Electronic Supplementary Information (ESI)

Galectin-targeting glycocalix[4]arenes can enter the cells

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1. MATERIALS

Lactose was purchased from Lachema NP (Brno, Czech Republic). tetra-*N*-Butylammonium bromide (TBAB), absolute methanol, absolute *N*,*N*-dimethylformamide (DMF), and acetic anhydride were obtained from Acros Organics (Geel, Belgium). 1,4-Dioxane, and *N*,*N*-diisopropylethyl amine (DIPEA) were bought from Alfa Aesar (Kandel, Germany). Dibutyltin oxide, Amberlite IR-120, and (3-dimethylaminopropyl)ethylcarbodiimide hydrochloride (EDC·HCl) were bought from Fluka (Steinheim, Germany). Calix[4]arene-25,26,27,28-tetrol, 1-hydroxybenzotriazole hydrate (HOBt), stannous chloride dihydrate, sodium nitrate, *N*-bromosuccinimide (NBS), 2,2′-azobis(2-methylpropionitrile (AIBN), tetrabutylammonium bromide, 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), and trifluoroacetic acid were purchased from Sigma-Aldrich (Germany, Steinheim). 4-Pentynoic acid, and deuterated solvents were from Fluorochem (United Kingdom, Hadfield). Triethylamine, and sodium hydrate were purchased from Merck (USA, Massachusetts). 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)

NMR experiments of compounds **11**, **12**, and **17** and their respective precursors were performed on an 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. $CDCl_3$ was used as a solvent for compound **11** and CD_3OD for compounds **12** and **17**. 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.

NMR spectrum of glycomimetic **8** was acquired on a Bruker Avance III 400 MHz spectrometer (Bruker BioSpin, Rheinstetten, Germany) at the indicated temperature in $CDCl_3$ using standard manufacturer's software. NMR spectra of glycocalix[4]arenes were acquired on a Bruker Avance III 700 MHz (compound **21**), 600 MHz (compound **27**), and 400 MHz (compound **23**) spectrometer (Bruker BioSpin, Rheinstetten, Germany) at indicated temperature using the same software. Compound **21** was measured in DMSO-d_6, compound **23** in DMF-d_7 **,** and compound **27** in CD_2Cl_2. Their precursors were measured as indicated in our previous work.[1] Spectra were referenced to residual solvent signals (DMSO-d_6: δ_H 2.499 ppm and δ_C 39.46 ppm, DMF-d_7: δ_H 2.745 ppm and δ_C 30.09 ppm, CD_2Cl_2: δ_H 5.323 ppm and δ_C 53.87 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 elongation of calix[4]arene side chains by lactosyl and coumaryl moieties, some signals were substantially broadened, thus complicating unambiguous structural assignments. Compounds **24** and **28** were available in very small amounts, and HMBC experiments did not provide full diagnostic correlations due to the very broad signals, so the structure of these compounds was determined from their peracetylated versions (peracetates **23** and **27**, respectively).

2.2. High-resolution mass spectrometry (HRMS)

The electrospray-ionization (ESI) spectra of compound **17** were measured using an LTQ Orbitrap Velos hybrid - Hybrid Ion-Trap-Orbitrap FT mass spectrometer (Thermo Fisher 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 compound **8** 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 **21, 24**, and **28** were recorded using UltrafleXtreme™ MALDI-TOF/TOF (time-of-flight) mass spectrometer (Bruker Daltonik, Bremen, Germany) operated in the reflectron mode. The sample was prepared using a dried 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).

2.3. High-performance liquid chromatography (HPLC)

HPLC 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 of compounds **11**, **12**, **19**, **21**, **24**, **26**, and **28** 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) 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. Compound **8** was analyzed on Chromolith RP-18e column (100×3 mm, monolith) preceded by Chromolith RP 18-e guard column (5 × 4.6 mm, Merck, Germany), with gradient elution (A = 5% acetonitrile, 0.1% formic acid, B = 80% acetonitrile, 0.1% formic acid): 0% B for 0-2 min, 0-90% B for 2-7 min, 95% B for 7-8 min, and 90-0% B for 8-11, 11-14 min for column equilibration, flow rate 1.2 mL/min, 25 °C, injection volume 1 μL. The sample of lactosyl azide (**5**) was analyzed on a TSK gel Amide-80 column (250 × 4.6 mm, 5 μm) preceded by TSKgel Amide-80 Guardgel (3.2 × 15 mm, Tosoh corp., Japan) in acetonitrile/ water (4/1, *v*/*v*), with gradient elution as follows (A = acetonitrile, B = water): 22% B for 0-7 min, 22-31% B for 7-16 min, 31-22% B for 16-17 min, and 22% B for 17-22 min for column equilibration; at a flow rate of 1 mL/min at 25 °C; injection volume 1 μL. PDA data were acquired at 200-380 nm and the maximum signal for the measured compound was extracted. Lactose was analyzed on

Chromolith RP-18e column (100×3 mm, monolith) preceded by Chromolith RP 18-e guard column (5 × 4.6 mm, Merck, Germany), with gradient elution (A = 5% acetonitrile, 0.1% formic acid, B = 80% acetonitrile, 0.1% formic acid): 0% B for 0-2 min, 0-90% B for 2-7 min, 95% B for 7-8 min, and 90-0% B for 8-11, 11-14 min for column equilibration, flow rate 0.5 mL/min, 25 °C, injection volume 1 μL. Detection was performed by MS-ESI, parameters were as follows: positive and negative mode; ESI interface voltage, 4.5 kV, - 3.5 kV; detector voltage, 1.15 kV; nebulizing gas flow, 1.5 mL/min; drying gas flow, 15 ml/min; heat block temperature, 200 °C; the temperature of desolvation line pipe, 250 °C, SCAN mode 200-600 *m*/*z*; chromatograms were analyzed using software LabSolutions ver. 5.75 SP2 (Shimadzu, Kyoto, Japan).

2.4. Infrared spectrometry (IR)

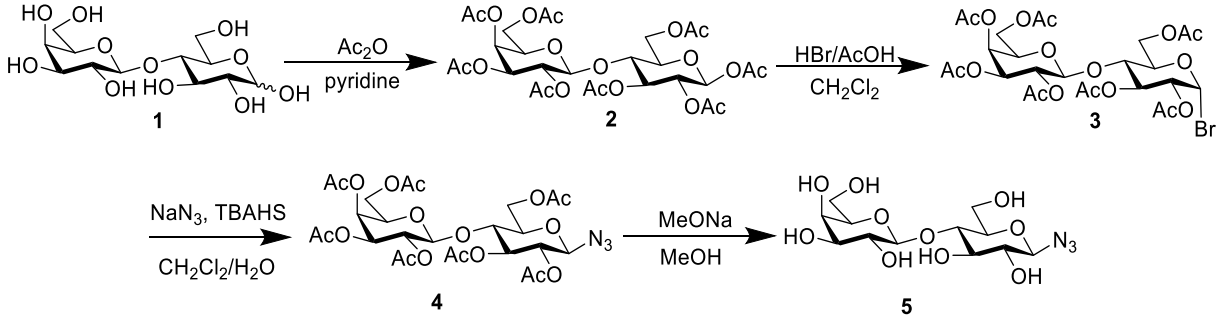
The analysis was performed by reflectance measurement (DRIFT) on a Nicolet 6700 FTIR spectrometer (Thermo-Nicolet, USA) in conjunction with a GladiATR diamond attenuated total-reflectance (ATR) attachment (PIKE, USA), DTG detector with KBr window. Measurement parameters: spectral range 4000 - 400 cm^{-1},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

3. SYNTHETIC PROCEDURES

3.1. Synthesis of glycosyl azides

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (5)

The title compound **5** was prepared as described previously.[1] Briefly, peracetylation of lactose **1** with acetic anhydride/pyridine, bromination of peracetate **2** with 33% HBr/AcOH in absolute dichloromethane, the introduction of azido moiety at C-1 of bromide **3** using NaN_3 in dichloromethane/saturated $NaHCO_3$ aq. under the catalysis by tetrabutylammonium hydrogen sulfate afforded azide peracetate **4** (7.18 g, 68%) as yellowish oil. The results of NMR analyses were in accord with the literature.[1, 2] Peracetate **4** was deprotected with MeONa/MeOH to afford azide **5** (1.6 g, 96%) as a white solid. Its analysis by HPLC is shown in Section 4.1.



Scheme S1. Synthesis of β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (**5**).

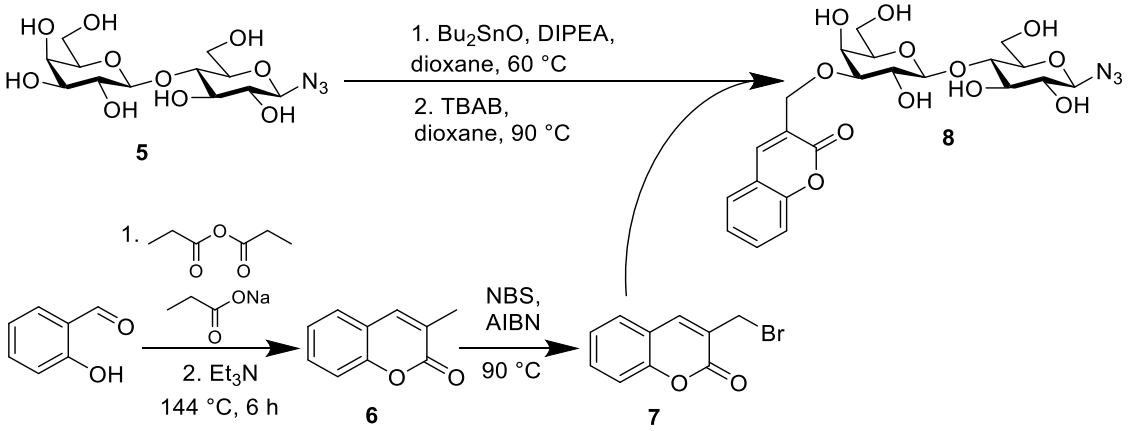
3′-*O*-(Coumarylmethyl)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (8)

3-Methylcoumarine (**6**) was prepared according to the literature with some modifications.[3, 4] Briefly, salicyl aldehyde (1.7 mL, 15.9 mmol) was heated to 144 °C. At 40 °C, propionic anhydride (3.23 mL, 24.8 mmol) and sodium propionate (0.79 g, 8.22 mmol) were added. At 70 °C, Et_3N (1.15 mL) was added. Then, the reaction mixture was refluxed at 144 °C for 6 h and monitored by TLC (hexane/ethyl acetate, 8/2). After completion, the reaction mixture was evaporated and separated on silica gel (cyclohexane/ethyl acetate, 9/1) to afford 3-methylcoumarine (**6**) (1.12 g, 45%) as yellowish powder.

3-(Bromomethyl)coumarin (**7**) was prepared according to the literature with some modifications.[3, 4] Briefly, 3-methylcoumarine (**6**; 4.2 g, 26.2 mmol) was dissolved in dichlorethane (10 mL) and heated to 60 °C. *N*-Bromosuccinimide (NBS, 2.38 g, 23.6 mmol) and 2,2′-azobis(2-methylpropionitrile) (AIBN, 0.3 g, 2 mmol) were added, the reaction mixture was heated to 90 °C and refluxed at 90 °C for 3 h. After that, another portion of NBS (2.38 g, 23.6 mmol) and AIBN (0.3 g, 2 mmol) were added, and the reaction mixture was refluxed at 90 °C overnight. The reaction was monitored by TLC (hexane/ethyl acetate, 8/2). After completion, the reaction mixture was evaporated and purified on silica gel (cyclohexane/ethyl acetate, 1/1) to afford compound **7** (5.42 g, 86%) as a yellowish powder.

Azide **5** (1.1 g, 3.0 mmol) and dibutyltin oxide (2.31 g, 9.3 mmol) were dissolved in absolute dioxane (20 mL) under argon, and heated to 60 °C. *N*,*N*-Diisopropylethylamine (2.75 mL, 15.8 mmol, DIPEA) was added and the reaction mixture was stirred at 60 °C for 65 min. Then, the reaction mixture was lyophilized, dissolved in absolute dioxane (20 mL) under argon, and tetrabutylammonium bromide (0.55 g, 1.7 mmol) with 3-(bromomethyl)coumarin (**7**; 2.2 g, 9.2 mmol) were added. The reaction mixture was stirred at 90 °C for 19 h under monitoring by TLC ($CHCl_3$/CH_3OH, 9/1 → 3/1). After completion, the reaction mixture was concentrated *in vacuo* and purified on silica gel (CH_2Cl_2/CH_3OH, 1/0 → 9/1) to afford compound **8** (1.2 g, 76%)

as a white powder. The structural integrity of azide **8** was confirmed by NMR, HRMS, and HPLC. Respective data and spectra are shown in Section 4.1.

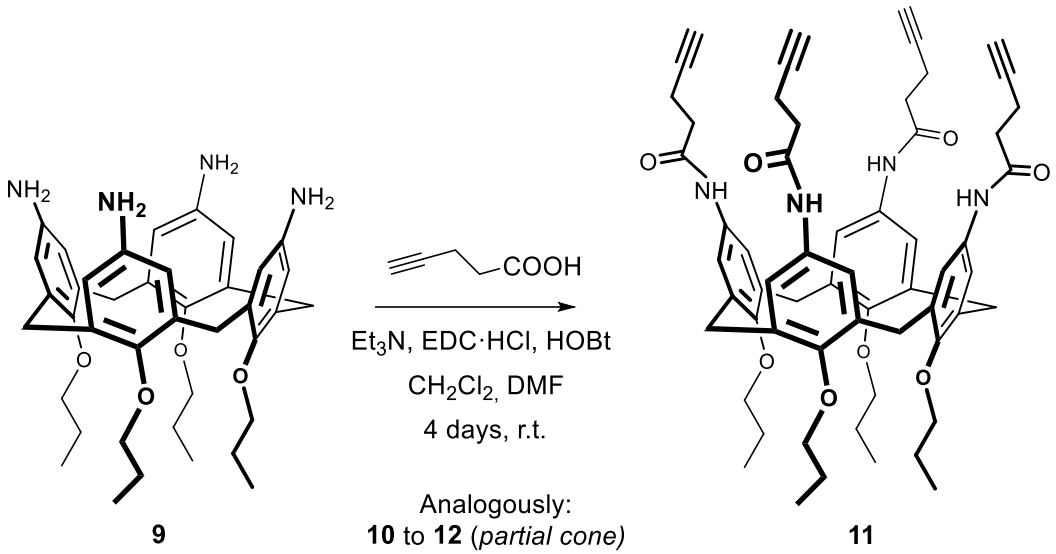


Scheme S2. Synthesis of 3′-*O*-(coumarylmethyl)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (**8**).

3.2. Synthesis of calix[4]arene cores

5,11,17,23-tetrakis(4-Pentynoylamino)-25,26,27,28-tetrapropoxycalix[4]arene (11, 12)

The precursors 5,11,17,23-tetraamino-25,26,27,28-tetrapropoxycalix[4]arenes (**9**, **10**) were prepared as described previously.[1] Their characterization by NMR confirmed their structural integrity and was in accord with the literature.[1, 5] 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 **9** or **10** (190 mg, 0.291 mmol) and Et_3N (340 mg, 2.44 mmol) in dry dichloromethane (18 mL) and dry DMF (2 mL) was added to the reaction mixture, which was allowed to reach room temperature and stirred for 4 days. After completion, the reaction mixture was diluted with 0.5M HCl (20 mL), a saturated solution of $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$. Compound **11** (*cone*) was isolated using silica gel column chromatography (acetone/CH_2Cl_2, 6/1) as yellowish crystals (85 mg, 30%). The NMR spectrum was identical to that in the literature.[1, 6] Compound **12** (*partial cone*) was isolated using silica gel column chromatography (*i*PrOH/CH_2Cl_2, 1/19) as yellowish crystals (66 mg, 27%). It was characterized by NMR, HRMS and IR spectrometry and the found data are in accordance with the literature.[1]



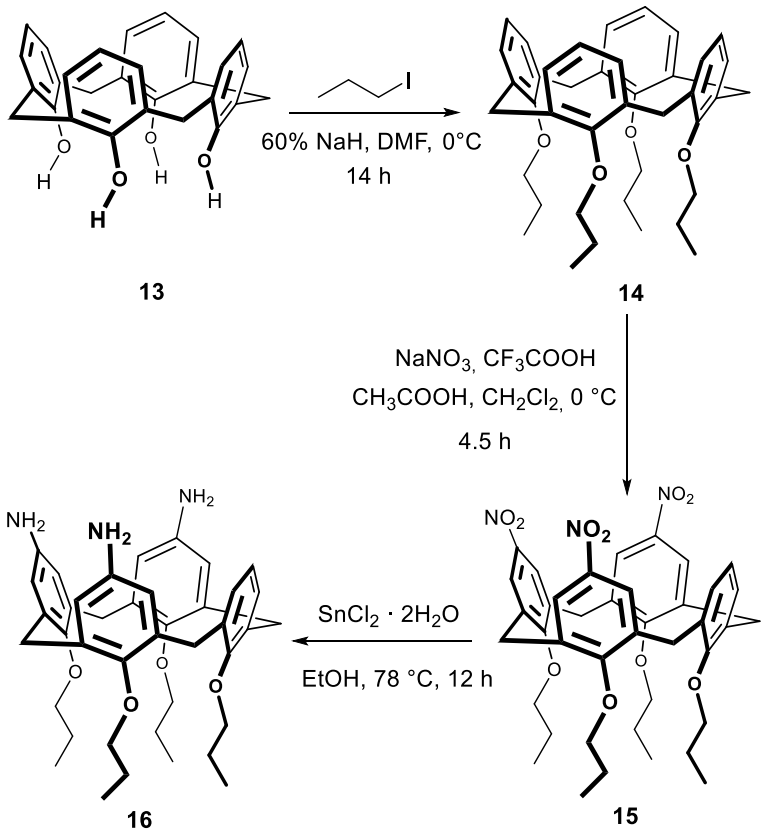
Scheme S3. Synthesis of 5,11,17,23-tetrakis(4-pentynoylamino)-25,26,27,28-tetrapropoxycalix[4]arenes **11** and **12** (exemplified by *cone* conformer **11**).

cone 5,11,17-Triamino-25,26,27,28-tetrapropoxycalix[4]arene (16)

Commercial calix[4]arene-25,26,27,28-tetrol **13** (10.0 g, 0.24 mol) was dissolved in dry DMF (150 mL) and cooled to 0 °C. Then, 60% NaH (7.5 g, 188 mmol) was carefully added. After 30 min, propyl iodide (27.0 mL, 282 mmol) was slowly added, and the reaction mixture was stirred at room temperature under a calcium chloride drying tube for 14 h. 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 25,26,27,28-tetrapropoxycalix[4]arene **14** (11.27 g, 81%). The NMR spectrum was identical to that reported in the literature.[7]

Compound **14** (6.0 g, 10.1 mmol) was dissolved in dry CH_2Cl_2 (200 mL) and ice-cold acetic acid (24 mL) was added. Then, $NaNO_3$ (17.2 g, 0.20 mol) and CF_3COOH (15 mL, 0.20 mol) were carefully added at 0 °C. The cooling bath was removed, and the reaction mixture was stirred for 4.5 h at room temperature. After completion, the reaction mixture was diluted with water (100 mL), the aqueous phase was twice extracted with CH_2Cl_2, and the combined organic phases were washed with water, brine and dried over $MgSO_4$. Silica gel flash chromatography (cyclohexane/CH_2Cl_2, 1/3) afforded 5,11,17-trinitro-25,26,27,28-tetrapropoxycalix[4]arene **15** (yellowish crystals, 0.45 g, 6%) as a side product. The NMR spectrum was identical to that reported in the literature.[8]

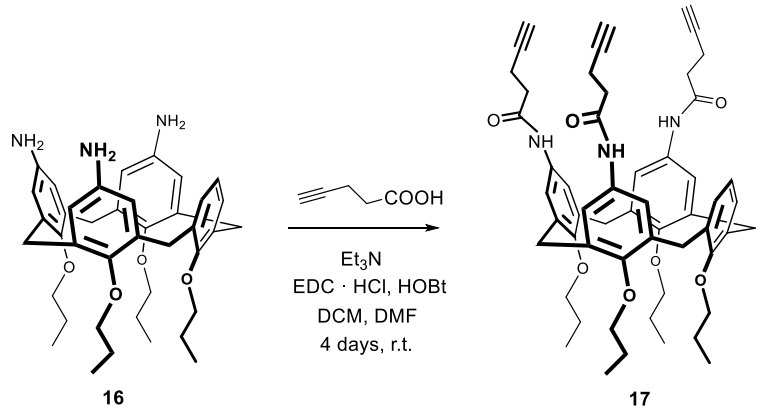
Compound **15** (350 mg, 0.48 mmol) was dissolved in ethanol (30 mL), $SnCl_2 \cdot 2H_2O$ (0.43 g, 7.22 mmol) was added and the reaction mixture was stirred for 12 h at 78 °C. After completion, the reaction mixture was cooled to room temperature, and poured into crushed ice. The pH value was adjusted to 9-10 using 1M KOH. The mixture was extracted with CH_2Cl_2 (4×50 mL), combined organic phases were washed with water, brine, and dried over $MgSO_4$. Solvents were evaporated and the title compound **16** was obtained as red-brown crystals (250 mg, 80%). The NMR spectrum was identical to that reported in the literature.[9]



Scheme S4. Synthesis of 5,11,17-triamino-25,26,27,28-tetrapropoxycalix[4]arene (**16**).

***cone* 5,11,17-tris(4-Pentynoylamino)-25,26,27,28-tetrapropoxy-calix[4]arene (17)**

EDC·HCl (555 mg, 2.89 mmol), HOBt (329 mg, 2.43 mmol), and 4-pentynoic acid (240 mg, 2.45 mmol) were dissolved in dry CH_2Cl_2 (9 mL) and dry DMF (1 mL), and the mixture was cooled to 0 °C. After 20 min, the mixture of **16** (247 mg, 0.387 mmol) and Et_3N (207 mg, 2.05 mmol) in dry CH_2Cl_2 (18 mL) and dry DMF (2 mL) was added. The reaction mixture was allowed to reach room temperature, and stirred for 4 days. After completion, the reaction mixture was diluted with 0.5M HCl (20 mL), a saturated solution of $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$. The title compound **17** was isolated using silica gel chromatography (acetone/CH_2Cl_2, 1/6) as orange crystals (163 mg, 48%). It was characterized by NMR, HRMS and IR spectrometry. Respective data and spectra are given in Section 4.2.

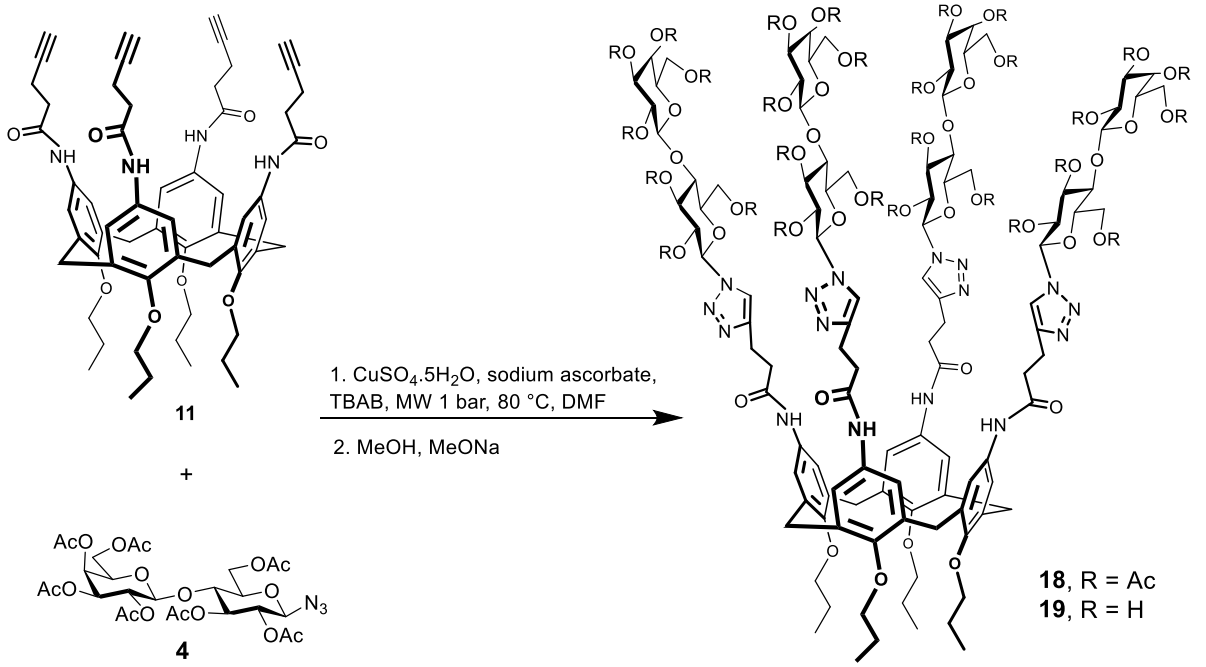


Scheme S5. Synthesis of 5,11,17-tris(4-pentynoylamino)-25,26,27,28-tetrapropoxycalix[4]arene (**17**).

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 (19)**

The title compound **19** was prepared as described previously.[1] Briefly, azide **4** (0.10 g, 0.15 mmol), calix[4]arene **11** (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 tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (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. 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 (dichloromethane/methanol, 1/0 → 95/5) to afford glycocalix[4]arene peracetate **18** (20 mg, 36% yield) as brownish solid. Peracetate **18** (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 to afford glycocalix[4]arene **19** (13 mg, 99%) as a brownish powder. The structural integrity of compound **19** was confirmed by NMR and was in accord with the literature.[1] Analysis of **19** by HPLC is shown in Section 4.3.



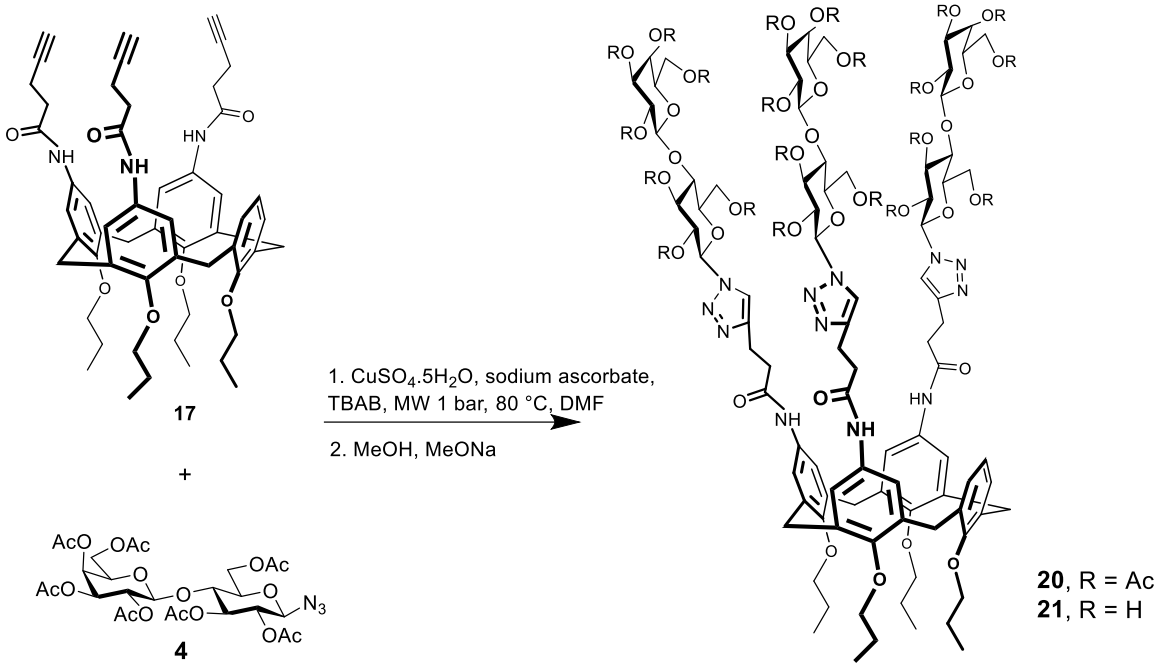
Scheme S6. Synthesis of *cone*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (**19**).

cone **5,11,17-tris{[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 **4** (0.100 g, 0.15 mmol), calix[4]arene **17** (0.020 g, 0.02 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

(dichloromethane/methanol, 1/0 → 95/5) to afford glycocalix[4]arene peracetate **20** (0.058 g, 20%) as a yellow solid.

Glycocalix[4]arene peracetate **20** (0.058 g, 20 μmol) was dissolved in absolute methanol (5 mL) under argon, and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 1 h. The reaction was monitored by TLC (dichloromethane/methanol, 95/25). After completion, the reaction mixture was evaporated to afford glycocalixarene **21** (0.030 g, 75%) as a yellowish powder. The structural integrity of compound **21** was confirmed by NMR, HRMS, and HPLC. The respective data and spectra are shown in Section 4.3.



Scheme S7. Synthesis of *cone*-5,11,17-*tris*{[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**).

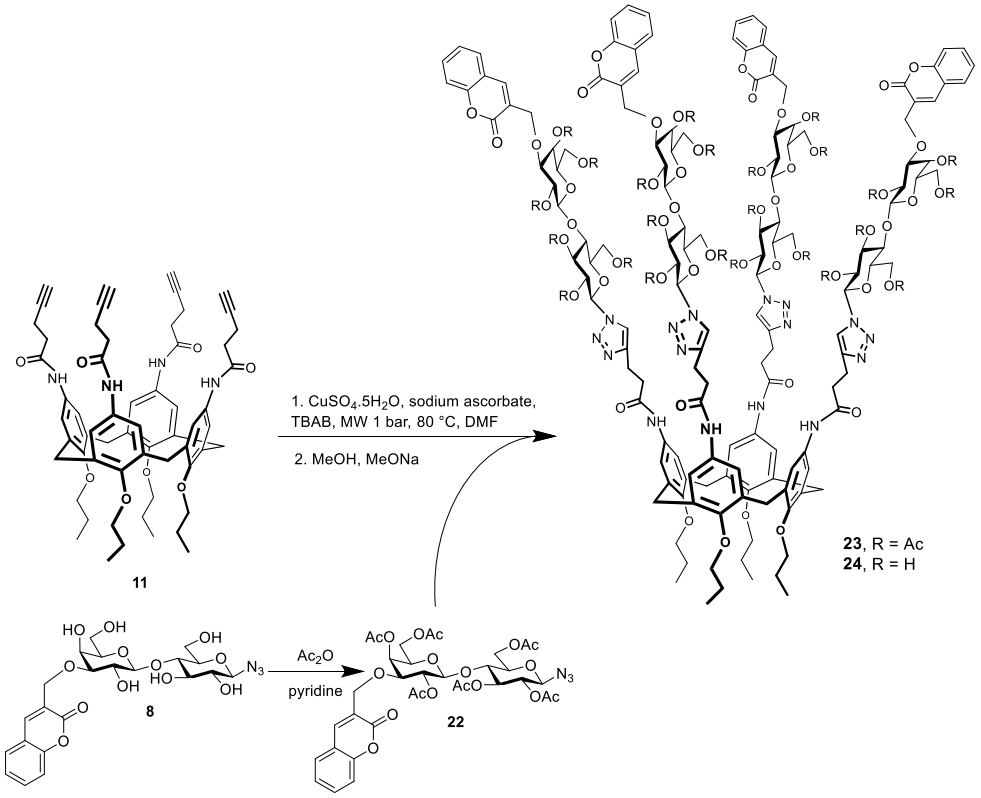
cone 5,11,17,23-tetrakis{[3′-*O*-(Coumarylmethyl)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxycalix[4]arene (24)

Compound **8** (1.1 g, 2.1 mmol) was dissolved in pyridine (5 mL) under argon, and acetic acid anhydride (4 mL, 42.3 mmol) was added. The reaction mixture was stirred for 24 h under monitoring by TLC (cyclohexane/ethyl acetate, 1/1). After completion, the reaction was quenched by the addition of water (5 mL) at 0 °C. The solution was diluted with ethyl acetate and extracted with water, 1M HCl, and brine. Combined organic phases were dried over Na_2SO_4, filtered, evaporated, desalinated on acidic ion exchange resin in the H^+ cycle, and purified on silica gel (cyclohexane/ethyl acetate, 2/1) to afford azide peracetate **22** (0.588 g, 36%) as colorless-white oil.

Compound **22** (0.122 g, 0.16 mmol), calixarene **11** (0.0195 g, 0.02 mmol), 2M water solution of sodium ascorbate (320 μL, 0.64 mmol), 2M water solution of $CuSO_4.5H_2O$ (160 μL, 0.32 mmol), and a catalytic amount of TBTA were dissolved in absolute dimethylformamide (4 mL) in a microwave tube. The mixture was heated at 80 °C by microwave irradiation (medium irradiation, 1 bar) for 8 h. The reaction was monitored by TLC (dichloromethane/methanol, 9/1). After completion, the reaction mixture was diluted with water, extracted with ethyl acetate, dried with Na_2SO_4, filtered, evaporated, and purified on silica gel (dichloromethane/methanol,

1/0 → 98/2) to afford glycocalix[4]arene peracetate **23** (0.019 g, 23%) as a yellow-brown solid. Its structural integrity and full substitution of all four propargyls by glycomimetics was confirmed by NMR. Respective data and spectra are available in Section 4.3.

Peracetate **23** (0.019 g, 4.7 μmol) was dissolved under argon in absolute methanol (5 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred overnight. The reaction was monitored by TLC (dichloromethane/methanol, 8/2). After completion, the reaction mixture was evaporated to afford glycocalix[4]arene **24** (0.013 g, 92%) as a slightly brown powder. The structural integrity of compound **24** was confirmed by HRMS, and HPLC. Respective data and spectra are available in Section 4.3. We observed that during deprotection under basic conditions as above, the *o*-coumaryl moiety on the lactosyl may reversibly hydrolyze to afford the corresponding *o*-coumaric acid.[10] The presence of the *o*-coumaric acid-bearing species (hydrolyzed form) was clearly indicated in the NMR spectra by a down-field shift of the carbon in the oxymethylene group bound to galactose and a significant up-field shift of the isolated methine, and it was also observed in the HRMS spectrum (Figure S19). The lactonization of *o*-coumaric acid back to the more stable six-membered coumaryl lactone occurred by dehydration by repeated lyophilization in dry DMSO.

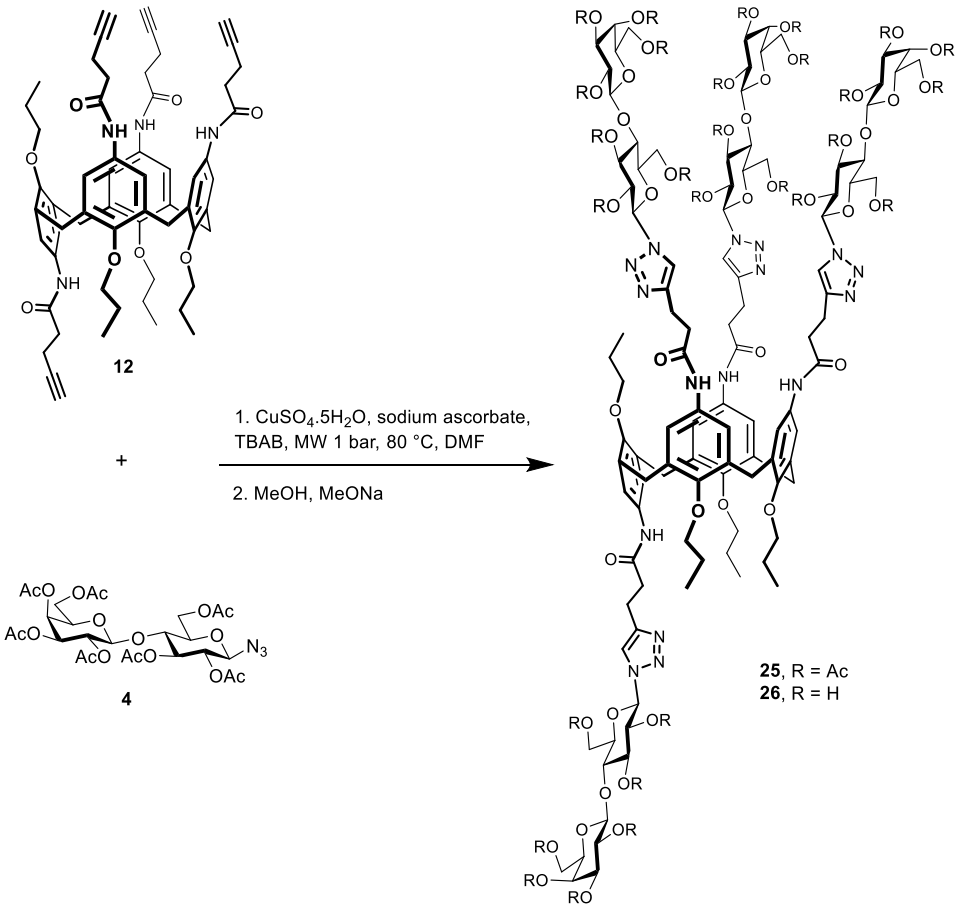


Scheme S8. Synthesis of *cone*-5,11,17,23-tetrakis{[3-*O*-(coumarylmethyl)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxy-calix[4]arene (**24**).

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 (26)

The title compound **26** was prepared as described previously.[1] Briefly, azide **4** (0.2 g, 0.30 mmol), calix[4]arene **12** (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 for 2 h. 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 to afford glycocalix[4]arene peracetate **25** (51 mg, 46%) as brownish oil. Peracetate **25** (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. After completion, the reaction was quenched with Amberlite IR 120/H^+, and stirred until neutral pH, filtered and evaporated to afford glycocalix[4]arene **26** (25 mg, 74%) as brownish powder. The structural integrity of compound **26** was confirmed by NMR and was in accord with the literature.[1] HPLC analysis of **26** is available in Section 4.3.



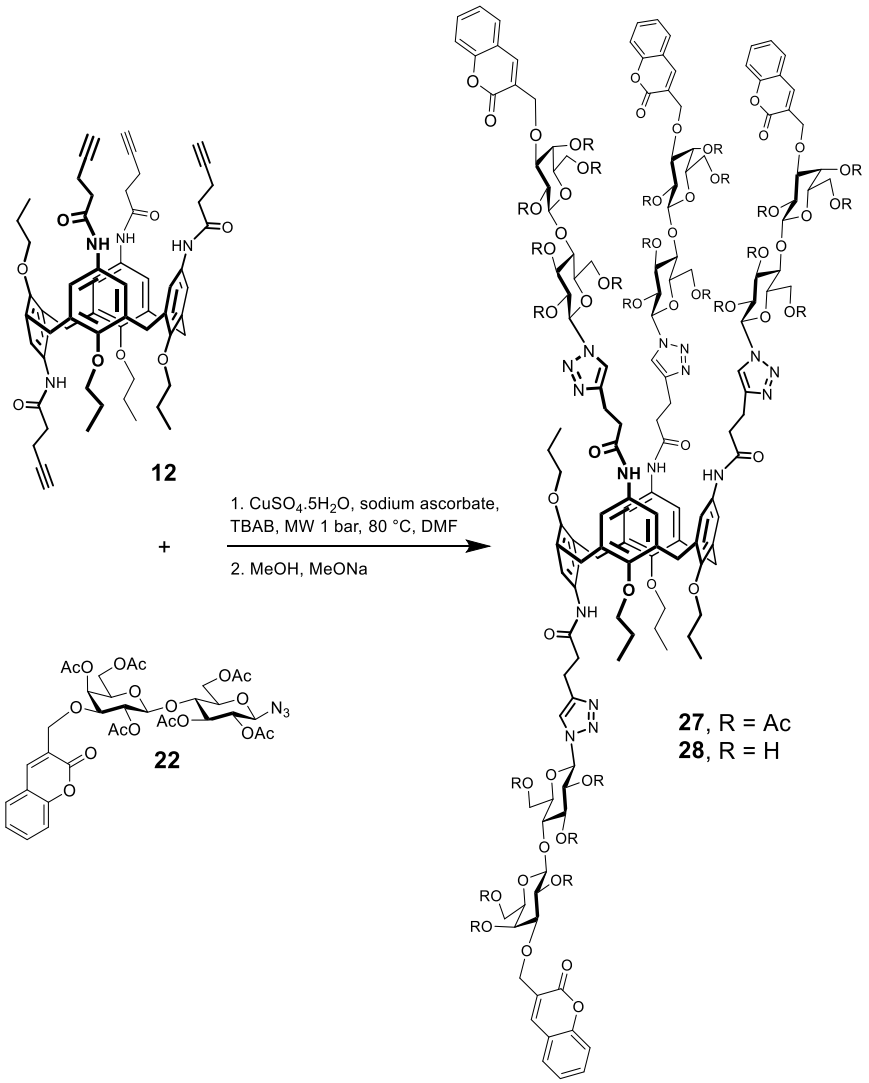
Scheme S9. Synthesis of *partial cone*-5,11,17,23-tetrakis{[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxy-calix[4]arene (**26**).

partial cone 5,11,17,23-tetrakis{[3´-*O*-(Coumarylmethyl)-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 (28)

Azide peracetate **22** (0.030 g, 0.039 mmol), calixarene **12** (3 mg, 3.1 μmol), 2M water solution of sodium ascorbate (100 μL, 0.20 mmol), 2M water solution of $CuSO_4.5H_2O$ (160 μL, 0.32 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, 12/1). 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 (dichloromethane/methanol, 1/0 → 97/3) to afford glycocalixarene peracetate **27** (0.006 g, 48%) as a yellow-brown solid. Its structural integrity and full substitution of all four

propargyls by glycomimetics was confirmed by NMR. Respective data and spectra are available in Section 4.3.

Peracetate **27** (0.006 g, 1.5 μmol) was dissolved under argon and heated to 42 °C in absolute methanol (2 mL), and a catalytic amount of freshly prepared MeONa was added. The reaction mixture was stirred for 2 h and monitored by TLC (dichloromethane/methanol, 9/1). After completion, the reaction mixture was evaporated to afford glycocalixarene **28** (4.6 mg, 99%) as a slightly brown powder. The structural integrity of compound **28** was confirmed by HRMS, and HPLC. Respective data and spectra are available in Section 4.3. We observed that during deprotection under basic conditions as above, the *o*-coumaryl moiety on the lactosyl may partially reversibly hydrolyze to afford the corresponding *o*-coumaric acid.[10] The presence of the *o*-coumaric acid-bearing species (hydrolyzed form) was clearly indicated in the NMR spectra by a down-field shift of the carbon in the oxymethylene group bound to galactose and a significant up-field shift of the isolated methine. The lactonization of *o*-coumaric acid back to the more stable six-membered coumaryl lactone occurred by dehydration by repeated lyophilization in dry DMSO.



Scheme S10. Synthesis of *partial cone*-5,11,17,23-tetrakis{[3′-*O*-(coumarylmethyl)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxy-calix[4]arene (**28**).

4. STRUCTURAL CHARACTERIZATION OF PREPARED COMPOUNDS

4.1. Glycosyl azides

β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (5)

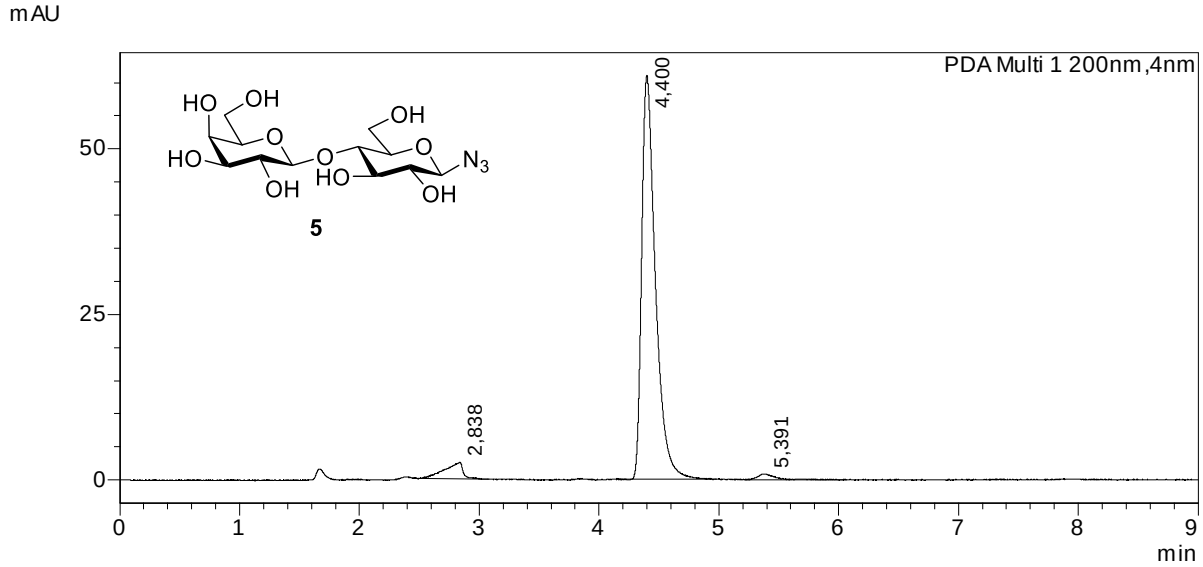


Figure S1. HPLC chromatogram of compound **5** (RT = 4.400 min, purity 93%).

3-*O*-(Coumarylmethyl)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl azide (8)

Table S1. ^{1}H and ^{13}C NMR data of compound **8** (399.87 MHz for ^{1}H, 100.55 MHz for ^{13}C, CD_3OD, 30 °C).

	Ato	δ_C	m	δ_H	n	m.	*J*[Hz]
Glc	**1**	91.97	d	4.543	1	d	8.7
	2	74.53	d	3.219	1	dd	8.9, 8.7
	3	76.57	d	3.551	1	dd	8.9, 8.6
	4	80.24	d	3.617	1	dd	9.6, 8.6
	5	78.69	d	3.523	1	ddd	9.6, 4.0, 2.4
	6	61.82	t	3.936	1	dd	12.3, 2.4
				3.875	1	dd	12.3, 4.0
Gal	**1**	105.03	d	4.435	1	d	7.8
	2	71.81	d	3.739	1	dd	9.6, 7.8
	3	83.39	d	3.485	1	dd	9.6, 3.2
	4	67.06	d	4.152	1	dd	3.2, 1.0
	5	76.94	D	3.614	1	ddd	7.4, 4.7, 1.0
	6	62.56	t	3.820	1	dd	11.4, 7.4
				3.743	1	dd	11.4, 4.7
coumaryl	**OC**	67.32	t	4.686	1	dd	14.3, 1.5
				4.561	1	dd	14.3, 1.4
	2'	162.61	s	-	0	-	-
	3'	127.09	s	-	0	-	-
	4'	141.34	d	8.178	1	dd	1.5, 1.4
	5'	120.80	s	-	0	-	-
	6'	129.39	d	7.636	1	dd	8.3, 1.6
	7'	125.90	d	7.35^{H}	1	m	-
	8'	132.64	d	7.575	1	ddd	1.6, Σ*J* = 15.8
	9'	117.35	d	7.36^{H}	1	m	-
	10'	154.62	s	-	0	-	-

^{H} … HSQC readout

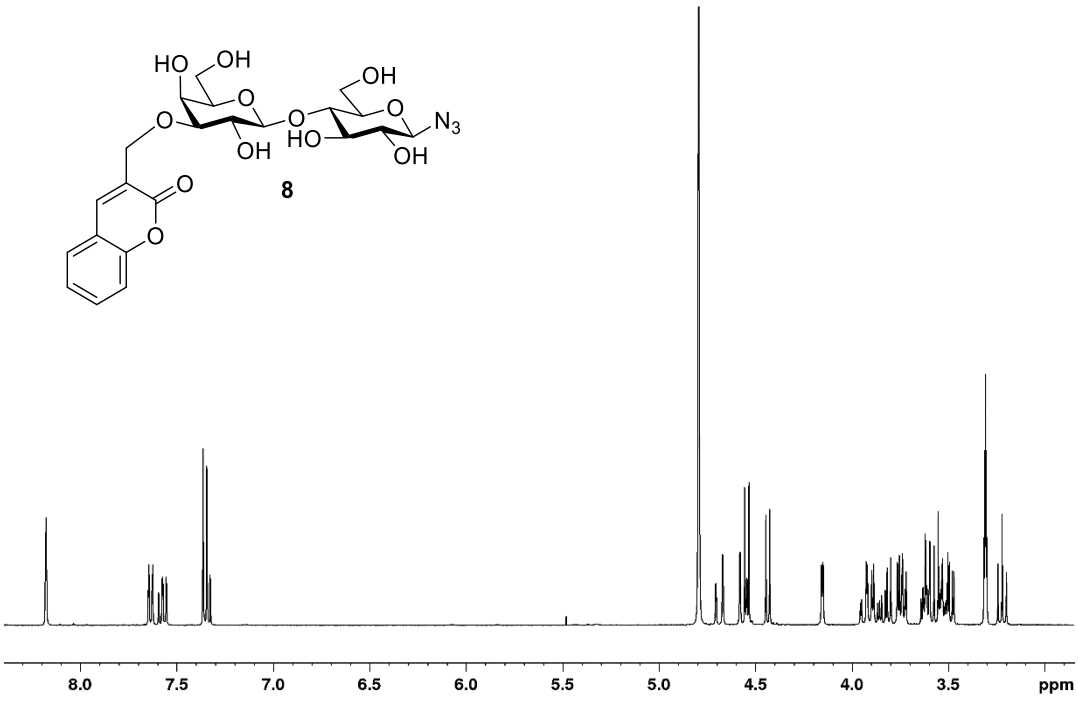


Figure S2. ^{1}H NMR spectrum of compound **8** (399.87 MHz, CD_3OD, 30 ˚C).

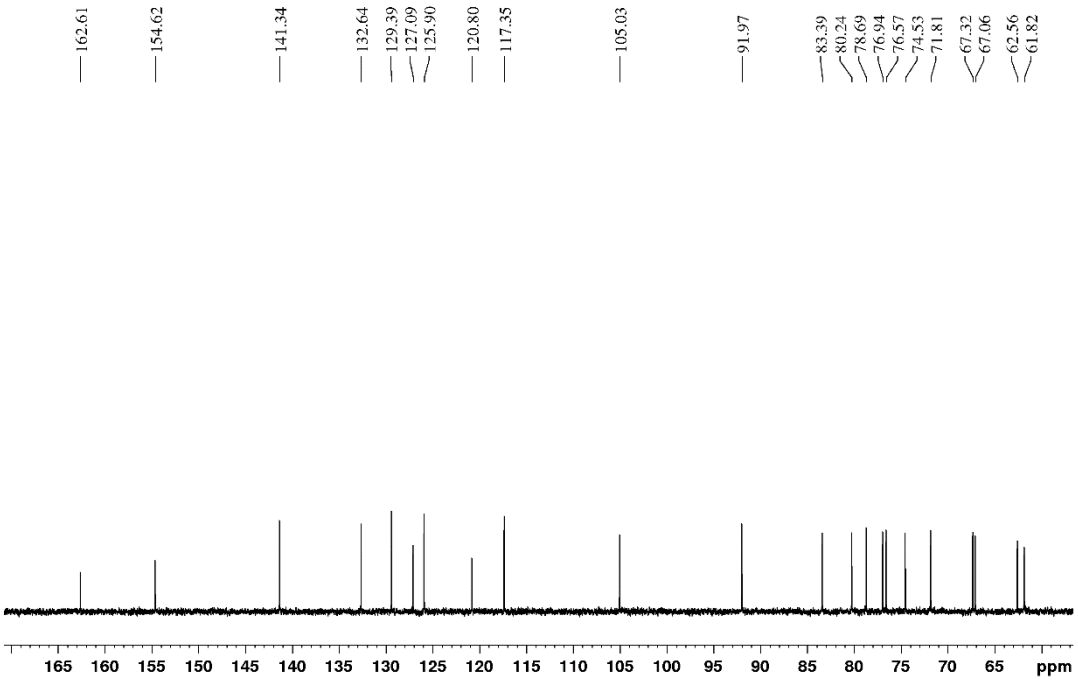


Figure S3. ^{13}C NMR spectrum of compound **8** (100.55 MHz, CD_3OD, 30 ˚C)**.**

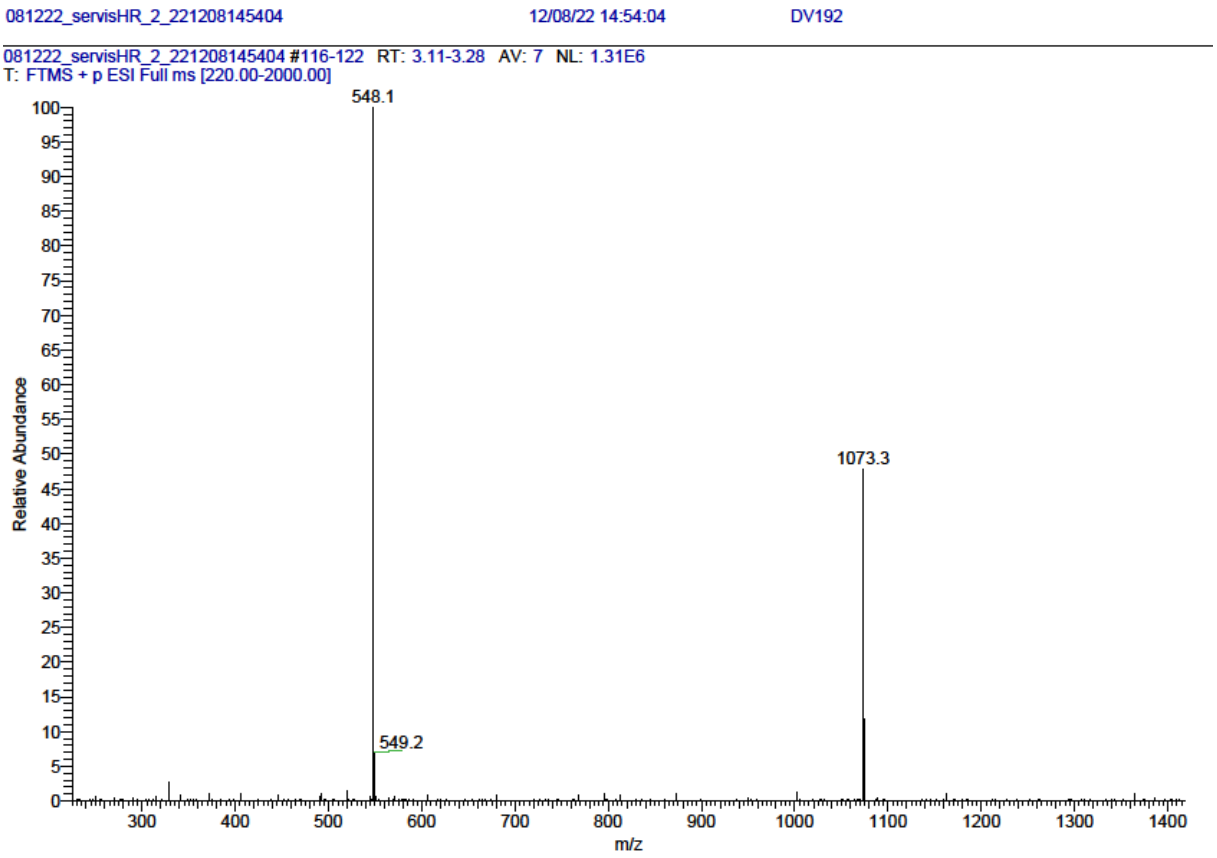


Figure S4. Mass spectrum (ESI$^+$) of compound **8**: $[M+Na]^+$, *m/z* 548.1; $[2M+Na]^+$, *m/z* 1073.3. HRMS (ESI$^+$): *m/z* for $C_{22}H_{27}O_{12}N_3Na^+$ calculated 548.14869, found 548.14878 (0.16 ppm).

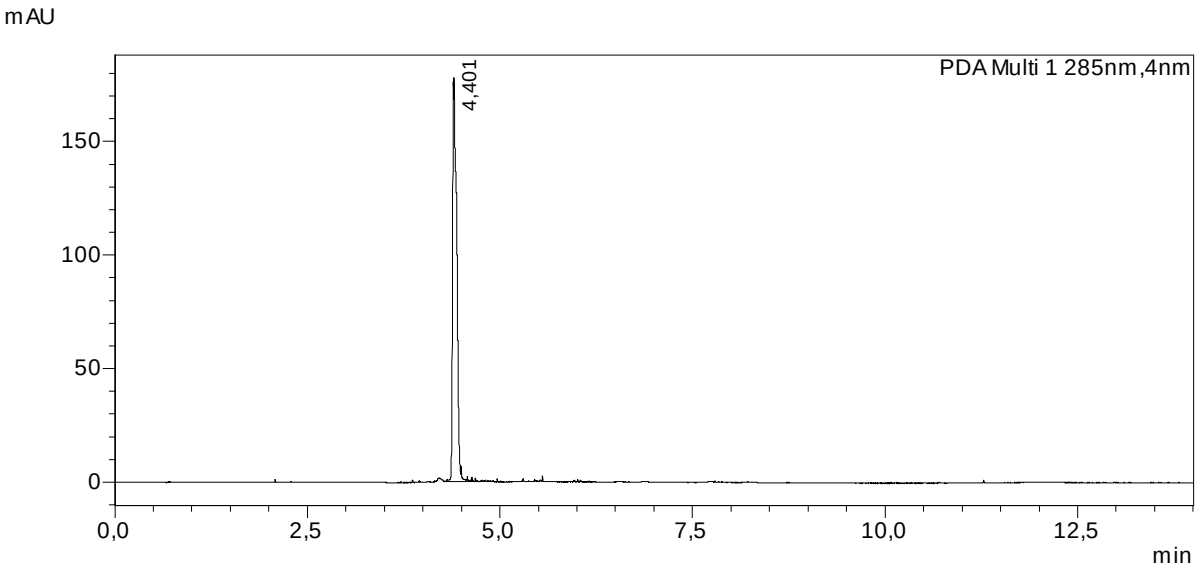


Figure S5. HPLC chromatogram of compound **8** (RT = 4.401 min, purity 99%).

4.2. Calix[4]arene cores

5,11,17,23-tetrakis(4-Pentynoylamino)-25,26,27,28-tetrapropoxycalix[4]arene (11, 12)

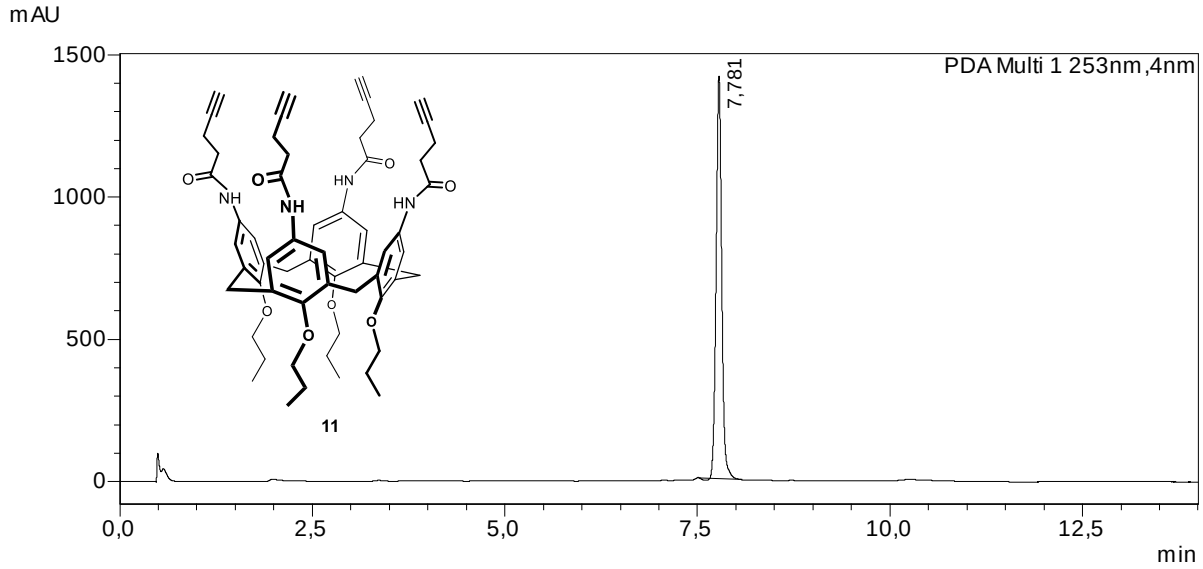


Figure S6. HPLC chromatogram of compound **11** (RT = 7.781 min, purity 98%).

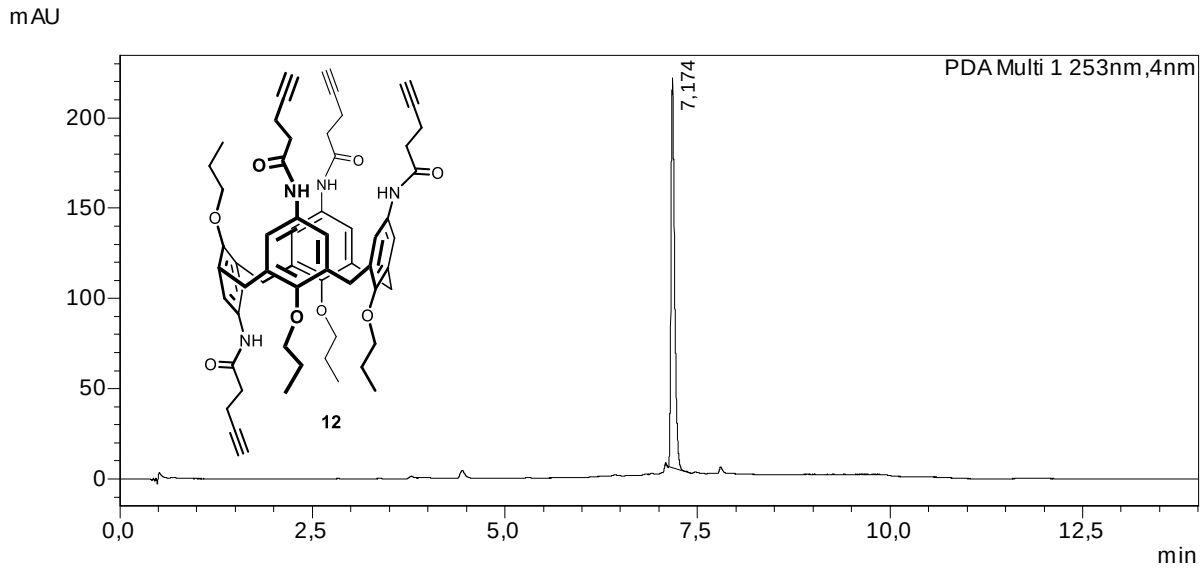


Figure S7. HPLC chromatogram of compound **12** (RT = 7.174 min, purity 96%).

***cone*-5,11,17-*tris*(4-Pentynoylamino)-25,26,27,28-tetrapropoxy-calix[4]arene (17)**

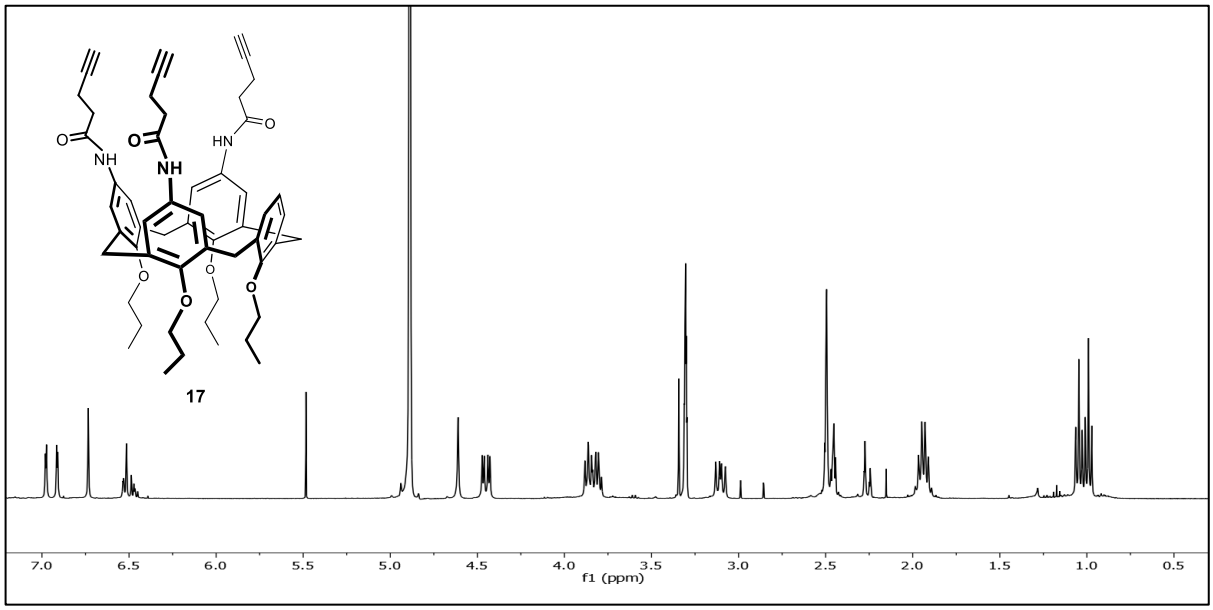


Figure S8. ^{1}H NMR spectrum of compound **17**. ^{1}H NMR (400 MHz, CD_3OD, 298 K) δ_H (ppm): 6.98 (d, 2H, *J* = 2.6 Hz, Ar-**H**), 6.91 (d, 2H, *J* = 2.6 Hz, Ar-**H**), 6.73 (s, 2H, Ar -**H**), 6.55 – 6.44 (m, 3H, Ar-**H**, Ar-**H**), 4.47 (d, 2H, *J* = 13.7 Hz, Ar-C**H$_2$**–Ar), 4.43 (d, 2H, *J* = 13.7 Hz, Ar–C**H$_2$**–Ar), 3.90 – 3.77 (m, 8H, -O-C**H$_2$**-CH_2-CH_3), 3.15 – 3.06 (m, 4H, Ar–C**H$_2$**-Ar), 2.53 -2.43 (m, 12H, -OC-C**H$_2$**-CH_2-C≡CH, -CO-CH_2-C**H$_2$**-C≡CH), 2,27 (brs, 2H, -C≡C**H**), 2.24 (brs, 1H, -C≡C**H**), 2.00 – 1.88 (m, 8H, -O-CH_2-C**H$_2$**-CH_3), 1.05 (t, 6H, *J* = 7.4 Hz, -O-CH_2-CH_2-C**H$_3$**), 0.99 (t, 6H, *J* = 7.4 Hz, -O-CH_2-CH_2-C**H$_3$**).

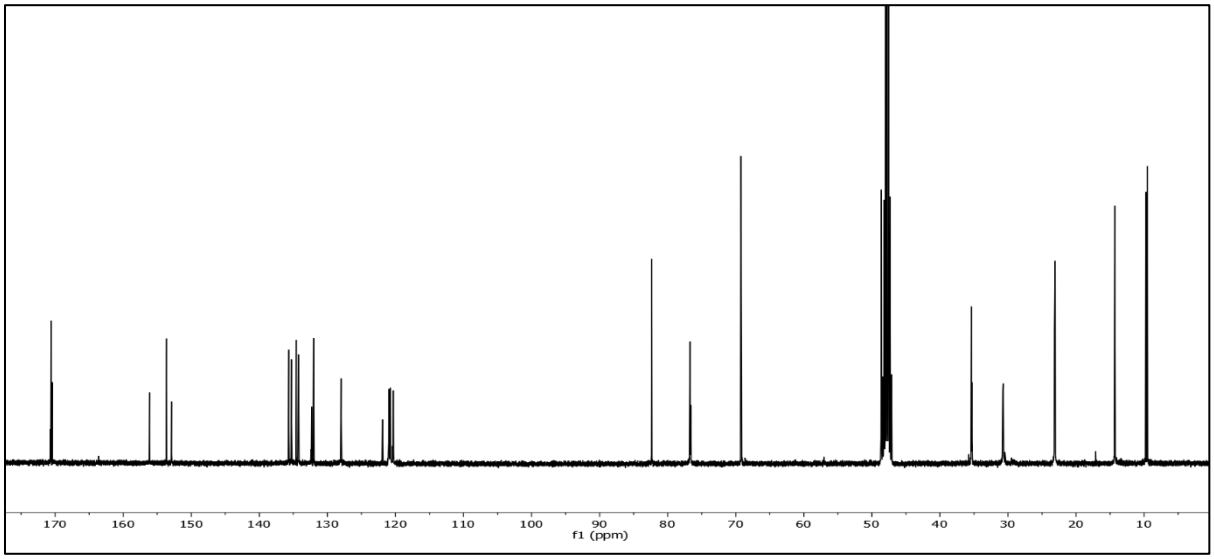


Figure S9. ^{13}C NMR spectrum of compound **17**. ^{13}C NMR (101 MHz, CD_3OD) δ_C (ppm): 170.60; 170.42; 156.14; 153.63; 152.90; 135.68; 135.27; 134.58; 134.26; 132.31; 132.02; 127.98; 121.90; 120.96; 120.73; 120.31; 82.35; 82.33; 76.73; 76.70; 76.61; 69.24;69.19; 47.54; 47.33; 47.12; 35.39; 35.29; 30.71;23.18; 23.08; 14.34; 14.31; 9.75; 9.52.

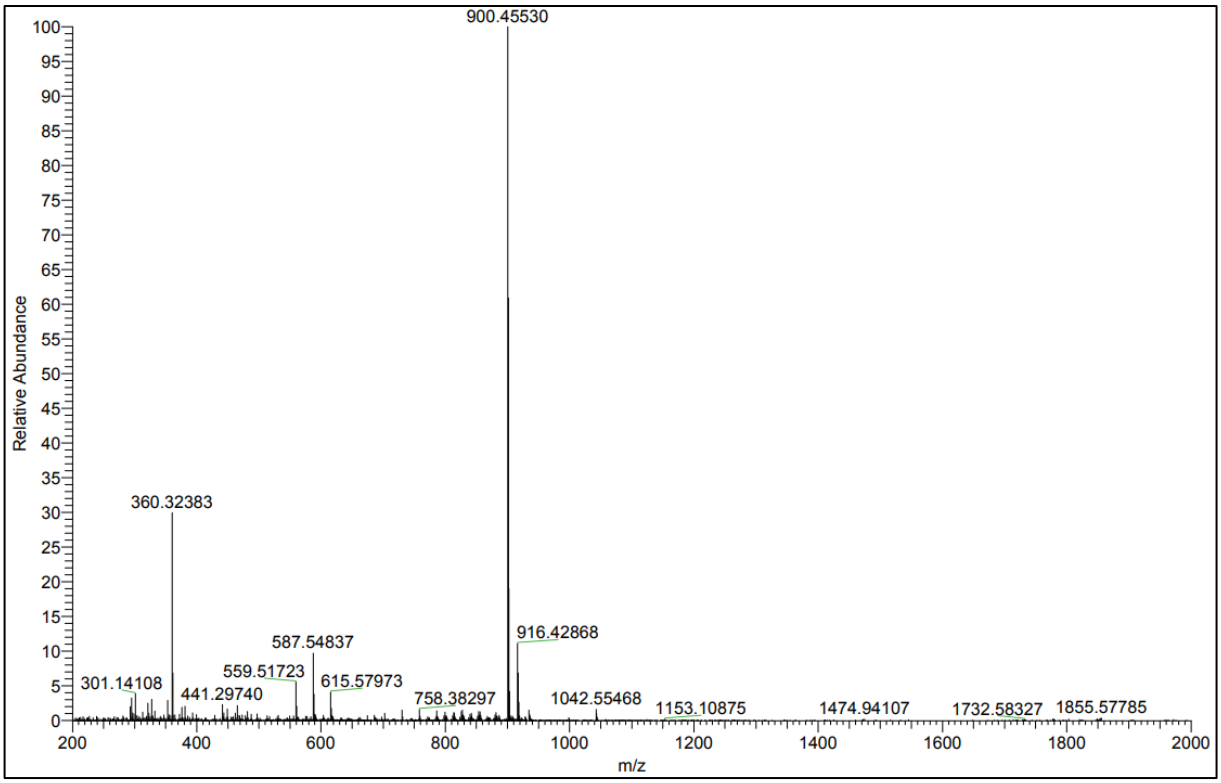


Figure S10. Mass spectrum (ESI$^+$) of compound **17.** HRMS (ESI$^+$): *m/z* for $C_{55}H_{63}N_3O_7Na^+$ calculated 900.45582, found 900.45530 and *m/z* for $C_{55}H_{63}N_3O_7K^+$ calculated 916.42976, found 916.42868.

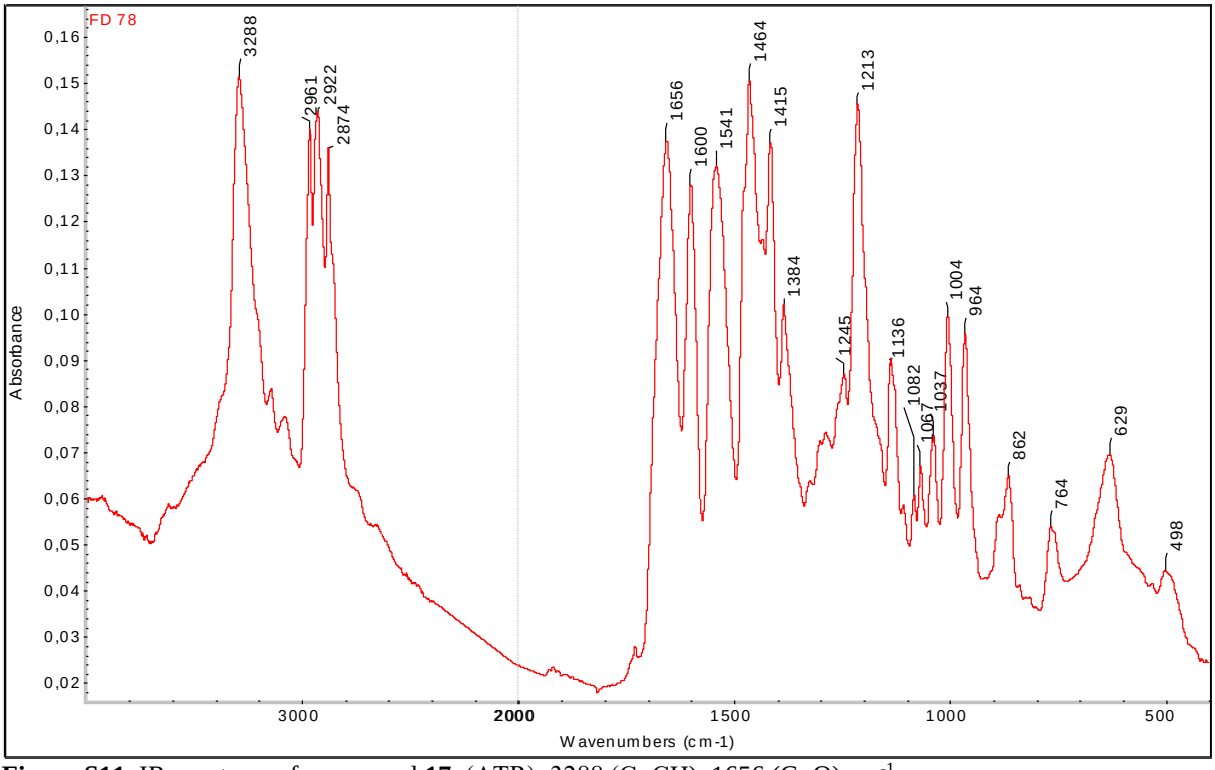


Figure S11. IR spectrum of compound **17.** (ATR): 3288 (C≡CH), 1656 (C=O) cm^{-1}.

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-tetrapropoxy-calix[4]arene (19)

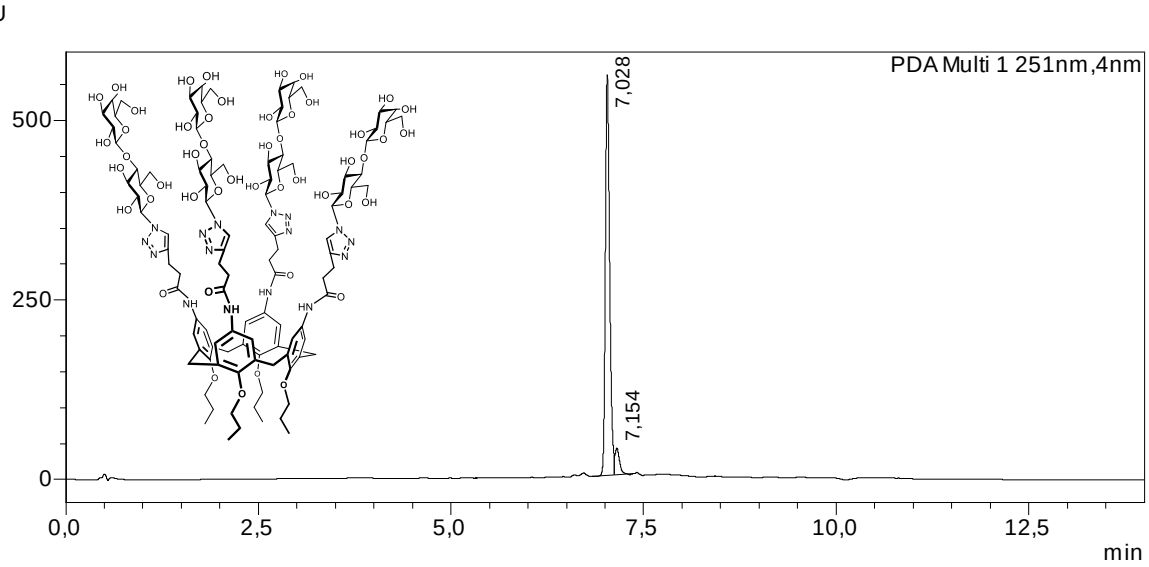


Figure S12. HPLC chromatogram of compound **19** (7.028 min, purity 94%).

cone-5,11,17-tris{[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	m.	*J*[Hz]
A-core	**ipso-**	155.34	s	-	0	-	
	ortho-	133.44	s	-	0	-	
	meta-	127.44	d	6.378	2	m	
	para-	121.71	d	6.378	1	m	
	1''	76.33	t	3.720	2	br t	
	2''	22.82	t	1.867	2	m	
	3''	10.36	q	1.027	3	t	7.4
B-core	**ipso-**	152.38	s	-	0	-	
	ortho	135.14	s	-	0	-	
	ortho	134.91	s	-	0		
	meta^{A}	119.60	d	7.222	1	d	1.8
				7.213	1	d	1.8
	meta^{C}	119.40	d	7.093	1	d	1.8
				7.084	1	d	1.8
	para-	133.03	s	-	0	-	
	1''	76.10	t	3.83^{H}	4	m	
	2''	22.49	t	1.878	4	m	
	3''	9.90	q	0.904	6	t	7.5
	NH	-	-	9.689	2	s	
	CO	169.42	s	-	0	-	
B-spacer	**1'**	35.27	t	2.651	4	br t	
	2'	20.85	t	2.921	4	br t	
	3'	145.84	s	-	0	-	
	4'	121.03	d	8.034	2	s	
B-Glc	**1**	86.80	d	5.581	2	d	9.3

	2	71.72	d	3.830	2	m	
	3	75.15	d	3.568	2	m	
	4	79.73	d	3.471	2	m	
	5	77.65	d	3.639	2	m	
	6	59.96	t	3.772	2	m	
				3.601	2	m	
B-Gal	**1**	103.71	d	4.256	2	d	7.4
	2	70.48	d	3.360	2	m	
	3	73.18	d	3.326	2	m	
	4	68.07	d	3.635	2	m	
	5	75.51	d	3.483	2	m	
	6	60.34	t	3.555	2	m	
				3.518	2	m	
C-core	**ipso-**	151.21	s	-	0	-	
	ortho-	133.23	s	-	0	-	
	meta-	118.86	d	6.689	1	d	1.9
				6.676	1	d	1.9
	para-	133.14	s	-	0	-	
	1’’	76.35	t	3.670	2	br t	
	2’’	22.73	t	1.852	2	m	
	3’’	10.33	q	1.006	3	t	7.4
	NH	-	-	9.442	1	s	
	CO	169.10	s	-	0	-	
C-spacer	**1’**	35.18	t	2.549	2	br t	
	2’	20.85	t	2.843	2	br t	
	3’	145.84	s	-	0	-	
	4’	120.95	d	7.972	1	s	
C-Glc	**1**	86.77	d	5.556	1	d	9.3
	2	71.70	d	3.805	1	m	
	3	75.15	d	3.568	1	m	
	4	79.73	d	3.547	1	m	
	5	77.64	d	3.624	1	m	
	6	59.96	t	3.759	1	m	
				3.587	1	m	
C-Gal	**1**	103.71	d	4.249	1	d	7.4
	2	70.48	d	3.354	1	m	
	3	73.18	d	3.321	1	m	
	4	68.07	d	3.631	1	m	
	5	75.51	d	3.478	1	m	
	6	60.34	t	3.551	1	m	
				3.514	1	m	
bridge AB	**-CH_2-**	30.45	t	4.347	2	br d	13.1
				3.110	2	br d	13.4
bridge BC	**-CH_2-**	30.66	t	4.312	2	br d	13.1
				3.040	2	br d	13.0

^{H} … HSQC readout

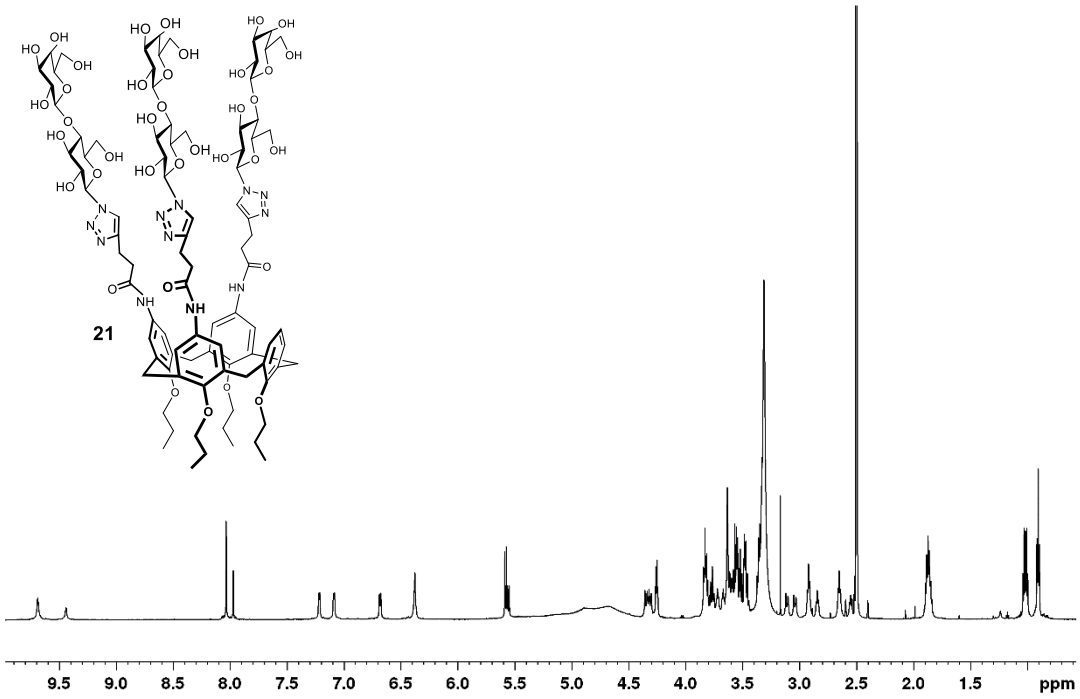


Figure S13. ^{1}H NMR spectrum of compound **21** (700.13 MHz, DMSO-d_6, 30 °C).

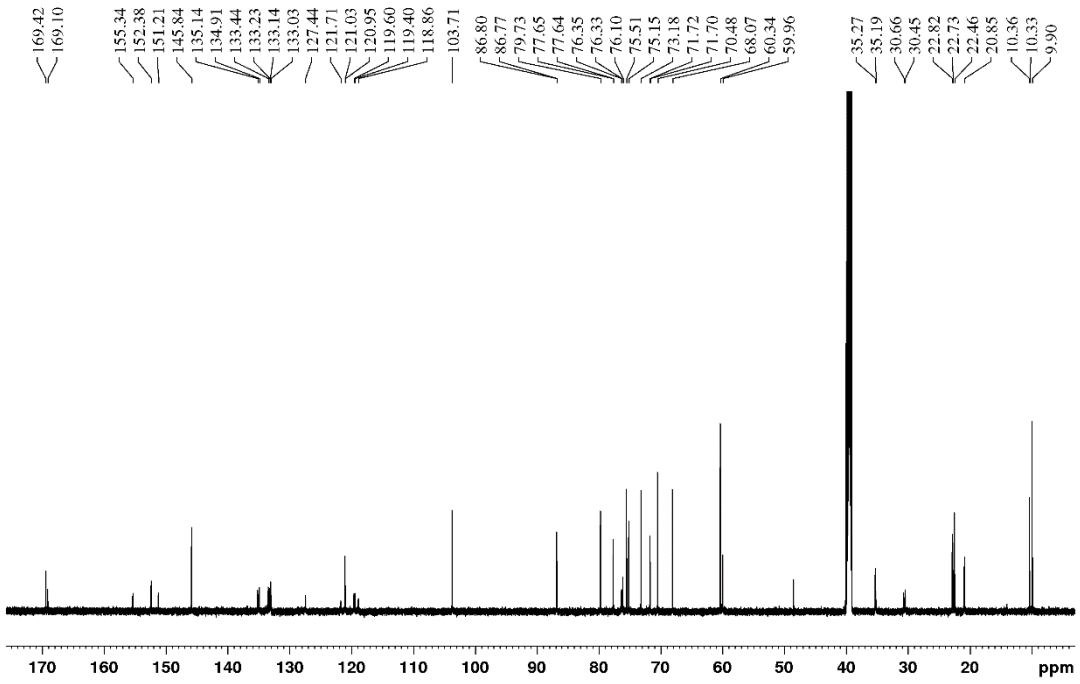


Figure S14. ^{13}C NMR spectrum of compound **21** (176.05 MHz, DMSO-d_6, 30 °C).

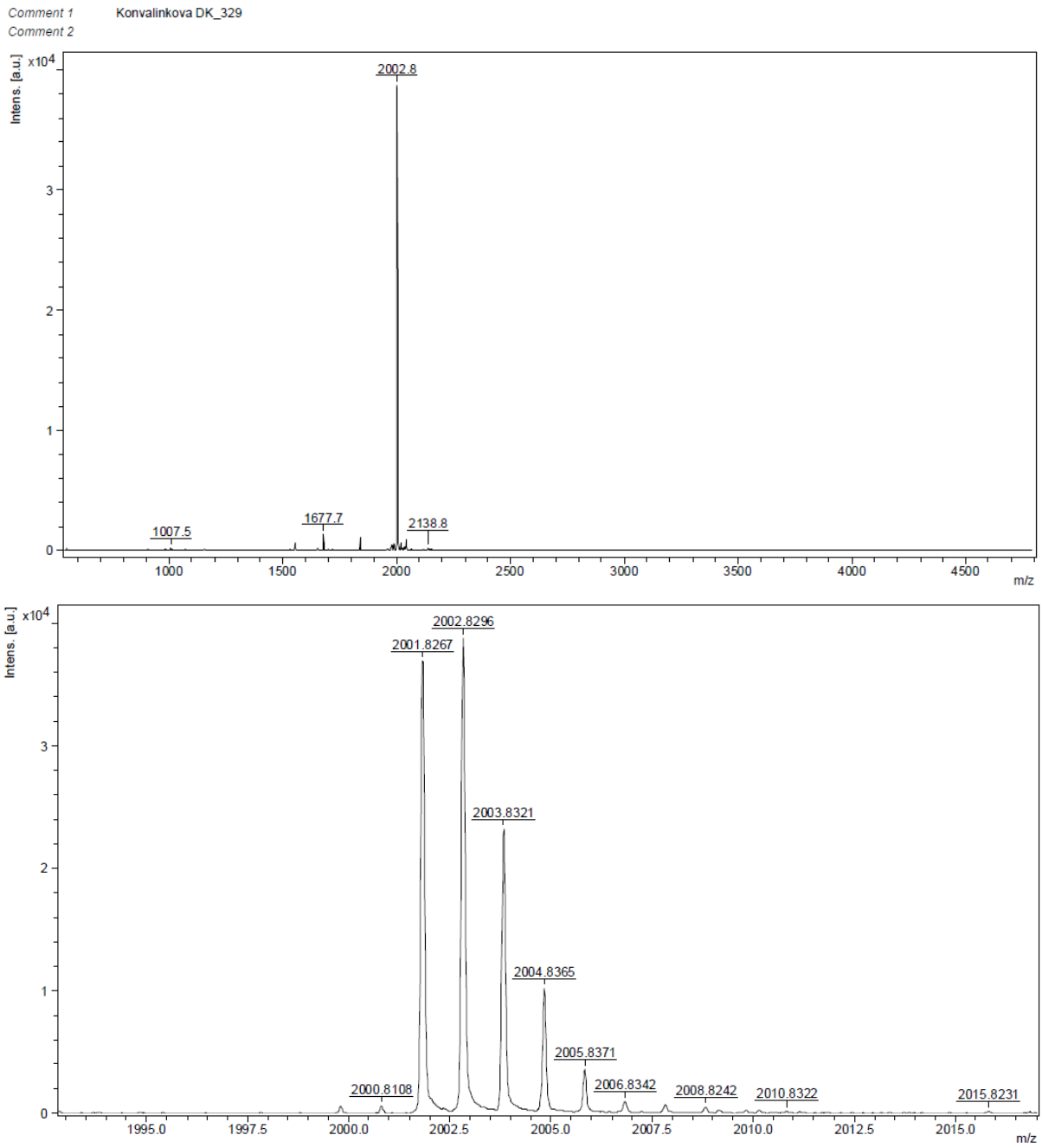


Figure S15. Mass spectrum (MALDI$^+$) of compound **21** ([M + Na]$^+$, *m*/*z* 2001.8; upper figure) and enlarged region showing a cluster of sodium adduct isotopic peaks (lower figure). **HRMS (MALDI$^+$)**: *m*/*z* for $C_{91}H_{126}O_{37}N_{12}Na^+$ calculated 2001.8239, found 2001.8267 (1.40 ppm).

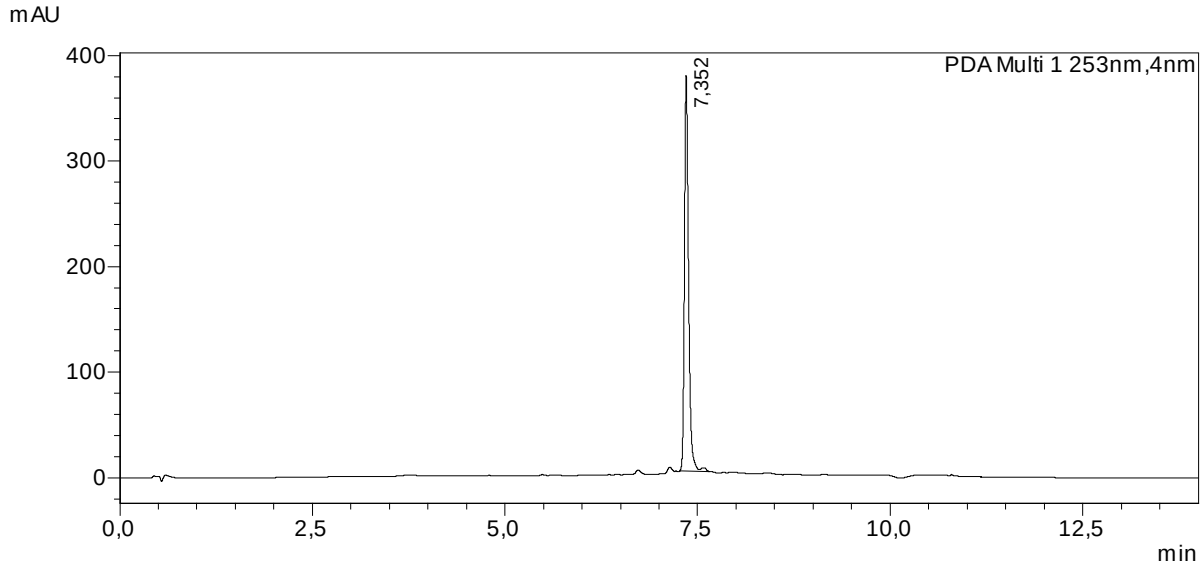


Figure S16. HPLC chromatogram of compound **21** (RT =7.352, purity 98%).

cone 5,11,17,23-tetrakis{[3-*O*-(Coumarylmethyl)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-1-(1,2,3-triazol-4-yl)prop-1-oyl]amino}-25,26,27,28-tetrapropoxy-calix[4]arene (24)

Table S3. ^{1}H and ^{13}C NMR data of compound **23** (peracetate of **24**) (399.87 MHz for ^{1}H, 100.55 MHz for ^{13}C, DMF-d_7, 30 °C).

	Atom	δ_C	m	δ_H	n	m.	*J*[Hz]
core	**ipso-**	152.95	s	-	0	-	
	ortho-	135.20	s	-	0	-	
	meta-	120.02	d	7.062	8	br s	
	para-	134.43	s	-	0	-	
	-CH$_2$-	31.82	t	4.44^{H}	4	br d	
				3.106	4	br d	12.4
	1''	77.32	t	3.848	8	br t	7.3
	2''	23.67	t	1.964	8	m	
	3''	10.58	q	1.010	1	t	7.4
	NH	-	-	9.571	4	s	
	CO	170.00	s	-	0	-	
spacer	**1'**	36.33	t	2.662	8	br t	7.5
	2'	21.74	t	2.959	8	br t	7.5
	3'	147.67	s	-	0	-	
	4'	121.94	d	8.056	4	br s	
Glc	**1**	84.99	d	6.290	4	d	9.0
	2	71.45	d	5.564	4	dd	9.6, 9.0
	3	73.62	d	5.500	4	dd	9.6, 8.8
	4	76.78	d	4.129	4	dd	9.9, 8.8
	5	75.94	d	4.311	4	ddd	9.9, 6.3, 1.7
	6	63.16	t	4.532	4	dd	11.9, 1.7
				4.19^{H}	4	m	
	2-CO	169.36	s	-	0	-	
	Ac	20.09	q	1.778	1	s	
	3-CO	170.14	s	-	0	-	
	Ac	20.84	q	2.070	1	s	
	6-CO	170.88	s	-	0	-	
	Ac	20.61	q	2.041	1	s	

Gal	**1**	101.63	d	4.843	4	d	8.1
	2	71.45	d	5.033	4	dd	10.1, 8.1
	3	78.40	d	4.086	4	dd	10.1, 3.5
	4	67.21	d	5.661	4	d	3.5
	5	71.26	d	4.21[H]	4	m	
	6	62.04	t	4.19[H]	8	m	
	2-CO	170.04	s	-	0	-	
	Ac	20.88	q	2.108	1	s	
	4-CO	170.69	s	-	0	-	
	Ac	20.61	q	2.133	1	s	
	6-CO	170.73	s	-	0	-	
	Ac	20.63	q	2.070	1	s	
coumaryl	**OCH_2**	67.05	t	4.556	1	dd	14.0, 1.4
				4.440	1	dd	14.0, 1.3
10	**2'**	160.29	s	-	0	-	
2	**3'**	126.28	s	-	0	-	
3	**4'**	139.86	d	7.867	1	br s	
4	**5'**	119.81	s	-	0	-	
5	**6'**	128.99	d	7.721	1	dd	7.7, 1.6
6	**7'**	125.33	d	7.398	1	ddd	7.7, 7.3, 1.0
7	**8'**	132.28	d	7.646	1	ddd	8.3, 7.3, 1.6
8	**9'**	116.80	d	7.418	1	dm	8.3
9	**10'**	153.91	s	-	0	-	

[H] … HSQC readout

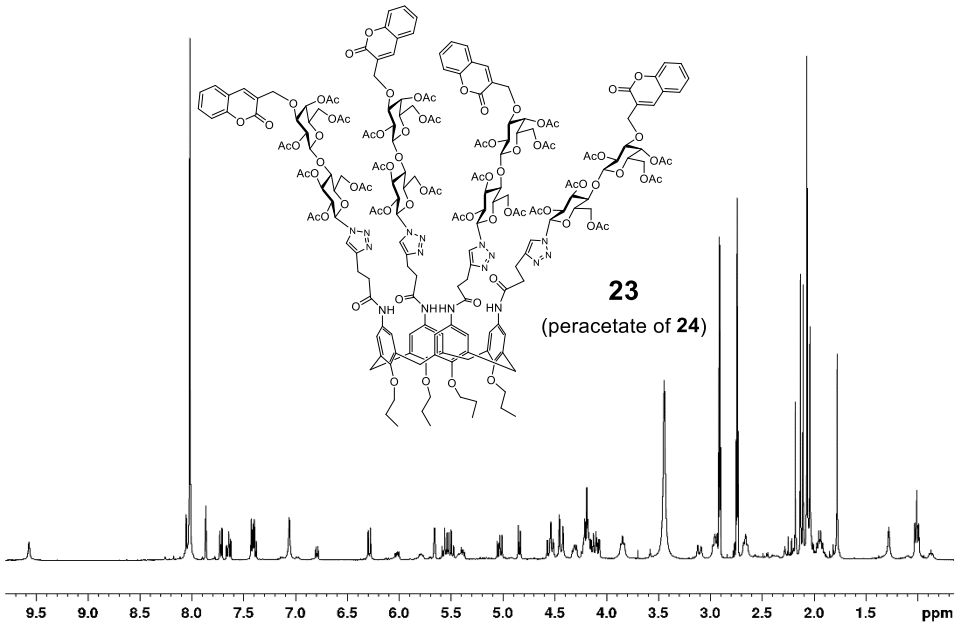


Figure S17 ^{1}H NMR spectrum of compound **23** (peracetate of **24**) (399.87 MHz, DMF-d_7, 30 °C**)**

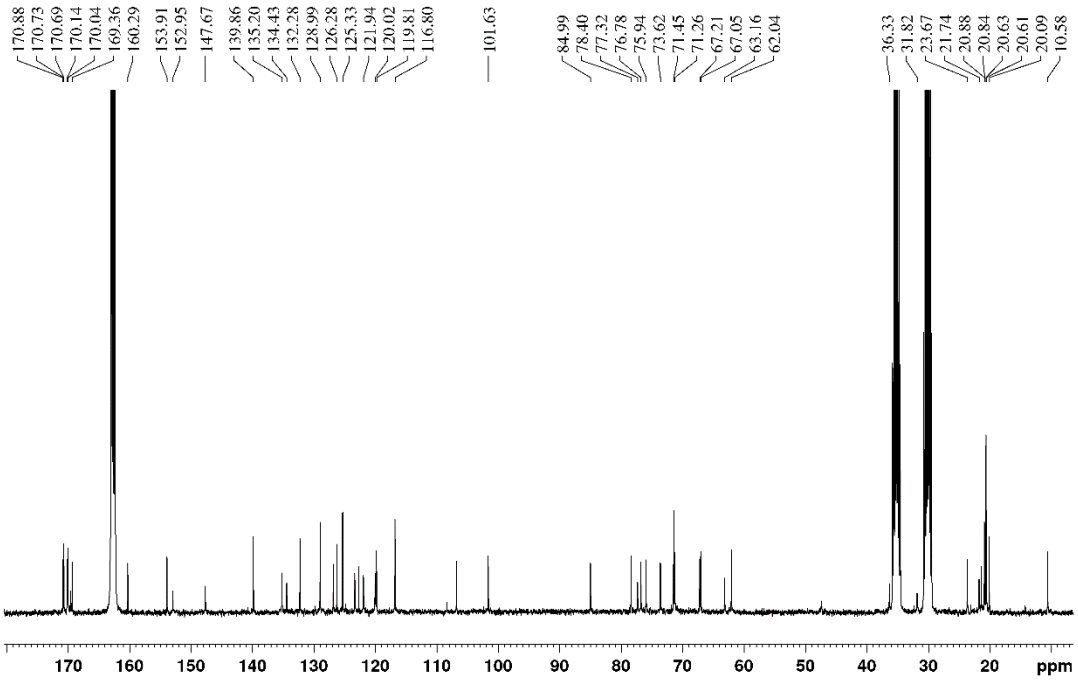
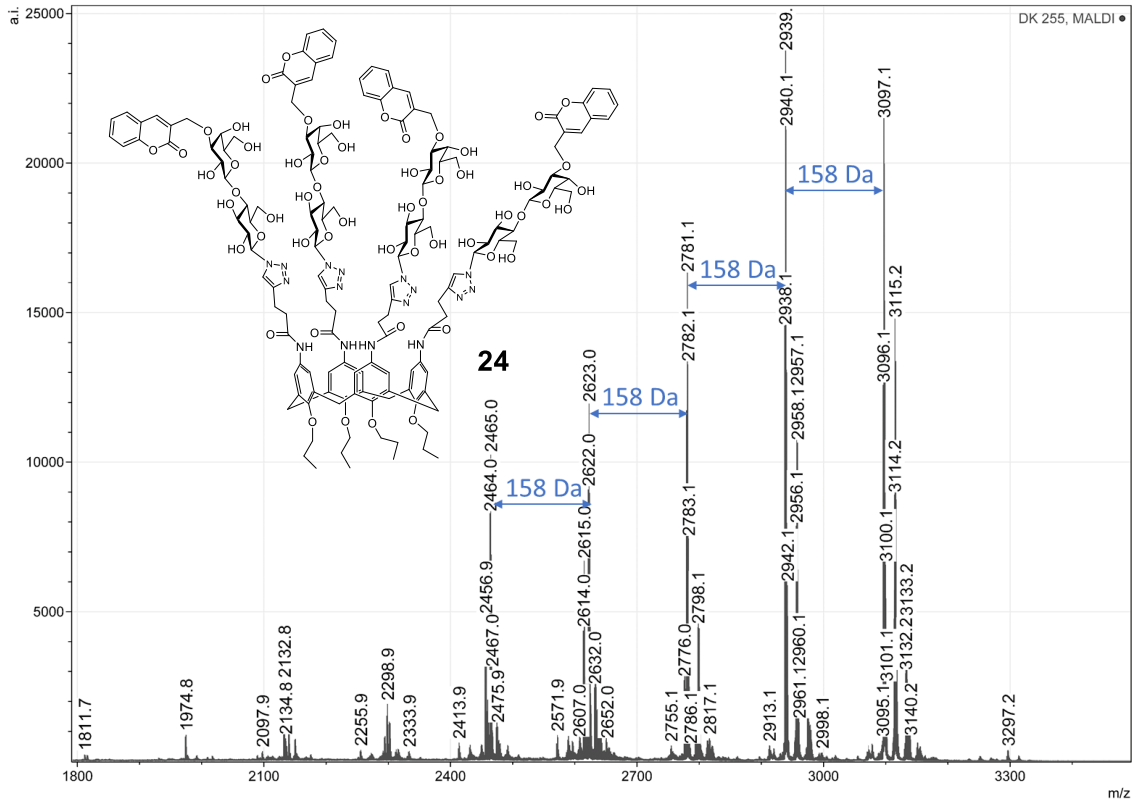


Figure S18. ^{13}C NMR spectrum of compound **23** (peracetate of **24**) (100.55 MHz, DMF-d_7, 30 °C)



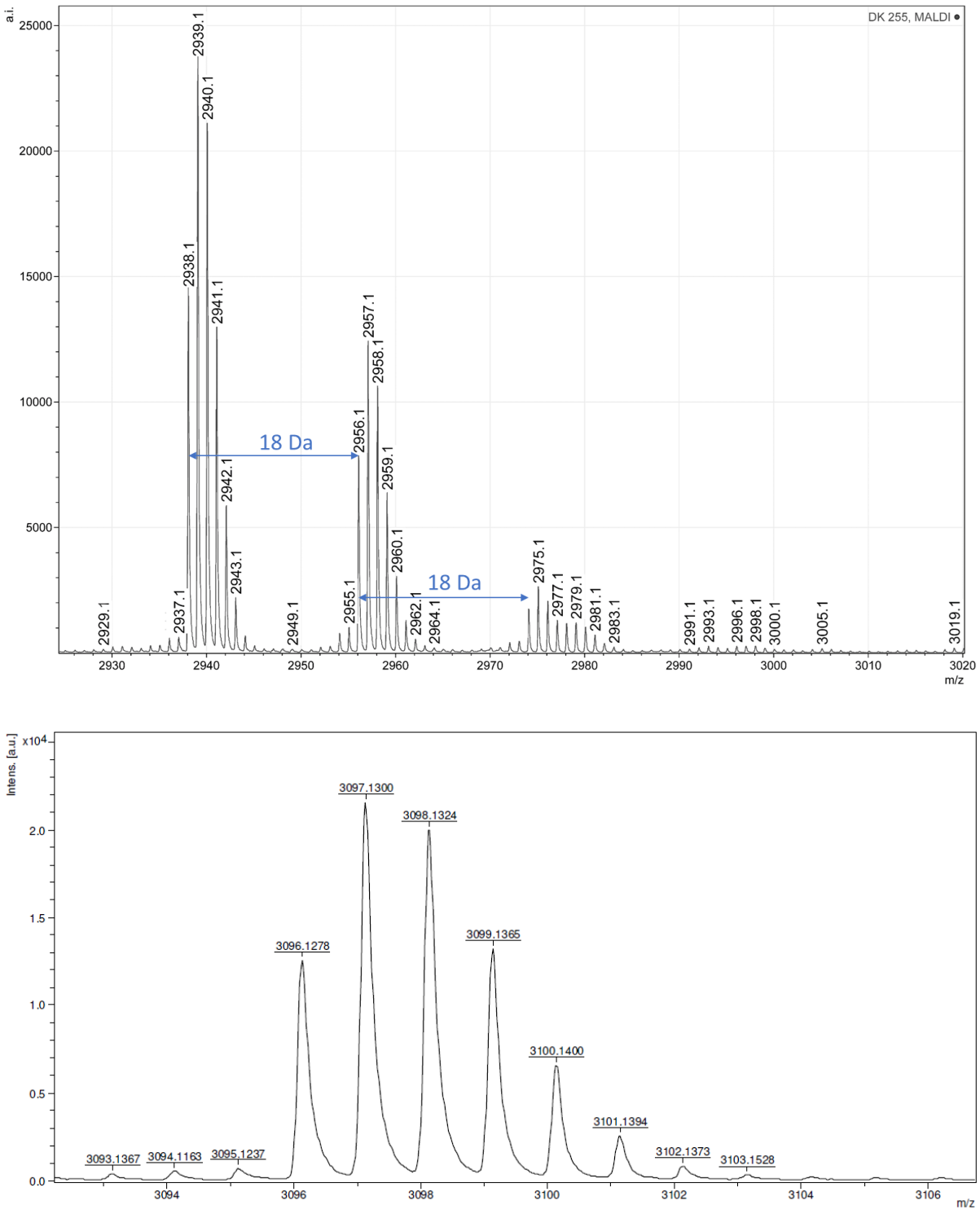


Figure S19. Mass spectrum (MALDI$^+$) of compound **24** (upper figure) and enlarged regions of the same spectrum (middle, lower figures). The upper spectrum shows a gradual loss of up to four *o*-coumaryl units (158 Da) from the sodium adduct $[M + Na]^+$ at *m/z* 3096.1. The middle spectrum shows peaks 18 mass units higher, which are consistent with the presence of *o*-coumaric acid-bearing species. The lower spectrum shows a cluster of sodium adduct isotopic peaks. **HRMS (MALDI$^+$)**: *m/z* for $C_{148}H_{176}N_{16}O_{56}Na^+$ calculated 3096.1308, found, 3096.1278 (-0.96 ppm).

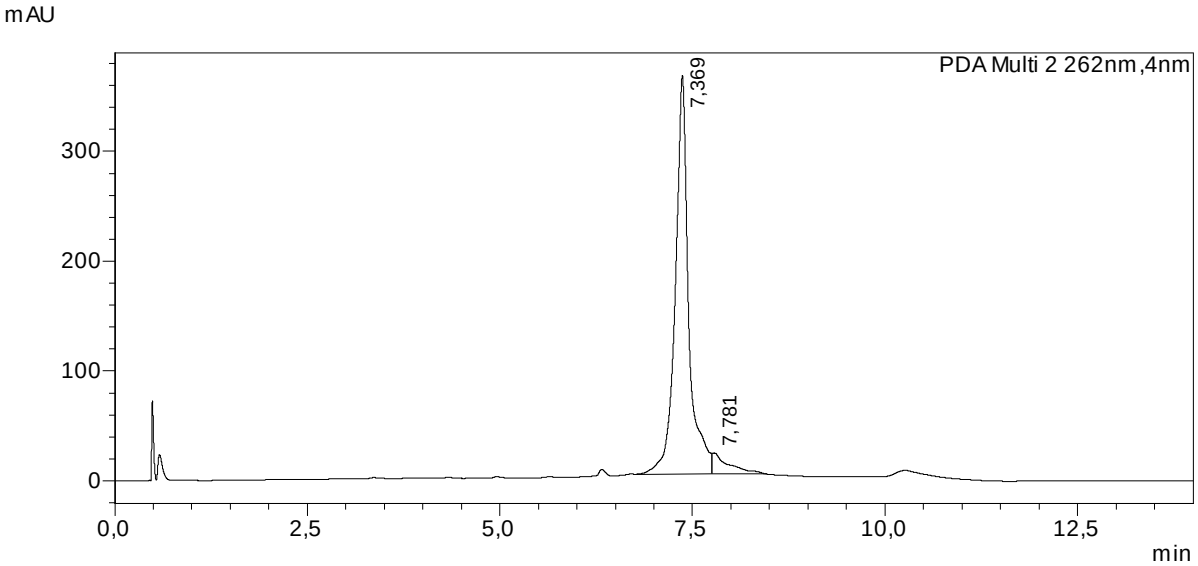


Figure S20. HPLC chromatogram of compound **24** (RT = 7.369, purity 94%). The peak at the beginning of elution belongs to DMSO (solvent).

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-tetrapropoxy-calix[4]arene (26)

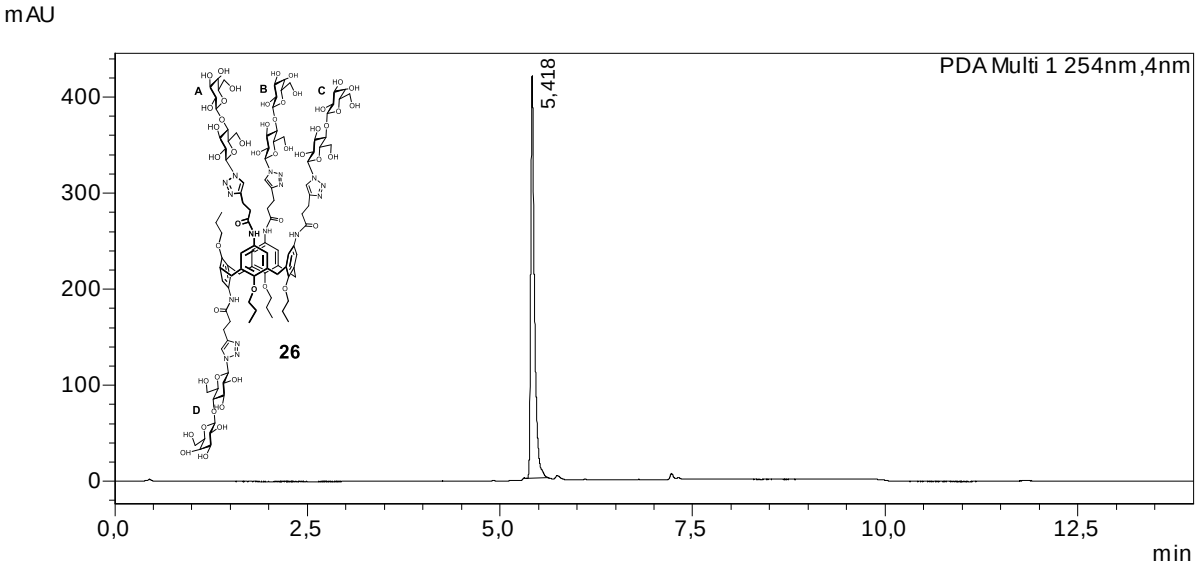


Figure S21. HPLC chromatogram of compound **26** (RT = 5.418 min, purity 99%).

partial cone 5,11,17,23-tetrakis{[3´-*O*-(Coumarylmethyl)-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 (28)

Table S4.^{1}H and ^{13}C NMR data of compound **27** (peracetate of **28**) (600.23 MHz for ^{1}H, 150.93 MHz for ^{13}C, CD_2Cl_2, 20 ºC)

	Atom	δ_C	m.	δ_H	m.	*J*[Hz]
core	***ipso-***	154.38[x], 153.34[x]	s	-	-	
	ortho-, para-	137.42[x], 134.25[x], 133.74[x],132.73[x], 132.59[xa], 131.54[xa]	s	-	-	

	meta-	122.36[x], 121.40[x]	d	7.385, 7.263, 7.163, 7.093, 6.987, 6.290, 6.214	br s	
	-CH_2-	30.79	t	4.01[H] 2.99[H]	m	
	-CH_2-	36.48[x]		3.605	br s	
	1’’	76.72, 76.14, 74.80[x]	t	3.75[H], 3.661, 3.476, 3.263	br m	
	2’’	24.24, 24.10, 22.23	t	1.87[H], 1.82[H], 1.421	br m	
	3’’	11.10, 10.70, 9.93	q	1.057, 1.053, 0.922, 0.680	t	7.4
	NH	-	-	n.e.	s	
	CO	169.52[x], 169.45[x]	s	-	-	
spacer	**1’**	36.91, 36.64[x], 36.08[x]	t	2.80[H], 2.781, 2.592	m	
	2’	21.79, 21.67[x], 21.51[x]	t	3.121, 3.013, 3.00[H]	m	
	3’	147.61, 147.52	s	-	-	
	4’	120.97[x], 120.85[x], 120.59	d	7.66[H], 7.64[H], 7.612	br s	
Glc	**1**	85.84, 85.73, 85.69[x], 85.63[x]	d	5.856, 5.793	br d, d	9.3
	2	70.74[x], 70.66	d	5.478, 5.470[T]	m, dd	9.6, 9.3
	3	73.15[x], 72.94, 72.91	d	5.41[H], 5.386[T]	m, dd	9.6, 9.0
	4	76.01, 75.99[x], 75.89	d	4.02[H], 3.990[T]	m, dd	9.8, 9.0
	5	76.38, 73.33[x]	d	4.00[H], 3.946[T]	m, ddd	9.8, 5.0, 1.7
	6	62.24[H], 62.10	t	4.52[H] 4.14[H]	m m	
Gal	**1**	101.59, 101.55, 101.44	d	4.53[H], 4.506	m, d	8.0
	2	70.88, 70.85	d	5.04[H], 5.039	m, dd	10.0, 8.0
	3	79.25	d	3.71[H], 3.702	m, dd	10.0, 3.4
	4	66.10	d	5.543	d	
	5	71.47, 71.39	d	3.90[H], 3.879	m	
	6	61.62, 61.59, 61.58, 61.56	t	4.16[H]	m	
coumaryl	**OCH_2**	67.50	t	4.586 4.363	br dd dd	13.6, 1.6
	2’	160.60	s	-	-	
	3’	125.74, 125.71	s	-	-	
	4’	139.63, 139.60	d	7.660	br s	
	5’	119.56	s	-	-	
	6’	128.41	d	7.53[H]	m	
	7’	124.90	d	7.30[H]	m	
	8’	131.78	d	7.53[H]	m	
	9’	116.79	d	7.33[H]	m	
	10’	153.75	s	-	-	

x … broad signal; a …tentative assignment; H … HSQC readout; T … 1D-TOCSY readout; n.e. … not extracted; Acetyls resonate in the regions δ_H 2.129-1.823 ppm; δ_C 170.73 - 169.40, 21.06 - 20.42 ppm.

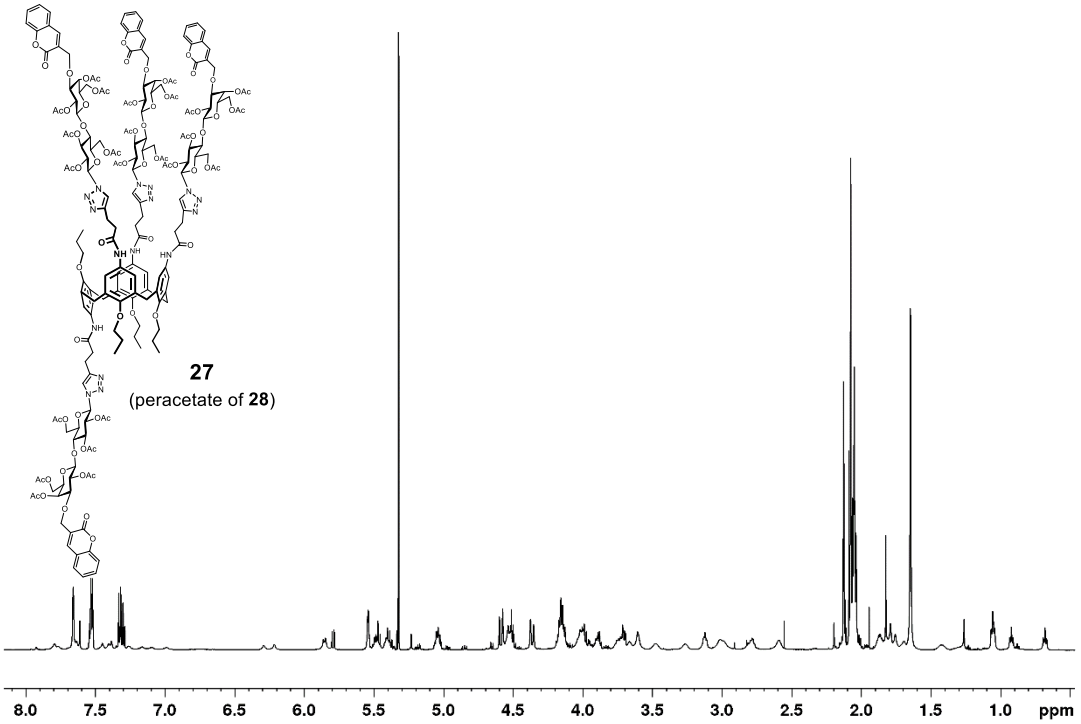
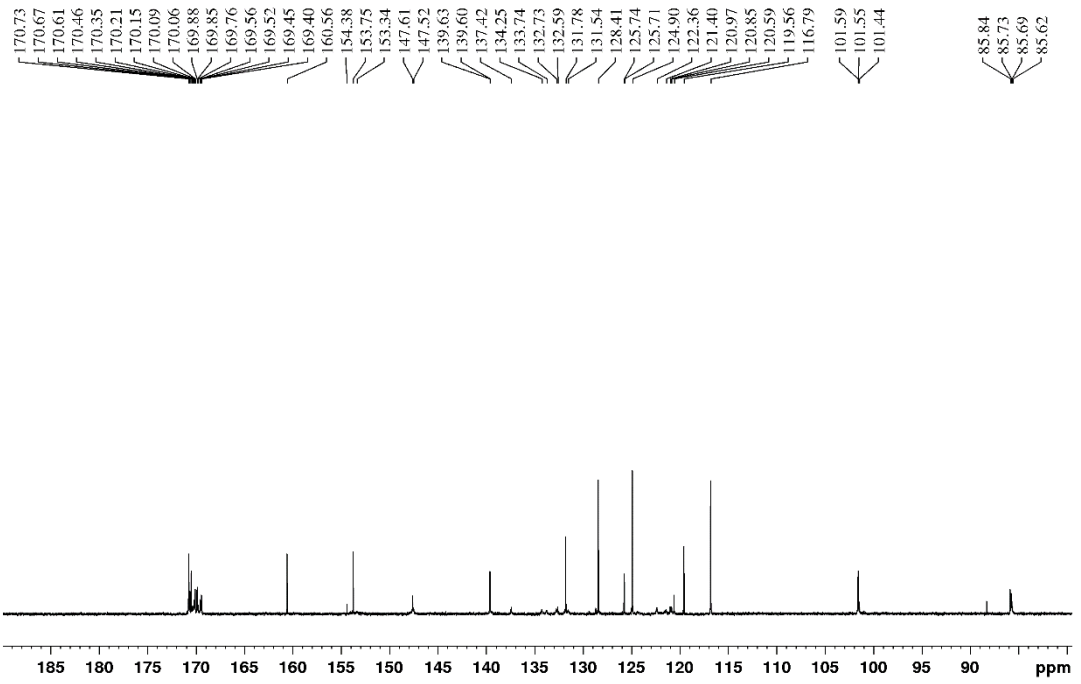


Figure S22.[1]H NMR spectrum of compound **27** (peracetate of **28**) (600.23 MHz for [1]H, CD_2Cl_2, 20 °C).



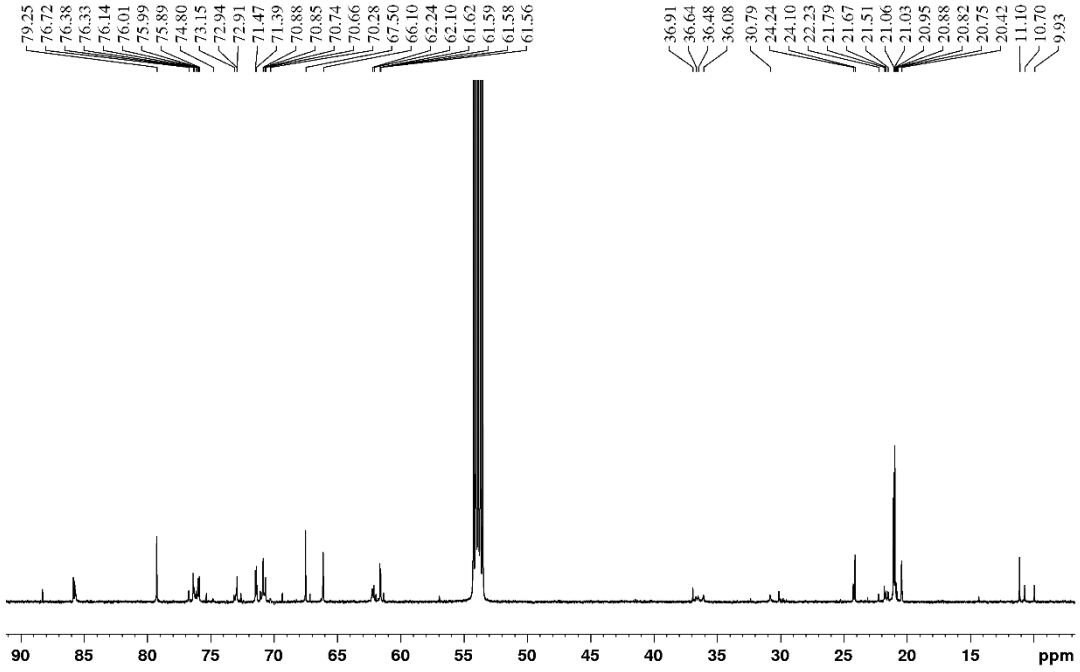
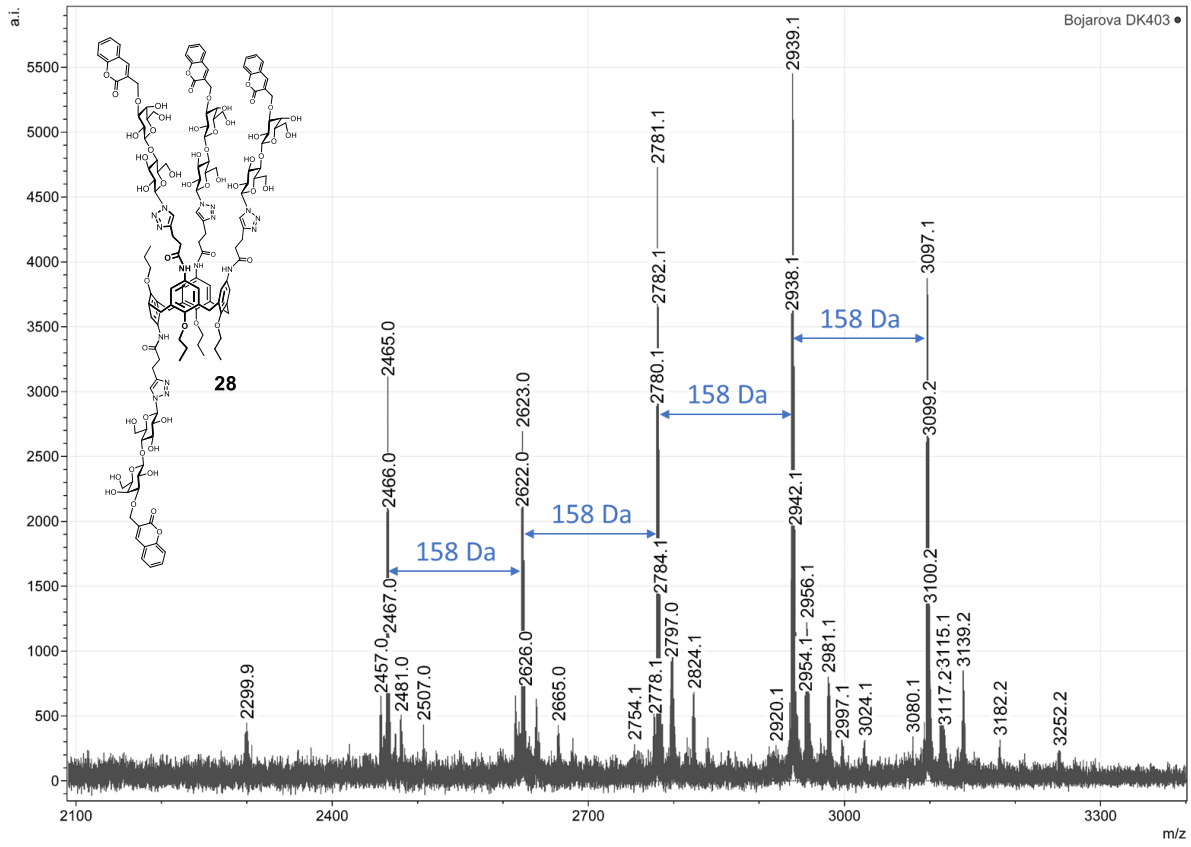


Figure S23. ^{13}C NMR spectrum of compound **27** (peracetate of **28**) (150.93 MHz, CD_2Cl_2, 20 °C).



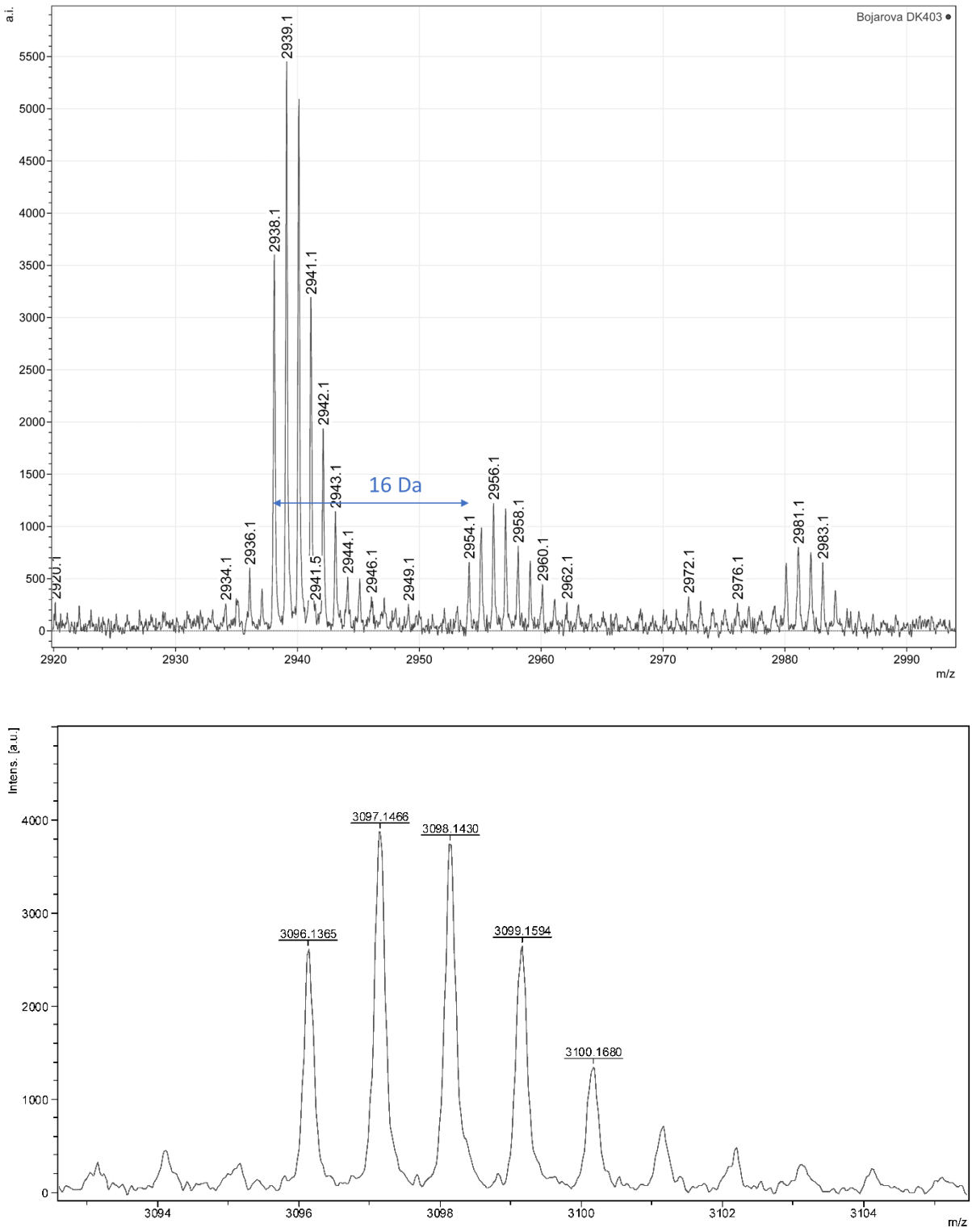


Figure S24. Mass spectrum (MALDI$^+$) of compound **28** (upper figure) and enlarged regions of the same spectrum (middle, lower figures). The upper spectrum shows a gradual loss of up to four *o*-coumaryl units (158 Da) from the sodium adduct $[M + Na]^+$ at *m/z* 3096.1. The middle spectrum shows that sodiated peaks are accompanied with potassiated species (16 mass units of difference between the masses of sodium and potassium). The lower spectrum shows a cluster of sodium adduct isotopic peaks. **HRMS (MALDI$^+$)**: *m/z* for $C_{148}H_{176}N_{16}O_{56}Na^+$ calculated 3096.1308, found, 3096.1365 (1.84 ppm).

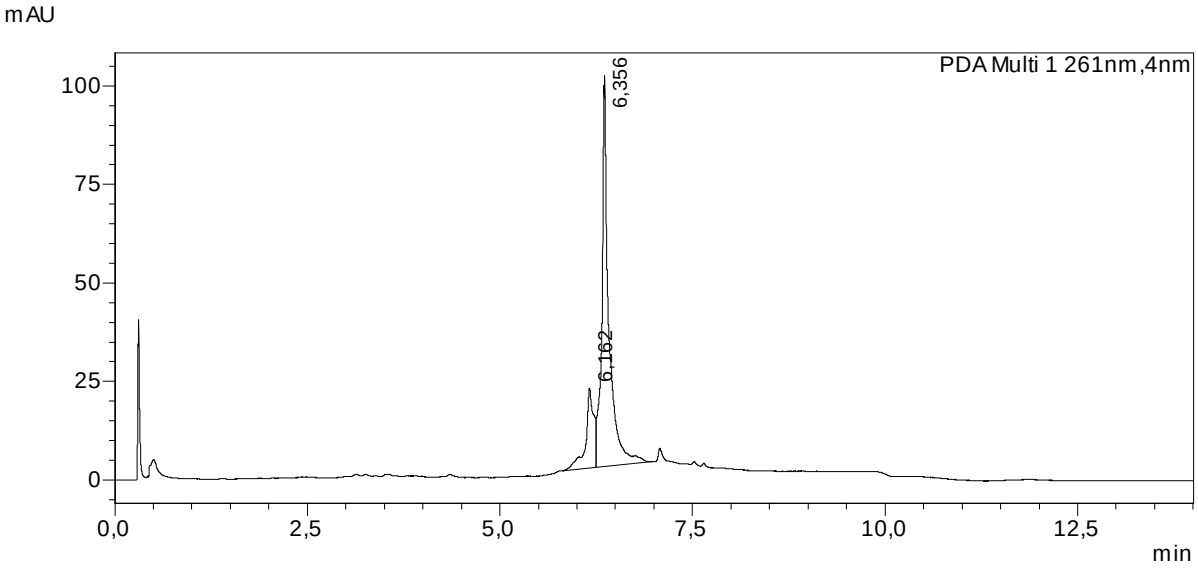


Figure S25. HPLC chromatogram of compound **28** (RT = 6.356 min, purity 81%). The peak at the beginning of elution belongs to DMSO (solvent).

5. BINDING OF PREPARED COMPOUNDS BY GAL-3

5.1. Production of human Gal-3-AVI

The construct of Gal-3 carrying an AVI-tag (Gal-3-AVI) for easy immobilization on the BLI biosensor, cloned into the vector pET-Duet1 (*Nco*I/*Asc*I), was expressed in *E. coli* BL21(λDE3) (Takara Bio, Kusacu, Japan) competent cells as described previously.[11, 12] The *E. coli* strain contained an IPTG-inducible plasmid carrying the gene of biotin ligase *birA* for selective *in vivo* mono-biotinylation of the AVI-tag. The cells were grown in MDO medium (20 g/L yeast extract, 20 g/L glycerol, 1 g/L KH_2PO_4, 3 g/L K_2HPO_4, 2 g/L NH_4Cl, 0.5 g/L Na_2SO_4, 0.01 g/L thiamine hydrochloride) supplemented with ampicillin (150 μg/mL), and chloramphenicol (10 μg/mL) and complemented with 50 μM D-biotin (12 μg/mL) before induction. After induction with 1 mM IPTG at an OD_{600} of 0.6, the cultures were grown for additional 4 h at 37 °C and harvested by centrifugation (8880 × g, 20 min, 4 °C).

For galectin purification, harvested cells were sonicated in equilibration buffer (20 mM phosphate/500 mM NaCl/20 mM imidazole, pH 7.4) for cell disruption (1 min pulse, 2 min pause, 6 repetitions). After centrifugation (20 230 × g, 20 min, 4 °C), the cell-free extract was used to load an equilibrated Ni-NTA column (GE Medical Systems, Prague, Czech Republic). The loaded column was washed with equilibration buffer, and the protein was eluted with an elution buffer containing 500mM imidazole. Fractions were analyzed for protein content using Bradford assay (calibrated for bovine serum albumin),[13] pooled and dialyzed overnight in PBS buffer (phosphate-buffered saline) pH 7.5 (7 L) containing 2 mM EDTA (ethylenediamine tetraacetic acid) followed by 4 h in PBS buffer (7 L). Gal-3-AVI protein was stable at 4 °C for *ca*. two months. Its purity was confirmed by SDS-PAGE and the presence of bound biotin was verified by Western blot as described previously.[11] The usual yield of Gal-3-AVI was *ca*. 3 g of cells per 1 L medium with *ca*. 1.5 mg of pure biotinylated galectin per 1 g cells.

5.2. Biolayer interferometry (BLI)

Measurements of affinity of Gal-3 with monovalent small-molecule compounds lactose, glycomimetic **8**, and tetravalent glycocalix[4]arenes **19**, **24**, **26**, and **28** were performed by BLI under constant conditions (25 ± 0.1 °C, 850 rpm) using interferometry device Octet® Red96e (FortéBio, Fremont, CA, USA). To measure glycocalix[4]arenes, Gal-3-AVI was diluted to a concentration of 2 μg/mL in PBS buffer containing 0.05% Tween-20 and immobilized on the streptavidin biosensor (Octet® SA Biosensors, Sartorius, Goettingen, Germany) *via* biotin-streptavidin interaction up to spectral shift of 0.6 nm. After the immobilization step (150 s), the interactions of the serially diluted (74 nM – 20 μM) glycocalix[4]arenes with Gal-3 were monitored for 500 s during the association and dissociation step. To measure small-molecule compounds, the biosensors were pre-incubated in the solution of Gal-3-AVI (10 μg/mL) in PBS with Tween-20 at 4 °C overnight. Before the association phase, the sensors were quenched with biocytin (10 μg/mL) for 120 s. Then, interactions of serially diluted (25 μM – 3 mM) monovalent ligands lactose or **8** were monitored under the same conditions as above. We verified that the galectin activity was not impaired by the immobilization of the construct on the biosensor. The acquired BLI data were investigated by Octet Analysis software (FortéBio, Fremont, CA, USA). The background of the nonspecific interaction of all ligands (maximum 10% of the total response) and the drift of the sensor itself were double-reference subtracted from the obtained data. The buffer solution before association and during dissociation contained

the same amount of DMSO as in the respective measured samples (0.002 – 0.4 % *v*/*v*). The amount of DMSO did not have a significant effect on the course of the measurement.

The affinity data obtained for all ligands were evaluated by steady-state analysis, in which the shift of the interference pattern was plotted versus ligand concentration according to Equation 1:

$$R_{\mathrm{eq}} = R_{\mathrm{max}} \frac{c}{K_{\mathrm{D}} + c} \tag{1}$$

where R_{eq} stands for the shift of the interference pattern in the equilibrium region of each sensogram curve, R_{max} stands for the maximal response in the equilibrium region, and c is the ligand concentration.

Additionally, the data for (multivalent) glycocalix[4]arenes were globally fitted to Langmuir one-to-one binding model according to Equation 2, presuming that ligand (L) and analyte (A) directly form the final complex (LA):

$$L + A \underset{k_d}{\overset{k_a}{\rightleftharpoons}} LA \tag{2}$$

where k_{a} stands for the association rate constant, and k_{d} stands for the dissociation rate constant. The equilibrium dissociation constant, K_{D}, was determined as the ratio between the dissociation and association rate constants. The relation between the constants is described by Equation 3:

$$K_{\mathrm{D}} = \frac{k_{\mathrm{d}}}{k_{\mathrm{a}}} \tag{3}$$

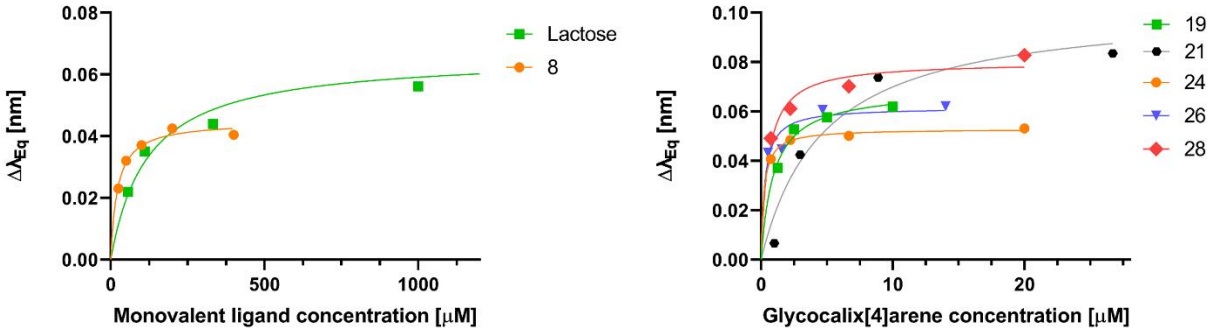


Figure S26. Steady-state analysis of the interaction between Gal-3 and small-molecule carbohydrate ligands lactose (**1**) and glycomimetic **8** (left panel) or glycocalix[4]arene ligands **19**, **21**, **24**, **26**, **28** (right panel) determined by BLI.

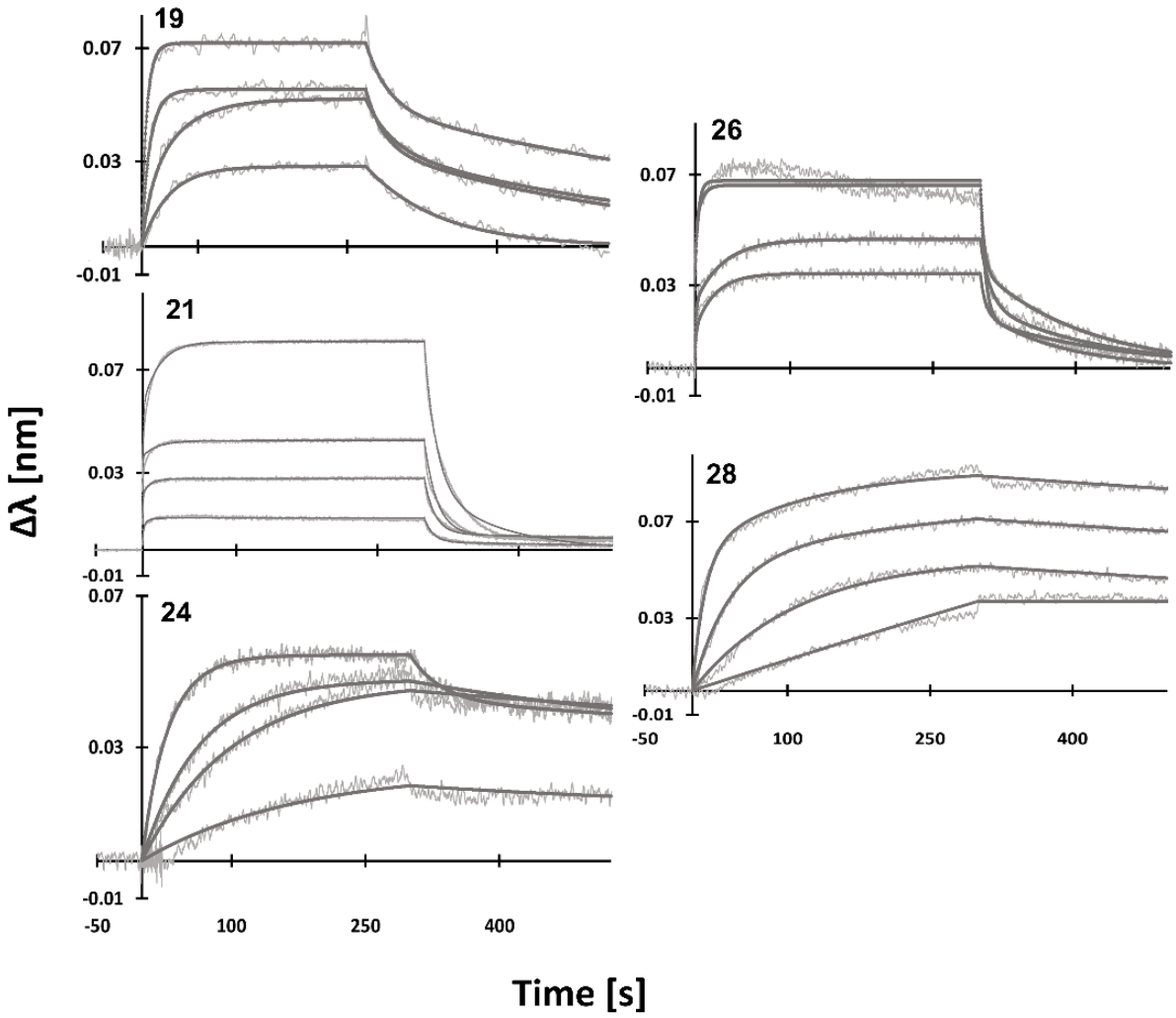


Figure S27. Kinetic analysis of the interaction between Gal-3 and glycocalix[4]arenes **19**, **21**, **24**, **26**, and **28** fitted with Langmuir one-to-one binding model. The data processing included Savitzky-Golay filtering as a part of the manufacturer´s software.

6. BIODISTRIBUTION OF GLYCOCALIX[4]ARENES

6.1. Caco-2 cell culture

The human colon adenocarcinoma cell line (Caco-2) was obtained from American Type Culture Collection (HTB-37™, ATCC, Manassas, VA, USA). The cells were grown as monolayer cultures in Petri dishes, incubated at 37 °C in a humidified atmosphere of 5% CO_2, and maintained in DMEM (supplemented with 10% *v*/*v* fetal bovine serum and 1% *v*/*v* of the antibiotic mixture (penicillin, 100 U/mL and streptomycin, 100 g/mL). Cells were detached for cell counting and subculture with a solution of 0.05% trypsin and 0.02% EDTA. The medium was changed every third day, and cells were passaged at approximately 90% confluence. At passages 30-50, Caco-2 cells were used in the transwell system.

6.2. Caco-2 cell processing

The cells were seeded in polycarbonate membrane inserts (0.4 μm pore diameter, 24well plate, Corning, USA) at 2×10^5 cells/cm^2.[14] The medium was changed every two days. On day 21 after post-seeding, the monolayers of differentiated Caco-2 cells that developed enterocyte characteristics were used for the experiments. Only the monolayers exhibiting a transepithelial flux of phenol red to the basolateral compartment of approximately 10^{-7} cm/s were used for the subsequent experiments.

For the biodistribution and qPCR assays, the differentiated monolayer of Caco-2 cells was washed with PBS and the apical-basolateral direction of transport was tested. The tested compounds (500 μM) were assayed in Hanks' Balanced Salt Solution (HBSS) containing 2% DMSO for 4 hours. The cell viability was not affected under these conditions. All media from the apical and basolateral compartments as well as Caco-2 cells were collected after treatment and stored at -20 °C before further analysis.

To harvest Caco-2 cells, the monolayer was washed with PBS and the cells were detached with trypsin/EDTA solution (0.2 mL). After 2 minutes of incubation at 37 °C, the neutralization solution (1 mL) was added, and the detached cells were transferred to a tube for centrifugation at 150 × g for 5 minutes. The pellet was collected and resuspended in 1 mL of PBS. Caco-2 cells were then separated by centrifugation at 150 × g for 5 minutes.

6.3. Detection of galectin-1 and -3 in Caco-2 cells

Western blot. Differentiated Caco-2 cells were lysed with RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Waltman, MA, USA) according to the manufacturer's instructions. Cells (approximately 6×10^5) were washed with PBS, and 500 μL of cold RIPA buffer was added to the cells. After 5 min on ice and occasional swirling, the lysate was gathered into a tube. The sample was centrifuged at 14,000 × g for 15 minutes to collect cell debris. The supernatant was transferred to a new tube, and the protein content was determined by the Bradford assay (calibrated for bovine serum albumin).[13] The proteins of the cell lysate (20 μg) were separated using SDS-PAGE, and then the galectins were detected by Western blot as described previously.[15] Sequential incubations with primary antibody against human Gal-1 or Gal-3 (D608T or D4I2R, respectively; dilution 1:1000; Cell Signaling Technology, Danvers, MA, USA) and horseradish peroxidase-linked secondary antibody (dilution 1:10,000; Thermo Fisher Scientific, Waltman, MA, USA) were followed by chemiluminescence detection. In contrast to Gal-3 (Figure S28), Gal-1 was not detected by Western blot.

Figure S28. Western blot of Gal-3 in Caco-2 cells. As expected,[16] a Gal-3 band was detected at a size of 28 kDa. Gal-1 was not detected.

qPCR Analysis. Total RNA was isolated from harvested Caco-2 cells using the RNeasy Plus Mini Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. Briefly, the cells were homogenized in RLT Plus lysis buffer (350 µL). Genomic DNA was removed by centrifugation (9000 × g, 30 s) using gDNA eliminator spin column, and after precipitation with ethanol (350 µL), total RNA was bound to RNeasy spin column. After purification, RNA was eluted with H_2O (30 µL). The concentration of total RNA was also measured spectrophotometrically (NanoDrop ONE, Thermo Fisher Scientific, Waltham, USA). The iScript™ cDNA Synthesis Kit (BIO-RAD, Hercules, CA, USA) was used for reverse transcription. RNA (max 1 µg) in a reaction (20 µL) for cDNA synthesis was incubated according to the manufacturer's protocol. Then, the quantitative PCR reaction was performed in the C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA) following the SYBR Green manufacturer's protocol. Technical duplicates were determined for each biological sample. The reaction mixture contained 2 µL of cDNA mixture, 10 µL of the iQ SYBR Green Supermix, and 1 µL of the PrimePCR™ SYBR Green Assay (all Bio-Rad, Hercules, CA, USA; *LGALS1*: qHsaCID0012142, *LGALS3*: qHsaCED0044416, *RPLP0*: qHsaCED0038653, *B2M*: qHsaCID0015347, *GAPDH*: qHsaCED0038674). All results were normalized to reference genes *B2M*, *GAPDH*, and *RPLP0*.[17] Relative expression was quantified using the ΔΔ threshold cycle (*Ct*) method.[18] Equation 4 for using multiple reference genes to calculate relative gene expression was as follows:

$$\frac{{E_{GOI}}^{\Delta Ct\ GOI}}{GeoMean[(E_{REF})^{\Delta Ct\ REF}]} \tag{4}$$

where *E* is the reaction efficiency, Δ*Ct* is the cycle threshold difference, *REF* is the reference gene, and *GOI* is the gene of interest. *E* was set to 2 because we used qPCR assays developed by the manufacturer, who guarantees the same *E* for all.

Under control conditions, the expression of the gene for Gal-1 (*LGALS1*) was 550-fold lower than that of the gene for Gal-3 (*LGALS3*). This result is consistent with the detection of galectins by Western blot, which also failed to detect Gal-1 due to its negligible expression in Caco-2 cells. Incubation of Caco-2 cells with compounds **11** (unsubstituted *cone* core) or **19** (tetralactosyl *cone* core) did not affect the expression of *LGALS3*, whereas incubation with compound **24** (tetracoumaryl *cone* core) resulted in a 74% decrease in *LGALS3* expression compared with the control of medium only ($P < 0.05$, ANOVA with Dunnett's test). The solvent (DMSO) had no effect on the course of the measurement.

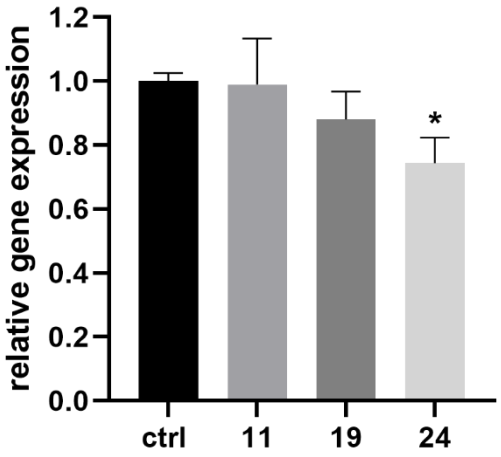


Figure S29. Altered expression of the *LGALS3* gene encoding for Gal-3 in Caco-2 cells in the presence of glycocalix[4]arenes. Gene expression in the control experiment (medium only) was set to 1.0. Data are presented as a mean ± SD of two independent biological experiments. * $P < 0.05$ (ANOVA with Dunnett's *post-hoc* test).

6.4. Biodistribution of glycocalix[4]arenes

Before HPLC analysis (Section 2.3), acetonitrile (30 µL) was added to samples from apical or basolateral medium (30 µL). Any precipitated proteins and salts were separated by centrifugation at 14 000 × g for 2 minutes. The supernatant (10 µL) was then used for HPLC analysis (respective methods are specified in Section 2.3). For samples from Caco-2 cells, 5% acetonitrile (50 µL) was added to the cells after the supernatant was removed. The cells were then sonicated (sonicator UP50H, Hielscher, Germany, 4 cycles (10 s, 20 s beaks between the cycles, 80% amplitude)). The sonicated cells were centrifuged (14 000 × g, 20 min), the supernatant was boiled (3 min), centrifuged again (14 000 × g, 10 min), and used for HPLC analysis as above – column "cytosol". DMSO (50 µL) was added to the pellet, vortexed, boiled (3 min), centrifuged again (14 000 × g, 10 min), and used for HPLC analysis as above – column "membrane". The PDA data were acquired in the 200-450 nm range and the signal at the absorption maximum of each compound was detected. The peak identification was performed by comparing the retention time and the UV spectrum of the compounds with the respective standards, whose characterization is given in Section 3. The peak purity was confirmed using the PDA detector scanning software. After recalculating the performed dilutions, the percentage distribution of the substance in media and cells was calculated according to Equation 5:

$$\%\ of\ distribution = 100 * \frac{peak\ area\ of\ compound\ in\ medium\ or\ cells}{\sum of\ peak\ areas\ in\ apical\ and\ basolateral\ media\ and\ cells} \quad (5)$$

Table S5. Biodistribution of calix[4]arene cores, monovalent carbohydrate ligands and glycocalix[4]arenes among apical, basolateral, and cellular compartments of Caco-2 cells.

Compound	Apical side	Basolateral side	Caco-2 cells		
			In total	Cytosol[a]	Membrane[a]
1 (lactose)	72 ± 3	9 ± 1	19 ± 4	19 ± 4	0
8 (coumaryl)	96 ± 1	4 ± 1	0	0	0
11 (*cone* core)	5.1 ± 0.5	0	94.9 ± 0.5	0.18 ± 0.05	94.8 ± 0.5
12 (*partial cone* core)	0.06 ± 0.02	0	99.94 ± 0.02	0.14 ± 0.02	99.80 ± 0.04
17 (trivalent core)	0.03 ± 0.02	0	99.96 ± 0.02	0.3 ± 0.3	99.6 ± 0.4

19 (tetralactosyl *cone*)	86 ± 7	0	14 ± 7	1.1 ± 0.8	13 ± 7
21 (trilactosyl *cone*)	84 ± 6	0	16 ± 6	8 ± 3	8 ± 4
24 (tetracoumaryl *cone*)	31 ± 5	0	69 ± 5****	0.08 ± 0.05	69 ± 5
26 (tetralactosyl *partial cone*)	83 ± 5	0.21 ± 0.06	16 ± 5	9 ± 6	8 ± 2
28 (tetracoumaryl *partial cone*)	0.06 ± 0.04	0	99.91 ± 0.05****	0.3 ± 0.1	99.6 ± 0.2

Data are expressed as a mean ± SD of three parallel experiments. **** $P < 0.0001$, tetracoumaryl *vs*. tetralactosyl glycocalixarene in the same conformation (ANOVA with Tukey post-test); [a] the sum of the compound in cytosolic and membrane fractions gives ist total content in Caco-2 cells.

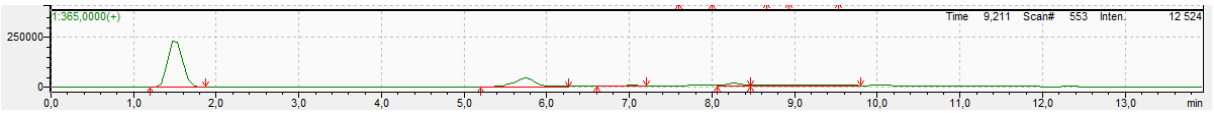


Figure S30. Mass spectrum (ESI$^+$) of lactose in apical medium (RT = 1.401 min).

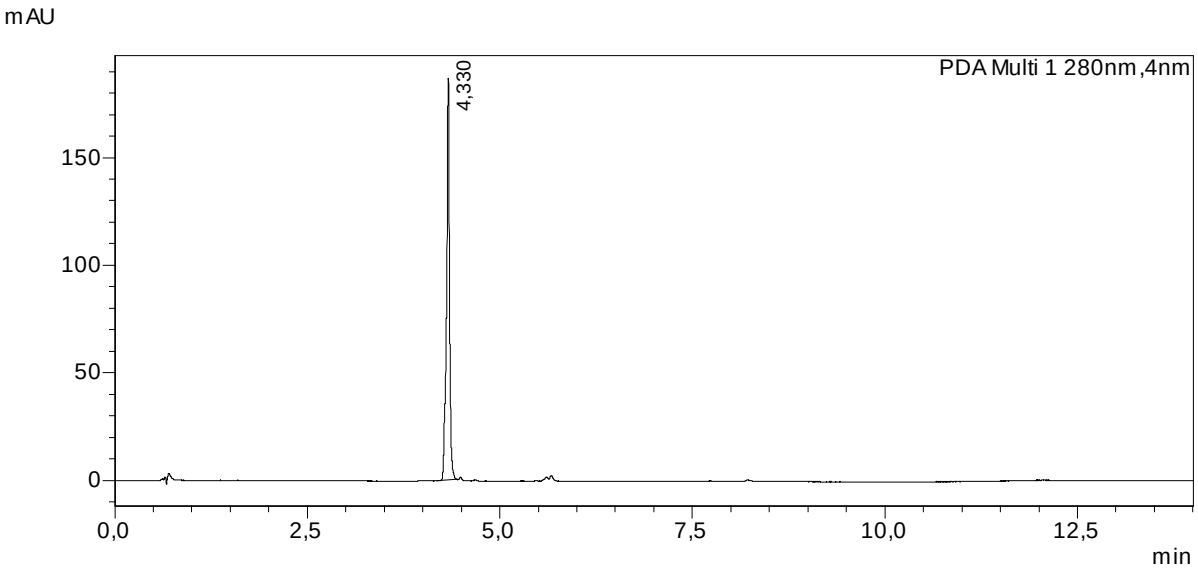


Figure S31. HPLC chromatogram of compound **8** (monovalent coumaryl glycomimetic) in the apical medium.

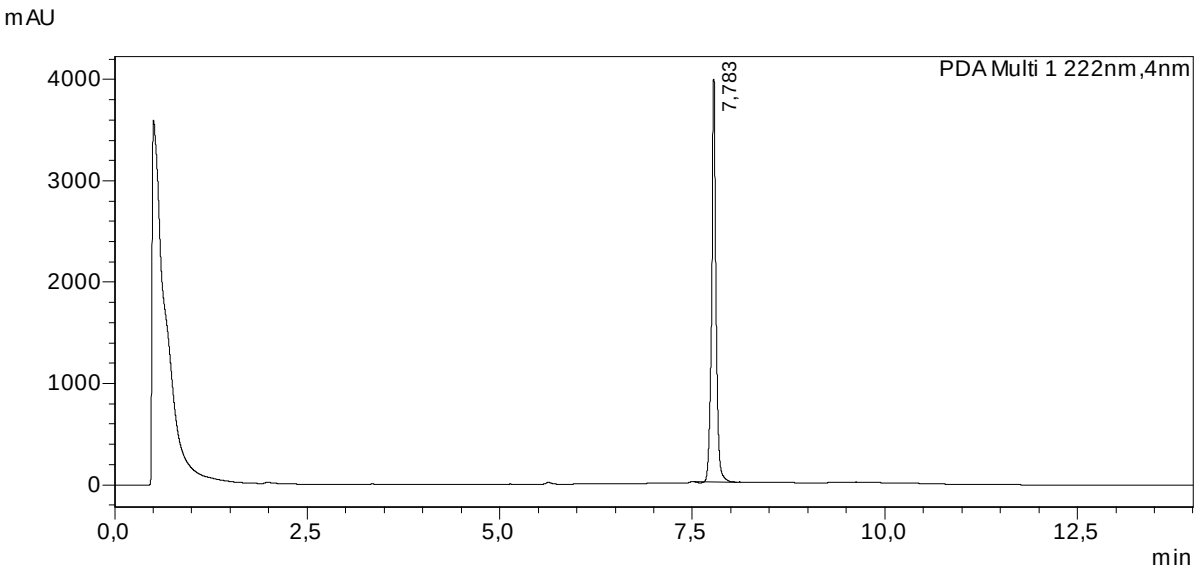


Figure S32. HPLC chromatogram of compound **11** (unsubstituted *cone* core) in Caco-2-cells. The peak at the beginning of the chromatogram belongs to dimethylsulfoxide, which was used as a solvent.

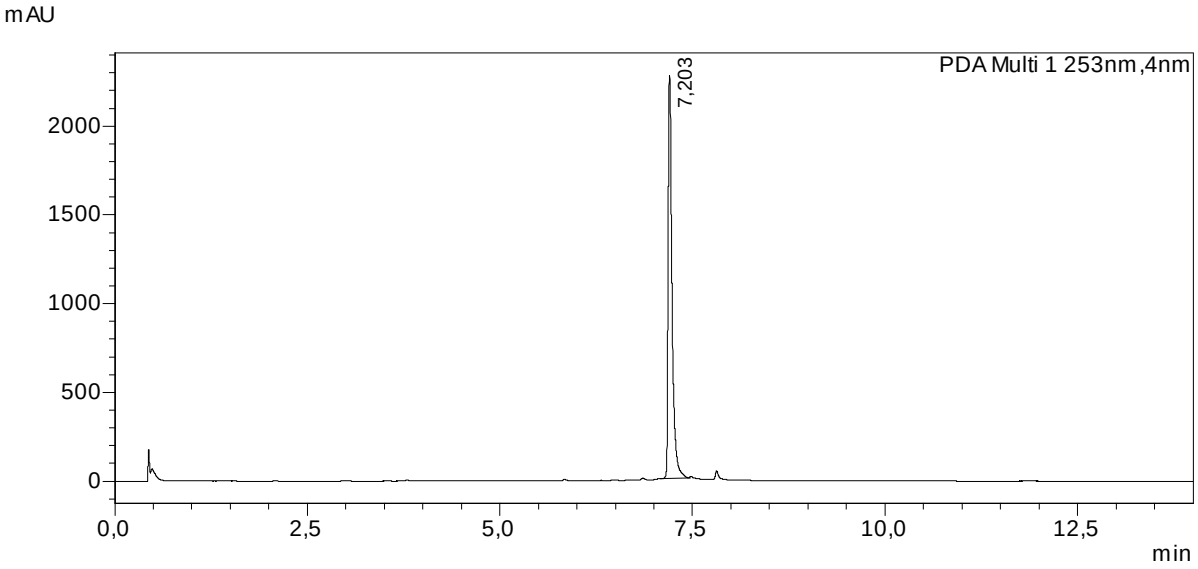


Figure S33. HPLC chromatogram of compound **12** (unsubstituted *partial cone* core) in Caco-2-cells.

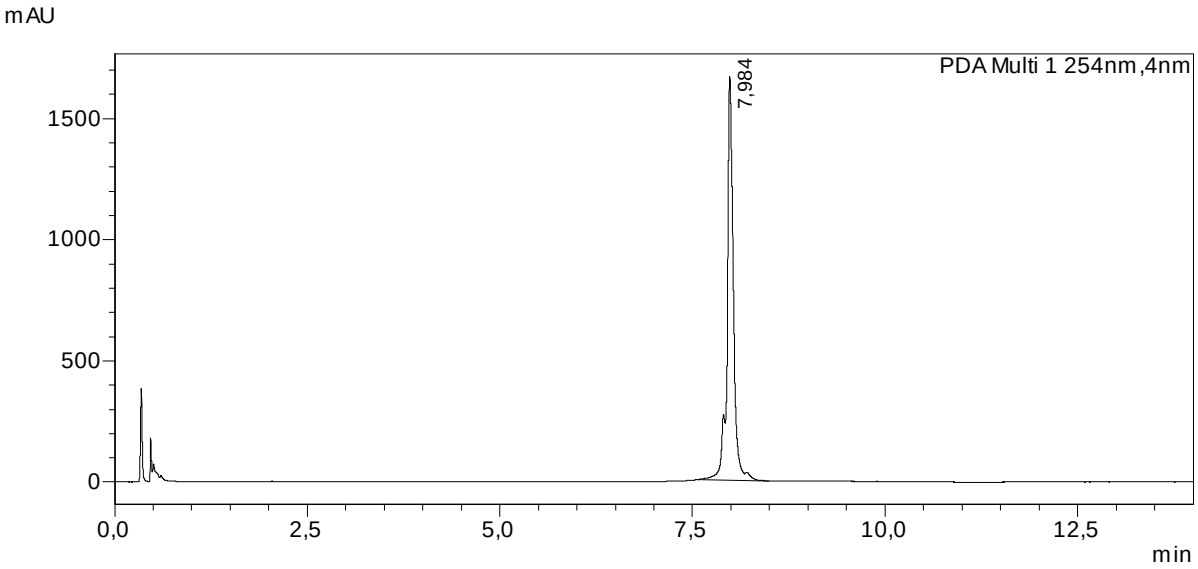


Figure S34. HPLC chromatogram of compound **17** (unsubstituted trivalent *cone* core) in Caco-2-cells.

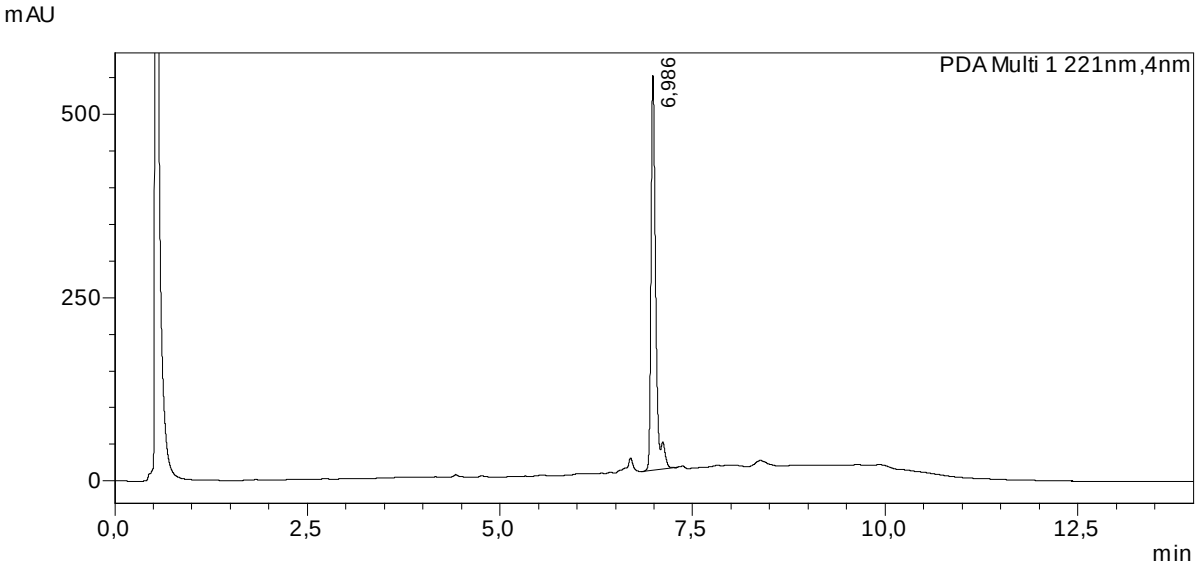


Figure S35. HPLC chromatogram of compound **19** (tetralactosyl *cone*) in apical medium. The peak at the beginning of the chromatogram belongs to dimethylsulfoxide, which was used as a co-solvent.

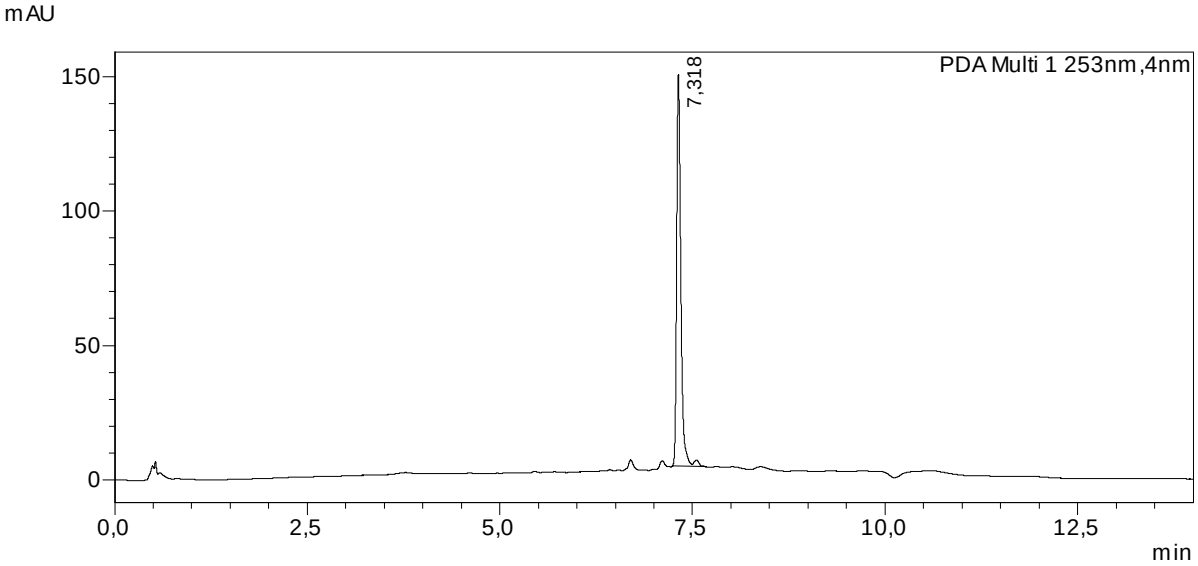


Figure S36. HPLC chromatogram of **21** (trilactosyl *cone*) in apical medium.

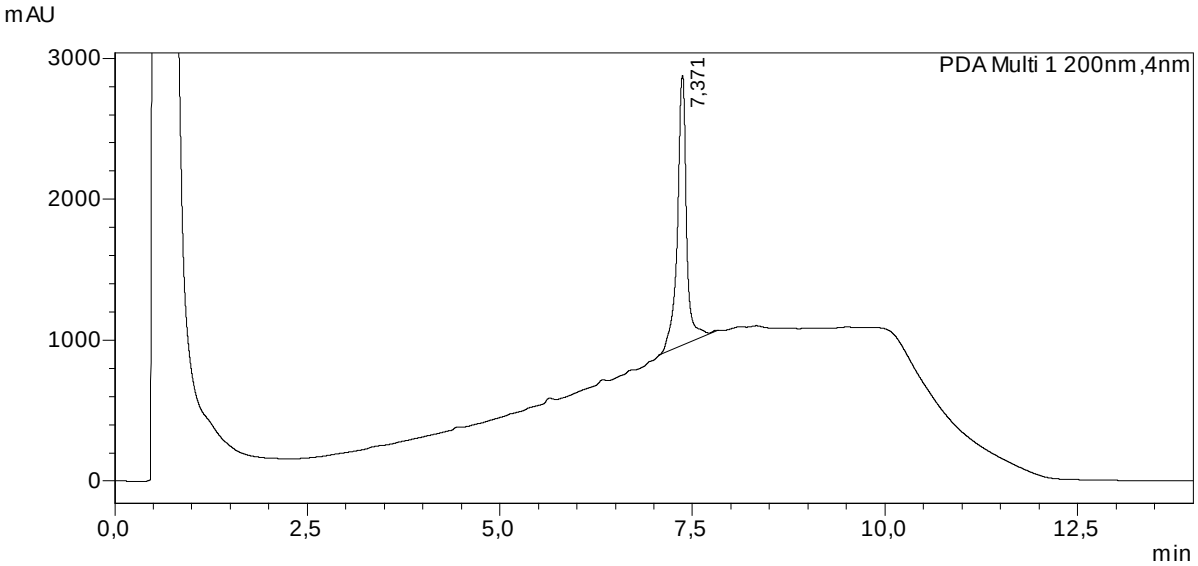


Figure S37. HPLC chromatogram of compound **24** (tetracoumaryl *cone*) in Caco-2-cells. The peak at the beginning of the chromatogram belongs to dimethylsulfoxide, which was used as a co-solvent.

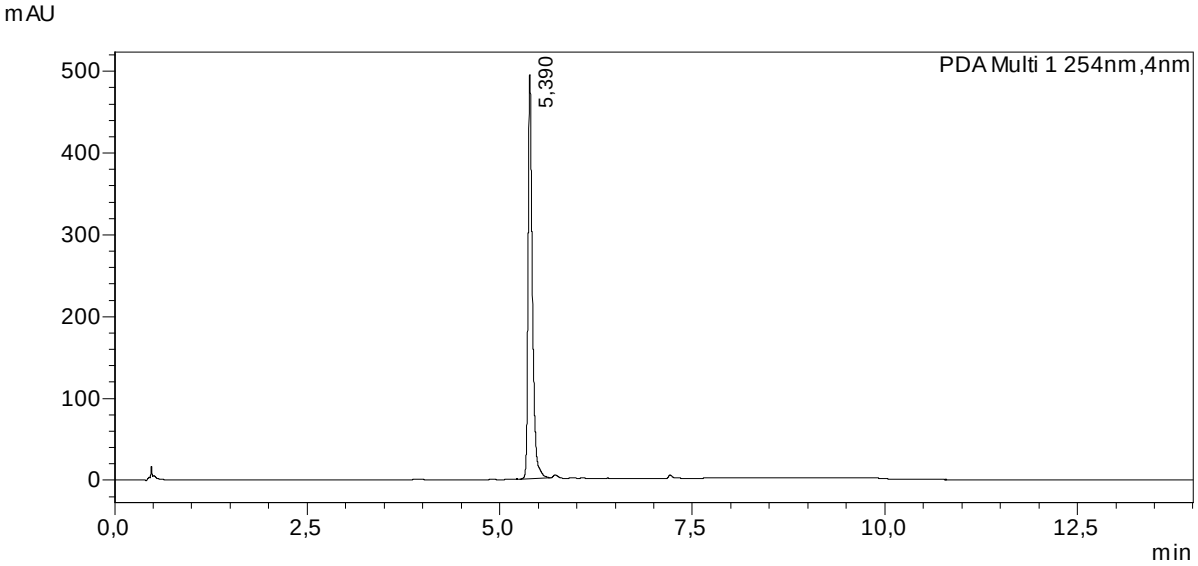


Figure S38. HPLC chromatogram of compound **26** (tetralactosyl *partial cone*) in the apical medium.

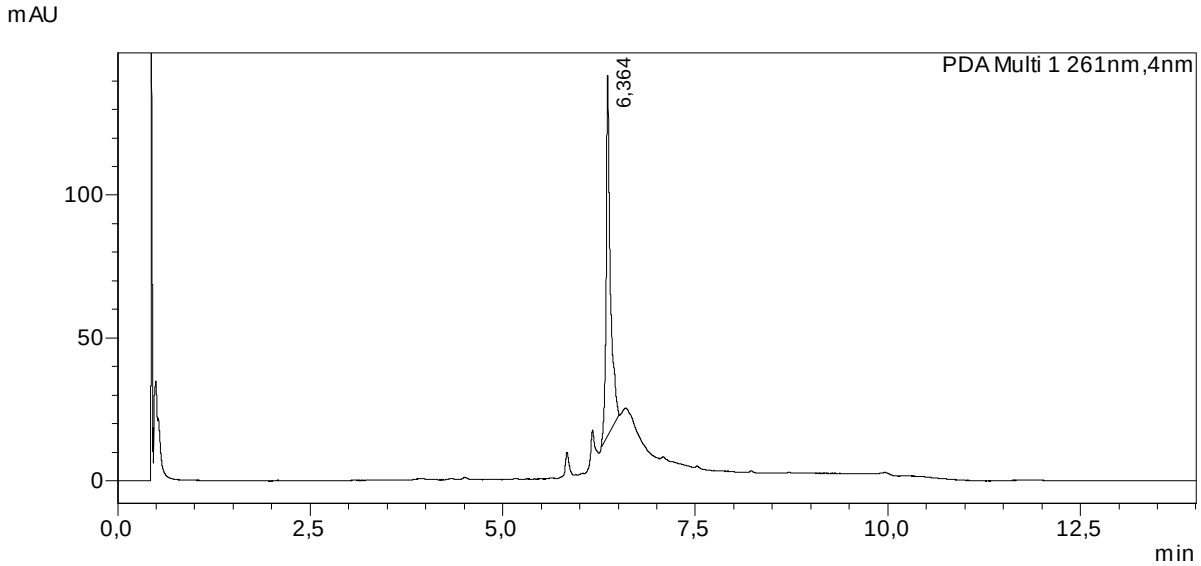


Figure S39. HPLC chromatogram of compound **28** (tetracoumaryl *partial cone*) in Caco-2-cells. The peak at the beginning of the chromatogram belongs to dimethylsulfoxide, which was used as a co-solvent.

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