Electronic supplementary information

An efficient and easy-accessible ligand for bioconjugation Cu(I)-catalyzed azide-alkyne cycloaddition

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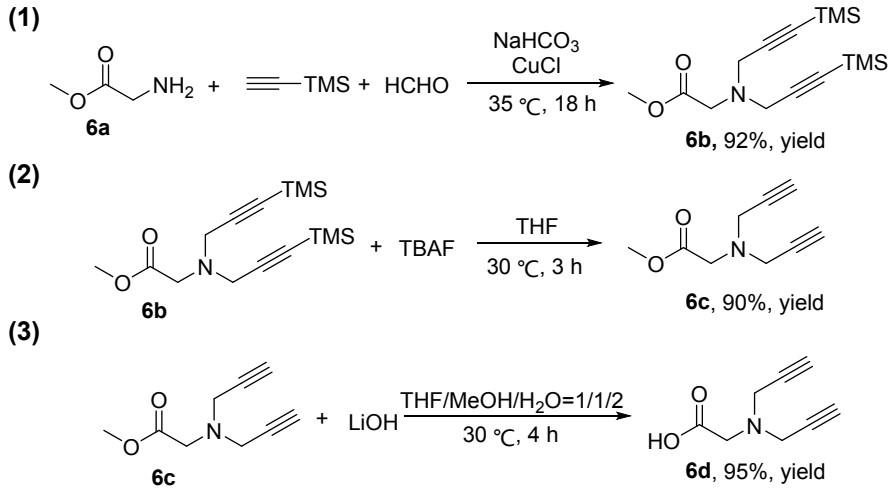
Experimental Procedural

1. **General information:**

Unless otherwise noted, all commercially available compounds and solvents were used directly without further purification. Isolation of all compounds related in this paper was carried out on silica gel column. TBTA was purchased from Aladdin Reagent Company. BTTPS was purchased from MeloPEG Company. RPMI-1640 medium was purchased from Thermo Fisher Inc. (USA). Ac_4ManNAl and bathocuproine disulfonte were purchased from Sigma-Aldrich company.7-AAD staining kit was purchased from Shanghai Sangon Biotechnology Company. FITC-PEG-azide was purchased from Aladdin Reagent Company. High resolution mass spectrum was obtained on Bruker compact mass spectrometer. NMR spectra were measured with Bruker Avane 400/600 MHz. Quantitative fluorescent analysis was conducted on Shimadzu RF-540 fluorespectra photometer. FITC and 7-AAD fluorescent images were captured on OLYMPUS FV1200MOE confocal microscopy. Flow cytometer analysis was carried out on FACS scan cytometer using CELLQUEST software.

2. **Synthesis of di(prop-2-yn-1-yl)glycine:**



(1) 20 mmol glycinemethylester (**6a**), 20 mmol $NaHCO_3$, 0.2 mmol CuCl, 50 mmol Trimethylsilylacetylene were added into 3.7 mL formaldehyde solution (37%), the mixture was stirred for 18 h at 35 ˚C. Then, the solution was extracted with 200 mL dichloromethane (DCM) for 3 times, the DCM phase was vacuum dried to give **6b** with 92% yield. The product **6b** is a colorless oil[1].

(2) 10 mmol **6b** was dissolved in 20 mL tetrahydrofuran (THF), then 25 mmol Tetrabutyl ammonium fluoride (TBAF) was added into the solution stepwise within 3 h at 30 ˚C. Then mixture was extracted with 200 mL DCM for 3 times, the DCM phase was vacuum dried to give **6c** with 90% yield. The product **6c** is a colorless oil. [1]

(3) 10 mmol **6c** was dissolved into 10 mL THF/methanol/H_2O, then 20 mmol LiOH was added into the solution, and stirred at 30 ˚C for 4 h, the resulting mixture was vacuum dried to give **6d** with 95% yield. The resulting product **6d** is a white solid.

3. **Synthesis of 6:**

10 mmol **6d** was dissolved into 10 mL acetonitrile, then 20 mmol 3-azidopropan-1-ol was added, after that 12 mmol DIPEA (N,N-diisopropylethylamine) and 1 mmol CuI was added, and the mixture was stirred at room temperature for 3 h. The solvent was removed under reduced press, and the 100 mL petroleum ether was added to the residue. The precipitate was collected after 24 hours, and washed with water and diethyl ether. After vacuum dried, 3.1 g **6** was obtained with the yield of 88%. **6** is a white solid.

4. **Model CuAAC reaction[2]:**

In typical experimental procedure, 50 μM 4-Pentynyl alcohol, 100 μM 3-azido-7-hydroxycoumarin, 75 μM CuSO4 and 300 μM ligand were mixed in 10 mL pH 7.4 PBS buffer successively, after that 2.5 mM sodium ascorbate was added to initiate the reaction, the reaction was carried out in air at room temperature.

5. **Catalytic constant calculation:**

The standard integrated rate law for a bimolecular reaction

$$\text{A (azide)} + \text{B (alkyne)} \rightarrow \text{P (triazole)}$$

was constructed for the present situation in which $[B_0] = n[A_0]$, ($n > 1$), as follows.

$$[A] = [A_0] - [P],\ [B] = [B_0] - [P]$$

$$\frac{d[P]}{dt} = k([A_0] - [P])([B_0] - [P])$$

$$k = \frac{d[P]}{dt([A_0] - [P])([B_0] - [P])}$$

The concentration of P was quantitative measured with fluorescent detector, and the initial concentrations of azide and alkyne were 100 μM and 50 μM, respectively.

Suppose: $K = \frac{[B_O] * [A_0] - [P]}{[A_O] * [B_0] - [P]}$, and $K_{obs} = \frac{1}{t * [A_0] - [B_0]} * lnK.$

6. **7-AAD staining procedure:**

The cultured cells were centrifuged at 1000 rpm for 5 min, and washed three times with PBS. The resulting cell was resuspended at 1×10^6 /ml in 7-AAD working solution and incubated 10-15 min at room temperature protected from light. After that, the cells were washed at least three times with PBS and analyzed with flow cytometry (Cytoflex, Beckman Counter).

Protocol for kinetic measurement.

Stock solutions:

$CuSO_4$: 10 mM in water, 100 mM in water.

Ligand: 10 mM in water for **3aa** ligands; 10 mM in water for **TBTA** ligands.

Sodium ascorbate: 25 mM in water.

Azido coumarin: 1.0 mM in dimethyl sulfoxide (DMSO).

Propargyl alcohol: 1.0 mM.

Buffer: 500 mM potassium phosphate pH 7.0

Final concentrations:

Buffer: 100 mM potassium phosphate. (pH 7.0)

Azido coumarin: 0.10 mM in DMSO.

Propargyl alcohol: 0.05 mM.

$CuSO_4$: 75 μM.

Sodium ascorbate: 2.5 mM.

Procedure for 200 μL reactions:

1.40 μL of 500 mM phosphate buffer pH 7.0.

2. 10 μL 1.0 mM Propargyl alcohol.

3. 10 μL of DMSO.

4. 6 μL of premixed $CuSO_4$ and ligand ([Cu] = 2.5 mM).

5. 20 μL 1.0 mM azido coumarin.

6. Add water to 180 μL volume.

7. 20 μL of 25 mM sodium ascorbate.

8. Read fluorescence (λex = 404 nm, λem = 477 nm, RFU).

Supplementary Figures

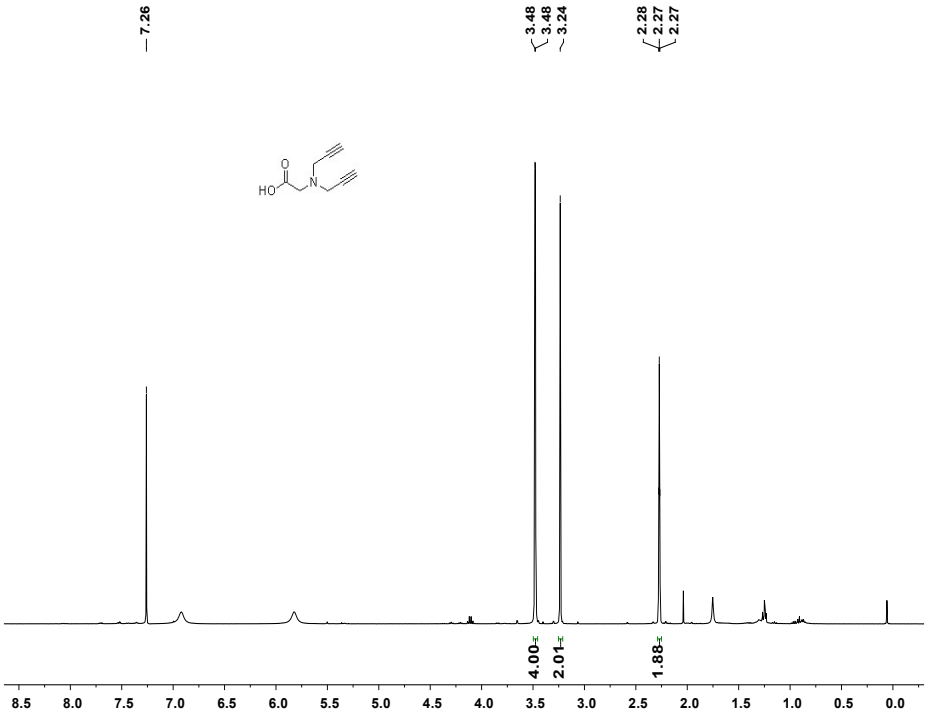


Figure S1. ^{1}H-NMR spectrum of Compound **6d.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.48 (d, J = 2.4 Hz, 4H), 3.24 (s, 2H), 2.27 (t, J = 2.4 Hz, 2H).

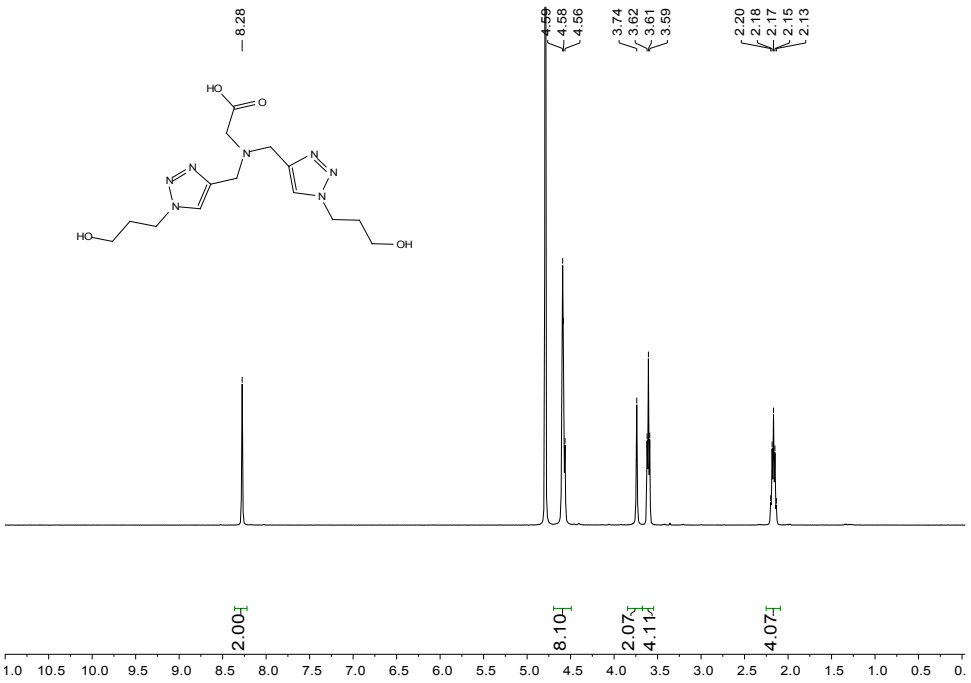


Figure S2. ^{1}H-NMR spectrum of Compound **6.** ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.28 (s, 2H), 4.58 (m, J = 12.6, 8H), 3.75 (s, 2H), 3.61 (t, J = 6.1 Hz, 4H), 2.16 (m, 4H).

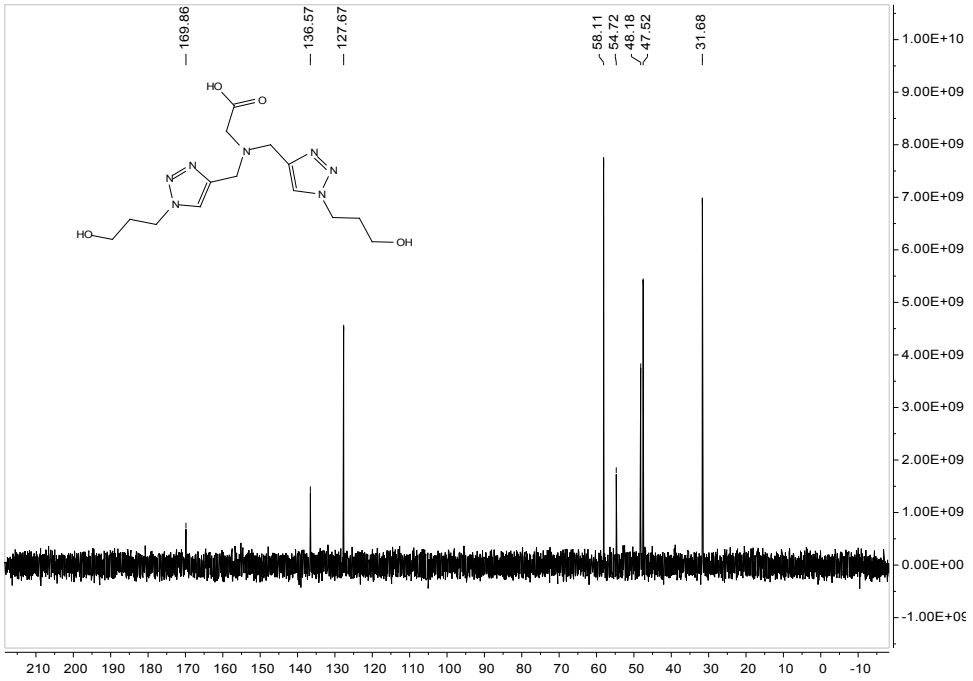


Figure S3. ^{13}C-NMR spectrum of Compound **6.**^{13}C NMR (101 MHz, Deuterium Oxide) δ 169.86, 136.57, 127.67, 58.11, 54.72, 48.18, 47.52, 31.68.

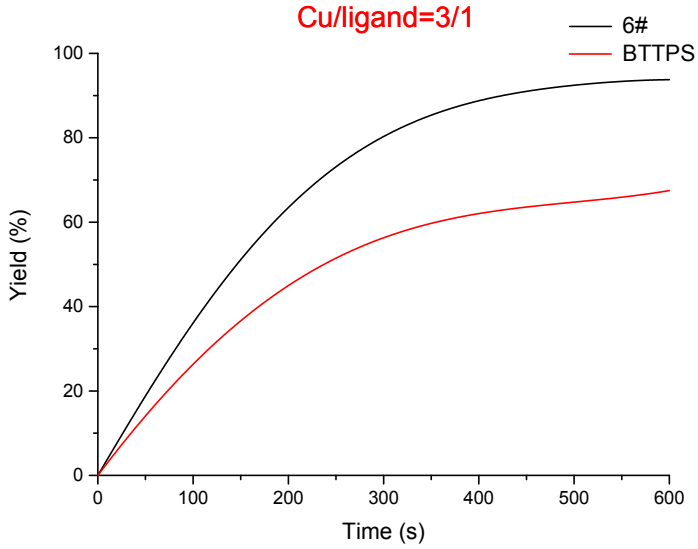


Figure S4. Catalytic performance of **6** and BTTPS at 3:1 Cu/**6** ratio. The reaction was carried out in PBS buffer (10 mM pH 7.4 PBS), 50 μM prop-2-yn-1-ol and 100 μM coumarin azide were added as substrates, 75 μM CuSO4 and 25 μM ligand were added as catalyst, 2.5 mM sodium ascorbate was added as reducing reagent, and the reaction was carried out in air.

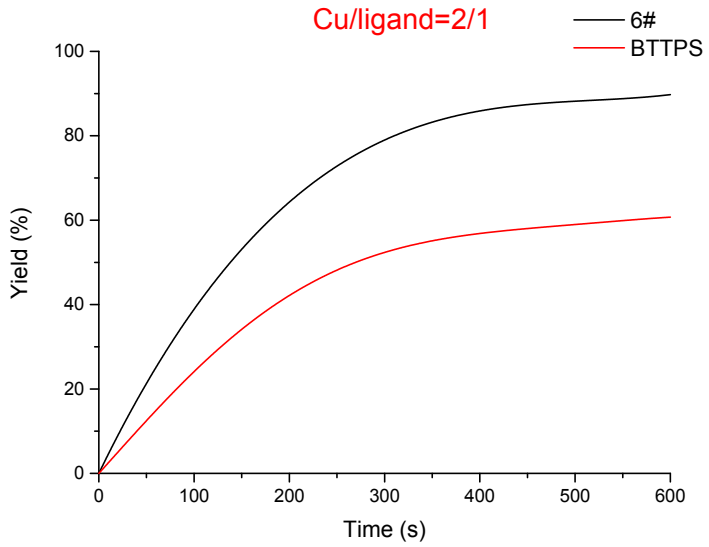


Figure S5. Catalytic performance of **6** and BTTPS at 2:1 Cu/**6** ratio. The reaction was carried out in PBS buffer (10 mM pH 7.4 PBS), 50 μM prop-2-yn-1-ol and 100 μM coumarin azide were added as substrates, 75 μM CuSO4 and 37.5 μM ligand were added as catalyst, 2.5 mM sodium ascorbate was added as reducing reagent, and the reaction was carried out in air.

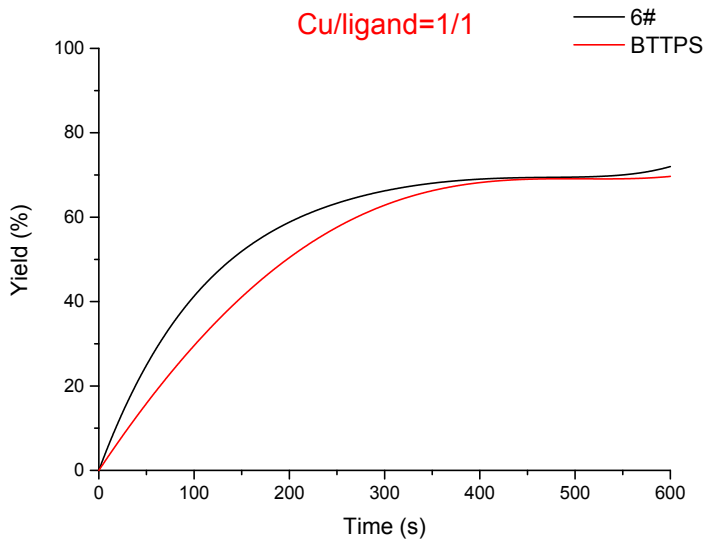


Figure S6. Catalytic performance of **6** and BTTPS at 1:1 Cu/**6** ratio. The reaction was carried out in PBS buffer (10 mM pH 7.4 PBS), 50 μM prop-2-yn-1-ol and 100 μM coumarin azide were added as substrates, 75 μM CuSO4 and 75 μM ligand were added as catalyst, 2.5 mM sodium ascorbate was added as reducing reagent, and the reaction was carried out in air.

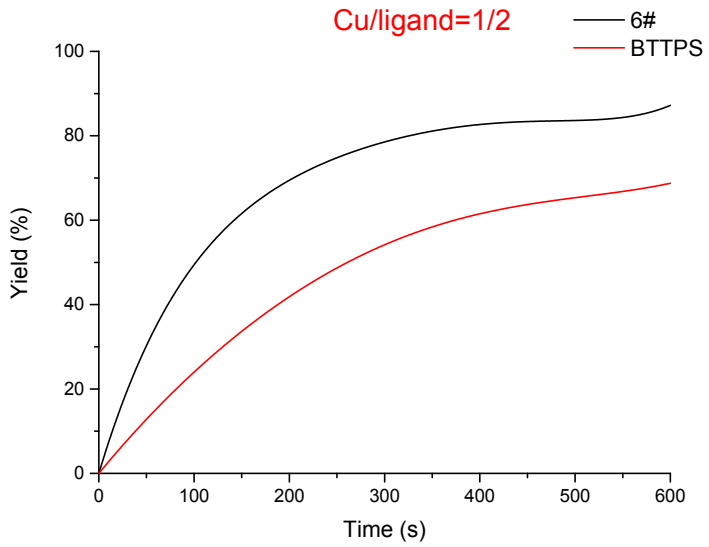


Figure S7. Catalytic performance of **6** and BTTPS at 1:2 Cu/**6** ratio. The reaction was carried out in PBS buffer (10 mM pH 7.4 PBS), 50 μM prop-2-yn-1-ol and 100 μM coumarin azide were added as substrates, 75 μM CuSO4 and 150 μM ligand were added as catalyst, 2.5 mM sodium ascorbate was added as reducing reagent, and the reaction was carried out in air.

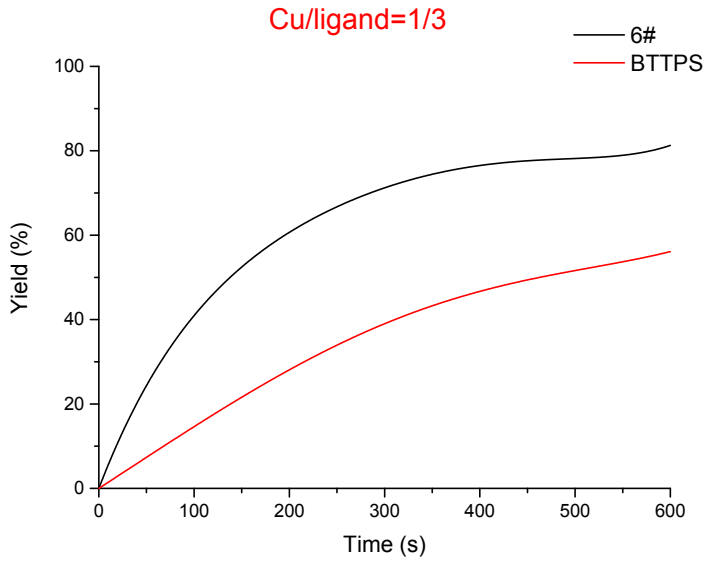


Figure 8. Catalytic performance of **6** and BTTPS at 1:3 Cu/**6** ratio. The reaction was carried out in PBS buffer (10 mM pH 7.4 PBS), 50 μM prop-2-yn-1-ol and 100 μM coumarin azide were added as substrates, 75 μM CuSO4 and 225 μM ligand were added as catalyst, 2.5 mM sodium ascorbate was added as reducing reagent, and the reaction was carried out in air.

References

1. A. Zhu, X. Xing, S. Wang, D. Yuan, G. Zhu, M. Geng, Y. Guo, G. Zhang and L. Li, *Green Chem.*, 2019, **21**, 3407-3412.
2. L. Li, S. Huang, T. Shang, B. Zhang, Y. Guo, G. Zhu, D. Zhou, G. Zhang, A. Zhu and L. Zhang, *J. Org. Chem.*, 2018, **83**, 13166-13177.