Electronic Supplementary Material (ESI) for RSC Advances.

Quinolizinium-based fluorescent probes for formaldehyde detection in aqueous solution, serum, and test strip via 2-aza-Cope rearrangement

Ajcharapan Tantipanjaporn,‡[b] Karen Ka-Yan Kung,‡[b] Hoi-Yi Sit,[a,b] and Man-Kin Wong*[a,b]

[a] The Hong Kong Polytechnic University Shenzhen Research Institute, Shenzhen, 518057, China

[b] State Key Laboratory of Chemical Biology and Drug Discovery, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hum, Hong Kong, China

SUPPORTING INFORMATION

Contents

Table S1 Reported representative ratiometric fluorescent probes based-on 2-aza Cope rearrangement reaction for FA detection.

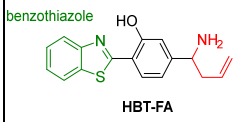
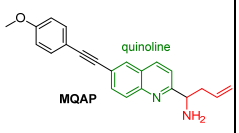
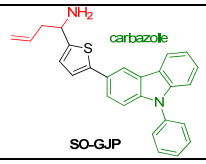
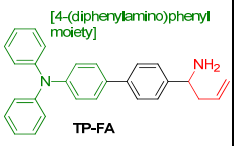
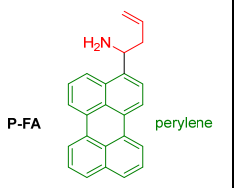
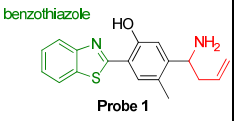
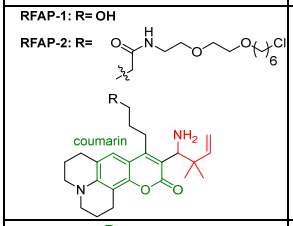
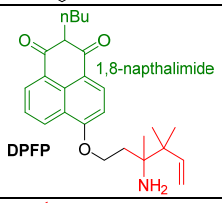
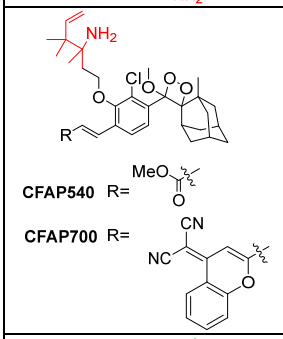
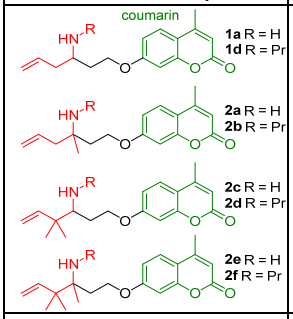
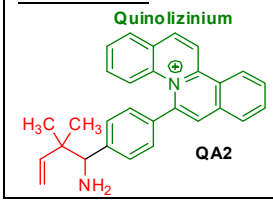
Probe Structure	Condition	Detection time	λ_{ex} (nm)	λ_{em} (nm)	LOD	Linear range	Application (Reference)
	PBS buffer-CH_3CN (10 mM, pH 7.4, 3:2, v/v), 37 °C	7 h	350	462 to 541	0.41 mM	0-30 mM	Detection of vaporized FA by test paper (Zhou, Yan et al. 2018)
	PBS solution (pH 7.4, 10 mM)	2.5 h	355	405 to 490	4.054 μM	0-1.0 mM	Cell imaging (MCF-7 cells, mice liver tissue and 5 days old zebra fish) (Yang, Fang et al. 2018)
	H_2O/DMSO mixture (3/7, v/v)	3 h (sol^n)	366	393 to 542	1.55 μM	10-800 μM	Cell imaging (HeLa cells), test strips (Gu, Li et al. 2020)
	1,4-Dioxane/water (2:8, v/v)	80 min (sol^n), 45 min (test cotton)	305	442 to 488	0.051 mM	0-5.6 mM	Fabric and cotton samples (Zhai, Zhang et al. 2019)
	DMSO solution & aqueous solution (nanoprobe)	90 min (DMSO), 120 min (nano probe	420 (DMSO) & 450 (nano probe)	452 to 550 (DMSO) 480 to 550 (nano probe)	6.1 μM (DMSO) & 0.96 μM (nanopro be)	0-0.8 mM (DMSO)	Cell imaging (HeLa cells) (nano probe) (Ji, Ma et al. 2019)
	Phosphate buffer:DMSO = 1:1 (v/v, pH 7.4, 10 mM)	90 min	430	492 to 552	0.58 μM	0-500 μM	Cell imaging (MGC-803 cells, zebra fish), water samples (Hao, Zhang et al. 2020)

Table S2 Reported representative aza-Cope based-fluorescent probes utilized the geminal dimethyl substitute homoallylic amine as a reaction site.

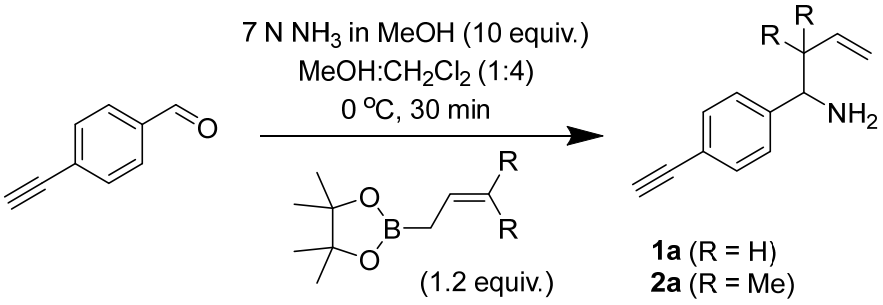
Probe Structure	Condition	Detection time	λ_{ex} (nm)	λ_{em} (nm)	LOD	Linear range	Type/Application (Reference)
	20 mM PBS, pH 7.4, 37 °C	2 h	420 to 470	510	0.3 μM	-	Ratiometric probe/ Live cell imaging (Brewer, Burgos-Barragan et al. 2017)
	PBS buffer (0.5% DMSO, 10 mM)	25 min	455	555	10 μM	0-0.25 mM	Turn-on probe/ Cell imaging (HeLa cells) (Xie, Yin et al. 2018)
	PBS buffer (10 mM, pH 7.4, 10% FBS)	60 min	-	540 (**CFAP540**) 700 (**CFAP700**)	-	-	Turn-on probe/ Cell imaging (HEK293 cells) (**CFAP540**), living mice (**CFAP700**) (Bruemmer, Green et al. 2018)
	PBS buffer (20 mM, pH 7.4), 37 °C	2 h	317	445	-	-	Turn-on probe/ Study N-substituent effect and the gem-dimethyl effect on aza-Cope reactivity or turn-on fluorescence (Du, Zhang et al. 2021)
This work 	PBS buffer (10 mM, pH 7.4), 37 °C	15 min	410	495 to 481	1 μM	0-1 mM	Fluorescence emission shift probe/ Serum and test strip

Reagents and Apparatus

All chemicals were purchased from commercial sources and used without further purification. Water was purified by a Milli-Q system (Millipore, USA). The reaction was monitored by TLC glass plates (0.25 mm) precoated with 60 Å silica gel with detection by UV-absorption (254 nm or 365 nm). Flash column chromatography was performed using silica gel 60 (230-400 mesh ASTM) in the indicated solvent mixture. High resolution mass spectra (HRMS) were recorded with an Agilent 6540 Q-TOF LC/MS instrument using electrospray ionization (ESI). NMR spectra were recorded on a Bruker DPX-400 MHz and Jeol ECZ500R 500 MHz spectrometer using TMS as internal standard. pH values were determined by using a JENWAY pH/mV temperature meter (Model 3510). Absorption and emission spectra were measured by Cary 8454 UV-Vis diode array system and Cary Eclipse fluorescence spectrophotometer, respectively.

Synthesis and Structural Characterization

Synthesis of 1a and 2a

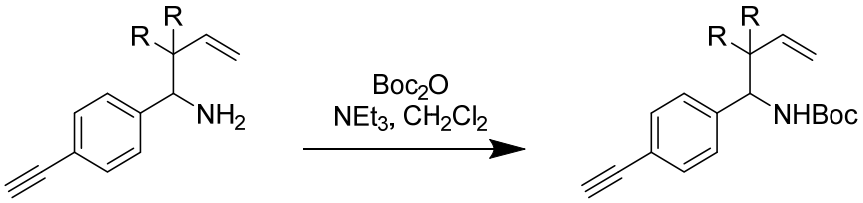


A round bottom flask was charged with 4-ethynylbenzaldehyde (130 mg, 1 mmol) in dichloromethane (6 mL), cooled to 0 °C and ammonia was added (1.43 mL, 7.0 M in MeOH). After stirring for 30 min, allylboronic acid pinacol ester (1.2 equiv.) was added dropwise, and the resulting solution was stirred at room temperature for 10 h. The volatiles were then removed under reduced pressure, and the residue was taken up in dichloromethane and washed with Milli-Q® water (2 × 20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. After that, the mixture was purified by flash column chromatography with 4-10% methanol in dichloromethane to obtain **1a** (or **2a**) as a product.

1a: Colorless oil. 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 5.71 (dq, J = 9.9, 7.3 Hz, 1H), 5.18 – 4.99 (m, 2H), 4.06 – 3.89 (m, 1H), 3.06 (s, 1H), 2.52 – 2.37 (m, 1H), 2.37 – 2.24 (m, 1H), 1.68 (br s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.69, 135.04, 132.26, 126.41, 120.68, 118.04, 83.66, 77.48, 77.16, 77.01, 76.84, 55.16, 44.07, 24.71. HRMS calcd. for $C_{12}H_{13}N$ $[M + H]^+$ 172.1121, found 172.1124; and $[M – NH_3 + H]^+$ 155.0855, found 155.0857.

2a: Colorless oil. 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 8.2 Hz, 2H), 5.83 (dd, J = 17.5, 10.8 Hz, 1H), 5.06 (ddd, J = 18.7, 14.1, 1.3 Hz, 2H), 3.75 (s, 1H), 3.05 (s, 1H), 1.49 (br s, 2H), 0.97 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.70, 145.53, 143.90, 131.41, 128.55, 120.70, 113.45, 83.79, 77.48, 77.16, 77.06, 76.84, 63.97, 41.59, 25.48, 21.75. HRMS calcd. for $C_{14}H_{17}N$ $[M + H]^+$ 200.1434, found 200.1435; and $[M – NH_3 + H]^+$ 183.1168, found 183.1190.

Synthesis of 1b and 2b



1a (R = H)
2a (R = Me)

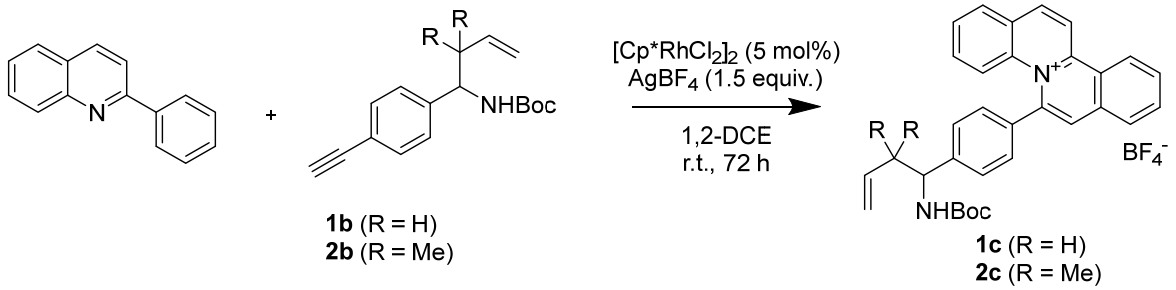
1b (R = H)
2b (R = Me)

A round bottom flask was charged with **1a** (or **2a**) (1 mmol) in dichloromethane (5 mL), cooled to 0 °C and triethylamine was added (420 µL, 3 equiv.). After stirring for 5 min, di-*tert*-butyl dicarbonate (350 µL,1.5 equiv.) was added dropwise, and the resulting solution stirred at room temperature for 3 h. After the reaction, Milli-Q® water (2 × 10 mL) was added to wash the crude mixture. The organic layer was collected, dried over Na_2SO_4, filtered and concentrated under reduced pressure. After that, the mixture was purified by flash column chromatography with 5-10% ethyl acetate in hexane to obtain **1b** (or **2b**) as a product.

1b: White powder. 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.40 (m, 2H), 7.22 (d, J = 8.1 Hz, 2H), 5.64 (ddt, J = 17.2, 10.2, 7.0 Hz, 1H), 5.09 (dd, J = 13.4, 5.4 Hz, 2H), 4.93 (d, J = 7.2 Hz, 1H), 4.72 (s, 1H), 3.09 – 3.02 (m, 1H), 2.48 (br s, 2H), 1.41 (br s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.18, 133.58, 132.31, 126.24, 120.86, 118.60, 83.54, 79.71, 77.43, 77.14, 77.11, 76.79, 53.82, 41.06, 28.37, 27.45. HRMS calcd. for $C_{17}H_{21}NO_2$ $[M + Na]^+$ 294.1465, found 294.1468.

2b: Colorless oil. 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 8.2 Hz, 2H), 7.14 (d, J = 8.2 Hz, 2H), 5.73 (dd, J = 17.5, 10.8 Hz, 1H), 5.14 (d, J = 10.8 Hz, 1H), 5.06 (d, J = 17.5 Hz, 2H), 4.44 (s, 1H), 3.06 (s, 1H), 1.42 (br s, 9H), 1.10 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.38, 143.40, 131.54, 128.21, 120.82, 114.50, 83.67, 79.64, 77.48, 77.23, 77.16, 76.84, 62.46, 40.80, 28.44, 27.80, 24.69, 12.48. HRMS calcd. for $C_{19}H_{25}NO_2$ $[M + Na]^+$ 322.1778, found 322.1783.

Synthesis of 1c and 2c



1b (R = H)
2b (R = Me)

1c (R = H)
2c (R = Me)

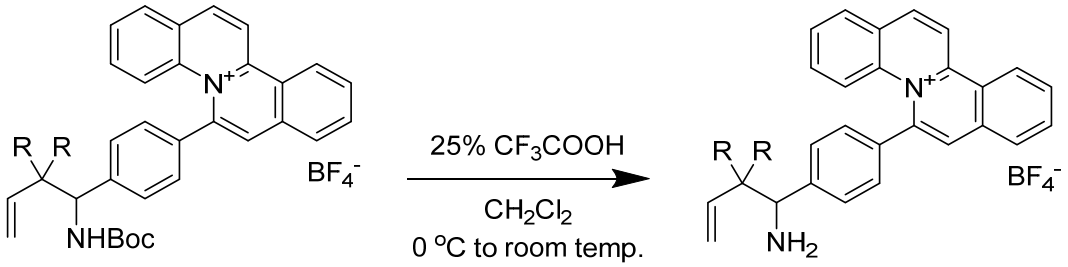
A mixture of 2-phenylquinoline (0.6 mmol, 1.2 equiv.), alkyne (0.5 mmol, 1 equiv.), $[Cp^*RhCl_2]_2$ (5 mol%), $AgBF_4$ (0.6 mmol, 1.2 equiv.) and 5 mL of 1,2-DCE was added into a 25 mL round bottom flask. The reaction mixture in the bottle was stirred at room temperature in open air for 72 h. After the reaction was completed, the mixture was concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 0.5-2.5% methanol in dichloromethane as the eluent and/or by preparative thin layer chromatography on silica gel using 7% methanol in dichloromethane as the developing solvent to give the product **1c** (or **2c**).

1c: Dark yellow powder. 56% yield. ^{1}H NMR (400 MHz, CD_3CN) δ 9.08 (d, J = 9.1 Hz, 1H), 8.98 (d, J = 8.5 Hz, 1H), 8.89 (d, J = 9.0 Hz, 1H), 8.34 (s, 1H), 8.33 – 8.28 (m, 1H), 8.26 (d, J = 8.5 Hz, 1H), 8.20 (d, J = 7.8 Hz, 2H), 8.10 (d, J = 8.3 Hz, 1H), 7.82 – 7.77 (m, 2H), 7.54 (dt, J = 14.5, 7.3 Hz, 3H), 7.43 – 7.37 (m, 3H), 5.81 – 5.70 (m, 1H), 5.09 (dd, J = 13.8, 6.6 Hz, 3H), 4.67 (s, 1H), 2.48 (t, J = 7.2 Hz, 2H), 1.38 (s, 13H). ^{13}C NMR (101 MHz, CD_3CN) δ 141.95, 137.94, 136.90, 135.54, 132.38, 131.27, 130.78, 130.52, 130.48, 130.23, 129.80, 129.30, 128.79, 128.69, 128.46, 128.24, 127.47, 127.40, 126.38, 119.69, 118.33, 66.27, 41.57, 28.57, 15.62, 1.94, 1.73, 1.53, 1.32, 1.11, 0.91, 0.70. HRMS calcd. for $C_{32}H_{31}N_2O_2^+$ $[M]^+$ 475.2380, found 475.2384; HRMS calcd. for BF_4^- $[M]^-$ 87.0035, found 87.0038.

2c: Yellow powder. 50% yield. ^{1}H NMR (400 MHz, CD_3CN) δ 9.11 (d, J = 9.2 Hz, 1H), 9.00 (d, J = 8.5 Hz, 1H), 8.91 (d, J = 9.1 Hz, 1H), 8.38 (s, 1H), 8.31 – 8.25 (m, 2H), 8.22 (d, J = 7.1 Hz, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.83 – 7.75 (m, 2H), 7.55 – 7.42 (m, 2H), 7.32 (d, J = 7.6 Hz, 3H), 5.87 (dd, J = 17.4, 10.8 Hz, 1H), 5.05 (dd, J = 25.5, 14.2 Hz, 2H),

4.54 (d, J = 9.0 Hz, 1H), 1.38 (d, J = 6.3 Hz, 9H), 1.06 (s, 3H), 0.98 (s, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ 141.98, 136.92, 134.78, 132.40, 130.59, 130.51, 129.31, 127.50, 126.47, 119.72, 118.33, 28.56, 1.94, 1.73, 1.53, 1.32, 1.11, 0.91, 0.70. HRMS calcd. for $C_{34}H_{35}N_2O_2$ $[M]^+$ 503.2693, found 503.2691; HRMS calcd. for BF_4^- $[M]^-$ 87.0035, found 87.0033.

Synthesis of Quinolizinium-based aza-Cope Probes QA1 and QA2



1c (R = H)
2c (R = Me)

QA1 (R = H)
QA2 (R = Me)

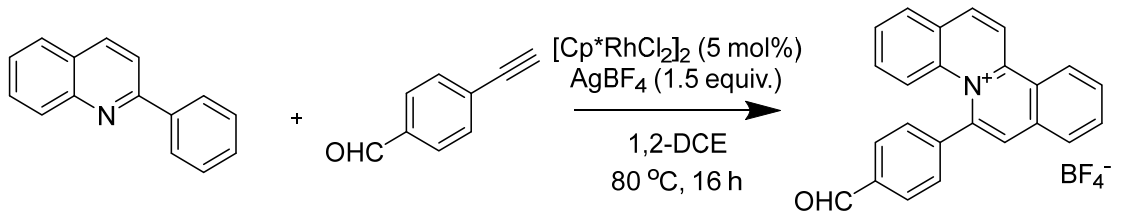
To a 25 mL round bottom flask was added **1c** (or **2c**) with 25% trifluoroacetic acid in dichloromethane (4 mL) at 0 °C and stirred at room temperature for 4 hours. The solvent was removed under reduced pressure, and triturated with toluene (3 × 5 mL). The resulting residue was purified by preparative thin layer chromatography on silica gel using 15% methanol in dichloromethane as the developing solvent to afford **QA1** (or **2**) as the product.

QA1: Brown powder. 50% yield. ^{1}H NMR (400 MHz, CD_3CN) δ 9.13 (d, J = 9.2 Hz, 1H), 9.02 (d, J = 8.5 Hz, 1H), 8.93 (d, J = 9.1 Hz, 1H), 8.39 (s, 1H), 8.30 (dd, J = 7.5, 4.5 Hz, 2H), 8.21 (t, J = 7.4 Hz, 1H), 8.12 (t, J = 7.7 Hz, 1H), 7.85 – 7.77 (m, 2H), 7.53 – 7.42 (m, 5H), 5.75 (dt, J = 16.7, 7.0 Hz, 1H), 5.16 – 5.05 (m, 2H), 4.17 (t, J = 6.9 Hz, 1H), 2.51 (t, J = 7.0 Hz, 2H). ^{13}C NMR (101 MHz, CD_3CN) δ 143.35, 142.05, 141.22, 140.26, 139.54, 139.38, 139.17, 138.26, 138.10, 137.68, 136.94, 135.36, 134.79, 131.37, 130.60, 130.55, 128.82, 128.56, 127.52, 126.42, 119.76, 118.85, 118.35, 60.16, 56.05, 54.23, 43.19, 1.94, 1.74, 1.53, 1.32, 1.12, 0.91, 0.70. HRMS calcd. for $C_{27}H_{23}N_2^+$ $[M]^+$ 375.1856, found 375.1859; HRMS calcd. for BF_4^- $[M]^-$ 87.0035, found 87.0036.

QA2: Yellow powder. 45% yield. ^{1}H NMR (400 MHz, CD_3CN) δ 9.12 (d, J = 9.1 Hz, 1H), 9.01 (d, J = 8.6 Hz, 1H), 8.91 (d, J = 9.1 Hz, 1H), 8.39 (s, 1H), 8.29 (d, J = 8.4 Hz, 2H),

8.20 (d, J = 7.4 Hz, 1H), 8.13 – 8.09 (m, 1H), 7.83 – 7.78 (m, 2H), 7.51 – 7.43 (m, 2H), 7.38 (s, 3H), 5.98 (dd, J = 17.6, 10.8 Hz, 1H), 5.02 – 4.90 (m, 2H), 4.48 (s, 1H), 1.03 (s, 3H), 0.99 (s, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ 149.50, 142.94, 142.70, 142.18, 137.18, 136.97, 134.61, 132.58, 131.44, 130.67, 130.57, 129.53, 129.40, 128.94, 128.20, 127.53, 126.48, 126.43, 119.70, 118.34, 117.10, 63.71, 41.10, 24.96, 22.57, 1.94, 1.73, 1.52, 1.32, 1.11, 0.91, 0.70. HRMS calcd. for $C_{29}H_{27}N_2^+$ $[M]^+$ 403.2169, found 403.2165; HRMS calcd. for BF_4^- $[M]^-$ 87.0035, found 87.0036.

Synthesis of QCHO



QCHO

A mixture of 2-phenylquinoline (1.2 mmol, 1.2 equiv.), 4-ethynylbenzaldehyde (1 mmol, 1 equiv.), $[Cp^*RhCl_2]_2$ (5 mol%), $AgBF_4$ (1 mmol, 1.2 equiv.) and 10 mL of 1,2-DCE was added into a 50 mL round bottom flask. The reaction mixture in the bottle was refluxed at 80 °C in open air for 16 h. After the reaction was completed, the mixture was concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using methanol in dichloromethane as the eluent to give **QCHO** as the product.

QCHO: Pale-yellow powder. 15% yield. ^{1}H NMR (400 MHz, CD_3CN) δ 10.07 (s, 1H), 9.17 (d, J = 9.1 Hz, 1H), 9.05 (d, J = 8.5 Hz, 1H), 8.98 (d, J = 9.1 Hz, 1H), 8.48 (s, 1H), 8.39 – 8.29 (m, 2H), 8.24 (t, J = 7.2 Hz, 1H), 8.19 – 8.10 (m, 1H), 8.00 (d, J = 8.4 Hz, 2H), 7.84 (t, J = 7.6 Hz, 1H), 7.79 (d, J = 8.9 Hz, 1H), 7.67 (d, J = 8.3 Hz, 2H), 7.54 (ddd, J = 8.8, 7.1, 1.5 Hz, 1H). ^{13}C NMR (101 MHz, CD_3CN) δ 192.94, 177.18, 149.25, 143.59, 142.45, 137.91, 137.08, 134.48, 132.88, 131.90, 131.49, 130.81, 130.69, 129.81, 129.62, 129.24, 127.61, 126.55, 119.80, 118.32, 116.52, 1.93, 1.72, 1.52, 1.31, 1.10, 0.90, 0.69. HRMS calcd. for $C_{24}H_{16}NO^+$ $[M]^+$ 334.1226, found 334.1233; HRMS calcd. for BF_4^- $[M]^-$ 87.0035, found 87.0036.

General Procedure for Spectral Measurement

Fluorescent Quantum Yield Measurement

The absorption and emission spectra were measured by Cary 8454 UV-Vis Diode Array System and Cary Eclipse Fluorescence Spectrophotometer, respectively. The excitation slit and emission slit for emission measurement were set at 5 nm with scan rate at 120 nm/min and medium PMT voltage. Fluorescent quantum yield of each compound was determined by a comparative method employing coumarin 153 (Φ = 0.54 in EtOH) as a standard and calculated with the following equation. (Rurack and Spieles 2011)

$$\Phi_{sample} = \Phi_{standard} \times \left(\frac{Grad_{sample}}{Grad_{standard}}\right) \times \left(\frac{n_{sample}}{n_{standard}}\right)^2$$

where Φ is the fluorescence quantum yield, Grad is the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and η is the refractive index of the solvent.

Determination of the Detection Limit

According to the titration experiment, the linear relationship between the conversion percentage (from 495 to 481) and the concentration of FA was plotted. The limit of detection (LOD, S/N = 3) was calculated using the following equation;

$$LOD = 3\sigma/slope$$

where "σ" was the standard deviation of the sensor solution and “slope” was the slope of the linear calibration curve.

General Procedure for Absorption and Emission Spectra Measurement

The stock solutions of **QA1-2** were prepared in CH_3CN. The stock solutions of formaldehyde, acetaldehyde, propionaldehyde, glyoxal, acetone, NaCl, KCl, $CaCl_2$, $FeSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $ZnCl_2$, L-cysteine, H_2O_2, KNO_3, and $NaNO_2$ were prepared in deionized water but butyraldehyde, and 2-furaldehyde were prepared in CH_3CN. To detect formaldehyde by spectrophotometer, the samples were prepared in 1.5 mL-Eppendorf by adding 100 μL of 0.1 mM probe (in CH_3CN) into 10 mM PBS buffer pH 7.4 followed by adding desired amount FA which the total volume was 1000 μL.

Mouse Serum Preparation

The mouse serum was obtained from C57BL/6 mouse (source from The Chinese University of Hong Kong). The whole blood was collected and allowed to clot by leaving it at room temperature without the disturbance for 1 h. The clotted blood was then centrifuged at 1000 g for 10 min. Sera were separated and stored at -80 °C. The mouse serum was diluted 1000-fold with PBS for the experiments.

UV-Vis and Fluorescence Experimental Results

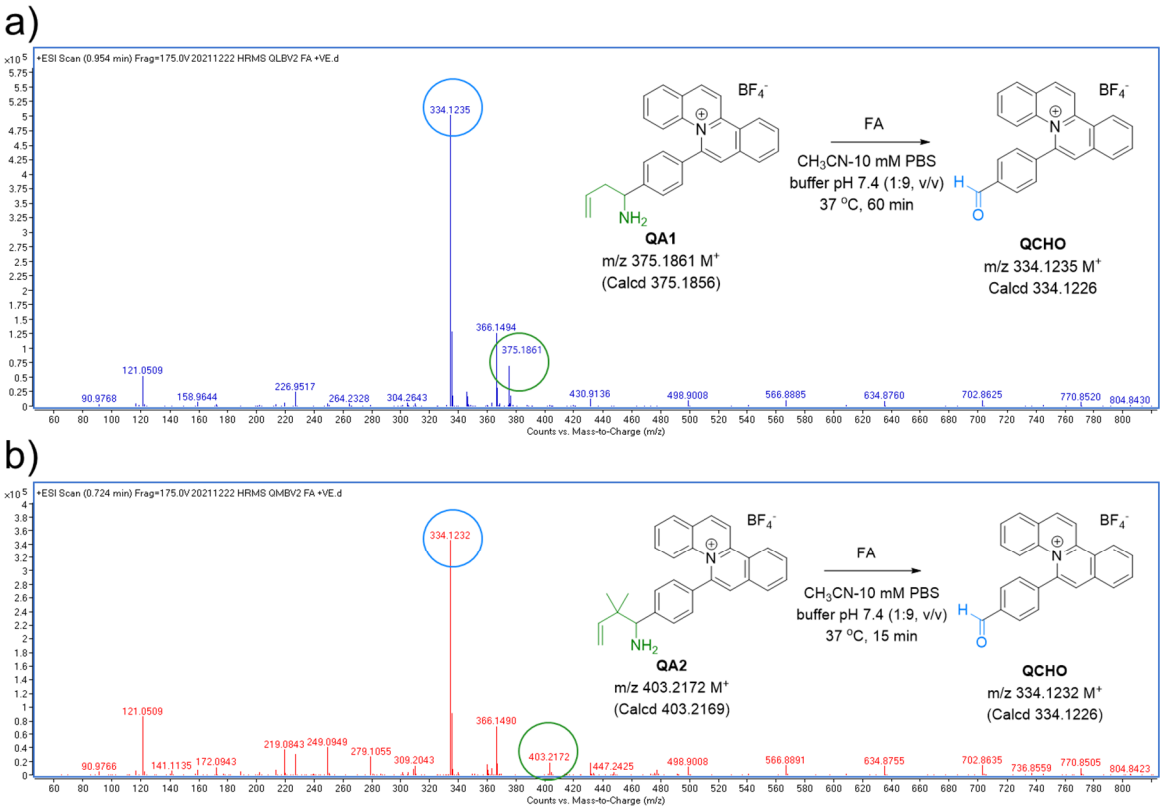


Figure S1. HR-MS spectra of (a) **QA1** and (b) **QA2** upon addition of FA.

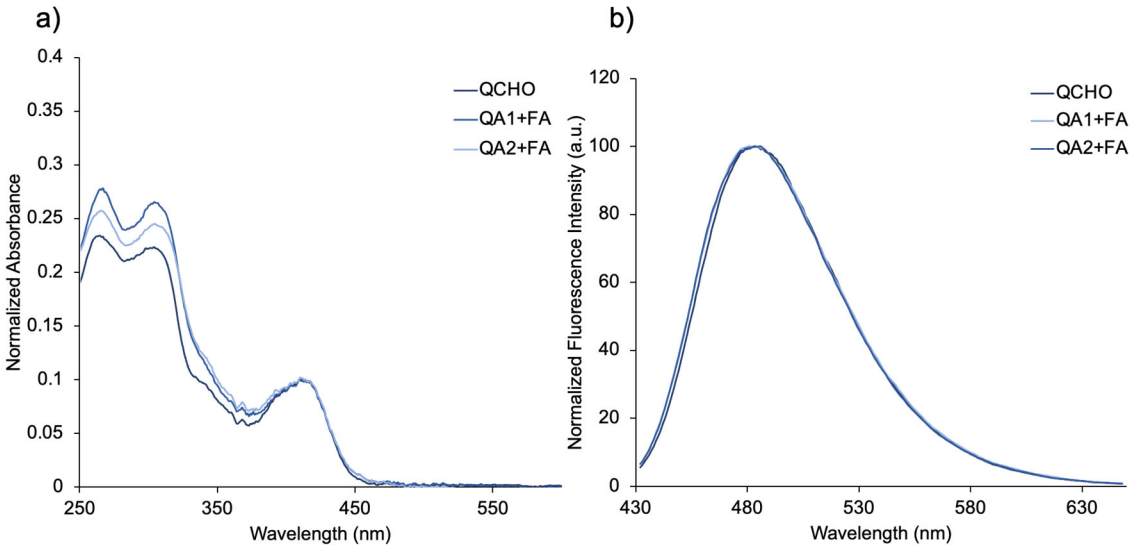


Figure S2. (a) Absorption and (b) fluorescence emission spectra of **QCHO** and **QA1-2** in the presence of FA in CH_3CN-10 mM PBS buffer pH 7.4 (1:9, v/v) at 37 °C. λ_{ex} 410 nm

Table S3. Absorption and emission properties of **QA1-2** in the presence and absence of FA and **QCHO** in CH_3CN-10 mM PBS buffer pH 7.4 (1:9, v/v) at 37 °C.

Probes	λ_{ex} (nm)	λ_{em} (nm)	Stokes Shift (cm^{-1})	Molar absorptivity ($M^{-1}cm^{-1}$)	Φ_F[a]
QA1	415	495	3894	1700	0.14
QA1+FA	413	481	3423	1900	0.14
QA2	415	495	3894	500	0.12
QA2+FA	413	481	3423	600	0.14
QCHO	413	481	3423	3500	0.14
[a] Quantum yields were measured using coumarin153 (Φ_F = 0.54 in ethanol) as the standard reference					

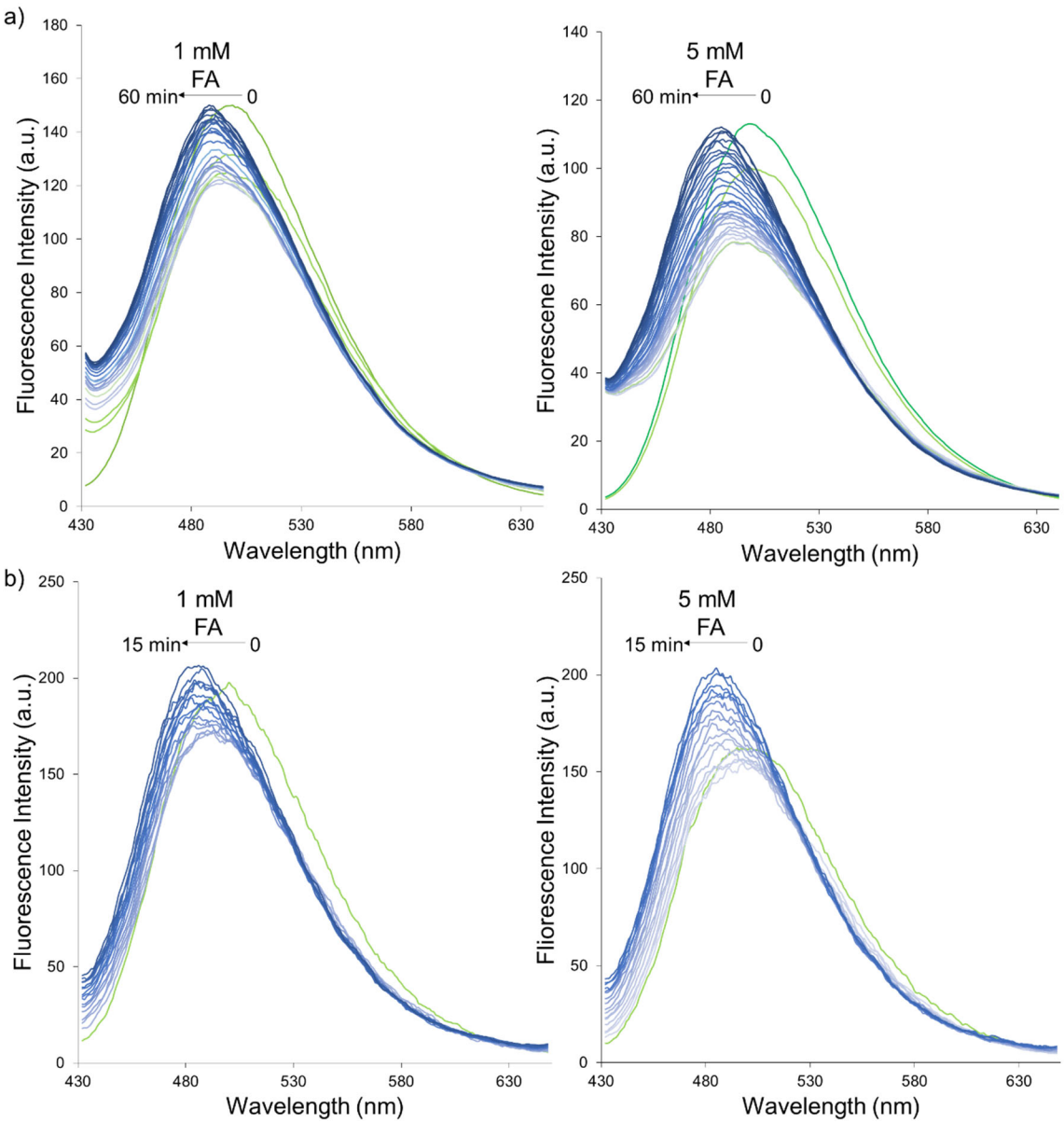


Figure S3. Time-dependence of the fluorescence emission shift of (a) **QA1** (10 μM) and (b) **QA2** (10 μM) upon the addition of FA (1 mM and 5 mM) in CH_3CN-10 mM PBS buffer pH 7.4 (1:9, v/v) at 37 °C. λ_{ex} = 410 nm.

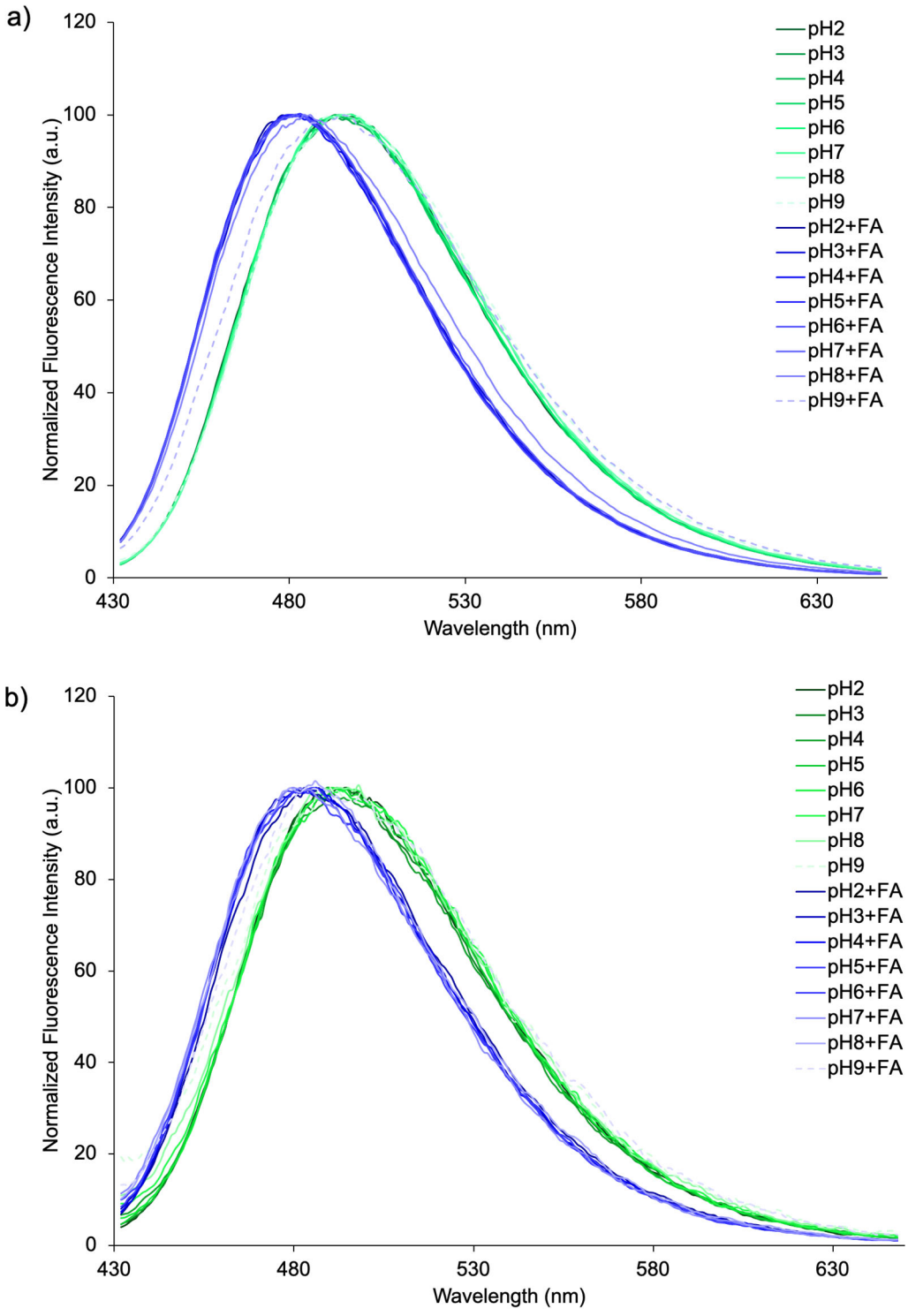


Figure S4. Fluorescence emission spectra of (a) **QA1** (10 μM) and (b) **QA2** (10 μM) in the absence and presence of FA (10 mM) in various pH values (2-9) with 10% CH_3CN at 37 °C. Incubation time of **QA1** and **QA2** were 60 min and 15 min, respectively. λ_{ex} = 410 nm.

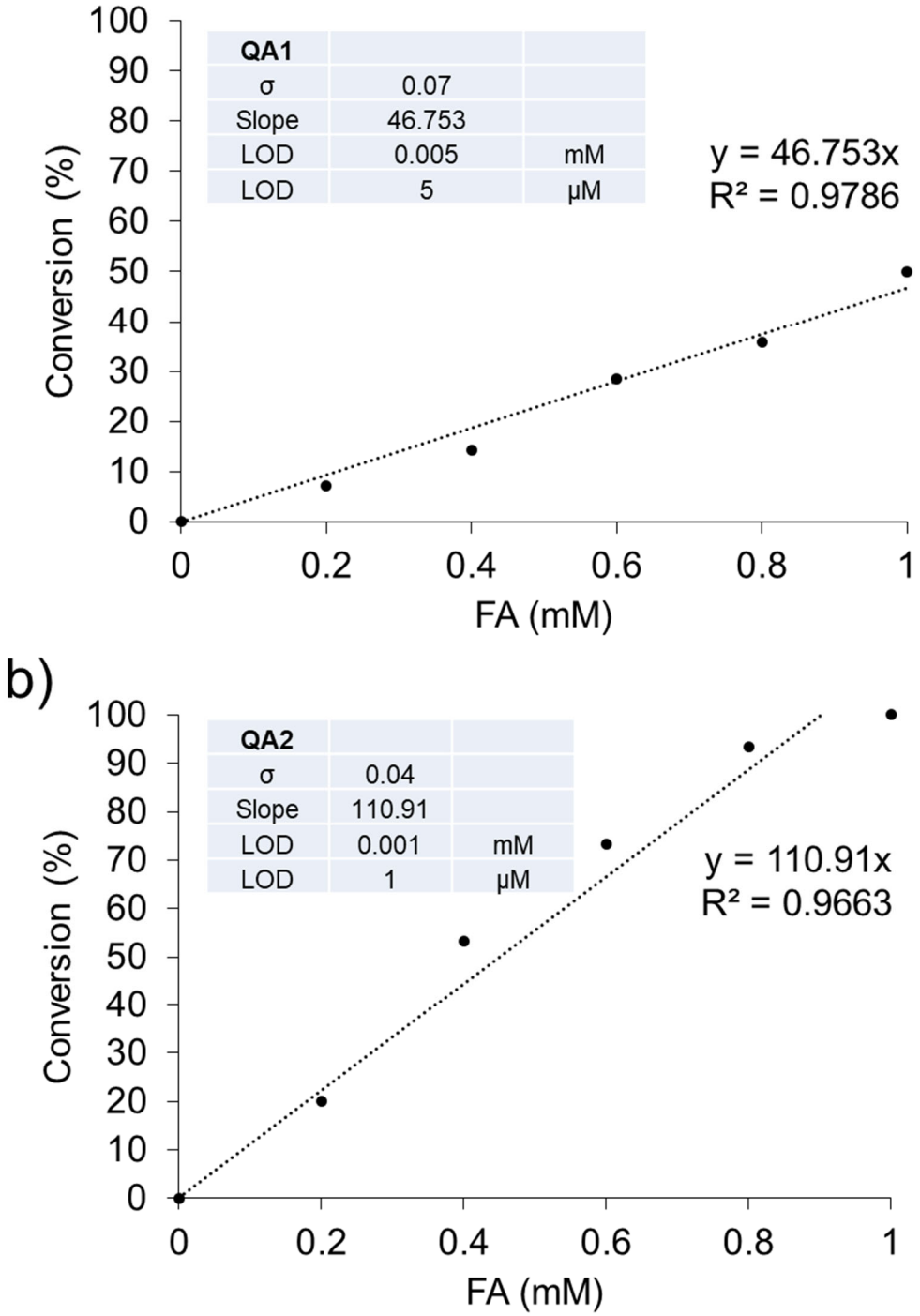


Figure S5. The linear relationship between conversion percentage (from 495 nm to 481 nm) of (a) **QA1** and (b) **QA2** and FA concentration. The graphs were plotted from the formaldehyde titration experiments of **QA1-2 (**10 µM) in CH_3CN-10 mM PBS buffer pH 7.4 (1:9, v/v) at 37 °C. Incubation time of **QA1** and **QA2** were 60 min and 15 min, respectively. λ_{ex} = 410 nm.

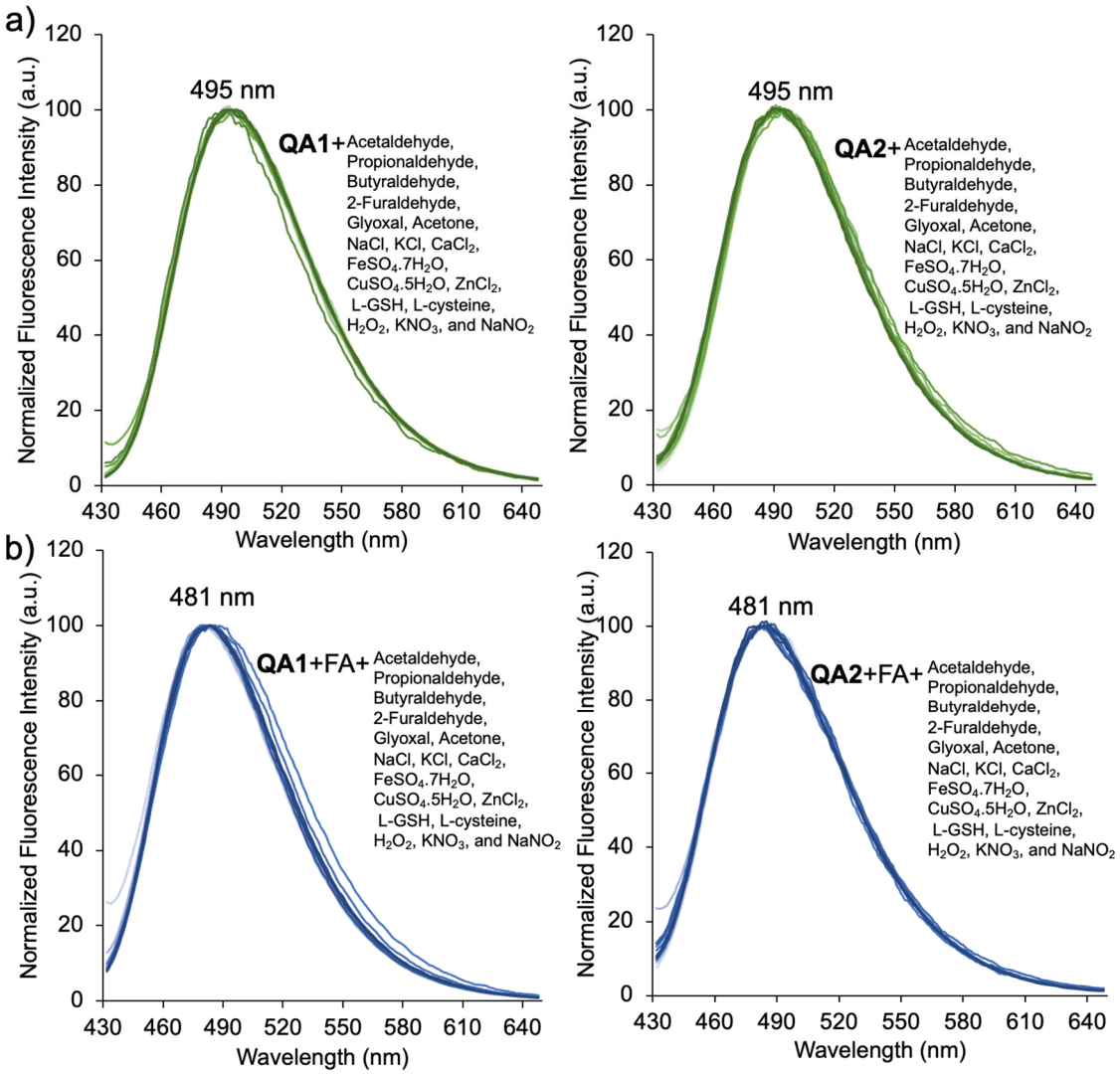


Figure S6. Fluorescence response of **QA1** (10 μM) and **QA2** (10 μM) incubated with various interfering compounds (5 mM) (a) in the absence and (b) presence of FA (5 mM) in CH_3CN-10 mM PBS buffer pH 7.4 (1:9, v/v) at 37 °C. Incubation time of **QA1** and **QA2** were 60 min and 15 min, respectively. λ_{ex} = 410 nm.

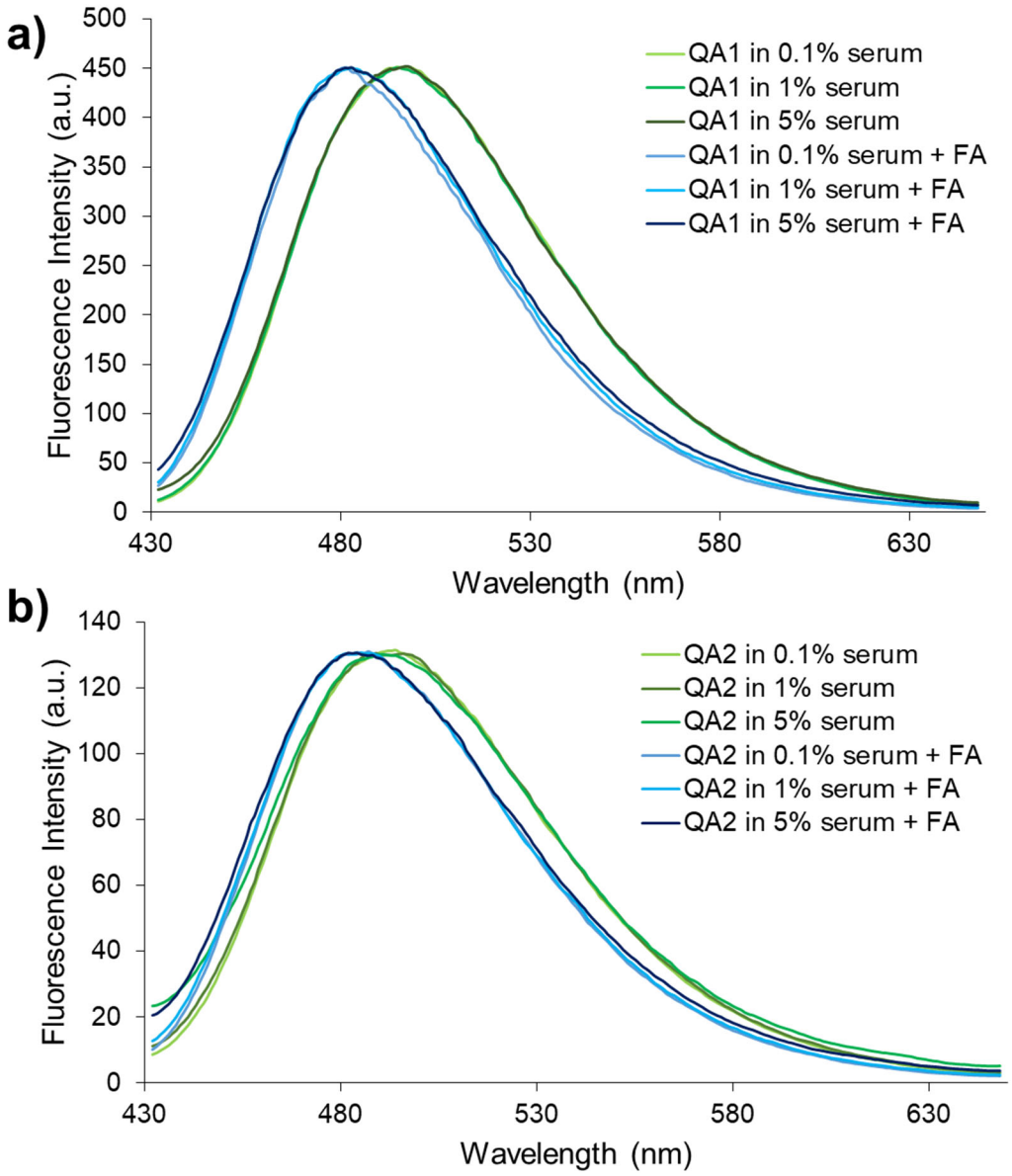
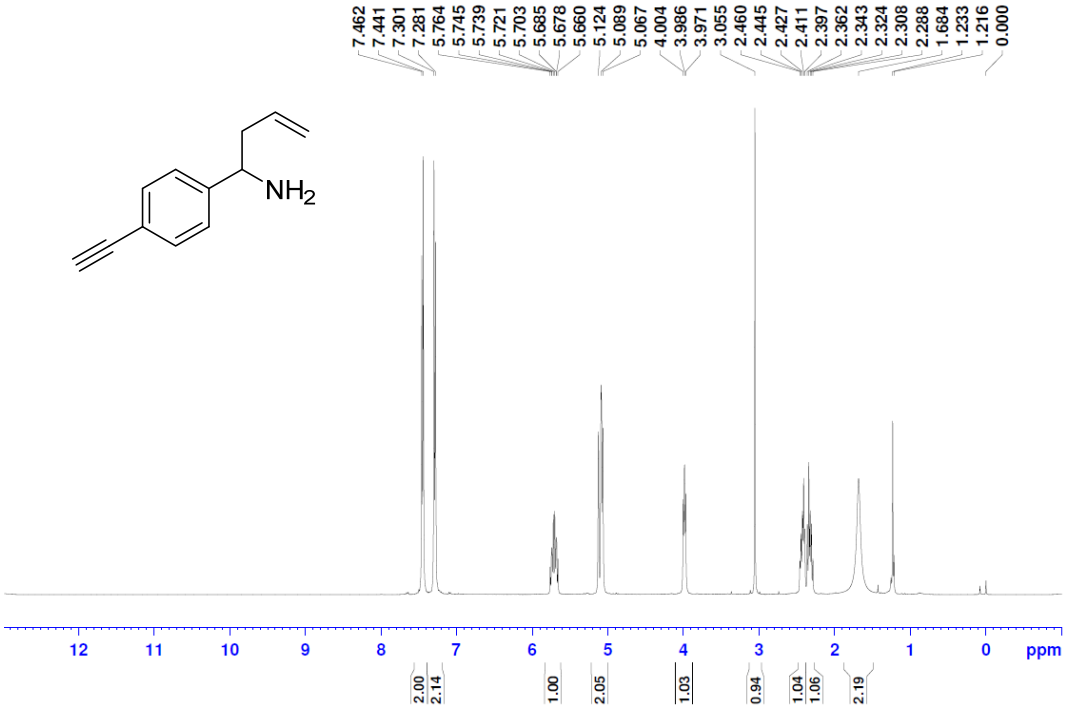


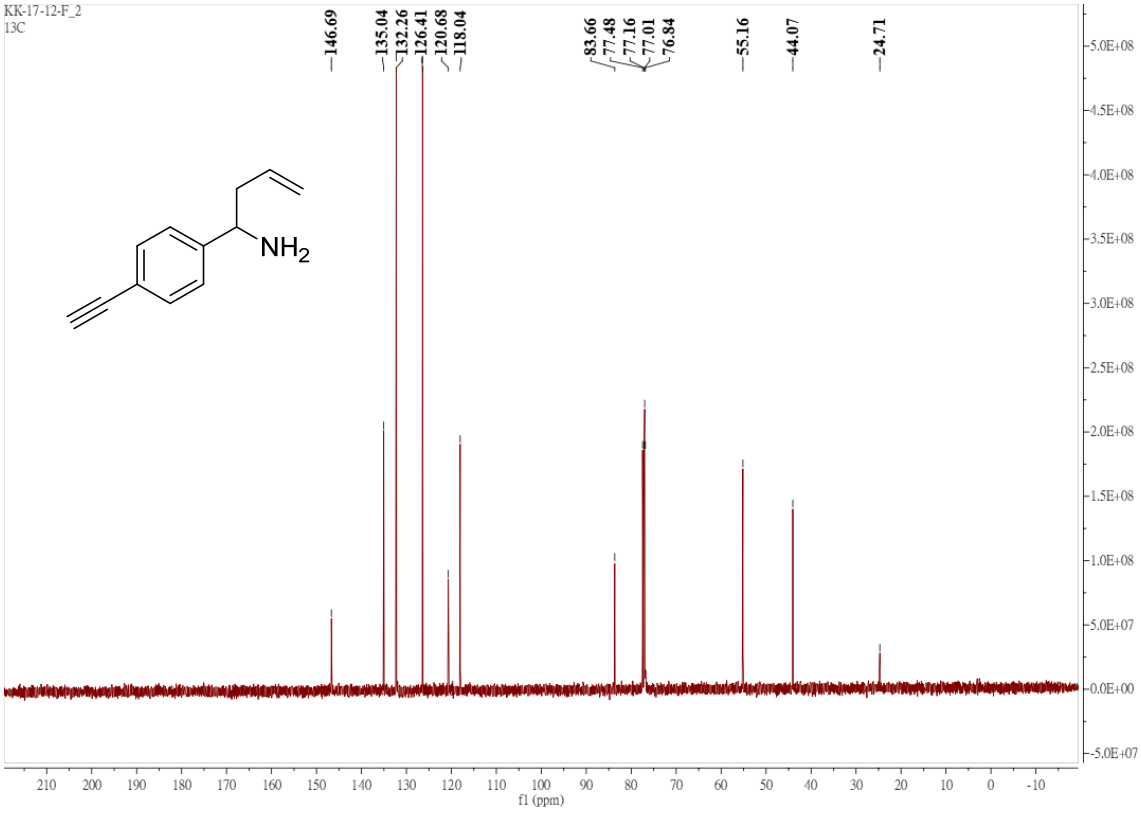
Figure S7. Fluorescence response of (a) **QA1** (10 μM) and (b) **QA2** (10 μM) in the absence and presence of FA (10 mM) with different percentage of mouse serum (0.1, 1, and 5%) in CH_3CN-10 mM PBS buffer pH 7.4 (1:9, v/v) at 37 °C. Incubation time of **QA1** and **QA2** were 60 min and 15 min, respectively. λ_{ex} = 410 nm.

NMR spectral data

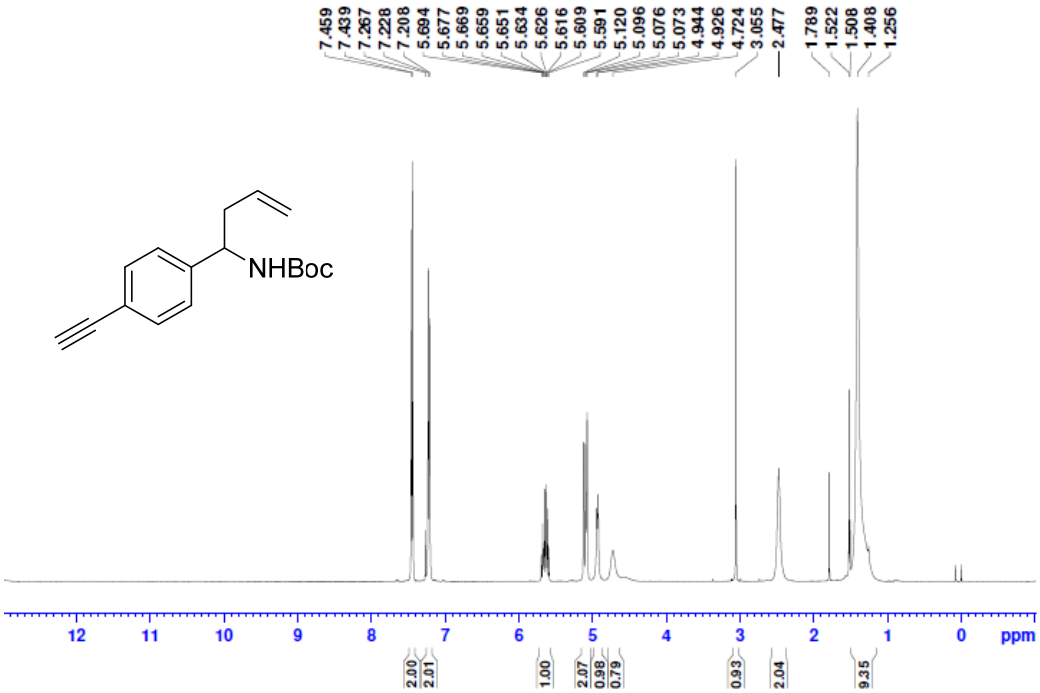
^{1}H NMR of 1a



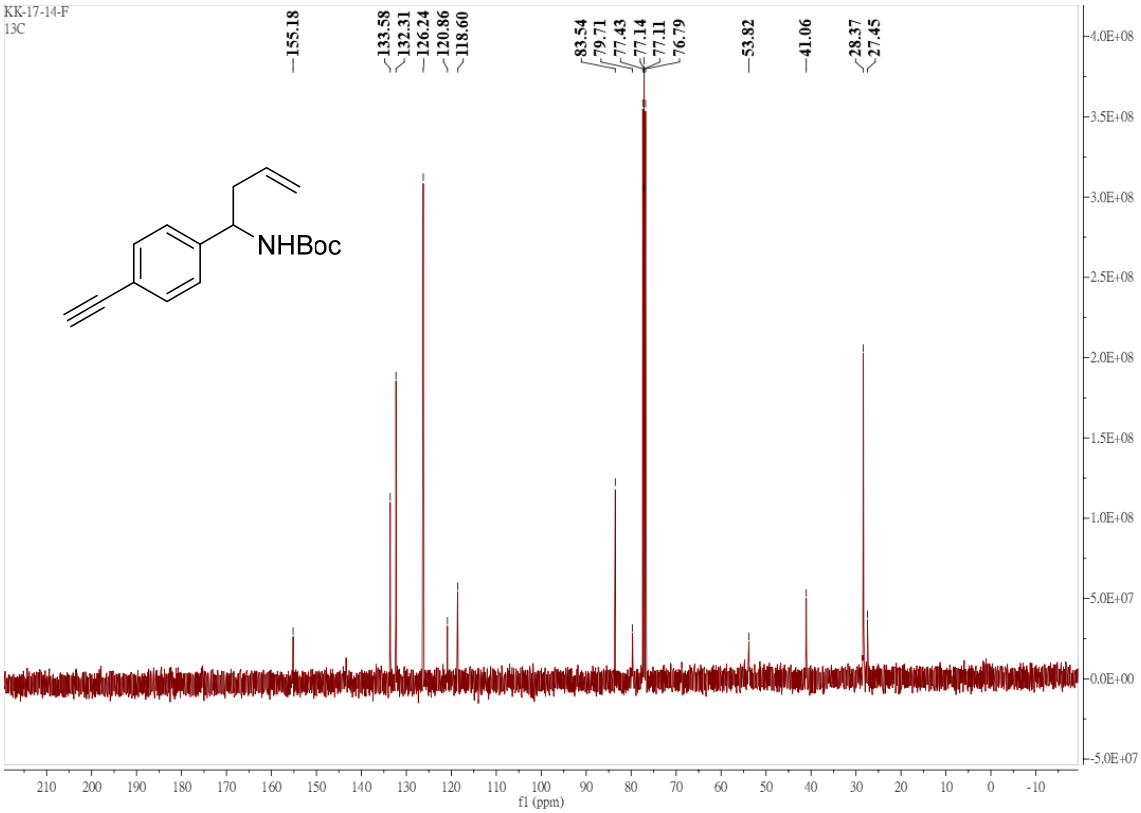
^{13}C NMR of 1a



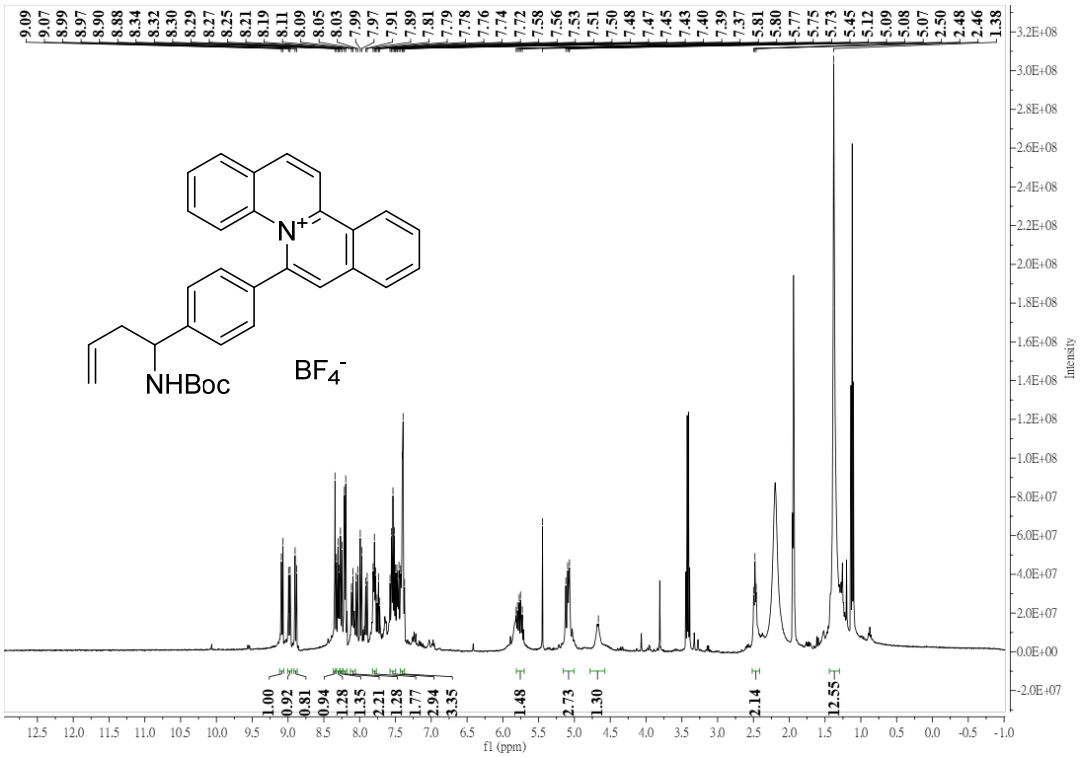
^{1}H NMR of 1b



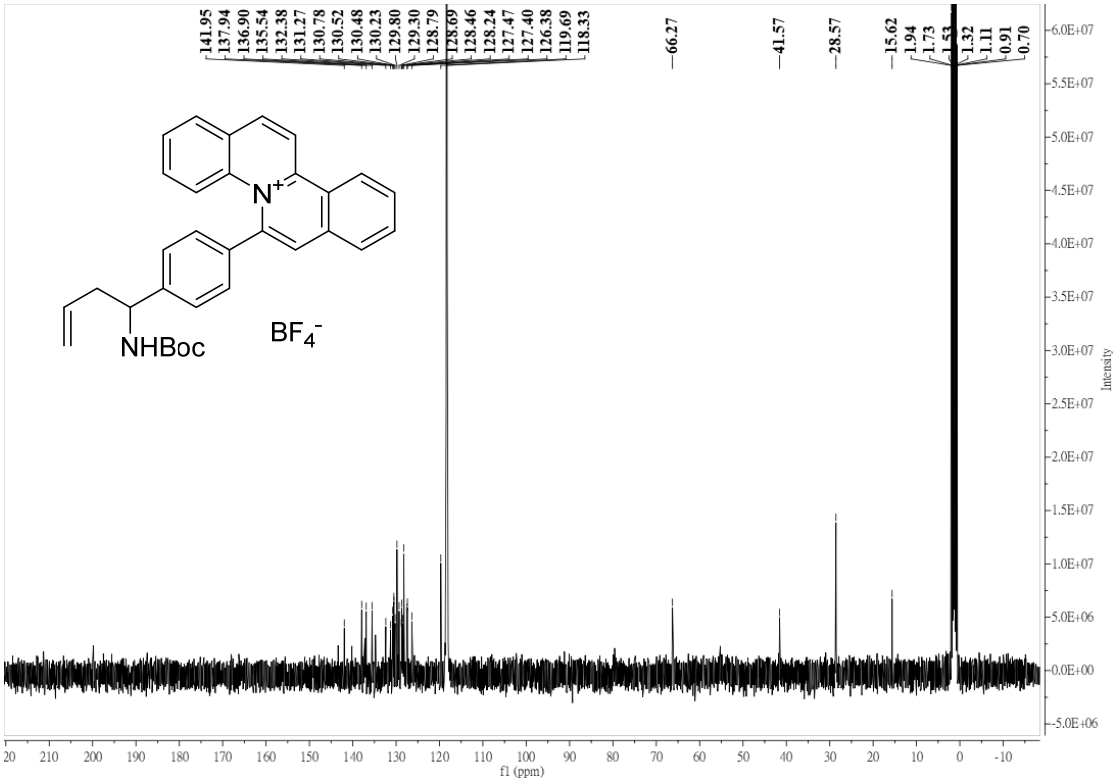
^{13}C NMR of 1b



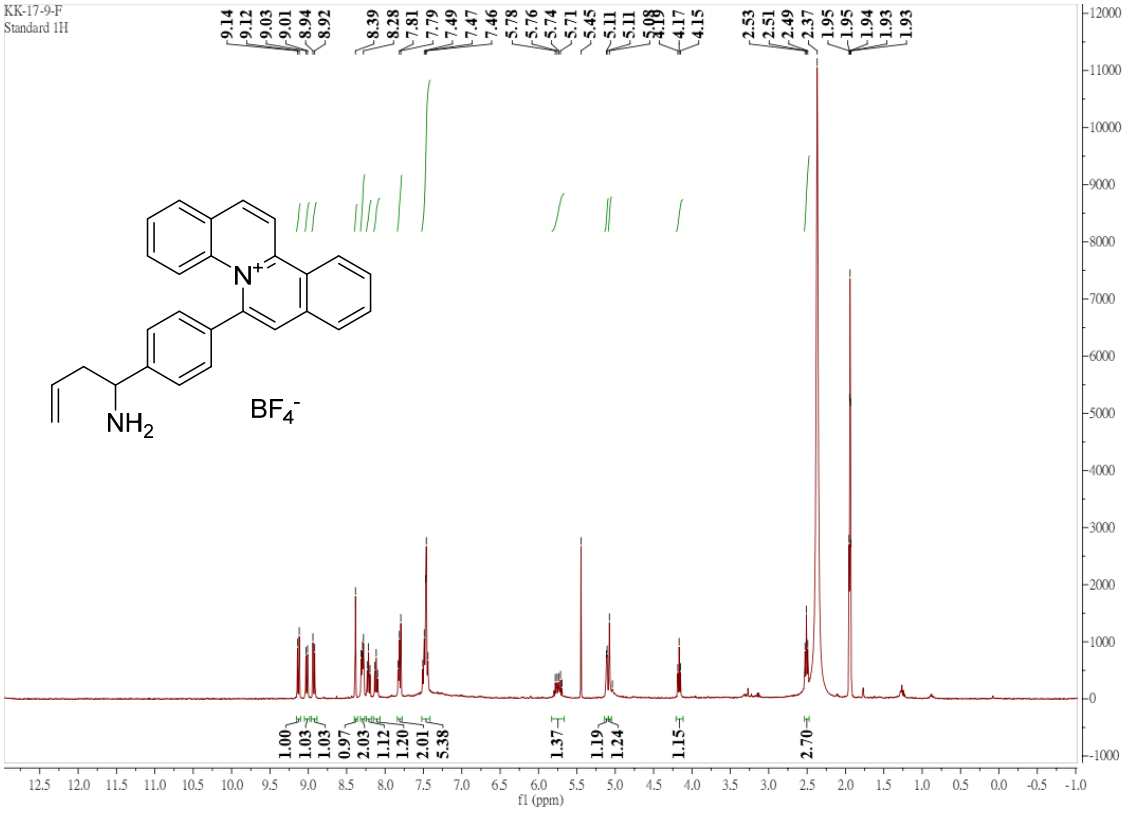
^{1}H NMR of 1c



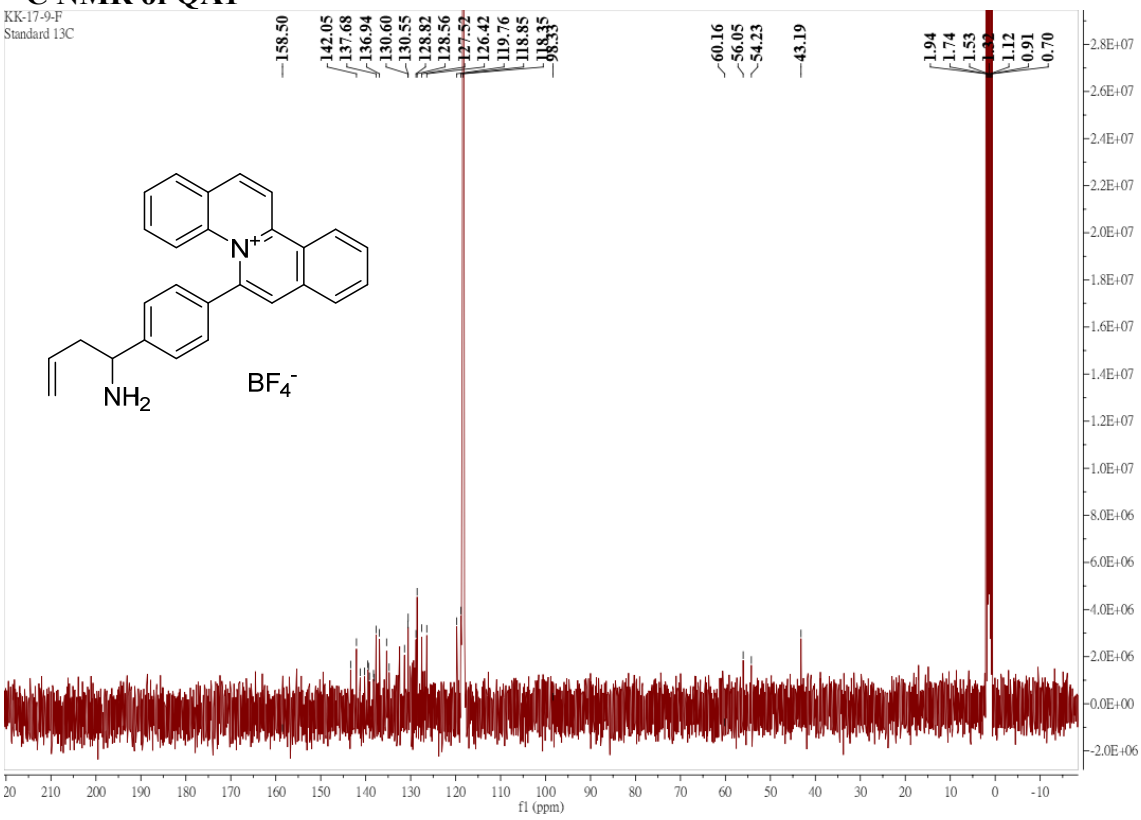
^{13}C NMR of 1c



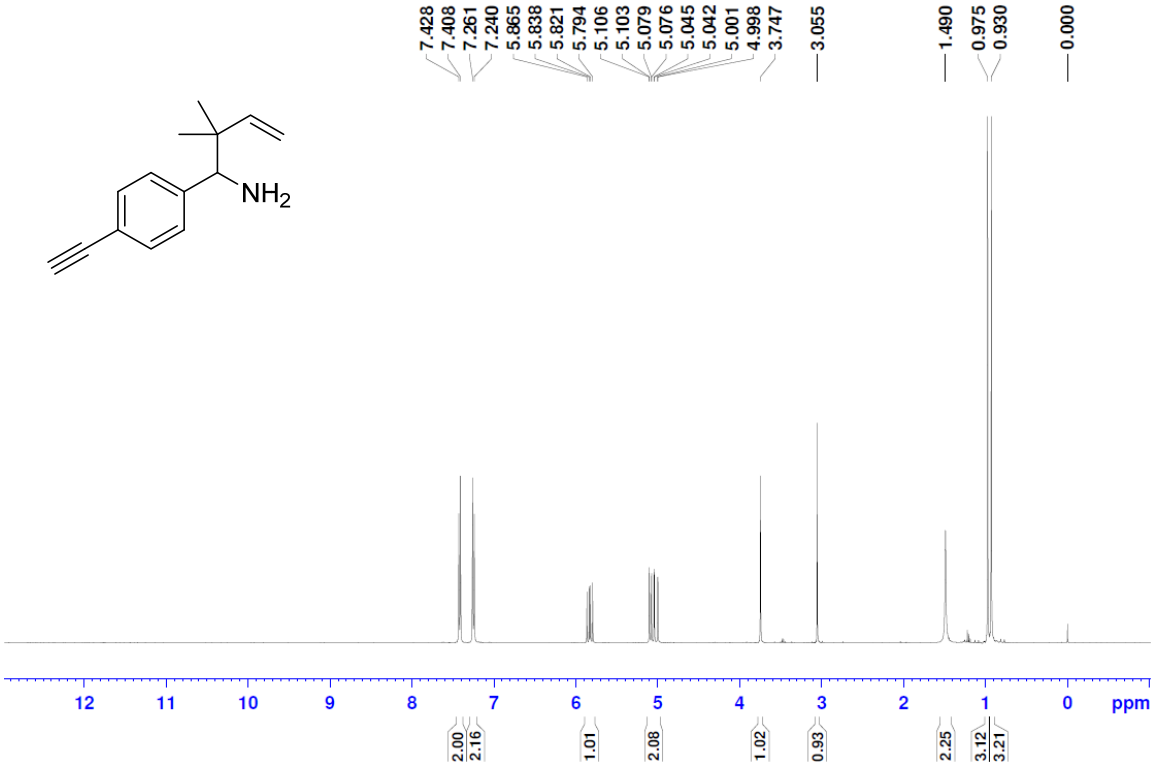
^{1}H NMR of QA1



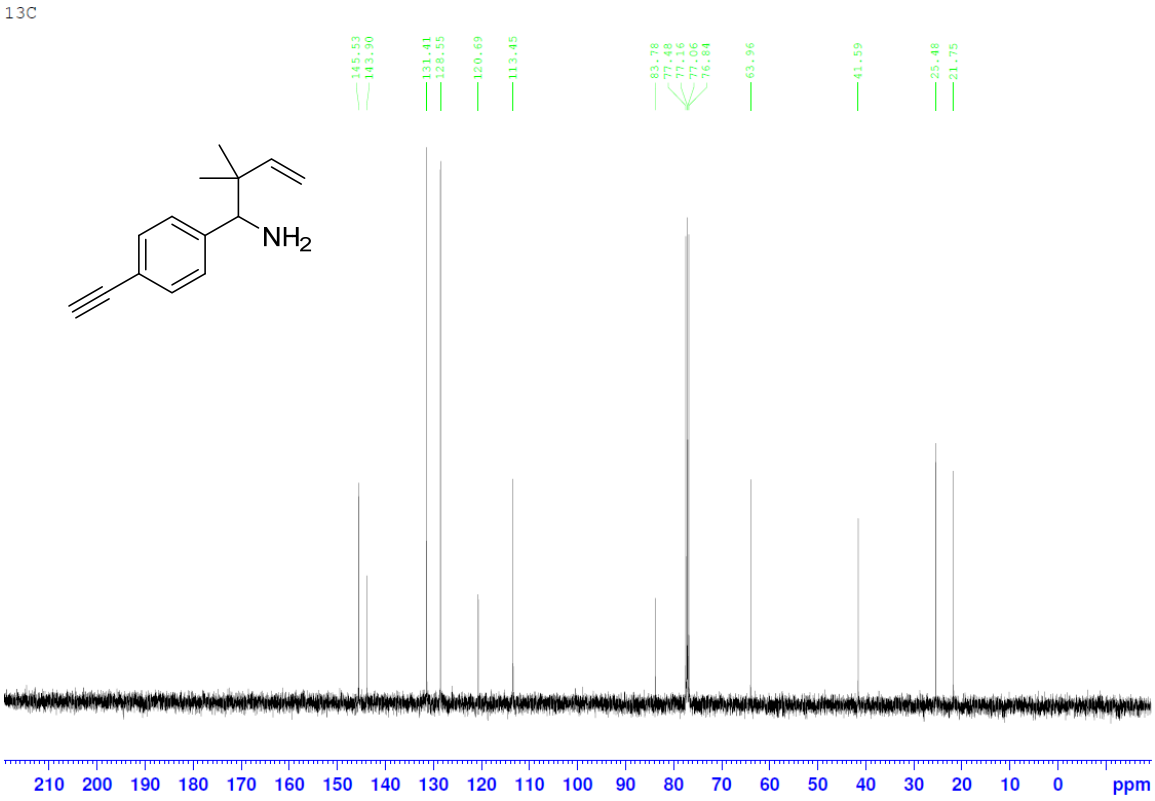
^{13}C NMR of QA1



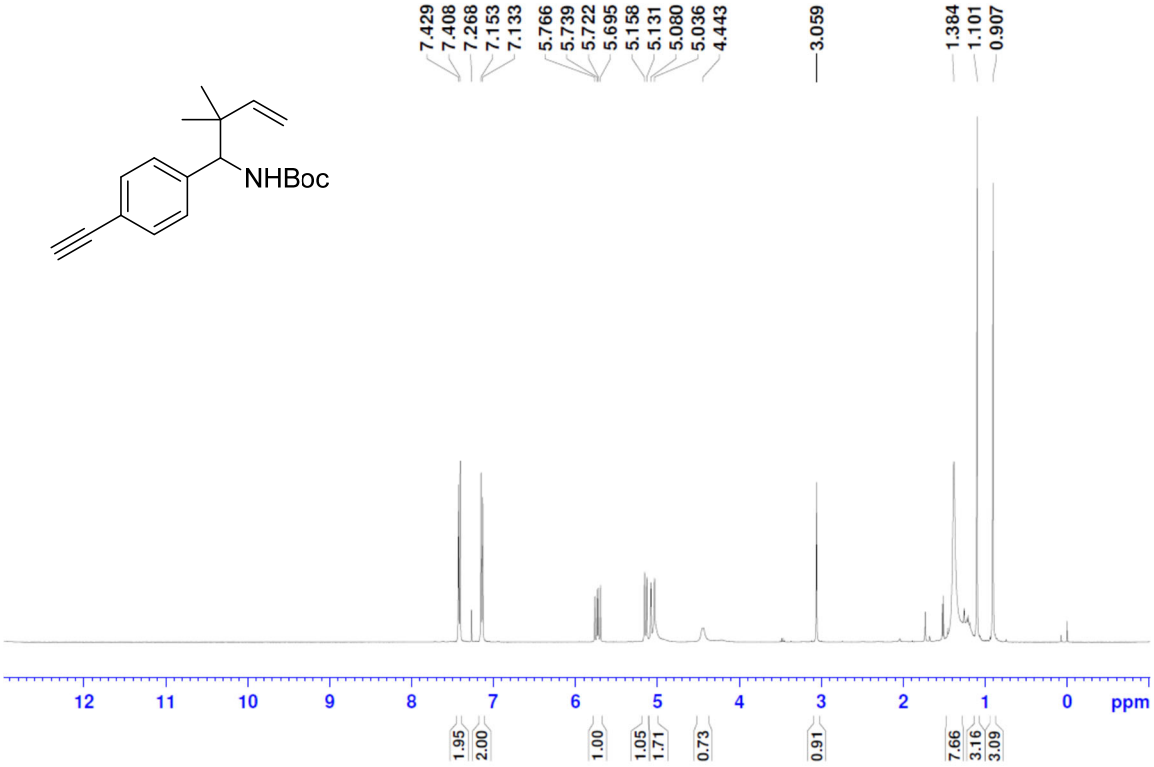
^{1}H NMR of 2a



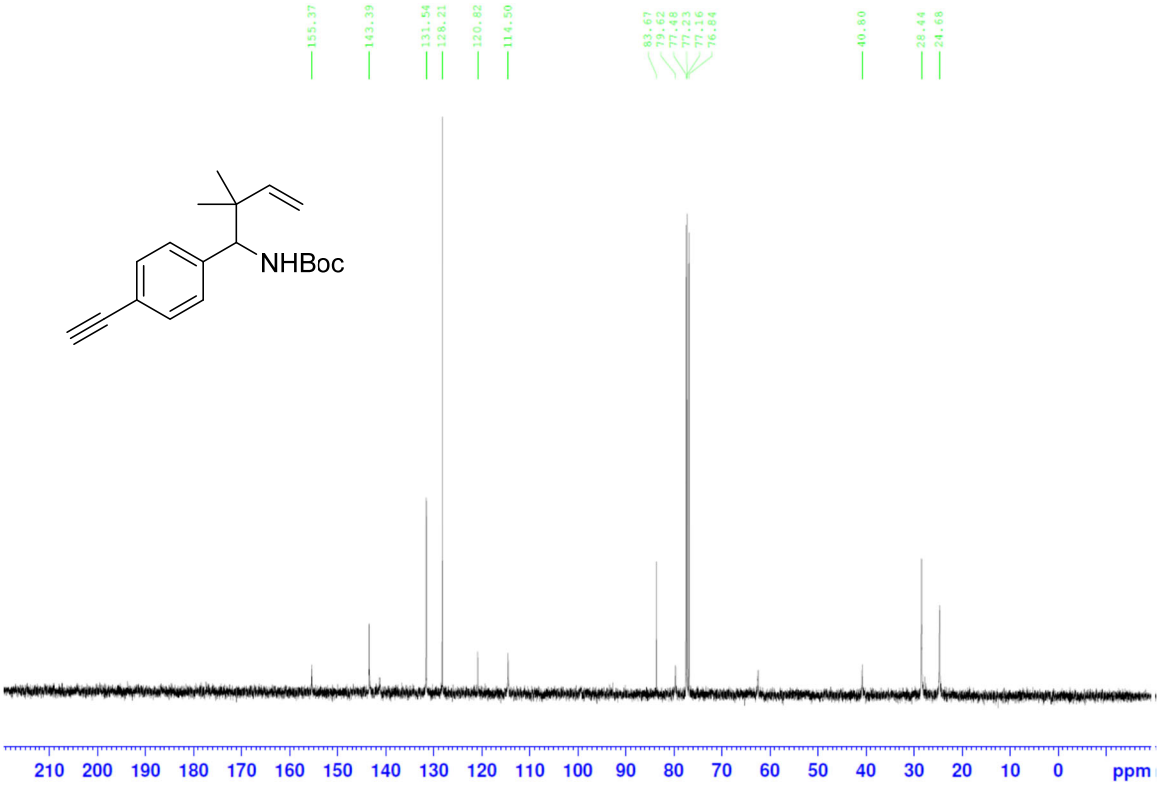
^{13}C NMR of 2a



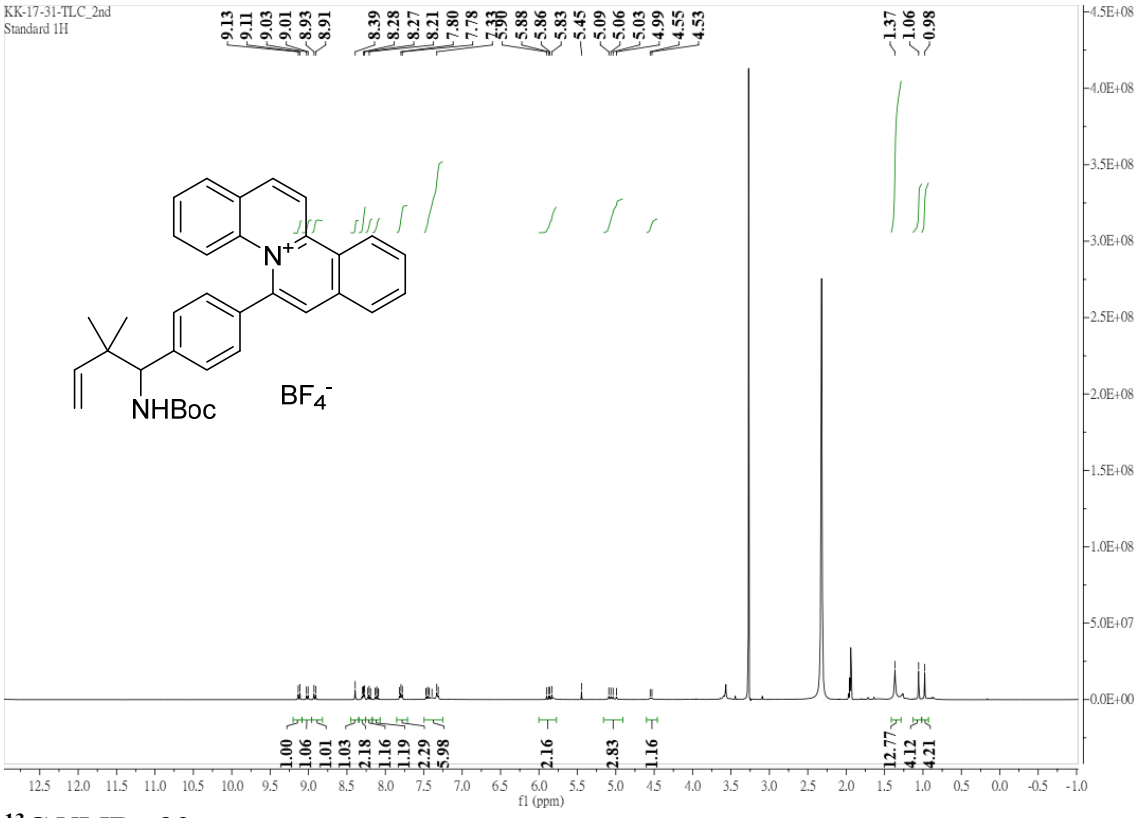
^{1}H NMR of 2b



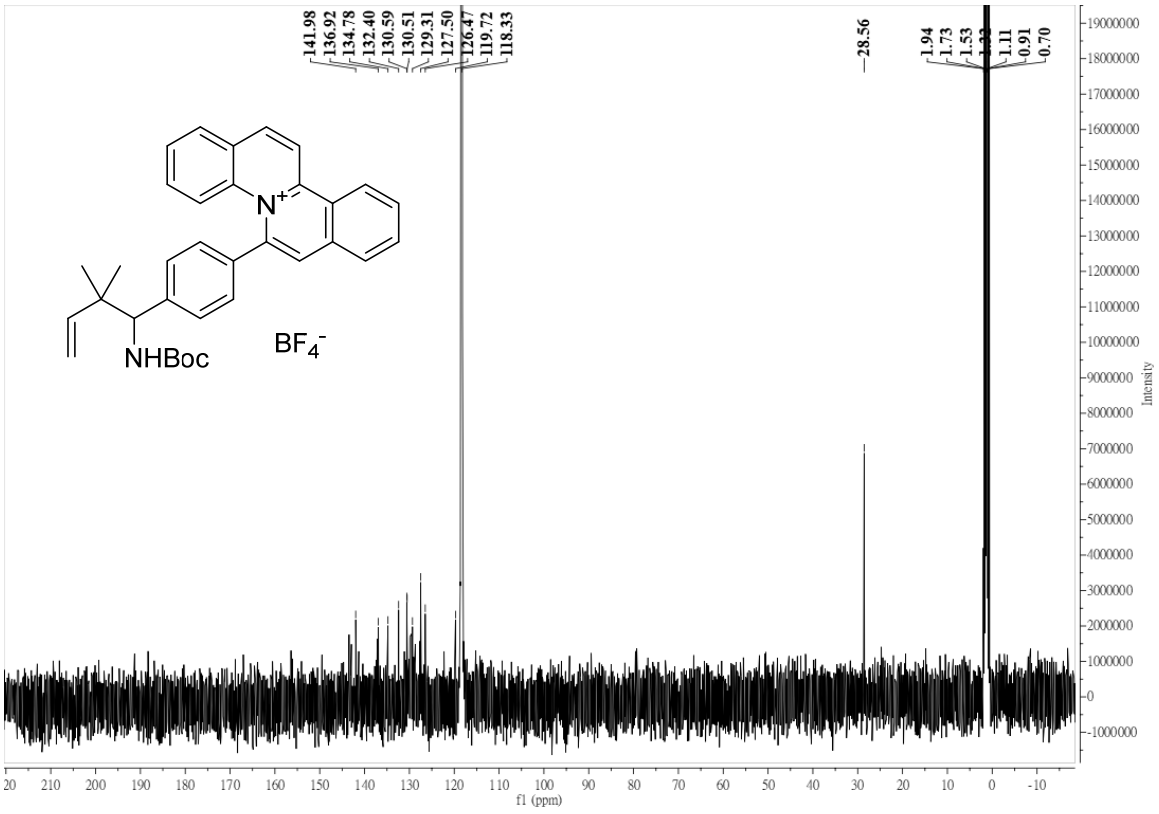
^{13}C NMR of 2b



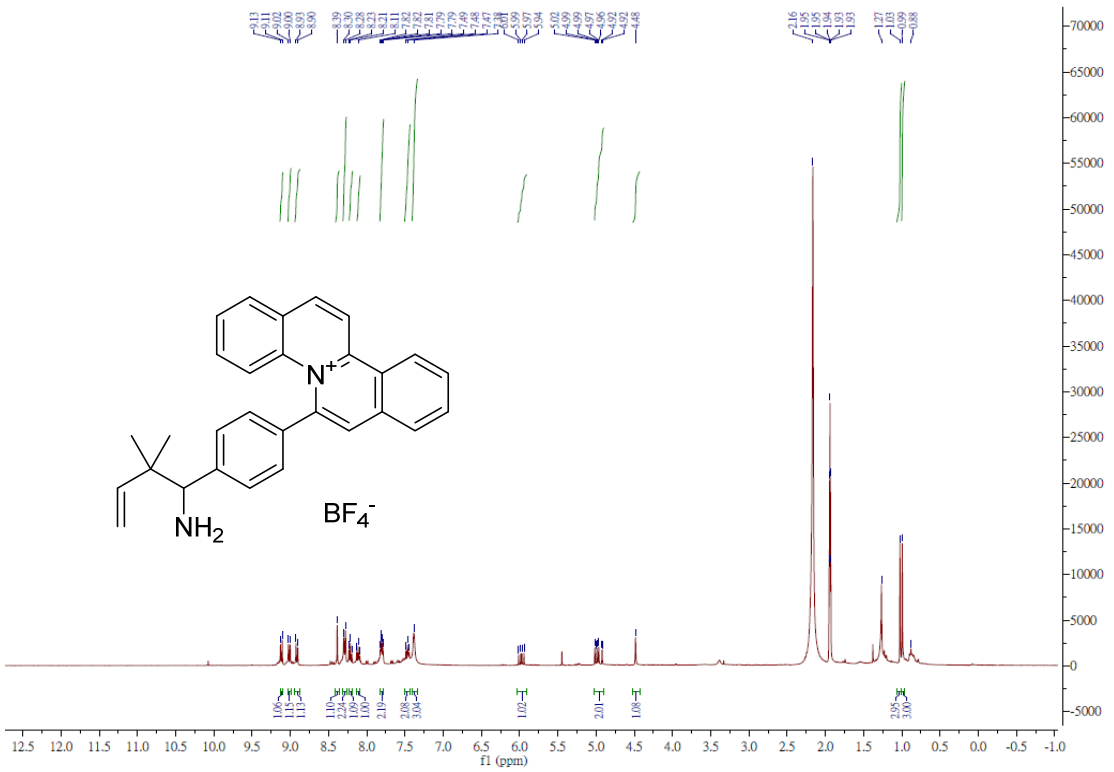
^{1}H NMR of 2c



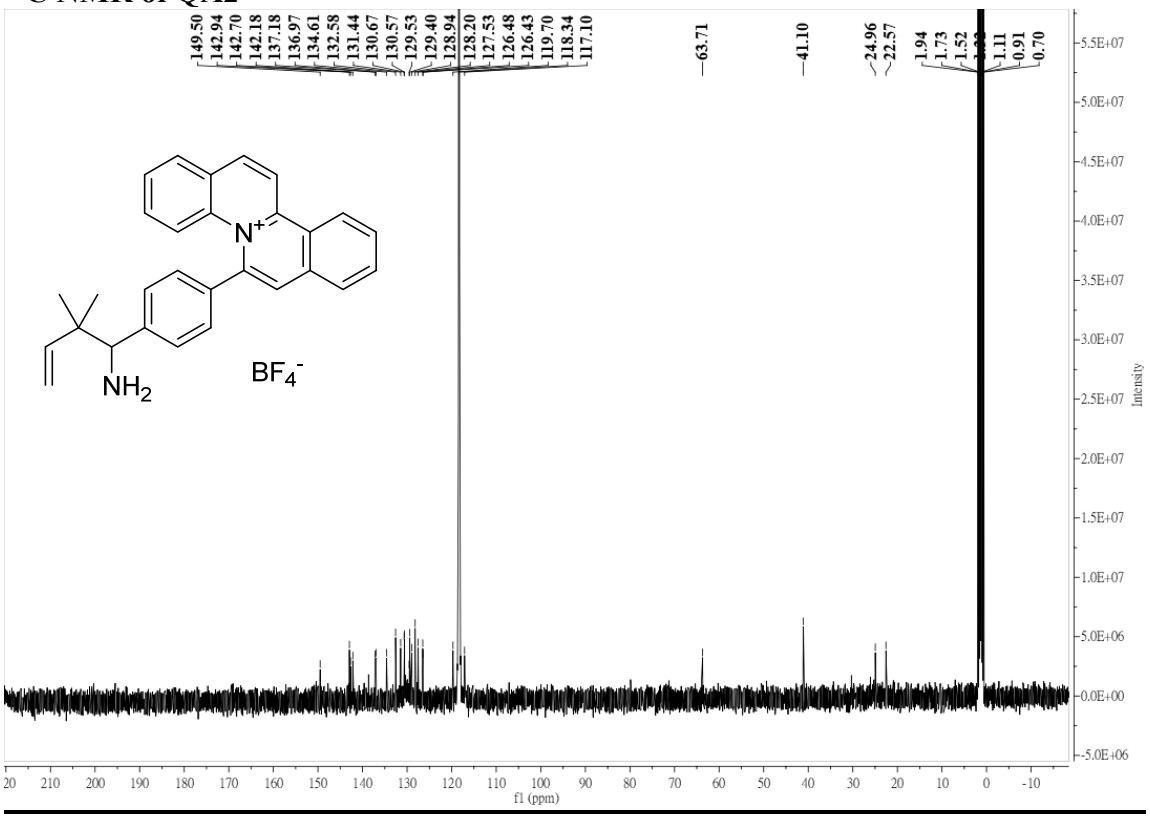
^{13}C NMR of 2c



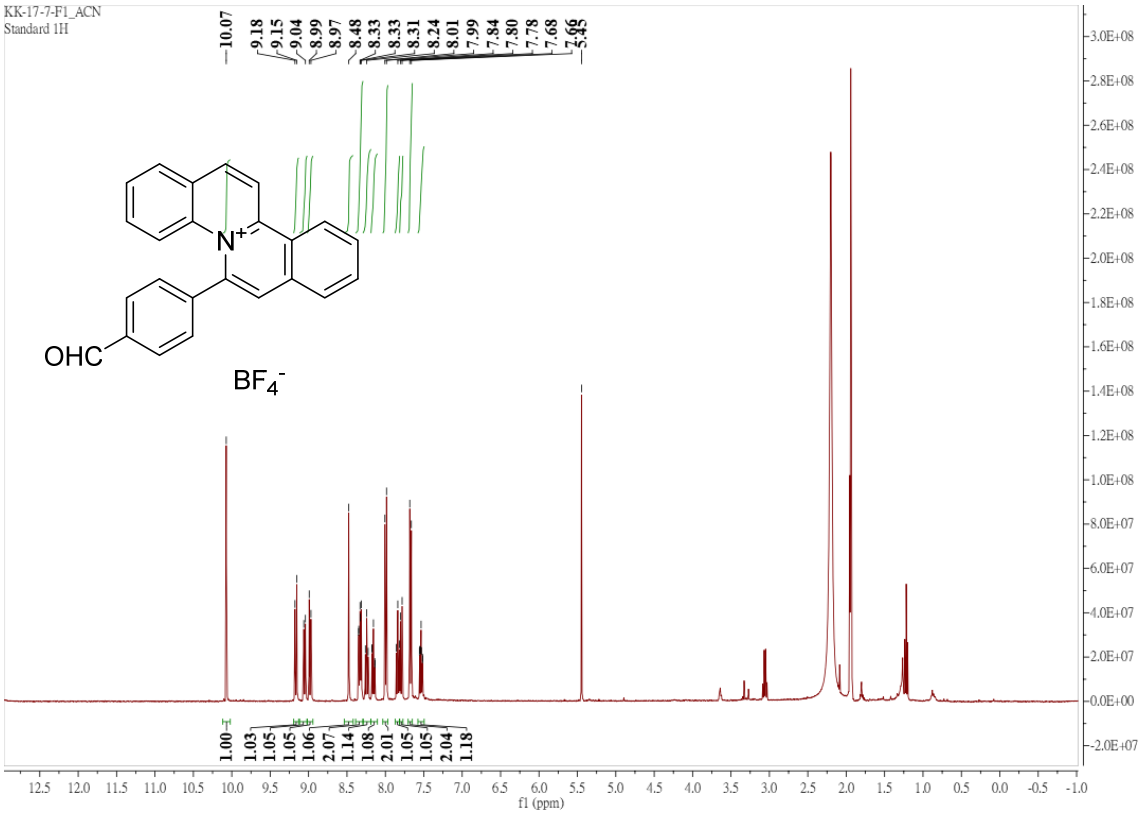
^{1}H NMR of QA2



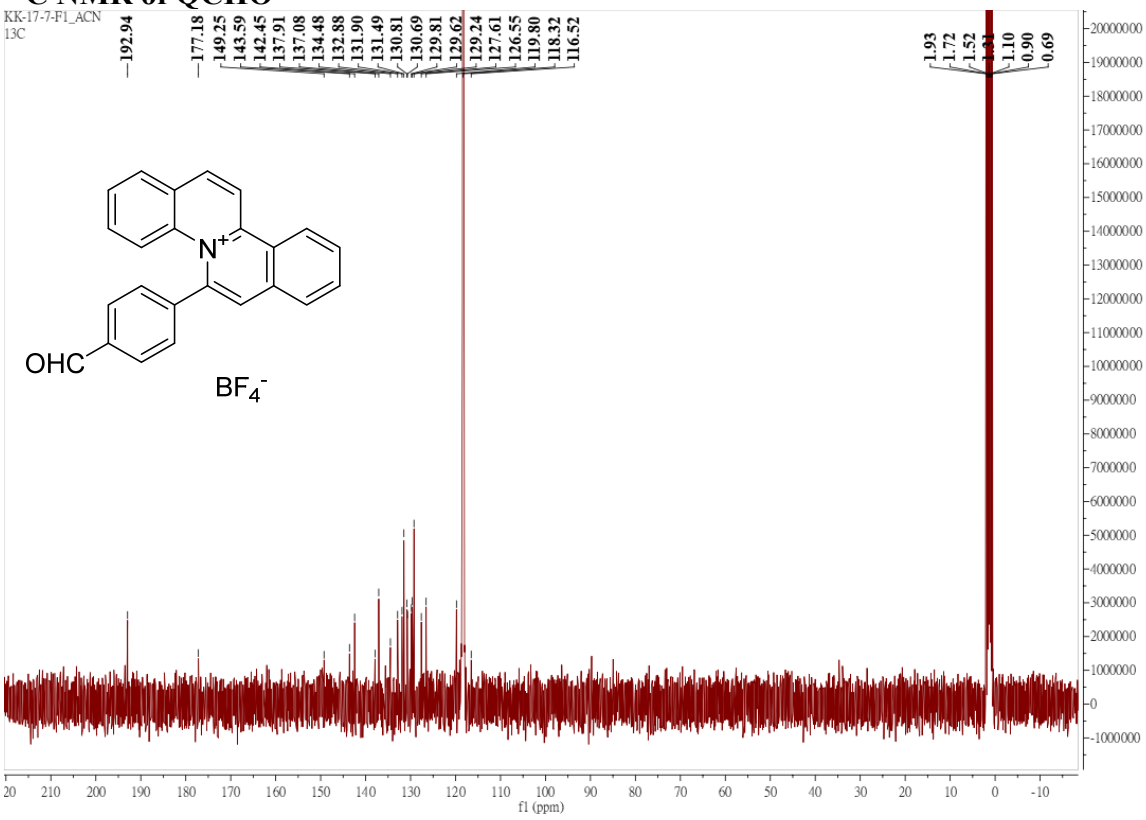
^{13}C NMR of QA2



^{1}H NMR of QCHO



^{13}C NMR of QCHO



References

Brewer, T. F., et al. (2017). "A 2-aza-Cope reactivity-based platform for ratiometric fluorescence imaging of formaldehyde in living cells." Chem. Sci. **8**(5): 4073-4081.

Bruemmer, K. J., et al. (2018). "Chemiluminescent Probes for Activity-Based Sensing of Formaldehyde Released from Folate Degradation in Living Mice." Angew. Chem. Int. Ed. **130**(25): 7630-7634.

Du, Y., et al. (2021). "Systematic investigation of the aza-Cope reaction for fluorescence imaging of formaldehyde in vitro and in vivo." Chem. Sci. **12**(41): 13857-13869.

Gu, J., et al. (2020). "A novel self-calibrating strategy for real time monitoring of formaldehyde both in solution and solid phase." J. Hazard. Mater. **386**: 121883.

Hao, Y., et al. (2020). "A benzothiazole-based ratiometric fluorescent probe for detection of formaldehyde and its applications for bioimaging." Spectrochim. Acta A Mol. Biomol. Spectrosc. **229**: 117988.

Ji, C., et al. (2019). "Perylene-Based Fluorescent Nanoprobe for Acid-Enhanced Detection of Formaldehyde in Lysosome." ACS Appl. Bio Mater. **2**(1): 555-561.

Rurack, K. and M. Spieles (2011). "Fluorescence quantum yields of a series of red and near-infrared dyes emitting at 600− 1000 nm." Anal. Chem. **83**(4): 1232-1242.

Xie, Z., et al. (2018). "A dual functional fluorogenic probe for visualization of intracellular pH and formaldehyde with distinct fluorescence signals." Org. Biomol. Chem. **16**(25): 4628-4632.

Yang, H., et al. (2018). "A ratiometric two-photon fluorescent probe for selective detection of endogenous formaldehyde via Aza-Cope reaction." Sens. Actuators B Chem. **270**: 318-326.

Zhai, B., et al. (2019). "A ratiometric fluorescent probe for the detection of formaldehyde in aqueous solution and air via Aza-Cope reaction." Dyes Pigm. **171**: 107743.

Zhou, Y., et al. (2018). "A ratiometric fluorescent probe for formaldehyde in aqueous solution, serum and air using aza-cope reaction." Sens. Actuators B Chem. **258**: 156-162.