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Supporting Information

Nucleic acid cleavage-based fluorescence probe for the detection of copper ion in pure aqueous solution

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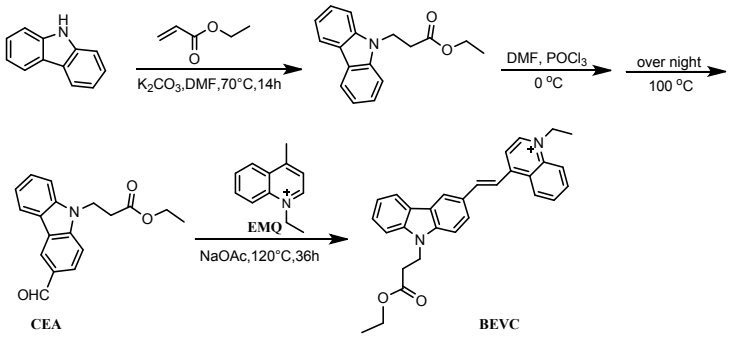
EXPERIMENTAL SECTION

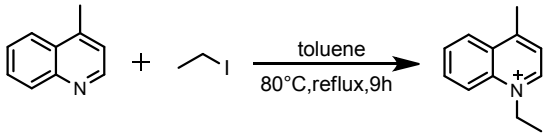
Chemicals and Apparatus. 4-methylquinoline, iodoethane, 9H-carbazole, ethyl acrylate, and phosphorus oxychloride were purchased from J&K Scientific Ltd. All chemicals were of analytical grade and used as received without further purification. G-quadruplex analogues with varying length were purchased from Sangon Biotech Company, Ltd. (Shanghai, China) without further purification, seeing Table S1 in supporting information. Sartorius ultrapure water (18.2 MΩ; Millipore Co., Billerica, MA) was used for the preparation of all buffers.

UV-vis absorption spectra were collected using a Hitachi U-4100 spectrophotometer (Kyoto, Japan). Mass spectra were performed using Bruker MicroTof-Q II hybrid quadrupole-time of flight. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. All fluorescence measurements were performed on a Hitachi F-4600 Fluorescence Spectrometer (Kyoto, Japan).

Synthesis of BEVC. The synthetic procedure of BEVC, 1-ethyl-4-methylquinolin (EMQ) and carbazole derivative (CEA) were first synthesized according to the reported methods.[S1,S2] Then, BEVC was obtained by condensation reaction of compound EMQ and CEA.

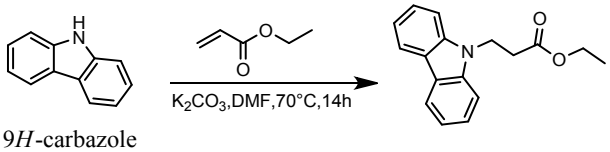


1-Ethyl-4-methylquinolin. To a solution of compound iodoethane (1.24g, 8mmol) in toluene (50ml), 4-methylquinoline (0.86g, 6mmol) was added. The mixture was refluxed at 80℃ for 19 h. After filtration, the solid was washed three times with ethyl ether and dried under vacuum to give dark green power (1.44 g, 80%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.35 (d, *J* = 6.0 Hz, 1H), 8.38-8.35 (m, 1H), 8.31 (d, *J* = 9.0 Hz, 1H), 8.19 (ddd, *J* = 8.9, 7.1, 1.4 Hz, 1H), 8.03-7.96 (m, 2H), 5.35 (q, *J* = 7.4 Hz, 2H), 3.01 (s, 3H), 1.81 (t, *J* = 7.3 Hz, 3H).



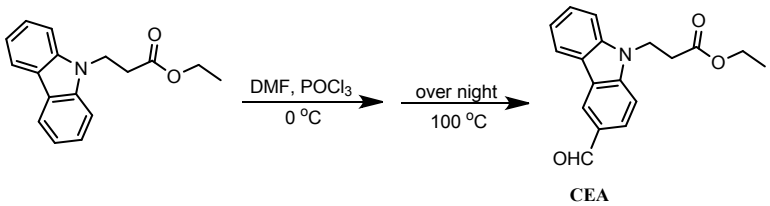
EMQ

1-Ethyl-3-(9H-carbazol-9-yl)propanoate. To a solution of compound 9H-carbazole (1.0 g, 6mmol) in DMF (50 mL), ethyl acrylate (0.8 mL, 7.0 mmol) and K_2CO_3 (1.0 g, 7.0 mmol) were added. The mixture was refluxed at 70℃, and monitored by TLC to confirm consumption of starting material. Upon completion, the mixture was cooled to room temperature, and poured into 80 mL ice water, extracted thrice with ethyl acetate (100 mL × 3), then the organic layer was dried by anhydrous Na_2SO_4. After filtration, organic solvents were evaporated to dryness and the residue was purified by column chromatography on silica gel (petroleum ether/ ethyl acetate = 4/1) to afford compound(1.1g, 70%) as a colourless liquid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (dt, *J* = 7.9, 0.9 Hz, 2H), 7.50-7.38 (m, 4H), 7.22 (ddd, *J* = 8.0, 6.3, 2.0 Hz, 2H), 4.61 (t, *J* = 7.2 Hz, 2H), 4.06 (q, *J* = 7.1 Hz, 2H), 2.81 (t, *J* = 7.1 Hz, 2H), 1.13 (t, *J* = 7.2 Hz, 3H).



9*H*-carbazole

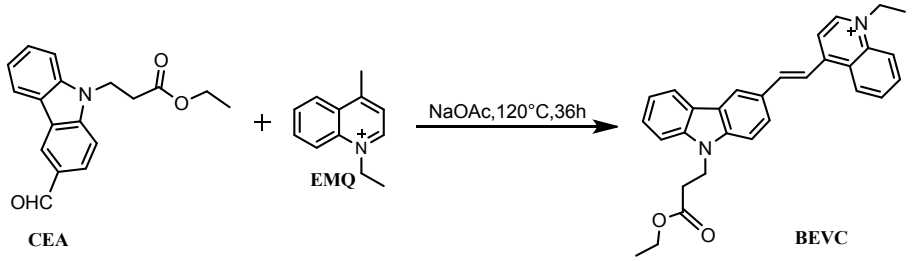
1-Ethyl-3-(3-formyl-9H-carbazol-9-yl)propanoate. To a solution of compound ethyl 3-(9H-carbazol-9-yl)propanoate (800 mg, 3.0 mmol) in dry DMF (40 mL) under N_2 atmosphere at 0℃, phosphorus oxychloride (0.7g mg, 4.6 mmol) was gradually added. The mixture was warmed up to room temperature, then refluxed at 100℃ for 24h. Then, the mixture was poured into cooled 5% NaOH solution and extracted thrice with dichloromethane (100 mL × 3). The organic layer was dried by anhydrous Na_2SO_4. After filtration, organic solvents were evaporated to dryness and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 3/1) to afford a yellow solid of ethyl 3-(3-formyl-9H-carbazol-9-yl)propanoate (800 mg, 54%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.06 (s, 1H), 8.55 (d, *J* = 1.6 Hz, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 7.98 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.35-7.28 (m, 1H), 7.26-7.20 (m, 1H), 7.15 (dd, *J* = 7.8, 2.7 Hz, 1H), 4.64 (t, *J* = 7.0 Hz, 2H), 4.07 (q, *J* = 7.1 Hz, 2H), 2.86-2.82 (m, 2H), 1.13 (t, *J* = 7.1 Hz, 3H).



CEA

(E)-3-(3-(2-(1-Ethylquinolin-1-ium-4-yl)vinyl)-9H-carbazol-9-yl)propanoate. To a solution of compound ethyl 3-(3-formyl-9H-carbazol-9-yl)propanoate (0.35g, 1.2mmol) in DMF (50 mL), 1-ethyl-4-methylquinolin-1-ium iodide (0.4g, 1.3mmol), NaOAc (100 mg) was added. The mixture was stirred and monitored by TLC to

confirm consumption of starting material. Upon completion, the mixture was cooled to room temperature, and evaporated to dryness and the residue was purified by column chromatography on silica gel (dichloromethane/methol = 20/1) to afford compound (0.28g, 55%) as a red solid, m.p. 182～185 ℃. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.77 (d, *J* = 8.5 Hz, 1H), 8.42 (d, *J* = 25.2 Hz, 2H), 8.21 (d, *J* = 7.7 Hz, 1H), 8.13-8.01 (m, 3H), 7.91 (td, *J* = 12.6, 11.1, 4.1 Hz, 3H), 7.54-7.45 (m, 3H), 7.37-7.29 (m, 2H), 4.99 (d, *J* = 7.6 Hz, 2H), 4.64 (t, *J* = 7.0 Hz, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 1.17 (t, *J* = 7.1 Hz, 3H); HRMS (ESI) m/z calcd for $C_{30}H_{29}N_2O_2^+$ ($[M]^+$): 449.2206. Found 449.2148.



Probe of G-quadruplex/BEVC constructing. To obtain G-quadruplex, solutions of 10 mM Tris-HCl (pH 7.4) and 150 mM KCl mixed with G-rich sequence DNA were heated to 95 ℃ for 10 min, cooled slowly to room temperature and then stored overnight at 4 ℃ before use. To perform ensemble, BMVC was incubated with G-quadruplex at room temperature for 10 min in the tris-HCl (pH 7.4, 10 mM) buffer solution containing 150 mM KCl.

Cu^{2+} Sensing. A stock solution was prepared by addition of G-quadruplex/BEVC in Tris-HCl buffer (10 mM, pH 7.4) containing 150 mM K^+ with final concentration of G-quadruplex at 200 nM and BEVC at 5.0 μM. For Cu^{2+} assay, 1.0 mL of G-quadruplex/BEVC was first added into a quartz cell, and then H_2O_2 solution was

added with final concentration of 2.0 mM, followed by treatment with Cu^{2+}. And the fluorescence signal was measured with excitation wavelength at 615 nm after incubation for 5 min at ambient temperature.

References

S1 J. Xu, Y. Zhang, H. Yu, X. Gao, S. Shao. *Anal. Chem*. 2016, **88**(2), 1455-1461.

S2 M. H. Hu, R. J. Guo, S. B. Chen, Z. S. Huang, J. H. Tan. *Dyes Pigm.* 2017, **137**, 191-199.

Table S1 Sequences of G-quadruplex

Type	Sequence (from 5′ to 3′)
L10	GGTGGTGGTG
L15	GGTGGTGGTGGTTGT
L20	GGTGGTGGTGGTTGTGGTGG
L26	GGTGGTGGTGGTTGTGGTGGTGGTGG
L32	GGTGGTGGTGGTTGTGGTGGTGGTGGTGGTGG

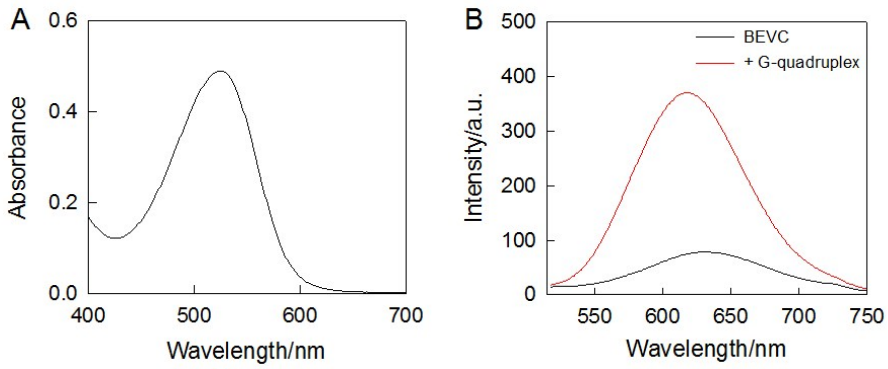


Fig. S1 (A) UV-visible absorption spectra of BEVC in Tris-HCl (10 mM, pH 7.4) buffer solution. (B) Fluorescence emission spectra of BEVC in the absence and presence of G-quadruplex in Tris-HCl (10 mM, pH 7.4) buffer solution containing 150 mM K^+.

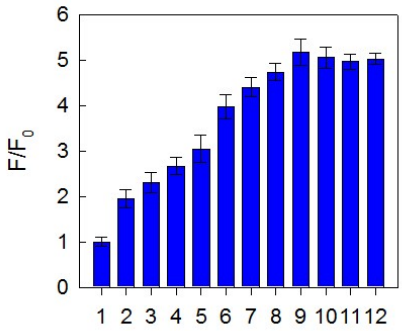


Fig. S2 Relationship between the fluorescence intensity ration as function of concentration ratio of BEVC to G-quadruplex. All of the samples reacted at room temperature for 5 min after treatment with different concentration of BEVC. F_0 is the fluorescence intensity of BEVC in Tris-HCl (10 mM, pH 7.4) buffer solution containing 150 mM K^+, where F is the fluorescence intensity of BEVC in the presence of different ratio of G-quadruplex. 1 to 12 are the concentration ration of BEVC to G-quadruplex. [G-quadruplex] = 0.2 μM

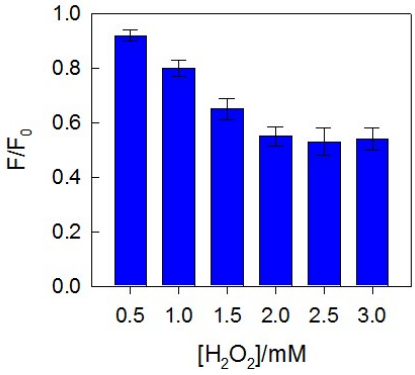


Fig. S3 The effect of H_2O_2 concentration to the fluorescence response for Cu^{2+} sensing. F_0 and F are the fluorescence intensity of G-quadruplex/BEVC in Tris-HCl (10 mM, pH 7.4) buffer solution containing 150 mM K^+ in the absence and presence of different concentration of H_2O_2. [G-quadruplex] = 0.2 μM; [BEVC] = 1.8 μM; [Cu^{2+}] = 100 μM.

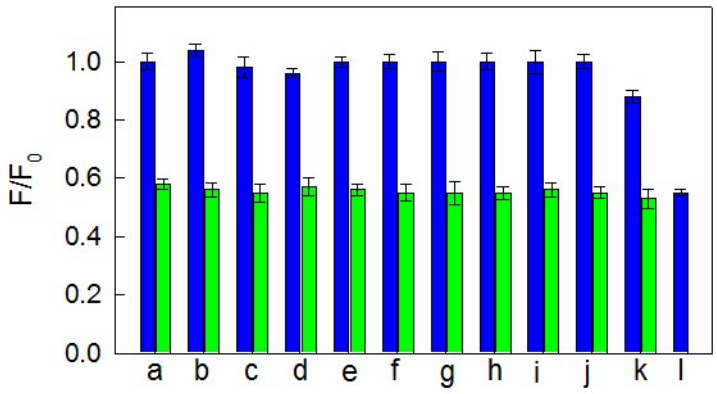
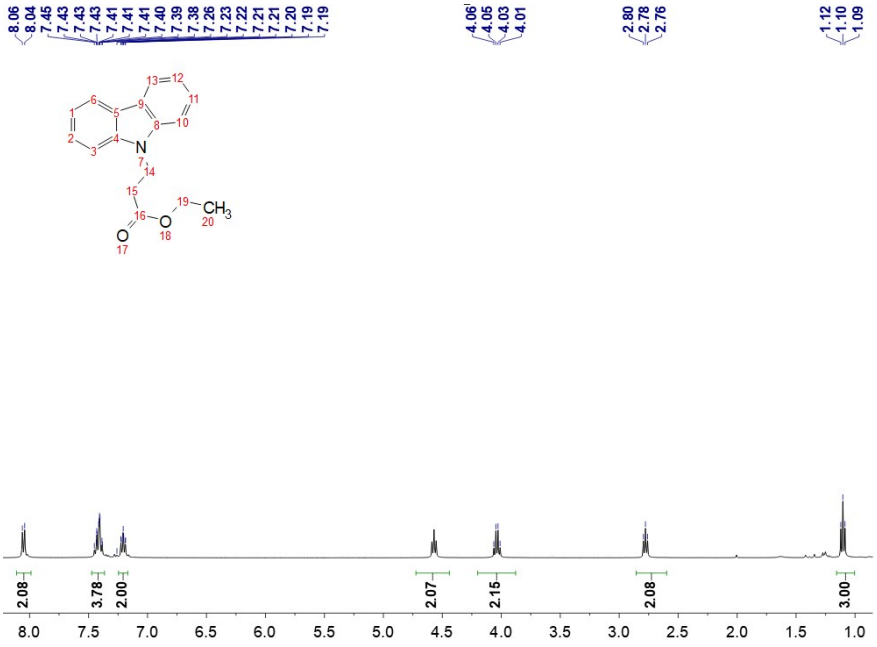


Figure S4. The selectivity of the proposed strategy for Cu^{2+} sensing. F_0 and F are the fluorescence intensity of G-quadruplex/BEVC in Tris-HCl (10 mM, pH 7.4) buffer solution containing 150 mM K^+ in the absence and presence of different metal ions and Cu^{2+}, respectively. From a to l are Sn^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, Ba^{2+}, Cd^{2+}, Fe^{3+}, Al^{3+} and Cu^{2+} before and after addition of Cu^{2+}. [G-quadruplex] = 0.2 μM; [BEVC] = 1.8 μM; [H_2O_2] = 2.0 mM; [Cu^{2+}] = 100 μM; [other control substances] = 500 μM.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7.23
7.22
7.21
7.21
7.20
7.19
7.19
4.06
4.05
4.03
4.01
2.80
2.78
2.76
1.12
1.10
1.09
2.08
3.78
2.00
2.07
2.15
2.08
3.00
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7.5
7.0
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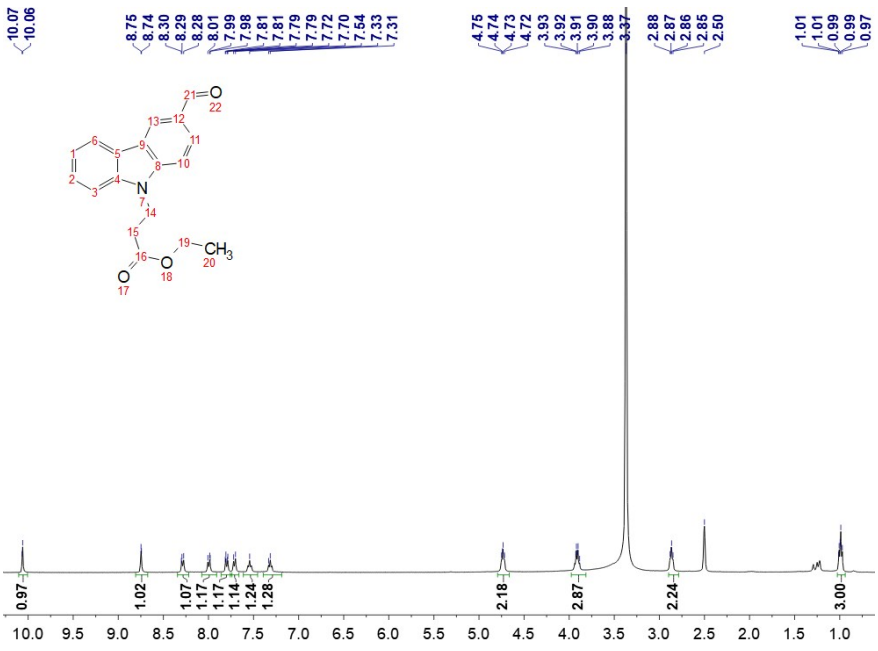
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7.79
7.72
7.70
7.64
7.33
7.31
4.75
4.74
4.73
4.72
3.93
3.92
3.91
3.90
3.88
3.37
2.88
2.87
2.86
2.85
2.50
1.01
1.01
0.99
0.99
0.97
0.97
1.02
1.07
1.17
1.17
1.14
1.24
1.28
2.18
2.87
2.24
3.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
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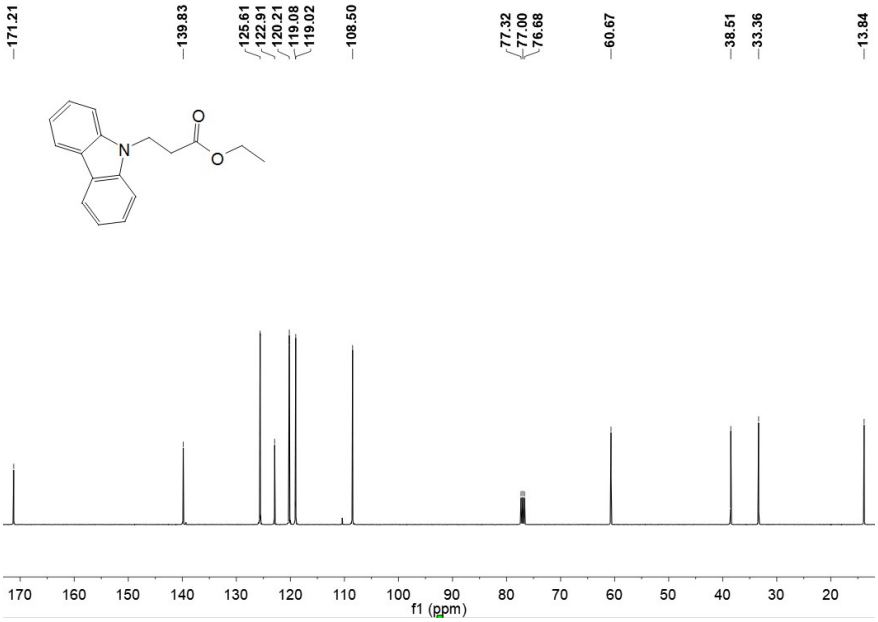
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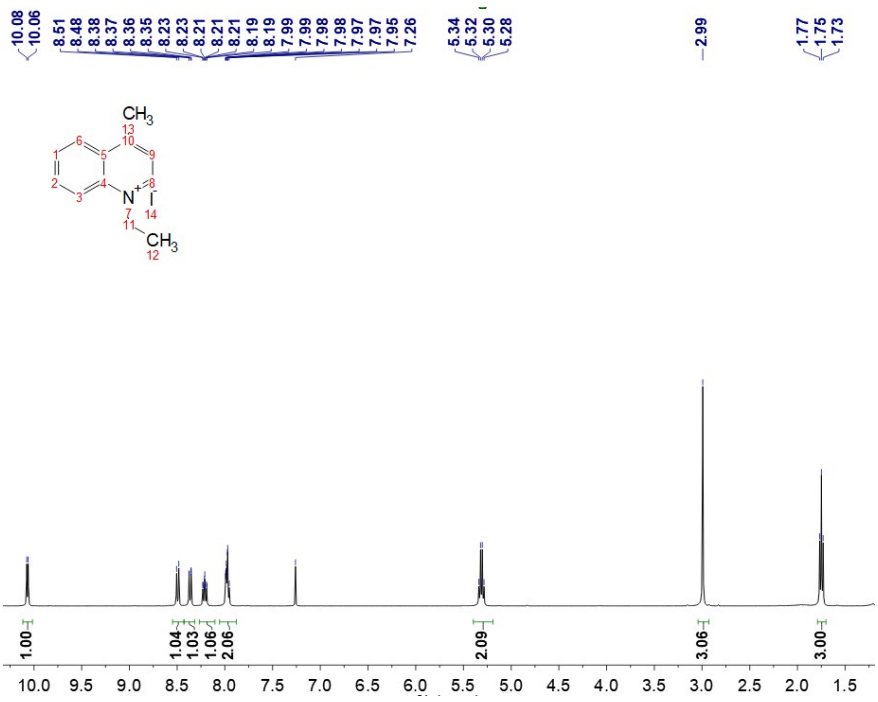
f1 (ppm)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7.26
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5.28
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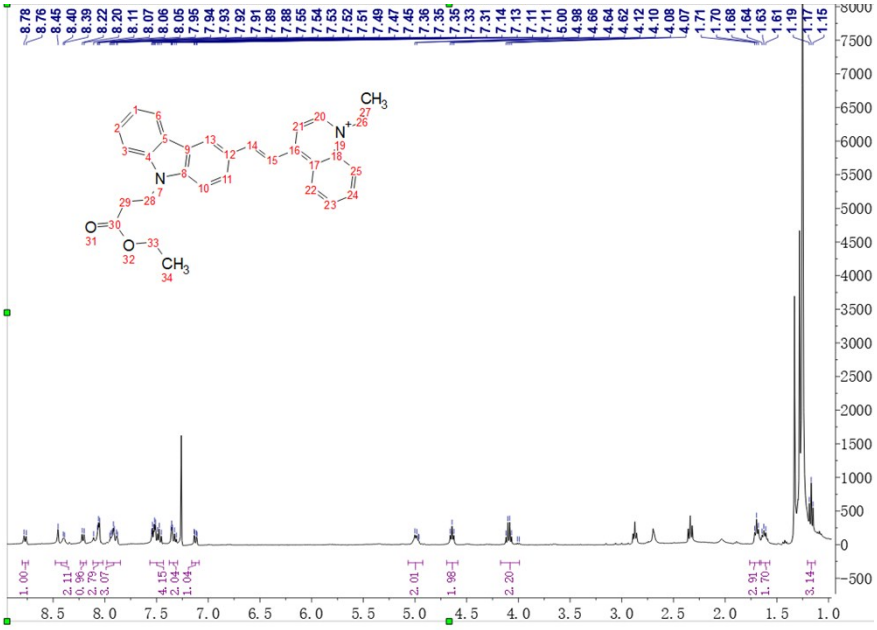

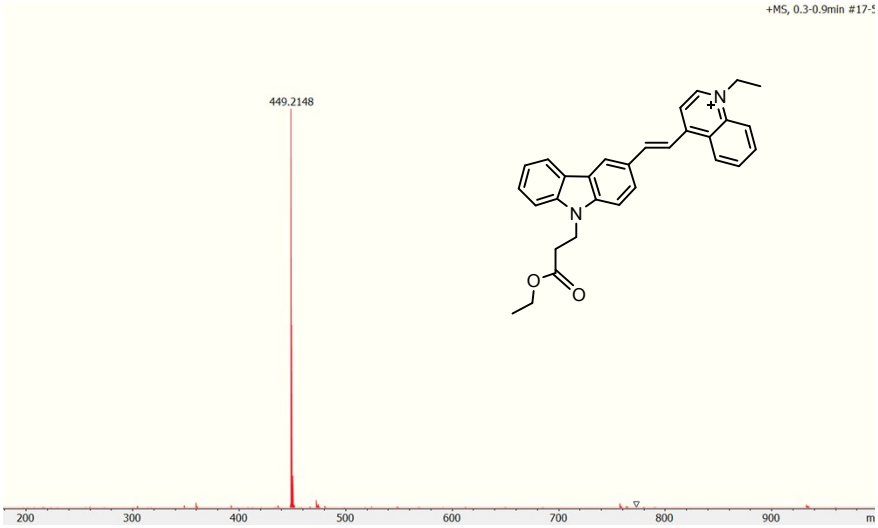
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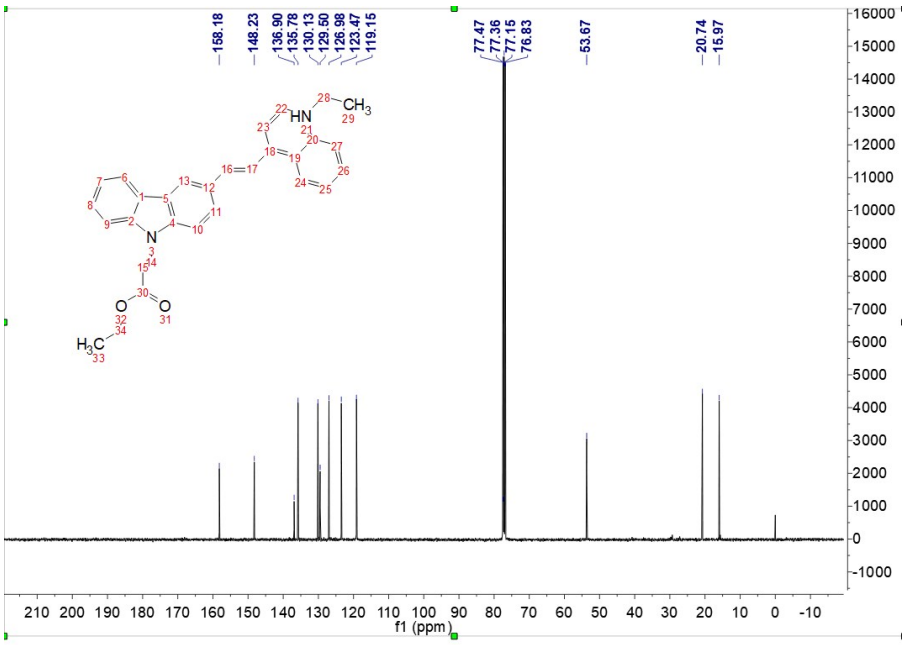
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H3C
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14000
13000
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11000
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9000
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6000
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f1 (ppm)