CHAPTER II

EXPERIMENTAL SECTION

2.1 General Procedures

2.1.1 Analytical instrument

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury Plus 400 nuclear magnetic resonance spectrometers. In all cases, samples were dissolved in deuterated chloroform. The chemical shifts were recorded in part per million (ppm) using a residue proton as internal reference. Elemental analysis was carried out on CHNS/O analyzer (Perkin Elmers PE 2400 series II). ESI mass spectra were recorded on a Micromass Platform II. Absorption spectra were measured by a Varian Cary 50 UV-Vis spectrophotometer equipped with a water-jacketed cell holder with temperature controlled at 25.0 ± 0.1°C. Electromotive force of the ion-selective electrode cell was measured on ORION 520A benchtop pH/ISE meter vs. double-junction reference Ag-AgCl electrode (Metrohm.6.0726.100) with 1 M KNO_3 salt-bridge.

2.1.2 Materials

Unless otherwise specified, the solvent and all materials were reagent grades purchased from Fluka, BDH, Aldrich, Carlo Erba, Merck or Lab scan and were used without further purification. Commercial grade solvents such as acetone, dichloromethane, hexane, methanol, toluene and ethyl acetate were purified by distillation before used. Acetonitrile and dichloromethane for set up the reaction were dried over calcium hydride and freshly distilled under nitrogen atmosphere prior to use. Tetrahydrofuran was freshly distilled from sodium/benzophenone.

Column chromatography was carried out on silica gel (Kieselgel 60, 0.063-0.200 mm, Merck). Thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60, F_{254}, 1 mm, Merck). Compounds on TLC plates were detected by the UV-light. Methanol for UV measurement (AR grade, Merck) was used as

received. Acetonitrile for UV measurement was dried over calcium hydride and freshly distilled under nitrogen atmosphere prior to use.

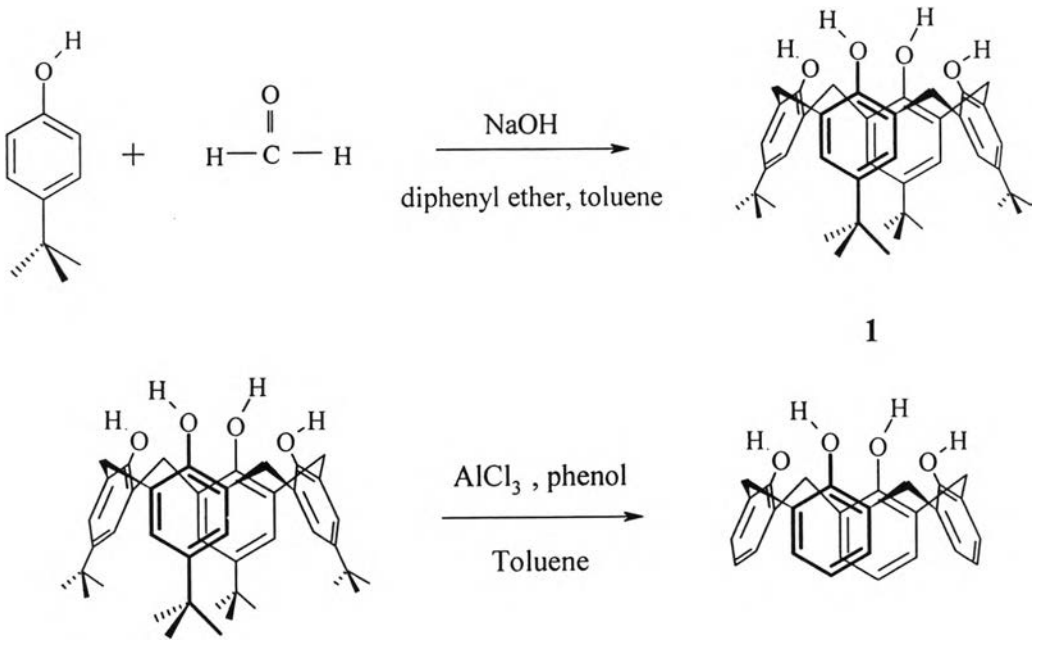
Materials for ISE-membrane (PVC, *o*-NPOE, KTClPB) were Selectophore grade from Fluka.

The synthesized compounds were characterized by ^{1}H-NMR spectroscopy, mass spectrometry and elemental analysis.

Water used in ISE experiments was ultrapure water from Milli-Q (Bedford, MA, USA) water purification system (Millipore).

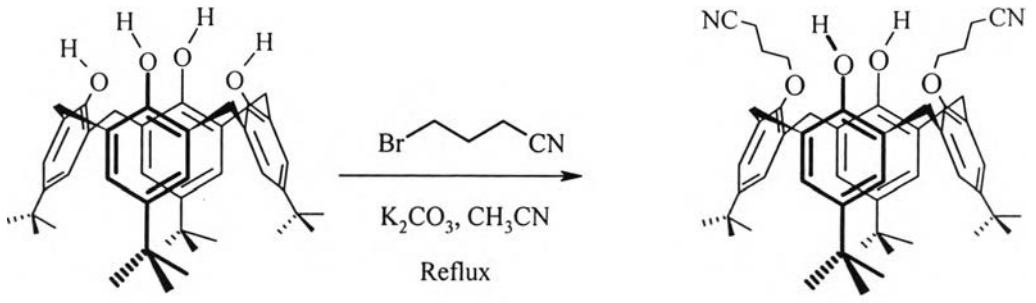
2.2 Synthesis

The preparation of *p-tert*-butylcalix[4]arene (**1**) and calixarene (**2**) were carried out as in literature procedure [61-62]. The reaction is outlined below.



1 **1** **2**

2.2.1 Preparation of 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(cyanopropoxy)calix[4]arene (3)



1 **3**

In a 250-mL two-necked round bottom flask equipped with a magnetic bar, *p*-*tert*-butylcalix[4]arene **1** (5.000 g, 7.70 mmol), potassium carbonate (1.330 g, 9.62 mmol) and acetonitrile (120 mL) were stirred under nitrogen atmosphere at room temperature for 1 h. Bromobutyronitrile (2.40 g, 16.2 mmol) was then added and the mixture was heated at reflux for 5 days. The mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (300 mL) and acidified with 1 M hydrochloric acid. The organic phase was dried over anhydrous sodium sulfate and concentrated. The concentrated solution was treated with methanol to yield 5,11,17,23-tetra-*p*-*tert*-butyl -25,27-bis(cyanopropoxy)calix[4]arene **3** (4.121 g, 68.3 %).

Characterization data for 3

^{1}*H-NMR spectrum* (400 MHz, $CDCl_3$) : δ (in ppm)

7.42 (s, 2H, ArO*H*)

7.06 (s, 4H, *m*-*H*ArOH)

6.86 (s, 4H, *m*- *H*ArOCH_2)

4.17 (d, *J* = 12.8 Hz, $4H_A$, ArCH_2Ar)

4.09 (t, *J* = 5.6 Hz, 4H, CH_2OAr)

3.37 (d, *J* = 12.8 Hz, $4H_B$, ArCH_2Ar)

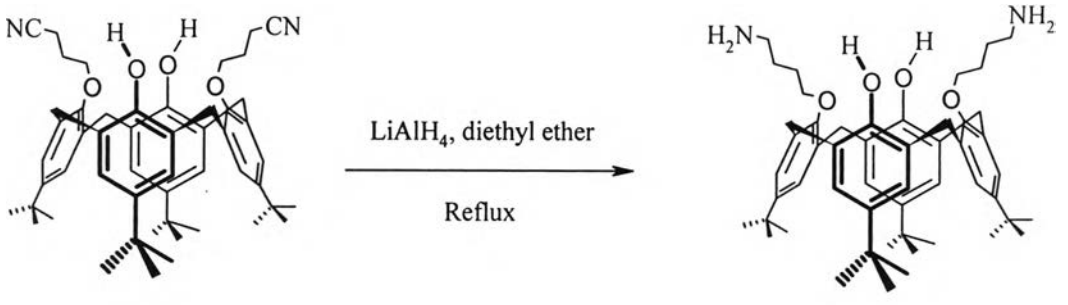
3.05 (t, *J* = 7.2 Hz, 4H, CH_2CN)

2.37 (qnt, *J* = 6.1 Hz, 4H, $CH_2CH_2CH_2$CN)

1.27 (s, 18H, *t*-C_4H_9)

1.00 (s, 18H, *t*-C_4H_9)

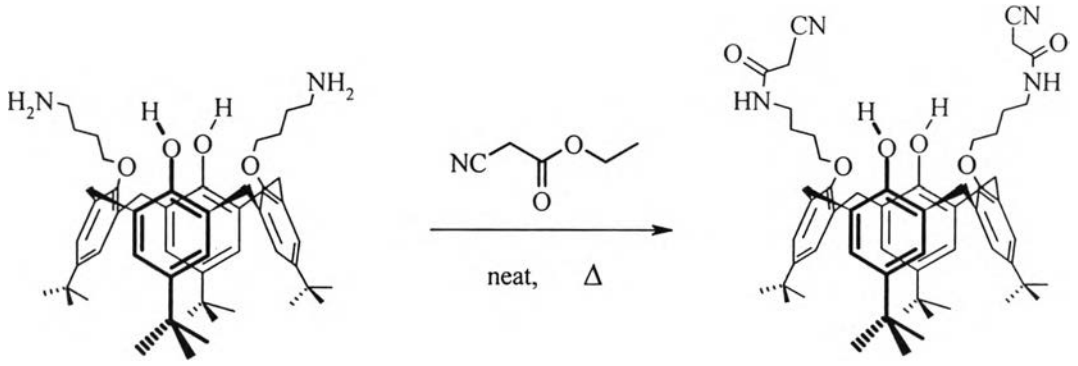
2.2.2 Preparation of 5,11,17,23-tetra-*p-tert*-butyl-25,27-bis(aminobutoxy)calix[4]arene (4)



3 4

In a 100-mL two-necked round bottom flask equipped with a magnetic bar, $LiAlH_4$ (0.233 g, 6.13 mmol) and diethyl ether (20 mL) were stirred under nitrogen atmosphere. The suspension was heated to about 40 °C in an oil bath. A suspension of 5,11,17,23-tetra-*p-tert*-butyl-25,27-bis(cyanopropoxy)calix[4]arene **3** (0.500 g, 0.638 mmol) in diethyl ether (50 mL) was then added dropwise. The mixture was refluxed for 5 h. The solvent was the evaporated and the residue was solubilized in CH_2Cl_2. A 20% (w/v) NaOH solution was then added dropwise until the grey suspension turned white and the organic phase was dried over anhydrous Na_2SO_4. The suspension was filtered and the filtrate was evaporated to dryness to yield 5,11,17,23-tetra-*p-tert*-butyl 25,27-bis(aminobutoxy)calix[4]arene **4** as a white solid (0.335g, 66.4%). The compound was used immediately for further reactions.

2.2.3 Preparation of 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(cyanoacetamidobutoxy)calix[4]arene (5)



4 **5**

In a 100 mL one-necked round bottom flask equipped with a magnetic bar, 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(aminobutoxy)calix[4]arene **4** (0.335 g, 0.423 mmol), ethyl cyanoacetate (0.335 g, 2.96 mmol) were stirred under nitrogen atmosphere and heated gradually to 80 °C for 5 h. The mixture was cooled to room temperature and the residue was solubilized with 10 mL CH_2Cl_2. The solution was chromatographed on a silica column with gradient elution (CH_2Cl_2-ethyl acetate) to give **5** as a white solid after evaporation (0.195 g, 49.9%).

Characterization data for 5

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

7.53 (s, 2H, N*H*)

7.11 (s, 4H, *m*-*H*ArOH)

6.70 (s, 4H, *m*-*H*ArOCH$_2$)

4.19 (d, *J* = 13.6 Hz, 4H$_A$, ArC*H*$_2$Ar)

4.01 (t, *J* = 6.0 Hz, 4H, C*H*$_2$OAr)

3.64 (q, *J* = 6.1 Hz, 4H, C*H*$_2$NH)

3.50 (s, 4H, C*H*$_2$CN)

3.39 (d, *J* = 13.2 Hz, 4H$_B$, ArC*H*$_2$Ar)

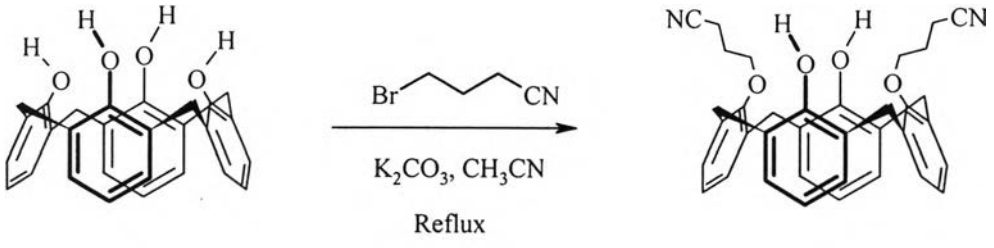
1.96 (qnt, *J* = 6.5 Hz, 4H, C*H*$_2$CH$_2$OAr)

1.88 (qnt, *J* = 6.5 Hz, 4H, C*H*$_2$CH$_2$CH$_2$OAr)

1.32 (s, 18H, *t*-C$_4$*H*$_9$)

0.87 (s, 18H, *t*-C$_4$*H*$_9$)

2.2.4 Preparation of 25,27-bis(cyanopropoxy)-26,28-dihydroxycalix[4]arene (6)



2 **6**

In a 250 mL two-necked round bottom flask equipped with a magnetic bar, 25,26,27,28-tetrahydroxycalix[4]arene **2** (5.000 g, 11.8 mmol), potassium carbonate (2.033 g, 14.7 mmol) and acetonitrile (120 mL) were stirred under nitrogen atmosphere at room temperature for 1 h. Bromobutyronitrile (3.67 g, 24.8 mmol) was then added and the mixture was heated at reflux for 5 days. The mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (300 mL) and acidified with 1 M hydrochloric acid. The organic phase was dried over anhydrous sodium sulfate and concentrated. The concentrated solution was treated with methanol to yield 25,27-bis(cyanopropoxy)-26,28-hydroxycalix[4]arene **6** as a white solid (4.870 g, 74.0 %).

Characterization data for 6

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

7.81 (s, 2H, ArO*H*)

7.08 (d, *J* = 7.2 Hz, 4H, *m*-*H*ArOH)

6.93 (d, *J* = 7.6 Hz, 4H, *m*-*H*ArOCH_2)

6.79 (t, *J* = 7.6 Hz, 2H, *p*-*H*ArOH)

6.69 (t, *J* = 7.4 Hz, 2H, *p*-*H*ArOCH_2)

4.19 (d, *J* = 13.2 Hz, $4H_A$, ArCH_2Ar)

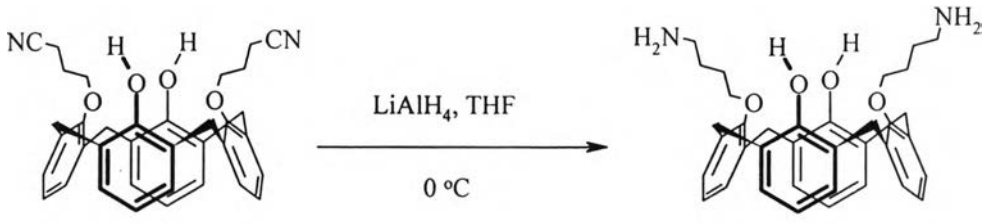
4.13 (t, *J* = 5.6 Hz, 4H, CH_2OAr)

3.45 (d, *J* = 13.2 Hz, $4H_B$, ArCH_2Ar)

3.08 (t, *J* = 7.2 Hz, 4H, CH_2CN)

2.39 (qnt, *J* = 6.3 Hz, 4H, CH_2CH_2OAr)

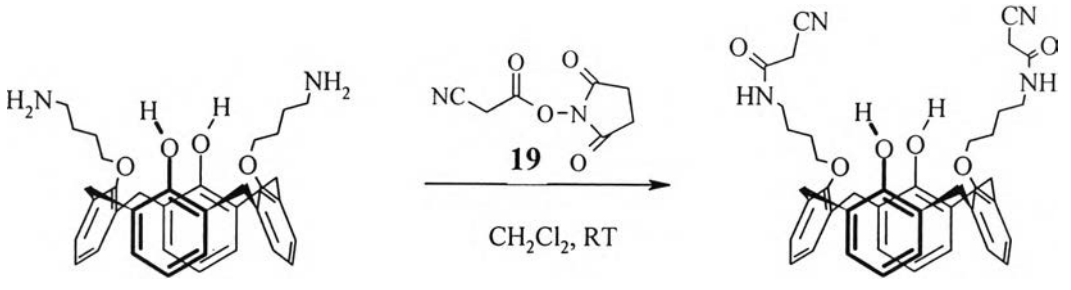
2.2.5 Preparation of 25,27-bis(aminobutoxy)-26,28-dihydroxy-calix[4]arene (7)



6 7

In a 250 mL two-necked round bottom flask equipped with a magnetic bar, $LiAlH_4$ (0.345 g, 9.09 mmol) and THF (20 mL) were stirred under nitrogen atmosphere. The flask was cooled to 0 °C in an ice bath. A solution of 25,27-bis(aminopropoxy)-26,28-dihydroxycalix[4]arene **6** (0.500 g, 0.89 mmol) in 50 mL THF was added dropwise for about 0.5 h. After 4 h, 50 mL THF was added followed by a 20% (w/v) NaOH solution dropwise until the grey suspension turned white. The white precipitated was filtered. The filtrate was dried over anhydrous Na_2SO_4 and evaporated to give a white solid (0.333 g, 65.6%). The compound was used immediately for further reaction.

2.2.6 Preparation of 25,27-bis(cyanoacetamidobutoxy)-26,28-dihydroxycalix[4]arene (8)



7 **8**

In a 250 mL two-necked round bottom flask equipped with a magnetic bar, cyanoacetic acid active ester **19** (0.225 g, 1.234 mmol) was dissolved in CH_2Cl_2 (100 mL) and stirred under nitrogen atmosphere at room temperature. A solution of 25,27-bis(aminobutoxy)-26,28-dihydroxycalix[4]arene **7** (0.333 g, 0.588 mmol) and *N*-ethyl-di-*iso*propylamine (0.161 g, 1.234 mmol) in CH_2Cl_2 (50 mL) was added dropwise. The mixture was left stirring for 48 h at room temperature. The solution was extracted once with 1 M HCl (50 mL), twice with water (50 mL). After drying over anhydrous Na_2SO_4, the organic part was evaporated to dryness under reduced pressure and the product was chromatographed on silica column with gradient elution (CH_2Cl_2-ethyl acetate) to give **8** as a white solid after evaporation (0.080 g, 19.4%).

Characterization data for 8

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

7.48 (s, 2H, ArO*H*)

7.32 (s, 2H, N*H*)

7.11 (d, *J* = 7.6 Hz, 4H, *m*-*H*ArOH)

6.85 (d, *J* = 7.6 Hz, 4H, *m*-*H*ArOCH$_2$)

6.76-6.70 (m, 4H, *p*-*H*ArOH and *p*-*H*ArOCH$_2$)

4.23 (d, *J* = 13.6 Hz, 4H$_A$, ArC*H*$_2$Ar)

4.02 (t, *J* = 6.0 Hz, 4H, C*H*$_2$OAr)

3.59 (q, *J* = 6.2 Hz, 4H, C*H*$_2$NH)

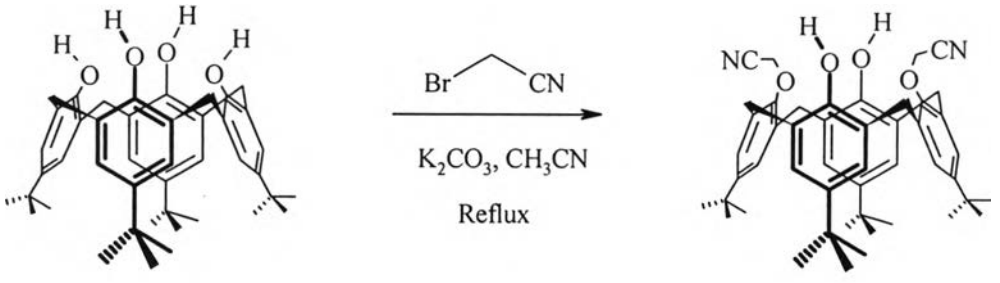
3.45 (s, 4H, C*H*$_2$CN)

3.43 (d, *J* = 11.2 Hz, 4H$_B$, ArC*H*$_2$Ar)

2.05 (qnt, *J* = 6.5 Hz, C*H*$_2$CH$_2$OAr)

1.94 (qnt, *J* = 6.8 Hz, 4H, C*H*$_2$CH$_2$NH).

2.2.7 Preparation of 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(cyanomethoxy)calix[4]arene (9)



1 **9**

In a 250-mL two-necked round bottom flask equipped with a magnetic bar, *p*-*tert*-butylcalix[4]arene **1** (5.000 g, 7.70 mmol), potassium carbonate (3.000 g, 21.7 mmol) and acetonitrile (120 mL) were stirred under nitrogen atmosphere at room temperature for 1 h. Bromoacetonitrile (4.000 g, 33.6 mmol) was then added and the mixture was heated at reflux for 7 h. The mixture was cooled to room temperature and filtered. The filtrate was evaporated to near dryness and treated with methanol to yield 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(cyanomethoxy)calix[4]arene **9** (3.103 g, 55.4%).

Characterization data for 9

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

7.12 (s, 4H, *m*-*H*ArOH)

6.72 (s, 4H, *m*-*H*ArOCH_2)

5.56 (s, 2H, ArO*H*)

4.81 (s, 4H, CH_2CN)

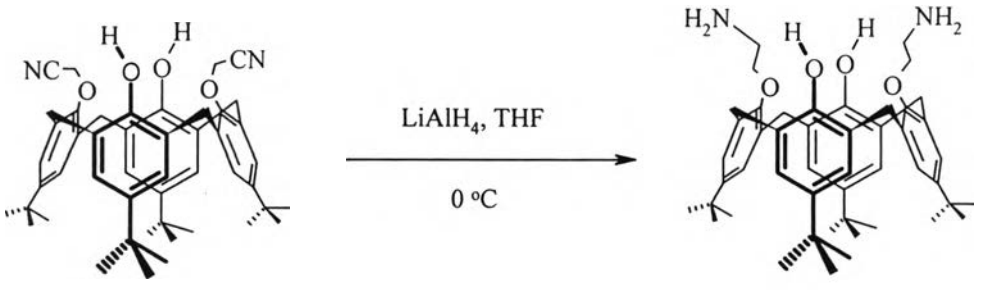
4.22 (d, J = 13.6 Hz, 4H_A, ArCH_2Ar)

3.45 (d, J = 13.2 Hz, 4H_B, ArCH_2Ar)

1.32 (s, 18H, *t*-C_4H_9)

0.88 (s, 18H, *t*-C_4H_9)

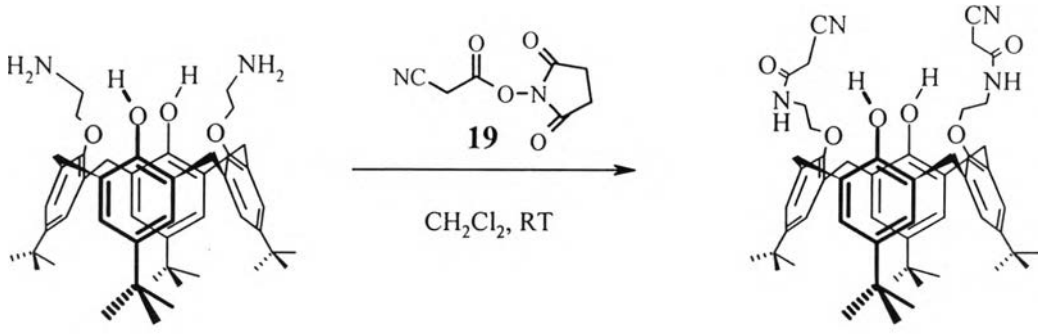
2.2.8 Preparation of 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(aminoethoxy)calix[4]arene (10)



9 **10**

In a 250-mL two-necked round bottom flask equipped with a magnetic bar, $LiAlH_4$ (0.104 g, 2.75 mmol) and THF (20 mL) were stirred under nitrogen atmosphere. The flask was cooled to 0 °C in an ice bath. A solution of 5,11,17,23 tetra-*p*-*tert*-butyl 25,27-bis(cyanomethoxy)calix[4]arene **9** (0.500 g, 0.688 mmol) in THF (50 mL) was added dropwise. After 4 h, 50 mL THF was added followed by a 20% (w/v) NaOH solution dropwise until the grey suspension turned white. The precipitated was filtered. The filtrate was dried over anhydrous Na_2SO_4 and evaporated to give a white solid (0.342 g, 67.7%). The compound was used immediately for further reactions.

2.2.9 Preparation of 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(acetamidoethoxy)calix[4]arene (11)



10 **11**

In a 250-mL two-necked round bottom flask equipped with a magnetic bar, cyanoacetic acid acive ester **19** (0.178 g, 0.977 mmol) was dissolved in CH_2Cl_2 (100 mL) and stirred under nitrogen atmosphere at room temperature. A solution of 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(aminoethoxy)calix[4]arene **10** (0.342 g, 0.465 mmol) and *N*-ethyl-di*iso*propylamine (0.126 g, 0.977 mmol) in CH_2Cl_2 (50 mL) was added dropwise. The mixture was left stirring for 48 h at room temperature. The solution was extracted once with 1 M HCl (50 mL), twice with water (50 mL). After drying over anhydrous Na_2SO_4, the organic part was evaporated to dryness under reduced pressure. The product was taken up with CH_2Cl_2 and **11** was precipitated from isopropyl alcohol as a white solid after filtration (0.136 g, 33.6%).

Charaterization data for 11

^1H NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

8.63 (t, *J* = 5.6 Hz, 2H, N*H*)

8.28 (s, 2H, ArO*H*)

7.07 (s, 4H, *m*-*H*ArOH)

6.95 (s, 4H, *m*-*H*ArOCH$_2$)

4.21 (d, *J* = 13.2 Hz, 4H$_A$, ArC*H*$_2$Ar)

4.11 (t, *J* = 4.8 Hz, 4H, C*H*$_2$OAr)

3.99 (q, *J* = 4.4 Hz, 4H, C*H*$_2$NH)

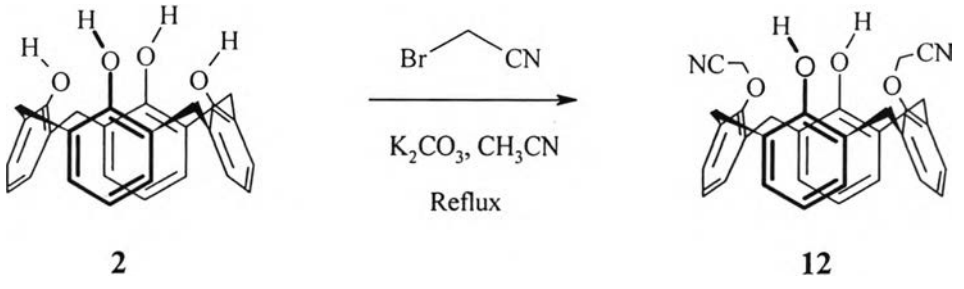
3.41 (d, *J* = 13.2 Hz, 4H$_B$, ArC*H*$_2$Ar)

3.41 (s, 4H, C*H*$_2$CN)

1.26 (s, 18H, *t*-C$_4$*H*$_9$)

1.07 (s, 18H, *t*-C$_4$*H*$_9$)

2.2.10 Preparation of 25,27-bis(cyanomethoxy)-26,28-dihydroxycalix[4]arene (12)



2 **12**

In a 250-mL two-necked round bottom flask equipped with a magnetic bar, 25,26,27,28-tetrahydroxycalix[4]arene **2** (5.000 g, 11.8 mmol), potassium carbonate (4.586 g, 33.2 mmol) and acetonitrile (120 mL) were stirred under nitrogen atmosphere at room temperature for 1 h. Bromoacetonitrile (6.115 g, 51.3 mmol) was then added and the mixture was heated at reflux for 7 h. The mixture was cooled to room temperature and filtered. The filtrate was evaporated to near dryness and treated with methanol to yield 25,27-bis(cyanomethoxy)-26,28-dihydroxycalix[4]arene **12** (3.564 g, 60.2 %).

Characterization data for 12

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

7.13 (d, J = 7.2 Hz, 4H, *m-H*ArOH)

6.83 (d, J = 7.6 Hz, 4H, *m-H*ArOCH$_2$)

6.79-6.73 (m, 4H, *p-H*ArOH and *p-H*ArOCH$_2$)

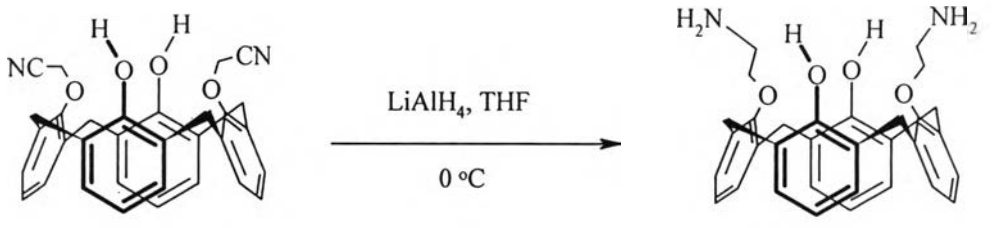
6.02 (s, 2H, ArO*H*)

4.85 (s, 4H, C*H*$_2$CN)

4.26 (d, J = 13.6 Hz, 4H$_A$, ArC*H*$_2$Ar)

3.52 (d, J = 13.6 Hz, 4H$_B$, ArC*H*$_2$Ar)

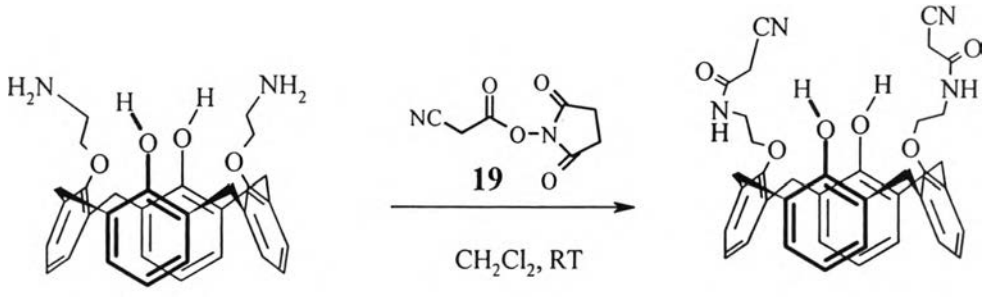
2.2.11 Preparation of 25,27-bis(aminoethoxy)-26,28-dihydroxycalix[4]arene (13)



12 **13**

In a 250-mL two-necked round bottom flask equipped with a magnetic bar, $LiAlH_4$ (0.203 g, 5.35 mmol) and THF (20 mL) were stirred under nitrogen atmosphere. The flask was cooled to 0 °C in an ice bath. A solution of 25,27-bis(cyanomethoxy)-26,28-dihydroxycalix[4]arene **12** (0.500 g, 0.995 mmol) in THF (50 mL) was added dropwise. After 4 h, 50 mL THF was added followed by a 20% (w/v) NaOH solution dropwise until the grey suspension turned white. The precipitated was filtered. The filtrate was dried over anhydrous Na_2SO_4 and evaporated to give a white solid (0.204 g, 40.2%). The compound was used immediately for further reaction.

2.2.12 Preparation of 25,27-bis(cyanoacetamidoethoxy)-26,28-dihydroxycalixarene (14)



13 **14**

In a 250 mL two-necked round bottom flask equipped with a magnetic bar, cyanoacetic acid acive ester **19** (0.153 g, 0.839 mmol) was dissolved in CH_2Cl_2 (100 mL) and stirred under nitrogen atmosphere at room temperature. A solution of 25,27-bis(aminoethoxy)-26,28-dihydroxycalix[4]arene **13** (0.204 g, 0.399 mmol) and *N*-ethyl-di-*iso*propylamine (0.108 g, 0.839 mmol) in CH_2Cl_2 (50 mL) was added dropwise. The mixture was left stirring for 48 h at room temperature. The solution was extracted once with 1 M HCl (50 mL), twice with water (50 mL). After drying over anhydrous Na_2SO_4, the organic part was evaporated to dryness under reduce pressure. The product was taken up with CH_2Cl_2 and **14** was precipitated from CH_2Cl_2-methanol as a white solid (0.103 g, 39.8%).

Characterization data for 14

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

8.39 (s, 2H, ArO*H*)

7.10 (d, *J* = 7.2 Hz, 4H, *m*-*H*ArOH)

6.98 (d, *J* = 7.6 Hz, 4H, *m*-*H*ArOCH$_2$)

6.83 (t, *J* = 7.6 Hz, 2H, *p*-*H*ArOH)

6.73 (t, *J* = 7.6 Hz, 2H, *p*-*H*ArOCH$_2$)

4.26 (d, *J* = 13.2 Hz, 4H$_A$, ArC*H*$_2$Ar)

4.14 (t, *J* = 4.8 Hz, 4H, C*H*$_2$OAr)

4.06 (q, *J* = 4.8 Hz, 4H, C*H*$_2$NH)

3.46 (d, *J* = 13.2 Hz, 4H$_B$, ArC*H*$_2$Ar)

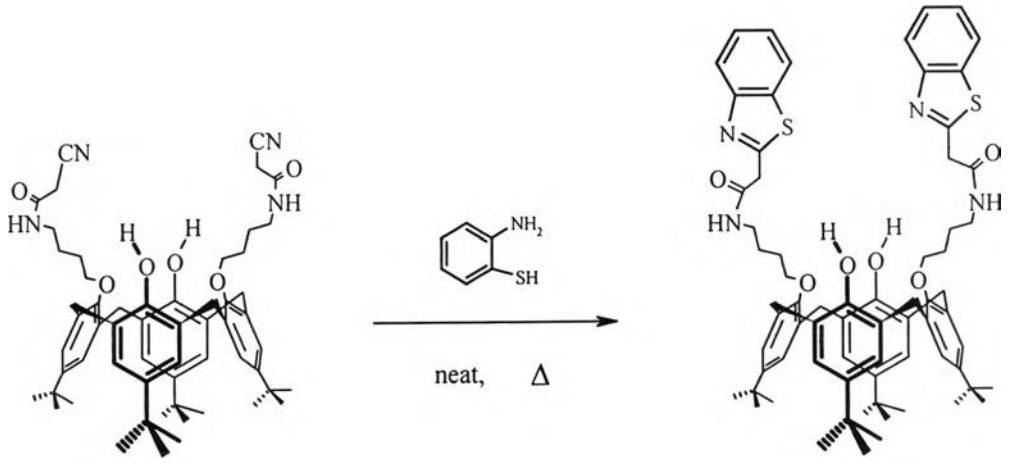
3.40 (s, 4H, C*H*$_2$CN)

2.2.13 General procedure for preparation of benzothiazolylacetamido-alkoxycalix[4]arene (15-18)

In a 100 mL one-necked round bottom flask equipped with a magnetic bar , a cyanoacetamido compound (0.200 g) was mixed with 2-aminothiophenol at 1:10 mol ratio and heated under N_2 atmosphere at 120 °C for 3 h. After cooling, the product was taken up with CH_2Cl_2 and loaded on silica column. The column was first washed with CH_2Cl_2 to remove excess 2-aminothiophenol after then gradient eluted with CH_2Cl_2-ethyl acetate. The fraction containing the benzothiazolylacetamido compound was evaporated to dryness under reduced pressure to give a solid.

2.2.13.1 5,11,17,23-Tetra-*p*-*tert*-butyl-25,27-bis(benzothiazolylacetamidobutoxy)calix[4]arene (15)

0.146 g (59.2%)



5 **15**

Characterization data for 15

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

7.93 (d, 2H, *J* = 7.6 Hz, BTAr*H*)

7.84 (br, 2H, N*H*)

7.77 (d, *J* = 7.6 Hz, 2H, BTAr*H*),

7.40 (t, *J* = 7.2 Hz, 2H, BTAr*H*)

7.39 (t, *J* = 8.0 Hz, 2H, BTAr*H*)

7.38 (s, 2H, ArO*H*)

7.04 (s, 4H, *m*-*H*ArOH)

6.76 (s, 4H, *m*-*H*ArOCH$_2$)

4.19 (d, *J* = 12.8 Hz, 4H$_A$, ArC*H*$_2$Ar)

4.14 (s, 4H, C*H*$_2$BT)

3.94 (t, 4H, *J* = 6.0 Hz, C*H*$_2$OAr)

3.50 (q, *J* = 6.4 Hz, 4H, C*H*$_2$NH)

3.28 (d, *J* = 13.2 Hz, 4H$_B$, ArC*H*$_2$Ar)

1.97 (q, *J* = 7.2 Hz, 4H, C*H*$_2$CH$_2$OAr)

1.88 (q, *J* = 7.2 Hz, 4H, C*H*$_2$CH$_2$NH)

1.29 (s, 18H, *t*-C$_4$*H*$_9$)

0.93 (s, 18H, *t*-C$_4$*H*$_9$)

ESI-MS for $C_{70}H_{84}N_4O_6S_2 + Cl^-$

Anal calc: m/z = 1177.1

Found: m/z = 1175.3

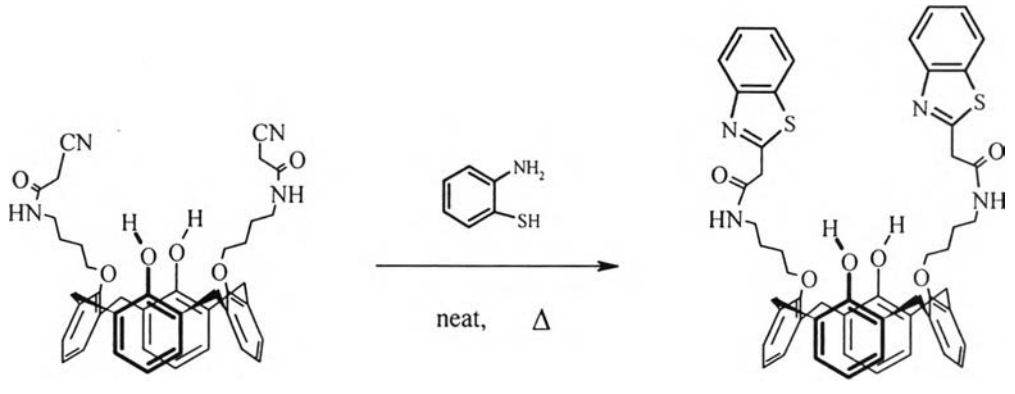
Elemental Analysis for $C_{70}H_{84}N_4O_6S_2$

Anal calc: C, 73.65; H, 7.42; N, 4.91

Found: C, 73.68; H, 7.45; N, 4.94

2.2.13.2 25,27-Bis(benzothiazolylacetamidobutoxy)-26,28-dihydroxycalix[4]arene (16)

0.167 g (63.7%)



8 **16**

Characterization data for 16

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

8.05 (br, 2H, N*H*)

8.00 (s, 2H, ArO*H*)

7.99 (d, *J* = 8.0 Hz, 2H, BTAr*H*)

7.82 (d, *J* = 7.2 Hz, 2H, BTAr*H*)

7.46 (t, *J* = 7.2 Hz, 2H, BTAr*H*)

7.42 (t, *J* = 8.0 Hz, 2H, BTAr*H*)

7.07 (d, *J* = 7.6 Hz, 4H, *m*-*H*ArOH)

6.91 (d, *J* = 8.0 Hz, 4H, *m*-*H*ArOCH$_2$)

6.75 (t, *J* = 7.2 Hz, 2H, *p*-*H*ArOH)

6.69 (t, *J* = 7.2 Hz, 2H, *p*-*H*ArOCH$_2$)

4.26 (s, 4H, C*H*$_2$BT)

4.25 (d, *J* = 13.6 Hz, 4H$_A$, ArC*H*$_2$Ar)

4.02 (t, *J* = 6.0 Hz, 4H, C*H*$_2$OAr)

3.54 (q, *J* = 6.4 Hz, 2H, C*H*$_2$NH)

3.38 (d, *J* = 13.2 Hz, 4H$_B$, ArC*H*$_2$Ar)

2.08-2.05 (m, 4H, C*H*$_2$CH$_2$OAr)

1.99 (q, *J* = 7.2 Hz, 4H, C*H*$_2$CH$_2$NH)

ESI-MS for $C_{54}H_{52}N_4O_6S_2$+Cl^-

Anal calc: m/z = 952.6

Found: m/z = 951.1

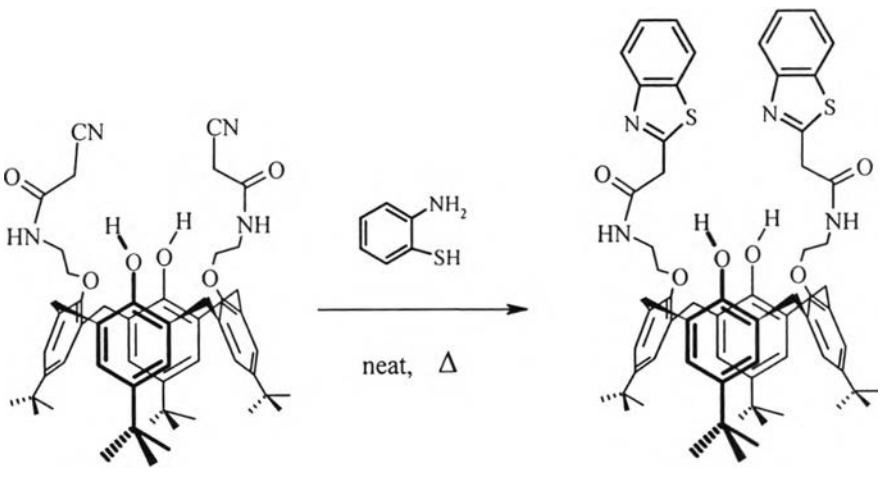
Elemental Analysis for $C_{54}H_{52}N_4O_6S_2$

Anal calc: C, 70.72; H, 5.71; N, 6.11

Found: C, 70.73; H, 5.69; N, 6.11

2.2.13.3 5,11,17,23-Tetra-*p*-*tert*-butyl-25,27-bis(benzothiazolylacetamidoethoxy)calix[4]arene (17)

0.160 g (64.2%)



11 **17**

Characterization data for 17

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

8.85 (br, 2H, N*H*)

8.37 (s, 2H, ArO*H*)

7.94 (d, *J* = 8.0 Hz, 2H, BTAr*H*)

7.80 (d, *J* = 7.6 Hz, 2H, BTAr*H*)

7.45 (t, *J* = 8.0 Hz, 2H, BTAr*H*)

7.37 (t, *J* = 8.0 Hz, 2H, BTAr*H*)

7.00 (s, 4H, *m*-*H*ArOH)

6.97 (s, 4H, *m*-*H*ArOCH$_2$)

4.24 (s, 4H, C*H*$_2$BT)

4.18 (d, *J* = 12.8 Hz, 4H$_A$, ArC*H*$_2$Ar)

4.08 (t, *J* = 4.8 Hz, C*H*$_2$OAr)

3.82 (q, *J* = 4.8 Hz, 4H, C*H*$_2$NH)

3.33 (d, *J* = 13.2 Hz, 4H$_B$, ArC*H*$_2$Ar)

1.28 (s, 18H, *t*-C$_4$*H*$_9$)

1.19 (s, 18H, *t*-C$_4$*H*$_9$)

ESI-MS for $C_{66}H_{76}N_4O_6S_2 + Cl^-$

Anal calc: m/z = 1120.94

Found: m/z 1120.5

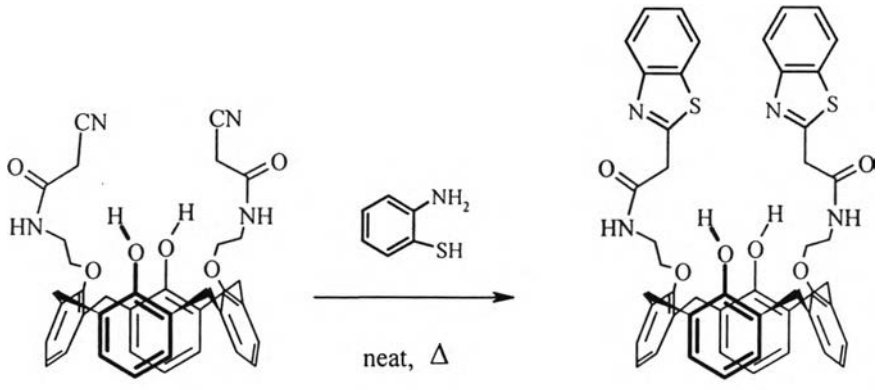
Elemental Analysis for $C_{66}H_{76}N_4O_6S_2$

Anal calc: C, 73.03; H, 7.06; N, 5.16

Found: C, 73.03; H, 7.08; N, 5.12

2.2.13.4 25,27-Bis(benzothiazolylacetamidoethoxy)-26,28-dihydroxycalix[4]arene (18)

0.054 g (20.1%)



14 **18**

Characterization data for 18

^{1}H-NMR spectrum (400 MHz, $CDCl_3$) : δ (in ppm)

8.70 (br, 2H, N*H*)

8.13 (s, 2H, ArO*H*)

7.95 (d, *J* = 8.0 Hz, 2H, BTAr*H*)

7.78 (d, *J* = 7.6 Hz, 2H, BTAr*H*)

7.47 (t, *J* = 7.2 Hz, 2H, BTAr*H*)

7.38 (t, *J* = 7.6 Hz, 2H, BTAr*H*)

6.95 (d, *J* = 7.2 Hz, 4H, *m*-*H*ArOH)

6.91 (d, *J* = 7.6 Hz, 4H, *m*-*H*ArOCH$_2$)

6.78 (t, *J* = 7.6 Hz, 2H, p-*H*ArOH)

6.61 (t, *J* = 7.6 Hz, 2H, p-*H*ArOCH$_2$)

4.27 (s, 4H, COC*H*$_2$)

4.17 (d, *J* = 14.0 Hz, 4H$_A$, ArC*H*$_2$Ar)

4.14 (t, *J* = 8.0 Hz, 4H, C*H*$_2$OAr)

3.89 (q, *J* = 4.4 Hz, C*H*$_2$NH)

3.32 (d, *J* = 13.3 Hz, 4H$_B$, ArC*H*$_2$Ar)

ESI-MS for m/z $C_{50}H_{44}N_4O_6S_2$+ Cl^-

Anal calc: 896.5

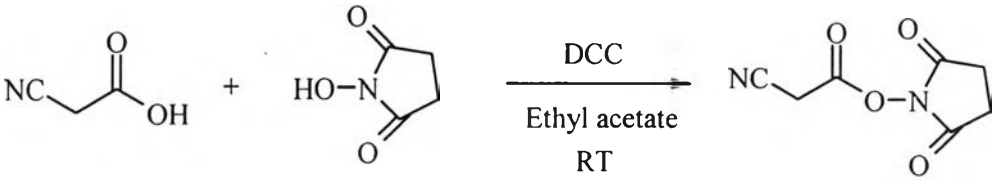
Found: 895.4

Elemental Analysis for $C_{50}H_{44}N_4O_6S_2$

Anal calc: C, 69.75; H, 5.15; N, 6.51

Found: C, 67.70; H, 5.16; N, 6.06

2.2.14 Preparation of cyanoacetic acid active ester (3-[(2,5-dioxopyrrolidin-1-yl)oxy-3-oxopropane nitrile) (19)



19

In a 250 mL two-necked round bottom flask equipped with a magnetic bar, cyanoacetic acid (0.851 g, 10.0 mmol) was dissolved in ethyl acetate (100 mL) and stirred under nitrogen atmosphere at room temperature. A solution of *N*-hydroxy succinimide (1.151 g, 10.0 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC, 2.063 g, 10.0 mmol) in ethyl acetate (50 mL) was added dropwise from the addition funnel. The mixture was left stirring overnight at room temperature and filtered. The filtrate was evaporated to near dryness under reduced pressure and treated with isopropanol to give **19** as a white solid (1.113 g, 61.1%).

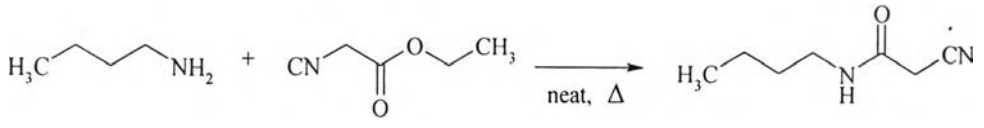
Characterization data for 19

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

3.87 (s, 2H, C*H*$_2$CN)

2.92 (s, 4H, C*H*$_2$CON)

2.2.15 Preparation of *N*-butyl-2-cyanoacetamide (20)



20

In a 250-mL two-necked round bottom flask equipped with a magnetic bar, ethyl cyanoacetate (1.547 g, 13.7 mmol) was mixed with *n*-butylamine (1.000 g, 13.7 mmol). The mixture was stirred and heated at 80 °C under nitrogen atmosphere for 3 h. After cooling, the residue was taken up with CH_2Cl_2 and treated with hexane to give **20** as a white solid (1.422 g, 74.2%)

Characterization data for 20

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

6.13 (s, 1H, N*H*)

3.36 (s, 2H, CH_2CN)

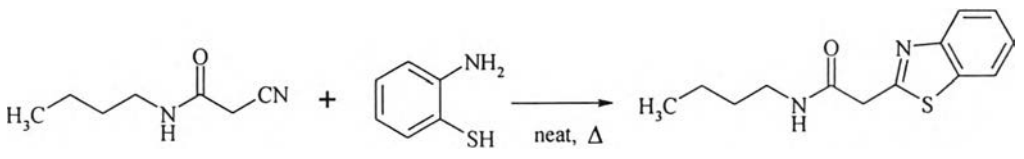
3.33 (q, *J* = 6.8 Hz, 2H, CH_2NH)

1.53 (qnt, *J* = 7.6 Hz, 2H, CH_2CH_2CH_3)

1.41-1.31 (m, 2H, CH_2CH_3)

0.94 (t, *J* = 7.2 Hz, 3H, CH_3)

2.2.16 Preparation of 2-(1,3-benzothiazol-2-yl)-*N*-butylacetamide (21)



21

In a 100-mL one-necked round bottom flask equipped with a magnetic bar , *N*-butyl-2-cyanoacetamide **20** (0.510 g, 3.64 mmol) was mixed with 2-aminothiophenol (0.540 g, 4.31 mmol) and heated under N_2 atmosphere at 120 °C for 3 h. After cooling, the product was taken up with CH_2Cl_2 and loaded on silica column. The column was first washed with CH_2Cl_2 to remove excess 2-aminothiophenol after then gradient eluted with CH_2Cl_2-ethyl acetate. The fraction containing the

benzothiazolylacetamido compound was evaporated to dryness under vacuum to give **21** as a white solid (0.767 g, 84.9%).

Characterization data for 21

^{1}H-NMR spectrum (400 MHz, $CDCl_3$): δ (in ppm)

8.00 (d, *J* = 8.4 Hz, 1H, Ar*H*)

7.88 (d, *J* = 8.0 Hz, 1H, Ar*H*)

7.50 (t, *J* = 7.2 Hz, 1H, Ar*H*)

7.41 (t, *J* = 7.6 Hz, 1H, Ar*H*)

7.18 (br, 1H, N*H*)

4.05 (s, 2H, CH_2BT)

3.30 (q, *J* = 7.6 Hz, 2H, CH_2NH)

1.51 (qnt, *J* = 7.6 Hz, 2H, CH_2CH_2NH)

1.39-1.30 (m, 2H, CH_2CH_3)

0.91 (t, *J* = 6.2 Hz, 3H, CH_3)

2.3 Cation Complexation Studies

2.3.1 Spectum change upon addition of metal ions

All solutions were prepared in 10% (v/v) acetonitrile in methanol. A stock solution of each ligand (2.5×10^{-4} M); supporting electrolyte (0.10 M); $CuCl_2$ (4.2×10^{-3} M); $NiCl_2$, $CdCl_2$, $HgCl_2$, $CaCl_2{\cdot}2H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, KCl, NaCl (5.00×10^{-2} M); $Pb(NO_3)_2$ (1.00×10^{-2} M) were prepared.

A supporting electrolyte was tetraethyl ammonium chloride for all metals except Pb^{2+} where the supporting electrolyte was tetrabutyl ammonium hexafluorophosphate.

The complexation studies were done by pipetting 1.00 mL of the stock ligand solution into each of a 5 mL volumetric flask followed by 0.50 mL of 0.10 M supporting electrolyte. A metal stock solution (0.30 mL for Cu^{2+}, 0.20 mL for Ni^{2+}, Cd^{2+}, Hg^{2+}; 0.50 mL for Ca^{2+}, Mg^{2+}, K^+, Na^+ ; 1.00 mL for Pb^{2+}) were then added to each flask and the content was diluted to the mark and mixed well. A blank (a metal solution with supporting electrolyte but *without ligand*) were prepared in the same

way. The solution was left at room temperature overnight and the spectrum was recorded from 250-400 nm against the solvent.

2.3.2 Studies of ligand spectrum

Four compounds were prepared in 10% (v/v) acetonitrile in methanol. 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(cyanoacetamidobutoxy)calix[4]arene (**5**) (5.0×10^{-5} M); 5,11,17,23-tetra-*p*-*tert*-butyl-25,27-bis(benzothiazolylacetamido-butoxy)calix[4]arene (**15**) (5.0×10^{-5} M); *N*-butyl-2-cyanoacetamide (**20**) (1.0×10^{-3} M); 2-(1,3-benzothiazol-2-yl)-*N*-butylacetamide (**21**) (5.0×10^{-5} M).

2.3.3 Determination of stoichiometry of metal complex by Jobs method

The Jobs method was performed at two *total* concentrations, 5.0×10^{-5} M and 1.0×10^{-4} M.

All solutions were prepared in 10% (v/v) acetonitrile in methanol. For total concentration of 5.0×10^{-5} M, the concentration of ligand and metal solution was 5.0×10^{-5} M in 0.01 M tetraethyl ammonium chloride. A 1.60 mL of ligand solution was pipetted into 1-cm cell containing magnetic bar followed by 0.40 mL of metal solution. The mixed solution was stirred for 30 seconds and the spectrum was recorded. This solution corresponds to mole fraction of metal of 0.20. A similar experiments were performed at ligand solution: metal solution volume of 1.40:0.60, 1.20:0.80, 1.00:1.00, 0.80:1.20, 0.60:1.40, 0.60:1.40, 0.40:1.60 which correspond to mole fraction of metal of 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, respectively.

In case of Cu^{2+} and Hg^{2+}, a metal spectrum corresponded to each mole fraction was recorded by replacing ligand solution with 0.01 M tetraethyl ammonium chloride.

2.3.4 Determination of stability constants by UV-vis titrations

All solutions were prepared in 10% (v/v) acetonitrile in methanol. A solution of 5.0×10^{-5} M of the ligands in a 0.01 M supporting electrolyte were prepared from a stock solution. The concentration of titrant (metal ion) was 4.0×10^{-4} M in 0.01 M

supporting electrolyte. A supporting electrolyte were tetraethyl ammonium chloride for Cu^{2+} and Hg^{2+} and tetrabutyl ammonium hexafluorophosphate for Pb^{2+}.

Ligand solution, 2 mL, was placed in a spectrophotometric cell of 1-cm path length. The spectrum was recorded from 250-400 nm. The solution of a metal was added successively into the cell from a microburette. The mixture was stirred for 30 seconds after each addition and its spectral variation was recorded. The volume added was 0.04 mL (10 additions), 0.08 mL (5 additions) and 0.10 mL (7 additions) which leads to 23 spectra including the ligand spectrum. The stability constants were calculated from spectrometric data using program SIRKO [63].

2.4 Ion-Selective Electrode Studies

2.4.1 Ion-selective electrode preparation

The ion-selective membrane was prepared as described by Craggs, Moody and Thomas [64]. The general procedure is as follows: the weighed amount of ligands, plasticizer, KTClPB and PVC were mixed in a vial with 6 mL THF (Table 2.1). The content was stirred and mixed thoroughly until dissolved. The solution was poured into glass casting ring resting on the glass plate. After placing a pad of filter papers weighted with a wooden block on top of the glass ring, the solvent was allowed to evaporate for two days at room temperature. The flexible, transparent membrane of about 0.4 mm was obtained. The weight percent composition of the membrane was 1.0:65.6:33.0:0.40 (ligand : *o*-NPOE : PVC : KTClPB).

Four to five PVC-tubes (5 mm i.d., 3 mm-wall thickness, 30 mm long) was glued at one end with PVC-THF solution and put on the membrane. After the solvent was evaporated, the membrane around each tube was cut with a knife to obtain a PVC-tube with membrane sealed at one end. The open-end of the PVC-tube was then fitted into a glass tube after which made into a ISE by filling with 0.10 M $Cu(NO_3)_2$ and putting a Ag/AgCl electrode in. The ISE was pre-conditioned by immersing in a 0.010 M $Cu(NO_3)_2$ solution for 12 h before used.

Table 2.1 Composition of ISE-membrane

ISE	Ligand	membrane composition/g			
		o-NPOE	PVC	KTClPB	ligand
1	**15**	0.2668	0.1339	0.0018	0.0044
2	**16**	0.2674	0.1335	0.0016	0.0044
3	**17**	0.2672	0.1325	0.0017	0.0044
4	**18**	0.2654	0.1321	0.0020	0.0041

2.4.2 Determination of potentiometric selectivity coefficient

Fifty milliliter of water was pipetted into a 50-mL beaker with magnetic bar and placed on a magnetic stirrer. The ISE and reference electrode were immersed. The emf was monitored while stirring until the steady emf was obtained (drift less than 0.1 mV in 5 seconds). The calibration curve was then constructed by adding different amount of stock solutions of $Cu(NO_3)_2$ successively from autopipette as follow: 5 μL of 1.00×10^{-3} M ; 50 μL of 1.00×10^{-3} M; 5 μL of 0.100 M ; 50 μL of 0.100 M; 50 μL of 1.00 M; 500 μL of 1.00 M. The steady state potentials were recorded after each addition. This resulted in a six potential corresponding to six concentration of 1.0×10^{-7}, 1.1×10^{-6}, 1.1×10^{-5} 1.1×10^{-4}, 1.1×10^{-3} and 1.1×10^{-2} M Cu^{2+}.

The determination of potentiometric selectivity coefficient by fixed interference method was determined as above but water was replaced by an aqueous solution of interfering ions:

1.00×10^{-4} M $Pb(NO_3)_2$,

1.00×10^{-3} M $Cd(NO_3)_2 \cdot 4H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$

1.00×10^{-2} M $Ca(NO_3)_2 \cdot 4H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, KNO_3, $NaNO_3$