

# Self-Assembly and Stability of Double Rosette Nanostructures with Biological Functionalities

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## Experimental

### General Methods:

<sup>1</sup>H NMR spectra were recorded on a Varian Unity 300 (<sup>1</sup>H NMR 300MHz) spectrometer in chloroform-*d*<sub>1</sub>, methanol-*d*<sub>4</sub> or dimethylsulphoxide-*d*<sub>6</sub>. Residual solvent protons were used as internal reference, and chemical shifts are given relatively to trimethylsilane (TMS). FAB spectra were measured on a Finnigan MAT 90 spectrometer with *m*-nitrobenzyl alcohol (NBA) matrix. MALDI-TOF mass spectra were recorded on a PerSpective Biosystems Voyager-De-RP spectrometer. A 337 nm UV nitrogen laser producing 3 ns pulses was used in the linear and reflectron modes. Elemental analyses were performed using a Carlo Erba EA1106. CD spectra were measured on a JASCO J-715 spectropolarimeter using a cell width of 0.01 cm. THF was distilled from Na/benzophenone and CH<sub>2</sub>Cl<sub>2</sub> from K<sub>2</sub>CO<sub>3</sub>. All commercially available reagents were reagent grade and used without further purification. Flash chromatography was performed on silica gel (SiO<sub>2</sub>, E. Merck 0.040 – 0.063 mm, 230 – 240 mesh). Bis(chlorotriazine)calix[4]arene **9**,<sup>8</sup> alkyl derivatives **1**,<sup>8</sup> aminoalkyl derivative **2g**,<sup>9</sup> ureido derivative **3**,<sup>9</sup> pyridyl derivatives **4**,<sup>10</sup> and tetra-O-acetyl-β-D-glucopyranosylisothiocyanate and hepta-O-acetyl-β-D-cellobiosyl-isothiocyanate<sup>11</sup> were synthesized according to literature procedures.

**General procedure for the preparation of calix[4]arene dimelamines 2a-f:** A solution of the calix[4]arene bis(chlorotriazine) **9** and the appropriate diamino compound (excess) in THF was stirred at 90°C overnight. After cooling down the solution to room temperature the product was precipitated in ice water. The white precipitate was filtered off and washed with water and/or methanol. Resulting in calix[4]arene dimelamines **2a-f** as a white powder. (In some cases, THF was added prior to precipitation in water for better removal of excess diamino compound).

**5,17-*N,N*-Bis[4-amino-6-(2-aminoethylamino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetrapropoxycalix[4]arene (2a)** was prepared from bis(chlorotriazine) **9** (250 mg, 0.28 mmol) and 1,2-ethanediamine (8 ml). The product was recrystallized from methanol. Pure dimelamine **2a** was obtained as white solid in 63% yield (165 mg, 0.18 mmol). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ=7.2–6.8 (br m, 6H), 6.5–6.1 (br m, 4H), 5.3–5.0 (br m, 6H), 4.38 and 3.05 (ABq, <sup>2</sup>J<sub>HH</sub>=13.4 Hz, 8H), 4.0–3.9 (m, 4H), 3.6–3.5 (m, 4H), 3.4–3.2 (br m,

4H), 2.6 (br m, 4H), 2.0–1.8 (m, 8H), 1.1 and 0.95 (t,  $^3J_{\text{HH}}=7.4$  Hz, 12H). MS (FAB):  $m/z = 927.3$  ( $[\text{M}+\text{H}^+]$ , calcd 927.5). Anal. Calcd for  $\text{C}_{50}\text{H}_{66}\text{N}_{14}\text{O}_4 \cdot 4\text{MeOH}$ : C 61.46, H 7.83, N 18.58; found C 61.29, H 6.98, N 17.82.

**5,17-*N,N*-Bis[4-amino-6-(3-aminopropylamino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetrapropoxycalix[4]arene (2b)** was prepared from bis(chlorotriazine) **9** (2.00 g, 2.1 mmol) and 1,3-propanediamine (25 ml). Pure dimelamine **2b** was obtained as white solid in 58% yield (1.26 g, 1.32 mmol).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta=8.6\text{--}8.4$  (br m, 2H),  $\delta=7.55\text{--}7.35$  (br m, 4H), 7.5–7.3 (br m, 2H),  $\delta=6.4\text{--}6.0$  (br m, 4H), 4.32 and 3.05 (ABq,  $^2J_{\text{HH}}=13.4$  Hz, 8H), 3.9–3.8 (m, 4H), 3.6–3.5 (m, 4H), 2.6 (br m, 4H), 2.0–1.8 (m, 8H), 1.6 (m, 4H), 1.1 and 0.95 (t,  $^3J_{\text{HH}}=7.4$  Hz, 12H). MS (FAB):  $m/z = 955.3$  ( $[\text{M}+\text{H}^+]$ , calcd 955.6). Anal. Calcd for  $\text{C}_{52}\text{H}_{70}\text{N}_{14}\text{O}_4 \cdot \text{H}_2\text{O}$ : C 64.18, H 7.46, N 20.15; found C 64.29, H 7.35, N 19.87.

**5,17-*N,N*-Bis[4-amino-6-(4-aminobutylamino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetrapropoxycalix[4]arene (2c)** was prepared from bis(chlorotriazine) **9** (250 mg, 0.28 mmol) and 1,4-butyldiamine (5 g). THF (8 ml) was added prior to precipitation in water (50 ml). Pure dimelamine **2c** was obtained as white solid in 89% (248 mg, 0.25 mmol).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta=7.0\text{--}6.7$  (br m, 6H), 6.4–6.0 (br m, 4H), 5.0–4.7 (br m, 6H), 4.36 and 3.05 (ABq,  $^2J_{\text{HH}}=13.7$  Hz, 8H), 3.9 (t,  $^3J_{\text{HH}}=8.0$  Hz, 4H), 3.65–3.55 (m, 4H), 3.3–3.2 (m, 4H), 2.7–2.6 (m, 4H), 1.9–1.7 (br m, 8H), 1.6–1.4 (br m, 8H), 1.0 and 0.85 (t,  $^3J_{\text{HH}}=7.4$  Hz, 12H). MS (FAB):  $m/z = 983.6$  ( $[\text{M}+\text{H}^+]$ , calcd 983.6). Anal. Calcd for  $\text{C}_{54}\text{H}_{74}\text{N}_{14}\text{O}_4$ : C 65.96, H 7.59, N 19.94; found C 65.90, H 7.61, N 19.82.

**5,17-*N,N*-Bis[4-amino-6-(5-aminopentylamino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetrapropoxycalix[4]arene (2d)** was prepared from bis(chlorotriazine) **9** (250 mg, 0.28 mmol) and 1,5-pentyldiamine (5 g). Pure dimelamine **2d** was obtained as white solid in 91% (260 mg, 0.26 mmol).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta=7.0\text{--}6.7$  (br m, 6H), 6.4–6.0 (br m, 4H), 4.9–4.6 (br m, 6H), 4.36 and 3.05 (ABq, 8H,  $^2J_{\text{HH}}=13.4$  Hz), 3.9 (m, 4H), 3.65–3.55 (m, 4H), 3.3–3.2 (m, 4H), 2.7–2.6 (m, 4H), 1.9–1.7 (br m, 8H), 1.6–1.2 (br m, 12H), 1.0 and 0.85 (t,  $^3J_{\text{HH}}=7.5$  Hz, 12H). MS (FAB):  $m/z = 1011.7$  ( $[\text{M}+\text{H}^+]$ , calcd 1011.6). Anal. Calcd for  $\text{C}_{56}\text{H}_{78}\text{N}_{14}\text{O}_4 \cdot 1.5\text{H}_2\text{O}$ : C 64.78, H 7.86, N 18.88; found C 64.71, H 7.87, N 18.87.

**5,17-*N,N*-Bis[4-amino-6-(6-aminohexylamino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetrapropoxycalix[4]arene (2e)** was prepared from bis(chlorotriazine) **9** (250 mg, 0.28 mmol) and 1,6-hexyldiamine (2 g). THF (4 ml) was added prior to precipitation in water (25 ml). Pure dimelamine **2e** was obtained as white solid in 92% (271 mg, 0.26 mmol).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta=7.0\text{--}6.7$  (br m, 6H), 6.4–6.0 (br m, 4H), 4.9–4.6 (br m, 6H), 4.36 and 3.05 (ABq,  $^2J_{\text{HH}}=13.4$  Hz, 8H), 3.9 (m 4H), 3.68–3.58 (m, 4H), 3.24 (q,  $^3J_{\text{HH}}=6.6$  Hz, 4H), 2.7–2.55 (m, 4H), 1.9–1.7 (br m, 8H), 1.6–1.2 (br m, 16H), 1.0 and 0.85 (t,  $^3J_{\text{HH}}=7.5$  Hz, 12H). MS (FAB):  $m/z = 1039.7$  ( $[\text{M}+\text{H}^+]$ , calcd 1039.7). Anal. Calcd for  $\text{C}_{58}\text{H}_{82}\text{N}_{14}\text{O}_4$ : C 67.02, H 7.95, N 18.87; found C 66.74, H 7.97, N 18.27.

**5,17-*N,N*-Bis[4-amino-6-(7-aminoheptylamino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetrapropoxycalix[4]arene (2f)** was prepared from bis(chlorotriazine) **9**

(250 mg, 0.28 mmol) and 1,7-pentylidiamine (1 g). Pure dimelamine **2f** was obtained as white solid in 53% (271 mg, 0.26 mmol). <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ=7.0–6.7 (br m, 6H), 6.4–6.0 (br m, 4H), 4.9–4.6 (br m, 6H), 4.36 and 3.05 (ABq, <sup>2</sup>J<sub>HH</sub>=13.4 Hz, 8H), 3.95–3.85 (m, 4H), 3.68–3.58 (m, 4H), 3.23 (q, 4H, <sup>3</sup>J<sub>HH</sub>=6.3 Hz), 2.7–6 (t, <sup>3</sup>J<sub>HH</sub>=6.8 Hz, 4H), 1.9–1.7 (br m, 8H), 1.6–1.2 (br m, 20H), 1.0 and 0.85 (t, <sup>3</sup>J<sub>HH</sub>=7.4 Hz, 12H). MS (FAB): *m/z* = 1067.7 ([M+H<sup>+</sup>], calcd 1067.7). Anal. Calcd for C<sub>60</sub>H<sub>86</sub>N<sub>14</sub>O<sub>4</sub>: C 67.51, H 8.12, N 18.37; found C 66.97, H 8.05, N 18.18.

**Calix[4]arene bis(tetra-O-acetyl-β-D-glucopyranosyl)dimelamine derivative (5a).** Dimelamine **2g** (424 mg, 0.42 mmol) was dissolved in freshly distilled THF (20 ml). Tetra-O-acetyl-β-D-glucopyranosylisothiocyanate (408 mg, 1.05 mmol) was added and the solution was stirred at room temperature for 4 hours. The solution was concentrated *in vacuo* and the product was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 95:4.5:0.5) to give pure **5a** as a white solid in 36% yield. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ = 7.2–6.8 (br m, 10H), 6.34 (s, 2H), 5.40–5.22 (m, 2H), 5.1–4.9 (m, 4H), , 4.38 and 3.05 (ABq, <sup>2</sup>J<sub>HH</sub>=13.4 Hz, 8H), 4.25 (m, 2H), 4.06–3.90 (br m, 8H), 3.85–3.75 (m, 4H), 2.65–2.45 (m, 2H), 2.2–1.8 (br m, 32H), 1.0 and 0.85 (m, 12H). MALDI-TOF-MS: *m/z* = 1790.7 ([M+H<sup>+</sup>], calcd 1789.8).

**Calix[4]arene bis(β-D-glucopyranosyl)dimelamine derivative (5b).** 1 ml of a freshly prepared sodium methoxide solution (23 mM) was added to a solution of dimelamine **5a** (100 mg, 0.06 mmol) in MeOH (4 ml). The solution was stirred for 3 hours at room temperature. Amberlite IR 120 (H<sup>+</sup>) washed with MeOH was added to the solution for exchange of Na<sup>+</sup> for H<sup>+</sup>. The Amberlite resin was filtered off and the product concentrated *in vacuo* to give pure **5b** as a white solid in 85% yield. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ = 9.0–8.0 (m, 2H), 7.6–7.3 (m, 4H), 6.27 (m, 6H), 5.4–4.8 (m, 6H), 4.43 (m, 2H), 4.33 (d, <sup>2</sup>J<sub>HH</sub>=12.0 Hz, 4H), 3.89 (m, 4H), 3.7–2.9 (br m, 20H), 1.90–1.74 (m, 8H), 1.06 (t, <sup>3</sup>J<sub>HH</sub>=7.4 Hz, 6H), 0.94–0.76 (m, 18H). MALDI-TOF-MS: *m/z* = 1457.9 ([M+H<sup>+</sup>], calcd 1454.8).

**Calix[4]arene bis(hepta-O-acetyl-β-D-cellobiosyl)dimelamine derivative (5c).** Dimelamine **2g** (424 mg, 0.42 mmol) was dissolved in freshly distilled THF (20 ml). Hepta-O-acetyl-β-D-cellobiosyl-isothiocyanate (711 mg, 1.05 mmol) was added and the solution was stirred at room temperature for 12 hours. The solution was concentrated *in vacuo* and the product was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 95:4.5:0.5) to give **5c** as a white solid in 88% yield. <sup>1</sup>H-NMR (CD<sub>3</sub>OD): δ = 7.0–6.2 (m, 10H), 5.78 (d, 4H), 5.20 (t, <sup>3</sup>J<sub>HH</sub>=8.8 Hz, 4H), 5.09 (t, <sup>3</sup>J<sub>HH</sub>=9.4 Hz, 4H), 4.96 (t, <sup>3</sup>J<sub>HH</sub>=9.8 Hz, 4H), 4.81 (t, <sup>3</sup>J<sub>HH</sub>=8.6 Hz, 4H), 4.53 and 3.05 (ABq, <sup>2</sup>J<sub>HH</sub>=13.4 Hz, 8H), 4.11 (d, <sup>2</sup>J<sub>HH</sub>=7.6 Hz, 4H), 3.99 (d, <sup>2</sup>J<sub>HH</sub>=7.6 Hz, 4H), 3.93 (m, 4H), 3.74–3.68 (m, 8H), 2.1–1.8 (m, 50H), 1.0 and 0.85 (m, 12H). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): δ = 184.6, 170.7, 170.5, 170.0, 169.8, 169.6, 169.5, 167.2, 164.7, 155.4, 152.2, 135.8, 134.7, 133.5, 127.8, 122.1, 120.9, 120.799.8, 82.0, 77.0, 76.8, 76.5, 73.9, 73.3, 72.8, 71.6, 71.4, 70.9, 68.3, 62.8, 62.0, 47.2, 37.3, 31.8, 31.0, 29.5, 29.2, 23.7, 23.5, 23.0, 22.6, 21.1, 20.9, 20.8, 20.7, 14.4, 11.1, 10.4 ppm. MS (FAB): *m/z* = 2368.1 ([M+H<sup>+</sup>], calcd 2365.0).

**Calix[4]arene bis( $\beta$ -D-cellobiosyl)dimelamine derivative (5d).** 1 ml of a freshly prepared sodium methoxide solution (23 mM) was added to a solution of dimelamine **5c** (100 mg, 0.06 mmol) in MeOH (4 ml). The solution was stirred for 3 hours at room temperature. Amberlite IR 120 ( $H^+$ ) washed with MeOH was added to the solution for exchange of  $Na^+$  for  $H^+$ . The Amberlite resin was filtered off and the product concentrated *in vacuo* to give pure **5d** as white solid in 85% yield.  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  = 9.0-8.0 (m, 2H), 7.7-7.3 (m, 4H), 6.27 (m, 6H), 5.38-08 (brm, 8H), 5.00 (m, 4H), 4.75 (m, 2H), 4.59 (m, 6H), 4.4-4.2 (br m, 8H), 3.88 (m, 4H), 3.8-2.8 (br m, 22H), 1.92-1.76 (m, 8H), 1.06 (t,  $^3J_{HH}=7.2$  Hz, 6H), 0.94-0.78 (m, 18H).  $^{13}C$ -NMR (DMSO- $d_6$ ):  $\delta$  = 155.4, 152.6, 136.0, 134.0, 133.3, 129.4, 128.7, 127.7, 122.1, 121.2, 103.6, 84.1, 80.7, 77.8, 77.1, 76.9, 76.5, 73.8, 72.8, 70.5, 61.5, 60.6, 51.4, 47.3, 37.1, 31.0, 23.8, 23.5, 23.0, 11.1, 10.4 ppm. MALDI-TOF-MS:  $m/z$  = 1780.6 ( $[M+H^+]$ , calcd 1776.8).

**Calix[4]arene bis(L-gulosyl)dimelamine derivative (5e).** A mixture of **2g** (295 mg, 0.29 mmol), (-)-2,3,4,6-di-O-isopropylidene-2-keto-L-gulonic acid (340 mg, 1.1 mmol), HBTU (443 mg, 1.1 mmol), HOBT (157 mg, 1.1 mmol) and DIPEA (0.4 ml, 2.3 mmol) in  $CH_2Cl_2$  (15 ml) was stirred at room temperature for 3 days.  $CH_2Cl_2$  was added and the organic layer was washed with 1M HCl, 10%  $Na_2CO_3$  and brine, and dried over  $Na_2SO_4$ . The product was concentrated *in vacuo* to give dimelamine **5e** as a solid in 41% yield.  $^1H$ -NMR ( $CDCl_3$ ):  $\delta$  = 8.01 (d,  $^2J_{HH}=8.1$  Hz, 4H), 7.4-6.8 (m, 6H), 5.39 (s, 2H), 5.18 (s, 2H), 4.77 (s, 2H), , 4.37 and 3.12 (ABq,  $^2J_{HH}=9.0$  Hz, 8H), 3.78-3.69 (m, 4H), 3.66-3.59 (m, 4H), 1.84-1.59 (m, 8H), 1.47 (s, 12H), 1.19 (s, 12H), 1.06-1.00 (m, 6H), 0.82-0.72 (m, 18H). MALDI-TOF-MS:  $m/z$  = 1525 ( $[M+H^+]$ , 1523.8 ).

**5,17-N,N-Bis[4-amino-6-(2-(ethanoic acid ethyl ester)amino)-1,3,5-triazin-2-yl] diamino-25,26,27,28-tetra propoxycalix[4]arene (6a)** A solution of calix[4]arene bis(chlorotriazine) **9** (250 mg, 0.28 mmol), glycine ethyl ester hydrochloride (400 mg, 2.8 mmol) and DIPEA (0.4 ml, 2.3 mmol) in freshly distilled THF (20 ml) was stirred at 90°C for 1 week. The product was precipitated in water, and recrystallized from methanol. Pure dimelamine **6a** was obtained as a white solid in 74% yield (213 mg, 0.21 mmol).  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  = 7.5-7.2 (br m, 6H), 6.4-6.0 (br m, 4H), 4.32 and 3.11 (ABq,  $^2J_{HH}=12.9$  Hz, 8H), 4.1-4.0 (m, 8H), 3.95-3.85 (m, 4H), 3.65-3.55 (m, 4H), 1.95-1.75 (br m, 8H), 1.25-1.05 (br m, 6H), 1.05 and 0.89 (t,  $^3J_{HH}=7.3$  Hz, 6H). MS (FAB):  $m/z$  = 1013.3 ( $[M+H^+]$ , calcd 1013.5). Anal. Calcd for  $C_{54}H_{68}N_{12}O_8$ : C 64.01 , H 6.76, N 16.59; found C64.43, H 6.94, N 16.62.

**5,17-N,N-Bis[4-amino-6-(2-(ethanoic acid)amino)-1,3,5-triazin-2-yl] diamino-25,26,27,28-tetra propoxycalix[4]arene (6a')**. To a solution of dimelamine **6a** (800 mg, 0.79 mmol) in THF/MeOH (20 ml; 1:1 v/v) a solution of 1 N NaOH (5 ml, 5 mmol) in water was added. The mixture was stirred at room temperature for 4 hours (TLC  $CH_2Cl_2$ :MeOH:AcOH = 90:9.5:0.5). The solution was quenched with aqueous citric acid solution (0.5 M). Then, the compound was extracted with  $CH_2Cl_2$  and the organic layer was separated and washed with brine. The organic layer was dried over  $Na_2SO_4$  and the product was concentrated *in vacuo*. Compound **6a'** was obtained as a solid in 85% yield (642 mg, 0.67 mmol).  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  = 12.4 (br s, 2H), 7.43 (s, 4H), 6.24 (m,

6H), 4.30 and 3.08 (ABq,  $^2J_{\text{HH}}=12.6$  Hz, 8H), 3.91 (m, 8H), 3.61 (m, 4H), 2.0-1.8 (m, 8H), 1.06 and 0.89 (t, 12H). MS (FAB):  $m/z = 957.4$  ( $[\text{M}+\text{H}^+]$ , calcd 956.5).

**5,17-*N,N*-Bis[4-amino-6-(2-(propanoic acid methyl ester)amino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetra propoxycalix[4]arene (6b)** A solution of calix[4]arene bis(chlorotriazine) **9** (250 mg, 0.28 mmol), *L*-alanine methyl ester hydrochloride (198 mg, 1.4 mmol) and DIPEA (1.0 ml, 5.7 mmol) in freshly distilled THF (10 ml) was stirred at 90°C for 1 week. The solution was concentrated *in vacuo* and the crude was redissolved in  $\text{CH}_2\text{Cl}_2$  and the organic layer was washed successively with 0.5 M HCl, 0.1 M  $\text{NaHCO}_3$  and brine. In addition, the organic layer was dried over  $\text{MgSO}_4$  and concentrated *in vacuo*, resulting in a light brown powder. Dimelamine **6b** was obtained in 74% yield (213 mg, 0.21 mmol).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta = 6.35\text{-}6.05$  (br m, 10H), 4.66-4.45 (br m, 2H), 4.3 and 3.1 (ABq,  $^2J_{\text{HH}}=12.9$  Hz, 8H), 3.9 (t,  $^3J_{\text{HH}}=7.8$  Hz, 4H), 3.68-3.49 (br m, 10H), 2.0-1.7 (br m, 8H), 1.35 (d,  $^2J_{\text{HH}}=7.2$  Hz, 6H), 1.07 and 0.88 (t,  $^3J_{\text{HH}}=7.4$  Hz, 12H). MS (FAB):  $m/z = 1013.6$  ( $[\text{M}+\text{H}^+]$ , calcd 1013.5). Anal. Calcd for  $\text{C}_{54}\text{H}_{68}\text{N}_{12}\text{O}_8 \cdot 2\text{H}_2\text{O}$ : C 61.82, H 6.92, N 16.02; found C 62.02, H 6.86, N 16.10.

**5,17-*N,N*-Bis[4-amino-6-(2-(4-(methylthio)butanoic acid methyl ester)amino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetra propoxycalix[4]arene (6c)** A solution of calix[4]arene bis(chlorotriazine) **9** (250 mg, 0.28 mmol), *L*-methionine methyl ester hydrochloride (560 mg, 2.8 mmol) and DIPEA (0.4 ml, 2.3 mmol) in freshly distilled THF (10 ml) was stirred at 90°C for 1 week. After completion of the reaction the solution was concentrated *in vacuo*, the crude was redissolved in  $\text{CH}_2\text{Cl}_2$  and the organic layer was washed successively with 0.5 M HCl, 0.1 M  $\text{NaHCO}_3$  and brine. In addition, the organic layer was dried over  $\text{MgSO}_4$  and concentrated *in vacuo*, resulting in a white powder. Pure dimelamine **6c** was obtained in 69% yield (221 mg, 0.19 mmol).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta = 6.43\text{-}6.04$  (br m, 10H), 4.82-4.55 (br m, 2H), 4.3 and 3.1 (ABq,  $^2J_{\text{HH}}=12.8$  Hz, 8H), 3.9 (t,  $^3J_{\text{HH}}=8.1$  Hz, 4H), 3.7-3.5 (br m, 10H), 2.65-2.4 (br m, 4H), 2.16-1.65 (br m, 18H), 1.64 and 0.87 (t,  $^3J_{\text{HH}}=7.4$  Hz, 12H). MS (FAB):  $m/z = 1133.5$  ( $[\text{M}+\text{H}^+]$ , calcd 1133.5).

**5,17-*N,N*-Bis[4-amino-6-(2-(6-*N*-Boc-aminohexanoic acid methyl ester)amino)-1,3,5-triazin-2-yl]diamino-25,26,27,28-tetra propoxycalix[4]arene (6d)** A solution of calix[4]arene bis(chlorotriazine) **9** (1.0 g, 1.14 mmol),  $\text{N}_\epsilon$ -BOC-*L*-lysine methyl ester hydrochloride (3.0 g, 10.2 mmol) and DIPEA (2.0 ml, 11.4 mmol) in freshly distilled THF (20 ml) was stirred at 90°C for 1 week. The solution was cooled down to room temperature. Then the product was precipitated in water, and recrystallized from methanol. Pure dimelamine **6d** was obtained as white powder in 85% yield (1.28 g, 0.96 mmol).  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ ):  $\delta = 6.3\text{-}6.05$  (br m, 10H), 4.58-4.40 (br m, 2H), 4.3 and 3.1 (ABq,  $^2J_{\text{HH}}=12.6$  Hz, 8H), 3.9 (t,  $^3J_{\text{HH}}=8.0$  Hz, 4H), 3.7-3.5 (br m, 10H), 2.96-2.78 (m, 4H), 2.0-1.8 (br m, 8H), 1.75-1.65 (m, 4H), 1.45-1.2 (br m, 26H), 1.07 and 0.87 (t,  $^3J_{\text{HH}}=7.4$  Hz, 12H). MS (FAB):  $m/z = 1327.9$  ( $[\text{M}+\text{H}^+]$ , calcd 1327.7). Anal. Calcd for  $\text{C}_{70}\text{H}_{98}\text{N}_{14}\text{O}_{12}$ : C 61.66, H 7.54, N 14.38; found C 61.89, H 7.50, N 14.42.

**5,17-*N,N*-Bis[4-amino-6-(2-(6-aminohexanoic acid methyl ester)amino)-1,3,5-triazin-2-yl]diamino-25, 26,27,28-tetra propoxycalix[4]arene (6e).** Dimelamine **6d**

(500 mg, 0.38 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (8 ml) and trifluoroacetic acid (8 ml). The solution was stirred for 2 hours at room temperature. The product was concentrated *in vacuo*. Toluene was used for azeotropic removal of residual trifluoroacetic acid. The product was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and washed twice with 1 N NaOH. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and the product was concentrated *in vacuo*. Pure dimelamine **6e** was obtained as a white solid in 24% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ = 7.1-6.1 (br m, 10H), 4.64-4.58 (br m, 2H), 4.3 and 3.1 (ABq, <sup>2</sup>J<sub>HH</sub>=12.6 Hz, 8H), 3.9 (br m, 4H), 3.7-3.5 (br m, 10H), 2.64-2.49 (m, 4H), 2.0-1.6 (br m, 12H), 1.45-1.2 (br m, 8H), 1.07 and 0.87 (t, <sup>3</sup>J<sub>HH</sub>=7.4 Hz, 12H). MALDI-TOF-MS: *m/z* = 1149.8 ([M+Na<sup>+</sup>], calcd 1149.6). Anal. Calcd for C<sub>60</sub>H<sub>82</sub>N<sub>14</sub>O<sub>8</sub>: C 63.92, H 7.33, N 17.39; found C 60.18, H 7.23, N 15.61.

**5,17-*N,N*-Bis[4-amino-6-(2-(6-*N*-Boc-aminohexanoic acid)amino)-1,3,5-triazin-2-yl] diamino-25, 26,27,28-tetra propoxycalix[4]arene (6f).** To a solution of dimelamine **6d** (500 mg, 0.38 mmol) in THF/MeOH (10 ml; 1/1 v/v) was added 1 N NaOH (1.5 ml, 1.5 mmol) in water. The mixture was stirred at room temperature for 14 hours (TLC CH<sub>2</sub>Cl<sub>2</sub>/MeOH/AcOH = 90:9.5:0.5). The solution was quenched with aqueous citric acid solution (0.5 M). Then CH<sub>2</sub>Cl<sub>2</sub> was added and the organic layer was separated. The organic layer was washed once more with aqueous citric acid solution (0.5 M), followed by 5% aqueous NaHCO<sub>3</sub> solution, and brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and the product was concentrated *in vacuo*. Dimelamine **6f** was obtained in 83% yield (0.41 g, 0.32 mmol). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ = 8.07 (s, 2H), 6.8-6.0 (br m, 10H), 4.31 and 3.08 (ABq, <sup>2</sup>J<sub>HH</sub>=12.6 Hz, 8H), 4.15 (m, 2H), 3.86 (m, 4H), 3.78 (m, 4H), 3.63 (m, 4H), 2.86 (m, 10H), 2.0-1.6 (br m, 16H), 1.4 (br s, 18H), 1.05 and 0.90 (m, 12H). MS (FAB): *m/z* = 1321.7 ([M+Na<sup>+</sup>], calcd 1321.7).

**Calix[4]arene bis(gly-gly-OEt)dimelamine (7a).** A solution of calix[4]arene bis(chlorotriazine) **9** (100 mg, 0.11 mmol), gly-gly-OEt-HCL (201 mg, 1.02 mmol) and DIPEA (0.4 ml, 2.3 mmol) in freshly distilled THF (10 ml) was stirred at 90°C for 1 week. The solution was cooled down and the product was precipitated in water, filtered off and washed with water. Pure **7a** was obtained as a white solid in 85% yield. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ = 7.45 (s, 4H), 6.25 (m, 6H), 4.29 and 3.07 (ABq, <sup>2</sup>J<sub>HH</sub>=12.6 Hz, 8H), 4.03 (m, 4H), 3.90-3.75 (m, 16H), 3.60 (m, 4H), 2.0-1.75 (m, 8H), 1.2-1.0 (m, 12H), 0.87 (t, 6H). MS (FAB): *m/z* = 1127.5 ([M+H<sup>+</sup>], calcd 1127.6).

**Calix[4]arene bis(gly-*L*-ala-OMe)dimelamine (7b).** A mixture of **6a'** (120 mg, 0.13 mmol), *L*-alanine methyl ester hydrochloride (35 mg, 0.25 mmol), HATU (106 mg, 0.28 mmol) and DIPEA (0.11ml) in CHCl<sub>3</sub> (10 ml) was stirred for 14 hours at room temperature. Then CHCl<sub>3</sub> was added and the organic layer was washed successively with 5% citric acid, 5% NaHCO<sub>3</sub>, brine and dried over MgSO<sub>4</sub>. The product was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 90/9.5/0.5) to give pure **7b** as a white solid in 50% yield. <sup>1</sup>H-NMR (CD<sub>3</sub>OD): δ = 6.9-6.4 (br m, 10H), 4.48 (s, 2H), 4.36 and 3.05 (ABq, <sup>2</sup>J<sub>HH</sub>=12.6 Hz, 8H), 3.91 (s, 4H), 3.85 (t, 4H), 3.67 (t, 4H), 3.57 (s, 6H), 1.9-1.8 (m, 8H), 1.19 (s, 6H), 0.98 and 0.89 (t, 12H). MS (FAB): *m/z* = 1127.5 ([M+H<sup>+</sup>], calcd. 1127.6).

**Calix[4]arene bis(gly-*L*-ser-OMe)dimelamine (7c).** A mixture of **6a'** (150 mg, 0.16 mmol), *L*-serine methyl ester hydrochloride (50 mg, 0.32 mmol), HATU (135 mg, 0.35 mmol) and DIPEA (0.14 ml) in CHCl<sub>3</sub> (10 ml) was stirred for 14 hours at room temperature. Then, CHCl<sub>3</sub> was added and the solution was washed successively with 5% citric acid, 5% NaHCO<sub>3</sub>, brine and dried over MgSO<sub>4</sub>. The product was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 90/9.5/0.5) to give **7c** as a white solid in 23% yield. <sup>1</sup>H-NMR (CD<sub>3</sub>OD): δ = 6.8-6.4 (br m, 10H), 4.38 and 3.05 (ABq, <sup>2</sup>J<sub>HH</sub>=12.6 Hz, 8H), 4.49 (m, 4H), 3.87 (t, 4H), 3.67 (m, 10H), 1.9-1.8 (m, 8H), 0.98 and 0.89 (t, 12H). MS (FAB): *m/z* = 1159.6 ([M+H<sup>+</sup>], calcd. 1159).

**Calix[4]arene bis(*L*-lys(Boc)-glu(OcHx)-NH<sub>i</sub>Pr)dimelamine (7d).** Dimelamine **9** (225 mg, 0.17 mmol), HOBT (70 mg, 0.52 mmol) and DIPEA (0.15 ml, 0.87 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) and the solution was cooled in an ice bath. EDC·HCl (100 mg, 0.52 mmol) was added to this solution and after stirring the solution for 10 minutes, *L*-lys(Boc)-glu(OcHx)-NH<sub>i</sub>Pr (142 mg, 0.52 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) was added. The mixture was stirred for 4 hours at room temperature, then concentrated *in vacuo* and the residue redissolved in ethylacetate (150 ml). The organic layer was washed successively with 0.5 M citric acid, 5% NaHCO<sub>3</sub>, brine and dried over Na<sub>2</sub>SO<sub>4</sub>. The product was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 90/9.5/0.5). Pure **7d** was obtained as a white solid in 48% yield. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ = 7.2-6.7 (br m, 10H), 4.8-4.6 (br m, 2H), 4.38 (m, 4H), 4.0-3.8 (br m, 8H), 3.7-3.5 (br m, 4H), 3.11 (m, 8H), 2.35 (m, 2H), 2.1-1.0 (br m, 64H), 0.85 (t, 6H). MALDI-TOF-MS: *m/z* = 1802.9 ([M+H<sup>+</sup>], calcd 1803.1).

**Calix[4]arene bis(*L*-lys(Boc)-tyr-OMe)dimelamine (7e).** A solution of **6f** (130 mg, 0.10 mmol), HOBT (54 mg, 0.40 mmol) and DIPEA (0.17 ml, 1.0 mmol) in freshly distilled THF (10 ml) was cooled in an ice bath. EDC·HCl (77 mg, 0.4 mmol) was added. After stirring for 10 minutes, *L*-tyrosine methyl ester (78 mg, 0.4 mmol) was added. The mixture was stirred for 8 hours at room temperature, then the solution was concentrated *in vacuo* and the residue redissolved in CH<sub>2</sub>Cl<sub>2</sub> (75 ml). The organic layer was washed successively with 0.5 M citric acid, 5% NaHCO<sub>3</sub> and brine, and dried over Na<sub>2</sub>SO<sub>4</sub>. The product was purified using column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 95/4.5/0.5) to give pure **7e** as a solid in 66% yield. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ = 7.2-6.4 (br m, 18H), 4.85-4.7 (m, 6H), 4.37 (d, 4H), 4.20 (m, 2H), 3.96 (m, 4H), 3.76-3.52 (m, 10H), 3.2-2.7 (m, 12H), 2.0-1.2 (m, 34H), 1.03 and 0.81 (t, 12H). MALDI-MS: *m/z* = 1654.0 ([M+H<sup>+</sup>], calcd 1653.9).

**Calix[4]arene bis(gly-gly-gly-OEt)dimelamine (8a).** A mixture of **6a'** (290 mg, 0.30 mmol), gly-gly-OEt·HCl (150 mg, 0.75 mmol), HATU (342 mg, 0.90 mmol) and DIPEA (0.26 ml, 1.5 mmol) in CHCl<sub>3</sub> (20 ml) was stirred at room temperature for 14 hours. The mixture was washed successively with 0.5 M HCl, NaHCO<sub>3</sub> (5%), brine and dried over Na<sub>2</sub>SO<sub>4</sub>. The crude product was purified by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH:NH<sub>4</sub>OH = 90/9.5/0.5) to give pure **8a** as a white solid in 68% yield. <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ = 6.9-6.3 (br m, 10H), 4.36 and 3.04 (ABq, <sup>2</sup>J<sub>HH</sub>=12.5 Hz, 8H), 3.90 (t, <sup>3</sup>J<sub>HH</sub>=8.0, 4H), 3.62 (t, <sup>3</sup>J<sub>HH</sub>=6.6, 4H), 3.90 (q, <sup>3</sup>J<sub>HH</sub>=6.6, 4H), 2.73 (s, 18H), 1.94-1.72 (m,

8H), 0.99 and 0.85 (t, 12H). MS (FAB):  $m/z = 957.4$  ( $[M+H]^+$ , calcd 1240.6). Anal. Calcd for  $C_{62}H_{80}N_{16}O_{12} \cdot 2H_2O$ : C 58.29, H 6.63, N 17.54; found C 58.36, H 6.50, N 17.25.

***L*-gln(Trt)-*L*-ser(O*t*Bu)-OMe.** *L*-Fmoc-gln(Trt)-OH (750 mg, 1.23 mmol), *L*-ser(O*t*Bu)-OMe (275 mg, 10.30 mmol) and DIPEA (0.4 ml, 2.46 mmol) were dissolved in acetonitrile (10 ml). HBTU (493 mg, 1.30 mmol) was added and the reaction mixture was stirred at room temperature for 1 hour. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography ( $CH_2Cl_2$ :MeOH: $NH_4OH = 95/4.5/0.5$ ) to give pure Fmoc-*L*-gln(Trt)-*L*-ser(O*t*Bu)-OMe as a white solid in 90% yield.  $^1H$ -NMR ( $CDCl_3$ ):  $\delta = 7.82$  (d, 2H), 7.64 (d, 2H), 7.52 (t, 2H), 7.29 (m, 17H), 7.10 (d, 1H), 5.83 (d, 1H), 4.65 (m, 1H), 4.44 (d, 2H), 4.18 (q, 2H), 3.82-3.78 (dd, 1H), 3.69 (s, 3H), 3.48-3.44 (dd, 1H), 2.42 (t, 2H), 2.18-2.12 (br m, 2H), 1.08 (s, 9H). MS (FAB):  $m/z = 768.3$  ( $[M+H]^+$ , calcd 768.4).

Subsequently, Fmoc-*L*-gln(Trt)-*L*-ser(O*t*Bu)-OMe (850 mg, 1.12 mmol) was dissolved in acetonitrile (10 ml) and diethylamine (5 ml) was added and the solution mixture was stirred for 1.5 hours at room temperature. The solvents were evaporated and the residue was triturated with diethylether. The obtained white solid was filtered off and washed twice with diethylether. The obtained product was used without further purification.

**Calix[4]arene bis(lys(Boc)-gln(Trt)-ser(O*t*Bu)-OMe)dimelamine (8b).** A solution of **6f** (182 mg, 0.14 mmol), HOBT (39 mg, 0.29 mmol) and DIPEA (0.1 ml, 0.5 mmol) in  $CH_2Cl_2$  (10 ml) was cooled to 0°C. EDC·HCl (59 mg, 0.31 mmol) in  $CH_2Cl_2$  (4 ml) was added. After 10 minutes of stirring *L*-gln(Trt)-*L*-ser(O*t*Bu)-OMe (158 mg, 0.29 mmol) and DIPEA (0.1 ml, 0.05 mmol) in  $CH_2Cl_2$  (8 ml) was added. The solution was stirred for 24 hours at room temperature after which the solvents were removed under reduced pressure. The residue was taken up in  $CH_2Cl_2$  and washed successively with 0.5 M citric acid, 1 N  $Na_2CO_3$ , 5%  $NaHCO_3$ , and brine. The organic layer was dried over  $Na_2SO_4$ , concentrated and the product was purified by column chromatography ( $CH_2Cl_2$ :MeOH: $NH_4OH = 95/4.5/0.5$ ) to give pure **8b** as a white solid in 45% yield.  $^1H$ -NMR ( $DMSO-d_6$ ):  $\delta = 7.4$ -6.8 (br m, 25H), 4.5 (m, 2H), 4.32 (d, 4H), 4.2 (m, 4H), 3.88 (t, 4H), 3.7-3.5 (m, 10H), 3.2-2.7 (m, 20H), 2.4-1.8 (m, 8H), 1.4-1.0 (m, 48H), 0.95-0.75 (m, 12H). MALDI-MS:  $m/z = 2356.4$  ( $[M+Ag]^+$ , calcd 2354.3).