SUPPLEMENTARY INFORMATION

Glycocalix[4]arenes and their affinity to a library of galectins: the linker matters

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Galectins are lectins that bind β-galactosides. They are involved in important extra- and intracellular biological processes such as apoptosis, and regulation of the immune system or the cell cycle. High-affinity ligands of galectins may introduce new therapeutic approaches or become new tools for biomedical research. One way of increasing a low affinity of β-galactoside ligands to galectins is their multivalent presentation, *e.g.*, using calixarenes. We report on the synthesis of glycocalix[4]arenes in cone, partial cone, 1,2-alternate, and 1,3-alternate conformations carrying a lactosyl ligand on three different linkers. The affinity of the prepared compounds to a library of human galectins was determined using competitive ELISA assay and biolayer interferometry. Structure-affinity relationships regarding the influence of the linker and of the core structure were formulated. Substantial differences were found between various linker lengths and the position of the triazole unit. The formation of supramolecular clusters was detected by atomic force microscopy. The present work gives a systematic insight into prospective galectin ligands based on the calix[4]arene core.

Content:

1. Materials

Dibutyltin oxide, Amberlite IR-120, and (3-dimethylamino-propyl)-ethyl-carbodiimide hydrochloride (EDC·HCl) were purchased from Fluka (Steinheim, Germany). 1,4-Dioxane, *N*,*N*-diisopropyl ethyl amine (DIPEA), and [2-(2-chloroethoxy)ethoxy]ethan-2-ol were bought from Alfa Aesar (Kandel, Germany). tetra-*N*-Butylammonium bromide (TBAB), absolute methanol, absolute *N*,*N*-dimethylformamide (DMF), and acetic anhydride were purchased from Acros Organics (Geel, Belgium). Lactose was bought from Lachema NP (Brno, Czech Republic). 1-Hydroxybenzotriazole hydrate (HOBt), potassium trimethylsilanolate, and stannous chloride dihydrate were purchased from Sigma-Aldrich (Germany, Steinheim). Triethylamine, sodium hydrate, and 100% fuming nitric acid were purchased from Merck (USA, Massachusetts). Cesium carbonate, 4-pentynoic acid, and deuterated solvents were purchased from Fluorochem (United Kingdom, Hadfield). If not specified otherwise, all other chemicals were purchased from Sigma-Aldrich (Germany, Steinheim) or VWR Chemicals (Czech Republic, Stříbrná Skalice).

2. Analytical methods

2.1. Nuclear magnetic resonance (NMR)

Compound **9** was measured on a Bruker Avance III 400 MHz spectrometer at 20 °C in $CDCl_3$; spectra were referenced to the residual signals of chlorofom (δ_H 7.263 ppm, δ_C 77.01 ppm). NMR experiments of compounds **14c**, **15b**, **15c**, **17** and **18** were performed on a Agilent 400-MR DDR2 spectrometer and Bruker Avance III 500 MHz spectrometer. ^{1}H NMR, and ^{13}C NMR spectra were recorded using standard manufacturer's software. Chemical shifts are given in δ-scale with digital resolution justifying the reported values to two decimal places. ^{1}H NMR spectra of compounds were measured using Agilent 400-MR DDR2 spectrometer (399.94 MHz) in $CDCl_3$ (compounds **14c**, **15b**, **15c**) or CD_3OD (compounds **17**, **18**) as a solvent. The ^{1}H NMR spectra of all samples were acquired in 5-mm NMR tubes and the conditions used for measurements were as follows: $\pi/2$ pulse width 4.75 μs, relaxation delay 1 s, acquisition time 2.556 s, spectral width 6410.3 Hz and usually 8 scans. ^{13}C NMR spectra of compounds **14c**, **15b**, **15c** and **17** were measured using Agilent 400-MR DDR2 spectrometer; compound **18** was measured using Bruker Avance III 126 MHz spectrometer. For compounds **14c**, **15b**, and **15c**, $CDCl_3$ was used as a solvent, and for compounds **17**, **18**, it was CD_3OD.

NMR spectra of glycocalix[4]arenes **21**-**31** were acquired on a Bruker Avance III 700 MHz spectrometer (Bruker BioSpin, Rheinstetten, Germany) at 30 °C using standard manufacturer's software. Compounds **21**, **23**, and **25** were measured in DMSO-d_6, compounds **27**, **29,** and **31** in D_2O (compound **27** after addition of 20% DMSO-d_6, compound **31** of 10% CD_3OD). Spectra were referenced to residual solvent signals (DMSO-d_6: δ_H 2.499 ppm and δ_C 39.46 ppm, D_2O: δ_H 4.732 ppm), carbon spectra in D_2O to the signal of acetone (δ_C 30.50 ppm). Individual spin systems in glycocalix[4]arenes were assigned by COSY and HSQC experiments and joined together with quaternary carbons using information extracted from the HMBC experiment. Due to the presence of lactosyl moiety in the molecules, some signals were substantially broadened, and thus complicated unambiguous structural assignment.

2.2. High-performance liquid chromatography (HPLC) and liquid chromatography with mass spectrometry detection (LC-MS)

HPLC and LCMS analyses were measured on Shimadzu Prominence LC analytical system comprising Shimadzu CBM-20A system controller, Shimadzu LC-20AD binary HPLC pump, Shimadzu CTO-10AS column oven, Shimadzu SIL-20ACHT cooling autosampler, Shimadzu SPD-20MA diode array detector, and Shimadzu LCMS-2020 mass detector (Shimadzu, Japan). HPLC analyses (samples **21**, **23**, **25**, **27**, **29**, and **31**) were performed on Chromolith FastGradient RP-18e column (50 × 3 mm, monolith) preceded by Chromolith RP 18-e guard column (5 × 4.6 mm, Merck, Germany) in water, with gradient elution (**A** = water, **B** = MeOH): 0% **B** for 0 min, 0-95% **B** for 0-7 min, 95% **B** for 7-9 min, and 95-0% **B** for 9-11, 11-14 min for column equilibration, flow rate 1.0 mL/min, 25 °C, injection volume 1 μL. PDA data were acquired at 200-380 nm and the maximum signal for each compound was extracted. For mass spectrometry, samples were dissolved in MeOH (MeOH/H_2O 4:1; 100 μL/min) and introduced into mobile phase flow (acetonitrile) using a 2 μL loop. Spray voltage, capillary voltage, tube lens voltage, and capillary temperature were 4.0 kV, −16 V, −120 V, and 275 °C, respectively.

2.3. High-resolution mass spectrometry (HRMS)

The electrospray-ionization (ESI) spectra of compounds **14b**, **15b**, **15c**, **17**, and **18** were measured using LTQ Orbitrap Velos hybrid - hybrid ion-trap-orbitrap FT Mass spectrometer (ThermoFisher Scientific, Waltham, USA) equipped with an electrospray ion source and ESI probe compatible with liquid flow rates of 1-1000 μL/min without splitting. Spray voltage, sheath gas, aux gas, sweep gas, source and capillary temperature were 3.0 kV, 18 flow units, 7 flow units, 0 flow units, 200 °C, and 300 °C, respectively. Samples were dissolved in methanol and injected using a 5-μL loop into the mobile phase flow. As a mobile phase was used methanol or dichloromethane at a 150 μL/min flow rate. Spectra were recorded at a resolution of 30,000.

The ESI spectra of compounds **9** and **21** were recorded using an LTQ Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific, Waltham, USA) operated in the positive ion mode. The mobile phase was methanol/water (4:1, *v*/*v*) at a 100 μL/min flow rate. The sample was dissolved in methanol and injected into the mobile phase flow using a 2-μL loop. The spray voltage, capillary voltage, tube lens voltage, and capillary temperature were 4.8 kV, 35 V, 145 V, and 275 °C, respectively. The spectra were recorded at a resolution of 100 000.

Matrix-assisted laser desorption/ionization (MALDI) spectra of compounds **23**, **25**, **27**, **29**, and **31** were recorded using UltrafleXtreme™ MALDI-TOF/TOF (time-of-flight) mass spectrometer (Bruker Daltonik, Bremen, Germany) operated in the reflectron mode. The samples were prepared using dried a droplet method with 2,5-dihydroxybenzoic matrix. Desorption and ionization were accomplished by a Smartbeam laser (Nd:YAG laser, 355 nm) operated at 1 kHz with optimized delayed extraction time. The ions were accelerated by a voltage of 25 kV. Data were collected with FlexControl software (Bruker Daltonics, Germany).

2.4. Infrared spectrometry (IR)

The analysis was performed by reflectance measurement (DRIFT) on a Nicolet 6700 FTIR spectrometer (Thermo-Nicolet, USA) in conjunction with a GladiATR diamond attenuated total-reflectance (ATR) attachment (PIKE, USA), DTG detector with KBr window. Measurement parameters: spectral range 4000 - 400 cm^{-1}, resolution 4 cm^{-1}, number of spectrum accumulations 64, Happ-Genzel apodization. The spectra were processed with software Omnic 9 (Thermo-Nicolet Instruments Co., USA).

3. Synthetic procedures

3.1 Synthesis of glycosyl azides

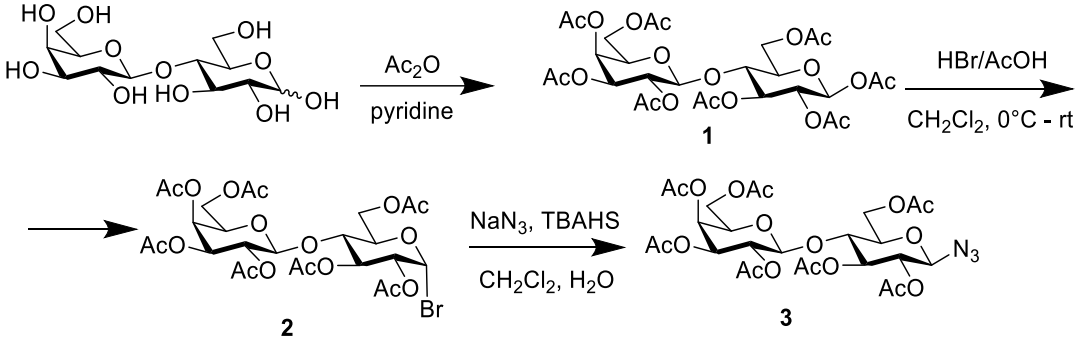
*3.1.1. Functionalization of lactose with linker **A***

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (3)

Acetic anhydride (16 mL, 169.3 mmol) and pyridine (10 mL) were added to lactose (5.6 g, 16.4 mmol) under argon, and the resulting suspension was stirred at room temperature for 6 days. After the suspension became a homogenous solution, the reaction was quenched by adding water (20 mL) at 0 °C. The solution was diluted with ethyl acetate and extracted with water, 1M HCl, and brine. The combined organic layer was dried over Na_2SO_4, filtered, and evaporated to afford peracetate **1** (10.9 g, 98% yield) as yellowish oil.

Peracetate **1** (10.9 g, 31.9 mmol) was dissolved under argon in absolute dichloromethane (20 mL) and cooled to 0 °C. Then, 33% HBr/AcOH (14 mL) was slowly added and the reaction mixture was stirred for 40 min at room temperature. The reaction mixture was monitored by TLC (cyclohexane/ethyl acetate, 1:1). After completion, the reaction was neutralized with a saturated solution of $NaHCO_3$ at 0 °C. The fractions were separated, and the organic phase was extracted with a saturated solution of $NaHCO_3$ and brine, filtered, and evaporated to afford crude product **2** (12.4 g) as yellow oil.

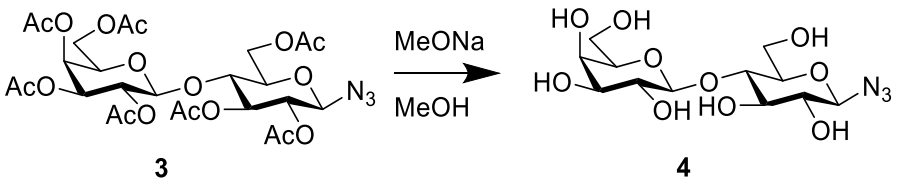
To crude product **2** (12.4 g) in absolute dichloromethane (20 mL), NaN_3 (4.3 g, 66.2 mmol), tetrabutylammonium hydrogen sulfate (4.26 g, 12.6 mmol, TBAHS), and saturated solution of $NaHCO_3$ (20 mL) were added. The reaction mixture was stirred vigorously at room temperature overnight. After completion, the organic and water phases were separated and the water phase was 3× extracted with dichloromethane. The combined organic phase was dried over Na_2SO_4, filtered, evaporated, and purified on silica gel (cyclohexane/ethyl acetate, 2:1) to afford azide **3** (7.18 g, 68% yield) as yellowish oil. The product was analyzed with ESI-MS, and NMR, and the results were in accord with the literature.[1]



Scheme S1. Synthesis of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (**3**).

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (4)

Peracetate **3** (3 g, 4.5 mmol) was dissolved under argon in absolute methanol (18 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 24 h and monitored by TLC (isopropyl alcohol/water/NH_4OH, 7:2:1). After completion, the reaction mixture was evaporated to afford azide **4** (1.6 g, 96% yield) as a white solid.



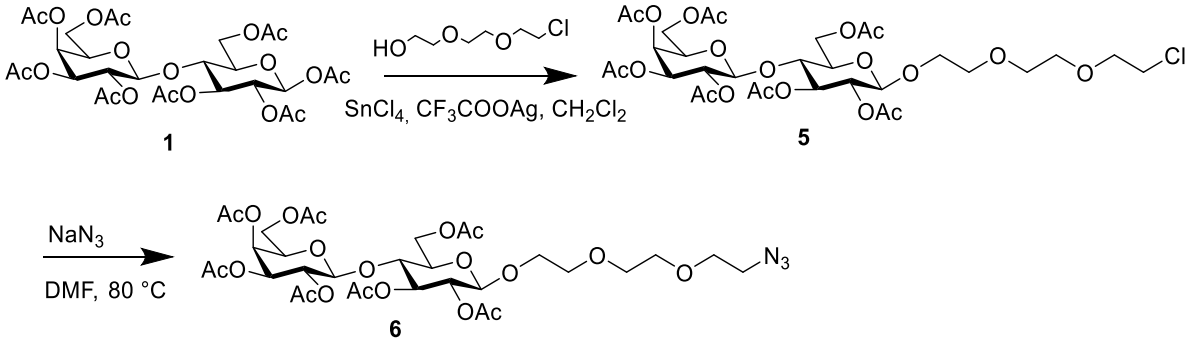
Scheme S2. Synthesis of β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (**4**).

*3.1.2. Functionalization of lactose with linker **B***

2-[2-(Azidoethoxy)ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (6)

Azide **6** was essentially prepared according to the literature with some modifications.[2] Peracetate **1** (3 g, 4.4 mmol) was dissolved in absolute dichloromethane (15 mL). [2-(2-Chloroethoxy)ethoxy]ethan-2-ol (1.53 mL, 10.5 mmol), CF_3COOAg (1.5 g, 6.8 mmol), and $SnCl_4$ (1M solution in methylene chloride; 1.36 mL, 13.6 mmol) were slowly added and the reaction mixture was stirred for 15 h under argon at room temperature. The reaction was monitored by TLC (ethyl acetate/cyclohexane, 3:1). The reaction was terminated by a slow addition of a saturated solution of $NaHCO_3$. The precipitate was filtered off through the Celite 545 pad. The organic and water phases were separated. The water phase was extracted with ethyl acetate and the organic phase was 3× extracted with water. The combined organic phases were dried over Na_2SO_4, filtered, and evaporated. The crude product **5** was used for the next reaction step without further purification.

The crude product **5** (4.7 g) was dissolved under argon in absolute DMF (13 mL) and NaN_3 was added (1.83 g, 28.1 mmol). The reaction mixture was stirred at 80 °C for 4 days. Subsequently, the reaction mixture was diluted with ethyl acetate and 3× extracted with water. The organic phase was separated, dried over Na_2SO_4, filtered, evaporated, and purified on silica gel. Three subsequent silica gel purifications were necessary to afford pure **6** (first and second chromatography: cyclohexane/ethylacetate, 1:1, third chromatography: dichloromethane) to afford product **6** (0.748 g, 21% yield over 2 reaction steps) as colorless oil. The product was analyzed with ESI-MS, and NMR and the results were in accord with the literature.[2]

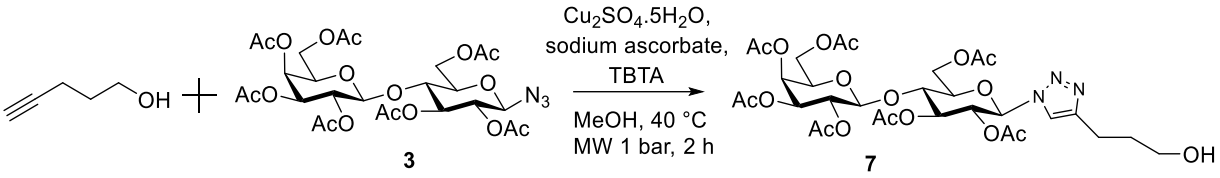


Scheme S3. Synthesis of 2-[2-(azidoethoxy)ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6**).

3.1.3. Functionalization of lactose with linker C

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)propan-1-ol (7)

Azide **3** (1 g, 1.51 mmol), pentynol (420 μL, 4.51 mmol), 1M aq. solution of sodium ascorbate (800 μL, 1.6 mmol), 2M aq. solution of sodium sulfate (200 μL, 0.8 mmol), and catalytic amount of 1-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-*N*,*N*-*bis*[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]methanamine (TBTA) were dissolved in methanol (2 mL) in a microwave tube. The mixture was heated at 40 °C by microwave irradiation (medium irradiation, 1 bar, Biotage, Technoprocur CZ) for 2 h. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). After completion, the reaction mixture was evaporated and purified on silica gel (dichloromethane) to afford product **7** (0.99 g, 78% yield) as yellow-white honey. The product was analyzed by ESI-MS, and NMR and the results were in accord with the literature.[3]



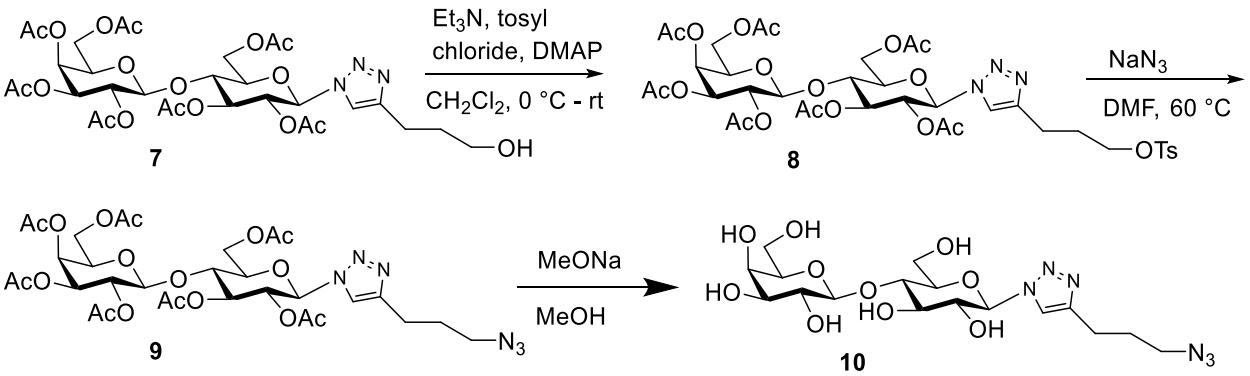
Scheme S4. Synthesis of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-1-(1,2,3 triazol-4-yl)propan-1-ol (**7**).

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-yl azide (10)

Alcohol **7** (0.99 g, 1.3 mmol) was dissolved under argon in absolute dichloromethane (10 mL) and cooled to 0 °C. Then, Et_3N (1.8 mL, 15.2 mmol), tosyl chloride (0.72 g, 3.8 mmol), and a catalytic amount of 4-dimethylaminopyridine (DMAP) were added. The reaction mixture was then heated to room temperature and stirred for 45 min. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). The reaction was terminated by the addition of water (10 mL). The phases were separated and the organic phase was extracted with water and brine. The combined organic phases were dried over Na_2SO_4, filtered, and evaporated to afford crude product **8** as a black foam (0.78 g). It was used in the next reaction step without further purification.

Crude product **8** (0.78 g, 1.05 mmol) was dissolved under argon in absolute dimethylformamide (DMF; 10 mL) and NaN_3 (0.8 g, 12.305 mmol) was added. The reaction mixture was stirred at 60 °C under argon for approximately 20 h. After completion, the reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4, filtered, evaporated, and purified on silica gel (cyclohexane/ethyl acetate, 1:1) to afford azide **8** (0.53 g, 52% yield) as yellowish oil. The structural integrity of azide **9** was confirmed with ESI-MS and NMR. Respective data are shown in Section 4.1.

Peracetate **9** (0.364 g, 0.47 mmol) was dissolved under argon in absolute methanol (4 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 21 h. The reaction was monitored by TLC (isopropyl alcohol/water/NH_4OH, 7:2:1). After completion, the reaction mixture was evaporated to afford deprotected alcohol **10** as colorless oil.

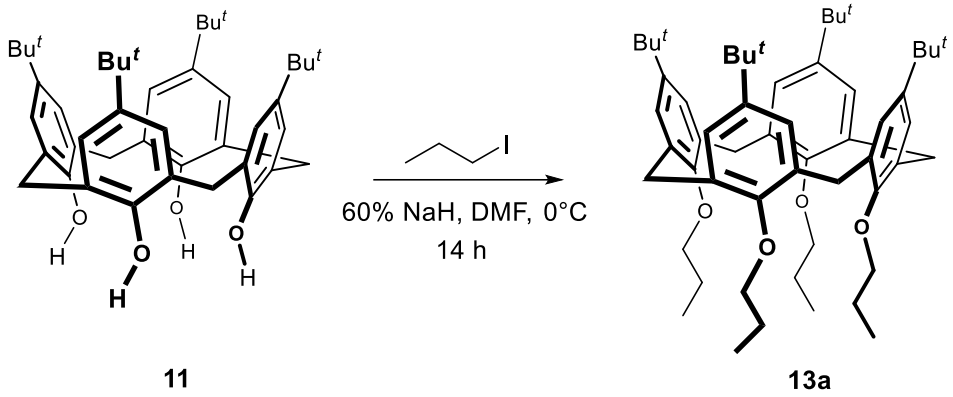


Scheme S5. Synthesis of β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-yl azide (**10**).

3.2 Synthesis of calix[4]arene cores

Cone-25,26,27,28-tetrapropoxycalix[4]arene (13a)

p-tert-Butylcalix[4]arene **11** (9.0 g, 0.014 mol) was dissolved in dry DMF (150 mL) and cooled to 0 °C. Then, 60% NaH (6.0 g, 0.15 mol) was carefully added. After 30 min, propyl iodide (13.3 mL, 0.136 mol) was slowly added and the reaction mixture was allowed to stir for 14 h at room temperature under calcium chloride drying tube. Then, 1M HCl was added and the reaction mixture was stirred for further 20 min. The aqueous phase was 7× extracted with chloroform and combined organic phases were washed with water, brine and dried over $MgSO_4$. Recrystallization with chloroform/methanol afforded the product **13a** (5.88 g, 78% yield). The ^{1}H-NMR spectrum was identical to that in the literature.[4]



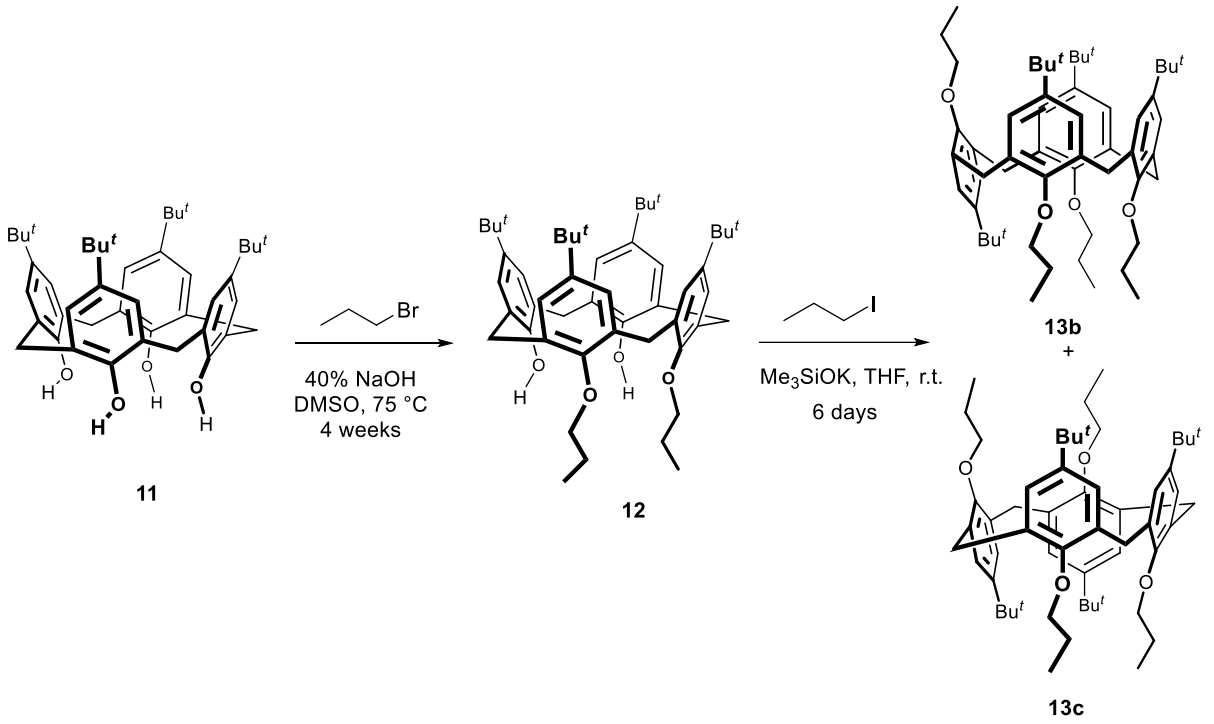
Scheme S6. Synthesis of *cone*-25,26,27,28-tetrapropoxycalix[4]arene (**13a**).

Partial cone- and *1,2- alternate*-25,26,27,28-tetrapropoxycalix[4]arene (13b, 13c)

p-tert-Butylcalix[4]arene **11** (5.0 g, 7.7 mmol) was dissolved in DMSO (75 mL). 40% Aqueous solution of NaOH (5.4 mL) was added and the reaction mixture was heated at 75 °C until the solution turned brown. Then, propyl iodide (3.8 mL, 0.042 mol) was added and the reaction mixture was heated for 4 weeks at 75 °C. After completion, the reaction mixture was poured into 10% aqueous solution of HCl (500 mL) and the resulting precipitate was filtered off and washed with 10% aqueous solution of HCl (2×50 mL). After recrystallization in acetonitrile, the mixture was purified using silica gel column chromatography (CH_2Cl_2/cyclohexane, 1:1) to

afford product **12** (1.33 g, 24% yield) as white crystals. The ^{1}H-NMR spectrum was identical to that in the literature.[4]

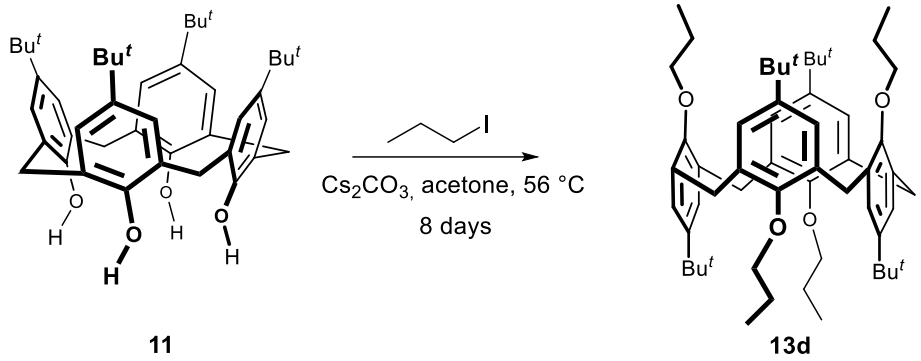
Compound **12** (4.0 g, 5.45 mmol) was dissolved in dry tetrahyfrofuran (250 mL), potassium trimethylsilanolate (3.49 g, 0.027 mol) was added and the mixture was stirred for 30 min at room temperature. Then, propyl iodide (2.66 mL, 0.027 mol) was slowly added and the reaction mixture was stirred for 6 days at room temperature. After completion, 10% aqueous solution of HCl (200 mL) was poured into the reaction mixture. Aqueous layer was extracted with CH_2Cl_2 (4×30 mL) and combined organic phases were washed with saturated solution of Na_2SO_3 (40 mL), water (40 mL), and dried over $MgSO_4$. Solvents were evaporated and both products were isolated using silica gel column chromatography (CH_2Cl_2/cyclohexane, 1:7). Then, compounds **13b** (0.78 g, 18% yield) and **13c** (1.74 g, 39% yield) were obtained as white powders. The ^{1}H NMR spectra were identical to those in the literature.[4]



Scheme S7. Synthesis of *partial cone*- and *1,2-alternate*-25,26,27,28-tetrapropoxycalix[4]arene (**13b**, **13c**).

1,3-Alternate-25,26,27,28-tetrapropoxycalix[4]arene (13d)

Compound **11** (6.0 g, 9.26 mmol) was dissolved in dry acetone (150 mL) and Cs_2CO_3 (12.06 g, 0.037 mol) was added. After 15 min, propyl iodide (10.9 mL, 0.112 mol) was added and the reaction mixture was boiled under reflux for 8 days. To the reaction mixure, 1M HCl (300 mL) was slowly added, the aqueous phase was 5× extracted with CH_2Cl_2 and combined organic phases were 2× washed with water, brine, and dried over $MgSO_4$. After crystallization in ethyl acetate, product **13d** (2.67 g, 36% yield) was obtained as colourless crystals. The ^{1}H NMR spectrum was identical to that in the literature.[4]



11 **13d**

Scheme S8. Synthesis of *1,3-alternate*-25,26,27,28-tetrapropoxycalix[4]arene (**13d**).

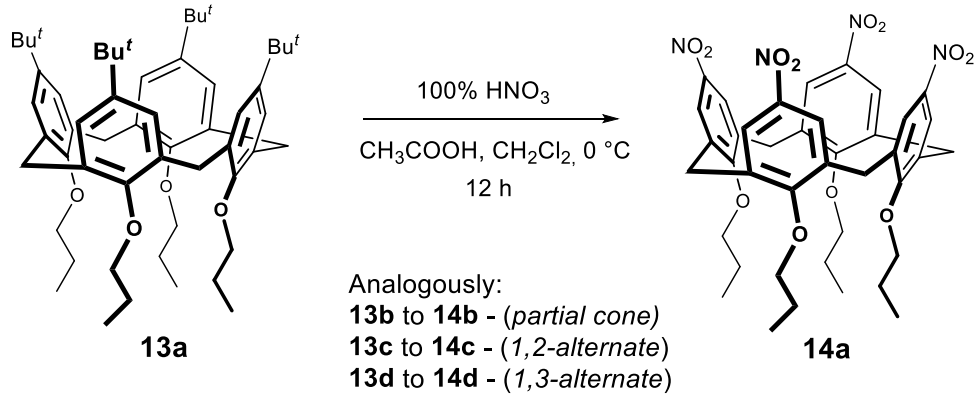
5,11,17,23-Tetranitro-25,26,27,28-tetrapropoxycalix[4]arene (14)

Compound **13a** (4.0 g, 4.89 mmol) was dissolved in dry CH_2Cl_2 (50 mL), and ice-cold acetic acid (48 mL) was added. Then, 100% HNO_3 (16 mL, 0.38 mol) was carefully added at 0 °C. The cooling bath was removed and the reaction mixture was stirred at room temperature until the black-violet colour of the mixture turned yellow (12 h). After completion, the reaction mixture was diluted with water (100 mL), the aqueous phase was 2× extracted with CH_2Cl_2 and combined organic phases were washed with water, brine and dried over $MgSO_4$. The solvent was evaporated to afford product **14a** (4.47 g, 99% yield) as yellow powder. The ^{1}H-NMR spectrum was identical to that in the literature.[5]

Compounds **14b-d** were synthesized in the same manner as compound **14a** but reaction time and the ratio of starting materials to the reagent were changed. The reaction mixture consisting of compound **13b** (0.81 mmol) and 100% HNO_3 (0.063 mol) was stirred at room temperature for 20 h. Silica gel flash chromatography (MeOH/$CHCl_3$/cyclohexane, 1.5:5:7.5) afforded product **14b** (43% yield) as yellowish crystals. The ^{1}H-NMR spectrum was identical to that in the literature.[5]

The reaction mixture consisting of compound **13c** (1.47 mmol) and 100% HNO_3 (0.029 mol) was stirred at room temperature for 90 min. Product **13c** was obtained as yellow powder in quantitave yield. It was characterized by ^{1}H-NMR, ^{13}C-NMR and HRMS spectrometry. Respective data and spectra are available in Section 4.2.

The reaction mixture consisting of compound **13d** (1.47 mmol) and 100% HNO_3 (0.029 mol) was stirred at room temperature for 2 h. Product **14d** was obtained as yellow powder in quantitave yield. The ^{1}H NMR spectrum was identical to that in the literature.[5]

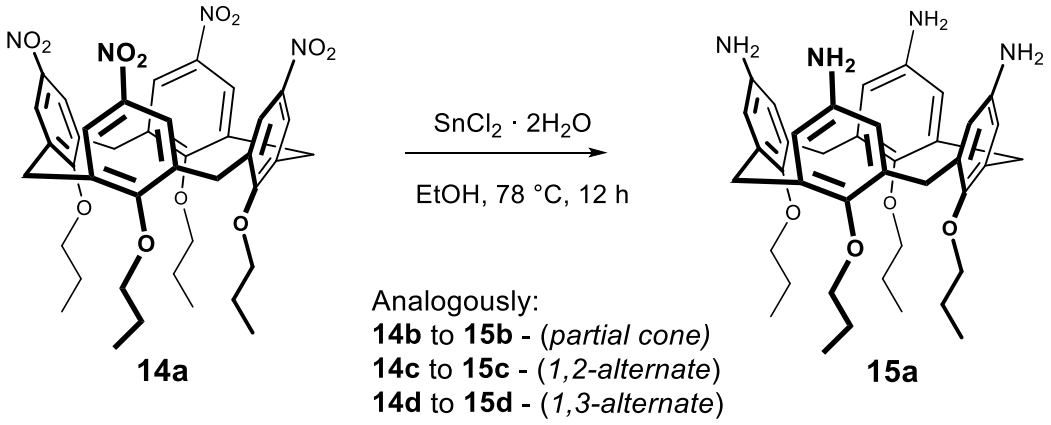


13a **14a**

Scheme S9. Synthesis of 5,11,17,23-tetranitro-25,26,27,28-tetrapropoxycalix[4]arenes **14** (exemplified by *cone* conformer **14a**).

5,11,17,23-Tetraamino-25,26,27,28-tetrapropoxycalix[4]arene (15)

Compound **14a-d** (2.0 g, 2.62 mmol) was dissolved in ethanol (140 mL), $SnCl_2 \cdot 2H_2O$ (11.89 g, 0.053 mol) was added and the reaction mixture was stirred for 12 h at 78 °C. After completion, the reaction mixture was cooled to room temperature and poured onto crushed ice. The pH value was adjusted to 9-10 using 1M KOH. The mixture was extracted with CH_2Cl_2 (4×100 mL) and combined organic phases were washed with water, brine, and dried over $MgSO_4$. Solvents were evaporated and product **15a-d** was obtained as red-brown crystals in 68% (1.15 g), 72%, 41%, and 82% yields, respectively. The ^{1}H-NMR spectra of **15a** and **15d** were identical to those in the literature.[6] Compounds **15b** and **15c** were characterized by ^{1}H NMR, ^{13}C NMR and HRMS spectrometry. Respective data and spectra are available in Section 4.2.



Scheme S10. Synthesis of 5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arenes **15** (exemplified by *cone* conformer **15a**).

5,11,17,23-Tetrakis(4-pentynoylamino)-25,26,27,28-tetrapropoxycalix[4]arene (16-19)

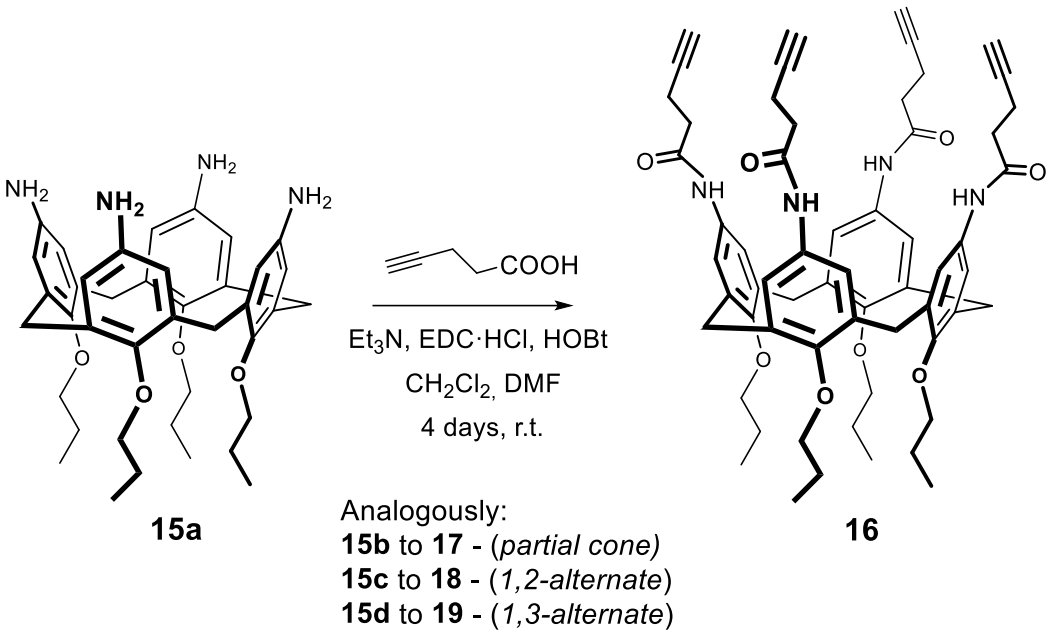
EDC·HCl (560 mg, 2.92 mmol), HOBt (400 mg, 2.96 mmol) and 4-pentynoic acid (24 mg, 2.45 mmol) were dissolved in dry dichloromethane (9 mL) and dry DMF (1 mL) and the mixture was cooled to 0 °C. After 20 min, the mixture of compound **15a-d** (190 mg, 0.291 mmol) and Et_3N (340 mg, 2.44 mmol), dissolved in dry dichloromethane (18 mL) and dry DMF (2 mL), was added to the reaction mixture, which was then allowed to reach room temperature and was stirred for 4 days. After completion, the reaction mixture was diluted with 0.5M HCl (20 mL), saturated solution of $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$. The products were isolated as follows:

Product **16** (*cone*) was isolated using silica gel column chromatography (acetone/ dichloromethane, 6:1) as yellowish crystals (85 mg, 30% yield). The ^{1}H NMR spectrum was identical to that in the literature.[2]

Product **17** (*partial-cone*) was isolated using silica gel column chromatography *i*PrOH: dichloromethane, 1:19) as yellowish crystals (27% yield). It was characterized by ^{1}H NMR, ^{13}C NMR, HRMS and IR spectrometry. Respective data and spectra are available in Section 4.2.

Product **18** (*1,2-alternate*) was isolated using silica gel column chromatography (acetonitrile/ dichloromethane, 1:3) as yellowish crystals (32% yield). It was characterized by ^{1}H NMR, ^{13}C NMR, HRMS and IR spectrometry. Respective data and spectra are available in Section 4.2.

Product **19** (*1,3-alternate*) was isolated using silica gel column chromatography (methanol/dichloromethane, 1:24) as yellowish crystals (21% yield). The ^{1}H NMR spectrum was identical to that in the literature.[2]



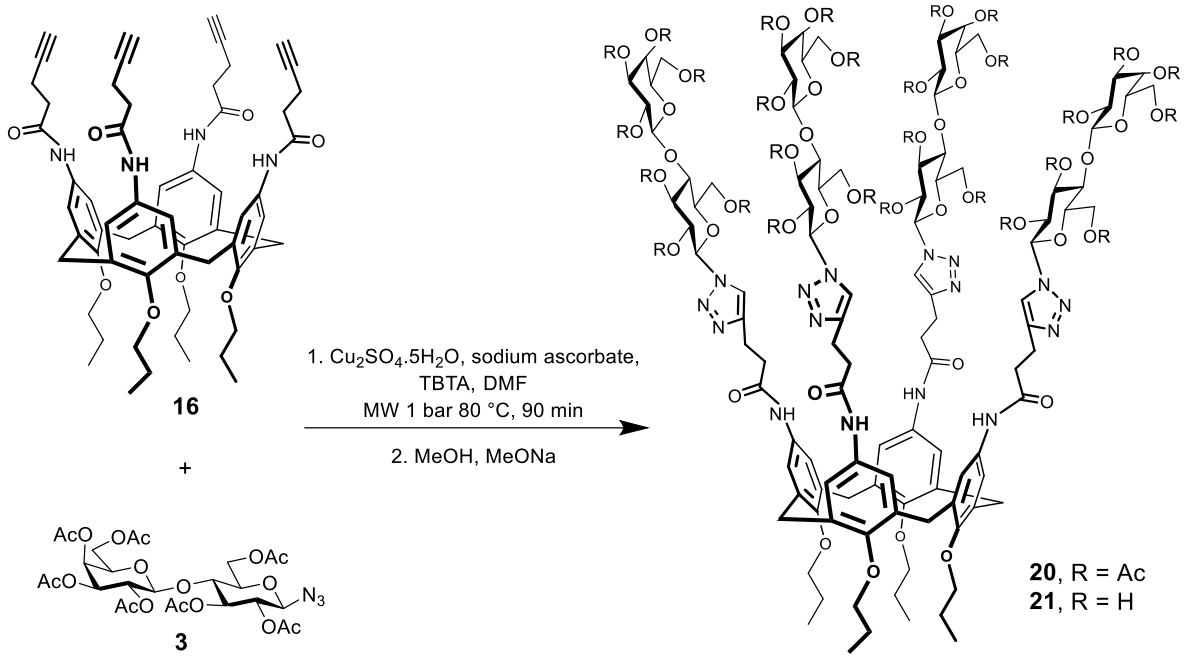
Scheme S11. Synthesis of 5,11,17,23-tetrakis(4-pentynoylamino)-25,26,27,28-tetrapropoxycalix[4]arenes **16-19** (exemplified by *cone* conformer **16**).

3.3. Synthesis of glycocalix[4]arenes

Cone-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (21)

Azide **3** (0.10 g, 0.15 mmol), calix[4]arene **16** (15 mg, 15 μmol), 2M aqueous solution of sodium ascorbate (80 μL, 0.16 mmol), 2M aqueous solution of $CuSO_4.5H_2O$ (40 μL, 0.08 mmol), and a catalytic amount of TBTA were dissolved in absolute dimethylformamide (3 mL) in a microwave tube. The mixture was heated at 80 °C by microwave irradiation (medium irradiation, 1 bar) for 90 min. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). After completion, the reaction mixture was diluted with water, extracted with ethyl acetate, dried over Na_2SO_4, filtered, evaporated, and purified on silica gel (gradient of dichloromethane to dichloromethane/methanol, 95:5) to afford glycocalix[4]arene peracetate **20** (20 mg, 36% yield) as brownish solid.

Peracetate **20** (20 mg, 5.53 μM) was dissolved under argon in absolute methanol (5 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 4 h. The reaction was monitored by TLC (dichloromethane/methanol, 90:10). After completion, the reaction mixture was evaporated to afford glycocalix[4]arene **21** (13 mg, 99% yield) as a brownish powder. The structural integrity of compound **21** was confirmed by HRMS, NMR, and HPLC. Respective data and spectra are available in Section 4.3.

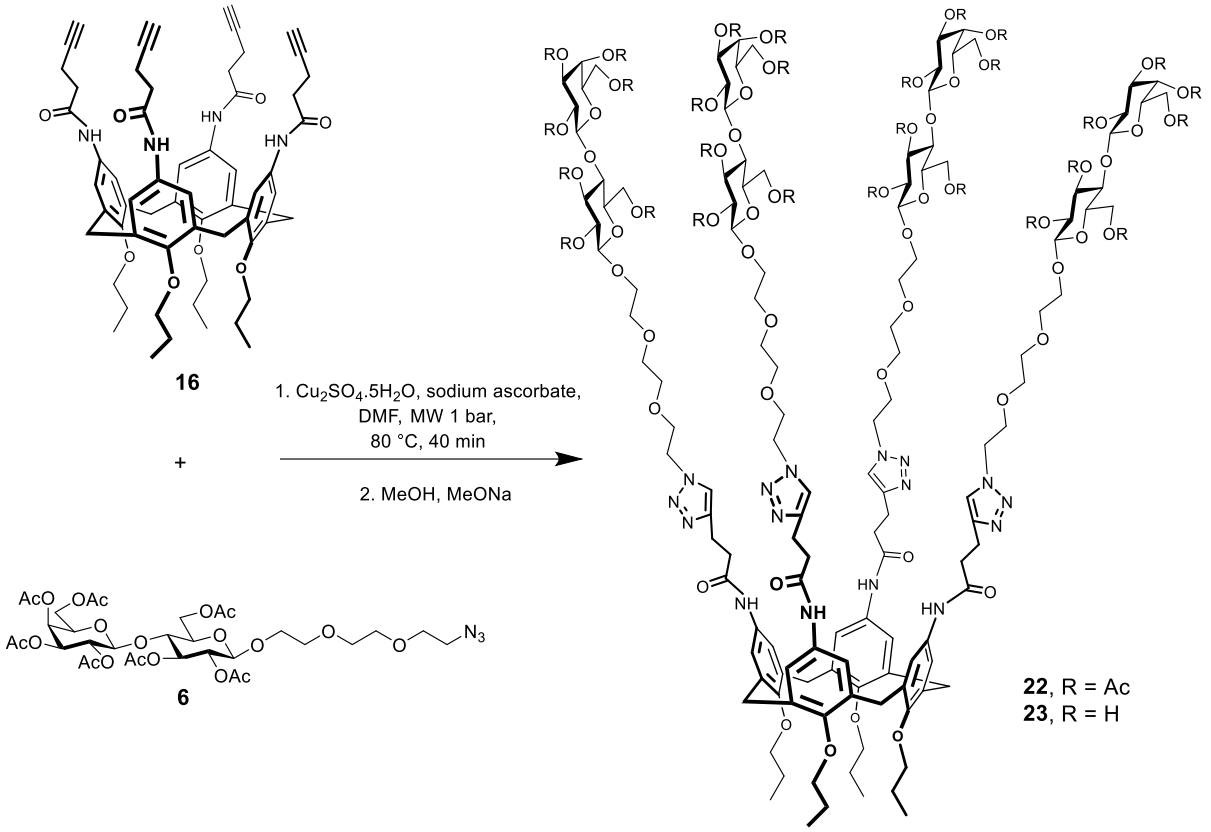


Scheme S12. Synthesis of *cone*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (**21**).

Cone-5,11,17,23-tetrakis{[2-(2-(azidoethoxy)ethoxy)ethyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (23)

Glycocalix[4]arene **23** was prepared essentially as described in the literature.[2] Azide **6 (**0.14 g, 0.18 mmol), calix[4]arene **16** (0.020 g, 0.02 mmol), 2M aqueous solution of sodium ascorbate (200 μL, 0.16 mmol), and 2M aqueous solution of $CuSO_4.5H_2O$ (100 μL, 0.08 mmol), and a catalytic amount of TBTA, were dissolved in absolute dimethylformamide (3 mL) in a microwave tube. The mixture was heated at 80 °C by microwave irradiation (medium irradiation, 1 bar) for 40 min. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). After completion, the reaction mixture was diluted with water, extracted with ethyl acetate, dried over Na_2SO_4, filtered, evaporated, and purified on silica gel (gradient of dichloromethane to dichloromethane/methanol, 94:6) to afford glycocalix[4]arene peracetate **22** (32 mg, 37% yield) as brownish foam.

Peracetate **22** (32 mg, 7.7 μM) was dissolved under argon in absolute methanol (2 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 4 h. The reaction was monitored by TLC (isopropyl alcohol/water/NH_4OH, 7:2:1). After completion, the reaction mixture was evaporated to afford glycocalix[4]arene **23** (20 mg, 90% yield) as a yellow-brown powder. The structural integrity of compound **23** was confirmed by HRMS, NMR, and HPLC. Respective data and spectra are available in Section 4.3.

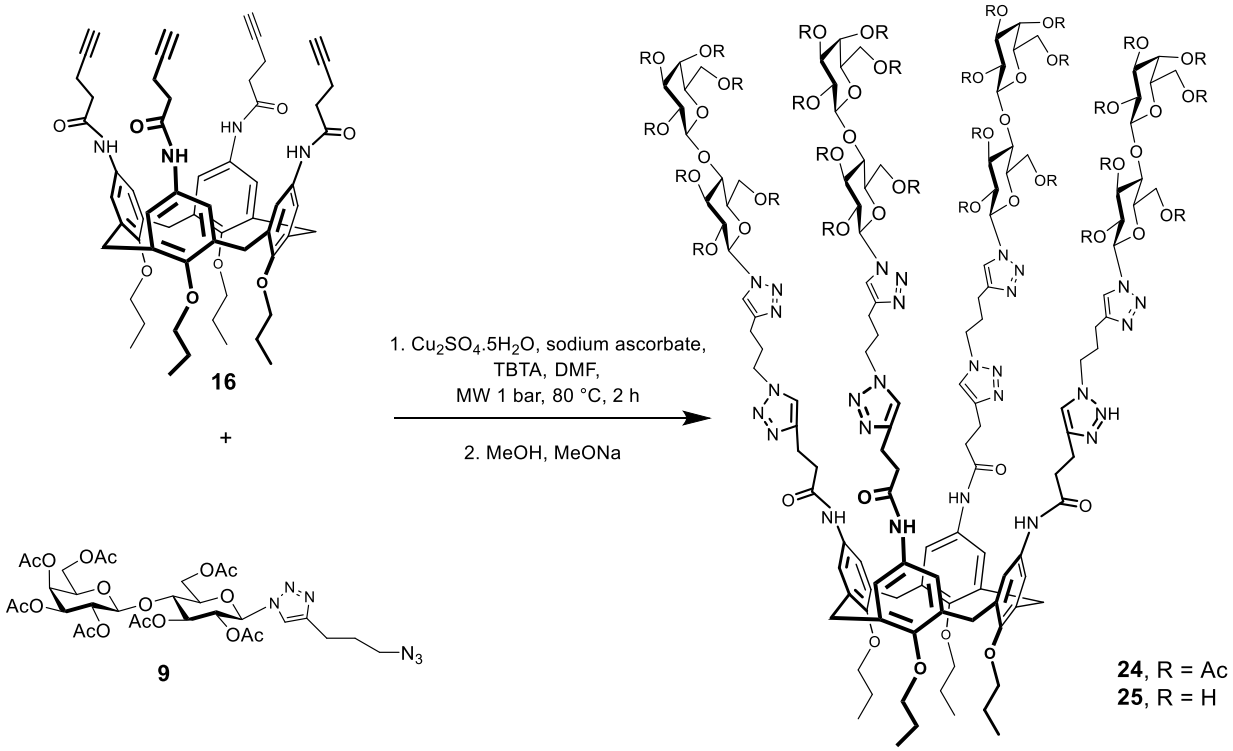


Scheme S13. Synthesis of *cone*-5,11,17,23-tetrakis{[2-(2-(azidoethoxy)ethoxy)ethyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (**23**).

Cone-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (25)

Azide **9** (66 mg, 0.086 mmol), calix[4]arene **16** (30 mg, 0.031 mmol), 2M aqueous solution of sodium ascorbate (400 μL, 0.8 mmol), 2M aqueous solution of $CuSO_4.5H_2O$ (200 μL, 0.4 mmol), and a catalytic amount of TBTA were dissolved in absolute dimethylformamide (2 mL) in a microwave tube. The mixture was heated at 80 °C by microwave irradiation (medium irradiation, 1 bar) for 2 h. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). After completion, the reaction mixture was diluted with water, extracted with dichloromethane, dried over Na_2SO_4, filtered, evaporated, and purified on silica gel (gradient, of dichloromethane to dichloromethane/methanol, 90:10) to afford glycocalix[4]arene peracetate **24** (33 mg, 26% yield) as a brownish solid.

Peracetate **24** (33 mg, 8.1 μM) was dissolved under argon in absolute methanol (3 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 5 h. The reaction was monitored by TLC (isopropyl alcohol/water/NH_4OH, 7:2:1). After completion, the reaction mixture was quenched with Amberlite IR 120/H^+ and stirred until neutral pH. Then, the reaction mixture was filtered and evaporated to afford glycocalix[4]arene **25** (6 mg, 26% yield) as a brownish powder. The structural integrity of compound **25** was confirmed by HRMS, NMR, and HPLC. Respective data and spectra are available in Section 4.3.

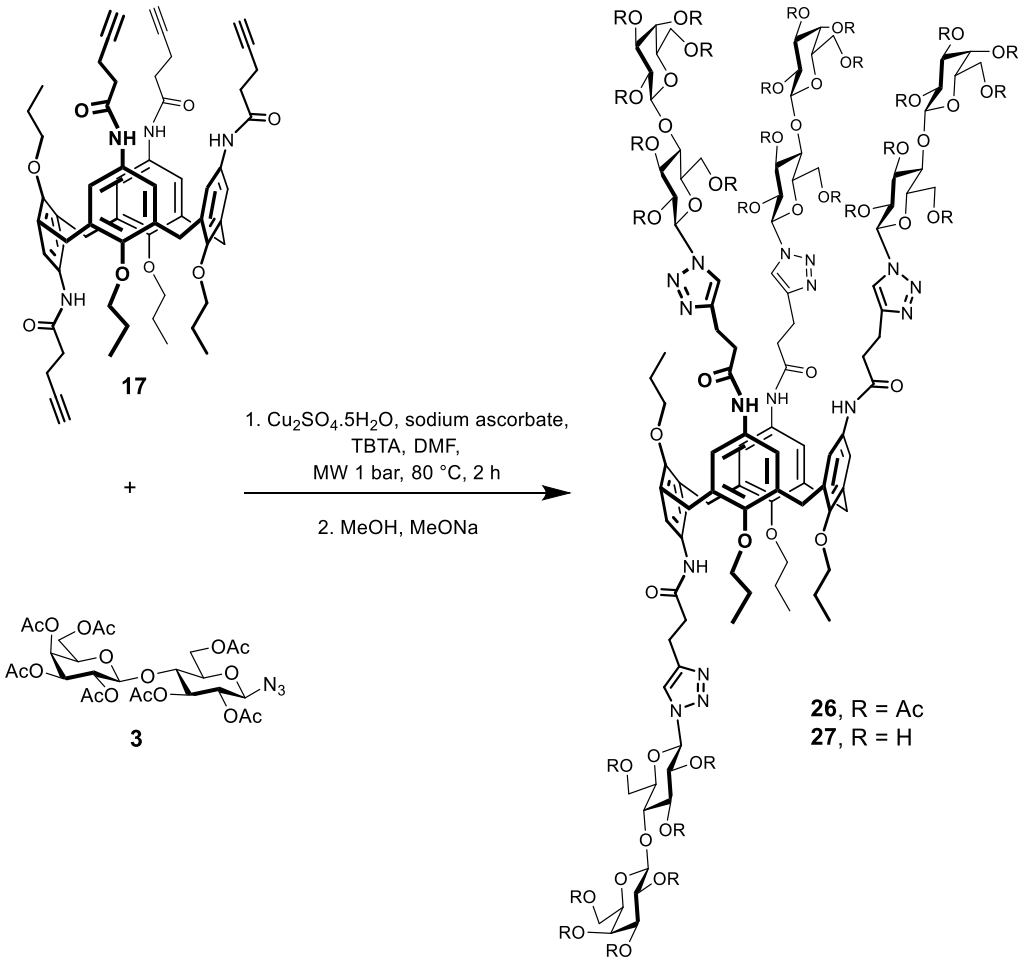


Scheme S14. Synthesis of *cone*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (**25**).

Partial cone-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (27)

Azide **3** (0.2 g, 0.30 mmol), calix[4]arene **17** (0.03 g, 31 μmol), 2M aqueous solution of sodium ascorbate (80 μL, 0.16 mmol), 2M aqueous solution of $CuSO_4.5H_2O$ (40 μl, 0.08 mmol), and a catalytic amount of TBTA were dissolved in absolute dimethylformamide (2 mL) in a microwave tube. The mixture was heated at 80 °C by microwave irradiation (medium irradiation, 1 bar) for 2 h. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). After completion, the reaction mixture was diluted with water, extracted with ethyl acetate, dried over Na_2SO_4, filtered, evaporated, and purified on silica gel (gradient of dichloromethane to dichloromethane/methanol, 94.5:4.5) to afford glycocalix[4]arene peracetate **26** (51 mg, 46% yield) as brownish oil.

Peracetate **26** (51 mg, 14 μM) was dissolved under argon in absolute methanol (6 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 2 h and monitored by TLC (isopropyl alcohol/water/NH_4OH, 7:2:1). After completion, the reaction mixture was quenched with Amberlite IR 120/H^+ and stirred until neutral pH. Then, the reaction mixture was filtered and evaporated to afford glycocalix[4]arene **27** (25 mg, 74% yield) as a brownish powder. The structural integrity of compound **27** was confirmed by HRMS, NMR, and HPLC. Respective data and spectra are available in Section 4.3.

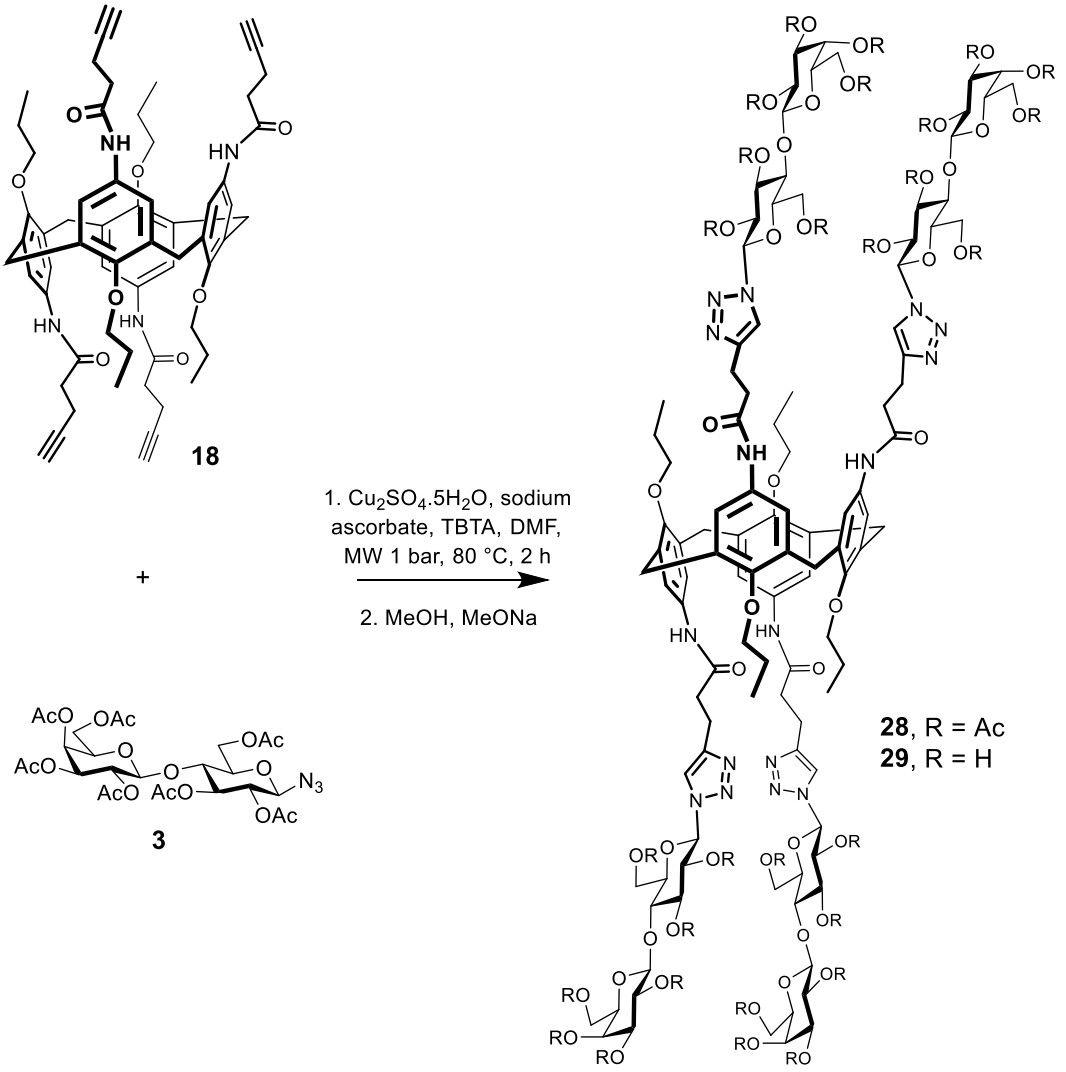


Scheme S15. Synthesis of *partial cone*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (**27**).

1,2-*Alternate*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (29)

Azide **3** (0.20 g, 0.30 mmol), calix[4]arene **18** (0.03 g, 0.031 mmol), 2M aqueous solution of sodium ascorbate (80 μL, 0.16 mmol), 2M aqueous solution of $CuSO_4.5H_2O$ (40 μL, 0.08 mmol), and a catalytic amount of TBTA were dissolved in absolute dimethylformamide (2 mL) in a microwave tube. The mixture was heated at 80 °C by microwave irradiation (medium irradiation, 1 bar) for 2 h. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). After completion, the reaction mixture was diluted with water, extracted with ethyl acetate, dried over Na_2SO_4, filtered, evaporated, and purified on silica gel (gradient of dichloromethane to dichloromethane/methanol, 96:4) to afford glycocalix[4]arene peracetate **28** (27 mg, 24% yield) as brownish oil.

Peracetate **28** (27 mg, 7.5 μM) was dissolved under argon in absolute methanol (6 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 2 h. The reaction was monitored by TLC (isopropyl alcohol/water/NH_4OH, 7:2:1). After completion, the reaction mixture was quenched with Amberlite IR 120/H^+ and stirred until neutral pH. Then, the reaction mixture was filtered and evaporated to afford glycocalix[4]arene **29** (16 mg, 88% yield) as brownish powder. The structural integrity of compound **29** was confirmed by HRMS, NMR, and HPLC. Respective data and spectra are available in Section 4.3.



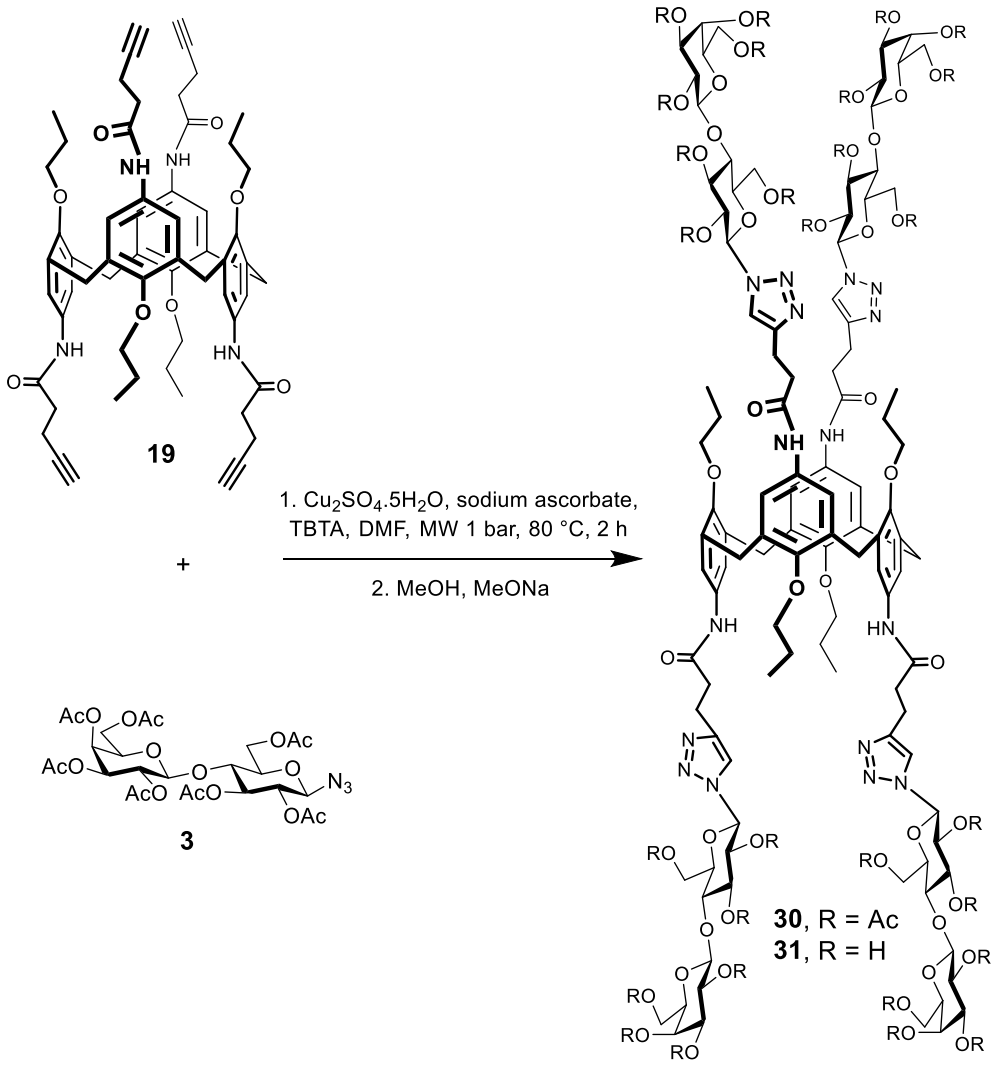
Scheme S16. Synthesis of 1,2-*alternate*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1- (1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (**29**).

1,3-Alternate-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (31)

Azide **3** (0.2 g, 0.30 mmol), calix[4]arene **19** (0.03 g, 31 μmol), 2M aqueous solution of sodium ascorbate (80 μL, 0.16 mmol), 2M aqueous solution of $CuSO_4.5H_2O$ (40 μL, 0.08 mmol), and a catalytic amount of TBTA were dissolved in absolute dimethylformamide (2 mL) in a microwave tube. The mixture was heated at 80 °C by microwave irradiation (medium irradiation, 1 bar) for 2 h. The reaction was monitored by TLC (dichloromethane/methanol, 95:5). After completion, the reaction mixture was diluted with water, extracted with ethyl acetate, dried over Na_2SO_4, filtered, evaporated, and purified on silica gel (gradient of dichloromethane to dichloromethane/methanol, 95:5) to afford glycocalix[4]arene peracetate **30** (25 mg, 23% yield) as brownish oil.

Peracetate **30** (25 mg, 6.9 μM) was dissolved under argon in absolute methanol (8 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 2 h. The reaction was monitored by TLC (isopropyl alcohol/water/NH_4OH, 7:2:1). After completion, the reaction mixture was evaporated, diluted in water/methanol, 87:13, and loaded onto the Bio-Gel P-6DG column (Bio-Rad Laboratories, USA) in a mobile phase of

water/methanol, 87:13, 7 mL/h, to afford glycocalix[4]arene **31** (10.5 mg, 63% yield) as a yellowish powder. The structural integrity of compound **31** was confirmed by HRMS, NMR, and HPLC. Respective data and spectra are available in Section 4.3.



Scheme S17. Synthesis of *1,3-alternate*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (**31**).

4. Structural characterization of prepared compounds

4.1. Glycosyl azides

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-1-(1,2,3 triazol-4-yl)prop-1-yl-azide (9)

Table S1. ^{1}H and ^{13}C NMR data of compound **9** (399.83 MHz for ^{1}H, 100.54 MHz for ^{13}C, $CDCl_3$, 20 °C).

	Atom	δ_C	m.	δ_H	n_H	m.	$J_{H,H}$
Glc	**1**	85.45	d	5.788	1	d	8.9
	2	70.47	d	5.343	1	dd	9.5, 8.9
	3	72.42	d	5.394	1	dd	9.5, 8.0
	4	75.60	d	3.945	1	dd	
	5	75.85	d	3.90^{H}	1	m	
	6	61.66	t	4.477	1	dd	
				4.140	1	dd	12.4, 5.0
	2-CO	169.16	s	-	0	-	
	Ac	20.17	q	1.858	3	s	
	3-CO	169.41	s	-	0	-	
	Ac	20.67	q	2.052	3	s	
	6-CO	170.18	s	-	0	-	
	Ac	20.77	q	2.099	3	s	
Gal	**1**	101.07	d	4.511	1	d	7.9
	2	68.94	d	5.121	1	dd	10.4, 7.9
	3	70.85	d	4.960	1	dd	10.4, 3.5
	4	66.49	d	5.354	1	dd	3.5, 0.8
	5	70.76	d	3.88^{H}	1	m	
	6	60.74	d	4.10^{H}	2	m	
	2-CO	169.01	s	-	0	-	
	Ac	20.59	q	2.046	3	s	
	3-CO	170.03	s	-	0	-	
	Ac	20.47	q	1.960	3	s	
	4-CO	170.07	s	-	0	-	
	Ac	20.61	q	2.153	3	s	
	6-CO	170.33	s	-	0	-	
	Ac	20.63	q	2.067	3	s	
aglycon	**1**	50.33	t	3.313	2	br t	6.7
	2	28.18	t	1.964	2	tt	7.4, 6.7
	3	22.48	t	2.797	2	dt	1.1, 7.4
	4	147.16	s	-	0	-	
	5	119.26	d	7.485	1	br s	

^{H} … HSQC readout

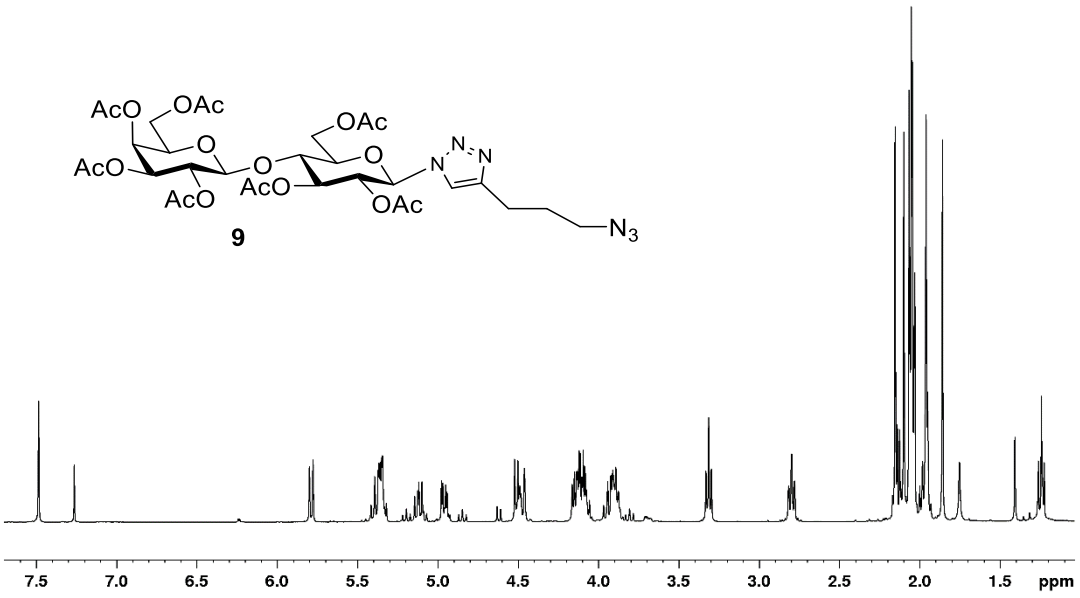


Figure S3. ^{1}H NMR spectrum of compound **9** (399.83 MHz, $CDCl_3$, 20 °C)**.**

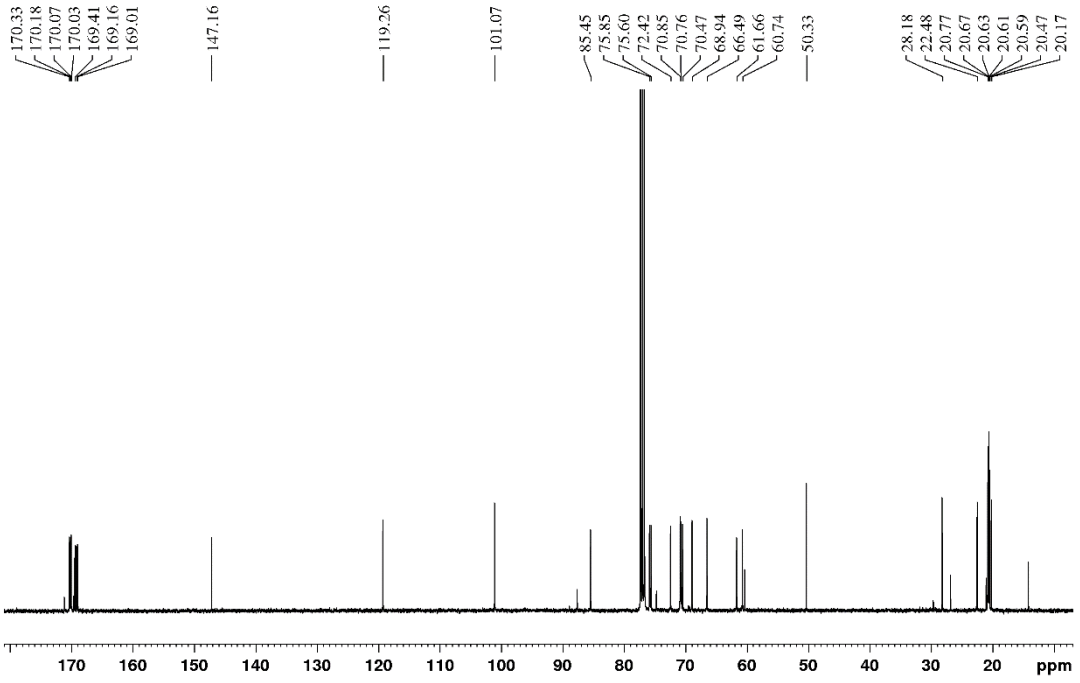


Figure S4. ^{13}C NMR spectrum of compound **9** (100.54 MHz, $CDCl_3$, 20 °C).

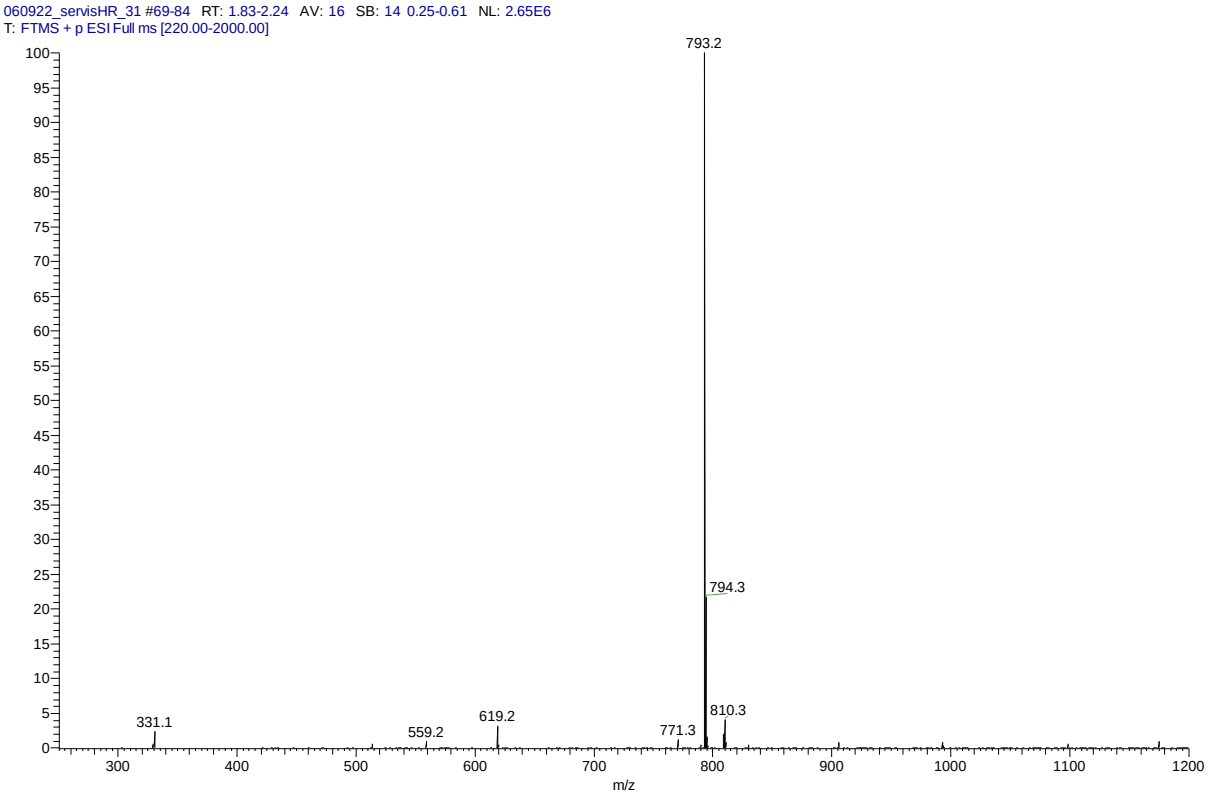


Figure S5. Mass spectrum (ESI$^+$) of compound **9:** [M + Na]$^+$, *m*/*z* 793.2; [M + H]$^+$, *m*/*z* 771.3. HRMS (ESI$^+$): *m*/*z* for $C_{31}H_{42}O_{17}N_6Na^+$ calculated 793.24986, found 793.24972 (−0.18 ppm).

4.2 Calix[4]arene cores

1,2-Alternate-5,11,17,23-tetranitro-25,26,27,28-tetrapropoxycalix[4]arene (14c)

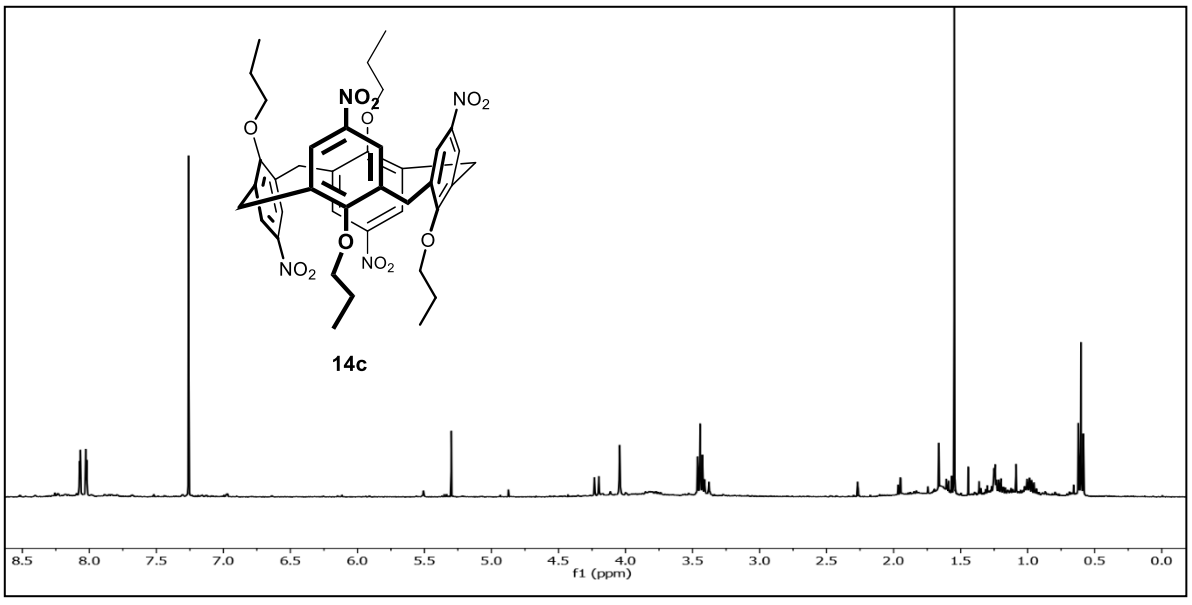


Figure S6. ^{1}H NMR spectrum of compound **14c.** **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K) δ_H (ppm): 8.07 (d, 4H, *J* = 2.8 Hz, Ar-**H**), 8.02 (d, 4H, *J* = 2.8 Hz, Ar-**H**), 4.22 (d, 2H, *J* = 13.2 Hz, Ar-C**H$_2$**-Ar), 4.04 (s, 4H, Ar-C**H$_2$**-Ar), 3.44-3.35 (m, -O-C**H$_2$**-CH_2-CH_3, Ar-C**H$_2$**-Ar), 1.33-1.14 (m, 4H, -O-CH_2-CH**H**-CH_3), 1.05-0.90 (m, 4H, -O-CH_2-CH**H**-CH_3), 0.60 (t, 12H, *J* = 7.5 Hz, -O-CH_2-CH_2-C**H$_3$**).

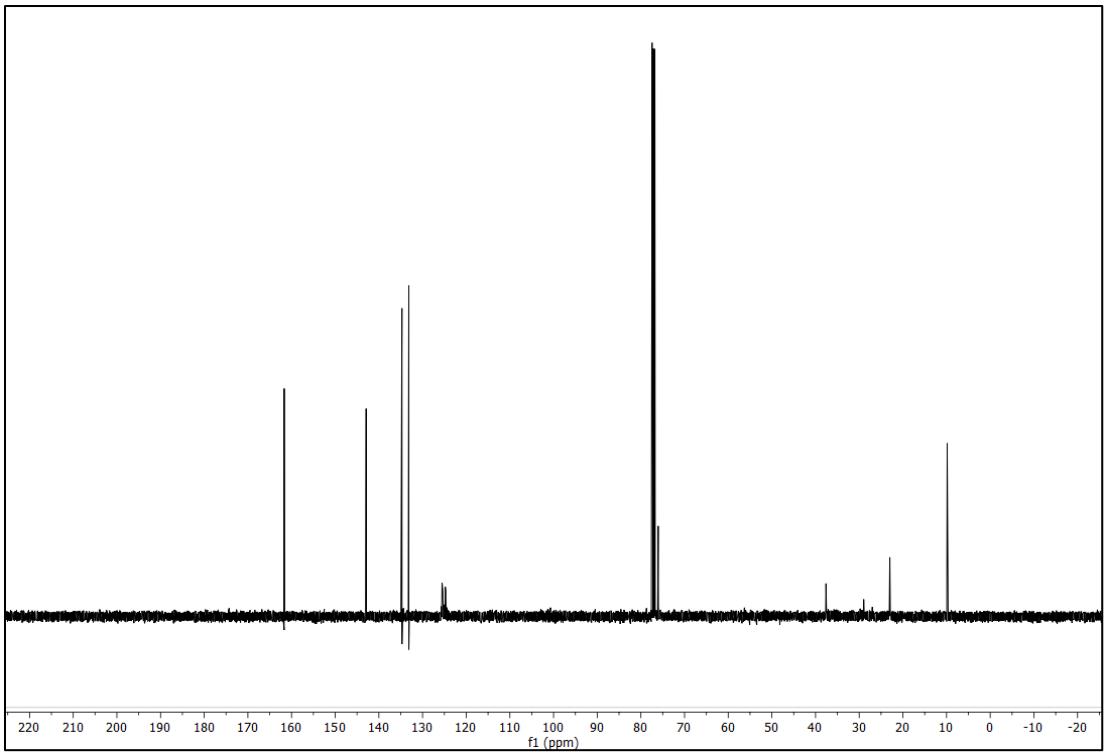


Figure S7. ^{13}C NMR spectrum of compound **14c.** **^{13}C NMR** (101 MHz, $CDCl_3$, 298 K) δ_C (ppm): 161.7 (*ipso*-), 142.9 (*para*-), 134.8 (*meta*-), 133.2 (*meta*-), 125.4 (*ortho*-), 124.7 (*ortho*-), 37.6 (-O-**C**H_2-CH_2-CH_3), 29.0 (-**C**H_2-), 23.0 (-O-CH_2-**C**H_2-CH_3), 9.9 (-O-CH_2-CH_2-**C**H_3).

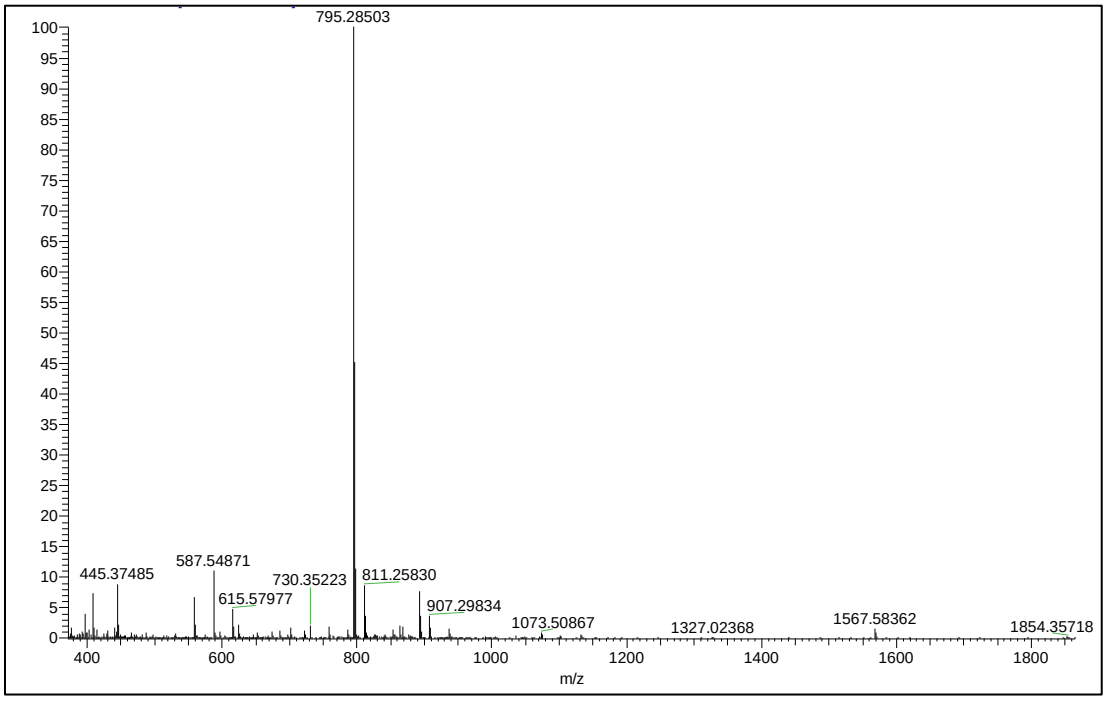


Figure S8. Mass spectrum (ESI$^+$) of compound **14c**. HRMS (ESI$^+$): *m*/*z* for $C_{40}H_{44}N_4O_{12}$ Na^+ calculated 795.2848, found 795.2850.

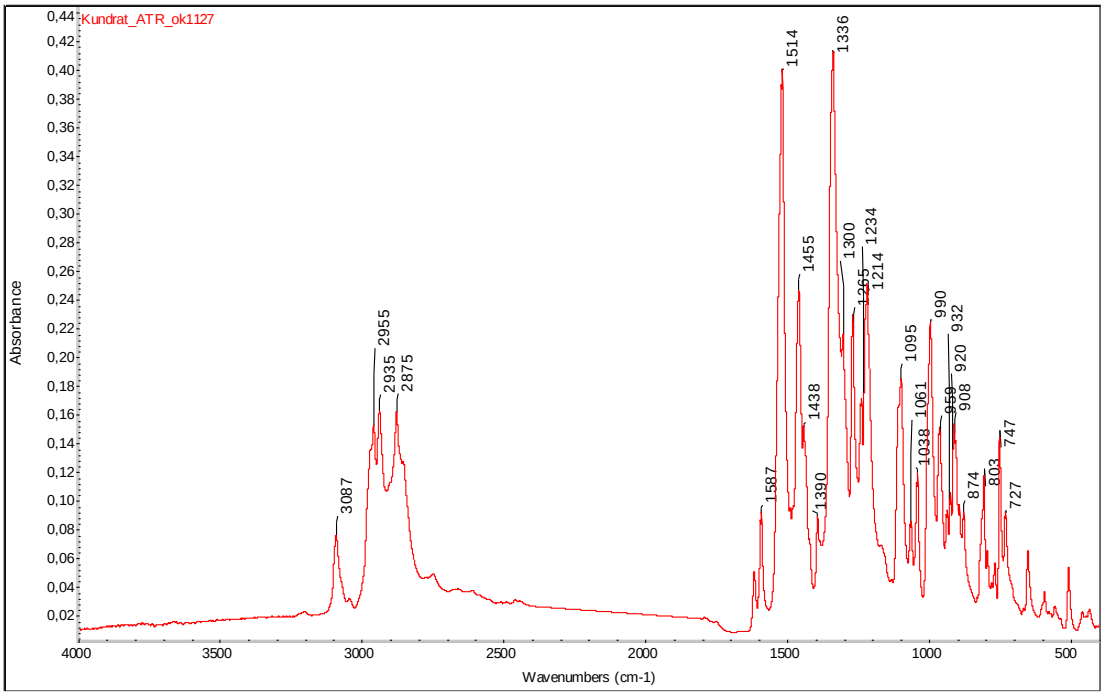


Figure S9. IR spectrum of compound **14c** (ATR): 1514 (Ar-NO_2), 1336 (Ar-NO_2) cm^{-1}.

Partial cone-5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arene (15b)

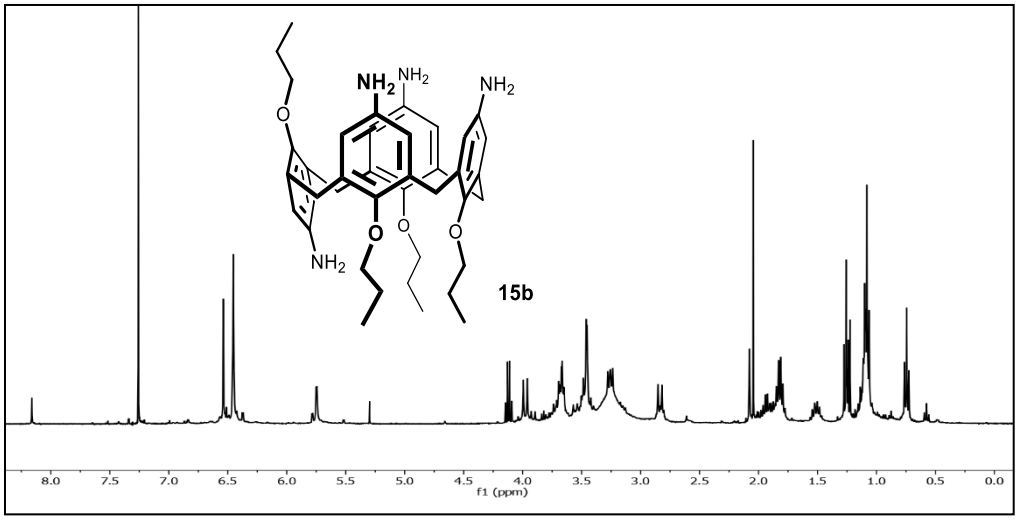


Figure S10. ^{1}H NMR spectrum of compound **15b**. **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K) δ_H (ppm): 6.53 (s, 2H, Ar-**H**), 6.45 (brs, 6H, Ar-**H**, Ar-**H**), 5.75 (d, 2H, *J* =2.8 Hz, Ar-**H**), 3.98 (d, 2H, *J* =13.3 Hz, Ar-C**H$_2$**-Ar), 3.75 -3.61 (m, 4H, -O-C**H$_2$**-CH_2-CH_3), 3.52-3.39 (m, 6H, -O-C**H$_2$**-CH_2-CH_3, Ar-C**H$_2$**-Ar), 3.25 (t, 2H, *J* =7.6 Hz, -O-C**H$_2$**-CH_2-CH_3), 2.83 (d, 2H, *J* =13.1 Hz, Ar-C**H$_2$**-Ar), 2.00-1.75 (m, 6H, -O-CH_2-C**H$_2$**-CH_3, -O-CH_2-C**H$_2$**-CH_3), 1.57-1.43 (m, 2H, -O-CH_2-C**H$_2$**-CH_3), 1.14-1.04 (m, 9H, -O-CH_2-CH_2-C**H$_3$**, -O-CH_2-CH_2-C**H$_3$**), 0.74 (t, 3H, *J* =7.6 Hz, -O-CH_2-CH_2-C**H$_3$**).

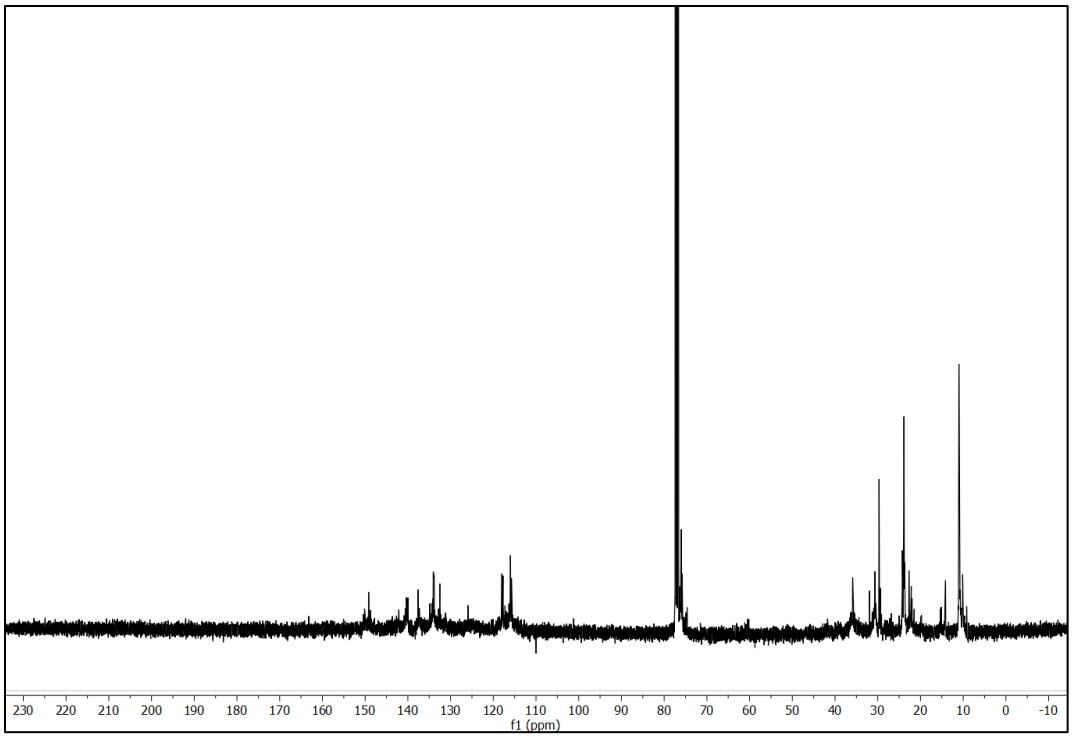


Figure S11. ^{13}C NMR spectrum of compound **15b.** **^{13}C NMR** (101 MHz, $CDCl_3$, 298 K) δ_C (ppm): 150.1 (*ipso*-B), 149.2 (*ipso*-A,C), 148.8 (*ipso*-D), 134.8 (*meta*-B), 134.0 (*meta*-D), 133.8 (*meta*-A,C), 132.5 (*meta*-A,C), 118.0 (*ortho*-B), 117.8 (*ortho*-D), 116.0 (*ortho*-A,C), 115.8 (*ortho*-A,C), 77.3 (-O-**C**H_2-CH_2-CH_3 B), 76.0 (-O-**C**H_2-CH_2-CH_3 A,C), 75.8 (-O-**C**H_2-CH_2-CH_3 D), 35.9 (Ar-**C**H_2-Ar AB,CB), 31.9 (Ar-**C**H_2-Ar AD,CD), 23.9 (-O-CH_2-**C**H_2-CH_3 B), 23.7 (-O-CH_2-**C**H_2-CH_3 A,C), 22.7 (-O-CH_2-**C**H_2-CH_3 D), 11.0 (-O-CH_2-CH_2-**C**H_3 B), 10.1 (-O-CH_2-**C**H_3 A,C), 9.2 (-O-CH_2-CH_2-**C**H_3 D).

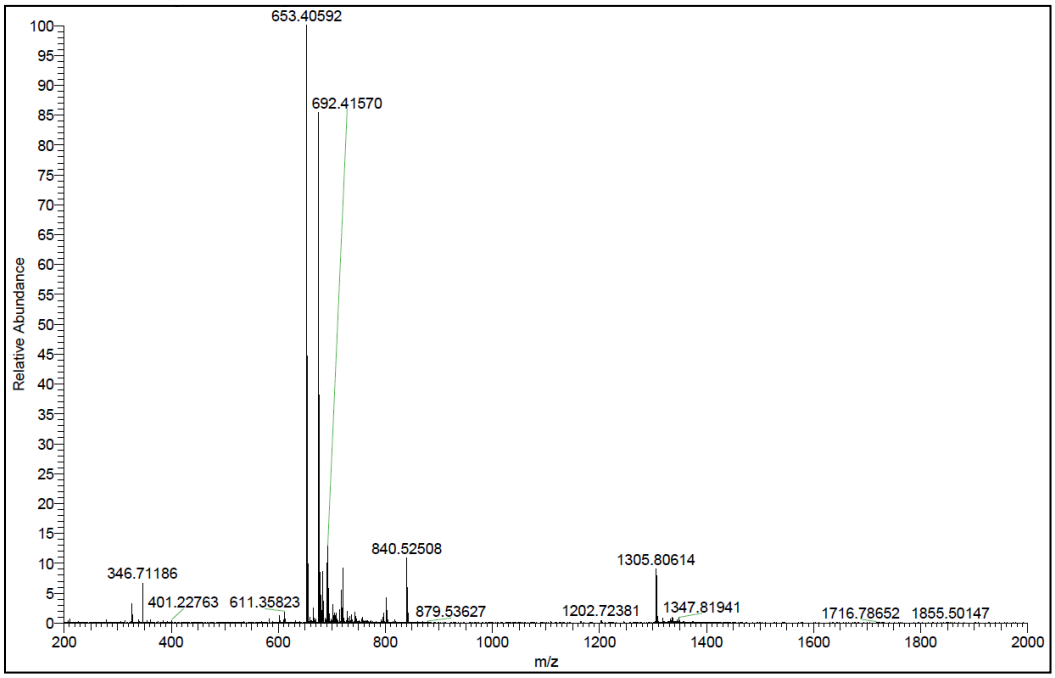


Figure S12. Mass spectrum (ESI$^+$) of compound **15b.** HRMS (ESI$^+$): *m/z* for $C_{40}H_{52}N_4O_4$ H^+ calculated 653.4061, found 653.4059, and *m/z* for $C_{40}H_{52}N_4O_4$ Na^+ calculated 675.3881, found 675.3875.

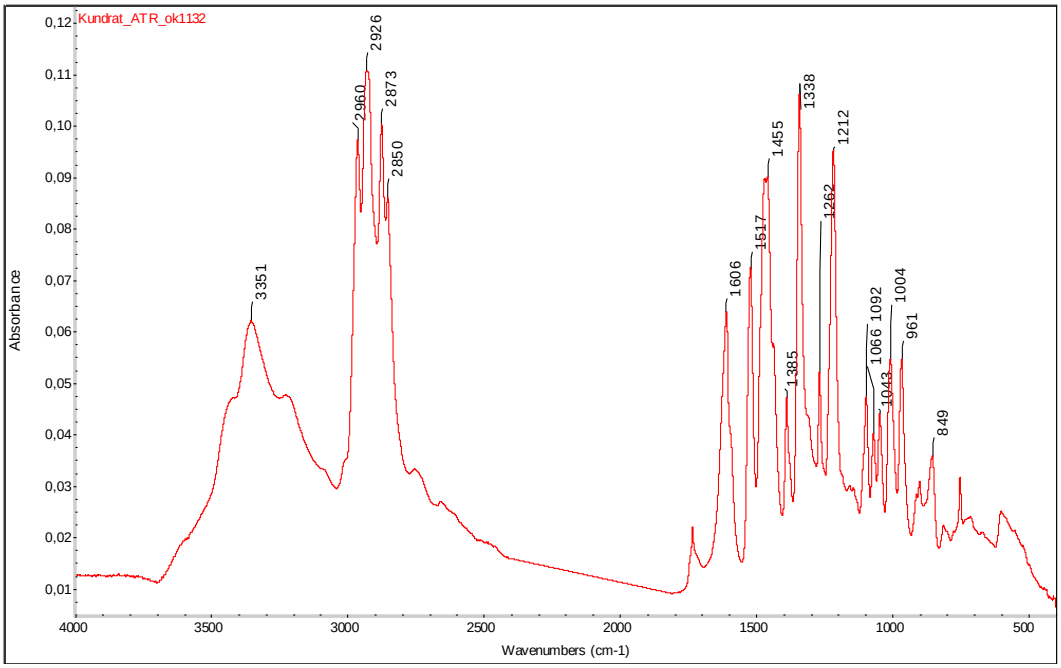


Figure S13. IR spectrum of compound **15b** (ATR): 3351 (Ar-NH_2) cm^{-1}.

1,2-Alternate-5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arene (15c)

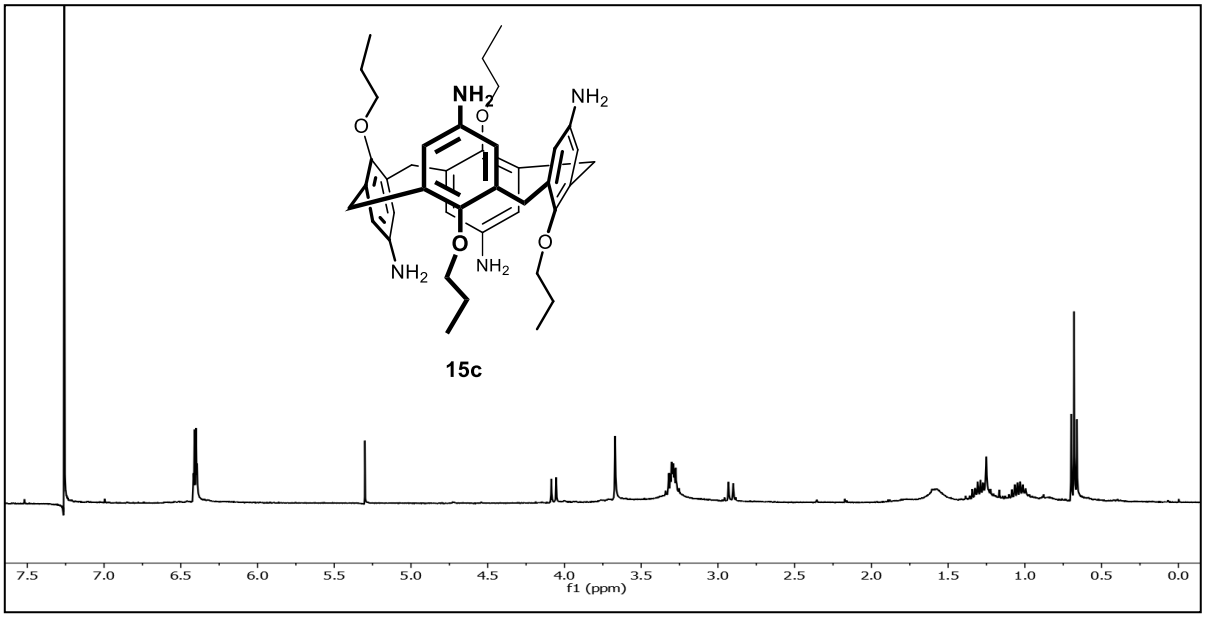


Figure S14. ^{1}H NMR spectrum of compound **15c. ^{1}H NMR** (400 MHz, $CDCl_3$, 298 K) δ_H (ppm): 6.42-6.38 (m, Ar-**H**, Ar-**H**), 4.07 (d, 2H, *J* = 12.3 Hz, Ar-C**H$_2$**-Ar), 3.67 (s, 4H, Ar-C**H$_2$**-Ar), 3.44-3.35 (m, -O-C**H$_2$**-CH_2-CH_3), 2.92 (d, 2H, *J* = 12.4 Hz, Ar-C**H$_2$**-Ar), 1.33-1.14 (m, 4H, -O-CH_2-C**H$_2$**-CH_3), 1.05-0.90 (m, 4H, -O-CH_2-C**H$_2$**-CH_3), 0.60 (t, 12H, *J* = 7.5 Hz, -O-CH_2-CH_2-C**H$_3$**).

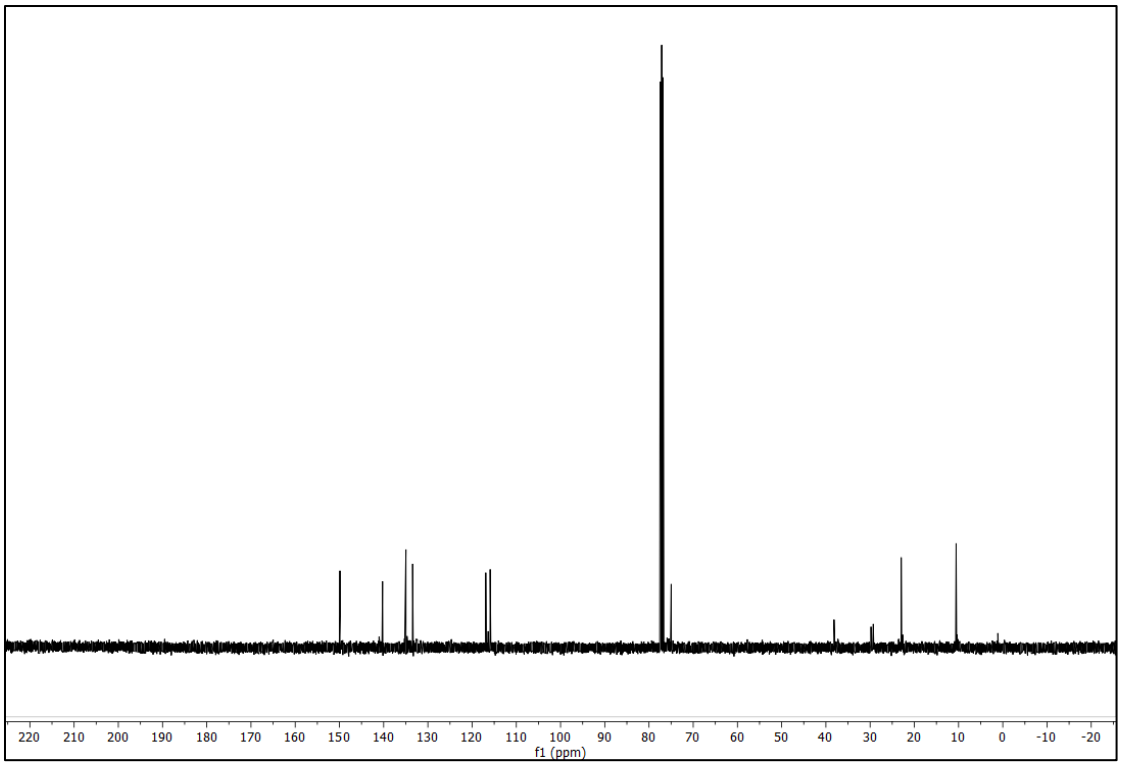


Figure S15. ^{13}C NMR spectrum of compound **15c.** **^{13}C NMR** (101 MHz, $CDCl_3$, 298 K) δ_C (ppm): 149.9 (*ipso*-), 140.3 (*para*-), 135.0 (*meta*-), 133.5 (*meta*-), 116.9 (*ortho*-), 115.9 (*ortho*-), 38.2 (-O-**C**H_2-CH_2-CH_3), 29.8 (-**C**H_2-),29.3 (-**C**H_2-), 23.0 (-O-CH_2-**C**H_2-CH_3), 10.6 (-O-CH_2-CH_2-**C**H_3).

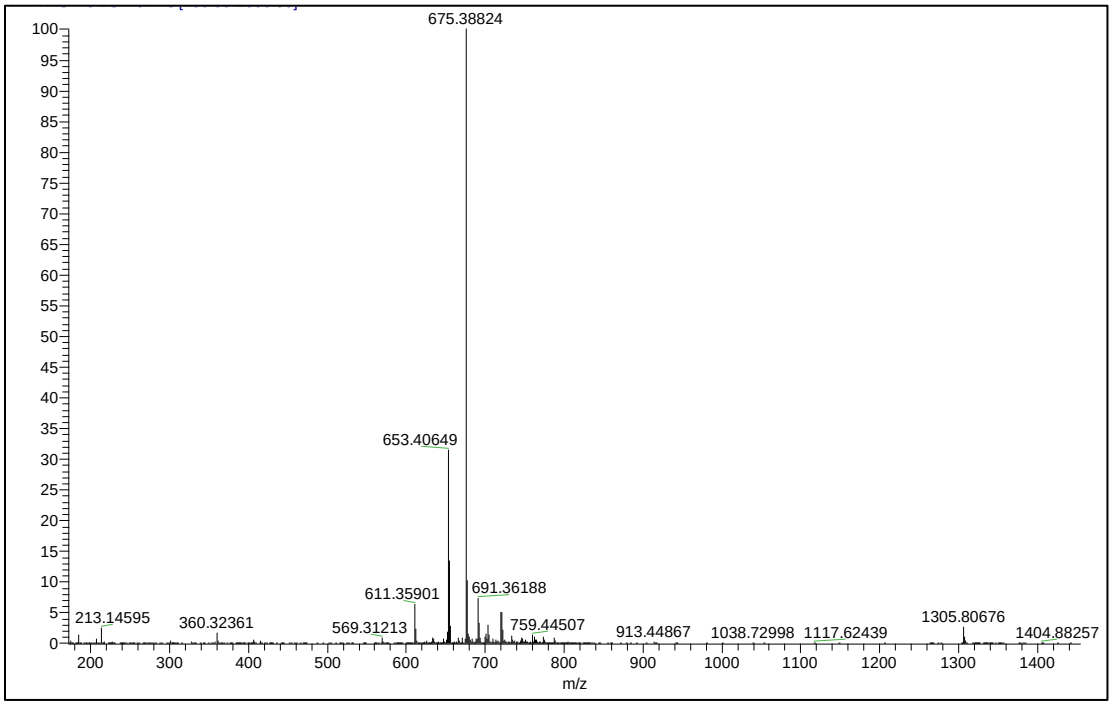


Figure S16. Mass spectrum (ESI$^+$) of compound **15c.** HRMS (ESI$^+$): *m*/*z* for $C_{40}H_{52}N_4O_4$ Na^+ calculated 675.3880, found 675.3882.

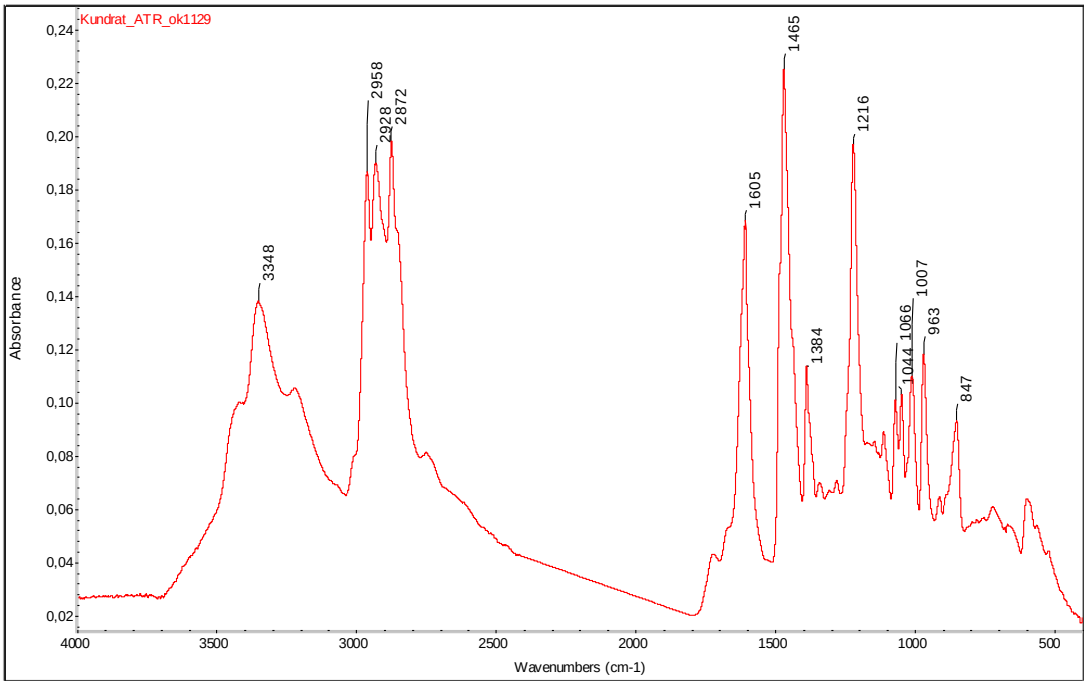


Figure S17. IR spectrum of compound **15c** (ATR): 3348 (Ar-NH_2) cm^{-1}.

Partial cone-5,11,17,23-tetrakis[4-pentynoylamino]-25,26,27,28-tetrapropoxycalix[4]arene (17)

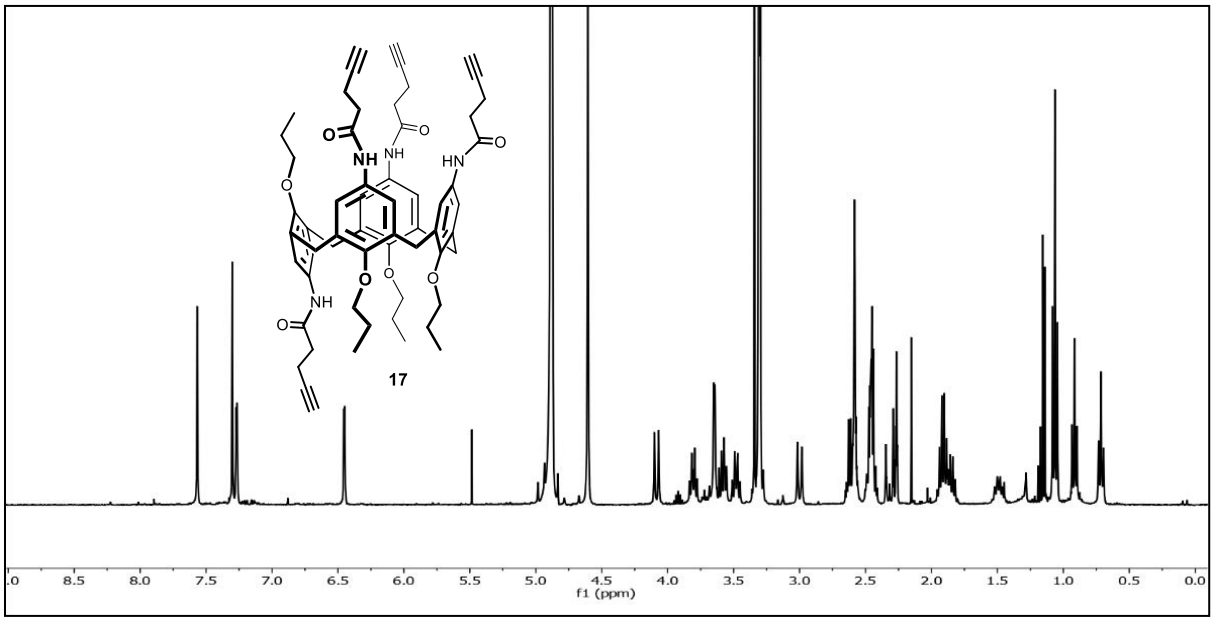


Figure S18. ^{1}H NMR spectrum of compound **17.** **^{1}H NMR** (400 MHz, CD_3OD, 298 K) δ_H (ppm): 7.57 (s, 2H, Ar-**H**), 7.30 (brs, 2H, Ar-**H**), 7.27 (d, 2H, *J* =2.7 Hz, Ar-**H**), 6.45 (d, 2H, *J* =2.7 Hz, Ar-**H**), 4.08 (d, 2H, *J* =13.1 Hz, Ar-C**H**$_2$-Ar), 3.85-3.76 (m, 2H, -O-C**H**$_2$-CH_2-CH_3), 3.70-3.54 (m, 8H, -O-C**H**$_2$-CH_2-CH_3, Ar-C**H**$_2$-Ar), 3.52-3.44 (m, 2H, -O-C**H**$_2$-CH_2-CH_3), 3.00 (d, 2H, *J* =13.1 Hz, Ar-C**H**$_2$-Ar), 2.66-2.54 (m, 8H, -O-CH_2-C**H**$_2$-CH_3), 2.52-2.40 (m, 8H, -OC-C**H**$_2$-CH_2-C≡CH), 2.35 (m, 1H, -C≡C**H**), 2.29 (t, 1H, -C≡C**H**), 2.27 (m, 2H, -C≡C**H**), 1.97-1.80 (m, 8H, -CO-CH_2-C**H**$_2$-C≡CH), 1.06 (t, 6H, -O-CH_2-CH_2-C**H**$_3$), 0.92 (t, 3H, -O-CH_2-CH_2-C**H**$_3$), 0.71 (t, 3H, *J* =7.6 Hz, -O-CH_2-CH_2-C**H**$_3$).

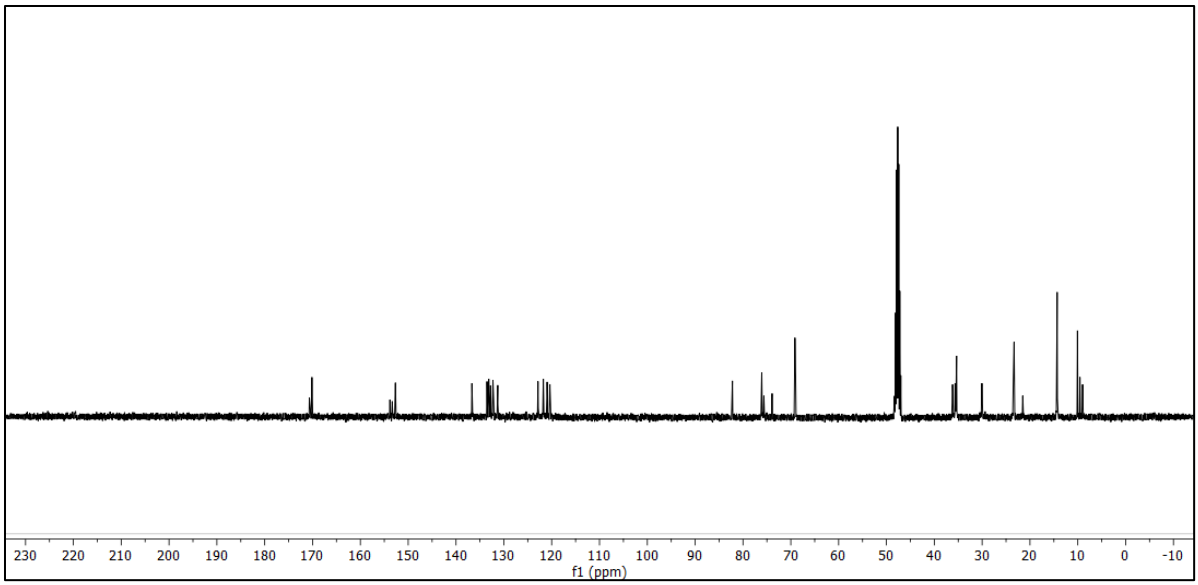


Figure S19. ^{13}C NMR spectrum of compound **17.** **^{13}C NMR** (101 MHz, CD_3OD) δ_C (ppm): 170.65; 170.12; 170.04; 153.81; 153.75; 153.32; 152.66; 136.67; 133.51; 133.15; 132.76; 132.27; 131.27; 122.84; 121.73; 120.93; 120.36; 82.31; 82.28; 82.26; 82.20; 76.07; 75.62; 73.91; 69.19; 69.13; 68.99; 36.17; 35.64; 35.43; 35.34; 30.03; 23.48; 23.31; 21.49; 14.41; 14.31; 10.06; 9.54; 8.99.

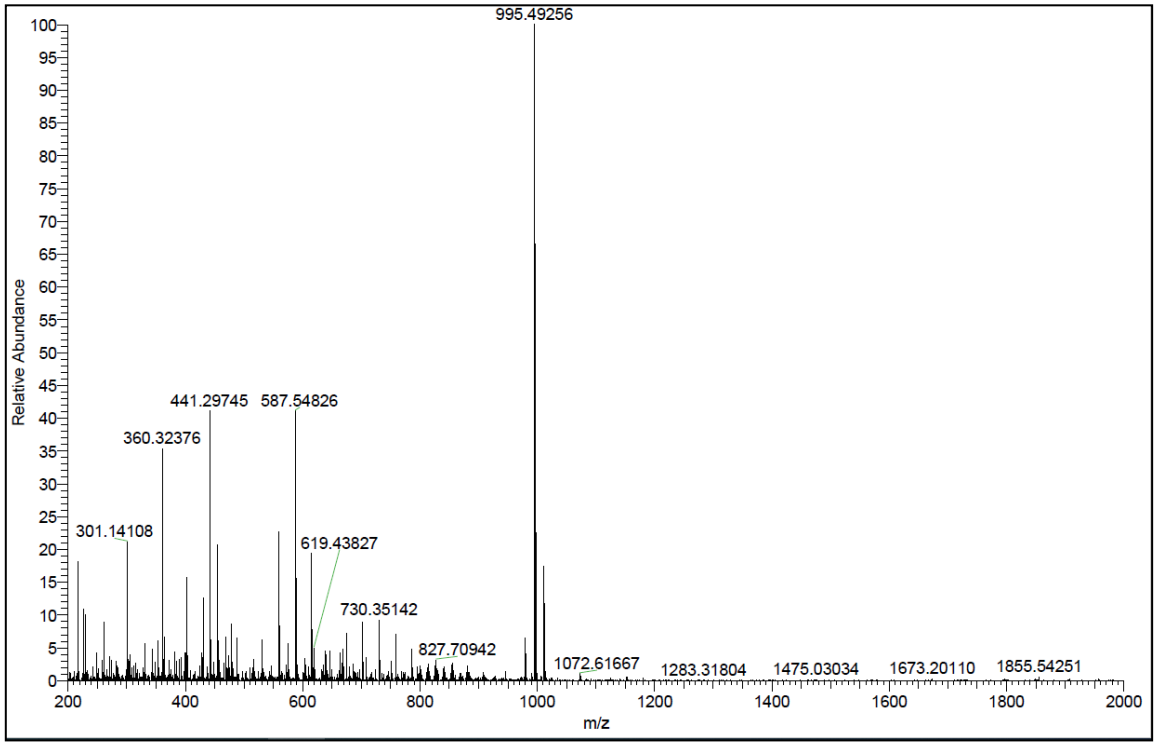


Figure S20. Mass spectrum (ESI$^+$) of compound **17**. HRMS (ESI$^+$): *m/z* for $C_{60}H_{68}N_4O_8Na^+$ calculated 995.4929, found 995.4927.

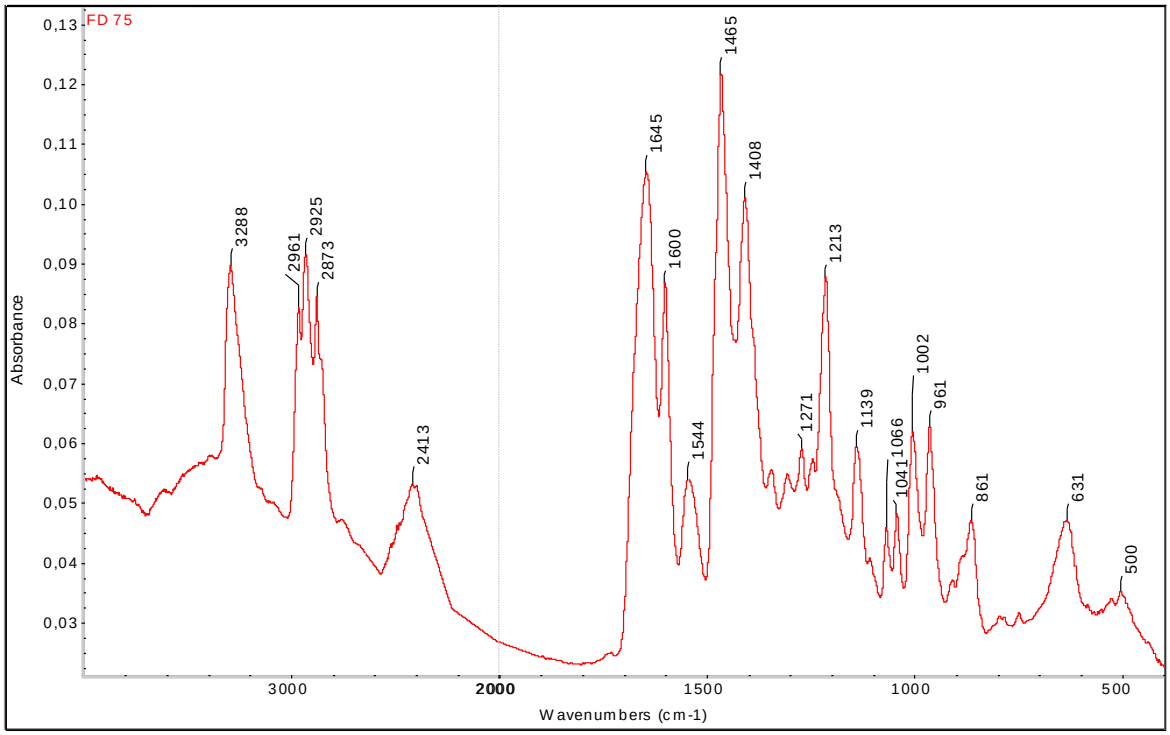


Figure S21. IR spectrum of compound **17** (ATR): 3288 (C≡CH), 1645 (C=O) cm^{-1}.

1,2 - Alternate - 5,11,17,23-tetrakis[4-pentynoylamino]-25,26,27,28-tetrapropoxycalix[4]arene

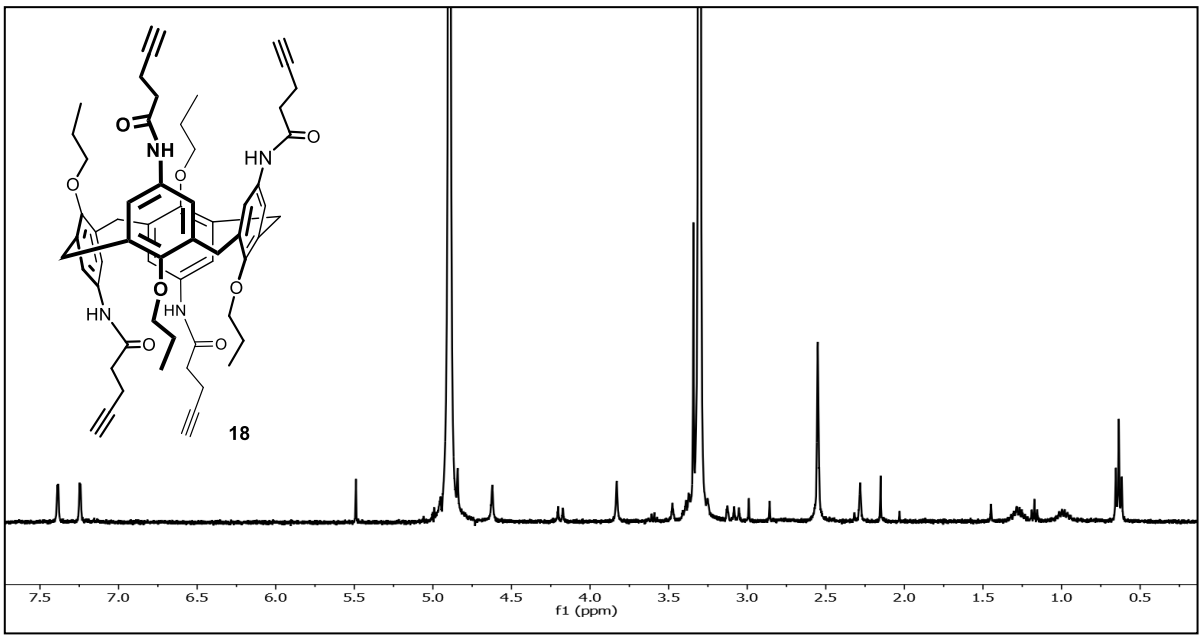


Figure S22. ^{1}H NMR spectrum of compound **18. ^{1}H NMR** (400 MHz, CD_3OD, 298 K) δ_H (ppm): 7.38 (s, 4H, Ar-**H**), 7.24 (s, 4H, Ar-**H**), 4.18 (d, 2H, *J* = 12.7 Hz, Ar-C**H**$_2$-Ar), 3.82 (s, 4H, Ar-C**H**$_2$-Ar), 3.42-3.24 (m, 8H, -O-C**H**$_2$-CH_2-CH_3), 3.07 (d, 2H, *J* = 10.3 Hz, Ar-C**H**$_2$-Ar), 2.32-2.23 (m, 16H, CO-C**H**$_2$-CH_2, CO-CH_2-C**H**$_2$), 2.15 (brs, 4H, -C≡C**H**), 1.46-1.13 (m, 4H, -O-CH_2-C**H**$_2$-CH_3), 1.06-0.86 (m, 4H, -O-CH_2-C**H**$_2$-CH_3), 0.63 (t, 12H, *J* = 7.5 Hz, -O-CH_2-CH_2-C**H**$_3$).

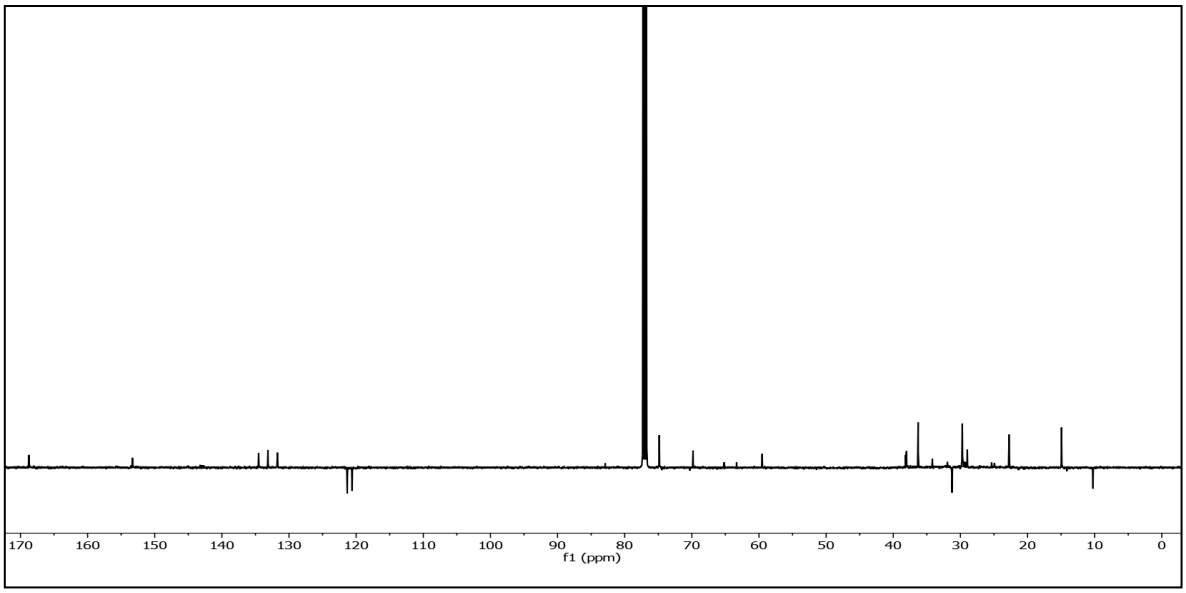


Figure S23. ^{13}C NMR spectrum of compound **18**. **^{13}C NMR** (125 MHz, CD_3OD, 298 K) δ_C (ppm): 168.74; 153.26; 134.72; 133.03; 131.80; 121.41; 120.59; 74.83; 69.80; 59.53; 38.06; 36.31; 31.26; 29.71; 28.97; 22.76; 14.96; 10.23.

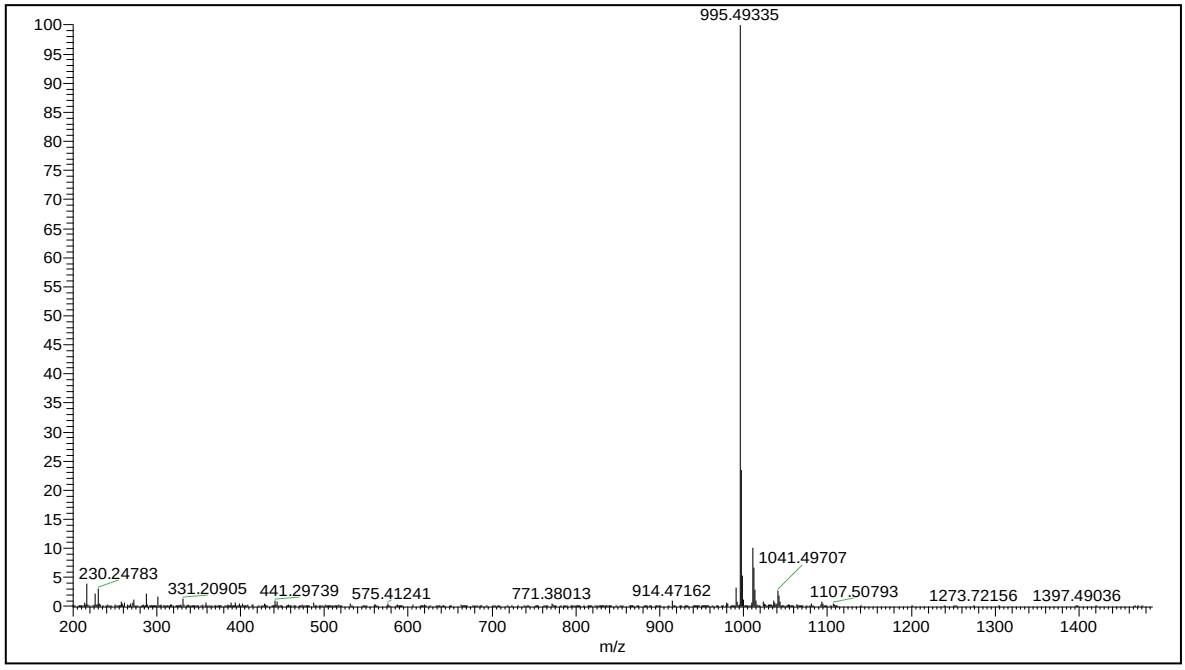


Figure S24. Mass spectrum (ESI$^+$) of compound **18**. HRMS (ESI$^+$): *m*/*z* for $C_{60}H_{68}N_4O_8Na^+$ calculated 995.4929, found 995.4933.

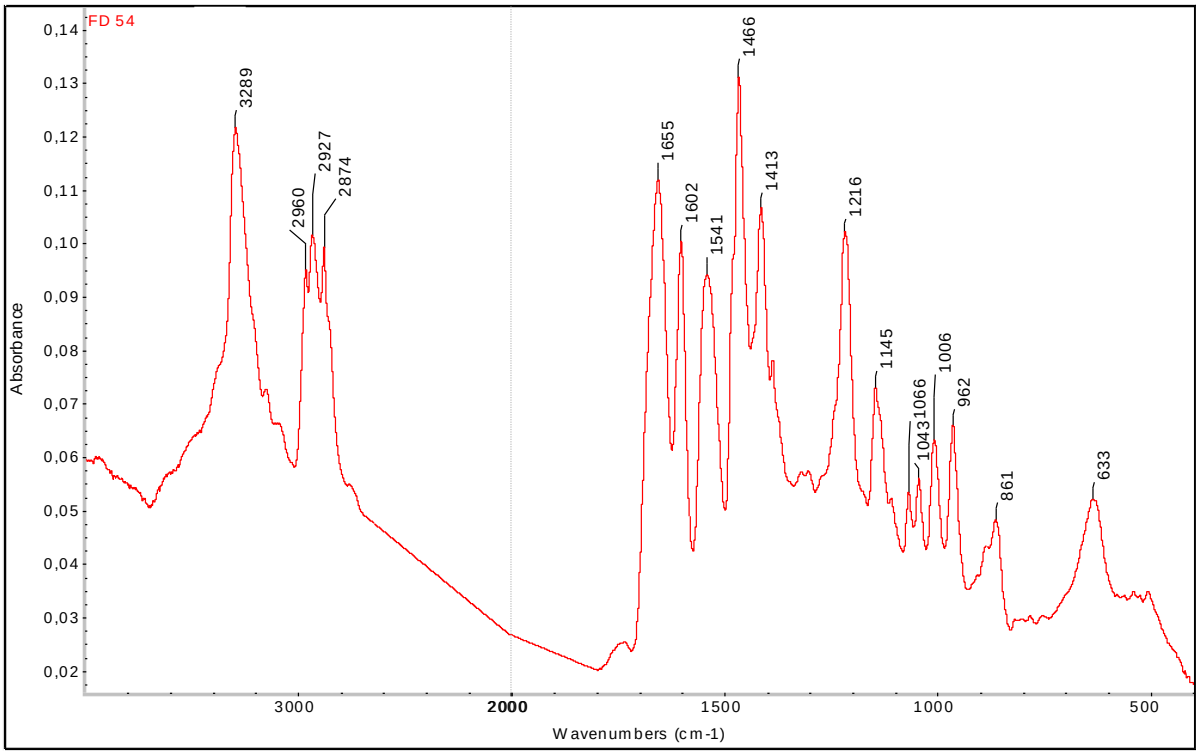


Figure S25. IR spectrum of compound **18** (ATR): 3289 (C≡CH), 1655 (C=O) cm^{-1}.

4.3 Glycocalix[4]arenes

Cone-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (21)

Table S2. ^{1}H and ^{13}C NMR data of compound **21** (700.13 MHz for ^{1}H, 176.05 MHz for ^{13}C, DMSO-d_6, 30 °C).

	Atom	δ_C	m.	δ_H	n_H	m.	*J*[Hz]
core	***ipso-***	151.77	s	-	0	-	
	ortho-	133.99	s	-	0	-	
	meta-	119.45	d	6.963, 6.953	8	s	
	para-	133.02	s	-	0	-	
	-CH_2-	30.74	t	4.340	4	br d	13.3
				3.071	4	br d	13.3
	1''	76.35	t	3.77^{H}	8	m	
	2''	22.55	t	1.893	8	m	
	3''	10.09	q	0.958	12	t	7.5
	NH	-	-	9.574	4	s	
	CO	169.36	s	-	0	-	
spacer	**1'**	35.15	t	2.600	8	br t	
	2'	20.82	t	2.883	8	br t	7.3
	3'	145.80	s	-	0	-	
	4'	121.00	d	8.000	4	s	
Glc	**1**	86.80	d	5.567	4	d	9.3
	2	71.70	d	3.821	4	dd	9.3, 9.0
	3	75.14	d	3.56^{H}	4	m	
	4	79.67	d	3.47^{H}	4	m	
	5	77.65	d	3.63^{H}	4	m	
	6	59.96	t	3.767	4	m	

				3.60[H]	4	m	
Gal	**1**	103.69	d	4.258	4	d	7.4
	2	70.49	d	3.359	4	dd	
	3	73.18	d	3.33	4	m	
	4	68.05	d	3.637	4	m	
	5	75.51	d	3.48[H]	4	m	
	6	60.33	t	3.53[H]	8	m	

[H] … HSQC readout

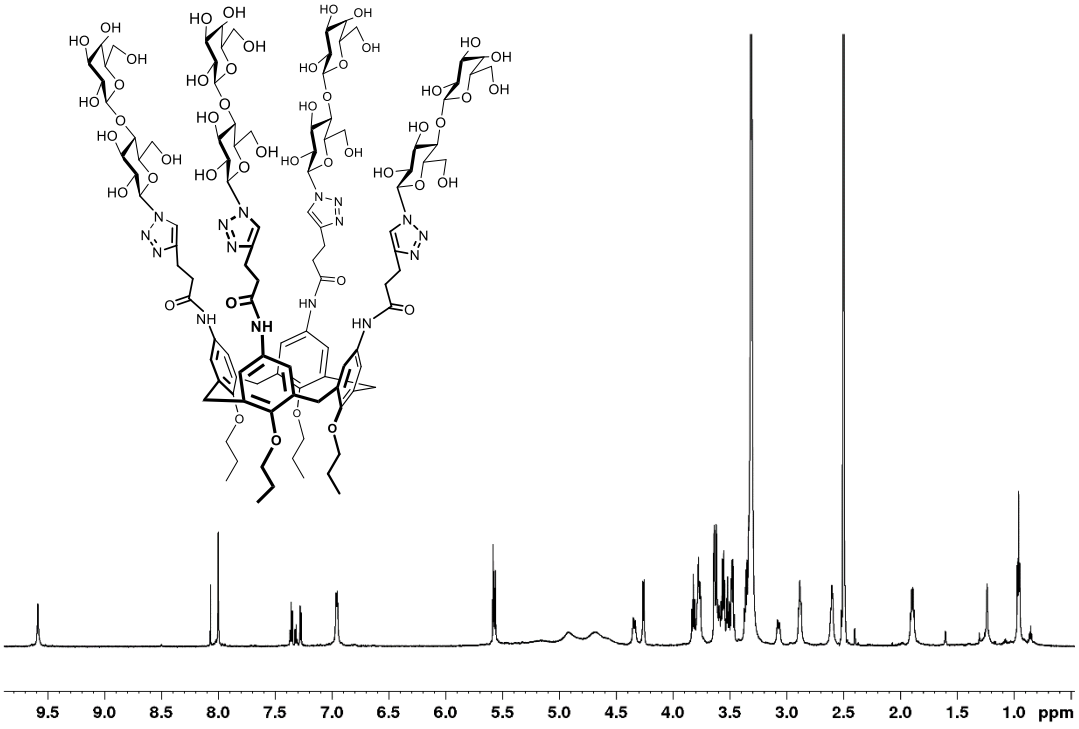


Figure S21. ^{1}H NMR spectrum of compound **21** (700.13 MHz, DMSO-d_6, 30 °C).

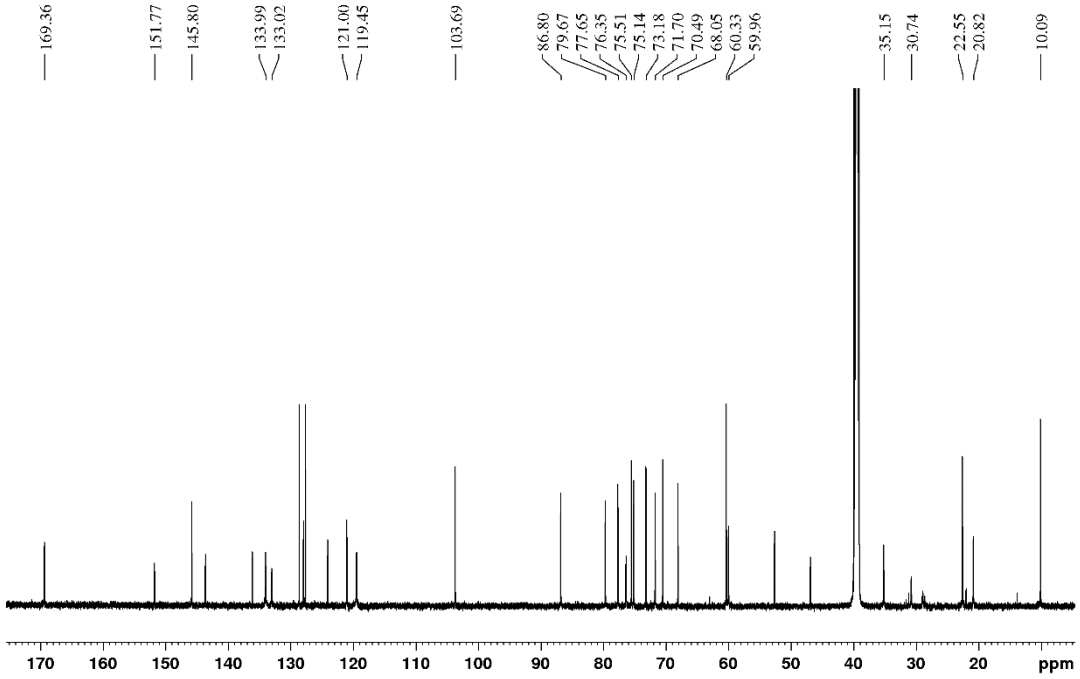
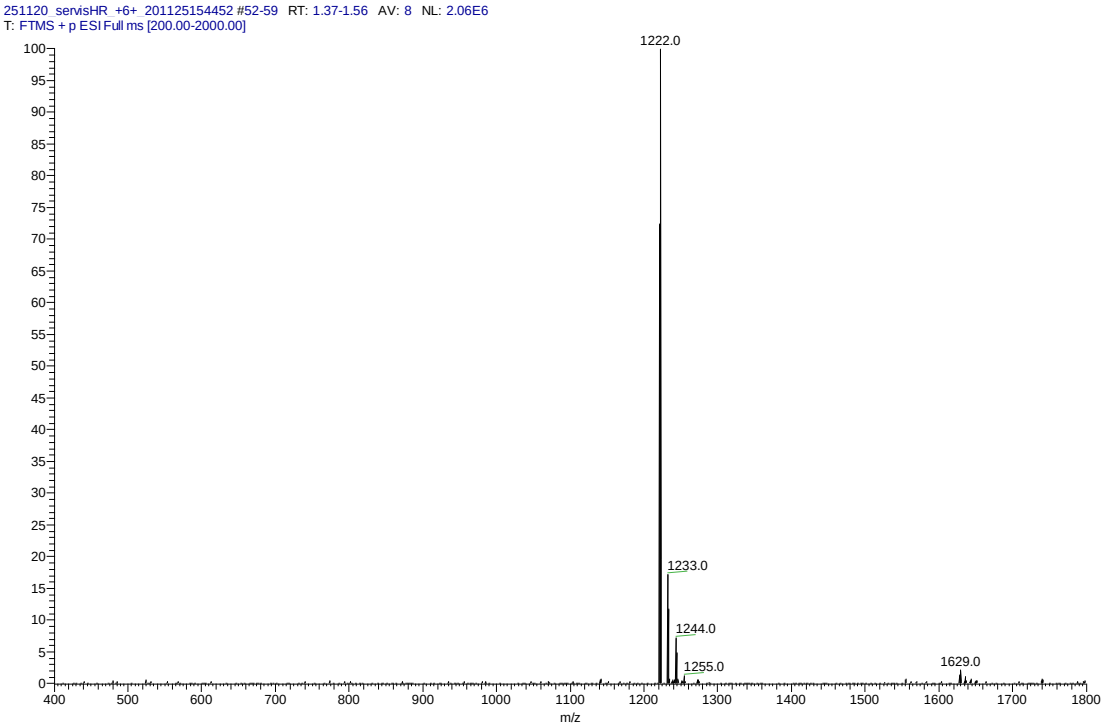


Figure S22. ^{13}C NMR spectrum of compound **21** (176.05 MHz, DMSO-d_6, 30 °C).



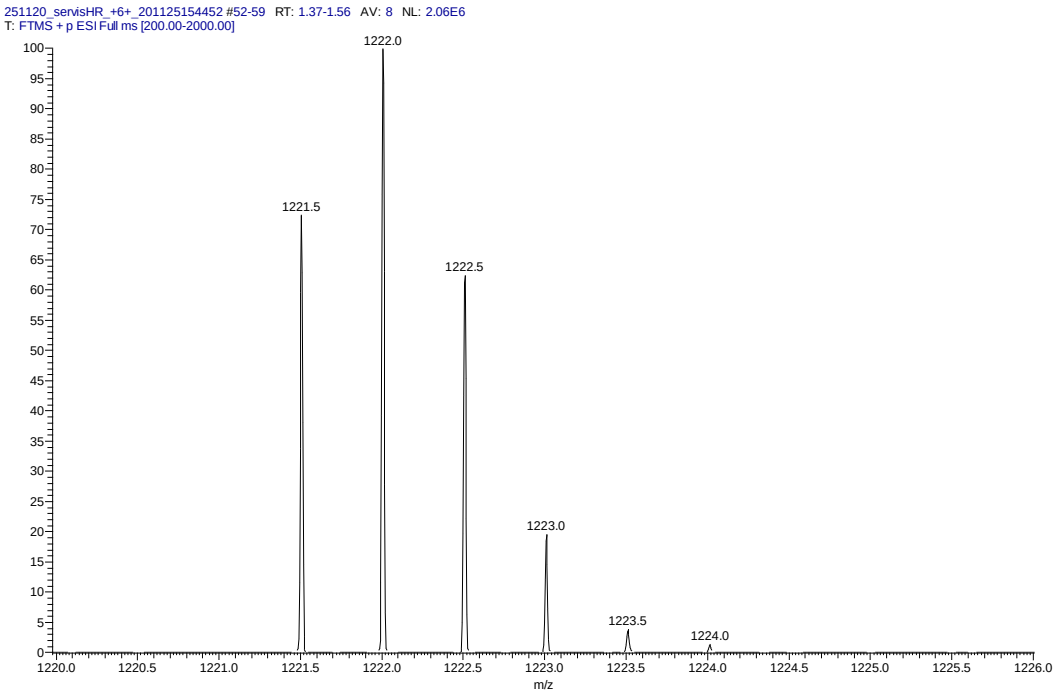


Figure S23. Mass spectrum (ESI$^+$) of compound **21** (upper figure) and enlarged region showing [M + 2Na]$^{2+}$, *m/z* 1221.5 (lower figure). HRMS (ESI$^+$): *m/z* for $C_{108}H_{154}O_{48}N_{16}^{2+}$ calculated 1221.50452, found 1221.50574 (1.00 ppm).

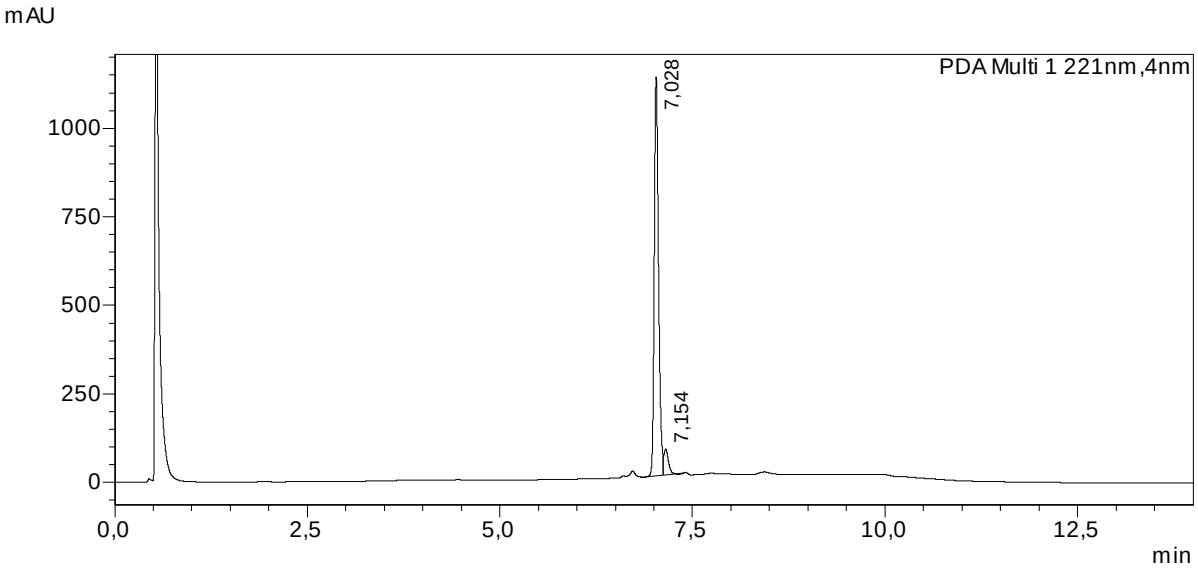


Figure S24. HPLC chromatogram of compound **21** (7.028 min, purity 94%; the peak at the beginning of the chromatogram is DMSO).

Cone-5,11,17,23-tetrakis{[2-(2-(azidoethoxy)ethoxy)ethyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (23)

Table S3. ^{1}H and ^{13}C NMR data compound **23** (700.13 MHz for ^{1}H, 176.05 MHz for ^{13}C, DMSO-d_6, 30 °C).

	Atom	δ_C	m.	δ_H	n_H	m.	*J*[Hz]
core	**ipso-**	151.80	s	-	0	-	
	ortho-	133.99	s	-	0	-	
	meta-	119.46	d	6.924	8	br s	-
	para-	133.02	s	-	0	-	
	-CH_2-	30.72	t	4.331	4	br d	13.2
				3.055	4	br d	13.2
	1''	76.33	t	3.77^{H}	8	m	
	2''	22.56	t	1.881	8	m	
	3''	10.11	q	0.955	12	t	7.54
	NH	-	-	9.552	4	s	
	CO	169.40	s	-	0	-	
spacer	**1'**	35.37	t	2.567	8	br t	7.5
	2'	20.88	t	2.859	8	br t	7.5
	3'	145.75	s	-	0	-	
	4'	122.19	d	7.781	4	s	
	5'	49.15	t	4.452	8	t	5.4
	6'	68.72	t	3.783	8	t	5.4
	7'	69.45	t	3.49	8	m	
	8'	69.57	t	3.47	8	m	
	9'	69.54	t	3.52	8	m	
	10'	67.91	t	3.831	4	ddd	
				3.567	4	ddd	
Glc	**1**	102.60	d	4.213	4	d	
	2	73.04	d	3.019	4	dd	Σ = 16.4
	3	74.91	d	3.30^{H}	4	m	
	4	80.61	d	3.29^{H}	4	m	
	5	74.78	d	3.27^{H}	4	m	
	6	60.47	t	3.53^{H}	4	m	
				3.48^{H}	4	m	
Gal	**1**	103.80	d	4.202	4	d	7.4
	2	70.53	d	3.32^{H}	4	dd	
	3	73.20	d	3.31^{H}	4	m	
	4	68.01	d	3.623	4	m	
	5	75.48	d	3.442	4	m	
	6	60.29	t	3.744	4	dd	
				3.601	4	dd	

^{H} … HSQC readout

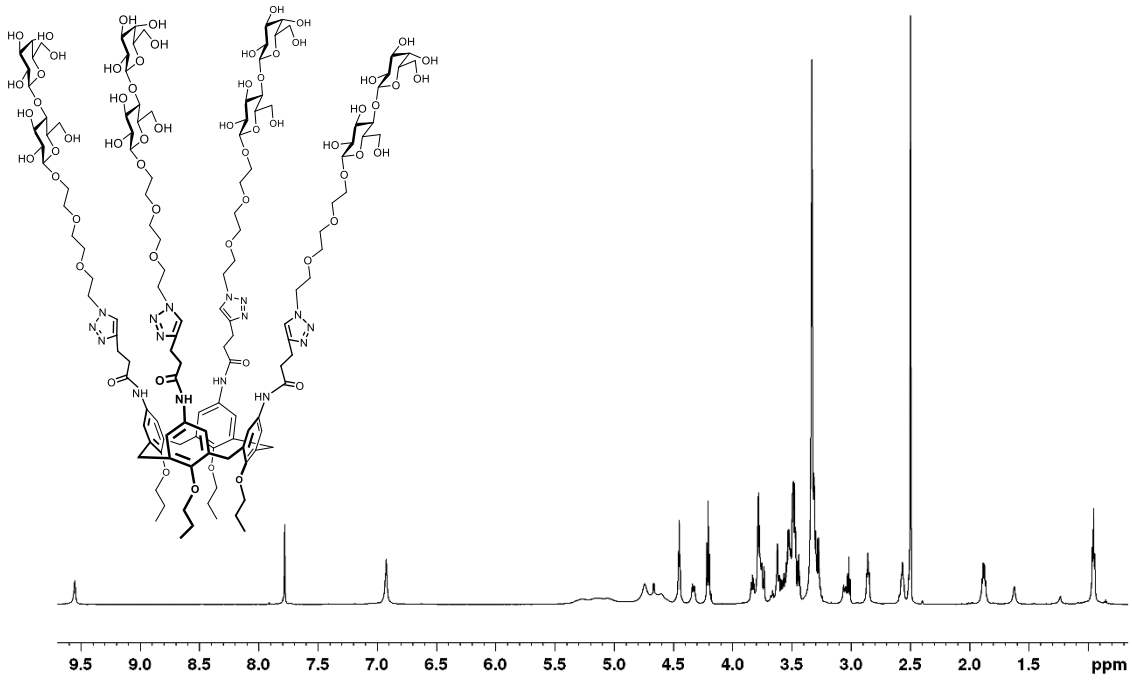


Figure S25. ^{1}H NMR spectrum of compound **23** (700.13 MHz, DMSO-d_6, 30 ºC).

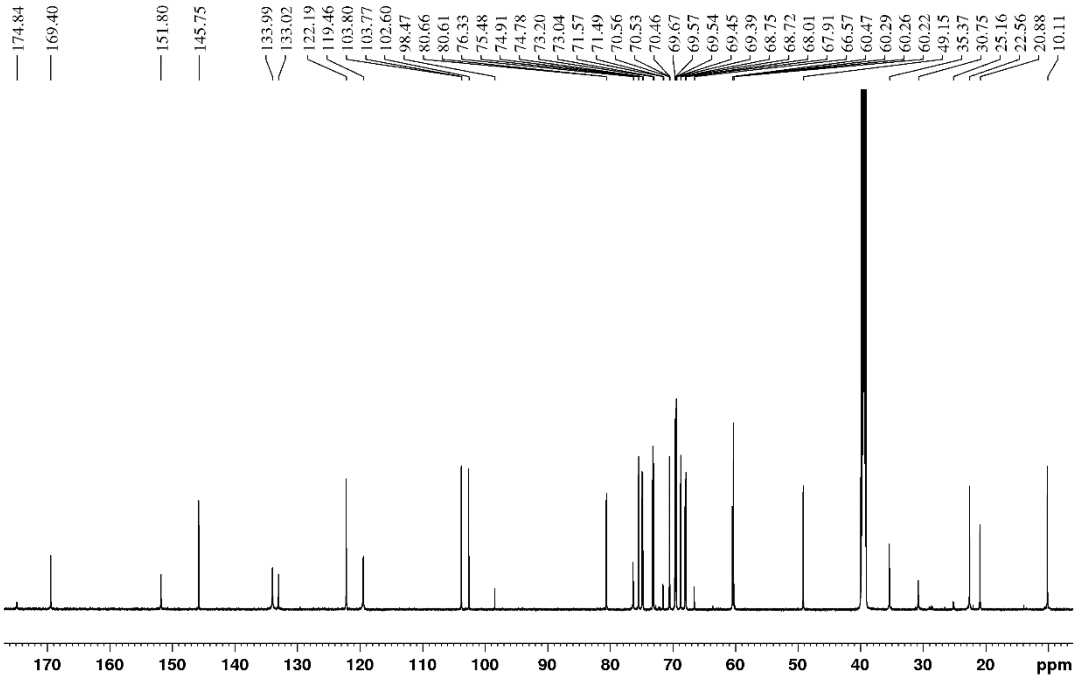


Figure S26. ^{13}C NMR spectrum of compound **23** (176.05 MHz, DMSO-d_6, 30 ºC).

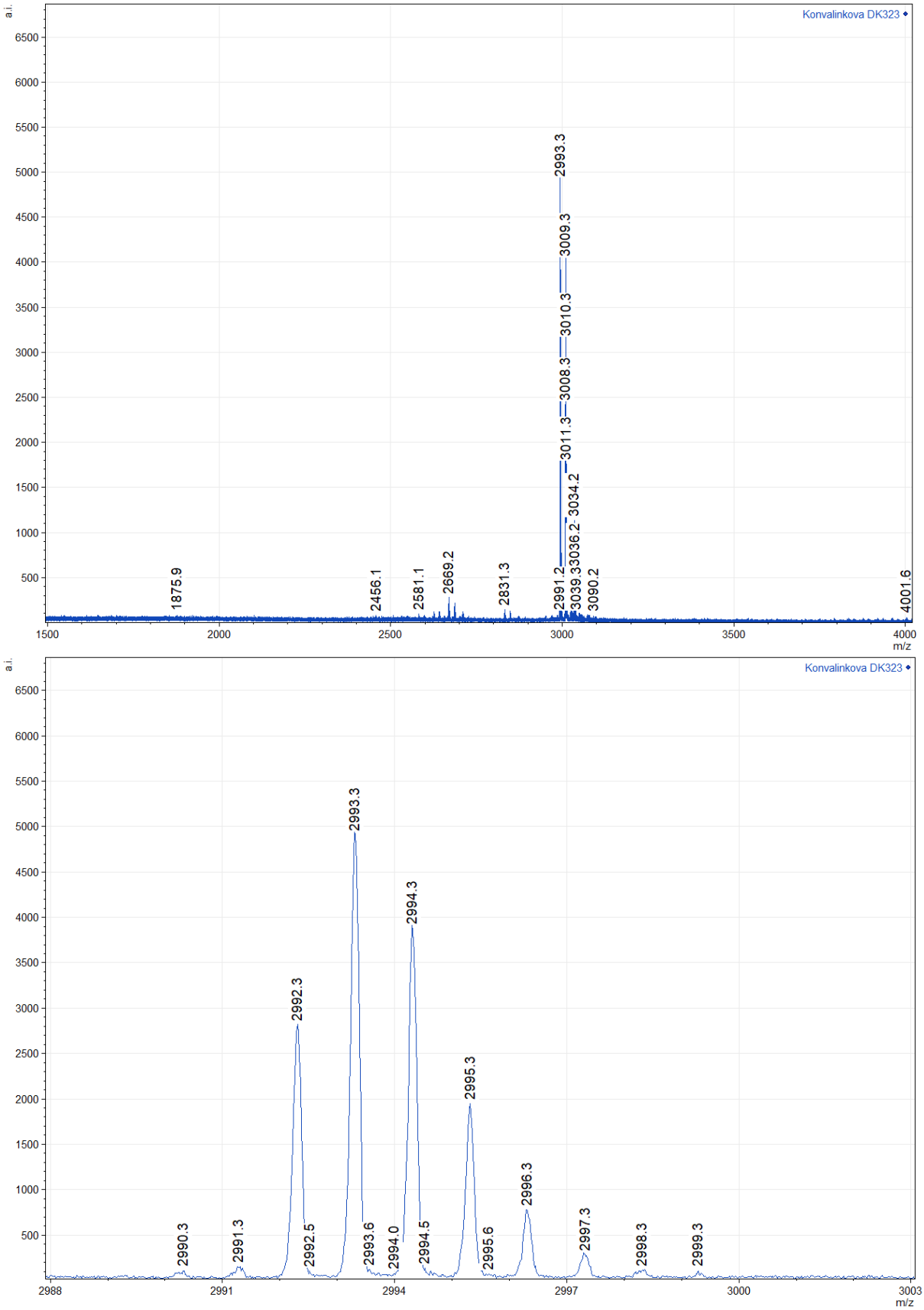


Figure S27. Mass spectrum (MALDI$^+$) of compound **23** (upper figure) and enlarged region showing [M + Na]$^+$, *m*/*z* 2992.3 (lower figure). HRMS (MALDI$^+$): *m*/*z* for $C_{132}H_{200}O_{60}N_{16}Na^+$ calculated 2992.2983, found 2992.3028 (1.50 ppm).

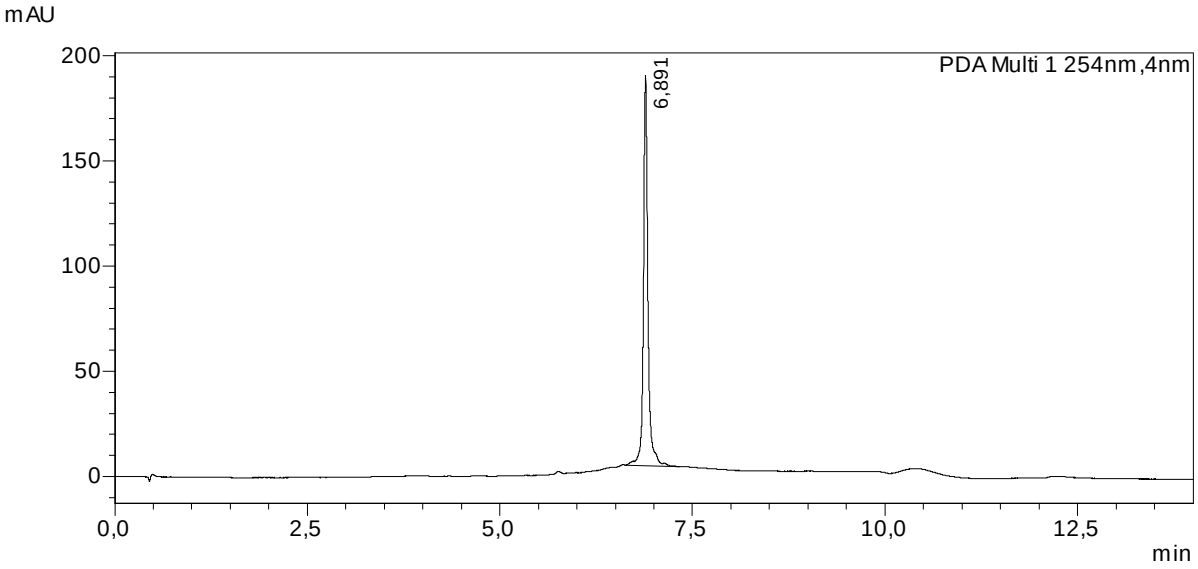


Figure S28. HPLC chromatogram of isolated compound **23** (6.891 min, 99% purity).

Cone-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (25)

Table S4. ^{1}H and ^{13}C NMR data compound **25** (700.13 MHz for ^{1}H, 176.05 MHz for ^{13}C, DMSO-d_6, 30 °C).

	Atom	δ_C	m.	δ_H	n_H	m.	*J*[Hz]
core	**ipso-**	151.80	s	-	0	-	
	ortho-	133.98	s	-	0	-	
	meta-	119.39	d	6.922	8	br s	
	para-	132.98	s	-	0	-	
	-CH_2-	30.73	t	4.329	4	br d	
				3.055	4	br d	12.3
	1^x	76.30	t	3.77^H	8	m	
	2^x	22.53	t	1.878	8	m	
	3^x	10.07	q	0.953	12	t	7.3
	NH	-	-	9.519	4	s	
	CO	169.35	s	-	0	-	
spacer	**1'**	35.41	t	2.570	8	br t	7.7
	2'	20.93	t	2.864	8	br t	7.7
	3'	145.86	s	-	0	-	
	4'	121.83	d	7.846	4	s	
	1''	48.56	t	4.369	8	t	7.1
	2''	29.27	t	2.133	8	m	
	3''	21.92	t	2.607	8	br t	7.3
	4''	145.49	s	-	0	-	
	5''	121.04	d	8.084	4	s	
Glc	**1**	86.85	d	5.572	4	d	9.3
	2	71.74	d	3.819	4	dd	9.3, 9.0
	3	75.06	d	3.571	4	dd	9.0, 8.8
	4	79.72	d	3.467	4	dd	9.7, 8.8
	5	77.60	d	3.642	4	ddd	9.7, 4.9, 2.3
	6	59.99	t	3.78^H	4	m	
				3.60^H	4	m	
Gal	**1**	103.68	d	4.259	4	d	7.5

	2	70.47	d	3.361	4	br dd	
	3	73.17	d	3.33[H]	4	m	
	4	68.06	d	3.64[H]	4	m	
	5	75.50	d	3.488	4	m	
	6	60.33	t	3.53[H]	8	m	

[H] … HSQC readout

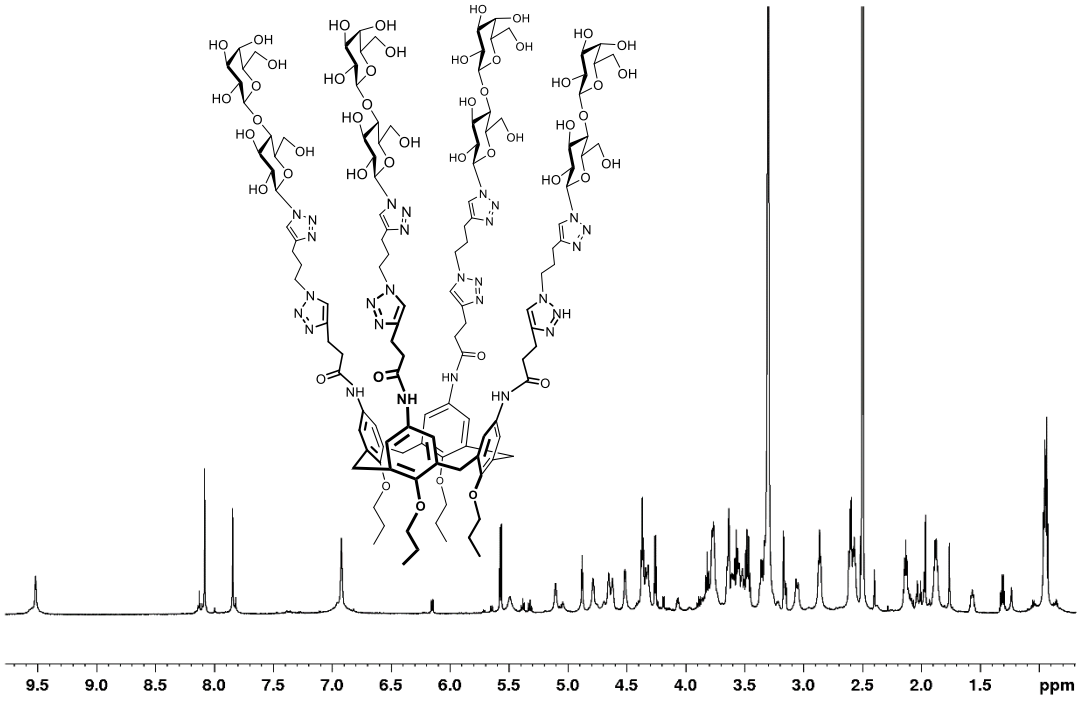


Figure S29. [1]H NMR spectrum of compound **25** (700.13 MHz, DMSO-d_6, 30 °C).

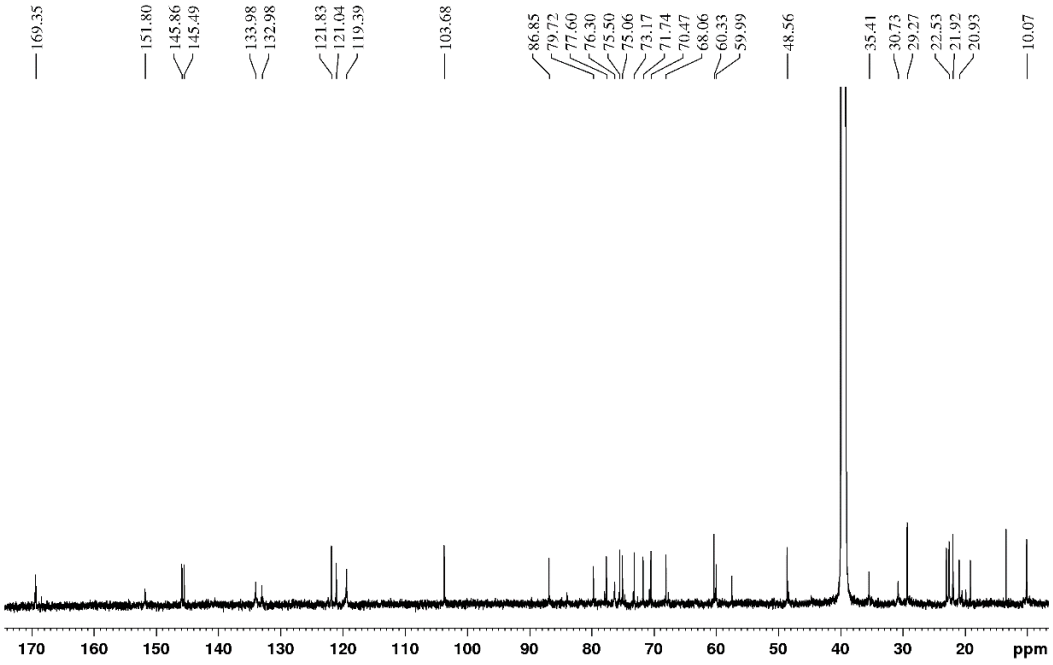


Figure S30. [13]C NMR spectrum of compound **25** (176.05 MHz, DMSO-d_6, 30 °C).

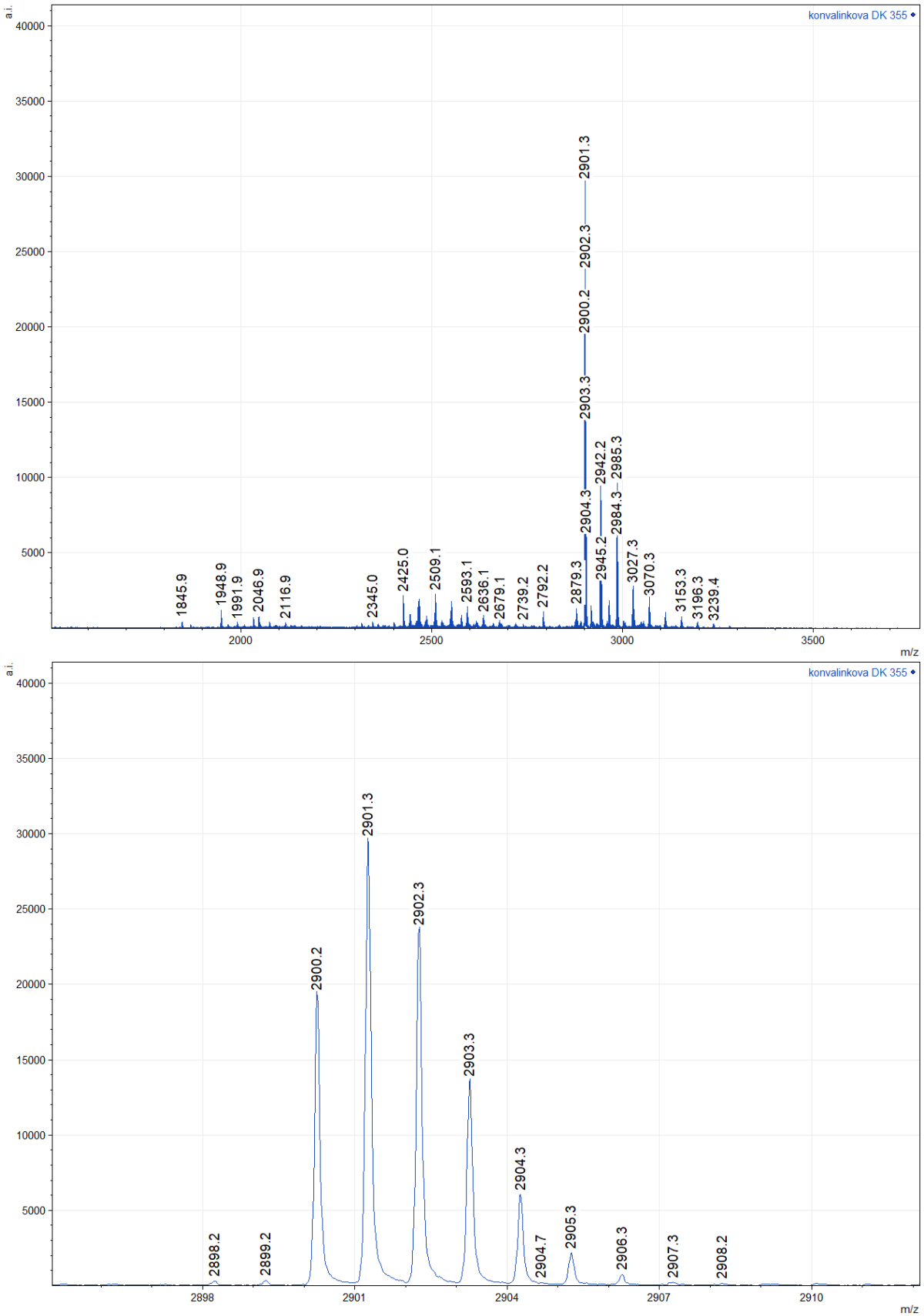


Figure S31. Mass spectrum (MALDI$^+$) of compound **25** (upper figure) and enlarged region showing [M + Na]$^+$, *m*/*z* 2900.2 (lower figure). HRMS (MALDI$^+$): *m*/*z* for $C_{128}H_{180}O_{48}N_{28}Na^+$ calculated 2900.2397, found 2900.2498 (3.48 ppm).

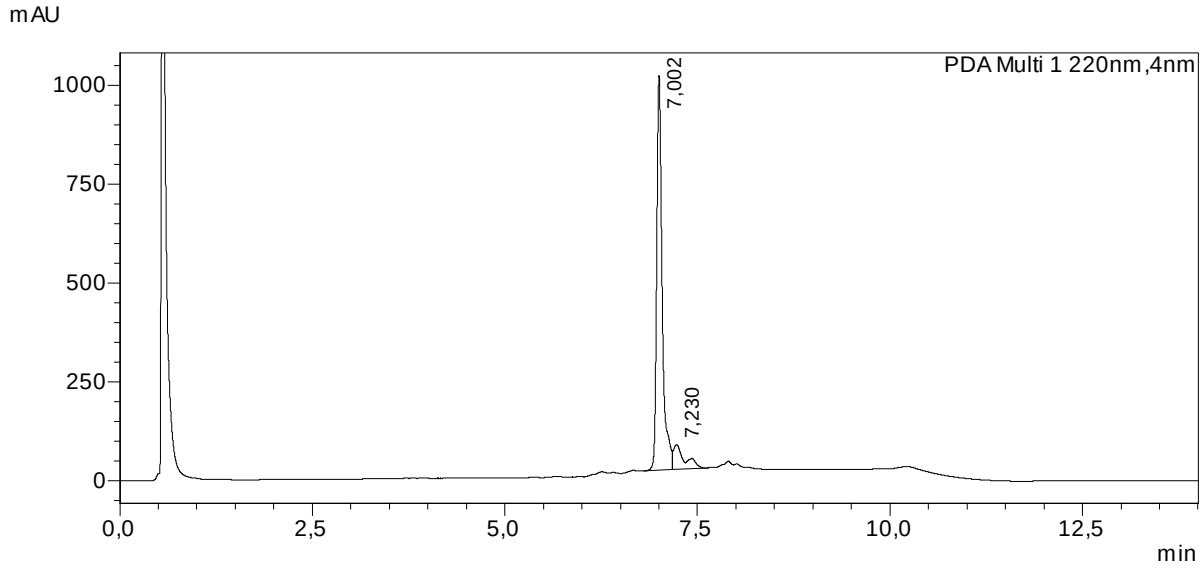


Figure S32. HPLC chromatogram of compound **25** (7.002 min, 89% purity).

Partial cone-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (27)

Table S5. ^{1}H and ^{13}C NMR data compound **27** (700.13 MHz for ^{1}H, 176.05 MHz for ^{13}C, D_2O + DMSO-d_6, 30 °C).

	Atom	δ_C	m.	δ_H	n_H	m.	*J*[Hz]
A, C core	***ipso-***	152.97	s	-	0	-	
	ortho-	n.e.	s	-	0	-	
	meta-	122.61, 120.05	d	7.525, 6.757	4	m	
	para-	n.e.	s	-	0	-	
	1''	76.46	t	4.07^{H}	2	m	
				3.684	2	-	
	2''	23.42	t	2.076	4	m	
	3''	11.17	q	1.289	6	t	7.2
	NH	-	-	n.d.	-	-	
	CO	171.13	s	-	0	-	
spacer	**1'**	35.49	t	2.89^{H}	4	br s	
	2'	21.23	t	3.221	4	br s	
	3'	146.76	s	-	0	-	
	4'	122.28, 122.24	d	8.273	2	s	
Glc	**1**	87.49	d	5.904	2	d	9.3
	2	72.13	d	4.21^{H}	2	m	
	3	75.14	d	4.00^{H}	2	m	
	4	78.40	d	4.00^{H}	2	m	
	5	77.94	d	4.02^{H}	2	m	
	6	60.02	t	4.111	2	m	
				4.04^{H}	2	m	
Gal	**1**	103.47	d	4.669	2	d	
	2	71.09	d	3.756	2	m	
	3	73.04	d	3.793	2	m	
	4	68.72	d	4.07^{H}	2	m	
	5	75.73	d	3.891	2	m	

	6	61.16	t	3.94[H]	4	m	
B, D core	***ipso-***	153.76, 152.97	s	-	0	-	
	ortho-	n.e.	s	-	0	-	
	meta-	122.11, 121.03	d	7.826, 7.587	4	m	
	para-	n.e.	s	-	0	-	
	1''	75.75, 73.64	t	3.515, n.d.	4	m	
	2''	23.58, 21.49	t	1.892, 1.675	4	m	
	3''	10.20, 9.66	q	0.990, 0.890	6	t	7.2
	NH	-	-	n.d.	-	-	
	CO	171.61, 171.00	s	-	0	-	
spacer	**1'**	36.03, 35.67	t	3.07[H], 3.06[H]	4	br s	
	2'	21.53, 21.15	t	3.36[H], 3.34[H]	4	br s	
	3'	146.91, 146.84	s	-	0	-	
	4'	122.42, 122.37	d	8.370, 8.357	2	s	
Glc	**1**	87.49	d	5.925, 5.914	2	d	9.2
	2	72.13	d	4.21[H]	2	m	
	3	75.14	d	4.00[H]	2	m	
	4	78.40	d	4.00[H]	2	m	
	5	77.94	d	4.02[H]	2	m	
	6	60.02	t	4.111	2	m	
				4.04[H]	2	m	
Gal	**1**	103.47	d	4.66[H]	2	d	
	2	71.09	d	3.756	2	m	
	3	73.04	d	3.793	2	m	
	4	68.72	d	4.07[H]	2	m	
	5	75.73	d	3.891	2	m	
	6	61.16	t	3.94[H]	4	m	
AB, CB	**-CH_2-**	36.76	t	4.276	2	m	
				3.290	2	m	
AD, CD	**-CH_2-**	30.34	t	n.d.	-	-	

[H] … HSQC readout; n.e. … not extracted; n.d. … not detected

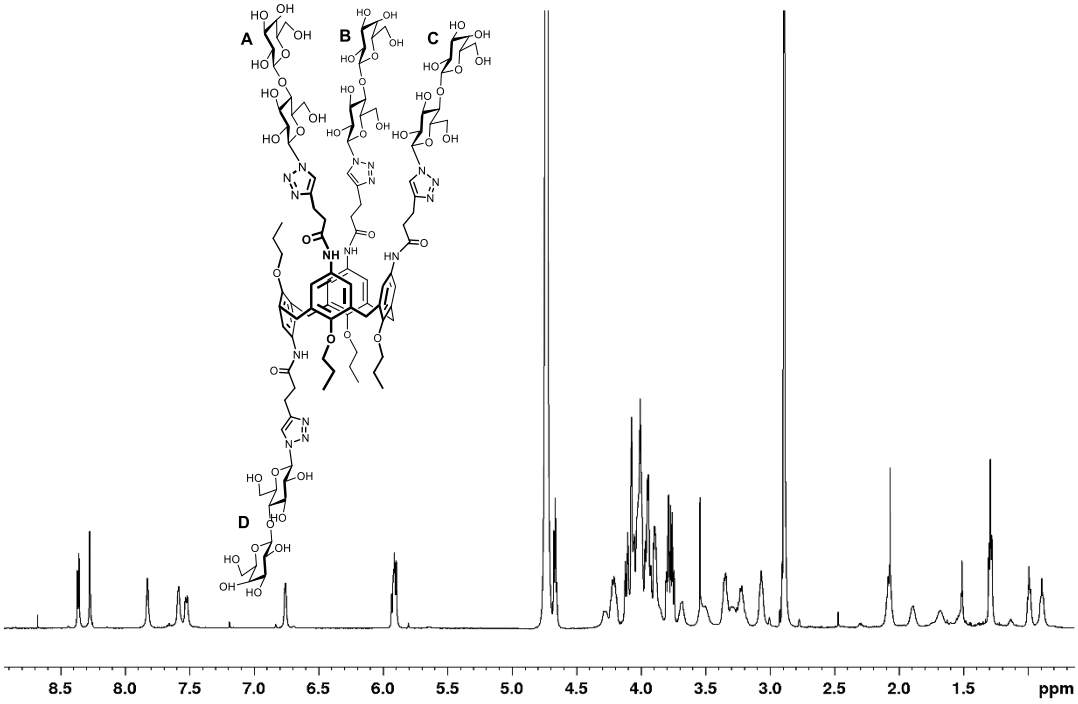


Figure S33. ^{1}H NMR spectrum of compound **27** (700.13 MHz, D_2O + DMSO-d_6, 30 °C).

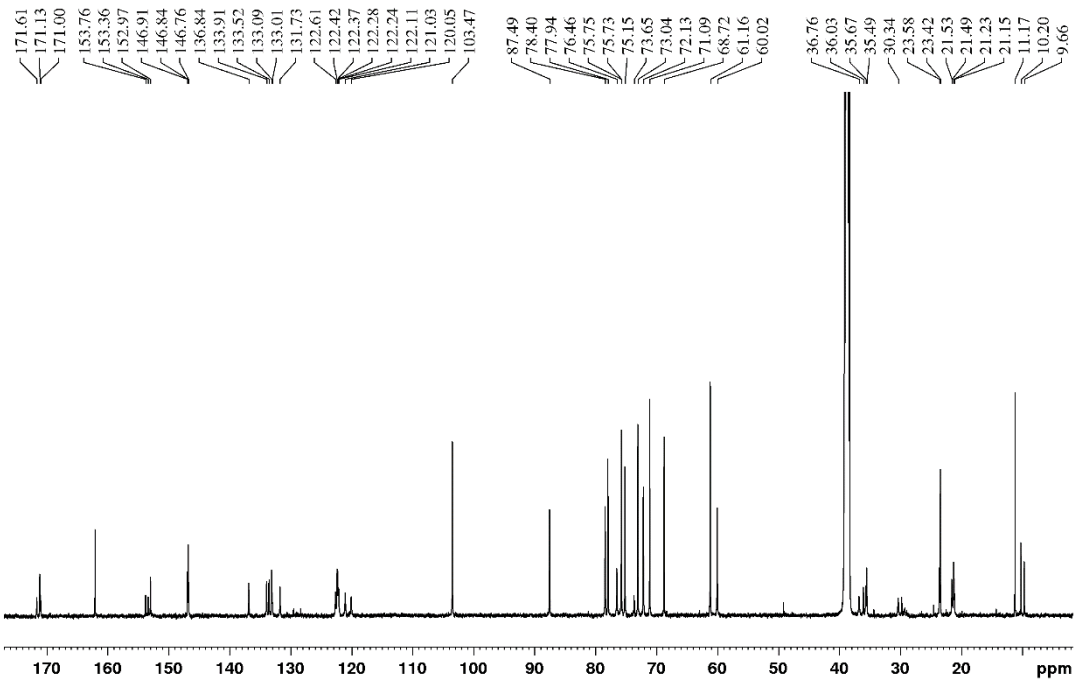


Figure S34. ^{13}C NMR spectrum of compound **27** (176.05 MHz, D_2O + DMSO-d_6, 30 °C).

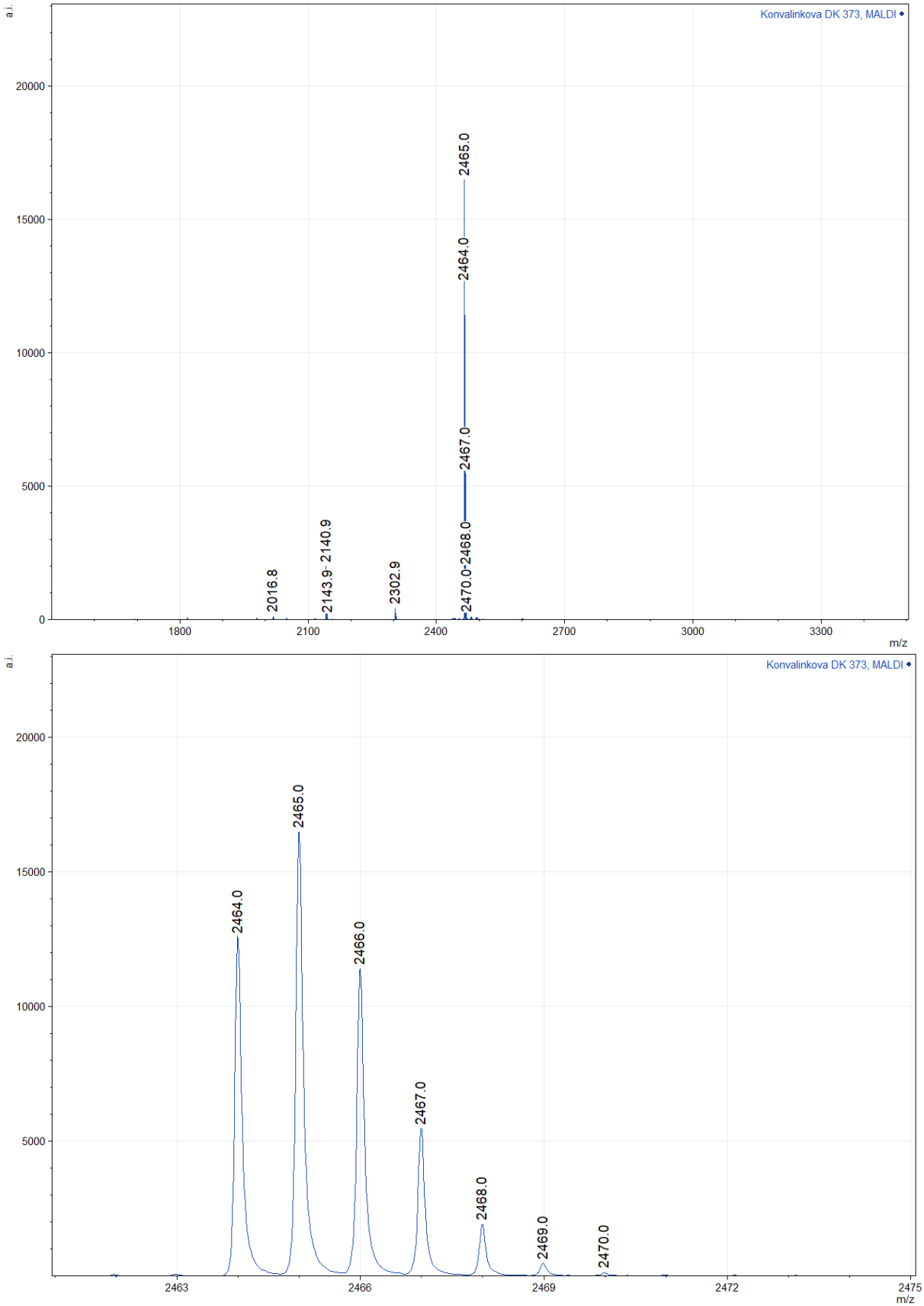


Figure S35. Mass spectrum (MALDI$^+$) of compound **27** (upper figure) and enlarged region showing [M + Na]$^+$, *m*/*z* 2464.0 (lower figure). HRMS (MALDI$^+$): *m*/*z* for $C_{108}H_{152}O_{48}N_{16}Na^+$ calculated 2463.9837, found 2463.9864 (1.10 ppm).

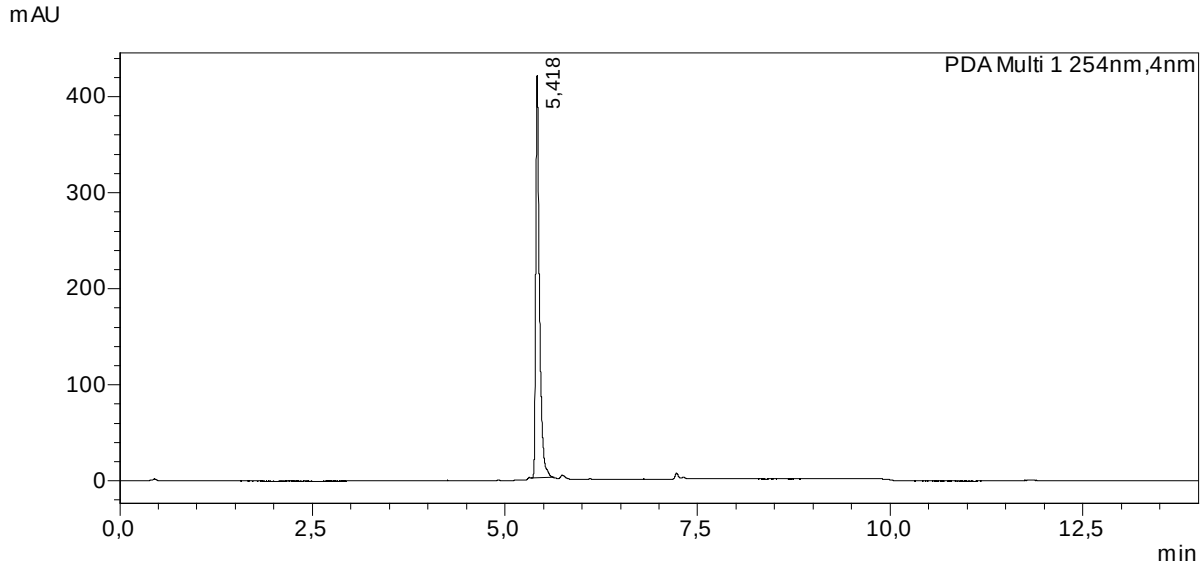


Figure S36. HPLC chromatogram of compound **27** (5.418 min, purity 99%).

1,2-*Alternate*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3/triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (29)

Table S6. ^{1}H and ^{13}C NMR data of compound **29** (700.13 MHz for ^{1}H, 176.05 MHz for ^{13}C, D_2O, 30 °C).

	Atom	δ_C	m.	δ_H	n_H	m.	*J*[Hz]
core	***ipso-***	153.22[x]	s	-	0	-	
	ortho-	133.28[x]	s	-	0	-	
	meta-	121.3[x]	d	7.23[H], 7.16[H]	8	m	
	para-	132.05[x]	s	-	0	-	
	-CH_2-	n.d.	t	n.d.	-	-	
	1''	n.d.	t	n.d.	8	m	
	2''	22.68[ax]	t	n.d.	8	m	
	3''	10.22	q	0.49	12	br s	
	NH	-	-	n.d.	-	-	
	CO	171.92[x]	s	-	0	-	
spacer	**1'**	35.57[a]	t	2.68[ax]	8	br s	
	2'	21.34[ax]	t	2.99[ax]	8	br s	
	3'	146.92	s	-	0	-	
	4'	122.49	d	7.904	4	s	
Glc	**1**	87.49	d	5.624	4	br s	
	2	72.28	d	3.99[H]	4	dd	
	3	74.80	d	3.84[H]	4	m	
	4	77.79[b]	d	3.85[H]	4	m	
	5	77.85[b]	d	3.88[H]	4	m	
	6	60.05	t	3.97[H]	4	m	
				3.87[H]	4	m	
Gal	**1**	103.21	d	4.469	4	br s	
	2	71.16	d	3.583	4	m	
	3	72.79	d	3.65[H]	4	m	
	4	68.76	d	3.93[H]	4	d	
	5	75.56	d	3.72[H]	4	m	
	6	61.20	t	3.77[H]	8	m	

n.d. … not detected; [x] … broad signal; [a] … tentative assignment; [b] … might be interchanged; [H] … HSQC readout

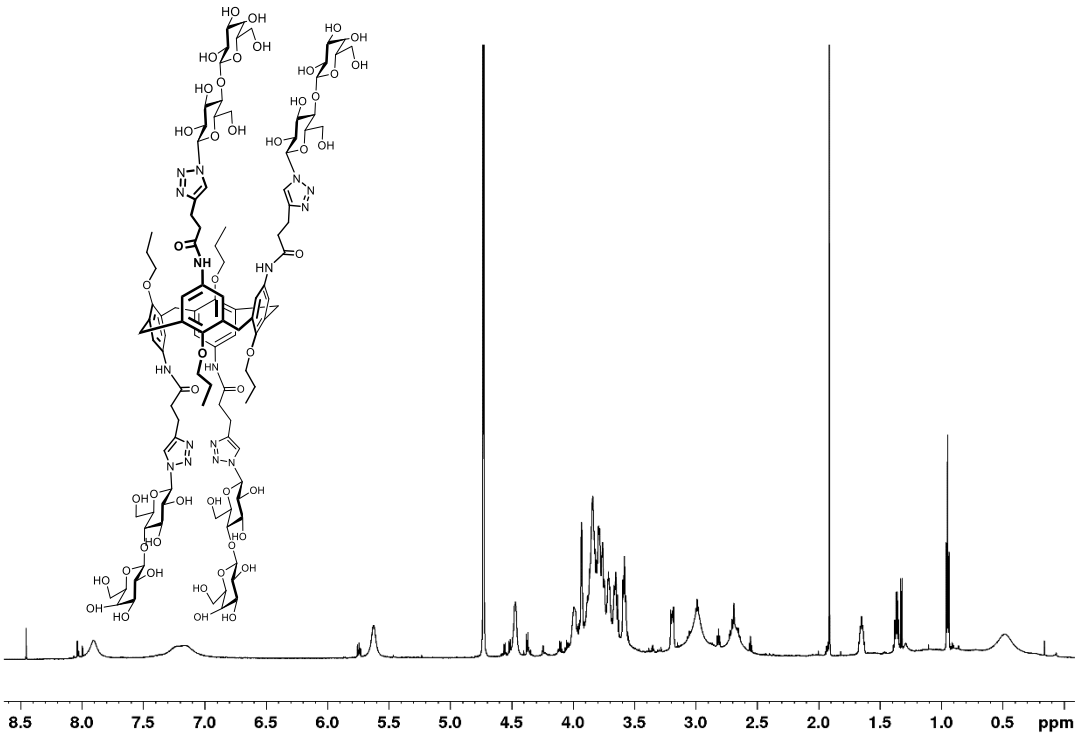


Figure S37. ^{1}H NMR spectrum of compound **29** (700.13 MHz, D_2O, 30 ºC).

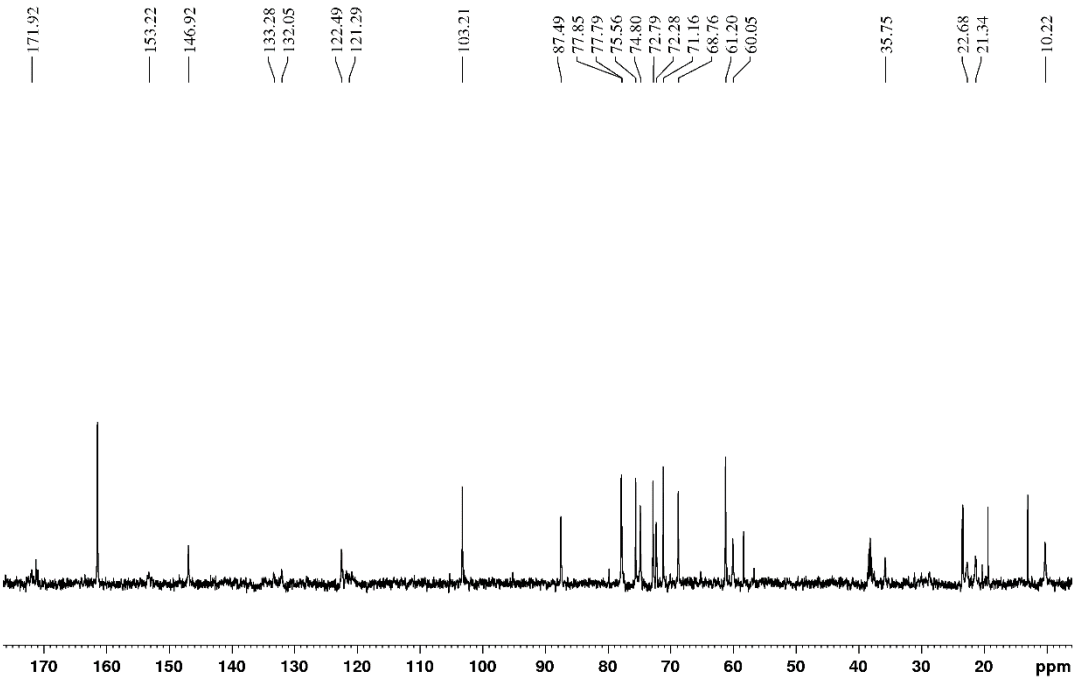


Figure S38. ^{13}C NMR spectrum of compound **29** (176.05 MHz, D_2O, 30 ºC).

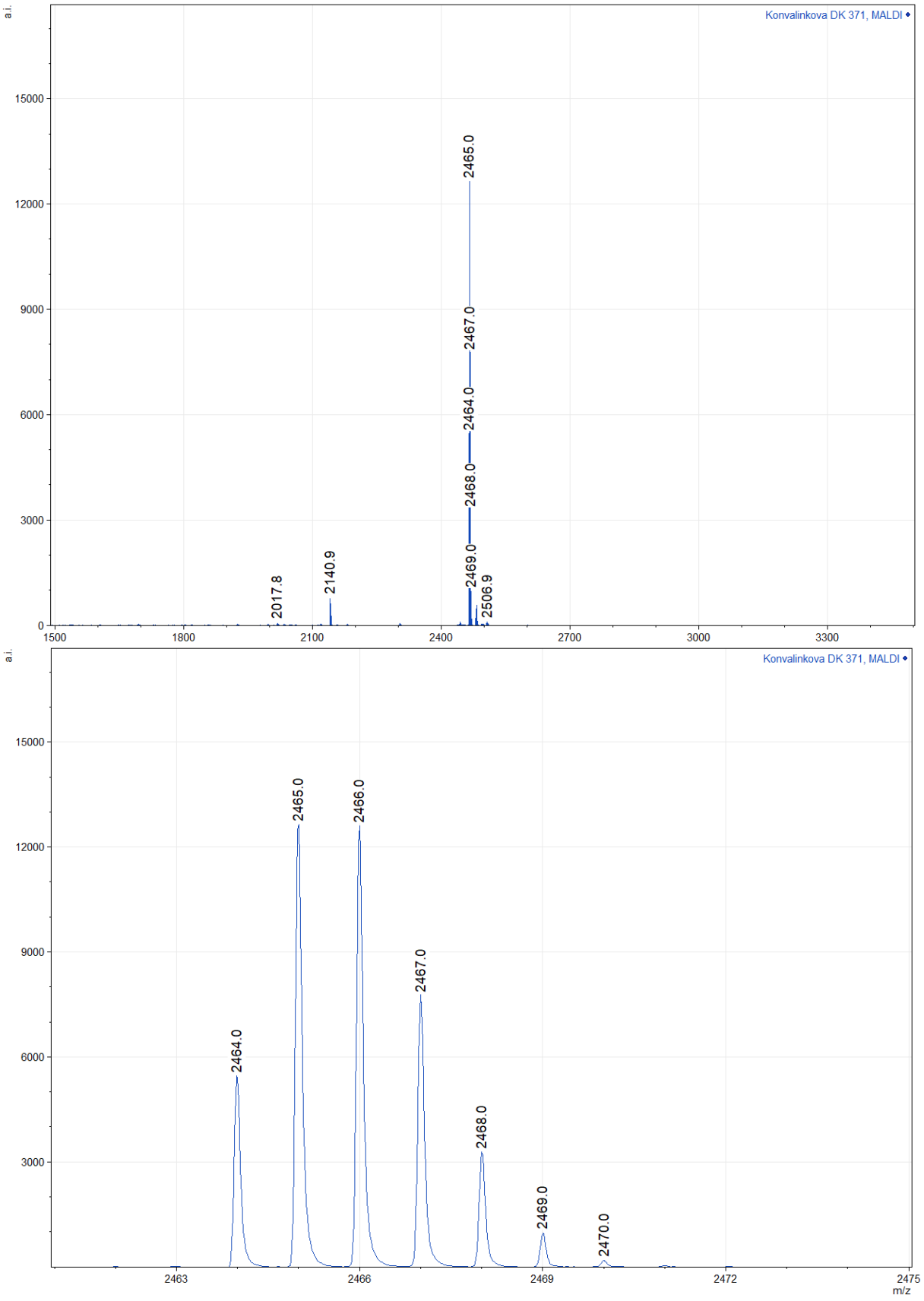


Figure S39. Mass spectrum (MALDI$^+$) of compound **29** (upper figure) and enlarged region showing [M + Na]$^+$, *m*/*z* 2464.0 (lower figure). HRMS (MALDI$^+$): *m*/*z* for $C_{108}H_{152}O_{48}N_{16}Na^+$ calculated 2463.9837, found 2463.9863 (1.04 ppm).

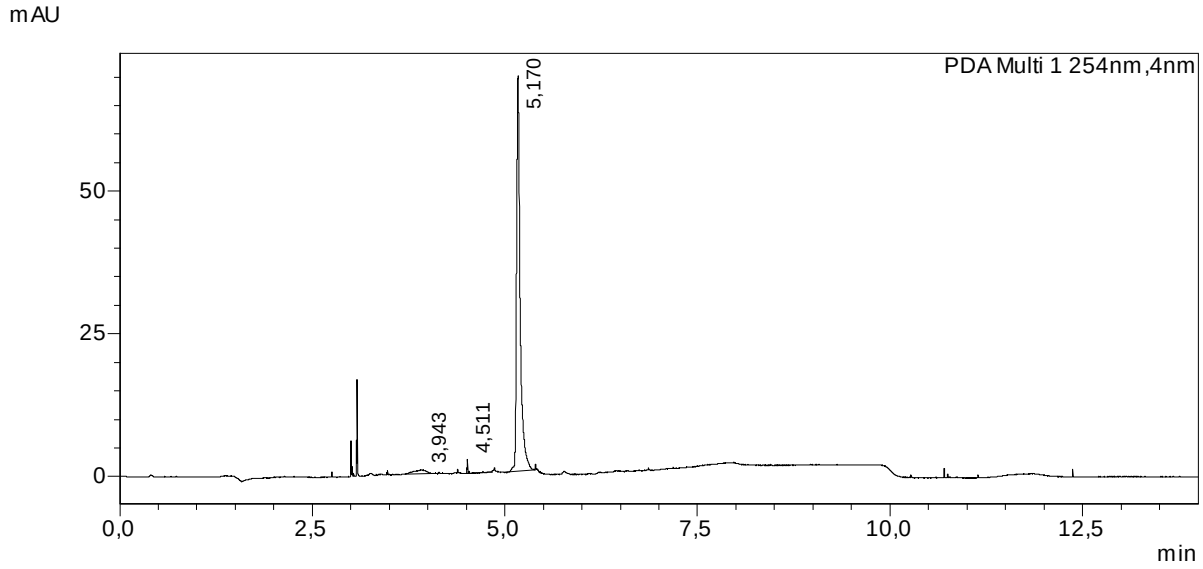


Figure S40. HPLC chromatogram of isolated compound **29** (5.17 min, 96% purity).

1,3-Alternate-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (31)

Table S7. ^{1}H and ^{13}C NMR data of compound **31** (700.13 MHz for ^{1}H, 176.05 MHz for ^{13}C, D_2O/CD_3OD, 30 °C).

	Atom	δ_C	m.	δ_H	n_H	m.	*J*[Hz]
core	***ipso-***	152.98	s	-	0	-	
	ortho-	134.88^{x}	s	-	0	-	
	meta-	122.97	d	7.189	8	m	
	para-	132.96^{x}	s	-	0	-	
	-CH_2-	37.44^{x}	t	n.d.	-	-	
	1''	73.95	t	3.248	8	m	
	2''	22.66	t	1.377	8	m	
	3''	9.75	q	0.705	12	t	7.1
	NH	-	-	n.d.	-	-	
	CO	172.65^{x}	s	-	0	-	
spacer	**1'**	35.15	t	2.709	8	br s	
	2'	21.28	t	3.005	8	br s	
	3'	147.02	s	-	0	-	
	4'	122.45	d	7.942	4	s	
Glc	**1**	87.47	d	5.638	4	d	9.1
	2	72.23	d	3.970	4	dd	9.1, 9.0
	3	74.81	d	3.81^{H}	4	m	
	4	77.69	d	3.83^{H}	4	m	
	5	77.84	d	3.79^{H}	4	m	
	6	59.99	t	3.85^{H}	8	m	
Gal	**1**	103.17	d	4.460	4	d	7.6
	2	71.16	d	3.550	4	dd	9.9, 7.6
	3	72.78	d	3.630	4	dd	9.9, 3.3
	4	68.78	d	3.897	4	d	3.3
	5	75.58	d	3.693	4	m	
	6	61.22	t	3.75^{H}	8	m	

^{H} … HSQC readout; ^{x} … tentative assignment; n.d. … not detected

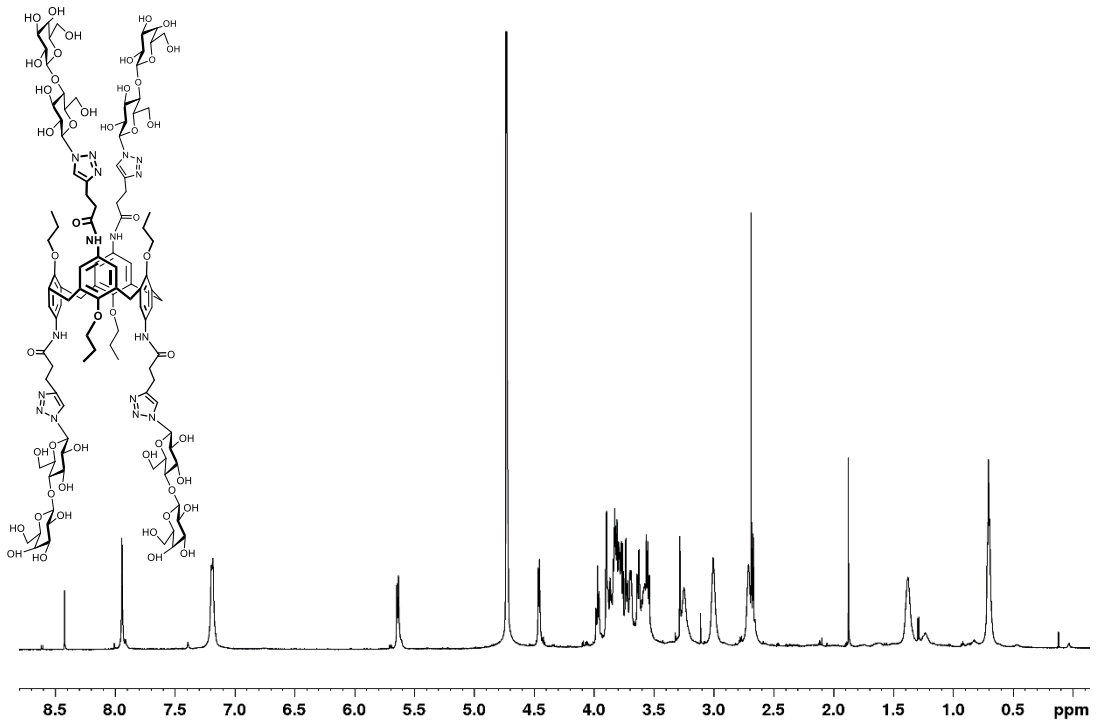


Figure S41. ^{1}H NMR spectrum of compound **31** (700.13 MHz, D_2O/CD_3OD, 30 °C).

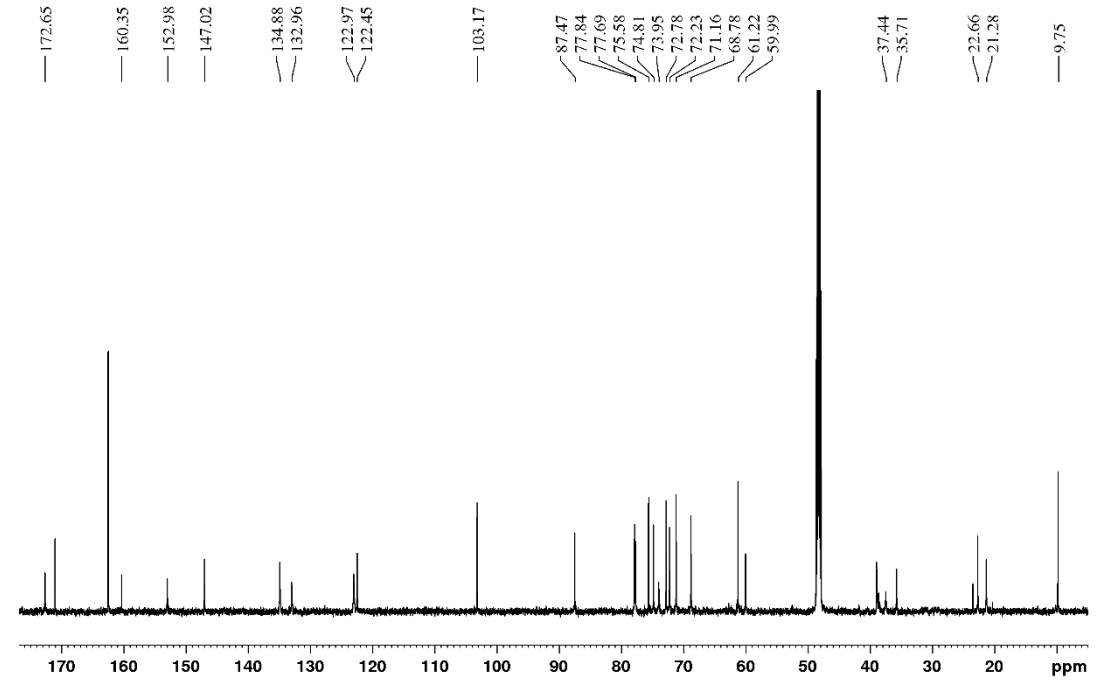


Figure S42. ^{13}C NMR spectrum of compound **31** (176.05 MHz, D_2O/CD_3OD, 30 °C).

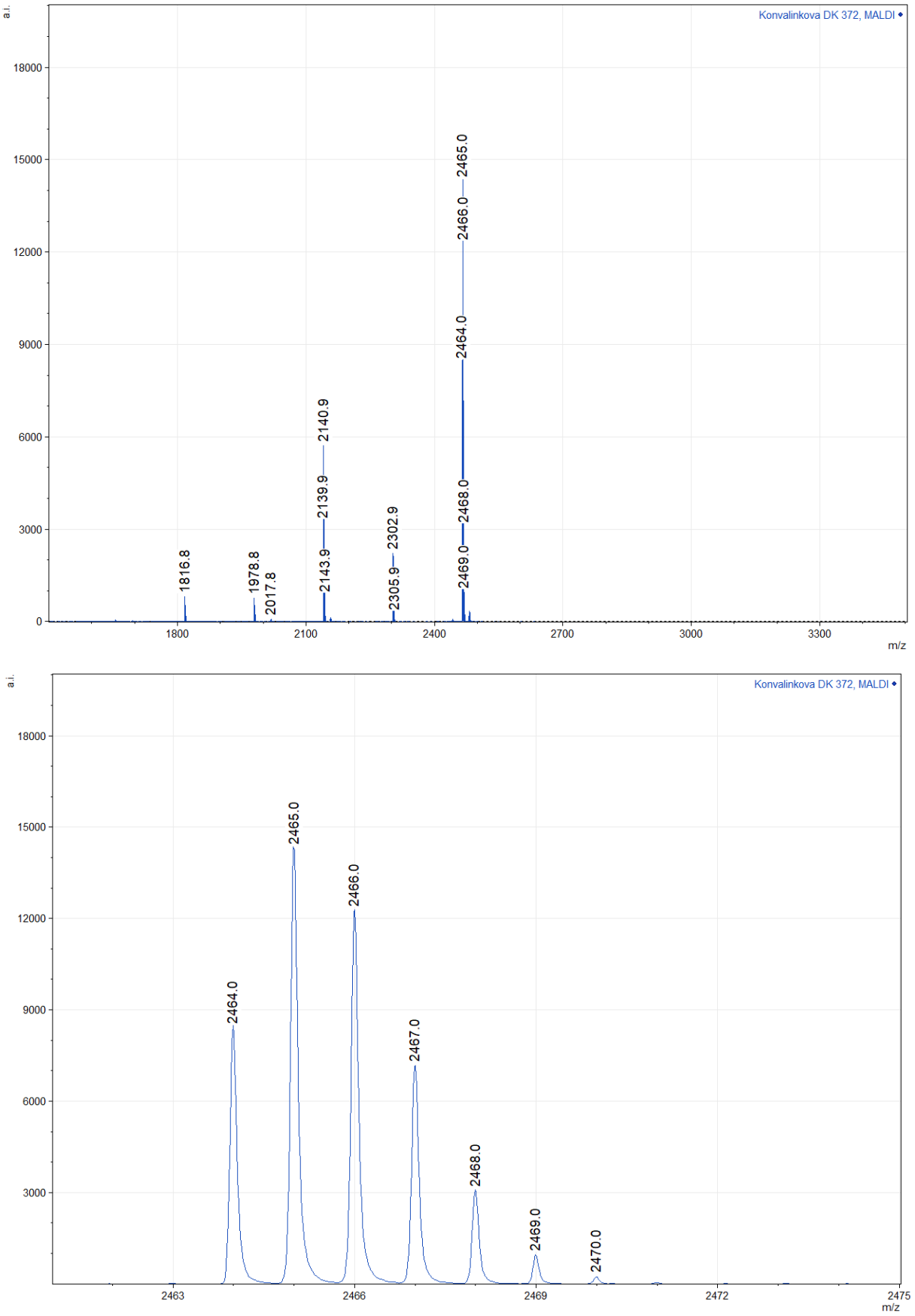


Figure S43. Mass spectrum (MALDI$^+$) of compound **31** (upper figure) and enlarged region showing [M + Na]$^+$, *m*/*z* 2464.0 (lower figure). HRMS (MALDI$^+$): *m*/*z* for $C_{108}H_{152}O_{48}N_{16}Na^+$ calculated 2463.9837, found 2463.9868 (1.27 ppm).

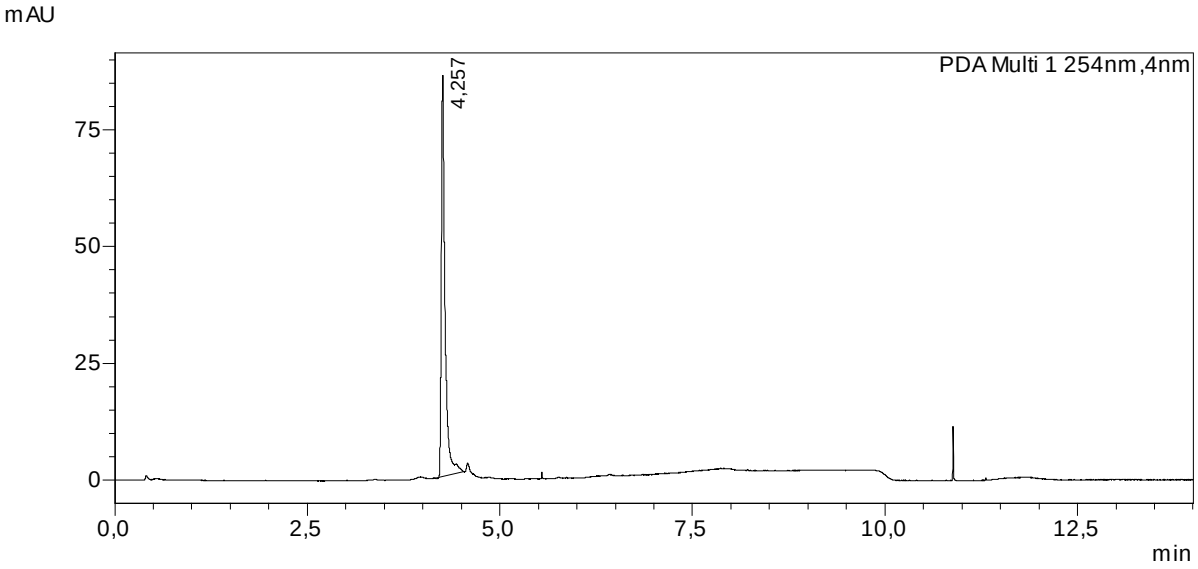


Figure S44. HPLC chromatogram of isolated compound **31** (4.257 min, 99% purity).

5. Production and purification of galectins

5.1. Purification of galectins

Table S7. Parameters of purification of recombinant His-tagged constructs of Gal-1, Gal-3, Gal-8, and Gal-9 by Ni-NTA affinity chromatography.

Protein	*m* cells from 1L media [g]	*m* proteins from 1 g cells [mg]	K_D for ASF [μM] [a]
Galectin-1	5.8	29	3.5 ± 1.2
Galectin-3	5.3	3.9	3.0 ± 0.7
Galectin-8	6.9	20	0.44 ± 0.09
Galectin-9	6.7	1.6	0.56 ± 0.26

[a] determined by direct ELISA assay in a procedure analogous to the competitive ELISA assay as detailed in the main text. In the direct ELISA assay, the incubation step comprises only serial dilution of the respective galectin in EPBS (50 μL/well).

5.2. Protein characterization

Protein concentration was determined by Bradford assay[7] using Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Hercules, USA). The assay solution was calibrated for bovine serum albumin (Sigma-Aldrich, Darmstadt, Germany). The purity of proteins and their molecular weights were analyzed by SDS-PAGE in 12% gel. Samples were loaded with *ca*. 2 μg protein per lane. Electrophoresis was performed with a 130 V constant voltage.

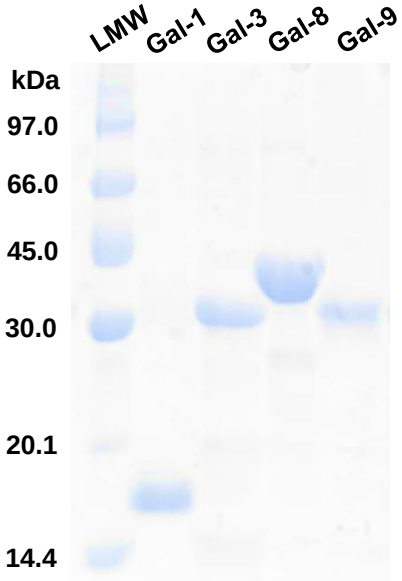


Figure S45. SDS-PAGE analysis of prepared galectins. LMW – Amersham Low Molecular Weight Calibration Kit for SDS Electrophoresis (GE Healthcare, Chicago, USA): 97 kDa – phosphorylase b from rabbit muscle; 66 kDa – bovine serum albumin; 45 kDa – chicken egg-white ovalbumin; 30 kDa – carbonic anhydrase from bovine erythrocyte; 20.1 kDa – trypsin inhibitor from soybean; 14.4 kDa – α-lactalbumin from bovine milk. Molecular weights of galectins: Gal-1 = 16.5 kDa, Gal-3 = 28.0 kDa, Gal-8 = 38.6 kDa, Gal-9 = 36.0 kDa.

6. BLI sensograms

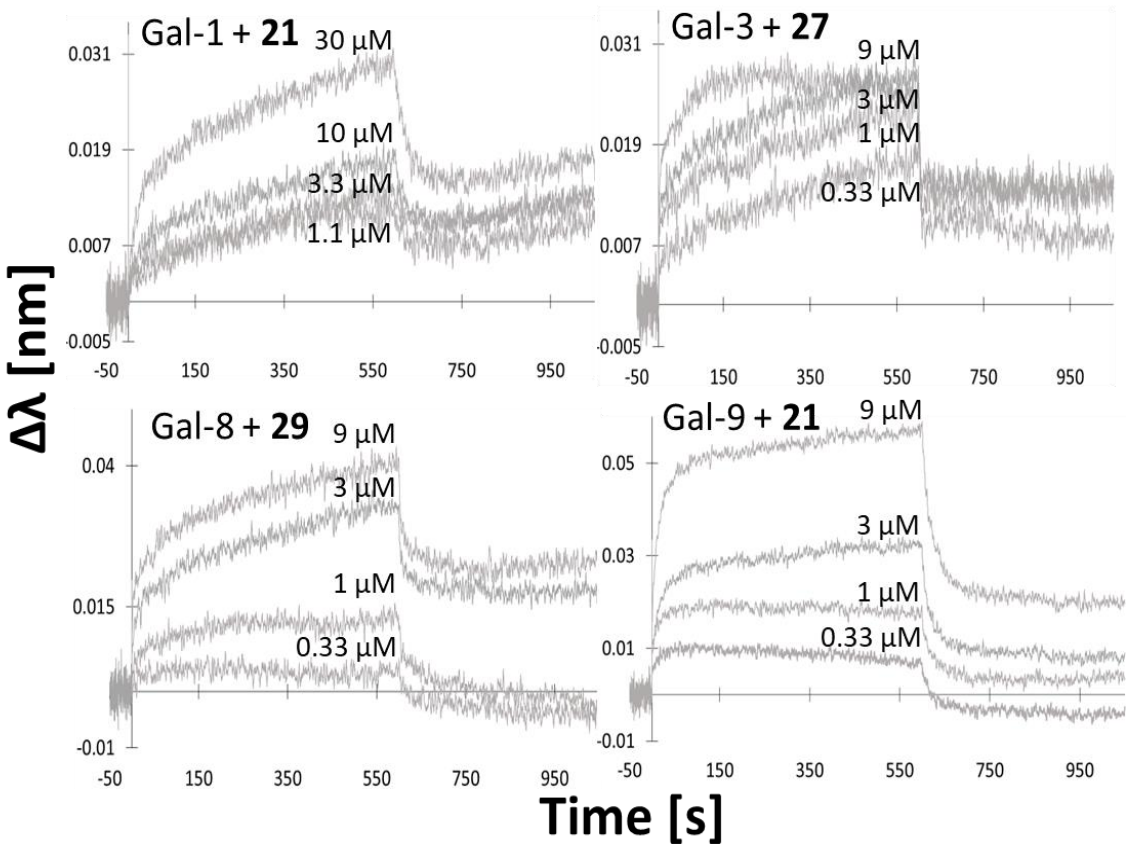


Figure S46. BLI sensograms of interaction of His-tagged galectins with selected glycocalix[4]arenes.

7. Additional AFM images

7.1. Gal-1 and glycocalix[4]arene 21

7.1.1. On HOPG substrate

Negative controls of Gal-1 without ligand showed the formation of monolayer "stains" and small oligomer clusters (*ca* < 10 units). Crystals of buffer salts (PBS) were observed too, but they were considerably larger and with *quasi*-prismatic aspect ratio.

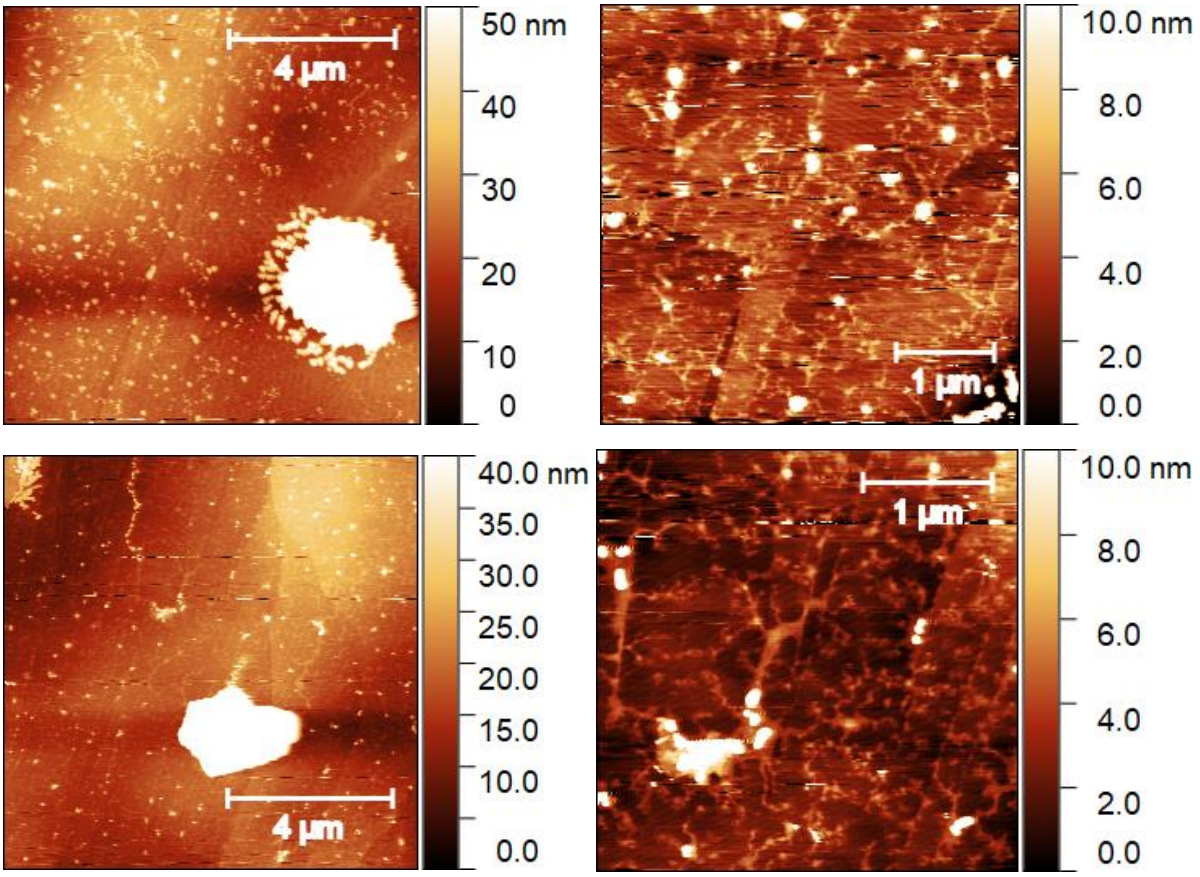


Figure S47. AFM analysis of Gal-1 negative control on HOPG substrate.

A different scenario of aggregation was observed with ligand **21**. Lamellar assemblies suggested chain-like association of proteins with occasional bundling and terminations. The height of such fibrils was *ca* 3 nm suggesting a monomolecular thickness. At certain concentration of fibrils on the surface the AFM visualizes them as "sheets".

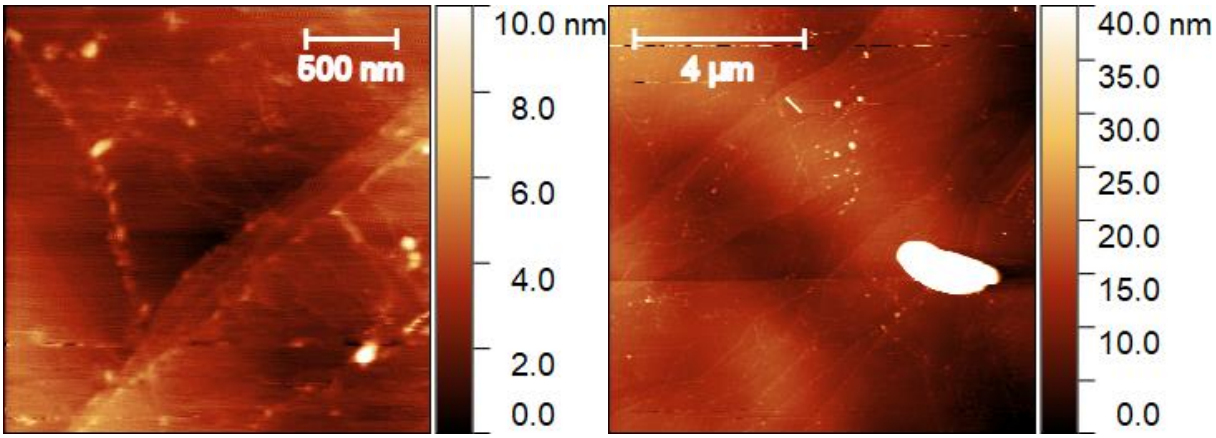


Figure S48. AFM analysis of Gal-1 with ligand **21** on HOPG substrate.

7.1.2. On mica substrate

Negative controls of Gal-1 did not show any sign of aggregate formation. However, the protein was present because the sample was "sticky" and individual spots of < 4 nm height were observed. Also, negative controls of ligands **21** alone did not show any sign of crystallization or aggregation.

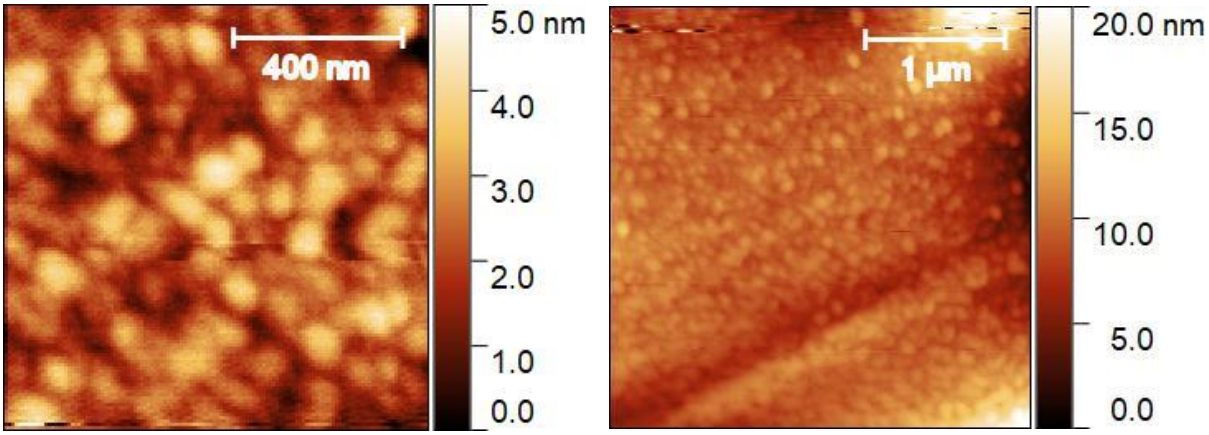


Figure S49. AFM analysis of Gal-1 negative control on mica substrate.

With ligand **21**, Gal-1 proteins aggregated into separated clusters with quantized height (5, 10, 15 and 20 nm), which suggests a maximum of 10 molecules aggregated in one cluster (assuming pyramid-like ordering). Close to buffer salts crystals, layers of denatured proteins appeared to deposit on the surface.

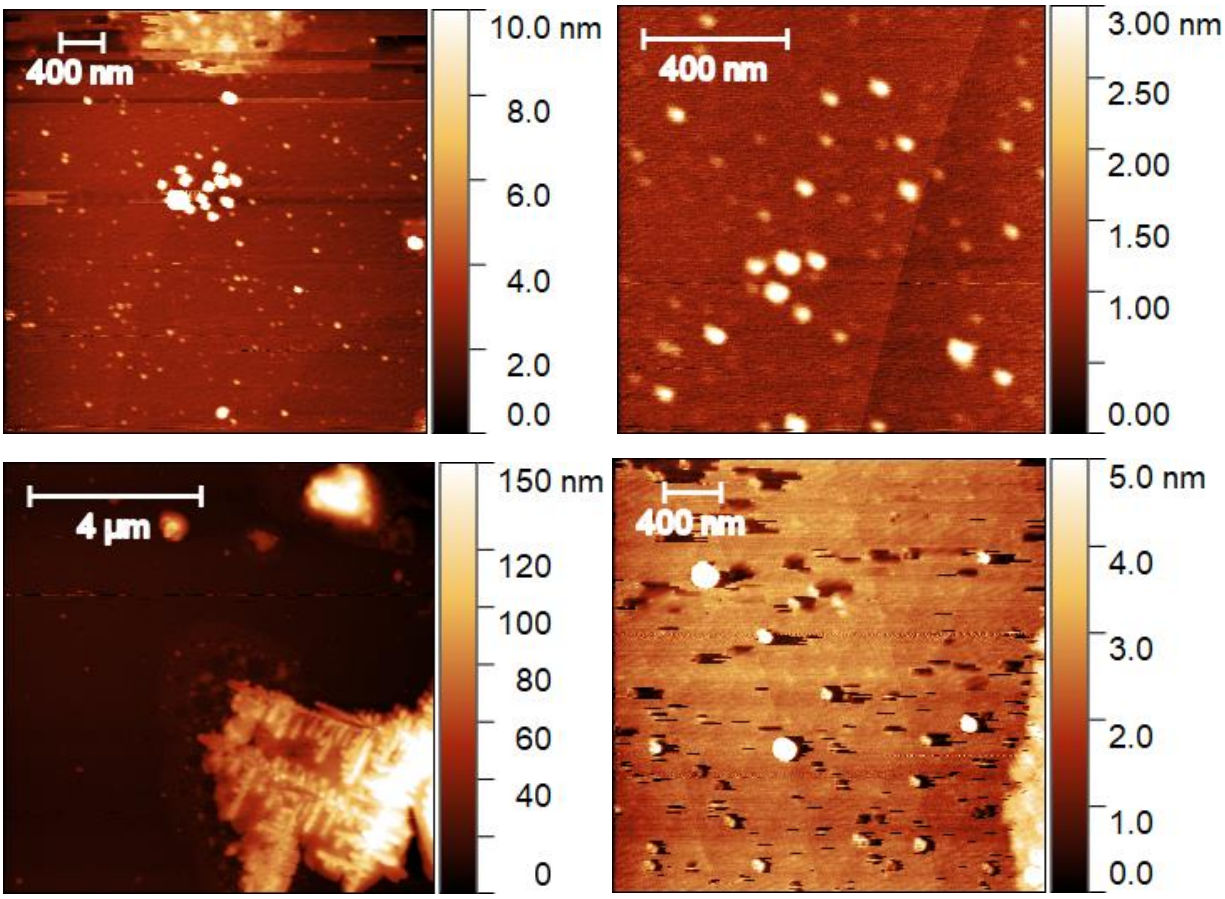
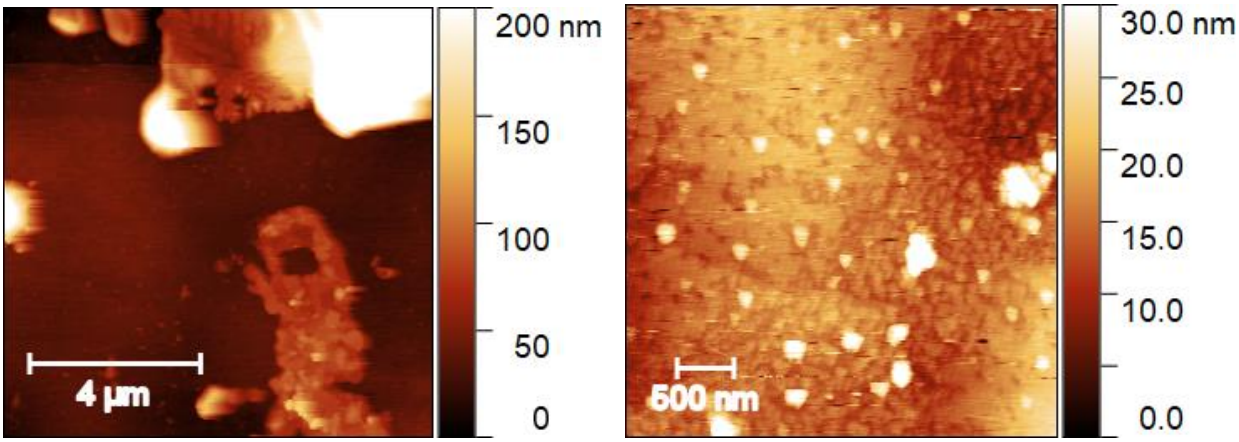


Figure S50. AFM analysis of Gal-1 with ligand **21** on mica substrate.

7.2. Gal-3 and glycocalix[4]arene 27

7.2.1. On HOPG substrate

In Gal-3 negative controls, the sample was very sticky and extensive stamping was observed, which lowered the informative value of images recorded. However, no protein aggregates were observed.



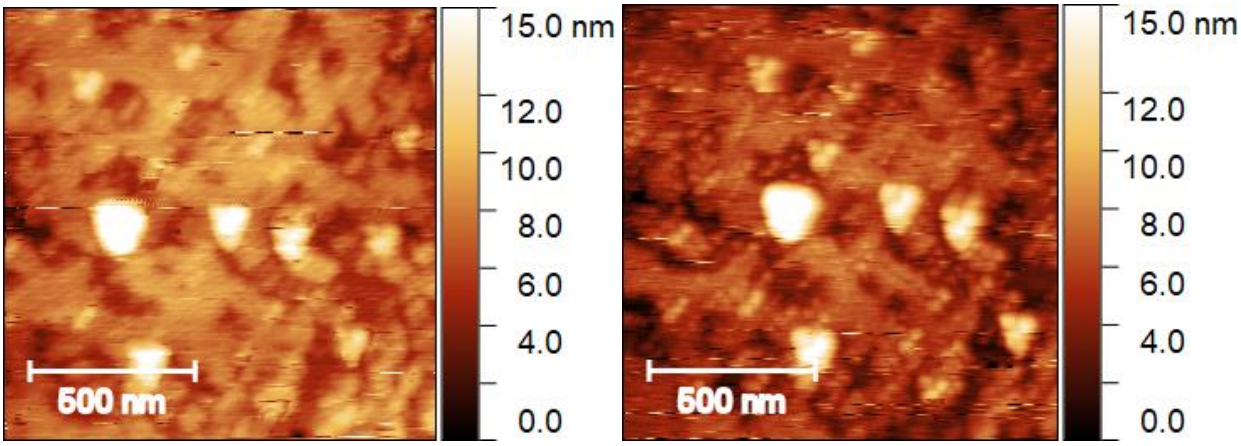


Figure S51. AFM analysis of Gal-3 negative control on HOPG substrate.

With ligand **27**, individual clusters with heights of tens of nanometers (a large number of aggregated species) formed uniformly all over the sample area, clearly suggesting pre-assembly in solution prior to deposition.

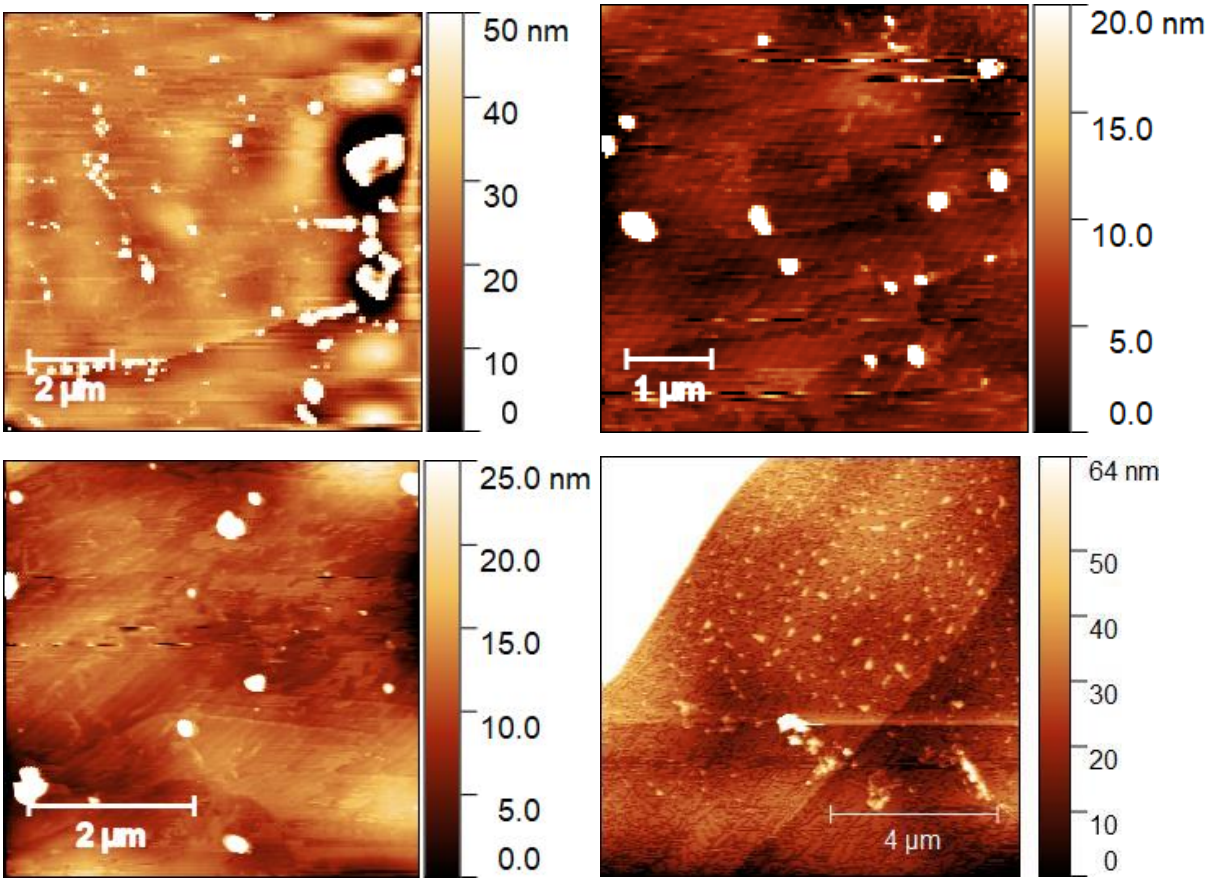


Figure S52. AFM analysis of Gal-3 with ligand **27** on HOPG substrate.

7.2.2. On mica substrate

Negative control of Gal-3 showed selective deposition of the material in coffee-ring stains over the surface as the sample was drying. Between the coffee rings, no sign of protein deposits could be detected; however, close to the ring features, individual aggregates could be detected

with a height below 20 nm, indicating *ca* < 10 protein molecules associated in one cluster (pyramid-like). This indicates a lower interaction energy between the protein and the surface than propensity towards self-association. The bow-tie pattern in some images is an instrumental artefact of the AFM piezo system, which is very shallow and typically not observed if aggregates are present.

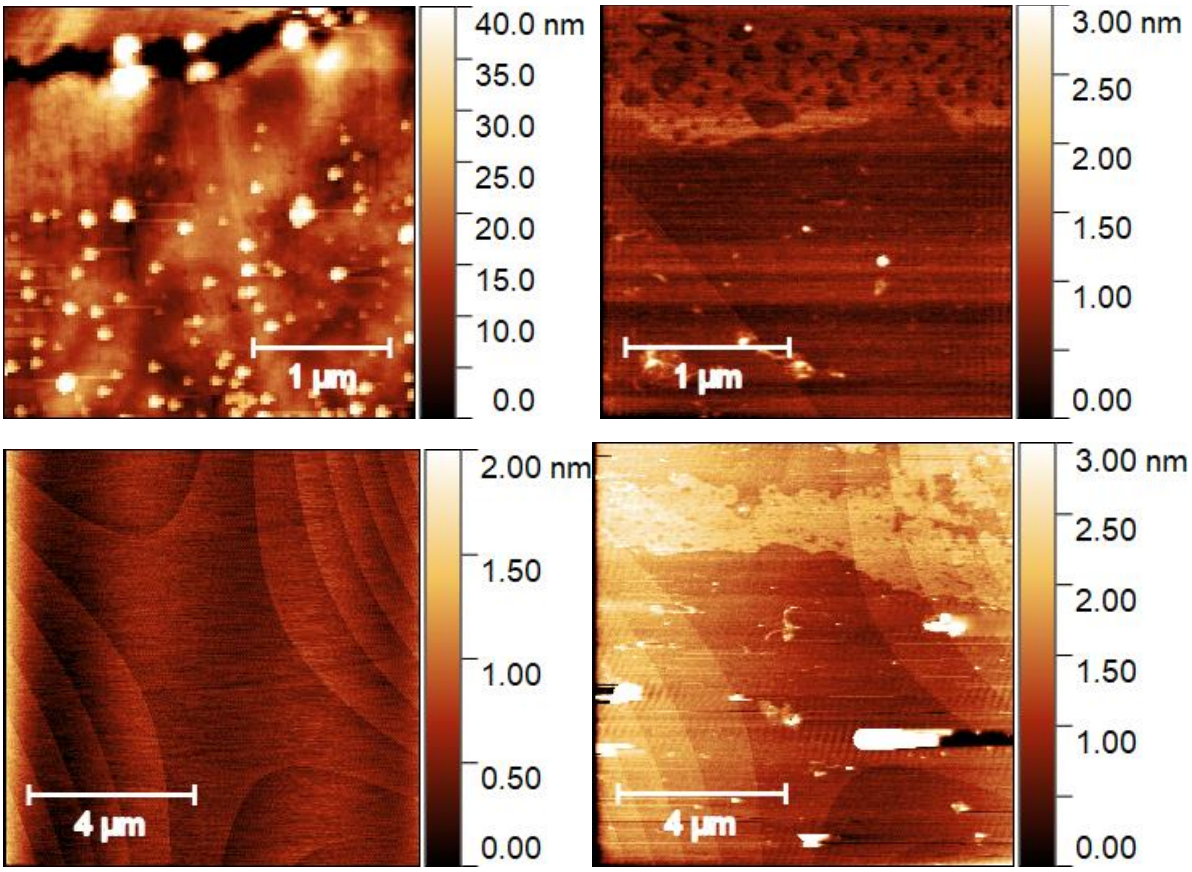


Figure S53. AFM analysis of Gal-3 negative control on mica substrate.

With ligand **27**, a large number of smaller aggregates was observed across the whole surface, with their heights quantized by increments of *ca* 7 nm. This suggest preorganization in solution prior to deposition and drying on the surface.

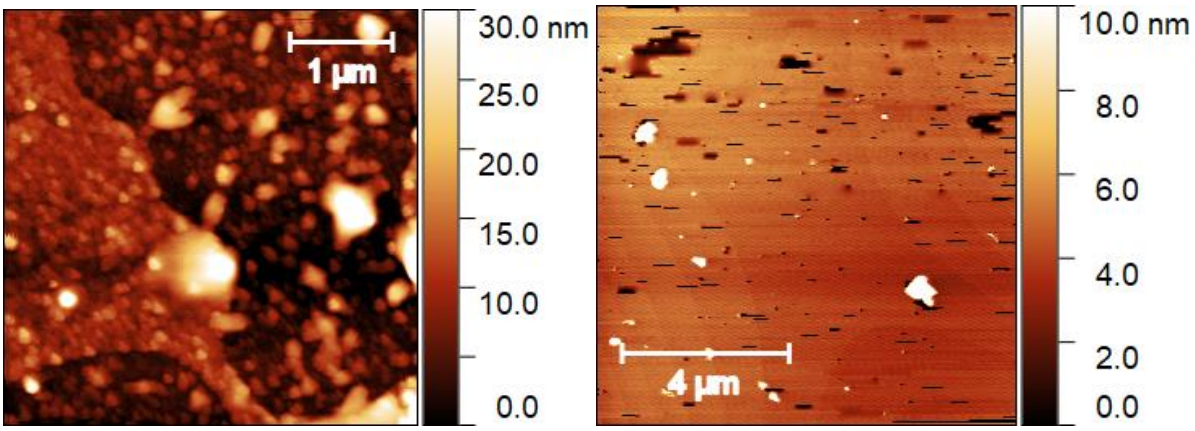


Figure S54. AFM analysis of Gal-3 with ligand **27** on mica substrate.

8. References

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