Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Antioxidant phospholipid calix[4]arene mimics as micellular delivery systems

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SUPPLEMENTARY INFORMATION

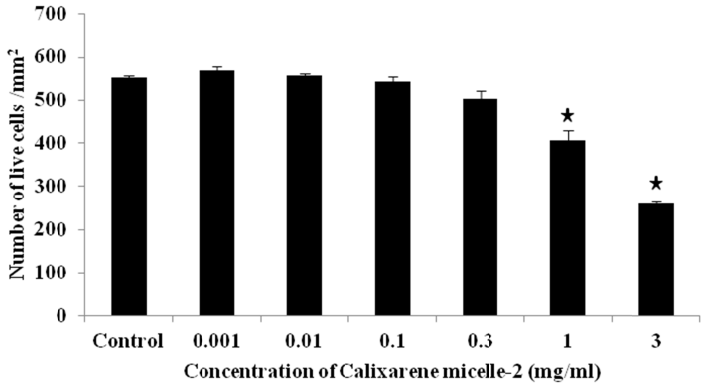


Figure S1: Cytotoxic effect of calixarene micelle-2 on PC12 cells viabilities (mean_SEM) after 24 h in culture (Live/Dead Assay). Statistically significant reductions in viability of cultures treated with calixarene preparations compared to untreated cells are indicated by * ($p<0.05$).

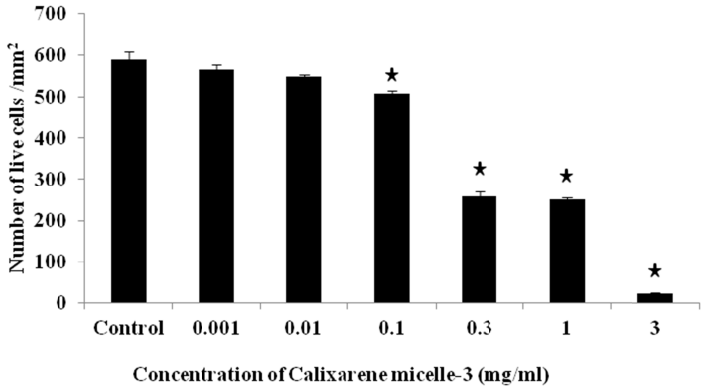


Figure S2: Cytotoxic effect of calixarene micelle-3 on PC12 cells viabilities (mean_SEM) after 24 h in culture (Live/Dead Assay). Statistically significant reductions in viability of cultures treated with calixarene preparations compared to untreated cells are indicated by * ($p<0.05$).

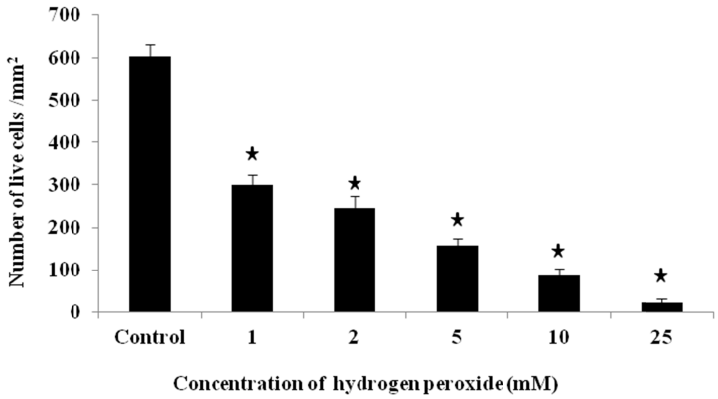


Figure S3: Dose dependent cytotoxic effect of H_2O_2 on PC12 cell viabilities (mean_SEM) after 60 min in culture (Live/Dead Assay). Statistically significant reductions in viability of cultures treated with calixarene preparations compared to untreated cells are indicated by * ($p<0.05$).

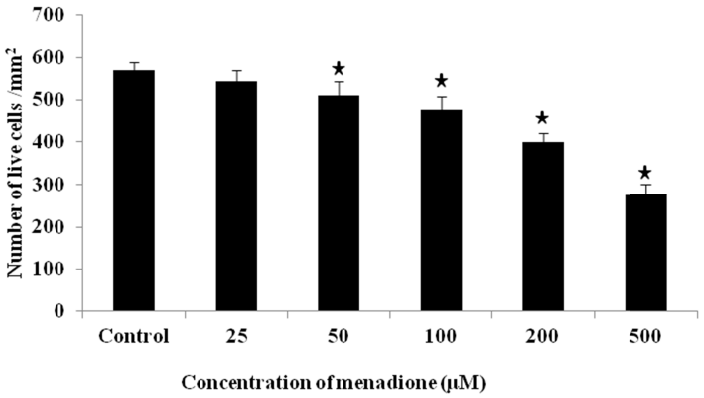


Figure S4: Dose dependent cytotoxic effect of menadione on PC12 cell viabilities (mean_SEM) after 60 min in culture (Live/Dead Assay). Statistically significant reductions in viability of cultures treated with calixarene preparations compared to untreated cells are indicated by * ($p<0.05$).

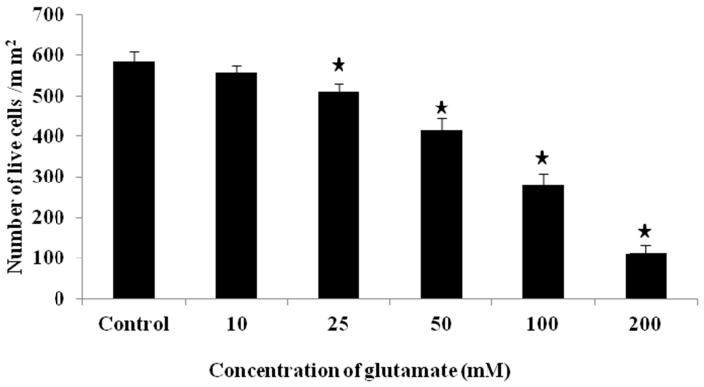


Figure S5: Dose dependent cytotoxic effect of glutamate on PC12 cell viabilities (mean_SEM) after 60 min in culture (Live/Dead Assay)v Statistically significant reductions in viability of cultures treated with calixarene preparations compared to untreated cells are indicated by * ($p<0.05$).

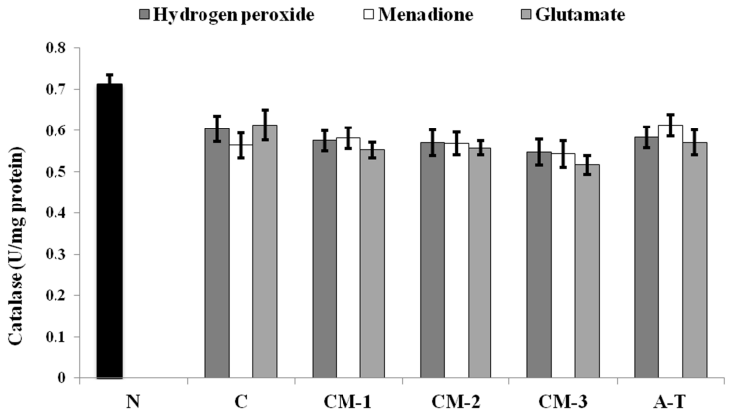


Figure S6. Effect of Calixarene micelles at 0.01 mg on Catalase activity in PC12 cells with different stressors like H_2O_2 at 1 mM, Menadione at 500 µM and Glutamate at 100 mM concentration for 60 min. C- Control group treated with 3 different stressors without calixarene micelles, N- Normal cells without oxidants and antioxidants treatment, CM1- Calixarene micelles with Phosphate group at 0.01 mg/mL concentration, CM2- Calixarene micelles with phosphate and methylene group at 0.01 mg/mL concentration, CM3 - Calixarene micelles with trimethyl amine group at 0.01 mg/mL concentration, A-T – α-Tocopherol at 0.01 mg/mL concentration. Values are expressed as mean±SEM of three determinations.

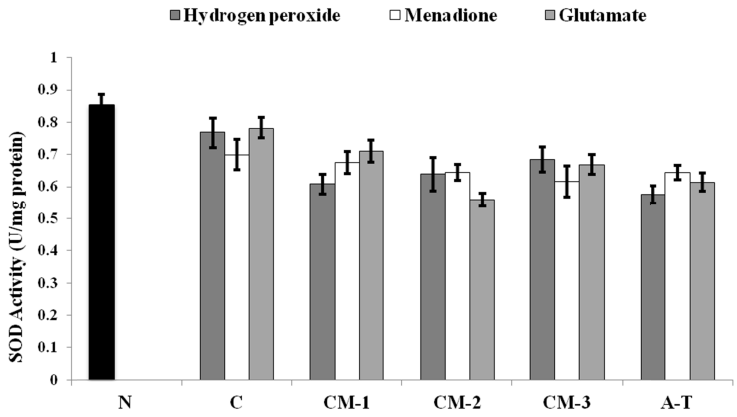


Figure S7. Effect of calixarene micelles at 0.01mg on superoxide dismutase activity in PC12 cells with different stressors like H_2O_2 at 1 mM, Menadione at 500 µM and Glutamate at 100 mM concentration for 60 min. C- Control group treated with 3 different stressors without calixarene micelles, N- Normal cells without oxidants and antioxidants treatment, CM1- Calixarene micelles with Phosphate group at 0.01mg/mL concentration, CM2- Calixarene micelles with phosphate and methylene group at 0.01mg/mL concentration, CM3 - Calixarene micelles with trimethyl amine group at 0.01 mg/mL concentration, A-T – α-Tocopherol at 0.01 mg/mL concentration. Values are expressed as mean±SEM of three determinations * indicate significant difference ($p<0.05$) from control group.

Preparation of Calixarenes

Preparation of 5,11,17,23-Tetra-formyl-25,26,27,28-tetra-octyloxy-calix[4]arene

Under inert gas atmosphere a mixture of dry 25,26,27,28-tetra-octyloxy-calix[4]arene (8..0 g 9.2 mmol) and hexamethylenetetramine (17 g, 120 mmol) in CF_3COOH (300 mL) was stirred for 96 hours under reflux. The mixture was cooled to room temperature and then poured into a stirring solution of 2 M HCl (400 mL) and CH_2Cl_2 (400 mL), and vigorously stirred for 1 hour. The mixture was extracted with CH_2Cl_2 (2 x 200 mL) and the combined organic layers were washed with saturated aqueous Na_2CO_3 (2 x 200 mL) and brine (2 x 200 mL), dried over $NaSO_4$ and the solvent was then removed under reduced pressure. The raw product was further purified by column chromatography (silica gel 60; hexane:ethyl acetate, 7:3) to give a white solid (5.26 g 58 %).

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.87 (t, ^3J=7.05 Hz, 12H), 1.27 (m, 20H), 1.87 (m, 8H), 3.33, 4.47 (2d, ^2J=13.8 Hz, 2 x 4H) 3.95 (t, ^3J=7.40 Hz, 8H), 7.13 (s, 8H), 9.55 (s, 4H).
^{13}C NMR ($CDCl_3$, 125.8 MHz) δ: 14.0 (CH_3), 22.6 (CH_2), 26.2 (CH_2), 29.5 (CH_2), 29.7 (CH_2), 30.3 (CH_2), 30.9 (CH_2), 31.9 (CH_2), 75.7 (CH_2), 130.2 (CH), 131.3 (C), 135.6 (C), 161.9 (C), 191.2 (CH).

Preparation of 5,11,17,23-Tetra-methylhydroxy-25,26,27,28-tetra-octyloxy-calix[4]arene

Under an inert gas atmosphere ethanol (100 mL) was added to a stirring solution of 5,11,17,23-tetra-formyl-25,26,27,28-tetra-octyloxy-calix[4]arene (2.0 g 2.0 mmol) in THF (20 mL). $NaBH_4$ (2.27 g 60 mmol) was then added and the mixture stirred for 18 hrs at room temperature. The mixture was then concentrated under vacuum and the resulting solid dissolved in CH_2Cl_2 (100 mL). 2 M HCl (100 mL) was added slowly and the solution was stirred for 1 hr. The reaction mixture was then extracted with CH_2Cl_2. The combined organic fractions were washed with 2M HCl (3 x 100 mL) and saturated aqueous Na_2CO_3(3 x 100 mL) and dried over Na_2SO_4 .The solvent was removed *in vacuo* to obtain a white solid (1.8 g , 89 %).

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.92 (t, ^3J=6.90 Hz, 12H), 1.35 (m, 20H), 1.95 (m, 8H), 3.17, 4.45 (2d, ^2J=13.1 Hz, 2 x 4H), 3.90 (t, ^3J=7.48 Hz, 8H), 4.36 (s, 8H), 6.70 (s, 8H).
^{13}C NMR ($CDCl_3$, 125.8 MHz) δ: 14.1 (CH_3), 22.7 (CH_2), 26.4 (CH_2), 29.6 (CH_2), 29.9 (CH_2), 30.3 (CH_2), 30.3 (CH_2), 31.0 (CH_2), 32.0 (CH_2), 64.7 (CH_2), 75.4 (CH_2), 127.1 (CH), 134.6 (C), 134.9 (C), 160.0 (C).

Preparation of 5,11,17,23-Tetra-methylchloride-25,26,27,28-tetra-octyloxy-calix[4]arene

Under an inert gas atmosphere thionylchloride (10 mL) was added to 5,11,17,23-tetra-methylhydroxy-25,26,27,28-tetra-octyloxy-calix[4]arene (2.0 g 2.0 mmol) and then stirred at room temperature for 18 hrs. The mixture was concentrated under vacuum and the resulting solid was dissolved in CH_2Cl_2 (100 mL), washed with saturated aqueous Na_2CO_3 (3 x 100 mL) and dried over Na_2SO_4 .The solvent was removed under reduced pressure to obtain an off-white solid (2.0 g, 93 %).

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.93 (t, ^3J=7.14 Hz, 12H), 1.34 (m, 20H), 1.92 (m, 8H), 3.16, 4.43 (2d, ^2J=13.3 Hz, 2 x 4H) 3.90 (t, ^3J=7.44 Hz, 8H), 4.33 (s, 8H), 6.68 (s, 8H).
^{13}C NMR ($CDCl_3$, 125.8 MHz) δ: 14.1 (CH_3), 22.7 (CH_2), 26.3 (CH_2), 29.6 (CH_2), 29.9 (CH_2), 30.3 (CH_2), 30.9 (CH_2), 32.0 (CH_2), 46.6 (CH_2), 75.4 (CH_2), 128.6 (CH), 130.9 (C), 135.2 (C), 156.8 (C).

Preparation of 5,11,17,23-Tetra-trimethyamoniumchloridemethyl-25,26,27,28-tetra-octyloxy-calix[4]arene

Under an inert gas atmosphere trimethylamine (5 mL) was transferred *via* cannula to a cooled pressure tube (-15 ºC) containing 5,11,17,23-tetra-methylchloride-25,26,27,28-tetra-octyloxy-calix[4]arene (0.50 g, 0.34 mmol) in dry DMF (30 mL). The pressure tube was sealed, the solution allowed to warm to room temperature and was then stirred at 100 ºC for 16 hrs. An off-white solid was collected by filtration, washed with water (10 mL) and dried *in vacuo*. To produce a white solid in quantitative yield.

^{1}H NMR (CD_3OD, 500 MHz) δ: 0.92 (t, ^3J=7.00 Hz, 12H), 1.34 (m, 20H), 2.00 (m, 8H), 2.99 (s, 36H), 3.39, 4.53 (2d, ^2J=13.2 Hz, 2 x 4H), 3.99 (t, ^3J=7.38 Hz, 8H),, 4.44 (s, 8H), 6.99 (s, 8H).
^{13}C NMR (CD_3OD, 125.8 MHz) δ: 13.1 (CH_3), 22.4 (CH_2), 26.2 (CH_2), 29.4 (CH_2), 29.7 (CH_2), 29.9 (CH_2), 30.2 (CH_2), 31.8 (CH_2), 51.5 (CH_3), 68.8 (CH_2), 75.5 (CH_2), 121.8 (C), 133.2 (CH), 135.5 (C), 158.3 (C).

Preparation of 5,11,17,23-Tetra-diethylphosphonomethyl-25,26,27,28-tetra-octyloxy-calix[4]arene

Dry 5,11,17,23-Tetra-methylchloride-25,26,27,28-tetra-octyloxy-calix[4]arene (2.0 g, 1.9 mmol) was added with stirring triethylphosphite (100 mL) and the mixture was then refluxed for 16 hours. The solution was cooled to room temperature

and the triethylphosphite removed under reduced pressure. The raw product was purified by column chromatography (silica gel 60; chloroform:methanol, 10:1) to give a white solid (3.71 g, 95 %).

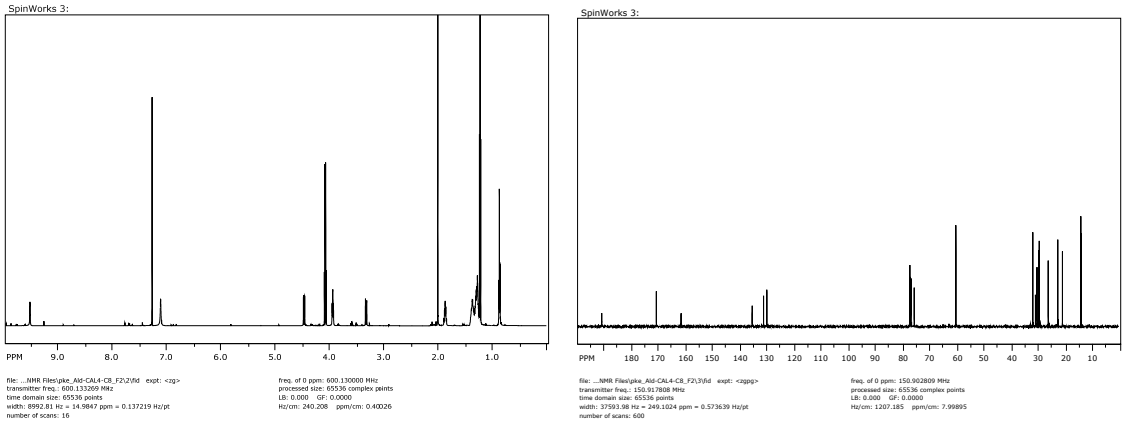
^{1}H NMR ($CDCl_3$, 500 MHz) δ: 0.89 (t, ^{3}J=6.95 Hz, 12H), 1.22 (t, ^{3}J=7.10 Hz, 24H), 1.31 (m, 20H), 1.85 (m, 8H), 2.78 (d, ^{2}J=21.2 Hz, 8H), 3.07, 4.35 (2d, ^{2}J=13.2 Hz, 2 x 4H), 3.81 (t, ^{3}J=7.5 Hz, 8H), 3.96 (m, 16H), 6.50 (s, 8H).
^{13}C NMR ($CDCl_3$, 125.8 MHz) δ: 14.1 (CH_3), 16.0 (d, CH_3, 5.8 Hz), 22.4 (CH_2), 26.1 (CH_2), 29.6 (CH_2), 29.9 (CH_2), 30.2 (CH_2), 31.0 (CH_2), 31.9 (CH_2), 32.5 (CH_2), 33.0 (d, CH_2, 138 Hz) 61.9 (d, CH_2, 6.9 Hz), 75.1 (CH_2), 124.0 (d, C, 9.2 Hz), 129.4 (d, CH, 6.4 Hz), 135.0 (d, C, 3.2 Hz), 155.8 (d, C, 4.1 Hz).

Preparation of 5,11,17,23-Tetra-phosphonomethyl-25,26,27,28-tetra-octyloxy-calix[4]arene

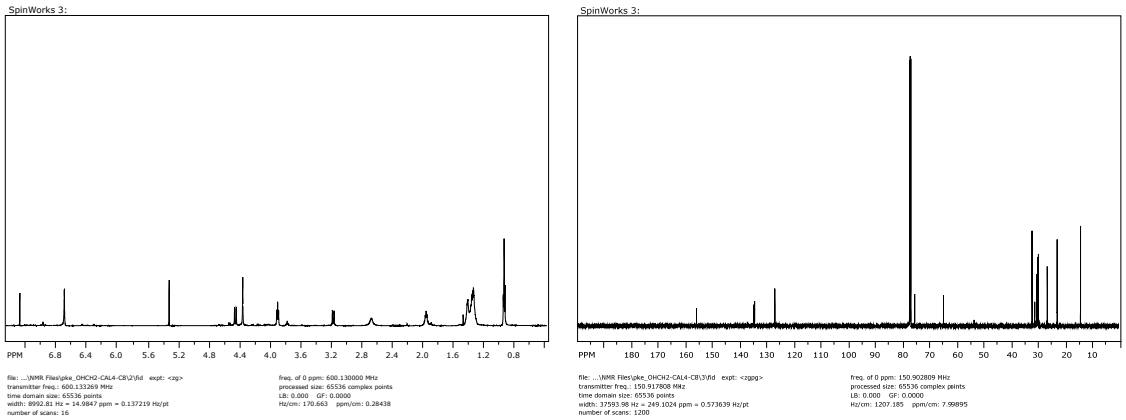
Under an inert gas atmosphere dry 5,11,17,23-tetra- methylchloride-25,26,27,28-tetra-octyloxy-calix[4]arene (264 mg, 0.179 mmol) was added to bromotrimethylsilane (3 mL) and stirred at room temperature for 5 hours. The bromotrimethylsilane was removed under reduced pressure. The resulting solid was dissolved in acetonitrile (10 mL), water was added (1mL) and stirred for 2 hours. The resultant white solid that formed was collected *via* vacuum filtration (210 mg, 94 %).

^{1}H NMR (MeOD/$CDCl_3$, 600.1 MHz) δ: 0.95 (t, ^{3}J=7.02 Hz, 12H), 1.39 (m, 20H), 1.96 (m, 8H), 2.83 (d, ^{2}J=20.9 Hz, 8H, 3.10, 4.42 (2d, ^{2}J=13.0 Hz, 2 x 4H), 3.85 (t, ^{3}J=7.03 Hz, 8H), 6.67 (s, 8H).
^{13}C NMR (MeOD/$CDCl_3$, 150.9 MHz) δ: 13.4 (CH_3), 22.6 (CH_2), 26.5 (CH_2), 29.5 (CH_2), 29.6 (CH_2), (CH_2), 30.0 (CH_2), 30.3 (CH_2), 32.0 (CH_2), 33.5 (d, CH_2, 135 Hz), 75.1 (CH_2), 125.5 (d, C, 9.4 Hz), 129.6 (d, CH, 5.7 Hz), 134.7 (C), 155.2 (C).

5,11,17,23-Tetra-formyl-25,26,27,28-tetra-octyloxy-calix[4]arene



5,11,17,23-Tetra-methylhydroxy-25,26,27,28-tetra-octyloxy-calix[4]arene



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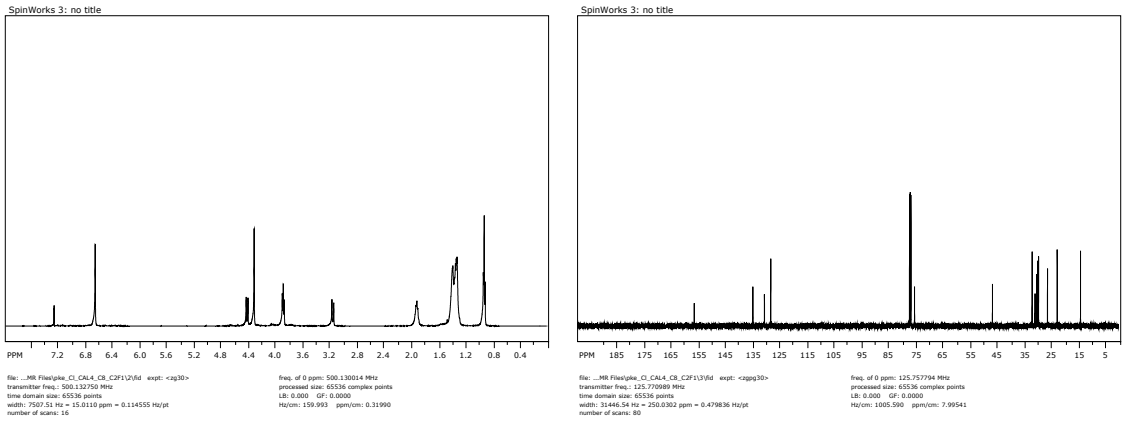

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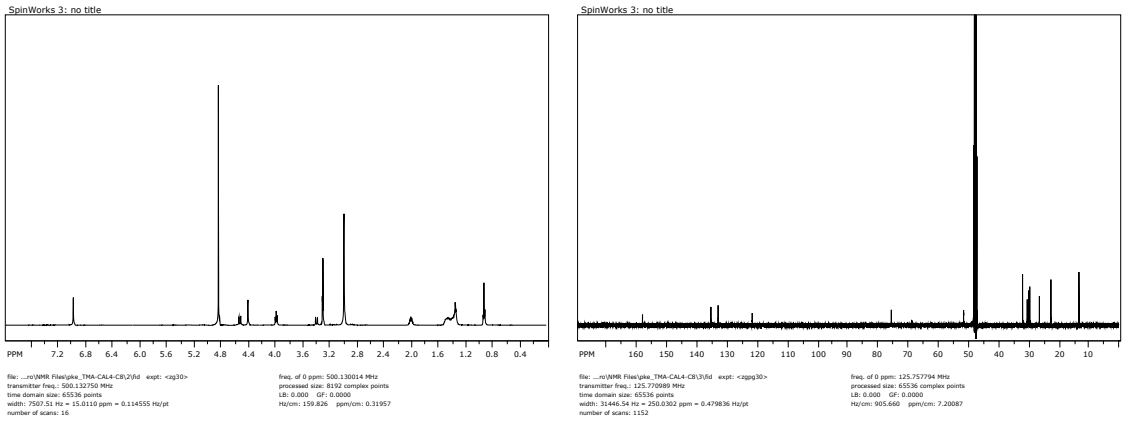
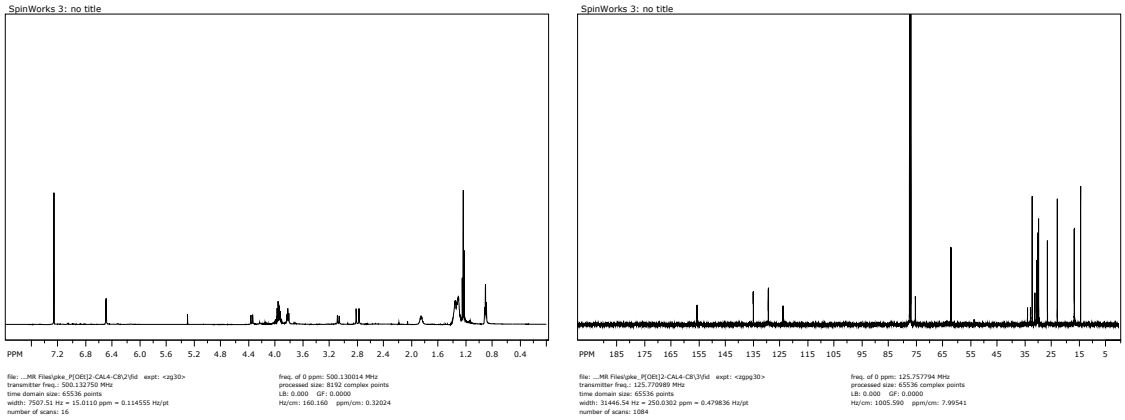


5,11,17,23-Tetra-diethylphosphonomethyl-25,26,27,28-tetra-octyloxy-calix[4]arene



5,11,17,23-Tetra-phosphonomethyl-25,26,27,28-tetra-octyloxy-calix[4]arene