Pentavalent pillar[5]arene-based glycoclusters and their multivalent binding to pathogenic bacterial lectins

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Contents

General procedures

All reagents for synthesis commercially available (highest purity available for reagent grade compounds) were used without further purification. Solvents were distilled over CaH_2 (CH_2Cl_2), Mg/I_2 (MeOH), Na/benzophenone (THF) or purchased dry. Reactions under microwave activation were performed on a Biotage Initiator system. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F_{254} (Merck). TLC plates were inspected by UV light (λ = 254 nm, 365 nm) and developed by treatment with a mixture of 10% H_2SO_4 in $EtOH/H_2O$ (95:5 v/v) followed by heating. Silica gel column chromatography was performed with silica gel Si 60 (40–63 μm). Optical rotation was measured using a Perkin Elmer polarimeter. NMR spectra were recorded at 293 K, unless stated otherwise. Chemical shifts are referenced relative to deuterated solvent residual peaks. The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; p, pseudo and bs, broad singlet. Complete signal assignments were based on 1D and 2D NMR correlations COSY and HSQC. High resolution (HR-ESI-QToF) mass spectra were recorded using a Bruker MicroToF-Q II XL spectrometer and MALDI-ToF-LD+ were recorded using a Waters QToF1 spectrometer. Infrared spectra were recorded using a Perkin Elmer RX 1 FT-IR spectrometer.

General procedure for 1,3-dipolar cycloadditions (Method A)

The alkyne-functionalized compound (1 eq.), CuI (0.5 eq.), DIPEA (1 eq. more than valency) and azido- or alkyl-derivative (1 eq. more than valency) in DMF were introduced into a Biotage Initiator 2-5 mL vial. The vial was sealed with a septum cap and heated at 110°C for 15 min under microwave irradiation (solvent absorption level : High). The crude mixture was concentrated and co-evaporated with toluene 6 times then purified by silica gel flash column chromatography to afford the desired cycloadducts.

General procedure for 1,3-dipolar cycloadditions (Method B)

The alkyne-functionalized compound (1 eq.), $CuSO_4$ (1 eq. per alkyne), sodium ascorbate (2 eq. per alkyne) and azido- or alkyl-derivative (1 eq. more than valency) in DMF were introduced into a Biotage Initiator 2-5 mL vial. The vial was sealed with a septum cap and heated at 110°C for 15 min under microwave irradiation (solvent absorption level : High). The crude mixture was concentrated and co-evaporated with toluene 6 times then purified by silica gel flash column chromatography to afford the desired cycloadducts.

General procedure for the Zemplén deacetylation (Method C)

To a suspension of acetylated glycoside or glycocluster (1 eq.) in distilled MeOH, was added MeONa (0.2 eq.). The mixture was stirred at r.t. for 16 h, neutralized with Amberlite IR 120 resin (H^+ form), filtrated and concentrated to afford the corresponding deprotected glycocluster.

General procedure for cyclisation of pillar[5]arene (Method D)

A suspension of corresponding monomer (10 mmol, 1 eq.) and paraformaldehyde (2 eq.) in 1,2-dichloroethane (30 mL) was placed at room temperature under argon. Boron trifluoride diethyl etherate (1 eq.) was added over 30 min and the reaction was stirred under argon for 3 h. The mixture became black and the reaction was followed by TLC. After addition of water (30 mL), the mixture was then concentrated to afford a black residue which was directly purified by silica gel flash chromatography (40 to 90% CH_2Cl_2 in *n*-hexane) to give the target product.

Warning about sodium azide (NaN_3)

Sodium azide, when inhaled, is highly toxic and may cause *death* (MSDS J.T. Baker). Precautions must be taken when weighing the material such as using a powder mask and a *teflon spatula* (metallic spatula may cause explosion). Azidation reactions were performed behind a plastic shield due to potential explosion. DMF is used as a polar solvent favoring the reaction but also to maintain a slightly basic pH (> 8) of the solution. With acidic pH *hydrazoic acid* (HN_3) may be formed, which may explode and/or, when inhaled, may cause intoxication, damage of the central nervous system and blood pressure effects.

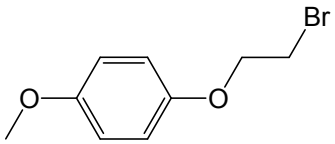
Hemagglutination Inhibition Assays (HIA). *Determination of lectin concentration:* Hemagglutination inhibition assays (HIA) were performed in U-shaped 96-well microtitre plates. Rabbit erythrocytes were purchased from Biomérieux and used without further washing. Erythrocytes were diluted to a 4% solution in NaCl (150 mM). Lectin solutions of 2 mg/mL were prepared in TRIS-HCl 20 mM, NaCl 100 mM and $CaCl_2$ 100 µM. The hemagglutination unit (HU) was first obtained by the addition of 25 µL of the 4% erythrocyte solution to 25 µL aliquots of sequential (two-fold) lectin dilutions. The mixture was incubated at 25°C for 60 minutes. The HU was measured as the minimum lectin concentration required for hemagglutination. Lectin concentrations of 4 HU were used for the lectin inhibition assays. For LecA, this concentration was found to be 8 µg/mL. *Determination of minimum inhibitory concentration (MIC):* Subsequent inhibition assays were then carried out by the addition of 12.5 µL lectin solution (at the required concentration) to 25 µL of sequential dilutions of glycoclusters, monomer molecules and controls. These solutions were then incubated at 25°C for 2 h then 12.5 µL of 4% erythrocyte solution was added followed by an additional incubation at 25°C for 30 minutes. The minimum inhibitory concentration (MIC) for each molecule was determined for each duplicate.

Enzyme-Linked Lectin Assays (ELLA). *Determination of lectin concentration:* 96-Well microtitre plates (Nunc Maxisorb) were coated with α-PAA-Gal for LecA: 100 µL of 5 µg/mL in carbonate buffer, pH 9.6 for 1h at 37°C then blocking at 37°C for 1h with 100 µL per well of 3% (w/v) BSA in PBS. Lectin solution (100 µL) was diluted (1/2) starting from 30 µg/mL. After 1h incubation at 37°C and 3 washings with T-PBS (PBS containing 0,05% Tween 20), 100 µL of horseradish streptavidin–peroxidase (HRP) conjugate (dilution 2:5000; Boehringer-Mannheim) was added and left for 1h at 37°C. Colouration was developed using 100 µL per well of 0.05 M phosphate/citrate buffer containing o-phenylenediamine dihydrochloride (0.4 mg/mL) and urea hydrogen peroxide (0.4 mg/mL) (OPD kit, Sigma-Aldrich) for 15 minutes and stopped with 50 µL of 30% sulfuric acid. Absorbance at 490 nm was then read using a microtiter plate reader (BioRad 680). Biotinylated lectins concentration were determined by plotting the relative absorbance versus lectin concentration. The concentration which leads to the highest response in the linear area was selected as the standard lectin concentration for the subsequent inhibition experiments. *Determination of inhibition potency (IC_{50}):* ELLAs were conducted in the same conditions as above. Inhibitor solutions (50 µL) were submitted to serial dilutions (1/3) with PBS-BSA 0.3% (w/v). Then, 50 µL of biotinylated LecA solution (0.12 µg/mL) was added in each well and the plates were incubated for 1h at 37°C. Plots of inhibition percentage versus inhibitor concentration and sigmoidal fitting provided IC_{50} determination.

Surface plasmon resonance. The studies were conducted using a Biacore X100 instrument (GE Healthcare) at 25°C with a CM5 sensor chip. A continuous flow of HEPES buffer (10 mM HEPES, 150 mM NaCl, 100 µM $CaCl_2$, twin 20 0.05 %, pH 7.5) was maintained over the sensor surface at a flow rate of 10 $\mu L.min^{-1}$. The CM5 sensor chip was activated with an injection of a solution containing *N*-ethyl-*N*'-(3-diethyl-aminopropyl)-carbodiimide (EDC) (0.2 M) and N-hydroxysuccinimide (NHS) (0.05 M) for 7 minutes. LecA (100 $\mu g.mL^{-1}$) in

NaOAc buffer (pH 4.2) was injected over the activated flow cell at flow rate of 10 μL.min^{-1} for 10 minutes to achieve a ~1000 RU. The same procedure was applied for BambL achieving 200 RU capture The immobilization procedure was completed by an injection of ethanolamine hydrochloride (1 M) (70 μL), followed by a flow of the buffer (100 μL.min^{-1}) in order to eliminate physically adsorbed compounds. Ethanolamine alone was used in channel 1 as a reference. For the LecA chip, Compounds **5a** and **9** were injected using the single-cycle mode with 5 concentrations varying from 0.5 nm to 5 μM concentration. By the end of measures the sensor chip was regenerated with 100 seconds pulse of methyl β-D-galactopyranoside (100 mM) followed by an injection of running buffer for 300 seconds.

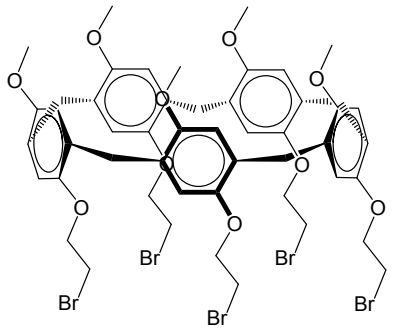
Microcalorimetry. Recombinant lyophilized lectin was dissolved in buffer (**LecA**: 20 mM TRIS-HCl, 100 μM $CaCl_2$, pH 7.5, NaCl 100 mM; **LecB**: 20 mM TRIS-HCl, 100 μM $CaCl_2$, pH 7.5, NaCl 100 mM; **BambL**: 20 mM TRIS-HCl, pH 7.5, NaCl 150 mM) and degassed. The protein concentration was checked by measuring A_{280} by using a theoretical molar extinction coefficient (**LecA**: 28000, **LecB**: 6990, **BambL**: 40450). Carbohydrate ligands were dissolved in the same buffer, degassed, and loaded in the injection syringe. ITC was performed with a VP-ITC microcalorimeter (Microcal, GE Healthcare). The lectin solution was placed in a 1.4478-mL sample cell at 25°C. Titration was performed with 10 μL injections of carbohydrate ligands every 400 s. Data were fitted with MicroCal Origin 7 software according to standard procedures. The fitted data yielded the stoichiometry (N), association constant (K_a), and enthalpy of binding (ΔH). Other thermodynamic parameters (i.e. changes in free energy (ΔG) and entropy (ΔS)) were calculated from the equation $\Delta G = \Delta H - T\Delta S = -RT\ln K_a$, in which T is the absolute temperature and R = 8.314 J.mol^{-1}.K^{-1}. Two independent titrations were performed for each ligand tested.



1-(2-bromoethoxy)-4-methoxybenzene (1): A heterogeneous mixture of 1, 2-dibromoethane (38.2 g mL, 21 mmol, 1 eq.) and K_2CO_3 (17.5 g, 13 mmol, 0.63 eq.) in 100 mL of acetone. The mixture was refluxed and 4-methoxyphenol (5.05 g, 40 mmol, 2 eq.) in 150 mL of acetone was added during 12 h and the reaction was refluxed for 12 h more. The mixture is black and the reaction was followed by TLC. The mixture was then filtrated and the solid was washed with acetone (250 mL). The filtrate was evaporated under vacuum to give a black residue which was then diluted in 200 mL of dichloromethane and washed with 100 mL of HCl 1M, 100 mL of NaOH 1M and 100 of brine. The orange solid residue was then treated with hexane/dichloromethane 9/1 to precipitate the dimeric coumpound which was eliminated by filtration. The filtrate was then concentrated and recristallized from hexane/ethyl acetate to get **1** as translucent sheet-like crystals (1.96 g, 6%).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.94-6.70 (m, 4H, H-ar), 4.25 (t, J = 6.3 Hz, 2H, OCH_2), 3.77 (s, 3H, CH_3), 3.61 (t, J = 6.3 Hz, 2H, CH_2Br).

D.M. Schultz *et al.*, *Bioorg. Med. Chem.* **2008**, *16*, 6242-6251. (Procedure)
Pomilio, A. B.; Tettamanzi, M. C. *Magn. Reson. Chem.***1996**, *34*, 165-171. (NMR data)



Compound 2: Obtained as a pale brown solid following method D: **1** (1.85 g, 8 mmol, 1 eq.), paraformaldehyde (501 mg, 16 mmol, 2 eq.), 1,2-dichloroethane (95 mL), boron trifluoride diethyl etherate (1 mL, 8 mmol, 1 eq.). The black reaction mixture was monitored by TLC. Purified by silica gel flash column chromatography (Hexane/CH_2Cl_2 4/1).

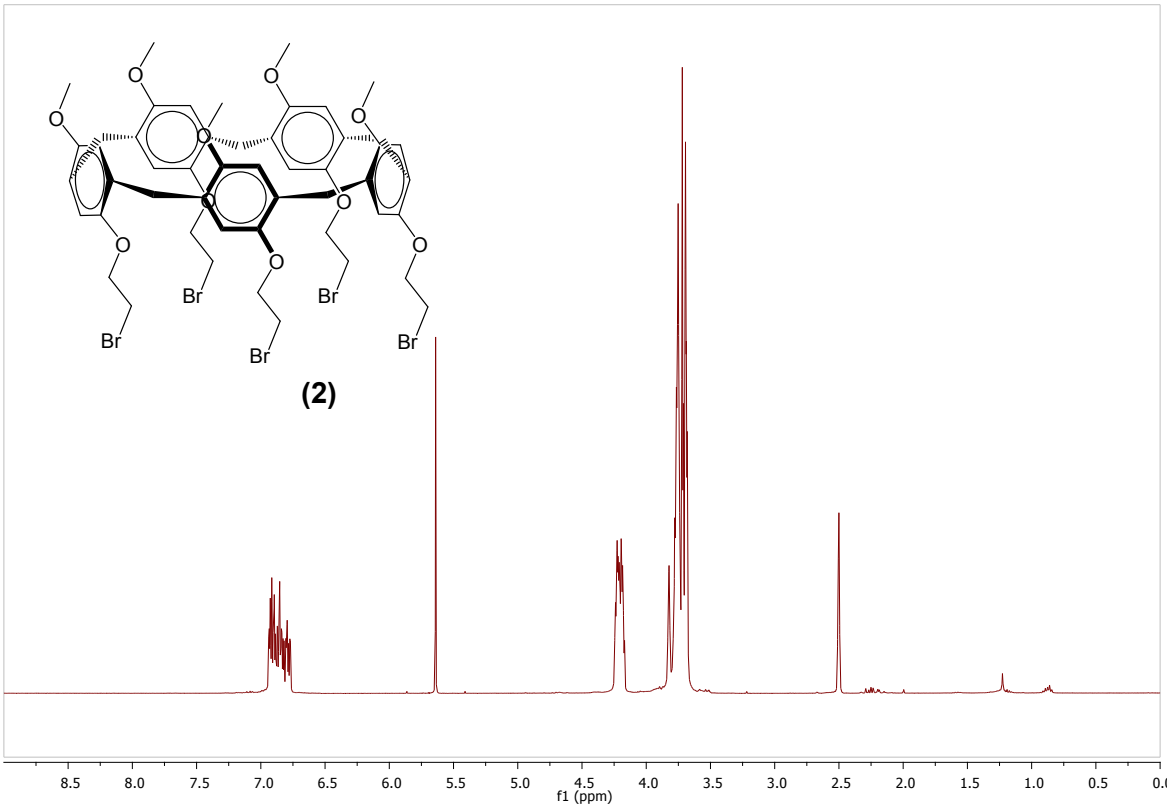
Yield = 84%, 1.62 g

$\mathbf{R_f}$ = 0.35 (Petroleum Ether/ethyl acetate 4/1).

^{1}H NMR (400 MHz, DMSO-d$_6$, 80°C) δ (ppm): 6.97-6.71 (m, 10H, H-ar), 4.29-4.11 (m, 10H, $ArCH_2Ar$), 3.88-3.57 (m, 35H, OCH_2CH_2Br, OCH_3).

^{13}C NMR (100 MHz, DMSO-d$_6$, 80°C) δ (ppm): 150.8-150.3, 148.5-148.1 (2m, 2×C^{IV}-O-ar), 128.7- 128.2, 127.9-127.6 (2M, 2×C^{IV}-ar), 115.9-115.1, 114.1-113.4 (2m, 2×CH-ar), 69.0-68.2, 55.7-54.9, (2m, 2×OCH_2), 54.2 (OCH_3), 31.5-31.1, 28.75-28.38 (2m, $ArCH_2Ar$).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{50}H_{56}Br_5O_{10}$ $[M+H]^+$ 1210.9785, found 1210.9792.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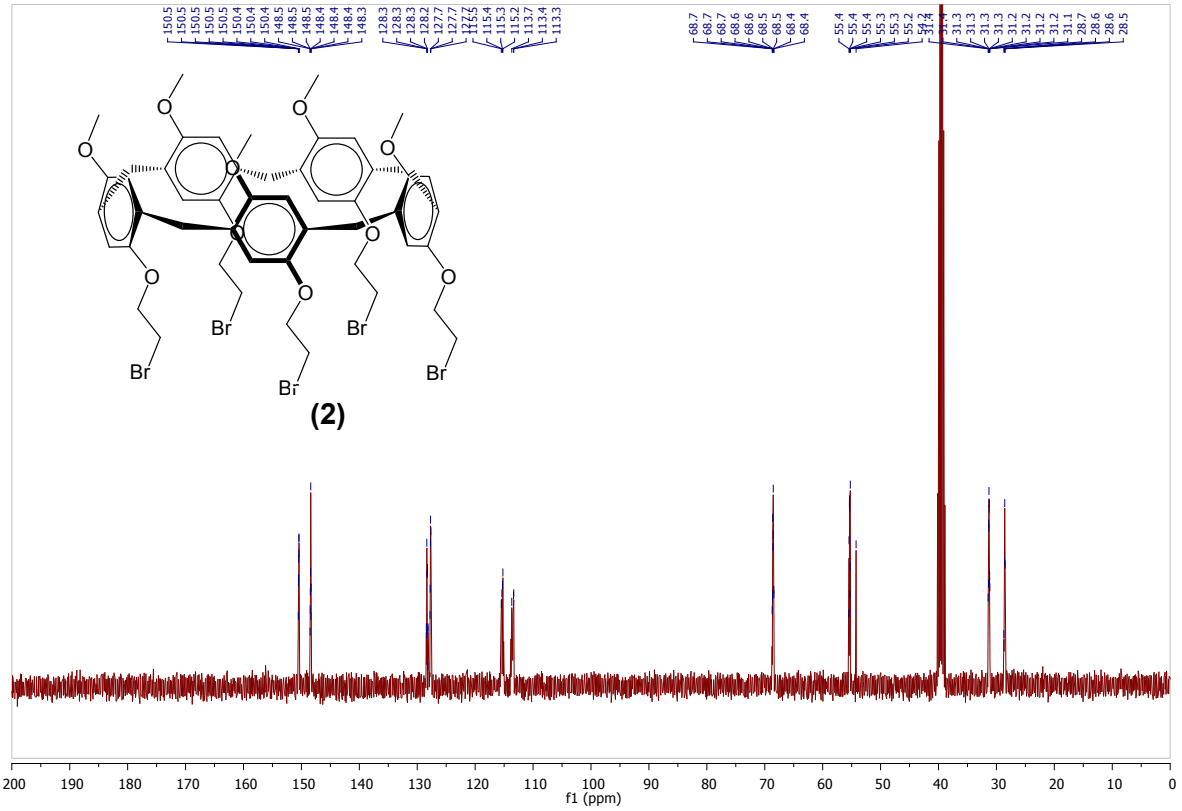
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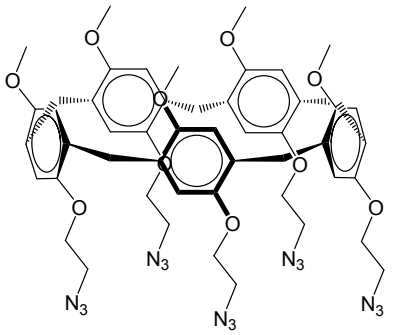


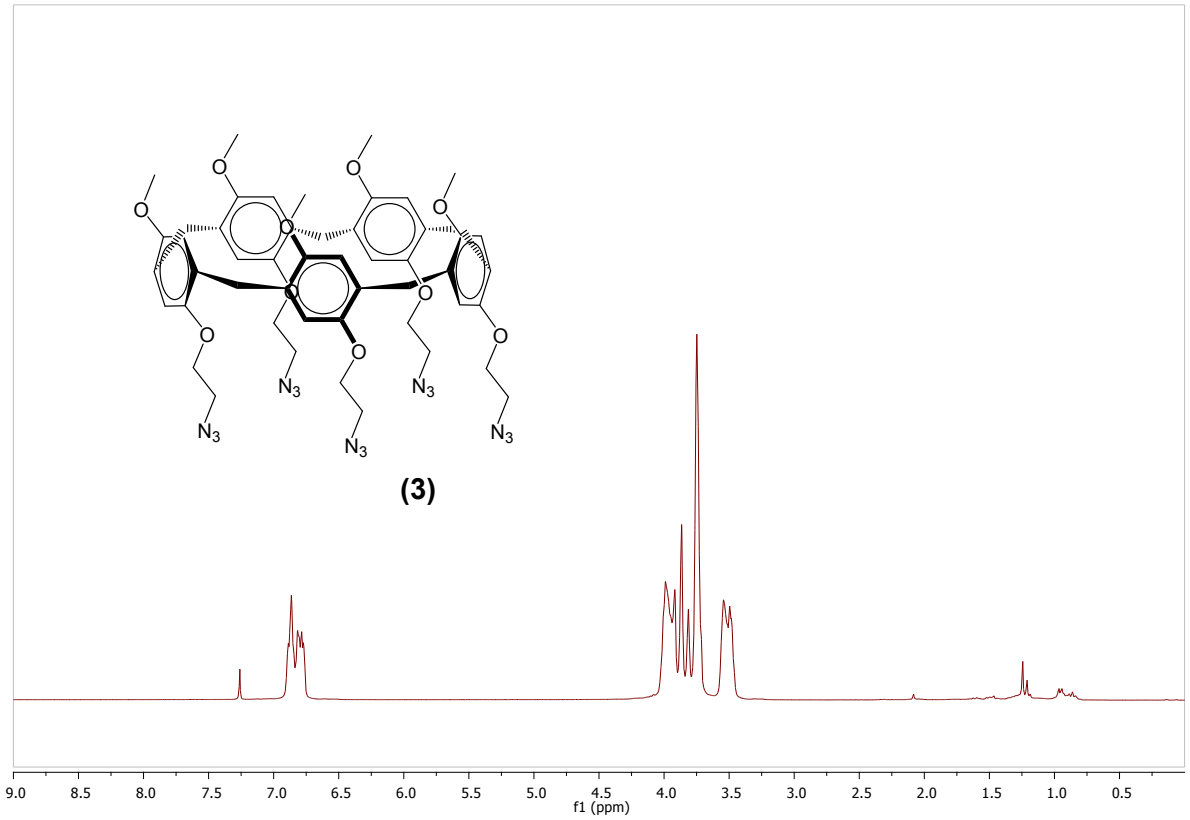
Compound 3: A solution of **2** (300 mg, 0.247 mmol, 1 eq.) and sodium azide (0.402 mg, 6.18 mmol, 25 eq.) in DMF (5 mL) was placed under argon and heated for 16 hours. After cooling at room temperature, the mixture was diluted with water (50 mL) and the aqueous layer was extracted with ethyl acetate (3×20 mL). Organic layers was then combined and dried over $MgSO_4$ before being concentrated. The brown residue was then purified by silica gel flash column chromatography (Hexane/ethyl acetate 4/1) to afford **3** as white foam (231 mg, 91%).

$\mathbf{R_f}$ = 0.35 (Petroleum ether/ethyl acetate 4/1).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.93-6.75 (m, 10H, H-ar), 4.07-3.93 (m, 10H, OCH_2), 3.87 (t, J = 15.7 Hz, 10H, $ArCH_2Ar$), 3.75 (s, 15H, OCH_3), 3.61-3.45 (m, 10H, CH_2N_3).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 151.6-151.3, 149.5-149.0 (2m, 2×C^{IV}-O-ar), 129.2-128.2 (m, 2×C^{IV}-ar), 116.1-115.4 (m, C^{IV}-ar), 114.2 (s, CH-ar), 67.9-67.4 (m, OCH_2), 56.0-55.7 (m, OCH_3), 51.1-50.7 (m, CH_2N_3), 30.2-29.3 (m, $ArCH_2Ar$).

HR-ESI-QTof (positive mode) m/z: calcd for $C_{50}H_{55}N_{15}NaO_{10}$ $[M+Na]^+$ 1048.4149, found 1048.4134.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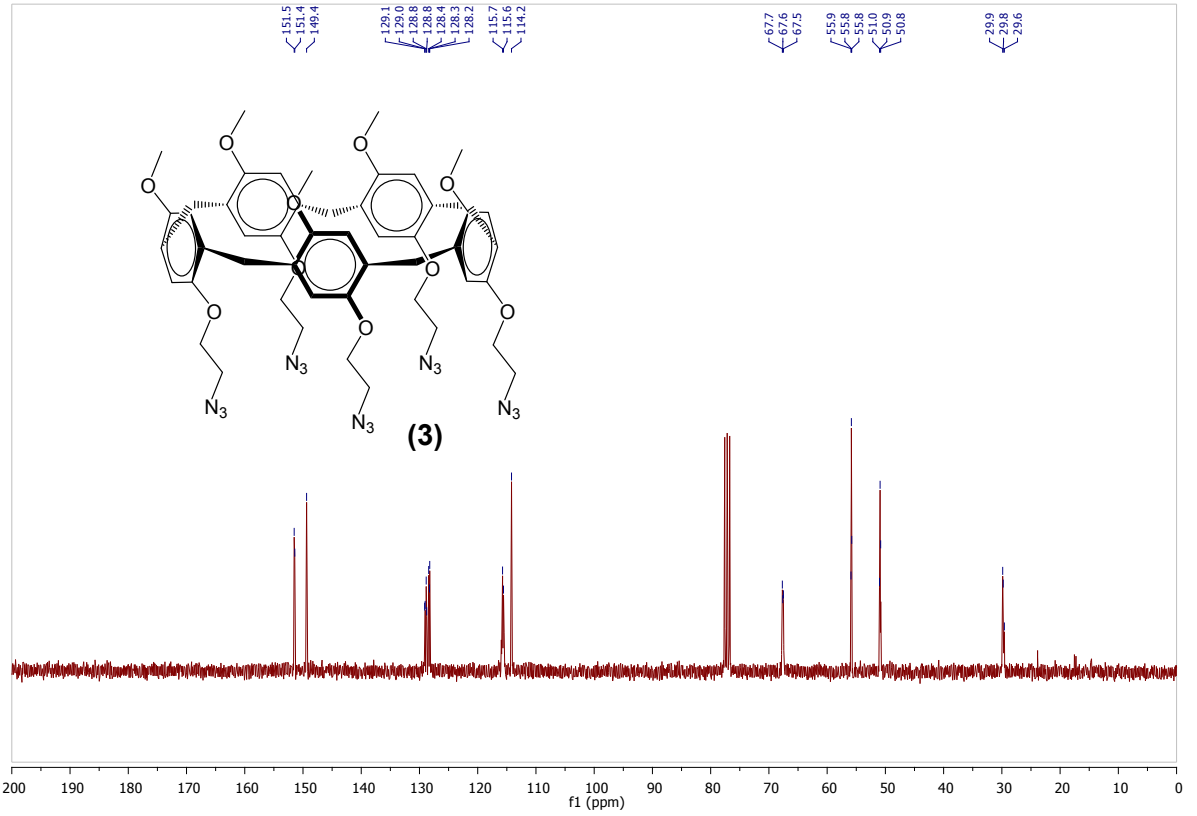
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50.8
29.9
29.8
29.6
(3)
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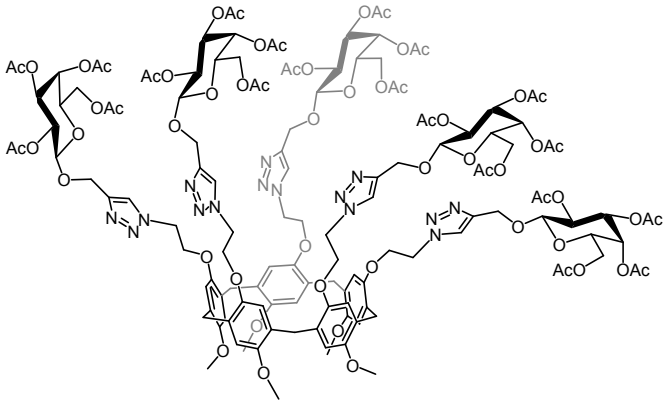


Glycocluster 4a: Obtained as a brown foam following Method A: **3** (82 mg, 0.080 mmol, 1 eq.), propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (282 mg, 0.601 mmol, 7.5 eq.), CuI (8 mg, 0.040 mmol, 0.5 eq.) and DIPEA (99 µL, 0.601 mmol, 7.5 eq.). Purified by silica gel flash column chromatography (DCM/MeOH 99/1 to 95/5).

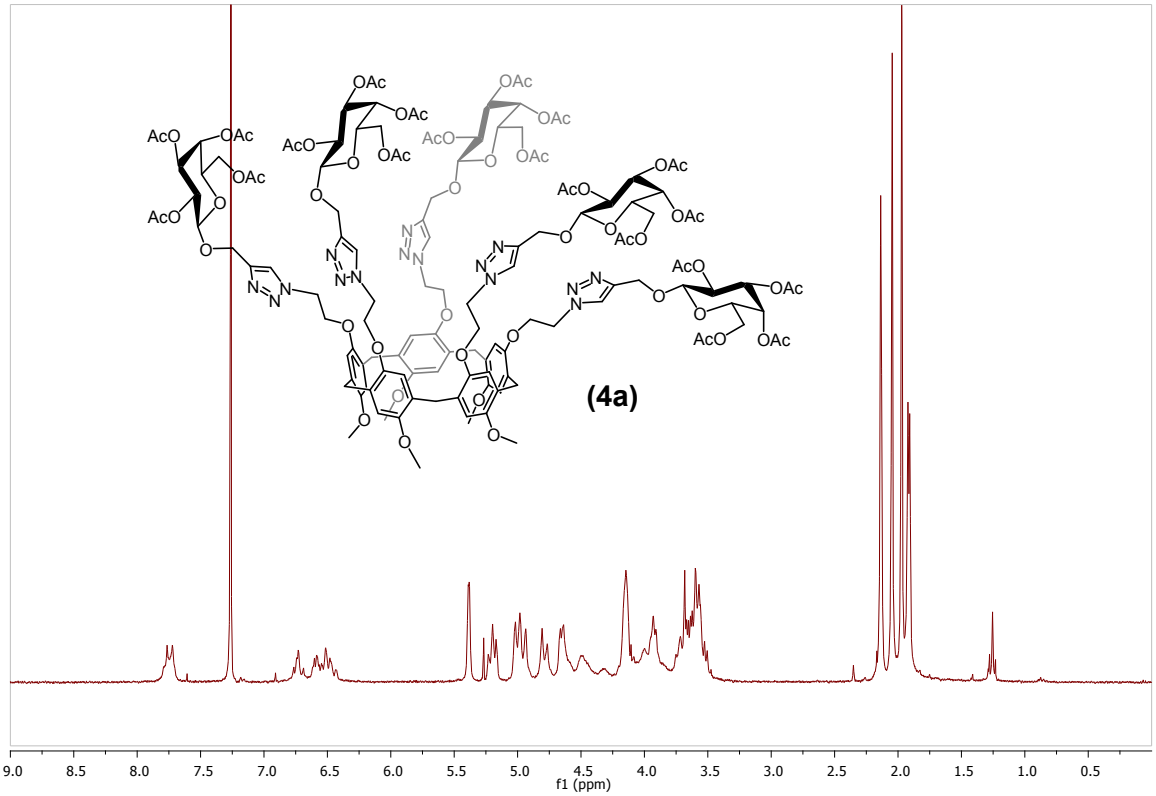
Yield = 87%, 205 mg

$\mathbf{R_f}$ = 0.65 (DCM/MeOH 9/1).

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.85-7.66 (m, 5H, H-triaz), 6.82-6.38 (m, 10H, H-ar), 5.43-5.35 (m, 5H, H-4), 5.29-5.14 (m, 5H, H-2), 5.06-4.89 (m, 10H, H-3, OCH_2-triaz), 4.86-4.72 (m, 5H, OCH_2-triaz), 4.71-4.60 (m, 5H, H-1), 4.60-4.21 (m, 10H, CH_2N), 4.22-4.08 (m, 10H, H-6), 4.04-3.79 (m, 15H, H-5, NCH_2CH_2), 3.77-3.46 (m, 25H, $ArCH_2Ar$, OCH_3), 2.14, 2.05, 1.97 (3s, 45H, 4×$COCH_3$), 1.92, 1.91 (2s, 15H, $COCH_3$).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.3, 170.1, 169.9, 169.4 (4×*C*OCH_3), 151.8-150.9, 149.5-148.6 (2m, C^{IV}-O-ar), 144.5-143. 6 (m, C^{IV}-triaz), 129.2-127.9 (m, C^{IV}-ar), 123.7-123.2 (m, CH-triaz), 116.1-115.1, 114.5-113.4 (2m, 2×CH-ar), 100.1 (C-1), 70.7 (2C, C-3, C-5), 68.6 (C-2), 68.1-67.2 (m, NCH_2CH_2), 67.0 (C-4), 62.7-62.1 (OCH_2-triaz), 61.1 (C-6), 56.4-55.3 (m, OCH_3), 50.2-49.5 (m, NCH_2), 30.3-29.0 ($ArCH_2Ar$), 20.53, 20.48, 20.4 (4×CO*C*H_3).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{135}H_{167}N_{15}O_{60}$ $[M+2H]^{2+}$ 1479.0233, found 1479.0231.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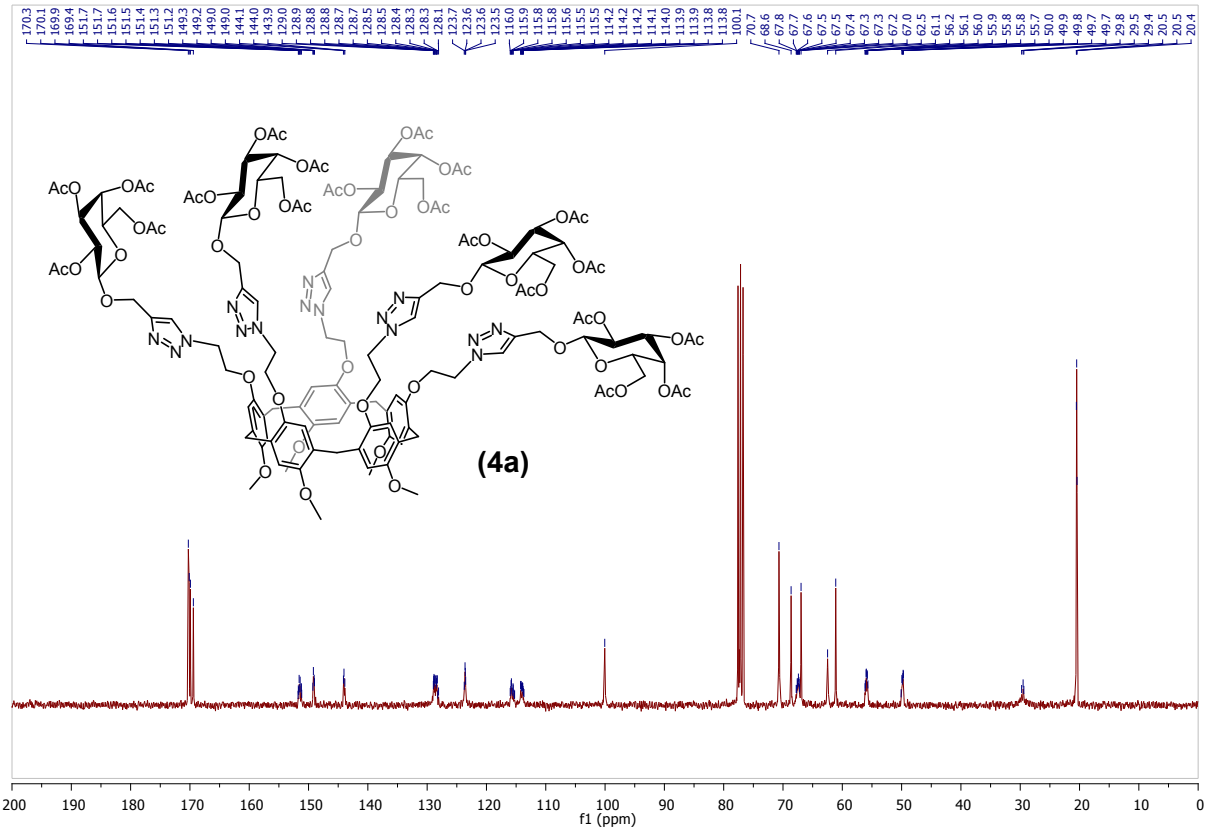
f1 (ppm)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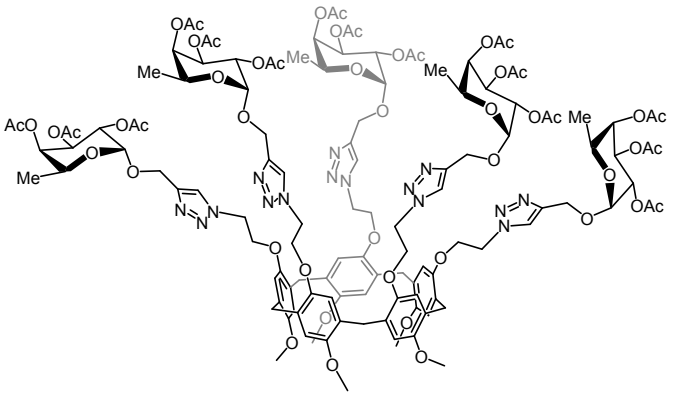


Glycocluster 4b: Obtained as a brown foam following Method A: **3** (65 mg, 0.063 mmol, 1 eq.), propargyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranoside (125 mg, 0.380 mmol, 6 eq.), CuI (6 mg, 0.032 mmol, 0.5 eq.) and DIPEA (62 µL, 0.380 mmol, 6 eq.). Purified by silica gel flash column chromatography (DCM/MeOH 99/1 to 96/4).

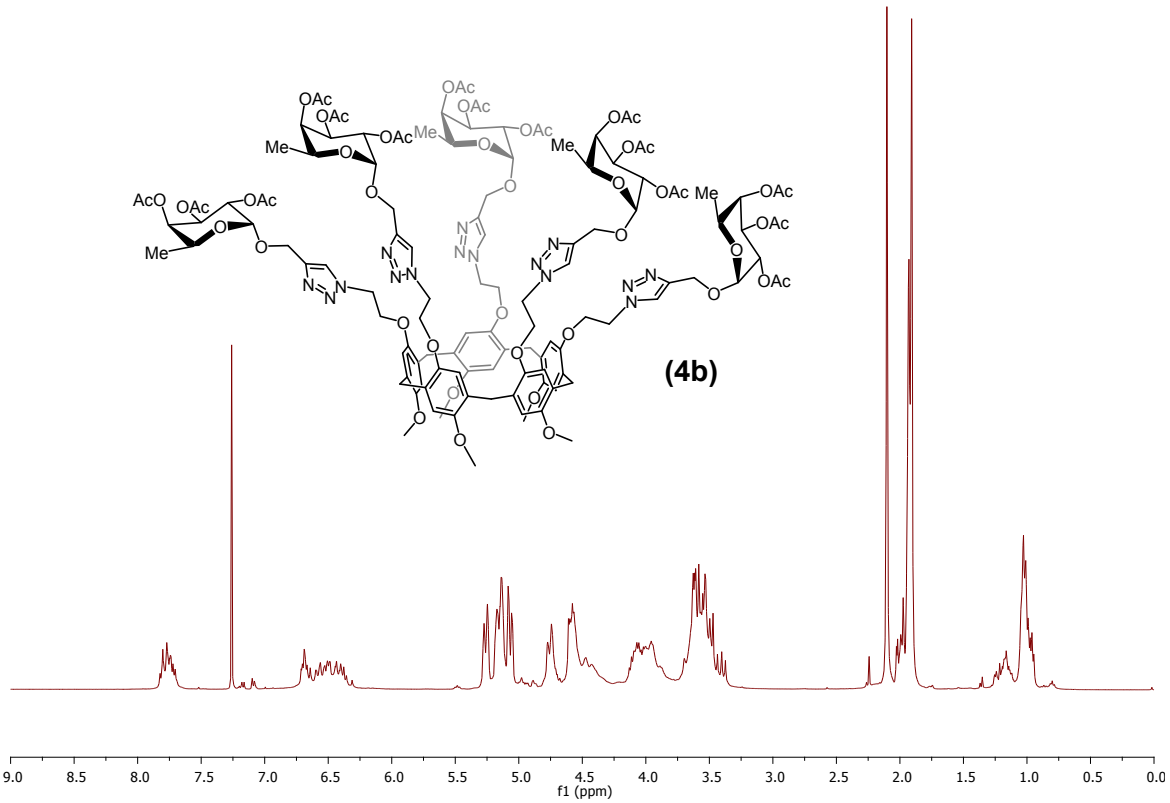
Yield = 93%, 158 mg

$\mathbf{R_f}$ = 0.60 (DCM/MeOH 9/1).

^{1}H NMR (300 MHz, acetone-d_6) δ (ppm): 7.86-7.67 (m, 5H, H-triaz), 6.75-6.27 (m, 10H, H-ar), 5.31-5.22 (m, 5H, H-3), 5.20-5.11 (m, 10H, H-1, H-4), 5.11-5.03 (m, 5H, H-2), 4.82-4.68, 4.65-4.30 (2M, 20H, OCH_2-triaz, CH_2N), 4.17-3.81 (m, 15H, OCH_2CH_2N, H-5), 3.73-3.35 (m, 25H, OCH_3, $ArCH_2Ar$), 2.10 (s, 15H, CH_3CO), 1.96-1.92 (m, 15H, CH_3CO), 1.91 (s, 15H, CH_3CO), 1.09-0.93 (m, 15H, H-6).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.6-170.5, 170.4-170.3, 170.0-169.8 (3M, 3×$COCH_3$), 151.9-151.0, 149.3-148.7 (2M, 2×C^{IV}-O-ar), 144.2-143.9 (C^{IV}-triaz), 129.1-128.0 (m, C^{IV}-ar), 124.0-123.2 (m, CH-triaz), 116.2-115.1, 114.5-113.5 (2M, 2×CH-ar), 95.7 (C-1), 71.0 (C-4), 67.9 (C-2), 67.8 (C-3), 67.4 (NCH_2CH_2), 64.6 (C-5), 61.2 (OCH_2-triaz), 56.2-55.6 (m, OCH_3), 50.1-49.5 (m, NCH_2), 30.5-28.7 (m, $ArCH_2Ar$), 20.7-20.5 (m, 3×$COCH_3$), 15.7 (C-6).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{125}H_{155}N_{15}Na_2O_{50}$ $[M+2Na]^{2+}$ 1355.9916, found 1355.9915.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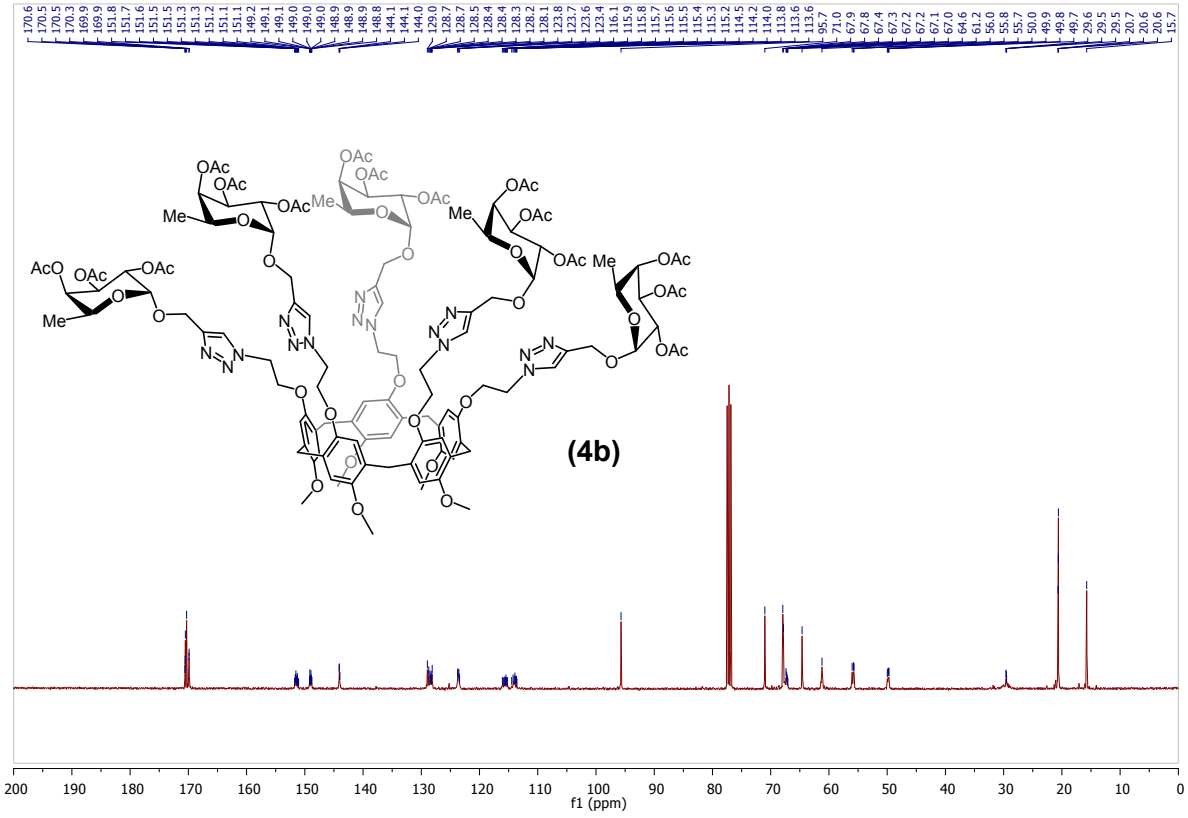
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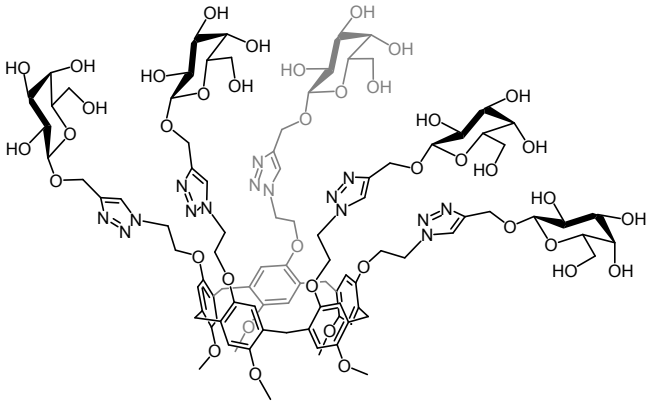


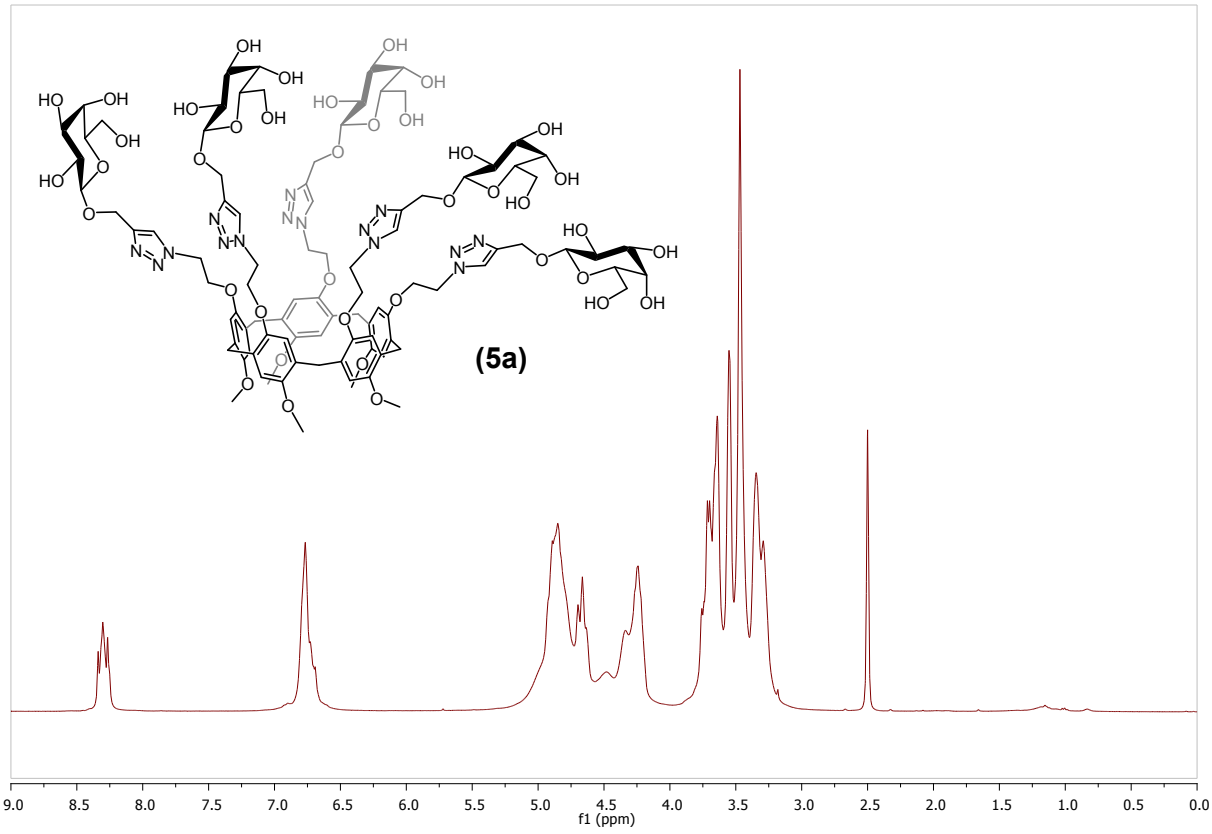
Glycocluster 5a: Obtained from **4a** (140 mg, 0.047 mmol) as a brown foam following Method C.

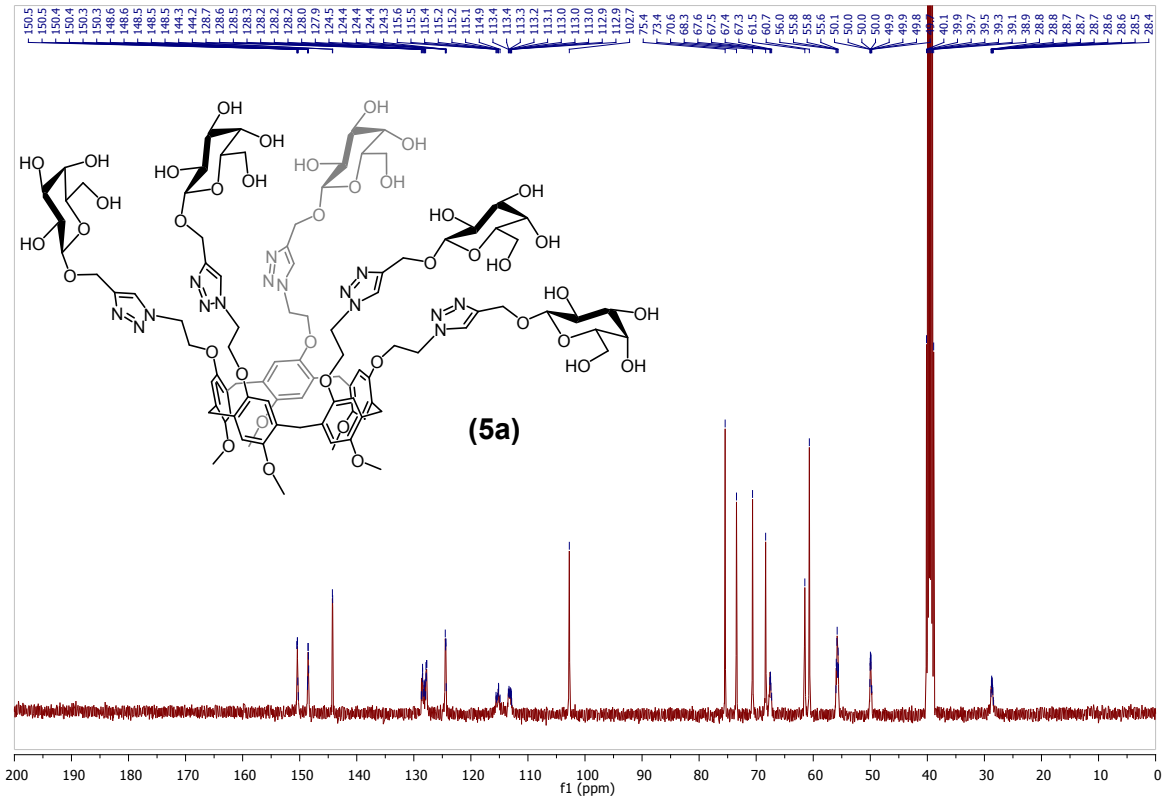
Yield = quantitative, 100 mg

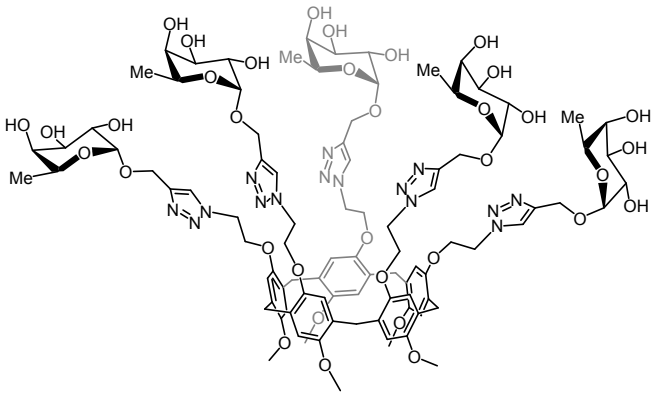
^{1}H NMR (400 MHz, DMSO-d$_6$ + εD$_2$O) δ (ppm): 8.38-8.21 (m, 5H, H-triaz), 6.86-6.60 (m, 10H, H-ar), 5.08-4.60 (m, 20H, OCH_2-triaz, NCH_2), 4.42-4.13 (m, 15H, NCH_2CH_2, H-1), 3.82-3.22 (m, 55H, H-2, H-3, H-4, H-5, H-6, $ArCH_2Ar$, OCH_3).

^{13}C NMR (100 MHz, DMSO-d$_6$ + εD$_2$O) δ (ppm): 150.6-150.2, 148.7-148.3 (2M, 2×C^{IV}-O-ar), 144.-144.2 (m, 2×C^{IV}-triaz), 128.7-127.6 (m, 2×C^{IV}-ar), 124.6-124.3 (m, CH-triaz), 115.8-114.7, 113.6-112.6 (2M, 2×CH-ar), 102.8 (C-1), 75.4 (C-3), 73.5 (C-2), 70.6 (C-5), 68.3 (C-4), 67.8-67.1 (m, NCH_2CH_2), 61.5 (OCH_2-triaz), 60.7 (C-6), 56.2-55.5 (m, OCH_3), 50.2-49.6 (m, NCH_2), 29.0-28.3 ($ArCH_2Ar$).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{95}H_{125}N_{15}Na_2O_{40}$ $[M+2Na]^{2+}$ 1080.8996, found 1080.9029.


(5a)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


(5a)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



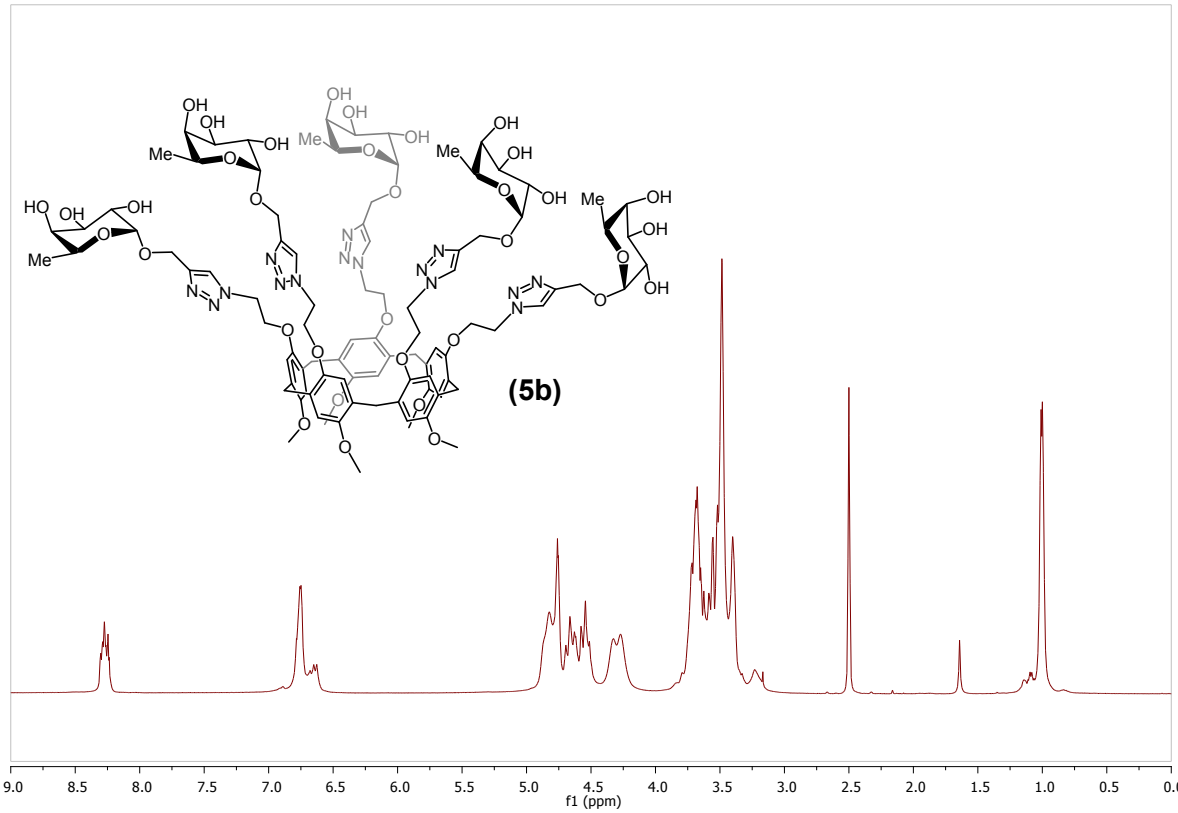
Glycocluster 5b: Obtained from **4b** (134 mg, 0.050 mmol) as a brown following Method C.

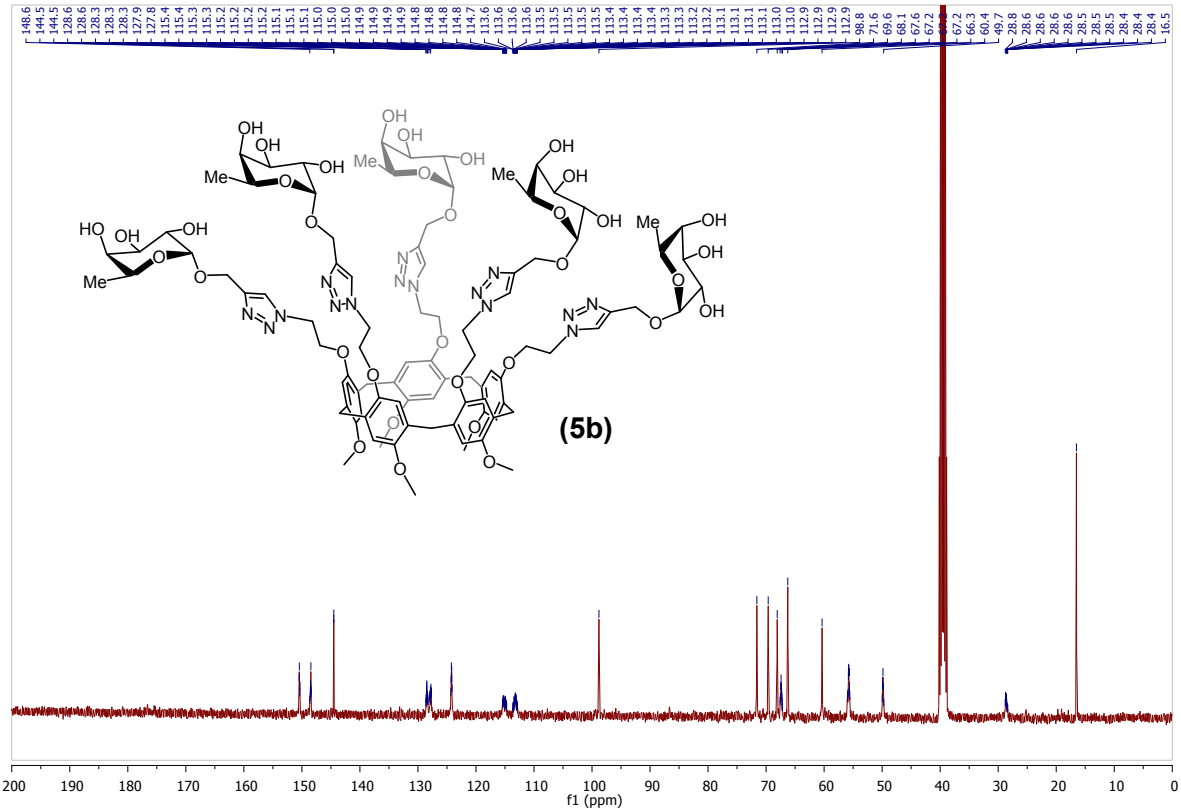
Yield = quantitative, 101 mg.

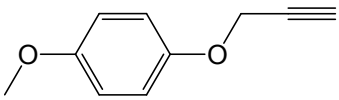
^{1}H NMR (500 MHz, DMSO-d$_6$ + εD$_2$O) δ (ppm): 8.34-8.18 (m, 5H, H-triaz), 6.87-6.58 (m, 10H, H-ar), 4.93-4.71 (m, 15H, NCH_2, H-1), 4.71-4.59, 4.50-4.47 (2M, 10H, OCH_2-triaz), 4.40-4.20 (m, 10H, NCH_2CH_2), 3.85-3.16 (m, 45H, H-2, H-3, H-4, H-5, OCH_3, $ArCH_2Ar$), 1.00 (d, J = 4.1 Hz, 15H).

^{13}C NMR (125 MHz, DMSO-d$_6$ + εD$_2$O) δ (ppm): 150.5-150.3, 148.7-148.4 (2M, 2×C^{IV}-O-ar), 144.6-144.4 (m, C^{IV}-triaz), 128.7-128.1, 128.0-127.5 (2M, 2×C^{IV}-ar), 124.5-124.0 (m, CH-triaz), 115.5-114.6, 113.6-112.7 (2M, 2×CH-ar), 98.8 (C-1), 71.6 (C-4), 69.6 (C-3), 68.1 (C-2), 67.7-67.1 (m, NCH_2CH_2), 66.3 (C-5), 60.4 (OCH_2-triaz), 56.1-55.5 (m, OCH_3), 50.0-49.6 (m, NCH_2), 28.8-28.4 (m, $ArCH_2Ar$), 16.5 (C-6).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{95}H_{127}N_{15}O_{35}$ $[M+2H]^{2+}$ 1018.9304, found 1018.9330.


(5b)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


(5b)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



1-Methoxy-4-(2-propynyloxy)benzene (6): Propargyl bromide (32.3 mL, 362 mmol, 3 eq.) was added to a heterogeneous mixture of 4-methoxyphenol (15.7 g, 121 mmol, 1 eq.) and K_2CO_3 (50.1 g, 362 mmol, 3 eq.) in acetone (500 mL). The reaction was refluxed (56°C) for 16 h. The mixture black reaction mixture was monitored by TLC then filtered and the solid was washed with acetone (500 mL). The filtrate was evaporated under vacuum to give a black residue which was purified by silica gel flash column chromatography (Hexane/CH_2Cl_2 4/1 to 1/1) to give **6** as an orange oil (18.0 g, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.96-6.89 (m, 2H, H-ar), 6.87-6.82 (m, 2H, H-ar), 4.64 (d, J = 2.4 Hz, 2H, CH_2), 3.78 (s, 1H, CH_3), 2.50 (t, J = 2.4 Hz, 1H, ≡CH).

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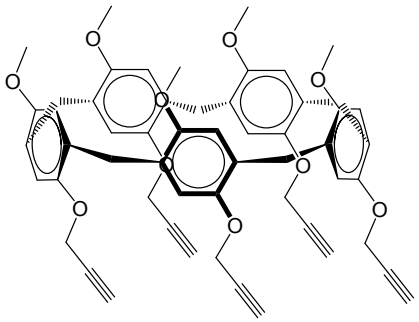


Compound 7: Obtained as a white powder following method D: **6** (4.03 g, 25 mmol, 1 eq.), paraformaldehyde (948 mg, 27 mmol, 1.1 eq.), 1,2-dichloroethane (125 mL), boron trifluoride diethyl etherate (3 mL, 25 mmol, 1 eq.). The black reaction mixture was monitored by TLC. Purified by silica gel flash column chromatography (Hexane/CH_2Cl_2 4/1).

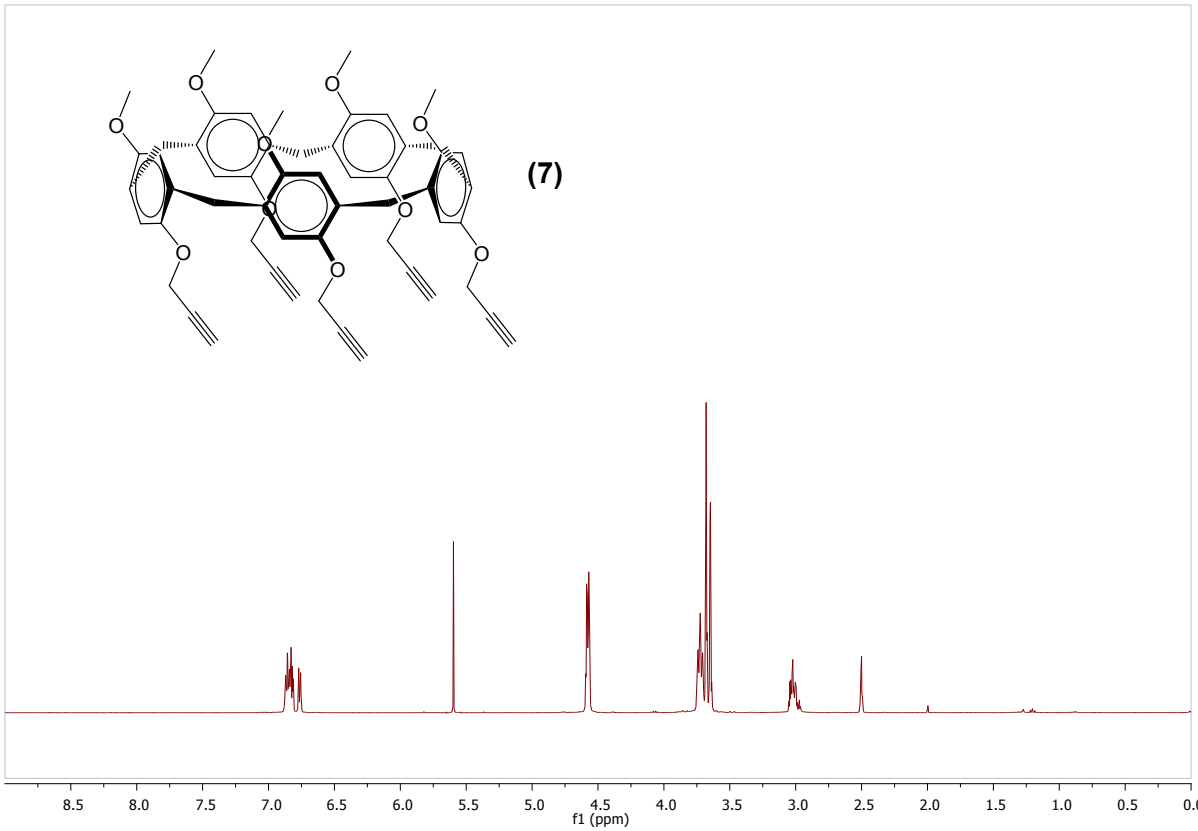
Yield = 50%, 2.18 g

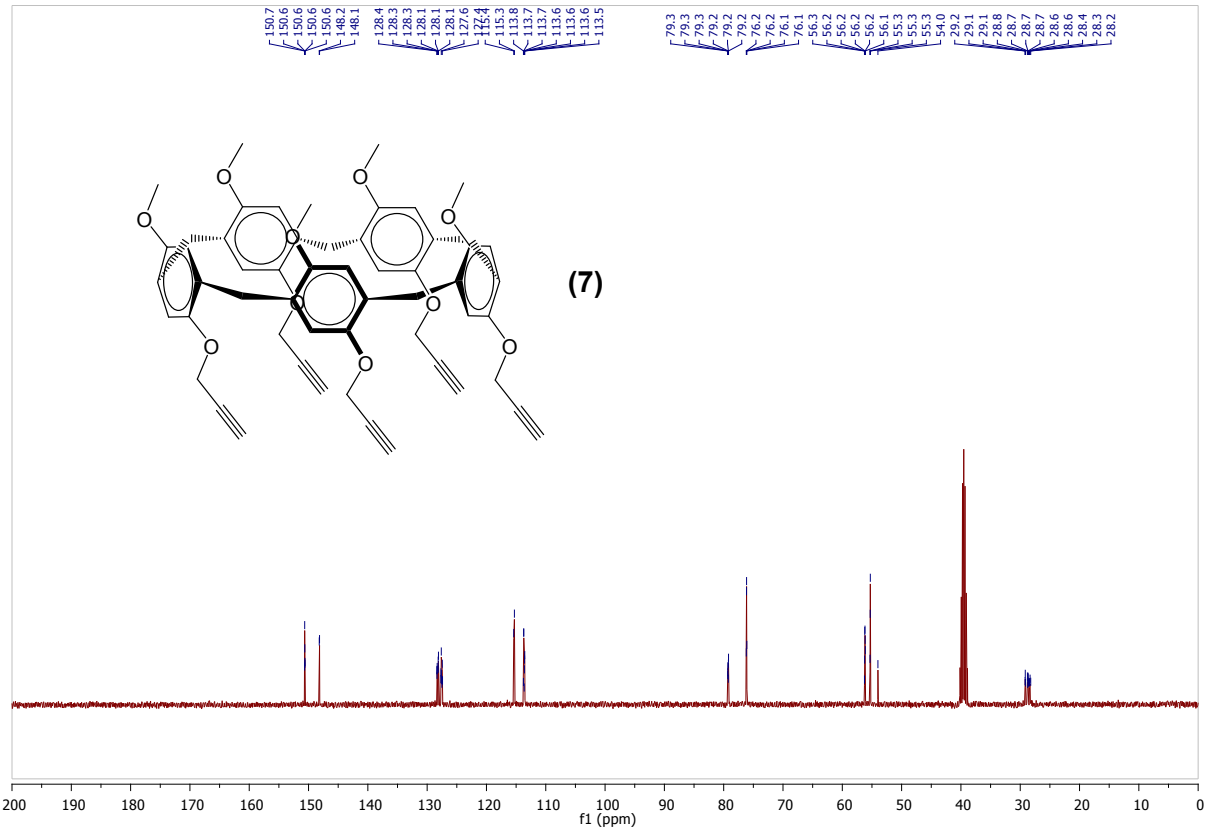
$\mathbf{R_f}$ = 0.2 (Hexane/ CH_2Cl_2 4/1).

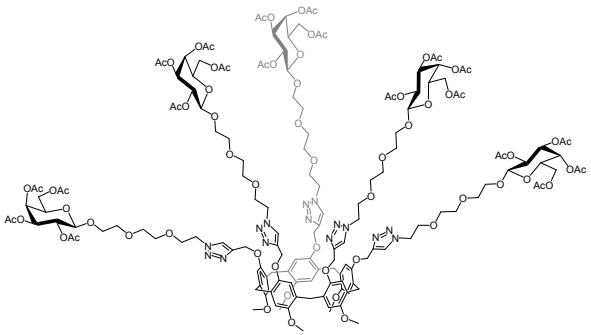
^{1}H NMR (400 MHz, DMSO-d$_6$, 100°C) δ (ppm): 6.92-6.73 (m, 10H, H-ar), 4.64-4.44 (m, 10H, OCH_2), 3.78-3.70 (m, 10H, $ArCH_2Ar$), 3.69-3.63 (m, 15H, OCH_3), 3.08-2.94 (m, 5H, CH).

^{13}C NMR (100 MHz, DMSO-d$_6$, 100°C) δ (ppm): 150.7-150.5, 148.3-148.1 (2M, 2×C^{IV}-O-ar), 128.4-128.3, 128.2-128.1, 127.71-127.65, 127.65-127.54, 127.54-127.39 (5M, 2×C^{IV}-ar), 115.46-115.35, 115.35-115.22 (2M, CH-ar), 113.84-113.67, 113.67-113.50 (2M, CH-ar), 79.34-79.25, 79.24-79.14 (2m, *C*≡CH), 76.2-76.1 (m, C≡*C*H), 56.30-56.06 (m, OCH_2), 55.38-55.20 (m, OCH_3), 54.06-53.94 (m, OCH_2), 29.26-29.02, 28.84-28.54, 28.50-28.17 (3M, $ArCH_2Ar$).

Huacheng Zhang, Xing Ma, Kim Truc Nguyen, and Yanli Zhao, *ACS Nano* **2013**, *7*, 7853-7863.


(7)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


150.7
150.6
150.6
150.6
150.6
148.2
148.1
128.4
128.3
128.3
128.1
128.1
128.1
127.6
115.3
113.8
113.7
113.7
113.6
113.6
113.6
113.5
79.3
79.3
79.3
79.2
79.2
79.2
76.2
76.2
76.1
76.1
56.3
56.2
56.2
56.2
56.2
56.1
55.3
55.3
55.3
54.0
29.2
29.1
29.1
28.8
28.7
28.7
28.7
28.6
28.6
28.4
28.3
28.2
(7)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



Glycocluster 8: Obtained as a brown foam following Method B: **7** (100 mg, 0.115 mmol, 1 eq.), 1-azido-3,6-dioxaoctyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (348 mg, 0.694 mmol, 6 eq.), $CuSO_4$•$5H_2O$ (71 mg, 0.288 mmol, 2.5 eq.) and sodium ascorbate (113 mg, 0.575 mmol, 5 eq.). Purified by silica gel flash column chromatography (DCM/MeOH 99/1 to 90/10).

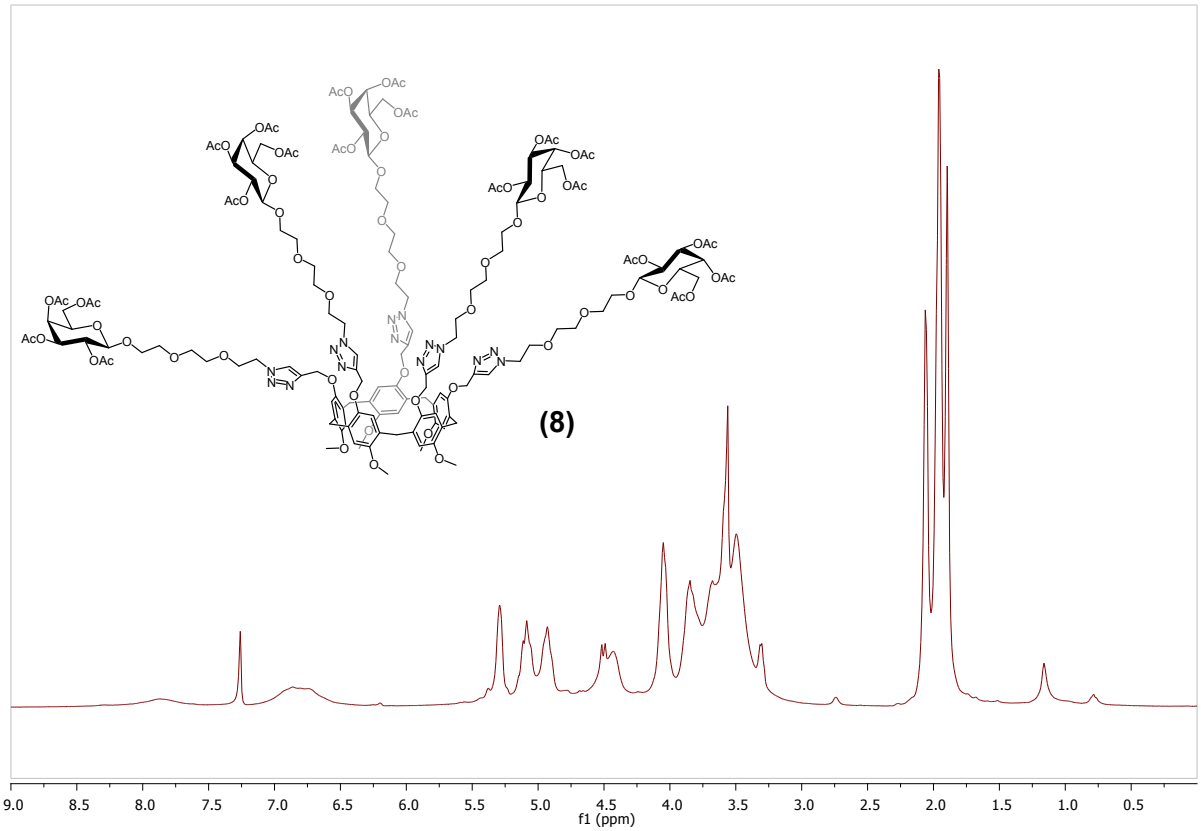
Yield = 76%, 300 mg

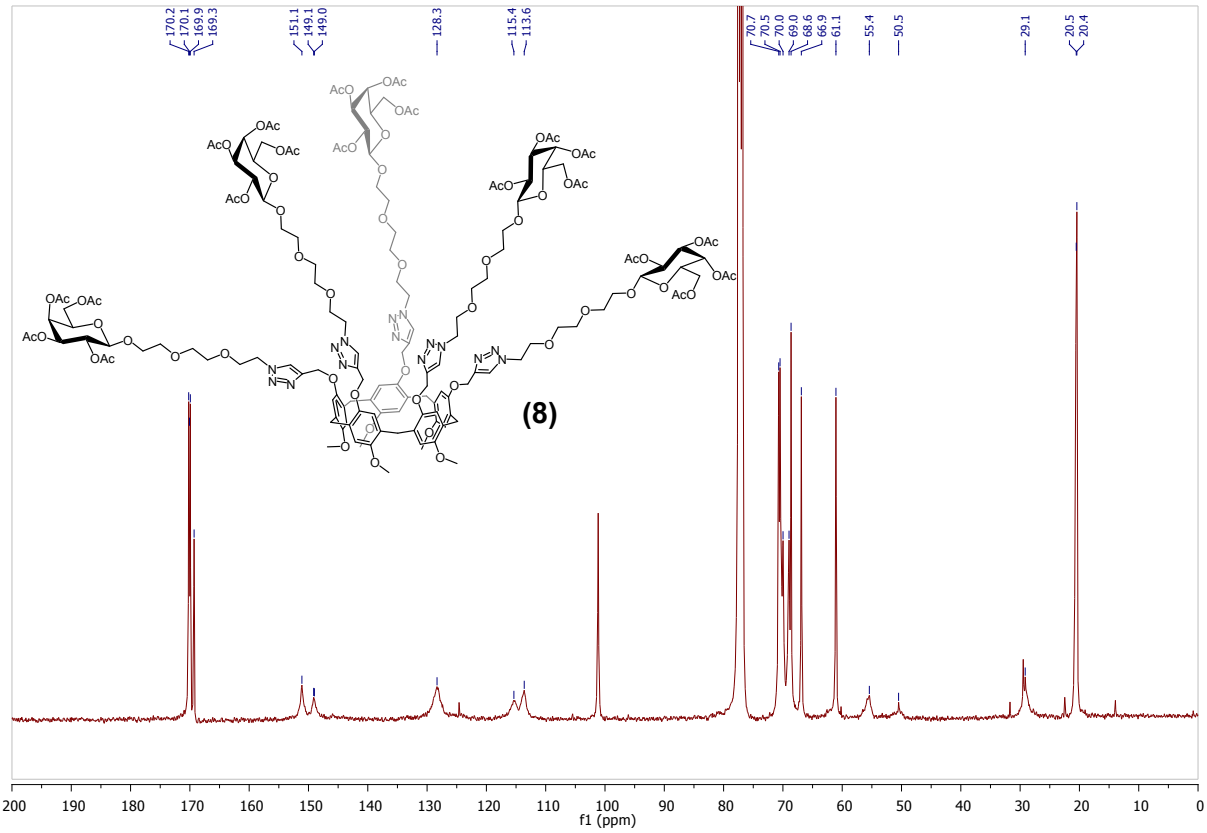
$\mathbf{R_f}$ = 0.35 (DCM/MeOH 95/5).

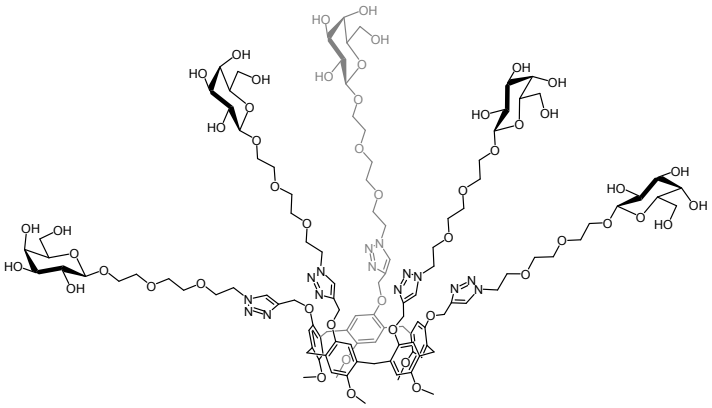
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.07-7.62 (m, 5H, H-triaz), 6.86 (s, 10H, H-ar), 5.38-5.21 (m, 5H, H-4), 5.21-5.02 (m, 15H, H-2, OCH_2triaz), 5.02-4.86 (m, 5H, H-3), 4.62-4.35 (m, 15H, H-1, CH_2N), 4.16-3.97 (m, 10H, H-6), 3.97-3.35 (m, 80H, H-5, 5×OCH_2, OCH_3, $ArCH_2Ar$).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.2, 170.1, 169.9, 169.3 (4×*C*OCH_3), 152.0-150.2 (m, C^{IV}-ar), 149.7-148.5 (m, C^{IV}-triaz), 129.6-127.0 (m, CH-triaz), 116.5-114.5, 114.5-112.7 (2M, 2×CH-ar), 101.2 (C-1), 71.7 (C-3), 70.4 (C-5), 70.1-69.7, 69.4-68.8 (2M, 5×OCH_2), 68.2 (C-2), 66.9 (C-4), 61.2-63.1 (OCH_2-triaz), 61.1 (C-6), 56.6-54.8 (m, OCH_3), 51.4-49.6 (m, NCH_2), 29.9-28.5 (m, $ArCH_2Ar$), 20.8-20.4 (m, 4×CO*C*H_3).

HR-ESI-QTof (positive mode) *m*/*z*: calcd for $C_{155}H_{207}N_{15}O_{70}$ $[M+2H]^{2+}$ 1699.1544, found 1699.1467.


(8)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


170.2
170.1
169.9
169.3
151.1
149.1
149.0
128.3
115.4
113.6
70.7
70.5
70.0
69.0
68.6
66.9
61.1
55.4
50.5
29.1
20.5
20.4
(8)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



Glycocluster 9: Obtained from **8** (200 mg, 0.059 mmol) as a brown foam following method C.

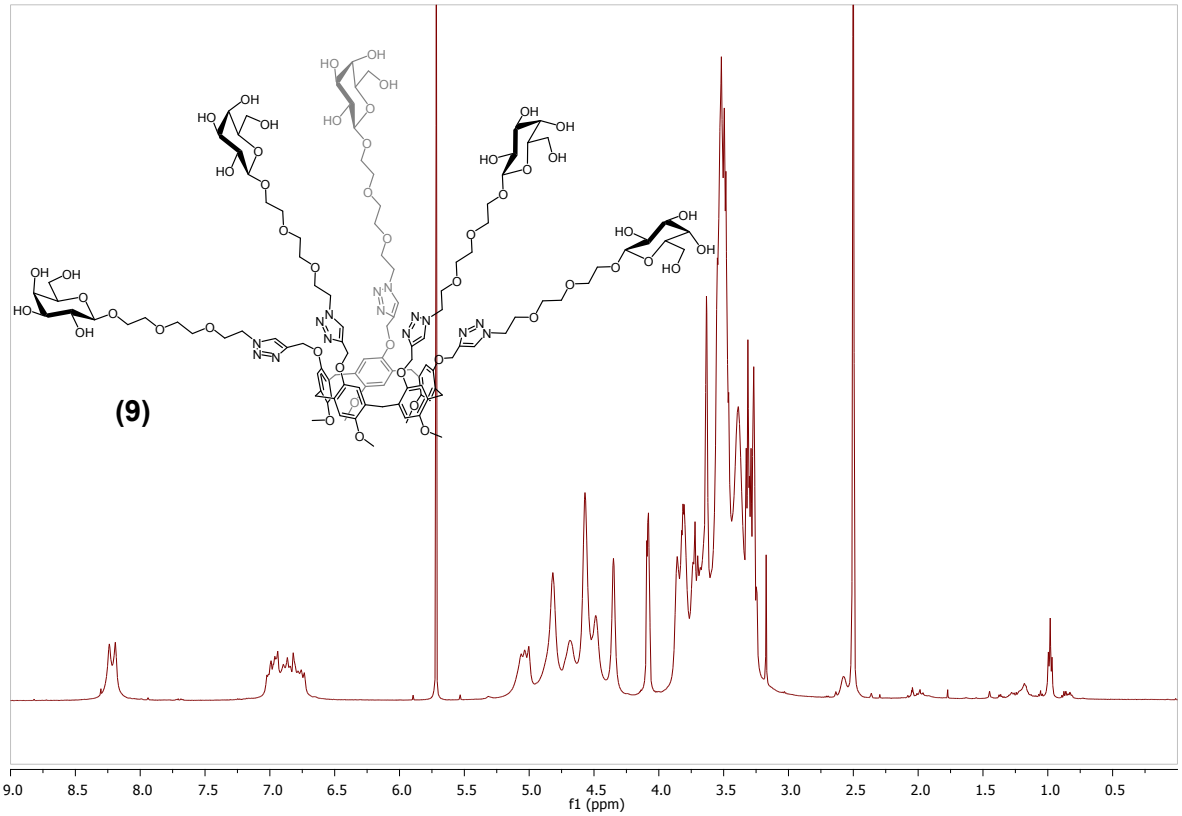
Yield = quantitative, 149 mg.

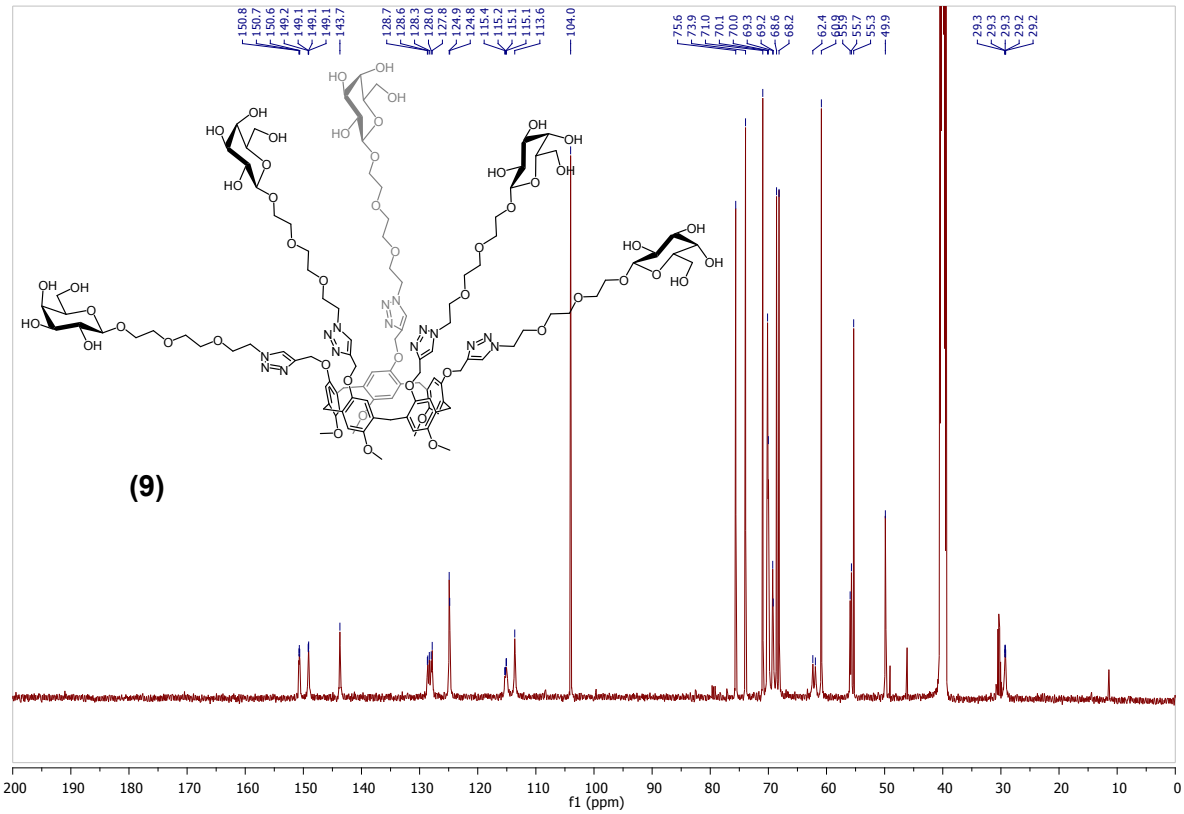
^{1}H NMR (500 MHz, DMSO-d$_6$ + εD$_2$O) δ (ppm): 8.29-8.12 (m, 5H, H-triaz)*, 7.05-6.68 (m, 10H, H-ar), 5.21-4.96 (m, 5H, OCH_2-triaz), 4.8-4.75 (m, 5H, OCH_2-triaz), 4.74-4.23 (m, 15H, OCH_3), 4.10-4.06 (m, 5H, H-1), 3.96-3.21 (m, 100H, NCH_2, 5×OCH_2, H-2, H-3, H-4, H-5, H-6, $ArCH_2Ar$).

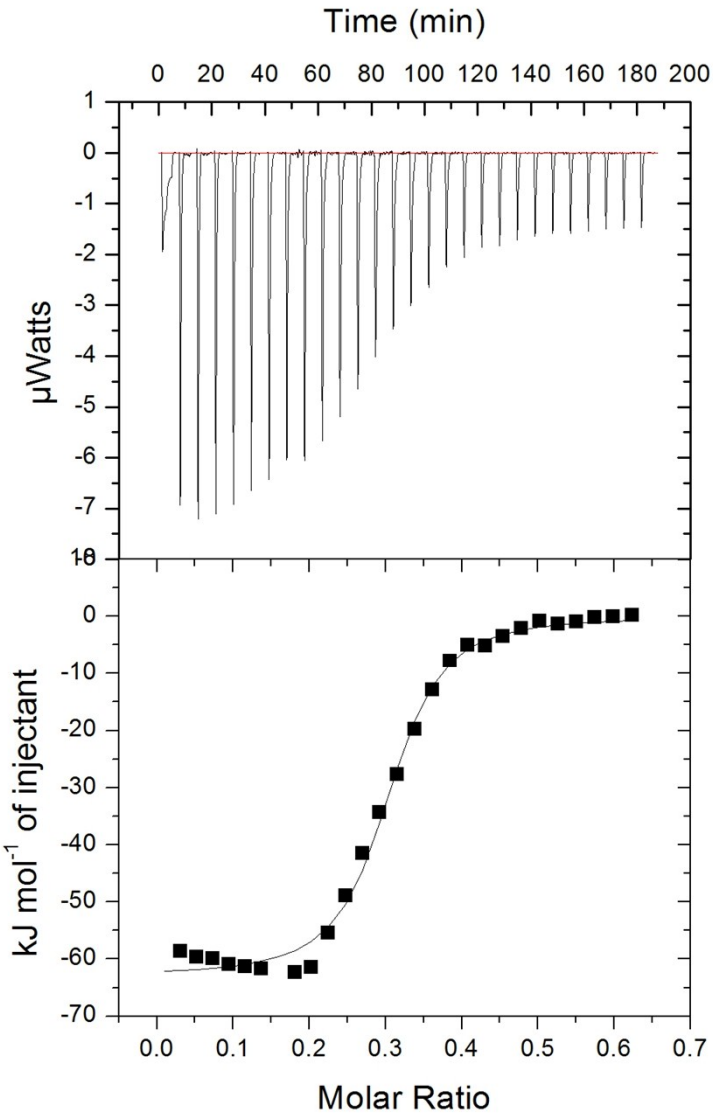
* This signal appears as two singlets due to the racemic mixture of the pillar[5]arene precursor.

^{13}C NMR (125 MHz, DMSO-d$_6$ + εD$_2$O) δ (ppm): 150.5-150.0, 148.9-148.4 (2M, 2×C^{IV}-O-ar), 143.5-143.1 (m, C^{IV}-triaz), 130.7-126.0 (m, 2×C^{IV}-ar), 124.6-124.2 (m, CH-triaz), 116.8-114.0, 113.5-112.8 (2M, CH-ar), 103.6 (C-1), 75.2 (C-3), 73.5 (C-2), 70.5 (C-5), 70.0-69.3 (m, 3×OCH_2), 69.0-68.5 (m, OCH_2), 68.2 (C-4), 67.7 (GalOCH_2), 62.2-61.7, 61.7-61.1 (2M, OCH_2-triaz), 60.5 (C-6), 54.9 (OCH_3), 49.5 (NCH_2), 29.1-28.6 (m, $ArCH_2Ar$).

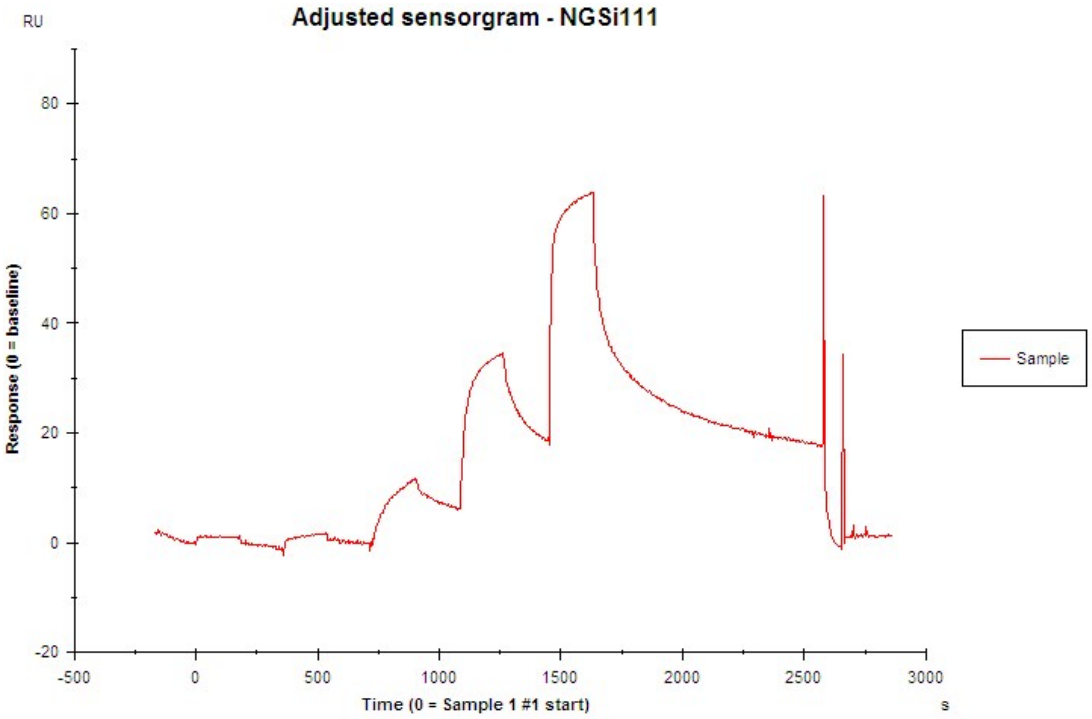
HR-ESI-QTof (positive mode) *m/z*: calcd for $C_{115}H_{167}N_{15}O_{50}$ $[M+2H]^{2+}$ 1279.0488, found 1279.0484.


(9)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

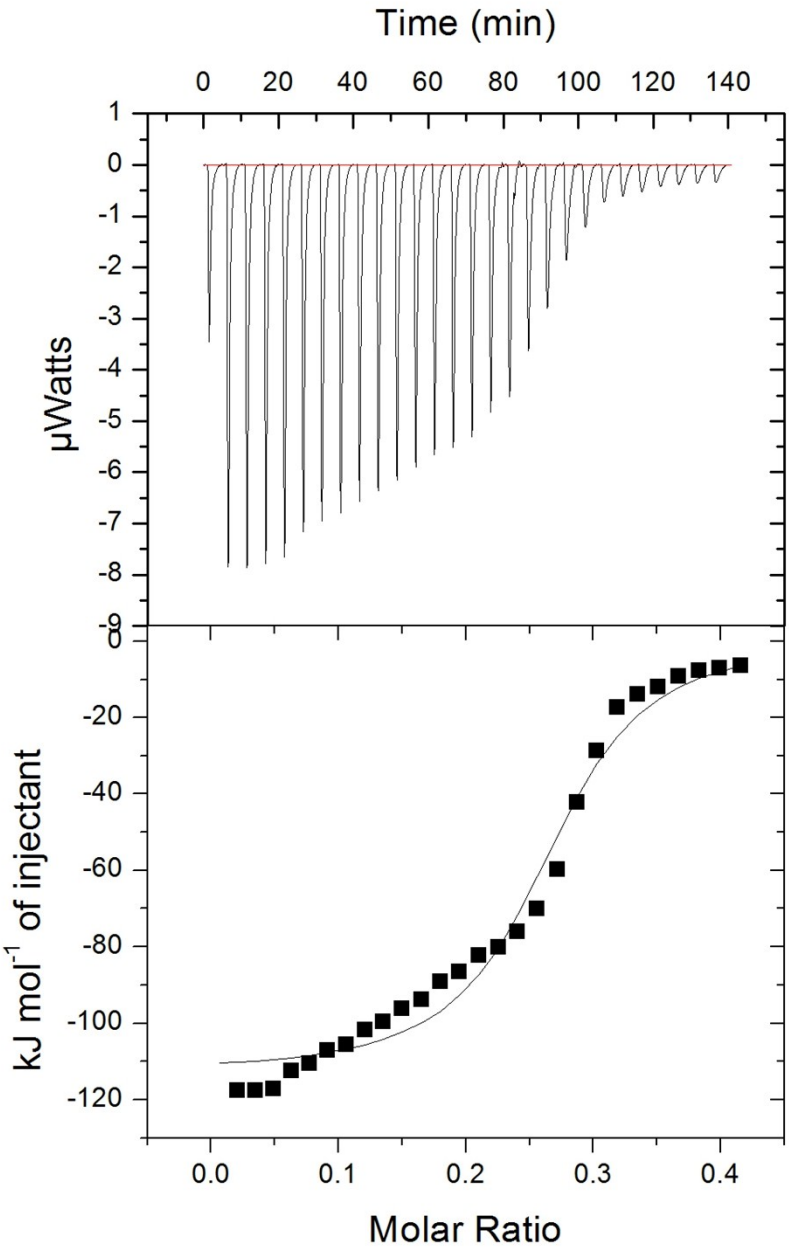

150.8 150.7 150.6 149.2 149.1 149.1 149.1 143.7 128.7 128.6 128.3 128.0 127.8 124.9 124.8 115.4 115.2 115.1 115.1 113.6 104.0 75.6 73.9 71.0 70.1 70.0 69.3 69.2 68.6 68.2 62.4 55.7 55.3 49.9 29.3 29.3 29.3 29.2 29.2
(9)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



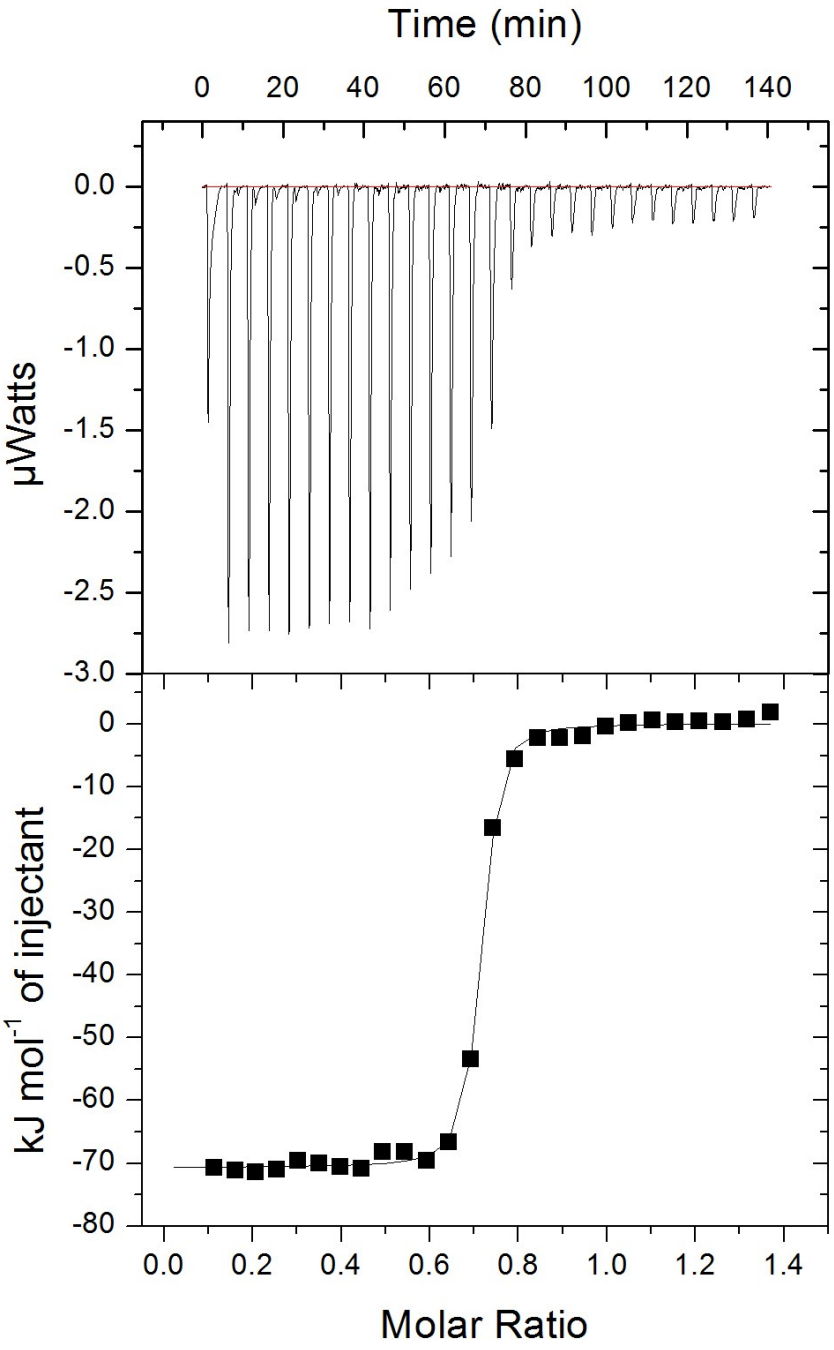
Raw ITC data (top) obtained by injections of **5a** (300 µM) in a solution of LecA (75 µM) and the corresponding integrated titration curve (bottom)



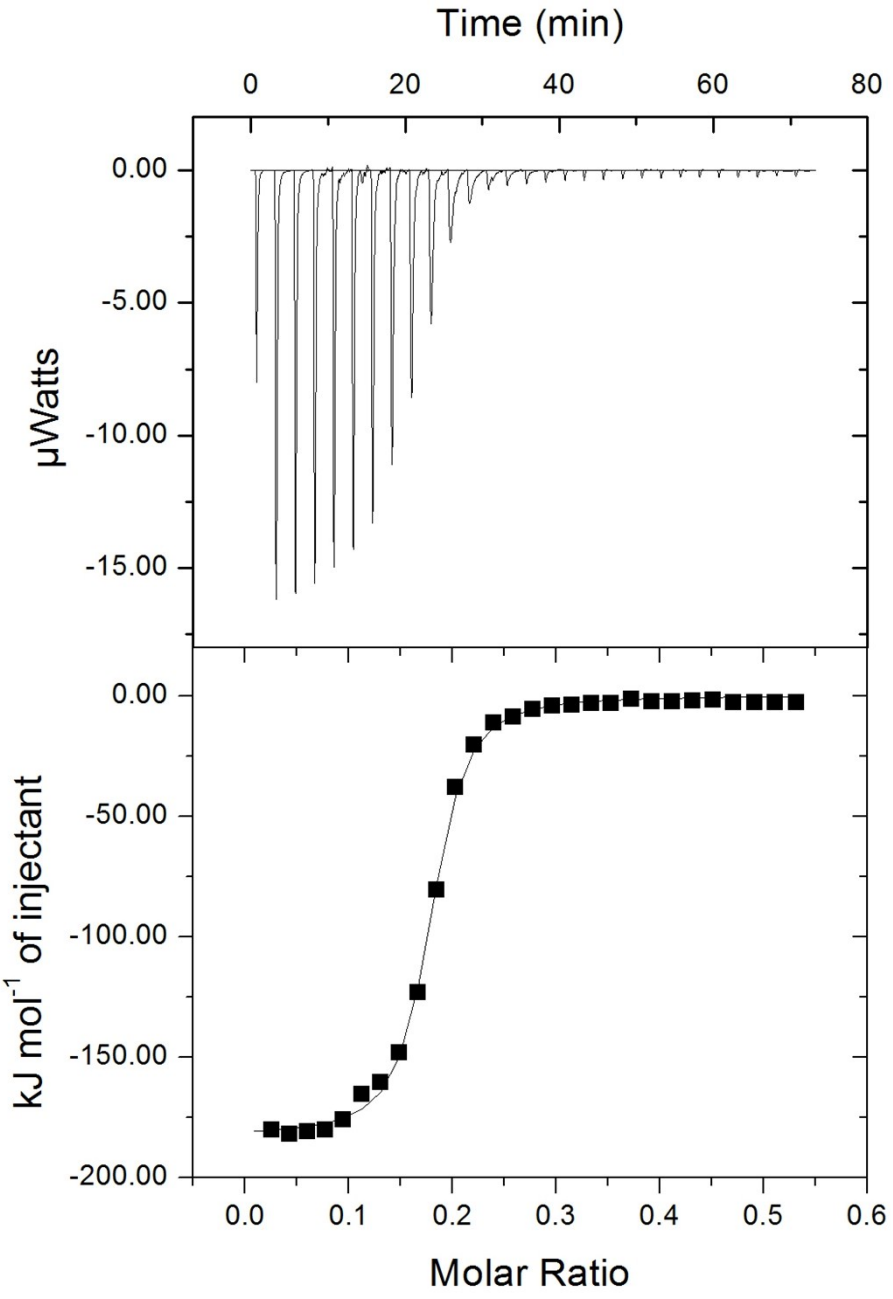
Sensorgramm of **5a** binding to a LecA sensorchip with increasing concentrations from 0.5 nM to 5 µM



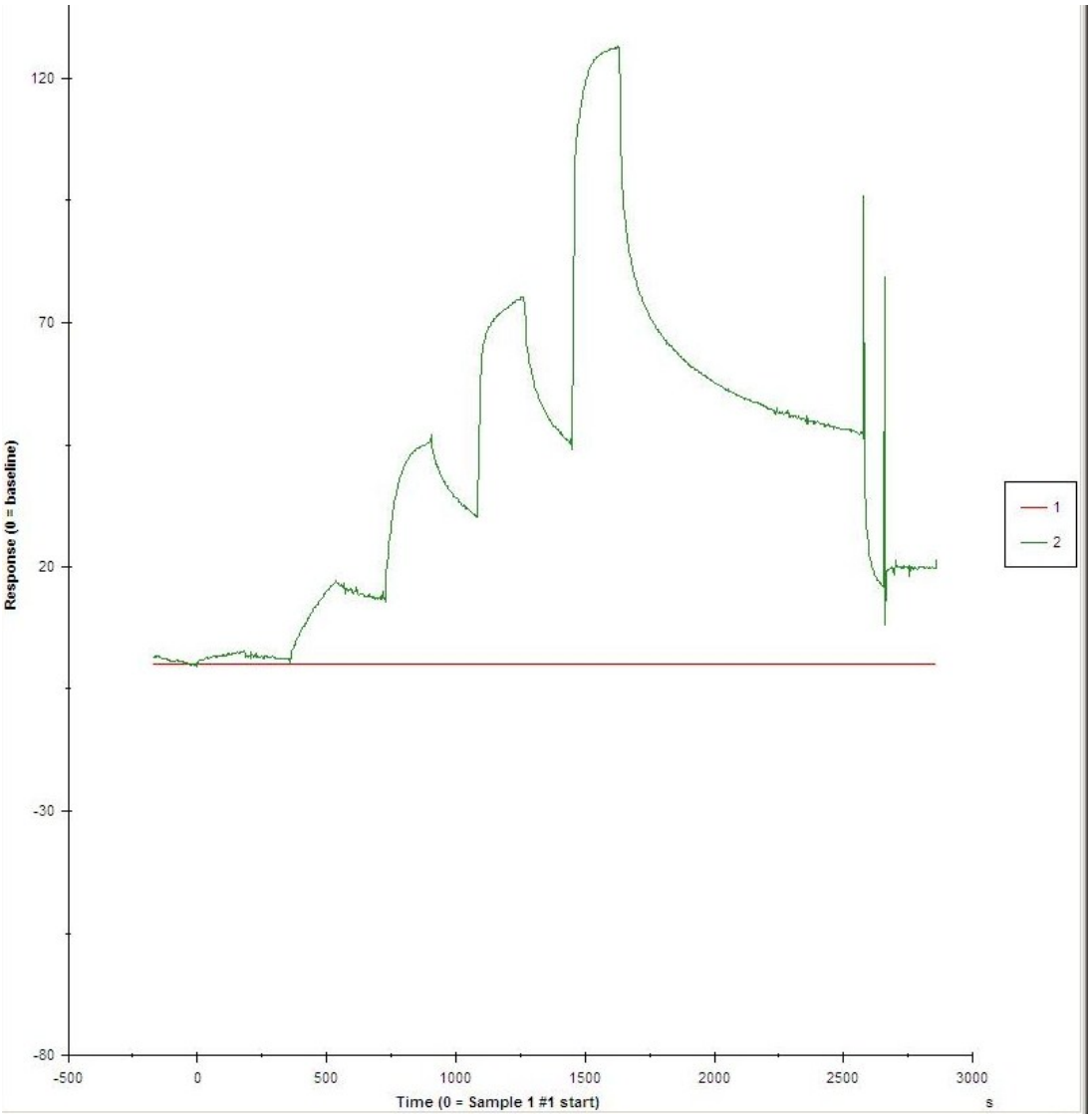
Raw ITC data (top) obtained by injections of **5b** (300 µM) in a solution of LecB (150 µM) and the corresponding integrated titration curve (bottom)



Raw ITC data (top) obtained by injections of **5b** (112 µM) in a solution of BambL (17 µM) and the corresponding integrated titration curve (bottom)



Raw ITC data (top) obtained by injections of **9** (300 µM) in a solution of LecA (122 µM) and the corresponding integrated titration curve (bottom)



Sensorgramm of **9** binding to a LecA sensorchip with increasing concentrations of **9** (from 0.5 nM to5 μM).

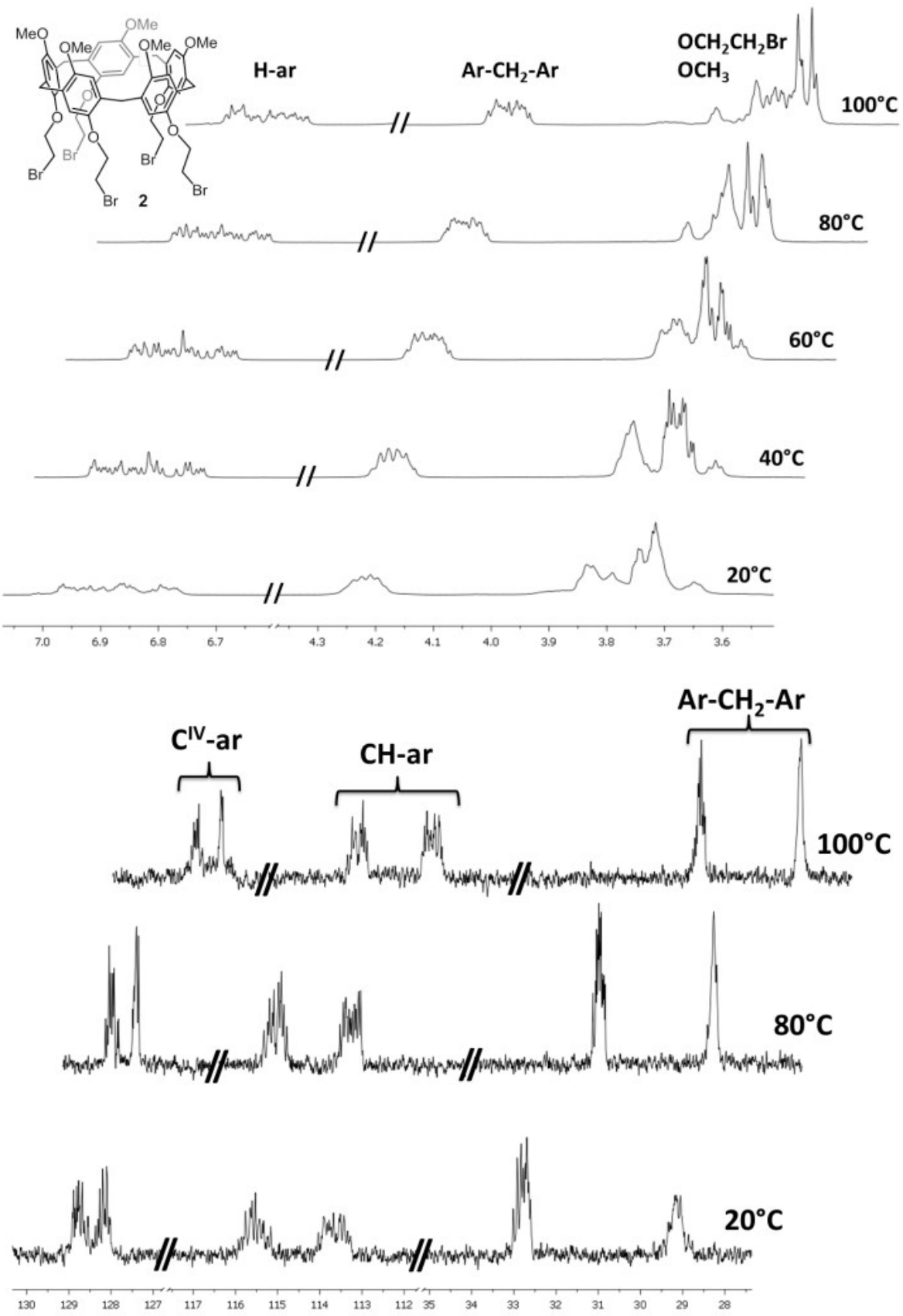


Figure S1. Partial ^{1}H (top) and ^{13}C (bottom) NMR data (400 MHz and 100 MHz respectively, DMSO-d_6) measured for the brominated compound **2**.

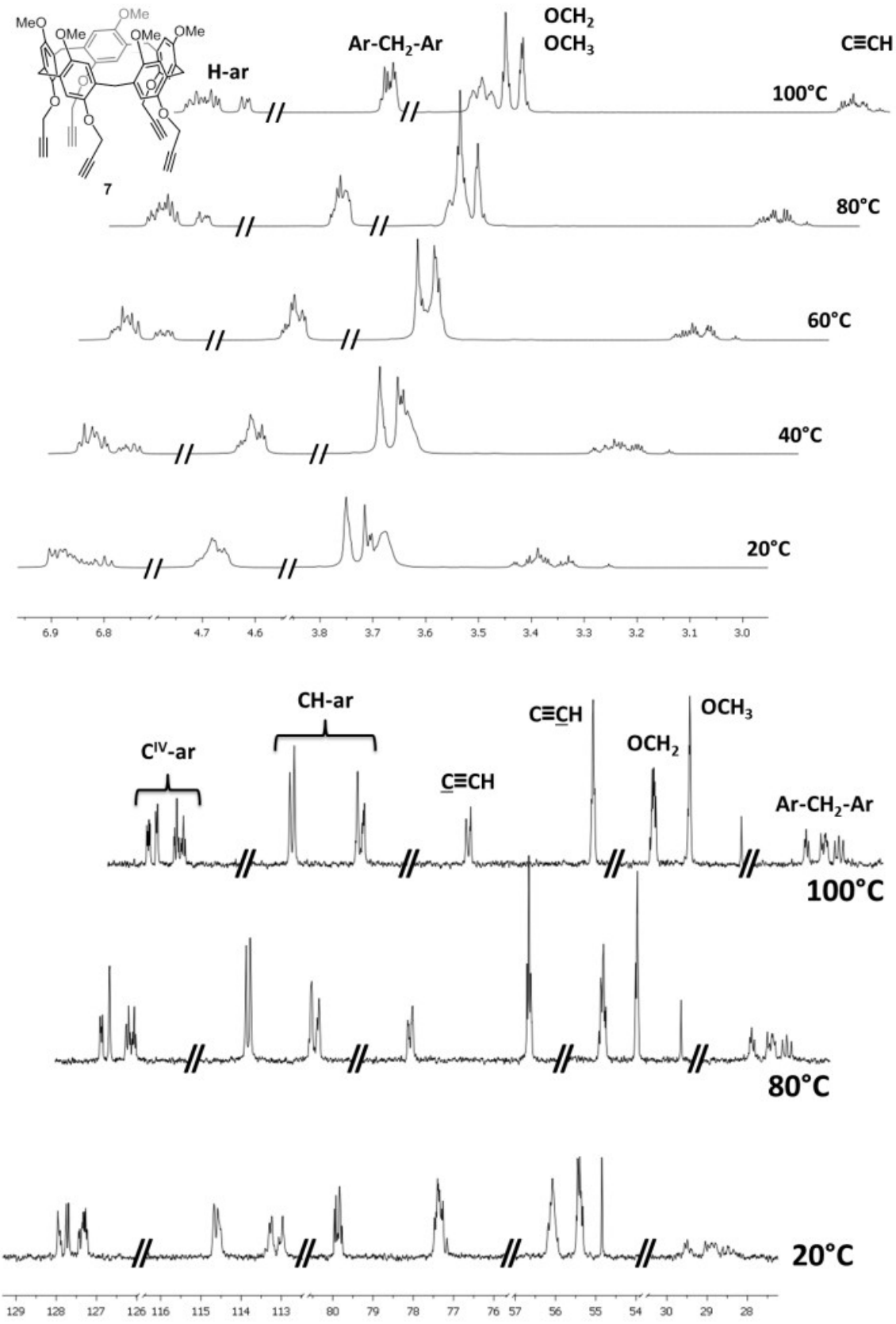


Figure S2. Partial ^{1}H (top) and ^{13}C (bottom) NMR data (400 MHz and 100 MHz respectively, DMSO-d_6) measured for the propargylated compound **7**.