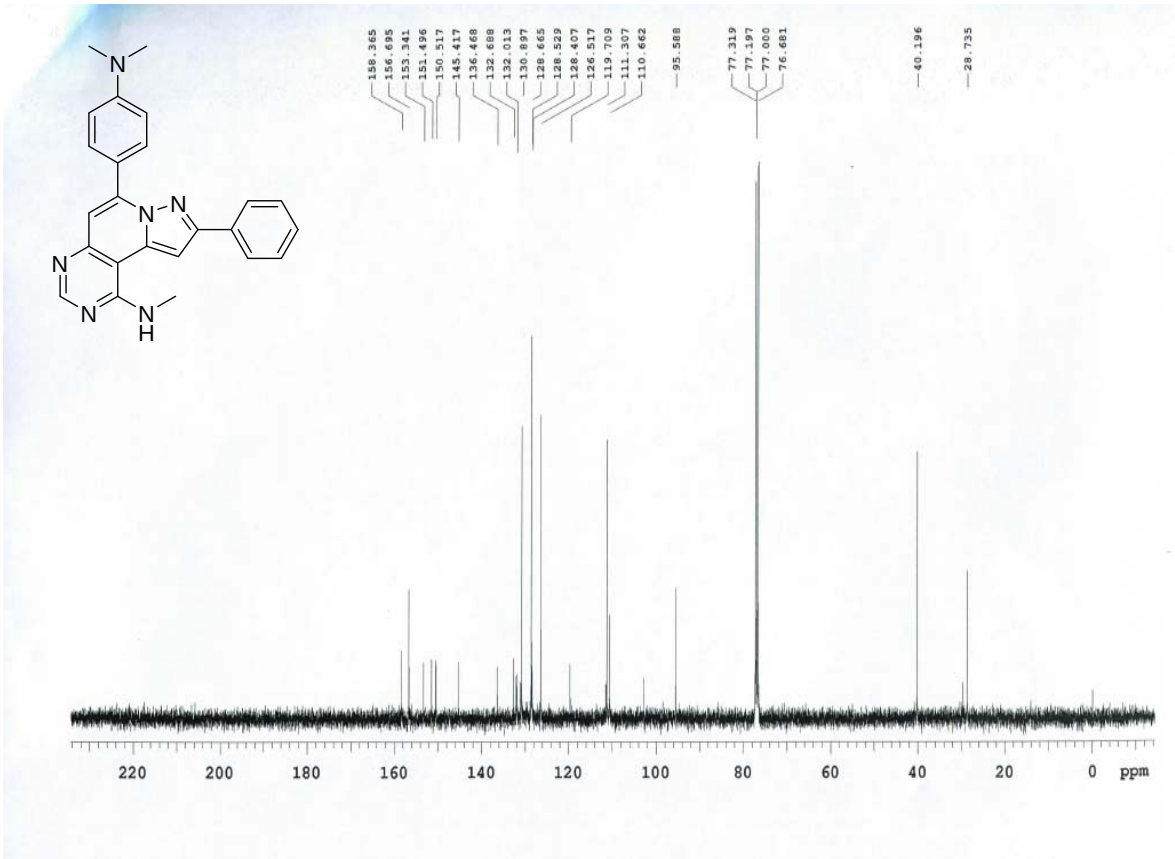

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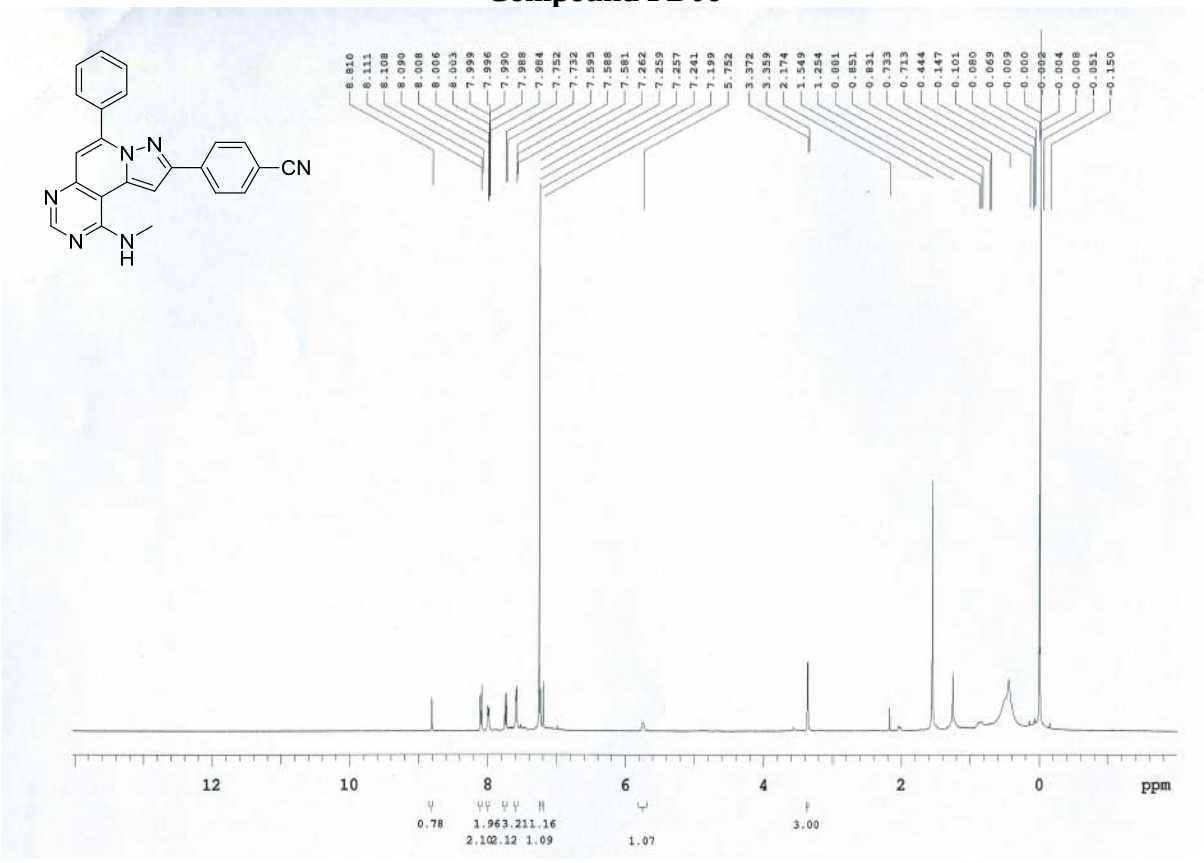

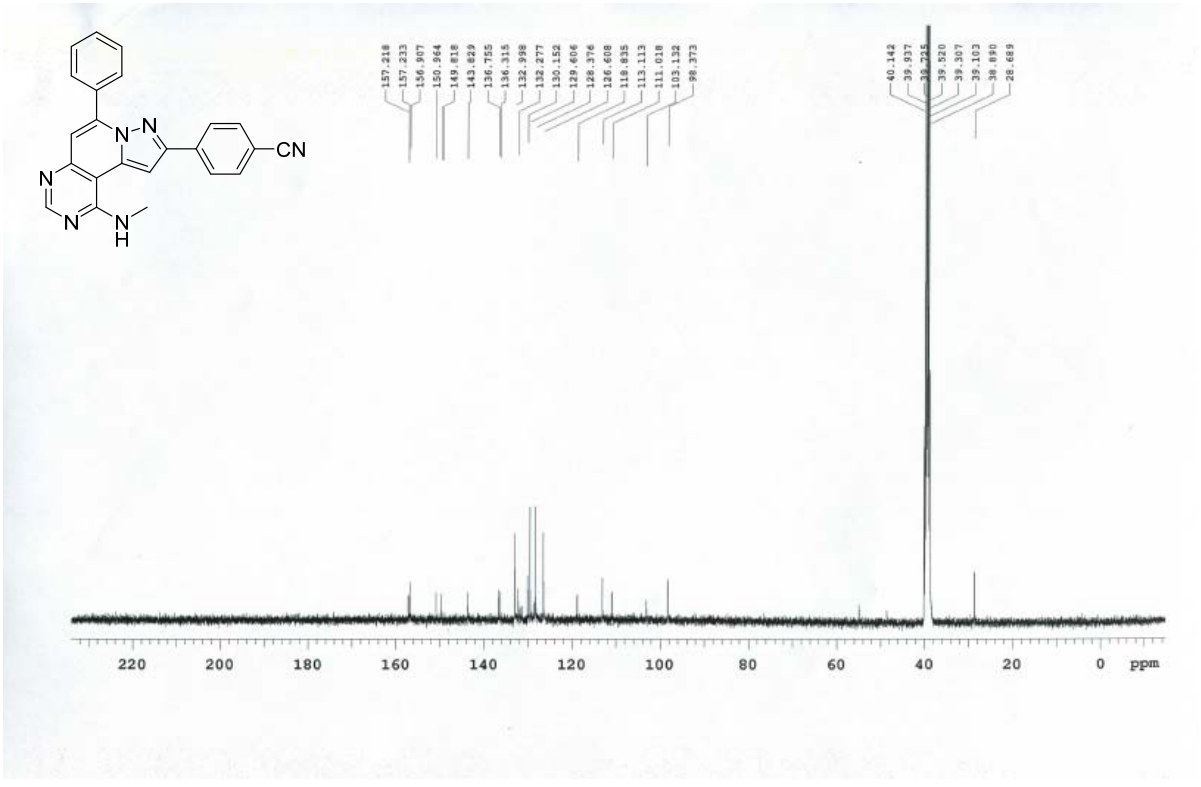

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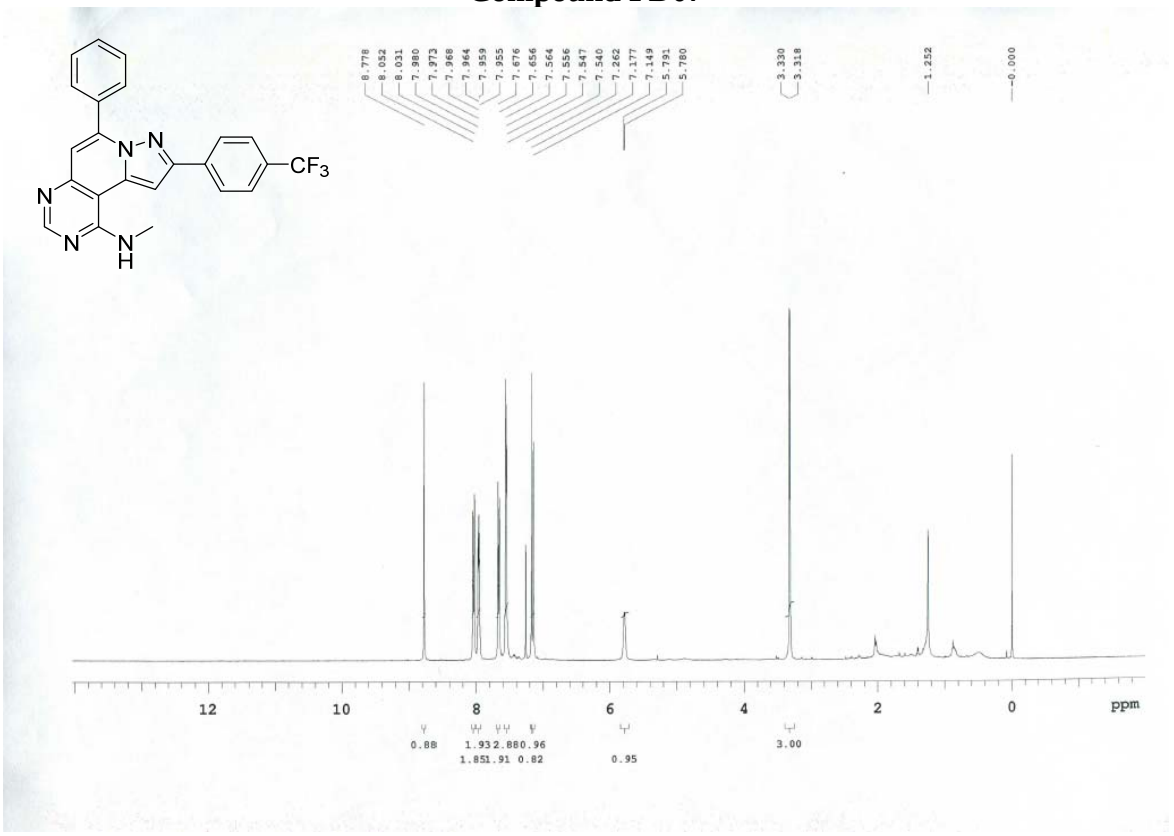
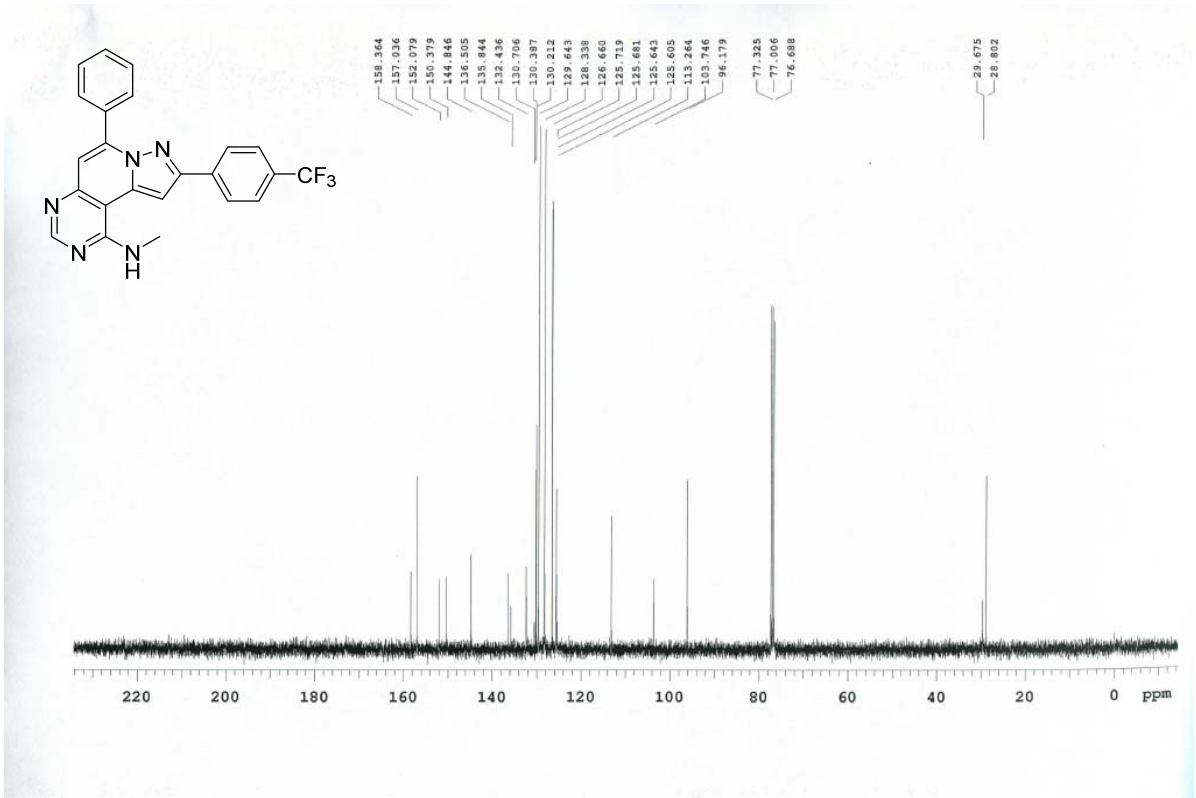
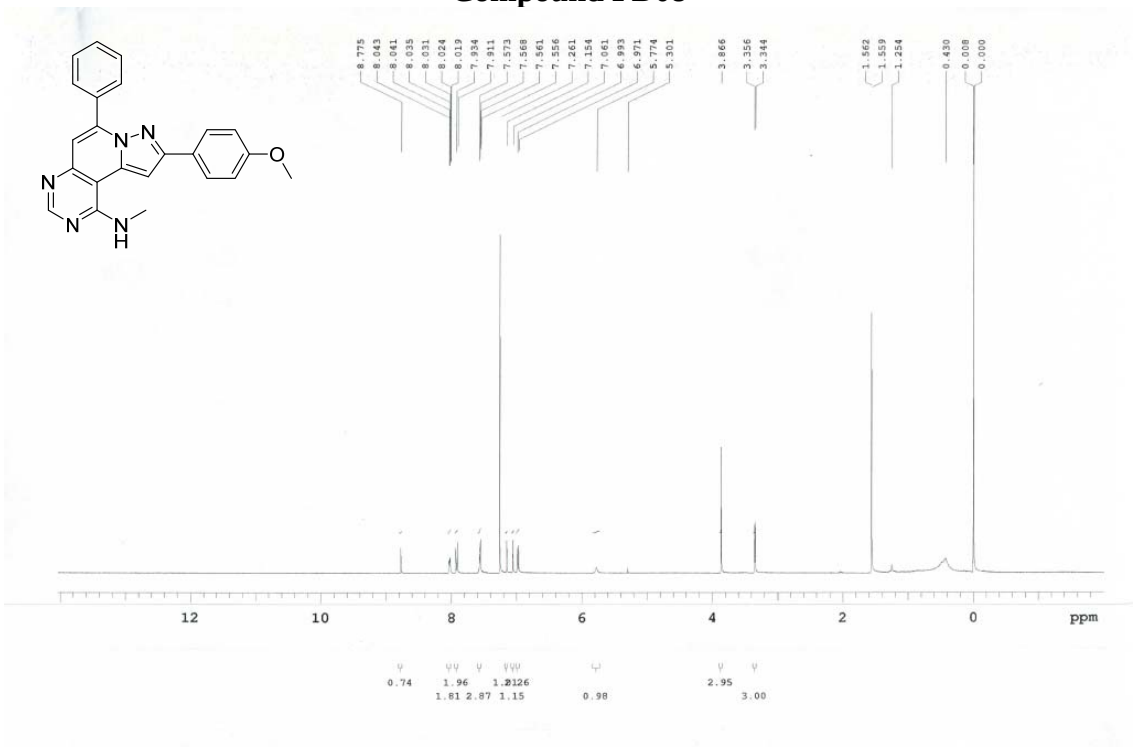
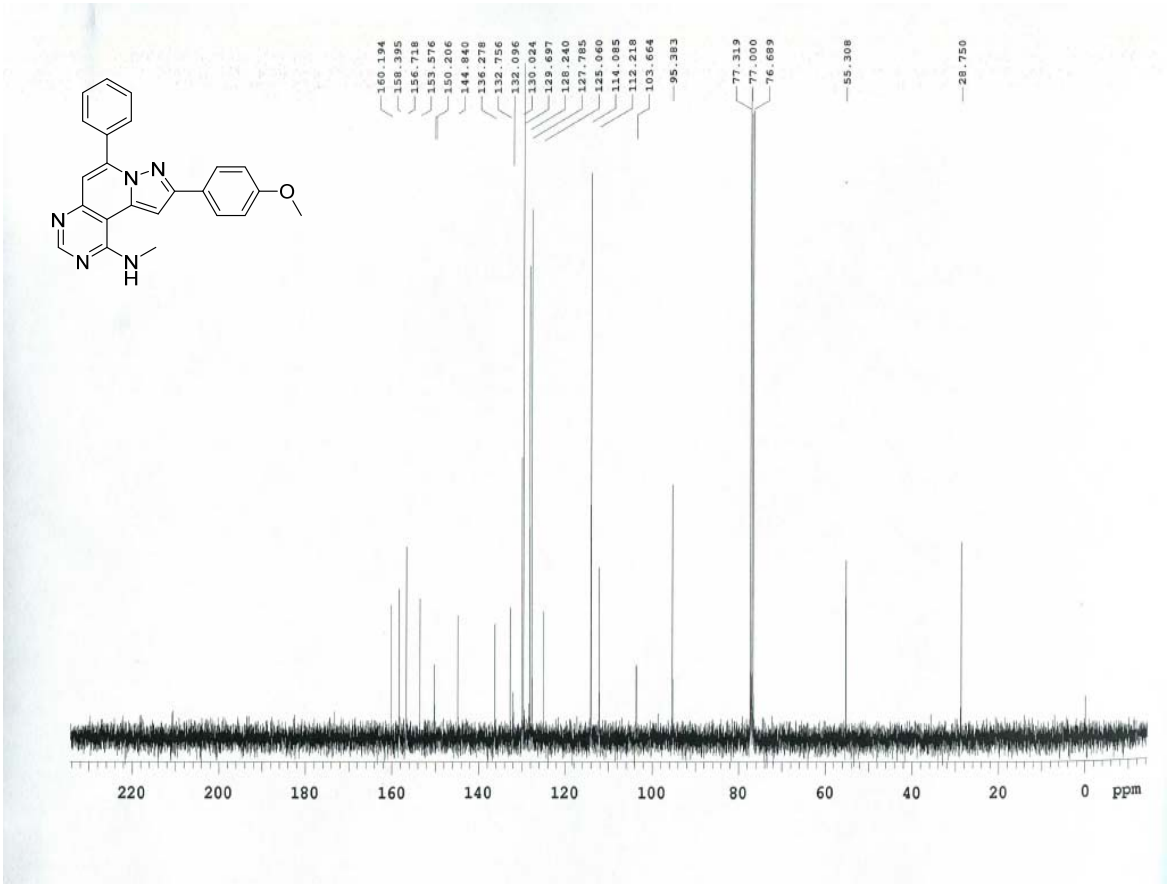


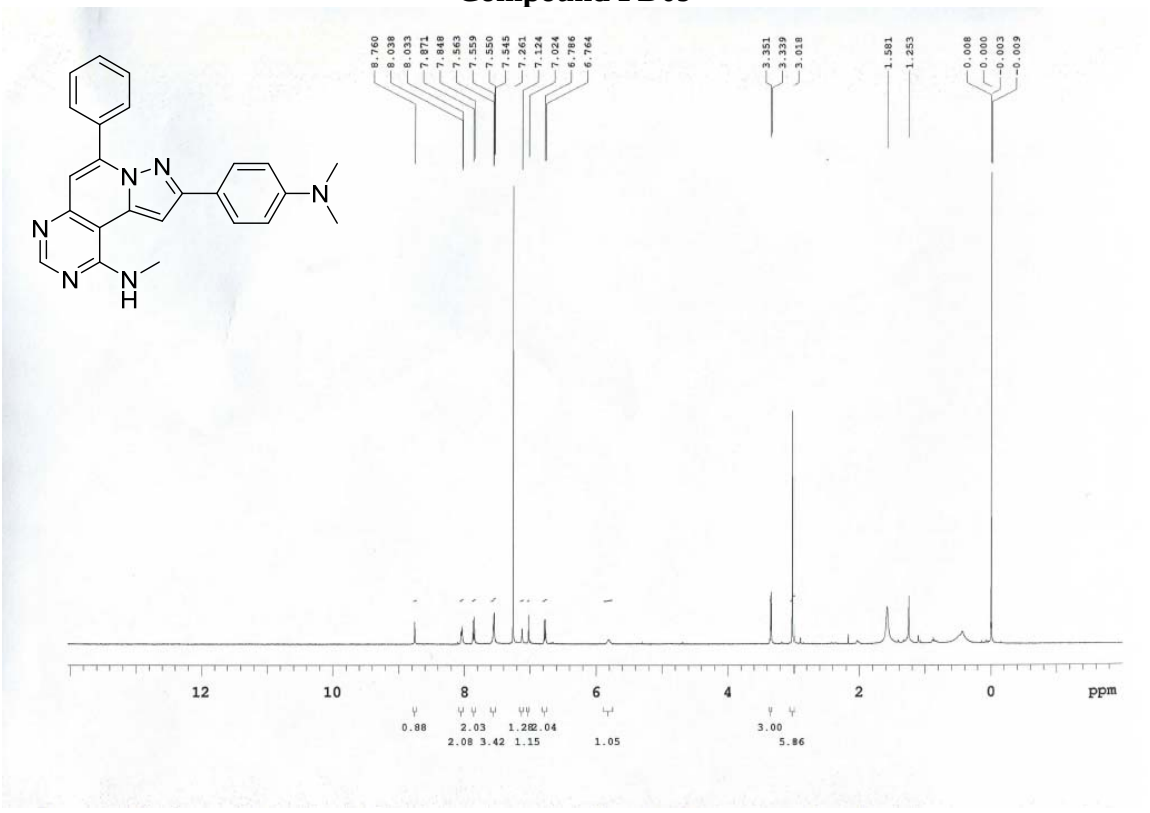


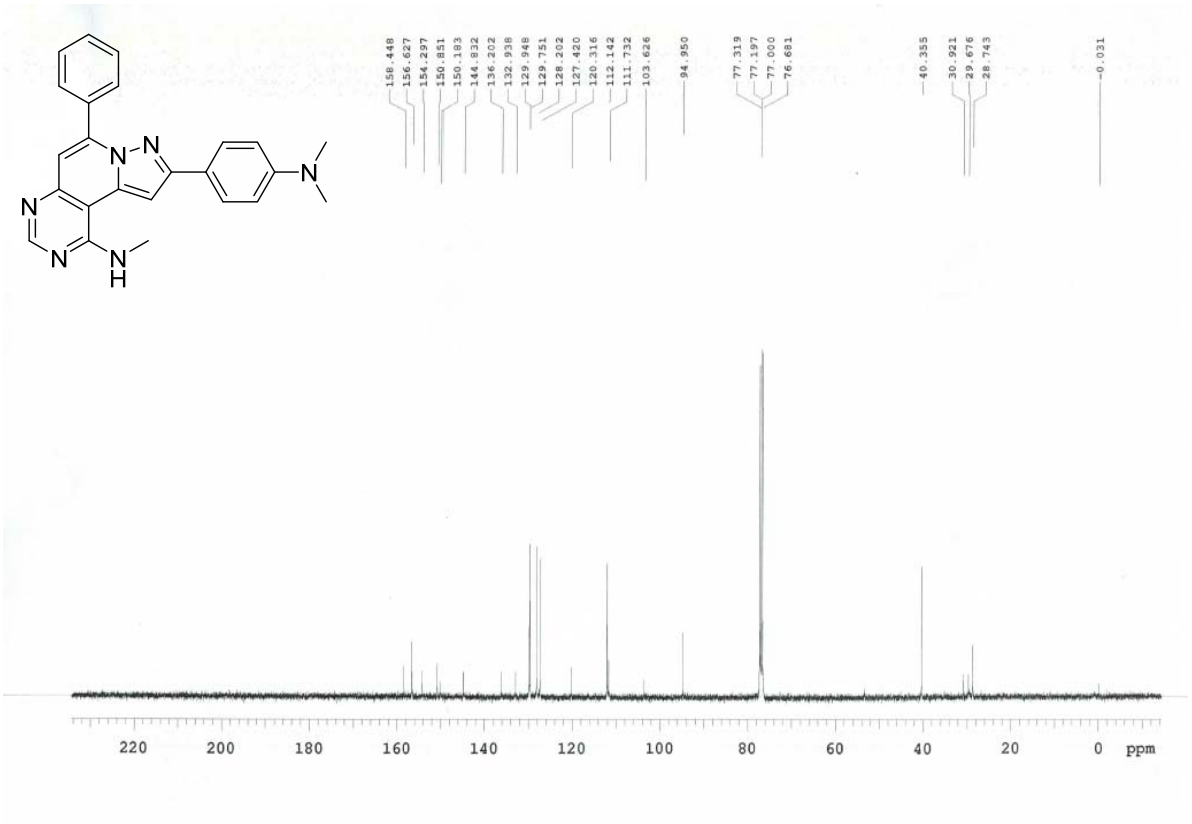
Compound FD08



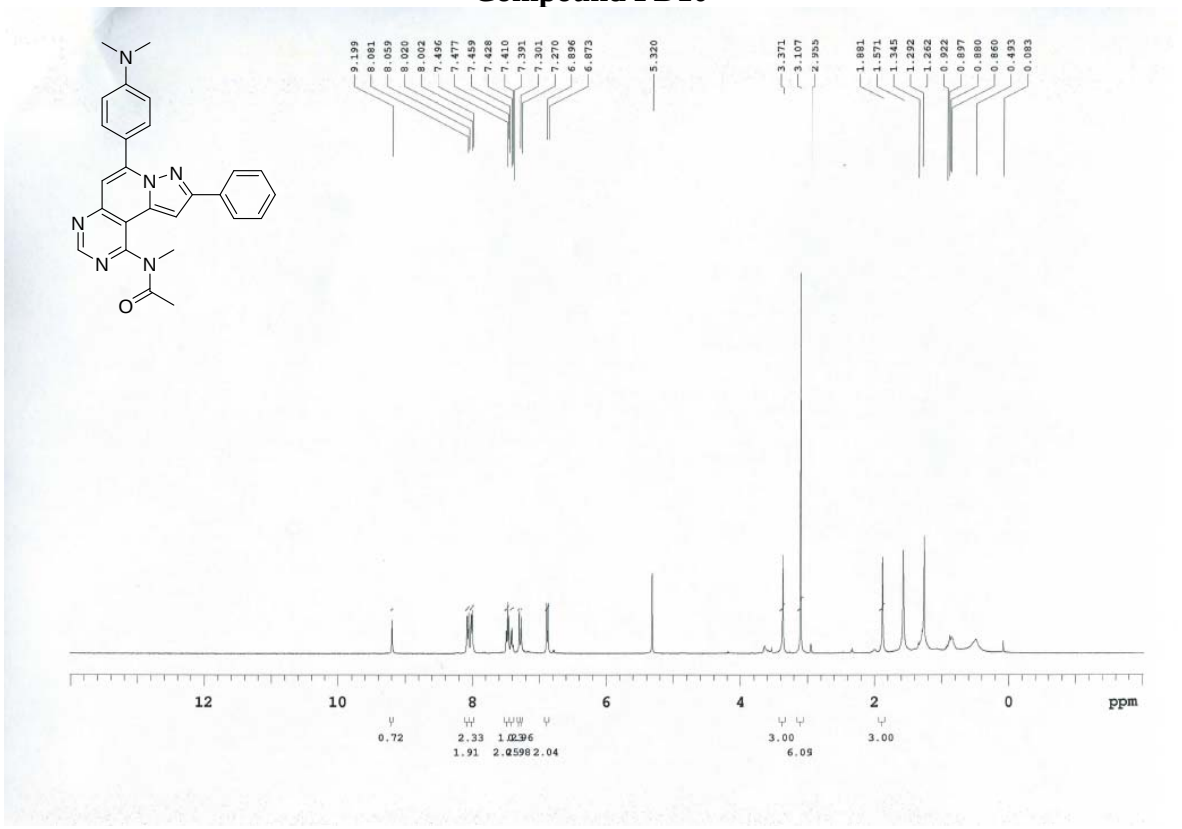


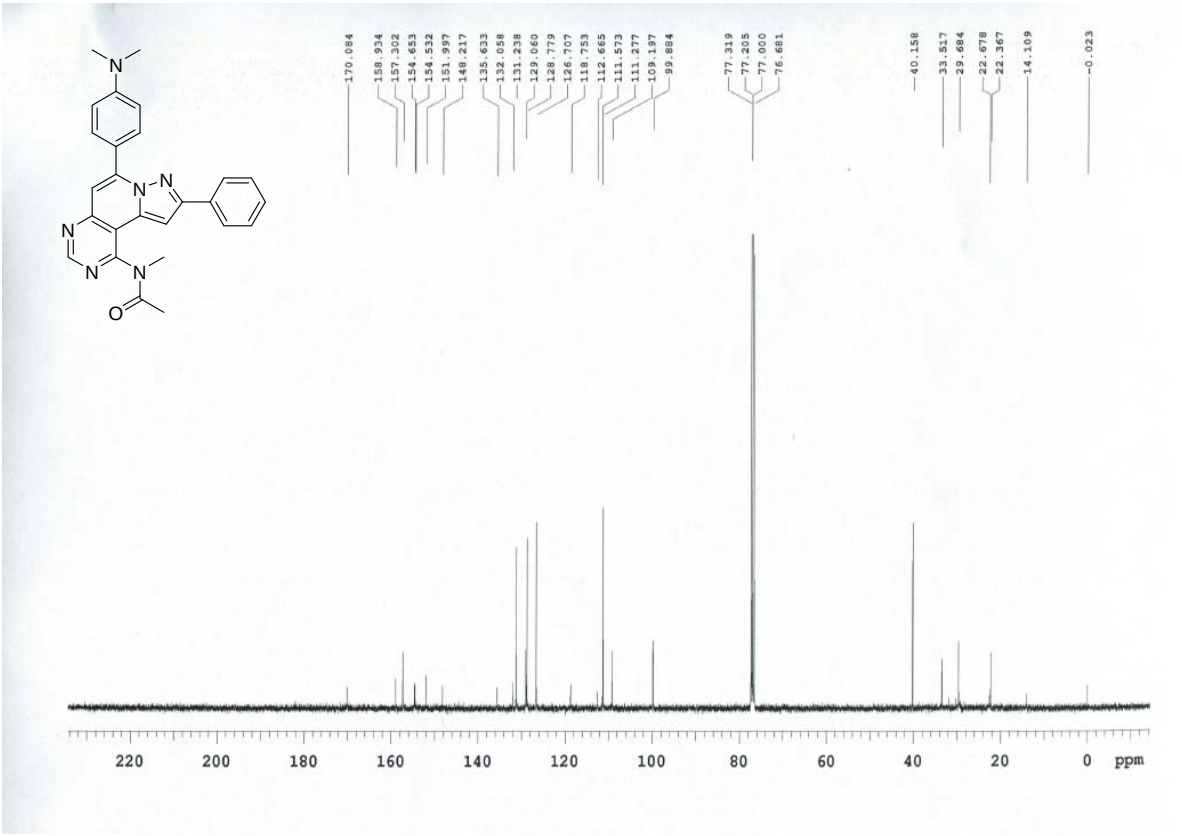
Compound FD09



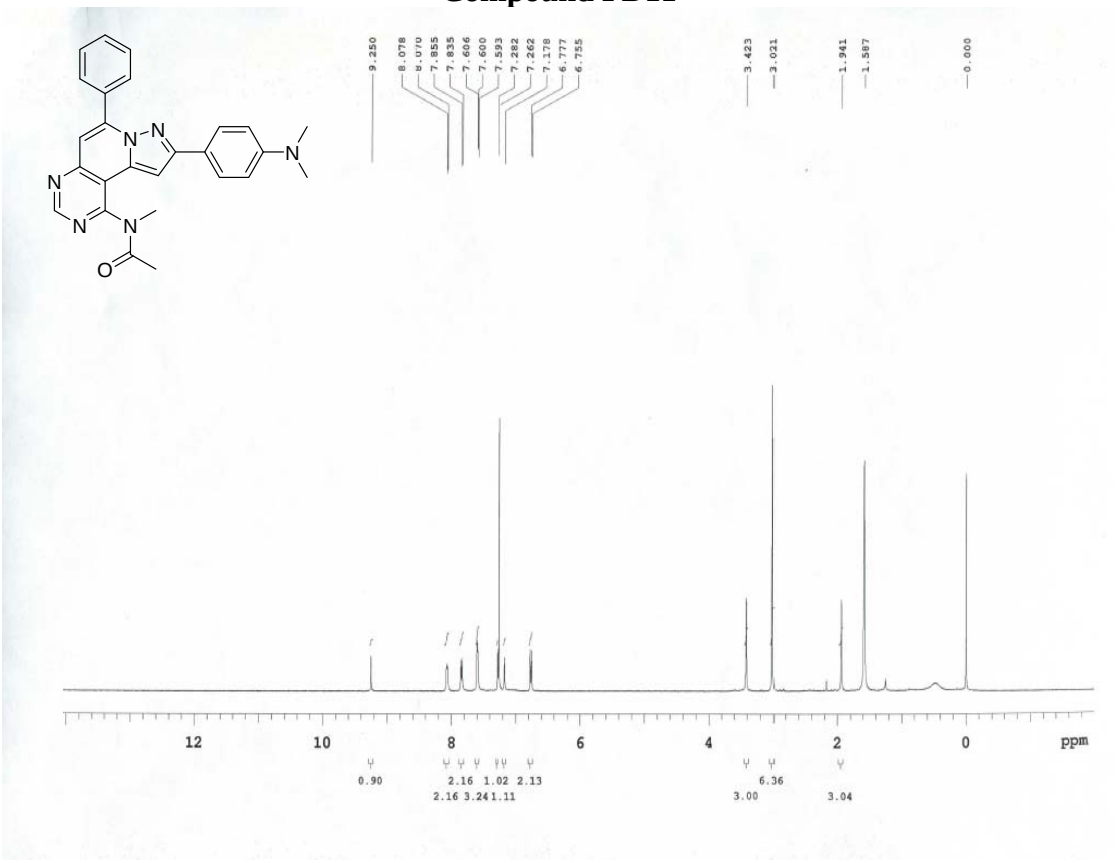


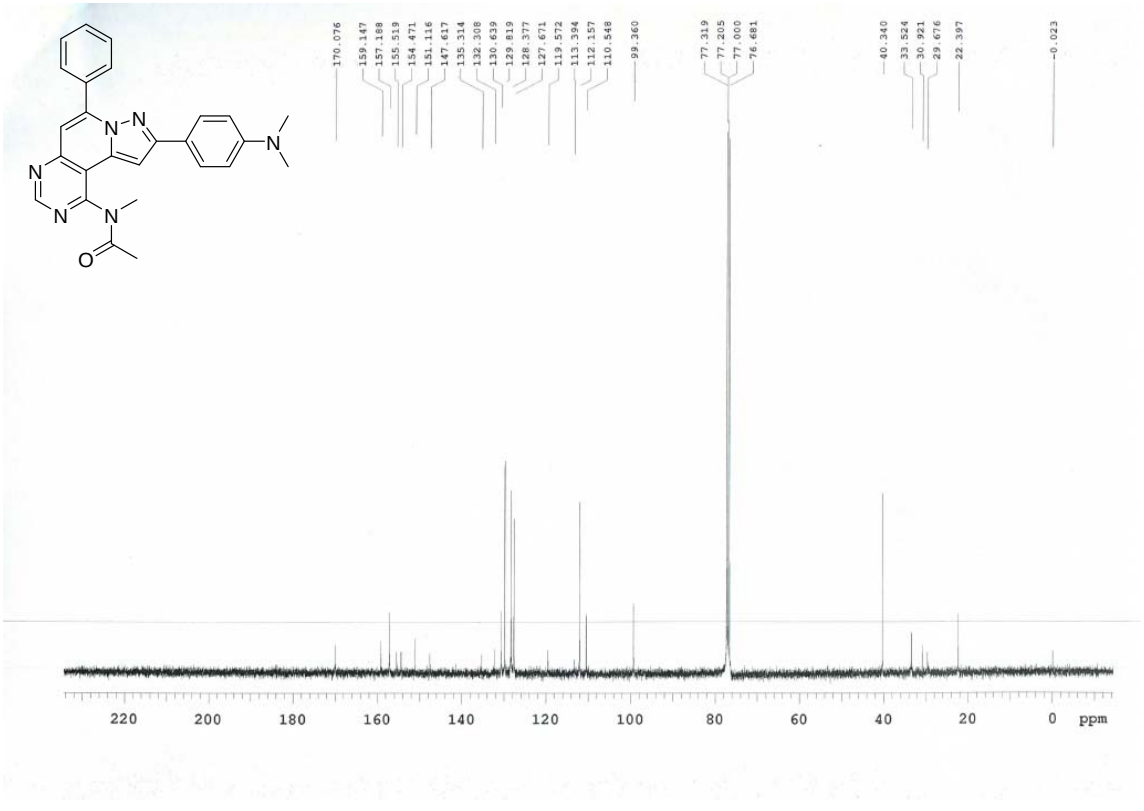
Compound FD10



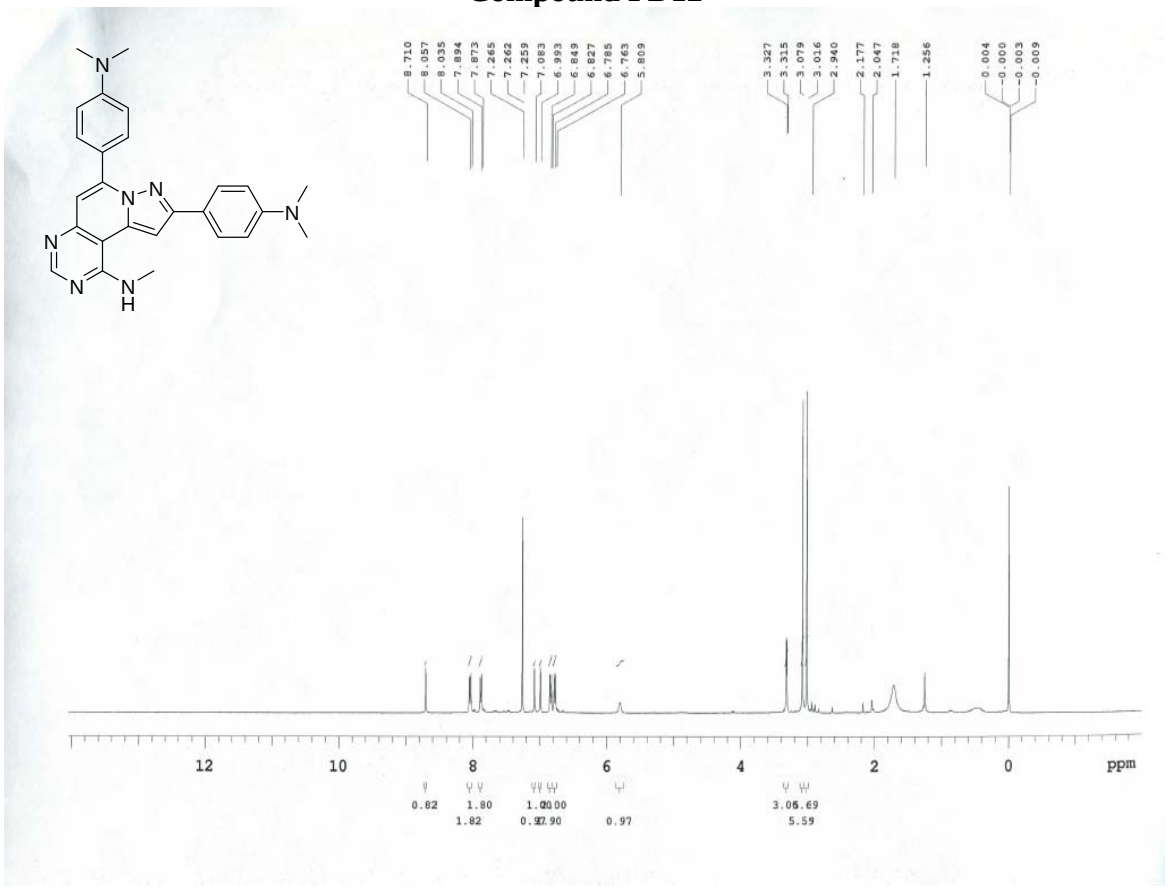


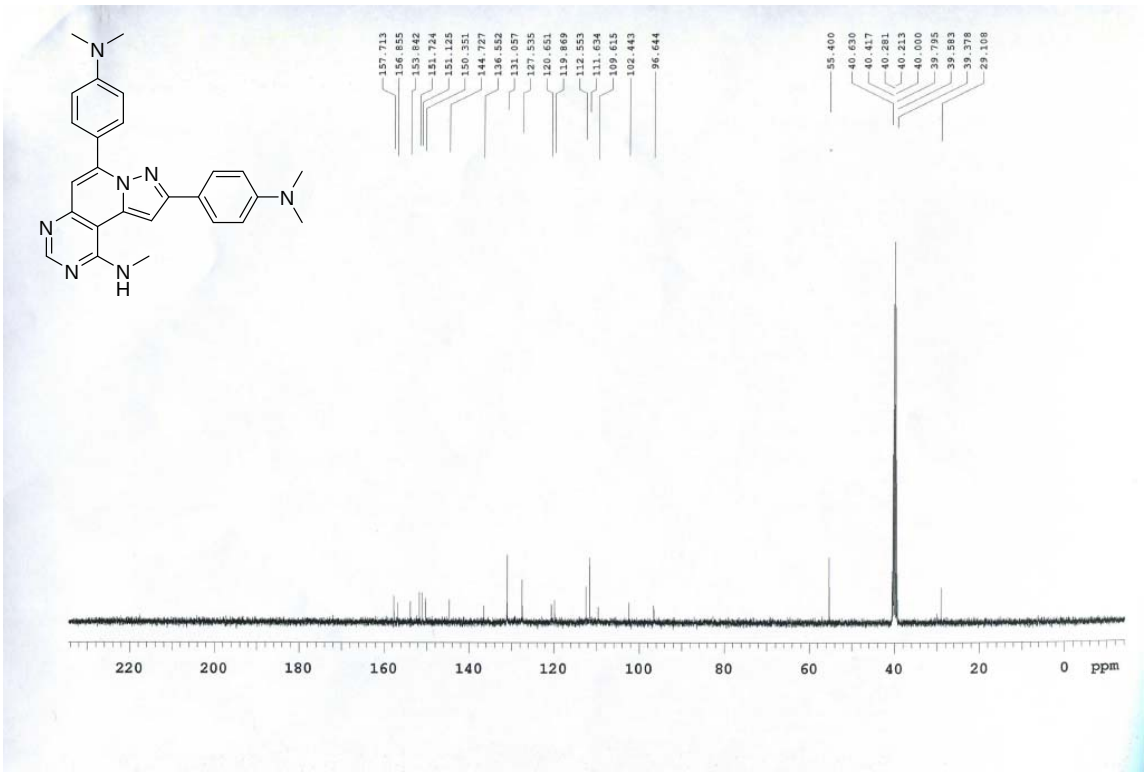
Compound FD11





Compound FD12





Compound FD13