Supporting Information

A Double Photocage Strategy to Construct Light-Controllable and Spatiotemporally Trackable Cathepsin B Activity-Based Probes

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1. General Methods and Instrumentation

Synthetic Materials. Most of the chemicals and solvents were purchased from ECHO Chemical, Alfa Aeser, AK Scientific, Acros, Showa, Merck, Nova Materials, and Thermo Fisher Scientific. All chemicals were reagent grade and used without further purification unless mentioned otherwise. The anhydrous solvents were obtained from the solvent purification system (LC Technology Solutions Inc.). Glass plates coated with 0.25 mm silica gel 60 F_{254} were purchased from Merck to conduct thin-layer chromatography (TLC), and the compounds were visualized by UV lamps or visualizing agents, including phosphomolybdic acid (PMA), potassium permanganate, ninhydrin, iodine and 4-anisaldehyde. Purification with flash column chromatography was performed on Merck Geduran silica gel 60 (particle size: 0.040-0.063 mm). The eluents used in flash column chromatography were the mixtures containing ethyl acetate (EA), n-hexane (HEX), dichloromethane (DCM) and methanol (MeOH).

Instrumentation. The experiments using high performance liquid chromatography were performed on either Agilent 1260 infinity quaternary LC system or Waters 1525 binary pump system. The purification was performed on a semi-preparative column (YMC-Triart C18, 5 µm pore size, 250x10 mm), and the analyses were performed on an analytical column (YMC-Triart C18, 5 µm pore size, 4.6x150 mm). Absorbances at 254 nm and 390 nm (**DEACM** absorption) were monitored. All LC/MS and MS experiments were performed on Agilent 1260 infinity quaternary LC system coupled with a Bruker MicroQTOF-Q II mass spectrometer. ^{1}H and ^{13}C NMR data were obtained with Varian Unity Plus-400 (^{1}H: 400 MHz, ^{13}C: 100 MHz). Chemical shifts (δ) were recorded in part per million (ppm), the residuals of non-deuterated solvents were used as references. Coupling constants (J) were recorded in hertz (Hz), and the splitting patterns were reported as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), dt (double triplet), td (triple doublet), m (multiplet), and br (broad). The analyses of photophysical properties were performed on SpectraMax i3x Multi-mode microplate reader (Molecular Devices, Hong Kong). The microscopic images were obtained from inverted microscopy Nikon Ti2 with 40x and 4x objective lens. The filters were abbreviated as EX (excitation filter), DM (dichroic filter), and BA (barrier filter). The UV LEDs were purchased from KOODYZ Technology (Taiwan), including the 310 nm LED (1 LED chip, 3.5 W) and the 365 nm LED (16 LED chips arranged in a 4x4 array inside a 1.5 cmx1.5 cm square, 50 W total). The samples were placed 2.5 cm below the LED light source on ice during irradiation experiments unless mentioned otherwise.

Photoactivation Efficiency Determination. To determine the photoactivation efficiency of the **2PPG-FK-AcRha (1)** and **2PPG-FK-H (2)**, the substrates (final 100 μM) were dissolved in MES buffer (25 mM pH 5.0 and 10 % (v/v) DMSO) in a 96-well plate. The resulting solutions were irradiated using LEDs with wavelength of either 310 nm or 365 nm under ambient temperature. The samples were collected at indicated times and analyzed by LC-HRMS, and the quantity of substrates and products were determined by the area under curve of absorbance at 254 nm. The results were shown in **Figure 2b-2d** and **S2-S4**.

Photophysical Properties Characterization. To measure the absorption spectra of **2PPG-FK-AcRha (1)**, **2PPG-FK-H (2)**, and **DEACM-OH (14)**, the substrates (final 100 μM) were dissolved in ddH_2O in a 96-well plate respectively, and the photophysical properties were measured by the microplate reader. The absorption spectra were recorded with the gap of 1 nm. To measure the excitation and emission spectrum of **AcRha (3)** and **DEACM-OH (14)**, the substrates (final 10 μM) were dissolved in ddH2O in a 96-well plate respectively, and the excitation and emission spectra were measured by the microplate reader with gap of 1 nm. The emission spectra of **DEACM** was recorded from 420 to 700 nm with excitation wavelength at 390 nm, and the excitation spectra of **DEACM** was recorded from 300 to 440 nm with emission wavelength at 470 nm. The emission spectra of **AcRha** was recorded from 500 to 700 nm with excitation wavelength at 470 nm, and the excitation spectra of **AcRha** was recorded from 300 to 505 nm with emission wavelength at 535 nm. The results were shown in **Figure S5**.

Measurement of the DEACM Emission Spectra after Photolysis. To measure the emission spectra of **DEACM** after **2PPG-FK-AcRha (1)** or **2PPG-FK-H (2)** was irradiated by various UV radiation, the substrates (final 10 μM) were dissolved in MES buffer (25 mM pH 5.0 and 1 % (v/v) DMSO) in a 96-well plate. The resulting solutions were irradiated using LEDs with wavelength of either 310 nm or 365 nm under ambient temperature. Afterward, the emission spectra of **DEACM** were measured by the microplate reader with the gap of 1 nm. The results were shown in **Figure 2e-2g**.

Activation of Cathepsin B. The pro-CTSB stock solution (108 μg/mL) was added to the activation buffer containing DTT (5 mM) in MES buffer (25 mM pH 5.0) and incubated under 37 ^{o}C for an hour to obtain the activated CTSB (1 μM).

HepG2 Cell Lysate Preparation. 10-cm culturing dishes were seeded with about 5 x 10^6 cells in 1 mL EMEM medium with 10 % FBS and 1 % PS (penicillin and streptomycin). The 10-cm plates were incubated under 37 ^oC and 5 % CO_2 for 72 hours. The cell culture of HepG2 was centrifuged for 5 minutes at 1000 RCF at 4 ^oC. The obtained pellet was washed three times by ice-cold 1x PBS and then added with chilled RIPA buffer (100 µL per 10^6 cells) with protease inhibitor PMSF (1 mM). After then, the suspension was ground for 5 minutes. The sample was then centrifuged at 10000 RCF at 4 ^oC to spin down the cell debris. The supernatant was then transferred to Eppendorf tubes for protein concentration determination. The concentration of the cell lysate was determined using standard Bradford assay.

Determination of Enzymatic Stability of 2PPG-FK-H (2). The solution of **2PPG-FK-H (2)** (100 µM) in MES buffer (25 mM pH 5.0 and 10 % (v/v) DMSO) was added with activated CTSB stock solution (final 40 nM) or cell lysate of HepG2 (5 µg/mL). The efficiency assay of enzymatic hydrolysis was performed under 37 ^oC for various time periods and the equal volume of ACN was then added to quench CTSB. The solutions were then centrifuged at 15000 rpm for 5 minutes. The supernatants were analyzed by LC-HRMS, and the quantity of substrates were determined by the area under curve of absorbance at 254 nm. The results were shown in **Figure S7**.

Determination of *in vitro* CTSB Degradation Efficiency of 2PPG-FK-AcRha (1). The solution of **2PPG-FK-AcRha (1)** (10 µM) in MES buffer was added with activated CTSB stock solution (final 40 nM), both activated CTSB stock solution (final 40 nM) and CTSB inhibitor **E64** (10 µM), or nothing. With or without UV irradiation (365 nm, 1 minute), the fluorescence intensity of **AcRha** from 0 to 120 minutes was monitored at Ex/Em: 470/535 nm to determine the CTSB degradation performance. The results were shown in **Figure 3b** and **3c**. After 120 minutes treatments, the emission spectra of **DEACM** was recorded from 420 to 700 nm with excitation wavelength at 390 nm, and the emission spectra of **AcRha** was recorded from 500 to 700 nm with excitation wavelength at 470 nm. The results were shown in **Figure 3d** and **3e**.

Fluorescence Microscopy of Cell Cultures. 96-well plates with poly-*L*-lysine coating were seeded with 20000 cells per well in 100 µL EMEM medium with 10 % FBS and 1 % PS (penicillin and streptomycin). The 96-well plates were incubated under 37 ^oC and 5 % CO_2 for 24 hours. After then, the medium was replaced with 100 µL serum free EMEM with 1 % (v/v) DMSO and **2PPG-FK-AcRha** (**1**) (final 10 µM) and incubated for further 1.5 hours. Afterward, the media were replaced by 1x PBS.

The resulting HepG2 cell cultures were treated with (**+UV**) or without (**-UV**) 365 nm UV radiation for 1 minute, and the images of the cell cultures were then recorded by fluorescence microscopy (**0 h**). After 1x PBS was replaced by serum free EMEM, the HepG2 cell cultures were further incubated for 4 hours. The medium was then replaced by 1x PBS again, and the images of the cell cultures were recorded again by fluorescence microscopy. For the experiments (**+E64**), additional cathepsin B inhibitor **E64** (final 100 μM) was added to the EMEM medium in every step. The images were recorded by the fluorescence microscopy: bright field images were recorded to determine distribution and morphology of the cells, the **DEACM** channel was recorded using the **DAPI** setting (EX: 361-389 nm, DM: 415 nm, BA: 430-490 nm) to visualize the distribution of released **DEACM**, and the **AcRha** channel was recorded using the **FITC** setting (EX: 465-495 nm, DM: 505 nm, BA: 512-558 nm) to visualize the distribution of **AcRha**. The results were shown in **Figure 4a-4c and S8**.

Regional Activation of 2PPG-FK-AcRha (1) in HepG2 cell cultures. 3.5-cm plates were seeded with 10^6 cells in 500 μL EMEM medium with 10 % FBS and 1 % PS (penicillin and streptomycin) and incubated for 24 hours under 37 °C and 5 % CO_2. After then, the medium was replaced with 500 μL serum free EMEM with 1 % (v/v) DMSO and **2PPG-FK-AcRha (1)** (final 10 μM) and incubated for further 1.5 hours. After then, the medium was replaced by 500 μL 1x PBS. By utilizing a handmade grating device (**Figure 4d**), the cells were regionally irradiated with 365 nm UV for 1 minute. The images were recorded by the fluorescence microscopy: bright field images were recorded to determine distribution and morphology of the cells, the **DEACM** channel was recorded using the **DAPI** setting (EX: 361-389 nm, DM: 415 nm, BA: 430-490 nm) to visualize the distribution of released **DEACM**, and the **AcRha** channel was recorded using the **FITC** setting (EX: 465-495 nm, DM: 505 nm, BA: 512-558 nm) to visualize the distribution of **AcRha**. The results were shown in **Figure 4d**.

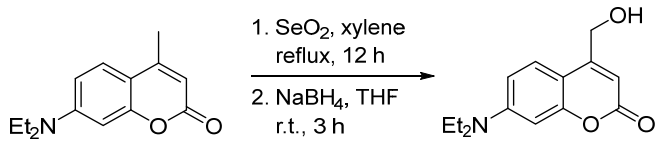
MTT Assay to Determine the Cytotoxicity of 2PPG-FK-AcRha (1). 96-well plates with poly-*L*-lysine coating were seeded with 8000 cells per well in 100 μL EMEM medium with 10 % FBS and 1 % PS (penicillin and streptomycin). The 96-well plates were incubated under 37 °C and 5 % CO_2 for 24 hours. After then, the medium was replaced with 100 μL serum free EMEM with 1 % (v/v) DMSO and added various concentrations of **2PPG-FK-AcRha** (**1**). The cells were then incubated for 48 hours under the same condition (37 °C, 5 % CO_2). The medium was replaced by 50 μL 1x PBS containing MTT (0.5 mg/mL), and the cells were incubated under the same condition (37 °C, 5 % CO_2) for further 3 hours. After removing the supernatants, the cells were added with 50 μL of DMSO, and the

absorbance at 570 nm and 690 nm were measured to determine the cell viability. The results were shown in **Figure S9a**.

MTT Assay to Determine the Cytotoxicity of 365 nm irradiation. 96-well plates with poly-*L*-lysine coating were seeded with 8000 cells per well in 100 μL EMEM medium with 10 % FBS and 1 % PS (penicillin and streptomycin). The 96-well plates were incubated under 37 ^{o}C and 5 % CO_2 for 24 hours. After then, the medium was replaced with 100 μL 1x PBS and exposed to UV irradiation of 365 nm for 0, 0.5, 1, or 2 minutes on ice. 1x PBS was then replaced by serum free EMEM, and the cells were then incubated for 48 hours under the same condition (37 ^{o}C, 5 % CO_2). The medium was replaced by 50 μL 1x PBS containing MTT (0.5 mg/mL), and the cell cultures were incubated under the same condition (37 ^{o}C, 5 % CO_2) for further 3 hours. After removing the supernatants, the cells were added with 50 μL of DMSO, and the absorbance at 570 and 690 nm were measured to determine the cell viability. The results were shown in **Figure S9b**.

2. Synthesis and Characterization of Compounds

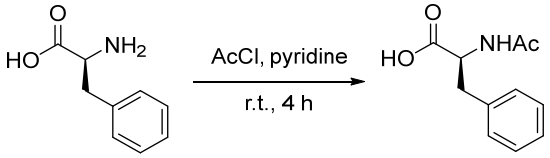
7-diethylamino-4-hydroxymethylcoumarin, 14 (DEACM-OH)



DEACM-OH, 14 (42 %)

Synthesis of **14** was performed according to literature[1]. 7-diethylamino-4-methylcoumarin (1.14 g, 2.38 mmol) and selenium dioxide (828 mg, 7.46 mmol) were dissolved in xylene (100 mL), and the mixture was refluxed for 12 h. Afterward, the solvent was evaporated under reduced pressure. The mixture was added with sodium borohydride (828 mg, 21.9 mmol) portionwise at ambient temperature and stirred for 2 hours. Upon completion of the reaction, the mixture was concentrated under reduced pressure and purified by flash column chromatography to afford **14** as a light-yellow solid in 42 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (1H, d, J=1.2 Hz), 6.54 (1H, dd, J=1.0, 0.4 Hz), 6.46 (1H, d, J=0.4 Hz), 6.24 (1H, s), 4.81 (2H, s), 3.38 (4H, q, J=0.8 Hz), 1.18 (6H, t, J=0.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.66, 156.14, 154.75, 150.50, 124.36, 108.56, 106.29, 105.42, 105.40, 97.74, 60.96, 44.70, 12.42.

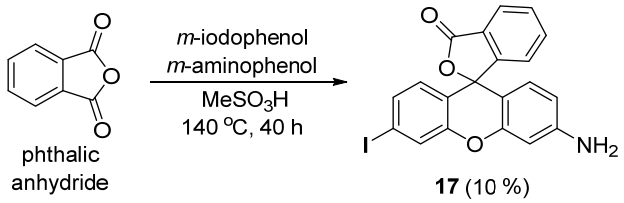
***N*-acetyl-*L*-phenylalanine, 11**



11 (50 %)

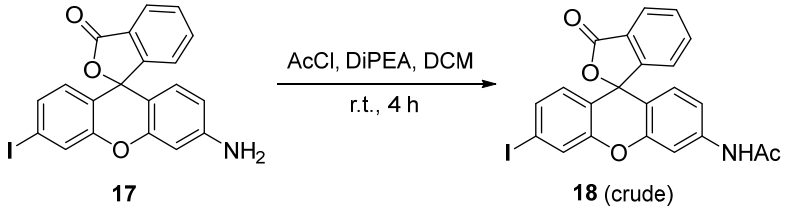
Synthesis of **11** was performed according to literature**[2].** A solution of *L*-phenylalanine (4.95 g, 30.0 mmol) in pyridine (20 mL) was added with acetyl chloride (2.75 g, 35.0 mmol) dropwise under ice bath. The resulting mixture was allowed to warm to ambient temperature and stirred for 4 hours. Afterward, the solvent was removed under reduced pressure, and a small amount of deionized water was added and then evaporated. The crude was dissolved in ethyl acetate and washed several times with 1 N $HCl_{(aq)}$ and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting crude was further purified by flash column chromatography to afford **11** as a white solid in 50 % yield. ^{1}H NMR (d6-DMSO, 400 MHz) δ 8.16 (1H, d, J=8.0 Hz), 7.28-7.16 (5H, m), 4.40-4.33 (1H, m), 3.01 (1H, dd, J=1.6, 0.4 Hz), 2.80 (1H, dd, J=1.2, 0.8 Hz), 1.75 (3H, s); ^{13}C NMR (d6-DMSO, 100 MHz) δ 173.16, 169.18, 137.73, 129.03, 128.15, 126.37, 53.50, 36.77, 22.33.

3'-amino-6'-iodo-3*H*-spiro[isobenzofuran-1,9'-xanthen]-3-one, 17



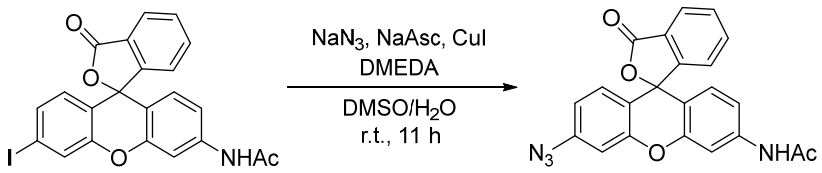
Synthesis of **17** was performed according to literature[3]. Phthalic anhydride (2.71 g, 18.32 mmol), 3-iodophenol (4.03 g, 18.32mmol), and 3-aminophenol (2.00 g, 18.32 mmol) were dissolved in methanesulfonic acid (16 mL), and the mixture was refluxed for 40 hours. After then, the mixture was poured into 400 g of ice, and adjusted to neutral by sodium hydroxide and concentrated hydrochloric acid. The resulting aqueous solution was extracted with dichloromethane. The organic layer was washed by 10 % $NaOH_{(aq)}$, dried over $MgSO_4$, concentrated under reduced pressure, and purified with flash column chromatography to afford compound **17** as a pink solid in 10 % yield. ^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.98 (1H, dt, J=7.6, 1. Hz), 7.78 (1H, dd, J=7.2, 1.2 Hz), 7.75 (1H, t, J=1.2 Hz), 7.69 (1H, td, J=7.2, 1 Hz), 7.40 (1H, dt, J=7.6, 1.2 Hz), 7.27 (1H, dt, J=8.0, 0.7 Hz), 6.48 (1H, d, J=8 Hz), 6.42 (1H, d, J=2 Hz), 6.37 (1H, d, J=8.6 Hz), 6.33 (1H, dd, J=8.6, 2.0 Hz), 5.70 (2H, s); ^{13}C NMR (d_6-DMSO, 100 MHz) δ 168.58, 152.18, 151.56, 151.44, 151.28, 135.63, 132.48, 130.16, 129.54, 128.47, 126.01, 125.38, 124.65, 123.99, 119.08, 111.53, 104.67, 99.04, 96.23.

***N*-(3'-iodo-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthen]-6'-yl)acetamide, 18**



17 (88.4 mg, 0.2 mmol) was dissolved in anhydrous DCM (2 mL), and the solution was added with acetyl chloride (29 µL, 31.4 mg, 0.4 mmol). The resulting mixture was stirred at ambient temperature for 4 hours. After then, the mixture was dissolved in ethyl acetate, washed by 1 N $HCl_{(aq)}$, dried over $MgSO_4$, concentrated under reduced pressure, and further dried under high vacuum to afford compound **18** as a pink solid. The product was then used without further purification. HRMS (ESI, m/z): $[M+H]^+$, calculated: 484.0040, found: 484.0048.

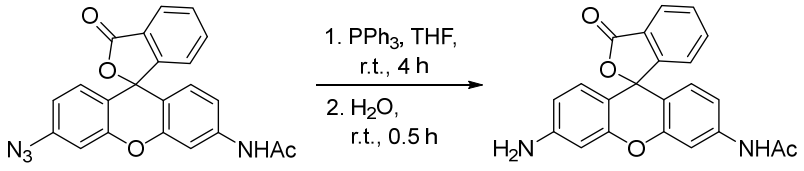
***N*-(3'-azido-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthen]-6'-yl)acetamide, 19**



18 (crude) **19** (crude)

Sodium azide (26.8 mg, 0.412 mmol), sodium ascorbate (2.04 mg, 0.0103 mmol), and copper(I) iodide (8 mg, 0.0412 mmol) were dried under high vacuum. **18** (100 mg crude) was dissolved in DMSO/H_2O (1 mL/0.2 mL), and the resulting solution was degassed under vacuum. The solution was slowly added to the mixture of sodium azide, sodium ascorbate, and copper(I) iodide. The resulting mixture was then added with *N*,*N'*-dimethylethylenediamine (DMEDA, 3.3 µL, 0.0309 mmol) and stirred at ambient temperature for 11 hours. Afterward, the mixture was diluted with ethyl acetate, washed with brine, dried over $MgSO_4$, concentrated under reduced pressure, and further dried under high vacuum to afford compound **19** as a pink solid. The product was then used without further purification. HRMS (ESI, m/z): $[M+H]^+$, calculated: 398.1015, found: 398.0996.

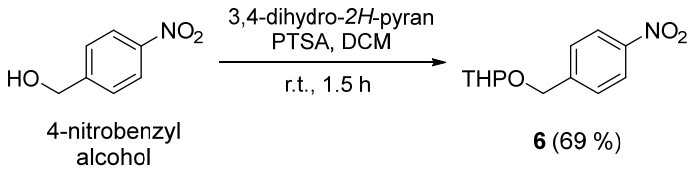
Rhodamine 110 mono-acetamide, 3 (AcRha)



19 (crude) **AcRha, 3** (40 %)

19 (90 mg crude) in THF (20 mL) was added with the solution of PPh_3 (119 mg, 0.45 mmol) in THF (0.45 mL), and the resulting solution was stirred at ambient temperature for 3 hours. After then, H_2O (3.5 mL) was added to the mixture, and the solution was stirred at ambient temperature for further 30 minutes. The mixture was diluted with ethyl acetate, washed by brine, dried over $MgSO_4$, concentrated under reduced pressure, and purified by flash column chromatography to afford compound **3** as a pink solid in 40 % yield. ^{1}H NMR (d_6-DMSO, 400 MHz) δ 10.20 (1H, s), 7.98 (1H, dt, J=7.6, 0.8 Hz), 7.80 (1H, d, J=1.6 Hz), 7.77 (1H, dd, J=7.2, 1.2 Hz), 7.70 (1H, td, J=7.6, 0.8 Hz), 7.25 (1H, dt, J=7.6, 0.8 Hz), 7.07 (1H, dd, J=8.8, 2.4 Hz), 6.64 (1H, d, J=8.8 Hz), 6.45 (1H, d, J=2.0 Hz), 6.37 (1H, d, J=8.4 Hz), 6.33 (1H, dd, J=8.4, 2.0 Hz), 5.66 (2H, br), 2.06 (3H, s). ^{13}C NMR (d_6-DMSO, 100 MHz) δ 168.81, 168.74, 152.45, 151.95, 151.32, 151.14, 141.12, 135.44, 129.93, 128.43, 128.17, 126.29, 124.49, 123.96, 114.64, 113.42, 111.22, 106.02, 105.19, 99.11, 83.44, 24.05. HRMS (ESI, m/z): $[M+H]^+$, calculated: 373.1190, found: 373.1222.

4-Nitrobenzyl-THP ether, 6

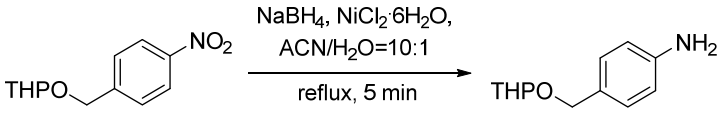


4-nitrobenzyl alcohol

6 (69 %)

A solution of 4-nitrobenzyl alcohol (3.06 g, 20.0 mmol) in DCM (100 mL) was added with 3,4-dihydro-2*H*-pyran (2.52 g, 30.0 mmol) and *p*-toluenesulfonic acid monohydrate (500 mg, 2.90 mmol) at ambient temperature. The resulting mixture was stirred at ambient temperature for 1.5 hours, washed with saturated $NaHCO_{3(aq)}$, dried over $MgSO_4$, and concentrated under reduced pressure. The resulting crude was further purified by flash column chromatography to afford **6** as yellow liquid in 69 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (2H, d, J=8.8 Hz), 7.51 (2H, d, J=8.8 Hz), 4.85 (1H, t, J=12.8 Hz), 4.71 (1H, t, J=3.6 Hz), 4.58 (1H, d, J=12.8 Hz), 3.90-3.83 (1H, m), 3.57-3.51 (1H, m), 19.2-1.53 (6H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 146.09, 127.74, 126.96, 123.57, 98.28, 67.61, 62.26, 30.43, 25.32, 19.23.

4-Aminobenzyl-THP ether, 7

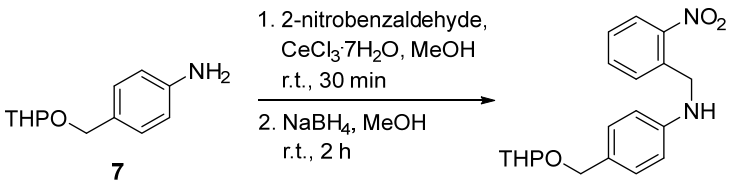


6

7 (quant.)

A solution of **6** (979 mg, 4.12 mmol) and nickel(II) chloride hexahydrate (200 mg, 0.84 mmol) in ACN/H_2O (22 mL, ACN:H_2O=10:1) was added with sodium borohydride (638 mg, 16.9 mmol) at ambient temperature, and the resulting mixture was refluxed for 5 minutes. The resulting mixture was then diluted with dichloromethane, washed with H_2O several times, dried over $MgSO_4$, and concentrated under reduced pressure to afford **7** as clear liquid in quantitative yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.16 (2H, d, J=8.0 Hz), 6.66 (2H, d, J=8.0 Hz), 4.67 (1H, t, J=3.6 Hz), 4.65 (1H, s), 4.38 (1H, d, J=12.0 Hz), 3.96-3.88 (1H, m), 3.57-3.55 (1H, m), 1.88-1.80 (1H, m), 1.92-1.53 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.93, 129.63, 128.08, 114.97, 97.31, 68.74, 62.14, 30.61, 25.51, 19.45.

***N*-(2-Nitrobenzyl)-4-aminobenzyl-THP ether, 8**



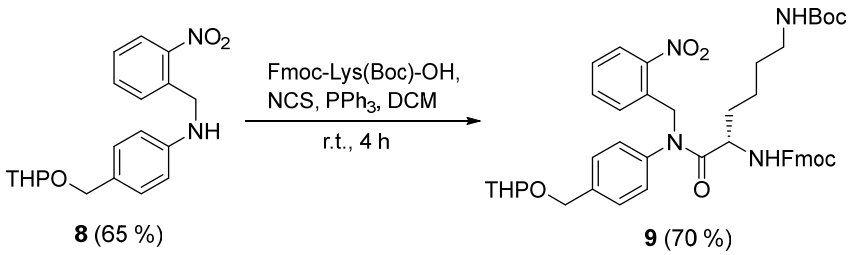
7

8 (65 %)

A solution of **7** (207 mg, 1.00 mmol) in methanol (5 mL) was added with 2-nitrobenzaldehyde

(166 mg, 1.10 mmol) and cerium(III) chloride heptahydrate (15 mg, 0.040 mmol), and stirred at ambient temperature for 30 minutes. After then, sodium borohydride (94.6 mg, 2.50 mmol) was added to the mixture and stirred for further 2 hours. Upon completion of the reaction (monitored by thin layer chromatography), the residue was diluted with ethyl acetate, washed with saturated $NaHCO_{3(aq)}$, dried over $MgSO_4$, and concentrated under reduced pressure. The crude was then purified by flash column chromatography to afford **8** as orange oil in 65 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (1H, dd, J=8.0, 1.2 Hz), 7.68 (1H, dd, J=8.0, 1.2 Hz), 7.58 (1H, td, J=8.0, 1.2 Hz), 7.44 (1H, td, J=7.6, 1.6 Hz), 7.18 (2H, d, J=8.8 Hz), 6.56 (2H, d, J=8.8 Hz), 4.75 (2H, s), 4.71-4.65 (2H, m), 4.38 (2H, d, J=11.2 Hz), 3.97-3.90 (1H, m), 3.95-3.52 (1H, m), 1.92-1.80 (1H, m), 1.76-1.52 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.16, 146.87, 135.51, 133.56, 129.70, 129.61, 127.89, 127.45, 125.09, 112.66, 97.36, 68.70, 62.06, 45.68, 30.53, 25.41, 19.37.

Fmoc-Lys(Boc)-PAB(oNB)-THP ether, 9

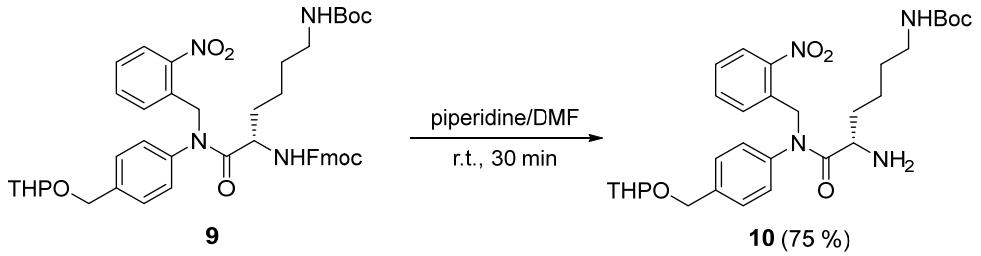


8 (65 %) **9** (70 %)

N-Chlorosuccinimide (80.1 mg, 0.6 mmol) and triphenylphosphine (157.4 mg, 0.6 mmol) were dried over vacuum. After then, 5 mL of dichloromethane was added to the mixture, followed by the addition of **8** (171.2 mg, 0.5 mmol). The resulting solution was stirred at ambient temperature until the solution became clear. Then, triethylamine (84 µL, 61.0 mg, 0.6 mmol) and Fmoc-Lys(Boc)-OH (281.1 mg, 0.6 mmol) were added to the solution respectively, and the resulting mixture was stirred at ambient temperature for an hour. Subsequently, the mixture was diluted with dichloromethane, and washed by 1N $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$, and brine. The organic layer was then dried over MgSO4, concentrated under reduced pressure, and purified with flash column chromatography to afford **9** as clear oil in 70 % yield. ^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.94 (1H, d, J=8.4 Hz), 7.88 (2H, d, J=7.6 Hz), 7.72 (2H, t, J=8.4 Hz), 7.67 (2H, t, J=8.0 Hz), 7.62 (1H, d, J=7.6 Hz), 7.48 (1H, t, J=7.6 Hz), 7.41-7.28 (7H, m), 6.68 (1H, s), 5.26 (1H, d, J=16.8 Hz), 4.49 (1H, d, J=16.8 Hz), 4.63-4.60 (2H, m), 4.39 (1H, d, J=12.4 Hz), 4.24-4.20 (3H, m), 4.07 (1H, q, J=6.8 Hz), 3.74-3.68 (1H, m), 3.42-3.40 (1H, m), 2.74 (2H, br), 1.70-1.60 (2H, m), 1.52-1.42 (6H, m), 1.34 (9H, s), 1.24-1.21 (1H, m), 1.126 (2H, br), 1.02-0.98 (1H, m). ^{13}C NMR (d6-DMSO, 100 MHz) δ 172.88, 156.19, 155.53, 148.25, 143.80, 140.71, 140.31, 139.20, 138.33, 133.57, 131.81, 129.46, 128.46, 128.05, 127.63, 127.06,

125.35, ,124.61, 120.10, 97.30, 77.32, 67.71, 67.35, 65.76, 61.22, 51.43, 50.06, 46.61, 30.38, 30.05, 28.75, 28.25, 24.95, 22.63, 18.95.

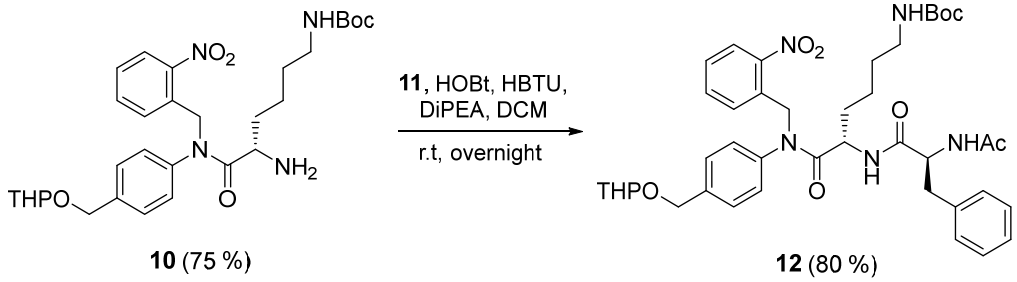
Lys(Boc)-PAB(oNB)-THP ether, 10



9 **10** (75 %)

A solution of **9** (103.7 mg, 0.13 mmol) in dimethylformamide (4 mL) was added with piperidine (1 mL), and the resulting solution was stirred at ambient temperature for 30 minutes. After then, the mixture was diluted with 50 mL of dichloromethane, concentrated under reduced pressure, and purified with flash column chromatography to afford **10** as clear oil in 75 % yield. ^{1}H NMR (d_6-DMSO, 400 MHz) δ 7.94 (1H, d, J=7.6 Hz), 7.70 (1H, t, J=8.0 Hz), 7.60 (1H, d, J=7.6 Hz), 7.49 (1H, t, J=8.0 Hz), 7.34-7.27 (4H, m), 6.69 (1H, t, J=5.6 Hz), 5.22 (1H, d, J=16.4 Hz), 5.03 (1H, d, J=16.8 Hz), 4.65-4.62 (2H, m), 4.42 (1H, d, J=13.2 Hz), 3.73 (1H, t, J=8.8 Hz), 3.45-3.41 (1H, m), 3.20 (1H, t, J=6.8 Hz), 2.79 (2H, q, J=6.0 Hz), 1.74-1.54 (3H, m), 1.53-1.37 (6H, m), 1.34 (9H, s), 1.23-1.00 (5H, m); ^{13}C NMR (d6-DMSO, 100 MHz) δ 176.18, 155.53, 148.30, 140.74, 138.12, 133.53, 132.00, 129.51, 128.48, 128.37, 127.86, 124.53, 97.39, 77.24, 67.42, 61.24, 50.81, 49.45, 39.65, 34.77, 30.07, 29.27, 28.23, 24.95, 22.58, 18.95.

Ac-Phe-Lys(Boc)-PAB(oNB)-THP ether, 12

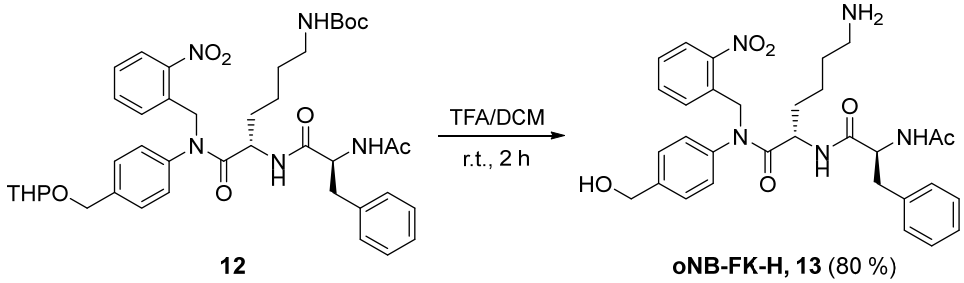


10 (75 %) **12** (80 %)

The solution of **10** (71.2 mg, 0.125 mmol) in DCM (5 mL) was added with **11** (52.0 mg, 0.250 mmol), DiPEA (66.0 µL, 0.375 mmol), HOBt (51.1 mg, 0.375 mmol), and HBTU (144 mg, 0.375 mmol), respectively. The resulting mixture was then stirred under ambient temperature overnight. After then, the solution was washed with brine and sat. $NaHCO_{3(aq)}$, dried over $MgSO_4$, and purified with flash column chromatography to afford **12** as a clear oil in 80 % yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.93 (1H, d, J=7.6 Hz), 7.60 (1H, t, J=7.6 Hz), 7.52 (1H, d, J=8.0 Hz), 7.40 (1H, d, J=7.6 Hz), 7.35 (1H,

d, J=7.6 Hz), 7.30-7.17 (3H, m), 7.17-7.10 (2H, m), 6.56 (1H, d, J=7.6 Hz), 6.09 (1H, d, J= 7.6 Hz), 5.36 (1H, d, J=16.8 Hz), 5.12 (1H, dd, J=16.4, 2.8 Hz), 4.76 (1H, d, J=12.4 Hz), 4.71-4.62 (3H, m), 4.54 (1H, br), 4.46 (1H, d, J=12.4 Hz), 3.86 (1H, t, J=10.8 Hz), 3.56-3.47 (1H, m), 3.14-2.85 (4H, m), 1.94 (3H, s), 1.87-1.78 (1H, m), 1.78-1.68 (3H, m), 1.68-1.64 (6H, m), 1.41 (9H, s), 1.27-1.05 (4H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.56, 170.90, 170.25, 156.43, 148.78, 140.11, 133.78, 132.33, 129.94, 129.83, 129. 62, 129.26, 128.84, 128.67, 128.45, 128.13, 127.25, 125.18, 109.78, 98.31, 98.31, 98.19, 68.19, 68.12, 62.39, 54.38, 38.84, 38.18, 32.06, 30.70, 29.26, 28.69, 25.62, 23.37, 19.50.

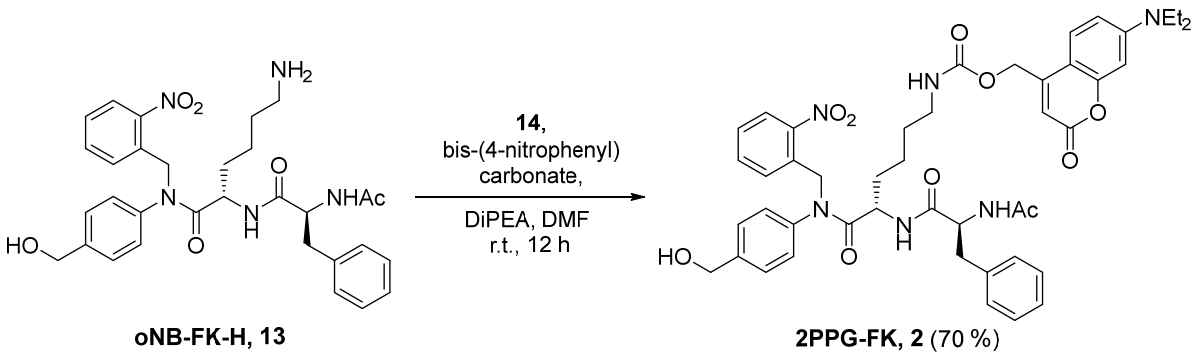
Ac-Phe-Lys-PAB(oNB)-OH, 13 (oNB-FK-H)



12 **oNB-FK-H, 13** (80 %)

12 (100 mg, 0.13 mmol) was dissolved in the co-solvent of TFA/DCM (3 mL/3 mL), and the resulting mixture was then stirred under ambient temperature for two hours. After then, the solvent was removed under high vacuum, and the crude was purified with flash column chromatography to afford **13** as light-yellow oil in 80 % yield. ^{1}H NMR (d6-DMSO, 400 MHz) δ 8.37 (1H, d, J=6.7 Hz), 8.12-8.04 (1H, m), 7.97 (1H, dd, J=8.1, 2.7 Hz), 7.78 (2H, s), 7.69 (2H, t, J=7.6 Hz), 7.58 (1H, d, J=7.9 Hz), 7.52 (1H, t, J=7.6 Hz), 7.36-7.30 (4H, m), 7.30-7.23 (4H, m), 7.23-7.14 (1H, m), 5.31 (1H, d, J=16.4 Hz), 5.00 (1H, d, J=16.5 Hz), 4.53 (1H, td, J=9.3, 4.1 Hz), 4.48 (2H, s), 2.67-2.61 (1H, m), 1.73 (3H, s), 1.66-1.47 (2H, m), 1.38-1.18 (3H, m), 1.13-1.02 (1H, m); ^{13}C NMR (d6-DMSO, 100 MHz) δ 172.18, 171.54, 169.20, 148.38, 142.54, 139.57, 137.93, 133.49, 131.70, 129.62, 129.21, 128.57, 127.99, 127.73, 127.38, 126.22, 124.62, 62.24, 53.51, 49.91, 49.58, 38.54, 37.48, 30.52, 26.42, 22.41, 22.17.

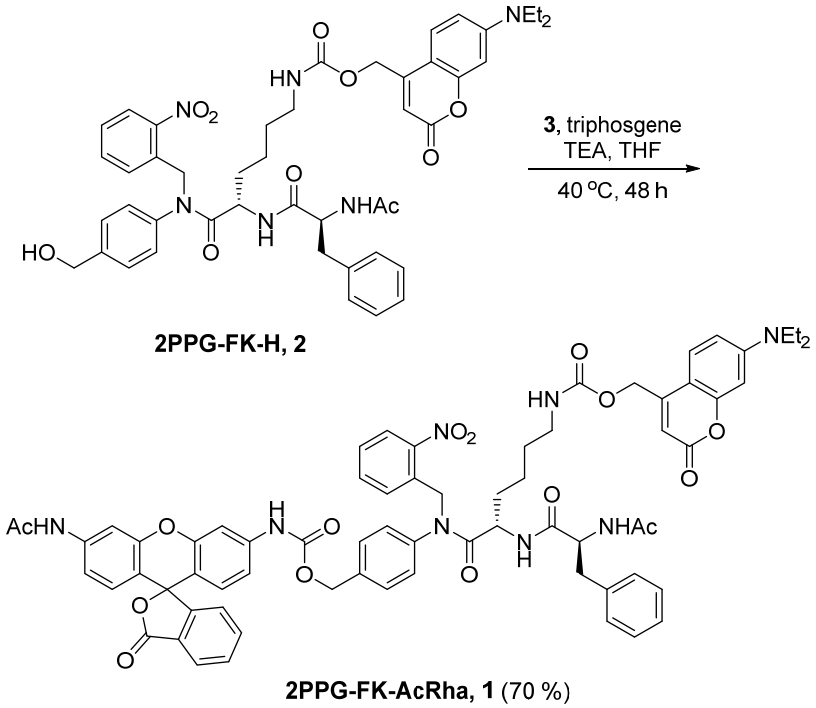
Ac-Phe-Lys(DEACM)-PAB(oNB)-OH, 2 (2PPG-FK-H)



oNB-FK-H, 13 **2PPG-FK, 2** (70 %)

14 (37.1 mg, 0.15 mmol), bis-(4-nitrophenyl)carbonate (45.6 mg, 0.15 mmol), and DiPEA (35 µL, 0.2 mmol) were dissolved in anhydrous DMF (3 mL), and the mixture was stirred under ambient temperature for 12 hours. **13** (100 mg, 0.132 mmol) was added to the solution of activated **14**. The resulting solution was then stirred under ambient temperature for further 12 hours. Afterward, the mixture was diluted with DCM, washed with brine, dried over $MgSO_4$, concentrated under reduced pressure, and purified with flash column chromatography to afford **2** as a light-yellow solid in 70 % yield. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.92 (1H, d, J=8.8 Hz), 7.69-7.56 (2H, m), 7.52-7.34 (4H, m), 7.33-7.16 (7H, m), 6.69 (1H, dd, J=8.8, 2.8 Hz), 6.52 (1H, d, J=2.4 Hz), 6.05 (1H, s), 5.35 (1H, d, J=16.0 Hz), 6.26 (2H, s), 5.10 (1H, d, J=16 Hz), 4.64 (1H, dd, J=5.2, 4.4 Hz), 4.45 (1H, dd, J=4.8, 4.4 Hz), 3.46 (4H, q, J=7.2 Hz), 3.16 (1H, dd, J=14.0, 5.2 Hz), 3.09-2.95 (2H, m), 2.91-2.81 (1H, m), 1.87 (3H, s), 1.72-1.56 (3H, m), 1.38-1.25 (6H, m), 1.19 (6H, t, J=7.2 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.62, 173.58, 173.31, 164.70, 158.21, 157.62, 154.21, 152.62, 150.48, 143.94, 141.29, 139.26, 138.69, 137.91, 134.61, 132.96, 131.75, 130.48, 129.78, 129.58, 129.37, 127.90, 126.21, 126.21, 125.87, 110.60, 107.29, 105.60, 98.44, 64.55, 62.86, 56.01, 51.80, 51.66, 45.77, 41.45, 38.86, 32.27, 30.11, 23.75, 22.52, 12.88.

Ac-Phe-Lys(DEACM)-PABC(oNB)-AcRha, 1 (2PPG-FK-AcRha)



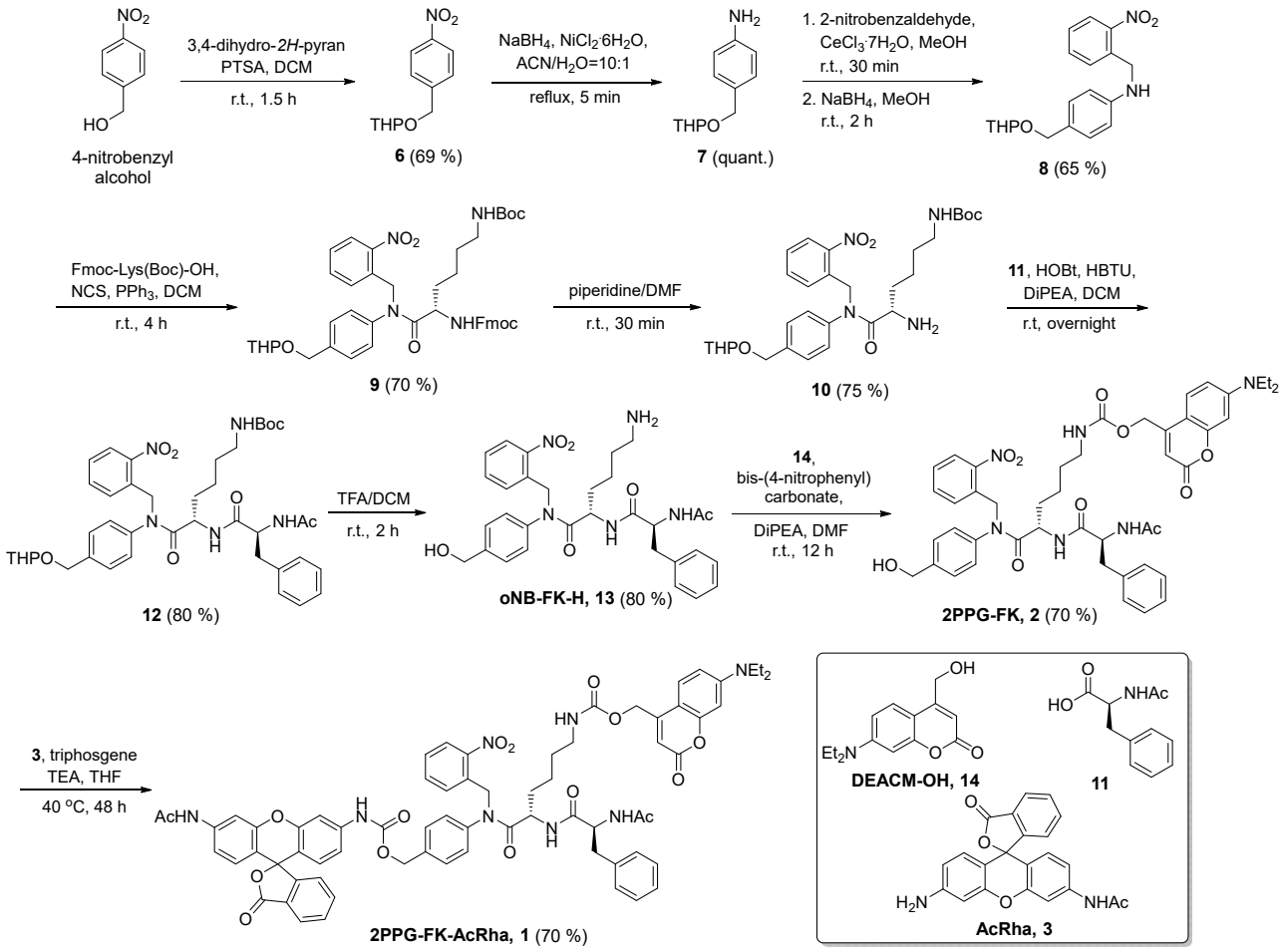
2PPG-FK-H, 2

2PPG-FK-AcRha, 1 (70 %)

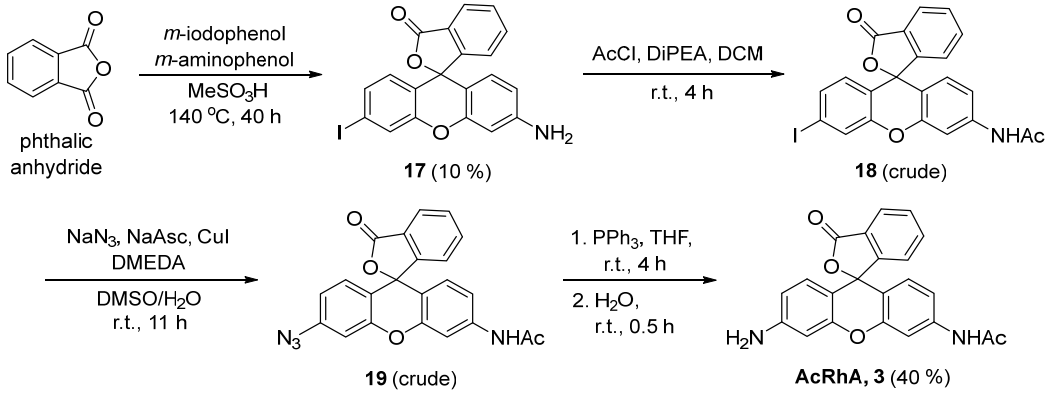
3 (7.4 mg, 20 µmol) and triphosgene (2.0 mg, 6.7 µmol) were dissolved in anhydrous THF (300 µL), and the resulting solution was added with anhydrous TEA (9 µL, 100 µmol) and 4 Å molecular sieve. The mixture was then stirred under ambient temperature for an hour. After then, **2** (9 mg, 10 µmol), anhydrous TEA (9 µL, 100 µmol) and 4 Å molecular sieve were added to the mixture. The

solution was then heated to 40 °C and stirred for further 48 hours. After then, the mixture was purified with high performance liquid chromatography to afford **1** as an orange solid in 70 % yield. ^{1}H NMR (d6-DMSO, 400 MHz) δ 8.32 (1H, d, J=7.2 Hz), 8.04-7.93 (2H, m), 7.81 (1H, s), 7.76 (1H, t, J=7.6 Hz), 7.69 (2H, q, J=7.6 Hz), 7.58 (1H, d, J=8.0 Hz), 7.54-7.49 (2H, m), 7.49-7.36 (7H, m), 7.25-7.20 (5H, m), 7.16 (1H, d, J=6.0 Hz), 7.11 (2H, d, J=9.2 Hz), 6.68 (2H, t, J=7.6 Hz), 6.62 (1H, d, J=9.2 Hz), 6.49 (1H, d, J=2.4 Hz), 5.92 (1H, s), 5.29 (1H, d, J=16.4 Hz), 5.17 (2H, s), 5.12 (2H, s), 4.99 (1H, d, J=16.8 Hz), 4.55-4.48 (1H, br), 4.27-4.21 (1H, br), 3.37 (4H, q, J=7.2 Hz), 3.00-2.85 (4H, m), 2.73-2.64 (1H, m), 2.52-2.51 (1H, m), 2.04 (3H, s), 1.71 (3H, s), 1.23-1.11 (5H, m), 1.07 (6H, t, J=7.2 Hz); ^{13}C NMR (d6-DMSO, 100 MHz) δ 172.23, 172.21, 171.56, 169.02, 168.84, 168.61, 160.68, 155.69, 155.37, 153.08, 151.88, 150.93, 150.76, 150.37, 141.36, 141.33, 137.89, 136.10, 136.04, 135.67, 135.63, 133.44, 131.65, 130.90, 130.16, 129.43, 129.25, 129.14, 128.43, 128.25, 128.17, 127.92, 127.86, 126.13, 125.68, 125.18, 124.57, 123.89, 112.87, 112.41, 111.91, 108.66, 106.90, 106.02, 105.23, 105.23, 104.36, 96.81, 81.93, 69.83, 60.75, 57.81, 49.68, 43.91, 40.42, 38.75, 37.45, 31.45, 28.76, 24.04, 22.37, 12.23. HRMS (ESI, m/z): $[M+H]^{+}$, calculated: 1247.4720, found: 1247.4196.

3. Supporting Schemes and Figures



Scheme S1. The synthetic scheme of **2PPG-FK-AcRha** (**1**).



Scheme S2 The synthetic scheme of the fluorophore, **AcRha** (**3**).

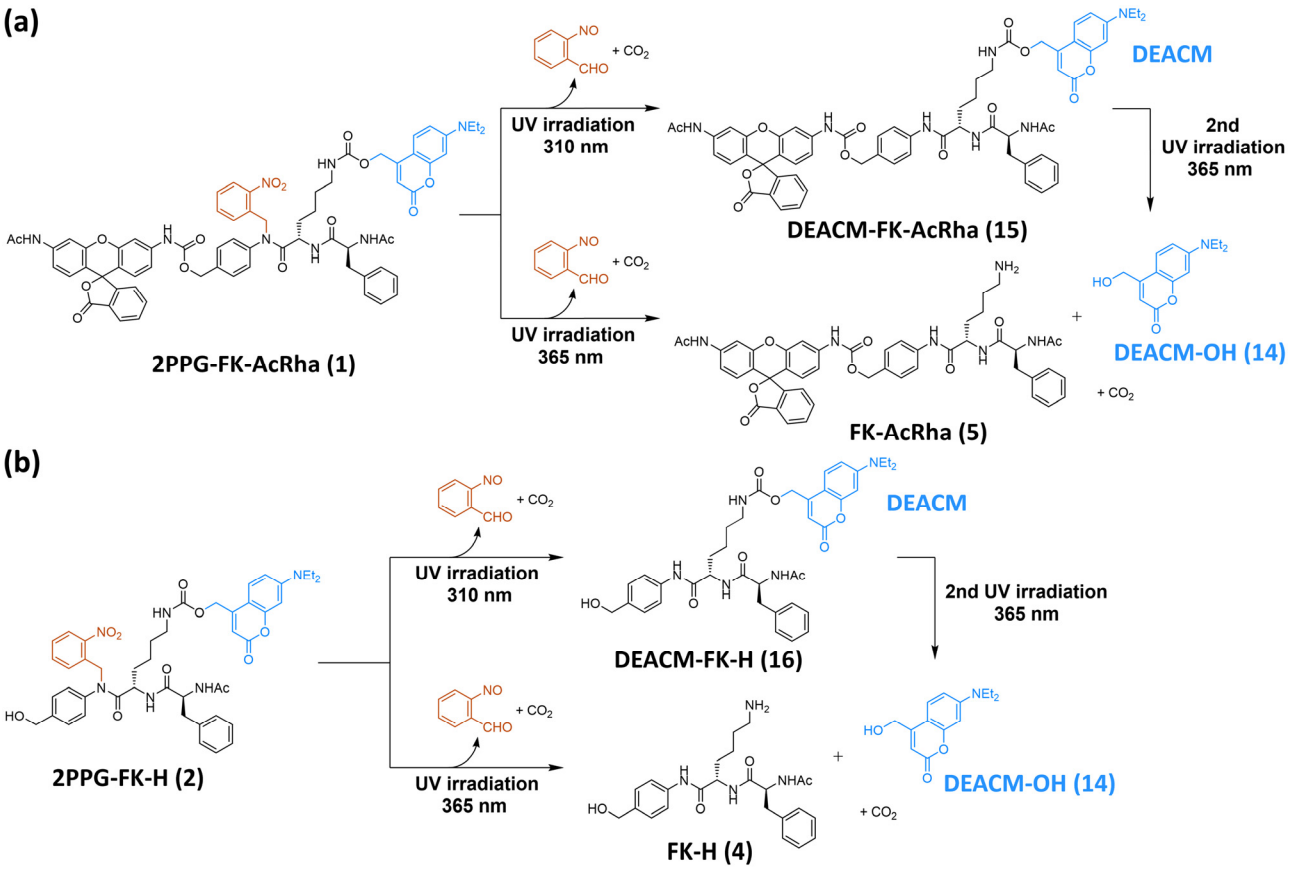


Figure S1. Schematics of sequential/direct photolysis of (a) **2PPG-FK-AcRha (1)** and (b) **2PPG-FK-H (2)**.

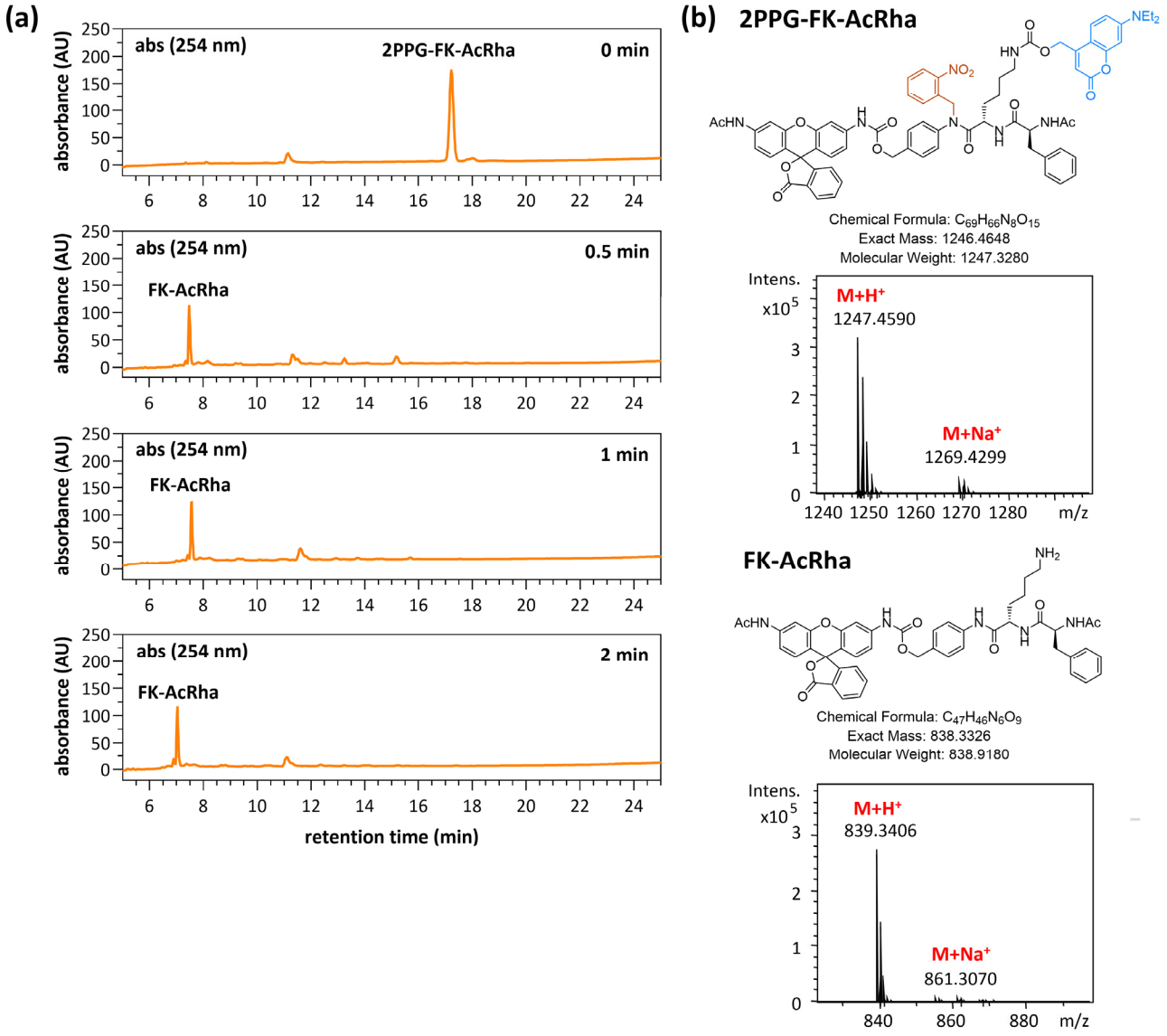


Figure S2. (a) The LC-HRMS chromatograms of **2PPG-FK-AcRha** (**1**) (100 µM) with 365 nm irradiation for various irradiation time periods (**Figure 2b**). (monitored by UV absorbance at 254 nm) (b) The HRMS spectra of **2PPG-FK-AcRha** (**1**) (retention time: 17.2 min) and the HRMS spectra of **FK-AcRha** (retention time: 7.0 min).

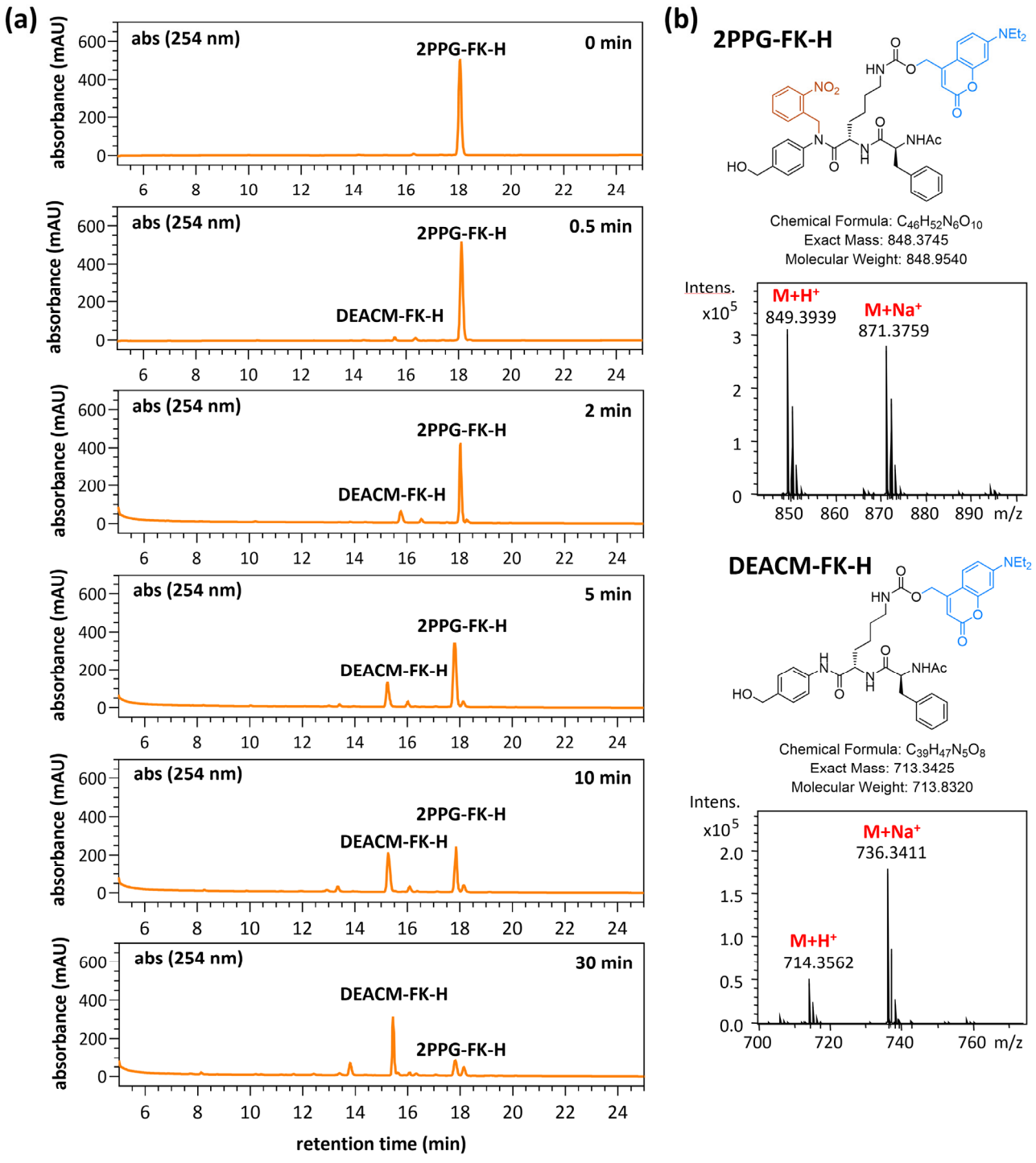


Figure S3. (a) The LC-HRMS chromatograms of **2PPG-FK-H** (**2**) (100 µM) with 310 nm irradiation for various irradiation time periods (**Figure 2c**). (monitored by UV absorbance at 254 nm) (b) The HRMS spectra of **2PPG-FK-H** (**2**) (retention time: 18.0 min) and the HRMS spectra of **DEACM-FK-H** (**16**) (retention time: 15.4 min).

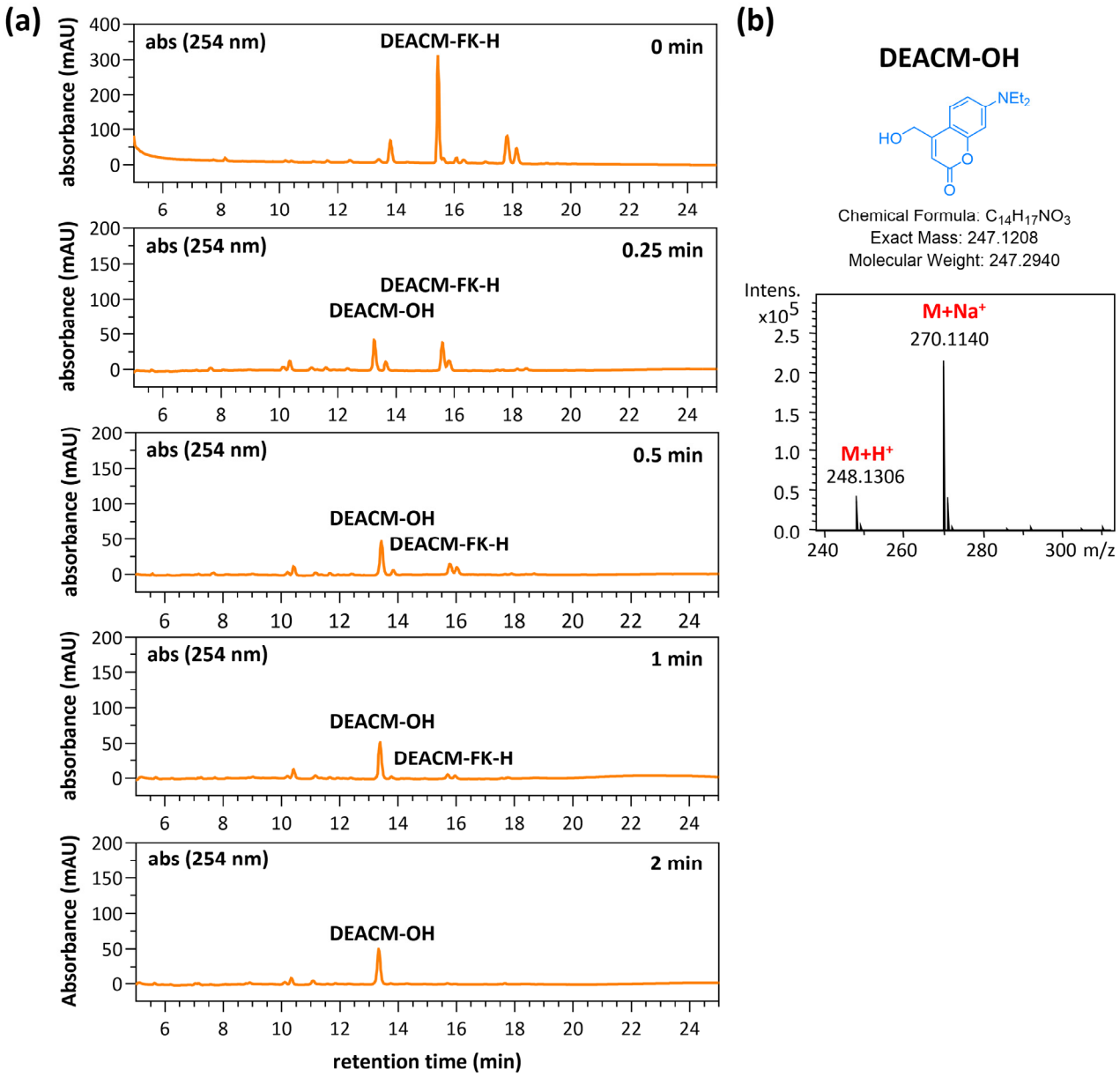


Figure S4. (a) The LC-HRMS chromatograms of **2PPG-FK-H** (**2**) (100 µM) with 310 nm irradiation for 30 minutes, then followed by 365 nm irradiation for various time periods (**Figure 2d**). (monitored by UV absorbance at 254 nm) (b) The HRMS spectra of **DEACM-OH** (**14**) (retention time: 13.3 min).

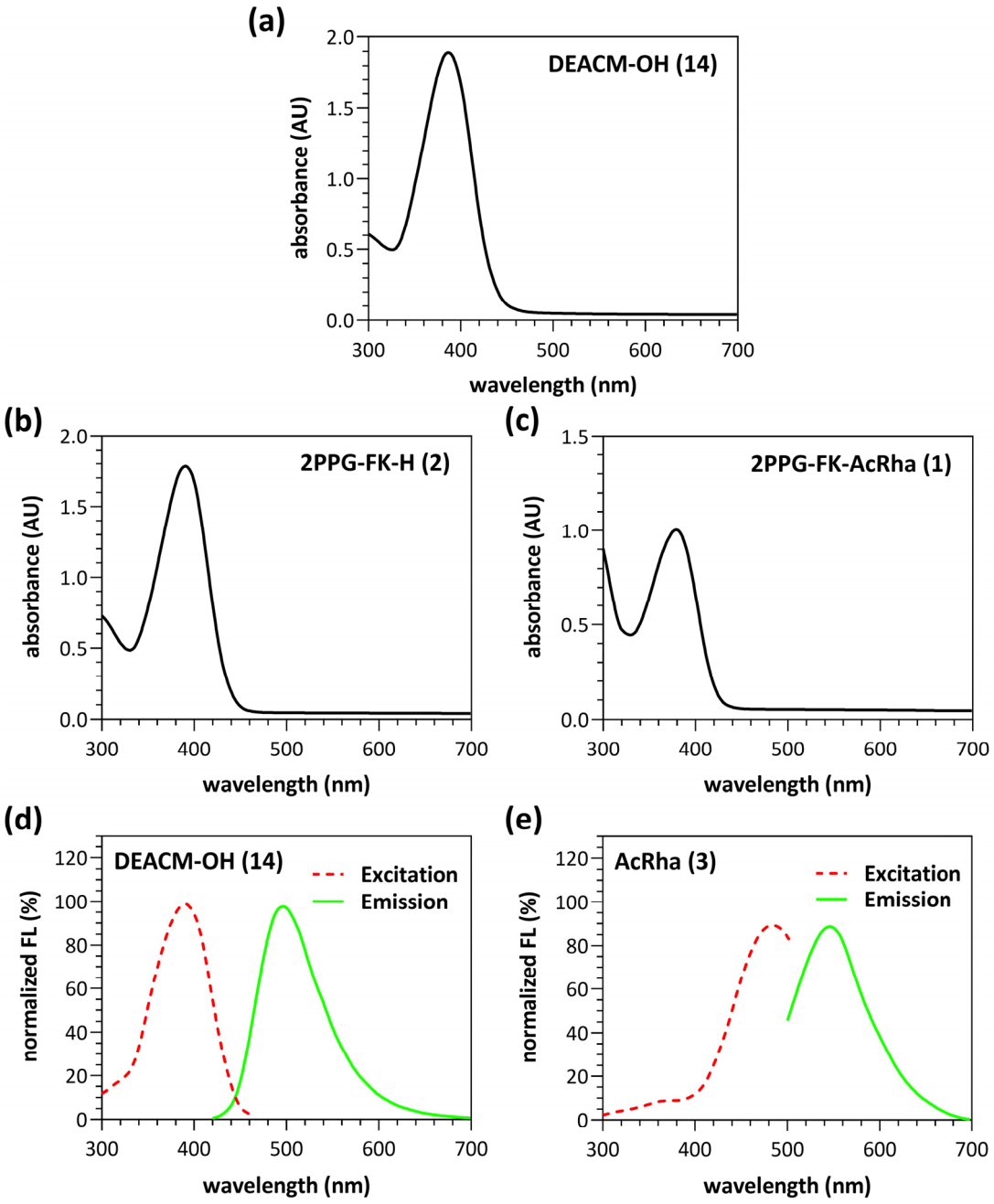


Figure S5. Absorbance spectrum of (a) **DEACM-OH (14)** (500 µM), (b) **2PPG-FK-H (2)** (500 µM), and (c) **2PPG-FK-AcRha (1)** (500 µM). Excitation and emission spectrum of (d) **DEACM-OH (14)** (10 µM), and (e) **AcRha (3)** (10 µM).

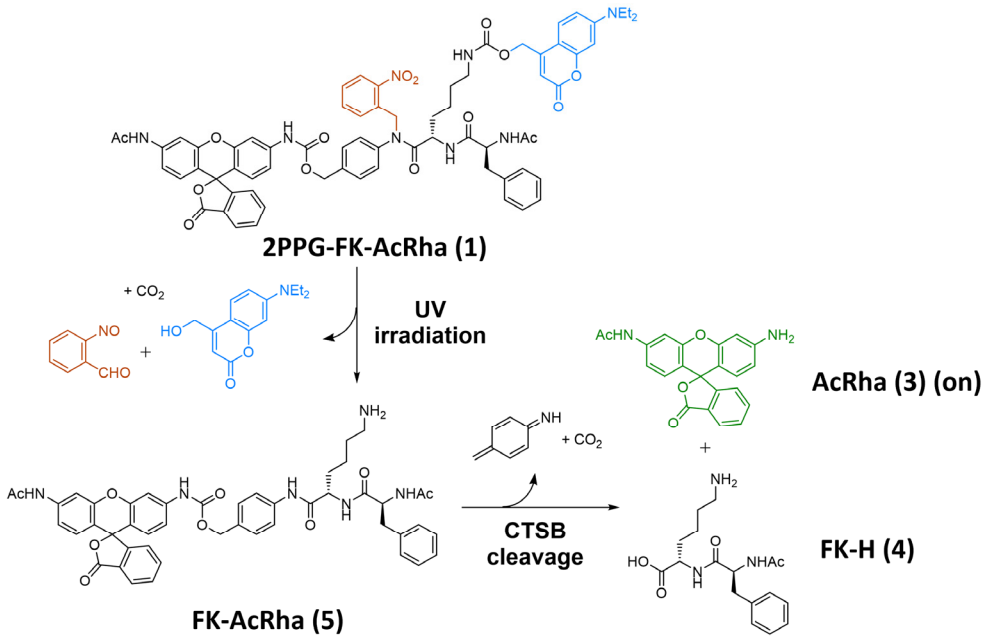


Figure S6. Schematics of sequential photorelease and CTSB activation of **2PPG-FK-AcRha** (**1**).

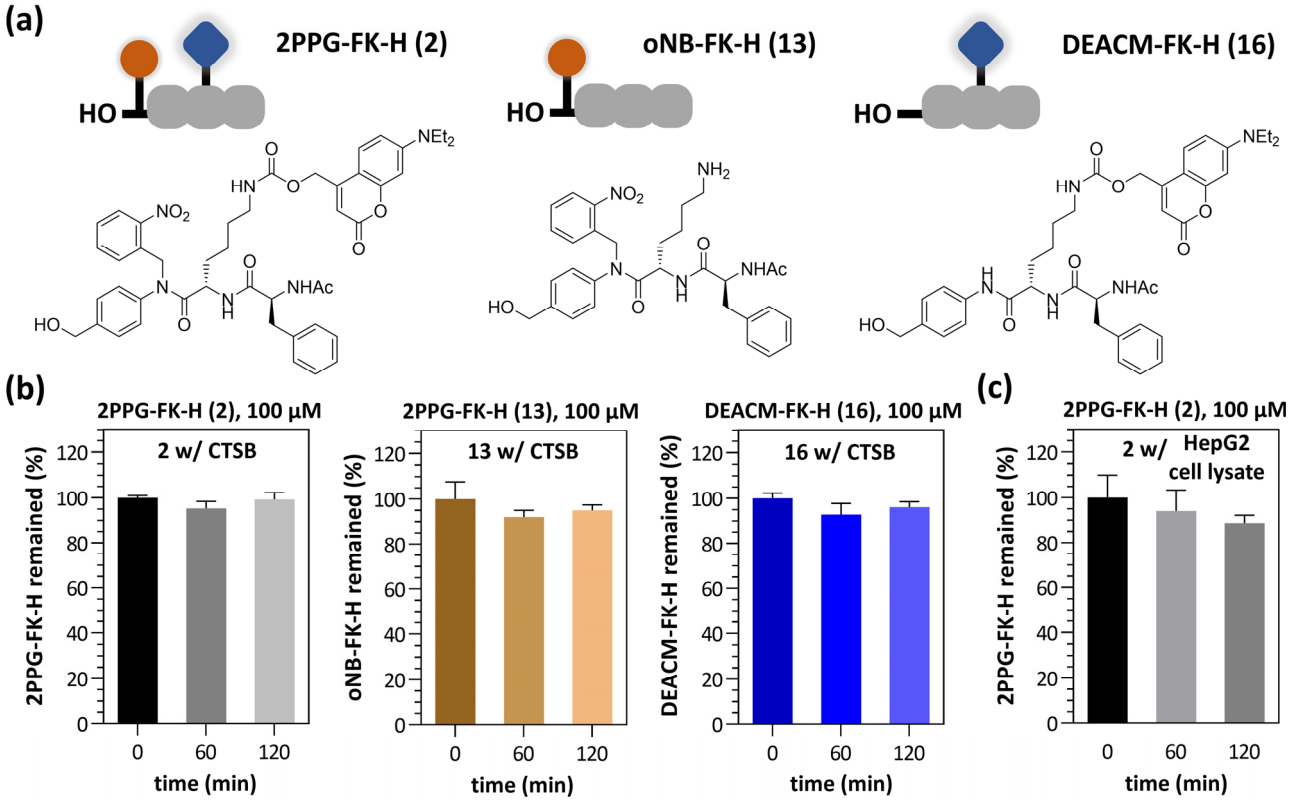


Figure S7. (a) Illustrations and structures of test compounds. (b) Stability of the double-caged dipeptide, **2PPG-FK-H** (**2**) (100 µM at 37 °C, CTSB, 40 nM), the cleavage site-caged dipeptide, **oNB-FK-H** (**13**). (100 µM at 37 °C, CTSB, 40 nM), and the **P1** side chain-caged dipeptide, **DEACM-FK-H** (**16**) (100 µM at 37 °C, CTSB, 40 nM) under CTSB treatment. (c) Stability of **2PPG-FK-H** (**2**) under HepG2 cell lysate treatment (100 µM at 37 °C, cell lysate: 5 µg/mL).

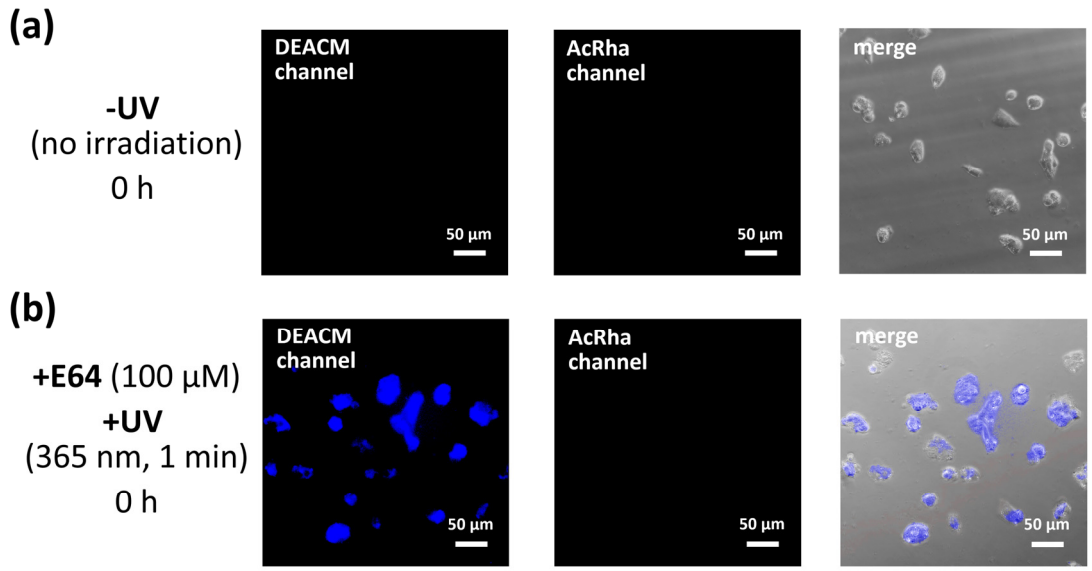


Figure S8. Fluorescence microscopic images of HepG2 treated with **2PPG-FK-AcRha** (**1**) (10 µM): (a) without irradiation and 0 h incubation; (b) with **E64** (100 µM), 365 nm irradiation for 1 minute, and 0 h incubation.

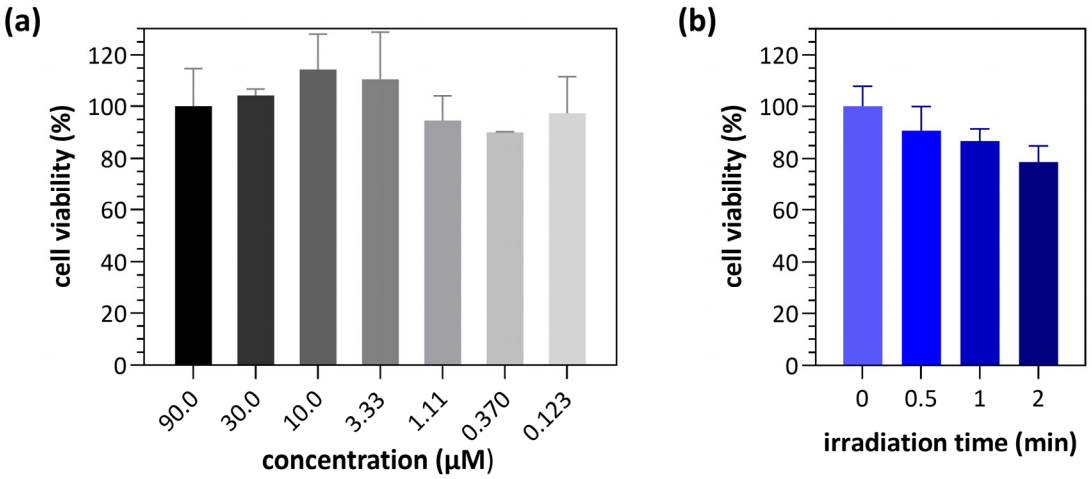


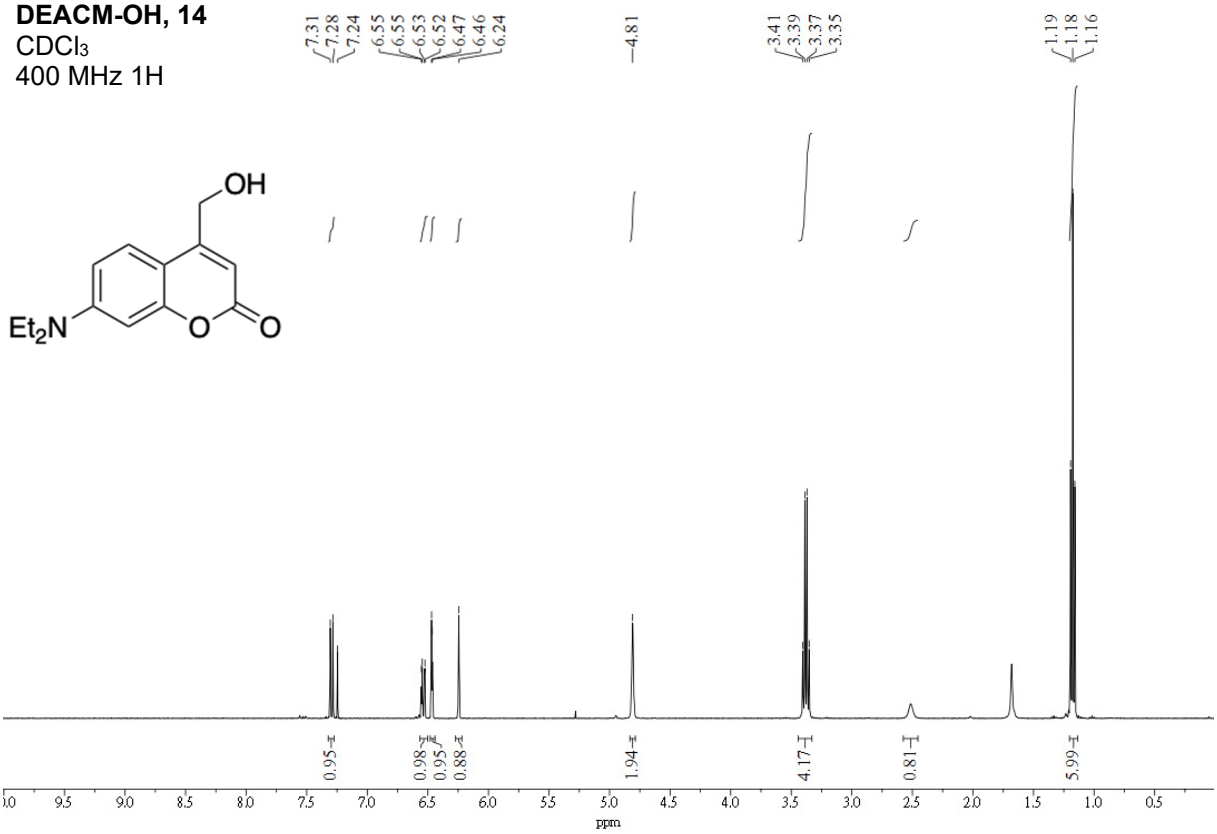
Figure S9. Cell viability assay of HepG2 cells treated with (a) **2PPG-FK-AcRha** (**1**) at various concentrations and (b) UV irradiation of 365 nm for various time periods.

4. References

1. Lankalapalli, R. S., Ouro, A., Arana, L., Gómez-Muñoz, A., & Bittman, R. Caged Ceramide 1-Phosphate Analogues: Synthesis and Properties. *J. Org. Chem.* **2009**, *74*, 8844-8847.
2. Maity, S. K., Kumar, R., Ambast, D. K., Pal, B., & Haldar, D. Self-Assembly and Nonlinear Optical Properties of a Synthetic Dipeptide. *J. Mater. Chem* **2012**, *22*, 22198-22203.
3. Gorska, K., Manicardi, A., Barluenga, S., & Winssinger, N. DNA-Templated Release of Functional Molecules with an Azide-Reduction-Triggered Immolative Linker. *Chem. Commun.* **2011**, *47*, 4364-4366.

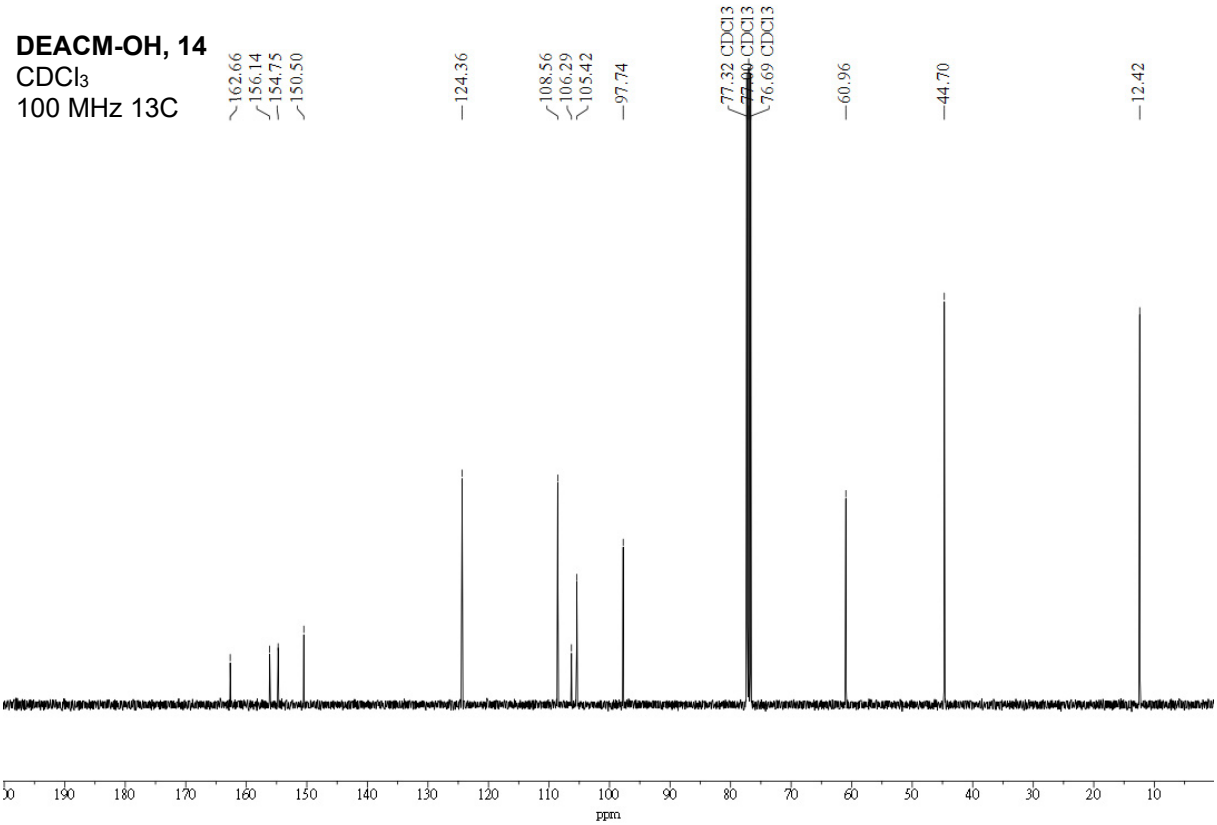
5. Appendix: NMR Spectroscopic Data

DEACM-OH, 14
$CDCl_3$
400 MHz 1H



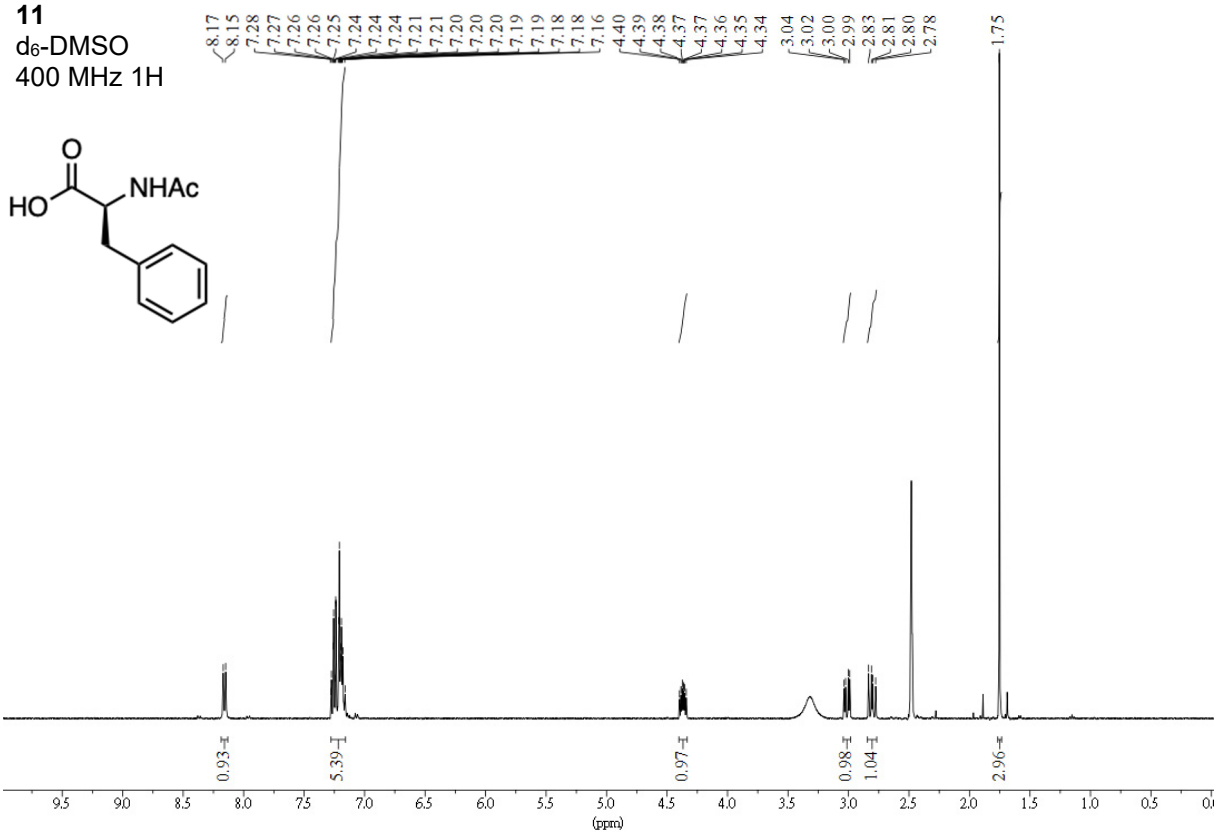
^{1}H NMR of compound 14

DEACM-OH, 14
$CDCl_3$
100 MHz 13C



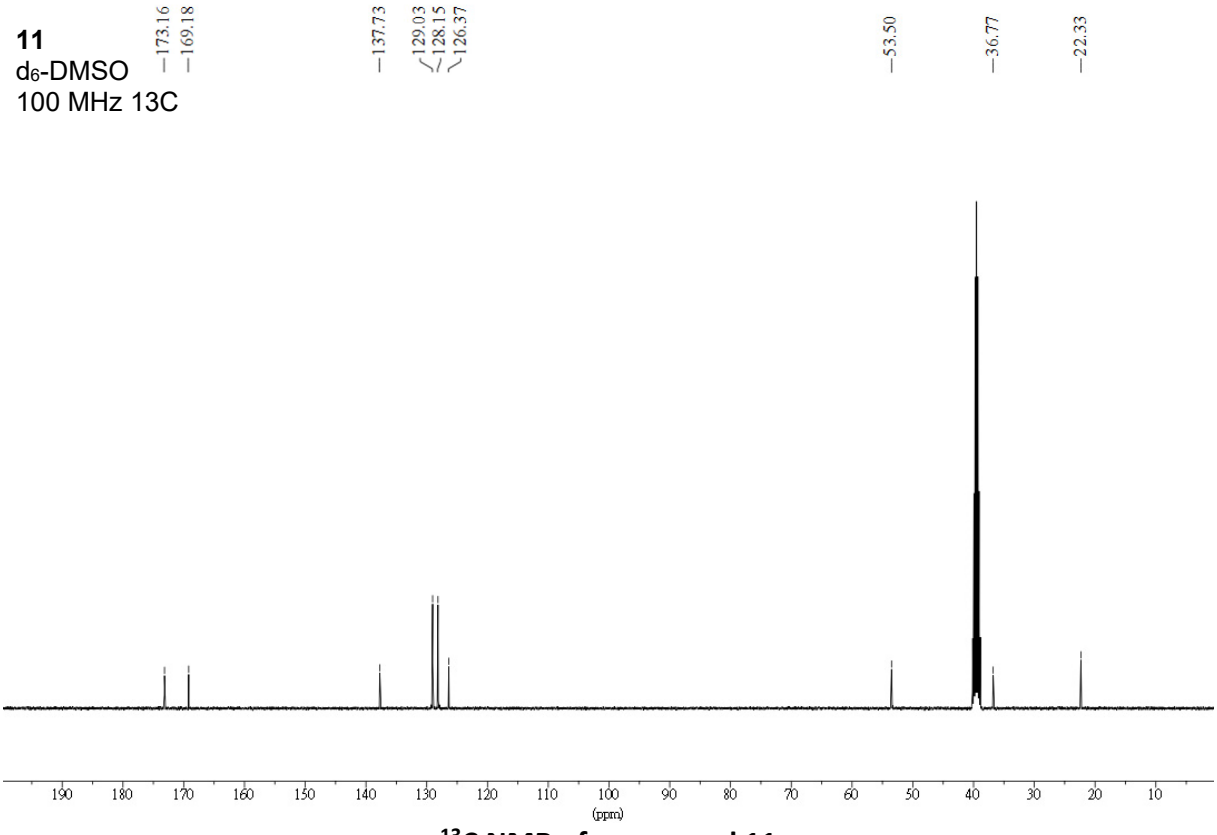
^{13}C NMR of compound 14

11
d_6-DMSO
400 MHz 1H

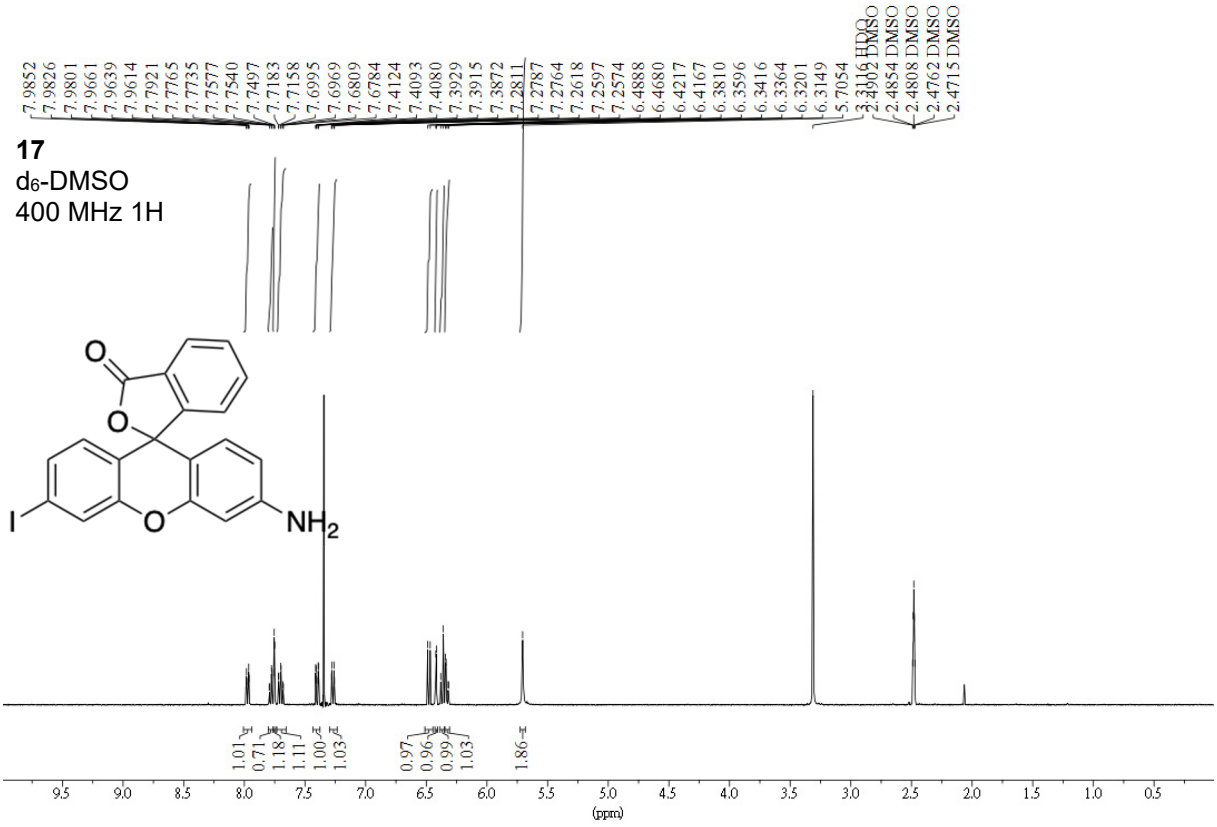


^{1}H NMR of compound 11

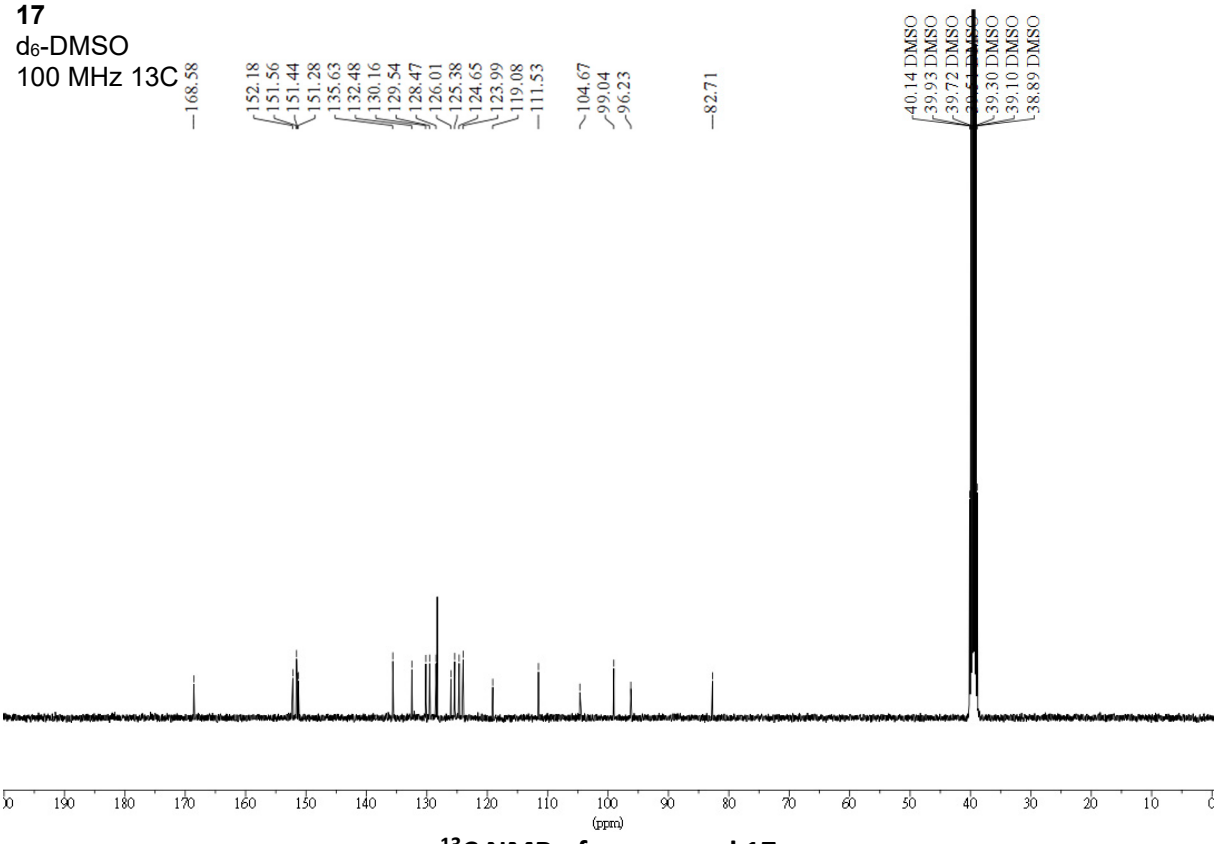
11
d_6-DMSO
100 MHz 13C



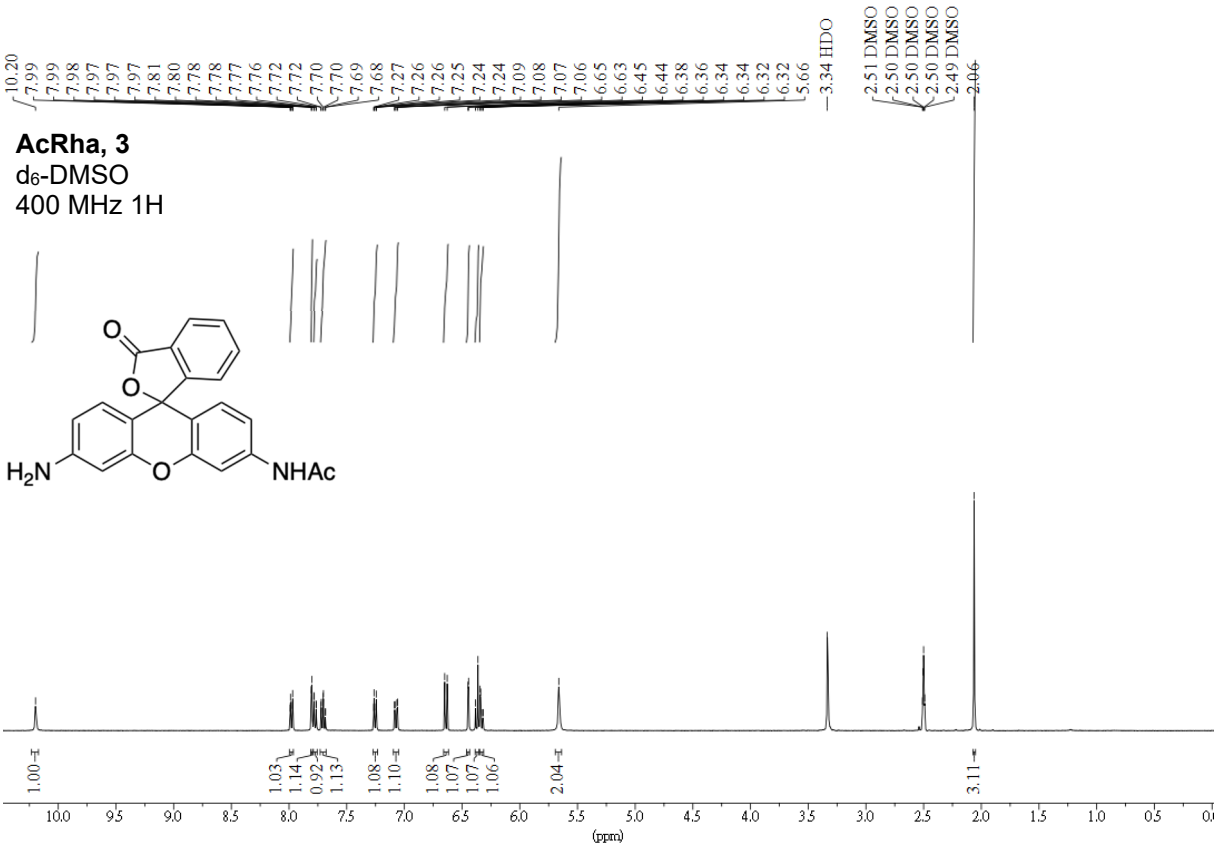
^{13}C NMR of compound 11



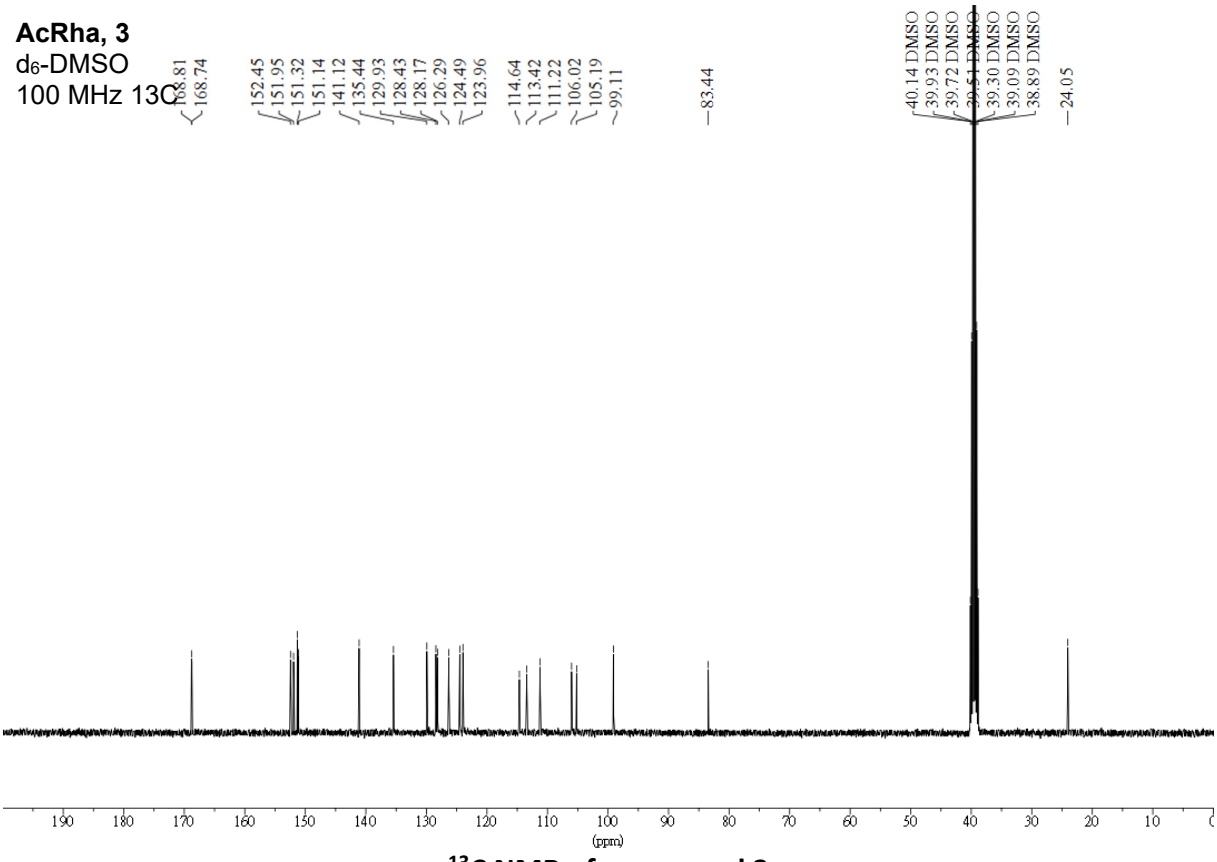
^{1}H NMR of compound 17



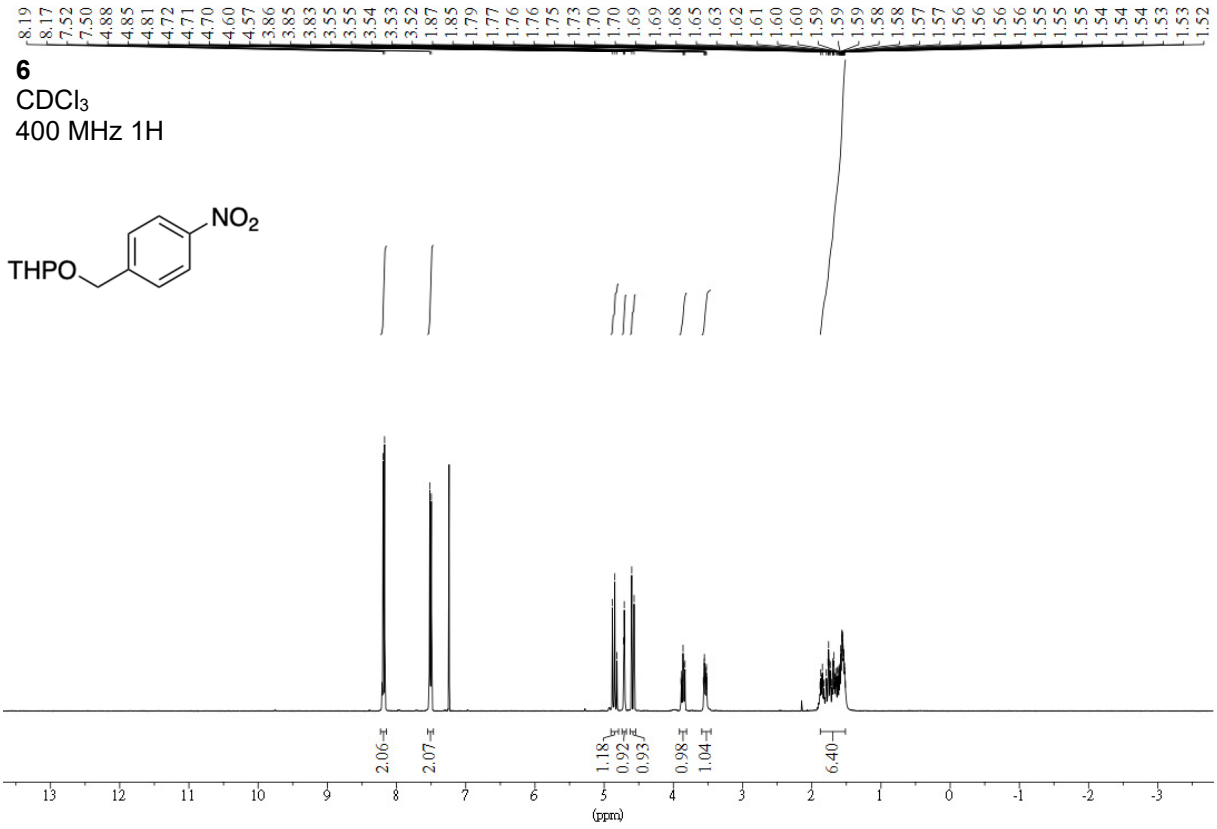
^{13}C NMR of compound 17



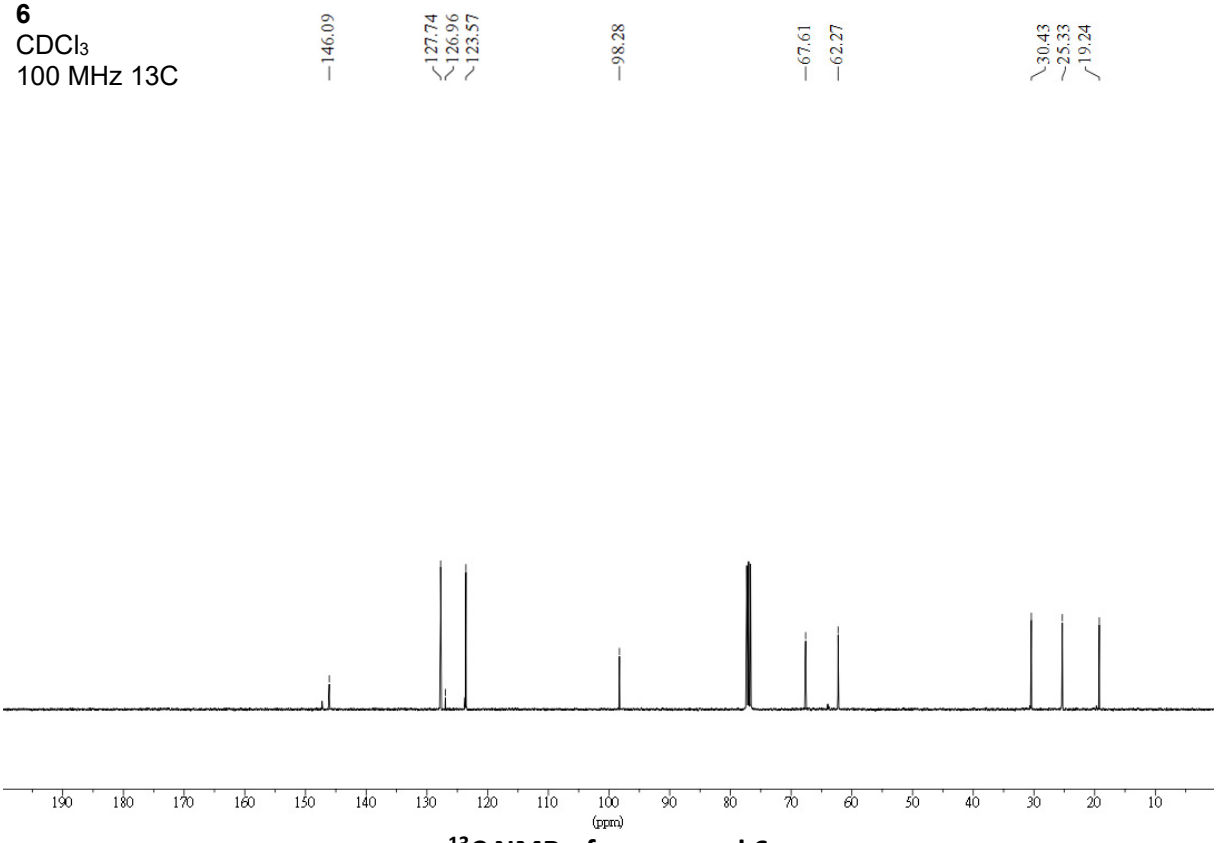
^{1}H NMR of compound 3



^{13}C NMR of compound 3

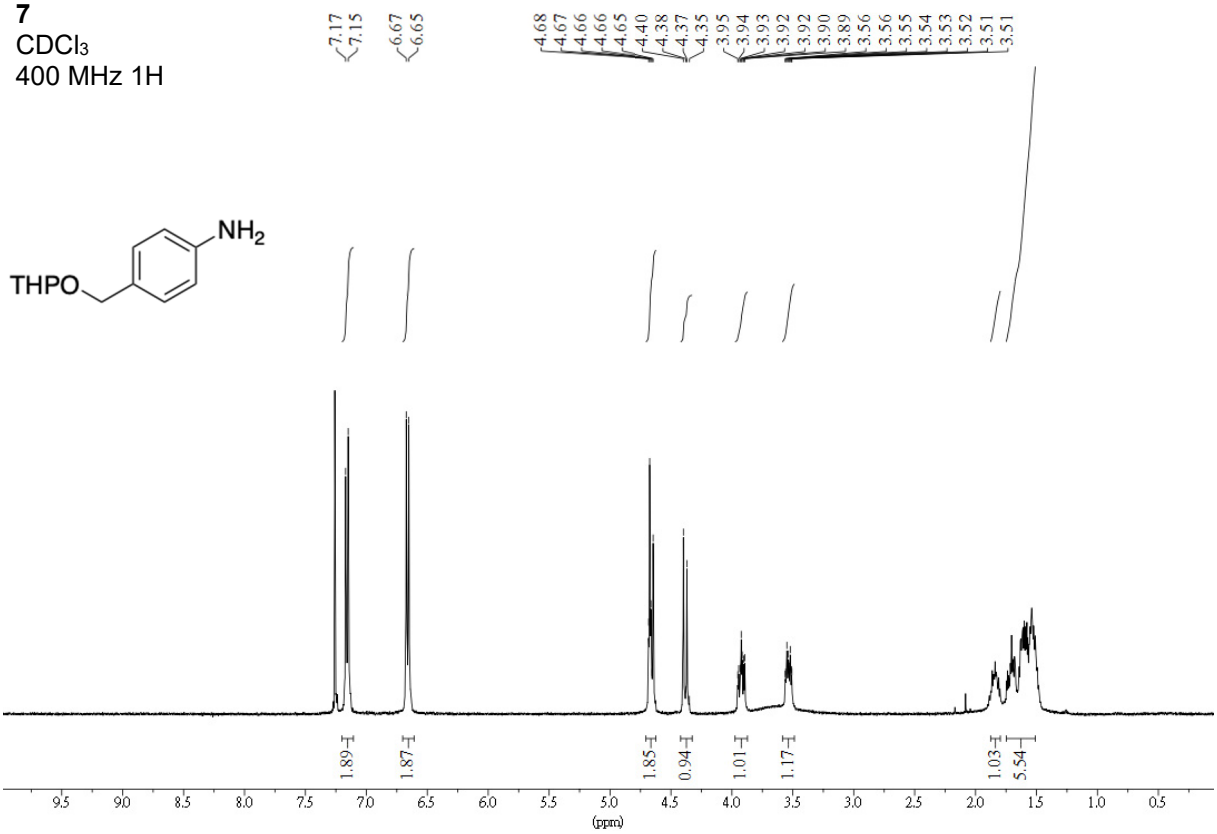


^{1}H NMR of compound 6



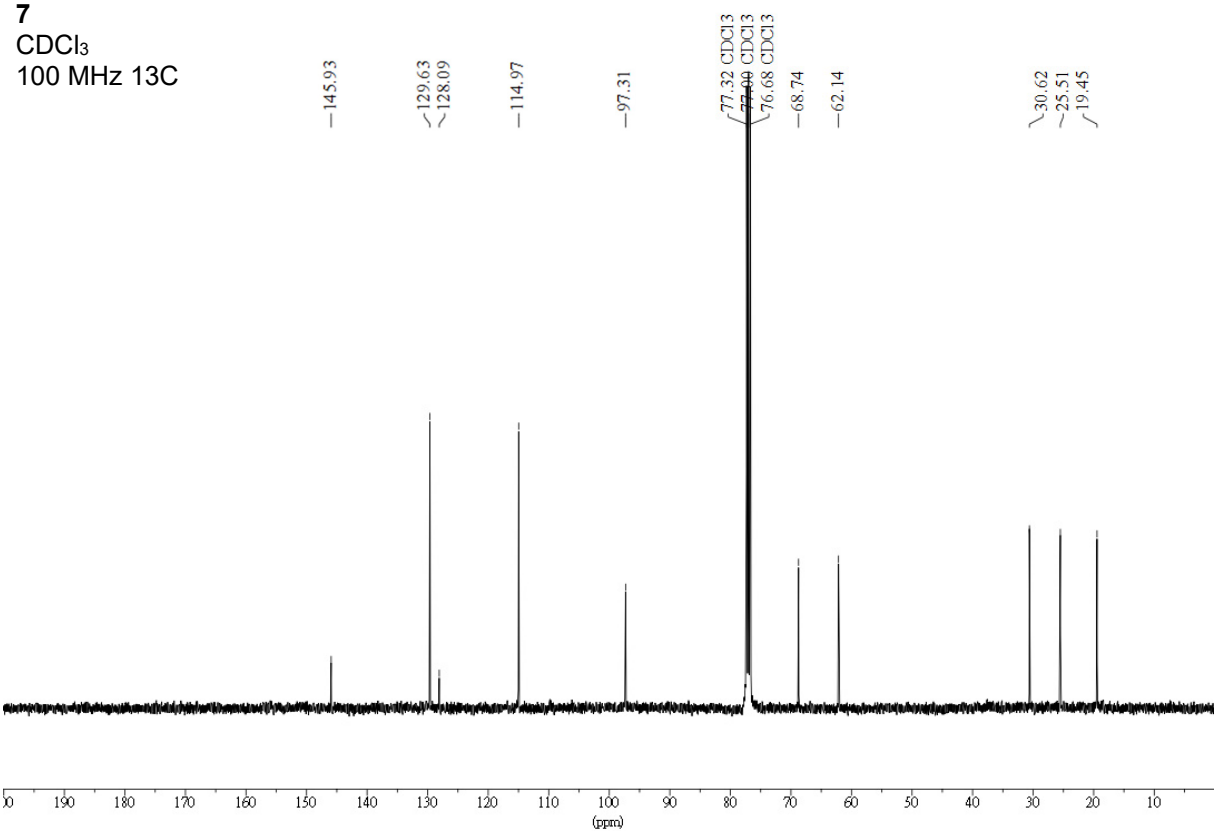
^{13}C NMR of compound 6

7
$CDCl_3$
400 MHz 1H

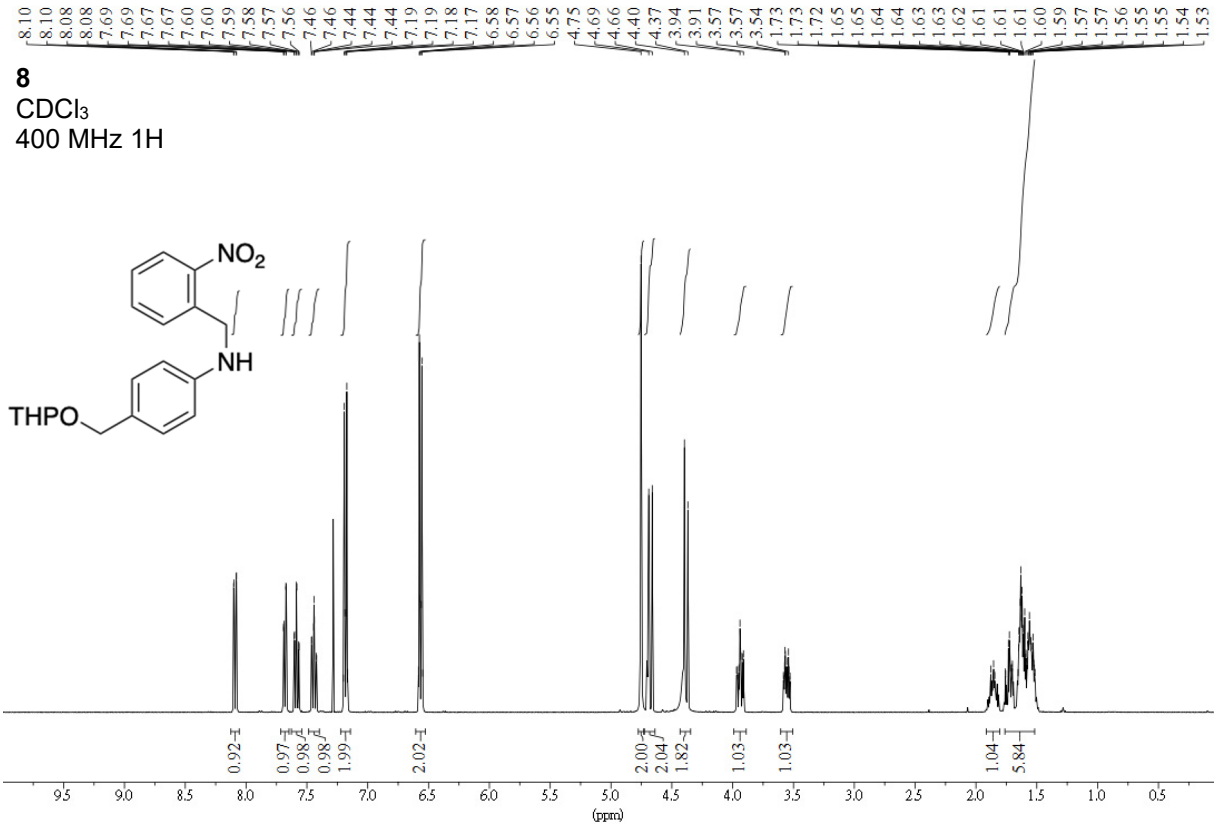


^{1}H NMR of compound 7

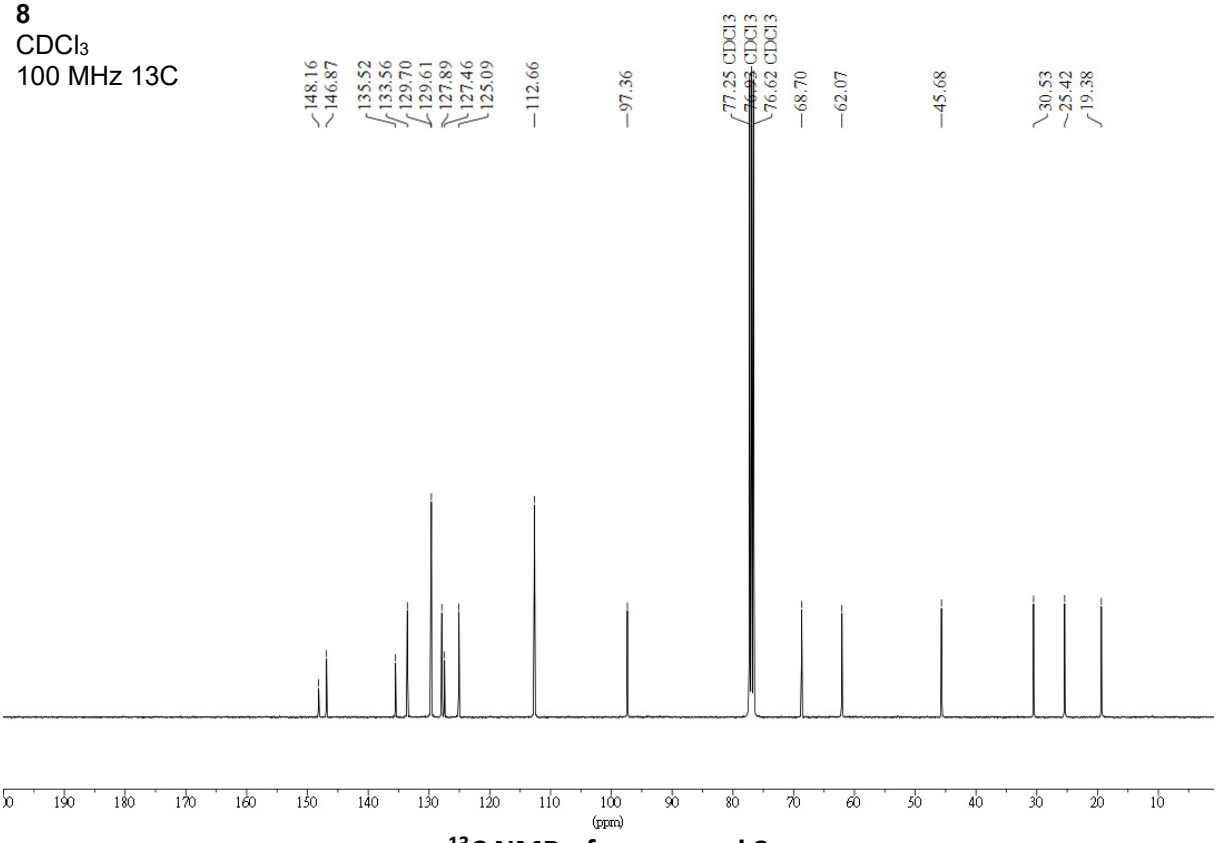
7
$CDCl_3$
100 MHz 13C



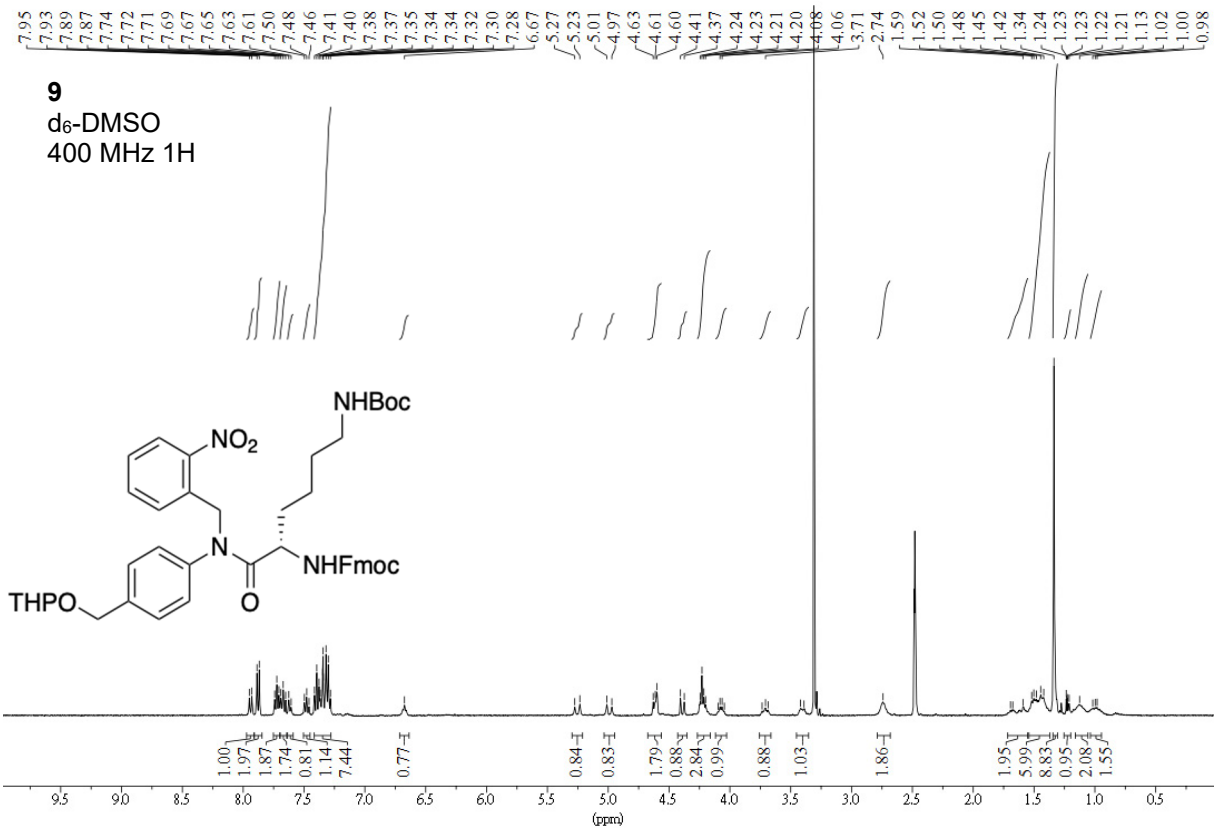
^{13}C NMR of compound 7



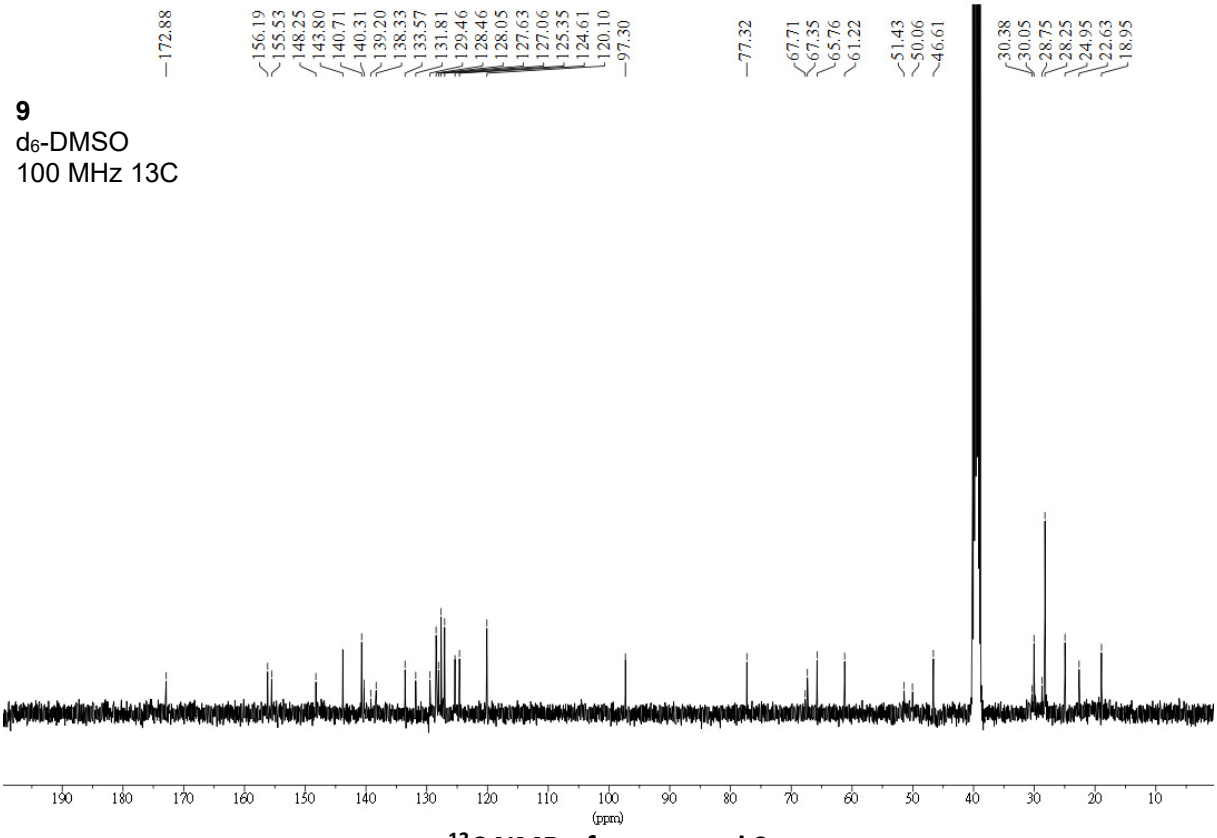
^{1}H NMR of compound 8



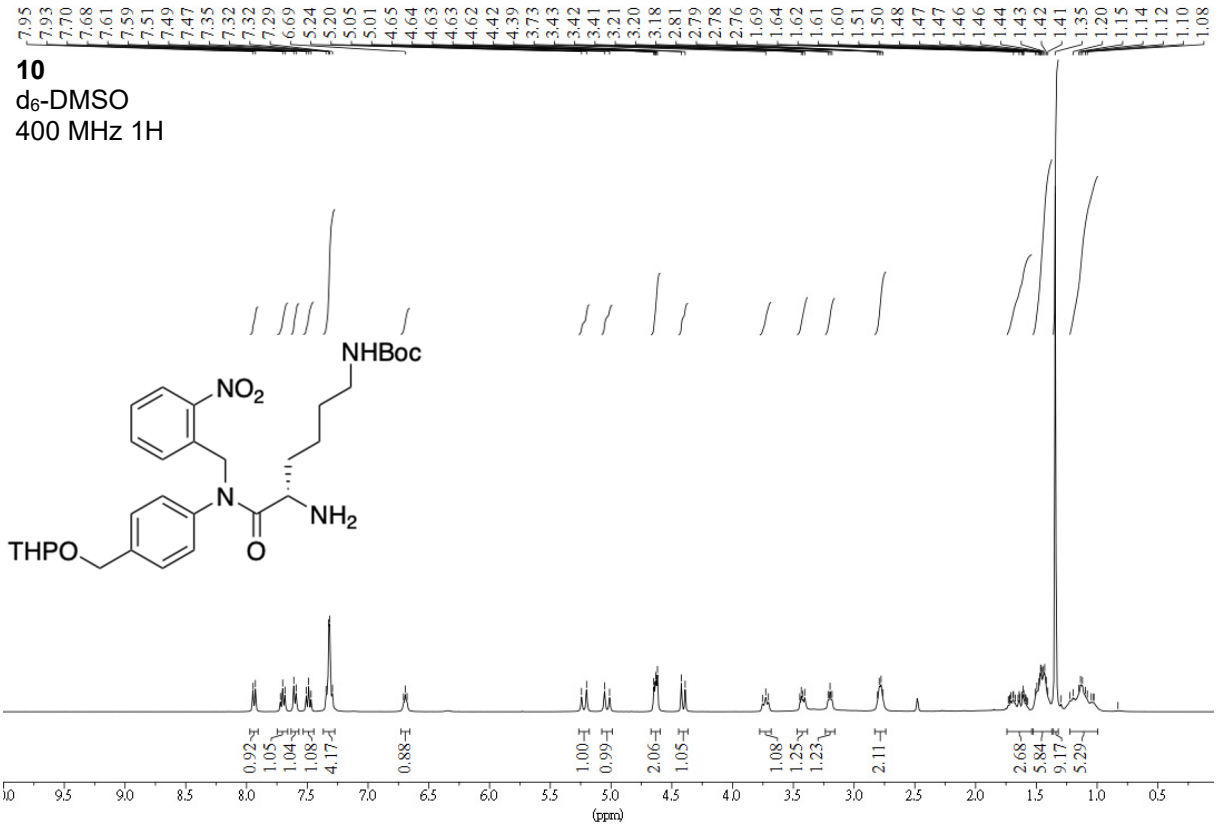
^{13}C NMR of compound 8



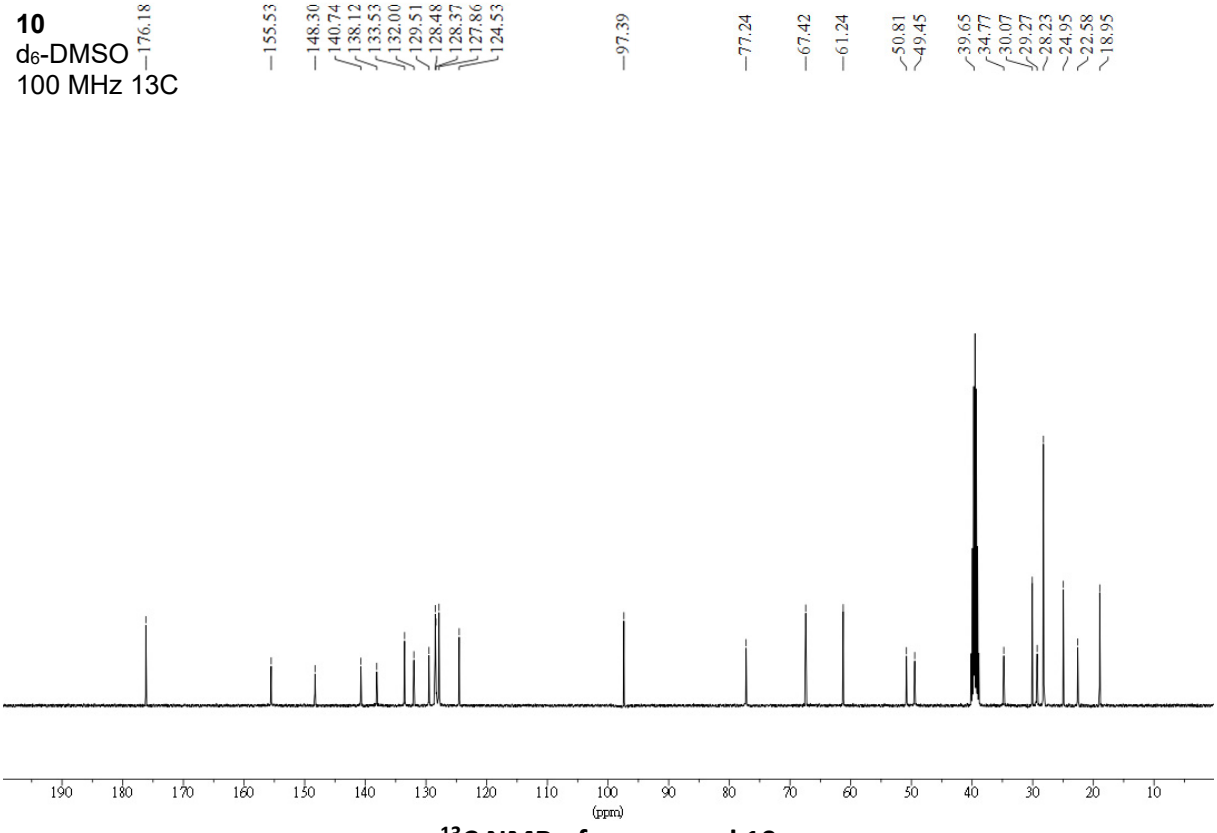
^{1}H NMR of compound 9



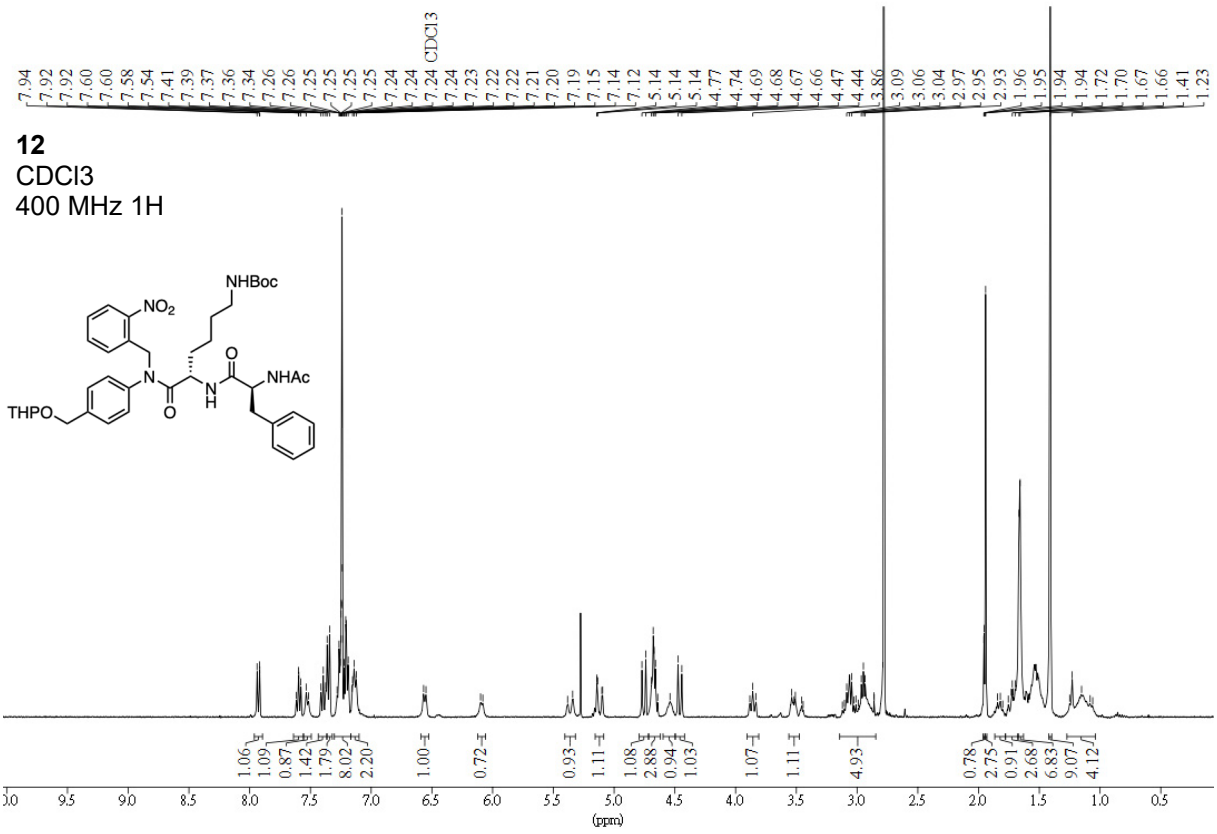
^{13}C NMR of compound 9



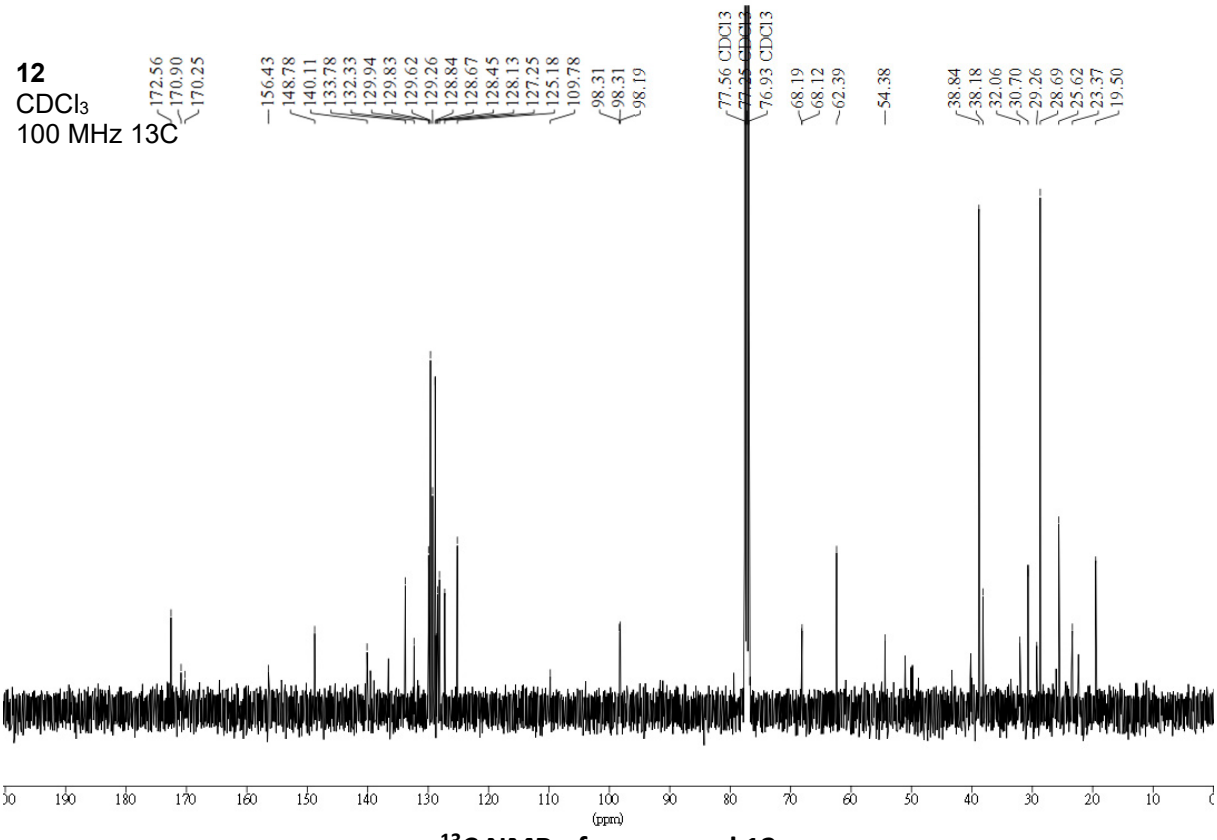
^{1}H NMR of compound 10



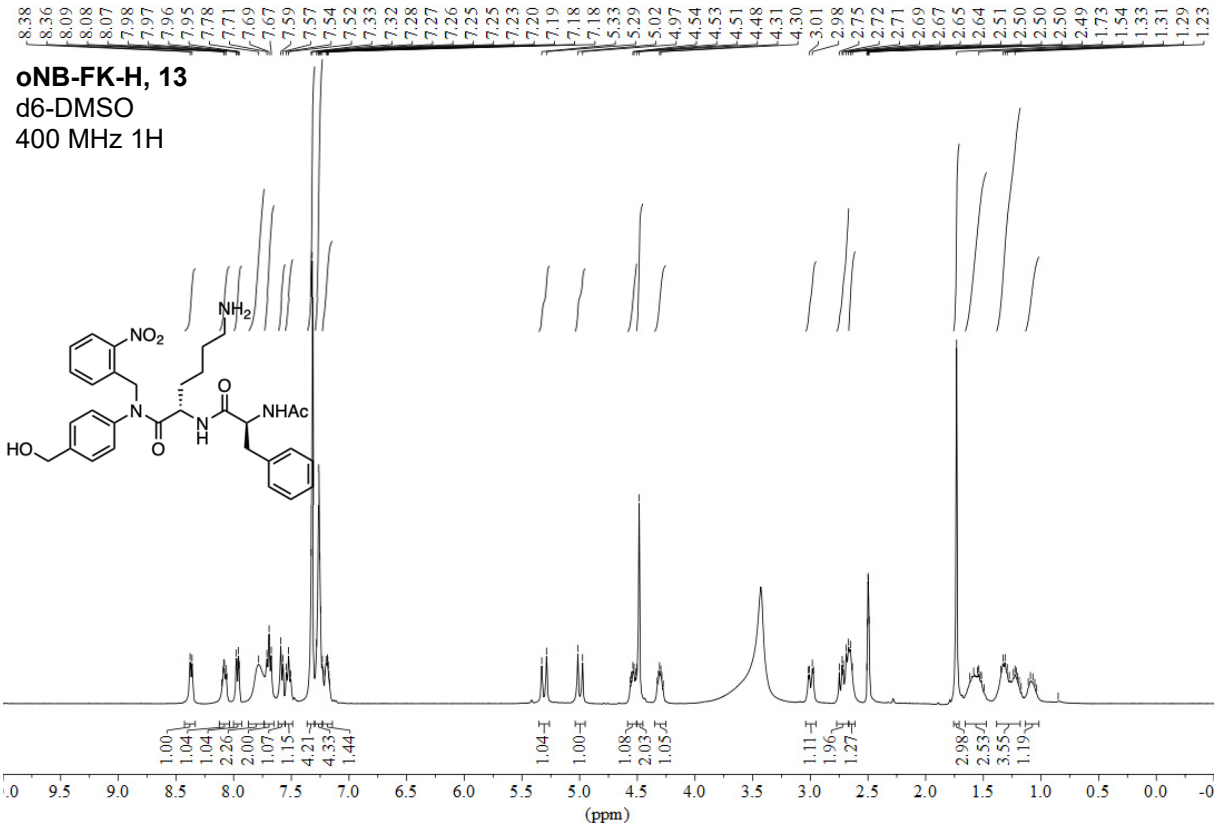
^{13}C NMR of compound 10



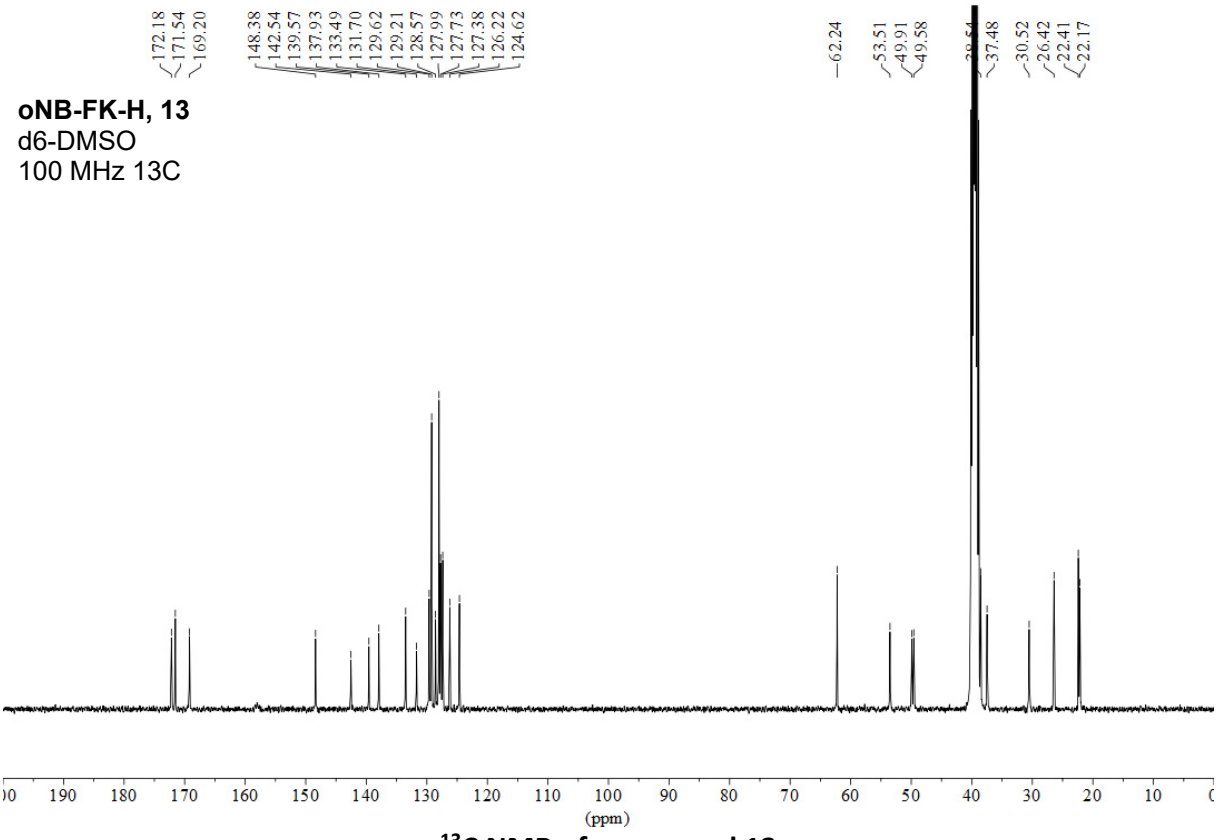
^{1}H NMR of compound 12



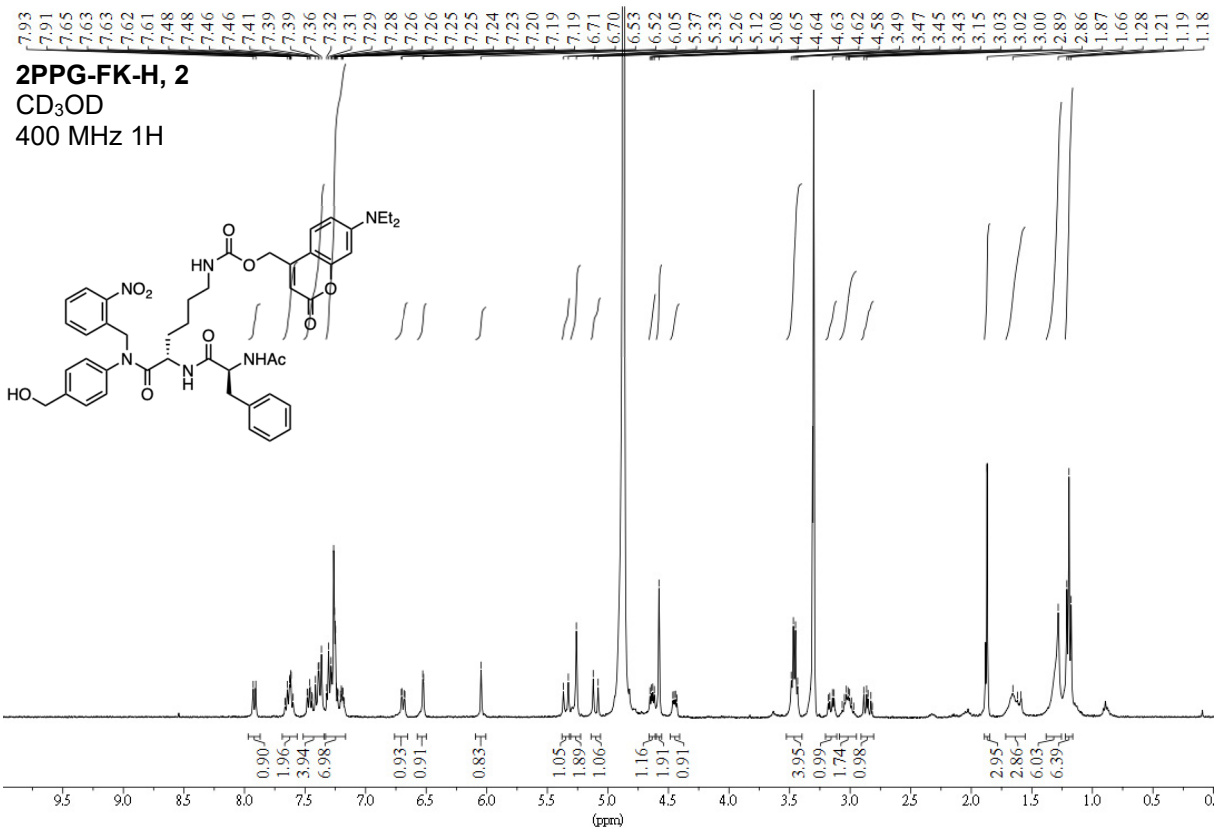
^{13}C NMR of compound 12



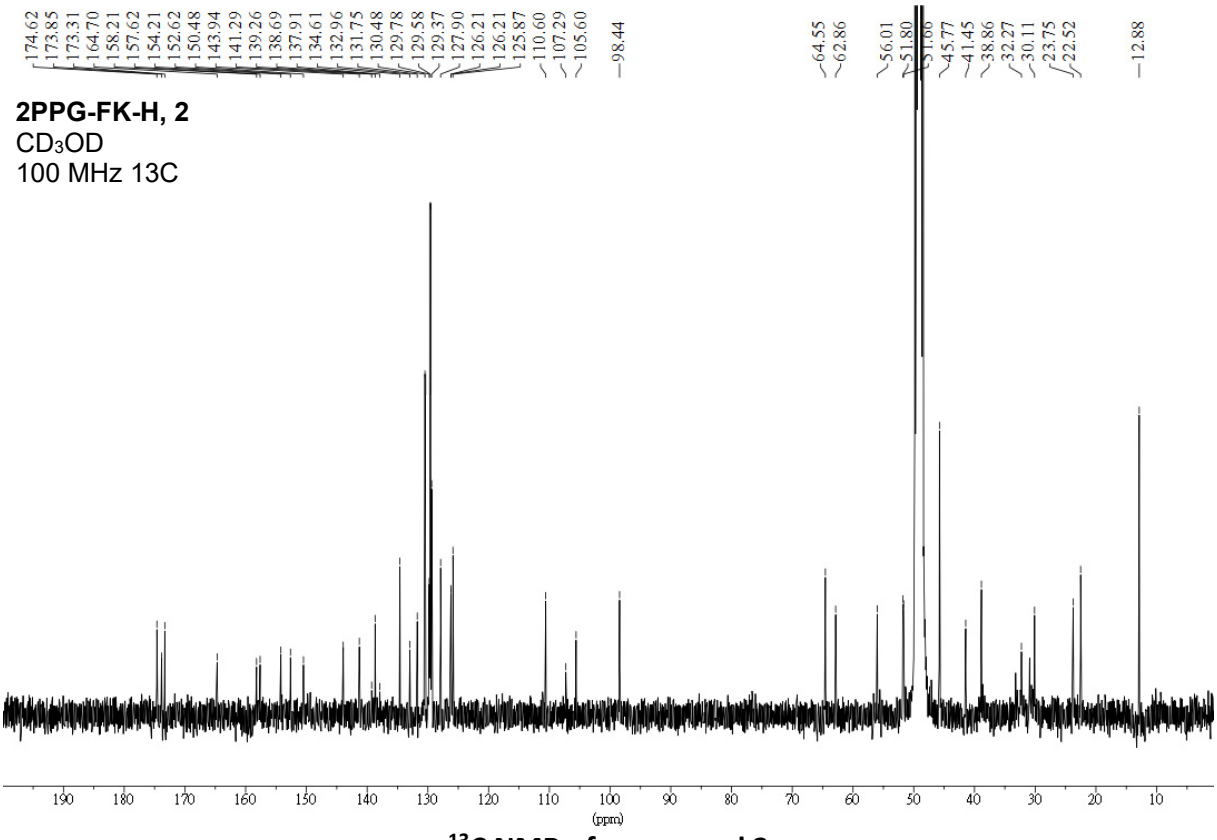
^{1}H NMR of compound 13



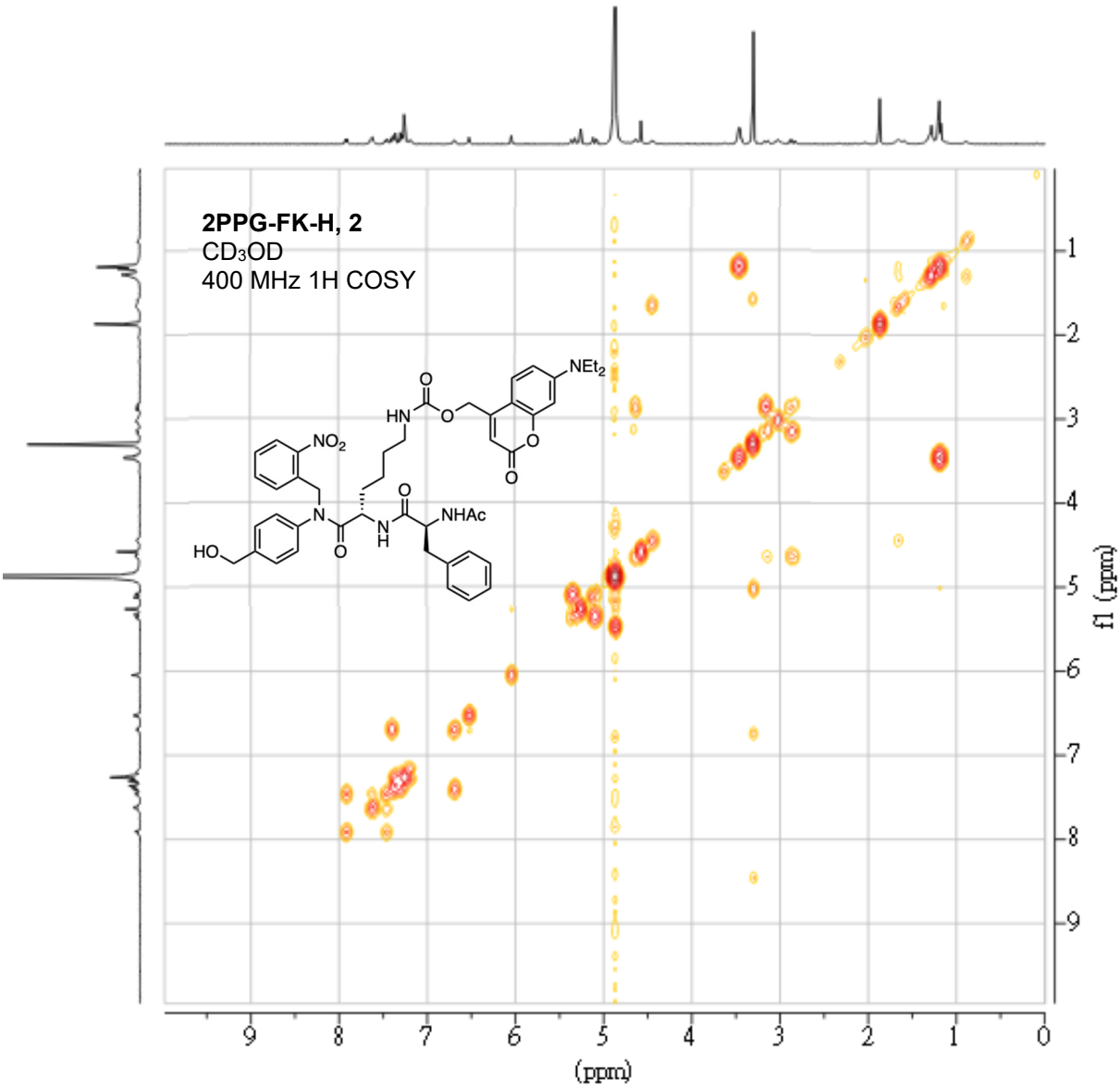
^{13}C NMR of compound 13



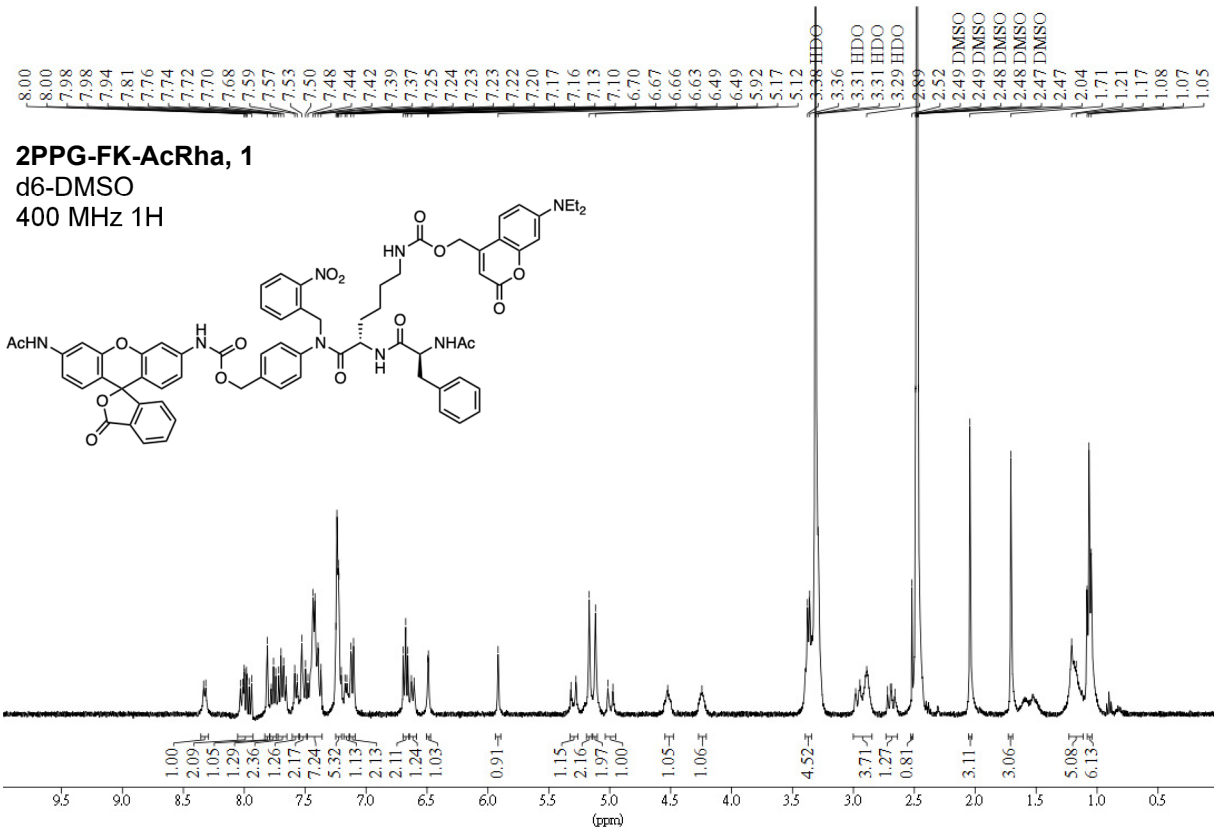
^{1}H NMR of compound 2



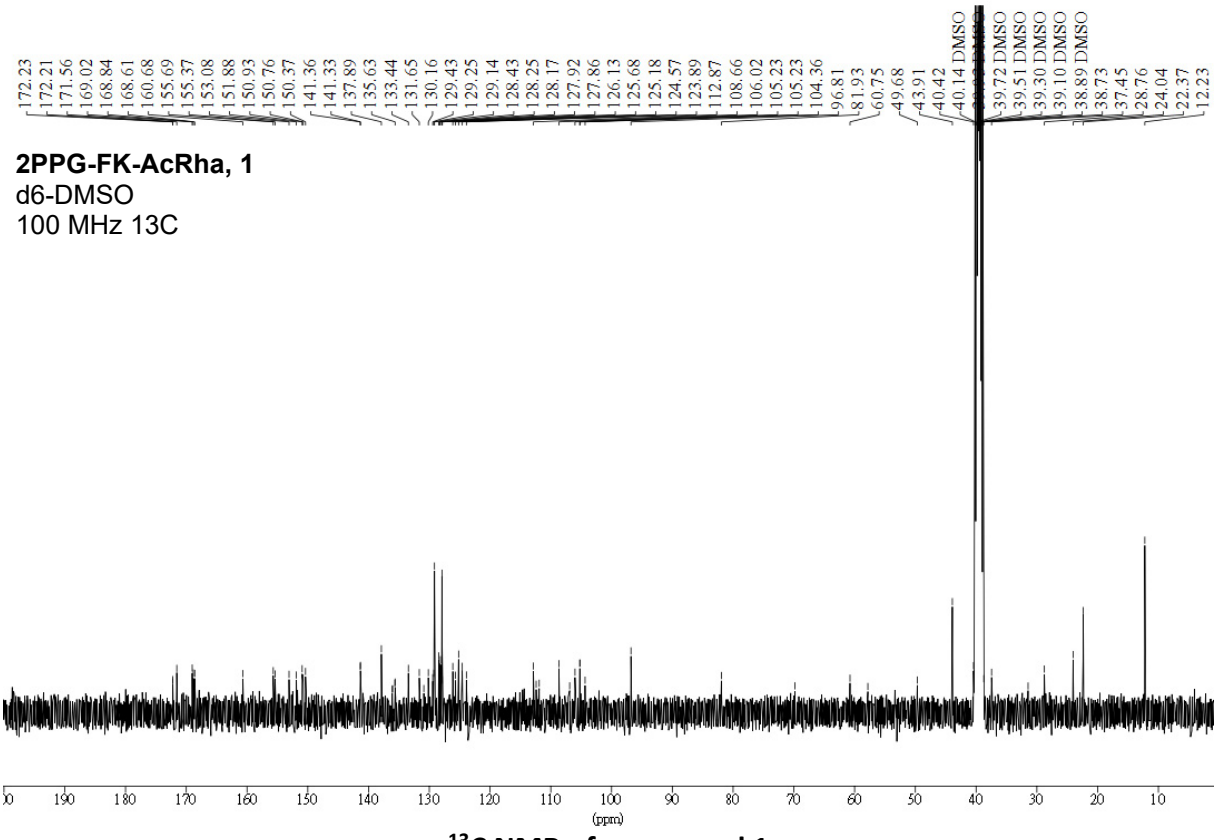
^{13}C NMR of compound 2



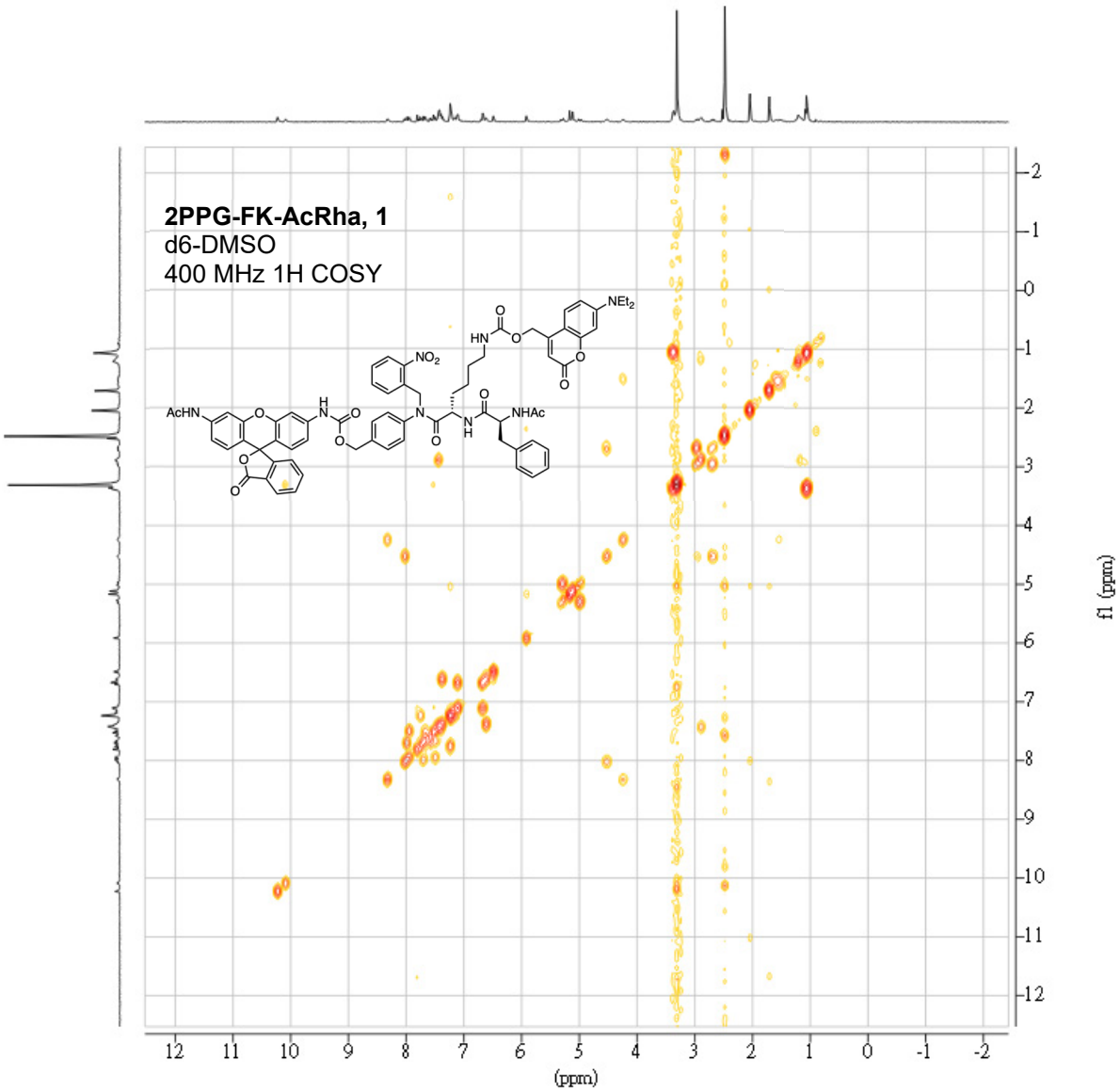
^{1}H COSY of compound 2



^{1}H NMR of compound 1



^{13}C NMR of compound 1



^{1}H COSY of compound 1