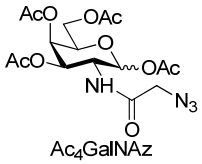
Supporting Information for

Rapid Labeling of Metabolically Engineered Cell-Surface Glycoconjugates with a Carbamate-Linked Cyclopropene Reporter

*Anne-Katrin Späte, Holger Bußkamp, Andrea Niederwieser, Verena F. Schart, Andreas Marx, and Valentin Wittmann**

University of Konstanz, Department of Chemistry and Konstanz Research School Chemical Biology (KoRS-CB), Universitätsstraße 10, 78457 Konstanz (Germany)

Fluorescence Microscopy with Tz-Cy3 for Dual Labeling with Ac_4GalNAz. HEK 293T cells (7500 cells/cm^2) were seeded in 8-well ibiTreat µ-Slides (ibidi) and allowed to attach for 12 h. Cells were then incubated with 100 µM Ac_4ManNCyoc **13** and 50 µM Ac_4GalNAz **25** for 48 h. No sugar or only one sugar was added as negative control. Cells were washed two times with PBS and then treated with a mixture of Tz-Cy3 **22** (25 µM) and AlexaFluor®488-DIBO **24** (20 µM) for 15 min at 37 °C. Cells were washed twice with PBS and nuclei were stained with Hoechst 33342 (10 µg mL^{-1}) for 20 min at room temperature in the dark. Cells were washed twice with PBS, and DMEM was added for microscopy. Microscopy was performed as described above.



25

Fluorescence Microscopy with Tz-Biotin for Dual Labeling with Ac_4GlcNAz. HEK 293T cells (7500 cells/cm^2) were seeded in 8-well ibiTreat µ-Slides (ibidi) and allowed to attach for 12 h. Cells were then incubated with 100 µM Ac_4ManNCyoc **13** and 50 µM Ac_4GlcNAz **23** for 48 h. No sugar or only one sugar was added as negative control. Cells were washed two times with PBS and then treated with a mixture of Tz-biotin **17** (25 µM) and AlexaFluor®488-DIBO **24** (20 µM) for 15 min at 37 °C. After two washes with PBS, cells were incubated with AlexaFluor®647-labeled streptavidin (6.6 µg mL^{-1}) and Hoechst 33342 (10 µg mL^{-1}) for 20 min at room temperature in the dark. Cells were washed twice with PBS, and DMEM was added for microscopy. Microscopy was performed as described above.

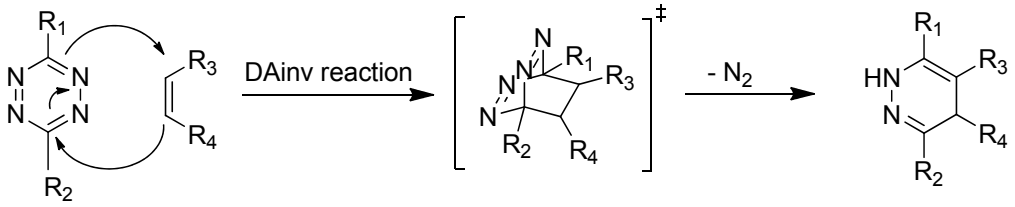


Figure S1. Mechanism of the DAinv reaction.

Stability of tetrazine 15. To determine the stability of Tz-PEG **15**, solutions of **15** (5 mM) in acetate buffer (pH 4.8)[1] and PBS (pH 7.2) were prepared. Decomposition of **15** was followed at room temperature by measuring its absorption at 522 nm over time (Figure S2).

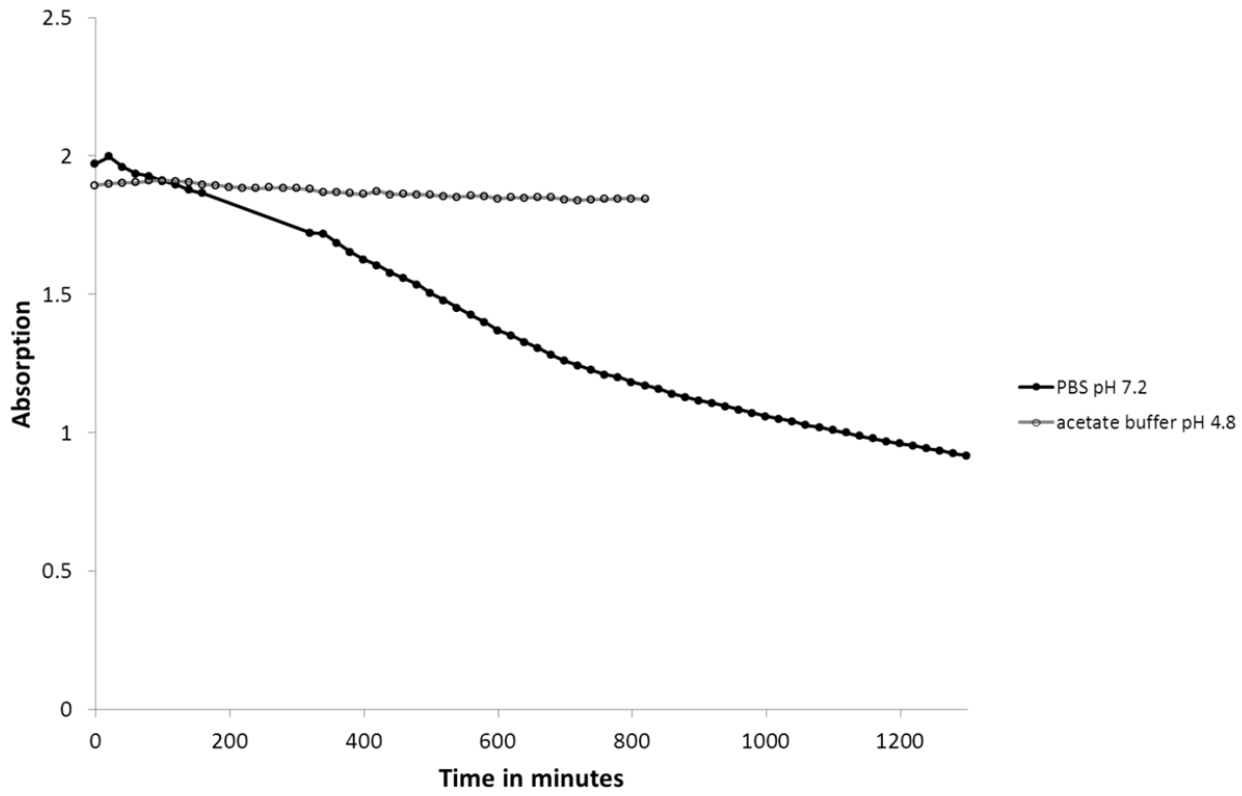
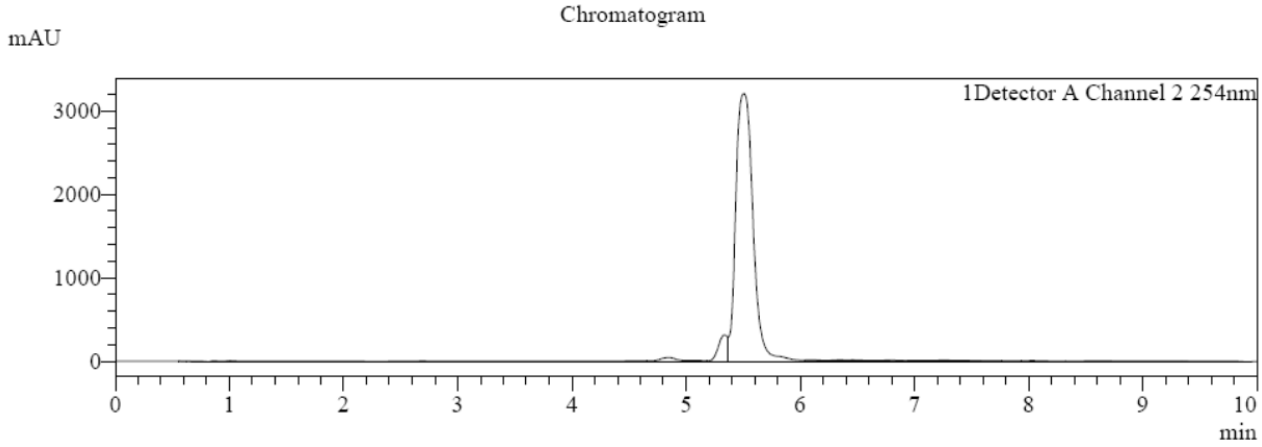


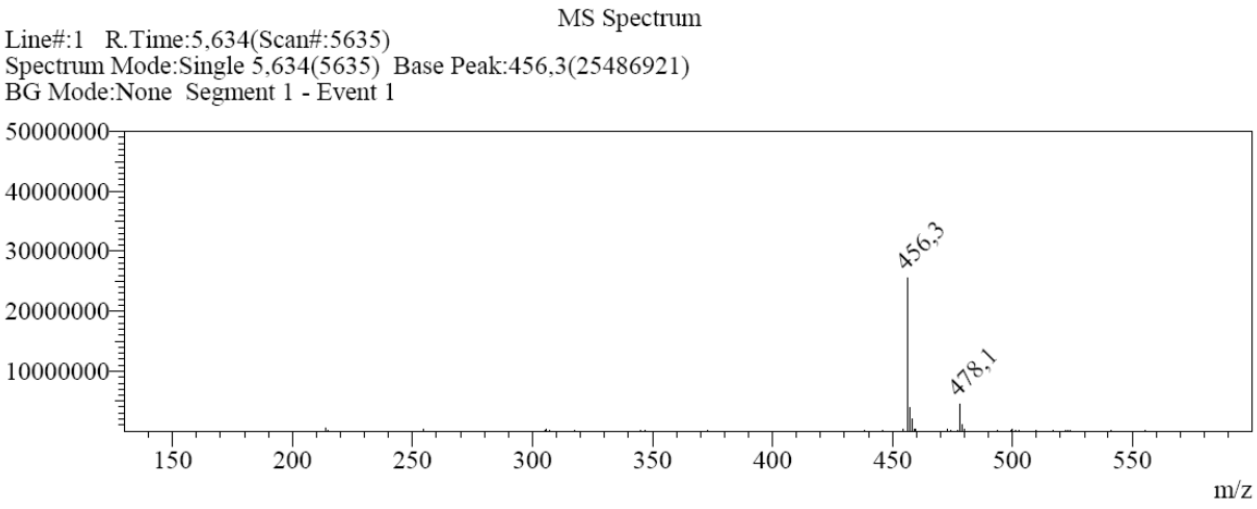
Figure S2. Decrease of the absorption at 522 nm over time of solutions of Tz-PEG **15** (5 mM) in acetate buffer (pH 4.8)[1] and PBS (pH 7.2).

[1] Niederwieser, A., Späte, A.-K., Nguyen, L. D., Jüngst, C., Reutter, W., and Wittmann, V. (2013) Two-Color Glycan Labeling of Live Cells by a Combination of Diels-Alder and Click Chemistry. *Angew. Chem., Int. Ed. 52*, 4265-4268.

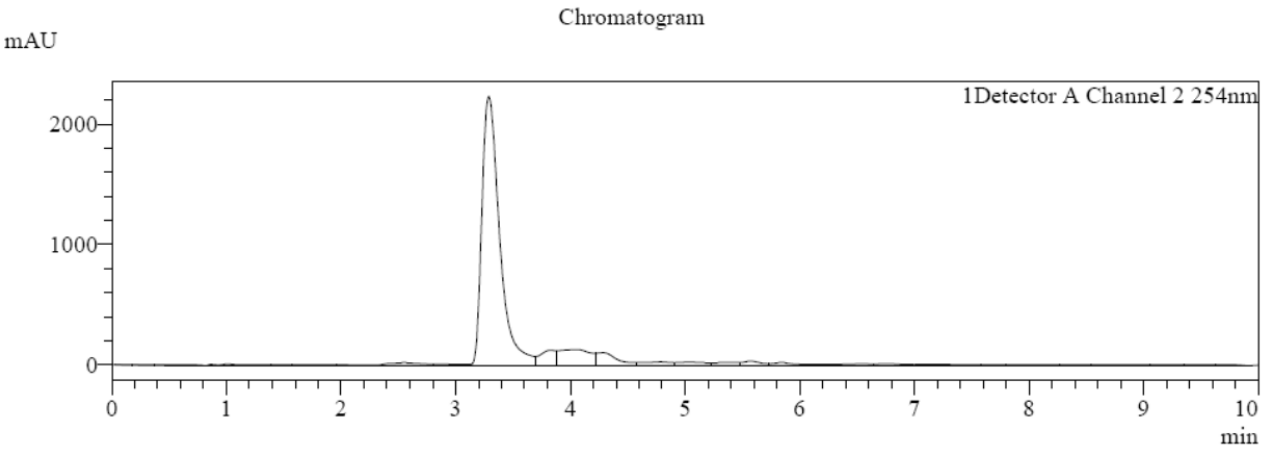
A


Chromatogram
mAU
1Detector A Channel 2 254nm
3000
2000
1000
0
0
1
2
3
4
5
6
7
8
9
10
min

B


MS Spectrum
Line#:1 R.Time:5,634(Scan#:5635)
Spectrum Mode:Single 5,634(5635) Base Peak:456,3(25486921)
BG Mode:None Segment 1 - Event 1
50000000
40000000
30000000
20000000
10000000
456,3
478,1
150
200
250
300
350
400
450
500
550
m/z

C



D

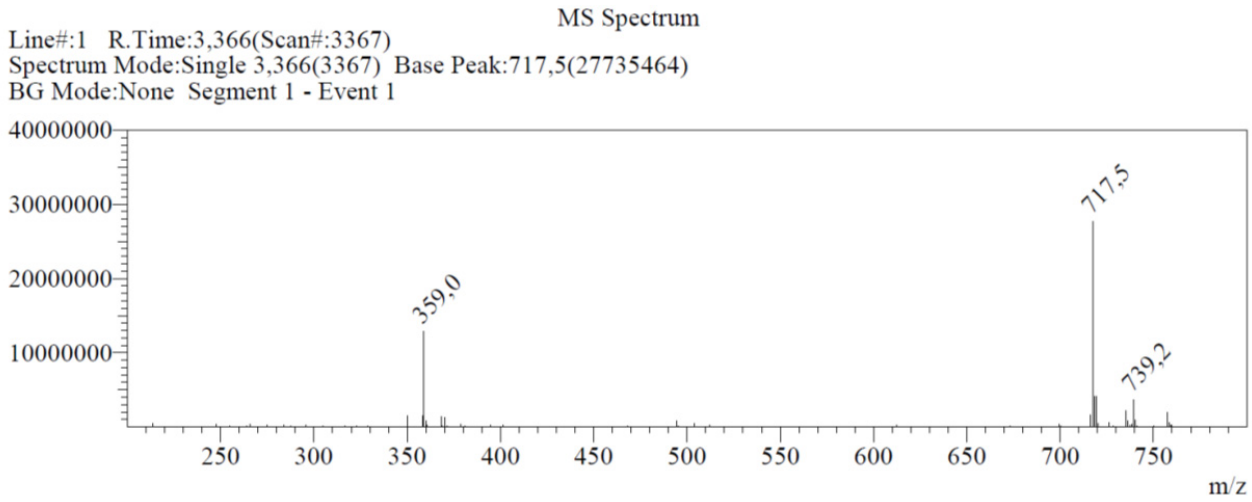


Figure S3. (A) HPLC analysis of Tz-PEG **15** using a gradient of CH_3CN in H_2O (with 0.1% formic acid, 20-90% in 10 min) and (B) Peak analyzed by ESI-MS. Calcd: $[M + H]^+$: 456.20, $[M + Na]^+$: 478.18 found: $[M + H]^+$: 456.35, $[M + Na]^+$: 478.05. (C) HPLC analysis (gradient as described under (A)) of ligation product **16** after the Diels-Alder reaction in solution for kinetic measurements. All Tz-PEG **15** (retention time: 5.6 min) has reacted. Ligation product formation **16** can be observed (retention time 3.3 min). (D) ESI-MS of **16** calcd. 359.16 $[M + 2H]^{2+}$, 717.31 $[M + H]^+$, 739.29 $[M + Na]^+$, found: 359.00 $[M + 2H]^{2+}$, 717.50 $[M + H]^+$, 739.15 $[M + Na]^+$.

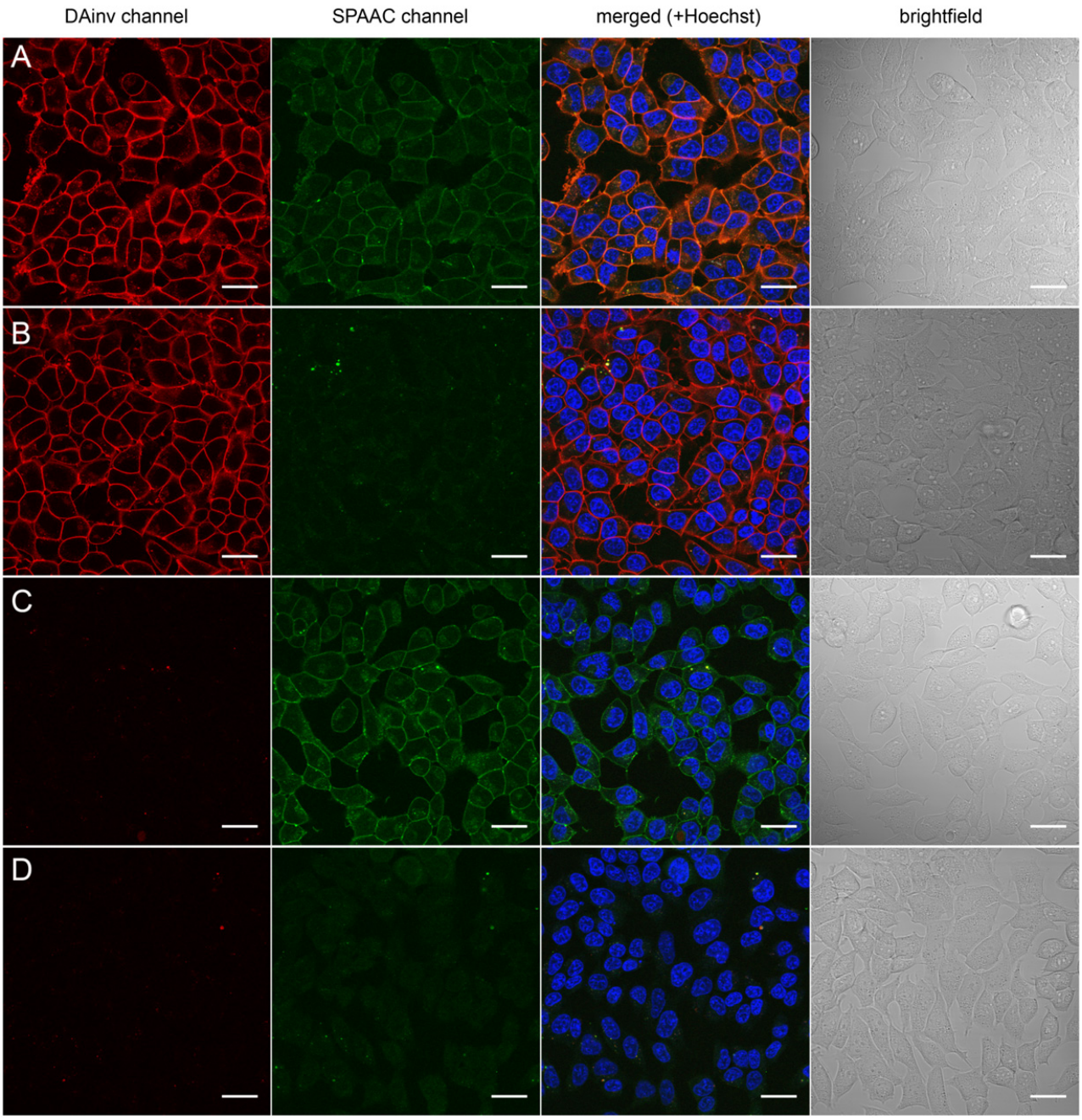


Figure S4. HEK 293T cells were grown with (A) 100 µM Ac_4ManNCyoc **13** and 50 µM Ac_4GalNAz **25**, (B) 100 µM Ac_4ManNCyoc **13**, (C) 50 µM Ac_4GalNAz **25**, and (D) without non-natural sugar for 48 h and incubated with a mixture of Tz-Cy3 **22** (25 µM) and DIBO-488 **24** (20 µM) for 15 min at 37 °C. Nuclei were stained with Hoechst33342. Scale bar: 30 µm.

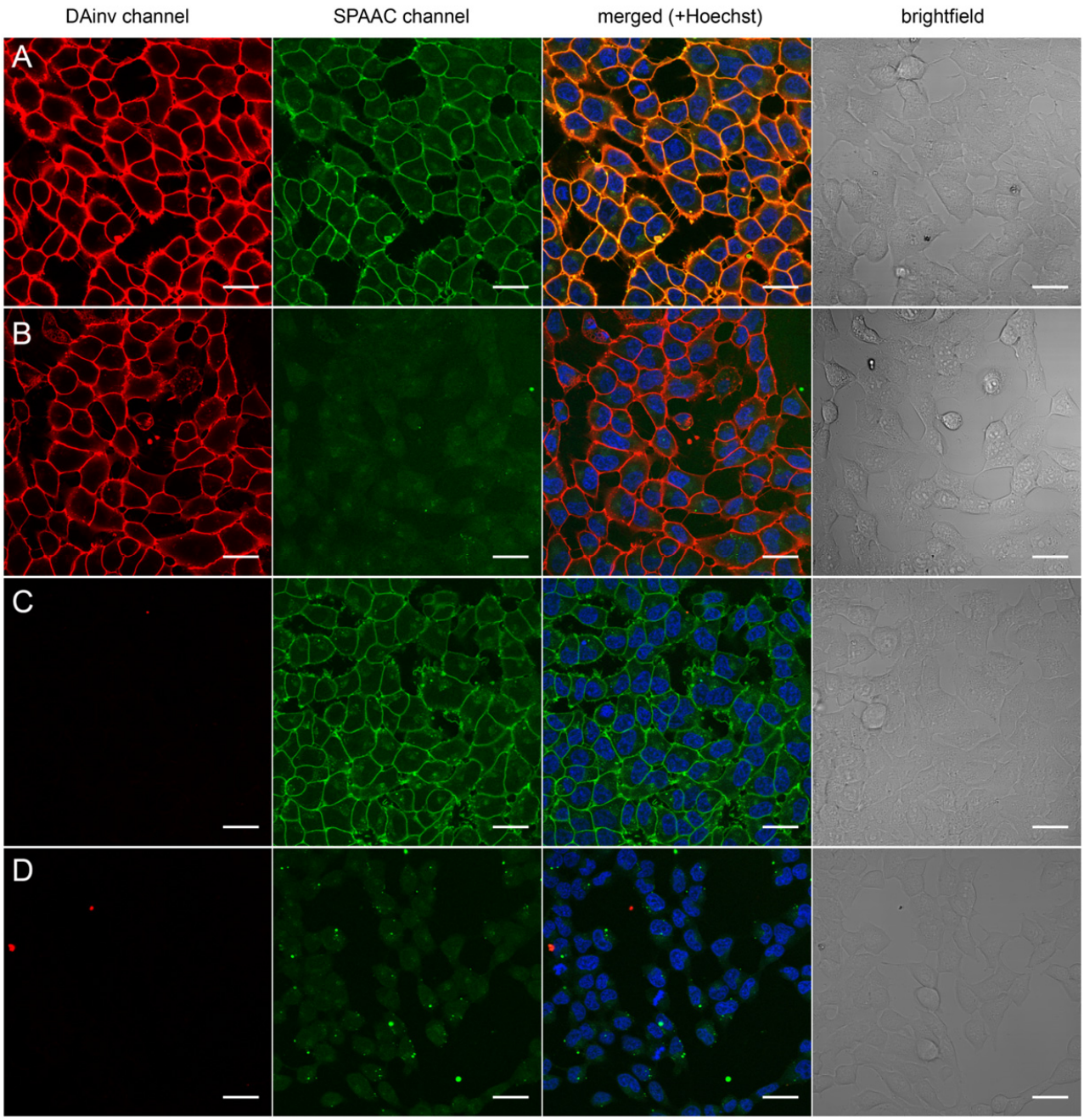


Figure S5. HEK 293T cells were grown with (A) 100 µM Ac_4ManNCyoc **13** and 50 µM Ac_4GlcNAz **23**, (B) 100 µM Ac_4ManNCyoc **13**, (C) 50 µM Ac_4GlcNAz **23**, and (D) without non-natural sugar for 48 h and incubated with a mixture of Tz-biotin **17** (25 µM) and DIBO-488 **24** (20 µM) for 15 min at 37 °C followed by labeling with Streptavidin-AlexaFluor647. Nuclei were stained with Hoechst33342. Scale bar: 30 µm.

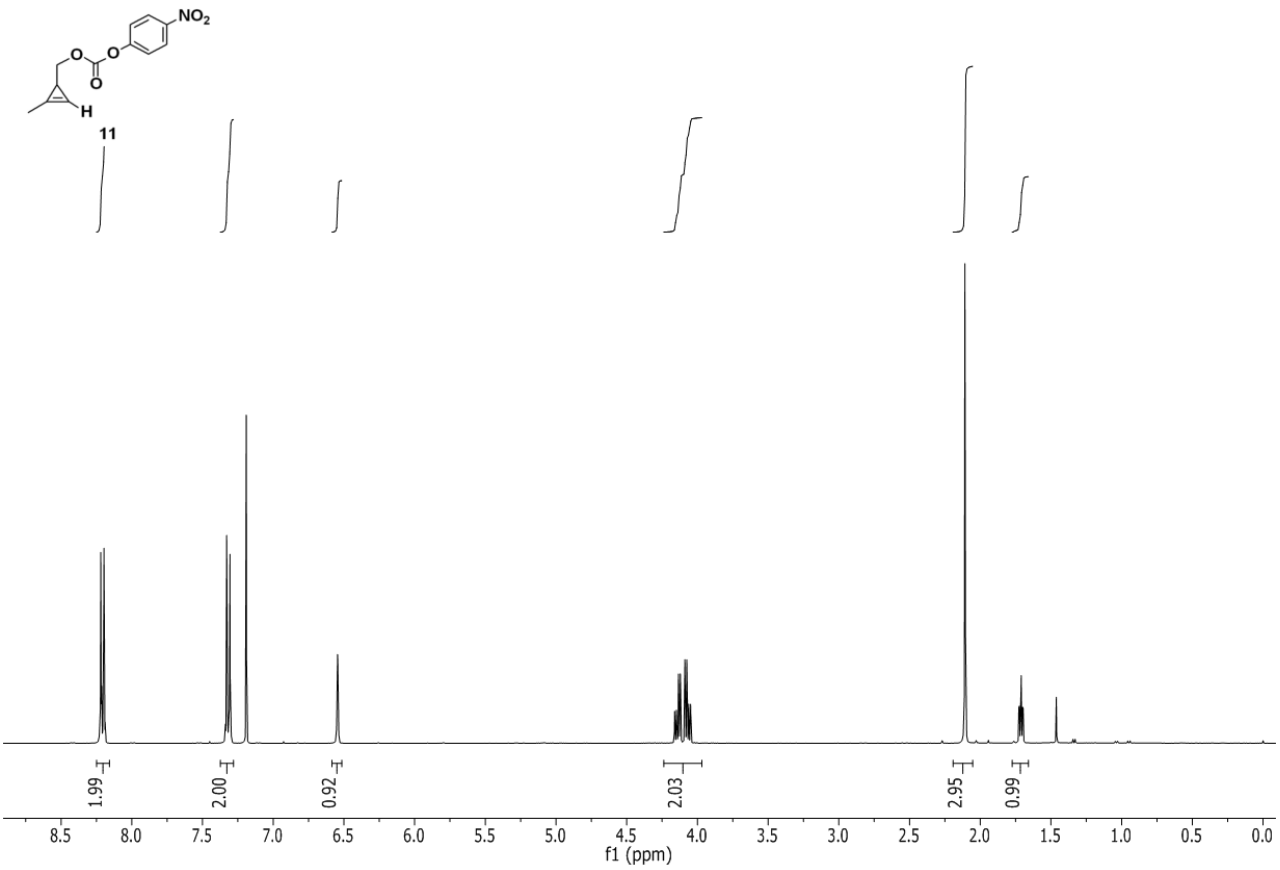


Figure S6. ^{1}H NMR spectrum ($CDCl_3$, 400.1 MHz) of **11**.

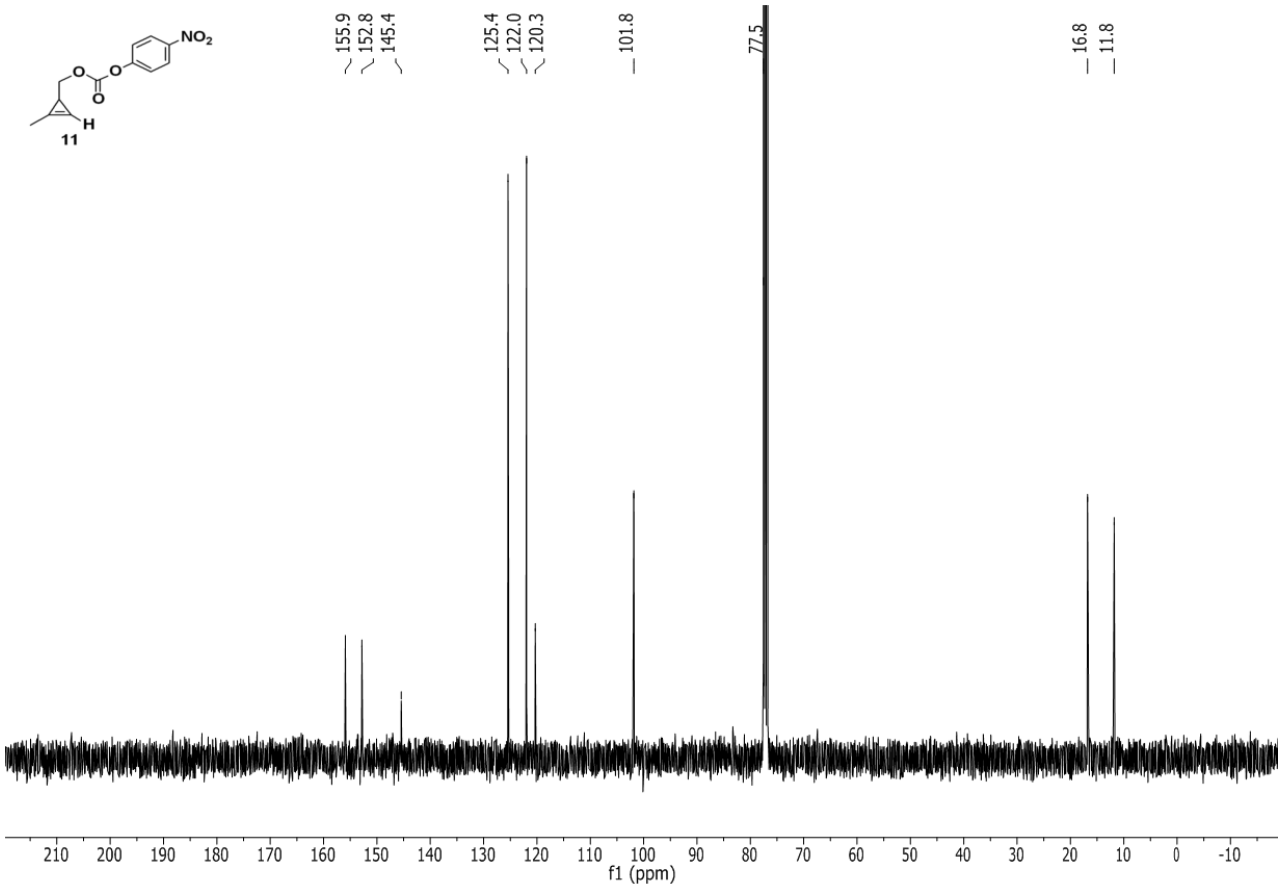


Figure S7. ^{13}C NMR spectrum ($CDCl_3$, 100.6 MHz) of **11**.

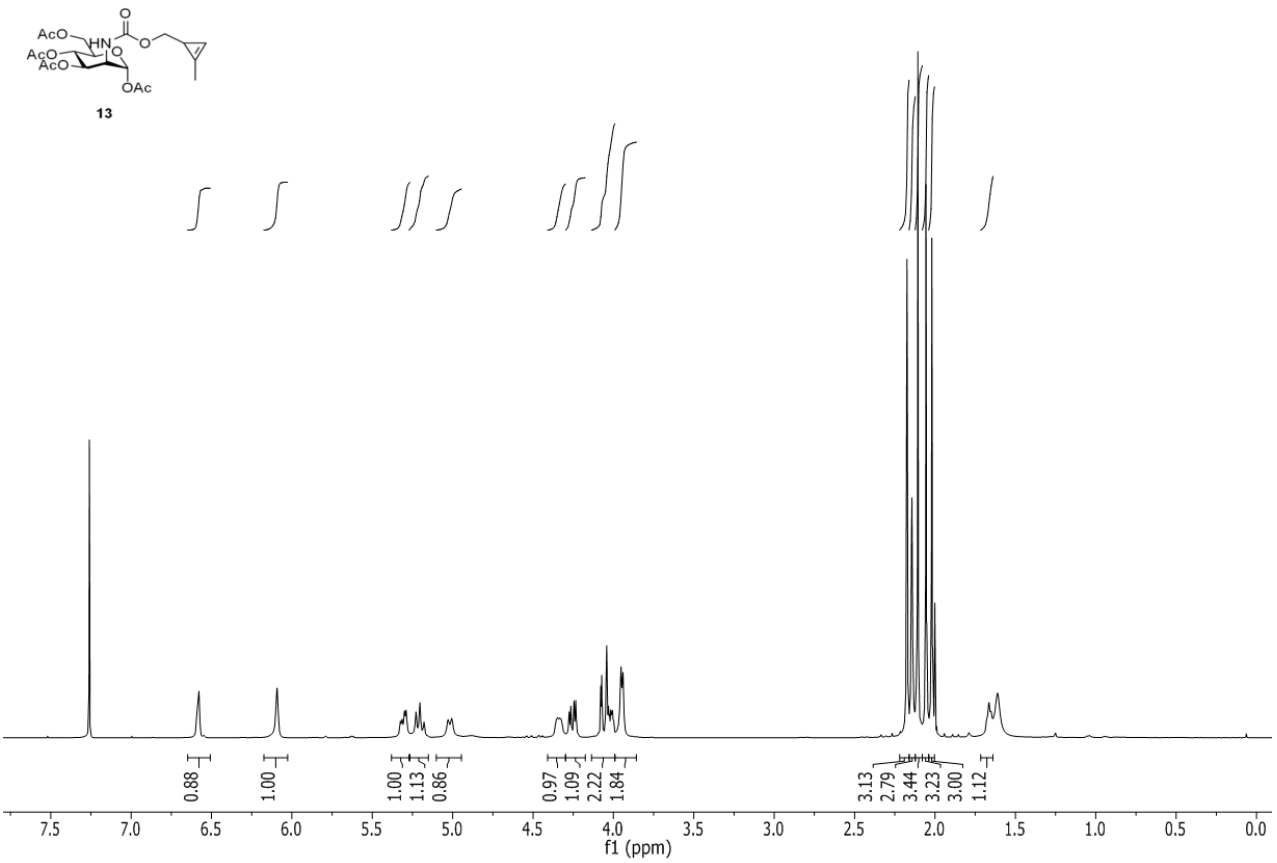


Figure S8. ^{1}H NMR spectrum ($CDCl_3$, 400.1 MHz) of **13**.

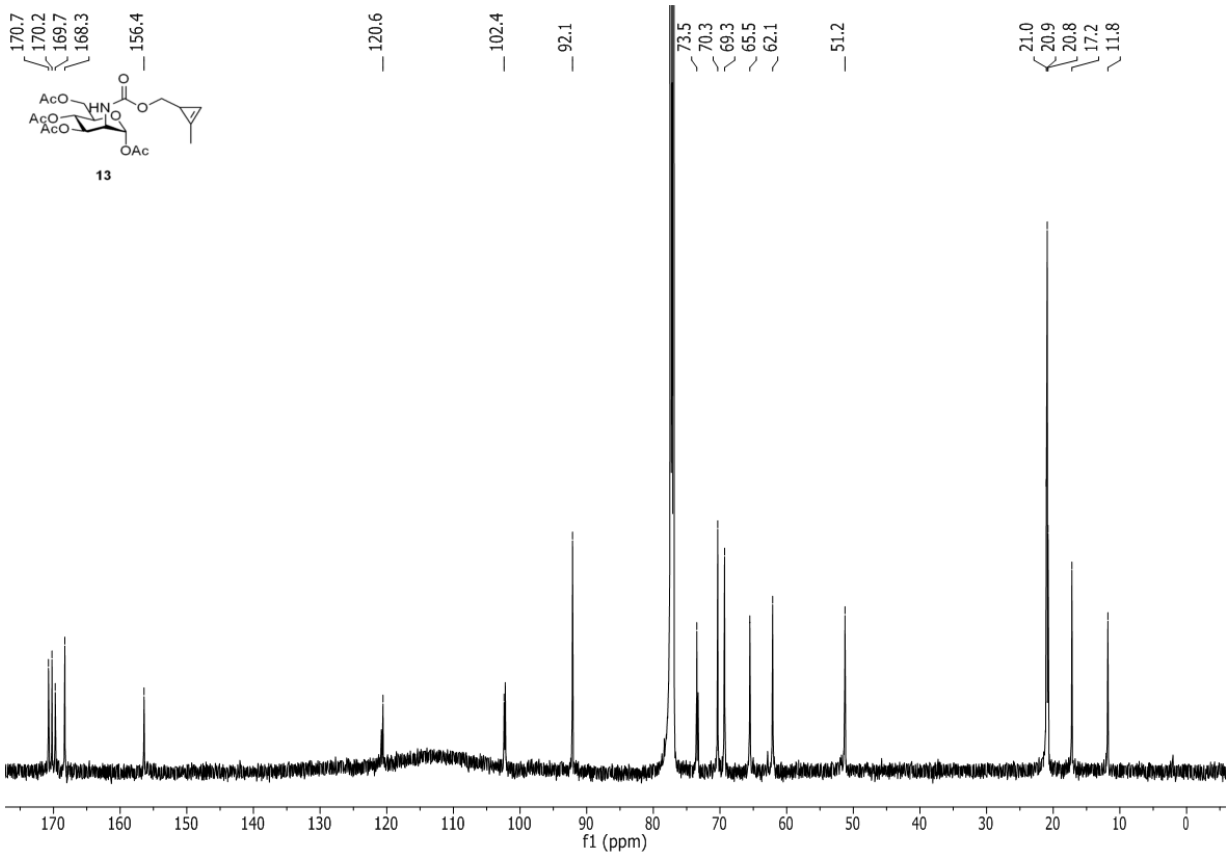


Figure S9. ^{13}C NMR spectrum ($CDCl_3$, 100.6 MHz) of **13**.