CHAPTER III

EXPERIMENT

3.1 Synthesis of 25,27-[*N*,*N*′-di-((2-ethoxy)benzyl)propylenediamine]-26,28-dimethoxy-*p*-*tert*-butylcalix[4]arene dihydrochloride (7)

3.1.1 Chemicals

1. Acetone	Commercial grade, Mallinckrodt, U.S.A.
2. Acetonitrile	Analytical Reagent grade, Lab-Scan, Ireland
3. Barium oxide	Practical grade, Fluka, Switzerland
4. 1,3-Diaminopropane	Analar grade, Fluka, Switzerland
5. 1,2-Dibromoethane	Analar grade, Merck, Germany
6. Benzophenone	Purum grade, Fluka, Switzerland
7. Deuterochloroform	Puriss grade, Fluka, Switzerland
8. Dichloromethane	Commercial grade, Mallinckrodt, U.S.A.
9. Diethyl ether	Analar grade, J.T.Baker, U.S.A.
10. Ethanol	Absolute grade, Merk, Germany
11. Ethyl acetate	Analytical Reagent grade, Lab-Scan, Ireland
12. Hexane	Analytical Reagent grade, Lab-Scan, Ireland
13. Hydrochloric acid	Analar grade, J.T.Baker, U.S.A.
14. Methanol	Analytical Reagent grade, Lab-Scan, Ireland

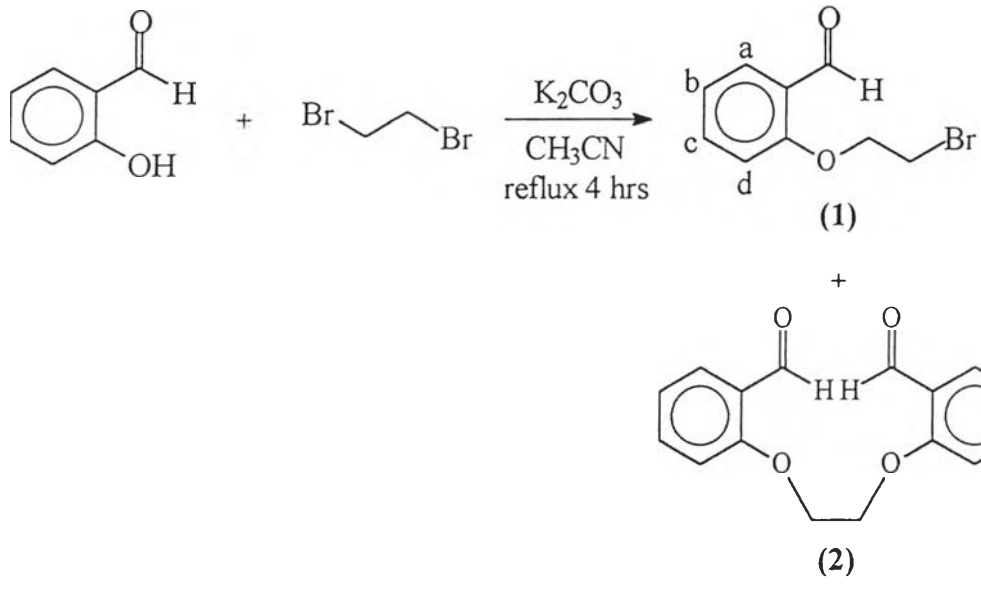
15. Methyl iodide	Analar grade, Fluka, Switzerland
16. Potassium carbonate	Analar grade, Merck, Germany
17. Potassium *tert*-butoxide	Practical grade, Fluka, Switzerland
18. Salicylaldehyde	Analar grade, Fluka, Switzerland
19. Sea sand	Fluka, Switzerland
20. Silica gel	No.7734, Fluka, Switzerland
21. Sodium	Purum grade, Fluka, Switzerland
22. Sodium borohydride	Analar grade, Fluka, Switzerland
23. Sodium sulphate anhydrous	Analar grade, Fluka, Switzerland
24. Tetrahydrofuran	Pro Analysis grade, Merck, Germany
25. Tetradeuteromethanol	Puriss grade, Fluka, Switzerland
26. *p-tert*-butylcalix[4]arene	Synthesized according to the published procedure [66]
27. Nitrogen gas	Ultrahigh purity grade

3.1.2 Instruments

1. CHNS/O Analyser PE2400 Series II, Perkin Elmer, U.S.A.
2. Eyela Magnetic Stirrer RC-2, Eyela, Japan
3. Fourier Transform NMR Spectrometer ACF 200 MHz, Bruker, Switzerland
4. Fourier Transform NMR Spectrometer 500 MHz, Jeol, Japan
5. Matrix Assisted Laser Description Ionization-Time of Flight Mass Spectrometer (Biflax)
6. Rotary Evaporator, Eyela, Japan
7. Vacuum-system B-169, Buchi, Switzerland

3.1.3 Preparation Methods

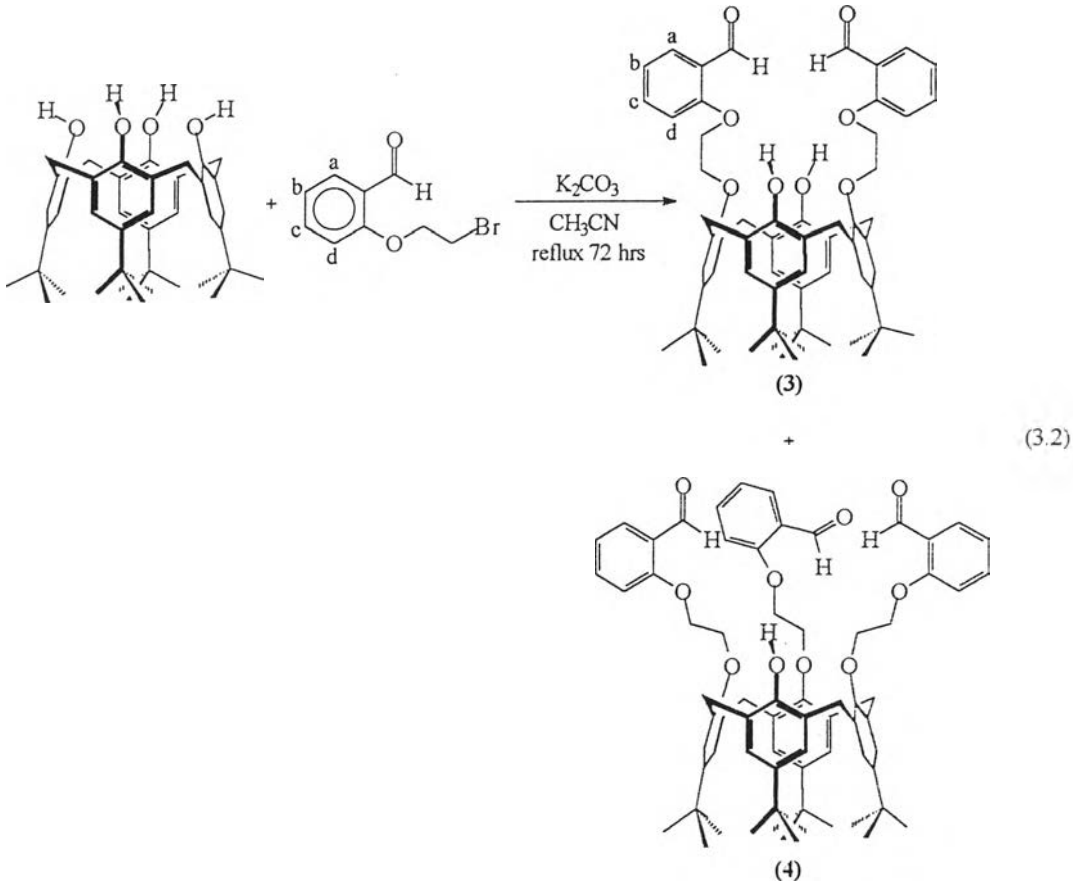
3.1.3.1 Preparation of 2(2′-bromoethoxy)benzaldehyde (1)



(3.1)

Into a 1 L two-necked round bottom flask containing a mixture of K_2CO_3 (46.00 g, 334.80 mmol), salicylaldehyde (25.4 mL, 242.4 mmol) and acetonitrile (500 mL) was slowly added 1,2-dibromoethane (205.5 mL, 2.2 mol). The reaction mixture was heated at reflux under nitrogen atmosphere for 4 hours. The mixture was cooled to room temperature and filtered off K_2CO_3. The yellow filtrate was then evaporated in vacuo to yield a mixture of **1** and **2** which were separated on a silica gel column. CH_2Cl_2 was used as eluant. The product **1** was crystallized by addition of hexane into its CH_2Cl_2 solution. (18.10 g, 33%). δ_H (200 MHz ; $CDCl_3$), 10.51 (1H, s, C*H*O), 7.83 (1H, d, J = 7.8, H_a), 7.52 (1H, t, J = 7.9, H_b), 7.05 (1H, t, J = 7.5, H_c), 6.94 (1H, d, J = 8.4, H_d), 4.40 (2H, t, J = 6.1, O$CH_2$$CH_2$Br) and 3.69 (2H, t, J = 6.0, OCH$_2$CH_2OBr). The ^{1}H NMR spectrum of **1** is shown in Figure A.1.

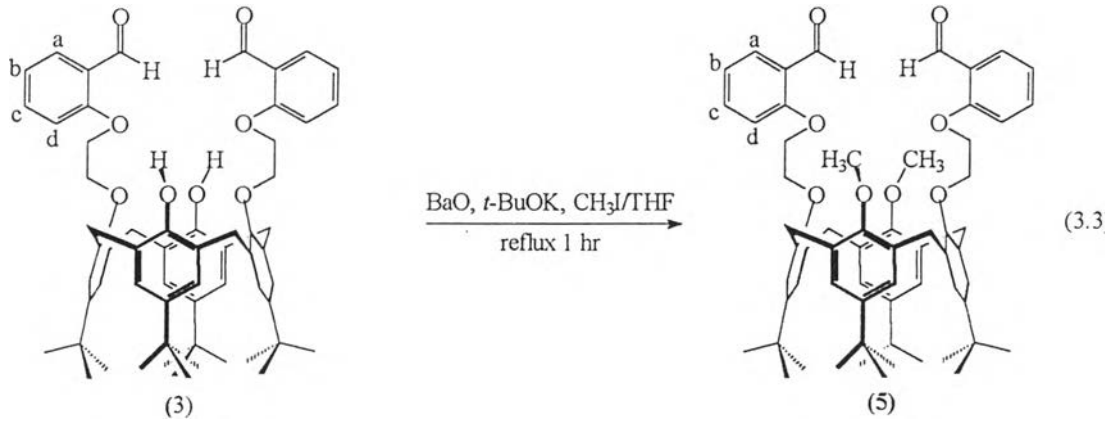
3.1.3.2 Preparation of 25,27-di-(2-ethoxy)benzaledehyde-*p-tert*-butyl-calix[4]arene (3)



(3.2)

Into a 1 L two-necked round bottom flask containing K_2CO_3 (23.00 g, 167.40 mmol) *p-tert*-butylcalix[4]arene (11.70 g, 18.10 mmol) and CH_3CN (350 mL) was added dropwise a solution of **1** (9.34 g, 40.86mmol) in CH_3CN (100 mL). The mixture was heated at reflux under nitrogen atmosphere for 72 hours. The reaction was subsequently cooled to room temperature and filtered. The pale yellow filtrate was then concentrated by reduced pressure and was added CH_3OH to precipitate **3**, 1,3-di-substistution derivative of *p-tert*-butylcalix[4]arene, and tri-substitution derivative **4**. The white solid **3** was separated from **4** by column chromatography employing CH_2Cl_2 as eluant. (8.48 g, 50%). δ_H (200 MHz ; $CDCl_3$), 10.47 (2H, s, C*H*O), 7.81 (2H, d, J = 7.7, H_a), 7.53-7.54 (12H, m, H_b), 7.45 (2H, s, ArO*H*), 6.98-6.94 (4H, m, H_c and H_d), 7.00 (4H, s, HOAr*H*), 6.84 (4H, s, ROAr*H*), 4.40-4.38 (8H, m, OCH_2CH_2O), 4.29,3.29 (8H, 2d, J = 13.0, $ArCH_2Ar$), 1.24 (18H, s, HOAr-*t*-C_4H_9) and 1.00 (18H, s, ROAr-*t*-C_4H_9). The ^{1}H NMR spectrum of **3** is shown in Figure A.2.

3.1.3.3 Preparation of 25,27-di-((2-ethoxy)benzaledehyde)-26,28-dimethoxy-*p*-*tert*-butylcalix[4]arene (5)



(3.3)

Under nitrogen atmosphere, a solution of compound **3** (1.12 g, 1.19 mmol) in dry THF* (80 mL) was stirred with BaO (0.19 g, 1.21 mmol) for 1 hour and 30 minutes in a 250 mL two-necked round bottom flask. Then, *t*-BuOK (0.41 g, 3.63 mmol) and CH_3I (0.39 ml, 6.24 mmol) were added to the mixture. The reaction was then heated at reflux for 1 hour. When the reaction was cooled to room temperature, THF was evaporated by reduced pressure to dryness. The residue was dissolved in CH_2Cl_2 and washed with 1 M aqueous HCl. The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silicagel column using 10% EtOAc in hexane as eluant to separate a crude product of **5**, which was purified again by column chromatography using 1% CH_3OH in CH_2Cl_2 as eluant. (0.33 g, 28%). δ_H (200 MHz ; $CDCl_3$), 10.44 (2H, br s, C*H*O), 7.82 (2H, d, J = 7.7, H_a), 7.52 (2H, t, J = 8.3, H_b), 7.05-6.98 (8H, m, H_c,H_d and ROAr*H*), 6.50 (4H, br s, CH_3OAr*H*), 4.50-4.03 (12H, m, OCH_2CH_2O and $ArCH_2Ar$), 3.82 (6H, s, OCH_3), 3.14 (4H, br s, $ArCH_2Ar$), 1.27 (18H, br s, ROAr-*t*-C_4H_9), 1.04 (9H, br, s, CH_3OAr-*t*-C_4H_9) and 0.79 (9H, br s, CH_3OAr-*t*-C_4H_9). The ^1H NMR spectrum of **5** is shown in Figure A.3. Anal.Cald for $C_{64}H_{76}O_8$; C, 78.98% ; H, 7.87%. Found : C, 78.97% ; H, 7.77%.

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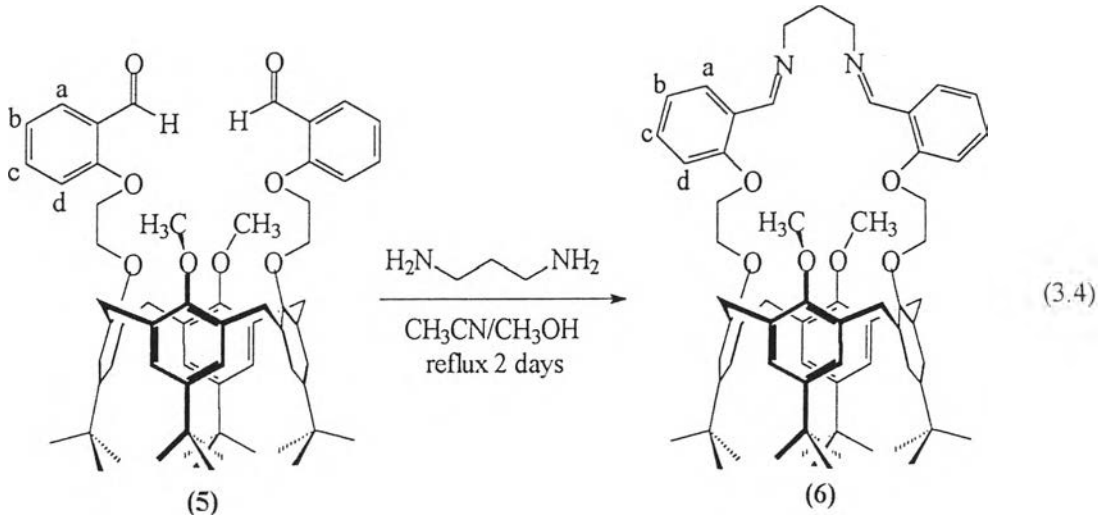
* THF was fresh distilled with Na metal and benzophenone (Na : benzophenone = 1:3 by weight) under nitrogen atmosphere.

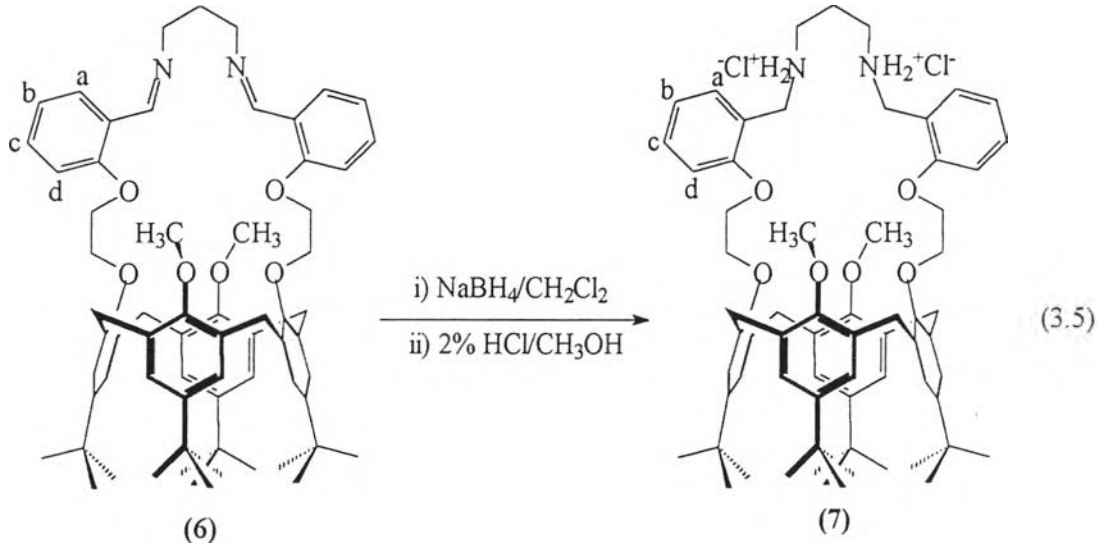
3.1.3.4 Preparation of 25,27-[*N,N'*-di-((2-ethoxy)benzyl)propylene-diimine]-26,28-dimethoxy-*p-tert*-butylcalix[4]arene (6)



(3.4)

Into a stirred solution of compound **5** (0.56 g, 0.58 mmol) in CH_3CN (60 mL) was added dropwise a solution of 1,3-diaminopropane (0.08 mL, 0.96 mmol) in methanol (12 mL). The reaction was heated at reflux for 24 hours. White solid of **6**, precipitated after the reaction was cooled to room temperature. It was isolated by filtration, dried and washed with cold methanol. (0.32 g, 55%). δ_H (200 MHz ; $CDCl_3$), 8.65 (2H, br s, *H*C=N), 7.90 (2H, d, J = 7.2, H_a), 7.32-7.27 (2H, m, H_b), 7.10-6.70 (8H, m, H_c,H_d and ROAr*H*), 6.50, 6.43 (4H, br s, CH_3OArH), 4.50-3.90 (12H, m, OCH_2CH_2O and $ArCH_2Ar$), 3.74 (3H, s, OCH_3), 3.61-3.32 (3H, m, OCH_3), 3.32-2.80 (8H, m, NCH_2CH2 and $ArCH_2Ar$), 2.05-1.85 (1H, m, $NCH_2CH_2CH_2N$), 1.70-1.52 (1H,m , $NCH_2CH_2CH_2N$), 1.32, 1.27 (1H, br s, ROAr-*t*-C_4H_9), 1.03 (9H, br, s, CH_3OAr-*t*-C_4H_9) and 0.79 (9H, br s, CH_3OAr-*t*-C_4H_9). The ^1H NMR spectrum of **6** is shown in Figure A.4. Anal.Cald for $C_{67}H_{82}O_6N_2$; C, 79.57% ; H, 8.17% ; N, 2.77%. Found : C, 79.49% ; H, 8.03% ; N, 2.62.

3.1.1.5 Preparation of 25,27-[*N*,*N*'-di-((2-ethoxy)benzyl)propylene-diamine]-26,28–dimethoxy–*p*-*tert*-butylcalix[4]arene dihydro chloride (7)



The compound **6** (0.47 g, 0.46 mmol) was stirred with $NaBH_4$ (0.48 g, 12.64 mmol) in CH_2Cl_2 (100 ml) under nitrogen atmosphere for 2 days. Excess $NaBH_4$ was then destroyed by a cupious amount of water. The organic layer was extracted, dried over Na_2SO_4 and removed solvent to yield a white residue. The residue was added 2% HCl/CH_3OH until pH of the solution reached 1. Upon removal of CH_3OH white solid precipitated. (0.39 g, 77%). δ_H (200 MHz ; CD_3OD), 7.49-7.40 (4H, m, H_a and H_b), 7.21 (4H, s, and ROAr*H*), 7.14-7.01 (4H, s, H_c, and H_d), 6.71 (4H, br s, CH_3OAr*H*), 4.45 (4H, br s, ArCH_2NH), 4.28-4.23 (16H , br m, ArCH_2Ar and OCH_2CH_2O), 3.52 (6H, br s, OCH_3), 3.34-3.28 (4H, *, ArCH_2Ar), 2.90 (4H, br s, NCH_2CH_2) 2.01 (2H, br s, $NCH_2CH_2CH_2N$), 1.34 (18H, s, ROAr-*t*-C_4H_9), 0.99 (18H, s, CH_3OAr-*t*-C_4H_9). The ^{1}H-NMR spectrum of 7 is shown in Figure A.6. Anal.calcd for $C_{67}H_{88}O_6N_2Cl_2.2CH_3OH.2H_2O$; C, 69.73% ; H, 8.48% ; N, 2.36%. Found : C, 69.84% ; H, 7.87% ; N, 2.39%. MALDI-TOF MS for [M^+] ; 1014.2 m/z.

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* The observed signal aggregates with the solvent peak

3.2 ^{1}H NMR studies of the compound 7

3.2.1 Addition of CD_3OD and DMSO-d_6 in the $CDCl_3$ solution of the compound 7

Typically, in a NMR tube, the compound 7 (0.01 g, 0.009 mmol) was dissolved in $CDCl_3$ (0.4 ml). CD_3OD or DMSO-d_6 (5 μL) was added into the tube. The spectrum was then recorded. A series of experiment was carried out by increasing the amount of CD_3OD or DMSO-d_6 to 10, 15, 20, 25, 30, 40 and 100 μL.

3.2.2 Low temperature NMR experiments

3.2.2.1 In the $CDCl_3$ solution

In a NMR tube, the compound 7 (0.01 g, 0.009 mmol) was dissolved in $CDCl_3$ (0.5 ml). CD_3OD (20 μL) was then added. The spectra were recorded at 27, 0, -15, -25, -35, and -40 °C.

3.2.2.2 In the mixture of $CDCl_3$ and CD_3OD solution

In a NMR tube, the compound 7 (0.01g, 0.009 mmol) was dissolved in $CDCl_3$ (0.5 ml). CD_3OD (20 μL) was then added. The spectra were recorded at 27, 0, -15, -25, -35 and -40 °C.

3.3 ^{1}H NMR studies of the compound 25,27-[*N,N'*-di-((2-ethoxy)benzyl) propylenediamine]–*p-tert*-butylcalix[4]arene dihydrochloride (9)

In a NMR tube, the compound 9 (0.02 g, 0.019 mmol) was dissolved in $CDCl_3$ (0.5 ml). 5 μL of CD_3OD was added into the tube. The spectrum was then recorded. A series of experiment was carried out by increasing the amount of CD_3OD to 10, 15, 20, 25, 30, 40, 50, 60, 80, 100 and 200 μL.

3.4 Basicity of 25,27-[*N,N'*-di-((2-ethoxy)benzyl)propylenediamine]-26,28-dimethoxy-*p-tert*-butylcalix[4]arene (L) and complexation of ligand L with transition metals

3.4.1 Chemicals

1. Tetrabutylammonium trifluoromethanesulfonate	Electrochemical grade, Fluka, Switzerland
2. Tetrabutylammoniumhydroxide 1.0 M solution in methanol	Analar grade, Aldrich, U.S.A.
3. Copper (II) trifluoromethanesulfonate	Analar grade, Aldrich, U.S.A.
4. Zinc (II) trifluoromethanesulfonate	Analar grade, Aldrich, U.S.A.
5. Potassium hydrogen phthalate	Analar grade, Carlo Erba, Italy
6. Perchloric acid 70-72 %	Analar grade, Merck, Germany
7. Methanol	Actual Analysis grade, J.T.Baker, U.S.A.
8. 25,27-[*N,N'*-di-((2-ethoxy)benzyl) propylenediamine]-26,28-dimethoxy -*p-tert*-butylcalix[4]arene dihydrochloride (7, **L.2HCl**)	Obtained from synthesis
9. Argon gas	Ultra high pure grade

3.4.2 Instruments

1. Automatic titrator, Mettler, Model DL 25, Switzerland
2. Combined pH electrode, Mettler, Model DG113-SC, Switzerland
3. Thermostat, Heto, Model DT-2, Denmark
4. Microcomputer, Model 486/DX4

3.5 Preparation of solutions

3.5.1 Potentiometric method

3.5.1.1 Primary standard solution of potassium hydrogen phthalate (KHP) was prepared by dissolving a weighted quantity of KHP in double distilled water.

3.5.1.2 Stock solution of 1 M perchloric acid ($HClO_4$) in methanol was prepared by diluting the concentrated $HClO_4$.

3.5.1.3 Stock solution of $1x10^{-1}$ M perchloric acid ($HClO_4$) in methanol was prepared by diluting the stock solution of 1 M $HClO_4$ with methanol.

3.5.1.4 Solution of $5x10^{-2}$ M $HClO_4$ in $1x10^{-2}$ M tetrabutylammonium trifluoromethanesulfonate ($Bu_4NCF_3SO_3$) was prepared by dilution of the stock solution of $1x10^{-1}$ M $HClO_4$ in methanol; a certain amount of dried $Bu_4NCF_3SO_3$ was added before diluting by methanol.

3.5.1.5 pH standard solution of pH 2 and 3 were prepared by dilution of the stock solution of $1x10^{-1}$ M $HClO_4$ in methanol; a weighted quantity of dried $Bu_4NCF_3SO_3$ was included to make $1x10^{-2}$ M $Bu_4NCF_3SO_3$.

3.5.1.6 $1x10^{-3}$ M 25,27-[*N,N'*-di-((2-ethoxy)benzyl)propylenediamine]-26,28-dimethoxy-*p-tert*-butylcalix[4]arene dihydrochloride (**7, L.2HCl**), $5x10^{-2}$ M Cu $(CF_3SO_3)_2$ and $5x10^{-2}$ M $Zn(CF_3SO_3)_2$ in methanolic solution of $1x10^{-2}$ M $Bu_4NCF_3SO_3$ were obtained by dissolving $Bu_4NCF_3SO_3$ and the mentioned substances and diluted with methanol.

3.5.1.7 Electrolyte solution of $1x10^{-2}$ M $Bu_4NCF_3SO_3$ in methanol was prepared by dissolving an exact quantity of $Bu_4NCF_3SO_3$ in methanol.

3.5.1.8 Solution of $5x10^{-2}$ M Bu_4NOH in methanolic solution of $1x10^{-2}$M $Bu_4NCF_3SO_3$ was prepared by dilution of the 1.0 M Bu_4NOH in methanol; a certain amount of dried $Bu_4NCF_3SO_3$ was added before diluting by methanol.

3.6 The Calibration of Electrode

An automatic titrator including combined pH electrode was used in the titration. The pH electrode was calibrated by two different standard pH. The pH of the standard solution of pH 2 was assigned to be 2.00 ± 0.005 pH units by adjusting the Nernstian slope as defined the ratio of pH to millivolt at isopotential point of pH 8.30 = 0.0 millivolt. The pH of solution can be corrected by the following equation (3.1).

$$pH_{corrected} = pH_{measured} + a + b[H^+]_{measured} \qquad (3.1)$$

where a and b are constants. The standard pH 3 was currently measured by the same electrodes and was used in the calculation for a and b constants.

3.7 Calculations

The titration data for determination of basicity constants of 25,27-[*N,N'*-di-((2-ethoxy)benzyl)propylenediamine-26,28-dimethoxy-*p-tert*-butylcalix[4]arene (**L**) and stability constants of complex between ligand **L** with transition metal ions were evaluated by the refinement program. The calculations were performed on the microcomputer. The titration data obtained from the measurements were used in the evaluation and the optimization process by the SUPERQUAD program [**65**].

3.8 Potentiometric Titration

Experiments for determination of basicity constants of 25,27-[*N,N'*-di((2-ethoxy)benzyl)propylenediamine]-26,28-dimethoxy-*p-tert*-butylcalix[4]arene (**L**) by means of potentiometric titrations were carried out at various constant temperature, namely 20, 23, 25, 27 and 30 °C ± 0.1 °C. The stability constants of its complex with transition metal ions were carried out at 25 °C. Argon gas saturated by the electrolyte solution of $1x10^{-2}$ M $Bu_4NCF_3SO_3$ in methanol was flowed through the titration chamber. The temperature of the titration chamber was kept by the external

circulation of thermostat bath. The solution of $5x10^{-2}$ M $HClO_4$ in $1x10^{-2}$ M $Bu_4NCF_3SO_3$ used for adjustment the pH of titration system was standardized with Bu_4NOH titrant. At least three titrations of each experiment were employed in the computer refinement, except the experiment of complexation studies of **L** with Zn^{2+}, only 2 titrations were used. The pH range and initial concentration of the ligand **L** at 20, 23, 25, 27 and 30 °C are shown in Tables 3.1, 3.2, 3.3, 3.4 and 3.5, respectively. The pH range and initial concentration of the ligand **L** with Cu^{2+} and with Zn^{2+} are shown in Tables 3.6 and 3.7, respectively.

Table 3.1 Experimental data used in computer simulations for determining the protonation constants of **L** in the methanolic solution of $1.0x10^{-2}$ M $Bu_4NCF_3SO_3$ at 20 ± 0.1 °C.

Titration	Initial concentration (mM)		pH range	Data points
	L	proton		
1	0.500	5.682	2.69-13.02	58
2	0.914	6.084	2.52-12.89	63
3	0.603	1.206	5.62-13.14	39

Table 3.2 Experimental data used in computer refinement for determining the protonation constants of **L** in the methanolic solution of $1.0x10^{-2}$ M $Bu_4NCF_3SO_3$ at 23 ± 0.1 °C.

Titration	Initial concentration (mM)		pH range	Data points
	L	proton		
1	0.456	4.892	2.58-12.90	54
2	0.460	4.568	2.58-12.78	54
3	0.301	0.602	5.88-12.97	30

Table 3.3 Experimental data used in computer refinement for determining the protonation constants of **L** in the methanolic solution of $1.0x10^{-2}$ M $Bu_4NCF_3SO_3$ at 25 ± 0.1 °C.

Titration	Initial concentration (mM)		pH range	Data points
	L	proton		
1	0.455	0.909	5.37-12.51	34
2	0.909	6.165	2.44-12.33	64
3	0.542	5.621	2.43-12.27	51

Table 3.4 Experimental data used in computer refinement for determining the protonation constants of **L** in the methanolic solution of $1.0x10^{-2}$ M $Bu_4NCF_3SO_3$ at 27 ± 0.1 °C.

Titration	Initial concentration (mM)		pH range	Data points
	L	proton		
1	0.459	4.845	2.51-12.85	52
2	0.463	4.526	2.44-12.75	57
3	0.303	0.606	5.45-12.97	31

Table 3.5 Experimental data used in computer refinement for determining the protonation constants of **L** in the methanolic solution of $1.0x10^{-2}$ M $Bu_4NCF_3SO_3$ at 30 ± 0.1 °C.

Titration	Initial concentration (mM)		pH range	Data points
	L	proton		
1	0.297	4.372	2.56-13.01	57
2	0.459	4.695	2.56-12.73	52
3	0.505	1.011	4.82-13.05	45

Table 3.6 Experimental data used in computer refinement for determining the stability constants of **L** with Cu^{2+} in the methanolic solution of 1.0 $x10^{-2}$ M $Bu_4NCF_3SO_3$ at 25 ± 0.1 °C.

Titration	Initial concentration (mM)			pH range	Data points
	L	proton	Cu^{2+}		
1	0.776	5.231	0.780	2.53-11.00	60
2	0.841	5.667	0.423	2.39-11.02	58
3	0.788	4.674	0.396	2.64-11.15	61

Table 3.7 Experimental data used in computer refinement for determining the stability constants of **L** with Zn^{2+} in the methanolic solution of $1.0x10^{-2}$ M $Bu_4NCF_3SO_3$ at 25 ± 0.1 °C.

Titration	Initial concentration (mM)			pH range	Data points
	L	proton	Zn^{2-}		
1	0.833	6.264	0.860	2.40-12.39	72
2	0.874	5.772	0.449	2.54-12.46	69