Electronic Supplementary Information

Novel mono substituted pyridoimidazoisoquinoliniums via a silver-catalyzed intramolecular cyclization and their applications in cellular imaging

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1. General information

All the chemicals including organic solvents were obtained from commercial vendors and used as received without further purification. All chromatographic separations were accomplished with Silica Gel 60N (Kanto Chemical Co., Inc.) or CHROMATOREX PSQ60B (Fuji Silysia Chemical LTD). Thin-layer chromatography (TLC) was performed using Macherey-Nagel Pre-coated TLC plates Sil G25 UV_{254}. 2-(2-Ethynylphenyl)imidazo[1,2-*a*]pyridines (**1**) were prepared according to the reported procedures.[1] Melting points measurements were conducted on a Yanagimoto micro melting point hot-stage apparatus (MP-S3) and reported as uncorrected values. ^{1}H NMR (TMS: δ = 0.00 ppm as an internal standard), ^{13}C NMR (CD_3OD: δ = 49.00 and DMSO-d_6: δ = 39.52 ppm as an internal standard), and ^{19}F NMR (376 MHz, benzotrifluoride; δ = −64.0 ppm as an external standard) spectra were recorded on JEOL ECZ-400S (for ^{1}H-, ^{13}C-, and ^{19}F NMR, 400, 100 and 376 MHz, respectively) spectrometers. Mass spectra were obtained an Agilent 5977E Diff-SST MSD-230V instrument (EI) and Agilent 6230 Accurate-Mass TOF LC/MS system (ESI). X-ray were recorded on Rigaku XtaLAB Synergy with HyPix3000 diffractometer. IR spectra were recorded on a FTIR-8400S or IRAffinity-1S system from Shimadzu spectrometer and were reported in frequency of absorption (cm^{-1}). Only selected IR absorbencies are reported. UV/Vis spectra were recorded at room temperature on a HITACHI U-2800A spectrophotometer ($C = 2.5\times10^{-5}$–5.1×10^{-5} M in CH_3OH) and fluorescence spectra on a JASCO FP-8300 luminescence spectrometer ($C = 2.2\times10^{-6}$–4.2×10^{-6} M in CH_3OH). Fluorescence signals were observed under a confocal laser scanning microscope (LSM-800; Carl Zeiss) and analyzed by ZEN2 software.

2. General procedure for the cyclization of 2-(2-ethynylphenyl)imidazo[1,2-*a*]pyridines

2-(2-Ethynylphenyl)imidazo[1,2-*a*]pyridine (**1**) (0.5 mmol), silver trifluoromethanesulfonate (13 mg, 0.05 mmol, 10 mol%), lithium trifluoromethanesulfonate (78 mg, 0.5 mmol, 1 eq.), and silica gel (1.27 g) was dissolved in CH_2Cl_2 (4 mL) and stirred at room temperature under air. After reaction completed, the reaction mixture was filtered and washed with CH_3OH. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography using CH_2Cl_2/CH_3OH as eluent to give **2**.

3. Characterization data

6-(4-Methoxyphenyl)pyrido[1′,2′;2,3]imidazo[5,1-*a*]isoquinolin-7-ium trifluoromethanesulfonate (**2a**)

Yield: 236 mg (99%); Pale yellow powder (CH_3OH); mp 231–232.5 °C; R_f = 0.3 (CH_2Cl_2-CH_3OH, 10:1); ^{1}H NMR (400 MHz, CD_3OD): δ = 9.28 (s, 1 H, Ar-H), 9.05 (d, *J* = 6.9 Hz, 1 H, Ar-H), 8.46 (t, *J* = 3.7 Hz, 1 H, Ar-H), 7.91–7.89 (m, 1 H, Ar-H), 7.80–7.62 (m, 6 H, Ar-H), 7.25 (t, *J* = 8.7 Hz, 3 H, Ar-H), 6.80 (d, *J* = 9.6 Hz, 1 H, Ar-H), 3.96 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, CD_3OD): δ = 163.2 (C), 136.2 (C), 135.8 (C), 134.1 (C), 132.5 (CH), 132.3 (CH), 132.2 (CH), 130.72 (CH), 130.68 (C), 130.5 (CH), 129.0 (CH), 126.1 (C), 125.3 (CH), 121.9 (C), 120.2 (CH), 119.3 (CH), 116.1 (CH), 115.1 (CH), 108.7 (CH), 56.1 (CH_3); ^{19}F NMR (376 MHz, CD_3OD): δ = −81.34; IR (KBr) *v* = 3123, 3082, 1256, 1032, 638 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{22}H_{17}N_2O$ $[M-OTf]^+$: 325.1335, found 325.1332.

6-(4-Methylphenyl)pyrido[1′,2′;2,3]imidazo[5,1-*a*]isoquinolin-7-ium trifluoromethanesulfonate (**2b**)

Yield: 213 mg (93%); Yellow plate (CH_3OH); mp 232–233.5 °C; R_f = 0.3 (CH_2Cl_2-CH_3OH, 10:1); ^{1}H NMR (400 MHz, CD_3OD): δ = 9.29 (s, 1 H, Ar-H), 9.05 (dt, *J* = 6.9, 1.4 Hz, 1 H, Ar-H), 8.47 (dd, *J* = 5.5, 3.2 Hz, 1 H, Ar-H), 7.92–7.89 (m, 1 H, Ar-H), 7.82–7.77 (m, 2 H, Ar-H), 7.73–7.59 (m, 4 H, Ar-H), 7.53 (d, *J* = 7.8 Hz, 2 H, Ar-H), 7.28 (s, 1 H, Ar-H), 6.76 (d, *J* = 9.1 Hz, 1 H, Ar-H), 2.56 (s, 3 H, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ = 142.9 (C), 136.2 (C), 136.0 (C), 134.1 (C), 132.6 (CH), 132.2 (CH), 131.4 (CH), 131.3 (C), 130.81 (CH), 130.77 (CH), 130.6 (C), 130.5 (CH), 129.1 (CH), 125.4 (CH), 122.0 (C), 120.2 (CH), 119.3 (CH), 115.0 (CH), 108.7 (CH), 21.6 (CH_3); ^{19}F NMR (376 MHz, CD_3OD): δ = −81.36; IR (KBr) *v* = 3100, 1258, 1169, 1028, 637 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{22}H_{17}N_2$ $[M-OTf]^+$: 309.1386, found 309.1393.

6-Phenyl-pyrido[1′,2′;2,3]imidazo[5,1-*a*]isoquinolin-7-ium trifluoromethanesulfonate (**2c**)

Yield: 222 mg (99%); Yellow plate (CH_3OH); mp 242–243.5 °C; R_f = 0.3 (CH_2Cl_2-CH_3OH, 10:1); ^{1}H NMR (400 MHz, CD_3OD): δ = 9.30 (s, 1 H, Ar-H), 9.06 (d, *J* = 6.4 Hz, 1 H, Ar-H), 8.49–8.47 (m, 1 H, Ar-H), 7.93–7.91 (m, 1 H, Ar-H), 7.83–7.63 (m, 9 H, Ar-H), 7.32 (s, 1 H, Ar-H), 6.68 (d, *J* = 9.1 Hz, 1 H, Ar-H); ^{13}C NMR (100 MHz, CD_3OD): δ = 136.2 (C), 135.8 (C), 134.3 (C), 134.0 (C), 132.6 (CH), 132.3 (CH), 132.2 (CH), 130.91 (CH), 130.88 (CH), 130.56 (C), 130.56 (CH), 129.1 (CH), 125.4 (CH), 122.0 (C), 120.2 (CH), 119.4 (CH), 114.9 (CH), 108.8

(CH); ^{19}F NMR (376 MHz, CD_3OD): δ = −81.35; IR (KBr) *v* = 3092, 1275, 1250, 1028, 638 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{21}H_{15}N_2$ $[M-OTf]^+$: 295.1230, found 295.1226.

6-(4-Fluorophenyl)pyrido[1′,2′;2,3]imidazo[5,1-*a*]isoquinolin-7-ium trifluoromethanesulfonate (**2d**)

Yield: 227 mg (98%); Colorless plate (CH_3OH); mp 283–284.5 °C; R_f = 0.6 (CH_2Cl_2-CH_3OH, 5:1); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.56 (s, 1 H, Ar-H), 9.22 (d, *J* = 6.9 Hz, 1 H, Ar-H), 8.58–8.56 (m, 1 H, Ar-H), 8.00–7.98 (m, 1 H, Ar-H), 7.88–7.81 (m, 5 H, Ar-H), 7.74 (t, *J* = 6.4 Hz, 1 H, Ar-H), 7.58 (tt, *J* = 11.0, 2.3 Hz, 2 H, Ar-H), 7.43 (s, 1 H, Ar-H), 6.71 (d, *J* = 9.1 Hz, 1 H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 163.4 (d, $^1J_{C,F}$ = 247.6 Hz, C), 134.4 (C), 133.3 (C), 132.2 (d, $^3J_{C,F}$ = 8.7 Hz, CH), 131.8 (C), 131.6 (CH), 131.4 (CH), 129.8 (CH), 129.6 (CH), 129.2 (C), 128.8 (C), 127.9 (CH), 124.3 (CH), 120.6 (C), 119.1 (CH), 117.8 (CH), 116.8 (d, $^2J_{C,F}$ = 22.2 Hz, CH), 113.5 (CH), 107.9 (CH); ^{19}F NMR (376 MHz, DMSO-d_6): δ = −79.08 (3 F), −110.61 (1 F); IR (ATR) *v* = 3084, 1250, 1153, 1028, 637 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{21}H_{14}FN_2$ $[M-OTf]^+$: 313.1136, found 313.1130.

6-[4-(Trifluoromethyl)phenyl]pyrido[1′,2′;2,3]imidazo[5,1-*a*]isoquinolin-7-ium trifluoromethanesulfonate (**2e**)

Yield: 197 mg (77%); Pale yellow plate (CH_3OH); mp >300 °C; R_f = 0.3 (CH_2Cl_2-CH_3OH, 10:1); ^{1}H NMR (400 MHz, CD_3OD): δ = 9.34 (s, 1 H, Ar-H), 9.09 (d, *J* = 6.4 Hz, 1 H, Ar-H), 8.51 (t, *J* = 5.0 Hz, 1 H, Ar-H), 8.03 (d, *J* = 8.2 Hz, 2 H, Ar-H), 7.98–7.94 (m, 3 H, Ar-H), 7.86–7.76 (m, 3 H, Ar-H), 7.68 (t, *J* = 6.9 Hz, 1 H, Ar-H), 7.39 (s, 1 H, Ar-H), 6.83 (d, *J* = 9.6 Hz, 1 H, Ar-H); ^{13}C NMR (100 MHz, CD_3OD): δ = 138.2 (C), 136.1 (C), 134.3 (C), 134.1 (C), 133.9 (q, $^2J_{C,F}$ = 35.6 Hz, C), 132.73 (CH), 132.71 (CH), 131.8 (CH), 131.3 (CH), 130.7 (CH), 130.3 (C), 129.4 (CH), 127.7 (q, $^3J_{C,F}$ = 3.9 Hz, CH), 125.4 (CH), 125.3 (q, $^1J_{C,F}$ = 271.7 Hz, C), 122.3 (C), 120.4 (CH), 120.3 (CH), 114.8 (CH), 108.9 (CH); ^{19}F NMR (376 MHz, CD_3OD): δ = −65.54 (3 F), −81.38 (3 F); IR (KBr) *v* =3134, 1325, 1279, 1138, 637 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{22}H_{14}F_3N_2$ $[M-OTf]^+$: 363.1104, found 363.1097.

6-(3-Thienyl)pyrido[1′,2′;2,3]imidazo[5,1-*a*]isoquinolin-7-ium trifluoromethanesulfonate (**2f**)

Yield: 224 mg (99%); Yellow prism (CH_3OH); mp 235–236 °C; R_f = 0.3 (CH_2Cl_2-CH_3OH, 10:1); ^{1}H NMR (400 MHz, CD_3OD): δ = 9.29 (s, 1 H, Ar-H), 9.06 (d, *J* = 6.2 Hz, 1 H, Ar-H), 8.51–8.46 (m, 1 H, Ar-H), 8.00–7.76 (m, 6

H, Ar-H), 7.67 (t, *J* = 6.7 Hz, 1 H, Ar-H), 7.41 (dd, *J* = 4.6, 0.9 Hz, 1 H, Ar-H), 7.37 (s, 1 H, Ar-H), 6.81 (d, *J* = 9.2 Hz, 1 H, Ar-H); ^{13}C NMR (100 MHz, CD_3OD): δ = 134.9 (C), 132.8 (C), 132.7 (C), 131.3 (CH), 131.1 (CH), 129.8 (C), 129.7 (CH), 129.2 (C), 129.1 (CH), 128.4 (CH), 128.34 (CH), 128.25 (CH), 127.8 (CH), 124.1 (CH), 120.8 (C), 118.9 (CH), 118.5 (CH), 113.4 (CH), 107.4 (CH); ^{19}F NMR (376 MHz, CD_3OD): δ = −81.38; IR (KBr) *v* = 3123, 1275, 1258, 1030, 640 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{19}H_{13}N_2S$ $[M-OTf]^+$: 301.0794, found 301.0788.

6-(1-Cyclohexenyl)pyrido[1′,2′;2,3]imidazo[5,1-*a*]isoquinolin-7-ium trifluoromethanesulfonate (**2g**)

Yield: 218 mg (97%); Colorless needle (CH_3OH); mp 270.5–272 °C; R_f = 0.3 (CH_2Cl_2-CH_3OH, 10:1); ^{1}H NMR (400 MHz, CD_3OD): δ = 9.23 (s, 1 H, Ar-H), 9.07 (d, *J* = 6.4 Hz, 1 H, Ar-H), 8.42–8.37 (m, 2 H, Ar-H), 8.11 (t, *J* = 8.2 Hz, 1 H, Ar-H), 7.87 (d, *J* = 6.0 Hz, 1 H, Ar-H), 7.77–7.73 (m, 3 H, Ar-H), 7.19 (s, 1 H, Ar-H), 6.44 (s, 1 H, Ar-H), 2.44–2.29 (m, 4 H, Ar-H_2), 1.99–1.79 (m, 4 H, Ar-H_2); ^{13}C NMR (100 MHz, CD_3OD): δ = 138.1 (C), 135.8 (CH), 133.8 (C), 133.2 (C), 133.1 (CH), 132.5 (CH), 130.9 (C), 130.6 (CH), 130.5 (CH), 128.9 (CH), 125.3 (CH), 121.8 (C), 120.3 (CH), 117.7 (CH), 115.1 (CH), 108.6 (CH), 29.4 (CH_2), 26.4 (CH_2), 23.0 (CH_2), 22.4 (CH_2); ^{19}F NMR (376 MHz, CD_3OD): δ = −81.36; IR (KBr) *v* = 3111, 2943, 1285, 1032, 638 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{21}H_{19}N_2$ $[M-OTf]^+$: 299.1543, found 299.1536.

6-(*n*-Butyl)pyrido[1′,2′;2,3]imidazo[5,1-*a*]isoquinolin-7-ium trifluoromethanesulfonate (**2h**)

Yield: 203 mg (95%); Pale yellow prism (CH_3OH); mp 206–207 °C; R_f = 0.3 (CH_2Cl_2-CH_3OH, 10:1); ^{1}H NMR (400 MHz, CD_3OD): δ = 9.17 (s, 1 H, Ar-H), 9.04 (d, *J* = 7.8 Hz, 1 H, Ar-H), 8.59 (d, *J* = 9.6 Hz, 1 H, Ar-H), 8.30–8.28 (m, 1 H, Ar-H), 8.11 (t, *J* = 7.3 Hz, 1 H, Ar-H), 7.78–7.62 (m, 4 H, Ar-H), 7.18 (s, 1 H, Ar-H), 3.42–3.30 (m, 2 H, CH_2), 1.94–1.86 (m, 2 H, CH_2), 1.71–1.62 (m, 2 H, CH_2), 1.09 (t, *J* = 7.3 Hz, 3 H, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ =137.8 (C), 135.9 (C), 133.9 (C), 133.2 (CH), 132.4 (CH), 130.5 (CH), 130.4 (C), 130.1 (CH), 128.2 (CH), 124.9 (CH), 121.2 (C), 120.3 (CH), 116.2 (CH), 108.6 (CH), 33.8 (CH_2), 30.3 (CH_2), 23.1 (CH_2), 14.3 (CH_3); ^{19}F NMR (376 MHz, CD_3OD): δ = −79.72; IR (KBr) *v* =3123, 2942, 1250, 1028, 637 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{19}H_{19}N_2$ $[M-OTf]^+$: 275.1543, found 275.1538.

4. Single crystal X-ray diffraction experiment

The X-ray diffraction measurements of compounds **2b** were carried out using an XtaLAB Synergy, Single source at home/near, HyPix3000 diffractometer. The crystal was kept at 103 K during data collection. Using Olex2,[2] the structure was solved with the SHELXT[3] structure solution program using Intrinsic Phasing and refined with the SHELXL[4] refinement package using Least Squares minimization.

Crystal data and structure refinement.

The colorless plate crystal (0.32 × 0.272 × 0.147 mm^3) of **2b** obtained from CH_2Cl_2/hexane. $C_{23}H_{17}N_2O_3F_3S$ (M = 458.44 g/mol): triclinic, space group *P*-1 (no. 2), a = 7.5716(2) Å, b = 10.5701(3) Å, c = 13.3435(4) Å, α = 81.216(2)°, β = 83.633(2)°, γ = 76.137(2)°, V = 1021.57(5) $Å^3$, Z = 2, T = 103 K, μ(Cu Kα) = 1.919 mm^{-1}, D_{calc} = 1.490 g/cm^3, 8540 reflections measured (6.724° ≤ 2Θ ≤ 136.664°), 3708 unique (R_{int} = 0.0321, R_{sigma} = 0.0310) which were used in all calculations. The final R_1 was 0.0367 (I > 2σ(I)) and wR_2 was 0.0986 (all data). CCDC 2324904 contains the supplementary crystallographic data which can be obtained free of charge from the Cambridge Crystallographic Data Center via https://www.ccdc.cam.ac.uk/structures/

5. Optical property of 2c

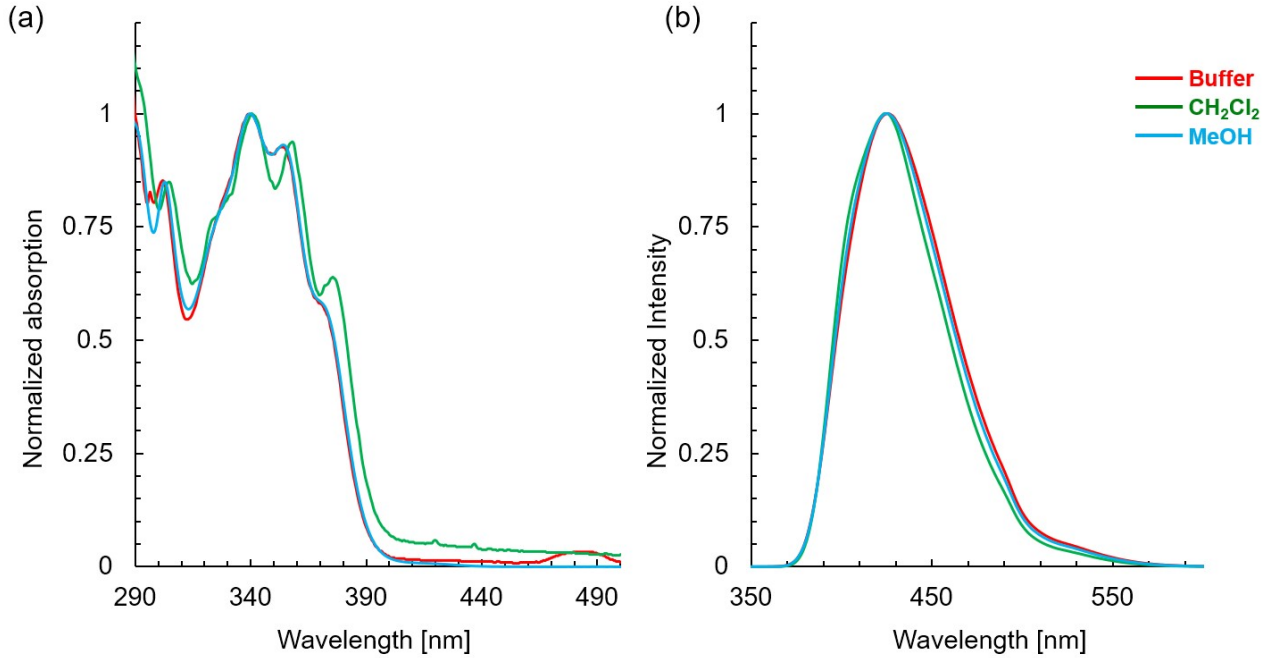


Figure S1: Normalized (a) absorption spectra and (b) fluorescence spectra of **2c** in MeOH, CH_2Cl_2 and HEPES buffer (same condition of staining solutions).

6. Cell culture and imaging

Cell culture

HeLa cells were cultured at 37°C in a humid atmosphere of 5% CO_2 in Eagle's MEM (minimum essential medium) supplemented with 10% fetal bovine serum (FBS).

Preparing stock solutions

To prepare a stock solution, dissolve the synthesized compounds **2a-h** in high-quality, anhydrous dimethylsulfoxide (DMSO) to a final concentration of 2 mM.

Preparing staining solutions

Dilute 2mM compounds **2a-h** (see Preparing stock solutions) to the final working concentration in appropriate buffer or growth medium.

Cell staining study

To investigate the cell staining efficiency of compounds **2a–h**, cells were incubated with compounds **2a–h** (2 μM) in an observation chamber for 30 min and washed using HEPES buffer. The compounds **2a–h** into cells were excited at 405 nm and fluorescence was detected by a band pass filter (400-590 nm) using a confocal laser scanning microscopy. The intracellular fluorescence intensity was measured by ZEN software (Zeiss).

Intracellular localization study

The cellular localization of compounds **2c** was determined by the colocalization of organelle specific dyes including ER-Tracker Green (Endoplasmic reticulum specific dye), MitoTracker Green (Mitochondria specific dye) and LysoTracker Green (Lysosome specific dye). Cells were incubated with compounds **2c** (2 μM) and organelle-specific dyes (concentration of each dye follows the protocol from suppliers) in an observation chamber for 30 min and washed using HEPES Buffer. The compounds **2c** into cells were excited at 405 nm and fluorescence was detected by a band pass filter (400-495 nm) using a confocal laser scanning microscopy. ER-Tracker Green, MitoTracker Green

and LysoTracker Green into cells were excited at 488 nm and fluorescence was detected by a band pass filter (400-590 nm).

Cytotoxicity assay

The cells cultured until they reached confluency were treated with compounds **2a–h** (2 μM) for 30 min. The cytotoxic effects of the compounds on these cells were investigated using 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay.

Statistical analysis

Tukey–Kramer's test and Dunnett's test were used to compare the differences between groups. Results were considered statistically significant at $p < 0.05$.

CCCP treatment

Hela cells treated with CCCP (100 μM) for 1 h and untreated cells were incubated with compound **2c** for 30 min in an observation chamber. They then observed it with a confocal laser scanning microscope and compared the images obtained.

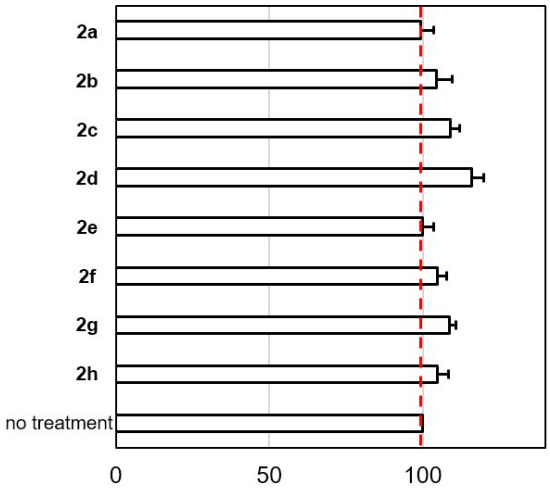


Figure S2. Cell viability was measured by MTT assay. HeLa cells incubated with **2a–h** (2 μM) at 37 °C for 30 min. Mean ± SE, n = 6, **p<0.05 vs. no treatment (Dunnett's test)

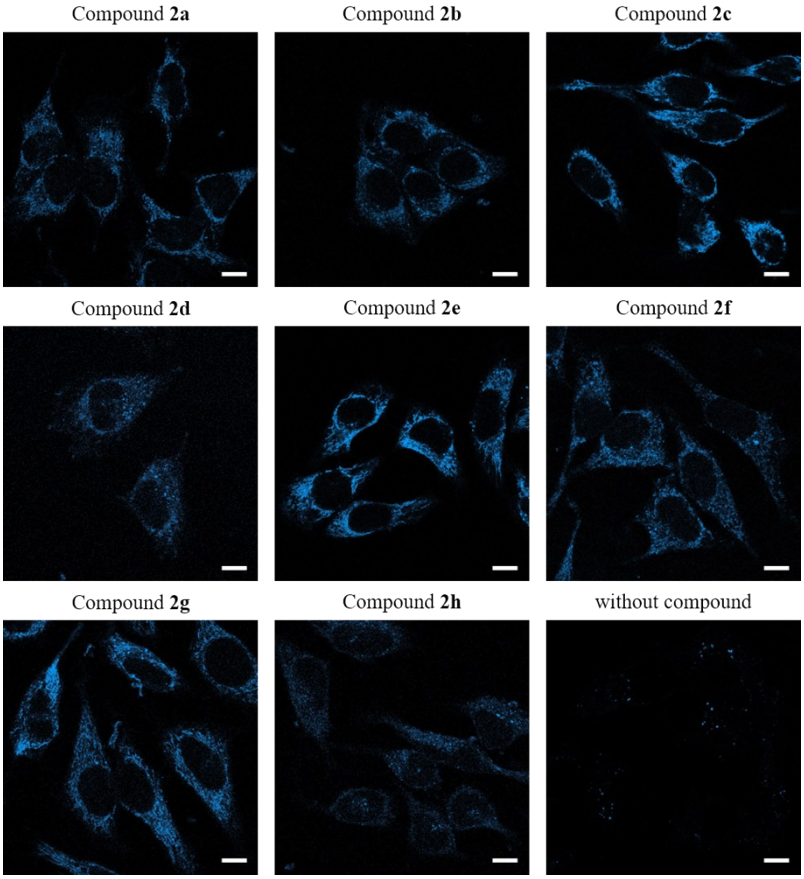


Figure S3. CLSM images of HeLa cells incubated with **2a–h** (2 μM) and without compound for 30 min. Scale bar: 10 μm.

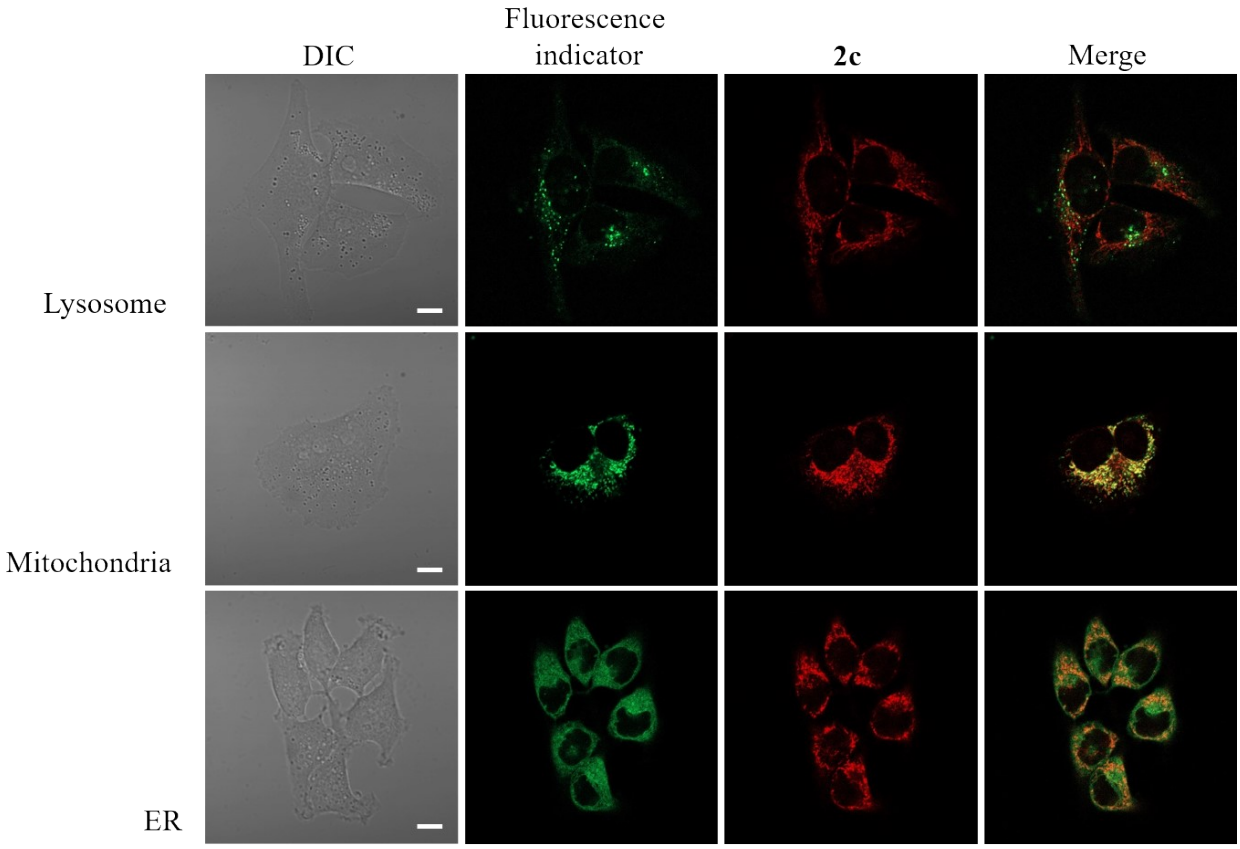


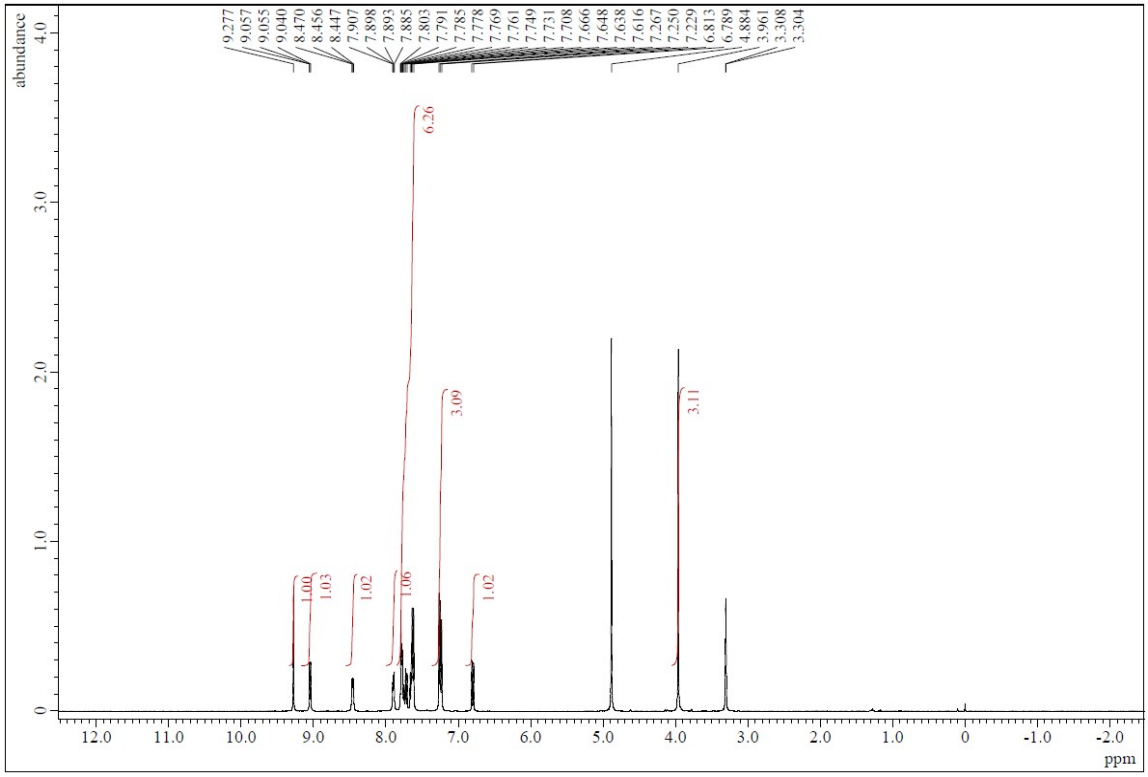
Figure S4. HeLa cells incubated with **2c** (2 μM) and fluorescence indicator at 37 ℃ for 30 min. Scale bar: 10 μm.

7. References

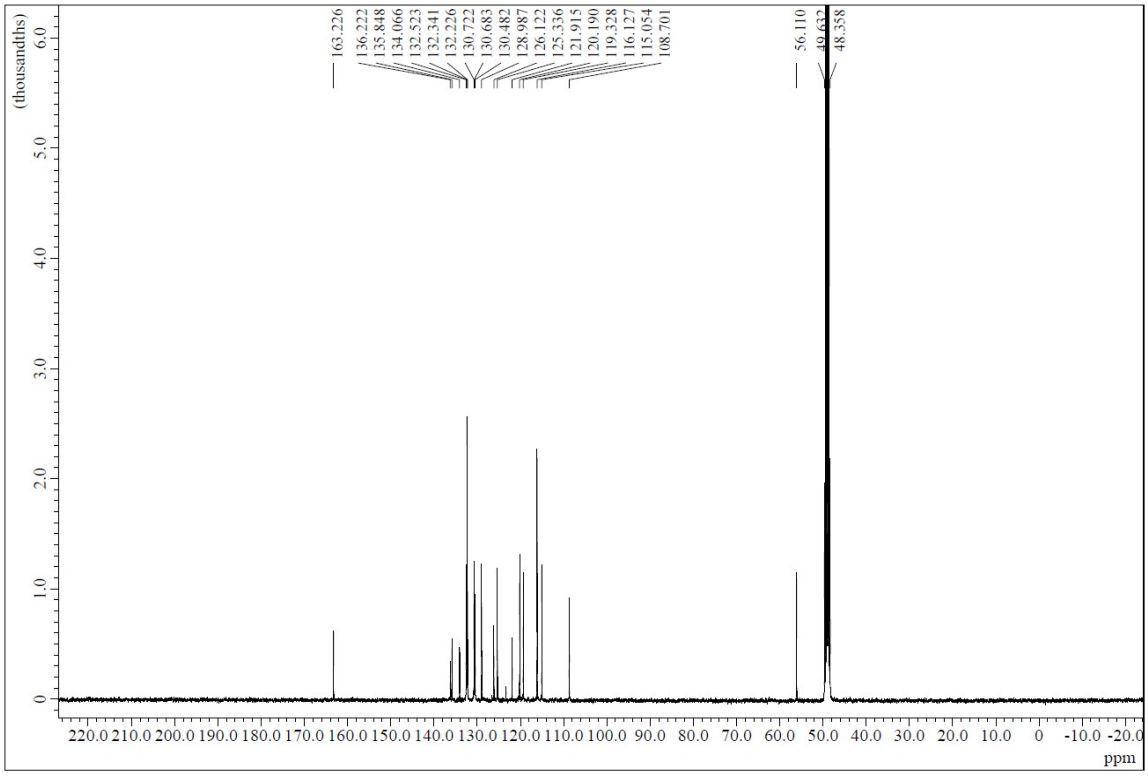
1. M. Kawakubo, Y. Inaguma, Y. Murata, M. Matsumura and Yasuike, *Tetrahedron Lett.*, 2022, **105**, 154054.
2. O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard and H. Puschmann, *J. Appl. Cryst.*, 2009, **42**, 339–341.
3. G.M. Sheldrick, *Acta Cryst.*, 2015, **A71**, 3–8.
4. G.M. Sheldrick, *Acta Cryst.*, 2015, **C71**, 3–8.

8. Copies of ^{1}H and ^{13}C NMR spectra of novel compounds

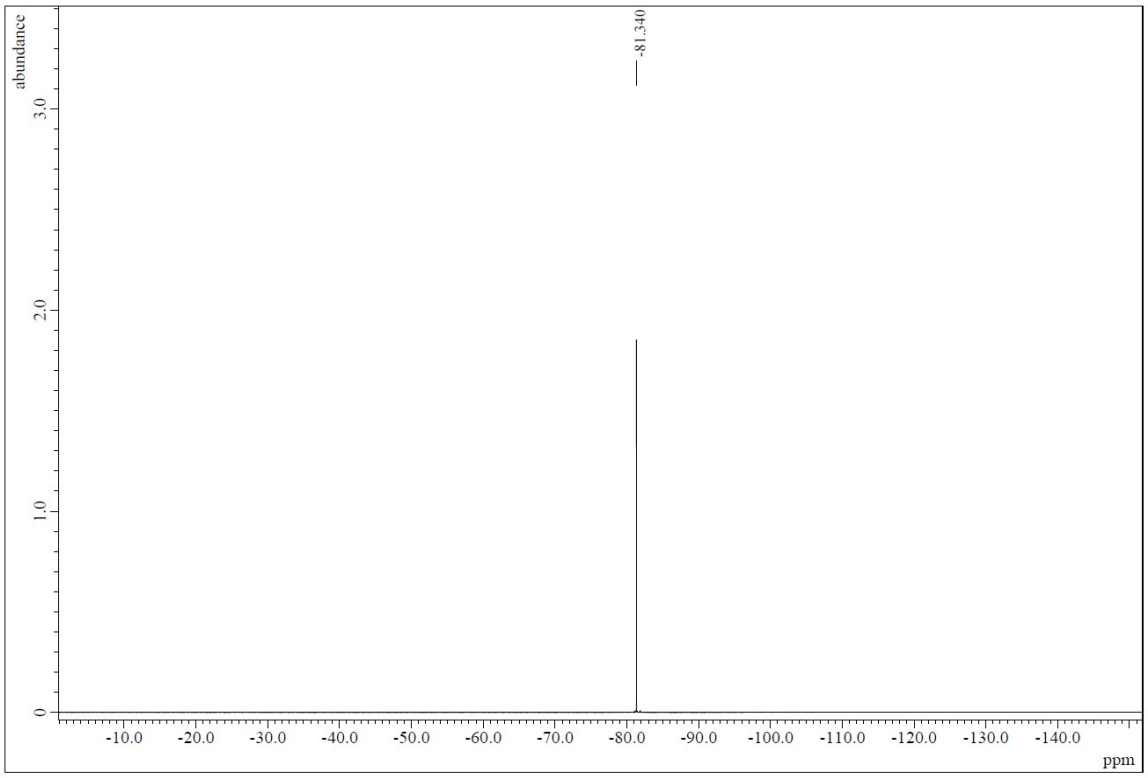
^{1}H NMR of **2a**



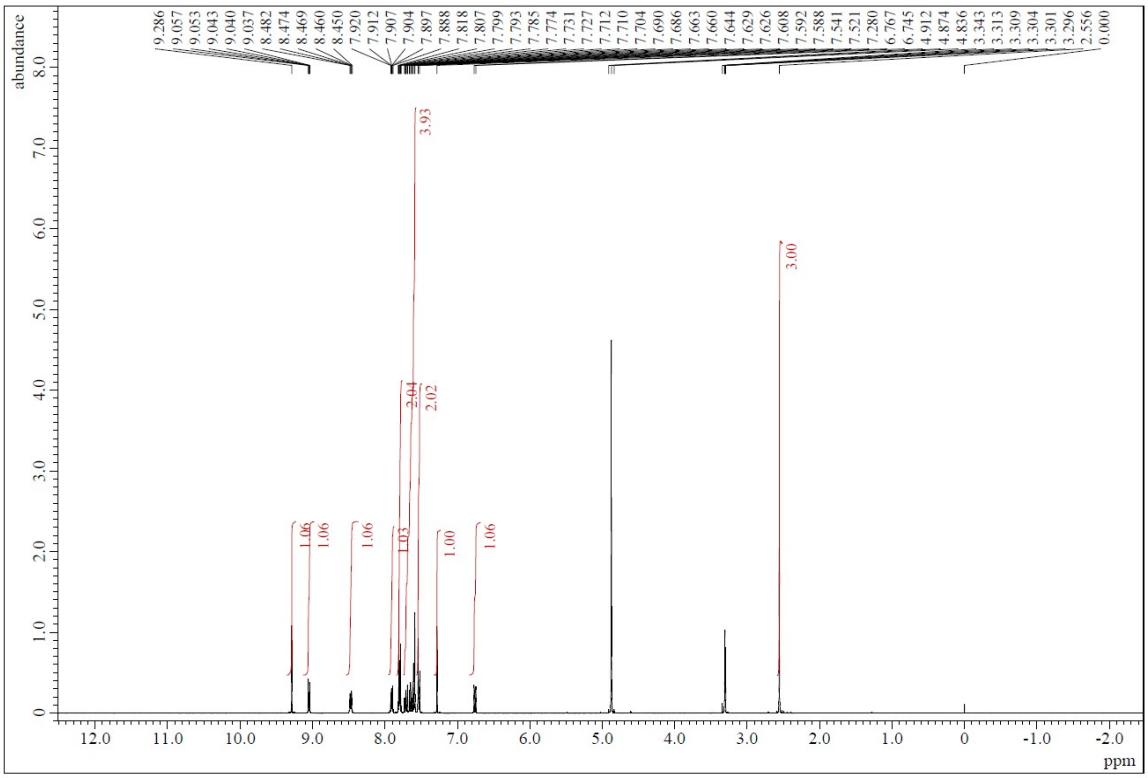
^{13}C NMR of **2a**



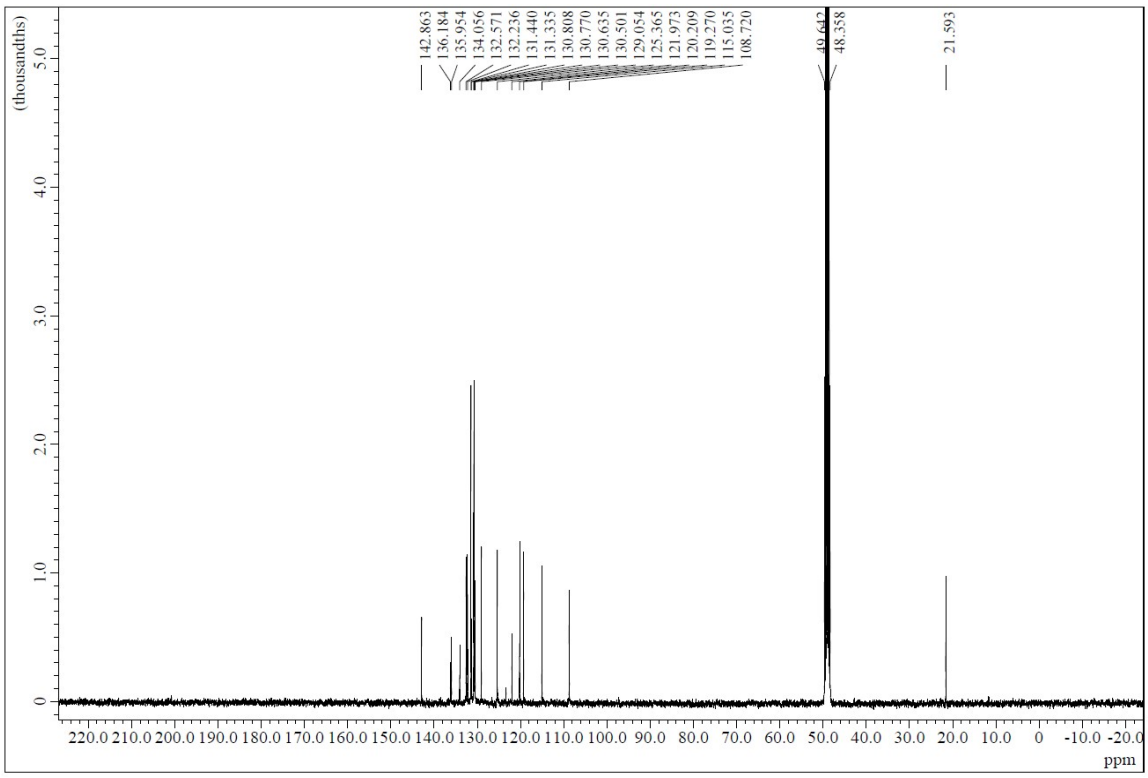
^{19}F NMR of **2a**



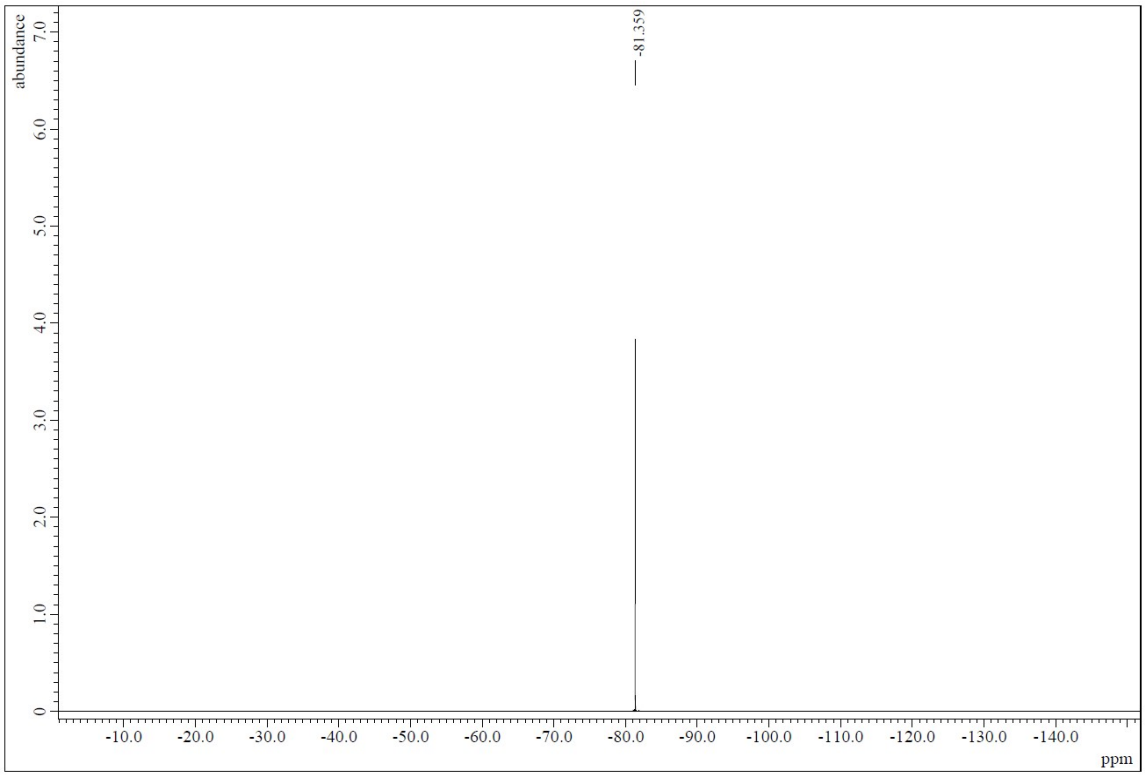
^{1}H NMR of **2b**



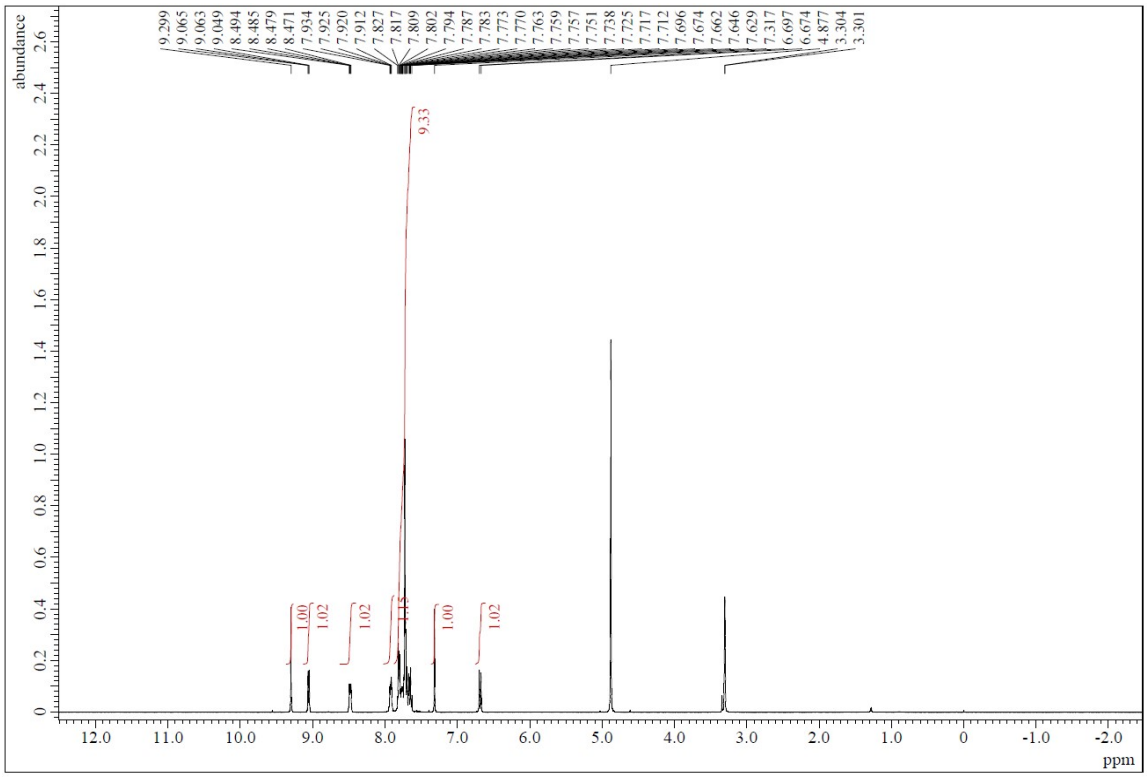
^{13}C NMR of **2b**



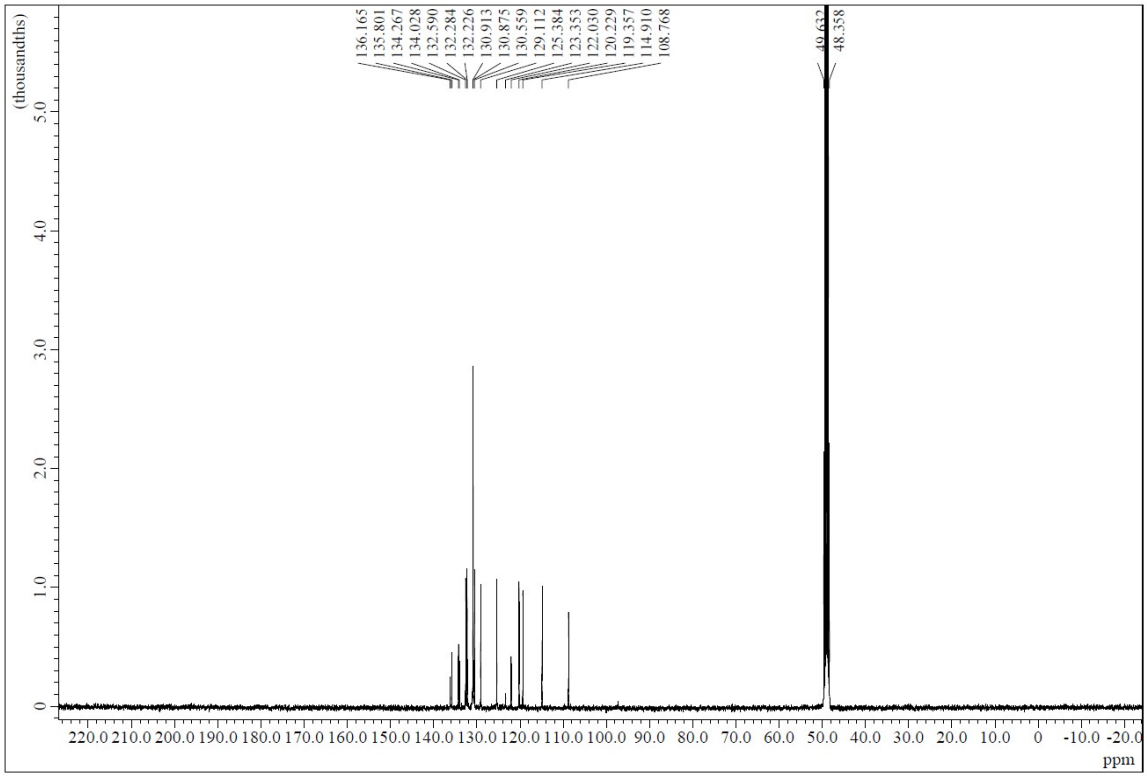
^{19}F NMR of **2b**



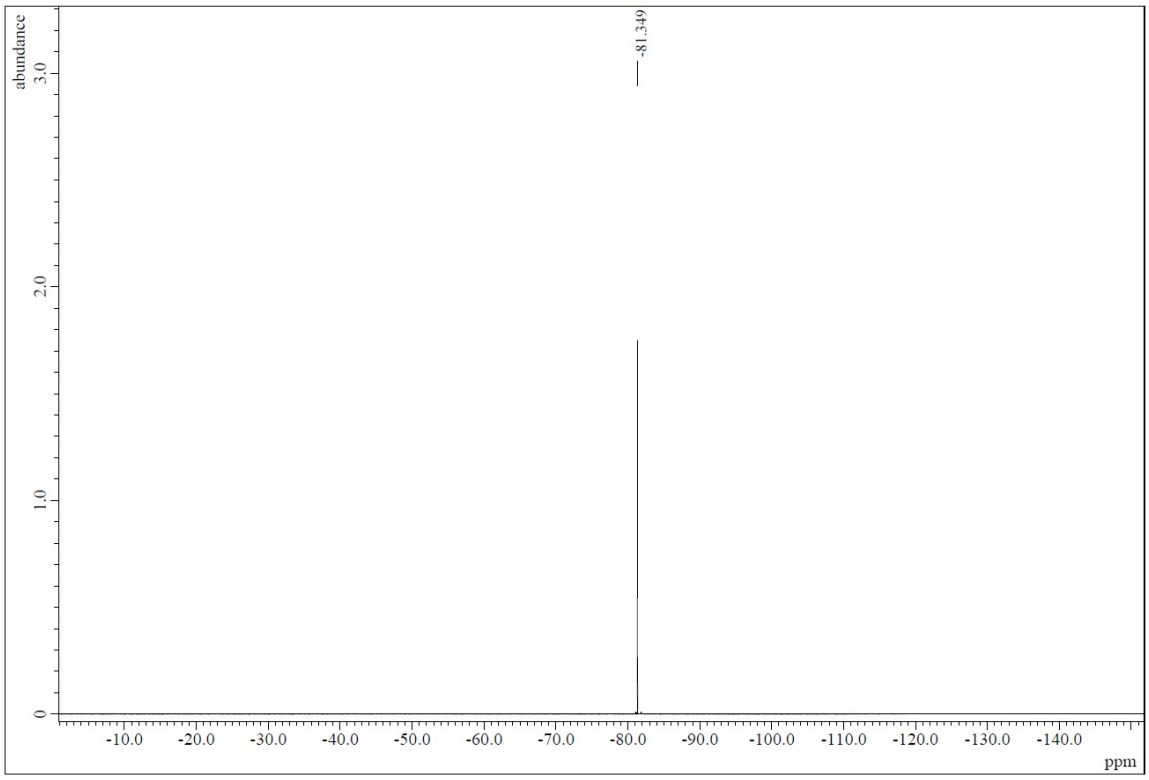
^{1}H NMR of **2c**



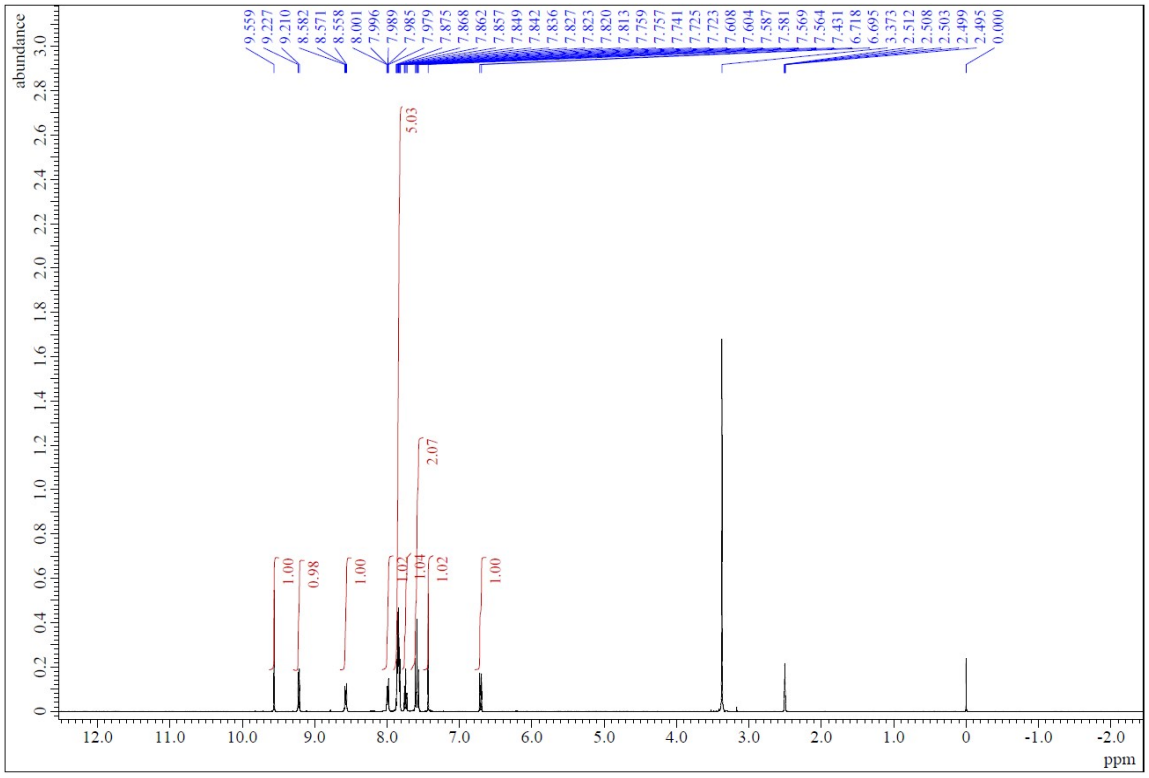
^{13}C NMR of **2c**



^{19}F NMR of **2c**



^{1}H NMR of **2d**



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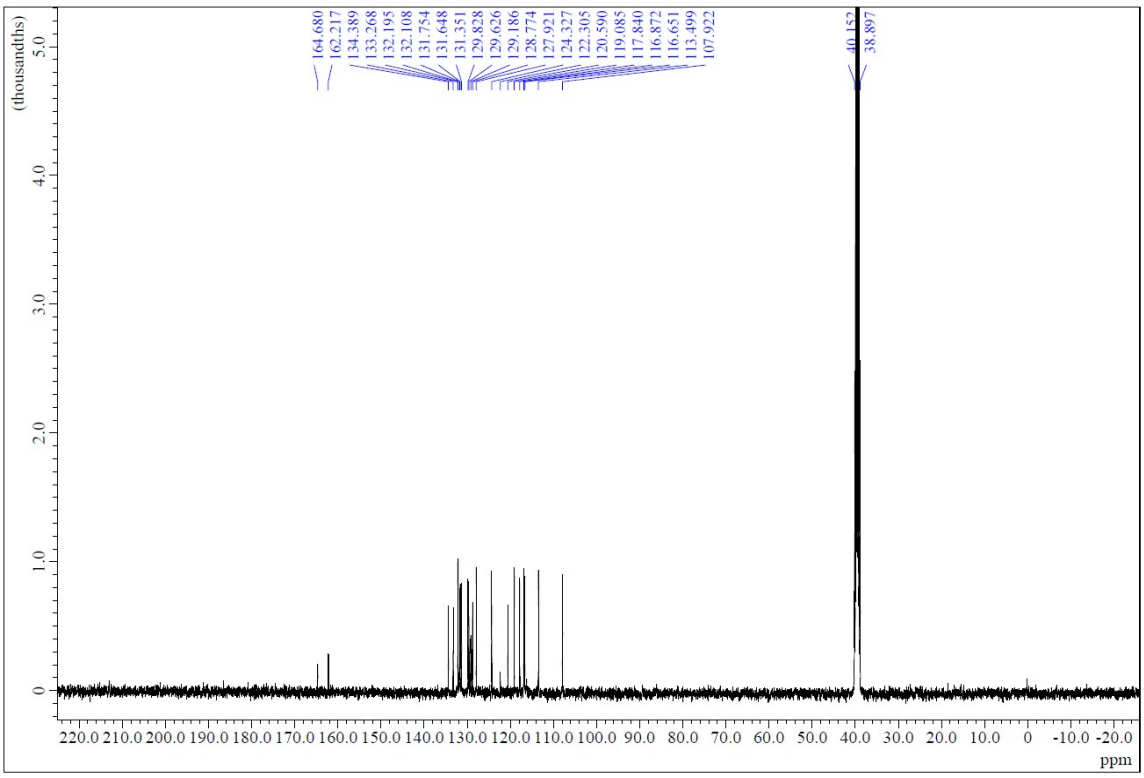

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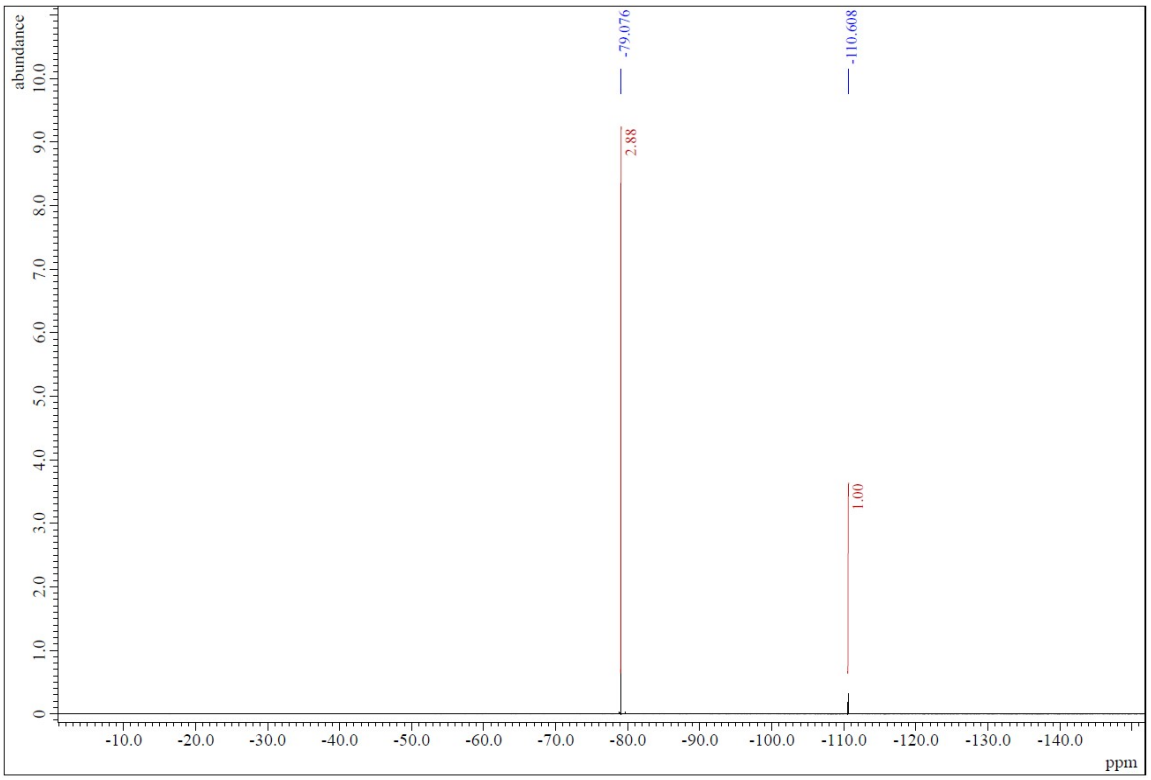
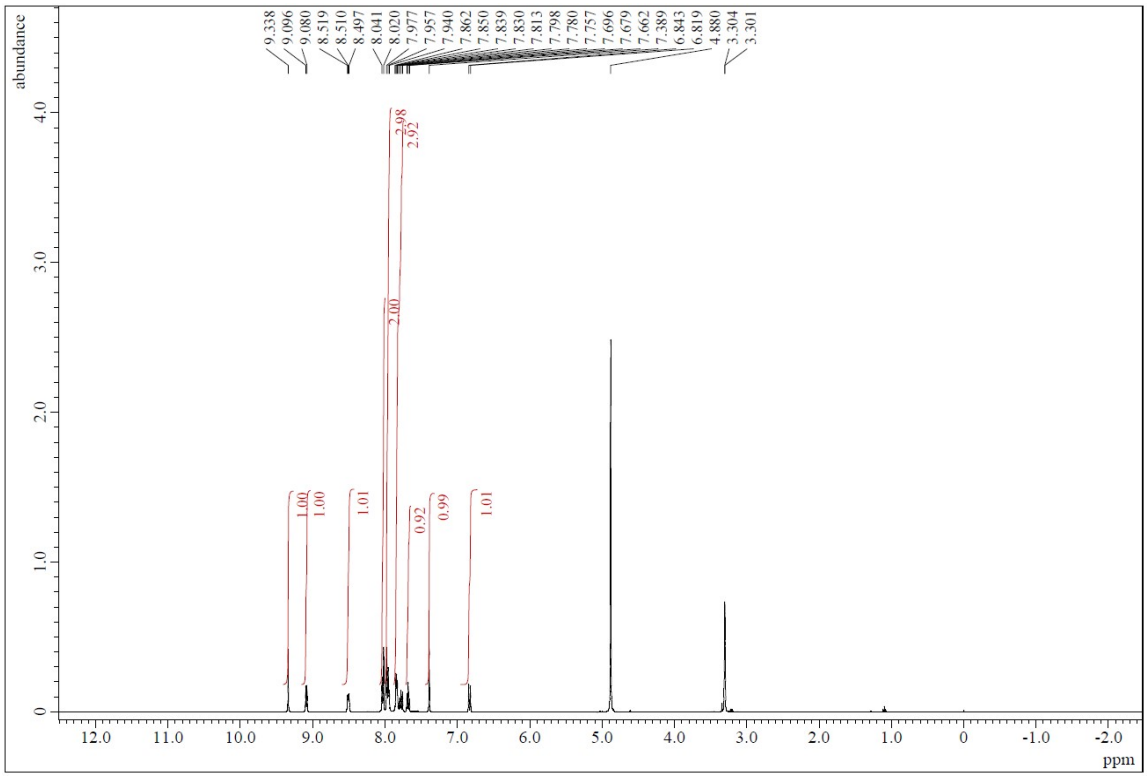


^{1}H NMR of **2e**



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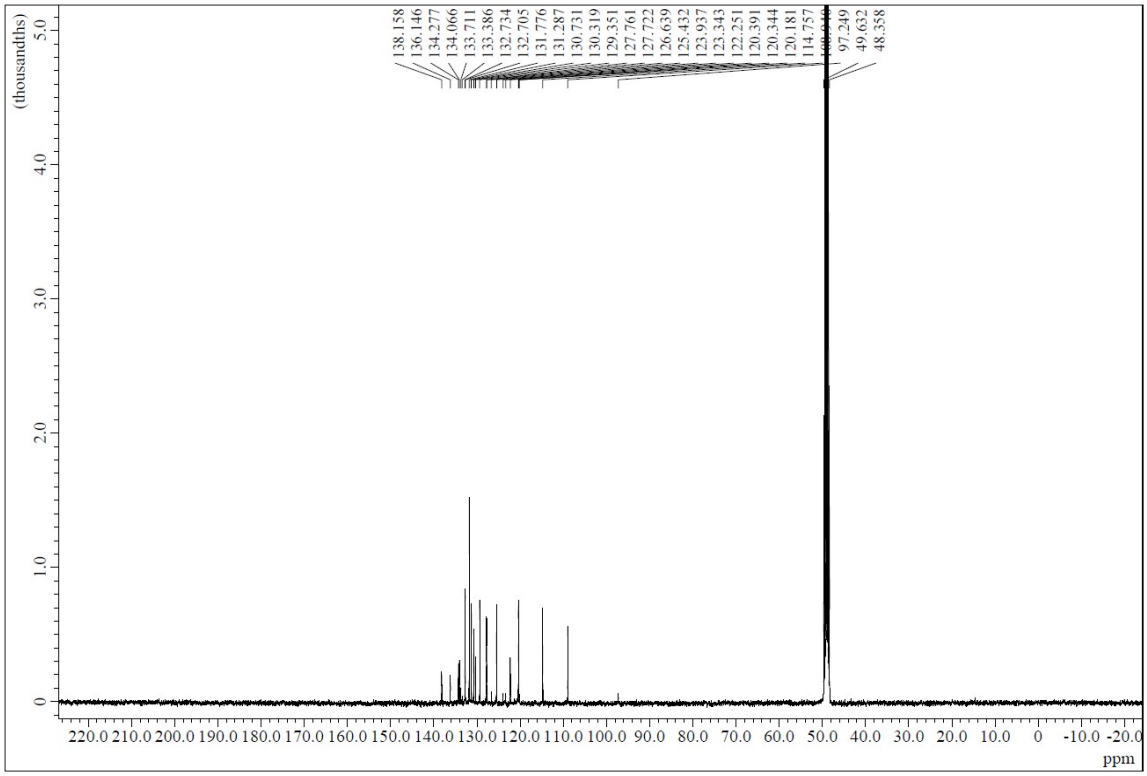
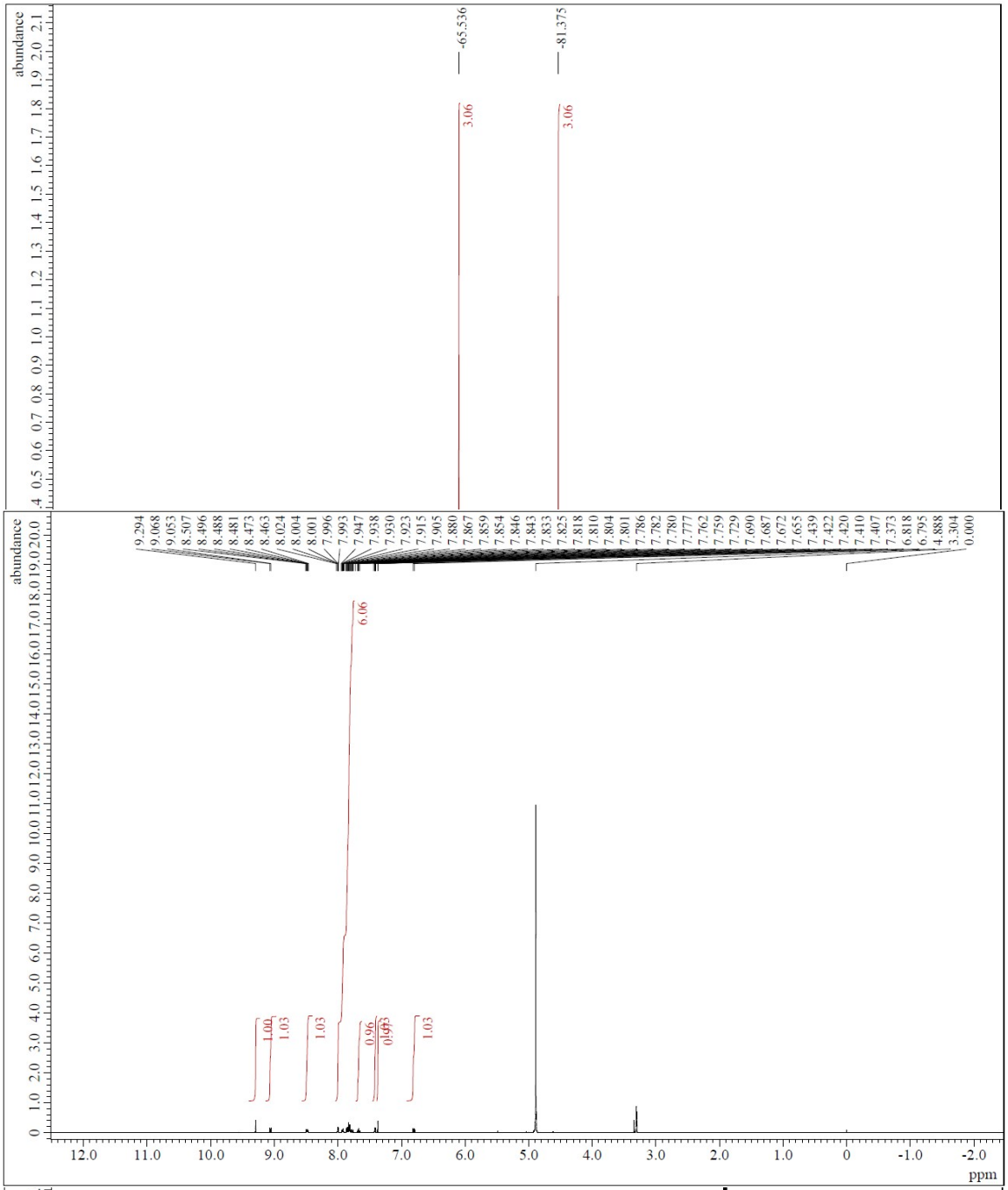
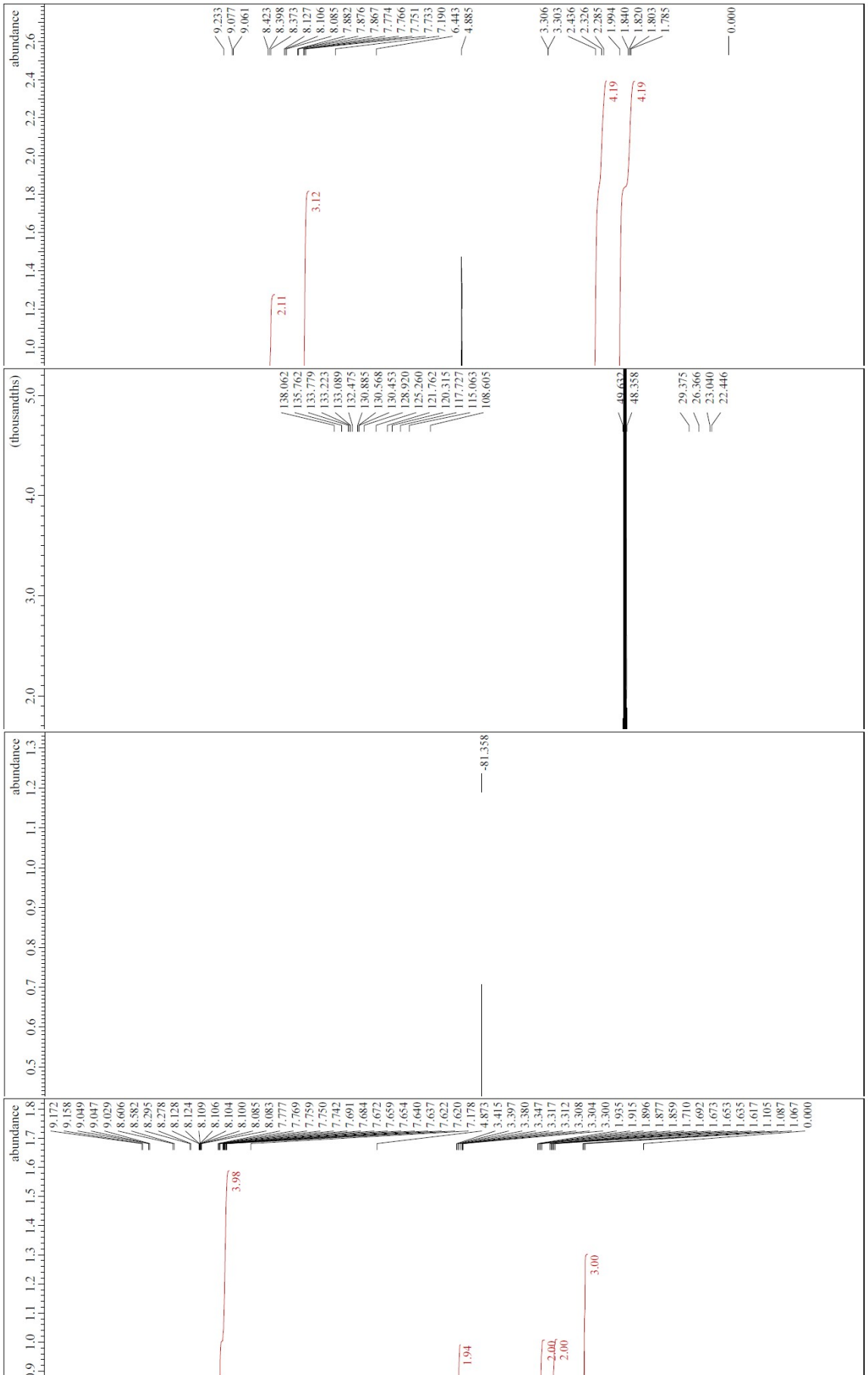


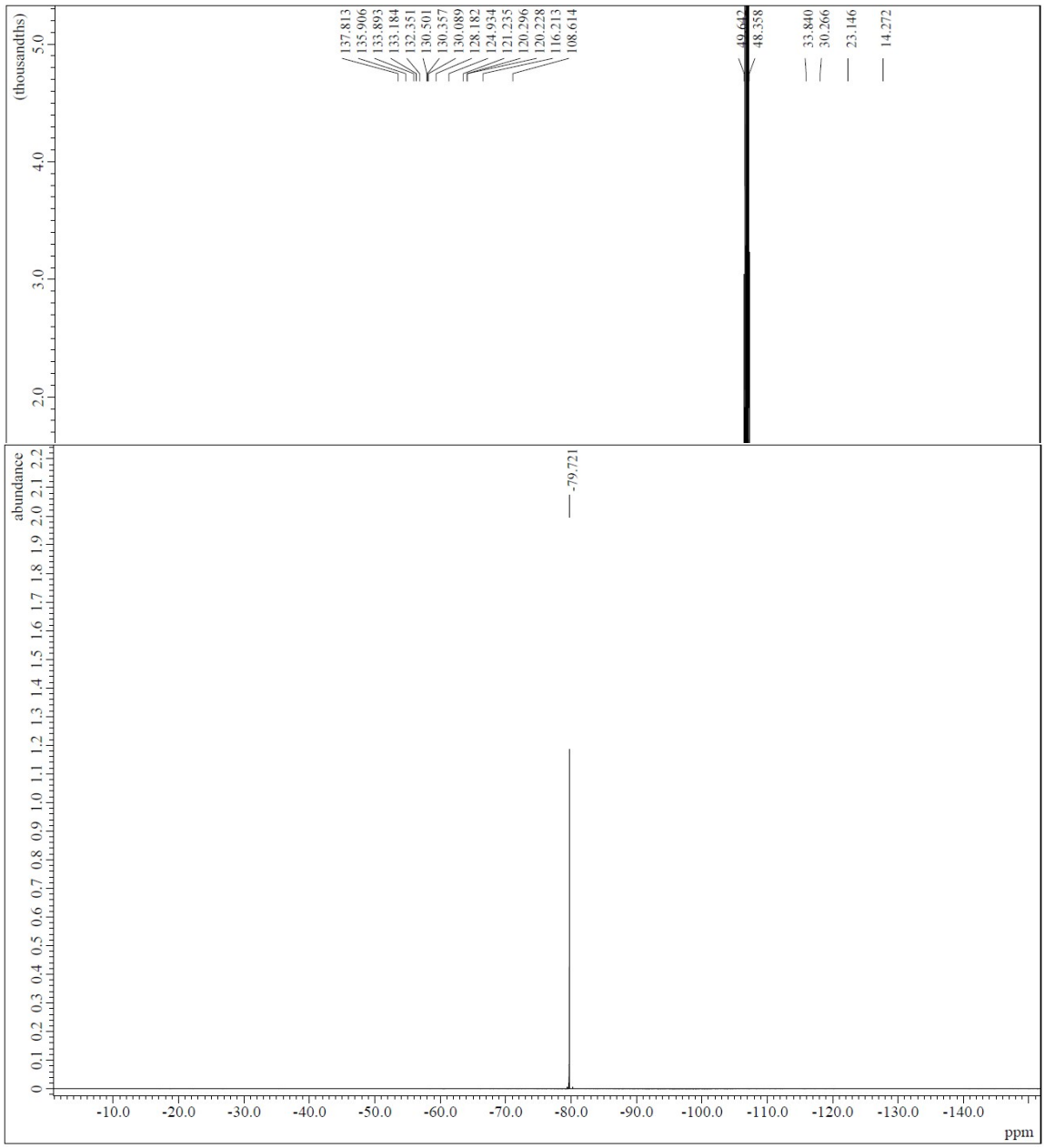
^{19}F NMR of **2e**



^{1}H NMR of **2g**



^{13}C NMR of **2h**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133.184
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124.934
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120.228
116.213
108.614
49.612
48.358
33.840
30.266
23.146
14.272
(thousandths)
-79.721
abundance
ppm