CHAPTER II

EXPERIMENTAL SECTION

2.1 Synthesis of Calix[4]arene Derivatives

2.1.1 General Procedure

2.1.1.1 Analytical Measurement

Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker ACF 200 MHz and JMN (Jeol) 500 MHz spectrometer. All chemical shifts were reported in part per million (ppm) using the residual proton or carbon signal in deuterated solvents as internal references. All protons were correctly assigned by means of two dimension NMR techniques such as ^{1}H-^{1}H COSY and ^{1}H-^{13}C- HMQC. Assignments of ^{13}C-NMR spectra were carried out by DEPT-90 and DEPT-135 techniques.

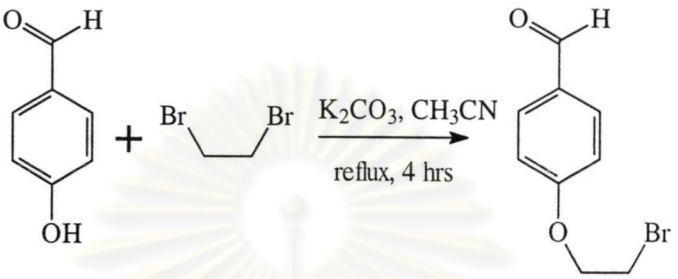
Elemental analyses were carried out on a Perkin-Elmer CHON/S analyzer (PE 2400 series II) by ignition combustion gas chromatography separated by frontal analysis and quantitatively detected by thermal conductivity detector. All melting points were obtained on an Electrothermal 9100 apparatus and uncorrected. MALDI-TOF mass spectra were recorded on Bruker BIFLEX^{Tm} using doubly recrystallized 2-cyano-4-hydroxy cinnamic acid (CCA) as matrix. Infrared spectra were obtained on a Nicolet Impact 410 using KBr pellet in the range of 4000-400 cm^{-1}.

2.1.1.2 Materials

Unless otherwise noted, all materials and solvents were standard analytical grade, purchased form Fluka, BDH, Aldrich, Carlo Erba, Merck or J.T Baker. They were used without further purification. Commercial grade solvents such as acetone, dichloromethane, hexane, methanol and ethyl acetate were purified by distillation. Acetonitrile and toluene were dried over CaH_2 and freshly distilled under nitrogen atmosphere prior to use. DMF was dried with CaH_2, distilled under reduced pressure and stored over molecular sieves 3Å or 4Å under nitrogen. All operations including DMF were succeeded by syringe. Column chromatographic operations were carried out on silica gel (Kieselgel 60, 0.063-0.200 mm, Merck). All eluents for column chromatography were stored over molecular sieves 3Å or 4Å prior to use. Thin-layer chromatography (TLC) were performed on silica gel plates (Kieselgel 60 F_{254}, 1 mm, Merck). Compounds on TLC plates were detected by the UV-light. All manipulations were carried out under nitrogen atmosphere. Starting material such as *p-tert*-butylcalix[4]arene was prepared according to literature procedure.[76] The synthetic procedures of 2-(2′-bromoethoxy)benzaldehyde **1a**, 25,26,27-tri((2-ethoxy) benzaldehyde)-*p-tert*-butylcalix[4]arene, **3a**, 25,26,27-*N*, *N*′, *N*″-tris((2-ethoxy) benzyl)ethylenetriimine-*p-tert*-butylcalix[4]arene, **4a**, 25,26,27-*N*, *N*′, *N*″-tris((2-ethoxy)benzyl)ethylenetretraamine-*p-tert*-butylcalix[4]arene.4HCl, **5a**, and 25,26,27-*N*, *N*′, *N*″-tris((2-ethoxy)benzyl)ethylenetretraamine-*p-tert*-butylcalix[4]arene, **6a**, have been previously reported by our research group.[77,78] All compounds were characterized by ^{1}H- NMR spectroscopy, mass spectrometry and elemental analyses.

2.1.2 Experimental Procedure

2.1.2.1 Preparation of 4-(2′-bromoethoxy)benzaldehyde (1b).



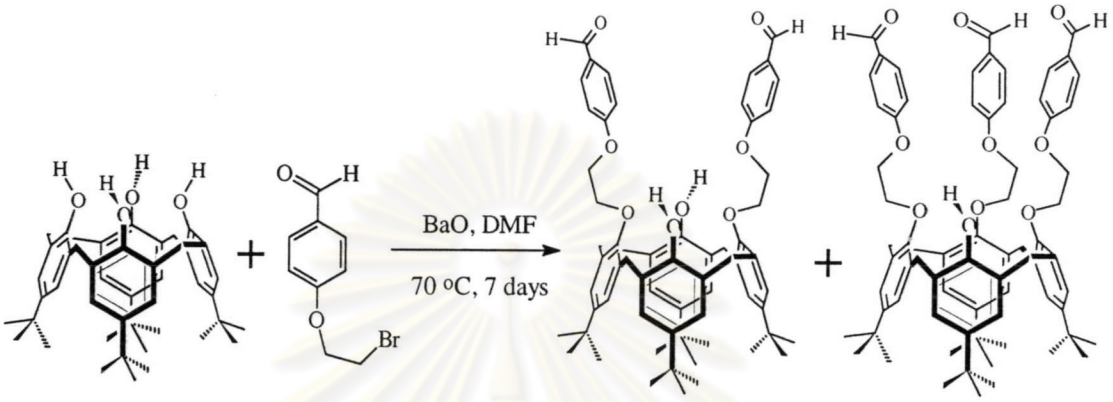
Into a 1-L two-necked round bottom flask equipped with a magnetic bar and a reflux condenser, 4-hydroxybenzaldehyde (14.86 g, 0.121 mol), potassium carbonate anhydrous (24.21 g, 0.175 mol) and acetonitrile (300 mL) were mixed and stirred. 1,2-Dibromoethane (247.03 g, 1.315 mol) in acetonitrile (50 mL) was then added dropwise through the addition funnel over 30 minutes into the mixture. The reaction mixture was refluxed with stirring under nitrogen atmosphere for 4 hours and then allowed to cool to room temperature. Potassium carbonate was filtered off and washed with a large amount of acetone and dichloromethane. The combined filtrate was concentrated on a rotary evaporator to obtain yellowish orange oil. The residue was placed on a silica gel column and eluted with dichloromethane. The desired product, 4-(2′-bromoethoxy)benzaldehyde, **1b**, was obtained as yellowish white solid after the addition of cold diethyl ether (18.24 g, 66% yield).

Characterization data for (1b):

^{1}H-NMR spectrum (200 MHz, $CDCl_3$): δ (in ppm)

δ= 9.84 (s, 1H, C**H**O), 7.83 (d, 2H, $J_{H\text{-}H}$ = 8.9 Hz, -OAr**H**$_a$), 6.97 (d, 2H, $J_{H\text{-}H}$ = 8.8 Hz, -OAr**H**$_b$), 4.33 (t, 2H, $J_{H\text{-}H}$ = 6.1 Hz, OC**H**$_2$CH$_2$Br), 3.63 (t, 2H, $J_{H\text{-}H}$= 6.1 Hz, OCH$_2$C**H**$_2$Br)). (Figure **A.2**)

2.1.2.2 Preparation of 25,26,27-tri((4-ethoxy)benzaldehyde)-*p*-*tert*-butylcalix[4]arene (3b)



2b **3b**

Into a 250 mL two-necked round bottom flask containing a suspension of *p-tert*-butylcalix[4]arene (7.03 g, 10.83 mmol) in dry DMF (150 mL) was treated with barium oxide (5.44 g, 35.63 mmol). The mixture was stirred for 2 hours. A solution of 4-(2′-bromoethoxy)benzaldehyde (7.47 g, 32.63 mmol) in dry DMF (50 mL) was then added dropwise over 2 hours. The mixture was stirred and heated at 70 °C under nitrogen atmosphere for 168 hours (7 days). The solution was allowed to cool to room temperature and evaporated to dryness under reduced pressure to yield a crude product. The residue was dissolved in dichloromethane (100 mL) and the aqueous solution of 3M hydrochloric acid was subsequently added until the pH of the solution reached 1. A copious amount of water was added and the mixture was stirred overnight at ambient pressure. The mixture was extracted with dichloromethane (3x50 mL) and washed with water until the aqueous phase contained no barium ion. The organic layer was dried over sodium sulfate anhydrous, filtered and evaporated to dryness to obtain a yellowish orange residue. The residue was dissolved in a minimum amount of dichloromethane and placed on a silica gel column using the ratio of the residue and silica gel of 1:30. Unreacted reactants were eluted with dichloromethane. Both of the desired products, 25,27-di((4-ethoxy)benzaldehyde)-*p*-

tert-butylcalix[4]arene (**2b**) and 25,26,27-tri((4-ethoxy)benzaldehyde)-*p-tert*-butylcalix[4]arene (**3b**) were eluted with a mixture of dichloromethane and ethyl acetate (98:2). The trialdehyde calix[4]arene was eluted from the column after its dialdehyde analogue. The collected fraction of dialdehyde compound was slowly evaporated and added methanol was added to precipitate a yellowish white powder (0.20 g, 2% yield). The trialdehyde *p-tert*-butylcalix[4]arene solution was concentrated on a rotary evaporator and ethanol was subsequently added to afford a yellowish crystalline solid (5.53 g, 46% yield). Both of the compounds were kept in a desiccator and dried *in vacuo*.

Characterization data for (2b)

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz): δ (in ppm)

δ = 10.06 (s, 2H, **CHO**), 7.98 (d, 4H, $J_{H\text{-}H}$ = 9.0 Hz, -OAr**H**$_a$), 7.41 (s, 2H, **H**OAr), 7.19 (d, 4H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr**H**$_b$), 7.02 (s, 2H, ROAr**H**), 7.01 (s, 2H, HOAr**H**), 4.54 and 3.49 (each d, 4H each, $J_{H\text{-}H}$ = 13.3 Hz, AB system, Ar**CH**$_A$**H**$_B$Ar), 4.51 (m, 4H, $OCH_2\mathbf{CH_2}O$), 4.48 (m, 4H, $O\mathbf{CH_2}CH_2O$), 1.47 (s, 18H, ROAr-*t*-$C_4\mathbf{H}_9$), 1.17 (s, 18H, HOAr-*t*-$C_4\mathbf{H}_9$). (Figure **A.3**)

^{13}C-NMR spectrum ($CDCl_3$, 125 MHz): δ (in ppm)

δ = 31.02 (q, C(**C**H$_3$)$_3$), 31.54 (t, Ar**C**H$_2$Ar), 31.66 (q, C(**C**H$_3$)$_3$), 33.81 (s, **C**(CH$_3$)$_3$), 33.96 (s, **C**(CH$_3$)$_3$), 66.86 (t), 73.00 (t), 115.07 (d), 125.15 (d), 125.67 (d), 127.77 (s), 130.17 (s), 131.91 (d), 132.62 (s), 141.60 (s), 147.21 (s), 149.55 (s), 150.43 (s), 163.50 (s), 190.76 (d, **C**H=O).

MALDI-TOF mass spectrum: 945.0 (M + 1) m/z.

Elemental Analysis: Anal Calcd for $C_{62}H_{72}O_8$: C 78.77; H 7.67.

Found: C 78.77; H 7.69.

Characterization data for (3b)

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz): δ (in ppm)

δ = 9.76 (s, 2H, C**H**O), 9.68 (s, 1H, C**H**O), 7.58 (d, 4H, $J_{H\text{-}H}$ = 9.0 Hz, -OAr**H**$_a$), 7.43 (d, 2H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr**H**$_a$), 7.21 (s, 2H, HOAr**H**), 7.16 (s, 2H, ROAr**H**), 6.70 (d, 4H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr**H**$_b$), 6.65 (d, 2H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr**H**$_b$), 6.57 (d, 2H, $J_{H\text{-}H}$ = 2.5 Hz, ROAr**H**$_a$), 6.55 (d, 2H, $J_{H\text{-}H}$ = 2.5 Hz, ROAr**H**$_b$), 5.43 (s, 1H, **H**OAr), 4.88 (t, 2H, $J_{H\text{-}H}$ = 8.0 Hz, OC**H**$_2$CH$_2$O), 4.47 and 3.33 (d, 4H, $J_{H\text{-}H}$ = 13.5 Hz, AB system, ArC**H**$_A$**H**$_B$Ar), 4.46 and 3.25 (d, 4H, $J_{H\text{-}H}$ = 13.0 Hz, AB system, ArC**H**$_A$**H**$_B$Ar), 4.30 (t, 2H, $J_{H\text{-}H}$ = 7.8 Hz, OCH$_2$C**H**$_2$O), 4.18-4.10 (m, 8H, OC**H**$_2$ C**H**$_2$O), 1.37 (s, 18H, HOAr-*t*-C$_4$**H**$_9$ and ROAr-*t*-C$_4$**H**$_9$), 0.85 (s, 18H, ROAr-*t*-C$_4$**H**$_9$). (Figure **A.4**)

^{13}C-NMR spectrum ($CDCl_3$, 125 MHz): δ (in ppm)

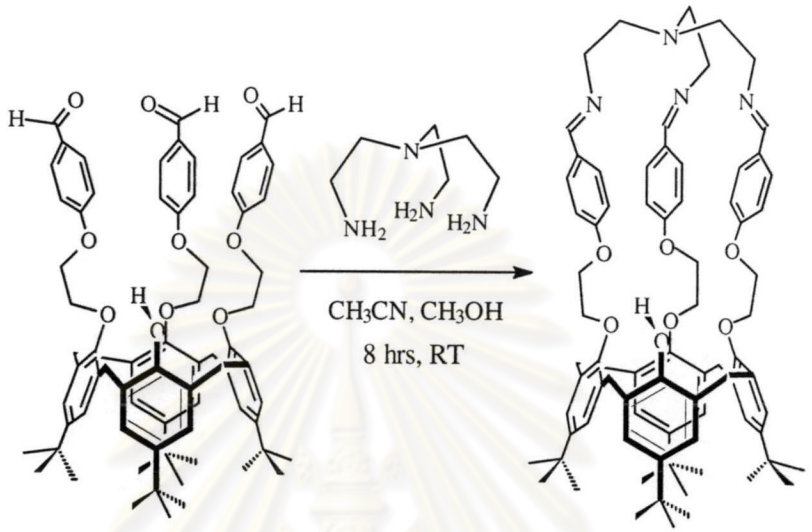
δ = 30.67 (t, ArC**H**$_2$Ar), 30.89 (q, C(C**H**$_3$)$_3$), 31.58 (q, C(C**H**$_3$)$_3$), 31.68 (q, C(C**H**$_3$)$_3$), 33.65 (s, **C**(CH$_3$)$_3$), 33.86 (s, **C**(CH$_3$)$_3$), 34.16 (s, **C**(CH$_3$)$_3$), 66.72 (t), 66.85 (t), 70.16 (t), 73.58 (d), 114.23 (d), 114.29 (d), 124.92 (d), 125.15 (d), 125.88 (d), 129.25 (s), 129.28 (s), 129.89 (s), 131.47 (d), 131.50 (d), 131.63 (d), 132.03 (s), 142.08 (s), 146.08 (s), 146.65 (s), 150.47 (s), 152.99 (s), 163.10 (s), 163.66 (s), 190.56 (d, **C**H=O).

MALDI-TOF mass spectrum: 1093.6 (M + 1) m/z.

Elemental Analysis: Anal Calcd for $C_{71}H_{80}O_{10}$: C 77.99; H 7.37.

Found: C 77.91; H 7.52.

2.1.2.3 Preparation of 25,26,27-*N*, *N'*, *N''*-tris((4-ethoxy)benzyl)ethylene triimine-*p*-*tert*-butylcalix[4]arene (4b).



3b **4b**

25,26,27-tri((4-ethoxy)benzaldehyde)-*p*-*tert*-butylcalix[4]arene (3.05 g, 2.79 mmol) was dissolved in dry acetonitrile (250 mL) in a 500 mL two necked round bottom flask. A solution of *tris*-(2 aminoethyl)amine (0.50 g, 3.43 mmol) in dry methanol (50 mL) was added dropwise into the solution over 2 hours. The solution was stirred and heated at 60°C under nitrogen atmosphere for another 6 hours to afford a creamy white precipitate. The product was filtered and washed with methanol. The filtrate was concentrated on a rotary evaporator and methanol was subsequently added to precipitate the remaining Schiff base calix[4]arene. The white solid was also filtered and washed with methanol to give imine calix[4]arene (3.23 g, 97% yield). The product was kept in a desiccator and dried *in vacuo*.

Characterization data for (4b)

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz): δ (in ppm)

δ = 8.08 (s, 2H, C**H**=N), 7.92 (s, 1H, C**H**=N), 7.38 (d, 4H, $J_{H\text{-}H}$ = 9.0 Hz, -OAr**H**$_a$), 7.20 (s, 2H, HOAr**H**), 7.18 (s, 2H, ROAr**H**), 6.74 (d, 4H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr**H**$_b$), 6.63 (d, 2H, $J_{H\text{-}H}$ = 2.5 Hz, ROAr**H**$_a$), 6.53 (d, 2H, $J_{H\text{-}H}$ = 2.0 Hz, ROAr**H**$_b$), 6.33 (s, 1H, **H**OAr), 6.13 (d, 2H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr**H**$_a$), 6.01 (d, 2H, $J_{H\text{-}H}$ = 9.0 Hz, -OAr**H**$_b$), 4.93 and 3.34 (d, 4H, $J_{H\text{-}H}$ = 13.3 Hz, AB system, ArC**H**$_A$**H**$_B$Ar), 4.58-4.55 (m, 2H, OC**H**$_2$CH$_2$O), 4.34 and 3.24 (d, 4H, $J_{H\text{-}H}$ = 12.5 Hz, AB system, ArC**H**$_A$**H**$_B$Ar), 4.26-4.18 (m, 4H, OC**H**$_2$CH$_2$O), 4.13-4.03 (m, 6H, OCH$_2$C**H**$_2$O), 3.78 (m, 2H, CH=NC**H**$_2$CH$_2$N), 3.72 (m, 2H, CH=NC**H**$_2$CH$_2$N), 3.65 (m, 2H, CH=NC**H**$_2$-CH$_2$N), 2.90 (m, 2H, CH=NCH$_2$C**H**$_2$N), 2.79 (m, 2H, CH=NCH$_2$C**H**$_2$N), 2.60 (m, 2H, CH=NCH$_2$C**H**$_2$N), 1.40 (s, 9H, HOAr-*t*-C$_4$**H**$_9$), 1.37 (s, 9H, ROAr-*t*-C$_4$**H**$_9$), 0.85 (s, 18H, ROAr-*t*-C$_4$**H**$_9$). (Figure **A.5**)

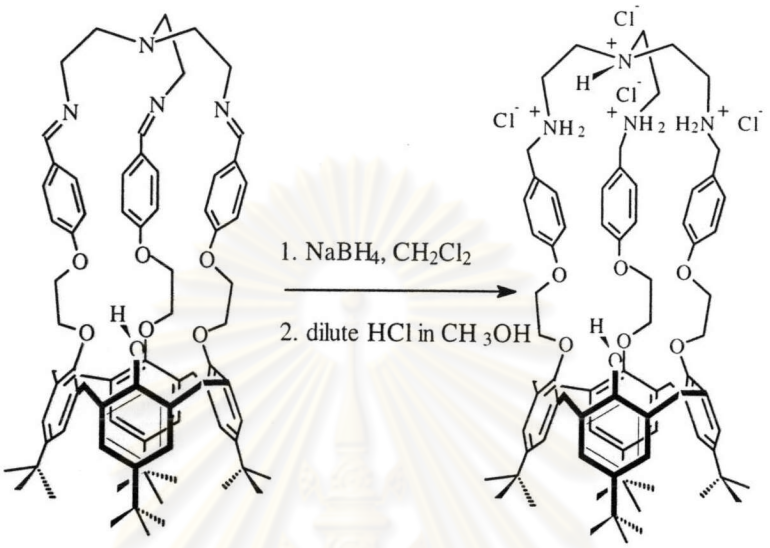
^{13}C-NMR spectrum ($CDCl_3$, 125 MHz): δ (in ppm)

δ = 30.47 (t, ArCH$_2$Ar), 30.69 (t, ArCH$_2$Ar), 31.03 (q, C(**C**H$_3$)$_3$), 31.68 (q, C(**C**H$_3$)$_3$), 31.87 (q, C(**C**H$_3$)$_3$), 33.71 (s, **C**(CH$_3$)$_3$), 33.90 (s, **C**(CH$_3$)$_3$), 34.23 (s, **C**(CH$_3$)$_3$), 52.12 (t), 53.95 (t), 54.66 (t), 58.66 (t), 65.66 (t), 66.72 (t), 70.85 (t), 74.26 (t), 113.03 (d), 114.62 (d), 124.59 (d), 125.15 (d), 125.56 (d), 125.79 (d), 128.66 (s), 129.13 (s), 129.40 (s), 129.58 (d), 131.88 (s), 132.35 (s), 135.78 (s), 140.83 (s), 145.78 (s), 146.37 (s), 150.81 (s), 151.31 (s), 153.45 (s), 159.09 (s), 160.32 (s), 161.46 (d, **C**H=N), 165.42 (d, **C**H=N).

MALDI-TOF mass spectrum: 1185.7 (M + 1) m/z.

Elemental Analysis: Anal Calcd for $C_{77}H_{92}O_7N_4$: C 78.01; H 7.82; N 4.73.
Found: C 77.95; H 7.66; N 4.77.

2.1.2.4 Preparation of 25,26,27-*N*, *N′*, *N″*-tris((4-ethoxy)benzyl)ethylene tetraamine-*p*-*tert*-butylcalix[4]arene.4HCl (5b).



4b **5b**

Into a 500 mL two-necked round bottom flask containing a solution of 25,26,27-*N*, *N′*, *N″* -tris((4-ethoxy)benzyl)ethylenetriimine-*p*-*tert*-butylcalix[4]arene (1.52 g, 1.283 mmol) in dichloromethane (300 mL) was added excess sodium borohydride (0.92 g, 24.35 mmol). The reaction mixture was purged with nitrogen and stirred under nitrogen atmosphere for 4 hours. A copious amount of water was subsequently added to destroy excess sodium borohydride. The organic layer was washed with several portions of water until the pH of the aqueous phase became neutral. The organic phase was separated and dried over sodium sulfate anhydrous. The solvent was then removed under reduced pressure to obtain a white solid. The residue was dissolved in a minimum amount of dichloromethane and acidified with HCl/CH_3OH (0.74% v/v) solution. The resultant solution was allowed to stand at room temperature for many hours. Upon evaporation of the solvent, the product was slowly recrystallized from the solution as a white solid (1.44 g, 84% yield).

Characterization data for (5b)

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz): δ (in ppm)

δ = 8.64 (s, broad, 4H, $ArCH_2N\mathbf{H}_2^+$), 8.24 (s, broad, 2H, $ArCH_2N\mathbf{H}_2^+$), 7.78 (d, 4H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr$\mathbf{H}_a$), 7.36 (d, 2H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr$\mathbf{H}_a$), 7.13 (s, 2H, HOAr**H**), 7.09 (s, 2H, ROAr**H**), 6.92 (d, 4H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr$\mathbf{H}_b$), 6.59 (d, 2H, $J_{H\text{-}H}$ = 2.5 Hz, ROAr$\mathbf{H}_a$), 6.57 (d, 2H, $J_{H\text{-}H}$ = 3.0 Hz, ROAr$\mathbf{H}_b$) 6.54 (d, 2H, $J_{H\text{-}H}$ = 8.5 Hz, -OAr$\mathbf{H}_b$), 6.11 (s, 1H, **H**OAr), 4.55 and 3.29 (d, 4H, $J_{H\text{-}H}$ = 13.5 Hz, AB system, $ArC\mathbf{H}_A\mathbf{H}_BAr$), 4.51 (m, 2H, $OC\mathbf{H}_2C\mathbf{H}_2O$), 4.48 (m, 4H, $OC\mathbf{H}_2C\mathbf{H}_2O$), 4.45 and 3.24 (d, 4H, $J_{H\text{-}H}$ = 13.0 Hz, AB system, $ArC\mathbf{H}_A\mathbf{H}_BAr$), 4.40-4.37 (m, 2H, $OC\mathbf{H}_2CH_2O$), 4.29-4.23 (m, 4H, $OCH_2C\mathbf{H}_2O$ and $CH_2NH_2^+C\mathbf{H}_2CH_2N$), 4.16-4.06 (m, 8H, $ArC\mathbf{H}_2NH_2^+$ and $OCH_2C\mathbf{H}_2O$), 4.00 (m, 2H, $CH_2NH_2^+C\mathbf{H}_2CH_2N$), 3.70 (s, broad, 2H, $CH_2NH_2^+C\mathbf{H}_2CH_2N$), 3.43 (s, broad, 2H, $CH_2NH_2^+$ $C\mathbf{H}_2CH_2N$), 3.37 (s, broad, 2H, $CH_2NH_2^+CH_2C\mathbf{H}_2N$), 3.09 (s, broad, 4H, $CH_2NH_2^+CH_2C\mathbf{H}_2N$), 1.33 (s, 9H, HOAr-*t*-$C_4\mathbf{H}_9$), 1.32 (s, 9H, ROAr-*t*-$C_4\mathbf{H}_9$), 0.82 (s, 18H, ROAr-*t*-$C_4\mathbf{H}_9$).(Figure **A.6**)

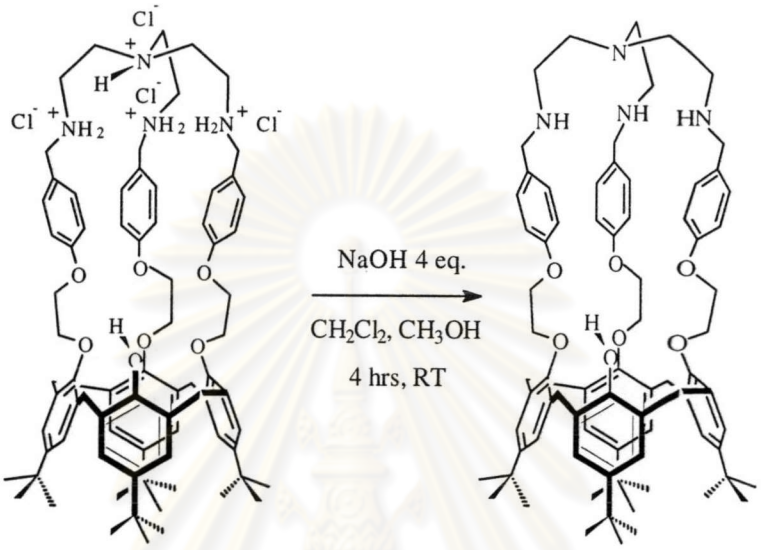
^{13}C-NMR spectrum ($CDCl_3$, 125 MHz): δ (in ppm)

δ = 30.92 (t, $ArC\mathbf{H}_2Ar$), 30.99 (q, $C(\mathbf{C}H_3)_3$), 31.21 (t, $ArC\mathbf{H}_2Ar$), 31.63 (q, $C(\mathbf{C}H_3)_3$), 31.76 (q, $C(\mathbf{C}H_3)_3$), 33.70 (s, $\mathbf{C}(CH_3)_3$), 33.85 (s, $\mathbf{C}(CH_3)_3$), 34.14 (s, $\mathbf{C}(CH_3)_3$), 43.95 (t), 44.13 (t), 50.81 (t), 52.24 (t), 66.81 (t), 67.28 (t), 71.59 (t), 74.09 (t), 114.15 (d), 114.39 (d), 122.76 (s), 123.06 (s), 124.86 (d), 125.13 (d), 125.30 (d), 125.74 (d), 128.65 (s), 131.98 (d), 132.08 (d), 133.15 (d), 135.53 (s), 141.31 (s), 145.88 (s), 146.04 (s), 150.75 (s), 150.91 (s), 153.38 (s), 159.45 (s), 159.65 (s).

MALDI-TOF mass spectrum: 1191.8 (M + 1– 4HCl) m/z.

Elemental Analysis: Anal Calcd for $C_{77}H_{102}O_7N_4Cl_4$: C 69.15; H 7.69; N 4.19.
Found: C 69.19; H 7.76; N 4.16.

2.1.2.5 Preparation of 25,26,27-*N*, *N′*, *N″*-tris((4-ethoxy)benzyl)ethylene tetraamine-*p*-*tert*-butylcalix[4]arene (6b).



5b **6b**

Into a 250 mL round bottom flask containing a solution of 25,26,27-*N*, *N′*, *N″*-tris((4-ethoxy)benzyl)ethylenetetraamine-*p*-*tert*-butylcalix[4]arene.4HCl (1.69 g, 1.262 mmol) in dry dichloromethane (100 mL) was added a solution of sodium hydroxide (0.20 g, 5.048 mmol) in methanol (40 mL). The reaction mixture was stirred under nitrogen atmosphere for 2 hours. The mixture was evaporated to dryness. The residue was added with water and dichloromethane. The organic layer was washed with several portions of water until the aqueous phase contained no chloride ion. The organic phase was separated and dried over sodium sulfate anhydrous. The solvent was then removed under reduced pressure to obtain a white solid. The residue was dissolved in a minimum amount of dichloromethane and added with ethanol. The solution was allowed to stand at room temperature for many hours. During this period, the neutral product was slowly recrystallized from the solution as a yellowish white-needle solid (1.12 g, 74% yield).

Characterization data for (6b)

^{1}H-NMR spectrum ($CDCl_3$, 500 MHz): δ (in ppm)

δ = 7.15 (s, 2H, HOAr**H**), 7.08 (s, 2H, ROAr**H**), 7.04 (d, 4H, $J_{H\text{-}H}$ = 8.4 Hz, -OAr$\mathbf{H}_a$), 6.66 (d, 4H, $J_{H\text{-}H}$ = 8.0 Hz, -OAr$\mathbf{H}_b$), 6.62 (d, 2H, $J_{H\text{-}H}$ = 2.4 Hz, ROAr$\mathbf{H}_a$), 6.57 (d, 2H, $J_{H\text{-}H}$ = 2.4 Hz, ROAr$\mathbf{H}_b$), 6.44 (d, 2H, $J_{H\text{-}H}$ = 9.0 Hz, -OAr$\mathbf{H}_a$), 6.37 (d, 2H, $J_{H\text{-}H}$ = 8.3 Hz, -OAr$\mathbf{H}_b$), 6.12 (s, 1H, **H**OAr), 4.78 and 3.26 (d, 4H, $J_{H\text{-}H}$ = 13.0 Hz, AB system, ArC$\mathbf{H}_A\mathbf{H}_B$Ar), 4.67 (m, 2H, OC$\mathbf{H}_2$C$\mathbf{H}_2$O), 4.40 and 3.27 (d, 4H, $J_{H\text{-}H}$ = 12.6 Hz, AB system, ArC$\mathbf{H}_A\mathbf{H}_B$Ar), 4.24 (m, 4H, OC$\mathbf{H}_2$CH$_2$O), 4.13-3.39 (m, 8H, OCH$_2$C$\mathbf{H}_2$O and ArC$\mathbf{H}_2$NH), 3.66 (m, 4H, ArC$\mathbf{H}_2$NH), 3.50 (s, broad, 2H, CH$_2$NHC$\mathbf{H}_2$CH$_2$N), 2.83 (s, broad, 6H, CH$_2$NHC$\mathbf{H}_2$CH$_2$N and CH$_2$NHCH$_2$C$\mathbf{H}_2$N), 2.66 (s, broad, 4H, CH$_2$NHCH$_2$C$\mathbf{H}_2$N), 1.85 (s, broad, 3H, ArCH$_2$N**H**CH$_2$ exchange with D_2O), 1.34(s, 9H, HOAr-*t*-C$_4\mathbf{H}_9$), 1.33 (s, 9H, ROAr-*t*-C$_4\mathbf{H}_9$), 0.85 (s, 18H, ROAr-*t*-C$_4\mathbf{H}_9$). (Figure **A.7**)

MALDI-TOF mass spectrum: 1191.2 (M + 1) m/z.

Elemental Analysis: Anal Calcd for $C_{77}H_{98}O_7N_4$: C 77.61; H 8.29; N 4.70
Found: C 77.58; H 8.22; N 4.66.

2.2 Studies of Binding Abilities by Potentiometric Titration

2.2.1 General Procedure

2.2.1.1 Apparatus

All potentiometric titrations were performed on an automatic titrator model DL25 (Mettler, Switzerland). The temperature during all titration experiments was controlled at 25 ± 0.1°C by external circulation of Heto DT-2 thermostat (Denmark). The concentrations of free proton [H^+] in observed solution were measured by combined pH electrode model DG 113-SC (Mettler, Switzerland) connected to an automatic titrator.

2.2.1.2 Chemicals

All materials and solvents were standard analytical grade purchased from Aldrich, Fluka and Merck, and used without further purification unless otherwise noted. Transition metal salts such as $Co(ClO_4)_2.6H_2O$, $Cu(CF_3SO_3)_2$ and $Zn(CF_3SO_3)_2$ were dried under reduced pressure and stored in a desiccator. Due to the extremely hygroscopic properties of $Co(ClO_4)_2$, it was firstly washed with diethyl ether to removed all remaining water and further dried in *vacuo* prior to use. Potassium hydrogen phthalate (KHP) was dried at 120°C for 2 hours and allowed to cool to room temperature in a desiccator. Synthetic ammonium salt *p-tert*-butylcalix[4]arene derivatives, 25,26,27-*N*, *N′*, *N″*-tris((2-ethoxy)benzyl)ethylenetetraamine-*p-tert*-butylcalix[4]arene.4HCl **5a** and 25,26,27-*N*, *N′*, *N″*-tris((4-ethoxy)benzylethylene tetraamine-*p-tert*-butylcalix[4]arene.4HCl **5b**, were dried under vacuum and kept away from direct sunlight to avoid the decomposition.

2.2.2 Preparation of the Solutions

Tetrabutylammonium trifluoromethanesulfonate ($Bu_4NCF_3SO_3$) was used as an inert background electrolyte for all experiments in this part. This electrolyte was prepared by dissolving weighted quantity of $Bu_4NCF_3SO_3$ in methanol. The concentration of the inert background electrolyte was kept constant at 1.00×10^{-2} M. Tetrabutylammonium hydroxide (Bu_4NOH 0.1 M in methanol/2-propanol), of which concentration 5.00×10^{-2} M, was utilized as a titrant base. This solution was prepared by dilution of the aforementioned solution in 1.00×10^{-2} M $Bu_4NCF_3SO_3$ in methanol. The primary standard solution of potassium hydrogen phthalate (KHP) was prepared by dissolved a weighted quantity of KHP in distilled water and used for standardization of Bu_4NOH solution. The stock solution of 1.0 M $HClO_4$ was prepared by dilution of the commercial concentrated $HClO_4$ (70-72% in water) in methanol. The standard solution of $HClO_4$ (5.00×10^{-2} M) was made by dilution of 1.00 M $HClO_4$ in 1.00×10^{-2} M $Bu_4NCF_3SO_3$ solution. The $HClO_4$ standard solutions (1.00×10^{-2} and 1.00×10^{-3} M in 1.00×10^{-2} M $Bu_4NCF_3SO_3$) were prepared from 5.00×10^{-2} M $HClO_4$ and used for electrode calibration. All $HClO_4$ solutions were standardized with the Bu_4NOH solution prior to use. Solutions of metal ions Co^{2+}, Cu^{2+} and Zn^{2+} were prepared by dissolution weighted quantities of metal salts in the inert background solution. Concentrations of each metal solutions were approximately 1.0×10^{-2} M. The solutions of 25,26,27-*N*, *N'*, *N''*-tris((2-ethoxy)benzyl)ethylene tetraamine-*p*-*tert*-butylcalix[4]arene.4HCl **5a** and 25,26,27-*N*, *N'*, *N''*-tris((4-ethoxy) benzyl)ethylene tetraamine-*p*-*tert*-butylcalix[4]arene.4HCl **5b** were prepared by dissolving weighted quantities of compounds **5a** and **5b** in 1.00×10^{-2} M $Bu_4NCF_3SO_3$. Concentrations of these ligand solutions were about 1.00×10^{-3} M.

2.2.3 Determination of Protonation and Stability Constants

The compounds **5a** and **5b** were used for basicity and binding ability studies of their neutral derivatives, 25,26,27-*N*, *N′*, *N″*-tris((4-ethoxy)benzyl)ethylenetetra amine-*p-tert*-butylcalix[4]arene **6a** and 25,26,27-*N*, *N′*, *N″*-tris((4-ethoxy)benzyl) ethylenetetraamine-*p-tert*-butylcalix[4]arene **6b**. All titrations were carried out at 25 °C under argon atmosphere saturated with the inert background solution. The concentration of the free proton, $[H^+]$ was denoted in the term of pH, which equivalent to –log $[H^+]$. The working electrode was calibrated at pH = 2 with a solution of 1.00 x 10^{-2} M $HClO_4$ in 1.00 x 10^{-2} M $Bu_4NCF_3SO_3$ prior to use. As the junction potentials vary exponentially with pH, [79] the relationship (**1**) was used.

$$pH_{real} = pH_{measured} + a + b[H^+]_{measured} \qquad (1)$$

The values for *a* and *b* were determined by measuring the pH of a solution of 1.00 x 10^{-3} M $HClO_4$ in 1.00 x 10^{-2} M $Bu_4NCF_3SO_3$. Both *a* and *b* values were included to the treatment program in order to obtain the accurate pH value. For each titration, typically 10 mL of the ligand solution was added to various amounts of 5.00 x 10^{-2} M $HClO_4$ solution to obtain various ratio of ligand to proton. The solution was titrated with the Bu_4NOH solution. The experimental data were shown in Table 2.1. For determination of stability constants of metal complexes, typically 10 mL of the ligand solution was added with the metal solution by varying the mole ratio of metal to ligand. The pH of solution was adjusted by adding varied amount of 5.00 x 10^{-2} M $HClO_4$ solution. Finally, the solution was titrated with standard Bu_4NOH solution. The experimental data for complexation studies with Co^{2+}, Cu^{2+} and Zn^{2+} were shown in Tables 2.2, 2.3 and 2.4, respectively.

Table 2.1 Experimental data for determining the protonation constants of compounds **6a** and **6b** in 1.00 x 10^{-2} M $Bu_4NCF_3SO_3$ (25 °C).

Ligand	Titration	Initial concentration (mM)		pH range	number of data points
		Ligand	Proton		
5a	1	1.014	4.056	3.710-11.001	59
	2	0.922	8.912	2.700-11.032	67
	3	0.845	11.638	2.786-10.691	65
	4	0.922	8.192	2.817-10.861	64
5b	1	1.007	4.028	3.924-11.333	75
	2	1.007	4.028	4.043-11.527	86
	3	0.839	11.557	2.435-10.897	54
	4	0.915	8.134	2.954-11.083	69

Table 2.2 Experimental data for determining the stability constants of compounds **6a** and **6b** with Co^{2+} in 1.00 x 10^{-2} M $Bu_4NCF_3SO_3$ (25 °C).

Ligand	Titration	Initial concentration (mM)			pH range	Number of data points
		Ligand	Proton	Co^{2+}		
5a	1	0.876	5.655	0.871	2.962-11.169	76
	2	0.839	7.479	0.835	2.933-11.112	71
	3	0.839	7.479	0.835	3.045-10.909	69
	4	0.805	5.202	1.602	2.995-11.070	77
5b	1	0.873	5.646	0.883	3.072-10.857	69
	2	0.837	7.475	0.847	2.815-10.935	73
	3	0.873	5.646	0.869	2.940-10.832	68
	4	0.772	10.967	0.771	2.940-10.924	69

Table 2.3 Experimental data for determining the stability constants of compounds **6a** and **6b** with Cu^{2+} in 1.00 x 10^{-2} M $Bu_4NCF_3SO_3$ (25 °C).

Ligand	Titration	Initial concentration (mM)			pH range	Number of data points
		Ligand	Proton	Cu^{2+}		
5a	1	0.882	5.677	0.883	3.018-10.834	56
	2	0.845	7.501	0.846	3.071-10.939	59
	3	0.811	5.223	1.625	3.002-10.874	74
	4	0.780	6.924	1.562	3.078-10.848	73
5b	1	0.922	3.687	0.923	3.653-11.000	64
	2	0.845	7.480	0.846	2.407-11.155	82
	3	0.845	7.480	0.846	2.667-11.252	79
	4	0.780	6.904	1.562	2.548-11.175	86

Table 2.4 Experimental data for determining the stability constants of compounds **6a** and **6b** with Zn^{2+} in 1.00 x 10^{-2} M $Bu_4NCF_3SO_3$ (25 °C).

Ligand	Titration	initial concentration (mM)			pH range	Number of data points
		ligand	proton	Zn^{2+}		
5a	1	0.875	5.655	0.885	3.114-10.163	60
	2	0.839	7.479	0.848	3.114-10.078	60
	3	0.805	5.202	1.629	3.027-10.130	62
	4	0.774	6.904	1.566	3.097-9.968	63
5b	1	0.876	5.602	0.871	2.904-10.865	71
	2	0.840	7.356	0.835	2.929-10.930	73
	3	0.806	5.153	1.629	3.051-10.908	79
	4	0.775	6.808	1.566	2.988-10.930	82

The experimental data were evaluated by the computer program SUPERQUAD.[75] The results were reported in terms of the logarithm of overall equilibrium constants (log β). For determination of stability constants of complexation of ligands and transition metal ions, these formation constants were calculated together with protonation constants of each ligand. The protonation constants of each ligand were fixed as constants during the refinement process. The metal hydroxide species, MOH^+, and autoprotolysis constants of methanol (K_{MeOH}) were also introduced into the computational procedure in order to obtain reasonable stability constants of the complexes.