Supporting Information

A Reaction-Based Fluorescent Probe for Imaging of Formaldehyde in Living Cells

Aaron Roth†, Hao Li†, Chelsea Anorma, Jefferson Chan*

Department of Chemistry, Roger Adams Laboratory, University of Illinois, 600 South Mathews, Urbana, Illinois 61801, United States

Correspondence should be addressed to J.C. (jeffchan@illinois.edu). † denotes equal contribution from A.R. and H.L.

Materials and Methods

Thin-layer chromatography (TLC) was performed on glass-backed TLC plates pre-coated with silica gel containing an UV_{254} fluorescent indicator (Macherey-Nagel). Compounds were visualized with a 254/365nm, handheld UV lamp (UVP). Flash chromatography was performed using 230-400 mesh silica gel P60 (SiliCycle Inc). Solvents used for anhydrous reactions were dried over 3 Å molecular sieves activated via heating under vacuum at 300 °C. All glassware used in anhydrous reactions were flame-dried or heated overnight in an oven at 160 °C and cooled immediately prior to use. 3-Bromoaniline, 3-bromoanisole, 4-bromo-3-methylaniline, 4-nitrobenzaldehyde, *N*-iodosuccinimide, potassium allyltrifluoroborate, sodium triacetoxyborohydride, tetrakis(triphenylphosphine)palladium(0), trifluoromethanesulfonic acid, and trimethylacetylene were purchased from Oakwood Products and used as received. 1-Bromo-3-chloropropane, aluminum trichloride, boron tribromide, copper iodide, dichlorodimethylsilane, formaldehyde solution, methyl chloroformate, phosphorous oxychloride, *sec*-butyllithium solution, and *tert*-butyllithium solution were purchased from Sigma-Aldrich and used without purification. Deuterated solvents were purchased from Cambridge Isotope Laboratories. ER-

Tracker™ Green, LysoTracker® Green DND-26, MitoTracker® Green FM, DAPI, Trypan Blue solution were purchased from Life Technologies.

All buffers used for pH titrations were prepared in deionized water and brought to the appropriate pH with aqueous HCl or NaOH. The buffers used were 50 mM glycine (pH range 2.00-3.50), 50 mM NaOAc (pH range 4.00-5.60), 50 mM MES (pH range 5.60-6.80), 50 mM HEPES (pH range 6.80-8.20), 50 mM Tris (pH range 8.20-9.50) and 50 mM glycine (pH range 9.50-10.40). A 10 mM stock solution of formaldehyde for in vitro titration experiments was prepared by heating a suspension of paraformaldehyde (>88% w/w, 17.6 mg, 0.50 mmol, TCI, lot #NXR2B) in phosphate buffered saline (PBS) buffered to pH 7.4 (50mL) at 88 °C for 1 hr. The solution was cooled to room temperature and filtered through a 0.22 μm syringe filter (Millex).

Spectroscopic Methods

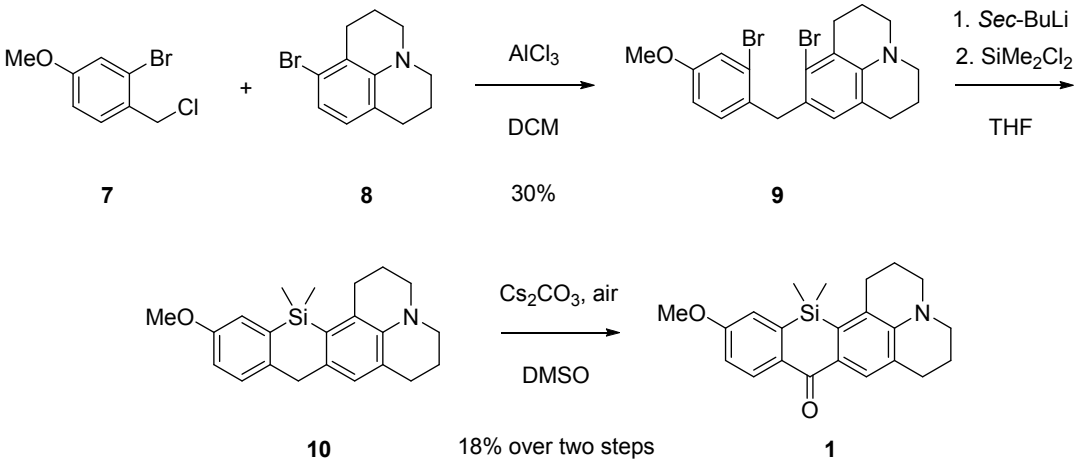
NMR spectra were recorded on Varian 400 or 500 MHz spectrometers at 25 °C. Chemical shifts are reported in ppm (δ) and are referenced to residual protic peaks. The following abbreviations are used to describe coupling constants: singlet (s), doublet (d), triplet (t), quartet (q), doublet doublet (dd), doublet triplet (dt), doublet quartet (dq), doublet doublet triplet (ddt), multiplet (m), and broad singlet (bs). IR spectra were recorded with a PerkinElmer Spectrum Two IR spectrometer. High-resolution mass spectra were acquired with a Waters Q-TOF Ultima ESI mass spectrometer and a Waters Synapt G2-Si ESI/LC-MS mass spectrometer. UV-visible spectra were recorded on a Cary 60 spectrometer. Fluorescence spectra were acquired on a QuantaMaster-400 scanning spectrofluorometer with micro fluorescence quartz cuvettes

(Science Outlet). Flow cytometry was performed on a BD Biosciences LSR II (San Jose, CA, USA), and the data were analyzed as described using FACSDiva software.

Cell Culture and Imaging Materials and Methods

HEK293TN cells were obtained from Prof. Paul Hergenrother (UIUC, Chemistry) and cultured in phenol-red free Dulbecco's modified eagle medium (DMEM, Corning) supplemented with 10% fetal bovine serum (FBS, Sigma Aldrich), and 1% penicillin/streptomycin (Corning). Neuroscreen-1 cells were obtained from Prof. Kai Zhang (UIUC, Biochemistry) and cultured in Ham's F-12K medium (Sigma Aldrich) supplemented with 15% horse serum (Hyclone), 2.5% fetal bovine serum (FBS, Sigma Aldrich), and 1% penicillin/streptomycin (Corning). Cells were incubated at 37°C and 5% CO_2. One day before imaging, cells were passed and plated on 8-well chambered coverglasses (Lab-Tek) at a density of 40,000 cells per mL (or 20,000 cells per well). Cells would reach 70-80% confluency before imaging. Immediately before the experiments, cells were washed with serum-free DMEM, incubated with FP1 in serum-free DMEM, rinsed with fresh media and imaged. Samples of HEK293TN and NS1 cells for flow cytometry were prepared by passaging and seeding each well of a 6-well cluster culture dish (Cyto-One) with 300,000 cells one day before experiments. Cells had reached 70% confluency before staining with 2 μM FP1 for 8 mins. Stained cells were aspirated and serum-free DMEM added. Subsequently, a 10x concentrated FA solution was added to give a final concentration of 1.0 mM. 2.5 mM and 5 mM. After incubation cells with FA, the media was removed and replaced with serum-free DMEM. Finally, cells were trypsinized, pelleted via centrifugation, resuspended in PBS for flow cytometric analysis.

Syntheses



Scheme 1. Synthesis of Si-xanthone (1).

2-Bromo-4-methoxybenzyl chloride (7). A two-neck round-bottom flask was charged with 3-bromoanisole (35.0 mL, 276.5 mmol, 1.0 eq.) and formaldehyde (37 wt % in H_2O, 38.0 mL, 1.38 mol, 5.0 eq.). Hydrogen chloride gas, generated from conc. hydrochloric acid and conc. sulfuric acid, was bubbled through the reaction mixture which was stirred at 50 °C for 6 hrs. Upon cooling to room temperature, the reaction was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic fractions were washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude residue was purified by fractional distillation to afford the title compound as a colorless oil (43.1 g, 183.1 mmol, 66.3% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, J = 8.5 Hz, 1H), 7.13 (d, J = 2.6 Hz, 1H), 6.85 (dd, J = 8.5, 2.6 Hz, 1H), 4.68 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.5, 132.0, 129.0, 125.0, 118.6, 114.0, 55.8, 46.5. IR (neat): 3008, 2966, 2937, 1602, 1492, 1263, 1242,

1028, 865, 843, 729, 661 cm^{-1}. HR-MS calculated for C_8H_8BrClO $[M\text{-}HCl]^+$ *m/z* 198.9753, found 198.9759.

8-Bromo-1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinoline (8). A round-bottom flask was charged with 3-bromoaniline (8.0 mL, 73.1 mmol, 1.0 eq.), 1-bromo-3-chloropropane (58.5 mL, 584.6 mmol, 8.0 eq.), and Na_2CO_3 (31.0 g, 292.3 mmol, 4.0 eq.). After stirring at 140 °C for 48 hrs, the reaction cooled to room temperature, transferred to a separatory funnel, and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Residual 1-bromo-3chloropropane was removed at 60 °C, 1 Torr. The crude dialkylated intermediate was dissolved in DMF (15.0 mL) and stirred at 160 °C for 24 hrs. After cooling to room temperature, the solution was concentrated under reduced pressure, washed with H_2O (200 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified via flash chromatography on a silica column (1:99 v/v EtOAc:Hexanes) to afford the title compound as a light yellow oil (12.0 g, 47.6 mmol, 65.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (d, *J* = 8.0 Hz, 1H), 6.66 (d, *J* = 8.0 Hz, 1H), 3.14 (dt, *J* = 12.5, 5.7 Hz, 4H), 2.80 (t, *J* = 6.7 Hz, 2H), 2.72 (t, *J* = 6.5 Hz, 2H), 2.07–1.87 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.8, 128.0, 123.2, 120.9, 120.8, 119.6, 50.2, 49.9, 28.8, 27.8, 22.2, 22.1. IR (neat): 3009, 2935, 1583, 1488, 1456, 1440, 1388, 1327, 1207, 1186, 1067, 1038, 792, 755, 578, 463 cm^{-1}. HR-MS calculated for $C_{12}H_{14}BrN$ $[M+H]^+$ *m/z* 252.0388, found 252.0393.

8-Bromo-9-(2-bromo-4-methoxybenzyl)-2,3,6,7-tetrahydro-1H,5H-pyrido[3,2,1-ij]quinoline (**9**). A flame-dried round-bottom flask was charged with compound **8** (5.0 g, 19.8 mmol, 1.0 eq.)

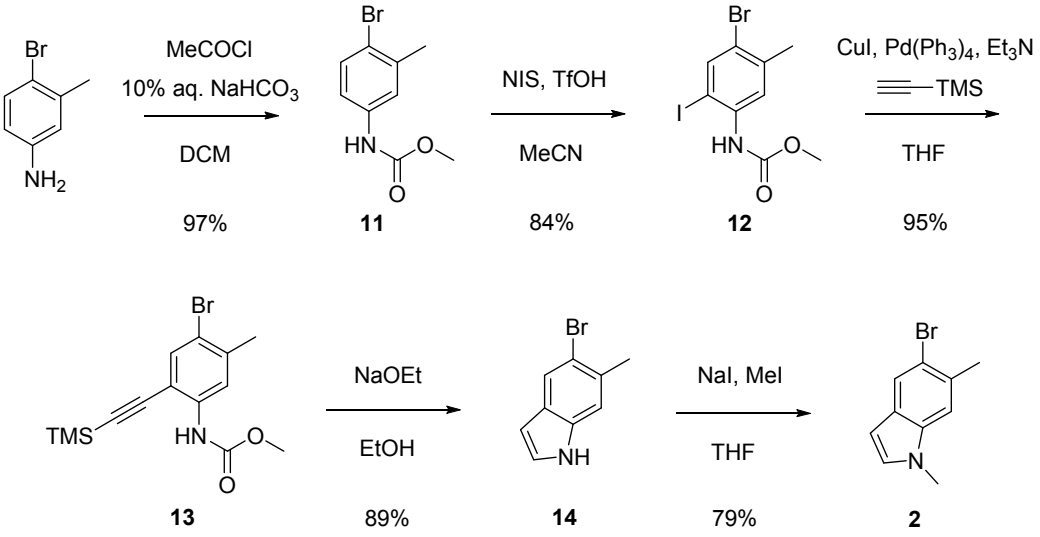
and anhydrous CH_2Cl_2 (200 mL). $AlCl_3$ (3.17 g, 23.8 mmol, 1.2 eq.) was added to the reaction in one portion. The resultant mixture was sonicated under a nitrogen atmosphere for 30 min. A solution of compound **8** (6.07 g, 25.8 mmol, 1.4 eq.) in anhydrous CH_2Cl_2 (7.0 mL) was transferred to the reaction via dropwise syringe addition. After overnight stirring at room temperature, the reaction was quenched by slow addition of sat. $NaHCO_3$ (~200 mL) and filtered through a bed of celite which was washed with CH_2Cl_2 (100 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic fractions were washed with brine (100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified via flash chromatography on a silica column (4:96 v/v EtOAc:Hexanes) to afford the title compound as a brown oil. (2.71 g, 6.0 mmol, 30.0% yield). Unreacted compound **8** was recovered via flash chromatography on a silica column. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (d, *J* = 2.6 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.79 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.51 (s, 1H), 4.04 (s, 2H), 3.80 (s, 3H), 3.22–3.13 (m, 2H), 3.13–3.08 (m, 2H), 2.86 (t, *J* = 6.7 Hz, 2H), 2.67 (t, *J* = 6.6 Hz, 2H), 2.07–2.00 (m, 2H), 2.00–1.94 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 143.4, 132.3, 131.1, 128.9, 126.5, 125.9, 125.2, 121.6, 121.0, 118.0, 113.7, 55.7, 50.3, 49.8, 41.4, 29.7, 27.8, 22.5, 22.2. IR (neat): 3006, 2938, 2834, 1488, 1458, 1441, 1331, 1305, 1283, 1233, 1205, 1183, 1037, 858, 751, 725, 667, 547, 463 cm^{-1}. HR-MS calculated for $C_{20}H_{21}Br_2NO$ $[M+H]^+$ *m/z* 450.0068, found 450.0055.

12-methoxy-14,14-dimethyl-2,3,6,7,9,14-hexahydro-1H,5H-benzo[5,6]silino[2,3-f]pyrido[3,2,1-ij]quinoline (**10**): A flame-dried round-bottom flask was charged with compound **9** (2.71 g, 6.0 mmol, 1.0 eq.) and anhydrous THF (100 mL). An oven-dried addition funnel was attached to the flask and the system was flushed with nitrogen. The reaction was cooled to -78

°C and treated with 1.4 M *sec*-butyllithium in cyclohexane (12.9 mL, 18.0 mmol, 3.0 eq.) via funnel addition over 30 min. After stirring at the same temperature for 10 min, a solution of $SiMe_2Cl_2$ (1.36 mL, 11.5 mmol, 1.9 eq.) in anhydrous THF (11.5 mL) was added dropwise over 20 min. The reaction was warmed to room temperature and stirred overnight. The volatiles were removed under reduced pressure to obtain the crude product which was washed with sat. $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic fractions were washed with brine (100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure to afford the title compound as a brown oil which was sufficiently pure to use without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 2.7 Hz, 1H), 6.88 (dd, *J* = 8.3, 2.7 Hz, 1H), 6.85 (s, 1H), 4.00 (s, 2H), 3.86 (s, 3H), 3.16 (s, 2H), 2.99 (s, 1H), 2.81 (s, 1H), 2.20 – 1.96 (m, 4H), 0.56 (d, *J* = 1.3 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 141.4, 138.6, 137.4, 134.2, 131.3, 128.6, 127.9, 127.7, 123.9, 118.6, 114.3, 55.5, 50.8, 50.2, 40.3, 29.9, 28.2, 22.9, 22.4, -0.4. IR (neat): 2933, 2833, 1682, 1596, 1548, 1304, 1246, 1039, 820, 768, 649, 453 cm^{-1}. HR-MS calculated for $C_{22}H_{27}NOSi$ $[M+H]^+$ *m/z* 350.1940, found 350.1940.

12-Methoxy-14,14-dimethyl-2,3,5,6,7,14-hexahydro-1H,9H-benzo[5,6]silino[2,3-f]pyrido[3,2,1-ij]quinolin-9-one (**1**): A round-bottom flask was charged with compound **10** (2.1 g, 6.0 mmol, 1.0 eq.), Cs_2CO_3 (5.9 g, 18.0 mmol, 3.0 eq.), and DMSO (20 mL). The reaction mixture was heated at 90 °C with the flask open to the atmosphere for 3 days. After cooling to room temperature, the reaction was diluted with CH_2Cl_2 (50 mL) and filtered through a bed of celite. The filtrate was diluted with brine (150 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic fractions were washed with brine (100 mL), dried (Na_2SO_4), filtered, and

concentrated under reduced pressure. The crude product was purified via flash chromatography on a silica column to afford the title compound as a yellow powder (412 mg, 1.1 mmol, 18.3% yield over two-steps beginning from compound **3**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 8.9 Hz, 1H), 8.09 (s, 1H), 7.08 (d, *J* = 2.8 Hz, 1H), 7.02 (dd, *J* = 8.9, 2.8 Hz, 1H), 3.90 (s, 3H), 3.30 (m, 4H), 2.93 (t, *J* = 6.3 Hz, 2H), 2.83 (t, *J* = 6.4 Hz, 2H), 2.13–1.88 (m, 4H), 0.53 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.7, 170.6, 161.8, 145.7, 142.2, 135.4, 134.2, 131.9, 130.6, 128.6, 124.8, 123.1, 117.6, 115.1, 55.5, 50.6, 50.1, 29.1, 28.4, 22.0, 21.7, -0.1. m.p. = 130 °C (decomp). IR (neat): 3012, 2972, 1739, 1585, 1366, 1231, 834, 765 cm^{-1}. HR-MS calculated for $C_{22}H_{25}NO_2Si$ $[M+H]^+$ *m/z* 364.1733, found 364.1740.



Scheme 2. Synthesis of 5-Bromo-1,6-dimethyl-1*H*-indole (2).

Methyl (4-bromo-3-methylphenyl)carbamate (**11**). A solution of 4-bromo-3-methylaniline (5.25 g, 28.2 mmol, 1.0 eq.), CH_2Cl_2 (50 mL), and 10% aq. $NaHCO_3$ (40 mL) was cooled in an ice-bath. Methyl chloroformate (4.0 mL, 51.8 mmol, 1.8 eq.) was added dropwise to the rapidly

stirred solution. The reaction was subsequently warmed to room temperature and stirred for 2 hrs. The reaction was then diluted with CH_2Cl_2 (50 mL) and sequentially washed with sat. NH_4Cl (50 mL), sat. $NaHCO_3$ (50 mL) and brine (50 mL). The organic fraction was dried (Na_2SO_4), filtered and concentrated to afford the crude residue as a brown solid which was purified via flash chromatography on a silica column (1:1 v/v EtOAc:Hexanes) to afford the title compound as a white solid (6.65 g, 27.3 mmol, 96.6% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.6 Hz, 1H), 7.28 (s, 1H), 7.18 (s, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 3.75 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.5, 138.7, 137.4, 132.8, 121.3, 118.8, 118.1, 77.7, 77.4, 77.2, 52.7, 23.2. m.p. = 71-72 °C. IR (neat): 3328, 1705, 1584, 1275, 1233, 1071, 1023, 826, 765, 679, 653 cm^{-1}. HR-MS calculated for $C_9H_{10}BrNO_2$ $[M+H]^+$ *m/z* 243.9973, found 243.9977.

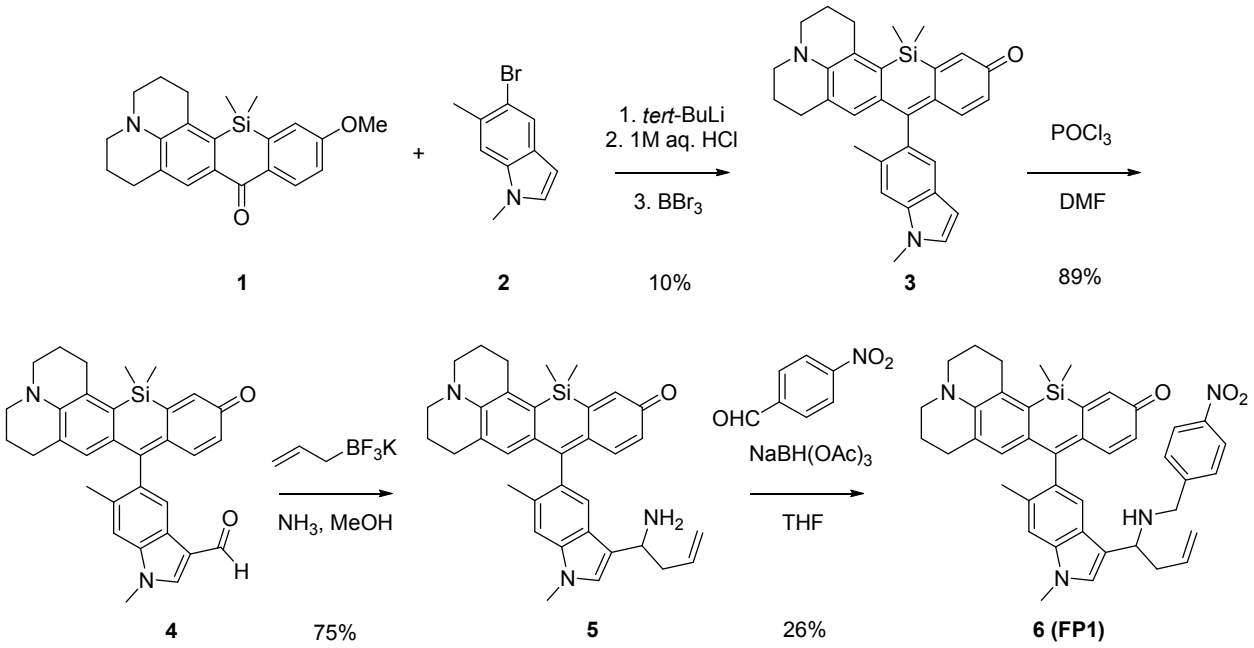
Methyl (4-bromo-2-iodo-5-methylphenyl)carbamate (12). A solution of compound **11** (32.0 g, 131.1 mmol, 1.0 eq.) in MeCN (150 mL) was cooled to 0°C in an ice-bath. *N*-iodosuccinimide (30.1 g, 137.6 mmol, 1.05 eq.) was added in one portion to the stirred solution. Trifluoromethanesulfonic acid (1.2 mL, 13.1 mmol, 0.1 eq.) was then added dropwise. The reaction was subsequently warmed to room temperature and stirred overnight. After overnight stirring, the resulting white solid was filtered, washed with cold MeCN, and dried under reduced pressure (42.2 g, 110.1 mmol, 84.0% yield). The compound was used in the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.85 (s, 1H), 6.87 (s, 1H), 3.80 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.9, 141.0, 139.5, 137.8, 121.9, 119.5, 85.5, 52.9, 23.3. m.p. 130-131 °C. IR (neat): 3276, 2939, 1690, 1603, 1491, 1275, 1241, 1042, 873, 863, 772, 606 cm^{-1}. HR-MS calculated for $C_9H_{10}BrINO_2$ $[M+H]^+$ *m/z* 369.8940, found 369.8945.

Methyl (4-bromo-5-methyl-2-((trimethylsilyl)ethynyl)phenyl)carbamate (13). A flame-dried round-bottom flask was charged with compound **12** (5.38 g, 14.5 mmol, 1.0 eq.), CuI (0.28 g, 1.5 mmol, 0.1 eq.), and $Pd(PPh_3)_4$ (0.84 g, 0.7 mmol, 0.05 eq.) and then dried under vacuum for 30 min. The solids were then dissolved in anhydrous THF (35 mL) and treated sequentially with trimethylsilylacetylene (2.3 mL, 16.0 mmol, 1.1 eq.) and anhydrous triethylamine (8.1 mL, 58.2 mmol, 4.0 eq.). The reaction was stirred at room temperature for 1 hr. All volatiles were removed under reduced pressure and the resultant residue was dissolved in EtOAc (50 mL) and washed with brine (3 × 50 mL). The organic fraction was dried (Na_2SO_4), filtered and concentrated to afford the crude product as a dark brown solid which was purified via flash chromatography on a silica column (3:97 v/v EtOAc:Hexanes) to afford the title compound as an orange solid (4.72g, 13.9 mmol, 95.4% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.39 (s, 1H), 7.24 (s, 1H), 3.71 (s, 3H), 2.28 (s, 3H), 0.24 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.4, 140.0, 138.7, 134.6, 119.4, 117.0, 110.6, 102.6, 99.0, 52.5, 23.6, 0.1. m.p. = 73-74 °C. IR (neat): 3394, 2959, 2150, 1740, 1509, 1220, 1070, 863, 839, 762, 574 cm^{-1}. HR-MS calculated for $C_{14}H_{18}BrNO_2Si$ $[M+Na]^+$ *m/z* 362.0182, found 362.0193.

5-Bromo-6-methyl-*1H*-indole (14). To a freshly prepared, 0.7 M sodium ethoxide solution (150 mL, 8.0 eq.) was added compound **13** (4.5 g, 13.3 mmol, 1.0 eq.) The reaction was stirred at 80°C until all starting material was consumed and then the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with brine (3 × 50 mL). The organic fraction was dried (Na_2SO_4), filtered and concentrated to afford the crude residue as a dark brown solid which was purified via flash chromatography on a silica column (3:17 v/v

EtOAc:Hexanes) to afford the title compound as a yellow solid (2.48 g, 11.8 mmol, 88.8% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (bs, NH), 7.88 (s, 1H), 7.26 (s, 1H), 7.16 (t, 1H), 6.52-6.48 (m, 1H), 2.54 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.5, 131.0, 128.0, 125.0, 124.0, 116.7, 112.7, 102.1, 23.8. m.p. = 85-86 °C. IR (neat): 2916, 1738, 1614, 1577, 1506, 1467, 1415, 1315, 1270, 1241, 1202, 964, 881, 842, 761, 730, 692 cm^{-1}. HR-MS calculated for C_9H_8BrN $[M+H]^+$ *m/z* 209.9918, found 209.9915.

5-Bromo-1,6-dimethyl-1H-indole (2). A flame-dried round-bottom flask was charged with compound **14** (5.41 g, 25.8 mmol, 1.0 eq.) and anhydrous THF (50 mL). The solution was cooled in an ice-bath and treated with NaH as a 60% dispersion in mineral oil (1.24 g, 30.9 mmol, 1.2 eq.) and methyl iodide (3.2 mL, 51.5 mmol, 2.0 eq.). After stirring at 0 °C for 2 hrs, the volatiles were removed under reduced pressure. The residue was dissolved with CH_2Cl_2 (100 mL) and washed with brine (50 mL). The organic fraction was (Na_2SO_4), filtered and concentrated to afford the crude product which was purified via flash chromatography on a silica column (7:193 v/v EtOAc:Hexanes) to afford the title compound as a yellow solid (4.56 g, 20.3 mmol, 79.1% yield**).** ^{1}H NMR (500 MHz, Acetone-d_6) δ 10.27 (bs, NH), 7.82 (s, 1H), 7.41 (s, 1H), 7.31 (dd, *J* = 3.2, 2.4 Hz, 1H), 6.45 (td, *J* = 2.1, 1.0 Hz, 1H), 2.48 (d, *J* = 0.8 Hz, 3H). ^{13}C NMR (125 MHz, Acetone-d_6) δ 136.1, 129.7, 128.5, 126.0, 123.5, 115.6, 113.2, 101.1, 23.1. m.p. = 90-91 °C. IR (neat): 33143118, 3095, 1467, 1338 1753, 1705, 1614, 1507, 1468, 1270, 993, 881, 842, 730, 693, 606 cm^{-1}. HR-MS calculated for $C_{10}H_{10}BrN$ $[M+H]^+$ *m/z* 224.0075, found 224.0079.



Scheme 3. Synthesis of FP1 (16).

9-(1,6-dimethyl-1H-indol-5-yl)-14,14-dimethyl-2,3,5,6,7,14-hexahydro-1H,12H-benzo[5,6]silino[2,3-f]pyrido[3,2,1-ij]quinolin-12-one (**3**). A solution of compound **2** (2.77 g, 12.4 mmol, 8.0 eq.) in 25 mL anhydrous THF was cooled to -78 °C and treated with a solution of *tert*-butyllithium in pentane (7.27 mL, 12.4 mmol, 8.0 eq.) which was added dropwise. The reaction was stirred at the same temperature for 8 min and then treated with a solution of compound **1** (556 mg, 1.5 mmol, 1.0 eq.) in anhydrous THF (15 mL). The reaction was warmed to room temperature and stirred for 3 hrs. The reaction was quenched by the addition of 10% aq. HCl (10 mL) and stirred at room temperature for 1 hr. The reaction was poured into sat. $NaHCO_3$ (25 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was eluted through a silica plug and concentrated to afford a blue film which was used without further purification. A solution of this intermediate in 20 mL anhydrous CH_2Cl_2 was cooled to 0 °C and

treated with a solution of 1 M BBr_3 in CH_2Cl_2 (2.6 mL, 2.6 mmol, 6.0 eq.) which was added dropwise. The reaction was warmed to room temperature, stirred for 2 hrs, and then quenched by addition of sat. $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography on a silica column (8:92 v/v MeOH/CH_2Cl_2) to afford the title compound as a blue film (45 mg, 0.1 mmol, 10% yield over two-steps beginning from compound **2**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (s, 1H), 7.23 (s, 1H), 7.08 (d, *J* = 3.1 Hz, 1H), 6.88 (d, *J* = 2.3 Hz, 1H), 6.84 (d, *J* = 9.9 Hz, 1H), 6.57 (s, 1H), 6.44 (d, *J* = 3.1 Hz, 1H), 6.20 (dd, *J* = 9.9, 2.3 Hz, 1H), 3.85 (s, 3H), 3.35 (t, *J* = 5.9 Hz, 2H), 3.30 (dt, *J* = 5.7, 2.9 Hz, 2H), 2.94 (t, *J* = 6.3 Hz, 2H), 2.41 (t, *J* = 6.2 Hz, 2H), 2.18 (s, 3H), 2.05 (q, *J* = 5.5 Hz, 2H), 1.84 (q, *J* = 6.1 Hz, 2H), 0.56 (d, *J* = 5.7 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.4, 162.3, 148.1, 145.5, 142.2, 136.9, 136.7, 136.1, 133.9, 132.3, 130.1, 129.2, 129.2, 128.7, 127.3, 126.2, 125.6, 122.0, 121.6, 109.9, 109.9, 100.8, 50.9, 50.3, 33.1, 29.9, 29.2, 28.1, 21.7, 21.2, 20.4, 0.0. IR (neat): 2924, 1612, 1578, 1509, 1228, 1210, 833, 765, 677, 556, 469 cm^{-1}. HR-MS calculated for $C_{31}H_{33}N_2OSi$ $[M+H]^+$ *m/z* 477.2362, found 477.2365

5-(14,14-dimethyl-12-oxo-2,3,6,7,12,14-hexahydro-1H,5H-benzo[5,6]silino[2,3-f]pyrido[3,2,1-ij]quinolin-9-yl)-1,6-dimethyl-1H-indole-3-carbaldehyde (**4**). Anhydrous DMF (3.0 mL) was cooled in an ice-bath and treated with dropwise addition of $POCl_3$ (10 uL, 0.11 mmol, 1.5 eq.). After 30 min, a solution of compound **3** (32 mg, 0.07 mmol, 1.0 eq.) in anhydrous DMF (2.0 mL) was added. The reaction was stirred 0 °C for 1 hr. The reaction was then syringed into a second reaction vessel where anhydrous DMF (3.0 mL) was cooled in an ice-bath and treated with dropwise addition of $POCl_3$ (10 uL, 0.11 mmol, 1.5 eq.). After 30 min,

reaction was treated with additional $POCl_3$ (10 uL, 0.11 mmol, 1.5 eq.) and stirred for 3 hrs. The reaction was poured in $NaHCO_3$ (50 mL) and extracted wit EtOAc (4 × 30 mL). The combined organic fractions were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography on a silica column (1:33 v/v MeOH/CH_2Cl_2) to afford the title compound as a dark blue film (30 mg, 0.06 mmol, 89.0 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (s, 1H), 7.99 (s, 1H), 7.73 (s, 1H), 7.28 (s, 1H), 6.86 (d, *J* = 2.3 Hz, 1H), 6.72 (dd, *J* = 9.9, 0.8 Hz, 1H), 6.43 (s, 1H), 6.17 (dd, *J* = 10.0, 2.3 Hz, 1H), 3.94 (s, 3H), 3.31 (dt, *J* = 27.4, 5.9 Hz, 4H), 2.93 (dd, *J* = 7.4, 5.1 Hz, 2H), 2.39 (dd, *J* = 7.3, 5.2 Hz, 2H), 2.21 (s, 3H), 2.10–2.01 (m, 2H), 1.83 (p, *J* = 6.2 Hz, 2H), 0.55 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.5, 183.8, 183.3, 147.9, 145.3, 141.6, 139.6, 138.0, 136.2, 135.6, 134.4, 132.9, 128.6, 128.2, 126.9, 126.0, 123.2, 122.6, 121.9, 118.2, 110.7, 50.8, 50.2, 34.0, 29.9, 29.2, 28.1, 21.7, 21.2, 20.5, 0.1, -0.1. IR (neat): 2927, 1737, 1574, 1353, 1301, 1203 cm^{-1}. HR-MS calculated for $C_{32}H_{32}N_2O_2Si$ $[M+H]^+$ *m/z* 505.2311, found 505.2319.

9-(3-(1-aminobut-3-en-1-yl)-1,6-dimethyl-1H-indol-5-yl)-14,14-dimethyl-2,3,5,6,7,14-hexahydro-1H,12H-benzo[5,6]silino[2,3-f]pyrido[3,2,1-ij]quinolin-12-one (5). Potassium allyltrifluoroborate (17.6 mg, 0.12 mmol, 2.0 eq.) was dissolved in a 7N solution of NH_3 in MeOH (3.0 mL) and stirred at room temperature for 15 min. A solution of compound **4** (9 mg, 0.016 mmol, 1 eq.) in 7N solution of NH_3 in MeOH (2.0 mL) and H_2O (10 µL) were added sequentially to the reaction which was stirred for 16 hrs. The reaction was poured into sat. $NaHCO_3$ (50 mL) and extracted with EtOAc (4 × 30 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography on a silica column (1:19 v/v MeOH/CH_2Cl_2) to afford the title

compound as a dark blue film (24 mg, 0.014 mmol, 74.5% yield). ^{1}H NMR (500 MHz, MeOD/$CDCl_3$) δ 7.24 (dd, *J* = 11.3, 8.6 Hz, 2H), 7.19 (s, 1H), 6.85–6.80 (m, 2H), 6.55 (d, *J* = 3.6 Hz, 1H), 6.15 (ddd, *J* = 10.1, 8.0, 2.4 Hz, 1H), 5.75–5.63 (m, 1H), 5.17–4.99 (m, 2H), 4.38 (td, *J* = 7.0, 4.7 Hz, 1H), 3.83 (s, 3H), 3.40 (t, *J* = 5.9 Hz, 2H), 3.35 (t, *J* = 6.1 Hz, 2H), 2.94 (t, *J* = 6.2 Hz, 2H), 2.74–2.66 (m, 1H), 2.61 (dd, *J* = 14.2, 7.2 Hz, 1H), 2.43–2.31 (m, 2H), 2.14 (s, 3H), 2.09–1.98 (m, 2H), 1.88–1.78 (m, 2H), 0.55 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.4, 147.9, 144.8, 142.0, 137.4, 136.1, 136.0, 135.5, 134.7, 132.0, 131.1, 130.4, 129.0, 128.4, 127.2, 126.2, 125.7, 121.9, 119.9, 117.7, 110.0, 50.7, 50.2, 48.0, 43.7, 43.5, 33.0, 29.9, 29.2, 28.2, 21.8, 21.3, 20.4, 0.2, 0.0. IR (neat): 3012, 2972, 1739, 1585, 1366, 1231, 834, 765 cm^{-1}. HR-MS calculated for $C_{35}H_{39}N_3OSi$ $[M+H]^+$ *m/z* 546.2941, found 546.2947.

FP1 (6). A solution of compound **5** (120.0 mg, 0.22 mmol, 1.0 eq.) in anhydrous THF (10.0 mL) was cooled in an ice-bath. To the solution, 4-nitrobenzaldehyde (100 mg, 0.66 mmol, 3.0 eq.), glacial AcOH (100 μL, 1.6 mmol, 10 eq.), and sodium triacetoxyborohydride (186 mg, 0.88 mmol, 4.0 eq.) were added sequentially. The reaction was warmed to room temperature and stirred for 16 hrs. Upon completion, the reaction was poured into sat. $NaHCO_3$ (50 mL) and extracted with EtOAc (4 × 30 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography on a silica column (1:33 MeOH/CH_2Cl_2) to afford the title compound as a dark blue film. (32.0 mg, 0.057 mmol, 26.0 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (d, *J* = 7.8 Hz, 1H), 8.20 (d, *J* = 8.7 Hz, 2H), 7.87 (dd, *J* = 8.6, 6.1 Hz, 2H), 7.38 (d, *J* = 51.2 Hz, 1H), 7.22 (s, 1H), 7.14 (d, *J* = 23.1 Hz, 1H), 6.88 (dd, *J* = 4.7, 2.3 Hz, 1H), 6.83 (dd, *J* = 22.0, 10.0 Hz, 1H), 6.53 (d, *J* = 50.1 Hz, 1H), 6.18 (ddd, *J* = 26.9, 9.9, 2.3 Hz, 1H), 5.79 (ddtd, *J* = 17.3, 10.1,

7.1, 4.5 Hz, 1H), 5.11–4.96 (m, 2H), 4.77 (td, *J* = 8.3, 5.5 Hz, 1H), 3.84 (d, *J* = 5.3 Hz, 3H), 3.43–3.33 (m, 2H), 3.3–3.21 (m, 2H), 2.96 (dt, *J* = 12.4, 5.9 Hz, 2H), 2.90–2.77 (m, 2H), 2.47–2.30 (m, 2H), 2.18 (d, *J* = 4.6 Hz, 3H), 2.14–2.02 (m, 2H), 1.91–1.71 (m, 2H), 0.59 (d, *J* = 7.1 Hz, 3H), 0.56 (d, *J* = 1.8 Hz, 3H).^{13}C NMR (125 MHz, $CDCl_3$) δ 183.9, 157.8, 157.7, 149.0, 148.0, 145.1, 142.1, 142.0, 137.3, 136.3, 135.8, 135.74, 135.70, 135.6, 134.4, 132.3, 132.2, 130.6, 129.1, 128.6, 128.5, 127.2, 126.6, 126.5, 126.0, 125.9, 124.5, 124.2, 123.97, 123.93, 121.9, 121.8, 120.5, 120.1, 117.7, 117.5, 116.6, 116.1, 110.25, 110.21, 68.1, 67.7, 50.8, 50.7, 50.29, 50.25, 42.2, 41.6, 33.16, 33.13, 29.9, 29.2, 29.2, 28.18, 28.10, 21.7, 21.28, 21.21, 20.5, 20.4, 0.15, 0.13, 0.06, 0.04. IR: 2925, 1737, 1571, 1347, 1301, 1204 cm^{-1}. HR-MS calculated for $C_{42}H_{44}N_4O_3Si$ $[M+H]^+$ *m/z* 681.3261, found 681.3272.

Compound	Φ	ε ($M^{-1} \cdot cm^{-1}$)	λ_{abs} (nm)	λ_{em} (nm)
3	ND[a]	8.6×10^4	633	649
4	0.13	10.6×10^4	633	649
5	0.02	10.0×10^4	633	649
FP1	ND[a]	2.9×10^4	620	649

Table S1. Fluorescence properties of compound **3**, **4**, **5** and **FP1**. a) ND = not determinable.

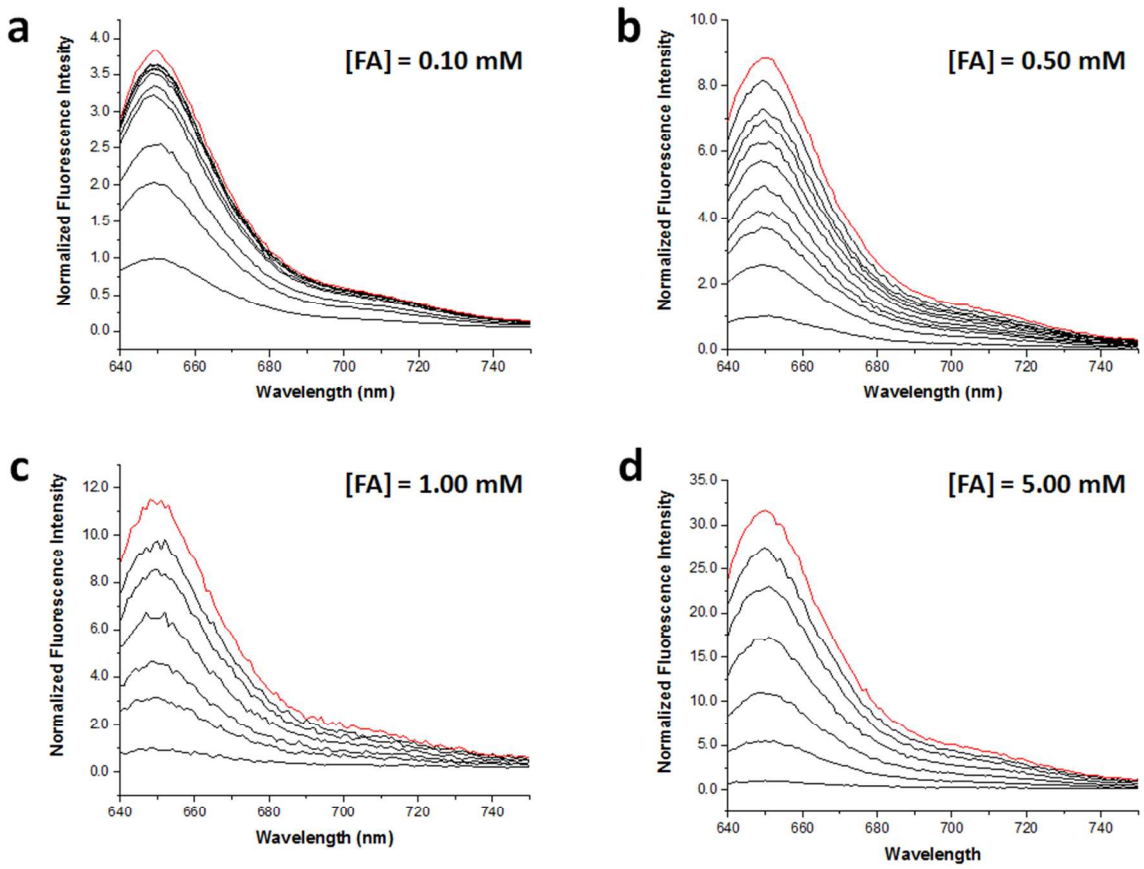


Figure S1. Normalized fluorescence emission of 1 µM FP1 in PBS (pH 7.4) reacted with a) 0.1 mM, b) 0.5 mM, c) 1 mM, and d) 5 mM FA. FP1 was excited at 633 nm and the emission was collect between 640 and 750 nm. All experiments were performed at 37 °C for 3 h. Time points on graphs a-b are recorded every 15 min. The time points for graphs c-d represent every 30 min. A concentration dependent increase in fluorescence intensity was observed with a fold increase of 3.9, 8.5, 11.6, and 33.5 for 0.1 mM, 0.5 mM, 1 mM, and 5 mM FA, respectively.

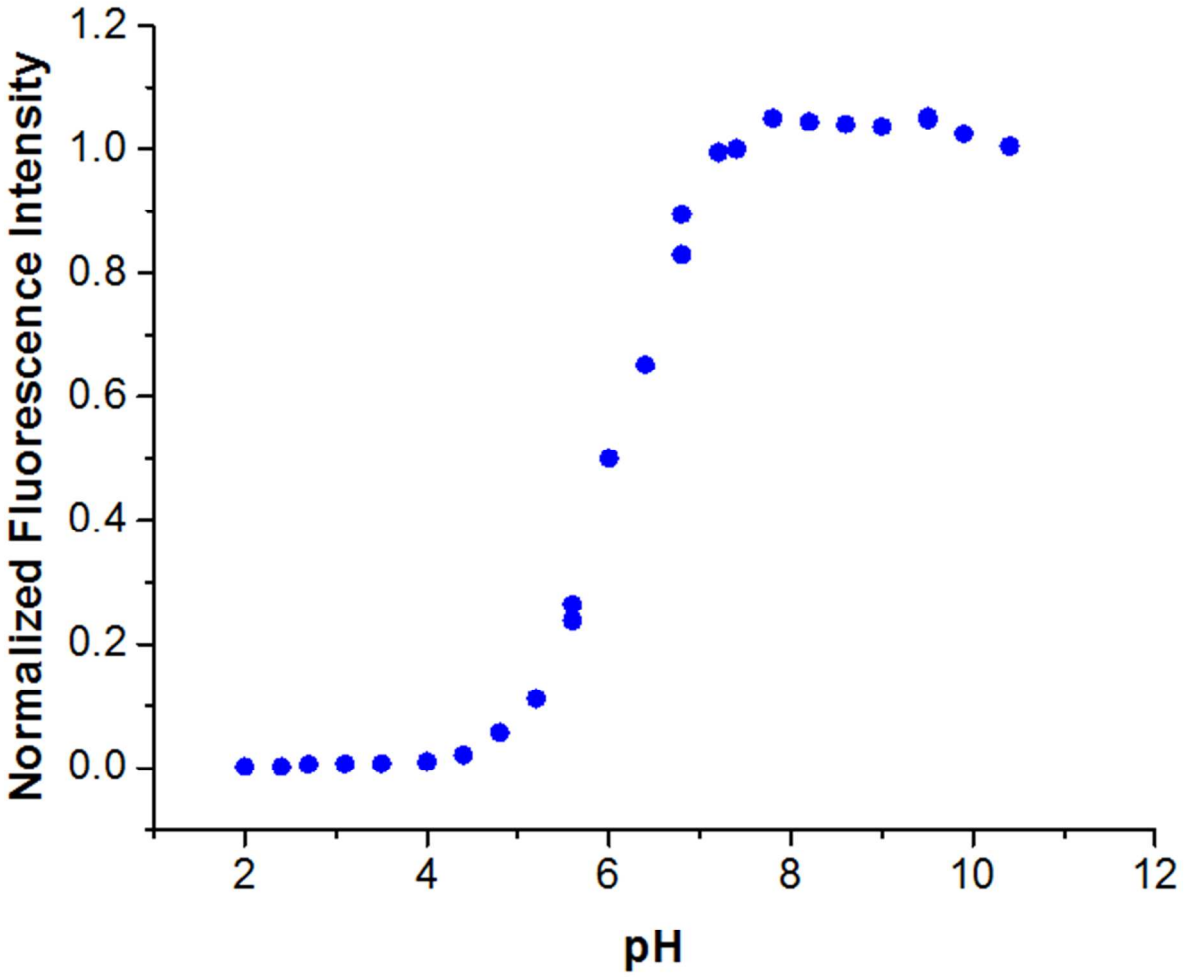


Figure S2. pH-Fluorescence profile of compound **4**. Fluorescence intensity was measured at 25 °C for the following pH values and indicated buffers: 50 mM Glycine (2.0, 2.4, 2.7, 3.1, 3.5), 50 mM sodium acetate (4.0, 4.4, 4.8, 5.2, 5.6), 50 mM MES (5.6, 6.0, 6.4, 6.8), 50 mM HEPES (6.8, 7.2, 7.4, 7.8, 8.2), 50 mM Tris (8.2, 8.6, 9.0, 9.5) and 50 mM glycine (9.5, 9.9, 10.4). Compound **4** was excited at 633 nm and the emission was collect between 640 and 750 nm.

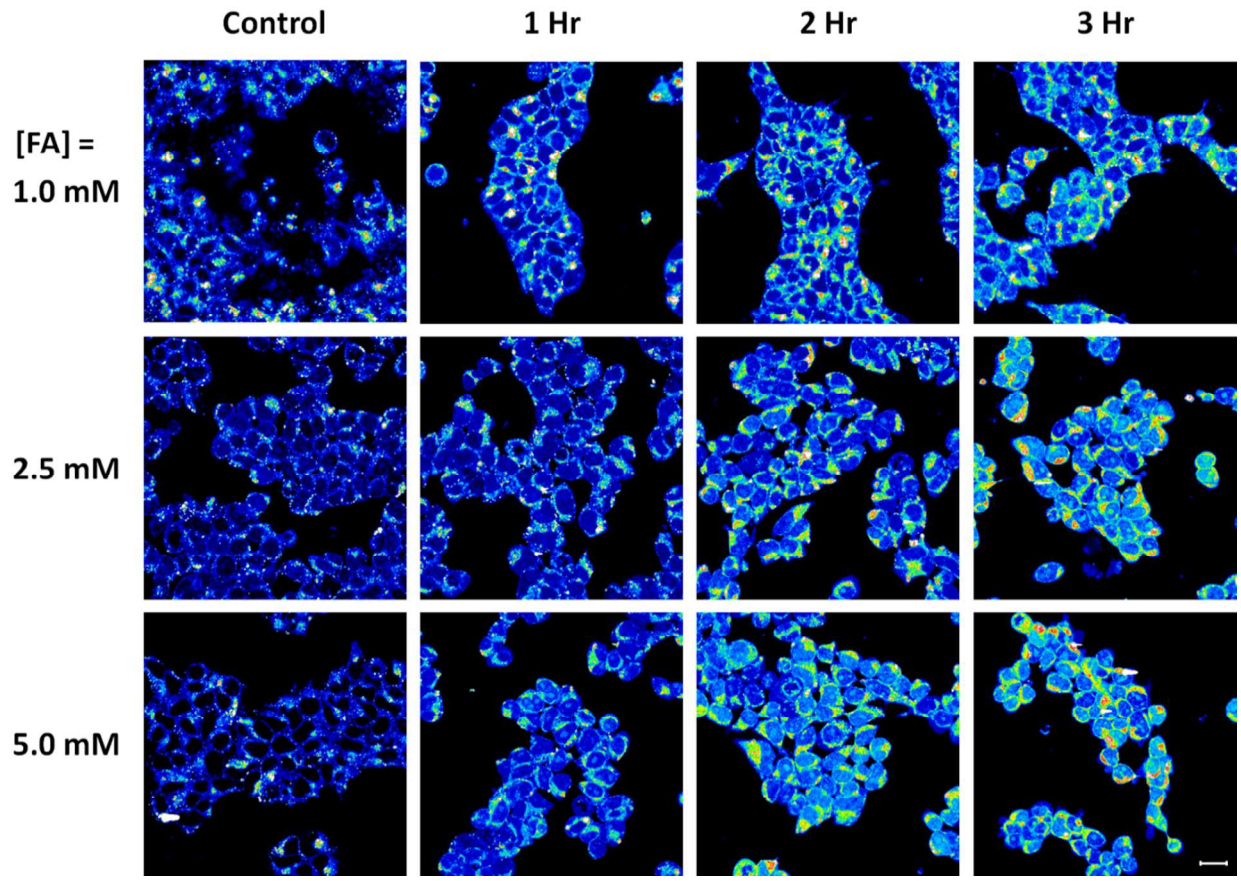


Figure S3. Live-cell imaging of HEK293TN cells. Cells were stained with a solution of 2 μM FP_1 in serum-free DMEM for 8 min, washed with fresh DMEM to remove excess dye and then incubated with 1, 2.5, or 5 mM FA at 37 °C for 1, 2, and 3 h. Cells were irradiated with the 633 nm HeNe laser set at 3% power with a pinhole size of 1 airy unit. The emission was collected between 645 and 800 nm. Scale bar represents 20 μm. Pseudo-coloring represents intensity distribution from highest intensity indicated by white to the lowest intensity designated by black.

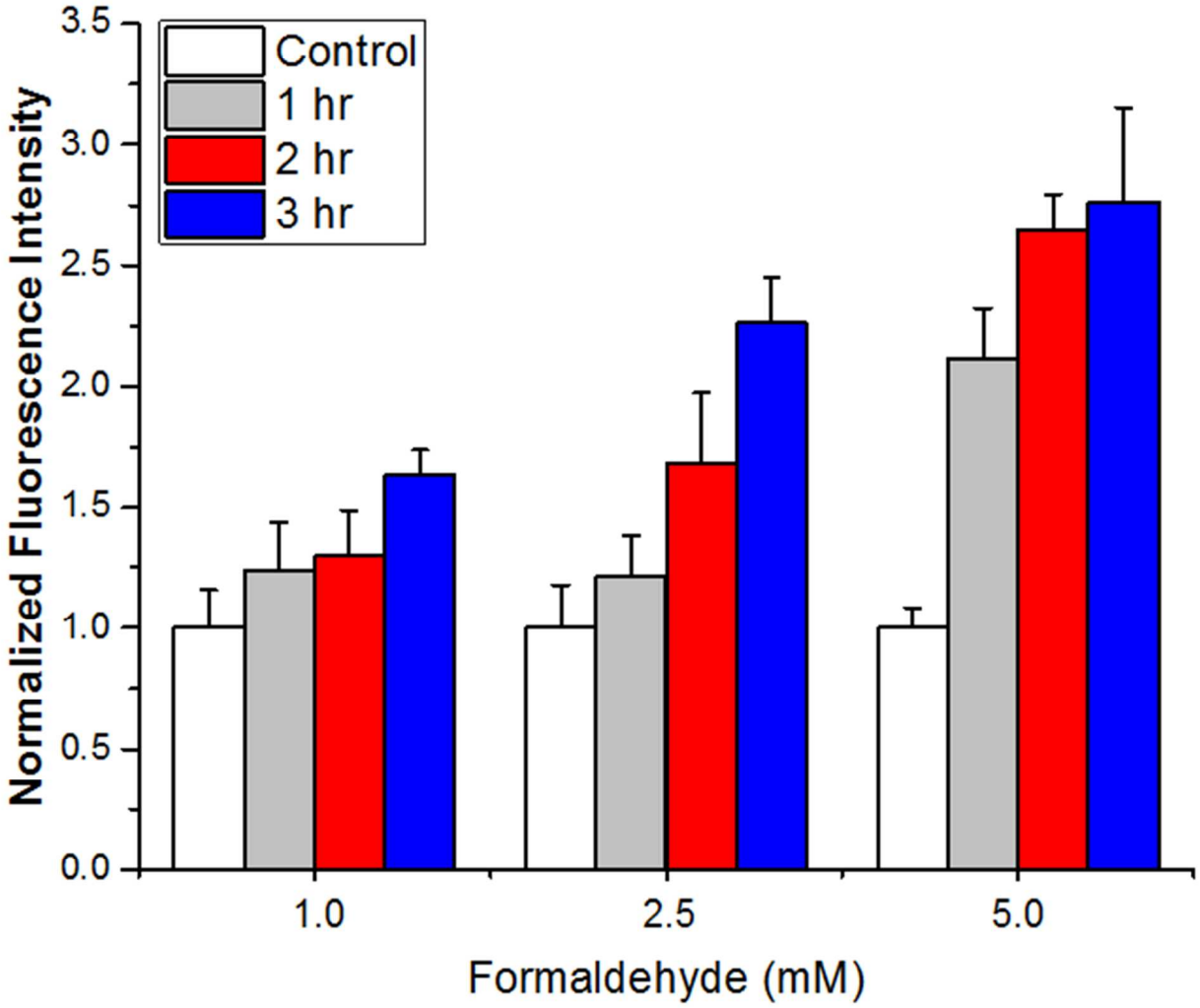


Figure S4. Quantification of observed fluorescence intensity by confocal imaging in HEK293TN cells after incubation with 1 mM, 2.5 mM or 5 mM FA for 1 (light grey bars), 2 (red bars) and 3 hrs (blue bars). A DMEM vehicle was added to control cells which was normalized to 1 (white bars). Cells incubated with 1 mM FA for 1, 2 and 3 hrs exhibited a 1.2-, 1.3-, and 1.6-fold increase in fluorescence intensity, respectively. Cells incubated with 2.5 mM FA for 1, 2 and 3 hrs resulted in a 1.2-, 1.7-, and 2.2-fold increase in fluorescence intensity, respectively. Cells incubated with 5 mM FA for 1, 2 and 3 hrs resulted in a 2.1-, 2.6-, and 2.8-fold increase in fluorescence intensity, respectively. For each condition, a minimum of 5 images were averaged ($n > 5$). Errors represent standard deviation.

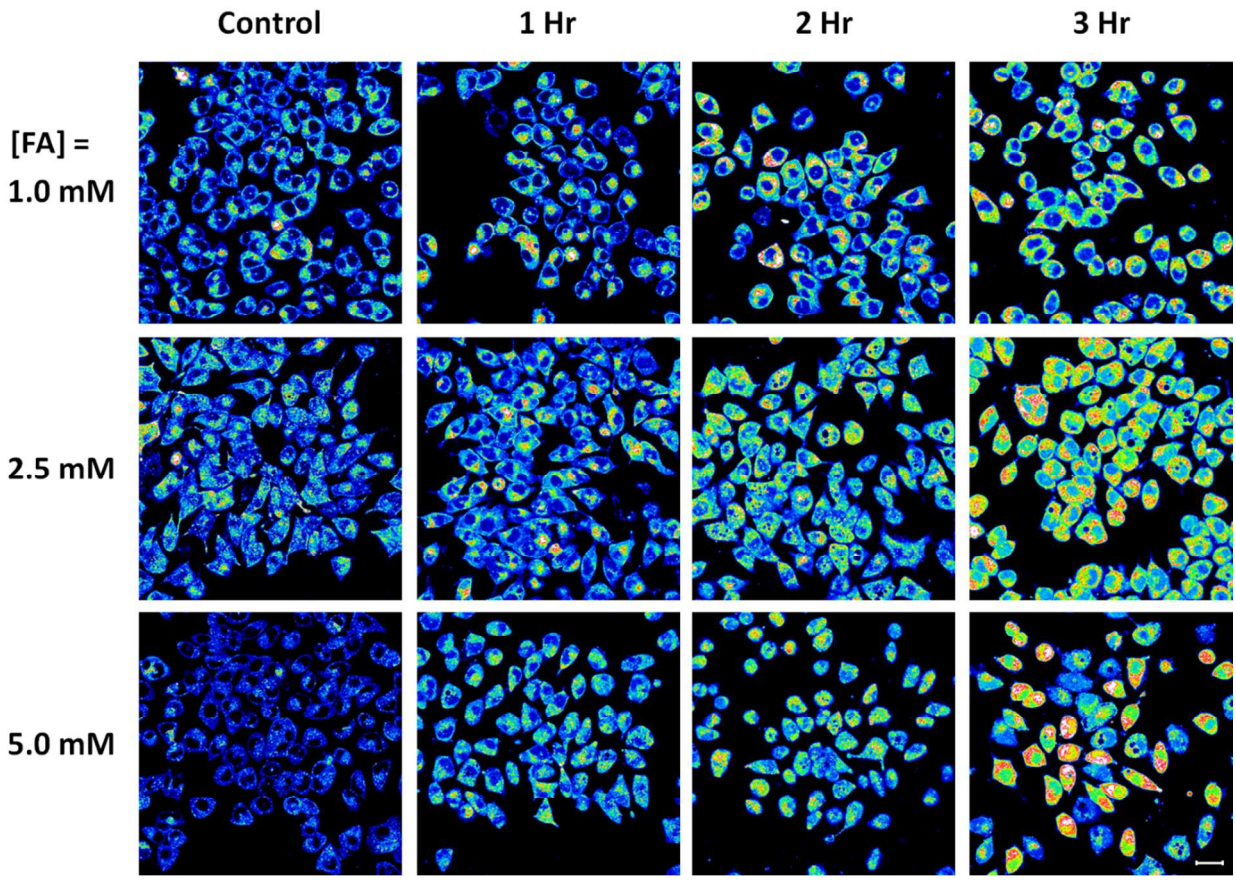


Figure S5. Live-cell imaging of NS1 cells. Cells were stained with a solution of 2 μM FP_1 in Ham's F-12K serum-free media for 8 min, washed with fresh media to remove excess dye and then incubated with 1, 2.5, or 5 mM FA at 37 °C for 1, 2, and 3 hrs. Cells were irradiated with the 633 nm HeNe laser set at 3% power with a pinhole size of 1 airy unit. The emission was collected between 645 and 800 nm. Scale bar represents 20 μm. Pseudo-coloring represents intensity distribution from highest intensity indicated by white to the lowest intensity designated by black.

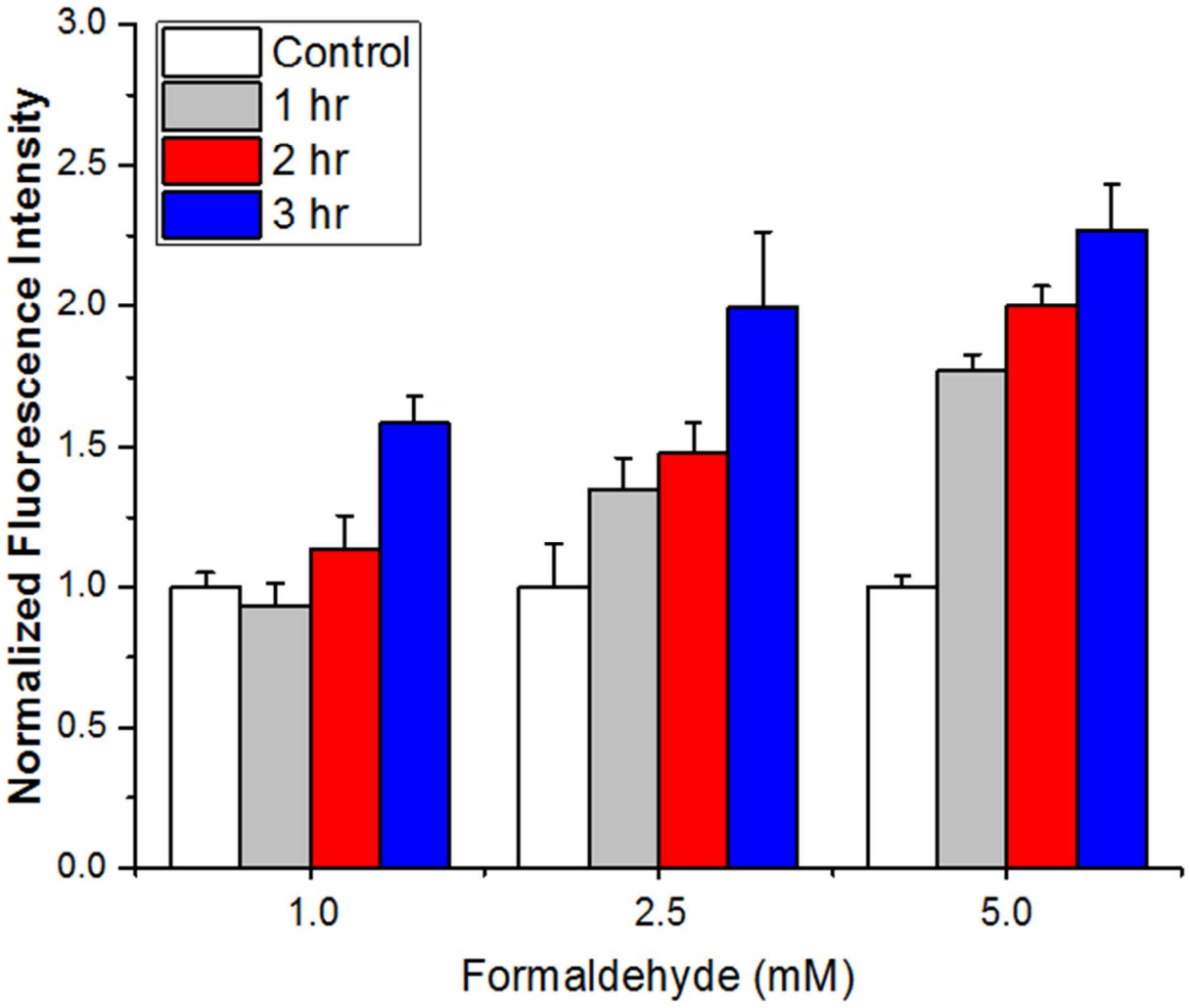


Figure S6. Quantification of observed fluorescence intensity by confocal imaging in NS1 cells after incubation with 1 mM, 2.5 mM or 5 mM FA for 1 (light grey bars), 2 (red bars) and 3 h (blue bars). A Ham's F-12K vehicle was added to control cells which was normalized to 1 (white bars). Cells incubated with 1 mM FA for 1, 2 and 3 hrs exhibited a 0-, 1.1-, and 1.6-fold increase in fluorescence intensity, respectively. Cells incubated with 2.5 mM FA for 1, 2 and 3 hrs resulted in a 1.3-, 1.5-, and 2.0-fold increase in fluorescence intensity, respectively. Cells incubated with 5 mM FA for 1, 2 and 3 hrs resulted in a 1.8-, 2.0-, and 2.3-fold increase in fluorescence intensity, respectively. For each condition, a minimum of 5 images were averaged ($n > 5$). Errors represent standard deviation.

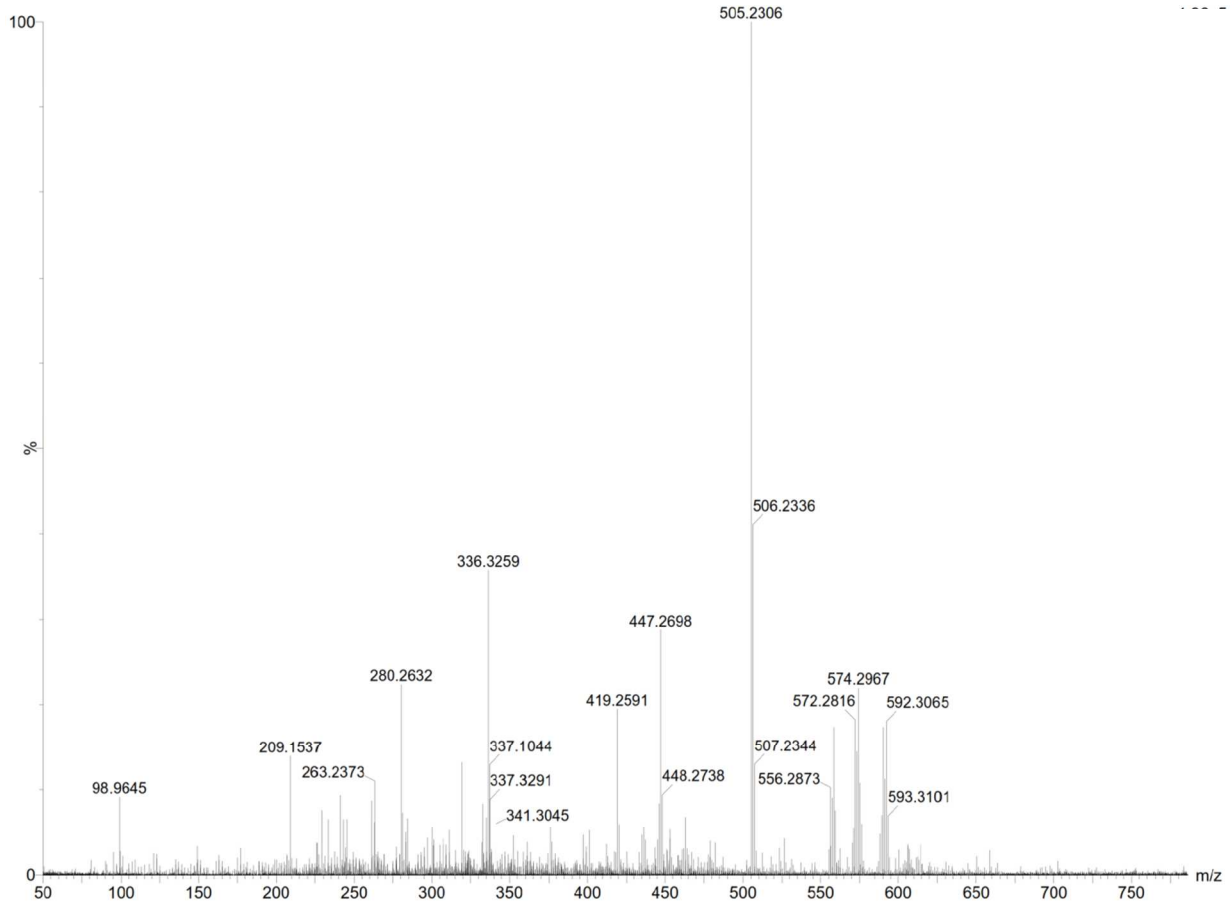


Figure S7. ESI-HRMS spectrum of a crude reaction mixture after FP_1 (25 μM) was reacted with 5 mM FA at 37 °C for 48 h in the presence of HEK293TN cellular lysates to yield compound **4**. HR-MS calculated for $C_{32}H_{33}N_2O_2Si$ $[M+H]^+$ *m/z* 505.2311, found 505.2306.

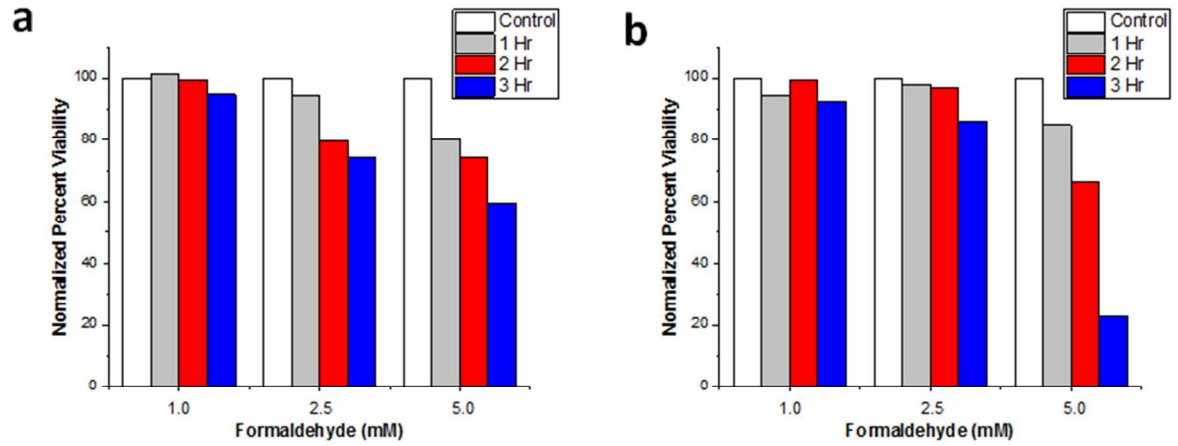


Figure S8. Trypan blue dye exclusion assay to determine cell viability of a) HEK293TN cells and b) NS-1 cells treated with FA. Both cell lines were treated 1 mM, 2.5 mM and 5 mM FA for 1, 2 and 3 hrs. For each condition, a 10 µL sample of cells was mixed with 10 µL of a 2× trypan blue solution. Live and dead cells were counted at each of the four 4×4 quadrants of a hemocytometer using a light microscope equipped with a 10× objective. Control samples treated with a vehicle control were normalized to 100% viability.

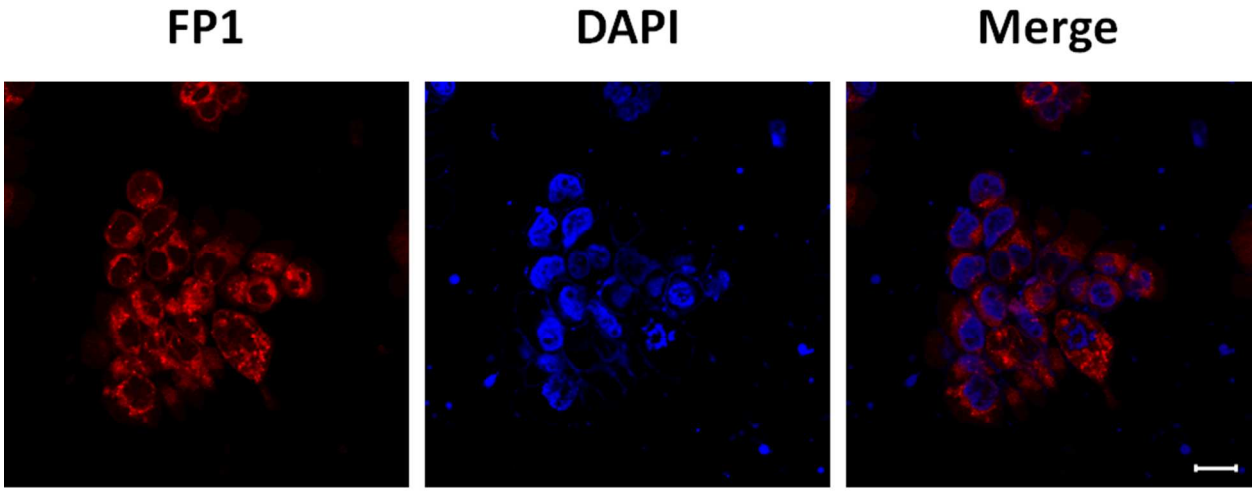


Figure S9. Nuclear staining of HEK293TN cells with DAPI demonstrates cell viability by showing intact nuclei after FP1 stained cells were treatment with 5 mM FA for 3 hrs. DAPI was applied as a 300 nM solution in serum-free DMEM for 5 min. Left: Fluorescent signal from FP1 obtained by irradiation with the 633 nm HeNe laser. Middle: Fluorescent signal from DAPI obtained by irradiation with the 405 nm laser line. Right: Merged image of both signals. Scale bar represents 20 µm.

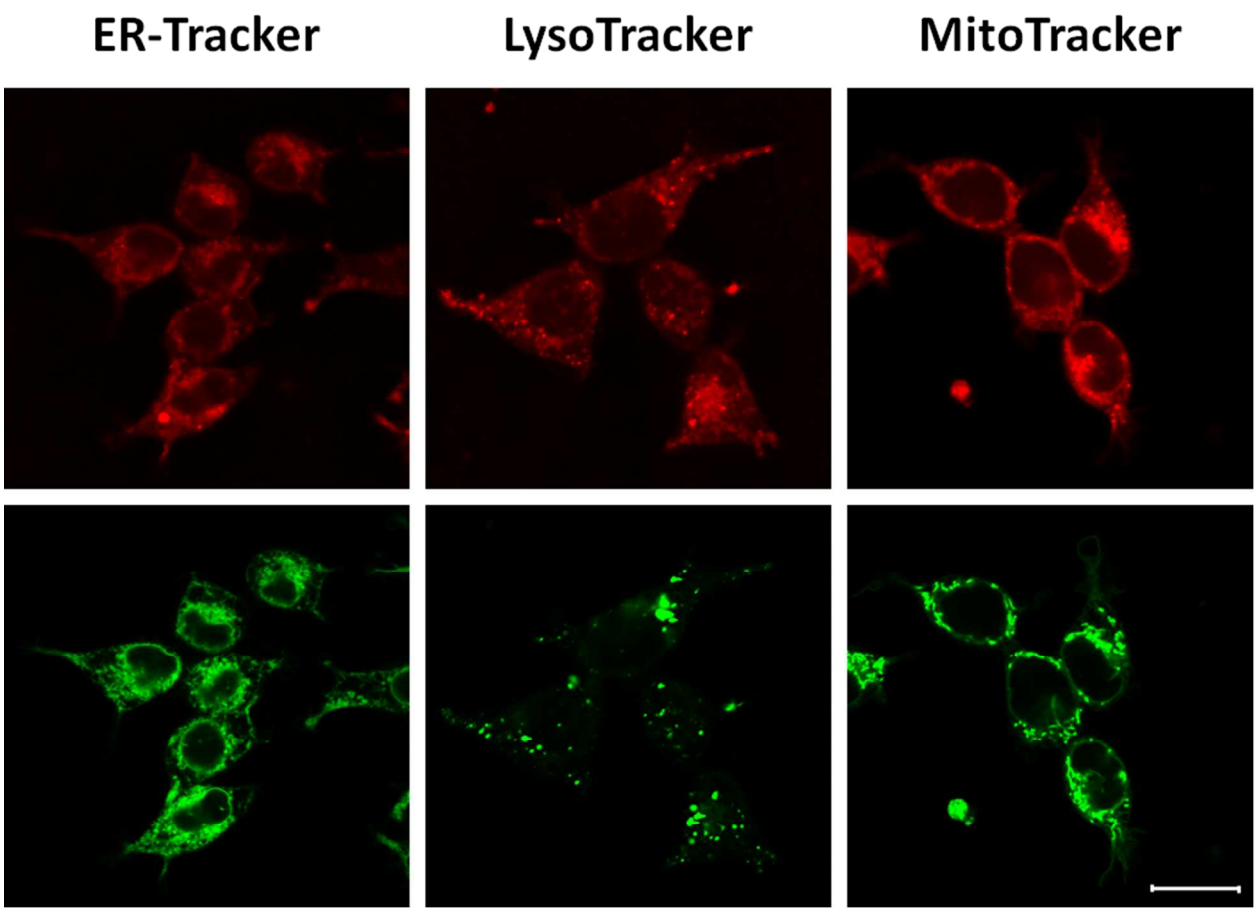


Figure S10. Confocal microscopy images of co-localization experiments using HEK293TN cells. Cells were co-incubated for 5 min with 2 μM FPI and 1 μM of each tracker in serum-free DMEM, then washed with dye-free DMEM and imaged. Top row of images show fluorescent signal from FP1 obtained by irradiation with the 633 nm HeNe laser. Bottom row of images show fluorescent signal from ER-Tracker Green, LysoTracker, and MitoTracker Green FM obtained by irradiation with the 488 nm laser. Scale bar represents 20 μm.

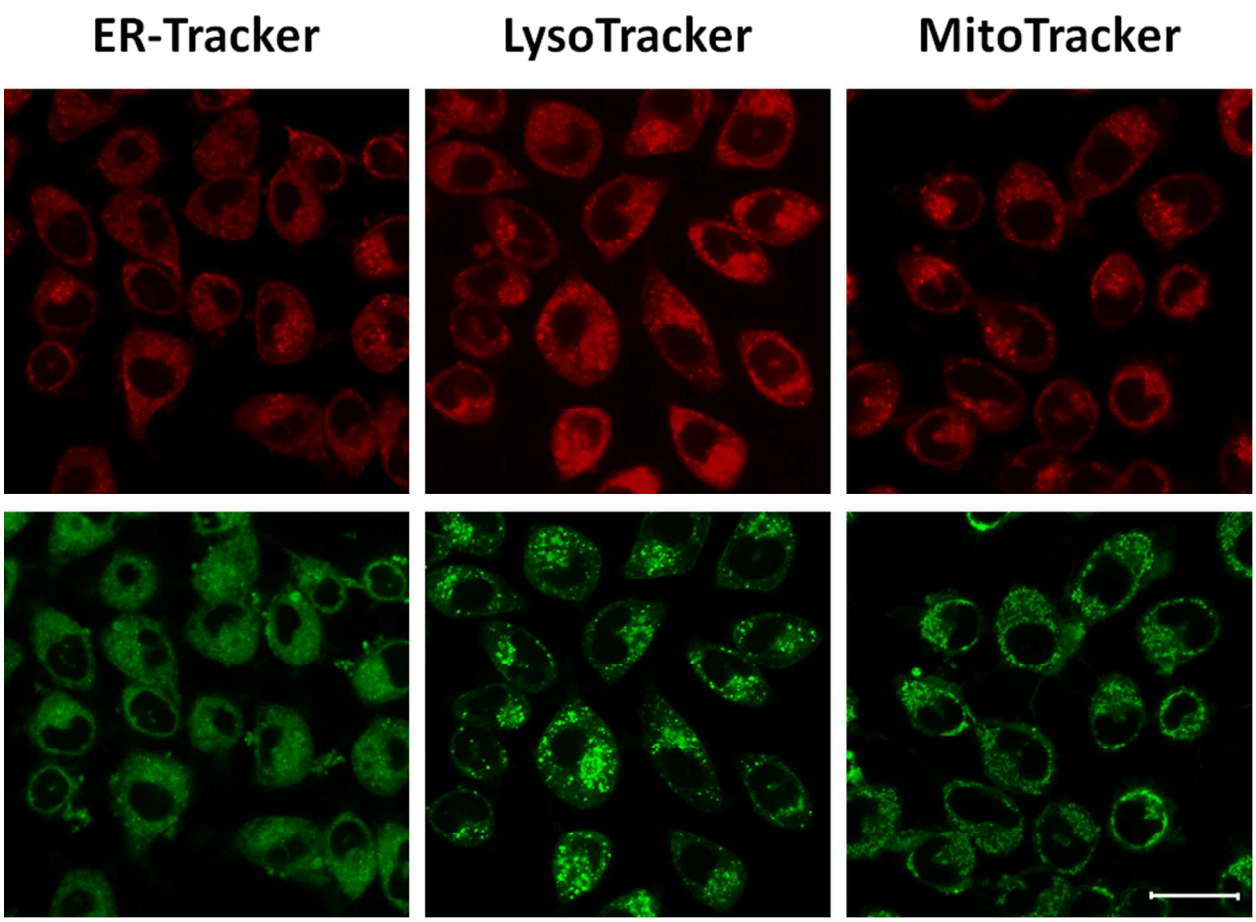


Figure S11. Confocal microscopy images of co-localization experiments using NS1 cells. Cells were co-incubated for 5 min with 2 μM FPI and 1 μM of each tracker in serum-free DMEM, then washed with dye-free DMEM and imaged. Top row of images show fluorescent signal from FP1 obtained by irradiation with the 633 nm HeNe laser. Bottom row of images show fluorescent signal from ER-Tracker Green, LysoTracker, and MitoTracker Green FM obtained by irradiation with the 488 nm laser. Scale bar represents 20 μm.

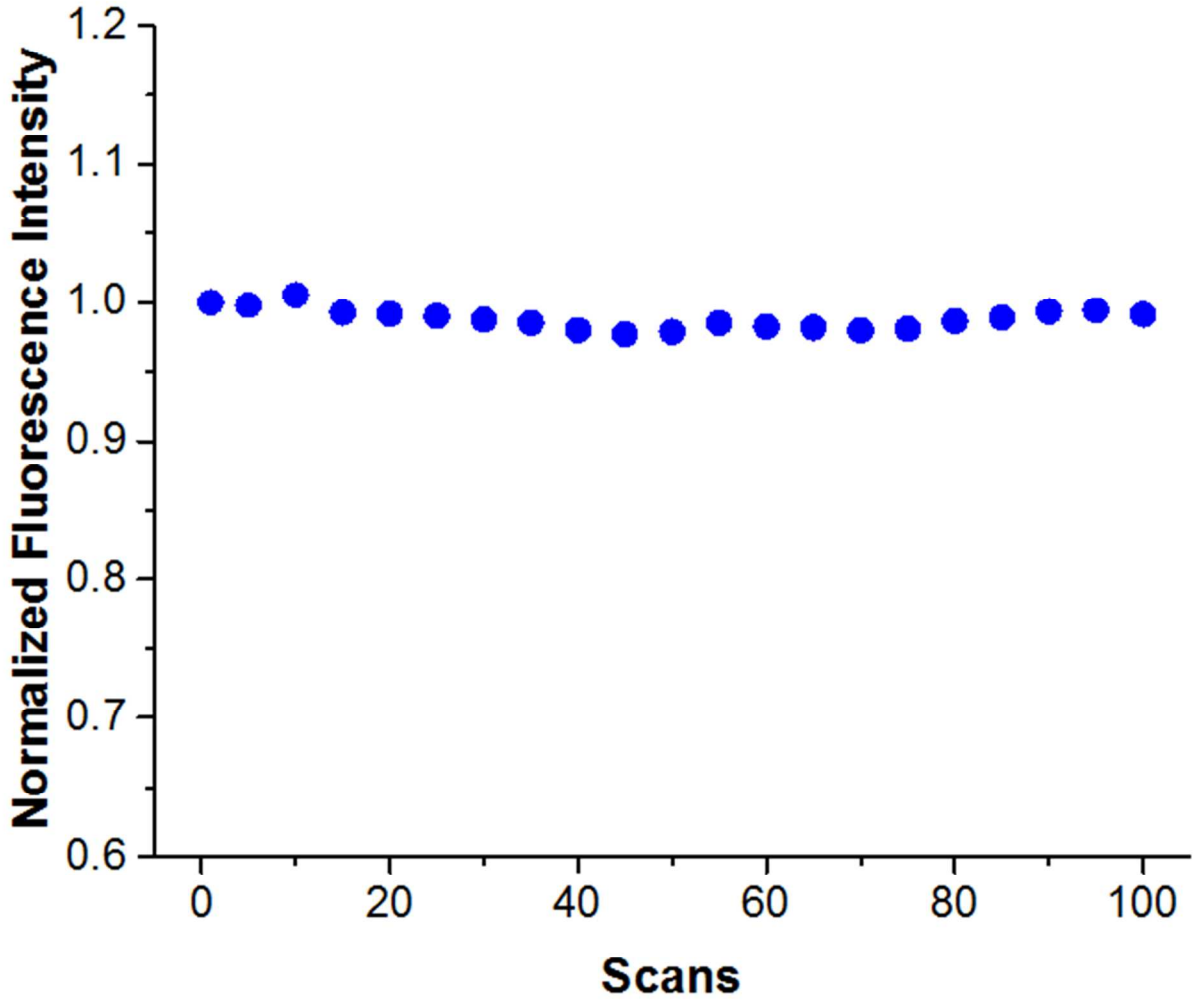


Figure S12. Photobleaching assay of FP1 in live cells. HEK293TN cells were stained with a solution of 2 μM FP1 in DMEM for 8 min, rinsed with fresh DMEM. After 30 min, a field of cells were subjected to irradiation for 100 scans with the 633 nm HeNe laser set at 25% power with a pinhole size of 1 airy unit. A data point is plotted for every 5 scans.

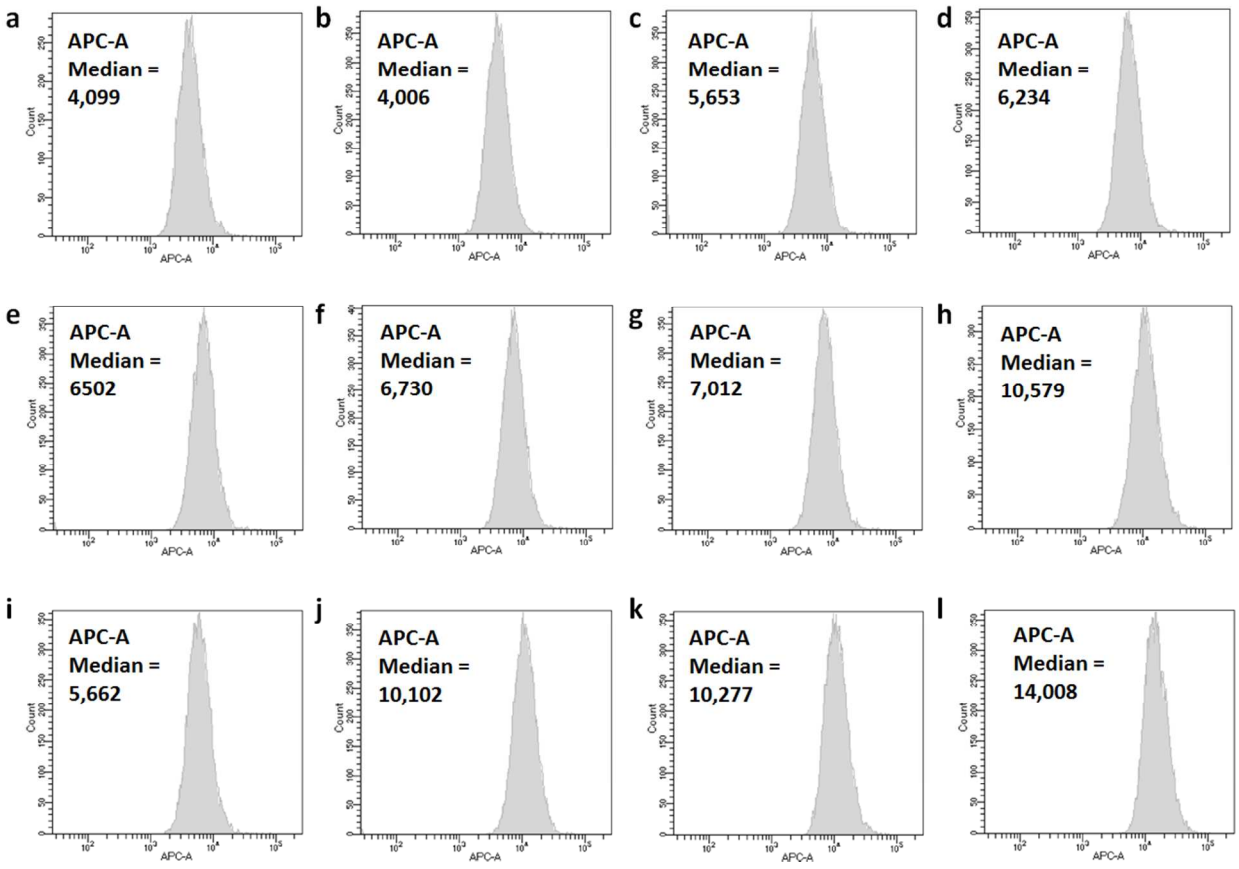


Figure S13. Flow cytometry analysis of HEK293TN cells. Cells were stained with a solution of 2 μM FP1 in DMEM for 8 min, rinsed with fresh DMEM and then treated with a) a DMEM vehicle control; b) 1 mM FA, 1 hr incubation; c) 1 mM FA, 2 hrs incubation; d) 1 mM FA, 3 hrs incubation; e) a DMEM vehicle control; f) 2.5 mM FA, 1 hr incubation; g) 2.5 mM FA, 2 hrs incubation; h) 2.5 mM FA, 3 hrs incubation; i) a DMEM vehicle control; j) 5 mM FA, 1 hr incubation; k) 5 mM FA, 2 hrs incubation; and l) 5 mM FA, 3 hrs incubation.

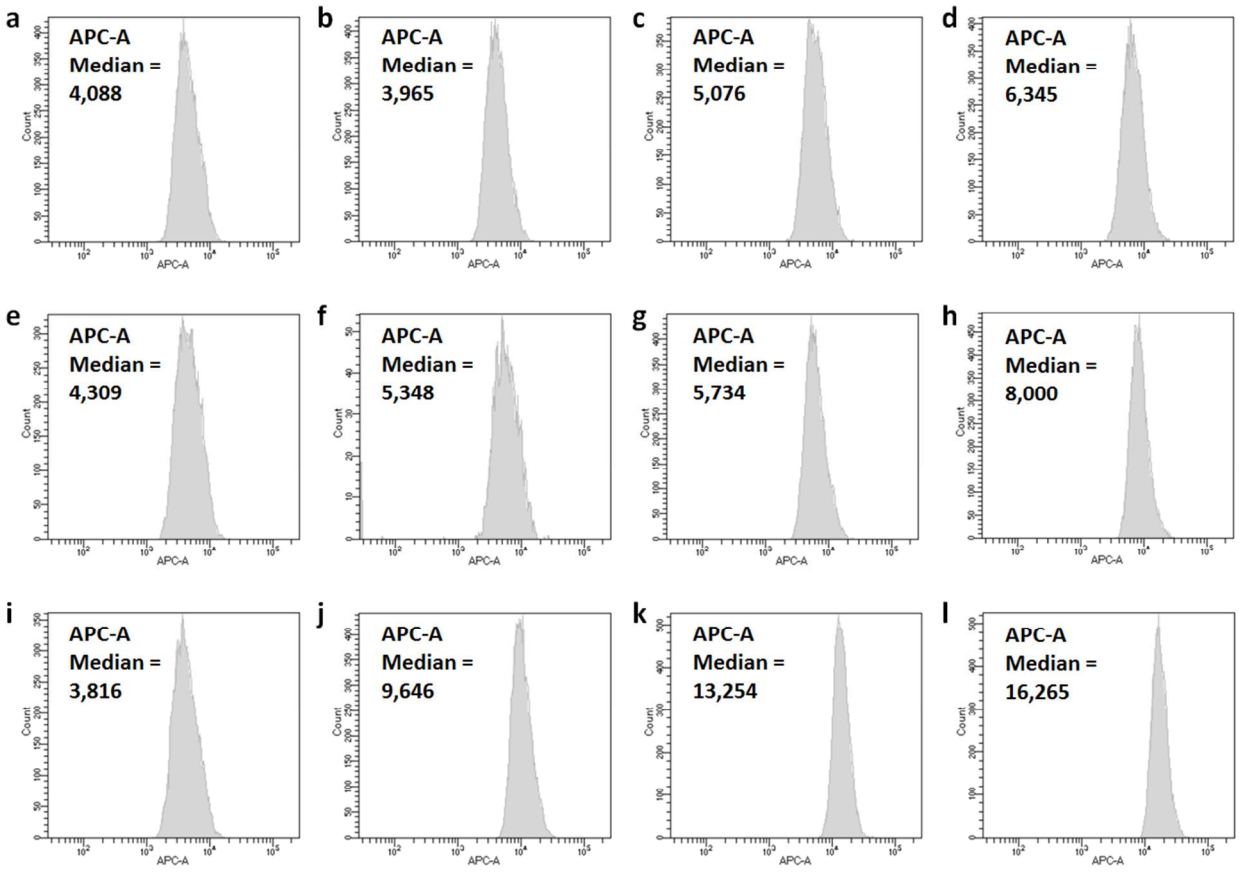


Figure S14. Flow cytometry analysis of NS1 cells. Cells were stained with a solution of 2 µM FP1 in Ham's F-12K media for 8 min, rinsed with fresh media and then treated with a) a DMEM vehicle control; b) 1 mM FA, 1 hr incubation; c) 1 mM FA, 2 hrs incubation; d) 1 mM FA, 3 hrs incubation; e) a DMEM vehicle control; f) 2.5 mM FA, 1 hr incubation; g) 2.5 mM FA, 2 hrs incubation; h) 2.5 mM FA, 3 hrs incubation; i) a DMEM vehicle control; j) 5 mM FA, 1 hr incubation; k) 5 mM FA, 2 hrs incubation; and l) 5 mM FA, 3 hrs incubation.

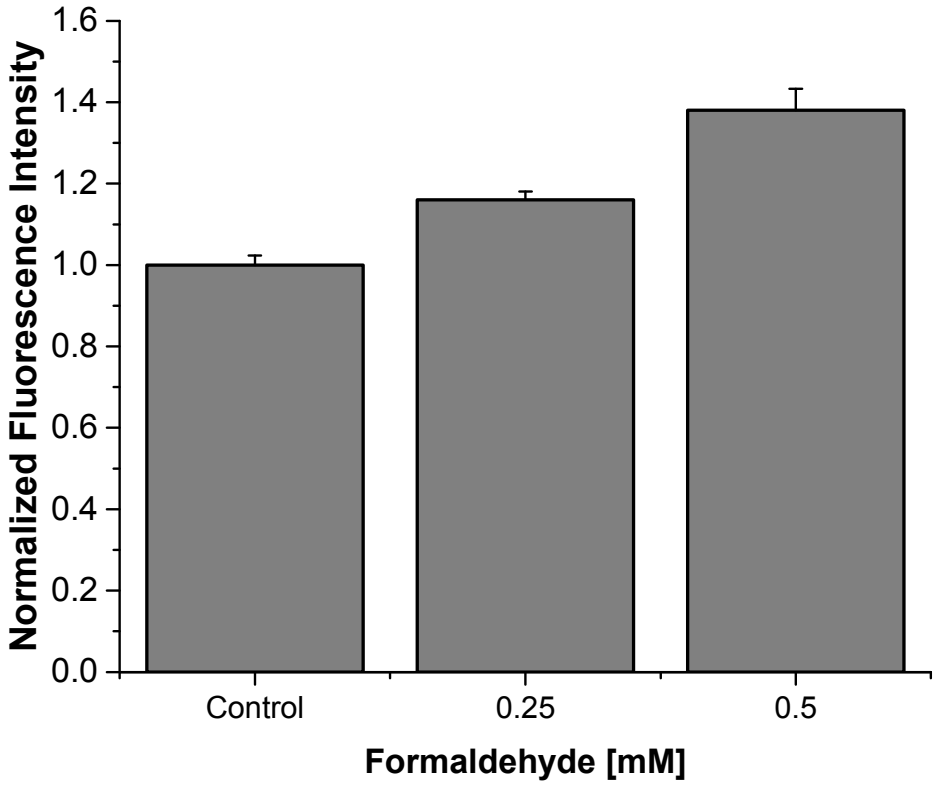


Figure S15. Quantification of data obtained from confocal microscopy images acquired by irradiation of NS1 cells treated with a vehicle control, 0.25 mM and 0.5 mM FA at 37 °C for 3 hrs with the 633 nm HeNe laser. Cells incubated with 0.25 mM FA resulted in a 16% fluorescence increase, whereas, cells incubated with 0.5 mM FA gave rise to a 38% signal enhancement. For each condition, a minimum of 5 images were averaged ($n > 5$). Errors represent standard deviation.

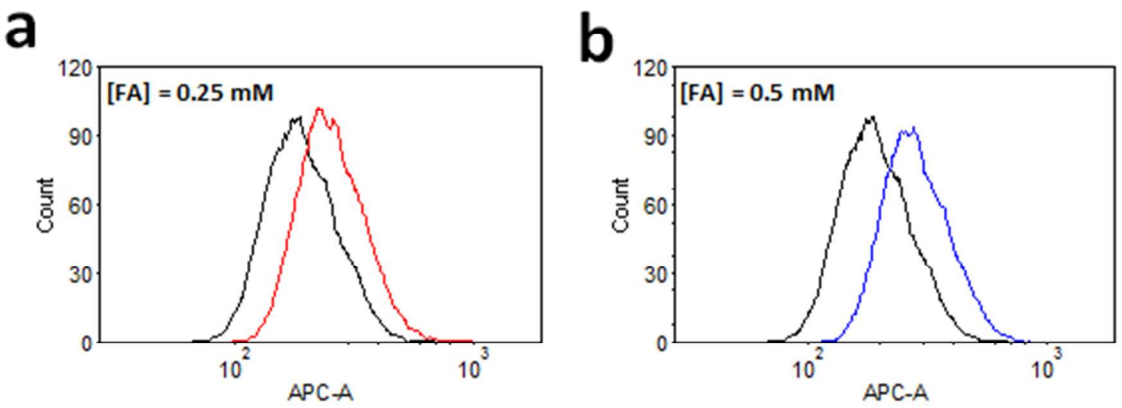


Figure S16. Flow cytometry analysis of NS1 cells stained with 1 µM FP1 and incubated with a) 0.25 mM and b) 0.5 mM FA at 37 °C for 3 hrs. Excitation was provided by the 633 nm HeNe laser and an APC-A filter set was applied. Only live cells were counted.

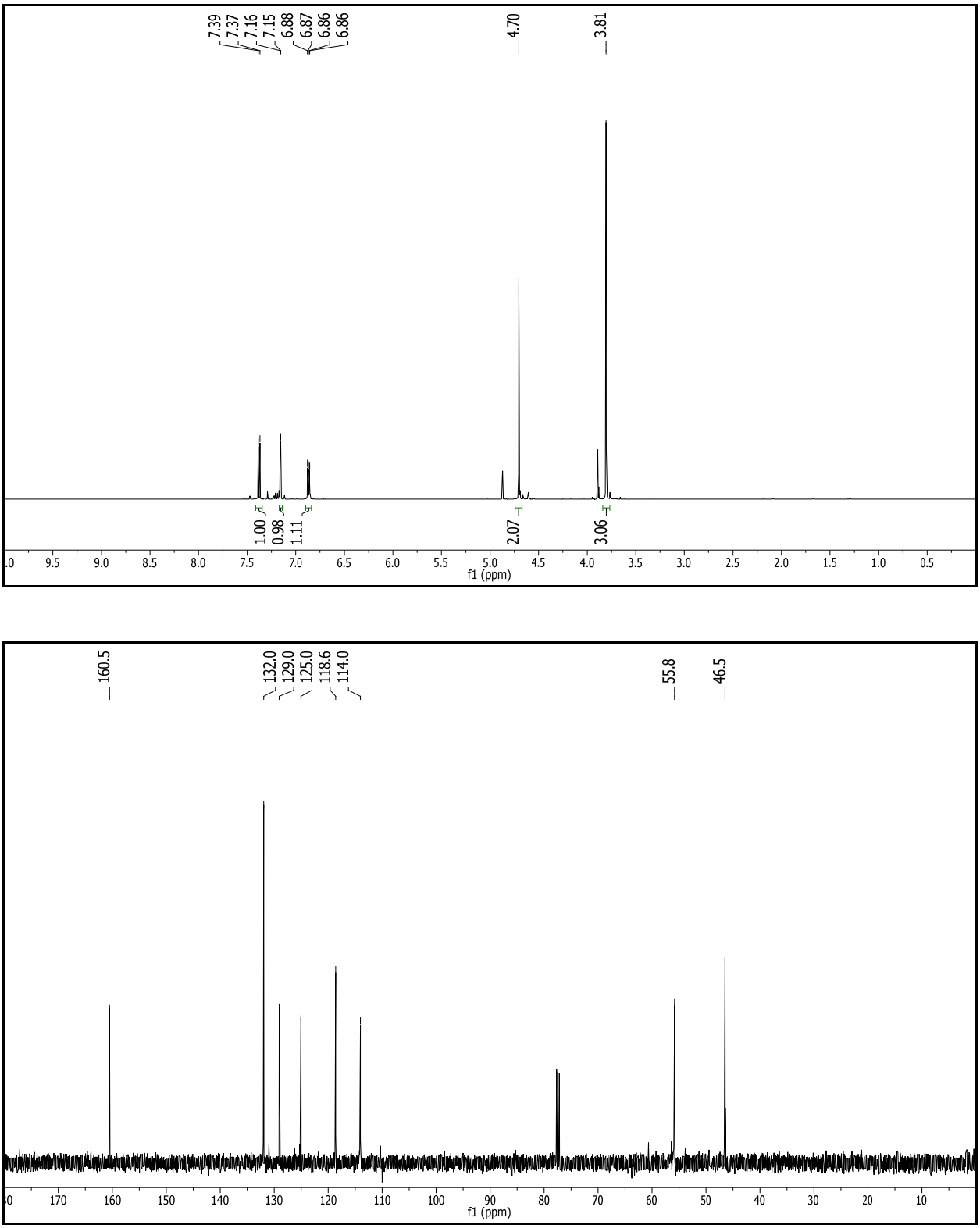


Figure S17. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **7**.

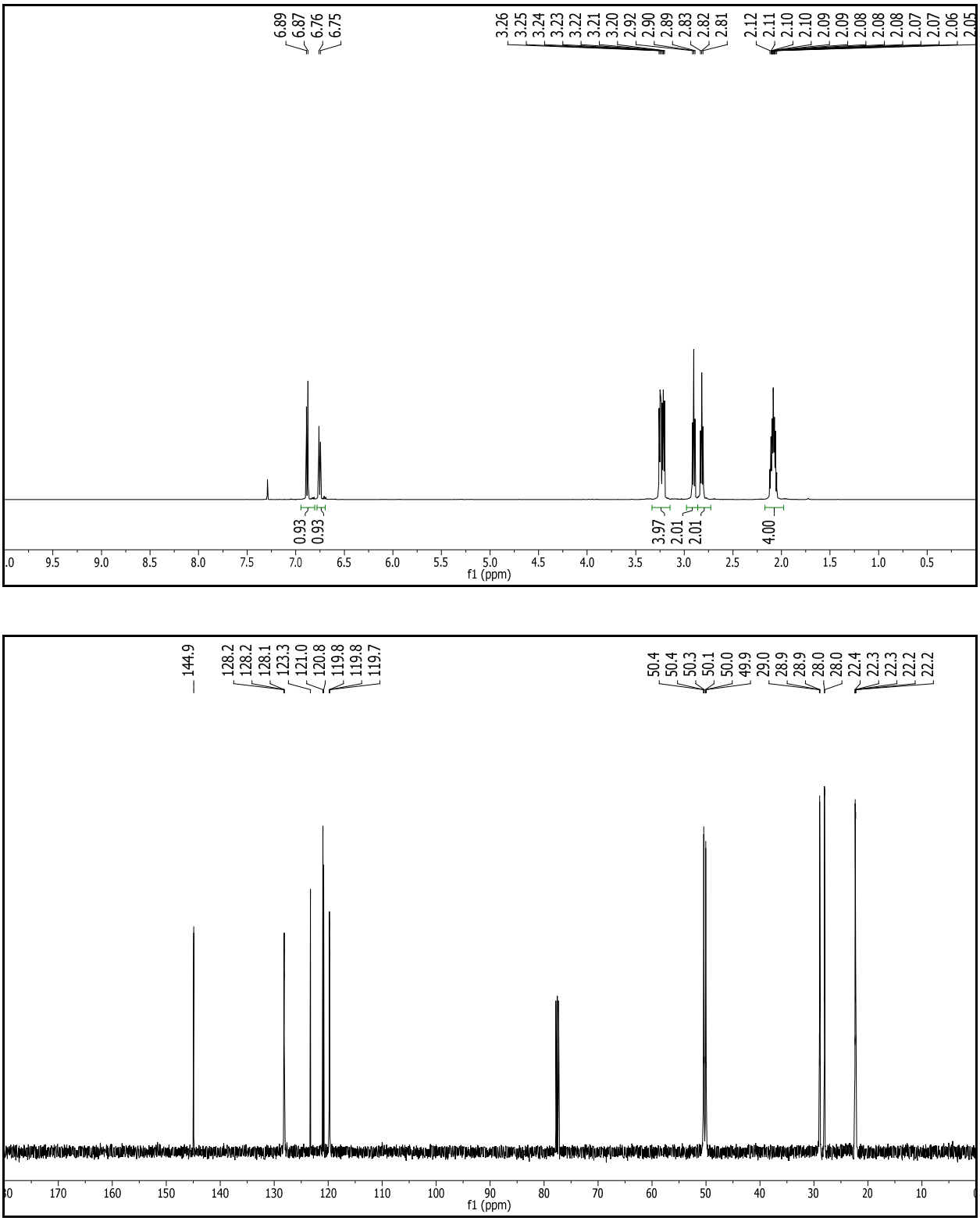


Figure S18. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **8**.

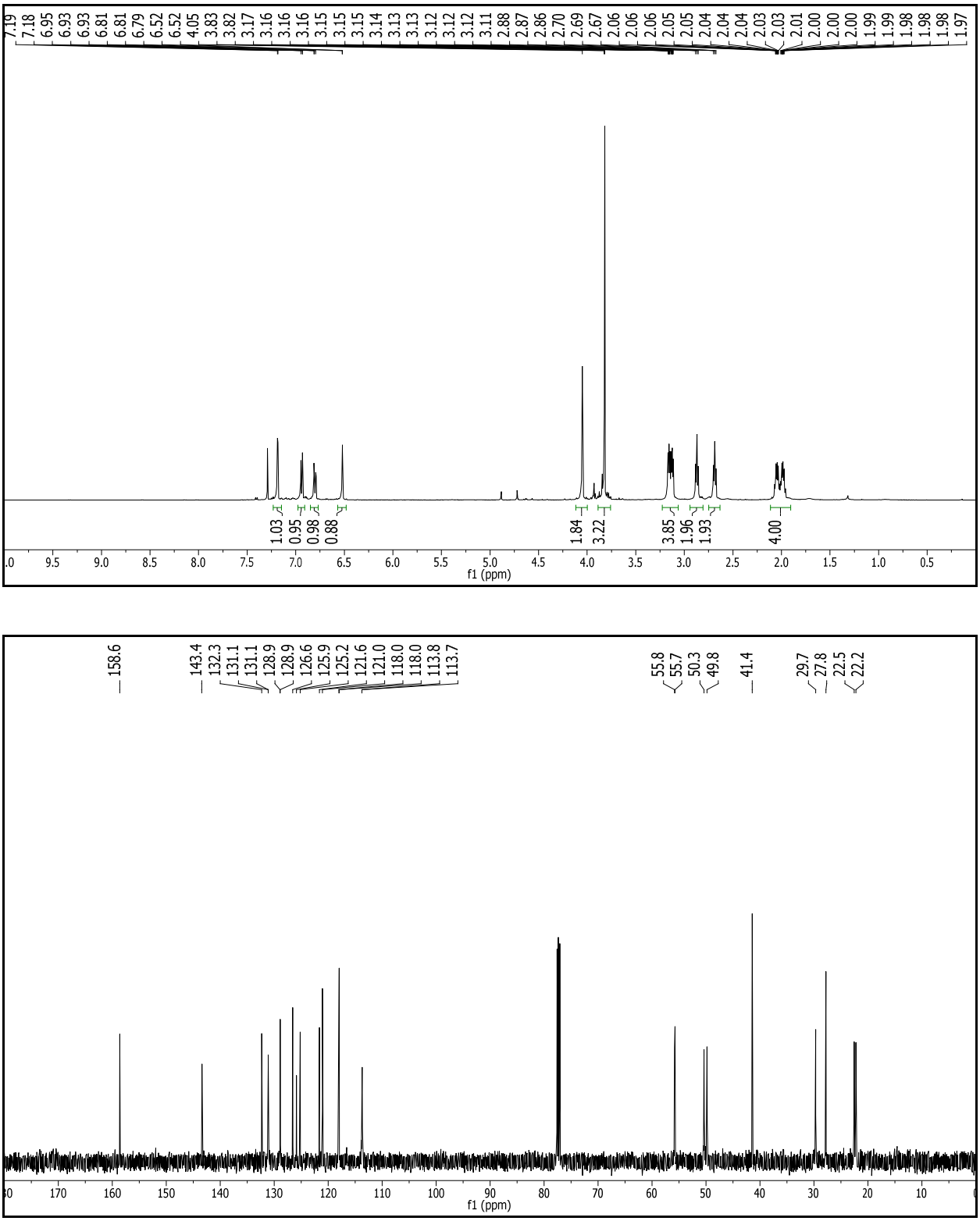


Figure S19. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **9**.

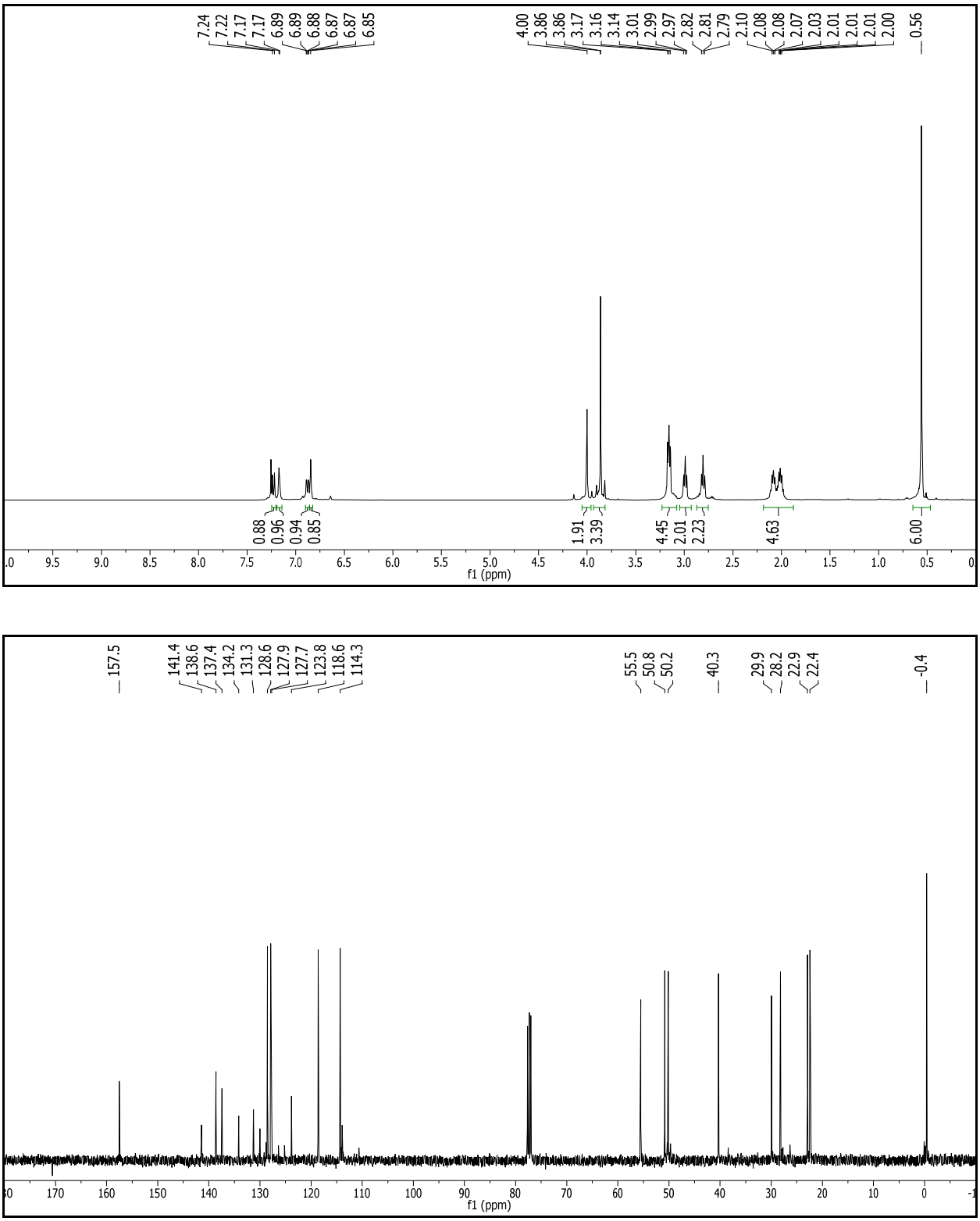


Figure S20. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **10**.

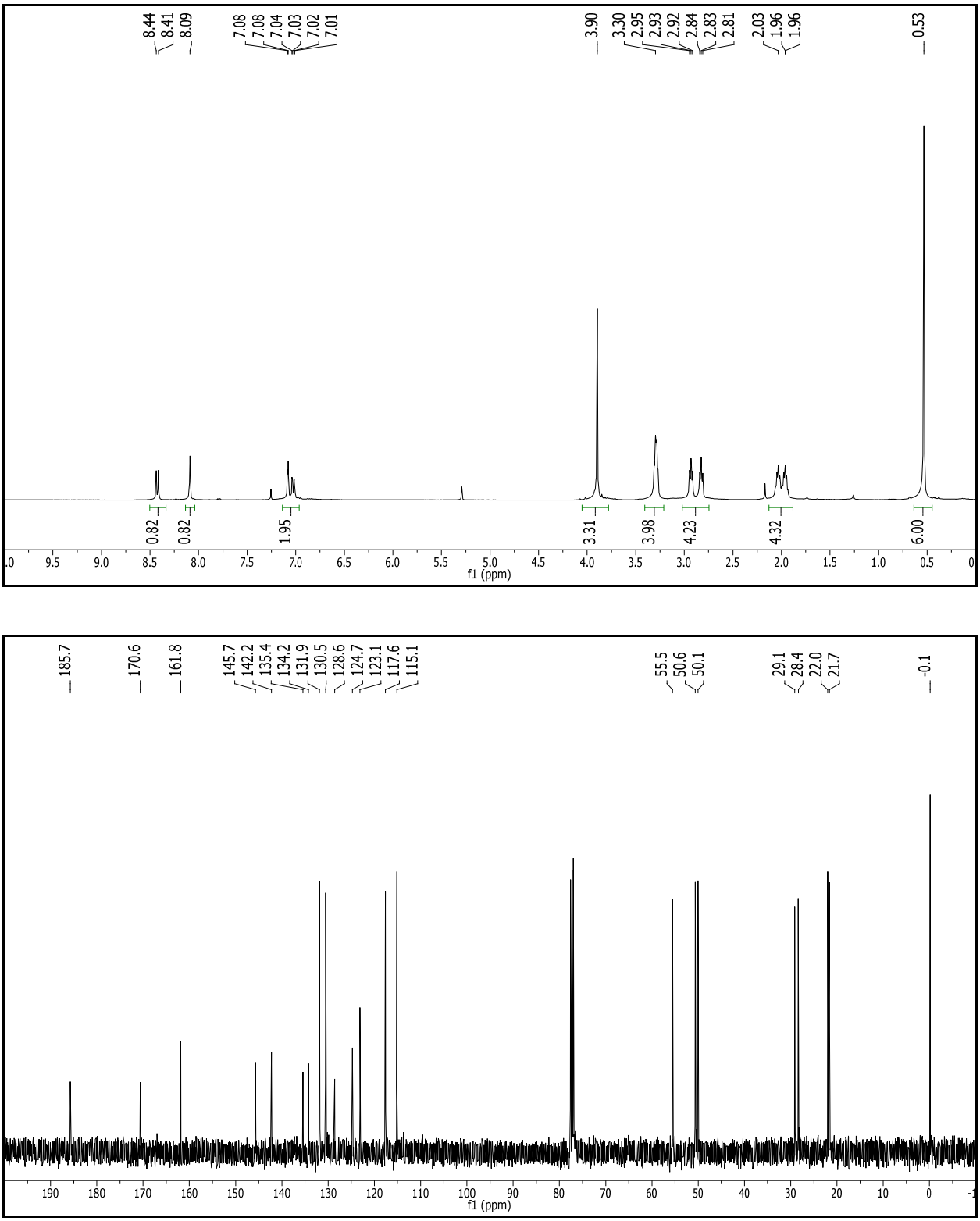


Figure S21. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **1**.

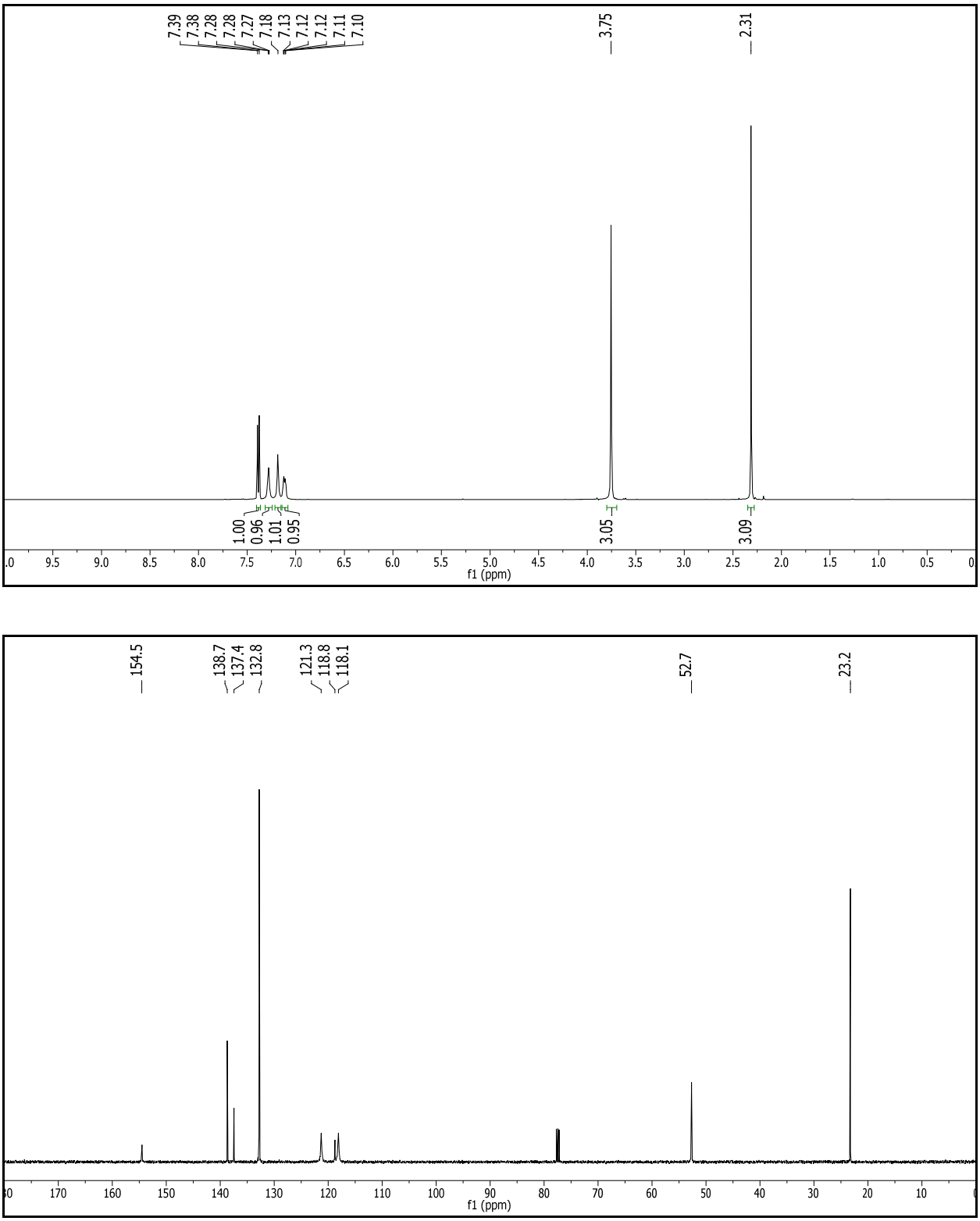


Figure S22. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **11**.

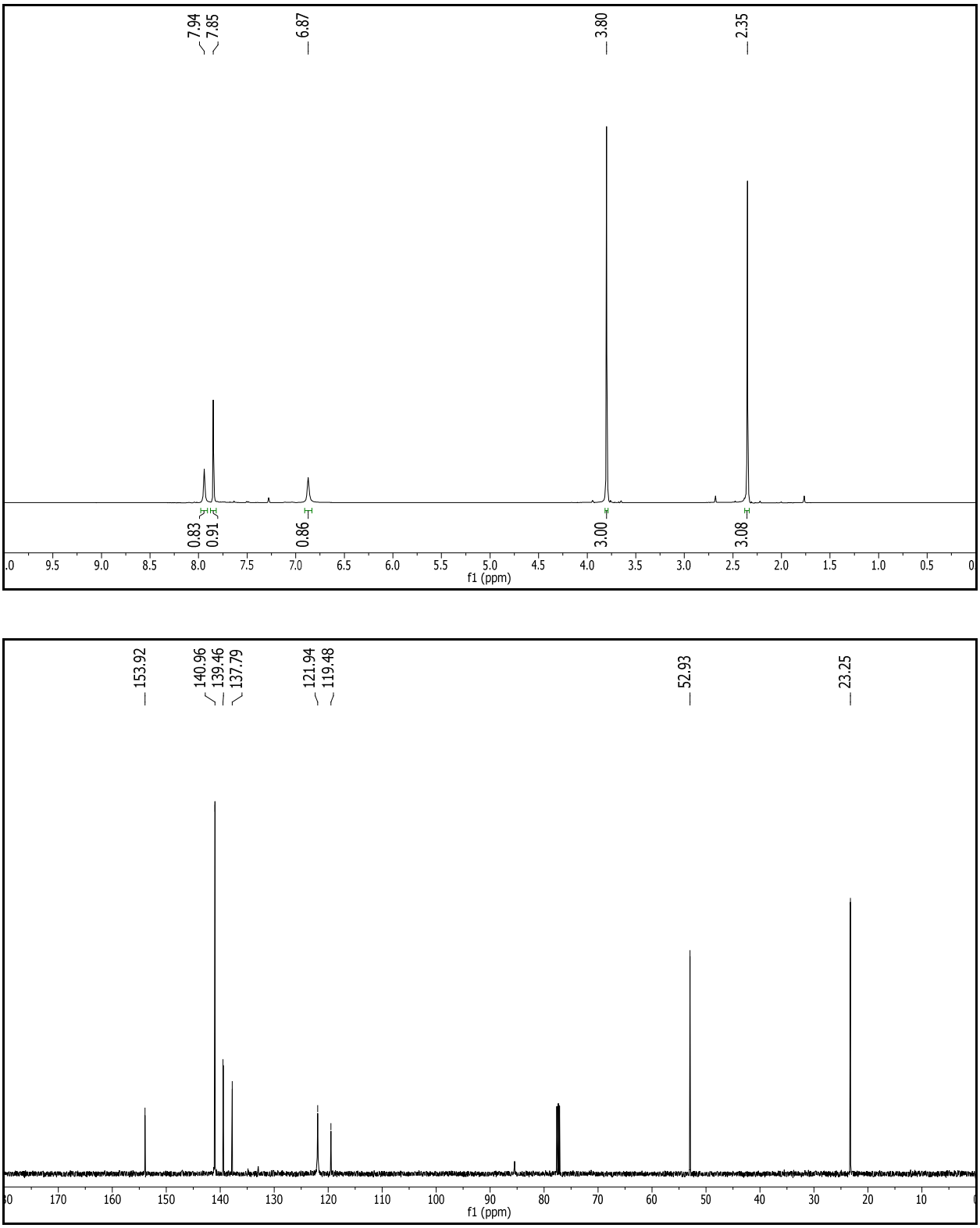


Figure S23. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **12**.

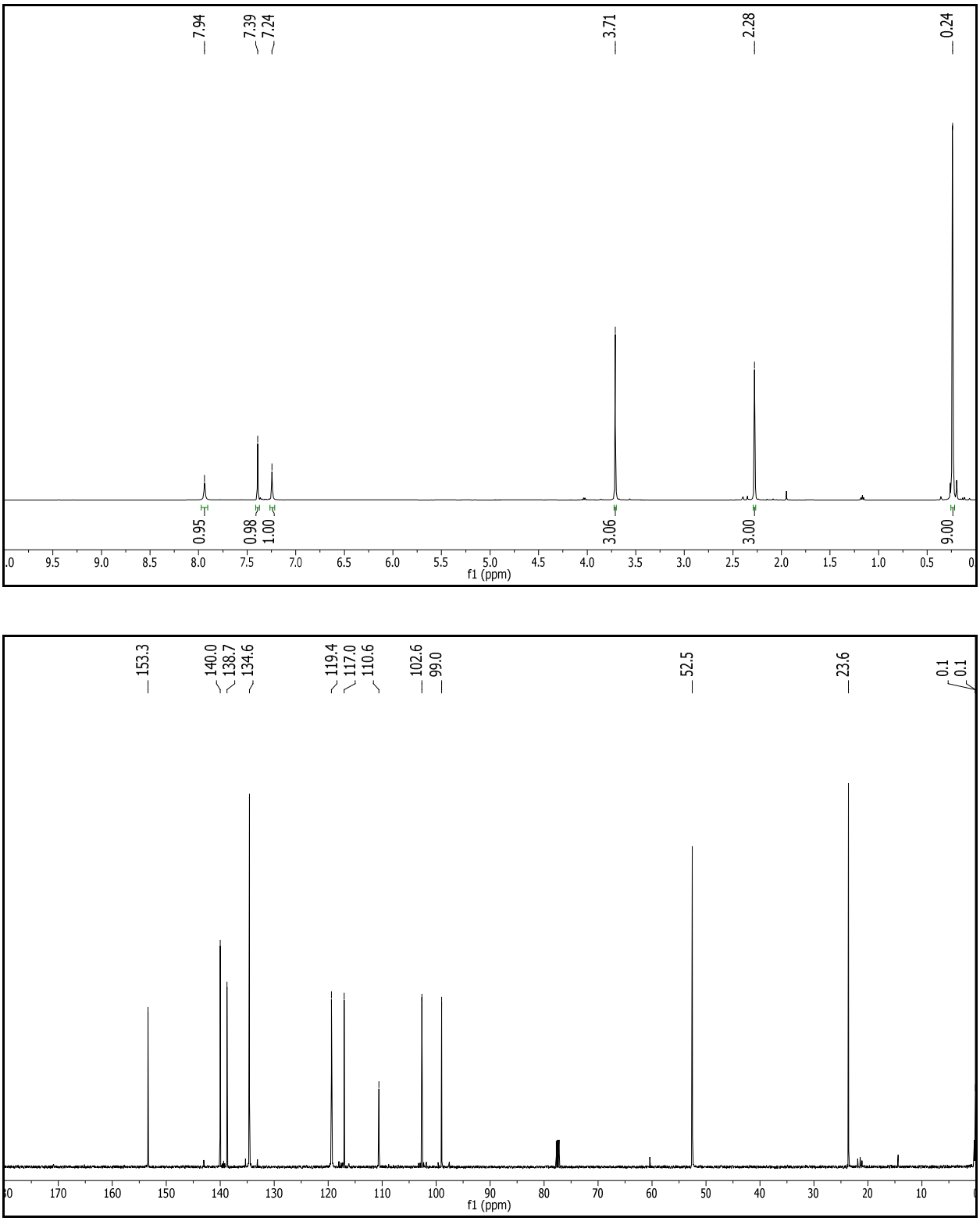


Figure S24. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **13**.

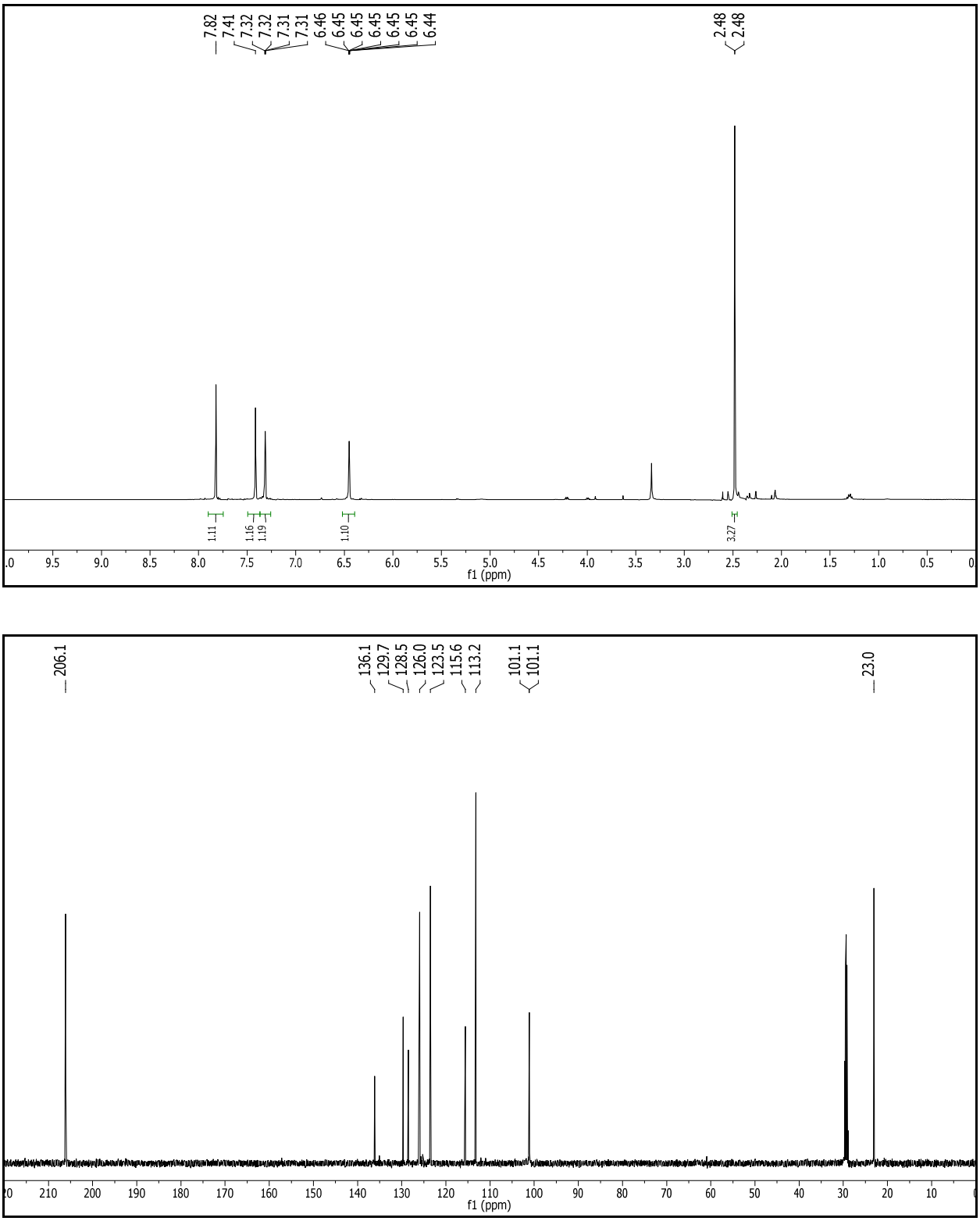


Figure S25. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **14**.

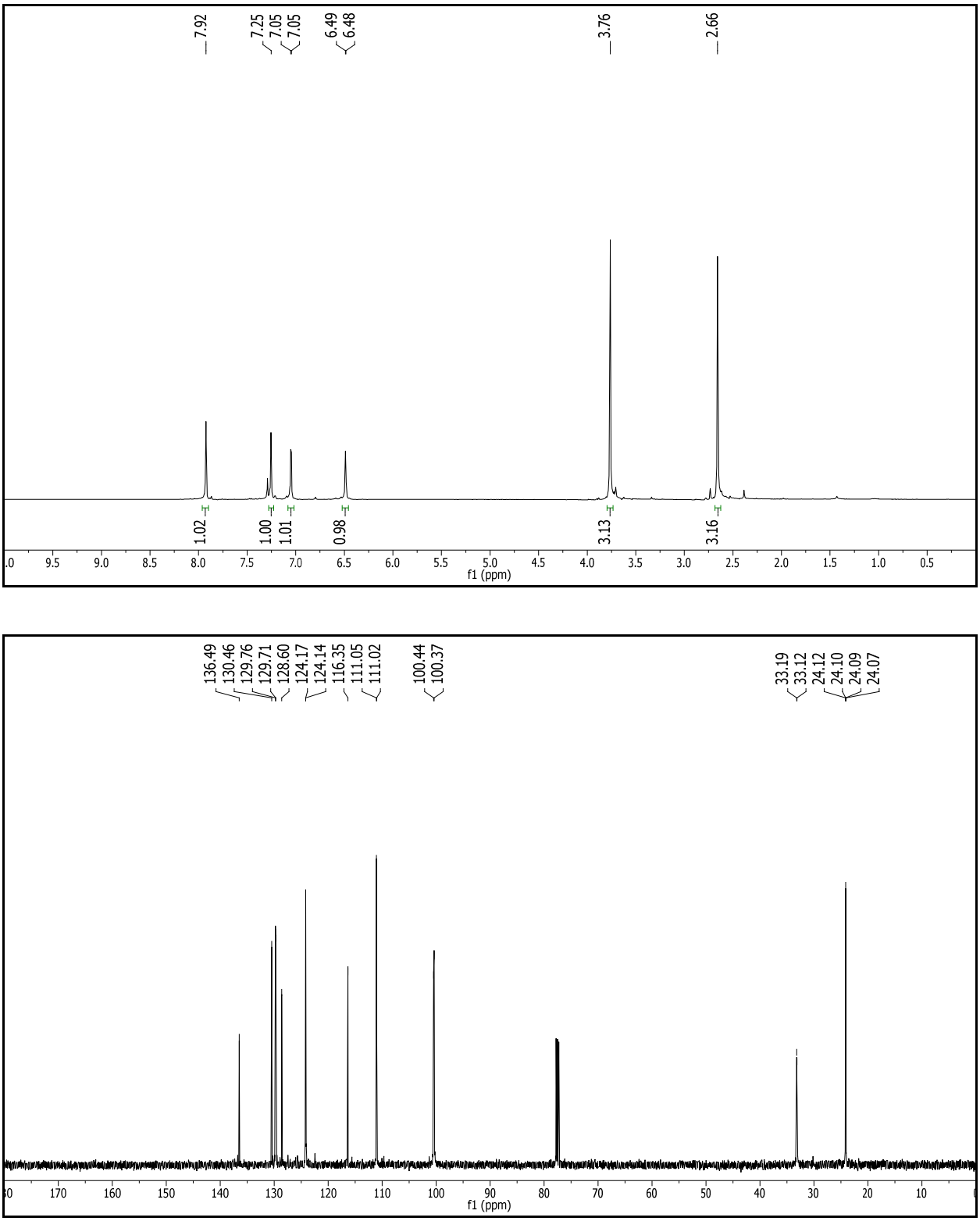


Figure S26. ^{1}H NMR (500 MHz, Acetone-d_6) and ^{13}C NMR (125 MHz, Acetone-d_6) NMR spectra of **2**.

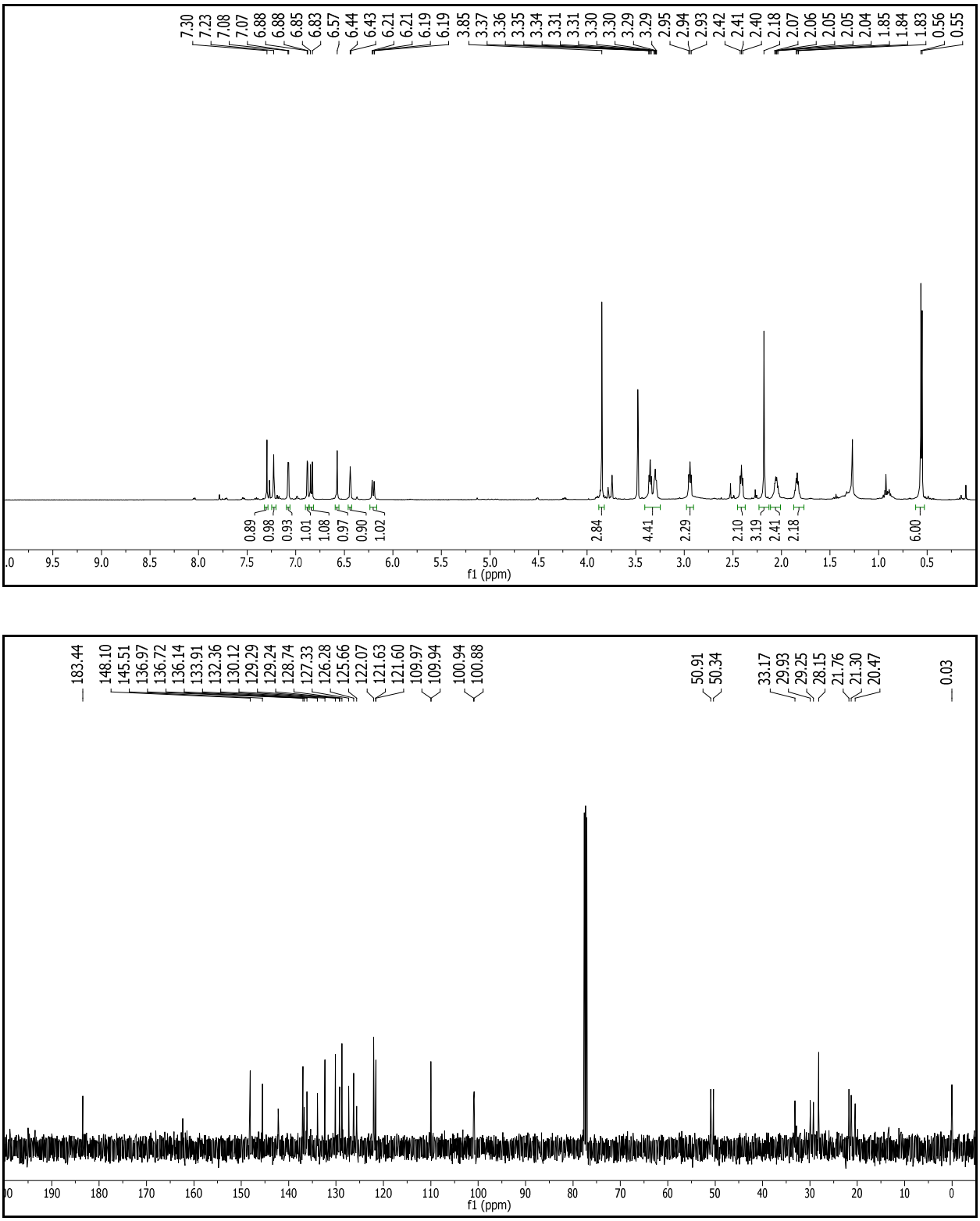


Figure S27. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **3**.

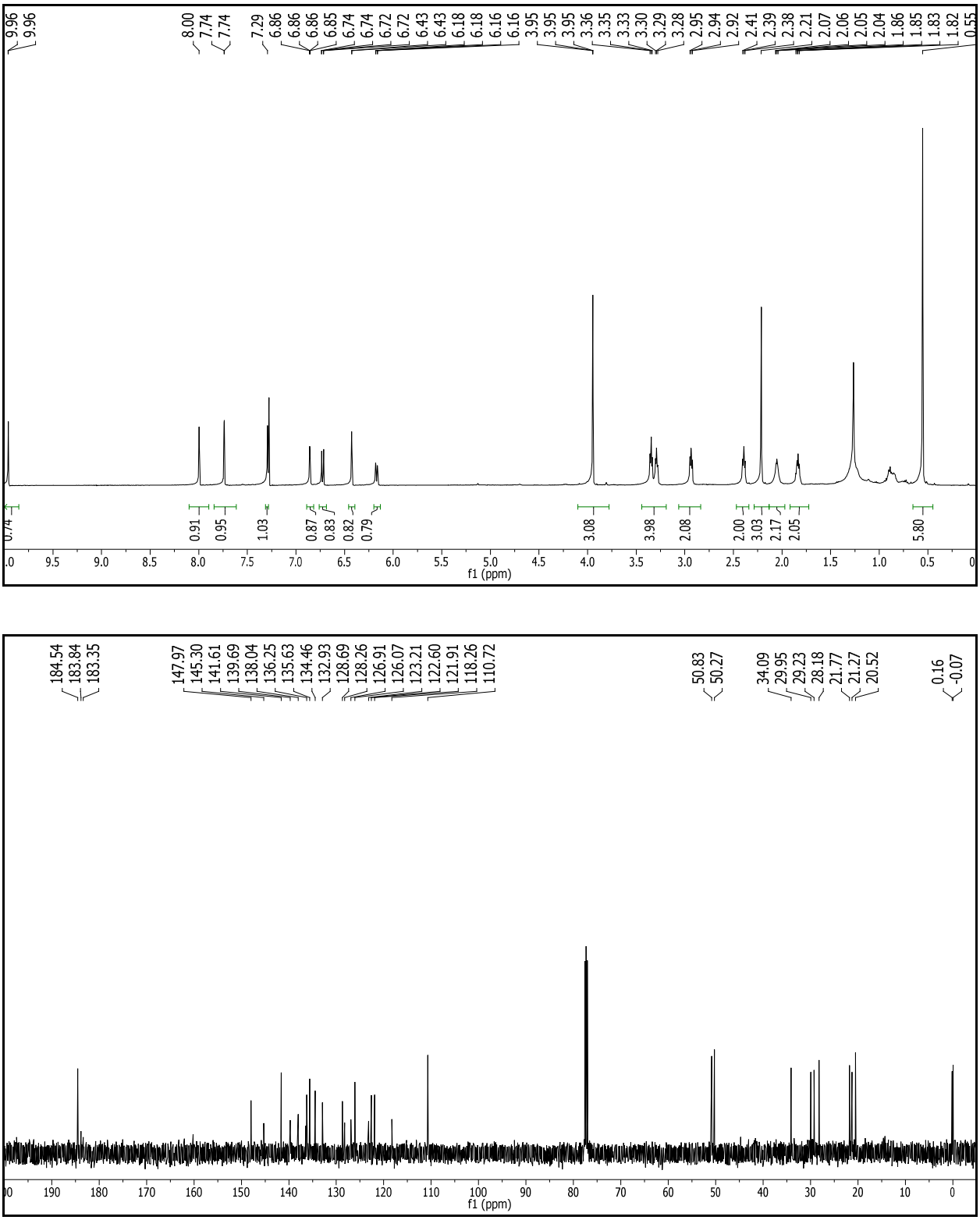


Figure S28. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **4**.

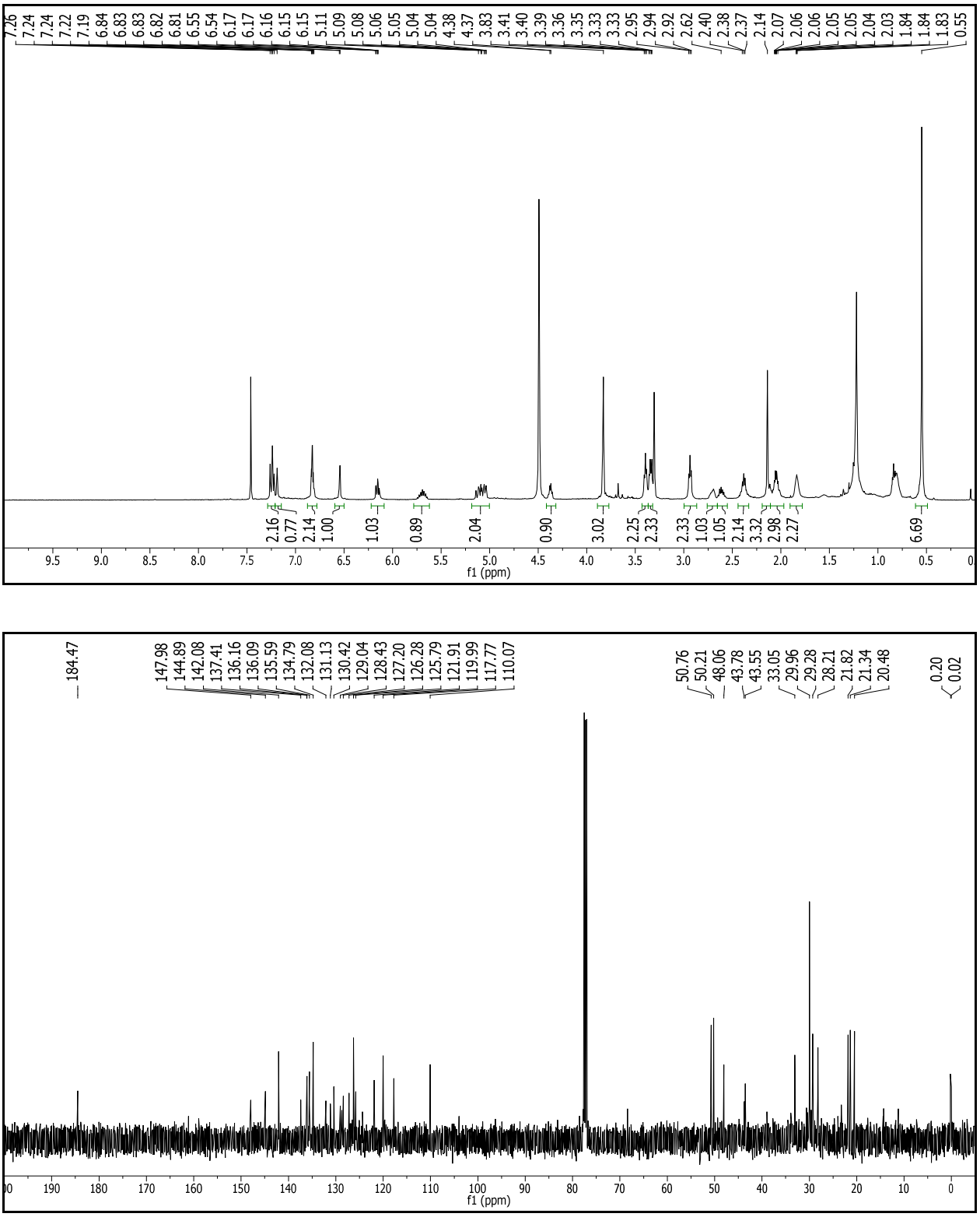


Figure S29. ^{1}H NMR (500 MHz, $CDCl_3$/MeOD) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **5**.

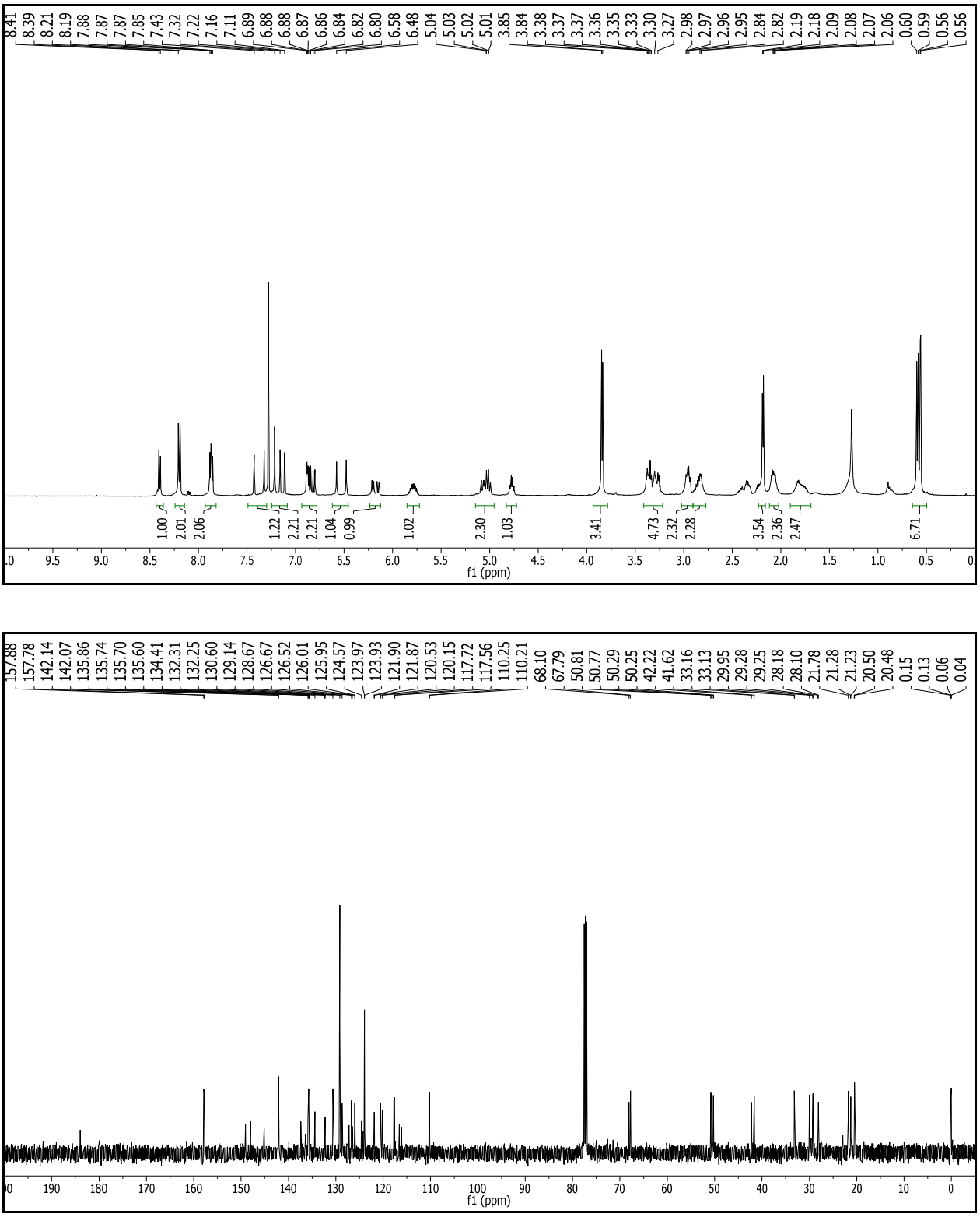


Figure S30. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **FP1**.