Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

All-In-One Azides: Empowered Click Reaction for *in vivo* Labeling and Imaging of Biomolecules

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General materials and methods

All chemical reagents were used as supplied by Sigma-Aldrich, J&K and Alfa AesarChemicals. DCM, DMF, acetonitrile were distilled from calcium hydride; THF was distilled from sodium/benzophenone ketyl prior to use. Methyl 2-cyano-2- (prop-2-yn-1-yl) pent-4-ynoate (**7**), [1](3-azidopropoxy) (tert-butyl)dimethylsilane (**8**), [2] 2-(2-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)ethyl)isoindoline-1,3-dione(**13**), [3] tert-butyl-(2-azidoethyl)-carbamate(**16**)[4] and N-(3-chloro-4-fluorophenyl)-7-fluoro-6-nitroquin azolin-4-amine(**21**)[5] were prepared according to the literature reported procedures. Srepavidin-HRP(#3999), HER2 XP® Rabbit mAb(#4290s) were bought from Cell Signalling Technology. Alexa Fluor® 555 conjugated Goat anti-Rabbit IgG (A-21428) was bought Life Technology. Anti β-Actin Mouse Monoclonal Anitibody (CW0096), HRP Conjugated Goat Anti-Mouse IgG (CW0102), HRP Conjugated Goat Anti-Rabbit IgG (CW0103) were bought from CWbiotech. CellTiter-Glo® Luminescent Cell Viability Assay (G7573) was bought from Promega. RPMI 1640 Medium(22400105), Fetal Bovine Serum (12483-020) and Penicillin-Streptomycin(15140-122) were bought from Life Technology. WESTERN LIGHTNING™ Plus-ECL (NEL103001EA) was bought from Perkin Elmer. DiI dye (C1036) and Antifade Mounting Medium (P0126) were bought from Beyotime. Hoechst dye (H1399)was bought from Life Technology. High Capacity Strpavidin agrose (20361) was bought from Thermo Scientific.

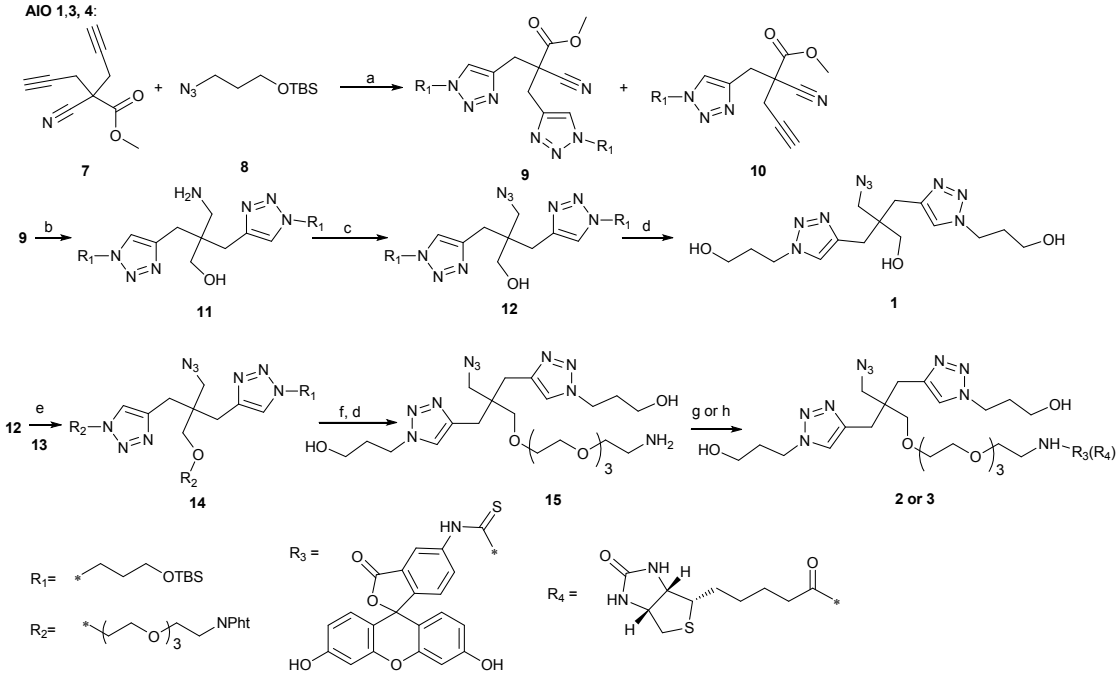
^{1}HNMR spectra were recorded on a Varian 400 MHz spectrometer at ambient temperaturewith $CDCl_3$ as the solvent unless otherwise stated. ^{13}C NMR spectra were recorded on a Varian 100 MHz spectrometer (with complete proton decoupling) at ambient temperature. Chemical shifts are reported in parts per million relative to chloroform (1H, δ 7.26; 13C, δ77.00). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constants. Infrared spectra were recorded on a Thermo Fisher FT-IR200 spectrophotometer. High-resolution mass spectra were obtained at Peking University Mass Spectrometry Laboratory using a Bruker APEX Flash chromatography. The samples were analyzed by HPLC/MS on a Waters Auto Purification LC/MS system (3100 Mass Detector, 2545 Binary Gradient Module, 2767 Sample Manager, and 2998 Photodiode Array (PDA)Detector). The system was equipped with a Waters C_{18} 5μm SunFire separation column(150*4.6 mm), equilibrated with HPLC grade water (solvent A) and HPLC grade acetonitrile (solvent B) with a flow rate of 0.3 mL/min.

Kinetic measurements using 2-azidoethanol and 7-ethynyl-2H-chromen-2-one as the model system were performed using a 96-well BioTek Synergy Hybrid Plate Reader. Standard curve, kinetic measurements and cell viability test were carried out by the Enspire™ 2300 Multilabel Reader from PerkinElmer. In-gel fluorescence was detected by ChemiDoc™ MP system (Bio-Rad) using 530/28 filter. Jurkat and H878Y cell imaging were conducted by Nikon A1-R Confocal, with 405nm, 488nm and 561 nm lasers. C. elegans live imaging were conducted by zeiss LSM 510 Pascal

inverted confocal microscope with 488 lasers (Carl Zeiss). Isothermal titration calorimetry was performed by iTC200 from GE Healthcare.

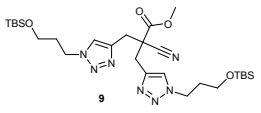
Synthetic Procedures

Synthesis of compound 1、2 and 3



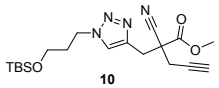
Scheme 1: Reagents and conditions: (a)$CuSO_4.5H_2O$, NaAsc, tBuOH/H_2O. (b) $LiAlH_4$, THF, 0°C, 30min, rt, 2h. (c) Imidazole-1-sulfonyl azide,$CuSO_4.5H_2O$, K_2CO_3, MeOH. (d) 1%HCl/EtOH, rt, 2h;(e) R_2I(**13**), LHDMS, MW, 80°C, 30min; (f) $N_2H_4 \cdot H_2O$, 78°C, overnight; (g) FITC, TEA, DMF, rt 16h. (h) Biotin-OSu, TEA, DMF, rt, 16h.

Synthetic procedures and characterization details:

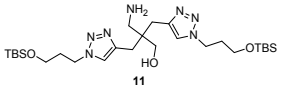


Methyl3-(1-(3-((tert-butyldimethylsilyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)-2-((1-(3-((tert-butyldimethylsilyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-2-cyanopropanoate (9): Compound 7(1.96 g, 11.16 mmol), compound **8** (1.2 g, 5.58 mmol), copper(II) sulfate pentahydrate (0.28 g, 1.12 mmol) and L-ascorbic acid sodium salt (0.44 g, 2.24 mmol)were in terburanol/water (1:1, v/v, 64 mL) and the mixture was stirred for 2h. Then ammonium hydroxide (5 mL) and water (5 mL) were added and the mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with bine. After dried over Na_2SO_4, the solution was concentrated *in vacuo* and purified by flash chromatography (silica gel, 50% EtOAc in petrol ether) to afford the desired product **9** as a white power (0.772 g, 45%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.69 (s, 2H), 4.48 (t, J = 7.0 Hz, 4H), 3.84 (s, 3H), 3.62 (t, J = 5.7 Hz, 4H), 3.48 – 3.33 (m, 4H), 2.15 – 2.07 (m, 4H), 0.90 (s, 18H), 0.05 (s, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 167.82, 140.55, 123.64, 118.54, 59.25, 53.92, 50.18, 47.27, 33.00, 32.05, 25.97, 18.31, -5.34; IR(neat) ν_{max} 2954, 2926, 2855, 1748, 1658, 1465, 1437,1255, 1103, 836 cm^{-1}; HRMS (ESI): $[M+H]^+$ calculated for

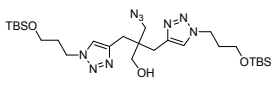
$C_{28}H_{52}N_7O_4Si_2$: 606.3614, found: 606.3602.



2-{1-[3-(tert-Butyl-dimethyl-silanyloxy)-propyl]-1H-[1,2,3]triazol-4-ylmethyl}-2-cyano-pent-4-ynoic acid methyl ester (10): Compound **10** was prepared in the same way that was previously described for **9** in 17% yield**. ^{1}H NMR** (400 MHz, $CDCl_3$) : δ 7.59 (s, 1H), 4.47 (t, *J* = 7.0 Hz, 2H), 3.85 (s, 3H), 3.61 (t, *J* = 5.7 Hz, 2H), 3.42 (s, 2H), 2.94 (dd, *J* = 16.9, 2.6 Hz, 1H), 2.81 (dd, *J* = 16.9, 2.7 Hz, 1H), 2.24 (t, *J* = 2.6 Hz, 1H), 2.13 – 2.06 (m, 2H), 0.90 (s, 9H), 0.05 (s, 6H). **^{13}C NMR** (100 MHz, $CDCl_3$) : δ 166.91, 139.72, 123.17, 117.37, 76.55, 73.29, 58.81, 53.59, 48.80, 46.72, 32.53, 31.74, 25.88, 25.54, 17.84, -5.77 . LCMS (ESI): $[M+H]^+$ calculated for $C_{19}H_{31}N_4O_3Si$, 391.21; found: 391.23.

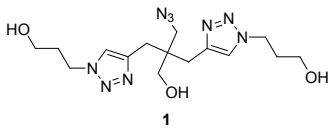


3-amino-2,2-bis((1-(3-((tert-butyldimethylsilyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)propan-1-ol (11): To a stirred suspension of $LiAlH_4$ (357 mg, 9.4 mmol) in dry THF at 0^{o}C under N_2 was slowly added a solution of compound **9** (1.9 g, 3.13 mmol) in THF. The reaction mixture was stirred 0^{o}C for 30 min and allowed to warm to room temperature and stirred for 2h. Next, the mixture was cooled to 0^{o}C and carefully quenched by water. Stirring at room temperature for a further 30 min yielded a turbid solution with a white precipitate, which was then removed by filtration through a Celite pad. The filtrate was extracted with EtOAc (30 mL × 3). The combined organic layers were wash with bine. After dried over anhydrous Na_2SO_4, the solution was concentrated *in vacuo* and purified by Biotage flash chromatography (C_{18}, 50% MeCN in water) to afford the desired product **11** as a colorless oil (638 mg, 35%).^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.61 (s, 2H), 4.43 (t, *J* = 7.0 Hz, 4H), 3.60 (t, *J* = 5.7 Hz, 4H), 3.44 (s, 2H), 3.22 (brs, 4H), 2.70 (d, *J* = 14.4 Hz, 3H), 2.61 (brs, 1H), 2.54 (d, *J* = 14.4 Hz, 2H), 2.13 – 2.04 (m, 4H), 0.87 (s, 18H), 0.02 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.70 (s), 123.80 (s), 68.54 (s), 59.46 (s), 47.17 (s), 33.14 (s), 27.76 (s), 26.01 (s), 18.37 (s), -5.30 (s); IR(neat) ν_{max} 2952, 2927, 2855, 1462, 1252,1099, 1046, 1006, 832, 773, 732, 661 cm^{-1}; HRMS (ESI): $[M+H]^+$ calculated for $C_{27}H_{56}N_7O_3Si_2$: 582.3978, found: 582.3973.



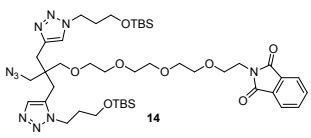
3-azido-2,2-bis((1-(3-((tert-butyldimethylsilyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)propan-1-ol (12):
Imidazole-1-sulfonyl azide (246 mg, 1.32 mmol) was added to a stirred suspension of **11** (638 mg, 1.1 mmol), potassium carbonate (167 mg,1.21 mmol), and copper(II) sulfate pentahydrate (55 mg, 20 mol %) in methanol (140 mL). The mixture was stirred at room temperature for 6h and partitioned with EtOAc and water. After separation, the aqueous layer was extracted with EtOAc (30 mL × 3) and the organic layers were combined , dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to provide a crude product. Further purification by flash chromatography (silica gel, 50% EtOAc in petrol ether) afford the desired product **12** as a colorless oil (320 mg, 49%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.59 (s, 2H), 4.42 (t,

J = 7.0 Hz, 4H), 3.58 (t, *J* = 5.7 Hz, 4H), 3.29 (s, 2H), 3.20 (s, 2H), 2.68 – 2.45 (m, 4H), 2.17 – 1.97 (m, 4H), 0.85 (s, 18H), 0.00 (s, 12H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 142.98, 123.84, 64.17, 59.31, 54.45, 47.09, 43.76, 32.99, 27.39, 25.90, 18.25, -5.42. HRMS (ESI): $[M+H]^+$ calculated for $C_{27}H_{54}N_9O_3Si_2$: 608.3872; found: 608.3874.



1

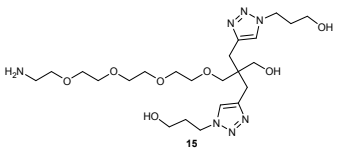
3,3'-(4,4'-(2-(azidomethyl)-2-(hydroxymethyl)propane-1,3-diyl)bis(1H-1,2,3-triazole-4,1-diyl))bis(propan-1-ol) (1): Compound **12** (100 mg) was dissolved in 2mL of 5%HCl/EtOH and the mixture was stirred at room temperature for 2h. After the reaction was completed as judged by LC-MS, the reaction soultion was neutralized with saturated aqueous $NaHCO_3$ and concentrated *in vacuo*. The resulting residue was purified by pre-HPLC to yield the desired product **1** (60 mg, 95%) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.67 (s, 2H), 4.53 (t, *J* = 6.7 Hz, 4H), 3.64 (t, *J* = 5.8 Hz, 4H), 3.33 (s, 2H), 3.26 (s, 2H), 2.58 (s, 4H), 2.19 – 2.10 (m, 4H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 157.18, 143.17, 124.28, 64.29, 58.80, 54.66, 47.09, 43.88, 32.63, 27.45; IR(neat) ν_{max} 3355, 2922, 2851, 1654, 1632, 1467, 1424, 668; HRMS (ESI): $[M+H]^+$calculated for $C_{15}H_{26}N_9O_3$: 380.2142, found: 380.2148.



14

2-(15-azido-14,14-bis((1-(3-((tert-butyldimethylsilyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-3,6,9,12-tetraoxapentadecyl)isoindoline-1,3-dione(14):

A mixture of compound **12** (100 mg, 0.16 mmol) and 2-(2-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)ethyl)iso-indoline-1,3-dione **13** (284 mg, 0.64 mmol) were placed in Biotage microwave tube and sealed, and 4mLof dry MeCN was added and stirring for 5 min, and then LHDMS (1M in hexane, 0.64 mmol, 0.64 mL) was added. The vial was carried out in the microwave reactor by microwave irradiation at 80^{o}C for 30min. After repeat the procedure four times, the reaction soultion was combined and quenched with water and extracted with EtOAc (10mL × 3). The organic layers were concentrated and purified by Biotage flash chromatography (C_{18}, 70%-100% MeCN in water) to yield the desired product **14**(237mg, 42%) as a colorless oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.82 (dd, J = 5.4, 3.1 Hz, 2H), 7.69 (dd, J = 5.5, 3.0 Hz, 4H), 4.43 (t, J = 7.1 Hz, 4H), 3.86 (t, J = 5.9 Hz, 2H), 3.69 (t, J = 5.9 Hz, 2H), 3.65 – 3.52 (m, 16H), 3.27 (s, 2H), 3.17 (s, 2H), 2.66 (s, 4H), 2.09 (dt, J = 12.6, 6.2 Hz, 4H), 0.88 (s, 18H), 0.03 (s, 12H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 168.24, 143.13, 133.99, 132.18, 124.13, 123.26, 72.56, 70.71, 70.68, 70.53, 70.49, 70.40, 70.14, 67.95, 59.53, 54.94, 47.05, 42.86, 37.28, 33.16, 27.87, 25.96, 18.31, -5.34; HRMS (ESI): $[M+H]^+$ calculated for $C_{43}H_{73}N_{10}O_8Si_2$: 913.5146, found: 913.5149.

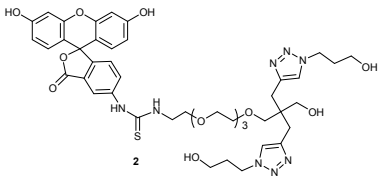


15

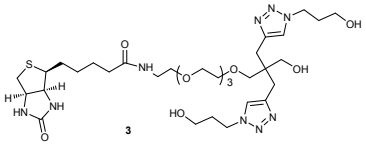
3,3'-(4,4'-(2-(13-amino-2,5,8,11-tetraoxatridecyl)-2-(azidomethyl)propane-1,3-diyl)bis(1H-1,2,3-triazole-4,1-diyl))bis(propan-1-ol) (15): To a solution of compound **14**(237 mg) in EtOH (9 ml) was added 85%

hydrazine hydrate (1.5 mL). The mixture was stirred at 78°C for overnight, and then concentrated *in vacuo*. 10 mL of EtOAc was added to the residue and the resulting white insoluble solid was filtered. The filtrate was concentrated by reduced pressure to give a curde amide compound (300 mg) as a colorless oil, which was used for next step without further purification.

The above obtained compound was dissolved in 1%HCl in EtOH(4mL) and stirred for 2h. Upon completion of the reaction, the mixture was neutralized with sat.$NaHCO_3$ solution and concentrated *in vacuo* and purified by Biotage flashchromatography (C_{18}, 10%-100% MeCN in water) to afford the desired product **15** as a colorless oil (88 mg, 61% in two steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.76 (s, 2H), 4.50 (t, *J* = 6.6 Hz, 4H), 3.87 – 3.53 (m, 20H), 3.28 (s,2H), 3.18 (s,2H), 2.61 (m,4H), 2.15 – 2.07 (m, 4H); **^{13}C NMR** (100 MHz, CD_3OD) δ 144.03, 125.87, 73.61, 71.73, 71.39, 71.32, 71.06, 67.76, 59.32, 55.97, 48.21, 43.48, 40.53, 33.98, 29.34; LCMS (ESI): $[M+H]^+$ calculated for $C_{23}H_{43}N_{10}O_6$: 555.33, found: 555.35.



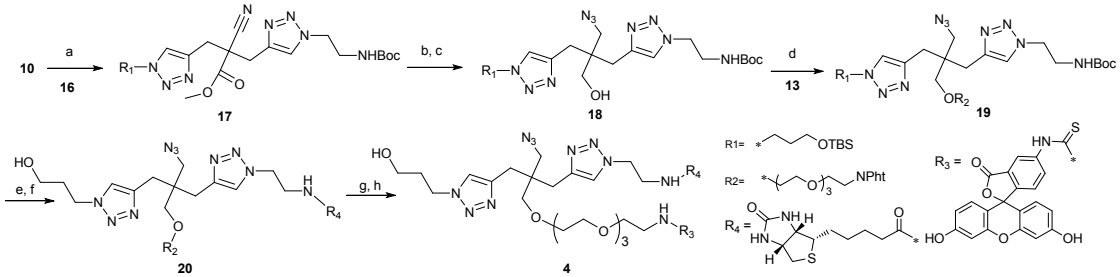
1-(15-azido-14,14-bis((1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl)methyl)-3,6,9,12-tetraoxapentadecyl)-3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thiourea (2): A mixture of amide **15** (68 mg), fluorescein isothiocyanate(47.74 mg) and trithylamine (0.03 mL) in dry DMF(1 mL) was stirred at room temperature for overnight. The mixture was concentrated by reduced pressure and purified by Biotage flash chromatography (C_{18}, 20%-100% MeCN in water) to afford the desired product **2**(64 mg, 55%) as a yellow solid. ^{1}H NMR (400 MHz, $CDOD_3$) δ 8.09 (s, 1H), 7.90 (s, 2H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 2H), 6.64 (d, *J* = 1.9 Hz, 2H), 6.56 (d, *J* = 8.9 Hz, 2H), 4.46 (t, *J* = 7.0 Hz, 4H), 3.85 – 3.69 (m, 2H), 3.71 – 3.59 (m, 11H), 3.53 (t, *J* = 6.0 Hz, 6H), 3.23 (s, 2H), 3.10 (s, 2H), 2.69 (s, 4H), 2.13 – 2.02 (m, 4H). ^{13}C NMR (100 MHz, DMSO) δ 180.91, 169.09, 161.23, 152.78, 142.43, 141.66, 129.63, 129.08, 124.93, 124.54, 117.42, 113.74, 110.49, 102.71, 72.42, 70.55, 70.23, 70.17, 70.07, 69.95, 68.82, 59.72, 57.89, 54.80, 46.90, 44.04, 42.42, 33.43, 28.32, 26.24.HRMS (ESI): $[M+H]^+$ calculated for $C_{44}H_{54}N_{11}O_{11}S$: 944.3719, found: 944.3715.



N-(15-azido-14,14-bis((1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl)methyl)-3,6,9,12-tetraoxapentadecyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (3): A mixture of **15** (54 mg), Biotin-OSu(50 mg) and trithylamine(0.026 mL) in dry DMF(1 mL) was stirred at room temperature for overnight. The mixture was concentrated by reduced pressure and purified by RP-HPLC(solvent A: H_2O; solvent B: acetonitrile; method: 20% B to 100% B over 20 min) . HPLC fractions containing the product were combined and lyophilized to provide the desired product **3** as a colorless oil (64 mg, 84%). **^{1}H NMR** (400 MHz,

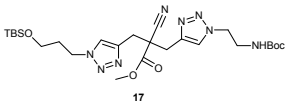
$CDOD_3$) δ 7.93 (s, 2H), 4.45-4.50 (m, 5H), 4.28 (dd, *J* = 7.9, 4.4 Hz, 1H), 3.66 (s, 4H), 3.65 – 3.60 (m, 4H), 3.57 – 3.52 (m, 7H), 3.47 (t, *J* = 5.5 Hz, 2H), 3.32 (d, *J* = 6.4 Hz, 2H), 3.27 (s, 2H), 3.21 – 3.14 (m, 1H), 3.13 (s, 2H), 2.90 (dd, J = 12.7, 5.0 Hz, 1H), 2.72 (s, 4H), 2.67 (d, *J* = 12.7 Hz, 1H), 2.18 (t, *J* = 7.4 Hz, 2H), 2.13 – 2.04 (m, 4H), 1.77 – 1.49 (m, 5H), 1.40 (m, 2H); **^{13}C NMR** (100 MHz, $CDOD_3$) δ 174.64 , 164.61, 142.75, 124.55, 72.26 , 70.28, 70.18, 70.07, 70.03, 69.83, 69.13, 61.92, 60.18, 57.94, 55.59, 54.66, 42.04, 39.65, 38.86 , 35.29, 32.64, 28.35, 28.13, 28.07, 25.43; IR (neat) ν_{max} 3358, 2921, 2851, 2101, 1694, 1659, 1457, 1092, 731; HRMS (ESI): $[M+H]^+$ calculated for $C_{33}H_{57}N_{12}O_8S$: 781.4138, found: 781.4139.

Synthesis of compound 4

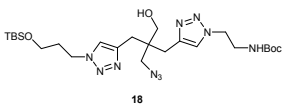


Scheme 2: Reagents and conditions: (a) 2-((tert-butyldime-thylsilyl)oxy)ethanamine(**16**), $CuSO_4.5H_2O$, NaAsc, tBuOH/H_2O; (b)$LiAlH_4$, THF, 0°C, 30min, rt, 2h; (c) Imidazole-1-sulfonyl azide,$CuSO_4.5H_2O$, K_2CO_3, MeOH; (d) R_2I(**13**), LHDMS, MW, 80°C, 30min; (e) TFA, DCM, rt, 4h; (f) Biotin-OSu, TEA, DMF, rt, 16h; (g) $N_2H_4 \cdot H_2O$, 78°C, overnight; (h) FITC, TEA, DMF, rt 16h.

Synthetic procedures and characterization details:



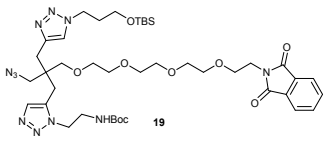
Methyl3-(1-(2-((tert-butoxycarbonyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)-2-((1-(3-((tert-butyldimethylsilyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-2-cyanopropanoate(15): Compound **10**(1.355 g, 3.46 mmol), tert-butyl (2-azidoethyl)carbamate **16**(0.773 g, 4.16 mmol), copper(II) sulfate pentahydrate (0.43 g, 1.73 mmol) and L-ascorbic acid sodium salt (0.69 g, 3.46 mmol) were in terburanol/water(1:1, v/v, 40 mL) and the mixture was stirred for 2h. Then ammonium hydroxide (5 mL) and water (5 mL) were added and the mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with bine. After dried over Na_2SO_4, the solution was concentrated *in vacuo* and the residue was purified by flash chromatography (silica gel, 30% EtOAc in petrol ether) to afford the desired product **17** as a white power (1.14 g, 57%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.49 (s, 1H), 5.50 (s, 2H), 4.33 –4.21 (m, 4H), 3.54 (s, 3H), 3.44–3.32 (m, 4H), 3.17 (dd, *J* = 35.0, 14.8 Hz, 4H), 1.94–1.82 (m, 2H), 1.17 (s, 9H), 0.67 (s, 9H), -0.18 (s, 6H).**^{13}C NMR** (100 MHz, $CDCl_3$) δ 167.51, 155.50, 140.12,123.69, 123.19 , 117.87 , 78.86 , 77.48 , 77.16 , 76.84 , 58.74 , 53.26 , 49.79 , 49.43 , 46.62 , 40.08 , 32.46 , 31.76 , 27.86 , 25.44 , 17.73 , -5.87. LCMS (ESI): $[M+H]^+$ calculated for $C_{26}H_{45}N_8O_5Si$: 577.33; found: 577.34.



tert-butyl(2-(4-(3-azido-2-((1-(3-((tert-

butyldimethylsilyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(hydroxymethyl)propyl)-1H-1,2,3-triazol-1-yl)ethyl)carbamate(18): To a stirred suspension of $LiAlH_4$ (225.72 mg, 5.94 mmol) in dry THF(12 mL) at 0°C under N_2 was slowly added a solution of the compound **17** (1.14 g, 1.98 mmol) in THF(6 mL). The reaction mixture was stirred 0°C for 30 min and allowed to warm to room temperature and stirred 2h. Next, the mixture was cooled to 0°C and carefully quenched by water**.** Stirring at room temperature for a further 30 min yielded a colorless solution with a white precipitate, which was removed by filtration through a Celite pad. The filtrate was extracted with EtOAc (30 mL × 3). The combined organic layers were wash with bine, dried over Na_2SO_4, filtered and evaporated in vacuo to give the key intermediate as a pale yellow oil (1.11 g), which was used for next step without purification.

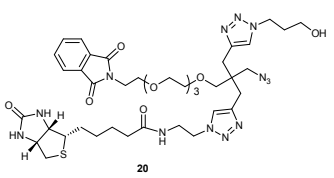
To a suspension of the above obtained intermediate (1.11 g), potassium carbonate (0.33 g, 2.41 mmol) and copper(II) sulfate pentahydrate (0.10 g, 0.40 mmol) in methanol (50 mL) was added imidazole-1-sulfonyl azide (0.382 g, 2.21 mmol). The mixture was stirred at room temperature for 6 h and concentrated in vacuo, and the residue was partitioned with EtOAc and water. After separation, the aqueous layer was extracted with EtOAc (30 mL × 3) and the combined organic layers were washed with ammonia and brine . After dried over Na_2SO_4, the solution was concentrated and the residue was purified by flash chromatography (silica gel, 50% EtOAc in petrol ether) to afford the desired product **18** as a colorless oil (0.30 g, 26% in two steps). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 12.4 Hz, 1H), 4.45 (t, *J* = 6.3 Hz, 4H), 3.68 – 3.56 (m, 4H), 3.28 (d, *J* = 30.2 Hz, 4H), 2.64 – 2.51 (m, 4H), 2.16 – 2.04 (m, 2H), 1.40 (s, 9H), 0.90 – 0.84 (m, 9H), 0.05 – 0.01 (m, 5H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 155.76, 142.92, 142.73, 124.17,123.90, 79.37, 77.48, 77.16, 76.84, 63.66, 59.08, 54.11, 49.75, 46.85, 43.37, 40.38, 32.75, 28.12, 27.26, 25.68, 18.95, 18.01, -5.62 . LCMS (ESI): $[M+H]^+$ calculated for $C_{25}H_{47}N_{10}O_4Si$: 579.35; found, 579.36.



19

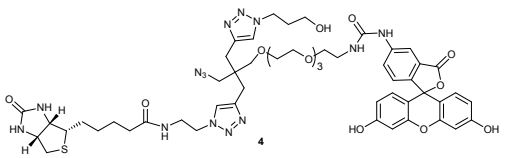
Tert-butyl(2-(4-(14-(azidomethyl)-14-((1-(3-((tert-butyldimethylsilyl)oxy)propyl)-1H-1,2,3-triazol-4-yl)methyl)-1-(1,3-dioxoisoindolin-2-yl)-3,6,9,12-tetraoxapentadecan-15-yl)-1H-1,2,3-triazol-1-yl)ethyl)carbamate(19): A mixture of compound **18** (100 mg, 0.17 mmol) and compound **13** (299 mg, 0.69 mmol) were placed in Biotage microwave tube and sealed, and 4mL of dry MeCN was added with stirring for 5 min, and then LHDMS (1M in hexane, 0.64 mmol, 0.69 mL) was added. The vial was carried out in the microwave reactor by microwave irradiation at 80°C for 30min. After repeat the procedure three times, the combined reaction was quenched with water and extracted with EtOAc (10 mL x 3). The organic layers were concentrated and purified by Biotage flash chromatography (C_{18}, 70%-100% MeCN in water) to yield the desired product **19**(60 mg, 40%) as a colorless oil. **^{1}H NMR** (400 MHz,$CDCl_3$) δ 7.83 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.79 (s, 1H), 7.71 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.68 (s, 1H), 4.44 (t, *J* = 7.0 Hz, 4H), 3.86 (t, *J* = 5.8 Hz, 2H), 3.70 (d, *J* = 5.7 Hz, 2H), 3.66 – 3.53 (m, 16H), 3.29 (s, 2H), 3.15 (s, 2H), 2.67 (s, 4H), 2.10 (m, 2H), 1.41 (s, 9H), 0.89 (s, 9H), 0.04

(s, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 168.35, 143.47, 143.11, 134.09, 132.21, 124.87, 124.17, 123.36, 72.54, 70.72, 70.49, 70.15, 68.01, 59.57, 54.92, 50.04, 47.13, 42.93, 40.55, 37.30, 33.21, 28.47, 27.92, 26.02, 18.39, -5.27. HRMS (ESI): $[M+H]^+$ calculated for $C_{41}H_{66}N_{11}O_9Si$: 884.4809; found: 884.4785.



20

N-(2-(4-(14-(azidomethyl)-1-(1,3-dioxoisoindolin-2-yl)-14-((1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl)methyl)-3,6,9,12-tetraoxapentadecan-15-yl)-1H-1,2,3-triazol-1-yl)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide(20): A mixture of compound **19**(60 mg)、TFA(0.6 mL) and DCM(6 mL) were stirred for 4h. The mixture was neutralized with sat. $NaHCO_3$ solution and the aqueous layer was extracted with EtOAc (10 mL × 3). The combined organic layers were wash with bine, dried over Na_2SO_4, filtered and evaporated *in vacuo* to afford the crude product(56 mg), which was used in the next step without further purification. The above obtained compound (56 mg) and Biotin-OSu (34.8 mg) were dissolved in DMF(1 mL)and 28 uL of trithylamine was added. The mixture was stirred for overnight and avaporated *in vacuo* and the residue was purified by pre-HPLC to give the product **20** (31 mg, 57% in two steps). **^{1}H NMR** (400 MHz, CD_3OD) δ 8.02 (s, 1H), 7.98 (s, 1H), 7.80 (ddd, *J* = 20.1, 5.9, 3.4 Hz, 4H), 4.58 – 4.44 (m, 4H), 4.39 (t, *J* = 6.2 Hz, 1H), 4.28 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.80 (t, *J* = 5.7 Hz, 2H), 3.69 – 3.50 (m, 14H), 3.27 (s, 1H), 3.13 (s, 2H), 2.89 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.72 (s, 3H), 2.67 (d, *J* = 12.7 Hz, 1H), 2.38 (dd, *J* = 12.9, 6.5 Hz, 1H), 2.17 – 2.05 (m, 3H), 1.73 – 1.62 (m, 1H), 1.55 (qd, *J* = 13.5, 6.4 Hz, 3H), 1.42 – 1.33 (m, 2H); **^{13}C NMR** (100 MHz, CD_3OD) δ 176.29, 169.69, 143.97, 135.40, 133.36, 126.45, 124.14, 73.52, 71.60, 71.52, 71.38, 71.21, 63.29, 61.64, 56.91, 55.92, 50.87, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 43.57 , 38.36, 36.65, 33.94, 29.83, 29.67, 29.42, 29.32, 26.70. LCMS (ESI): $[M+H]^+$ calculated for $C_{36}H_{50}N_{13}O_7S$: 808.37; found, 808.38.

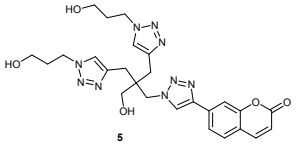


4

N-(2-(4-(2-(azidomethyl)-4-(2-(2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)ureido)ethoxy)ethoxy)-2-((1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl)methyl)butyl)-1H-1,2,3-triazol-1-yl)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide(5): To a solution of compound **20**(31 mg) in EtOH (6 mL) was added 85% hydrazine hydrate (0.5 mL). The mixture was stirred at 78^{o}C for overnight, and then concentrated *in vacuo.* 5 mL of EtOAc was added to the residue and the resulting white solid was filtered. The filtrate was concentrated *in vacuo* to give a pale yellow oil (28 mg), which was used in the next step without further purification. The above obtained product(28 mg) and FITC(12.9 mg) were dissolved in DMF(1 mL) and 14 uLof trithylamine was added. The mixture was stirred for overnight and avaporated in vacuo and the residue was purified by pre-HPLC to give the desired product **5** as a reddish yellow solid(12 mg, 27% in two steps

). **^{1}H NMR** (400 MHz, CD_3OD) δ 8.14 (s, 1H), 8.05-7.99 (m, 2H), 7.75 (d, *J* = 7.9 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 1H), 6.73 – 6.60 (m, 3H), 6.52 (dd, *J* = 8.7, 2.3 Hz, 2H), 4.51-4.48 (m, 2H), 4.46-4.43 (m, 1H), 4.29 – 4.24 (m, 1H), 3.82 – 3.58 (m, 9H), 3.54 (t, *J* = 5.9 Hz, 2H), 3.24(s, 1H), 3.16-3.10 (m, 2H), 2.92 – 2.83 (m, 2H), 2.71 – 2.58 (m, 3H), 2.21 – 2.00 (m, 3H), 1.69 – 1.42 (m, 4H), 1.42 – 1.19 (m, 6H), 1.00-0.94 (m, 1H), 0.88 (t, *J* = 6.8 Hz, 1H). HRMS (ESI): $[M+H]^+$ calculated for $C_{53}H_{67}N_{14}O_{12}S_2$:1155.4499; found: 1155.4474.

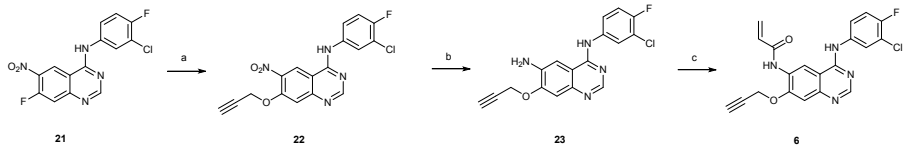
Synthesis of compound 5



5

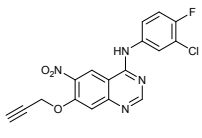
7-(1-(3-hydroxy-2,2-bis((1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl)methyl)propyl)-1H-1,2,3-triazol-4-yl)-2H-chromen-2-one(5): Compound **1** (40 mg), 7-ethynyl-2H-chromen-2-one(17.7 mg), sodium asorbate(208 mg) and copper(II) sulfate pentahydrate (39 mg) and were dissolved in a mixture of terbutanol/water(1:1, v/v, 4 mL). The mixture was stirred at room temperature for overnight. The mixture was diluted with ethyl acetate(20 mL) and ammonium hydroxide(5 mL). The aqueous extracts were extracted with ethyl acetate (20 mL × 3) and the combined organic extracts were wash with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give a yellow solid . The residue was purified by pre-HPLC to yield the desired product **5** as a yellow solid(45 mg, 78%). **^{1}H NMR** (400 MHz, DMSO) δ 8.84 (s, 1H), 8.09 (d, J = 9.6 Hz, 1H), 8.03 (s, 2H), 7.91–7.83 (m, 2H), 7.80 (d, J = 7.9 Hz, 1H), 6.49 (d, J = 9.6 Hz, 1H), 4.41 (m, 6H), 3.39 (t, J = 6.1 Hz, 4H), 3.12 (s, 2H), 2.62 (s, 4H), 1.96 (m, 4H). **^{13}C NMR** (100 MHz, DMSO) δ 160.41, 154.53, 145.01, 144.42, 142.48, 134.64, 129.60, 125.05, 124.81, 121.66, 118.55, 116.33, 112.64, 63.23, 57.87, 53.35, 46.99, 42.98, 33.42, 28.27; IR (neat) ν_{max} 3328, 2924, 2930, 1715, 1619, 1020, 939; HRMS (ESI): $[M+H]^+$ calculated for $C_{26}H_{32}N_9O_5$: 550.2521, found: 550.2519.

Synthesis of compound 6



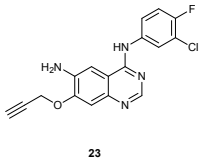
21 22 23 6

Scheme 3. Reagents and conditions: (a) $SOCl_2$, DMF, reflux, overnight, 92%; (b) 3-chloro-4-fluoroaniline, *t*-BuOH, THF, 65 °C, overnight, 94%; (c) Propargyl alcohol, *t*-BuOK, THF, r.t, 5h, 92%; (d) Fe, NH_4Cl, EtOH, H_2O, 80 °C, 4h, 98%; (e) Acrylyl chloride, Et_3N, DMF, 0 °C, 1h, 77%.

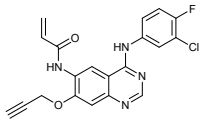

22

***N*-(3-chloro-4-fluorophenyl)-6-nitro-7-(prop-2-yn-1-yloxy)quinazolin-4-amine(22):** To a solution of **21** (700 mg, 2.1 mmol) in THF (10 mL), propargyl alcohol (236 mg, 4.2 mmol) was added, the reaction mixture was stirred at r.t for 5 min, then potassium tert-butanolate (236 mg, 2.1 mmol) was added under 0 °C. After stirring at room temperature for 5 h, water (100 mL) was added and the mixture was extracted with ethyl acetate (3×100 mL). The combined organic fractions were dried (Na_2SO_4)

and concentrated in vacuo to give **22**(711mg, 92%) as a yellow solid which was used in the next step without further purification. **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 3.77 (t, J =2.4 Hz, 1H), 5.18 (d, J =2.4 Hz, 2H), 7.43-7.48 (m, 1H), 7.54 (s, 1H), 7.74-7.78 (m, 1H), 8.13 (dd, J =2.8Hz, 6.8 Hz, 1H), 8.65 (s, 1H), 9.23 (s, 1H), 10.29 (bar, 1H). LC-MS (ESI) *m/z* : calcd. for $C_{17}H_{10}ClFN_4O_3$ $[M+H]^+$: 373.04, found: 372.81.

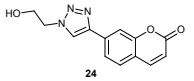

23

***N*^{4}-(3-chloro-4-fluorophenyl)-7-(prop-2-yn-1-yloxy)quinazoline-4,6-diamine(23):**A suspension of **22** (500 mg, 1.3 mmol) , iron powder (376 mg, 6.5 mmol) and NH_4Cl(215 mg, 3.9 mmol) in EtOH(30 mL)/H_2O(1 mL) was stirred at 80 ^{o}C for 4 h. The precipitate (iron powder) was separated by filtration and the filtrate was concentrated under reduced pressure. Purification of the crude product by column chromatography (petroleum ether : EtOAc =1 : 1) to give **23** (450 mg, 98%) as a yellow solid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 3.69 (t, J =2.4 Hz, 1H), 5.03 (d, J =2.4 Hz, 2H), 5.38 (s, 2H), 7.21 (s, 1H), 7.37-7.41 (m, 2H), 7.78-7.82 (m, 1H), 8.18 (dd, J =2.4Hz, 6.8 Hz, 1H), 8.38 (s, 1H), 9.44 (s, 1H). LC-MS (ESI) *m/z* : calcd. for $C_{17}H_{12}ClFN_4O$ $[M+H]^+$: 343.07, found: 343.29.


6

***N*-(4-((3-chloro-4-fluorophenyl)amino)-7-(prop-2-yn-1-yloxy)quinazolin-6-yl)acrylamide(6)**: To a solution of **23** (165 mg, 0.48 mmol) , Et_3N (97 mg, 0.96 mmol) in anhydrous DMF (2 mL), acrylyl chloride (43 mg, 0.48 mmol) was added under ice-bath, the reaction mixture was stirred at 0 ^{o}C for 1 h. water (10 mL) was added and the mixture was extracted with ethyl acetate (3×10 mL), the combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo. Purification of the crude product by column chromatography (petroleum ether : EtOAc =1 : 1) to give **6** (136 mg, 77%) as a pale-yellow solid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 3.81 (t, J =2.4 Hz, 1H), 5.17 (d, J =2.4 Hz, 2H), 5.85 (dd, J =2.0Hz, 10.0 Hz, 1H), 6.34 (dd, J =2.0Hz, 16.8 Hz, 1H), 6.77 (dd, J =10.0Hz, 16.8 Hz, 1H), 7.45 (s, 1H), 7.51 (t, J =9.2 Hz, 1H), 7.69-7.73 (m, 1H), 8.03 (dd, J =2.4Hz, 6.8 Hz, 1H), 8.76 (s, 1H), 9.09 (s, 1H), 9.96 (s, 1H). **^{13}C NMR**(100 MHz,DMSO-d_6): δ 56.9 ,78.8 , 79.9, 108.7, 109.7, 117.0 , 117.4 , 119.1, 119.3, 122.9, 124.1, 127.5, 132.1, 137.2, 149.1, 152.4, 153.7, 154.5, 157.3, 164.1. HRMS: calcd. for $C_{20}H_{14}ClFN_4O_2$ $[M+H]^+$: 397.0789, found: 397.0857.

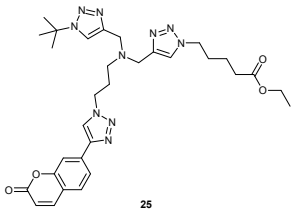
Synthesis of compound 24


24

7-(1-(2-hydroxyethyl)-1H-1,2,3-triazol-4-yl)-2H-chromen-2-one (24): To a mixture of 7-ethynyl-2H-chromen-2-one (100 mg) and 2-azidoethanol (62 mg) in THF (1 mL) was added copper(I) iodide(23 mg) and trimethylamine(0.08 mL). The mixture was stirred and refluxed for overnight. Then ammonium hydroxide (2 mL) was added and the mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with bine. After dried over Na_2SO_4, the solution was concentrated *in vacuo* and the residue was purified by flash chromatography (silica gel, 100% EtOAc) to afford the desired product **24** as a pale yellow power (100 mg, 61%). **^{1}H NMR** (400 MHz, CD_3OD) δ 8.51 (s, 1H), 7.96 (d, J = 9.5 Hz, 1H), 7.86 – 7.78 (m, 2H), 7.69 (d, J = 7.8 Hz, 1H), 6.42 (d, J = 9.5 Hz, 1H), 4.55 (t, J = 5.2 Hz, 2H), 3.98 (t, J = 5.2Hz, 2H); **^{13}C NMR**

(100 MHz, DMSO) δ 160.01, 154.12, 144.78, 143.99, 134.42, 129.18, 123.42, 121.18, 118.15, 115.80, 112.11, 59.79, 52.64; IR(neat) ν_{max} 3441, 3360, 2921, 2581, 1687, 1660, 1615, 1458, 1197, 847; HRMS (ESI): $[M+H]^+$ calculated for $C_{13}H_{12}N_3O_3$: 258.0873, found: 258.0872.

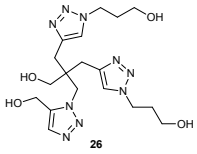
Synthesis of compound 25



Ethyl 5-(4-((((1-(tert-butyl)-1H-1,2,3-triazol-4-yl)methyl)(3-(4-(2-oxo-2H-chromen-7-yl)-1H-1,2,3-triazol-1-yl)propyl)amino)methyl)-1H-1,2,3-triazol-1-yl)pentanoate(25)

The compound was prepared according to the reported literatur[6]. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.83 (dd, J = 8.0, 1.6 Hz, 1H), 7.79 (d, J = 1.5 Hz, 1H), 7.69 (d, J = 9.3 Hz, 1H), 7.65 (s, 2H), 7.52 (d, J = 8.1 Hz, 1H), 6.40 (d, J = 9.5 Hz, 1H), 4.54 (t, J = 6.6 Hz, 2H), 4.34 (d, J = 7.1 Hz, 2H), 4.09 (q, J = 7.1 Hz, 4H), 3.74 (d, J = 5.5 Hz, 4H), 2.53 (t, J = 6.2 Hz, 2H), 2.31 (t, J = 7.3 Hz, 4H), 1.98 – 1.88 (m, 2H), 1.65 (s, 9H), 1.22 (t, J = 7.1 Hz, 3H). LCMS (ESI): $[M+H]^+$ calculated for $C_{31}H_{41}N_{10}O_4$: 617.33, found: 617.35.

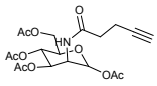
Synthesis of compound 26



3,3'-(4,4'-(2-(hydroxymethyl)-2-((5-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)methyl)propane-1,3-diyl)bis(1H-1,2,3-triazole-4,1-diyl))bis(propan-1-ol)(26): The compound was prepared according to the

method described for **5. ^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.97 (s, 2H), 4.68 (s, 2H), 4.49 (t, *J* = 7.0 Hz, 4H), 4.41 (s, 2H), 3.55 (t, *J* = 6.0 Hz, 4H), 3.22 (s, 2H), 2.67 (q, *J* = 14.7 Hz, 4H), 2.13 – 2.05 (m, 4H). **^{13}C NMR** (100 MHz, DMSO) δ 148.65, 143.98, 126.39, 126.12, 64.45, 59.34, 56.47, 53.93, 44.45, 34.00, 29.09. LCMS (ESI): $[M+H]^+$ calculated for $C_{18}H_{30}N_9O_4$: 436.24, found: 436.26.

Synthesis of (3S,4R,5S,6R)-6-(acetoxymethyl)-3-(pent-4-ynamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate



The compound was prepared according to the reported literature[7]. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.05 (d, J = 1.8 Hz, 1H), 6.02 (s,1H), 6.00 (s, 1H), 5.87 (d, J = 1.8 Hz,0.5H), 5.33 (dd, J = 10.2, 4.4 Hz,1H), 5.22 (t, J = 10.1 Hz, 1H), 5.17 (t, J = 9.8 Hz, 0.5H), 5.04 (dd, J = 9.9, 4.0 Hz, 0.5H), 4.81 (ddd, J = 9.2, 4.0, 1.8 Hz, 1H), 4.67 (ddd, J = 9.3, 4.4, 1.9 Hz, 1H), 4.31 – 4.25 (m, 2H), 4.13 – 4.01 (m, 3H), 3.83 – 3.77 (m, 0.5H), 2.59 – 2.45 (m, 6H), 2.20 (t, J = 2.6 Hz, 1H), 2.18 (s, 3H), 2.11 (s, 1.5H), 2.10 (s, 3H), 2.06 (s, 4.5H), 2.01 (s, 1.5H), 2.01 (s, 3H). LCMS (ESI): $[M+H]^+$ calculated for $C_{19}H_{26}NO_{10}$: 428.16, found: 428.18.

Experimental Protocols

Kinetic experiments and k_{obs} calculation

Reactions were performed with 10 μM **1**, 100 μM 7-ethynylcoumarin (EC), 100 mM sodium ascorbate, and accordingly appropriate equivalences of $CuSO_4$. All reactions are carried out in DMF: H_2O 1:1 solution, except Figure S8 (DMF: H_2O 1:3). $CuSO_4$, **1** and sodium ascorbate were first pre-mixed and incubated for 10min prior to addition of 7-ethynylcoumarin. For control reactions with ligand, 50 μM TBTA/BTTES/THPTA was premixed with 50 μM $CuSO_4$ and sodium ascorbate for 10min, followed by the addition of 50 μM 2-azidoethenal and 500 μM compound 7-ethynylcoumarin. For control reaction with A19, reaction was performed as with **1**. **1**, 7-ethynylcoumarin, 2-azidoethenal, A19, TBTA, BTTES, and THPTA were kept as stocks (4×) in DMF; $CuSO_4$ and sodi-um ascorbate were kept as stocks in H_2O (4×) . The fluorescence of triazole products were measured every 6 seconds during a total time course of 5 minutes. Product concentrations and yields were calculated according to the standard curves presented in Figures S1, S2 and S3. A pseudo-first order reaction model was used for the calculation of k_{obs}.

$$-d[\mathbf{1}]/dt = k_{obs}[EC_0][\mathbf{1}] \quad (1)$$

Integrate:

$$\ln[\mathbf{1}] = \ln[\mathbf{1}_0] - k_{obs}[EC_0]t \quad (2)$$

Points of t and ln[**1**] were plotted and linearly fitted by Graphpad software.
For control experiment, k_{obs} was calculated in the same method.

Isothermal titration calorimetry (ITC)

Titrations were performed at 25℃ following the instruction of ITC200 under indicated concentration and solvent condition as illustrated in Figure S8.

Halide tolerance experiments and k_{obs} calculation

Reactions were performed with 10 μM **1**, 10 μM $CuSO_4$, 100 μM 7-ethynylcoumarin (EC), 100 mM sodium ascorbate, and accordingly appropriate equivalences of NaX (1 eq or 100 eq of NaCl, NaBr or NaI). All reactions are carried out in DMF: H_2O 1:1 solution. $CuSO_4$, **1,** NaX and sodium ascorbate were first pre-mixed and incubated for 10min prior to addition of 7-ethynylcoumarin. **1**, and 7-ethynylcoumarin were kept as stocks (4×) in DMF; $CuSO_4$, NaX and sodium ascorbate were kept as stocks in H_2O (8×) . The fluorescence of triazole products were measured every 6 seconds during a total time course of 5 minutes. Product concentrations and yields were calculated according to the standard curves presented in Figures S1. A pseudo-first order reaction model was used for the calculation of k_{obs}.

Cell viability test

Jurkat and HER2-transformed BA/F3 cells were grown in medium supplemented with 10% fetal bovine serum, L-Glutamine (2 mM), Penicillin (100 units/mL), and Streptomycin (100 μg/mL). Cell lines were maintained in a 5% CO2 humidified

atmosphere at 37°C. 12 h before the experiment, cells were plated in 96 well plates at 3000 cells/well in 100 μL of medium. Cells were incubated with the 20 μM, 50 μM, and 100 μM concentrations of **1**, A19, TBTA, BTTES, THPTA or their complexes with Cu(I) for 4 h. The Cu(I)/AIO reagent complex and the Cu(I)/ligand complex were premixed with equal amounts of the reagents and $CuSO_4$, followed by addition of an excessive amount of sodium ascorbate. CellTiter 96® AQueous One Solution Cell Proliferation Assays (Promega, Madison, WI, USA) were performed to determine cell viability, according to the product instructions. Luminescence was measured using a PE Enspire VICTORTM X plate reader.

In vivo labeling and imaging of live cell membrane glycoproteins

100 μM Ac4ManNAl in a solution of ethanol was added to the culture plates. After the evaporation of solvent, Jurkat cells were seeded at a density of 10^6 cells/mL in 10 mL medium. Cells were cultured for 72 h before use. Alkyne labeled and unlabled Jurkat cells were washed and resuspended in fresh medium and incubated with 100 μM of the **2**/Cu complex and sodium ascorbate for 1h. Cells were diluted with PBS and centrifuged 3 times at 150 rpm for 4 min, stained with 5 ug/mL Hoechst and 2 ug/mL DiI together for 5 min, and then washed again with PBS. Cells were mounted in PBS buffer immediately after the fluorescence staining procedure. Confocal imaging was carried out with a Nikon A1-R con-focal microscope with 405nm, 488nm, and 561 nm lasers. Mean fluorescence intensity was calculated by velocity software.

Glycoprotein Pulldown Assay

Ac_4ManNAl treated or untreated Jurkat cells were lysated, then bifunctional azides assisted CuAAC reaction was performed with 100 μM premixed **3**/$CuSO_4$ or **4**/$CuSO_4$ complex and 10mM sodium L-ascorbate for 1hr at 37°C in 1mL 1mg/mL cell lysate. Lysates were then precipitated using 5 volumes of methanol, washed 3 times with methanol and then redissolved with 1mL PBS buffer containing 0.2% SDS. 100uL strpavidin agrose (Thermo) was added to each sample. Samples were incubated for 2 hr at room temperature, centrifuged at 3000 rpm for 3 min and then washed 3 times with PBS buffer. 30ul 1× loading buffer was used for elution.

Western blot for biotin

Reaction mixture was mixed with 5x loading buffer and directly loaded on SDS gel for electrophoresis. Gels were then transferred to nitrocellulose membranes. NC membranes were blocked with PBS buffer containing 5% nonfat powdered milk and 1% triton for 1 h, incubated with HRP-anti-biotin antibody(1:2000 dilution) for 1hr, washed with 1× PBST buffer 5min 3 times, and developed using WESTERN LIGHTNING™ *Plus*-ECL(PE).

Alkyne labeling and *in vivo* imaging of Her2

HER2/H878Y-transformed BA/F3 cells were treated with 10 μM **6** for 3 h. Cells were then centrifuged at 1500 rpm for 5 min. The supernatant was discarded and cells were washed with PBS buffer and centrifuged 3 more times at 1500 rpm. The cells were then resuspended with medium. After resuspension, compound **6**-treated HER2/H878Y-transformed BA/F3 cells were incubated with 100 μM of the **2**/Cu complex and sodium ascorbate for 2 h, washed with PBS, and centrifuged 3 times at 1500 rpm for 4 min.

Cells were then fixed with 4% formaldehyde, permeabilized with 0.1% Triton X-100, and then immunostained with HER2 Rabbit mAb (1:2000 dilution) and Alexa Fluor® 555 conjugated Goat anti-Rabbit IgG (1:2000 dilution). After immunostaining, cells were stained with 5 μg/mL Hoechst, washed with PBS buffer, and mounted with Antifade Mounting Medium. Confocal imaging was carried out with a Nikon A1-R confocal mi-croscope, with 405nm, 488nm, and 561 nm lasers.

HER2 Pulldown Assay

Compound **6** treated or untreated HER2/H878Y-transformed BA/F3 cells were lysated, then bifunctional azides assisted CuAAC reaction was performed with 100 μM premixed **3**/$CuSO_4$ complex and 10mM sodium L-ascorbate for 1hr at 37^{o}C in 1mL 1mg/ml cell lysate. Lysates were then precipitated using 5 volumes of methanol, washed 3 times with methanol and then redissolved with 1mL PBS buffer containing 0.2% SDS. 100uL strpavidin agrose was added to each sample. Samples were incubated for 2 hr at room temperature, centrifuged at 3000 rpm for 3 min and then washed 3 times with PBS buffer. 30ul 1× loading buffer was used for elution.

Western blot for HER2

Samples were mixed with 5× loading buffer and directly loaded on SDS gel for electrophoresis. Gels were then transferred to nitrocellulose membranes. NC membranes were blocked with PBS buffer containing 5% nonfat powdered milk and 1% triton for 1 h, incubated with anti-HER2 antibody(1:2000 dilution) for 1hr, washed with 1×PBST buffer 5min 3 times, then incubated with secondary antibody(anti-rabbit, 1:4000 dilution) for 30min, washed with 1× PBST buffer 5min 3 times and developed using WESTERN LIGHTNING^{TM} *Plus*-ECL(PE).

Live Imaging of Ac_4GalNAl -Labeled *C. elegans*.

Mixed-stage strain N2 *C. elegans* that had been cultured on NGM plates containing 5 mM, 250 μM or without Ac_4GalNAl were reacted with 100 μM or 50 μM of the **2**/Cu(I) complex for 40 min at 20°C. The AIO reagent complex was treated with pre-mixed **2**/CuCl (1:1) in DMSO, and then diluted with ddH_2O to the working concentration. After 40min, the reaction was quenched by dilution with ddH_2O to a

volume of 200 µL. Worms were washed once by centrifugation and then transferred to new plates. After recovery for 4 h, the imaging experiments were performed using a Zeiss LSM 510 Pascal inverted confocal microscope with a 488 laser (Carl Zeiss). Images were processed and viewed using LSM Image Browser software.

Supporting Figures and Discussion

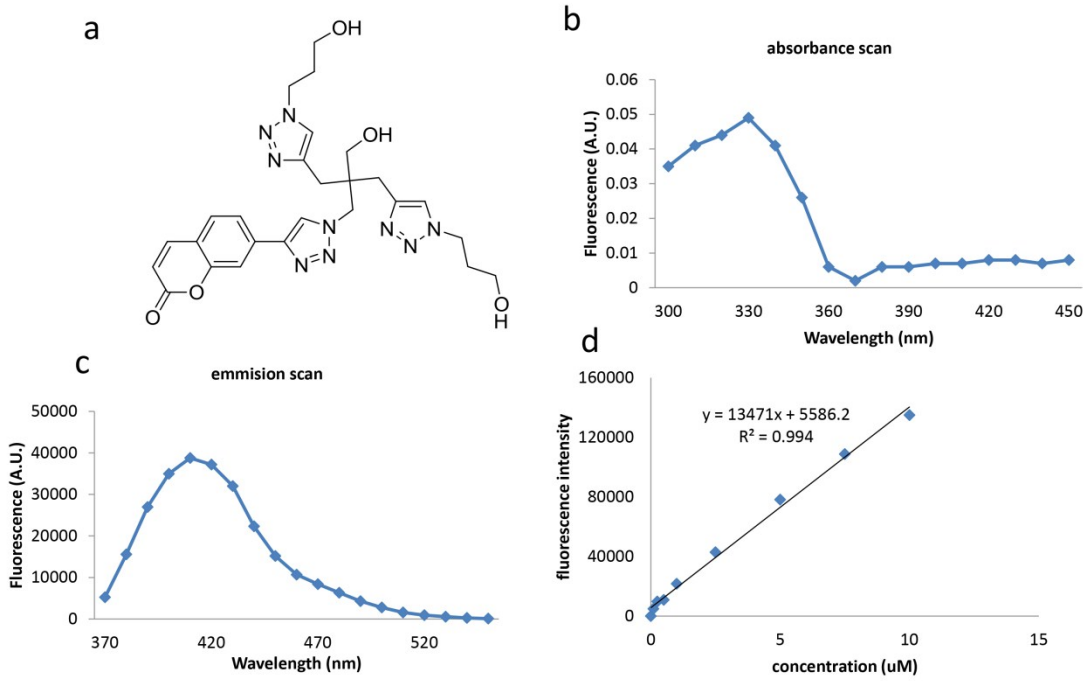


Figure S1. Standard curve for triazole product 5.
(a), structure of compound **5**. (b), absorbance scan of compound **5**. (c), emission scan of compound **5**. (d) standard curve of compound **5**. Absorbance and emission scan is carried out with 10 µM solution in DMF/H_2O(1/1). Points for standard curve are obtained with λ_{ex}= 320 nm and λ_{em}= 400 nm.

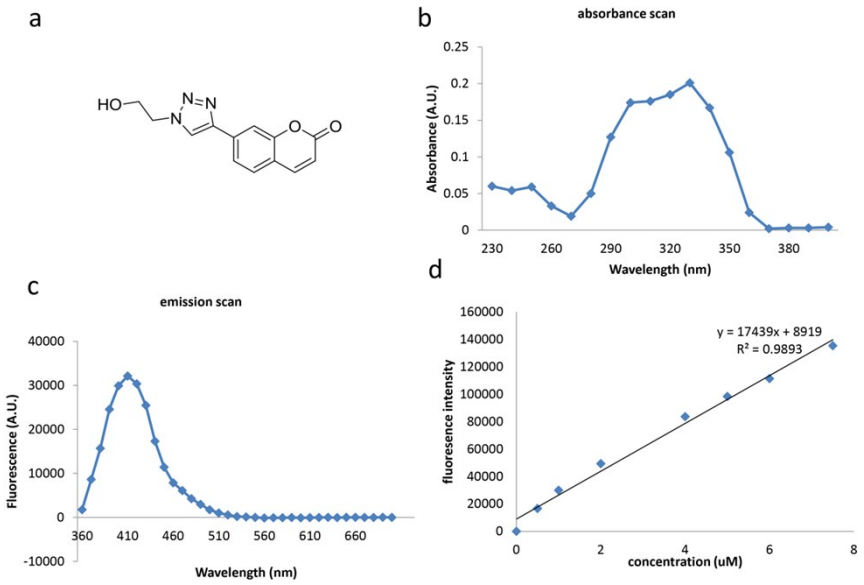


Figure S2. Standard curve of compound 24.

(a), structure of compound **24**. (b), absorbance scan of compound **24**. (c) emission scan of compound **24**. (d) standard curve of compound **24**. Absorbance and emission scan is carried out with 10 μM solution in DMF/H_2O(1/1) . Spots for standard curve are obtained with λ_{ex}= 320 nm and λ_{em}= 400 nm.

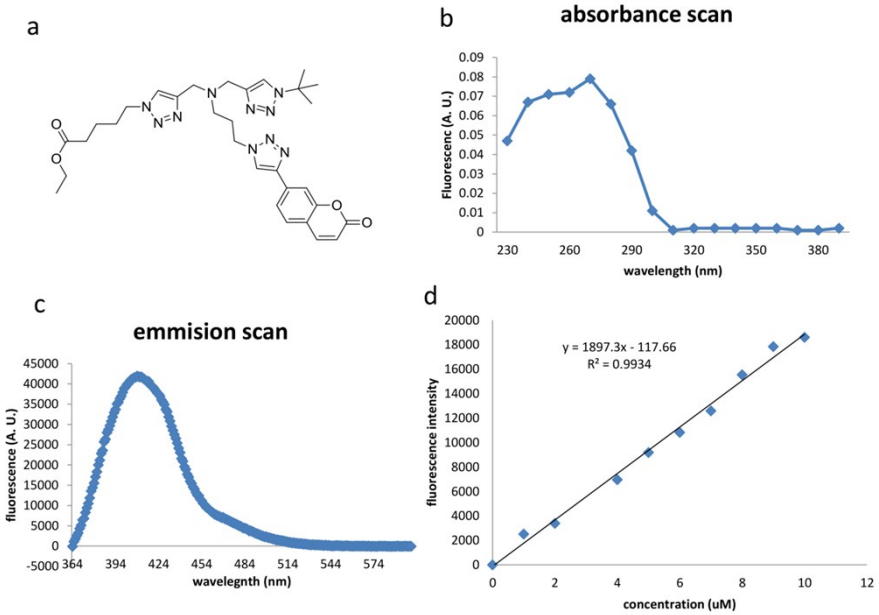
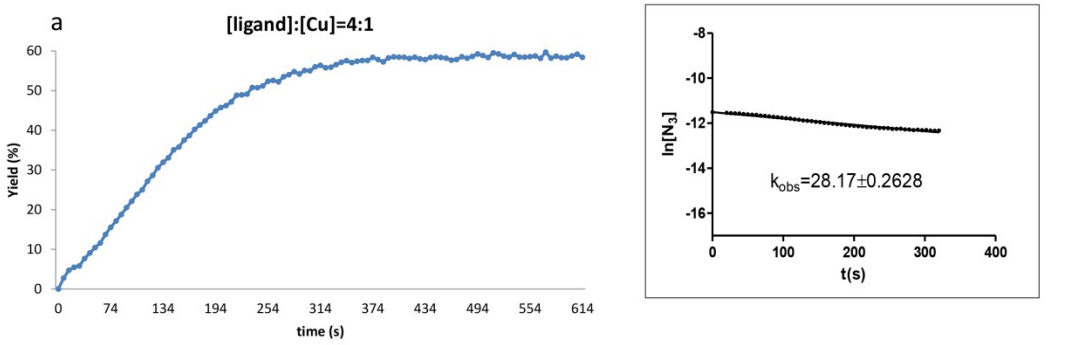


Figure S3. Standard curve of compound 25.

(a), structure of compound **25**. (b), absorbance scan of compound **25**. (c) emission scan of compound **25**. (d) standard curve of compound **25**. Absorbance and emission scan is carried out with 10 μM solution in DMF/H_2O(1/1) . Spots for standard curve are obtained with λ_{ex}= 320 nm and λ_{em}= 400 nm.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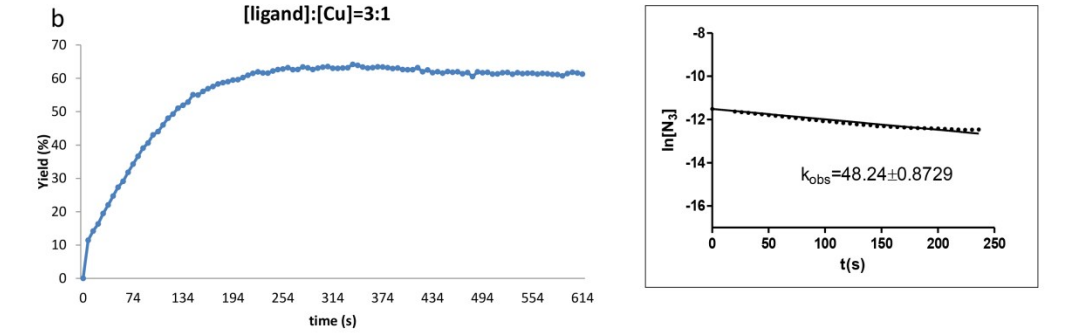
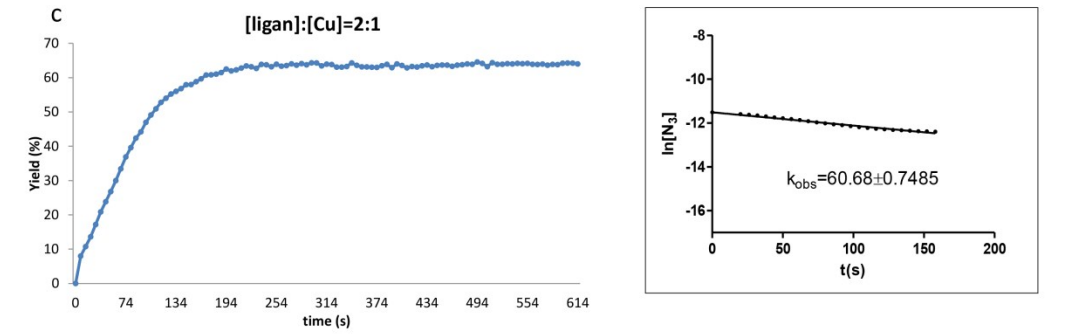
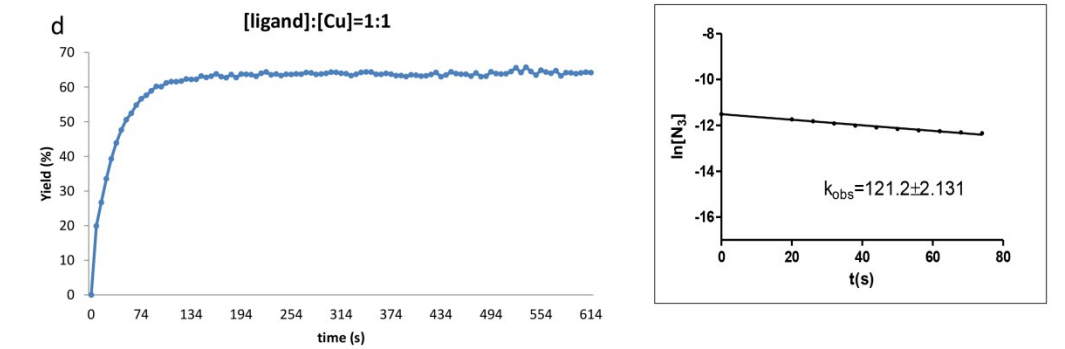
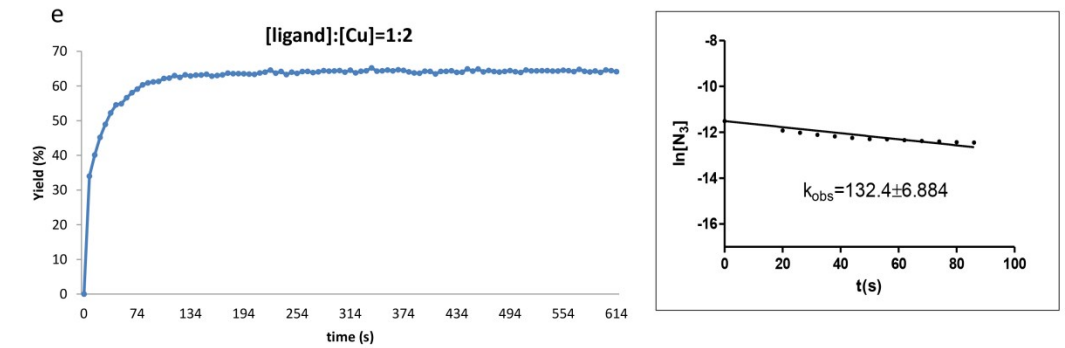
[ligand]:[Cu]=4:1
Yield (%)
time (s)
ln[N3]
kobs=28.17±0.2628
t(s)

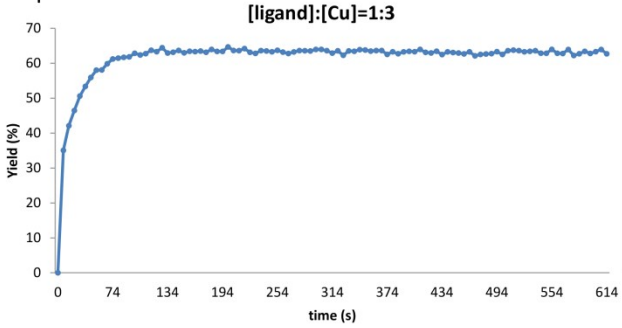

b
[ligand]:[Cu]=3:1
Yield (%)
time (s)
ln[N3]
kobs=48.24±0.8729
t(s)

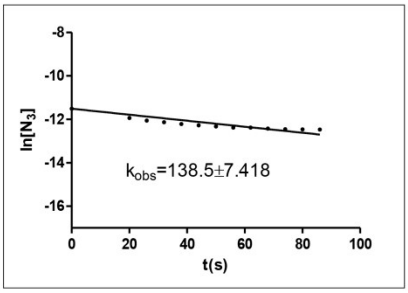

c
[ligan]:[Cu]=2:1
Yield (%)
time (s)
ln[N3]
kobs=60.68±0.7485
t(s)


d
[ligand]:[Cu]=1:1
Yield (%)
time (s)
ln[N3]
kobs=121.2±2.131
t(s)

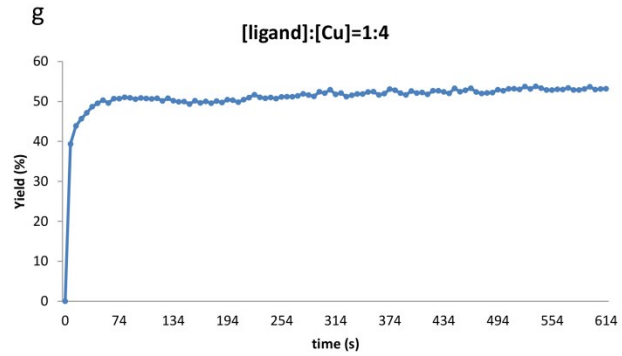

e
[ligand]:[Cu]=1:2
Yield (%)
time (s)
ln[N3]
kobs=132.4±6.884
t(s)

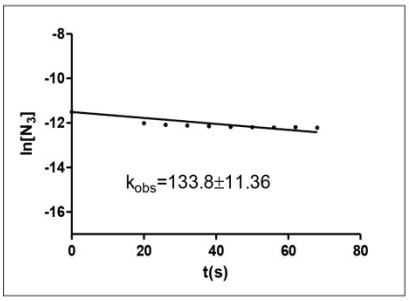
f


[ligand]:[Cu]=1:3
Yield (%)
time (s)

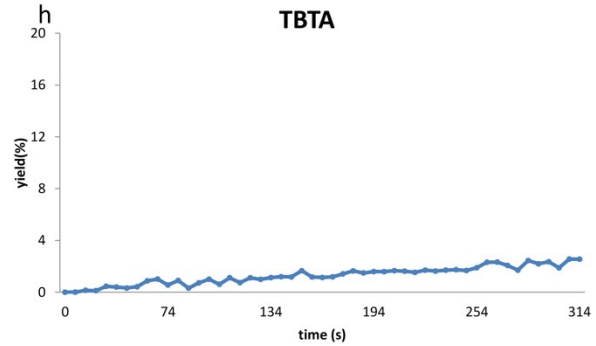

ln[N3]
kobs=138.5±7.418
t(s)

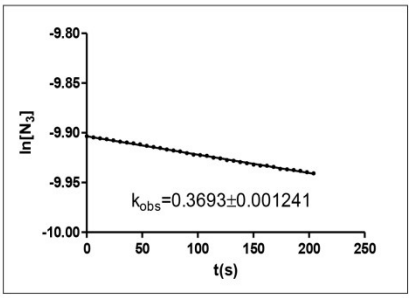
g


[ligand]:[Cu]=1:4
Yield (%)
time (s)

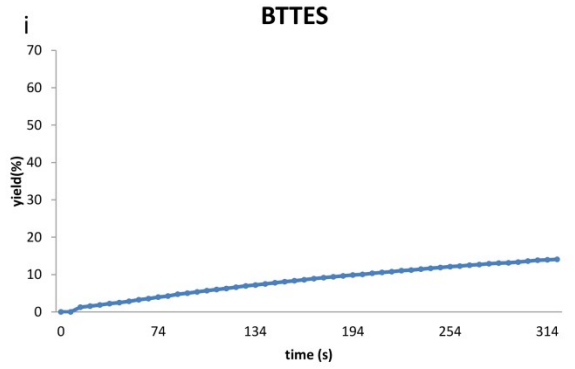

ln[N3]
kobs=133.8±11.36
t(s)

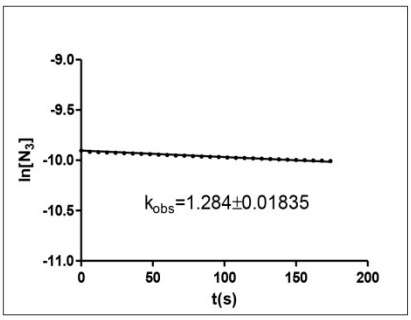
h


TBTA
yield(%)
time (s)

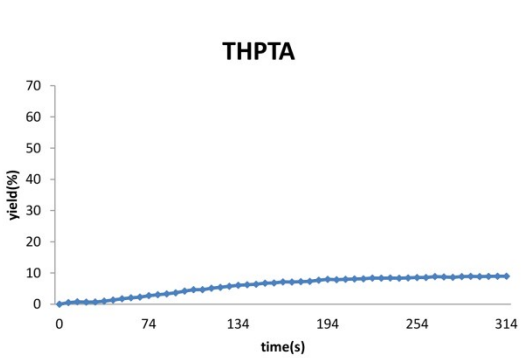

ln[N3]
kobs=0.3693±0.001241
t(s)

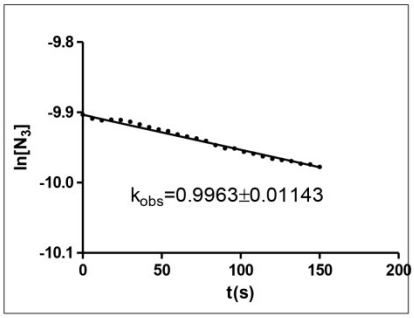
i


BTTES
yield(%)
time (s)


ln[N3]
kobs=1.284±0.01835
t(s)

j


THPTA
yield(%)
time(s)


ln[N3]
kobs=0.9963±0.01143
t(s)

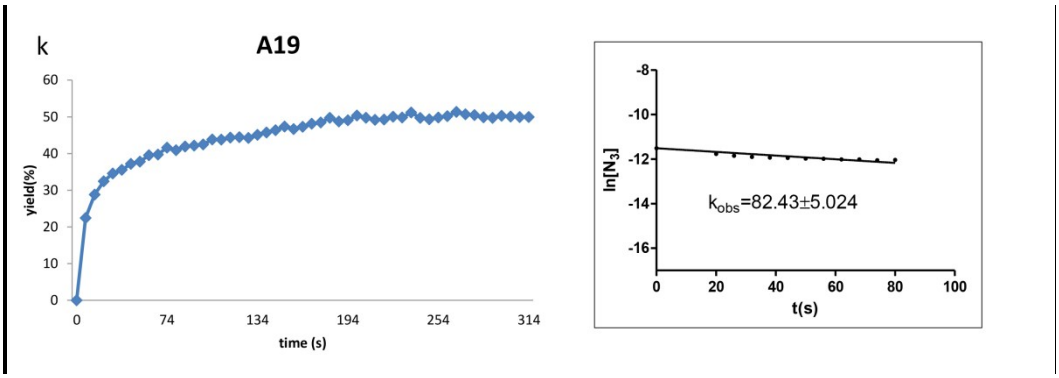


Figure S4. Time course and kinetics of model reaction.

(a)~(g), time course and k_{obs} calculation of the model reaction at different $CuSO_4$ varients.(left: time course of the reaction; right: k_{obs} calculation using pseudo-first order reaction model. Reactions were performed with 10 μM **1**, 100 μM 7-ethynylcoumarin, 100 mM sodium ascorbate and different equivalence of $CuSO_4$ accordingly.) (h)-(j), time course and k_{obs} calculation of the control model reaction using TBTA/BTTES/THPTA as ligand. (left: time course of the reaction; right: k_{obs} calculation. Reactions were performed with 50 μM 2-azidoethenal, 50 μM $CuSO_4$, 500 μM 7-ethynylcoumarin, 100 mM sodium ascorbate.) (k), time course and k_{obs} calculation of the control model reaction using A19. (left: time course of the reaction; right: k_{obs} calculation. Reactions were performed with 10 μM A19, 10 μM $CuSO_4$, 100 μM 7-ethynylcoumarin, 100 mM sodium ascorbate and different equivalence of $CuSO_4$ accordingly.) All reactions are carried out in DMF: H_2O 1:1 solution.

Table S1. Kinetics of model reactions

entry	[ligand]:[Cu]	$k_{obs}(M^{-1}\cdot s^{-1})$	Cu order
1	4:1	28.17	1
2	3:1	48.24	1
3	2:1	60.68	1
4	1:1	121.2	1
5	1:2	132.4	0.081
6	1:3	138.5	0.081
7	1:4	133.8	0.081
TBTA	1:1	0.369	--
BTTES	1:1	1.284	--
TJHPTA	1:1	0.9963	--
A19	1:1	82.43	--

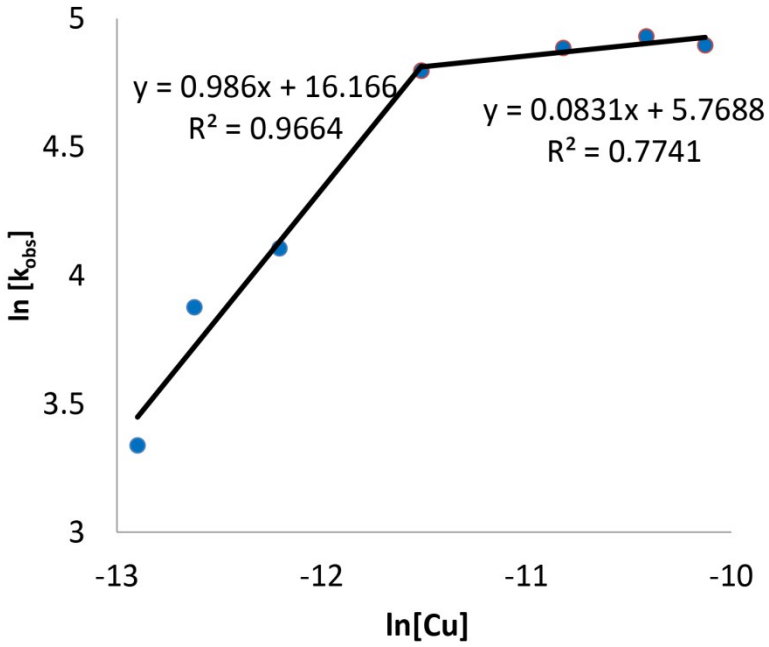


Figure S5. Determination of reaction order of Cu.

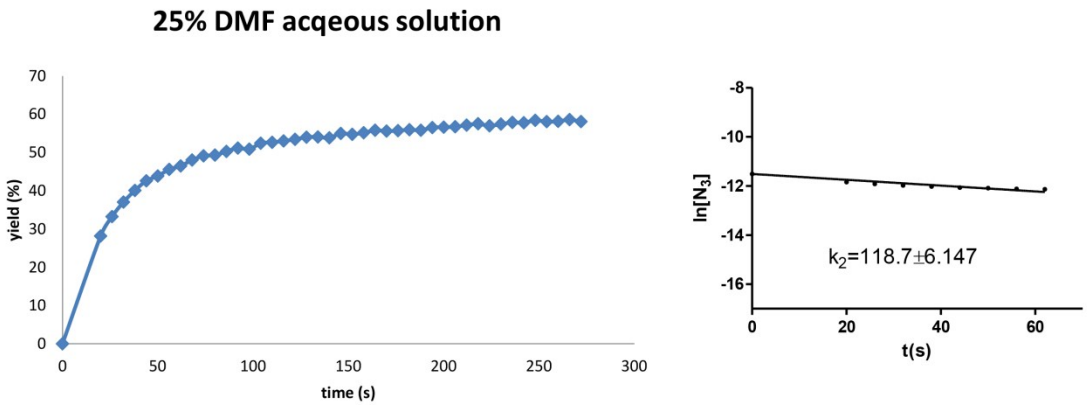


Figure S6. Time course and kinetics of model reaction under DMF: H_2O 1:3 solvant condition. (left: time course of the reaction; right: k_{obs} calculation using pseudo-first order reaction model. Reactions were performed with 10 μM **1**, 10 μM $CuSO_4$, 100 μM 7-ethynylcoumarin, 100 mM sodium ascorbate.)

Discussion of the proposed reaction mechanism of AIO-assisted CuAAC

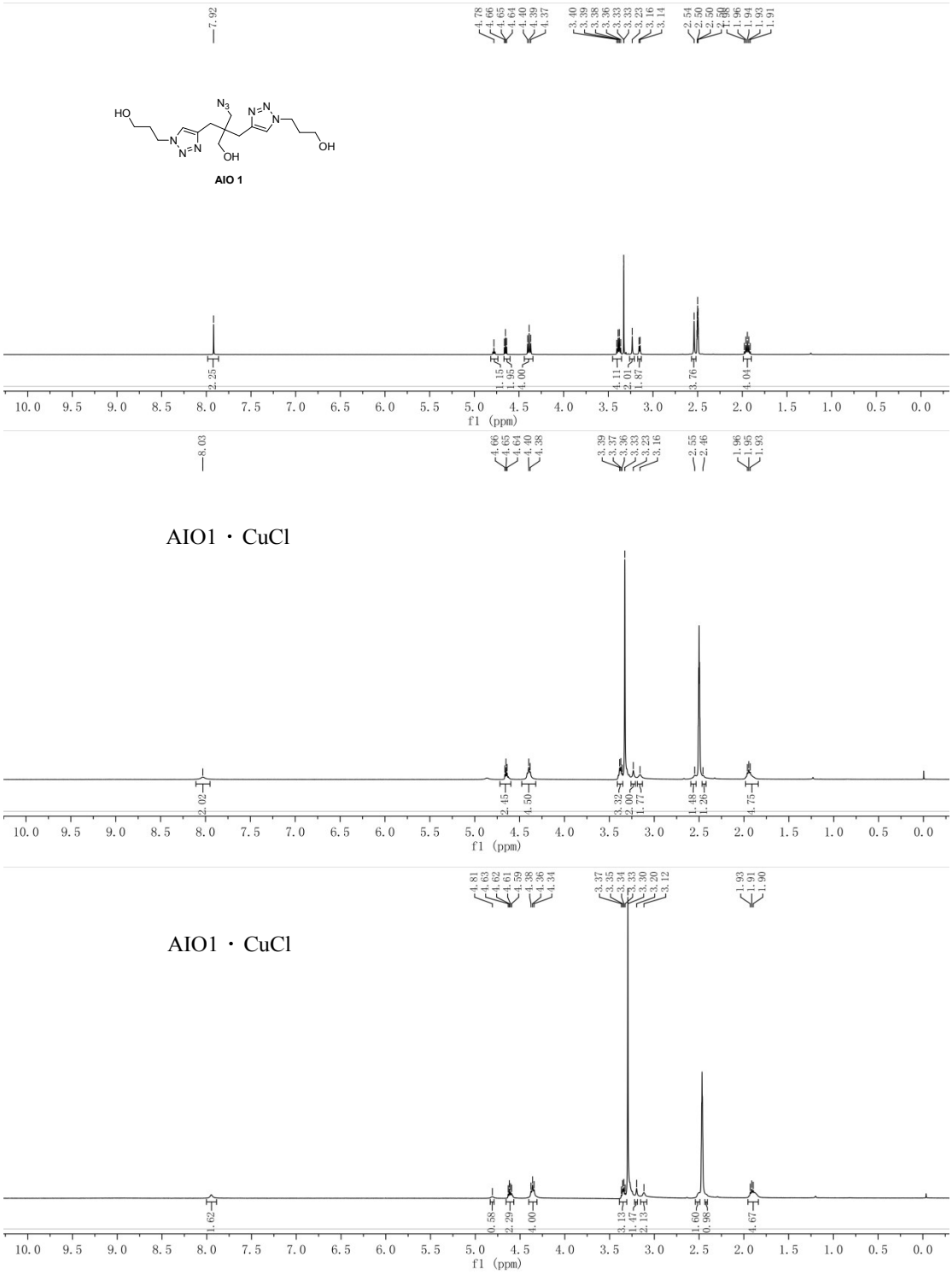


Figure S7. ^{1}H NMR of AIO 1(a) and a mixture of AIO 1 with 0.2 eq CuCl(b) or 1.0eq CuCl (c) in DMSO-d6.

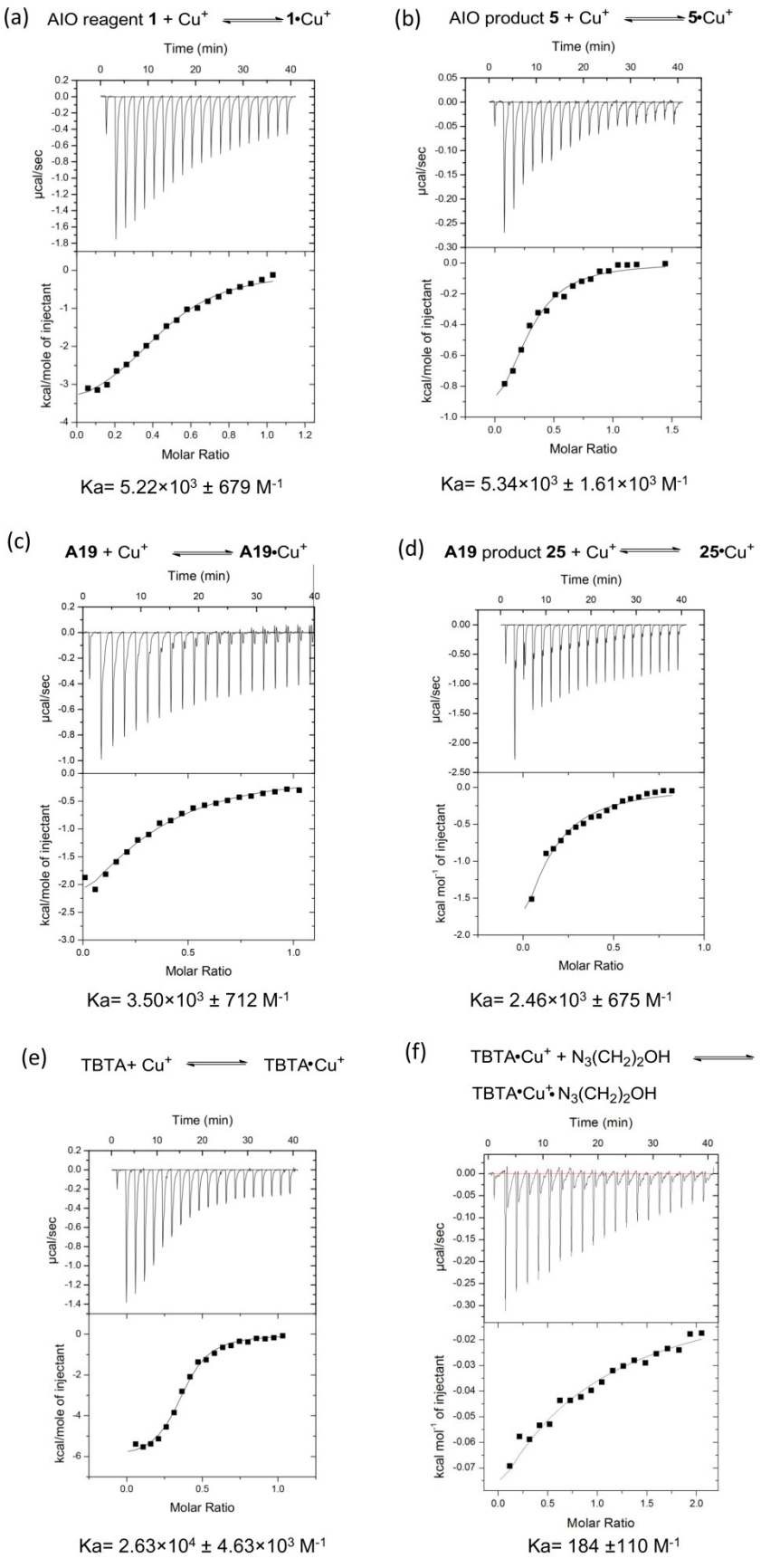
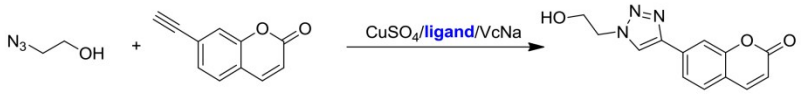


Figure S8. Measure of association constant (Ka) of copper chelating ligands (AIO1, 5, A19, 25 and TBTA) with Cu(I) using isothermal titration calorimetry

(ITC).

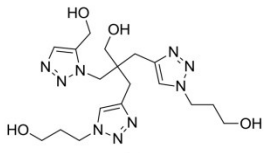
(a), 5 mM solution of compound **1** in DMSO was titrated to 1 mM solution of CuCl in in DMSO at 25℃. (b), 2.8 mM solution of compound **5** in DMSO was titrated to 0.4 mM solution of CuCl in in DMSO at 25℃. (c), 5 mM solution of compound **A19** in DMSO was titrated to 1 mM solution of CuCl in in DMSO at 25℃. (d), 8 mM solution of compound **25** in DMSO was titrated to 2 mM solution of CuCl in in DMSO at 25℃. (e), 2 mM solution of TBTA in DMSO was titrated to 0.4 mM solution of CuCl in in DMSO at 25℃. (f), 20 mM solution of $N_3(CH_2)_2OH$ in DMSO/ H_2O (1/1) was titrated to 2 mM solution of TBTA/$CuSO_4$ complex in DMSO/ H_2O (1/1) at 25℃.

(a)



ligand: TBTA or compound **26**

(b)



26

(c)

No.	TBTA	26	alkyne	azide	Sodium Ascorbate
1	--	100uM	1mM	100uM	5mM
2	100uM	--	1mM	100uM	5mM

(d)

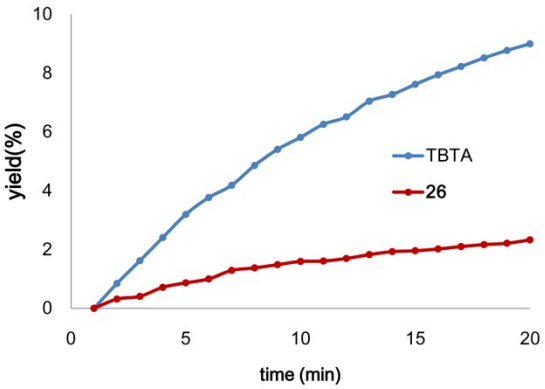


Figure S9. Model reaction catalyzed by TBTA and analogue 26 of AIO1 product. (a), model reaction. (b), structure of compound **26**. (c), model reaction condition.(solvent: DMF/ H_2O(1/1)). (d), yield and time course of the reaction.

From these results we speculated:

(1) The affinity of AIO **1** with Cu(I) was similar as **A19** , which was consistent with the results that their reaction rate constant were in the same level. Also, the Ka value of AIO **1** with Cu(I) was slightly higher than **A19**, which might explain why AIO **1** showed slightly faster kinetics than **A19**.

(2) The Ka level of CuAAC reaction products (compound **5** and **25**) were nearly equivalent affinity as the reactants. Since the products contains three triazole rings

and might function as ligand to facilitate the reaction, we synthesized analogue of compound **5** (compound **26**, see Supporting Information Figure S9 and at the end of this part) and found it was 4 times worse than TBTA. This excluded the possibility that product contributes to the fast kinetics.

(3) TBTA had a significantly higher Ka than AIO **1**, **A19** and their products, though TBTA assited CuAAC reaction was about 300 times slower than AIO assisted CuAAC reaction. Combining these results, chelating azides such as AIO **1** and **A19** should follow different reaction mechanism compared with ligands assisted CuAAC reacrions.

(4) In the mechanism of ligand assisted CuAAC reaction, the formation of the metallacycle intermediate is a stepwise process, in which coordination of the azide to the Cu(I) center might be a key step. We therefore measured the Ka constant between a standard azide ($N_3CH_2CH_2OH$) and TBTA-Cu^+ complex. The Ka level of azide binding step was 2 orders of magbitudes smaller than that between TBTA and Cu^+. This results suggested that coordination of azides to the Cu(I) center was a relatively difficult process. While AIO reagents, containing both the azide group and internal copper chelating moiety, form the AIO-Cu^+ in one step. The Ka of AIO**1** chelating with Cu^+ was over 20 times higher than that of formation of TBTA-Cu^+-$N_3(CH_2)_2OH$. The results suggested the tendency of chelating azides(AIO**1** and **A19**) binding to the Cu(I) center was stronger than standard azides.

(5) Thus, we speculated the strong affinity of AIO reagents with Cu(I), which would simplify the reaction, from multiple components down to only two components (the alkyne and the copper-aizde complexes) and therefore facilitate the formation of metallacycle intermediate in the rate-determining step. And this was consistent with the reported concept of copper chelating-assistted metal catalysis.[8-9]

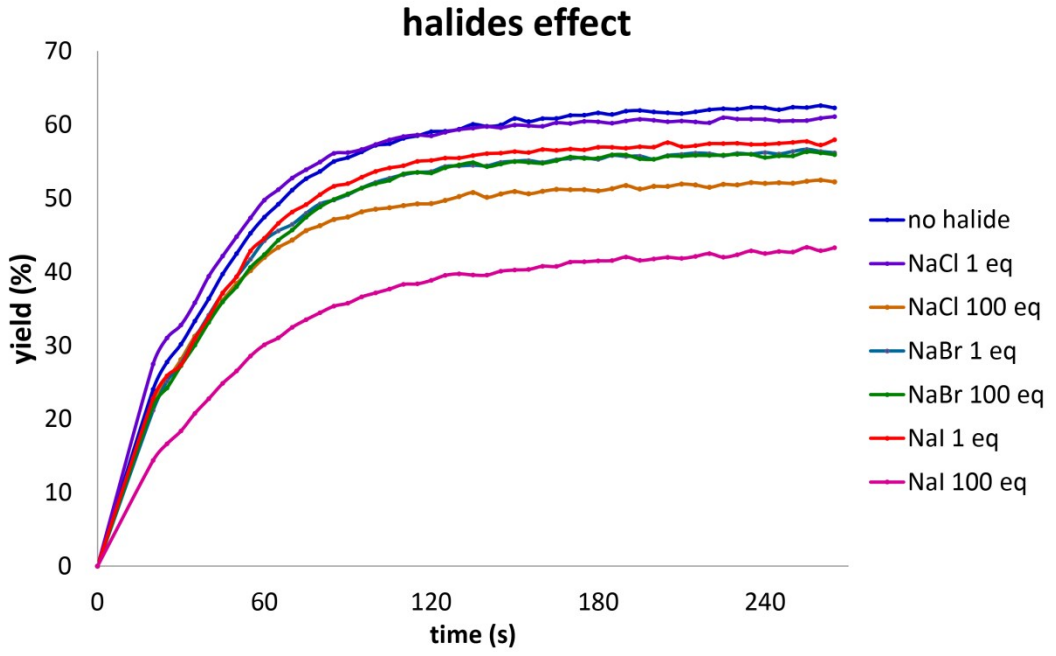
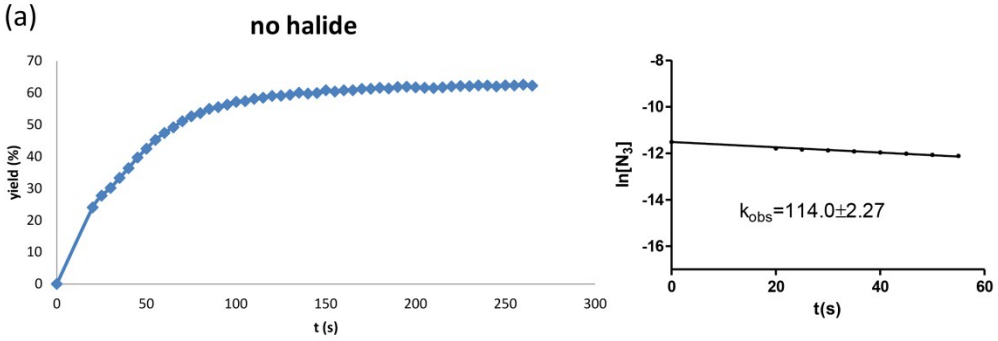
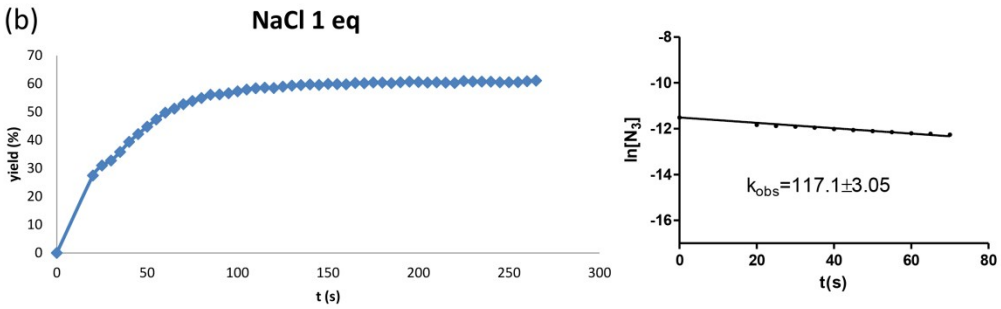


Figure S10. Yield and time course of model reaction under halide addition.

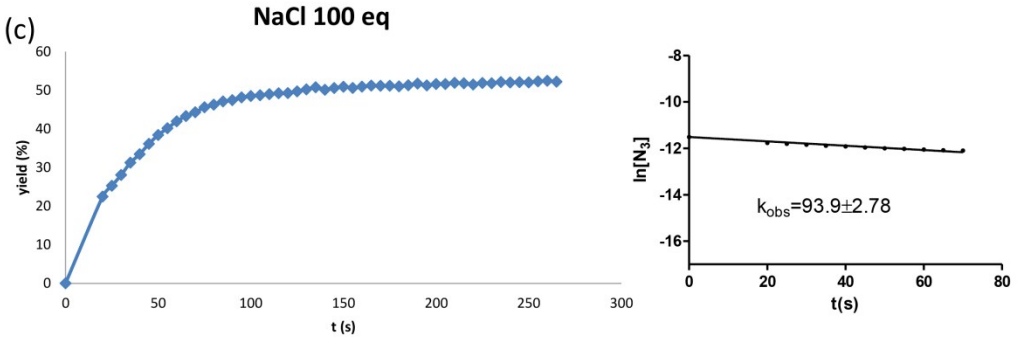
(a)


no halide
yield (%)
t (s)
ln[N3]
kobs=114.0±2.27
t(s)

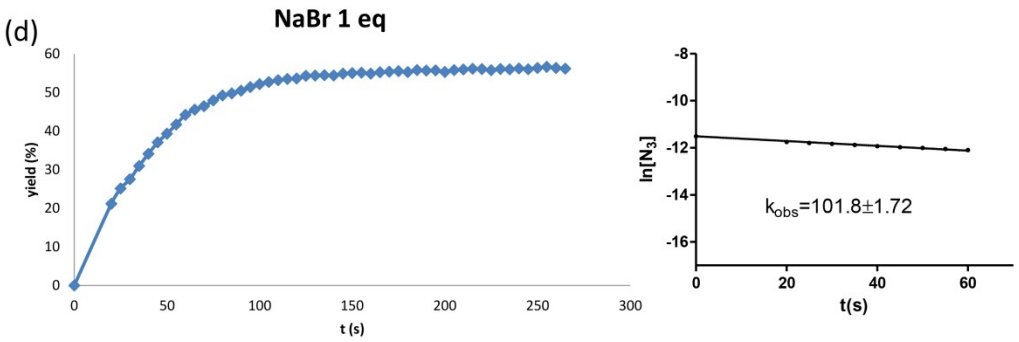
(b)


NaCl 1 eq
yield (%)
t (s)
ln[N3]
kobs=117.1±3.05
t(s)

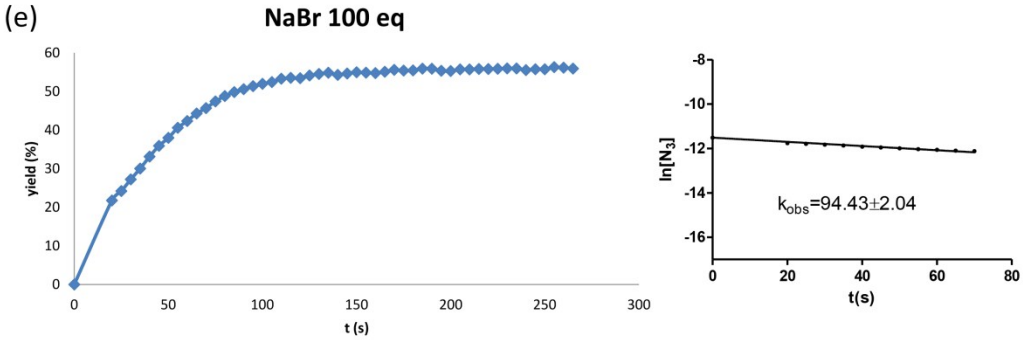
(c)


NaCl 100 eq
yield (%)
t (s)
ln[N3]
kobs=93.9±2.78
t(s)

(d)


NaBr 1 eq
yield (%)
t (s)
ln[N3]
kobs=101.8±1.72
t(s)

(e)


NaBr 100 eq
yield (%)
t (s)
ln[N3]
kobs=94.43±2.04
t(s)

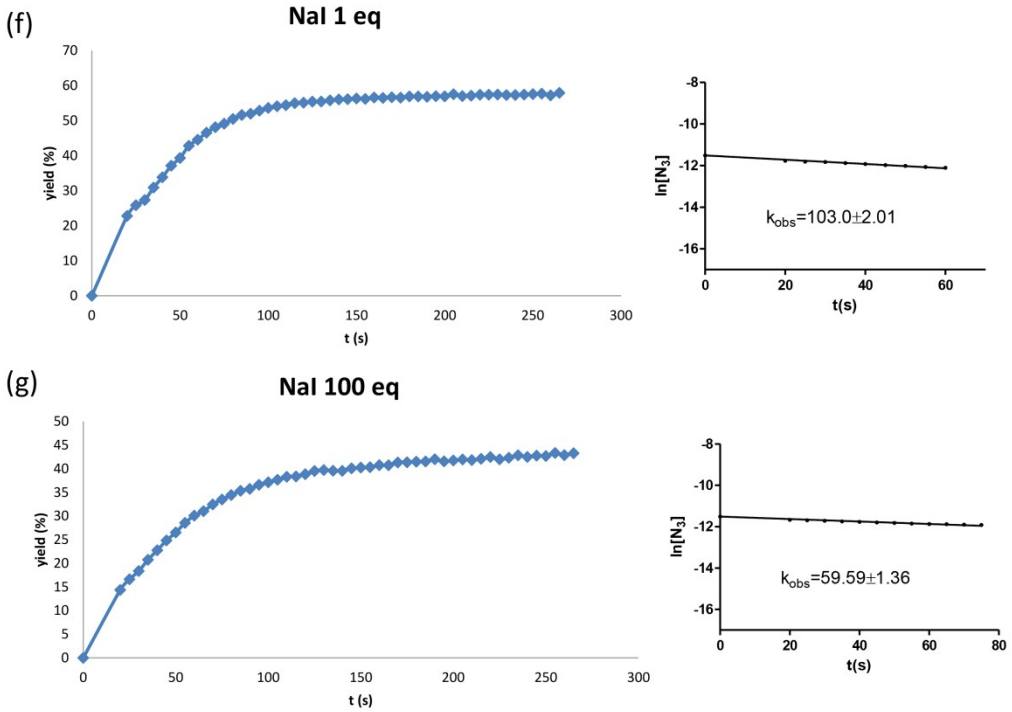


Figure S11. Time course and kinetics of model reaction under halide addition. (a)~(g), time course and k_{obs} calculation of the model reaction at different halide addition.(left: time course of the reaction; right: k_{obs} calculation using pseudo-first order reaction model. Reactions were performed with 10 μM **1**, 10 μM $CuSO_4$, 100 μM 7-ethynylcoumarin, 100 mM sodium ascorbate and 1 or 100 equivalent of NaCl, NaBr, or NaI, accordingly.) All reactions are carried out in DMF: H_2O 1:1 solution.

Table S2. Kinetics of model reactions under halide addition

condition	k_{obs} ($M^{-1}\cdot s^{-1}$)
No halide	114.0
NaCl (1 eq)	117.1
NaCl (100 eq)	93.9
NaBr (1 eq)	101.8
NaBr (100 eq)	94.4
NaI (1 eq)	103.0
NaI (100 eq)	59.6

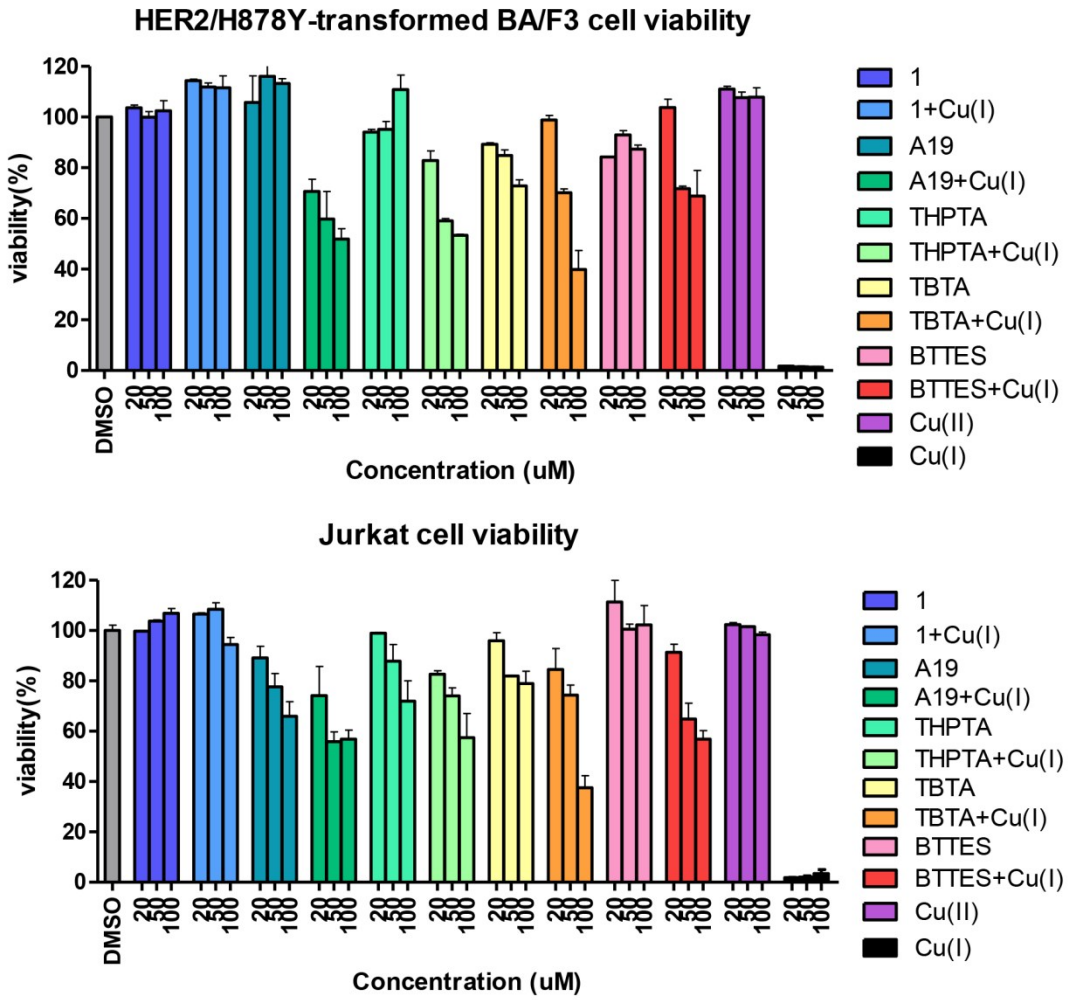


Figure S12. Protective effects of AIO reagents.

(a), Cell viability of HER2/H878Y-transformed BA/F3 cells. (b), Cell viability of Jurkat cells. Cells were incubated with the 20 μM, 50 μM and 100 μM **1**, A19, THPTA, TBTA, BTTES, and their complex with Cu(I) for 4 h. Cu(I)/AIO reagent complex, Cu(I)/A19 complex and the Cu(I)/ligand complex were premixed with equal amounts of the reagents and $CuSO_4$, followed by addition of an excessive amount of sodium ascorbate (5mM). Cell viability was measured by Cell Tilter-Glo Luminescence assay.

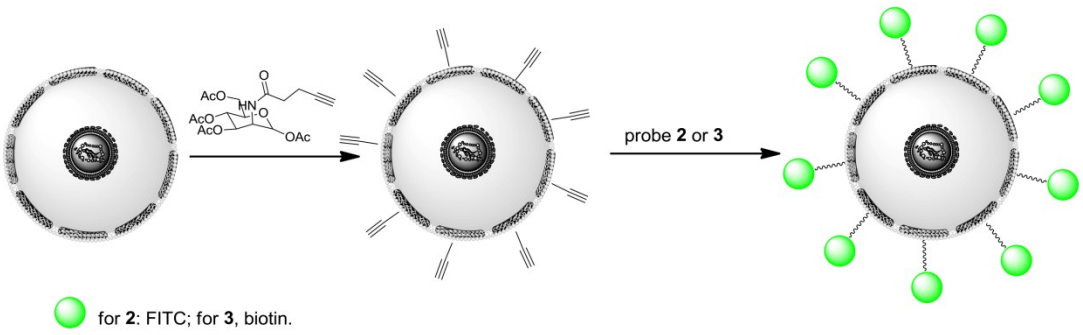


Figure S13. Schematic representation of the whole labeling process on Jurkat cells.

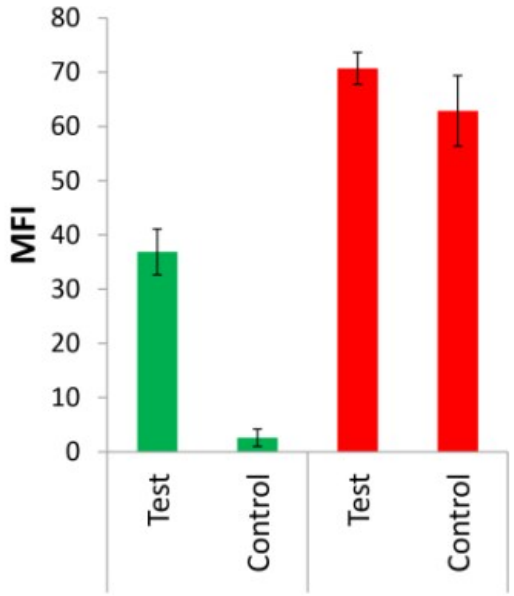


Figure S14. The mean fluorescence intensity (MFI) of the FITC channel and the DiI channel of Jurkat cells after AIO probe 2 labeling. (Test: Ac_4ManNAl treated; Control: Ac_4ManNAl untreated).

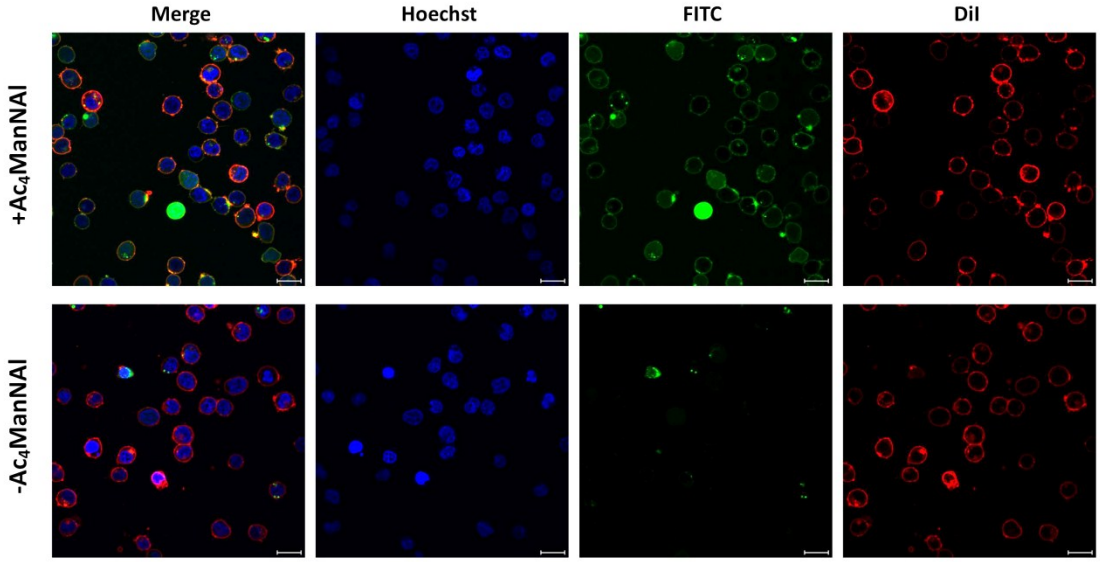


Figure S15. Fluorescence imaging of Jurkat cells after AIO probe 2 labeling. Scale bar: 20μm.

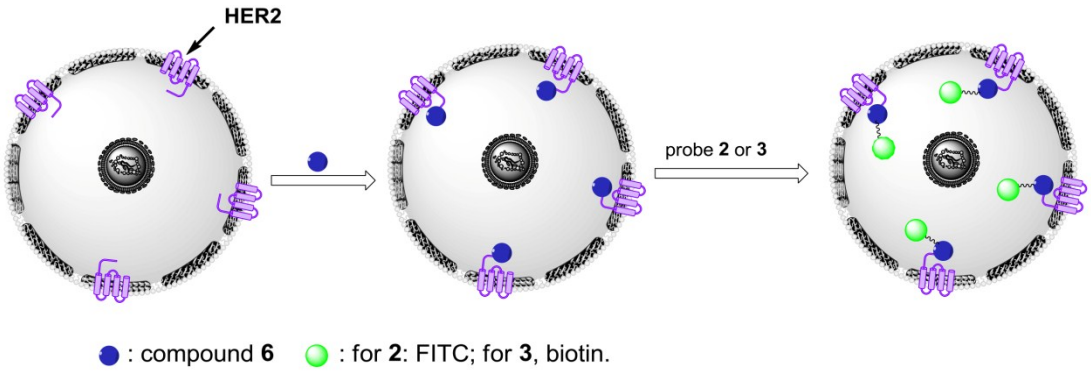


Figure S16. Schemetic representation of the labeling process of the whole labeling process on HER2/H878Y-transformed BA/F3 cells.

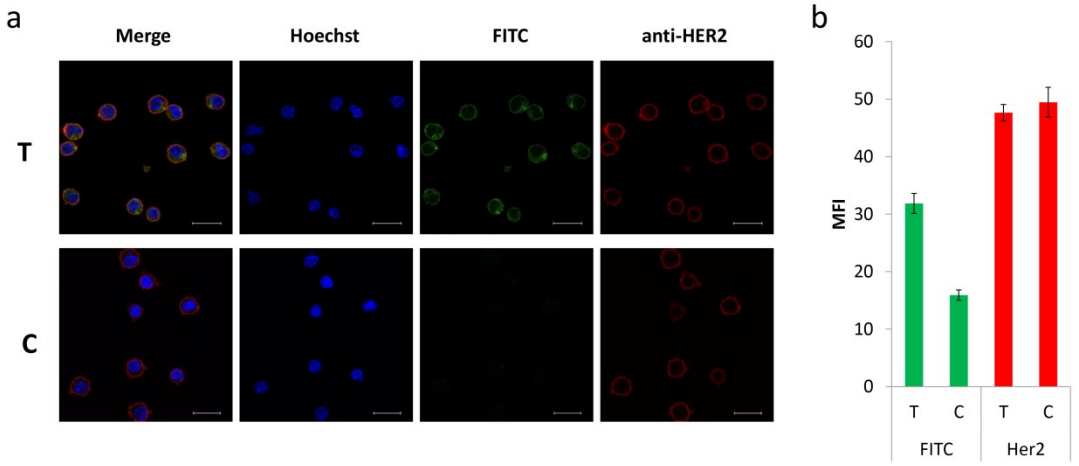


Figure S17. Fluorescence imaging of HER2/H878Y-transformed BA/F3 cells. (a), confocal imaging of HER2/H878Y-transformed BA/F3 cells after AIO probe **2** labeling. (b), mean fluorescence intensity of FITC channel and anti-HER2 channel. (T: compound **6** treated H878Y cells; C: untreated HER2/H878Y-transformed BA/F3 cells. Scale bar: 20 μm.)

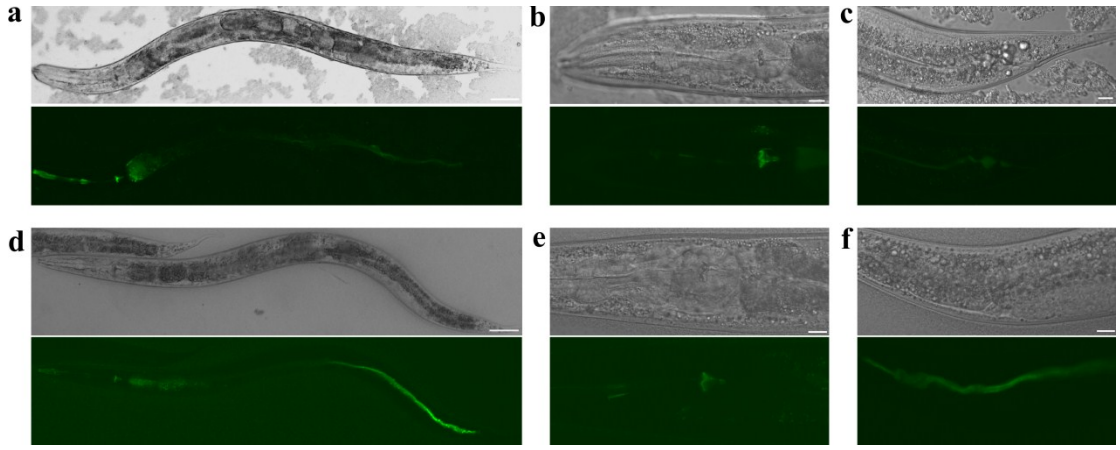
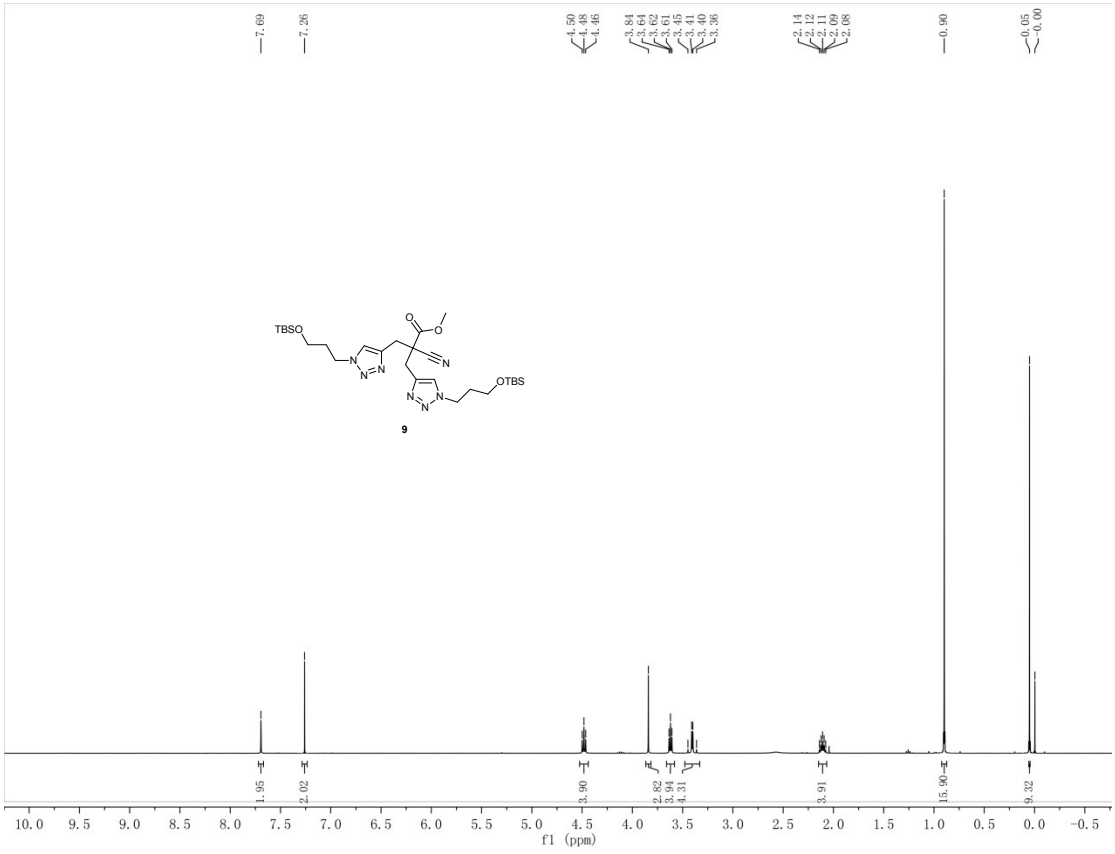
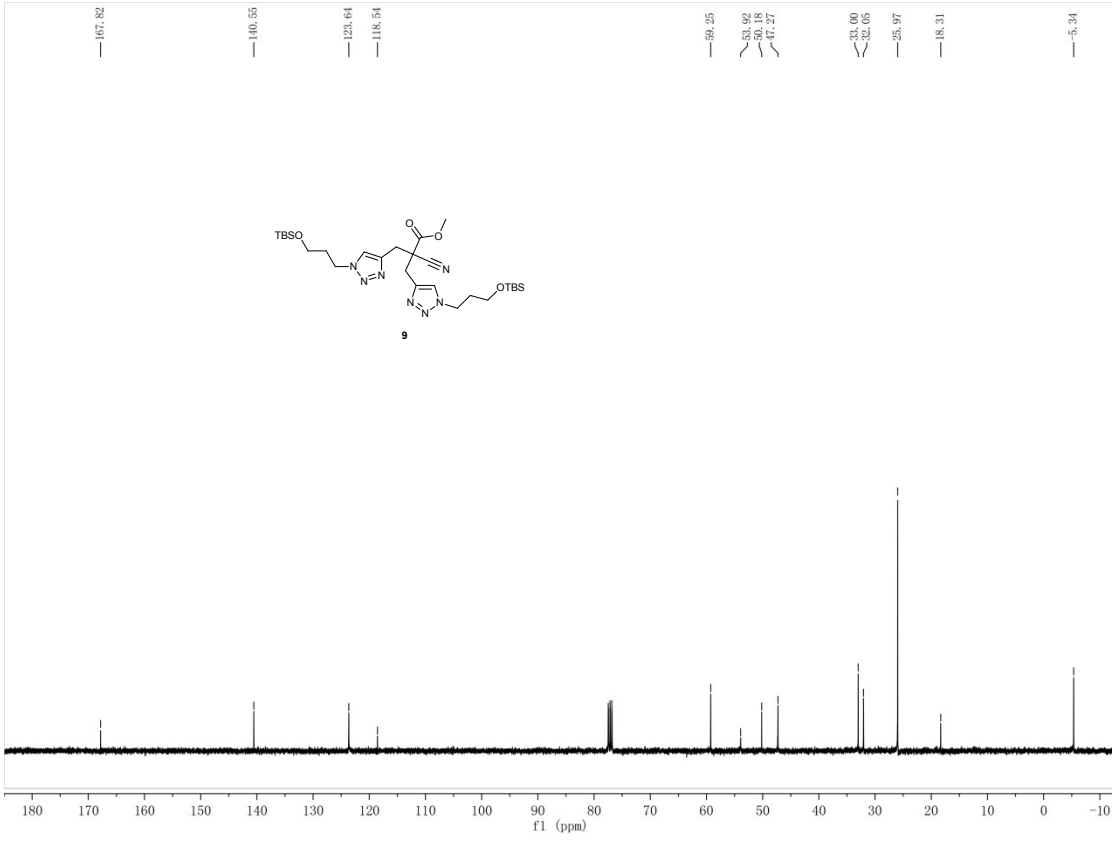
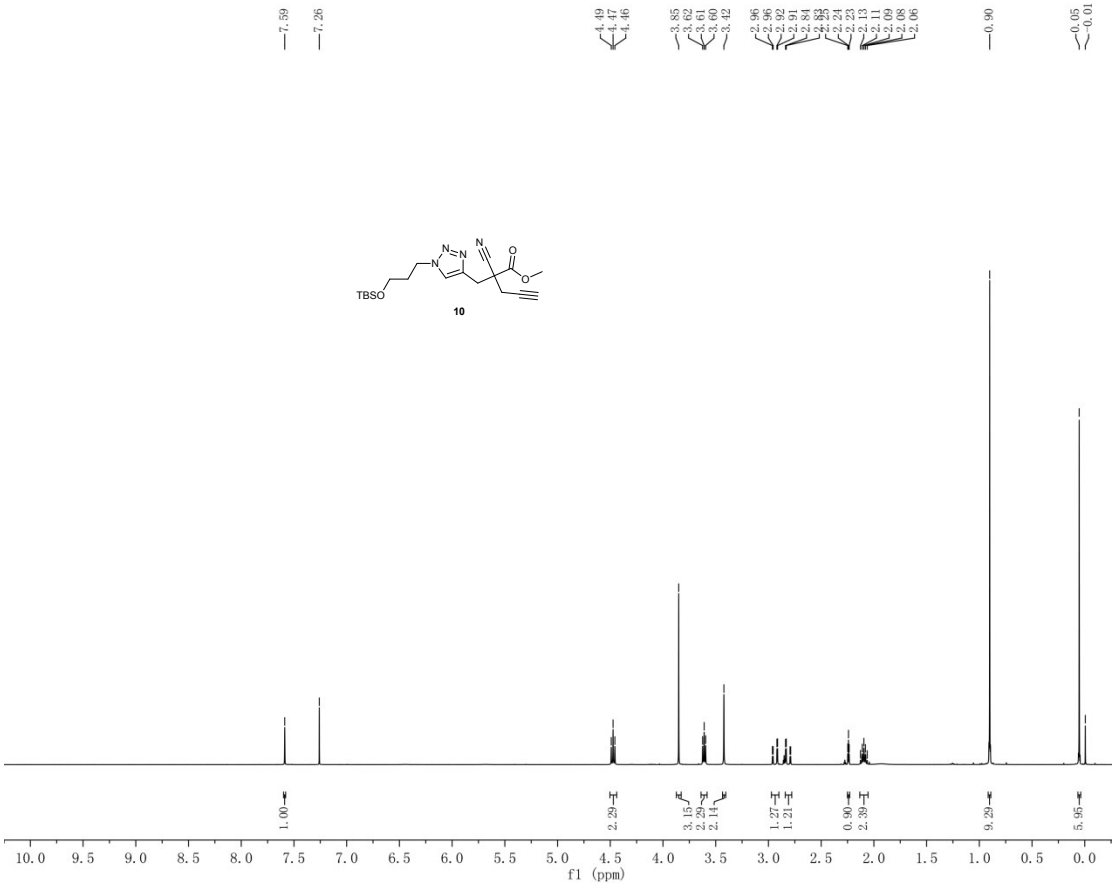


Figure S18. *In vivo* imaging of glycan distribution in *C. elegans*. (a-c) *C. elegans* young adult hermaphrodites that had been incubated with 5 mM Ac_4GalNAl were reacted with 50 μM AIO regent **2** and imaged alive. Fluorescence was observed in the pharynx and tail region. Higher magnification images of pharynx and tail region are shown in (b-c). (d-f) *C. elegans* young adult hermaphrodites that had been incubated with 250 μM Ac_4GalNAl were reacted with 100 μM AIO reagent **2** and imaged alive. Fluorescence was observed in the pharynx and tail region. Higher magnification images of pharynx and tail region are shown in (d-f). Scale bars: a and d 50μm, b-c and e-f: 10 μm.

^{1}H and ^{13}C NMR Spectra



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2.91
2.84
2.24
2.23
2.13
2.11
2.09
2.08
2.06
0.90
0.05
0.01
TBSO
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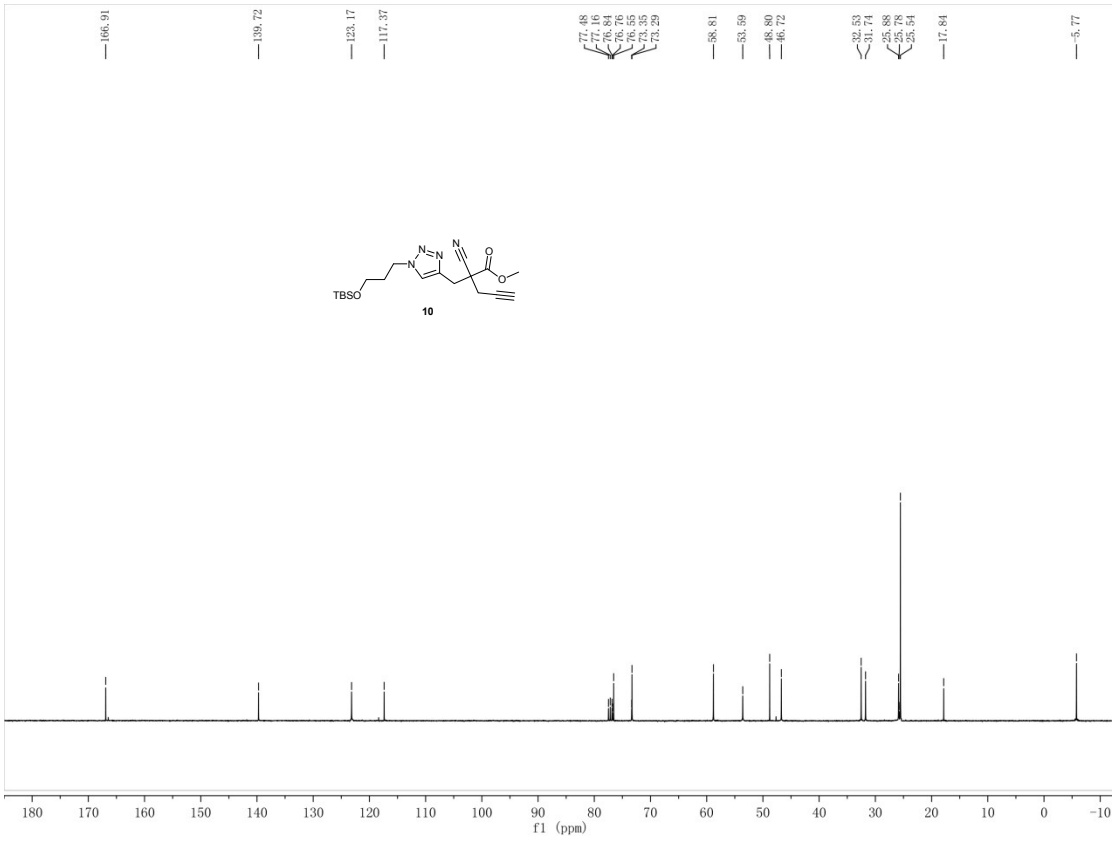
f1 (ppm)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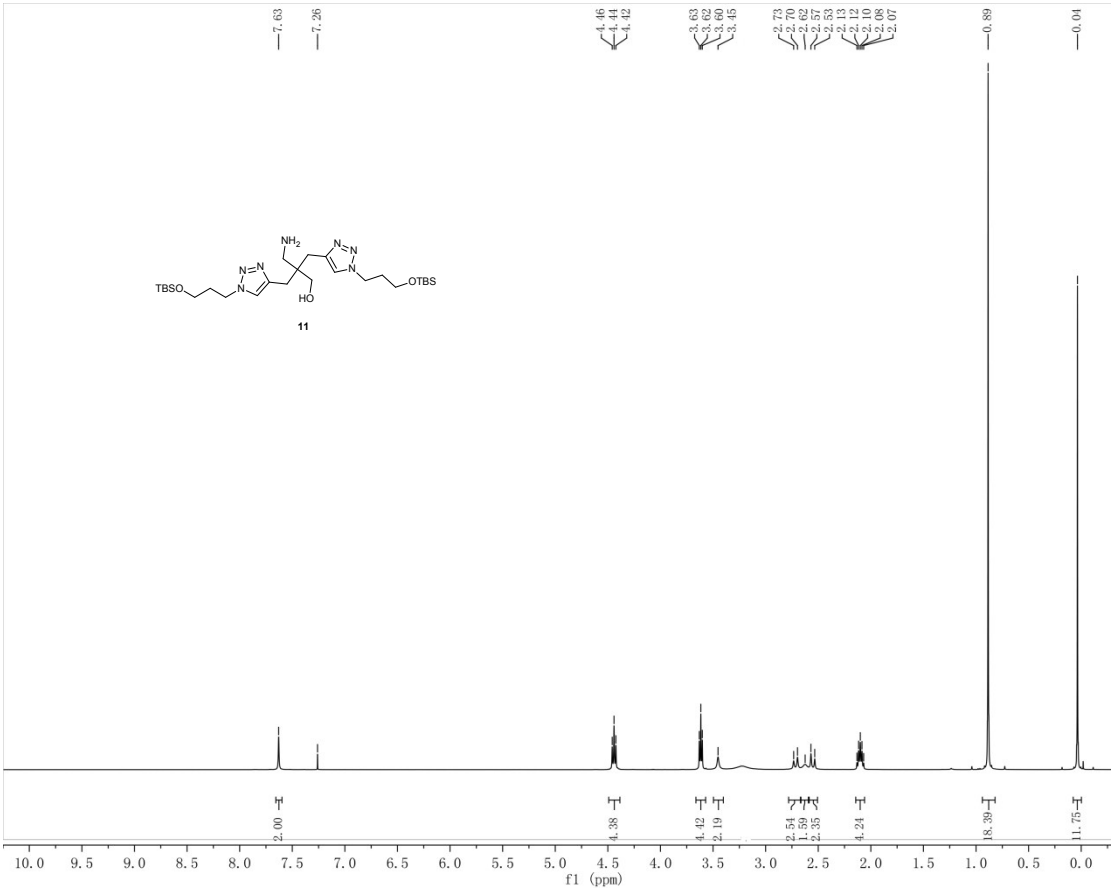
25.78
25.54
17.84
-5.77
TBSO
10
f1 (ppm)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2.10
2.08
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0.89
0.04
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TBSO
OTBS
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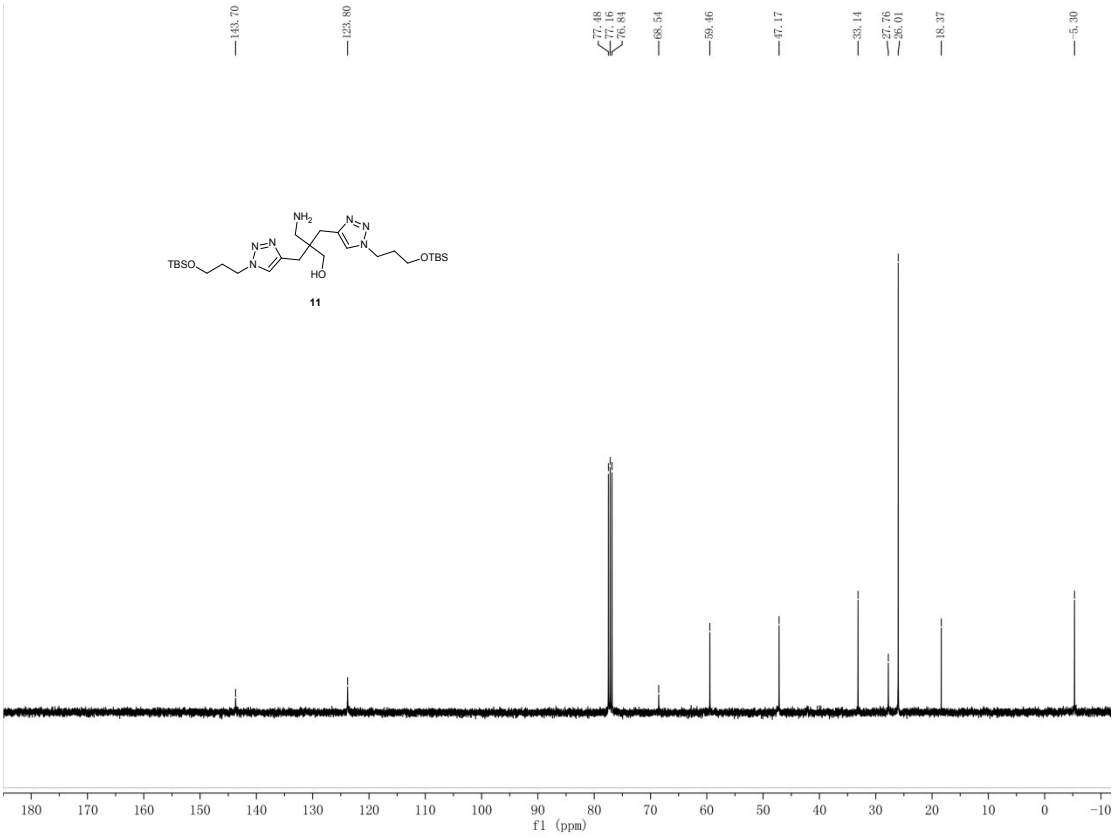
f1 (ppm)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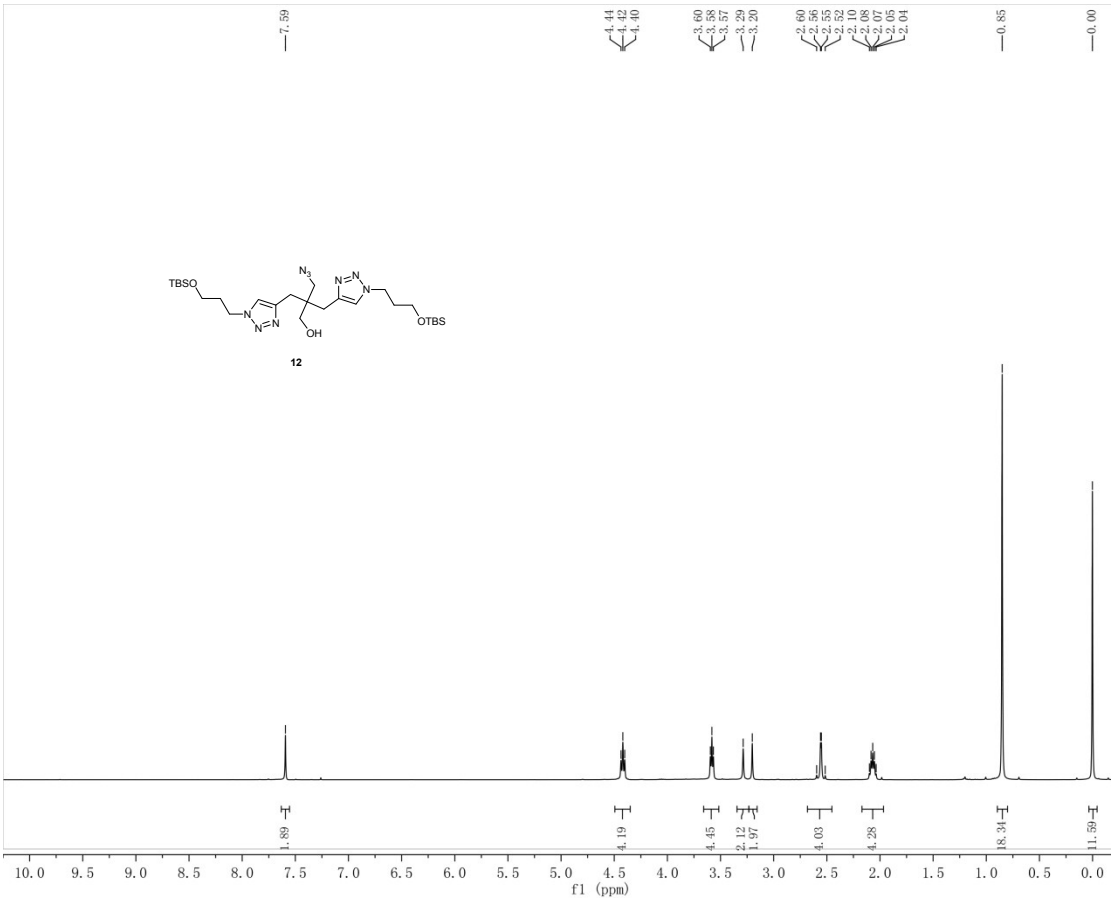
TBSO
OTBS
HO
11
f1 (ppm)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2.10
2.08
2.07
2.05
2.04
0.85
0.00
TBSO
N3
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4.45
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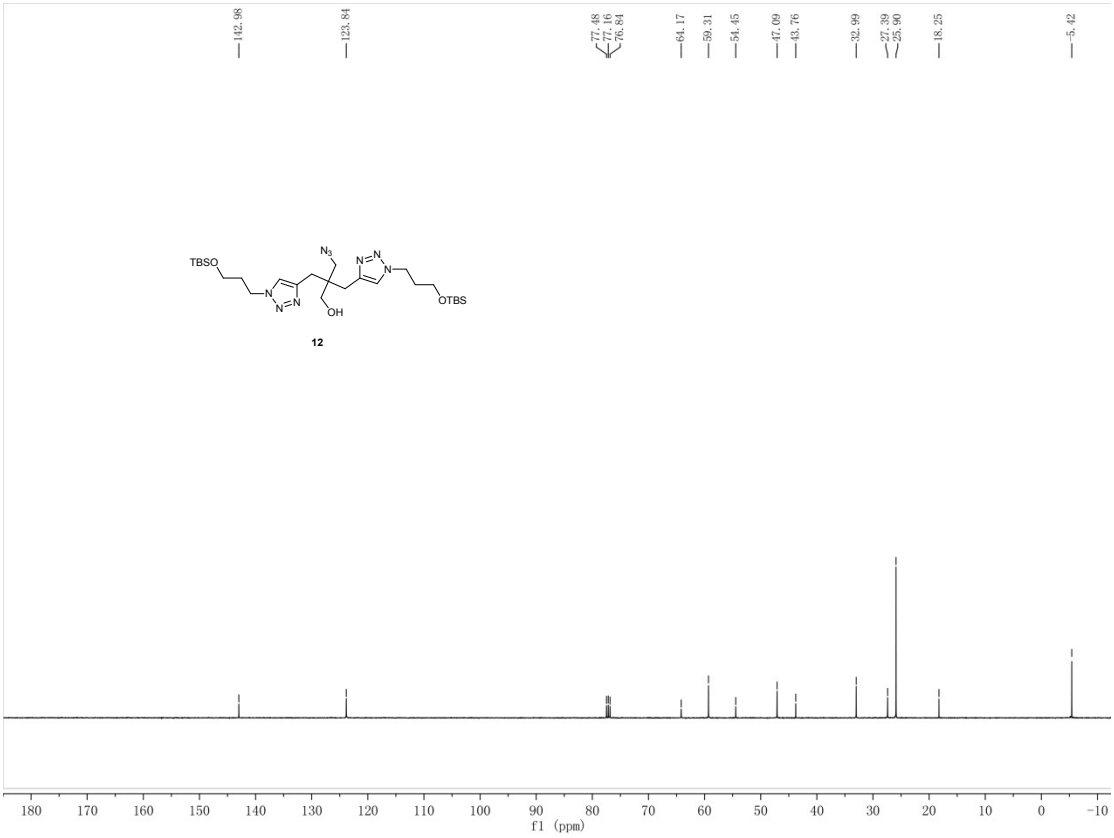
11.59
f1 (ppm)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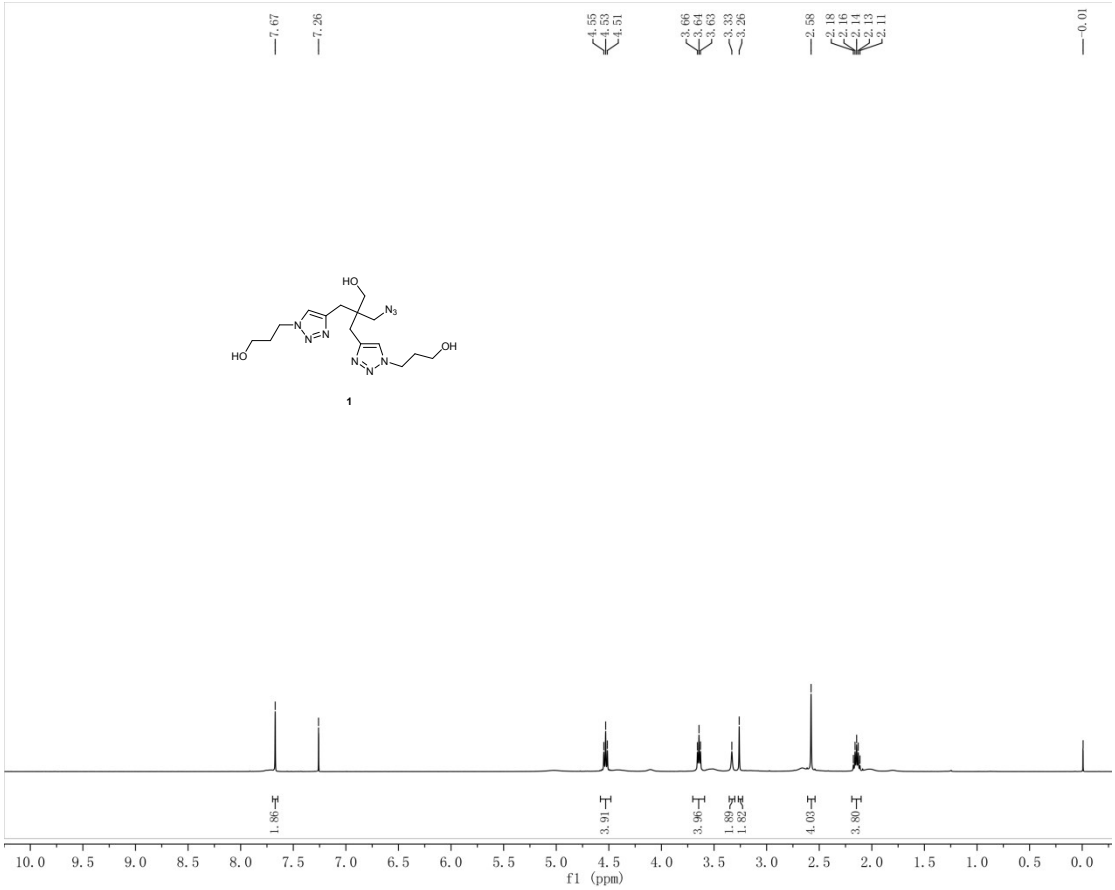
TBSO
N3
OH
OTBS
12
f1 (ppm)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OH
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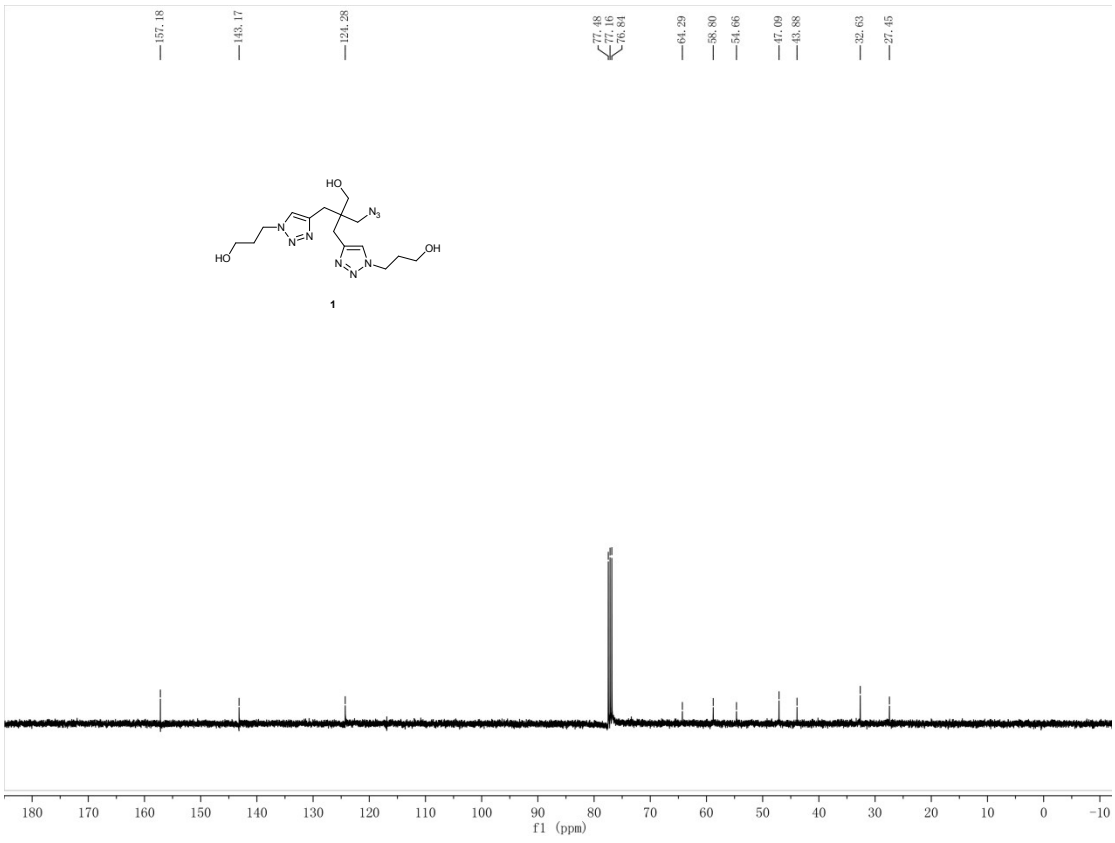
f1 (ppm)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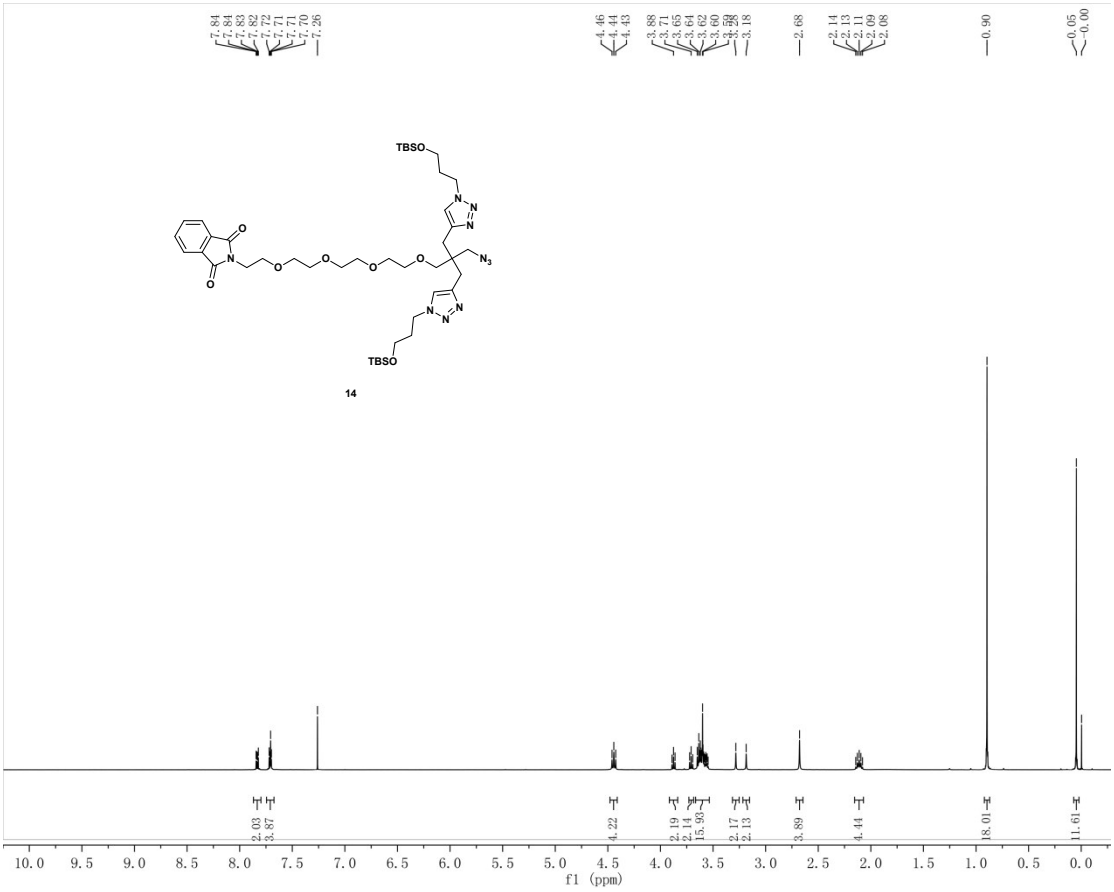
f1 (ppm)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2.08
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0.05
0.00
TBSO
TBSO
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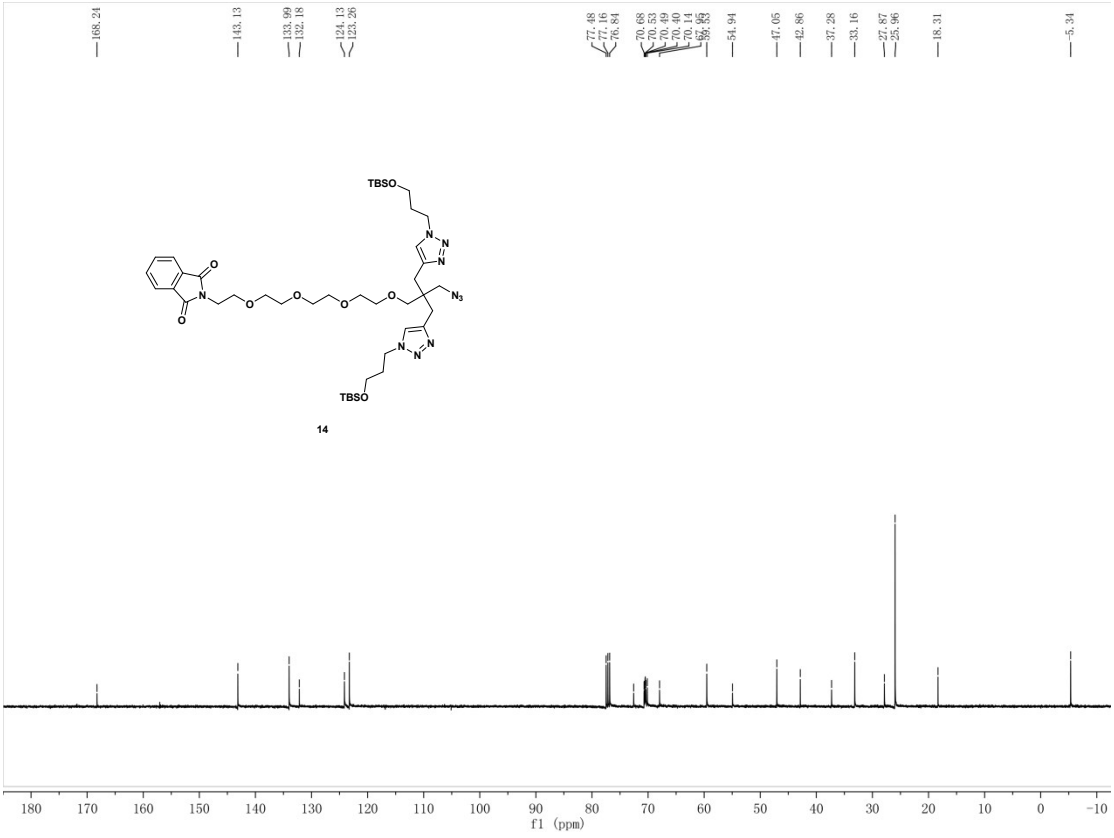
f1 (ppm)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TBSO
TBSO
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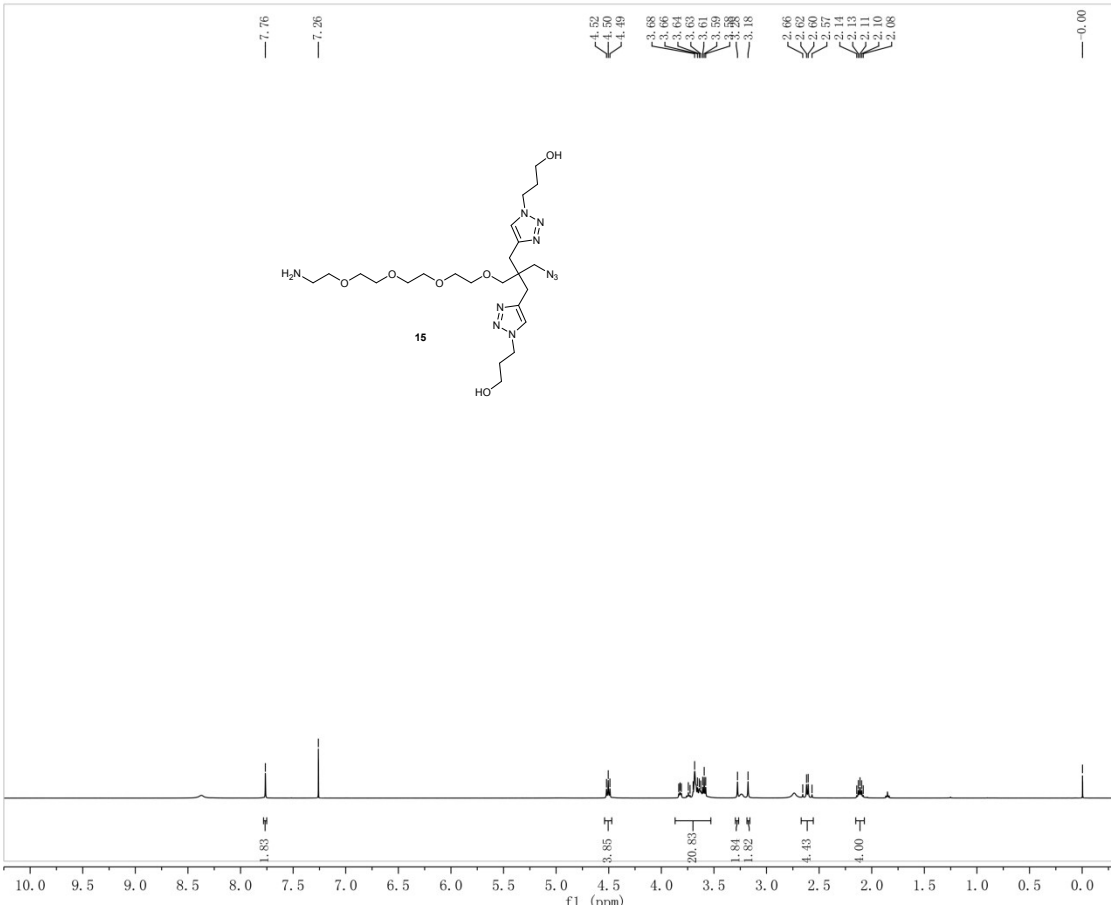
f1 (ppm)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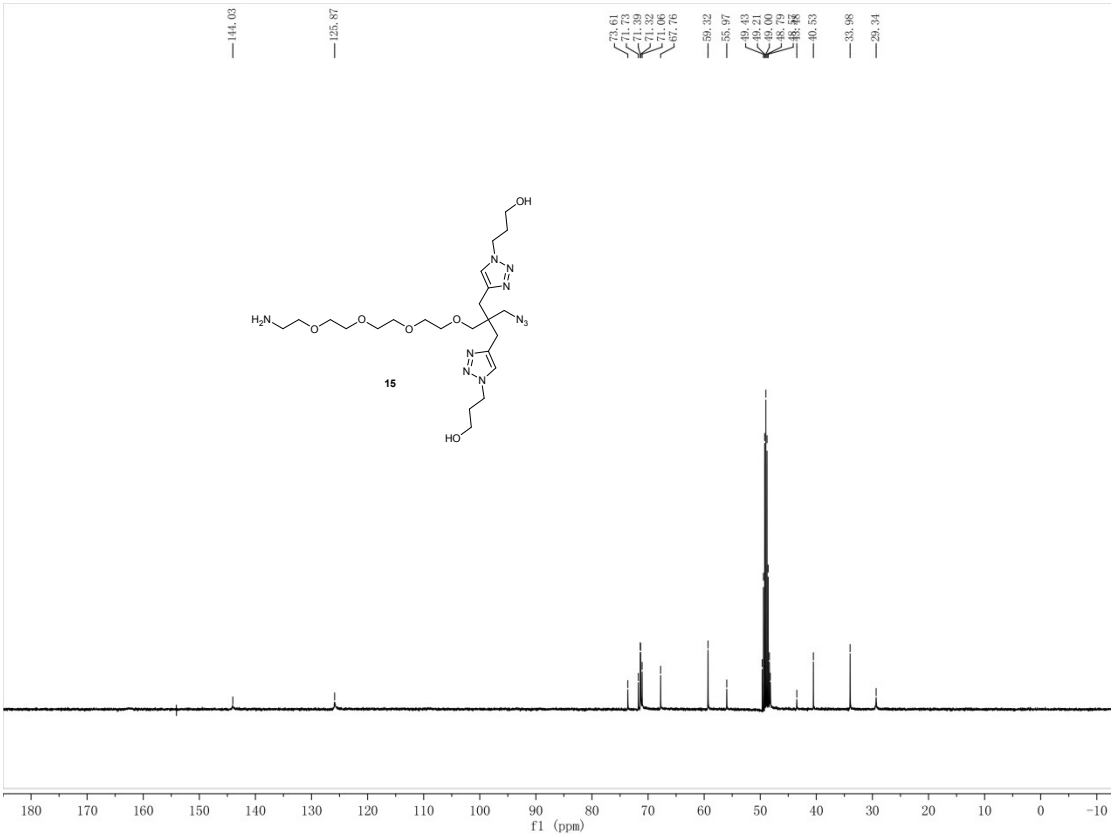
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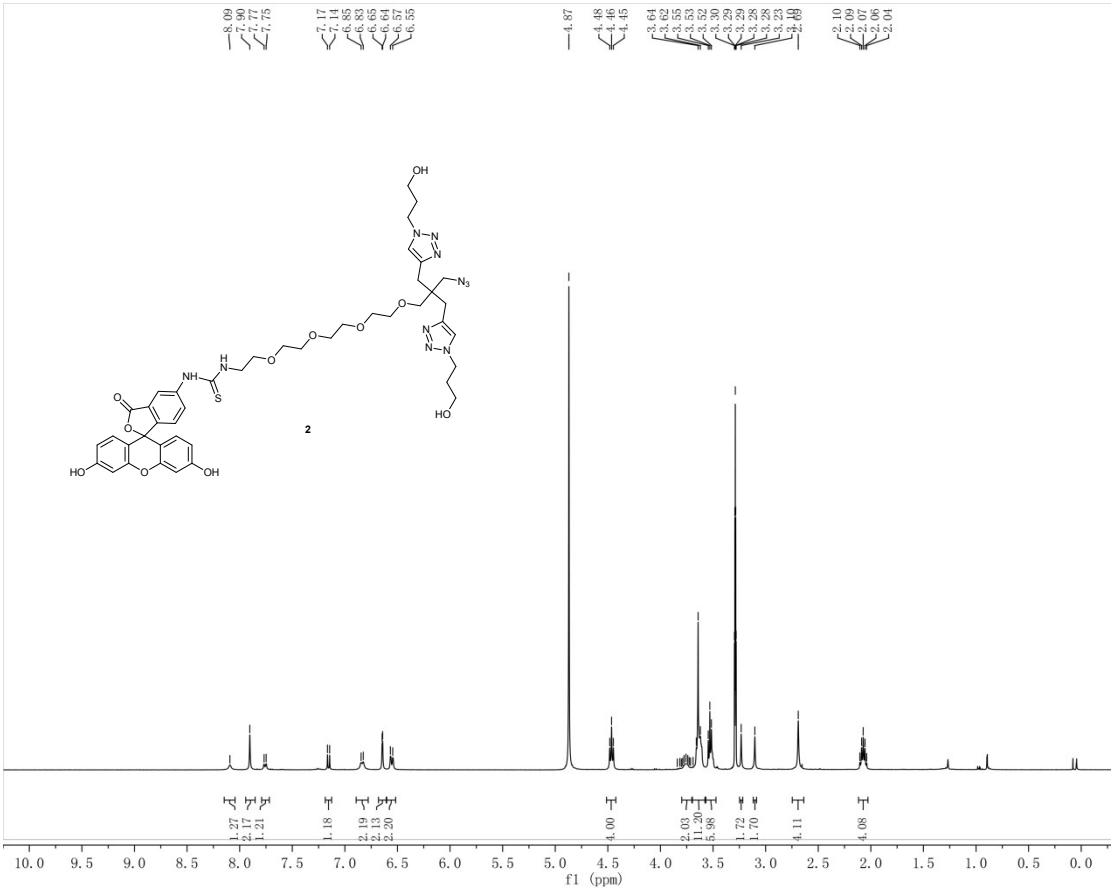
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6.55
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4.45
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2.07
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2.04
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N3
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S
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HO
OH
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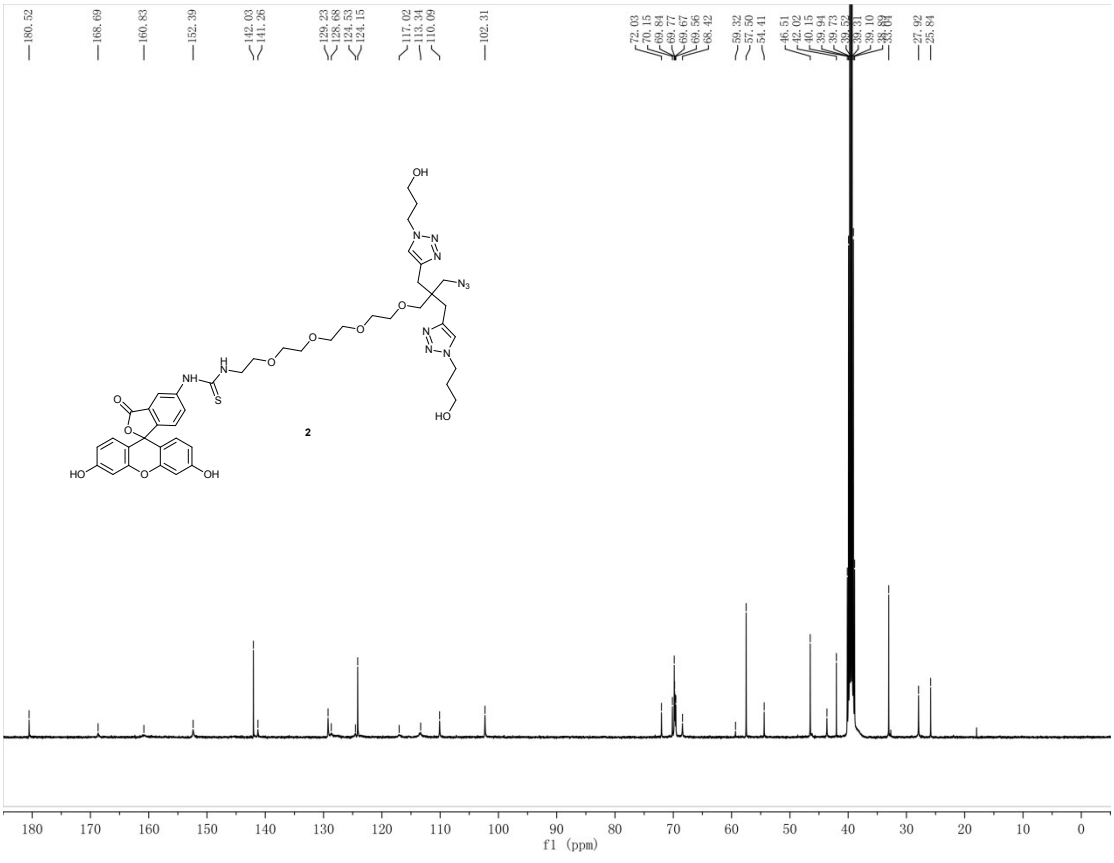
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59.32
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46.51
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OH
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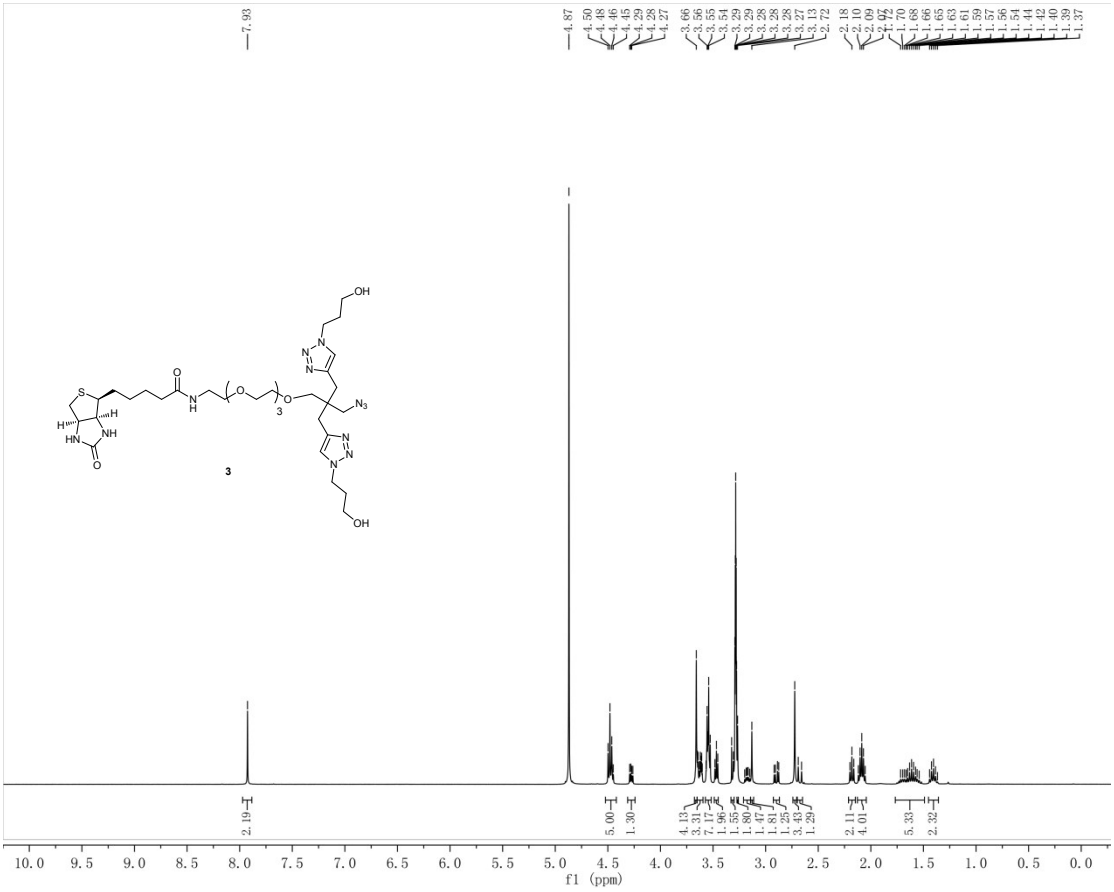
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3.13
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2.10
2.09
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1.63
1.61
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1.56
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1.39
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S
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NH
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N
H
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N
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1.29
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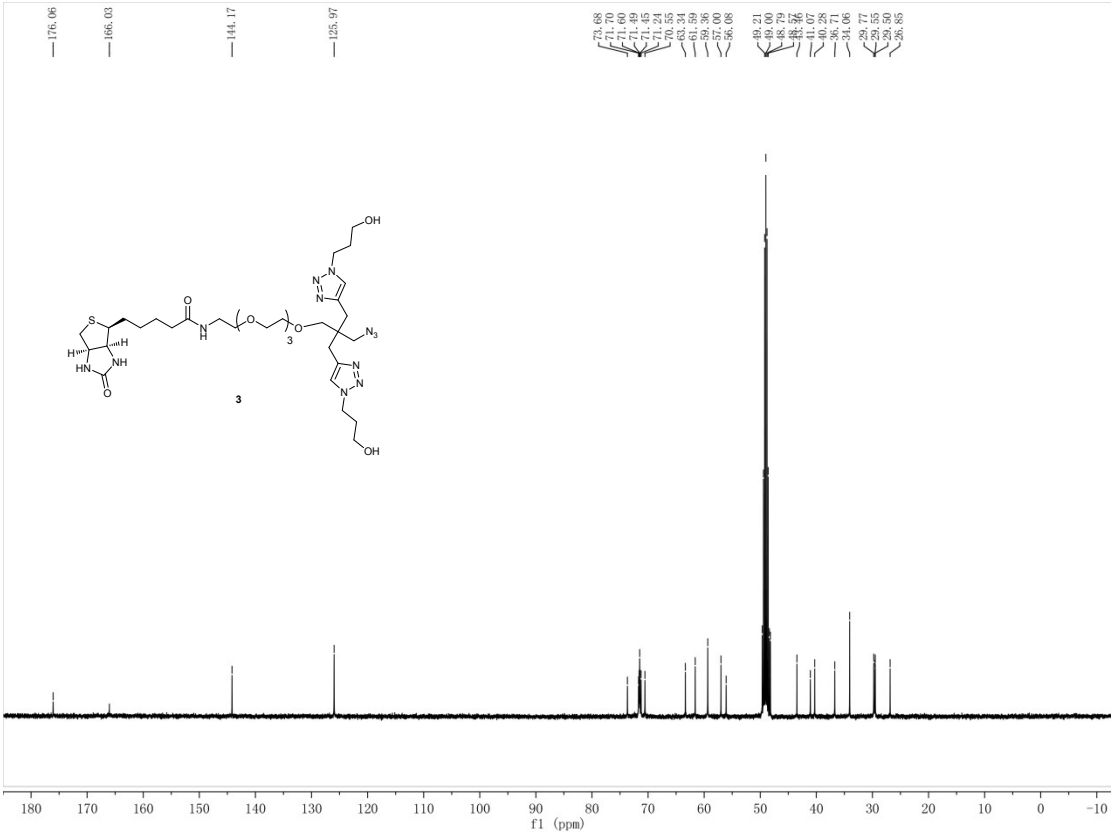
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S
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NH
O
O
N
H
O
3
N3
N
N
N
OH
180
170
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80
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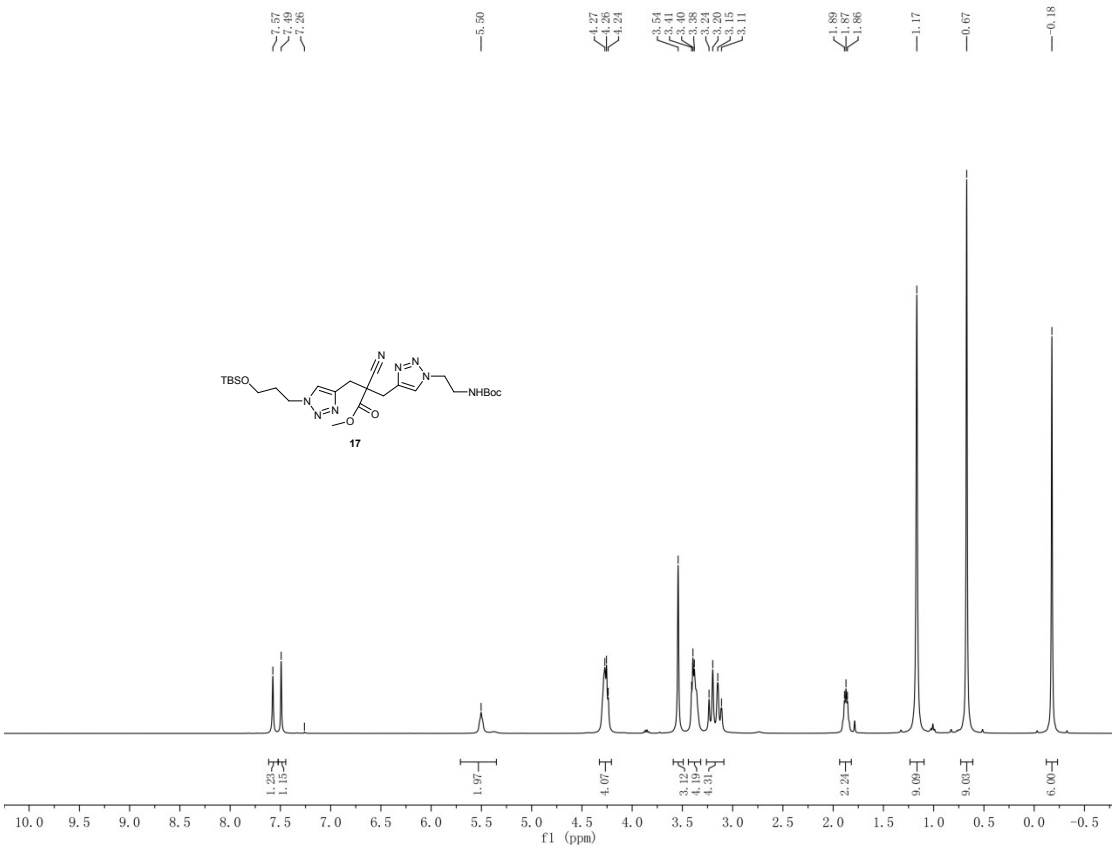
f1 (ppm)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3.15
3.11
1.89
1.87
1.86
1.17
0.67
-0.18
TBSO
NHBoc
17
1.23
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4.07
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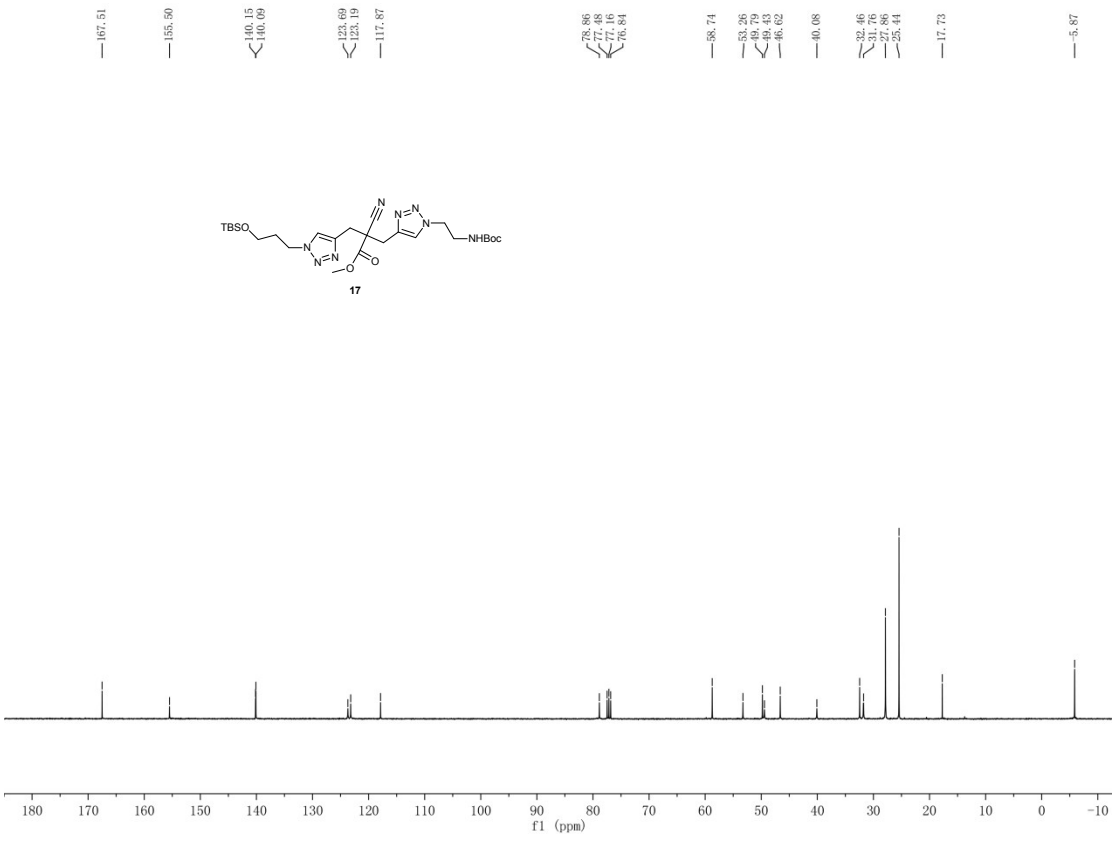
2.24
9.09
9.03
6.00
f1 (ppm)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53.26
49.79
49.43
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25.44
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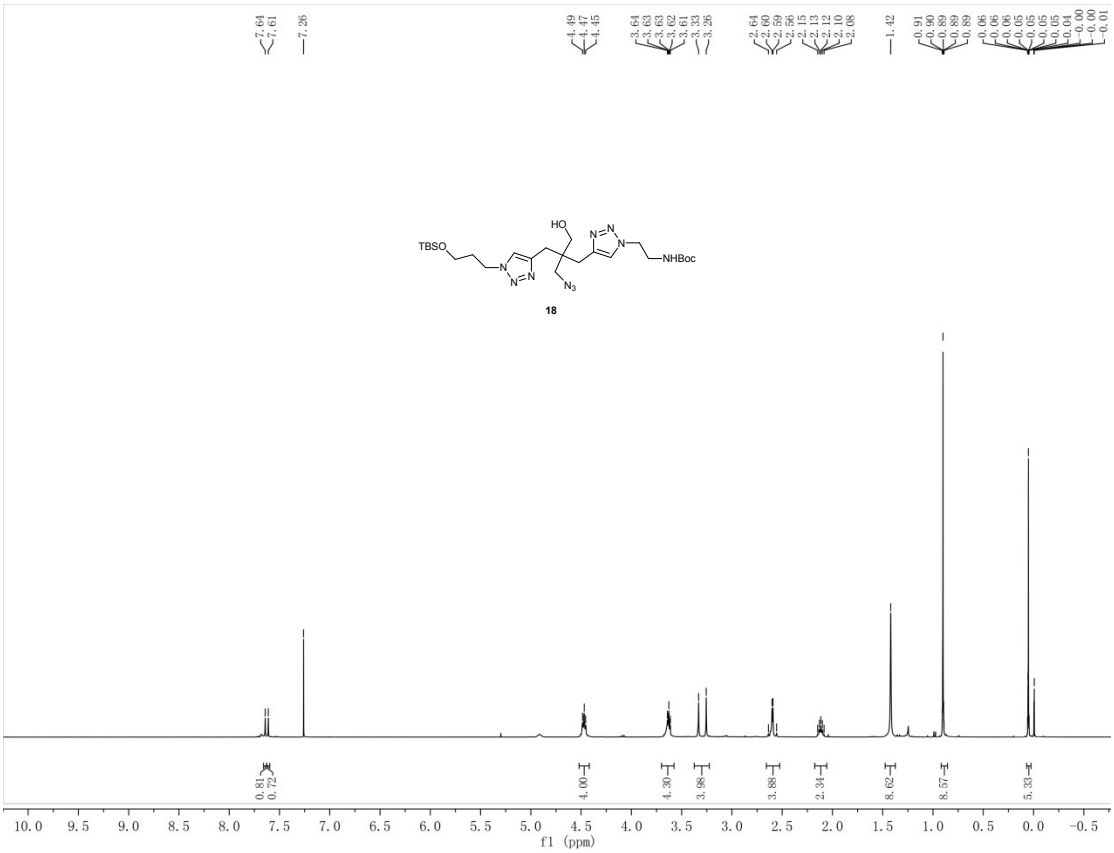
-5.87
TBSO
NHBoc
17
f1 (ppm)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2.59
2.56
2.15
2.13
2.12
2.10
2.08
1.42
0.91
0.90
0.89
0.89
0.89
0.06
0.06
0.06
0.05
0.05
0.05
0.05
0.04
0.00
0.00
0.01
HO
TBSO
NHBoc
N3
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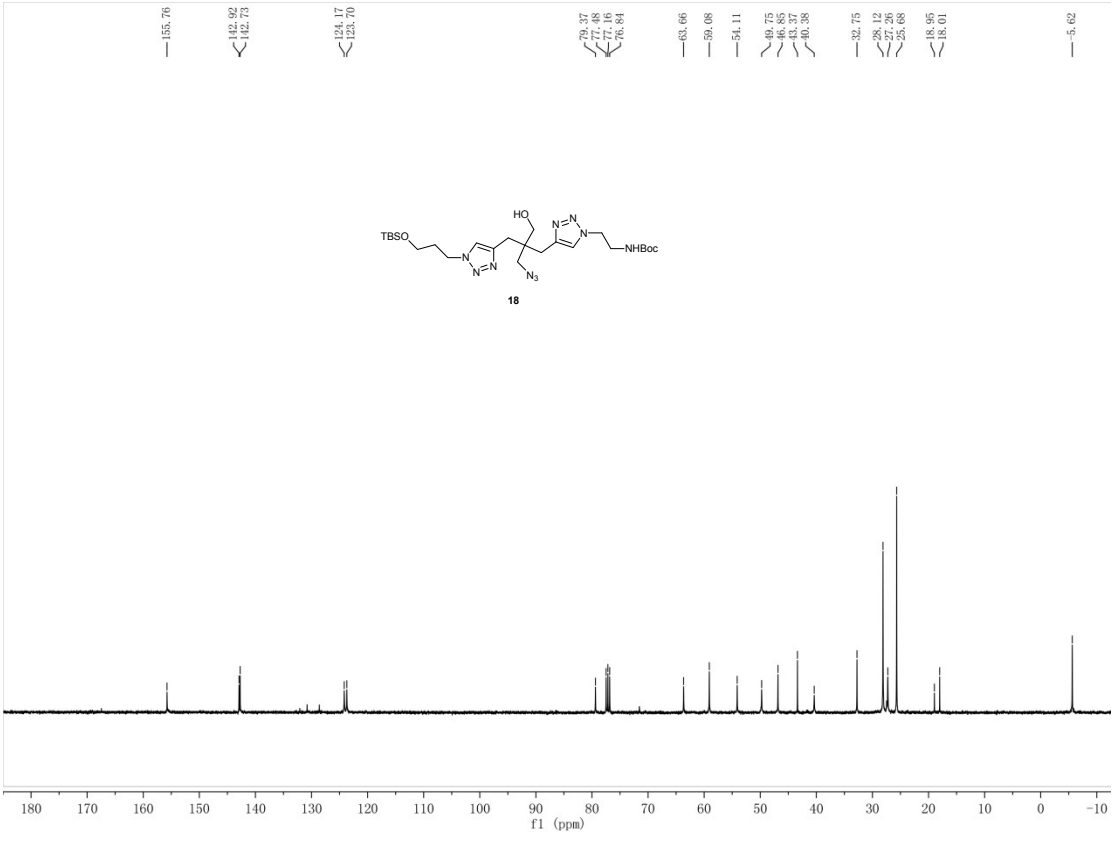
3.88
2.34
8.62
8.57
5.33
f1 (ppm)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63.66
59.08
54.11
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43.37
40.38
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25.68
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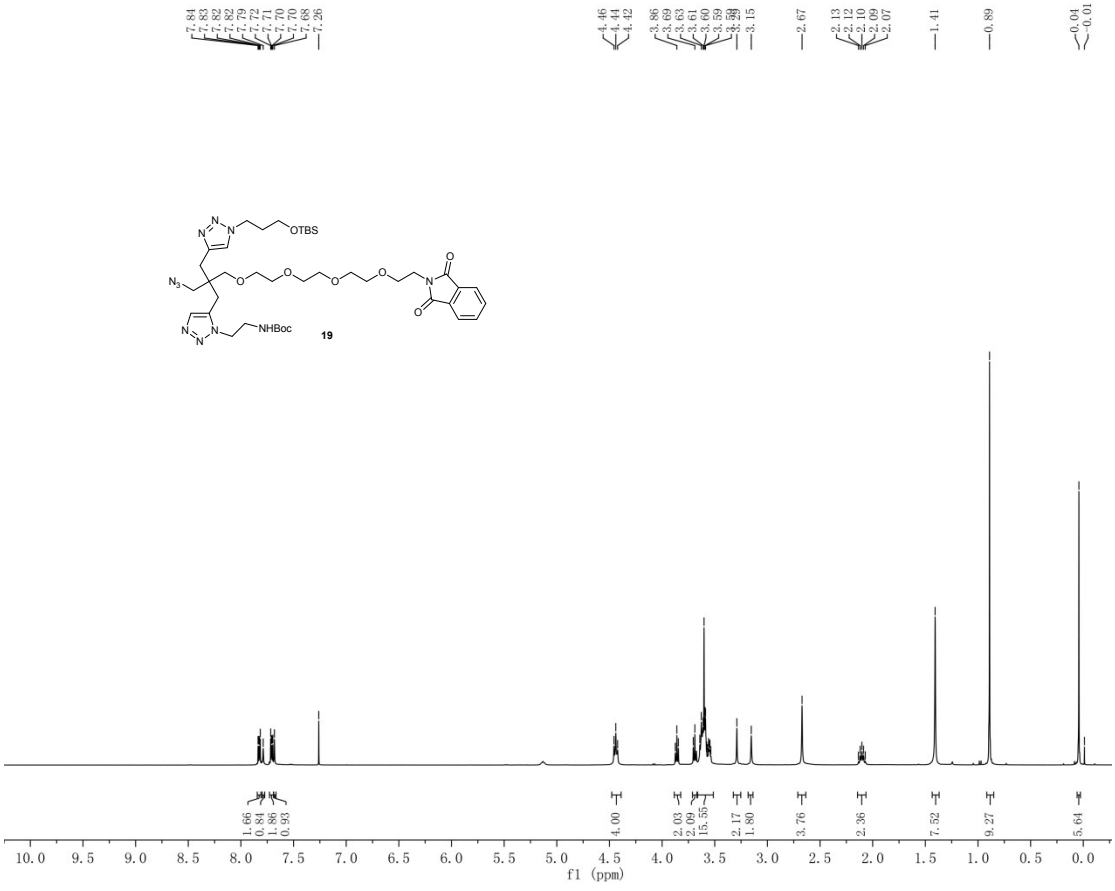
HO
TBSO
NHBoc
N3
18
f1 (ppm)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7.68
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4.42
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3.63
3.61
3.60
3.59
3.15
2.67
2.13
2.12
2.10
2.09
2.07
1.41
0.89
0.04
0.01
OTBS
N3
NHBoc
19
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1.86
0.93
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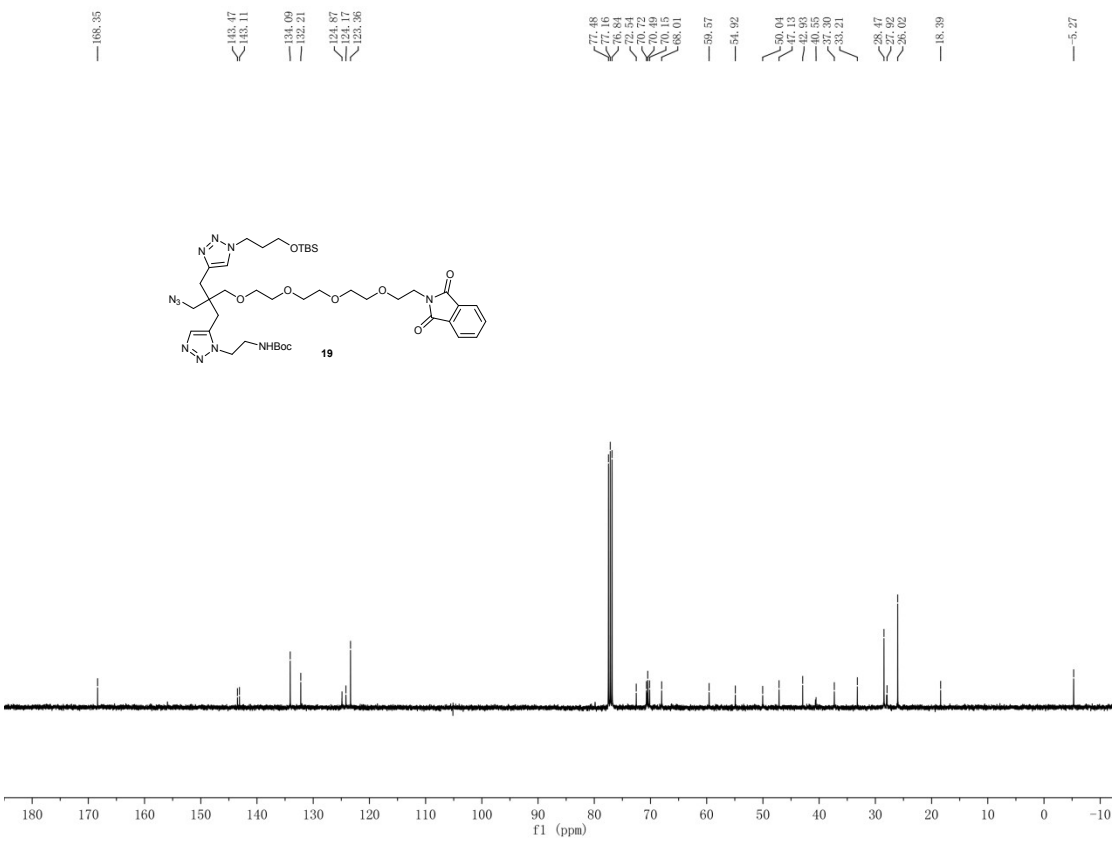
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70.49
70.15
68.01
59.57
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50.04
47.13
42.93
40.55
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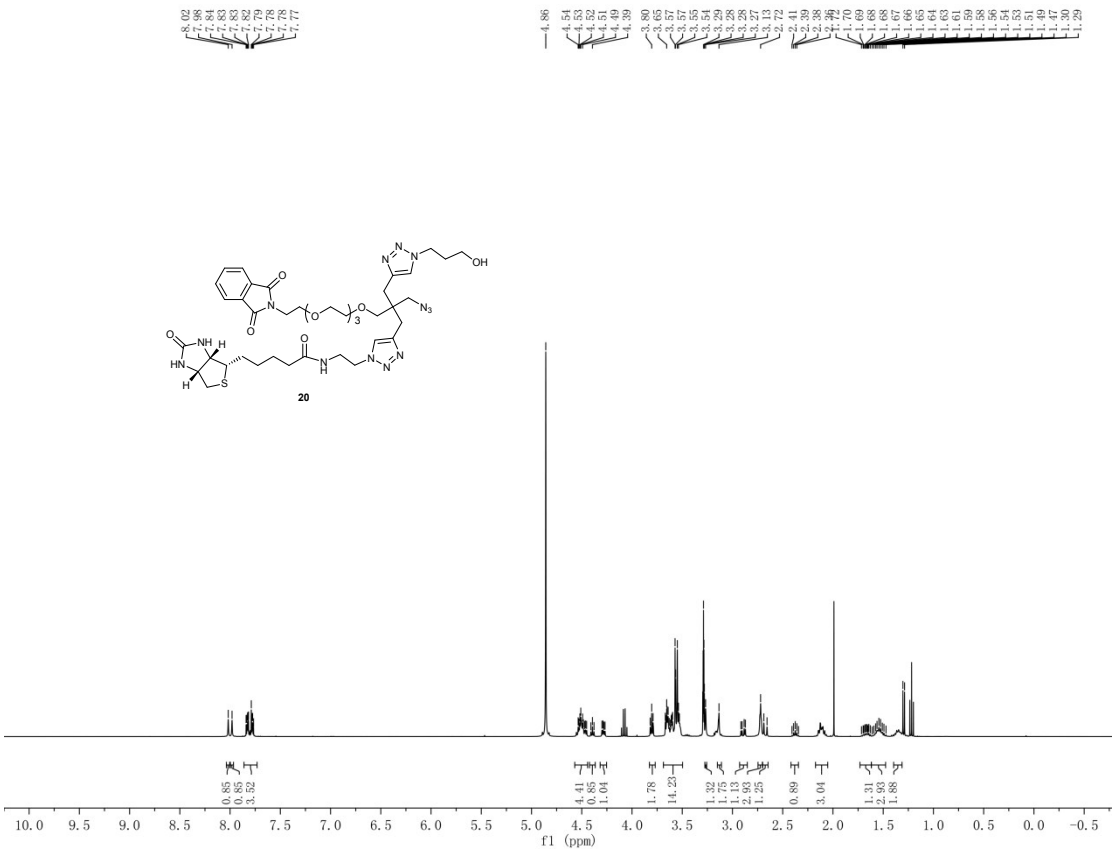
OTBS
N3
NHBoc
19
f1 (ppm)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4.53
4.52
4.51
4.49
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3.57
3.57
3.55
3.54
3.29
3.28
3.28
3.27
3.13
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1.68
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1.67
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1.59
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0.85
3.52
4.41
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1.31
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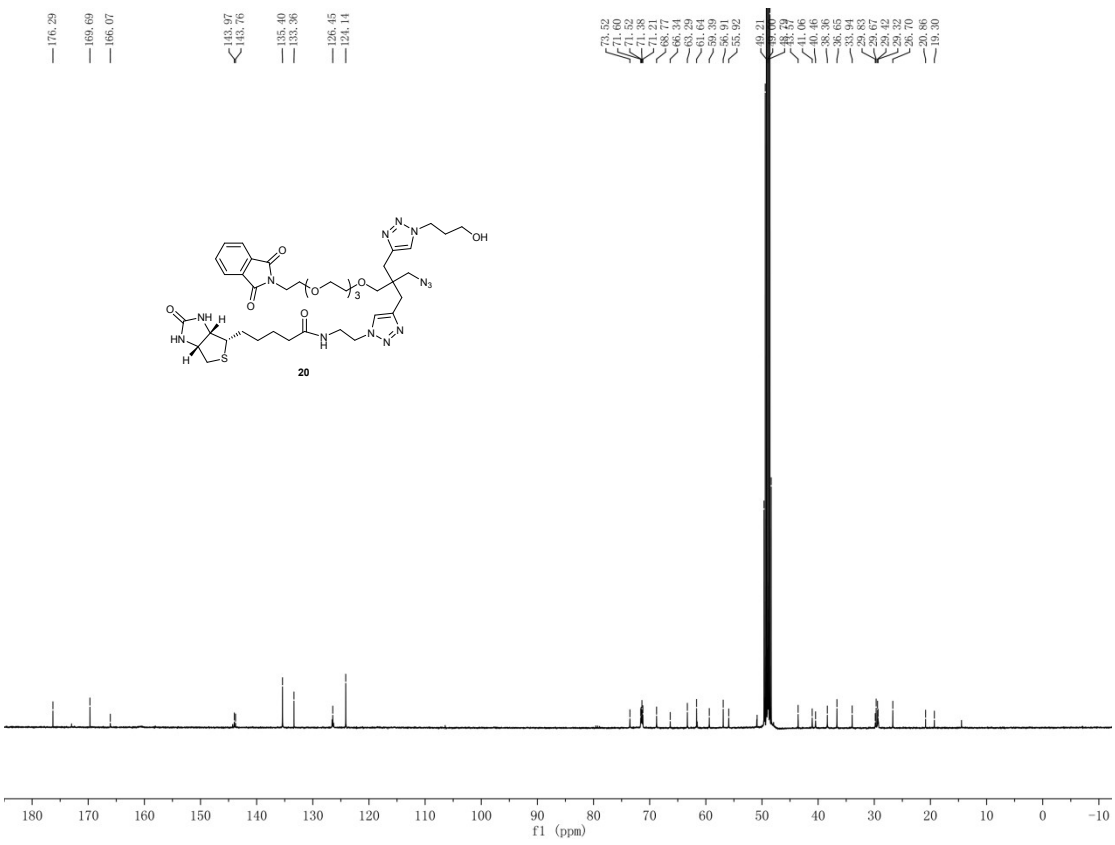
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68.77
66.34
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49.21
48.79
43.67
41.06
40.46
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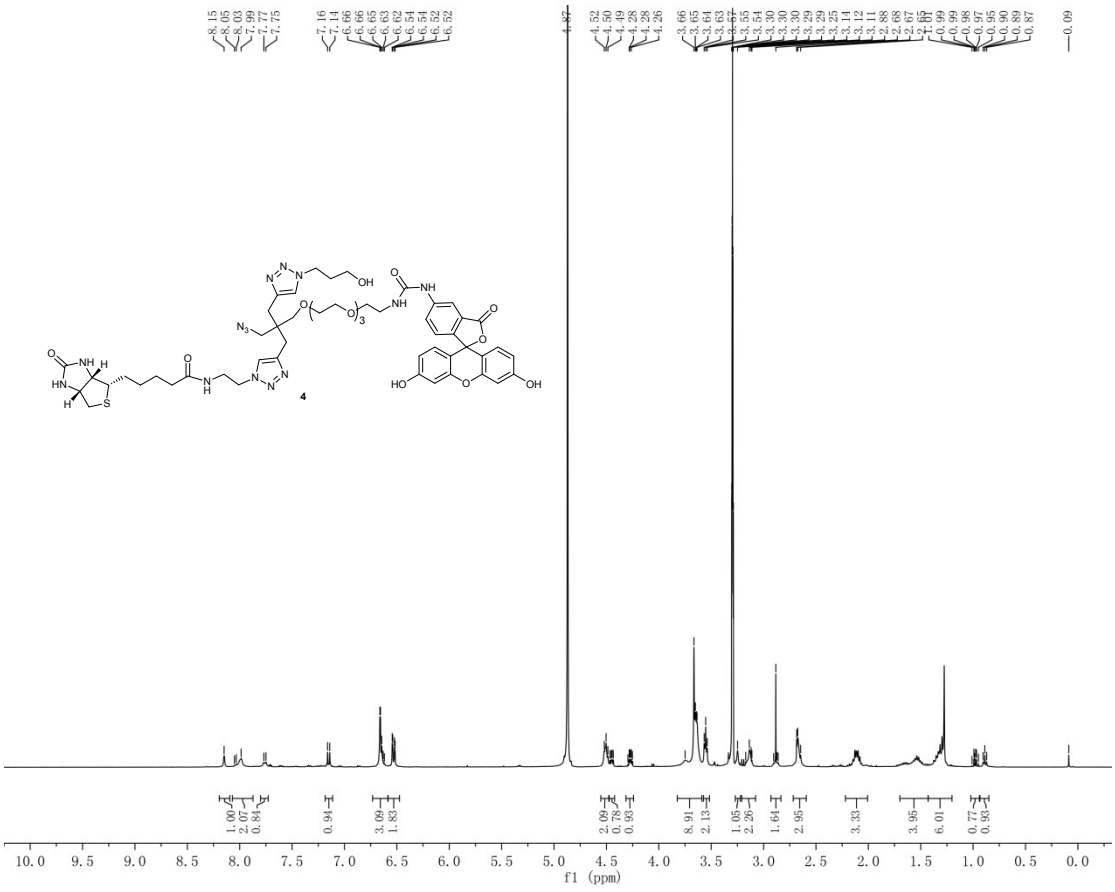
19.30
20
f1 (ppm)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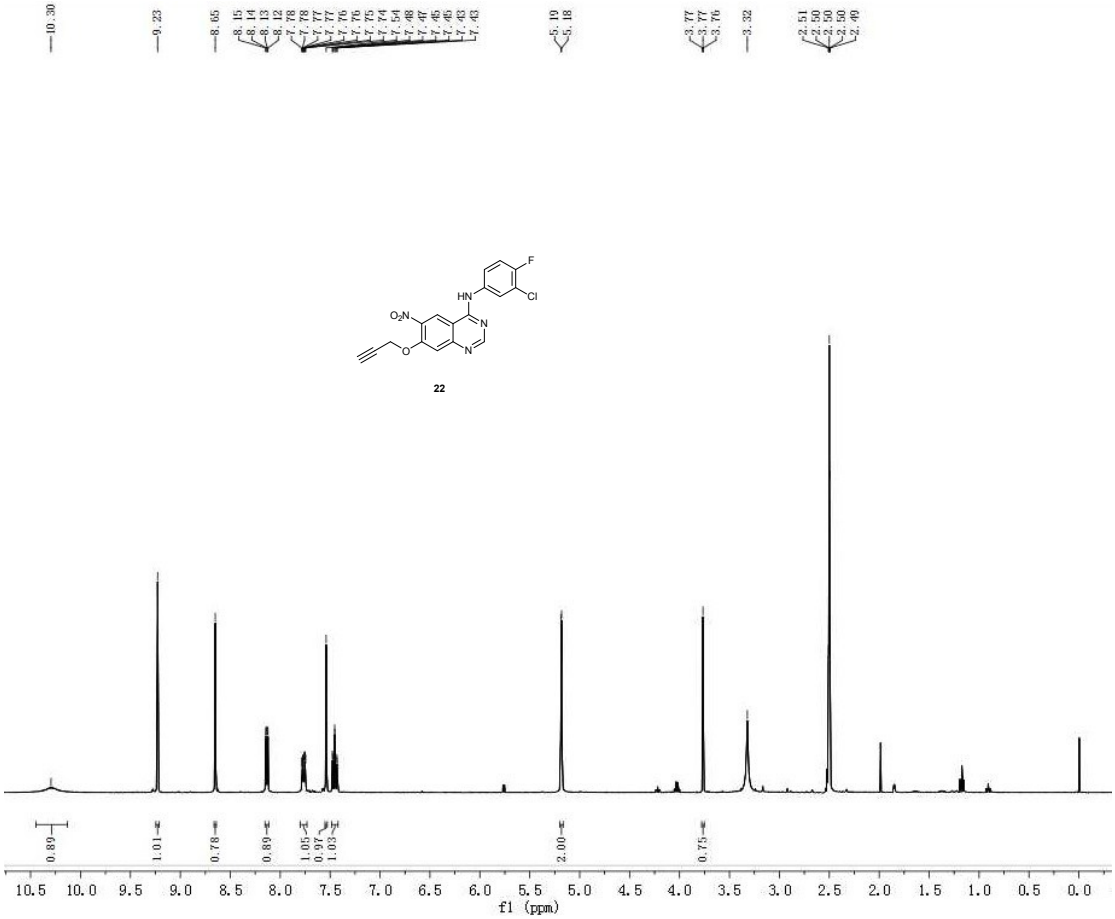
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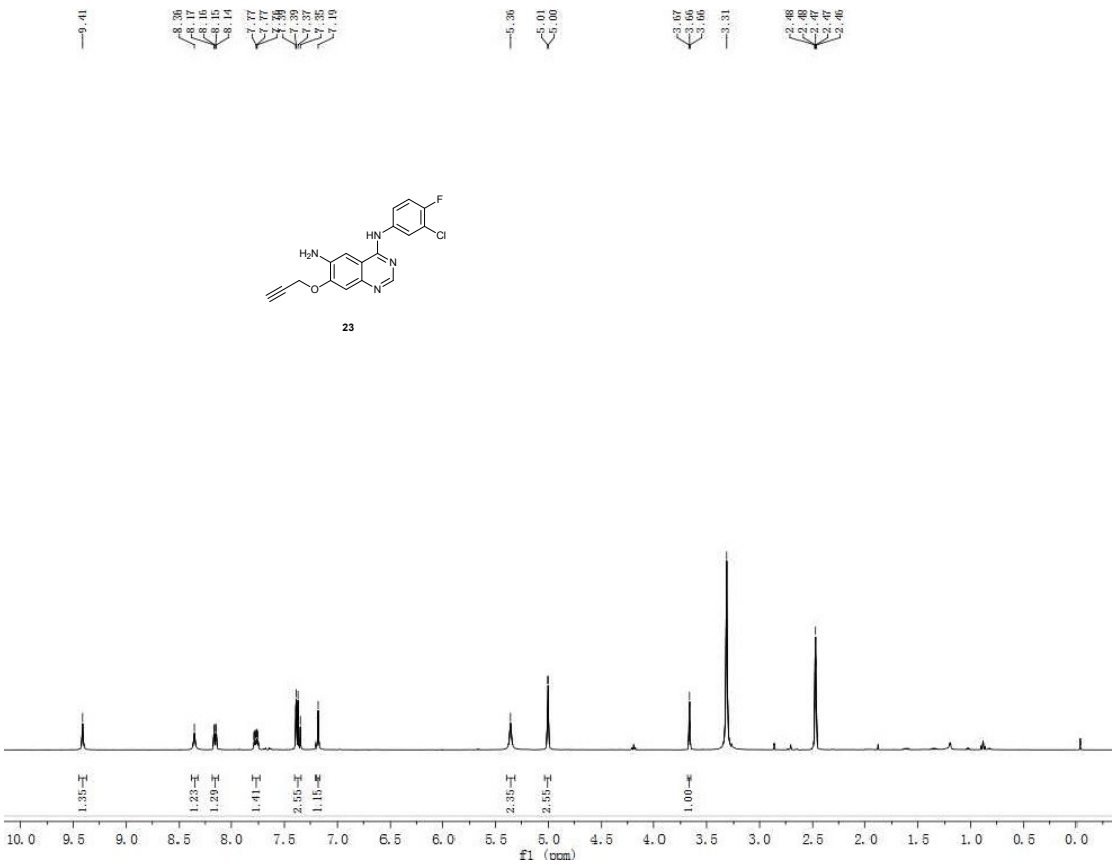
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7.35
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5.01
5.00
3.67
3.66
3.66
3.31
2.48
2.48
2.47
2.47
2.46
F
Cl
HN
N
N
O
H2N
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1.23
1.29
1.41
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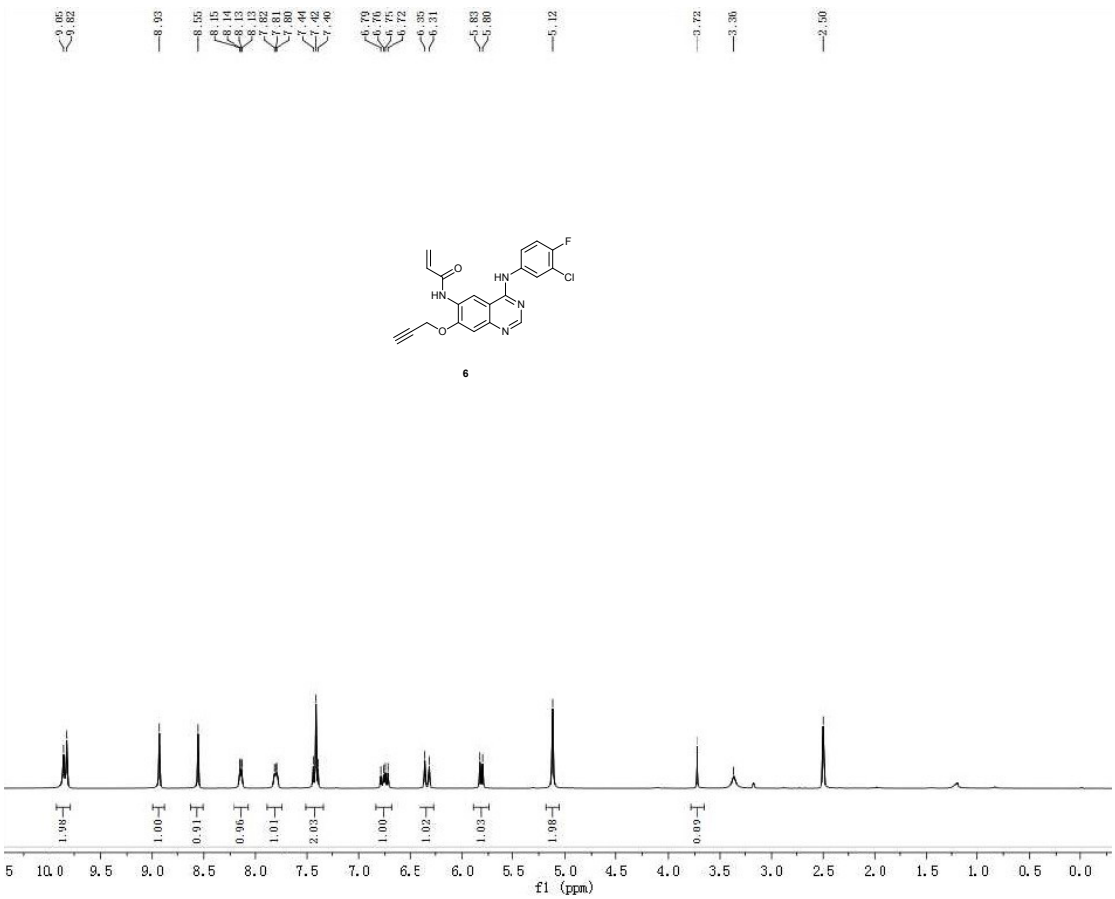
1.15
2.35
2.55
1.00
f1 (ppm)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6.76
6.75
6.72
6.35
6.31
5.83
5.80
5.12
3.72
3.36
2.50
F
Cl
HN
O
HN
N
N
O
6
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1.03
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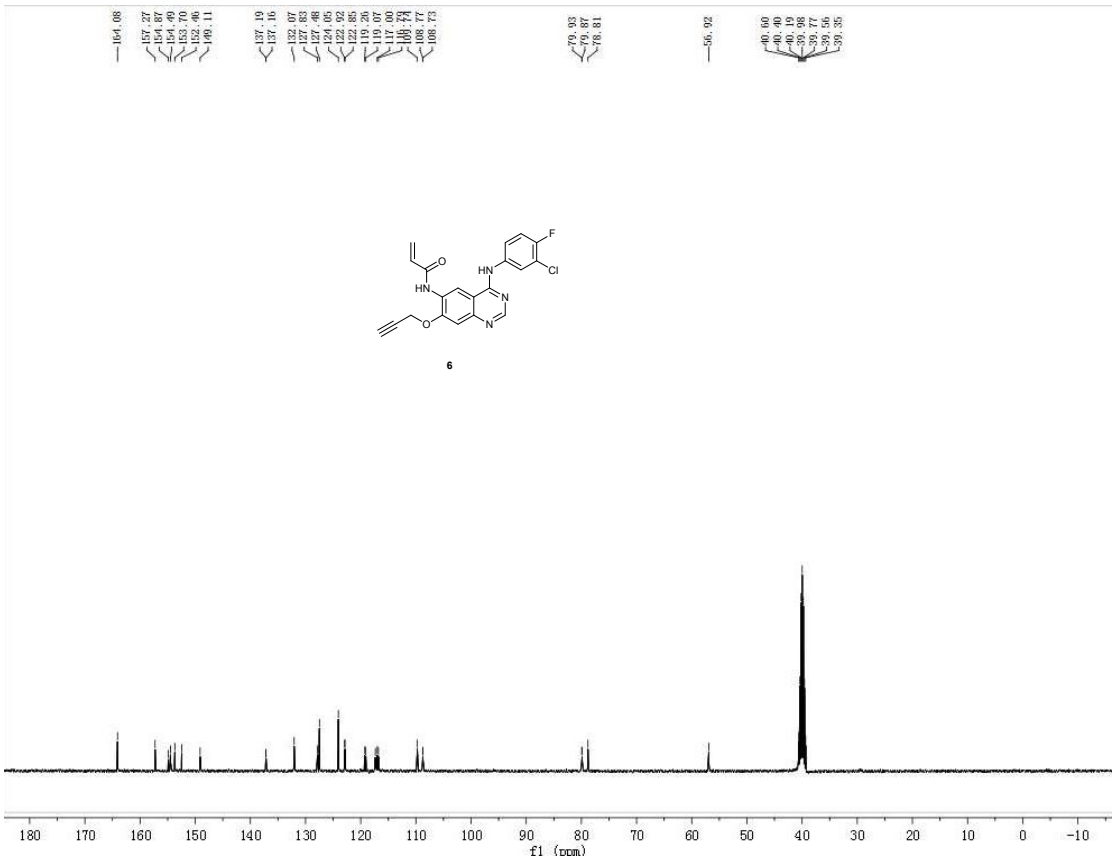
f1 (ppm)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122.85
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108.73
79.93
79.87
78.81
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40.60
40.40
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F
Cl
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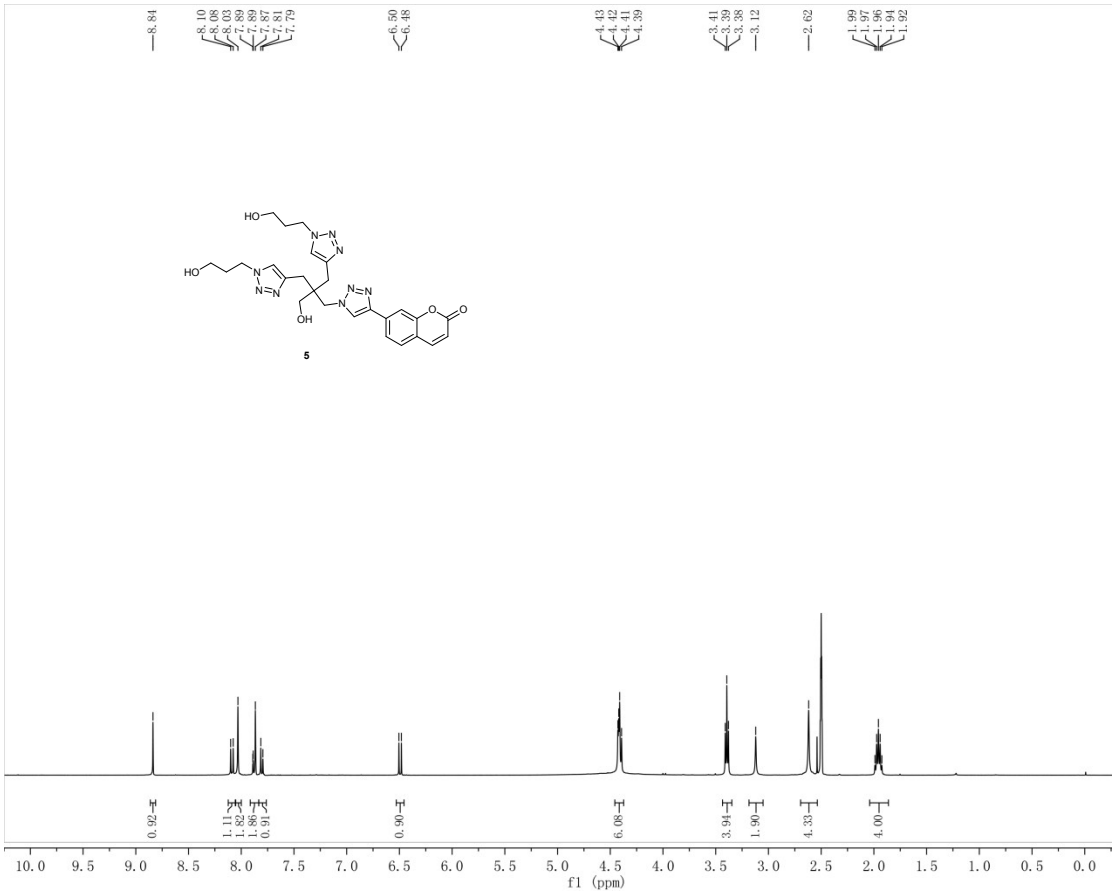
O
6
f1 (ppm)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4.41
4.39
3.41
3.39
3.38
3.12
2.62
1.99
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1.96
1.94
1.92
HO
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N
N
HO
N
N
N
OH
O
O
5
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6.08
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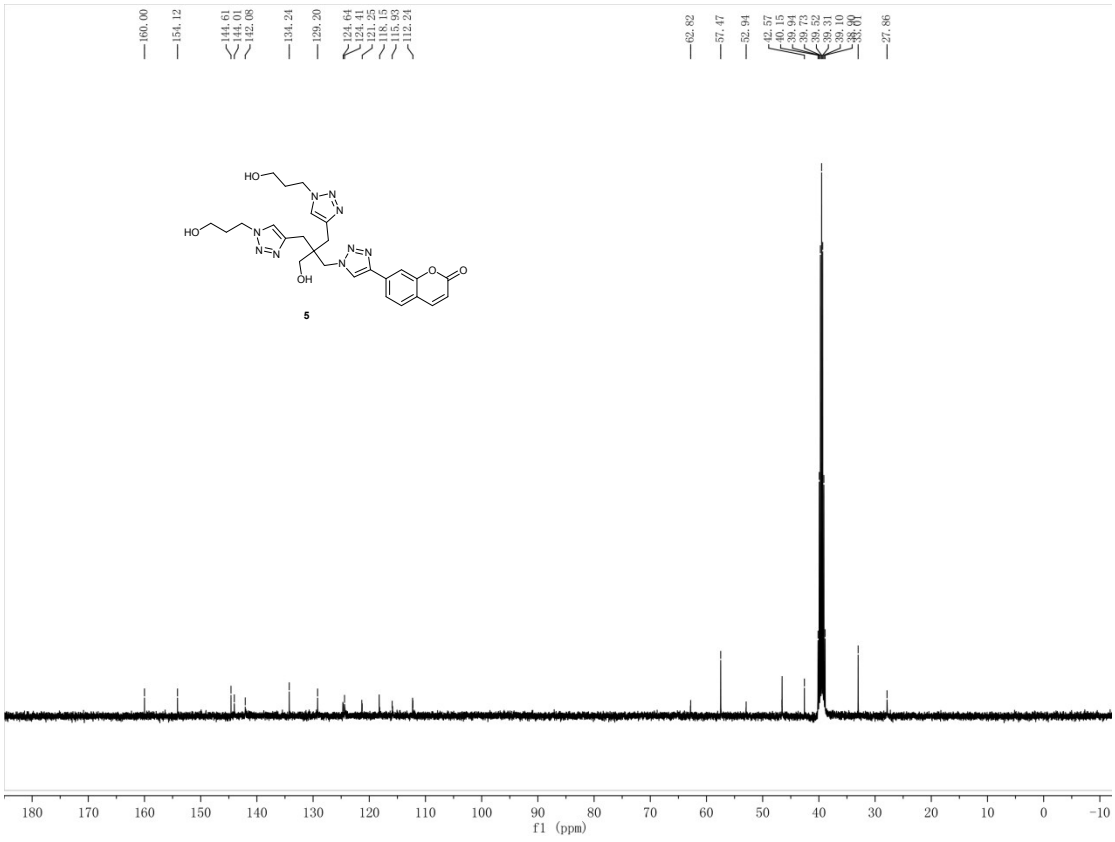
1.90
4.33
4.00
f1 (ppm)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42.57
40.15
39.94
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39.31
39.10
38.90
33.01
27.86
HO
HO
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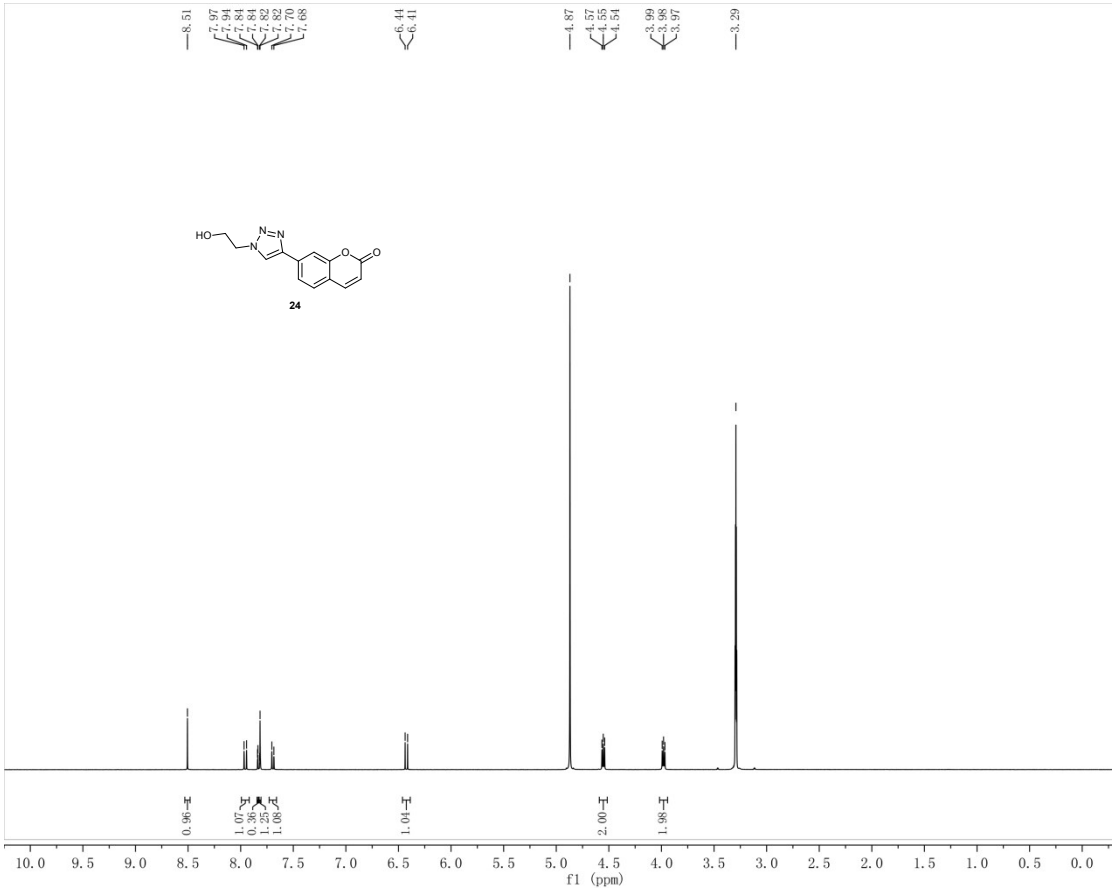
O
O
5
f1 (ppm)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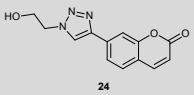
4.57
4.55
4.54
3.99
3.98
3.97
3.29
HO
O
O
24
0.96
1.07
0.36
1.25
1.06
1.04
2.00
1.98
f1 (ppm)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N
N
O
O

24


8.18
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4.68
4.51
4.49
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4.41
3.57
3.55
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2.66
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2.13
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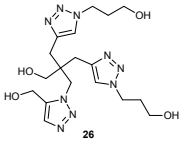
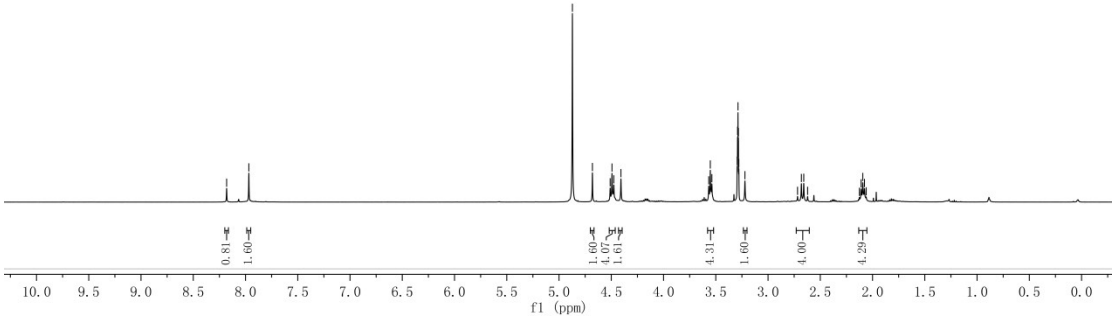
2.06


OH
HO
HO
OH

26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8.0
7.5
7.0
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6.0
5.5
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1.0
0.5
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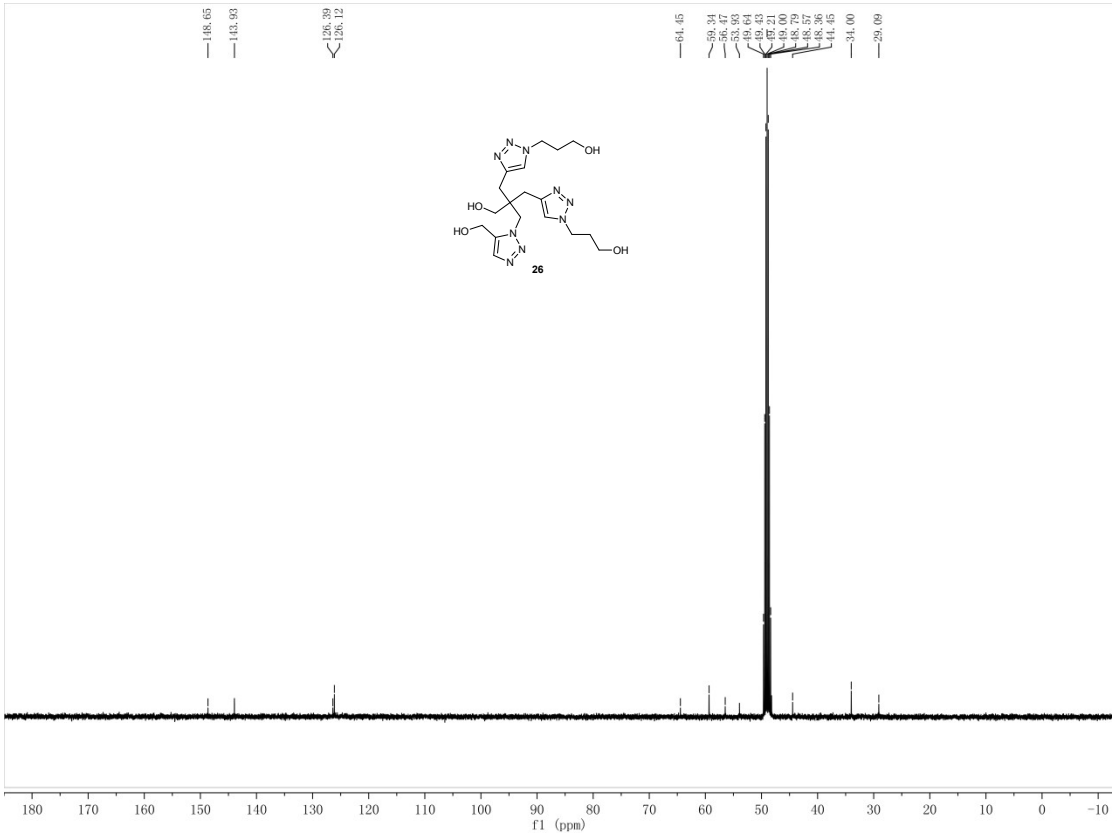
f1 (ppm)



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