Supplementary Information for:

Highly selective and sensitive fluorescence turn-on probe for a catalytic amount of Cu(I) ions in water through the click reaction

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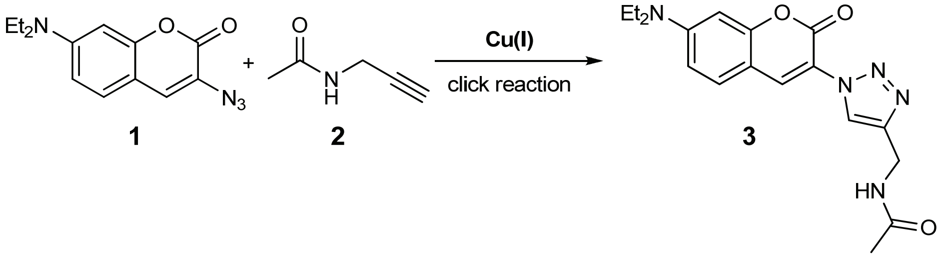
Contents

Supplementary Information

1. Instruments and reagents

All fluorescence and UV-vis absorption spectra were recorded in FP 6500 fluorescence spectrometer and HP 8453 absorption spectrometer, respectively. The $_1$H spectra were recorded at 200 MHz NMR spectroscopy. Mass spectra were recorded on G6401A MS-spectrometer. All experiments were carried out with commercially available reagents and solvents, and used without further purification, unless otherwise noted.

2. Synthesis



3-Azido-7-diethylaminochromen-2-one (3-azido-7-diethylaminocoumarin) (**1**)

3-Azido-7-diethylaminochromen-2-one was synthesized according to the modified literature procedure.[S1]
^{1}H NMR (200 MHz, $CDCl_3$): δ 7.19 (d, 1H, J = 8.8Hz), 7.10 (s, 1H), 6.59 (d, 1H, J = 8.8Hz), 6.50 (s, 1H), 3.41 (q, 4H, J = 6.4Hz) 1.21 (t, 6H, J = 6.8Hz).

N-(prop-2-ynyl)acetamide (**2**)

The proparagylamine hydrochloride (0.5 g, 5.46 mmol) was dissolved in 10 mL of MC and subsequently TEA (1.53 mL, 10.92 mmol) and acetyl chloride (0.505 mL, 7.10 mmol) were added at 0°C under N_2 gas. The reaction mixture was stirred for additional 1h and purification by silica gel column chromatography with EtOAc as an eluent gave the desired amide as a colorless liquid in 34 % yield (0.1775g).
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.10 (s, 1H), 3.95 (dd, 1H, J = 5.2, 2.4 Hz), 2.17 (t, 1H, J = 2.4 Hz), 1.95 (s, 3H).

Compound (**3**)

The 3-azido-7-diethylaminocoumarin (**1**, 0.0100 g , 0.039 mmol), *N*-(prop-2-ynyl)acetamide (**2**, 0.0038 g, 0.039 mmol) and sodium ascorbate (0.0153 g, 0.077 mmol) were dissolved in 4 mL of MeOH, followed by the addition of copper (II) sulfate (0.0974g, 0.039mmol). The reaction mixture was stirred vigorously at 0 °C in the dark for 1 h. Purification by silica gel column chromatography with DCM as eluent gave the desired product in 49 % yield (0.0063g).
^{1}H NMR (200 MHz, DMSO-d_6) δ 8.45 (s, 1H), 8.43(br, 1H), 8.35 (s, 1H), 7.64 (d, J = 9.0Hz), 6.84 (dd, 1H, J = 9.0, 2.6 Hz), 6.67 (d, 1H, J = 2.6 Hz), 4.37 (d, 2H, J = 5.4 Hz), 3.48 (q, J = 7.2Hz), 1.86 (s, 1H), 1.15 (t, J = 7.2Hz).
^{13}C NMR (50 MHz, DMSO-d_6): δ 169.9, 157.5, 156.4, 152.2, 145.8, 137.7, 131.3, 124.4, 117.0, 110.8, 107.3, 97.1, 45.0, 34.8, 23.2, 13.1.
LRMS (FAB$^+$, glycerol): obsd 356 ($[M+H]^+$, calcd 356 for $C_{18}H_{22}N_5O_3$)

General procedure for metal screening and competition assay

To a solution of azidocoumarin (**1**, 20 μM), alkyne (**2**, 10 mM), and sodium ascorbate (10 mM) in 2.0 mL HEPES buffer (0.10 M, pH 7.4) was added 4 μL stock solution of each metal ion (1 equiv, final concentration 20 μM). The stock solutions of each metal ion were prepared to be 10 mM in distilled water. After standing at rt for 1 h, the fluorescence spectra of each solution was measured by spectrophotometer. Competitive assay was also performed in a similar way. After metal ion and $CuSO_4 \cdot 5H_2O$ (1 equiv each) were added to the solution of **1** (20 μM), **2** (10 mM), and sodium ascorbate (10 mM) in HEPES buffer, and then the reaction mixture was incubated at rt for 1 h, their fluorescence spectra were monitored by spectrophotometer.

Reference

S1. Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603.

3. NMR spectra

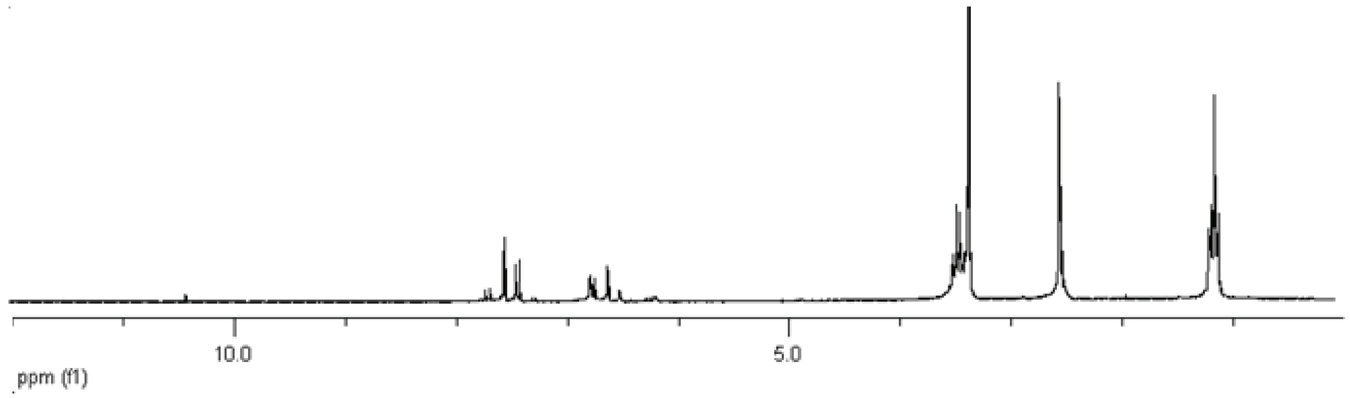


Fig. S1. ^{1}H spectrum of **1** in DMSO-d_6.

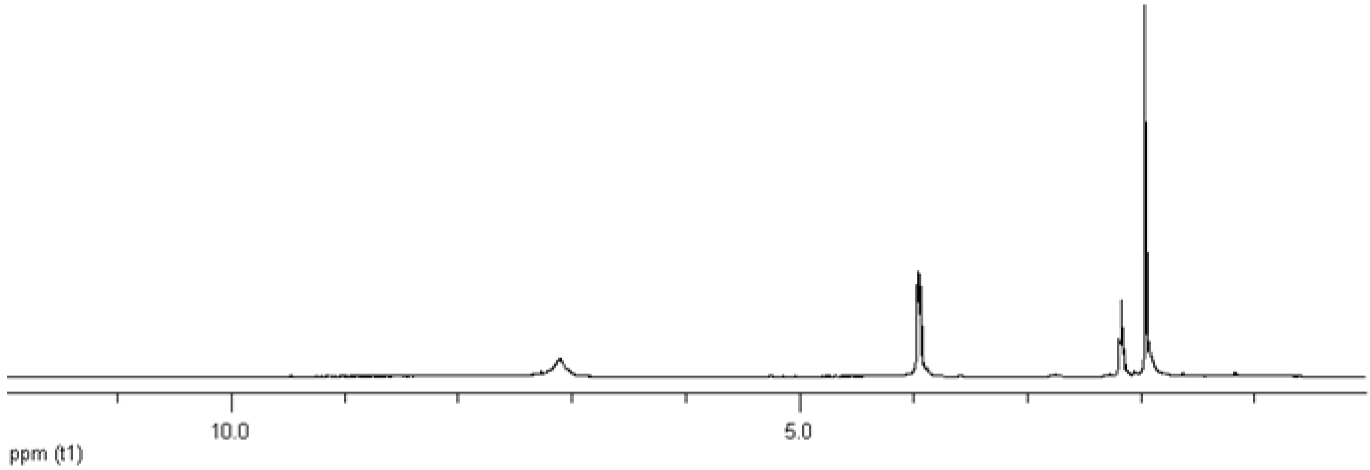
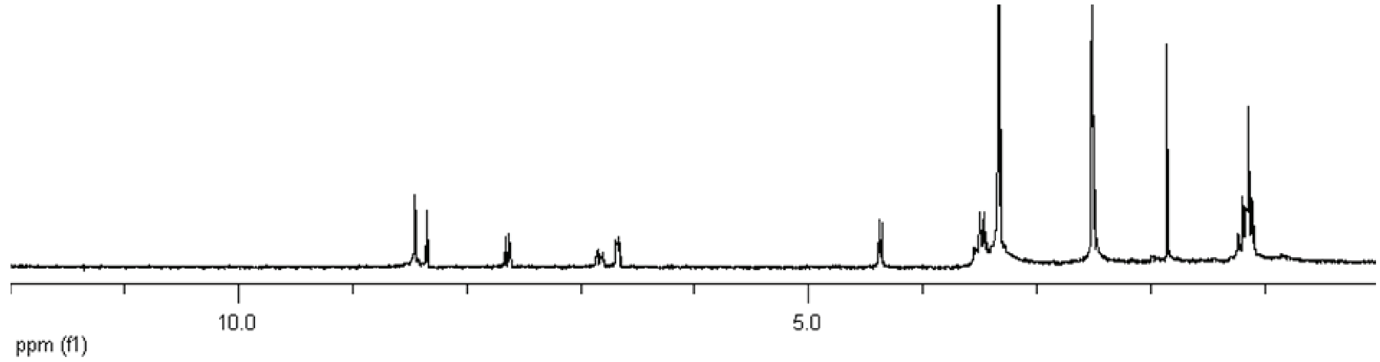


Fig. S2. ^{1}H spectrum of **2** in CDCl$_3$.



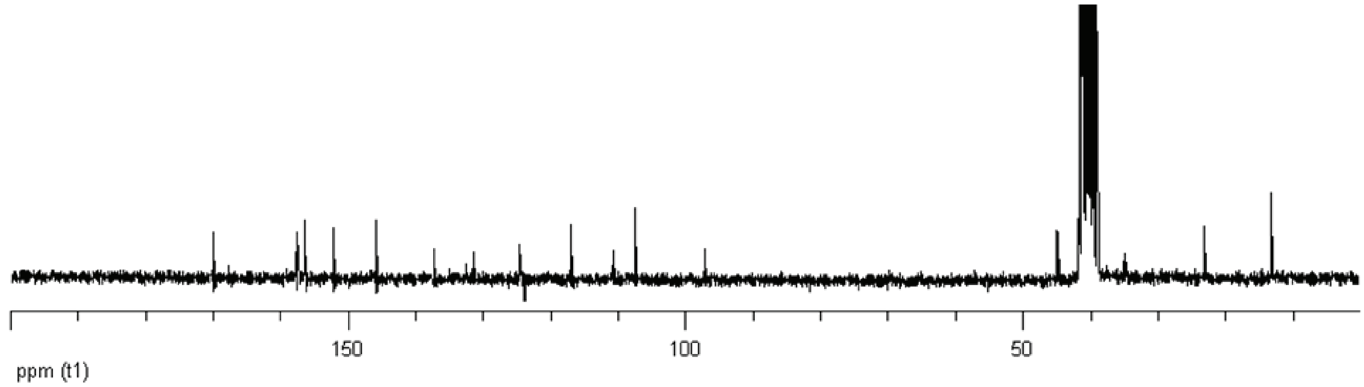


Fig. S3. ^{1}H and ^{13}C spectra of **3** in DMSO-d_6.

4. Rate analysis

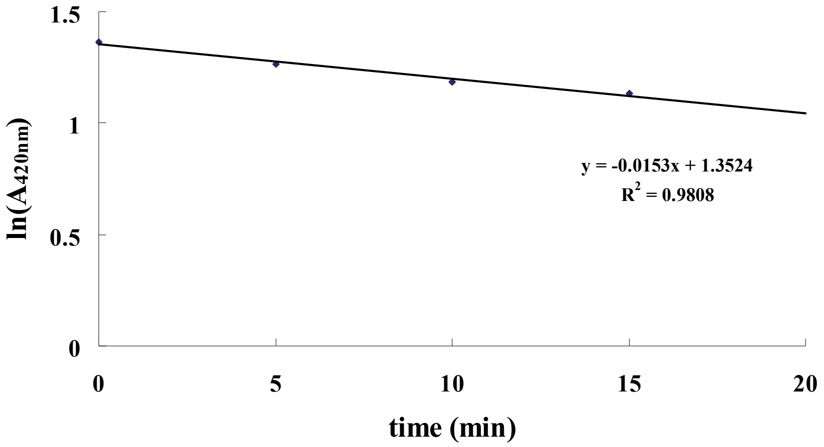


Fig. S4. The Second-order rate constant of chemical reaction of **1** (20 μM) and **2** (10 mM). $CuSO_4$ = 20 μM in HEPES buffer (0.1 M, pH 7.4).

5. Limit of detection

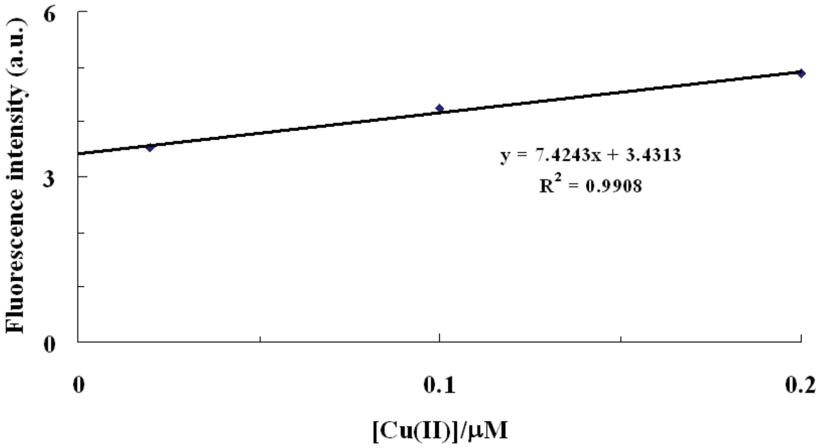
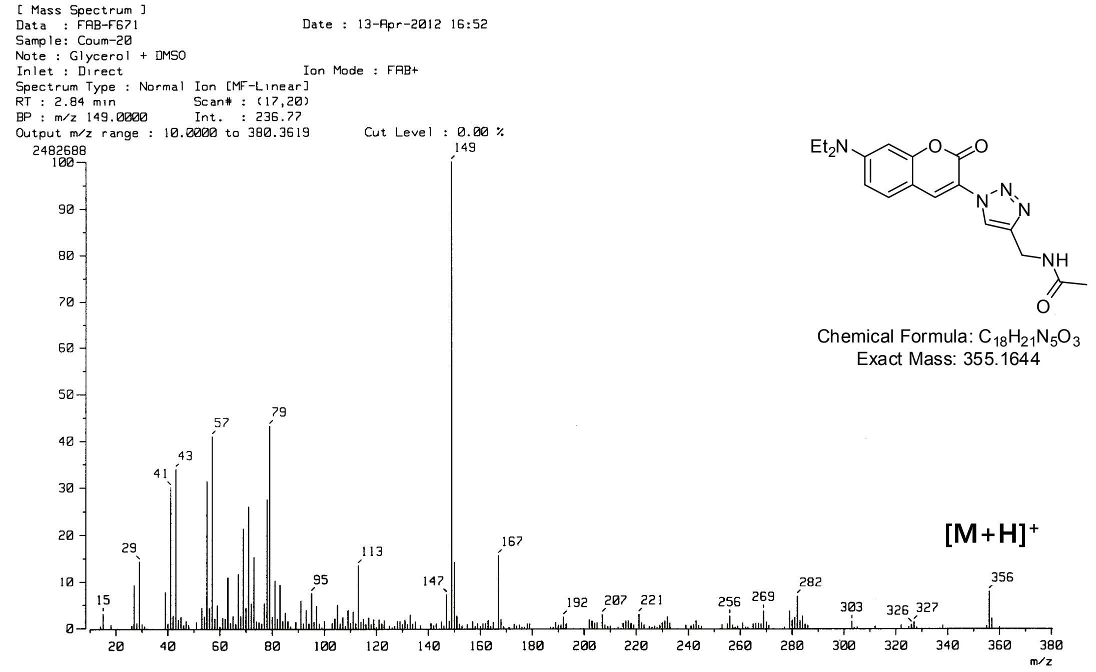


Fig. S5. The limit of detection of copper ions was measured between 0.001 and 0.01 equiv of copper ions. [**1**] = 20 μM, [NaAsc] =20 μM, and [alkyne] = 10 mM in HEPES buffer (0.1 M, pH 7.4).

6. Mass spectrum



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[ Mass Spectrum ]
Data   : FAB-F671                      Date : 13-Apr-2012 16:52
Sample: Coum-20
Note : Glycerol + DMSO
Inlet : Direct                         Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.84 min           Scan# : (17,20)
BP : m/z 149.0000       Int.   : 236.77
Output m/z range : 10.0000 to 380.3619       Cut Level : 0.00 %
```

Chemical Formula: $C_{18}H_{21}N_5O_3$
Exact Mass: 355.1644

Fig. S6. Low resolution mass spectrum of **3**.