ELECTRONIC SUPPLEMENTARY INFORMATION

Orthogonal, metal-free surface modification by strain-promoted azide-alkyne and nitrile oxide-alkene/alkyne cycloadditions

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Instrumentation and analysis

Nuclear magnetic resonance: NMR spectra were recorded on Bruker spectrometers (ARX 300, ARX 400). Deuterated solvents were used in all measurements. Chemical shifts (δ) are reported in units of parts per million (ppm). For referencing, nuclear magnetic resonance signals of the deuterated solvents were used. Coupling constants (J) are reported in Hertz (Hz). Complex spectra were additionally analyzed by two-dimensional NMR-spectroscopy. For the evaluation of NMR-data, MestReNova 6.0.3 was used. Signal description is carried out by the following nomenclature: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, b = broad.

Mass spectrometry: Mass spectra were recorded on the electronspray ionization spectrometers (ESI) MicroToF (Bruker Daltronics) and Thermo Scientific Orbi-Trap LTQ-XL.

Contact angle measurements: Water contact angles were measured by the sessile drop method on a DSA 100 goniometer (Krüss GmbH Wissenschaftliche Laborgeräte) on glass substrates. Every measurement was carried out three times on different positions of the same sample. In the case of surface modifications by microcontact chemistry, the surfaces were homogeneously reacted by printing with a flat PDMS stamp. Data evaluation was done using the software Drop Shape Analysis.

X-ray photoelectron spectroscopy: XPS spectra were recorded on Kratos Axis Ultra systems (Kratos Analytical). As excitation source monochromatized Al_{Ka} radiation (1486.6 eV) was used. The obtained data was analyzed by the software CasaXPS (version 2.2.0). Referencing was carried out by setting the C(1s)-peak of the saturated hydrocarbons to 285 eV. All measurements were carried out on silicon substrates. In the case of oxime-modified substrates, the surfaces were homogeneously reacted by printing with a flat PDMS stamp.

Time-of-flight secondary ion mass spectrometry: SIMS analysis was carried out using a type IV compatible TOF-SIMS instrument equipped with a liquid metal ion gun (IONTOF GmbH). Bi^{3+} clusters with energy of 25 keV were used as primary ions. The samples were imaged under a primary ion dose density of up to 1.2 x 10^{13} ions/cm^2. Image analysis was performed in a mode with high lateral resolution and in return with low mass

resolution. Therefore some ion images contain the intensities of two different secondary ions. For a definite identification of these ions a second analysis with high mass resolution was made. Ions with a higher proportion of the signal are listed first. All measurements were carried out on silicon substrates.

Atomic force microscopy: Measurements were carried out using a NanoWizard 3 system (JPK instruments) in the tapping mode. Silicon wafers were used as substrates. Data evaluation was done with the program gwyddion (version 2.1.9).

Light microscopy and fluorescence microcopy: Microscopy images were recorded on an Olympus inverted research microvope CKX41, which was modified with a DX 20 L-FW camera (Kappa opto-electronics GmbH). The camera was controlled by the program Kappa CameraControl (version 2.7.5.7032). Excitation light for fluorescence microscopy was produced by a mercury burner U-RFL-T. All investigations were carried out on glass substrates.

Synthesis

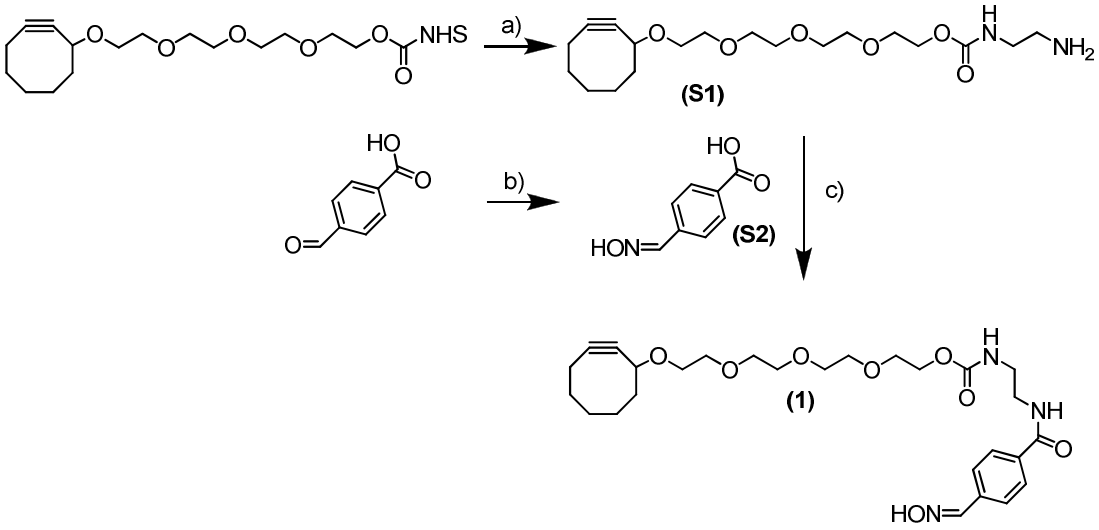
General Procedures and Materials

Chemicals were obtained from Sigma Aldrich, ABCR, AcrosOrganics or from Merck and used without further purification, unless otherwise noted. Dichloromethane was dried by distillation from calcium hydride. THF was dried by distillation from potassium / benzophenone. Methanol and dimethylformamide (DMF) were dried by molecular sieves (3 Å). Nitromethane was dried by distillation from P_2O_5. Analytical thin-layer chromatography (TLC) was performed on 0.2 mm Merck precoated silica gel 60 F254 aluminum sheets. Spots were detected by reaction with basic $KMnO_4$ solution. Purifications by column chromatography over silica were carried out using silica gel 60 (0.063-0.2 mm, Merck).

Synthesis of cyclooctyne oxime (1)

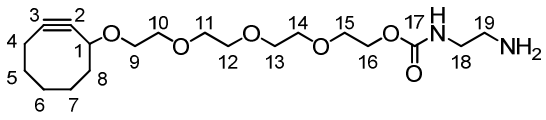
Cyclooctyne oxime (1) was synthesized according to the reaction described in Scheme ESI-1.

Scheme ESI-1:



Reaction conditions: a) ethylene diamine, DCM, 69 %; b) hydroxylamine hydrochloride, NaOAc, EtOH, 81 %; c) NMM, HOBt * H_2O, EDC * HCl, DMF, 47 %.

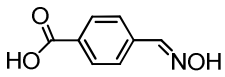
Cyclooctyne amine (S1)



Cyclooctyne modified succinimidyl carbonate (see scheme ESI-1, 300 mg, 0.680 mmol) was synthesized similarly to a reported procedure [1] and dissolved in DCM (6 mL). This solution was slowly dropped into an ice-bath cooled, vigorously stirred solution of ethylene diamine (817 mg, 13.6 mmol, 909µL) in DCM (2 mL) under an atmosphere of Ar. The reaction mixture was stirred for 2 h at rt and concentrated. Column chromatography over silica (DCM : MeOH : $NH_{3\,(aq)}$ 89 : 10 : 1, R_f = 0.18) yielded 180 mg (0.466 mmol, 69 %)

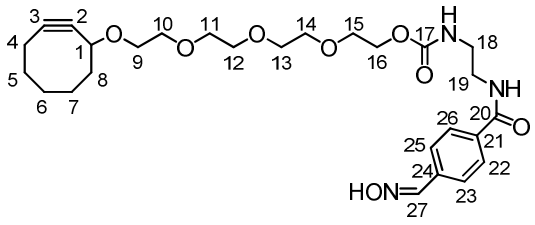
of the product. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.66 (bs, 1H, NH), 4.19 – 4.03 (m, 2H), 3.70 – 3.47 (m, 14H), 3.47 – 3.34 (m, 1H), 3.20 – 3.06 (m, 2H), 2.72 (m, 2H), 2.22 – 1.18 (m, 12H, 4,5,6,7,8-H + NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.79, 100.07, 92.76, 72.74, 70.56, 70.50, 70.50, 70.50, 70.38, 69.62, 68.44, 63.83, 43.49, 42.24, 41.59, 34.29, 29.73, 26.37, 20.69. HRMS (ESI): calculated for $[C_{19}H_{34}N_2O_6 + H^+]^+$ 387.2490, found 387.2493.

4-((Hydroxyimino)methyl)benzoic acid (S2)



The 4-formylbenzoic acid (1.00 g, 6.66 mmol) and hydroxylamine hydrochloride (834 mg, 12.0 mmol) were dissolved in EtOH (35 mL) and water (10 ml) to which sodium acetate (1.47 g, 18.0 mmol) was added. The mixture was refluxed for 9 hours. Following solvent removal under reduced pressure the crude product, obtained as a white solid, was washed with water (30 ml) and dried to yield 890 mg (5.39 mmol, 81%) product oxime [2] (DCM : MeOH 90 : 10, R_f = 0.20). ^{1}H NMR (400 MHz, DMSO d_6): δ = 11.49 (bs, 1H, COOH), 8.20 (s, 1H, HCN), 7.95 - 7.93 (m, 2H, Ar-H), 7.66 (d, J = 8.4 Hz, 2H, Ar-H). ^{13}C NMR (100 MHz, DMSO d_6): δ = 166.86, 147.48, 137.14, 131.06, 129.64, 126.37. HRMS (ESI) calculated for $[C_8H_7NO_3 – H^+]^-$ 164.0348, found 164.0348.

Cyclooctyne oxime (1)



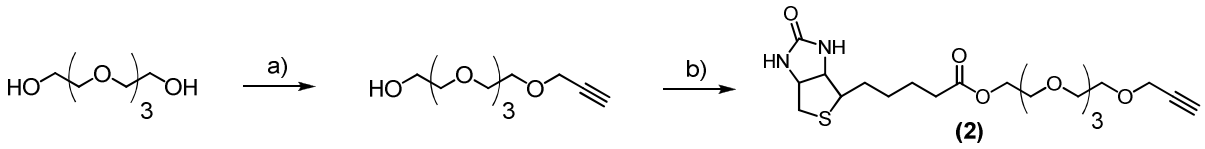
Cyclooctyne amine (S1) (130 mg, 0.336 mmol) was dissolved in dry DMF (3 mL) under Ar and 4-methylmorpholine (68.0 mg, 0.672 mmol, 73.9 µL), 4-((hydroxyimino)methyl)benzoic acid (S2) (49.9 mg, 0.302 mmol), 1-hydroxybenzotriazole hydrate (HOBt * H_2O) (5.2 mg, 34 nmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC * HCl) (64.4 mg, 0.336 mmol) were added. The reaction mixture was

stirred for 6 h at rt. After removal of the solvent by oil pump, the residue was purified by column chromatography over silica (ethyl acetate to ethyl acetate : methanol 97 : 3, R_f (ethyl acetate) = 0.23), yielding 85.0 mg (0.159 mmol, 47 %) of the pure product. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.10 (bs, 1H), 8.08 (s, 1H, 27-H), 7.78 (d, J = 8.3 Hz, 2H, Ar-H), 7.55 (d, J = 8.2 Hz, 2H, Ar-H), 5.72 (bs, 1H, NH), 4.27 – 4.07 (m, 2H), 3.84 – 3.26 (m, 19H), 2.40 – 1.09 (m, 10H, 4,5,6,7,8-H).^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.64, 157.96, 149.11, 135.57, 135.18, 127.79, 127.05, 100.43, 92.84, 73.00, 70.74, 70.71, 70.69, 70.66, 70.56, 69.63, 68.63, 64.43, 42.38, 41.58, 40.74, 34.45, 29.89, 26.55, 20.86. HRMS (ESI): calculated for $[C_{27}H_{39}N_3O_8 + Na^+]^+$ 556.2629, found 556.2629.

Synthesis of biotin alkyne (2)

Biotin alkyne (2) was synthesized according to the synthesis route described in Scheme ESI-2. Detailed experimental and analytical data is described in reference [3].

Scheme ESI-2:

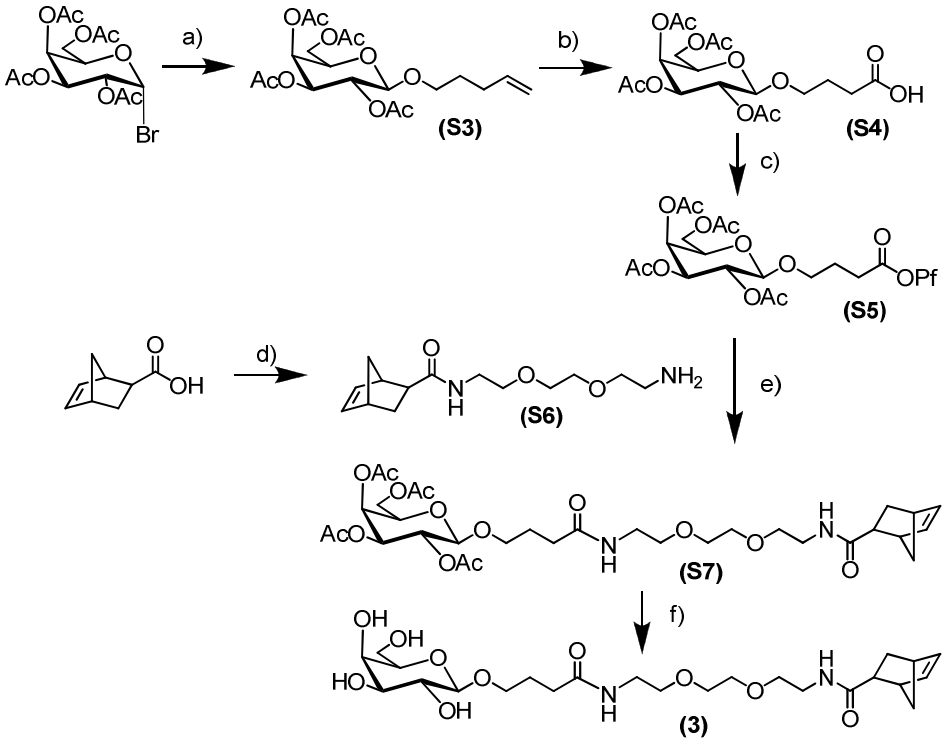


Reaction conditions: a) tert-BuOK, propargyl bromide, THF, 41 %; b) D-biotin, NMM, HOBt, EDC * HCl, DMF, 33 %.

Synthesis of ß-D-galactose norbornene conjugate (3)

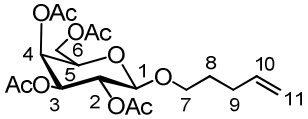
ß-D-Galactose norbornene conjugate (3) was synthesized according to the synthesis route described in Scheme ESI-3.

Scheme ESI-3:



Reaction conditions: a) 4-Penten-1-ol, $Hg(CN)_2$, CH_3NO_2, 66 %; b) $NaIO_4$, $RuCl_3$ * x H_2O, DCM / CH_3CN / H_2O, 87 %; c) Pentafluorophenol, EDC * HCl, NMM, DCM, 75 %; d) IBCF, NEt_3, 1,8-diamino-3,6-dioxaoctane, 82 %; e) NEt_3, DCM, 95 %; f) NaOMe, MeOH, 90 %.

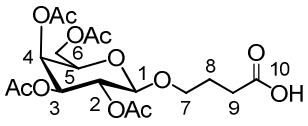
Pentenyl 2,3,4,6-tetra-O-acetyl-ß-D-galactopyranoside (S3)



To a solution of 1-bromo-2,3,4,6-tetra-O-acetyl-ß-D-galactopyranoside [4] (9.60 g, 23.3 mmol) in dry CH_3NO_2 (30 mL) under Ar was added 4-penten-1-ol (2.01 g, 23.3 mmol) and $Hg(CN)_2$ (5.89 g, 23.3 mmol). After the suspension was stirred for 2 d at 40 °C, the mixture was filtered and the filtrate concentrated. The residue was dissolved in ethyl acetate (200 mL) and the solution washed with aqueous LiBr (1 M, 2 x 200 mL), dried ($MgSO_4$) and concentrated. Purification was carried out by column chromatography over silica (cyclohexane : ethyl acetate 2 : 1, R_f = 0.33), yielding 6.40 g (15.4 mmol, 66 %) of product. ^{1}H NMR (400 MHz,

$CDCl_3$): δ = 5.73 (ddt, J = 16.9 Hz, J = 10.2 Hz, J = 6.7 Hz, 1H, 10-H), 5.33 (dd, J = 3.4 Hz, J = 1.0 Hz, 1H, 4-H), 5.15 (dd, J = 10.5 Hz, J = 7.9 Hz, 1H, 2-H), 4.99 – 4.96 (m, 1H, 3-H), 4.96 – 4.89 (m, 2H, 11-H), 4.40 (d, J = 8.0 Hz, 1H, 1-H), 4.16 – 4.03 (m, 2H, 6-H), 3.89 – 3.80 (m, 2H, 5,7-H), 3.49 – 3.38 (m, 1H, 7´-H), 2.13 – 1.89 (m, 14H, 9-H + 4OAc), 1.73 – 1.52 (m, 2H, 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.52, 170.41, 170.31, 169.49, 137.94, 115.20, 101.47, 71.08, 70.69, 69.51, 69.05, 67.19, 61.41, 29.95, 28.72, 20.90, 20.81, 20.81, 20.74. HRMS (ESI): calculated for $[C_{19}H_{28}O_{10} + Na^+]^+$ 439.1575, found 439.1573; calculated for $[(C_{19}H_{28}O_{10})_2 + Na^+]^+$ 855.3257, found 855.3248. IR (neat): ν = 1744 (ester).

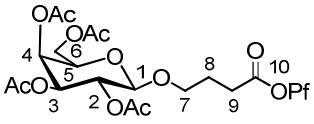
2,3,4,6-Tetra-O-acetyl-ß-D-galactopyranoyl-oxybutanoic acid (S4)



The reaction was carried out similarly to a reported procedure for the mannose analogue [5]. Galactopyranoside (S3) (5.17 g, 12.4 mmol) was dissolved in a mixture of DCM (20 mL), CH_3CN (20 mL) and water (30 mL). $NaIO_4$ (10.6 g, 49.7 mmol) and $RuCl_3$* x H_2O (35 – 40 % Ru, 73 mg) were added. The reaction mixture was stirred for 2 h at rt and another portion of $NaIO_4$ (10.6 g, 49.7 mmol) was added. The suspension was stirred for further 2 h. DCM (150 mL) and water (300 mL) were added and the layers separated. The aqueous layer was extracted with DCM (3 x 100 mL) and the combined organic layers were dried over $MgSO_4$. The solvent was removed by rotary evaporation and the residue purified by column chromatography over silica (ethyl acetate : cyclohexane : methanol 10 : 10 : 1, R_f = 0.20), yielding 4.70 g (10.8 mmol, 87 %) of product. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.34 (dd, J = 3.3 Hz, J = 0.8 Hz, 1H, 4-H), 5.15 (dd, J = 10.5 Hz, J = 7.9 Hz, 1H, 2-H), 4.97 (dd, J = 10.5 Hz, J = 3.4 Hz, 1H, 3-H), 4.42 (d, J = 7.9 Hz, 1H, 1-H), 4.18 – 4.02 (m, 2H, 6-H), 3.95 – 3.82 (m, 2H, 5,7-H), 3.60 - 3,45 (m, 1H, 7´-H), 2.39 (t, J = 7.2 Hz, 2H, 9-H), 2.14 – 1.91 (4s, 12H, 4OAc), 1.91 – 1.78 (m, 2H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 178.92, 170.64, 170.48, 170.37, 169.72, 101.41, 71.05, 70.79, 68.98, 68.73, 67.21, 61.44, 30.29, 24.66, 20.88, 20.83, 20.83, 20.74. HRMS (ESI): calculated for

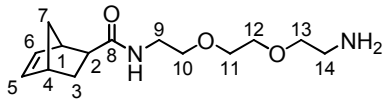
$[C_{18}H_{25}O_{12} + H^+]^+$ 433.1341, found 433.1374; calculated for $[(C_{18}H_{25}O_{12})_2 + H^+]^+$ 867.2765, found 867.2893. IR (neat): ν = 1741 (ester), 1711 (carboxylic acid).

2,3,4,6-Tetra-O-acetyl-ß-D-galactopyranosyl-oxybutanoic acid pentafluorophenol ester (S5)



To a solution of acid (S4) (3.80 g, 8.75 mmol) and pentafluorophenol (1.93 g, 10.5 mmol) in DCM (80 mL) under Ar was added N-methyl morpholine (88.5 mg, 0.875 mmol, 96.2 µL) and N-(3-dimethylaminopropyl)-N´-ethylcarbodiimide hydrochloride (EDC * HCl, 2.01 g, 10.5 mmol). The reaction mixture was stirred over night. Removal of the solvent by rotary evaporation and purification of the residue by column chromatography over silica (cyclohexane : ethyl acetate 2 : 1, $R_f = 0.28$) yielded 3.94 g (6.56 mmol, 75 %) of product. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.34 (dd, J = 3.4 Hz, J = 0.9 Hz, 1H, 4-H), 5.16 (dd, J = 10.5 Hz, J = 7.9 Hz, 1H, 2-H), 4.97 (dd, J = 10.5 Hz, J = 3.4 Hz, 1H, 3-H), 4.43 (d, J = 8.0 Hz, 1H, 1-H), 4.17 – 4.03 (m, 2H, 6-H), 4.00 – 3.83 (m, 2H, 5,7-H), 3.62 – 3.51 (m, 1H, 7´-H), 2.72 (t, J = 7.2 Hz, 2H, 9-H), 2.16 – 1.84 (m, 14H, 8-H + 4OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.57, 170.43, 170.30, 169.66, 169.23, 144.96 – 135.09 (m, OPf), 101.40, 71.02, 70.84, 68.92, 68.08, 67.17, 61.40, 29.69, 24.85, 20.77, 20.75, 20.73, 20.67. HRMS (ESI): calculated for $[C_{24}H_{25}O_{12}F_5 + Na^+]^+$ 623.1158, found 623.1156. IR (neat): ν = 1788 (Pf-ester), 1746 (ester).

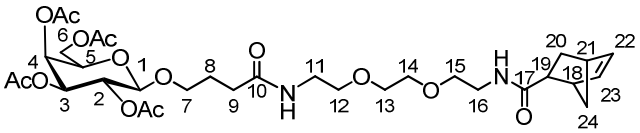
Norbornene amine (S6)



To a stirred solution of endo-5-norbornene-2-carboxylic acid (4.00 mmol, 553 mg) in DCM (20 mL) under Ar was added triethylamine (6.00 mmol, 607 mg, 836 µL) and isobutyl chloroformate (3.60 mmol, 492 mg, 467 µL)

at 0 °C. The reaction mixture was stirred for 30 min at the same temperature and then slowly dropped in a vigorously stirred solution of 1,8-diamino-3,6-dioxaoctane (24.0 mmol, 3.56 g, 3.43 mL) in DCM at 0 °C. After 10 min, the solution was allowed to reach room temperature and was stirred for an additional hour. Finally, the solvent was removed by rotary evaporation and the residue purified by column chromatography over silica (DCM : MeOH : $NH_{3\ (aq)}$ 89 : 10 : 1, $R_f = 0.27$), yielding 790 mg (2.94 mmol, 82 %) of product. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.37 (bs, 1H, NH), 6.05 (dd, J = 5.6 Hz, J = 2.9 Hz, 1H, 6-H), 6.01 (dd, J = 5.6 Hz, J = 3.0 Hz, 1H, 5-H), 3.61 – 3.41 (m, 8H, 10,11,12,13-H), 3.42 – 3.34 (m, 2H), 2.89 – 2.73 (m, 4H), 1.98 – 1.91 (m, 1H), 1.86 – 1.78 (m, 1H), 1.70 (bs, 2H, NH_2), 1.64 (d, J = 8.3 Hz, 1H), 1.28 – 1.16 (m, 2H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.82, 138.25, 136.11, 73.20, 70.28, 70.17, 70.06, 47.30, 46.36, 44.57, 41.69, 41.62, 39.30, 30.52. HRMS (ESI): calculated for $[C_{14}H_{24}N_2O_3 + H^+]^+$ 269.1860, found 269.1880.

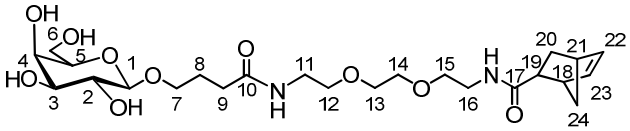
2,3,4,6-Tetra-O-acetyl-ß-D-galactose norbornene conjugate (S7)



The pentafluorophenol ester (S5) (600 mg, 1.00 mmol) was dissolved in DCM (20 mL) under Ar and a solution of norbornene amine (S6) (322 mg, 1.20 mmol) and triethylamine (202 mg, 2.00 mmol, 279 µL) in DCM was slowly added drop wise at 0 °C. The reaction mixture was stirred for further 15 min at 0 °C and for an additional hour at rt. Removal of the solvent and purification by column chromatography over silica (ethyl acetate : methanol 95 : 5, $R_f = 0.22$) yielded 649 mg (0.948 mmol, 95 %) of the product. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.24 (bs, 1H, NH), 6.17 (bs, 1H, NH), 6.11 – 6.01 (m, 2H, 22,23-H), 5.35 – 5.31 (m, 1H, 4-H), 5.15 – 5.03 (m, 1H, 2-H), 5.00 – 4.91 (m, 1H, 3-H), 4.41 (d, J = 7.9 Hz, 1H, 1-H), 4.17 – 4.01 (m, 2H, 6-H), 3.89 – 3.78 (m, 2H, 5,7-H), 3.60 – 3.34 (m, 9H, 7´,12,13,14,15-H), 3.46 – 3.35 (m, 4H), 2.88 – 2.81 (m, 2H), 2.19 (t, J = 7.2 Hz, 2H, 9-H), 2.13 – 1.89 (m, 14H, 8-H + 4OAc), 1.86 – 1.78 (m, 2H), 1.64 (d, J = 8.3 Hz, 1H), 1.28 – 1.16 (m, 2H,

24-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 176.10, 172.82, 170.56, 170.37, 170.26, 169.82, 138.35, 136.11, 101.43, 70.94, 70.75, 70.31, 70.28, 70.09, 69.94, 69.35, 69.08, 67.15, 61.36, 47.32, 46.41, 44.70, 41.67, 39.37, 39.32, 32.73, 30.59, 25.73, 20.94, 20.80, 20.80, 20.72. HRMS (ESI): calculated for $[C_{32}H_{48}N_2O_{14} + Na^+]^+$ 707.2998, found 707.2990.

ß-D-Galactose norbornene conjugate (3)

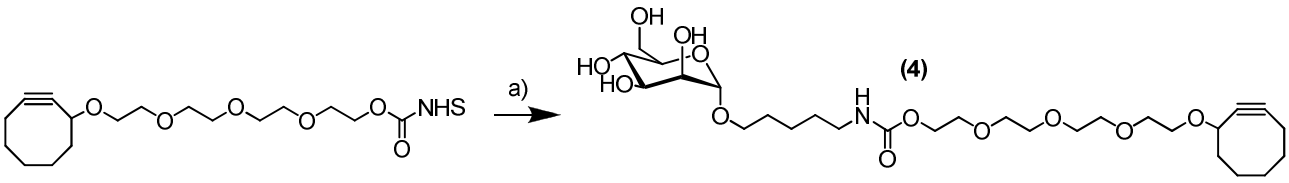


Tetra-O-acetyl-ß-D-galactose norbornene conjugate (S7) (600 mg, 0.877 mmol) was dissolved in dry methanol (20 mL) under Ar and catalytic amounts of NaOMe were added. The reaction mixture was stirred for 5 h at rt. Acetic acid (1 mL) was added and the solution concentrated. Salts were removed by column chromatography over a small amount of silica (DCM : methanol 95 : 5 to 85 : 15), yielding in 408 mg (0.790 mmol, 90 %) of the product. ^{1}H NMR (300 MHz, MeOD): δ = 6.15 – 6.10 (m, 2H, 22,23-H), 4.19 (d, J = 7.1, 1H, 1-H), 3.94 – 3.84 (m, 1H), 3.83 – 3.80 (m, 1H), 3.77 – 3.67 (m, 2H), 3.63 – 3.45 (m, 11H), 3.38 – 3.31 (m, 4H), 2.89 – 2.82 (m, 2H), 2.31 (t, J = 7.3, 2H, 9-H), 2.15 – 2.08 (m, 1H), 1.96 – 1.80 (m, 3H), 1.68 (d, J = 8.1, 1H), 1.34 – 1.21 (m, 2H, 24-H). ^{13}C NMR (75 MHz, MeOD): δ = 178.61, 176.24, 139.12, 137.45, 105.12, 76.74, 75.07, 72.69, 71.38, 71.37, 70.78, 70.69, 70.39, 69.83, 62.64, 48.74, 47.18, 45.23, 42.88, 40.49, 40.39, 33.79, 31.33, 27.33. HRMS (ESI): calculated for $[C_{24}H_{40}N_2O_{10} + Na^+]^+$ 539.2575, found 539.2573.

Synthesis of α-D-mannose cyclooctyne conjugate (4)

α -D-Mannose cyclooctyne conjugate (4) was synthesized according to the reaction described in Scheme ESI-4.

Scheme ESI-4:



Reaction conditions: a) 5-Aminopentyl-α-D-mannopyranoside, NEt_3, DMF, 76 %.

Cyclooctyne modified succinimidyl carbonate (see scheme ESI-4, 199 mg, 0.450 mmol) was synthesized similarly to a reported procedure [1] and dissolved in DMF (1 mL). This solution was slowly dropped in a vigorously stirred solution of 5-aminopentyl-α-D-mannopyranoside [6] (133 mg, 0.500 mmol) and triethylamine (152 mg, 1.50 mmol, 209 µL) in DMF (4 mL). After complete addition of the cyclooctyne derivative, the reaction mixture was stirred for further 2 h at rt. The solvent was removed by oil pump and the residue purified by column chromatography over silica (DCM : MeOH 90 : 10, R_f = 0.3), yielding 201 mg (0.340 mmol, 76 %) of the product. ^{1}H NMR (300 MHz, MeOD): δ = 4.70 (d, J = 1.3 Hz, 1H, anomeric-H), 4.23 – 4.07 (m, 3H), 3.84 – 3.52 (m, 19H), 3.52 – 3.32 (m, 3H), 3.24 – 3.12 (m, 2H), 3.11 – 3.01 (m, 2H), 2.26 – 1.32 (m, 16H). ^{13}C NMR (75 MHz, MeOD): δ = 158.92, 101.53, 100.92, 93.75, 74.60, 73.85, 72.69, 72.25, 71.57, 71.57, 71.43, 70.64, 69.54, 68.65, 68.41, 65.04, 62.95, 47.88, 43.40, 41.73, 37.13, 35.50, 31.00, 30.30, 27.53, 26.40, 24.62, 21.32. HRMS (ESI): calculated for $[C_{28}H_{49}NO_{12} + Na^+]^+$ 614.3147, found 614.3150.

Surface Chemistry

Light microscopy: Wetting experiments on the azide/oxime-terminated surface

A light microscopy image of water droplets, which are selectively condensed on the hydrophilic pattern of the immobilized cyclooctyne oxime linker (**1**) is shown in figure ESI-1.

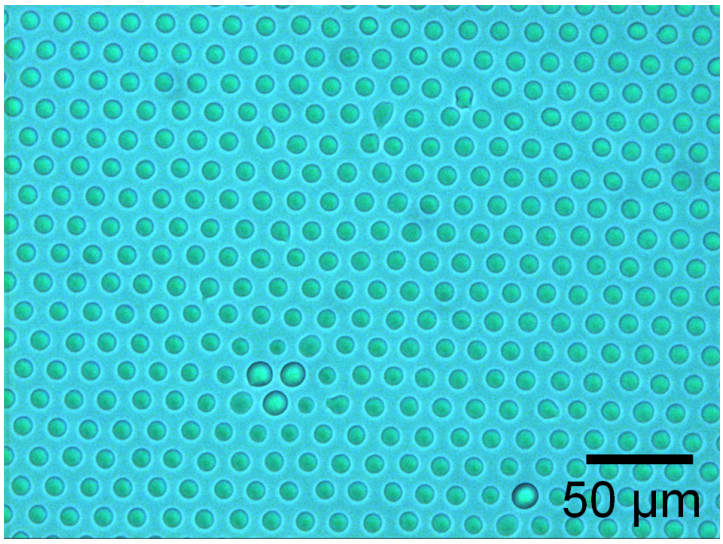


Figure ESI-1: Light microscopy image of water droplets condensed on the hydrophilic pattern of the oxime linker (**1**).

XPS measurements

The XPS analysis of undecylazide-terminated silicon substrates (blue) and of homogeneously modified undecylazide-terminated silicon substrates by µCP of cyclooctyne oxime (**1**) with a flat stamp (red) is shown in figure ESI-2. It was found that the XPS C1s spectra of surfaces modified with the oxime show characteristic peaks for carbons in a C-N/C-O (286.5 eV), in an amide (288 eV) an in a carbamate (290 eV) environment (figure ESI-2A, red) in addition to carbon atoms in a C-C environment. In the case of the undecylazide-SAM only saturated hydrocarbon atoms and carbon atoms that are connected to a nitrogens are present (figure ESI-2A, blue). The N1s region of the undecylazide-terminated surface shows two peaks for the azide nitrogen atoms (figure ESI-2B, blue), which vanish after µCP of the cyclooctyne oxime (**1**) and a new nitrogen peak is formed. This peak reflects the nitrogens in the triazole ring and in the linker molecule (figure ESI-2B, red).

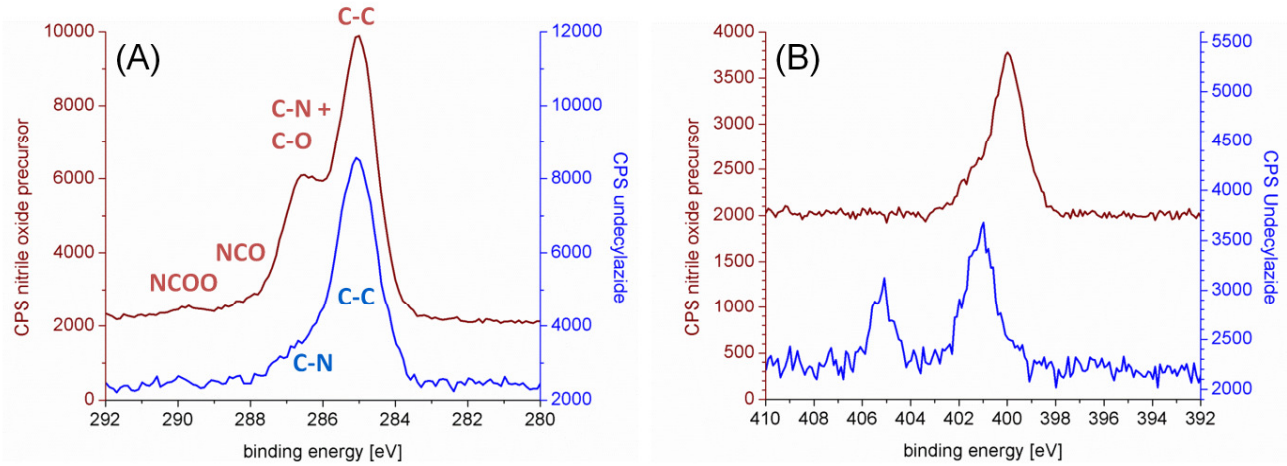


Figure ESI-2: XPS analysis of undecylazide-terminated silicon substrates (blue) after reaction with the cyclooctyne oxime linker (**1**) by µCP with a flat stamp. (A) C1s carbon and (B) N1s nitrogen signal of the undecylazide-modified silicon surface before (blue) and after immobilization of oxime (**1**).

ToF-SIMS measurements: Analysis of the azide/oxime-patterned surface

Analysis of azide/oxime-patterned silicon substrates by time-of-flight secondary ion mass spectrometry in the positive (figure ESI-3) and negative ion mode (figure ESI-4) verified the successful immobilization of cyclooctyne oxime linker (**1**). Characteristic oxygen-containing fragments such as $C_2H_5O^+$ in the positive ion mode and CNO^-, $C_2H_3O^-$ and $C_2H_3O_2^-$ in the negative ion mode are exclusively formed within the dotted pattern. The formation of the aromatic ions $C_7H_7^+$, $C_7H_4N^+$, $C_7H_6NO^+$ (figure ESI-3), $C_7H_4N^-$ and $C_7H_6NO^-$ (figure ESI-4) can be assigned to the presence of benzaldoxime-residues.

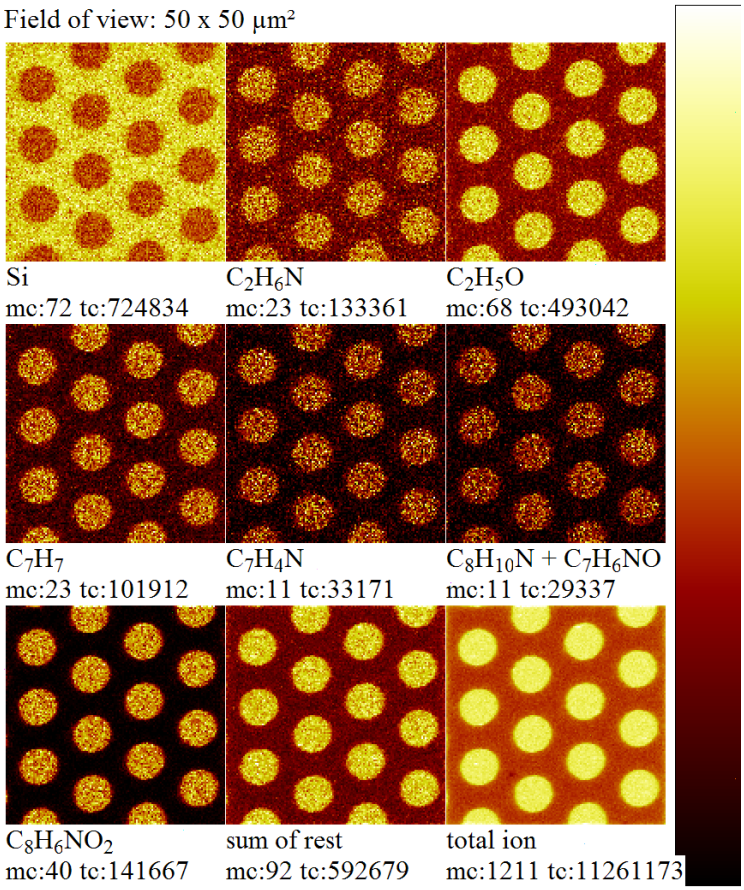


Figure ESI-3: Time-of-flight secondary ion mass spectrometry analysis of an azide/oxime-patterned silicon substrate in the positive ion mode (mc: maximum counts, tc: total counts).

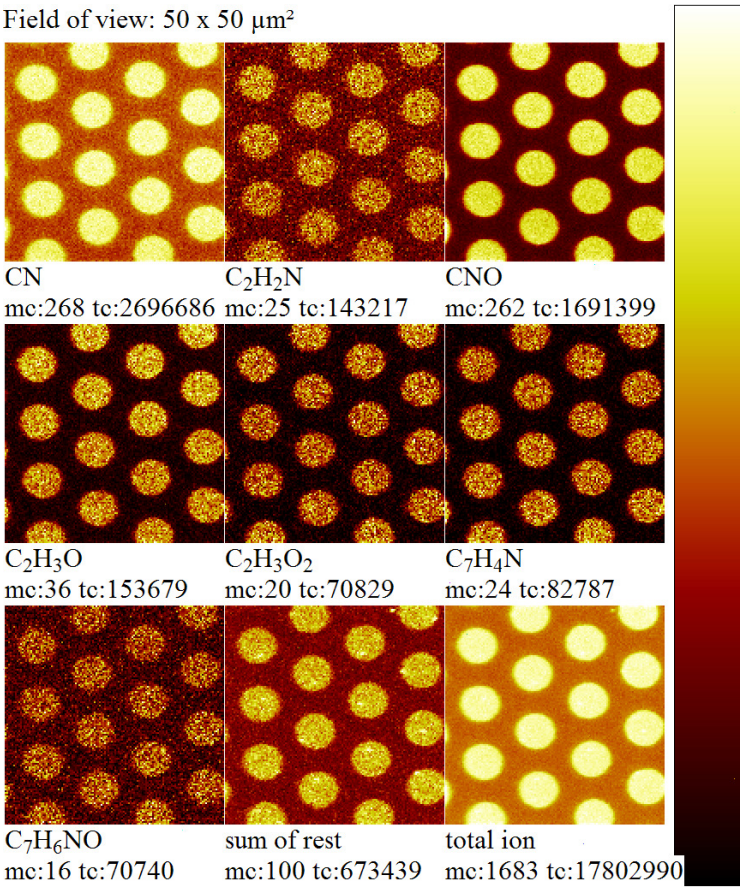


Figure ESI-4: Time-of-flight secondary ion mass spectrometry analysis of an azide/oxime-patterned silicon substrate in the negative ion mode (mc: maximum counts, tc: total counts).

ToF-SIMS measurements: Analysis of the azide/oxime-patterned surface after immobilization of biotin alkyne (2) by NOAC

Surface analysis by time-of-flight secondary ion mass spectrometry in the negative ion mode was carried out to verify the immobilization of biotin alkyne within the dotted areas by NOAC. Besides anionic fragments that were also found in the case of the azide/oxime-pattened surface, additionally characteristic sulfur-containing fragments such as S^-, HS^- and $34S^-$ were found, which proves the successful attachment of biotin residues.

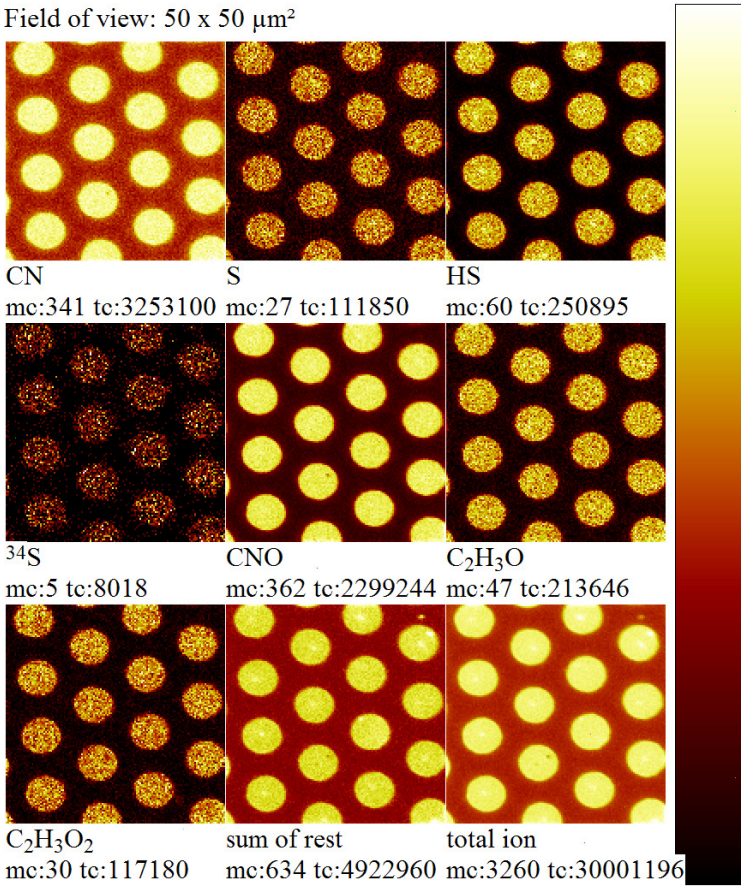


Figure ESI-5: Time-of-flight secondary ion mass spectrometry analysis of an azide/oxime-patterned silicon substrate in the negative ion mode after attachment of biotin alkyne (**2**) by alkyne-nitrile oxide cycloaddition (mc: maximum counts, tc: total counts).

Fluorescence microscopy: Immobilization of PAMAM G4 dendrimers by peptide coupling

PAMAM G4 dendrimers were attached via peptide chemistry directly after immobilization of pentenoic acid on nitrile oxide patterns. The immobilization reaction of 4-pentenoic acid was carried out as described for the norbornene and alkyne derivatives. After immobilization, the carboxylic acid functionalized surfaces were activated by immersion in a stirred solution of dicyclohexylcarbodiimide (1.0 M) and N-hydroxysuccinimide (1.0 M) in DMF (peptide synthesis grade) [7]. After 1h, the substrate surfaces were washed with DCM, dried in a stream of Ar and subsequently covered with a solution of PAMAM G4 dendrimers (1 % wt. in 9:1 ethanol-methanol). A cover slip was applied on the top of the solutions in order to prevent evaporation of the solvent. Incubation was carried out for 1 h. The substrates were cleaned by extensive washing with Milli-Q water and ethanol and dried. Fluorescent labeling was achieved by coverage of the surfaces with a solution of 5(6)-carboxyfluorescein-NHS ester (20 mM) and NEt_3 (20 mM) in DMF for 1 h. Finally, the surfaces were cleaned by washing with ethanol, dilute aqueous $NaHCO_3$, Milli-Q water and analyzed by fluorescence microscopy. The selective presence of the fluorescent dye in a dot pattern verifies the attachment of PAMAM dendrimers within areas with immobilized carboxylic acid groups (figure ESI-5).

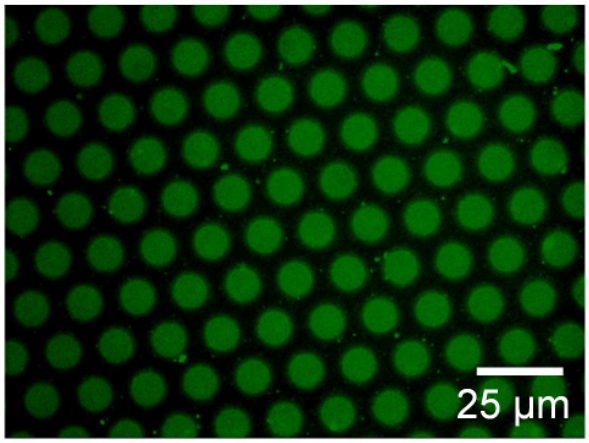


Figure ESI-6: Fluorescence microscopy image of fluorescein-labeled PAMAM G4 dendrimers attached to carboxylic acid groups via peptide coupling. The acid functionality was induced by reaction of nitrile oxides with 4-pentenoic acid.

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