Supporting Information for:

Biocompatible Copper(I) Catalysts for in Vivo Imaging of Glycans

David Soriano del Amo,[†] Wei Wang,[†] Hao Jiang,[†] Christen Besanceney,[†] Amy C. Yan,[†] Matthew Levy,[†] Yi Liu,[‡] Florence L. Marlow[#] and Peng Wu[†*]

[†]Department of Biochemistry and [#]Developmental and Molecular Biology, Albert Einstein College of Medicine, Yeshiva University, 1300 Morris Park Ave, Bronx, NY 10461

[‡]The Molecular Foundry, Lawrence Berkeley National Laboratory, One Cyclotron Road, MS 67R6110, Berkeley, CA 94720

[*]Correspondence: Peng Wu

Email: peng.wu@einstein.yu.edu

Materials and methods

All chemical reagents and solvents were obtained from Sigma-Aldrich and Acros and used without further purification unless otherwise noted. Flash chromatography was performed using a Sorbent 60 Å 230- to 400-mesh silica gel. Analytical thin layer chromatography (TLC) was performed on glass-backed Analtech Uniplate silica gel plates, and compounds were visualized by staining with *p*-anisaldehyde or phosphomolybdic acid or $KMnO_4$ stain. Organic extracts were dried over anhydrous $MgSO_4$ or Na_2SO_4, and the drying agent was removed by gravity filtration. Unless otherwise specified, all solvents were removed under reduced pressure, using a rotary evaporator. Rhodamine-dextran, Alexa Fluor dye-conjugated azides, alkynes and streptavidin were purchased from Invitrogen. Frosted microscope slides and wide-bore Pasteur pipets were purchased from Fisher Scientific and 18 × 18 mm square cover glasses were purchased from Corning. Melting point (mp) was taken on a Melt-Temp (Laborotary Device USA) apparatus using a Traceable® digital thermometer without calibration. RP-HPLC purifications were performed using a LC-6AA Shimadzu high performance liquid chromatographer equipped with a SPD-M20A Prominence Diode Array Detector and a Shimadzu Epic C18 column (ID: 23 mm). NMR spectra were obtained with Bruker DRX 300 and 600 spectrometers. ^1H chemical shifts (δ) are referenced to residual protic solvent (D_2O, 4.79 ppm; $CDCl_3$, 7.26 ppm) and coupling constants (*J*) are reported in hertz (Hz). ^{13}C NMR spectra were recorded at 75 MHz and proton decoupled. Electrospray ionization mass spectra (ESI-MS) were obtained at the Albert Einstein Laboratory for Macromolecular Analysis and Proteomics. Kinetic measurements using propargyl alcohol and 3-azido-7-hydroxycoumarin as the model

system were performed using a 96-well BioTek Synergy Hybrid Plate Reader. Microinjections were performed using a PV 820 Pneumatic PicoPump (World Precision Instruments) under Nikon SMZ1500 with lens Plan Apo 1× WD70.

Flow cytometry

Flow cytometry experiments were performed on a Becton Dickinson FACScan analog bench top analyzer flow cytometer using a 488 nm argon laser. At least 18000 cells were recorded for each sample. Flow cytometry data were analyzed using Flowjo. Mean fluorescence intensity (MFI) was calculated for live cells. Cell viability was ascertained by gating the sample on the basis of forward scatter (to sort by size) and FL3 (to sort by 7-AAD negative). Scatter plots are shown for the total cell population (**Figure S5**–**S9**).

Image acquisition and analysis

Whole animal images were acquired with an Olympus SZ16 fluorescent or SZ61 dissecting microscope equipped with a digital camera and pictureframe software. Confocal fluorescent images were acquired sequentially on a Leica SP5 AOBS confocal microscope with a 10×/0.4 air objective. A 488 nm Ar laser with a 505–550 nm bandpass filter for Alexa Fluor 488 was used. All embryo images were acquired using a 5 μm step size and 1 airy unit. Composite figures were prepared using ImageJ, Photoshop CS2 and Illustrator CS2 software (Adobe). Movies were assembled using ImageJ.

Tissue culture/cell growth conditions

Jurkat and LNCaP cells were grown in RPMI 1640 medium supplemented with 10% FCS (Sigma). HEK 293T cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS. Pro^{-}5 CHO cells were grown in suspension or monolayer in alpha-Minimum Essential medium supplemented with 10% FCS. In all cases, cells were incubated in a 5.0% carbon dioxide, water-saturated incubator at 37 °C.

Zebrafish husbandry and strains

Zebrafish husbandry was carried out in accordance with the Animal Institute protocol of the Albert Einstein College of Medicine. Casper mutant (a double mutant line that lacks melanocytes and iridophores described previously[1]) or AB/Tü wild-type strains were used in this study. Clutches of homozygous double mutant (*casper)* embryos were obtained by intercrossing homozygous adults that are double for mutations in *nacre,*[2] and *roy orbison (roy).*[1]

Synthetic procedures

Ac_4ManNAc,[3] Ac_4ManNAl,[4] Ac_4ManNAz,[3] GDP-Fuc[5], GDP-FucAl,[5] biotin-azide,[6] biotin-alkyne,[7] *tert*-butyl azide[8] were synthesized as previously described. The dendrimer precursor of ligand **15** was a gift from Prof. Craig Hawker. All final compounds were purified by either reverse phase HPLC or Bio-Gel P2 gel (Bio-Rad) filtration chromatography. The synthesis of other members of the ligand library (SI Fig. 1) will be reported in separated publications.

Synthesis of 1-*tert*-butyl-4-(diethoxymethyl)-1*H*-1,2,3-triazole

To a 20-mL screw-capped scintillation vial equipped with a stirring bar were added 3,3-diethoxy-1-propyne (1.50 g, 11.8 mmol, 1.0 eq) and *tert*-butyl azide (1.34 g, 13.5 mmol, 1.15 eq) in 10 mL 1:1 mixture of *tert*-butyl alcohol and water. Sodium bicarbonate (1.40 g, 16.7 mmol, 1.41 eq), copper(II) sulfate pentahydrate (0.143 g, 0.57 mmol, 5.0 mol%), and sodium ascorbate (0.47 g, 2.35 mmol, 20 mol%) were added to the mixture. The reaction was stirred vigorously overnight, and TLC analysis indicated the formation of a new product (R_f = 0.7 in ethyl acetate, $KMnO_4$ stain). EDTA (2 mL, 0.5 M, pH = 8) was added, the reaction mixture was diluted with EtOAc (90 mL), washed with sat aq $NaHCO_3$ (2 × 50 mL), water (2 × 10 mL), and brine (30 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concd *in vacuo* to provide 2.54 g of a light yellow oil (yield: 95%). The crude product was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 5.69 (s, 1H), 3.76-3.55 (m, 4H), 1.65 (s, 9H), 1.23 (t, J = 7.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) 146.5, 118.9, 97.2, 61.9, 59.4, 30.1, 15.2; HRMS calcd for $[M+Na]^+$ $C_{11}H_{21}N_3NaO_2$ 250.1531, found 250.1531.

Synthesis of 1-*tert*-butyl-1*H*-1,2,3-triazole-4-carbaldehyde

To a 50-mL round bottom flask was added a solution of 1-*tert*-butyl-4-(diethoxymethyl)-1*H*-1,2,3-triazole (1.28 g, 5.63 mmol) in dichloromethane (6.0 mL), followed by addition of water (3.0 mL) and trifluoroacetic acid (1.0 mL). The reaction was stirred vigorously under nitrogen for 3 h until TLC analysis indicated the complete disappearance of the starting material (10%

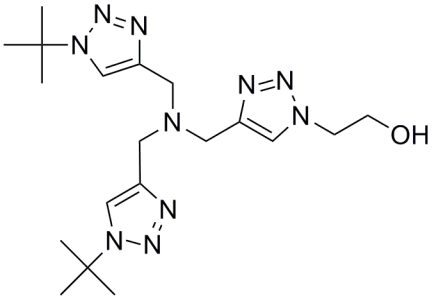
EtOAc in dichloromethane, starting material R_f 0.5, product R_f 0.6, $KMnO_4$ stain). The reaction mixture was diluted with EtOAc (100 mL), washed with sat aq $NaHCO_3$ (3 × 40 mL) and brine (40 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concd *in vacuo* to provide 0.71 g of a light yellow oil (yield: 82%). The crude product was used without purification.

Synthesis of *N*,*N*-bis((1-*tert*-butyl-1*H*-1,2,3-triazol-4-yl)methyl)prop-2-yn-1-amine

To a 250-mL round bottom flask was added a solution of 1-*tert*-butyl-1*H*-1,2,3-triazole-4-carbaldehyde (2.47 g, 16.1 mmol, 2.2 eq) in dichloroethane (84 mL, ~0.2 M), followed by addition of propargyl amine (361 mg, 7.2 mmol, 1.0 eq). To this mixture sodium triacetoxyborohydride (3.8 g, 17.9 mmol, 2.5 eq) was added in one portion with vigorous stirring. The reaction mixture was stirred at room temperature for 40 h. 1N H_2SO_4 (86 mL) was added to the reaction, and the mixture was stirred for 15 min. The pH was adjusted to >10 by addition of potassium carbonate. The reaction mixture was diluted with water (100 mL) and extracted with dichloromethane (3 × 300 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concd *in vacuo* to provide a crude product. Further purification by flash chromatography (100 g silica gel, 20% hexanes in EtOAc, R_f 0.2, $KMnO_4$ stain) provided 1.98 g of product (yield: 84%) as a white powder. mp 125.5-126.1 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (s, 2H), 3.87 (s, 4H), 3.40 (d, *J* = 2.4 Hz, 2H), 2.28 (t, *J* = 2.4 Hz, 1H), 1.67 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.6, 120.2, 78.8, 73.5, 59.2, 47.8, 42.2, 30.0; HRMS calcd for $[M+H]^+$ $C_{17}H_{28}N_7$

330.2406, found 330.2400.

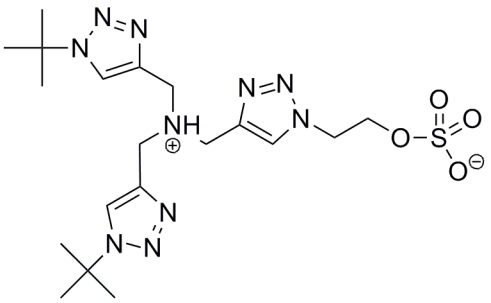
Synthesis of 2-(4-((bis((1-*tert*-butyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)ethanol:



To a 20-mL screw-capped vial equipped with a stirring bar were added *N,N*-bis((1-*tert*-butyl-1*H*-1,2,3-triazol-4-yl)methyl)prop-2-yn-1-amine (1.0 g, 3.04 mmol, 1.0 eq) and 2-azidoethanol (304 mg, 3.49 mmol, 1.15 eq) in THF (16 mL). To the mixture were added copper(I) acetate (20 mg, 0.16 mmol, 5.0 mol%) and sodium ascorbate (130 mg, 0.66 mmol, 20 mol%). The reaction mixture was stirred vigorously with the cap closed at 60 °C overnight. To the reaction mixture were added water (2 mL) and CupriSorb^{TM} (~0.2 g). The mixture was stirred for 30 additional min and then filtered. The crude product was concd *in vacuo* and was purified by RP-HPLC (solvent A: 0.1% TFA in H_2O; solvent B: 0.1% TFA in acetonitrile; method: 20% B to 100% B over 20 min). HPLC fractions containing the product (RT: 14 min–15.5 min) were combined and lyophilized to provide 1.2 g (95%) of product as a tan solid. mp 82.0-83.0 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.94 (s, 2H), 7.92 (s, 1H), 4.51 (t, J = 5.1 Hz, 2H), 3.96 (t, J = 5.2 Hz, 2H), 3.83 (s, 2H), 3.81 (s, 4H), 1.61 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.8, 143.3, 125.9, 123.0, 60.6, 60.5, 52.8, 48.4, 39.1, 29.3; HRMS calcd for $[M+H]^+$ $C_{19}H_{33}N_{10}O$ 417.2839, found 417.2839.

Synthesis of 2-(4-((bis((1-tert-butyl-1H-1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-

1,2,3-triazol-1-yl)ethanesulfonic acid (BTTES, 2)



To a solution of 2-(4-((bis((1-*tert*-butyl-1*H*-1,2,3-triazol-4-yl)methyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)ethanol (100 mg, 0.24 mmol, 1.0 eq) in pyridine (5 mL, ~0.05 M) was added sulfur trioxide pyridine complex (190 mg, 1.2 mmol, 5.0 eq). The reaction was stirred at 50 °C under argon overnight. Upon cooling to room temperature methanol (1 mL) was added. Stirring continued for an additional 30 min. The volatiles were then removed *in vacuo* to yield a solid that was purified via RP-HPLC (solvent A: 0.1% TFA in H_2O; solvent B: 0.1% TFA in acetonitrile; method: 20% B to 100% B over 20 min). HPLC fractions containing the product (RT: 14 min–15.5 min) were pooled and lyophilized to provide 86 mg of **2** white powder (75%). mp 160 °C (dec); ^{1}H NMR (300 MHz, D_2O) δ 7.99 (s, 1H), 7.96 (s, 2H), 4.73 (d, J = 4.8 Hz, 2H), 4.41 (d, J = 4.8 Hz, 2H), 3.84 (s, 2H), 3.81 (s, 4H), 1.62 (s, 18H); ^{13}C NMR (75 MHz, D_2O) δ 143.8, 143.3, 126.2, 123.0, 67.0, 60.6, 50.0, 48.4, 48.2, 29.3; HRMS calcd for $[M+H]^+$ $C_{19}H_{33}N_{10}O_4S$ 497.2407, found 497.2437.

A pH 7.0, 20 mM solution of the HPLC purified BTTES was prepared by dissolving the material in water, and neutralizing with NaOH. This stock was used for all kinetics and in vivo labeling experiments.

Protocol for kinetic measurement of CuAAC of 3-azido-7-hydroxycoumarin and propargyl alcohol[9]

Stock solutions:

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

Ligand: 20 mM in water for monomeric tris(triazolylmethyl)amine-based ligands; 10 mM in water for dimeric tris(triazolylmethyl)amine-based ligands

Sodium ascorbate: 25 mM in water

Azido coumarin: 1.0 mM in 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

Ligand: ligand to copper ratio is 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1

DMSO: 5%vol

Sodium ascorbate: 2.5 mM

Procedure for 200 μL reactions:

In a 96–well fluorescence plate, *add the reagents in the following order*:

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 (λ_{ex} = 404 nm, λ_{em} = 477 nm, RFU).

References:

1. White, R. M.; Sessa, A.; Burke, C.; Bowman, T.; LeBlanc, J.; Ceol, C.; Bourque, C.; Dovey, M.; Goessling, W.; Burns, C. E.; Zon, L. I., *Cell Stem Cell* **2008,** 2, 183-189.
2. Lister, J. A.; Robertson, C. P.; Lepage, T.; Johnson, S. L.; Raible, D. W., *Development* **1999,** 126, 3757-3767.
3. Laughlin, S. T.; Agard, N. J.; Baskin, J. M.; Carrico, I. S.; Chang, P. V.; Ganguli, A. S.; Hangauer, M. J.; Lo, A.; Prescher, J. A.; Bertozzi, C. R., *Methods Enzymol.* **2006,** 415, 230-250.
4. Chang, P. V.; Chen, X.; Smyrniotis, C.; Xenakis, A.; Hu, T.; Bertozzi, C. R.; Wu, P., *Angew. Chem. Int. Ed.* **2009,** 48, 4030-4033.
5. Wang, W.; Hu, T.; Frantom, P. A.; Zheng, T.; Gerwe, B.; Del Amo, D. S.; Garret, S.; Seidel, R. D., 3rd; Wu, P., *Proc. Natl. Acad. Sci. U. S. A.* **2009,** 106, 16096-16101.
6. Hang, H. C.; Yu, C.; Pratt, M. R.; Bertozzi, C. R., *J. Am. Chem. Soc.* **2004,** 126, 6-7.
7. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R., *J. Am. Chem. Soc.* **2004,** 126, 15046-15047.
8. Bottaro, J. C.; Penwell, P. E.; Schmitt, R. J., *Synthetic Commun.* **1997,** 27, 1465-1467.
9. Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G., *Angew. Chem. Int. Ed.* **2009,** 48, 9879-9883.

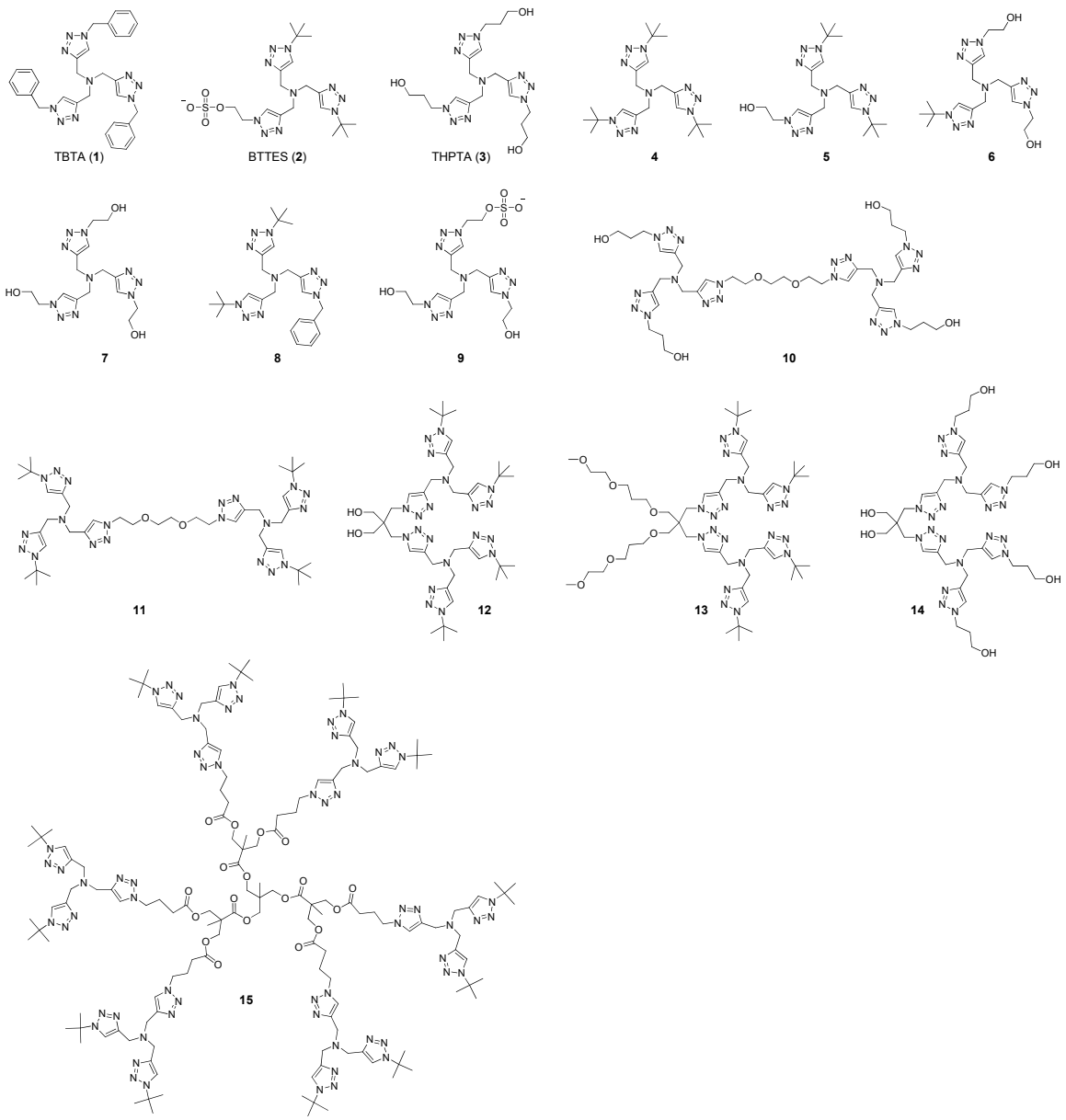


Figure S1: Structures of the library of TBTA analogues screened.

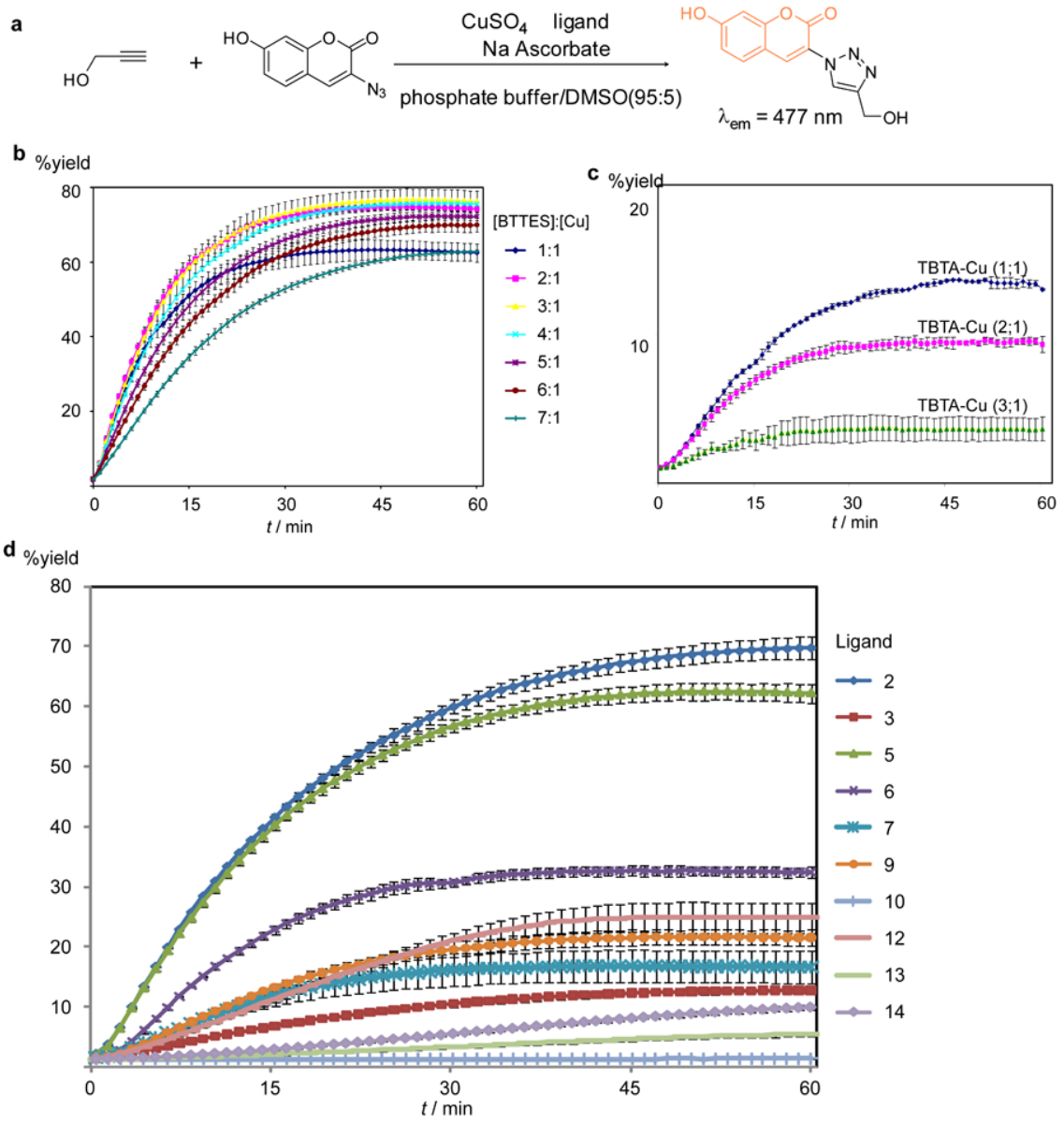


Figure S2: Effects of Cu(I)-stablizing ligands on the cycloaddition rate of CuAAC established using a fluorogenic assay. (**a**) The reaction scheme of the fluorogenic assay for screening CuAAC accelerating ligands. (**b**) Conversion–time profiles of CuAAC as a function of BTTES/Cu ratio. (**c**) Conversion–time profiles of CuAAC as a function of TBTA/Cu ratio. (**d**) Comparison of the efficiencies of rate-acceleration of different TBTA analogs ([ligand]:[Cu] = 6:1 for monovalent tris(triazolylmethyl)amine-based ligand and [ligand]:[Cu] = 3:1 for divalent tris(triazolylmethyl)amine-based ligand). Reaction conditions: propargyl alcohol (50 μM), 3-azido-7-hydroxycoumarin (100 μM), $CuSO_4$ (75 μM), 0.1 M potassium phosphate buffer (pH 7.0)/DMSO 95:5, sodium

ascorbate (2.5 mM), rt. Error bars represent the standard deviation of three replicate experiments.

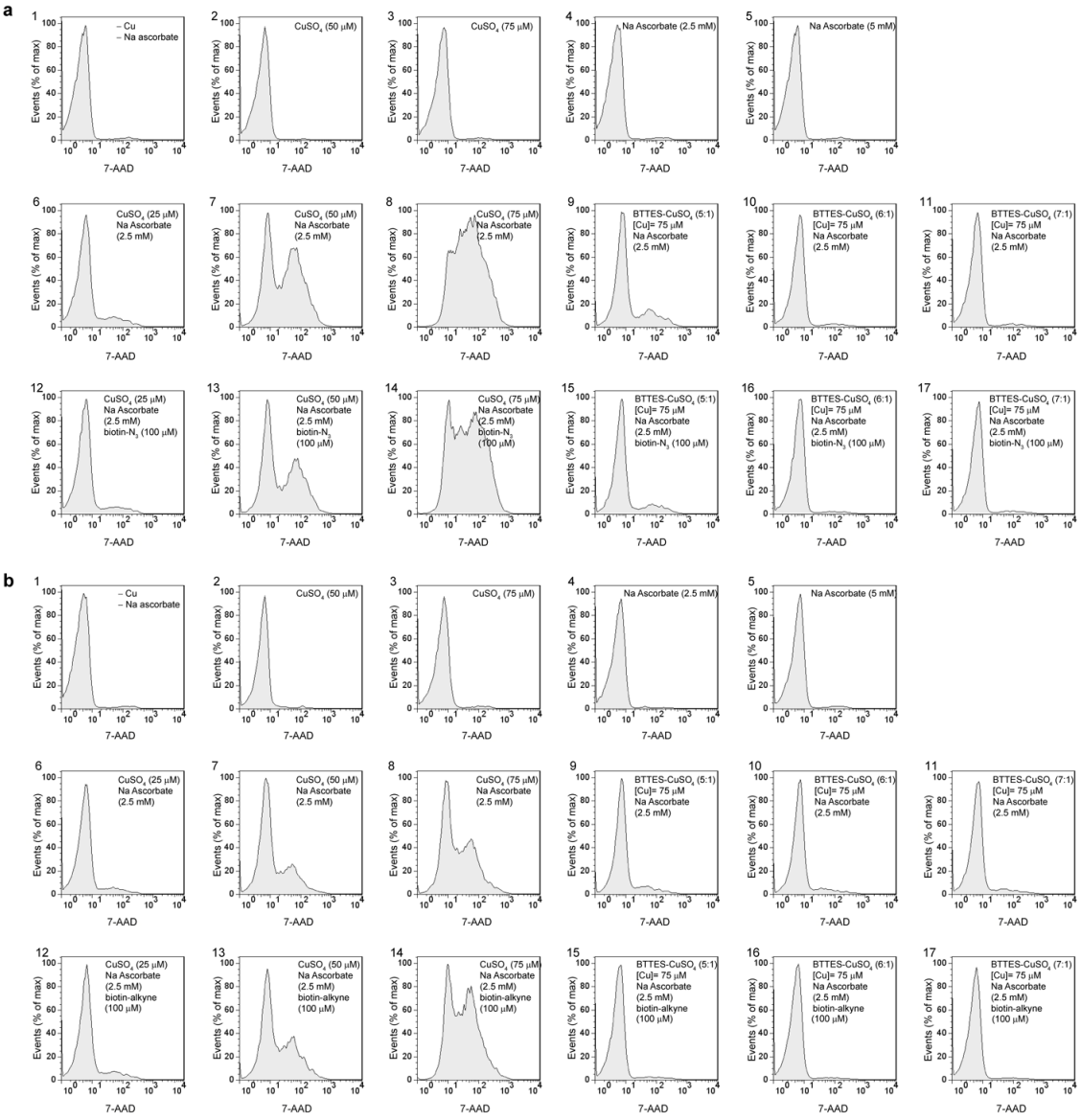


Figure S3: Cytotoxicity analysis of CuAAC (Jurkat cells). Jurkat cells were cultured in medium supplemented with 50 μM (**a**) Ac_4ManNAl or (**b**) Ac_4ManNAz. After 3 days, the cells were incubated with $CuSO_4$ (2, 3) or sodium ascorbate alone (4, 5) for 30 min or sodium ascorbate + $CuSO_4$ in the absence (6–8, 12–14) or presence (9–11, 15–17) of BTTES for 5 min (ligand and $CuSO_4$ were premixed). Reactions were quenched with

BCS. The cells were washed and diluted in FACS buffer supplemented with 2 μg/mL 7-AAD prior to flow cytometry analysis.

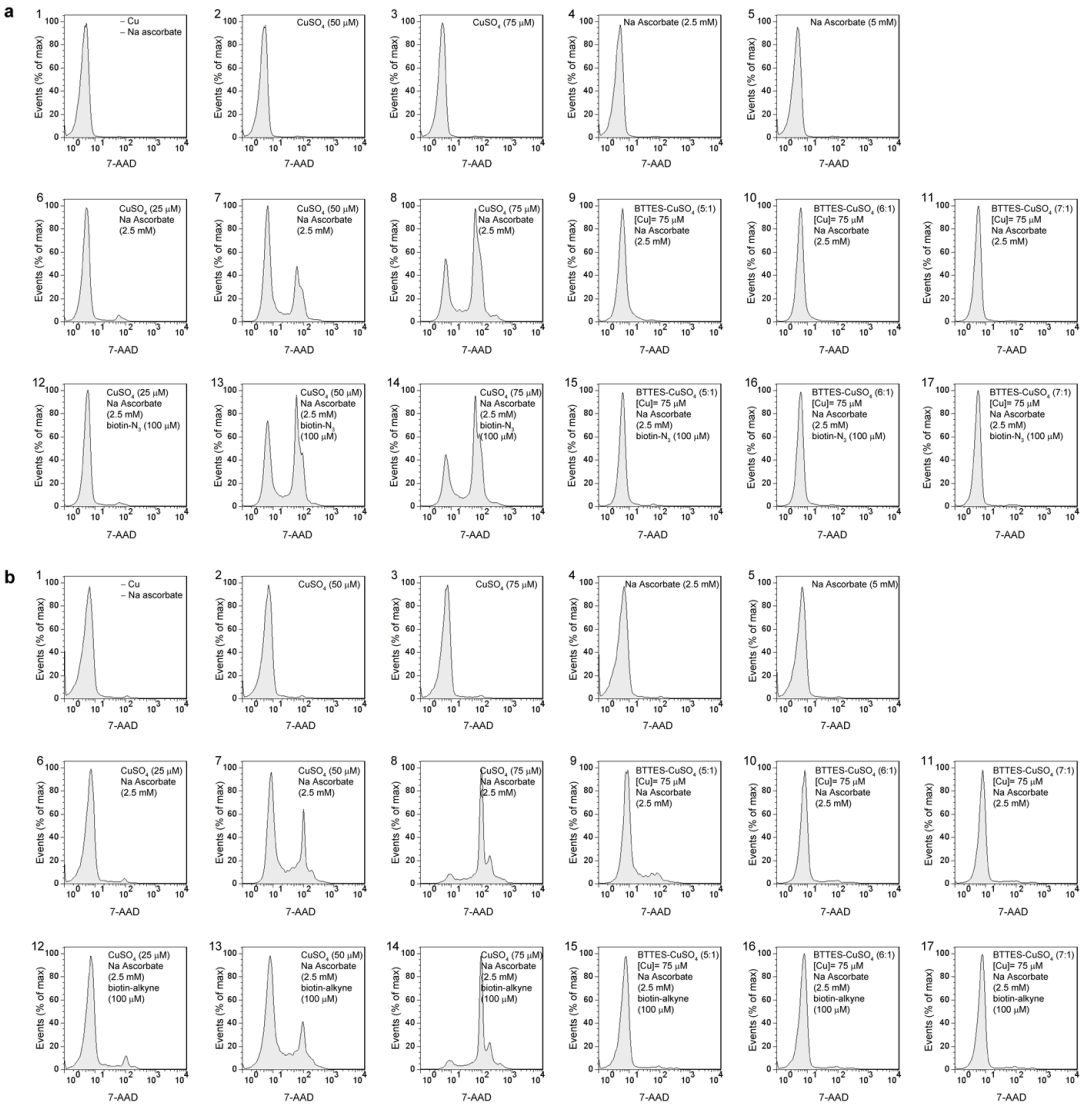


Figure S4: Cytotoxicity analysis of CuAAC (CHO cells). Pro$^-$5 CHO cells were cultured in medium supplemented with 50 μM (**a**) Ac_4ManNAl or (**b**) Ac_4ManNAz. After 3 days, the cells were incubated with $CuSO_4$ (2, 3) or sodium ascorbate alone (4, 5) for 30 min or sodium ascorbate + $CuSO_4$ in the absence (6–8, 12–14) or presence (9–11, 15–17) of BTTES for 5 min (ligand and $CuSO_4$ were premixed). Reactions were

quenched with BCS. The cells were washed and diluted in FACS buffer supplemented with 2 μg/mL 7-AAD prior to flow cytometry analysis.

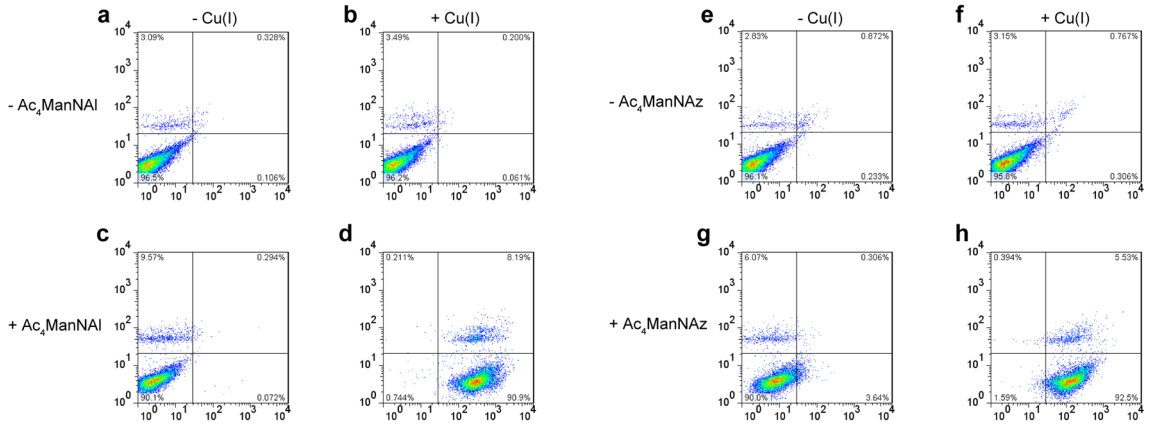


Figure S5: Representative FL3 *vs.* FL1 scatter plots for the labeling experiments described in **Fig. 2b**. In all plots, the x-axis shows the degree of cell-surface sialic acid labeling as measured by Alexa Fluor 488 fluorescence (FL1) and the y-axis shows the degree of cell-nucleus labeling as measured by 7-AAD fluorescence. LNCaP cells were cultured in untreated medium (**a**, **b**, **e**, **f**) or medium supplemented with 50 μM Ac_4ManNAl (**c**, **d**) or Ac_4ManNAz (**g**, **h**). After 3 days, cells were labeled with 100 μM biotin-azide (**a**–**d**) or biotin-alkyne (**e**–**h**) in the absence (**a**, **c**, **e**, **g**) or presence (**b**, **d**, **f**, **h**) of BTTES-$CuSO_4$ 6:1 complex ([Cu] = 75 μM) and 2.5 mM sodium ascorbate. The reactions were quenched with BCS and probed with streptavidin-Alexa Fluor 488 conjugates. The cells were then treated with 7-AAD and analyzed by flow cytometry.

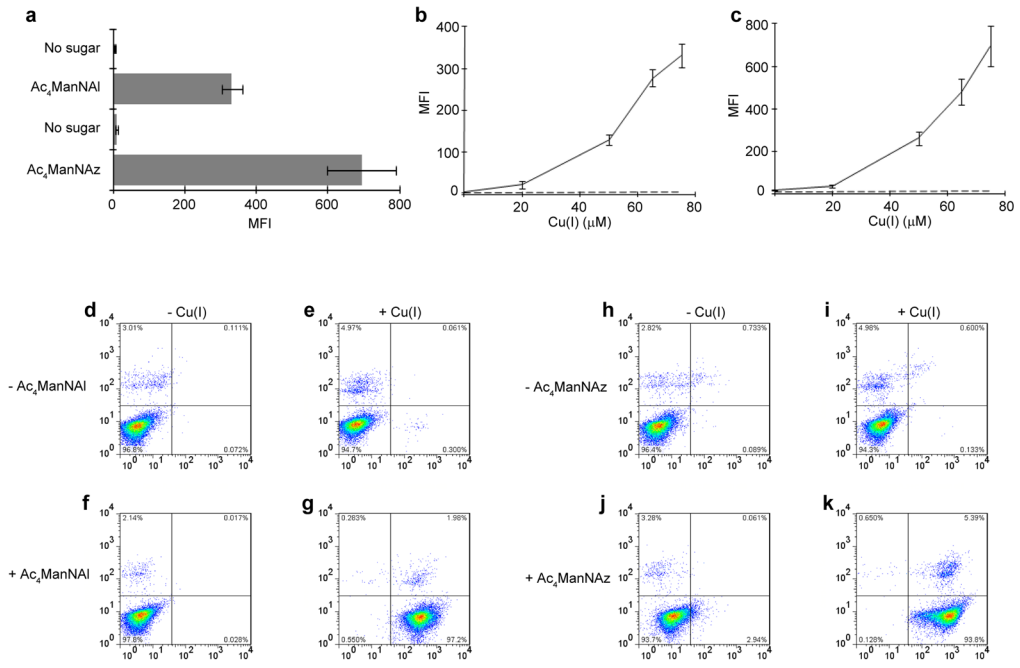


Figure S6: Flow cytometry data of cell surface labeling experiments described in **Fig. 2a** using HEK 293T cells.[a] (**a**) Cells were treated with biotin-azide or biotin-alkyne (100 μM) in the presence of the BTTES-Cu(I) catalyst ([Cu] = 75 μM) for 1 or 2.5 min respectively before probing with streptavidin-Alexa Fluor 488 conjugates. In all cases, cells cultured in the absence of sugar displayed mean fluorescence intensity (MFI, arbitrary units) values < 15. (**b**) Cells were labeled with biotin-azide (100 μM) for 1 min in the presence of 25–75 μM Cu(I). (**c**) Cells were labelled with biotin-alkyne (100 μM) for 2.5 min in the presence of 25–75 μM Cu(I). Error bars represent the standard deviation of three replicate experiments. Solid line, + Ac_4ManNAl (**b**) or Ac_4ManNAz (**c**); dashed line, no sugar. (**d**–**k**) Representative FL3 *vs.* FL1 scatter plots for the labeling experiments. In all plots, the x-axis shows the degree of cell-surface sialic acid labeling as measured by Alexa Fluor 488 fluorescence (FL1) and the y-axis shows the degree of cell-nucleus labeling as measured by 7-AAD fluorescence.

[a] When the SiaNAl-bearing HEK cells were treated with biotin-azide (100 μM) and the BTTES-Cu(I) catalyst ([Cu] = 75 μM) for 90 s, the MFI is ~ 780.

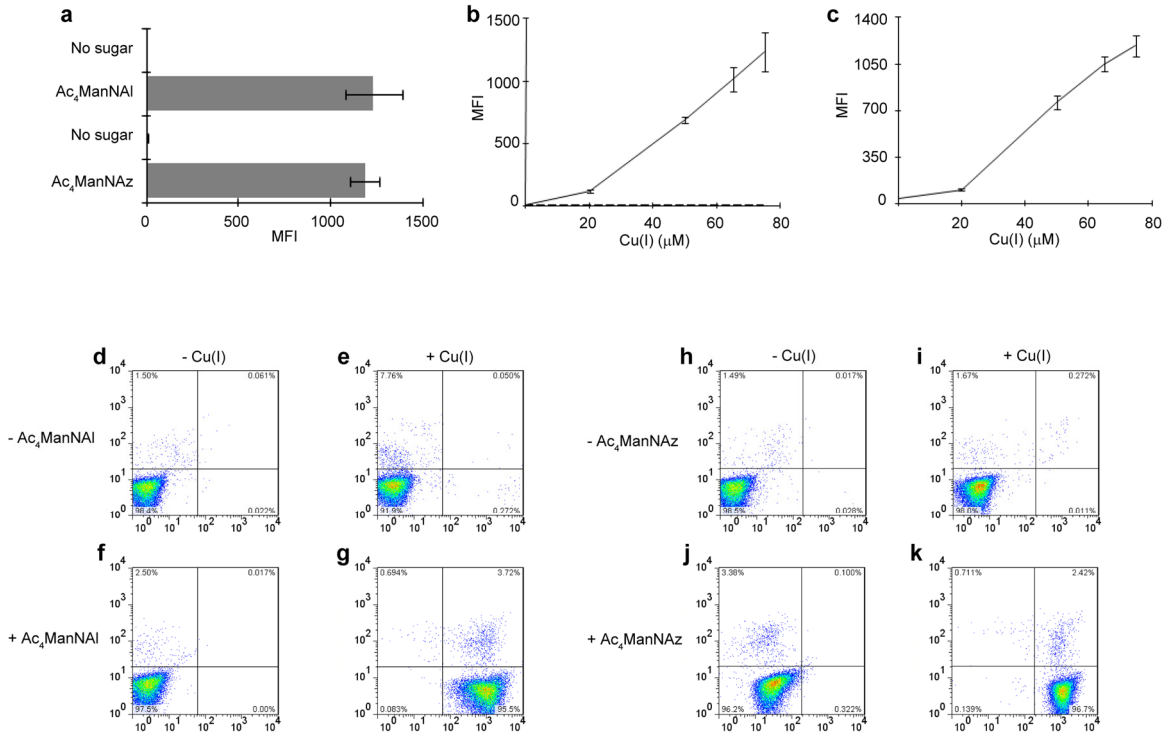


Figure S7: Flow cytometry data of cell surface labeling experiments described in **Fig. 2a** using Jurkat cells.[a] (**a**) Cells were treated with biotin-azide or biotin-alkyne (100 μM) in the presence of the BTTES-Cu(I) catalyst ([Cu] = 75 μM) for 1 or 2.5 min respectively before probing with streptavidin-Alexa Fluor 488 conjugates. In all cases, cells cultured in the absence of sugar displayed mean fluorescence intensity (MFI, arbitrary units) values < 15. (**b**) Cells were labeled with biotin-azide (100 μM) for 1 min in the presence of 25–75 μM Cu(I). (**c**) Cells were labeled with biotin-alkyne (100 μM) for 2.5 min in the presence of 25–75 μM Cu(I). Error bars represent the standard deviation of three

replicate experiments. Solid line, + Ac_4ManNAl (**b**) or Ac_4ManNAz (**c**); dashed line, no sugar. (**d**–**k**) Representative FL3 *vs.* FL1 scatter plots for the labeling experiments. In all plots, the x-axis shows the degree of cell-surface sialic acid labeling as measured by Alexa Fluor 488 fluorescence (FL1) and the y-axis shows the degree of cell-nucleus labeling as measured by 7-AAD fluorescence.

[a]When the SiaNAl-bearing Jurkat cells were treated with biotin-azide (100 μM) and the BTTES-Cu(I) catalyst ([Cu]= 75 μM) for 90 s, the MFI is ~5200.

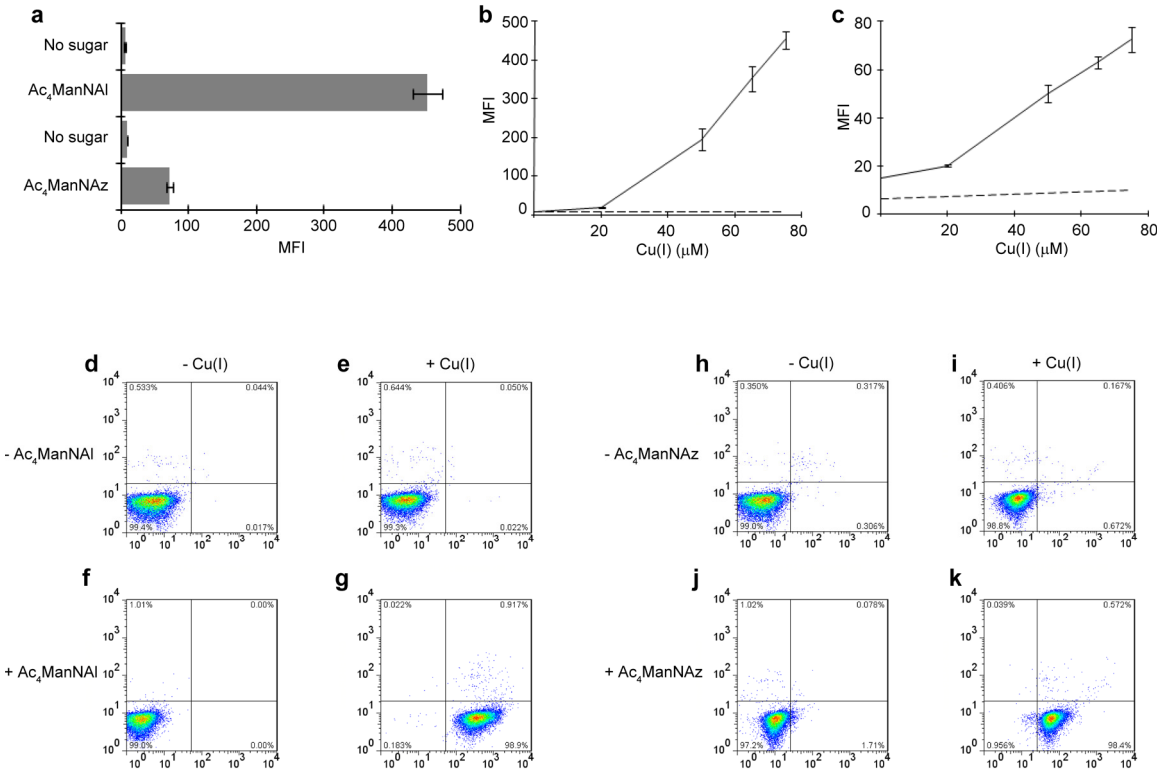


Figure S8: Flow cytometry data of cell surface labeling experiments described in Fig. 2a using Pro$^-$5 CHO cells. (a) Cells were treated with biotin-azide or biotin-alkyne (100 μM) in the presence of the BTTES-Cu(I) catalyst ([Cu] = 75 μM) for 1 or 2.5 min respectively before probing with streptavidin-Alexa Fluor 488 conjugates. In all cases, cells cultured in the absence of sugar displayed mean fluorescence intensity (MFI, arbitrary units)

values < 15. (b) Cells were labeled with biotin-azide (100 μM) for 1 min in the presence of 25–75 μM Cu(I). (c) Cells were labeled with biotin-alkyne (100 μM) for 2.5 min in the presence of 25–75 μM Cu(I). Error bars represent the standard deviation of three replicate experiments. Solid line, + Ac_4ManNAl (b) or Ac_4ManNAz (c); dashed line, no sugar. (d–k) Representative FL3 *vs.* FL1 scatter plots for the labeling experiments. In all plots, the x-axis shows the degree of cell-surface sialic acid labeling as measured by Alexa Fluor 488 fluorescence (FL1) and the y-axis shows the degree of cell-nucleus labeling as measured by 7-AAD fluorescence.

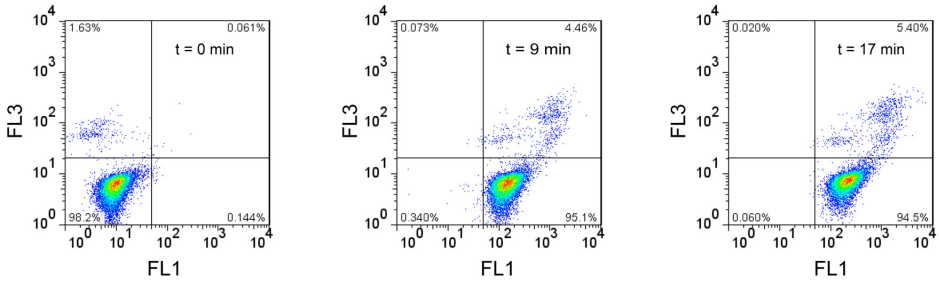


Figure S9: Representative FL3 *vs.* FL1 scatter plots for the labeling experiments described in **Fig. 3**. In all plots, the x-axis shows the degree of cell-surface sialic acid labeling as measured by Alexa Fluor 488 fluorescence (FL1) and the y-axis shows the degree of cell-nucleus labeling as measured by 7-AAD fluorescence.

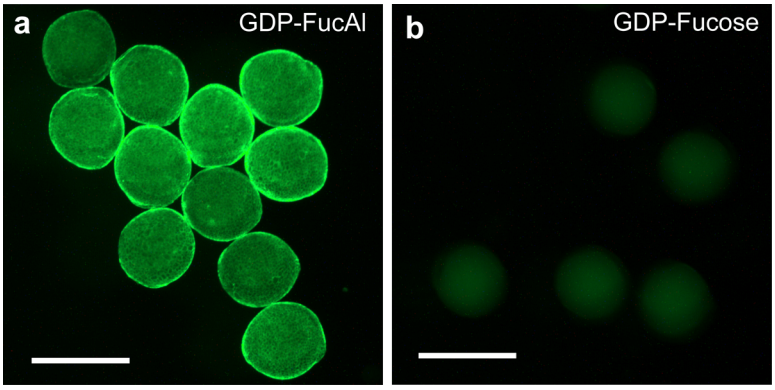


Figure S10. In vivo imaging of fucosylated glycans during early zebrafish embryogenesis via BTTES-Cu(I)-catalyzed click chemistry. One-cell embryos microinjected with a single dose (20 pmol) of GDP-FucAl (left) or GDP-fucose (right) were allowed to develop to 10 hpf. The embryos were then reacted with 488-azide catalyzed by BTTES-Cu(I) and imaged using fluorescence microscopy. Scale bar: 1 mm.

Figure S11: Minor developmental defects were observed for the zebrafish embryos treated with GDP-FucAl through four days post click reaction. The experimental group consisted of 40 embryos. Three embryos exhibited impaired posterior body development characterized by a shorter anterior-posterior axis (day 3). The bright field images for day 1 embryos were acquired immediately after the click reaction. Scale bar: 500 μm.

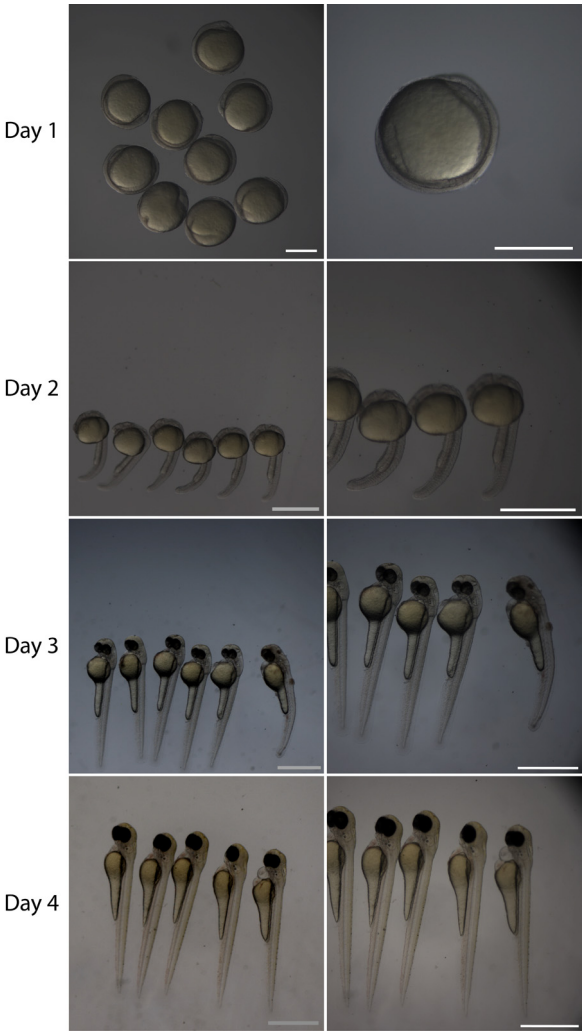


Figure S12: Minor developmental defects were observed for the zebrafish embryos microinjected with 1-2 nL of rhodamine-dextran (5% w/v) in 0.2 M KCl, but not subjected to the click reaction. The experimental group consisted of ~1000 embryos. Around 10% embryos exhibited impaired posterior body development characterized by a shorter anterior-posterior axis (day 3). These results suggested that the defects observed for the experimental group (**Figure S11**) may due to the microinjection rather than the click reaction. Scale bar: 500 μm.

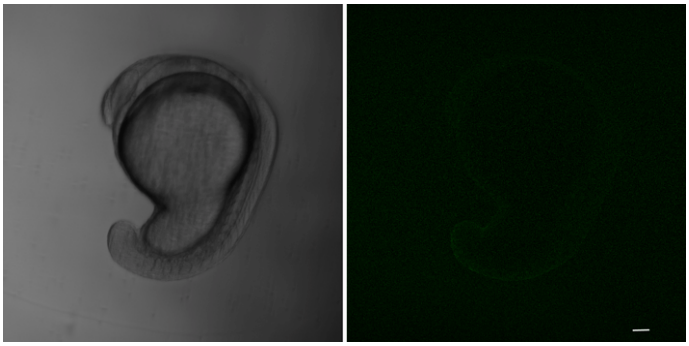
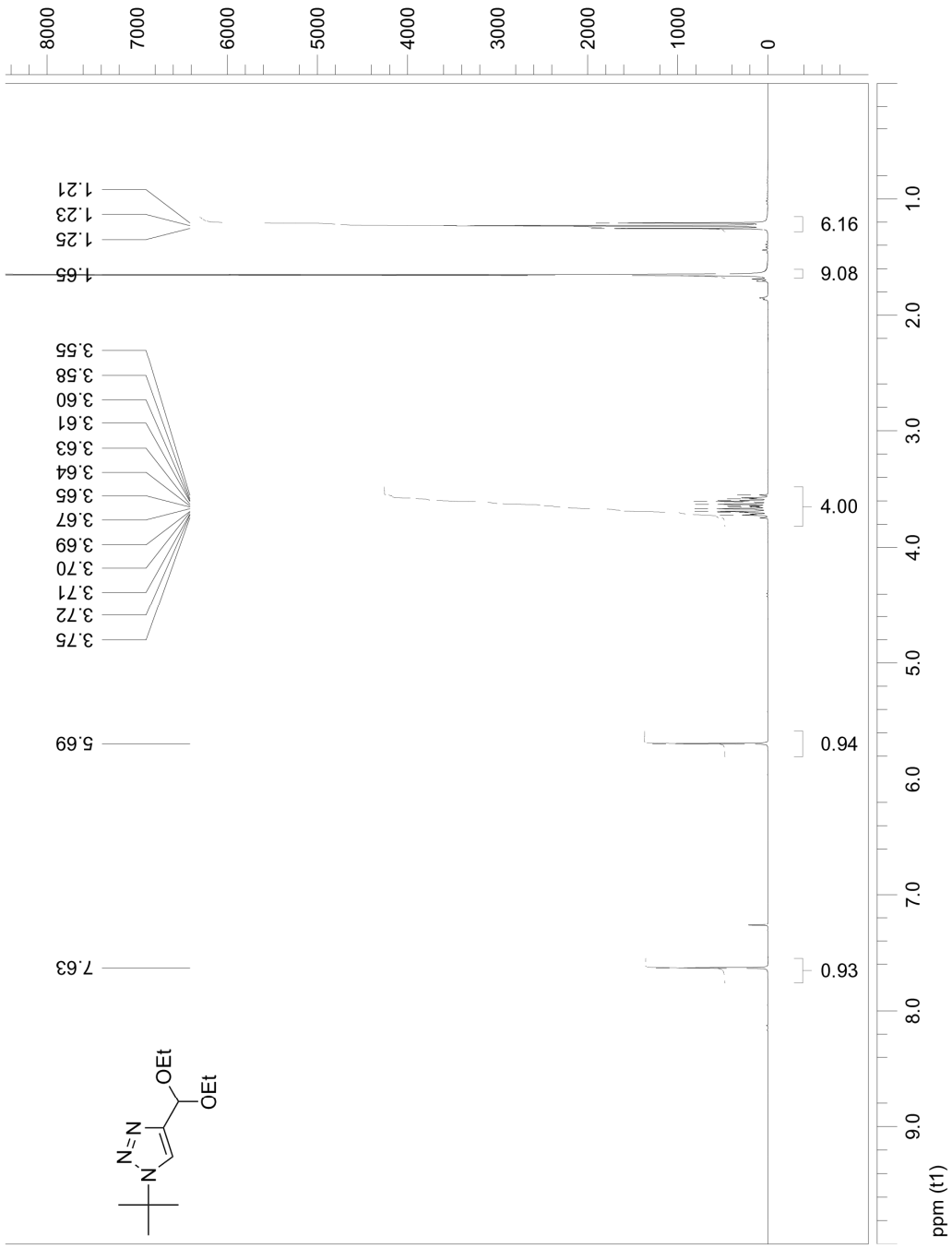
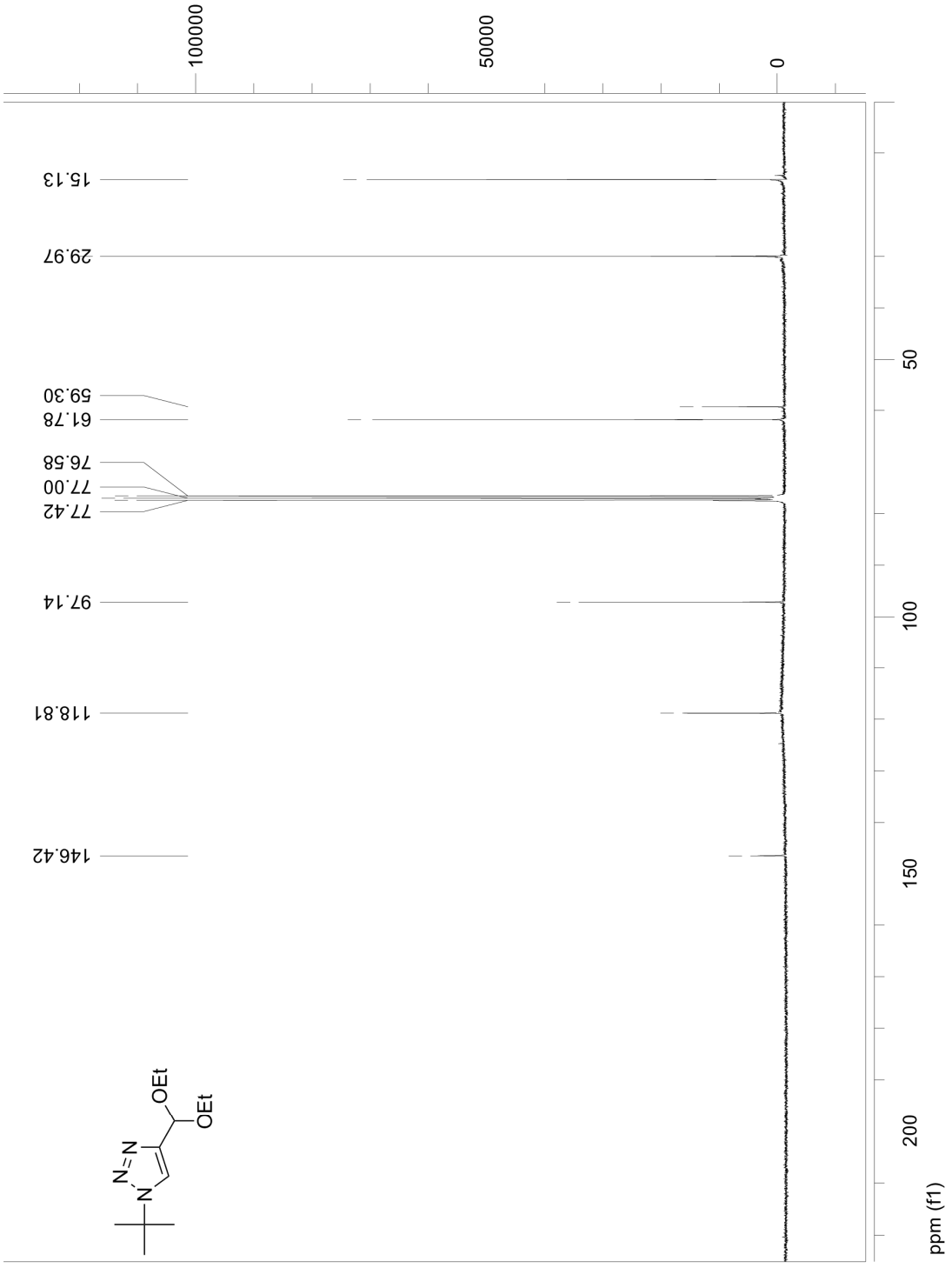


Figure S13. In vivo labeling of fucosylated glycans during zebrafish embryogenesis via THPTA-Cu(I)-catalyzed click chemistry. One-cell embryos microinjected with a single dose of GDP-FucAl (20 pmol) were allowed to develop to various stages. The embryos were then reacted with Alexa Fluor 488-azide (100 μM) catalyzed by THPTA-Cu(I) ([THPTA]:[CuSO4] = 6:1, [$CuSO_4$] = 50 μM, [sodium ascorbate] = 2.5 mM). The reaction was quenched by BCS after 3 min, and the embryos were imaged using confocal fluorescence microscopy. Left: brightfield; Right: maximum intensity z-projection images of Alexa Fluor 488 fluorescence. The embryo shown was at ~18 hpf. Similar results were obtained for embryos at 10 and 80 hpf (data not shown). Scale bar: 100 μm.

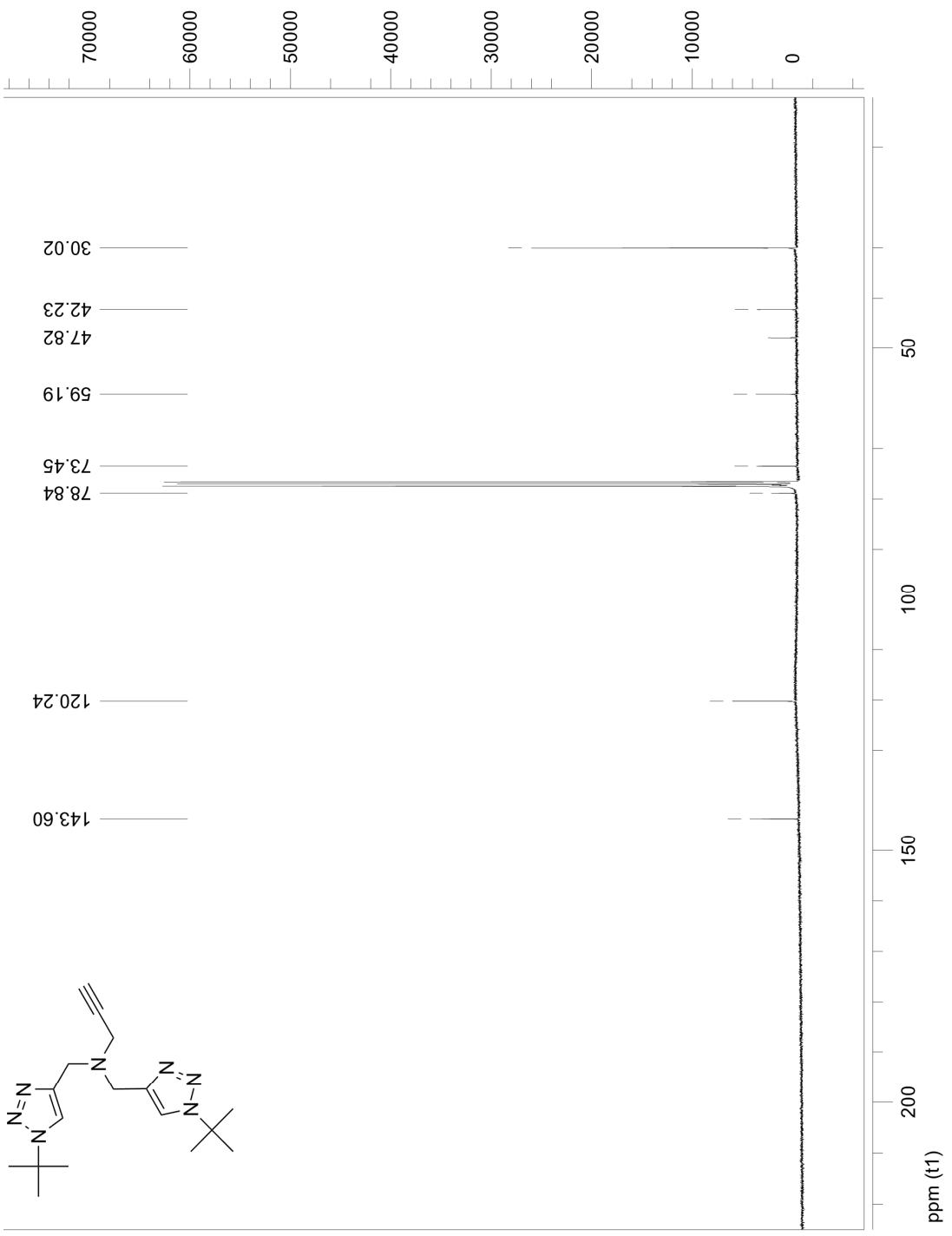
Movie S1. z-series of Alexa Fluor 488-labeling of zebrafish embryo shown in **Fig. 5c** revealed that the labeled FucAl are located on the enveloping layer of the embryo (lateral view). One-cell stage zebrafish embryos were microinjected with a single dose of GDP-FucAl (20 pmol) and allowed to develop to 10 hpf. The embryos were then reacted with 488-azide (green) catalyzed by BTTES-Cu(I) and imaged using confocal microscopy at 11 hpf. Scale bar: 100 μm.

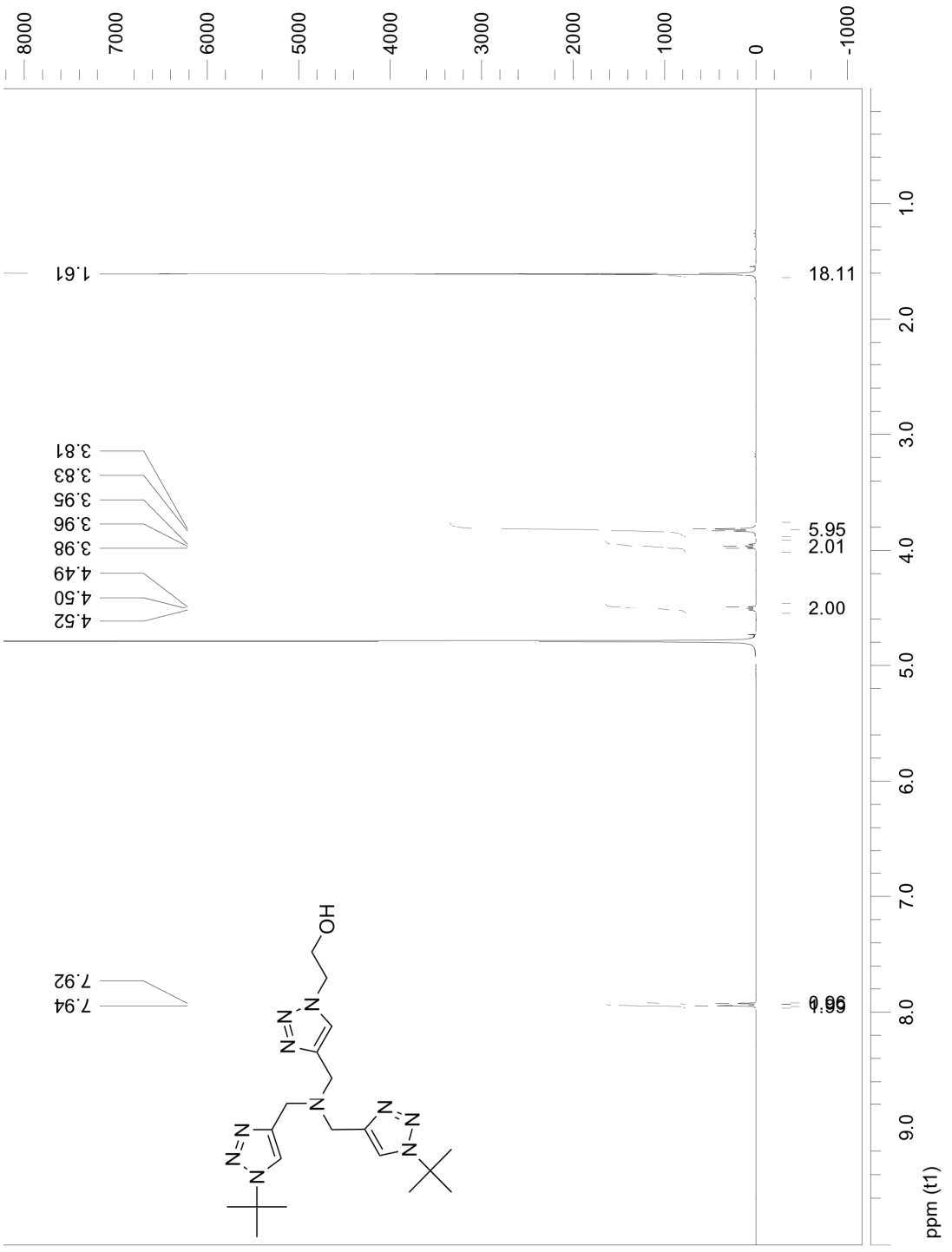
NMR Spectra:

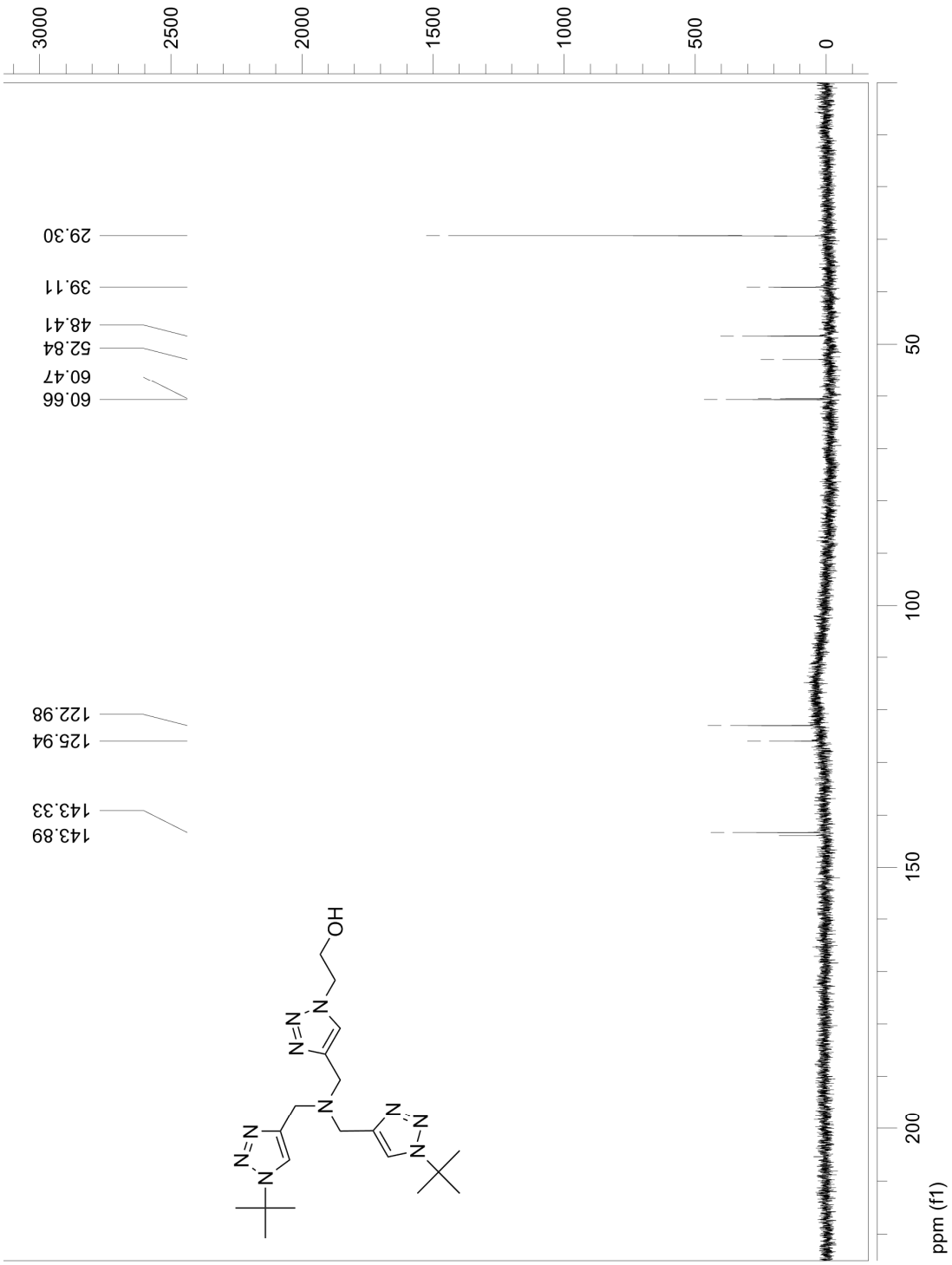

8000
7000
6000
5000
4000
3000
2000
1000
0
1.21
1.23
1.25
1.65
3.55
3.58
3.60
3.61
3.63
3.64
3.65
3.67
3.69
3.70
3.71
3.72
3.75
5.69
7.63
6.16
9.08
4.00
0.94
0.93
OEt
OEt
N
N
N
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
ppm (t1)

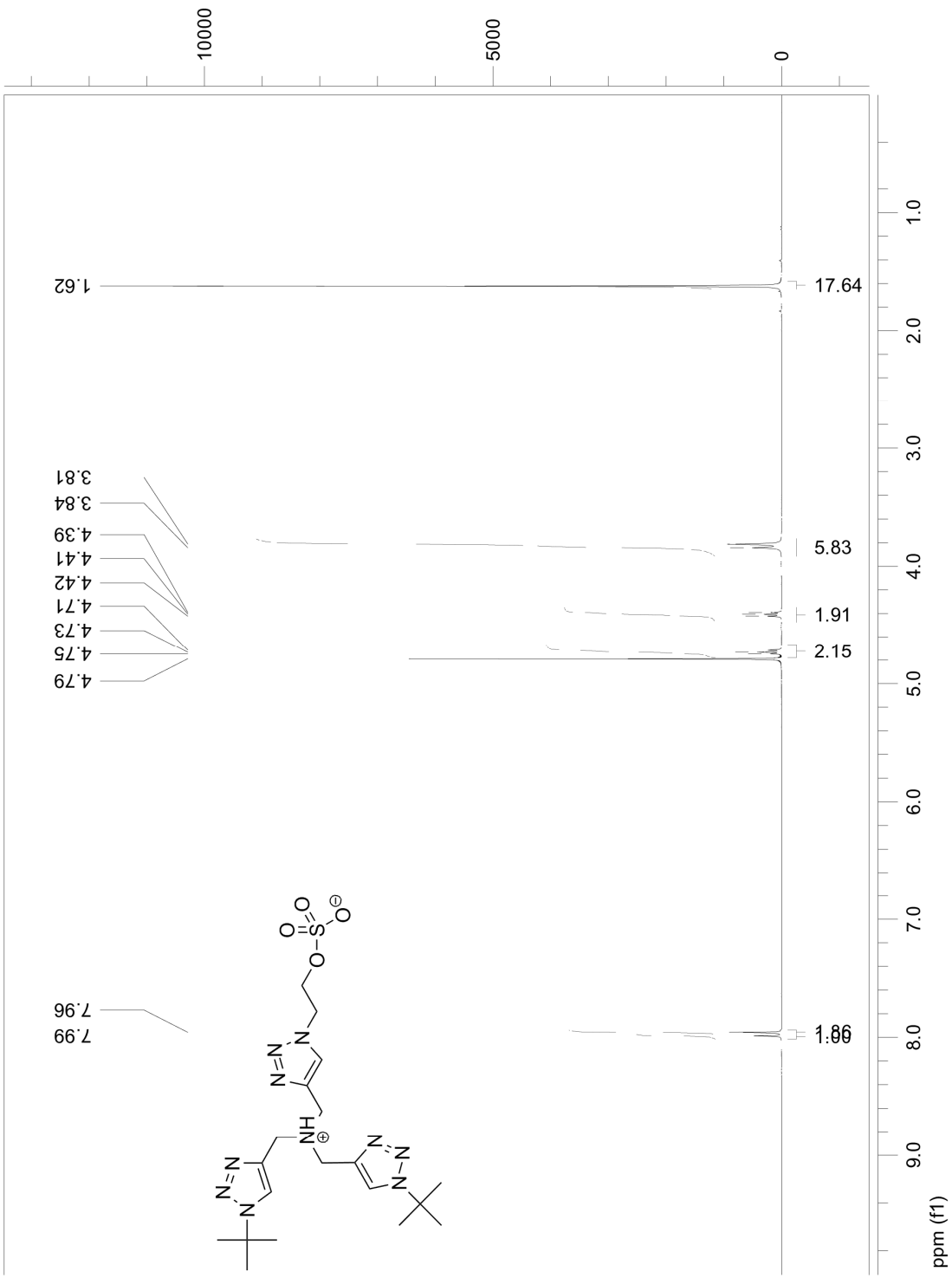

100000
50000
0
15.13
29.97
59.30
61.78
76.58
77.00
77.42
97.14
118.81
146.42
OEt
OEt
N
N
N
50
100
150
200
ppm (f1)

20000
15000
10000
5000
0
1.665
2.273
2.281
2.289
3.390
3.398
3.866
7.260
7.667
18.49
0.94
2.09
4.21
2.00
5.0
ppm (t1)


70000
60000
50000
40000
30000
20000
10000
0
30.02
42.23
47.82
59.19
73.45
78.84
120.24
143.60
50
100
150
200
ppm (t1)


8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
1.61
3.81
3.83
3.95
3.96
3.98
4.49
4.50
4.52
7.92
7.94
18.11
5.95
2.01
2.00
1.00
1.00
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
ppm (t1)
OH
N
N=N


3000
2500
2000
1500
1000
500
0
29.30
39.11
48.41
52.84
60.47
60.66
122.98
125.94
143.33
143.89
OH
N
N
N
N
N
N
N
N
N
N
50
100
150
200
ppm (f1)


10000
5000
0
1.62
3.81
3.84
4.39
4.41
4.42
4.71
4.73
4.75
4.79
7.96
7.99
17.64
5.83
1.91
2.15
1.86
1.00
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
ppm (f1)

9000
8000
7000
6000
5000
4000
3000
2000
1000
29.31
48.19
48.38
49.98
60.64
66.99
123.04
126.23
143.32
143.81
50
100
150
200
ppm (f1)