

Supporting Information

Clustering zwitterionic amino acids at the upper rim of cone calix[4]arene triggers the selective recognition of Gram-negative bacterial envelope.

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Experimental section

General procedures for calixarene syntheses and characterization.

Chemicals were purchased from commercial sources and used without further purification, unless otherwise indicated. When anhydrous conditions were required, the reactions were performed in oven-dried glassware under argon atmosphere. Anhydrous solvents over molecular sieves were purchased from Acros Organics® with a content of water $\leq$ 50 ppm. Thin-layer chromatography (TLC) was performed on Silica Gel F₂₅₄ plates or RP-C18 Silica plates (Merck) with UV detection (254 nm and 365 nm) or using appropriate developing solutions. Flash column chromatography was performed on silica gel 230–400 mesh (Merck), according to the procedure described in the literature. Automated flash chromatography was performed on a Biotage® Isolera™ Prime system. NMR experiments were recorded on a Bruker AV400 or a Bruker Avance III 600 MHz NMR spectrometer equipped with HR-MAS probe at 298 K. Chemical shifts (δ) are reported in ppm downfield from the residual solvent peak, whereas coupling constants (*J*) are stated in Hz. The ¹H and ¹³C-NMR resonances of compounds were assigned by means of COSY and HSQC experiments. NMR data processing was performed with MestReNova v14.2.0 software (Mestrelab Research, Santiago de Compostela, Spain). Mass spectra were recorded on a Waters SQ-Detector or a Waters Xevo G2XS MS spectrometer (ESI-MS). All the HR-MS were acquired using a Xevo G2-XS QToF spectrometer in direct infusion at a 10 μ M concentration in H₂O:MeCN 1:1 obtained from a 10 mM stock solution of the ligands in DMSO.

Synthesis of 5,11,17,23-Tetrakis(methyl-N-L-proline)-25,26,27,28-tetrahydroxycalix[4]arene 1.

In a two-neck round bottom flask, p-Hcalix[4]arene (2 g, 4.8 mmol), tetrahydrofuran (45 mL), L-proline (6 mmol for each aromatic of the scaffold) dissolved in water (7 mL), glacial acetic acid (5.6 mL) and formaldehyde (2.0 mL, 37% w/v, 27 mmol) were combined and left stirring at room temperature for 96 hours. The liquid was decanted and drained well from the solid that deposited on the flask wall. The white solid was then triturated with acetone and collected by filtration. The precipitate was washed with acetone and then recrystallized from water/ethanol/acetone (1:1:2)- to yield a white solid (3.7 g; yield 77%). ¹H NMR (D₂O): δ (ppm) 7.03 (s, 8H, ArH), 3.88–3.83 (broad s, 8H, ArCH₂Ar), 3.82–3.67 (s, 8H, ArCH₂N), 3.67–3.54 (broad t, 4H, NCH), 3.35–3.18 (broad m, 4H, CH₂N), 2.98–2.75 (broad m, 4H, CH₂N), 2.20–1.82 (broad m, 4H, CHCH₂), 1.82–1.40 (broad m, 12H CHCH₂). The compound showed the same physico-chemical properties as those reported in literature.¹

Synthesis of 5,11,17,23-Tetrakis(methyl-N-glycine)-25,26,27,28-tetrahydroxycalix[4]arene (2).

In a two-neck round bottom flask p-Hcalix[4]arene (0.5 g, 1.18 mmol), ethanol (3 mL), toluene (10 mL), glycine (5 mmol for each aromatic of the scaffold) dissolved in water (2 mL), and formaldehyde (0.5 mL, 37% w/v, 27 mmol) were combined and left stirring at 100 °C for 72 hours. After cooling to room temperature, the white solid was filtered and triturated with hot methanol to give the pure product as a white solid (0.630 g, yield 69%) mp >280 °C dec, ¹H NMR (400 MHz, D₂O+NaOD) δ (ppm) 6.96 (s, 8H, ArH), 4.15 (broad s, 4H, ArCH_{ax}Ar), 3.83 (s, 8H, NH-CH₂-COOH), 3.24 (broad s, 12H, ArCH₂N+ArCH_{eq}Ar); ¹³C NMR (100 MHz, D₂O+NaOD) δ (ppm) 171.4 (C=O), 153.5 (C–OH), 131.3 (Ar), 129.8 (Ar), 122.1 (Ar), 49.9 (NH-CH₂-COOH), 47.7 (Ar-CH₂-N), 31.4 (Ar-CH₂-Ar). ESI-MS: m/z calcd for C₄₀H₄₅N₄O₁₂ [(2+H)⁺] 773.30, found 773.15, calcd for C₈₀H₈₉N₈O₂₄ [(2+2+H)⁺] 1545.59 found 1545.15. HR-MS: m/z calcd for C₄₀H₄₅N₄O₁₂ [(2+H)⁺] 773.3028, found 773.3041.

Synthesis of 5,11,17,23-Tetrakis(methyl-N-sarcosine)-25,26,27,28-tetrahydroxycalix[4]arene (3).

In a two-neck round bottomed flask p-Hcalix[4]arene (0.5 g, 1.18 mmol), methanol (3 mL), THF (10 mL), sarcosine (5 mmol for each aromatic of the scaffold) dissolved in water (2 mL), and formaldehyde (0.5 mL, 37% w/v, 27 mmol) were combined and left stirring at 80 °C for 96h. After cooling to room temperature the white solid formed was filtered and triturated with cold methanol and then acetone to give a white solid (0.200 g, yield 54%) mp >293 °C dec, ¹H NMR (400 MHz, D₂O+NaOD) δ (ppm) 6.93 (s, 8H, ArH), 4.12 (broad s, 4H, ArCH_{ax}Ar), 3.27 (broad s, 12H, ArCH₂N+ArCH_{eq}Ar), 2.88 (s, 8H, N-CH₂-COOH), 2.01 (s, 12H, OCH₃); ¹³C NMR (100 MHz, D₂O+NaOD) δ (ppm) 178.6 (C=O), 152.8 (C–OH), 130.4 (Ar), 129.8 (Ar), 127.3 (Ar), 59.8 (N-CH₂-COOH), 59.5 (Ar-CH₂-N), 40.6 (N-CH₃), 31.5 (Ar-CH₂-Ar); ESI-MS: m/z calcd for C₄₄H₅₃N₄O₁₂ [(3+H)⁺] 829.36, found 829.09, calcd for C₄₄H₅₂N₄NaO₁₂ [(3+Na)⁺] 851.34, found 851.24. HR-MS: m/z calcd for C₄₄H₅₃N₄O₁₂ [(3+H)⁺] 829.3654, found 829.3671.

General procedure for synthesis of 4, 5 and 6.

In a two-neck round-bottom flask the p-Hcalix[4]arene (1 mmol), tetrahydrofuran (15 mL) and the appropriated amino acid (6 mmol for each aromatic of the calixarene) were dissolved in water (2 mL). Glacial acetic acid (2.8 mL) and formaldehyde (0.5 mL, 37% w/v, 7 mmol) were combined and left stirring at room temperature for 96 hours. The precipitated solid was filtered and washed with cold water.

Synthesis of 5,11,17,23-Tetrakis(methyl-N-L-valine)-25,26,27,28-tetrahydroxycalix[4]arene (4).

The pure product was obtained after trituration with methanol and then acetone to give a white solid (0.350 g, yield 31%) mp >300 °C dec; ¹H NMR (400 MHz, D₂O+NaOD) δ (ppm) 6.89 (s, 8H, ArH), 4.17 (broad s, 4H, ArCH_{ax}Ar), 3.38–3.12 (m, 12H, ArCH₂N+ArCH_{eq}Ar), 2.67–2.66 (d, *J* = 6 Hz, 4H, NHCHCOOH), 1.69–1.61 (m, 4H, CH(CH₃)₂), 0.74–0.70 (dd,

$J = 7$ Hz, 24H, $(CH_3)_2$; ^{13}C NMR (100 MHz, $D_2O + NaOD$) δ (ppm) 181.8 (C=O), 152.2 (C–OH), 130.6 (Ar), 129.7 (Ar), 128.4 (Ar), 68.5 (N–CH–COOH), 50.8 (Ar–CH₂–N), 31.6 (Ar–CH₂–Ar), 30.5 ($CH(CH_3)_2$), 18.6 (CH_3), 18.3 (CH_3). ESI-MS: m/z calcd for $C_{52}H_{69}N_4O_{12}$ $[(4+H)^+]$ 941.49, found 941.34, calcd for $C_{52}H_{68}N_4NaO_{12}$ $[(4+Na)^+]$ 963.47 found 963.25. HR-MS: m/z calcd for $C_{52}H_{69}N_4O_{12}$ $[(4+H)^+]$ 941.4907, found 941.4944.

Synthesis of 5,11,17,23-Tetrakis(methyl-N-L-phenylalanine)-25,26,27,28-tetrahydroxycalix[4]arene (5).

The pure product was obtained after trituration with tetrahydrofuran and then methanol to give a pale-yellow solid (0.450g, yield 35%) mp $>320^\circ C$ dec; 1H NMR (400 MHz, $D_2O + NaOD$) δ (ppm) 6.67–6.59 (broad m, 28 H, ArH), 4.09 (broad s, 4H, $ArCH_{ax}Ar$), 3.15–2.98 (broad m, 16H, $ArCH_2N + ArCH_{eq}Ar + NCHCOOH$), 2.62–2.43 (m, 8H, CH_2 Phe); ^{13}C NMR (100 MHz, $D_2O + NaOD$) 181.3 (C=O), 151.8 (C–OH), 137.8 (Ar), 130.9 (Ar), 129.5 (Ar), 128.6 (Ar), 128.1 (Ar), 126.2 (Ar), 63.1 (N–CH–COOH), 50.2 (Ar–C–N), 38.6 (Ar–CH₂–CH), 31.6 (Ar–CH₂–Ar). ESI-MS m/z calcd for $C_{68}H_{69}N_4O_{12}$ $[(5+H)^+]$ 1133.49, found 1133.27, calcd for $C_{68}H_{68}N_4NaO_{12}Na$ $[(5+Na)^+]$ 1155.47, found 1155.24. HR-MS: m/z calcd for $C_{68}H_{69}N_4O_{12}$ $[(5+H)^+]$ 1133.4907, found 1133.4913.

Synthesis of 5,11,17,23-Tetrakis(methyl-N-L-leucine)-25,26,27,28-tetrahydroxycalix[4]arene (6).

The pure product was obtained by trituration with methanol and then tetrahydrofuran to give a white solid (0.320g, yield 29%) mp $>310^\circ C$ dec, 1H NMR (400 MHz, $D_2O + NaOD$) δ (ppm) 6.73 (s, 8H, ArH), 4.06 (broad s, 4H, $ArCH_{ax}Ar$), 3.25–2.99 (m, 12H, $ArCH_2N + ArCH_{eq}Ar$), 2.75–2.71 (t, $J = 7$ Hz, 4H, $NHCHCOOH$), 1.11–0.99 (m, 8H, $CH_2(CH_3)_2$), 0.47 (d, $J = 6.3$ Hz, 12H, CH_3), 0.23 (d, $J = 6.3$ Hz, 12H, CH_3); ^{13}C NMR (100 MHz, $D_2O + NaOD$) δ (ppm) 183.2 (C=O), 152.8 (C–OH), 130.4 (Ar), 129.1 (Ar), 128.4 (Ar), 61.1 (N–CH–COOH), 50.5 (Ar–CH₂–N), 42.3 ($CH-CH_2-CH(CH_3)_2$), 31.4 (Ar–CH₂–Ar), 24.4 ($CH(CH_3)_2$), 21.8 (CH_3), 21.5 (CH_3); ESI-MS: m/z calcd for $C_{56}H_{77}N_4O_{12}$ $[(6+H)^+]$ 997.55, found 997.27, calcd for $C_{56}H_{76}N_4NaO_{12}$ $[(6+Na)^+]$ 1019.54, found 1019.24, calcd for $C_{56}H_{75}Na_2N_4O_{12}$ $[(6-H+2Na)^+]$ 1041.52 found 1041.37. HR-MS: m/z calcd for $C_{56}H_{77}N_4O_{12}$ $[(6+H)^+]$ 997.5533, found 997.5504.

Synthesis of 5,11,17,23-tetrakis(bis-Boc-guanidino)-tetrakis[25,26,27,28-(ethoxycarbonylmethoxy)]calix[4]arene (13).

Compound **12**² (436 mg, 0.526 mmol) was dissolved in 10 mL of DMF, then Bis-Boc-thiourea (872mg, 3.16 mmol), $HgCl_2$ (1.14 g, 4.21 mmol) and TEA (0.6 mL) were added in sequence. The mixture was stirred for 16 hours and monitored by TLC (hexane/ethyl acetate 7/3) and upon completion it was quenched by addition of water (20 mL). The product was extracted with DCM (2x20 mL). The combined organic phases were dried over anhydrous Na_2SO_4 , then the solvent was removed under reduced pressure. The crude was purified by column chromatography (eluent, hexane/ethyl acetate 8/2) and pure product **13** was obtained as a yellowish solid in 35% yield (385 mg, 0.21 mmol). 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 11.56 (s, 4H, NHBoc), 9.82 (s, 4H, $NHC(=NBoc)NHBoc$), 6.94 (bs, 8H, ArH), 4.86 (d, $J = 13.3$ Hz, 4H, $ArCH_{ax}H_{eq}Ar$), 4.71 (bs, 8H, $ArOCH_2$), 4.17 (q, $J = 7.0$ Hz, 8H, OCH_2), 3.21 (d, $J = 13.3$ Hz, 4H, $ArCH_{ax}H_{eq}Ar$), 1.50 – 1.40 (m, 72H, $OC(CH_3)_3$), 1.27 (t, $J = 7.1$ Hz, 12H, OCH_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.1 ($COOEt$), 163.5 (Cguanidinium), 153.4, 153.0, 134.2, 131.4, 123.3 (Ar), 83.1 and 79.1 ($C(CH_3)_3$), 71.4 (OCH_2CO), 60.4 (OCH_2CH_3), 31.8 ($ArCH_2Ar$), 28.1, 28.2 ($C(CH_3)_3$), 14.2 (OCH_2CH_3). ESI-MS: m/z calcd for $C_{88}H_{124}N_{12}O_{28}$ $[M+H]^+$ 1796.86, obs 1798.02; calc for $C_{83}H_{117}N_{12}O_{26}$ $[(M-Boc) + H]^+$ 1697.82, obs 1697.92, calc for $C_{78}H_{109}N_{12}O_{24}$ $[(M-2Boc) + H]^+$ 1597.77, obs 1597.95.

Synthesis of 5,11,17,23-tetra-guanidino-tetrakis[25,26,27,28-(hydroxycarbonylmethoxy)]calix[4]arene, tetrahydrochloride (7).

In a round-bottom flask compound **13** (100 mg, 0.056 mmol) was dissolved in 1,4-dioxane (1 mL) then HCl (37% in water, 1 mL) was added to the solution. The mixture was stirred for 1 hour, then the solvent was evaporated. Trituration in Et_2O of the yellowish solid afforded the product **7** as a white powder in 99% yield (35 mg, 0.039 mmol). 1H NMR (400 MHz, D_2O) δ (ppm) 6.89 (s, 8H, ArH), 4.85 (s, 8H, $ArOCH_2$), 4.75 (partially overlapping with HOD, d, 4H, $ArCH_{ax}H_{eq}Ar$), 3.45 (d, $J = 13.6$ Hz, 4H, $ArCH_{ax}H_{eq}Ar$). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 173.6 ($COOH$), 156.2 ($C(NH)_3$), 154.0, 136.2, 129.7 (Ar), 126.1 (Armeta), 71.5 (OCH_2), 30.5 ($ArCH_2Ar$). ESI-MS: m/z calcd for $C_{40}H_{45}N_{12}O_{12}$ $[M-3H]^+$ 885.33, obs 885.19; calcd for $C_{40}H_{46}N_{12}O_{12}$ $[M-2H]^+$ 885.33, obs 885.19; calcd for $C_{40}H_{46}N_{12}O_{12}$ $[M-2H]^+$ 443.16, obs 443.29. HR-MS: m/z calcd for $C_{40}H_{45}N_{12}O_{12}$ $[(M-3H)^+]$ 885.3274, found 885.3294.

Synthesis of 5,11,17,23-Tetrakis[(bis-Boc-guanidino)methyl]-25,26,27,28-tetrahydroxycalix[4]arene (11).

Compound **10**³ (500mg, 0.63 mmol) was dissolved in 15 mL of DMF, then potassium carbonate (504 mg, 3.65 mmol) and bis-Boc-guanidine (947 mg, 3.65 mmol) were added in sequence. The mixture was heated at $70^\circ C$ and stirred for 48 hours. Upon completion, the reaction mixture was quenched by addition of water (40 mL) and then extracted with DCM (2x30 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and then the solvent was removed under reduced pressure. The crude was purified by column chromatography (eluent, hexane/ethyl acetate 7/3) thus obtaining the product as a yellowish solid in 34% yield (124 mg, 0.085 mmol). 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.4–9.1 (bs, 4H, OH), 6.7–7.1 (bs, 8H, ArH), 4.4 – 4.2 (m, 8H, $ArCH_2N$), 3.5 – 3.1 (m, 8H, $ArCH_2Ar$), 1.75 – 1.25 (m, 72H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) (most significant peaks) 163–157 (CO), 154 (Ar), 130.7 (Ar), 127.7 (ArH), 78.0 and 76.3 ($C(CH_3)_3$), 44.5 ($ArCH_2N$), 32.9 ($ArCH_2Ar$), 28.7–28.0 ($C(CH_3)_3$). ESI-MS: m/z calcd for $C_{76}H_{109}N_{12}O_{20}$ $[M+H]^+$

1509.79, obs 1509.70; calc for $C_{71}H_{101}N_{12}O_{18}[(M-Boc) + H]^+$ 1409.74, obs 1409.58; calc for $C_{66}H_{93}N_{12}O_{16}[(M-2Boc) + H]^+$ 1309.68, obs 1309.62.

Synthesis of 5,11,17,23-tetrakis[guanidinomethyl]-25,26,27,28-tetrahydroxycalix[4]arene, tetratrifluoroacetate (9).

Compound **11** (100 mg, 0.133 mmol) was dissolved in DCM (3 mL) then TFA (3 mL) was added to the solution. After 1 hour the solvent was removed under reduced pressure and a brown solid was obtained. The product was purified by trituration in Et₂O/EtOH and dried under vacuum to afford a yellow solid (21 mg, 0.029 mmol) in 22% yield. ¹H NMR (400 MHz, CD₃OD, 328K) δ (ppm) 7.11 (s, 8H, ArH), 4.20 (s, 8H, ArCH₂N), 3.94 (bs, 8H, ArCH₂Ar). ¹³C NMR (100 MHz, D₂O) δ (ppm) 163.0 (CF₃COO, J_{CF} = 35Hz), 156.7 (C(NH)₃), 148.4 (Ar), 128.9 (Ar), 127.6 (Ar), 116.3 (CF₃, J = 292 Hz), 43.9 (ArCH₂N), 30.4 (ArCH₂Ar). HR-MS: m/z calcd for C₃₆H₄₅N₁₂O₄ [M-3H]⁺ 709.3681, obs 709.3714.

Synthesis of 5,11,17,23-Tetrakis(methyl-N-L-proline methylester)-25,26,27,28-tetrahydroxycalix[4]arene (1a), tetrahydrochloride.

Compound **1** (100 mg, 0.035 mmol) was dissolved in MeOH (10 mL) and the solution was cooled to 0°C. Then oxalyl chloride (1.1 mmol) and DMF (1 drop) were added. The solution was stirred for 20 h at room temperature after which the solvent removed under reduced pressure. The obtained oil was triturated with diethyl ether 2 times, obtaining a yellow solid in a 58% yield (60 mg). ¹H NMR (400 MHz, D₂O) δ (ppm) 7.20 (s, 8H, ArH), 4.27 (d, J = 13.0 Hz, 4H, ArCHHN), 4.19 – 4.09 (m, 4H, NCH), 4.06 (d, J = 13.0 Hz, 4H, ArCHHN), 3.62 (s, 12H, NCH₂, ArCH_{ax}Ar), 3.21 (bs, 4H, ArCHH_{eq}Ar), 2.80 (s, 12H, OCH₃), 2.37 (m, 4H, NCHCHH), 2.08 (m, 4H, NCH₂CHH), 1.99 – 1.82 (m, 8H, NCHCHH, NCH₂CHH). ¹³C NMR (100 MHz, D₂O) δ (ppm) 169.5 (COO), 150.1 (C-OH), 131.6 (CH_{meta}), 128.9 (C_{ortho}), 123.0 (C_{para}), 65.35 (NCH), 58.0 (ArCH₂N), 55.4 (NCH₂), 52.5 (OCH₃), 29.9 (ArCH₂Ar), 28.2 (NCHCH₂), 22.4 (NCH₂CH₂). ESI-MS: m/z calcd for C₅₆H₆₉N₄O₁₂ [M+H]⁺ 989.48, obs 989.463. HR-MS: m/z calcd for C₅₆H₆₉N₄O₁₂ [M+H]⁺ 989.4907, obs 989.4954.

Synthesis of 5,11,17,23-Tetrakis(carbonyl-N-L-proline methylester)-25,26,27,28-tetrabenzyloxycalix[4]arene, partial cone (15).

Compound **14** (300 mg, 0.31 mmol) was dissolved in dry DCM (4 mL) then oxalyl chloride (1.19 g, 9.36 mmol) was added to the solution. The mixture was stirred for one hour at room temperature. The solvent was then removed under reduced pressure obtaining a pale-yellow solid. This was then immediately subjected to the following reaction, by dissolving it in dry DCM (4 mL) and DIPEA (0.82 mL). Then L-proline methyl ester (201 mg, 1.56 mmol) was added to the solution. The mixture was stirred for 16 hours at room temperature. Upon completion the reaction was quenched by addition of water (5 mL). The organic phase was separated from the aqueous one and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude was purified on silica (eluent: DCM/MeOH 98/2). A pale-yellow solid was obtained in 13% yield (50 mg). ¹H NMR (400 MHz, CD₃OD, 300K) δ (ppm) 7.69 and 7.62 (d, J = 4 Hz, 1H each, ArH), 7.4-7.3 (m, 18H, ArH), 7.25-6.95 (m, 6H, ArH), 6.80 and 6.78 (d, J = 4 Hz, 1H each, ArH), 4.95-4.8 (m, 8H, OCH₂Ar), 4.75-4.35 (m, 4H, CHN), 4.05-3.40 (m, 26H, CH₂N, OCH₃, ArCH₂Ar), 3.05-2.85 (m, 2H, ArCH₂Ar), 2.6-1.3 (m, 16H, CH₂CH₂CH₂N). The ¹³C NMR spectrum of this compound was not recorded, due to the mixture of conformations present as shown by its ¹H NMR spectrum. ESI-MS: m/z calc for C₈₄H₈₅N₄O₁₆ [M+H]⁺, 1405.61, obs 1406.41; 1428.35 [38%, M+Na]⁺

Synthesis of 5,11,17,23-Tetrakis(carbonyl-N-L-proline)-25,26,27,28-tetrahydroxycalix[4]arene, tetra lithium salt (1b).

Compound **15** (50 mg, 0.035 mmol) and Pd/C (10 mg) were dissolved in MeOH (10 mL) and poured in a Parr flask filled with H₂ gas (3 PSI). The suspension was stirred for 16 h after which it was filtered on celite, and the solvent removed under reduced pressure. The obtained oily compound was readily subjected to the hydrolysis reaction. It was dissolved in water (1 mL) to which LiOH monohydrate (5 mg, 0.11 mmol) was previously added. The reaction was stirred for 16 hours. The solvent was then removed under reduced pressure obtaining the lithium salt in a 90% yield (25 mg) over the two steps. ¹H NMR (400 MHz, D₂O) δ (ppm) 7.17 (bs, 8H, ArH), 4.5 – 4.0 (m, ArCH_{ax}H_{eq}Ar + NCH, 8H), 3.60 – 3.20 (m, CHHN, ArCH_{ax}H_{eq}Ar, 8H), 2.80-2.60 (m, CHHN, 4H), 2.2-1.8 (m, CHCH₂, 8H), 1.7-1.3 (m NCH₂CH₂, 8H). ¹³C NMR (100 MHz, D₂O) δ (ppm) 179.70 (COOH), 171.39 (CONH), 155.23 (Ar), 130.63 (Ar), 127.9 (Ar), 126.47 (Ar), 62.10 (NCH(COOH)CH₂), 50.77 (NCH₂), 31.33 (NCHCH₂), 29.85 (ArCH₂Ar), 25.14 (NCH₂CH₂). HR-MS: m/z calcd for C₅₂H₄₉N₄O₁₆ [M+5H-4Li]⁺ 989.3451, obs 989.3484.

Single Crystal X-ray Diffraction.

A summary of data collection and structure refinement for the reported structures is reported in **Table S1**. Single crystal data were collected with Bruker D8 equipped with a PhotonII area detector (Cu Ka: λ = 1.54184 Å). The intensity data were integrated from several series of exposure frames, collected at 200 K, covering the sphere of reciprocal lattice (Bruker AXS Inc., Madison, Wisconsin, 2012). An absorption correction was applied using the program SADABS.⁴ The structures were solved with the ShelXT⁵ structure solution program and refined with ShelXL⁶ on F² with full-matrix least squares, using the Olex2 software.⁷ Non-hydrogen atoms were refined with anisotropic thermal parameters except for the minor component of the disordered dimethylformamide fragment. Hydrogen atoms were placed in their calculated

positions except for the hydrogen atoms of the phenol groups, which were located from the difference Fourier map, and they were refined with bond distance restraints. The hydrogen atoms of the protonated amino moieties were located from the difference Fourier map and they were then placed at their calculated positions. A carboxylate group was disordered in two positions, which were refined with site occupancy factors of 0.7 and 0.3, respectively. The major component of the disordered carboxylate acted as hydrogen bond acceptor towards a water molecule (O5w), whereas the minor component acted as hydrogen bond acceptor towards a protonated amino group (N1) of a symmetry related calixarene molecule. Graphical material was prepared with the Mercury program.⁸

Table S1. Crystal data and structure refinement for compound 2.

Empirical formula	C ₄₃ H _{64.4} N ₅ NaO _{20.2}
Formula weight	997.58
Temperature/K	200.0
Crystal system	monoclinic
Space group	P2 ₁ /n
a/Å	9.8654(3)
b/Å	27.7900(9)
c/Å	17.9538(6)
α/°	90
β/°	102.504(2)
γ/°	90
Volume/Å ³	4805.5(3)
Z	4
ρ _{calc} /g/cm ³	1.379
μ/mm ⁻¹	1.003
F(000)	2120.0
Crystal size/mm ³	0.08 × 0.07 × 0.02
Radiation	CuKα (λ = 1.54184 Å)
2θ range for data collection/°	6.362 to 140.732
Index ranges	-12 ≤ h ≤ 12, -33 ≤ k ≤ 33, -21 ≤ l ≤ 18
Reflections collected	88994
Independent reflections	9120 [R _{int} = 0.1094, R _{sigma} = 0.0404]
Data/restraints/parameters	9120/7/702
Goodness-of-fit on F ²	1.035
Final R indexes [I > 2σ (I)]	R ₁ = 0.0668, wR ₂ = 0.1791
Final R indexes [all data]	R ₁ = 0.1003, wR ₂ = 0.2056
Largest diff. peak/hole / e Å ⁻³	0.63/-0.40

STD NMR experiments.

Bacterial strains and media.

Pseudomonas putida (ATCC® 47054™), *Escherichia coli* MG1655 (ATCC® 700926™) and *Mycobacterium smegmatis* (ATCC® 700084™) were grown in LB broth at 37 °C aerobically under agitation in orbital shaker at 150 rpm, for 24 hours; *Staphylococcus epidermidis* (ATCC® 12228™) was grown in Nutrient Broth at 37 °C aerobically under agitation in orbital shaker at 150 rpm, for 24 hours. Bacterial cells were harvested and washed 2 times with PBS 10 mM at 1500 ×g for 15 minutes at 4 °C. Pellets were resuspended in PBS 10 mM (OD 600nm = 1.5) and freeze-dried in 750 μL aliquots.

Purification of *Pseudomonas putida* and *E. coli* LPS.

Bacterial strains and media. *Pseudomonas putida* KT2440 (ATCC® 47054™) and *Escherichia coli* MG1655 (ATCC® 700926™) were grown as described above. Bacterial cells were harvested and washed with NaCl 100 mM at 7500 ×g (Eppendorf Centrifuge 5430 R) for 10 minutes and then freeze-dried.

Extraction of *Escherichia coli* MG1655 LOS.⁹ Freeze-dried cells (2.7 g) were resuspended in PCP solution (2.5% w/v),¹⁰ stirred vigorously for 1 hour and extracted 3 times. PCP solution consists of aqueous 90% Phenol-Chloroform-light Petroleum (2:5:8 v/v/v), to which solid phenol was added until limpidness. Light solvents were removed under vacuum and LOS was precipitated from the remaining phenolic phase by adding Milli-Q water drop by drop, maintaining at 4 °C. The solid was harvested by centrifugation at 7500 ×g for 10 minutes, suspended in water and dialysed (MWCO 1000 Da) against distilled water until there was no longer phenol smell. Sample was then freeze-dried (Martin Christ Alpha 1-2 LDplus).

Extraction of *Pseudomonas putida* KT2440 LPS. Freeze-dried cells (1.7 g) were resuspended in PCP solution (2.5% w/v) and extracted as described for *E. coli* cells. The remaining cell pellet from PCP procedure was extracted according to the hot phenol-water protocol.¹¹ Cells were treated with 50 mL of water and an equal volume of 90% phenol; the solution was incubated at 68 °C for 30 minutes. The separation between phenol and water phases was performed by spinning at 7500 $\times g$, 4 °C for 15 minutes. The extraction was repeated three times. The water supernatants were pooled and dialysis (MWCO 1000 Da) against distilled water was used for phenol removal from the water phase, which was finally freeze-dried.

LPS characterization. Samples were screened by Kdo assay, ¹H NMR spectroscopy and discontinuous SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Electrophoresis).¹² Gels were realised with 15% separating gel (lower gel), 5% stacking gel (upper gel).

Samples were prepared from aqueous solutions (1 mg/mL) of cell extracts and were conveniently denatured (100 °C, 5 minutes) adding 25% (v/v) denaturing sample buffer (2-mercaptoethanol (0.5 mL); glycerol (2.5 mL); 3% bromophenol blue (33 μ L); Tris 1 M pH 6.8 (0.6 mL); SDS 20% (2 mL); Milli-Q water until 10 mL). The electrophoretic analyses were performed on Mini Protean III Bio-Rad system, at 150 V (constant voltage) in electrophoresis buffer (TRIS (3 g), glycine (14.4 g), SDS (1 g) in 1L Milli-Q water). Gels were stained according to the Alcian Blue and/or Silver Stain procedures. **Alcian Blue:**¹³ The gel was left 1 hour in the fixing solution (40% ethanol, 5% acetic acid). Then, it was treated with the Alcian Blue solution overnight (0.05 w/v Alcian Blue in fixing solution). The gel was washed with the fixing solution, to remove the background blue colour.

Silver Stain:¹⁴ The gel was left 1 hour in the fixative solution (40% ethanol, 5% acetic acid). Then, it was kept for 20 minutes in the oxidative solution of NaIO₄ (0.1 % in fixative solution) and washed three times with Milli-Q water (10 min) and treated with aqueous silver nitrate (0.1 %) for 30 minutes and subsequently it was quickly washed with Milli-Q water. The colouration was developed with an aqueous solution of Na₂CO₃ (3%) and formaldehyde (0.02%). The stain development was stopped with a solution of acetic acid (1%).

Thiobarbiturate assay for Kdo. Samples were tested for the content of 2-keto-3-deoxymanno-octulosonic acid (Kdo),¹⁵ an LPS marker. Briefly, a solution of 10 mM Kdo was used for calibration and 5 mg/mL solutions of samples were tested. Samples were incubated at 100 °C for 2 hours with an equal volume of 2 M HCl. Then the assay was performed treating with periodate 0.025 M in H₂SO₄ 0.063 M (30 min), 2% NaAsO₂ in HCl 0.5 M and 0.6% thiobarbituric acid (100 °C, 15 min). After adding DMSO, the sample absorbance was read at 549 nm with a spectrophotometer (Varian Cary® 50 UV-Vis Spectrophotometer).

NMR sample preparation.

8 μ L of 10 mM stock solution in d₆-DMSO of test molecule were added to disposable Kel-F inserts for 4 mm ZrO 2 MAS rotor (final concentration 2 mM). For experiments with ligand alone, 32 μ L of deuterated PBS (d-PBS) 10 mM were added to the disposable insert (total volume 40 μ L). For experiments with cells, 2 aliquots of freeze-dried cells (OD 600 nm = 1.5 in 750 μ L) were resuspended in distilled water and then washed in d-PBS 10 mM, by centrifuging for 7.5 min at 3000 $\times g$. Pellets were reunited and suspended in 32 μ L of d-PBS and added to the disposable insert (total volume 40 μ L).

For experiments with LPS, 3.2 μ L of 5 mg/mL in d-PBS 10 mM stock solution of LPS from *E. coli* or *P. Putida* were added to the disposable insert (final LPS concentration 0.4 mg/mL). 28.8 μ L of d-PBS 10 mM were then added to the disposable insert to reach the total volume of 40 μ L.

For experiments with LPS dispersed in d₂₅-SDS micelles, 3.2 of stock solution 5 mg/mL in d-PBS 10 mM of LPS and 4 μ L of 100 mM in d-PB (20 mM) stock solution of d-SDS were added to the disposable insert (final LPS concentration 0.4 or 0.8 mg/mL; final d-SDS concentration 10 mM). 24.8 μ L of d-PBS 10 mM were then added to the disposable insert to reach the total volume of 40 μ L.

NMR spectra acquisition.

NMR spectra were acquired by using a Bruker Avance III 600 MHz NMR spectrometer, equipped with a HR-MAS probe (Bruker Inc.; Billerica, MA, US).¹⁶ Ligand resonances were assigned through 1- and 2D standard spectra (¹H, ¹H-¹H-COSY, ¹H, ¹H-TOCSY, ¹H, ¹H-NOESY and ¹H, ¹³C-HSQC) acquired with the pulse sequences provided by the manufacturer. ¹H-NMR spectra were acquired with 16 scans. STD NMR¹⁷ spectra were acquired by applying the standard pulse sequence with 256 scans, selective irradiation frequency 5.15, 0.5 or -1.0 ppm, off-resonance irradiation frequency 30 ppm, at 37 °C and at a spinning rate of 3 kHz. Each STD NMR experiment was repeated at least three times (biological triplicate). Spectra were processed and analyzed with MestreNova software (Mestrelab, Spain).

Determination of the K_D value for the binding of compound 1 to *E. coli* LPS.

K_D value for the binding of compound 1 to *E. coli* LPS was determined applying the method of the binding isotherm of initial growth rates developed by Angulo *et al.*¹⁸ Five NMR samples containing LPS 50 μ M and a different concentration of compound 1 (0.125, 0.25, 0.5, 0.75 and 1.5 mM) were prepared in d-PBS, pH 7.4, 25 °C. For each ligand concentration, a series of STD spectra was recorded at the liquid state with a TXI probe, varying the saturation time (0.65, 1.0, 1.5, 2.0 and 2.5 s). Both ligand concentration and saturation time parameters were optimized. For each compound 1 concentration, STD-AF values for Pro H δ_b proton were obtained at the different saturation times (STD-AF = $\epsilon(I_0 - I)/I_0$ where (I₀-I) is the

peak intensity in the STD spectrum, I_0 is the peak intensity of an unsaturated reference spectrum and $\varepsilon = [\text{compound } \mathbf{1}]_0/[\text{LPS}]_0$ and fit by using the equation $\text{STD-AF}(t_{\text{sat}}) = \text{STD-AF}_{\text{max}}[1 - \exp(-k_{\text{sat}}t_{\text{sat}})]$. The initial slopes, STD-AF_0 , were obtained from $\text{STD-AF}_0 = \text{STD-AF}_{\text{max}}k_{\text{sat}}$. STD-AF_0 values were then represented as a function of the ligand concentration, and the mathematical fit to a Langmuir isotherm ($y = B_{\text{max}}x/(K_D + x)$) allowed to obtain B_{max} and K_D values.

General schemes for the synthesis of the ligands 2-7 and 9

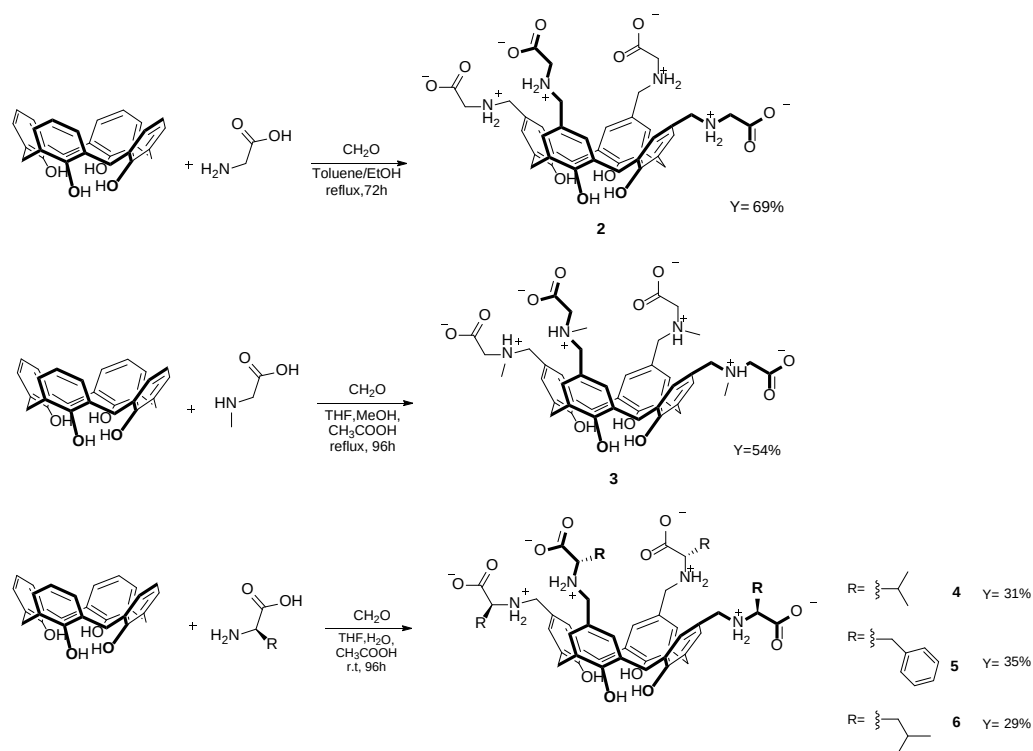


Figure S1. Scheme of synthesis of calix[4]arenes 2, 3, 4, 5 and 6.

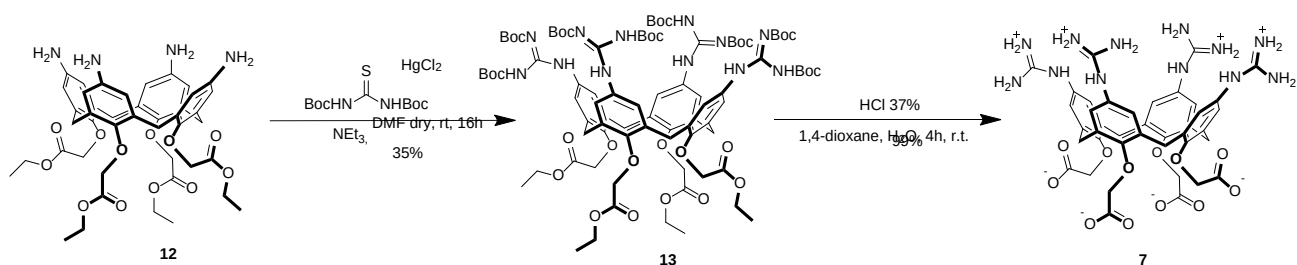


Figure S2. Scheme of synthesis of calix[4]arene 7.

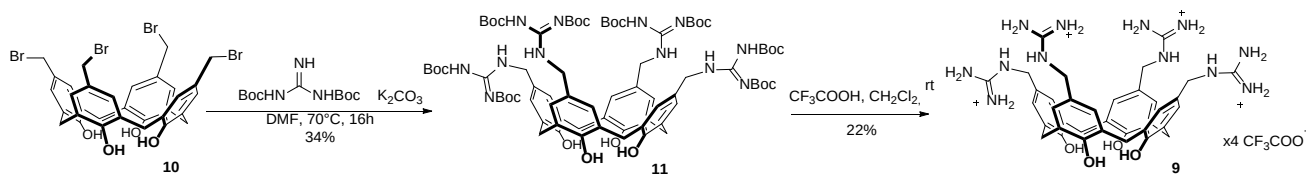


Figure S3. Scheme of synthesis of calix[4]arene 9.

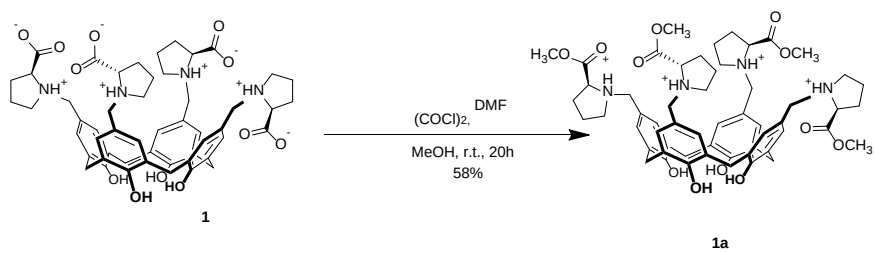


Figure S4. Scheme of synthesis of calix[4]arene **1a**

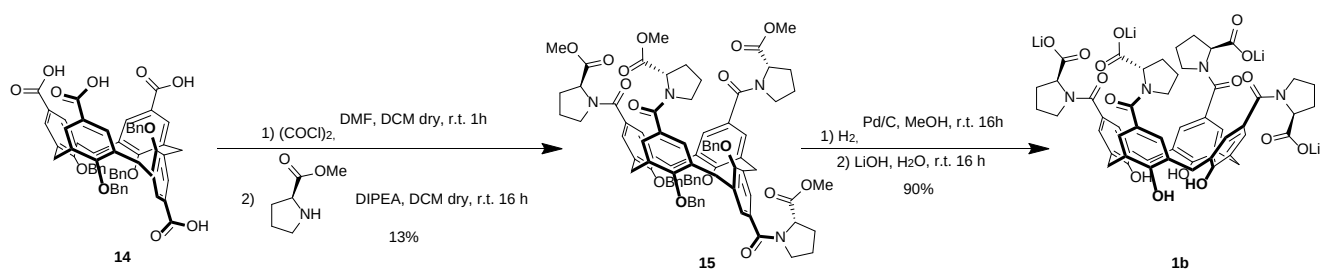


Figure S5. Scheme of synthesis of calix[4]arene **1b**.

NMR and MS spectra of the calixarene ligands 1a,b, 2-7 and 9, and their intermediates

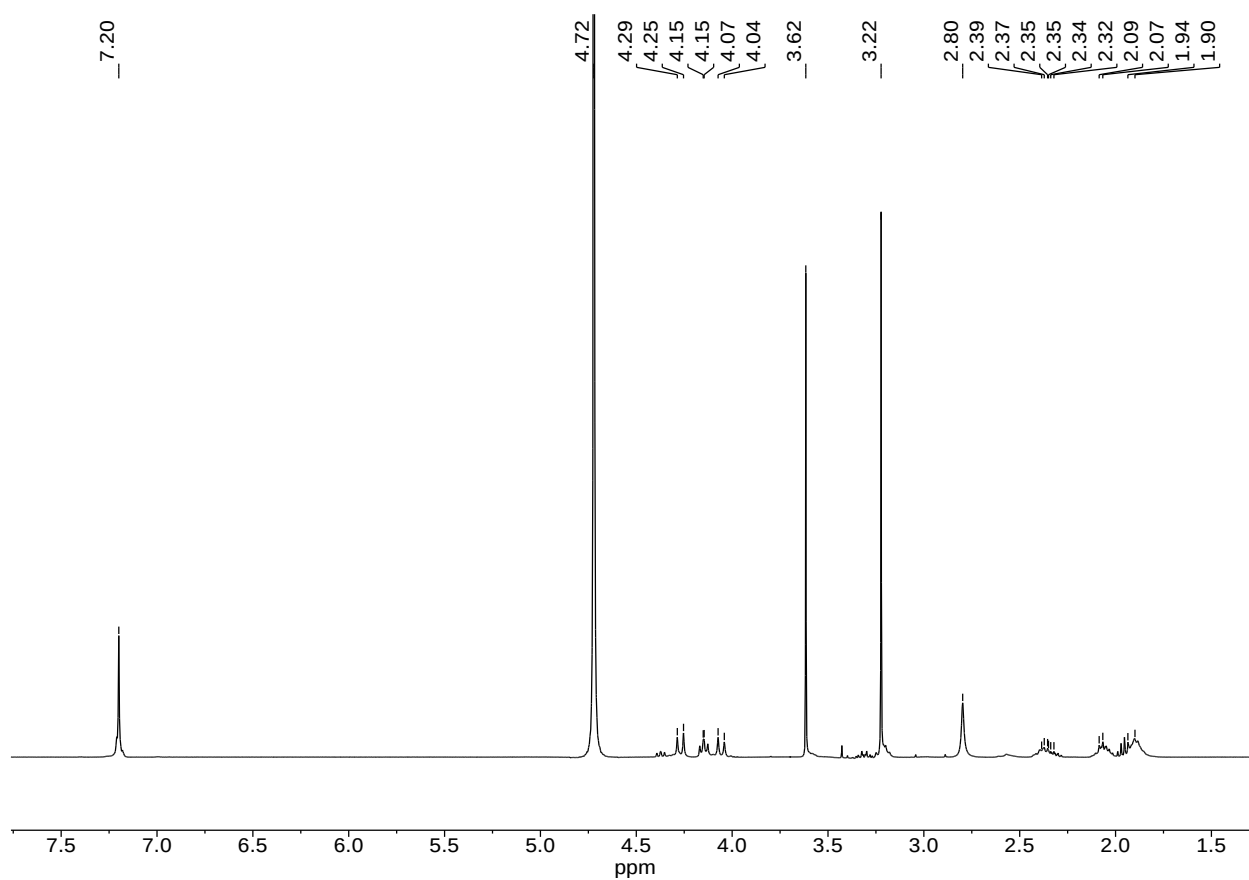


Figure S6. ¹H NMR spectrum (400 MHz, D₂O) of calixarene **1a**.

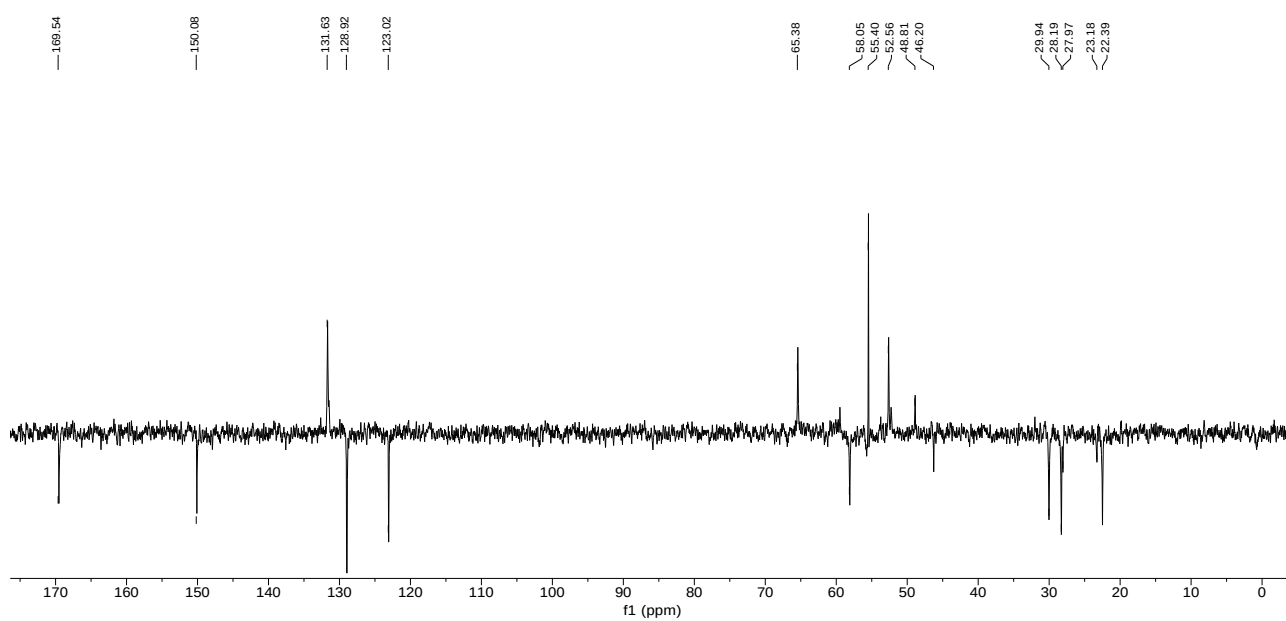


Figure S7. APT spectrum (100 MHz, D₂O) of calixarene **1a**.

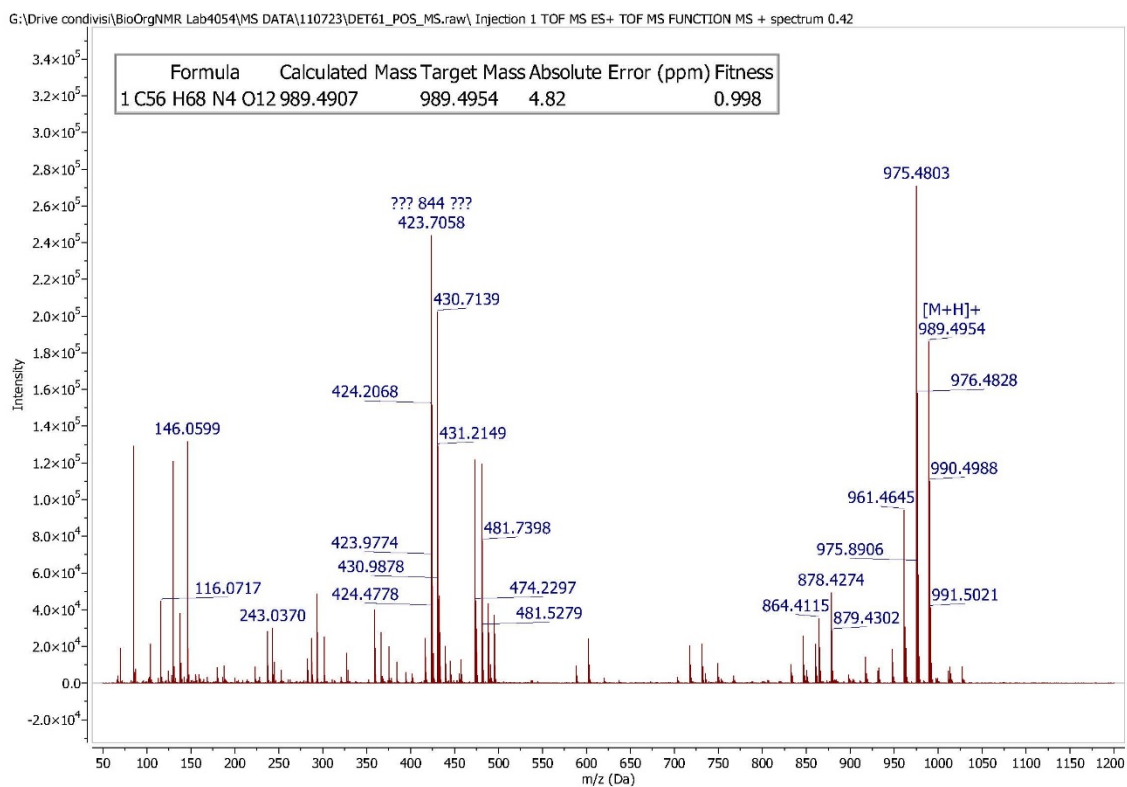


Figure S8. HR-MS (+) spectrum of calixarene **1a**.

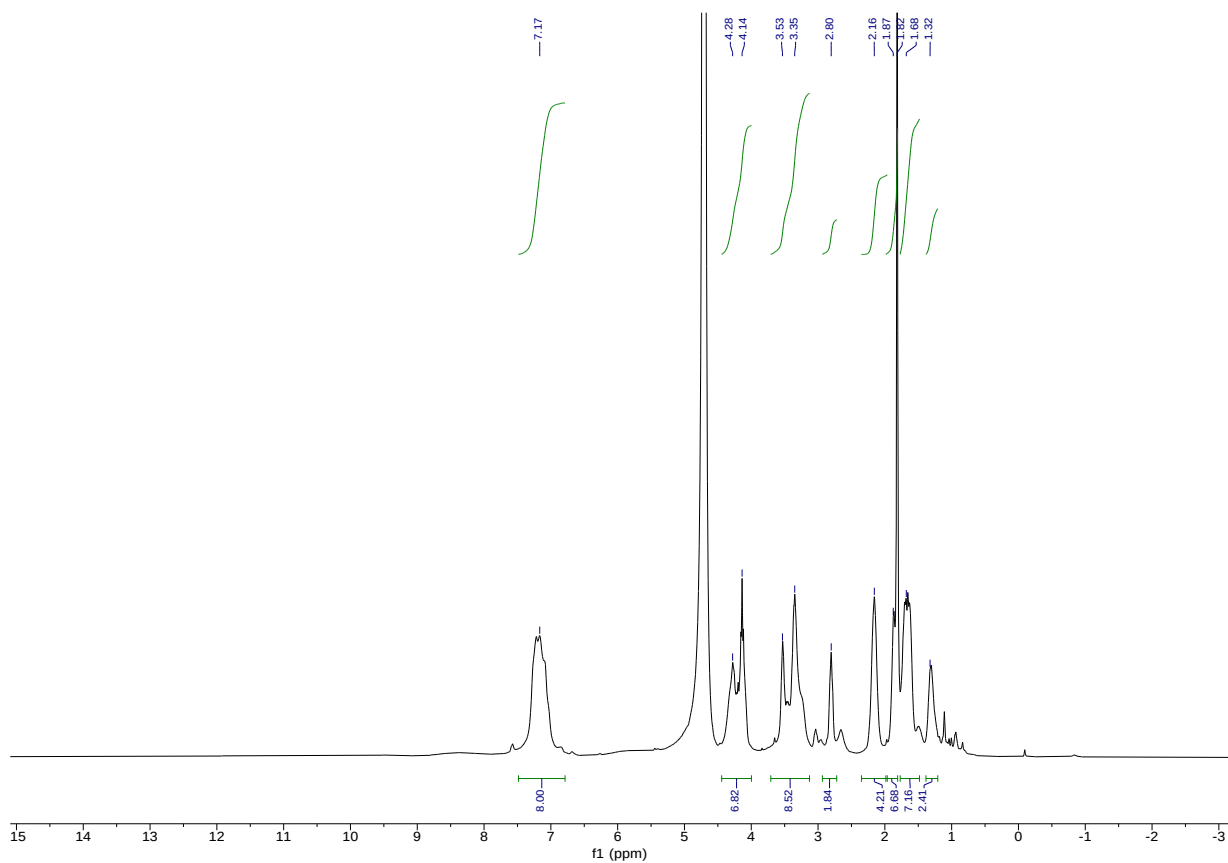


Figure S9. ¹H NMR spectrum (400 MHz, D₂O) of calixarene **1b**.

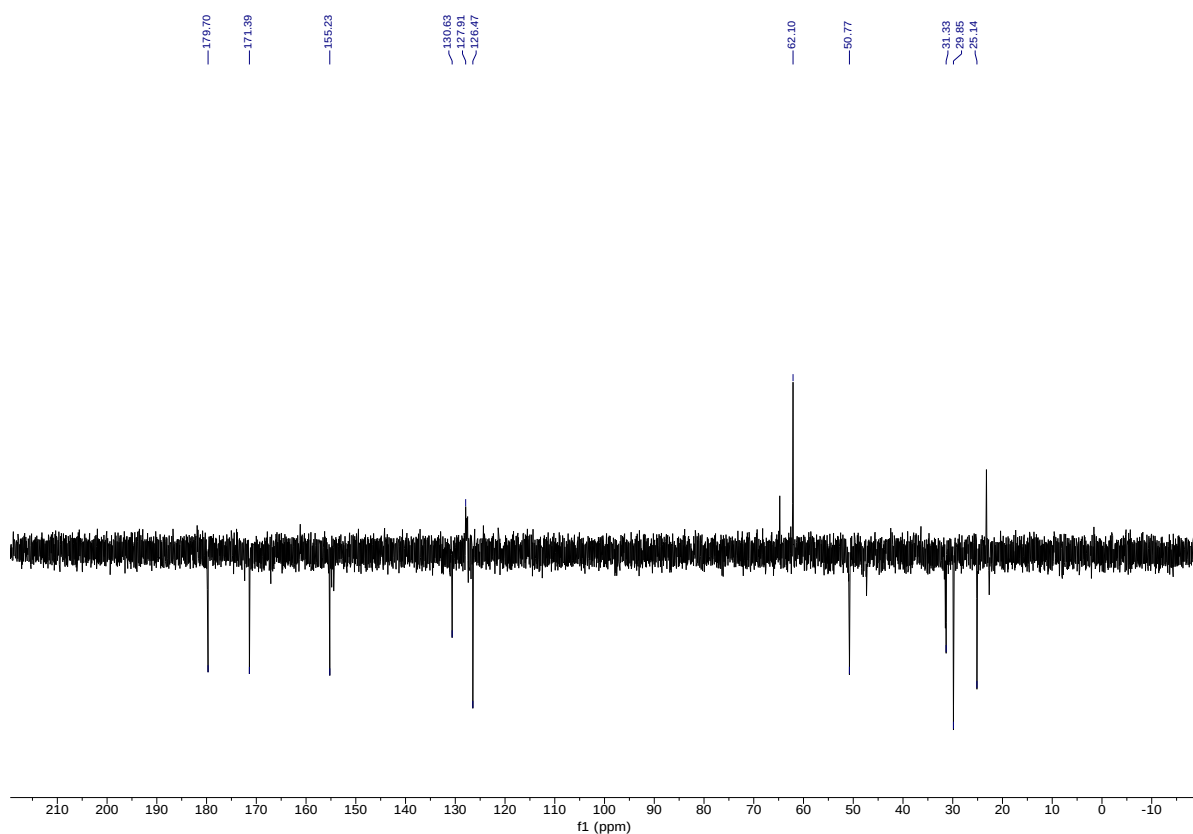


Figure S10. APT spectrum (100 MHz, D₂O) of calixarene **1b**.

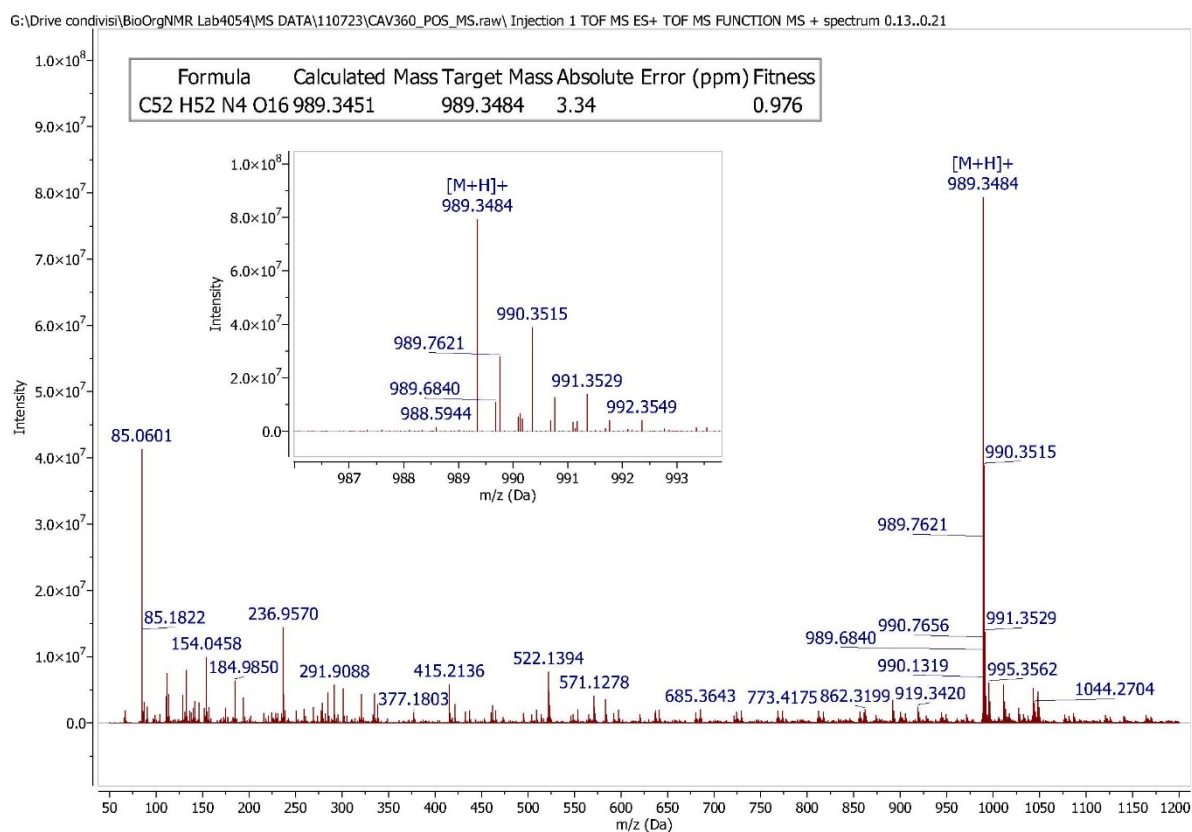


Figure S11. HR-MS (+) spectrum of calixarene **1b**.

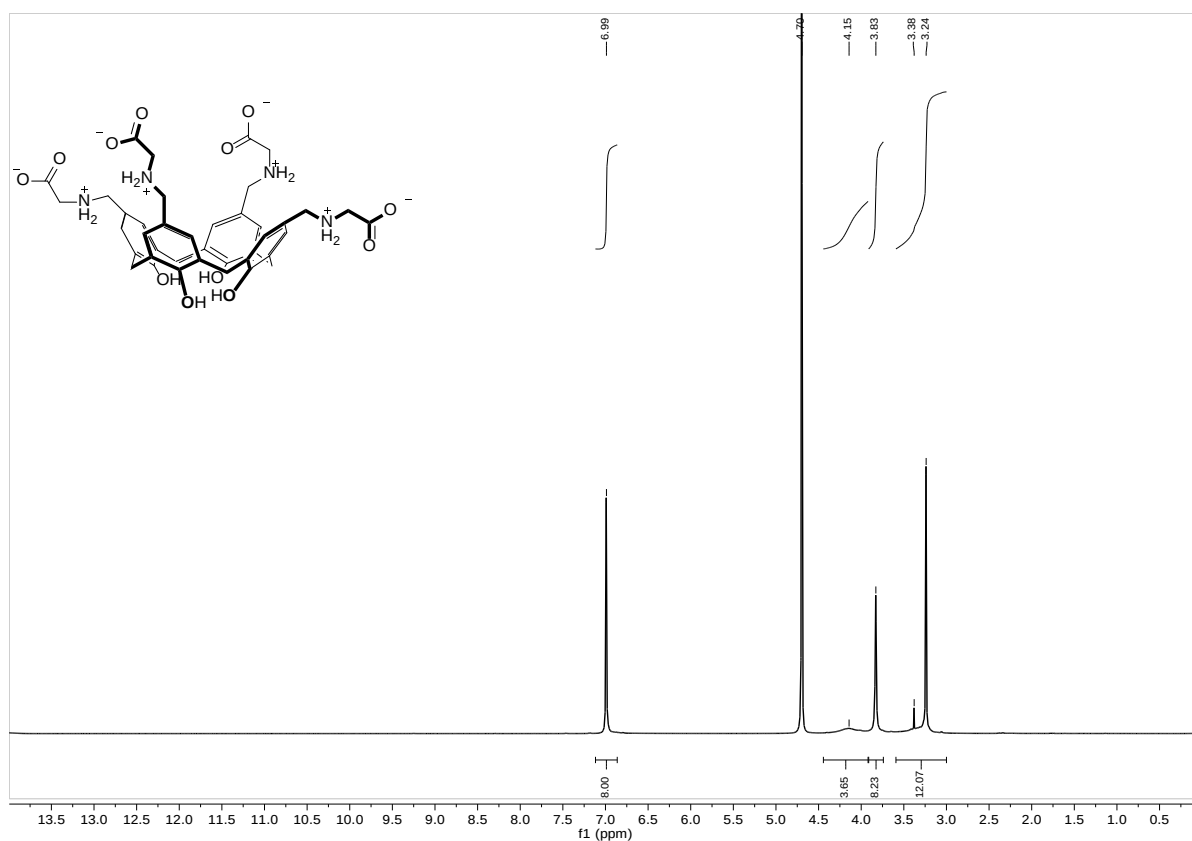


Figure S12. ^1H NMR spectrum (400 MHz, D_2O +NaOD) of calixarene **2**. The peak at 3.38 ppm is due to residual methanol.

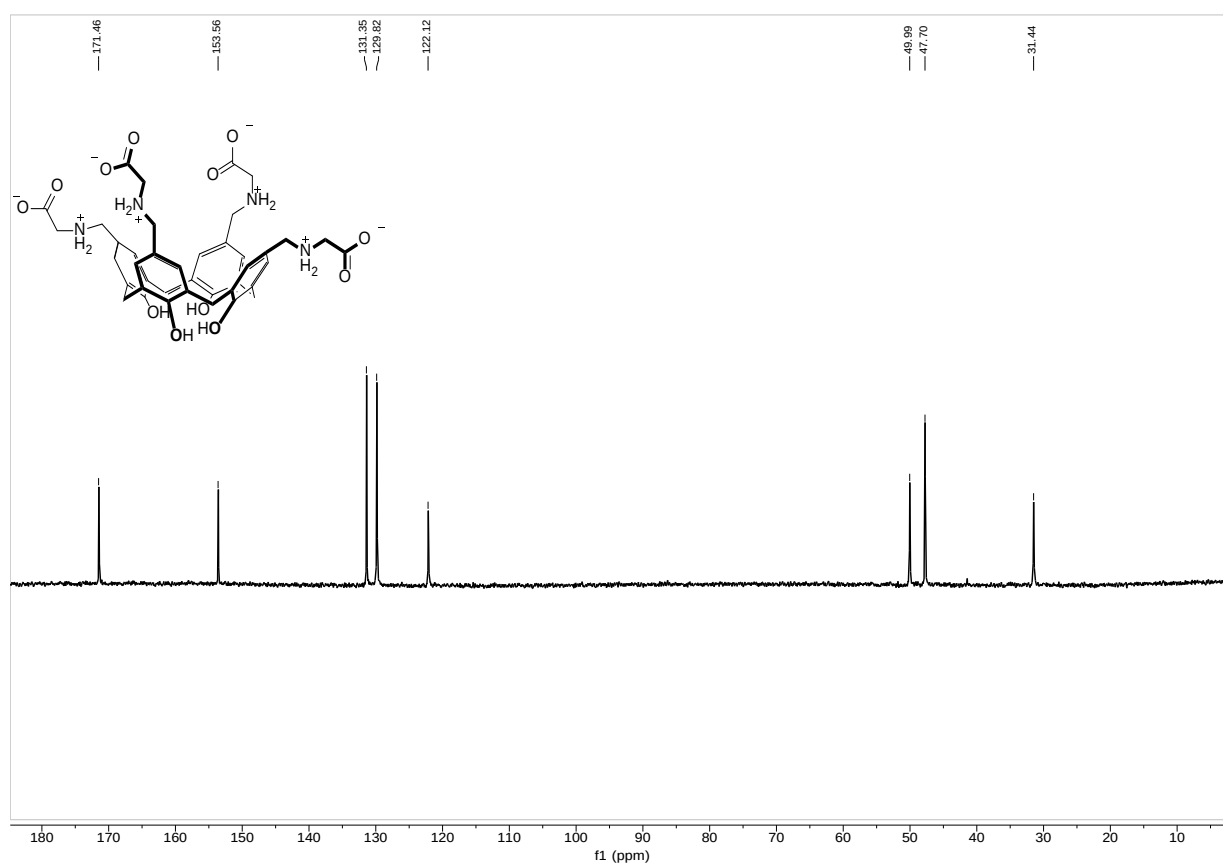


Figure S13. ^{13}C NMR spectrum (100 MHz, D_2O +NaOD) of calixarene **2**

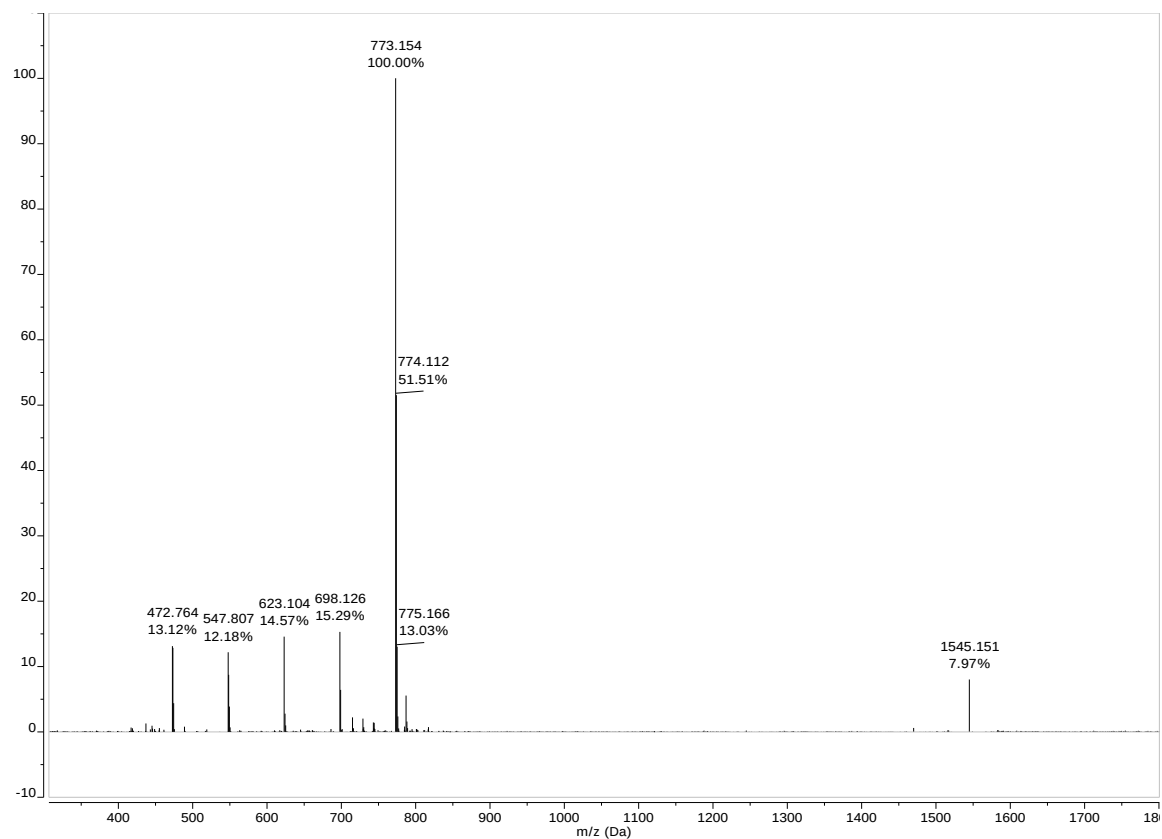


Figure S14. ESI-MS (+) spectrum of calixarene 2.

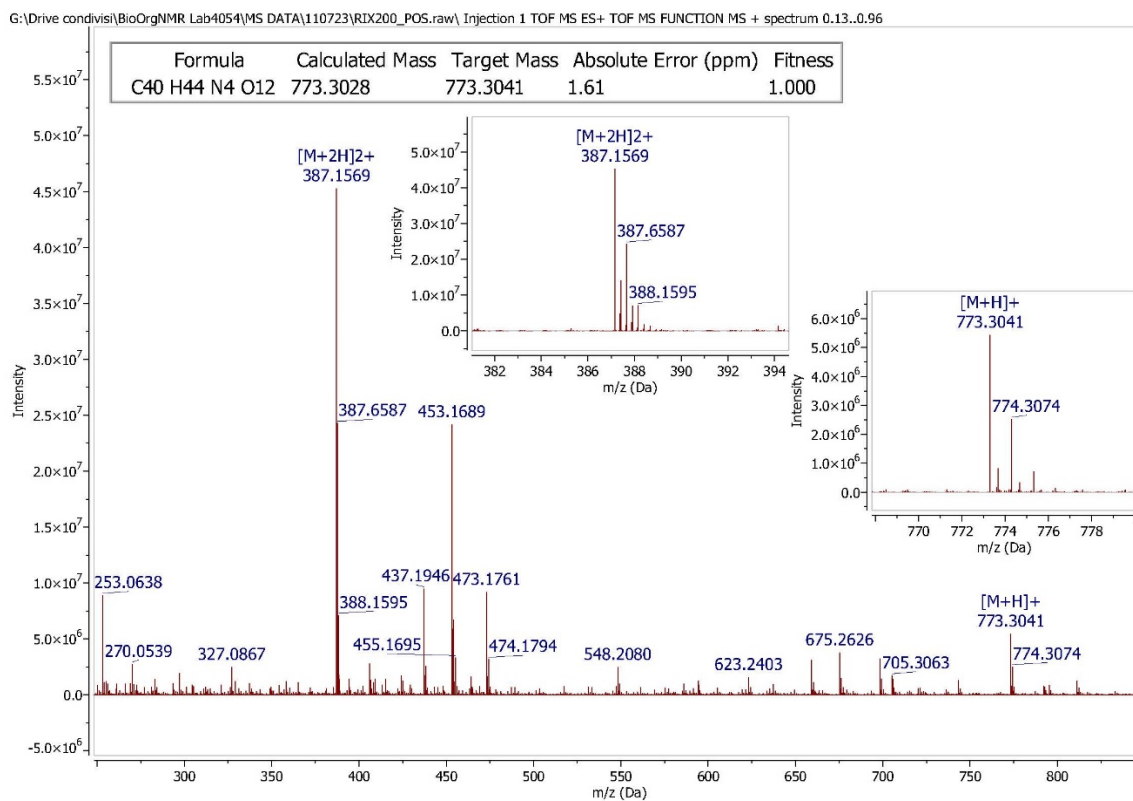


Figure S15. HR-MS (+) spectrum of calixarene 2.

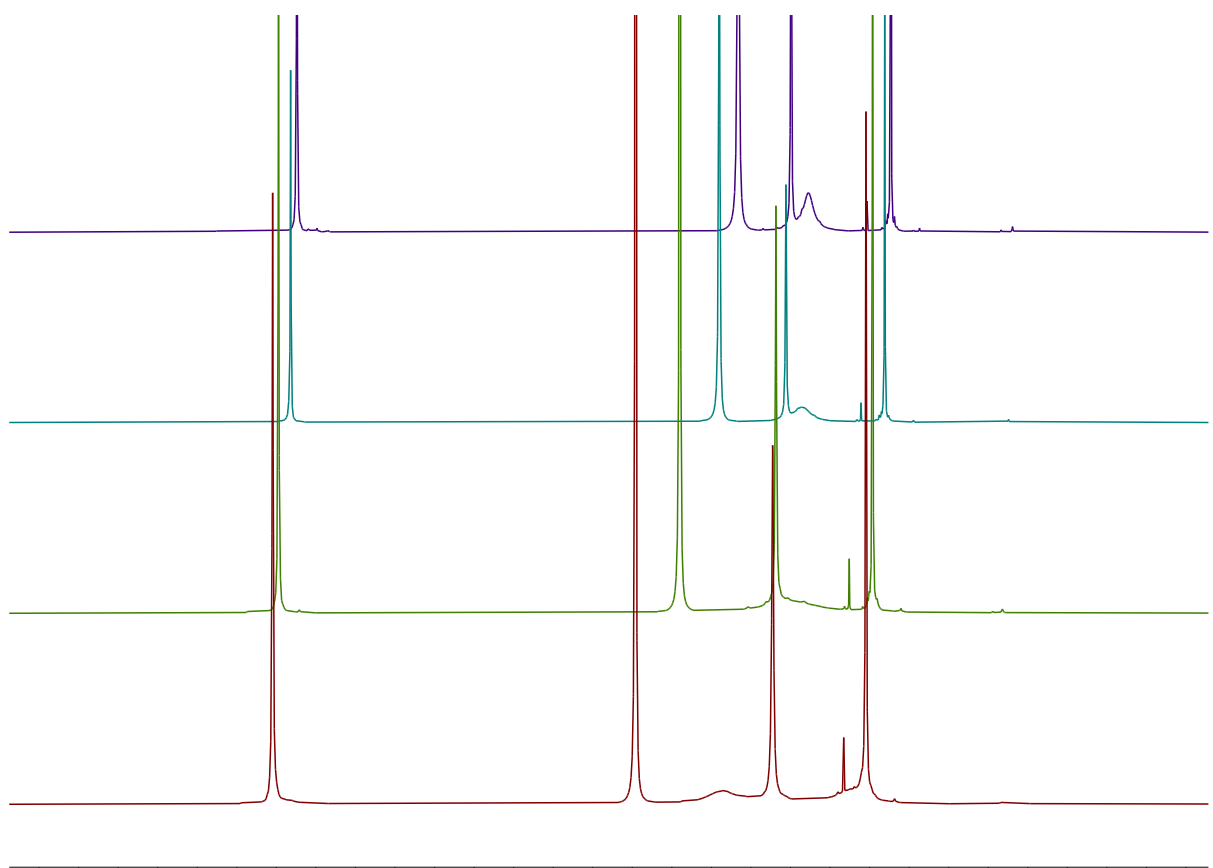


Figure S16. VT- ^1H NMR spectrum (400 MHz, D_2O) of calixarene **2**.

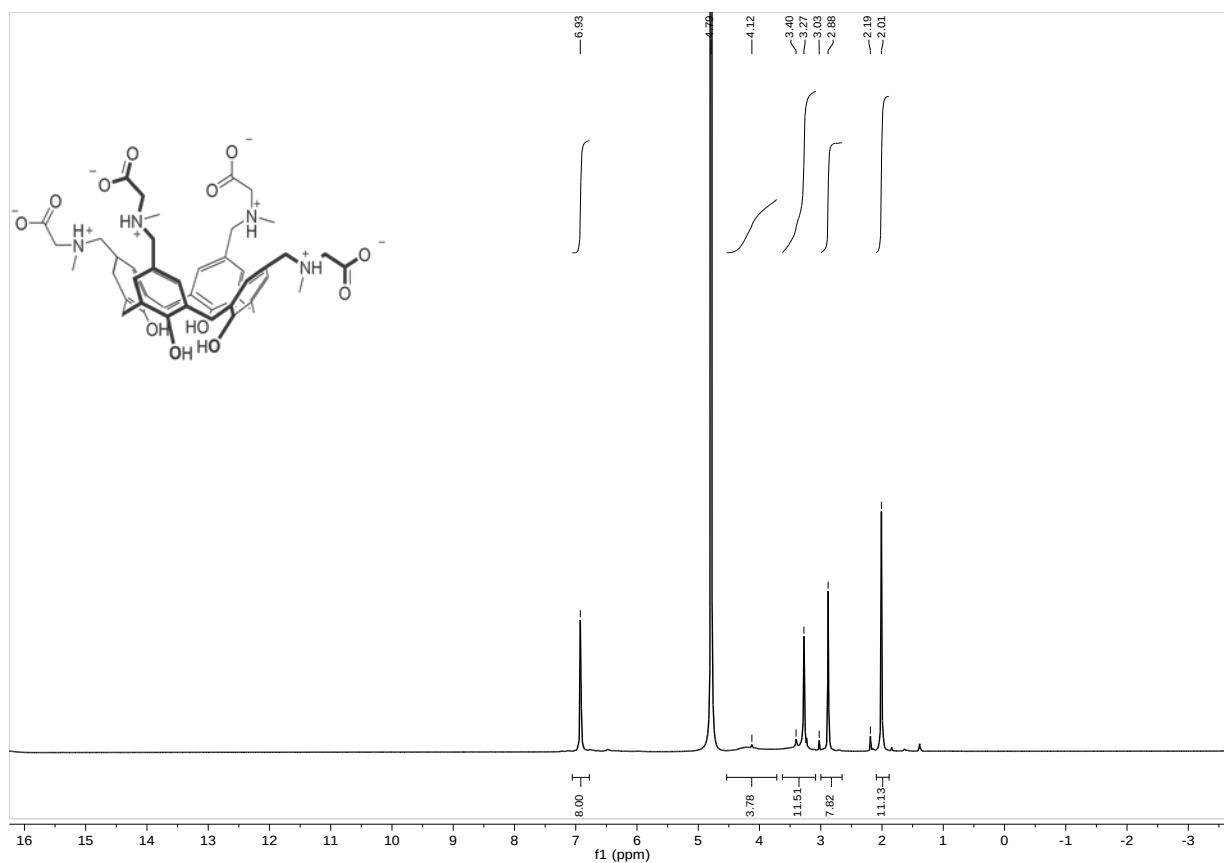


Figure S17. ^1H NMR spectrum (400 MHz, $\text{D}_2\text{O} + \text{NaOD}$) of calixarene **3**.

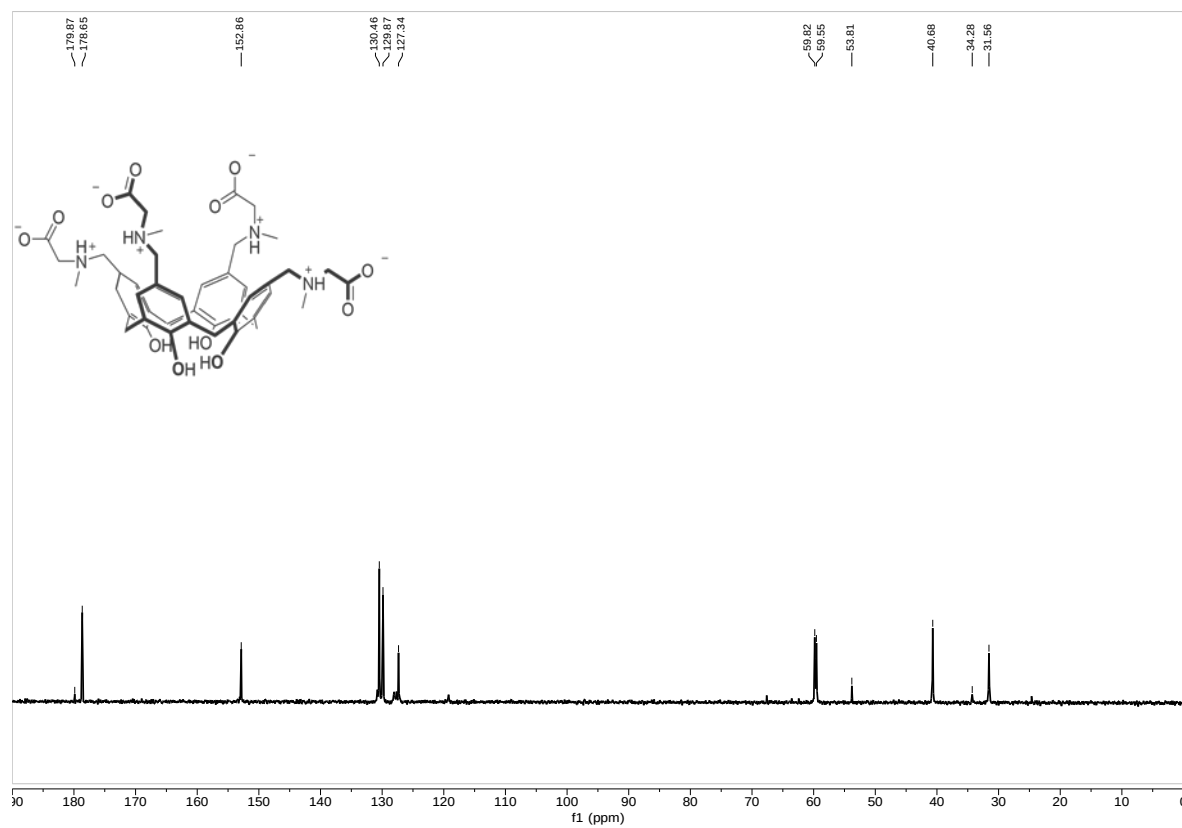


Figure S18. ^{13}C NMR spectrum (400 MHz, $\text{D}_2\text{O} + \text{NaOD}$) of calixarene **3**. The small peaks at 34.28, 53.81 and 179.87 ppm are due to residual sarcosine.

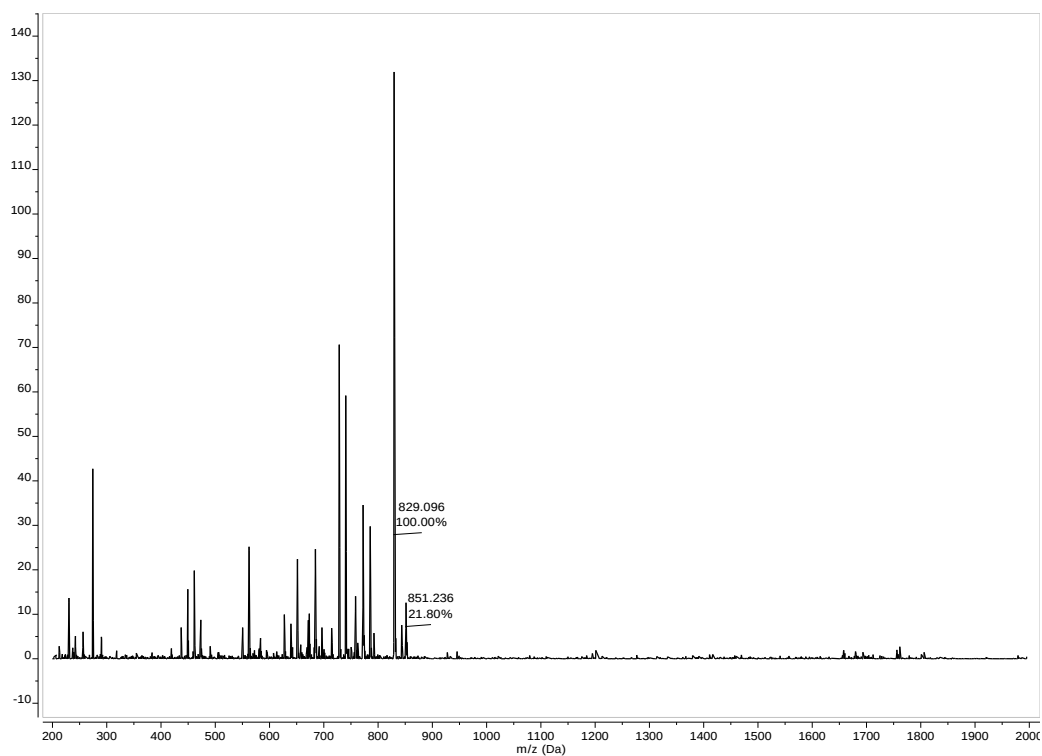


Figure S19. ESI-MS (+) spectrum of calixarene **3**.

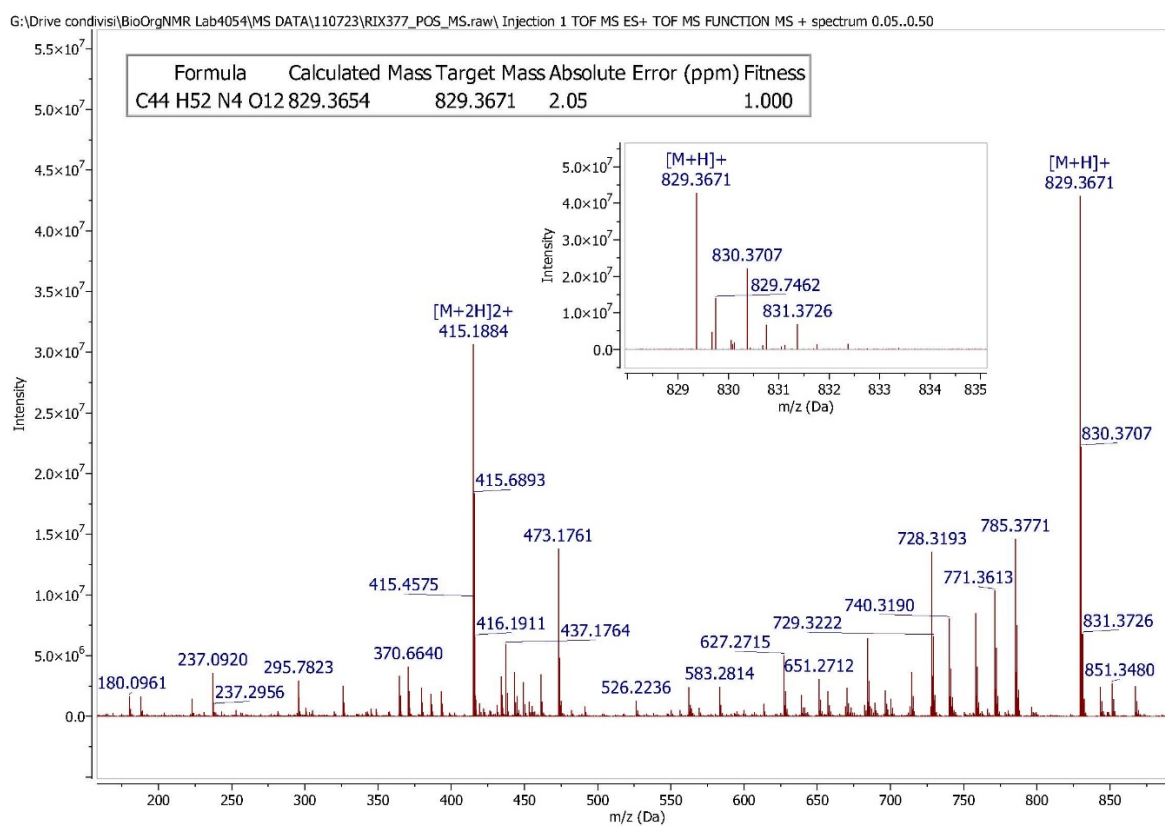


Figure S20. HR-MS (+) spectrum of calixarene **3**.

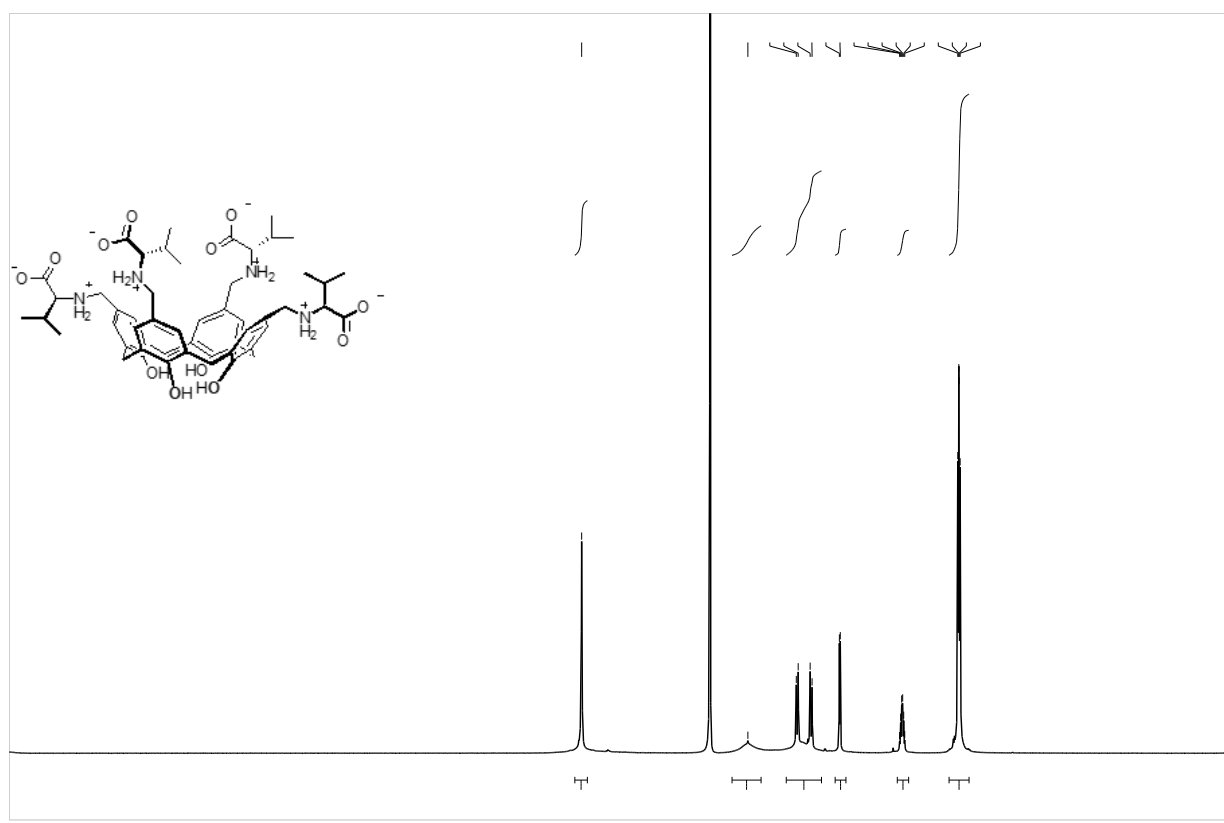


Figure S21. ^1H NMR spectrum (400 MHz, D_2O +NaOD) of calixarene **4**.

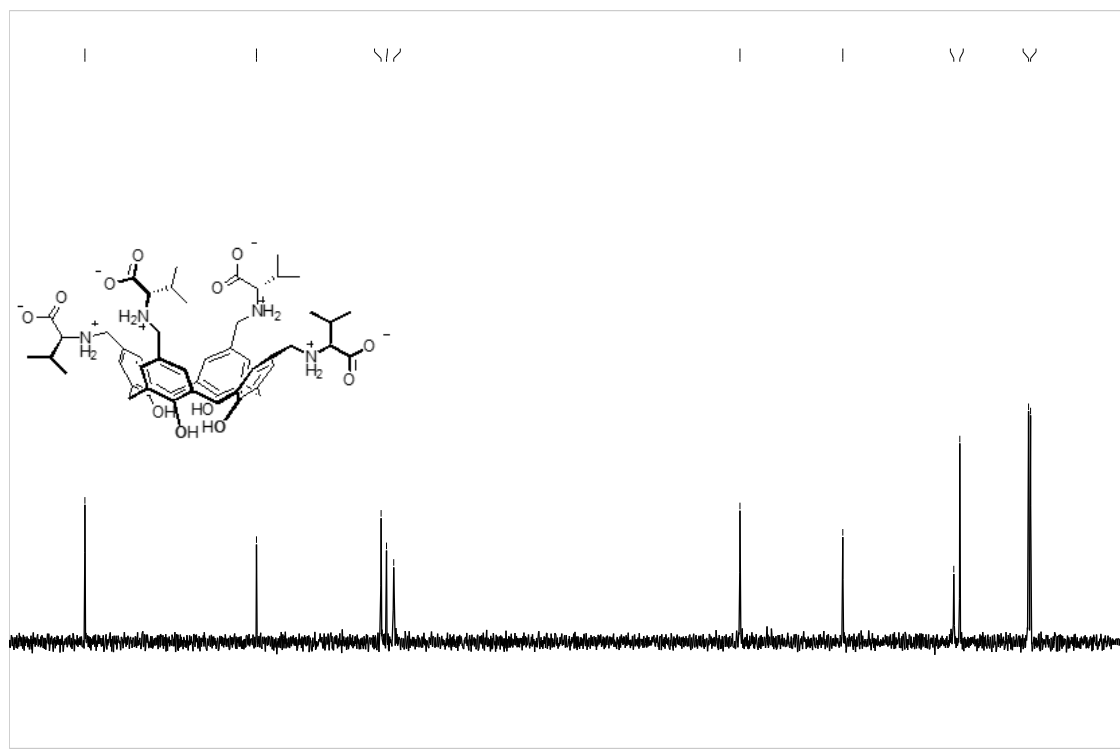
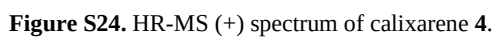
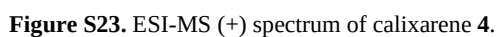


Figure S22. ^{13}C NMR spectrum (100 MHz, D_2O +NaOD) of calixarene **4**.



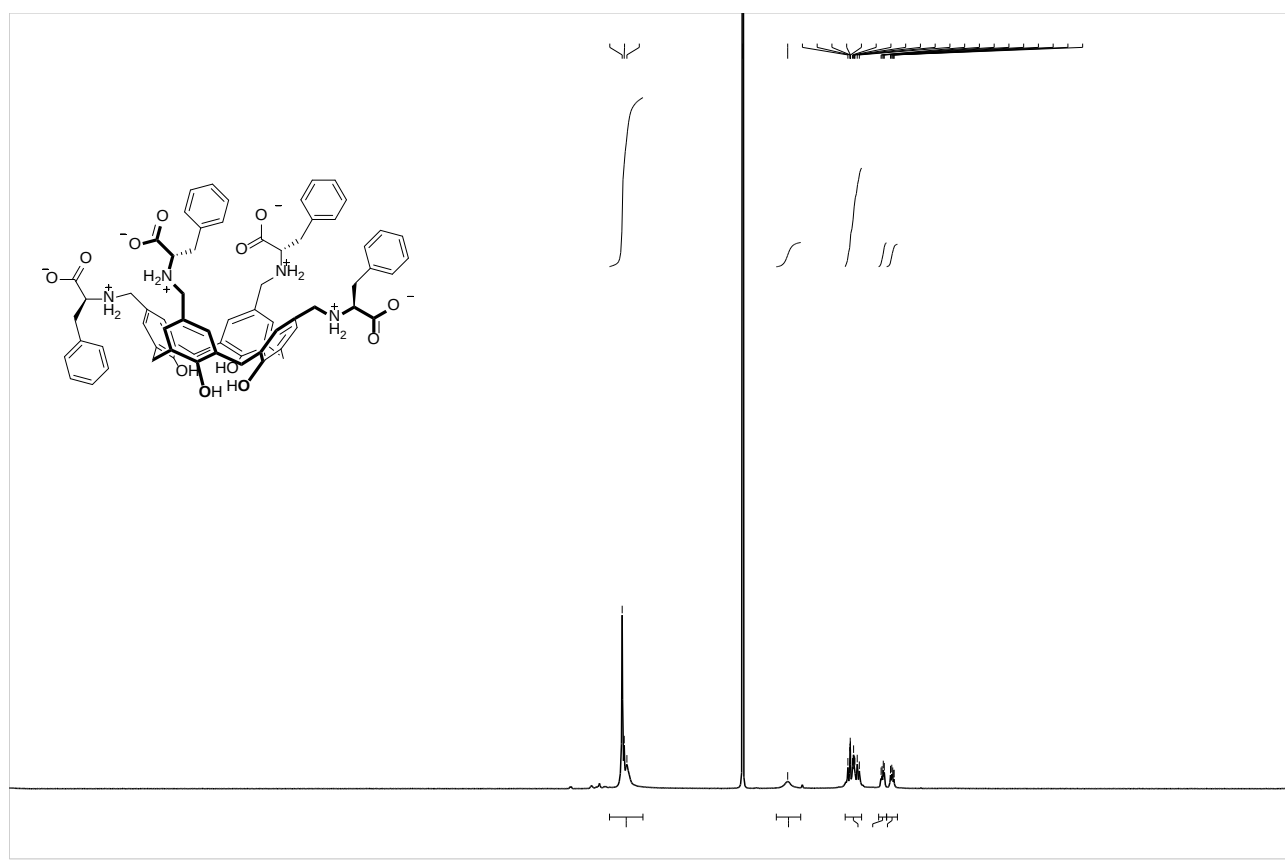


Figure S25. ¹H NMR spectrum (400 MHz, D₂O +NaOD) of calixarene 5.

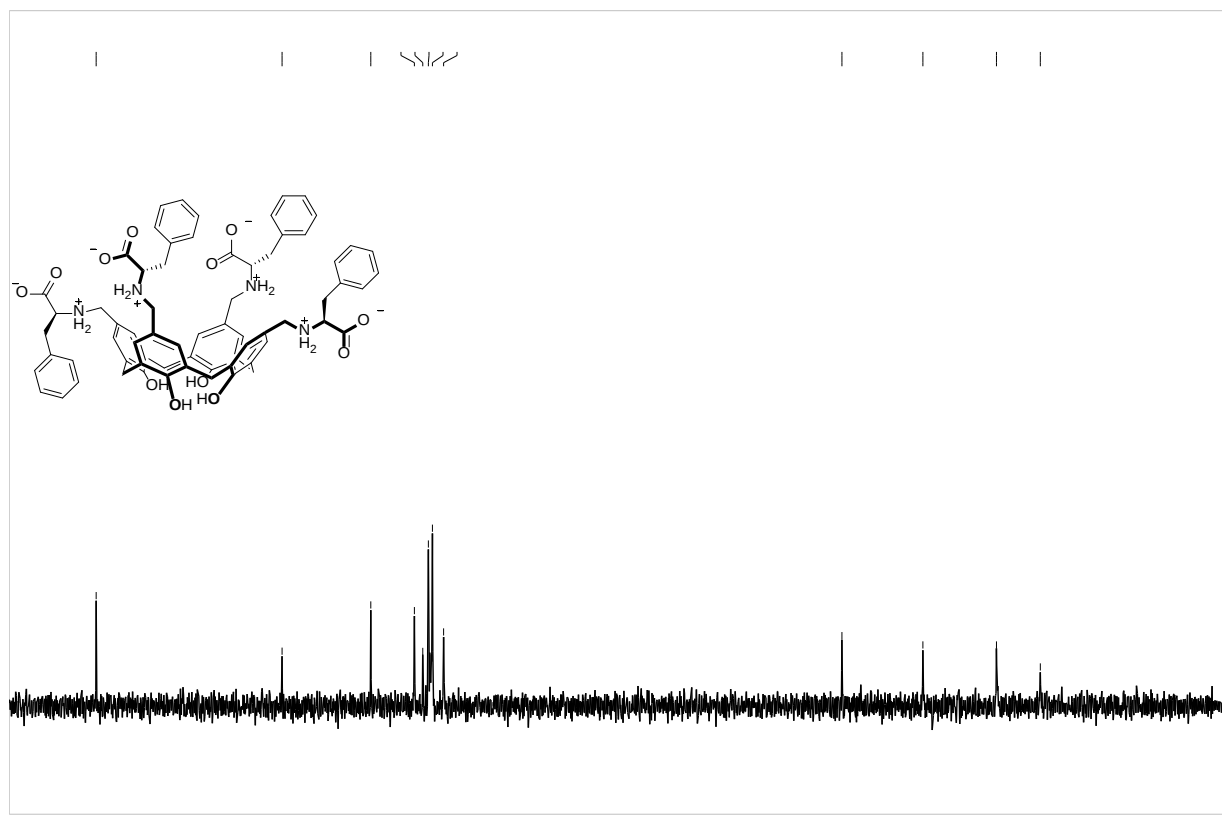


Figure S26. ¹³C NMR spectrum (100 MHz, D₂O +NaOD) of calixarene 5.

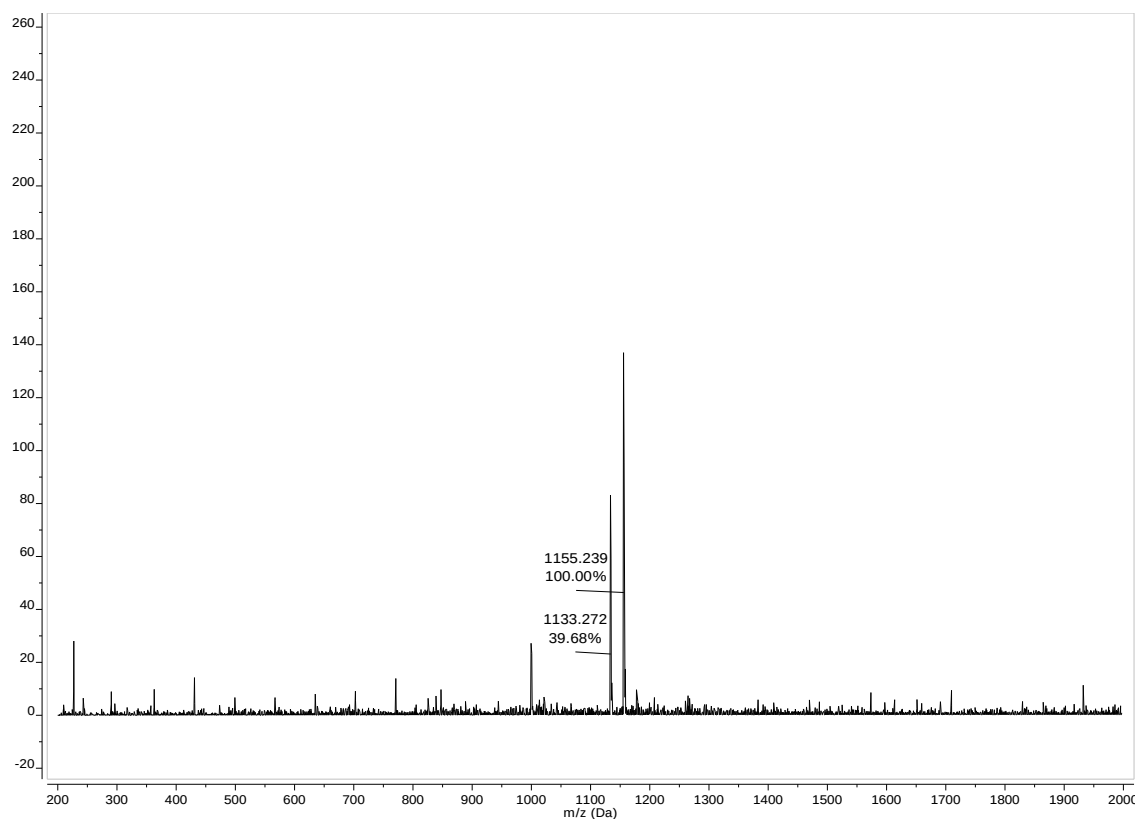


Figure S27. ESI-MS (+) spectrum of calixarene 5.

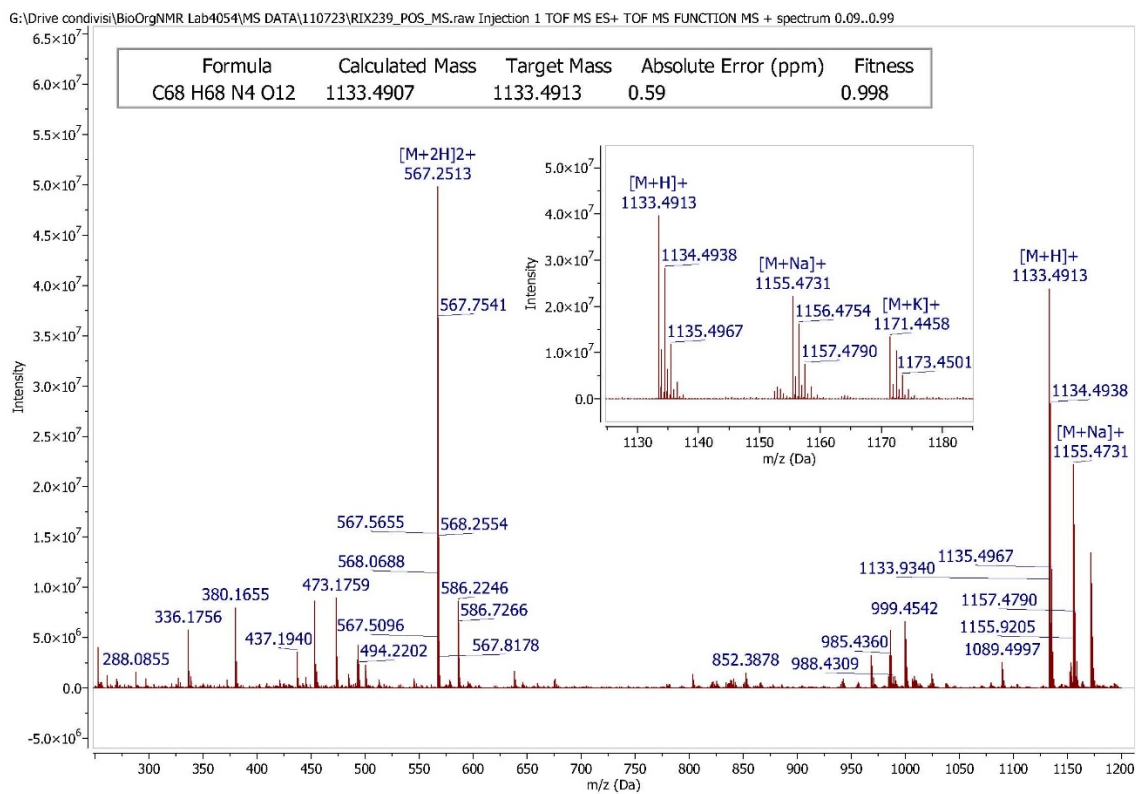


Figure S28. HR-MS (+) spectrum of calixarene 5.

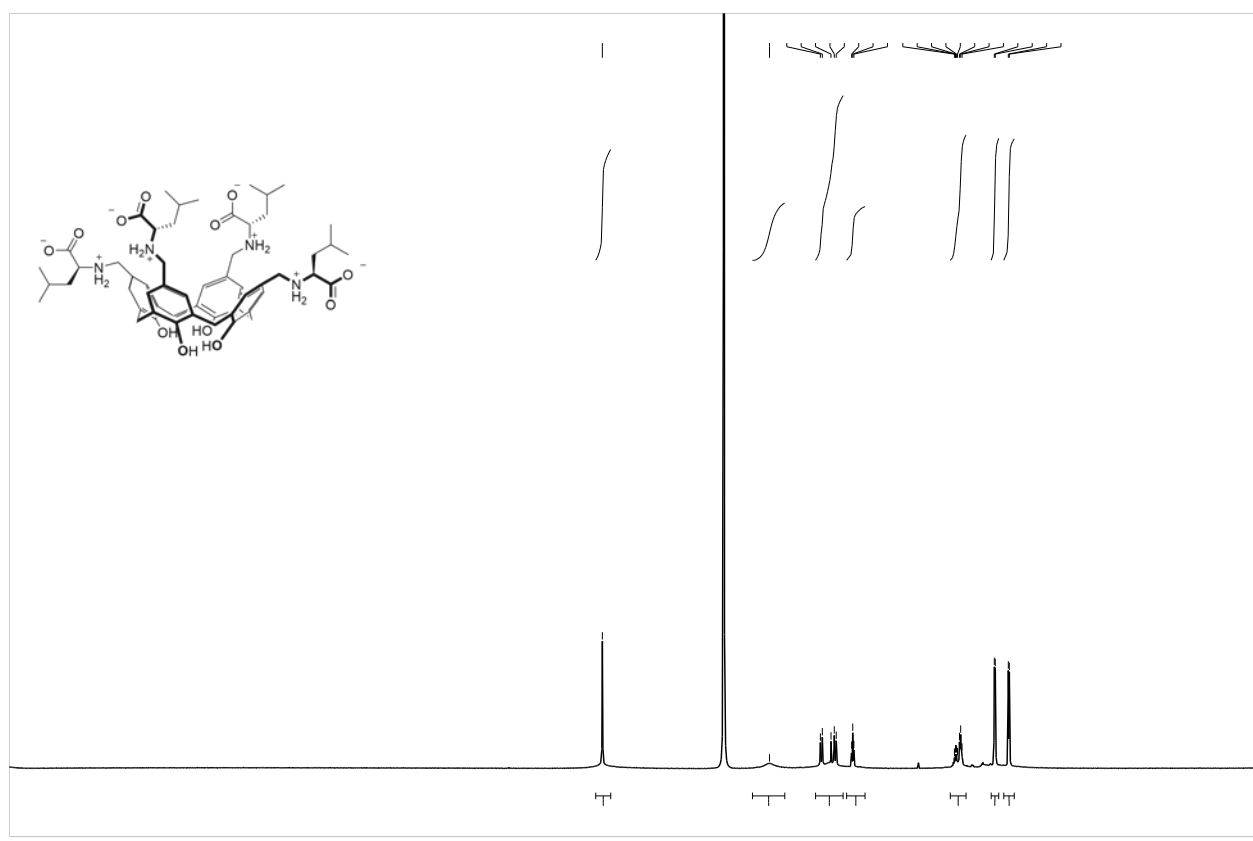


Figure S29. ¹H NMR spectrum (400 MHz, D₂O +NaOD) of calixarene **6**.

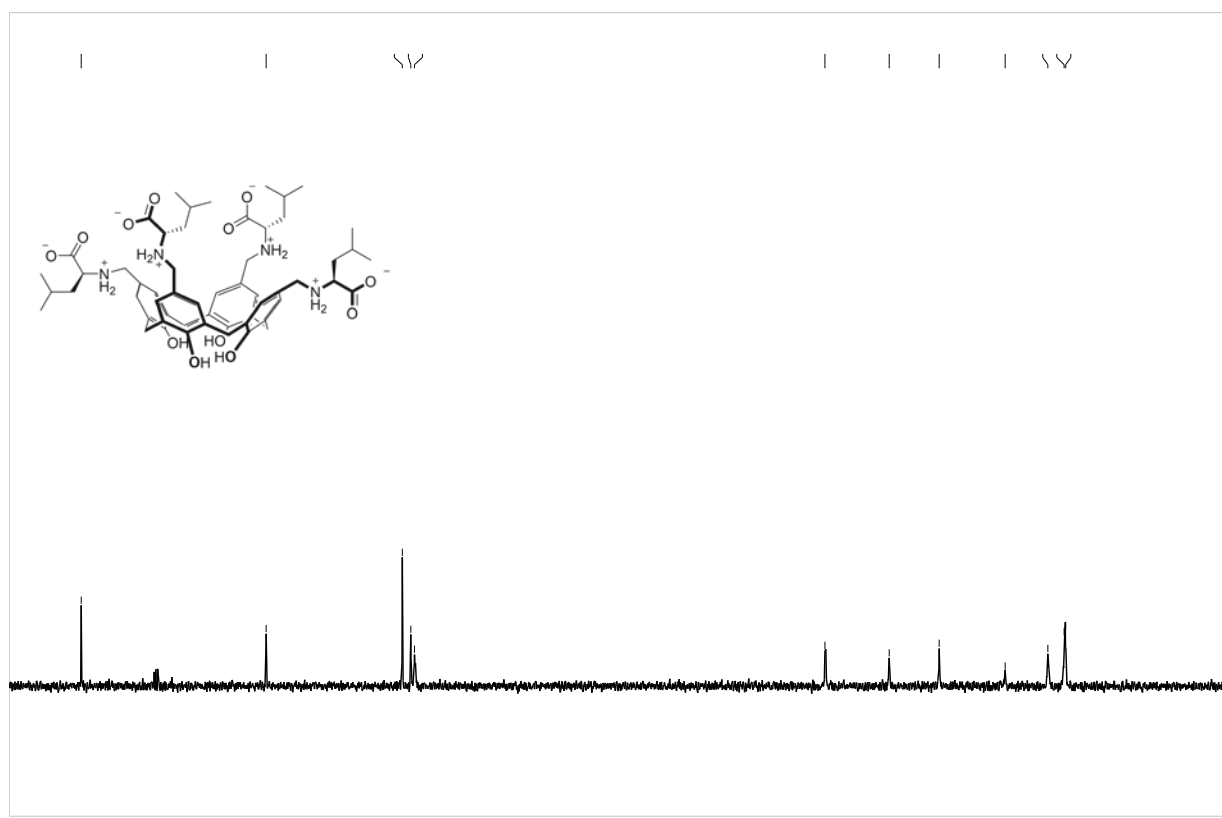


Figure S30. ¹³C NMR spectrum (100 MHz, D₂O +NaOD) of calixarene **6**.

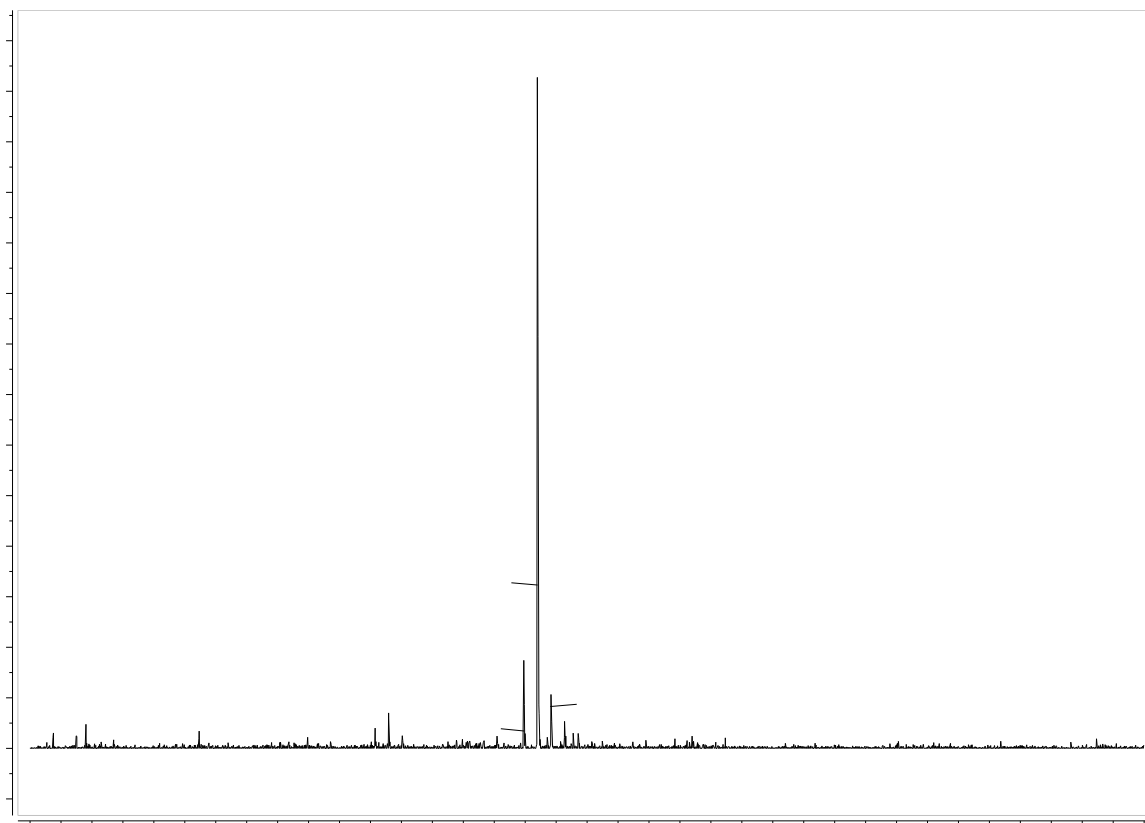


Figure S31. ESI-MS (+) spectrum of calixarene **6**.

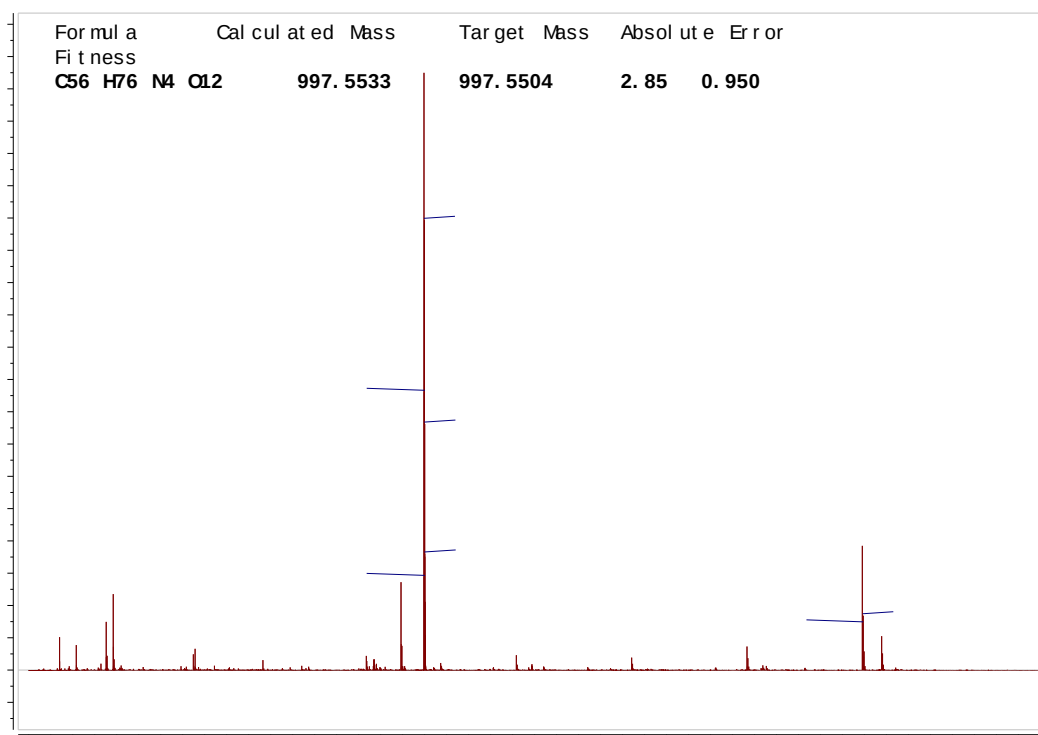


Figure S32. HR-MS (+) spectrum of calixarene **6**.

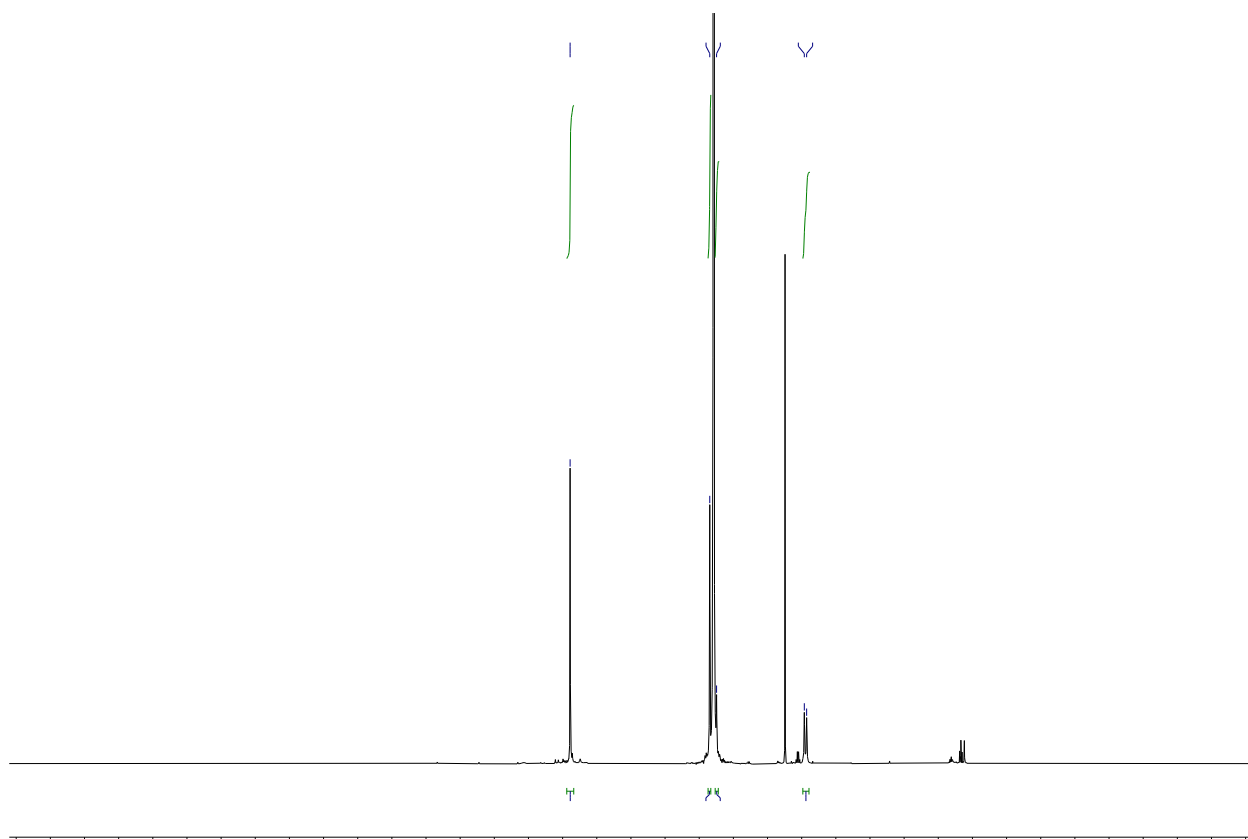


Figure S33. ¹H NMR spectrum (400 MHz, D₂O) of calixarene 7.

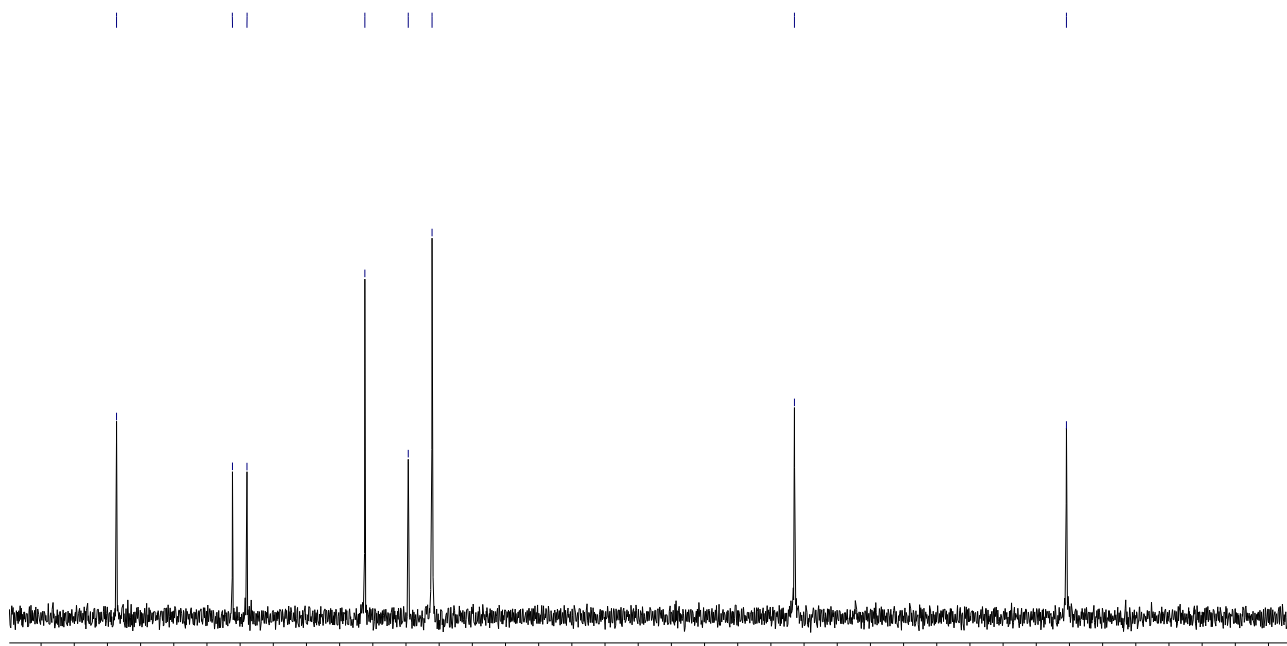


Figure S34. ¹³C NMR spectrum (100 MHz, D₂O) of calixarene 7.

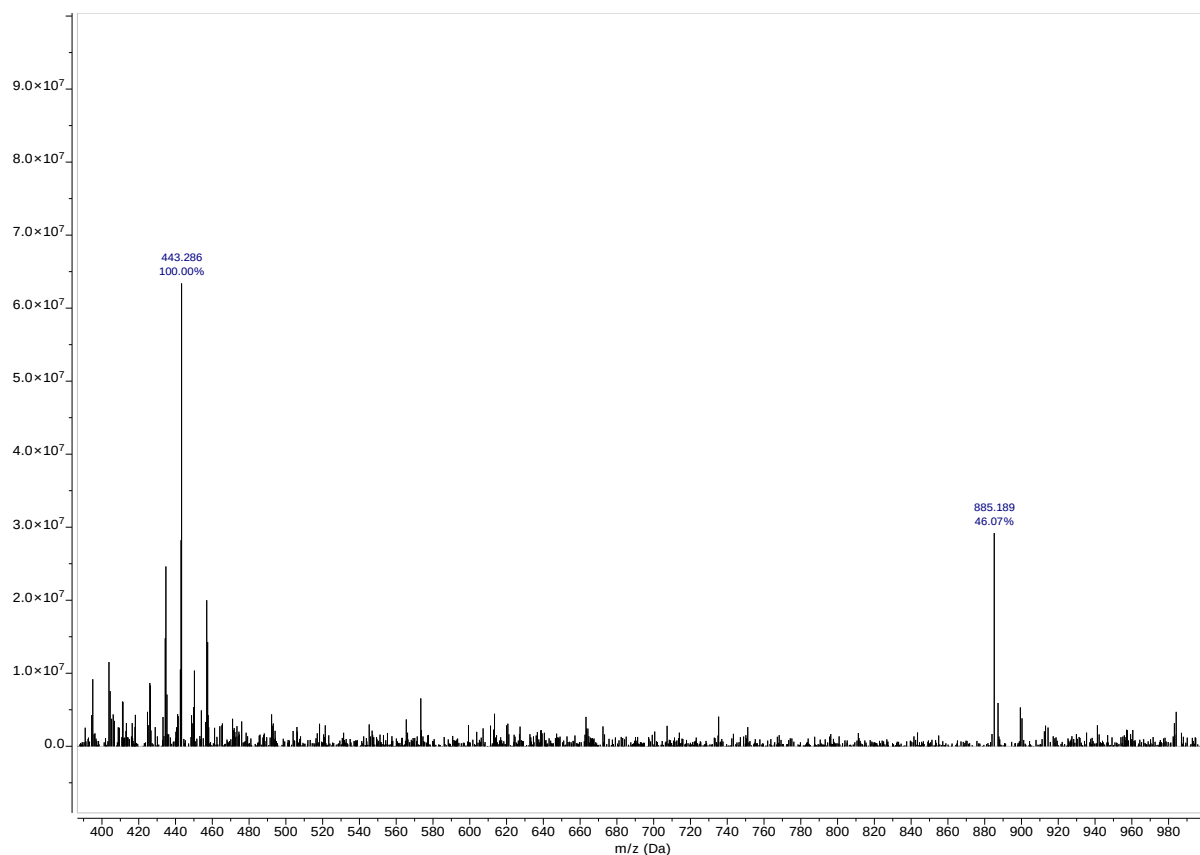


Figure S35. ESI-MS (+) spectrum of calixarene 7.

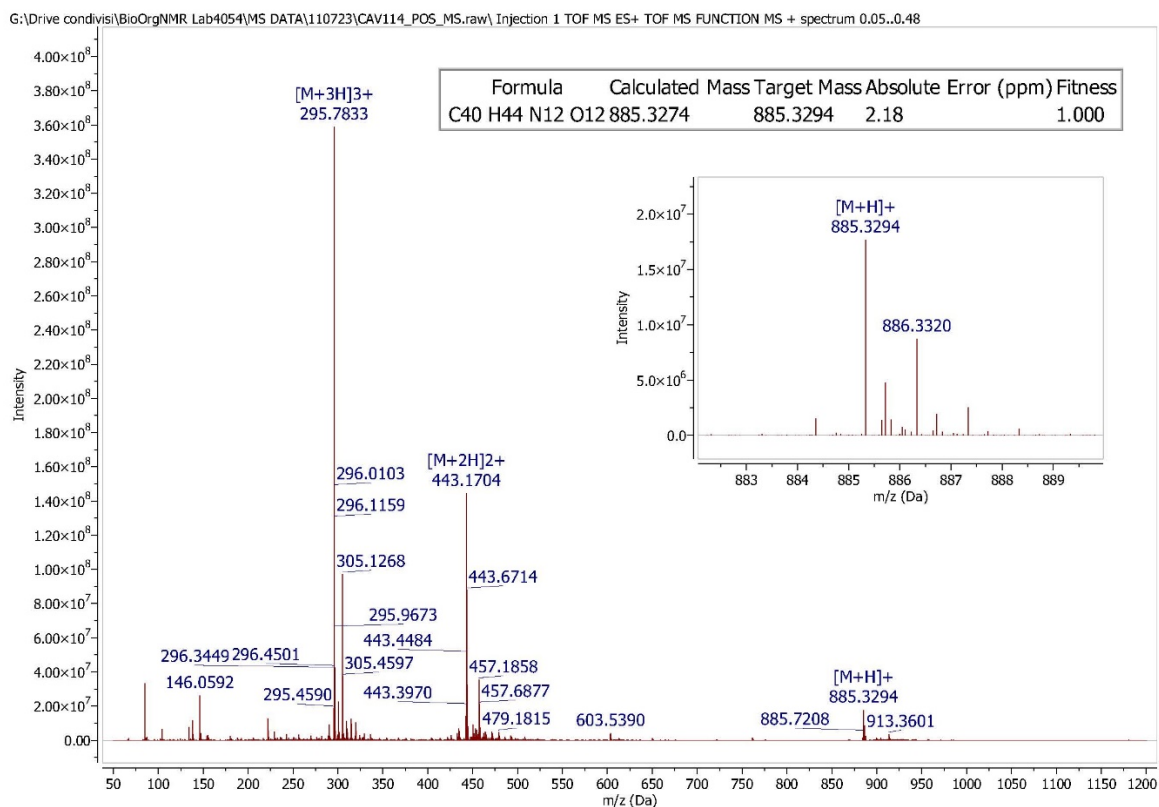


Figure S36. HR-MS (+) spectrum of calixarene 7.

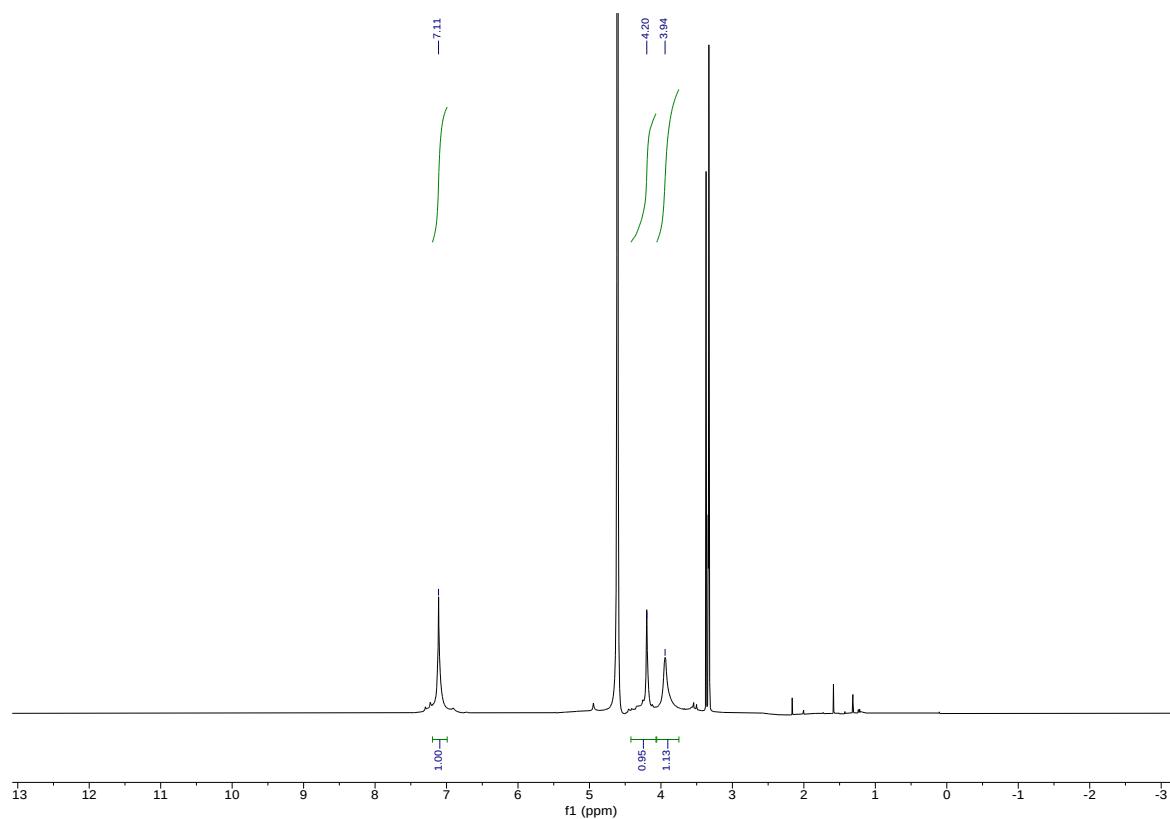


Figure S37. ¹H NMR spectrum (400 MHz, CD₃OD, 328K) of calixarene **9**.

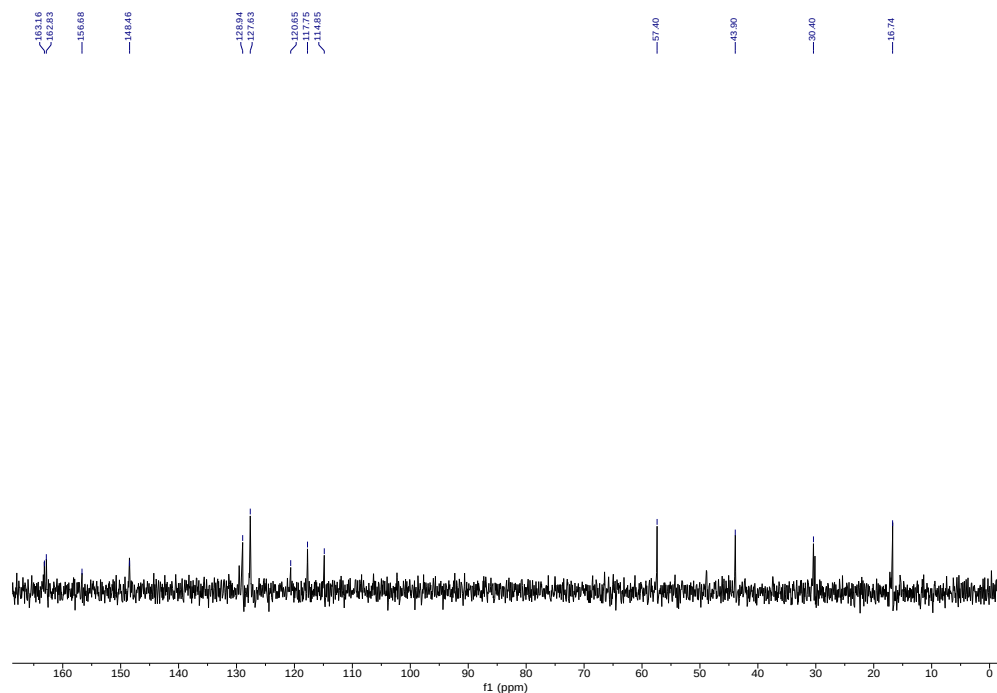


Figure S38. ¹³C NMR spectrum (100 MHz, D₂O) of calixarene **9** (57.4 and 16.7 ppm ethanol from crystallization).

G:\Drive condvisi\BioOrgNMR Lab4054\MS DATA\110723\CAV178_POS_MS.raw\ Injection 1 TOF MS ES+ TOF MS FUNCTION MS + spectrum 0.37

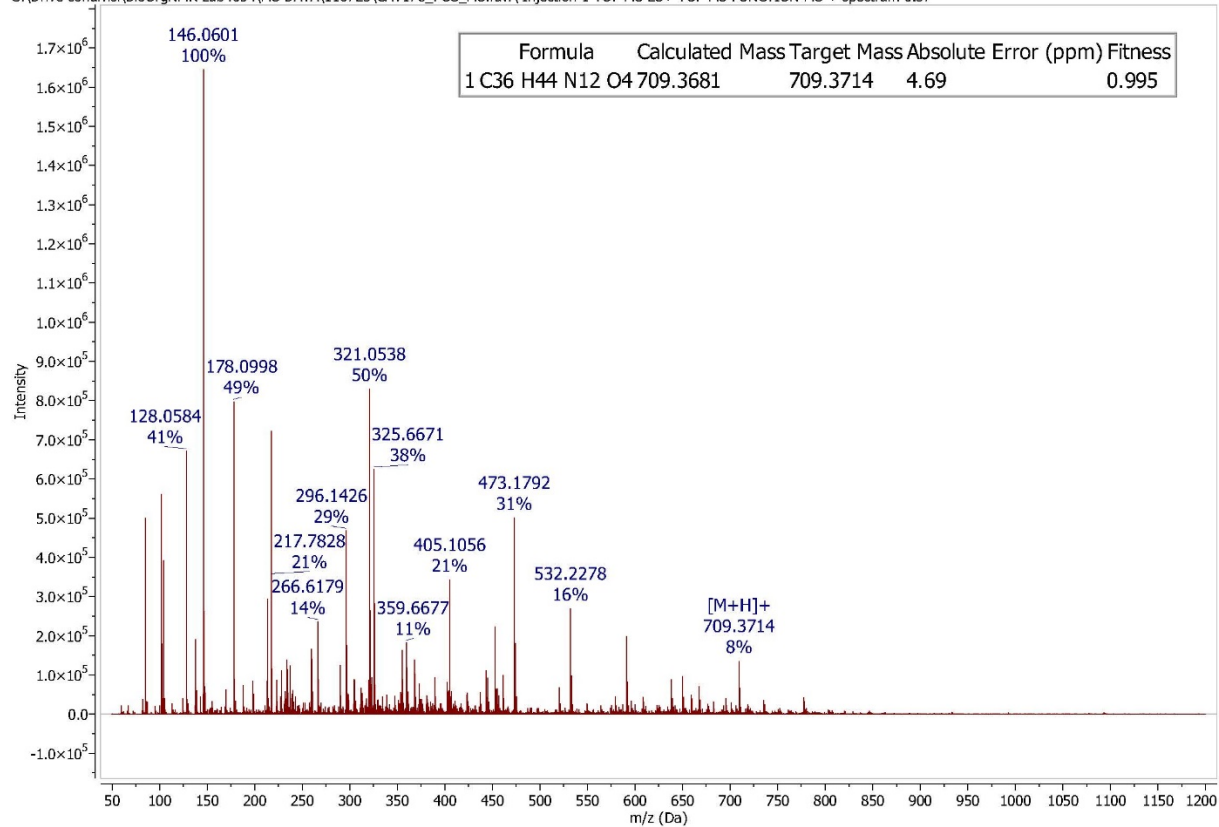


Figure S39. ESI-MS (+) spectrum of calixarene **9**.

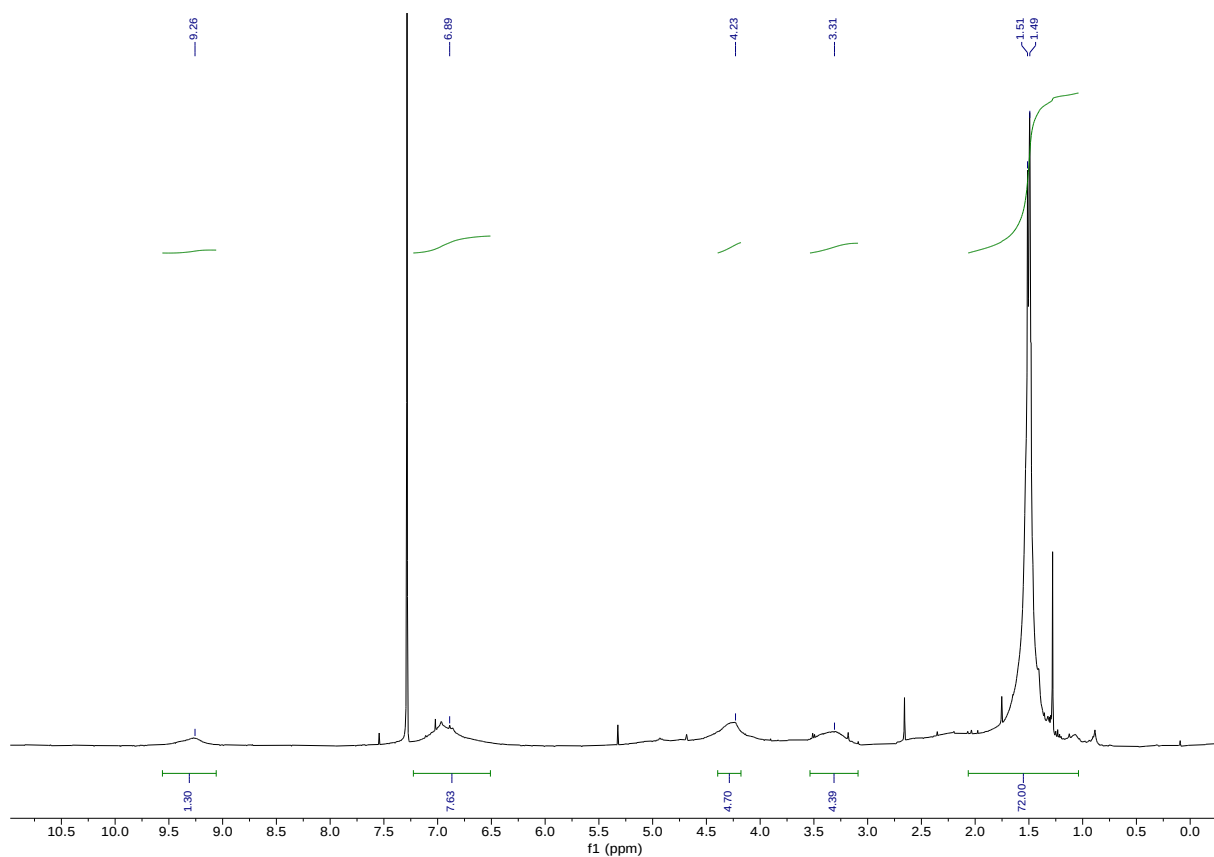


Figure S40. ¹H NMR spectrum (400 MHz, CDCl₃) of calixarene **11**.

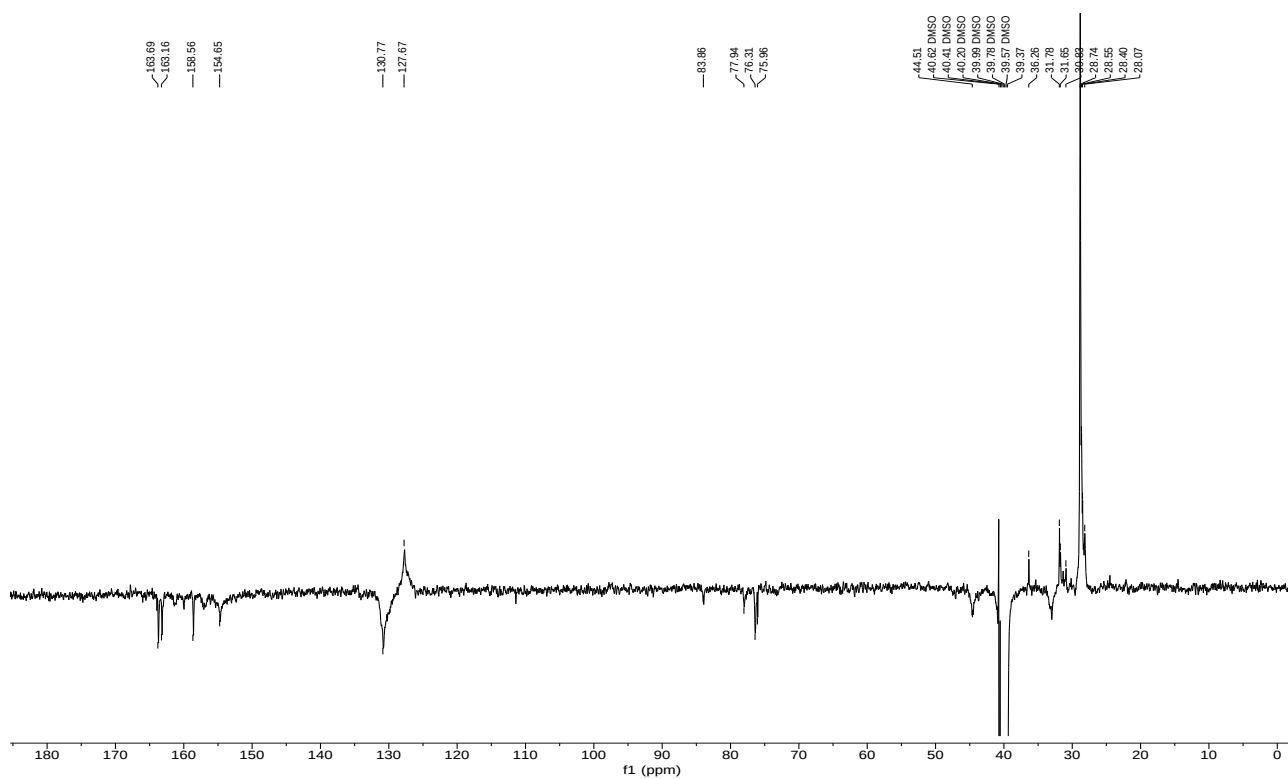


Figure S41. APT NMR spectrum (100 MHz, DMSO-d₆) of calixarene **11**.

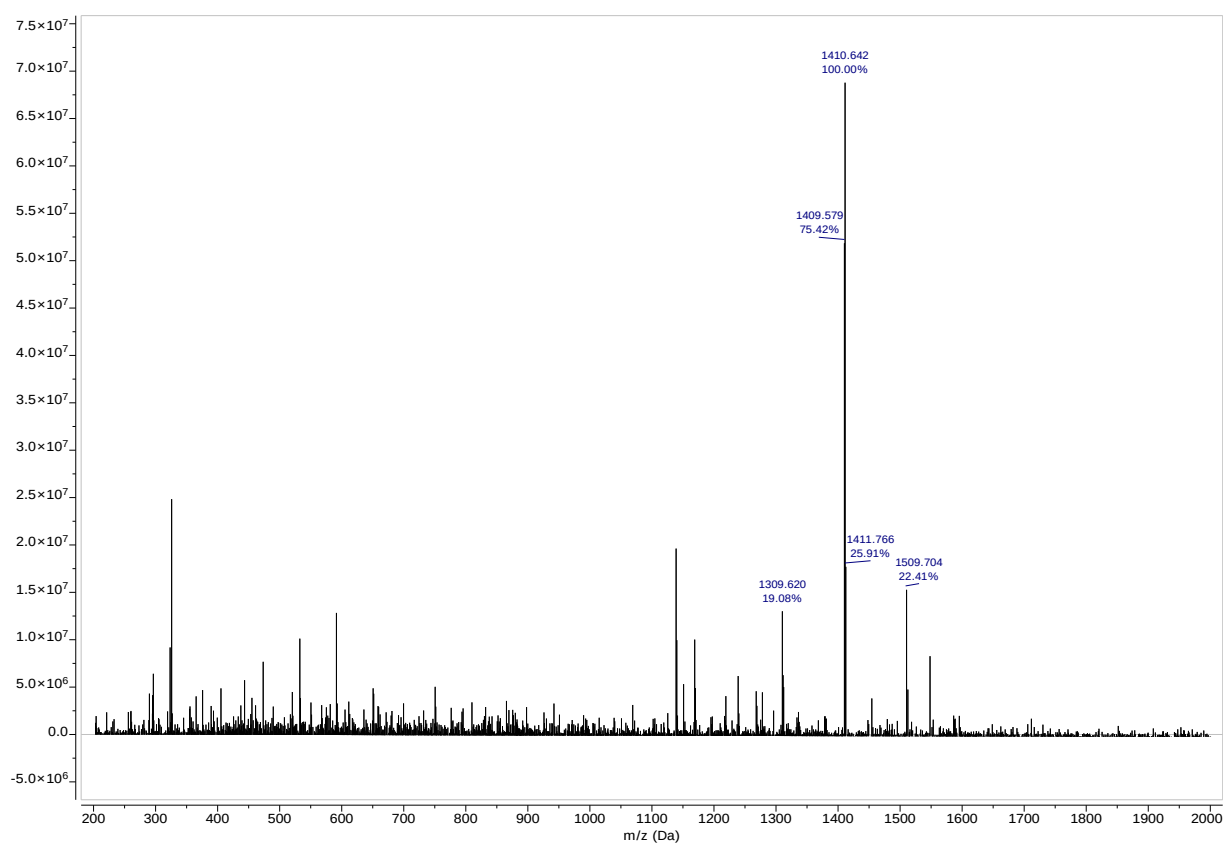


Figure S42. ESI-MS (+) spectrum of calixarene **11**.

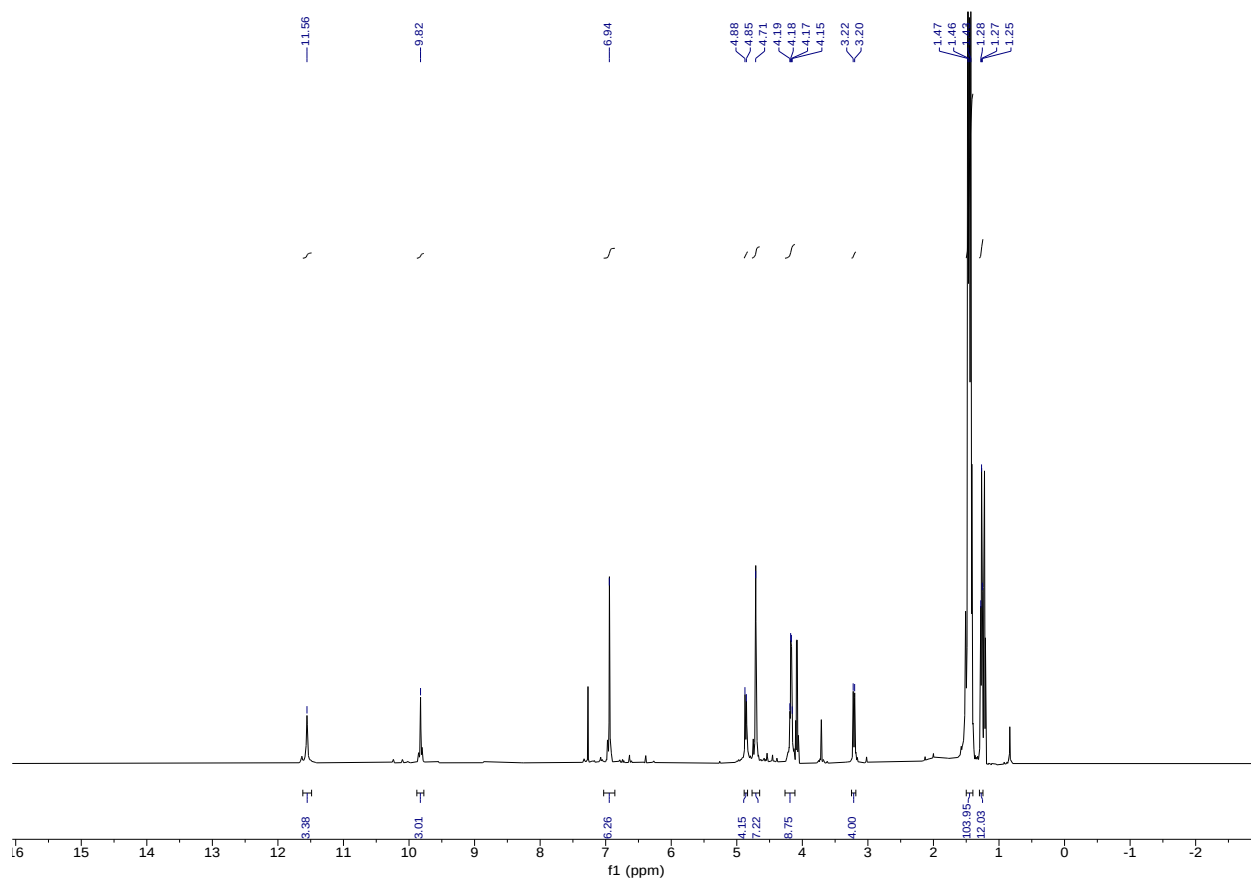


Figure S43. ^1H NMR spectrum (400 MHz, CDCl_3) of calixarene **13**.

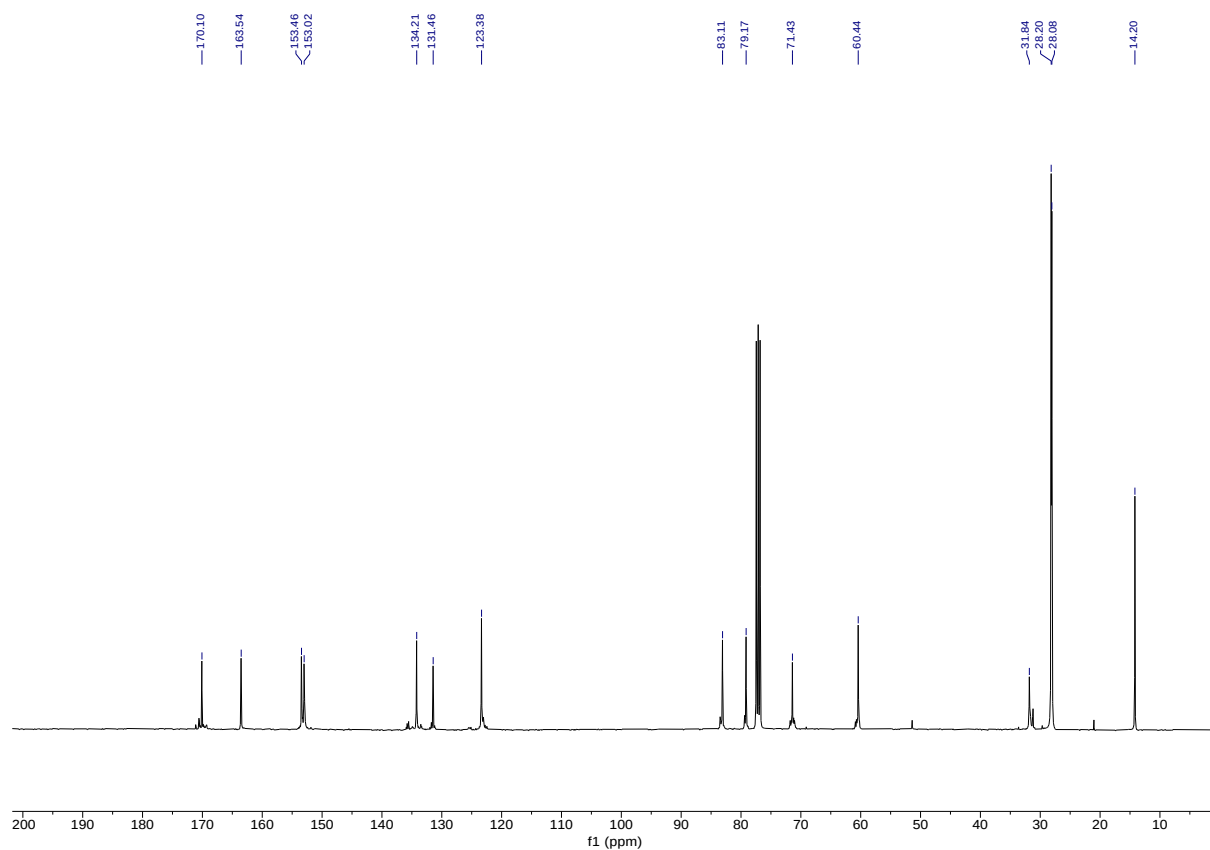


Figure S44. ^{13}C NMR spectrum (100 MHz, CDCl_3) of calixarene **13**.

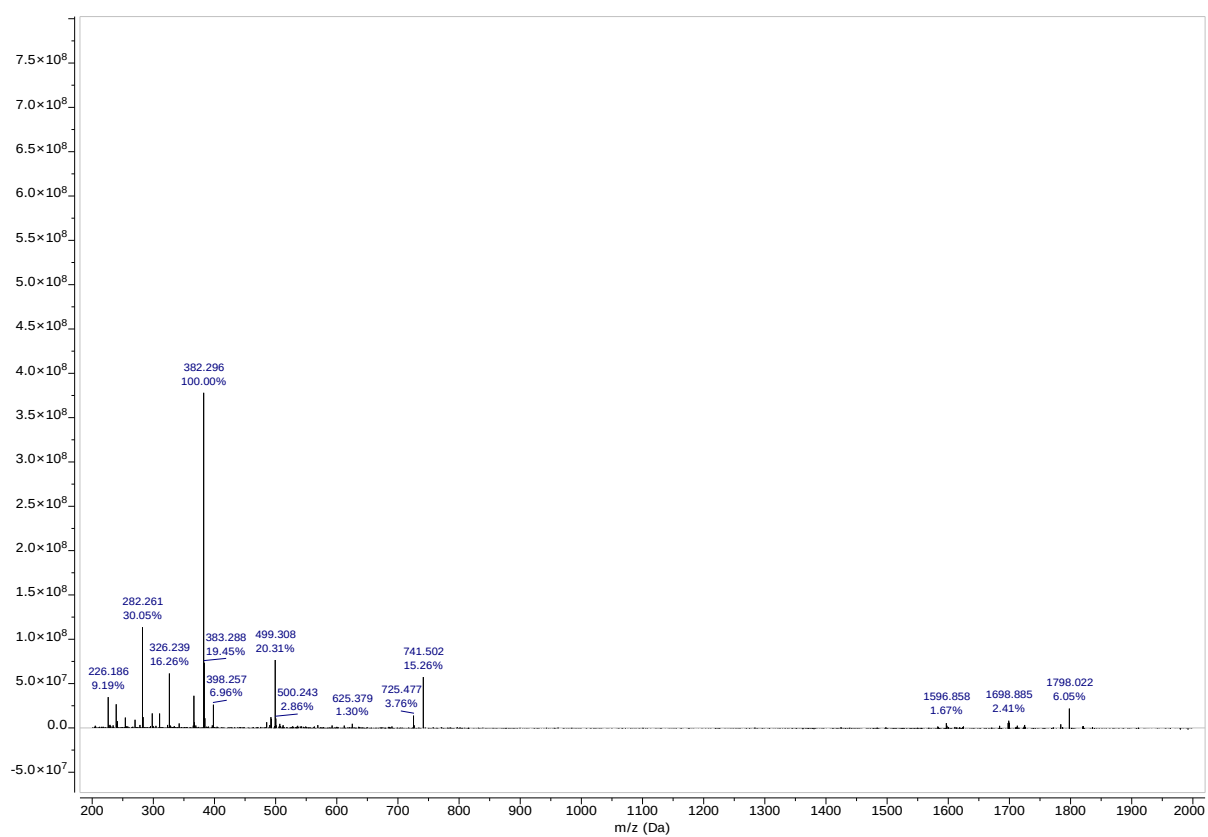


Figure S45. ESI-MS (+) spectrum of calixarene **13**.

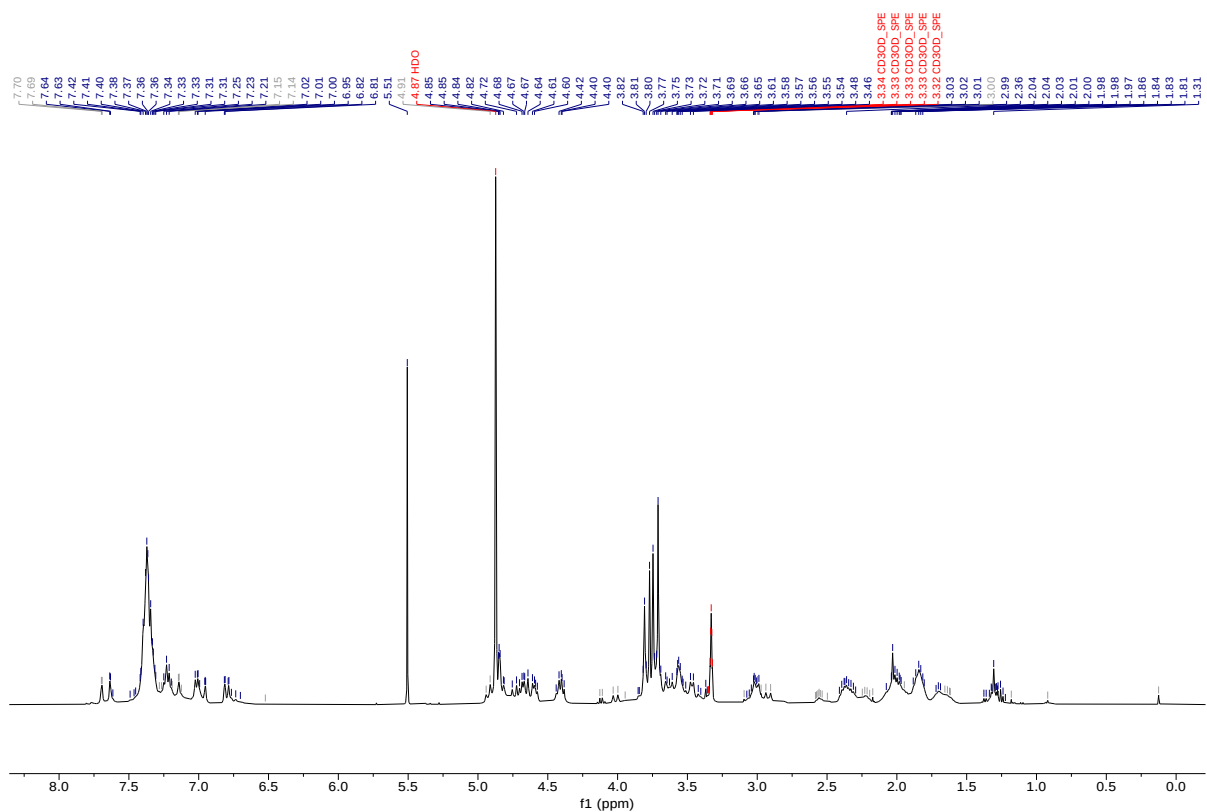


Figure S46. ^1H NMR spectrum (400 MHz, CD_3OD) of calixarene **15**.



Figure S47. ESI-MS (+) spectrum of calixarene **15**.

X-Ray crystal structure of compound 2.

Crystal data and structure refinement details are provided in **Table S1**. CCDC 2375133 contains the supporting crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

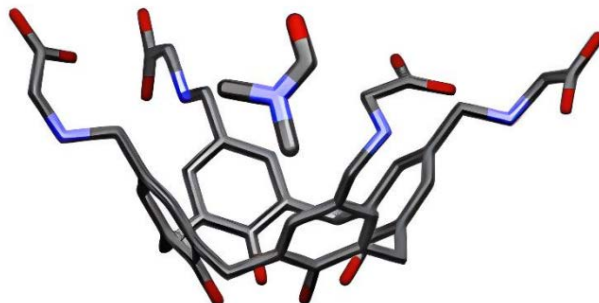


Figure S48. Crystal structure of compound 2 showing the inclusion of one DMF molecule.

In more detail, two adjacent carboxylates (O9 and O11) are pinching a sodium cation and, together with an additional carboxylate (O5) moiety from a different calixarene molecule, define a dinuclear fragment embedded between four calixarene moieties. The sodium ion exhibits a distorted square pyramidal geometry with Na-O bond distances in the 2.27-2.40 Å range, with the O9 atom acting as a bridge between two metal centres, **Figure S48**. The fourth carboxylic group on the upper rim exchanges a hydrogen bond with the protonated amino group of an adjacent calixarene molecule ($N4 \cdots O8$, 2.79 Å).

The crystal packing shows the presence of antiparallel arrays of calixarene columns, which are connected by the sodium dimeric system and by an extensive net of hydrogen bonds involving the metal-free zwitterionic arm of the calixarene with water molecules of crystallization, **Figure S49**.

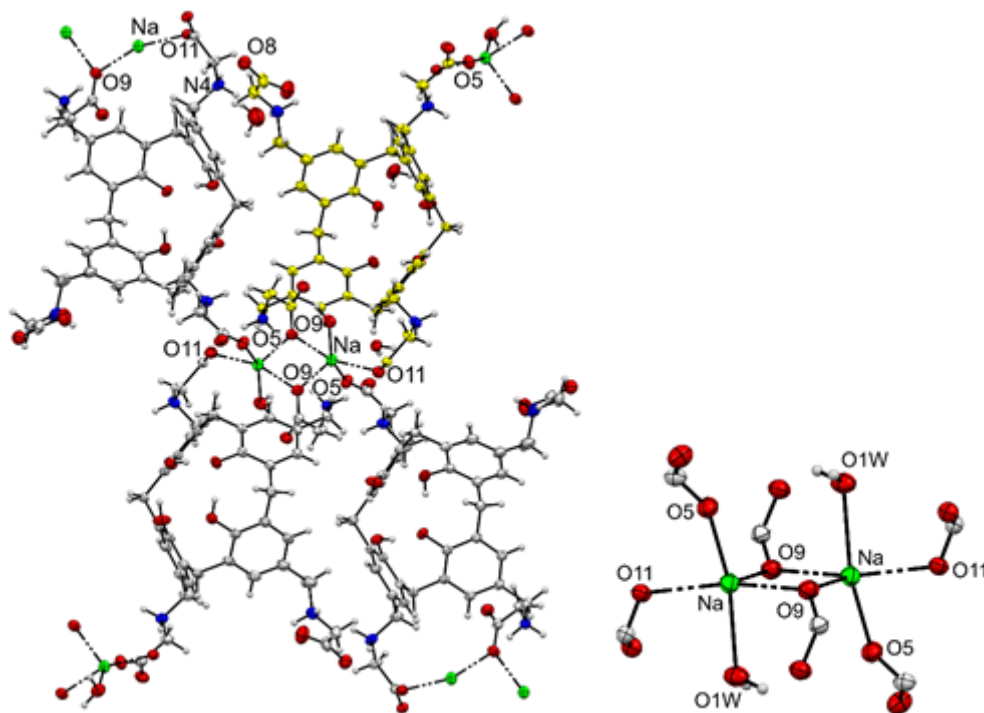


Figure S49. Above, portion of the crystal packing of 2, showing the sodium dimeric moiety in the center. Below, highlight on the sodium dimeric fragment.

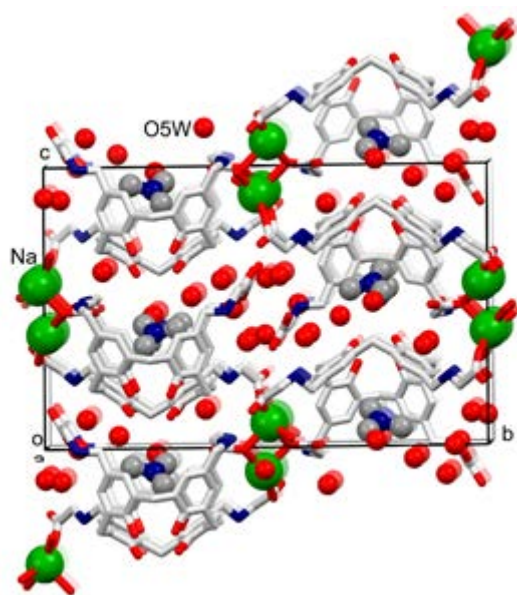


Figure S50. Crystal packing of **2** as viewed along the *a* crystallographic axis. Hydrogen atoms were omitted for clarity.

Bacterial strains survival to HR-MAS NMR conditions.

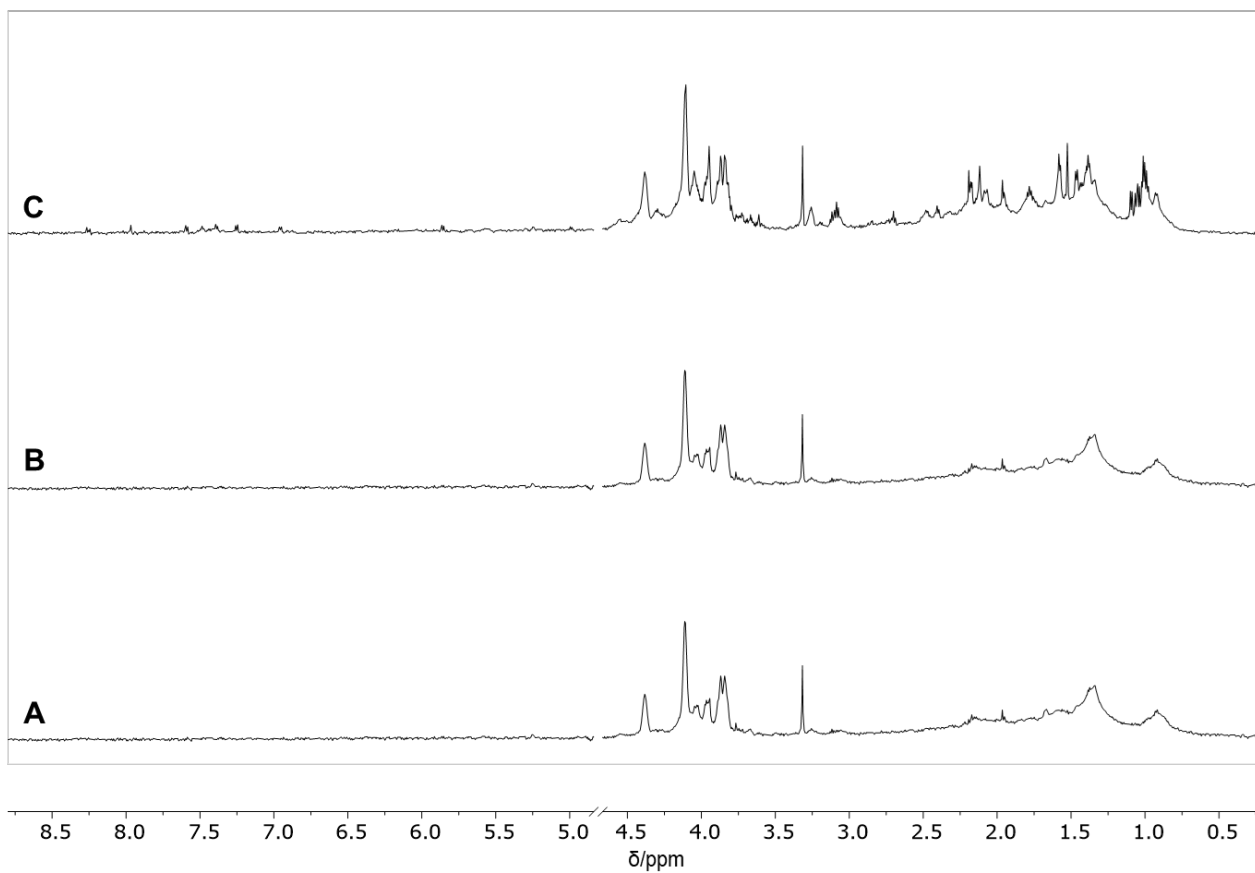


Figure S51. ¹H NMR spectra acquired on a solution containing 8 mg of *S. epidermidis* suspended in d-PBS (final sample volume, 40 µl) immediately after sample preparation (A), and after 4 h (B) and 12 h (C). Spectra were recorded with 120 scans at 37 °C and with a spin rate of 3 kHz.

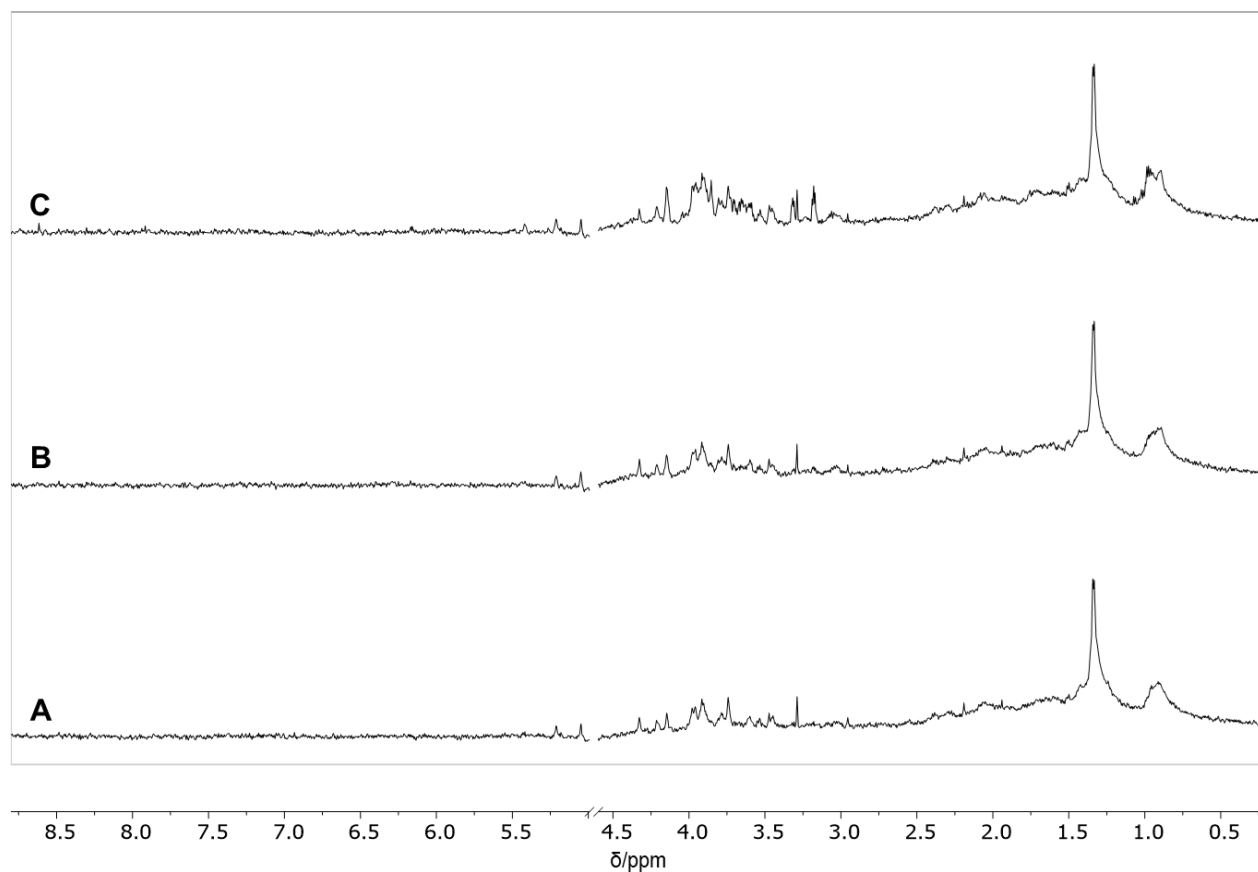


Figure S52. ^1H NMR spectra acquired on a solution containing 8 mg of *P. putida* suspended in d-PBS (final sample volume, 40 μl) immediately after sample preparation (**A**), and after 4 h (**B**) and 20 h (**C**). Spectra were recorded with 120 scans at 37 $^{\circ}\text{C}$ and with a spin rate of 3 kHz.

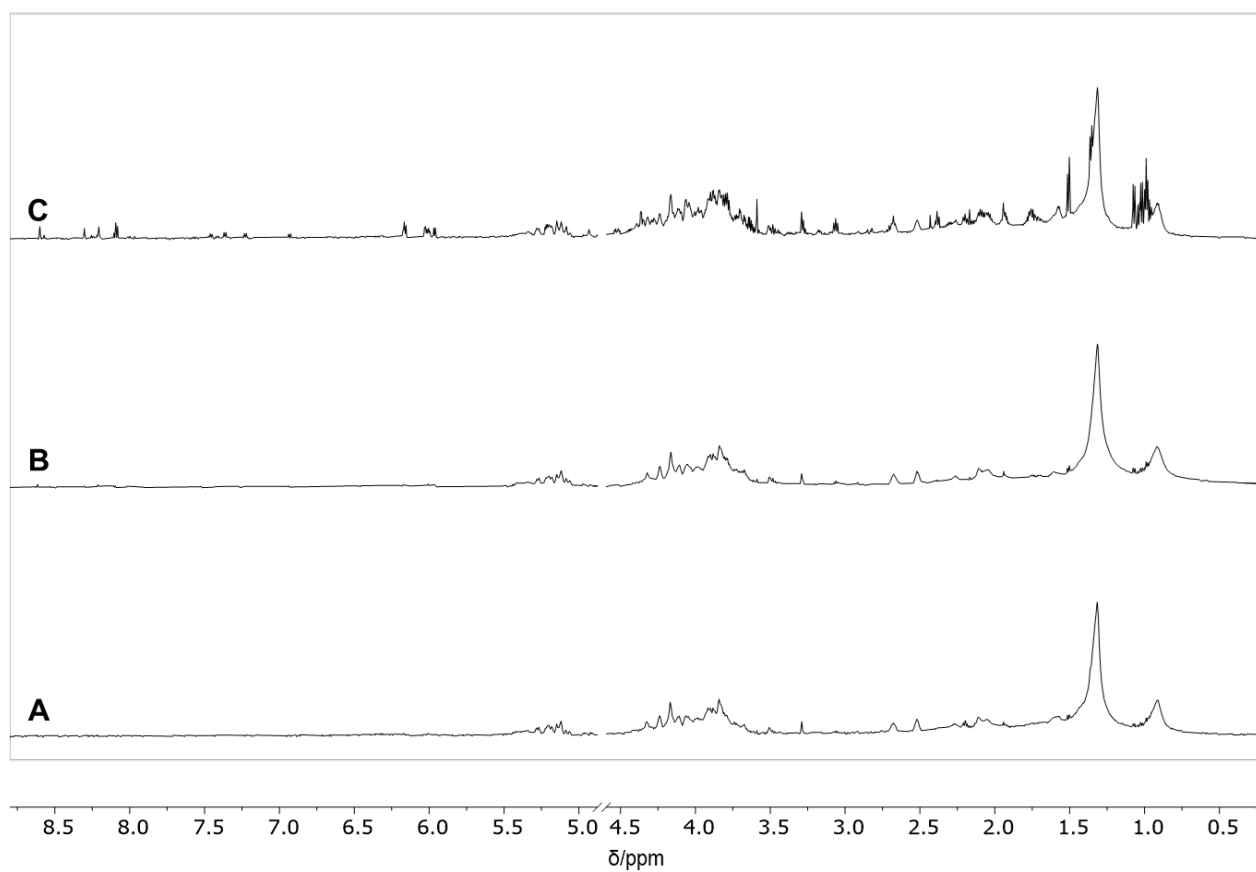


Figure S53. ^1H NMR spectra acquired on a solution containing 8 mg of *M. smegmatis* suspended in d-PBS (final sample volume, 40 μl) immediately after sample preparation (A), and after 4 h (B) and 18 h (C). Spectra were recorded with 120 scans at 37 $^\circ\text{C}$ and with a spin rate of 3 kHz.

On cell STD NMR experiments – additional figures

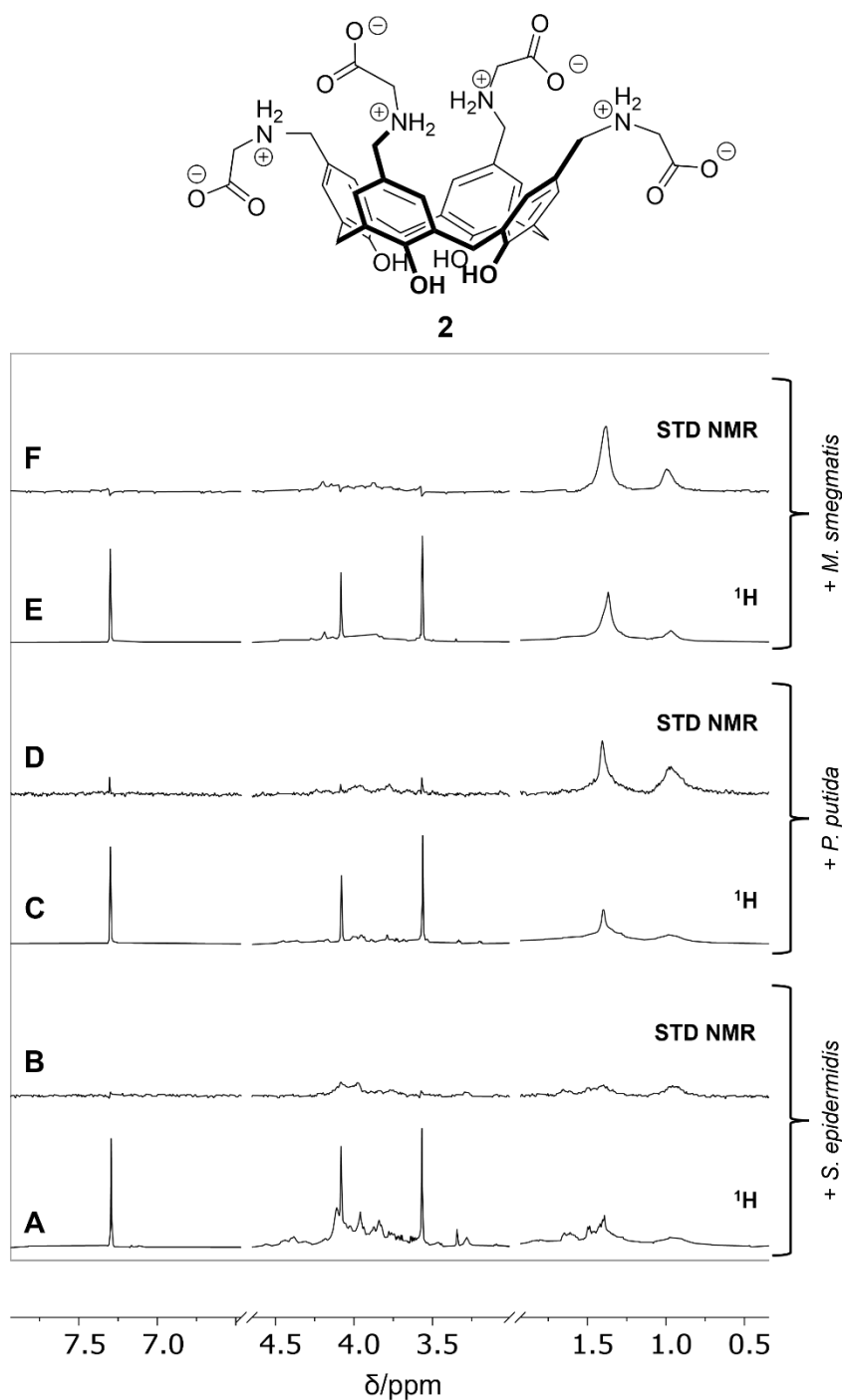


Figure S54. Representative STD NMR spectra acquired for compound **2**. **A)** ^1H spectrum of a sample containing compound **2** (2 mM) and *S. epidermidis* cells (ca. 8 mg), deuterated PBS, pH 7.4; 80 scans; **B)** 1D STD NMR spectrum of a sample containing compound **2** (2 mM) and *S. epidermidis* cells (ca. 8 mg), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x; **C)** ^1H spectrum of a sample containing compound **2** (2 mM) and *P. putida* cells (ca. 8 mg), deuterated PBS, pH 7.4; 80 scans; **D)** 1D STD NMR spectrum of a sample containing compound **2** (2 mM) and *P. putida* cells (ca. 8 mg), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x; **E)** ^1H spectrum of a sample containing compound **2** (2 mM) and *M. smegmatis* cells (ca. 8 mg), deuterated PBS, pH 7.4; 80 scans; **F)** 1D STD NMR spectrum of a sample containing compound **2** (2 mM) and *M. smegmatis* cells (ca. 8 mg), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x. STD NMR spectra were acquired with 3 s of saturation time, on-resonance frequency of 0.5 ppm, off-resonance frequency of 30 ppm. All spectra were recorded at 37 °C at 600 MHz.

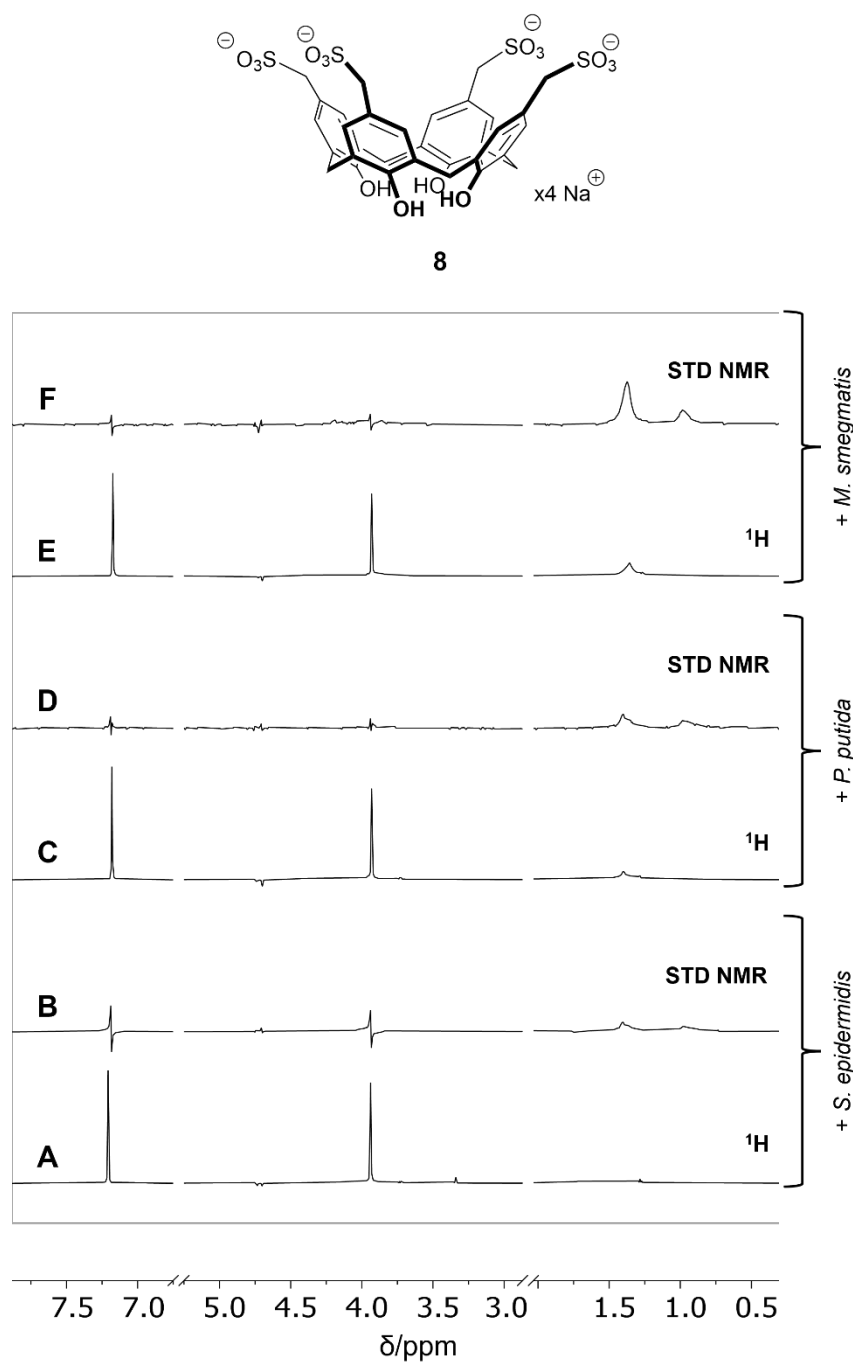


Figure S55. Representative STD NMR spectra acquired for compound **8**. **A**) ^1H spectrum of a sample containing compound **8** (2 mM) and *S. epidermidis* cells (ca. 8 mg), deuterated PBS, pH 7.4; 80 scans; **B**) 1D STD NMR spectrum of a sample containing compound **8** (2 mM) and *S. epidermidis* cells (ca. 8 mg), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x; **C**) ^1H spectrum of a sample containing compound **8** (2 mM) and *P. putida* cells (ca. 8 mg), deuterated PBS, pH 7.4; 80 scans; **D**) 1D STD NMR spectrum of a sample containing compound **8** (2 mM) and *P. putida* cells (ca. 8 mg), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x; **E**) ^1H spectrum of a sample containing compound **8** (2 mM) and *M. smegmatis* cells (ca. 8 mg), deuterated PBS, pH 7.4; 80 scans; **F**) 1D STD NMR spectrum of a sample containing compound **8** (2 mM) and *M. smegmatis* cells (ca. 8 mg), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x. STD NMR spectra were acquired with 3 s of saturation time, on-resonance frequency of 0.5 ppm, off-resonance frequency of 30 ppm. All spectra were recorded at 37 °C at 600 MHz.

When compared with the broad aliphatic cell resonances (1.5-0.8 ppm), compound **8** signals appearing in the STD spectra **B** and **F** are half positive and half negative, with a null total integral.¹⁹

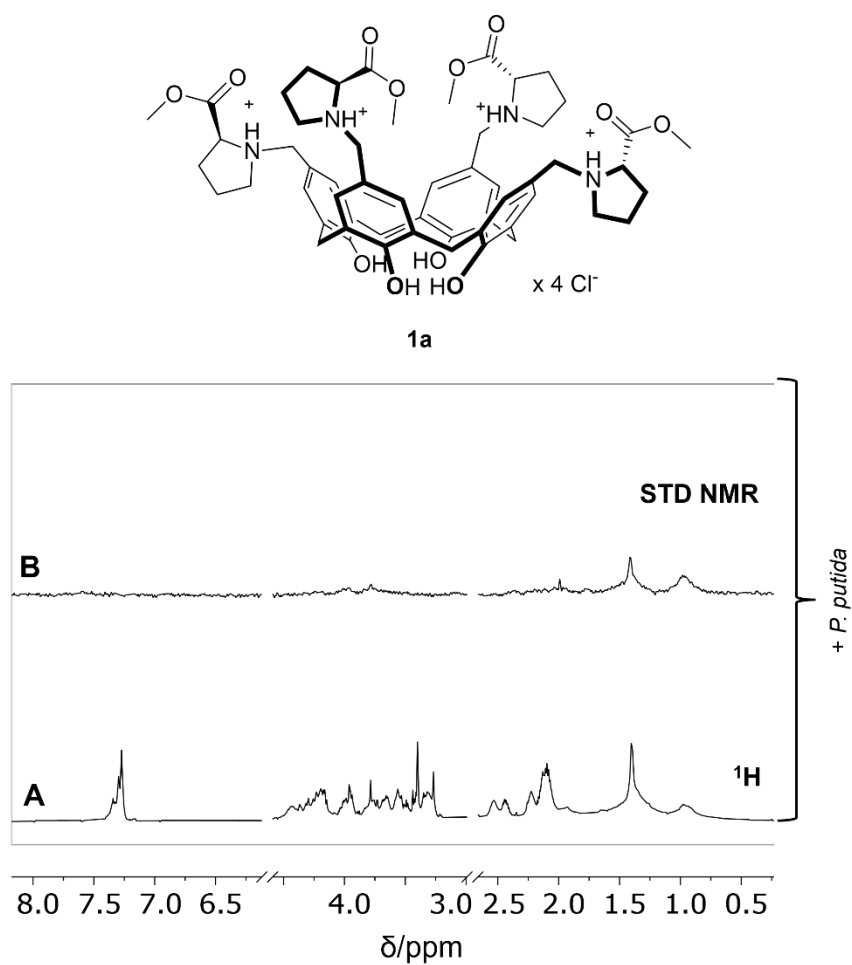


Figure S56. Representative STD NMR spectra acquired for compound **1a**. **A**) ¹H spectrum of a sample containing compound **1a** (2 mM) and *P. putida* cells (ca. 8 mg), deuterated PBS, pH 7.4; 80 scans; **B**) 1D STD NMR spectrum of a sample containing compound **1a** (2 mM) and *P. putida* cells (ca. 8 mg), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x. STD NMR spectra were acquired with 3 s of saturation time, on-resonance frequency of 0.5 ppm, off-resonance frequency of 30 ppm. All spectra were recorded at 37 °C at 600 MHz.

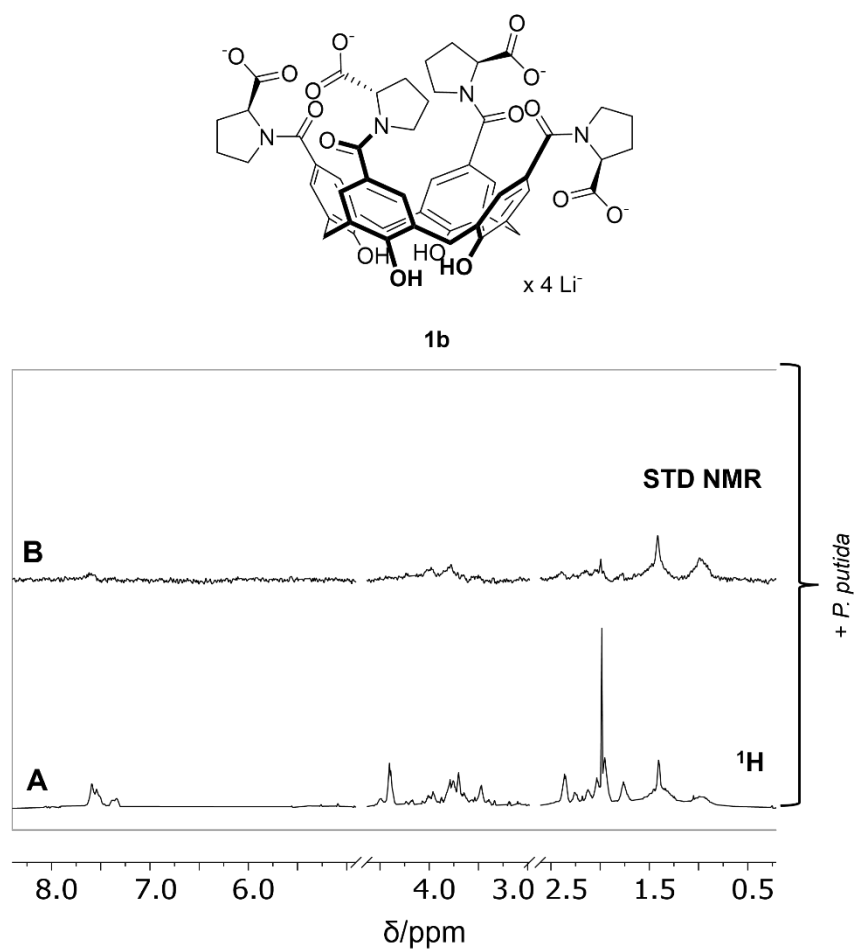


Figure S57. Representative STD NMR spectra acquired for compound **1b**. **A**) ^1H spectrum of a sample containing compound **1b** (2 mM) and *P. putida* cells (ca. 8 mg), deuterated PBS, pH 7.4; 80 scans; **B**) 1D STD NMR spectrum of a sample containing compound **1b** (2 mM) and *P. putida* cells (ca. 8 mg), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x. STD NMR spectra were acquired with 3 s of saturation time, on-resonance frequency of 0.5 ppm, off-resonance frequency of 30 ppm. All spectra were recorded at 37 °C at 600 MHz.

Isolation of LPS from *E. coli* and *P. putida* KT2440.

Table S2. Yields of cellular extracts.

Sample	Type of extraction	Dry cells (mg)	LPS (mg)	yield (%)
<i>E. coli</i> MG1655	PCP	2700	66.1	2.45
<i>P. putida</i> KT2440	PCP	1700	55.86	3.29
<i>P. putida</i> KT2440	H ₂ O/PhOH	1700	109.2	6.42

Electrophoretic analyses with SDS PAGE (15% separating gel and 5% stacking gel) technique^{19–21} were performed on cellular extracts. Gels were stained according to different procedures, i.e. Silver Stain, specific to reveal R- and S-type LPS, and Alcian Blue followed by Silver Stain, used to detect possible anionic polysaccharidic components. The gel coloured with Silver Stain procedure is showed in **Figure S58**.

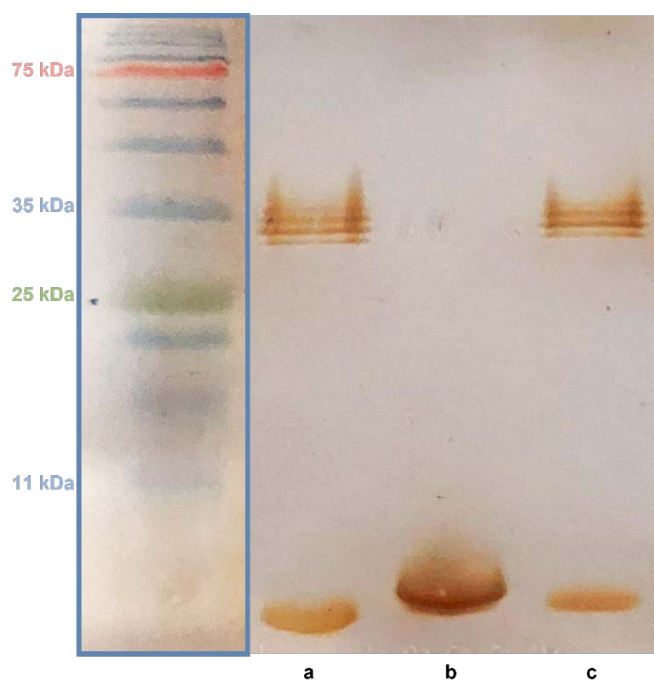


Figure S58: Discontinuous SDS-PAGE electrophoresis gel (separating gel 15%), coloured according to Silver Stain procedure. a) PCP precipitate from *P. putida* KT2440 (5 µg); b) PCP precipitate from *E. coli* MG1655 (5 µg); c) H₂O/PhOH extract from *P. putida* KT2440 (30 µg). The lane of the molecular weight marker (BLUeye Pre-stained Protein Ladder, Sigma-Aldrich, St. Louis, MO, USA) is indicated by the blue box.

LOS banding of *E. coli* was compared to that obtained from the same *E. coli* strain in previous work²⁰. Banding observed for *P. putida* are compatible with those reported in literature.^{21,22}

¹H-NMR was used to verify the absence of other saccharide material or contaminants from the extraction. The recovery of LPS was confirmed using the thiobarbiturate assay, which allows to evaluate the level of Kdo (3-deoxy-D-manno-oct-2-ulosonic acid) in the sample. In fact, being a ubiquitous acid monosaccharide found in all LPS core regions, Kdo is a marker for LPSs. Kdo assay was used to compare the efficiency of LPS extraction from *P. putida* KT2440 to LOS obtained from *E. coli* MG1655 and previously reported²⁰ (data not shown).

STD NMR experiments with *E. coli* LPS – additional experiments

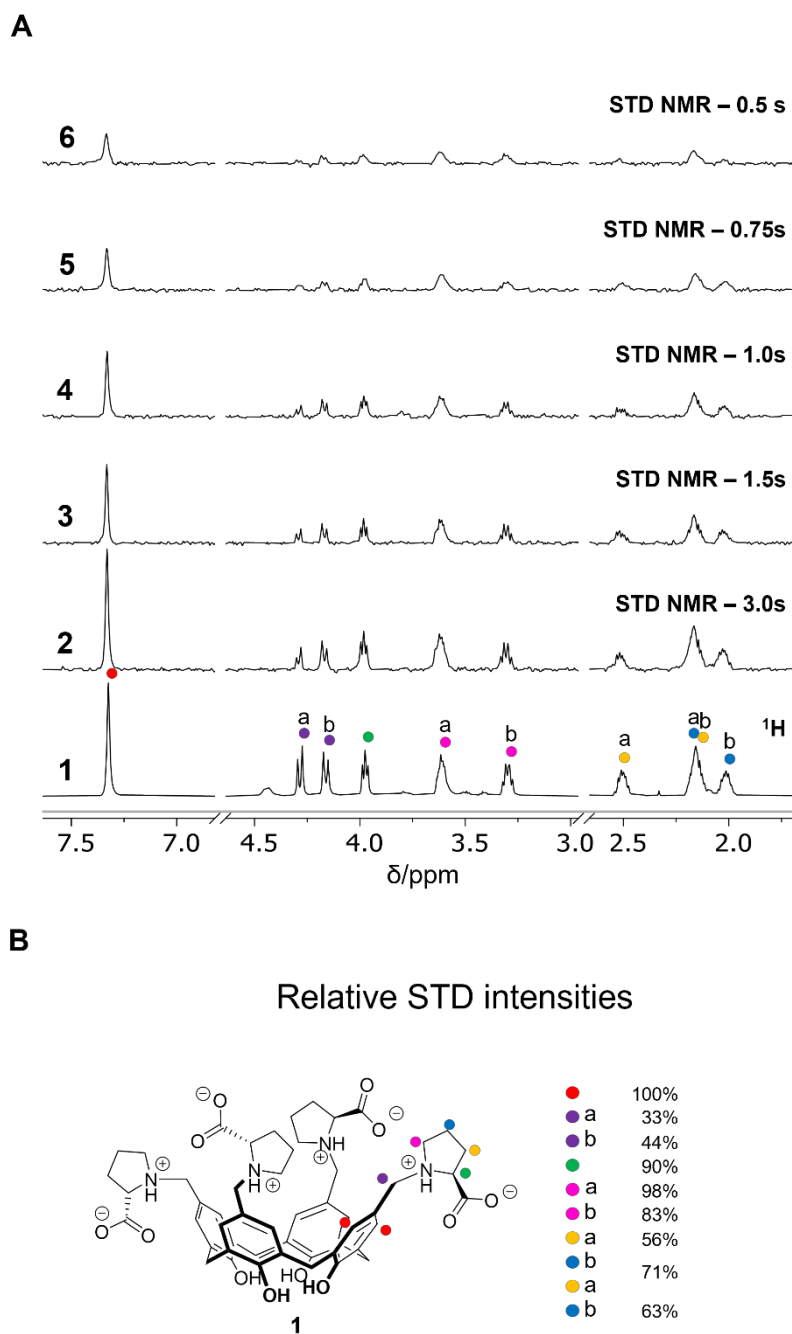


Figure S59. Representative STD NMR spectra acquired for compounds **1** in the presence of *E. coli* LPS. **Panel A.** **1**) ^1H spectrum of a sample containing compound **1** (2 mM) and *E. coli* LPS (0.4 mg/ml), deuterated PBS, pH 7.4; 80 scans; **2-6**) 1D STD NMR spectra of a sample containing compound **1** (2 mM) and *E. coli* LPS (0.4 mg/ml), deuterated PBS, pH 7.4; 256 scans, signal enhanced 10 x. STD NMR spectra were acquired with 3.0 s (**2**), 1.5 s (**3**), 1.0 s (**4**), 0.75 s (**5**) or 0.5 s (**6**) of saturation time, on-resonance frequency of 0.5 ppm, off-resonance frequency of 30 ppm. All spectra were recorded at 37 °C at 600 MHz.

Panel B. Relative STD intensities for each proton in compound **1**. The ligand epitope map was obtained from STD NMR spectrum acquired with 0.75 s of saturation time. Fractional STD effects for each proton was calculated by $(I_0 - I)/I_0$, where $(I_0 - I)$ is the peak intensity in the STD spectrum and I_0 is the peak intensity of an unsaturated reference spectrum. Then the whole set of STD values was normalized against the highest one and the result expressed in percentage.

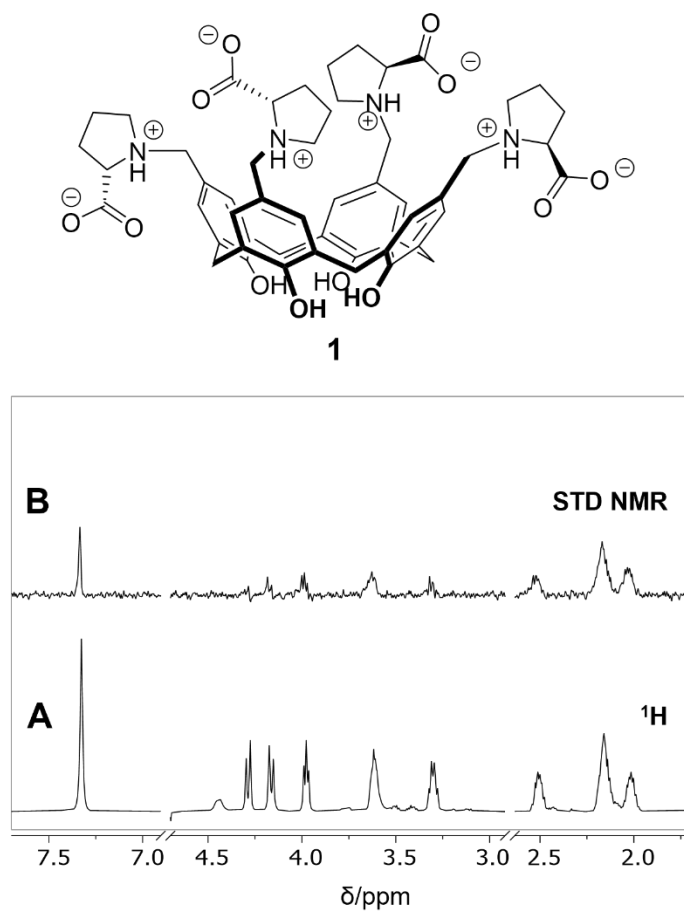
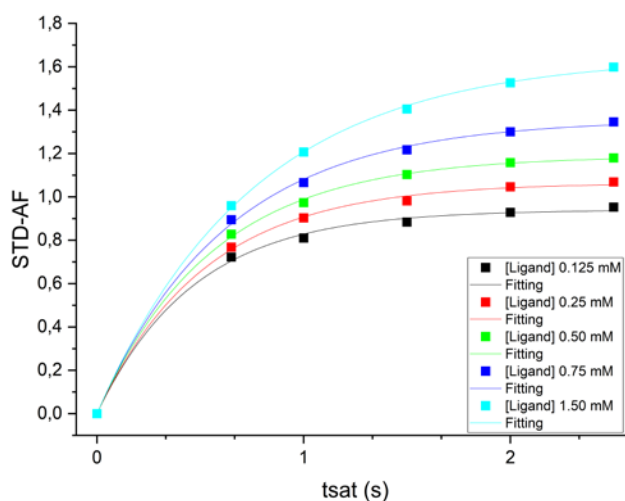


Figure S60. Representative STD NMR spectra acquired for compound **1** in the presence of *E. coli* LPSs dispersed in d_{25} -SDS micelles. **A)** ^1H spectrum of a sample containing compound **1** (2 mM) and *E. coli* LPS (0.4 mg/ml), d_{25} -SDS 10 mM, deuterated PBS, pH 7.4; 80 scans; **B)** 1D STD NMR spectrum of a sample containing compound **1** (2 mM) and *E. coli* LPS (0.4 mg/ml), d_{25} -SDS 10 mM, deuterated PBS, pH 7.4; 768 scans, signal enhanced 10 x. STD NMR spectra were acquired with 2.0 s of saturation time, on-resonance frequency of 0.5 ppm, off-resonance frequency of 30 ppm. All spectra were recorded at 37 °C at 600 MHz.

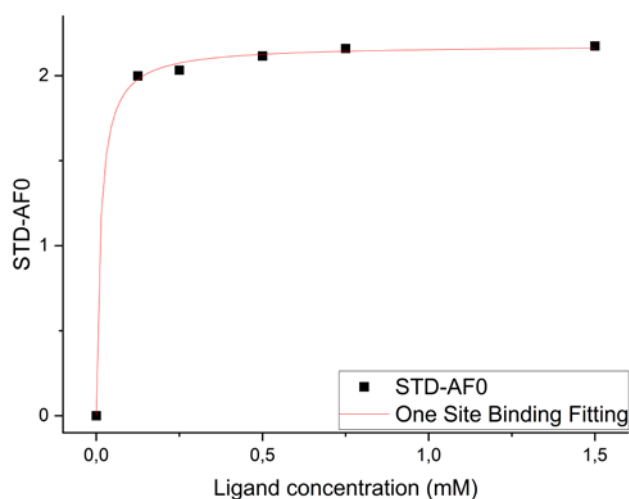
Determination of K_D value for compound 1 binding to *E. coli* LPS by the binding isotherm of STD-AF initial growth rates approach.

A)



[Compound 1] (mM)	STD-AF _{max}	k _{sat} (s ⁻¹)	Reduced Chi-Sqr	Adj. R-Square
0.125	0.94014 ± 0.01304	2.12672 ± 0,13398	3.20268E-4	0.99854
0.25	1.06517 ± 0.01141	1.90853 ± 0,08369	2.11196E-4	0.99871
0.50	1.18916 ± 0.00869	1.77938 ± 0,04995	1.09796E-4	0.99945
0.75	1.35718 ± 0.01500	1.59166 ± 0,06137	2.69074E-4	0.99984
1.50	1.64763 ± 0.01218	1.32040 ± 0,02925	1.20015E-4	0.99966

B)



	B _{max}	K _D (mM)	Reduced Chi-Sqr	Adj. R-Square
STD-AF ₀	2.18004 ± 0.01927	0.01274 ± 0.00241	6.80257E-4	0.99908

Figure S61. K_D value determination by the binding isotherm of STD-AF initial growth rates approach. **Pannel A.** For each concentration of compound 1 (0.125, 0.25, 0.5, 0.75, 1.5 mM; *E. coli* LPS concentration 50 μ M), STD-AF values for Pro H δ_b proton were obtained at different saturation times (0.65, 1.0, 1.5, 2.0, 2.5 s) and fit by using the equation $\text{STD-AF}(t_{\text{sat}}) = \text{STD-AF}_{\text{max}}[1 - \exp(-k_{\text{sat}}t_{\text{sat}})]$. The initial slopes, STD-AF₀, were obtained from $\text{STD-AF}_0 = \text{STD-AF}_{\text{max}}k_{\text{sat}}$. **Pannel B.** STD-AF₀ values were represented as a function of the ligand concentration, and the mathematical fit to a Langmuir isotherm ($y = B_{\text{max}}x/(K_D + x)$) allowed to obtain B_{max} and K_D values.

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