Supporting Information for

Visible-light Responsive Defluorination-Acyl Fluoride Exchange for Photoclick Labeling Based on Phenoxazine Chromophore

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1. Supplementary Methods

1.1 General Information–chemical synthesis

Unless otherwise indicated, all solvents and starting materials were purchased from commercial sources and used directly without further purification. Flash column chromatography was performed by using 100-200 or 200-300 mesh silica gel. Anhydrous solvents, purchased from Acros Organics (dioxane, DMF, and THF), commercially available chemicals were obtained from Adamas, Acros Organics, Aldrich Chemical Co., Alfa, Aesar and TCI. The ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Brüker Avance 400 spectrometer (^{1}H: 400 MHz, ^{13}C: 101 MHz, ^{19}F: 376 MHz). Chemical shifts (δ) for ^{1}H and ^{13}C NMR spectra are given in ppm relative to TMS. The residue solvent signals were used as references for ^{1}H and ^{13}C NMR spectra and the chemical shifts converted to the TMS scale ($CDCl_3$, 7.26 ppm for ^{1}H NMR and 77.16 ppm for ^{13}C NMR; CD_3CN, 1.94 ppm for ^{1}H NMR; DMSO-*d*6, 2.50 ppm for ^{1}H NMR and 39.5 ppm for ^{13}C NMR). Shifts multiplicity was reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs. = broad.

Abbreviations used: Et_3N = triethylamine; EtOAc = ethyl acetate; PE = petroleum ether; DMF = dimethylformamide; THF = tetrahydrofuran; TLC = thin layer chromatography; HPLC = High Performance Liquid Chromatography; ACN = acetonitrile; PBS = phosphate buffer solution.

1.2 General information–spectra acquisition

UV/Vis absorbance spectra were recorded by using 0.2 × 1.0 cm quartz cuvettes (0.2 cm optical path for photo-stimulation and 1.0 cm optical path for UV/Vis absorption measurement on a Thermo NANODROP 2000C Spectrophotometer. Exact ESI mass spectra were recorded on a SHIMADZU LCMS-IT-TOF, flow rate = 1.0 mL/min. LC-ESI-MS were obtained on a Thermo LTQ-XL mass spectrometer.

1.3 General information–light sources

The photo-irradiation power density of various light sources in photo-chemical transformation experiments were measured by an optical power meter produced by Thorlabs:

A 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after an optical filter) was utilized for exciting target compounds HPLC-MS analysis and photo-quantum yields.

A 365 nm LED (166.8 mW cm^{-2}) was utilized for exciting target compounds HPLC-MS analysis and photo-quantum yields.

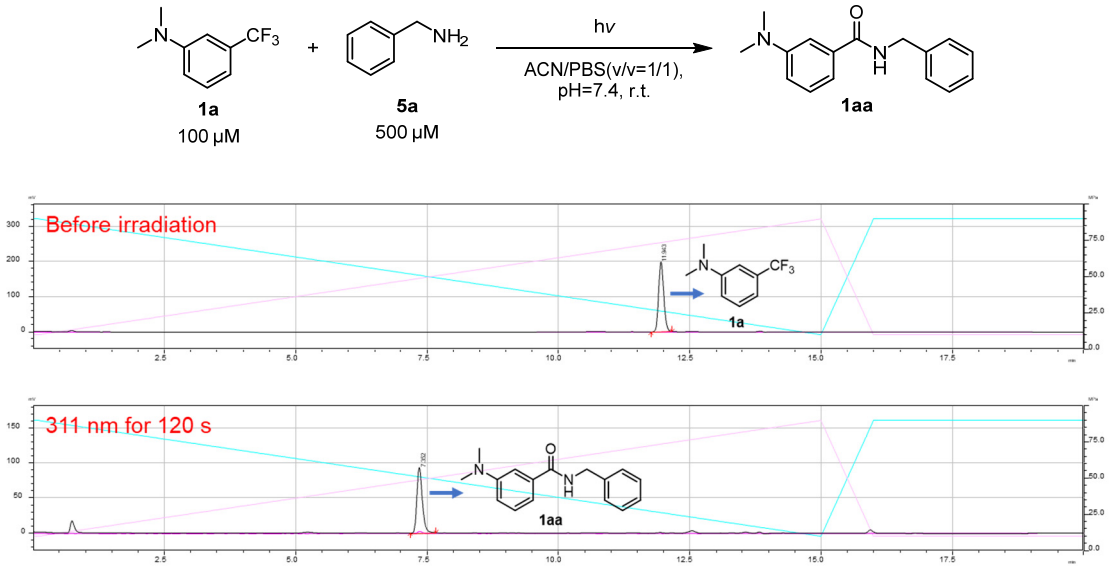
A 405nm LED (21.0 mW cm^{-2}) was utilized for protein photo-affinity labeling in *E. coli* lysate.

A 405 nm laser (73.1 mW cm^{-2}) was utilized for exciting target compounds HPLC-MS analysis and photo-quantum yields.

2. Supplementary Figures and Tables

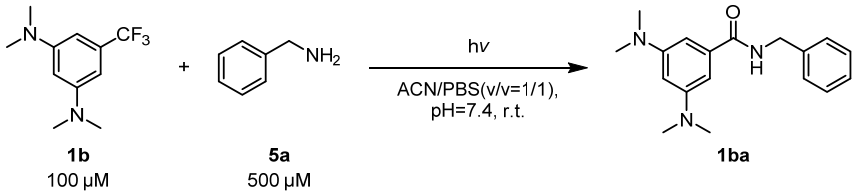
2.1 HPLC analysis for the photo-Defluorination-Acyl Fluoride Exchange Reaction for the four types of TFMA reagents with benzylamine

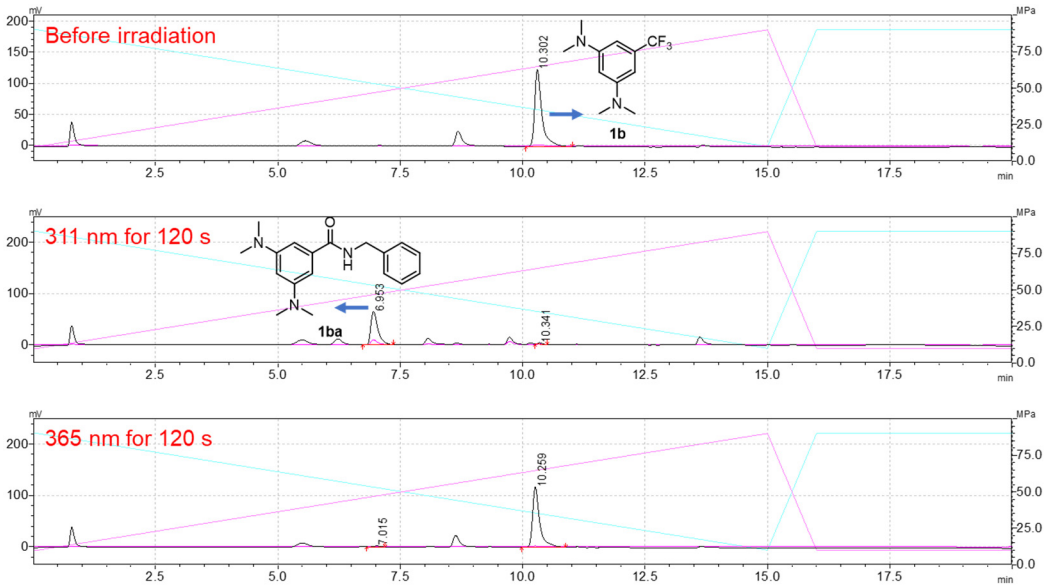
Note: The pink climbing line in the traces represents the linear increase of acetonitrile (1 ‰ formic acid) in eluent solvent over time, while the blue linear falling line indicates the corresponding linear decrease of water (1 ‰ formic acid) in the eluent. To maintain clarity in the diagrams, all liquid phase experiments adhere to this solvent gradient. Consequently, in some of the diagrams, grid lines and gradient indicators have been omitted for a more streamlined presentation. **Area**: The area of each peak was accurately determined through the integration of its peak signal across its entire appearance duration within the HPLC traces.



Item	Peak	Time	Area
Before irradiation	**1a**	**11.943**	**1342105**
311 nm for 120 s	**1aa**	**7.352**	**665559**

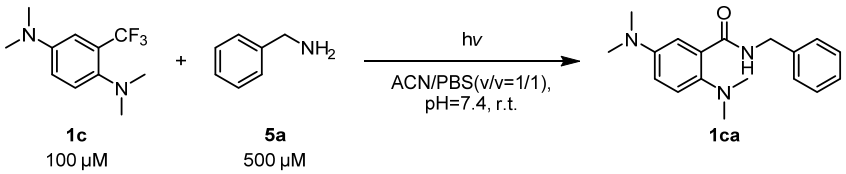
Figure S1. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **1a** and benzylamine **5a**. Reaction conditions: **1a** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.





Item	Peak	Time	Area
Before irradiation	**1b**	**10.302**	**1271239**
311 nm for 120 s	**1ba**	**6.953**	**634699**
365 nm for 120 s	**1b**	**10.302**	**1189056**
	1ba	**7.015**	**16431**

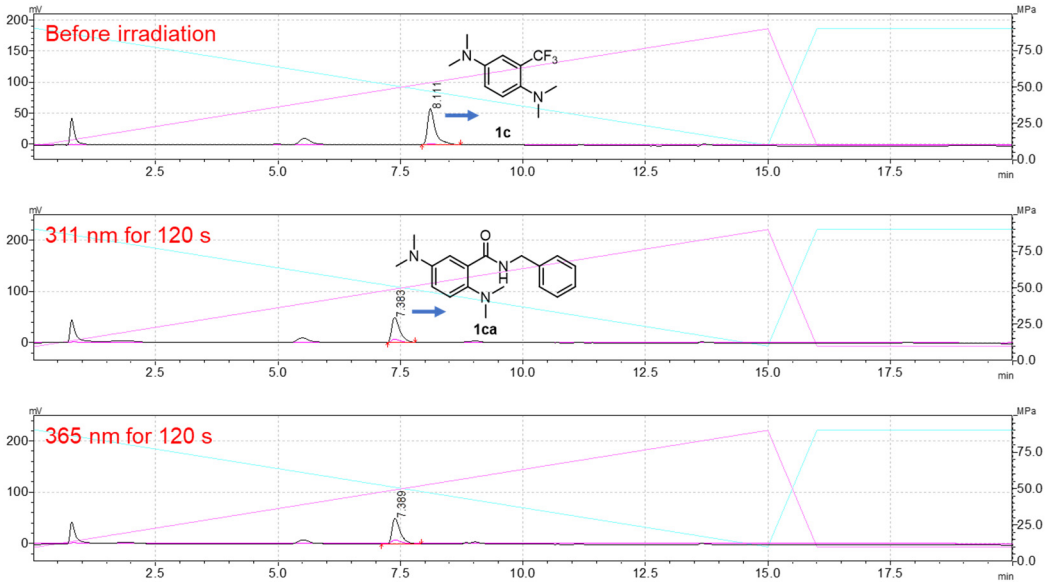
Figure S2. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **1b** and benzylamine **5a**. Reaction conditions: **1b** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



1c
100 μM

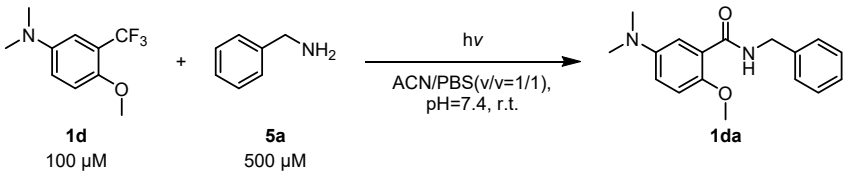
5a
500 μM

1ca



Item	Peak	Time	Area
Before irradiation	**1c**	**8.111**	**646719**
311 nm for 120 s	**1ca**	**7.383**	**559334**
365 nm for 120 s	**1ca**	**7.389**	**581308**

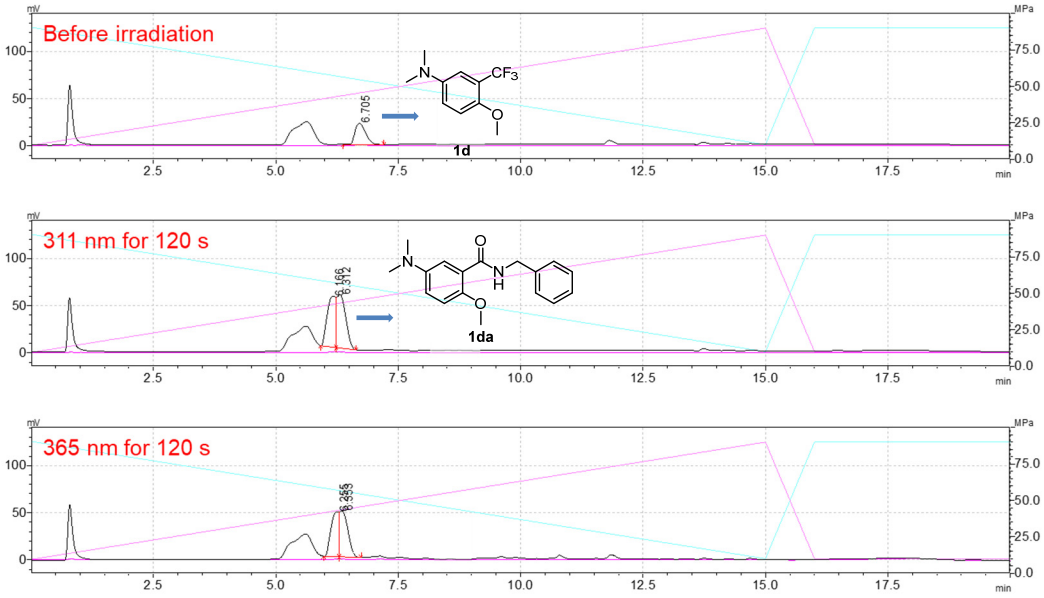
Figure S3. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **1c** and benzylamine **5a**. Reaction conditions: **1c** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



1d
100 μM

5a
500 μM

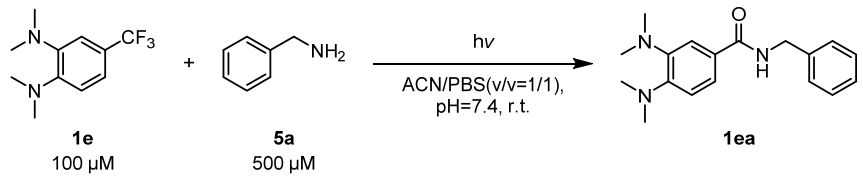
1da

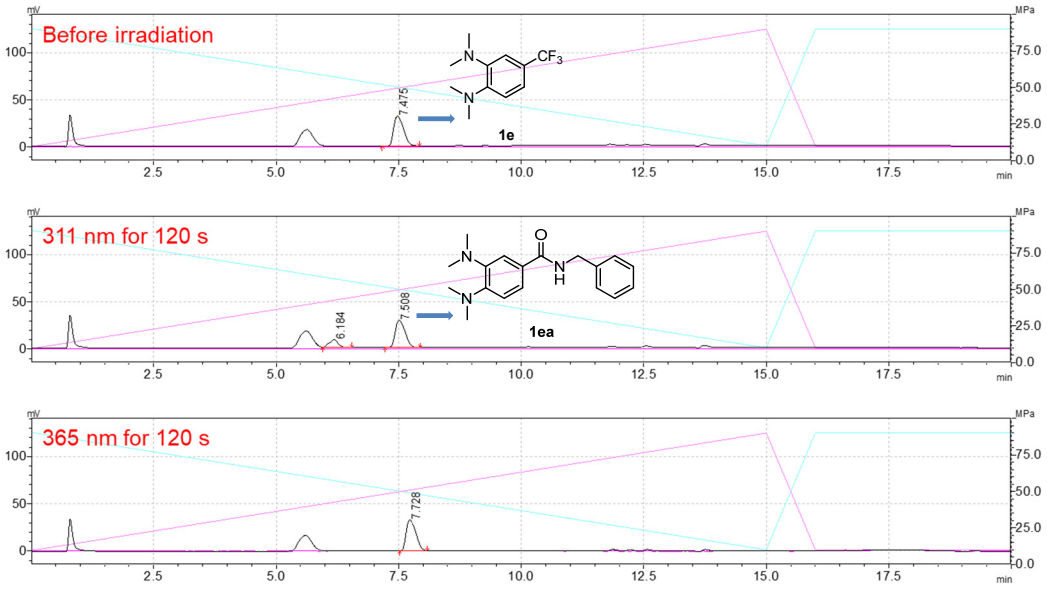


Item	Peak	Time	Area
Before irradiation	**1d**	**6.705**	**333754**
311 nm for 120 s	**1da**	**6.312**	**658949**
365 nm for 120 s	**1da**	**6.353**	**527780**

Figure S4. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **1d** and benzylamine **5a**. Reaction conditions: **1d** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

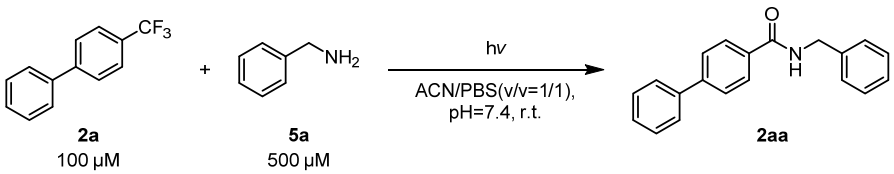
Note: The peak observed at 5.5 minutes in each trace corresponds to benzylamine. The peak detected at 6.166 minutes in the second trace and the peak at detected at 6.255 minutes in the third trace represent mass peaks of carboxylic acid formed from hydrolysis of acyl fluoride.

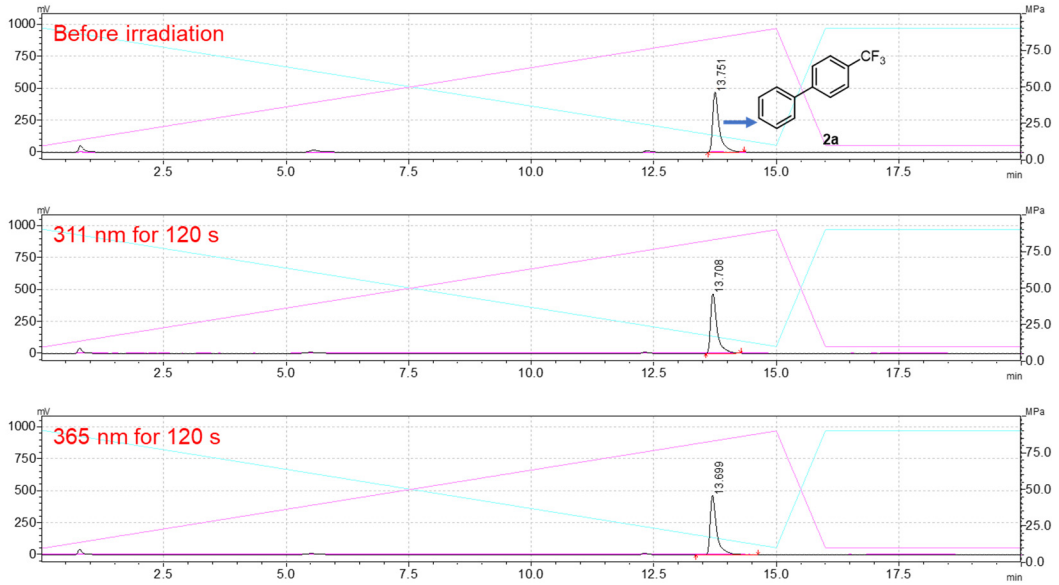




Item	Peak	Time	Area
Before irradiation	**1e**	**7.475**	**439388**
311 nm for 120 s	**1e**	**7.508**	**404298**
	1ea	**6.184**	**100484**
365 nm for 120 s	**1e**	**7.728**	**446891**

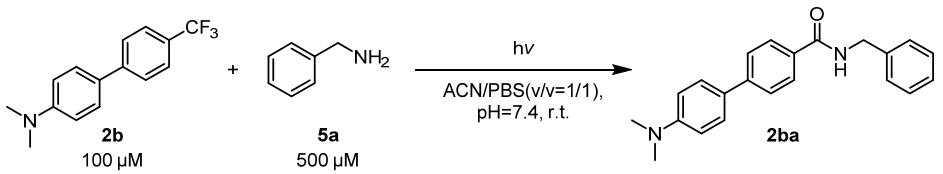
Figure S5. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **1e** and benzylamine **5a**. Reaction conditions: **1e** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

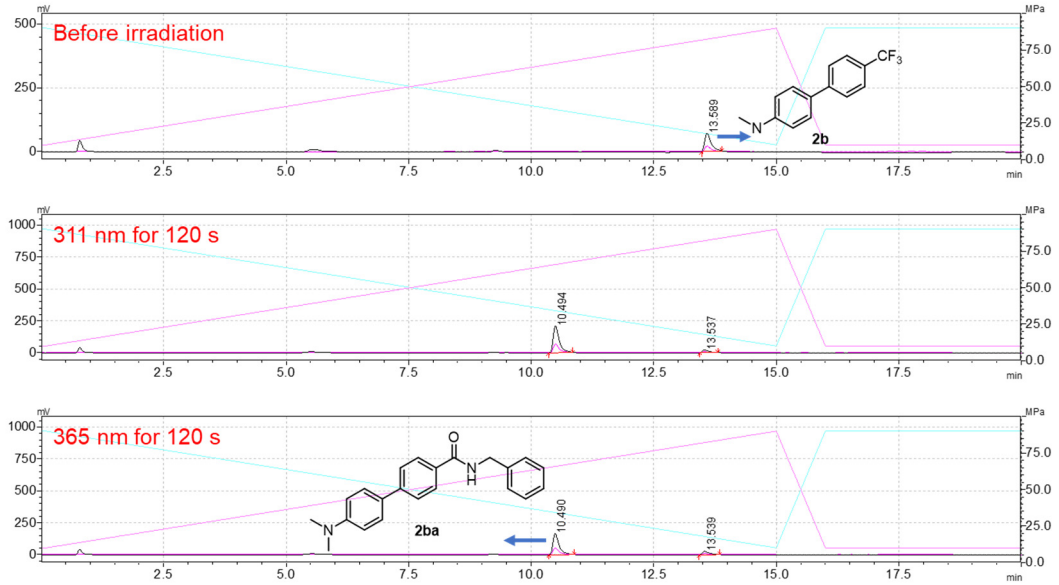




Item	Peak	Time	Area
Before irradiation	**2a**	**13.751**	**4356960**
311 nm for 120 s	**2a**	**13.708**	**4227677**
365 nm for 120 s	**2a**	**13.699**	**4253347**

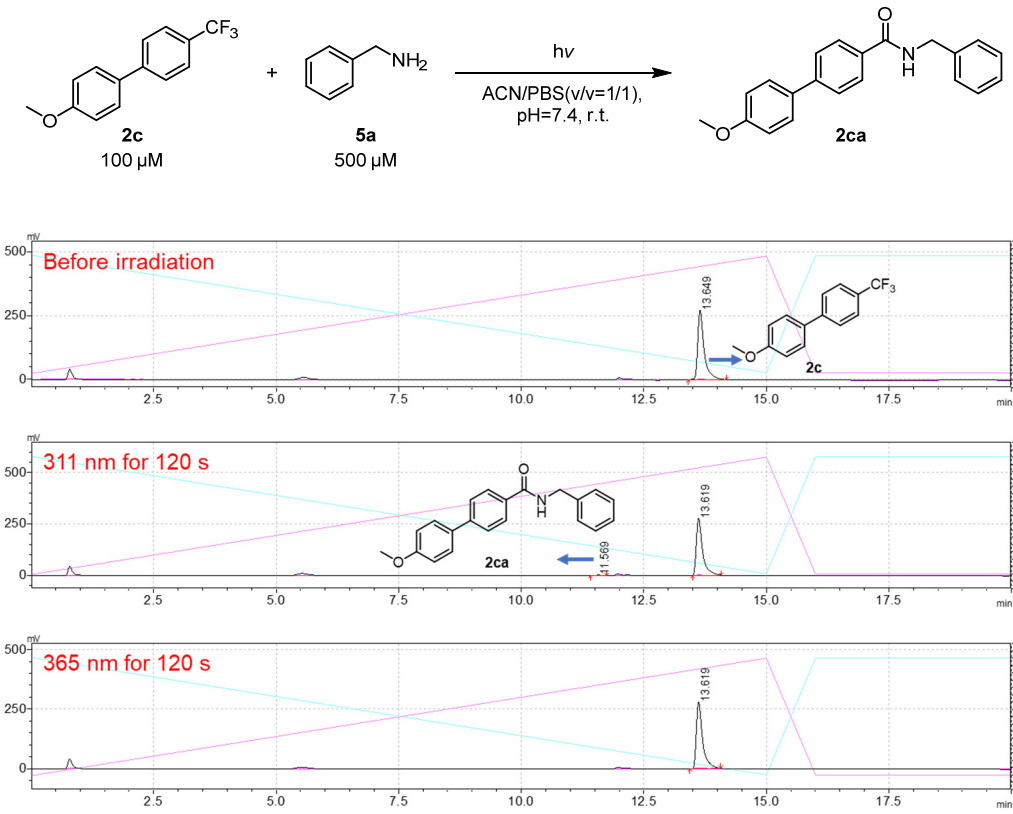
Figure S6. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **2a** and benzylamine **5a**. Reaction conditions: **2a** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.





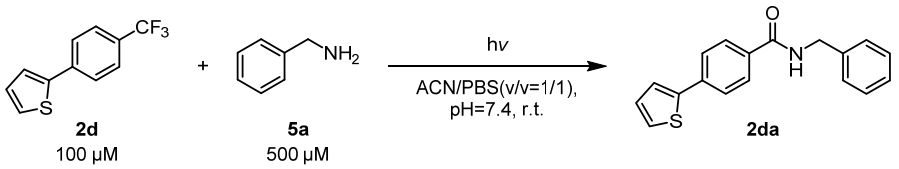
Item	Peak	Time	Area
Before irradiation	**2b**	**13.589**	**639258**
311 nm for 120 s	**2b**	**13.537**	**195244**
	2ba	**10.494**	**1800835**
365 nm for 120 s	**2b**	**13.539**	**250129**
	2ba	**10.490**	**1437596**

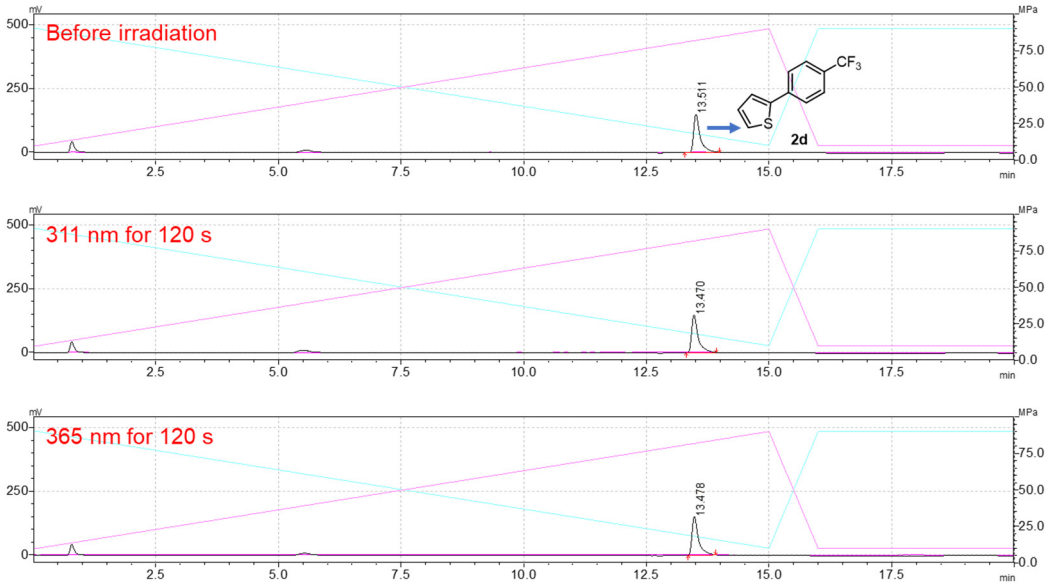
Figure S7. HPLC analysis of the Photo-induced Defluorination-Acyl Fluoride Exchange Reaction between **2b** and Benzylamine **5a**. Reaction conditions: **2b** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v=1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



Item	Peak	Time	Area
Before irradiation	**2c**	**13.649**	**2473213**
311 nm for 120 s	**2c**	**13.619**	**2468124**
	2ca	**11.569**	**20981**
365 nm for 120 s	**2c**	**13.619**	**2501024**

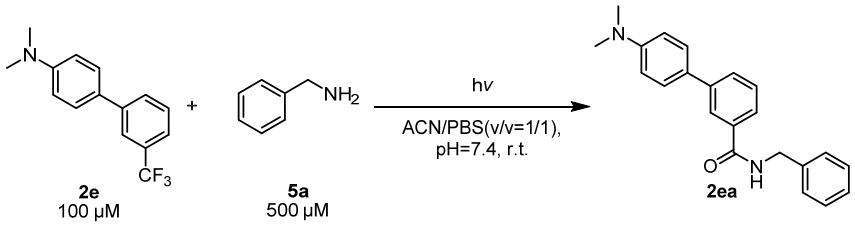
Figure S8. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **2c** and benzylamine **5a**. Reaction conditions: **2c** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

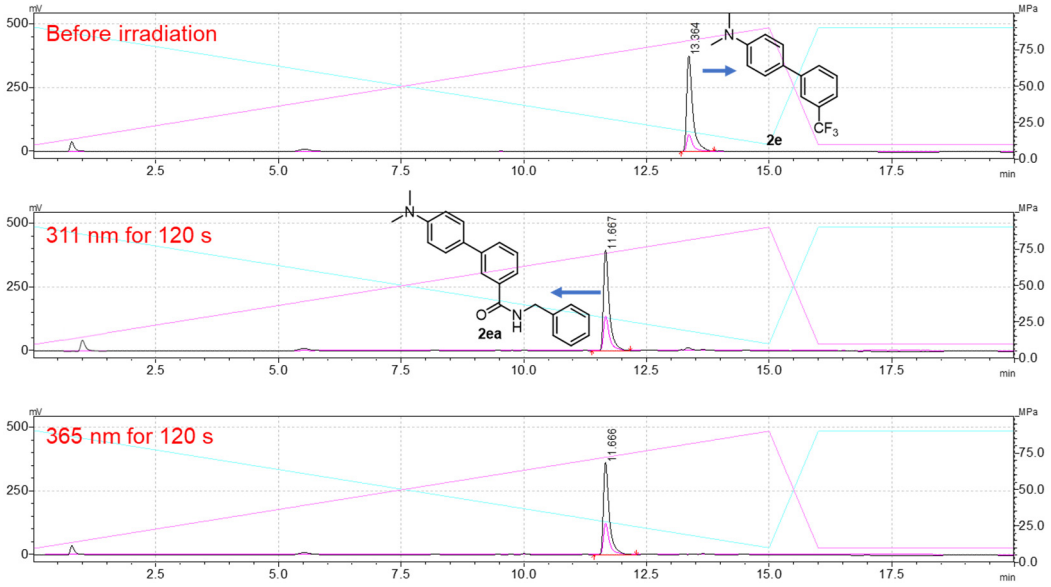




Item	Peak	Time	Area
Before irradiation	**2d**	**13.511**	**1372931**
311 nm for 120 s	**2d**	**13.470**	**1353649**
365 nm for 120 s	**2d**	**13.478**	**1373281**

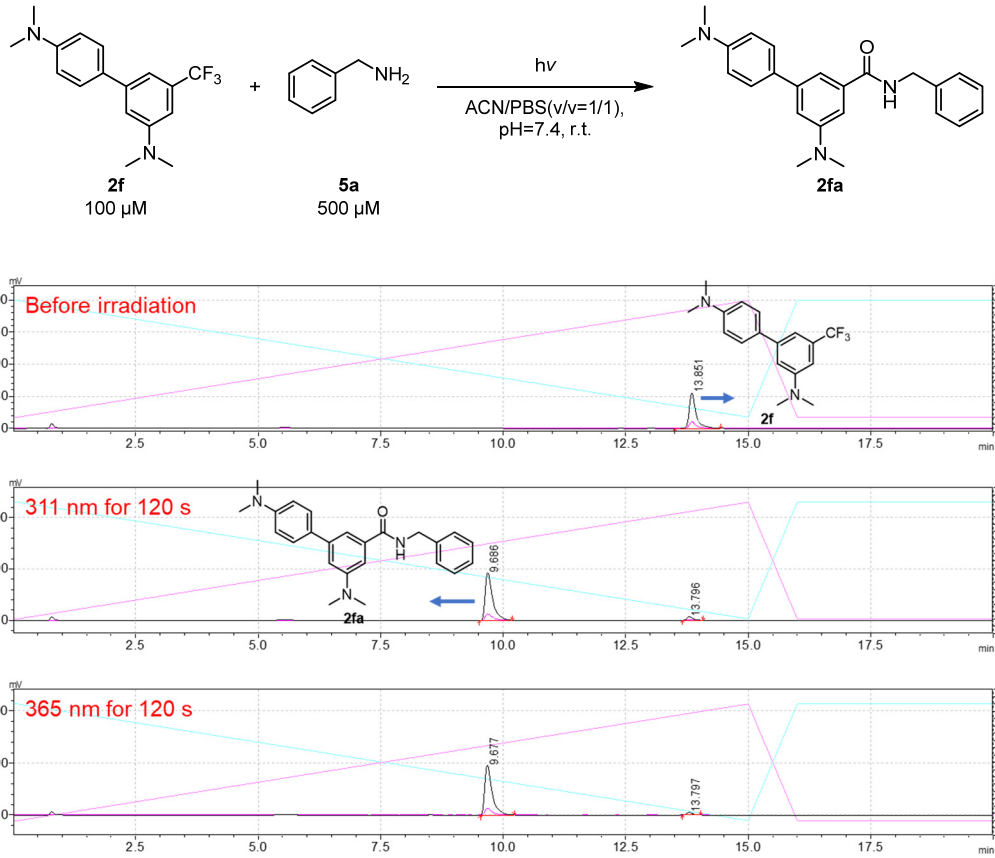
Figure S9. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **2d** and benzylamine **5a**. Reaction conditions: **2d** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.





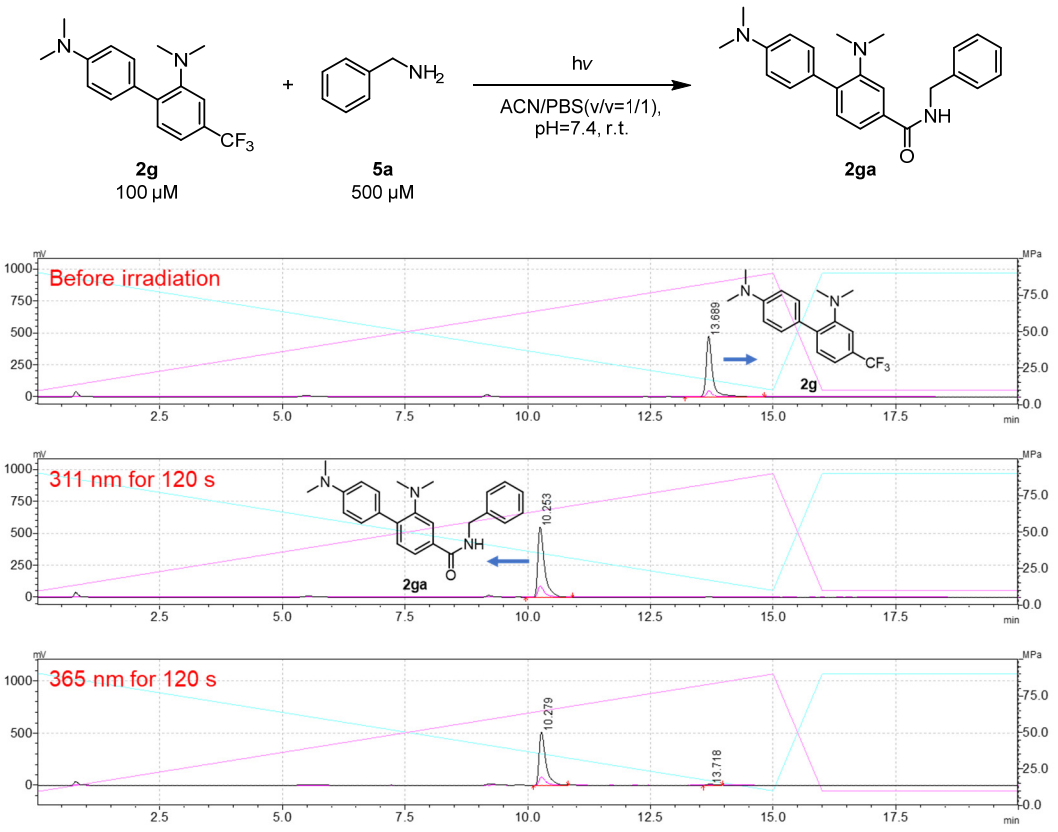
Item	Peak	Time	Area
Before irradiation	**2e**	**13.364**	**3195670**
311 nm for 120 s	**2ea**	**11.667**	**3227000**
365 nm for 120 s	**2ea**	**11.666**	**2999405**

Figure S10. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **2e** and benzylamine **5a**. Reaction conditions: **2e** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



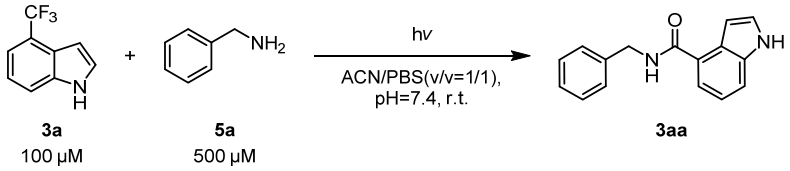
Item	Peak	Time	Area
Before irradiation	**2f**	**13.851**	**2688670**
311 nm for 120 s	**2f**	**13.796**	**305372**
	2fa	**9.686**	**4889923**
365 nm for 120 s	**2f**	**13.797**	**212413**
	2fa	**9.677**	**5017197**

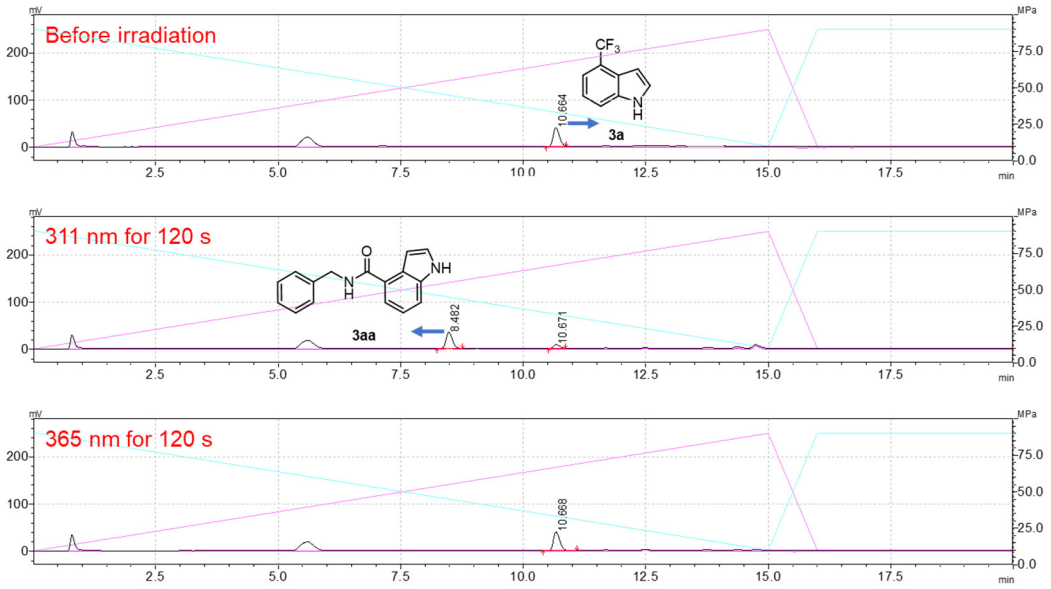
Figure S11. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **2f** and benzylamine **5a**. Reaction conditions: **2f** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



Item	Peak	Time	Area
Before irradiation	**2g**	**13.689**	**4221387**
311 nm for 120 s	**2ga**	**10.253**	**5185959**
365 nm for 120 s	**2g**	**13.718**	**129136**
	2ga	**10.279**	**4835609**

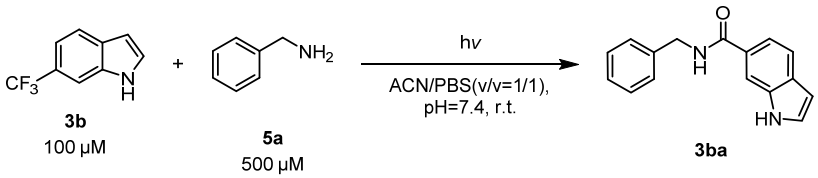
Figure S12. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **2g** and benzylamine **5a**. Reaction conditions: **2g** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

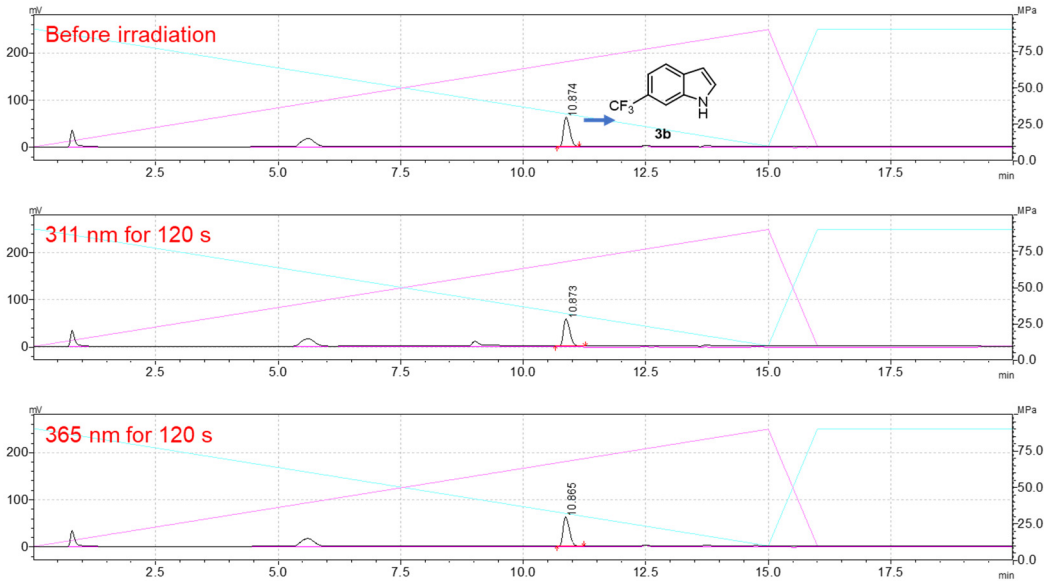




Item	Peak	Time	Area
Before irradiation	**3a**	**10.664**	**335455**
311 nm for 120 s	**3a**	**10.671**	**66863**
	3aa	**8.482**	**304512**
365 nm for 120 s	**3a**	**10.668**	**341918**

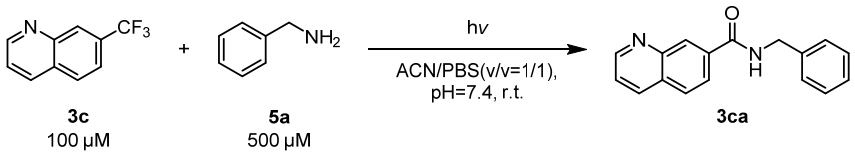
Figure S13. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **3a** and benzylamine **5a**. Reaction conditions: **3a** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

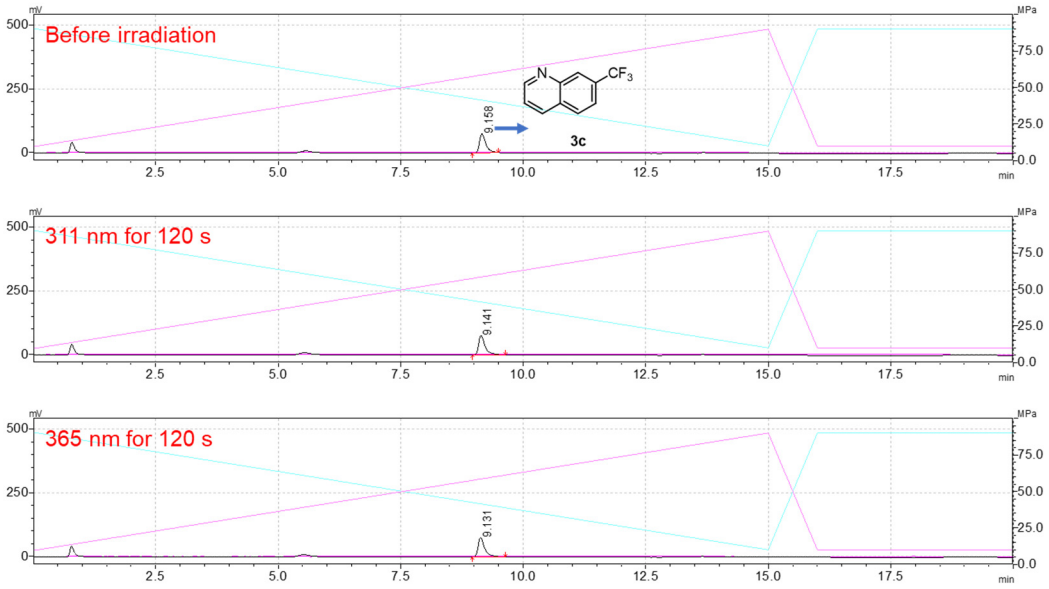




Item	Peak	Time	Area
Before irradiation	**3b**	**10.874**	**546442**
311 nm for 120 s	**3b**	**10.873**	**507282**
365 nm for 120 s	**3b**	**10.865**	**551049**

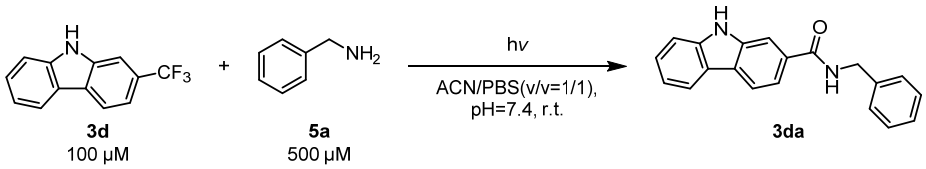
Figure S14. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **3b** and benzylamine **5a**. Reaction conditions: **3b** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

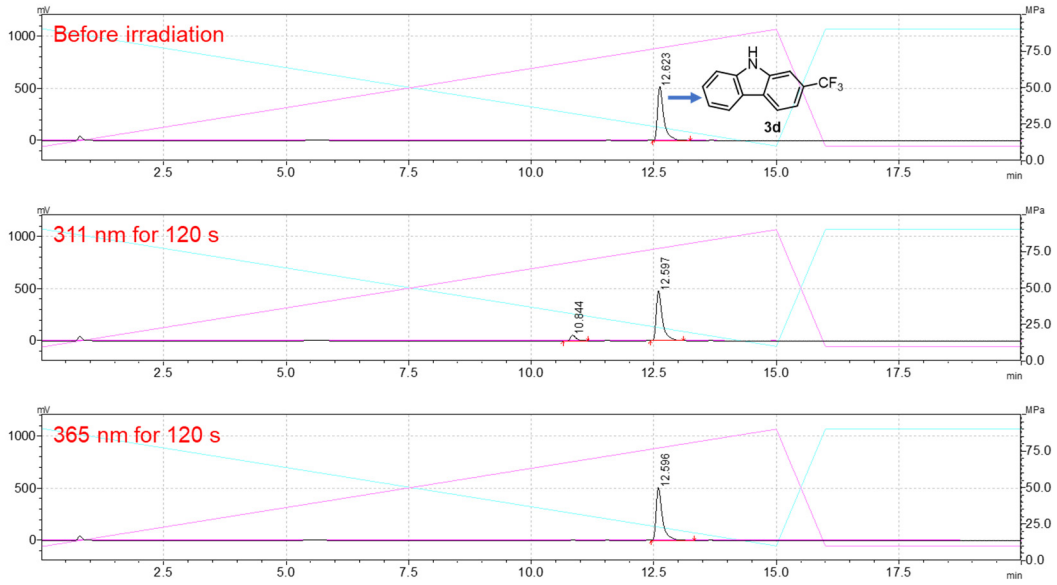




Item	Peak	Time	Area
Before irradiation	**3c**	**9.158**	**667488**
311 nm for 120 s	**3c**	**9.141**	**706888**
365 nm for 120 s	**3c**	**9.131**	**706817**

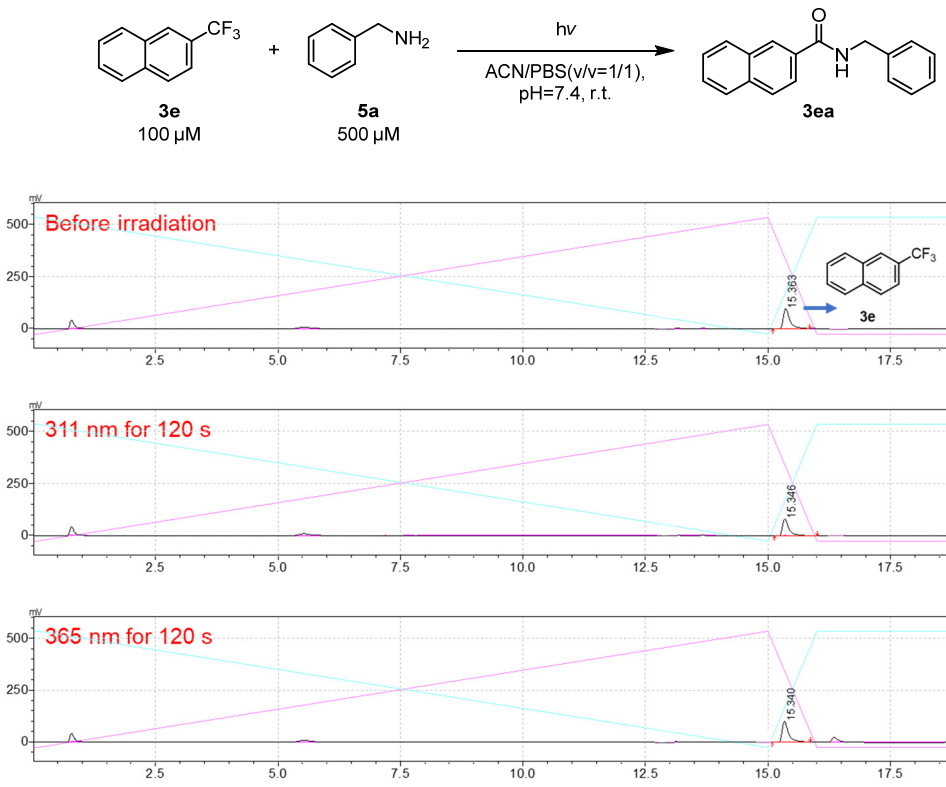
Figure S15. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **3c** and benzylamine **5a**. Reaction conditions: **3c** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.





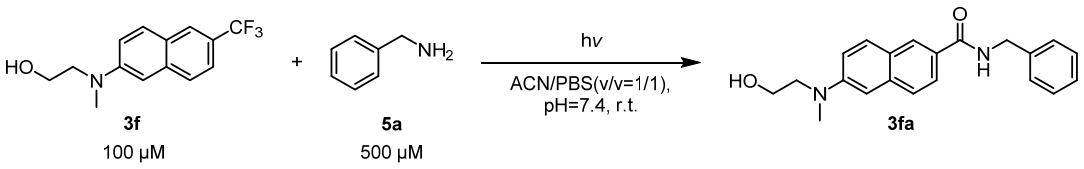
Item	Peak	Time	Area
Before irradiation	**3d**	**12.623**	**4543435**
311 nm for 120 s	**3d**	**12.597**	**4150277**
365 nm for 120 s	**3d**	**12.596**	**4454097**

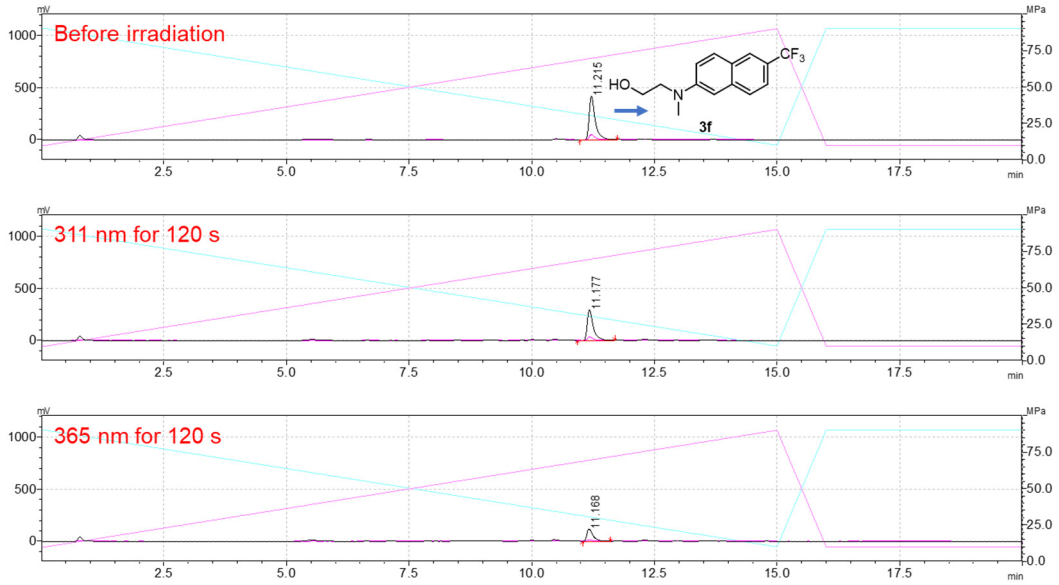
Figure S16. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **3d** and benzylamine **5a**. Reaction conditions: **3d** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



Item	Peak	Time	Area
Before irradiation	**3e**	**15.363**	**888161**
311 nm for 120 s	**3e**	**15.346**	**774299**
365 nm for 120 s	**3e**	**15.340**	**906006**

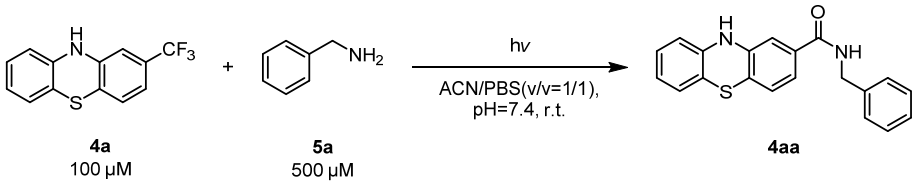
Figure S17. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **3e** and benzylamine **5a**. Reaction conditions: **3e** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

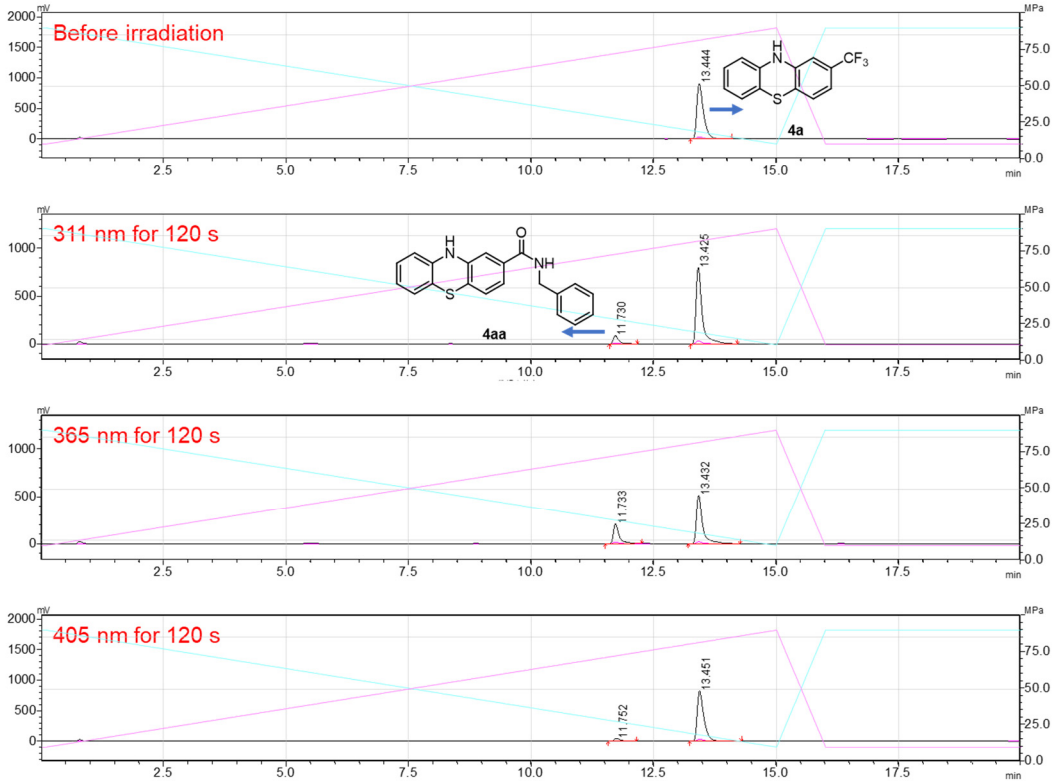




Item	Peak	Time	Area
Before irradiation	**3f**	**11.215**	**3605884**
311 nm for 120 s	**3f**	**11.177**	**2502755**
365 nm for 120 s	**3f**	**11.168**	**1041430**

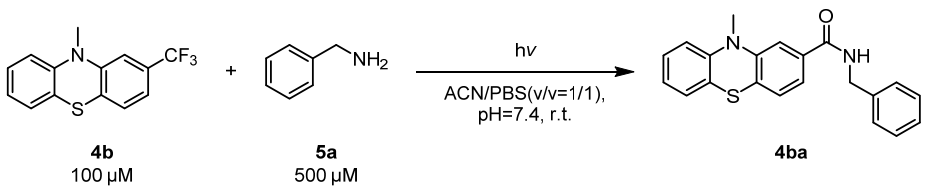
Figure S18. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **3f** and benzylamine **5a**. Reaction conditions: **3f** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

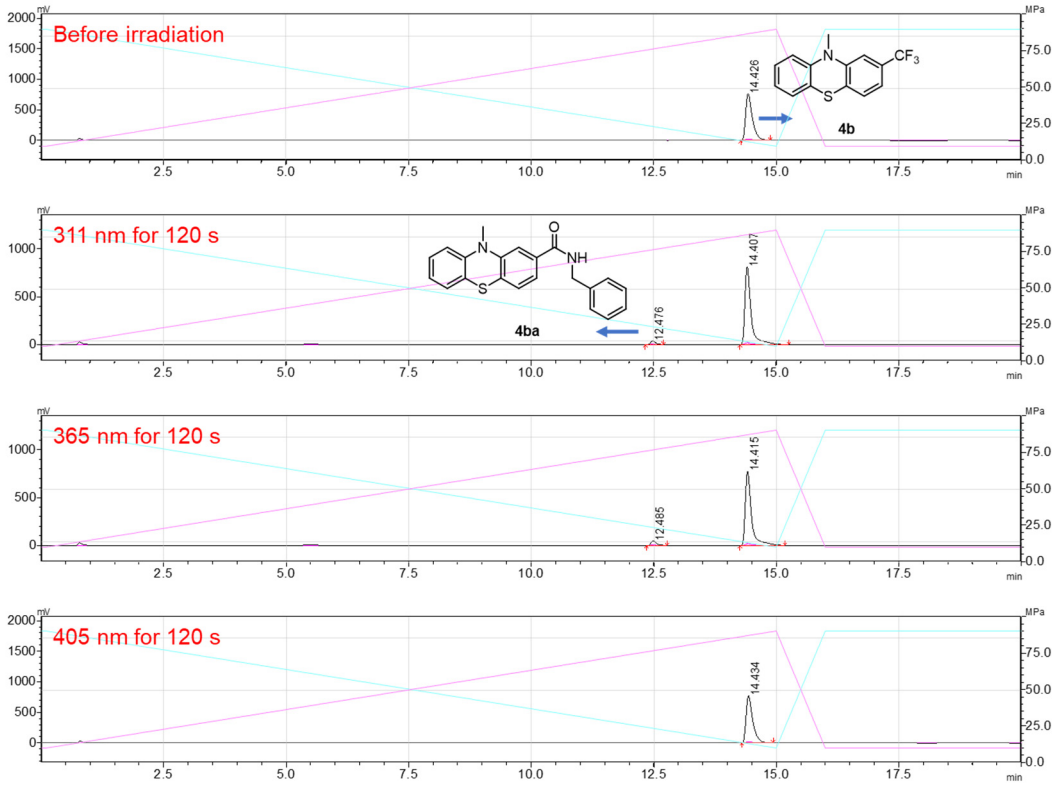




Item	Peak	Time	Area
Before irradiation	**4a**	**13.444**	**8577615**
311 nm for 120 s	**4a**	**13.425**	**6918741**
	4aa	**11.730**	**658419**
365 nm for 120 s	**4a**	**13.432**	**4465774**
	4aa	**11.733**	**1710655**
405 nm for 120 s	**4a**	**13.451**	**7748736**
	4aa	**11.752**	**401279**

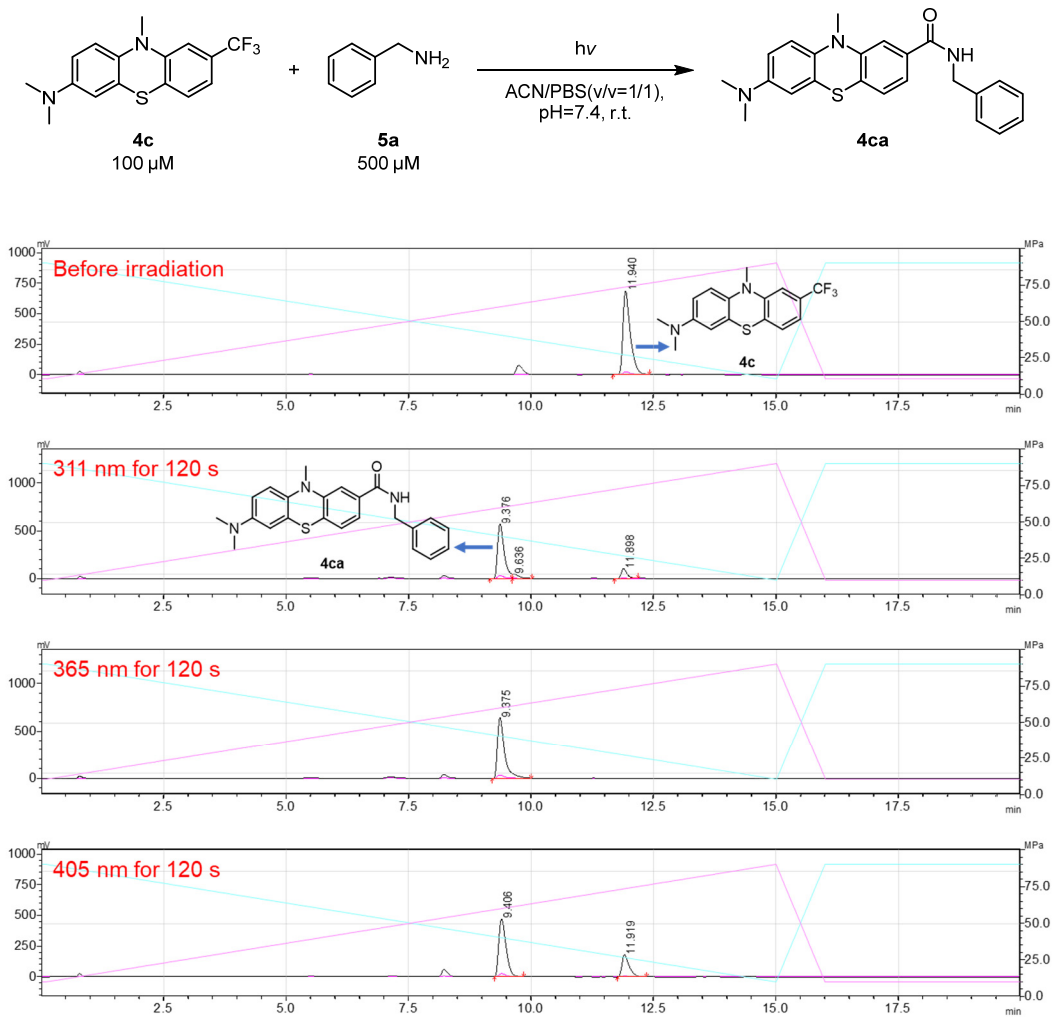
Figure S19. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **4a** and benzylamine **5a**. Reaction conditions: **4a** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) or 405 nm laser (73.1 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.





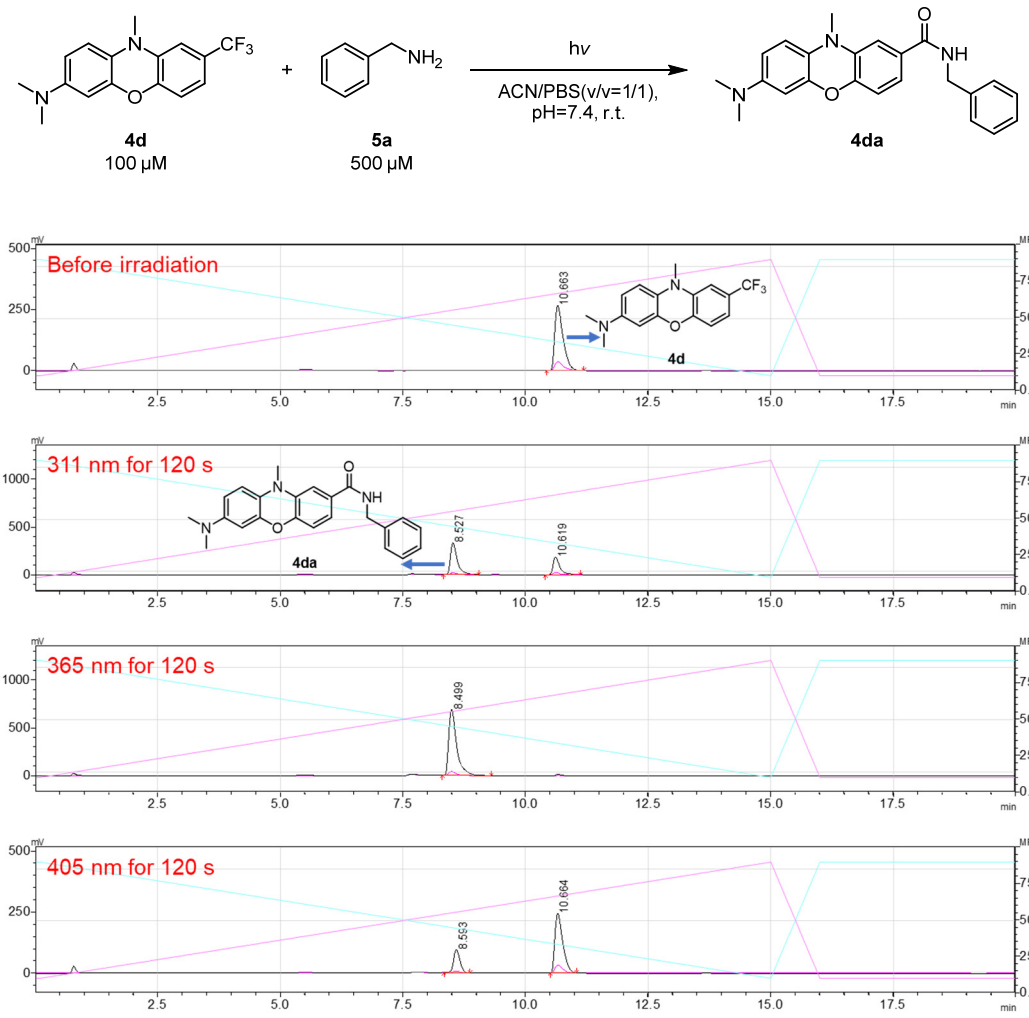
Item	Peak	Time	Area
Before irradiation	**4b**	**14.426**	**7200354**
311 nm for 120 s	**4b**	**14.407**	**7116530**
	4ba	**12.476**	**265673**
365 nm for 120 s	**4b**	**14.415**	**6811783**
	4ba	**12.485**	**364577**
405 nm for 120 s	**4b**	**14.434**	**7223069**

Figure S20. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **4b** and benzylamine **5a**. Reaction conditions: **4b** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) or 405 nm laser (73.1 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



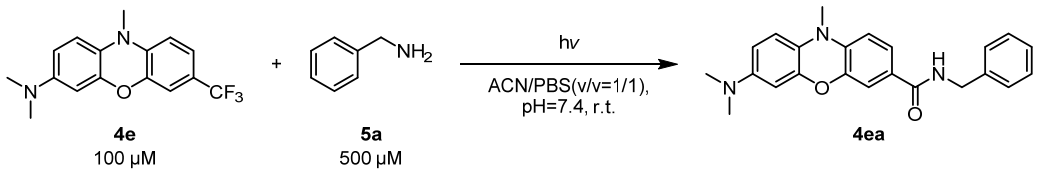
Item	Peak	Time	Area
Before irradiation	**4c**	**11.940**	**7128221**
311 nm for 120 s	**4c**	**11.898**	**828664**
	4ca	**9.376**	**5342244**
365 nm for 120 s	**4ca**	**9.375**	**6473261**
405 nm for 120 s	**4c**	**11.919**	**1844807**
	4ca	**9.406**	**4888885**

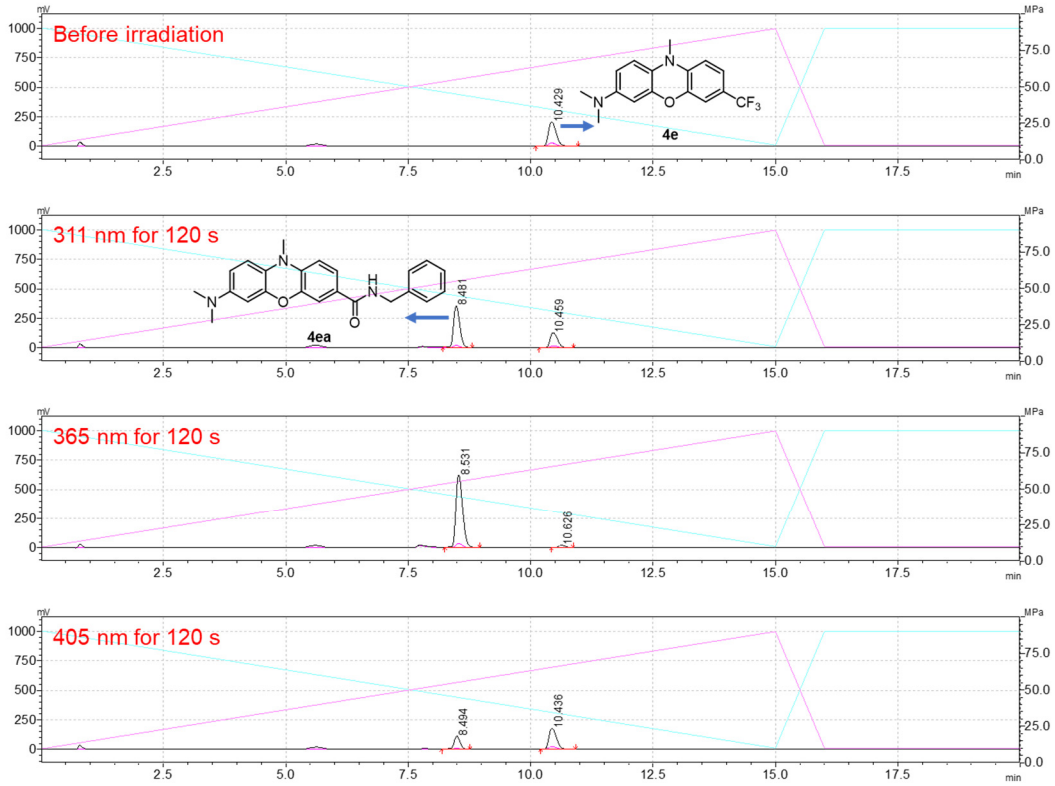
Figure S21. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **4c** and benzylamine **5a**. Reaction conditions: **4c** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) or 405 nm laser (73.1 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



Item	Peak	Time	Area
Before irradiation	**4d**	**10.663**	**3119251**
311 nm for 120 s	**4d**	**10.619**	**1751426**
	4da	**8.527**	**3163781**
365 nm for 120 s	**4da**	**8.499**	**7701165**
405 nm for 120 s	**4d**	**10.664**	**2721168**
	4da	**8.593**	**852457**

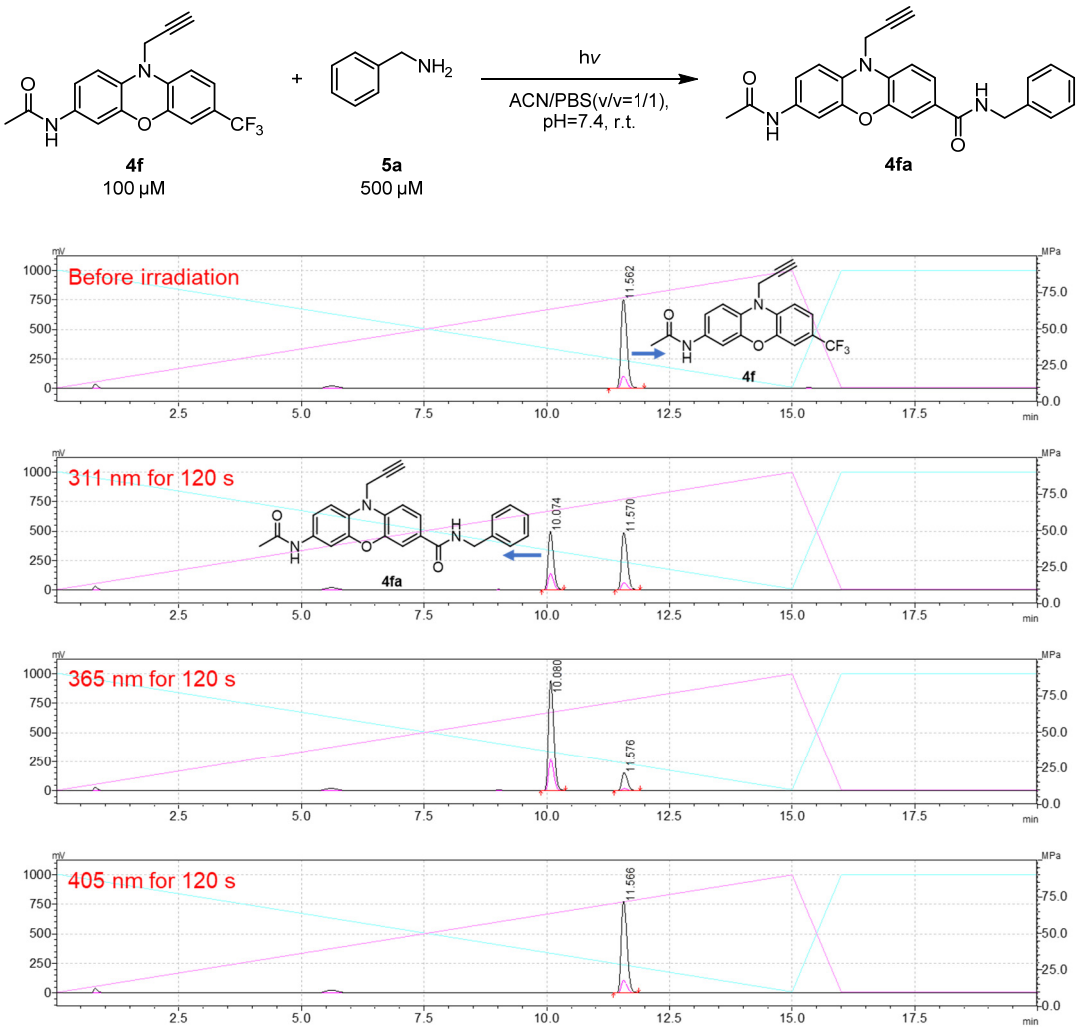
Figure S22. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **4d** and benzylamine **5a**. Reaction conditions: **4d** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) or 405 nm laser (73.1 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.





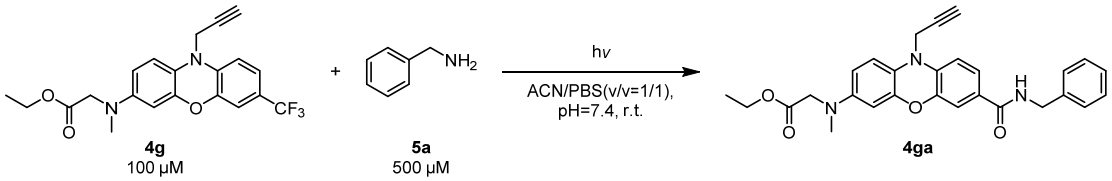
Item	Peak	Time	Area
Before irradiation	**4e**	**10.429**	**2256454**
311 nm for 120 s	**4e**	**10.459**	**1315840**
	4ea	**8.481**	**3081728**
365 nm for 120 s	**4e**	**10.626**	**219149**
	4ea	**8.531**	**5882404**
405 nm for 120 s	**4e**	**10.436**	**1866194**
	4ea	**8.494**	**903560**

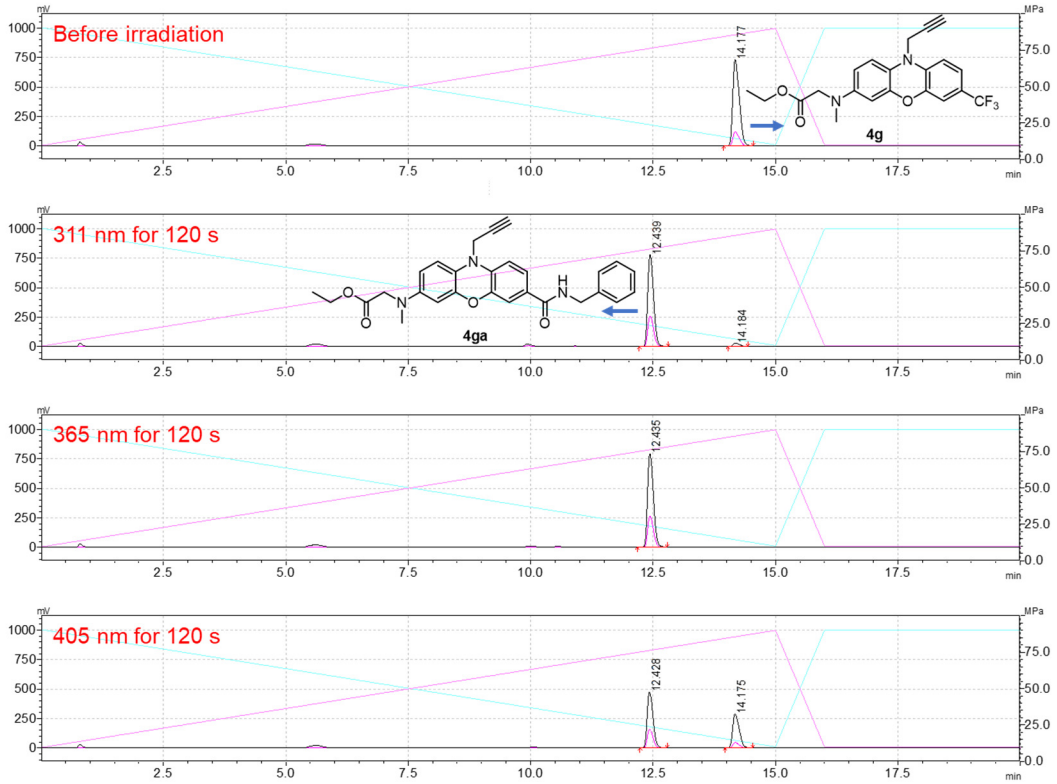
Figure S23. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **4e** and benzylamine **5a**. Reaction conditions: **4e** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) or 405 nm laser (73.1 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.



Item	Peak	Time	Area
Before irradiation	**4f**	**11.562**	**6286951**
311 nm for 120 s	**4f**	**11.570**	**3978029**
	4fa	**10.074**	**3763041**
365 nm for 120 s	**4f**	**11.576**	**1236397**
	4fa	**10.080**	**7148918**
405 nm for 120 s	**4f**	**11.566**	**6421293**

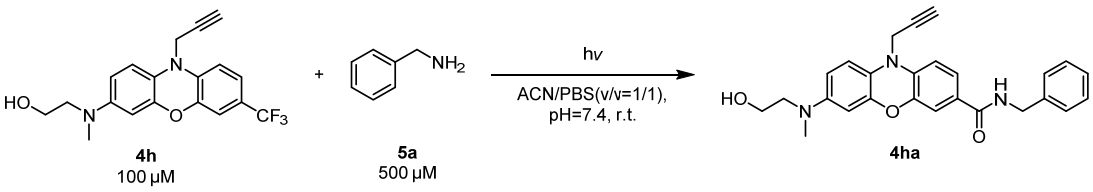
Figure S24. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **4f** and benzylamine **5a**. Reaction conditions: **4f** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) or 405 nm laser (73.1 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

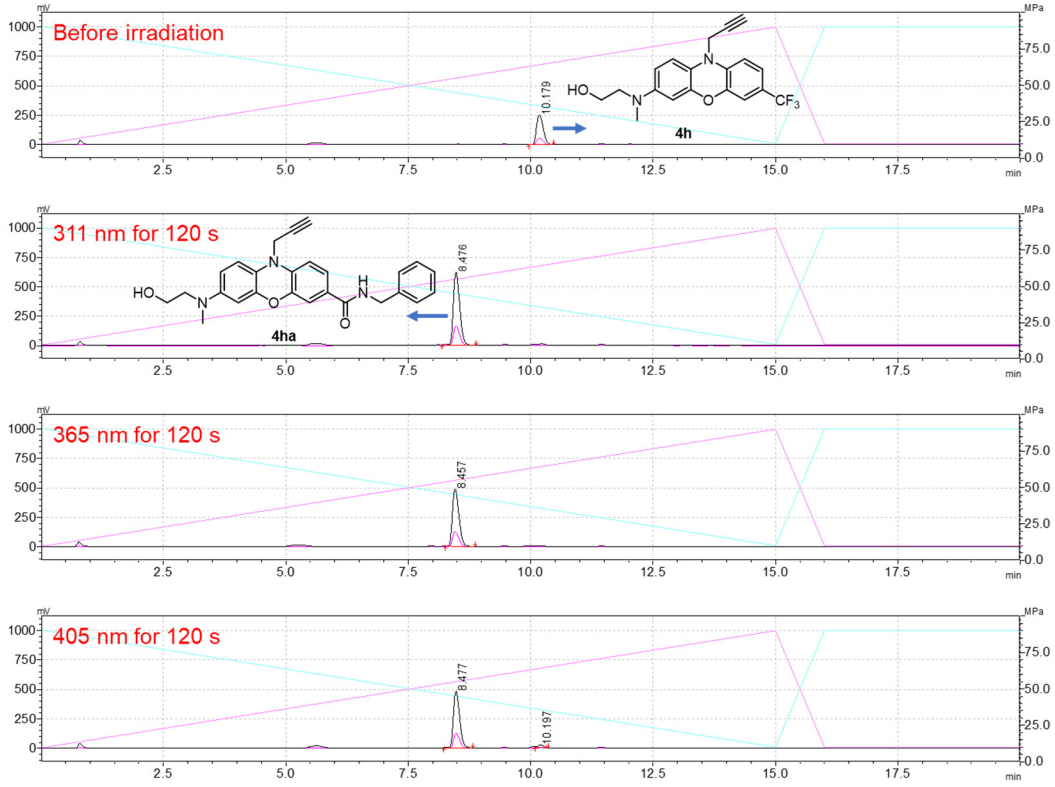




Item	Peak	Time	Area
Before irradiation	**4g**	**14.177**	**6713142**
311 nm for 120 s	**4g**	**14.184**	**252424**
	4ga	**12.439**	**6413684**
365 nm for 120 s	**4ga**	**12.435**	**6550716**
405 nm for 120 s	**4g**	**14.175**	**2642152**
	4ga	**12.428**	**3913118**

Figure S25. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **4g** and benzylamine **5a**. Reaction conditions: **4g** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) or 405 nm laser (73.1 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.





Item	Peak	Time	Area
Before irradiation	**4h**	**10.179**	**2365988**
311 nm for 120 s	**4ha**	**8.476**	**5561736**
365 nm for 120 s	**4ha**	**8.457**	**4467460**
405 nm for 120 s	**4h**	**10.197**	**157296**
	4ha	**8.477**	**4216390**

Figure S26. HPLC analysis of the photo-induced Defluorination-Acyl Fluoride Exchange reaction between **4h** and benzylamine **5a**. Reaction conditions: **4h** (100 μM) and **5a** (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4), 311 nm UV lamp (5.9 mW cm^{-2}, single wavelength output after passing through an optical filter) or 365 nm LED (166.8 mW cm^{-2}) or 405 nm laser (73.1 mW cm^{-2}) for 120 seconds. The samples before and after the irradiation procedures were analyzed by HPLC-MS.

2.2 The chemoselectivity of the photo-DAFEx reaction towards natural amino acid (AAs) residues

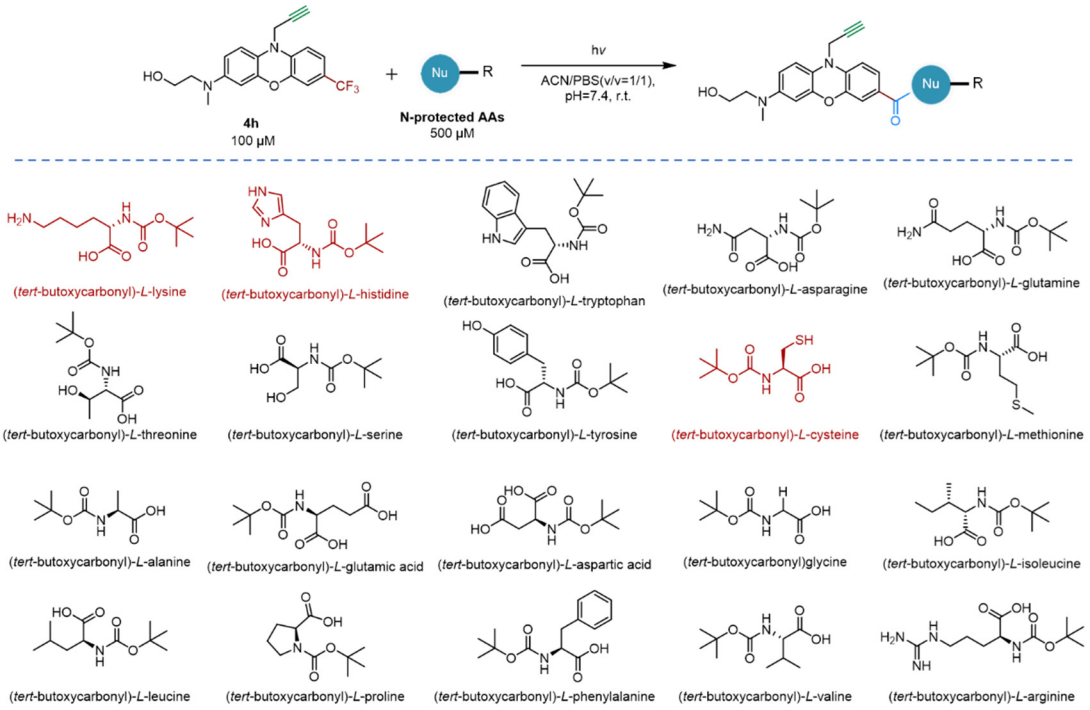
Stocks:

✓ *N*-terminal protected AAs in PBS (50 mM for each one);

✓ **4h** (10.0 mM) in ACN.

10 μL of **4h** stock solution, 10 μL of *N*-terminal protected amino acid (AA) stock solution, and 980 μL of an ACN/PBS solution (v/v = 1/1, pH = 7.4) were mixed and irradiated with a 405 nm LED (21.0 mW cm^{-2}) for 180 seconds in sealed clamp-on vials. The sample was collected and prepared for HPLC-MS analysis.

Table S1. Reactivity screening of the photo-DAFEx reaction with natural amino acid residues



A mixture of **4h** (100 μM) and one selected natural amino acid (500 μM) in 1.0 mL of ACN/PBS (v/v = 1/1, pH = 7.4) was treated with a 405 nm LED (21.0 mW cm^{-2}) for 180 seconds. The sample was collected and prepared for HPLC-MS analysis, noting that only the red-labeled amino acids were capable of reacting with the TFMA reagent **4h**.

Stocks:

√ **4h** (10.0 mM) in ACN.

10 μL of **4h** stock solution, 990 μL ACN/PBS solution (v/v = 1/1, pH = 7.4) were mixed and irradiated with a 405 nm LED (21.0 mW cm^{-2}) for 180 seconds in sealed clamp-on vials. The sample was collected and prepared for HPLC-MS analysis.

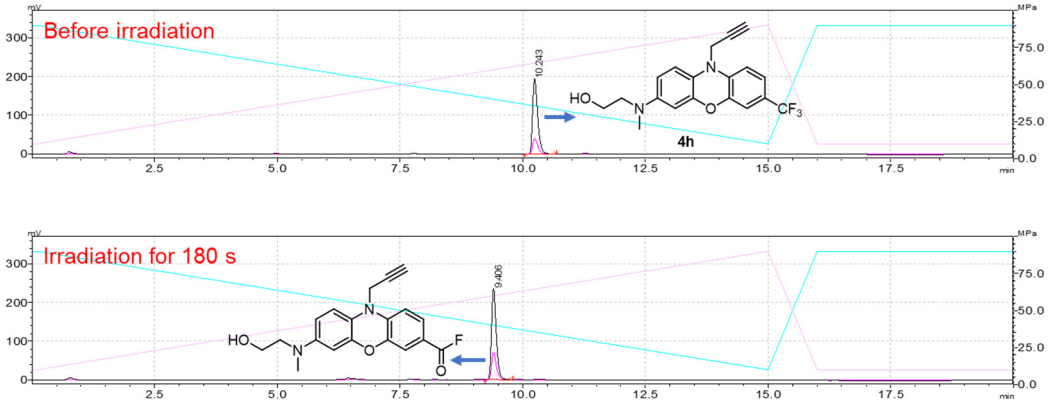


Figure S27. Photo-defluorination of **4h** to obtain the acyl fluoride intermediate. **4h** (100 μM) in 1.0 mL of ACN/PBS (v/v = 1/1, pH = 7.4) solution were treated with a 405 nm LED (21.0 mW cm^{-2}) for 180 seconds to transform into the corresponding acyl fluoride, analyzed via HPLC-MS.

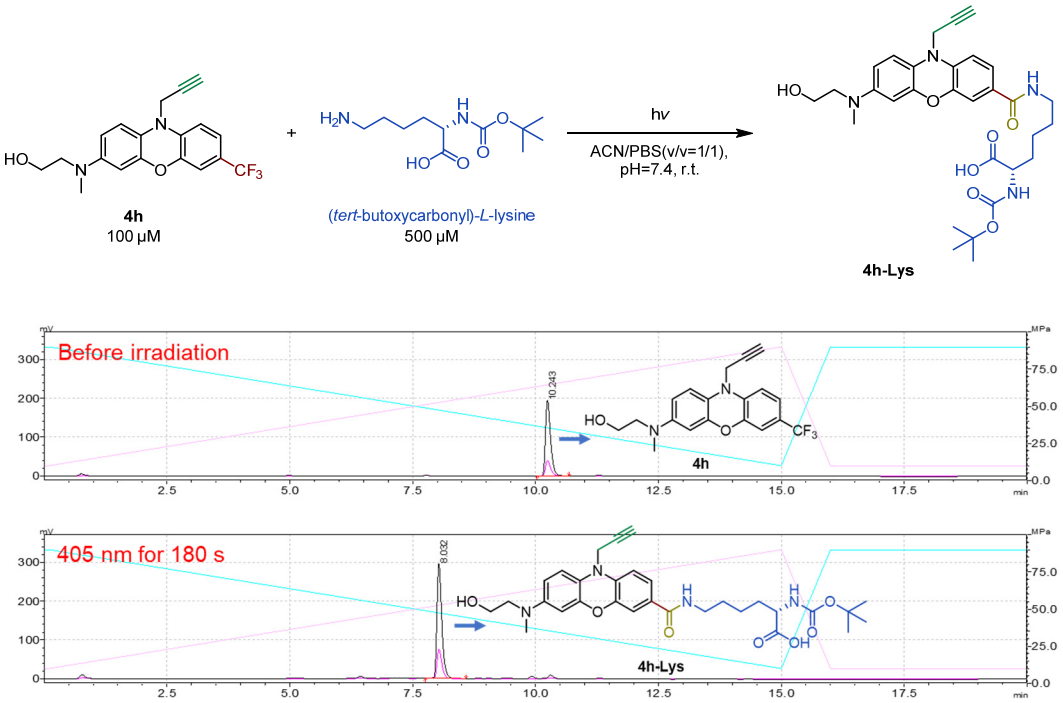


Figure S28. The photo-DAFEx reaction between **4h** and (*tert*-butoxycarbonyl)-*L*-lysine. **4h** (100 μM) and (*tert*-butoxycarbonyl)-*L*-lysine (500 μM) in 1.0 mL of ACN/PBS (v/v = 1/1, pH = 7.4) were irradiated with a 405 nm LED (21.0 mW cm^{-2}) for 180 seconds.

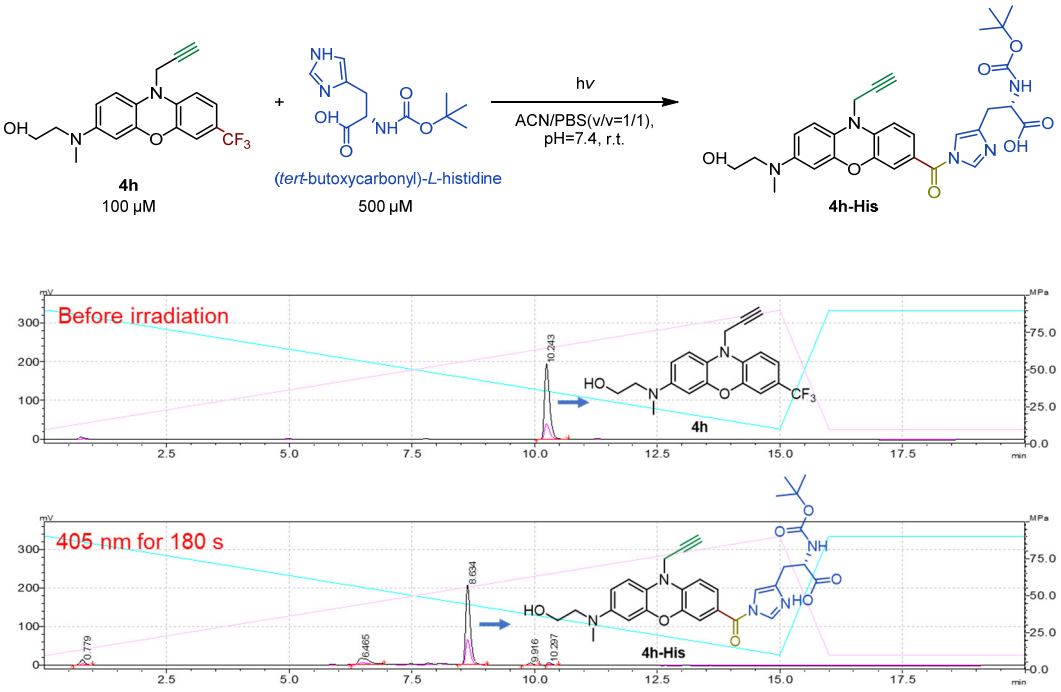


Figure S29. The photo-DAFEx reaction between **4h** and (*tert*-butoxycarbonyl)-*L*-histidine. **4h** (100 μM) and (*tert*-butoxycarbonyl)-*L*-histidine (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4) were irradiated with a 405 nm LED (21.0 mW cm^{-2}) for 180 seconds.

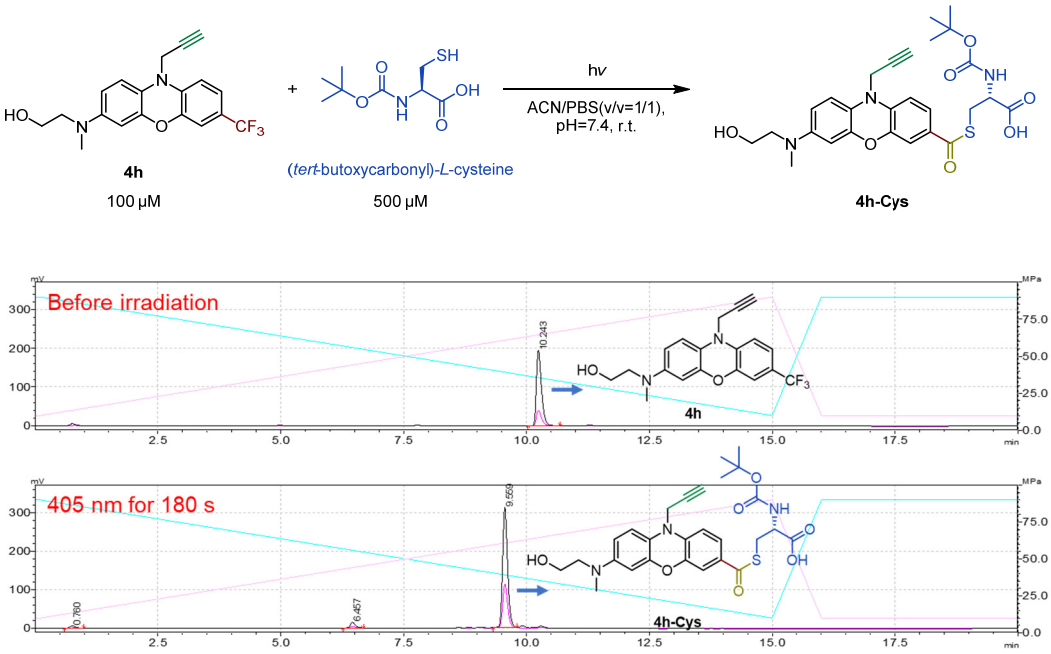


Figure S30. The photo-DAFEx reaction between **4h** and (*tert*-butoxycarbonyl)-*L*-cysteine. **4h** (100 μM) and (*tert*-butoxycarbonyl)-*L*-cysteine (500 μM) in 1.0 mL of ACN/PBS (v/v = 1:1, pH = 7.4) were irradiated with a 405 nm LED (21.0 mW cm^{-2}) for 180 seconds.

2.3 Determination of the photoreaction quantum yields (Φ_R) for the photo-defluorination of 1a-4h

The photo-defluorination quantum yields of the photo-defluorination of the TFMA reagents were determined by using potassium ferrioxalate based chemical actinometer.[1] In brief, a 250 μL fresh solution of 6.0 mM potassium ferrioxalate in 0.1 N H_2SO_4 aqueous solution was irradiated with the 311 nm lamp (5.9 mW cm^{-2}, single wavelength output after an optical filter) in a quartz cuvette (0.2 cm × 1.0 cm optical path) for specified times before quenching by addition of 4.75 mL of NaOAc/HOAc buffer (pH = 4.3) and 5.0 mL of 0.1% 1,10-phenanthroline solution in water to develop the characteristic colour at 510 nm. The mixture was stirred for 30 min before UV-Vis measurement. All the works were carried out in the dark and the samples were also protected from light with aluminum foil during handling. All the operating procedures were the same for both the chemical actinometer and tested samples (direct measurement without developing process), including light sources, experimental setup, volume of the solution, and the cuvette. The quantum yield for a test compound was calculated based on the following equations:

The incident monochromic photon flux I_0:

$$I_0 = \frac{d[\mathrm{Act}]}{dt} \times [1/(1-10^{-\mathrm{Abs_c}})]/\Phi_c = \frac{d[\mathrm{photo-DAFEx}]}{dt} \times [1/(1-10^{-\mathrm{Abs_t}})]/\Phi_t \quad \text{2-3}$$

Because at the initial photo-conversion stage: $\frac{d[Act]}{dt} = \frac{dAbs_{product}}{dt}(\frac{1}{\varepsilon_{product}})$, therefore:

$$I_0 = (40 \times k_c/\varepsilon_{510} l) \times [1/(1-10^{-\mathrm{Abs_c}})]/\Phi_c = (k_t/\Delta\varepsilon_p l) \times [1/(1-10^{-\mathrm{Abs_t}})/\Phi_t$$

The subscript "***c***" represents to the parameters of the chemical actinometer;

The subscript "***t***" represents to the parameters of the tested compound **1a-4h**, and "***p***" for the corresponding acyl fluorides. Therefore:

$$\Phi_{reac.} = \Phi_t = [(1-10^{-\varepsilon_c c_c l})]/(1-10^{-\varepsilon_t c_t l})] \times [k_t/(40 \times k_c)] \times (\varepsilon_{510}/\Delta\varepsilon_p) \times \Phi_c$$

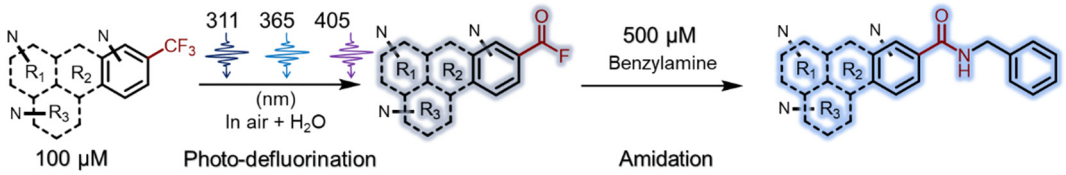
where ε_c and ε_t were extinction coefficients of the standard chemical actinometer and test samples (**1a-4h** at 311 nm), respectively. l = 1.0 cm;

k_t and k_c were slopes of the linear fitting line of product formation in plots of absorbance changes versus time at the observing wavelength for the test compound and the standard chemical actinometer, respectively. The zeroth-order photo-conversion rate (k_t and k_c) could be only applied at very low conversion (as low as possible) of the starting materials because the absorption of light by the products formed under such conditions is minimal. Noteworthy, the addition of the buffer solution and the developer during the color readout of the actinometer conversion resulted in a 40-fold dilution, therefore the k_c need to be multiplied by 40;

c_c and c_t were concentrations of the standard actinometer and the test compound, respectively;

ε_{510} [4] and $\Delta\varepsilon_p$ were extinction coefficients of the Fe^{2+}-(1,10-phenanthroline)$_3$ complex at 510 nm for the actinometer and the difference in extinction coefficients of the acyl fluorides compared to the sample **1a-4h** at the monitoring wavelength, respectively. The photo-defluorination quantum yields for **1a-4h** in ACN/H_2O (v/v = 1/1) with designated irradiation were obtained.

Table S1. The summary for the photo-defluorination quantum yield for the TFMA reagents under various irradiation wavelengths



Compound	$\lambda_{ex.}$ (nm)	Φ_R at $\lambda_{ex.}$(%)
1a	311	14
1b	311	11
1c	311	13
1d	311	9.1
2b	365	8.3
2e	365	32
2f	365	13
2g	365	11
3a	311	29
4a	365	11
4c	365/405	9.3/3.9
4d	365	8.6
4e	365/405	16/11
4g	405	9.6
4h	405	9.6

2.4 Determination of half-life ($t_{1/2}$) of the acyl fluoride intermediate from photo-defluorination of the TFMA reagents

For determination of half-life ($t_{1/2}$) of the acyl fluoride from **2f**, **2g**, **4c**, **4e**, and **4h**, the hydrolysis of acyl fluoride in ACN/PBS (v/v = 1/1, pH = 7.4) at room temperature can be regarded as a pseudo-first-order reaction due to the constant concentration of water. In brief, 100 μM of the TFMA reagents in ACN/PBS (v/v = 1/1, pH = 7.4) was placed in a capped sample vial, and irradiated with either 365 nm or 405 nm LED to reach the complete photo-defluorination at room temperature. After a specific time, the remaining concentration of the acyl fluoride in the vial was analyzed by HPLC-MS.

The rate constant k (s^{-1}) was calculated based on the following equations:

$$\ln\left(\frac{a}{c}\right) = kt$$

a represents to the initial concentration of acyl fluoride; ***c*** represents to the concentration of the reagent after a specific time. Therefore:

$$t_{1/2} = \frac{ln2}{k}$$

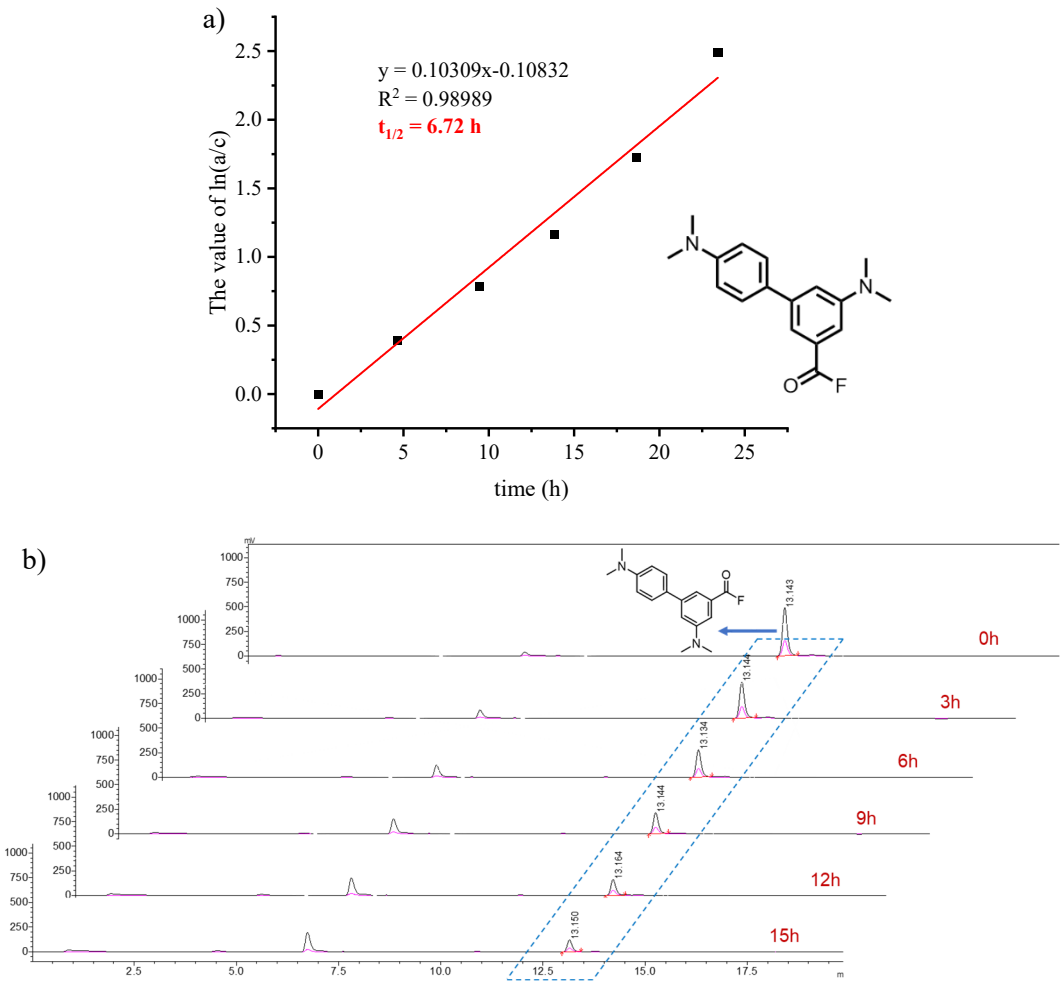


Figure S31. a) Time-course plot of the acyl fluoride from photo-defluorination from **2f** to the value of $\ln\left(\frac{a}{c}\right)$ with a linear fitting curve; b) HPLC traces of the acyl fluoride from photo-defluorination from **2f** stored after a specific time.

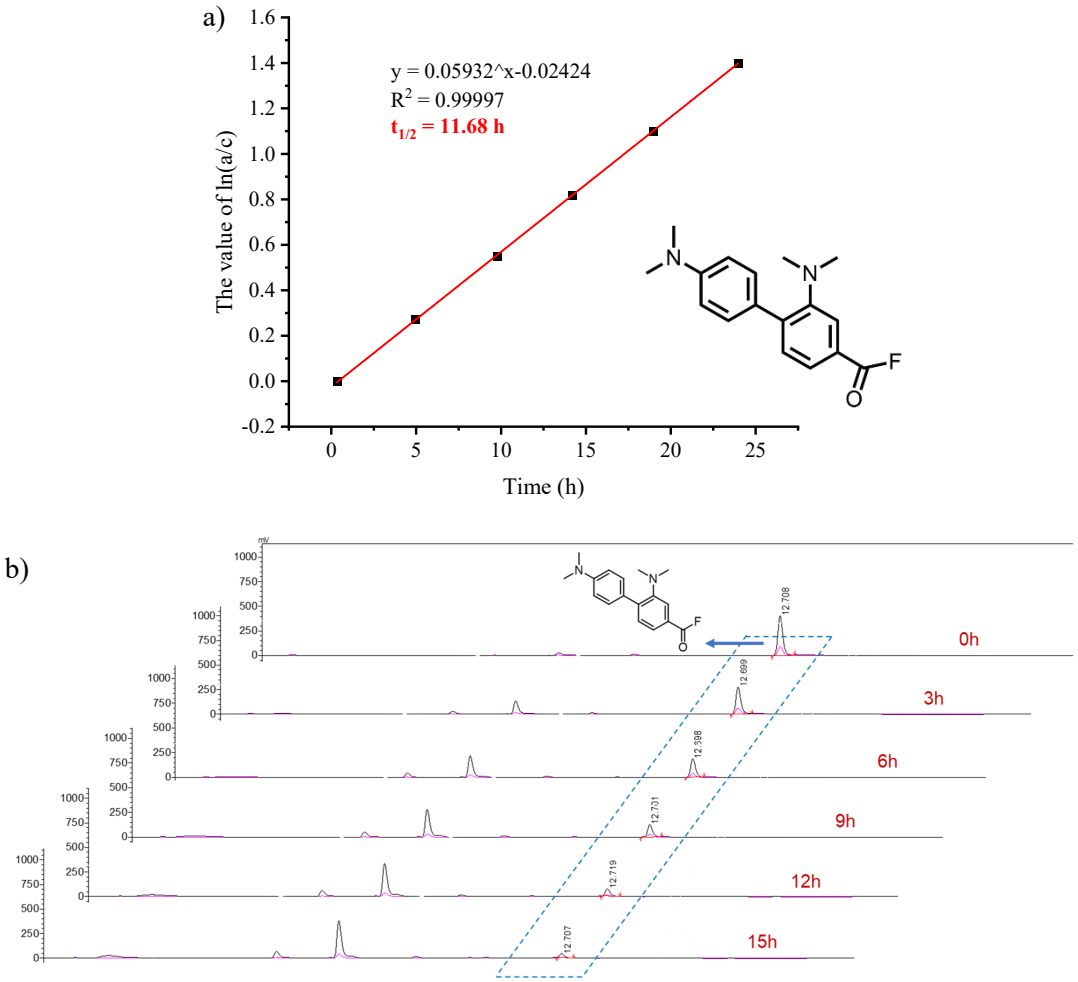


Figure S32. a) Time-course plot of the acyl fluoride from photo-defluorination from **2g** to the value of $\ln\left(\frac{a}{c}\right)$ with a linear fitting curve; b) HPLC traces of the acyl fluoride from photo-defluorination from **2g** stored after a specific time.

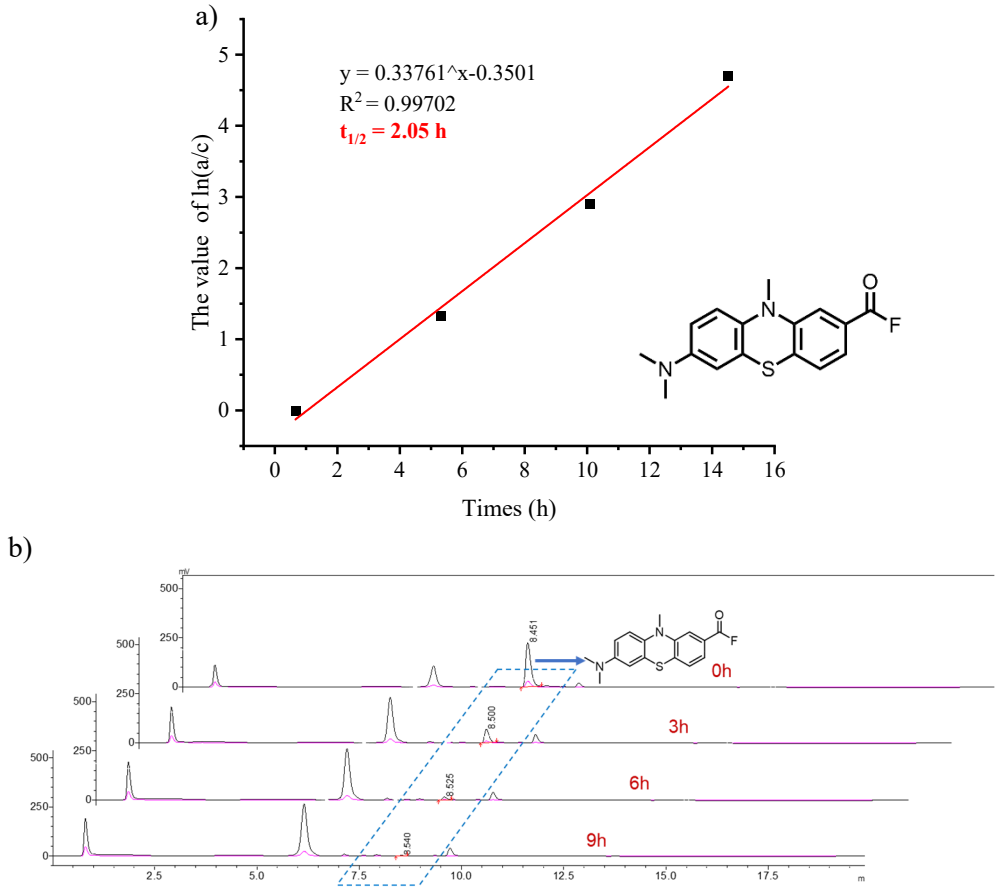
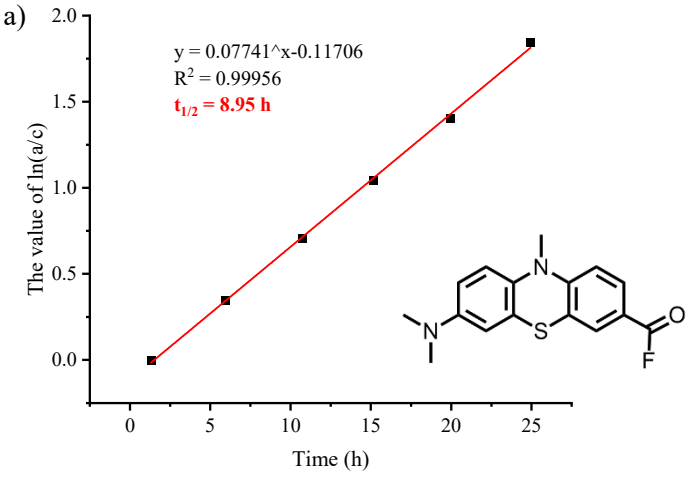


Figure S33. a) Time-course plot of the acyl fluoride from photo-defluorination from **4c** to the value of $\ln\left(\frac{a}{c}\right)$ with a linear fitting curve; b) HPLC traces of the acyl fluoride from photo-defluorination from **4c** stored after a specific time.



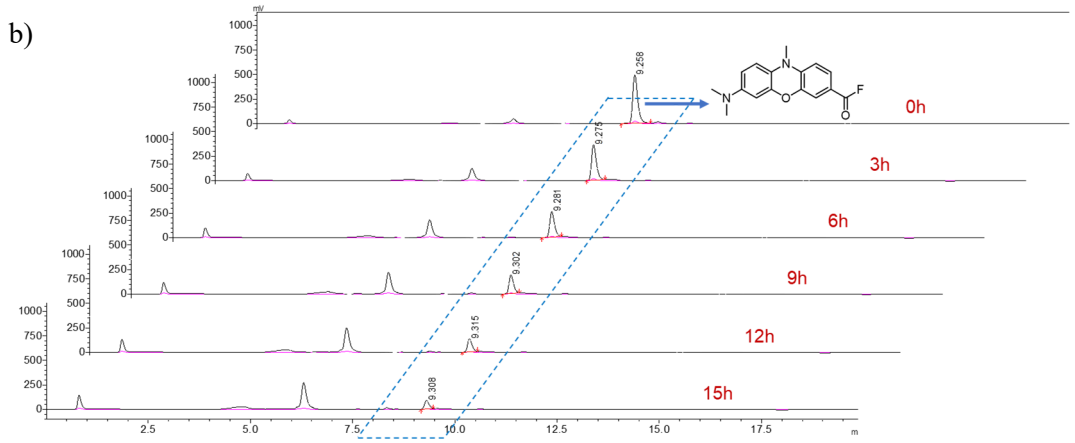


Figure S34. a) Time-course plot of the acyl fluoride from photo-defluorination from **4e** to the value of $\ln\left(\frac{a}{c}\right)$ with a linear fitting curve; b) HPLC traces of the acyl fluoride from photo-defluorination from **4e** stored after a specific time.

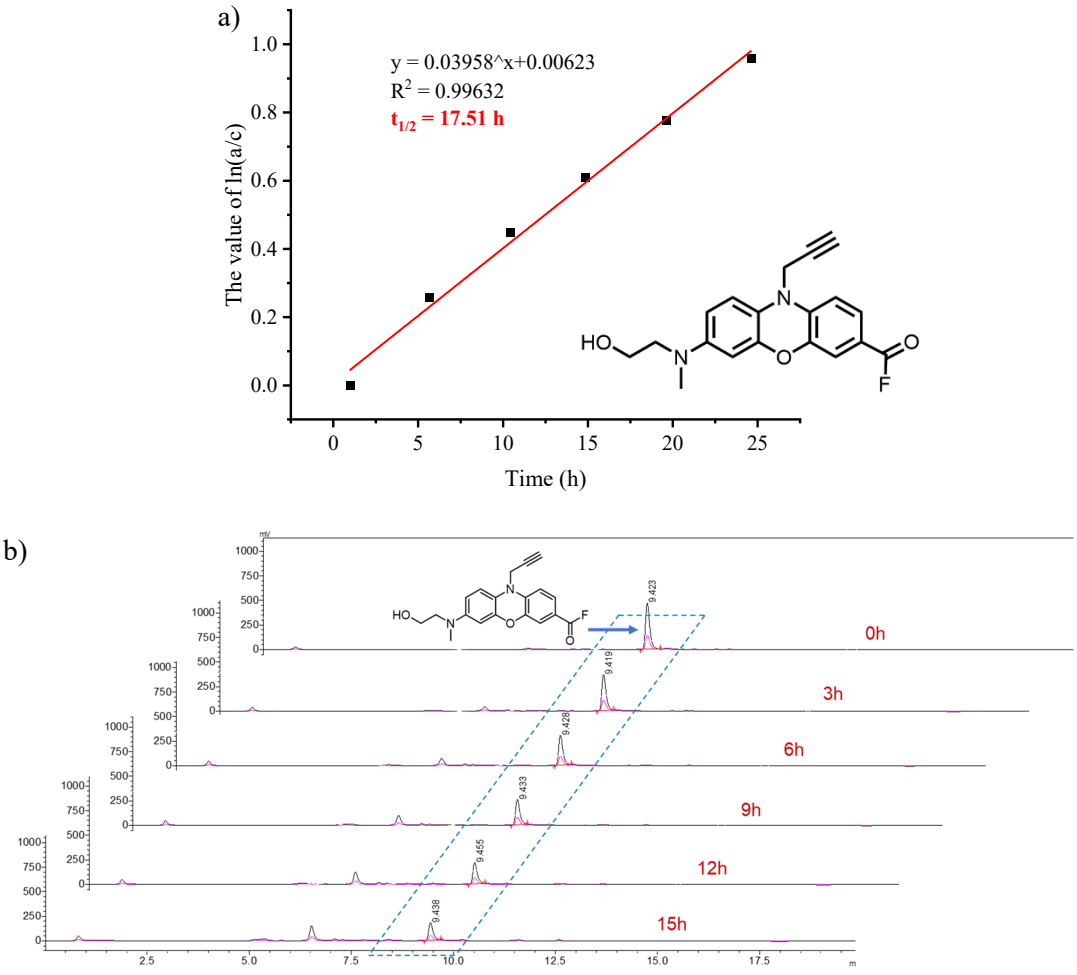
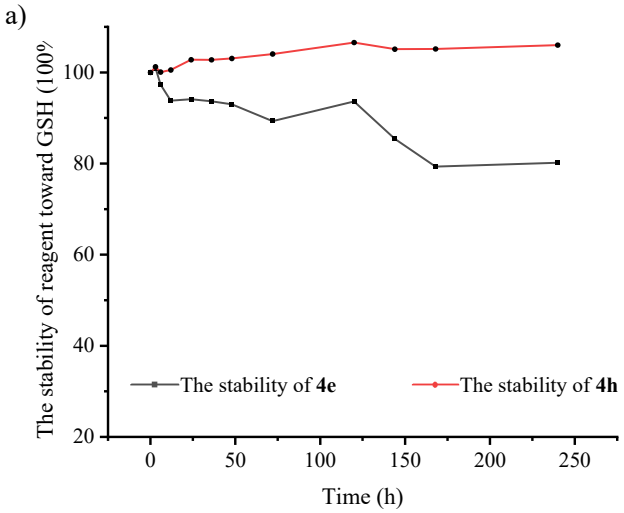
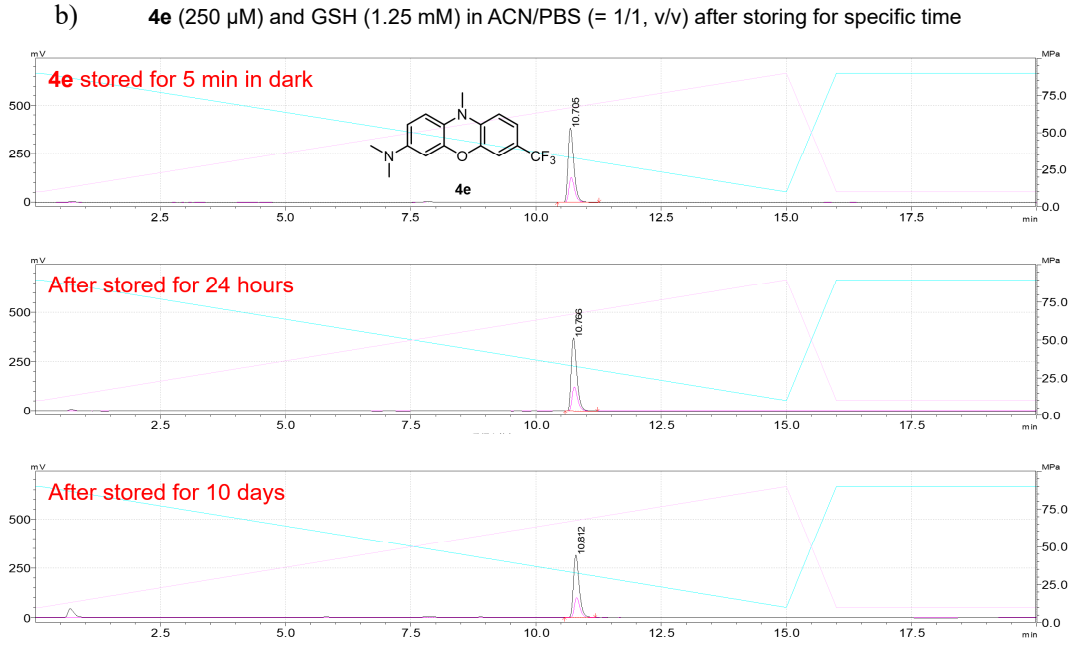


Figure S35. a) Time-course plot of the acyl fluoride from photo-defluorination from **4h** to the value of $\ln\left(\frac{a}{c}\right)$ with a linear fitting curve; b) HPLC traces of the acyl fluoride from photo-defluorination from **4h** stored after a specific time.





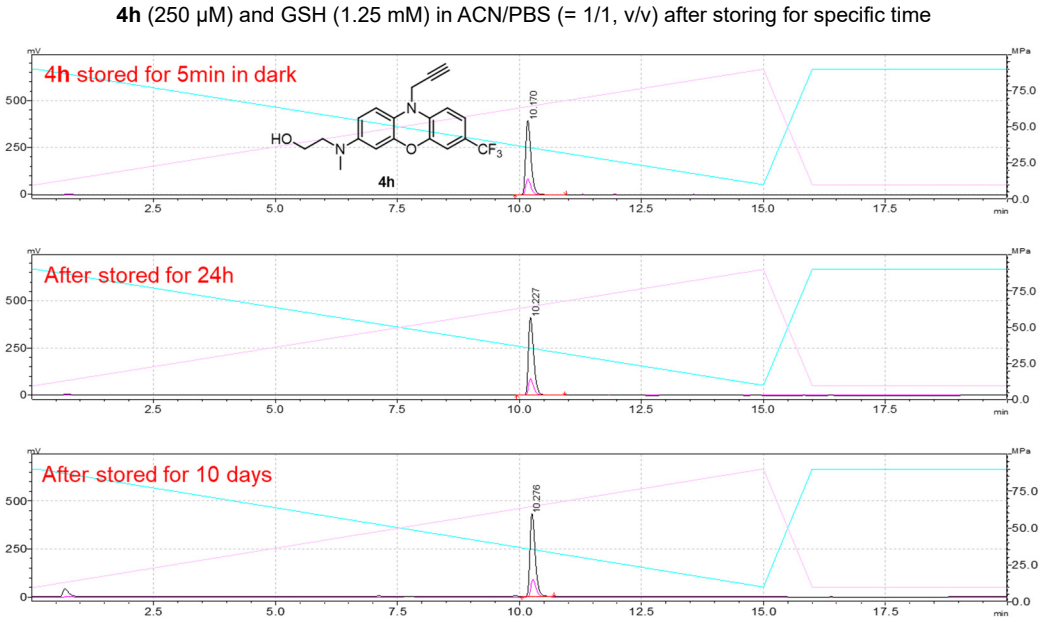
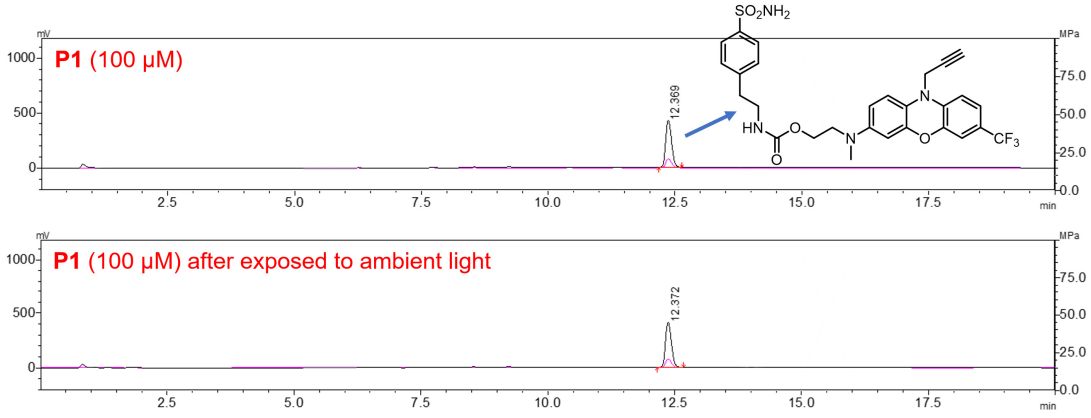


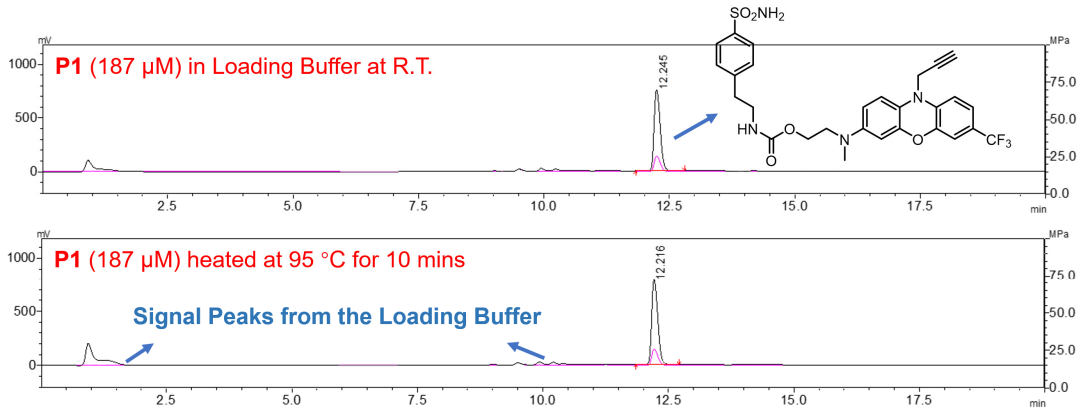
Figure S36. The TFMA reagent stability tests evaluated against thiol nucleophilic addition. a) The plot for the stability of **4e**/**4h** (250 μM) towards GSH (1.25 mM) assessed by analyzing the decaying of integrated peak areas based on HPLC traces. A slight upward trend in the concentration of the remaining TFMA reagent was observed after the incubation, attributing to solvent evaporation during the storage. b) HPLC traces of **4e**/**4h** (250 μM) and GSH (1.25 mM) in ACN/PBS (v/v = 1/1), stored in the dark for 5 min, 24 hours, 10 days.

a)



Conditions:	Retention Time (mins)	Area
P1 (100 μM)	**12.369**	**3454252**
P1 after exposure to ambient light for 1 hour via daylight fluorescent tubes	**12.372**	**3323352**

b)



Conditions:	Retention Time (mins)	Area
P1 (187 μM)	**12.245**	**6375394**
P1 subjected to heating at 95 ºC for 10 mins in SDS-PAGE Loading Buffer with DTT	**12.216**	**6622837**

Figure S37. Stability tests of **P1** probe under exposure to ambient light lamp or subject to heating under reducing conditions. a) HPLC trace of **P1** (100 μM) in ACN/PBS (v/v = 1/1) before exposure; HPLC trace of **P1** (100 μM) in ACN/PBS (v/v = 1/1) after exposure to ambient light for 1 hour; b) HPLC trace of **P1** (187 μM) stored at room temperature; HPLC trace of **P1** (187 μM) after subjection to heating at 95 ℃ for 10 mins in the SDS-PAGE Loading Buffer.

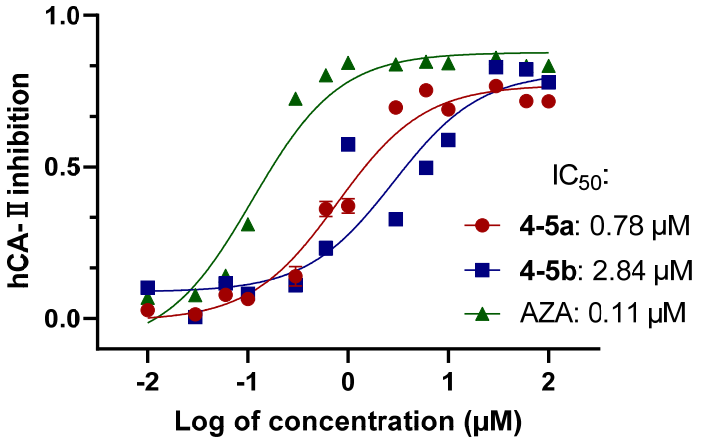


Figure S38. The enzymatic activity of hCA-II (1.0 μM) in catalyzing the hydrolysis of *p*-nitrophenyl acetate (2.5 mM), plotted against inhibitor concentration. Error bars indicate SD, $n = 3$.

3. Computational details

All DFT and TD-DFT calculations were performed using Gaussian 09 program package.[1] Geometry optimizations and natural bond orbital (NBO) analysis[2-3] were performed at the M06-2X/6-31+G**/SMD(acetonitrile/water=1/1, volume ratio) level of theory, combining the D3 version of Grimme's dispersion.[4-7] Excited states were calculated at the TD-PBE0(D3)/6-31+G**/SMD(acetonitrile : water=1:1, volume ratio) level.[8] The natural transition orbitals (NTO) analysis[9] were performed using Multiwfn3.8(dev) program[10] and visualized by VMD 1.9.3 program.[11]

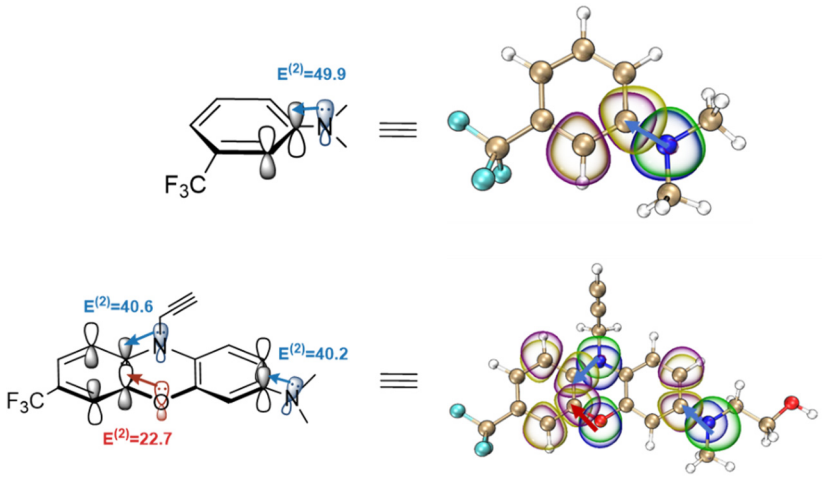


Figure S39. Orbital interaction and the results of second order perturbation theory analysis ($E^{(2)}$, in kcal mol^{-1}) for key NBO pairs in **1a** and **4h**.

Cartesian coordinates of optimized geometries.

1a

C	-0.96945300	0.34814800	-0.01286400
C	-0.93269300	1.73785700	0.03344400
C	0.32193200	2.34885600	0.02462700
C	1.49111900	1.60180000	-0.03591700
C	0.18640500	-0.42783900	-0.07208400
C	1.45396100	0.18838900	-0.10358800
C	-2.28152200	-0.37870300	0.00825800
H	-1.84293500	2.32436100	0.08153800
H	0.39201600	3.43161700	0.07225200
H	2.44013500	2.12424800	-0.03182600
H	0.09226600	-1.50763800	-0.09752900
N	2.61173400	-0.55998100	-0.20920800

F	-2.45366500	-1.15548000	-1.08433000
F	-3.34042500	0.44832800	0.05984900
F	-2.39034600	-1.20902500	1.06959300
C	3.87880200	0.08886800	0.09761500
C	2.53040500	-1.98505000	0.07671700
H	2.17501700	-2.18501800	1.09901600
H	3.52134300	-2.42382000	-0.04073800
H	4.06739800	0.91873500	-0.58851300
H	4.68273300	-0.63583200	-0.03354000
H	3.91405900	0.47139400	1.12885600
H	1.86202500	-2.48654100	-0.62831700

Zero-point correction = 0.179703 (Hartree/Particle)
Thermal correction to Energy = 0.191803
Thermal correction to Enthalpy = 0.192747
Thermal correction to Gibbs Free Energy = 0.140300

4h

C	3.07142000	-0.41795200	-0.04238300
C	3.05754200	0.98859400	-0.05654800
C	1.88400300	1.70681900	-0.29560400
C	0.68258900	1.05824400	-0.56613200
C	0.70729800	-0.33799400	-0.56190800
C	1.84999000	-1.07196800	-0.31246300
N	-0.54440400	1.70238700	-0.87072200
C	-1.70091400	0.99603300	-0.52840400
C	-1.62620500	-0.41123000	-0.51716200
O	-0.44870600	-1.04068100	-0.86445900
C	-2.92726300	1.59145800	-0.22277100
C	-4.04689800	0.80667200	0.05987100
C	-3.94160800	-0.57682300	0.06274700
C	-2.71863500	-1.19665700	-0.21935600
N	4.22898300	-1.14893100	0.25190900
C	4.24730200	-2.53724400	-0.19772800
C	5.48832000	-0.41736300	0.12283000
C	-0.58079200	3.15640400	-0.86836100
C	-5.11838800	-1.44348000	0.37480400
F	-4.89406400	-2.24778000	1.43854600
F	-5.43468500	-2.27167700	-0.64692300
F	-6.22758900	-0.73711300	0.65337400
H	3.95861000	1.55865800	0.12675200
H	1.93677000	2.79009600	-0.27727700
H	1.75560300	-2.15009600	-0.34050300

H	-3.02871000	2.66994100	-0.20362000
H	-4.99125500	1.29044700	0.28474000
H	-2.61033200	-2.27766400	-0.22420500
H	3.41944600	-3.09061600	0.24754800
H	4.18705200	-2.62520700	-1.29378300
H	5.15910500	-3.02445700	0.14422200
H	5.46724200	0.43638500	0.80619700
H	5.62101800	-0.02501000	-0.89970300
H	-1.46990500	3.48543600	-1.41260900
H	0.27863300	3.52466000	-1.43478000
C	6.71267000	-1.23378700	0.50849600
H	6.96666200	-1.97323000	-0.25839200
H	6.53937800	-1.75368900	1.46018600
O	7.77808000	-0.29613800	0.64160100
H	8.60608300	-0.78187600	0.75261500
C	-0.57582500	3.73675500	0.48808600
C	-0.57237700	4.18563100	1.60980800
H	-0.56877900	4.58305100	2.60525200

Zero-point correction = 0.332888 (Hartree/Particle)
Thermal correction to Energy = 0.356047
Thermal correction to Enthalpy = 0.356991
Thermal correction to Gibbs Free Energy = 0.279345

4. Protein modification

4.1 Deconvoluted MS spectra of hCA-II after PAL with P1/P2

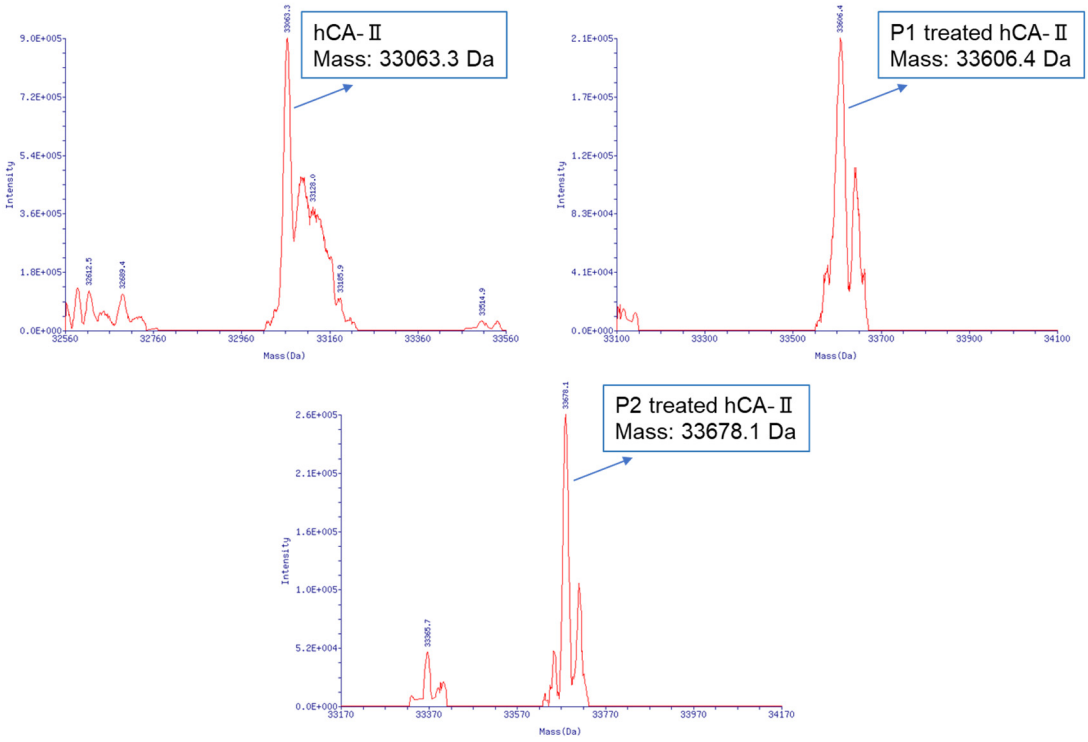


Figure S40. Deconvoluted MS spectra to identify purified hCA-II over-expressed in *E. coli* cells (BL21(DE3)) and after the photo-DAFEx treatment with **P1**/**P2**. hCA-II (4.0 mg/mL) and probe (200 μM) in PBS buffer (pH = 7.4) was irradiated with a 405 nm LED (21.0 mW cm $^{-2}$) for 20 seconds.

4.2 Photo-DAFEx reaction of P1/P2/P3/P4 to conduct PAL with concentration gradient screening, irradiation time screening toward isolated proteins in PBS and PAL of proteins in bacterial lysate.

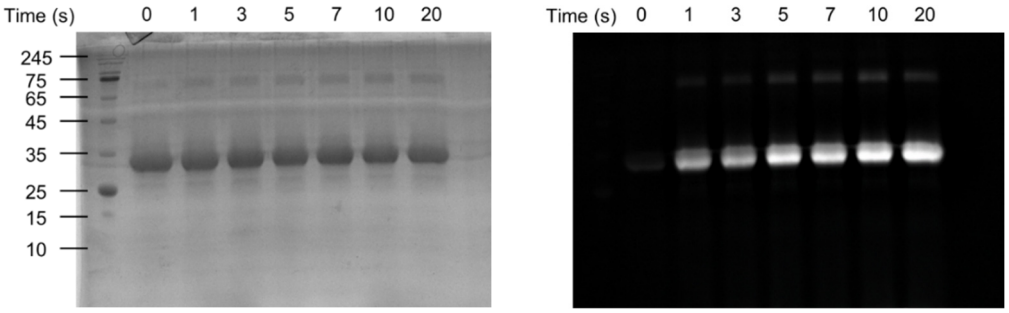


Figure S41. In-gel fluorescence analysis and Coomassie brilliant blue (CBB) staining of SDS-PAGE of the resolved protein mixtures containing hCA-II (130 μM, 4.0 mg/mL) after photo-labelling with **P1** (50 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) exposure for various durations: 0 s, 1 s, 3 s, 5 s, 7 s, 10 s, and 20 s. The fluorophore (Alexa-647) decoration was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).

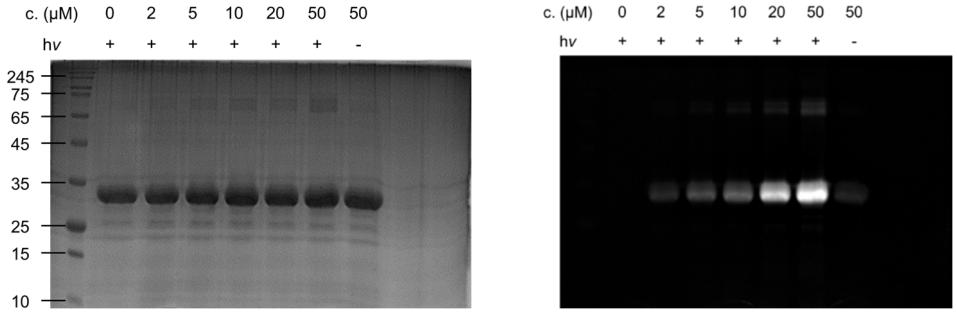


Figure S42. In-gel fluorescence analysis and Coomassie brilliant blue (CBB) staining of SDS-PAGE of the resolved hCA-II photo-labeled with **P1** at various final concentrations (2.0 μM, 5.0 μM, 10 μM, 20 μM, and 50 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) for 20 seconds. The fluorophore (Alexa-647) decoration was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).

There was some unknown in-gel fluorescence labeling of the targeting proteins by the PAL probe under conditions where high concentrations of probes were used and kept in dark. This unexpected labeling phenomenon might be attributed to potential thermal defluorination or an accelerated photo-defluorination by weak ambient light, followed by subsequent crosslinking to the targeting proteins in the microenvironment where the probe and proteins are in close proximity.

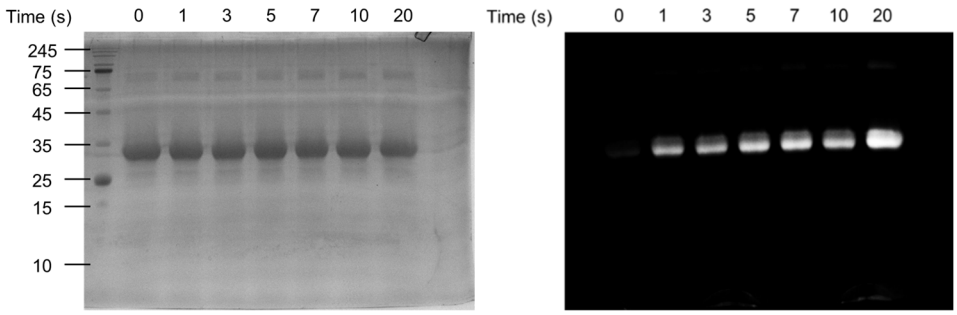


Figure S43. In-gel fluorescence analysis and Coomassie brilliant blue (CBB) staining of SDS-PAGE of the resolved protein mixtures containing hCA-II (130 μM, 4.0 mg/mL) photo-labeled with **P2** (50 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) exposure for various durations: 0 s, 1 s, 3 s, 5 s, 7 s, 10 s, and 20 s. The fluorophore (Alexa-647) decoration was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).

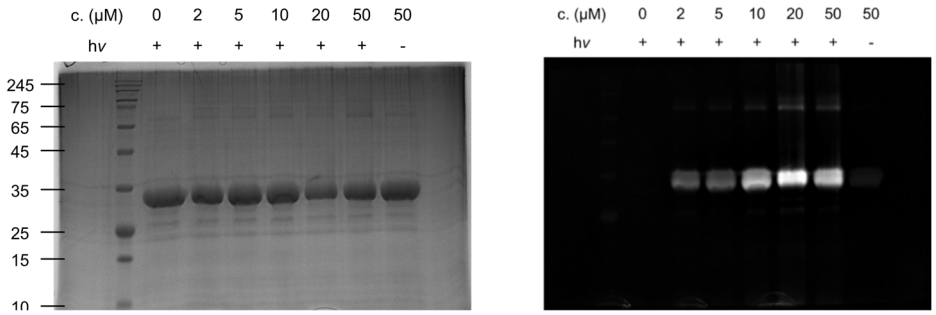


Figure S44. In-gel fluorescence analysis and Coomassie brilliant blue (CBB) staining of SDS-PAGE of the resolved hCA-II photo-labeled with **P2** at various final concentrations (2.0 μM, 5.0 μM, 10 μM, 20 μM, and 50 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) irradiation for 20 seconds. The fluorophore (Alexa-647) decoration was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).

There was some unknown in-gel fluorescence labeling of the targeting proteins by the PAL probe under conditions where high concentrations of probes were used and kept in dark. This unexpected labeling phenomenon might be attributed to potential thermal defluorination or an accelerated photo-defluorination by weak ambient light, followed by subsequent crosslinking to the targeting proteins in the microenvironment where the probe and proteins are in close proximity.

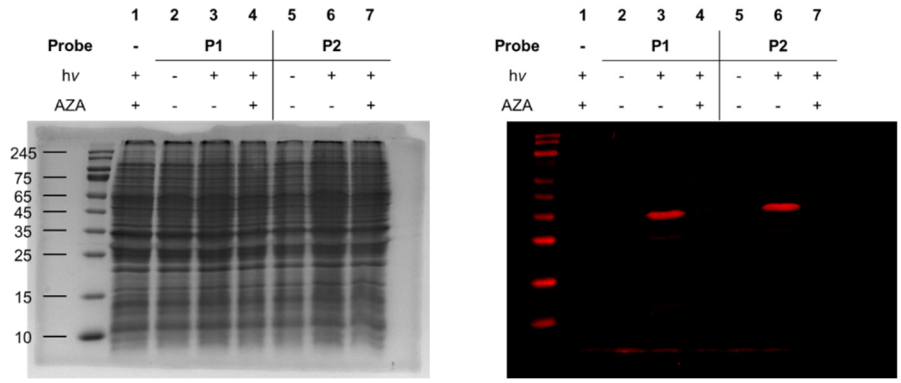


Figure S45. Photoaffinity-labeling of the recombinant hCA-II in *E. coli* lysate by using **P1**/**P2** (20 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm light irradiation for 20 seconds. AZA (200 μM) as a competitive inhibitor. Fluorophore (Alexa-647) decoration of the labeled protein was achieved by using CuAAc with an N_3-Alexa-647 conjugate. The labeled protein was then resolved by SDS-PAGE and imaged via a fluorescence gel imager (left image: CBB staining; right image: in-gel fluorescence).

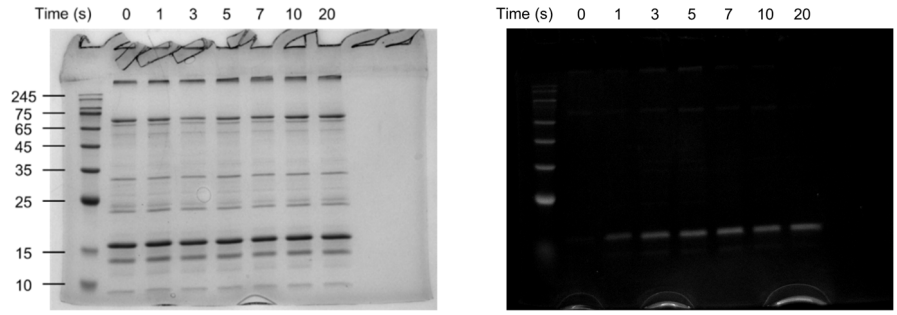


Figure S46. In-gel fluorescence analysis and Coomassie brilliant blue (CBB) staining of SDS-PAGE of the resolved protein mixtures containing BRD4 (60 μM, 4.0 mg/mL) photo-labeled with **P3** (50 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) exposure for various durations: 0 s, 1 s, 3 s, 5 s, 7 s, 10 s, and 20 s. The fluorophore (Alexa-647) decoration was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).

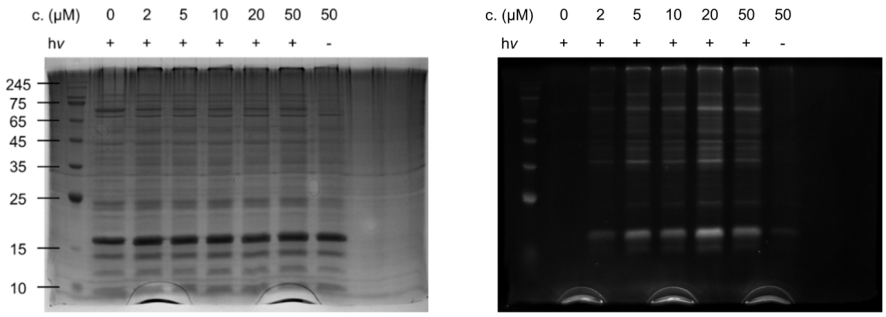


Figure S47. In-gel fluorescence analysis and Coomassie brilliant blue (CBB) staining of SDS-PAGE of the resolved protein mixtures containing BRD4 (60 μM, 4.0 mg/mL) photo-labeled with **P3** at various final concentrations (2.0 μM, 5.0 μM, 10 μM, 20 μM, and 50 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) irradiation for 20 seconds. The fluorophore (Alexa-647) decoration was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).

There was some unknown in-gel fluorescence labeling of the targeting proteins by the PAL probe under conditions where high concentrations of probes were used and kept in dark. This unexpected labeling phenomenon might be attributed to potential thermal defluorination or an accelerated photo-defluorination by weak ambient light, followed by subsequent crosslinking to the targeting proteins in the microenvironment where the probe and proteins are in close proximity.

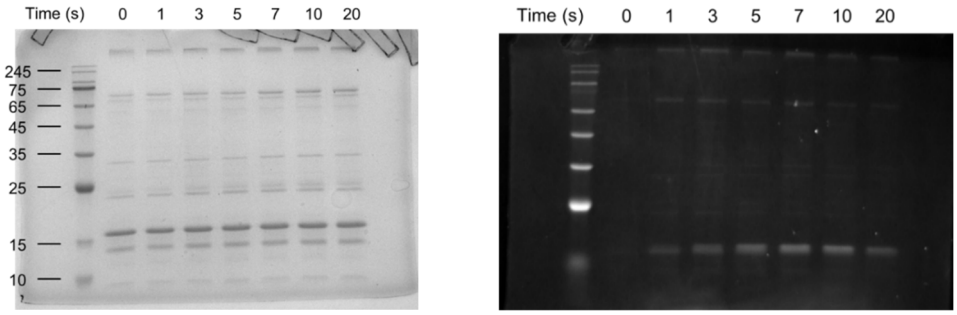


Figure S48. In-gel fluorescence analysis and Coomassie brilliant blue (CBB) staining of SDS-PAGE of the resolved protein mixtures containing BRD4 (60 μM, 4.0 mg/mL) photo-labeled with **P4** (50 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) exposure for various durations: 0 s, 1 s, 3 s, 5 s, 7 s, 10 s, and 20 s. The fluorophore (Alexa-647) decoration was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).

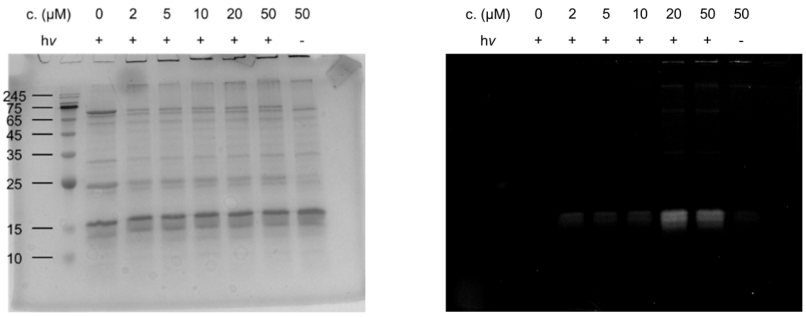


Figure S49. In-gel fluorescence analysis and Coomassie brilliant blue (CBB) staining of SDS-PAGE of the resolved protein mixtures containing BRD4 (60 μM, 4.0 mg/mL) photo-labeled with **P4** at various final concentrations (2.0 μM, 5.0 μM, 10 μM, 20 μM, and 50 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) for 20 seconds. The fluorophore (Alexa-647) decoration was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).

There was some unknown in-gel fluorescence labeling of the targeting proteins by the PAL probe under conditions where high concentrations of probes were used and kept in dark. This unexpected labeling phenomenon might be attributed to potential thermal defluorination or an accelerated photo-defluorination by weak ambient light, followed by subsequent crosslinking to the targeting proteins in the microenvironment where the probe and proteins are in close proximity.

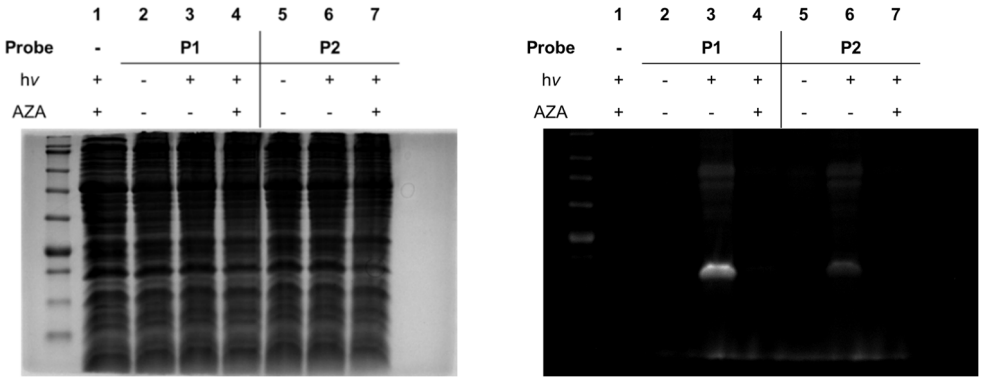
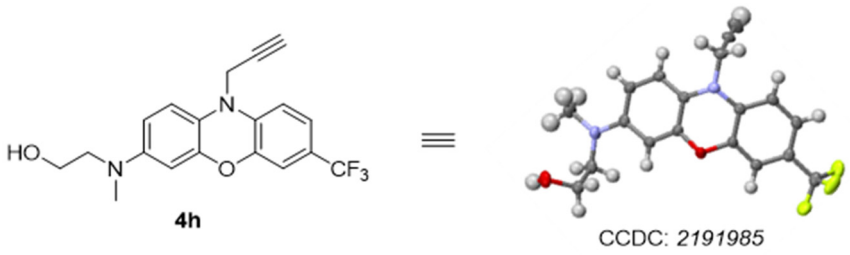


Figure S50. Photoaffinity labeling of the recombinant BRD4 in *E. coli* lysate with **P3**/**P4** (20 μM) through the photo-DAFEx chemistry in PBS buffer (pH = 7.4) under 405 nm LED (21.0 mW cm^{-2}) irradiation for 20 seconds. Native (+)-JQ1 (200 μM) was used as a competitive inhibitor. The fluorophore (Alexa-647) decoration of the photoaffinity labeled proteins was achieved by using CuAAc with an N_3-Alexa-647 conjugate (left: CBB staining; right: in-gel fluorescence).



The colorless crystal in rod-shape, with approximate dimensions of 0.063 × 0.076 × 0.630 mm^3, was selected and mounted for the single-crystal X-ray diffraction. The data set was collected by Bruker D8 Venture Photon II diffractometer at 173(2)K equipped with micro-focus Cu radiation source (Kα = 1.54178 Å). Applied with face-indexed numerical absorption correction, the structure solution was solved and refinement was processed by SHELXTL (version 6.14) and OLEX 2.3 program package a, b, c, d. The structure was analyzed by ADDSYM routine implemented in PLATON suite and no higher symmetry was suggested.

Identification code	**4h**
Empirical formula	$C_{19}H_{17}F_3N_2O_2$
Formula weight	362.35
Temperature/K	173
Crystal system	monoclinic
Space group	C 2/c
a/Å	32.3188(11)
b/Å	4.4791 (2)
c/Å	23.8716 (8)
α/°	90
β/°	107.0298 (13)
γ/°	90
Volume/Å3	3304.1(2)
Z	8
ρ_{calcg}/cm^3	1.457
μ/mm^{-1}	1.009
F(000)	1504.0
Crystal size/mm^3	0.063 × 0.076 × 0.630
Radiation	MoKα (λ = 1.54178)
2Θ range for data collection/°	68.274
Index ranges	40, 5, 29
Reflections collected	0.0493 (2990)
Independent reflections	0.1358 (3188)
Goodness-of-fit on F2	1.123
Final R indexes [I >= 2σ (I)]	R1 = 0.0493, wR2 = 0.1358

Figure S51. Data for the **4h** X-ray crystal diffraction analysis and refinement.

5. Methods

5.1 Expression and purification of the recombinant hCA-II

Escherichia coli BL21 (DE3) with the hCA-II expression constructs (plasmid: pET-hCA-II-His6) were grown in 5.0 mL LB broth supplemented with ampicillin (100 μg/mL) overnight at 37 °C. An aliquot of this culture was then used to inoculate a fresh culture (1:100 dilution). When the culture grew to an OD of 0.5, it was supplemented with 50 μM $ZnSO_4$, and protein production was induced with 1 mM IPTG. After 8 h, cells were pelletized in 50 mL conical tubes by centrifugation at 4000 g for 30 min at 4 °C and resuspended in a fresh 3.0 mL of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH = 8.0). The lysates were further treated with ultrasonication in an ice/water bath. Following centrifuge, the supernatants were incubated with 200 μL Ni-NTA resin (Thermo HisPur) at 4 °C for 2 h with gentle shaking. The resin was centrifuged briefly and washed three times with washing buffer. Finally, the protein was eluted with elution buffer. The eluted fractions were collected, concentrated and subjected to buffer exchange to PBS buffer (pH = 7.4) using an Amicon Ultra-15 Centrifugal Filter (10k MWCO, Millipore). The protein purity was analyzed by SDS-PAGE and LC/ESI-MS, and the protein mass was obtained by deconvoluting the raw charge ladder in the MS spectra.

5.2 Mass analysis for PAL of the recombinant hCA-II

Protein stock: hCA-II (130 μM, 4.0 mg/mL) in PBS (pH = 7.4) buffer;
Reactant stocks: **P1/P2** (200 μM) in DMSO;

10 μL of hCA-II stock solution (130 μM, 4 mg/mL) and 10 μL of probe stock solution (200 μM) were mixed in 80 μL of PBS buffer (pH = 7.4) to a final volume of 100 μL. The mixture was incubated for 30 minutes at room temperature in a dark environment, followed by irradiation with a 405 nm LED (21.0 mW cm^{-2}) light source for 20 seconds. The specimens were collected and subjected to LC/ESI-MS mass spectrometry analysis.

5.3 In-gel fluorescence analysis for PAL of the recombinant hCA-II

Protein stock: hCA-II (130 μM, 4.0 mg/mL) in PBS buffer (pH = 7.4);
Probe stocks: **P1/P2** in DMSO with five concentrations of stocks: 20 μM, 50 μM, 100 μM, 200 μM, 500 μM;

10 μL of hCA-II stock solution, 10 μL of the probe stock solution, and 80 μL of PBS buffer (pH = 7.4) were mixed to a total volume of 100 μL, and incubated in the dark for 30 minutes at room temperature. The mixture was exposed to a 405 nm LED light source (21.0 mW cm^{-2}) in Eppendorf tubes for varying durations or at concentrations required for screening, again at room temperature. Fluorophore decoration of the labeled protein was achieved using CuAAc with an N_3-Alexa-647 conjugate. The specimens were collected, resolved and analyzed by SDS-PAGE.

5.4 Enzymatic activity assay of the recombinant hCA-II

The enzymatic activity of hCA-II can be determined through the catalytic hydrolysis of *p*-nitrophenyl acetate into *p*-nitrophenol, which exhibits strong absorbance at 405 nm. A mixture of the recombinant hCA-II (10 μM) and *p*-nitrophenyl acetate (2.5 mM) was incubated in a 96-well plate, in the presence of various concentrations of inhibitor, in PBS buffe (pH = 7.4) (final volume:

100 μL). Following incubation at 25 °C for 30 minutes, the absorbance at 405 nm was recorded by using a multimode microplate reader (iMark, BIO-RAD). The percentage inhibition was calculated relative to the maximal activity measured in the presence/absence of the inhibitors. The IC_{50} value was derived from an inhibition plot of the percent inhibition versus inhibitor concentration, utilizing the GraphPad Prism software.

5.5 Photoaffinity-labeling of the recombinant hCA-II in bacterial lysate

Protein stock: hCA-II (130 μM, 4 mg/mL) in PBS buffer (pH = 7.4);
Probe stocks: **P1**/**P2** (200 μM) in DMSO, AZA (2000 μM) in DMSO;

Escherichia coli BL21 (DE3) strains containing the hCA-II expression constructs (plasmid: pET-hCA-II-His6) were grown overnight in 5.0 mL of LB broth supplemented with 100 μg/mL ampicillin at 37 °C. An aliquot of this culture was used to inoculate a fresh 50 mL culture (1:100 dilution). After 8 hours of growth, the cells were harvested by centrifugation in 50 mL conical tubes at 4000 g for 30 minutes at 4 °C. The pelleted cells were then resuspended in 3.0 mL of fresh PBS buffer (pH = 7.4). The resuspended cells in the buffer were further treated with ultrasonication in an ice/water bath. Following centrifugation, the resulting supernatants were collected as cell lysates for further analyses.

40 μL of cell lysates, 3.0 μL of hCA-II solution, 10 μL of the probe solution (**P1**/**P2**, 200 μM), 10 μL of AZA stock (2000 μM), and 37 μL of PBS buffer (pH = 7.4) buffer were mixed as the control groups.

All groups were incubated under darkness for 30 min at room temperature, and treated with a 405 nm LED (21.0 mW cm^{-2}) in eppendorf tubes for 20 s at room temperature. After 30 min incubation, followed by CuAAC reaction with N_3-AlexaFlour 647 fluorescent dye, specimens were collected, resolved and analyzed by SDS-PAGE. The labeled proteins were visualized by illumination with 365 nm UV on a gel imager (ChampChemi^{TM} 610Plus, SAGECREATION).

40 μL of cell lysates, 3.0 μL of hCA-II solution, 10 μL of the probe solution (**P1**/**P2**, 200 μM), and 47 μL of PBS buffer (pH = 7.4) were mixed as the positive test groups.

40 μL of cell lysates, 3.0 μL of hCA-II solution, 10 μL of the probe solution (**P1**/**P2**, 200 μM), 10 μL of AZA stock (2000 μM), and 37 μL of PBS buffer (pH = 7.4) were combined as the competitive inhibition test groups.

All groups were incubated in the dark for 30 minutes at room temperature, and subjected to illumination with a 405 nm LED (21.0 mW cm^{-2}) in eppendorf tubes for 20 seconds at room temperature. The samples were further incubated for an additional 30 minutes to allow for the CuAAC reaction with N_3-Alexa Fluor 647 fluorescent dye. Following the incubation period, specimens were collected and analyzed by SDS-PAGE. The labeled proteins were visualized by illumination with 365 nm UV light on a gel imager (ChampChem^{iTM} 610Plus, SAGECREATION).

5.6 Expression and purification of the recombinant BRD4

Escherichia coli BL21 (DE3) with the BRD4 expression constructs (plasmid: pET-BRD4-His6) were grown in 5.0 mL LB broth supplemented with kanamycin (100 μg/mL) overnight at 37 °C. An aliquot of this culture was then used to inoculate a fresh culture (1:100 dilution). When the culture grew to an OD of 0.5, cells were incubated at 18°C until an OD of 0.8, the protein production was induced with 1 mM IPTG. After 8 h, cells were pelletized in 50 mL conical tubes by centrifugation at 4000 g for 30 min at 4 °C and resuspended in a fresh 3.0 mL of lysis buffer (50 mM NaH_2PO_4,

300 mM NaCl, 10 mM imidazole, pH = 8.0). The lysates were further treated with ultrasonication in an ice/water bath. Following centrifuge, the supernatants were incubated with 200 μL Ni-NTA resin (Thermo HisPur) at 4 °C for 2 h with gentle shaking. The resin was centrifuged briefly and washed three times with washing buffer. Finally, the protein was eluted with elution buffer. The eluted fractions were collected, concentrated and subjected to buffer exchange to PBS buffer (pH = 7.4) using an Amicon Ultra-15 Centrifugal Filter (10k MWCO, Millipore). The protein purity was analyzed by SDS-PAGE and LC/ESI-MS, and the protein mass was obtained by deconvoluting the raw charge ladder in the MS spectra.

5.7 In-gel fluorescence analysis of BRD4 labeling

Protein stock: BRD4 (60 μM, 4 mg/mL) in PBS (pH = 7.4) buffer;
Reactant stocks: **P3**/**P4** (200 μM) in DMSO were given five concentrations stocks: 20 μM, 50 μM, 100 μM, 200 μM, 500 μM;

10 μL of BRD4 stock solution (60 μM, 4 mg/mL) and 10 μL of probe stocks (500 μM) were combined in 80 μL of PBS buffer (pH = 7.4) to make a total volume of 100 μL. The mixture was incubated in the dark at room temperature for 30 minutes. Following incubation, the mixture was irradiated for 20 seconds using a 405 nm LED (21.0 mW cm^{-2}) light source within eppendorf tubes. After 30 minutes of incubation, the sample underwent a CuAAC reaction with N_3-AlexaFluor 647 fluorescent dye. The specimens were collected, resolved and analyzed by SDS-PAGE.

5.8 In-gel fluorescence analysis of photoaffinity-labeling the recombinant BRD4 in bacterial lysate

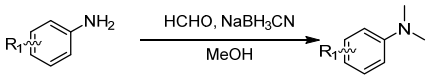
Protein stock: BRD4 (130 μM, 4.0 mg/mL) in PBS buffer (pH = 7.4);
Probe stocks: **P3**/**P4** (200 μM) in DMSO; (+)-JQ1 (2000 μM) in DMSO;

40 μL of cell lysates, 5.0 μL of BRD4 protein solution, 10 μL of the probe solution (**P3**/**P4**, 200 μM), and 45 μL of PBS buffer (pH = 7.4) as the test group. 40 μL of cell lysates, 5 μL of BRD4 solution, 10 μL of the same probe solution (**P3**/**P4**, 200 μM), 10 μL of AZA stock (2000 μM), and 35 μL of PBS buffer (pH = 7.4). All groups were incubated in the dark at room temperature for 30 minutes, then treated with a 405 nm LED light source (21.0 mW cm^{-2}) for 20 seconds in eppendorf tubes at room temperature. Following an additional 30 minutes of incubation, the samples underwent a CuAAC reaction with N_3-AlexaFluor 647 fluorescent dye. The specimens were collected, resolved and analyzed by SDS-PAGE. The photo-labeled proteins were visualized by illumination with 365 nm UV light on a gel imager (ChampChem^{iTM} 610Plus, SAGECREATION).

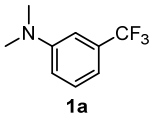
6. Synthesis procedures and compounds characterization

General Procedure for the TFMA reagent

Procedure I:



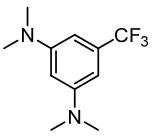
A mixture of trifluoromethylaniline derivatives (2.0 mmol, 1.0 equiv.), 40% aqueous solution of formaldehyde (758 μL, 20.0 mmol, 10.0 equiv.), and sodium cyanoborohydride (252 mg, 4.0 mmol, 2.0 equiv.) in anhydrous methanol was prepared. To this mixture, a few drops of acetic acid were added, and the solution was stirred overnight at room temperature. The reaction was quenched by the addition of water (20 mL) and subsequently extracted with ethyl acetate three times (30 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (PE/EA = 10/1) to yield the corresponding products **1a-1e**.



1a

***N,N*-dimethyl-3-(trifluoromethyl)aniline** (**1a**):

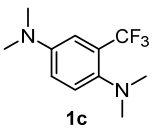
1a was synthesized as a yellow oil using **Procedure I** (309 mg, 82 %). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.31 (ddt, J = 8.4, 7.6, 0.6 Hz, 1H), 6.94 (ddd, J = 7.4, 1.6, 0.8 Hz, 1H), 6.89 (d, J = 1.8 Hz, 1H), 6.85 (dd, J = 8.4, 2.6 Hz, 1H), 2.99 (s, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 150.46, 131.36 (q, J = 31.3 Hz), 129.43, 124.57 (q, J = 272.1 Hz), 115.18, 112.67 (q, J = 4.2 Hz), 108.47 (q, J = 3.8 Hz), 40.37. **^{19}F NMR (376 MHz, $CDCl_3$)** δ -63.79. **HRMS (ESI)** calculated $C_9H_{11}F_3N^+$ $[M+H]^+$ m/z 190.0844, found 190.0836.



1b

***N*^{1},*N*^{1},*N*^{3},*N*^{3}-tetramethyl-5-(trifluoromethyl)benzene-1,3-diamine (1b)**:

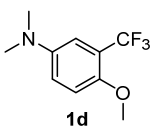
1b was synthesized as a white solid using **Procedure I** (338 mg, 73 %). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 6.37 (d, J = 2.2 Hz, 2H), 6.13 (t, J = 2.2 Hz, 1H), 2.99 (s, 12H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 151.62, 131.85 (q, J = 30.8 Hz), 124.89 (q, J = 272.6 Hz), 99.02 (d, J = 1.4 Hz), 98.21 (q, J = 4.0 Hz), 40.60. **^{19}F NMR (376 MHz, $CDCl_3$)** δ -62.81. **HRMS (ESI)** calculated $C_{11}H_{16}F_3N_2^+$ $[M+H]^+$ m/z 233.1260, found 233.1264.



1c

***N*^{1},*N*^{1},*N*^{4},*N*^{4}-tetramethyl-2-(trifluoromethyl)benzene-1,4-diamine (1c)**:

1c was synthesized as a white solid using **Procedure I** (371 mg, 80 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.32 (d, J = 8.8 Hz, 1H), 6.93 (d, J = 3.1 Hz, 1H), 6.86 (dd, J = 8.9, 3.1 Hz, 1H), 2.96 (s, 6H), 2.65 (s, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 147.61, 142.97, 127.54 (q, J = 28.0 Hz), 124.48, 124.29 (q, J = 273.4 Hz), 116.28, 110.18 (q, J = 5.8 Hz), 46.55, 40.71. **^{19}F NMR (376 MHz, $CDCl_3$)** δ -60.69. **HRMS (ESI)** calculated $C_{11}H_{16}F_3N_2^+$ $[M+H]^+$ m/z 233.1260, found 233.1257.

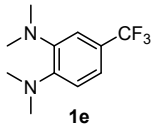


1d

4-methoxy-*N,N*-dimethyl-3-(trifluoromethyl)aniline (4-1d):

1d was synthesized as a yellow oil using **Procedure I** (341 mg, 78 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.00 – 6.92 (m, 2H), 6.87 (dd, J = 9.0, 3.1 Hz, 1H), 3.84 (s, 3H), 2.91 (s, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 149.22 (d, J = 1.8 Hz),

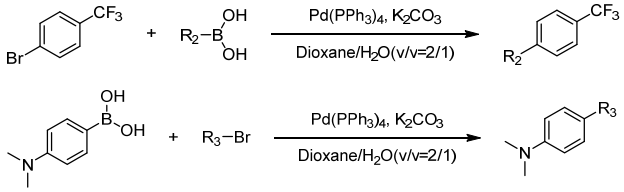
144.97, 123.92 (q, J = 272.5 Hz), 119.25 (q, J = 30.1 Hz), 117.31, 114.06, 111.82 (q, J = 5.5 Hz), 56.78, 41.32. **^{19}F NMR (376 MHz, CDCl$_3$)** δ -62.08. **HRMS (ESI)** calculated $C_{10}H_{13}F_3NO^+$ $[M+H]^+$ m/z 220.0944, found 220.0940.



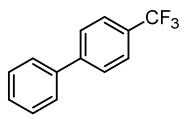
1e

***N*^{1},*N*^{1},*N*^{2},*N*^{2}-tetramethyl-4-(trifluoromethyl)benzene-1,2-diamine (1e)**:
1e was synthesized as a white solid using **Procedure I** (370 mg, 80 % yield). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.15 (dd, J = 8.3, 2.1 Hz, 1H), 7.07 (d, J = 2.1 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 2.84 (s, 6H), 2.79 (s, 6H). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 147.61, 144.55, 124.89 (q, J = 271.2 Hz), 122.76 (q, J = 32.0 Hz), 118.53 (q, J = 4.0 Hz), 117.23, 114.58 (q, J = 3.6 Hz), 40.83 (d, J = 13.6 Hz). **^{19}F NMR (376 MHz, CDCl$_3$)** δ -61.47. **HRMS (ESI)** calculated $C_{11}H_{16}F_3N_2^+$ $[M+H]^+$ m/z 233.1260, found 233.1255.

Procedure II:

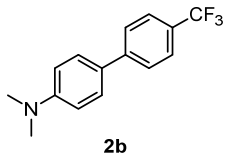


Arylboronic acid compounds (2.0 mmol, 1.0 equiv.), bromo-substituted aromatic compounds (2.0 mmol, 1.0 equiv.), *tetrakis*(triphenylphosphine)palladium (115.6 mg, 0.1 mmol, 0.05 equiv.), and K_2CO_3 (552 mg, 4.0 mmol, 2.0 equiv.) were stirred in a mixed solvent of 1,4-dioxane/H_2O (v/v = 2/1, total 15 mL) overnight at 85°C under a nitrogen atmosphere. Water (30 mL) was added to the reaction mixture, which was then extracted with ethyl acetate three times (50 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (PE/EA = 10/1) to yield the corresponding products **2a-2g**.



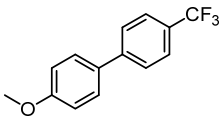
2a

4-(trifluoromethyl)-1,1'-biphenyl (2a):
2a was synthesized as a white solid using **Procedure II** (337 mg, 76 % yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 7.89 – 7.83 (m, 2H), 7.81 – 7.76 (m, 2H), 7.74 – 7.68 (m, 2H), 7.55 – 7.47 (m, 2H), 7.46 – 7.40 (m, 1H).**^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 144.62, 139.07, 129.54, 128.87, 128.82, 128.35 (q, J = 31.9 Hz), 128.24 (q, J = 222.5 Hz) 127.90, 127.90, 127.84, 127.48, 126.17 (q, J = 3.9 Hz), 123.48, 120.77, 40.63, 40.42, 40.21, 40.00, 39.79, 39.58, 39.37. **^{19}F NMR (376 MHz, DMSO-*d*$_6$)** δ -61.05. **HRMS (ESI)** calculated $C_{13}H_{10}F_3^+$ $[M+H]^+$ m/z 223.0729, found 223.0727.



2b

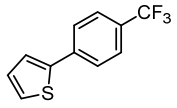
***N*,*N*-dimethyl-4'-(trifluoromethyl)-[1,1'-biphenyl]-4-amine (2b)**:
2b was synthesized as a white solid using **Procedure II** (429 mg, 81 % yield). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.69 – 7.60 (m, 4H), 7.55 – 7.50 (m, 2H), 6.85 – 6.77 (m, 2H), 3.02 (s, 6H). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 150.52, 144.67, 127.88, 127.85 (q, J = 32.2 Hz), 127.30, 126.21, 125.60 (q, J = 3.8 Hz), 124.54 (q, J = 271.7 Hz), 112.64, 40.43. **^{19}F NMR (376 MHz, CDCl$_3$)** δ -62.19. **HRMS (ESI)** calculated $C_{15}H_{15}F_3N^+$ $[M+H]^+$ m/z 266.1151, found 266.1151.



2c

4-methoxy-4'-(trifluoromethyl)-1,1'-biphenyl (2c):

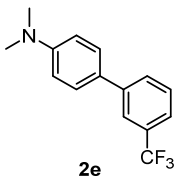
2c was synthesized as a yellow oil using **Procedure II** (398 mg, 79 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 7.86 – 7.79 (m, 2H), 7.78 – 7.72 (m, 2H), 7.72 – 7.64 (m, 2H), 7.10 – 7.00 (m, 2H), 3.81 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 160.15, 144.24 (d, J = 1.5 Hz), 131.26, 128.68, 127.55 (q, J = 31.8 Hz), 127.24, 126.14 (q, J = 3.8 Hz), 124.92 (d, J = 271.8 Hz), 115.01, 55.69. **^{19}F NMR (376 MHz, DMSO)** δ -60.86. **HRMS (ESI)** calculated $C_{14}H_{12}F_3O^+$ $[M+H]^+$ m/z 253.0835, found 253.0827.



2d

2-(4-(trifluoromethyl)phenyl)thiophene (2d):

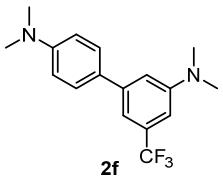
2d was synthesized as a white solid using **Procedure II** (378 mg, 83 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 7.91 – 7.84 (m, 2H), 7.78 – 7.73 (m, 2H), 7.70 – 7.65 (m, 2H), 7.19 (dd, J = 5.0, 3.7 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 141.90, 138.08, 129.29, 128.01 (q, J = 32.1 Hz), 127.92, 126.50 (q, J = 3.8 Hz), 126.34, 126.06, 121.99 (q, J = 271.8 Hz). **^{19}F NMR (376 MHz, DMSO)** δ -61.03. **HRMS (ESI)** calculated $C_{11}H_7F_3NaS^+$ $[M+Na]^+$ m/z 251.0113, found 251.0122.



2e

***N,N*-dimethyl-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-amine (2e)**:

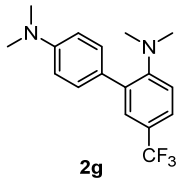
2e was synthesized as a white solid using **Procedure II** (424 mg, 80 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.84 – 7.80 (m, 1H), 7.79 – 7.69 (m, 1H), 7.56 – 7.49 (m, 4H), 3.03 (s, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 150.44, 142.01, 131.03 (q, J = 31.7 Hz), 129.40, 129.10, 127.79, 127.46, 124.43 (q, J = 272.1 Hz), 122.89 (q, J = 3.8 Hz), 122.53 (q, J = 3.9 Hz), 112.73, 40.46. **^{19}F NMR (376 MHz, $CDCl_3$)** δ -62.55. **HRMS (ESI)** calculated $C_{15}H_{15}F_3N^+$ $[M+H]^+$ m/z 266.1151, found 266.1154.



2f

***N^3,N^3,$N^{4'}$,$N^{4'}$*-tetramethyl-5-(trifluoromethyl)-[1,1'-biphenyl]-3,4'-diamine (2f)**:

2f was synthesized as a white solid using **Procedure II** (511 mg, 83 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.56 – 7.47 (m, 2H), 7.15 (s, 1H), 7.01 (t, J = 2.0 Hz, 1H), 6.85 – 6.78 (m, 3H), 3.05 (s, 6H), 3.01 (s, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 150.84, 150.32, 142.90, 131.63 (q, J = 31.1 Hz), 128.88, 127.91, 124.72 (q, J = 272.6 Hz), 113.21, 112.68, 111.34 (q, J = 3.9 Hz), 106.47 (q, J = 4.0 Hz), 40.57, 40.55. **^{19}F NMR (376 MHz, $CDCl_3$)** δ -62.59. **HRMS (ESI)** calculated $C_{17}H_{20}F_3N_2^+$ $[M+H]^+$ m/z 309.1573, found 309.1569.



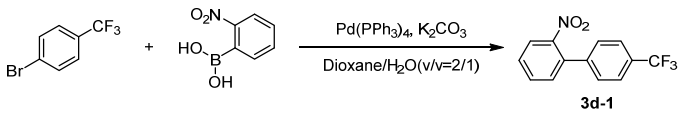
2g

***N^2,N^2,$N^{4'}$,$N^{4'}$*-tetramethyl-4-(trifluoromethyl)-[1,1'-biphenyl]-2,4'-diamine (2g)**:

2g was synthesized as a yellow solid using **Procedure II** (468 mg, 76 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.50 – 7.45 (m, 2H), 7.26 (t, J = 8.0 Hz, 1H), 7.22 – 7.16 (m, 2H), 6.79 – 6.75 (m, 2H), 2.99 (s, 6H), 2.58 (s, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 151.51, 149.70, 137.42, 131.65, 129.07, 129.02 (q, J = 31.8 Hz), 128.57 (q, J = 4.6 Hz), 124.54 (q, J = 272.1 Hz), 117.82 (q, J = 4.0 Hz), 114.03 (q, J = 3.7 Hz), 112.36, 42.93, 40.49. **^{19}F NMR (376 MHz, $CDCl_3$)** δ -62.29. **HRMS (ESI)** calculated $C_{17}H_{20}F_3N_2^+$ $[M+H]^+$ m/z

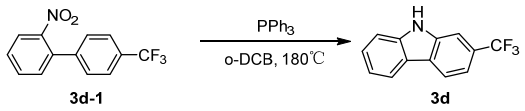
309.1573, found 309.1569.

Procedure III:



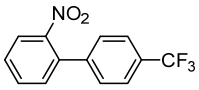
3d-1

1-Bromo-4-(trifluoromethyl)benzene (447 mg, 2.0 mmol, 1.0 equiv.), (2-nitrophenyl)boronic acid (334 mg, 2.0 mmol, 1.0 equiv.), *tetrakis*(triphenylphosphine)palladium (115 mg, 0.1 mmol, 0.05 equiv.), and K_2CO_3 (552 mg, 4.0 mmol, 2.0 equiv.) were stirred in a mixed solvent of 1,4-dioxane/H_2O (v/v = 2/1, total 15 mL) overnight at 85°C under a nitrogen atmosphere. Water (50 mL) was added to the reaction mixture, which was then extracted with ethyl acetate three times (50 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (PE/EA = 20/1) to yield the corresponding product **3d-1** (83% yield).



3d-1 **3d**

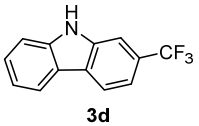
3d-1 (534 mg, 2.0 mmol, 1.0 equiv.) and triphenylphosphine (1.310 g, 5.0 mmol, 2.5 equiv.) were stirred in anhydrous 1,2-dichlorobenzene (20 mL) at 185°C under a nitrogen atmosphere for 15 hours. The reaction was then quenched by the addition of water (30 mL) and extracted with ethyl acetate (50 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (PE/EA = 10/1) to afford the corresponding product **3d** (56% yield).



3d-1

2-nitro-4'-(trifluoromethyl)-1,1'-biphenyl (3d-1):

3d-1 was synthesized as a yellow solid using **Procedure III** (443 mg, 83 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.16 – 8.11 (m, 1H), 7.88 (m, 1H), 7.61 (dd, J = 8.1, 1.0 Hz, 1H), 7.50 – 7.44 (m, 3H), 7.35 – 7.30 (m, 2H). **HRMS (ESI)** calculated $C_{13}H_9F_3NO_2^+$ $[M+H]^+$ m/z 268.0580, found 268.0577.

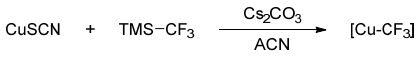


3d

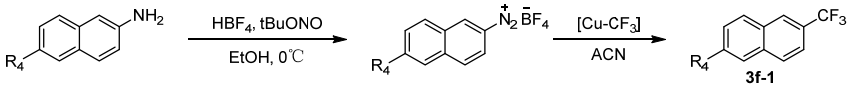
2-(trifluoromethyl)-9*H*-carbazole (3d):

3d was synthesized as a yellow solid using **Procedure III** (263 mg, 56 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 11.62 (s, 1H), 8.33 (dd, J = 8.1, 1.1 Hz, 1H), 8.23 (dd, J = 7.9, 1.1 Hz, 1H), 7.82 (dt, J = 1.7, 0.8 Hz, 1H), 7.59 (dt, J = 8.2, 1.0 Hz, 1H), 7.48 (m, 2H), 7.24 (m, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 141.27, 139.21, 127.50, 126.05 (q, J = 31.2 Hz), 125.78, 125.55 (q, J = 271.8 Hz), 121.87, 121.52, 121.50, 119.78, 115.23 (q, J = 3.7 Hz), 111.97, 108.42 (q, J = 4.4 Hz). **^{19}F NMR (376 MHz, DMSO)** δ -59.25. **HRMS (ESI)** calculated $C_{13}H_9F_3N^+$ $[M+H]^+$ m/z 236.0682, found 236.0691.

Procedure IV:

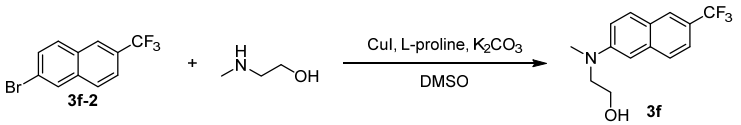


Trifluoromethyl)trimethylsilane (852 mg, 6.0 mmol, 3.0 equiv.) was dissolved in anhydrous acetonitrile (15 mL). Subsequently, cuprous thiocyanate (244 mg, 2.0 mmol, 1.0 equiv.) and cesium carbonate (1.950 g, 6.0 mmol, 3.0 equiv.) were slowly added to the solution at room temperature, and the resulting mixture was stirred for 10 minutes under a nitrogen atmosphere for protection.

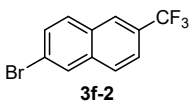


A mixture of naphthalen-2-amine or 6-bromonaphthalen-2-amine (2.0 mmol, 1.0 equiv.) and 40% fluoroboric acid aqueous solution (460 μL, 4.0 mmol, 2.0 equiv.) in ethanol (10 mL) was prepared. Tert-butyl nitrite (476 μL, 4.0 mmol, 2.0 equiv.) was then added to this mixture at 0°C. The resulting solution was stirred in an ice-water bath for 1 hour. Subsequently, diethyl ether (20 mL) was added, brownish-red production was precipitated. The suspension was filtered, and the solid residue was washed with diethyl ether. The resulting diazonium salt was dried under vacuum for further use.

The diazonium salt solid, obtained from the reaction in the previous step, was dissolved in anhydrous acetonitrile. This solution was then slowly added to the reaction mixture, which was subsequently stirred for 16 hours at room temperature. The reaction was quenched by the addition of water (30 mL) and the mixture was extracted with ethyl acetate three times (50 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (PE/EA = 10/1) as the eluent to afford the corresponding product **3f-1**.

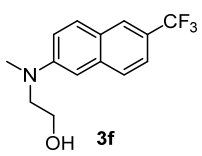


3f-2 (273 mg, 2.0 mmol, 1.0 equiv.), 2-(methylamino)ethan-1-ol (184 mg, 3.0 mmol, 1.5 equiv.), potassium carbonate (552 mg, 4.0 mmol, 2.0 equiv.), cuprous iodide (39 mg, 0.2 mmol, 0.1 equiv.), and *L*-proline (46 mg, 0.4 mmol, 2.0 equiv.) were dissolved in anhydrous DMSO (10 mL) under a nitrogen atmosphere. The mixture was stirred and heated to 80 °C, where it was allowed to react overnight. After completion, the reaction was quenched by the addition of water (30 mL) and the mixture was extracted with ethyl acetate three times (50 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting concentrate was purified by silica gel chromatography (PE/EA = 3/1) as the eluent to afford the product **3f** (53% yield).



2-bromo-6-(trifluoromethyl)naphthalene (3f-2):

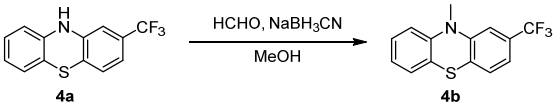
3f-2 was synthesized as a white solid using **Procedure IV** (225 mg, 48 % yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 8.44 (d, J = 1.8 Hz, 1H), 8.32 (d, J = 2.0 Hz, 1H), 8.05 (d, J = 5.7 Hz, 1H), 8.03 (d, J = 5.9 Hz, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H).



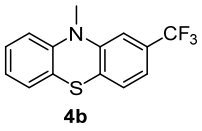
2-(methyl(6-(trifluoromethyl)naphthalen-2-yl)amino)ethan-1-ol (3f):

3f was synthesized as a yellow solid using **Procedure IV** (285 mg, 48 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.16 – 8.04 (m, 1H), 7.88 (d, J = 9.1 Hz, 1H), 7.83 – 7.75 (m, 1H), 7.50 (dd, J = 8.7, 2.0 Hz, 1H), 7.32 (dd, J = 9.2, 2.6 Hz, 1H), 6.97 (d, J = 2.6 Hz, 1H), 4.73 (t, J = 5.3 Hz, 1H), 3.61 (m, 2H), 3.55 (m, 2H), 3.07 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 149.44, 136.96, 130.11, 127.25, 125.65 (d, J = 4.6 Hz), 125.53 (q, J = 271.1 Hz), 124.50, 121.62 (q, J = 3.3 Hz), 121.32 (q, J = 28.2 Hz), 117.42, 104.62, 58.78, 54.53, 39.23. **^{19}F NMR (376 MHz, DMSO)** δ -59.84. **HRMS (ESI)** calculated $C_{14}H_{15}F_3NO^+$ $[M+H]^+$ m/z 270.1100, found 270.1098.

Procedure V:



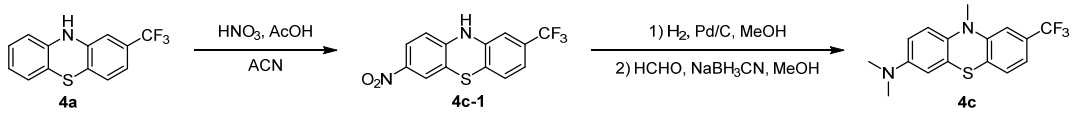
4a (534 mg, 2.0 mmol, 1.0 equiv.) was dissolved in anhydrous MeOH (20 mL), and added 40% aqueous solution of formaldehyde (758 μL, 20.0 mmol, 10.0 equiv.), sodium cyanoborohydride (252 mg, 4.0 mmol, 2.0 equiv.), and a few drops of acetic acid. The mixture was stirred overnight at room temperature. After completion, the reaction was quenched by the addition of water (30 mL) and extracted with ethyl acetate three times (30 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (PE/EA = 10/1) to afford the corresponding product **4b** (70% yield).



10-methyl-2-(trifluoromethyl)-10*H*-phenothiazine (4b):

4b was synthesized as a yellow solid using **Procedure V** (393 mg, 70 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 7.33 (dd, J = 8.0, 0.9 Hz, 1H), 7.28 – 7.21 (m, 2H), 7.16 (dd, J = 7.6, 1.5 Hz, 1H), 7.11 (d, J = 1.8 Hz, 1H), 7.03 – 6.94 (m, 2H), 3.35 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 146.35, 144.96, 128.92 (q, J = 31.9 Hz), 128.63, 128.17, 127.79, 127.37, 124.66 (q, J = 272.3 Hz), 123.60, 121.46, 119.43 (q, J = 4.0 Hz), 115.55, 111.01 (q, J = 3.8 Hz), 35.68. **^{19}F NMR (376 MHz, DMSO)** δ -61.03. **HRMS (ESI)** calculated $C_{14}H_{11}F_3NS^+$ $[M+H]^+$ m/z 282.0559, found 282.0554.

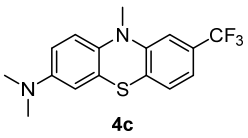
Procedure VI:



4a (534 mg, 2.0 mmol, 1.0 equiv.) and acetic acid (1.15 mL, 20.0 mmol, 10.0 equiv.) were dissolved in acetonitrile (40 mL). Concentrated nitric acid (835 μL, 20.0 mmol, 10.0 equiv.) was added slowly and dropwise to the solution. The mixture was stirred for approximately 30 minutes until complete conversion of **4a** to **4c-1**, monitored by TLC. After completion, the reaction mixture was poured into water (50 mL), and the resulting precipitate was filtered, washed with ethanol, and

recrystallized to afford the intermediate **4c-1**.

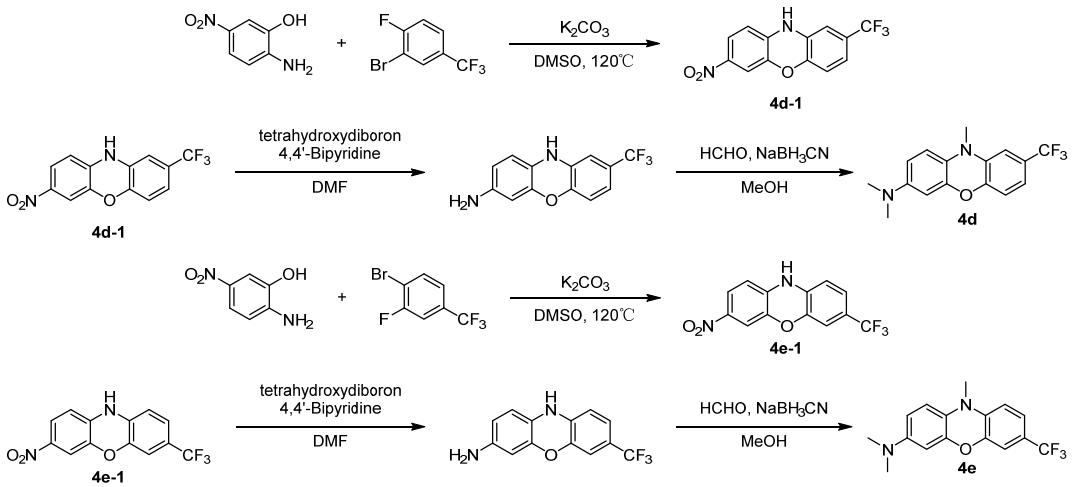
4c-1 (624 mg, 2.0 mmol, 1.0 equiv.) and 10% aqueous Pd/C (22 mg, 0.2 mmol, 0.1 equiv.) were suspended in methanol (10 mL), and the mixture was stirred and reacted under a hydrogen atmosphere at room temperature for approximately 2 hours. After complete conversion was confirmed by TLC, the Pd/C was removed by filtration. The filtrate was subsequently treated with the addition of 40% formaldehyde solution (758 μL, 20.0 mmol, 10.0 equiv.), sodium cyanoborohydride (252 mg, 4.0 mmol, 2.0 equiv.), and several drops of acetic acid. The resulting mixture was stirred overnight. The reaction was quenched by the addition of water (30 mL) and extracted with ethyl acetate three times (50 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/EA = 10/1) to afford the corresponding product **4c** (72% yield).



4c

***N,N*,10-trimethyl-8-(trifluoromethyl)-10*H*-phenothiazin-3-amine (4c)**: **4c** was synthesized as a yellow solid using **Procedure VI** (466 mg, 72 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.19 (dd, J = 8.0, 0.9 Hz, 1H), 7.11 (m, 1H), 6.92 (d, J = 1.7 Hz, 1H), 6.77 – 6.72 (m, 1H), 6.62 – 6.57 (m, 2H), 3.35 (s, 3H), 2.88 (s, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 147.50, 146.89, 135.50, 129.59 (q, J = 32.2 Hz), 127.99, 127.03, 124.28 (q, J = 272.2 Hz), 123.30, 118.25 (q, J = 4.0 Hz), 114.96, 112.27, 112.10, 109.82 (q, J = 3.8 Hz), 41.14, 35.21. **^{19}F NMR (376 MHz, $CDCl_3$)** δ -62.50. **HRMS (ESI)** calculated $C_{16}H_{16}F_3N_2S^+$ $[M+H]^+$ m/z 325.0981, found 325.0983.

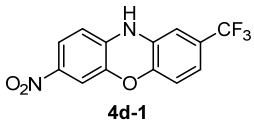
Procedure VII:



2-amino-5-nitrophenol (308 mg, 2.0 mmol, 1.0 equiv.) was reacted with 2-bromo-1-fluoro-4-(trifluoromethyl)benzene (482 mg, 2.0 mmol, 1.0 equiv.) or 1-bromo-2-fluoro-4-(trifluoromethyl)benzene (482 mg, 2.0 mmol, 1.0 equiv.), along with potassium carbonate (552 mg, 4.0 mmol, 2.0 equiv.), in DMSO (15 mL). The mixture was heated to 120 °C and stirred for 12 hours under a nitrogen atmosphere. After completion, the reaction was quenched by the addition of water (30 mL), ethyl acetate was then added, and the mixture was washed with saturated saline solution three times (20 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting concentrate was purified by silica gel chromatography (PE/EA = 2/1) to afford the intermediate **4d-1** or **4e-1** (55% yield / 67% yield).

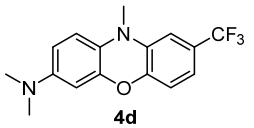
4d-1 or **4e-1** (592 mg, 2.0 mmol, 1.0 equiv.), was reacted with *tetra*hydroxydiboron (541 mg, 6.0 mmol, 3.0 equiv.) and 4,4'-bipyridine (156 mg, 1.0 mmol, 0.5 equiv.) in DMF (10 mL). The mixture was stirred at room temperature for approximately 5 minutes. Completion of the reaction was confirmed by TLC monitoring. The mixture was then quenched by the addition of water (5 mL), and the product was extracted with ethyl acetate (20 mL × 2). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure.

To the resulting residue, 15 mL of anhydrous MeOH, 40% formaldehyde solution (758 μL, 20.0 mmol, 10.0 equiv.), sodium cyanoborohydride (252 mg, 4.0 mmol, 2.0 equiv.), and several drops of acetic acid were added. The mixture was stirred overnight. The reaction was quenched by the addition of water (30 mL) and extracted with ethyl acetate three times (50 mL each). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/EA = 10/1) to afford the corresponding product **4d** or **4e** (66% yield / 71% yield)



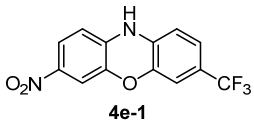
4d-1

7-nitro-2-(trifluoromethyl)-10*H*-phenoxazine (4d-1): **4d-1** was synthesized as a red solid using **Procedure VII** (325 mg, 55 % yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 9.50 (s, 1H), 7.66 (dd, J = 8.7, 2.5 Hz, 1H), 7.30 (d, J = 2.5 Hz, 1H), 6.97 (ddd, J = 8.3, 2.2, 0.9 Hz, 1H), 6.75 (d, J = 8.3 Hz, 1H), 6.67 (d, J = 2.2 Hz, 1H), 6.49 (d, J = 8.7 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 145.69, 142.25, 140.52, 138.98, 131.18, 125.48 (q, J = 32.2 Hz), 124.17 (q, J = 271.6 Hz), 122.58, 119.99 (q, J = 4.3 Hz), 116.23, 112.88, 110.82 (q, J = 3.6 Hz), 110.78. **^{19}F NMR (376 MHz, DMSO-*d*$_6$)** δ -61.46. **HRMS (ESI)** calculated $C_{13}H_8F_3N_2O_3^+$ $[M+H]^+$ m/z 297.0482, found 297.0482



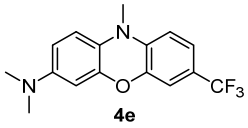
4d

***N,N*,10-trimethyl-8-(trifluoromethyl)-10*H*-phenoxazin-3-amine (4d)**: **4d** was synthesized as a yellow solid using **Procedure VII** (406 mg, 66 % yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 6.85 – 6.79 (m, 1H), 6.70 – 6.63 (m, 2H), 6.49 (d, J = 9.1 Hz, 1H), 6.13 (d, J = 2.3 Hz, 2H), 2.87 (s, 3H), 2.65 (s, 6H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 142.92, 141.89, 140.65, 123.57, 121.16 (q, J = 32.0 Hz), 120.87, 119.72, 116.83 (q, J = 271.6 Hz), 112.46, 110.16, 107.48, 102.71, 97.38, 36.36, 26.18. **^{19}F NMR (376 MHz, DMSO)** δ -67.06. **HRMS (ESI)** calculated $C_{16}H_{16}F_3N_2O^+$ $[M+H]^+$ m/z 309.1209 found 309.1201.



4e-1

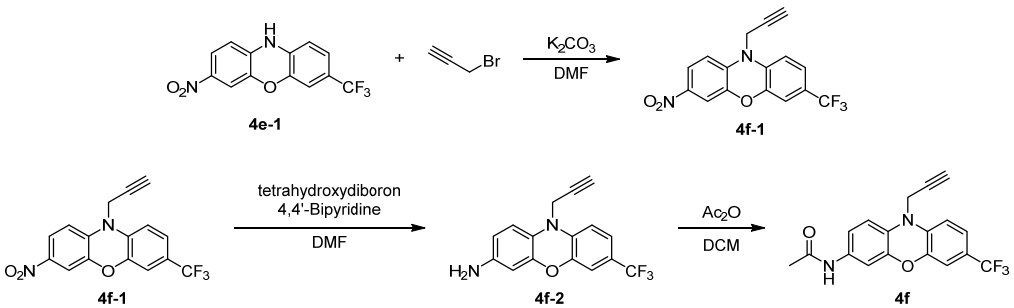
3-nitro-7-(trifluoromethyl)-10*H*-phenoxazine (4e-1): **4e-1** was synthesized as a red solid using **Procedure VII** (396 mg, 67 % yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 9.65 (s, 1H), 7.65 (dd, J = 8.7, 2.5 Hz, 1H), 7.25 (d, J = 2.6 Hz, 1H), 7.08 (dd, J = 8.3, 2.0 Hz, 1H), 6.83 (s, 1H), 6.57 (d, J = 8.2 Hz, 1H), 6.49 (d, J = 8.7 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 142.83, 142.45, 140.75, 138.79, 134.03, 126.96 (q, J = 271.0 Hz), 122.75 (q, J = 33.0 Hz), 122.44 (q, J = 4.3 Hz), 122.36, 114.55, 113.05, 112.44 (q, J = 3.7 Hz), 110.58. **^{19}F NMR (376 MHz, DMSO)** δ -60.85. **HRMS (ESI)** calculated $C_{13}H_8F_3N_2O_3^+$ $[M+H]^+$ m/z 297.0482, found 297.0481.



4e

***N,N*,10-trimethyl-7-(trifluoromethyl)-10*H*-phenoxazin-3-amine (4e)**: **4e** was synthesized as a yellow solid using **Procedure VII** (437 mg, 71 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.07 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.86 (d, *J* = 2.1 Hz, 1H), 6.46 (dd, *J* = 8.7, 3.6 Hz, 2H), 6.26 (d, *J* = 2.2 Hz, 2H), 3.02 (s, 3H), 2.86 (s, 6H). **^{13}C NMR (101 MHz, $CDCl_3$)** *δ* 146.89, 145.61, 144.88, 138.42, 124.25 (q, *J* = 270.7 Hz), 124.16, 121.54 (q, *J* = 33.2 Hz), 121.17 (q, *J* = 4.2 Hz), 112.36, 112.06 (q, *J* = 3.9 Hz), 110.16, 107.50, 102.03, 41.07, 30.97. **^{19}F NMR (376 MHz, $CDCl_3$)** *δ* -61.89. **HRMS (ESI)** calculated $C_{16}H_{16}F_3N_2O^+$ $[M+H]^+$ m/z 309.1209, found 309.1203.

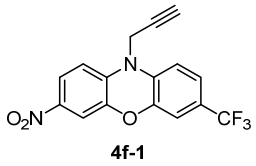
Procedure VIII:



4e-1 (592 mg, 2.0 mmol, 1.0 equiv.), potassium carbonate (552 mg, 4.0 mmol, 2.0 equiv.), and propargyl bromide (344 μL, 4.0 mmol, 2.0 equiv.) were sequentially added to DMF (10 mL). The mixture was stirred at room temperature overnight. After completion of the reaction, the mixture was quenched by the addition of water (30 mL). Ethyl acetate was then added, and the resulting mixture was extracted with saturated brine (50 mL × 3). The organic layer was separated, washed with saturated brine three times (50 mL each), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product obtained was purified by silica gel chromatography (PE/EA = 2/1) to afford the intermediate **4f-1** (80% yield).

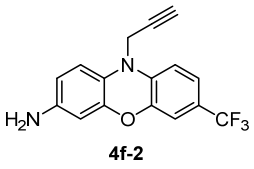
4f-1 (668 mg, 2.0 mmol, 1.0 equiv.), *tetra*hydroxydiboron (541 mg, 6.0 mmol, 3.0 equiv.), and 4,4'-Bipyridine (156 mg, 1.0 mmol, 0.5 equiv.) were dissolved in DMF (5 mL). The mixture was stirred for about 5 minutes at room temperature and monitored by TLC until the conversion was complete. The mixture was filtered, and the residue was washed with ethyl acetate (20 mL × 2). The filtrate was concentrated under vacuum, and the residue was purified by silica gel chromatography (PE/EA = 2/1) to afford the corresponding product **4f-2** (55% yield).

4f-2 (608 mg, 2.0 mmol, 1.0 equiv.) was dissolved in DCM (20 mL). Acetic anhydride (1.962 mL, 20.0 mmol, 10.0 equiv.) was added, and the mixture was stirred for about 4 hours at room temperature and monitored by TLC until the conversion was complete. The reaction was quenched with water (30 mL) and extracted with ethyl acetate three times (50 mL each). The organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by silica gel chromatography (PE/EA = 2/1) to afford the corresponding product 4f (66% yield).



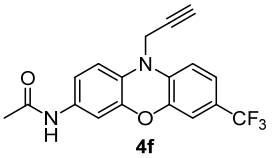
4f-1

3-nitro-10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10H-phenoxazine (4f-1): **4f-1** was synthesized as a red solid using **Procedure VIII** (534 mg, 71 % yield). **^{1}H NMR (400 MHz, CDCl$_3$)** δ 7.87 (dd, J = 8.9, 2.5 Hz, 1H), 7.58 (d, J = 2.5 Hz, 1H), 7.22 (m, J = 8.4, 2.0, 0.9 Hz, 1H), 7.00 (d, J = 2.1 Hz, 1H), 6.81 (dd, J = 8.5, 1.0 Hz, 1H), 6.74 (d, J = 8.9 Hz, 1H), 4.35 (d, J = 2.5 Hz, 2H), 2.36 (t, J = 2.4 Hz, 1H). **^{13}C NMR (101 MHz, CDCl$_3$)** δ 144.87, 144.85, 142.57, 138.34, 134.25, 130.48 (q, J = 271.6 Hz), 125.69 (q, J = 33.6 Hz), 121.97 (q, J = 4.2 Hz), 121.20, 113.21 (q, J = 3.7 Hz), 112.60, 111.42, 111.28, 75.81, 74.02, 34.86. **^{19}F NMR (376 MHz, CDCl$_3$)** δ -62.54. **HRMS (ESI)** calculated $C_{16}H_{10}F_3N_2O_3^+$ $[M+H]^+$ m/z 335.0638, found 335.0632.



4f-2

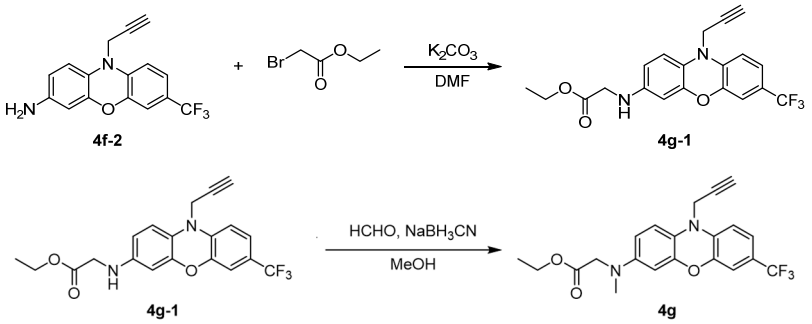
10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10H-phenoxazin-3-amine (4f-2): **4f-2** was synthesized as a gray solid using **Procedure VIII** (334 mg, 55 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 7.23 (dd, J = 8.5, 2.2 Hz, 1H), 7.01 (d, J = 2.1 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 6.61 (d, J = 8.5 Hz, 1H), 6.17 (dd, J = 8.5, 2.5 Hz, 1H), 6.13 (d, J = 2.4 Hz, 1H), 4.91 (s, 2H), 4.43 (d, J = 2.4 Hz, 2H), 3.21 (t, J = 2.2 Hz, 1H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 145.28, 145.08, 144.68, 137.46, 124.65 (q, J = 270.7 Hz), 121.80 (q, J = 4.2 Hz), 121.03, 120.92 (q, J = 32.5 Hz), 114.36, 112.51, 112.24 (q, J = 3.9 Hz), 109.16, 102.62, 79.07, 75.13, 33.67. **^{19}F NMR (376 MHz, DMSO)** δ -60.24. **HRMS (ESI)** calculated $C_{16}H_{12}F_3N_2O^+$ $[M+H]^+$ m/z 305.0896, found 305.0895.



4f

***N*-(10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10*H*-phenoxazin-3-yl)acetamide (4f)**: **4f** was synthesized as a yellow solid using **Procedure VIII** (456 mg, 66 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 9.86 (s, 1H), 7.34 – 7.25 (m, 1H), 7.23 (d, J = 2.3 Hz, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.92 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 8.7 Hz, 1H), 4.68 – 4.32 (m, 2H), 3.25 (d, J = 2.4 Hz, 1H), 2.01 (s, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 168.50, 144.74, 144.20, 136.78, 135.08, 127.07, 124.51 (q, J = 270.7 Hz), 122.11 (q, J = 3.9 Hz), 121.79 (q, J = 28.9 Hz), 114.76, 113.59, 113.06, 112.51 (q, J = 3.4 Hz), 107.43, 78.68, 75.40, 33.70, 24.37. **^{19}F NMR (376 MHz, DMSO)** δ -60.44. **HRMS (ESI)** calculated $C_{18}H_{14}F_3N_2O_2^+$ $[M+H]^+$ m/z 347.1002, found 347.1001.

Procedure IX:



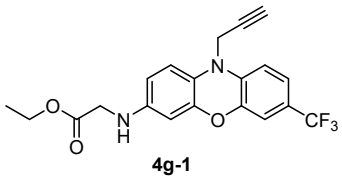
4f-2 4g-1 4g

4f-2 (608 mg, 2.0 mmol, 1.0 equiv.), potassium carbonate (552 mg, 4.0 mmol, 2.0 equiv.), and ethyl bromoacetate (266 μL, 2.4 mmol, 1.2 equiv.) were sequentially added to DMF (10 mL) and

the mixture was stirred at room temperature overnight. The reaction was quenched by water (30 mL), then ethyl acetate was added (30 mL × 3) and washed three times with saturated brine (50 mL × 3), and the organic phase obtained was dried with anhydrous Na_2SO_4 and then concentrated in vacuum. The obtained concentrate was purified by silica gel chromatography (PE/EA = 2/1) to give the intermediate **4g-1** (61 % yield).

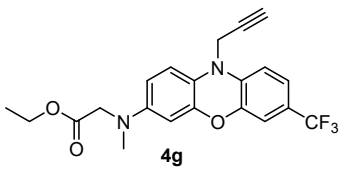
4f-2 (608 mg, 2.0 mmol, 1.0 equiv.), potassium carbonate (552 mg, 4.0 mmol, 2.0 equiv.), and ethyl bromoacetate (266 μL, 2.4 mmol, 1.2 equiv.) were sequentially added to DMF (10 mL). The mixture was stirred at room temperature overnight. After completion of the reaction, the mixture was quenched by the addition of water (30 mL), and the mixture was extracted with saturated brine (30 mL × 3) and ethyl acetate (50 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting concentrate was purified by silica gel chromatography (PE/EA = 2/1) to afford the intermediate **4g-1** (61% yield).

The reaction was progressed by sequentially adding compound **4g-1** (780 mg, 2.0 mmol, 1.0 equiv.), 40% formaldehyde solution (758 μL, 20.0 mmol, 10.0 equiv.), sodium cyanoborohydride (252 mg, 4.0 mmol, 2.0 equiv.), and a few drops of acetic acid to a solution of methanol (10 mL). The resulting mixture was stirred overnight at room temperature. Upon completion of the reaction, the mixture was quenched by the addition of water (30 mL). The reaction mixture was then extracted with ethyl acetate (50 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered to remove any solid residue, and concentrated under reduced pressure. The residue obtained was purified by silica gel chromatography (PE/EA = 2/1) to afford the corresponding product **4g** (77% yield).



4g-1

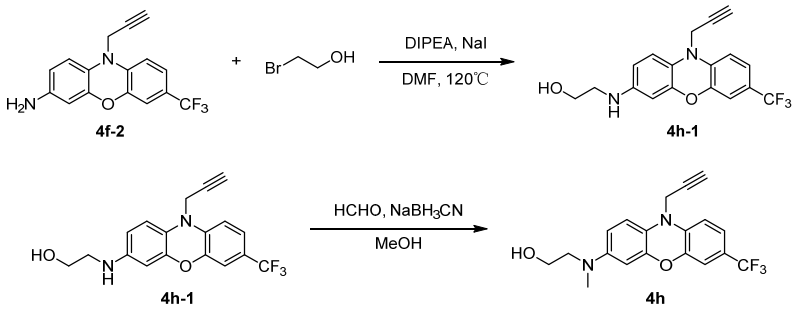
ethyl(10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10*H*-phenoxazin-3-yl)glycinate (4g-1): **4g-1** was synthesized as a yellow solid using **Procedure IX** (475 mg, 61 % yield). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.16 – 7.10 (m, 1H), 6.93 (d, J = 2.1 Hz, 1H), 6.66 (d, J = 8.3 Hz, 1H), 6.59 (d, J = 8.4 Hz, 1H), 6.22 – 6.13 (m, 2H), 4.28 – 4.19 (m, 4H), 3.83 (s, 2H), 2.24 (t, J = 2.4 Hz, 1H), 1.30 (t, J = 7.2 Hz, 3H). **^{13}C NMR (101 MHz, $CDCl_3$)** δ 171.03, 146.10, 145.11, 143.22, 136.64, 124.07 (q, J = 270.9 Hz), 123.62, 122.81 (q, J = 33.2 Hz), 121.25 (q, J = 4.1 Hz), 113.14, 112.54 (d, J = 3.8 Hz), 111.11, 107.61, 102.21, 77.50, 72.64, 61.39, 46.22, 34.24, 14.20. **^{19}F NMR (376 MHz, $CDCl_3$)** δ -62.05. **HRMS (ESI)** calculated $C_{21}H_{20}F_3N_2O_3^+$ $[M+H]^+$ m/z 405.1421 found 405.1420.



4g

ethyl-N-methyl-N-(10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10H-phenoxazin-3-yl)glycinate (4g): **4g** was synthesized as a yellow solid using **Procedure IX** (622 mg, 77 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 7.25 (ddd, J = 8.5, 2.2, 1.0 Hz, 1H), 7.00 (d, J = 2.1 Hz, 1H), 6.91 – 6.85 (m, 1H), 6.74 (d, J = 8.7 Hz, 1H), 6.27 (dd, J = 8.7, 2.9 Hz, 1H), 6.23 (d, J = 2.8 Hz, 1H), 4.47 (d, J = 2.4 Hz, 2H), 4.13 (s, 2H), 4.08 (q, J = 7.1 Hz, 2H), 3.25 – 3.19 (m, 1H), 2.91 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 170.93, 145.89, 145.13, 144.66, 137.23, 124.60 (d, J = 270.8 Hz), 121.92 (q, J = 4.2 Hz), 121.84, 121.19 (q, J = 32.6 Hz), 114.34, 112.68, 112.20 (q, J = 3.7 Hz), 107.56, 101.10, 78.96, 75.17, 60.67, 53.78, 39.45, 33.59, 14.59. **^{19}F NMR (376 MHz, DMSO)** δ -60.33. **HRMS (ESI)** calculated $C_{21}H_{20}F_3N_2O_3^+$ $[M+H]^+$ m/z 405.1421 found 405.1420.

Procedure X:

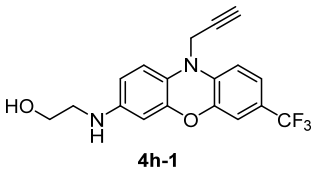


4f-2 (608 mg, 2.0 mmol, 1.0 equiv.), DIPEA (1.043 mL, 6.0 mmol, 3.0 equiv.), and sodium iodide (450 mg, 3.0 mmol, 1.5 equiv.) were added to DMF (15 mL), and the mixture was stirred and reacted for 8h at 120 °C under nitrogen protection. The reaction was quenched with water (10 mL), then ethyl acetate was added (30 mL × 3) and washed three times with saturated saline (50 mL × 3), the organic phase obtained was dried with anhydrous Na_2SO_4 and then concentrated in vacuum. The obtained concentrate was purified by silica gel chromatography (PE/EA = 2/1) to give the intermediate **4h-1** (56 % yield).

4f-2 (608 mg, 2.0 mmol, 1.0 equiv.), DIPEA (1.043 mL, 6.0 mmol, 3.0 equiv.), and sodium iodide (450 mg, 3.0 mmol, 1.5 equiv.) were added to dry DMF (15 mL) in a single-necked flask under nitrogen atmosphere. The mixture was stirred and reacted for 8 h at 120 °C. After cooling to room temperature, the reaction was quenched with water (10 mL). The reaction mixture was then extracted with ethyl acetate (50 mL × 3) and saturated saline solution (50 mL × 3), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (PE/EA = 2/1) to afford the intermediate **4h-1** (56% yield).

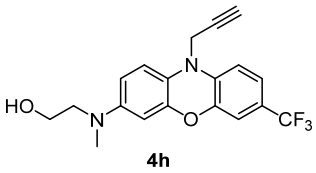
4h-1 (696 mg, 2.0 mmol, 1.0 equiv.), 40% formaldehyde solution (758 μL, 20.0 mmol, 10.0 equiv.), sodium cyanoborohydride (252 mg, 4.0 mmol, 2.0 equiv.), and a few drops of acetic acid was added to methanol (10 mL) and stirred overnight. The reaction was quenched with water (30 mL) and extracted with ethyl acetate three times (50 mL × 3). The organic layers were dried over anhydrous Na_2SO_4, and concentrated in vacuum. The residue was purified by silica chromatography (PE/EA = 2/1) to afford product **4h** (54 % yield).

4h-1 (696 mg, 2.0 mmol, 1.0 equiv.), 40% formaldehyde solution (758 μL, 20.0 mmol, 10.0 equiv.), sodium cyanoborohydride (252 mg, 4.0 mmol, 2.0 equiv.), and a few drops of acetic acid were added to methanol (10 mL) in a suitable reaction vessel. The mixture was stirred overnight at room temperature or a specified temperature if necessary. After completion of the reaction, it was quenched by the addition of water (30 mL). The resulting mixture was then extracted with ethyl acetate three times (50 mL each). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue obtained was purified by silica gel chromatograph (PE/EA = 2/1) to afford product **4h** (54% yield).



4h-1

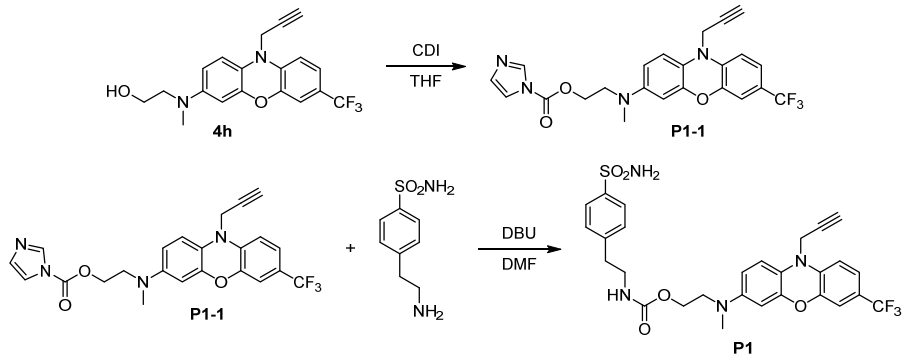
2-((10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10H-phenoxazin-3-yl)amino)ethan-1-ol (4h-1): **4h-1** was synthesized as a yellow solid using **Procedure X** (389 mg, 56 % yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 7.27 – 7.21 (m, 1H), 7.01 (d, *J* = 2.1 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 6.67 (d, *J* = 8.4 Hz, 1H), 6.23 – 6.15 (m, 2H), 5.39 (t, *J* = 5.8 Hz, 1H), 4.68 (t, *J* = 5.4 Hz, 1H), 4.45 (d, *J* = 2.4 Hz, 2H), 3.52 (q, *J* = 5.8 Hz, 2H), 3.24 – 3.19 (m, 1H), 3.02 (q, *J* = 5.9 Hz, 2H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 145.83, 145.21, 144.65, 137.39, 124.65 (q, *J* = 270.7 Hz), 121.83 (q, *J* = 4.3 Hz), 120.96, 120.94 (q, *J* = 32.7 Hz), 114.42, 112.56, 112.22 (q, *J* = 3.7 Hz), 107.00, 101.06, 79.07, 75.13, 60.09, 46.32, 33.66. **^{19}F NMR (376 MHz, DMSO-*d*$_6$)** δ -60.24. **HRMS (ESI)** calculated $C_{18}H_{16}F_3N_2O_2^+$ $[M+H]^+$ m/z 349.1158, found 349.1154.



4h

2-(methyl(10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10*H*-phenoxazin-3-yl)amino)ethan-1-ol (4-4h): **4h** was synthesized as a yellow solid using **Procedure X** (390 mg, 54 % yield). **^{1}H NMR (400 MHz, Chloroform-*d*)** δ 7.28 – 7.21 (m, 1H), 6.99 (d, *J* = 2.1 Hz, 1H), 6.87 (dd, *J* = 8.5, 0.9 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 1H), 6.31 – 6.22 (m, 2H), 4.66 (t, *J* = 5.4 Hz, 1H), 4.47 (d, *J* = 2.4 Hz, 2H), 3.51 (q, *J* = 6.0 Hz, 2H), 3.29 (t, *J* = 6.2 Hz, 2H), 3.24 – 3.19 (m, 1H), 2.85 (s, 3H). **^{13}C NMR (101 MHz, Chloroform-*d*)** δ 150.99, 149.93, 149.42, 142.05, 129.38 (q, *J* = 270.7 Hz), 126.61 (q, *J* = 4.2 Hz), 125.79 (q, *J* = 32.5 Hz), 125.65, 119.17, 117.38, 116.93 (q, *J* = 3.8 Hz), 111.92, 105.55, 83.78, 79.87, 63.27, 59.73, 43.92, 38.36. **^{19}F NMR (376 MHz, CDCl$_3$)** δ -55.53. **HRMS (ESI)** calculated $C_{19}H_{18}F_3N_2O_2^+$ $[M+H]^+$ m/z 363.1315 found 363.1322.

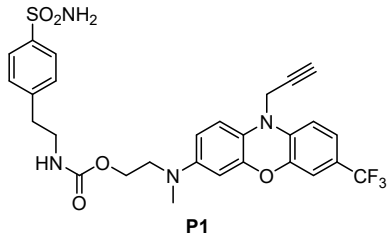
Procedure XI:



4h (725 mg, 2.0 mmol, 1.0 equiv.) and 1,1'-carbonyldiimidazole (972 mg, 6.0 mmol, 3.0 equiv.) were dissolved in tetrahydrofuran (15 mL) and the resulting solution was stirred overnight at room temperature. Following completion, the reaction mixture was concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (PE/EA = 2/1) to afford the intermediate **P1-1**.

Intermediate **P1-1** (912 mg, 2.0 mmol, 1.0 equiv.), 4-(2-aminoethyl)benzenesulfonamide (800 mg, 4.0 mmol, 2.0 equiv.), and DBU (608 mg, 4.0 mmol, 2.0 equiv.) were dissolved in DMF (15 mL) in a suitable reaction vessel. The mixture was stirred at room temperature for approximately 6 hours. Upon completion, the reaction was quenched by the careful addition of water (20 mL). The

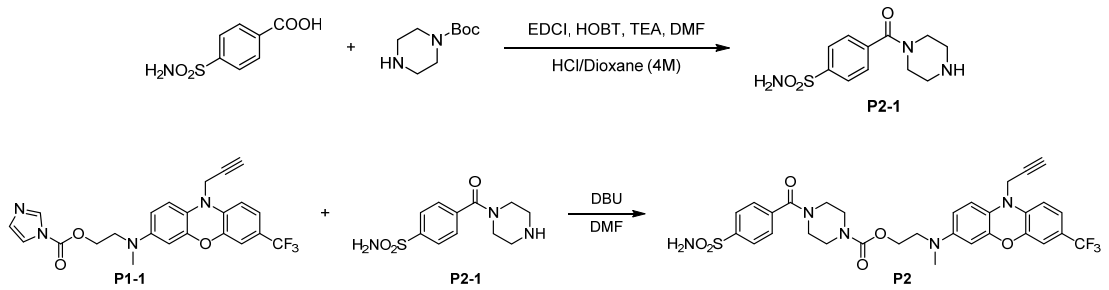
aqueous layer was then extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed three times with saturated brine (50 mL × 3). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was further purified by silica gel chromatography (DCM/MeOH = 10/1) to afford product **P1** (47% yield).



P1

2-(methyl(10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10*H*-phenoxazin-3-yl)amino)ethyl-(4-sulfamoylphenethyl)carbamate (P1): **P1** was synthesized as a yellow solid using **Procedure XI** (552 mg, 47 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 7.77 – 7.70 (m, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.31 – 7.20 (m, 3H), 6.98 (d, J = 2.1 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.75 (d, J = 8.8 Hz, 1H), 6.32 (dd, J = 8.8, 2.8 Hz, 1H), 6.27 (d, J = 2.7 Hz, 1H), 4.47 (d, J = 2.5 Hz, 2H), 3.48 – 3.41 (m, 2H), 3.28 – 3.14 (m, 3H), 2.83 (s, 3H), 2.79 (t, J = 7.3 Hz, 2H), 1.23 (s, 2H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 156.56, 145.82, 145.26, 144.69, 143.99, 142.52, 137.27, 129.56, 126.14, 124.60 (q, J = 271.0 Hz), 121.89, 121.34, 120.96 (q, J = 32.6 Hz), 114.45, 112.66, 112.16, 107.45, 100.98, 78.97, 75.10, 61.34, 51.47, 41.90, 39.05, 35.57, 33.65. **^{19}F NMR (376 MHz, DMSO)** δ -60.35. **HRMS (ESI)** calculated $C_{28}H_{28}F_3N_4O_5S^+$ $[M+H]^+$ m/z 589.1727, found 589.1733.

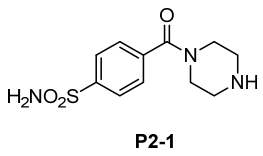
Procedure XII:



4-Sulfamoylbenzoic acid (402 mg, 2.0 mmol, 1.0 equiv.), EDCI (768 mg, 4.0 mmol, 2.0 equiv.), HOBT (541 mg, 4.0 mmol, 2.0 equiv.), and triethylamine (516 μL, 3.7 mmol, corrected for volume to molarity, 1.85 equiv.) were dissolved in DMF (10 mL) in a suitable reaction vessel. After stirring for half an hour, *tert*-butyl piperazine-1-carboxylate (484 mg, 2.6 mmol, 1.3 equiv.) was added. The reaction mixture was then stirred overnight at room temperature. Upon completion, the reaction was quenched by then addition of water (10 mL). The aqueous layer was then extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed three times with saturated brine (50 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4.

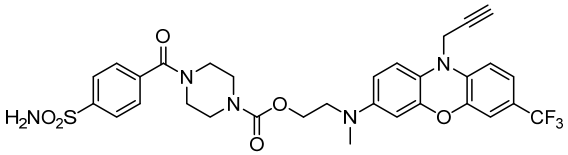
The obtained concentrate was added to the solvent mixture of hydrochloric acid (4.0 M) and 1,4-dioxane, and purified by silica gel chromatography (DCM/MeOH=10/1) to give the intermediate **P2-1** (77 % yield). The **P1-1** (912 mg, 2.0 mmol, 1.0 equiv.), **P2-1** (1.076 g, 4.0 mmol, 2.0 equiv.), and DBU (608 mg, 4.0 mmol, 2.0 equiv.) were continued to be dissolved in DMF (10 mL) and the mixture was stirred for about 6 hours at room temperature. The reaction was quenched with water (10 mL), then ethyl acetate was added (30 mL × 3) and washed three times with saturated brine (50 mL × 3), and the organic phase obtained was dried with anhydrous Na_2SO_4 and then

concentrated in vacuum. The obtained concentrate was purified by silica gel chromatography (DCM/MeOH = 10/1) to give the final product **P2** (42% yield).



P2-1

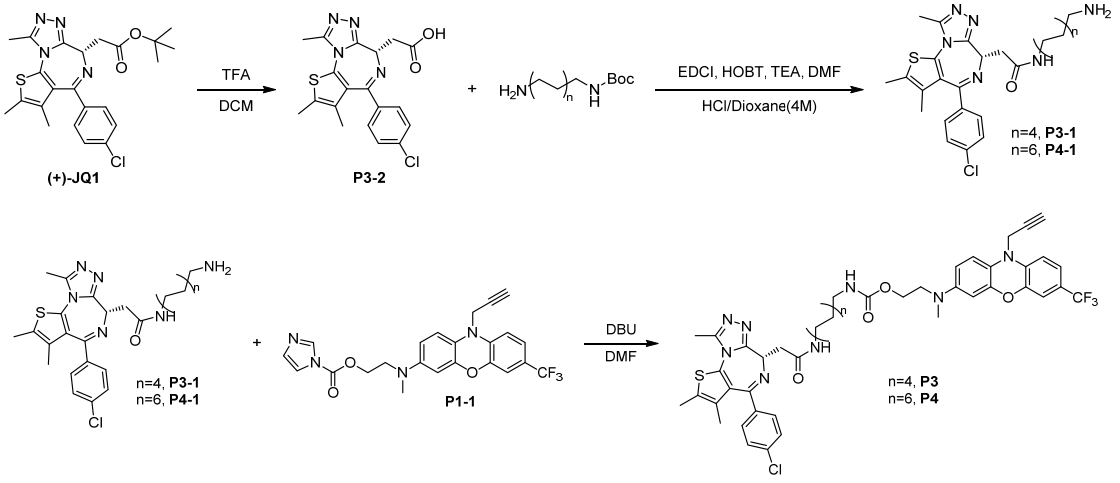
4-(piperazine-1-carbonyl)benzenesulfonamide (P2-1): **P2-1** was synthesized as a yellow solid using **Procedure XII** (414 mg, 77 % yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 7.92 – 7.86 (m, 2H), 7.68 – 7.63 (m, 2H), 7.51 (s, 2H), 3.69 (d, J = 123.4 Hz, 4H), 3.14 (s, 4H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 168.50, 145.59, 138.59, 128.22, 126.30, 42.79. **HRMS (ESI)** calculated $C_{11}H_{16}N_3O_3S^+$ $[M+H]^+$ m/z 270.0907, found 270.0901.



P2

2-(methyl(10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10*H*-phenoxazin-3-yl)amino)ethyl-4-(4-sulfamoylbenzoyl)piperazine-1-carboxylate (P2): **P2** was synthesized as a yellow solid using **Procedure XII** (551 mg, 42 % yield). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 7.86 (d, J = 8.1 Hz, 2H), 7.56 – 7.51 (m, 2H), 7.47 (s, 2H), 7.29 – 7.21 (m, 1H), 6.99 (d, J = 2.1 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.75 (d, J = 8.7 Hz, 1H), 6.39 – 6.24 (m, 2H), 4.46 (s, 2H), 4.15 (t, J = 5.4 Hz, 2H), 3.54 (t, J = 5.6 Hz, 4H), 3.26 – 3.10 (m, 7H), 2.85 (s, 3H). **^{13}C NMR (101 MHz, DMSO-*d*$_6$)** δ 168.44, 154.82, 146.01, 145.34, 145.18, 144.66, 139.27, 137.22, 127.96, 126.32 (d, J = 3.3 Hz), 124.60 (d, J = 270.8 Hz), 121.94, 121.30, 121.18 (d, J = 32.5 Hz), 114.42, 112.69, 112.17, 107.59, 101.13, 78.97, 75.04, 62.89, 51.12, 41.79, 38.70, 33.63, 19.01. **^{19}F NMR (376 MHz, DMSO-*d*$_6$)** δ -60.28. **HRMS (ESI)** calculated C31H31F3N5O6S$^+$ $[M+H]^+$ m/z 658.1942, found 658.1945.

Procedure XIII:



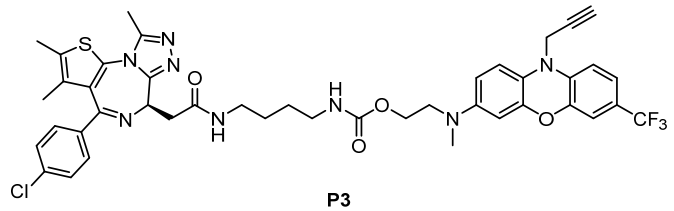
(+)-JQ1 P3-2 P1-1

The synthesis procedure for (+)-**JQ1** and its derivatives was adapted from reference.[12] **P3-2** (800 mg, 2.0 mmol, 1.0 equiv.), EDCI (768 mg, 4.0 mmol, 2.0 equiv.), HOBT (541 mg, 4.0 mmol, 2.0 equiv.), and triethylamine (516 μL, 4.0 mmol, 2.0 equiv.) were dissolved in 15 mL of DMF. After allowing the mixture to stir for half an hour, either *tert*-butyl (9-aminononyl)carbamate or *tert*-butyl (13-aminotridecyl)carbamate (2.6 mmol, 1.3 equiv.) was added. The reaction was then stirred

overnight at room temperature. To quench the reaction, 10 mL of water was added. The aqueous layer was then extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed three times with saturated brine (50 mL × 3).

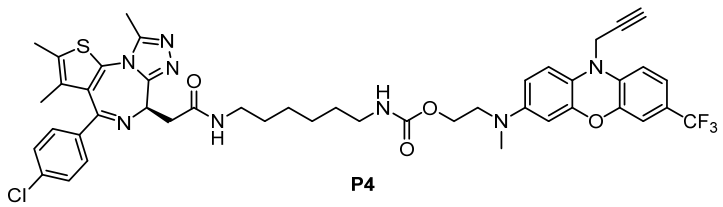
The organic layers were dried over anhydrous Na_2SO_4, and concentrated under vacuum. The resulting concentrate was purified by silica gel chromatography (DCM/MeOH = 10/1) to give the product. Subsequently, the obtained intermediate product was dissolved in a 4.0 M HCl/Dioxane solution, and the reaction was allowed to proceed at room temperature for approximately 2 hours until complete conversion was achieved. The reaction mixture was concentrated under vacuum, affording the intermediate product **P3-1**/**P4-1**.

The compounds **P3-1**/**P4-1** (2.0 mmol, 1.0 equiv.), **P1-1** (1.824 g, 4.0 mmol, 2.0 equiv.), and DBU (608 mg, 4.0 mmol, 2.0 equiv.) were dissolved in 15 mL of DMF, and the resulting mixture was stirred for approximately 6 hours at room temperature. The reaction was then quenched by the addition of 10 mL of water. The aqueous layer was then extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed three times with saturated brine (50 mL × 3). The organic phase was dried over anhydrous Na_2SO_4 and subsequently concentrated under vacuum. The concentrate obtained was purified by silica gel chromatography (DCM/MeOH = 10/1) as the eluent, to give the final product **P3** and **P4** (38% and 33%).



P3

2-(methyl(10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10*H*-phenothiazin-3-yl)amino)ethyl-(*R*)-(4-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)butyl)carbamate (P3): **P3** was synthesized as a yellow solid using **Procedure XIII** (664 mg, 38 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.18 (t, J = 5.7 Hz, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.44 – 7.37 (m, 2H), 7.28 – 7.21 (m, 1H), 7.19 – 7.10 (m, 1H), 6.98 (d, J = 2.2 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.74 (d, J = 8.8 Hz, 1H), 6.31 (dd, J = 8.8, 2.8 Hz, 1H), 6.26 (d, J = 2.8 Hz, 1H), 4.58 – 4.43 (m, 3H), 4.05 (t, J = 6.0 Hz, 2H), 3.49 – 3.41 (m, 2H), 3.30 – 3.10 (m, 5H), 3.03 – 2.97 (m, 2H), 2.84 (s, 3H), 2.58 (s, 3H), 2.41 – 2.37 (m, 3H), 1.61 (s, 3H), 1.43 (q, J = 3.4 Hz, 4H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 169.84, 163.47, 156.63, 155.60, 150.26, 145.82, 145.25, 144.69, 137.27, 137.23, 135.69, 132.73, 131.14, 130.57, 130.30, 130.03, 128.93, 124.61 (q, J = 270.7 Hz), 121.85, 121.34, 120.94 (q, J = 32.7 Hz), 114.45, 112.65, 112.17, 107.46, 100.98, 78.99, 75.13, 61.20, 56.50, 54.35, 51.52, 49.07, 39.07, 38.70, 38.11, 27.35, 27.03, 14.49, 13.12, 11.75. **^{19}F NMR (376 MHz, DMSO-d_6)** δ -60.31. **HRMS (ESI)** calculated $C_{43}H_{43}ClF_3N_8O_3S_2^+$ $[M+H]^+$ m/z 875.2535, found 875.2539.



P4

2-(methyl(10-(prop-2-yn-1-yl)-7-(trifluoromethyl)-10*H*-phenothiazin-3-yl)amino)ethyl-(*R*)-(6-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)hexyl)carbamate (P4): **P4** was synthesized

as a yellow solid using **Procedure XIII** (685 mg, 33 % yield). **^{1}H NMR (400 MHz, DMSO-d_6)** δ 8.15 (t, J = 5.6 Hz, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.44 – 7.39 (m, 1H), 7.27 – 7.22 (m, 1H), 7.11 (t, J = 5.8 Hz, 1H), 6.97 (d, J = 2.1 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 6.74 (d, J = 8.8 Hz, 1H), 6.31 (dd, J = 8.8, 2.8 Hz, 2H), 6.26 (d, J = 2.8 Hz, 1H), 4.57 – 4.40 (m, 3H), 4.07 – 4.04 (m, 1H), 3.43 (t, J = 6.0 Hz, 2H), 3.30 – 2.90 (m, 2H), 2.84 (s, 3H), 2.58 (s, 3H), 2.41 – 2.37 (m, 3H), 1.61 (s, 3H), 1.50 – 1.24 (m, 5H). **^{13}C NMR (101 MHz, DMSO-d_6)** δ 169.80, 163.46, 156.57, 155.60, 150.25, 145.84, 145.24, 144.69, 137.27, 137.23, 135.71, 132.72, 131.14, 130.55, 130.29, 130.04, 128.90, 124.61 (q, J = 270.5 Hz), 121.84, 121.32, 121.13 (q, J = 38.4 Hz), 114.43, 112.64, 112.14, 107.43, 100.97, 78.97, 75.11, 61.19, 54.38, 51.53, 39.01, 38.86, 38.13, 33.66, 29.86, 29.66, 29.49, 26.50, 26.40, 14.47, 13.11, 11.74. **^{19}F NMR (376 MHz, DMSO-d_6)** δ -60.34. **HRMS (ESI)** calculated $C_{45}H_{47}ClF_3N_8O_{3\ s2}^{+}$ $[M+H]^+$ m/z 903.2848, found 903.2841.

7. Supplementary reference

[1] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09 Rev. D.01*, Gaussian, Inc.: Wallingford, CT, 2013.

[2] J. P. Foster and F. Weinhold. Natural hybrid orbitals. *J. Am. Chem. Soc.* **1980**, 102, 7211–7218.

[3] A. E. Reed and F. Weinhold. Natural bond orbital analysis of near-Hartree-Fock water dimer. *J. Chem. Phys.* **1983**, 78, 4066–4073.

[4] Y. Zhao and D. G. Truhlar. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **2008**, 120, 215-241.

[5] M. J. Frisch, J. A. Pople, and J. S. Binkley. Self-Consistent Molecular Orbital Methods. 25. Supplementary Functions for Gaussian Basis Sets. *J. Chem. Phys.* **1984**, 80, 3265-3269.

[6] T. Clark, J. Chandrasekhar, G. W. Spitznagel, and P. v. R. Schleyer. Efficient diffuse function-augmented basis sets for anion calculations. 3. The 3-21+G basis set for 1 st-row elements, Li-F. *J. Comp. Chem.* **1983**, 4, 294-301.

[7] A. V. Marenich, C. J. Cramer, and D. G. Truhlar. Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B.* **2009**, 113, 6378-6396.

[8] C. Adamo, V. Barone, Toward Reliable Density Functional Methods Without Adjustable Parameters: The PBE0 Model. *J. Chem. Phys.* **1999**, 110, 6158-6170.

[9] R. L. Martin. Natural transition orbitals. *J. Chem. Phys.* **2003**, 118, 4775.

[10] T. Lu, F. Chen. Multiwfn: A multifunctional wavefunction analyzer. *J. Comput. Chem.* **2012**, 33, 580-592.

[11] W. Humphrey, A. Dalke, K. Schulten., VMD: Visual molecular dynamics. *J. Mol. Graph. Model.* **1996**, 14, 33-38.

[12] Guo, Z.; Sun, Y.; Liang, L.; Lu, W.; Luo, B.; Wu, Z.; Huo, B.; Hu, Y.; Huang, P.; Wu, Q., Design and Synthesis of Dual EZH2/BRD4 Inhibitors to Target Solid Tumors. *Journal of Medicinal Chemistry* **2022,** *65*, 6573-6592.

[a] Sheldrick, G. M. *Acta Cryst.* **2008**, *A64*, 112–122.

[b] Sheldrick, G. M. *Acta Cryst.* **2015**, *A71*, 3–8.

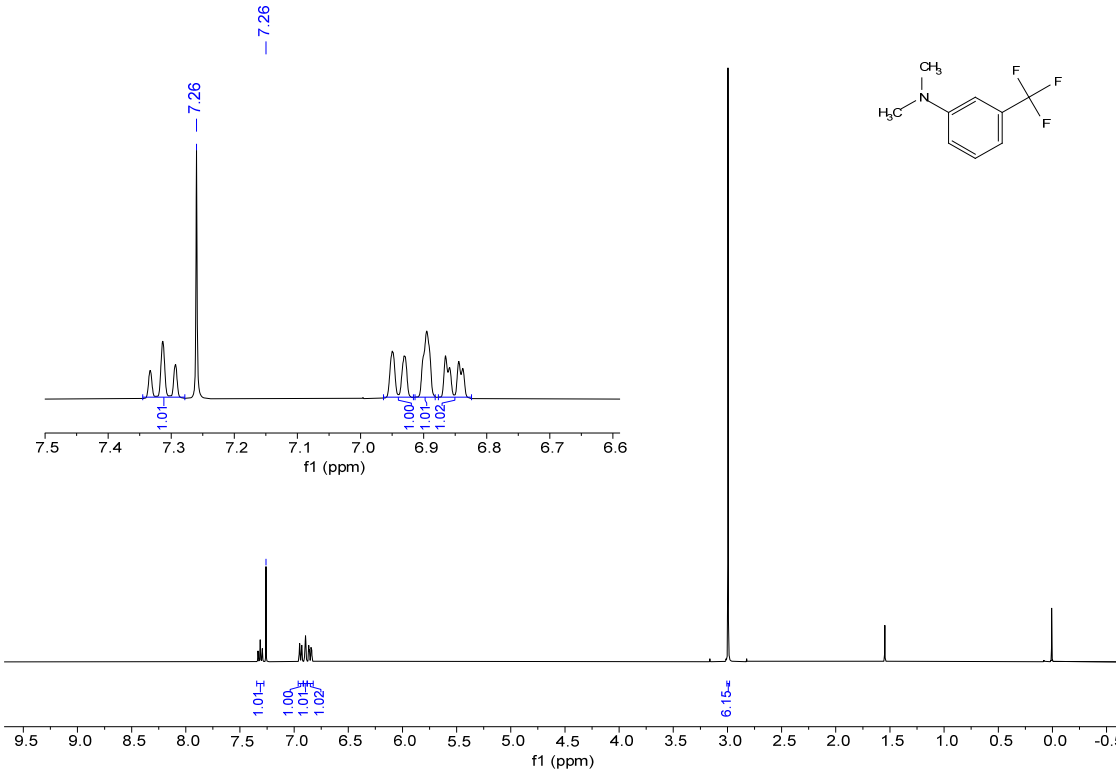
[c] Sheldrick, G. M. *Acta Cryst*. **2015**, *C71*, 3–8.

[d] Dolomanov, O.V., Bourhis, L.J., Gildea, R.J., Howard, J. A. K., Puschmann, H. *J. Appl. Cryst*. **2009**, *42*, 339-341.

[e] Spek, A. L. *J. Appl. Cryst.* **2003**, *36*, 7–13.

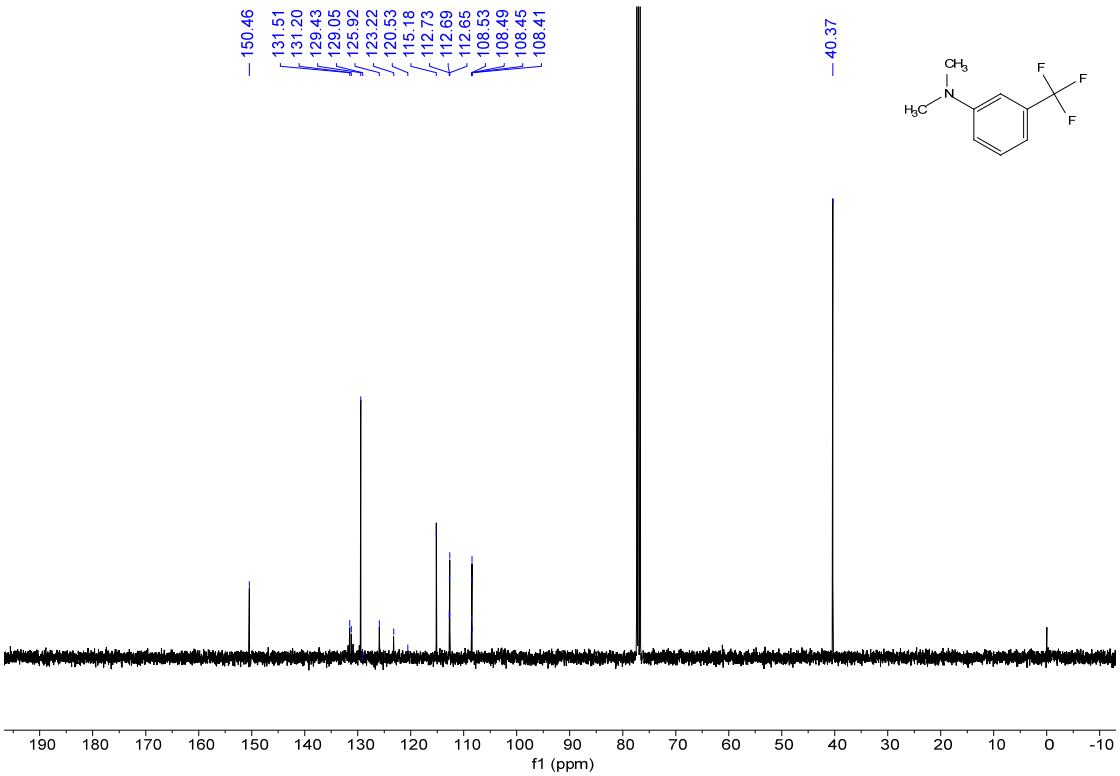
8. NMR spectra

^{1}H NMR (400 MHz, $CDCl_3$)



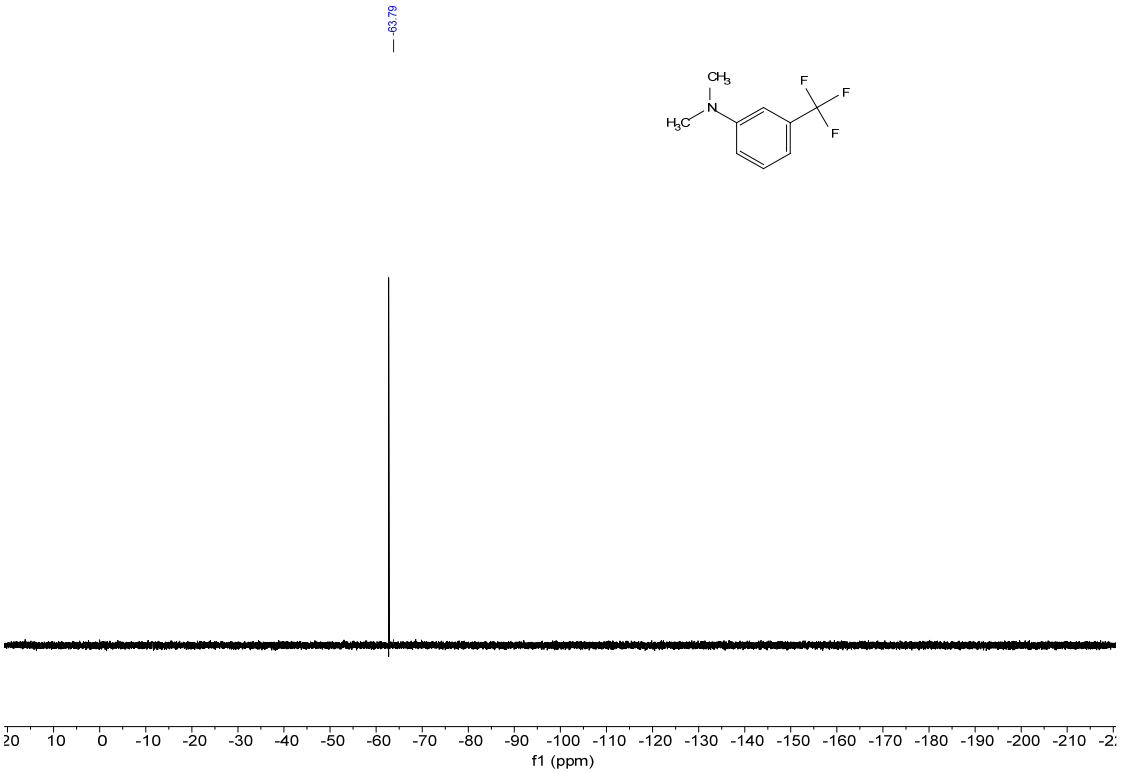
^{1}H NMR spectrum of **1a**

^{13}C NMR (101 MHz, $CDCl_3$)



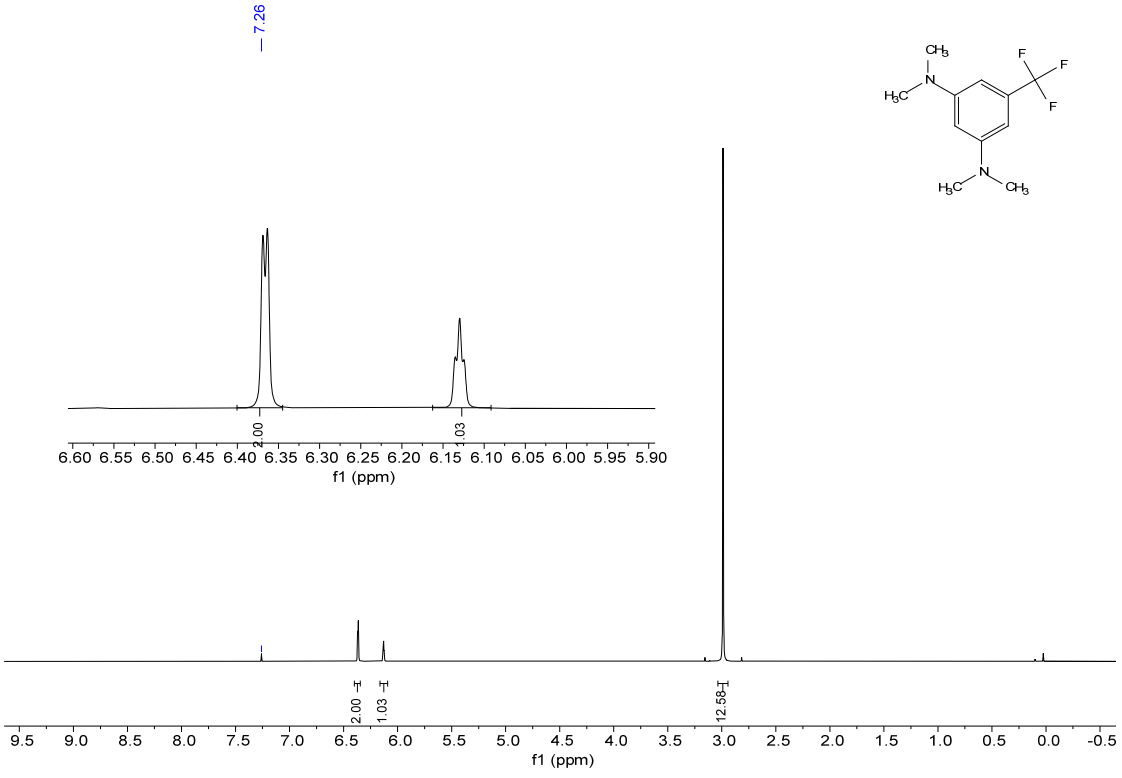
^{13}C NMR spectrum of **1a**

^{19}F NMR (376 MHz, $CDCl_3$)



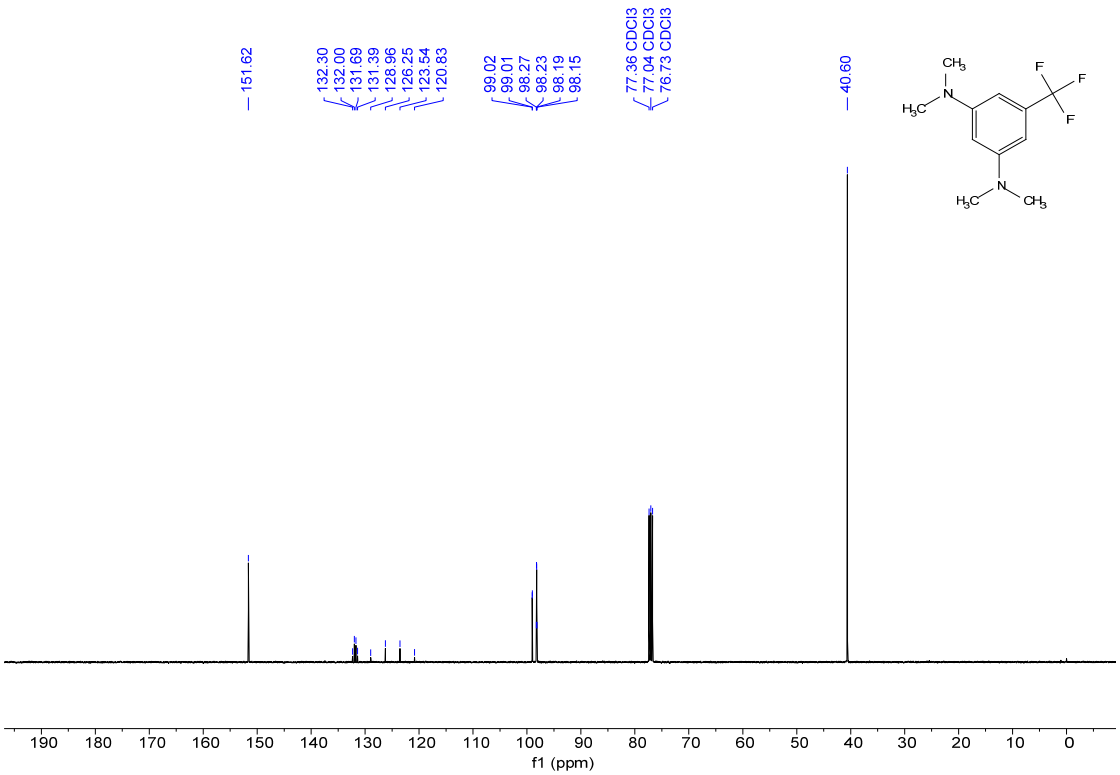
^{19}F NMR spectrum of **1a**

^{1}H NMR (400 MHz, $CDCl_3$)



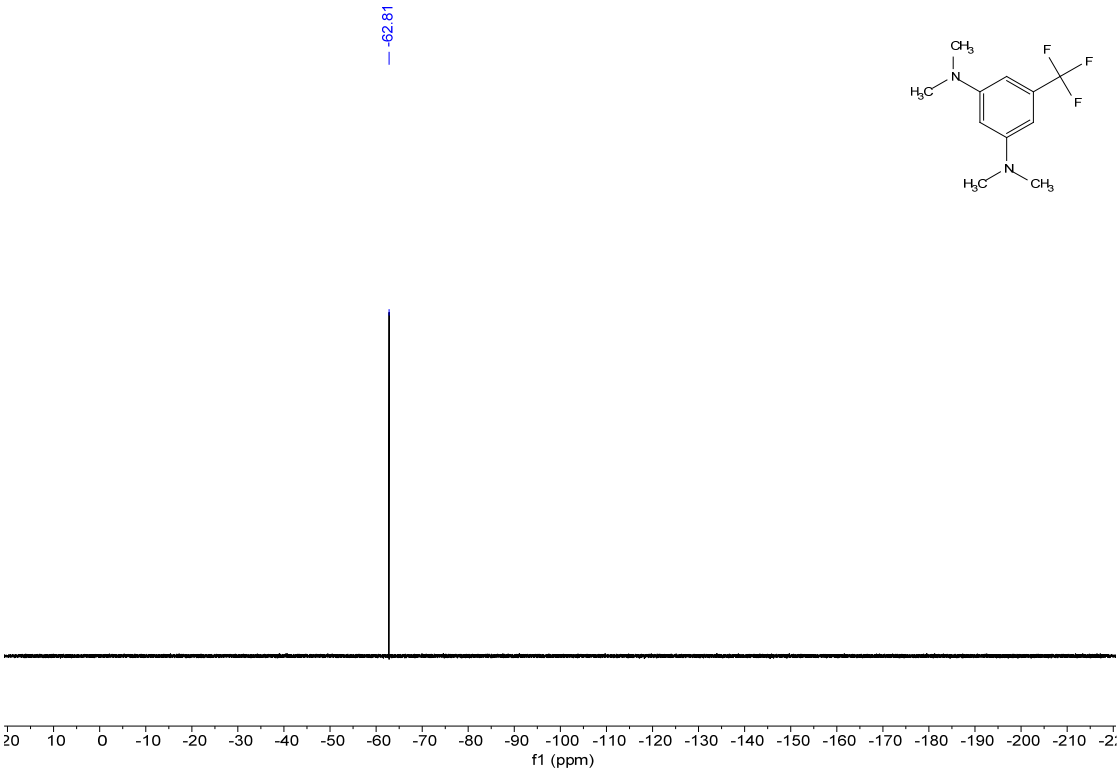
^{1}H NMR spectrum of **1b**

^{13}C NMR (101 MHz, $CDCl_3$)



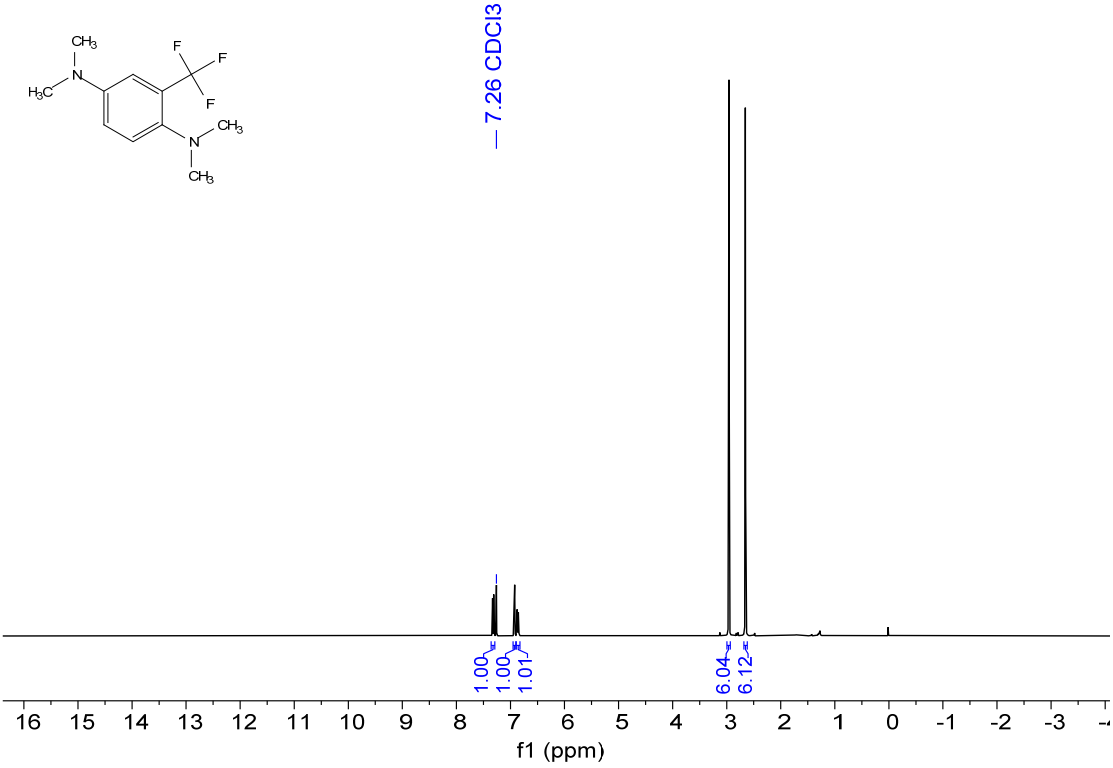
^{13}C NMR spectrum of **1b**

^{19}F NMR (376 MHz, $CDCl_3$)



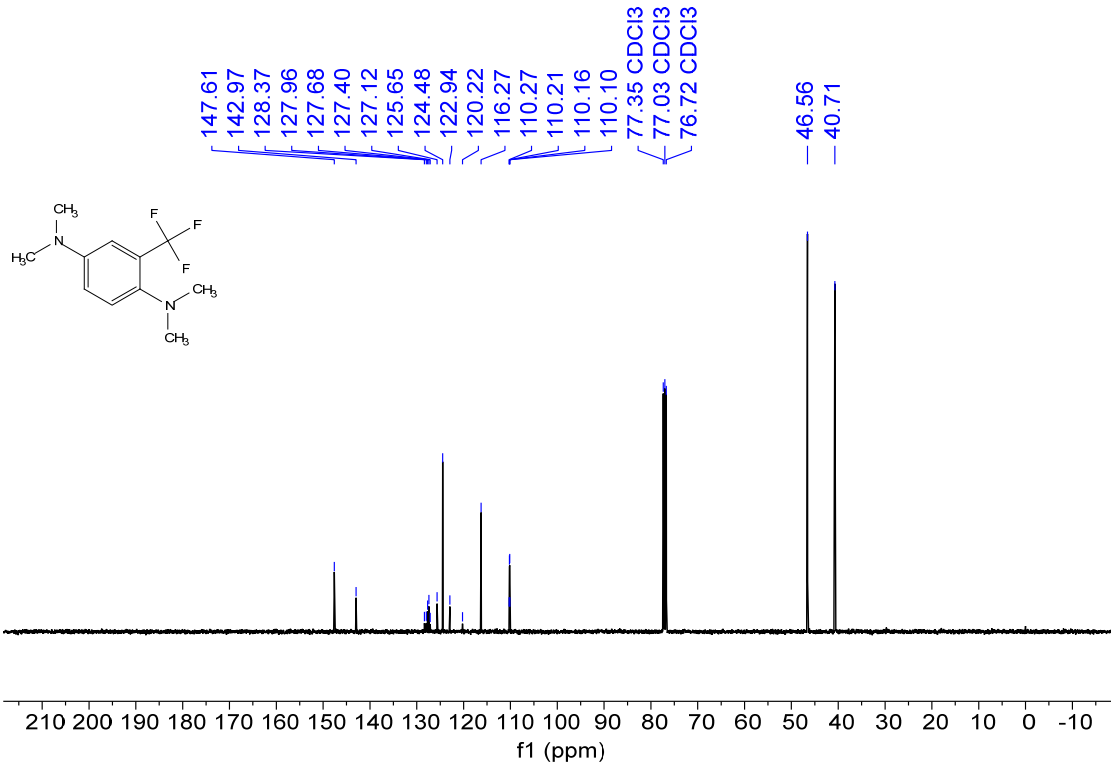
^{19}F NMR spectrum of **1b**

^{1}H NMR (400 MHz, $CDCl_3$)



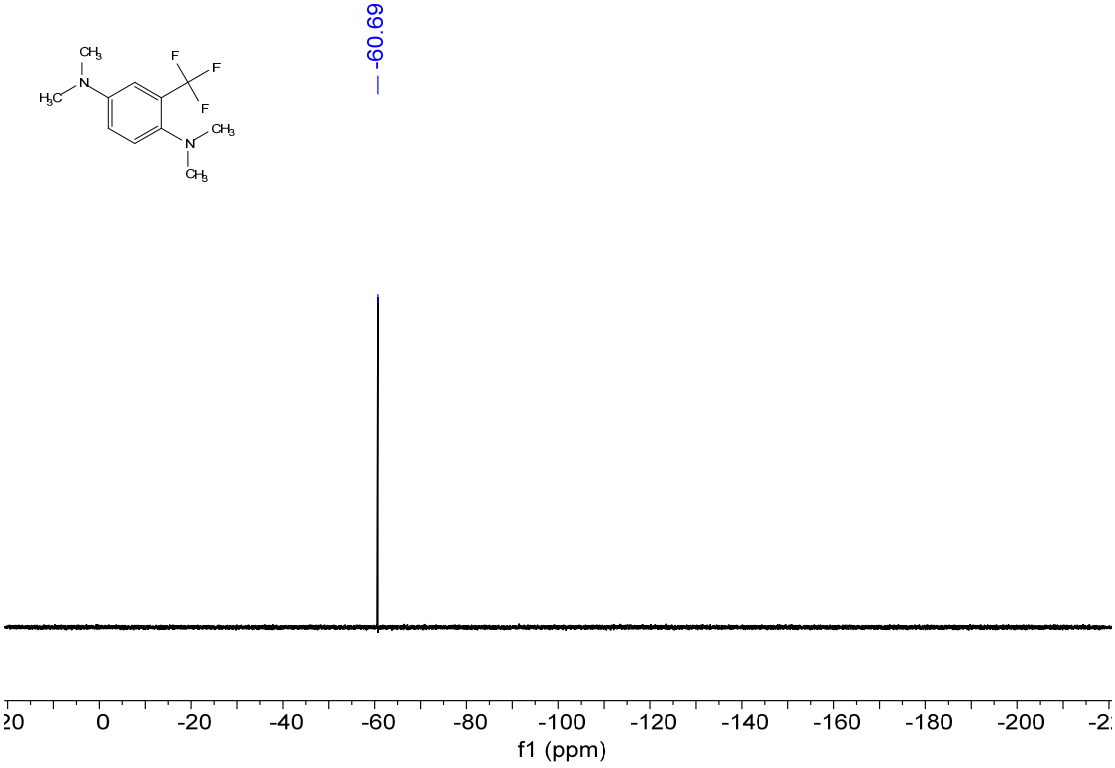
^{1}H NMR spectrum of **1c**

^{13}C NMR (101 MHz, $CDCl_3$)



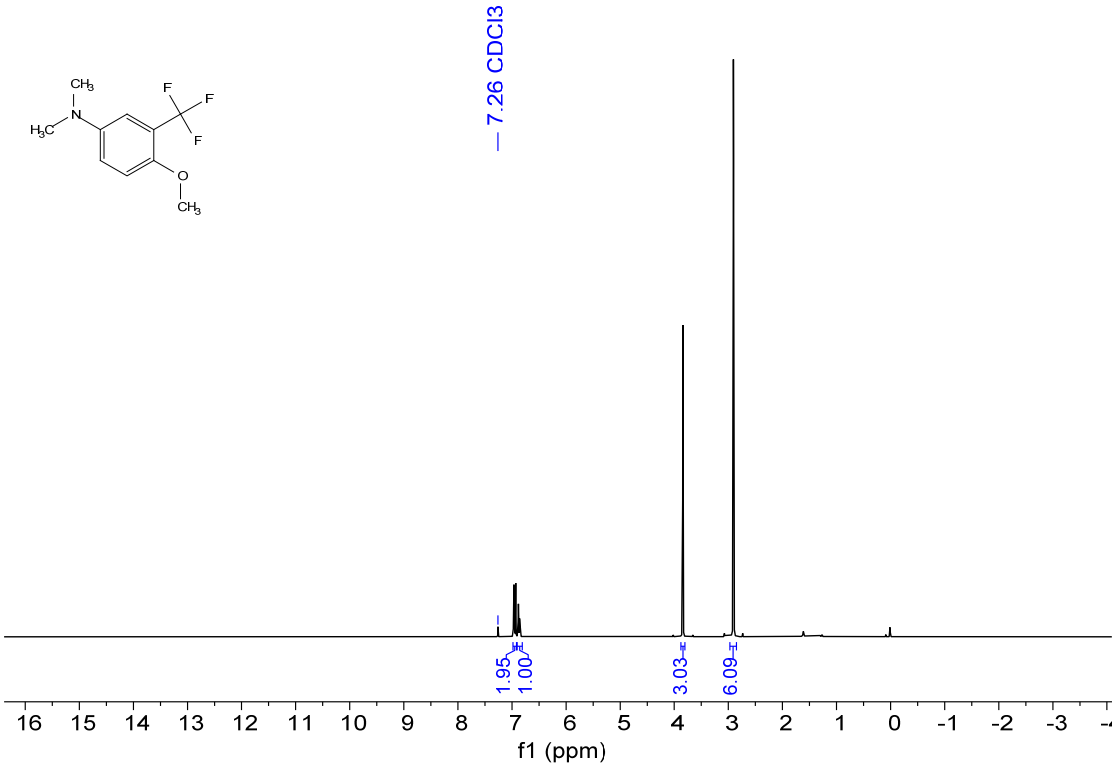
^{13}C NMR spectrum of **1c**

^{19}F NMR (376 MHz, $CDCl_3$)



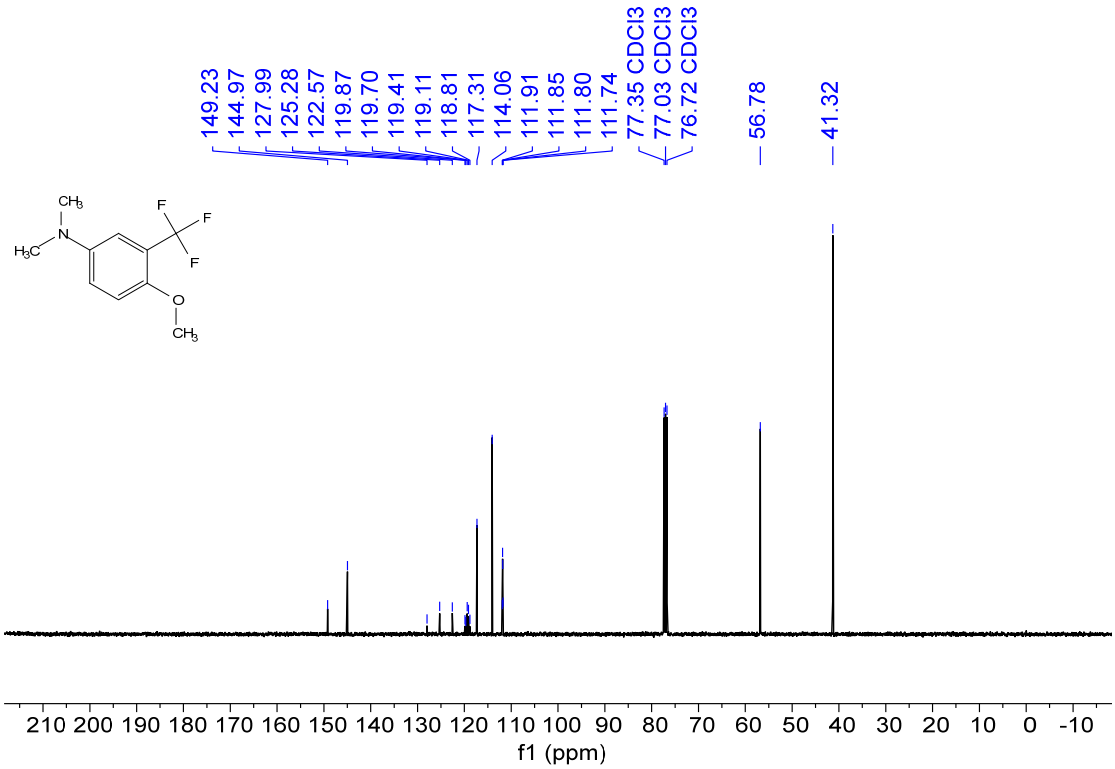
^{19}F NMR spectrum of **1c**

^{1}H NMR (400 MHz, $CDCl_3$)



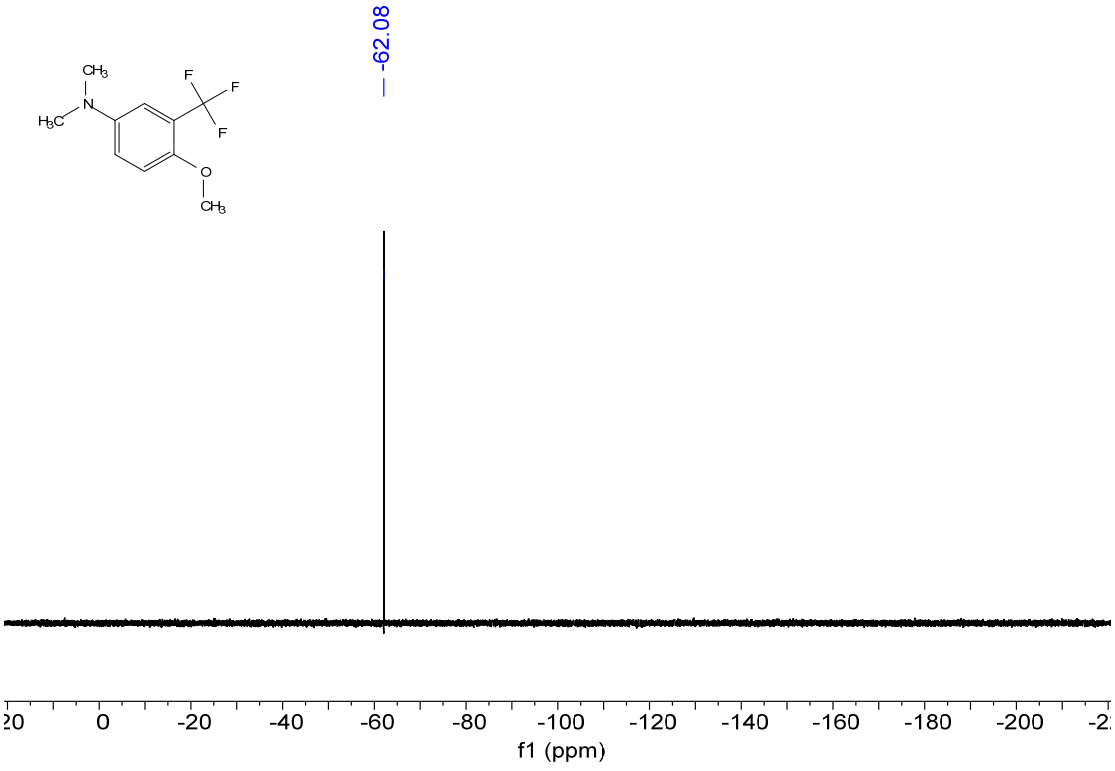
^{1}H NMR spectrum of **1d**

^{13}C NMR (101 MHz, $CDCl_3$)



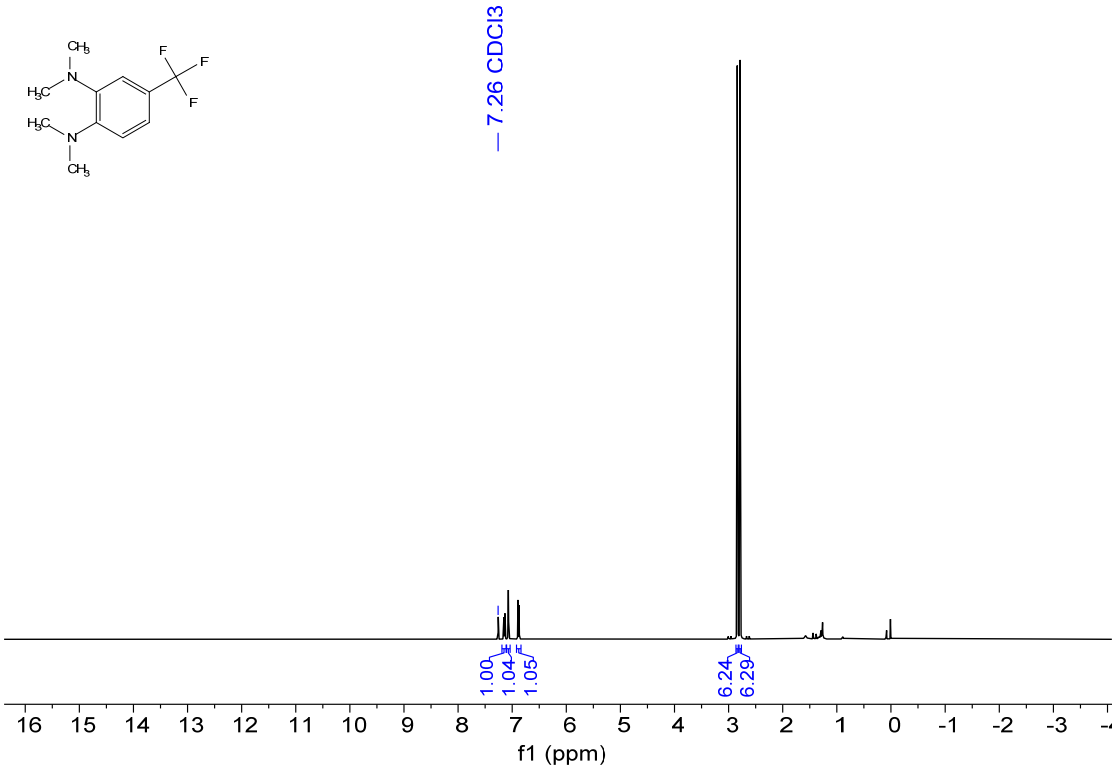
^{13}C NMR spectrum of **1d**

^{19}F NMR (376 MHz, $CDCl_3$)



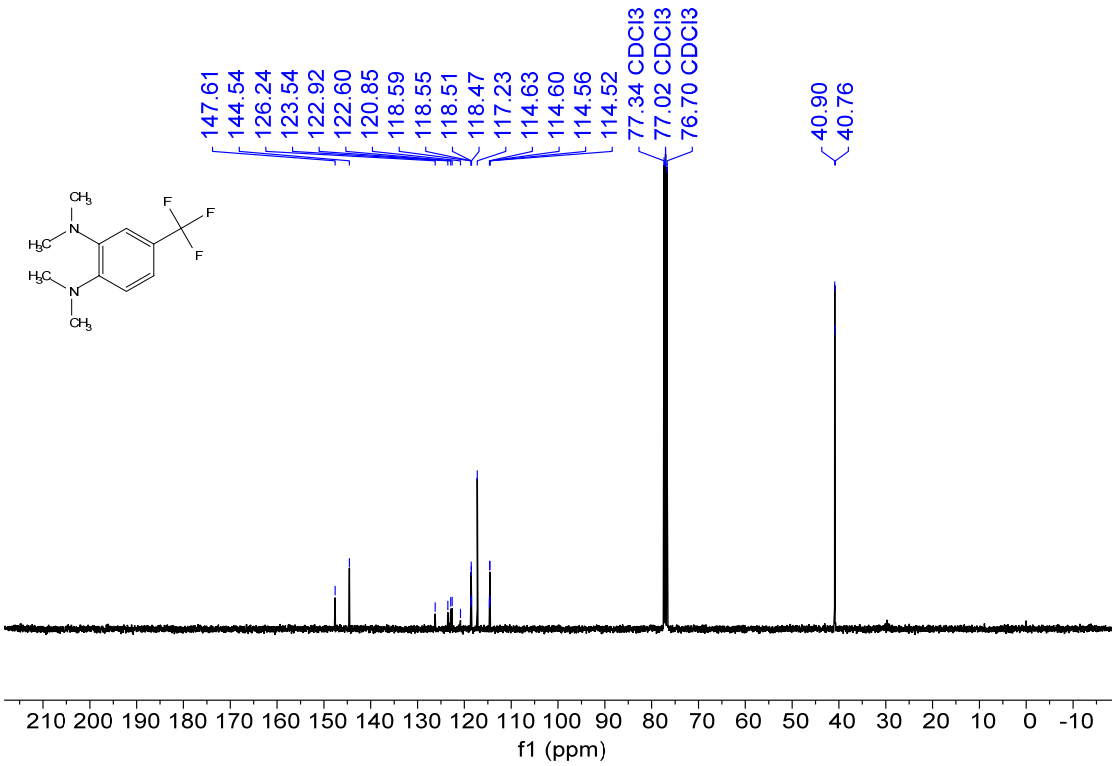
^{19}F NMR spectrum of **1d**

^{1}H NMR (400 MHz, $CDCl_3$)



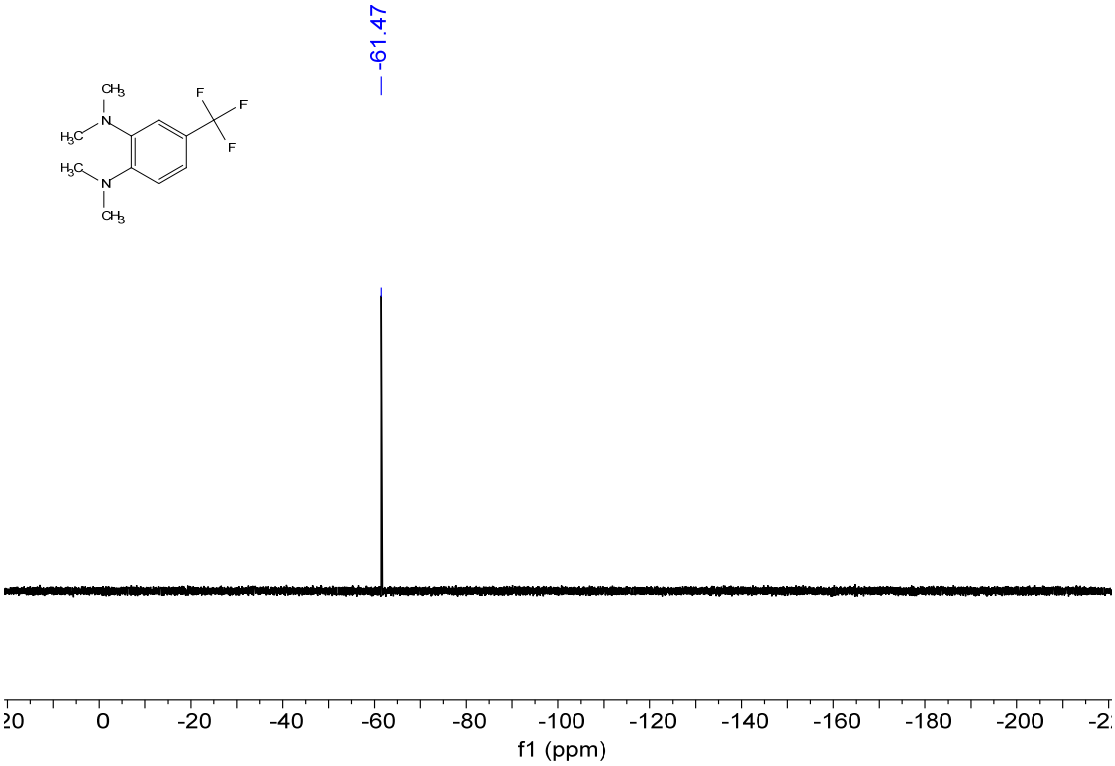
^{1}H NMR spectrum of **1e**

^{13}C NMR (101 MHz, $CDCl_3$)



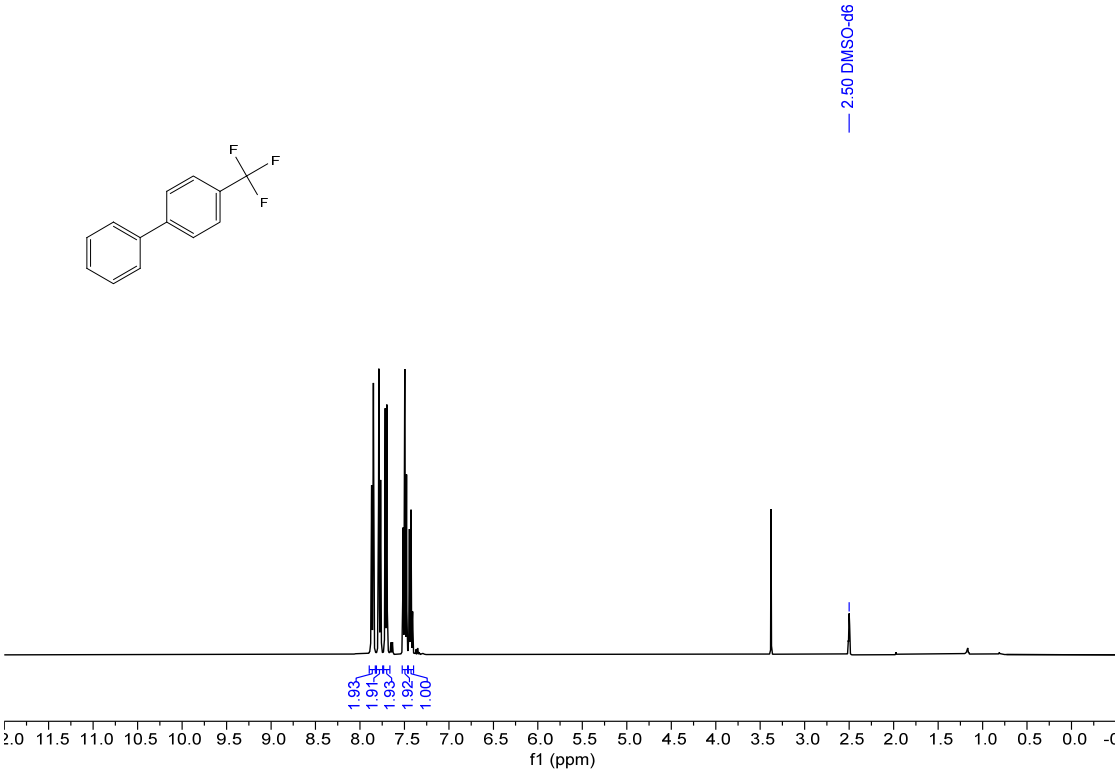
^{13}C NMR spectrum of **1e**

^{19}F NMR (376 MHz, $CDCl_3$)



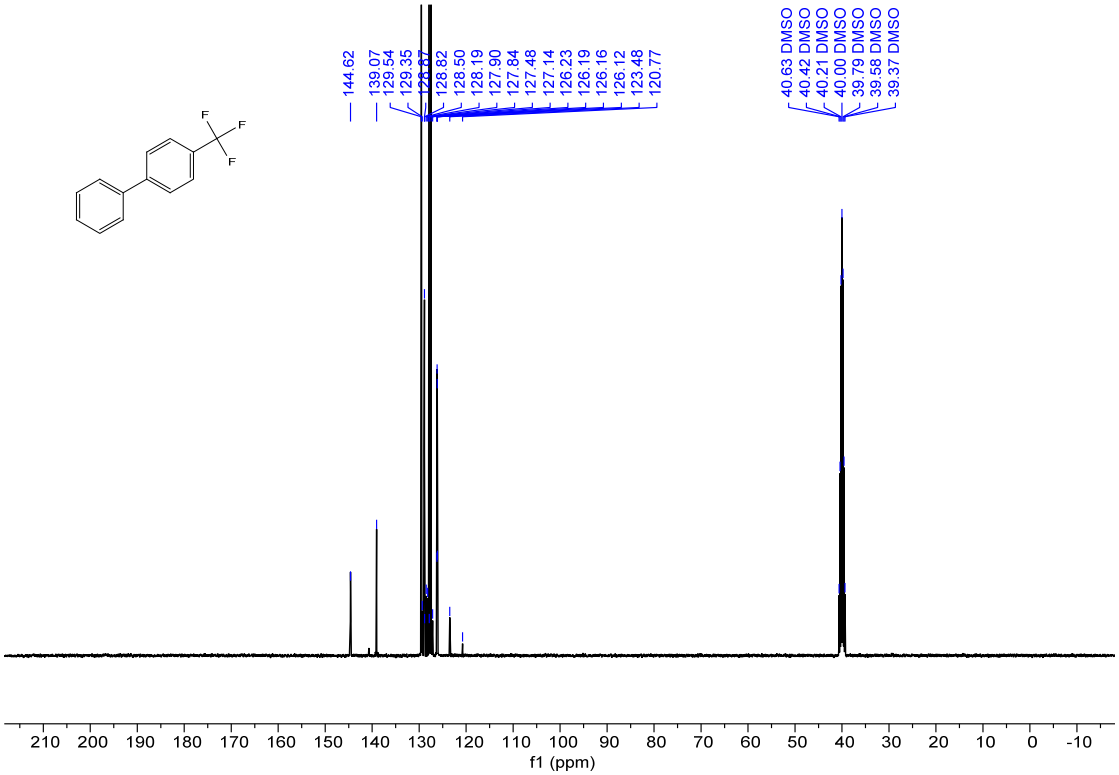
^{19}F NMR spectrum of **1e**

^{1}H NMR (400 MHz, DMSO-d_6)



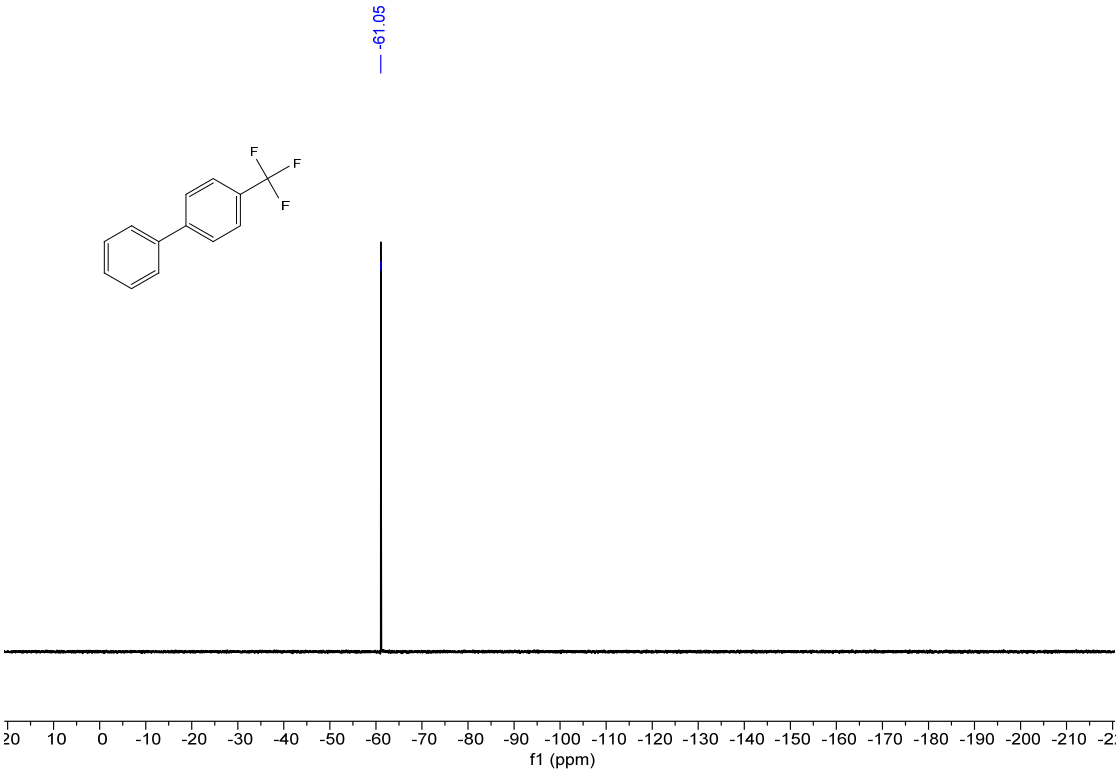
^{1}H NMR spectrum of **2a**

^{13}C NMR (101 MHz, DMSO-d_6)



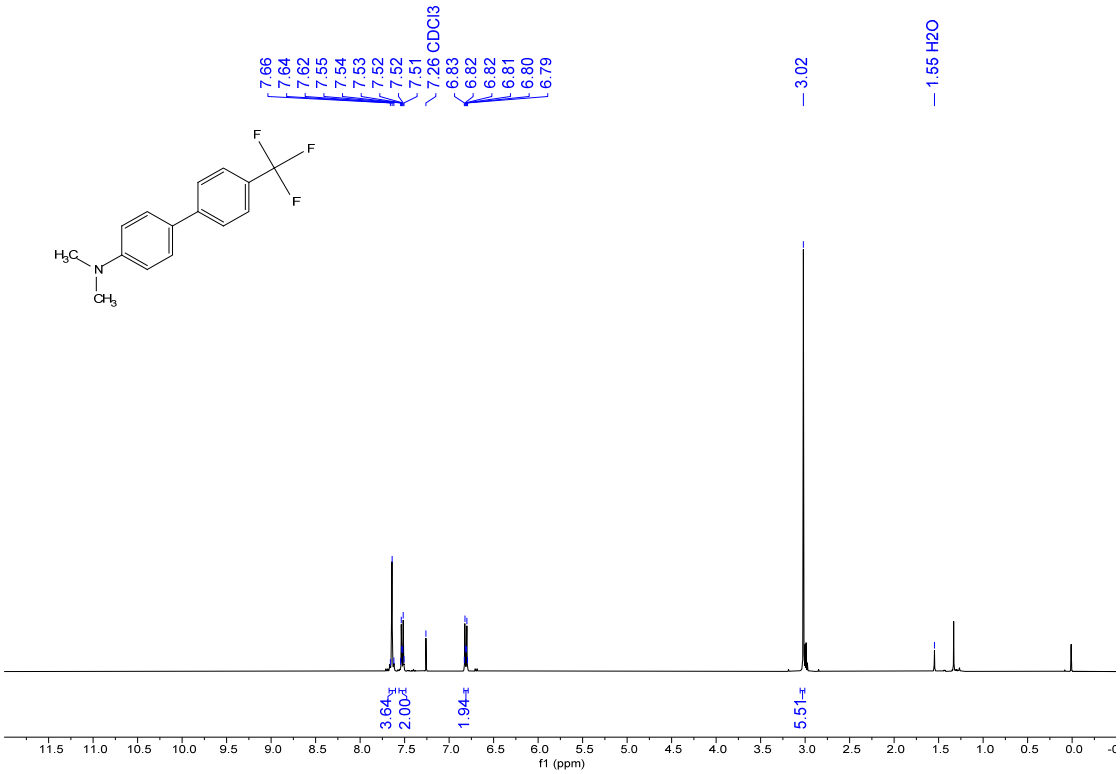
^{13}C NMR spectrum of **2a**

^{19}F NMR (376 MHz, DMSO-d_6)



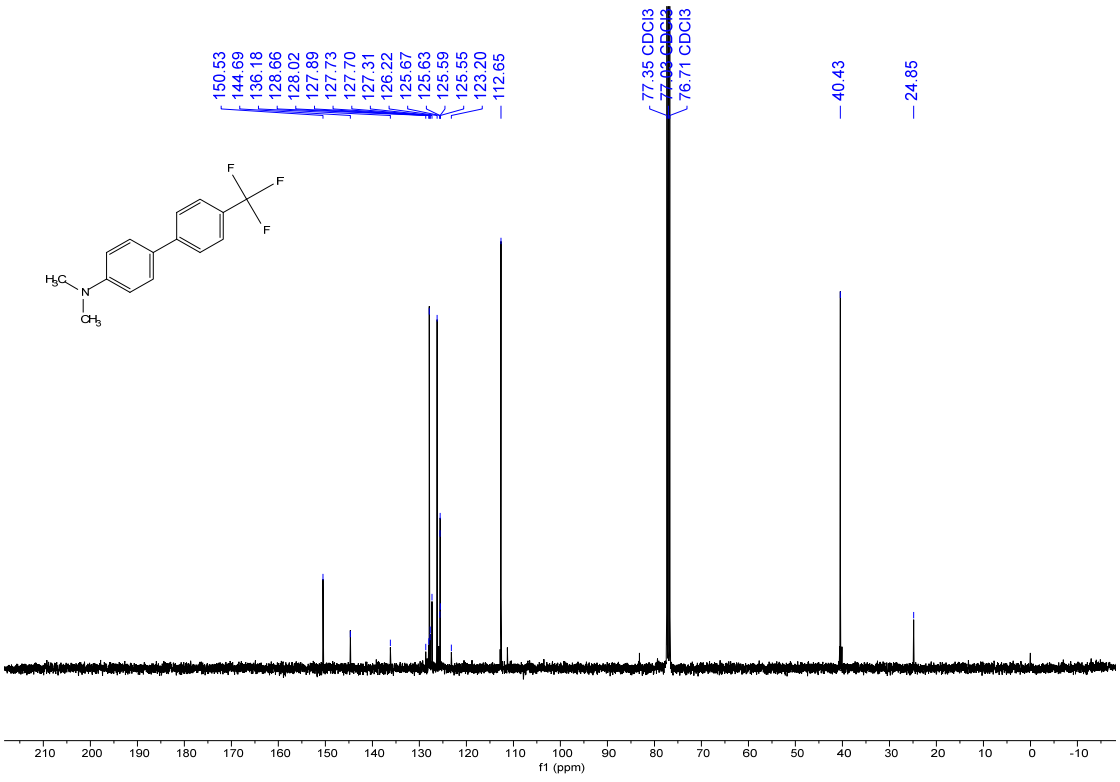
^{19}F NMR spectrum of **2a**

^{1}H NMR (400 MHz, $CDCl_3$)



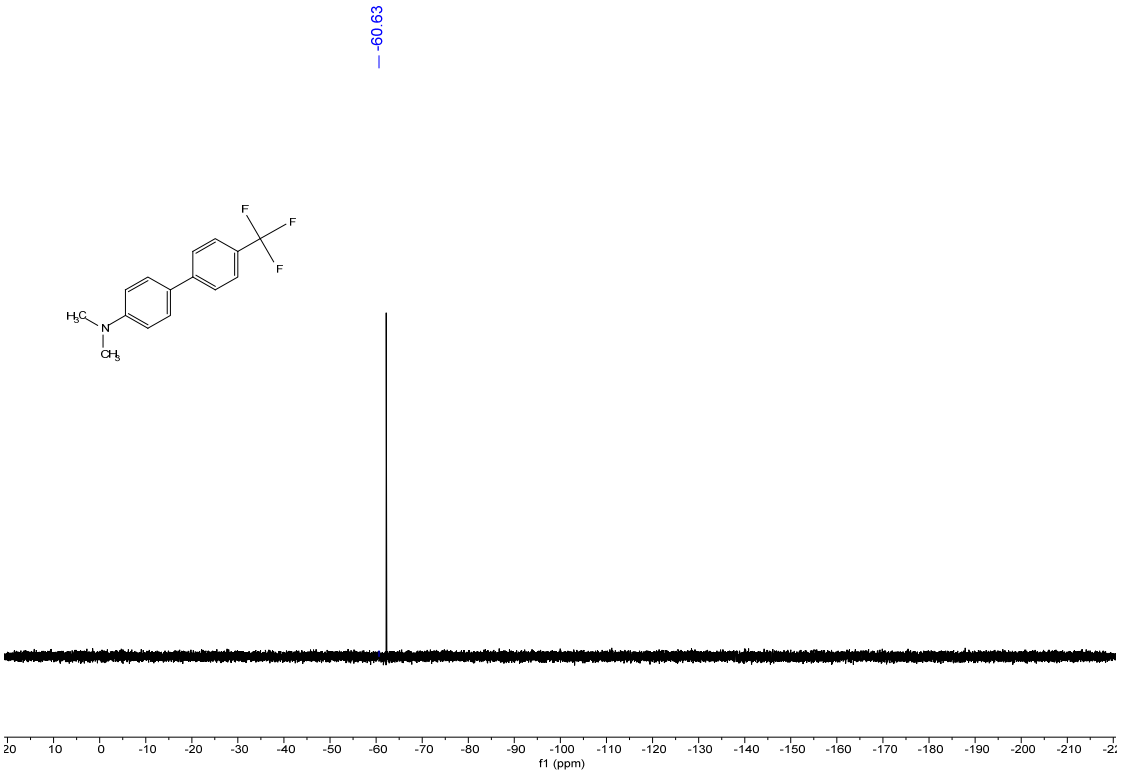
^{1}H NMR spectrum of **2b**

^{13}C NMR (101 MHz, $CDCl_3$)



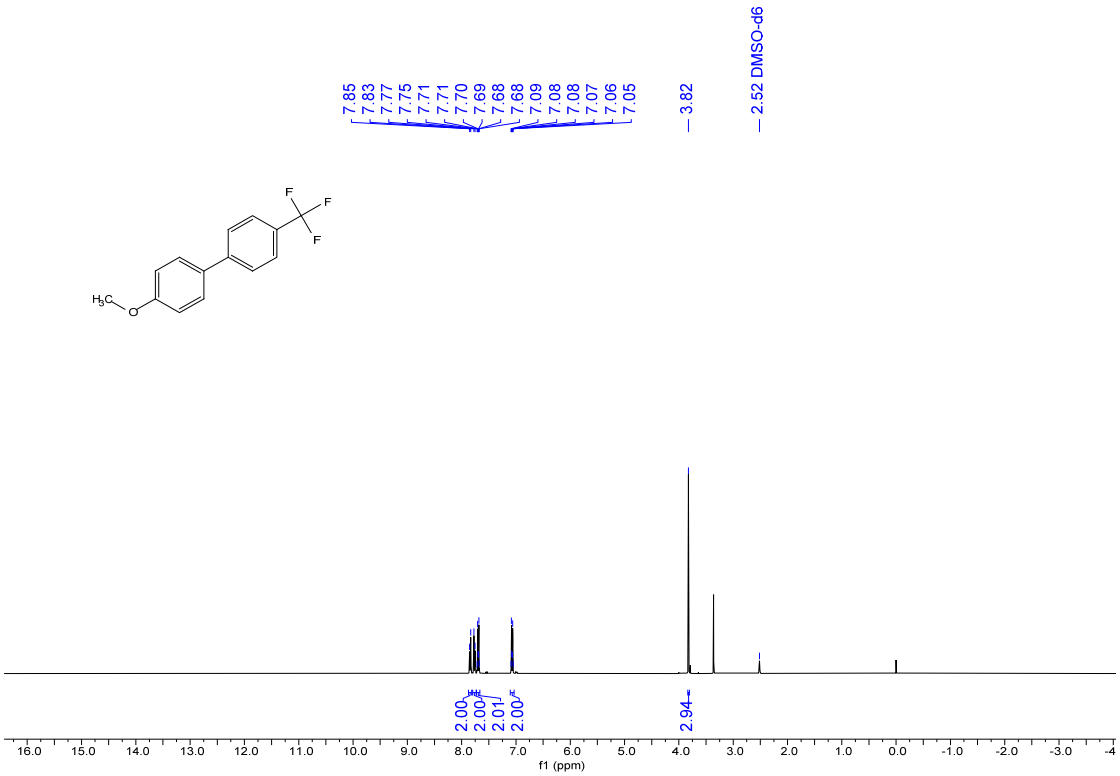
^{13}C NMR spectrum of **2b**

^{19}F NMR (376 MHz, $CDCl_3$)



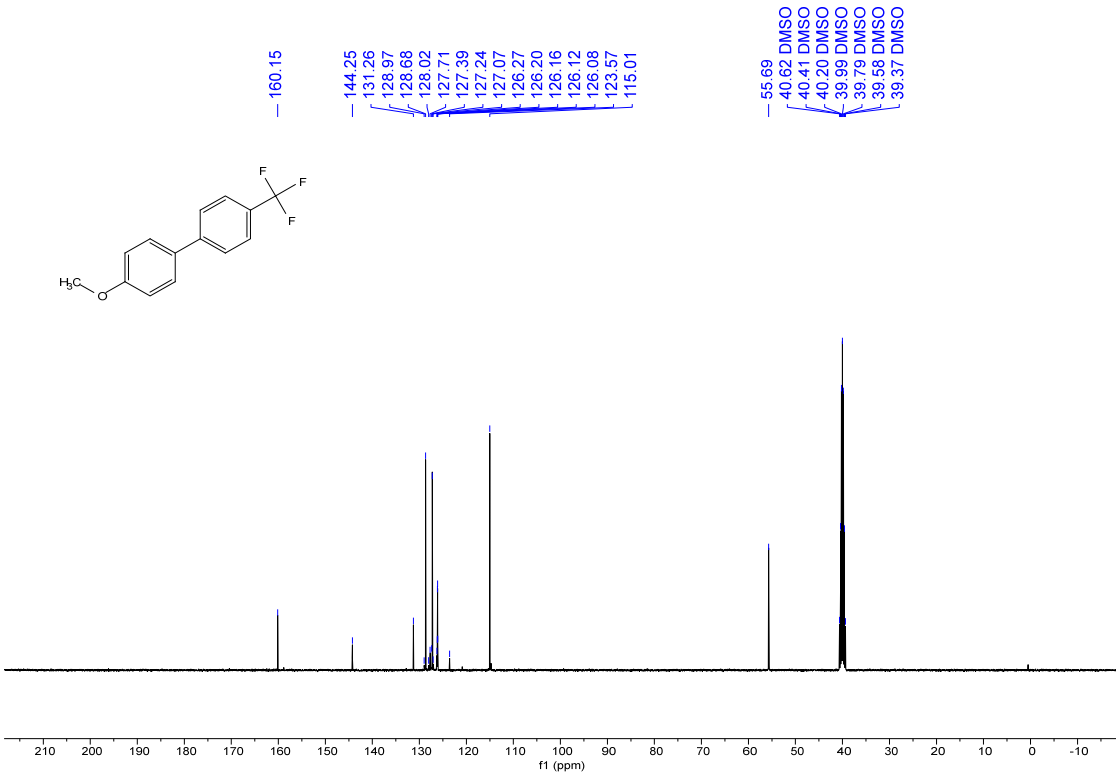
^{19}F NMR spectrum of **2b**

^{1}H NMR (400 MHz, DMSO-d_6)



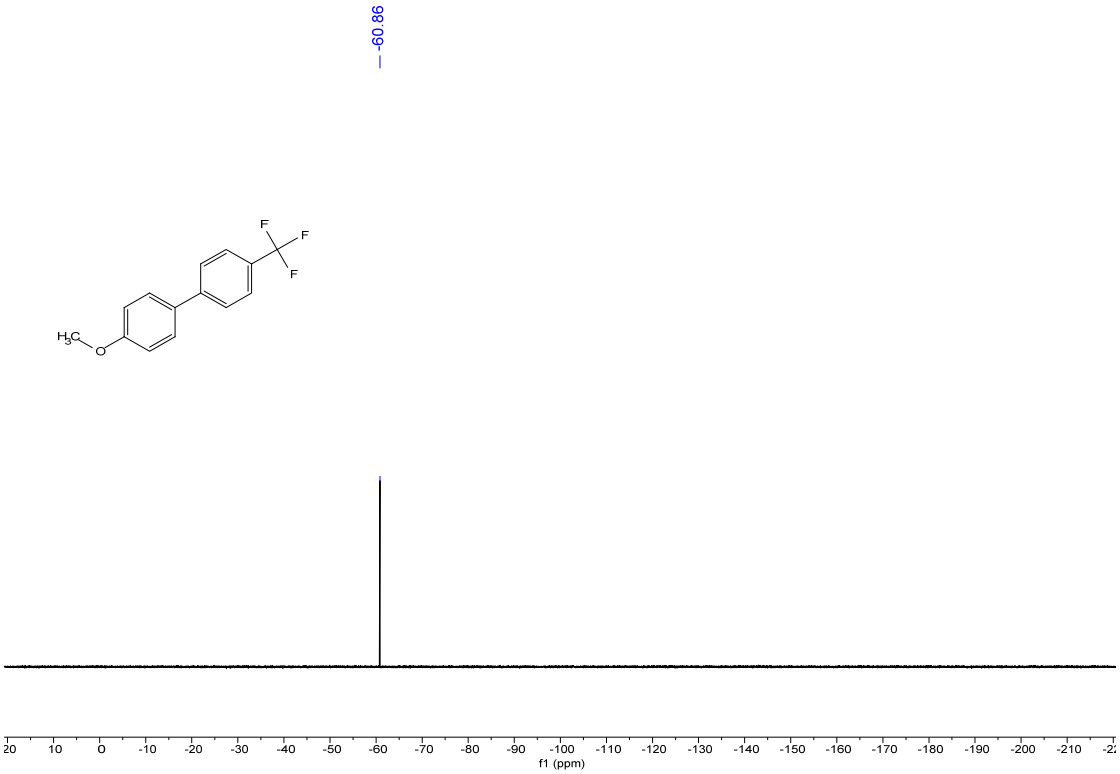
^{1}H NMR spectrum of **2c**

^{13}C NMR (101 MHz, DMSO-d_6)



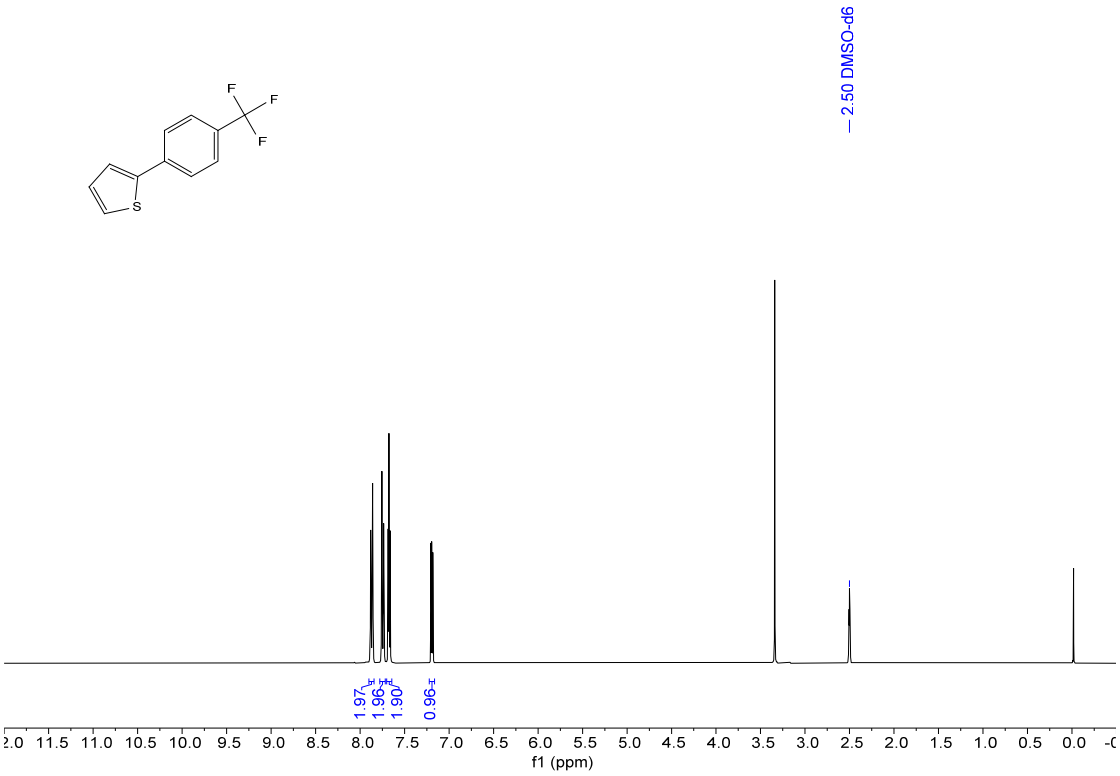
^{13}C NMR spectrum of **2c**

^{19}F NMR (376 MHz, DMSO-d_6)



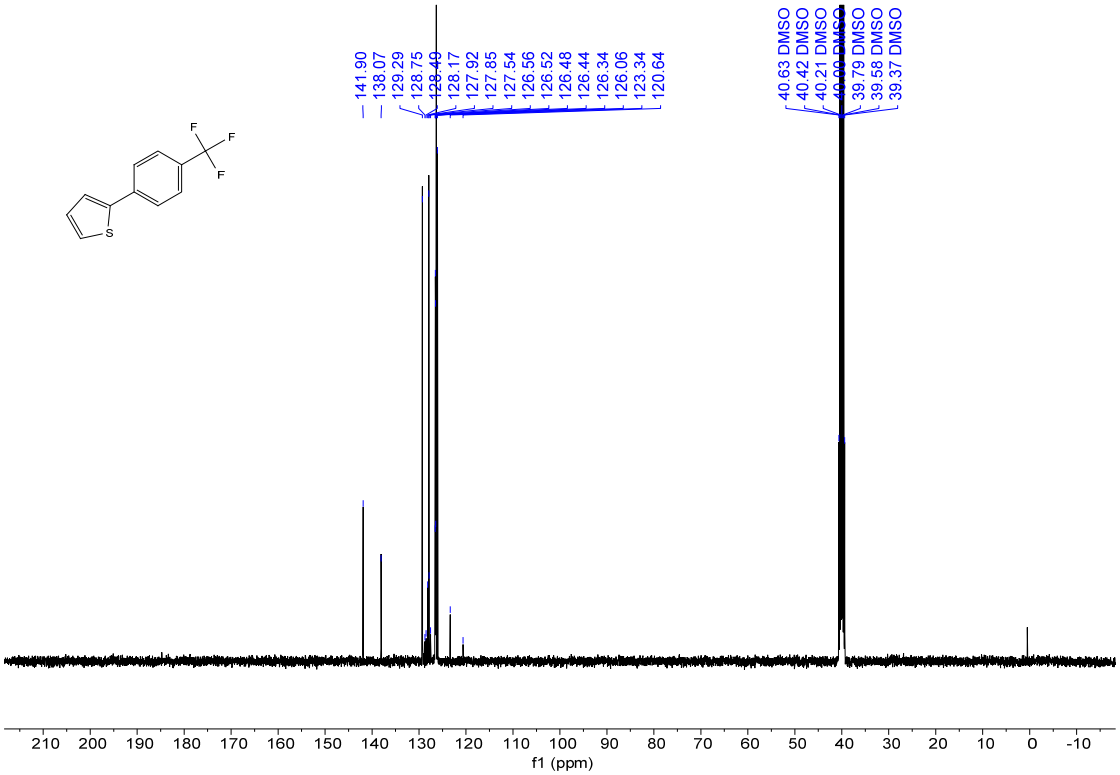
^{19}F NMR spectrum of **2c**

^{1}H NMR (400 MHz, DMSO-d_6)



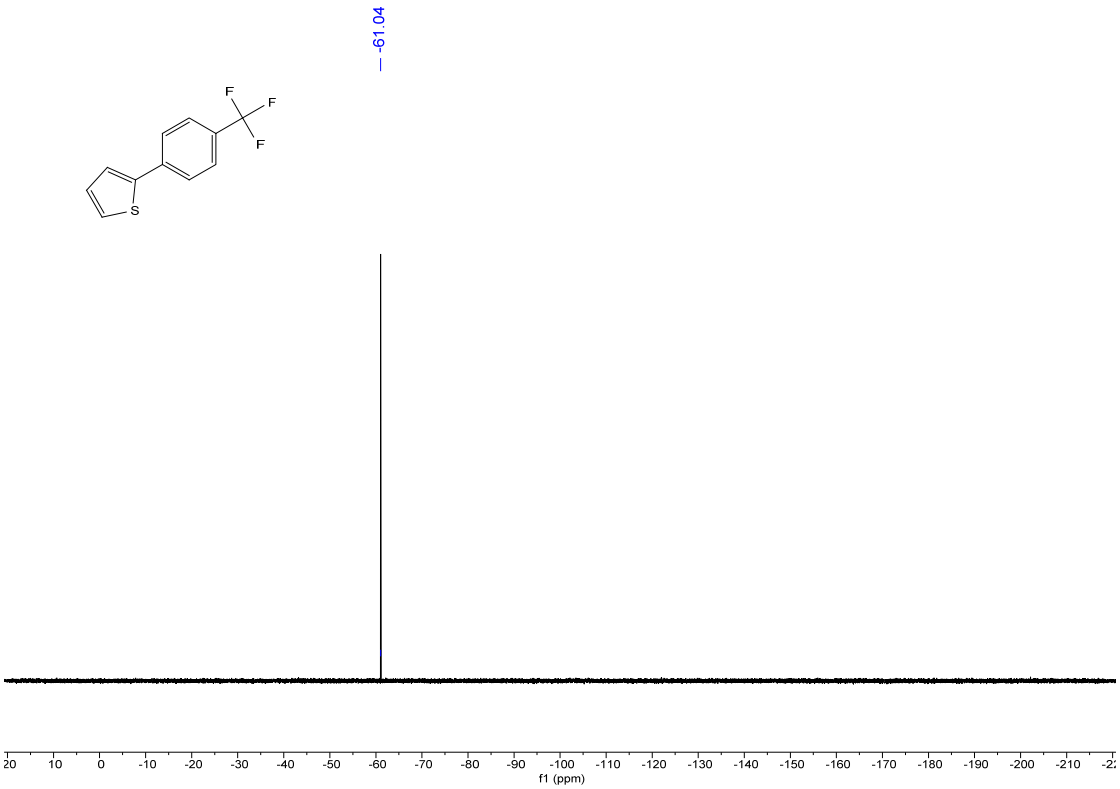
^{1}H NMR spectrum of **2d**

^{13}C NMR (101 MHz, DMSO-*d*$_6$)



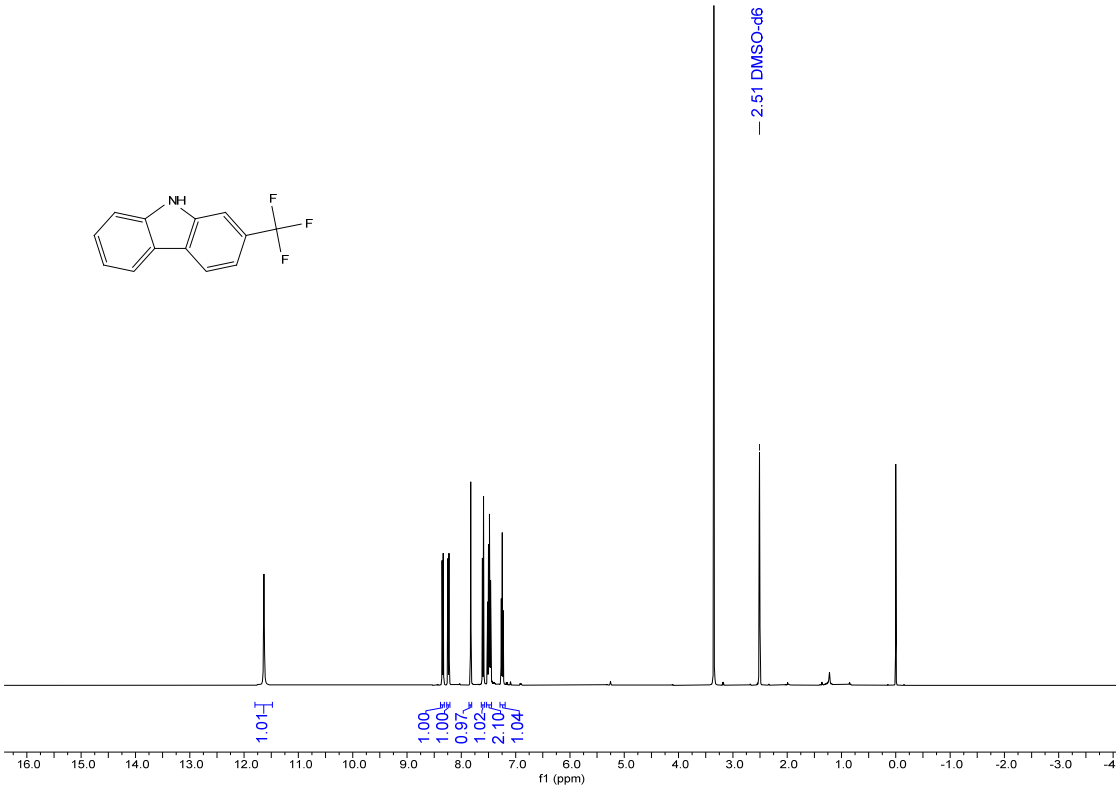
^{13}C NMR spectrum of **2d**

^{19}F NMR (376 MHz, DMSO-*d*$_6$)



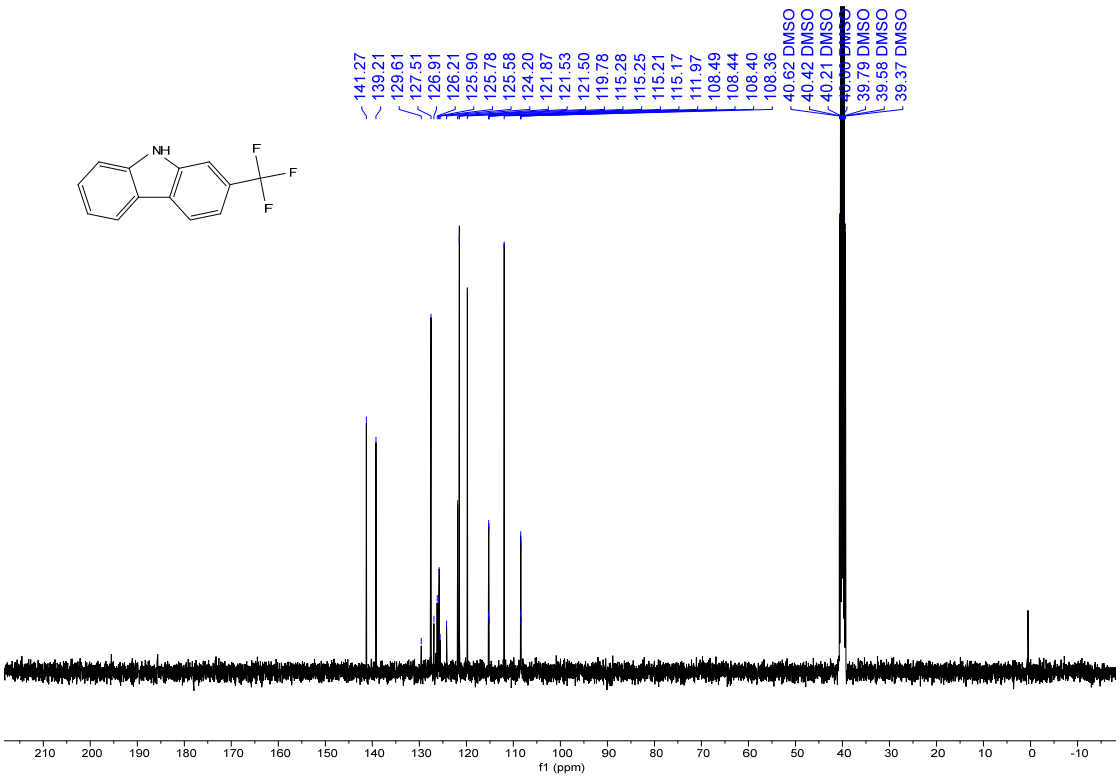
^{19}F NMR spectrum of **2d**

^{1}H NMR (400 MHz, DMSO-d_6)



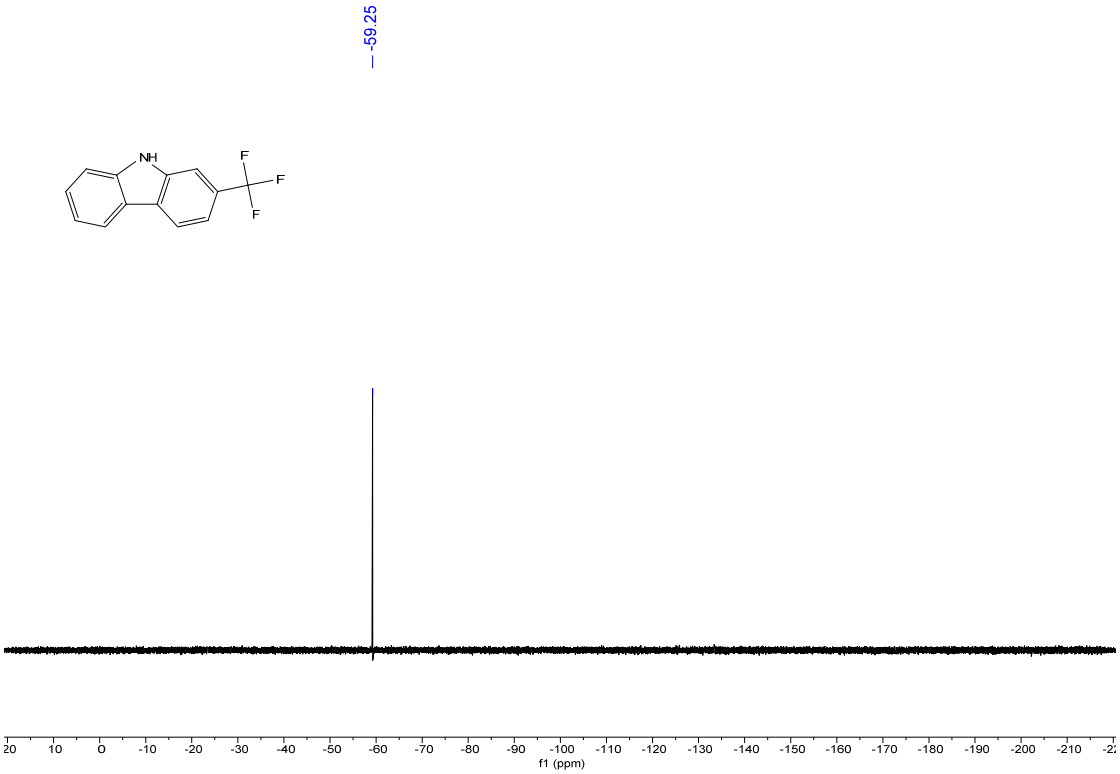
^{1}H NMR spectrum of **3d**

^{13}C NMR (101 MHz, DMSO-d_6)



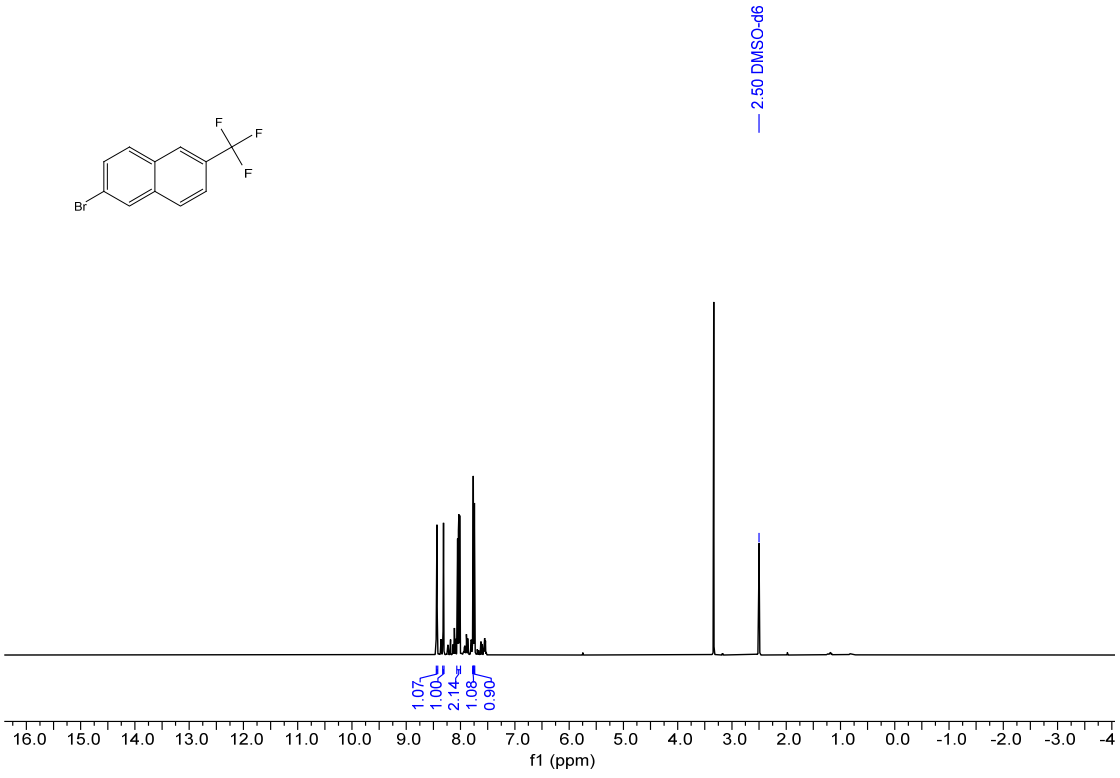
^{13}C NMR spectrum of **3d**

^{19}F NMR (376 MHz, DMSO-d_6)



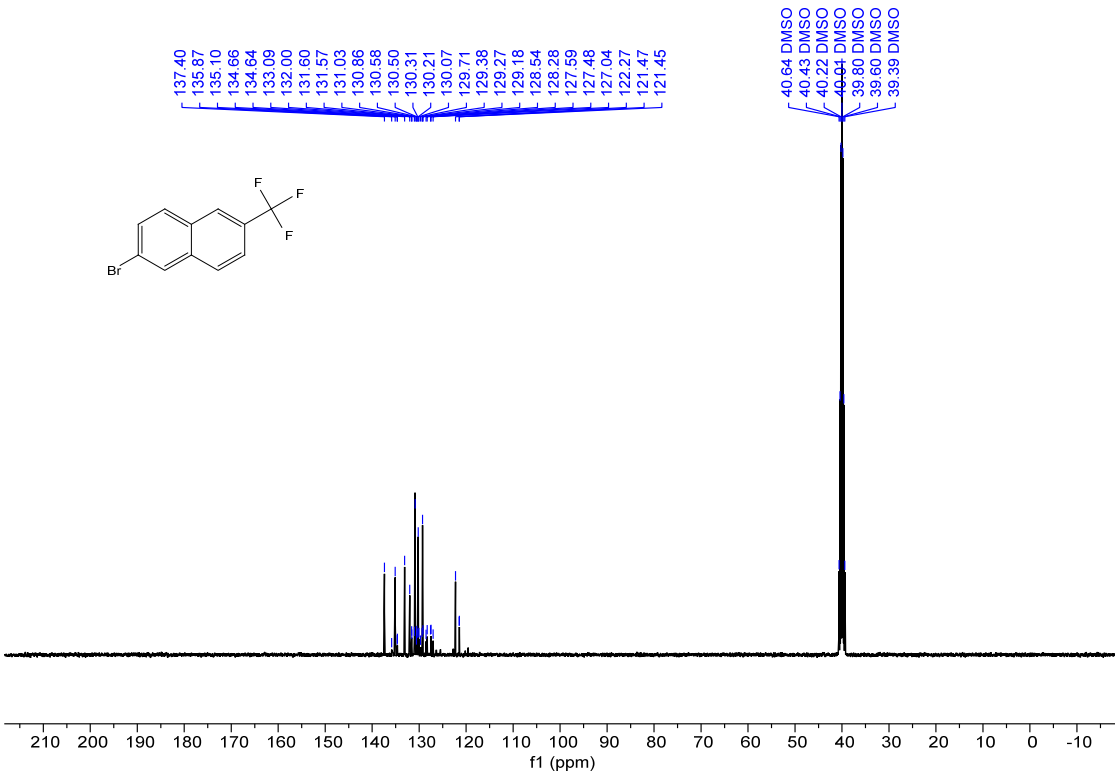
^{19}F NMR spectrum of **3d**

^{1}H NMR (400 MHz, DMSO-d_6)



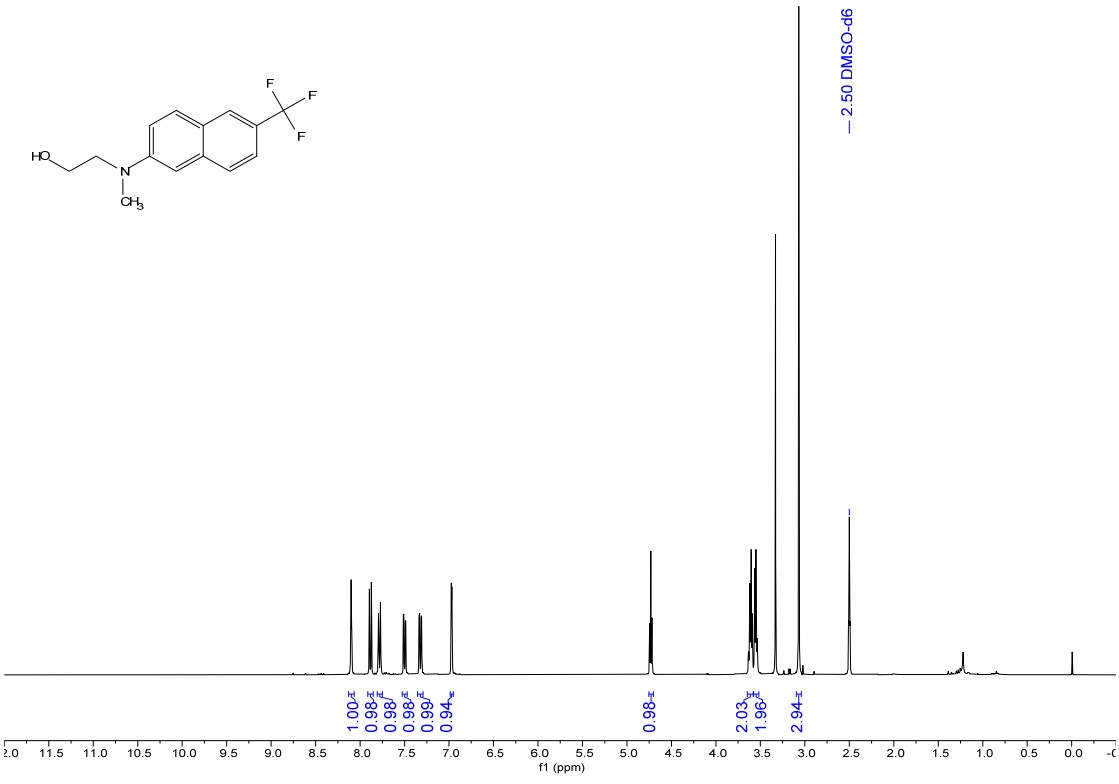
^{1}H NMR spectrum of **3f-2**

^{13}C NMR (101 MHz, DMSO-*d*$_6$)



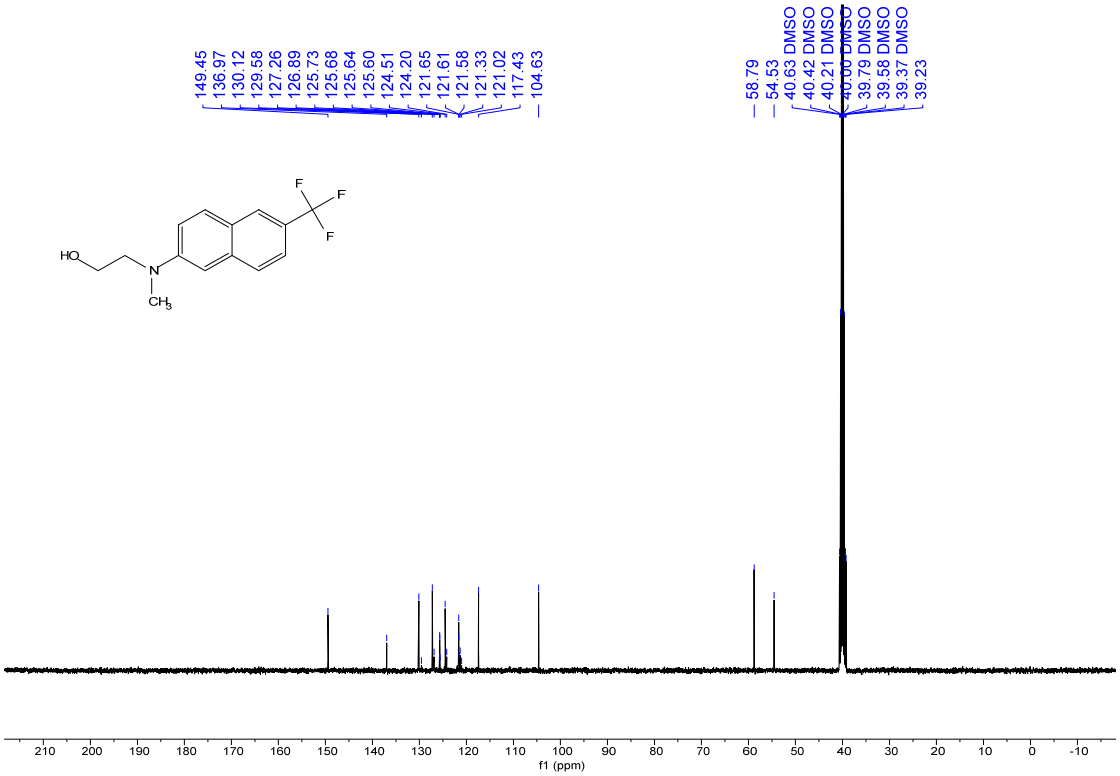
^{13}C NMR spectrum of **3f-2**

^{1}H NMR (400 MHz, DMSO-*d*$_6$)



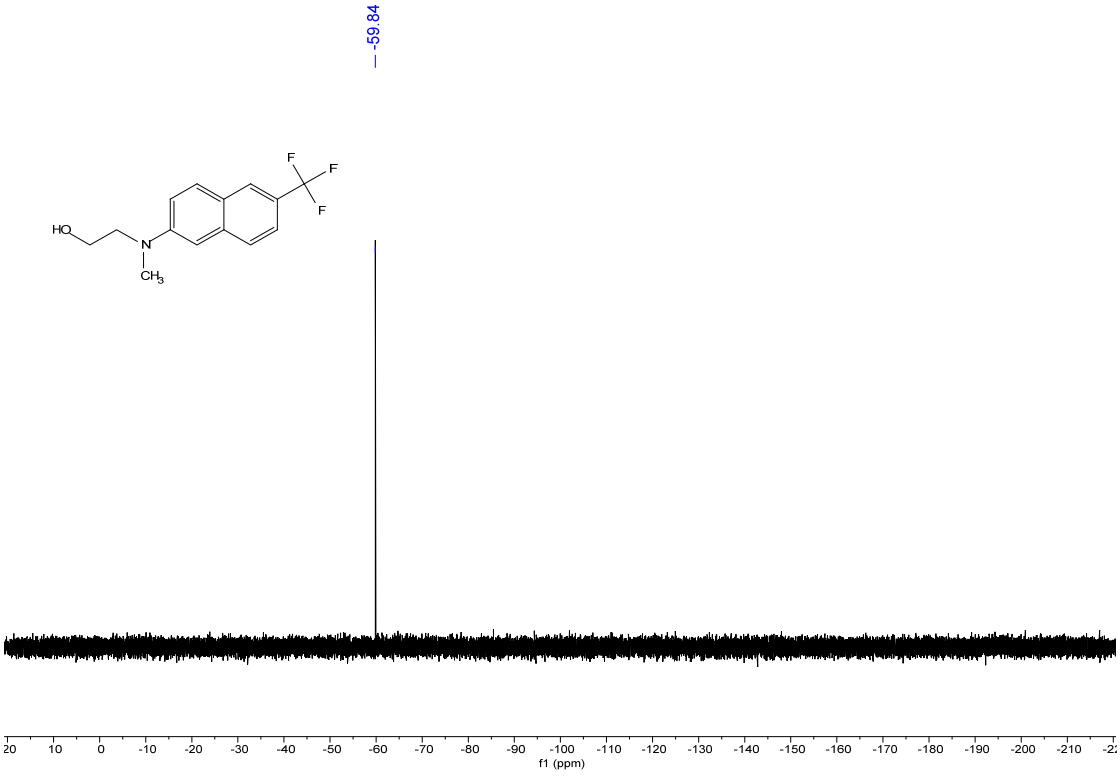
^{1}H NMR spectrum of **3f**

^{13}C NMR (101 MHz, DMSO-*d*$_6$)



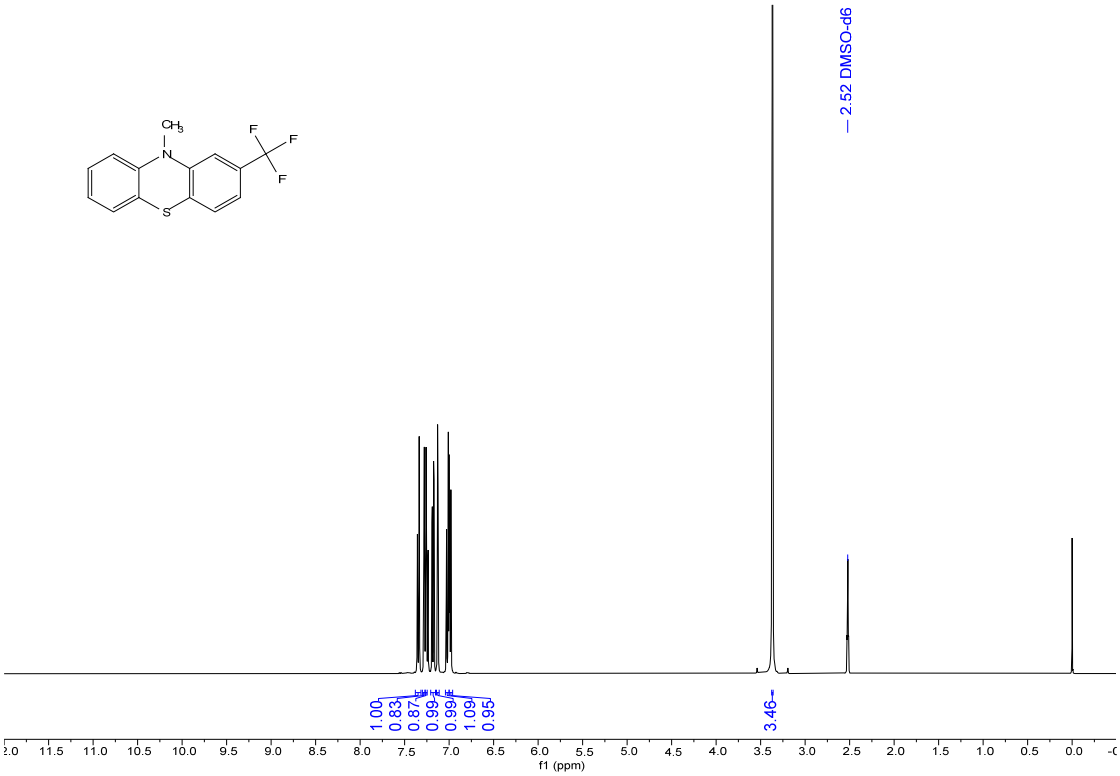
^{13}C NMR spectrum of **3f**

^{19}F NMR (376 MHz, DMSO-*d*$_6$)



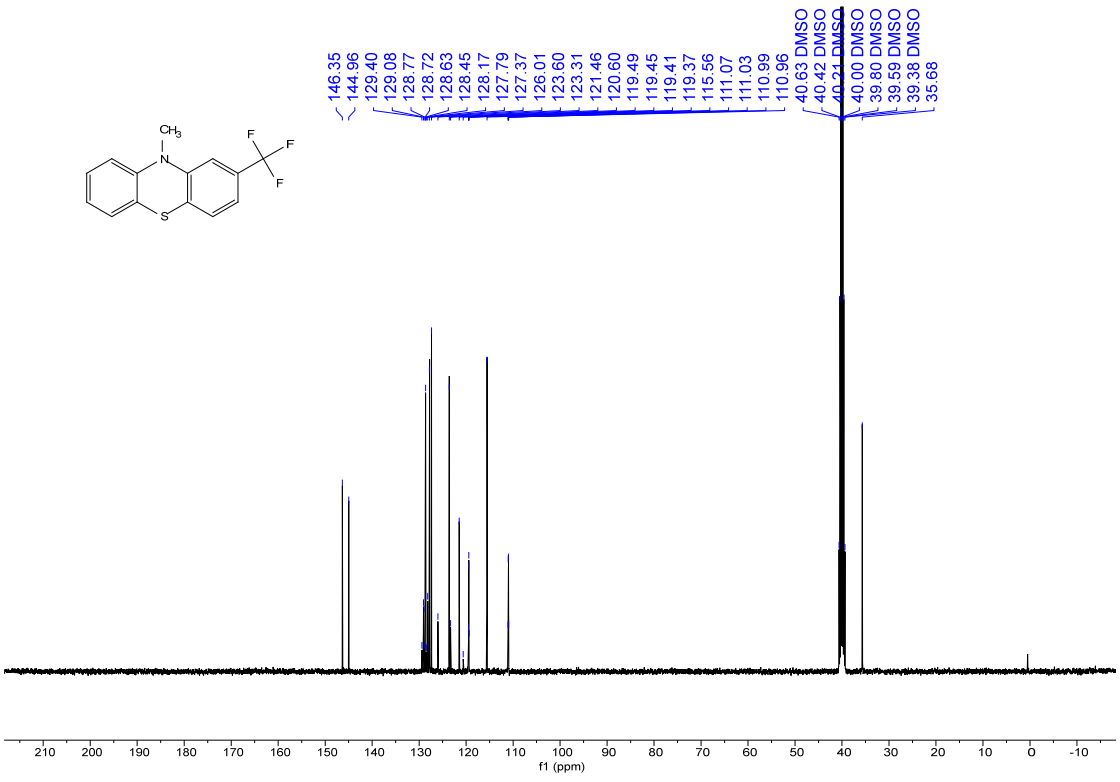
^{19}F NMR spectrum of **3f**

^{1}H NMR (400 MHz, DMSO-d_6)



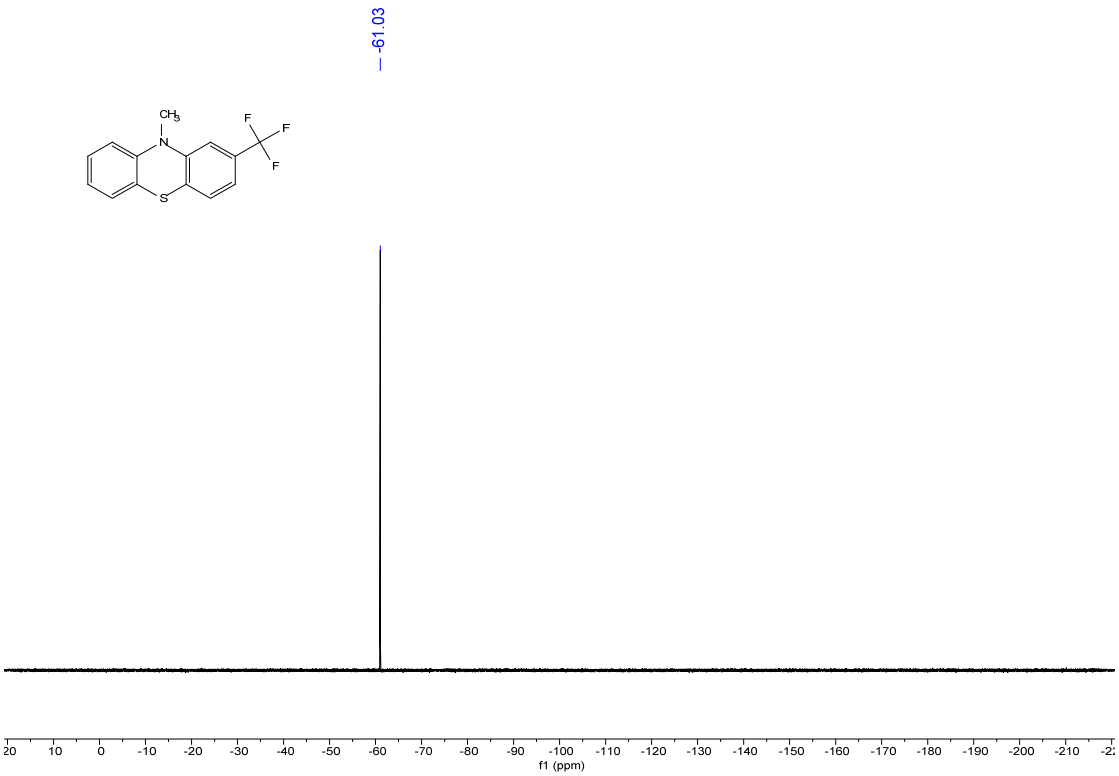
^{1}H NMR spectrum of **4b**

^{13}C NMR (101 MHz, DMSO-d_6)



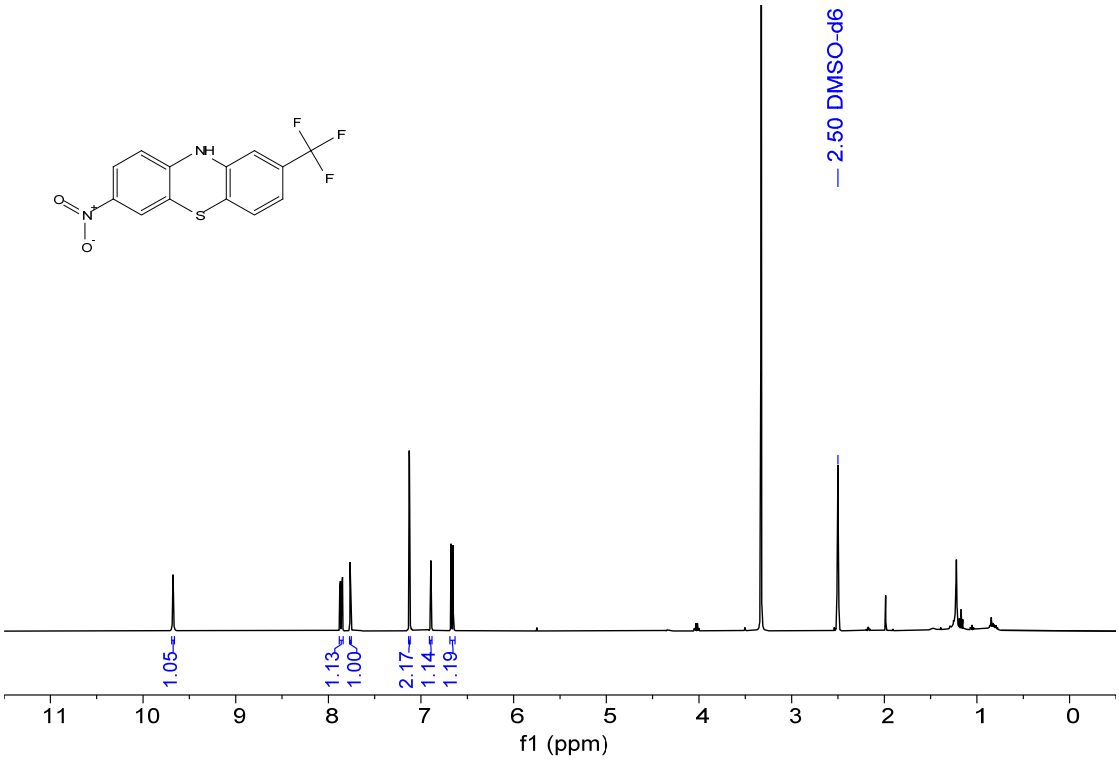
^{13}C NMR spectrum of **4b**

^{19}F NMR (376 MHz, DMSO-d_6)



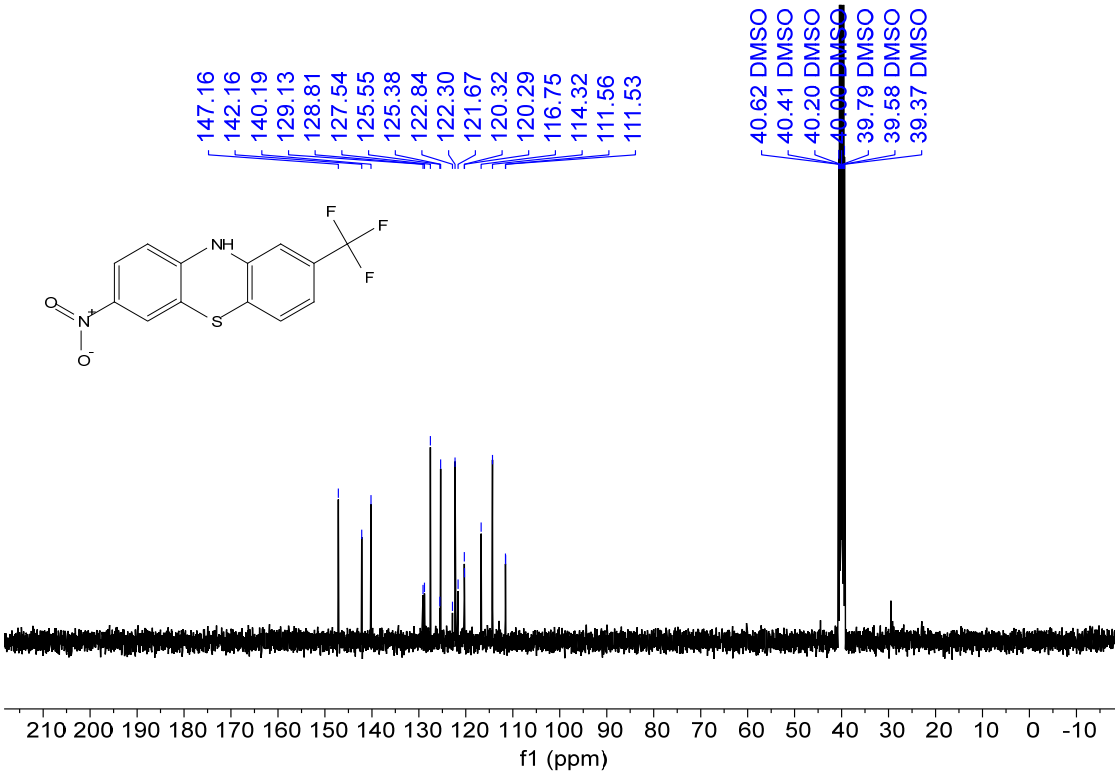
^{19}F NMR spectrum of **4b**

^{1}H NMR (400 MHz, $CDCl_3$)



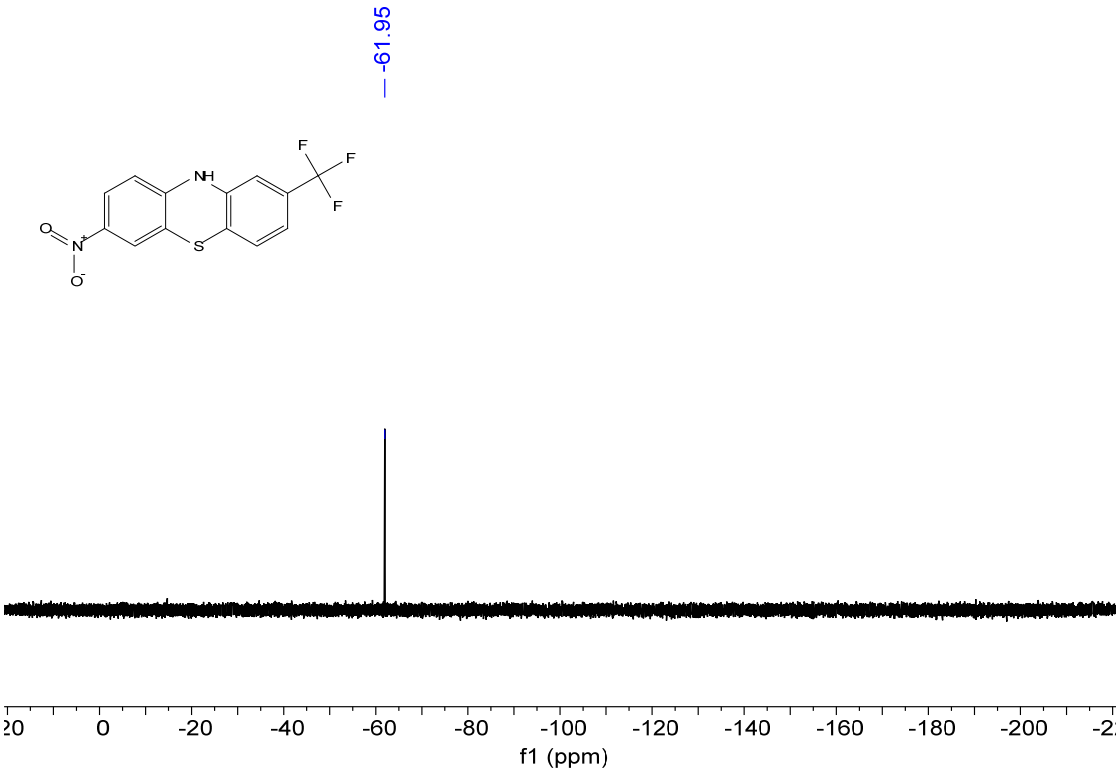
^{1}H NMR spectrum of **4c-1**

^{13}C NMR (101 MHz, $CDCl_3$)



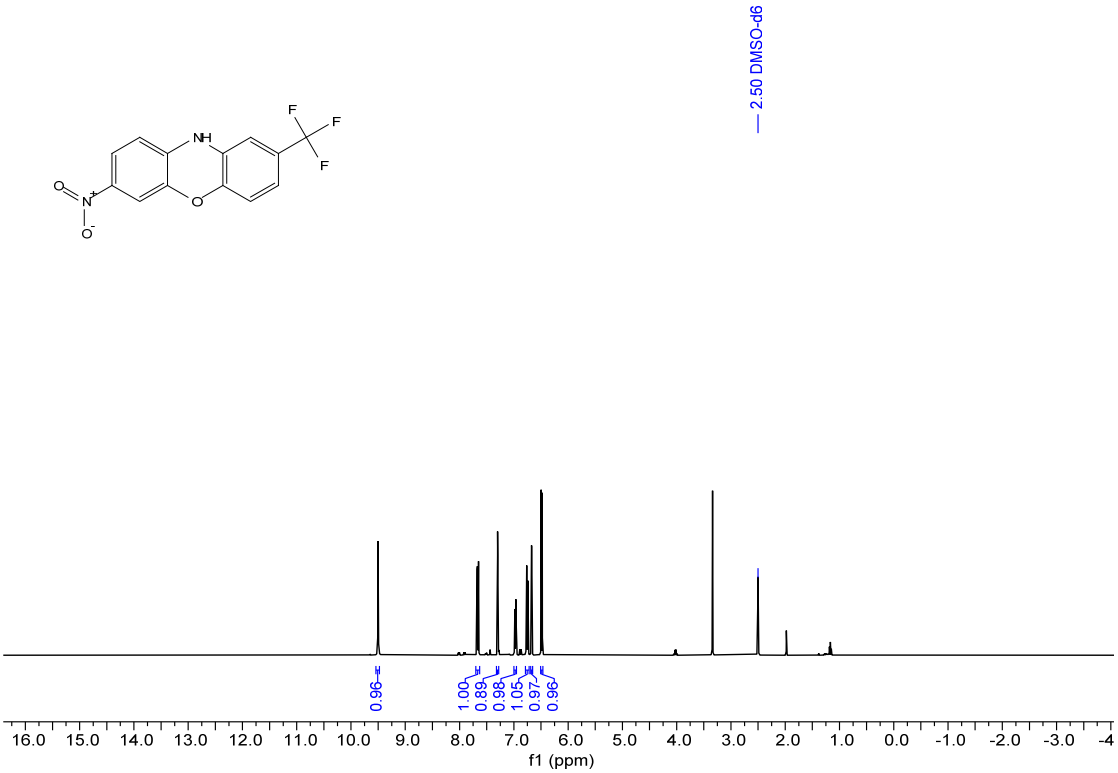
^{13}C NMR spectrum of **4c-1**

^{19}F NMR (376 MHz, $CDCl_3$)



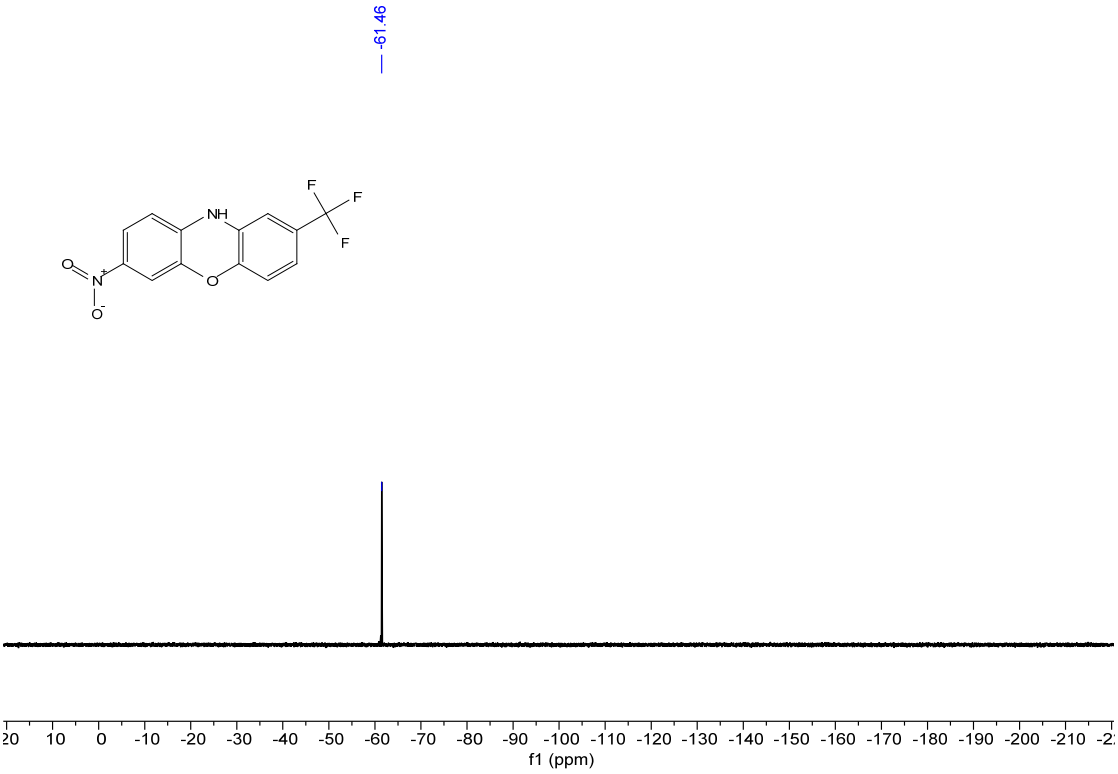
^{19}F NMR spectrum of **4c-1**

^{1}H NMR (400 MHz, DMSO-d_6)



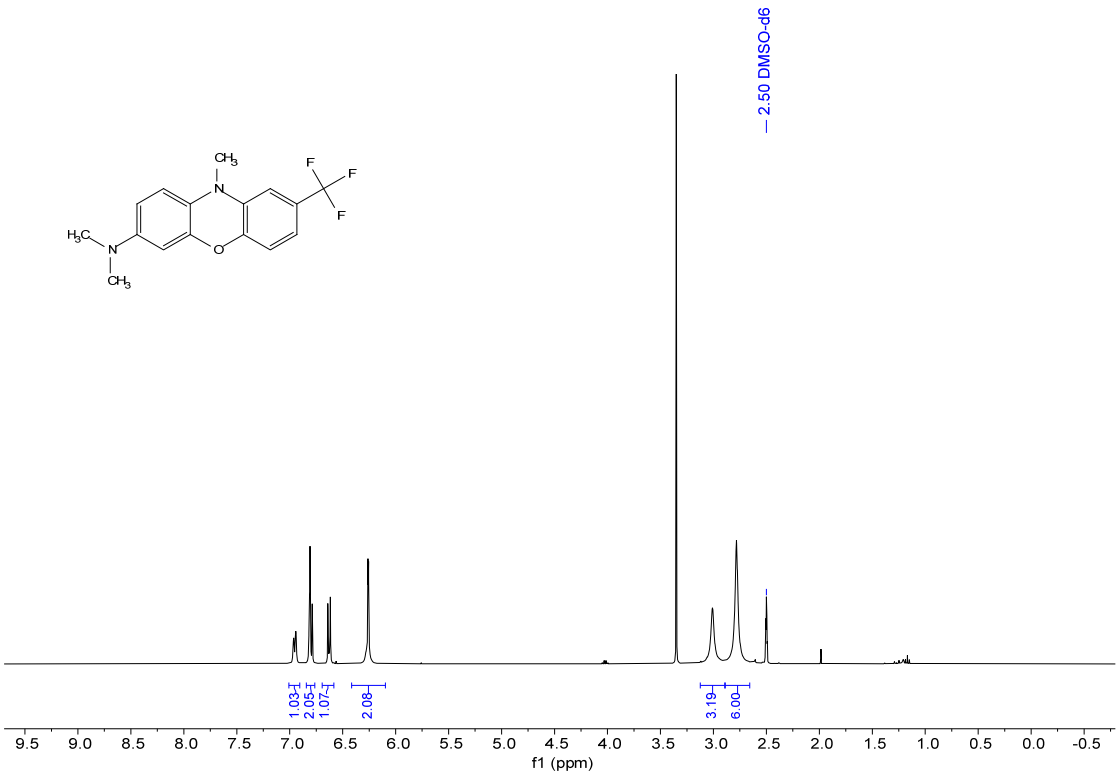
^{1}H NMR spectrum of **4d-1**

^{19}F NMR (376 MHz, $CDCl_3$)



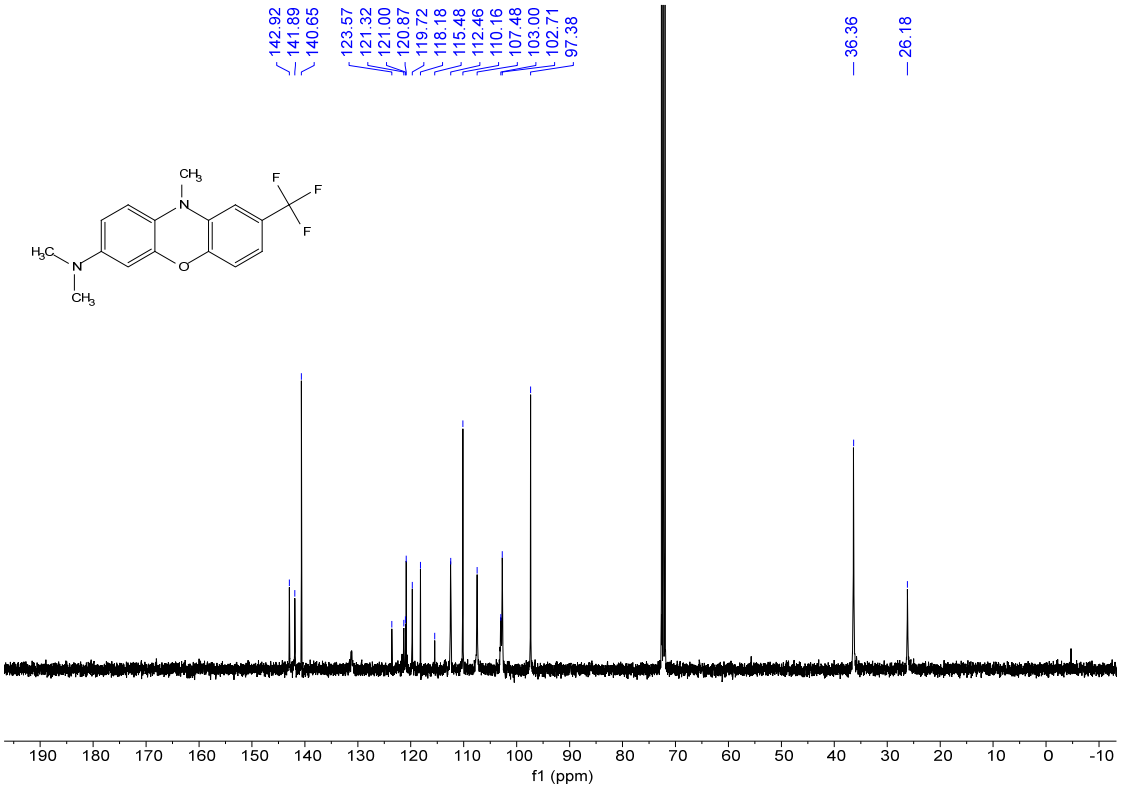
^{19}F NMR spectrum of **4d-1**

^{1}H NMR (400 MHz, DMSO-d_6)



^{1}H NMR spectrum of **4d**

^{13}C NMR (101 MHz, DMSO-d_6)

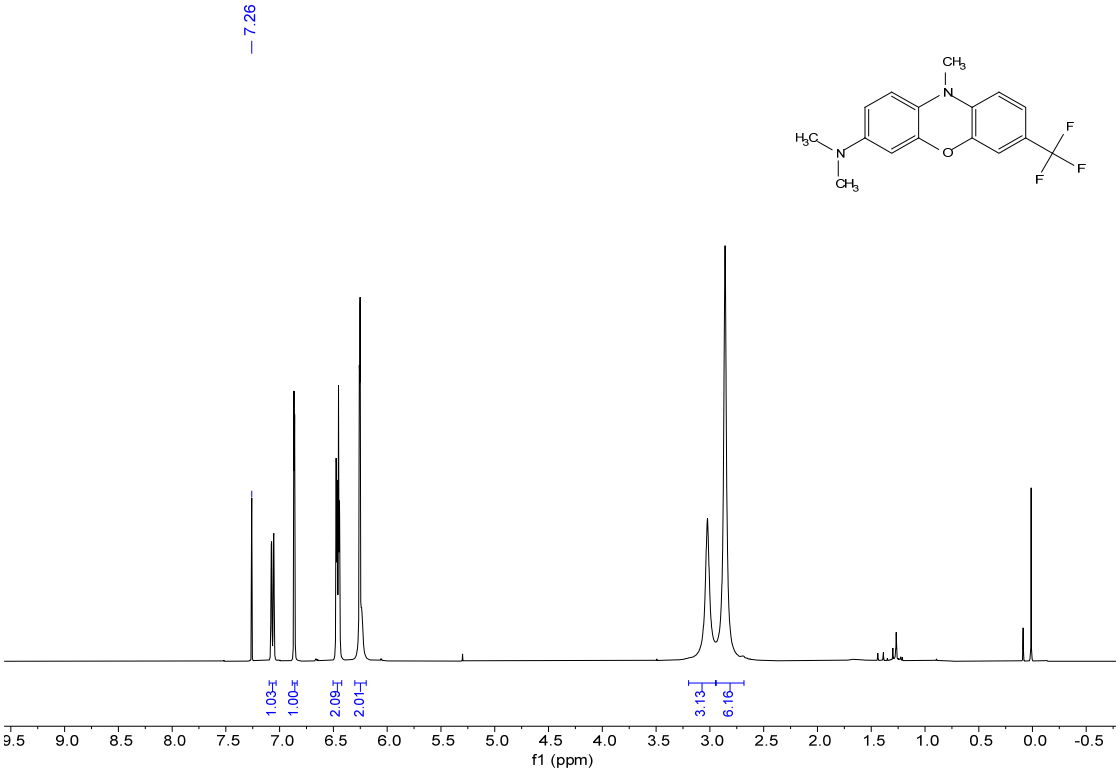


^{13}C NMR spectrum of **4d**

^{19}F NMR (376 MHz, DMSO-d_6)

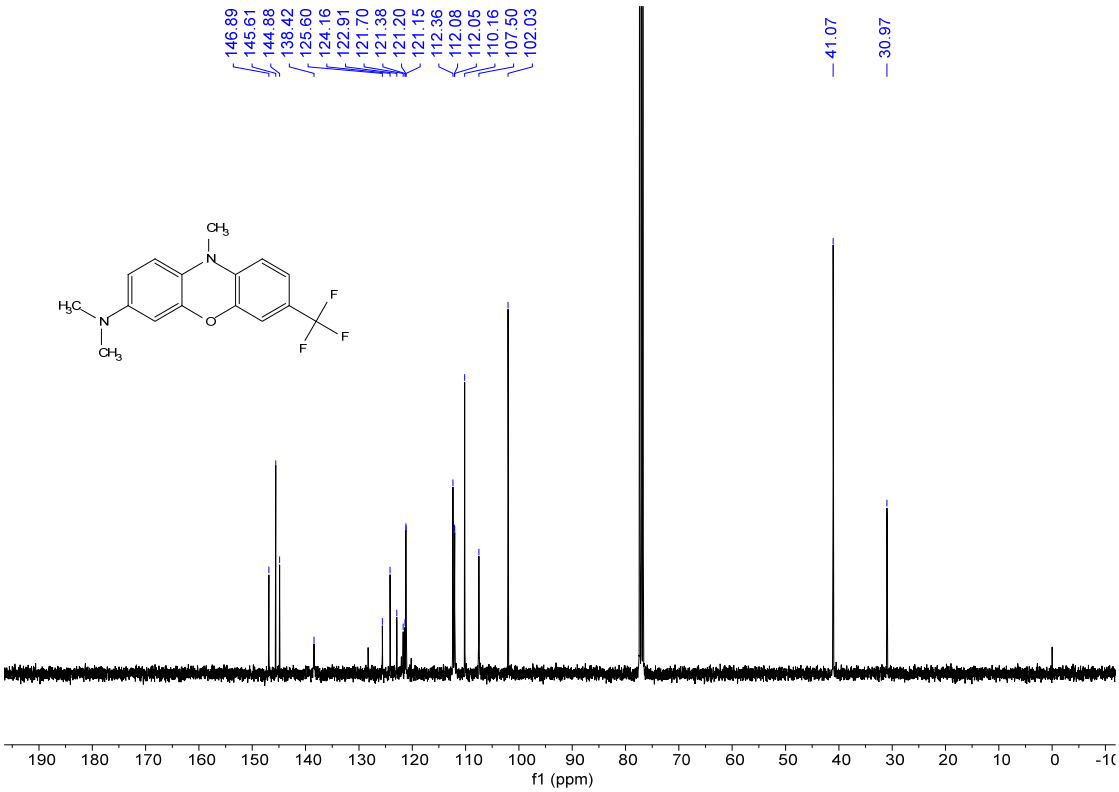
^{19}F NMR spectrum of **4d**

^{1}H NMR (400 MHz, $CDCl_3$)



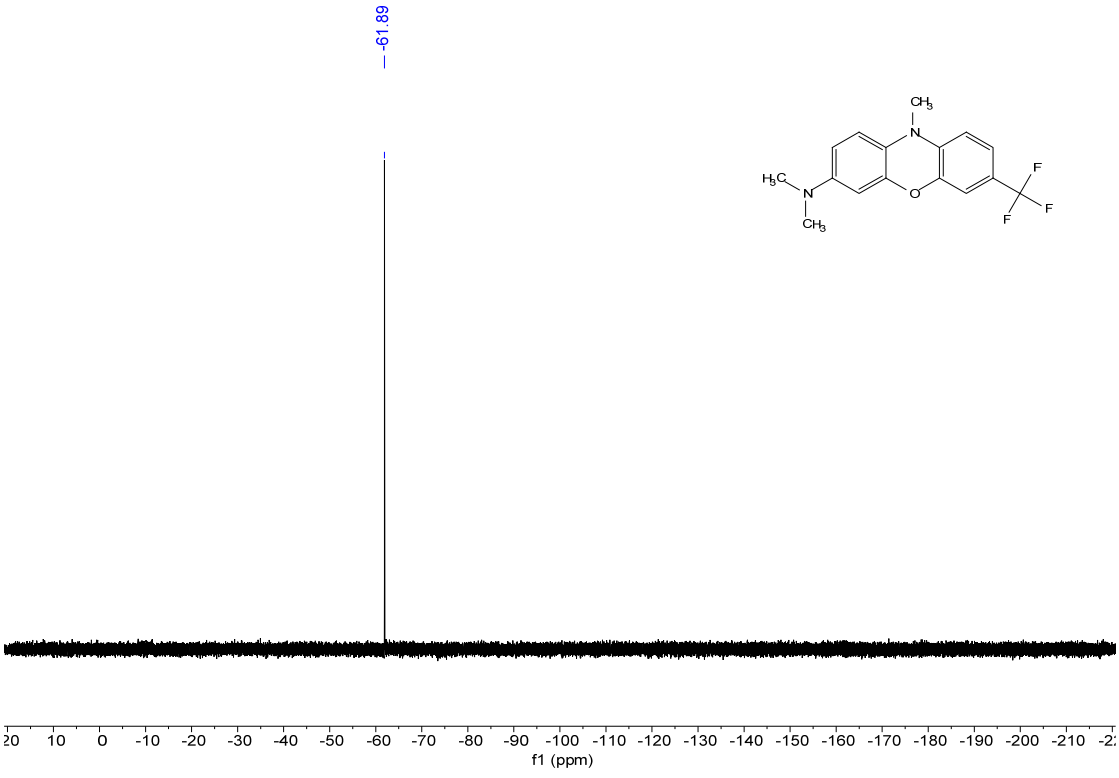
^{1}H NMR spectrum of **4e**

^{13}C NMR (101 MHz, $CDCl_3$)



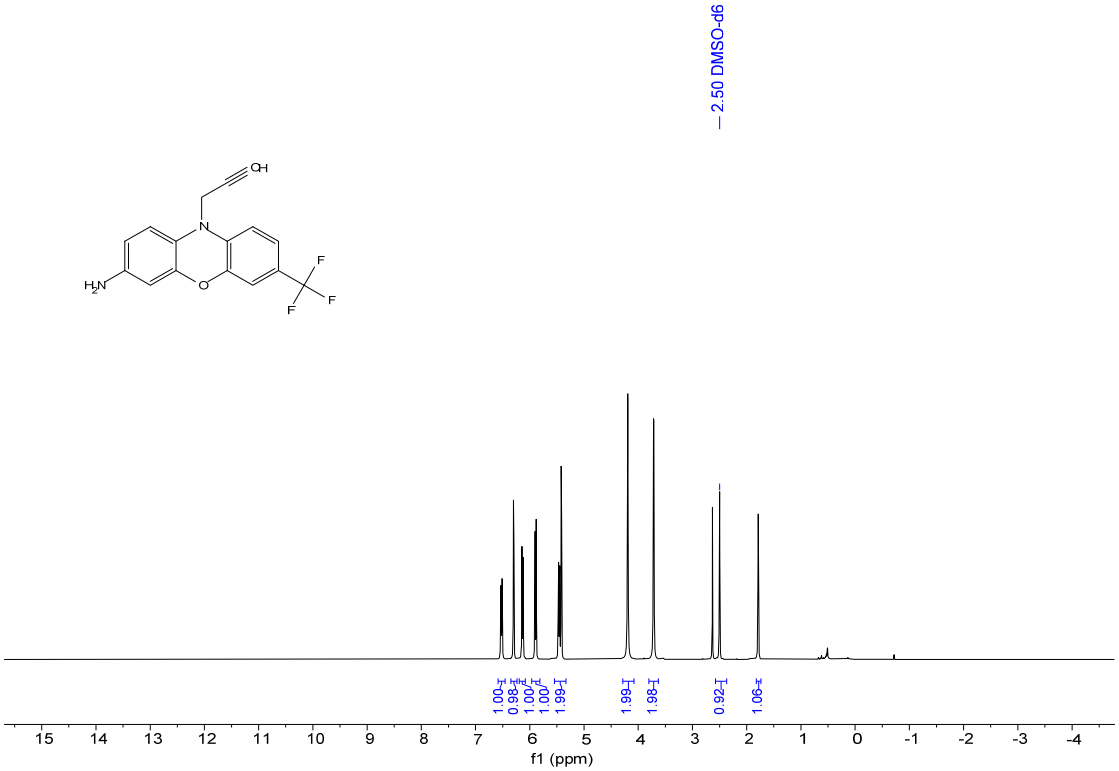
^{13}C NMR spectrum of **4e**

^{19}F NMR (376 MHz, $CDCl_3$)



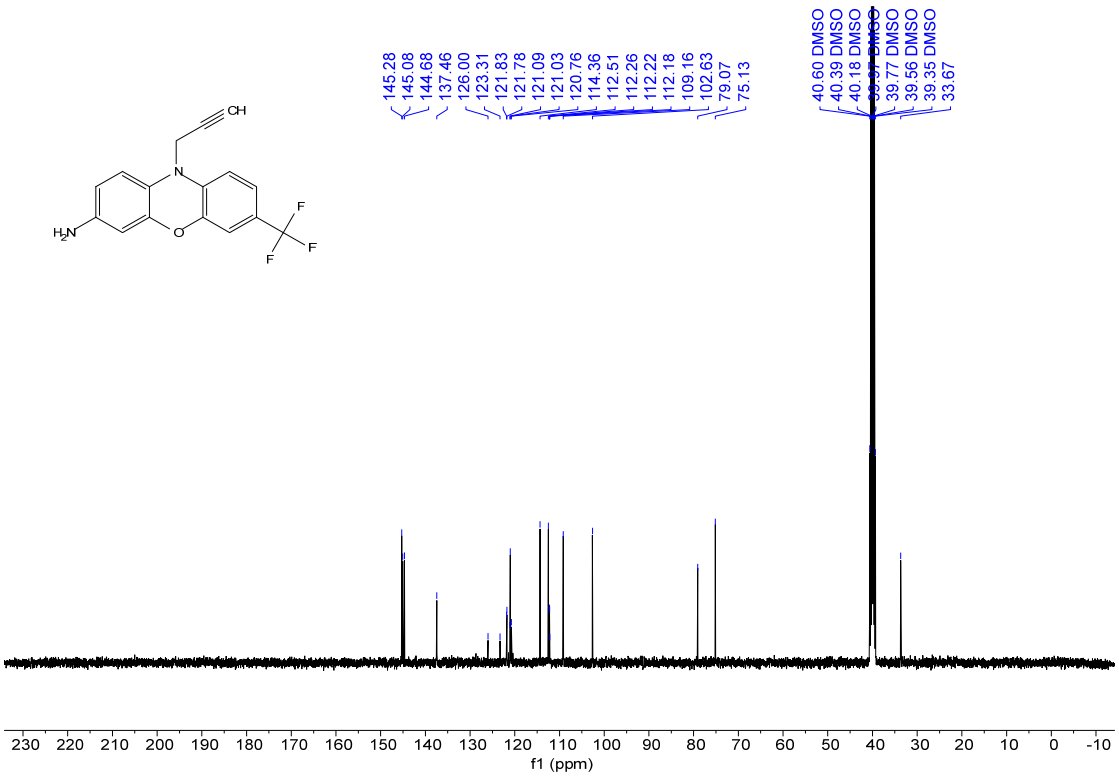
^{19}F NMR spectrum of **4e**

^{1}H NMR (400 MHz, DMSO-d_6)



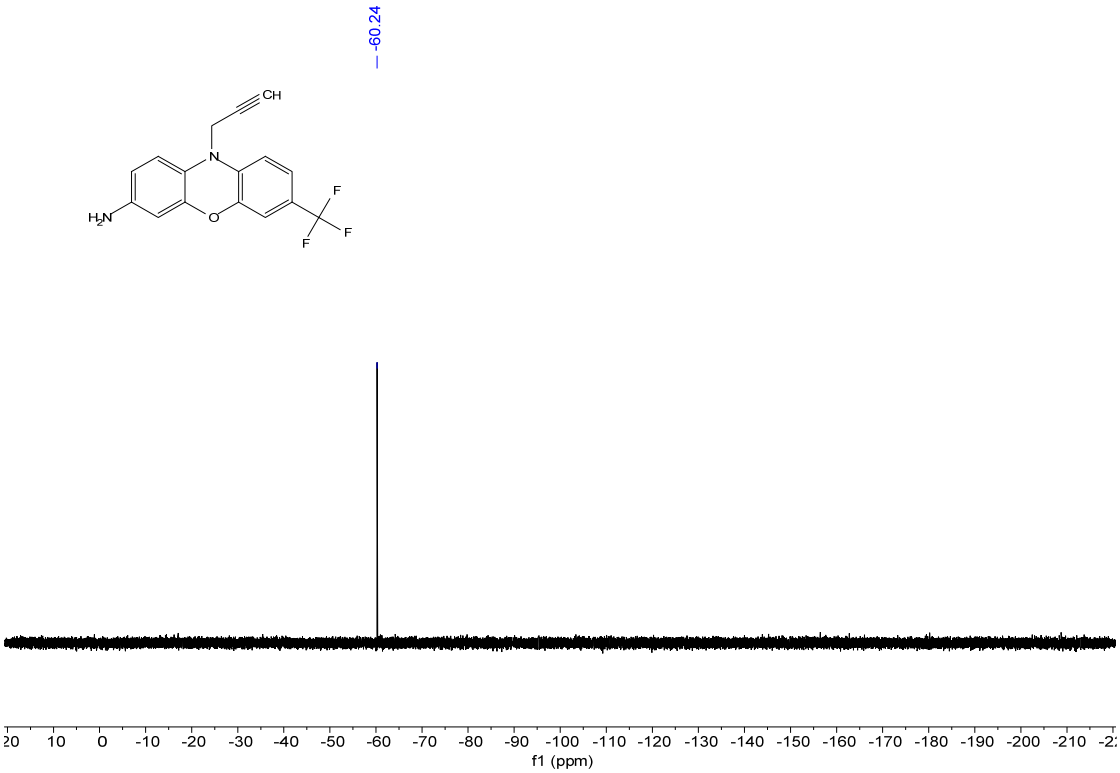
^{1}H NMR spectrum of **4f-2**

^{13}C NMR (101 MHz, DMSO-d_6)



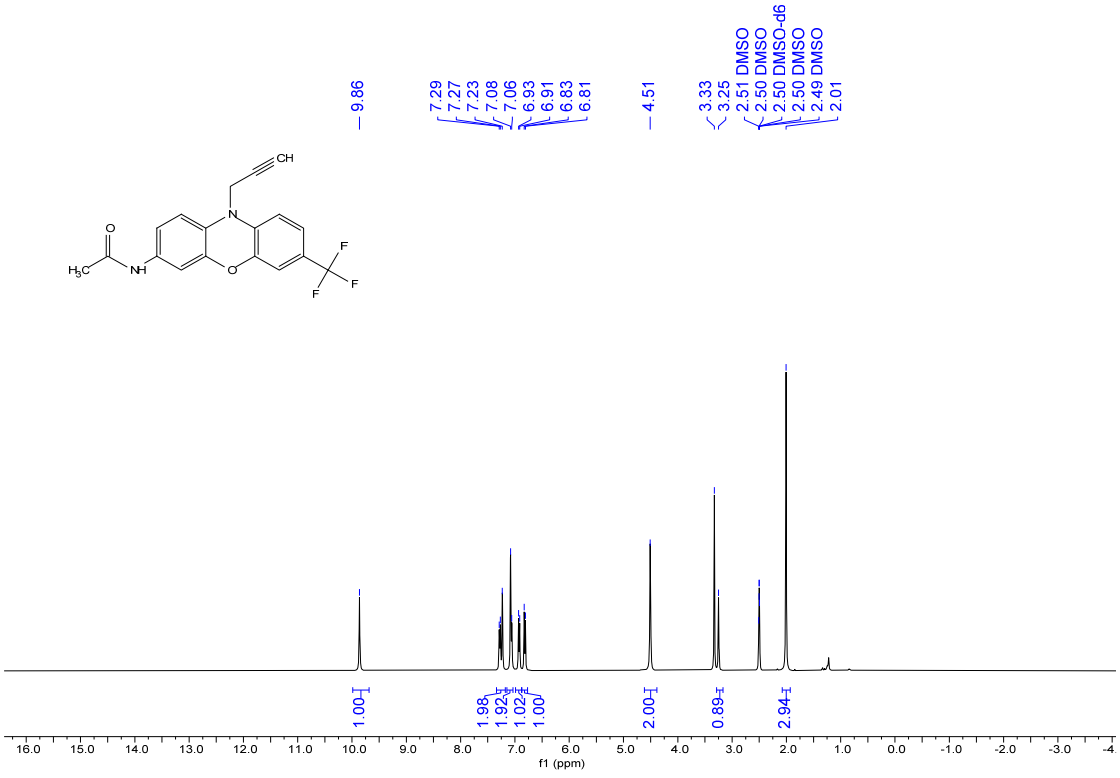
^{13}C NMR spectrum of **4f-2**

^{19}F NMR (376 MHz, DMSO-d_6)



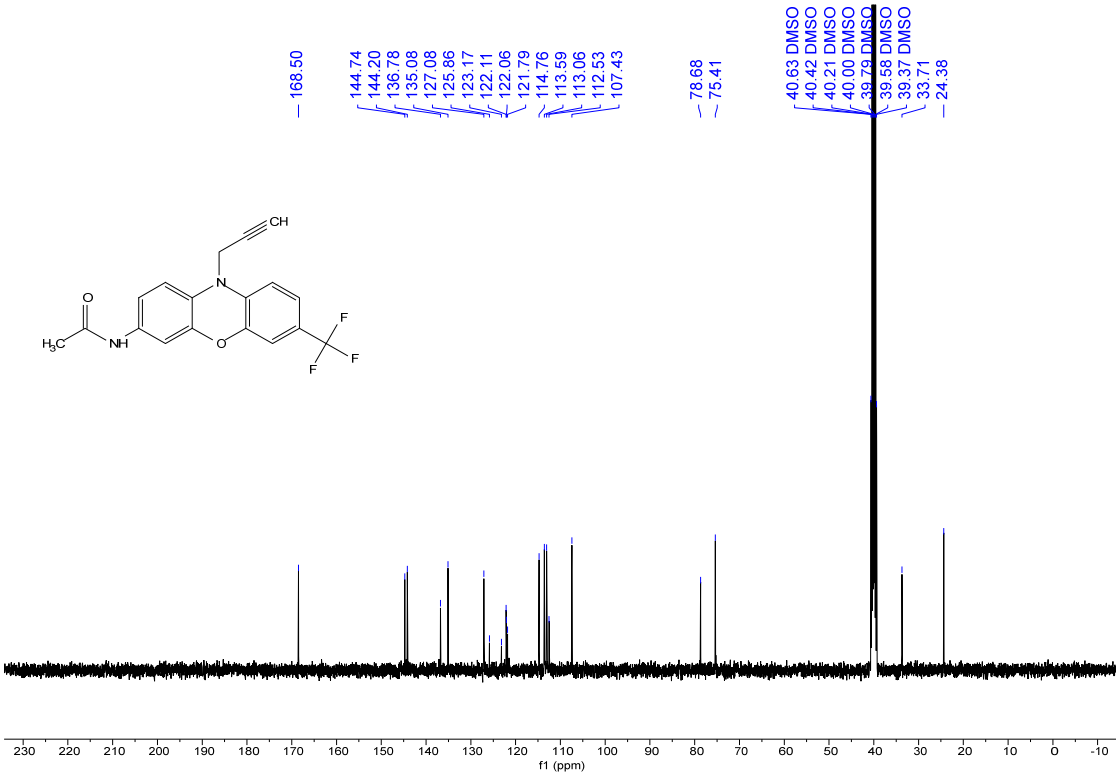
^{19}F NMR spectrum of **4f-2**

^{1}H NMR (400 MHz, DMSO-d_6)



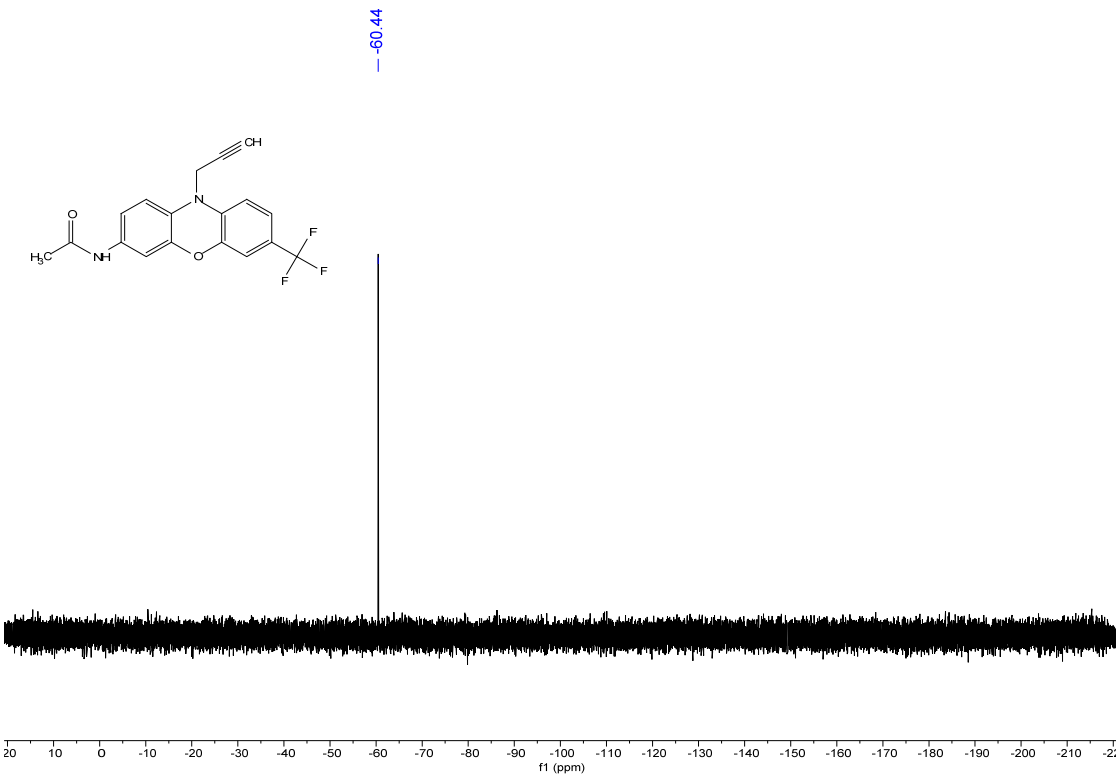
^{1}H NMR spectrum of **4f**

^{13}C NMR (101 MHz, DMSO-*d*$_6$)



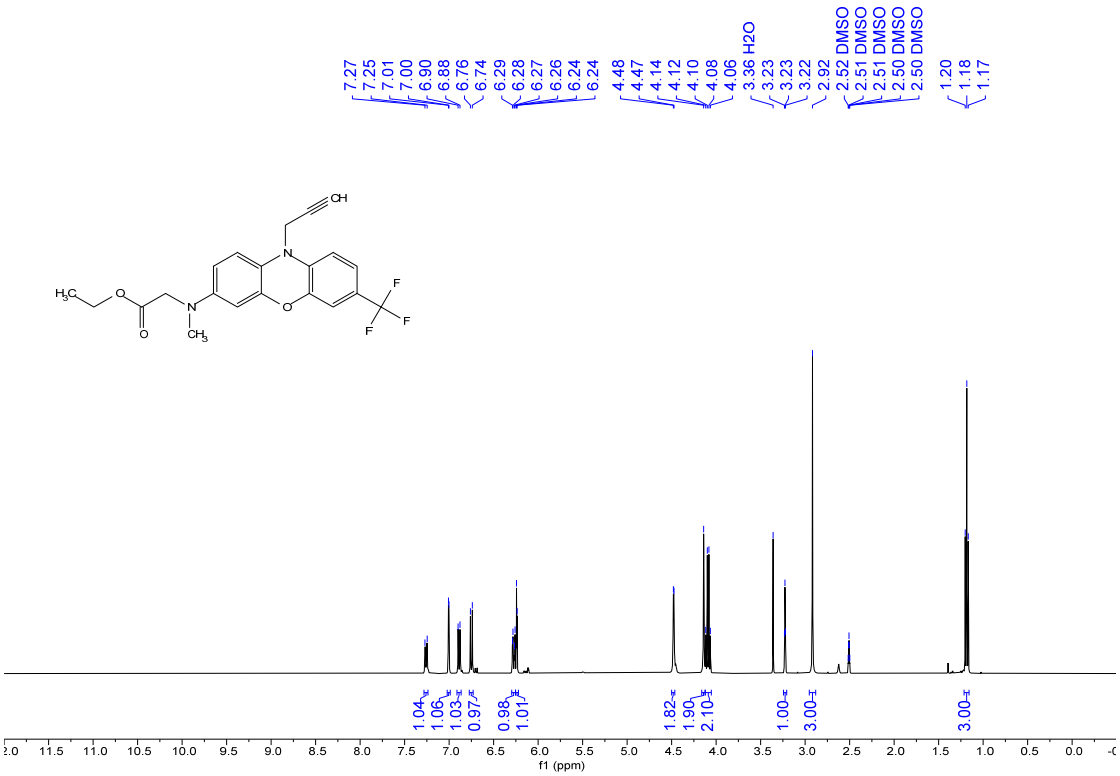
^{13}C NMR spectrum of **4f**

^{19}F NMR (376 MHz, DMSO-*d*$_6$)



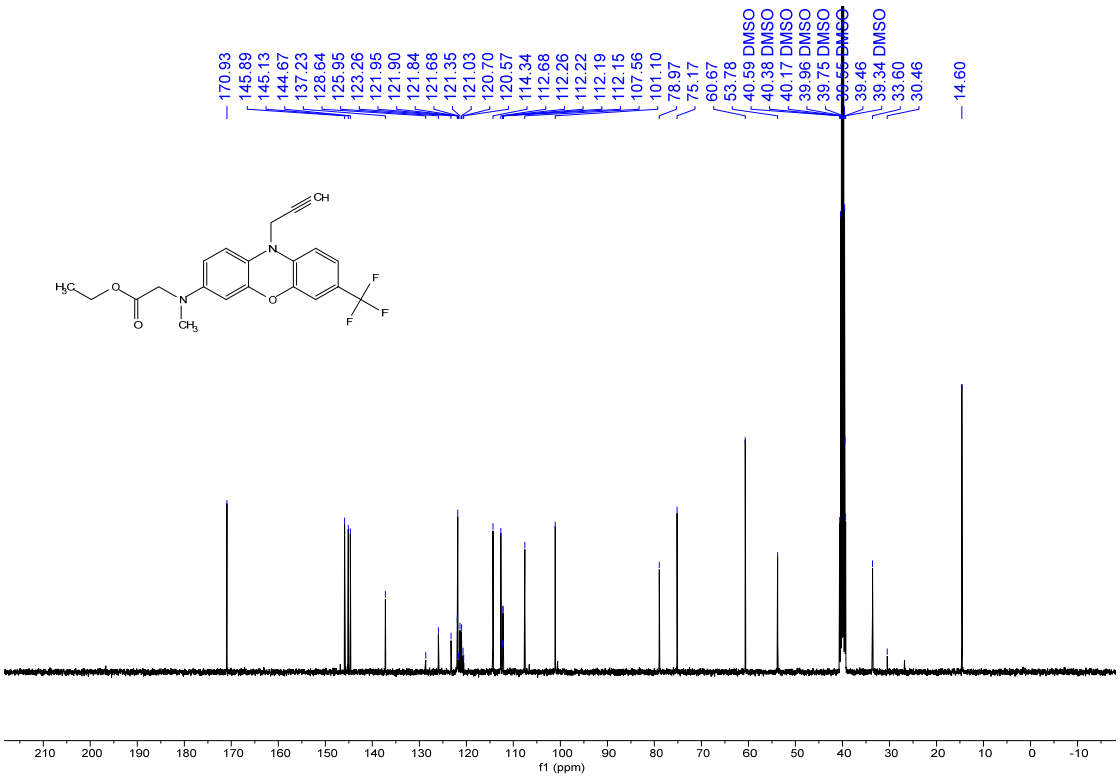
^{19}F NMR spectrum of **4f**

^{1}H NMR (400 MHz, DMSO-d_6)



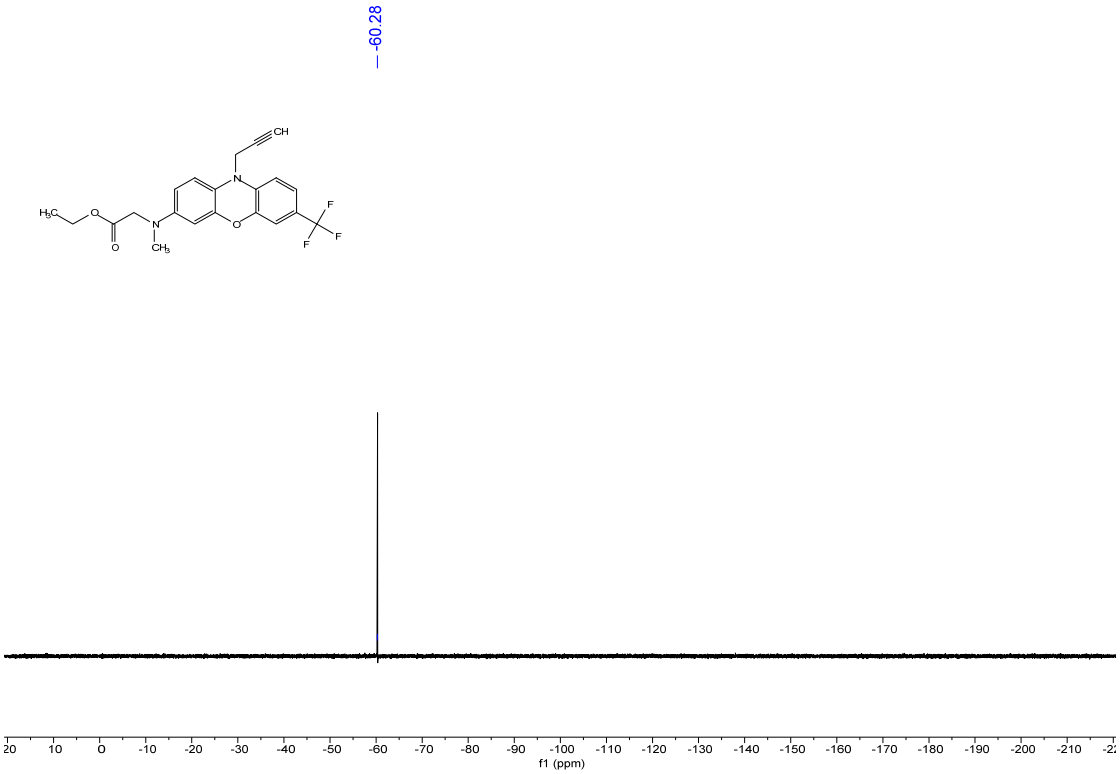
^{1}H NMR spectrum of **4g**

^{13}C NMR (101 MHz, DMSO-d_6)



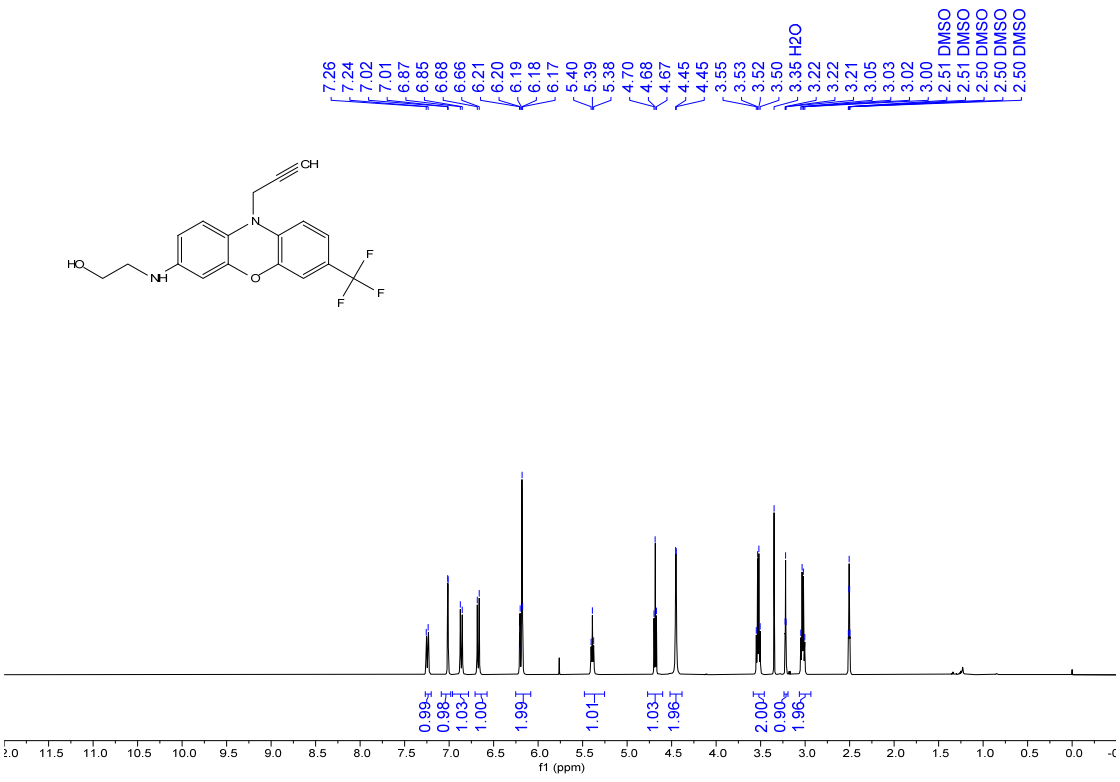
^{13}C NMR spectrum of **4g**

^{19}F NMR (376 MHz, DMSO-d_6)



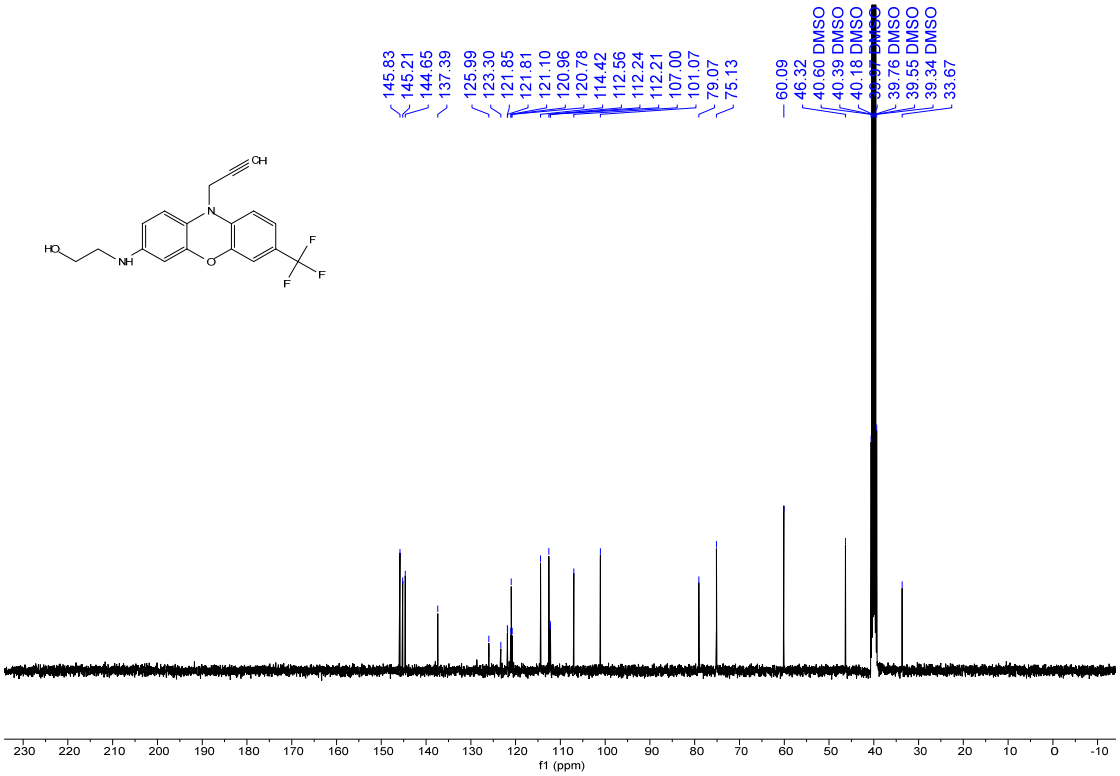
^{19}F NMR spectrum of **4g**

^{1}H NMR (400 MHz, DMSO-d_6)



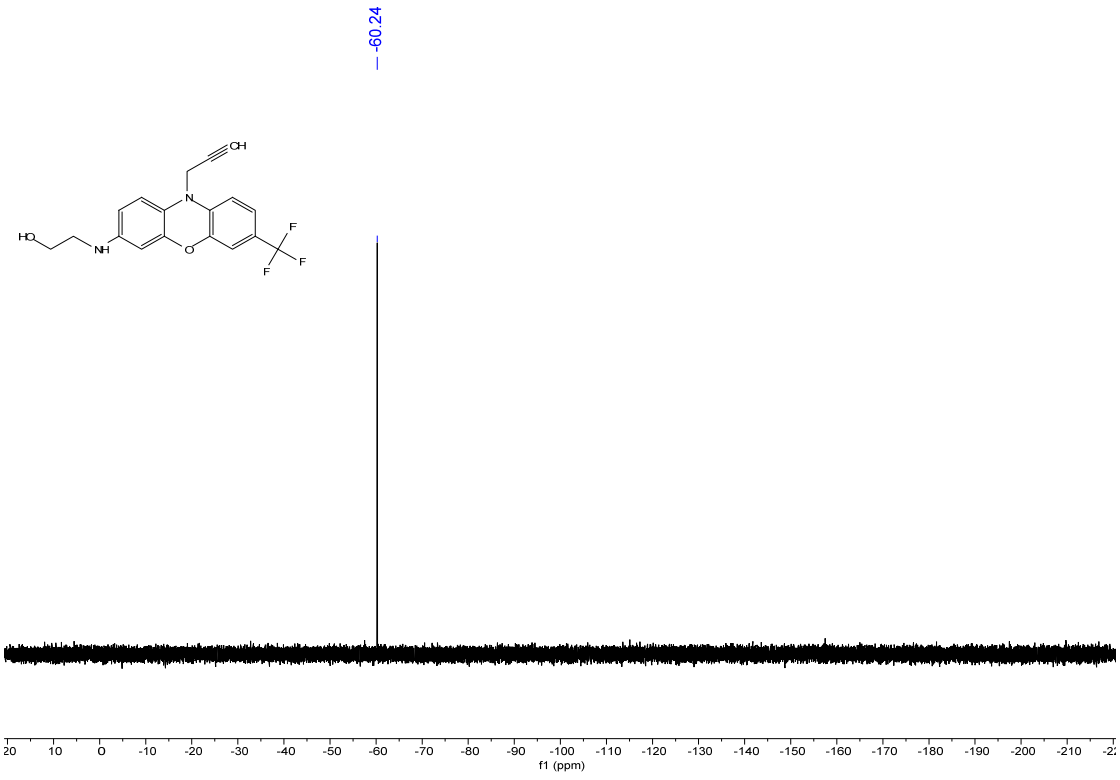
^{1}H NMR spectrum of **4h-1**

^{13}C NMR (101 MHz, DMSO-d_6)



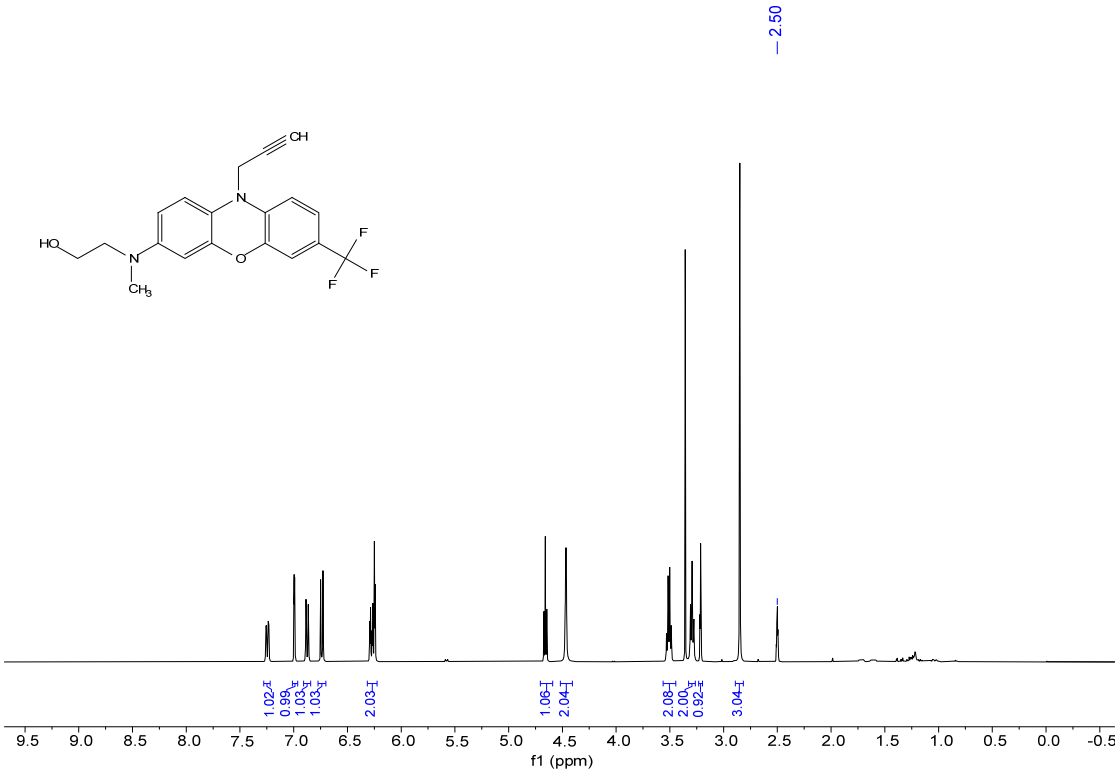
^{13}C NMR spectrum of **4h-1**

^{19}F NMR (376 MHz, DMSO-d_6)



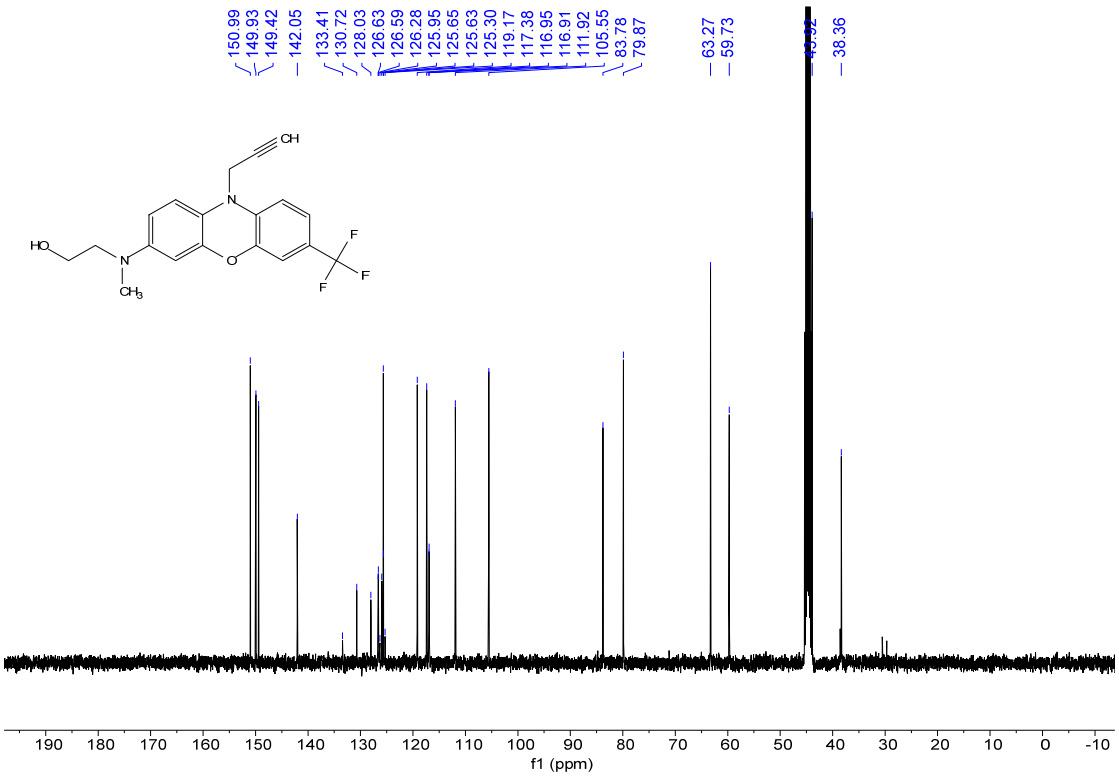
^{19}F NMR spectrum of **4h-1**

^{1}H NMR (400 MHz, DMSO-d_6)



^{1}H NMR spectrum of **4h**

^{13}C NMR (101 MHz, DMSO-d_6)

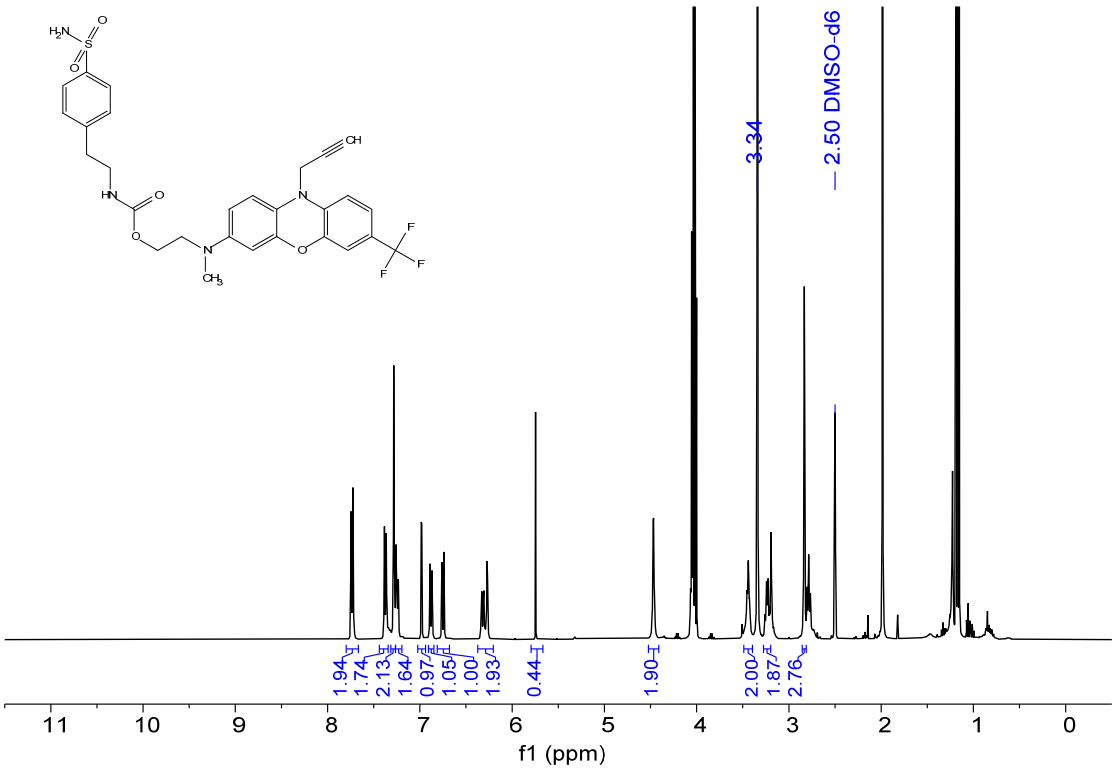


^{13}C NMR spectrum of **4h**

^{19}F NMR (376 MHz, DMSO-d_6)

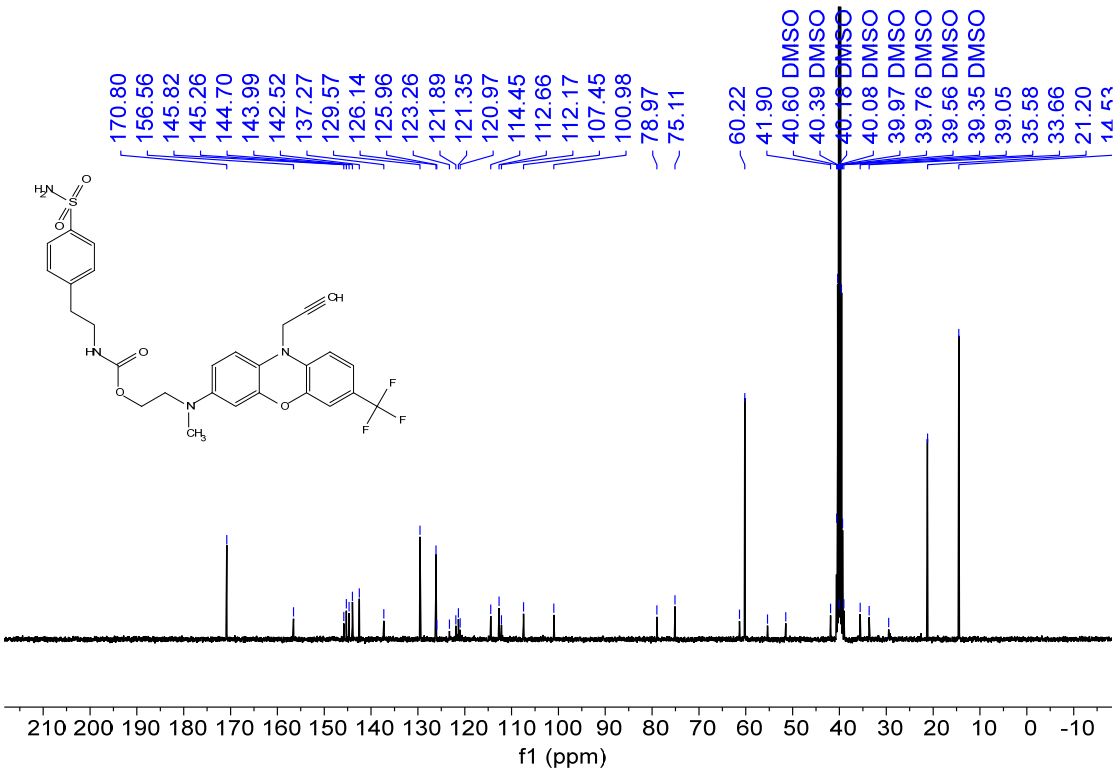
^{19}F NMR spectrum of **4h**

^{1}H NMR (400 MHz, DMSO-d_6)



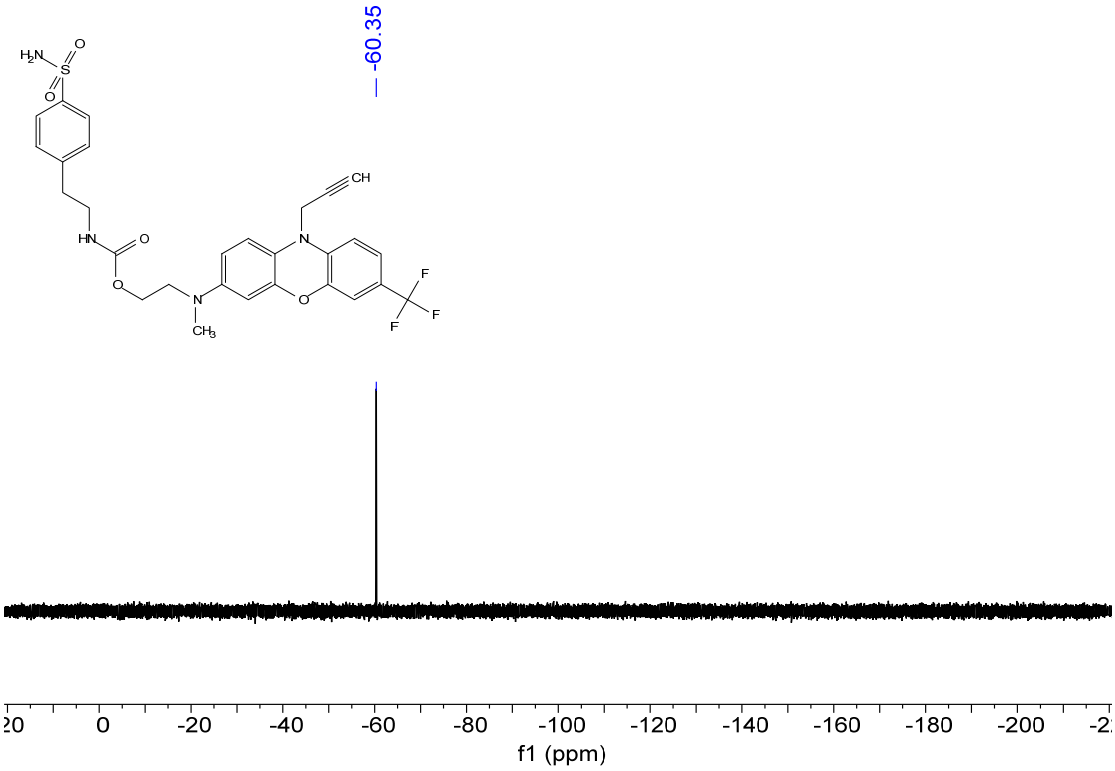
^{1}H NMR spectrum of **P1**

^{13}C NMR (101 MHz, DMSO-d_6)



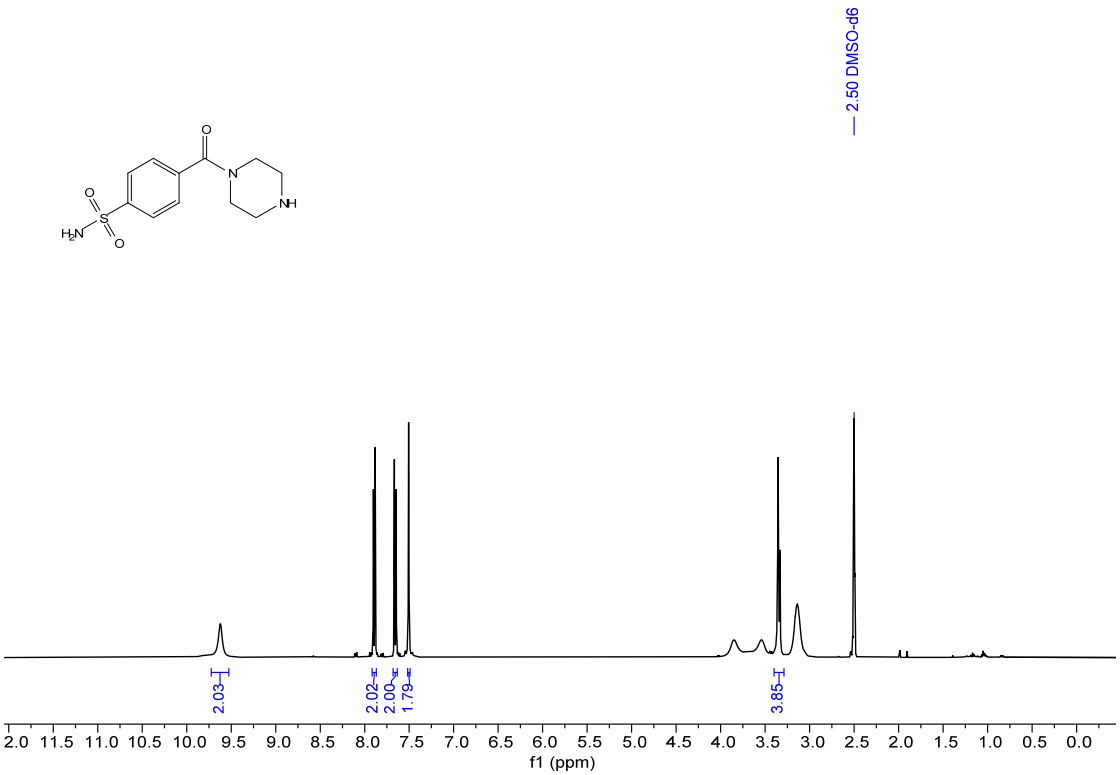
^{13}C NMR spectrum of **P1**

^{19}F NMR (376 MHz, DMSO-d_6)



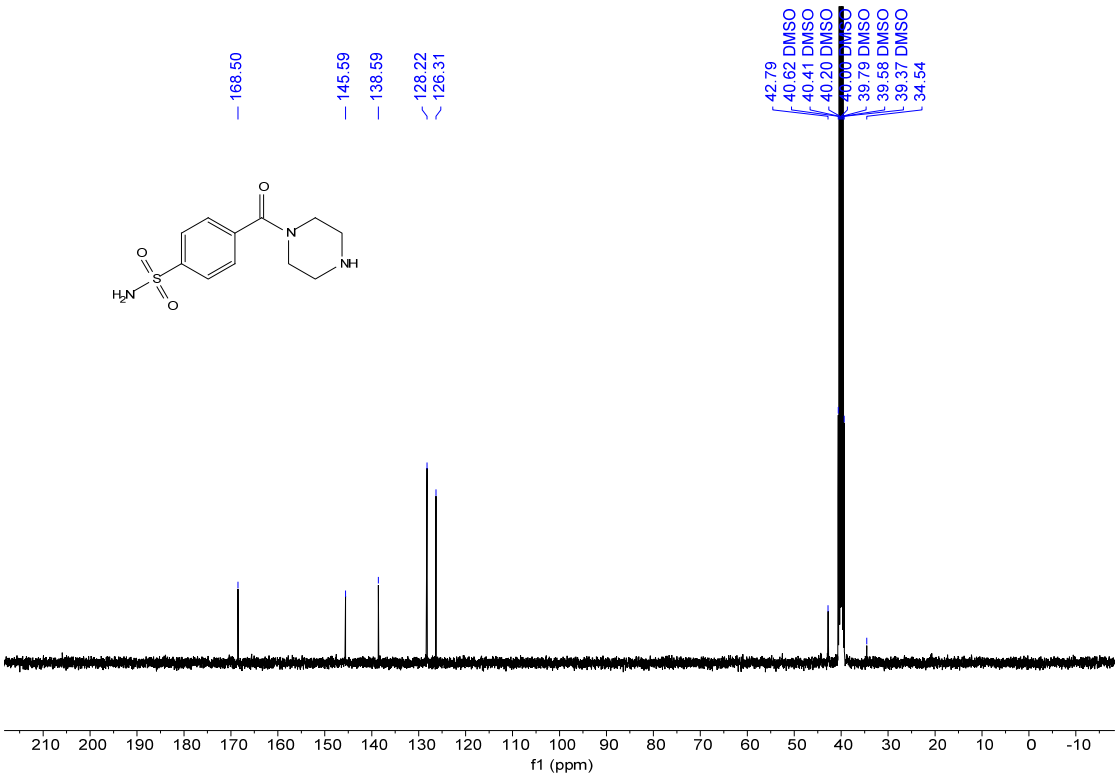
^{19}F NMR spectrum of **P1**

^{1}H NMR (400 MHz, DMSO-d_6)



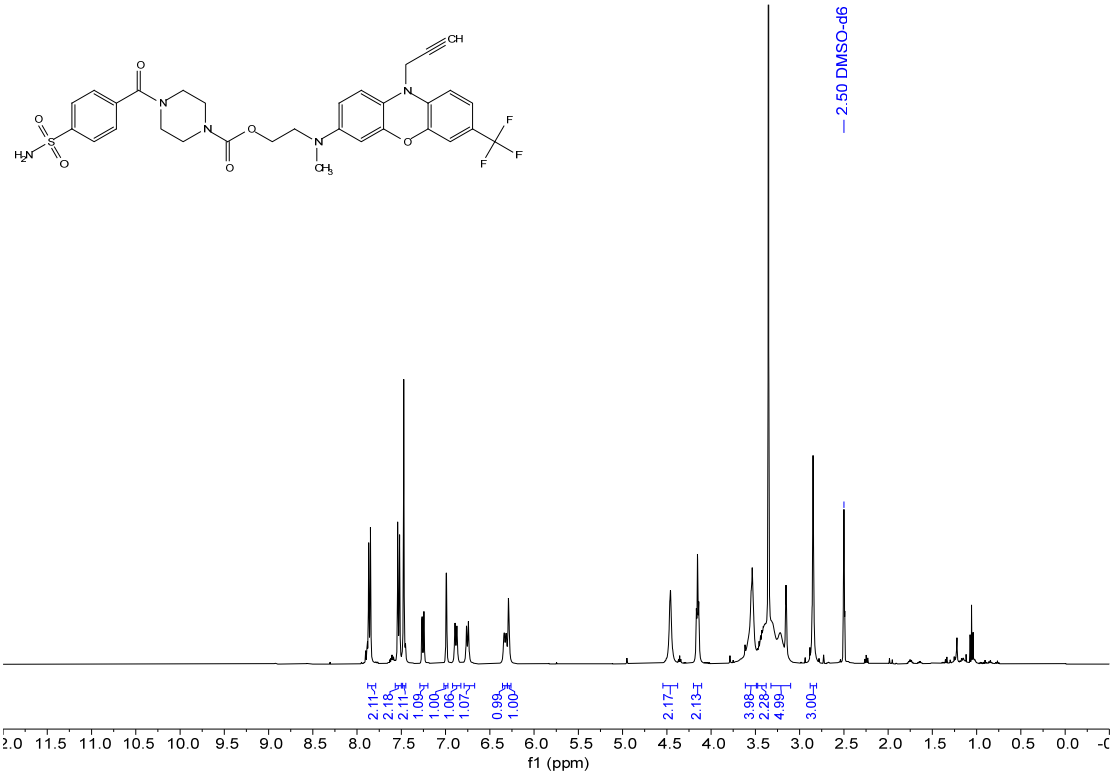
^{1}H NMR spectrum of **P2-1**

^{13}C NMR (101 MHz, DMSO-d_6)



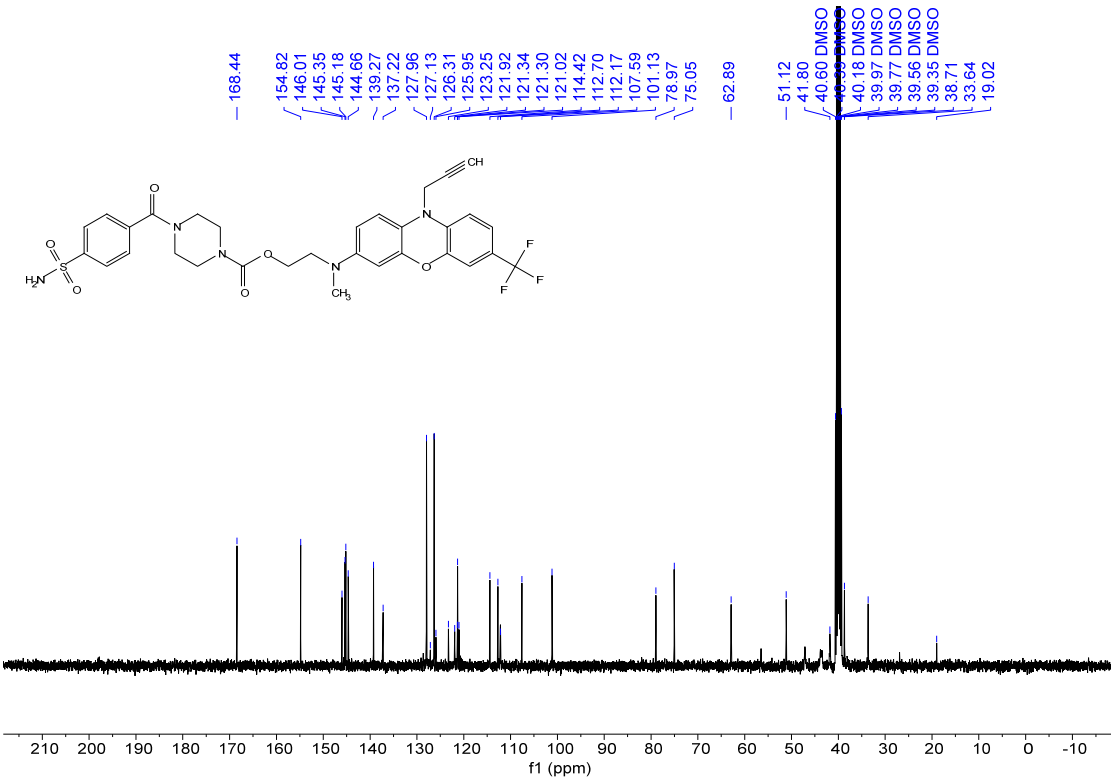
^{13}C NMR spectrum of **P2-1**

^{1}H NMR (400 MHz, DMSO-d_6)



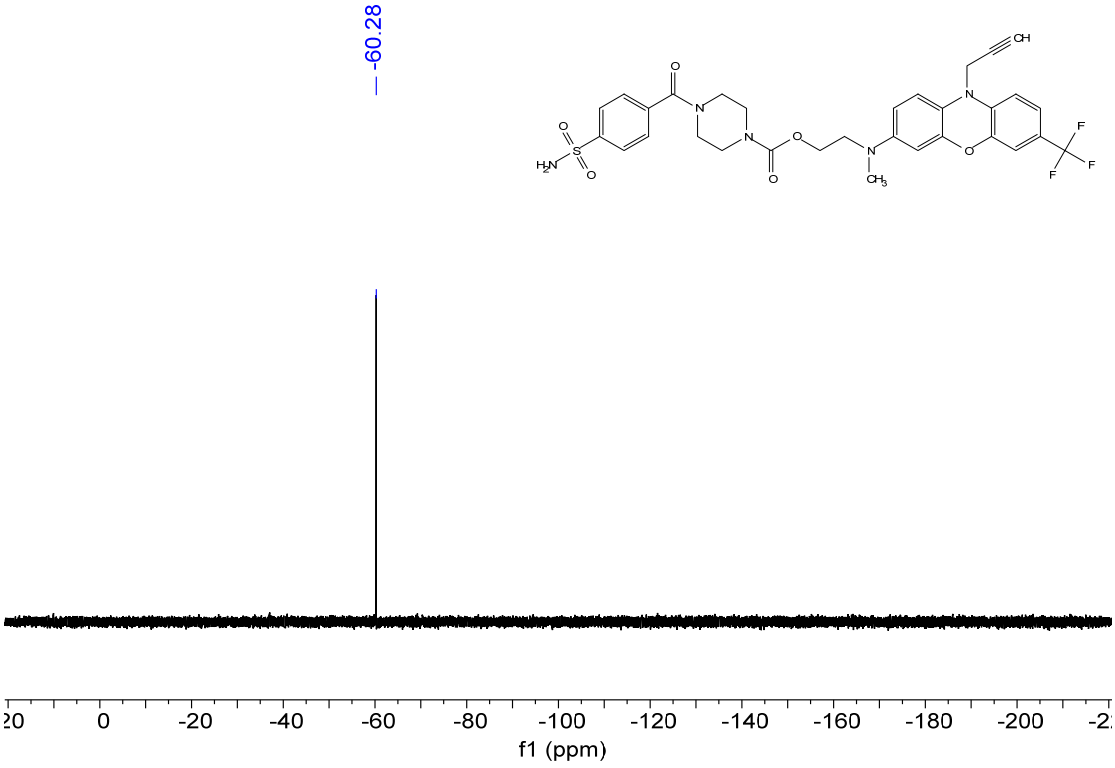
^{1}H NMR spectrum of **P2**

^{13}C NMR (101 MHz, DMSO-d_6)



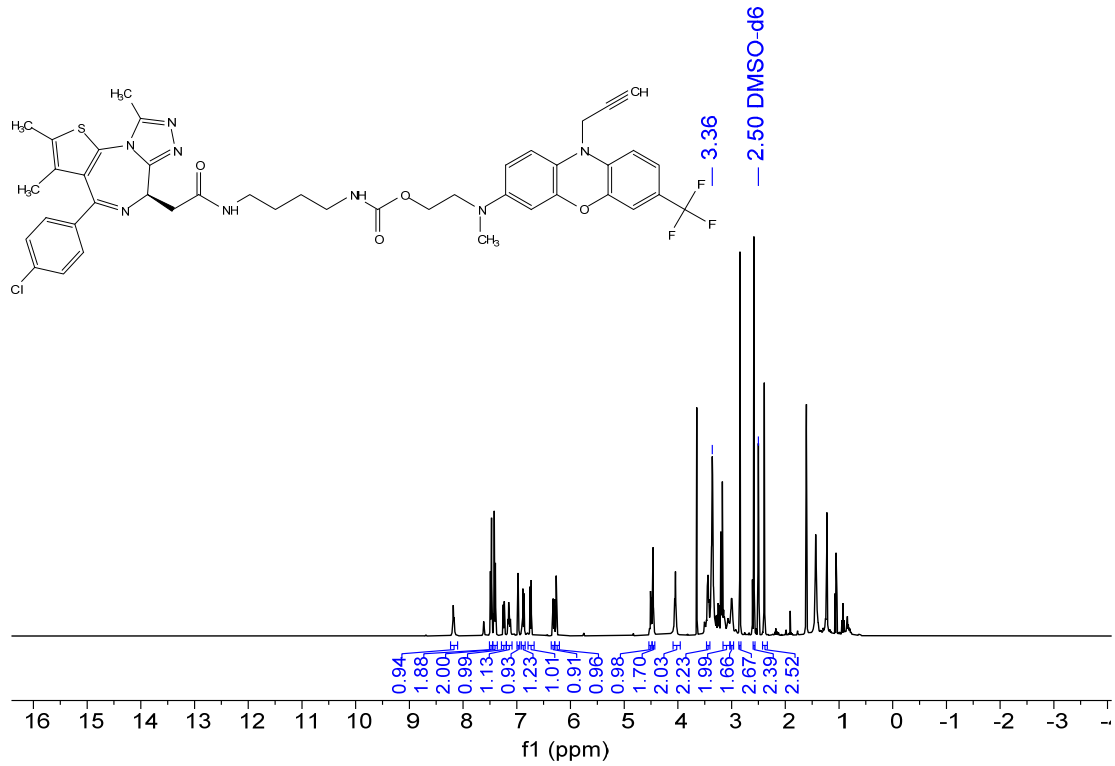
^{13}C NMR spectrum of **P2**

^{19}F NMR (376 MHz, DMSO-d_6)



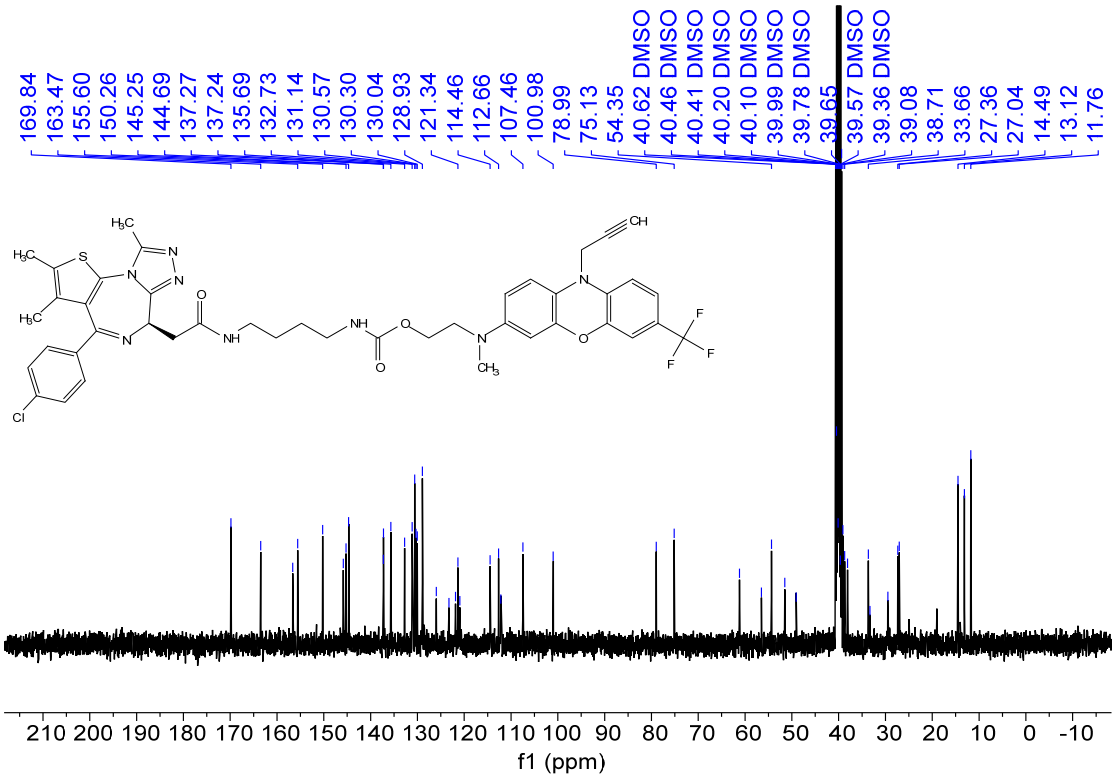
^{19}F NMR spectrum of **P2**

^{1}H NMR (400 MHz, DMSO-d_6)



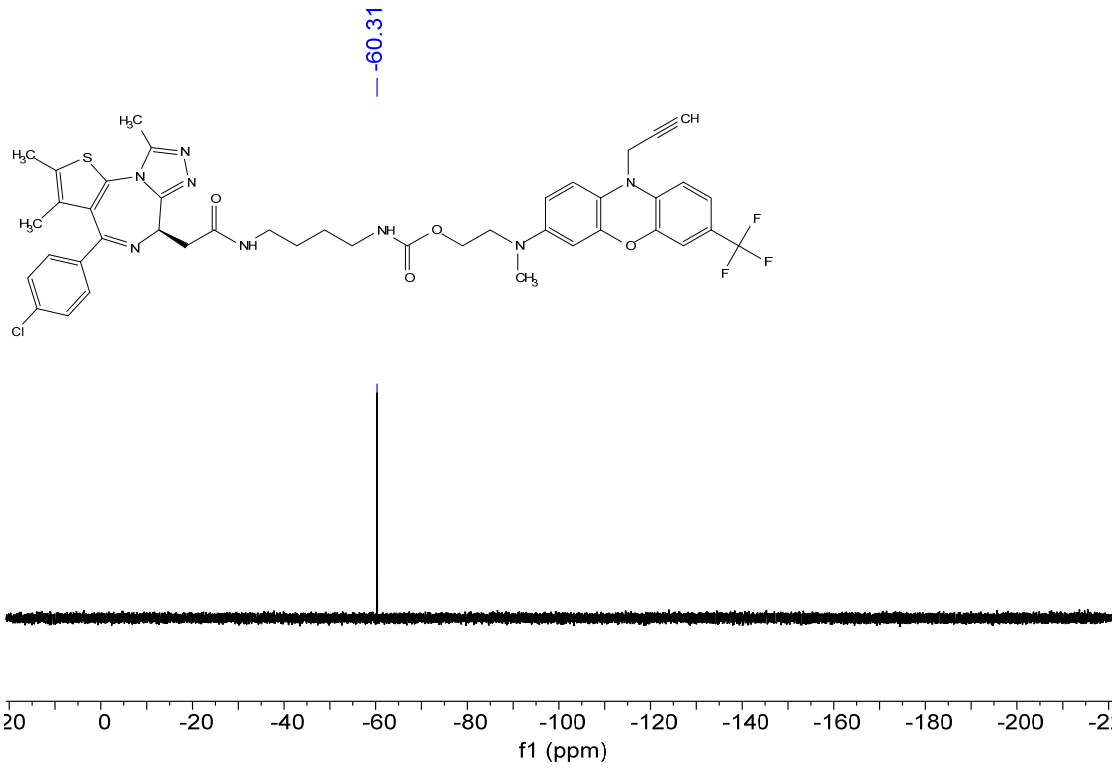
^{1}H NMR spectrum of **P3**

^{13}C NMR (101 MHz, DMSO-d_6)



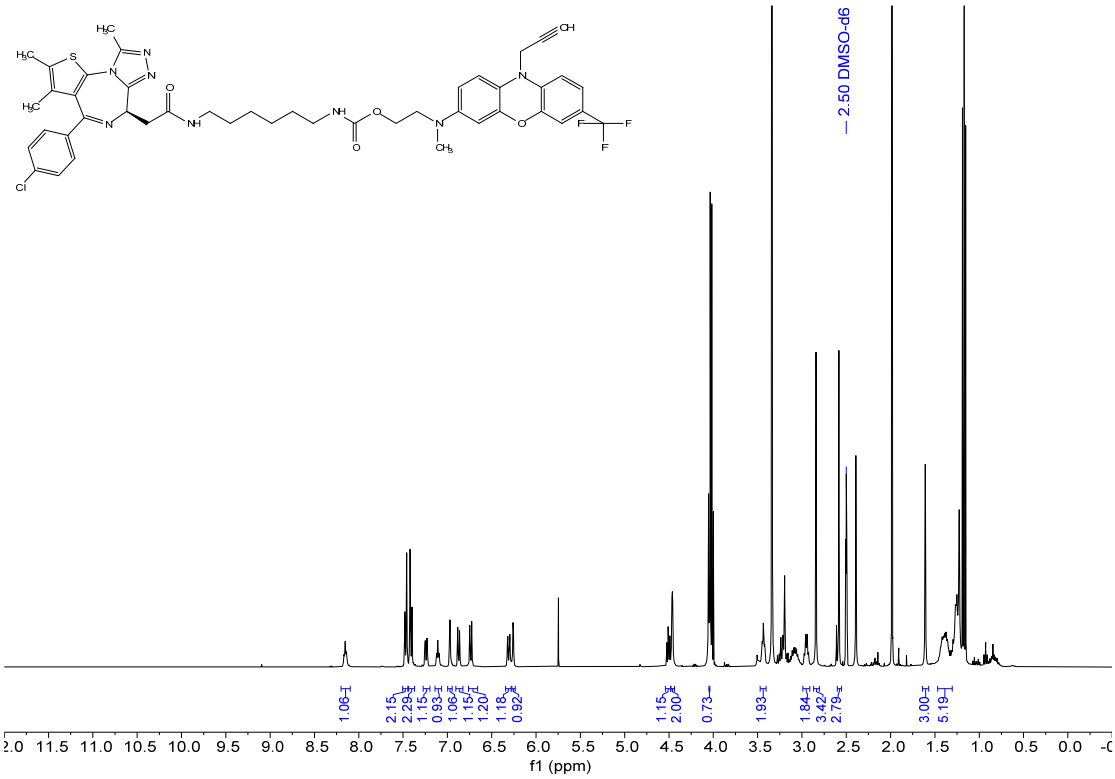
^{13}C NMR spectrum of **P3**

^{19}F NMR (376 MHz, DMSO-d_6)



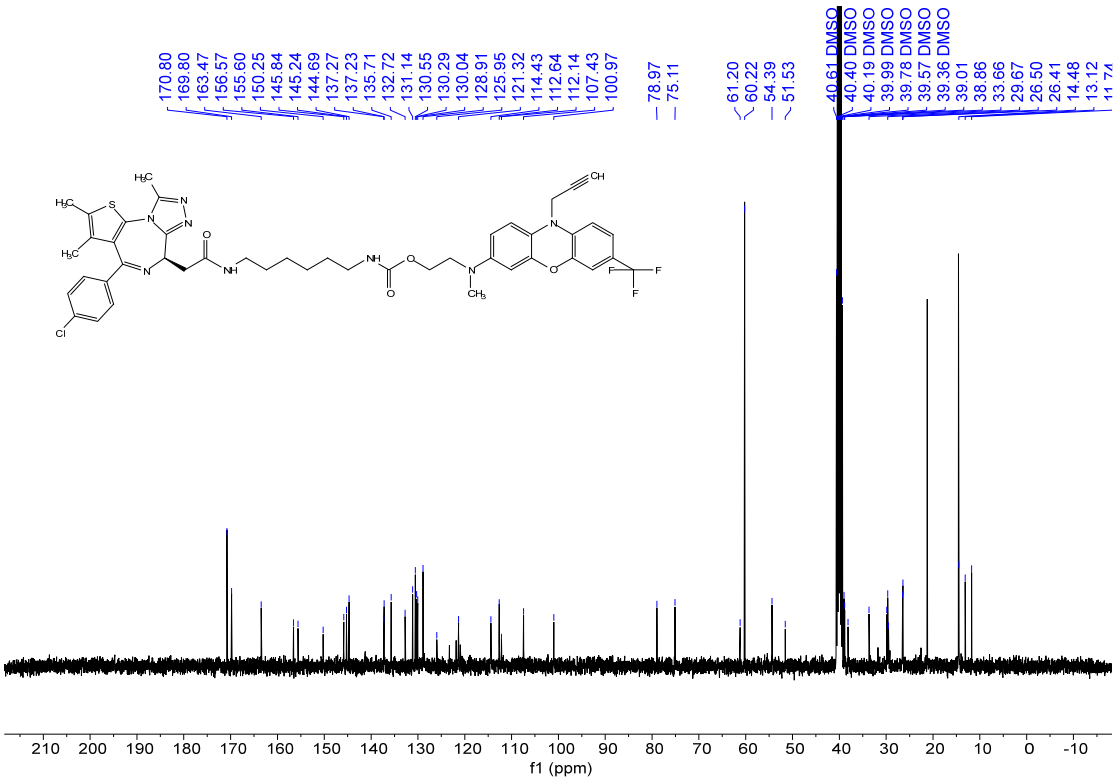
^{19}F NMR spectrum of **P3**

^{1}H NMR (400 MHz, DMSO-d_6)



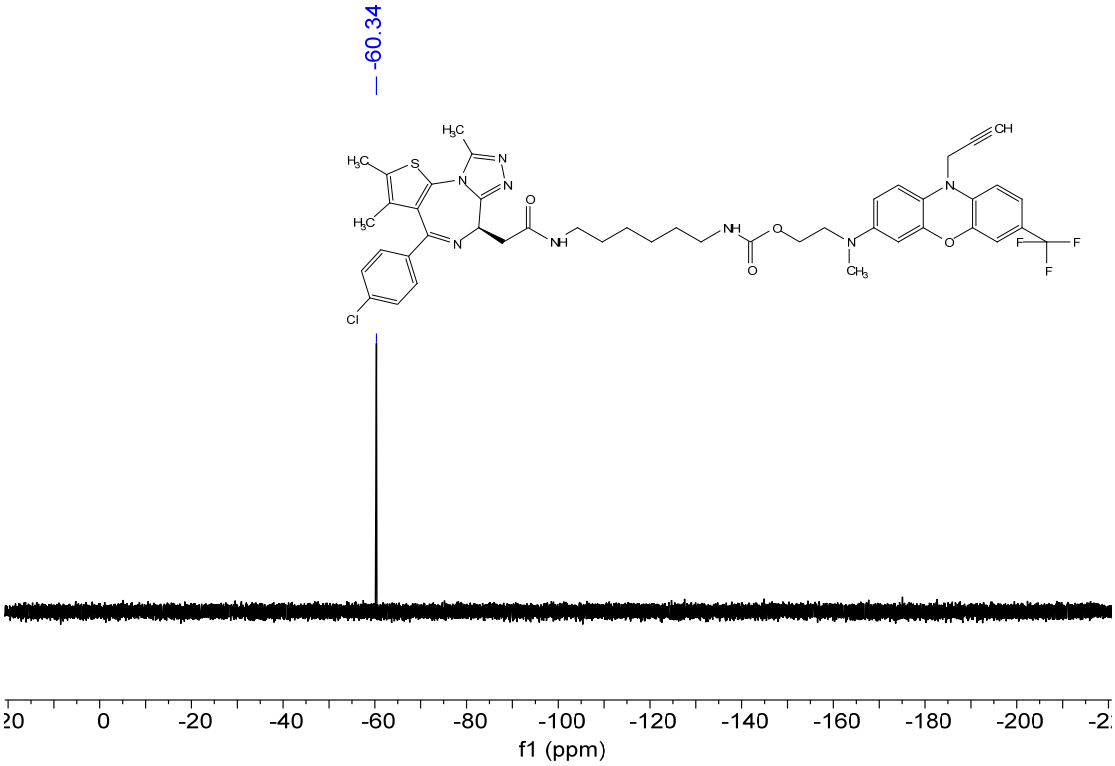
^{1}H NMR spectrum of **P4**

^{13}C NMR (101 MHz, DMSO-d_6)



^{13}C NMR spectrum of **P4**

^{19}F NMR (376 MHz, DMSO-d_6)



^{19}F NMR spectrum of **P4**