Electronic Supporting Information

Controlled Density Glycodendron Microarrays for Studying Carbohydrate-Lectin Interactions

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General Methods

Chemicals were purchased from Sigma-Aldrich, Merck, Dextra or CarboSynth and used without further purification, unless otherwise indicated. Solvents were purchased from Fisher scientific, ScharLab and Carlo Erba. Anhydrous solvents were purchased from Sigma-Aldrich® with a content of water ≤ 0.005 %. H_2O was purified with a Milli-Q purification system from Millipore (18.3 MΩ).

All reactions that needed dry conditions were carried out using standard techniques under an inert atmosphere of oxygen-free Argon or Nitrogen, unless otherwise stated. TLC were performed using pre-coated aluminum chromate-plates Silica Gel 60 F254 Merck of 0.25 mm thick. Compounds were visualized by using UV light (254 nm) and revealed by immersion into solutions of molybdenum blue (20 g of ammonium molybdate (VI) tetrahydrate, 0.4 g of sulphate of hydrated cerium and 10% sulfuric acid in 400 mL of H_2O) and heating at 100 °C, or 0.1% ninhydrin in ethanol, or a basic solution (10 % w/w K_2CO_3 in water) of $KMnO_4$. Purifications by Flash column chromatography were performed using Silica gel 60 (particle size 0,063-0,200 nm or 0,015-0,040 mm), from Merck, eluting by gravity or subjecting it to light pressure. Purifications by Size-Exclusion Chromatography has been carried out in columns filled with Sephadex LH-20 or G-25 (GE Healthcare Life Science), eluting by gravity.

NMR spectra, ^{1}H and ^{13}C, were recorded at 298 K on a Bruker DRX300, DRX400 and DRX500 spectrometers. All chemical shifts were reported in ppm (δ) using the residual proton solvent peaks as internal standards. These abbreviations were used to indicate the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, td = triplet of doublets, m = multplet.

ESI Mass spectra were obtained with an Esquire 6000 ESI-Ion Trap (Bruker Daltonics) at the Centro de investigaciòn isla de la Cartuja, CICCartuja. ESI-HRMS Mass spectra were obtained with an Q Exactive MS/MS (Thermo Scientific) at the Centro de Investigación, Tecnología e Innovación (CITIUS).

MALDI-TOF MS analysis were performed on an Ultraflextreme III time-of-flight mass spectrometer equipped with a pulsed Nd:YAG laser (355 nm) and controlled by FlexControl 3.3 and FlexImaging 2.1 softwares (Bruker Daltonics, Bremen , Germany). The acquisitions (total of 2000-3000) were carried out in positive reflector ion mode with pulse duration of 50 ns, laser fluence of 40 % and laser frequency of 500 Hz. Laser intensity was set marginally above the threshold of ionization to avoid fragmentation (less than 10 % for all the cases). All the peaks were detected as sodium adducts with high intensity signal (> 1000 U.A). The m/z range was chosen according to the mass of the sample. The acquired data was processed (baseline substraction and normalized) using the Bruker software FlexAnalysis 3.3. MALDI-TOF analysis was performed in positive ionization mode with a LIFT cell voltage of 18.9 kV and a final acceleration voltage set at 29.3 kV. The parent mass ion was assigned manually (monoisotopic peak). The MS/MS spectra were acquired from 2000-3000 laser shots. High resolution mass measurements were carried out using the calibration standards of similar mass (Lamivudine m/z 230.0594, Leucine-enkephalin m/z 556.2766, des-Arg- Bradykinin m/z 904.4681, Angiotensin, m/z 1296.6853 and Glu-Fibrinopeptide B m/z 1570.6774) to achieve high mass accuracy. Contact angle measurements were performed at r.t. using DSA 100 contact angle meter (Krüss). Microarrays were printed employing a robotic piezoelectric SciFLEXARRAYER spotter S11 (Scienion, Berlin, Germany). Indium tin oxide (ITO) coated slides (75 mm x 25 mm) were obtained from Hudson Surface Technology, Inc. (Fort Lee, NJ). The slides have a nominal transmittance of >78 % and an ITO thickness of 130 nm. Modified surfaces were stored under vacuum conditions until its use. Plant lectins were purchased from Vector Laboratories and Sigma Aldrich while human lectins from institut de Biologie Structurale-Grenoble**.** All proteins were labeled with Alexafluor-647, Alexafluor-555 and Cy3 from Thermo Fisher Scientific according to the manufactureŕ s instructions. Cyclooctynes immobilization and lectin incubations were performed using the Hybridization gasket slide kit® from Agilent and 16-well Proplate module/6x7 mm from EMS ProSciTech. Fluorescence measurements were performed in an Agilent G265BA microarray scanner system (Agilent

Technologies, Santa Clara, USA). Quantification was performed with ProScanArray® Express software (Perkin Elmer, Shelton, USA).

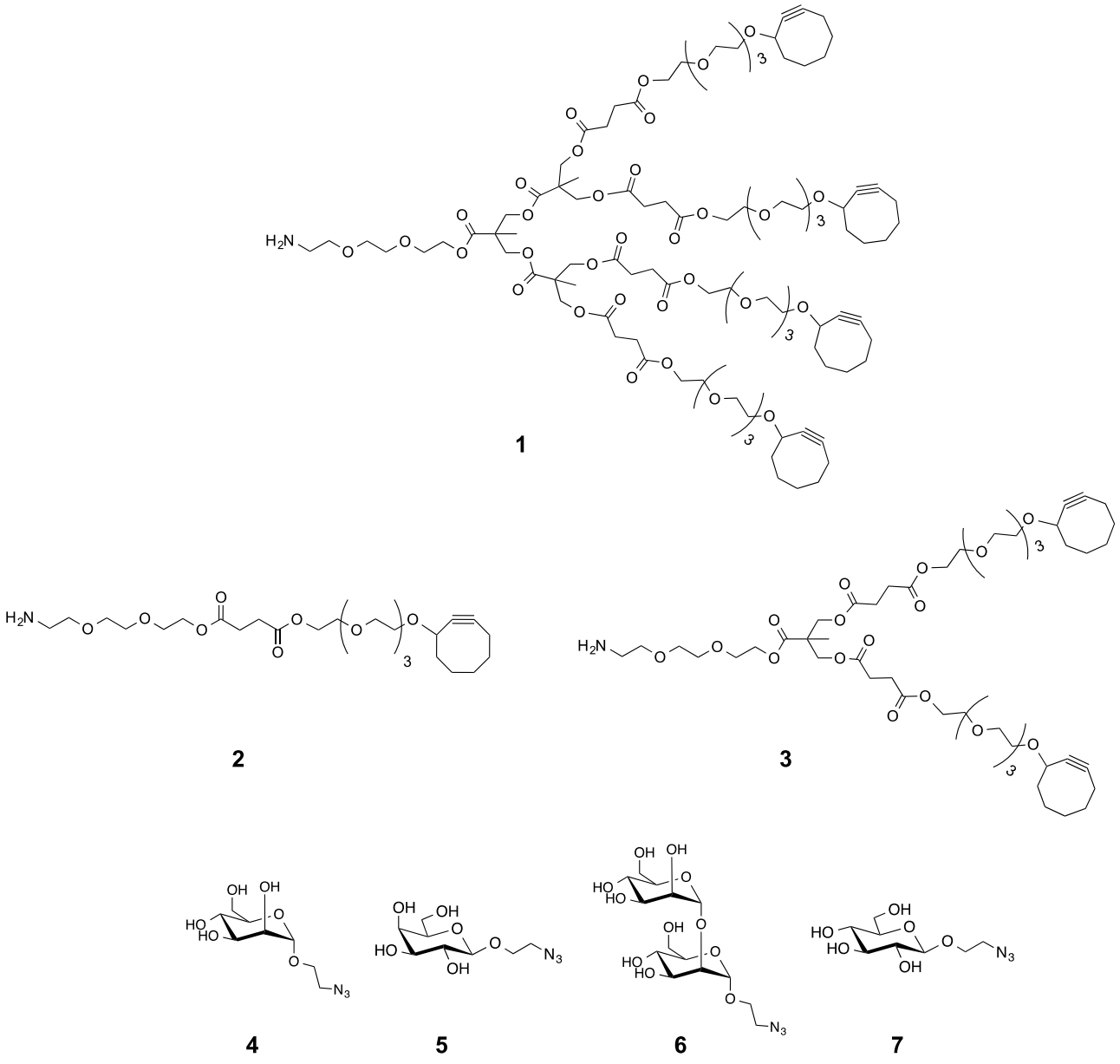
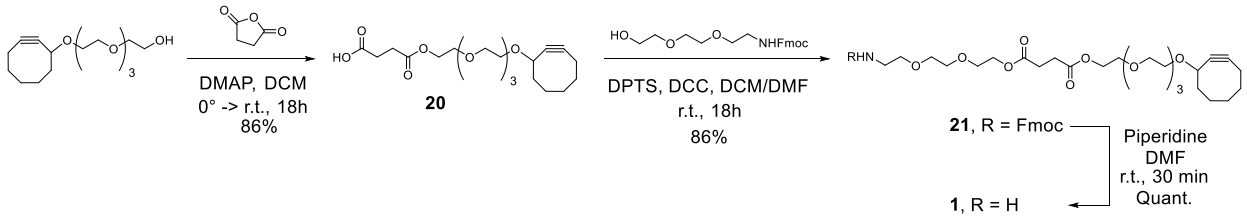
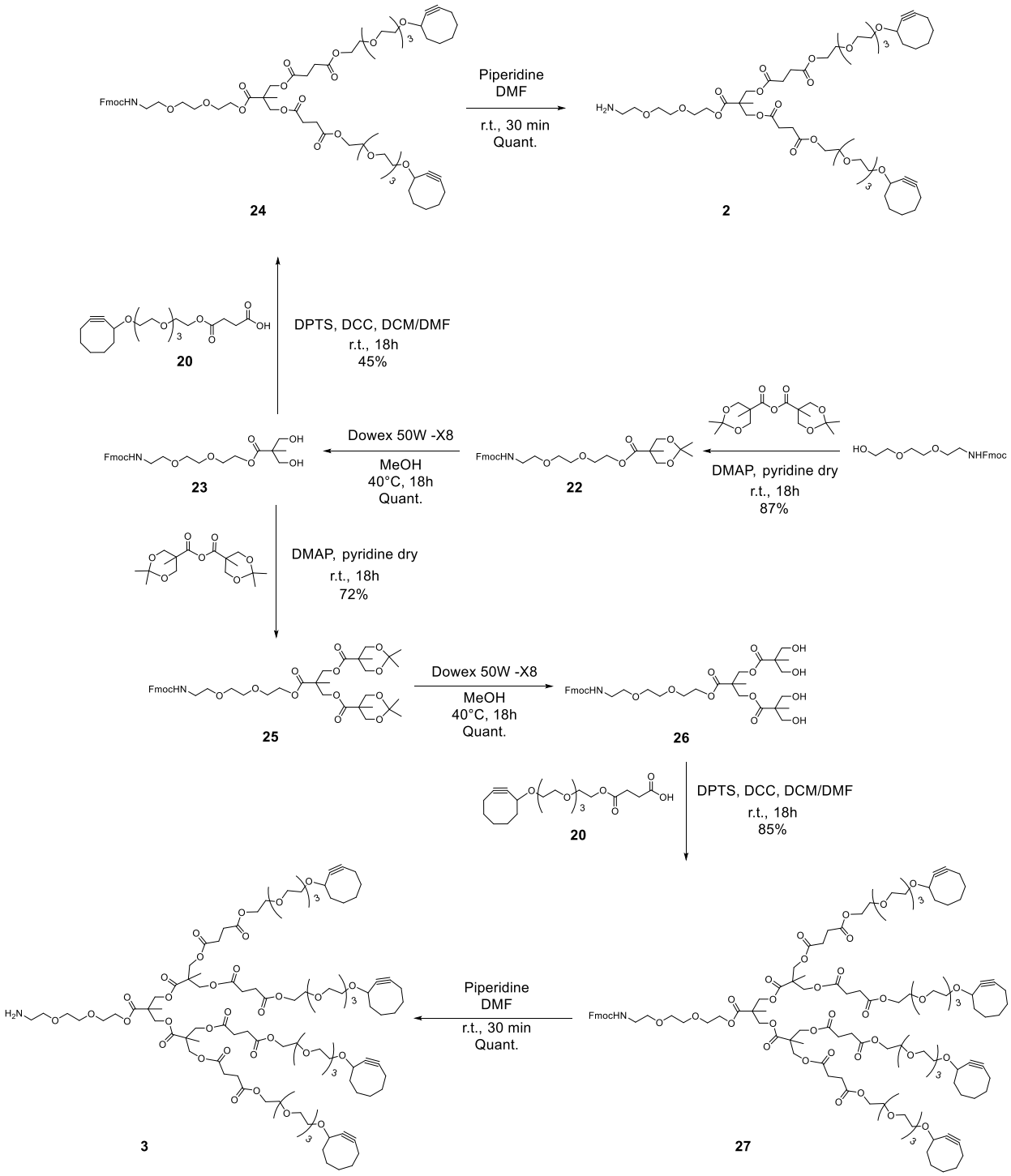


Figure S1. Cyclooctyne dendrons compounds **1-3** and 2-azido ethyl carbohydrates **4-7**.

1. Synthesis of cyclooctyne dendrons 1-3

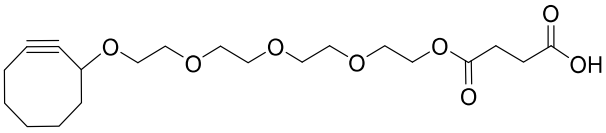


Scheme S.1 Synthesis of Mono-cyclooctyne **1**.



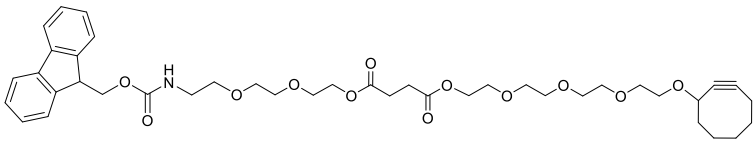
Scheme S.2 Synthesis of bi- (**2**) and tetra-cyclooctyne (**3**) dendrons.

Compound 20



To a solution of 2-[2-[2-[2-(2-Cyclooctyn-1-yloxy)ethoxy]ethoxy]ethoxy]ethanol compound, previously synthetized following the procedure described in literature[1] (0.83 g, 2.8 mmol) and succinic anhydride (0.33 g, 3.2 mmol) in dry CH_2Cl_2 (19 mL) under dry Ar atmosphere, DMAP (0.34 g, 2.8 mmol) was added. Then, the mixture was stirred at room temperature for 18h. Afterwards, the solvent was evaporated under vacuum and Et_2O (20 mL) was added. The organic phase was washed with 5% Na_2CO_3 solution (2 X 40 mL) and the aqueous layer was acidified until pH 4 by adding 1M HCl solution (40 mL). The combined organic layers were washed with brine (180 mL) and H_2O (180 mL), dried with Na_2SO_4 to give compound **20** (0.96 g 86%) as a light-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.33 – 4.14 (m, 3H, CH_2OCO and CH-ring), 3.79 – 3.38 (m, 14H CH_2PEG), 2.64 (s, 4H, CH_2succ.), 2.35 – 2.04 (m, 3H, CH_2, CH_2-ring), 2.02 – 1.86 (m, 2H, CH_2, CH_2-ring), 1.86 – 1.74 (m, 2H, CH_2, CH_2-ring), 1.74 – 1.50 (m, 1H, CH_2, CH_2-ring), 1.44-1.41 (m, 1H, CH_2, CH_2-ring), 1.29-1.25 (m, 1H, CH_2, CH_2-ring). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.15 (COOH), 172.1 ($COOCH_2$), 100.1 (C-triple bond), 92.7 (C-triple bond), 72.7 (CO-ring), 70.5, 70.4, 70.4, 70.3, 68.9, 68.3, 63.8 (CPEG), 42.2 (C-ring), 34.2 (C-ring), 29.7 (C-ring), 29.1 (Csucc), 28.9 (C-ring), 26.3 (C-ring), 20.6 (C-ring). ESI-MS: m/z calcd. for $C_{20}H_{32}O_8$: 400.2 $[M]^+$; found: 423.3 $[M+Na]^+$, 438.3 $[M+K]^+$; ESI-HRMS: m/z calcd. for $C_{20}H_{32}O_8Na$: 423.1994; found: 423. 1985.

Compound 21

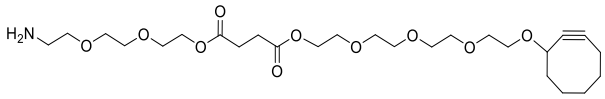


Compound **20** (0.150 g, 0.374 mmol) and the commercial [2-[2-(2-Hydroxyethoxy)ethoxy]ethyl]carbamic acid 9H-fluoren-9-ylmethyl ester (0.124 g, 0.339 mmol) were solubilize with DMAP (0.010 g, 0.085 mmol) and DPTS (0.091 g, 0.37 mmol) in a mixture of dry DCM/DMF (10 mL/10 mL) under argon atmosphere. To this solution, DCC (0.140 g, 0.68 mmol) in dry DCM (5 mL) was added dropwise. After 18h of stirring at r.t., the insoluble residue of DCU was removed by filtration on Celite pad with DCM. The solvent was removed under vacuum to give a light-yellow oil. Purification was performed by flash chromatography on silica gel (gradient DCM/MeOH 9:1) to give **21** (0.217 g, 86%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.62 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.42 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 7.33 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 5.39 (s, 1H, NH), 4.43 (d, *J*= 6.9 Hz, 2H, CH_2-Fmoc), 4.28-4.22 (m, 6H, CH_2OCO, CH-Fmoc and CH-ring), 3.76 – 3.50 (m, 22H, CH_2PEG) 3.44-3.40 (m, 2H, CH_2NH), 2.66 (s, 4H, CH_2succ), 2.30-2.10 (m, 3H, CH_2-ring), 2.06 – 1.90 (m, 2H, CH_2-ring), 1.90 – 1.76 (m, 2H, CH_2-ring), 1.76 – 1.54 (m, 1H, CH_2-ring), 1.44-1.41 (m, 1H, CH_2-ring), 1.29-1.25 (m, 1H, CH_2, CH_2-ring). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.2 (CCOO), 156.5 (NCOO), 144.0 (Carom-Fmoc), 141.3 (Carom-Fmoc), 127.6 (Carom-Fmoc), 127.0 (Carom-Fmoc), 125.1 (Carom-Fmoc), 119.9 (Carom-Fmoc), 100.0 (C-triple bond), 92.8 (C-triple bond), 72.8 (CO-ring), 70.6, 70.6,

[1] E Lallana, E Fernandez-Megia, R Riguera, J. Am. Chem. Soc., 2009, **131**, 5748–5750.

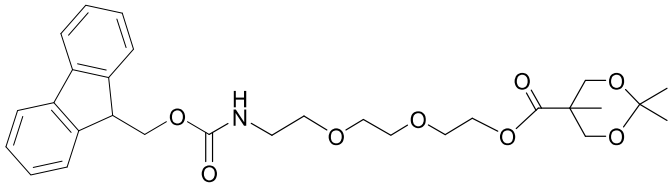
70.5, 70.4, 70.3, 70.1, 69.0, 68.5 (CPEG), 66.6 (CH_2-Fmoc), 63.9 ($\underline{C}H_2COO$), 47.3 (CH-Fmoc), 42.3 (C-ring), 40.9 (CNH), 34.3 (C-ring), 29.7 (C-ring), 28.9 (Csucc), 26.4 (C-ring), 20.7 (C-ring). ESI-MS m/z calcd. for $C_{41}H_{55}NO_{12}$: 753.37 $[M]^+$; found: 776.37 $[M + Na]^+$; MALDI-TOF MS m/z: calcd. for $C_{41}H_{55}NO_{12}$: 753.37 $[M]^+$; found 777.31 $[M+Na]^+$, 793.30 $[M+K]^+$, (matrix DHB); MALDI-TOF-HRMS: m/z calcd. for $C_{41}H_{55}NO_{12}Na$: 776.3621; found: 776.3666.

Compound 1



To a solution of **21** (0.058 g, 0.077 mmol) in DMF (4 mL), under Argon atmosphere, a solution (20%) of Piperidine (0.016 mL) was added dropwise. The mixture was stirring at room temperature for 30 minutes. Afterwards, a TLC plate was performed (DCM:MeOH 9/1) to verify the complete absence of the starting product. Purification was performed by Sephadex LH-20 in MeOH affording compound **1** (0.037 g, quant.) as a light-yellow oil. ^{1}H NMR (500 MHz, MeOH- d_4): δ 4.14 – 4.10 (m, 5H, CH_2PEG and CH-ring), 3.63 – 3.52 (m, 19H, CH_2PEG), 3.45 – 3.37 (m, 2H CH_2PEG-O-ring), 3.21 – 3.19 (m, 2H, CH_2NH), 2.55 (s, 4H, CH_2succ), 2.23 – 1.91 (m, 3H, CH_2-ring), 1.88 – 1.78 (m, 2H, CH_2-ring), 1.78 – 1.67 (m, 2H, CH_2-ring), 1.65 – 1.46 (m, 2H, CH_2-ring), 1.46 – 1.29 (m, 1H, CH_2-ring).^{13}C NMR (126 MHz, MeOH-d_4): δ 172.5 (C$\underline{C}$OO), 99.4 (C-triple bond), 92.2 (C-triple bond), 72.4 (C-ring), 71.3, 70.2, 70.1, 70.0, 69.9, 68.7, 68.6, 68.1 (CH_2PEG), 63.5 ($\underline{C}H_2COO$), 41.9 (C-ring), 40.6 (CHNH), 34.0 (C-ring), 29.6 (C-ring), 28.5 (Csucc), 26.1 (C-ring), 19.8 (C-ring). MALDI-TOF MS m/z: calcd. for $C_{26}H_{45}NO_{10}$: 531.643 $[M]^+$; found 533.11 $[M+H]^+$, 555.07 $[M+Na]^+$, 571.05 $[M+K]^+$, (matrix DHB); MALDI-TOF-HRMS: m/z calcd. for $C_{26}H_{45}NO_{10}Na$: 554.2940; found: 554.2928.

Compound 22

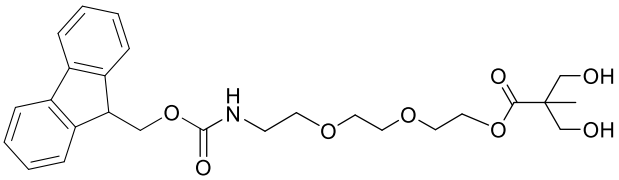


The commercial [2-[2-(2-Hydroxyethoxy)ethoxy]ethyl]carbamic acid 9H-fluoren-9-ylmethyl ester (0.40 g, 1.08mmol) and DMAP (0.06 g, 0.54 mmol) were dissolved in pyridine (10 mL) at room temperature. To this mixture after 10 minutes, a solution of 1,3-Dioxane-5-carboxylic acid, 2,2,5-trimethyl-, 5,5'-anhydride, previously synthetized following the procedure described in literature[2] (0.53 g, 1.6 mmol) in 5 mL of DCM was added. After 18 h of stirring at room temperature, the excess of anhydride was quenched with 1 mL of water under vigorous stirring. The reaction was then diluted with 100 mL of DCM and washed with a solution of 10% of Na_2CO_3 (3 X 25 mL), and brine (25 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under vacuum. Purification was performed by column chromatography on silica gel (gradient EtOAc/DCM 6:4 to EtOAc/DCM 8:2) to afford **22** as a light-yellow oil (0.50 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.58 (d, *J*= 7.6 Hz, 2H, CHarom-Fmoc), 7.36 (t, *J*= 7.4 Hz, 2H, CHarom-

[2] M. Malkoch, E Malmstrom, A Hult, Macromolecules, 2002, **35**, 8307 – 8314.

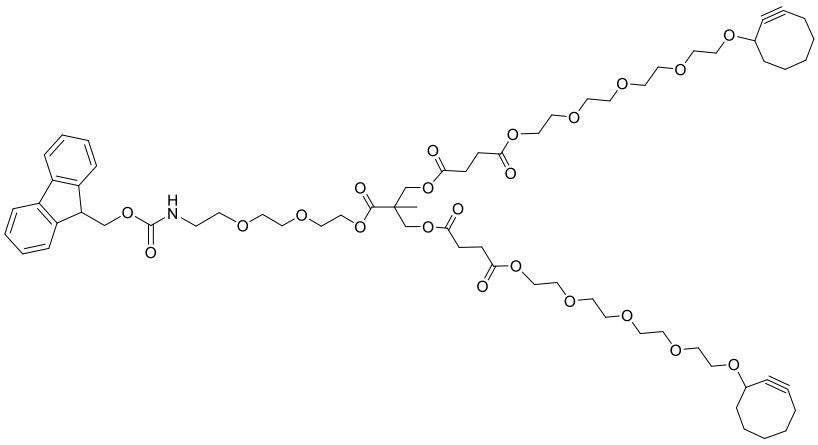
Fmoc), 7.28 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 5.47 (s, 1H, NH), 4.39 (d, *J*= 6.9 Hz, 2H, CH_2-Fmoc), 4.31 – 4.23 (m, 1H, CH-Fmoc), 4.17 (d, J = 11.7 Hz, 2H, CC$\underline{H}_2$O), 3.77 – 3.50 (m, 12H, CH_2PEG and CC$\underline{H}_2$O), 3.38-3.34 (m, 2H, C$\underline{H}_2$NH), 1.38 (s, 6H, CH_3), 1.23 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.9 (C$\underline{C}$OO), 156.3 (N$\underline{C}$OO), 143.7 (Carom-Fmoc), 141.1 (Carom-Fmoc), 127.4 (Carom-Fmoc), 126.8 (Carom-Fmoc), 124.8 (Carom-Fmoc), 119.7 (Carom-Fmoc), 97.8 ($\underline{C}(CH_3)_2$), 70.3, 70.1, 69.9, 68.8 (CPEG), 66,3 (CH_2-Fmoc) 65.7 ($\underline{C}H_2$-bisMPA), 63.5 (CH-Fmoc), 47.1 ($\underline{C}$CO), 41.6 (CNH), 24.4, 22.4, 18.4 (CH_3). ESI-MS m/z calcd. for $C_{29}H_{37}NO_8$: 527.25 $[M]^+$; found: 550.34 $[M + Na]^+$. ESI-HRMS m/z calcd. for $C_{29}H_{37}NO_8Na$: 550.2417; found: 550.2408.

Compound 23



To a solution of compound **22** (0.5g, 0.95mmol) in 20 mL of MeOH, two teaspoons of DOWEX 50W-X8 was added. The mixture was stirred at 40 °C for 18h. Afterwards, the resin was filtered off and washed with MeOH. The filtrate was evaporated under vacuum and a Sephadex LH-20 in MeOH was performed to afford **23** as white crystals (0.44 g, quant). ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.77 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.63 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.38 (t, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.30 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 4.34 (d, *J*= 6.9 Hz, 2H, CH_2-Fmoc), 4.30-4-23 (m, 1H, CHFmoc), 3.74-3.49 (m, 14H, CH_2PEG and CC$\underline{H}_2$O), 3.37 (s, 1H, NH), 3.29 (m, 2H, CH_2NH), 1.18 (s, 3H, CH_3). ^{13}C NMR (101 MHz, MeOH- d_4): δ 175.1 (C$\underline{C}$OO), 157.4 (N$\underline{C}$OO), 143.9 (Carom-Fmoc), 141.2 (Carom-Fmoc), 127.4 (Carom-Fmoc), 126.8 (Carom-Fmoc), 124.8 (Carom-Fmoc), 119.6 (Carom-Fmoc), 70.1, 69.8, 69.6, 68.6 (CPEG), 66.3 (CH_2-Fmoc), 64.5 ($\underline{C}H_2$-bisMPA), 63.3 (CH-Fmoc), 50.2 ($\underline{C}$CO), 40.3 (CNH), 16.1 (CH_3). Calcd. for $C_{26}H_{33}NO_8$: 487.55 $[M]^+$; found: 488.31 $[M + H]^+$ and 510.32 $[M + Na]^+$. ESI-HRMS m/z calcd. for $C_{26}H_{33}NO_8Na$: 510.2104; found: 510.2096.

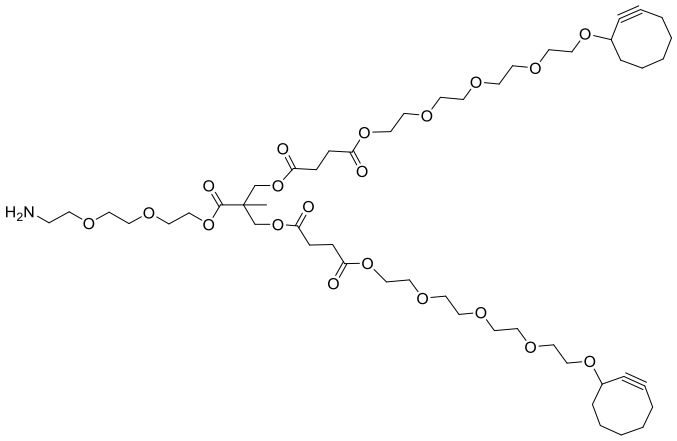
Compound 24



Compound **23** (0.55 g, 0.113 mmol) and compound **20** (0.136 g, 0.339 mmol) was solubilize with DMAP (0.005 g, 0.04 mmol) and DPTS (0.044 g, 0.176 mmol) in a mixture of dry DCM/DMF (5 mL/5 mL) under argon atmosphere. To this solution, DCC (0.050 g, 0.24mmol) in dry DCM (5 mL) was added dropwise. After 18h of

stirring at room temperature, the insoluble residue of DCU was removed by filtration on Celite pad with DCM. The solvent was removed under vacuum to give a light-yellow oil. Purification was performed by flash chromatography on silica gel (gradient DCM/MeOH 9:1) to give compound **24** (0.114 g, 45%) as a light-brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.63 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.42 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 7.33 (t, *J*= 7.6 Hz, 2H, CHarom-Fmoc), 5.44 (s, 1H, NH), 4.42 (d, *J*= 7.0 Hz, 2H, CH_2-Fmoc), 4.36 – 4.20 (m, 11H, CH_2OCO, CH-Fmoc, CH_2PEG and CH-ring), 3.79 – 3.47 (m, 38H, CH_2PEG and CCH_2CO), 3.42 – 3.40 (m, 2H, CH_2NH), 2.65 (s, 8H, CH_2succ), 2.34 – 2.08 (m, 6H, CH_2-ring), 2.05 – 1.88 (m, 4H, CH_2-ring), 1.91 – 1.77 (m, 4H, CH_2-ring), 1.78 – 1.55 (m, 2H, CH_2-ring), 1.53 – 1.38 (m, 2H, CH_2-ring), 1.27 – 1.24 (m, 5H, CH_2-ring and CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.5 (CCOO-bisMPA), 172.1 (CCOO-CPEG), 171.7 (CCOO-CH_2), 156.5 (NCOO), 144.0 (Carom-Fmoc), 141.3 (Carom-Fmoc), 127.6 (Carom-Fmoc), 127.0 (Carom-Fmoc), 125.1 (Carom-Fmoc), 119.9 (Carom-Fmoc), 100.0 (C-triple bond), 92.8 (C-triple bond), 72.7 (CO-ring), 70.6, 70.5, 70.5, 70.4, 70.3, 69.0, 68.5 (CPEG), 66.6 (CH-Fmoc), 65.5 (CH_2-bisMPA), 64.1 (CH_2COO-linker) 63.9 (CH_2COO-branch), 47.3 (CH-Fmoc), 46.3 (CCO-bisMPA), 42.3 (C-ring), 40.9 (CNH), 34.3 (C-ring), 29.7 (C-ring), 28.9 (Csucc), 26.4 (C-ring), 20.7 (C-ring), 17.7 (CH_3). ESI-MS m/z calcd. for $C_{66}H_{93}NO_{22}$: 1251.62 $[M]^+$; found: 1274.46 $[M + Na]^+$; MALDI-TOF MS m/z: calcd. for $C_{66}H_{93}NO_{22}$: 1252.45 $[M]^+$; found 1274.68 $[M+Na]^+$, 1290.06 $[M+K]^+$, (matrix DHB); MALDI-TOF-HRMS: m/z calcd. for $C_{66}H_{93}NO_{22}Na$: 1274.6083; found: 1274.6055.

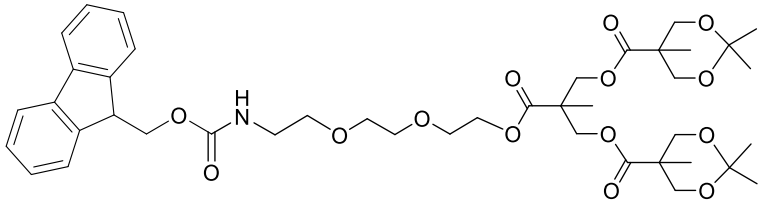
Cyclooctyne dendron 2



To a solution of **24** (0.043 g, 0.035 mmol) in DMF (2 mL), under Argon atmosphere, a solution (20%) of Piperidine (10 µL) was added. The mixture was stirring at room temperature for 30 minutes. Afterwards, a TLC plate was performed (DCM/MeOH 9:1) to verify the complete absence of the starting product. Purification was performed by Sephadex LH-20 in MeOH affording **2** (0.037g, quant.) as a light-brown oil. ^{1}H NMR (500 MHz, MeOH-d_4): δ 4.21 – 4.11 (m, 10H, CH_2OCO, CH_2PEG, CH-ring CH_2), 3.66 – 3.46 (m, 38H, CH_2PEG and CCH_2CO), 3.22 – 3.20 (m, 2H, CH_2NH_2), 2.53 (s, 8H, CH_2succ), 2.17-1.97 (m, 6H, CH_2-ring), 1.83 (m, 4H, CH_2-ring), 1.78 – 1.69 (m, 4H, CH_2-ring), 1.66 – 1.47 (m, 2H, CH_2-ring), 1.39-1.33 (m, 2H, CH_2-ring), 1.16 – 1.14 (m, 5H, CH_2-ring and CH_3). ^{13}C NMR (126 MHz, MeOH- d_4): δ 172.7 (CCOO-bisMPA), 172.4 (CCOO-CPEG), 171.9 (CCOO-CH_2), 99.4 (C-triple bond), 92.3 (C-triple bond), 72.4 (CO-ring), 72.2, 71.2, 70.1, 70.02, 69.9, 68.6, 68.1 (CPEG), 65.2 (CH_2-bisMPA), 64.0 (CH_2COO-linker), 63.6 (CH_2COO-branch), 60.8 (CNH_2), 46.2 (CCO-bisMPA), 41.9 (C-ring), 40.5 (CNH2), 34.0 (C-ring), 29.5 (C-ring), 28.4 (Csucc), 26.1 (C-ring), 19.85 (C-ring), 16.7 (CH_3).

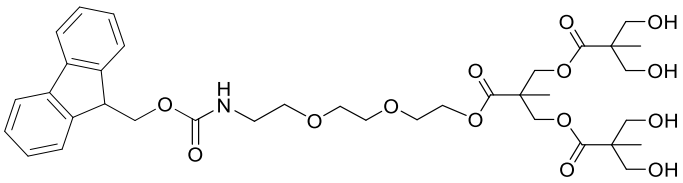
MALDI-TOF MS m/z: calcd. for $C_{51}H_{83}NO_{20}$: 1029.55 $[M]^+$; found 1031.62 $[M+H]^+$, 1053.63 $[M+Na]^+$ (matrix DHB); MALDI-TOF HRMS: m/z calcd. for $C_{51}H_{83}NO_{20}Na$: 1052.5402; found: 1052.5422.

Compound 25



1,3-Dioxane-5-carboxylic acid, 2,2,5-trimethyl-, 5,5'-anhydride, previously synthetized following the procedure described in literature,[2] (0.362 g, 0.74 mmol) and DMAP (0.09 g, 0.74 mmol) were dissolved in pyridine (10 mL) at room temperature. At this mixture after 10 minutes, a solution of **23** (0.738 g, 2.23 mmol) in 5 mL of DCM was added. After 18h of stirring at room temperature, the excess of anhydride was quenched with 1 mL of water under vigorous stirring. The reaction was then diluted with 100 mL of DCM and washed with a solution of 10% of Na_2CO_3 (3 X 25 mL), and brine (25 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under vacuum. Purification was performed by column chromatography on silica gel (gradient EtOAc/DCM 4:6 to EtOAc/DCM 6:4) to afford **25** as a light-yellow oil (0.42 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.62 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.42 (t, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.33 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 5.40 (s, 1H, NH), 4.42 (d, *J*= 6.9 Hz, 2H, CH_2-Fmoc), 4.36-4.34 (m, 4H, CC$\underline{H}_2$O), 4.30-4.24 (m, 1H, CH-Fmoc), 4.17 (d, *J*= 11.7 Hz, 8H, CC$\underline{H}_2$O), 3.70 – 3.54 (m, 10H, CH_2PEG), 3.47 – 3.37 (m, 2H, CH_2NH), 1.40 (s, 12H, CH_3), 1.32 (s, 3H, CH_3), 1.17 (s, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.5 (C$\underline{C}$OO-bisMPA$_{ext}$), 172.5 (C$\underline{C}$OO-bisMPA$_{int}$), 156.5 (N$\underline{C}$OO), 144.0 (Carom-Fmoc), 141.3 (Carom-Fmoc), 127.6 (Carom-Fmoc), 127.0 (Carom-Fmoc), 125.0 (Carom-Fmoc), 119.9 (Carom-Fmoc), 98.1 ($\underline{C}(CH_3)_2$), 70.5, 70.3, 70.1, 68.9 (CPEG), 66.6 (CH_2-Fmoc), 65.9 ($\underline{C}H_2$-bisMPA$_{ext}$), 65.3 ($\underline{C}H_2$-bisMPA$_{int}$), 64.2 (CH-Fmoc), 47.3 ($\underline{C}$CO-bisMPA$_{ext}$), 46.7 ($\underline{C}$CO-bisMPA$_{int}$), 40.9 (CNH), 25.1, 18.5, 17.7 (CH_3). ESI-MS m/z calcd. for $C_{42}H_{57}NO_{14}$: 799.91 $[M]^+$; found: 822.34 $[M + Na]^+$. ESI-HRMS m/z calcd. for $C_{42}H_{57}NO_{14}Na$: 822.3637; found: 822.3639.

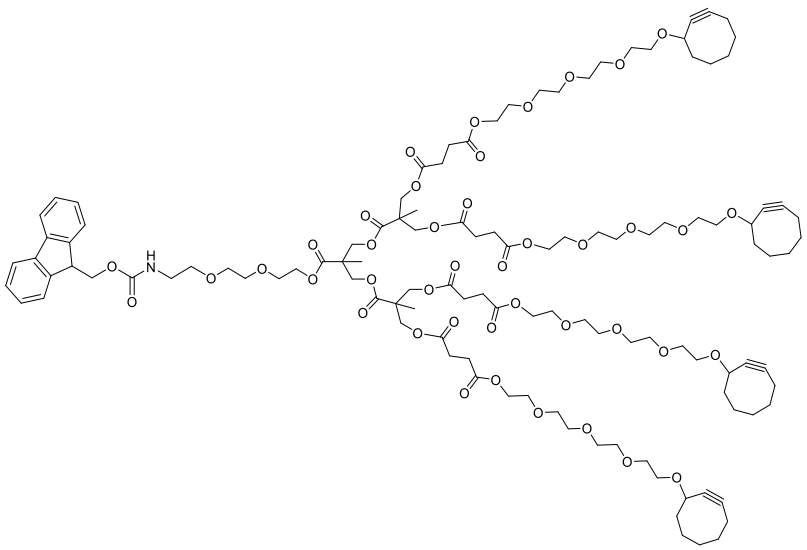
Compound 26



To a solution of compound **25** (0.28g, 0.35 mmol) in 20 mL of MeOH, two teaspoons of DOWEX 50W-X8 was added. The mixture was stirred at 40 °C for 18h. Afterwards, the resin was filtered off and washed with MeOH. The filtrate was evaporated under vacuum to afford **26** as white crystals (0.23 g, quant). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.61 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.40 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 7.31 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 5.74 (s, 1H, NH), 4.44 – 4.33 (m, 4H, CH_2-Fmoc and CC$\underline{H}_2$O), 4.33 – 4.19 (m, 5H, CC$\underline{H}_2$O and CH-Fmoc), 3.80 – 3.35 (m, 18H, CH_2PEG, CC$\underline{H}_2$O and CH_2NH), 1.30 (s,

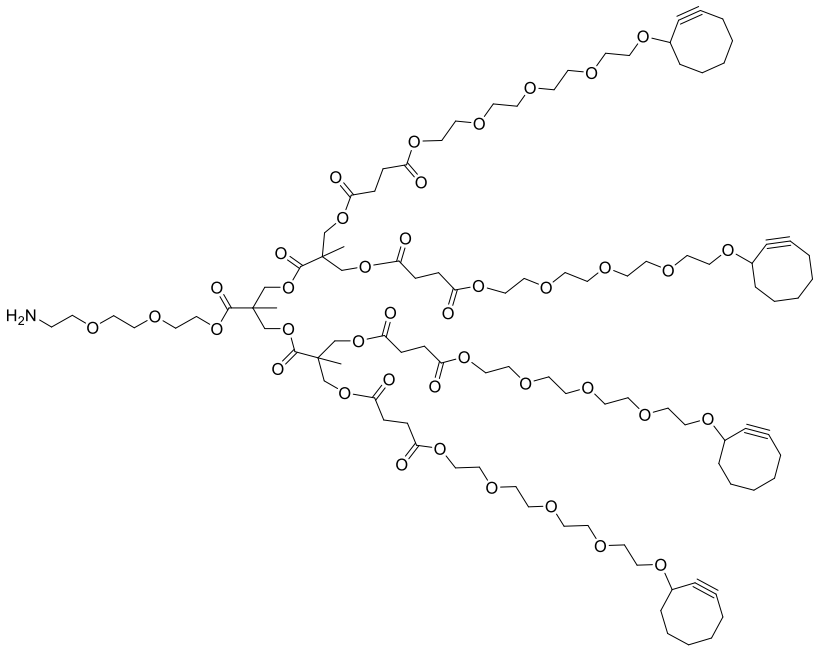
3H, CH_3), 1.08 (s, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.0 (C<u>C</u>OO-bisMPA$_{ext}$), 173.0 (C<u>C</u>OO-bisMPA$_{int}$), 156.7 (N<u>C</u>OO), 143.9 (Carom-Fmoc), 141.2 (Carom-Fmoc), 127.6 (Carom-Fmoc), 127.0 (Carom-Fmoc), 125.0 (Carom-Fmoc), 119.9 (Carom-Fmoc), 70.2, 70.1, 68.8, 67.3, 66.8, 66.7 (CPEG), 66.6 (CH_2-Fmoc), 65.0 (<u>C</u>H$_2$-bisMPA$_{ext}$), 64.1 (CH-Fmoc), 47.2 (<u>C</u>CO-bisMPA$_{ext}$), 46.4 (<u>C</u>CO-bisMPA$_{int}$), 40.9 (CNH), 18.0 (CH_3), 17.1 (CH_3). ESI-MS calcd. for $C_{36}H_{49}NO_{14}$: 719.78 $[M]^+$; found: 742.22 $[M + Na]^+$. ESI-HRMS m/z calcd. for $C_{36}H_{49}NO_{14}Na$: 742.3051; found: 742.3042.

Compound 27



Compound **26** (0.92 g, 0.128 mmol) and compound **20** (0.307 g, 0.77 mmol) was solubilize with DMAP (0.005 g, 0.04 mmol) and DPTS (0.127 g, 0.512 mmol) in a mixture of dry DCM/DMF (5 mL/5 mL) under argon atmosphere. To this solution, DCC (0.050 g, 0.24mmol) in dry DCM (5 mL) was added dropwise. After 18h of stirring at r.t., the insoluble residue of DCU was removed by filtration on Celite pad with DCM. The solvent was removed under vacuum to give a light-yellow oil. Purification was performed by flash chromatography on silica gel (gradient DCM/MeOH 95:5) to give **27** (0.247 g, 85%) as a brown oil. ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.60 (d, *J*= 7.5 Hz, 2H, CHarom-Fmoc), 7.39 (t, *J*= 7.4 Hz, 2H, CHarom-Fmoc), 7.35 – 7.26 (t, *J*= 7.6 Hz, 2H, CHarom-Fmoc), 5.44 (s, 1H, NH), 4.39 (d, *J*= 6.9 Hz, 2H, CH_2-Fmoc), 4.31 – 4.13 (m, 21H, C<u>H</u>$_2$OCO, CH-Fmoc, CH_2PEG and CH-ring), 3.77 – 3.44 (m, 68H, CH_2PEG and CC<u>H</u>$_2$CO), 3.42 – 3.39 (m, 2H, CH_2NH), 2.63 (s, 16H, CH_{2succ}), 2.31 – 2.06 (m, 12H, CH_2-ring), 2.03 – 1.88 (m, 8H, CH_2-ring), 1.88 – 1.75 (m, 8H, CH_2-ring), 1.73 – 1.52 (m, 8H, CH_2-ring), 1.49 – 1.35 (m, 4H, CH_2-ring), 1.31 – 1.24(m, 3H, CH_3), 1.22 (s, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.1 (C<u>C</u>OO-bisMPA), 171.9 (C<u>C</u>OO-CPEG), 171.7 (C<u>C</u>OO-CH_2), 156.5 (N<u>C</u>OO), 143.9 (Carom-Fmoc), 141.2 (Carom-Fmoc), 127.6 (Carom-Fmoc), 127.0 (Carom-Fmoc), 125.0 (Carom-Fmoc), 119.9 (Carom-Fmoc), 99.9 (C-triple bond), 92.8 (C-triple bond), 72.7 (<u>C</u>O-ring), 70.5, 70.5, 70.3, 70.3, 69.0, 68.4 (CH_2PEG) 66.6 (CH-Fmoc), 65.7 (<u>C</u>H$_2$-bisMPA$_{ext}$), 65.3 (<u>C</u>H$_2$-bisMPA$_{int}$) 64.2 (<u>C</u>H$_2$COO-linker) 63.8, (<u>C</u>H$_2$COO-branch) 47.3 (CH-Fmoc) 46.4 (<u>C</u>CO-bisMPA), 42.3 (C-ring), 40.9 (CNH), 34.3 (C-ring), 29.7 (C-ring), 28.8 (Csucc), 26.4 (C-ring), 20.7 (C-ring), 17.7 (CH_3), 17.5 (CH_3). ESI-MS m/z calcd. for $C_{116}H_{169}NO_{42}$: 2248.11 $[M]^+$; found: 1144.18 $[M + 2Na]^{2+}$ and 2271.41 $[M + Na]^+$; MALDI-TOF MS m/z calcd. for $C_{116}H_{169}NO_{42}$: 2248.11 $[M]^+$; found 2272.10 $[M+Na]^+$, 2288.10 $[M+K]^+$ (matrix DHB); MALDI-TOF-HRMS: m/z calcd. for $C_{116}H_{169}NO_{42}Na$: 2271.1013; found: 2271.1011.

Cyclooctyne dendron 3



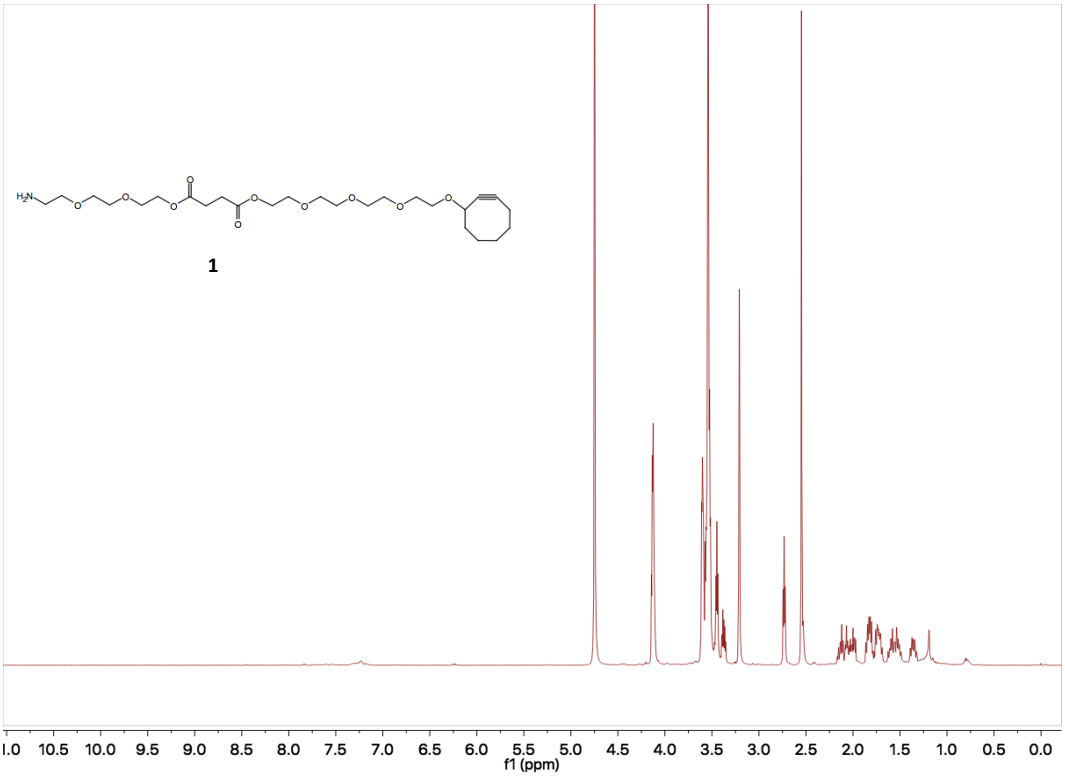
To a solution of **27** (0.080 g, 0.036 mmol) in DMF (2 mL), under Argon atmosphere, a solution (20%) of Piperidine (15 µL) was added. The mixture was stirring at room temperature for 30 minutes. Afterwards, a TLC was performed (DCM/MeOH 9:1) to verify the complete absence of the starting product. Purification was performed by Sephadex LH-20 in MeOH affording **3** (0.067 g, quant.) as a light-brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.35 – 4.11 (m, 20H, C$\underline{H}_2$OCO, CH_2PEG and CH-ring) 3.79 – 3.41 (m, 72H, CH_2PEG, CH_2NH_2 and CC$\underline{H}_2$CO), 2.62 (s, 16H, CH_2succ), 2.30 – 2.04 (m, 12H, CH_2-ring), 2.02 – 1.86 (m, 8H, CH_2-ring), 1.85 – 1.72 (m, 8H, CH_2-ring), 1.72 – 1.47 (m, 8H, CH_2-ring), 1.46 – 1.34 (m, 4H, CH_2-ring), 1.23 – 1.15 (m, 3H, CH_3), 1.22 (s, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.1 (C$\underline{O}$O-bisMPA), 171.9 (C$\underline{C}$OO-CPEG), 171.7 (C$\underline{C}$OO-CH_2) 99.9 (C-triple bond), 92.8 (C-triple bond), 72.7 (C$\underline{O}$-ring), 70.6, 70.5, 70.4, 69.0, 68.4 (CH_2PEG), 65.2 (C$\underline{H}_2$-bisMPA$_{ext}$), 65.1 (C$\underline{H}_2$-bisMPA$_{int}$) 63.9 (C$\underline{H}_2$COO-linker), 63.8 (C$\underline{H}_2$COO-branch) 46.5 (C$\underline{C}$O-bisMPA), 46.3 (C_{quat}, C18 bis MPA), 42.2 (C-ring), 39.6 (CNH), 34.3 (C-ring), 29.7 (C-ring), 28.8 (Csucc), 26.3 (C-ring), 20.7 (C-ring), 17.7 (CH_3), 17.5 (CH_3). MALDI-TOF MS: m/z calcd. for $C_{101}H_{159}NO_{40}$: 2026,04 $[M]^+$ found 2028,69 $[M+H]^+$, 2050,96 $[M+Na]^+$, 2066.90 $[M+K]^+$, (matrix DHB); MALDI-TOF-HRMS m/z calcd. for $C_{101}H_{159}NO_{40}Na$: 2049.0332; found: 2049.0301.

Azido Carbohydrates

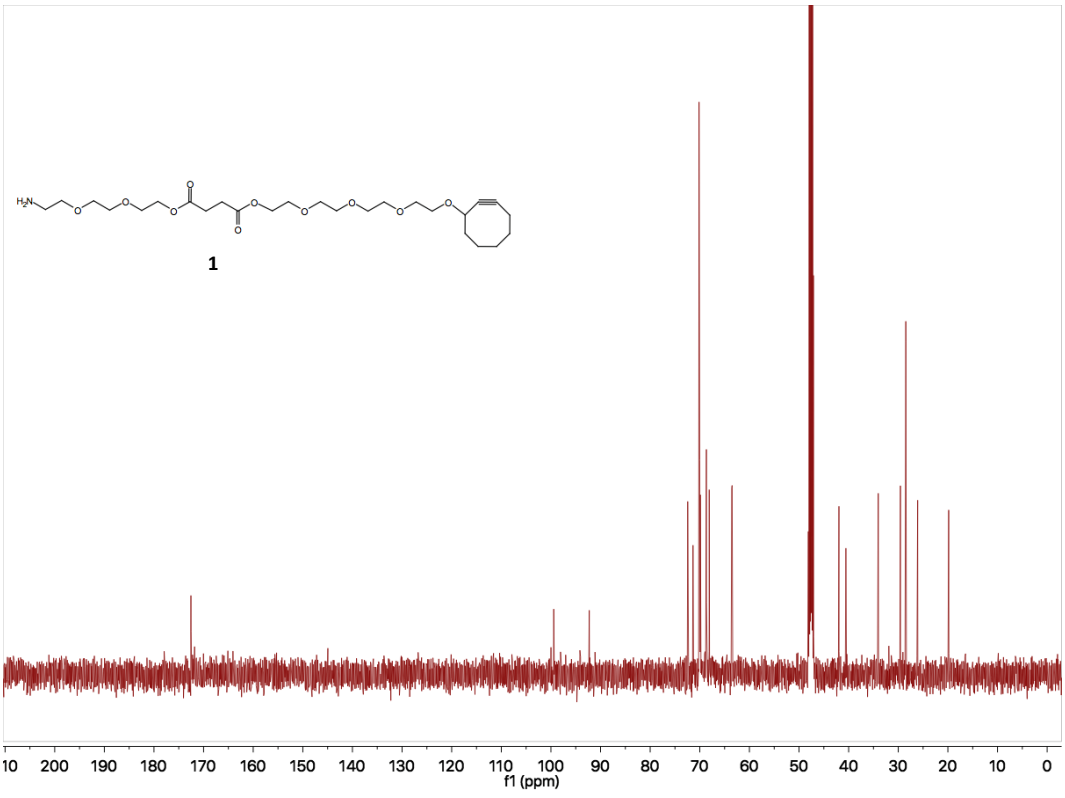
The preparation of the 2-azido ethyl mannose, (**4**) galactose (**5**) and glucose (**7**) derivatives was achieved using a procedure reported in literature.[3] The preparation of 2-azido ethyl mannose α1,2 mannose (**6**) was carried on using a procedure reported in literature.[4]

[3] E. Arce, P. M. Nieto, V. Díaz, R. García Castro, A. Bernad and J. Rojo, *Bioconjug. Chem.*, 2003, **14**, 817–823.
[4] J. J. Reina, A. Di Maio, J. Ramos-Soriano, R. C. Figueiredo and J. Rojo, *Org. Biomol. Chem.*, 2016, **14**, 2873–2882.

1.1 ^{1}H NMR (top), ^{13}C NMR (middle) and MS (MALDI-TOF or ESI, bottom) spectra



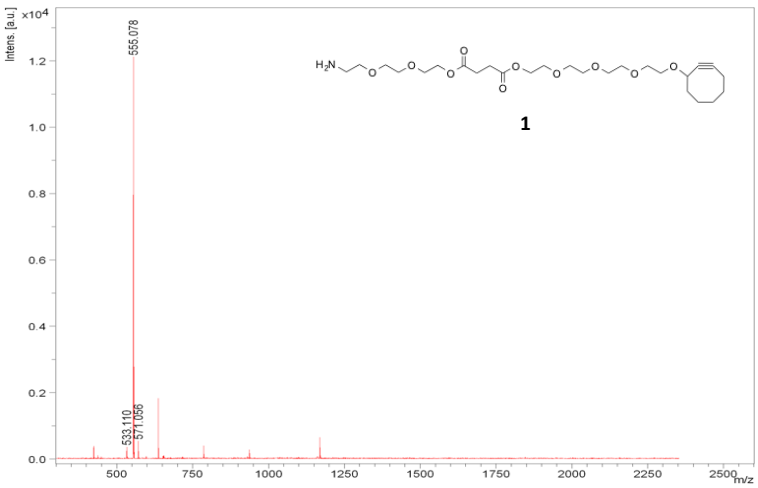
^{1}H-NMR (400 MHz, MeOH –*d4*): compound 1



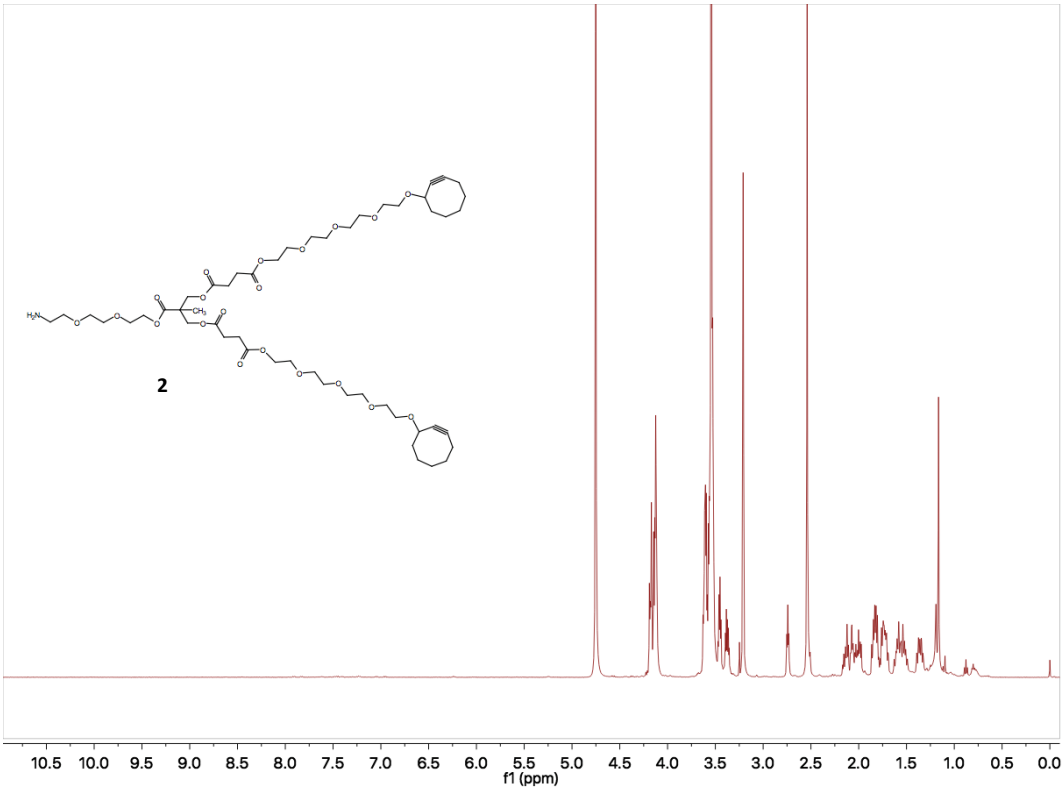
^{13}C-NMR (101 MHz, MeOH –*d4*): compound 1

MALDI-TOF

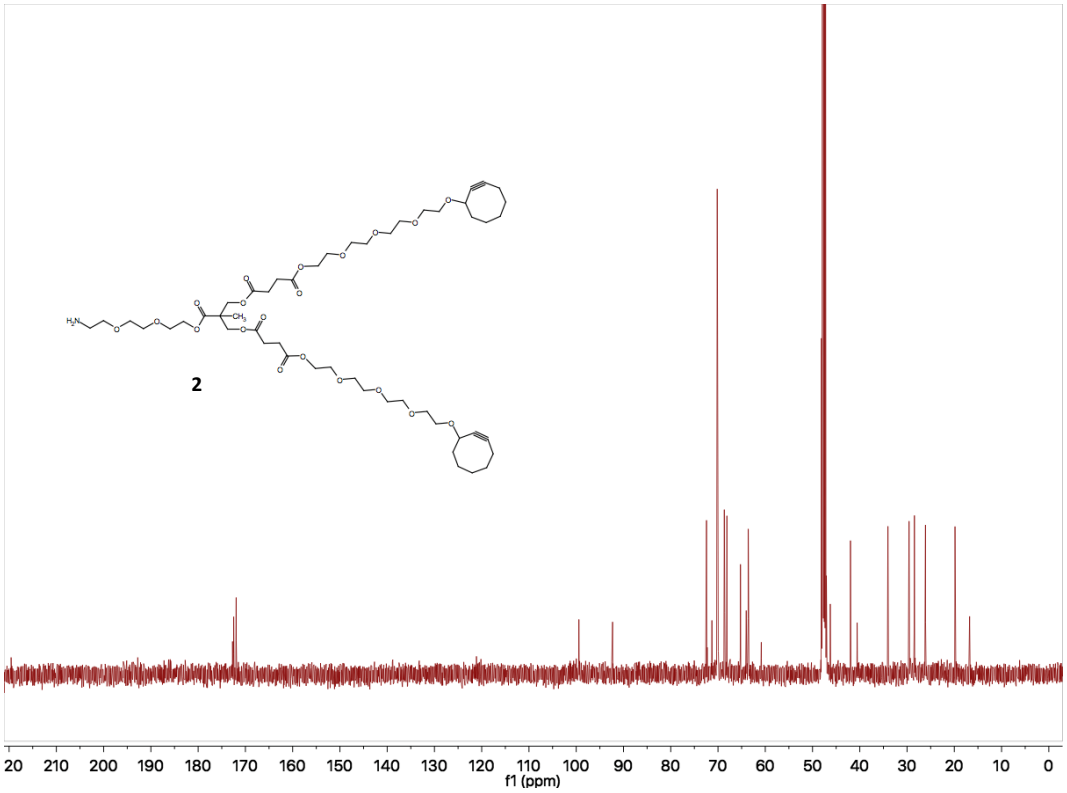
MS



MALDI-TOF spectrum of compound 1

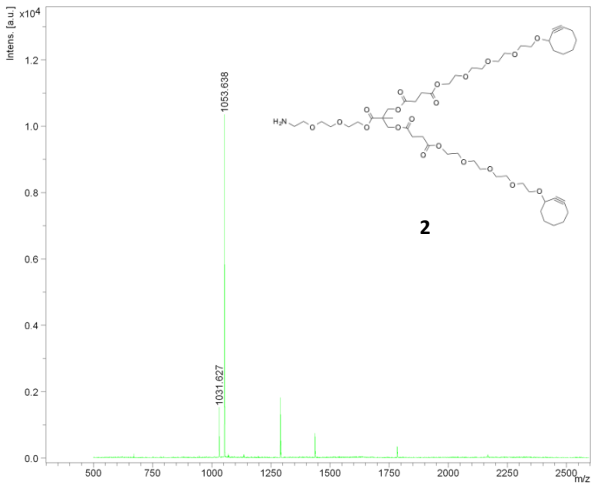


^{1}H-NMR (400 MHz, MeOH –*d4*): compound 2

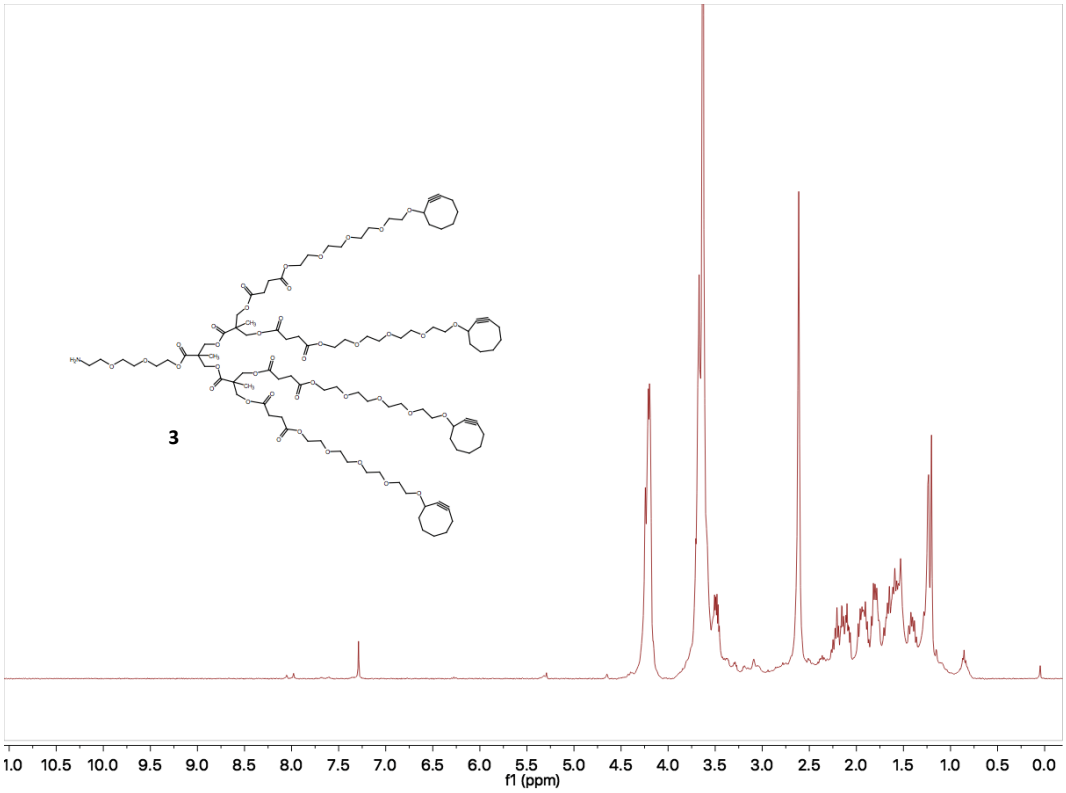


^{13}C-NMR (101 MHz, MeOH –*d4*): compound 2

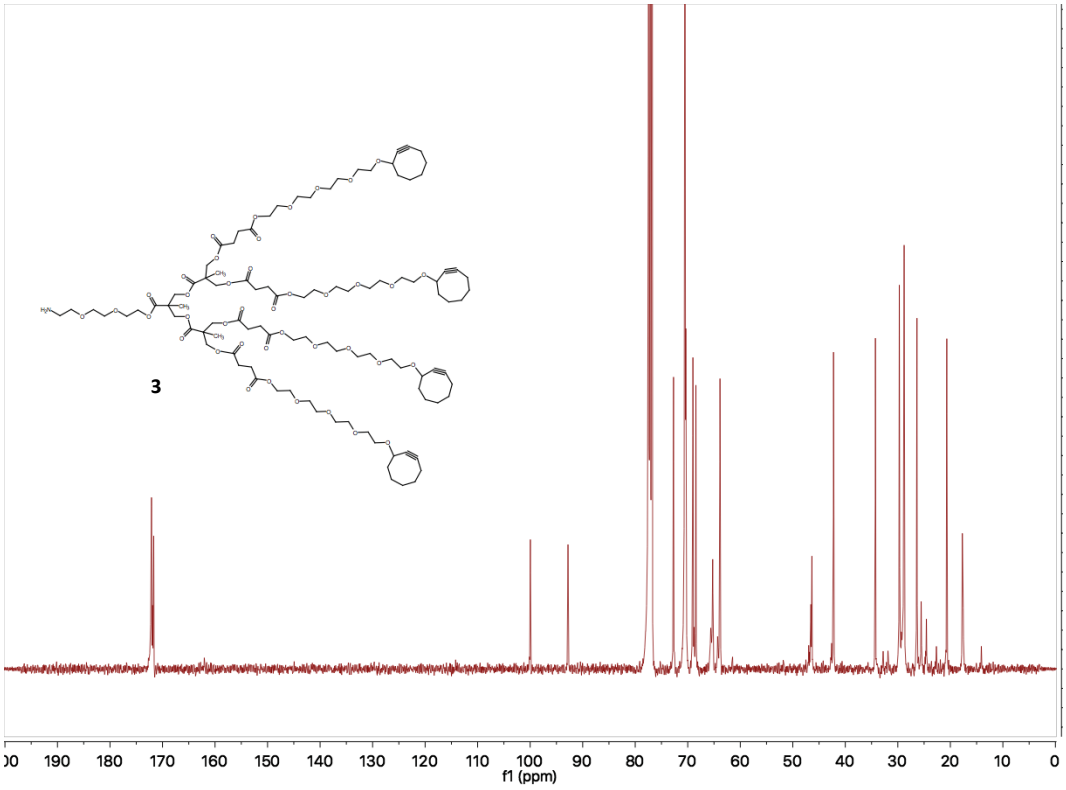
MALDI-TOF MS



MALDI-TOF spectrum of compound 2



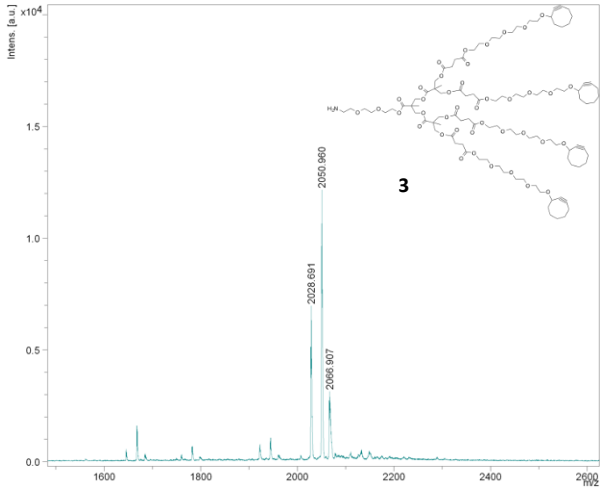
^{1}H-NMR (400 MHz, $CDCl_3$): compound 3

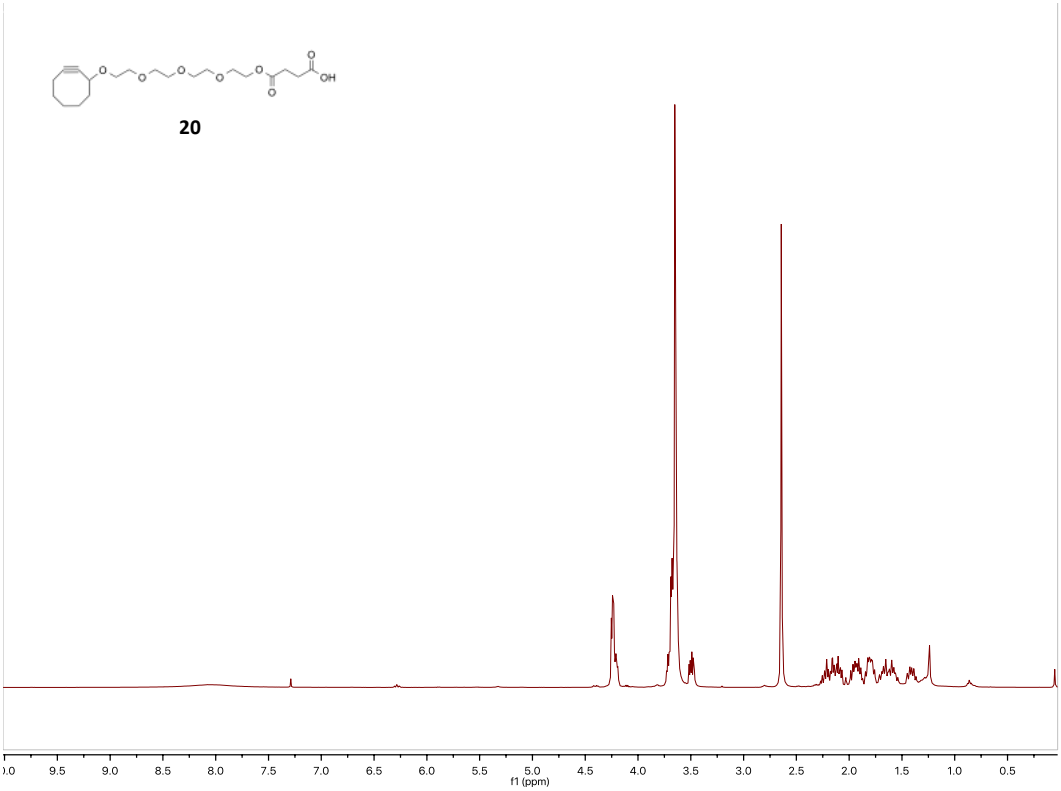


^{13}C-NMR (101 MHz, $CDCl_3$): compound 3

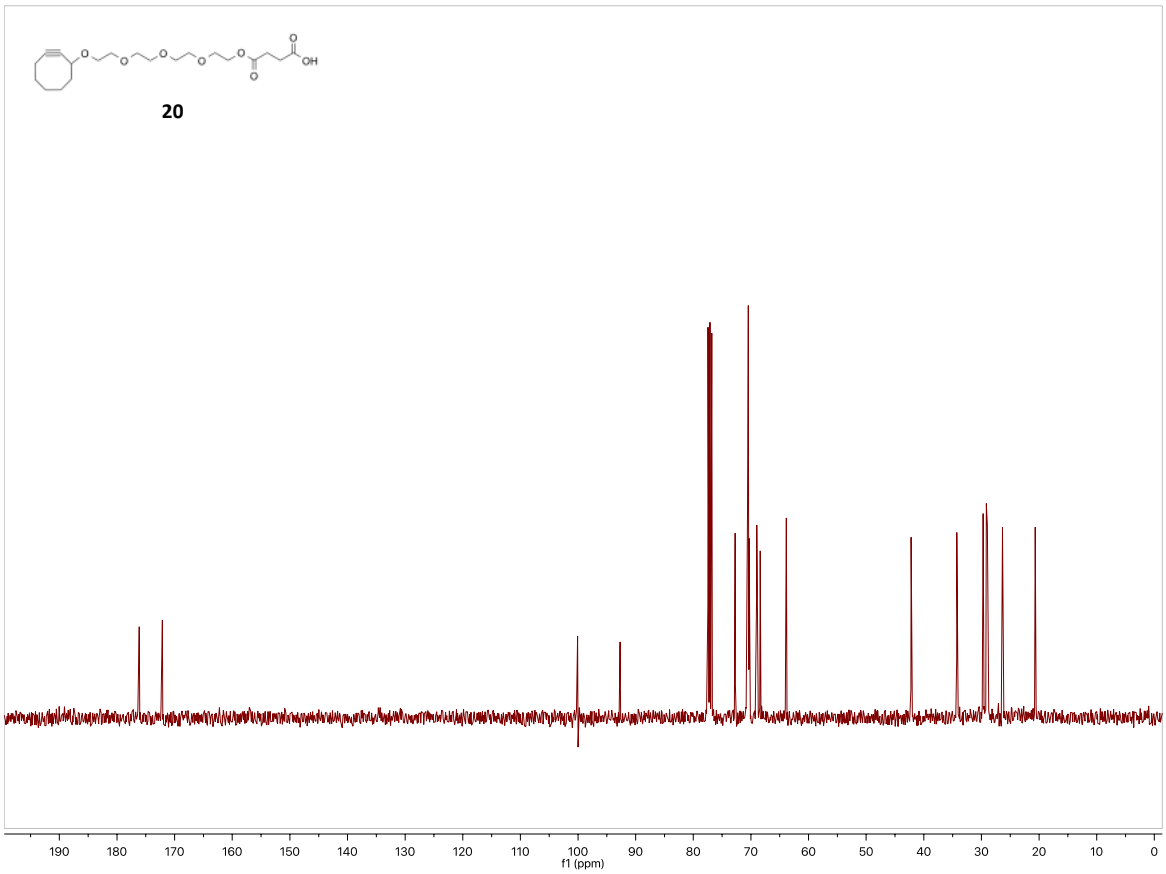
MALDI-TOF

MS

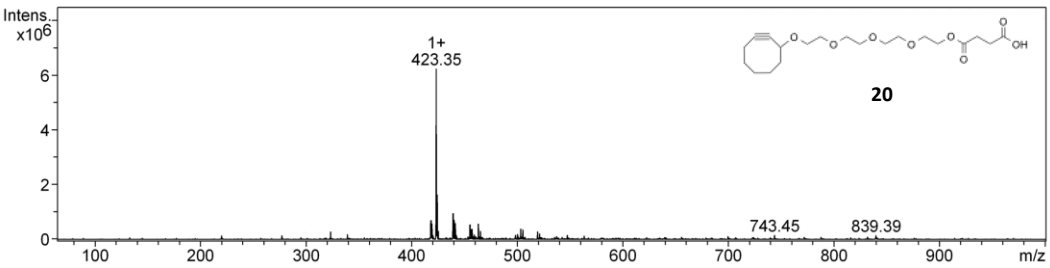




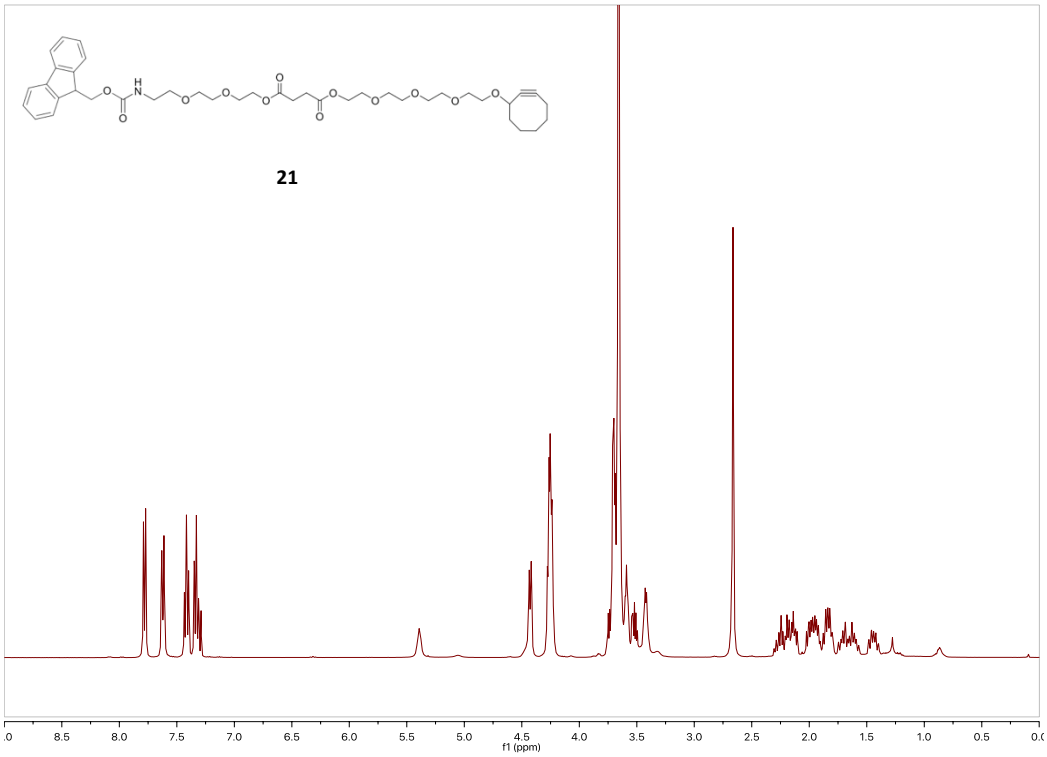
^{1}H-NMR (400 MHz, CDCl3): compound 20



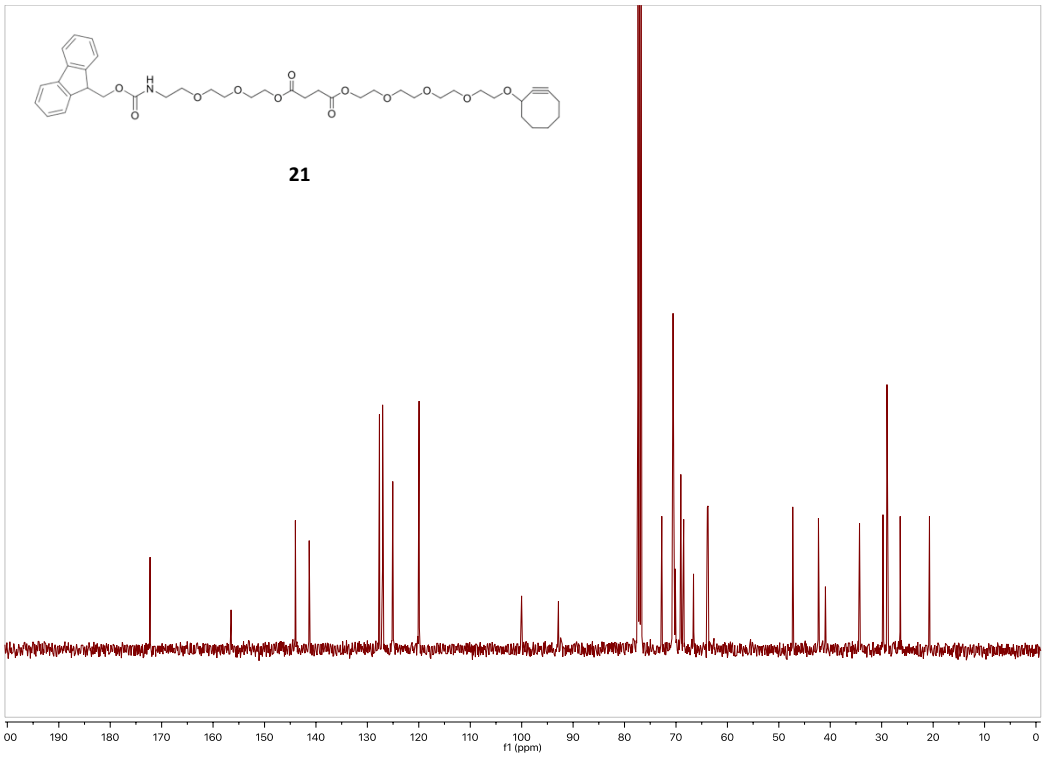
^{13}C-NMR (101 MHz, $CDCl_3$): compound 20



ESI-MS spectrum of compound 20



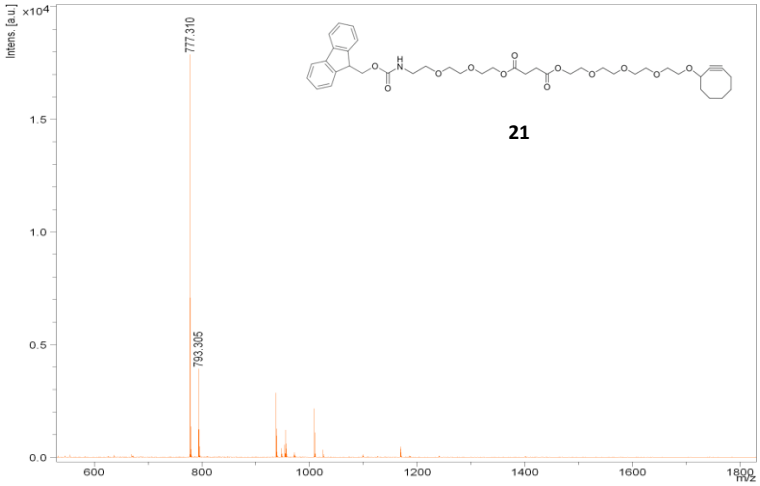
^{1}H-NMR (400 MHz, $CDCl_3$): compound 21



^{13}C-NMR (101 MHz, $CDCl_3$): compound 21

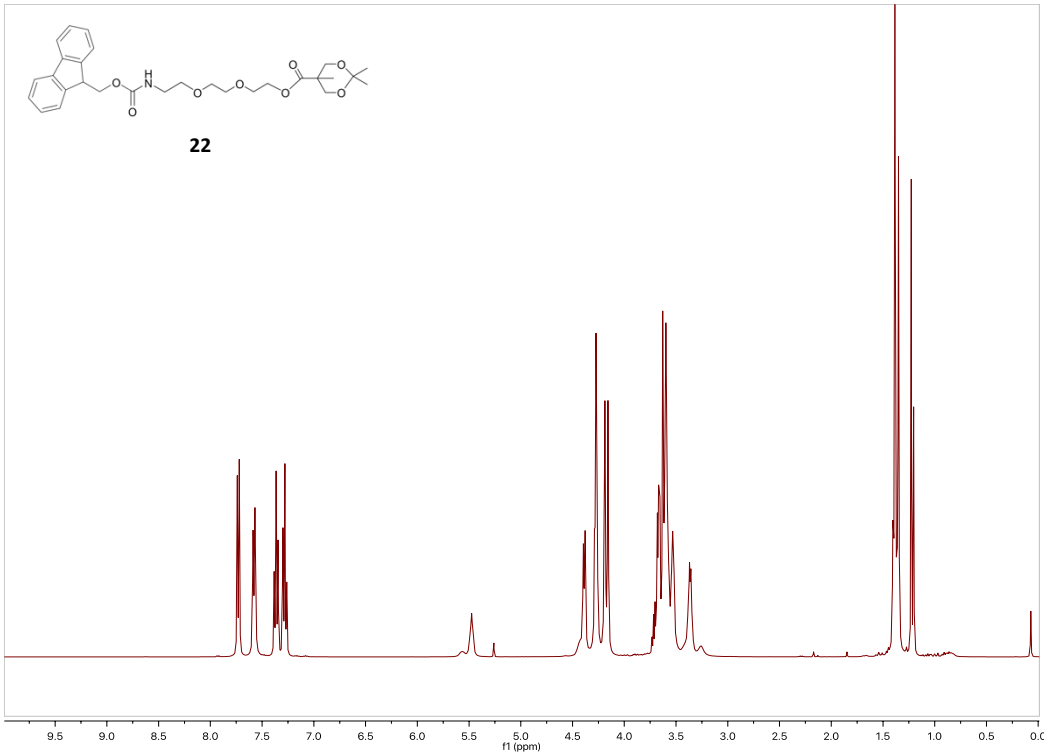
MALDI-TOF

MS



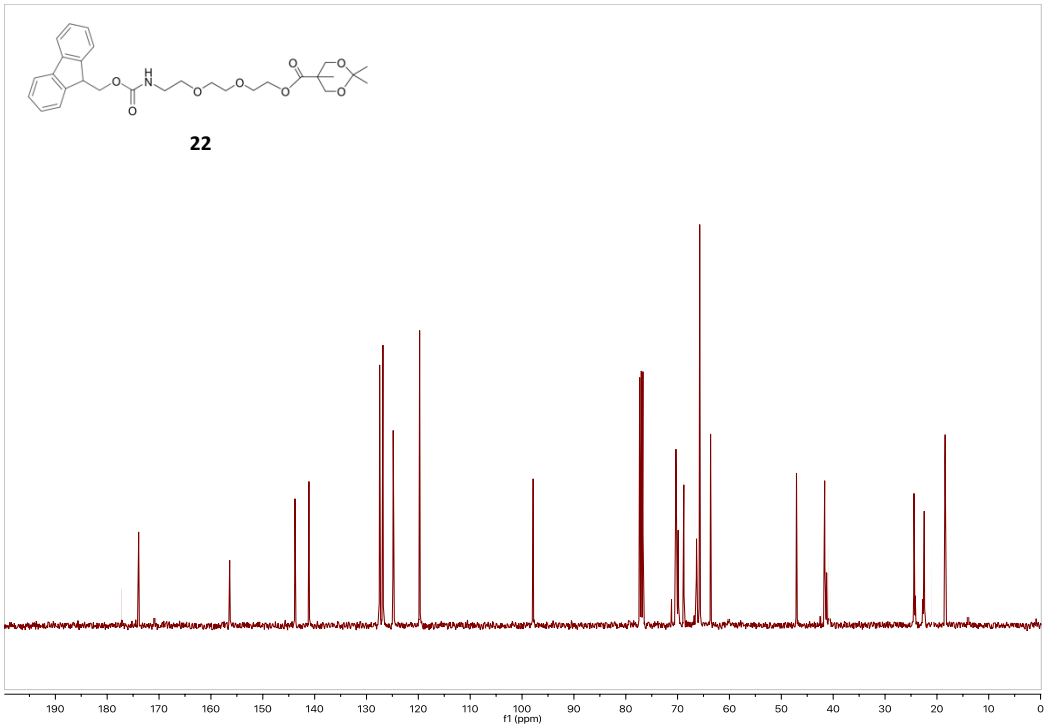
21

MALDI-TOF spectrum of compound 21

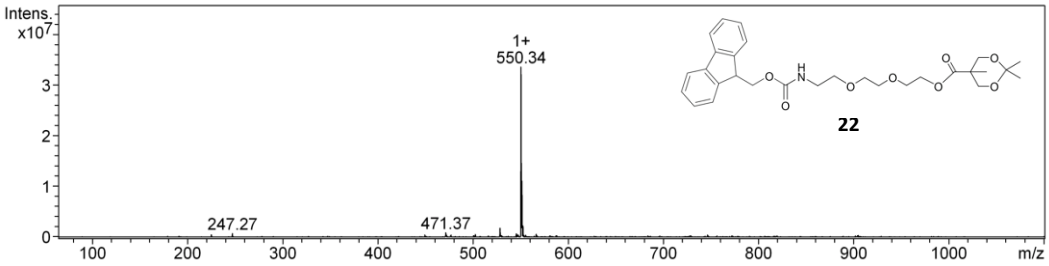


22

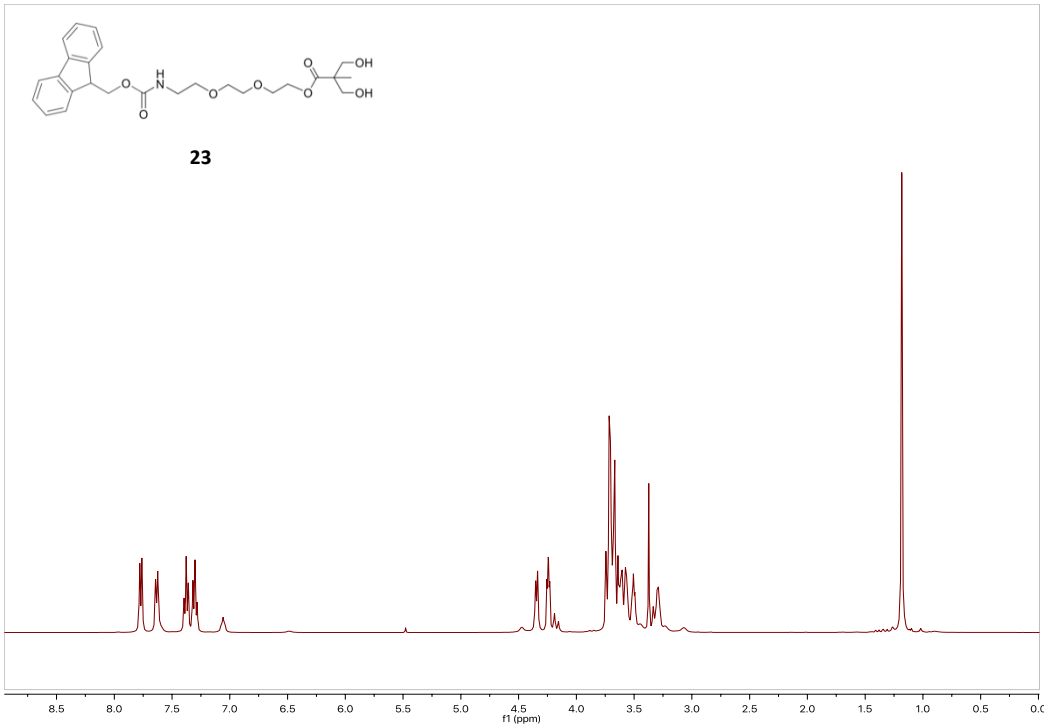
^{1}H-NMR (400 MHz, $CDCl_3$): compound 22



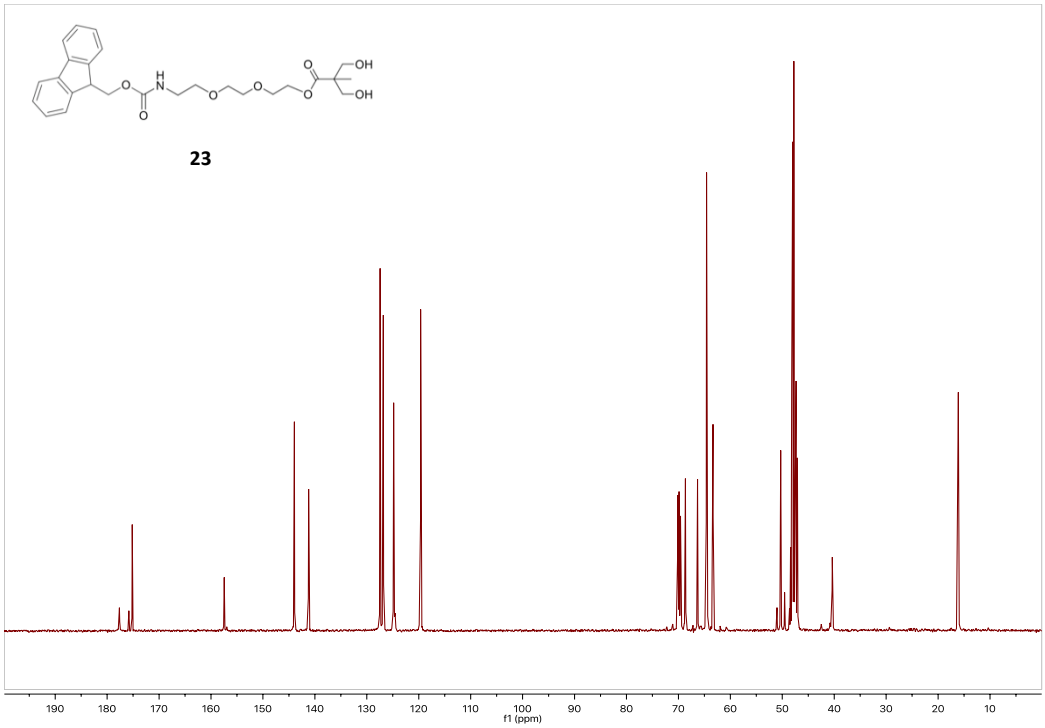
^{13}C-NMR (101 MHz, $CDCl_3$): compound 22



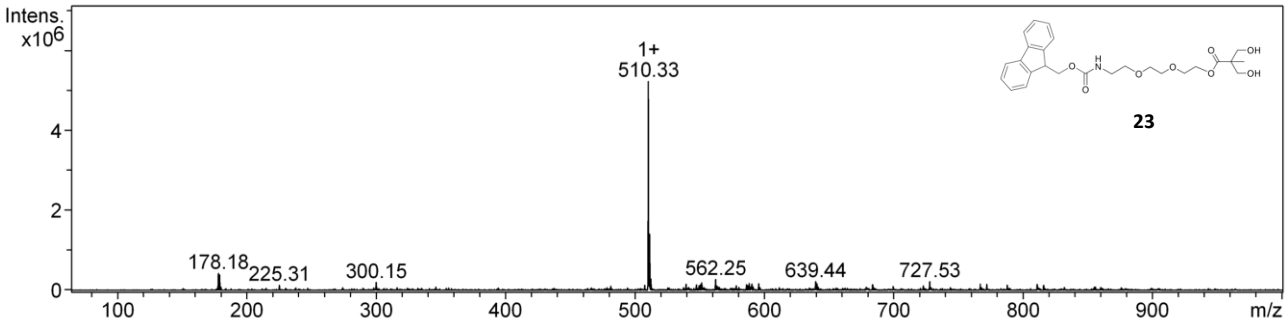
ESI-MS spectrum of compound 22



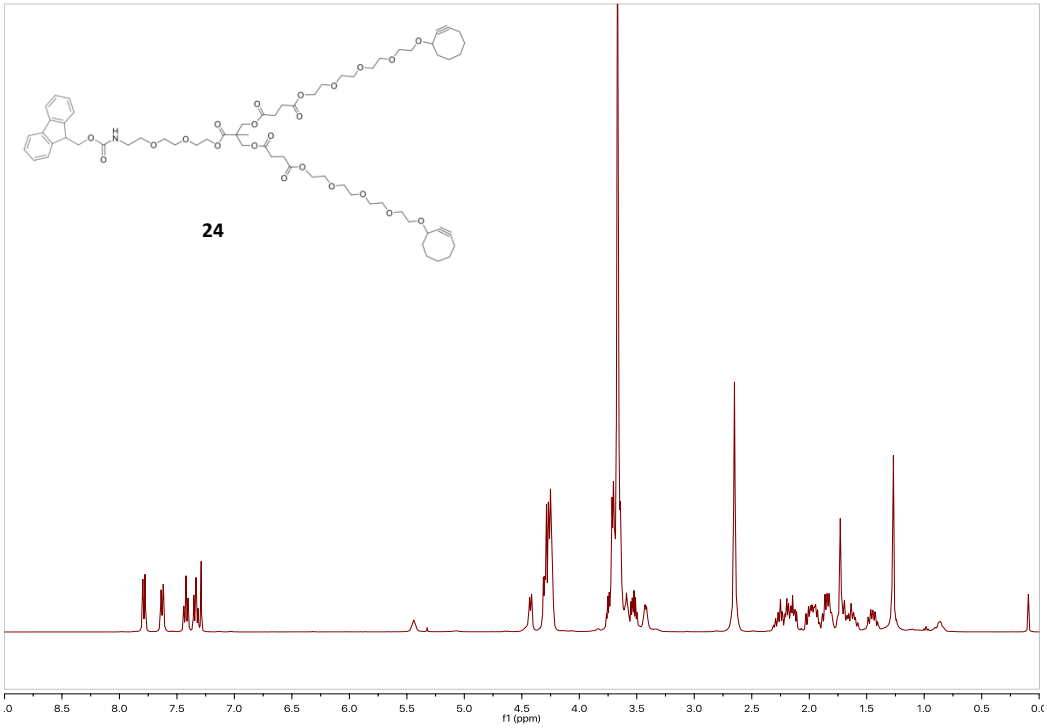
^{1}H-NMR (400 MHz, MeOH –*d4*): compound 23



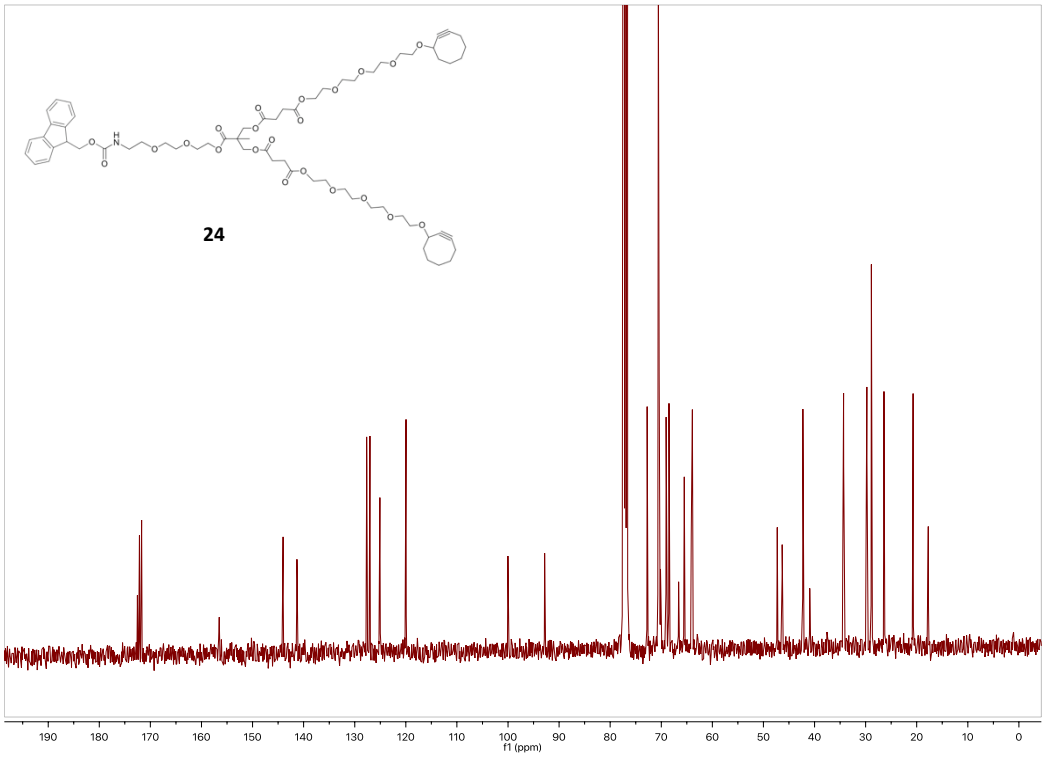
^{13}C-NMR (101 MHz, MeOH –*d4*): compound 23



ESI-MS spectrum of compound 23

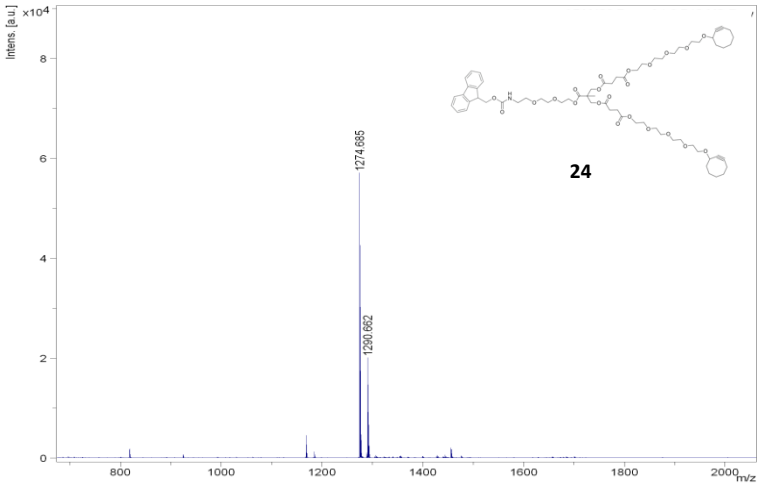


^{1}H-NMR (400 MHz, $CDCl_3$): compound 24

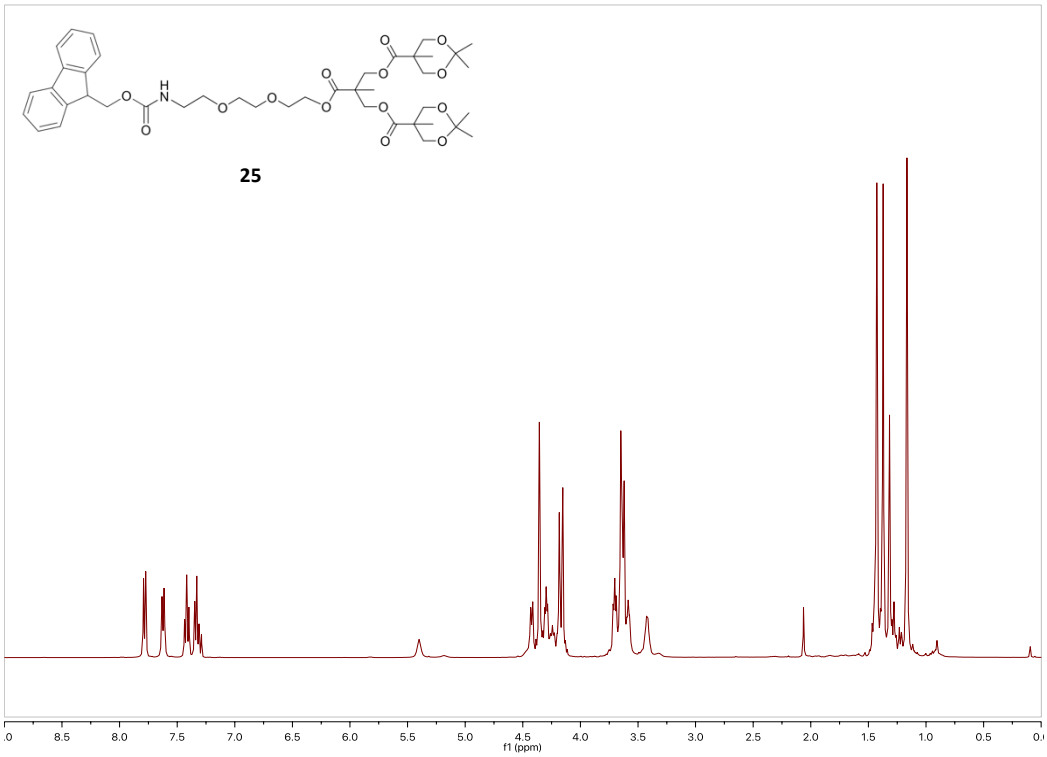


^{13}C-NMR (101 MHz, $CDCl_3$): compound 24

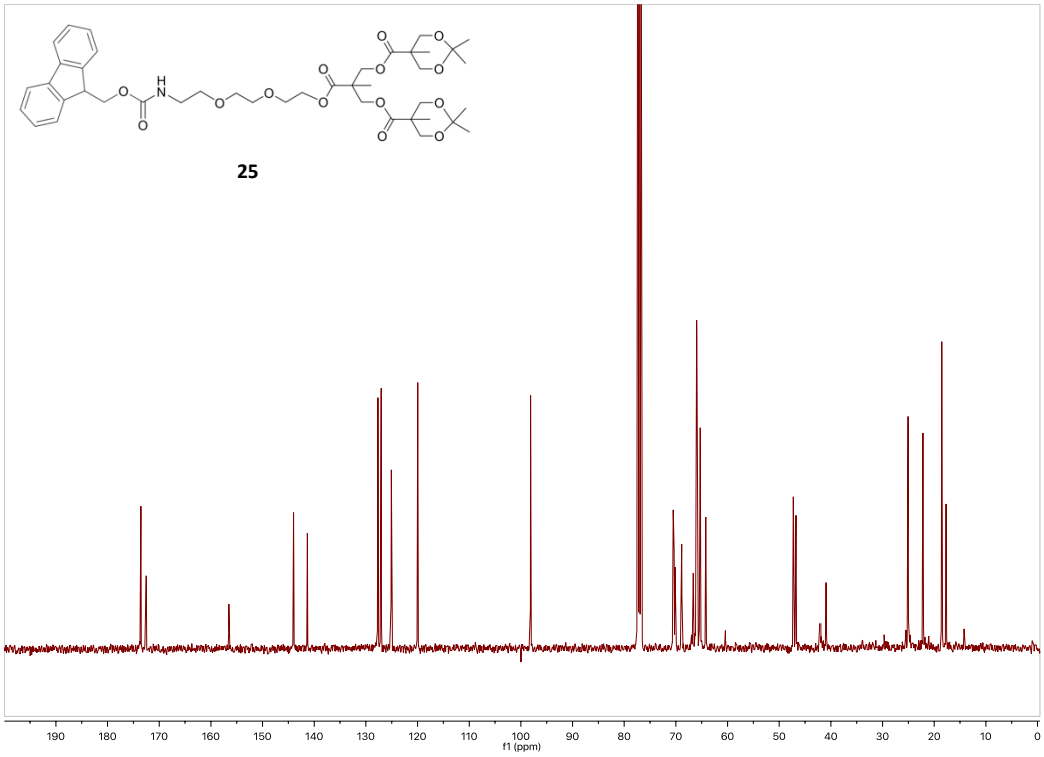
MALDI-TOF MS



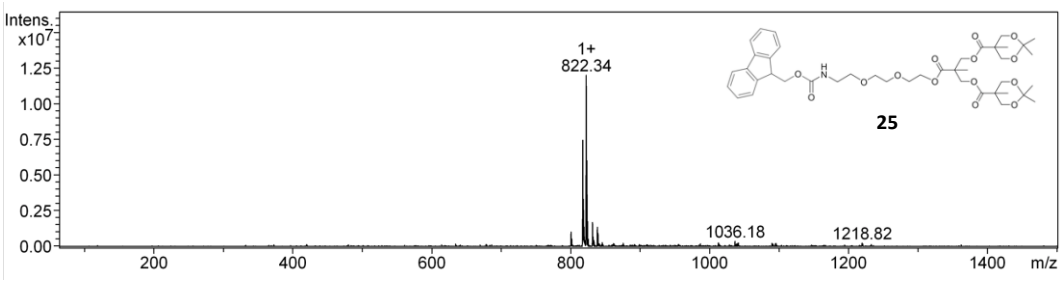
MALDI-TOF spectrum of compound 24



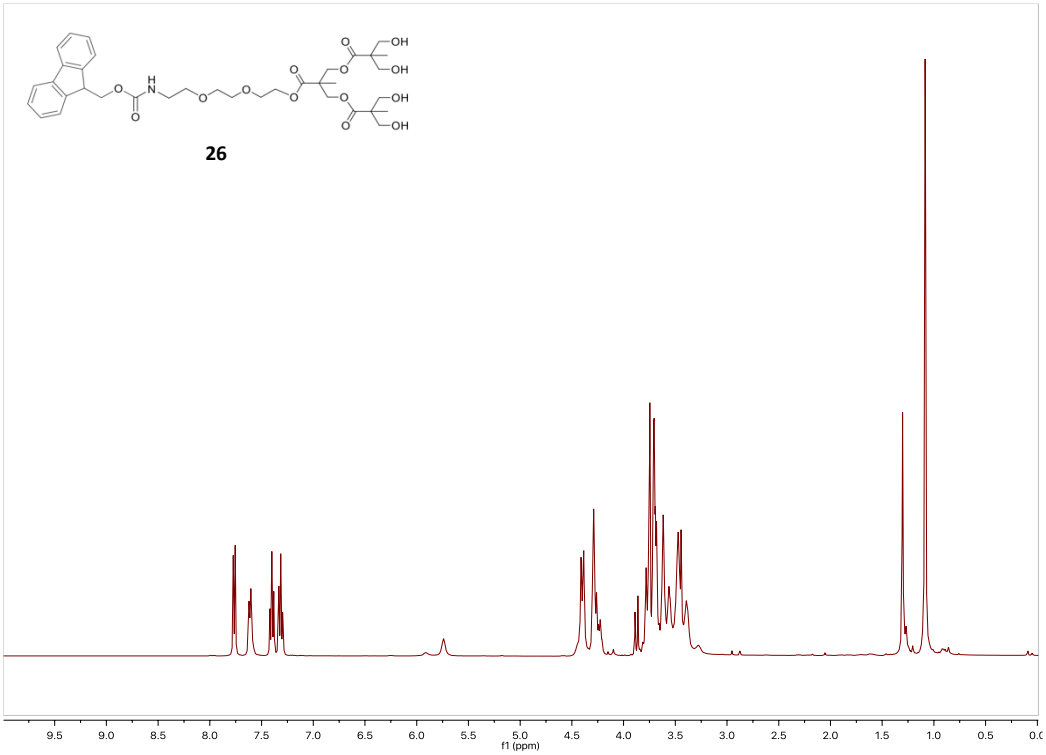
^{1}H-NMR (400 MHz, $CDCl_3$): compound 25



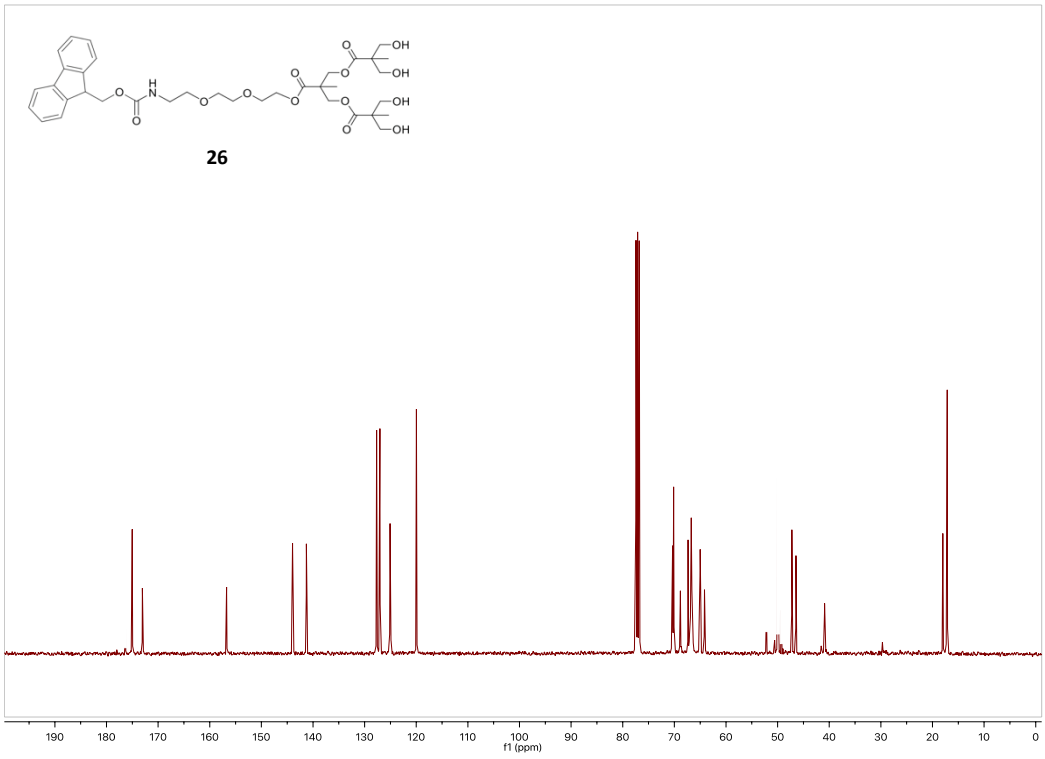
^{13}C-NMR (101 MHz, $CDCl_3$): compound 25



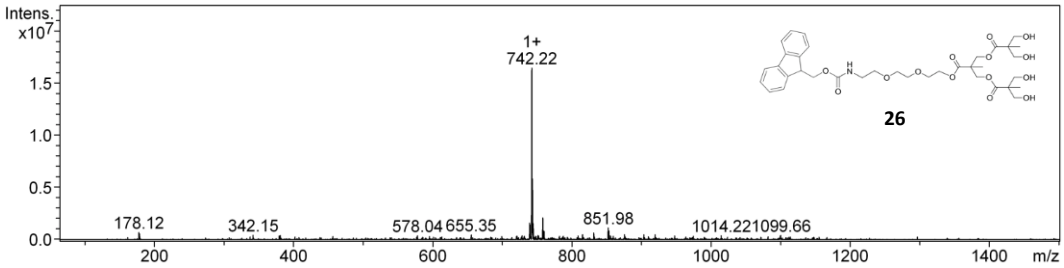
ESI-MS spectrum of compound 25



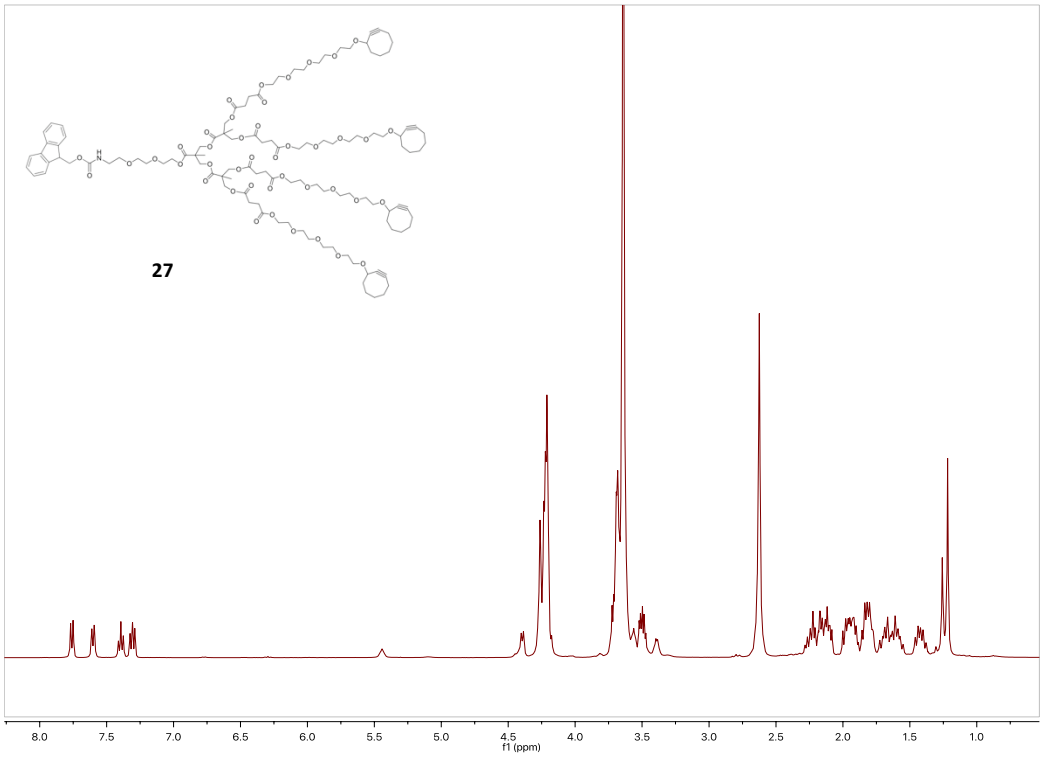
^{1}H-NMR (400 MHz, $CDCl_3$): compound 26



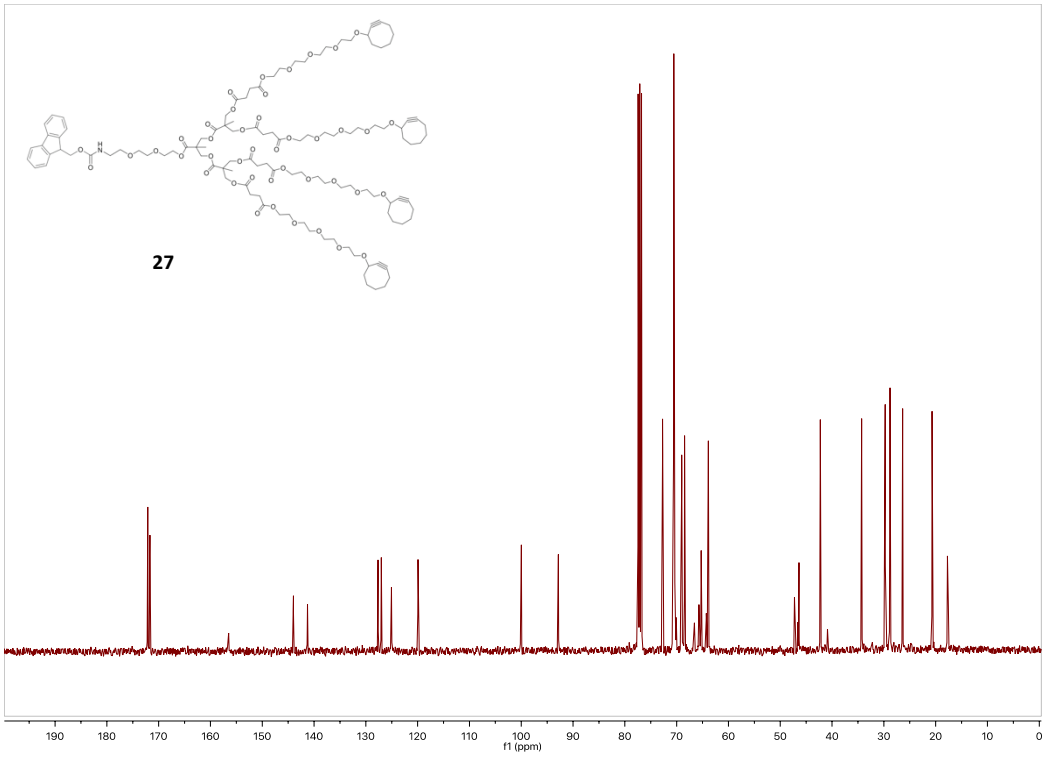
^{13}C-NMR (101 MHz, $CDCl_3$): compound 26



ESI-MS spectrum of compound 26

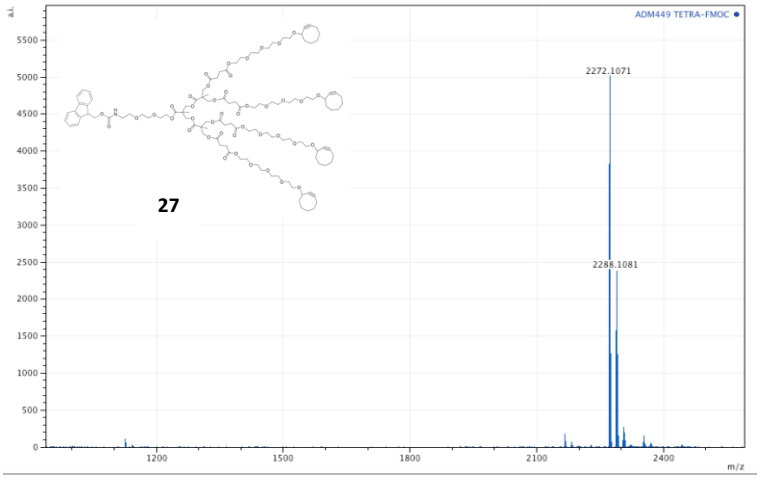


^{1}H-NMR (400 MHz, $CDCl_3$): compound 27



^{13}C-NMR (101 MHz, $CDCl_3$): compound 27

MALDI-TOF MS



MALDI-TOF spectrum of compound 27

2. On-Chip synthesis of glycodendrons via SPAAC and binding assays with fluorescently labeled lectins

The general strategy for the on-chip synthesis of glycondendrons consists to first functionalize the commercial ITO-coated glass slide with an octadecylphosphonic chain for the following hydrophobic immobilization of NHS-activated linker (**Figure S1a**). After that, cyclooctyne dendrons at different valences are immobilized on the surface via amine-based covalent bond (**Figure S1b**) and several glycodendrons are then prepared via spotwise SPAAC reaction with a selection of azide-carbohydrates (**Figure S1c**). The binding properties of the newly synthesized glycodendrons are tested against several types of fluorescently labeled lectins (**Figure S1d**).

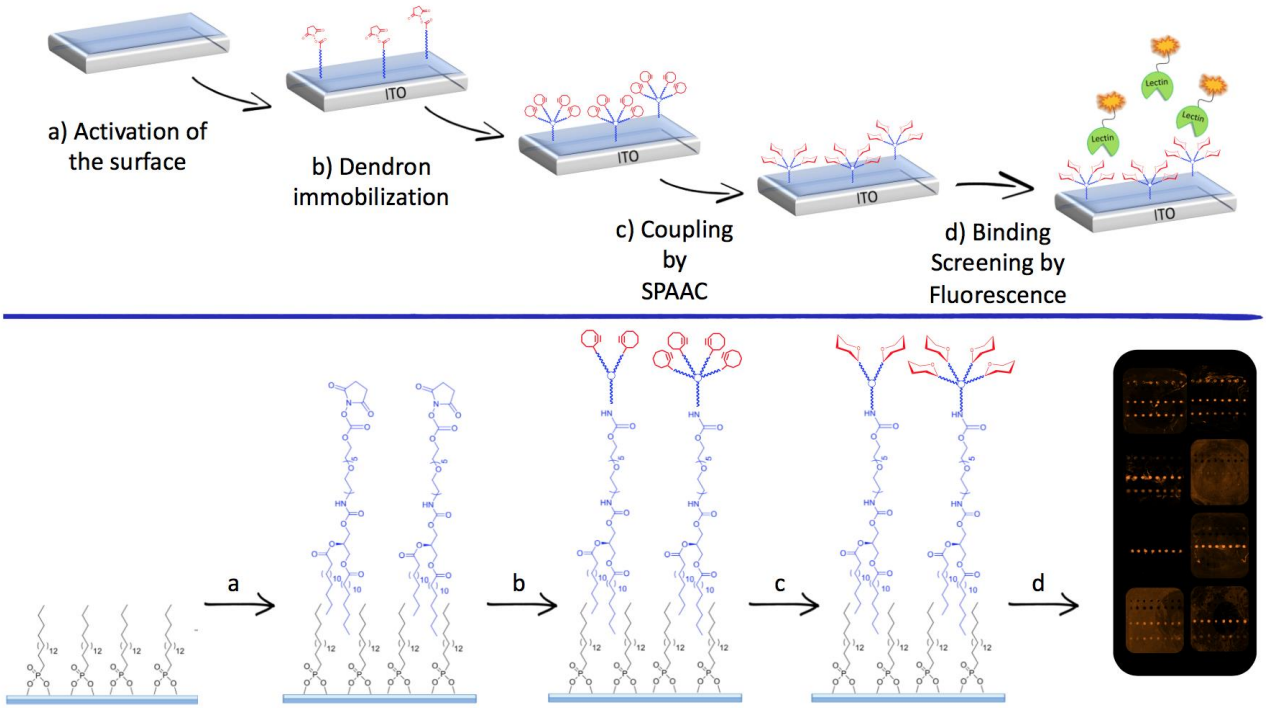


Figure S1. Schematic representation of the direct on-chip approach for the preparation of glycodendrons at different valences.

2.1 Preparation of NHS-activated hydrophobic ITO-coated glass slides

The NHS-activated hydrophobic ITO-coated glass slides were prepared according to a procedure in reference.[5]

2.2 Immobilization of Cyclooctyne-dendrons (1-3) onto NHS-activated hydrophobic ITO-glass slide

The NHS-activated hydrophobic ITO-coated glass slide was physically divided in subarrays by using *16-well proplate® Module/6x7mm*; each subarray was incubated with aqueous solution containing: 60 mM of phosphate buffer pH=7.8 and the desired cyclooctyne-dendron (**1**, **2** or **3**).

[5] A. Cioce, M. Thépaut, F. Fieschi, N. C. Reichardt, *Chem. Eur. J.*, 2020, doi:10.1002/chem.202000026

To perform density quantification studies, different coating concentrations of cyclooctyne-dendrons have been employed (5 µM, 10 µM, 20 µM, 30 µM) while, for all the other assays, a concentration of 5µM has been selected.

The reaction was left 2h at r.t. and washed with aqueous solution containing 0.1%TFA and 0.05% AcCN; after that, the unreacted NHS-surface was quenched with 50 mM ethanolamine solution by 20 min incubation at r.t.

Substrate	Theoretical m/z $[M+Na]^+$
NHS-linker	1049,3
1	1456,5
2	1954,2
3	2951,7

Table S1 Theoretical ion $[M+Na]^+$ mass peaks of NHS-linker and immobilized Cyclooctynes-dendrons (**1-3**).

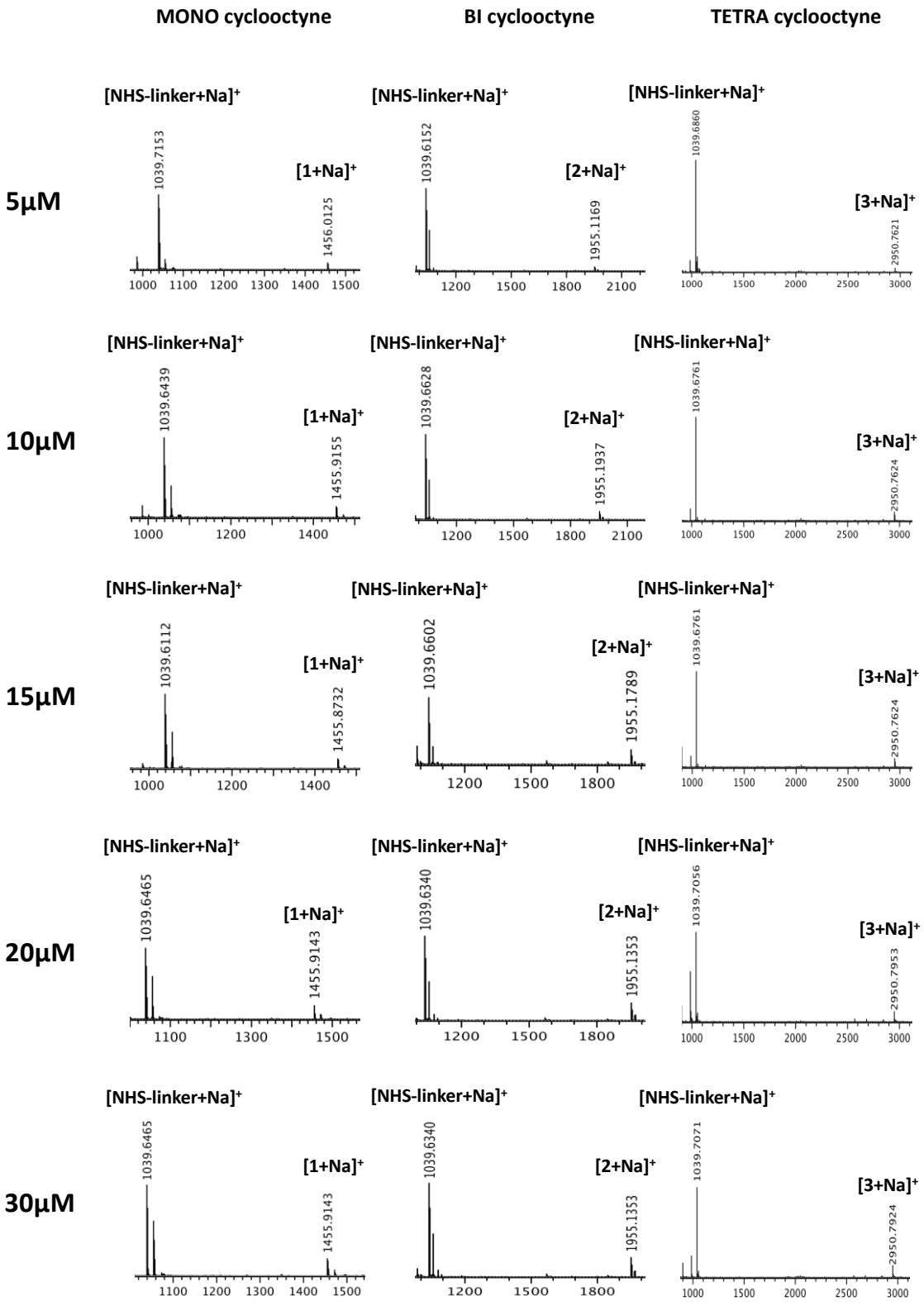


Figure S2 MALDI-TOF MS analysis of immobilized cyclooctyne-dendrons (**1-3**) by adopting different coating concentrations

2.3 Surface characterization by contact angle (Θ)

After modifying the NHS-hydrophobic ITO-coated glass surface with cyclooctyne-dendrons, the contact angle was measured by placing a droplet of water on the surface by *Drop Shape analyzer-DSA100*; by immobilization of cyclooctyne dendrons (**1-3**) onto NHS-surface, no significant changes in term of surface hydrophobicity have been observed (Θ= 74°-84°).

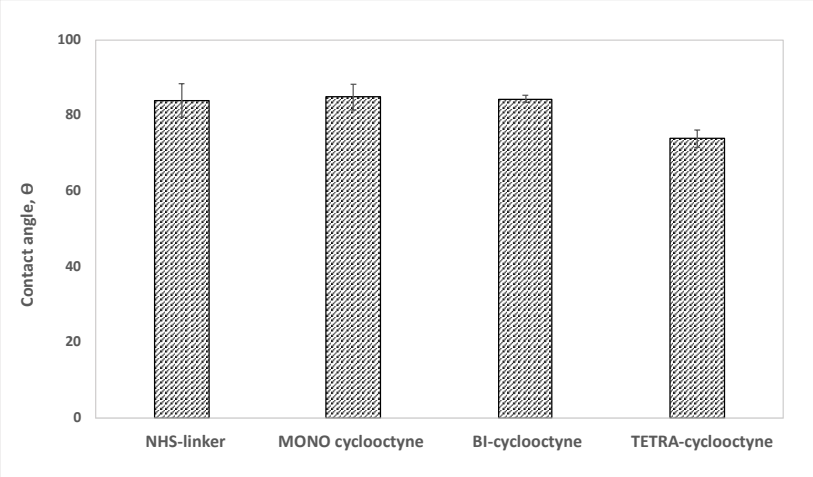


Figure S3 Comparison of contact angle values of hydrophobic ITO-glass surface before and after cyclooctyne immobilization.

2.4 On-chip Strain-promoted alkyne azido cycloaddition (SPAAC) by using azidoethyl carbohydrates (4-7)

The on-chip SPAAC was performed by robotic dispensing, via *automated non-contact dispensing system of ultra-low volumes (sciFLEXARRAYER)*, 5mM solution (10% DMSO in water) of azidoethyl glycosides (**4-7**, 20 drops, 5.12 nL) on cyclooctyne surfaces (**1-3**); for statistical studies, 8 replicates for each carbohydrate azide were printed. The slide was placed 2h at 19°C under controlled humidity of 70% and then dried at 40°C for 16h; after that, it was washed with nanowater and dried under stream of air. DHB matrix, containing 0.01 mM of sodium citrate, was spotted on each spot to quantify reaction conversion by MALDI-TOF analysis. Almost complete conversion (>95%) was achieved for all azidoethyl glycosides (**4-7**) on all cyclooctyne surfaces (**1-3**) (**Table S2**).

	Substrate	Theoretical m/z $[M+Na]^+$	Percentage of conversion
MONO	**8**	1705,6	98%
	11	1705,6	96%
	14	1867,7	95%
	17	1705,6	98%
BI	**9(1)**	2203,3	1%
	9(2)	2452,4	99%
	12(1)	2203,3	2%
	12(2)	2452,4	98%
	15(1)	2365,7	3%
	15(2)	2777,2	97%
	18(1)	2203,3	2%
	18(2)	2452,4	98%
TETRA	**10(1)**	3200,8	nd
	10(2)	3449,9	nd
	10(3)	3699	5%
	10(4)	3948,1	95%
	13(1)	3200,8	nd
	13(2)	3449,9	nd
	13(3)	3699	4%
	13(4)	3948,1	95%
	16(1)	3362,85	nd
	16(2)	3774	nd
	16(3)	4185,15	3%
	16(4)	4596,3	97%
	19(1)	3200,8	nd
	19(2)	3449,9	nd
	19(3)	3699	3%
	19(4)	3948,1	97%

Table S2 SPAAC conversion achieved on the three cyclooctyne-dendron surfaces **(1-3)** with azidoethyl glycosides **(4-7)**. (dx.y(1) =mono-coupled product; dx.y(2) =bi- coupled product; dx.y(3) =tri- coupled product; dx.y(4) =tetra- coupled product; nd= not detected).

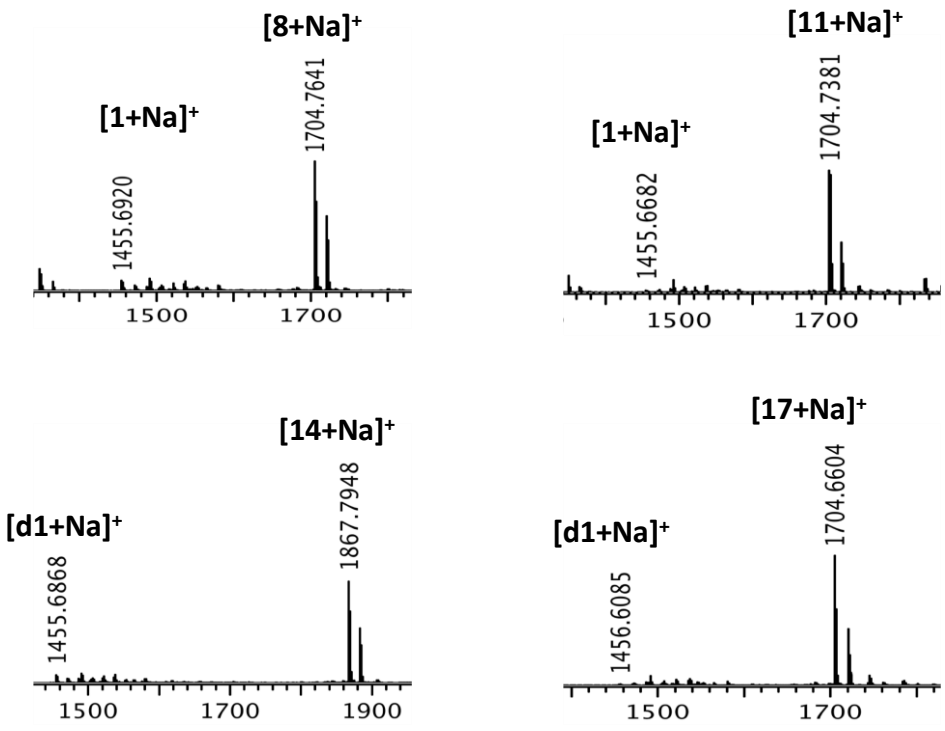


Figure S5 MALDI-TOF mass spectra detected after on-chip SPAAC with azidoethyl glucosides (**4-7**) on Mono-cyclooctyne functionalized surface **(1)**

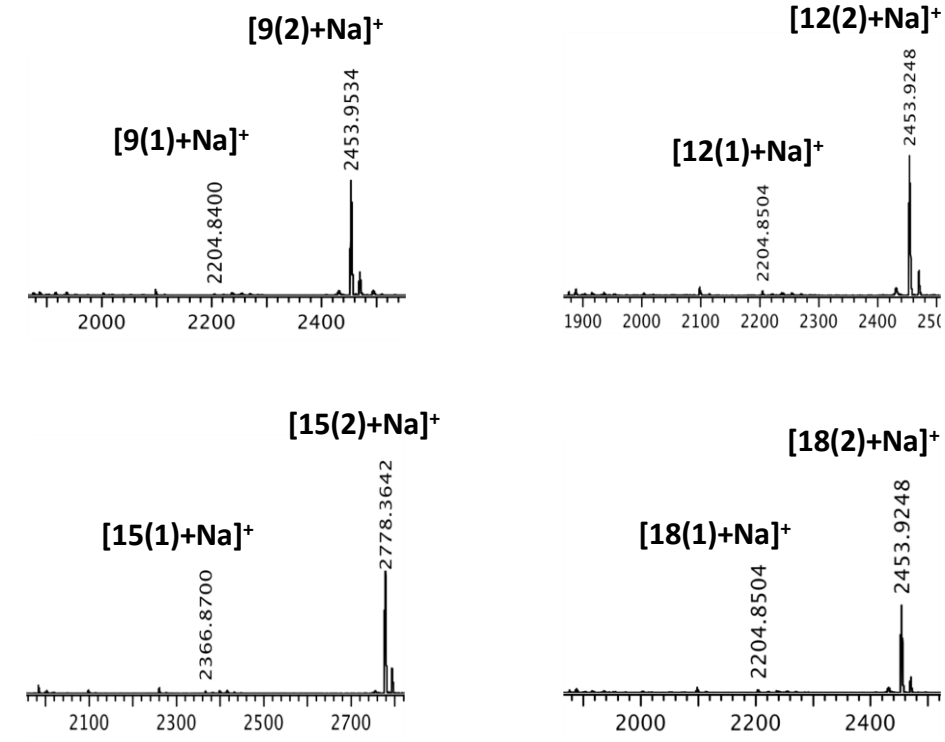


Figure S6 MALDI-TOF mass spectra detected after on-chip SPAAC with azidoethyl glucosides (**4-7**) on Bi-cyclooctyne functionalized surface **(2)**.

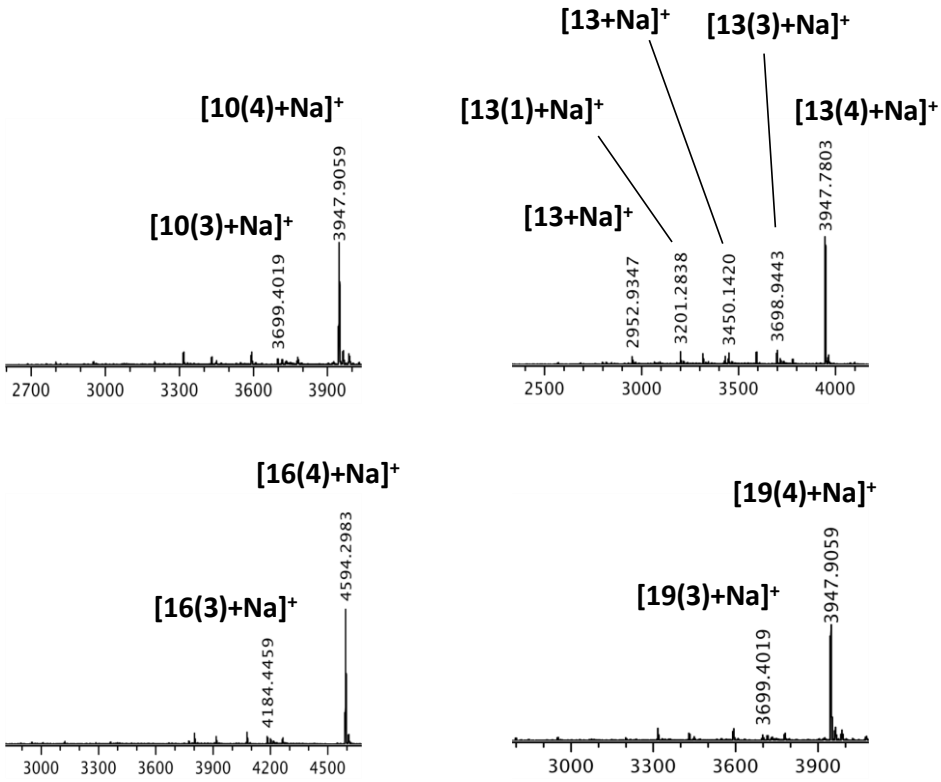


Figure S7 MALDI-TOF mass spectra detected after on-chip SPAAC with azidoethyl glucosides (**4-7**) on Tetra-cyclooctyne functionalized surface **(3)**.

2.5 On-chip binding assays of glycodendrons with fluorescently labelled lectins

Glycodendrons arrays were incubated, by using *16-well proplate® Module/6x7mm*, with fluorescently labelled lectins in binding buffer (Tris-HCl 100 mM, pH=7.5 with 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.2% BSA); the incubation was left 1h in the dark at r.t. for plant lectins as PSA, WFA and ConA or 1h at 4°C for human lectins as DC-SIGN ECD, DC-SIGNR ECD, Langerin ECD and Dectin-2 ECD. Arrays were washed with nanowater, dried and scanned with *Agilent G2565AA microarray scanner system* at 10 µm resolution.

Here are reported the fluorescent images of the glycodendrons arrays after incubation with the fluorescently labeled lectins.

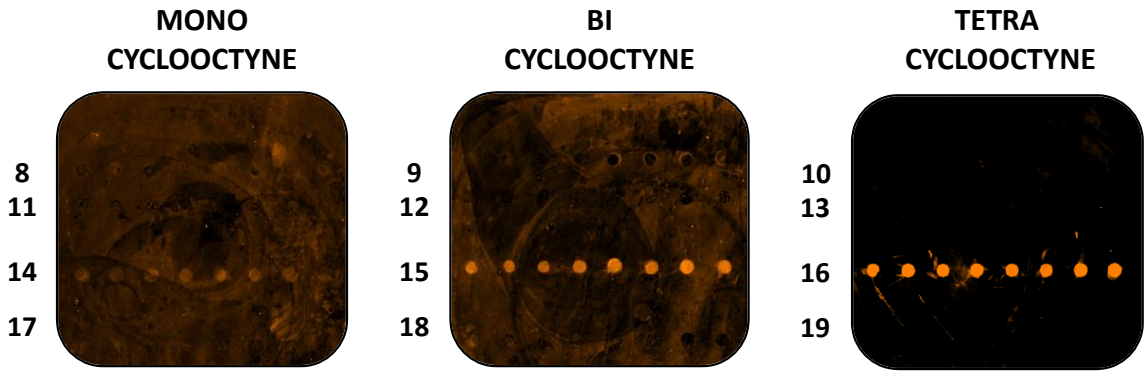


Figure S8 Fluorescence image of the neo-glycodendrons' arrays after incubation with *PSA-Alexafluor555* (50µg/mL, degree of labelling (DOL)L=0.37)

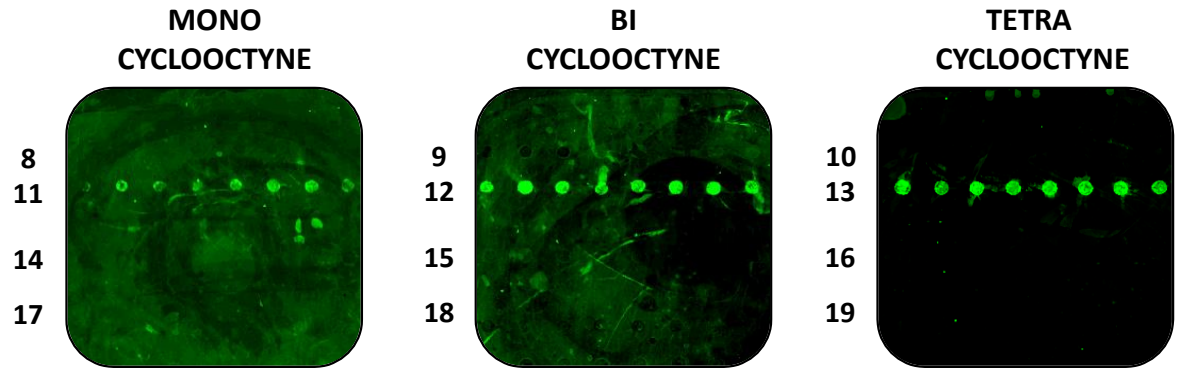


Figure S9 Fluorescence image of the neo-glycodendrons' arrays after incubation with *WFA-Alexafluor647* (50µg/mL, DOL= 0.71).

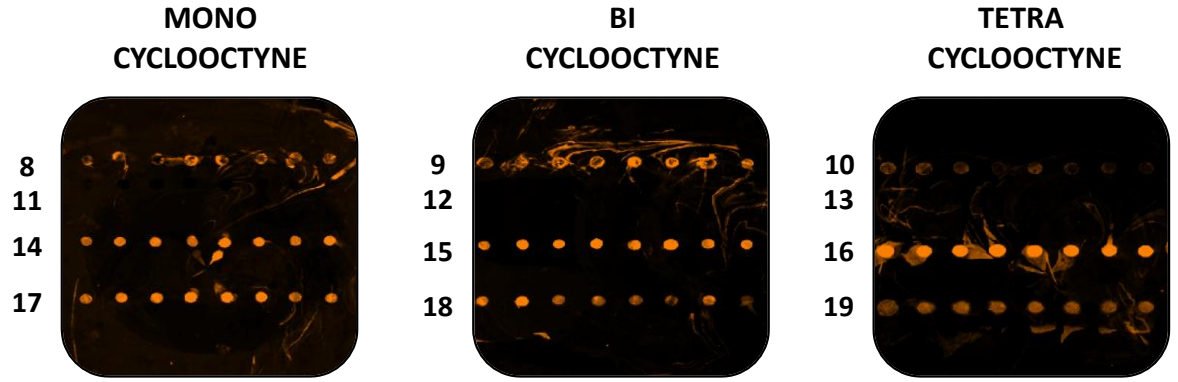


Figure S10 Fluorescence image of the neo-glycodendrons' arrays after incubation with *DC SIGN ECD-Cy3* (10µg/mL, DOL= 0.95).

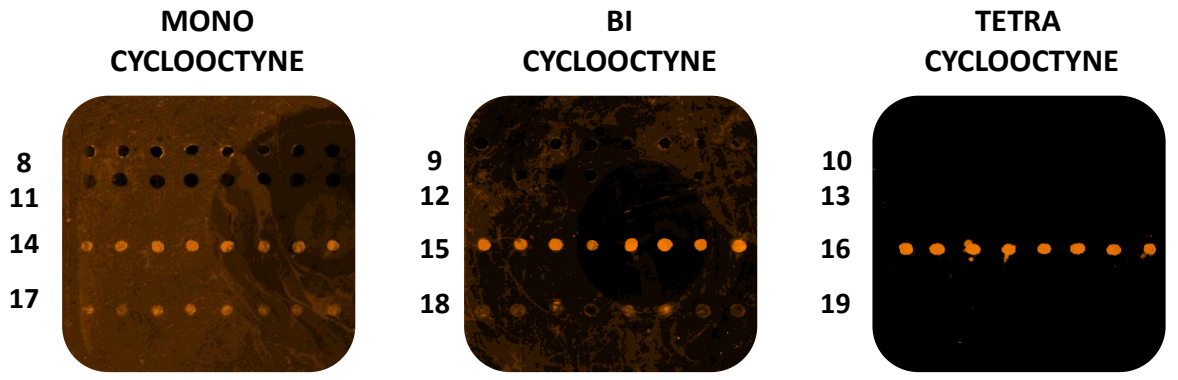


Figure S11 Fluorescence image of the neo-glycodendrons' arrays after incubation with *DCR ECD-Cy3* (10µg/mL, DOL= 0.40).

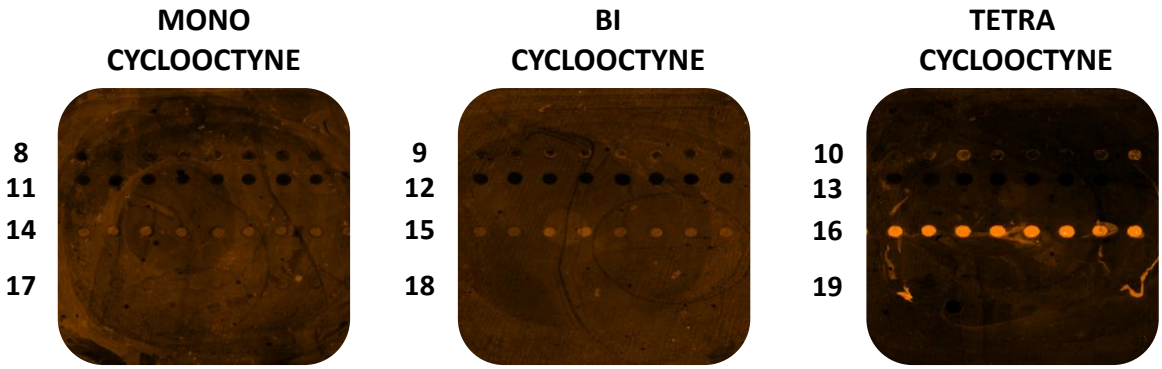


Figure S12 Fluorescence image of the neo-glycodendrons' arrays after incubation with *Langerin ECD-Cy3* (10µg/mL, DOL= 0.70).

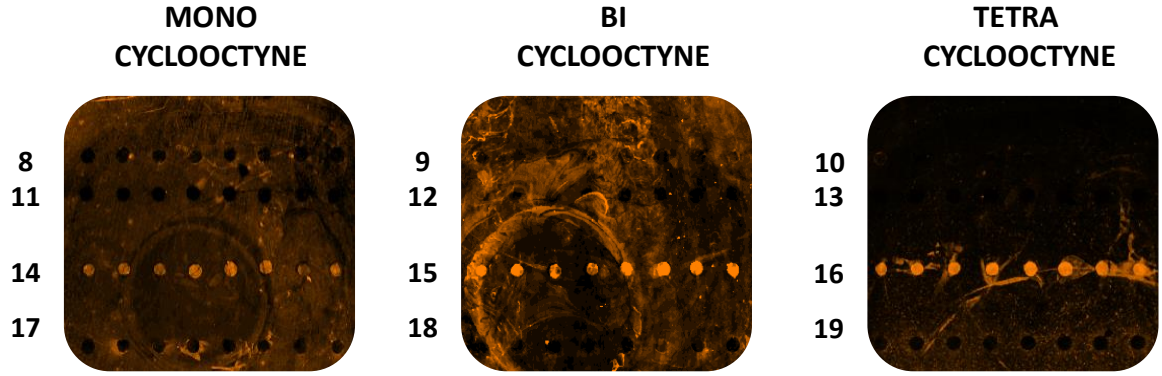


Figure S13 Fluorescence image of the neo-glycodendrons' arrays after incubation with *Dectin-2 ECD-Cy3* (10µg/mL, DOL= 0.40).

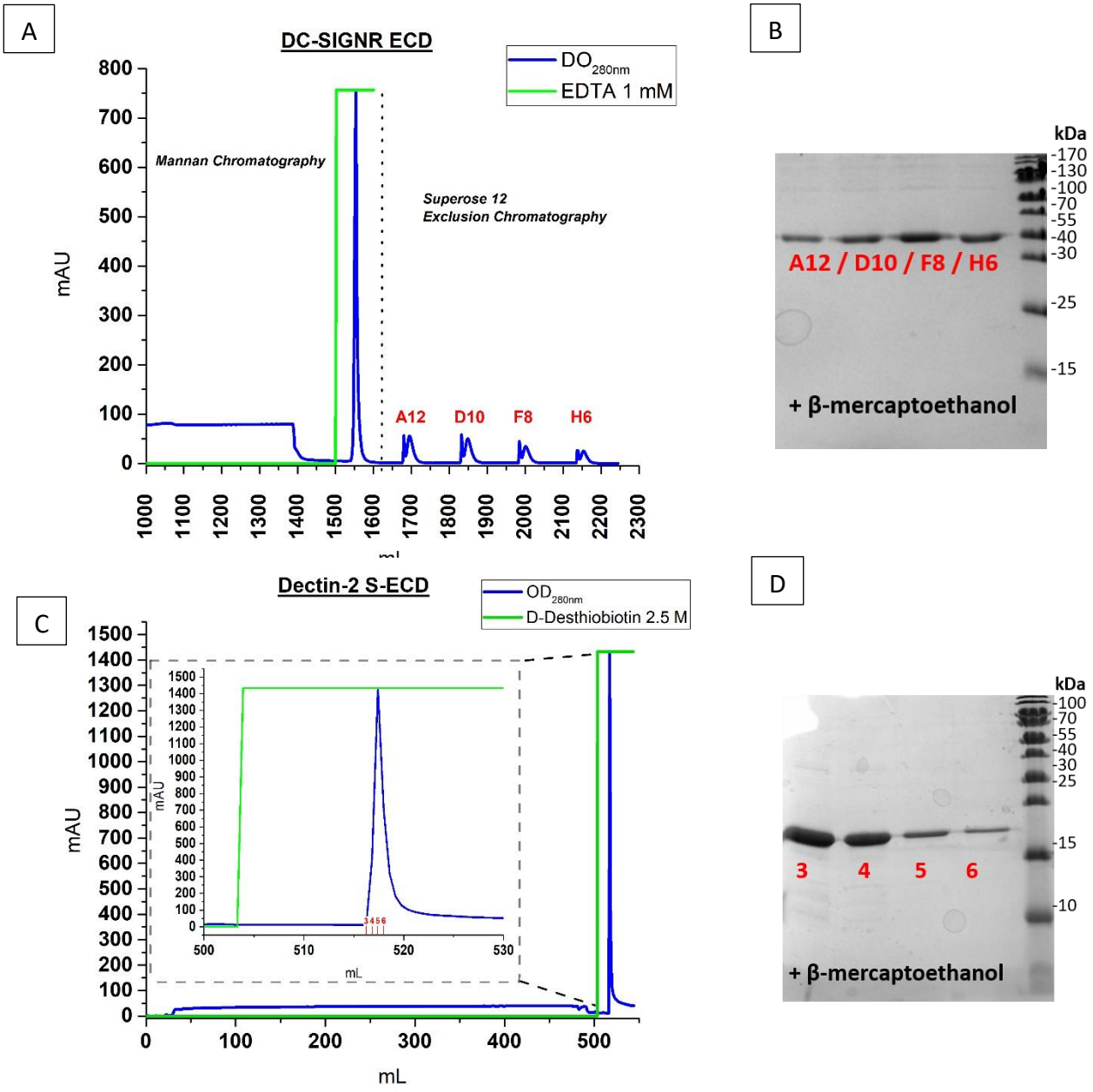
3. Production of C-Type Lectin Receptors

DC-SIGN extracellular domain (DC-SIGN ECD) and langerin extracellular domain (langerin ECD) constructs were produced and purified as previously described. [6,7]

DC-SIGNR ECD (amino acids 78-399) and Dectin-2 ECD (amino acids 42-209) were expressed in *E. coli* BL21(DE3) in 1 liter of LB medium supplemented with 50 µg/mL kanamycin at 37 °C. Expression was induced by addition of 1 mM isopropyl 1-thio-Dgalactopyranoside (IPTG) when the culture had reached an A600 nm of 0.8 and maintained for 3h. The protein was expressed in the cytoplasm as inclusion bodies. Cells were harvested by a 20-min centrifugation at 5000 g at 4 °C. The pellet was resuspended in 30 mL of a solution containing 150 mM NaCl, 25 mM Tris-HCl, pH 8 and one anti-protease mixture tablet (Complete EDTA free, Roche). Cells were disrupted by sonication and cell debris eliminated by centrifugation at 100,000 g for 45 min at 4 °C in a Beckman 45Ti rotor. The pellet was solubilized in 30 mL of 6 M guanidine-HCl containing 25 mM Tris-HCl pH 8, 150 mM NaCl and 0,01% β-mercaptoethanol. The mixture was centrifuged at 100,000g for 45 min at 4°C and the supernatant was diluted 5-fold, by slow addition with stirring, with 1.25 M NaCl, 25 mM CaCl2 and 25 mM or 200 mM Tris-HCl pH 8 for DC-SIGNR and dectin-2 ECD, respectively. The diluted mixture was dialyzed against 10 volumes of 25 mM Tris-HCl, pH 8, 150 mM NaCl, 4 mM CaCl2 (buffer A) with 3 buffer changes. After dialysis, insoluble precipitate was removed by centrifugation at 100,000g for 1h at 4°C. The supernatant containing DC-SIGNR ECD was loaded on Mannan agarose column (Sigma) for purification by affinity chromatography equilibrated with buffer A. After loading, DC-SIGNR ECD was tightly bound to the column and eluted in the same buffer without CaCl2 but supplemented with 1 mM EDTA (buffer B). This step was followed by SEC (Size Exclusion Chromatography) using a Superose 6 column (GE Heathcare) equilibrated with buffer A. Fractions were analyzed by SDS-PAGE (12%) and DC-SIGNR ECD containing fractions were pooled and concentrated by ultrafiltration (YM10 membrane from Amicon). The supernatant containing the Strep tagged dectin-2 ECD was loaded onto a StrepTrap HP column (GE Heathcare) at 4°C. Unbound proteins were washed away with buffer A before dectin- 2 ECD was eluted with buffer C (150 mM NaCl, 25 mM Tris-HCl, pH 8, 4 mM CaCl2, 2.5 mM D-desthiobiotin). Eluted fractions were analyzed by SDS-PAGE (15%) and dectin-2 ECD containing fractions were pooled and concentrated by ultrafiltration (YM10 membrane from Amicon).

[6] M. Thépaut, J. Valladeau, A. Nurisso, R. Kahn, B. Arnou, C. Vivès, S. Saeland, C. Ebel, C. Monnier, C. Dezutter-Dambuyant, A. Imberty, F. Fieschi, *Biochemistry*, 2009, **48**, 12, 2684–2698.

[7] G. Tabarani, M Thépaut, D Stroebel, C Ebel, C Vivés, P Vachette, D Durand, F Fieschi, *J. Biol. Chem*., 2009, **284**, 21229–21240.



FigureS14. A) Chromatogram of DC-SIGNR ECD purification on a Mannan-Agarose column coupled to four consecutive Superose 12 columns and B) the SDS-PAGE analysis of DC-SIGNR ECD purification (37.28 kDa) in reductive (+ β-mercaptoethanol) conditions. C) Chromatogram of Dectin-2 Strep-ECD purification on a StrepTactin column and D) the SDS-PAGE analysis of Dectin-2 Strep-ECD purification (21.4 kDa) in reductive (+ β-mercaptoethanol) conditions.