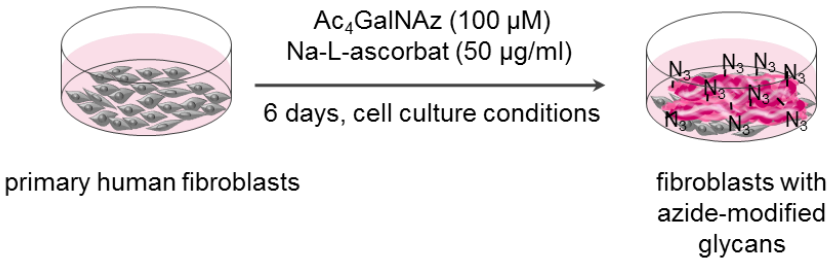
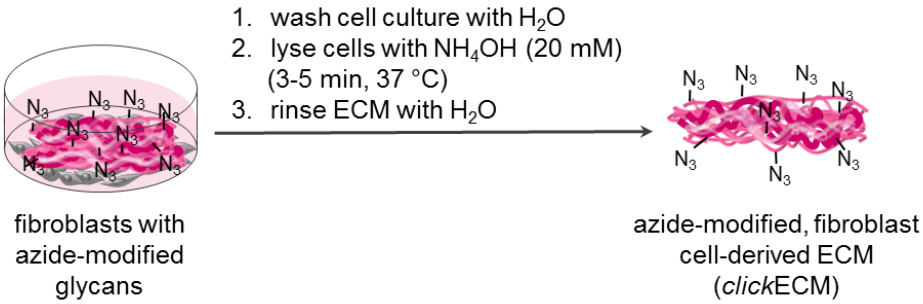
Supplementary Scheme 1: Metabolic oligosaccharide engineering.

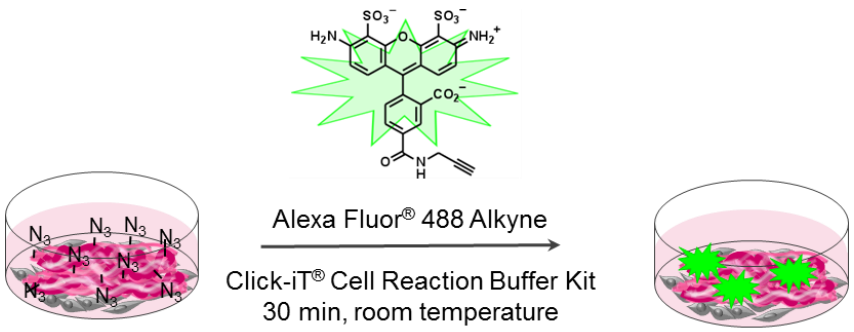


Supplementary Scheme 2: Isolation of azide-modified *click*ECM.

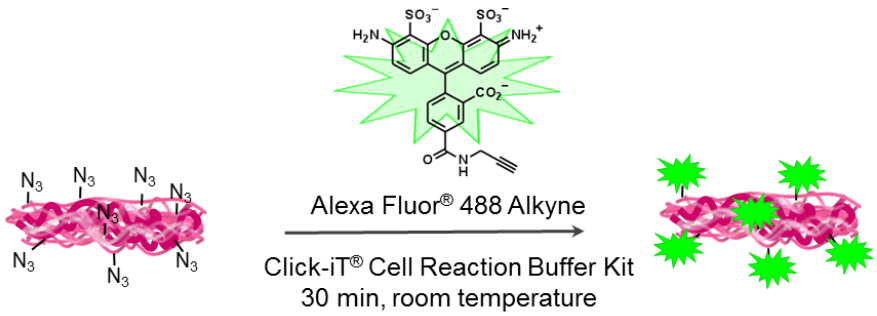


Supplementary Scheme 3: Detection of azide groups in formalin-fixed samples via copper-catalyzed azide-alkyne cycloaddition with an alkyne-modified fluorophore.

A Fibroblast cell cultures

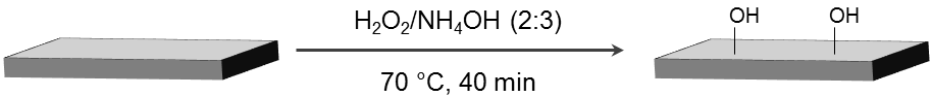


B *click*ECM

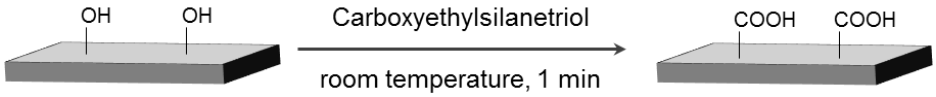


Supplementary Scheme 4: Alkyne modification of artificial surfaces (silicon wafers).

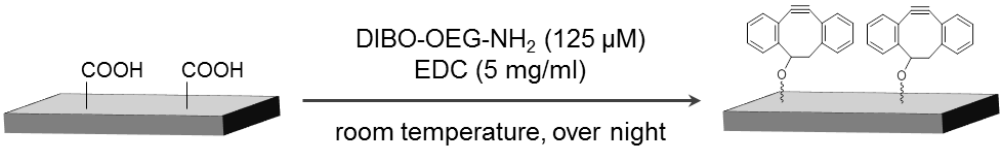
A Surface activation with ammonium hydroxide and hydrogen peroxide



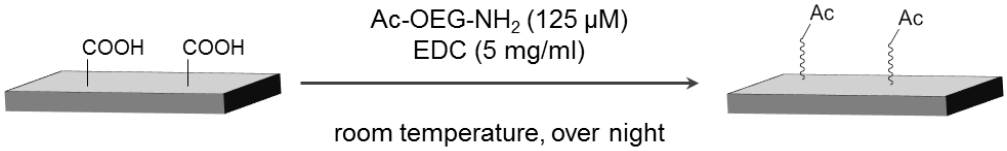
B Silanization



C Functionalization with alkyne groups (DIBO-functionalized surfaces)

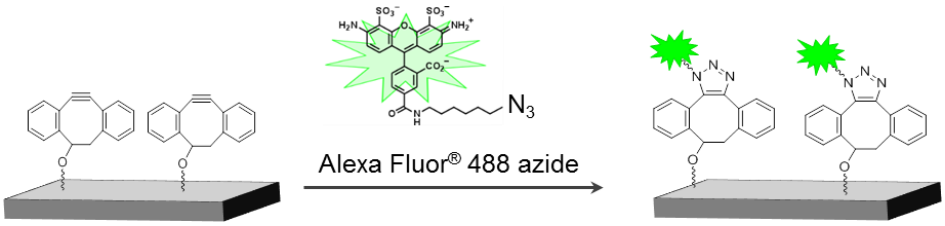


D Functionalization with Ac-OEG-linker (control)

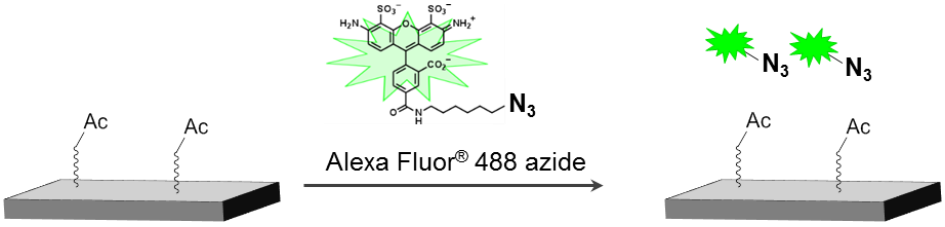


Supplementary Scheme 5: Detection of the immobilized DIBO group with an azide-modified fluorophore (copper-free azide-alkyne cycloaddition).

A DIBO-functionalized surfaces

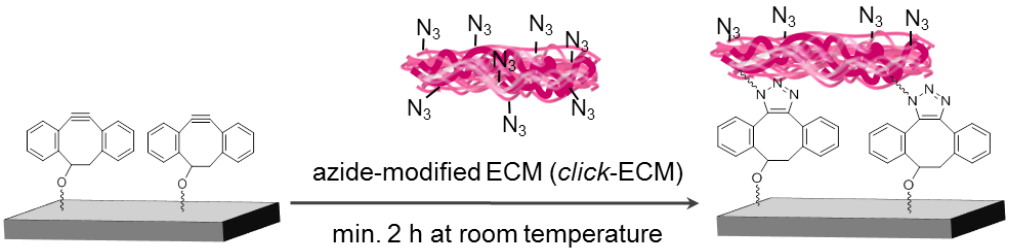


B Control: unspecific interaction of an immobilized Ac-OEG-linker with the azide-modified fluorophore.

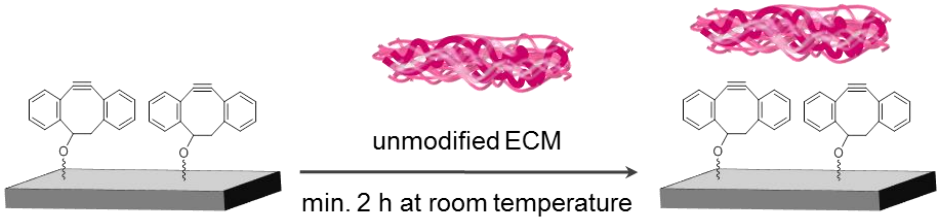


Supplementary Scheme 6:

A Covalent immobilization of azide-modified *click*ECM on DIBO-functionalized artificial surfaces (silicon-wafers).



B Preparation of control substrates via physisorption of unmodified ECM on DIBO-functionalized artificial surfaces (silicon-wafers).

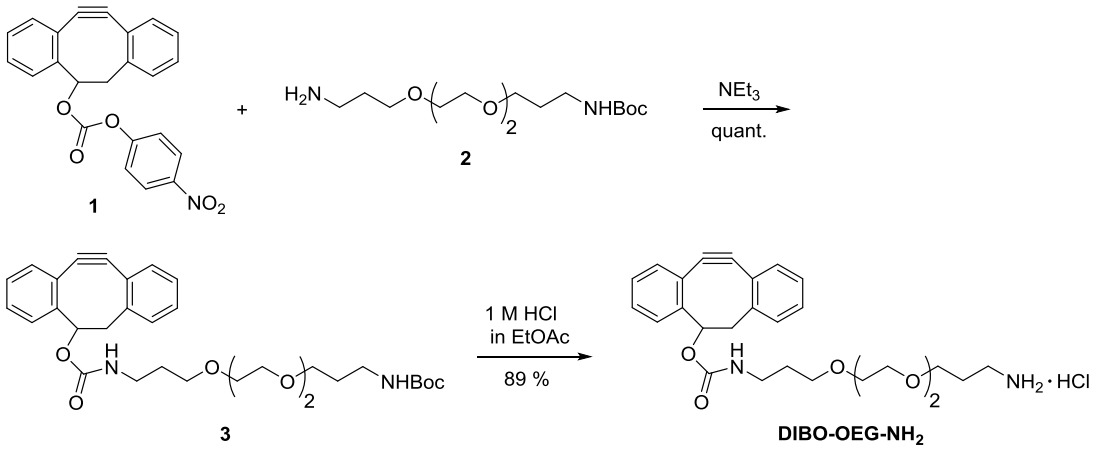


Chemical Syntheses

General Methods. NEt_3 was distilled from CaH_2 before usage. Analytical thin layer chromatography (TLC) was carried out on silica gel 60 F_{254} coated aluminum sheets from *Merck* with detection under UV light (λ = 254 nm). Additionally, the TLC plates were charred using ethanolic ninhydrin solution (3 % w/v) or *p*-anisaldehyde (3.7 mL *p*-anisaldehyde, 5 mL conc. H_2SO_4 and 1.4 mL HOAc in 135 mL dry EtOH) staining solution. Flash column chromatography (FC) was performed on silica 60 (40-63 µm) from *Merck*. NMR spectra were recorded on Bruker Avance III 400 instruments. Chemical shifts are given in ppm and referenced to the solvent signal ($CDCl_3$: δ_H = 7.26 δ_C = 77.16, CD_3OD: δ_H = 3.31, δ_C = 49.0). Assignment of proton and carbon resonances was achieved by two-dimensional COSY, HSQC, and HMBC experiments. LC-ESI-MS analyses were conducted on a LCMS2020 instrument from *Shimadzu* (pumps LC-20 AD, autosampler SIL-20AT HAT, column oven CTO-20AC, UV-Vis detector SPD-20A, controller CBM-20, ESI detector and software LCMS-solution) with an EC 125/4 Nucleodur C18, 3 µM column (*Machery-Nagel*). HRMS analyses were performed on a *micrOTOF II* instrument from *Bruker* in positive mode.

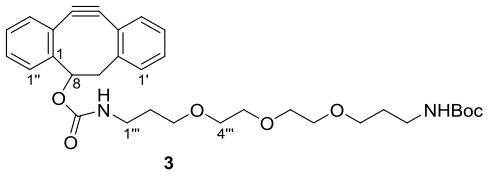
$Ac_4GalNAz$[1] and $Ac_4GalNAc$[2] were synthesized according to published procedures. From both monosaccharides stock solutions (100 mM) in DMSO were prepared. Prior to cell treatment, these stock solutions were diluted to 10 mM in PBS and sterile filtered.

Synthesis of DIBO-OEG-NH_2



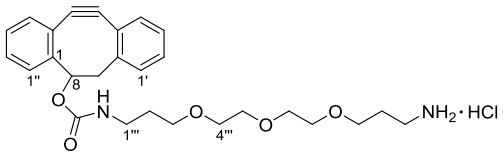
Supplementary Scheme 7: Synthesis of DIBO-OEG-NH_2

7,8-Didehydro-1,2:5,6-dibenzocyclooctene-3-yl (3-(2-(2-(3-*N*-Boc-aminopropoxy)-ethoxy)ethoxy)propyl)carbamate (3)



Carbonate **1**[3] (46 mg, 119 µmol) was dissolved in dry DMF. Tri(ethylene glycol) derivative **2** (107 mg, 334 µmol) and dry NEt_3 (50 µL, 360 µmol) were added and the solution was stirred at RT. When TLC showed complete consumption of **1**, the solvent was removed under reduced pressure and the residue coevaporated 3 times with toluene. FC (petroleum ether/EtOAc 1:1 to 1:3) afforded **3** (68 mg, quant.) as pale oil. TLC (petroleum ether/EtOAc, 1:3): R_f = 0.33; ^{1}H NMR (400.1 MHz, CD_3OD): δ = 7.56 (d, J = 7.8 Hz, 1H, Ar-H), 7.44-7.25 (m, 7H, Ar-H), 5.43 (m, 1H, H-8), 3.70-3.51 (m, 10H, OC$\underline{H}_2$$CH_2$ 3’’’,4’’’,5’’’,6’’’,7’’’), 3.49 (t, 2H, CH_2 8’’’), 3.25-3.17 (m, 3H, H-7a and CH_2 1’’’), 3.10 (t, J = 6.8 Hz, 2H, CH_2 10’’’), 2.83 (dd, J = 14.9, 3.8 Hz, 1H, H-7b) 1.77 (quint, J = 6.4 Hz, 2H, CH_2 2’’’), 1.70 (quint, J = 6.5 Hz, 2H, CH_2 9’’’), 1.42 (s, 9H, 3 x CH_3); ^{13}C NMR (100.6 MHz, CD_3OD): δ = 158.4 (C=O), 157.9 (C=O), 153.7 (quart.), 152.4 (quart.), 131.0 (C-1’), 129.3, 129.2, 128.3 (C-3’’), 128.2, 127.2, 126.9, 125.0 (C-1’), 124.9, 122.4 (C-2), 113.8 (C-4), 111.0 (C-3), 79.9 ($\underline{C}(CH_3)_3$), 77.8 (C-8), 2 x 71.5, 71.3, 71.2 (C-4’’’-C-7’’’), 69.9, 69.8 (C-3’’’ and C-8’’’), 47.2 (C-7), 39.2 (C-1’’’), 38.7 (C-10’’’), 2 x 30.9 (C-2’’’ and C-9’’’), 3 x 28.8 (CH_3); HRMS: *m/z* calcd for $C_{32}H_{43}N_2O_7$: 567.3065 $[M+H]^+$, found: 567.3040.

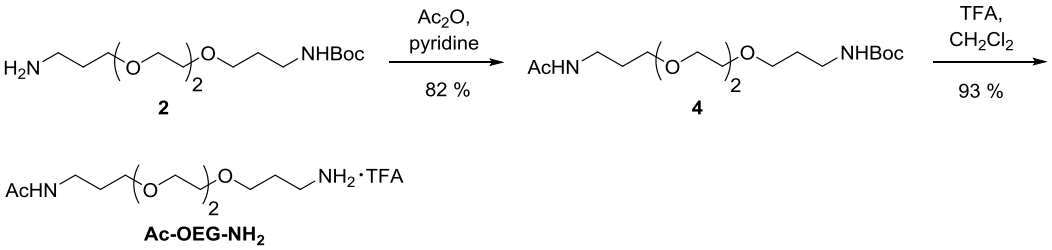
DIBO-OEG-NH_2



DIBO-OEG-NH_2

Compound **3** (66 mg, 116 µmol) was dissolved in 2 M HCl in EtOAc (2.5 mL) and the solution was stirred at RT. After complete consumption of starting material, the reaction mixture was diluted with toluene (4 mL) and then the solvent was removed under reduced pressure. DIBO-OEG-NH_2 (52 mg, 89 %) was obtained as pale yellow oil. TLC (CH_2Cl_2/MeOH 9:1) R_f = 0.22; ^{1}H NMR (399.8 MHz, CD_3OD): δ = 7.90 (s, 1H, C(O)NH), 7.57 (d, J = 7.7 Hz, 1H, Ar-H), 7.44-7.25 (m, 7H, Ar-H), 5.43 (m, 1H, H-8), 3.70-3.45 (m, 12H, OC$\underline{H_2}$$CH_2$ 3’’’, 4’’’, 5’’’, 6’’’, 7’’’, 8’’’), 3.26-3.16 (m, 3H, H-7a and CH_2 1’’’), 2.89-2.76 (m, 3H, H-7b and CH_2 10’’’), 1.82-1.69 (m, 4H, CH_2 2’’’ and CH_2 9’’’); ^{13}C NMR (100.5 MHz, CD_3OD): δ = 158.0 (C=O), 153.6 (quart.), 152.4 (quart.), 131.0 (C-1’), 129.3, 129.2, 128.3 (C-3’’), 128.2, 127.2, 126.9, 2 x 124.9, 122.4 (C-2), 113.8 (C-4), 111.0 (C-3), 77.8 (C-8), 71.4, 71.2, 71.1, 71.0 (C-4’’’-C-7’’’), 70.4, 69.5 (C-3’’’ and C-8’’’), 47.2 (C-7), 40.1 (C-1’’’), 39.1 (C-10’’’), 30.9, 30.2 (C-2’’’ and C-9’’’); HRMS: *m/z* calcd for $C_{27}H_{34}N_2O_5$: 467.2540 $[M+H]^+$, found: 467.2527.

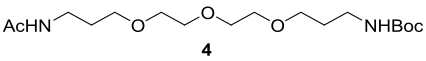
Synthesis of Ac-OEG-NH_2



2 **4** **Ac-OEG-NH_2**

Supplementary Scheme 8: Synthesis of Ac-OEG-NH_2

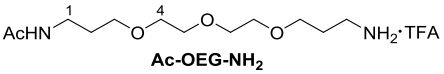
tert-Butyl(2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)carbamate (4)



4

Tri(ethylene glycol) derivative **2** (270 mg, 843 µmol) was dissolved in pyridine (2 mL) and Ac_2O (172 mg, 1.69 mmol) was added. According to TLC the reaction was completed after 45 min. The solvent was removed under reduced pressure and the residue 3 times coevaporated with toluene. FC (CH_2Cl_2 to CH_2Cl_2/MeOH 19:1) afforded **4** (250 mg, 82 %) as pale oil. TLC (EtOAc/MeOH 9:1): R_f = 0.6; ^{1}H NMR (400.1 MHz, $CDCl_3$,): δ = 6.64 (br s, 1H, NHAc), 5.08 (br s, 1H, NHBoc), 3.57-3.35 (m, 12 H, 6 x CH_2), 3.20 (q, J = 6.1 Hz, 2H, C<u>H</u>$_2$NHAc), 3.07 (q. J = 6.0 Hz, 2H, C<u>H</u>$_2$NHBoc), 1.82 (s, 3H, CH_3CO), 1.70-1.56 (m, 4H, 2 x CH_2), 1.29 (s, 9H, 3 x CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$): 170.1 (<u>C</u>(O)CH_3), 155.9 (C(O)O), 78.6 (C quart., C-2), 2 x 70.3, 69.9, 69.8, 69.3 (5 x CH_2), 38.3, 37.7 (2 x CH_2NH), 29.5, 28.8 (2 x CH_2), 28.3 (3 x (<u>C</u>H_3)$_3$CO), 23.0 (<u>C</u>H_3CO); HRMS: *m/z* calcd for $C_{17}H_{35}N_2O_6$: 363.2457 $[M+H]^+$, found: 363.2474.

Ac-OEG-NH_2



Ac-OEG-NH_2

Compound **4** (249 mg, 687 µmol) was dissolved in dry DCM (1.6 mL) and cooled to 0 °C. TFA (800 µL) was added and the mixture stirred at RT. After 1 h TLC showed complete conversion of starting material. The solvent was removed under reduced pressure and the residue was coevaporated 3 times with toluene. The residue was dissolved in H_2O (2 mL) and lyophilized. This was repeated 2 times. Ac-OEG-NH_2 (241 mg, 93 %) was obtained as pale oil. TLC (CH_2Cl_2/MeOH 3:1): R_f = 0.05; ^{1}H NMR (400.1 MHz, CD_3OD): δ = 3.69-3.61 (m, 8H, 4 x CH_2), 3.61-3.57 (m, 2H, CH_2), 3.51 (t, J = 6.1 Hz, 2H, CH_2), 3.24 (t, J = 7.0 Hz, 2H,

CH_2), 3.09 (t, J = 6.4 Hz, 2H, CH_2), 1.96-1.88 (m, 5H, CH_3 and CH_2), 1.75 (m, 2 H, CH_2); ^{13}C NMR (100.6 MHz, CD_3OD): δ = 173.3 (C=O), 71.4, 71.1, 2 x 71.0 (C-4 – C7), 70.4, 69.6 (C-3 and C-8), 40.1 (C-1), 37.8 (C-10), 30.5 (C-2), 28.1 (C-9), 22.6 (CH_3); HRMS: *m/z* calcd for $C_{17}H_{27}N_2O_4$: 263.1965 $[M+H]^+$, found: 263.1956.

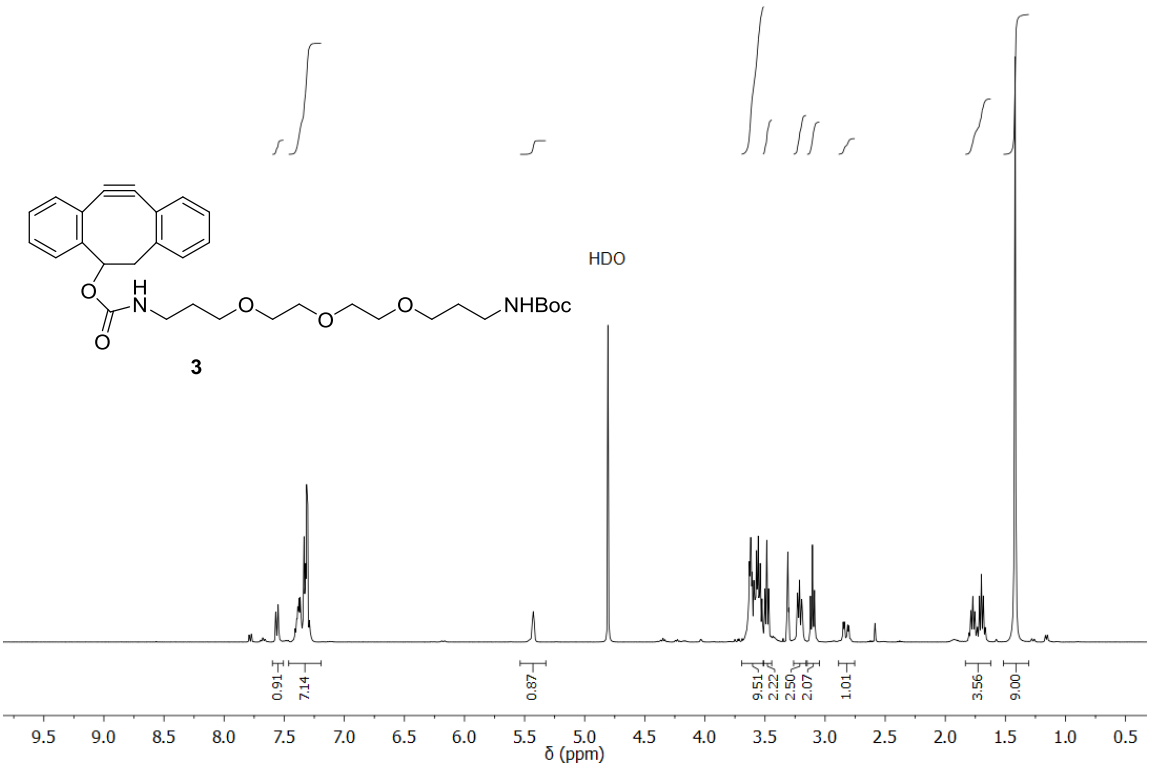
From both DIBO-OEG-NH_2 and Ac-OEG-NH_2 stock solutions (125 mM) in DMSO were prepared.

References

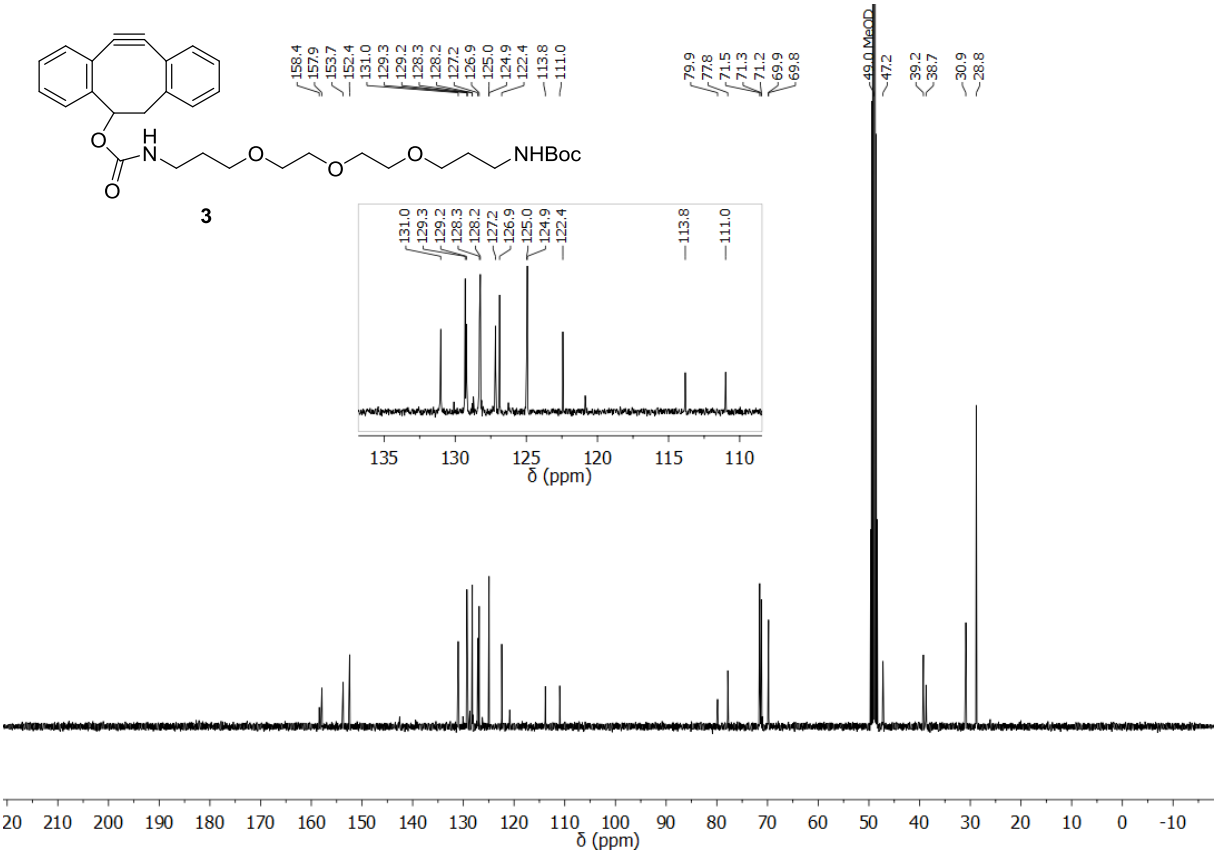
1 Hang, H. C., Yu, C., Kato, D. L. & Bertozzi, C. R. A metabolic labeling approach toward proteomic analysis of mucin-type O-linked glycosylation. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14846-14851, doi:10.1073/pnas.2335201100 (2003).

2 Traar, P., Belaj, F. & Francesconi, K. A. Synthesis of methyl 2-acetamido-2-deoxy-1-seleno-beta-D-gluco- and galacto-pyranoside: Selenium metabolites in human urine. *Aust. J. Chem.* **57**, 1051-1053, doi:10.1071/ch04176 (2004).

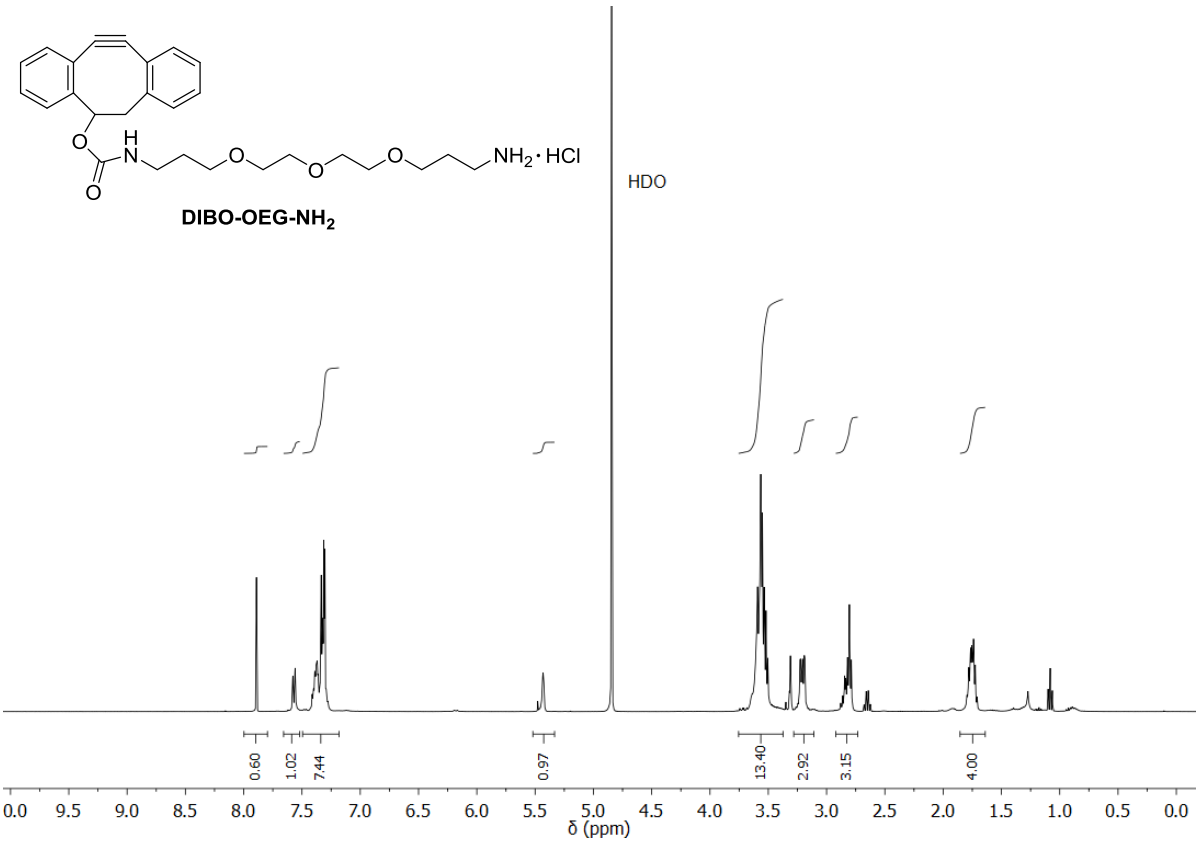
3 Ning, X., Guo, J., Wolfert, Margreet A. & Boons, G.-J. Visualizing Metabolically Labeled Glycoconjugates of Living Cells by Copper-Free and Fast Huisgen Cycloadditions. *Angew. Chem., Int. Ed.* **47**, 2253-2255, doi:10.1002/anie.200705456 (2008).



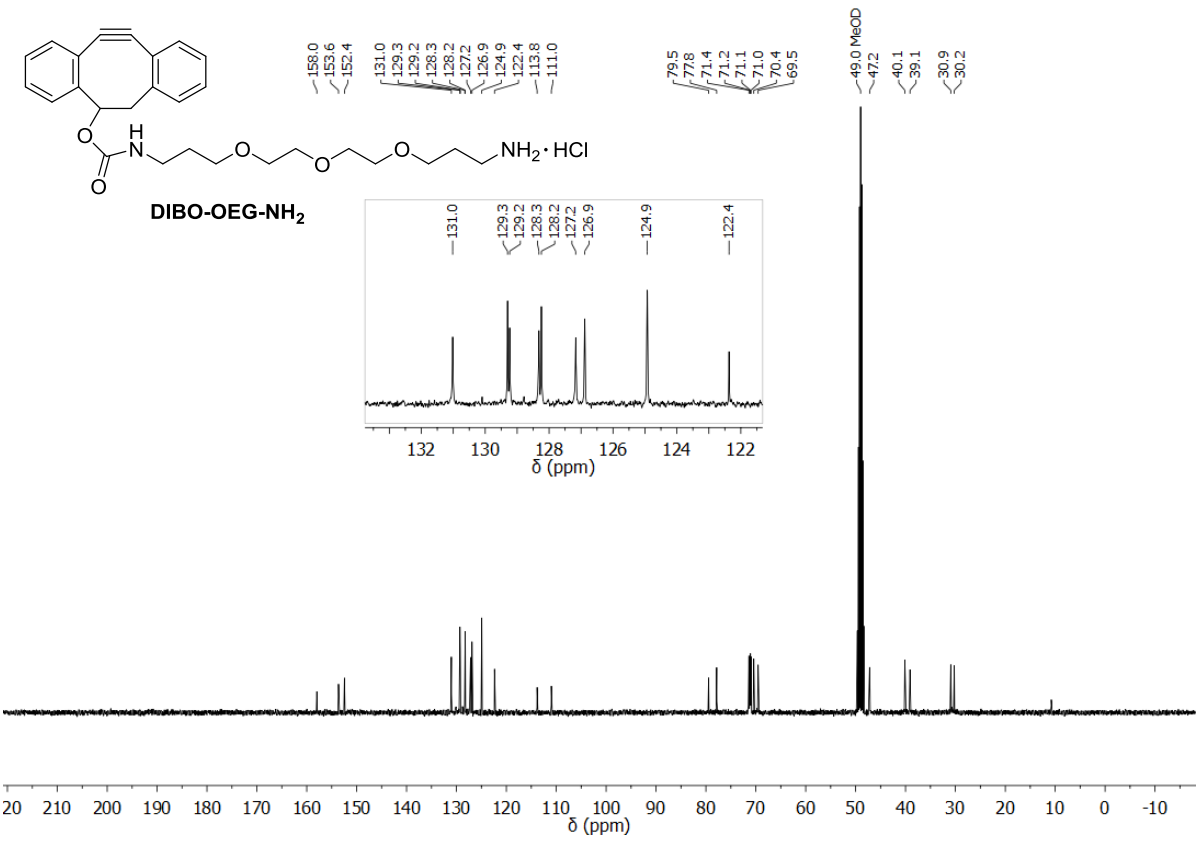
^{1}H NMR spectrum (400.1 MHz, CD_3OD) of compound **3**



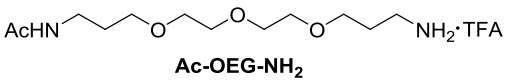
^{13}C NMR spectrum (100.6 MHz, CD_3OD) of compound **3**



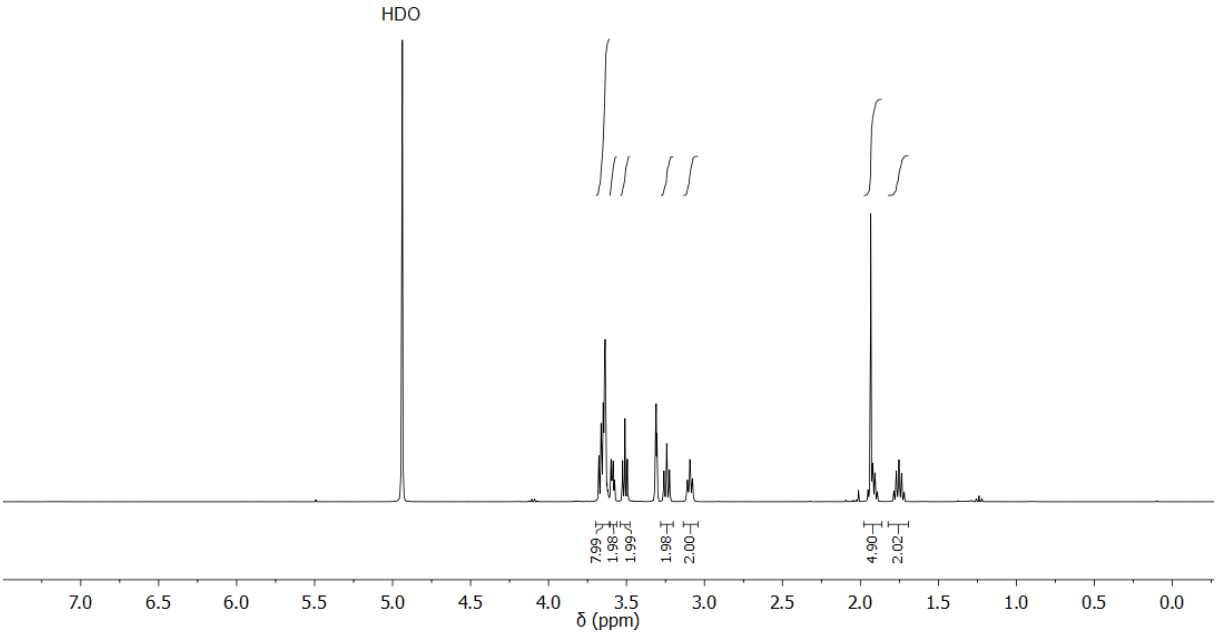
^{1}H NMR spectrum (399.8 MHz, CD_3OD) of DIBO-OEG-NH_2



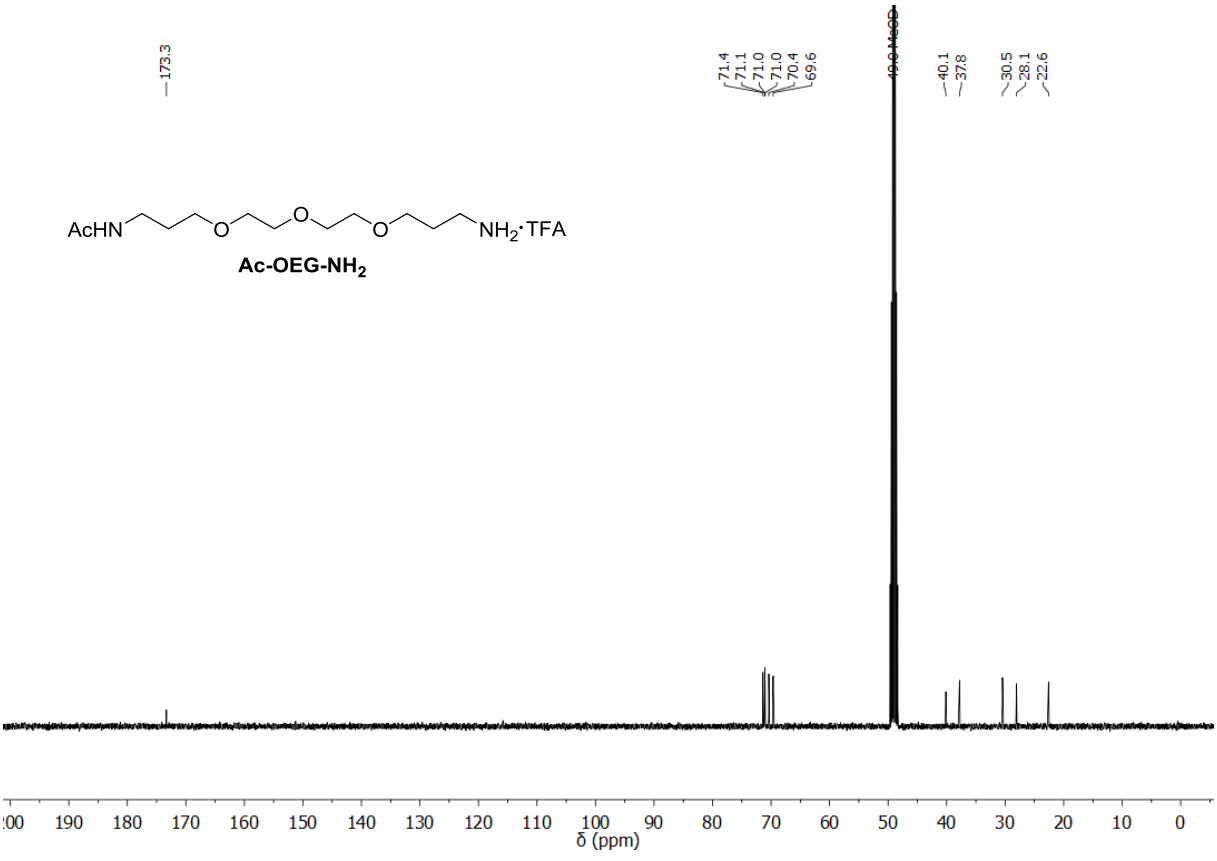
^{13}C NMR spectrum (100.5 MHz, CD_3OD) of DIBO-OEG-NH_2



Ac-OEG-NH_2



^{1}H NMR spectrum (400.1 MHz, CD_3OD) of Ac-OEG-NH_2



Ac-OEG-NH_2

^{13}C NMR spectrum (100.6 MHz, CD_3OD) of Ac-OEG-NH_2