Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2018

Electronic Supplementary Information

A Toolbox Approach for Multivalent Presentation of Ligand-Receptor Recognition on a Supramolecular Scaffold

Svenja Ehrmann,[a,d†] Chih-Wei Chu,[b†] Shalini Kumari,[a†] Kim Silberreis,[c] Christoph Böttcher,[d] Jens Dernedde,[c] Bart Jan Ravoo,[*b] and Rainer Haag[*a]

[a] *Institute for Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany. *Email: haag@chemie.fu-berlin.de*
[b] *Organic Chemistry Institute and Center for Soft Nanoscience, Westfälische Wilhelms-Universität Münster, Corrensstr. 40, 48149 Münster, Germany. *Email: b.j.ravoo@uni-muenster.de*
[c] *Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health; Institute of Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, CVK Augustenburger Platz 1, 13353 Berlin, Germany*
[d] *Research Center of Electron Microscopy, Freie Universität Berlin, Fabeckstr. 36a, 14195 Berlin, Germany*
[†] *The authors contributed equally.*

Table of contents

1. Methods and Materials

Synthesis. All reagents and solvents were obtained from commercial suppliers and used without further purification unless stated otherwise. Reactions requiring dry and oxygen-free conditions were carried out in oven-dried glassware with septa using usual Schlenk techniques. Millipore water (Milli-Q) was obtained from a Merck Millipore Milli-Q Integral System. NMR spectra were recorded on JEOL ECX400, JEOL ECP500, BRUKER AV500 and BRUKER AV700 spectrometers at 400 MHz, 500 MHz and 700 MHz for ^{1}H NMR spectra and 100 MHz, 125 MHz and 175 MHz for ^{13}C NMR spectra, respectively. Chemical shifts are given in parts per million (ppm) in relation to deuterated solvent peak calibration.

Flash columns were either performed with Macherey-Nagel silica gel 60 M or at a Combiflash R_f system from Teledyne ISCO. HPLC purification was conducted using a Puriflash PF-50C18HP RP-cartridge (20 g) from Interchim (France) and UV detection (254 nm). Ultrafiltration was carried out under 5 bar N_2, using cellulose membranes (MWCO = 1000 Da).

Surface Plasmon Resonance (SPR). Experiments were performed on a Biacore X100 instrument (GE Healthcare Europe, Freiburg, Germany) at 25 °C, using HBS-Ca-Mn buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 0.1 mM $CaCl_2$ and 0.01 mM $MnCl_2$) in all cases. Biotinylated ConA (Vector Labs, Biozol, Eching, Germany) was immobilised on a streptavidin sensor chip (GE Healthcare, final response 2600 RU), whereas the reference lane was left unfunctionalised. Each cycle consisted of a 120 s period of sample contact time (association phase) followed by a 600 s dissociation phase. The regeneration was achieved in two steps (regeneration buffer 1) 800 mM methyl-α-D-mannopyranoside (60 s), buffer 2 10 mM MES (pH 6.1 + 1 mM $CaCl_2$ + 1 mM $MnCl_2$). β-CD (10 mM) was added to the running buffer during the measurements of mannose-adamantyl conjugates to ConA to shield the adamantyl moiety efficiently from unspecific binding to the dextran layer of the sensor chip. For the measurements of the ligand-decorated vesicles, γ-CD (10 mM) was added as a surfactant. All sample measurements were analysed with single cycle kinetics. Therefore, a concentration series of each sample was measured in triplicates. The determination of K_D values was performed with response unit (RU) data points taken at 15 s before injection stop using built-in software of the Biacore X100. Corresponding binding isotherms were plotted.

L-selectin binding measurements using label-free microscale thermophoresis (MST). Label-free microscale thermophoresis was used to measure the binding interactions between L-selectin and adamantane-coupled sulphated dendrons in the presence and absence of cyclodextrin vesicles according to the following protocol. For each measurement, a dilution series with constant L–selectin concentration but varying ligand concentrations was prepared in PBS. No significant ligand-derived autofluorescence was detected at 280 nm wavelength. The final L–selectin concentration was 100 μM. Approximately 1.5 μL of each sample was loaded in a premium capillary. All measurements were performed at 22 °C. The thermophoretic movement of fluorescent L-selectin was monitored with a laser on for 30 s and off for 5 s keeping the MST power at 40% and LED power at 20%. Fluorescence was measured before laser heating ($F_{Initial}$) and after 30 s of laser irradiation (F_{Hot}). The K_D values were then calculated from three independent thermophoresis measurements using the NanoTemper software (NanoTemper Technologies, Munich, Germany).

Cryogenic transmission electron microscopy (Cryo-TEM). *Cryo-sample preparation:* Droplets (5 μL) of sample solution were placed on hydrophilised holey carbon-filmed grids (Quantifoil R1/4) at room temperature. The grids were surface plasma treated just prior to use (BALTEC MED 020 device at 8.5 mA for 60 s). Vitrified films were prepared in a 'Vitrobot' (PC controlled vitrification robot, FEI) at 22 °C and a humidity of 100%. Excess sample was removed by blotting using two filter papers for

3.5 s and the thin film thus formed was shot (acceleration about 3 g) into liquid ethane just above its freezing point.

Cryo-TEM: The vitrified samples were transferred under liquid nitrogen into a Talos Arctica transmission electron microscope (FEI, Thermo Fisher), using the microscope´s autoloader protocol. Micrographs were recorded at a sample temperature of around 100 K using the microscope's low-dose protocol at a primary magnification of 28000x and an acceleration voltage of 200 kV. Image recording was done using a falcon direct electron detector (FEI Company, Oregon, USA). The defocus was chosen to be 6.5 µm in all cases to create enough phase contrast.

Dynamic Light Scattering (DLS). DLS measurements were performed on a Malvern Zetasizer Nano in quartz suprasil or disposable UV micro cuvettes (Brand). Samples were prepared in Milli-Q, PBS or HEPES buffer and filtered through a 0.45 µm membrane prior to the measurement.

Optical density (OD_{400}). The aggregation of the guest decorated CDV and ConA were analysed by optical density measurements at λ = 400 nm (OD_{400}) using a V-650 double-beam spectrophotometer with a PAC-743 automatic 6-position cell block. Samples were prepared in disposable PMMA cuvettes with a path length of 1 cm and sample volume of 1 mL. The spectra were plotted and analysed using the software spectra analysis. Typically, 1 mL of a 20 µM CDV solution in HEPES buffer (pH 7.4, 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MnCl_2$) was put in a cuvette. After an equilibration period of 5 min, 10 µL of the ligand solution (stock solutions of 1 mM for Ad_1-systems and 0.5 mM for Ad_2-systems) were added and gently mixed. The artificial glycocalyx was then formed within another 5 min. After the addition of 10 µL of ConA (10 mg/mL from stock solution) the agglutination occurred immediately and the solution turned turbid.

Isothermal titration calorimetry (ITC). ITC was carried out from a TA Instruments Nano ITC Low Volume with a cell volume of 170 µL using ITCRun version 2.1.7.0 Firmware version 1.31. All titrations were performed by using a 50 µL syringe and 20 injections of 2.5 µL at a temperature of 25 °C. A stirring rate of 350 rpm was applied while titrating the CD to the ligands solution. All samples were dissolved in distilled deionised water (ddH_2O). All data were corrected by subtraction of a blank titration of CD into pure water before analysis.

Cell experiments – bacteria detaching assay. The potential of detaching uropathogenic bacteria of human urinary bladder epithelial cells using functionalised synthetic structures was analysed in a bacterium detaching assay. Therefore, functionalised cyclodextrin vesicles (CDV) with different two-adamantane ligands, including mono-, tri- and octavalent mannose were investigated. As a control the inactive ligand Ad-TEG-OH was used. To reach a half and a full coverage of the CDV, the adamantane ligands were set in a 100-fold higher concentration than the CDV in ratios between 1:2 for one-adamantane systems and 1:4 in two-adamantane systems.

The human uroepithelial RT-4 cells (DSMZ-German collection of microorganisms and cell cultures, Braunschweig, Germany) were seeded in a 24-well plate (TPP) at a cell density of 70,000 cells/cm^2 in RPMI 1640 (Gibco, Darmstadt, Germany), 10% FCS (Biochrom, Berlin, Germany) and 1% penicillin/streptavidin (Biochrom, Berlin, Germany). An incubation overnight (37 °C, 5% CO_2) allowed the cells to adhere. The uropathogenic *Escherichia coli* (*E. coli*) strain 178 was grown in LB-medium with 10 µg/ml tetracycline until the culture reached an optical density at a wavelength of 600 nm (OD_{600}) of 1, measured by using a spectrophotometer (Ultrospec 300 by Pharmacia Biotec, Munich, Germany). Bacteria were centrifuged at 4000 rpm, 21 °C, 10 min (Megafuge 2.0R, Heraeus Instruments, Hanau, Germany). Afterwards they were washed two times by using TBS-Ca-Mn-Buffer containing 50 mM Tris, 150 mM NaCl, 1 mM $CaCl_2$ and 1 mM $MnCl_2$ (all Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The bacteria suspension was diluted to a final OD_{600} of 0.001.

RT-4 cells were washed two times with the same TBS-Ca-Mn buffer before they were incubated with 100 µl of the bacteria suspension (OD_{600} = 0.001) for 1 h, 37 °C on a plate rocker. Again, the cells were washed two times with the TBS-Ca-Mn buffer to get rid of the unbound bacteria. Subsequently, 100 µl of prepared functionalised CDV loaded with adamantane-mannose ligands were applied to detach bound bacteria from cell surfaces during a 15 min incubation at 37 °C. For quantification, the supernatant was collected and dilutions spread on selective LB-agar plates. After an overnight incubation (37 °C), the grown colonies were counted.

2. Synthesis

2a. Synthesis of Ad_1-Man_1, Ad_2-Man_1 and Ad_2Man_3

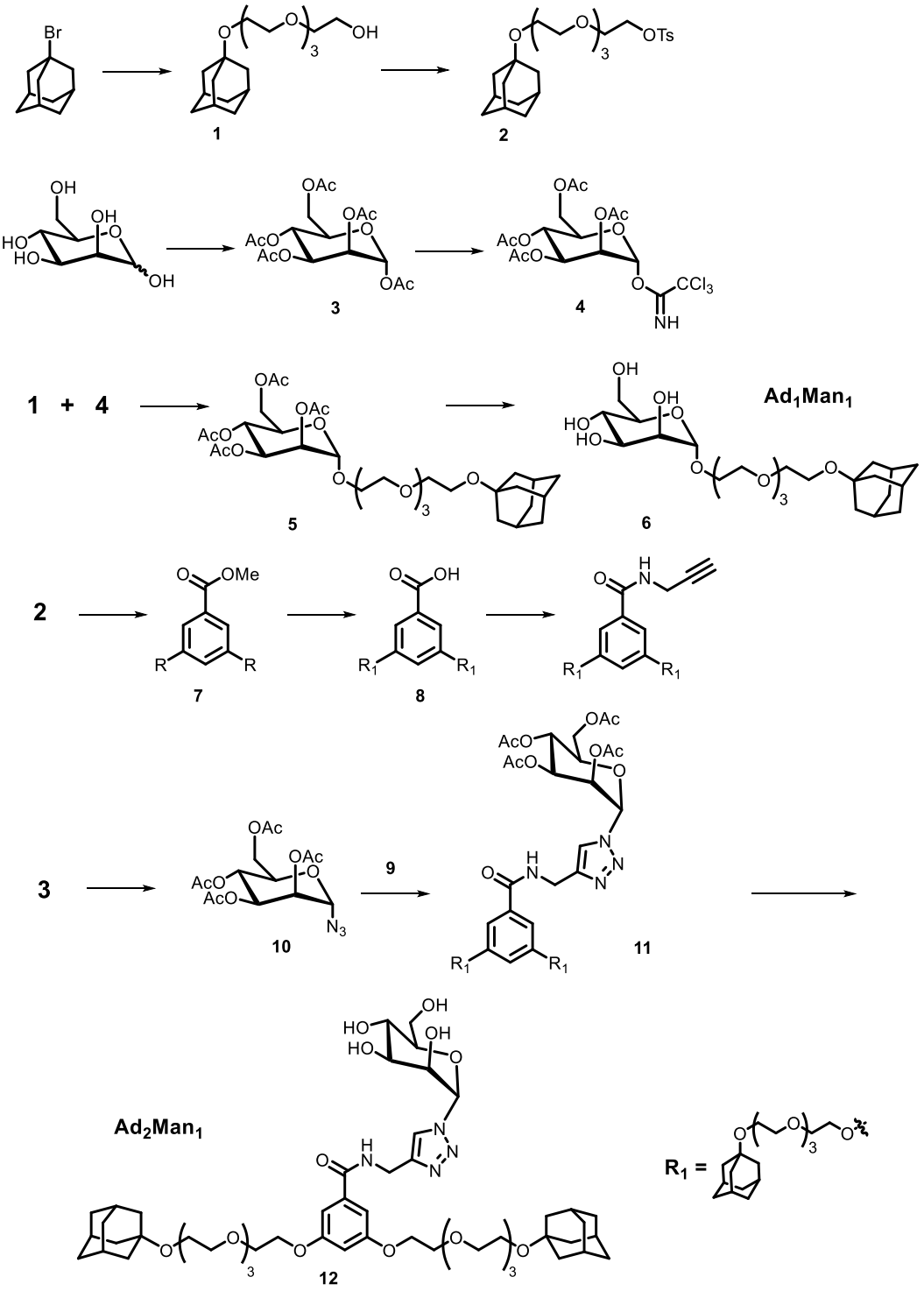


Figure S1. Synthesis routes of mono-mannoside ligands (**Ad_1Man_1** and **Ad_2Man_1**).

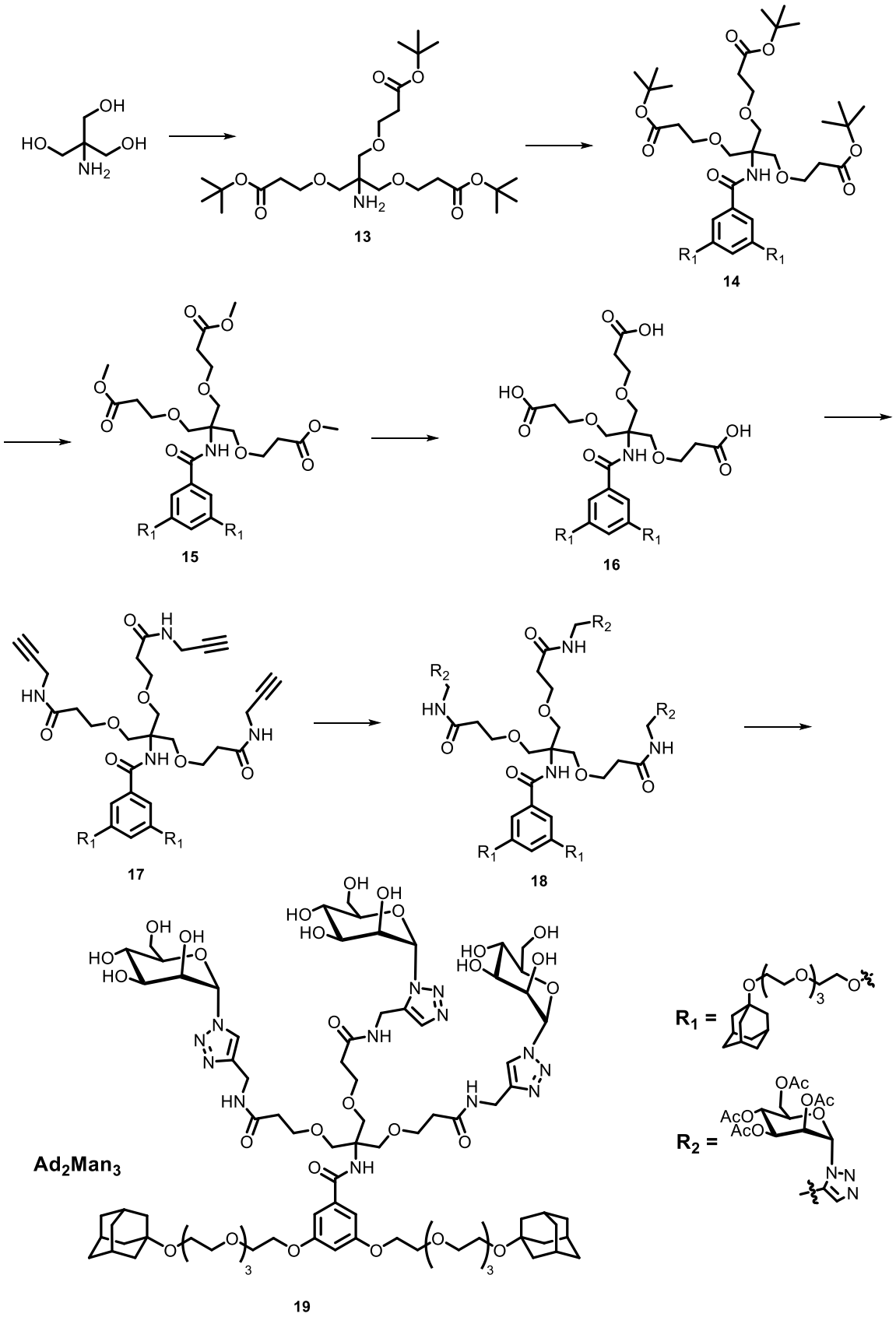
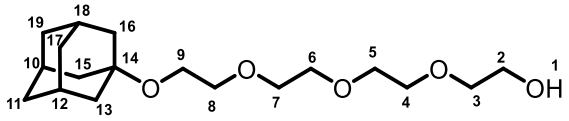


Figure S2. Synthesis routes of tri-mannoside ligand **Ad$_2$Man$_3$**.

Compound 1 – Ad-TEG-OH



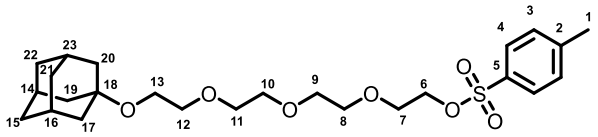
To a solution of 1-bromoadamantane (6.543 g, 30.4 mmol, 1 eq.) in tetraethylene glycol (150 mL) under argon atmosphere, triethylamine (3 eq., 90 mmol, V = 12.7 mL) was added. After stirring at 180 °C overnight, the mixture was extracted with DCM (200 mL) and water (200 mL). The organic phase was further washed with water (1 x 250 mL) and brine (1 x 250 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the title product was obtained as brown oil (9.474 g, 95%).

Molecular formula: $C_{18}H_{32}O_5$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 3.76-3.53 (m, 16H, 2-9-H), 2.83 (s, 1H, 1-H), 2.13 (s, 3H, 10-, 12-, 18-H), 1.74 (d, 6H, 13-, 15-, 16-H), 1.60 (m, 6H, 11-, 17-, 19-H) ppm.

HRMS (*m*/*z*): calculated for $[C_{18}H_{32}O_5Na]^+$: 351.2142, found: 351.2148.

Compound 2



To a stirring solution of **1** (1.66 g, 5.06 mmol, 1 eq.) in DCM (70 mL) at 0 °C, triethylamine (1.5 eq., 7.6 mmol, 1.06 mL) was added. Then, a solution of 4-toluenesulfonyl chloride (1.1 eq., 5.57 mmol, 1.06 g) and catalytic amount of DMAP (62 mg) in DCM (50 mL) was added to above solution. The solution was stirred overnight at room temperature. The solution was quenched by 10% of HCl solution, then washed and extracted with $NaHCO_3$ (1 x 100 mL) solution and brine (1 x 100 mL) The organic phase was combined and dried over $MgSO_4$. After removal of solvent under reduced pressure, the mixture was further purified by column chromatography (ethyl acetate, R_f = 0.75). The title product was obtained as colourless oil (1.74 g, 71%).

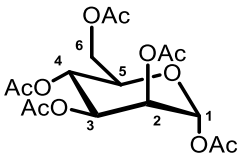
Molecular formula: $C_{25}H_{38}O_7S$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 7.80 (d, 2H, 4-H), 7.34 (d, 2H, 3-H), 4.16 (t, 2H, 6-H), 3.72-3.54 (m, 14H, 7-13-H), 2.44 (s, 3H, 1-H), 2.13 (s, 3H, 14-, 16-, 23-H), 1.73 (d, 6H, 17-, 19-, 20-H), 1.60 (m, 6H, 15-, 21-, 22-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 114.90 (C_q, 5-C), 133.18 (C_q, 2-C), 129.95, 128.13 (2 CH, 3-, 4-C), 72.41 (C_q, 18-C), 71.43, 70.90, 70.78, 70.72, 70.68, 69.38, 68.83 (7 CH_2, 6-12-C), 59.39 (CH_2, 13-C), 41.62 (3 CH_2, 17-, 19-, 20-C), 36.61 (3 CH_2, 15-, 21-, 22-C), 30.65 (3 CH, 14-, 16-, 23-C), 21.79 (CH_3, 1-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{25}H_{38}O_7SNa]^+$: 505.2236, found: 505.2224.

Compound 3



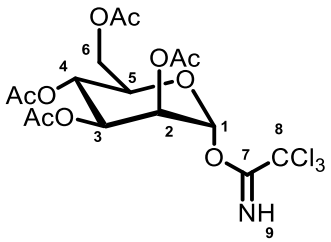
To a solution of D-(+)-mannose (5.4 g, 30 mmol, 1 eq.) in acetate anhydride (6 eq., 180 mmol, V = 17 mL) at 0 °C, 1,4-diazabicyclo[2.2.2]octane (1 eq., 30 mmol, 3.36 g) was added portion by portion over 1 hour. Then, the mixture was warmed up to room temperature and stirred for additional 18 h. The reaction mixture was poured into ice-water mixture (300 mL) and extracted with DCM (2 x 150 mL). The organic phase was further washed with $NaHCO_3$ (3 x 100 mL) and brine (1 x 100 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the title product was obtained as colourless sticky oil (10.997 g, 94%).

Molecular formula: $C_{16}H_{22}O_{11}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 6.03 (d, 1H, 1-H), 5.33-5.17 (m, 3H, 2-, 3-, 4-H), 4.30-3.94 (m, 3H, 5-, 6-H), 2.20-1.89 (m, 15H, OAc) ppm.

HRMS (*m*/*z*): calculated for $[C_{16}H_{22}O_{11}Na]^+$: 413.1060, found: 413.1058.

Compound 4



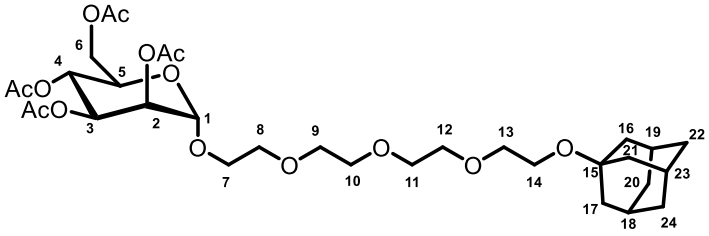
To a solution of **3** (2.278 g, 5.8 mmol, 1 eq.) in dry DCM (20 mL) at room temperature, dimethylaminopropylamine (5 eq., 29.2 mmol, V = 3.6 mL) was added and stirred at this temperature for 3 h. Then trichloroacetonitrile (10 eq., 58 mmol, V = 5.8 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.8 eq., 4.64 mmol, V = 0.69 mL) was added dropwise at 0 °C and stirred for 30 min and additional 2 h at room temperature. The reaction mixture was then diluted with DCM (100 mL) and extracted and washed with 10% HCl (1 x 100 mL), saturated Na_2CO_3 (1 x 100 mL) and brine (1 x 100 mL). The organic phase was combined and dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was further purified by column chromatography (pentane : ethyl acetate = 1 : 1, R_f = 0.2). The title product was obtained as yellow oil (609 mg, 21%).

Molecular formula: $C_{16}H_{20}NO_{10}Cl_3$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 8.79 (s, 1H, 9-H), 6.28 (d, 1H, 1-H), 5.47 (m, 1H, 2-H), 5.43-5.37 (m, 2H, 3-, 4-H), 4.33-4.07 (m, 3H, 5-, 6-H), 2.23-1.98 (m, 12H, OAc) ppm.

HRMS (*m*/*z*): calculated for $[C_{16}H_{20}NO_{10}Cl_3Na]^+$: 514.0050, found: 514.0050.

Compound 5



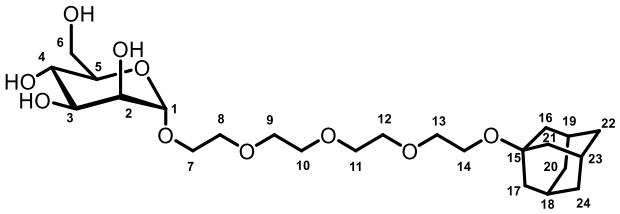
To a solution of **1** (300 mg, 0.92 mmol, 1.5 eq.) in dry DCM (5 mL), compound **4** (300 mg, 0.61 mmol, 1 eq.) was added and stirred at -5 °C until fully dissolved. Boron trifluoride diethyl etherate (1.5 eq., 0.918 mmol, V = 0.11 mL) was added dropwise and this temperature for 2 h. After additional 2 h stirring at 0 °C, the reaction was quenched by saturated $NaHCO_3$ solution (100 mL) and extracted with DCM (100 mL). The organic phase was washed with 10% HCl (1 x 100 mL), water (1 x 100 mL) and brine (1 x 100 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was further purified by column chromatography (ethyl acetate : pentane = 1 : 1, R_f = 0.18). The title product was obtained as colourless oil (165 mg, 37%).

Molecular formula: $C_{32}H_{50}O_{14}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 5.40-5.22 (m, 3H, 2-, 3-, 4-H), 4.87 (d, 1H, 1-H), 4.35-4.24 (m, 1H, 5-H), 4.12-4.02 (m, 2H, 6-H), 3.87-3.54 (m, 16H, 7-14-H), 2.18-1.96 (m, 15H, 18-, 19-, 23-H OAc), 1.73 (d, 6H, 16-, 17-, 21-H), 1.60 (m, 6H, 20-, 22-, 24-H) ppm.

HRMS (*m*/*z*): calculated for $[C_{32}H_{50}O_{14}Na]^+$: 681.3098, found: 681.3074.

Compound 6 - Ad_1Man_1



To a solution of **5** (165 mg, 0.25 mmol, 1 eq.) in dry methanol (5 mL), sodium methoxide (1.5 eq., 0.375 mmol, 20 mg) was added. After stirring for 6 h at room temperature, the mixture was diluted with methanol (p.a.) and neutralised by Dowex ion exchange resin. The title product was obtained after filtration and concentration under reduced pressure as colourless oil (85 mg, 69%).

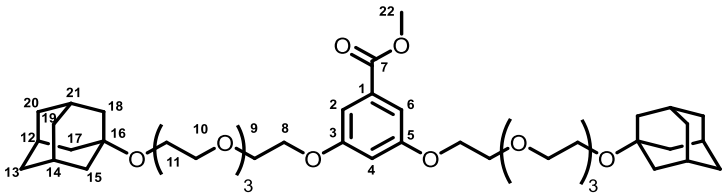
Molecular formula: $C_{24}H_{42}O_{10}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 4.80 (d, 1H, 1-H), 3.88-3.79 (m, 3H, 2-, 3-, 4-H), 3.75-3.55 (m, 19H, 5-, 6-, 7-14-H), 3.87-3.54 (m, 16H, 7-14-H), 2.18-2.09 (s, 3H, 18-, 19-H), 1.78 (d, 6H, 16-, 17-, 21-H), 1.67 (m, 6H, 20-, 22-, 24-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 101.73 (CH, 1-C), 74.59 (CH, 5-C), 73.73(CH, 2-C), 72.51 (C_q, 15-C), 72.17 (CH, 3-C), 72.09 (CH, 4-C), 71.58, 71.52, 71.38 (5 CH_2, 9-13-C), 68.57 (CH_2, 8-C), 67.77 (CH_2, 7-C), 62.87 (CH_2, 6-C), 60.38 (CH_2, 14-C), 42.51 (3 CH_2, 16-, 17-, 21-C), 37.48 (3 CH_2, 20-, 22-, 24-C), 31.98 (3 CH, 18-, 19-, 23-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{24}H_{42}O_{10}Na]^+$: 513.2676, found: 513.2679.

Compound 7



To a solution of **2** (1.156 g, 2.4 mmol, 2.4 eq.) in dry acetonitrile (10 mL) under argon atmosphere, methyl 3,5-dihydroxybenzoate (1 eq., 1.0 mmol, 168 mg) and K_2CO_3 (10 eq., 10 mmol, 1.38 g) were added. The reaction mixture was then stirred at refluxing temperature for 48 h. The solvent was removed and the residue was re-dissolved in DCM before extracted with water and washed with brine. The organic phase was combined and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the mixture was further purified by column chromatography (DCM : MeOH = 98 : 2, R_f = 0.3). The title product was obtained as colourless oil (773 mg, 98%).

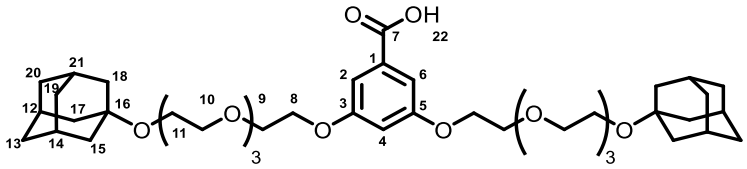
Molecular formula: $C_{44}H_{68}O_{12}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 7.18 (d, 2H, 2-, 6-H), 6.68 (t, 1H, 4-H), 4.12 (t, 4H, 8-H), 3.87 (s, 3H, 22-H), 3.84 (t, 4H, 9-H), 3.75-3.52 (m, 24H, 10-, 11-H), 2.12 (s, 6H, 12-, 14-, 21-H), 1.73 (d, 12H, 15-, 17-, 18-H), 1.59 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 166.86 (C_q, 7-C), 159.83 (C_q, 1-C), 131.95 (2 C_q, 3-, 5-C), 108.10 (CH, 4-C), 106.97 (2 CH, 2-, 6-C), 72.37 (2 C_q, 16-C), 71.36, 70.94, 70.75, 70.73, 70.68, 69.69, 67.83 (14 CH_2, 8-, 9-, 10-C), 59.34 (2 CH_2, 11-C), 52.34 (CH_3, 22-C), 41.55 (6 CH_2, 15-, 17-, 18-C), 36.55 (6 CH_2, 13-, 19-, 20-C), 30.59 (6 CH, 12-, 14-, 21-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{44}H_{68}O_{12}Na]^+$: 811.4603, found: 811.4606

Compound 8



To a solution of **7** (1.6 g, 2 mmol, 1 eq.) in methanol, an aqueous solution of lithium hydroxide (4.5 eq., 9 mmol, 213 mg) was added dropwise. The mixture was then stirred at room temperature overnight before being quenched by 10% HCl (2 x 100 mL). The mixture was extracted with DCM (100 mL) and washed with water (1 x 100 mL) and brine (1 x 100 mL). The organic phase was combined and dried over $MgSO_4$. After removal of solvent under reduced pressure, the title product was obtained as colourless oil (1.517 g, 98%).

Molecular formula: $C_{43}H_{66}O_{12}$

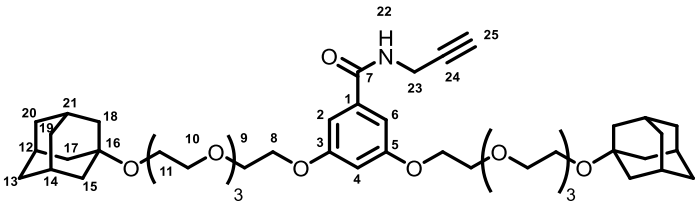
^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 7.24 (d, 2H, 2-, 6-H), 6.71 (t, 1H, 4-H), 4.14 (t, 4H, 8-H), 3.85 (t, 4H, 9-H), 3.75-3.55 (m, 24H, 10-, 11-H), 2.12 (s, 6H, 12-, 14-, 21-H), 1.74 (d, 12H, 15-, 17-, 18-H), 1.60 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 169.90 (C_q, 7-C), 159.90 (C_q, 1-C), 131.53 (2 C_q, 3-, 5-C), 108.63 (CH, 4-C), 107.68 (2 CH, 2-, 6-C), 72.58 (2 C_q, 16-C), 71.37, 70.98, 70.74, 70.71, 69.78, 67.90 (14 CH_2,

8-, 9-, 10-C), 59.40 (2 CH_2, 11-C), 41.56 (6 CH_2, 15-, 17-, 18-C), 36.58 (6 CH_2, 13-, 19-, 20-C), 30.64 (6 CH, 12-, 14-, 21-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{43}H_{66}O_{12}Na]^+$: 797.4446, found: 797.4445.

Compound 9



To a solution of **8** (0.5 g, 0.65 mmol, 1 eq.) in dry DMF under argon atmosphere, EDC hydrochloride (1.5 eq., 0.975 mmol, 187 mg) and 1-hydroxybenzotriazole hydrate (1.5 eq., 0.975 mmol, 132 mg) were added. After stirring for 30 min, N-methylmorpholine (1.5 eq., 0.975 mmol, V = 1.1 mL) and propargylamine (1.5 eq., 0.975 mmol, V = 0.06 mL) were added and stirred at room temperature overnight. The reaction mixture was quenched by saturated $NaHCO_3$ solution (100 mL) and extracted with DCM (100 mL). The organic phase was further washed with 10% HCl (1 x 100 mL), water (1 x 100 mL), and brine (1 x 100 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was further purified by column chromatography (ethyl acetate, R_f = 0.25). The title product was obtained as colourless oil (350 mg, 62%).

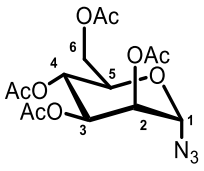
Molecular formula: $C_{46}H_{69}NO_{11}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 6.97 (d, 2H, 2-, 6-H), 6.62 (t, 1H, 4-H), 6.58 (s, 1H, 22-H), 4.22 (m, 2H, 23-H), 4.14 (t, 4H, 8-H), 3.84 (t, 4H, 9-H), 3.75-3.54 (m, 24H, 10-, 11-H), 2.27 (dd, 1H, 25-H), 2.12 (s, 6H, 12-, 14-, 21-H), 1.73 (d, 12H, 15-, 17-, 18-H), 1.60 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 166.93 (C_q, 7-C), 160.10 (2 C_q, 3-, 5-C), 135.89 (C_q, 1-C), 106.12 (CH, 4-C), 106.34 (2 CH, 2-, 6-C), 79.69 (C_q, 24-C), 79.65 (C_q, 25-C) 72.46 (2 C_q, 16-C), 71.88, 71.38, 70.95, 70.72, 69.77, 67.91 (14 CH_2, 8-, 9-, 10-C), 59.38 (2 CH_2, 11-C), 41.58 (6 CH_2, 15-, 17-, 18-C), 36.57 (6 CH_2, 13-, 19-, 20-C), 30.62 (6 CH, 12-, 14-, 21-C), 29.90 (CH_2, 23-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{46}H_{69}NO_{11}Na]^+$: 834.4768, found: 834.4739.

Compound 10



To a solution of **3** (840 mg, 2.16 mmol, 1 eq.) in dry DCM under argon atmosphere, trimethylsilyl azide (2.5 eq., 5.4 mmol, V = 0.71 mL) was added dropwise. After stirring for 30 min, $SnCl_4$ (0.3 eq., 0.65 mmol, V = 0.1 mL) was added dropwise and stirred at room temperature overnight. The mixture was diluted with DCM (100 mL) and stirred vigorously with saturated $NaHCO_3$ (100 mL) for 30 min. The organic phase was then extracted with water (1 x 100 mL) and brine (1 x 100 mL) and dried over MgSO4. After removal of solvent under reduced pressure, the residue was further purified by column

chromatography (cyclohexane : ethyl acetate = 1 : 1, R_f = 0.4). The title product was obtained as colourless oil (718 mg, 89%).

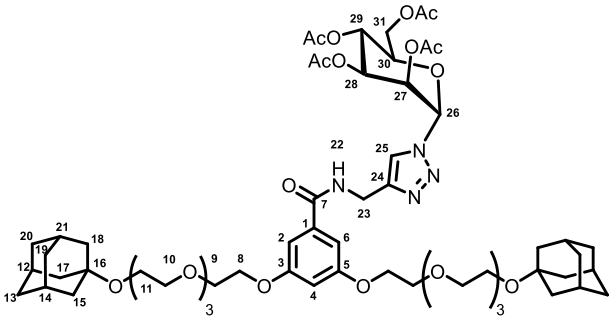
Molecular formula: $C_{14}H_{19}N_3O_9$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 5.39 (d, 1H, 1-H), 5.32-5.11 (m, 3H, 2-, 3-, 4-H), 4.36-4.06 (m, 3H, 5, 6-H), 2.21-1.95 (m, 12H, OAc) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 170.60, 169.86, 169.75, 169.62 (4 C_q, OAc), 87.43 (CH, 1-C), 70.59, 69.14, 68.20, 65.56 (4 CH, 2-, 3-, 4-, 5-C), 62.11 (CH_2, 6-C), 20.82, 20.72, 20.67, 20.61 (4 CH_3, OAc) ppm.

HRMS (*m*/*z*): calculated for $[C_{14}H_{19}N_3O_9Na]^+$: 369.1019, found: 369.1020.

Compound 11



Compound **9** (236 mg, 0.29 mmol, 1 eq.) and **10** (1.2 eq., 0.35 mmol, 130 mg) were dissolved in dry DMF under argon atmosphere. Copper(I) iodine (0.15 eq., 0.0435 mmol, 8 mg) was added to above solution. After stirring at 60 °C for 18 h, the mixture was quenched by 10% HCl (100 mL) and extracted with DCM (150 mL). The organic phase was further washed with 10% HCl (1 x 100 mL), water (1 x 100 mL), and brine (1 x 100 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was further purified by column chromatography (DCM : methanol = 95 : 5, R_f = 0.47). The title product was obtained as colourless oil (300 mg, 87%).

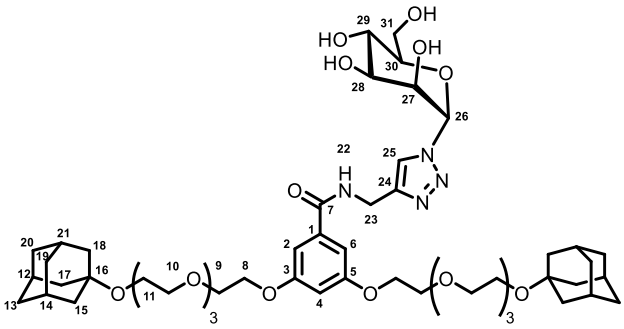
Molecular formula: $C_{60}H_{88}N_4O_{20}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 7.84 (s, 1H, 22-H), 7.09 (s, 1H, 25-H), 6.96 (d, 2H, 2-, 6-H), 6.62 (t, 1H, 4-H), 5.96 (d, 1H, 26-H), 5.90 (m, 1H, 27-H), 5.38 (m, 2H, 28, 29-H), 4.73 (d, 2H, 23-H), 4.37 (m, 1H, 31'-H), 4.30 (m, 1H, 30-H), 4.13 (m, 4H, 8-H), 4.04 (m, 1H, 31-H), 3.84 (m, 4H, 9-H), 3.73-3.54 (m, 24H, 10-, 11-H), 2.20-1.93 (m, 18H, 12-, 14-, 21-H OAc), 1.73 (d, 12H, 15-, 17-, 18-H), 1.60 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 170.66, 169.76, 169.74, 169.45 (C_q, OAc), 169.28 (C_q, 7-C), 160.17 (C_q, 1-C), 144.60 (C_q, 24-C), 135.96 (C_q, 3-, 5-C), 125.38 (CH, 25-C), 106.09 (CH, 4-C), 105.50 (2 CH, 2-, 6-C), 87.60 (CH, 23-C), 83.82 (CH_2, 26-C), 72.43 (C_q, 16-C), 71.41, 70.97, 70.75, 69.78, 68.88, 68.37, 67.98 (CH_2, 8-10-C), 66.20 (CH, 27-C), 65.76 (CH, 28-C), 62.28 (CH, 29-C), 61.67 (CH, 30-C), 59.41 (CH_2, 11-C), 53.57 (CH_2, 31-C), 41.61 (6 CH_2, 15-, 17-, 18-C), 36.60 (6 CH_2, 13-, 19-, 20-C), 30.64 (6 CH, 12-, 14-, 21-C), 20.95, 20.89, 20.85, 20.74 (4 CH_3, OAc) ppm.

HRMS (*m*/*z*): calculated for $[C_{60}H_{88}N_4O_{20}Na]^+$: 1207.5890, found: 1207.5856.

Compound 12 - Ad_2Man_1



To a solution of **11** (105 mg, 0.089 mmol, 1 eq.) in dry methanol (5 mL) under argon atmosphere, sodium methoxide (1.5 eq., 0.133 mmol, 7 mg) was added. After stirring for 6 h at room temperature, the mixture was diluted with methanol (p.a.) and neutralised by Dowex ion exchange resin. The title product was obtained after filtration and concentration under reduced pressure as colourless oil (48 mg, 53%).

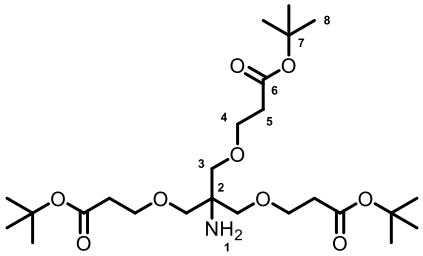
Molecular formula: $C_{52}H_{80}N_4O_{16}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 8.10 (s, 1H, 22-H), 7.04 (d, 2H, 2-, 6-H), 6.70 (t, 1H, 4-H), 6.00 (d, 1H, 26-H), 4.65 (m, 3H, 27-, 28-, 29-H), 4.15 (m, 4H, 8-H), 4.08 (m, 1H, 30-H), 3.84 (m, 4H, 9-H), 3.80-3.50 (m, 26H, 10-, 11-, 31-H), 2.21 (s, 6H, 12-, 14-, 21-H OAc), 1.74 (d, 12H, 15-, 17-, 18-H), 1.64 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 169.56 (C_q, 7-C), 161.50 (C_q, 1-C), 160.17 (C_q, 1-C), 143.16 (C_q, 24-C), 137.29 (C_q, 3-, 5-C), 124.37 (CH, 25-C), 107.19 (CH, 4-C), 106.20 (2 CH, 2-, 6-C), 88.33 (CH, 23-C), 78.60 (CH_2, 26-C), 73.66 (C_q, 16-C), 72.57, 72.21, 71.77, 71.60, 71.58, 71.53, 70.75 (CH_2, 8-10-C), 70.09 (CH, 27-C), 69.00 (CH, 28-, 29-C), 68.62 (CH, 30-C), 62.52 (CH_2, 31-C), 60.42 (CH_2, 11-C), 42.52 (6 CH_2, 15-, 17-, 18-C), 36.21 (6 CH_2, 13-, 19-, 20-C), 31.97 (6 CH, 12-, 14-, 21-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{52}H_{80}N_4O_{16}Na]^+$: 1039.5467, found: 1039.5444.

Compound 13



To a solution of TRIS (2.42 g, 20 mmol, 1eq.) in DMSO (5 mL), 5 M of NaOH aqueous solution (2 mmol, V = 0.4 mL) was added. *tert*-butyl acrylate (3.5 eq., 70 mmol, V = 10 mL) was added before the mixture was stirring at room temperature overnight. After removal of solvent under reduced pressure, the residue was further purified by column chromatography (ethyl acetate : cyclohexane = 2 : 1 + 0.05 v/v% methanol, R_f = 0.3). The title product was obtained as colourless oil (2.97 g, 29%).

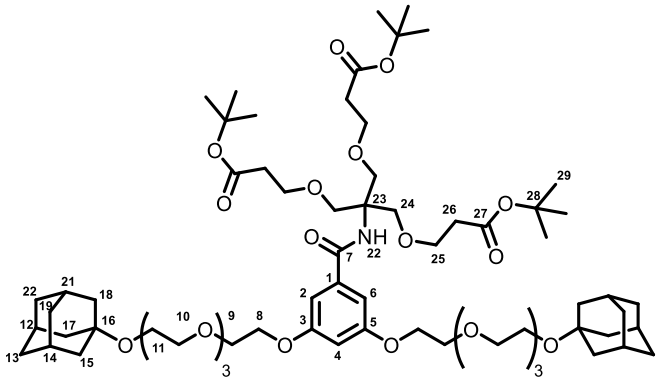
Molecular formula: $C_{25}H_{47}NO_9$

^{1}H (400 MHz, $CDCl_3$, 298 K): δ = 3.62 (t, 6H, 4-H), 3.29 (s, 6H, 3-H), 2.43 (t, 6H, 5-H), 1.42 (s, 27H, 8-H) ppm.

^{13}C (100 MHz, $CDCl_3$, 298 K): δ = 171.01 (3 C_q, 6-C), 80.51 (3 C_q, 7-C), 72.97 (3 CH_2, 4-C), 67.23 (3 CH_2, 3-C), 56.04 (C_q, 2-C), 36.43 (3 CH_2, 5-C), 28.21 (9 CH_3, 29-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{25}H_{47}NO_9H]^+$: 506.3329, found: 506.3331.

Compound 14



Compound **8** (123 mg, 0.16 mmol, 1.1 eq.) and compound **13** (73 mg, 0.144 mmol, 1 eq.) were dissolved in dry THF (5 mL) under argon atmosphere. PyBOP (1 eq., 0.16 mmol, 83 mg) and DIPEA (4 eq., 0.64 mmol, V = 0.11 mL) were added. After stirring at room temperature for 18 h, the mixture was quenched by 10% HCl (100 mL) and extracted with DCM (150 mL). The organic phase was washed with 10% HCl (1 x 100 mL), $NaHCO_3$ (1 x 100 mL), and brine (1 x 100 mL) and dried over $MgSO_4$. After removal of solvent, the residue was further purified by column chromatography (ethyl acetate : methanol = 90 : 5, R_f = 0.75). The title product was obtained as colourless oil (170 mg, 84%).

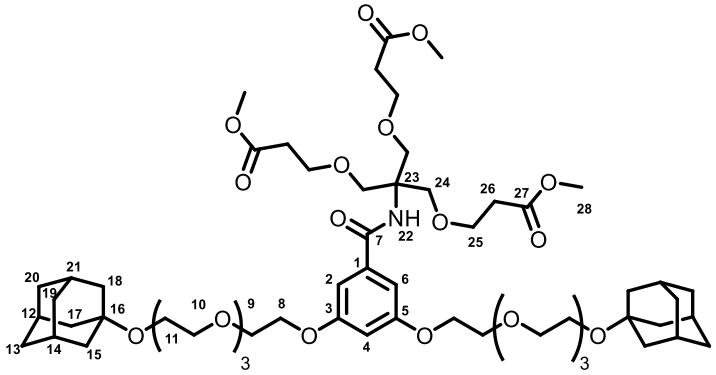
Molecular formula: $C_{68}H_{111}NO_{20}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 6.89 (d, 2H, 2-, 6-H), 6.58 (t, 1H, 4-H), 6.47 (br, 1H, 22-H), 4.13 (m, 4H, 8-H), 3.86-3.55 (m, 40H, 9-, 10-, 11-, 24-, 25-H), 2.44 (t, 6H, 26-H), 2.13 (s, 6H, 12-, 14-, 21-H), 1.74 (d, 12H, 15-, 17-, 18-H), 1.60 (m, 12H, 13-, 19-, 22-H), 1.40 (s, 27H, 29-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 170.90 (3 C_q, 27-C), 167.49 (C_q, 7-C), 159.87 (C_q, 1-C), 137.60 (2 C_q, 3-, 5-C), 105.93 (CH, 4-C), 104.64 (2 CH, 2-, 6-C), 80.52 (3 C_q, 28-C), 72.35 (2 C_q, 16-C), 71.37, 70.89, 70.75, 70.72, 70.70, 69.73, 69.20, 67.69, 67.18 (20 CH_2, 8-, 9-, 10-, 24-, 25-C), 60.27 (C_q, 23-C), 59.35 (2 CH_2, 11-C), 41.57 (6 CH_2, 15-, 17-, 18-C), 36.56 (6 CH_2, 13-, 19-, 20-C), 36.32 (3 CH_2, 26-C), 30.60 (6 CH, 12-, 14-, 21-C), 28.17 (9 CH_3, 29-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{68}H_{111}NO_{20}Na]^+$: 1284.7592, found: 1284.7563

Compound 15



To a solution of **14** (170 mg, 0.135 mmol, 1 eq.) in dry methanol (10 mL) under argon atmosphere, acetyl chloride (10 eq., V = 0.1 mL) was added dropwise. After stirring for 18 h, the mixture was quenched by 10% HCl (50 mL) and extracted with DCM (50 mL). The organic phase was washed with 10% HCl (1 x 50 mL) and brine (1 x 50 mL) and dried over $MgSO_4$. The title product was obtained after removal of solvent under reduced pressure (130 mg, 85%).

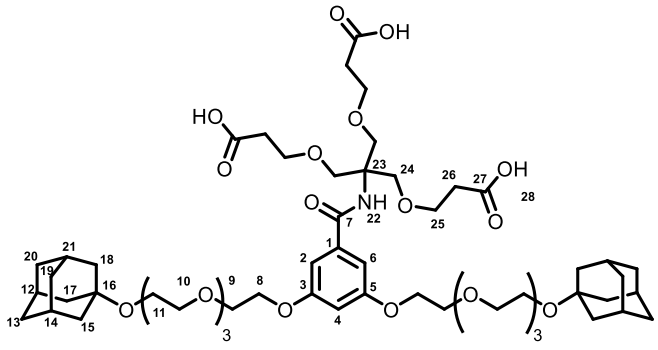
Molecular formula: $C_{59}H_{93}NO_{20}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 6.89 (d, 2H, 2-, 6-H), 6.59 (t, 1H, 4-H), 6.43 (s, 1H, 22-H), 4.14 (m, 4H, 8-H), 3.86-3.55 (m, 49H, 9-, 10-, 11-, 24-, 25-, 28-H), 2.55 (t, 6H, 26-H), 2.13 (s, 6H, 12-, 14-, 21-H), 1.74 (d, 12H, 15-, 17-, 18-H), 1.60 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 172.15 (3 C_q, 27-C), 167.35 (C_q, 7-C), 159.94 (C_q, 1-C), 137.50 (2 C_q, 3-, 5-C), 105.91 (CH, 4-C), 104.72 (2 CH, 2-, 6-C), 72.40 (2 C_q, 16-C), 71.40, 70.91, 70.77, 70.74, 70.71, 69.76, 69.26, 67.75, 66.86 (20 CH_2, 8-, 9-, 10-, 24-, 25-C), 60.20 (C_q, 23-C), 59.37 (2 CH_2, 11-C), 51.82 (3 CH_3, 28-C), 41.59 (6 CH_2, 15-, 17-, 18-C), 36.58 (6 CH_2, 13-, 19-, 20-C), 34.92 (3 CH_2, 26-C), 30.62 (6 CH, 12-, 14-, 21-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{59}H_{93}NO_{20}Na]^+$: 1158.6183, found: 1158.6168.

Compound 16



To a solution of **15** (130 mg, 0.115 mmol, 1 eq.) in methanol (10 mL), the same volume of lithium hydroxide (13 eq., 1.5 mmol, 36 mg) aqueous solution was added. After stirring at room temperature for 18 h, the mixture was quenched by 10% HCl (50 mL) and extracted with DCM (50 mL). The organic phase was washed with 10% HCl (1 x 50 mL) and brine (1 x 50 mL) and dried over $MgSO_4$. The title product was obtained after removal of solvent under reduced pressure (92 mg, 73%).

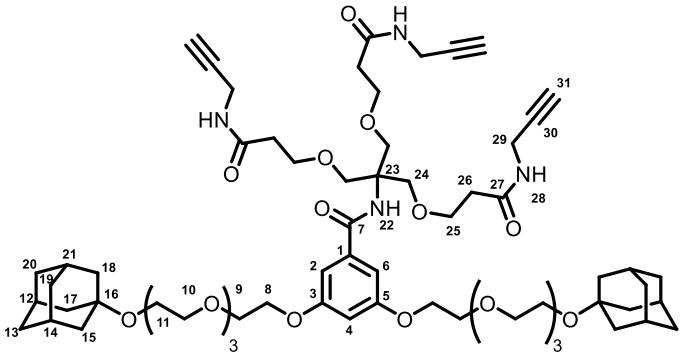
Molecular formula: $C_{56}H_{87}NO_{20}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 6.93 (d, 2H, 2-, 6-H), 6.71 (s, 1H, 22-H), 6.58 (t, 1H, 4-H), 4.17 (m, 4H, 8-H), 3.89-3.54 (m, 40H, 9-, 10-, 11-, 24-, 25-H), 2.57 (t, 6H, 26-H), 2.11 (s, 6H, 12-, 14-, 21-H), 1.72 (d, 12H, 15-, 17-, 18-H), 1.59 (m, 12H, 13-, 19-, 22-H).

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 175.22 (3 C_q, 27-C), 167.54 (C_q, 7-C), 159.86 (C_q, 1-C), 137.22 (2 C_q, 3-, 5-C), 105.99 (CH, 4-C), 105.54 (2 CH, 2-, 6-C), 72.61 (2 C_q, 16-C), 71.29, 70.81, 70.66, 70.62, 70.59, 69.77, 69.70, 67.65, 66.77 (20 CH_2, 8-, 9-, 10-, 24-, 25-C), 60.06 (C_q, 23-C), 59.34 (2 CH_2, 11-C), 53.57 (3 CH_2, 26-C), 41.51 (6 CH_2, 15-, 17-, 18-C), 36.55 (6 CH_2, 13-, 19-, 20-C), 30.61 (6 CH, 12-, 14-, 21-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{56}H_{86}NO_{20}]^-$: 1092.5749, found: 1092.5773.

Compound 17



To a solution of **16** (213 mg, 0.19 mmol, 1 eq.) in dry THF (15 mL) under argon atmosphere, PyBOP (3.5 eq., 0.68 mmol, 354 mg) was added. After propargyl amine (3.5 eq., 0.68 mmol, V = 0.043 mL) and DIPEA (12 eq., 2.28 mmol, V = 0.4 mL) were added, the mixture was stirred at room temperature overnight. The mixture was quenched by 10% HCl (100 mL) and extracted with DCM (100 mL). The organic phase was washed with 10% HCl (1 x 100 mL), saturated $NaHCO_3$ (1 x 100 mL), and brine (1 x 100 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was further purified by column chromatography (DCM : methanol = 95 : 5, R_f = 0.3). The title product was obtained as colourless oil (165 mg, 72%).

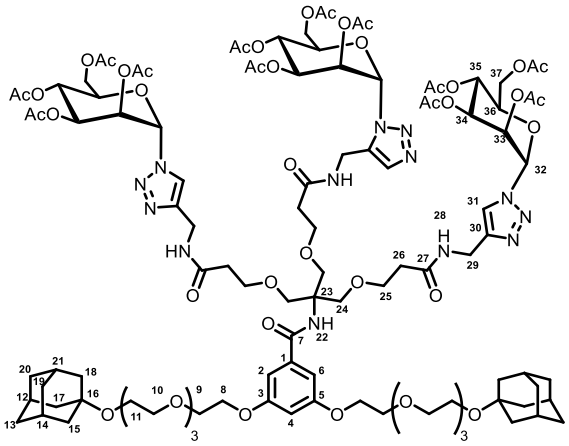
Molecular formula: $C_{65}H_{96}N_4O_{17}$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 6.98 (t, 3H, 28-H), 6.91 (d, 2H, 2-, 6-H), 6.65 (s, 1H, 22-H), 6.60 (t, 1H, 4-H), 4.14 (m, 4H, 8-H), 3.97 (m, 6H, 29-H), 3.85-3.53 (m, 40H, 9-, 10-, 11-, 24-, 25-H), 2.47 (t, 6H, 26-H), 2.20 (t, 3H, 31-H), 2.13 (s, 6H, 12-, 14-, 21-H), 1.72 (d, 12H, 15-, 17-, 18-H), 1.60 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (75.5 MHz, $CDCl_3$, 298 K): δ = 171.32 (3 C_q, 27-C), 167.44 (C_q, 7-C), 160.01 (C_q, 1-C), 137.03 (2 C_q, 3-, 5-C), 106.13 (CH, 4-C), 104.65 (2 CH, 2-, 6-C), 80.19 (3 C_q, 30-C), 72.50 (2 C_q, 16-C), 71.36, 70.88, 70.72, 70.70, 70.64, 69.69, 69.65, 67.75, 67.29 (20 CH_2, 8-, 9-, 10-, 24-, 25-C), 59.91 (C_q, 23-C), 59.35 (2 CH_2, 11-C), 41.58 (6 CH_2, 15-, 17-, 18-C), 36.56 (6 CH_2, 13-, 19-, 20-C), 36.48 (3 CH_2, 26-C), 30.61 (6 CH, 12-, 14-, 21-C), 29.08 (3 CH_2, 29-C) ppm.

MALDI-MS (*m*/*z*): calculated for $[C_{65}H_{96}N_4O_{17}Na]^+$: 1227.67, found: 1227.55.

Compound 18



Compound **10** (206 mg, 0.55 mmol, 3.6 eq.) and **17** (185 mg, 0.154 mmol, 1 eq.) were dissolved in dry DMF (15 mL) under argon atmosphere. Copper (I) iodine (0.5 eq., 0.077 mmol, 15 mg) was added and the mixture was stirred at 70 °C overnight. After removal of DMF, the residue was extracted with DCM (100 mL) and 10% HCl (100 mL). The organic phase was washed with water (1 x 100 mL) and brine (1 x 100 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was further purified by column chromatography (DCM : methanol = 90 :10, R_f = 0.25). The title product was obtained as colourless oil (244 mg, 68%).

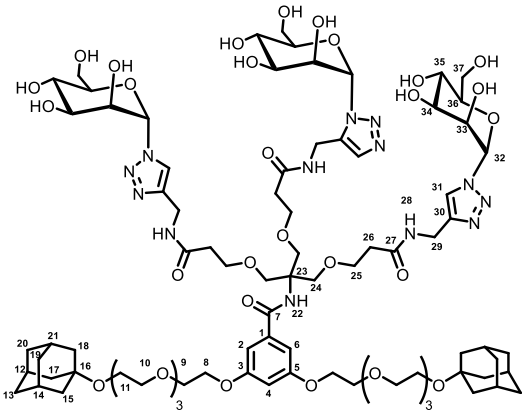
Molecular formula: $C_{107}H_{153}N_{13}O_{44}$

^{1}H (400 MHz, $CDCl_3$, 298 K): δ = 7.78 (s, 3H, 31-H), 7.65 (t, 3H, 28-H), 6.83 (d, 2H, 2-, 6-H), 6.72 (s, 1H, 22-H), 6.55 (t, 1H, 4-H), 5.98 (d, 3H, 32-H), 5.91-5.84 (m, 3H, 33-H), 5.37 (dt, 3H, 34-H), 4.54-4.24 (m, 9H, 29-, 36-H), 4.13-4.05 (m, 4H, 8-H), 4.04-3.96 (m, 3H, 35-H), 3.85-3.52 (m, 40H, 9-, 10-, 11-, 24-, 25-, 29-, 37-H), 2.50 (t, 6H, 26-H), 2.18-1.97 (m, 42H, 12-, 14-, 21-H, OAc), 1.71 (d, 12H, 15-, 17-, 18-H), 1.58 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (100 MHz, $CDCl_3$, 298 K): δ = 171.94 (3 C_q, 27-C), 170.65, 169.88, 169.76, 169.72 (12 C_q, OAc), 162.66 (C_q, 7-C), 159.93 (C_q, 1-C), 145.73 (C_q, 30-C), 137.06 (2 C_q, 3-, 5-C), 123.39 (3 CH, 31-C), 106.06 (CH, 4-C), 104.79 (2 CH, 2-, 6-C), 83.91 (3 CH, 32-C), 72.42 (2 C_q, 16-C), 71.94, 69.16, 68.35, 65.84 (12 CH, 33, 34, 35, 36-C), 71.38, 70.81, 70.70, 70.67, 70.65, 69.68, 69.57, 67.79, 67.56 (23 CH_2, 8-, 9-, 10-, 24-, 25, 29-C), 61.77 (3 CH_2, 37-C), 59.35 (2 CH_2, 11-C), 41.58 (6 CH_2, 15-, 17-, 18-C), 36.57 (6 CH_2, 13-, 19-, 20-C), 34.73 (3 CH_2, 26-C), 30.61 (6 CH, 12-, 14-, 21-C), 20.87, 20.83, 20.81, 20.71 (4 CH_3, OAc) ppm.

MALDI-MS (*m*/*z*): calculated for $[C_{107}H_{153}N_{13}O_{44}Na]^+$: 2347.00, found: 2346.99.

Compound 19 - Ad_2Man_3



To a solution of **18** (74 mg, 0.032 mmol, 1 eq.) in dry methanol (5 mL) under argon atmosphere, sodium methoxide (1.5 eq, 0.048 mmol, 3 mg) was added. After stirring at room temperature for 6 h, the mixture was diluted with methanol (p. a.) and neutralised by Dowex ion exchange resin. The title product was obtained after filtration and concentration under reduced pressure as colourless solid (50 mg, 53%).

Molecular formula: $C_{83}H_{129}N_{13}O_{32}$

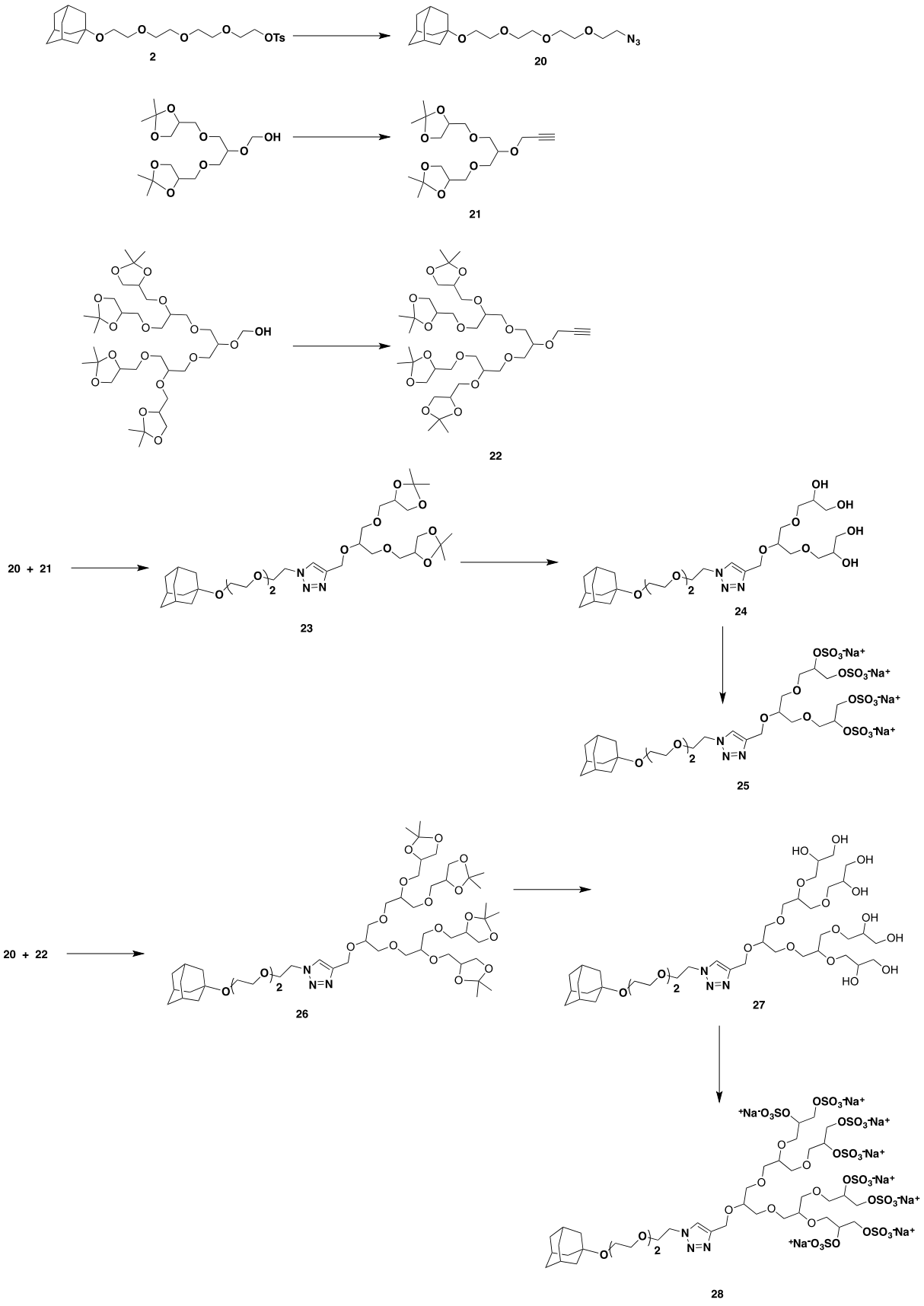
^{1}H (600 MHz, CD_3OD, 298 K): δ = 7.98 (s, 3H, 31-H), 6.87 (d, 2H, 2-, 6-H), 6.63 (t, 1H, 4-H), 5.97 (d, 3H, 32-H), 4.61 (m, 3H, 33-H), 4.41 (s, 6H, 29-H), 4.12 (dt, 3H, 33-H), 4.05 (dt, 3H, 35-H), 3.81 (t, 4H, 8-H), 3.79-3.49 (m, 61H, 9-, 10-, 11-, 24-, 25-, 29-, 36-, 37-H, 12 -OH), 2.46 (t, 6H, 26-H), 2.10 (br, 6H, 12-, 14-, 21-H), 1.73 (d, 12H, 15-, 17-, 18-H), 1.58 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (150 MHz, CD_3OD, 298 K): δ = 174.02 (3 C_q, 27-C), 169.92 (C_q, 7-C), 161.33 (C_q, 1-C), 146.45 (3 C_q, 30-C), 138.50 (2 C_q, 3-, 5-C), 130.75, 127.08, 124.25 (3 C_q, 30-C), 107.28 (CH, 4-C), 105.54 (2 CH, 2-, 6-C), 88.29 (3 CH, 32-C), 78.56, 73.69, 72.57, 70.73 (12 CH, 33, 34, 35, 36-C), 72.20, 71.70, 71.55, 71.53, 71.70, 71.55, 71.53, 71.51, 70.10, 70.05, 68.96, 68.60, 68.58 (20 CH_2, 8-, 9-, 10-, 24-, 25-C), 62.49 (3 CH_2, 37-C), 61.98 (3 CH_2, 29-C), 60.42 (2 CH_2, 11-C), 42.54 (6 CH_2, 15-, 17-, 18-C), 37.49 (6 CH_2, 13-, 19-, 20-C), 35.66 (3 CH_2, 26-C), 31.97 (6 CH, 12-, 14-, 21-C) ppm.

MALDI-MS (*m*/*z*): calculated for $[C_{83}H_{129}N_{13}O_{32}Na]^+$: 1842.88, found: 1842.68.

2b. Synthesis route of Ad_1Su_4, Ad_1Su_8 and Ad_2Su_8

a)



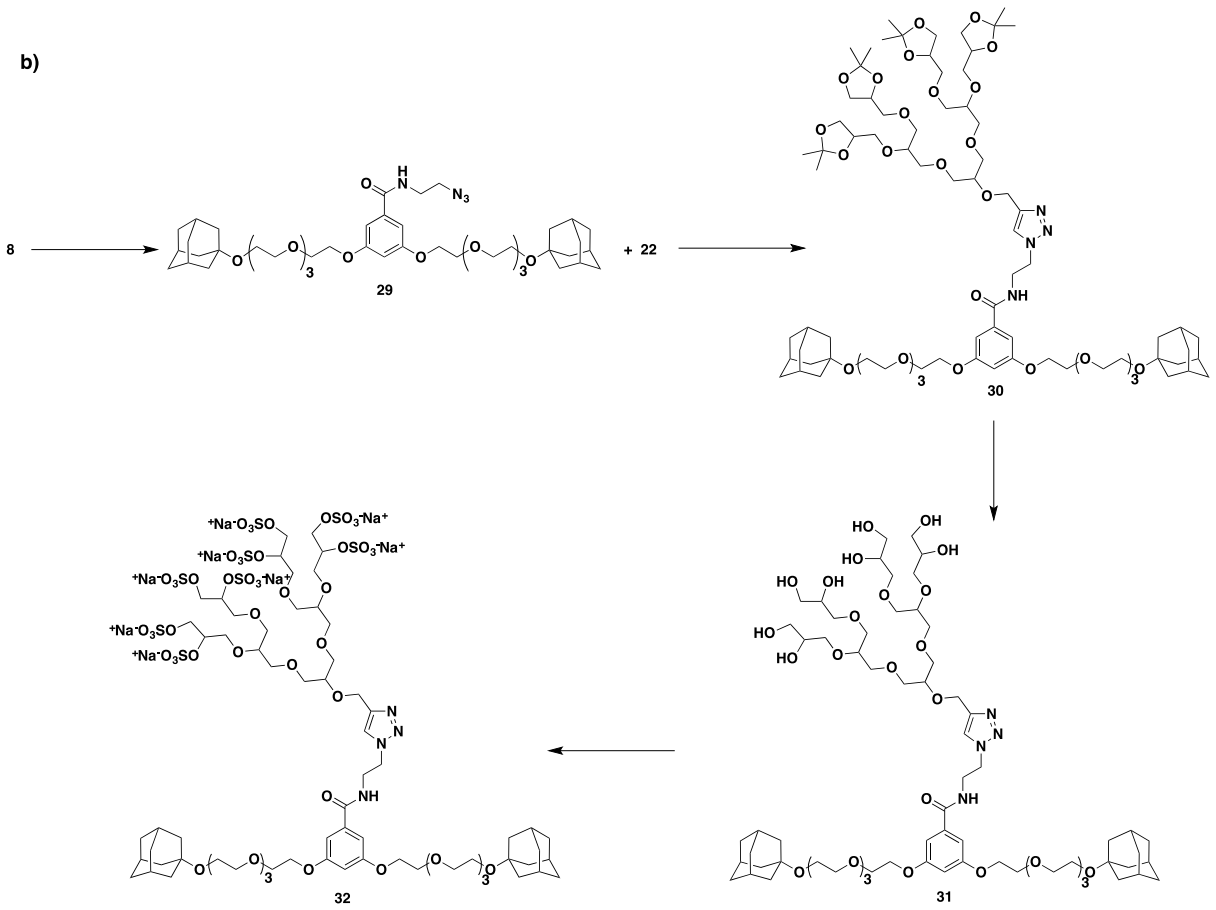
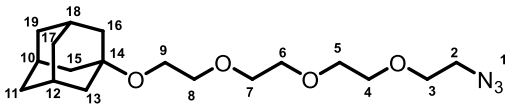


Figure S3. Synthesis routes of (a) sulphated ligands **Ad_1Su_4** and **Ad_1Su_8** and (b) **Ad_2Su_8**

Compound 20



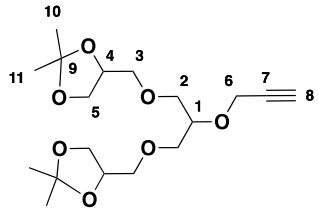
To a solution of **2** (0.858 g, 1.78 mmol, 1 eq.) in DMF, sodium azide (5 eq., 8.9 mmol, 0.58 g) was added in one portion. The reaction mixture was stirred at 80 °C for overnight before evaporation of solvent. The residue was dissolved in 100 mL DCM and extracted with water (1 x 100 mL) and brine (2 x 100 mL). The organic phase was collected and dried over $MgSO_4$ before removal of solvent under reduced pressure. The product was obtained as colourless oil (537 mg, 85%).

Molecular formula: $C_{18}H_{31}N_3O_4$

^{1}H (300 MHz, $CDCl_3$, 298 K): δ = 3.71-3.54 (m, 14H, 3-9-H), 3.39 (t, 2H, 2-H), 2.13 (s, 3H, 10-, 12-, 18-H), 1.79-1.70 (m, 6H, 13-, 15-, 16-H), 1.61 (m, 6H, 11-, 17-, 19-H) ppm.

HRMS (*m*/*z*): calculated for $[C_{18}H_{31}N_3O_4Na]^+$: 376.2207, found: 376.2212.

Compound 21



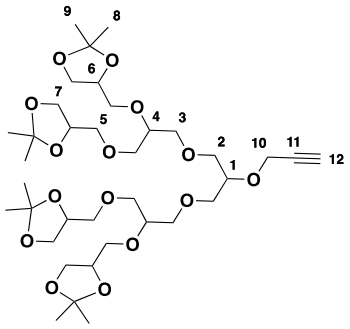
To a solution of protected dendron [G1-OH] (5 g, 15.61 mmol) in THF, sodium hydride (1.123 g, 46.82 mmol) was added and stirred at 50 °C for 1 h. Propargyl bromide (4.43 mL, 46.82 mmol) was added slowly to the reaction mixture and stirred at room temperature overnight. The progress of the reaction was analysed by TLC; the excess of NaH was quenched by the dropwise addition of water while keeping the reaction flask in an ice bath. The reaction mixture was concentrated under reduced pressure and diluted with water. The compound was extracted with DCM and the organic layer was combined and dried over $MgSO_4$. The reaction mixture was concentrated and purified by column chromatography to obtain a pale yellow oily product. (yield: 4.25 g, 76%).

Molecular formula: $C_{18}H_{30}O_7$

^{1}H NMR (500 MHz, acetone-d_6) δ 4.33-4.16 (m, 4H, 5-H), 4.03-4.0 (m, 2H, 4-H), 3.83-3.46 (m, 11H, 1-, 2-, 3-, 6-H), 2.97-2.91 (m, 1H, 8-H), 1.33 (s, 6H, 11-H), 1.28 (s, 6H, 10-H) ppm.

MS (ESI-TOF) m/z = calculated for $[C_{18}H_{30}O_7Na]^+$: 381.1884; found: 381.2093.

Compound 22



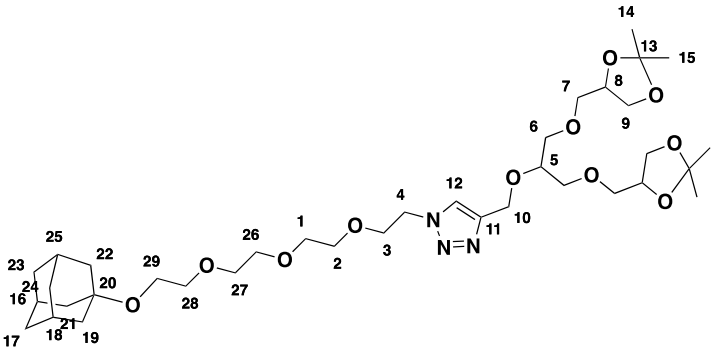
22 was synthesised according to the procedure described for **21**, using the G2-dendron analogue. (yield: 4.05 g, 76%)

Molecular formula: $C_{36}H_{62}O_{15}$

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.32-4.30 (m, 2H, 10-H), 4.25-4.22 (m, 4H, 6-H), 4.05-4.02 (m, 4H, 7-H), 3.73-3.67 (m, 4H, 7-H), 3.69-3.45 (m, 23H, 1-, 2-, 3-, 4-, 5-H), 2.42-2.41 (m, 1H, 12-H), 1.40 (s, 12H, 9-H), 1.34 (s, 12H, 8-H) ppm.

MS (ESI) m/z = calculated for $[C_{36}H_{62}O_{15}Na]^+$: 757.3981; found: 757.3964.

Compound 23



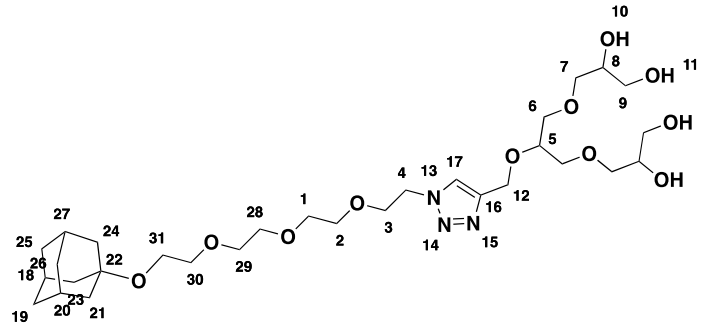
To a mixture of **20** (1 g, 2.82 mmol) and **21** (2 g, 5.65 mmol) in DMF, copper sulphate solution (0.022 g, 0.14 mmol, aq. 0.1 M) and sodium ascorbate (0.11 g, 0.56 mmol, 0.2 M) were added. The reaction mixture was stirred at room temperature overnight. After complete consumption of the azide, checked by IR spectroscopy, the stirring was stopped and solvent was removed under reduced pressure and diluted with water. Organic compound was extracted from the aqueous phase using DCM. The combined organic phase was washed with saturated EDTA solution and water and dried over Na_2SO_4. The reaction mixture was concentrated and purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 3:2) to obtain the pale yellow oily product (1.42 g, yield: 80%).

Molecular formula: $C_{36}H_{61}N_3O_{11}$

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (s, 1H, 12-H), 4.53 (s, 2H, 10-H), 4.24-4.19 (m, 2H, 8-H), 4.03-3.99 (m, 2H, 4-H), 3.88-3.87 (m, 2H, 3-H), 3.73-3.43 (m, 26H, 1-, 2-, 3-, 5-, 6-, 7-, 8-, 9-, 26-, 27-, 28-, 29-H), 2.11 (s, 3H, 16-, 18-, 25-H), 1-71-1.70 (d, 6H, 19-, 21-, 22-H), 1.62-1.54 (m, 6H, 17-, 23-, 24-H), 1.38 (s, 6H, 14-H), 1.32 (s, 6H, 15-H) ppm.

ESI-MS (*m*/*z*): calculated for $[C_{36}H_{61}N_3O_{11}Na]^+$: 734.4306; found 734.7810.

Compound 24 – Ad_1OH_4



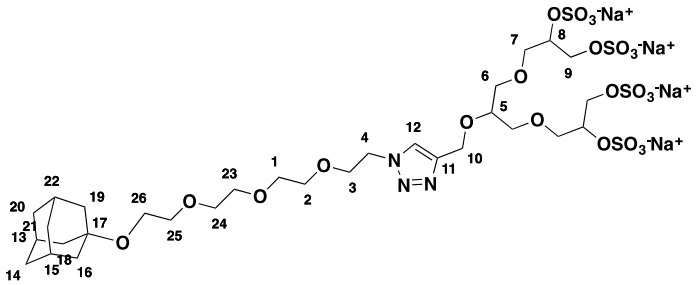
23 (1 g, 1.40 mmol) was dissolved in 1% aq. TFA (10 ml) and stirred at room temperature overnight. Deprotection reaction was monitored by using NMR. After complete deprotection, the solvent was removed and the compound was dissolved in water and dialyzed. Freeze-drying yielded a colourless solid (yield: 0.66 g, 76%).

Molecular formula: $C_{30}H_{53}N_3O_{11}$

^{1}H NMR (300 MHz, MeOD-d_4) δ 8.59 (s, 1H, 17-H), 4.95-4.82 (m, 2H, 12-H), 4.75-4.69 (m, 2H, 8-H), 3.99-3.95 (m, 2H, 4-H), 3.69-3.55 (m, 21H, 3-, 2-, 1-, 28-, 29-, 30-, 31-, 5-, 9-, 7-H), 3.53-3.45 (m, 4H, 6-H), 3.33-3,32 (m, 2H, 7-H), 2.14 (s, 3H, 18-, 20-, 27-H), 1-77 (d, 6H, 21-, 23-, 24-H), 1.72-1.62 (m, 6H, 19-, 26-, 25-H) ppm.

ESI-MS (*m*/*z*): calculated for $[C_{30}H_{53}N_3O_{11}Na]^+$: 654.3680; found 654.3611.

Compound 25 – Ad_1Su_4



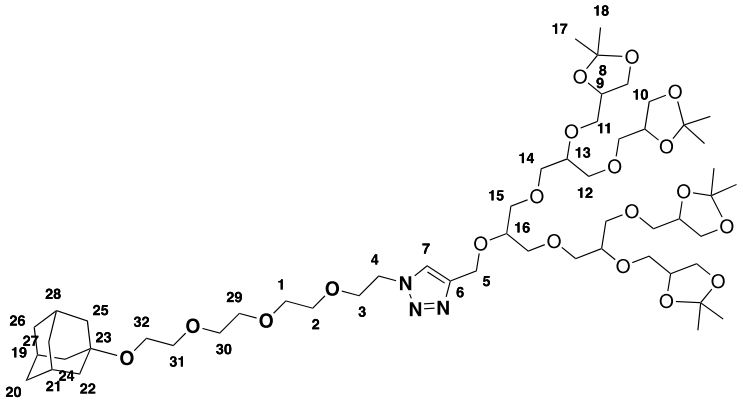
After drying for 18 h under high vacuum at 60 °C, **24** (0.5 g, 0.79 mmol) was dissolved in dry DMF under inert conditions. The solution was heated to 60 °C and sulphur trioxide pyridine complex (2.5 g, 15.8 mmol), dissolved in a minimum amount of dry DMF, was added dropwise. The reaction mixture was stirred for 18 h at 60 °C and further for 48 h at room temperature. Then, the reaction was quenched by adding 10 mL deionized water. To the aqueous solution, 1 M NaOH was added immediately until pH 11 was reached. Solvent was removed under vacuum and was further purified by dialysis (MWCO = 500-1000 Da) in saturated aqueous NaCl solution and water. After dialysis the product was obtained by freeze-drying as colourless solid (yield: 0.52 g, 64%).

Molecular formula: $C_{30}H_{49}N_3Na_4O_{23}S_4$

^{1}H NMR (500 MHz, D_2O) δ 8.18 (s, 1H, 12-H), 4.87-4.80 (m, 2H, 8-H), 4.74-4.67 (m, 4H, 9-H), 4.31-3.28 (m, 2H, 10-H), 4.26-4.22 (m, 2H, 4-H), 4.03-4.02 (t, 2H, 3-H), 3.80-3.64 (m, 21H, 1-, 2-, 23-, 24-, 25-, 26-, 5-, 6-, 7-H), 2.17 (s, 3H, 13-, 15-, 22-H), 1.79 (d, 6H, 16-, 18-, 19-H), 1.70-1.61 (m, 6H, 14-, 21-, 20-H) ppm.

ESI-MS (*m*/*z*): calculated for $[C_{30}H_{49}N_3Na_4O_{23}S_4Na]^+$: 1062.1230; found 1062.1153.

Compound 26



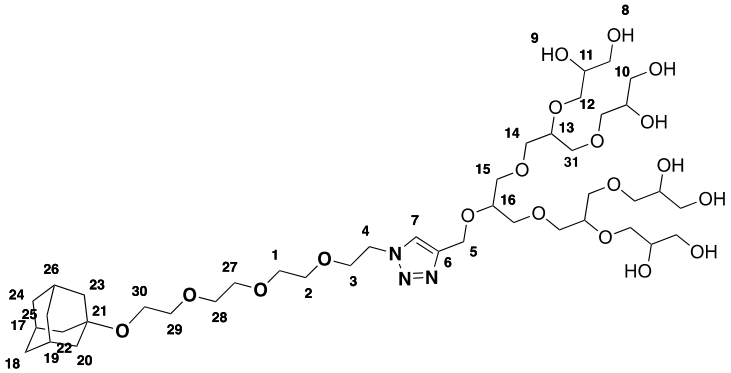
Similar procedure as for **23: 20** (0.5 g, 1.41 mmol) and **22** (2.0 g, 2.82 mmol) were coupled using copper sulphate (0.01 g, 0.07 mmol) and sodium ascorbate (0.05 g, 0.28 mmol) assisted click reaction (yield: 1.19 g, 78%).

Molecular formula: $C_{54}H_{93}N_3O_{19}$

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (s, 1H, 7-H), 4.60 (s, 2H, 5-H), 4.23-4.20 (m, 4H, 20-, 11-H), 4.03-3.92 (m, 8H, 3-, 4-, 10-H), 3.71-3.45 (m, 40H, 1-, 2-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 29-, 30-, 31-, 32-H), 2.11 (s, 3H, 19-, 21-, 28-H), 1-73-1,71 (d, 6H, 22-, 24-, 25-H), 1.63-1.55 (m, 6H, 20-, 26-, 27-H), 1.38 (s, 12H, 17-H), 1.32 (s, 12H, 18-H) ppm.

ESI-MS (*m*/*z*): calculated for $[C_{54}H_{93}N_3O_{19}Na]^+$: 1110.6403; found 1110.4400.

Compound 27 – Ad_1OH_8



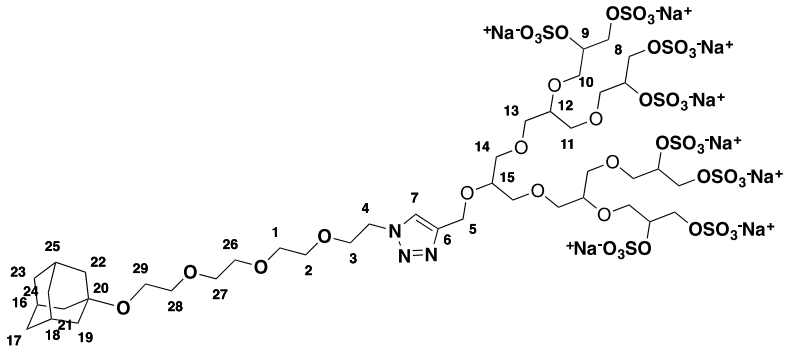
The deprotection was performed by similar procedure as described for **24** (yield: 0.57 g, 68%).

Molecular formula: $C_{42}H_{77}N_3O_{19}$

^{1}H NMR (300 MHz, CD_3OD) δ 8.10 (s, 1H, 7-H), 4.83-4.80 (m, 2H, 5-H), 4.64-4.61 (t, 2H, 4-H), 3.95-3.93 (t, 2H, 3-H), 3.82-3.78 (m, 4H, 11-H), 3.74-3.70 (m, 4H, 10-H), 3.66-3.50 (m, 37H, 10-, 12-, 13-, 31-, 14-, 15-, 16-, 1-, 2-, 27-, 28-, 29-, 30-H), 3.35-3.34 (m, 2H, 31-H), 2.17-2-16 (m, 3H, 17-, 19-, 26-H), 1.80 (d, 6H, 20-, 22-, 23-H), 1.74-1.66 (m, 6H, 18-, 25-, 24-H) ppm.

ESI-MS (*m*/*z*): calculated for $[C_{42}H_{77}N_3O_{19}Na]^+$: 950.5151; found 950.5009.

Compound 28 – Ad_1Su_8



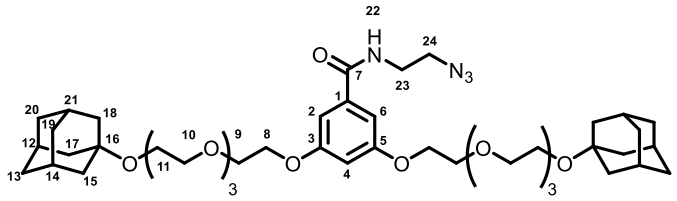
The sulphation was accomplished by using a similar procedure as for **25** using **28** (0.50 g, 0.53 mmol). (yield: 0.55 g, 60%).

Molecular formula: $C_{42}H_{69}N_3Na_8O_{43}S_8$

^{1}H NMR (500 MHz, D_2O) δ 8.18 (s, 1H, 7-H), 4.88 (m, 4H, 9-H), 4.72-4.71 (m, 6H, 5-, 8-H), 4.33-3.24 (m, 6H, 4-, 8-H), 4.04-4.02 (m, 2H, 3-H), 3.84-3.81 (m, 11H, 10-, 11-, 12-, 13-, 15-H), 3.74-3.67 (m, 24H, 1-, 2-, 10-, 11-, 13-, 14-, 26-, 27-, 28-, 29-H), 2.18 (s, 3H, 16-, 18-, 25-H), 1.80 (s, 6H, 19-, 21-, 22-H), 1.71-1.62 (m, 6H, 17-, 23-, 24-H) ppm.

ESI-MS (*m*/*z*): calculated for $[C_{42}H_{69}N_3Na_8O_{43}S_8Na]^+$: 1766.0252; found 1766.8288.

Compound 29



To a solution of **8** (0.5 g, 0.65 mmol, 1eq.) in dry DMF under argon atmosphere, EDC hydrochloride (1.5 eq., 0.975 mmol, 187 mg) and 1-hydroxybenzotriazole hydrate (1.5 eq., 0.975 mmol, 132 mg) were added. After stirring for 30 minutes, N-methylmorpholine (1.5 eq., 0.975 mmol, V = 1.1 mL) and 2-azidoethan-1-amine (1.5 eq., 0.975 mmol, 84 mg) were added and stirred at room temperature overnight. The reaction mixture was quenched by saturated $NaHCO_3$ solution (100 mL) and extracted with DCM (100 mL). The organic phase was further washed with 10 % HCl (1 x 100 mL), water (1 x 100 mL), and brine (1 x 100 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was further purified by column chromatography (ethyl acetate, $R_f = 0.25$). The title product was obtained as colourless oil (400 mg, 73%).

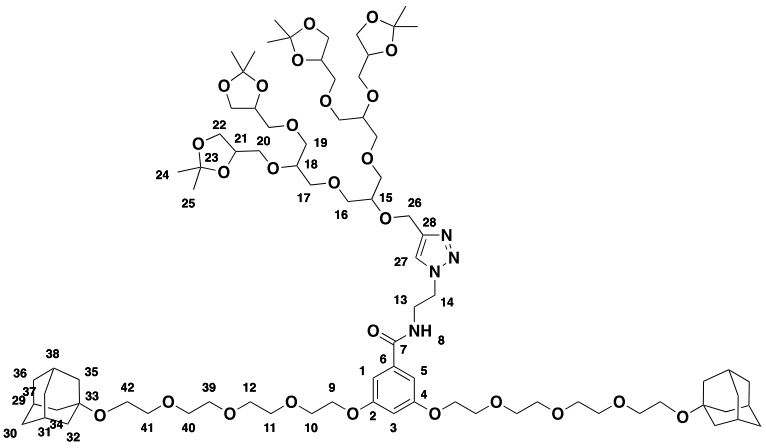
Molecular formula: $C_{45}H_{70}N_4O_{11}$

^{1}H (400 MHz, $CDCl_3$, 298 K): δ = 6.95 (d, 2H, 2-, 6-H), 6.64 (s, 1H, 22-H), 6.62 (m, 1H, 4-H), 4.14 (t, 4H, 8-H), 3.84 (t, 4H, 9-H), 3.73-3.52 (m, 24H, 10-, 11-, 23- and 24-H), 2.12 (s, 6H, 12-, 14-, 21-H), 1.73 (d, 12H, 15-, 17-, 18-H), 1.60 (m, 12H, 13-, 19-, 22-H) ppm.

^{13}C (100 MHz, $CDCl_3$, 298 K): δ = 167.58 (C_q, 7-C), 160.14 (2 C_q, 3-, 5-C), 136.27 (C_q, 1-C), 106.06 (CH, 4-C), 105.23 (2 CH, 2-, 6-C), 72.43 (2 C_q, 16-C), 71.88, 71.38, 70.95, 70.72, 69.77, 67.91 (14 CH_2, 8-, 9-, 10-C), 59.39 (2 CH_2, 11-C), 50.97 (CH_2, 24-C), 41.60 (6 CH_2, 15-, 17-, 18-C), 39.59 (CH_2, 23-C), 36.58 (6 CH_2, 13-, 19-, 20-C), 30.63 (6 CH, 12-, 14-, 21-C) ppm.

HRMS (*m*/*z*): calculated for $[C_{45}H_{70}N_4O_{11}Na]^+$: 865.4939, found: 865.4928.

Compound 30



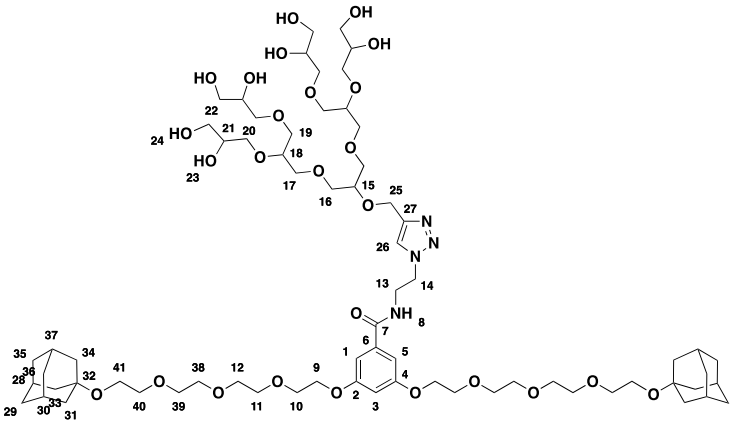
A similar procedure as for **23** was followed for the coupling of **29** (0.50 g, 0.59 mmol) and **22** (1.29 g, 1.77 mmol) using copper sulphate (0.004 g, 0.02 mmol) and sodium ascorbate (0.02 g, 0.11 mmol) assisted click reaction (yield: 0.69 g, 75%).

Molecular formula: $C_{81}H_{132}N_4O_{26}$

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.67 (m, 1H, 27-H), 6.95-6.87 (m, 2H, 1-, 5-H), 6.65-6.56 (m, 1H, 3-H), 4.78-4.74 (m, 2H, 14-H), 4.57-4.55 (m, 2H, 9-H), 4.31-4.30 (m, 2H, 9-H), 4.25-4.19 (m, 6H, 21-, 26-H), 4.12-4.10 (m, 4H, 22-H), 4.04-3.99 (m, 5H, 15-, 18-, 13-H), 3.83-3.80 (m, 4H, 22-H), 3.73-3.59 (m, 36H, 10-, 11-, 12-, 39-, 40-, 41-, 42-, 17-, 19-, 20-H), 3.57-3.46 (m, 12H, 16-, 17-, 19-, 20-H), 2.19-2.03 (m, 6H, 29-, 31-, 38-H), 1.76-1.67 (m, 12H, 32-, 34-, 35-H), 1.63-1.54 (m, 12H, 30-, 37-, 36-H), 1.39-1.38 (m, 12H, 24-H), 1.34-1.33 (m, 12H, 25-H) ppm.

ESI-MS (*m*/*z*): calculated for $[C_{81}H_{132}N_4O_{26}Na]^+$: 1599.9130; found 1599.9100.

Compound 31



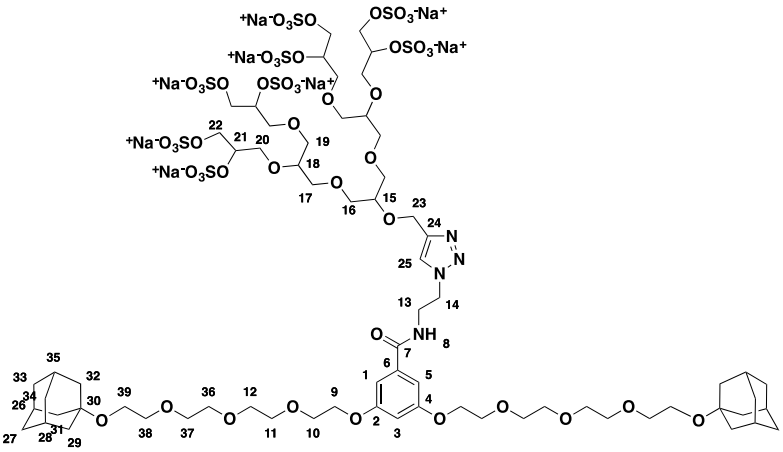
The deprotection was performed by similar procedure as **24** (yield: 0.40 g, 70%).

Molecular formula: $C_{69}H_{116}N_4O_{26}$

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.85 (s, 1H, 26-H), 6.98-6.92 (m, 2H, 1-, 5-H), 6.61 (s, 1H, 3-H), 4.85-4.65 (m, 2H, 14-H), 4.12 (bs, 6H, 9-, 25-H), 4.02-3.53 (m, 65H, 13-, 15-, 16-, 17-, 18-, 19-, 20-, 21-, 22-, 10-, 11-, 12-, 38-, 39-, 40-, 41-H), 2.13 (s, 6H, 28-, 30-, 37-H), 1.79-1.72 (m, 12H, 31-, 33-, 34-H), 1.67-1.56 (m, 12H, 29-, 36-, 35-H) ppm.

ESI-MS (*m*/*z*): calculated for $[C_{81}H_{132}N_4O_{26}Na]^+$: 1439.7878; found 1439.7818.

Compound 32 (Ad_2Su_8)



The sulphation was done by using a similar procedure as for **25** (yield: 60%).

Molecular formula: $C_{69}H_{108}N_4Na_8O_{50}S_8$

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.10 (s, 1H, 25-H) 7.00-6.97 (m, 2H, 1-, 5-H), 6.84 (s, 1H, 3-H), 4.39-4.18 (m, 12H, 9-, 22-H), 3.79-3.51 (m, 56H, 10-, 11-, 12-, 36-, 37-, 38-, 39- 13-, 14-, 23-, 15-, 16-, 17-, 18-, 19-, 20-, 21-H), 2.05-1.98 (m, 6H, 26-, 28-, 35-H), 1.68-1.59 (m, 12H, 29-, 31-, 32-H), 1.49-1.47 (m, 12H, 27-, 33- 34-H) ppm.

2c. Synthesis route of Ad_2Man_8

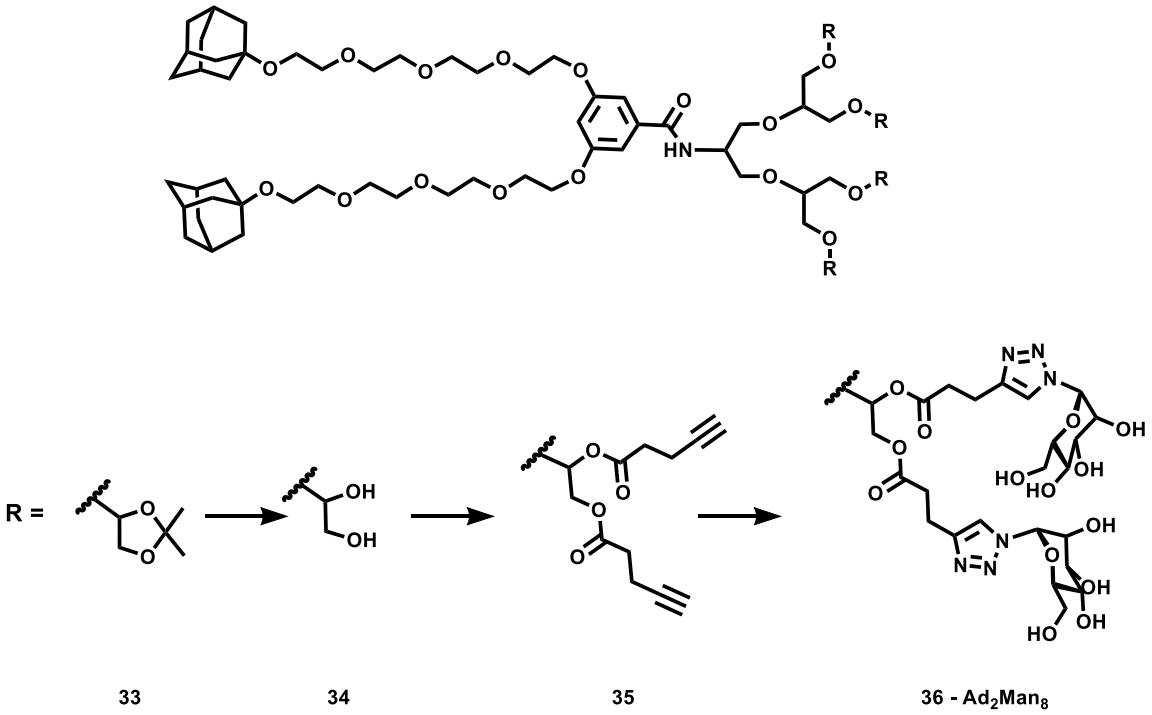
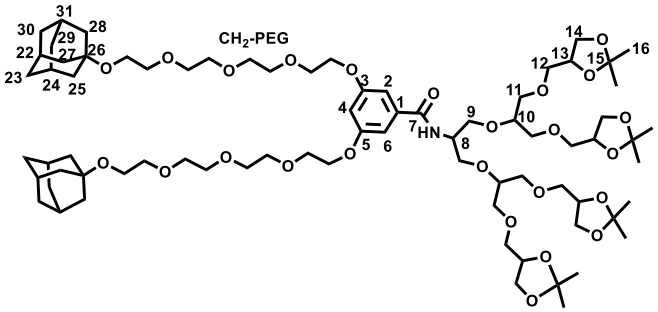


Figure S4. Synthesis routes of octa-mannoside ligand **Ad_2Man_8**.

Compound 33



Diadamantane-carboxylic acid **8** (400 mg, 0.52 mmol) and G2-Amine[1] (539 mg, 0.77 mmol) were dissolved in dry THF (10mL) under argon atmosphere. After cooling to 0°C, EDC•HCl (148 mg, 0.77 mmol) and catalytic amounts of DMAP were added to the reaction mixture, which was kept stirring overnight while warming to room temperature. Afterwards, the solvent was removed under reduced pressure. The residue was dissolved in DCM and washed with $NaHCO_3$ solution, brine and water. The organic layer was dried over Na_2SO_4 before being concentrated in vacuo. The crude product was purified

by column chromatography (Silica, DCM/MeOH (0-4%)), which yielded the desired product as yellow oil (523 mg, 69%).

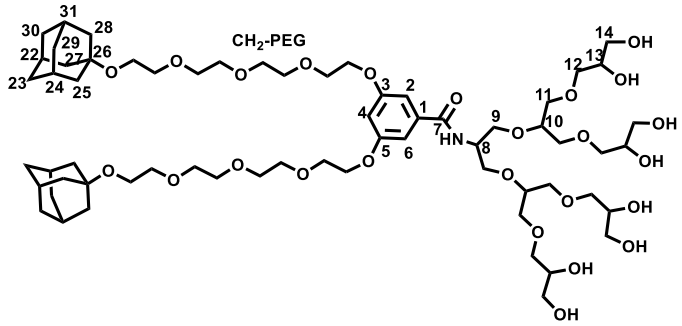
Molecular formula: $C_{76}H_{125}NO_{25}$

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.28-1.32 (m, 12H, H-16), 1.34-1.38 (m, 12H, H-16), 1.64 (q, J = 12.6 Hz, 12H, H-23,-29,-30), 1.75 (d, J = 2.7 Hz, 12H, H-25,-27,-28), 2.11 (s, 6H, H-22,-24,-31), 3.45-3.71 (m, 50H, CH_2-PEG/H-8-12,-14), 3.84-3.85 (m, 4H, CH_2-PEG), 3.97-4.04 (m, 4H, H-14), 4.15-4.17 (m, 4H, CH_2-PEG), 4.18-4.23 (m, 4H, H-13), 6.70-6.71 (m, 1H, H-4), 7.01-7.03 (m, 2H, H-2,-6) ppm.

^{13}C NMR (100 MHz, Methanol-d_4): δ = 24.43, 25.87, 30.66, 36.21, 41.26, 48.56, 59.15, 66.29, 66.41, 67.70, 69.47, 70.29, 70.33, 70.51, 70.93, 71.22, 72.19, 72.29, 74.82, 74.95, 78.58, 106.06, 109.17, 136.49, 160.22, 168.18 ppm.

ESI-TOF (*m*/*z*): calculated for $[C_{76}H_{125}NO_{25}H]^+$: 1452.8613, found 1452.8633; $[C_{76}H_{125}NO_{25}Na]^+$: 1474.8433, found 1474.8448.

Compound 34



Acetal-protected diadamantane-G2 **33** (490 mg, 0.34 mmol) was dissolved in 10 mL methanol. After addition of Dowex-H (2 eq, by weight), the reaction mixture was kept stirring until complete conversion was reached. The reaction was monitored by TLC and NMR. After completion, Dowex-H was filtered off and washed with methanol. The filtrate was concentrated in vacuo yielding the title compound as slight yellow oil (415 mg, 95%).

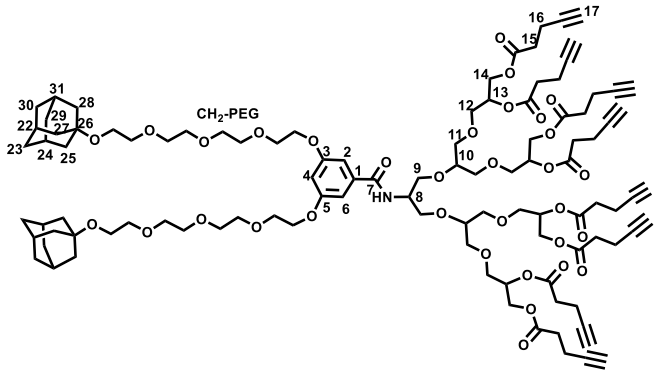
Molecular formula: $C_{64}H_{109}NO_{25}$

^{1}H NMR (400 MHz, Methanol-d_4): δ = 1.64 (q, J = 12.4 Hz, 12H, H-23,-29,-30), 1.75 (d, J = 2.7 Hz, 12H, H-25,-27,-28), 2.11 (s, 6H, H-22,-24,-31), 3.44-3.76 (m, 50H, CH_2-PEG/H-8-14), 3.84-3.86 (m, 4H, CH_2-PEG), 4.15-4.17 (m, 4H, CH_2-PEG), 6.70-6.71 (m, 1H, H-4), 7.01-7.02 (m, 2H, H-2,-6) ppm.

^{13}C NMR (100 MHz, Methanol-d_4): δ = 30.66, 36.19, 41.23, 59.13, 63.14, 67.66, 69.46, 70.30, 70.46, 70.89, 71.09, 71.64, 72.33, 72.67, 78.55, 105.98, 136.44, 160.18, 168.41 ppm.

ESI-TOF (*m*/*z*): calculated for $[C_{64}H_{109}NO_{25}Na]^+$: 1314.7181; found 1314.7256.

Compound 35



34 (50 mg, 0.039 mmol) was dissolved in dry THF under argon atmosphere. After cooling to 0°C, 4-pentynoic acid (61 mg, 0.62 mmol), EDC•HCl (119 mg, 0.62 mmol) and catalytic amounts of DMAP were added. The reaction solution was kept stirring for 16 h while warming to room temperature. Afterwards, the solvent was removed under reduced pressure. The residue was dissolved in DCM and washed with $NaHCO_3$ solution and water. The organic layer was dried over Na_2SO_4 before being concentrated in vacuo. The crude product was purified by column chromatography (Silica, ethyl acetate). The pure product was obtained as slight yellow oil (72 mg, 93%).

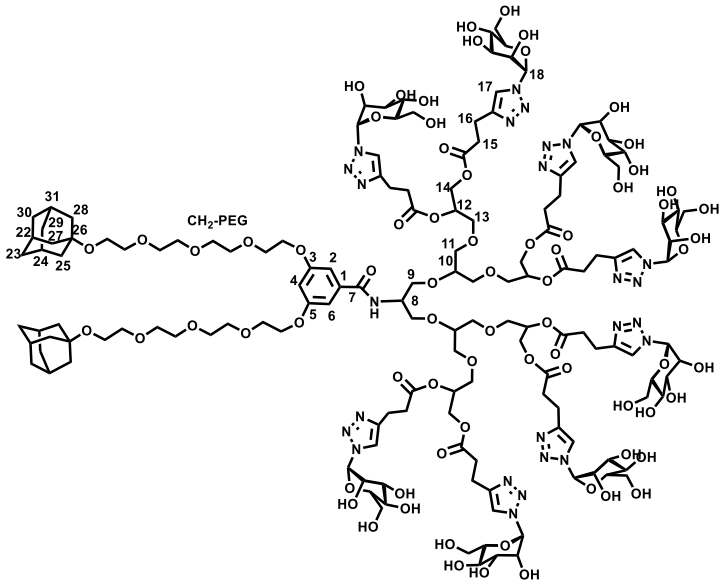
Molecular formula: $C_{104}H_{141}NO_{33}$

^{1}H NMR (700 MHz, Methanol-d_4/Aceton-d_6): δ = 1.65 (q, J = 12.2 Hz, 12H, H-23,-29,-30), 1.76 (s, 12H, H-25,-27,-28), 2.13 (s, 6H, H-22,-24,-31), 2.33 (s, 8H, H-17), 2.44-2.50 (m, 16H, H-16), 2.53-2.59 (m, 16H, H-15), 3.59-3.87 (m, 50H, CH_2-PEG/H-8-14), 4.19-4.42 (m, 13H, CH_2-PEG/H-8-14), 5.18-5.21 (m, 4H, H-13), 6.72 (s, 1H, H-4), 7.03 (s, 2H, H-2,-6) ppm.

^{13}C NMR (176 MHz, Methanol-d_4/Aceton-d_6): δ = 13.72, 30.58, 32.92, 33.07, 33.09, 36.16, 41.21, 59.10, 62.65, 67.66, 68.76, 68.98, 69.23, 69.39, 70.30, 70.42, 70.56, 70.88, 71.11, 71.30, 72.14, 78.74, 82.24, 104.28, 106.00, 136.45, 160.14, 167.81, 171.29, 171.56 ppm.

ESI-TOF (*m*/*z*): calculated for $[C_{104}H_{141}NO_{33}Na]^+$: 1954.9278; found 1954.9352; $[C_{104}H_{141}NO_{33}Na_2]^{2+}$: 988.9585; found 988.9656.

Compound 36 – Ad_2Man_8



α-D-mannopyranosyl monoazide[2] (51 mg, 0.248 mmol) and **35** (30 mg, 0.015 µmol) were dissolved in 2 mL of dry DMF. A small piece of copper wire was added to the solution. Copper(II)sulphate pentahydrate (4 mg, 0.015 µmol) and sodium ascorbate (12 mg, 0.060 mmol) were dissolved in 0.5 mL water each and mixed before being added to the reaction solution, which was stirred at 50°C for 4d. Afterwards, the solvent was evaporated and the residue was re-dissolved in water. Remaining salts were removed by ultrafiltration (MWCO = 1 kDa) in water. Purification of the crude product by HPLC (H_2O/MeOH 20-80%) and subsequent lyophilisation yielded the desired product as a white solid (23 mg, 43%).

Molecular formula: $C_{152}H_{229}N_{25}O_{73}$

^{1}H NMR (700 MHz, D_2O): δ = 1.54-1.62 (m, 12H, H-23,-29,-30), 1.71 (s, 12H, H-25,-27,-28), 2.09 (s, 6H, H-22,-24,-31), 2.82 (s, 16H, H-15), 3.06 (s, 16H, H-16), 3.37-4.80 (m, 112H, H-PEG, -Dendron, -Mannose), 5.24 (s, 4H, H-12), 6.14 (s, 8H, H-18), 6.82 (s, 1H, H-4), 7.11 (s, 2H, H-2/-6), 8.04 (s, 8H, H-17) ppm.

^{13}C NMR (176 MHz, MeOD): δ = 20.23, 20.30, 30.51, 32.76, 32.96, 35.99, 41.00, 58.98, 60.88, 62.81, 66.32, 66.99, 67.60, 68.66, 69.26, 69.34, 69.95, 70.04, 70.19, 70.63, 70.76, 71.05, 72.87, 73.40, 76.82, 79.82, 86.73, 106.08, 112.37, 136.16, 146.41, 159.96, 172.58, 172.91 ppm.

ESI-TOF (*m*/*z*): calculated for $[C_{152}H_{229}N_{25}O_{73}Na_2]^{2+}$: 1809.2380; found 1809.2501; $[C_{152}H_{229}N_{25}O_{73}Na_3]^{3+}$: 1213.8217; found 1213.8320.

3. ITC plots for all ligands

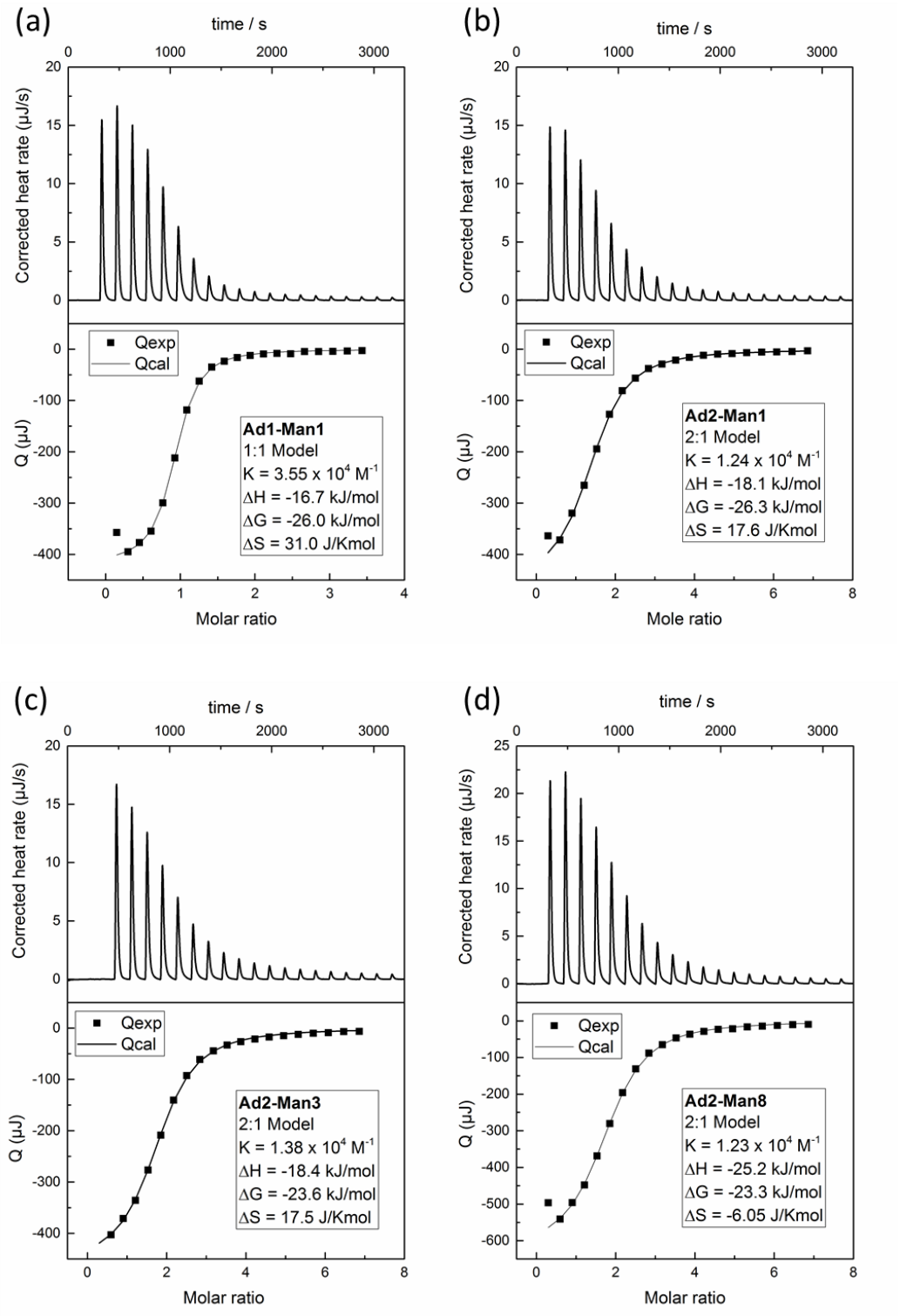


Figure S5. Physicochemical properties of adamantane-mannose ligands determined by ITC (a) **Ad_1Man_1**, (b) **Ad_2Man_1**, (c) **Ad_2Man_3** and (d) **Ad_2Man_8**.

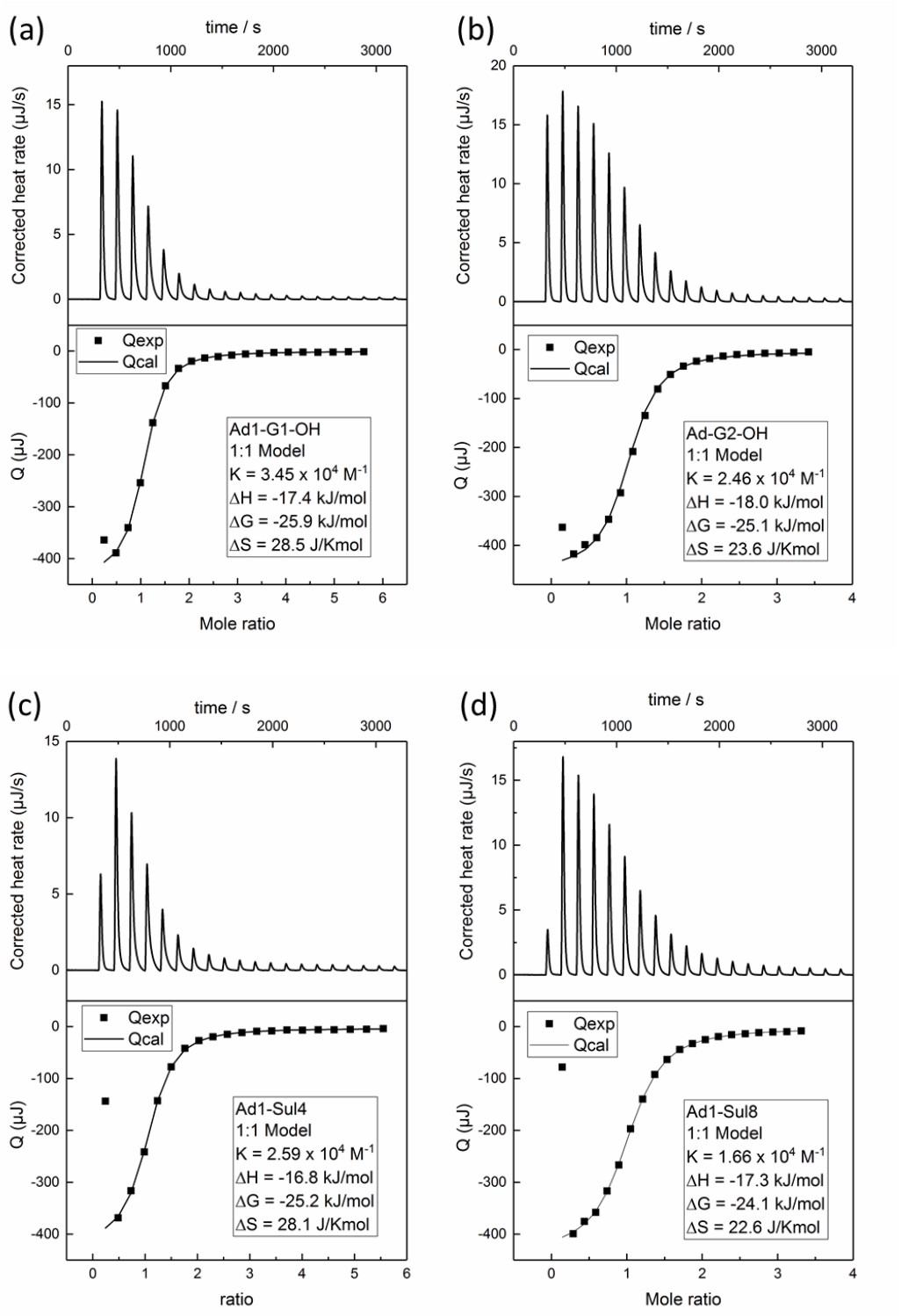


Figure S6. Physicochemical properties of adamantane-dendron and adamantane-sulphates ligands determined by ITC (a) **Ad$_1$OH$_4$**, (b) **Ad$_1$OH$_8$**, (c) **Ad$_1$Su$_8$** and (d) **Ad$_1$Su$_8$**.

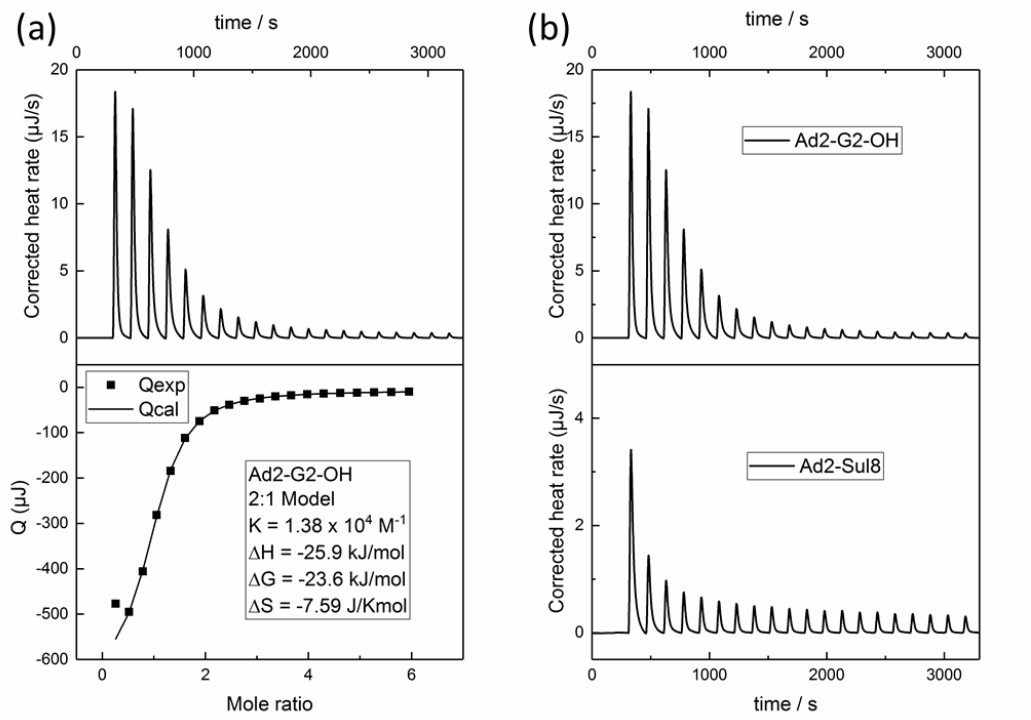


Figure S7. (a) ITC of **Ad_2OH_8** (Ad2-G2-OH) and (b) comparison of ITC plots of **Ad_2OH_8** and **Ad_2Su_8**.

4. OD_{400} agglutination assay for surface coverage dependent measurements

Binding properties of mannoside-functionalised vesicles were further analysed with respect to the dependence between the vesicle's surface coverage with ligands and their ability to form agglomerates. The agglutination of vesicles, which was mediated by binding to ConA, was observed by optical density measurements at a fixed wavelength of λ = 400 nm (OD_{400}). Concentrations of vesicles and mannoside ligands were chosen so that, in general, every cyclodextrin formed an inclusion complex with an adamantyl residue. This concentration relation is referred to as 100% surface coverage.

In a first experiment, the agglutination of the vesicles with different ligands was tested (Figure S8b). Here, all vesicles exhibited a surface coverage of 100%. All tested ligand-vesicles complexes showed high capabilities to provoke agglutination, as can be seen by the increasing optical absorption with time. Additionally, Ad_2-Man_1 not only showed agglutination but also an additional cross-linking of CDVs even in absence of ConA (area between green and red arrow in Figure S8c. This behaviour was observed and described before by Kauscher et al.[3] and can be understood from the ability of the two adamantyl residues to bind to two different vesicles, hence, inducing intervesicular crosslinking. For larger mannoside complexes (**Ad_2Man_3** and **Ad_2Man_8**), the self-aggregation from CDVs was no longer observed due to the highly branched and sterically demanding mannoside structures.

In order to investigate the dependence between CDV's surface coverage and their aggregation behaviour, a second set of experiments with changing carbohydrate concentrations on the CDVs was performed. A lower concentration of ligands on the vesicle's surface was achieved by replacing the mannosides with inactive adamantyl tetraethyleneglycol (**Ad-TEG-OH**). The ratio between active and inactive ligands was varied between 10% and 100%, in order to receive the desired surface coverage. Ligand-concentration dependent aggregation was observed in all cases (Figures S9). Figure S8c compares the measured absorbance at a fixed time (t = 40 min) for different surface coverage and different ligands. A bisected behaviour with two distinct regions was observed, which are indicated by grey lines as guide to the eye in Figure S8c. For surface coverage below 20%, the gain in absorbance is rather steep for all ligands. With further surface coverage, the curve flattens drastically and no substantial increase of aggregation is observed. Hence, a threshold of around 20% surface coverage for effective aggregation can be deduced from the measured data. Furthermore, no significant difference was observed for the three ligands tested. This behaviour can be explained by considering the assay setup, i.e., to initiate aggregation, two functionalised CDVs need to bind through their ligands to one of the four binding sites of the same ConA. Here, the valency of the ligand plays an inferior role, as a simple monovalent binding in both cases already leads to aggregation. By considering the dimensions of the CDVs compared to ConA, it appears to be obvious that an increased ligand concentration after a certain threshold does not affect the aggregation substantially due to steric reasons. At the utmost, the strength and stability of the aggregate might be increased due to additional crosslinking. The selectivity of our supramolecular scaffold was confirmed by additional OD400 experiments. A similar setup as before was applied, while the lectin PNA instead of Con A was added to the guest functionalised CDV solutions. Peanut agglutinin (PNA) is a plant lectin protein and binds to galactose. As can be seen in Fig. S8b, the addition of PNA induced only a slightly increased optical density, which can be attributed to light scattering by PNA at 400 nm. The optical density remained constant without further increase, showing that our system does not show unspecific binding, but bound specifically to mannose receptors at Con A.

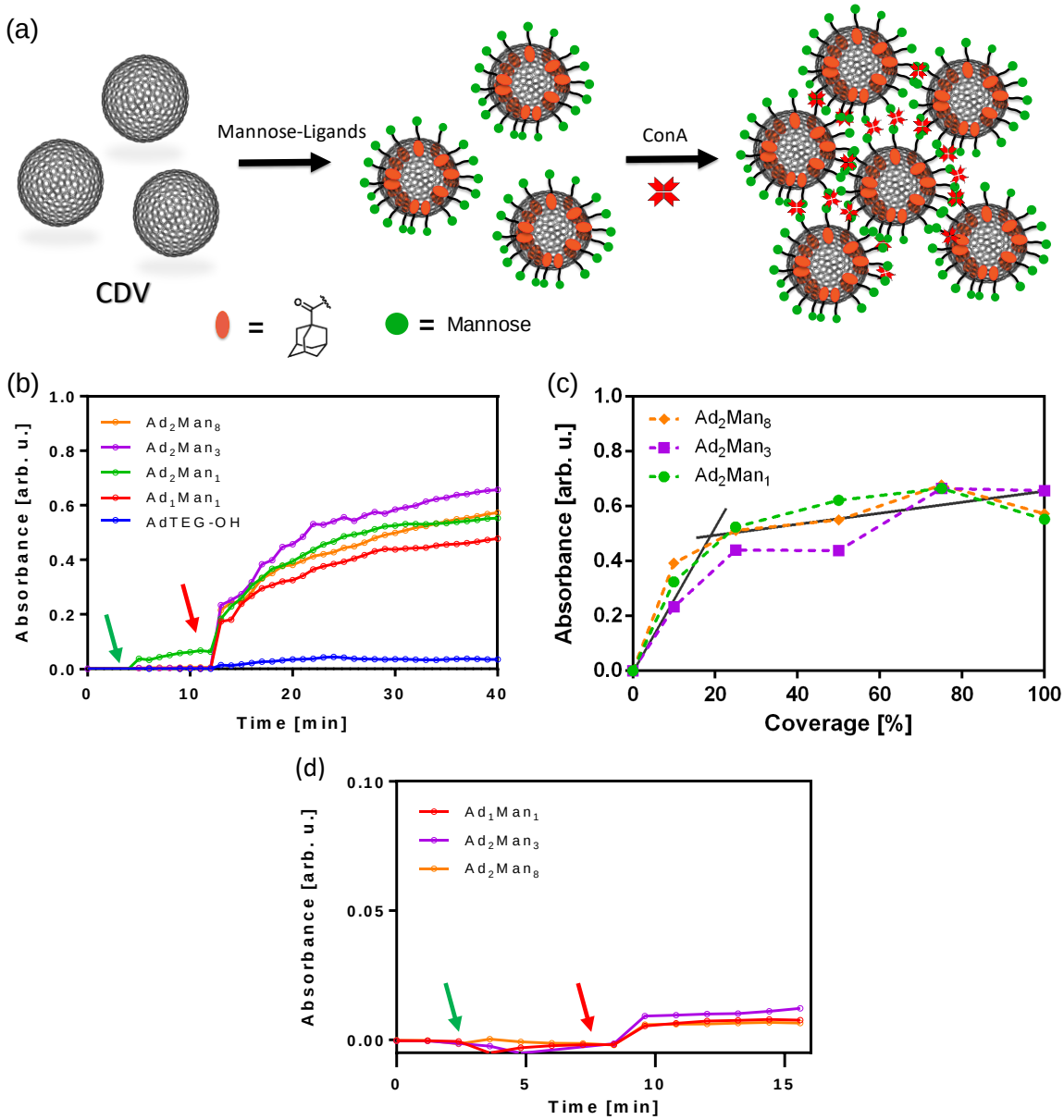


Figure S8. Optical density measurements at λ = 400 nm. (a) Schematic presentation of the aggregation experiment. (b) Agglutination of vesicles with 100% surface coverage of different ligands in the presence of 0.1 mg/mL ConA over time. Arrows represent the addition of ligands (green) and ConA (red). (c) Agglutination at t = 40 min of CDV + mannoside ligands in relation to the ligand surface coverage. Connection lines and grey. lines are guides to the eye. (d) Agglutination experiment with functionalised vesicles and PNA. Arrows represent the addition of ligands (green) and PNA (red) - Note the expanded y-scale. All measurements were carried out in HEPES buffer (pH 7.4, 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MnCl_2$).

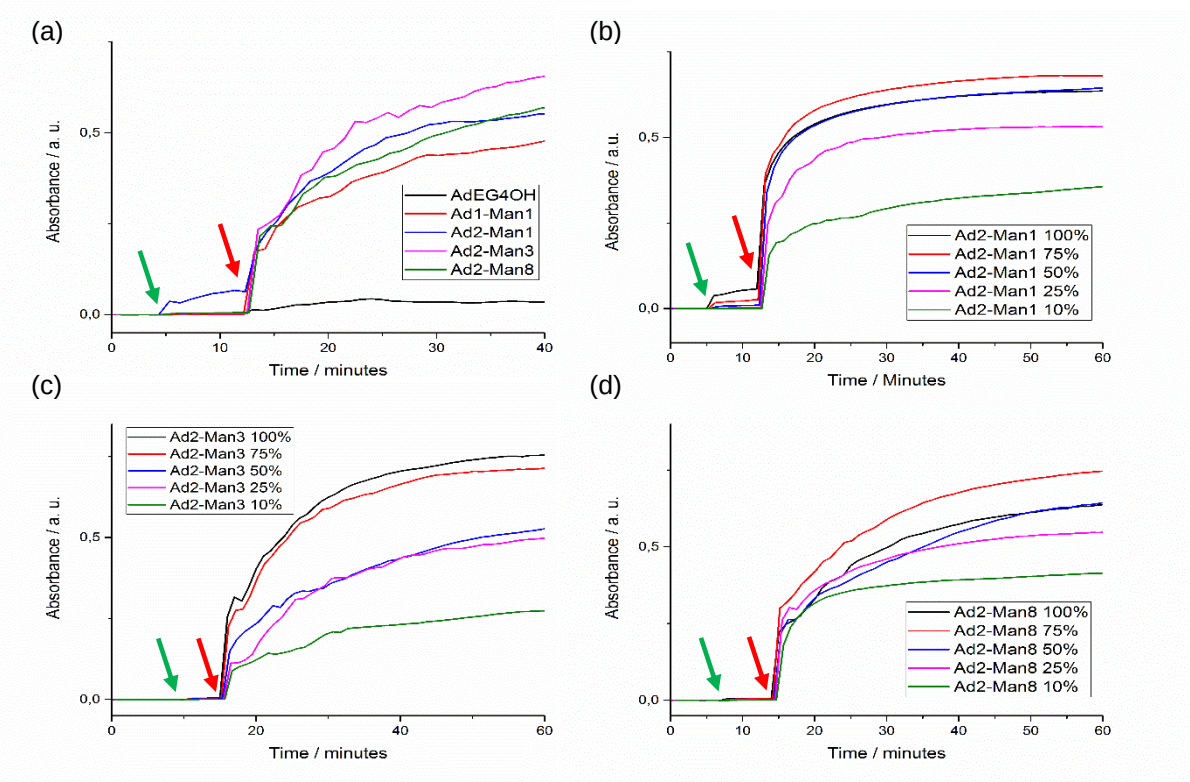


Figure S9. (a) Agglutination of 100% coverage of different ligands on vesicles. Reduced agglutination with respect to decreased surface coverage of (b) **Ad$_2$Man$_1$**, (c) **Ad$_2$Man$_3$,** and (d) **Ad$_2$Man$_8$** functionalised vesicles. Percentage values were obtained by mixtures with guest **Ad-TEG-OH**. All measurements were carried out in the presence of 0.1 mg/mL ConA.

5. Additional information for SPR experiments

5a. Binding isotherms for all ligands and CDV ligand complexes

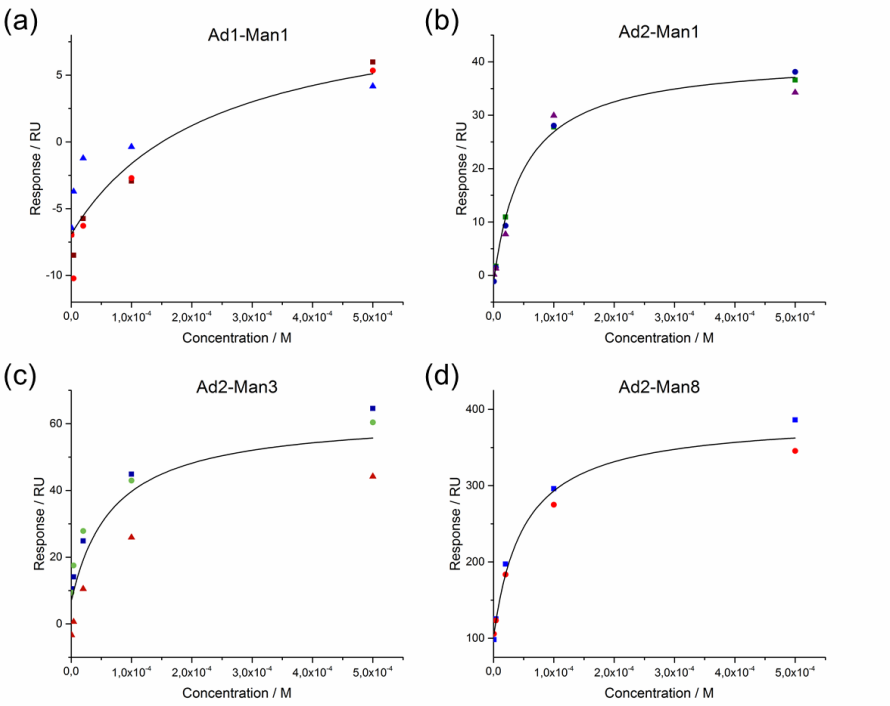


Figure S10. Resulting binding isotherms derived from single-cycle kinetic measurements of (a) **Ad$_1$Man$_1$**, (b) **Ad$_2$Man$_1$**, (c) **Ad$_2$Man$_3$** and (d) **Ad$_2$Man$_8$**.

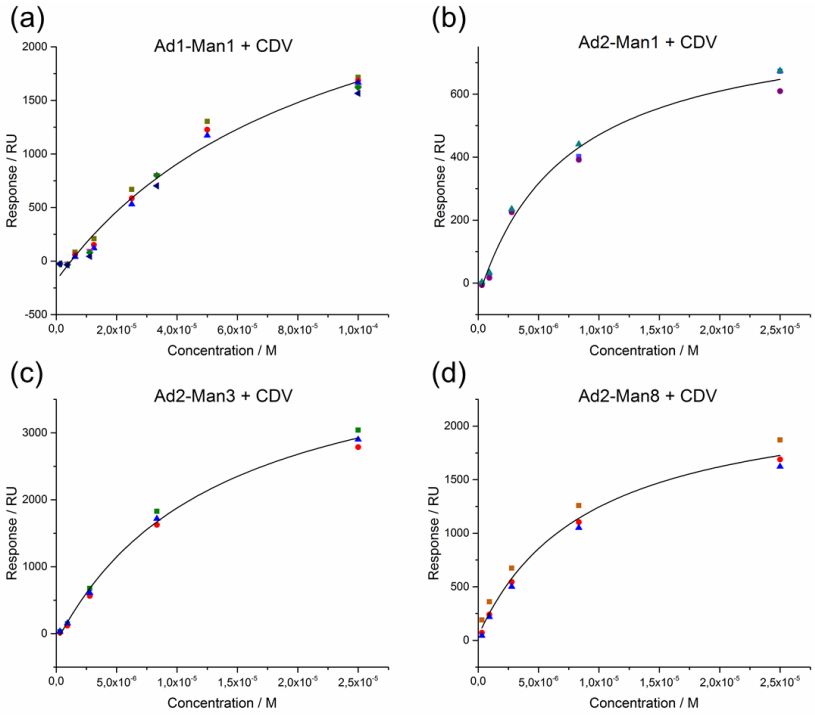


Figure S11. Resulting binding isotherms derived from single-cycle kinetic measurements of guest-functionalised CDVs (a) **Ad$_1$Man$_1$**, (b) **Ad$_2$Man$_1$**, (c) **Ad$_2$Man$_3$**, and (d) **Ad$_2$Man$_8$**.

5b. Addition of γ-CD

γ-CD was able to prevent binding of adamantyl moieties to the dextran layer, however, reversible binding to the CDVs was still favoured due to the much higher binding affinity of adamantane to β-CD. According to reported literature, the affinity between adamantane and γ-CD (K_a = ~10^2 M^{-1}) was two orders lower than for β-CD (K_a = ~10^4 M^{-1}).[4] In order to prove that the addition of γ-CD did not affect our supramolecular system, control experiments were performed. Shape and size consistency of the vesicles after the addition of γ-CD was confirmed by DLS and Cryo-TEM experiments. Determined hydrodynamic diameters were found to remain unchanged, which indicates morphological stability upon addition of γ-CD (Fig. S12a). The corresponding micrographs obtained from cryo-TEM experiments showed likewise mono- and multilayer vesicles with intact membranes. Secondly, the influence of addition γ-CD on agglutination of ligand-decorated CDV in the presence of ConA was studied by OD_{400} measurements (Figure S12b). **Ad_2Man_3** and **Ad_2Man_8** were applied as test ligands. It can be seen that the effect of additional γ-CD is of minor influence since all of the measurements show a similar absorbance and aggregation rate. These experiments supported our claims that our SPR setup gave us reliable results.

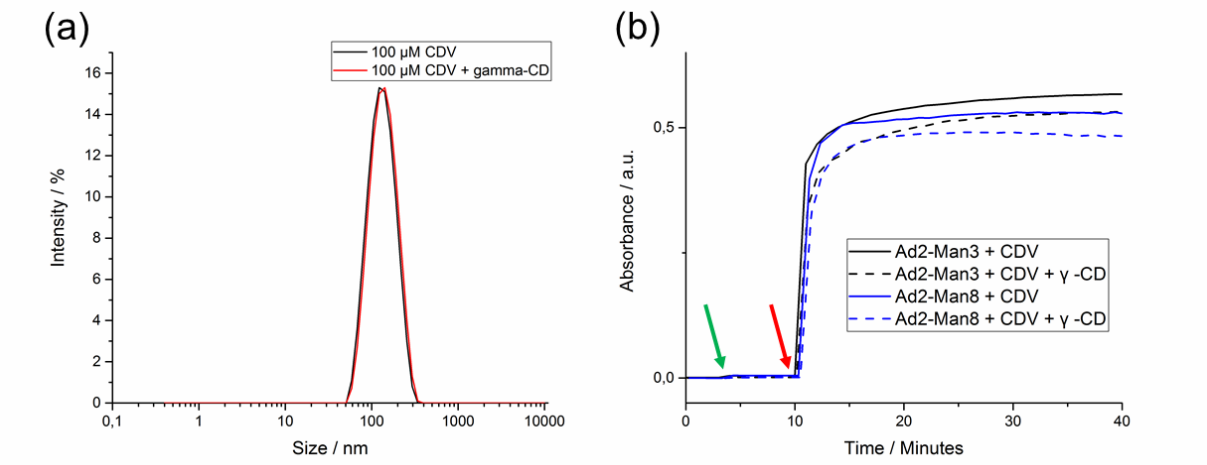


Figure S12. (a) Size of CDV and in presence of 10 mM γ-CD determined by DLS. (b) Agglutination of ligand-decorated CDV in presence and absence of 10 mM γ-CD with 0.1 mg/mL lectin ConA (OD_{400}). Arrows indicate the addition of ligands (green) and ConA (red).

[1] M. Wyszogrodzka, K. Möws, S. Kamlage, J. Wodzińska, B. Plietker, and R. Haag, *Eur. J. Org. Chem.* **2008**, 53-63.

[2] P. Bojarová, L. Petrásková, E. E. Ferrandi, D. Monti, H. Pelantová, M. Kuzma, P. Simerská, and V. Křen, *Adv. Synth. Catal.* 2007, **349**, 1514-1520.

[3] U. Kauscher and B. J. Ravoo, *Beilstein J. Org. Chem.*, 2012, **8**, 1543–1551.

[4] N. Taulier and T. V. Chalikian, *J. Phys. Chem. B.* 2008, **112**, 9546-9549.