The first account of a structurally persistent micelle

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Synthesis and spectroscopic characterisation of the amphiphilic dendro-calixarene 1

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4 \xrightarrow{\text{C}_{12}H_{25}Br} \xrightarrow{\text{NaOH} / \text{TBAB}} 5
\text{conc. HNO}_3 / \text{CH}_3\text{COOH}

7 \xrightarrow{\text{Raney-Ni} / \text{H}_2} 6

8 \xrightarrow{\text{NaOH}} 9
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\[
\begin{align*}
\text{DCC / HOBT} & \\
\begin{align*}
R & = \text{t-Bu.} \\
R & = \text{H}
\end{align*}
\end{align*}
\]

1: \( R = \text{H} \)

10: \( R = \text{t-Bu.} \)
**Tert-butyl-calix[4]arene 4**

The synthesis of tert-butyl-calix[4]arene 4 was performed according to Gutsche & Iqbal.\[S1]\n

Calix[4]arene 4 (14 g, 0.02 mol) was dissolved in 1000 ml of toluene in a 2 l round-bottomed flask. Subsequently 25 g NaOH in 25 ml water, 1.4 g (4.35 mmol) tetrabutyl-
ammoniumbromide and 96.1 ml (0.4 mol) dodecylbromide were added. The mixture was stirred at 110 °C for 24 hours. After cooling to room temperature the resulting yellow liquid was washed with 500 ml water, 560 ml 1 M HCl and twice with 500 ml of a saturated solution of sodium chloride in water. After evaporation of the solvent a yellow oil was obtained. For further purification the oil was dissolved in a small amount of CHCl$_3$ from which the reaction product was precipitated by the addition of ethanol. The resulting white powder was dried under reduced pressure.

**Yield:** 24.46 g (0.019 mol, 95%)

**MS** (FAB, NBA): $m/z = 1321$ [M+H]$^+$.  

$^1$H-NMR (400 MHz, CDCl$_3$, RT): $\delta$ [ppm] = 0.88 (t, $^3$J = 7.1, 12H, CH$_3$), 1.07 (s, 36H, t-Bu-CH$_3$), 1.27 (m, 56H, CH$_2$), 1.38 (m, 16H, CH$_2$), 2.01 (m, 8H, CH$_2$), 3.08 (d, $^2$J = 12.5, 4H, Ar-CH$_2$), 3.83 (t, $^3$J = 7.6, 8H, O-CH$_2$), 4.38(d, $^2$J = 12.5, 4H, Ar-CH$_2$), 6.76 (s, 8H, Ar-H).

$^{13}$C-NMR (100.50 MHz, CDCl$_3$, RT): $\delta$ [ppm] = 14.11 (CH$_3$), 22.72, 26.41, 29.46, 29.79, 29.86, 29.92, 30.10, 30.43 (CH$_2$), 31.11 (Ar-CH$_2$), 31.47 (t-Bu-CH$_3$), 32.00 (CH$_2$), 33.79 (t-Bu-Cq), 75.47 (OCH$_2$), 124.85 (C$_{Ar}$-H), 133.87 (C$_{Ar}$-CH$_2$), 144.11 (C$_{Ar}$-t-Bu), 153.79 (C$_{Ar}$-O).

**IR** (KBr, $\bar{v}$ [cm$^{-1}$]): 2919.2, 2850.6, 1482.2, 1467.6,1390.9,1361.5, 1300.2, 1248.2, 1202.0, 1123.5, 1019.4, 869.2, 721.2, 636.2.

C$_{92}$H$_{152}$O$_4$ **Calculate:** C 83.57, H 11.59 **Found:** C 83.38, H 11.47.

5,17,11,23-Tetra(tert-butyl)-25,26,27,28-tetradecyloxyalix[4]arene 5 (5g, 3.79 mmol) was dissolved in 100 ml CH₂Cl₂ in a 250 ml round-bottomed flask. Subsequently 12.5 ml 65% nitric acid and 8.5 ml acetic acid were added. The solution was stirred until the colour changed from dark blue to red-brown. The organic phase was washed with 75 ml water, 100 ml of a saturated solution of sodium hydrogencarbonate in water and 100 ml of a saturated solution of sodium chloride in water. The resulting organic solution was dried over Na₂SO₄. After filtration and evaporation of the solvent a brown oil was obtained. The crude product was purified by flash chromatography (SiO₂, toluene / cyclohexane 80:20, Rf (6) = 0.15). For further purification the oil was dissolved in a small amount of CHCl₃ from which the reaction product was precipitated by the addition of ethanol. The resulting light yellow powder was dried under reduced pressure.

**Yield:** 1.1 g (0.83 mmol, 22 %)

**MS (FAB, NBA):** m/z = 1300 [M]+.

**¹H-NMR** (400 MHz, CDCl₃, RT): δ [ppm] = 0.87 ( t, ³J=7.1, 12H, CH₃), 1.27 (m, 56H, CH₂), 1.39 (s, 18H, t-Bu-CH₃), 1.55 (m, 8H, CH₂) 1.87 (m, 16H, CH₂), 3.16 ( d, ²J=13.2 , 4H, Ar-CH₂ ), 3.76 (t, ³J=6.5, 4H, O-CH₂), 3.97 (t, ³J= 8.3, 4H, O-CH₂), 4.42 (d, ²J= 13.7, 4H, Ar-CH₂), 6.97 (s, 4H, H_AR-t-Bu), 7.16 (s, 4H, H_AR-NO₂).

**¹³C-NMR** (100.50 MHz, CDCl₃, RT): δ [ppm] = 14.10 (CH₃), 22.69, 25.98, 26.51, 29.40, 29.78, 29.81, 29.86, 30.12, 30.39 (CH₂), 31.29 (Ar-CH₂), 31.61 (t-Bu-CH₃), 31.94 (CH₂), 34.27 (t-Bu-Cq), 75.15 (O-CH₂), 75.68 (O-CH₂), 122.68, 126.38 (C_AR-H), 135.01, 135.19 (C_AR-CH₂), 142.57 (C_AR-NO₂), 146.09 (C_AR-t-Bu), 154.71, 160.37 (C_AR-O).

**IR (KBr, ν [cm⁻¹]):** 2918.8, 2850.4, 1531.4, 1468.0, 1347.5, 1309.1, 1260.9, 1210.1, 1112.4, 1090.8, 1016.0, 937.5, 900.7, 767.9, 745.1 721.0, 546.7.

C₈₄H₁₃₄N₂O₈ **Calculate:** C 77.61, H 10.39, N 2.15 **Found:** C 77.51, H 10.49 N 2.15.

For the hydrogenation of 6 to 7 commercial Raney-nickel was washed twice with 100 ml ethanol and then 2x with 100 ml toluene. To 10 g of this Raney-nickel 4.2 g (3.4 mmol) of 6 dissolved in 200 ml toluene was added. The reaction mixture was hydrogenated at 60 °C under intensive stirring until the consumption of H₂ was finished. Subsequently the Raney-nickel was removed by filtration. Evaporation of the solvent gave a brown oil which was dissolved in a small amount of CHCl₃. Precipitation with ethanol gave a brown powder, which was dried under reduced pressure.

**Yield:** 4.01 g (3.23 mmol, 95 %)

**MS (FAB, NBA):** \(m/z = 1240\) [M]+.

**1H-NMR (400 MHz, CDCl₃, RT):** \(\delta [ppm] = 0.86 (t, J=7.1, 12H, CH₃), 1.26 (m, 68H, CH₂), 1.36 (s, 18H, t-Bu-CH₃), 1.54 (m, 4H, CH₂), 1.80 (m, 4H, CH₂), 1.87 (m, 4H, CH₂), 2.82 (b, 4H, NH₂), 2.98 (d, J=13.2, 4H, Ar-CH₂), 3.61 (t, J=6.5, 4H, O-CH₂), 3.97 (t, J=8.3, 4H, O-CH₂), 4.35 (d, J=13.7, 4H, Ar-CH₂), 5.43 (s, 4H, H₁₆-NH₂), 7.04 (s, 4H, H₁₆-t-Bu).

**13C-NMR (100.50 MHz, CDCl₃, RT):** \(\delta [ppm] = 14.14\) (CH₃), 22.72, 26.07, 26.79, 29.44, 29.76, 29.81, 29.90, 30.21, 30.59 (CH₂), 31.39 (Ar-CH₂), 31.78 (t-Bu-CH₃), 31.97 (CH₂), 34.08 (t-Bu-Cq), 74.88 (O-CH₂), 75.19 (O-CH₂), 115.61, 125.61 (C₁₆-H), 134.07, 136.15 (C₁₆-CH₂), 140.46 (C₁₆-NH₂), 144.01 (C₁₆-t-Bu), 149.19, 155.84 (C₁₆-O).

**IR (KBr, \(\tilde{\nu}\) [cm⁻¹]):** 2917.9, 2849.3, 1613.4, 1465.5, 1362.4, 1308.1, 1218.2, 1141.2, 1108.6, 1020.0, 849.5, 801.5, 719.4, 595.9.

C₈₄H₁₃₈N₂O₄ **Calculate:** C 81.36, H 11.22 N 2.26 **Found:** C 81.23, H 11.18 N 2.26.

To 2.31 g (2.0 mmol) of 7 dissolved in 50 ml dry THF in a 100 ml round-bottomed flask, 2.6 ml (24.23 mmol) of methyl malonyl chloride were added. After stirring for five hours at room temperature the solvent was evaporated. The residue was dissolved in 100 ml CHCl₃ and washed with 100 ml of a saturated solution of sodium hydrogencarbonate in water and 100 ml of a saturated solution of sodium chloride in water. After drying over MgSO₄, the organic phase was filtered and evaporated. The residue was dried under reduced pressure to yield 8 as a white powder.

**Yield:** 2.68 g (1.86 mmol, 93 %)

**MS** (FAB, NBA): \(m/z = 1440 \ [M]^+\).

**¹H-NMR** (400 MHz, CDCl₃, RT): \(\delta \ [ppm] = 0.86 \ (t, ^3J=7.1, 12H, CH₃), 1.26 \ (m, 68H, CH₂), 1.32 \ (s, 18H, t-Bu-CH₃), 1.52 \ (m, 4H, CH₂), 1.82 \ (m, 4H, CH₂), 1.93 \ (m, 4H, CH₂), 3.06 \ (d, ^2J=13.0, 4H, Ar-CH₂), 3.19 \ (s, 4H, CO-CH₂-CO), 3.61 \ (t, ^3J=6.5, 4H, O-CH₂), 3.67 \ (s, 6H, OCH₃), 3.97 \ (t, ^3J= 8.3, 4H, O-CH₂), 4.37 \ (d, ^2J= 13.7, 4H, Ar-CH₂), 6.15 \ (s, 4H, H_AR-NH), 7.03 \ (s, 4H, H_AR-t-Bu) 7.92 \ (b, 2H, NH).

**¹³C-NMR** (100.50 MHz, CDCl₃, RT): \(\delta \ [ppm] = 14.23 \ (CH₃), 22.80, 26.18, 26.77, 29.52, 29.56, 29.83, 29.89, 30.26, 30.60 \ (CH₂), 31.35 \ (Ar-CH₂), 31.75 \ (t-Bu-CH₃), 32.05 \ (CH₂), 34.20 \ (t-Bu-Cq), 42.40 \ (CO-CH₂-CO), 52.50 \ (OCH₃), 75.03 \ (O-CH₂), 75.31 \ (O-CH₂), 121.52, 125.67 \ (C_AR-H), 130.34 \ (C_AR-NH), 133.95, 135.34 \ (C_AR-CH₂), 144.56 \ (C_AR-t-Bu), 153.00, 155.04 \ (C_AR-O), 162.58 \ (C=O), 169.73 \ (C=O).

**IR** (KBr, \(\tilde{\nu} \ [cm^{-1}]): 3317.0, 2962.5, 2923.6, 2853.0, 1749.5, 1664.4, 1605.4, 1551.4, 1467.2, 1417.2, 1381.7, 1362.6, 1261.9, 1216.9, 1097.0, 1020.7, 869.6, 799.9, 703.0, 593.7, 547.8.

**C_{92}H_{146}N_{2}O_{10}** Calculate: C 76.73, H 10.22, N 1.95 Found: C 76.60, H 10.32, N 1.95.

5,17-Di[(3-methoxy-3-oxopropanoyl)amino]-11,23-di(tert-butyl)-25,26,27,28-tetradodecyl-oxycalix[4]arene 8 (1g, 0.64 mmol) was dissolved in 30 ml THF in a 100 ml round-bottomed flask, 180 mg NaOH dissolved in 5 ml water was added and the mixture was stirred for twelve hours. After that time 25 ml 1M HCl were added and most of the solvent was removed. The residue was washed with 50 ml water to give an orange powder after filtration and drying in reduced pressure.

Yield: 0.89 g (0.63 mmol, 98 %)

MS (FAB, NBA): m/z = 1412 [M]+.

$^1$H-NMR (400 MHz, CDCl$_3$, RT): $\delta$ [ppm] = 0.86 ( t, $^3$J=7.1, 12H, CH$_3$), 1.26 (m, 68H, CH$_2$), 1.32 (s, 18 H, t-Bu-CH$_3$), 1.52 (m, 4H, CH$_2$), 1.82 (m, 4H, CH$_2$), 1.93 (m, 4H, CH$_2$), 3.06 (d, $^2$J=13.0, 4H, Ar-CH$_2$), 3.20 (s, 4H, CO-CH$_2$-CO), 3.63 (t, $^3$J=6.5, 4 H, O-CH$_2$), 4.00 (t, $^3$J=8.3, 4H, O-CH$_2$), 4.38 (d, $^2$J= 13.7, 4H, Ar-CH$_2$), 6.14 (s, 4H, H$_{AR}$-NH), 7.06 (s, 4H, H$_{AR}$-t-Bu), 8.55 (b, 2H, NH).

$^{13}$C-NMR (100.50 MHz, CDCl$_3$, RT): $\delta$ [ppm] = 14.10 (CH$_3$), 22.70, 26.06, 26.69, 29.47, 29.73, 29.85, 29.88, 30.19, 30.53 (CH$_2$), 31.31 (Ar-CH$_2$), 31.65 (t-Bu-CH$_3$), 32.98 (CH$_2$), 34.11 (t-Bu-Cq), 43.08 (CO-CH$_2$-CO), 74.98 (O-CH$_2$), 75.50 (O-CH$_2$), 121.42, 125.85 (C$_{AR}$-H), 130.34 (C$_{AR}$-NH), 134.06, 135.50 (C$_{AR}$-CH$_2$), 144.93 (C$_{AR}$-t-Bu), 153.43, 155.22 (C$_{AR}$-O), 156.73 (C=O), 172.31 (C=O).

IR (KBr, $\tilde{\nu}$ [cm$^{-1}$]): 3318.9, 2961.6, 2923.1, 2852.5, 1738.1, 1606.0, 1557.7, 1466.5, 1381.1, 1362.9, 1311.2, 1262.0, 1216.3, 1102.2, 1019.5, 964.8, 871.6, 800.2, 720.2, 684.3, 545.7, 493.8.

C$_{90}$H$_{142}$N$_2$O$_{10}$ Calculate: C 76.55, H 10.14, N 1.98 Found: C 76.45, H 10.03, N 1.95.
In a 250 ml round-bottomed flask 0.48 g (0.34 mmol) 9, 0.42 g (2.04 mmol) dicyclohexylcarbodiimide and 0.32 g (2.04 mmol) HOBT(1-hydroxybenzotriazole) were dissolved in 100 ml dry DMF and stirred continuously. After one hour 3.0 g (2.04 mmol) 11 were added. After another hour dicyclohexylurea began to precipitate. After 48 hours the solvent was evaporated. The residue was dissolved in cold diethyl acetate and the insoluble urea was filtered off. The resulting organic solution was washed with 50 ml of 10% citric acid, 50 ml of a 8% solution of sodium hydrogencarbonate in water and 50 ml of a saturated solution of sodium chloride in water. The resulting organic solution was dried over MgSO₄, then filtered and evaporated to give a brown oil. The crude product was purified by flash chromatography (SiO₂, acetic ester / cyclohexane 65:35, Rₛ (10) = 0.8). After evaporation of the solvent 10 was obtained as a brown solid which was subsequently dried under reduced pressure.

**Yield:** 0.30 g (0.07 mmol, 20 %)

**MS** (FAB, NBA): \( m/z = 4256 \ [\text{M+H}]^+ \).

**¹H-NMR** (400 MHz, CDCl₃, RT): \( \delta \ [\text{ppm}] = 0.83 \ (t, ^3J=7.1, 12H, \text{CH}_3), 1.21 \ (m, 68H, \text{CH}_2), 1.29 \ (s, 18H, \text{t-Bu-CH}_3), 1.36 \ (s, 162H, -C(\text{CH}_3)_3), 1.52 \ (m, 4H, \text{CH}_2), 1.90 \ (m, 44H, \text{CH}_2), 2.11 \ (m, 60H, \text{CH}_2), 3.10 \ (d, ^2J=13.0, 4H, \text{Ar-CH}_2), 3.20 \ (s, 4H, \text{CO-CH}_2-\text{CO}), 3.63 \ (t, ^3J=6.5, 4H, \text{O-CH}_2), 4.00 \ (t, ^3J= 8.3, 4H, \text{O-CH}_2), 4.38 \ (d, ^2J= 13.7, 4H, \text{Ar-CH}_2), 6.18 \ (s, 4H, \text{H}_{\text{AR-NH}}), 7.13 \ (s, 4H, \text{H}_{\text{AR-t-Bu}}).

**¹³C-NMR** (100.50 MHz, CDCl₃, RT): \( \delta \ [\text{ppm}] = 13.97 \ (\text{CH}_3), 22.57, 25.89, 26.49 \ (\text{CH}_2), 27.98 \ (-C(\text{CH}_3)_3), 28.32, 29.32, 29.66, 29.71, 29.73, 30.04, 30.22, 30.42 \ (\text{CH}_2), 31.37 \ (\text{Ar-CH}_2), 31.66 \ (\text{t-Bu-CH}_3), 32.98, 33.88 \ (\text{CH}_2), 34.00 \ (\text{t-Bu-Cq}), 42.60 \ (\text{CO-CH}_2-\text{CO}), 57.38 \ (\text{NHCCCH}_2), 74.96 \ (\text{O-CH}_2), 75.08 \ (\text{O-CH}_2), 80.28(-C(\text{CH}_3), 119.28, 125.43 \ (\text{C}_{\text{AR-H}}), 130.01
(C<sub>AR</sub>-NH), 134.44, 135.55 (C<sub>AR</sub>-CH<sub>2</sub>), 144.44 (C<sub>AR</sub>-t-Bu), 152.44, 154.60 (C<sub>AR</sub>-O), 155.41 (C=O), 172.34 (C=O), 172.59 (C=O), 172.83 (C=O).

**IR** (KBr, $\tilde{\nu}$ [cm$^{-1}$]): 3363.7, 2927.9, 2855.3, 1733.8, 1682.8, 1541.5, 1465.6, 1368.1, 1314.9, 1260.4, 1216.4, 1153.0, 1102.4, 1022.4, 956.2, 848.4, 801.6, 757.9.

C<sub>242</sub>H<sub>406</sub>N<sub>10</sub>O<sub>50</sub> **Calculate:** C 68.30, H 9.62, N 3.29  **Found:** C 68.15, H 9.70, N 3.30.


To a solution of 0.1 g (0.02 mmol) 10 in 5 ml toluene 1.5 ml trifluoroacetic acid were added and stirred for 24 hours. After that time the solvent was evaporated and the crude brown product was stirred with 10 ml diethyl ether to remove remaining trifluoroacetic acid. The product was filtered off to give a yellow ochre powder after drying under reduced pressure.

**Yield:** 0.06 g (0.019 mmol, 98 %)

**MS** (FAB, NBA): m/z = 3246 [M$^+$].

**$^1$H-NMR** (400 MHz, D<sub>2</sub>O, RT): $\delta$ [ppm] = 0.89 ( t, $^3$J=7.1, 12H, CH<sub>3</sub>), 1.29 (m, 86H, CH<sub>2</sub>), 1.54 (m, 4H, CH<sub>2</sub>), 1.91 (m, 44H, CH<sub>2</sub>), 2.13 (m, 60H, CH<sub>2</sub>), 3.21 (b, 8H), 3.70 (b, 4H, O-CH<sub>2</sub>), 4.04 (b, 4H, O-CH<sub>2</sub>), 4.43 (b, 4H, Ar-CH<sub>2</sub>), 6.74 (s, 4H, H<sub>AR</sub>-NH), 7.19 (s, 4H, H<sub>AR</sub>-t-Bu).

**$^{13}$C-NMR** (100.50 MHz, D<sub>2</sub>O, RT): $\delta$ [ppm] = 14.15 (CH<sub>3</sub>), 22.57, 25.89, 26.49 (CH<sub>2</sub>), 28.32, 29.32, 29.66, 29.71, 29.73, 30.04, 30.22, 30.42 (CH<sub>2</sub>), 31.37 (Ar-CH<sub>2</sub>), 31.66 (t-Bu-CH<sub>3</sub>), 32.98, 33.88 (CH<sub>2</sub>), 34.00 (t-Bu-Cq), 42.60 (CO-CH<sub>2</sub>-CO), 58.57 (NHCH<sub>2</sub>), 74.96 (O-CH<sub>2</sub>), 75.08 (O-CH<sub>2</sub>), 121.02, 125.43 (C<sub>AR</sub>-H), 131.01 (C<sub>AR</sub>-NH), 134.95, 135.55 (C<sub>AR</sub>-CH<sub>2</sub>), 144.44 (C<sub>AR</sub>-t-Bu), 152.44, 154.60 (C<sub>AR</sub>-O), 155.41 (C=O), 172.34 (C=O), 172.90 (C=O), 175.35 (COOH).

**IR** (KBr, $\tilde{\nu}$ [cm$^{-1}$]): 3442.5, 2925.7, 2854.5, 1732.9, 1654.8, 1541.7, 1459.9, 1367.7, 1261.1, 1156.0, 1105.0, 1027.9, 801.0, 472.4.
$\text{C}_{170}\text{H}_{262}\text{N}_{10}\text{O}_{50}$  \textbf{Calculate:} C 62.90, H 8.14, N 4.32  \textbf{Found:} C 62.75, H 8.05, N 4.30.

9-Cascade:aminomethan[3]:(2-aza-3-oxopentylidy)propanacid-\textit{tert}-butylester 11

The synthesis of 11 was performed according to Brettreich & Hirsch.$^{[S2]}$
Electron cryo-microscopy

**Principles:** To obtain information on the structure of assemblies in their native fully hydrated state, dehydration, crystallisation or even rearrangements of the assemblies must be prevented. By quick-freezing ultra thin sample layers in an adequate coolant, cooling rates in the order of $10^4$ K/s can be achieved allowing the amorphous solidification (“vitrification”) of the solvent.\[^{[S3, S4]}\] Assemblies embedded in a solid glass-like layer of the solvent can thus be directly visualised in a TEM, using phase contrast imaging techniques.\[^{[S5, S6]}\] No additional contrast enhancing material is necessary for imaging. Maintaining the sample temperature constantly at $\sim 94$ K not only preserves the native structure information but also significantly reduces the radiation damage.

**Preparation:** Droplets of the sample (5 µl) were applied to perforated (1 µm hole diameter) carbon film covered 200 mesh grids (R1/4 batch of Quantifoil Micro Tools GmbH, Jena, Germany), which had been hydrophilized before use by 60 s plasma treatment at 8 W in a BALTEC MED 020 device. The supernatant fluid was removed with a filter paper until an ultra-thin layer of the sample solution was obtained spanning the holes of the carbon film. The samples were immediately vitrified by propelling the grids into liquid ethane at its freezing point (90 K) and operating a guillotine-like plunging device using the CEVS humidity and temperature controlled vitrification system.\[^{[S7]}\] The vitrified samples were subsequently transferred under liquid nitrogen into a Philips CM12 transmission electron microscope (FEI Company, Oregon, USA) using the Gatan (Gatan Inc., California, USA) cryoholder and -stage (Model 626). Microscopy was carried out at 94 K sample temperature using the low dose protocol of the microscope at a primary magnification of 58,300× and an accelerating voltage of 100 kV (LaB$_6$ - illumination). In all cases the defocus was set to 1.2 µm, which corresponds to a first zero of the phase contrast transfer function at 2.1 nm.
Note: Though the determination of the native structure of the micellar assemblies was the major goal of this study, we were interested to know if the structure of the microcontainers after removal of the solvent remains intact. We therefore repeated the described procedure with air-dried samples.

**Dried sample preparation and room temperature electron microscopy**

Sample solution (5 µl) was absorbed onto hydrophilised Formvar®-supported carbon film that covered the copper grids (400 mesh). The supernatant fluid was removed by blotting with a filter paper and the sample was allowed to dry in air. Contrast enhancing heavy metal stain solution (1% Phospho-Tungstic-Acid (PTA) at pH 7.4) was subsequently applied for 45 s and blotted again (“negative stain” method). A standard holder was used to transfer the dried samples into a Philips CM 12 TEM (FEI company, Oregon, USA) equipped with a LaB₆ cathode. Images were taken at a defocus value of 0.6 µm using a primary magnification of $58,300 \times$ at an accelerating voltage of 100 kV ($C_s=2$ mm).

**Image processing**

**Principles:** Owing to the low signal-to-noise ratio of the images the direct structural information from individual particles is very restricted. By aligning and summing a large number of projection images of individual particles in the identical spatial orientation, the signal (i.e. the inherent structural information) is enhanced with respect to the random background noise. A set of such noise free sum images (“class averages”), representing projections in different spatial orientations, can be used to determine the three-dimensional information of the object.

**Procedure:** Prior to processing, micrographs were checked for absence of astigmatism or drift by laser optical diffraction. Optical sound micrographs were digitised using the Heidelberg "Primescan" drum scanner (Heidelberger Druckmaschinen AG, Heidelberg,
Germany) at a nominal pixel resolution of 0.68 Å in the digitised images (scanning resolution 4 µm). Individual particles were interactively selected and extracted from the digitised micrographs as 200×200 pixel fields. After suppression of high frequency background noise by filtering well established non-biased “reference-free” alignment and automatic classification procedures were performed using multivariate statistical analysis and automated hierarchical classification schemes with the aid of IMAGIC-5 software (Image Science GmbH, Berlin, Germany). Random orientation of the assemblies within the matrix of vitrified water yield a set of ‘class averages’, which represent ‘typical views’ of noise-reduced two-dimensional projection images. A precondition for a successful three-dimensional structure reconstruction is an isomorphic data set, e.g. all particle images have to be derived from one distinct structurally determined object. Inhomogenities in the data set would lead to a drastic loss of resolution or would even prevent a coherent reconstruction procedure. Assignment of the angular relation between the obtained various ‘class averages’ (“Euler angle” determination) was performed by using the common line projection theorem ("angular reconstitution" technique (van Heel, 1987)). A first rough three-dimensional reconstruction was then calculated following the back projection algorithm procedure. After several iterative procedure cycles it became evident that $C_2$ is an inherent symmetry element in the data set and subsequent calculations were thus performed under $C_2$ symmetry restraint. Further refinements were obtained by using “anchorset” reprojection images as references (= reference image set of angle-defined projections into the 3D-volume) in a series of subsequent multireference alignment procedures. The final resolution achieved in the reconstruction of 2600 individual particles was determined by a Fourier shell correlation method (FSC) and amounts to ~12 Å, considering the 3 $\sigma$ threshold criterion (~16 Å at 0.5 criterion) and the $C_2$ point-group symmetry.

**Note:** The described procedure was applied independently to data sets taken from vitrified samples (cryo-TEM) as well as from air-dried-samples (cp. Supplement figure 6). In both
cases, the three-dimensional reconstruction calculations reproduced the described structure with high consistency.

**Fitting of atomic structures into EM density maps**

A low energy conformer of 1 was obtained from molecular mechanics calculations using the MM+ force field implemented on HyperChem 6.01 (Hypercube, Inc., Gainesville, USA). Corresponding pdb-data have been imported in AMIRA software (Indeed - Visual Concepts GmbH, Berlin, Germany) and were visually fitted into the EM density data with the aid of stereo glasses (CrystalEyes®, StereoGraphics Corporation, San Rafael, USA).

**References for supporting material**


Supporting figure 1

Fourier Shell Correlation (FSC) of the final 3D reconstruction, plotted as a function of the spatial frequency, together with the 3 threshold curve. Resolution values corresponding to the 0.5 and 3 -criterion are marked by arrows.

Supporting figure 2

A) Definition of Euler angles $\alpha$, $\beta$ and $\gamma$ as used for the 3D reconstruction.

B) Distribution of Euler angles beta and gamma (alpha = 0°) of class averages used for the final 3D reconstruction. Note, that one hemisphere of the angle distribution is redundant due to the imposed $C_2$ symmetry (grey points).

Supporting figure 3

Movie of the surface rendered three-dimensional density map of dendro-calixaren micelles as reconstructed from cryo-TEM data.

Supporting figure 4

Movie of the 3D arrangement of seven calixarene molecules (head groups in the low energy conformation; alkyl chains omitted) resulting from the best fit into the reconstructed density map. Structural information on the flexible alkyl chains is unavailable from the reconstruction (see manuscript) and thus only head groups were used for the fitting.

Supporting figure 5

Movie of seven calixarene molecules (head groups only) in an alternative $C_{2v}$ symmetry arrangement. The model shows the molecules in a chemically equivalent environment, but they appear to fit less accurately into the EM density map.
Supplement figure 6

Stereo view of the surface-rendered three-dimensional density map of the dendro-calixarene micelles as reconstructed from data of air-dried samples. Bar=25 Å
Supporting Figures

Supporting figure 1

Supporting figure 2
Supporting figure 3

Supporting figure 4

Supporting figure 5
Reconstruction from dried sample (stereo view)

Supporting figure 6