Supporting Information for:

Monitoring Dynamic Glycosylation in Vivo Using Super-sensitive Click Chemistry

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Notes

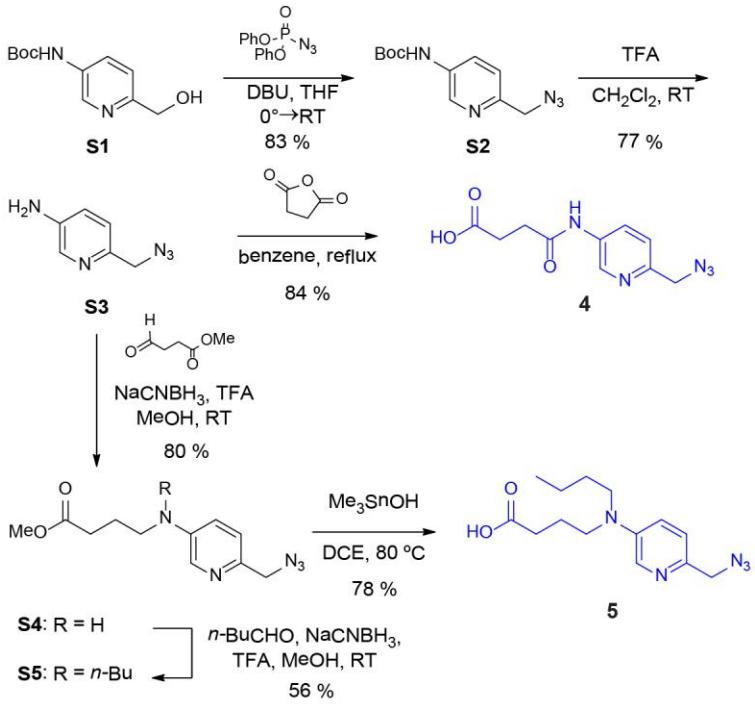
The authors declare no competing financial interest.

Materials and Methods for the Chemical Syntheses

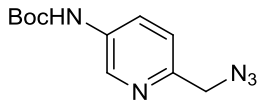
All chemical reagents and solvents were obtained from commercial sources (*Aldrich*, *Acros*, *Fisher*) and used without further purification unless otherwise noted. Anhydrous solvents (tetrahydrofurane, toluene, dichloromethane, diethyl ether) were obtained using a *Pure Solv™* AL-258 solvent purification system. Dimethyl formamide was dried over activated 4 Å molecular sieves. Chromatography was performed either on a *Teledyne ISCO CombiFlash R_f 200i* using disposable silica cartridges (4, 12, and 24 g) or using *Alfa Aesar Silica 60 0.019–0.037 mm, 400–600 mesh* silica gel. Analytical thin layer chromatography (TLC) was performed on aluminum-backed *Silicycle* silica gel plates (250 μm film thickness, indicator F254). Compounds were visualized using a dual wave length (254 nm and 365 nm) UV lamp, and/or staining with CAM (cerium ammonium molybdate) or $KMnO_4$ stains. NMR spectra were recorded on *Bruker DRX 300* and *DRX 600* spectrometers. ^{1}H and ^{13}C chemical shifts (δ) are reported relative to tetramethyl silane (TMS, 0.00/0.00 ppm) as internal standard or to residual solvent (CD_3OD: 3.31 ppm/49.00 ppm; $CDCl_3$: 7.26/77.16 ppm). Mass spectra were recorded on a *Shimadzu LCMS 2010EV* (direct injection unless otherwise noted). LCMS was obtained using a *Agilent 6130 Quadrupole LC/MS* with a *Waters XBridge OST C18 Column 2.5 μm (4.6x50 mm).* Semi-preparative scale HPLC was performed using a *Shimadzu LC-6AD Liquid Chromatograph with Waters XBridge OST C18 Column 2.5 μm (10x50 mm).* Analytical LCMS was obtained with a flow rate of 0.3 mL/min. Semi-preparative scale HPLC was performed with a flow rate of 3 mL/min. Unless otherwise noted, analytical as well as semi-preparative separations were run with a gradient of 1 to 50% B in A (A: 0.1 M aqueous triethylammonium acetate (TEAA), pH = 7; B: acetonitrile). IR spectra were recorded on an *Agilent Cary 630 FTIR* on a ZnSe crystal. Wavenumbers are reported in cm^{-1}.

4-(Azidomethyl)benzoic acid (**1**) and 6-(azidomethyl)nicotinic acid (**3**),[1] *tert*-butyl (6-(hydroxylmethyl)pyridin-3-yl)carbamate (**S1**)[2] and *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)-propyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide[3] were synthesized according to literature procedures.

[1] Uttamapinant, C.; Tangpeerachaikul, A.; Grecian, S.; Clarke, S.; Singh, U.; Slade, P.; Gee, K. R.; Ting, A.Y.; *Angew. Chem. Int. Ed.* **2012**, *51*, 5852–5856.
[2] Mitsuya, M.; Kobayashi, K.; Kawakami, K.; Satoh, A.; Ogino, Y.; Kakikawa, T.; Ohtake, N.; Kimura, T; Hirose, H.; Sato, A.; Numazawa, T.; Hasegawa, T.; Noguchi, K.; Mase, T.; *J. Med. Chem.* **2000**, *43*, 5017–5029.
[3] Hang, H. C.; Yu, C.; Pratt, M. R.Bertozzi, C. R; *J. Am. Chem. Soc.* **2004**, *126*, 6–7.



Scheme S1: Synthesis of the Cu(I)-chelating azides **4** and **5**. DCE = 1,2-dichloroethane, TFA = trifluoroacetic acid.



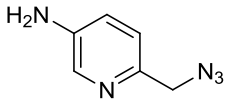
***tert*-Butyl (6-(azidomethyl)pyridin-3-yl)carbamate (S2)**:[4] In a flame-dried and argon-flushed 250 mL round-bottom flask, *tert*-butyl (6-(hydroxymethyl)pyridin-3-yl)carbamate (**S1**, 760 mg, 3.39 mmol) was dissolved in tetrahydrofuran (9.9 mL) and cooled to 0 °C. DBU (1.02 mL, 6.78 mmol) and diphenyl phosphorazidate (1.46 mL, 6.78 mmol) were added drop wise, simultaneously *via* syringes over ca 2–3 min. The mixture was stirred at 0 °C for ca 5 min, then allowed to warm to room temperature. After a couple of minutes, a precipitate forms (presumably the benzylic phosphate), that re-dissolves after a few more minutes at room temperature. TLC analysis of a reaction aliquot (H_2O/EtOAc micro-workup) after 1 h at room

[4] This reaction has been performed before on a similar substrate, see: Mochizuki, A.; Nagate, T.; Kanno, H.; Suzuki, M.; Ohta, T.; *Bioorg. Med. Chem.* **2011**, *19*, 1623–1642

temperature indicated full conversion. Ethyl acetate (50 mL) and water (50 mL) were added and the mixture was stirred vigorously at room temperature for ca 10 min. The layers were separated and the aqueous layer was extracted with EtOAc (2×50 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$) and evaporated.

The crude residue was absorbed on 4 g silica gel for dry loading and purified by flash column chromatography on the *CombiFlash* (5 →30 % EtOAc in hexanes). *tert*-Butyl (6-(azidomethyl)pyridin-3-yl)carbamate was obtained as colorless, crystalline solid (700 mg, 2.81 mmol, 83 %).

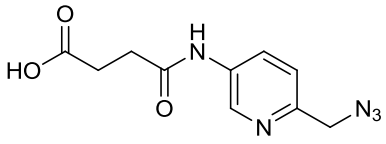
TLC: R_f 0.22 (4:1 hexanes/EtOAc). **IR** (ZnSe, film): 3319 (br, N-H st), 2979 (w, C-H st), 2933 (w, C-H st), 2099 (s, N_3 st asym), 1724 (m, C=O st), 1699 (m, N-H δ), 1589 (m), 1524 (s), 1384 (m), 1368 (m), 1345 (w), 1335 (s; shoulder at 1266), 1151 (vs), 1055 (s), 1019 (m), 837 (w), 772 (w). **^{1}H-NMR** (600 MHz, $CDCl_3$): δ 8.41 (d, J = 2.4 Hz, 1H), 8.02 (s, br, 1H), 7.28 (d, J = 8.5 Hz, 1H), 6.64 (s, br, 1H), 4.42 (s, 2H), 1.53 (s, 9H). **^{13}C-NMR** (151 MHz, $CDCl_3$): δ 152.5, 149.8, 139.9, 134.7, 126.3, 122.3, 81.5, 55.3, 28.3. **ESI-MS** *m/z* (rel int): (pos) 249.9 ($[M+H]^+$, 100); (neg) 248.0 ($[M–H]^–$, 100).



6-(Azidomethyl)pyridin-3-amine (**S3**): In a flame-dried round bottom flask equipped with a stir bar and rubber septum, *tert*-Butyl (6-(azidomethyl)pyridin-3-yl)carbamate (**S2**, 650 mg, 2.61 mmol) was dissolved in anhydrous dichloromethane (19.0 mL) under an argon atmosphere. Trifluoroacetic acid (3.01 mL, 39.1 mmol) was added at room temperature. After 2 h at room temperature, TLC analysis of a reaction aliquot ($NaHCO_3$ satd aq/EtOAc micro-workup) indicated full conversion. Satd aq sodium bicarbonate (50 mL) was added and the mixture was stirred vigorously for 10 min at room temperature, until the CO_2-formation ceased. The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and evaporated *in vacuo* to give the crude product as an almost colorless oil. The crude residue was absorbed on

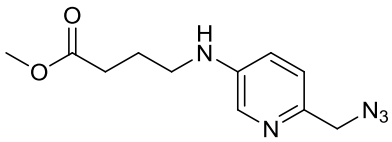
2 g silica gel for dry loading and purified by flash column chromatography on the *CombiFlash* (MeOH in CH_2Cl_2, 2→15 %). 6-(Azidomethyl)pyridin-3-amine (300 mg, 2.01 mmol, 77 %) was isolated as colorless oil that turns yellow with time. It should be stored at –20 ˚C to avoid decomposition.

TLC: R_f 0.44 (19:1, CH_2Cl_2/MeOH). **IR** (ZnSe, film): 3445 (w), 3341 (br, N-H st), 3199 (br, N-H st), 2925 (m, C-H st), 2855 (C-H st), 2095 (vs, N_3 st asym), 1624 (m, N-H δ), 1596 (m), 1574 (m), 1491 (s), 1419 (m), 1343 (w), 1300 (s), 1251 (s), 1138 (m), 1021 (m), 878 (w), 832 (m), 798 (w). **^{1}H-NMR** (600 MHz, $CDCl_3$): δ 8.06 (dd, *J* = 2.8, 0.5 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 1H), 6.98 (dd, *J* = 8.3, 2.8 Hz, 1H), 4.34 (s, 2H), 3.87-3.86 (m, 2H). **^{13}C-NMR** (151 MHz, $CDCl_3$): δ 145.0, 142.3, 137.1, 122.9, 122.1, 55. 5. **ESI-MS** *m/z* (rel int): (pos) 150.0 ($[M+H]^+$, 100).



4-((6-(Azidomethyl)pyridin-3-yl)amino)-4-oxobutanoic acid (**4**): In a flame-dried 10 mL round-bottom flask, equipped with a reflux condenser, 6-(azidomethyl)pyridin-3-amine (**S3**; 50 mg, 0.34 mmol) was dissolved in benzene (0.56 mL), succinic anhydride (40.3 mg, 0.40 mmol) was added under an argon atmosphere, and the resulting mixture was heated at 90 °C. After 10–15 min at reflux, the formation of a white precipitate was observed. TLC analysis of a reaction aliquot after 1 h indicated full conversion to the desired product. The solvent was evaporated *in vacuo* and the crude product was dried in high vacuum. The crude residue was absorbed on silica gel (350 mg) for dry loading and purified on the *CombiFlash* (MeOH in CH_2Cl_2, 3→10 %). 4-((6-(Azidomethyl)pyridin-3-yl)amino)-4-oxobutanoic acid (69.8 mg, 0.28 mmol, 84 %) was obtained as white powder (*NOTE*: extended drying in high vacuum is necessary to completely remove remaining solvent!)

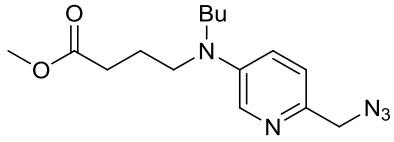
TLC: R_f 0.05 (19:1, CH_2Cl_2/MeOH). **IR** (ZnSe, film): 3342 (m, COO-H st), 3309 (w), 3176 (br), 3105 (br), 2927 (w), 2111 (vs, N_3 st asym), 1714 (s, C=O st), 1697 (vs, C=O st), 1664 (s, N-H δ), 1609 (w), 1541 (s), 1525 (s), 1423 (w), 1378 (m), 1349 (s), 1305 (m), 1247 (m), 1197 (m), 1165 (vs)1130 (w), 1033 (w), 955 (w), 836 (w), 820 (w), 685 (w). **^{1}H-NMR** (600 MHz, DMSO-d_6): δ 12.15 (s, 1H), 10.25 (s, 1H), 8.72 (d, J = 2.3 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 4.44 (s, 2H), 2.60 (t, J = 6.6 Hz, 2H), 2.53 (t, J = 6.6 Hz, 2H). **^{13}C-NMR** (151 MHz, DMSO-d_6): δ 173.7, 170.8, 149.6, 140.2, 135.3, 126.5, 122.6, 54.1, 30.9, 28.6. **ESI-MS** *m/z* (rel int): (pos) 249.9 ($[M+H]^+$, 100); (neg) 497.1 ($[2M–H]^–$, 45), (248.0 ($[M–H]^–$, 100).



Methyl 4-((6-(azidomethyl)pyridin-3-yl)amino)butanoate (**S4**): In a 50 mL conical flask, 6-(azidomethyl)pyridin-3-amine (**S3**; 200 mg, 1.34 mmol) was azeotroped from benzene (2 ×). A stir bar was added, the flask evaporated in high vacuum and re-filled with argon. The substrate was then dissolved in dry methanol (2.9 mL). A few crumbs of methyl orange, as well as molecular sieves (4 Å; 150 mg, freshly activated in high vacuum in a separate flask) were added. To the stirred mixture was added neat sodium cyanoborohydride (59.0 mg, 0.94 mmol), followed by methyl 4-oxobutanoate (0.31 mL, 2.68 mmol) and trifluoroacetic acid (0.34 mL, 4.42 mmol) (indicator turns red). The flask was sealed with a stopper (Teflon® grease) and the mixture stirred at room temperature. The given amount of acid should be sufficient to maintain acidic pH over the reaction time, as indicated by the color of the reaction mixture. More TFA may be added as needed to maintain acidic pH. After 15 h at room temperature, TLC analysis of a reaction aliquot ($NaHCO_3$ satd aq/EtOAc micro-workup) indicated full conversion. The reaction mixture was filtered through a plug of Celite. The plug and the molecular sieves were rinsed thoroughly with EtOAc (ca 10 mL) and CH_2Cl_2 (ca 5 mL). The organic layers were washed thoroughly with satd aq sodium biocarbonate (CO_2 formation), and brine. The organic layer was dried ($MgSO_4$), filtered and evaporated *in vacuo*. The crude residue was absorbed on silica (1.0 g) for dry loading and purified on the *CombiFlash* (EtOAc in hexanes, 15 →70 %). Methyl

4-((6-(azidomethyl)pyridin3-yl)amino)butanoate (269 mg, 1.08 mmol, 80 %) was obtained as colorless oil that partially crystallizes upon storage at low temperature.

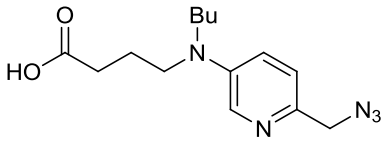
TLC: R_f 0.58 (19:1, CH_2Cl_2/MeOH). **IR** (ZnSe, film): 3387 (w, N-H st), 3302 (w), 2952 (w, C-H st), 2870 (w, C-H st), 2092 (vs, N_3 st asym), 1726 (vs, C=O st), 1596 (m), 1578 (m), 1508 (m), 1437 (w), 1319 (w), 1250 (s), 1201 (s), 1168 (vs), 1139 (s), 1100 (w), 1015 (w), 879 (w), 826 (m), 668 (w). **^{1}H-NMR** (600 MHz, $CDCl_3$): δ 7.99 (d, *J* = 2.8 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 6.88 (dd, *J* = 8.4, 2.8 Hz, 1H), 4.33 (s, 2H), 3.97 (s, 1H), 3.69 (s, 3H), 3.20 (q, *J* = 6.4 Hz, 2H), 2.45 (t, *J* = 7.0 Hz, 2H), 1.97 (quintet, *J* = 7.0 Hz, 2H). **^{13}C-NMR** (151 MHz, $CDCl_3$): δ 173.8, 143.8, 143.7, 135.8, 123.0, 118.9, 55.6, 51.9, 43.1, 31.6, 24.4. **ESI-MS** *m/z* (taken from main LCMS peak, as trace impurities of the doubly alkylated species rendered direct injection unfeasible) (rel int): (pos) 249.9 ($[M+H]^+$, 100).



Methyl 4-((6-(azidomethyl)pyridin-3-yl)(butyl)amino)butanoate (**S5**): In a 25 mL conical flask, methyl 4-((6-(azidomethyl)pyridin-3-yl)amino)butanoate (**S4**, 65.0 mg, 0.30 mmol) was azeotroped from benzene (2 ×) and dried in high vacuum. After re-filling the flask with argon, a dry stir bar was added and the starting material was dissolved in dry methanol (642 µl). 100 mg freshly activated molecular sieves (4 Å) were added followed by sodium cyanoborohydride (95.0 mg, 1.50 mmol), as well as a few crumbs methyl orange. Then, with stirring, butyraldehyde (539 µl, 6.02 mmol) was added, followed by dropwise addition of trifluoroacetic acid (185 µl, 2.41 mmol). The solution turned from yellow to intense red immediately, strong gas formation was observed. The exothermic reaction was perform in a tightly sealed flask (glass stopper, PTFE grease), and the reaction was stirred at room temperature. TLC analysis of a reaction aliquot (satd aq $NaHCO_3$/EtOAc micro-workup), taken after 1 h 40 min, indicated full conversion. After 2 h, the reaction mixture was filtered through plug of *Celite*, the plug was rinsed thoroughly with EtOAc and CH_2Cl_2 (ca 15 mL combined volume). The combined organic layers were treated with sat aq $NaHCO_3$ under vigorous stirring and the mixture was stirred until gas formation ceased. The aqueous layer was extracted (3 ×) with CH_2Cl_2, the combined organic

layers were dried ($MgSO_4$), filtered and evaporated *in vacuo*. The crude residue was absorbed on 400 mg of silica gel for dry loading, then purified on the *CombiFlash* (EtOAc in hexanes, 5 →30→70 %). Methyl 4-((6-(azidomethyl)pyridin-3-yl)(butyl)amino)butanoate (51.5 mg, 0.169 mmol, 56 %) was obtained as a yellow oil.

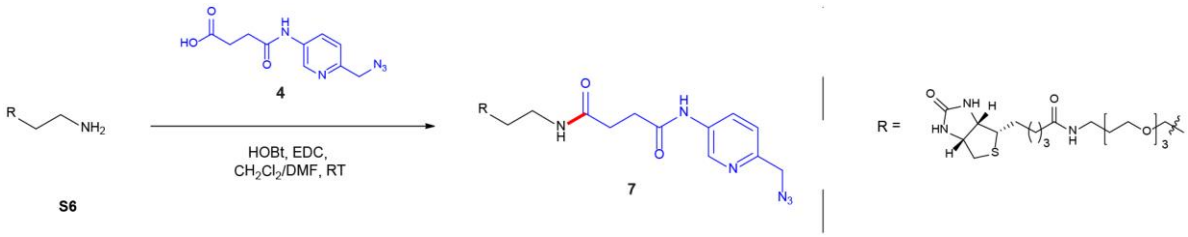
TLC: R_f 0.19 (4:1, hexanes:EtOAc). **IR** (ZnSe, film): 2956 (m, C-H st), 2935 (m, C-H st), 2873 (w), 2894 (vs, N_3 st asym), 1735 (vs, C=O st), 1591 (m), 1559 (m), 1500 (s), 1460 (w), 1437 (w), 1411 (w), 1367 (m), 1254 (m), 1221 (s), 1198 (s), 1170 (s), 1127 (w), 1082 (w), 1012 (w), 879 (w), 816 (w). **^{1}H-NMR** (600 MHz, $CDCl_3$): δ 8.06 (d, *J* = 3.0 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 6.95 (dd, *J* = 8.6, 3.0 Hz, 1H), 4.33 (s, 2H), 3.69 (s, 3H), 3.34 (t, *J* = 7.6 Hz, 2H), 3.29 (t, *J* = 7.7 Hz, 2H), 2.37 (t, *J* = 7.1 Hz, 2H), 1.91 (quintet, *J* = 7.4 Hz, 2H), 1.56 (dt, *J* = 15.3, 7.6 Hz, 2H), 1.35 (dq, *J* = 15.0, 7.5 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). **^{13}C-NMR** (151 MHz, $CDCl_3$): δ 173.6, 143.4, 142.0, 134.4, 123.0, 118.7, 55.6, 51.8, 50.8, 50.0, 31.2, 29.2, 22.4, 20.3, 14.1. **ESI-MS** *m/z* (rel int): (pos) 306.0 ($[M+H]^+$, 100) 263.0 (15).



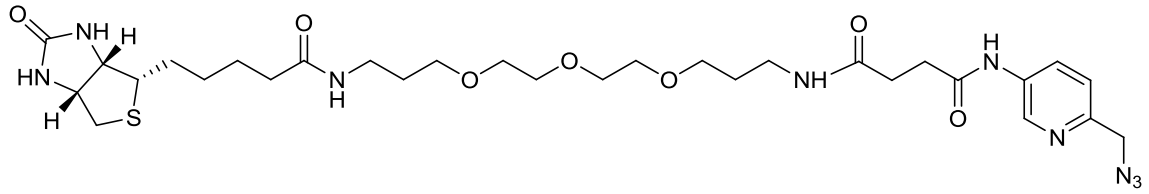
4-((6-(Azidomethyl)pyridin-3-yl)(butyl)amino)butanoic acid (**5**): In a 10 mL screw-cap tube, methyl 4-((6-(azidomethyl)pyridin-3-yl)(butyl)amino)butanoate (**S5**; 15.5 mg, 0.05 mmol) was dissolved in dry 1,2-dichloroethane (1.27 mL) under an argon atmosphere. To this solution, trimethylstannanol (55.1 mg, 0.31 mmol) was added in one portion, the tube was sealed tightly and heated at 80 °C. After 15 h, TLC analysis of a reaction aliquot indicated almost complete conversion (traces of starting material). The reaction mixture was transferred into a 25 mL pear-shaped flask, the reaction vessel rinsed thoroughly with CH_2Cl_2. 150 mg silica gel, as well as 0.3 mL deionized water were added. The slurry was agitated briefly at room temperature, then all solvent was removed *in vacuo*. The silica containing the crude product was dried in high vacuum. The thus absorbed crude product was dry loaded directly on the *CombiFlash* and purified by flash column chromatography (acetone in ethyl acetate, 10 →30 %). 4-((6-

(Azidomethyl)pyridin-3-yl)(butyl)amino)butanoic acid (11.5 mg, 0.04 mmol, 78 %) was obtained as colorless oil that solidifies by time (purity ≥95).

TLC: R_f 0.30 (19:1, CH_2Cl_2/MeOH). **IR** (ZnSe, film): 2956 (m, C-H st), 2929 (m, C-H st), 2870 (m, C-H st), 2093 (vs, N_3 st asym), 1705 (s, C=O st), 1594 (s), 1559 (s), 1502 (s), 1461 (w), 1409 (m), 1366 (m), 1349 (m), 1254 (s), 1194 (vs), 1168 (vs), 1147 (s), 1124 (m), 1022 (w), 875 (w), 816 (s), 763 (w). **^{1}H-NMR** (600 MHz, $CDCl_3$): δ 8.35 (d, *J* = 2.9 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 1H), 6.98 (dd, *J* = 8.7, 2.9 Hz, 1H), 4.40 (s, 2H), 3.45 (t, *J* = 7.9 Hz, 2H), 3.28 (t, *J* = 7.6 Hz, 2H), 2.44 (t, *J* = 6.4 Hz, 2H), 1.93 (dt, *J* = 14.8, 7.1 Hz, 2H), 1.56 (dt, *J* = 15.2, 7.6 Hz, 2H), 1.36 (dq, *J* = 14.8, 7.4 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). **^{13}C-NMR** (151 MHz, $CDCl_3$): δ 176.2, 144.1, 140.4, 133.1, 123.6, 119.3, 54.4, 50.6, 50.2, 31.3, 29.2, 22.1, 20.4, 14.1. **ESI-MS** *m/z* (rel int): (pos) 292.1 ($[M+H]^+$, 100); (neg) 581.3 ($[2M–H]^-$, 30), 290.0 ($[M–H]^-$, 100).



Scheme S2: Coupling of azide **4** to *N*-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (**S6**) according to Bertozzi *et al.*[1] HOBt = *1H*-benzo[d][1,2,3]triazol-1-ol. EDC = 1-Ethyl-3-(3-diethylaminopropyl)carbodiimide hydrochloride

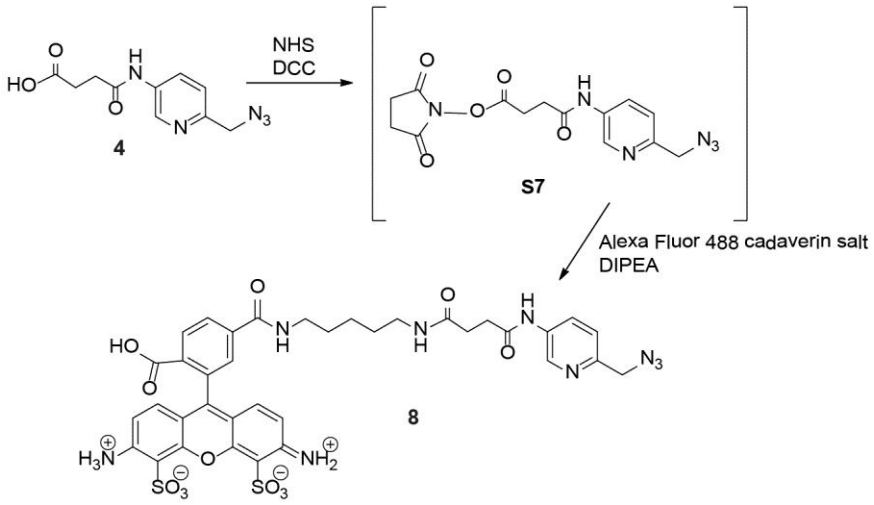


***N*^{1}-(6-(azidomethyl)pyridin-3-yl)-*N*^{4}-(15-oxo-19-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno-[3,4-*d*]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)succinamide** (**7**): In a flame-dried 10-mL screw-cap tube with cross-shaped stir bar, 4-((6-(azidomethyl)pyridin-3-yl)amino)-4-oxobutanoic acid (**4**, 6.45 mg, 0.026 mmol) was suspended in dichloromethane (0.3 mL) under

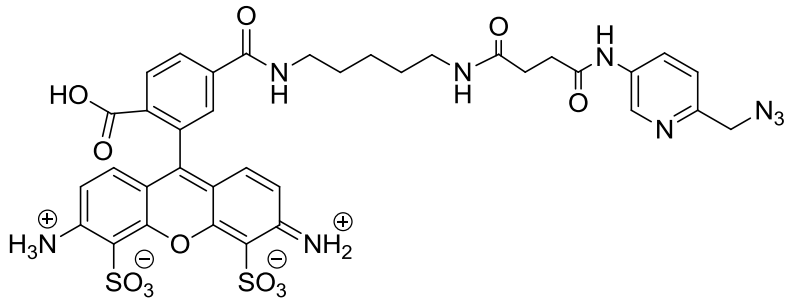
an argon atmosphere. *1H*-benzo[d][1,2,3]triazol-1-ol (3.49 mg, 0.026 mmol) was added neat, followed by EDC (4.96 mg, 0.026 mmol). Sonication was applied to aid the dissolving process. After ca 10 min at room temperature, complete dissolution of all material was observed.
The mixture was allowed to stir for 40 min at room temperature. This solution was added to a solution of N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (**S6**, 10.5 mg, 0.024 mmol) in dry DMF (0.3 mL) previously prepared in a separate tube. The first tube was rinsed with CH_2Cl_2 (0.2 mL). The reaction was monitored by TLC analysis of reaction aliquots. After 5 h, the solvents were evaporated *in vacuo*. The crude residue was absorbed on 200 mg of silica gel for dry loading, then purified on the *CombiFlash* (EtOAc:MeOH:H_2O, 17:3:1). N^1-(6-(azidomethyl)pyridin-3-yl)-N^4-(15-oxo-19-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)succinamide (2.0 mg, 0.003 mmol, 13 %) was obtained as colorless resin.
[*NOTE*: The purification conditions have not yet been fully optimized. Better yields have been observed in some experiments. Multiple chromatographies may be necessary to obtain pure material]

TLC: R_f 0.12 (EtOAc:MeOH:H_2O, 17:3:1). **^{1}H-NMR** (600 MHz, $CDCl_3$): δ 8.76 (d, *J* = 2.6 Hz, 1H), 8.14 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 4.51 (dd, *J* = 7.9, 4.7 Hz, 1H), 4.46 (s, 2H), 4.32 (dd, *J* = 7.9, 4.6 Hz, 1H), 3.67-3.65 (m, 4H), 3.60 (ddd, *J* = 8.7, 5.7, 3.2 Hz, 4H), 3.54 (t, *J* = 6.1 Hz, 4H), 3.30-3.28 (m, 4H), 3.24-3.21 (m, 1H), 2.95 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.73 (dt, *J* = 12.8, 6.4 Hz, 3H), 2.60 (t, *J* = 6.9 Hz, 2H), 2.22 (t, *J* = 7.3 Hz, 2H), 1.80–1.74 (m, 4H), 1.74–1.59 (m, 4H), 1.48–1.43 (m, 2H). **^{13}C-NMR** (151 MHz, $CDCl_3$): δ 176.0, 174.4, 173.4, 166.1, 151.6, 141.7, 136.9, 129.3, 124.1, 71.5 (2C),[5] 71.2 (2C),[5] 70.0, 69.9, 63.9, 61.6, 57.0, 55.8, 41.0, 37.9, 37.8, 36.9, 32.8, 31.7, 30.4, 30.4, 29.8, 29.5, 26.9. **ESI-MS** *m/z* (rel int): (pos) 701.3 ($[M+Na]^+$, 50), 678.3 ($[M+H]^+$, 100), 551.1 (15), 511.2 (30); (neg) 676.4 ($[M–H]^–$, 100).

[5] As confirmed by an HSQC experiment.



Scheme S3: Synthesis of the azide **4**-conjugated Alexa Fluor 488 (**8**). NHS = N-Hydroxy succinimide, DCC = N,N'-Dicyclohexylcarbodiimide, DIPEA = N,N-Diisopropylethylamine.



Azide **4**-conjugated Alexa Fluor 488 (**8**): Azide **4** (2.1 mg, 8.4 µmol) was dissolved in dry THF (500 µL) and DCC (3.48 mg, 16.8 µmol) and NHS (1.94 mg, 16.8 µmol) were added to the solution. The mixture was stirred at room temperature and followed by TLC analysis (3:1 EtOAc:MeOH). No starting material could be detected after 3 h. The reaction mixture was then filtered through glass wool and the solvent removed *in vacuo*. The crude activated ester **S7** was then dissolved in dry DMF (800 µL) and added to the Alexa Fluor 488 cadaverin salt (1.0 mg, 1.6 µmol). DIPEA (20 µL) was added to the solution, and the reaction mixture was stirred at room temperature overnight. The solvent was then evaporated and the residue was purified by semi-preparative scale HPLC to yield **8** as an orange powder after lyophilization (0.06 mg, 0.07 mmol, 5 %).

ESI-MS *m/z* (rel int): (pos) 850.2 ($[M+H]^+$, 100) (Figure S1).

Materials and methods for cell culture, flow cytometry and zebrafish husbandry

Kinetics

Kinetic measurements using benzothiazole alkyne and azides as the model system were performed using a *96-well BioTek Synergy Hybrid Plate Reader*.

Tissue culture/cell growth conditions

Jurkat cell were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) (Sigma). Wild-type and LEC2 CHO cells were grown in suspension or monolayer in alpha-Minimum Essential medium supplemented with 10 % FBS. In all cases, cells were incubated in a 5.0 % carbon dioxide, water-saturated incubator at 37 °C.

Flow cytometry

Flow cytometry experiments were performed on an *Eclipse iCyt* flow cytometer using a 488 nm argon laser. At least 15000 cells were recorded for each sample. Flow cytometry data were analyzed using *ec800 version 1.3.3* software. Mean fluorescence intensity (MFI) was calculated for live cells. Cell viability was ascertained by gating samples on the basis of forward scatter (to sort by size) and FL4 channel (to sort by 7-AAD negative).

Zebrafish husbandry, strains and microinjection

Zebrafish husbandry was carried out in accordance with the Animal Institute protocol of the Albert Einstein College of Medicine. The wild-type strain was used in this study. Microinjections were performed using a *PV 820 Pneumatic PicoPump* (World Precision Instruments) under a *Nikon SMZ1500* with *Plan Apo 1 ×WD70* lens.

Image acquisition and analysis

Confocal fluorescent images were acquired sequentially on a *Leica SP5 AOBS confocal microscope* with a 10×/0.4 air objective. All embryo images were acquired using a 3 μm step size and 1 airy unit. Composite figures were prepared using *ImageJ*, *Photoshop CS2* and *Illustrator CS5* software (Adobe).

Protocol for kinetic measurement of CuAAC using 2-ethynylbenzo[d]thiazole and azides (Figure S2)

The semi-quantitative measurement of the kinetics of CuAAC was performed as described in Scheme 1. As the conversion rate (%) is correlated with the fluorescence intensity of each product, the standard curves for each of the triazole products were generated by measuring the maximum fluorescence in 100% reactions ([Azide]:[2-ethynylbenzo[d]thiazole]=4:1, [Cu]=50 μM) at different concentrations of 2-ethynylbenzo[d]thiazole, to calibrate the conversion rate (%) for each azide. The fluorescence intensity generated by each triazole product reached a maximum when one equivalent of 2-ethynylbenzo[d]thiazole and four equivalents of azide were used in the presence of 50 μM of copper (I). No further increase was observed, even when adding more azide and copper (I), at which point, the disappearance of the starting 2-ethynylbenzo[d]thiazole and the formation of the triazole product was confirmed by LC-MS analysis. The fluorescence intensity has an excellent linear correlation (R^2=0.99) with the concentration of the triazole product below 25 μM, which is the theoretical maximum concentration of the cycloaddition product formed in this assay. The corresponding products of azide **1**, **2**, **3** and **4** have similar maximum fluorescence intensities (with only a slight variation of < 7%), which were used to define the 100% conversion for each reaction.

Stock solutions:

Buffer: 500 mM potassium phosphate, pH = 7.0

2-Ethynylbenzo[d]thiazole: 5 mM

$CuSO_4$: 2 mM in water

BTTPS: 4 mM in water

Azide 1, **2**, **3, 4** or **5**: 5 mM in DMSO

Sodium ascorbate: 25 mM in water

Final concentrations:

Buffer: 100 mM potassium phosphate (pH = 7.0)

2-Ethynylbenzo[d]thiazole: 50 μM

$CuSO_4$: 25 μM (50 μM in 100% rxn)

Catalyst:ligand ratio: [BTTPS] : [$CuSO_4$] = 2:1

Azide 1, **2**, **3, 4** or **5**: 25 μM

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. 2 μL 5 mM 2-ethynylbenzo[d]thiazole.

3. 5 μL of the pre-mixed $CuSO_4$ and BTTPS ([Cu] = 1 mM, [BTTPS]= 2 mM) (10 μL in 100 % rxn).

4. 1 μL 5 mM azide **1**, **2**, **3, 4** or **5**.

5. Add water to 180 μL volume.

6. 20 μL of 25 mM sodium ascorbate.

7. Read fluorescence (λ_{ex} = 285 nm, $\lambda_{em\ (max)}$ = 363 nm, RFU).

The general procedure for the metabolic labeling of sialylated glycans in Jurkat cells, detection of alkynl sialic acids via the CuAAC, and analysis by flow cytometry (Figure S4-5)

For the CuAAC labeling efficiency and biocompatibility evaluation experiment, Jurkat cells were seeded at 0.15 M/mL and incubated for 3 days in untreated RPMI or RPMI medium containing 50 μM Ac_4ManNAl. On day three, the cells were harvested.

The harvested cells were washed 2× with cold labeling buffer (PBS, pH = 7.4, 1 % FBS), and were aliquoted into a 96-well round bottom tissue culture plate (0.4 million cells in 90 μL/well) (Corning Inc.). For CuAAC, 50 μM biotin-azide **6** or **7**, the BTTPS-$CuSO_4$ complex ([BTTPS]:[$CuSO_4$] = 5:1) and 2.5 mM sodium ascorbate were added to each well. After reaction for various time periods at room temperature, the reactions were quenched with 1 mM BCS.

Following the reactions, the cells were pelleted, washed 3× with 200 μL cold labeling buffer and re-suspended in the same buffer containing 1 μg/mL streptavidin-Alexa Fluor 488 (Invitrogen). The plate was covered with aluminum foil and incubated at 4 °C for 30 min (re-suspension once after 15 min). The cells were then washed 3× with 200 μL cold labeling buffer and re-suspended in 250 μL cold FACS buffer (Hank's Balanced Salt Solution, pH = 7.4, 1 % FBS, 2 μg/mL 7-AAD, 0.2 % NaN_3) for flow cytometry analysis. The cell survival rate was

calculated by gating the sample on the basis of forward scatter (to sort by size) and FL4 (to sort by 7-AAD negative).

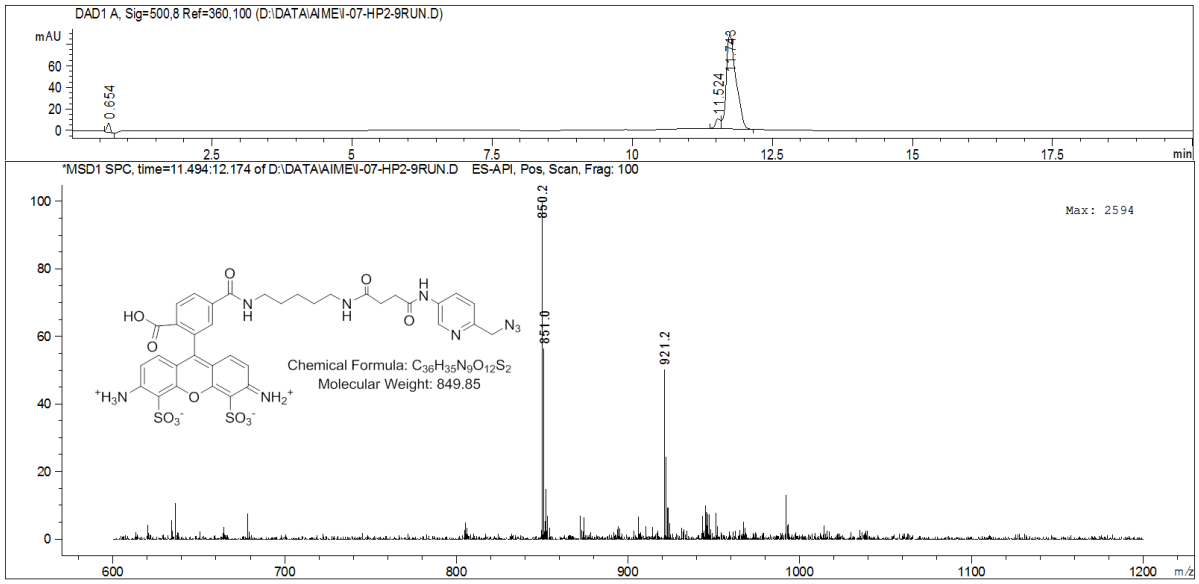


Figure S1: ESI-MS for azide **4**-conjugated Alexa Fluor 488 (**8**)

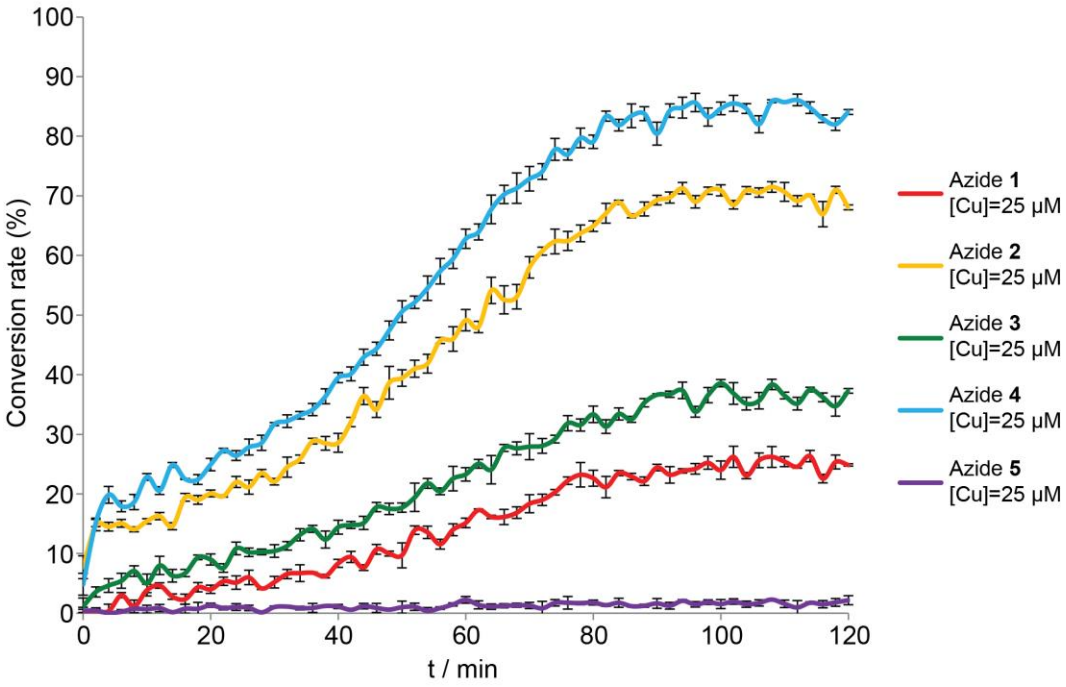


Figure S2: Conversion-time profiles of CuAAC in the presence of various azides using the fluorogenic assay outlined in **Scheme 1B**. Error bars represent the standard deviation of three replicate experiments.

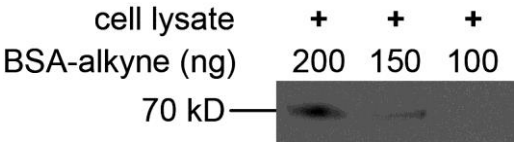


Figure S3: Evaluation of the detection limit of biotin azide **6** for labeling the alkyne-modified BSA in cell lysates.

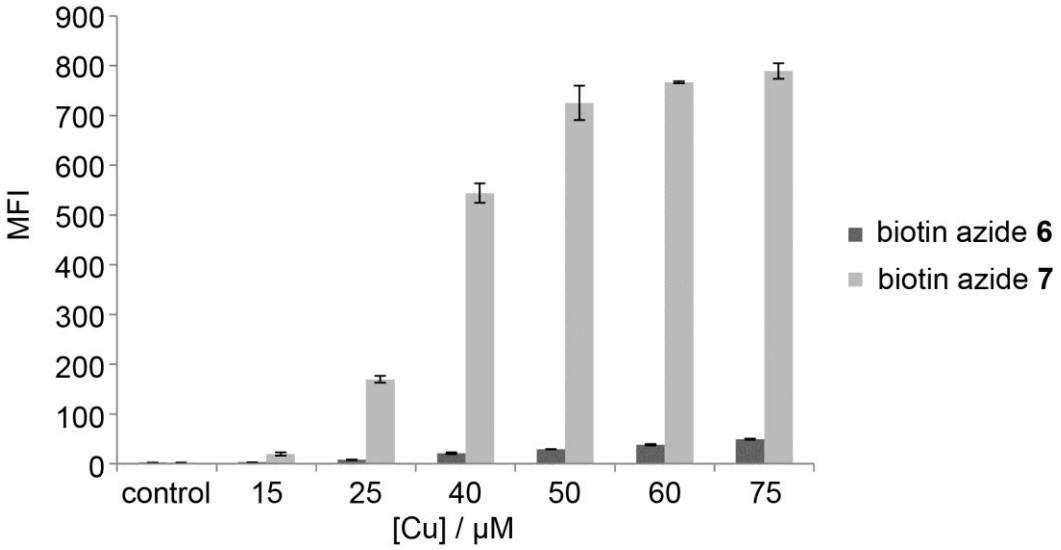


Figure S4: Comparison of the reactivity of biotin azide **6** and **7** in the labeling of metabolically incorporated SiaNAl on the cell-surface of live Jurkat cells at different Cu(I) concentrations. Cells were cultured with 10 μM Ac_4ManNAl for 3 days. For CuAAC, 50 μM biotin-azide, BTTPS-$CuSO_4$ complex ([BTTPS]:[$CuSO_4$] = 5:1) and 2.5 mM sodium ascorbate were used. Labeling reactions were allowed to proceed for 5 min before quenching the reactions with BCS. Error bars represent the standard deviation of three replicate experiments.

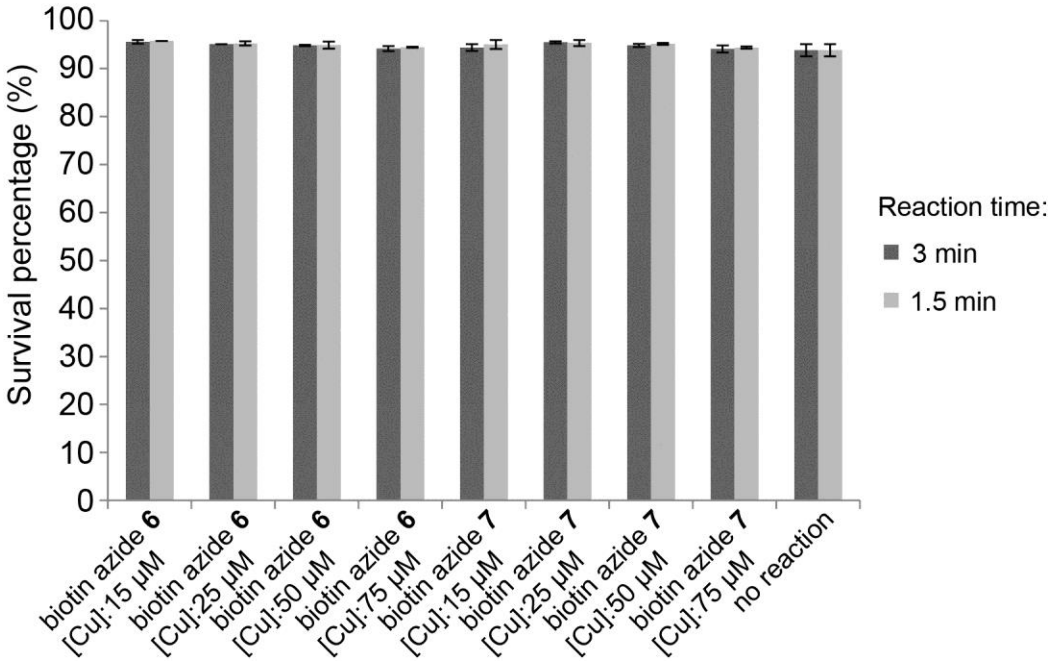


Figure S5: Biocompatibility analysis of CuAAC with biotin azide **6** and **7** (Jurkat cells). Jurkat cells (0.15 million) were incubated in untreated RPMI medium (10 % FBS) or RPMI medium (10 % FBS) containing 50 μM Ac_4ManNAl for 3 days. The cells were harvested and reacted with biotin **6** (50 μM) and biotin azide **7** (50 μM) in the presence of BTTPS-Cu(I) complex ([BTTPS]:[$CuSO_4$] = 5:1, [Cu] = 15, 25, 50 or 75 μM) for 3 min or 1.5 min at room temperature. The cell survival rate was calculated by gating the sample on the basis of forward scatter (to sort by size) and FL4 (to sort by 7-AAD negative). Error bars represent the standard deviation of three replicate experiments.